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# **SYNTHESIS OF N-HYDROXYPYRAZINONES AS POTENTIAL HIV INHIBITORS**

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**“***Imagination is more important than knowledge.”*

- *Albert Einstein* -



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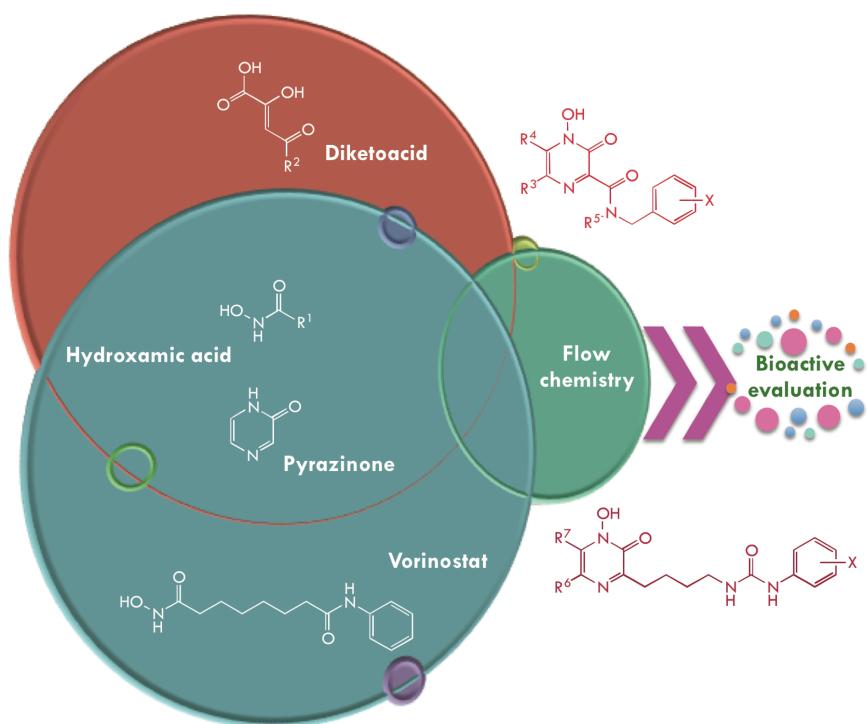
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Thank you,  
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Anh Hung Mai

## Summary

Hydroxamic acids, with the two bioactive representatives aspergillic acid (naturally occurring) and vorinostat (synthetic), are well known for their strong binding ability with many metal ions. This explains their potential to inhibit specific metal-containing enzymes involved in e.g. human immunodeficiency virus and cancer progression.

Our group has previously been working on the design and synthesis of highly functionalized pyrazinones. Therefore, it was of interest to us to combine these two classes of compounds into new compounds resembling integrase inhibitor diketoacid-based structures and histone deacetylase inhibitors.



Functionalization of C-3 of *N*-hydroxypyrazinones has been thoroughly investigated in this thesis resulting in the successful synthesis of a library containing three series of

novel compounds, including 3-alkyl-/carboxamide-/ureidoalkyl-1-hydroxypyrazinones.

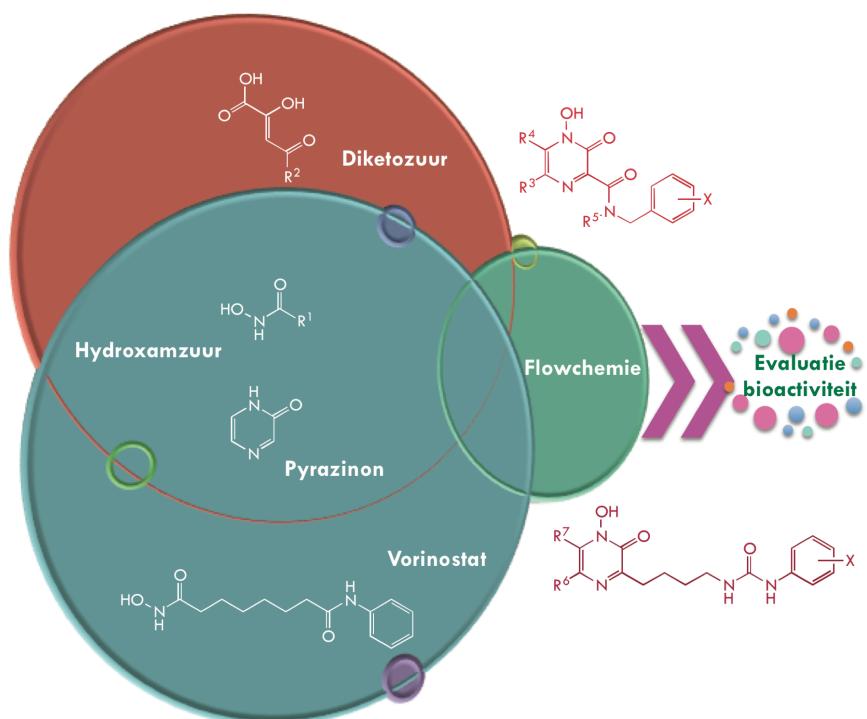
With the implementation of flow chemistry technology and catalytic transfer hydrogenation, we have succeeded in delivering a reproducible methodology for the selective debenzylation of O-benzyl protected N-hydroxypyrazinones, with a potential for scaling up to gram scale on suitable flow apparatus.

Numerous N-hydroxypyrazinones have been synthesized with HIV inhibitory activity in mind. However, *in vitro* evaluation of anti-HIV-1 and HIV-2 replication on human T-lymphocyte cells using an MTT assay showed that none of the synthesized compounds exhibits the inhibitory activity. Some compounds do however show inhibition in an HIV-1 reverse transcriptase polymerase assay.

## Samenvatting

Hydroxamzuren, met als typerende voorbeelden het bioactieve aspergilluszuur (natuurlijk) en vorinostat (synthetisch), zijn welbekend vanwege hun vermogen om verschillende metaalionen sterk te binden. Deze eigenschap verklaart ook ondermeer hun potentieel om metaal bevattende enzymen, waaronder deze die betrokken zijn in de proliferatie van het HIV en het verloop van kancers, specifiek te inhiberen.

Aangezien onze onderzoeksgroep een uitgebreide *know-how* bezit met betrekking tot de bereiding van hoog-gefunctionaliseerde pyrazinonen, verdiende het onze aandacht om deze twee klassen van verbindingen (hydroxamzuren en pyrazinonen) te combineren tot nieuwe verbindingen die een sterke chemisch-structurele gelijkenis vertonen met diketozuur gebaseerde integrase-inhibitoren en histondeacetylase-inhibitoren.



De C3-functionalisatie van *N*-hydroxypyrazinonen werd in deze thesis grondig bestudeerd. Dit leidde tot de succesvolle synthese van een bibliotheek met drie nieuwe klassen van verbindingen, waaronder 3-alkyl-, 3-carboxamide-, en 3-ureidoalkyl-1-hydroxypyrazinonen.

Met behulp van *flow* chemie en katalytische transfer-hydrogenering werd een reproduceerbare methodologie ontwikkeld voor de selectieve debenylering van O-benzyl-beschermde *N*-hydroxypyrazinonen. Deze methodologie kan opgeschaald worden van milligramschaal naar gramschaal met behulp van gepaste *flow*-instrumenten.

Talrijke *N*-hydroxypyrazinonen werden bereid met het oog op HIV inhibitie activiteit. De resultaten verkregen uit de *in vitro* biologische evaluatie van HIV-1 en HIV-2 replicatie in humane T-lymfocytcellen (door middel van een MTT-test) hebben echter aantoond dat geen enkele van de verbindingen inhibitorische activiteit vertonen. Een aantal verbindingen daarentegen vertoont echter wel inhibitie activiteit in een HIV-1 reverse transcriptase polymerase test.

## List of Abbreviations and Symbols

<b>δ</b>	Chemical shift in parts per million downfield from tetramethylsilane
<b>Ø</b>	Mole fraction of a solvent in an aqueous solution
<b>ABTS</b>	2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
<b>Ac</b>	Acetyl
<b>ACN</b>	Acetonitrile
<b>AIDS</b>	Acquired immune deficiency syndrome
<b>All</b>	Allyl
<b>AMD3100</b>	1,1'-[1,4-Phenylenebis(methylene)]bis-1,4,8,11-tetraazacyclotetradecane
<b>API-ES</b>	Atmospheric pressure ionization electrospray
<b>aq</b>	Aqueous
<b>Ar</b>	Aryl
<b>ART</b>	Antiretroviral therapy
<b>Asp</b>	Aspartic acid (D)
<b>AZT</b>	Azidothymidine
<b>B3LYP</b>	3-Parameter hybrid Becke exchange/Lee-Yang-Parr correlation functional
<b>brd</b>	Broad doublet (in NMR spectrometry)
<b>Bn</b>	Benzyl
<b>Boc</b>	<i>tert</i> -Butoxycarbonyl
<b>BPR</b>	Backpressure regulator
<b>brs</b>	Broad singlet (in NMR spectrometry)
<b>calcd</b>	Calculated
<b>Cbz</b>	Carbobenzyloxy
<b>CCID50</b>	Cell culture infectious dose
<b>CCR5</b>	Chemokine receptor type 5
<b>CD4</b>	Cluster of differentiation 4 (a glycoprotein on surface of immune cells)
<b>CDCl<sub>3</sub></b>	Deuterated chloroform
<b>CD<sub>3</sub>OD</b>	Deuterated methanol
<b>Compd.</b>	Compound
<b>CXCR4</b>	Chemokine receptor type 4
<b>Cy</b>	Cyclohexyl
<b>CYP3A4</b>	Enzyme responsible for oxidizing foreign organic molecules in metabolism

<b>d</b>	Doublet (in NMR spectrometry)
<b>DCE</b>	1,2-Dichloroethane
<b>DCM</b>	Dichloromethane
<b>dd</b>	Doublet of doublet (in NMR spectrometry)
<b>DFT</b>	Density functional theory
<b>DPEA</b>	<i>N,N</i> -Diisopropylethylamine
<b>DMAP</b>	4-( <i>N,N</i> -Dimethylamino)pyridine
<b>DMF</b>	Dimethylformamide
<b>DMSO-d<sub>6</sub></b>	Deuterated dimethyl sulfoxide
<b>DNA</b>	Deoxyribonucleic acid
<b>dNTP(s)</b>	Deoxyribonucleotide triphosphate(s)
<b>DPP</b>	Diphenylphosphine
<b>dt</b>	Doublet of triplet (in NMR spectrometry)
<b>DTG</b>	Dolutegravir
<b>EC<sub>50</sub></b>	Half maximal effective concentration
<b>EDCI</b>	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
<b>EI</b>	Electron impact (ionization)
<b>equiv</b>	Equivalent
<b>ESI</b>	Electrospray ionization
<b>Et</b>	Ethyl
<b>EtOAc</b>	Ethyl acetate
<b>EtOH</b>	Ethanol
<b>eV</b>	Electron volt
<b>EVG</b>	Elvitegravir
<b>FDA</b>	US Food and Drug Administration
<b>Fmoc</b>	Fluorenylmethyloxycarbonyl
<b>FT</b>	Fourier transform
<b>G9a</b>	Methyltransferase (an enzyme methylates lysine 9 in histone H3)
<b>Glu</b>	Glutamic acid (E)
<b>Gly</b>	Glycine (G)
<b>HAART</b>	Highly active antiretroviral therapy
<b>HCV</b>	Hepatitis C virus
<b>HDAC</b>	Histone deacetylase
<b>His</b>	Histidine (H)
<b>HIV</b>	Human immunodeficiency virus
<b>HMBC</b>	Heteronuclear multiple bond correlation
<b>HOBr</b>	Hydroxybenzotriazole

<b>HPLC</b>	High performance (pressure) liquid chromatography
<b>HRMS</b>	High resolution mass spectrometry
<b>HSE</b>	Health, Safety, and Environment
<b>Hz</b>	Hertz
<b>I</b>	Ionic strength
<b>i-Bu</b>	Isobutyl
<b>i-Pr</b>	Isopropyl
<b>IC<sub>50</sub></b>	Half-maximum inhibitory concentration
<b>ID</b>	Inner diameter
<b>IDA</b>	Iminodiacetic acid
<b>IN</b>	Integrase
<b>J</b>	Coupling constant (in NMR spectrometry)
<b>LC</b>	Liquid chromatography
<b>LiHMDS</b>	Lithium hexamethyldisilazane or lithium bis(trimethylsilyl)amide
<b>LRMS</b>	Low resolution mass spectrometry
<b>Lys</b>	Lysine (K)
<b>m</b>	Multiplet (in NMR spectrometry)
<b>M</b>	Molar (moles per liter)
<b>m/z</b>	Mass-to-charge ratio
<b>Me</b>	Methyl
<b>MHz</b>	Megahertz
<b>MIC</b>	Minimum inhibitory concentration
<b>MilliQ</b>	Ultrapure water of type 1 (from Millipore Corporation)
<b> mM</b>	Millimolar (millimoles per liter)
<b>MMP</b>	Matrix metalloproteinase (also mitochondrial membrane permeability)
<b>mol</b>	Mole(s)
<b>MS</b>	Mass spectrometry
<b>MT-4</b>	Human T-lymphocyte cells
<b>MTT</b>	Tetrazolium-based colorimetric assay [using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]
<b>Mw</b>	Molecular weight
<b>NaHMDS</b>	Sodium bis(trimethylsilyl)amide
<b>NMM</b>	<i>N</i> -Methylmorpholine
<b>NMP</b>	<i>N</i> -Methyl-2-pyrrolidone
<b>NMR</b>	Nuclear magnetic resonance
<b>NNRTIs</b>	Non-nucleoside reverse transcriptase inhibitors
<b>NOE</b>	Nuclear Overhauser effect

<b>NRTIs</b>	Nucleoside reverse transcriptase inhibitors
<b>OD</b>	Outer diameter; optical density
<b>OM-10.1</b>	Latently HIV-infected cell line
<b>OTf</b>	Triflate or Trifluoromethanesulfonate
<b>PDB</b>	Protein data bank
<b>Pent</b>	Pentyl
<b>PG</b>	Protecting group
<b>Ph</b>	Phenyl
<b>PMB</b>	p-Methoxybenzyl
<b>PML</b>	Promyelocytic leukemia
<b>ppm</b>	Part(s) per million
<b>PTFE</b>	Polytetrafluoroethylene
<b>q</b>	Quartet (in NMR spectrometry)
<b>quint</b>	Quintet (in NMR spectrometry)
<b>RAL</b>	Raltegravir
<b>RNA</b>	Ribonucleic acid
<b>RP</b>	Reversed phase
<b>rpm</b>	Revolutions per minute (frequency of a rotation)
<b>rt</b>	Room temperature
<b>R<sub>t</sub></b>	Residence time
<b>RT</b>	Reverse transcriptase
<b>s</b>	Singlet (in NMR spectrometry)
<b>SAR</b>	Structure-activity relationship
<b>sec-Bu</b>	sec-Butyl
<b>t</b>	Triplet (in NMR spectrometry)
<b>t-Bu</b>	tert-Butyl
<b>TFA</b>	Trifluoroacetic acid
<b>THF</b>	Tetrahydrofuran
<b>TLC</b>	Thin-layer chromatography
<b>TPA</b>	Tetradecanoyl phorbol acetate (PMA)
<b>Tyr</b>	Tyrosine (Y)
<b>UNAIDS</b>	The Joint United Nations Programme on HIV and AIDS
<b>UV-VIS</b>	Ultraviolet-Visible spectrophotometry
<b>v/v</b>	Volume-to-volume ratio
<b>VPA</b>	Vaproic acid
<b>w/w</b>	Weight-to-weight ratio
<b>WHO</b>	World Health Organization

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# Chapter 1

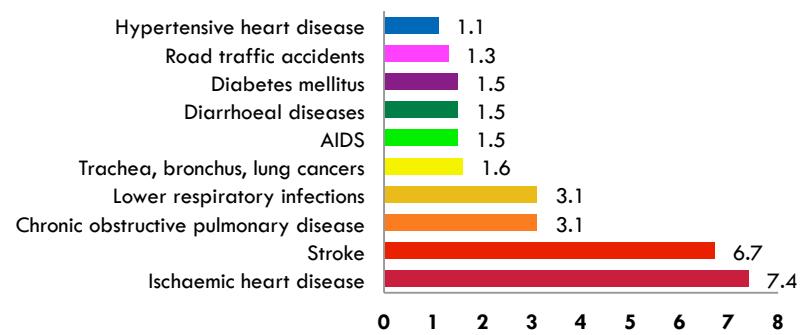
## Introduction

- 1.1 HIV and enzyme inhibitors
  - 1.1.1 What is HIV/AIDS?
  - 1.1.2 HIV life cycle
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  - 1.1.4 Current ART strategies
  - 1.1.5 IN inhibitors and HDAC inhibitors – Mechanism of action
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- 1.4 Continuous flow techniques as tools for organic synthesis
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## 1.1 HIV and enzyme inhibitors

According to estimates by WHO and UNAIDS, at the end of 2013, globally about 35 million people were infected with the human immunodeficiency virus (HIV), an additional 2.1 million people were diagnosed, and 1.5 million died of acquired immunodeficiency syndrome (AIDS) related causes.<sup>1</sup> Together with cardiovascular diseases and cancers, this makes AIDS disease one of the leading causes of death in the world (**Figure 1.1**).<sup>2</sup>



**Figure 1.1.** Causes of death in the world (WHO 2012, estimated number of deaths in millions).

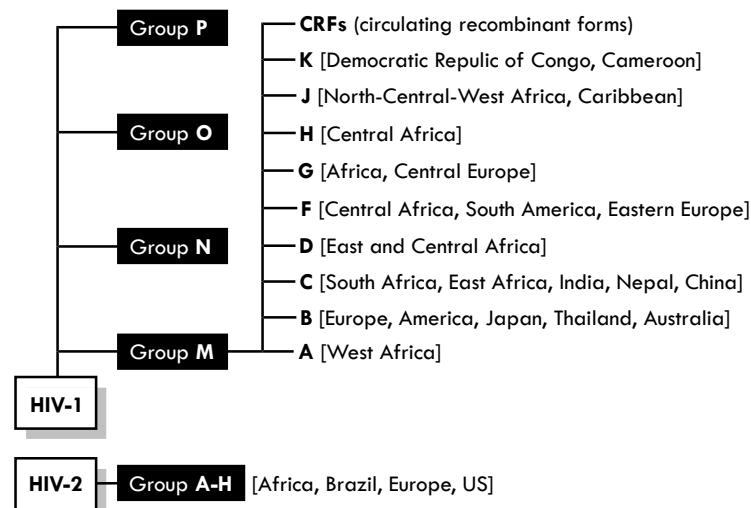
Due to the high genetic variability of HIV and its ability to escape the host immune response, it is very difficult to develop therapeutic and preventive drugs.<sup>3</sup> To date, neither an AIDS' cure nor an efficient HIV vaccine has been available.<sup>4</sup>

### 1.1.1 What is HIV/AIDS?

After infecting cells of the immune system, HIV destroys or damages their function, weakening the immune system and leading to immune deficiency. Opportunistic infections associated with immunodeficiency will then take place.

HIV infection is characterized by three stages: acute infection (2-4 weeks, flu-like symptoms), clinical latency (about 10 years without treatment, no significant symptoms), and AIDS (about 3 years without treatment). AIDS is the most progressive stage of HIV infection. It is defined by the occurrence of any of more than 20 opportunistic infections or HIV-related cancers.

There are two major types of HIV, HIV-1 and HIV-2.<sup>5</sup> The origin of HIV-1 is still unclear, it is however believed to be closely related to SIV (simian immunodeficiency virus, found in chimpanzees and gorillas) in genetic organization.<sup>6-11</sup> While with HIV-2, there were evidences that it was original from sooty mangabeys, and it has somehow transmitted to human in the rural area of West Africa.<sup>7,8,10,12</sup> Although both HIV-1 and HIV-2 are known to cause AIDS, HIV-2 is considered less virulent and less infective as compared to HIV-1, the latter being the majority cause of global HIV infections. Each is further divided into groups and subtypes, which are summarized in **Figure 1.2.**<sup>11</sup> The term HIV used in this thesis will refer to HIV-1 unless noted otherwise.



**Figure 1.2.** HIV types, groups, and subtypes [and their distributions].

### 1.1.2 HIV life cycle

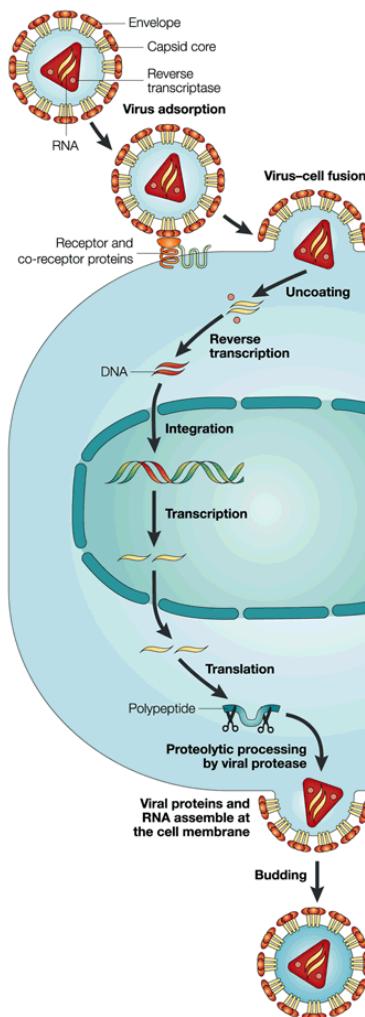
The main target of HIV when infecting the human body is the CD4 cell (T-cell or CD4 lymphocyte). The virus will go through multiple steps to reproduce itself and create many more virus particles (**Figure 1.3**).<sup>13-17</sup>

#### Step 1. Binding and fusion

HIV binds to receptors and co-receptors on the surface of the CD4 cell, “unlocks” the cell membrane, and releases its genetic material into the cell.

### Step 2. Reverse transcription

Reverse transcriptase (enzyme from HIV) transcribes the HIV-RNA into HIV-DNA so that HIV can enter the nucleus of the CD4 cell.



### Step 3. Integration

HIV-DNA uses an enzyme (integrase) to integrate itself with the CD4 cell DNA.

### Step 4. Transcription

When HIV is integrated, the virus starts using the machinery of the CD4 cell to create long HIV protein strands as building blocks for more HIV.

### Step 5. Assembly

A protease cuts up the long HIV protein strands. These smaller HIV proteins combine with HIV-RNA to assemble a new virus.

### Step 6. Budding

The new virus pushes itself out of the CD4 cell, taking with it part of the membrane of the cell. This outer part covers the virus and contains all of the structures necessary for binding to CD4 cells and entering a new life cycle.

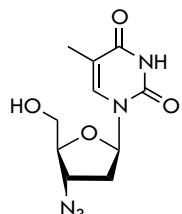
**Figure 1.3.** Replication cycle of HIV. Reprinted with permission from ref [13]. Copyright © 2002 Nature Publishing Group.

#### 1.1.3 HIV treatment

Nowadays, HIV infection is manageable with the use of proper HIV therapies such as antiretroviral therapy (ART). ART helps people infected

with HIV live longer and healthier lives, and decreases the number of people dying from AIDS-related diseases.<sup>18</sup> The more drugs are licensed, the lower the cost of ART treatment for HIV becomes. If the annual cost of standard HIV treatment per patient was over \$10,000 in the late 1990s, it is now going down by more than 99% (about \$60/patient/year).<sup>19</sup>

AZT (azidothymidine, 1987, **Figure 1.4**) was the first FDA approved drug for treatment of HIV infection.<sup>20,21</sup> More than 30 HIV medicines are currently being used for treatment of HIV infection and many more are under development.<sup>22,23</sup> They are divided into several different classes based on their function in blocking HIV at different stages in its life cycle.



**Figure 1.4.** Structure of AZT (azidothymidine).

#### 1.1.4 Current ART strategies

Based on their inhibitory mechanisms, antiretroviral agents used in current ART strategies can be divided into two main groups: (i) inhibitors that directly target the virus and (ii) inhibitors that target CD4 cell factors.<sup>16,17,22,24-33</sup>

##### Inhibitors that directly-target the virus:

- Entry and fusion inhibitors

Entry inhibitors prevent the virus from entering CD4 cells by blocking the interaction between HIV and CD4 cell receptors/co-receptors. Fusion inhibitors target the interaction sites on either HIV or CD4 cells and prevent the conformational changes that are required for the membrane fusion.

- Integrase inhibitors

Integrase (IN) inhibitors block the integrase enzyme, and prevent the virus from adding its DNA into the CD4 cells' DNA. The replication and virus creation process is thus inhibited.

- Polymerase inhibitors

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) directly block the reverse transcriptase enzyme, and prevent HIV from functioning correctly in making copies of its own DNA.

Nucleoside reverse transcriptase inhibitors (NRTIs) work as faulty building blocks that prevent the virus from using reverse transcriptase to correctly build new DNA in the reproduction process of HIV-DNA.

- Protease inhibitors

Protease inhibitors block the protease enzyme, and prevent the long HIV protein strands from being cut up into functional pieces.

- HDAC inhibitors

Histone deacetylase (HDAC) inhibitors reactivate latent HIV resting within CD4 cells; hence, the virus is able to continue the new virus production and replication, which can be prevented with the use of ongoing ART.

#### **Inhibitors that target the host cell factors:**

- CYP3A4 inhibitor

Cytochrome P450 3A4 (CYP3A4) is an important enzyme and its function is to oxidize foreign organic molecules so that they can be removed from the body. Hence, inhibition of CYP3A4 could increase absorption and decrease hepatic metabolism of protease inhibitors.

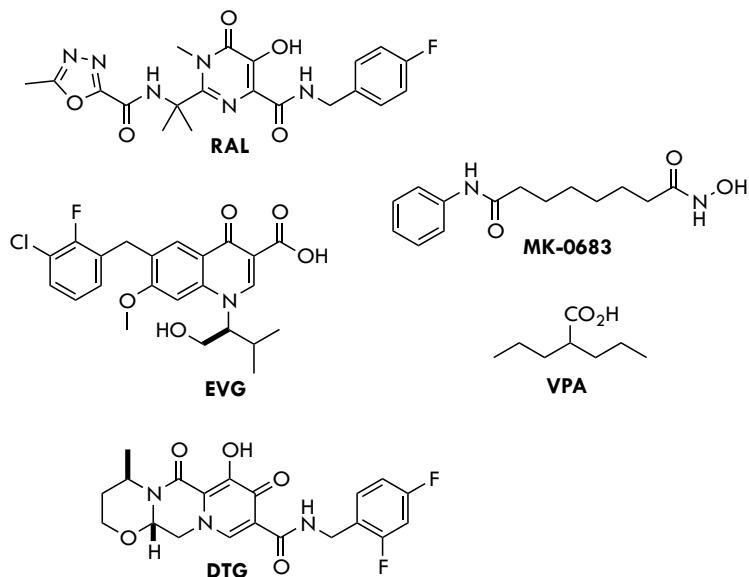
- HIV co-receptors antagonists

These inhibitors block the interaction of HIV and CCR5 and/or CXCR4 receptors.

Despite the fact that HIV ART has shown significant progress lately and that diverse antiretroviral drugs have been licensed for HIV treatment and that many more are in the late stages of clinical trials, antiretroviral drug development and improvement are still necessary to overcome the issues of viral resistance, drug-related side effects, and high doses as well as to make new drugs become more effective, accessible, and affordable.<sup>13,18,34,35</sup> Because even with highly active ART (HAART, a

therapy that combines several antiretroviral medicines from at least two different classes to suppress the replication process of virus in at least two different ways) annually costs drop to less than hundreds (not thousands) of US dollars per patient, the therapy still seems unaffordable in many underdeveloped and developing countries.

The main principle of drug discovery is to explore the molecular targets of existing drugs to design new improved drugs. HIV integrase and HIV latency are important targets due to their crucial roles in the integration, a fundamental step in the reproduction cycle of HIV. While there are three known FDA approved IN inhibitors: raltegravir (RAL, 2007), elvitegravir (EVG, 2012), and dolutegravir (DTG, 2013), HDAC inhibitors including valproic acid (VPA) and vorinostat (MK-0683) are still under phase II studies (**Figure 1.5**).<sup>18</sup>

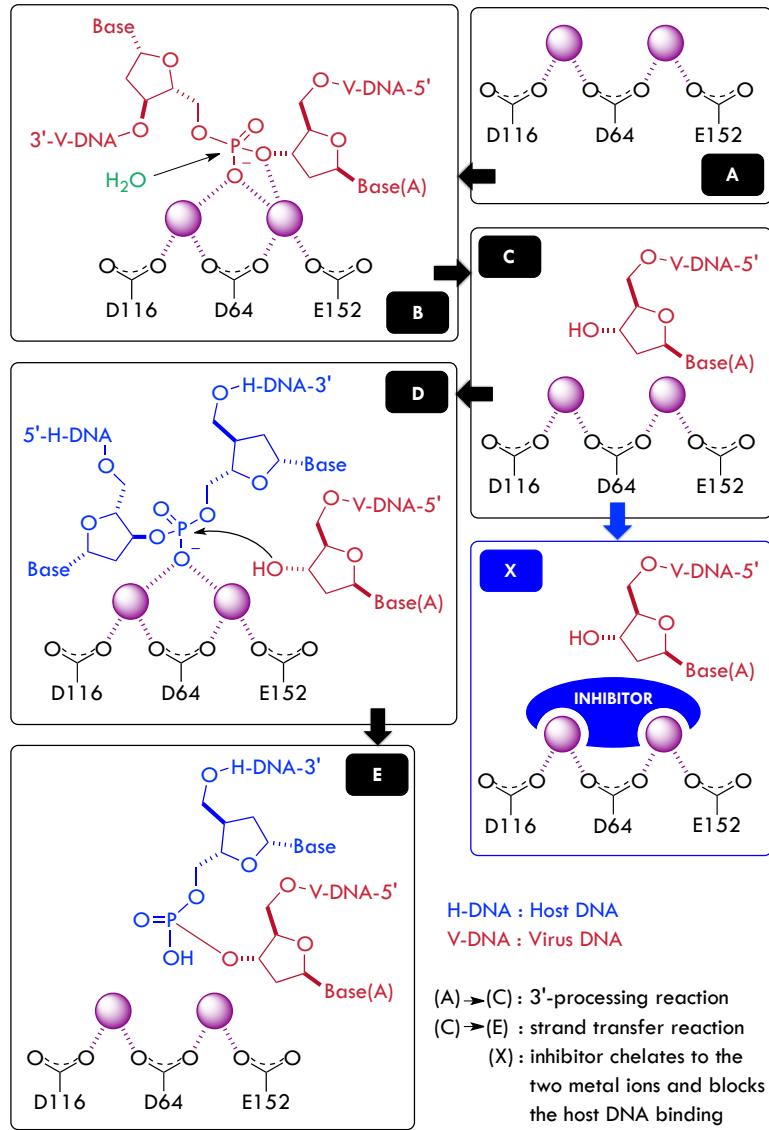


**Figure 1.5.** Structure of IN inhibitors and HDAC inhibitors.

### 1.1.5 IN inhibitors and HDAC inhibitors - Mechanism of action

#### IN inhibition mechanism

During the integration process, IN catalyzes the 3'-processing reaction and strand transfer reaction (**Figure 1.6**).<sup>36-40</sup>



**Figure 1.6.** Two-metal-ion catalysis and chemical structural IN inhibition mechanism. Copyright © 2006 Elsevier Ltd. Re-adapted from ref [36].

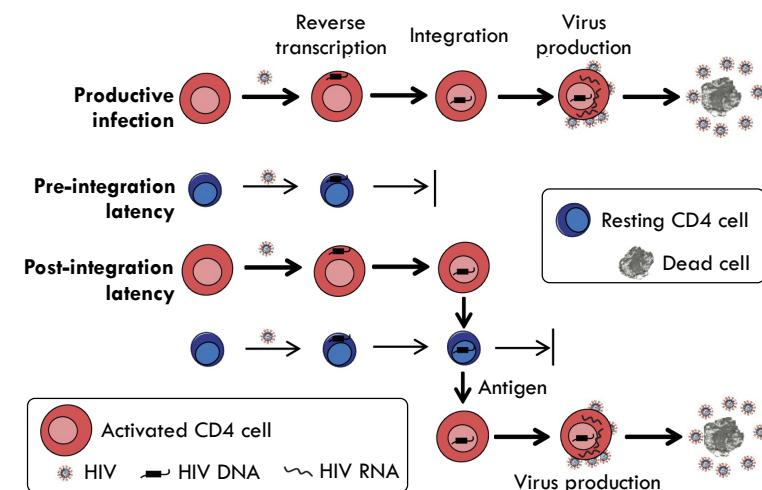
At first, IN recognizes the adenine base conserved in the third position from 3'-end of the viral DNA, and then activates the next phosphate ester with the two metals (A-B). The pre-integration complex is formed (B-C) and transported into the nucleus of CD4 cell, where it binds to the

CD4 DNA, activating a phosphate ester by the two  $Mg^{2+}$  ions (C-D). The viral DNA joins the CD4 DNA via an  $S_{N}2$ -like nucleophilic reaction (D-E).<sup>41-43</sup>

In the presence of an IN inhibitor, this process is stopped at stage (C), where it binds to the catalytic triad (D64, D116, E152) on the active site of the pre-integration complex, blocking the CD4 DNA binding of viral DNA (stage X).

#### HDAC inhibition mechanism

During its replication life cycle, pre-/post-integrated HIV DNA can turn into a latent state in which it is transcriptionally silent in the CD4 reservoir and becomes unaffected with ART. Two forms of HIV latency are described in **Figure 1.7**.<sup>44,45</sup> The formation of HIV latency is a complex process that might involve multiple mechanisms restricting productive viral transcription.<sup>46</sup>

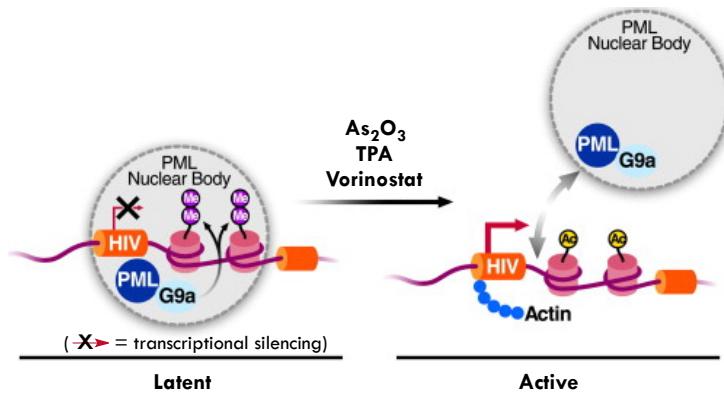


**Figure 1.7.** Latent HIV infection. Copyright © 2013 Wiley Periodicals, Inc. Re-adapted from ref [45].

It is still unclear how vorinostat or other antigens (e.g. arsenic trioxide  $As_2O_3$ , tetradecanoyl phorbol acetate TPA) exactly reactivate latent HIV; however, the mechanism of the reactivation of latent HIV might involve in the colocalization of latent HIV with the promyelocytic

leukemia (PML) nuclear body, which is thought to play a certain role in the regulation of gene expression.<sup>47-50</sup>

As described in **Figure 1.8**, PML is closely located to the latent HIV, producing methyltransferase G9a and leading to the dimethylation of lysine 9 in histone H3. These processes would be interrupted under the presence of latent antigens. The latent HIV therefore becomes active and binds to actin, which prevents the relocalization of HIV to PML nuclear bodies.<sup>48,49,51-53</sup>



**Figure 1.8.** Reactivation of latent HIV. Copyright © 2013 Elsevier Inc. Re-adapted from ref [48].

## 1.2 Metal-complexing small molecules as drug compounds

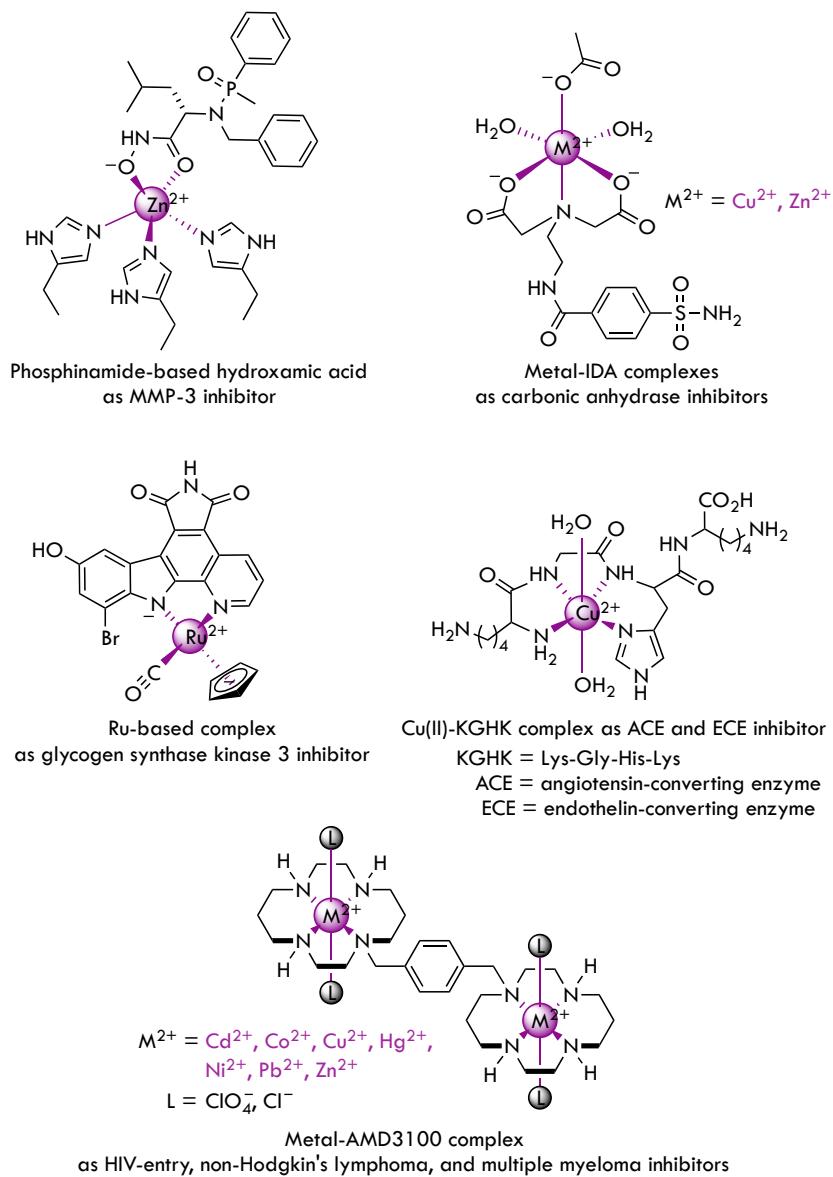
Metal ions play vital functions in biological systems, e.g. by enabling electron transport and charge distribution, by organizing and stabilizing protein structure, by binding and activating/inhibiting substrate or subdomain or protein, by catalyzing enzymatic transformations, etc.<sup>54-57</sup> Recently, the application of metal complexes in biology was thoughtfully reviewed by Haas and Franz.<sup>55</sup> Some essential metal ions in biological systems are summarized in **Table 1.1.**<sup>58</sup>

In the human body, metal ions (except for  $\text{Na}^+$  and  $\text{K}^+$ ) are usually complexed by enzymes and form the active site within the protein. Whereas the metal ion binding to a protein generally influences its bioactivity, surrounding chelating agents (or ligands) are able to alter the reactivity of metal ion center.<sup>54,59</sup>

**Table 1.1.** Important metal ions and their functions in biological system.<sup>58</sup>

Metal ion	Coordination number & Geometry	Ligand preference	Function
Na <sup>+</sup>	6 Octahedral	Carboxylate, ether, hydroxyl	Charge carrier, osmotic balance, nerve impulses
K <sup>+</sup>	6 7 Flexible 8	Carboxylate, ether, hydroxyl	Charge carrier, osmotic balance, nerve impulses
Mg <sup>2+</sup>	6 Octahedral	Carboxylate, phosphate	Structural role in hydrolases, isomerases, and phosphate transfer
Ca <sup>2+</sup>	6 7 Flexible 8	Carbonyl, carboxylate, phosphate	Structural, charge balance, reaction initiator, and phosphate transfer
Zn <sup>2+</sup>	4 or 5 Tetrahedral, square pyramid	Carbonyl, carboxylate, imidazole, thiolate	Structure in zinc fingers, gene regulation, anhydrases, dehydrogenases, and peptidases
Mn <sup>2+/3+</sup>	6 Tetragonal, octahedral	Carboxylate, hydroxide, imidazole, phosphate	Structural role in oxidases, photosynthesis
Fe <sup>2+/3+</sup>	4 or 6 Tetrahedral, octahedral	Carboxylate, oxide, phenolate, thiolate, imidazole, pyrrole	Electron transfer in oxidases, nitrogen fixation in nitrogenases, dioxygen transport in hemoglobin and myoglobin
Cu <sup>+2+</sup>	3 Trigonal planar, tetrahedral, 4 square planar, 5 square pyramid, 6 tetragonal	Carboxylate, imidazole, thioether, thiolate	Electron transfer, oxidases, and hydroxylases, dioxygen transport in hemocyanin
Co <sup>+2+/3+</sup>	4 or 6 Tetrahedral, octahedral	Carboxylate, imidazole, thioether, thiolate	Enzyme catalysis (alkyl group transfer in vitamin B12), oxidases
Ni <sup>2+</sup>	4 or 6 Square planar, octahedral	Imidazole, polypyrrrole, thioether, thiolate	Enzyme catalysis, hydrogenases, hydrolases
Mo <sup>4+/5+/6+</sup>	6 Octahedral	Carboxylate, oxide, phenolate, sulfide, thiolate	Enzyme catalysis, nitrogen fixation in nitrogenases, oxo transfer in oxidases
Cr <sup>3+</sup>	6 Octahedral	Oxygen-donors	Essential to carbohydrate, lipid metabolism

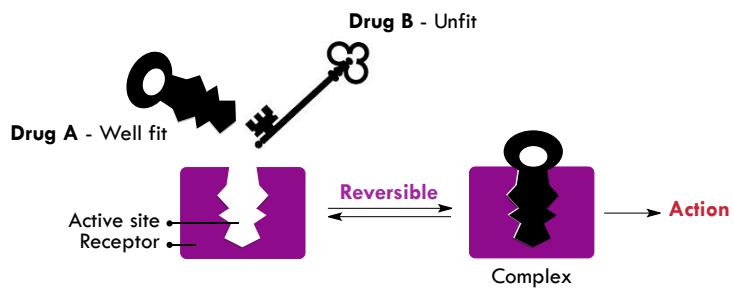
These properties in principle provide a powerful tool for inhibiting a particular enzyme by using synthetic small molecules as appropriate chelating agents. Current strategies in drug design and development are established based on this principle.<sup>55</sup> Some representative examples of metal-complexing small molecules as enzyme inhibitors are given in **Figure 1.9**.<sup>55,60-69</sup>



**Figure 1.9.** Selective examples of metal complexes as enzyme inhibitors.

Currently the pursuit of small molecules as effective enzyme inhibitors is the main focus in medicinal and biological chemistry.<sup>70,71</sup> Two major approaches in drug design have been developed so far.

In the traditional approach, small molecules are designed as drugs that can interact with disease-related protein (enzymes, receptors), the drugs bind to the active sites in an attempt to saturate these targets and then to make them either inactivate or to stimulate them, whichever has a therapeutic effect.<sup>72,73</sup> However, the limitation of these strategies is that the interaction of drug and protein is stoichiometric within a ‘lock-key’ theory (Emil Fisher, 1894, **Figure 1.10**).<sup>74</sup> The reversible nature of this binding leads to unacceptable issues of increased drug dose as well as side effects.<sup>74-77</sup>



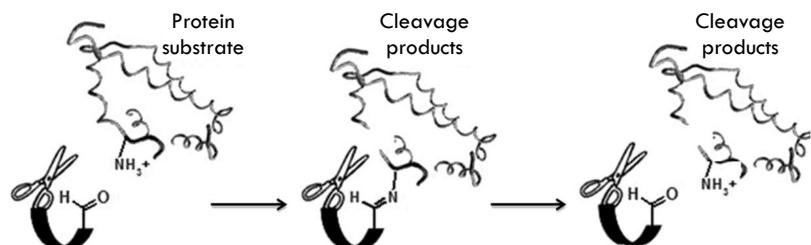
**Figure 1.10.** Illustration of the mechanism of action of traditional drugs.

Other approaches including the design and synthesis of catalytic metallodrugs are developed towards the promising irreversible inactivation or “suicide” inhibition or peptide-cleaving catalytic drugs,<sup>72,73</sup> are supposed to contain both a targeting domain (to selectively interact with the active site of receptor) and a catalytic metal center (to catalyze the irreversible reaction at the binding site).<sup>75,77,78</sup> In catalytic metallodrugs, metal ions play an important role, they help metallodrugs simultaneously accomplish many reactions, including oxidation, hydroxylation, and carbonylation of amino acid side chains, leading to the cleavage of the target-protein strand.<sup>77,79</sup>

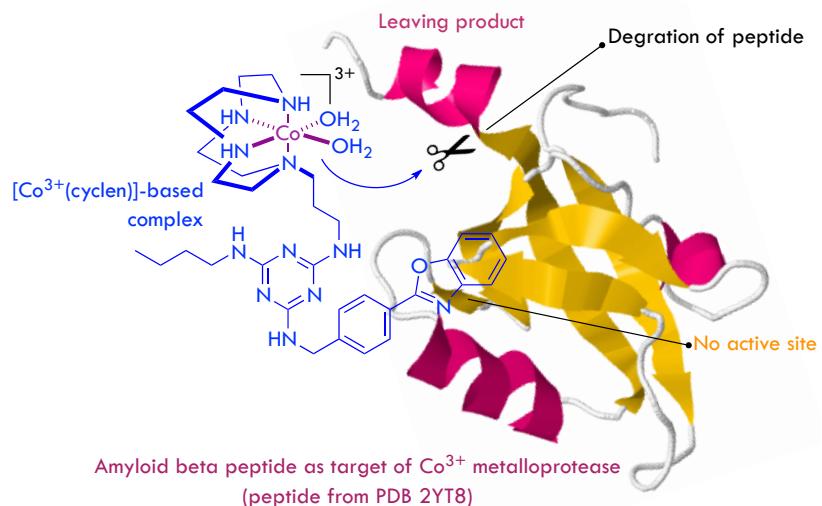
The mode of action involves the formation of a covalent bond between the inhibitor and the active site of the receptor (which may also potentially cause the target-inactivation as conventional drugs). Then a selective irreversible cleavage and/or destruction of the disease-target follows (**Figure 1.11**).<sup>80,81</sup>

With this mechanism, catalytic metallodrugs allow to enhance the possibility of lowering the doses and increasing the selectivity.<sup>75</sup> Another benefit of this

approach is that it can be applied to disease-target protein/receptors without an active site (**Figure 1.12**).<sup>79,82</sup>



**Figure 1.11.** Illustration of the mechanism of action of an irreversible catalytic metallodrug using an imine bond (reversible) to the active site of protein. Copyright © 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. Re-adapted from ref [81].



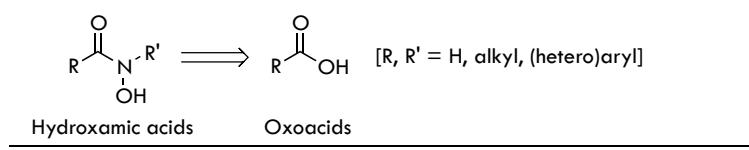
**Figure 1.12.** Illustration of the mechanism of action of an irreversible catalytic metallodrug without covalent bond to the active site of peptide/protein. Copyright © 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. Re-adapted from ref [82].

The advantages of catalytic metallodrugs notwithstanding, their potential to inhibit a target becomes effective only if they possess a high enough affinity binding with the target domain and a proper alignment of the metal ion center to the metallodrug-target complex to enhance the catalytic reaction, destroy the bioactivity and so feasibly cure the target disease.<sup>75,79,80,83,84</sup>

### 1.3 Hydroxamic acid and *N*-hydroxypyrazinone scaffolds

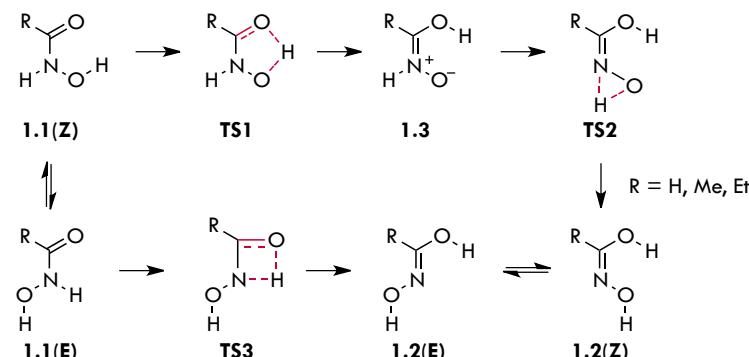
#### 1.3.1 Hydroxamic acids – Properties and synthesis strategies

Hydroxamic acids, represented by the general formula in **Figure 1.13**, are derived from oxoacids where  $-\text{OH}$  is replaced by  $-\text{NHOH}$  or  $-\text{N}(\text{R}')\text{OH}$ .<sup>85</sup>



**Figure 1.13.** Structural formula of hydroxamic acids.

Oxalohydroxamic acid was first discovered by Lossen in 1869, the synthesis and structures of hydroxamic acids as well as their bioactivities were not fully understood until the 1980s.<sup>86</sup> Hydroxamic acids can exhibit a keto-imino tautomerism.<sup>87</sup> The stability of these tautomers was determined via theoretical calculations and experimental studies on formohydroxamic acid (**1.1** where  $\text{R} = \text{H}$ ), e.g. X-ray and  $^{17}\text{O}$  NMR (**Figure 1.14**).<sup>88-90</sup>



**1.1(Z) > 1.1(E) > 1.2(Z) > 1.2(E) > 1.3** : DFT calculations (gas phase)  
B3LYP/6-311++G\*\*//B3LYP/6-31G\*

**1.1(E) >>** : crystal  
**1.1(Z) >>** : solution

**1.1(E/Z) :** non-planar  
**1.2(E/Z) :** nearly planar

**Figure 1.14.** Keto-imino tautomerism of hydroxamic acid. Copyright © 1998 American Chemical Society. Re-adapted from ref [87].

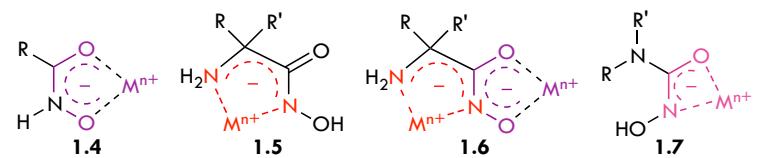
One of the most important properties and applications of hydroxamic acids is their metal ion complexing ability, explaining their essential role in biological chemistry. Hydroxamic acids can coordinate with a variety of metal ions throughout the periodic table (**Figure 1.15**).<sup>91</sup>

The periodic table shows the following trends in coordination studies:

- In solid state:** Elements highlighted in purple include Sc, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Br, Kr, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po, At, Rn, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Th, Pa, U, Np, Pu, Am, Cm, Bk, Cf, Es, Fm, Md, No, Lr.
- In solution:** Elements highlighted in light blue include Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, In, Sn, Sb, Te, I, Xe, Rf, Db, Sg, Bh, Hs, Mt, Ds, Rg, Cn, Uut, Fl, Uup, Lv, Uus.
- Central metals in heterometallic-polyfunctional hydroxamic acid complexes:** Elements highlighted in teal include Be, Al, Si, P, S, Cl, Ar, Y, Zr, Nb, Mo, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Te, I, Xe, Ba, \*Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po, At, Rn.

**Figure 1.15.** Studies of homo-/heterometallic hydroxamic acid complexes.  
Copyright © 2007 Elsevier B.V. Re-adapted from ref [91].

The presence of one nitrogen atom and two oxygen atoms in hydroxamate ions varies the possibility of coordination complex with metal ions via (N,N), (N,O) and (O,O) bidentate binding modes (depending on the metal ion and the pH of solution); however, most experimental data on characterization of these complexes in the solid state and in solution proposed a favored (O,O) bidentate binding mode (**Figure 1.16**).<sup>86,92-98</sup>



**Figure 1.16.** Possible metal ion  $M^{n+}(X,Y)$  coordination of (amino)hydroxamic acid.

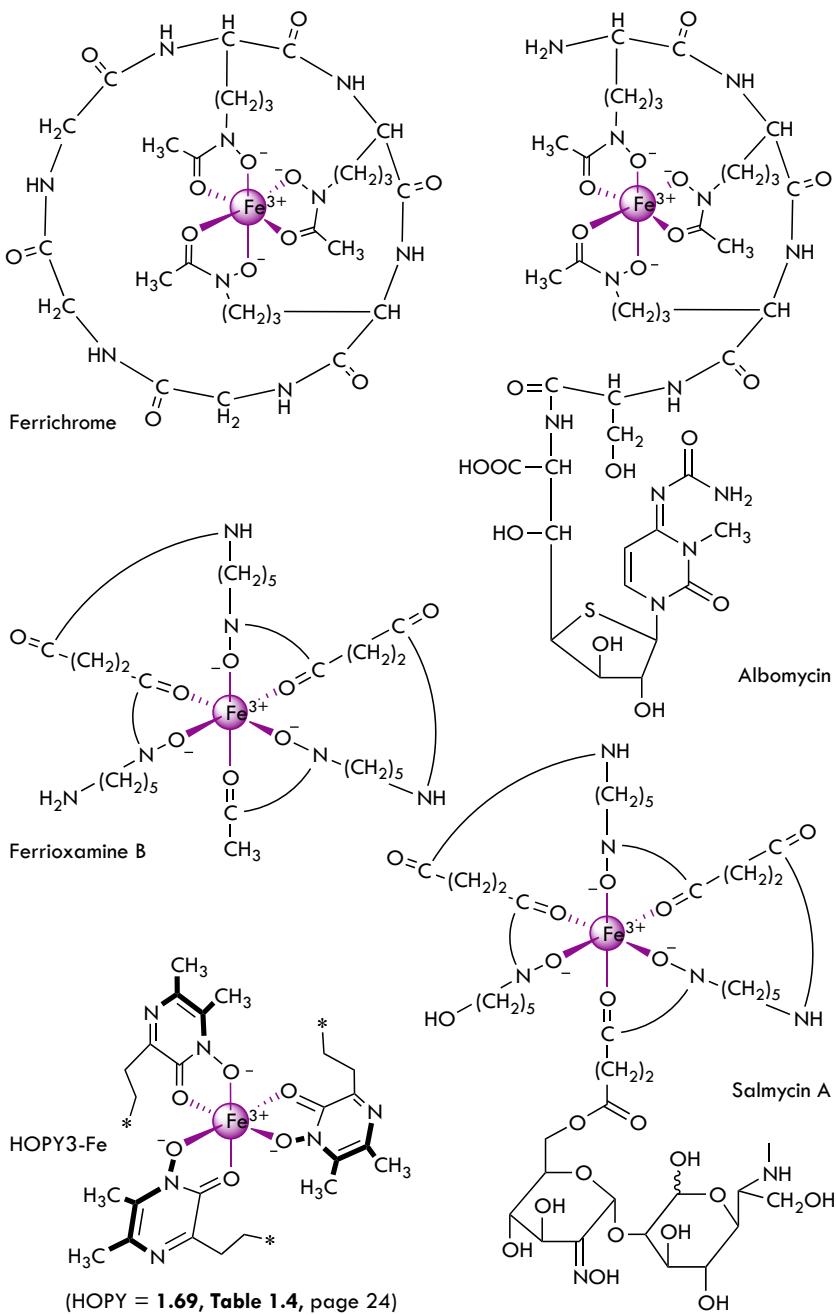
The lack of evidence for (N,O) chelating mode of structure **1.7** in the solid state could be explained by the unstable 4-membered chelate ring.

Due to the strong binding to metal ions, hydroxamic acids are well known for their numerous biological and pharmacological properties, such as scavenging metals, inhibiting metallo-enzymes, and generating nitric oxide.<sup>99</sup>

The ability of hydroxamic acid derivatives, especially siderophores, to scavenge metals (particularly iron) is largely applied in treatment of iron overload in patients.<sup>99</sup> Some examples of hydroxamic acid complexed ferric ion are presented in **Figure 1.17**.<sup>100-102</sup>

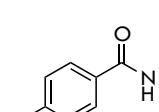
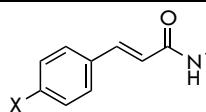
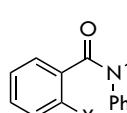
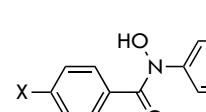
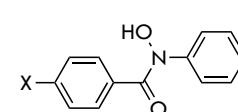
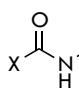
The complexation properties of hydroxamic acids may be most influenced by their acidity, which depends on the medium and substituent effects. Though there are two possible acidic sites, NH and OH, the theoretical and experimental data show that in aqueous solution, hydroxamic acids behave as oxygen acids. Nitrogen acids are only favored in the gas state or solid state or in solutions of less polar solvents (dioxane, DMSO, MeOH). pK<sub>a</sub> values of some hydroxamic acids are given in **Table 1.2**.<sup>103</sup>

As a consequence of their important role in biology (e.g. antibacterial, antifungal, antiinflammatory, antiasthmatic, and anticancer) and in particular their potency towards inhibition of matrix metalloproteinase and histone deacetylase, synthesis of bioactive hydroxamic acids is an interesting field of study. **Table 1.3** gives a brief summary of several common synthetic methodologies that have been developed and reported so far.



**Figure 1.17.** Chemical structures of some ferric complexes with hydroxamic acid. Copyright © 2008, Springer Science & Business Media, LLC. Re-adapted from refs [100,101].

**Table 1.2.**  $pK_a$  values of some hydroxamic acids.<sup>103</sup>

Hydroxamic acid	X	$pK_a$ (25 °C)
	H	aq. I = 0 8.91 aq. EtOH ( $\phi = 0.0405$ ), I = 0.08 8.84
	Me	9.05 -
	F	8.81 -
	Cl	8.70 8.60
	Br	8.57 -
	OMe	9.15 9.05
	CN	8.26 -
	NO <sub>2</sub>	8.13 8.03
	H	aq. EtOH ( $\phi = 0.0405$ ), I = 0.08 8.74
	OMe	8.85
	H	aq. dioxane ( $\phi = 0.0503$ ), I = 0 9.23
	Me	9.12
	F	8.92
	Cl	8.85
	Br	8.86
	I	8.92
	OMe	9.24
	NO <sub>2</sub>	8.87
	H	aq. dioxane ( $\phi = 0.0503$ ), I = 0 9.38
	F	9.32
	Cl	9.22
	Br	9.21
	OMe	9.53
	NO <sub>2</sub>	8.87
	H	aq. dioxane ( $\phi = 0.0503$ ), I = 0 9.37
	F	9.39
	Cl	9.18
	Br	9.16
	OMe	9.68
	H	aq., I = 0 8.78
	Me	9.40
	Pr	9.46
	Pent	9.88
	Cy	9.92
	CH <sub>2</sub> Cl	8.53
	Ph	8.89
	Bn	9.33
	4-ClBn	8.85
	4-NO <sub>2</sub> Ph	8.02
	NH <sub>2</sub>	10.5
	All	8.90

**Table 1.3.** Summary of common hydroxamic acid synthetic methodologies.

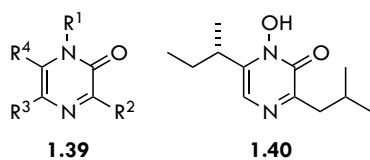
Methodology	Reaction	Ref
	$\text{R}^1\text{C}(=\text{O})\text{OH}$ $\xrightarrow[\text{DCM}/-20^\circ\text{C to rt}]{\text{Oxalyl chloride/DMF/NMP/R}^2\text{NH}_2\text{OH}}$ $\text{R}^1\text{C}(=\text{O})\text{N}^{\text{-}}(\text{R}^2)\text{OH}$ <b>1.14</b>	$\text{R}^1 = \text{aryl, amino acid}$ $\text{R}^2 = \text{alkyl, aryl}$ 104
	$\text{R}^1\text{C}(=\text{O})\text{OH}$ $\xrightarrow[\text{Et}_2\text{O}/\text{MeOH}/0^\circ\text{C to rt}]{\text{Ethyl chloroformate/H}_2\text{N-OH (fresh)}}$ $\text{R}^1\text{C}(=\text{O})\text{N}^{\text{-}}(\text{H})\text{OH}$ <b>1.16</b>	$\text{R} = \text{alkyl, aryl}$ 105
From carboxylic acids	$\text{R}^1\text{C}(=\text{O})\text{OH}$ $\xrightarrow[\text{THF/r.t.}]{\text{BzSO}_2\text{CH}_3/\text{Et}_3\text{N/R}^2\text{NH}_2\text{OH}}$ $\text{R}^1\text{C}(=\text{O})\text{N}^{\text{-}}(\text{H})\text{OH}$ <b>1.18</b>	$\text{R}^1 = \text{n-Bu, Ph, 2-furyl, 2-pyridyl}$ $\text{R}^2 = \text{H, Me}$ 106
	$\text{R}^1\text{C}(=\text{O})\text{OH}$ $\xrightarrow[\text{(81)-92\%}]{\text{TCT/NMM/HO-NH}_2\text{HCl}}$ $\text{R}^1\text{C}(=\text{O})\text{N}^{\text{-}}(\text{H})\text{OH}$ <b>1.19</b>	$\text{TCT} = \begin{array}{c} \text{Cl} \\   \\ \text{N}=\text{N}-\text{C}_6\text{H}_3-\text{N}=\text{N} \\   \\ \text{Cl} \end{array}$ $\text{RCO}_2\text{H} = \text{protected amino acids}$ $(\text{Boc, Cbz, Fmoc})$ or aromatic acids 107
	$\text{R}^1\text{C}(=\text{O})\text{OH}$ $\xrightarrow[\text{(80-98\%)}]{\text{DMAP (cat)/DMF/r.t.}}$ $\text{R}^1\text{C}(=\text{O})\text{N}^{\text{-}}(\text{H})\text{OH}$ <b>1.20</b>	$\text{TCT} = \begin{array}{c} \text{Cl} \\   \\ \text{N}=\text{N}-\text{C}_6\text{H}_3-\text{N}=\text{N} \\   \\ \text{Cl} \end{array}$ $\text{RCO}_2\text{H} = \text{protected amino acids}$ $(\text{Boc, Cbz, Fmoc})$ or aromatic acids 108
	$\text{R}^1\text{C}(=\text{O})\text{OH}$ $\xrightarrow[\text{THF/r.t.}]{\text{ZnCl}_2\text{-Et}_2\text{O}}$ $\text{R}^1\text{C}(=\text{O})\text{N}^{\text{-}}(\text{H})\text{OR}^2$ $\xrightarrow[\text{(50-90\%)}]{\text{H}_2/\text{Pd-BaSO}_4}$ $\text{R}^1\text{C}(=\text{O})\text{N}^{\text{-}}(\text{H})\text{OH}$ <b>1.22</b> <b>1.23</b>	$\text{R}^1\text{CO}_2\text{H} = \text{aliphatic/aromatic or protected amino acids}$ $\text{R}^2 = \text{H, Bu}$ $\text{R}^3 = \text{H, alkyl, Ph}$ $\text{R}^4 = \text{alkyl, aryl, cyclohexyl}$ 108
	$\text{R}^1\text{C}(=\text{O})\text{OH}$ $\xrightarrow[\text{(27-95\%)}]{\text{NH}_2\text{CN-R}^4}$ $\text{R}^1\text{C}(=\text{O})\text{N}^{\text{-}}(\text{H})\text{OR}^2$ <b>1.25</b>	$\text{R}^1\text{C}(=\text{O})\text{N}^{\text{-}}(\text{H})\text{OH}$ <b>1.26</b>
		$\text{R}^1\text{C}(=\text{O})\text{N}^{\text{-}}(\text{H})\text{OH}$ <b>1.27</b>

Table 1.3. (cont.)

Methodology	Reaction	Ref
	$\text{Ar}-\text{C}(=\text{O})-\text{OMe} \xrightarrow[\text{KCN (cat.)/THF/MeOH/r}]{\text{H}_2\text{N-OH (50% aqueous)}} \text{Ar}-\text{C}(=\text{O})-\text{NH-OH}$	109
	<b>1.28</b>	
From ester of carboxylic acids	$\text{R}^1-\text{C}(=\text{O})-\text{OR}^2 \xrightarrow[\text{Flow/-78 } ^\circ\text{C}]{\text{LiHMDS/BnONHNH}_2\text{HCl}} \text{R}^1-\text{C}(=\text{O})-\text{NH-Bn} \quad \left[ \begin{array}{l} \xrightarrow{[\text{H}]} \text{R}^1-\text{C}(=\text{O})-\text{NH-OH} \\ \text{R}^1-\text{C}(=\text{O})-\text{NH-OH} \end{array} \right]$	110 R <sup>1</sup> = alkyl, aryl, ester of protected amino acids R <sup>2</sup> = Me, Et
	<b>1.30</b>	
	$\text{R}^1-\text{C}(=\text{O})-\text{OMe} \xrightarrow[\text{Flow/70 } ^\circ\text{C}]{\text{H}_2\text{N-OH/MeONa/MeOH}} \text{R}^1-\text{C}(=\text{O})-\text{NH-OH}$	R = aryl or methoxy ester of N-Boc amino acids
	<b>1.33</b>	111
	$\text{R}^1-\text{C}(=\text{O})-\text{OR}^2 \xrightarrow[\text{MeOH/H}_2\text{O}/-5 } ^\circ\text{C}]{\text{H}_2\text{N-OH/KOH}} \text{R}^1-\text{C}(=\text{O})-\text{NH-OH}$	112 R <sup>1</sup> = (hydroxy)aminoalkyl R <sup>2</sup> = Me, Et
	<b>1.35</b>	113
Via carbonylation	$\text{R}^1-\text{X} \xrightarrow[\text{Et}_3\text{N/DMF}/60 } ^\circ\text{C}]{\text{CO/Pd(OAc)}_2/\text{PPh}_3/\text{R}^2\text{NHOOH}} \text{R}^1-\text{C}(=\text{O})-\text{N}(\text{R}^2)-\text{OH}$	114 R <sup>1</sup> = steroidyl R <sup>2</sup> = Me, t-Bu X = I, OTf
	<b>1.37</b>	
	<b>1.38</b>	

### 1.3.2 *N*-Hydroxypyrazinones

The pyrazinone (**1.39**, **Figure 1.18**) is a valuable class of scaffold in medicinal chemistry. It is present amongst others in the inhibition of HCV NS3 protease, neutrophil elastase, prolyl oligopeptidase, TF-FVIIa, and thrombin.<sup>115-121</sup> A lot of research in our group has been directed towards the development of synthetic strategies for highly functionalized pyrazinones.<sup>122-129</sup>



**Figure 1.18.**  
Pyrazinone and aspergillic acid.

*N*-Hydroxypyrazinones are cyclic hydroxamic acid containing scaffolds derived from pyrazinones. A typical example is the naturally occurring aspergillic acid (**1.40**, **Figure 1.18**). These *N*-hydroxypyrazinones are of potential interest due to their bioactivity.<sup>130-132</sup>

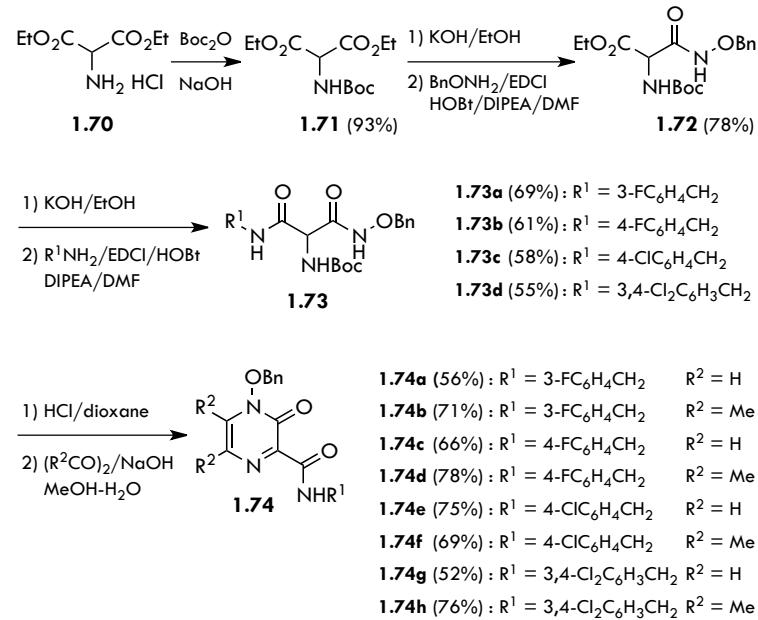
Some examples of bioactive compounds found to contain the *N*-hydroxypyrazinone core are presented in **Table 1.4**.

In recent years ground-laying work was done within our research group on the synthesis of *N*-hydroxypyrazinones. O-Protected *N*-hydroxypyrazinone-3-carboxamide scaffolds were prepared from diethyl aminomalonate ester (**1.70**) via multi-step synthesis, including saponification, amidation, and cyclization, in moderate to good yields (**Scheme 1.1**).<sup>133</sup>

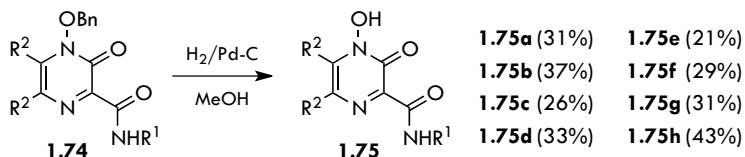
Protecting groups were involved in this work and therefore deprotection was a crucial step to obtain the final products. Several conditions were applied to remove the *N*-hydroxy protecting groups; however, the yields of these reactions were low (**Scheme 1.2**) and at times hard to reproduce.<sup>133</sup> Changing the protecting group from Bn to PMB or *t*-Bu did not solve the problem as the by-products (*N*-H pyrazinones, over-reduced compounds) were always obtained as the major products instead of the desired ones.<sup>133</sup>

**Table 1.4.** Some examples of *N*-hydroxypyrazinones and their bioactivities.

Compd.	Structure	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	Antimicrobial	MIC ( $\mu$ g/mL)	Ref
1.41		<i>i</i> -Bu	H	sec-Bu	<i>S. pneumonia</i>	15.0	134
1.42		H	Me	Me	<i>S. pneumonia</i>	25.0	134
1.43		C <sub>2</sub> H <sub>4</sub> SCH <sub>3</sub>	Me	Me	<i>S. pneumonia</i>	12.5	134
1.44		Bn	Me	Me	<i>S. pneumonia</i>	6.3	134
1.45		H	Ph	Me	<i>S. pneumonia</i>	50.0	134
1.46		<i>i</i> -Bu	H	<i>i</i> -Bu	<i>S. aureus</i>	1.0	135
					<i>S. epidermidis</i>	0.5	135
					<i>B. subtilis</i>	2.0	135
					<i>B. dysenteriae</i>	7.8	135
					<i>B. proteus</i>	7.8	135
					<i>E. coli</i>	15.6	135
1.47		Bn	Ph	Ph		6.0	136
1.48		Bn	Ph	Me	Anthrax lethal factor	70.0	136
1.49		Bn	Ph	H		15.0	136
1.50		4-Ph-Bn	Ph	Me	inhibitory activity	15.0	136
1.51		Bn	4-Cy-Ph	H		8.0	136
1.52		4-Ph-Bn	4-Ph-Ph	H	(IC <sub>50</sub> $\mu$ M)	12.0	136
1.53		4-Ph-Bn	4-Br-Ph	H		5.0	136
1.54		Pent	H	H			
1.55		H	Pent	H	Anti-hypertension treatment		137
1.56		H	H	Pent			
1.57		Et	H	Et			
1.58		<i>i</i> -Bu	<i>i</i> -Pr	H	Inhibition of endonuclease	5.1	138
1.59		<i>i</i> -Bu	Ph	H		4.8	139
1.60		<i>i</i> -Bu	2-F-Ph	H	Inhibition of influenza virus (IC <sub>50</sub> $\mu$ M)	7.3	138
1.61		<i>i</i> -Bu	3-F-Ph	H		1.5	138
1.62		<i>i</i> -Bu	4-F-Ph	H		0.9	139
1.63		Bn	4-MeO-Bn	H		2.8	139
1.64		Bn	4-F-Bn	H		3.5	139
1.65		4-F-Ph	Ph	H		6.5	139
1.66		Me	4-HO-Ph	H		17.0	140
1.67		<i>i</i> -Bu	4-HO-Ph	H	(IC <sub>50</sub> $\mu$ M)	4.5	140
1.68		Bn	Ph	OH	Cytotoxicity against cancer cell line NCI-H187 (IC <sub>50</sub> $\mu$ M)	1.8	141
					Antimalarial activity (IC <sub>50</sub> $\mu$ M)	28.8	141
1.69						Iron removal from human transferrin	101
		R = Me/ <i>i</i> -Bu (L and D forms)					



**Scheme 1.1.** Synthesis of O-benzyl N-hydroxypyrazinone-3-carboxamides.



**Scheme 1.2.** Deprotection of O-benzyl N-hydroxypyrazinone-3-carboxamides.

#### 1.4 Continuous flow techniques as tools for organic synthesis

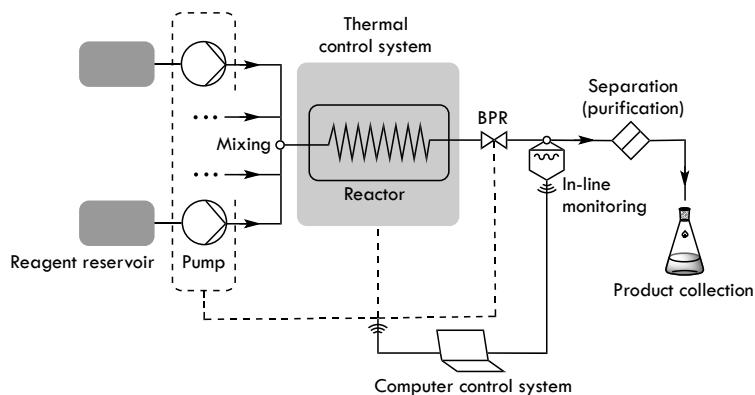
Continuous micro- or milliflow technology (performing a chemical process in a continuous manner) is lately receiving much attention both in academic and industrial chemistry.<sup>142</sup>

The benefits, namely the enhanced performance and efficiency, bring continuous flow techniques to a wide range of applications in pharmaceutical, agrochemical, petrochemical, fine and specialty chemicals. The shift from batch to continuous flow has become a recurrent trend in both pharmaceutical early stage in the research and development and manufacturing.<sup>143</sup>

### 1.4.1 General experimental set-up

The general set-up of most of the continuous flow systems is described in

**Figure 1.19.**<sup>144-146</sup>



Reagent reservoirs: Storing the solutions of starting materials.

Pumps: Controlling the main flow stream (flow rate or residence time) as well as varying the reagent stoichiometry.

Mixing: Mixing the flow streams of reagents.

Reactor: Where the chemical reaction will occur (chip-based or tube-based reactors).

Thermal control system: Efficiently heating or cooling the reactor at a desired temperature.

Backpressure regulator: Regulating the pressure (fixed or controllable pressure).

Separation (or purification): Scavengers (capturing side products), continuous liquid-liquid extraction, solvent evaporation, distillation, ...

Product collection: Manually or automatically collecting, or directly feeding to another flow set-up.

In-line monitoring: Following the reaction course (UV-VIS, FTIR, Raman, MS, NMR).

Computer control system: Collecting and analyzing data from the in-line monitoring, enabling the possibility of integration some or all components of the flow system to make it as a fully automated or self-optimization process.

**Figure 1.19.** Schematic overview of a continuous flow process in general. Re-adapted from ref [145].

### 1.4.2 Advantages

Flow chemistry is well described as being a high yielding chemical synthesis technique with fewer by-products. Some obvious advantages of the flow technique can be summarized as follows.

- **Thermal control**

Temperature is an important factor in either endothermic or exothermic reaction.

As a result of the optimum ratio of reactor-wall surface area to reaction volume by using the small diameter and circular channels in a plate-type reactor or tubing reactor (glass or stainless steel), the heat transfer in the flow system is maximized and efficiently controlled. An advanced flow system may have an extra pre-mixing temperature-controlled sector, which provides a precise thermal control of the mixing process as well as uniform and reproducible temperature profiles.<sup>144</sup>

- **Mixing**

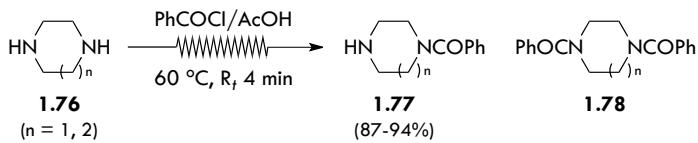
The fact that only small amount of reagents are mixed together at any time allows the continuous flow process to be easily controlled.

An excellent mixing behavior in a flow reactor is usually obtained by either splitting the reagent flow streams into many microchannels before the mixing point, or varying the shape and dimensions of the flow channels, or even introducing the addition static mixers.<sup>144</sup>

- **Stoichiometric control and selectivity**

Stoichiometric imbalance of a reactant in a fast kinetic reaction with highly reactive chemicals causes the formation of by-products.<sup>144,147</sup>

Well mixing and good stoichiometric control enhance the selectivity of the formation of the desired product, which is normally isolated from the flow reactor, reducing or even avoiding the possibility of over-reaction in certain situations, e.g. selective debenzylation of O-benzyl N-hydroxypyrazinones (**Chapter 3**) or monoacetylation of symmetrical diamines (**Scheme 1.3**).<sup>148,149</sup>

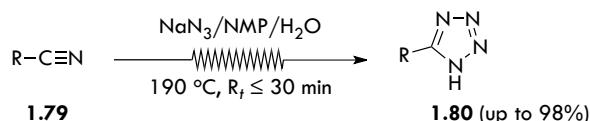


**Scheme 1.3.** Monoacetylation of piperazines and homopiperazines in a microflow reactor.

- **Safety**

The more flexible process control and the use of only small quantities of reagents at any time as well as the *in situ* formation of hazardous reagents for only a short amount of time during the course of the reaction minimize most of the hazardous risks related to azides, diazo compounds, hydrogen, carbon monoxide, ozone, ...

A safe and efficient methodology for the synthesis of tetrazoles in a microflow reactor was reported by Palde *et al.* (**Scheme 1.4**).<sup>150</sup>



**Scheme 1.4.** Synthesis of 5-substituted 1*H*-tetrazoles in a microflow reactor.

- **Enhanced process parameters**

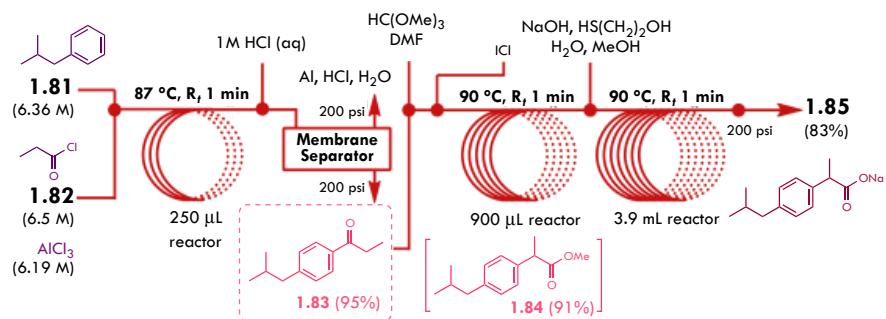
The combination of using high pressures and superheated solvents allows many reactions to occur above the solvent boiling points (**Table 1.5**).

**Table 1.5.** Boiling points of some common solvents

Solvent	Boiling point (°C)	
	1 bar	3 bar
Dichloromethane	40	62
Methanol	65	91
Tetrahydrofuran	66	92
Ethyl acetate	77	105
Toluene	110	144
Dimethylformamide	153	195

- **Multi-step process**

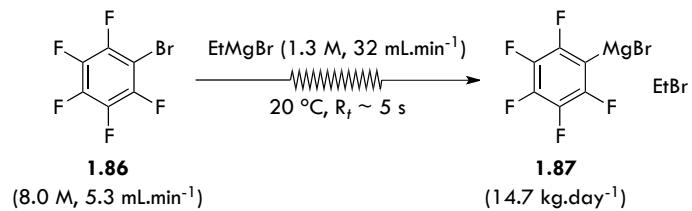
Being highly selective, flow chemistry is cleaner and therefore allows two or more synthetic steps to be joined together in one efficient and complete sequence. A schematic illustration for the synthesis and purification of ibuprofen is presented in **Figure 1.20**.<sup>151</sup>



**Figure 1.20.** Schematic illustration of a three-minute flow set-up for the multistep synthesis of ibuprofen. Copyright © 2015 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. Re-adapted from ref [151].

- **Scalability**

In principle, flow chemistry is infinitely scalable and has time as its determining restrictive parameter. Wakami and colleague reported an efficient methodology for the synthesis of pentafluorophenylmagnesium bromide, a Grignard reagent, in a pilot flow reactor with a scale of 14.7 kg/day (**Scheme 1.5**).<sup>152</sup>



**Scheme 1.5.** Scale-up in synthesis of pentafluorophenylmagnesium bromide.

### 1.4.3 Limitations

Despite many advantages, flow chemistry itself definitely has some limitations that should be looked into before any flow reactions are considered. Solubility is one of those. The issue could come from either the reactants or the products, especially at elevated temperatures.<sup>144</sup> Precipitation or crystallization potentially causes reactor blockage. Suitable solvents and temperatures (even pre-/post-reactor heating) could, in principle, solve this problem.

Highly corrosive reagents (damaging the reactors) and outgassing (emptying the reactor) also need to be taken care of.<sup>144</sup>

The optimization of the flow process (temperature, flow rate or residence time, and any other crucial parameters) is required before the reaction could be performed. And this makes the flow system become less flexible for different types of reactions.<sup>153</sup>

The formation of gases in a reaction may also cause a serious problem (unreliable residence times and draining of the reactor); however, the installation of a suitable backpressure regulator on the outlet of the reactor usually solves the problem.

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## Chapter 2

# Goals and Objectives



With regard to the previous results obtained in the lab and in view of the more general importance of metal coordinating compounds for drug design, we set ourselves the following goals for this thesis.

**A. Further optimization of the synthesis of N-hydroxypyrazinones**

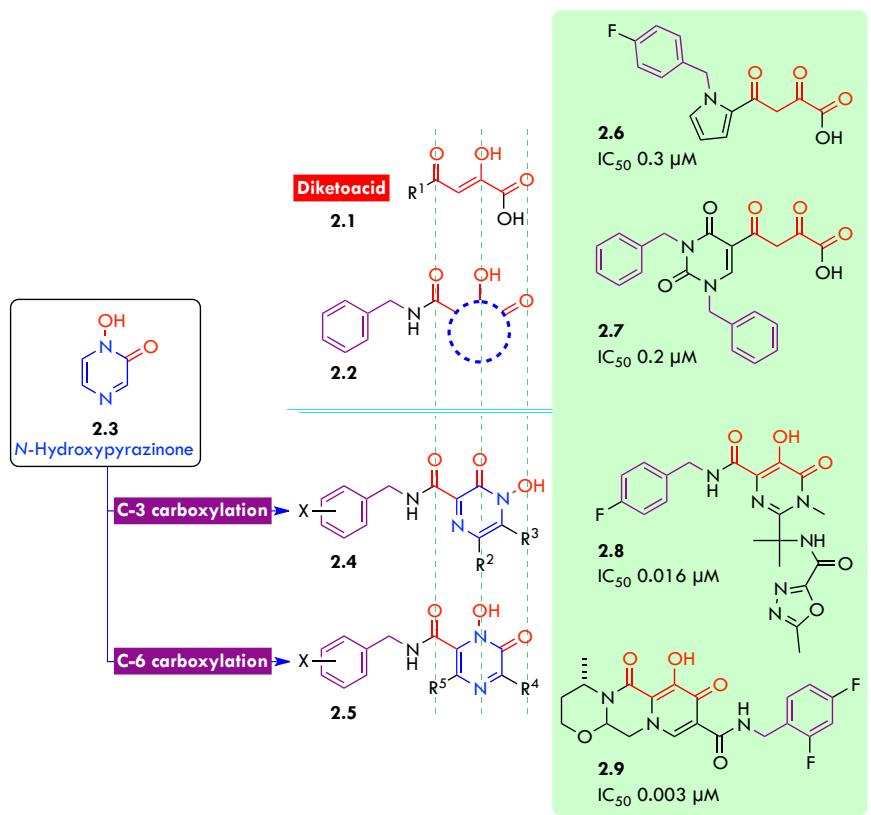
As mentioned in the introduction, the previously developed synthesis of *N*-hydroxypyrazinones suffered from some serious drawbacks in terms of reproducing the yields of the final compounds due to overreduction in the last step of the synthesis. A major problem here was a sufficient lack of control of the reaction parameters during the deprotection step. We envisaged to render the synthesis more robust in preparation of making a library of *N*-hydroxypyrazinone derivatives. We planned to implement flow chemistry techniques in order to improve control over the reaction parameters.

**B. Synthesis of a library of compounds based on the hydroxypyrazinone scaffold**

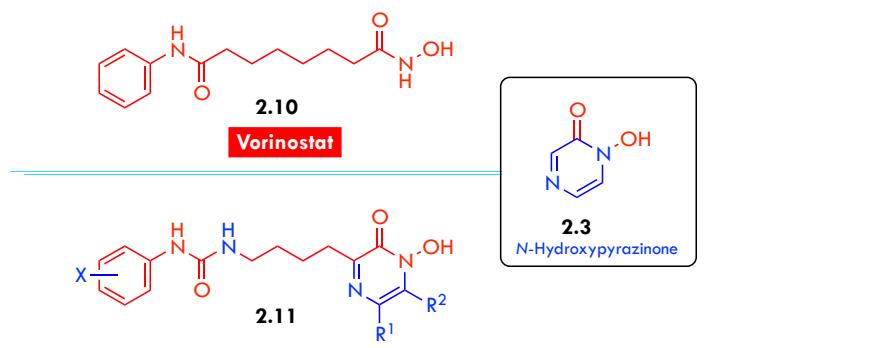
In view of the potential interest in metal complexing molecules for drug design and especially in view of the similarity of the *N*-hydroxypyrazinones with known metal complexing bioactive compounds, we proposed to generate a library of *N*-hydroxypyrazinones with a specific focus towards analogues of anti-HIV drugs.

Analogues of diketoacid-based integrase inhibitors were conceptually created (**Figure 2.1**) by transferring the binding pattern of the diketoacid (**2.1**, **2.6**, and **2.7**) to a heterocyclic core (**2.4**, **2.5**, **2.8**, and **2.9**).<sup>1</sup> Variation of the functionality involved in binding is possible and the classes **2.4** and **2.5** seemed of interest as target compounds. In view of the previous experience with the suboptimal synthesis of compounds **2.4**, we planned to first tackle these as targets though the resemblance between the diketoacids and compounds **2.5** is more striking.

Also analogues **2.11** of the HDAC inhibitor vorinostat (**Figure 2.2**) were planned as these seemed to be within easy reach, that is, if the general strategy for the *N*-hydroxypyrazinone synthesis could be optimized.



**Figure 2.1.** Conceptual design of novel diketoacid-based scaffolds as potential integrase inhibitors via C-3/C-6 carboxylation of *N*-hydroxypyrazinones.



**Figure 2.2.** Design of novel vorinostat-based *N*-hydroxypyrazinone scaffolds.

**C. Biological evaluation of the compounds in an *in vitro* anti-HIV assay**

All compounds were submitted for biological evaluation in the HIV research at Rega Institute for Medical Research (KU Leuven, Belgium). The bioactivities of the compounds were evaluated on both anti-HIV replication inhibitory activities (MT-4/MTT assay) and HIV-1 reverse transcriptase polymerase inhibition (HIV-1 RT kit assay).<sup>2,3</sup>

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# Chapter 3

# Results and Discussion

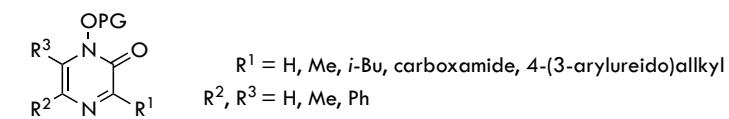
- 3.1 Synthesis of 1-benzyloxyprazin-2(1*H*)-ones
  - 3.1.1 3-Alkyl-1-benzyloxyprazin-2(1*H*)-ones
  - 3.1.2 1-Benzyloxyprazin-2(1*H*)-one-3-carboxamides
  - 3.1.3 3-[4-(3-Arylureido)butyl]-1-benzyloxyprazin-2(1*H*)-ones
- 3.2 Synthesis of 1-hydroxypyrazin-2(1*H*)-ones
  - 3.2.1 Debenylation of 1-benzyloxyprazin-2(1*H*)-ones in batch
  - 3.2.2 Methodology for debenylation of 1-benzyloxyprazin-2(1*H*)-ones in flow
  - 3.2.3 Synthesis of *N*-hydroxypyrazinones via debenylation in flow
- 3.3 Biological evaluation



### 3.1 Synthesis of 1-benzyloxypyrazin-2(1*H*)-ones

The results concerning the synthesis of 3-alkyl-/carboxamide-1-benzyloxy-pyrazin-2(1*H*)-ones described in this subchapter were recently published by our research group.<sup>1</sup> In addition to a summary of this work, we present here results obtained for the synthesis of 3-[4-(3-arylureido)butyl]-1-benzyloxypyrazin-2(1*H*)-ones.

The precursors for the synthesis of *N*-hydroxypyrazin-2(1*H*)-ones are depicted in **Figure 3.1** and were achieved via the synthetic procedures explained in the subchapters **3.1.1**, **3.1.2**, and **3.1.3**.



**Figure 3.1.** General O-protected *N*-hydroxypyrazin-2(1*H*)-one precursors.

Some common O-protecting groups are known for hydroxamic acids such as the benzoyl, *tert*-butyl, *tert*-butyldimethylsilyl, 2,4-dimethoxybenzyl, 5,5-dimethyl-1,4,2-dioxazole, 2-methylpropenyl, 4-nitrophenyl, trimethylsilyl, 2-(trimethylsilyl)-ethyl, and trityl group.<sup>2-11</sup> The choice of the O-protecting group of hydroxamic acids is in part associated with the commercial availability of the corresponding O-protected hydroxylamines. From our point of view, the benzyl-protecting group is an interesting option due to its good tolerance towards moisture and mildly acidic or basic conditions, which are usually essential requirements in multi-step synthesis. Also, it is easy to cleave off the benzyl protective group by reductive hydrogenolysis or Lewis acid-based deprotection.<sup>12</sup>

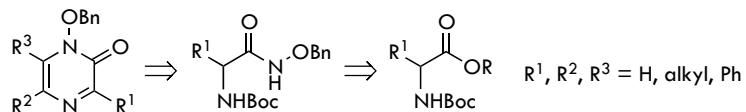
In order to avoid potential selectivity problems when preparing complex systems (like the 3-carboxamide derivatives described in the following subchapters) and in order to avoid difficulties in purification steps, we chose to work via the O-benzyl-protected precursors. Thus O-benzylhydroxylamine (or its hydrochloric salt form) was used as starting component to introduce to the formation of hydroxamates.

While the variation in R<sup>2</sup> and R<sup>3</sup> groups is kept limited due to the scarce availability of commercial glyoxal derivatives, the R<sup>1</sup> group has been well explored because it is introduced via easily available amino acids, such as

glycine ( $R^1 = H$ ), alanine ( $R^1 = Me$ ), and leucine ( $R^1 = i\text{-}Bu$ ). Also, a wide range of carboxamide groups, in the form of primary and secondary amides, as well as 4-(3-arylureido)butyl groups were introduced at the 3-position.

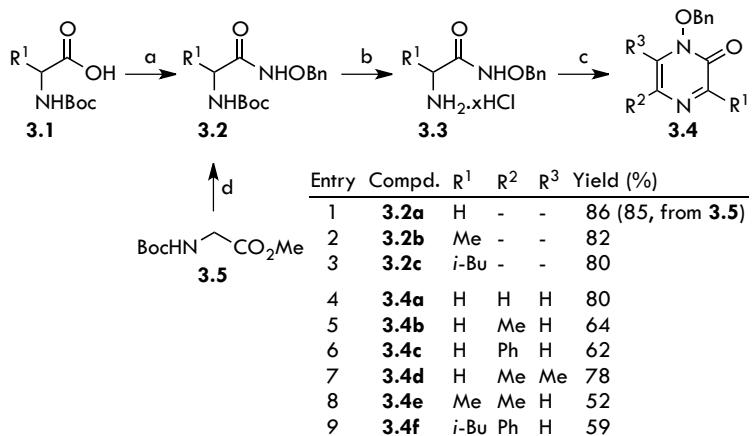
### 3.1.1 3-Alkyl-1-benzyloxypyrazin-2(1*H*)-ones

1-Benzylxypyrazin-2(1*H*)-ones are synthesized in several synthetic steps according to the retrosynthetic **Scheme 3.1**.



**Scheme 3.1.** Retrosynthesis of 3-alkyl-1-benzyloxypyrazin-2(1*H*)-ones.

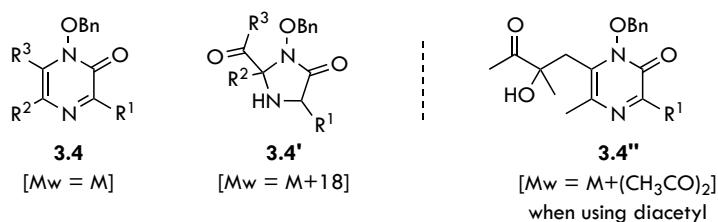
The synthesis of 3-alkyl-1-benzyloxypyrazin-2(1*H*)-ones **3.4** was achieved by amidation of *N*-Boc protected amino acids **3.1** using O-benzyl hydroxylamine in combination with HOBr/EDCI/DIPEA in anhydrous DMF, followed by Boc-deprotection by concentrated HCl in dioxane and condensation (**Scheme 3.2**).



**Scheme 3.2.** Synthetic pathway for preparation of 3-alkyl-1-benzyloxypyrazin-2(1*H*)-ones **3.4**. Reagents and conditions: (a)  $BnONH_2 \cdot HCl$  (1 equiv), HOBr (1.3 equiv), EDCI (1.3 equiv), DIPEA (2.3 equiv), DMF,  $-10\text{ }^\circ C$  then rt, 16 h; (b) 4 M HCl (10 equiv) in dioxane, rt, 30 min; (c)  $R^2R^3(CO)_2$  (0.9 equiv), 2 M NaOH, pH 8–10,  $MeOH\text{-}H_2O$  (2:1),  $-35\text{ }^\circ C$  then rt, overnight; (d)  $BnONH_2$  (1.1 equiv), LiHMDS (3.1 equiv), THF,  $-78\text{ }^\circ C$ , 2 h.

Our synthetic scheme for the synthesis of 3-alkyl-1-benzyloxypyrazin-2(1*H*)-ones improves upon reported procedures<sup>13-16</sup> and is similar to chemistry we already applied in the preparation of 1-alkyl-/aryl-pyrazinones.<sup>17,18</sup> It relies on the base catalyzed condensation of a glyoxal derivative with an amino acid hydroxamate. The condensation with phenyl glyoxal needs a higher reaction temperature (50-70 °C) as compared to those with glyoxal, methyl glyoxal, and diacetyl. In order to avoid excessive side reactions resulting in very complex mixtures, it was important to use the glyoxal derivative as a limiting reagent (0.9 equiv or less) and to add it slowly to the cooled reaction mixture (-35 °C) via a syringe pump over the course of 30-120 min.

The condensation of glyoxal derivatives and amino acid amides has previously been studied in literature for the synthesis of pyrazinones. Konakahara *et al.* reported that the key factors affecting the yield of pyrazinone products are reaction temperature, pH dependence, as well as the base-addition rate.<sup>19</sup> When doing the variation of pH from 8-10 in this cyclization, Ohkanda and colleagues have shown that there is a possibility of forming complex reaction mixtures (**Figure 3.2**).<sup>20</sup> In some occasions during the cyclization step we encountered by-products **3.4'** and/or **3.4''** (when using diacetyl) as evidenced by ESI-MS consequently resulting in low reaction yields.

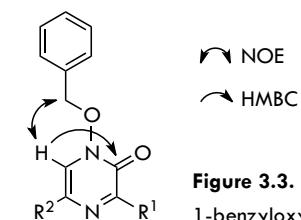


**Figure 3.2.** Possible product and by-products in the cyclization of glyoxal derivative and amino acid *N*-benzyloxamide.

O-Benzyl hydroxamate **3.2a** could also be prepared via direct amidation of the glycine methyl ester **3.5** using LiHMDS base in THF.<sup>21</sup> A comparable yield and a shorter reaction time are observed in this instance (**Scheme 3.2**, Entry 1). With the same substrate and conditions, LiHMDS gave a better yield in comparison with NaHMDS (68% yield of

**3.2a** from **3.5**). The better yield in the case of using LiHMDS might be explained by the higher reactivity of lithium salt of O-benzyl hydroxylamine over the sodium derivative, which depends on its higher solubility in THF at low temperature.<sup>21</sup>

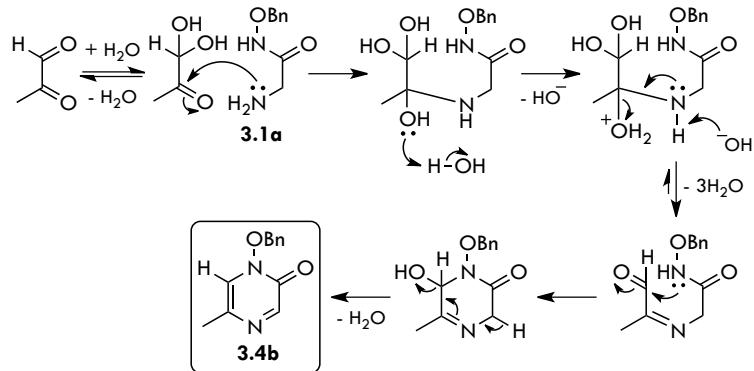
The regioselectivity of this reaction with an unsymmetrical glyoxal derivative (methyl/phenyl glyoxal) was reported before and was confirmed by us via NMR analysis. Proton H-6 in compounds **3.4a,b,c,e,f** (unsubstituted at position 6, but substituted at position 5) shows a clear NOE correlation with the methylene protons of the O-benzyl group, as well as an HMBC correlation with C-2 (**Figure 3.3**).<sup>13,14</sup>



**Figure 3.3.** NOE and HMBC correlations of C6-unsubstituted 1-benzyloxy-2(1H)-ones **3.4**.

The regioselectivity could come as a surprise as it might be expected that in general the reaction proceeds via an initial condensation of the amine with the more electrophilic aldehyde. However, in the case of methylglyoxal the aldehyde group can become inactive due to the hydration (or solvation).<sup>22</sup> Therefore, the methyl carbonyl group and not the aldehyde group is the target of the first nucleophilic attack of the primary amino group in glycine N-benzyloxyamide despite the fact of the steric hindrance.<sup>23</sup> This could explain the observed regioselectivity. The course of the reaction is continued with the dehydration, tautomerization, and cyclization together with the cleaving of the second water molecule (**Scheme 3.3**).<sup>24</sup> Phenylglyoxal may also behave in a similar fashion in this kind of reaction.

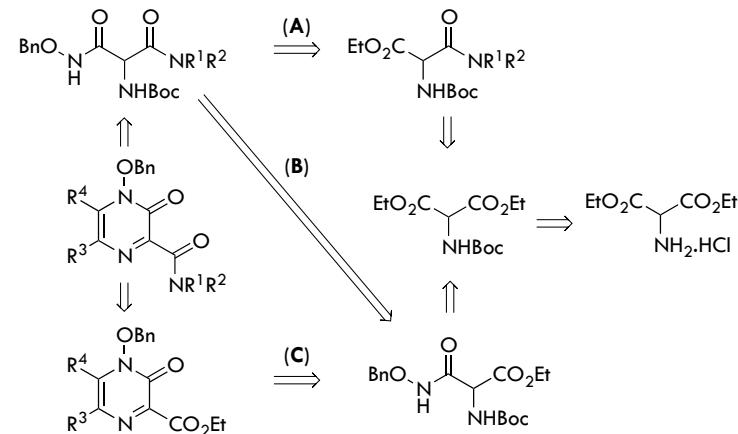
Using the aforementioned approach of slow addition, with glyoxal as the limiting reagent, the previously reported 1-benzyloxy-2(1H)-ones **3.4a**, **3.4b** and **3.4d** were obtained in better yields as compared to those mentioned in the literature.<sup>15,16</sup>



**Scheme 3.3.** Potential reaction mechanism for cyclization in the synthesis of pyrazin-2(1*H*)-ones.

### 3.1.2 1-Benzylxypyrazin-2(1*H*)-one-3-carboxamides

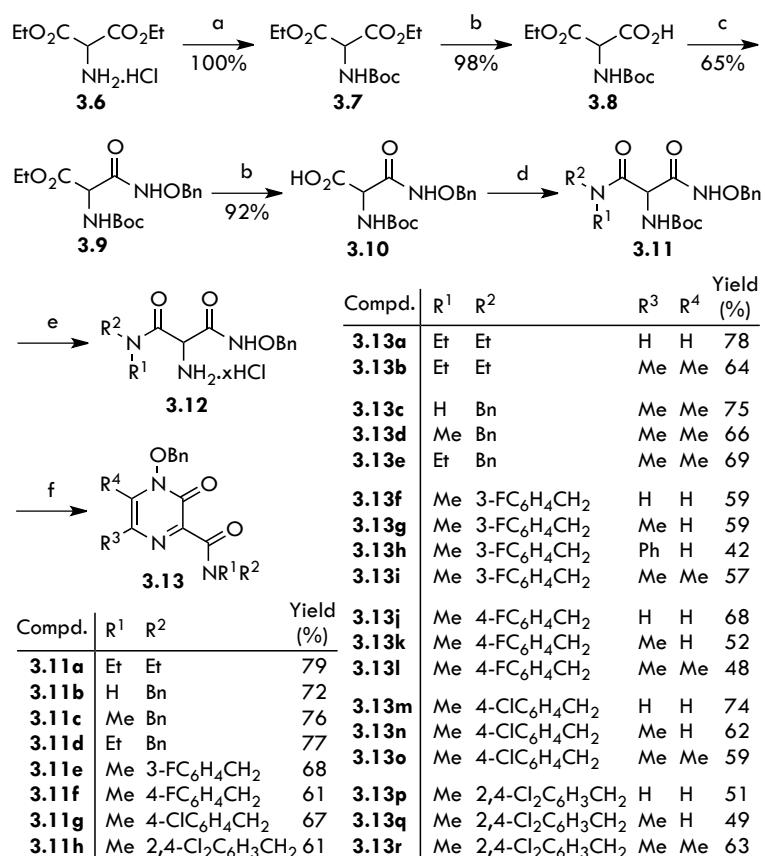
Several retrosynthetic approaches were investigated in order to synthesize the structural more complex 1-benzylxypyrazin-2(1*H*)-one-3-carboxamides (**Scheme 3.4**).



**Scheme 3.4.** Retrosynthesis of 1-benzylxypyrazin-2(1*H*)-one-3-carboxamides ( $R^1, R^2, R^3, R^4 = H, \text{alkyl, aryl}$ ) via different approaches (A – **Scheme 3.7**; B – **Scheme 3.5**; and C – **Scheme 3.8**).

The synthesis of the novel 1-benzylxypyrazin-2(1*H*)-one-3-carboxamides **3.13** is described in **Scheme 3.5**. In this pathway, the amino group in diethyl aminomalonate ester hydrochloride (**3.6**) is first

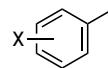
protected with a Boc-group to form **3.7**. This reaction is accelerated by using a catalytic amount of DMAP.<sup>25</sup> This is followed by iterative monosaponification and amidation to generate **3.11**.<sup>26,27</sup> These compounds are then converted to pyrazine-2(1*H*)-ones **3.13a-r** in moderate to good yields after Boc-removal.



**Scheme 3.5.** Synthesis of 1-benzyloxy pyrazin-2(1*H*)-one-3-carboxamides **3.13**.

Reagents and conditions: (a) Boc<sub>2</sub>O (1.05 equiv), NaHCO<sub>3</sub> (1.05 equiv), DMAP (0.01 equiv), H<sub>2</sub>O-dioxane, rt, overnight; (b) KOH (1 equiv), EtOH, rt, overnight; (c) BnONH<sub>2</sub>.HCl (1 equiv), HOBT (1.3 equiv), EDCI (1.3 equiv), DIPEA (2.3 equiv), DMF, -10 °C then rt, 16 h; (d) R<sup>1</sup>R<sup>2</sup>NH (1 equiv), HOBT (1.3 equiv), EDCI (1.3 equiv), DIPEA (1.3 equiv), DMF, -10 °C then rt, 16 h; (e) 4 M HCl (16 equiv) in dioxane, rt, 30 min; (f) R<sup>3</sup>R<sup>4</sup>(CO)<sub>2</sub> (0.9 equiv), 2 M NaOH, pH 8-10, MeOH-H<sub>2</sub>O (2:1), -35 °C then rt, overnight.

Non-commercially available secondary aromatic amines were prepared via an unmodified method described by Merritt and coworkers (**Scheme 3.6**).<sup>28</sup>

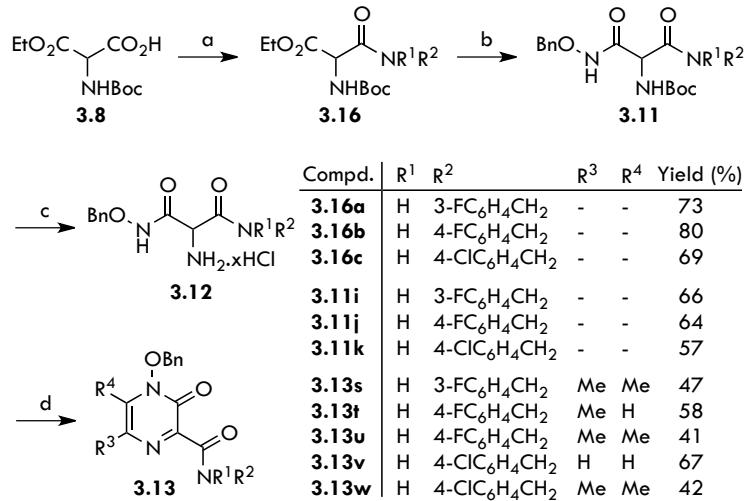
			Yield (%)
	Compd.	X	
	<b>3.15a</b>	3-F	84
	<b>3.15b</b>	4-F	78
	<b>3.15c</b>	4-Cl	69
	<b>3.15d</b>	2,4-Cl <sub>2</sub>	72

**Scheme 3.6.** Synthesis of secondary aromatic amines **3.15**. Reagents and conditions: (i) **3.14** (1 equiv), MeNH<sub>2</sub> (1.2 equiv), NaHCO<sub>3</sub> (2 equiv), MeOH, reflux, 4 h; (ii) NaBH<sub>4</sub> (1.2 equiv, 2 h), 10 °C, 2 h.

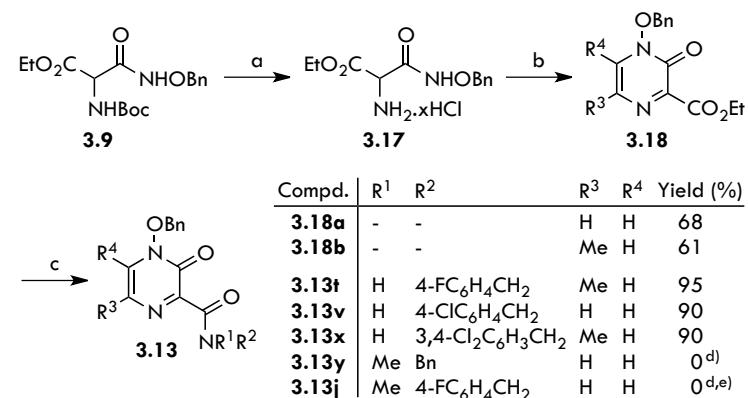
Compounds **3.11** can alternatively directly be obtained via conversion of ethyl ester **3.16** using LiHMDS and BnONH<sub>2</sub> (**Scheme 3.7**). In the case of 3-carboxylated derivatives of 1-benzyloxypyrazin-2(1*H*)-one, the latter approach is able to reduce the number of reaction steps leading to the final products **3.13s-w**; however, it does limit the late stage diversification at C-3 of the target compounds, which in terms of library generation is a drawback.

In a further effort to shorten the synthetic protocol in the synthesis of 1-benzyloxypyrazin-2(1*H*)-ones **3.13**, we performed the cyclization of the product of Boc-deprotection of **3.9** with glyoxals to generate 1-benzyloxypyrazin-2(1*H*)-one 3-carboxyl ethyl esters **3.18**, which could be used as precursors in a one-step amidation to form **3.13** (**Scheme 3.8**) using MgCl<sub>2</sub> as Lewis acid catalyst.<sup>29</sup>

The desired secondary amide products (**3.13t**, **3.13v** and **3.13x**) could be obtained in high yields by treating ethyl esters **3.18** with primary amines. Unfortunately, no conversion was detected in case of secondary amines even after prolonged reaction time and heating at a temperature of 50 °C (**3.13j** and **3.13y**). However, compound **3.13j** can be synthesized via methods described in **Scheme 3.5** with 38% yield from **3.9**).



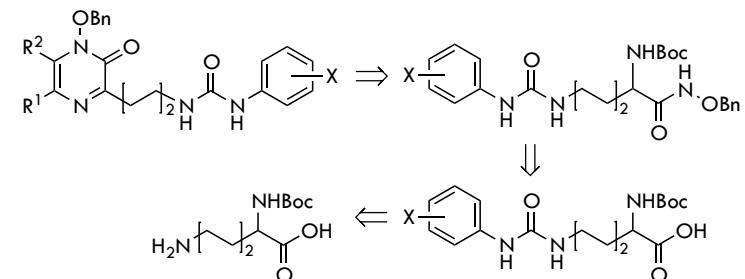
**Scheme 3.7.** Synthesis of 1-benzyloxy pyrazin-2(1*H*)-one-3-carboxamides via direct amidation of **3.16**. Reagents and conditions: (a)  $\text{R}^1\text{R}^2\text{NH}$  (1 equiv), HOBr (1.3 equiv), EDCI (1.3 equiv), DIPEA (1.3 equiv), DMF, -10 °C then rt, 16 h; (b)  $\text{BnONH}_2$  (1.1 equiv), LiHMDS (4.1 equiv), THF, -78 °C, 2 h; (c) 4 M HCl (16 equiv) in dioxane, rt, 30 min; (d)  $\text{R}^3\text{R}^4(\text{CO})_2$  (0.9 equiv), 2 M NaOH, pH 8-10, MeOH-H<sub>2</sub>O (2:1), -35 °C then rt, overnight.



**Scheme 3.8.** Synthesis of 1-benzyloxy pyrazin-2(1*H*)-one-3-carboxamides via amidation of **3.18**. Reagents and conditions: (a) 4 M HCl (16 equiv) in dioxane, rt, 30 min; (b)  $\text{R}^3\text{R}^4(\text{CO})_2$  (0.9 equiv), 2 M NaOH, pH 7-8, MeOH-H<sub>2</sub>O (2:1), -35 °C then rt, overnight; (c) (i)  $\text{MgCl}_2$  (2 equiv), THF, rt, 5 min; (ii)  $\text{R}^1\text{R}^2\text{NH}$  (2.5 equiv), rt, 16 h; (d) No conversion, **3.18** was recovered (by HPLC-UV-MS); (e) 38% yield of **3.13j** from **3.9** (Scheme 3.5).

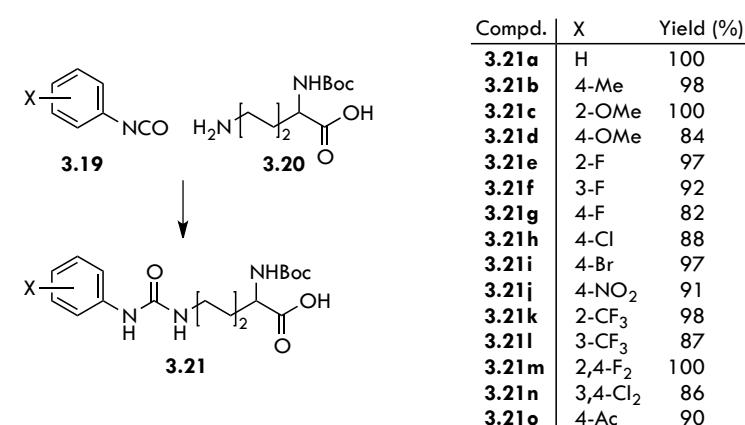
### 3.1.3 3-[4-(3-Arylureido)butyl]-1-benzyloxypyrazin-2(1*H*)-ones

Another O-benzyl N-hydroxypyrazinone precursor mimicking the vorinostat skeleton was simply achieved from *N*-alpha-protected lysine via the strategy described in the **Scheme 3.9**.



**Scheme 3.9.** Retrosynthesis of 3-[4-(3-arylureido)butyl]-1-benzyloxypyrazin-2(1*H*)-ones ( $R^1, R^2 = H, Me, Ph; X = H, Me, OMe, \text{halogen}, NO_2, CF_3, Ac$ ).

The urea derivatives **3.21** were obtained from the reaction of the commercial *N*-alpha-Boc protected lysine **3.20** and a variety of aryl isocyanates **3.19** under reflux.<sup>30</sup> The short reaction time and possibility of non-chromatographic purification facilitated the achievement of a large set of these compounds (**Scheme 3.10**). Quantitative yields were obtained in many cases.



**Scheme 3.10.** Synthesis of *N*-Boc lysine urea derivatives **3.21**. Reagents and conditions: *N*-Boc lysine **3.20** (1 equiv), isocyanate **3.19** (1.2 equiv),  $\text{NaHCO}_3$  (4 equiv), dioxane- $\text{H}_2\text{O}$ ,  $80^\circ\text{C}$ , 1-3 h.

The *N*-Boc lysine urea derivatives **3.21** were then successively converted to *N*-Boc lysine urea O-benzyl hydroxamate **3.22** and 3-[4-(3-arylureido)-butyl]-1-benzyloxypyrazin-2(1*H*)-ones **3.23** via conventional amidation with O-benzylhydroxylamine (**Scheme 3.11**) and base catalyzed cyclization after Boc-removal with glyoxal derivatives (**Scheme 3.12**). The chemistry of these syntheses is similar to those of the preparation of previous precursors **3.4** and **3.13**.

Compd.	X	Yield (%)
<b>3.22a</b>	H	79
<b>3.22b</b>	4-Me	80
<b>3.22c</b>	2-OMe	92
<b>3.22d</b>	4-OMe	97
<b>3.22e</b>	2-F	99
<b>3.22f</b>	3-F	93
<b>3.22g</b>	4-F	99
<b>3.22h</b>	4-Cl	90
<b>3.22i</b>	4-Br	79
<b>3.22j</b>	4-NO <sub>2</sub>	94
<b>3.22k</b>	2-CF <sub>3</sub>	99
<b>3.22l</b>	3-CF <sub>3</sub>	100
<b>3.22m</b>	2,4-F <sub>2</sub>	99
<b>3.22n</b>	3,4-Cl <sub>2</sub>	97
<b>3.22o</b>	4-Ac	99

**Scheme 3.11.** Synthesis of *N*-Boc lysine urea O-benzyl hydroxamate derivatives **3.22**. Reagents and conditions: *N*-Boc lysine urea derivative **3.21** (1 equiv), HOBr (1.3 equiv), EDCI (1.3 equiv), DIPEA (2.3 equiv), BnONH<sub>2</sub>·HCl (1.3 equiv), DMF, -10 °C then rt, overnight.

Due to the low solubility in water of the amidation products **3.22**, they could be obtained as pure via several extraction and washing steps. The yields of compounds **3.22** are usually excellent. Boc-removal was carried out in concentrated hydrochloric acid in dioxane at room temperature with stirring. Alternatively the reaction was accelerated in the sonicator bath.

TFA was used for *N*-Boc deprotection of compound **3.22o** since HCl/dioxane conditions failed.

In general the conversion of *N*-alpha-Boc lysine **3.20** to its O-benzyl hydroxamate derivatives after Boc-removal smoothly occurred in three

synthetic steps and was then followed by the condensation with glyoxals to form 1-benzyloxypyrazin-2(1*H*)-ones **3.23**.

Because of the steric hindrance of the long 4-(3-arylureido)butyl side-chain, the cyclization does not proceed to complete at room temperature, not even after prolonging the reaction time (monitored by ESI-MS). However, completion of the reaction was effected by heating the mixture at 70 °C for 1-2 h after being stirred at ambient temperature for 5-8 h.

O=C(Nc1ccccc1)CC(C)C[C@H](N[C@@H](C)C(=O)Nc2ccccc2)C(=O)N[C@@H](C)C(=O)Nc3ccccc3  
**3.22**

O=C1N(Oc2ccccc2)C(=O)C[C@H]2C[C@H](N[C@@H](C)C(=O)Nc3ccccc3)C(=O)N1Cc4ccccc4  
**3.23**

Compd.	R <sup>1</sup>	R <sup>2</sup>	X	Yield (%)
<b>3.23p</b>	H	H	2-F	67
<b>3.23q</b>	Me	H	2-F	59
<b>3.23r</b>	Ph	H	2-F	22
<b>3.23s</b>	Me	Me	2-F	48
<b>3.23t</b>	H	H	3-F	60
<b>3.23u</b>	Me	H	3-F	66
<b>3.23v</b>	Me	Me	3-F	54
<b>3.23w</b>	H	H	4-F	74
<b>3.23x</b>	Me	H	4-F	55
<b>3.23y</b>	Me	Me	4-F	50
<b>3.23z</b>	H	H	4-Cl	52
<b>3.23za</b>	Me	H	4-Cl	69
<b>3.23zb</b>	Ph	H	4-Cl	24
<b>3.23zc</b>	Me	Me	4-Cl	57
<b>3.23zd</b>	H	H	4-Br	63
<b>3.23ze</b>	Me	Me	4-Br	74
<b>3.23zf</b>	H	H	4-NO <sub>2</sub>	61
<b>3.23zg</b>	Me	Me	4-NO <sub>2</sub>	43
<b>3.23zh</b>	H	H	2-CF <sub>3</sub>	49
<b>3.23zi</b>	Me	Me	2-CF <sub>3</sub>	64
<b>3.23zj</b>	H	H	3-CF <sub>3</sub>	46
<b>3.23zk</b>	Me	Me	3-CF <sub>3</sub>	49
<b>3.23zl</b>	H	H	2,4-F <sub>2</sub>	49
<b>3.23zm</b>	Me	Me	2,4-F <sub>2</sub>	51
<b>3.23zn</b>	H	H	3,4-Cl <sub>2</sub>	42
<b>3.23zo</b>	Me	Me	3,4-Cl <sub>2</sub>	37
<b>3.23zp</b>	H	H	4-Ac	71 <sup>a)</sup>
<b>3.23zq</b>	Me	H	4-Ac	64 <sup>a)</sup>
<b>3.23zr</b>	Me	Me	4-Ac	80

**Scheme 3.12.** Synthesis of 3-[4-(3-arylureido)butyl]-1-benzyloxypyrazin-2(1*H*)-ones **3.23**. Reagents and conditions: (i) 4 M HCl (16 equiv) in dioxane, rt, 30 min; (ii) R<sup>1</sup>R<sup>2</sup>(CO)<sub>2</sub> (0.9 equiv), 2 M NaOH, pH 8-10, MeOH-H<sub>2</sub>O (2:1), -35 °C then rt (5-8 h), and 70 °C (1-2 h). <sup>a)</sup> TFA (16 equiv) in DCM was used in the N-Boc deprotection step.

The yields of compounds **3.23** were not impressive; however, we did not further investigate the optimization of this reaction and prioritized the diversification of the library of compounds instead. In the case of phenyl glyoxal, the reaction mixture was heated during the addition of excess phenyl glyoxal (portionwise addition), yields of these products **3.23** were very low and furthermore the mixture appeared to be contaminated with many by-products, requiring a great deal of purification steps through column chromatographies. Due to this problem, only a limited number of 3-[4-(3-arylureido)butyl]-1-benzyloxy-5-phenylpyrazin-2(1*H*)-ones **3.23** were prepared.

### 3.2 Synthesis of 1-hydroxypyrazin-2(1*H*)-ones

Part of this subchapter is based on the results described in our recent publication in the Journal of Flow Chemistry (DOI: 10.1556/JFC-D-14-00036).<sup>31</sup> Following is a summary of the work presented in the paper, expanded with a set of new results dealing with the synthesis of 3-[4-(3-arylureido)butyl]-1-hydroxypyrazin-2(1*H*)-ones.

#### 3.2.1 Debenzylation of 1-benzyloxypyrazin-2(1*H*)-ones in batch

Reductive debenzylation of O-benzyl hydroxamates has been reported before in literature, though yields are sometimes low and overreduction to amides is a known problem; however, an appropriate catalyst system selection (Pd-BaSO<sub>4</sub>) can solve this problem in a number of cases.<sup>32-41</sup>

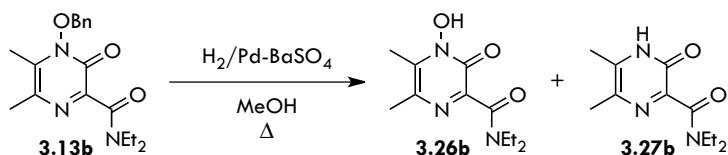
We started our debenzylation studies with 1-benzyloxypyrazin-2(1*H*)-one (**3.4a**) using batch chemistry (**Table 3.1**) with hydrogen gas and different palladium catalysts (e.g. Pd-C and Pd-BaSO<sub>4</sub>). This showed that the hydrogenolysis is fast and more selective for the desired compounds **3.24a** with the less reactive Pd-based catalysts at high temperature (Entry 6, **Table 3.1**). Since a characteristic ultraviolet (UV) absorption maximum of the core pyrazinone scaffolds is present in the range of 320-370 nm (depending on pH), a high-performance liquid chromatography-ultraviolet-mass spectrometry (HPLC-UV-MS) method has been used to quantify the relative conversion and selectivity of the reactions in this study.<sup>24,42,43</sup> It is based on the integration of the relative peak areas of the reactant and the product and the by-product UV-absorption signals at the indicated wavelength.

**Table 3.1.** Batch debenzylation of 1-benzyloxypyrazin-2(1*H*)-one **3.4a**.

Entry	Catalyst	Condition	Ratio <sup>a)</sup>	
			3.24a	3.25a
1		2 bar / rt / 5 min	0	100
2		1 bar / rt / 10 min	25	75
3	5% Pd-C	1 bar / rt / 30 min	20	80
4		1 bar / reflux / 5 min	60	40
5 <sup>b)</sup>	5% Pd-BaSO <sub>4</sub>	1 bar / reflux / 5 min	80	20
6 <sup>c)</sup>	5% Pd-BaSO <sub>4</sub>	1 bar / reflux / 5 min	95	5

Reagents and conditions: **3.4a** (0.1 mmol, 0.1 M), H<sub>2</sub> (in a Parr apparatus or with a balloon), palladium catalyst (5 %wt.). <sup>a)</sup> Quantification at 350 nm via RP HPLC (with MS confirmation of compound identity). <sup>b)</sup> Pd-BaSO<sub>4</sub> reduced form. <sup>c)</sup> Pd-BaSO<sub>4</sub> unreduced form.

However, when the same conditions were applied to 1-benzyloxypyrazin-2(1*H*)-one-3-carboxamide derivatives **3.13**, removal of the benzyl group most of the time met with failure due to overreduction. Exceptionally, an excellent conversion (91%, HPLC-UV-MS, 350 nm) was only once obtained when performing the debenzylation of **3.13b** with 5% Pd-BaSO<sub>4</sub> (unreduced form) under hydrogen gas (1 bar, balloon, **Scheme 3.13**). Moreover, the selectivity obtained in all these reactions was not reproducible even with the same substrates.



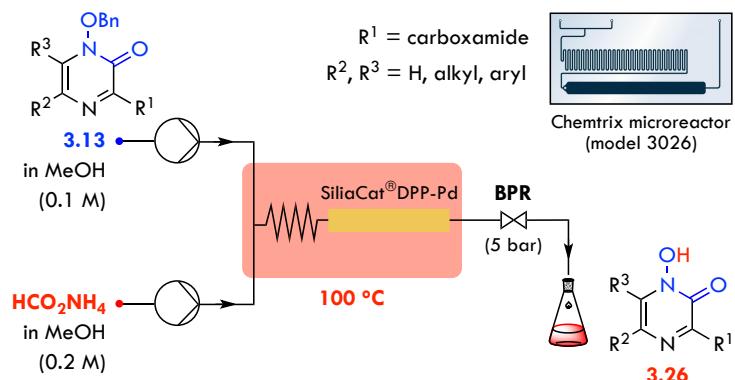
**Scheme 3.13.** Batch debenzylation of 1-benzyloxypyrazin-2(1*H*)-one-3-carboxamide **3.13b**. Reagents and conditions: **3.13b** (33 mg, 0.1 mmol), MeOH (2.5 mL), H<sub>2</sub> (balloon), 5% Pd-BaSO<sub>4</sub> (unreduced form, 1.65 mg), reflux, 30 min. Selectivity at 350 nm (via HPLC-UV-MS). Quantitative conversion.

Efficient control of the contact time between the reagents and the products and the catalyst seemed to be of the utmost importance. So we decided to switch from conventional batch hydrogenation conditions to

flow technology using a catalytic transfer hydrogenation where residence times are more easily tuned and controlled.

### 3.2.2 Methodology of debenzylation of 1-benzyloxypyrazin-2(1H)-ones in flow

The reactions under flow conditions were performed in the Labtrix®Start system using a Chemtrix Catalyst microreactor, PTFE tubing, syringe pump, heating controller, and a 5 bar backpressure regulator (**Figure 3.4**). We carefully screened and optimized the factors that could affect the selectivity and the conversion of the debenzylation process under flow conditions.



**Figure 3.4.** Schematic representation for the debenzylation experimental set-up in flow.

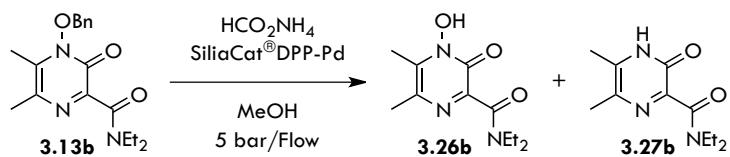
As mentioned in subchapter 1.4, low product solubility is a limiting factor in flow chemistry in general; the formation of precipitate during the reaction may block the microchannels and build up pressure, which could break the glass reactor.<sup>44,45</sup> After screening different solvents we found methanol being the best candidate: it could properly dissolve reasonable amounts of most of our substrates as well as the resulting debenzylated products. Besides that, its polarity is also favorable for the hydrogenolysis. Ethanol and isopropanol were able to dissolve the substrate benzyl hydroxamates, but not the hydroxamic acid products.

Many hydrogen donors can be used in catalytic transfer hydrogenation, such as formic acid and its salts, hydrazine, isopropanol, cyclohexane,

cyclohexadiene, phosphinic acid, etc.<sup>46,47</sup> *In situ* hydrogen formation increases the gas-liquid-solid phase interaction, helps to control the stoichiometry of the reagents, and most importantly helps to avoid the risk of fire and explosion associated with using hydrogen gas (especially when dealing with larger scale synthesis). We used ammonium formate, which shows more advantages in comparison to the others, as a hydrogen donor.<sup>48-53</sup> Ammonium formate acts as a hydrogen equivalent that may be presented either in the adsorbed form on palladium or in the palladium hydride form.<sup>53</sup> Study on the decomposition of ammonium formate shows that it brings to the reaction not only hydrogen but also ammonia and carbon dioxide.<sup>49,52-54</sup> These gases were observed as bubbles formed downstream of the backpressure regulator.

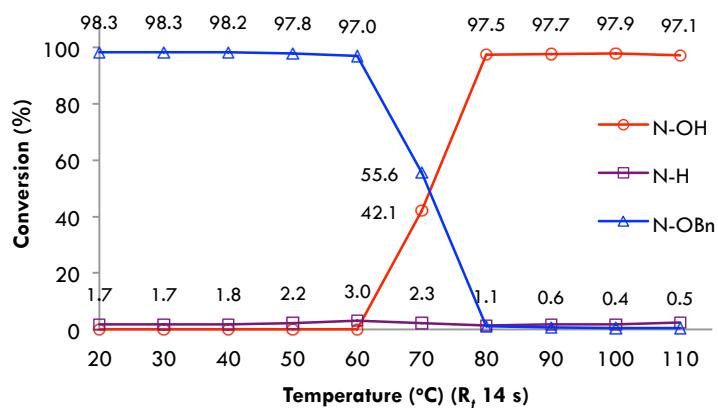
The pressure in the reaction mixture was fixed using a backpressure regulator set at 5 bar. This also allowed us to work above the boiling point of methanol up to 110 °C.

An appropriate catalyst was needed for the debenylation, both in terms of reactivity and in terms of reactor specifications. Horn and Cerato-Noyerie recently reported the development of a convenient *in situ* PdCl<sub>2</sub>-based catalyst for hydrogenation in glass microreactors.<sup>55</sup> We found an alternative Pd source with appropriate particle size specifications in the form of SiliaCat®DPP-Pd which has been shown to be a useful catalyst for many kinds of Pd-catalyzed reactions (Heck, Sonogashira, Kumada, Suzuki, Stille reactions and Buchwald aminations) in flow.<sup>56-58</sup> This catalyst was used in the optimization of temperature and residence time for the debenylation in flow using the model system 1-benzyloxypyrazin-2(1H)-one-3-carboxamide **3.13b** with ammonium formate in methanol (**Scheme 3.14**).



**Scheme 3.14.** Debenylation of representative precursor **3.13b** in flow. Reagents and conditions: **3.13b** (0.25 mmol, 0.1 M, 2.5 mL), HCO<sub>2</sub>NH<sub>4</sub> (0.5 mmol, 0.2 M, 2.5 mL, 2 equiv), MeOH (solvent), SiliaCat®DPP-Pd (12 mg).

The optimization of the temperature and the residence time was performed using the setup described in **Figure 3.4** and **Scheme 3.14**. The two solutions were pumped into the reactor containing SiliaCat®DPP-Pd catalyst. Product, by-product and starting material (if not fully converted) passed through the backpressure regulator before being collected in vials, which were used for HPLC-UV-MS analysis. Temperature (20–110 °C) and residence time ( $R$ , 14–36 s) were varied and the results are summarized in **Figure 3.5** (temperature effect) and **Figure 3.6** (residence time effect).

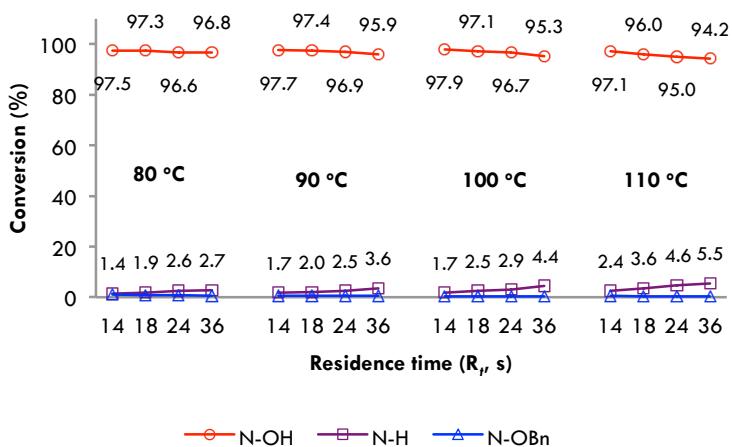


**Figure 3.5.** Temperature effect on debenzylation of **3.13b** in flow (at residence time of 14 s, about 6 dead volumes were allowed to pass after modifying the temperature before the conversion was determined, conversion at 350 nm, HPLC-UV-MS).

As shown in **Figure 3.5**, the reaction only occurs with acceptable conversions above 60 °C, the temperature above which ammonium formate efficiently decomposes in the presence of Pd. Without catalyst ammonium formate decomposes at above 180 °C. The conversion is more or less constant and complete within a wide range of temperatures (80–110 °C) at a residence time of 14 s without losing selectivity for the formation of the desired product (**3.26b**, N-OH form, 97–98%) over the overreduced product (**3.27b**, N-H form, 1–2%).

At 80 °C, complete conversion is seen with the residence time of 14 s. Increasing the residence time favors the formation of overreduced

species (**Figure 3.6**). The same trend is observed at higher temperatures, with relatively larger amounts of overreduced compounds formed at longer residence time (up to 5.5 % at 110 °C using a residence time of 36 s).

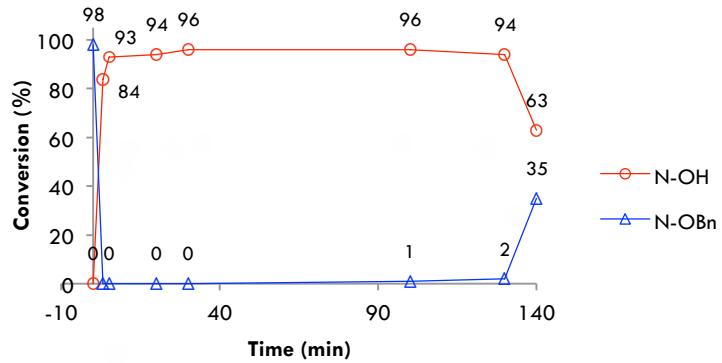


**Figure 3.6.** Residence time effect on debenzylation of **3.13b** in flow (at different temperatures, conversion at 350 nm, HPLC-UV-MS)

Based on these results, it is considered that the optimal condition for debenzylation of 1-benzyloxypyrazin-2(1*H*)-one-3-carboxamide **3.13b** in this flow setup with  $\text{HCO}_2\text{NH}_4$ /SiliaCat®DPP-Pd in methanol are a temperature above 80 °C and a residence time of 14 s. Using these conditions, the turnover number of the catalyst was determined to be at least 100 in a long-run debenzylation of compound **3.13b** (**Figure 3.7**).

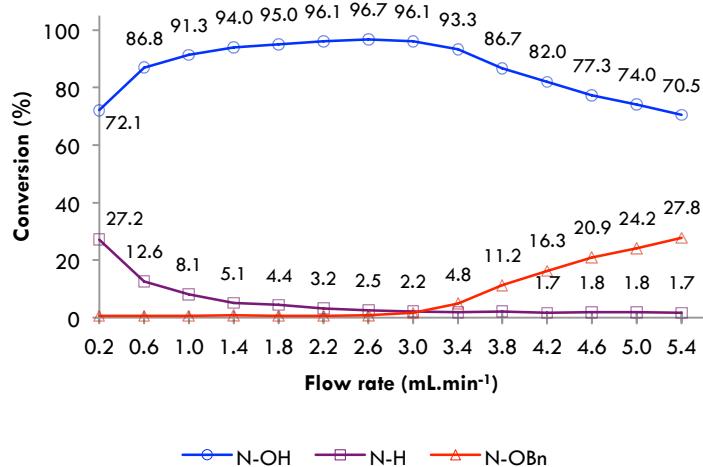
To test the scalability of the protocol, a follow-up experiment was performed with compound **3.13b** in a ThalesNano X-Cube milliflow reactor system equipped with a catalyst cartridge containing SiliaCat®DPP-Pd. Excellent conversion (96%) and selectivity (98%) were obtained at a flow rate of 3.0  $\text{mL}\cdot\text{min}^{-1}$  (**Figure 3.8**).

In this way 2.2 g of **3.13b** has been converted into the corresponding 1-hydroxypyrazin-2(1*H*)-one-3-carboxamide **3.26b** (1.4 g, 90% yield) at 110 °C, 30 bar backpressure with a flow rate of 3.0  $\text{mL}\cdot\text{min}^{-1}$ .



**Figure 3.7.** Long-term performance of debenzylation of compound **3.13b** in Labtrix®Start (conversion at 350 nm, HPLC-UV-MS). Reagents and conditions: **3.13b** (0.1 M),  $\text{HCO}_2\text{NH}_4$  (0.2 M; 2 equiv), SiliaCat®DPP-Pd (13.4 mg, 0.2 mmol.g<sup>-1</sup> loading), MeOH (solvent), 100 °C, residence time R, 14 s (total 50  $\mu\text{L}\cdot\text{min}^{-1}$  flow rate). Turnover number of the catalyst was calculated as follows:

$$\text{TON} = \frac{\text{mmol}_{(3.26b)}}{\text{mmol}_{\text{catalyst}}} = \frac{125 \text{ (min)} \times 25 \text{ ( $\mu\text{L}\cdot\text{min}^{-1}$ )} \times 10^{-3} \times 0.1 \text{ (mmol.mL}^{-1})}{0.2 \text{ (mmol.g}^{-1}) \times 10^{-3} \times 13.4 \text{ (mg)}} \approx 116$$



**Figure 3.8.** Flow rate effect on debenzylation of **3.13b** on X-Cube at 110 °C and 30 bar backpressure (conversion at 350 nm, HPLC-UV-MS). Reagents and conditions: **3.13b** (0.1 M),  $\text{HCO}_2\text{NH}_4$  (0.2 M; 2 equiv), SiliaCat®DPP-Pd (188 mg), MeOH (solvent).

### 3.2.3 Synthesis of *N*-hydroxypyrazinones via debenzylation in flow

For further testing the scope of the procedure, we preferred to perform the reactions at 100 °C since the higher temperature would be beneficial in keeping all of the reaction partners in solution.

In this way we managed to convert the compounds **3.4** into a library of aspergillic acid-like hydroxamic acids in good to excellent yields (**Table 3.2**). All the compounds **3.24** show satisfactory analytical data and test positive for the hydroxamate function in an aqueous ferric chloride solution (dark red color) and an aqueous copper (II) acetate solution (green color).

**Table 3.2.** Debenzylation of 1-benzyloxypyrazin-2(1*H*)-ones **3.4** in flow.

	HCO <sub>2</sub> NH <sub>4</sub>	SiliaCat®DPP-Pd	MeOH	100 °C/5 bar/R, 14 s		+	
Entry	Precursor	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	Yield of <b>3.24</b> (%) <sup>a)</sup>	Selectivity (%) <sup>b)</sup>	
					3.24	3.24	3.25
1	<b>3.4a</b>	H	H	H	87	100 0	
2	<b>3.4b</b>	H	Me	H	91	100 0	
3	<b>3.4c</b>	H	Ph	H	89	94 6	
4	<b>3.4d</b>	H	Me	Me	90	99 1	
5	<b>3.4e</b>	Me	Me	H	93	100 0	
6	<b>3.4f</b>	<i>i</i> -Bu	Ph	H	95	100 0	

Reagents and conditions: **3.4** (0.1 mmol, 0.1 M), HCO<sub>2</sub>NH<sub>4</sub> (0.2 mmol, 0.2 M, 2 equiv), MeOH (solvent), SiliaCat®DPP-Pd (12 mg), 100 °C, R, 14 s. <sup>a)</sup> Isolated yield. <sup>b)</sup> Quantitative conversion (quantification at 350 nm, HPLC-UV-MS).

Though the structures of 3-carboxamide O-benzyl precursors **3.13** are more complex than the **3.4** analogues, most of them react in the same fashion when being treated with a combination of HCO<sub>2</sub>NH<sub>4</sub>/SiliaCat®DPP-Pd. A family of *N*-hydroxypyrazin-2(1*H*)-one-3-carboxamides **3.26** was synthesized by debenzylation of the corresponding precursors **3.13** using flow methodology as shown in **Table 3.3**. These reactions were excellent both in terms of yield and selectivity.

**Table 3.3.** Debenzylation of 1-benzyloxypyrazin-2(1*H*)-ones **3.13** in flow.

The reaction scheme shows the conversion of 1-benzyloxypyrazin-2(1*H*)-ones (**3.13**) to two products: *N*-hydroxypyrazinones (**3.26**) and tertiary amide products (**3.27**). The starting material **3.13** is a pyrazinone derivative with a benzyl group (OBn) at position 1 and an amide group (NR<sup>1</sup>R<sup>2</sup>) at position 2. It reacts with HCO<sub>2</sub>NH<sub>4</sub> (0.2 mmol, 0.2 M, 2 equiv) in MeOH (solvent) over SiliaCat®DPP-Pd (12 mg) at 100 °C/5 bar for R<sub>f</sub> 14 s. Product **3.26** is the *N*-hydroxypyrazinone where the benzyl group is replaced by a hydroxyl group (OH). Product **3.27** is the tertiary amide where the benzyl group is replaced by an amide group (NR<sup>1</sup>R<sup>2</sup>).

Entry	Precursor	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	Yield of		Selectivity (%) <sup>b</sup>
						(%) <sup>a</sup>	<b>3.26</b>	
1	<b>3.13a</b>	Et	Et	H	H	92	100	0
2	<b>3.13b</b>	Et	Et	Me	Me	90	98	2
3	<b>3.13c</b>	H	Bn	Me	Me	72	85	15 <sup>c,d)</sup>
4	<b>3.13d</b>	Me	Bn	Me	Me	91	96	4
5	<b>3.13e</b>	Et	Bn	Me	Me	94	97	3
6	<b>3.13f</b>	Me	3-FC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	H	H	93	100	0
7	<b>3.13g</b>	Me	3-FC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Me	H	96	100	0
8	<b>3.13h</b>	Me	3-FC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Ph	H	73	87	13 <sup>e)</sup>
9	<b>3.13i</b>	Me	3-FC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Me	Me	81	89	11
10	<b>3.13j</b>	Me	4-FC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	H	H	94	100	0
11	<b>3.13k</b>	Me	4-FC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Me	H	89	97	3
12	<b>3.13l</b>	Me	4-FC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Me	Me	90	95	5
13	<b>3.13m</b>	Me	4-ClC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	H	H	98	100	0
14	<b>3.13n</b>	Me	4-ClC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Me	H	89	97	3
15	<b>3.13o</b>	Me	4-ClC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Me	Me	91	95	5
16	<b>3.13p</b>	Me	2,4-Cl <sub>2</sub> C <sub>6</sub> H <sub>3</sub> CH <sub>2</sub>	H	H	95	100	0
17	<b>3.13q</b>	Me	2,4-Cl <sub>2</sub> C <sub>6</sub> H <sub>3</sub> CH <sub>2</sub>	Me	H	92	98	2
18	<b>3.13r</b>	Me	2,4-Cl <sub>2</sub> C <sub>6</sub> H <sub>3</sub> CH <sub>2</sub>	Me	Me	82	87	13 <sup>e)</sup>
19	<b>3.13s</b>	H	3-FC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Me	Me	53	62	38 <sup>c,d)</sup>
20	<b>3.13s</b>	H	3-FC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Me	Me	91	93	7 <sup>c,d,f)</sup>
21	<b>3.13t</b>	H	4-FC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Me	H	93	96	4 <sup>c,d,f)</sup>
22	<b>3.13u</b>	H	4-FC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Me	Me	92	96	4 <sup>c,d,f)</sup>
23	<b>3.13w</b>	H	4-ClC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Me	Me	91	94	6 <sup>c,d,f)</sup>

Reagents and conditions: **3.13** (0.1 mmol, 0.1 M), HCO<sub>2</sub>NH<sub>4</sub> (0.2 mmol, 0.2 M, 2 equiv), MeOH (solvent), SiliaCat®DPP-Pd (12 mg), 100 °C, R<sub>f</sub> 14 s. <sup>a)</sup> Isolated yield. <sup>b)</sup> Quantitative conversion (quantification at 350 nm, HPLC-UV-MS). <sup>c)</sup> Quantification at 375 nm (HPLC-UV-MS). <sup>d)</sup> Precipitation could be avoided by diluting starting materials 3 times or using a mixture of MeOH-toluene. <sup>e)</sup> Substrate contained impurities (~8%, HPLC-UV-MS, 350 nm). <sup>f)</sup> Reactions were performed at 80 °C and R<sub>f</sub> 10 s.

In case of secondary amides, **3.13c,s-w** (Entries 3 and 19-23, **Table 3.3**), the solubility behavior of the product *N*-hydroxypyrazinones **3.26** is completely different in comparison with that of tertiary amide products. These secondary amide *N*-hydroxypyrazinones are less soluble in MeOH and are easily precipitated right after forming inside the reactor/tubing, which causes the problem of microchannel clogging. An

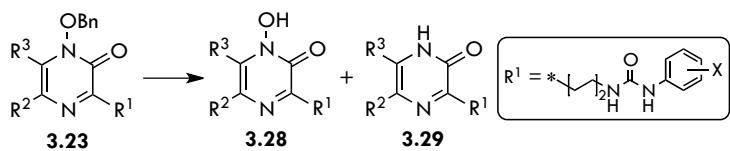
organic acid additive, for instance formic acid or acetic acid, solves the issue of poor solubility; however, it also drives the reaction to the overreduction pathway to form pyrazinones **3.27**. Working with more dilute solution of the reactants or changing the solvent system (e.g., MeOH-toluene) is ultimately required, but, even so, the yield and selectivity of these conversions were still not as good as expected (Entries 3 and 19, **Table 3.3**). A quick optimization was performed on the example **3.13s** and we found that the best conditions for debenzylation of secondary amide 1-benzyloxypyrazin-2(1*H*)-one-3-carboxamides are a temperature of 80 °C and a residence time of 10 s. By applying these conditions, the yield and selectivity of **3.26s-w** were improved dramatically (Entries 20-23, **Table 3.3**).

Furthermore, selective N-/O-debenzylation may also be an issue of some specific substrates containing an N-benzyl amide (**3.13c-e**, Entries 3-5, **Table 3.3**); several catalyst systems have been developed to promote either N-debenzylation or O-debenzylation.<sup>33,59-62</sup> Using HCO<sub>2</sub>NH<sub>4</sub>/SiliaCat®DPP-Pd, neither N-debenzylation of the 1-benzyloxypyrazin-2(1*H*)-one-3-carboxamides nor dehalogenation was observed as a side reaction in our examples (**3.13c-e**, Entries 3-5, and **3.13f-w**, Entries 6-23, **Table 3.3**) even at high temperature and high pressure, which are known selectivity hampering factors.<sup>63</sup>

Applying the same flow methodology for debenzylation as was used in preparation of N-hydroxypyrazin-2(1*H*)-ones **3.24** and **3.26**, an analogue of 3-[4-(3-arylureido)butyl]-1-hydroxypyrazin-2(1*H*)-ones **3.28** was synthesized as shown in **Table 3.4** and **Table 3.5**. Due to the poor solubility of 3-[4-(3-arylureido)butyl]-1-benzyloxypyrazin-2(1*H*)-ones **3.23** in pure MeOH, a mixture of MeOH-toluene (1:1 v/v) was used to make a solution of precursors **3.23** at a low concentration (0.04 M).

The experiments in **Table 3.4** show excellent results in terms of yield and selectivity for compounds **3.28**. The impurities from the starting material are again noticed as the factor that lowers the yield in the examples of **3.23c,g,j,n,r** (Entries 3, 7, 10, 14 and 18, **Table 3.4**).

**Table 3.4.** Debenzylation of 1-benzyloxypyrazin-2(1*H*)-ones **3.23** in flow.



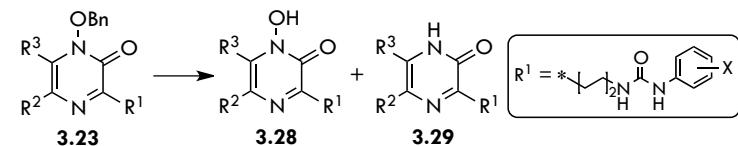
Entry	Precursor	$R^2$	$R^3$	X	Yield of <b>3.28</b> (%) <sup>a)</sup>	Selectivity (%) <sup>b)</sup>	
						<b>3.28</b>	<b>3.29</b>
1	<b>3.23a</b>	H	H	H	90	97	3
2	<b>3.23b</b>	Me	H	H	92	100	0
3	<b>3.23c</b>	Ph	H	H	74	80	20
4	<b>3.23d</b>	Me	Me	H	93	99	1
5	<b>3.23e</b>	H	H	4-Me	98	100	0
6	<b>3.23f</b>	Me	H	4-Me	90	93	7
7	<b>3.23g</b>	Ph	H	4-Me	81	90	10
8	<b>3.23h</b>	Me	Me	4-Me	92	94	6
9	<b>3.23i</b>	H	H	2-OMe	100	100	0
10	<b>3.23j</b>	Me	H	2-OMe	78	81	19
11	<b>3.23k</b>	Me	Me	2-OMe	91	95	5
12	<b>3.23l</b>	H	H	4-OMe	92	100	0
13	<b>3.23m</b>	Me	H	4-OMe	88	100	0
14	<b>3.23n</b>	Ph	H	4-OMe	80	85	15
15	<b>3.23o</b>	Me	Me	4-OMe	95	97	3
16	<b>3.23p</b>	H	H	2-F	100	100	0
17	<b>3.23q</b>	Me	H	2-F	98	100	0
18	<b>3.23r</b>	Ph	H	2-F	83	88	12
19	<b>3.23s</b>	Me	Me	2-F	91	93	7
20	<b>3.23t</b>	H	H	3-F	100	100	0
21	<b>3.23u</b>	Me	H	3-F	90	100	0
22	<b>3.23v</b>	Me	Me	3-F	92	96	4
23	<b>3.23w</b>	H	H	4-F	99	100	0
24	<b>3.23x</b>	Me	H	4-F	97	100	0
25	<b>3.23y</b>	Me	Me	4-F	94	100	0
26	<b>3.23zh</b>	H	H	2-CF <sub>3</sub>	89	100	0
27	<b>3.23zi</b>	Me	Me	2-CF <sub>3</sub>	90	92	8
28	<b>3.23zj</b>	H	H	3-CF <sub>3</sub>	99	100	0
29	<b>3.23zk</b>	Me	Me	3-CF <sub>3</sub>	98	100	0
30	<b>3.23zl</b>	H	H	2,4-F <sub>2</sub>	98	100	0
31	<b>3.23zm</b>	Me	Me	2,4-F <sub>2</sub>	95	100	0
32	<b>3.23zn</b>	H	H	3,4-Cl <sub>2</sub>	90	100	0
33	<b>3.23zo</b>	Me	Me	3,4-Cl <sub>2</sub>	100	100	0
34	<b>3.23zp</b>	H	H	4-Ac	91	100	0
35	<b>3.23zq</b>	Me	H	4-Ac	90	100	0
36	<b>3.23zr</b>	Me	Me	4-Ac	88	92	8

Reagents and conditions: **3.23** (0.1 mmol, 0.04 M, in MeOH-toluene 1:1 v/v), HCO<sub>2</sub>NH<sub>4</sub> (0.2 mmol, 0.08 M, 2 equiv, in MeOH), SiliaCat®DPP-Pd (12 mg), 100 °C, R, 14 s. <sup>a)</sup> Isolated yield. <sup>b)</sup> Quantification at 320 nm (HPLC-UV-MS).

No dehalogenation was observed during the hydrogenative debenzylation of dichloro-substituted **3.23zn,zo** in flow (Entries 32 and 33, **Table 3.4**); however, it turned to be less obvious to deprotect the

monochloro- and monobromo-substituted precursors **3.23z-ze** (Entries 1-6, **Table 3.5**). Because of the lack of the selectivity in the case of bromo-compounds, we did not isolate any of these products.

**Table 3.5.** Debenzylation of 1-benzyloxypyrazin-2(1*H*)-ones **3.23** (cont).



Entry	Precursor	R <sup>2</sup>	R <sup>3</sup>	X	Isolated yield (vs. selectivity) (%)	
					3.28	3.29
1	<b>3.23z</b>	H	H	Cl	<b>3.28z</b> <b>90</b> (93)	<b>3.29z</b> (0)
				H	<b>3.28a</b> (7)	<b>3.29a</b> (0)
2	<b>3.23za</b>	Me	H	Cl	<b>3.28za</b> <b>86</b> (90)	<b>3.29za</b> (0)
				H	<b>3.28b</b> (10)	<b>3.29b</b> (0)
3	<b>3.23zb</b>	Ph	H	Cl	<b>3.28zb</b> <b>54</b> (63)	<b>3.29zb</b> (7)
				H	<b>3.28c</b> (30)	<b>3.29c</b> (0)
4	<b>3.23zc</b>	Me	Me	Cl	<b>3.28zc</b> <b>59</b> (64)	<b>3.29zc</b> (0)
				H	<b>3.28d</b> (36)	<b>3.29d</b> (0)
5	<b>3.23zd</b>	H	H	Br	<b>3.28zd</b> (0)	<b>3.29zd</b> (20)
				H	<b>3.28a</b> (60)	<b>3.29a</b> (20)
6	<b>3.23ze</b>	Me	Me	Br	<b>3.28ze</b> (20)	<b>3.29ze</b> (30)
				H	<b>3.28b</b> (20)	<b>3.29b</b> (20)
7 <sup>a,b)</sup>	<b>3.23zf</b>	H	H	NO <sub>2</sub>	<b>3.28zf</b> <b>90</b> (91)	<b>3.29zf</b> (0)
				NH <sub>2</sub>	<b>3.28zs</b> (9)	<b>3.29zs</b> (0)
8 <sup>a,c)</sup>	<b>3.23zf</b>	H	H	NO <sub>2</sub>	<b>3.28zf</b> (16)	<b>3.29zf</b> (0)
				NH <sub>2</sub>	<b>3.28zs</b> <b>82</b> (84)	<b>3.29zs</b> (0)
9 <sup>a,b)</sup>	<b>3.23zg</b>	Me	Me	NO <sub>2</sub>	<b>3.28zg</b> <b>75</b> (80)	<b>3.29zg</b> (15)
				NH <sub>2</sub>	<b>3.28zt</b> (5)	<b>3.29zt</b> (0)
10 <sup>a,c)</sup>	<b>3.23zg</b>	Me	Me	NO <sub>2</sub>	<b>3.28zg</b> (0)	<b>3.29zg</b> (0)
				NH <sub>2</sub>	<b>3.28zt</b> <b>93</b> (100)	<b>3.29zt</b> (0)

Reagents and conditions: **3.23** (0.1 mmol, 0.04 M, in MeOH-toluene 1:1 v/v), HCO<sub>2</sub>NH<sub>4</sub> (0.2 mmol, 0.08 M, 2 equiv, in MeOH), SiliaCat®DPP-Pd (12 mg), 100 °C, R<sub>f</sub> 14 s. <sup>a)</sup> Reactions in batch [**3.23** (0.1 mmol), H<sub>2</sub> (balloon), 5% Pd-BaSO<sub>4</sub> (unreduced form, 1.65 mg), MeOH (2.5 mL), refluxed]. <sup>b)</sup> In 20 min. <sup>c)</sup> In 120 min. Percentages in brackets (quantification at 320 nm, HPLC-UV-MS) present the selectivity of the reaction. The values in **bold** are isolated yields, which refer to the sum of all fractions collected (%).

The simultaneous reduction of the nitro substituent to an amino functional group during the debenzylation was detected in flow conditions. Nevertheless, performing the reaction in batch conditions under hydrogen atmosphere using a different catalyst system helped to control the selectivity (5% Pd-BaSO<sub>4</sub>, unreduced form). Within the course of 20

min refluxing, **3.23zf** and **3.23zg** were successfully converted to the corresponding debenzylation products without a significant reduction of the nitro group (Entries 7 and 9, **Table 3.5**). Prolonging the reaction time up to 2 h led to the formation of the amino-substituted urea *N*-hydroxypyrazinones **3.28zs** and **3.28zt** (Entries 8 and 10, **Table 3.5**) due to the completed debenzylation and reduction of the nitro group. We did not try this procedure in flow because the particle size of the Pd-BaSO<sub>4</sub> was not adapted to our microflow reactor chip.

All *N*-hydroxypyrazin-2(1*H*)-one products were purified by RP-HPLC before submitting for biological testing.

### 3.3 Biological evaluation

This part of the work has been performed by the group of Prof. Christophe Pannecouque (Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven). Two methodologies for screening HIV inhibitory activity were used, including an MT-4/MTT assay for analysis of HIV replication inhibitory activity and an HIV-1 RT assay for evaluation of HIV-1 RT polymerase inhibition.<sup>64,65</sup> A brief summary of these two assay protocols was given in the subchapters **5.4.1** and **5.4.2**.

Three series of compounds **3.24**, **3.26**, and **3.28** were initially screened for inhibitory activity against HIV-1 (strain IIIB) and HIV-2 (ROD strain) replication by using an MT-4/MTT assay. Unfortunately, all *N*-hydroxypyrazinone scaffolds submitted for anti-HIV replication evaluation show poor activity and exhibit no selectivity (**Tables 3.6** and **3.7**).

Further, an extra biological evaluation of 3-[4-(3-arylureido)butyl]-1-hydroxypyrazin-2(1*H*)-ones **3.28** and some other 1-hydroxypyrazin-2(1*H*)-ones **3.24f**, **3.26h**, **3.26t** against HIV-1 RT was performed by using the RT assay (EnzChek® RT assay kit). Some of the compounds show a limited inhibitory activity. Selected results were presented in **Table 3.8**. It is noticed that most of the compounds showing activity (**3.28c,g,s,zb**) contain the phenyl group at C-5 of the *N*-hydroxypyrazinone. This is in agreement with the results that Heeres and colleagues reported for *N*-methyl/benzyl pyrazinones with aryl substituents at C-5.<sup>66</sup> Further molecular modeling and SAR study are required to improve the inhibitory activity of these *N*-hydroxypyrazinones structures.

**Table 3.6.** Anti-HIV activity and cytotoxicity of *N*-hydroxypyrazinones **3.24** and **3.26**.

Entry	Compound	HIV-1 (IIIB strain) ( $\mu\text{g/mL}$ )		HIV-2 (ROD strain) ( $\mu\text{g/mL}$ )		SD	SI
		IC <sub>50</sub>	CC <sub>50</sub>	IC <sub>50</sub>	CC <sub>50</sub>		
1	<b>3.24a</b>	>50	>50	>50	>50		×1
2	<b>3.24b</b>	>50	>50	>50	>50		×1
3	<b>3.24c</b>	>50	>50	>50	>50		×1
4	<b>3.24f</b>	>8.46	8.46	>8.46	8.46	0.65	<1
5	<b>3.26a</b>	>50	>50	>50	>50		×1
6	<b>3.26b</b>	>50	>50	>50	>50		×1
7	<b>3.26c</b>	>50	>50	>50	>50		×1
8	<b>3.26d</b>	>50	>50	>50	>50		×1
9	<b>3.26e</b>	>50	>50	>50	>50		×1
10	<b>3.26f</b>	>50	>50	>50	>50		×1
11	<b>3.26g</b>	>50	>50	>50	>50		×1
12	<b>3.26h</b>	>40.20	≥40.20	>40.20	≥40.20		*
13	<b>3.26i</b>	>50	>50	>50	>50		×1
14	<b>3.26j</b>	>50	>50	>50	>50		×1
15	<b>3.26k</b>	>50	>50	>50	>50		×1
16	<b>3.26l</b>	>50	>50	>50	>50		×1
17	<b>3.26m</b>	>50	>50	>50	>50		×1
18	<b>3.26n</b>	>50	>50	>50	>50		×1
19	<b>3.26o</b>	>50	>50	>50	>50		×1
20	<b>3.26p</b>	>50	>50	>50	>50		×1
21	<b>3.26q</b>	>50	>50	>50	>50		×1
22	<b>3.26r</b>	>50	>50	>50	>50		×1
23	<b>3.26t</b>	>100	>100	>100	>100		×1
3.6.1	<b>BOE/BIRG587</b>	0.083	>4.00			0.015	48
				>4.00	>4.00		×1
3.6.2	<b>CLO/3TC</b>	0.51	>20.00			0.19	>39
				2.02	>20.00	0.67	>10
3.6.3	<b>DDN/AZT</b>	0.0019	>25.00			0.0008	>13144
				0.0018	>25.00	0.0004	>14245
3.6.4	<b>DDN/DDI</b>	5.48	>50.00			1.79	>9
				10.61	>50.00	4.59	>5

IC<sub>50</sub> : 50% Inhibitory concentration (concentration at which 50% inhibition of virus replication is observed)

CC<sub>50</sub> : 50% Cytotoxic concentration (concentration at which 50% adverse effect is observed on the host cell)

SD : Standard deviation

SI : Selectivity Index (CC<sub>50</sub>/IC<sub>50</sub>)

\* : < or ×1

BOE/BIRG587 : Nevirapine, an antiretroviral drug (NNRTI)

CLO/3TC : Lamivudine, an antiretroviral drug (NNRTI)

DDN/AZT : 2',3'-Dideoxynucleoside/3'-azido-3'-deoxythymidine

DDN/DDI : 2',3'-Dideoxynucleoside/2',3'-dideoxyinosine

**Table 3.7.** Anti-HIV activity and cytotoxicity of the *N*-hydroxypyrazinones 3.28.

Entry	Compound	HIV-1 (IIIB strain) ( $\mu$ g/mL)		HIV-2 (ROD strain) ( $\mu$ g/mL)		SD	SI
		IC <sub>50</sub>	CC <sub>50</sub>	IC <sub>50</sub>	CC <sub>50</sub>		
1	<b>3.28a</b>	>100.00	>100.00	>100.00	>100.00		×1
2	<b>3.28b</b>	>45.65	45.65	>45.65	45.65	6.64	<1
3	<b>3.28c</b>	>9.07	9.07	>9.07	9.07	0.77	<1
4	<b>3.28d</b>	>54.58	54.58	>54.58	54.58	5.78	<1
5	<b>3.28e</b>	>74.50	≥74.50	>74.50	≥74.50		*
6	<b>3.28f</b>	>41.93	41.93	>41.93	41.93	4.62	<1
7	<b>3.28g</b>	>7.40	7.40	>7.40	7.40	2.49	<1
8	<b>3.28h</b>	>9.05	9.05	>9.05	9.05	1.24	<1
9	<b>3.28i</b>	>91.00	≥91.00	>91.00	≥91.00		*
10	<b>3.28j</b>	>42.90	42.90	>42.90	42.90	3.10	<1
11	<b>3.28k</b>	>22.51	22.51	>22.51	22.51	16.92	<1
12	<b>3.28l</b>	>100.00	>100.00	>100.00	>100.00		×1
13	<b>3.28m</b>	>100.00	>100.00	>100.00	>100.00		×1
14	<b>3.28n</b>	>35.10	35.10	>35.10	35.10	14.47	<1
15	<b>3.28o</b>	>31.01	31.01	>31.01	31.01	24.86	<1
16	<b>3.28p</b>	>100.00	>100.00	>100.00	>100.00		×1
17	<b>3.28q</b>	>44.60	44.60	>44.60	44.60	4.99	<1
18	<b>3.28r</b>	>5.44	5.44	>5.44	5.44	3.28	<1
19	<b>3.28s</b>	>8.80	8.80	>8.80	8.80	0.87	<1
20	<b>3.28t</b>	>100.00	>100.00	>100.00	>100.00		×1
21	<b>3.28u</b>	>47.35	47.35	>47.35	47.35	3.77	<1
22	<b>3.28v</b>	>9.18	9.18	>9.18	9.18	1.52	<1
3.7.1 <b>BOE/BIRG587</b>		0.083	>4.00			0.015	48
				>4.00	>4.00		×1
3.7.2 <b>CLO/3TC</b>		0.51	>20.00			0.19	>39
				2.02	>20.00	0.67	>10
3.7.3 <b>DDN/AZT</b>		0.0019	>25.00			0.0008	>13144
				0.0018	>25.00	0.0004	>14245
3.7.4 <b>DDN/DDI</b>		5.48	>50.00			1.79	>9
				10.61	>50.00	4.59	>5

IC<sub>50</sub> : 50% Inhibitory concentrationCC<sub>50</sub> : 50% Cytotoxic concentration

SD : Standard deviation

SI : Selectivity Index

\* : &lt; or ×1

BOE/BIRG587 : Nevirapine

CLO/3TC : Lamivudine

DDN/AZT : 2',3'-Dideoxynucleoside/3'-azido-3'-deoxythymidine

DDN/DDI : 2',3'-Dideoxynucleoside/2',3'-dideoxyinosine

**Table 3.7. (cont.)**

Entry	Compound	HIV-1 (IIIB strain) ( $\mu\text{g/mL}$ )		HIV-2 (ROD strain) ( $\mu\text{g/mL}$ )		SD	SI
		IC <sub>50</sub>	CC <sub>50</sub>	IC <sub>50</sub>	CC <sub>50</sub>		
23	<b>3.28w</b>	>100.00	>100.00	>100.00	>100.00		×1
24	<b>3.28x</b>	>42.83	42.83	>42.83	42.83	2.75	<1
25	<b>3.28y</b>	>9.02	9.02	>9.02	9.02	0.44	<1
26	<b>3.28z</b>	>45.53	45.53	>45.53	45.53	5.03	<1
27	<b>3.28za</b>	>31.06	31.06	>31.06	31.06	26.22	<1
28	<b>3.28zb</b>	>6.77	6.77	>6.77	6.77	2.31	<1
29	<b>3.28zc</b>	>8.72	8.72	>8.72	8.72	1.28	<1
30	<b>3.28zf</b>	>100.00	>100.00	>100.00	>100.00		×1
31	<b>3.28zg</b>	>9.35	9.35	>9.35	9.35	2.08	<1
32	<b>3.23zh</b>	>100.00	>100.00	>100.00	>100.00		×1
33	<b>3.23zi</b>	>7.47	7.47	>9.35	9.35	1.46	<1
34	<b>3.23zj</b>	>40.55	40.55	>40.55	40.55	5.50	<1
35	<b>3.23zk</b>	>7.98	7.98	>7.98	7.98	1.35	<1
36	<b>3.23zl</b>	>47.10	≥47.10	>47.10	≥47.10		*
37	<b>3.23zm</b>	>7.99	7.99	>7.99	7.99	2.00	<1
38	<b>3.23zn</b>	>31.83	31.83	>31.83	31.83	23.37	<1
39	<b>3.23zo</b>	>7.53	7.53	>7.53	7.53	3.90	<1
40	<b>3.23zp</b>	>100.00	>100.00	>100.00	>100.00		×1
41	<b>3.23zq</b>	>43.98	43.98	>43.98	43.98	4.07	<1
42	<b>3.23zr</b>	>52.30	52.30	>52.30	52.30	9.58	<1
43	<b>3.28zt</b>	>100.00	>100.00	>100.00	>100.00		×1
3.7.1 <b>BOE/BIRG587</b>		0.083	>4.00			0.015	48
				>4.00	>4.00		×1
3.7.2 <b>CLO/3TC</b>		0.51	>20.00			0.19	>39
				2.02	>20.00	0.67	>10
3.7.3 <b>DDN/AZT</b>		0.0019	>25.00			0.0008	>13144
				0.0018	>25.00	0.0004	>14245
3.7.4 <b>DDN/DDI</b>		5.48	>50.00			1.79	>9
				10.61	>50.00	4.59	>5

IC<sub>50</sub> : 50% Inhibitory concentrationCC<sub>50</sub> : 50% Cytotoxic concentration

SD : Standard deviation

SI : Selectivity Index

\* : &lt; or ×1

BOE/BIRG587 : Nevirapine

CLO/3TC : Lamivudine

DDN/AZT : 2',3'-Dideoxynucleoside/3'-azido-3'-deoxythymidine

DDN/DDI : 2',3'-Dideoxynucleoside/2',3'-dideoxyinosine

**Table 3.8.** Activity against HIV-1 RT polymerase of some *N*-hydroxypyrazinones.

Entry	Compound	IC <sub>50</sub> (μg/mL)	SD
1	<b>3.24f</b>	>120	
2	<b>3.26h</b>	>120	
3	<b>3.26t</b>	>120	
4	<b>3.28b</b>	>120	
5	<b>3.28c</b>	54.45	7.61
6	<b>3.28e</b>	>120	
7	<b>3.28g</b>	44.10	7.77
8	<b>3.28i</b>	>120	
9	<b>3.28l</b>	>120	
10	<b>3.28n</b>	>120	
11	<b>3.28p</b>	>120	
12	<b>3.28r</b>	>120	
13	<b>3.28s</b>	105.07	15.28
14	<b>3.28t</b>	>120	
15	<b>3.28w</b>	>120	
16	<b>3.28z</b>	>120	
17	<b>3.28zb</b>	36.13	3.18
18	<b>3.28zf</b>	>120	
19	<b>3.23zg</b>	>120	
20	<b>3.23zh</b>	>120	
21	<b>3.23zi</b>	>120	
22	<b>3.23zl</b>	>120	
23	<b>3.23zn</b>	>120	
24	<b>3.23zp</b>	>120	
25	<b>3.28zt</b>	>120	
3.8.1	<b>BOE/BIRG587</b>	0.81	0.29
3.8.2	<b>DMP266</b>	0.03	0.02

IC<sub>50</sub> : 50% Inhibitory concentration

SD : Standard deviation

BOE/BIRG587 : Nevirapine

DMP266 : Efavirenz

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## Chapter 4

# Conclusions and Perspectives

### 4.1 Conclusions

#### 4.1.1 Synthesis of *N*-hydroxypyrazinone analogue

#### 4.1.2 Biological evaluation of the compounds

### 4.2 Future perspectives



## 4.1 Conclusions

### 4.1.1 Synthesis of *N*-hydroxypyrazinone analogue

No real problems were met during the synthesis of the pyrazinone precursors. Most of intermediate products were prepared in good to excellent yields. Though the cyclization in preparation of O-benzyl *N*-hydroxypyrazinones seems not to have a very high yield, it might be further optimized to obtain better yields especially in case of reactions involving phenyl glyoxal (for example, by refluxing in methanol as described by Jones in synthesis of pyrazinones).<sup>1</sup>

While attempts of C-3 functionalization of O-benzyl *N*-hydroxypyrazinones were maximized with either C-3 alkyl (**3.4a-f**) or C-3 carboxamide group [as both secondary amide (**3.13c,s-w,x**) and tertiary amide (**3.13a-b,d-r,y**)] or 3-[4-(3-arylureido)butyl] group (**3.23a-zr**), the functionalization at C-5 and C-6 were limited to H, Me, and Ph due to the availability of glyoxal derivatives. Although regioselectivity in the cyclization in the synthesis of pyrazinones has been mentioned in the literature, no specific explanation was found in the available literature for this system.<sup>2</sup> Therefore, we put forward a mechanistic explanation for this reaction.

With the precursors in hand, the final step in the synthetic pathway, the debenzylation, was thoroughly investigated to prepare *N*-hydroxypyrazinones. Batch hydrogenolysis debenzylation requires a deactivated Palladium-based catalyst (e.g. 5% Pd-BaSO<sub>4</sub>, unreduced form) in order to avoid over-reduction leading to the formation of lactams instead of hydroxamic acid pyrazinones; however, the reproducibility in this batch reaction remains problematic.

With the application of flow technology and catalytic transfer hydrogenation, we have succeeded in delivering a reproducible methodology for the selective debenzylation of O-benzyl protected cyclic hydroxamates, with a potential of scaling up from milligram scale to gram scale on a suitable flow apparatus. This finally allowed the efficient, safe, and reproducible synthesis of a library of *N*-hydroxypyrazinone analogues for anti-HIV research.

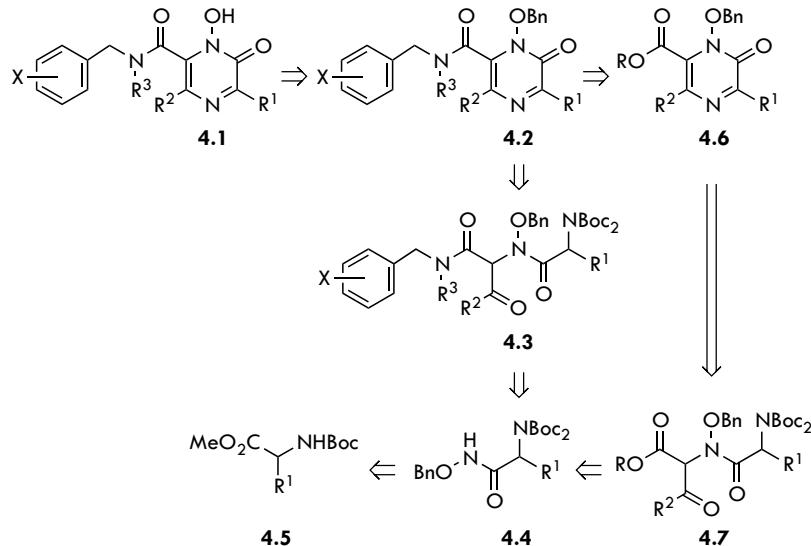
#### 4.1.2 Biological evaluation of the compounds

All the tested compounds do not or only partially inhibit either HIV-1 (strain IIIB) or HIV-2 (ROD strain) replication on human T-lymphocyte cells as evidenced by an MTT assay. Some compounds do however show inhibition in an HIV-1 reverse transcriptase polymerase assay. This might be related to flexibility and the possibility of *cis/trans* isomerisation of the amide bond in the C-3 carboxamide series and the length of aliphatic linkage in the 3-[4-(3-aryureido)butyl]-1-hydroxypyrazinone analogues.

#### 4.2 Future perspectives

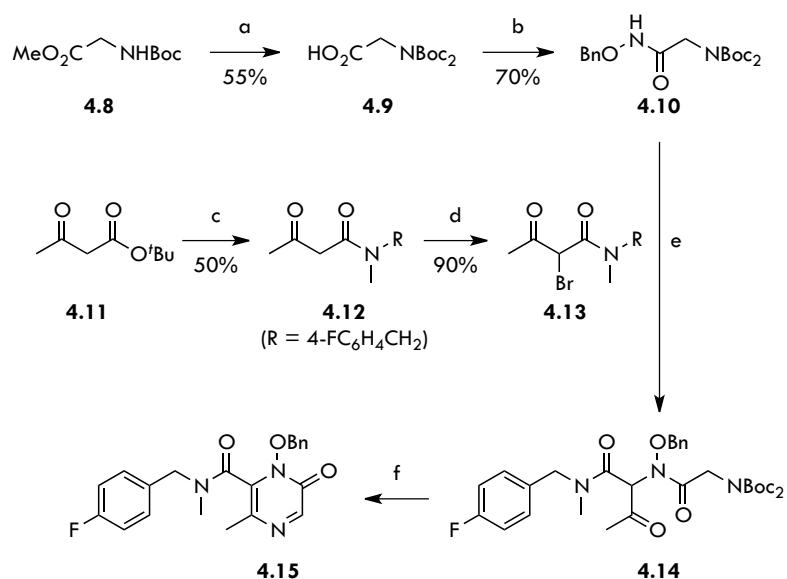
Although C-3 functionalization of *N*-hydroxypyrazinones does not show the expected anti-HIV activity, using the information from this *in vitro* assay and further molecular modeling may help improve HIV inhibition.

The synthesis of *N*-hydroxypyrazinone 6-carboxamide analogues has been investigated. This series of compounds could be synthesized in several synthetic steps according to the retrosynthetic **Scheme 4.1**.



**Scheme 4.1.** Retrosynthesis of 6-carboxamide-1-hydroxypyrazin-2(1H)-ones.

The first derivative **4.15**, obtained via the intermediate **4.14** was observed by LC-MS, showing that the product seems viable via the strategy depicted in **Scheme 4.2**. Further optimizations are needed to fully explore the scope of C-6 functionalization of *N*-hydroxypyrazinones. The work is currently the subject of another project.



**Scheme 4.2.** Synthesis of a 6-carboxamide-1-benzylxypyrazin-2(1*H*)-one. Reagents and conditions: (a) (i)  $\text{Boc}_2\text{O}$  (2 equiv), DMAP (0.01 equiv), ACN,  $55^\circ\text{C}$ , 5 h;<sup>3</sup> (ii)  $\text{NaOH}$  (1.5 equiv),  $\text{MeOH}$ , rt, 7 h; (b)  $\text{BnONH}_2$  (1 equiv),  $\text{HOBT}$  (1.3 equiv), EDCI (1.3 equiv), DIPEA (2.3 equiv), DMF,  $-10^\circ\text{C}$  then rt, overnight; (c)  $\text{RNHCH}_3$  (1.2 equiv), xylene,  $120^\circ\text{C}$ , 15 min;<sup>4</sup> (d)  $\text{KBr}$  (1.2 equiv), 1N  $\text{HCl}$  (1.2 equiv),  $\text{H}_2\text{O}_2$  (2 equiv), toluene, rt, 5 h;<sup>5</sup> (e)  $\text{NaH}$  (1.2 equiv), DMF,  $50^\circ\text{C}$ , 3 h; (f)  $\text{TFA}$  (20 equiv), DCE, reflux, 5 min.<sup>6</sup> Compound **4.15** was detected by LC-MS.

On the other hand, determination of  $\text{p}K_a$  values of the synthetic *N*-hydroxypyrazinones (by NMR titration) and study their metal complexation properties are also the subjects of the other projects.

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# Chapter 5

# Experimental

- 5.1 General
- 5.2 Safety
  - 5.2.1 General safety aspects
  - 5.2.2 Specific safety risks
- 5.3 Synthetic procedures and characterization of the compounds
  - 5.3.1 General synthetic procedures
  - 5.3.2 Synthesis and characterization of 3-alkyl-1-hydroxypyrazin-2(1*H*)-ones and intermediates
  - 5.3.3 Synthesis and characterization of 1-hydroxypyrazin-2(1*H*)-one-3-carboxamides and intermediates
  - 5.3.4 Synthesis and characterization of 3-[4-(3-arylureido)-butyl]-1-hydroxypyrazin-2(1*H*)-ones and intermediates
- 5.4 Biological evaluation



## 5.1 General

Chemicals were bought from commercial vendors (Sigma-Aldrich, Acros, TCI, Strem, Fluka, Iris Biotech) and were used without prior purification. All solvents were analytical or HPLC or LC-MS grade and were used as supplied. Heptane was purified by distillation before usage. Dried solvents were bought, except for THF, which was dried using an Mbraun SPS 800 drying setup.

Most of palladium-based catalysts were purchased from Sigma-Aldrich. SiliaCat®DPP-Pd was bought from SiliCycle and sieved one more time to obtain the particle size 80-120 mesh before packing into the microreactor.

All moisture-sensitive reactions were carried out under argon atmosphere and in flame-dried glassware. Debenylation reactions under batch conditions were magnetically stirred under a hydrogen atmosphere (balloon) or shaken in a Parr hydrogenation apparatus.

Reactions under continuous flow conditions were performed in a Labtrix®Start (Chemtrix) and an X-Cube (ThalesNano) system. The Labtrix®Start unit is equipped with a reactor holder, a temperature controller (Laird Technologies MTTC 1410), two standard syringe pumps (Chemyx Fusion 200, using 1.0 and 2.5 mL syringes), PTFE tubing (1/32" OD × 0.009" ID), an IDEX 5 bar backpressure regulator, and a catalyst reactor model 3026 (Chemtrix). The X-Cube system (ThalesNano) is equipped with two HPLC pumps (giving a flow rate of 0.1 to 3.0 mL·min<sup>-1</sup>), inlet and outlet pressure systems (max 150 bar), a heater unit, a SiliaCat®DPP-Pd CatCart column, stainless steel tubing (0.5 mm ID), PTFE tubing (1/8" OD × 1/16" ID).

All reactions were monitored by TLC or LC-UV-MS (Prevail C8 2.1×150 mm 5 µm column, quadrupole ion trap, 350 nm) using atmospheric pressure ionization electrospray (API-ES) in positive mode. TLC was performed using Machery-Nagel SIL G-25 UV<sub>254</sub> pre-coated glass silica plates.

Flash chromatography was performed using a Büchi Sepacore™ flash apparatus, consisting of a C-660 Büchi fraction collector, C-615 Pump manager, C-635 UV-Photometer, two C-605 pump modules and a Linseis D120S plotter. Pre-packed Grace™ Reveleris™ silica flash cartridges or self-packed with 40-63 mesh silica gel (Fisher Scientific). Preparative HPLC (Luna

C18 21.2×150 mm 5  $\mu$ m column, photodiode array detector) was used to purify the N-hydroxypyrazinone products. Solvents were evaporated on rotavapor at 40 °C. A benchtop lyophilizer was used to dry water samples. Yields refer to the amount of isolated compound after chromatography or to the integration of the corresponding UV absorption peak in the LC-UV-MS spectrum.

NMR spectra were recorded at room temperature on either 300 MHz, 400 MHz or 600 MHz FT-NMR spectrometer in the indicated deuterated solvents; chemical shifts are expressed in  $\delta$  scale (ppm) using tetramethylsilane as an internal standard, and coupling constants  $J$  are in hertz (Hz).

Low-resolution MS spectra were recorded on the LC-MS (API-ES) in positive mode. High-resolution ESI-MS spectra were performed on a micrOTOF-Q instrument in positive mode (70 eV) with a resolution of 10,000.

## 5.2 Safety

### 5.2.1 General safety aspects

All the experiments in this thesis were performed in agreement of the code of practice for safety in the lab and the departmental safety brochure. Special precautions were taken into account when handling hazardous compounds or dealing with precarious lab techniques, such as manipulations involving pressure apparatus. Specific information regarding personal protection and precautions are available on the HSE website, the Molecular Design and Synthesis website, and departmental safety website.

### 5.2.2 Specific safety risks

The most hazardous chemicals were avoided where possible and replaced with less dangerous ones as much as possible; however, in certain cases we still have to handle reagents that show high risks. The most important ones (E4+ class) are declared below.

**Hydrogen gas** is a highly flammable gas. It is usually stored as gas under pressure and may explode when heated. Storing the substance in a well-ventilated place and keeping it away from heat, sparks, open flames, hot surfaces, sunlight, combustible materials, oxidizing agents, and halogens are strictly required.

**Pd-based catalysts** are usually highly flammable solids and should be kept away from heat, sparks, open flames, hot surfaces.

**HOBt** belongs to the group of explosives with the risk of explosion by shock, friction, fire or other sources of ignition.

**o-(Trifluoromethyl)phenyl isocyanate** is a flammable liquid, which may cause serious eye and skin irritation in case of direct interaction as well as breathing difficulties when inhaled.

### 5.3 Synthetic procedures and characterization of the compounds

#### 5.3.1 General synthetic procedures

**Procedure A: Amidation of carboxylic acids using HOBr/EDCI.** To a stirring solution of carboxylic acid (10 mmol, 1 equiv) in dry DMF (10 mL) in a two-neck flask under argon atmosphere at -10 °C, HOBr (hydrate form, 1.76 g, 13 mmol, 1.3 equiv) was added followed by the addition of DIPEA (2.26 mL, 13 mmol, 1.3 equiv) and EDCI (2.02 g, 13 mmol, 1.3 equiv). The reaction mixture was stirred at -10 °C for 30 min before an appropriate amine (10 mmol, 1 equiv) was added and allowed to warm to room temperature. After overnight stirring, the reaction mixture was treated with 10% citric acid solution (25 mL) and extracted with EtOAc. The organic phase was washed with a saturated NaHCO<sub>3</sub> solution (2×), followed by water and brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated by evaporation *in vacuo*. The crude product was purified by silica gel flash chromatography (EtOAc-heptane).

**Procedure B: Preparation of O-benzyl hydroxamates from carboxylic acids.** The experiment was performed according to procedure A, using O-benzylhydroxylamine hydrochloride (1.60 g, 10 mmol, 1 equiv) as amine and an extra equivalent of DIPEA.

**Procedure C: Synthesis of some N,N-arylmethylamines.** Non-commercial secondary amines were prepared via the procedure described by Merritt *et al.*<sup>1</sup> To a solution of appropriate benzaldehyde (30 mmol, 1 equiv) in MeOH (50 mL), methylamine (4.43 mL, 36 mmol, 1.2 equiv, 33% in MeOH) and NaHCO<sub>3</sub> (5.04 g, 60 mmol, 2.0 equiv) was added. After refluxing for 4 h, the reaction mixture was cooled to 5 °C followed by a portionwise addition of NaBH<sub>4</sub> (1.36 g, 36 mmol, 1.2 equiv). After the addition was completed (within 2 h, 10 °C), the reaction mixture was stirred at room temperature for 2 h, then quenched by water, evaporated to remove MeOH, diluted with DCM, washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated by evaporation *in vacuo*. The product was used as such without further purification.

**Procedure D: Boc-deprotection of N-Boc O-benzyl hydroxamates.** A solution of 4 M HCl in dioxane (16 equiv) was added to a two-neck flask containing *N*-Boc O-benzyl hydroxamate under argon atmosphere at 0 °C. The reaction mixture was then stirred at room temperature for 30 min (or in the sonicator bath for 15 min) and evaporated *in vacuo*. The crude product was used as such without further purification. Alternatively to HCl in dioxane TFA in DCM can also be used.

**Procedure E: Synthesis of 1-benzyloxypyrazin-2(1*H*)-ones.** To a solution of *N*-Boc deprotected hydroxamate (2.2 mmol, 1.1 equiv, procedure D) in MeOH-H<sub>2</sub>O (2:1, 15 mL) at below -35 °C, an aqueous 2 M NaOH solution was added dropwise while stirring. The mixture was adjusted to pH 8-10 followed by the dropwise addition of an appropriate glyoxal derivative solution (2.0 mmol, 1 equiv, in 2 mL of MeOH) via a syringe pump over the course of 30-120 minutes. The reaction was allowed to warm to room temperature and was kept stirring overnight. After the reaction was complete (monitored by TLC or ESI-MS), the mixture was evaporated to remove MeOH and subsequently extracted with EtOAc. The organic phase was washed with water and brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated by evaporation *in vacuo*. The crude product was purified by silica gel flash chromatography (EtOAc-heptane).

In the case of reactive reactants, the reaction was stirred overnight in the thermostat at -30 °C. Reactions with phenyl glyoxal were usually heated to 70 °C and an extra portion of phenyl glyoxal was added.

The condensation of glyoxal derivatives with less reactive urea compounds usually required 1-2 h heating at 70 °C after being stirred at room temperature for 5-8 h. After cooling, the precipitate formed was filtered and washed with cold water. The crude product was further purified by silica gel flash chromatography (MeOH-DCM).

**Procedure F: Direct amidation of esters using LiHMDS.** To a stirring solution of O-benzylhydroxylamine (3 mmol, 1 equiv) in dry THF (10 mL) in a two-neck flask under argon atmosphere at -78 °C, LiHMDS (1M in toluene, 12.3 mL, 12.3 mmol, 4.1 equiv) was added. The solution was

stirred at this temperature for 15 min before a solution of the appropriate ester (3 mmol, 1 equiv) in a minimum amount of THF was added dropwise. The reaction mixture was kept stirring at the given temperature for 2 h, then quenched with saturated NH<sub>4</sub>Cl, and extracted with EtOAc. The organic phase was washed with water and brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated by evaporation *in vacuo*. The crude product was purified by silica gel flash chromatography (EtOAc-heptane).

**Procedure G: Direct amidation of esters using MgCl<sub>2</sub>.** To a solution of the appropriate ethyl ester (0.5 mmol, 1 equiv) in THF (2 mL), MgCl<sub>2</sub> (93 mg, 1.0 mmol, 2 equiv) was added. The mixture was stirred at room temperature for 5 min followed by the addition of a solution of the appropriate amine (1.25 mmol, 2.5 equiv) in THF (0.5 mL). The reaction mixture was kept stirring for 16 h, then extracted with a mixture of H<sub>2</sub>O-EtOAc (10:10 mL). The organic phase was washed with an aqueous solution of 0.25 M HCl (or 15% citric acid), then NaHCO<sub>3</sub> and brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated *in vacuo*. The crude product was further purified by silica gel flash chromatography (EtOAc-heptane).

**Procedure H: General procedure for the debenzylation of 1-benzyloxypyrazin-2(1H)-ones in flow.** These reactions were performed on a Labtrix®Start system which was implemented with a 5 bar backpressure regulator. The catalyst reactor was packed with 12-15 mg of SiliaCat®DPP-Pd. Two solutions of 1-benzyloxypyrazin-2(1H)-one derivative (0.1 mmol, 0.1 M, in MeOH) and HCO<sub>2</sub>NH<sub>4</sub> (12.6 mg, 0.2 mmol, 0.2 M, in MeOH) were prepared in two gastight syringes and pumped through the reactor with a flow rate of 50 µL·min<sup>-1</sup> (or residence time R<sub>f</sub> 14 s) while heating (temperature program: 25-60-80-100 °C within 2 min). The product was collected in vials (Treated with an aqueous solution of FeCl<sub>3</sub> for identification of the target material) and purified by RP HPLC using ACN/MilliQ water (containing 0.1% HCOOH). The flow system was cleaned by pumping MeOH (2 mL) through the tubing and reactor at 80 °C.

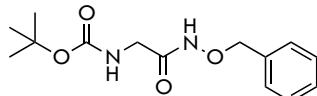
Less soluble 3-[4-(3-arylureido)butyl]-1-benzyloxypyrazin-2(1*H*)-ones and secondary amide 1-benzyloxypyrazin-2(1*H*)-one-3-carboxamides were dissolved in MeOH-toluene (1:1 v/v).

**Procedure I: Debenylation of 1-benzyloxypyrazin-2(1*H*)-ones in batch with hydrogen gas (balloon).** To a three-neck flask, purged with argon gas, a catalytic amount of 5% Pd-BaSO<sub>4</sub> (unreduced form, 1.65 mg), dry MeOH (2 mL), and a stirring bar were added. After the catalyst was saturated with hydrogen, a solution of 1-benzyloxypyrazin-2(1*H*)-one (0.1 mmol, in MeOH, 0.5 mL) was injected. The reaction mixture was stirred under reflux for 15-30 min and then cooled to room temperature. The catalyst was filtered through a layer of celite and washed with MeOH (or filtered through a disposable syringe filter Chromafil®O-45/15 MS in case of small scale samples). The filtrate was evaporated *in vacuo*. The crude product was purified via RP HPLC using ACN/MilliQ water (containing 0.1% HCOOH) to obtain the desired product.

**Procedure J: Preparation of N-Boc lysine urea derivatives from isocyanate and amine.** To a stirring solution of Boc-protected lysine (1.0 g, 4.06 mmol, 1 equiv) and sodium bicarbonate (1.344 g, 16.24 mmol, 4 equiv) in dioxane-water (125 mL, 60/65 v/v) at 80 °C, an appropriate aryl isocyanate (4.87 mmol, 1.2 equiv) in dioxane (5 mL) was added dropwise. The reaction was complete within the course of 1-3 hours. After 60% of the solvent volume was evaporated, the reaction mixture was diluted with a solution of 1M NaHCO<sub>3</sub> and extracted with DCM. The aqueous phase was acidified to pH 2 with 1M HCl in an ice-bath and extracted with EtOAc. The organic phase was washed with water (2×) and brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated by evaporation *in vacuo*. The product was used as such without further purification.

### 5.3.2 Synthesis and characterization of 3-alkyl-1-hydroxypyrazin-2(1H)-ones and intermediates

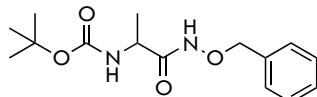
#### *tert*-Butyl 2-benzyloxyamino-2-oxoethylcarbamate (3.2a)



Compound **3.2a** was prepared via procedure B, using *N*-Boc glycine (1.75 g, 10 mmol). Yield 86% (2.41 g). **1H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 11.02 (brs, 1H), 7.39-7.33 (m, 5H), 6.95 (brs, 1H), 4.78 (s, 2H), 3.45 (s, 2H), 1.38 (s, 9H). **13C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 167.16, 156.23, 136.44, 129.23, 128.78, 128.71, 78.52, 78.37, 41.52, 28.66. **HRMS** (ESI, positive mode): *m/z* 303.1355 [M+Na]<sup>+</sup>, calcd for [C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>Na]<sup>+</sup>: 303.1315.

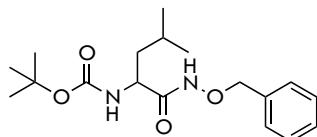
Compound **3.2a** could also be prepared by direct amidation of *N*-Boc glycine methyl ester (10 mmol) via procedure F. Yield 85% (2.38 g).

#### *tert*-Butyl 1-benzyloxyamino-1-oxopropan-2-ylcarbamate (3.2b)



Compound **3.2b** was prepared via procedure B, using *N*-Boc alanine (1.89 g, 10 mmol). Yield 82% (2.40 g). **1H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 11.09 (brs, 1H), 7.38-7.36 (m, 5H), 6.93 (d, *J* = 7.0 Hz, 1H), 4.76 (s, 2H), 3.82 (quint, *J* = 7.0 Hz, 1H), 1.37 (s, 9H), 1.13 (d, *J* = 7.0 Hz, 3H). **13C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 170.20, 155.43, 136.44, 129.31, 128.73, 128.69, 78.47, 77.16, 48.05, 28.66, 18.55. **LRMS** (ESI, positive mode): *m/z* 317.4 [M+Na]<sup>+</sup>.

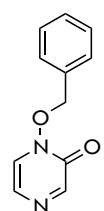
#### *tert*-Butyl 1-benzyloxyamino-4-methyl-1-oxopentan-2-ylcarbamate (3.2c)



Compound **3.2c** was prepared via procedure B, using *N*-Boc leucine (3.36 g, 10 mmol). Yield 80% (2.40 g). **1H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 11.19 (brs, 1H), 7.39-

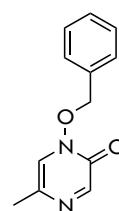
7.35 (m, 5H), 6.87 (d,  $J = 7.7$  Hz, 1H), 4.78 (s, 2H), 3.85 (dt,  $J_1 = 7.7$  Hz,  $J_2 = 7.7$  Hz, 1H), 1.58-1.49 (m, 1H), 1.42-1.31 (overlapped, 11H), 0.85 (d,  $J = 7.6$  Hz, 3H), 0.83 (d,  $J = 7.6$  Hz, 3H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  169.80, 155.65, 136.40, 129.26, 128.67, 78.42, 77.17, 50.93, 41.16, 28.64, 24.62, 23.11, 22.22. **LRMS** (ESI, positive mode): *m/z* 359.3 [M+Na]<sup>+</sup>.

#### 1-Benzylxypyrazin-2(1*H*)-one (3.4a)



Compound **3.4a** was prepared by deprotection of **3.2a** (616 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (228  $\mu\text{L}$ , 2.0 mmol). Yield 80% (323 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.20 (s, 1H), 7.86 (d,  $J = 4.6$  Hz, 1H), 7.51-7.48 (m, 2H), 7.43-7.41 (m, 3H), 7.26 (d,  $J = 4.6$  Hz, 1H), 5.27 (s, 2H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  151.82, 150.54, 133.26, 129.76, 129.33, 129.23, 128.49, 122.49, 78.10. **HRMS** (ESI, positive mode): *m/z* 225.0611 [M+Na]<sup>+</sup>, calcd for [C<sub>11</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub>Na]<sup>+</sup>: 225.0634.

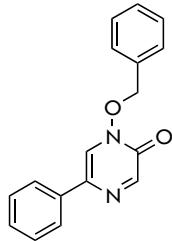
#### 1-Benzylxypyrazin-2(1*H*)-one (3.4b)



Compound **3.4b** was prepared by deprotection of **3.2a** (616 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (307  $\mu\text{L}$ , 2.0 mmol). Yield 64% (275 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.14 (s, 1H), 7.76 (s, 1H), 7.50-7.49 (m, 2H), 7.43-7.42 (m, 3H), 5.24 (s, 2H), 2.16 (s, 3H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  151.30, 150.00, 133.95, 131.32, 130.25, 129.73, 129.03, 126.46, 78.65, 19.18. **HRMS** (ESI, positive mode): *m/z* 239.0740 [M+Na]<sup>+</sup>, calcd for [C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>Na]<sup>+</sup>: 239.0791.

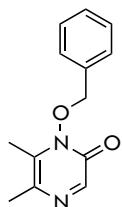
#### 1-Benzylxypyrazin-2(1*H*)-one (3.4c)

Compound **3.4c** was prepared by deprotection of **3.2a** (616 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using phenylglyoxal monohydrate (304 mg, 2.0 mmol). Yield 62% (345 mg).



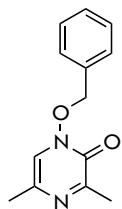
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.40 (s, 1H), 7.53-7.33 (m, 11H), 5.38 (s, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  151.75, 150.43, 134.73, 132.96, 130.18, 129.92, 129.03, 128.91, 128.35, 125.09, 124.39, 78.93. **HRMS** (ESI, positive mode): *m/z* 301.0866 [M+Na]<sup>+</sup>, calcd for [C<sub>17</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>Na]<sup>+</sup>: 301.0947.

#### 1-Benzyl-5,6-dimethylpyrazin-2(1H)-one (3.4d)



Compound **3.4d** was prepared by deprotection of **3.2a** (616 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using diacetyl (175  $\mu$ L, 2.0 mmol). Yield 78% (360 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.00 (s, 1H), 7.55-7.52 (m, 2H), 7.45-7.43 (m, 3H), 5.23 (s, 2H), 2.28 (s, 3H), 2.23 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  152.00, 145.63, 136.24, 134.03, 130.32, 129.80, 129.26, 129.08, 77.42, 19.72, 13.23. **HRMS** (ESI, positive mode): *m/z* 253.0915 [M+Na]<sup>+</sup>, calcd for [C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>Na]<sup>+</sup>: 253.0947.

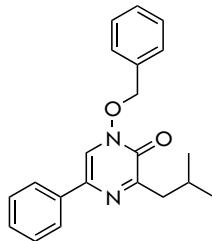
#### 1-Benzyl-3,5-dimethylpyrazin-2(1H)-one (3.4e)



Compound **3.4e** was prepared by deprotection of **3.2b** (647 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (307  $\mu$ L, 2.0 mmol). Yield 52% (240 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  7.59 (s, 1H), 7.51-7.48 (m, 2H), 7.43-7.41 (m, 3H), 5.21 (s, 2H), 2.34 (s, 3H), 2.11 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  158.58, 151.17, 134.13, 130.18, 129.66, 129.41, 129.00, 124.28, 78.42, 20.93, 19.36. **HRMS** (ESI, positive mode): *m/z* 253.0887 [M+Na]<sup>+</sup>, calcd for [C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>Na]<sup>+</sup>: 253.0947.

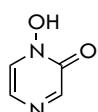
#### 1-Benzyl-3-isobutyl-5-phenylpyrazin-2(1H)-one (3.4f)

Compound **3.4f** was prepared by deprotection of **3.2c** (740 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using phenylglyoxal monohydrate (304 mg, 2.0 mmol). Yield 59% (397 mg).



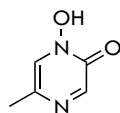
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 8.38 (s, 1H), 7.84-7.82 (m, 2H), 7.56-7.54 (m, 2H), 7.44-7.41 (m, 5H), 7.33-7.30 (m, 1H), 5.32 (s, 2H), 2.71 (d, *J* = 6.8 Hz, 2H), 2.24 (nonet, *J* = 6.8 Hz, 1H), 0.96 (d, *J* = 6.8 Hz, 6H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 160.64, 151.33, 135.77, 133.97, 130.38, 129.73, 129.15, 128.98, 128.14, 125.21, 124.02, 78.68, 42.12, 26.63, 22.93. **HRMS** (ESI, positive mode): *m/z* 357.6 [M+Na]<sup>+</sup>.

#### 1-Hydroxypyrazin-2(1H)-one (3.24a)



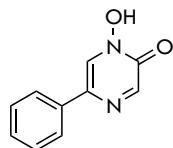
Compound **3.24a** was prepared by debenzylation of **3.4a** (20.2 mg, 0.1 mmol) in flow via procedure H. Yield 87% (9.7 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 8.09 (s, 1H), 7.96 (d, *J* = 4.4 Hz, 1H), 7.32 (d, *J* = 4.4 Hz, 1H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 153.13, 147.55, 129.20, 123.54. **HRMS** (ESI, positive mode): *m/z* 135.0191 [M+Na]<sup>+</sup>, calcd for [C<sub>4</sub>H<sub>4</sub>N<sub>2</sub>O<sub>2</sub>Na]<sup>+</sup>: 135.0165.

#### 1-Hydroxy-5-methylpyrazin-2(1H)-one (3.24b)



Compound **3.24b** was prepared by debenzylation of **3.4b** (21.6 mg, 0.1 mmol) in flow via procedure H. Yield 91% (11.5 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 8.01 (s, 1H), 7.82 (s, 1H), 2.20 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 151.95, 146.22, 131.87, 126.22, 19.31. **HRMS** (ESI, positive mode): *m/z* 149.0342 [M+Na]<sup>+</sup>, calcd for [C<sub>5</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub>Na]<sup>+</sup>: 149.0321.

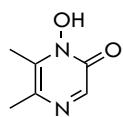
#### 1-Hydroxy-5-phenylpyrazin-2(1H)-one (3.24c)



Compound **3.24c** was prepared by debenzylation of **3.4c** (27.8 mg, 0.1 mmol) in flow via procedure H. Yield 89% (16.7 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 8.62 (s, 1H), 8.22 (s, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.43 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 1H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 152.10, 146.54, 135.60, 132.55, 129.19, 128.17, 125.58, 125.18.

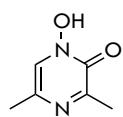
**HRMS** (ESI, positive mode):  $m/z$  211.0476 [M+Na]<sup>+</sup>, calcd for [C<sub>10</sub>H<sub>8</sub>N<sub>2</sub>O<sub>2</sub>Na]<sup>+</sup>: 211.0478.

**1-Hydroxy-5,6-dimethylpyrazin-2(1H)-one (3.24d)**



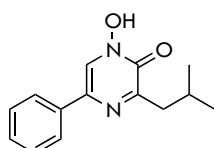
Compound **3.24d** was prepared by debenzylation of **3.4d** (23.0 mg, 0.1 mmol) in flow via procedure H. Yield 90% (12.6 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  7.87 (s, 1H), 2.32 (s, 3H), 2.27 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  152.07, 140.24, 135.63, 130.63, 19.98, 13.30. **HRMS** (ESI, positive mode):  $m/z$  163.0494 [M+Na]<sup>+</sup>, calcd for [C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>O<sub>2</sub>Na]<sup>+</sup>: 163.0478.

**1-Hydroxy-3,5-dimethylpyrazin-2(1H)-one (3.24e)**



Compound **3.24e** was prepared by debenzylation of **3.4e** (23.0 mg, 0.1 mmol) in flow via procedure H. Yield 93% (13.0 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  7.62 (s, 1H), 2.28 (s, 3H), 2.13 (s, 3H). **LRMS** (API-ES, positive mode):  $m/z$  141.2 [M+H]<sup>+</sup>.

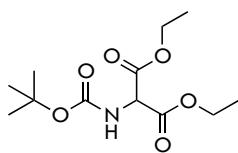
**1-Hydroxy-3-isobutyl-5-phenylpyrazin-2(1H)-one (3.24f)**



Compound **3.24f** was prepared by debenzylation of **3.4f** (33.4 mg, 0.1 mmol) in flow via procedure H. Yield 95% (23.2 mg). **<sup>1</sup>H NMR** (600 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  8.46 (s, 1H), 7.90 (d, *J* = 6.9 Hz, 2H), 7.43-7.40 (m, 2H), 7.32-7.29 (m, 1H), 2.69 (d, *J* = 6.7 Hz, 2H), 2.26-2.22 (m, 1H), 0.95 (d, *J* = 6.7 Hz, 6H). **<sup>13</sup>C NMR** (150 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  157.68, 151.78, 135.98, 130.46, 129.13, 127.97, 125.14, 124.88, 123.33, 42.11, 26.67, 22.93. **LRMS** (API-ES, positive mode):  $m/z$  245.2 [M+H]<sup>+</sup>.

### 5.3.3 Synthesis and characterization of 1-hydroxypyrazin-2(1H)-one 3-carboxamides and intermediates

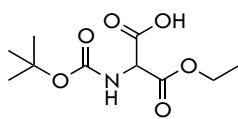
#### Diethyl 2-*tert*-butoxycarbonylaminomalonate (3.7)



To a suspension of diethyl aminomalonate hydrochloride (25 g, 118.12 mmol, 1 equiv) in a mixture of water (150 mL) and dioxane (220 mL) in a round bottom flask with magnetic bar, NaHCO<sub>3</sub> (10.42 g,

124.03 mmol, 1.05 equiv) was slowly added while stirring at room temperature. When the solution became clear, a catalytic amount of DMAP (1 mol%, 144 mg) was added followed by dropwise addition of a solution of Boc<sub>2</sub>O (27.07 g, 124.03 mmol, 1.05 equiv) in dioxane (80 mL). After the reaction was complete (monitored by TLC), the solvents were evaporated *in vacuo*. The residue was dissolved in EtOAc. The organic phase was washed with solutions of 5% KHSO<sub>4</sub>, satd. NaHCO<sub>3</sub>, water brine and subsequently dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, then filtered and evaporated *in vacuo*. The desired product was NMR pure without the need for purification via column chromatography. Quantitative yield (32.51 g). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 7.67 (d, J = 8.1 Hz, 1H), 4.80 (d, J = 8.1 Hz, 1H), 4.22-4.09 (m, 4H), 1.39 (s, 9H), 1.20 (t, J = 7.1 Hz, 6H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ 167.04, 155.54, 79.51, 61.96, 57.89, 28.48, 12.28. HRMS (ESI, positive mode): m/z 298.1323 [M+Na]<sup>+</sup>, calcd for [C<sub>12</sub>H<sub>21</sub>NO<sub>6</sub>Na]<sup>+</sup>: 298.1261.

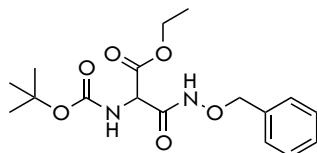
#### 2-*tert*-Butoxycarbonylamo-3-ethoxy-3-oxopropanoic acid (3.8)



To a solution of diester 3.7 (27.51 g, 100 mmol) in EtOH (100 mL) in a round bottom flask, a solution of KOH (5.60 g, 100 mmol, 1 equiv) in EtOH (60 mL) was added dropwise while stirring at room temperature. The reaction mixture was kept stirring overnight. After the reaction was complete, 90% of solvent was removed by evaporation *in vacuo*. The residue was dissolved in a solution of 1M NaHCO<sub>3</sub> (100 mL) and extracted with EtOAc. The aqueous phase was then acidified by KHSO<sub>4</sub> (powder) at 0 °C and extracted with EtOAc. The organic phase was dried over anhydrous MgSO<sub>4</sub>, filtered, and evaporated to obtain the pure product 3.8 without

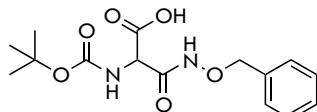
the need for purification via column chromatography. Yield 98% (24.11 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  13.31 (brs, 1H), 7.44 (d,  $J$  = 7.8 Hz, 1H), 4.72 (d,  $J$  = 7.8 Hz, 1H), 4.15 (q,  $J$  = 7.0 Hz, 2H), 1.39 (s, 9H), 1.20 (t,  $J$  = 7.0 Hz, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  168.20, 167.63, 155.52, 79.37, 61.75, 58.01, 28.53, 14.43. **HRMS** (ESI, positive mode): *m/z* 270.0970 [M+Na]<sup>+</sup>, calcd for [C<sub>10</sub>H<sub>17</sub>NO<sub>6</sub>Na]<sup>+</sup>: 270.0948.

**Ethyl 3-benzyloxyamino-2-tert-butoxycarbonylamino-3-oxopropanoate (3.9)**



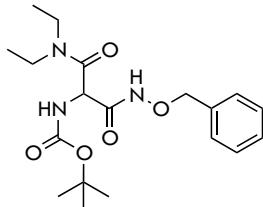
Compound **3.9** was prepared via procedure B using 18.70 g (75.62 mmol) of **3.8**. Yield 65% (17.35 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  11.63 (s, 1H), 7.40-7.35 (m, 5H), 7.26 (d,  $J$  = 8.3 Hz, 1H), 4.81 (s, 2H), 4.65 (d,  $J$  = 8.3 Hz, 1H), 4.13 (q,  $J$  = 7.0 Hz, 2H), 1.39 (s, 9H), 1.18 (t,  $J$  = 7.0 Hz, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  167.93, 162.89, 155.52, 136.12, 129.33, 128.81, 128.76, 79.42, 77.39, 61.87, 56.26, 28.51, 14.38. **HRMS** (ESI, positive mode): *m/z* 375.1573 [M+Na]<sup>+</sup>, calcd for [C<sub>17</sub>H<sub>24</sub>N<sub>2</sub>O<sub>6</sub>Na]<sup>+</sup>: 375.1527.

**3-Benzylamino-2-tert-butoxycarbonylamino-3-oxopropanoic acid (3.10)**



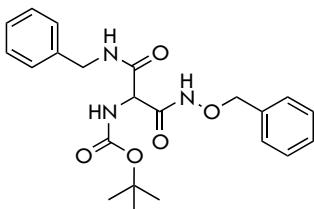
The saponification of ester **3.9** (18.00 g, 51.08 mmol) to obtain compound **3.10** was done via the same procedure to prepare **3.8**. Yield 92% (15.21 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  13.15 (brs, 1H), 11.58 (s, 1H), 7.40-7.37 (m, 5H), 6.98 (d,  $J$  = 8.3 Hz, 1H), 4.81 (s, 2H), 4.58 (d,  $J$  = 8.3 Hz, 1H), 1.40 (s, 9H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  169.20, 163.57, 155.42, 136.13, 129.36, 128.80, 128.77, 79.28, 77.39, 56.14, 28.54. **HRMS** (ESI, positive mode): *m/z* 347.1165 [M+Na]<sup>+</sup>, calcd for [C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>O<sub>6</sub>Na]<sup>+</sup>: 347.1214.

**tert-Butyl 1-benzyloxyamino-3-diethylamino-1,3-dioxopropan-2-ylcarbamate (3.11a)**



Compound **3.11a** was prepared from **3.10** (3.24 g, 10 mmol) via procedure A using 1.03 mL (10 mmol) of diethylamine. Yield 79% (3.0 g).  **$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.42 (brs, 1H), 7.38-7.33 (m, 5H), 6.15 (s, 1H), 4.96 (d,  $J = 6.0$  Hz, 1H), 4.89 (s, 2H), 3.56-3.48 (m, 2H), 3.34-3.20 (m, 2H), 1.42 (s, 9H), 1.19 (t,  $J = 7.0$  Hz, 3H), 1.14 (t,  $J = 7.0$  Hz, 3H).  **$^{13}\text{C NMR}$**  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  165.38, 164.61, 155.68, 134.99, 129.21, 128.75, 128.55, 81.00, 78.27, 54.23, 41.92, 40.81, 28.24, 13.91, 12.61. **HRMS** (ESI, positive mode):  $m/z$  402.1970 [ $\text{M}+\text{Na}]^+$ , calcd for  $[\text{C}_{19}\text{H}_{29}\text{N}_3\text{O}_5\text{Na}]^+$ : 402.1999.

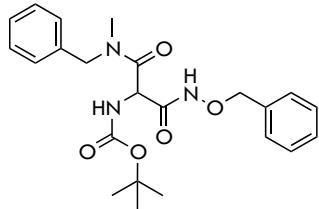
**tert-Butyl 1-benzylamino-3-benzyloxyamino-1,3-dioxopropan-2-ylcarbamate (3.11b)**



Compound **3.11b** was prepared from **3.10** (3.24 g, 10 mmol) via procedure A using 1.09 mL (10 mmol) of benzyl-amine. Yield 72% (2.98 g).  **$^1\text{H NMR}$**  (400 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  11.42 (s, 1H), 8.47 (t,  $J = 5.9$  Hz, 1H), 7.38-7.37 (m, 5H), 7.33-7.22 (m, 5H), 6.84 (d,  $J = 7.8$  Hz, 1H), 4.78 (s, 2H), 4.60 (d,  $J = 7.8$  Hz, 1H), 4.37-4.26 (m, 2H), 1.41 (s, 9H).  **$^{13}\text{C NMR}$**  (100 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  166.92, 164.33, 155.21, 139.34, 136.14, 129.32, 128.77, 128.66, 127.56, 127.28, 79.51, 77.46, 57.40, 42.84, 28.52. **LRMS** (ESI, positive mode):  $m/z$  436.5 [ $\text{M}+\text{Na}]^+$ .

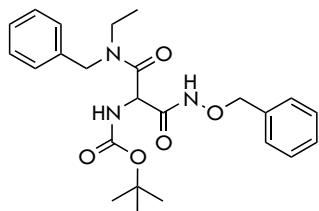
**tert-Butyl 1-benzylmethylamino-3-benzyloxyamino-1,3-dioxopropan-2-ylcarbamate (3.11c)**

Compound **3.11c** was prepared from **3.10** (3.24 g, 10 mmol) via procedure A using 1.21 g (10 mmol) of *N*-methylbenzylamine (procedure C). Yield 76% (3.25 g).  **$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ ): ( $\alpha : \beta$  =



$\alpha : \beta = 100 : 46$ )  $\alpha$  rotamer:  $\delta$  11.43 (s, 1H), 7.37-7.23 (m, 10H), 7.02 (d,  $J = 7.1$  Hz, 1H), 5.03 (d,  $J = 7.1$  Hz, 1H), 4.79 (s, 2H), 4.64 (d,  $J = 15.1$  Hz, 1H), 4.42 (d,  $J = 15.1$  Hz, 1H), 2.91 (s, 3H), 1.40 (s, 9H);  $\beta$  rotamer:  $\delta$  11.43 (s, 1H), 7.37-7.23 (m, 10H), 7.02 (overlapped, 1H), 5.05 (overlapped, 1H), 4.75 (s, 2H), 4.73 (overlapped, 1H), 4.39 (overlapped, 1H), 2.72 (s, 3H), 1.34 (s, 9H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\alpha$  rotamer:  $\delta$  167.11, 164.28, 155.33, 137.49, 136.25, 129.34, 128.86, 128.75, 127.86, 127.54, 79.47, 77.33, 54.55, 51.13, 35.07, 28.53;  $\beta$  rotamer:  $\delta$  167.02, 164.40, 155.18, 136.93, 136.25, 129.34, 129.04, 128.75, 127.86, 127.54, 79.47, 77.33, 54.55, 52.52, 3.73, 28.45. LRMS (ESI, positive mode):  $m/z$  450.6 [ $\text{M}+\text{Na}]^+$ .

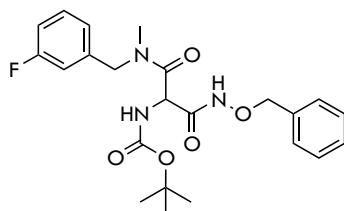
**tert-Butyl 1-benzylethylamino-3-benzyloxymethyl-1,3-dioxopropan-2-ylcarbamate (3.11d)**



Compound **3.11d** was prepared from **3.10** (3.24 g, 10 mmol) via procedure A using 1.49 mL (10 mmol) of *N*-ethylbenzylamine. Yield 77% (3.39 g).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ): ( $\alpha : \beta = 100 : 53$ )  $\alpha$  rotamer:  $\delta$  9.41 (s, 1H), 7.37-7.16 (m, 10H), 6.18 (brs, 1H), 5.04 (d,  $J = 5.6$  Hz, 1H), 4.89 (s, 2H), 4.88 (d,  $J = 15.5$  Hz, 1H), 4.41 (d,  $J = 15.5$  Hz, 1H), 3.45 (brs, 1H), 3.21-3.16 (m, 1H), 1.42 (s, 9H), 1.14 (t,  $J = 7.0$  Hz, 3H);  $\beta$  rotamer:  $\delta$  9.31 (s, 1H), 7.37-7.16 (m, 10H), 6.18 (brs, 1H), 4.97 (d,  $J = 5.6$  Hz, 1H), 4.84 (s, 2H), 4.70 (d,  $J = 15.5$  Hz, 1H), 4.37 (d,  $J = 15.5$  Hz, 1H), 3.63 (brs, 1H), 3.21-3.16 (m, 1H), 1.39 (s, 9H), 1.10 (t,  $J = 7.0$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\alpha$  rotamer:  $\delta$  165.62, 165.41, 165.32, 155.76, 136.45, 134.98, 129.21, 128.93, 128.69, 128.59, 127.61, 127.39, 126.96, 81.18, 78.33, 54.30, 48.25, 41.55, 28.26, 13.43;  $\beta$  rotamer:  $\delta$  165.62, 165.41, 165.32, 155.67, 135.86, 134.98, 129.21, 128.93, 128.79, 128.69, 127.91, 127.39, 126.96, 81.11, 78.28, 54.30, 50.48,

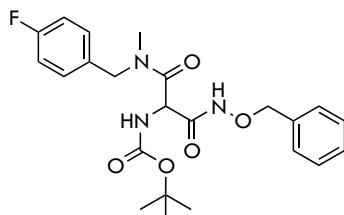
41.43, 28.22, 12.08. **LRMS** (ESI, positive mode):  $m/z$  464.5 [ $M+Na$ ]<sup>+</sup>.

**tert-Butyl 1-benzyloxyamino-3-(3-fluorobenzylmethylamino)-1,3-dioxopropan-2-ylcarbamate (3.11e)**



Compound **3.11e** was prepared from **3.10** (3.24 g, 10 mmol) via procedure A using 1.39 g (10 mmol) of *N*-(3-fluorobenzyl)-*N*-methylamine (procedure C). Yield 68% (3.04 g). **<sup>1</sup>H NMR** (400 MHz, DMSO- $d_6$ ): ( $\alpha$  :  $\beta$  = 100 : 36)  $\alpha$  rotamer:  $\delta$  11.41 (s, 1H), 7.38-7.35 (m, 6H), 7.10-7.05 (m, 4H), 5.04 (d,  $J$  = 7.3 Hz, 1H), 4.80 (s, 2H), 4.66 (d,  $J$  = 15.3 Hz, 1H), 4.43 (d,  $J$  = 15.3 Hz, 1H), 2.93 (s, 3H), 1.40 (s, 9H);  $\beta$  rotamer:  $\delta$  11.39 (s, 1H), 7.38-7.35 (m, 6H), 7.10-7.05 (m, 4H), 5.04 (d,  $J$  = 7.3 Hz, 1H), 4.75 (s, 2H), 4.66 (d,  $J$  = 15.3 Hz, 1H), 4.43 (d,  $J$  = 15.3 Hz, 1H), 2.74 (s, 3H), 1.32 (s, 9H). **<sup>13</sup>C NMR** (100 MHz, DMSO- $d_6$ ):  $\alpha$  rotamer:  $\delta$  167.34, 164.27, 162.85 (d,  $J_{CF}$  = 243.6 Hz), 155.43, 140.57 (d,  $J_{CF}$  = 7.3 Hz), 136.22, 130.78 (d,  $J_{CF}$  = 8.3 Hz), 129.34, 128.76, 123.85, 114.40 (d,  $J_{CF}$  = 20.0 Hz), 114.30 (d,  $J_{CF}$  = 21.0 Hz), 79.48, 77.36, 54.57, 50.77, 35.24, 28.53;  $\beta$  rotamer:  $\delta$  167.23, 164.45, 162.92 (d,  $J_{CF}$  = 243.6 Hz), 155.24, 140.05 (d,  $J_{CF}$  = 7.3 Hz), 137.32, 130.97 (d,  $J_{CF}$  = 8.3 Hz), 129.34, 128.76, 123.64, 114.67 (overlapped), 79.48, 77.36, 54.47, 52.12, 33.79, 28.41. **LRMS** (ESI, positive mode):  $m/z$  468.3 [ $M+Na$ ]<sup>+</sup>.

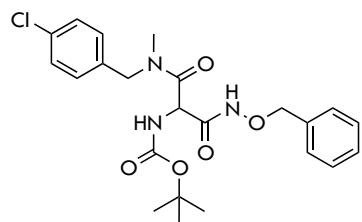
**tert-Butyl 1-benzyloxyamino-3-(4-fluorobenzylmethylamino)-1,3-dioxopropan-2-ylcarbamate (3.11f)**



Compound **3.11f** was prepared from **3.10** (3.24 g, 10 mmol) via procedure A using 1.39 g (10 mmol) of *N*-(4-fluorobenzyl)-*N*-methylamine (procedure C). Yield 61% (2.71 g). **<sup>1</sup>H NMR** (400 MHz, DMSO- $d_6$ ): ( $\alpha$  :  $\beta$  = 100 : 39)  $\alpha$  rotamer:  $\delta$  11.41 (s, 1H), 7.38-7.12

(m, 9H), 7.03 (d,  $J = 7.3$  Hz, 1H), 5.03 (d,  $J = 7.3$  Hz, 1H), 4.79 (s, 2H), 4.62 (d,  $J = 14.8$  Hz, 1H), 4.41 (d,  $J = 14.8$  Hz, 1H), 2.90 (s, 3H), 1.40 (s, 9H);  $\beta$  rotamer:  $\delta$  11.39 (s, 1H), 7.45-7.20 (m, 9H), 7.09 (overlapped, 1H), 5.07 (d,  $J = 7.3$  Hz, 1H), 4.75 (s, 2H), 4.62 (d,  $J = 14.8$  Hz, 1H), 4.41 (d,  $J = 14.8$  Hz, 1H), 2.72 (s, 3H), 1.34 (s, 9H).  $^{13}\text{C}$  NMR (100 MHz, DMSO-d<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  167.14, 164.31, 161.85 (d,  $J_{\text{CF}} = 244.4$  Hz), 155.36, 136.22, 133.67, 129.93 (d,  $J_{\text{CF}} = 8.1$  Hz), 129.34, 128.75, 115.59 (d,  $J_{\text{CF}} = 21.0$  Hz), 79.48, 77.35, 54.57, 50.44, 34.98, 28.51;  $\beta$  rotamer:  $\delta$  167.03, 164.50, 162.02 (d,  $J_{\text{CF}} = 244.4$  Hz), 155.19, 136.22, 133.09, 129.64 (d,  $J_{\text{CF}} = 8.1$  Hz), 129.34, 128.75, 115.79 (d,  $J_{\text{CF}} = 21.0$  Hz), 79.48, 77.35, 54.57, 51.83, 33.60, 28.43. LRMS (ESI, positive mode):  $m/z$  468.6 [M+Na]<sup>+</sup>.

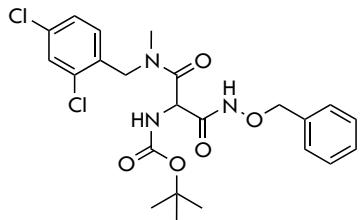
**tert-Butyl 1-benzyloxyamino-3-(4-chlorobenzylmethylamino)-1,3-dioxopropan-2-ylcarbamate (3.11g)**



Compound **3.11g** was prepared from **3.10** (3.24 g, 10 mmol) via procedure A using 1.56 g (10 mmol) of *N*-(4-chlorobenzyl)-*N*-methylamine (procedure C). Yield 67% (3.11 g).  $^1\text{H}$  NMR (400 MHz, DMSO-d<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 41)  $\alpha$  rotamer:  $\delta$  11.41 (s, 1H), 7.37-7.25 (m, 9H), 7.04 (d,  $J = 8.0$  Hz, 1H), 5.02 (d,  $J = 8.0$  Hz, 1H), 4.79 (s, 2H), 4.61 (d,  $J = 15.1$  Hz, 1H), 4.41 (d,  $J = 15.1$  Hz, 1H), 2.90 (s, 3H), 1.40 (s, 9H);  $\beta$  rotamer:  $\delta$  11.39 (overlapped, 1H), 7.37-7.23 (m, 9H), 7.09 (overlapped, 1H), 5.04 (overlapped, 1H), 4.74 (s, 2H), 4.61 (d,  $J = 15.1$  Hz, 1H), 4.41 (d,  $J = 15.1$  Hz, 1H), 2.73 (s, 3H), 1.32 (s, 9H).  $^{13}\text{C}$  NMR (100 MHz, DMSO-d<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  167.20, 164.29, 155.38, 136.59, 136.20, 132.19, 129.81, 129.35, 128.97, 128.80, 128.76, 79.51, 77.36, 54.56, 50.56, 35.10, 28.53;  $\beta$  rotamer:

$\delta$  167.13, 164.47, 155.17, 136.20, 136.02, 132.48, 129.90, 129.40, 128.97, 128.80, 128.76, 79.51, 77.36, 54.49, 51.91, 33.81, 28.42. LRMS (ESI, positive mode):  $m/z$  484.7 [M+Na]<sup>+</sup>.

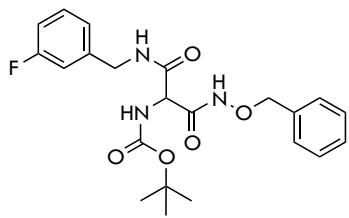
**tert-Butyl (1-benzyloxyamino-3-(2,4-dichlorobenzylmethylamino)-1,3-dioxopropan-2-ylcarbamate (3.11h)**



Compound **3.11h** was prepared from **3.10** (3.24 g, 10 mmol) via procedure A using 1.90 g (10 mmol) of *N*-(2,4-dichlorobenzyl)-*N*-methylamine (procedure C). Yield 61% (3.01 g). **<sup>1</sup>H NMR**

(400 MHz, DMSO-d<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 37)  $\alpha$  rotamer:  $\delta$  11.40 (s, 1H), 7.63-7.26 (m, 8H), 7.08 (d,  $J$  = 7.8 Hz, 1H), 5.07 (d,  $J$  = 7.8 Hz, 1H), 4.81 (s, 2H), 4.65 (d,  $J$  = 16.0 Hz, 1H), 4.45 (d,  $J$  = 16.0 Hz, 1H), 2.98 (s, 3H), 1.40 (s, 9H);  $\beta$  rotamer:  $\delta$  11.40 (s, 1H), 7.58-7.21 (m, 8H), 7.05 (overlapped, 1H), 4.99 (d,  $J$  = 7.8 Hz, 1H), 4.72 (s, 2H), 4.65 (d,  $J$  = 16.0 Hz, 1H), 4.45 (d,  $J$  = 16.0 Hz, 1H), 2.79 (s, 3H), 1.28 (s, 9H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  167.54, 164.17, 155.43, 136.29, 133.84, 133.56, 132.84, 129.93, 129.33, 129.25, 128.74, 127.83, 79.49, 77.33, 54.59, 49.15, 35.73, 28.54;  $\beta$  rotamer:  $\delta$  167.69, 164.43, 154.99, 136.29, 133.56, 133.46, 133.12, 129.93, 129.33, 129.25, 128.74, 128.01, 79.36, 77.28, 54.49, 50.44, 34.24, 28.34. **LRMS** (positive mode): *m/z* 520.2 [M+Na]<sup>+</sup>.

**tert-Butyl 1-benzyloxyamino-3-(3-fluorobenzylamino)-1,3-dioxopropan-2-ylcarbamate (3.11i)**

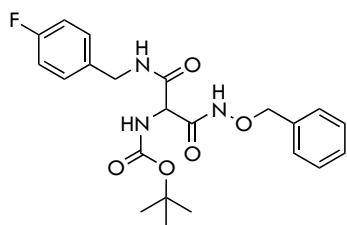


Compound **3.11i** was prepared from **3.16a** (1.06 g, 3 mmol) via procedure F using 384  $\mu$ L (3.3 mmol, 1.1 equiv) of O-benzyl-hydroxylamine and 12.3 mL (12.3 mmol, 4.1 equiv) of a solution of 1M LiHMDS in toluene.

Yield 66% (854 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  11.44 (brs, 1H), 8.54 (t,  $J$  = 6.3 Hz, 1H), 7.38-7.03 (m, 9H), 6.92 (d,  $J$  = 7.7 Hz, 1H), 4.78 (s, 2H), 4.59 (d,  $J$  = 7.7 Hz, 1H), 4.38-4.27 (m, 2H), 1.40 (s, 9H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  167.20, 164.28, 162.74 (d,  $J_{CF}$  = 243.6 Hz), 155.28, 142.53 (d,  $J_{CF}$  = 7.0 Hz), 136.11, 130.55 (d,  $J_{CF}$  =

8.3 Hz), 129.33, 128.78, 123.46, 114.09 (d,  $J_{CF} = 21.6$  Hz), 113.95 (d,  $J_{CF} = 21.1$  Hz), 79.55, 77.48, 57.46, 42.28, 28.52. **HRMS** (ESI, positive mode):  $m/z$  454.1698 [M+Na]<sup>+</sup>, calcd for [C<sub>22</sub>H<sub>26</sub>FN<sub>3</sub>O<sub>5</sub>Na]<sup>+</sup>: 454.1749.

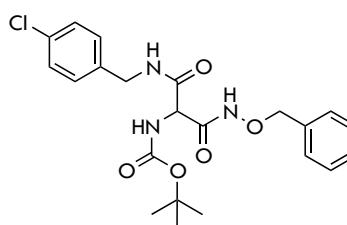
**tert-Butyl 1-benzyloxyamino-3-(4-fluorobenzylamino)-1,3-dioxopropan-2-ylcarbamate (3.11j)**



Compound **3.11j** was prepared from **3.16b** (1.06 g, 3 mmol) via procedure F using 384  $\mu$ L (3.3 mmol, 1.1 equiv) of O-benzylhydroxylamine and 12.3 mL (12.3 mmol, 4.1 equiv) of a solution of 1M LiHMDS in toluene.

Yield 64% (828 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  11.42 (brs, 1H), 8.49 (brs, 1H), 7.37-7.10 (m, 9H), 6.85 (d,  $J = 7.8$  Hz, 1H), 4.77 (s, 2H), 4.57 (d,  $J = 7.8$  Hz, 1H), 4.33-4.23 (m, 2H), 1.39 (s, 9H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  167.98, 164.32, 161.67 (d,  $J_{CF} = 244.5$  Hz), 155.24, 136.17, 135.59 (d,  $J_{CF} = 2.7$  Hz), 129.56 (d,  $J_{CF} = 8.3$  Hz), 129.32, 128.77, 115.36 (d,  $J_{CF} = 21.4$  Hz), 79.52, 77.43, 57.39, 42.16, 28.52. **HRMS** (ESI, positive mode):  $m/z$  454.1715 [M+Na]<sup>+</sup>, calcd for [C<sub>22</sub>H<sub>26</sub>FN<sub>3</sub>O<sub>5</sub>Na]<sup>+</sup>: 454.1749.

**tert-Butyl 1-benzyloxyamino-3-(4-chlorobenzylamino)-1,3-dioxopropan-2-ylcarbamate (3.11k)**

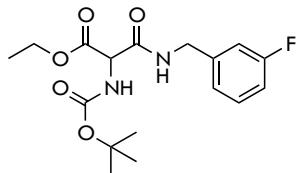


Compound **3.11k** was prepared from **3.16c** (1.11 g, 3 mmol) via procedure F using 384  $\mu$ L (3.3 mmol, 1.1 equiv) of O-benzylhydroxylamine and 12.3 mL (12.3 mmol, 4.1 equiv) of a solution of LiHMDS 1M in toluene.

Yield 57% (766 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  11.43 (brs, 1H), 8.52 (brs, 1H), 7.37-7.27 (m, 9H), 6.88 (d,  $J = 8.2$  Hz, 1H), 4.77 (s, 2H), 4.58 (d,  $J = 8.2$  Hz, 1H), 4.34-4.24 (m, 2H), 1.40 (s, 9H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  167.07, 164.31, 155.25, 138.50, 136.14, 131.84, 129.46, 129.32, 128.77, 128.59, 79.54, 77.46, 57.41, 42.21, 28.52.

**HRMS** (ESI, positive mode):  $m/z$  470.1418 [M+Na]<sup>+</sup>, calcd for [C<sub>22</sub>H<sub>26</sub>ClN<sub>3</sub>O<sub>5</sub>Na]<sup>+</sup>: 470.1453.

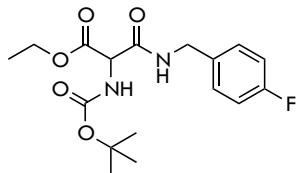
**Ethyl 2-tert-butoxycarbonylamino-3-(3-fluorobenzylamino)-3-oxopropanoate (3.16a)**



Compound **3.16a** was prepared from **3.8** (1.62 g, 5 mmol) via procedure A using 0.57 mL (5 mmol) of 3-fluorobenzylamine. Yield 73% (1.29 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.88 (dd,  $J_1 = 6.2$  Hz,  $J_2 = 5.5$  Hz, 1H),

7.38-7.04 (m, 5H), 4.83 (d,  $J = 8.2$  Hz, 1H), 4.40 (dd,  $J_1 = 15.6$  Hz,  $J_2 = 6.2$  Hz, 1H), 4.27 (dd,  $J_1 = 15.6$  Hz,  $J_2 = 5.4$  Hz, 1H), 4.14 (q,  $J = 6.9$  Hz, 2H), 1.39 (s, 9H), 1.18 (t,  $J = 6.9$  Hz, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  167.73, 165.58, 162.18 (d,  $J_{CF} = 244.3$  Hz), 154.97, 141.83 (d,  $J_{CF} = 6.7$  Hz), 130.04 (d,  $J_{CF} = 8.1$  Hz), 122.91, 113.49 (d,  $J_{CF} = 22.5$  Hz), 113.45 (d,  $J_{CF} = 21.1$  Hz), 78.84, 61.232, 57.92, 41.64, 27.96, 13.79. **HRMS** (ESI, positive mode):  $m/z$  377.1461 [M+Na]<sup>+</sup>, calcd for [C<sub>17</sub>H<sub>23</sub>FN<sub>2</sub>O<sub>5</sub>Na]<sup>+</sup>: 377.1483.

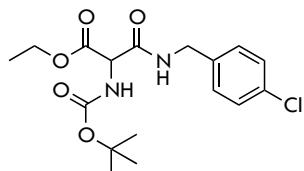
**Ethyl 2-tert-butoxycarbonylamino-3-(4-fluorobenzylamino)-3-oxopropanoate (3.16b)**



Compound **3.16b** was prepared from **3.8** (1.62 g, 5 mmol) via procedure A using 0.57 mL (5 mmol) of 4-fluorobenzylamine. Yield 80% (1.42 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.83 (dd,  $J_1 = 6.4$  Hz,  $J_2 = 5.7$  Hz, 1H),

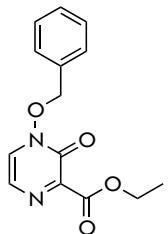
7.31-7.11 (m, 5H), 4.81 (d,  $J = 8.5$  Hz, 1H), 4.35 (dd,  $J_1 = 15.3$  Hz,  $J_2 = 6.4$  Hz, 1H), 4.23 (dd,  $J_1 = 15.3$  Hz,  $J_2 = 5.7$  Hz, 1H), 4.13 (q,  $J = 7.0$  Hz, 2H), 1.39 (s, 9H), 1.16 (t,  $J = 7.0$  Hz, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  168.33, 165.94, 161.71 (d,  $J_{CF} = 242.4$  Hz), 155.52, 135.47, 129.55 (d,  $J_{CF} = 8.0$  Hz), 115.40 (d,  $J_{CF} = 21.3$  Hz), 79.42, 61.78, 58.47, 42.08, 28.52, 14.38. **HRMS** (ESI, positive mode):  $m/z$  377.1466 [M+Na]<sup>+</sup>, calcd for [C<sub>17</sub>H<sub>23</sub>FN<sub>2</sub>O<sub>5</sub>Na]<sup>+</sup>: 377.1483.

**Ethyl 2-*tert*-butoxycarbonylamino-3-(4-chlorobenzylamino)-3-oxopropanoate (3.16c)**



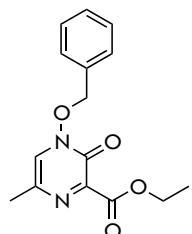
Compound **3.16c** was prepared from **3.8** (1.62 g, 5 mmol) via procedure A using 0.67 mL (5 mmol) of 4-chlorobenzylamine. Yield 69% (1.28 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 8.85 (dd, *J*<sub>1</sub> = 6.1 Hz, *J*<sub>2</sub> = 5.6 Hz, 1H), 7.37 (d, *J* = 8.3 Hz, 2H), 7.28 (d, *J* = 8.3 Hz, 2H), 7.14 (d, *J* = 8.1 Hz, 1H), 4.81 (d, *J* = 8.1 Hz, 1H), 4.36 (dd, *J*<sub>1</sub> = 15.5 Hz, *J*<sub>2</sub> = 6.1 Hz, 1H), 4.23 (dd, *J*<sub>1</sub> = 15.5 Hz, *J*<sub>2</sub> = 5.6 Hz, 1H), 4.13 (q, *J* = 7.0 Hz, 2H), 1.39 (s, 9H), 1.17 (t, *J* = 7.0 Hz, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 167.75, 165.44, 154.95, 137.82, 131.32, 128.87, 128.05, 78.82, 61.20, 57.90, 41.56, 27.96, 13.84. **HRMS** (ESI, positive mode): *m/z* 393.1139 [M+Na]<sup>+</sup>, calcd for [C<sub>17</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>5</sub>Na]<sup>+</sup>: 393.1188.

**Ethyl 1-benzyloxyprazin-2(1*H*)-one-3-carboxylate (3.18a)**



Compound **3.18a** was prepared by deprotection of **3.9** (2.72 g, 9.9 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (1.03 mL, 9.0 mmol). Yield 68% (1.68 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 8.17 (d, *J* = 4.3 Hz, 1H), 7.53-7.43 (m, 5H), 7.36 (d, *J* = 4.3 Hz, 1H), 5.30 (s, 2H), 4.32 (q, *J* = 7.0 Hz, 2H), 1.30 (t, *J* = 7.0 Hz, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 163.50, 150.13, 148.53, 133.66, 133.43, 130.38, 129.89, 129.10, 121.98, 79.22, 61.95, 14.46. **LRMS** (ESI, positive mode): *m/z* 297.3 [M+Na]<sup>+</sup>.

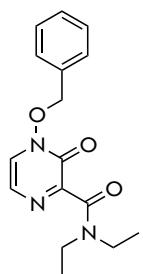
**Ethyl 1-benzyloxy-5-methylprazin-2(1*H*)-one-3-carboxylate (3.18b)**



Compound **3.18b** was prepared by deprotection of **3.9** (525 mg, 1.49 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (207 μL, 1.35 mmol). Yield 61% (238 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-

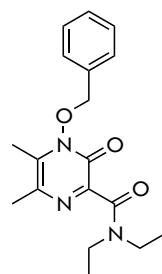
$\delta$ :  $\delta$  8.09 (s, 1H), 7.53-7.43 (m, 5H), 5.27 (s, 2H), 4.32 (q,  $J$  = 7.1 Hz, 2H), 2.19 (s, 3H), 1.30 (t,  $J$  = 7.1 Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  163.64, 149.13, 147.52, 133.75, 130.43, 130.31, 129.84, 129.62, 129.07, 79.20, 61.95, 19.09, 14.48. LRMS (ESI, positive mode):  $m/z$  311.3 [M+Na]<sup>+</sup>.

**1-Benzyl-N,N-diethylpyrazin-2(1H)-one-3-carboxamide (3.13a)**



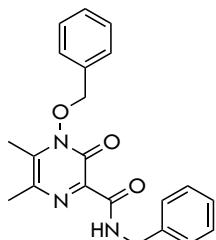
Compound **3.13a** was prepared by deprotection of **3.11a** (834 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (228  $\mu\text{L}$ , 2.0 mmol). Yield 78% (468 mg).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  7.98 (d,  $J$  = 4.5 Hz, 1H), 7.51-7.48 (m, 2H), 7.43-7.41 (m, 3H), 7.28 (d,  $J$  = 4.5 Hz, 1H), 5.31 (s, 2H), 3.42 (q,  $J$  = 7.1 Hz, 2H), 3.12 (q,  $J$  = 7.1 Hz, 2H), 1.13 (t,  $J$  = 7.1 Hz, 3H), 1.03 (t,  $J$  = 7.1 Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  164.48, 155.15, 150.16, 133.64, 130.65, 130.39, 129.87, 129.06, 122.12, 78.88, 42.41, 39.00, 14.44, 13.17. LRMS (ESI, positive mode):  $m/z$  324.4 [M+Na]<sup>+</sup>.

**1-Benzyl-N,N-diethyl-5,6-dimethylpyrazin-2(1H)-one-3-carboxamide (3.13b)**



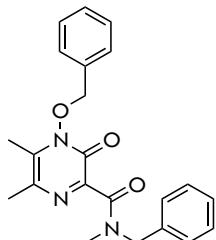
Compound **3.13b** was prepared by deprotection of **3.11a** (834 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using diacetyl (175  $\mu\text{L}$ , 2.0 mmol). Yield 64% (420 mg).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  7.55-7.53 (m, 2H), 7.45-7.44 (m, 3H), 5.26 (s, 2H), 3.42 (q,  $J$  = 7.1 Hz, 2H), 3.16 (q,  $J$  = 7.1 Hz, 2H), 2.34 (s, 3H), 2.24 (s, 3H), 1.14 (t,  $J$  = 7.1 Hz, 3H), 1.06 (t,  $J$  = 7.1 Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  164.86, 149.89, 149.65, 137.44, 133.89, 130.38, 129.88, 129.11, 128.41, 77.61, 42.52, 39.00, 19.69, 14.49, 13.54, 13.25. HRMS (ESI, positive mode):  $m/z$  352.1544 [M+Na]<sup>+</sup>, calcd for [C<sub>18</sub>H<sub>23</sub>N<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 352.1632.

**N-Benzyl-1-benzyloxy-5,6-dimethylpyrazin-2(1H)-one-3-carboxamide (3.13c)**



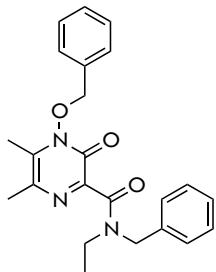
Compound **3.13c** was prepared by deprotection of **3.11b** (910 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using diacetyl (175  $\mu$ L, 2.0 mmol). Yield 75% (546 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  9.58 (t,  $J$  = 5.4 Hz, 1H), 7.57-7.55 (m, 2H), 7.45-7.44 (m, 3H), 7.36-7.25 (m, 5H), 5.27 (s, 2H), 4.53 (d,  $J$  = 5.4 Hz, 2H), 2.39 (s, 3H), 2.32 (s, 3H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO- $d_6$ ):  $\delta$  162.61, 151.93, 142.41, 140.67, 139.53, 133.80, 130.40, 130.03, 129.90, 129.11, 128.83, 127.87, 127.37, 77.87, 42.88, 20.06, 13.98. **HRMS** (ESI, positive mode):  $m/z$  386.1442 [M+Na] $^+$ , calcd for [C<sub>21</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>Na] $^+$ : 386.1475.

**N-Benzyl-1-benzyloxy-N,5,6-trimethylpyrazin-2(1H)-one-3-carboxamide (3.13d)**



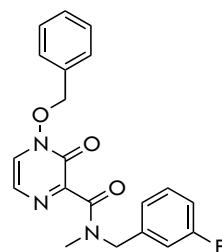
Compound **3.13d** was prepared by deprotection of **3.11c** (941 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using diacetyl (175  $\mu$ L, 2.0 mmol). Yield 66% (499 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ): ( $\alpha$  :  $\beta$  = 100 : 73)  $\alpha$  rotamer:  $\delta$  7.56-7.30 (m, 10H), 5.29 (s, 2H), 4.68 (s, 2H), 2.81 (s, 3H), 2.35 (s, 3H), 2.26 (s, 3H);  $\beta$  rotamer:  $\delta$  7.56-7.30 (m, 10H), 5.25 (s, 2H), 4.42 (s, 2H), 2.84 (s, 3H), 2.32 (s, 3H), 2.22 (s, 3H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO- $d_6$ ):  $\alpha$  rotamer:  $\delta$  165.68, 149.61, 149.48, 137.92, 137.17, 133.89, 130.36, 129.88, 129.12, 128.99, 128.18, 128.01, 127.69, 77.71, 49.68, 35.29, 19.76, 13.60;  $\beta$  rotamer:  $\delta$  165.66, 149.82, 149.23, 137.92, 137.01, 133.87, 130.36, 129.88, 129.09, 128.93, 128.58, 128.18, 127.69, 77.61, 53.55, 32.01, 19.72, 13.58. **HRMS** (ESI, positive mode):  $m/z$  400.1611 [M+Na] $^+$ , calcd for [C<sub>23</sub>H<sub>23</sub>N<sub>3</sub>O<sub>3</sub>Na] $^+$ : 400.1632.

**N-Benzyl-1-benzyloxy-N-ethyl-5,6-dimethylpyrazin-2(1*H*)-one-3-carboxamide (3.13e)**



Compound **3.13e** was prepared by deprotection of **3.11d** (971 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using diacetyl (175  $\mu$ L, 2.0 mmol). Yield 69% (543 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 64)  $\alpha$  rotamer:  $\delta$  7.55-7.27 (m, 10H), 5.29 (s, 2H), 4.68 (s, 2H), 3.13 (q, *J* = 7.0 Hz, 2H), 2.35 (s, 3H), 2.26 (s, 3H), 1.02 (t, *J* = 7.0 Hz, 3H);  $\beta$  rotamer:  $\delta$  7.55-7.27 (m, 10H), 5.23 (s, 2H), 4.41 (s, 2H), 3.32 (q, *J* = 7.0 Hz, 2H), 2.31 (s, 3H), 2.22 (s, 3H), 1.05 (t, *J* = 7.0 Hz, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  165.73, 149.76, 149.57, 137.77, 137.76, 133.89, 130.36, 129.88, 129.12, 128.92, 128.31, 127.95, 127.55, 77.69, 46.71, 42.61, 19.75, 14.04, 13.60;  $\beta$  rotamer:  $\delta$  165.30, 149.76, 149.43, 137.76, 137.41, 133.89, 130.36, 129.86, 129.09, 128.84, 128.67, 127.97, 127.55, 77.60, 51.18, 39.30, 19.69, 13.56, 12.63. **HRMS** (ESI, positive mode): *m/z* 414.1773 [M+Na]<sup>+</sup>, calcd for [C<sub>23</sub>H<sub>25</sub>N<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 414.1788.

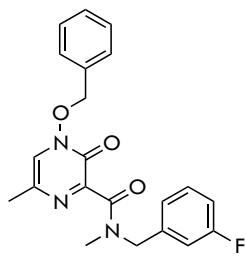
**1-Benzyl-1-benzyloxy-N-(3-fluorobenzyl)-N-methylpyrazin-2(1*H*)-one-3-carboxamide (3.13f)**



Compound **3.13f** was prepared by deprotection of **3.11e** (980 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (228  $\mu$ L, 2.0 mmol). Yield 59% (436 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 69)  $\alpha$  rotamer:  $\delta$  8.05 (d, *J* = 4.5 Hz, 1H), 7.36 (d, *J* = 4.5 Hz, 1H), 7.53-7.38 (m, 6H), 7.22-7.11 (m, 3H), 5.34 (s, 2H), 4.71 (s, 2H), 2.80 (s, 3H);  $\beta$  rotamer:  $\delta$  8.00 (d, *J* = 4.5 Hz, 1H), 7.30 (d, *J* = 4.5 Hz, 1H), 7.53-7.38 (m, 6H), 7.22-7.11 (m, 3H), 5.29 (s, 2H), 4.42 (s, 2H), 2.86 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  165.46, 162.88 (d, *J*<sub>CF</sub> = 243.5 Hz), 154.61, 150.14, 140.05 (d, *J*<sub>CF</sub> = 7.3 Hz), 136.66, 131.04 (d, *J*<sub>CF</sub> = 7.7 Hz), 131.05, 130.39, 129.87, 129.08, 123.86 (d, *J*<sub>CF</sub> = 3.0 Hz), 122.53, 114.50 (d, *J*<sub>CF</sub> = 20.9 Hz), 114.45 (d,

$J_{CF} = 21.8$  Hz), 79.08, 49.25, 35.40;  $\beta$  rotamer:  $\delta$  165.34, 162.75 (d,  $J_{CF} = 243.5$  Hz), 154.54, 150.35, 139.96 (d,  $J_{CF} = 7.3$  Hz), 136.64, 130.96 (d,  $J_{CF} = 7.7$  Hz), 131.00, 130.33, 129.86, 129.04, 124.18 (d,  $J_{CF} = 3.0$  Hz), 122.33, 114.90 (d,  $J_{CF} = 20.9$  Hz), 114.82 (d,  $J_{CF} = 21.8$  Hz), 78.95, 52.88, 32.13. **LRMS** (ESI, positive mode):  $m/z$  390.2 [M+Na]<sup>+</sup>.

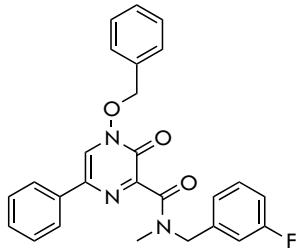
**1-Benzylxy-N-(3-fluorobenzyl)-N,5-dimethylpyrazin-2(1H)-one-3-carboxamide (3.13g)**



Compound **3.13g** was prepared by deprotection of **3.11e** (980 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (307  $\mu$ L, 2.0 mmol). Yield 59% (436 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): ( $\alpha$  :  $\beta$  = 100 : 67)  $\alpha$  rotamer:  $\delta$  7.42-6.96 (m, 10H), 5.33 (s, 2H), 4.77 (s, 2H), 2.87 (s, 3H), 2.20 (s, 3H);  $\beta$  rotamer:  $\delta$  7.42-6.96 (m, 10H), 5.30 (s, 2H), 4.38 (s, 2H), 3.00 (s, 3H), 2.16 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>):  $\alpha$  rotamer:  $\delta$  165.55, 163.13 (d,  $J_{CF} = 246.5$  Hz), 154.05, 149.23, 138.64 (d,  $J_{CF} = 7.2$  Hz), 132.82, 131.30, 130.33 (d,  $J_{CF} = 8.2$  Hz), 130.08, 129.80, 128.91, 126.25, 123.45 (d,  $J_{CF} = 2.9$  Hz), 114.82 (d,  $J_{CF} = 21.1$  Hz), 114.44 (d,  $J_{CF} = 21.1$  Hz), 79.01, 49.99, 35.08, 19.20;  $\beta$  rotamer:  $\delta$  165.42, 163.19 (d,  $J_{CF} = 246.5$  Hz), 153.69, 149.42, 138.39 (d,  $J_{CF} = 7.2$  Hz), 132.79, 131.06, 130.36 (d,  $J_{CF} = 8.2$  Hz), 130.08, 129.78, 128.87, 126.26, 123.37 (d,  $J_{CF} = 2.9$  Hz), 114.97 (d,  $J_{CF} = 21.1$  Hz), 114.75 (d,  $J_{CF} = 21.1$  Hz), 78.92, 53.72, 32.26, 19.13. **LRMS** (ESI, positive mode):  $m/z$  404.2 [M+Na]<sup>+</sup>.

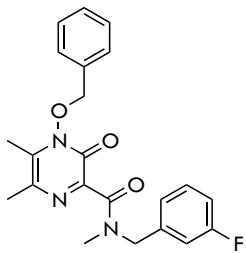
**1-Benzylxy-N-(3-fluorobenzyl)-N-methyl-5-phenylpyrazin-2(1H)-one-3-carboxamide (3.13h)**

Compound **3.13h** was prepared by deprotection of **3.11e** (980 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using a phenylglyoxal monohydrate (304 mg, 2.0 mmol). Yield 42% (369 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): ( $\alpha$  :  $\beta$  = 100 : 67)  $\alpha$  rotamer:  $\delta$  7.58-6.94 (m, 15H), 5.39 (s, 2H), 4.80 (s, 2H), 3.05 (s, 3H);  $\beta$  rotamer:  $\delta$  7.58-6.94 (m, 15H), 5.36 (s, 2H), 4.44 (s, 2H), 2.90 (s, 3H).



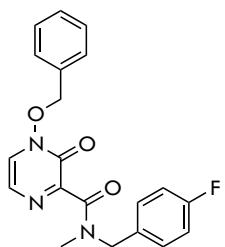
**<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>):  $\alpha$  rotamer:  $\delta$  165.44, 163.11 (d,  $J_{CF} = 246.4$  Hz), 153.73, 149.51, 138.65 (d,  $J_{CF} = 7.1$  Hz), 134.16, 132.70, 130.35 (d,  $J_{CF} = 8.1$  Hz), 130.20, 129.94, 129.70, 129.01, 128.89, 128.56, 125.24, 123.42 (d,  $J_{CF} = 2.9$  Hz), 114.92 (d,  $J_{CF} = 21.0$  Hz), 114.60 (d,  $J_{CF} = 21.0$  Hz), 79.27, 49.98, 35.23;  $\beta$  rotamer:  $\delta$  165.32, 163.14 (d,  $J_{CF} = 246.4$  Hz), 153.20, 149.31, 138.42 (d,  $J_{CF} = 7.1$  Hz), 134.04, 132.72, 130.35 (d,  $J_{CF} = 8.1$  Hz), 130.18, 129.91, 129.48, 128.96, 128.84, 128.51, 125.09, 123.20 (d,  $J_{CF} = 2.9$  Hz), 114.77 (d,  $J_{CF} = 21.0$  Hz), 114.46 (d,  $J_{CF} = 21.0$  Hz), 79.19, 53.74, 32.42. **LRMS** (ESI, positive mode):  $m/z$  466.3 [M+Na]<sup>+</sup>.

**1-Benzylxoy-N-(3-fluorobenzyl)-N,5,6-trimethylpyrazin-2(1H)-one-3-carboxamide (3.13i)**



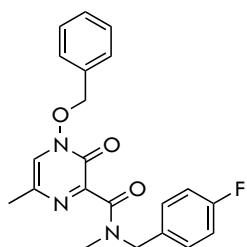
Compound **3.13i** was prepared by deprotection of **3.11e** (980 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using diacetyl (175  $\mu$ L, 2.0 mmol). Yield 57% (453 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): ( $\alpha : \beta = 100 : 72$ )  $\alpha$  rotamer:  $\delta$  7.49-6.95 (m, 9H), 5.31 (s, 2H), 4.78 (s, 2H), 2.90 (s, 3H), 2.31 (s, 3H), 2.25 (s, 3H);  $\beta$  rotamer:  $\delta$  7.49-6.95 (m, 9H), 5.28 (s, 2H), 4.43 (s, 2H), 3.01 (s, 3H), 2.26 (s, 3H), 2.23 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>):  $\alpha$  rotamer:  $\delta$  165.96, 163.14 (d,  $J_{CF} = 246.9$  Hz), 149.85, 149.67, 138.85 (d,  $J_{CF} = 7.2$  Hz), 136.86, 132.94, 130.28 (d,  $J_{CF} = 8.0$  Hz), 130.16, 129.78, 129.48, 128.87, 123.50 (d,  $J_{CF} = 2.8$  Hz), 114.85 (d,  $J_{CF} = 22.0$  Hz), 114.36 (d,  $J_{CF} = 22.0$  Hz), 78.11, 50.05, 35.20, 19.62, 13.41;  $\beta$  rotamer:  $\delta$  165.85, 163.67 (d,  $J_{CF} = 246.9$  Hz), 150.06, 149.30, 138.68 (d,  $J_{CF} = 7.2$  Hz), 136.91, 132.91, 130.28 (d,  $J_{CF} = 8.0$  Hz), 130.16, 129.78, 129.23, 128.83, 123.37 (d,  $J_{CF} = 2.8$  Hz), 114.85 (d,  $J_{CF} = 22.0$  Hz), 114.76 (d,  $J_{CF} = 22.0$  Hz), 78.05, 53.84, 32.29, 19.53, 13.38. **LRMS** (ESI, positive mode):  $m/z$  418.3 [M+Na]<sup>+</sup>.

**1-Benzylxy-N-(4-fluorobenzyl)-N-methylpyrazin-2(1*H*)-one-3-carboxamide (3.13j)**



Compound **3.13j** was prepared by deprotection of **3.11f** (980 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (228  $\mu$ L, 2.0 mmol). Yield 68% (499 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 76)  $\alpha$  rotamer:  $\delta$  8.04 (d,  $J$  = 4.6 Hz, 1H), 7.51-7.37 (m, 7H), 7.34 (d,  $J$  = 4.6 Hz, 1H), 7.25-7.18 (m, 2H), 5.33 (s, 2H), 4.67 (s, 2H), 2.77 (s, 3H);  $\beta$  rotamer:  $\delta$  8.00 (d,  $J$  = 4.6 Hz, 1H), 7.51-7.37 (m, 7H), 7.31 (d,  $J$  = 4.6 Hz, 1H), 7.25-7.18 (m, 2H), 5.30 (s, 2H), 4.37 (s, 2H), 2.83 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  165.34, 161.95 (d,  $J_{CF}$  = 243.6 Hz), 154.69, 150.10, 133.66, 133.22 (d,  $J_{CF}$  = 2.9 Hz), 130.99, 130.38, 129.97 (d,  $J_{CF}$  = 8.3 Hz), 129.87, 129.08, 122.47, 115.82 (d,  $J_{CF}$  = 21.4 Hz), 79.05, 48.94, 35.16;  $\beta$  rotamer:  $\delta$  165.23, 162.10 (d,  $J_{CF}$  = 243.6 Hz), 154.69, 150.34, 133.66, 133.03 (d,  $J_{CF}$  = 2.9 Hz), 130.95, 130.33, 130.20 (d,  $J_{CF}$  = 8.3 Hz), 129.87, 129.04, 122.30, 115.78 (d,  $J_{CF}$  = 21.4 Hz), 78.94, 52.62, 31.91. **HRMS** (ESI, positive mode): *m/z* 390.1119 [M+Na]<sup>+</sup>, calcd for [C<sub>20</sub>H<sub>18</sub>FN<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 390.1224.

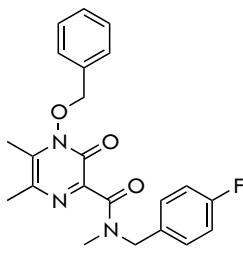
**1-Benzylxy-N-(4-fluorobenzyl)-N,5-dimethylpyrazin-2(1*H*)-one-3-carboxamide (3.13k)**



Compound **3.13k** was prepared by deprotection of **3.11f** (980 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (307  $\mu$ L, 2.0 mmol). Yield 52% (400 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 69)  $\alpha$  rotamer:  $\delta$  7.95 (s, 1H), 7.48-7.35 (m, 7H), 7.24-7.17 (m, 2H), 5.30 (s, 2H), 4.66 (s, 2H), 2.77 (s, 3H), 2.20 (s, 3H);  $\beta$  rotamer:  $\delta$  7.91 (s, 1H), 7.48-7.35 (m, 7H), 7.24-7.17 (m, 2H), 5.27 (s, 2H), 4.37 (s, 2H), 2.83 (s, 3H), 2.16 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  165.44, 161.90 (d,  $J_{CF}$  = 243.0 Hz),

153.64, 149.02, 133.77, 133.24, 130.94, 130.30, 130.04 (d,  $J_{CF} = 7.6$  Hz), 129.81, 129.04, 127.70, 115.82 (d,  $J_{CF} = 21.2$  Hz), 79.02, 48.95, 35.11;  $\beta$  rotamer:  $\delta$  165.20, 161.80 (d,  $J_{CF} = 243.0$  Hz), 153.51, 147.92, 133.77, 133.10, 130.65, 130.30, 130.04 (d,  $J_{CF} = 7.6$  Hz), 129.81, 129.04, 127.70, 115.73 (d,  $J_{CF} = 21.2$  Hz), 78.92, 52.67, 31.91. **HRMS** (ESI, positive mode):  $m/z$  404.1309 [M+Na]<sup>+</sup>, calcd for [C<sub>21</sub>H<sub>20</sub>FN<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 404.1381.

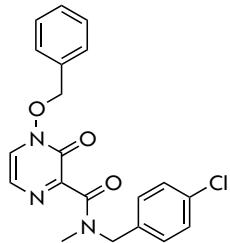
**1-Benzylxy-N-(4-fluorobenzyl)-N,5,6-trimethylpyrazin-2(1H)-one-3-carboxamide (3.13l)**



Compound **3.13l** was prepared by deprotection of **3.11f** (980 mg, 2.2 mmol) via procedure D and then cyclization via procedure E using diacetyl (175  $\mu$ L, 2.0 mmol). Yield 48% (382 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 77)  $\alpha$  rotamer:  $\delta$  7.56-7.17 (m, 9H), 5.29 (s, 2H), 4.66 (s, 2H), 2.81 (s, 3H), 2.35 (s, 3H), 2.26 (s, 3H);  $\beta$  rotamer:  $\delta$  7.56-7.17 (m, 9H), 5.25 (s, 2H), 4.41 (s, 2H), 2.83 (s, 3H), 2.32 (s, 3H), 2.23 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  165.70, 161.94 (d,  $J_{CF} = 242.8$  Hz), 149.61, 149.36, 137.97, 133.88, 133.38 (d,  $J_{CF} = 2.8$  Hz), 130.34, 130.06 (d,  $J_{CF} = 8.1$  Hz), 129.87, 129.11, 128.94, 115.81 (d,  $J_{CF} = 21.3$  Hz), 77.72, 49.00, 35.23, 19.74, 13.59;  $\beta$  rotamer:  $\delta$  165.61, 162.09 (d,  $J_{CF} = 242.8$  Hz), 149.84, 149.17, 137.97, 133.88, 133.21 (d,  $J_{CF} = 2.8$  Hz), 130.34, 130.28 (d,  $J_{CF} = 8.1$  Hz), 129.87, 129.09, 128.62, 115.71 (d,  $J_{CF} = 21.3$  Hz), 77.63, 52.78, 31.90, 19.69, 13.59. **HRMS** (ESI, positive mode):  $m/z$  418.1451 [M+Na]<sup>+</sup>, calcd for [C<sub>22</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 418.1537.

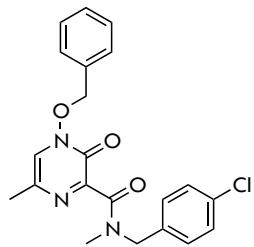
**1-Benzylxy-N-(4-chlorobenzyl)-N-methylpyrazin-2(1H)-one-3-carboxamide (3.13m)**

Compound **3.13m** was prepared by deprotection of **3.11g** (508 mg, 1.1 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (114  $\mu$ L, 1.0 mmol). Yield 74% (282 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): ( $\alpha$  :  $\beta$  = 100 : 67)  $\alpha$  rotamer:  $\delta$  7.41-7.10 (m, 11H), 5.35 (s, 2H), 4.74 (s, 2H), 2.83 (s, 3H);  $\beta$  rotamer:  $\delta$  7.41-7.10 (m, 11H),



5.32 (s, 2H), 4.35 (s, 2H), 2.98 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>):  $\alpha$  rotamer:  $\delta$  165.27, 155.13, 150.30, 134.56, 133.39, 132.63, 130.13, 129.93, 129.33, 129.08, 129.00, 128.95, 122.04, 79.07, 49.78, 34.97;  $\beta$  rotamer:  $\delta$  165.17, 154.89, 150.50, 134.12, 133.89, 132.61, 130.11, 129.89, 129.33, 129.08, 129.00, 128.95, 121.83, 78.99, 53.52, 32.17. **LRMS** (ESI, positive mode): *m/z* 406.2 [M+Na]<sup>+</sup>.

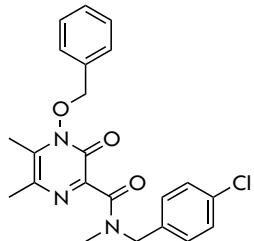
**1-Benzyl-N-(4-chlorobenzyl)-N,5-dimethylpyrazin-2(1H)-one-3-carboxamide (3.13n)**



Compound **3.13n** was prepared by deprotection of **3.11g** (508 mg, 1.1 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (154  $\mu$ L, 1.0 mmol). Yield 62% (246 mg). **<sup>1H NMR</sup>** (400 MHz, CDCl<sub>3</sub>): ( $\alpha$  :  $\beta$  = 100 : 66)  $\alpha$  rotamer:  $\delta$  7.42-7.27 (m, 9H), 6.97 (s, 1H), 5.33 (s, 2H), 4.74 (s, 2H), 2.84 (s, 3H), 2.20 (s, 3H);  $\beta$  rotamer:  $\delta$  7.42-7.27 (m, 9H), 6.96 (s, 1H), 5.30 (s, 2H), 4.36 (s, 2H), 2.98 (s, 3H), 2.16 (s, 3H). **<sup>13C NMR</sup>** (100 MHz, CDCl<sub>3</sub>):  $\alpha$  rotamer:  $\delta$  165.50, 154.09, 149.22, 134.56, 133.35, 132.79, 131.30, 130.07, 129.82, 129.36, 128.92, 126.21, 79.01, 49.80, 34.96, 19.20;  $\beta$  rotamer:  $\delta$  165.38, 153.78, 149.43, 134.20, 133.87, 132.77, 131.05, 130.05, 129.79, 129.16, 128.96, 128.87, 126.21, 78.93, 53.58, 32.16, 19.16. **LRMS** (ESI, positive mode): *m/z* 420.3 [M+Na]<sup>+</sup>.

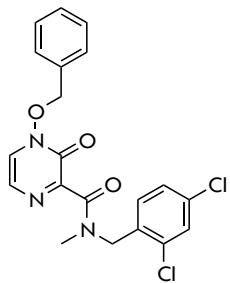
**1-Benzyl-N-(4-chlorobenzyl)-N,5,6-trimethylpyrazin-2(1H)-one-3-carboxamide (3.13o)**

Compound **3.13o** was prepared by deprotection of **3.11g** (508 mg, 1.1 mmol) via procedure D and then cyclization via procedure E using diacetyl (88  $\mu$ L, 1.0 mmol). Yield 59% (242 mg).



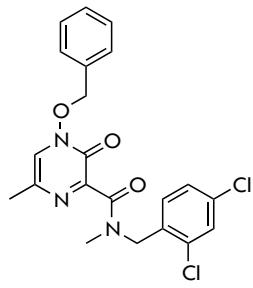
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): (α : β = 100 : 71) α rotamer: δ 7.48-7.34 (m, 9H), 5.33 (s, 2H), 4.78 (s, 2H), 2.89 (s, 3H), 2.33 (s, 3H), 2.27 (s, 3H); β rotamer: δ 7.48-7.34 (m, 9H), 5.31 (s, 2H), 4.43 (s, 2H), 3.01 (s, 3H), 2.28 (s, 3H), 2.25 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>): α rotamer: δ 165.91, 149.83, 149.68, 136.83, 134.76, 133.25, 132.89, 130.13, 129.78, 129.47, 129.39, 128.88, 128.86, 78.10, 49.83, 35.08, 19.61, 13.40; β rotamer: δ 165.81, 150.05, 149.35, 136.87, 134.46, 133.74, 132.88, 130.13, 129.78, 129.21, 129.00, 128.88, 128.83, 78.04, 53.66, 32.17, 19.54, 13.38. **HRMS** (ESI, positive mode): *m/z* 434.1195 [M+Na]<sup>+</sup>, calcd for [C<sub>22</sub>H<sub>22</sub>ClN<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 434.1242.

**1-Benzyl-N-(2,4-dichlorobenzyl)-N-methylpyrazin-2(1H)-one-3-carboxamide (3.13p)**



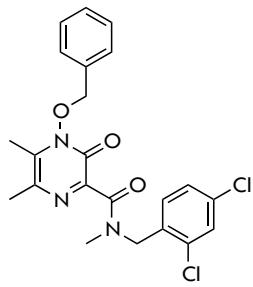
Compound **3.13p** was prepared by deprotection of **3.11h** (546 mg, 1.1 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (114 μL, 1.0 mmol). Yield 51% (212 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): (α : β = 100 : 46) α rotamer: δ 8.07 (d, *J* = 4.6 Hz, 1H), 7.68 (d, *J* = 1.8 Hz, 1H), 7.53-7.40 (m, 7H), 7.37 (d, *J* = 4.6 Hz, 1H), 5.34 (s, 2H), 4.72 (s, 2H), 2.86 (s, 3H); β rotamer: δ 7.96 (d, *J* = 4.6 Hz, 1H), 7.61 (d, *J* = 1.8 Hz, 1H), 7.53-7.40 (m, 7H), 7.24 (d, *J* = 4.6 Hz, 1H), 5.25 (s, 2H), 4.53 (s, 2H), 2.93 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): α rotamer: δ 165.58, 154.37, 150.19, 133.72, 133.61, 133.35, 131.18, 131.14, 130.39, 130.30, 129.87, 129.42, 129.09, 128.12, 122.62, 79.12, 47.58, 35.86; β rotamer: δ 165.63, 154.01, 150.24, 133.65, 133.44, 133.09, 131.18, 130.86, 130.39, 130.30, 129.87, 129.30, 129.04, 128.12, 122.23, 78.88, 50.48, 32.81. **HRMS** (ESI, positive mode): *m/z* 440.0472 [M+Na]<sup>+</sup>, calcd for [C<sub>20</sub>H<sub>17</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 440.0539.

**1-Benzylxy-N-(2,4-dichlorobenzyl)-N,5-dimethylpyrazin-2(1*H*)-one-3-carboxamide (3.13q)**



Compound **3.13q** was prepared by deprotection of **3.11h** (546 mg, 1.1 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (154  $\mu$ L, 1.0 mmol). Yield 49% (212 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ): ( $\alpha$  :  $\beta$  = 100 : 49)  $\alpha$  rotamer:  $\delta$  7.98 (s, 1H), 7.67 (d,  $J$  = 2.0 Hz, 1H), 7.54-7.39 (m, 7H), 5.32 (s, 2H), 4.72 (s, 2H), 2.87 (s, 3H), 2.22 (s, 3H);  $\beta$  rotamer:  $\delta$  7.88 (s, 1H), 7.60 (d,  $J$  = 2.0 Hz, 1H), 7.54-7.39 (m, 7H), 5.22 (s, 2H), 4.53 (s, 2H), 2.94 (s, 3H), 2.10 (s, 3H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO- $d_6$ ):  $\alpha$  rotamer:  $\delta$  165.67, 153.31, 149.11, 133.74, 133.71, 133.36, 131.13, 131.11, 130.31, 130.23, 129.82, 129.39, 129.05, 128.12, 127.92, 79.09, 47.61, 35.84, 19.18;  $\beta$  rotamer:  $\delta$  165.68, 152.80, 149.11, 133.58, 133.55, 133.07, 131.13, 130.58, 130.31, 130.23, 129.93, 129.22, 129.01, 128.03, 127.89, 78.86, 50.47, 32.94, 19.06. **HRMS** (ESI, positive mode):  $m/z$  454.0563 [M+Na] $^+$ , calcd for [C<sub>21</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>Na] $^+$ : 454.0696.

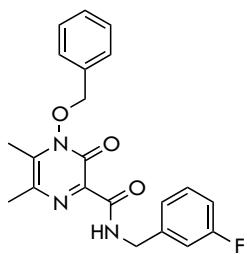
**1-Benzylxy-N-(2,4-dichlorobenzyl)-N,5,6-trimethylpyrazin-2(1*H*)-one-3-carboxamide (3.13r)**



Compound **3.13r** was prepared by deprotection of **3.11h** (546 mg, 1.1 mmol) via procedure D and then cyclization via procedure E using diacetyl (88  $\mu$ L, 1.0 mmol). Yield 63% (281 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ): ( $\alpha$  :  $\beta$  = 100 : 50)  $\alpha$  rotamer:  $\delta$  7.67 (brs, 1H), 7.56-7.43 (m, 7H), 5.30 (s, 2H), 4.72 (s, 2H), 2.90 (s, 3H), 2.36 (s, 3H), 2.28 (s, 3H);  $\beta$  rotamer:  $\delta$  7.59 (d,  $J$  = 2.0 Hz, 1H), 7.56-7.43 (m, 7H), 5.20 (s, 2H), 4.56 (s, 2H), 2.93 (s, 3H), 2.29 (s, 3H), 2.15 (s, 3H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO- $d_6$ ):  $\alpha$  rotamer:  $\delta$  165.97, 149.72, 148.99, 138.27, 133.69, 133.52, 133.03, 131.07, 130.35, 129.95, 129.88, 129.38, 129.13, 129.08, 128.13, 77.78, 47.64, 35.97, 19.79,

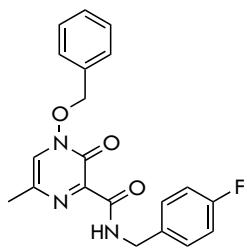
13.64;  $\beta$  rotamer:  $\delta$  165.96, 148.99, 148.46, 138.27, 133.87, 133.69, 133.27, 131.07, 130.35, 129.95, 129.88, 129.20, 129.13, 128.56, 128.02, 77.59, 50.60, 32.91, 19.62, 13.57. **HRMS** (ESI, positive mode):  $m/z$  468.0789 [M+Na]<sup>+</sup>, calcd for [C<sub>22</sub>H<sub>21</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 468.0852.

**1-Benzylxy-N-(3-fluorobenzyl)-5,6-dimethylpyrazin-2(1H)-one-3-carboxamide (3.13s)**



Compound **3.13s** was prepared by deprotection of **3.11i** (237 mg, 0.55 mmol) via procedure D and then cyclization via procedure E using diacetyl (44  $\mu$ L, 0.5 mmol). Yield 47% (89 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  9.62 (t,  $J$  = 6.0 Hz, 1H), 7.59-7.07 (m, 9H), 5.29 (s, 2H), 4.55 (d,  $J$  = 6.0 Hz, 2H), 2.41 (s, 3H), 2.32 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  162.83, 162.72 (d,  $J_{CF}$  = 242.6 Hz), 151.90, 142.70 (d,  $J_{CF}$  = 7.2 Hz), 142.45, 140.79, 133.82, 130.76 (d,  $J_{CF}$  = 8.2 Hz), 130.43, 129.94, 129.15, 123.82 (d,  $J_{CF}$  = 2.4 Hz), 114.49 (d,  $J_{CF}$  = 21.7 Hz), 114.09 (d,  $J_{CF}$  = 20.9 Hz), 79.89, 42.38, 20.08, 14.01. **LRMS** (API-ES, positive mode):  $m/z$  382.2 [M+H]<sup>+</sup>.

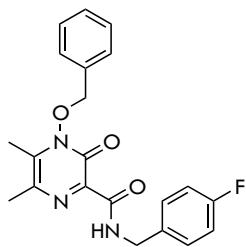
**1-Benzylxy-N-(4-fluorobenzyl)-5-methylpyrazin-2(1H)-one-3-carboxamide (3.13t)**



Compound **3.13t** was prepared by deprotection of **3.11j** (237 mg, 0.55 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (77  $\mu$ L, 0.5 mmol). Yield 58% (107 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  9.47 (t,  $J$  = 5.8 Hz, 1H), 8.07 (s, 1H), 7.54-7.52 (m, 2H), 7.44-7.39 (m, 5H), 7.18 (t,  $J$  = 8.7 Hz, 2H), 5.28 (s, 2H), 4.49 (d,  $J$  = 5.8 Hz, 2H), 2.23 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  162.75, 161.74 (d,  $J_{CF}$  = 242.6 Hz), 150.89, 148.38, 135.62 (d,  $J_{CF}$  = 2.9 Hz), 133.78, 131.15, 130.31, 129.87 (d,  $J_{CF}$  = 8.2 Hz), 129.84, 129.28, 129.08, 115.54 (d,  $J_{CF}$  = 21.3 Hz), 79.20, 42.13, 19.36. **LRMS** (ESI, positive mode):  $m/z$  390.1 [M+Na]<sup>+</sup>.

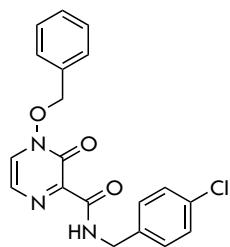
Compound **3.13t** was also prepared by amidation of ester **3.18b** (115 mg, 0.4 mmol) via procedure G using 115  $\mu$ L of 4-fluorobenzylamine (2.5 equiv, 1.0 mmol). Yield 95% (139 mg).

**1-Benzylxy-N-(4-fluorobenzyl)-5,6-dimethylpyrazin-2(1*H*)-one-3-carboxamide (3.13u)**



Compound **3.13u** was prepared by deprotection of **3.11j** (237 mg, 0.55 mmol) via procedure D and then cyclization via procedure E using diacetyl (44  $\mu$ L, 0.5 mmol). Yield 41% (78 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  9.58 (t, *J* = 6.4 Hz, 1H), 7.57-7.55 (m, 1H), 7.46-7.44 (m, 2H), 7.42-7.36 (m, 3H), 7.19-7.12 (m, 3H), 5.27 (s, 2H), 4.49 (d, *J* = 6.4 Hz, 2H), 2.40 (s, 3H), 2.32 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  162.68, 161.73 (d, *J*<sub>CF</sub> = 242.3 Hz), 151.92, 142.43, 140.75, 135.84 (d, *J*<sub>CF</sub> = 2.9 Hz), 133.77, 130.42, 130.03, 129.91 (d, *J*<sub>CF</sub> = 8.1 Hz), 129.14, 128.83, 115.54 (d, *J*<sub>CF</sub> = 21.6 Hz), 77.89, 42.18, 20.08, 14.03. **LRMS** (API-ES, positive mode): *m/z* 382.2 [M+H]<sup>+</sup>.

**1-Benzylxy-N-(4-chlorobenzyl)pyrazin-2(1*H*)-one-3-carboxamide (3.13v)**

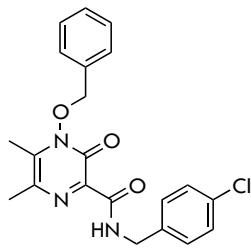


Compound **3.13v** was prepared by deprotection of **3.11k** (246 mg, 0.55 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (57  $\mu$ L, 0.5 mmol). Yield 67% (123 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  9.39 (t, *J* = 6.0 Hz, 1H), 8.12 (d, *J* = 4.3 Hz, 1H), 7.53-7.51 (m, 2H), 7.44-7.37 (m, 8H), 5.30 (s, 2H), 4.49 (d, *J* = 6.0 Hz, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  162.92, 151.71, 150.12, 138.51, 133.71, 132.25, 131.95, 130.38, 129.89, 129.67, 129.10, 128.75, 122.58, 79.19, 42.14. **LRMS** (ESI, positive mode): *m/z* 392.1 [M+Na]<sup>+</sup>.

Compound **3.13v** was also prepared by amidation of ester **3.18a** (110 mg, 0.4 mmol) via procedure G using 122  $\mu$ L of 4-chlorobenzylamine

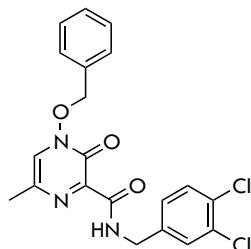
(2.5 equiv, 1.0 mmol). Yield 90% (132 mg).

**1-Benzylxy-N-(4-chlorobenzyl)-5,6-dimethylpyrazin-2(1*H*)-one-3-carboxamide (3.13w)**



Compound **3.13w** was prepared by deprotection of **3.11k** (246 mg, 0.55 mmol) via procedure D and then cyclization via procedure E using diacetyl (44  $\mu$ L, 0.5 mmol). Yield 42% (83 mg).  **$^1\text{H NMR}$**  (400 MHz, DMSO- $d_6$ ):  $\delta$  9.62 (t,  $J$  = 6.0 Hz, 1H), 7.58-7.55 (m, 2H), 7.46-7.36 (m, 7H), 5.28 (s, 2H), 4.53 (d,  $J$  = 6.0 Hz, 2H), 2.40 (s, 3H), 2.32 (s, 3H).  **$^{13}\text{C NMR}$**  (100 MHz, DMSO- $d_6$ ):  $\delta$  162.72, 151.92, 142.30, 140.81, 138.74, 133.81, 131.93, 130.41, 130.07, 129.93, 129.76, 129.14, 128.76, 77.90, 42.24, 20.08, 14.01. **LRMS** (ESI, positive mode):  $m/z$  421.9 [M+Na]<sup>+</sup>.

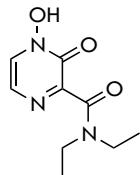
**1-Benzylxy-N-(3,4-dichlorobenzyl)-5-methylpyrazin-2(1*H*)-one-3-carboxamide (3.13x)**



Compound **3.13x** was prepared by amidation of ester **3.18b** (115 mg, 0.4 mmol) via procedure G using 133  $\mu$ L of 3,4-dichlorobenzylamine (2.5 equiv, 1.0 mmol). Yield 90% (150 mg).  **$^1\text{H NMR}$**  (400 MHz, DMSO- $d_6$ ):  $\delta$  9.97 (t,  $J$  = 6.0 Hz, 1H), 7.48 (s, 1H), 7.42-7.38 (m, 6H), 7.23 (d,  $J$  = 8.3 Hz, 1H), 7.14 (s, 1H), 5.32 (s, 2H), 4.64 (d,  $J$  = 6.0 Hz, 2H), 2.29 (s, 3H).  **$^{13}\text{C NMR}$**  (100 MHz, DMSO- $d_6$ ):  $\delta$  161.76, 152.19, 144.64, 138.46, 132.93, 132.58, 132.29, 131.39, 130.56, 130.07, 130.04, 129.87, 129.03, 128.68, 127.37, 79.39, 42.74, 19.59. **LRMS** (ESI, positive mode):  $m/z$  440.0 [M+Na]<sup>+</sup>.

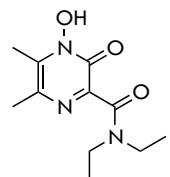
***N,N*-Diethyl-1-hydroxypyrazin-2(1*H*)-one-3-carboxamide (3.26a)**

Compound **3.26a** was prepared by debenzylation of **3.13a** (30.1 mg, 0.1 mmol) in flow via procedure H. Yield 92% (19.4 mg).  **$^1\text{H NMR}$**  (400 MHz, DMSO- $d_6$ ):  $\delta$  8.05 (d,  $J$  = 4.4 Hz, 1H), 7.33 (d,  $J$  = 4.4 Hz, 1H),



3.43 (q,  $J = 7.1$  Hz, 2H), 3.14 (q,  $J = 7.1$  Hz, 2H), 1.13 (t,  $J = 7.1$  Hz, 3H), 1.04 (t,  $J = 7.1$  Hz, 3H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO- $d_6$ ):  $\delta$  165.01, 152.00, 150.78, 129.97, 122.47, 41.44, 38.93, 14.49, 13.22. **HRMS** (ESI, positive mode):  $m/z$  234.1103 [M+Na]<sup>+</sup>, calcd for [C<sub>9</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 234.0849.

**N,N-Diethyl-1-hydroxy-5,6-dimethylpyrazin-2(1H)-one-3-carboxamide (3.26b)**



Compound **3.26b** was prepared by debenzylation of **3.13b** (32.9 mg, 0.1 mmol) in flow via procedure H. Yield 90% (21.5 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  3.41 (q,  $J = 7.1$  Hz, 2H), 3.14 (q,  $J = 7.1$  Hz, 2H), 2.34 (s, 3H), 2.27 (s, 3H), 1.13 (t,  $J = 7.1$  Hz, 3H), 1.04 (t,  $J = 7.1$  Hz, 3H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO- $d_6$ ):  $\delta$  165.41, 149.79, 145.18, 136.71, 129.25, 42.51, 38.94, 19.91, 14.53, 13.60, 13.30. **HRMS** (ESI, positive mode):  $m/z$  262.1404 [M+Na]<sup>+</sup>, calcd for [C<sub>11</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 262.1162.

**N-Benzyl-1-hydroxy-5,6-dimethylpyrazin-2(1H)-one-3-carboxamide (3.26c)**



Compound **3.26c** was prepared by debenzylation of **3.13c** (36.3 mg, 0.1 mmol) in flow via procedure H. Yield 72% (19.7 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  9.95 (brs, 1H), 7.34-7.24 (m, 5H), 4.54 (d,  $J = 4.5$  Hz, 2H), 2.37 (s, 6H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO- $d_6$ ):  $\delta$  163.41, 154.33, 141.88, 141.32, 139.70, 129.85, 127.71, 127.36, 42.82, 19.93, 14.20. **HRMS** (ESI, positive mode):  $m/z$  296.1186 [M+Na]<sup>+</sup>, calcd for [C<sub>14</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 296.1006.

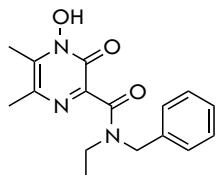
**N-Benzyl-1-hydroxy-N,5,6-trimethylpyrazin-2(1H)-one-3-carboxamide (3.26d)**

Compound **3.26d** was prepared by debenzylation of **3.13d** (37.7 mg, 0.1 mmol) in flow via procedure H. Yield 91% (26.1 mg).



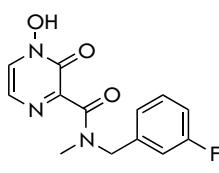
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): ( $\alpha : \beta = 100 : 78$ )  $\alpha$  rotamer:  $\delta$  7.38-7.28 (m, 5H), 4.67 (s, 2H), 2.76 (s, 3H), 2.36 (s, 3H), 2.28 (s, 3H);  $\beta$  rotamer:  $\delta$  7.38-7.28 (m, 5H), 4.37 (s, 2H), 2.80 (s, 3H), 2.33 (s, 3H), 2.25 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  166.18, 149.61, 144.69, 137.26, 137.04, 129.79, 128.96, 128.24, 127.86, 49.62, 35.30, 19.99, 13.63;  $\beta$  rotamer:  $\delta$  166.14, 149.90, 144.69, 137.04, 137.00, 129.47, 128.87, 127.96, 127.57, 53.61, 31.91, 19.94, 13.63. **HRMS** (ESI, positive mode): *m/z* 310.1327 [M+Na]<sup>+</sup>, calcd for [C<sub>15</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 310.1162.

***N*-Benzyl-*N*-ethyl-1-hydroxy-5,6-dimethylpyrazin-2(1*H*)-one-3-carboxamide (3.26e)**



Compound **3.26e** was prepared by debenzylation of **3.13e** (39.1 mg, 0.1 mmol) in flow via procedure H. Yield 94% (28.3 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): ( $\alpha : \beta = 100 : 64$ )  $\alpha$  rotamer:  $\delta$  7.42-7.26 (m, 5H), 4.68 (s, 2H), 3.10 (q, *J* = 6.6 Hz, 2H), 2.37 (s, 3H), 2.29 (s, 3H), 1.00 (t, *J* = 6.6 Hz, 3H);  $\beta$  rotamer:  $\delta$  7.42-7.26 (m, 5H), 4.36 (s, 2H), 3.30 (q, *J* = 6.6 Hz, 2H), 2.33 (s, 3H), 2.25 (s, 3H), 1.02 (t, *J* = 6.6 Hz, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  166.16, 149.67, 145.03, 137.85, 136.78, 129.60, 128.85, 127.82, 127.44, 46.61, 42.62, 19.97, 14.11, 13.61;  $\beta$  rotamer:  $\delta$  165.74, 149.67, 145.03, 137.43, 136.88, 129.46, 128.78, 128.39, 127.91, 51.23, 39.07, 19.91, 13.61, 12.62. **HRMS** (ESI, positive mode): *m/z* 324.1453 [M+Na]<sup>+</sup>, calcd for [C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 324.1319.

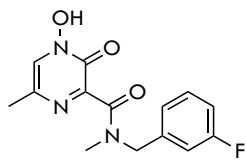
***N*-(3-Fluorobenzyl)-1-hydroxy-*N*-methylpyrazin-2(1*H*)-one-3-carboxamide (3.26f)**



Compound **3.26f** was prepared by debenzylation of **3.13f** (36.7 mg, 0.1 mmol) in flow via procedure H. Yield 93% (25.8 mg). **<sup>1</sup>H NMR** (600 MHz, DMSO-*d*<sub>6</sub>): ( $\alpha : \beta = 100 : 72$ )  $\alpha$  rotamer:  $\delta$  8.13 (d, *J* = 4.0 Hz, 1H),

7.43-7.36 (m, 2H), 7.22-7.10 (m, 3H), 4.71 (s, 2H), 2.80 (s, 3H);  $\beta$  rotamer:  $\delta$  8.07 (d,  $J = 4.0$  Hz, 1H), 7.43-7.36 (m, 2H), 7.22-7.10 (m, 3H), 4.41 (s, 2H), 2.84 (s, 3H).  **$^{13}\text{C}$  NMR** (150 MHz, DMSO- $d_6$ ):  $\alpha$  rotamer:  $\delta$  165.98, 162.92 (d,  $J_{\text{CF}} = 243.6$  Hz), 151.37, 150.67, 140.15 (d,  $J_{\text{CF}} = 6.7$  Hz), 130.98 (d,  $J_{\text{CF}} = 8.7$  Hz), 130.20, 123.76 (d,  $J_{\text{CF}} = 2.1$  Hz), 123.05, 114.39 (d,  $J_{\text{CF}} = 20.9$  Hz), 114.27 (d,  $J_{\text{CF}} = 21.9$  Hz), 49.15, 35.42;  $\beta$  rotamer:  $\delta$  165.85, 162.73 (d,  $J_{\text{CF}} = 243.6$  Hz), 151.37, 150.94, 140.00 (d,  $J_{\text{CF}} = 6.7$  Hz), 130.92 (d,  $J_{\text{CF}} = 8.7$  Hz), 130.20, 124.30 (d,  $J_{\text{CF}} = 2.1$  Hz), 122.79, 114.90 (d,  $J_{\text{CF}} = 21.9$  Hz), 114.87 (d,  $J_{\text{CF}} = 20.9$  Hz), 52.96, 32.01. **HRMS** (ESI, positive mode):  $m/z$  300.0712 [M+Na] $^+$ , calcd for [C<sub>13</sub>H<sub>12</sub>FN<sub>3</sub>O<sub>3</sub>Na] $^+$ : 300.0755.

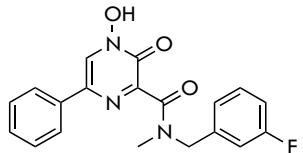
**N-(3-Fluorobenzyl)-1-hydroxy-N,N-dimethylpyrazin-2(1*H*)-one-3-carboxamide (3.26g)**



Compound **3.26g** was prepared by debenzylation of **3.13g** (38.1 mg, 0.1 mmol) in flow via procedure H. Yield 96% (27.9 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO- $d_6$ ):  $\alpha$  rotamer:  $\delta$  7.98 (s, 1H), 7.45-7.36 (m, 1H), 7.25-7.09 (m, 3H), 4.70 (s, 2H), 2.80 (s, 3H), 2.24 (s, 3H);  $\beta$  rotamer:  $\delta$  7.93 (s, 1H), 7.45-7.36 (m, 1H), 7.25-7.09 (m, 3H), 4.40 (s, 2H), 2.83 (s, 3H), 2.19 (s, 3H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO- $d_6$ ):  $\alpha$  rotamer:  $\delta$  166.06, 162.92 (d,  $J_{\text{CF}} = 243.4$  Hz), 150.10, 149.49, 140.18 (d,  $J_{\text{CF}} = 7.2$  Hz), 131.46, 130.94 (d,  $J_{\text{CF}} = 8.5$  Hz), 127.35, 123.82 (d,  $J_{\text{CF}} = 2.5$  Hz), 114.36 (d,  $J_{\text{CF}} = 20.8$  Hz), 114.35 (d,  $J_{\text{CF}} = 21.8$  Hz), 49.20, 35.38, 19.30;  $\beta$  rotamer:  $\delta$  165.93, 162.73 (d,  $J_{\text{CF}} = 243.4$  Hz), 150.10, 149.74, 140.03 (d,  $J_{\text{CF}} = 7.2$  Hz), 131.08, 130.83 (d,  $J_{\text{CF}} = 8.5$  Hz), 127.35, 124.41 (d,  $J_{\text{CF}} = 2.5$  Hz), 115.01 (d,  $J_{\text{CF}} = 21.8$  Hz), 114.84 (d,  $J_{\text{CF}} = 20.8$  Hz), 53.03, 32.02, 19.23. **LRMS** (API-ES, positive mode):  $m/z$  292.2 [M+H] $^+$ .

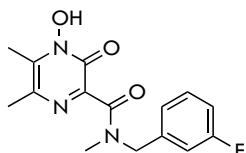
**N-(3-Fluorobenzyl)-1-hydroxy-N-methyl-5-phenylpyrazin-2(1*H*)-one-3-carboxamide (3.26h)**

Compound **3.26h** was prepared by debenzylation of **3.13h** (44.4 mg, 0.1 mmol) in flow via procedure H. Yield 73% (25.8 mg).



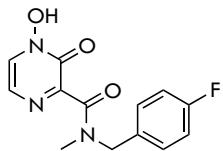
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 89)  $\alpha$  rotamer:  $\delta$  8.76 (s, 1H), 7.89 (d, *J* = 7.8 Hz, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.47-7.11 (m, 7H), 4.76 (s, 2H), 2.88 (s, 3H);  $\beta$  rotamer:  $\delta$  8.71 (s, 1H), 7.89 (d, *J* = 7.8 Hz, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.47-7.11 (m, 7H), 4.51 (s, 2H), 2.91 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  165.96, 162.97 (d, *J*<sub>CF</sub> = 243.3 Hz), 150.09, 149.74, 140.14 (d, *J*<sub>CF</sub> = 7.1 Hz), 135.20, 131.99, 130.98 (d, *J*<sub>CF</sub> = 8.2 Hz), 129.24, 128.44, 126.64, 125.38, 124.18 (d, *J*<sub>CF</sub> = 2.8 Hz), 114.79 (d, *J*<sub>CF</sub> = 21.9 Hz), 114.39 (d, *J*<sub>CF</sub> = 20.9 Hz), 49.28, 35.55;  $\beta$  rotamer:  $\delta$  165.88, 162.79 (d, *J*<sub>CF</sub> = 243.3 Hz), 150.09, 149.97, 140.14 (d, *J*<sub>CF</sub> = 7.1 Hz), 135.20, 131.51, 130.84 (d, *J*<sub>CF</sub> = 8.2 Hz), 129.14, 128.33, 126.64, 125.22, 123.79 (d, *J*<sub>CF</sub> = 2.8 Hz), 114.76 (d, *J*<sub>CF</sub> = 20.9 Hz), 114.28 (d, *J*<sub>CF</sub> = 21.9 Hz), 53.04, 32.27. **LRMS** (API-ES, positive mode): *m/z* 354.2 [M+H]<sup>+</sup>.

**N-(3-Fluorobenzyl)-1-hydroxy-N,5,6-trimethylpyrazin-2(1H)-one-3-carboxamide (3.26i)**



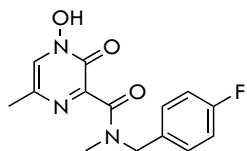
Compound **3.26i** was prepared by debenzylation of **3.13i** (39.5 mg, 0.1 mmol) in flow via procedure H. Yield 81% (24.7 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 68)  $\alpha$  rotamer:  $\delta$  7.44-7.09 (m, 4H), 4.70 (s, 2H), 2.80 (s, 3H), 2.37 (s, 3H), 2.30 (s, 3H);  $\beta$  rotamer:  $\delta$  7.44-7.09 (m, 4H), 4.40 (s, 2H), 2.83 (s, 3H), 2.33 (s, 3H), 2.25 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  166.24, 162.94 (d, *J*<sub>CF</sub> = 243.8 Hz), 149.50, 144.74, 140.31 (d, *J*<sub>CF</sub> = 7.2 Hz), 137.04, 130.91 (d, *J*<sub>CF</sub> = 8.4 Hz), 129.87, 123.79 (d, *J*<sub>CF</sub> = 2.4 Hz), 114.31 (d, *J*<sub>CF</sub> = 21.1 Hz), 114.30 (d, *J*<sub>CF</sub> = 21.7 Hz), 49.25, 35.47, 19.96, 13.63;  $\beta$  rotamer:  $\delta$  166.14, 162.74 (d, *J*<sub>CF</sub> = 243.8 Hz), 149.75, 144.42, 140.16 (d, *J*<sub>CF</sub> = 7.2 Hz), 137.04, 130.81 (d, *J*<sub>CF</sub> = 8.4 Hz), 129.49, 124.38 (d, *J*<sub>CF</sub> = 2.4 Hz), 114.97 (d, *J*<sub>CF</sub> = 21.7 Hz), 114.79 (d, *J*<sub>CF</sub> = 21.1 Hz), 53.13, 32.02, 19.88, 13.61. **LRMS** (API-ES, positive mode): *m/z* 306.2 [M+H]<sup>+</sup>.

**N-(4-Fluorobenzyl)-1-hydroxy-N-methylpyrazin-2(1*H*)-one-3-carboxamide (3.26j)**



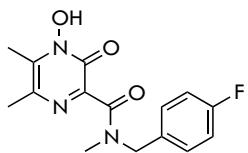
Compound **3.26j** was prepared by debenzylation of **3.13j** (36.7 mg, 0.1 mmol) in flow via procedure H. Yield 94% (26.1 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 78)  $\alpha$  rotamer:  $\delta$  8.13 (d, *J* = 4.2 Hz, 1H), 7.43-7.17 (m, 5H), 4.67 (s, 2H), 2.77 (s, 3H);  $\beta$  rotamer:  $\delta$  8.09 (d, *J* = 4.2 Hz, 1H), 7.43-7.17 (m, 5H), 4.38 (s, 2H), 2.81 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  165.88, 161.89 (d, *J*<sub>CF</sub> = 243.0 Hz), 151.38, 150.69, 133.28 (d, *J*<sub>CF</sub> = 2.3 Hz), 130.30 (d, *J*<sub>CF</sub> = 8.4 Hz), 130.22, 129.83 (d, *J*<sub>CF</sub> = 7.9 Hz), 122.96, 115.78 (d, *J*<sub>CF</sub> = 21.2 Hz), 48.84, 35.18;  $\beta$  rotamer:  $\delta$  165.75, 162.06 (d, *J*<sub>CF</sub> = 243.0 Hz), 151.38, 150.99, 133.07 (d, *J*<sub>CF</sub> = 2.3 Hz), 130.30 (d, *J*<sub>CF</sub> = 8.4 Hz), 130.22, 129.83 (d, *J*<sub>CF</sub> = 7.9 Hz), 122.76, 114.73 (d, *J*<sub>CF</sub> = 21.2 Hz), 52.68, 31.79. **HRMS** (ESI, positive mode): *m/z* 300.0703 [M+Na]<sup>+</sup>, calcd for [C<sub>13</sub>H<sub>12</sub>FN<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 300.0755.

**N-(4-Fluorobenzyl)-1-hydroxy-N,N-dimethylpyrazin-2(1*H*)-one-3-carboxamide (3.26k)**



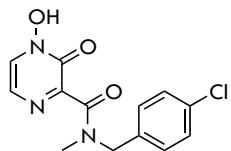
Compound **3.26k** was prepared by debenzylation of **3.13k** (38.1 mg, 0.1 mmol) in flow via procedure H. Yield 89% (25.9 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 76)  $\alpha$  rotamer:  $\delta$  7.97 (s, 1H), 7.44-7.15 (m, 4H), 4.66 (s, 2H), 2.77 (s, 3H), 2.23 (s, 3H);  $\beta$  rotamer:  $\delta$  7.93 (s, 1H), 7.44-7.15 (m, 4H), 4.36 (s, 2H), 2.81 (s, 3H), 2.20 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  165.90, 161.90 (d, *J*<sub>CF</sub> = 242.8 Hz), 150.34, 149.38, 133.31 (d, *J*<sub>CF</sub> = 2.9 Hz), 131.35, 129.92 (d, *J*<sub>CF</sub> = 8.2 Hz), 127.26, 115.75 (d, *J*<sub>CF</sub> = 21.3 Hz), 48.89, 35.13, 19.27;  $\beta$  rotamer:  $\delta$  165.78, 162.09 (d, *J*<sub>CF</sub> = 242.8 Hz), 150.15, 149.66, 133.10 (d, *J*<sub>CF</sub> = 2.9 Hz), 131.05, 130.39 (d, *J*<sub>CF</sub> = 8.2 Hz), 127.26, 115.66 (d, *J*<sub>CF</sub> = 21.3 Hz), 52.75, 31.80, 19.24. **HRMS** (ESI, positive mode): *m/z* 314.0847 [M+Na]<sup>+</sup>, calcd for [C<sub>14</sub>H<sub>14</sub>FN<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 314.0911.

**N-(4-Fluorobenzyl)-1-hydroxy-N,5,6-trimethylpyrazin-2(1H)-one-3-carboxamide (3.26l)**



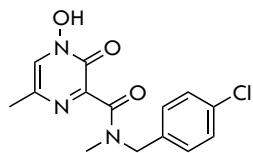
Compound **3.26l** was prepared by debenzylation of **3.13l** (39.5 mg, 0.1 mmol) in flow via procedure H. Yield 90% (27.5 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): ( $\alpha : \beta = 100 : 73$ )  $\alpha$  rotamer:  $\delta$  7.45-7.14 (m, 4H), 4.65 (s, 2H), 2.77 (s, 3H), 2.36 (s, 3H), 2.29 (s, 3H);  $\beta$  rotamer:  $\delta$  7.45-7.14 (m, 4H), 4.36 (s, 2H), 2.80 (s, 3H), 2.33 (s, 3H), 2.26 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  166.11, 161.88 (d, *J*<sub>CF</sub> = 242.7 Hz), 149.46, 144.61, 136.97, 133.44 (d, *J*<sub>CF</sub> = 2.9 Hz), 129.88 (d, *J*<sub>CF</sub> = 8.2 Hz), 129.81, 115.72 (d, *J*<sub>CF</sub> = 21.3 Hz), 48.94, 35.23, 19.95, 13.61;  $\beta$  rotamer:  $\delta$  166.02, 162.06 (d, *J*<sub>CF</sub> = 243.0 Hz), 149.75, 144.87, 136.97, 133.23 (d, *J*<sub>CF</sub> = 2.9 Hz), 130.36 (d, *J*<sub>CF</sub> = 8.2 Hz), 129.49, 115.62 (d, *J*<sub>CF</sub> = 21.3 Hz), 52.83, 31.80, 19.90, 13.61. **HRMS** (ESI, positive mode): *m/z* 328.0994 [M+Na]<sup>+</sup>, calcd for [C<sub>15</sub>H<sub>16</sub>FN<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 328.1068.

**N-(4-Chlorobenzyl)-1-hydroxy-N-methylpyrazin-2(1H)-one-3-carboxamide (3.26m)**



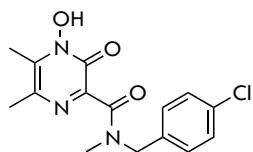
Compound **3.26m** was prepared by debenzylation of **3.13m** (38.4 mg, 0.1 mmol) in flow via procedure H. Yield 98% (28.8 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): ( $\alpha : \beta = 100 : 73$ )  $\alpha$  rotamer:  $\delta$  8.10 (d, *J* = 4.2 Hz, 1H), 7.45-7.32 (m, 5H), 4.67 (s, 2H), 2.77 (s, 3H);  $\beta$  rotamer:  $\delta$  8.05 (d, *J* = 4.2 Hz, 1H), 7.45-7.32 (m, 5H), 4.38 (s, 2H), 2.82 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  165.95, 151.25, 150.72, 136.23, 132.27, 139.24, 129.70, 128.96, 122.94, 48.99, 35.28;  $\beta$  rotamer:  $\delta$  165.85, 151.25, 150.72, 136.01, 132.63, 130.24, 130.08, 128.90, 122.72, 52.76, 31.94. **LRMS** (API-ES, positive mode): *m/z* 294.1 [M+H]<sup>+</sup>.

**N-(4-Chlorobenzyl)-1-hydroxy-N,5-dimethylpyrazin-2(1*H*)-one-3-carboxamide (3.26n)**



Compound **3.26n** was prepared by debenzylation of **3.13n** (39.8 mg, 0.1 mmol) in flow via procedure H. Yield 89% (27.4 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 67)  $\alpha$  rotamer:  $\delta$  7.97 (s, 1H), 7.45-7.34 (m, 4H), 4.66 (s, 2H), 2.77 (s, 3H), 2.23 (s, 3H);  $\beta$  rotamer:  $\delta$  7.92 (s, 1H), 7.45-7.34 (m, 4H), 4.38 (s, 2H), 2.82 (s, 3H), 2.19 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  165.95, 150.28, 149.38, 136.24, 132.28, 131.37, 129.89, 128.95, 127.28, 49.01, 35.24, 19.28;  $\beta$  rotamer:  $\delta$  165.86, 150.06, 149.63, 136.04, 132.63, 131.05, 130.18, 128.85, 127.28, 52.82, 31.96, 19.23. **LRMS** (API-ES, positive mode): *m/z* 308.1 [M+H]<sup>+</sup>.

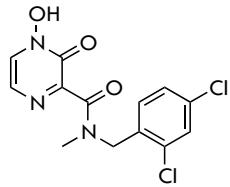
**N-(4-Chlorobenzyl)-1-hydroxy-N,5,6-trimethylpyrazin-2(1*H*)-one-3-carboxamide (3.26o)**



Compound **3.26o** was prepared by debenzylation of **3.13o** (41.2 mg, 0.1 mmol) in flow via procedure H. Yield 91% (29.3 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 82)  $\alpha$  rotamer:  $\delta$  7.42-7.34 (m, 4H), 4.65 (s, 2H), 2.77 (s, 3H), 2.35 (s, 3H), 2.28 (s, 3H);  $\beta$  rotamer:  $\delta$  7.42-7.34 (m, 4H), 4.36 (s, 2H), 2.80 (s, 3H), 2.32 (s, 3H), 2.24 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\alpha$  rotamer:  $\delta$  166.34, 151.03, 36.86, 136.87, 131.62, 129.20, 128.35, 48.48, 31.36, 19.49, 13.09;  $\beta$  rotamer:  $\delta$  166.15, 149.63, 138.86, 135.67, 131.98, 129.62, 128.25, 52.35, 34.76, 19.44, 13.09. **HRMS** (ESI, positive mode): *m/z* 344.1 [M+Na]<sup>+</sup>, calcd for [C<sub>15</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 344.1.

**N-(2,4-Dichlorobenzyl)-1-hydroxy-N-methylpyrazin-2(1*H*)-one-3-carboxamide (3.26p)**

Compound **3.26p** was prepared by debenzylation of **3.13p** (41.8 mg, 0.1 mmol) in flow via procedure H. Yield 95% (31.2 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): ( $\alpha$  :  $\beta$  = 100 : 44)  $\alpha$  rotamer:  $\delta$  8.09 (d, *J* = 4.2 Hz,



1H), 7.66 (brs, 1H), 7.55 (brd,  $J = 8.5$  Hz, 1H), 7.46 (brd,  $J = 8.5$  Hz, 1H), 7.38 (d,  $J = 4.2$  Hz, 1H), 4.70 (s, 2H), 2.86 (s, 3H);  $\beta$  rotamer:  $\delta$  8.01 (d,  $J = 4.2$  Hz, 1H), 7.58 (brs, 1H), 7.55 (brd,  $J = 8.5$  Hz, 1H), 7.46 (brd,  $J = 8.5$  Hz, 1H), 7.26 (d,  $J = 4.2$  Hz, 2H), 4.51 (s, 2H), 2.91 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO- $d_6$ ):  $\alpha$  rotamer:  $\delta$  166.50, 151.94, 150.00, 133.55, 132.94, 130.86, 130.72, 129.79, 129.36, 127.98, 123.30, 47.59, 35.90;  $\beta$  rotamer:  $\delta$  166.50, 151.94, 150.00, 133.64, 133.22, 130.72, 130.64, 129.79, 129.22, 128.07, 122.85, 50.47, 32.71. **HRMS** (ESI, positive mode):  $m/z$  350.00007 [M+Na]<sup>+</sup>, calcd for [C<sub>13</sub>H<sub>11</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 350.0070.

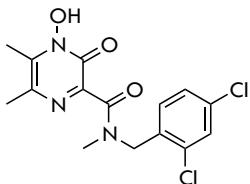
**N-(2,4-Dichlorobenzyl)-1-hydroxy-N,5-dimethylpyrazin-2(1H)-one-3-carboxamide (3.26q)**



Compound **3.26q** was prepared by debenzylation of **3.13q** (43.2 mg, 0.1 mmol) in flow via procedure H. Yield 92% (31.5 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO- $d_6$ ): ( $\alpha$  :  $\beta$  = 100 : 42)  $\alpha$  rotamer:  $\delta$  7.99 (s, 1H), 7.66 (d,  $J = 1.9$  Hz, 1H), 7.59 (d,  $J = 8.4$  Hz, 1H), 7.45 (dd,  $J_1 = 8.4$  Hz,  $J_2 = 1.9$  Hz, 1H), 4.70 (s, 2H), 2.87 (s, 3H), 2.24 (s, 3H);  $\beta$  rotamer:  $\delta$  7.90 (s, 1H), 7.57 (d,  $J = 1.9$  Hz, 1H), 7.59 (d,  $J = 8.4$  Hz, 1H), 7.45 (dd,  $J_1 = 8.4$  Hz,  $J_2 = 1.9$  Hz, 1H), 4.51 (s, 2H), 2.92 (s, 3H), 2.12 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO- $d_6$ ):  $\alpha$  rotamer:  $\delta$  166.33, 149.83, 149.36, 133.67, 133.52, 132.95, 131.07, 129.85, 129.35, 127.97, 127.73, 47.62, 35.87, 19.35;  $\beta$  rotamer:  $\delta$  166.33, 149.83, 149.36, 133.67, 133.64, 133.20, 131.68, 129.85, 129.14, 127.73, 127.69, 50.46, 32.85, 19.22. **HRMS** (ESI, positive mode):  $m/z$  364.0165 [M+Na]<sup>+</sup>, calcd for [C<sub>14</sub>H<sub>13</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 364.0226.

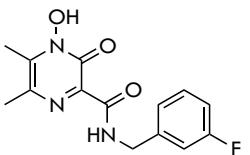
**N-(2,4-Dichlorobenzyl)-1-hydroxy-N,5,6-trimethylpyrazin-2(1H)-one-3-carboxamide (3.26r)**

Compound **3.26r** was prepared by debenzylation of **3.13r** (44.6 mg, 0.1 mmol) in flow via procedure H. Yield 80% (28.5 mg).



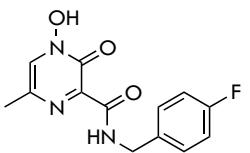
**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): (α : β = 100 : 48) α rotamer: δ 7.66 (d, *J* = 1.9 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.46-7.44 (m, 1H), 4.70 (s, 2H), 2.88 (s, 3H), 2.38 (s, 3H), 2.30 (s, 3H); β rotamer: δ 7.57 (d, *J* = 1.9 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.46-7.44 (m, 1H), 4.51 (s, 2H), 2.91 (s, 3H), 2.31 (s, 3H), 2.18 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>): α rotamer: δ 166.40, 149.62, 144.42, 137.29, 133.64, 132.92, 131.02, 129.78, 129.36, 127.93, 47.67, 35.98, 20.01, 13.66; β rotamer: δ 166.48, 149.72, 144.42, 137.29, 133.76, 133.49, 133.16, 131.02, 130.04, 129.14, 127.93, 50.59, 32.82, 19.84, 13.62. **HRMS** (ESI, positive mode): *m/z* 378.0320 [M+Na]<sup>+</sup>, calcd for [C<sub>15</sub>H<sub>15</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>Na]<sup>+</sup>: 378.0383.

**N-(3-Fluorobenzyl)-1-hydroxy-5,6-dimethylpyrazin-2(1H)-one-3-carboxamide (3.26s)**



Compound 3.26s was prepared by debenzylation of 3.13s (38.1 mg, 0.1 mmol) in flow via procedure H. Yield 91% (26.4 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 9.83 (s, 1H), 7.40-7.35 (m, 1H), 7.18-7.06 (m, 3H), 4.56 (s, 2H), 2.39 (s, 6H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>): δ 163.12, 162.72 (*d*, *J*<sub>CF</sub> = 243.3 Hz), 153.65, 153.41, 142.73 (*d*, *J*<sub>CF</sub> = 7.3 Hz), 140.91, 131.35, 130.77 (*d*, *J*<sub>CF</sub> = 8.1 Hz), 123.68, 114.35 (*d*, *J*<sub>CF</sub> = 21.5 Hz), 114.08 (*d*, *J*<sub>CF</sub> = 21.0 Hz), 42.34, 19.64, 14.10. **LRMS** (API-ES, positive mode): *m/z* 292.1 [M+H]<sup>+</sup>.

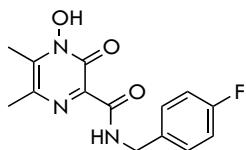
**N-(4-Fluorobenzyl)-1-hydroxy-5-methylpyrazin-2(1H)-one-3-carboxamide (3.26t)**



Compound 3.26t was prepared by debenzylation of 3.13t (36.7 mg, 0.1 mmol) in flow via procedure H. Yield 93% (25.7 mg). **<sup>1</sup>H NMR** (400 MHz, CD<sub>3</sub>OD): δ 8.16 (brs, 1H), 7.44-7.41 (m, 2H), 7.08 (t, *J* = 8.5 Hz, 2H), 4.64 (s, 2H), 2.44 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, CD<sub>3</sub>OD): δ 163.01, 161.16 (*d*, *J*<sub>CF</sub> = 243.8 Hz), 152.76, 152.71, 136.77, 134.24

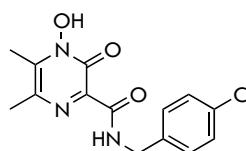
(d,  $J_{\text{CF}} = 3.1$  Hz), 133.31, 129.19 (d,  $J_{\text{CF}} = 8.2$  Hz), 114.84 (d,  $J_{\text{CF}} = 21.7$  Hz), 42.23, 17.78. **LRMS** (API-ES, positive mode):  $m/z$  278.2 [M+H]<sup>+</sup>.

**N-(4-Fluorobenzyl)-1-hydroxy-5,6-dimethylpyrazin-2(1*H*)-one-3-carboxamide (3.26u)**



Compound **3.26u** was prepared by debenzylation of **3.13u** (38.1 mg, 0.1 mmol) in flow via procedure H. Yield 92% (26.8 mg). **<sup>1</sup>H NMR** (400 MHz, CD<sub>3</sub>OD): ( $\alpha$  :  $\beta$  = 100 : 78)  $\alpha$  rotamer:  $\delta$  7.41 (dd,  $J_1 = 8.3$  Hz,  $J_2 = 5.5$  Hz, 2H), 7.07 (t,  $J = 8.3$  Hz, 2H), 4.64 (s, 2H), 2.47 (s, 3H), 2.26 (s, 3H);  $\beta$  rotamer:  $\delta$  7.29 (dd,  $J_1 = 8.7$  Hz,  $J_2 = 5.5$  Hz, 2H), 7.12 (t,  $J = 8.7$  Hz, 2H), 5.52 (s, 2H), 2.54 (s, 3H), 2.50 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, CD<sub>3</sub>OD):  $\alpha$  rotamer:  $\delta$  163.91, 163.10, 162.12 (d,  $J_{\text{CF}} = 245.1$  Hz), 141.45, 134.45 (d,  $J_{\text{CF}} = 3.0$  Hz), 132.61, 128.59 (d,  $J_{\text{CF}} = 8.5$  Hz), 114.80 (d,  $J_{\text{CF}} = 21.5$  Hz), 42.10, 19.01, 15.45;  $\beta$  rotamer:  $\delta$  163.10, 162.35 (d,  $J_{\text{CF}} = 245.1$  Hz), 161.54, 155.68, 141.45, 132.61, 130.70 (d,  $J_{\text{CF}} = 3.1$  Hz), 129.14 (d,  $J_{\text{CF}} = 8.1$  Hz), 115.37 (d,  $J_{\text{CF}} = 21.9$  Hz), 47.38, 18.26, 12.60. **LRMS** (API-ES, positive mode):  $m/z$  292.2 [M+H]<sup>+</sup>.

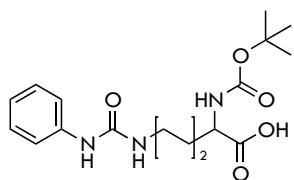
**N-(4-Chlorobenzyl)-1-hydroxy-5,6-dimethylpyrazin-2(1*H*)-one-3-carboxamide (3.26w)**



Compound **3.26w** was prepared by debenzylation of **3.13w** (39.8 mg, 0.1 mmol) in flow via procedure H. Yield 91% (28.0 mg). **<sup>1</sup>H NMR** (400 MHz, CD<sub>3</sub>OD):  $\delta$  7.40-7.34 (m, 4H), 4.65 (s, 2H), 2.55 (s, 3H), 2.51 (s, 3H). **<sup>13</sup>C NMR** (100 MHz, CD<sub>3</sub>OD):  $\delta$  163.84, 163.08, 151.86, 141.42, 137.28, 132.67, 132.54, 128.82, 128.24, 42.13, 18.22, 12.63. **LRMS** (API-ES, positive mode):  $m/z$  308.10 [M+H]<sup>+</sup>.

### 5.3.4 Synthesis and characterization of 3-[4-(3-arylureido)butyl]-1-hydroxypyrazin-2(1*H*)-ones and intermediates

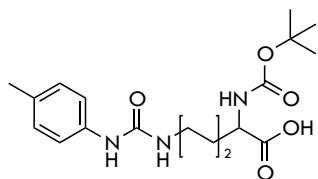
#### 2-*tert*-Butoxycarbonylamino-6-(3-phenylureido)hexanoic acid (3.21a)



Compound **3.21a** was prepared via procedure J using 1.0 g (4.06 mmol) of *N*-Boc lysine. Quantitative yield (1.48 g). **<sup>1</sup>H NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  12.40 (brs, 1H), 8.37 (s, 1H),

7.37 (d,  $J = 7.4$  Hz, 2H), 7.20 (t,  $J = 7.4$  Hz, 2H), 7.04 (d,  $J = 7.9$  Hz, 1H), 6.87 (t,  $J = 7.4$  Hz, 1H), 6.11 (t,  $J = 5.3$  Hz, 1H), 3.87-3.81 (m, 1H), 3.05 (dt,  $J_1 = 6.4$  Hz,  $J_2 = 5.3$  Hz, 2H), 1.71-1.52 (m, 2H), 1.37 (overlapped, 13H). **<sup>13</sup>C NMR** (100 MHz, DMSO- $d_6$ ):  $\delta$  174.67, 156.07, 155.64, 141.05, 129.05, 121.33, 118.03, 78.39, 53.94, 39.25, 30.99, 29.89, 28.67, 23.57. **LRMS** (API-ES, negative mode):  $m/z$  364.1 [M-H]<sup>-</sup>.

#### 2-*tert*-Butoxycarbonylamino-6-[3-(*p*-tolyl)ureido]hexanoic acid (3.21b)

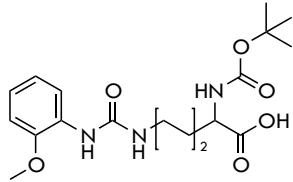


Compound **3.21b** was prepared via procedure J using 1.0 g (4.06 mmol) of *N*-Boc lysine. Yield 98% (1.51 g).

**<sup>1</sup>H NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  12.37 (brs, 1H), 8.23 (s, 1H), 7.25 (d,  $J = 8.1$  Hz, 2H), 7.02 (overlapped, 1H), 7.00 (d,  $J = 8.1$  Hz, 2H), 6.05 (t,  $J = 5.6$  Hz, 1H), 3.87-3.81 (m, 1H), 3.04 (dt,  $J_1 = 6.3$  Hz,  $J_2 = 5.6$  Hz, 2H), 2.20 (s, 3H), 1.71-1.52 (m, 2H), 1.37 (overlapped, 13H). **<sup>13</sup>C NMR** (100 MHz, DMSO- $d_6$ ):  $\delta$  174.68, 156.07, 155.74, 138.50, 130.03, 129.44, 118.17, 78.40, 53.95, 39.32, 31.02, 29.94, 28.43, 23.58, 20.74. **LRMS** (API-ES, negative mode):  $m/z$  378.2 [M-H]<sup>-</sup>.

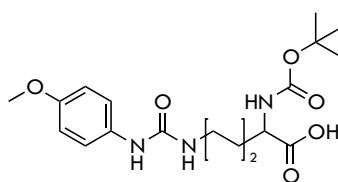
#### 2-*tert*-Butoxycarbonylamino-6-[3-(2-methoxyphenyl)ureido]-hexanoic acid (3.21c)

Compound **3.21c** was prepared via procedure J using 1.0 g (4.06 mmol) of *N*-Boc lysine. Quantitative yield (1.61 g). **<sup>1</sup>H NMR** (400 MHz, DMSO- $d_6$ ):  $\delta$  12.38 (brs, 1H), 8.08 (dd,  $J_1 = 7.5$  Hz,  $J_2 = 1.9$  Hz, 1H), 7.84 (s,



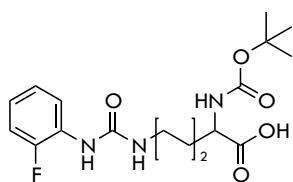
1H), 7.02 (d,  $J = 7.9$  Hz, 1H), 6.95 (dd,  $J_1 = 7.5$  Hz,  $J_2 = 1.9$  Hz, 1H), 6.87-6.80 (m, 3H), 3.86 (overlapped, 1H), 3.83 (s, 3H), 3.05 (dt,  $J_1 = 6.3$  Hz,  $J_2 = 5.8$  Hz, 2H), 1.71-1.52 (m, 2H), 1.38 (overlapped, 13H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  174.68, 156.07, 155.58, 147.66, 130.05, 121.23, 120.91, 118.30, 110.98, 78.40, 56.10, 53.95, 39.24, 31.00, 29.82, 28.67, 23.62. **LRMS** (API-ES, negative mode): *m/z* 394.1 [M-H]<sup>-</sup>.

**2-tert-Butoxycarbonylamino-6-[3-(4-methoxyphenyl)ureido]-hexanoic acid (3.21d)**



Compound **3.21d** was prepared via procedure J using 739 mg (3.0 mmol) of *N*-Boc lysine. Yield 84% (997 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  12.37 (brs, 1H), 8.16 (s, 1H), 7.27 (d,  $J = 8.8$  Hz, 2H), 7.02 (d,  $J = 7.9$  Hz, 1H), 6.79 (d,  $J = 8.8$  Hz, 2H), 6.00 (t,  $J = 5.6$  Hz, 1H), 3.87-3.82 (m, 1H), 3.68 (s, 3H), 3.04 (dt,  $J_1 = 6.3$  Hz,  $J_2 = 5.6$  Hz, 2H), 1.70-1.52 (m, 2H), 1.37 (overlapped, 13H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  174.68, 156.07, 155.90, 154.30, 134.21, 119.80, 114.31, 78.40, 55.59, 53.96, 39.37, 31.02, 29.97, 28.68, 23.58. **LRMS** (API-ES, negative mode): *m/z* 394.1 [M-H]<sup>-</sup>.

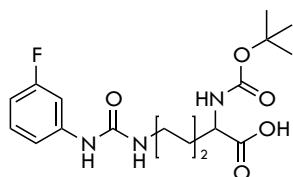
**2-tert-Butoxycarbonylamino-6-[3-(2-fluorophenyl)ureido]hexanoic acid (3.21e)**



Compound **3.21e** was prepared via procedure J using 1.0 g (4.06 mmol) of *N*-Boc lysine. Yield 97% (1.51 g).  **$^1\text{H}$  NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  12.39 (brs, 1H), 8.21 (d,  $J = 2.4$  Hz, 1H), 8.12 (dt,  $J_1 = 8.2$  Hz,  $J_2 = 1.3$  Hz, 1H), 7.15 (dt,  $J_1 = 11.7$  Hz,  $J_2 = 8.2$  Hz, dt,  $J_3 = 1.3$  Hz, 1H), 7.08-7.01 (m, 2H), 6.93-6.87 (m, 1H), 6.60 (t,  $J = 5.4$  Hz, 1H), 3.88-3.82 (m, 1H), 3.07 (dt,  $J_1 = 6.3$  Hz,  $J_2 = 5.4$  Hz, 2H), 1.71-1.53 (m, 2H), 1.38 (overlapped, 13H).

**<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  174.67, 156.08, 155.28, 151.99 (d,  $J_{CF}$  = 239.4 Hz), 128.88 (d,  $J_{CF}$  = 9.8 Hz), 124.78 (d,  $J_{CF}$  = 2.9 Hz), 121.79 (d,  $J_{CF}$  = 7.4 Hz), 120.52, 115.17 (d,  $J_{CF}$  = 19.1 Hz), 78.40, 53.93, 39.30, 31.00, 29.76, 28.67, 23.59. **LRMS** (API-ES, negative mode): *m/z* 382.1 [M-H]<sup>-</sup>.

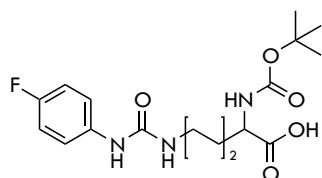
**2-tert-Butoxycarbonylamino-6-[3-(3-fluorophenyl)ureido]hexanoic acid (3.21f)**



Compound **3.21f** was prepared via procedure J using 1.0 g (4.06 mmol) of *N*-Boc lysine. Yield 92% (1.44 g).

**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  12.37 (brs, 1H), 8.63 (s, 1H), 7.44 (dt,  $J_1$  = 12.4 Hz,  $J_2$  = 2.2 Hz, 1H), 7.22 (dt,  $J_1$  = 8.2 Hz,  $J_2$  = 7.2 Hz, 1H), 7.03-6.99 (m, 2H), 6.67 (ddt,  $J_1$  = 8.5 Hz,  $J_2$  = 2.5 Hz,  $J_3$  = 0.8 Hz, 1H), 6.20 (t,  $J$  = 5.8 Hz, 1H), 3.87-3.82 (m, 1H), 3.06 (dt,  $J_1$  = 6.4 Hz,  $J_2$  = 5.8 Hz, 2H), 1.71-1.50 (m, 2H), 1.37 (overlapped, 13H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  174.68, 162.89 (d,  $J_{CF}$  = 239.9 Hz), 156.07, 155.42, 142.98 (d,  $J_{CF}$  = 11.5 Hz), 130.51 (d,  $J_{CF}$  = 9.8 Hz), 113.70, 107.57 (d,  $J_{CF}$  = 21.3 Hz), 104.66 (d,  $J_{CF}$  = 26.6 Hz), 78.40, 53.94, 39.32, 31.00, 29.81, 28.67, 23.55. **LRMS** (API-ES, negative mode): *m/z* 382.1 [M-H]<sup>-</sup>.

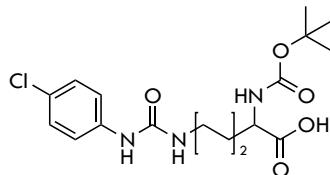
**2-tert-Butoxycarbonylamino-6-[3-(4-fluorophenyl)ureido]hexanoic acid (3.21g)**



Compound **3.21g** was prepared via procedure J using 1.0 g (4.06 mmol) of *N*-Boc lysine. Yield 82% (1.28 g).

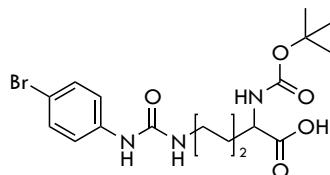
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  12.39 (brs, 1H), 8.40 (s, 1H), 7.39-7.36 (m, 2H), 7.06-7.00 (m, 3H), 6.10 (t,  $J$  = 5.7 Hz, 1H), 3.87-3.82 (m, 1H), 3.05 (dt,  $J_1$  = 6.4 Hz,  $J_2$  = 5.7 Hz, 2H), 1.71-1.53 (m, 2H), 1.37 (overlapped, 13H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  174.68, 157.26 (d,  $J_{CF}$  = 237.9 Hz), 156.07, 155.71, 137.42, 119.63 (d,  $J_{CF}$  = 7.5 Hz), 115.47 (d,  $J_{CF}$  = 22.0 Hz), 78.40, 53.95, 39.35, 31.01, 29.89, 28.67, 23.57. **LRMS** (API-ES, negative mode): *m/z* 382.1 [M-H]<sup>-</sup>.

**2-*tert*-Butoxycarbonylamino-6-[3-(4-chlorophenyl)ureido]hexanoic acid (3.21h)**



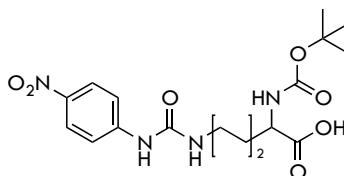
Compound **3.21h** was prepared via procedure J using 1.0 g (4.06 mmol) of *N*-Boc lysine. Yield 88% (1.43 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  12.40 (brs, 1H), 8.53 (s, 1H), 7.42 (d, *J* = 8.7 Hz, 2H), 7.23 (d, *J* = 8.7 Hz, 2H), 7.00 (d, *J* = 7.8 Hz, 1H), 6.17 (t, *J* = 5.5 Hz, 1H), 3.90-3.84 (m, 1H), 3.07 (dt, *J*<sub>1</sub> = 6.4 Hz, *J*<sub>2</sub> = 5.5 Hz, 2H), 1.72-1.53 (m, 2H), 1.38 (overlapped, 13H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  174.71, 156.07, 155.51, 140.04, 128.84, 124.84, 119.53, 78.40, 53.95, 39.34, 31.03, 29.84, 28.65, 23.55. **LRMS** (API-ES, negative mode): *m/z* 398.1 [M-H]<sup>-</sup>.

**6-[3-(4-Bromophenyl)ureido]-2-*tert*-butoxycarbonylaminohexanoic acid (3.21i)**



Compound **3.21i** was prepared via procedure J using 2.0 g (8.12 mmol) of *N*-Boc lysine. Yield 97% (3.50 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  12.38 (brs, 1H), 8.54 (s, 1H), 7.36 (s, 4H), 7.01 (d, *J* = 8.0 Hz, 1H), 6.17 (t, *J* = 5.7 Hz, 1H), 3.87-3.82 (m, 1H), 3.05 (dt, *J*<sub>1</sub> = 6.4 Hz, *J*<sub>2</sub> = 5.7 Hz, 2H), 1.71-1.52 (m, 2H), 1.37 (overlapped, 13H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  174.68, 156.07, 155.46, 140.50, 131.75, 119.96, 112.59, 78.40, 53.94, 39.39, 31.00, 29.83, 28.67, 23.56. **LRMS** (API-ES, negative mode): *m/z* 444.0 [M-H]<sup>-</sup>.

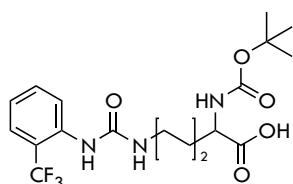
**2-*tert*-Butoxycarbonylamino-6-[3-(4-nitrophenyl)ureido]hexanoic acid (3.21j)**



Compound **3.21j** was prepared via procedure J using 1.0 g (4.06 mmol) of *N*-Boc lysine. Yield 91% (1.51 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  12.40 (brs, 1H), 9.22 (s, 1H), 8.13 (d, *J* = 9.3 Hz, 2H), 7.62 (d, *J* = 9.3 Hz, 2H), 7.02 (d, *J* =

8.0 Hz, 1H), 6.45 (t,  $J$  = 5.7 Hz, 1H), 3.89-3.83 (m, 1H), 3.10 (dt,  $J_1$  = 6.4 Hz,  $J_2$  = 5.7 Hz, 2H), 1.72-1.53 (m, 2H), 1.50-1.41 (m, 2H), 1.38 (overlapped, 11H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  174.68, 156.07, 154.85, 147.74, 140.78, 125.56, 117.22, 78.40, 53.93, 39.49, 30.99, 29.64, 28.66, 23.53. **LRMS** (API-ES, negative mode): *m/z* 409.1 [M-H]<sup>-</sup>.

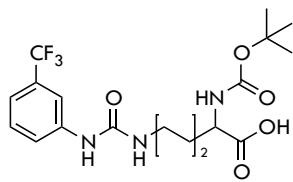
**2-tert-Butoxycarbonylamino-6-[3-(2-trifluoromethylphenyl)ureido]-hexanoic acid (3.21k)**



Compound **3.21k** was prepared via procedure J using 1.0 g (4.06 mmol) of *N*-Boc lysine. Yield 98% (1.72 g).

**$^1\text{H}$  NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  12.41 (brs, 1H), 7.97 (d,  $J$  = 8.3 Hz, 1H), 7.70 (s, 1H), 7.60 (d,  $J$  = 8.0 Hz, 1H), 7.55 (t,  $J$  = 8.0 Hz, 1H) 7.16 (t,  $J$  = 7.7 Hz, 1H), 7.02 (d,  $J$  = 7.7 Hz, 1H), 7.00 (t,  $J$  = 5.7 Hz, 1H), 3.89-3.83 (m, 1H), 3.08 (dt,  $J_1$  = 6.4 Hz,  $J_2$  = 5.7 Hz, 2H), 1.72-1.53 (m, 2H), 1.38 (overlapped, 13H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  174.68, 156.07, 155.33, 137.91, 133.15, 126.16 (q,  $J_{\text{CF}}$  = 5.5 Hz), 124.96, 124.58 (q,  $J_{\text{CF}}$  = 272.5 Hz), 122.88, 118.92 (q,  $J_{\text{CF}}$  = 28.7 Hz), 78.39, 53.91, 39.49, 31.00, 29.65, 28.65, 23.63. **LRMS** (API-ES, negative mode): *m/z* 432.1 [M-H]<sup>-</sup>.

**2-tert-Butoxycarbonylamino-6-[3-(3-trifluoromethylphenyl)ureido]-hexanoic acid (3.21l)**

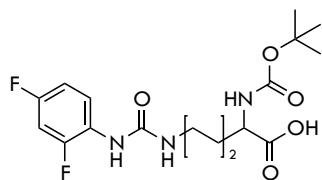


Compound **3.21l** was prepared via procedure J using 1.0 g (4.06 mmol) of *N*-Boc lysine. Yield 87% (1.53 g).

**$^1\text{H}$  NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  12.38 (brs, 1H), 8.78 (s, 1H), 7.96 (s, 1H), 7.49 (brd,  $J$  = 7.8 Hz, 1H), 7.43 (t,  $J$  = 7.8 Hz, 1H), 7.20 (brd,  $J$  = 7.8 Hz, 1H), 7.01 (d,  $J$  = 8.0 Hz, 1H), 6.27 (t,  $J$  = 5.7 Hz, 1H), 3.88-3.82 (m, 1H), 3.07 (dt,  $J_1$  = 6.4 Hz,  $J_2$  = 5.7 Hz, 2H), 1.72-1.53 (m, 2H), 1.38 (overlapped, 13H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  174.68, 156.07, 155.48, 141.92, 130.13, 129.86 (q,  $J_{\text{CF}}$  = 31.8 Hz), 124.75 (q,  $J_{\text{CF}}$  = 272.7 Hz), 121.53, 117.53 (q,  $J_{\text{CF}}$  = 3.7 Hz), 113.96 (q,  $J_{\text{CF}}$  = 3.9 Hz), 78.39, 53.94, 39.39, 31.02, 29.79, 28.66, 23.56. **LRMS** (API-ES,

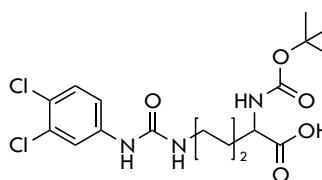
negative mode):  $m/z$  432.1 [M-H] $^-$ .

**2-*tert*-Butoxycarbonylamino-6-[3-(2,4-difluorophenyl)ureido]-hexanoic acid (3.21m)**



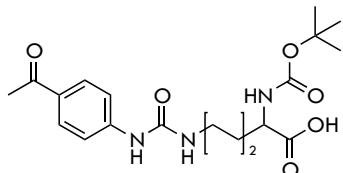
Compound **3.21m** was prepared via procedure J using 500 mg (2.03 mmol) of *N*-Boc lysine. Quantitative yield (815 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  12.38 (brs, 1H), 8.18 (brs, 1H), 8.10-8.03 (m, 1H), 7.24-7.18 (m, 1H), 7.02 (d,  $J$  = 8.0 Hz, 1H), 6.96 (t,  $J$  = 8.7 Hz, 1H), 6.54 (t,  $J$  = 5.7 Hz, 1H), 3.88-3.82 (m, 1H), 3.07 (dt,  $J_1$  = 6.4 Hz,  $J_2$  = 5.7 Hz, 2H), 1.71-1.53 (m, 2H), 1.38 (overlapped, 13H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  174.67, 157.74 (d,  $J_{CF}$  = 11.4 Hz), 156.07, 155.37, 151.97 (dd,  $J_{CF}$  = 243.7 Hz,  $J_{CF}$  = 12.5 Hz), 125.45 (dd,  $J_{CF}$  = 10.6 Hz,  $J_{CF}$  = 3.1 Hz), 121.68 (dd,  $J_{CF}$  = 8.6 Hz,  $J_{CF}$  = 2.1 Hz), 111.21 (dd,  $J_{CF}$  = 21.6 Hz,  $J_{CF}$  = 2.9 Hz), 103.93 (dd,  $J_{CF}$  = 26.6 Hz,  $J_{CF}$  = 24.1 Hz), 78.40, 53.93, 39.34, 30.99, 29.76, 28.67, 23.57. **LRMS** (API-ES, negative mode):  $m/z$  400.1 [M-H] $^-$ .

**2-*tert*-Butoxycarbonylamino-6-[3-(3,4-dichlorophenyl)ureido]-hexanoic acid (3.21n)**



Compound **3.21n** was prepared via procedure J using 1.0 g (4.06 mmol) of *N*-Boc lysine. Yield 86% (1.52 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  12.41 (brs, 1H), 8.74 (s, 1H), 7.84 (d,  $J$  = 2.4 Hz, 1H), 7.43 (d,  $J$  = 8.9 Hz, 1H), 7.24 (dd,  $J_1$  = 8.9 Hz,  $J_2$  = 2.4 Hz, 1H), 7.02 (d,  $J$  = 8.0 Hz, 1H), 6.28 (t,  $J$  = 5.7 Hz, 1H), 3.87-3.82 (m, 1H), 3.06 (dt,  $J_1$  = 6.4 Hz,  $J_2$  = 5.7 Hz, 2H), 1.71-1.52 (m, 2H), 1.39 (overlapped, 13H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  174.68, 156.07, 155.28, 141.33, 131.33, 130.83, 122.50, 119.09, 118.12, 78.40, 53.93, 39.39, 30.99, 29.74, 28.67, 23.55. **LRMS** (API-ES, negative mode):  $m/z$  432.1 [M-H] $^-$ .

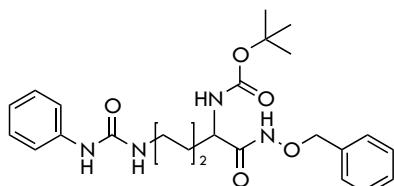
**6-[3-(4-Acetophenyl)ureido]-2-*tert*-butoxycarbonylaminohexanoic acid (3.21o)**



Compound **3.21o** was prepared via procedure J using 1.0 g (4.06 mmol) of N-Boc lysine. Yield 90% (1.49 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 12.41 (brs, 1H),

8.88 (s, 1H), 7.84 (d, *J* = 8.5 Hz, 2H), 7.51 (d, *J* = 8.5 Hz, 2H), 7.02 (d, *J* = 7.8 Hz, 1H), 6.33 (t, *J* = 5.7 Hz, 1H), 3.89-3.83 (m, 1H), 3.08 (dt, *J*<sub>1</sub> = 6.4 Hz, *J*<sub>2</sub> = 5.7 Hz, 2H), 2.49 (s, 3H), 1.72-1.54 (m, 2H), 1.38 (overlapped, 13H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 196.60, 174.69, 156.07, 155.17, 145.73, 130.13, 130.05, 116.95, 78.40, 53.94, 39.37, 31.00, 29.75, 28.67, 26.69, 23.56. **LRMS** (API-ES, negative mode): *m/z* 406.2 [M-H]<sup>-</sup>.

***tert*-Butyl [1-benzyloxyamino-1-oxo-6-(3-phenylureido)hexan-2-yl]carbamate (3.22a)**

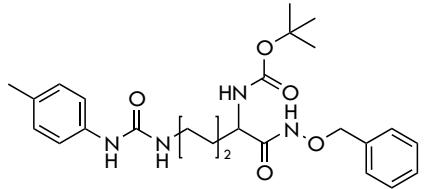


Compound **3.22a** was prepared via procedure B, using 1.47 g (4.02 mmol) of **3.21a**. Yield 79% (1.49 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 11.15 (s, 1H), 8.35 (s, 1H),

7.37-7.32 (m, 7H), 7.19 (dd, *J*<sub>1</sub> = 8.2 Hz, *J*<sub>2</sub> = 7.6 Hz, 2H), 6.88-6.85 (m, 2H), 6.08 (t, *J* = 5.6 Hz, 1H), 4.77 (s, 2H), 3.77-3.71 (m, 1H), 3.03 (dt, *J*<sub>1</sub> = 6.4 Hz, *J*<sub>2</sub> = 5.6 Hz, 2H), 1.54-1.48 (m, 2H), 1.40 (overlapped, 2H), 1.37 (s, 9H), 1.31-1.17 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 169.52, 155.65, 155.62, 141.05, 136.42, 129.27, 129.02, 128.67, 128.64, 121.34, 117.94, 78.47, 77.20, 52.53, 39.27, 32.02, 29.94, 28.63, 23.30. **LRMS** (API-ES, positive mode): *m/z* 471.2 [M+H]<sup>+</sup>.

***tert*-Butyl {1-benzyloxyamino-1-oxo-6-[3-(*p*-tolyl)ureido]hexan-2-yl}carbamate (3.22b)**

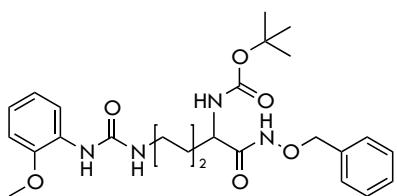
Compound **3.22b** was prepared via procedure B, using 1.50 g (3.95 mmol) of **3.21b**. Yield 80% (1.53 g).



**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  11.15 (s, 1H), 8.24 (s, 1H), 7.38-7.33 (m, 5H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.00 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 7.8 Hz, 1H), 6.03 (t, *J* = 5.7

Hz, 1H), 4.77 (s, 2H), 3.74 (dt, *J*<sub>1</sub> = 7.9 Hz, *J*<sub>2</sub> = 7.9 Hz, 1H), 3.02 (dt, *J*<sub>1</sub> = 6.4 Hz, *J*<sub>2</sub> = 5.7 Hz, 2H), 2.20 (s, 3H), 1.54-1.48 (m, 2H), 1.38 (s, 9H), 1.36 (overlapped, 2H), 1.30-1.16 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  169.53, 155.73, 155.70, 138.49, 136.41, 130.02, 129.44, 129.27, 128.70, 118.16, 78.47, 77.20, 52.53, 39.34, 32.02, 29.97, 28.66, 23.31, 20.74. **LRMS** (API-ES, positive mode): *m/z* 485.3 [M+H]<sup>+</sup>.

**tert-Butyl {1-benzylamino-6-[3-(2-methoxyphenyl)ureido]-1-oxohexan-2-yl}carbamate (3.22c)**

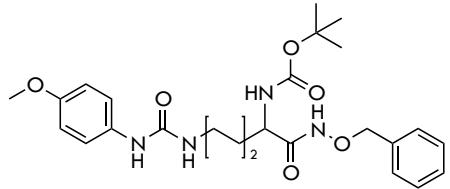


Compound **3.22c** was prepared via procedure B, using 1.60 g (4.04 mmol) of **3.21c**. Yield 92% (1.86 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  11.17 (s, 1H), 8.10 (d,

*J* = 7.3 Hz, 1H), 7.85 (s, 1H), 7.38-7.34 (m, 5H), 6.94 (d, *J* = 7.7 Hz, 1H), 6.90-6.80 (m, 4H), 4.78 (s, 2H), 3.82 (s, 3H), 3.78-3.73 (m, 1H), 3.04 (dt, *J*<sub>1</sub> = 6.4 Hz, *J*<sub>2</sub> = 5.6 Hz, 2H), 1.55-1.50 (m, 2H), 1.39 (s, 9H), 1.36 (overlapped, 2H), 1.31-1.23 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  169.54, 155.70, 155.60, 147.66, 136.41, 130.06, 129.27, 128.70, 121.23, 120.91, 118.31, 110.97, 78.48, 77.22, 56.09, 52.52, 39.27, 32.02, 29.85, 28.65, 23.33. **LRMS** (API-ES, positive mode): *m/z* 501.3 [M+H]<sup>+</sup>.

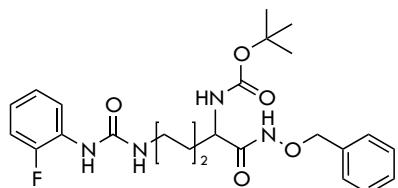
**tert-Butyl {1-benzylamino-6-[3-(4-methoxyphenyl)ureido]-1-oxohexan-2-yl}carbamate (3.22d)**

Compound **3.22d** was prepared via procedure B, using 0.99 g (2.50 mmol) of **3.21d**. Yield 97% (1.22 g). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  11.15 (s, 1H), 8.15 (s, 1H), 7.38-7.32 (m, 5H), 7.26 (d, *J* = 9.0 Hz, 2H), 6.87 (d, *J* = 7.7 Hz, 1H), 6.79 (d, *J* = 9.0 Hz, 2H), 5.97 (t, *J* = 5.9 Hz,



<sup>1</sup>H), 4.77 (s, 2H), 3.74 (dt, *J*<sub>1</sub> = 7.7 Hz, *J*<sub>2</sub> = 7.1 Hz, 1H), 3.68 (s, 3H), 3.02 (dt, *J*<sub>1</sub> = 6.4 Hz, *J*<sub>2</sub> = 5.9 Hz, 2H), 1.53-1.48 (m, 2H), 1.38 (s, 9H), 1.35 (overlapped, 2H), 1.29-1.18 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ 169.55, 155.90, 155.70, 154.30, 136.40, 134.20, 129.28, 128.86, 128.71, 119.80, 114.31, 78.48, 77.22, 55.60, 52.53, 39.38, 32.02, 30.01, 28.66, 23.30. LRMS (API-ES, positive mode): *m/z* 501.3 [M+H]<sup>+</sup>.

**tert-Butyl {1-benzyloxyamino-6-[3-(2-fluorophenyl)ureido]-1-oxohexan-2-yl}carbamate (3.22e)**

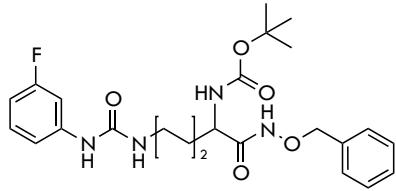


Compound **3.22e** was prepared via procedure B, using 1.50 g (3.91 mmol) of **3.21e**. Yield 99% (1.89 g). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ

11.17 (s, 1H), 8.22 (s, 1H), 8.14 (t, *J* = 8.2 Hz, 1H), 7.39-7.34 (m, 5H), 7.16 (dd, *J*<sub>1</sub> = 11.6 Hz, *J*<sub>2</sub> = 8.2 Hz, 1H), 7.06 (t, *J* = 7.9 Hz, 1H), 6.93-6.89 (m, 2H), 6.60 (t, *J* = 5.7 Hz, 1H), 4.78 (s, 2H), 3.76 (dt, *J*<sub>1</sub> = 7.3 Hz, *J*<sub>2</sub> = 7.0 Hz, 1H), 3.06 (dt, *J*<sub>1</sub> = 6.4 Hz, *J*<sub>2</sub> = 5.7 Hz, 2H), 1.55-1.50 (m, 2H), 1.40 (overlapped, 2H), 1.38 (s, 9H), 1.31-1.19 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ 169.53, 155.70, 155.28, 151.99 (d, *J*<sub>CF</sub> = 239.7 Hz), 136.40, 129.27, 128.70, 128.01 (d, *J*<sub>CF</sub> = 21.9 Hz), 124.77 (d, *J*<sub>CF</sub> = 2.9 Hz), 121.8 (d, *J*<sub>CF</sub> = 7.3 Hz), 120.50, 115.15 (d, *J*<sub>CF</sub> = 19.1 Hz), 78.47, 77.22, 52.50, 39.33, 32.00, 29.78, 28.65, 23.29. LRMS (API-ES, positive mode): *m/z* 489.3 [M+H]<sup>+</sup>.

**tert-Butyl {1-benzyloxyamino-6-[3-(3-fluorophenyl)ureido]-1-oxohexan-2-yl}carbamate (3.22f)**

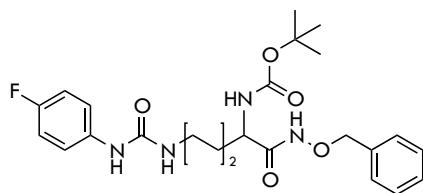
Compound **3.22f** was prepared via procedure B, using 1.40 g (3.65 mmol) of **3.21f**. Yield 93% (1.66 g). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 11.17 (s, 1H), 8.63 (s, 1H), 7.47 (d, *J* = 12.5 Hz, 1H), 7.38-7.34 (m, 5H),



7.22 (dd,  $J_1 = 15.3$  Hz,  $J_2 = 8.0$  Hz, 1H), 7.01 (d,  $J = 8.0$  Hz, 1H), 6.89 (d,  $J = 7.8$  Hz, 1H), 6.68 (dt,  $J_1 = 8.5$  Hz,  $J_2 = 1.5$  Hz, 1H), 6.19 (t,  $J = 5.7$  Hz, 1H),

4.78 (s, 2H), 3.76 (dt,  $J_1 = 7.8$  Hz,  $J_2 = 7.1$  Hz, 1H), 3.05 (dt,  $J_1 = 6.4$  Hz,  $J_2 = 5.7$  Hz, 2H), 1.55-1.50 (m, 2H), 1.41 (overlapped, 2H), 1.38 (s, 9H), 1.30-1.18 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  169.54, 162.90 (d,  $J_{\text{CF}} = 234.9$  Hz), 155.70, 155.43, 142.97 (d,  $J_{\text{CF}} = 11.4$  Hz), 136.39, 130.50 (d,  $J_{\text{CF}} = 9.8$  Hz), 129.27, 128.70, 113.69, 107.57 (d,  $J_{\text{CF}} = 21.3$  Hz), 104.66 (d,  $J_{\text{CF}} = 26.6$  Hz), 78.48, 77.22, 52.52, 39.36, 32.01, 29.84, 28.64, 23.27. LRMS (API-ES, positive mode): *m/z* 489.3 [M+H]<sup>+</sup>.

**tert-Butyl {1-benzyloxyamino-6-[3-(4-fluorophenyl)ureido]-1-oxohexan-2-yl}carbamate (3.22g)**

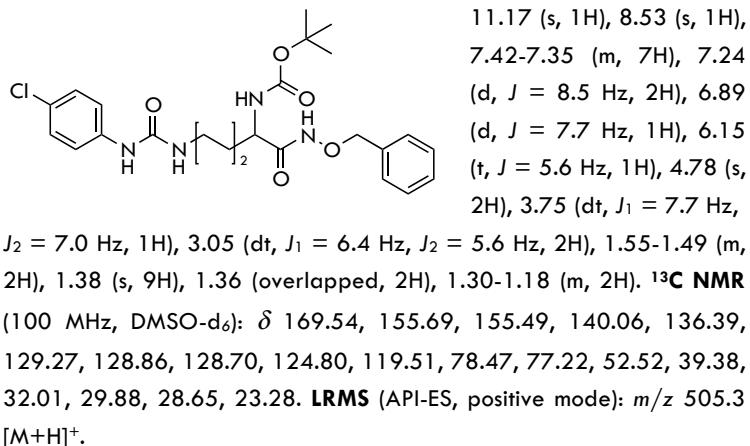


Compound **3.22g** was prepared via procedure B, using 1.25 g (3.26 mmol) of **3.21g**. Yield 99% (1.58 g).  $^1\text{H}$  NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  11.17 (s,

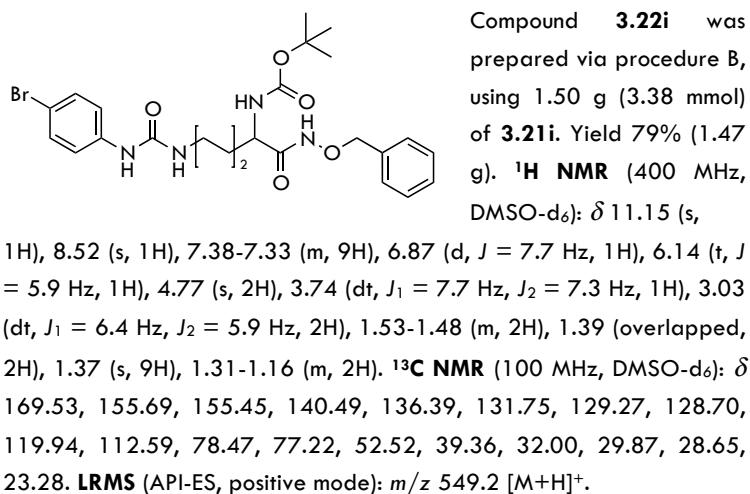
1H), 8.41 (s, 1H), 7.40-7.34 (m, 7H), 7.03 (t,  $J = 8.8$  Hz, 2H), 6.89 (d,  $J = 7.7$  Hz, 1H), 6.08 (t,  $J = 5.7$  Hz, 1H), 4.78 (s, 2H), 3.75 (dt,  $J_1 = 7.7$  Hz,  $J_2 = 7.1$  Hz, 1H), 3.04 (dt,  $J_1 = 6.4$  Hz,  $J_2 = 5.7$  Hz, 2H), 1.55-1.49 (m, 2H), 1.38 (s, 9H), 1.36 (overlapped, 2H), 1.30-1.18 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  169.54, 157.26 (d,  $J_{\text{CF}} = 236.8$  Hz), 155.71, 137.42, 136.39, 129.28, 128.70, 119.62 (d,  $J_{\text{CF}} = 7.3$  Hz), 115.47 (d,  $J_{\text{CF}} = 22.0$  Hz), 78.47, 77.22, 52.53, 39.38, 32.02, 29.93, 28.65, 23.29. LRMS (API-ES, positive mode): *m/z* 489.3 [M+H]<sup>+</sup>.

**tert-Butyl {1-benzyloxyamino-6-[3-(4-chlorophenyl)ureido]-1-oxohexan-2-yl}carbamate (3.22h)**

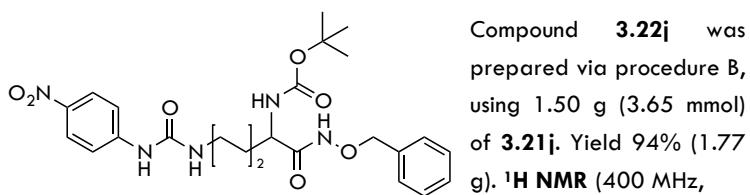
Compound **3.22h** was prepared via procedure B, using 1.40 g (3.50 mmol) of **3.21h**. Yield 90% (1.59 g).  $^1\text{H}$  NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$



***tert*-Butyl {1-benzyloxyamino-6-[3-(4-bromophenyl)ureido]-1-oxohexan-2-yl}carbamate (3.22i)**

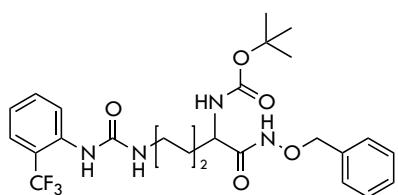


***tert*-Butyl {1-benzyloxyamino-6-[3-(4-nitrophenyl)ureido]-1-oxohexan-2-yl}carbamate (3.22j)**



DMSO-d<sub>6</sub>): δ 11.17 (s, 1H), 9.22 (s, 1H), 8.12 (d, *J* = 8.5 Hz, 2H), 7.61 (d, *J* = 8.5 Hz, 2H), 7.37-7.34 (m, 5H), 6.90 (d, *J* = 7.7 Hz, 1H), 6.42 (t, *J* = 5.7 Hz, 1H), 4.77 (s, 2H), 3.75 (dt, *J*<sub>1</sub> = 7.7 Hz, *J*<sub>2</sub> = 6.7 Hz, 1H), 3.08 (dt, *J*<sub>1</sub> = 6.4 Hz, *J*<sub>2</sub> = 5.7 Hz, 2H), 1.55-1.50 (m, 2H), 1.41 (overlapped, 2H), 1.38 (s, 9H), 1.31-1.19 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ 169.51, 155.69, 154.85, 147.72, 140.78, 136.37, 129.28, 128.69, 125.55, 118.45, 117.21, 78.48, 77.23, 52.51, 39.39, 31.99, 29.69, 28.64, 23.25. LRMS (API-ES, positive mode): *m/z* 416.2 [M-Boc+H]<sup>+</sup>.

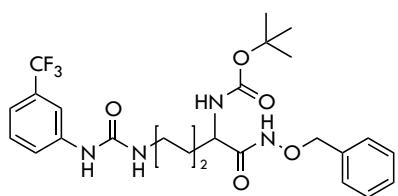
**tert-Butyl {1-benzyloxyamino-6-[3-(2-trifluoromethylphenyl)ureido]-1-oxohexan-2-yl}carbamate (3.22k)**



Compound **3.22k** was prepared via procedure B, using 1.70 g (3.92 mmol) of **3.21k**. Yield 99% (2.09 g). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 11.18 (s, 1H), 7.99 (d,

*J* = 8.1 Hz, 1H), 7.71 (s, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.54 (t, *J* = 7.9 Hz, 1H), 7.38-7.32 (m, 5H), 7.16 (t, *J* = 7.9 Hz, 1H), 7.00 (t, *J* = 5.6 Hz, 1H), 6.89 (d, *J* = 7.9 Hz, 1H), 4.78 (s, 2H), 3.77 (dt, *J*<sub>1</sub> = 7.9 Hz, *J*<sub>2</sub> = 7.0 Hz, 2H), 3.06 (dt, *J*<sub>1</sub> = 6.4 Hz, *J*<sub>2</sub> = 5.6 Hz, 2H), 1.56-1.50 (m, 2H), 1.41 (overlapped, 2H), 1.38 (s, 9H), 1.32-1.22 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ 169.53, 155.69, 155.32, 137.91, 136.39, 133.14, 129.27, 128.69, 126.15 (*q*, *J*<sub>CF</sub> = 5.2 Hz), 124.90, 124.59 (*q*, *J*<sub>CF</sub> = 273.0 Hz), 122.84, 118.85 (*q*, *J*<sub>CF</sub> = 29.7 Hz), 78.47, 77.22, 52.49, 39.52, 32.01, 29.67, 28.63, 23.33. LRMS (API-ES, positive mode): *m/z* 539.3 [M+H]<sup>+</sup>.

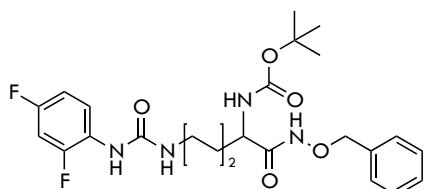
**tert-Butyl {1-benzyloxyamino-6-[3-(3-trifluoromethylphenyl)ureido]-1-oxohexan-2-yl}carbamate (3.22l)**



Compound **3.22l** was prepared via procedure B, using 1.50 g (3.46 mmol) of **3.21l**. Quantitative yield (1.86 g). <sup>1</sup>H NMR (400 MHz,

DMSO-d<sub>6</sub>):  $\delta$  11.17 (s, 1H), 8.79 (d,  $J$  = 6.0 Hz, 1H), 7.98 (d,  $J$  = 5.4 Hz, 1H), 7.51-7.33 (m, 7H), 7.21 (t,  $J$  = 7.1 Hz, 1H), 6.89 (t,  $J$  = 7.0 Hz, 1H), 6.27-6.23 (m, 1H), 4.78 (s, 2H), 3.80-3.73 (m, 1H), 3.10-3.04 (m, 2H), 1.55-1.50 (m, 2H), 1.41 (overlapped, 2H), 1.38 (s, 9H), 1.31-1.23 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  169.56, 155.71, 155.49, 141.92, 136.40, 130.13, 129.89 (q,  $J_{CF}$  = 31.8 Hz), 129.28, 128.71, 124.77 (q,  $J_{CF}$  = 271.5 Hz), 121.52, 117.54 (q,  $J_{CF}$  = 3.5 Hz), 113.97 (q,  $J_{CF}$  = 4.1 Hz), 78.48, 77.24, 52.54, 39.40, 32.03, 29.85, 28.65, 23.29. LRMS (API-ES, positive mode): *m/z* 439.2 [M-Boc+H]<sup>+</sup>.

**tert-Butyl {1-benzyloxyamino-6-[3-(2,4-difluorophenyl)ureido]-1-oxohexan-2-yl}carbamate (3.22m)**

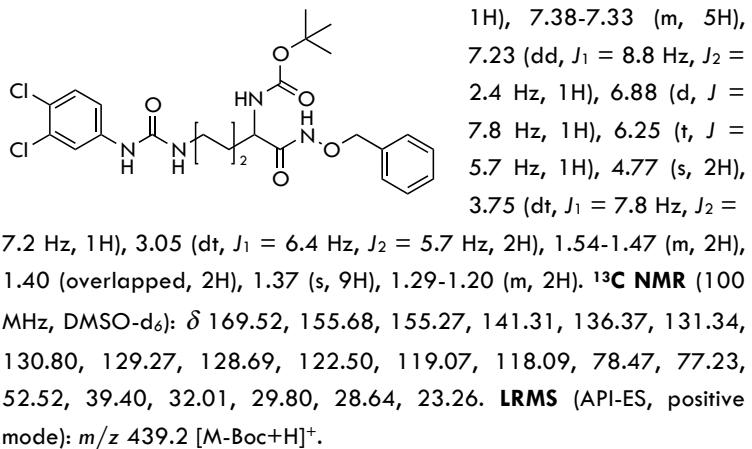


Compound **3.22m** was prepared via procedure B, using 0.81 g (2.02 mmol) of **3.21m**. Yield 99% (1.01 g). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  11.17 (s, 1H), 8.19

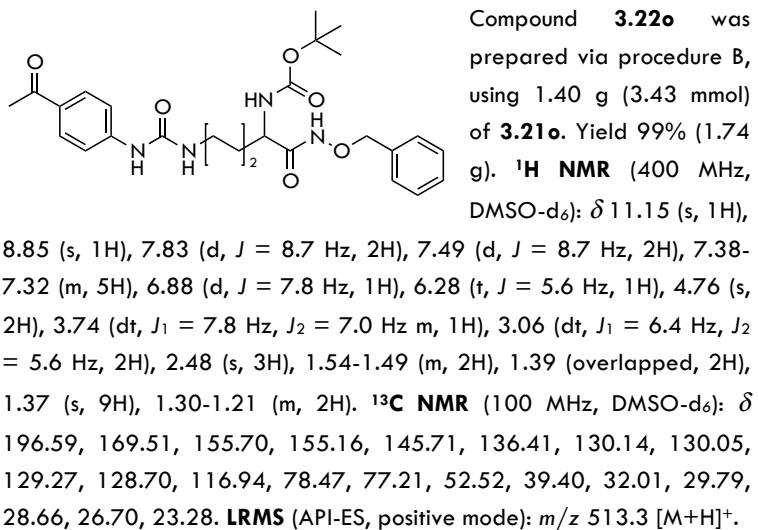
(brs, 1H), 8.07 (dt,  $J_1$  = 9.3 Hz,  $J_2$  = 6.4 Hz, 1H), 7.38-7.32 (m, 5H), 7.20 (ddd,  $J_1$  = 11.5 Hz,  $J_2$  = 9.0 Hz,  $J_3$  = 2.9 Hz, 1H), 6.98-6.93 (m, 1H), 6.89 (d,  $J$  = 7.7 Hz, 1H), 6.53 (t,  $J$  = 6.0 Hz, 1H), 4.77 (s, 2H), 3.75 (dt,  $J_1$  = 7.7 Hz,  $J_2$  = 7.2 Hz, 1H), 3.05 (dt,  $J_1$  = 6.4 Hz,  $J_2$  = 6.0 Hz, 2H), 1.55-1.49 (m, 2H), 1.40 (overlapped, 2H), 1.38 (s, 9H), 1.30-1.20 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  169.52, 156.54 (dd,  $J_{CF}$  = 239.6 Hz,  $J_{CF}$  = 12.0 Hz), 155.69, 155.37, 151.95 (dd,  $J_{CF}$  = 243.6 Hz,  $J_{CF}$  = 12.0 Hz), 136.38, 129.27, 128.70, 125.44 (dd,  $J_{CF}$  = 10.7 Hz,  $J_{CF}$  = 3.3 Hz), 121.65 (dd,  $J_{CF}$  = 9.5 Hz,  $J_{CF}$  = 2.8 Hz), 111.21 (dd,  $J_{CF}$  = 21.4 Hz,  $J_{CF}$  = 3.5 Hz), 103.91 (dd,  $J_{CF}$  = 26.6 Hz,  $J_{CF}$  = 23.8 Hz), 78.47, 77.22, 52.50, 39.38, 32.00, 29.78, 28.64, 23.27. LRMS (API-ES, positive mode): *m/z* 507.3 [M+H]<sup>+</sup>.

**tert-Butyl {1-benzyloxyamino-6-[3-(3,4-dichlorophenyl)ureido]-1-oxo-hexan-2-yl}carbamate (3.22n)**

Compound **3.22n** was prepared via procedure B, using 1.50 g (3.45 mmol) of **3.21n**. Yield 97% (1.81 g). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  11.16 (s, 1H), 8.72 (s, 1H), 7.84 (d,  $J$  = 2.4 Hz, 1H), 7.42 (d,  $J$  = 8.8 Hz,

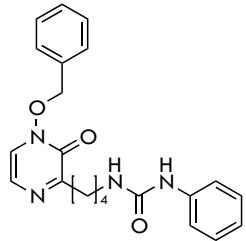


**tert-Butyl {6-[3-(4-acetophenyl)ureido]-1-benzylamino-1-oxohexan-2-yl}carbamate (3.22o)**



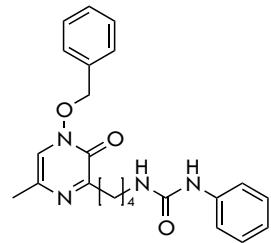
**1-Benzylamino-3-[4-(3-phenylureido)butyl]pyrazin-2(1H)-one (3.23a)**

Compound **3.23a** was prepared by deprotection of **3.22a** (350 mg, 0.74 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (76  $\mu\text{L}$ , 0.67 mmol). Yield 44% (116 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.38 (s, 1H), 7.72 (d,  $J = 4.5$  Hz, 1H), 7.50-7.48 (m, 2H), 7.43-7.41 (m, 3H), 7.37 (d,  $J = 7.9$  Hz, 2H), 7.20 (dd,  $J_1 = 7.2$  Hz, 1H), 6.88 (d,  $J = 7.8$  Hz, 1H), 6.25 (t,  $J = 5.7$  Hz, 1H), 4.77 (s, 2H), 3.75 (dt,  $J_1 = 7.8$  Hz,  $J_2 = 7.2$  Hz, 1H), 3.05 (dt,  $J_1 = 6.4$  Hz,  $J_2 = 5.7$  Hz, 2H), 1.54-1.47 (m, 2H), 1.40 (overlapped, 2H), 1.37 (s, 9H), 1.30-1.21 (m, 2H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  196.59, 169.51, 155.70, 155.16, 145.71, 136.41, 130.14, 130.05, 129.27, 128.70, 116.94, 78.47, 77.21, 52.52, 39.40, 32.01, 29.79, 28.66, 26.70, 23.28. **LRMS** (API-ES, positive mode): *m/z* 513.3 [M+H]<sup>+</sup>.



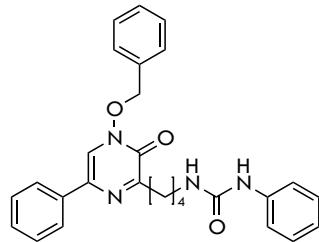
$\delta$  = 8.2 Hz,  $J_2$  = 7.9 Hz, 2H), 7.14 (d,  $J$  = 4.5 Hz, 1H), 6.87 (t,  $J$  = 7.5 Hz, 1H), 6.14 (t,  $J$  = 5.7 Hz, 1H), 5.25 (s, 2H), 3.14-3.09 (m, 2H), 2.76 (t,  $J$  = 7.5 Hz, 2H), 1.67 (quint,  $J$  = 7.5 Hz, 2H), 1.49 (quint,  $J$  = 7.5 Hz, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  162.38, 155.66, 152.10, 141.07, 133.98, 130.29, 129.72, 129.06, 129.02, 127.75, 121.36, 118.03, 78.44, 39.34, 32.91, 29.97, 23.90. **LRMS** (API-ES, positive mode): *m/z* 393.2 [M+H]<sup>+</sup>.

**1-Benzyl-5-methyl-3-[4-(3-phenylureido)butyl]pyrazin-2(1H)-one  
(3.23b)**



Compound 3.23b was prepared by deprotection of 3.22a (350 mg, 0.74 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (102  $\mu$ L, 0.67 mmol). Yield 53% (144 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.76 (brs, 1H), 7.36 (brs, 7H), 7.21-7.16 (m, 2H), 6.94-6.90 (m, 1H), 6.83 (brs, 1H), 5.88 (brs, 1H), 5.23 (s, 2H), 3.24 (brs, 2H), 2.83 (brs, 2H), 2.13 (s, 3H), 1.72 (brs, 2H), 1.55 (brs, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>):  $\delta$  161.85, 156.47, 151.42, 139.58, 133.01, 130.77, 129.89, 129.65, 128.89, 128.83, 122.96, 122.41, 119.55, 68.75, 39.81, 33.26, 29.78, 24.23, 19.49. **LRMS** (API-ES, positive mode): *m/z* 407.2 [M+H]<sup>+</sup>.

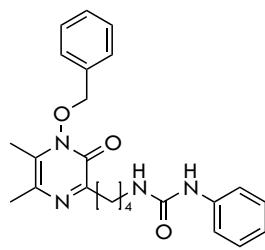
**1-Benzyl-5-phenyl-3-[4-(3-phenylureido)butyl]pyrazin-2(1H)-one  
(3.23c)**



Compound 3.23c was prepared by deprotection of 3.22a (350 mg, 0.74 mmol) via procedure D and then cyclization via procedure E using phenylglyoxal mono-hydrate (102 mg, 0.67 mmol). Yield 17% (53 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  9.04 (s, 1H),

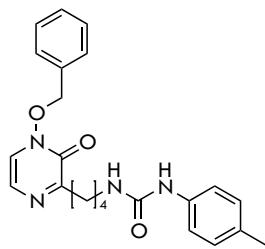
7.43-7.23 (m, 15H), 7.01 (t,  $J$  = 7.3 Hz, 1H), 5.15 (t,  $J$  = 5.7 Hz, 1H), 4.91 (s, 2H), 3.22 (dt,  $J_1$  = 6.5 Hz,  $J_2$  = 5.7 Hz, 2H), 2.57 (t,  $J$  = 7.3 Hz, 2H), 1.55-1.46 (m, 4H).  **$^{13}\text{C}$  NMR** (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  161.65, 155.91, 153.46, 139.04, 136.62, 134.82, 130.06, 129.31, 129.12, 128.91, 128.65, 128.57, 128.32, 128.17, 125.17, 123.19, 120.33, 78.65, 39.61, 29.31, 23.70, 23.14. **LRMS** (API-ES, positive mode):  $m/z$  475.2 [M+Li]<sup>+</sup>.

**1-Benzylxy-5,6-dimethyl-3-[4-(3-phenylureido)butyl]pyrazin-2(1H)-one (3.23d)**



Compound **3.23d** was prepared by deprotection of **3.22a** (350 mg, 0.74 mmol) via procedure D and then cyclization via procedure E using diacetyl (58  $\mu\text{L}$ , 0.67 mmol). Yield 46% (130 mg).  **$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  8.37 (s, 1H), 7.53-7.51 (m, 2H), 7.44-7.42 (m, 3H), 7.37 (d,  $J$  = 8.0 Hz, 2H), 7.20 (t,  $J$  = 8.0 Hz, 2H), 6.87 (t,  $J$  = 7.5 Hz, 1H), 6.13 (t,  $J$  = 5.9 Hz, 1H), 5.21 (s, 2H), 3.11 (dt,  $J_1$  = 6.6 Hz,  $J_2$  = 5.9 Hz, 2H), 2.72 (t,  $J$  = 7.6 Hz, 2H), 2.25 (s, 3H), 2.20 (s, 3H), 1.66 (quint,  $J$  = 7.6 Hz, 2H), 1.49 (quint,  $J$  = 7.6 Hz, 2H).  **$^{13}\text{C}$  NMR** (100 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  156.94, 155.65, 151.56, 141.06, 134.16, 133.30, 130.28, 129.73, 129.06, 127.14, 121.33, 118.02, 77.20, 39.39, 32.85, 30.09, 24.36, 19.82, 13.11. **LRMS** (API-ES, positive mode):  $m/z$  421.2 [M+H]<sup>+</sup>.

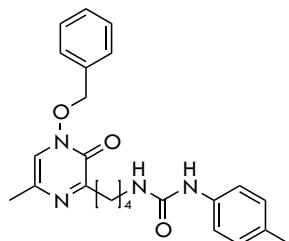
**1-Benzylxy-3-[4-[3-(*p*-tolyl)ureido]butyl]pyrazin-2(1H)-one (3.23e)**



Compound **3.23e** was prepared by deprotection of **3.22b** (375 mg, 0.77 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (80  $\mu\text{L}$ , 0.70 mmol). Yield 71% (202 mg).  **$^1\text{H}$  NMR** (400 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  8.25 (s, 1H), 7.70 (d,  $J$  = 4.5 Hz, 1H), 7.48 (brs, 2H), 7.41 (brs, 3H), 7.25 (d,  $J$  = 7.8 Hz, 2H), 7.13 (d,  $J$  = 4.5 Hz, 1H), 7.00 (d,  $J$  = 7.8 Hz, 2H), 6.07 (t,  $J$  = 5.8 Hz,

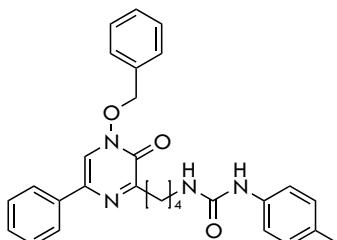
1H), 5.25 (s, 2H), 3.12-3.07 (m, 2H), 2.76 (t,  $J$  = 7.5 Hz, 2H), 2.20 (s, 3H), 1.70-1.62 (m, 2H), 1.51-1.44 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  162.39, 155.75, 152.10, 138.50, 133.98, 130.29, 130.04, 129.72, 129.46, 129.02, 127.75, 121.36, 118.16, 78.44, 39.39, 32.91, 30.00, 23.91, 20.74. LRMS (API-ES, positive mode):  $m/z$  407.2 [M+H] $^+$ .

**1-Benzylxyloxy-5-methyl-3-{4-[3-(*p*-tolyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23f)**



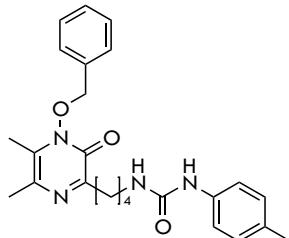
Compound **3.23f** was prepared by deprotection of **3.22b** (375 mg, 0.77 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (107  $\mu\text{L}$ , 0.70 mmol). Yield 64% (188 mg).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.38 (s, 5H), 7.22 (s, 1H), 7.20 (d,  $J$  = 8.3 Hz, 2H), 7.03 (d,  $J$  = 8.3 Hz, 2H), 6.83 (s, 1H), 5.50 (t,  $J$  = 5.7 Hz, 1H), 5.24 (s, 2H), 3.26 (dt,  $J_1$  = 6.7 Hz,  $J_2$  = 5.7 Hz, 2H), 2.85 (t,  $J$  = 7.6 Hz, 2H), 2.26 (s, 3H), 2.14 (s, 3H), 1.78-1.70 (m, 2H), 1.60-1.53 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.02, 156.51, 151.44, 136.46, 133.08, 132.72, 130.59, 129.88, 129.60, 128.82, 123.00, 120.77, 78.66, 39.83, 33.23, 29.57, 24.28, 20.73, 19.48. LRMS (API-ES, positive mode):  $m/z$  421.3 [M+H] $^+$ .

**1-Benzylxyloxy-5-phenyl-3-{4-[3-(*p*-tolyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23g)**



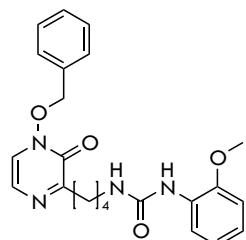
Compound **3.23g** was prepared by deprotection of **3.22b** (375 mg, 0.77 mmol) via procedure D and then cyclization via procedure E using phenylglyoxal monohydrate (106 mg, 0.70 mmol). Yield 9% (30 mg). LRMS (API-ES, positive mode):  $m/z$  483.3 [M+H] $^+$ .

**1-Benzylxy-5,6-dimethyl-3-{4-[3-(*p*-tolyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23h)**



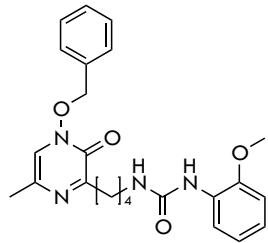
Compound **3.23h** was prepared by deprotection of **3.22b** (375 mg, 0.77 mmol) via procedure D and then cyclization via procedure E using diacetyl (61  $\mu$ L, 0.70 mmol). Yield 58% (176 mg).  **$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.48-7.46 (m, 2H), 7.41-7.38 (m, 3H), 7.19 (d,  $J$  = 8.3 Hz, 2H), 7.07 (d,  $J$  = 8.3 Hz, 2H), 6.55 (s, 1H), 5.26 (t,  $J$  = 6.0 Hz, 1H), 5.24 (s, 2H), 3.32 (dt,  $J_1$  = 6.7 Hz,  $J_2$  = 6.0 Hz, 2H), 2.85 (t,  $J$  = 7.6 Hz, 2H), 2.28 (s, 3H), 2.25 (s, 3H), 2.21 (s, 3H), 1.77 (quint,  $J$  = 7.6 Hz, 2H), 1.61 (overlapped, 2H).  **$^{13}\text{C}$  NMR** (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  157.68, 156.11, 152.17, 136.19, 133.23, 132.46, 129.90, 129.73, 129.60, 128.80, 128.33, 121.29, 77.66, 39.84, 32.80, 29.13, 24.47, 20.76, 19.63, 13.00. **LRMS** (API-ES, positive mode):  $m/z$  435.3 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(2-methoxyphenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23i)**



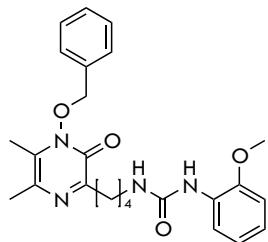
Compound **3.23i** was prepared by deprotection of **3.22c** (600 mg, 1.20 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (123  $\mu$ L, 1.08 mmol). Yield 66% (301 mg).  **$^1\text{H}$  NMR** (400 MHz,  $\text{CDCl}_3$ - $\text{CD}_3\text{OD}$ ):  $\delta$  8.01 (d,  $J$  = 7.5 Hz, 1H), 7.40 (s, 5H), 7.14 (d,  $J$  = 4.4 Hz, 1H), 7.07 (d,  $J$  = 4.4 Hz, 1H), 6.96-6.85 (m, 3H), 5.29 (s, 2H), 3.86 (s, 3H), 3.27 (t,  $J$  = 6.4 Hz, 2H), 2.89 (t,  $J$  = 7.5 Hz, 2H), 1.83-1.75 (m, 2H), 1.67-1.60 (m, 2H).  **$^{13}\text{C}$  NMR** (100 MHz,  $\text{CDCl}_3$ - $\text{CD}_3\text{OD}$ ):  $\delta$  163.47, 157.13, 153.27, 148.58, 133.28, 130.38, 130.11, 129.22, 127.32, 122.41, 122.13, 121.25, 119.57, 110.57, 79.21, 55.83, 39.79, 33.37, 29.88, 24.31. **LRMS** (API-ES, positive mode):  $m/z$  423.2 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(2-methoxyphenyl)ureido]butyl}-5-methyl-pyrazin-2(1*H*)-one (3.23j)**



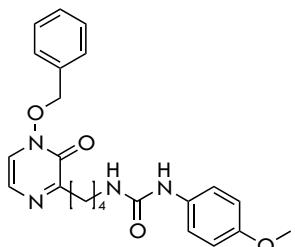
Compound **3.23j** was prepared by deprotection of **3.22c** (600 mg, 1.20 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (166  $\mu$ L, 1.08 mmol). Yield 67% (316 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  8.12-8.10 (m, 1H), 7.43-7.37 (m, 5H), 7.30 (s, 1H), 6.94-6.89 (m, 2H), 6.85 (s, 1H), 6.82-6.80 (m, 1H), 5.85 (t,  $J$  = 6.0 Hz, 1H), 5.27 (s, 2H), 3.76 (s, 3H), 3.32 (dt,  $J_1$  = 6.6 Hz,  $J_2$  = 6.0 Hz, 2H), 2.89 (t,  $J$  = 7.6 Hz, 2H), 2.16 (s, 3H), 1.83-1.76 (m, 2H), 1.67-1.60 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>):  $\delta$  162.04, 155.91, 151.39, 148.07, 133.16, 130.36, 129.85, 129.56, 129.01, 128.77, 123.03, 121.99, 121.08, 119.45, 110.12, 78.54, 55.57, 39.88, 33.15, 29.53, 24.20, 19.43. **LRMS** (API-ES, positive mode): *m/z* 437.2 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(2-methoxyphenyl)ureido]butyl}-5,6-dimethyl-pyrazin-2(1*H*)-one (3.23k)**



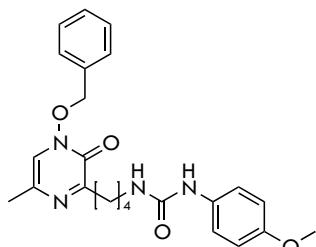
Compound **3.23k** was prepared by deprotection of **3.22c** (600 mg, 1.20 mmol) via procedure D and then cyclization via procedure E using diacetyl (94  $\mu$ L, 1.08 mmol). Yield 69% (336 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  8.10-8.08 (m, 1H), 7.46-7.44 (m, 2H), 7.38-7.35 (m, 3H), 7.23 (s, 1H), 6.90-6.88 (m, 2H), 6.79-6.77 (m, 1H), 5.78 (t,  $J$  = 5.7 Hz, 1H), 5.22 (s, 2H), 3.73 (s, 3H), 3.31 (dt,  $J_1$  = 6.6 Hz,  $J_2$  = 5.7 Hz, 2H), 2.84 (t,  $J$  = 7.5 Hz, 2H), 2.24 (s, 3H), 2.19 (s, 3H), 1.82-1.74 (m, 2H), 1.66-1.59 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>):  $\delta$  157.66, 155.79, 152.09, 147.98, 133.24, 132.41, 129.90, 129.54, 129.08, 128.75, 128.19, 121.88, 121.09, 119.33, 110.06, 77.59, 55.57, 39.92, 32.90, 29.48, 24.42, 19.59, 12.97. **LRMS** (API-ES, positive mode): *m/z* 451.2 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(4-methoxyphenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23l)**



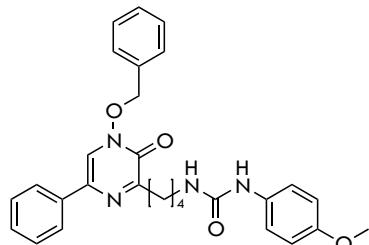
Compound **3.23l** was prepared by deprotection of **3.22d** (300 mg, 0.60 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (62  $\mu$ L, 0.54 mmol). Yield 32% (73 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.17 (s, 1H), 7.71 (d, *J* = 4.6 Hz, 1H), 7.50-7.40 (m, 5H), 7.27 (d, *J* = 8.9 Hz, 2H), 7.13 (d, *J* = 4.6 Hz, 1H), 6.80 (d, *J* = 8.9 Hz, 2H), 6.02 (t, *J* = 5.7 Hz, 1H), 5.25 (s, 2H), 3.68 (s, 3H), 3.10 (dt, *J*<sub>1</sub> = 6.6 Hz, *J*<sub>2</sub> = 5.7 Hz, 2H), 2.76 (t, *J* = 7.6 Hz, 2H), 1.70-1.62 (m, 2H), 1.51-1.44 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  162.40, 155.91, 154.30, 152.10, 134.21, 133.98, 130.29, 129.72, 129.02, 127.74, 121.36, 119.79, 114.32, 78.43, 55.60, 39.38, 32.92, 30.04, 23.91. **LRMS** (API-ES, positive mode): *m/z* 423.3 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(4-methoxyphenyl)ureido]butyl}-5-methyl-pyrazin-2(1*H*)-one (3.23m)**



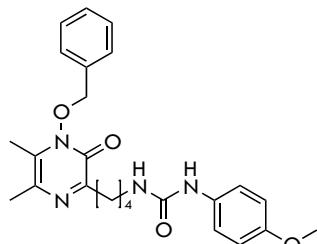
Compound **3.23m** was prepared by deprotection of **3.22d** (300 mg, 0.60 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (83  $\mu$ L, 0.54 mmol). Yield 61% (144 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.17 (s, 1H), 7.60 (s, 1H), 7.50-7.40 (m, 5H), 7.27 (d, *J* = 9.0 Hz, 2H), 6.79 (d, *J* = 9.0 Hz, 2H), 6.02 (t, *J* = 5.9 Hz, 1H), 5.22 (s, 2H), 3.68 (s, 3H), 3.09 (dt, *J*<sub>1</sub> = 6.7 Hz, *J*<sub>2</sub> = 5.9 Hz, 2H), 2.73 (t, *J* = 7.6 Hz, 2H), 2.13 (s, 3H), 1.68-1.60 (m, 2H), 1.51-1.44 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  161.28, 155.90, 154.29, 150.92, 134.22, 134.09, 130.21, 129.66, 129.47, 128.99, 124.23, 119.78, 114.32, 78.39, 55.60, 39.38, 33.07, 30.11, 24.16, 19.50. **LRMS** (API-ES, positive mode): *m/z* 437.3 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(4-methoxyphenyl)ureido]butyl}-5-phenyl-pyrazin-2(1H)-one (3.23n)**



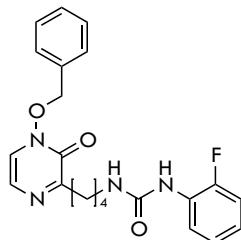
Compound **3.23n** was prepared by deprotection of **3.22d** (300 mg, 0.60 mmol) via procedure D and then cyclization via procedure E using phenylglyoxal monohydrate (82 mg, 0.54 mmol). Yield 18% (48 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 8.39 (s, 1H), 8.16 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.57-7.55 (m, 2H), 7.44-7.26 (m, 8H), 6.79 (d, *J* = 8.8 Hz, 2H), 6.04 (t, *J* = 5.9 Hz, 1H), 5.32 (s, 2H), 3.68 (s, 3H), 3.14 (dt, *J*<sub>1</sub> = 6.7 Hz, *J*<sub>2</sub> = 5.9 Hz, 2H), 2.86 (t, *J* = 7.5 Hz, 2H), 1.81-1.73 (m, 2H), 1.58-1.51 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 161.15, 155.93, 154.31, 151.13, 135.74, 134.22, 134.01, 130.36, 130.31, 129.72, 129.13, 128.99, 128.13, 125.23, 123.97, 119.81, 114.32, 78.75, 55.60, 39.39, 33.08, 30.06, 23.86. **LRMS** (API-ES, positive mode): *m/z* 499.3 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(4-methoxyphenyl)ureido]butyl}-5,6-dimethyl-pyrazin-2(1H)-one (3.23o)**



Compound **3.23o** was prepared by deprotection of **3.22d** (300 mg, 0.60 mmol) via procedure D and then cyclization via procedure E using diacetyl (47 μL, 0.54 mmol). Yield 78% (190 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD): δ 7.48-7.41 (m, 5H), 7.24 (dd, *J*<sub>1</sub> = 8.9 Hz, *J*<sub>2</sub> = 1.3 Hz, 2H), 6.82 (dd, *J*<sub>1</sub> = 8.9 Hz, *J*<sub>2</sub> = 1.3 Hz, 2H), 5.73 (t, *J* = 5.7 Hz, 1H), 5.24 (s, 2H), 3.77 (s, 3H), 3.27 (dt, *J*<sub>1</sub> = 6.6 Hz, *J*<sub>2</sub> = 5.7 Hz, 2H), 2.84 (t, *J* = 7.5 Hz, 2H), 2.27 (s, 3H), 2.24 (s, 3H), 1.80-1.73 (m, 2H), 1.66-1.59 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD): δ 157.78, 157.52, 155.86, 152.67, 133.51, 133.32, 132.66, 130.24, 129.99, 129.23, 129.10, 122.12, 114.49, 78.16, 55.70, 39.72, 33.21, 29.88, 24.76, 19.46, 13.17. **LRMS** (API-ES, positive mode): *m/z* 451.2 [M+H]<sup>+</sup>.

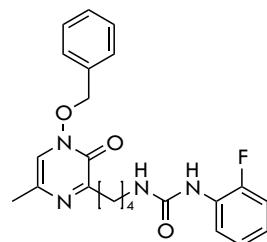
**1-Benzylxy-3-{4-[3-(2-fluorophenyl)ureido]butyl}pyrazin-2(1*H*)-one  
(3.23p)**



Compound **3.23p** was prepared by deprotection of **3.22e** (450 mg, 0.92 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (94  $\mu$ L, 0.83 mmol). Yield 67% (228 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  8.05 (dt,  $J_1$  =

8.2 Hz,  $J_2$  = 1.4 Hz, 1H), 7.41 (s, 5H), 7.13 (d,  $J$  = 4.4 Hz, 1H), 7.08-7.02 (m, 3H), 6.95-6.91 (m, 1H), 5.29 (s, 2H), 3.30-3.27 (m, 2H), 2.90 (t,  $J$  = 7.6 Hz, 2H), 1.83-1.75 (m, 2H), 1.68-1.61 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  163.43, 156.74, 153.24, 152.96 (d,  $J_{CF}$  = 241.8 Hz), 133.24, 130.36, 130.11, 129.21, 128.16 (d,  $J_{CF}$  = 10.4 Hz), 127.30, 124.58 (d,  $J_{CF}$  = 3.5 Hz), 122.66 (d,  $J_{CF}$  = 7.5 Hz), 122.12, 121.46, 114.97 (d,  $J_{CF}$  = 19.4 Hz), 79.21, 39.74, 33.35, 29.5, 24.25. **LRMS** (API-ES, positive mode): *m/z* 411.2 [M+H]<sup>+</sup>.

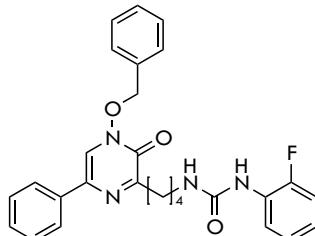
**1-Benzylxy-3-{4-[3-(2-fluorophenyl)ureido]butyl}-5-methylpyrazin-2(1*H*)-one (3.23q)**



Compound **3.23q** was prepared by deprotection of **3.22e** (450 mg, 0.92 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (127  $\mu$ L, 0.83 mmol). Yield 59% (208 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.23 (brs, 1H),

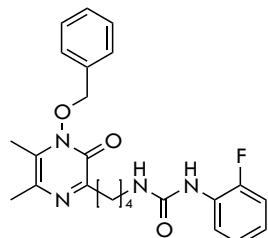
8.13 (dt,  $J_1$  = 8.3 Hz,  $J_2$  = 1.3 Hz, 1H), 7.61 (s, 1H), 7.51-7.48 (m, 2H), 7.43-7.41 (m, 3H), 7.19-7.14 (m, 1H), 7.07 (t,  $J$  = 7.5 Hz, 1H), 6.93-6.88 (m, 1H), 6.65-6.61 (m, 1H), 5.23 (s, 2H), 3.16-3.11 (m, 2H), 2.75 (t,  $J$  = 7.6 Hz, 2H), 2.13 (s, 3H), 1.69-1.62 (m, 2H), 1.53-1.46 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  161.24, 155.28, 151.99 (d,  $J_{CF}$  = 240.6 Hz), 150.93, 134.08, 130.21, 129.66, 129.47, 128.99, 128.89 (d,  $J_{CF}$  = 10.6 Hz), 124.79 (d,  $J_{CF}$  = 2.5 Hz), 124.24, 121.78 (d,  $J_{CF}$  = 6.9 Hz), 120.50, 115.17 (d,  $J_{CF}$  = 19.0 Hz), 78.39, 39.38, 33.02, 29.88, 24.13, 19.49. **LRMS** (API-ES, positive mode): *m/z* 425.2 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(2-fluorophenyl)ureido]butyl}-5-phenylpyrazin-2(1*H*)-one (3.23r)**



Compound **3.23r** was prepared by deprotection of **3.22e** (450 mg, 0.92 mmol) via procedure D and then cyclization via procedure E using phenylglyoxal monohydrate (126 mg, 0.83 mmol). Yield 22% (89 mg). **LRMS** (API-ES, positive mode): *m/z* 487.3 [M+H]<sup>+</sup>.

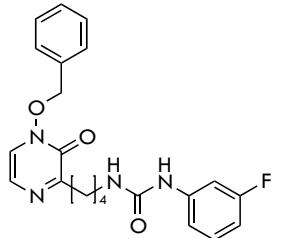
**1-Benzylxy-3-{4-[3-(2-fluorophenyl)ureido]butyl}-5,6-dimethyl-pyrazin-2(1*H*)-one (3.23s)**



Compound **3.23s** was prepared by deprotection of **3.22e** (450 mg, 0.92 mmol) via procedure D and then cyclization via procedure E using diacetyl (72  $\mu$ L, 0.83 mmol). Yield 48% (175 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  8.11 (dt, *J*<sub>1</sub> = 8.3 Hz, *J*<sub>2</sub> = 1.3 Hz, 1H), 7.45-7.37 (m, 6H), 7.06-6.85 (m, 3H), 6.05 (t, *J* = 5.9 Hz, 1H), 5.24 (s, 2H), 3.33 (dt, *J*<sub>1</sub> = 6.6 Hz, *J*<sub>2</sub> = 5.9 Hz, 2H), 2.85 (t, *J* = 7.7 Hz, 2H), 2.25 (s, 3H), 2.19 (s, 3H), 1.82-1.75 (m, 2H), 1.66-1.59 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>):  $\delta$  157.56, 155.57, 152.48 (d, *J*<sub>CF</sub> = 241.6 Hz), 152.25, 133.14, 132.55, 129.89, 129.59, 128.77, 128.68, 127.89 (d, *J*<sub>CF</sub> = 10.2 Hz), 124.44 (d, *J*<sub>CF</sub> = 3.3 Hz), 122.56 (d, *J*<sub>CF</sub> = 7.5 Hz), 121.25, 114.62 (d, *J*<sub>CF</sub> = 19.4 Hz), 77.75, 39.75, 32.86, 29.00, 24.47, 19.63, 13.01. **LRMS** (API-ES, positive mode): *m/z* 439.2 [M+H]<sup>+</sup>.

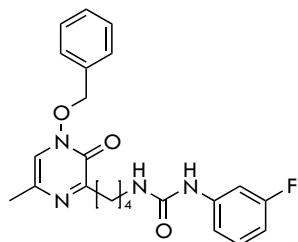
**1-Benzylxy-3-{4-[3-(3-fluorophenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23t)**

Compound **3.23t** was prepared by deprotection of **3.22f** (533 mg, 1.09 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (112  $\mu$ L, 0.98 mmol). Yield 60% (241 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.39 (s, 4H), 7.30 (s, 1H), 7.27 (s, 1H), 7.20-7.15 (m, 1H), 7.09-7.03 (m, 3H), 6.98 (d, *J* = 4.6 Hz, 1H), 6.71-6.66 (m,



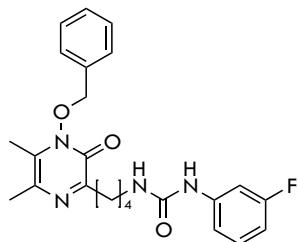
1H), 5.48 (t,  $J = 6.1$  Hz, 1H), 5.29 (s, 2H), 3.33 (dt,  $J_1 = 6.4$  Hz,  $J_2 = 6.1$  Hz, 2H), 2.89 (t,  $J = 7.5$  Hz, 2H), 1.82-1.75 (m, 2H), 1.66-1.59 (m, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  163.21 (d,  $J_{CF} = 243.7$  Hz), 163.18, 155.46, 152.76, 140.99 (d,  $J_{CF} = 11.1$  Hz), 132.82, 130.00 (d,  $J_{CF} = 7.0$  Hz), 129.96, 129.83, 128.95, 126.42, 121.93, 114.69, 109.31 (d,  $J_{CF} = 21.8$  Hz), 106.72 (d,  $J_{CF} = 25.6$  Hz), 78.83, 39.71, 32.86, 28.98, 23.94. LRMS (API-ES, positive mode): *m/z* 411.2 [M+H]<sup>+</sup>.

**1-Benzyl-3-{4-[3-(3-fluorophenyl)ureido]butyl}-5-methylpyrazin-2(1H)-one (3.23u)**



Compound 3.23u was prepared by deprotection of 3.22f (533 mg, 1.09 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (151  $\mu$ L, 0.98 mmol). Yield 66% (275 mg). LRMS (API-ES, positive mode): *m/z* 425.2 [M+H]<sup>+</sup>.

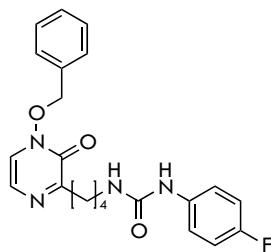
**1-Benzyl-3-{4-[3-(3-fluorophenyl)ureido]butyl}-5,6-dimethyl-pyrazin-2(1H)-one (3.23v)**



Compound 3.23v was prepared by deprotection of 3.22f (533 mg, 1.09 mmol) via procedure D and then cyclization via procedure E using diacetyl (86  $\mu$ L, 0.98 mmol). Yield 54% (232 mg). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.69 (s, 1H), 7.44-7.34 (m, 5H), 7.26 (s, 1H), 7.11-7.06 (m, 1H), 7.01 (d,  $J = 8.2$  Hz, 1H), 6.60 (dt,  $J_1 = 8.2$  Hz,  $J_2 = 2.0$  Hz, 1H), 5.93 (t,  $J = 5.7$  Hz, 1H), 5.23 (s, 2H), 3.30 (dt,  $J_1 = 6.6$  Hz,  $J_2 = 5.7$  Hz, 2H), 2.83 (t,  $J = 7.6$  Hz, 2H), 2.26 (s, 3H), 2.22 (s, 3H), 1.80-1.73 (m, 2H), 1.64-1.57 (m, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  163.16 (d,  $J_{CF} = 243.1$  Hz), 157.31, 155.85, 152.24, 141.45 (d,  $J_{CF} = 10.9$  Hz), 132.90, 132.50, 129.87, 129.83, 129.72, 129.14, 128.85,

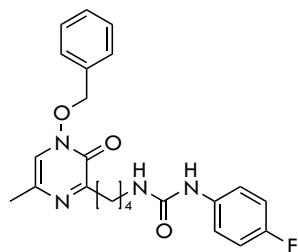
114.19 (d,  $J_{CF} = 2.6$  Hz), 108.61 (d,  $J_{CF} = 21.4$  Hz), 106.13 (d,  $J_{CF} = 26.1$  Hz), 77.93, 39.73, 32.94, 29.44, 24.52, 19.68, 13.02. **LRMS** (API-ES, positive mode):  $m/z$  439.2 [M+H]<sup>+</sup>.

**1-Benzylxyloxy-3-{4-[3-(4-fluorophenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23w)**



Compound **3.23w** was prepared by deprotection of **3.22g** (500 mg, 1.02 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (105  $\mu$ L, 0.92 mmol). Yield 74% (279 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.40 (s, 5H), 7.32-7.29 (m, 2H), 7.08-7.06 (m, 2H), 6.95 (t,  $J = 8.5$  Hz, 2H), 5.29 (s, 2H), 3.28 (t,  $J = 6.5$  Hz, 2H), 2.89 (dd,  $J_1 = 7.9$  Hz,  $J_2 = 7.1$  Hz, 2H), 1.81-1.73 (m, 2H), 1.66-1.59 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  163.12, 158.41 (d,  $J_{CF} = 240.6$  Hz), 156.64, 152.78, 135.42 (d,  $J_{CF} = 2.2$  Hz), 132.77, 129.92, 129.74, 128.84, 126.79, 121.67, 120.81 (d,  $J_{CF} = 7.6$  Hz), 115.21 (d,  $J_{CF} = 22.2$  Hz), 78.80, 39.29, 32.95, 29.42, 23.81. **LRMS** (API-ES, positive mode):  $m/z$  411.2 [M+H]<sup>+</sup>.

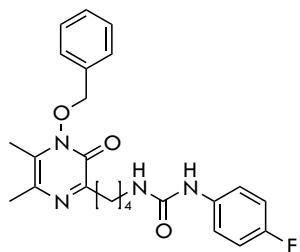
**1-Benzylxyloxy-3-{4-[3-(4-fluorophenyl)ureido]butyl}-5-methylpyrazin-2(1*H*)-one (3.23x)**



Compound **3.23x** was prepared by deprotection of **3.22g** (500 mg, 1.02 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (141  $\mu$ L, 0.92 mmol). Yield 55% (215 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.40 (brs, 5H), 7.31-7.27 (m, 2H), 6.99-6.93 (m, 3H), 6.86 (s, 1H), 5.36 (brs, 1H), 5.27 (s, 2H), 3.37-3.31 (m, 2H), 2.90-2.86 (m, 2H), 2.17 (s, 3H), 1.80-1.72 (m, 2H), 1.60-1.54 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>):  $\delta$  162.27, 158.81 (d,  $J_{CF} = 240.5$  Hz), 156.89, 151.88, 135.76, 133.17, 131.15, 130.16, 129.98, 129.09, 123.77, 121.07 (d,  $J_{CF} = 7.6$  Hz), 115.53 (d,

$J_{CF} = 22.3$  Hz), 79.07, 39.46, 33.45, 29.53, 24.53, 19.38. **LRMS** (API-ES, positive mode):  $m/z$  425.2 [M+H]<sup>+</sup>.

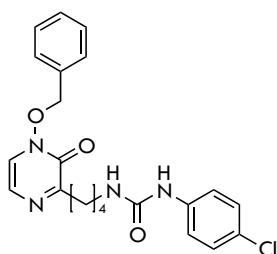
**1-Benzylxy-3-{4-[3-(4-fluorophenyl)ureido]butyl}-5,6-dimethyl-pyrazin-2(1*H*)-one (3.23y)**



Compound **3.23y** was prepared by deprotection of **3.22g** (500 mg, 1.02 mmol) via procedure D and then cyclization via procedure E using diacetyl (81  $\mu$ L, 0.92 mmol). Yield 50% (202 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.47-7.39 (m, 5H), 7.31-7.27

(m 2H), 6.95-6.89 (m, 2H), 6.80 (brs, 1H), 5.46 (t,  $J = 5.7$  Hz, 1H), 5.24 (s, 2H), 3.34 (dt,  $J_1 = 6.7$  Hz,  $J_2 = 5.7$  Hz, 2H), 2.85 (t,  $J = 7.3$  Hz, 2H), 2.26 (s, 3H), 2.23 (s, 3H), 1.82-1.75 (m, 2H), 1.63 (overlapped, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>):  $\delta$  158.86 (d,  $J_{CF} = 241.8$  Hz), 157.59, 155.95, 152.26, 135.16, 133.09, 132.57, 129.86, 129.70, 128.86, 128.72, 121.96 (d,  $J_{CF} = 7.8$  Hz), 115.58 (d,  $J_{CF} = 22.3$  Hz), 77.76, 53.42, 39.71, 32.70, 28.87, 24.56, 19.67, 13.03. **LRMS** (API-ES, positive mode):  $m/z$  439.2 [M+H]<sup>+</sup>.

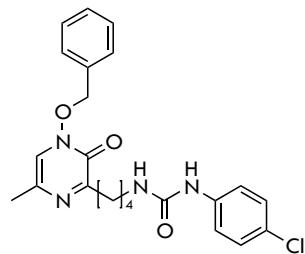
**1-Benzylxy-3-{4-[3-(4-chlorophenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23z)**



Compound **3.23z** was prepared by deprotection of **3.22h** (375 mg, 0.74 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (76  $\mu$ L, 0.67 mmol). Yield 52% (149 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.46 (s, 1H), 7.37 (s, 5H), 7.28 (d,  $J = 8.8$  Hz, 2H), 7.16 (d,  $J = 8.8$  Hz, 2H), 7.11 (d,  $J = 4.5$  Hz, 1H), 7.04 (d,  $J = 4.5$  Hz, 1H), 5.25 (s, 2H), 3.23 (t,  $J = 6.8$  Hz, 2H), 2.85 (t,  $J = 7.5$  Hz, 2H), 1.78-1.70 (m, 2H), 1.63-1.56 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  162.95, 156.45, 152.82, 138.29, 132.79, 129.92, 129.68, 128.78, 128.55, 126.90, 126.83, 121.72, 119.92, 78.79, 39.29, 32.89, 29.47, 23.78. **LRMS**

(API-ES, positive mode):  $m/z$  427.2 [M+H]<sup>+</sup>.

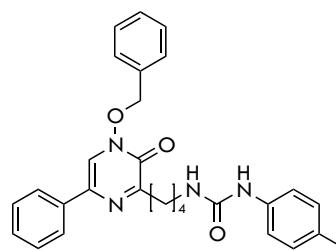
**1-Benzylxy-3-{4-[3-(4-chlorophenyl)ureido]butyl}-5-methylpyrazin-2(1*H*)-one (3.23za)**



Compound **3.23za** was prepared by deprotection of **3.22h** (375 mg, 0.74 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (103  $\mu$ L, 0.67 mmol). Yield 69% (204 mg). **1H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$

7.61 (s, 1H), 7.37 (s, 5H), 7.29 (d,  $J$  = 8.7 Hz, 2H), 7.14 (d,  $J$  = 8.7 Hz, 2H), 6.86 (s, 1H), 5.80 (t,  $J$  = 5.8 Hz, 1H), 5.23 (s, 2H), 3.27 (dt,  $J_1$  = 6.7 Hz,  $J_2$  = 5.8 Hz, 2H), 2.85 (t,  $J$  = 7.6 Hz, 2H), 2.16 (s, 3H), 1.80-1.70 (m, 2H), 1.61-1.55 (m, 2H). **13C NMR** (100 MHz, CDCl<sub>3</sub>):  $\delta$  161.76, 156.06, 151.50, 138.16, 132.82, 131.16, 129.86, 129.76, 128.88, 128.82, 127.25, 123.00, 120.52, 78.90, 39.72, 33.23, 29.49, 24.31, 19.52. **LRMS** (API-ES, positive mode):  $m/z$  441.1 [M+H]<sup>+</sup>.

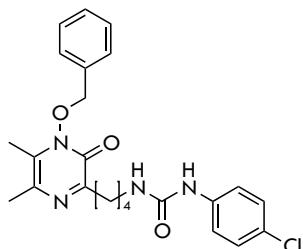
**1-Benzylxy-3-{4-[3-(4-chlorophenyl)ureido]butyl}-5-phenylpyrazin-2(1*H*)-one (3.23zb)**



Compound **3.23zb** was prepared by deprotection of **3.22h** (375 mg, 0.74 mmol) via procedure D and then cyclization via procedure E using phenylglyoxal monohydrate (102 mg, 0.67 mmol). Yield 24% (81 mg). **1H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$

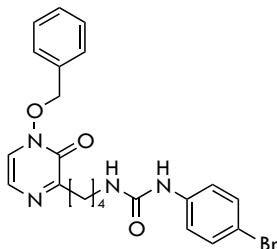
7.58 (d,  $J$  = 7.5 Hz, 2H), 7.45-7.41 (m, 6H), 7.37 (d,  $J$  = 7.5 Hz, 2H), 7.32 (d,  $J$  = 8.8 Hz, 2H), 7.20 (d,  $J$  = 8.8 Hz, 2H), 5.34 (s, 2H), 3.31 (t,  $J$  = 6.3 Hz, 2H), 2.98 (t,  $J$  = 7.6 Hz, 2H), 1.92-1.84 (m, 2H), 1.72-1.65 (m, 2H). **13C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  161.91, 156.67, 152.04, 138.63, 135.26, 133.16, 132.81, 130.32, 130.09, 129.18, 129.05, 128.92, 128.54, 127.15, 125.45, 122.74, 120.24, 79.28, 39.65, 33.41, 29.75, 24.07. **LRMS** (API-ES, positive mode):  $m/z$  503.2 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(4-chlorophenyl)ureido]butyl}-5,6-dimethyl-pyrazin-2(1*H*)-one (3.23zc)**



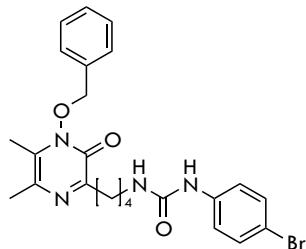
Compound **3.23zc** was prepared by deprotection of **3.22h** (375 mg, 0.74 mmol) via procedure D and then cyclization via procedure E using diacetyl (58  $\mu$ L, 0.67 mmol). Yield 57% (174 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.48-7.41 (m, 5H), 7.34-7.31 (m, 3H), 7.21-7.17 (m, 3H), 5.23 (s, 2H), 3.28 (overlapped, 2H), 2.83 (t, *J* = 7.6 Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.79-1.72 (m, 2H), 1.67-1.59 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  157.74, 156.30, 152.44, 138.50, 133.23, 133.06, 130.00, 129.86, 129.16, 129.07, 128.96, 126.95, 120.03, 77.99, 39.16, 33.10, 29.28, 24.58, 19.45, 13.10. **LRMS** (API-ES, positive mode): *m/z* 455.2 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(4-bromophenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23zd)**



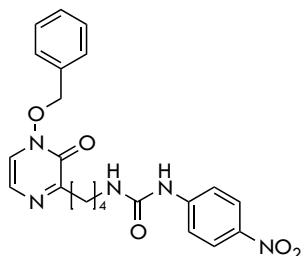
Compound **3.23zd** was prepared by deprotection of **3.22i** (700 mg, 1.27 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (131  $\mu$ L, 1.15 mmol). Yield 63% (341 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.43-7.39 (m, 5H), 7.34 (dd, *J*<sub>1</sub> = 9.0 Hz, *J*<sub>2</sub> = 1.9 Hz, 2H), 7.27 (dd, *J*<sub>1</sub> = 9.0 Hz, *J*<sub>2</sub> = 1.9 Hz, 2H), 7.06-7.05 (m, 2H), 5.28 (s, 2H), 3.28 (t, *J* = 6.5 Hz, 2H), 2.88 (t, *J* = 7.5 Hz, 2H), 1.80-1.72 (m, 2H), 1.65-1.58 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  163.34, 156.28, 152.91, 138.95, 132.90, 131.77, 130.08, 129.93, 129.03, 126.90, 121.85, 120.57, 120.45, 114.45, 78.98, 39.35, 33.14, 29.46, 23.98. **LRMS** (API-ES, positive mode): *m/z* 471.1 [M+H]<sup>+</sup>.

**1-Benzylxyloxy-3-{4-[3-(4-bromophenyl)ureido]butyl}-5,6-dimethyl-pyrazin-2(1*H*)-one (3.23ze)**



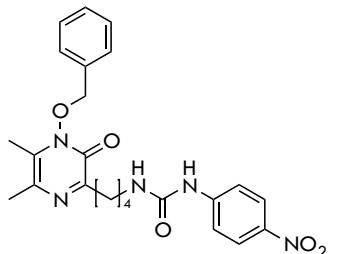
Compound **3.23ze** was prepared by deprotection of **3.22i** (700 mg, 1.27 mmol) via procedure D and then cyclization via procedure E using diacetyl (100 µL, 1.15 mmol). Yield 74% (425 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD): δ 7.47-7.41 (m, 5H), 7.33 (d, J = 8.8 Hz, 2H), 7.27 (d, J = 8.8 Hz, 2H), 5.23 (s, 2H), 3.29 (t, J = 6.4 Hz, 2H), 2.83 (t, J = 7.6 Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.79-1.72 (m, 2H), 1.66-1.60 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD): δ 157.72, 156.26, 152.44, 139.03, 133.23, 133.06, 131.75, 130.00, 129.86, 129.08, 128.96, 120.37, 114.36, 77.99, 39.18, 33.10, 29.31, 24.57, 19.44, 13.10. **LRMS** (API-ES, positive mode): *m/z* 499.1 [M+H]<sup>+</sup>.

**1-Benzylxyloxy-3-{4-[3-(4-nitrophenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23zf)**



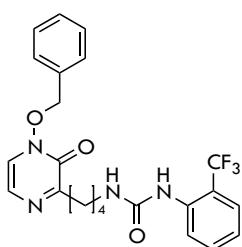
Compound **3.23zf** was prepared by deprotection of **3.22j** (850 mg, 1.65 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (169 µL, 1.49 mmol). Yield 61% (398 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD): δ 8.13 (d, J = 9.2 Hz, 2H), 7.56 (d, J = 9.2 Hz, 2H), 7.41 (s, 5H), 7.15 (d, J = 4.6 Hz, 1H), 7.09 (d, J = 4.6 Hz, 1H), 5.29 (s, 2H), 3.30 (t, J = 6.7 Hz, 2H), 2.90 (t, J = 7.6 Hz, 2H), 1.83-1.76 (m, 2H), 1.69-1.62 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD): δ 163.33, 155.87, 153.23, 146.93, 141.87, 133.19, 130.34, 130.13, 129.22, 127.32, 125.45, 122.17, 117.53, 79.24, 39.73, 33.32, 29.76, 24.18. **LRMS** (API-ES, positive mode): *m/z* 438.2 [M+H]<sup>+</sup>.

**1-Benzylxy-5,6-dimethyl-3-{4-[3-(4-nitrophenyl)ureido]butyl}-pyrazin-2(1*H*)-one (3.23zg)**



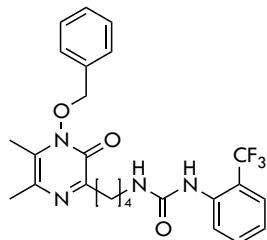
Compound **3.23zg** was prepared by deprotection of **3.22j** (850 mg, 1.65 mmol) via procedure D and then cyclization via procedure E using diacetyl (130  $\mu$ L, 1.49 mmol). Yield 43% (298 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  8.12 (d, *J* = 9.0 Hz, 2H), 7.55 (d, *J* = 9.0 Hz, 2H), 7.48-7.41 (m, 5H), 6.09 (t, *J* = 5.5 Hz, 1H), 5.24 (s, 2H), 3.31 (t, *J* = 6.2 Hz, 2H), 2.85 (t, *J* = 7.6 Hz, 2H), 2.28 (s, 3H), 2.25 (s, 3H), 1.82-1.75 (m, 2H), 1.70-1.63 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  157.59, 155.74, 152.62, 146.83, 141.74, 133.56, 133.20, 130.16, 129.97, 129.28, 129.05, 125.35, 117.40, 78.14, 39.54, 33.16, 29.60, 24.65, 19.41, 13.12. **LRMS** (API-ES, positive mode): *m/z* 466.2 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(2-trifluoromethylphenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23zh)**



Compound **3.23zh** was prepared by deprotection of **3.22k** (800 mg, 1.49 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (152  $\mu$ L, 1.34 mmol). Yield 49% (302 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.93 (d, *J* = 8.2 Hz, 1H), 7.56 (d, *J* = 8.2 Hz, 1H), 7.50 (t, *J* = 8.2 Hz, 1H), 7.42-7.40 (m, 5H), 7.15 (overlapped, 1H), 7.14 (d, *J* = 4.6 Hz, 1H), 7.08 (d, *J* = 4.6 Hz, 1H), 5.29 (s, 2H), 3.29 (t, *J* = 6.8 Hz, 2H), 2.90 (t, *J* = 7.6 Hz, 2H), 1.83-1.76 (m, 2H), 1.69-1.61 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  163.44, 156.82, 153.25, 137.12, 133.24, 132.78, 130.37, 130.12, 129.22, 127.33, 126.17 (*q*, *J*<sub>CF</sub> = 5.4 Hz), 125.50, 124.55 (*q*, *J*<sub>CF</sub> = 272.5 Hz), 123.47, 122.11, 120.94 (*q*, *J*<sub>CF</sub> = 29.6 Hz), 79.20, 39.92, 33.34, 29.75, 24.29. **LRMS** (API-ES, positive mode): *m/z* 461.2 [M+H]<sup>+</sup>.

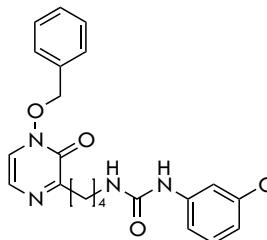
**1-Benzylxyloxy-5,6-dimethyl-3-{4-[3-(2-trifluoromethylphenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23zi)**



Compound **3.23zi** was prepared by deprotection of **3.22k** (800 mg, 1.49 mmol) via procedure D and then cyclization via procedure E using diacetyl (117  $\mu$ L, 1.34 mmol). Yield 64% (419 mg).

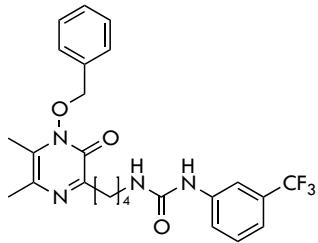
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.97 (d, *J* = 8.2 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.49 (t, *J* = 8.2 Hz, 1H), 7.48-7.46 (m, 2H), 7.42-7.40 (m, 3H), 7.30 (s, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 6.63 (t, *J* = 6.0 Hz, 1H), 5.24 (s, 2H), 3.31 (dt, *J*<sub>1</sub> = 6.6 Hz, *J*<sub>2</sub> = 6.0 Hz, 2H), 2.84 (t, *J* = 7.7 Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.81-1.73 (m, 2H), 1.69-1.62 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  157.56, 156.10, 152.23, 136.88, 133.04, 132.92, 132.38, 129.82, 129.59, 128.71, 128.66, 125.73 (q, *J*<sub>CF</sub> = 5.2 Hz), 124.71, 124.11 (q, *J*<sub>CF</sub> = 272.2 Hz), 122.79, 120.11 (q, *J*<sub>CF</sub> = 29.1 Hz), 77.73, 39.46, 32.85, 29.06, 24.50, 19.17, 12.87. **LRMS** (API-ES, positive mode): *m/z* 489.2 [M+H]<sup>+</sup>.

**1-Benzylxyloxy-3-{4-[3-(3-trifluoromethylphenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23zj)**



Compound **3.23zj** was prepared by deprotection of **3.22l** (900 mg, 1.67 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (171  $\mu$ L, 1.50 mmol). Yield 46% (318 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.75 (s, 1H), 7.54-7.50 (m, 1H), 7.41 (s, 5H), 7.36 (t, *J* = 8.2 Hz, 1H), 7.20 (d, *J* = 7.9 Hz, 1H), 7.15 (d, *J* = 4.4 Hz, 1H), 7.09 (d, *J* = 4.4 Hz, 1H), 5.29 (s, 2H), 3.29 (t, *J* = 6.6 Hz, 2H), 2.90 (t, *J* = 7.5 Hz, 2H), 1.83-1.75 (m, 2H), 1.68-1.61 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  163.39, 156.76, 153.29, 140.85, 133.25, 131.43 (q, *J*<sub>CF</sub> = 32.0 Hz), 130.40, 130.15, 129.62, 129.24, 127.37, 124.56 (q, *J*<sub>CF</sub> = 272.3 Hz), 122.20, 121.91, 118.70 (q, *J*<sub>CF</sub> = 3.7 Hz), 115.43 (q, *J*<sub>CF</sub> = 3.9 Hz), 79.26, 39.75, 33.36, 29.91, 24.24. **LRMS** (API-ES, positive mode): *m/z* 461.2 [M+H]<sup>+</sup>.

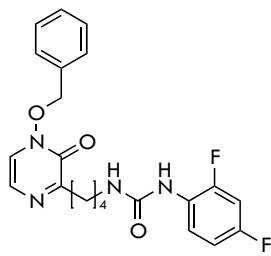
**1-Benzylxy-5,6-dimethyl-3-{4-[3-(3-trifluoromethylphenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23zk)**



Compound **3.23zk** was prepared by deprotection of **3.22l** (900 mg, 1.67 mmol) via procedure D and then cyclization via procedure E using diacetyl (132  $\mu$ L, 1.50 mmol). Yield 49% (359 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.72 (s, 1H), 7.54

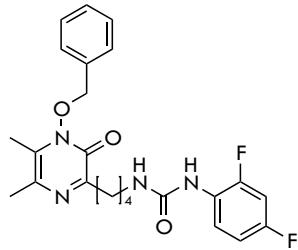
(dd,  $J_1$  = 8.0 Hz,  $J_2$  = 1.8 Hz, 1H), 7.48-7.40 (m, 5H), 7.34 (t,  $J$  = 8.0 Hz, 1H), 7.19 (d,  $J$  = 8.0 Hz, 1H), 5.24 (s, 2H), 3.30 (t,  $J$  = 6.5 Hz, 2H), 2.84 (t,  $J$  = 7.7 Hz, 2H), 2.27 (s, 3H), 2.24 (s, 3H), 1.81-1.74 (m, 2H), 1.68-1.61 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  157.71, 156.43, 152.58, 140.67, 133.43, 133.17, 131.26 (q,  $J_{CF}$  = 31.9 Hz), 130.13, 129.95, 129.44, 129.21, 129.04, 124.37 (q,  $J_{CF}$  = 272.2 Hz), 121.69, 118.52 (q,  $J_{CF}$  = 3.9 Hz), 78.11, 39.40, 33.16, 29.56, 24.66, 19.45, 13.14. **LRMS** (API-ES, positive mode): *m/z* 489.2 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(2,4-difluorophenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23zl)**



Compound **3.23zl** was prepared by deprotection of **3.22m** (500 mg, 0.99 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (101  $\mu$ L, 0.89 mmol). Yield 49% (187 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.97 (m, 1H), 7.41 (s, 5H), 7.14 (d,  $J$  = 4.6 Hz, 1H), 7.08 (d,  $J$  = 4.6 Hz, 1H), 6.85-6.79 (m, 2H), 5.29 (s, 2H), 3.28 (t,  $J$  = 6.8 Hz, 2H), 2.90 (t,  $J$  = 7.6 Hz, 2H), 1.83-1.75 (m, 2H), 1.68-1.60 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  163.40, 157.98 (dd,  $J_{CF}$  = 242.8 Hz,  $J_{CF}$  = 11.5 Hz), 156.81, 153.23, 153.05 (dd,  $J_{CF}$  = 244.3 Hz,  $J_{CF}$  = 11.5 Hz), 133.22, 130.35, 130.11, 129.21, 127.31, 124.40 (dd,  $J_{CF}$  = 11.0 Hz,  $J_{CF}$  = 3.7 Hz), 122.74 (dd,  $J_{CF}$  = 9.9 Hz,  $J_{CF}$  = 1.4 Hz), 122.12, 111.02 (dd,  $J_{CF}$  = 21.7 Hz,  $J_{CF}$  = 3.8 Hz), 103.55 (dd,  $J_{CF}$  = 26.8 Hz,  $J_{CF}$  = 23.9 Hz), 79.20, 39.76, 33.34, 29.85, 24.23. **LRMS** (API-ES, positive mode): *m/z* 429.2 [M+H]<sup>+</sup>.

**1-Benzylxy-3-{4-[3-(2,4-difluorophenyl)ureido]butyl}-5,6-dimethyl-pyrazin-2(1*H*)-one (3.23zm)**

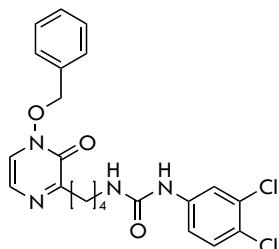


Compound **3.23zm** was prepared by deprotection of **3.22m** (500 mg, 0.99 mmol) via procedure D and then cyclization via procedure E using diacetyl (78  $\mu$ L, 0.89 mmol). Yield 51% (207 mg).

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.99-7.90 (m, 1H), 7.49-7.42 (m, 5H), 6.85-6.78 (m, 2H), 5.25 (s, 2H), 3.29 (t, *J* = 6.5 Hz, 2H), 2.85 (d, *J* = 7.6 Hz, 2H), 2.28 (s, 3H), 2.25 (s, 3H), 1.82-1.75 (m, 2H), 1.69-1.62 (m, 2H).

**<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  157.96 (dd, *J*<sub>CF</sub> = 244.1 Hz, *J*<sub>CF</sub> = 11.0 Hz), 157.76, 156.89, 154.78 (dd, *J*<sub>CF</sub> = 242.1 Hz, *J*<sub>CF</sub> = 12.0 Hz), 152.73, 133.65, 133.36, 130.29, 130.12, 130.03, 129.30, 129.13, 124.44 (dd, *J*<sub>CF</sub> = 8.1 Hz, *J*<sub>CF</sub> = 3.7 Hz), 122.69 (dd, *J*<sub>CF</sub> = 8.6 Hz, *J*<sub>CF</sub> = 1.8 Hz), 111.00 (dd, *J*<sub>CF</sub> = 21.8 Hz, *J*<sub>CF</sub> = 3.7 Hz), 103.53 (dd, *J*<sub>CF</sub> = 26.7 Hz, *J*<sub>CF</sub> = 23.8 Hz), 78.21, 39.72, 33.27, 29.86, 24.82, 19.44, 13.17. **LRMS** (API-ES, positive mode): *m/z* 457.2 [M+H]<sup>+</sup>.

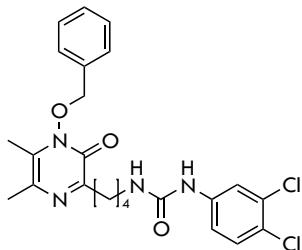
**1-Benzylxy-3-{4-[3-(3,4-dichlorophenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.23zn)**



Compound **3.23zn** was prepared by deprotection of **3.22n** (900 mg, 1.67 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (171  $\mu$ L, 1.50 mmol). Yield 42% (291 mg).

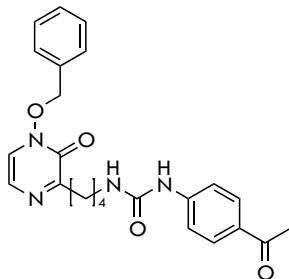
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.64 (d, *J* = 2.4 Hz, 1H), 7.41 (s, 5H), 7.28 (d, *J* = 8.8 Hz, 1H), 7.19 (dd, *J*<sub>1</sub> = 8.8 Hz, *J*<sub>2</sub> = 2.4 Hz, 1H), 7.12 (d, *J* = 4.7 Hz, 1H), 7.08 (d, *J* = 4.7 Hz, 1H), 5.29 (s, 2H), 3.27 (t, *J* = 6.7 Hz, 2H), 2.89 (t, *J* = 7.6 Hz, 2H), 1.82-1.74 (m, 2H), 1.67-1.60 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  163.36, 156.39, 153.18, 139.89, 133.14, 132.59, 130.48, 130.30, 130.09, 129.19, 127.24, 124.96, 122.10, 120.29, 118.13, 79.19, 39.64, 33.29, 29.78, 24.15. **LRMS** (API-ES, positive mode): *m/z* 461.1 [M+H]<sup>+</sup>.

**1-Benzylxyloxy-3-{4-[3-(3,4-dichlorophenyl)ureido]butyl}-5,6-dimethyl-pyrazin-2(1*H*)-one (3.23zo)**



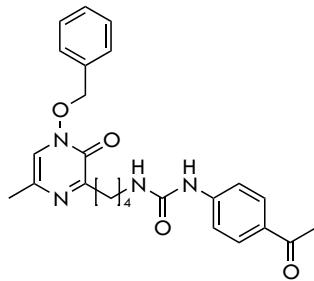
Compound **3.23zo** was prepared by deprotection of **3.22n** (900 mg, 1.67 mmol) via procedure D and then cyclization via procedure E using diacetyl (132  $\mu$ L, 1.50 mmol). Yield 37% (272 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.63 (d, *J* = 2.5 Hz, 1H), 7.48-7.42 (m, 5H), 7.27 (d, *J* = 8.8 Hz, 1H), 7.19 (dd, *J*<sub>1</sub> = 8.8 Hz, *J*<sub>2</sub> = 2.5 Hz, 1H), 5.24 (s, 2H), 3.28 (t, *J* = 6.5 Hz, 2H), 2.84 (t, *J* = 7.6 Hz, 2H), 2.27 (s, 3H), 2.24 (s, 3H), 1.80-1.73 (m, 2H), 1.67-1.61 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  157.71, 156.26, 152.61, 139.85, 133.50, 133.19, 132.53, 130.42, 130.16, 129.98, 129.25, 129.07, 124.85, 120.19, 118.02, 78.14, 39.44, 33.18, 29.60, 24.67, 19.45, 13.16. **LRMS** (API-ES, positive mode): *m/z* 489.1 [M+H]<sup>+</sup>.

**3-{4-[3-(4-Acetophenyl)ureido]butyl}-1-benzylxyloxy-pyrazin-2(1*H*)-one (3.23zp)**



Compound **3.23zp** was prepared by deprotection of **3.22o** (533 mg, 1.04 mmol) via procedure D and then cyclization via procedure E using a 40% glyoxal solution (107  $\mu$ L, 0.94 mmol). Yield 71% (290 mg). **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  7.88 (d, *J* = 8.7 Hz, 2H), 7.49 (d, *J* = 8.7 Hz, 2H), 7.40 (s, 5H), 7.15 (d, *J* = 4.5 Hz, 1H), 7.08 (d, *J* = 4.5 Hz, 1H), 5.29 (s, 2H), 3.29 (t, *J* = 6.6 Hz, 2H), 2.90 (t, *J* = 7.5 Hz, 2H), 2.55 (s, 3H), 1.83-1.75 (m, 2H), 1.68-1.61 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  198.78, 163.37, 156.32, 153.26, 145.46, 133.25, 130.82, 130.38, 130.31, 130.14, 129.23, 127.34, 122.19, 117.64, 79.25, 39.74, 33.35, 29.86, 26.32, 24.23. **LRMS** (API-ES, positive mode): *m/z* 435.2 [M+H]<sup>+</sup>.

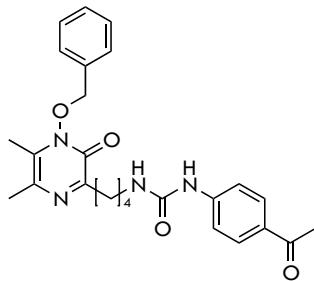
**3-{4-[3-(4-Acetophenyl)ureido]butyl}-1-benzyloxy-5-methylpyrazin-2(1*H*)-one (3.23zq)**



Compound **3.23zq** was prepared by deprotection of **3.22o** (533 mg, 1.04 mmol) via procedure D and then cyclization via procedure E using a 40% methylglyoxal solution (144 µL, 0.94 mmol). Yield 64% (270 mg). **<sup>1</sup>H NMR** (600 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$

7.88 (d, *J* = 8.8 Hz, 2H), 7.49 (d, *J* = 8.8 Hz, 2H), 7.43-7.40 (m, 5H), 7.01 (s, 1H), 5.26 (s, 2H), 3.30 (t, *J* = 6.7 Hz, 2H), 2.88 (t, *J* = 7.6 Hz, 2H), 2.56 (s, 3H), 2.18 (s, 3H), 1.81-1.76 (m, 2H), 1.68-1.63 (m, 2H). **<sup>13</sup>C NMR** (150 MHz, CDCl<sub>3</sub>-CD<sub>3</sub>OD):  $\delta$  198.78, 162.13, 156.31, 152.04, 145.46, 133.32, 131.39, 130.78, 130.31, 130.06, 129.17, 124.04, 117.62, 79.22, 39.68, 33.52, 29.87, 26.33, 24.59, 19.34. **LRMS** (API-ES, positive mode): *m/z* 449.2 [M+H]<sup>+</sup>.

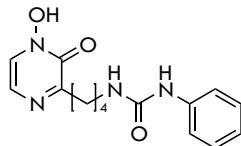
**3-{4-[3-(4-Acetophenyl)ureido]butyl}-1-benzyloxy-5,6-dimethyl-pyrazin-2(1*H*)-one (3.23zr)**



Compound **3.23zr** was prepared by deprotection of **3.22o** (533 mg, 1.04 mmol) via procedure D and then cyclization via procedure E using diacetyl (82 µL, 0.94 mmol). Yield 80% (348 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.87 (s, 1H), 7.83 (dd, *J*<sub>1</sub> = 8.5 Hz, *J*<sub>2</sub> = 1.8 Hz, 2H), 7.52-7.42 (m, 7H), 6.33 (t, *J* = 5.6 Hz, 1H),

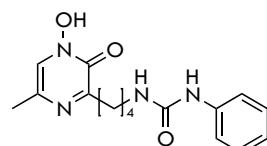
5.20 (s, 2H), 3.13 (overlapped, 2H), 2.71 (t, *J* = 7.2 Hz, 2H), 2.47 (s, 3H), 2.24 (s, 3H), 2.19 (s, 3H), 1.69-1.62 (m, 2H), 1.54-1.47 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  196.22, 156.46, 154.74, 151.13, 145.26, 133.68, 132.88, 129.84, 129.69, 129.62, 129.29, 128.61, 126.75, 116.51, 76.76, 38.97, 32.37, 29.47, 26.25, 23.87, 19.35, 12.65. **LRMS** (API-ES, positive mode): *m/z* 463.2 [M+H]<sup>+</sup>.

**1-Hydroxy-3-[4-(3-phenylureido)butyl]pyrazin-2(1*H*)-one (3.28a)**



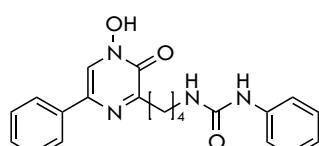
Compound **3.28a** was prepared by debenzylation of **3.23a** (39.3 mg, 0.1 mmol) in flow via procedure H. Yield 90% (27.2 mg). **<sup>1</sup>H NMR** (600 MHz, DMSO-d<sub>6</sub>): δ 8.37 (s, 1H), 7.81 (d, *J* = 3.9 Hz, 1H), 7.37 (d, *J* = 7.8 Hz, 2H), 7.21-7.19 (m, 3H), 6.87 (t, *J* = 7.3 Hz, 1H), 6.13 (t, *J* = 5.8 Hz, 1H), 3.10 (dt, *J*<sub>1</sub> = 6.7 Hz, *J*<sub>2</sub> = 5.8 Hz, 2H), 2.74 (t, *J* = 7.5 Hz, 2H), 1.68-1.63 (m, 2H), 1.50-1.45 (m, 2H). **<sup>13</sup>C NMR** (150 MHz, DMSO-d<sub>6</sub>): δ 158.7, 155.07, 152.05, 140.49, 128.49, 126.53, 121.17, 120.76, 117.44, 117.34, 38.76, 32.34, 29.42, 23.48. **LRMS** (API-ES, positive mode): *m/z* 303.1 [M+H]<sup>+</sup>.

**1-Hydroxy-5-methyl-3-[4-(3-phenylureido)butyl]pyrazin-2(1*H*)-one (3.28b)**



Compound **3.28b** was prepared by debenzylation of **3.23b** (40.7 mg, 0.1 mmol) in flow via procedure H. Yield 92% (29.1 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 8.38 (s, 1H), 7.65 (s, 1H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.20 (dd, *J*<sub>1</sub> = 8.0 Hz, *J*<sub>2</sub> = 7.5 Hz, 2H), 6.87 (t, *J* = 7.5 Hz, 1H), 6.15 (t, *J* = 5.9 Hz, 1H), 3.10 (dt, *J*<sub>1</sub> = 6.6 Hz, *J*<sub>2</sub> = 5.9 Hz, 2H), 2.72 (t, *J* = 7.5 Hz, 2H), 2.17 (s, 3H), 1.68-1.61 (m, 2H), 1.52-1.45 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 158.10, 155.66, 151.34, 141.06, 129.84, 129.06, 123.98, 121.33, 118.03, 39.58, 33.07, 30.07, 24.31, 19.58. **LRMS** (API-ES, positive mode): *m/z* 317.2 [M+H]<sup>+</sup>.

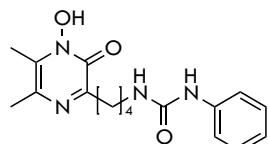
**1-Hydroxy-5-phenyl-3-[4-(3-phenylureido)butyl]pyrazin-2(1*H*)-one (3.28c)**



Compound **3.28c** was prepared by debenzylation of **3.23c** (46.9 mg, 0.1 mmol) in flow via procedure H. Yield 74% (28.0 mg). **<sup>1</sup>H NMR** (600 MHz, DMSO-d<sub>6</sub>): δ 8.40 (s, 1H), 7.90 (d, *J* = 7.5 Hz, 2H), 7.41-7.37 (m, 5H), 7.29 (t, *J* = 7.2 Hz, 1H), 7.20 (t, *J* = 7.5

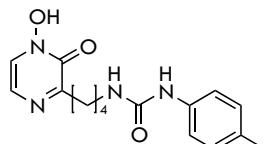
Hz, 2H), 6.87 (t,  $J$  = 7.5 Hz, 1H), 6.19 (t,  $J$  = 5.9 Hz, 1H), 3.15 (dt,  $J_1$  = 6.6 Hz,  $J_2$  = 5.9 Hz, 2H), 2.82 (t,  $J$  = 7.3 Hz, 2H), 1.77-1.72 (m, 2H), 1.57-1.52 (m, 2H).  **$^{13}\text{C}$  NMR** (150 MHz, DMSO-d<sub>6</sub>):  $\delta$  155.68, 141.09, 136.61, 129.09, 129.06, 128.88, 127.81, 125.13, 121.32, 118.02, 39.39, 33.01, 30.02, 24.15. **LRMS** (API-ES, positive mode): *m/z* 385.2 [M+Li]<sup>+</sup>.

**1-Hydroxy-5,6-dimethyl-3-[4-(3-phenylureido)butyl]pyrazin-2(1*H*)-one (3.28d)**



Compound **3.28d** was prepared by debenzylation of **3.23d** (42.1 mg, 0.1 mmol) in flow via procedure H. Yield 93% (30.7 mg).  **$^1\text{H}$  NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.9-7.22 (m, 5H), 7.05 (brs, 1H), 7.00 (t,  $J$  = 6.9 Hz, 1H), 3.27 (t,  $J$  = 6.4 Hz, 2H), 2.80 (t,  $J$  = 7.4 Hz, 2H), 2.38 (s, 3H), 2.33 (s, 3H), 1.76-1.69 (m, 2H), 1.58-1.52 (m, 2H).  **$^{13}\text{C}$  NMR** (100 MHz, CDCl<sub>3</sub>):  $\delta$  156.17, 150.81, 138.95, 131.55, 129.16, 123.25, 120.46, 120.18, 39.76, 32.43, 29.15, 24.66, 19.82, 12.81. **LRMS** (API-ES, positive mode): *m/z* 331.2 [M+H]<sup>+</sup>.

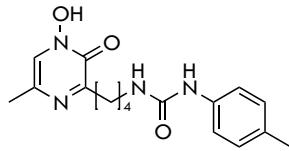
**1-Hydroxy-3-[4-[3-(*p*-tolyl)ureido]butyl]pyrazin-2(1*H*)-one (3.28e)**



Compound **3.28e** was prepared by debenzylation of **3.23e** (40.7 mg, 0.1 mmol) in flow via procedure H. Yield 98% (31.0 mg).  **$^1\text{H}$  NMR** (400 MHz, CD<sub>3</sub>OD):  $\delta$  8.25 (s, 1H), 7.81 (d,  $J$  = 4.2 Hz, 1H), 7.25 (d,  $J$  = 8.0 Hz, 2H), 7.20 (d,  $J$  = 4.2 Hz, 1H), 7.01 (d,  $J$  = 8.0 Hz, 2H), 6.08 (t,  $J$  = 5.7 Hz, 1H), 3.09 (dt,  $J_1$  = 6.7 Hz,  $J_2$  = 5.7 Hz, 2H), 2.74 (t,  $J$  = 7.4 Hz, 2H), 2.21 (s, 3H), 1.69-1.62 (m, 2H), 1.51-1.44 (m, 2H).  **$^{13}\text{C}$  NMR** (100 MHz, CD<sub>3</sub>OD):  $\delta$  159.32, 155.74, 152.64, 138.59, 130.03, 129.46, 127.12, 121.73, 118.15, 39.37, 32.91, 30.03, 24.06, 20.74. **LRMS** (API-ES, positive mode): *m/z* 317.2 [M+H]<sup>+</sup>.

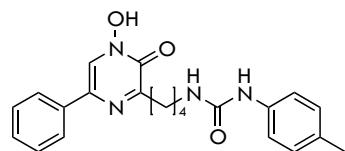
**1-Hydroxy-5-methyl-3-[4-[3-(*p*-tolyl)ureido]butyl]pyrazin-2(1*H*)-one (3.28f)**

Compound **3.28f** was prepared by debenzylation of **3.23f** (42.1 mg, 0.1 mmol) in flow via procedure H. Yield 90% (29.7 mg).



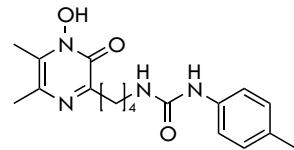
**<sup>1</sup>H NMR** (600 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.26 (s, 1H), 7.65 (s, 1H), 7.25 (d,  $J$  = 8.2 Hz, 2H), 7.00 (d,  $J$  = 8.2 Hz, 2H), 6.09 (t,  $J$  = 5.9 Hz, 1H), 3.09 (dt,  $J_1$  = 6.7 Hz,  $J_2$  = 5.9 Hz, 2H), 2.71 (t,  $J$  = 7.6 Hz, 2H), 2.20 (s, 3H), 2.17 (s, 3H), 1.66-1.61 (m, 2H), 1.49-1.44 (m, 2H). **<sup>13</sup>C NMR** (150 MHz, DMSO-d<sub>6</sub>):  $\delta$  158.16, 155.73, 151.31, 138.50, 137.80, 130.01, 129.8, 129.46, 123.96, 118.13, 39.36, 33.08, 30.11, 24.32, 20.74, 19.58. **LRMS** (API-ES, positive mode): *m/z* 331.2 [M+H]<sup>+</sup>.

**1-Hydroxy-5-phenyl-3-{4-[3-(p-tolyl)ureido]butyl}pyrazin-2(1H)-one (3.28g)**



Compound **3.28g** was prepared by debenzylation of **3.23g** (24.1 mg, 0.05 mmol) in flow via procedure H. Yield 81% (15.9 mg). **<sup>1</sup>H NMR** (600 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.46 (s, 1H), 8.27 (s, 1H), 7.91 (d,  $J$  = 7.5 Hz, 2H), 7.40 (t,  $J$  = 7.5 Hz, 2H), 7.30 (t,  $J$  = 7.5 Hz, 1H), 7.26 (d,  $J$  = 8.2 Hz, 2H), 7.00 (d,  $J$  = 8.2 Hz, 2H), 6.12 (t,  $J$  = 5.9 Hz, 1H), 3.13 (dt,  $J_1$  = 6.9 Hz,  $J_2$  = 5.9 Hz, 2H), 2.83 (t,  $J$  = 7.4 Hz, 2H), 2.21 (s, 3H), 1.78-1.73 (m, 2H), 1.56-1.51 (m, 2H). **<sup>13</sup>C NMR** (150 MHz, DMSO-d<sub>6</sub>):  $\delta$  157.56, 155.76, 151.96, 138.52, 136.10, 130.49, 130.00, 129.45, 129.11, 127.87, 125.14, 123.68, 118.14, 39.40, 33.07, 30.06, 24.07, 20.75. **LRMS** (API-ES, positive mode): *m/z* 393.2 [M+H]<sup>+</sup>.

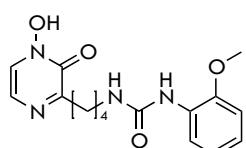
**1-Hydroxy-5,6-dimethyl-3-{4-[3-(p-tolyl)ureido]butyl}pyrazin-2(1H)-one (3.28h)**



Compound **3.28h** was prepared by debenzylation of **3.23h** (43.5 mg, 0.1 mmol) in flow via procedure H. Yield 92% (31.7 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.26 (s, 1H), 7.25 (d,  $J$  = 8.1 Hz, 2H), 7.00 (d,  $J$  = 8.1 Hz, 2H), 6.09 (t,  $J$  = 5.7 Hz, 1H), 3.08 (dt,  $J_1$  = 6.7 Hz,  $J_2$  = 5.7 Hz, 2H), 2.68 (t,  $J$  = 7.4 Hz, 2H),

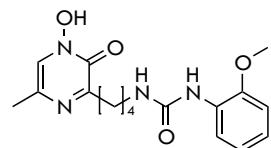
2.27 (s, 3H), 2.23 (s, 3H), 2.20 (s, 3H), 1.67-1.59 (m, 2H), 1.50-1.43 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  155.74, 152.37, 151.43, 138.50, 132.61, 130.01, 129.44, 128.11, 118.14, 39.36, 32.78, 30.11, 24.55, 20.73, 19.97, 13.18. **LRMS** (API-ES, positive mode): *m/z* 345.2 [M+H]<sup>+</sup>.

**1-Hydroxy-3-[4-[3-(2-methoxyphenyl)ureido]butyl]pyrazin-2(1H)-one (3.28i)**



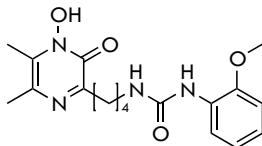
Compound **3.28i** was prepared by debenzylolation of **3.23i** (42.3 mg, 0.1 mmol) in flow via procedure H. Quantitative yield (33.2 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.08 (d, *J* = 7.3 Hz, 1H), 7.85 (brs, 1H), 7.81 (brs, 1H), 7.20 (brs, 1H), 6.94 (d, *J* = 7.2 Hz, 1H), 6.887-6.80 (m, 3H), 3.82 (s, 3H), 3.10 (dt, *J*<sub>1</sub> = 7.0 Hz, *J*<sub>2</sub> = 5.6 Hz, 2H), 2.74 (t, *J* = 7.0 Hz, 2H), 1.71-1.64 (m, 2H), 1.51-1.44 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  159.34, 155.59, 152.63, 147.67, 130.05, 127.13, 121.69, 121.24, 120.91, 118.31, 110.99, 56.10, 39.28, 32.90, 29.91, 24.11. **LRMS** (API-ES, positive mode): *m/z* 333.1 [M+H]<sup>+</sup>.

**1-Hydroxy-3-[4-[3-(2-methoxyphenyl)ureido]butyl]-5-methylpyrazin-2(1H)-one (3.28j)**



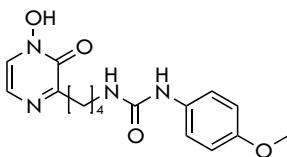
Compound **3.28j** was prepared by debenzylolation of **3.23j** (43.7 mg, 0.1 mmol) in flow via procedure H. Yield 78% (27.0 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.08 (dd, *J*<sub>1</sub> = 7.4 Hz, *J*<sub>2</sub> = 1.8 Hz, 1H), 7.83 (s, 1H), 7.63 (s, 1H), 6.94 (dd, *J*<sub>1</sub> = 7.4 Hz, *J*<sub>2</sub> = 1.8 Hz, 1H), 6.87-6.81 (m, 3H), 3.83 (s, 3H), 3.11 dt, *J*<sub>1</sub> = 6.6 Hz, *J*<sub>2</sub> = 5.8 Hz, 2H), 2.73 (t, *J* = 7.5 Hz, 2H), 2.18 (s, 3H), 1.70-1.63 (m, 2H), 1.51-1.45 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  158.23, 155.61, 151.28, 144.73, 130.10, 129.82, 123.86, 121.26, 120.93, 118.38, 56.14, 39.33, 33.04, 29.98, 24.35, 19.56. **LRMS** (API-ES, positive mode): *m/z* 347.2 [M+H]<sup>+</sup>.

**1-Hydroxy-3-{4-[3-(2-methoxyphenyl)ureido]butyl}-5,6-dimethyl-pyrazin-2(1*H*)-one (3.28k)**



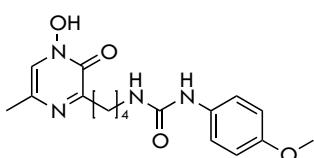
Compound **3.28k** was prepared by debenzylation of **3.23k** (45.1 mg, 0.1 mmol) in flow via procedure H. Yield 91% (32.8 mg). **<sup>1</sup>H NMR** (300 MHz, DMSO-d<sub>6</sub>): δ 8.07 (dd, *J*<sub>1</sub> = 7.0 Hz, *J*<sub>2</sub> = 2.5 Hz, 1H), 7.86 (s, 1H), 6.95-6.92 (m, 1H), 6.87-6.79 (m, 3H), 3.82 (s, 3H), 3.09 (dt, *J*<sub>1</sub> = 6.9 Hz, *J*<sub>2</sub> = 5.8 Hz, 2H), 2.68 (t, *J* = 7.5 Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.69-1.59 (m, 2H), 1.50-1.41 (m, 2H). **<sup>13</sup>C NMR** (75 MHz, DMSO-d<sub>6</sub>): δ 155.08, 152.07, 150.79, 147.13, 132.01, 129.51, 127.59, 120.72, 120.39, 117.75, 110.43, 55.57, 38.80, 32.26, 29.49, 24.07, 19.44, 12.67. **LRMS** (API-ES, positive mode): *m/z* 361.2 [M+H]<sup>+</sup>.

**1-Hydroxy-3-{4-[3-(4-methoxyphenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.28l)**



Compound **3.28l** was prepared by debenzylation of **3.23l** (42.3 mg, 0.1 mmol) in flow via procedure H. Yield 92% (30.6 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 8.17 (s, 1H), 7.81 (brs, 1H), 7.27 (d, *J* = 8.9 Hz, 2H), 7.21 (brs, 1H), 6.80 (d, *J* = 8.9 Hz, 2H), 6.02 (t, *J* = 5.7 Hz, 1H), 3.69 (s, 3H), 3.08 (dt, *J*<sub>1</sub> = 6.8 Hz, *J*<sub>2</sub> = 5.7 Hz, 2H), 2.73 (t, *J* = 6.8 Hz, 2H), 1.69-1.62 (m, 2H), 1.50-1.43 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 155.90, 154.30, 134.22, 127.10, 121.77, 119.79, 114.32, 55.60, 39.38, 32.94, 30.06, 24.09. **LRMS** (API-ES, positive mode): *m/z* 333.2 [M+H]<sup>+</sup>.

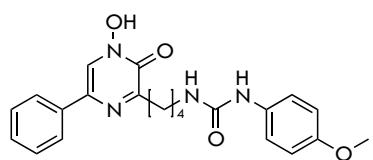
**1-Hydroxy-3-{4-[3-(4-methoxyphenyl)ureido]butyl}-5-methylpyrazin-2(1*H*)-one (3.28m)**



Compound **3.28m** was prepared by debenzylation of **3.23m** (43.7 mg, 0.1 mmol) in flow via procedure H. Yield 88% (30.5 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 8.21 (d, *J* = 4.7 Hz, 1H),

7.63 (brs, 1H), 7.27 (d,  $J = 8.9$  Hz, 2H), 6.80 (d,  $J = 8.9$  Hz, 2H), 6.08 (t,  $J = 5.7$  Hz, 1H), 3.69 (s, 3H), 3.08 (dt,  $J_1 = 6.8$  Hz,  $J_2 = 5.7$  Hz, 2H), 2.70 (t,  $J = 7.4$  Hz, 2H), 2.16 (s, 3H), 1.67-1.58 (m, 2H), 1.50-1.42 (m, 2H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  155.91, 154.27, 134.25, 129.93, 128.50, 124.51, 119.77, 114.49, 114.31, 55.60, 39.21, 33.08, 30.14, 24.41, 19.65. **LRMS** (API-ES, positive mode): *m/z* 347.2 [M+H]<sup>+</sup>.

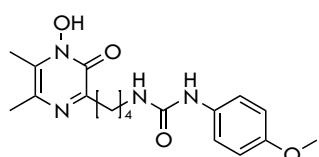
**1-Hydroxy-3-{4-[3-(4-methoxyphenyl)ureido]butyl}-5-phenylpyrazin-2(1*H*)-one (3.28n)**



Compound **3.28n** was prepared by debenzylation of **3.23n** (24.9 mg, 0.05 mmol) in flow via procedure H. Yield 80% (16.3 mg).  **$^1\text{H}$  NMR** (600

MHz, DMSO-d<sub>6</sub>):  $\delta$  8.44 (s, 1H), 8.21 (s, 1H), 7.90 (d,  $J = 7.7$  Hz, 2H), 7.40 (t,  $J = 7.7$  Hz, 2H), 7.29 (t,  $J = 7.7$  Hz, 1H), 7.28 (d,  $J = 8.8$  Hz, 2H), 6.79 (d,  $J = 8.8$  Hz, 2H), 6.09 (t,  $J = 5.7$  Hz, 1H), 3.69 (s, 3H), 3.13 (dt,  $J_1 = 6.8$  Hz,  $J_2 = 5.7$  Hz, 2H), 2.81 (t,  $J = 7.3$  Hz, 2H), 1.78-1.73 (m, 2H), 1.56-1.51 (m, 2H).  **$^{13}\text{C}$  NMR** (150 MHz, DMSO-d<sub>6</sub>):  $\delta$  155.93, 154.25, 136.28, 134.25, 129.09, 127.76, 125.10, 124.93, 124.12, 119.77, 114.30, 55.59, 39.45, 33.08, 30.11, 24.18. **LRMS** (API-ES, positive mode): *m/z* 409.2 [M+H]<sup>+</sup>.

**1-Hydroxy-3-{4-[3-(4-methoxyphenyl)ureido]butyl}-5,6-dimethyl-pyrazin-2(1*H*)-one (3.28o)**

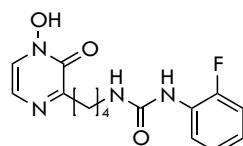


Compound **3.28o** was prepared by debenzylation of **3.23o** (45.1 mg, 0.1 mmol) in flow via procedure H. Yield 95% (34.2 mg).  **$^1\text{H}$  NMR** (300

MHz, DMSO-d<sub>6</sub>):  $\delta$  8.17 (s, 1H), 7.26 (d,  $J = 8.9$  Hz, 2H), 6.79 (d,  $J = 8.9$  Hz, 2H), 6.02 (t,  $J = 5.7$  Hz, 1H), 3.68 (s, 3H), 3.07 (dt,  $J_1 = 6.8$  Hz,  $J_2 = 5.7$  Hz, 2H), 2.67 (t,  $J = 7.6$  Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.67-1.57 (m, 2H), 1.50-1.40 (m, 2H).  **$^{13}\text{C}$  NMR** (75 MHz, DMSO-d<sub>6</sub>):  $\delta$  155.39, 153.76, 152.04, 150.82, 133.67, 132.03, 127.61, 119.25,

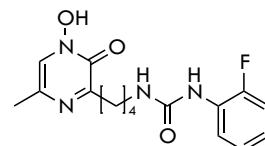
113.79, 55.07, 38.60, 32.28, 29.64, 24.04, 19.45, 12.68. **LRMS** (API-ES, positive mode): *m/z* 361.2 [M+H]<sup>+</sup>.

**3-{4-[3-(2-Fluorophenyl)ureido]butyl}-1-hydroxypyrazin-2(1*H*)-one  
(3.28p)**



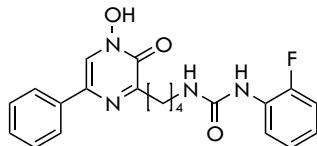
Compound **3.28p** was prepared by debenzylation of **3.23p** (41.0 mg, 0.1 mmol) in flow via procedure H. Quantitative yield (32.0 mg). **<sup>1</sup>H NMR** (300 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.24 (d, *J* = 2.0 Hz, 1H), 8.13 (dt, *J*<sub>1</sub> = 8.3 Hz, *J*<sub>2</sub> = 1.1 Hz, 1H), 7.82 (d, *J* = 4.4 Hz, 1H), 7.21 (d, *J* = 4.4 Hz, 1H), 7.16 (dd, *J*<sub>1</sub> = 11.7 Hz, *J*<sub>2</sub> = 8.9 Hz, 1H), 7.06 (t, *J* = 7.8 Hz, 1H), 6.94-6.87 (m, 1H), 6.63 (t, *J* = 5.7 Hz, 1H), 3.12 (dt, *J*<sub>1</sub> = 6.6 Hz, *J*<sub>2</sub> = 5.7 Hz, 2H), 2.75 (t, *J* = 7.5 Hz, 2H), 1.72-1.62 (m, 2H), 1.53-1.44 (m, 2H). **<sup>13</sup>C NMR** (75 MHz, DMSO-d<sub>6</sub>):  $\delta$  158.95, 154.77, 152.02, 151.46 (d, *J*<sub>CF</sub> = 240.3 Hz), 128.36 (d, *J*<sub>CF</sub> = 10.2 Hz), 126.57, 124.30 (d, *J*<sub>CF</sub> = 3.2 Hz), 121.27 (d, *J*<sub>CF</sub> = 8.1 Hz), 121.18, 119.95, 114.69 (d, *J*<sub>CF</sub> = 20.0 Hz), 39.81, 32.36, 29.32, 23.51. **LRMS** (API-ES, positive mode): *m/z* 321.1 [M+H]<sup>+</sup>.

**3-{4-[3-(2-Fluorophenyl)ureido]butyl}-1-hydroxy-5-methylpyrazin-2(1*H*)-one (3.28q)**



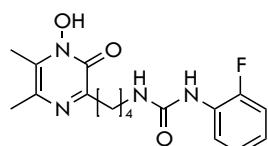
Compound **3.28q** was prepared by debenzylation of **3.23q** (42.5 mg, 0.1 mmol) in flow via procedure H. Yield 98% (33.7 mg). **<sup>1</sup>H NMR** (300 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.24 (d, *J* = 2.1 Hz, 1H), 8.13 (dt, *J*<sub>1</sub> = 8.5 Hz, *J*<sub>2</sub> = 1.4 Hz, 1H), 7.65 (s, 1H), 7.16 (ddd, *J*<sub>1</sub> = 11.8 Hz, *J*<sub>2</sub> = 8.0 Hz, *J*<sub>3</sub> = 1.0 Hz, 1H), 7.06 (t, *J* = 7.8 Hz, 1H), 6.91 (m, 1H), 6.64 (t, *J* = 5.8 Hz, 1H), 3.12 (dt, *J*<sub>1</sub> = 6.8 Hz, *J*<sub>2</sub> = 5.8 Hz, 2H), 2.72 (t, *J* = 7.6 Hz, 2H), 2.17 (s, 3H), 1.70-1.60 (m, 2H), 1.53-1.43 (m, 2H). **<sup>13</sup>C NMR** (75 MHz, DMSO-d<sub>6</sub>):  $\delta$  157.72, 154.78, 151.46 (d, *J*<sub>CF</sub> = 240.5 Hz), 150.75, 129.32, 128.36 (d, *J*<sub>CF</sub> = 10.2 Hz), 124.29 (d, *J*<sub>CF</sub> = 3.4 Hz), 123.41, 121.27 (d, *J*<sub>CF</sub> = 7.4 Hz), 119.95 (d, *J*<sub>CF</sub> = 1.4 Hz), 114.67 (d, *J*<sub>CF</sub> = 19.0 Hz), 38.83, 32.52, 29.40, 23.77, 19.05. **LRMS** (API-ES, positive mode): *m/z* 335.2 [M+H]<sup>+</sup>.

**3-{4-[3-(2-Fluorophenyl)ureido]butyl}-1-hydroxy-5-phenylpyrazin-2(1*H*)-one (3.28r)**



Compound **3.28r** was prepared by debenzylation of **3.23r** (48.7 mg, 0.1 mmol) in flow via procedure H. Yield 83% (32.9 mg). **<sup>1</sup>H NMR** (300 MHz, DMSO-d<sub>6</sub>): δ 8.47 (s, 1H), 8.24 (d, *J* = 2.0 Hz, 1H), 8.13 (t, *J* = 8.1 Hz, 1H), 7.91 (d, *J* = 7.6 Hz, 2H), 7.4 (t, *J* = 7.6 Hz, 2H), 7.29 (t, *J* = 7.1 Hz, 1H), 7.16 (dd, *J*<sub>1</sub> = 11.5 Hz, *J*<sub>2</sub> = 8.4 Hz, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.93-6.87 (m, 1H), 6.66 (t, *J* = 5.8 Hz, 1H), 3.16 (dt, *J*<sub>1</sub> = 6.8 Hz, *J*<sub>2</sub> = 5.8 Hz, 2H), 2.84 (t, *J* = 7.5 Hz, 2H), 1.82-1.72 (m, 2H), 1.59-1.50 (m, 2H). **<sup>13</sup>C NMR** (75 MHz, DMSO-d<sub>6</sub>): δ 157.54, 154.80, 151.45 (d, *J*<sub>CF</sub> = 240.3 Hz), 151.13, 135.46, 129.95, 128.61, 128.56, 128.38 (d, *J*<sub>CF</sub> = 10.4 Hz), 127.45, 124.65, 124.40, 124.30 (d, *J*<sub>CF</sub> = 3.3 Hz), 129.91, 121.27 (d, *J*<sub>CF</sub> = 7.3 Hz), 119.95 (d, *J*<sub>CF</sub> = 1.5 Hz), 114.68 (d, *J*<sub>CF</sub> = 19.0 Hz), 38.84, 32.50, 29.35, 23.46. **LRMS** (API-ES, positive mode): *m/z* 397.2 [M+H]<sup>+</sup>.

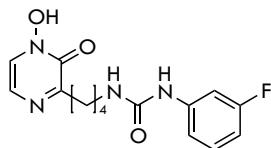
**3-{4-[3-(2-Fluorophenyl)ureido]butyl}-1-hydroxy-5,6-dimethyl-pyrazin-2(1*H*)-one (3.28s)**



Compound **3.28s** was prepared by debenzylation of **3.23s** (43.9 mg, 0.1 mmol) in flow via procedure H. Yield 91% (31.7 mg). **<sup>1</sup>H NMR** (300 MHz, CD<sub>3</sub>OD): δ 7.97 (t, *J* = 7.8 Hz, 1H), 7.09-6.93 (m, 3H), 3.25 (t, *J* = 6.2 Hz, 2H), 2.81 (t, *J* = 6.8 Hz, 2H), 2.40 (s, 3H), 2.33 (s, 3H), 1.81-1.71 (m, 2H), 1.64-1.55 (m, 2H). **<sup>13</sup>C NMR** (75 MHz, CD<sub>3</sub>OD): δ 165.57, 157.91, 154.19 (d, *J*<sub>CF</sub> = 240.9 Hz), 151.91, 134.66, 131.38, 129.03 (d, *J*<sub>CF</sub> = 10.5 Hz), 125.29 (d, *J*<sub>CF</sub> = 3.5 Hz), 123.75 (d, *J*<sub>CF</sub> = 8.2 Hz), 122.60 (d, *J*<sub>CF</sub> = 1.4 Hz), 115.78 (d, *J*<sub>CF</sub> = 19.6 Hz), 40.47, 33.30, 30.66, 25.80, 19.52, 13.05. **LRMS** (API-ES, positive mode): *m/z* 349.2 [M+H]<sup>+</sup>.

**3-[4-[3-(3-Fluorophenyl)ureido]butyl]-1-hydroxypyrazin-2(1*H*)-one**

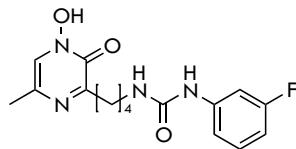
(**3.28t**)



Compound **3.28t** was prepared by debenzylation of **3.23t** (41.0 mg, 0.1 mmol) in flow via procedure H. Quantitative yield (32.0 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 8.67 (s, 1H),

7.81 (brs, 1H), 7.45 (d, *J* = 12.0 Hz, 1H), 7.25-7.20 (m, 2H), 7.01 (d, *J* = 8.3 Hz, 1H), 6.68 (t, *J* = 8.3 Hz, 1H), 6.26 (t, *J* = 5.8 Hz, 1H), 3.11 (dt, *J*<sub>1</sub> = 6.8 Hz, *J*<sub>2</sub> = 5.8 Hz, 2H), 2.74 (t, *J* = 7.5 Hz, 2H), 1.70-1.62 (m, 2H), 1.52-1.45 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 162.90 (d, *J*<sub>CF</sub> = 240.9 Hz), 159.17, 155.44, 152.84, 143.00 (d, *J*<sub>CF</sub> = 11.7 Hz), 130.52 (d, *J*<sub>CF</sub> = 9.8 Hz), 127.24, 121.78, 113.70, 107.56 (d, *J*<sub>CF</sub> = 21.2 Hz), 104.64 (d, *J*<sub>CF</sub> = 26.5 Hz), 39.38, 32.89, 29.89, 24.05. **LRMS** (API-ES, positive mode): *m/z* 321.1 [M+H]<sup>+</sup>.

**3-[4-[3-(3-Fluorophenyl)ureido]butyl]-1-hydroxy-5-methylpyrazin-2(1*H*)-one (**3.28u**)**

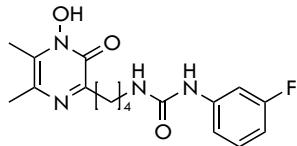


Compound **3.28u** was prepared by debenzylation of **3.23u** (42.5 mg, 0.1 mmol) in flow via procedure H. Yield 90% (31.0 mg). **<sup>1</sup>H NMR** (400 MHz,

DMSO-d<sub>6</sub>): δ 8.68 (s, 1H), 7.64 (s, 1H), 7.45 (dt, *J*<sub>1</sub> = 12.2 Hz, *J*<sub>2</sub> = 2.0 Hz, 1H), 7.22 (dd, *J*<sub>1</sub> = 15.4 Hz, *J*<sub>2</sub> = 8.0 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 1H), 6.67 (dt, *J*<sub>1</sub> = 8.4 Hz, *J*<sub>2</sub> = 2.0 Hz, 1H), 6.28 (t, *J* = 5.7 Hz, 1H), 3.11 (dt, *J*<sub>1</sub> = 6.9 Hz, *J*<sub>2</sub> = 5.7 Hz, 2H), 2.71 (t, *J* = 7.5 Hz, 2H), 2.17 (s, 3H), 1.68-1.60 (m, 2H), 1.52-1.45 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 162.89 (d, *J*<sub>CF</sub> = 240.3 Hz), 157.98, 155.44, 151.40, 143.00 (d, *J*<sub>CF</sub> = 11.4 Hz), 130.51 (d, *J*<sub>CF</sub> = 9.8 Hz), 129.86, 124.05, 113.70, 107.56 (d, *J*<sub>CF</sub> = 21.0 Hz), 104.64 (d, *J*<sub>CF</sub> = 26.3 Hz), 39.36, 33.05, 29.97, 24.29, 19.58. **LRMS** (API-ES, positive mode): *m/z* 335.2 [M+H]<sup>+</sup>.

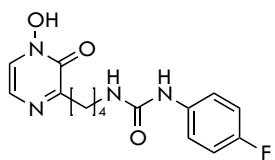
**3-[4-[3-(3-Fluorophenyl)ureido]butyl]-1-hydroxy-5,6-dimethyl-pyrazin-2(1*H*)-one (**3.28v**)**

Compound **3.28v** was prepared by debenzylation of **3.23v** (43.9 mg, 0.1 mmol) in flow via procedure H. Yield 92% (32.1 mg).



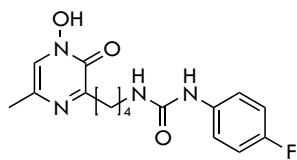
**<sup>1</sup>H NMR** (300 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.69 (s, 1H), 7.44 (d,  $J$  = 12.2 Hz, 1H), 7.22 (dd,  $J_1$  = 15.3 Hz,  $J_2$  = 7.8 Hz, 1H), 7.00 (d,  $J$  = 7.8 Hz, 1H), 6.67 (dt,  $J_1$  = 8.4 Hz,  $J_2$  = 2.0 Hz, 1H), 6.28 (t,  $J$  = 5.7 Hz, 1H), 3.09 (dt,  $J_1$  = 6.9 Hz,  $J_2$  = 5.7 Hz, 2H), 2.67 (t,  $J$  = 7.3 Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.67-1.58 (m, 2H), 1.51-1.42 (m, 2H). **<sup>13</sup>C NMR** (75 MHz, DMSO-d<sub>6</sub>):  $\delta$  162.38 (d,  $J_{CF}$  = 240.2 Hz), 154.92, 151.69, 151.02, 142.50 (d,  $J_{CF}$  = 11.5 Hz), 132.22, 130.03 (d,  $J_{CF}$  = 9.8 Hz), 127.63, 113.17 (d,  $J_{CF}$  = 2.2 Hz), 107.04 (d,  $J_{CF}$  = 21.0 Hz), 104.10 (d,  $J_{CF}$  = 26.5 Hz), 38.62, 32.26, 29.47, 24.03, 19.50, 12.71. **LRMS** (API-ES, positive mode): *m/z* 349.2 [M+H]<sup>+</sup>.

**3-{4-[3-(4-Fluorophenyl)ureido]butyl}-1-hydroxypyrazin-2(1H)-one  
(3.28w)**



Compound **3.28w** was prepared by debenzylation of **3.23w** (41.0 mg, 0.1 mmol) in flow via procedure H. Yield 99% (31.7 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.42 (s, 1H), 7.81 (d,  $J$  = 4.2 Hz, 1H), 7.38 (dd,  $J_1$  = 8.8 Hz,  $J_2$  = 5.0 Hz, 2H), 7.20 (d,  $J$  = 4.2 Hz, 1H), 7.04 (t,  $J$  = 8.8 Hz, 2H), 6.13 (t,  $J$  = 5.8 Hz, 1H), 3.10 (dt,  $J_1$  = 6.8 Hz,  $J_2$  = 5.8 Hz, 2H), 2.74 (t,  $J$  = 7.6 Hz, 2H), 1.70-1.62 (m, 2H), 1.51-1.44 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  159.39, 157.27 (d,  $J_{CF}$  = 236.7 Hz), 155.72, 152.59, 137.42 (d,  $J_{CF}$  = 2.2 Hz), 127.09, 121.71, 119.63 (d,  $J_{CF}$  = 7.5 Hz), 115.48 (d,  $J_{CF}$  = 22.1 Hz), 39.36, 32.90, 29.98, 24.04. **LRMS** (API-ES, positive mode): *m/z* 321.1 [M+H]<sup>+</sup>.

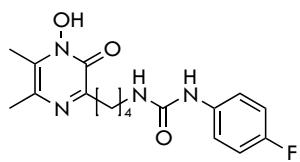
**3-{4-[3-(4-Fluorophenyl)ureido]butyl}-1-hydroxy-5-methylpyrazin-2(1H)-one (3.28x)**



Compound **3.28x** was prepared by debenzylation of **3.23x** (42.5 mg, 0.1 mmol) in flow via procedure H. Yield 97% (32.4 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.40 (s, 1H), 7.63 (s, 1H), 7.38 (dd,  $J_1$  = 8.7 Hz,  $J_2$  = 5.0 Hz, 2H), 7.03 (t,  $J$  = 8.7 Hz, 2H), 6.12 (t,

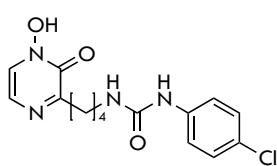
$J = 5.7$  Hz, 1H), 3.10 (dt,  $J_1 = 6.8$  Hz,  $J_2 = 5.7$  Hz, 2H), 2.72 (t,  $J = 7.5$  Hz, 2H), 2.17 (s, 3H), 1.69-1.61 (m, 2H), 1.52-1.45 (m, 2H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  158.06, 157.29 (d,  $J_{\text{CF}} = 236.5$  Hz), 155.73, 151.36, 137.44 (d,  $J_{\text{CF}} = 2.0$  Hz), 129.85, 123.93, 119.66 (d,  $J_{\text{CF}} = 7.5$  Hz), 115.46 (d,  $J_{\text{CF}} = 22.0$  Hz), 39.43, 33.03, 30.04, 24.30, 19.58. **LRMS** (API-ES, positive mode): *m/z* 335.2 [M+H]<sup>+</sup>.

**3-[4-[3-(4-Fluorophenyl)ureido]butyl]-1-hydroxy-5,6-dimethyl-pyrazin-2(1*H*)-one (3.28y)**



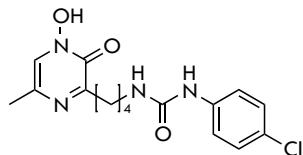
Compound **3.28y** was prepared by debenzylation of **3.23y** (43.9 mg, 0.1 mmol) in flow via procedure H. Yield 94% (32.7 mg).  **$^1\text{H}$  NMR** (600 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.47 (s, 1H), 7.38 (dd,  $J_1 = 8.7$  Hz,  $J_2 = 5.0$  Hz, 2H), 7.04 (t,  $J = 8.7$  Hz, 2H), 6.19 (t,  $J = 5.7$  Hz, 1H), 3.09 (dt,  $J_1 = 6.8$  Hz,  $J_2 = 5.7$  Hz, 2H), 2.67 (t,  $J = 7.6$  Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.65-1.60 (m, 2H), 1.49-1.44 (m, 2H).  **$^{13}\text{C}$  NMR** (150 MHz, DMSO-d<sub>6</sub>):  $\delta$  163.86, 157.22 (d,  $J_{\text{CF}} = 236.8$  Hz), 156.07, 155.72, 151.76, 137.47 (d,  $J_{\text{CF}} = 1.9$  Hz), 132.89, 128.18, 119.57 (d,  $J_{\text{CF}} = 7.3$  Hz), 115.48 (d,  $J_{\text{CF}} = 22.0$  Hz), 39.42, 32.78, 30.07, 24.59, 20.06, 13.25. **LRMS** (API-ES, positive mode): *m/z* 349.2 [M+H]<sup>+</sup>.

**3-[4-[3-(4-Chlorophenyl)ureido]butyl]-1-hydroxypyrazin-2(1*H*)-one (3.28z)**



Compound **3.28z** was prepared by debenzylation of **3.23z** (42.7 mg, 0.1 mmol) in flow via procedure H. Yield 90% (30.3 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.56 (s, 1H), 7.80 (d,  $J = 4.0$  Hz, 1H), 7.41 (d,  $J = 8.7$  Hz, 2H), 7.24 (d,  $J = 8.7$  Hz, 2H), 7.20 (d,  $J = 4.0$  Hz, 1H), 6.21 (t,  $J = 5.6$  Hz, 1H), 3.10 (dt,  $J_1 = 6.8$  Hz,  $J_2 = 5.6$  Hz, 2H), 2.73 (t,  $J = 7.5$  Hz, 2H), 1.69-1.62 (m, 2H), 1.51-1.44 (m, 2H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  155.50, 140.09, 128.87, 127.22, 124.77, 121.81, 119.51, 39.38, 32.88, 29.92, 24.06. **LRMS** (API-ES, positive mode): *m/z* 337.1 [M+H]<sup>+</sup>.

**3-[4-[3-(4-Chlorophenyl)ureido]butyl]-1-hydroxy-5-methylpyrazin-2(1*H*)-one (3.28za)**



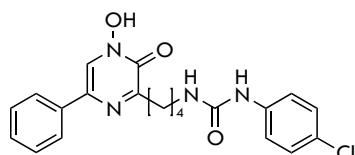
Compound **3.28za** was prepared by debenzylation of **3.23za** (44.1 mg, 0.1 mmol) in flow via procedure H. Yield 87% (30.5 mg).

**<sup>1</sup>H NMR** (400 MHz, CD<sub>3</sub>OD-DMSO-d<sub>6</sub>):  $\delta$  7.56 (s, 1H), 7.40 (d, J = 8.7 Hz, 2H), 7.22 (d, J = 8.7 Hz, 2H), 3.15 (t, J = 6.9 Hz, 2H), 2.76 (t, J = 7.7 Hz, 2H), 2.20 (s, 3H), 1.74-1.66 (m, 2H), 1.56-1.49 (m, 2H).

**<sup>13</sup>C NMR** (100 MHz, CD<sub>3</sub>OD-DMSO-d<sub>6</sub>):  $\delta$  156.51, 140.36, 131.25, 129.36, 126.11, 124.43, 120.30, 39.97, 33.53, 30.51, 24.98, 19.72.

**LRMS** (API-ES, positive mode): *m/z* 351.1 [M+H]<sup>+</sup>.

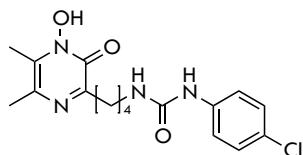
**3-[4-[3-(4-Chlorophenyl)ureido]butyl]-1-hydroxy-5-phenylpyrazin-2(1*H*)-one (3.28zb)**



Compound **3.28zb** was prepared by debenzylation of **3.23zb** (50.3 mg, 0.1 mmol) in flow via procedure H. Yield 54% (22.3 mg). **<sup>1</sup>H NMR** (600 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.66 (s, 1H), 8.42 (s, 1H), 7.89 (d, J = 7.5 Hz, 2H), 7.42 (d, J = 8.8 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.27 (t, J = 7.5 Hz, 1H), 7.24 (d, J = 8.8 Hz, 2H), 6.34 (t, J = 5.7 Hz, 1H), 3.14 (dt, J<sub>1</sub> = 6.8 Hz, J<sub>2</sub> = 5.7 Hz, 2H), 2.80 (t, J = 7.2 Hz, 2H), 1.77-1.72 (m, 2H), 1.56-1.51 (m, 2H). **<sup>13</sup>C NMR** (150 MHz, DMSO-d<sub>6</sub>):  $\delta$  163.86, 155.55, 140.17, 136.53, 130.82, 129.06, 128.85, 127.63, 125.05, 124.68, 119.49, 39.44, 33.04, 29.99, 24.27.

**LRMS** (API-ES, positive mode): *m/z* 413.2 [M+H]<sup>+</sup>.

**3-[4-[3-(4-Chlorophenyl)ureido]butyl]-1-hydroxy-5,6-dimethyl-pyrazin-2(1*H*)-one (3.28zc)**

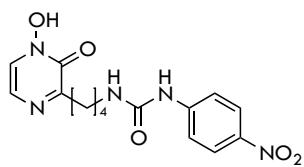


Compound **3.28zc** was prepared by debenzylation of **3.23zc** (45.5 mg, 0.1 mmol) in flow via procedure H. Yield 59% (21.5 mg).

**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$

8.54 (s, 1H), 7.40 (d,  $J$  = 8.0 Hz, 2H), 7.24 (d,  $J$  = 8.0 Hz, 2H), 6.20 (brs, 1H), 3.09 (dt,  $J_1$  = 6.9 Hz,  $J_2$  = 5.2 Hz, 2H), 2.67 (t,  $J$  = 7.2 Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.66-1.59 (m, 2H), 1.50-1.43 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  155.49, 152.25, 151.47, 140.09, 132.63, 128.87, 128.17, 124.76, 119.50, 39.38, 32.74, 29.99, 24.52, 19.98, 13.19. LRMS (API-ES, positive mode): *m/z* 365.1 [M+H]<sup>+</sup>.

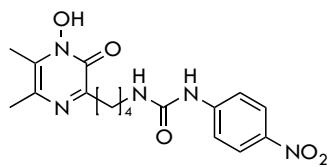
**1-Hydroxy-3-{4-[3-(4-nitrophenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.28zf)**



Compound **3.28zf** was prepared by debenzylation of **3.23zf** (43.7 mg, 0.1 mmol) in batch via procedure I in 20 min. Yield 90% (31.3 mg).  $^1\text{H}$  NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  9.23 (s, 1H), 8.13 (d,  $J$  = 8.4 Hz, 2H),

7.81 (brs, 1H), 7.61 (d,  $J$  = 8.4 Hz, 2H), 7.21 (brs, 1H), 6.48 (brs, 1H), 3.14 (dt,  $J_1$  = 8.6 Hz,  $J_2$  = 5.8 Hz, 2H), 2.74 (t,  $J$  = 7.6 Hz, 2H), 1.70-1.63 (m, 2H), 1.54-1.47 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  159.40, 154.86, 152.55, 147.73, 140.78, 127.07, 125.58, 121.71, 117.23, 39.46, 32.86, 29.71, 23.98. LRMS (API-ES, positive mode): *m/z* 348.1 [M+H]<sup>+</sup>.

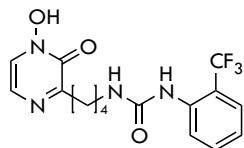
**1-Hydroxy-5,6-dimethyl-3-{4-[3-(4-nitrophenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.28zg)**



Compound **3.28zg** was prepared by debenzylation of **3.23zg** (46.6 mg, 0.1 mmol) in batch via procedure I in 20 min. Yield 75% (28.2 mg).  $^1\text{H}$  NMR (400 MHz, CD<sub>3</sub>OD):  $\delta$  9.26 (s, 1H), 8.12 (d,  $J$  = 8.3 Hz, 2H), 7.62

(d,  $J$  = 8.3 Hz, 2H), 6.51 (brs, 1H), 3.13 (dt,  $J_1$  = 7.3 Hz,  $J_2$  = 5.4 Hz, 2H), 2.68 (t,  $J$  = 7.5 Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.68-1.61 (m, 2H), 1.53-1.46 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz, CD<sub>3</sub>OD):  $\delta$  154.86, 152.28, 151.43, 147.76, 140.75, 132.62, 128.16, 125.56, 117.21, 39.50, 32.71, 29.79, 24.47, 19.96, 13.18. LRMS (API-ES, positive mode): *m/z* 376.2 [M+H]<sup>+</sup>.

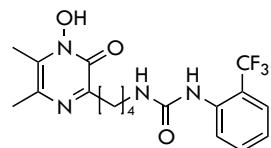
**1-Hydroxy-3-{4-[3-(2-trifluoromethylphenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.28zh)**



Compound **3.28zh** was prepared by debenzylation of **3.23zh** (46.0 mg, 0.1 mmol) in flow via procedure H. Yield 89% (33.0 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  7.97 (d, *J* = 8.2

Hz, 1H), 7.82 (d, *J* = 4.2 Hz, 1H), 7.72 (s, 1H), 7.60 (d, *J* = 8.2 Hz, 1H), 7.56 (t, *J* = 8.2 Hz, 1H), 7.21 (d, *J* = 4.2 Hz, 1H), 7.17 (t, *J* = 8.2 Hz, 1H), 7.02 (t, *J* = 5.8 Hz, 1H), 3.12 (dt, *J*<sub>1</sub> = 7.0 Hz, *J*<sub>2</sub> = 5.8 Hz, 2H), 2.75 (t, *J* = 7.5 Hz, 2H), 1.72-1.64 (m, 2H), 1.53-1.46 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  159.31, 155.33, 152.62, 137.90, 133.17, 127.13, 126.18 (q, *J*<sub>CF</sub> = 5.5 Hz), 124.96, 124.58 (q, *J*<sub>CF</sub> = 272.3 Hz), 122.89, 121.69, 118.90 (q, *J*<sub>CF</sub> = 28.2 Hz), 39.38, 32.85, 29.73, 24.08. **LRMS** (API-ES, positive mode): *m/z* 371.1 [M+H]<sup>+</sup>.

**1-Hydroxy-5,6-dimethyl-3-{4-[3-(2-trifluoromethylphenyl)ureido]-butyl}pyrazin-2(1*H*)-one (3.28zi)**

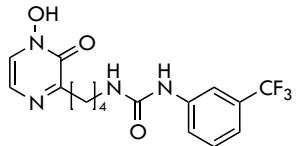


Compound **3.28zi** was prepared by debenzylation of **3.23zi** (48.9 mg, 0.1 mmol) in flow via procedure H. Yield 90% (35.9 mg). **<sup>1</sup>H NMR** (300 MHz, DMSO-d<sub>6</sub>):  $\delta$  7.96 (d, *J* = 8.4

Hz, 1H), 7.73 (s, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.55 (t, *J* = 7.6 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.02 (t, *J* = 5.6 Hz, 1H), 3.11 (dt, *J*<sub>1</sub> = 6.7 Hz, *J*<sub>2</sub> = 5.6 Hz, 2H), 2.69 (t, *J* = 7.4 Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.70-1.60 (m, 2H), 1.52-1.43 (m, 2H). **<sup>13</sup>C NMR** (75 MHz, DMSO-d<sub>6</sub>):  $\delta$  155.57, 154.81, 151.88, 150.90, 137.40, 132.68, 132.10, 127.58, 125.67 (q, *J*<sub>CF</sub> = 5.3 Hz), 124.41, 124.07 (q, *J*<sub>CF</sub> = 273.0 Hz), 122.37, 118.33 (q, *J*<sub>CF</sub> = 28.9 Hz), 39.46, 32.20, 29.30, 24.06, 19.46, 12.70. **LRMS** (API-ES, positive mode): *m/z* 399.2 [M+H]<sup>+</sup>.

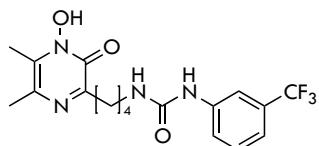
**1-Hydroxy-3-{4-[3-(3-trifluoromethylphenyl)ureido]butyl}pyrazin-2(1*H*)-one (3.28zj)**

Compound **3.28zj** was prepared by debenzylation of **3.23zj** (46.0 mg, 0.1 mmol) in flow via procedure H. Yield 99% (36.7 mg).



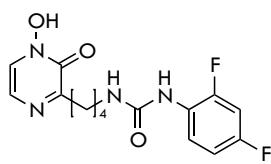
**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.80 (s, 1H), 7.97 (s, 1H), 7.82 (d,  $J$  = 4.0 Hz, 1H), 7.49 (d,  $J$  = 8.0 Hz, 1H), 7.44 (t,  $J$  = 7.9 Hz, 1H), 7.21 (overlapped, 2H), 6.30 (t,  $J$  = 5.7 Hz, 1H), 3.12 (dt,  $J_1$  = 6.7 Hz,  $J_2$  = 5.7 Hz, 2H), 2.75 (t,  $J$  = 7.4 Hz, 2H), 1.71-1.63 (m, 2H), 1.53-1.46 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  159.41, 155.49, 152.57, 141.92, 130.15, 129.86 (q,  $J_{CF}$  = 31.4 Hz), 127.07, 124.75 (q,  $J_{CF}$  = 272.3 Hz), 121.70, 121.53, 117.53 (q,  $J_{CF}$  = 3.8 Hz), 113.94 (q,  $J_{CF}$  = 3.8 Hz), 39.38, 32.89, 29.88, 24.01. **LRMS** (API-ES, positive mode): *m/z* 371.1 [M+H]<sup>+</sup>.

**1-Hydroxy-5,6-dimethyl-3-{4-[3-(3-trifluoromethylphenyl)ureido]butyl}pyrazin-2(1H)-one (3.28zk)**



Compound 3.28zk was prepared by debenzylation of 3.23zk (48.9 mg, 0.1 mmol) in flow via procedure H. Yield 98% (39.0 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.83 (s, 1H), 7.97 (s, 1H), 7.49 (d,  $J$  = 7.7 Hz, 1H), 7.43 (t,  $J$  = 7.7 Hz, 1H), 7.20 (d,  $J$  = 7.7 Hz, 1H), 6.34 (t,  $J$  = 5.8 Hz, 1H), 3.11 (dt,  $J_1$  = 6.8 Hz,  $J_2$  = 5.8 Hz, 2H), 2.68 (t,  $J$  = 7.5 Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.68-1.60 (m, 2H), 1.52-1.45 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  155.50, 152.22, 151.49, 141.94, 132.66, 130.13, 129.85 (q,  $J_{CF}$  = 31.2 Hz), 128.15, 124.76 (q,  $J_{CF}$  = 272.3 Hz), 121.52, 117.50 (q,  $J_{CF}$  = 3.8 Hz), 113.93 (q,  $J_{CF}$  = 3.9 Hz), 39.44, 32.75, 29.95, 24.51, 19.98, 13.18. **LRMS** (API-ES, positive mode): *m/z* 399.2 [M+H]<sup>+</sup>.

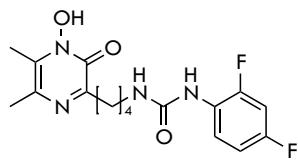
**3-{4-[3-(2,4-Difluorophenyl)ureido]butyl}-1-hydroxypyrazin-2(1H)-one (3.28zl)**



Compound 3.28zl was prepared by debenzylation of 3.23zl (42.8 mg, 0.1 mmol) in flow via procedure H. Yield 98% (33.2 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.21 (s, 1H), 8.10-8.03 (m, 1H), 7.80 (d,  $J$  = 3.6 Hz, 1H), 7.25-7.19 (m, 2H), 6.97 (t,  $J$  = 8.7 Hz,

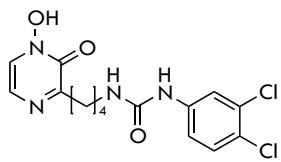
1H), 6.58 (t,  $J = 5.7$  Hz, 1H), 3.12 (dt,  $J_1 = 6.8$  Hz,  $J_2 = 5.7$  Hz, 2H), 2.73 (t,  $J = 7.4$  Hz, 2H), 1.70-1.63 (m, 2H), 1.52-1.45 (m, 2H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  163.74, 155.38, 156.56 (dd,  $J_{\text{CF}} = 240.2$  Hz,  $J_{\text{CF}} = 11.4$  Hz), 151.99 (dd,  $J_{\text{CF}} = 242.2$  Hz,  $J_{\text{CF}} = 11.8$  Hz), 153.03 (d,  $J_{\text{CF}} = 3.5$  Hz), 127.45, 125.47 (dd,  $J_{\text{CF}} = 10.8$  Hz,  $J_{\text{CF}} = 3.4$  Hz), 121.80, 121.70 (d,  $J_{\text{CF}} = 9.7$  Hz), 111.23 (dd,  $J_{\text{CF}} = 21.4$  Hz,  $J_{\text{CF}} = 3.4$  Hz), 103.94 (dd,  $J_{\text{CF}} = 26.7$  Hz,  $J_{\text{CF}} = 23.8$  Hz), 39.38, 32.86, 29.85, 24.10. **LRMS** (API-ES, positive mode):  $m/z$  339.1 [M+H]<sup>+</sup>.

**3-[4-[3-(2,4-Difluorophenyl)ureido]butyl]-1-hydroxy-5,6-dimethyl-pyrazin-2(1H)-one (3.28zm)**



Compound **3.28zm** was prepared by debenzylation of **3.23zm** (45.7 mg, 0.1 mmol) in flow via procedure H. Yield 95% (34.8 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.20 (d,  $J = 3.7$  Hz, 1H), 8.09-8.03 (m, 1H), 7.24-7.19 (m, 1H), 6.97 (t,  $J = 8.7$  Hz, 1H), 6.58 (t,  $J = 5.8$  Hz, 1H), 3.10 (dt,  $J_1 = 6.8$  Hz,  $J_2 = 5.8$  Hz, 2H), 2.67 (t,  $J = 7.5$  Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.67-1.60 (m, 2H), 1.50-1.43 (m, 2H).  **$^{13}\text{C}$  NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  163.95, 156.56 (dd,  $J_{\text{CF}} = 240.7$  Hz,  $J_{\text{CF}} = 11.2$  Hz), 155.37, 151.98 (dd,  $J_{\text{CF}} = 243.6$  Hz,  $J_{\text{CF}} = 11.5$  Hz), 151.90, 133.01, 128.17, 125.45 (dd,  $J_{\text{CF}} = 10.5$  Hz,  $J_{\text{CF}} = 3.4$  Hz), 121.68 (dd,  $J_{\text{CF}} = 8.7$  Hz,  $J_{\text{CF}} = 2.2$  Hz), 111.22 (dd,  $J_{\text{CF}} = 21.3$  Hz,  $J_{\text{CF}} = 3.6$  Hz), 103.93 (dd,  $J_{\text{CF}} = 26.8$  Hz,  $J_{\text{CF}} = 23.9$  Hz), 39.37, 32.74, 29.93, 24.59, 20.07, 13.26. **LRMS** (API-ES, positive mode):  $m/z$  367.2 [M+H]<sup>+</sup>.

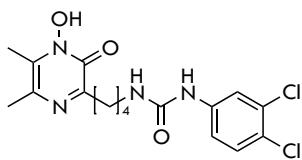
**3-[4-[3-(3,4-Dichlorophenyl)ureido]butyl]-1-hydroxypyrazin-2(1H)-one (3.28zn)**



Compound **3.28zn** was prepared by debenzylation of **3.23zn** (46.1 mg, 0.1 mmol) in flow via procedure H. Yield 90% (33.4 mg).  **$^1\text{H}$  NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.75 (s, 1H), 7.84-7.81 (m, 2H), 7.43 (d,  $J = 8.7$  Hz, 1H), 7.25-7.20 (m, 2H), 6.31 (t,  $J = 5.8$  Hz, 1H), 3.11 (dt,  $J_1 = 6.8$  Hz,  $J_2 = 5.8$  Hz, 2H), 2.74 (t,  $J = 7.5$  Hz, 2H),

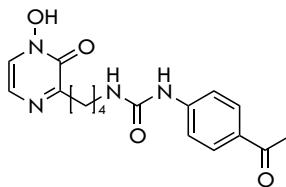
1.69-1.62 (m, 2H), 1.52-1.45 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  158.78, 155.29, 152.92, 141.33, 131.33, 130.83, 127.22, 122.48, 121.98, 119.07, 118.11, 39.38, 32.83, 29.80, 24.04. **LRMS** (API-ES, positive mode): *m/z* 371.1 [M+H]<sup>+</sup>.

**3-[4-[3-(3,4-Dichlorophenyl)ureido]butyl]-1-hydroxy-5,6-dimethyl-pyrazin-2(1*H*)-one (3.28zo)**



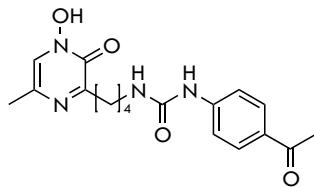
Compound **3.28zo** was prepared by debenzylation of **3.23zo** (48.9 mg, 0.1 mmol) in flow via procedure H. Quantitative yield (39.9 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.81 (s, 1H), 7.84 (d, *J* = 2.2 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.24 (dd, *J*<sub>1</sub> = 8.8 Hz, *J*<sub>2</sub> = 2.2 Hz, 1H), 6.38 (t, *J* = 5.8 Hz, 1H), 3.10 (dt, *J*<sub>1</sub> = 7.0 Hz, *J*<sub>2</sub> = 5.8 Hz, 2H), 2.67 (t, *J* = 7.4 Hz, 2H), 2.27 (s, 3H), 2.23 (s, 3H), 1.67-1.59 (m, 2H), 1.51-1.44 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  156.07, 155.30, 151.62, 141.37, 132.78, 131.31, 130.81, 128.20, 122.44, 119.06, 118.10, 39.45, 32.74, 29.91, 24.52, 20.01, 13.21. **LRMS** (API-ES, positive mode): *m/z* 399.1 [M+H]<sup>+</sup>.

**3-[4-[3-(4-Acetophenyl)ureido]butyl]-1-hydroxypyrazin-2(1*H*)-one (3.28zp)**



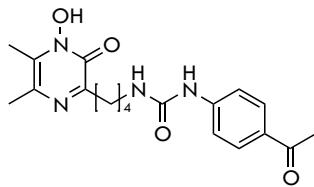
Compound **3.28zp** was prepared by debenzylation of **3.23zp** (43.5 mg, 0.1 mmol) in flow via procedure H. Yield 91% (31.3 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.90 (s, 1H), 7.84 (d, *J* = 8.6 Hz, 2H), 7.81 (d, *J* = 4.1 Hz, 1H), 7.51 (d, *J* = 8.6 Hz, 2H), 7.20 (d, *J* = 4.1 Hz, 1H), 6.38 (t, *J* = 5.7 Hz, 1H), 3.13 (dt, *J*<sub>1</sub> = 6.8 Hz, *J*<sub>2</sub> = 5.7 Hz, 2H), 2.74 (t, *J* = 7.4 Hz, 2H), 2.48 (s, 3H), 1.70-1.63 (m, 2H), 1.53-1.44 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  196.62, 158.95, 155.18, 152.83, 145.74, 130.11, 130.06, 127.26, 121.81, 116.94, 39.37, 32.87, 29.83, 26.70, 24.05. **LRMS** (API-ES, positive mode): *m/z* 345.2 [M+H]<sup>+</sup>.

**3-[4-[3-(4-Acetophenyl)ureido]butyl]-1-hydroxy-5-methylpyrazin-2(1*H*)-one (3.28zq)**



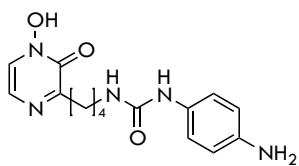
Compound **3.28zq** was prepared by debenzylation of **3.23zq** (44.9 mg, 0.1 mmol) in flow via procedure H. Yield 90% (32.3 mg). **<sup>1</sup>H NMR** (300 MHz, DMSO-d<sub>6</sub>): δ 8.93 (s, 1H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.65 (s, 1H), 7.50 (d, *J* = 8.5 Hz, 2H), 6.41 (t, *J* = 5.7 Hz, 1H), 3.11 (dt, *J*<sub>1</sub> = 6.8 Hz, *J*<sub>2</sub> = 5.7 Hz, 2H), 2.71 (t, *J* = 7.5 Hz, 2H), 2.48 (s, 3H), 2.16 (s, 3H), 1.69-1.59 (m, 2H), 1.53-1.44 (m, 2H). **<sup>13</sup>C NMR** (75 MHz, DMSO-d<sub>6</sub>): δ 196.14, 157.25, 154.67, 151.01, 145.26, 132.28, 129.56, 129.40, 123.68, 116.41, 38.60, 32.54, 29.40, 26.22, 23.80, 19.10. **LRMS** (API-ES, positive mode): *m/z* 359.2 [M+H]<sup>+</sup>.

**3-[4-[3-(4-Acetophenyl)ureido]butyl]-1-hydroxy-5,6-dimethyl-pyrazin-2(1*H*)-one (3.28zr)**



Compound **3.28zr** was prepared by debenzylation of **3.23zr** (46.3 mg, 0.1 mmol) in flow via procedure H. Yield 88% (32.8 mg). **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 8.90 (s, 1H), 7.84 (d, *J* = 8.1 Hz, 2H), 7.50 (d, *J* = 8.1 Hz, 2H), 6.38 (t, *J* = 5.5 Hz, 1H), 3.12 (dt, *J*<sub>1</sub> = 6.8 Hz, *J*<sub>2</sub> = 5.5 Hz, 2H), 2.48 (s, 3H), 2.27 (s, 3H), 2.23 (s, 3H), 1.67-1.60 (m, 2H), 1.52-1.45 (m, 2H). **<sup>13</sup>C NMR** (100 MHz, DMSO-d<sub>6</sub>): δ 196.62, 155.17, 152.06, 151.60, 145.76, 132.76, 130.10, 130.05, 128.17, 116.93, 39.37, 32.74, 29.91, 26.70, 24.54, 20.01, 13.22. **LRMS** (API-ES, positive mode): *m/z* 373.2 [M+H]<sup>+</sup>.

**3-[4-[3-(4-Aminophenyl)ureido]butyl]-1-hydroxypyrazin-2(1*H*)-one (3.28zs)**



Compound **3.28zs** was prepared by debenzylation of **3.23zf** (43.7 mg, 0.1 mmol) in batch via procedure I in 2 h. Yield 82% (26.0 mg). **<sup>1</sup>H NMR** (600 MHz, DMSO-d<sub>6</sub>): δ 8.18 (s, 2H),

7.85 (s, 1H), 7.79 (d,  $J$  = 4.1 Hz, 1H), 7.18 (d,  $J$  = 4.1 Hz, 1H), 6.98 (d,  $J$  = 8.7 Hz, 2H), 6.45 (d,  $J$  = 8.7 Hz, 2H), 5.92 (t,  $J$  = 5.6 Hz, 1H), 3.06 (dt,  $J_1$  = 6.8 Hz,  $J_2$  = 5.6 Hz, 2H), 2.71 (t,  $J$  = 7.4 Hz, 2H), 1.66-1.61 (m, 2H), 1.47-1.42 (m, 2H).  **$^{13}\text{C}$  NMR** (150 MHz, DMSO-d<sub>6</sub>):  $\delta$  163.91, 156.19, 153.28, 143.70, 130.20, 127.61, 125.58, 121.90, 120.65, 114.63, 112.27, 39.42, 32.94, 30.18, 24.18. **LRMS** (API-ES, positive mode): *m/z* 318.1 [M+H]<sup>+</sup>.

## 5.4 Biological evaluation

### 5.4.1 *In vitro* anti-HIV assay

The screening for anti-HIV replication activities was performed on human T-lymphocyte cells (MT-4) by using the standard tetrazolium-based colorimetric (MTT) assay.<sup>2</sup>

Stock solutions (10 times of final concentration) of test compounds were added in 25 µL volumes to two series of triplicate wells in order to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Series of fivefold dilution of test compounds were made directly in flat-bottomed 96-well microtiter plates using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1 (IIIB strain) or HIV-2 (ROD strain) stock (50 µL) at 100-300 CCID<sub>50</sub> (cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter plate. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells were centrifuged for 5 min at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at  $6 \times 10^5$  cells/mL, and 50 µL volumes were transferred to the microtiter plate wells. Five days after infection, the viability of mock- and HIV-infected cells was examined via spectrophotometer by the MTT assay.

The MTT assay is based on the reduction of yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide by mitochondrial dehydrogenase of metabolically active cells to a blue-purple formazan, which can be measured via spectrophotometer (**Scheme 5.1**). The absorbance was read in an eight-channel computer-controlled photometer, at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells.

The 50% cytotoxic concentration (CC<sub>50</sub>) was defined as the concentration of the test compound that reduced the absorbance (OD540) of the

mock-infected control sample by 50%. The 50% inhibitory concentration ( $IC_{50}$ ) was defined as the concentrations of compound at which 50% inhibition of virus replication is observed in MT-4 cells.



**Scheme 5.1.** Reduction of MTT during the MTT assay.

All the compounds were screened against HIV-1 and HIV-2 strains. The  $IC_{50}$  and  $CC_{50}$  values were obtained by using the aforementioned methodology. AZT, Dideoxynucleoside, Lamivudine, and Nevirapine were used as positive controls (Table 3.6 and Table 3.7).

#### 5.4.2 *In vitro* HIV-1 RT kit assay

The analysis of HIV-1 RT polymerase inhibition was evaluated by using an EnzChek<sup>®</sup> RT assay kit (Molecular Probes Inc.), following the manufacturer's protocol.<sup>3</sup>

In short, the reaction mixture contained 10  $\mu$ g/mL poly(A) ribonucleotide template, 1.5  $\mu$ M oligo d(T)<sub>16</sub> primer, 50 mM Tris-HCl (pH 7.6), 20% glycerol and 2 mM DTT. Subsequently, 5  $\mu$ L of the dilute enzyme with the testing compound at grade concentrations were added to the wells containing the reaction mixture and incubated at room temperature.

After 1 h, the reaction was terminated by adding 200 mM EDTA. The products were detected and quantified by using a fluorometer or a microplate reader at fluorescein wavelengths (excitation at  $\sim$ 480 nm and emission at  $\sim$ 520 nm). Efavirenz and Nevirapine were used as positive controls. The activity of the *N*-hydroxypyrazinones on HIV-1 RT inhibition was expressed via the  $IC_{50}$  values, which were presented in Table 3.8.

### References

1. Merritt, J. R.; Liu, J.; Quadros, E.; Morris, M. L.; Liu, R.; Zhang, R.; Jacob, B.; Postelnek, J.; Hicks, C. M.; Chen, W.; Kimble, E. F.; Rogers, W. L.; O'Brien, L.; White, N.; Desai, H.; Bansal, S.; King, G.; Ohlmeyer, M. J.; Appell, K. C.; Webb, M. L. *J. Med. Chem.* **2009**, *52*, 1295-1301.
2. Pannecouque, C.; Daelemans, D.; De Clercq, E. *Nat. Protoc.* **2008**, *3*, 427-434.
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## List of Publications and Attended Conferences

### Publications

1. Ceunen, S., De Borggraeve, W. M., Compernolle, F., Mai, A. H., Geuns, J. (2013). Diterpene glycosides from *Stevia phlebophylla* A. Gray. *Carbohydrate Research*, 379, 1-6.
2. Luu-Huynh, V. L., Vo, T. N., Nguyen, P. D., Mai, A. H., Tu, D. D., Ton, T. Q., Nguyen, K. P. P. (2013). Three new iridoid glucoside salts from *Hedyotis tenelliflora* growing in Vietnam. *Natural Product Communications*, 8(11), 1507-1508.
3. Mai, A. H.; Pawar, S.; De Borggraeve, W. M. (2014). Synthesis of 1-benzyloxy-pyrazin-2(1H)-one derivatives. *Tetrahedron Letters*, 55(33), 4664-4666.
4. Mai, A. H.; De Borggraeve, W. M. (2014). Synthesis of *N*-hydroxypyrazin-2(1H)-ones. *Journal of Flow Chemistry*, DOI: 10.1556/JFC-D-14-00036.

### Attended conferences

1. **16<sup>th</sup> Sigma-Aldrich Organic Synthesis Meeting**  
Spa (Belgium), 6-7 Dec 2012  
Participation (poster): Mai, A. H., De Borggraeve W. M., "Development of building blocks for integrase inhibitors."
2. **4<sup>th</sup> Conference on Frontiers in Organic Synthesis Technology - FROST4**  
Budapest (Hungary), 16-18 Oct 2013  
Participation (poster): Mai, A. H., De Borggraeve W. M., "Development of building blocks for integrase inhibitors."
3. **17<sup>th</sup> Sigma-Aldrich Symposium**  
Blankenberge (Belgium), 5-6 Dec 2013  
Participation (poster): Mai, A. H., De Borggraeve W. M., "Development of building blocks for integrase inhibitors."
4. **12<sup>th</sup> Chemistry Conference for Young Scientists - ChemCYS 2014**  
Blankenberge (Belgium), 27-28 Feb 2014  
Participation (poster): Mai, A. H., De Borggraeve W. M., "Development of building blocks for integrase inhibitors."
5. **14<sup>th</sup> Belgian Organic Synthesis Symposium - BOSS XIV**  
Louvain-La-Neuve (Belgium), 13-18 Jul 2014  
Participation (poster): Mai, A. H., De Borggraeve W. M., "Selective debenzylation of *N*-benzyloxy-pyrazinones in flow."