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6-Arylpyrazine-2-carboxamides: A New Core for *Trypanosoma brucei* Inhibitors

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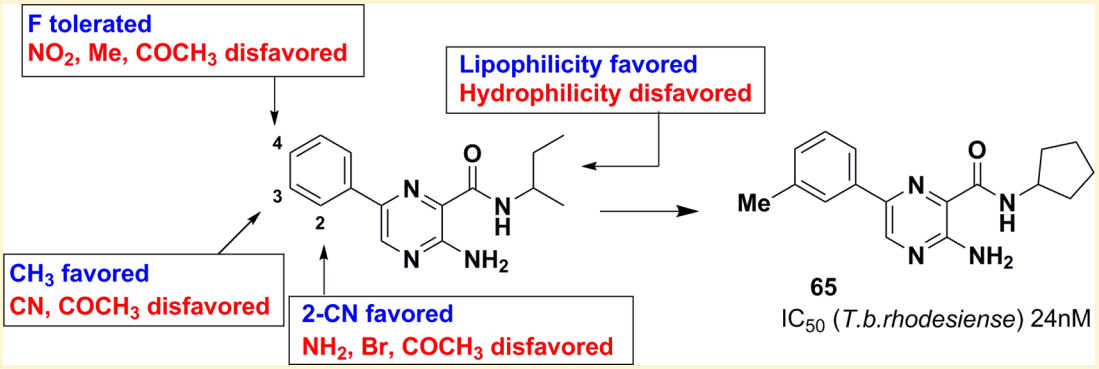
Supporting Information



ABSTRACT: From a whole-organism high throughput screen of approximately 87000 compounds against *Trypanosoma brucei brucei*, we recently identified eight new unique compounds for the treatment of human African trypanosomiasis. In an effort to understand the structure−activity relationships around these compounds, we report for the first time our results on a new class of trypanocides, the pyrazine carboxamides. Attracted by the low molecular weight (270 g·mol^{-1}) of our starting hit (**9**) and its potency (0.49 μM), the SAR around the core was explored, leading to compounds having an EC_{50} as low as 25 nM against *T. b. brucei* and being more than 1500 times less toxic against mammalian L6 and HEK293 cell lines. The most potent compounds in the series were exquisitely selective for *T. brucei* over a panel of other protozoan parasites, showing an excellent correlation with the human infective parasite *Trypanosoma brucei rhodesiense*, the most potent compound (**65**) having an EC_{50} of 24 nM. The compounds are highly drug-like and are able to penetrate the CNS, their only limitation currently being their rate of microsomal metabolism. To that effect, efforts to identify potential metabolites of selected compounds are also reported.

INTRODUCTION

Human African trypanosomiasis (HAT), also known as sleeping sickness, belongs to a group of diseases that are considered to be neglected. HAT is endemic to sub-Saharan Africa, covering 37 countries.[1] Two forms of HAT are known and are caused by different parasite subspecies, both of which are transmitted by the bite of the tsetse fly. In Western and Central Africa, *Trypanosoma brucei gambiense* is responsible for a chronic infection (>95% of reported cases), whereas *Trypanosoma brucei rhodesiense* causes an acute infection in Eastern and Southern Africa.[1] According to the World Health Organization (WHO), 70 million people living in endemic areas are at risk of contracting HAT and about 8000 new HAT cases are reported annually.[1−3] If untreated, this disease is ultimately fatal in almost all cases and only a few drugs are currently available for its treatment (Figure 1).[4] Suramin (**1**) and pentamidine (**2**) are used for the hemolymphatic or early stage of the infection which is characterized by the spread of trypanosomes in the blood and lymphatic systems. The second,

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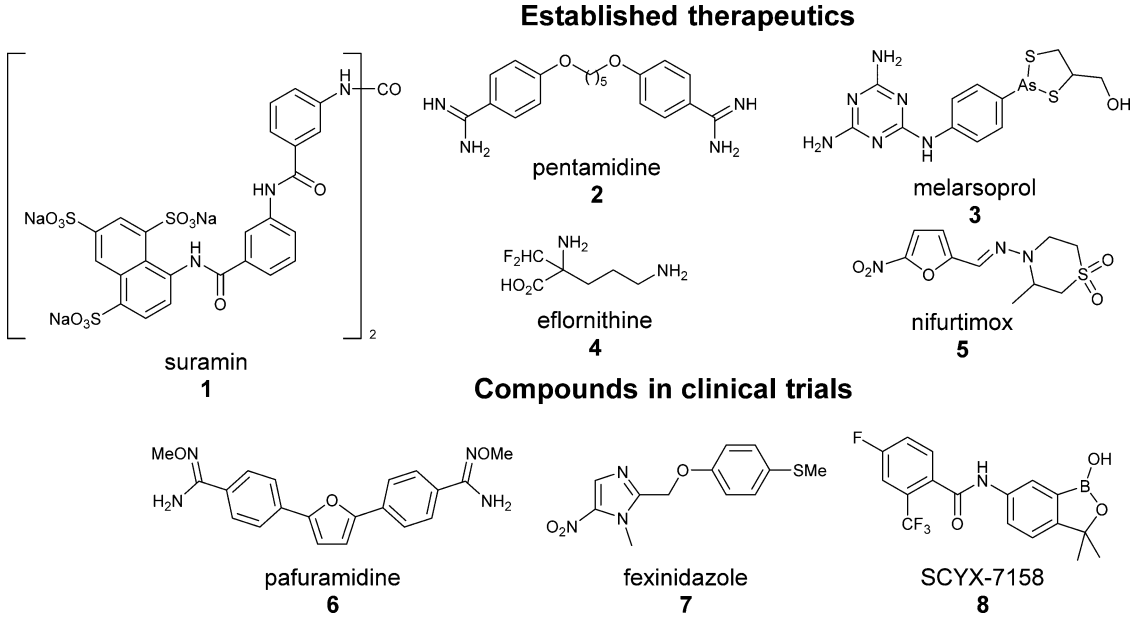

Figure 1. Structure of currently marketed HAT drugs and compounds in chemical trials.

encephalitic stage, is reached once the parasite crosses the blood–brain barrier (BBB) and enters the central nervous system (CNS). During this stage of the disease, chemotherapeutics are required to cross the BBB in order to be efficacious. A well-known treatment for the later stage of the infection, melarsoprol (**3**), was discovered in 1949; however, it shows unacceptable toxicity leading to a 5.9% fatality rate during treatment.[5] Recently, studies have also shown an increasing number of melarsoprol-resistant parasites.[6] Eflornithine (**4**) is reported to be a safer alternative to melarsoprol, but it is only effective against *T. b. gambiense*. It is also expensive and requires special equipment for dosing, making its use difficult in the endemic, rural areas.[7] Finally, combination therapies using eflornithine and nifurtimox (**5**) (NECT), a drug initially used to treat Chagas disease (another disease of the kinetoplastids but caused by infection with *Trypanosoma cruzi*), was found to be as efficacious as eflornithine (**4**) alone and its administration easier.[8] NECT also showed some activity against melarsoprol-resistant parasites.[9] During the last 20 years, only one molecule (eflornithine) has been approved by the FDA for the treatment of HAT, and therefore there is a lack of an efficacious and nontoxic treatment for this disease. Recently, a concerted effort has been made in the quest for finding new treatments for HAT,[10] with a few compounds entering clinical development. Pafuramidine (**6**) has been abandoned in phase I clinical trials due to liver toxicity and renal insufficiency.[11] Two drugs are still being studied in clinical trials, fexinidazole (**7**)[12] and SCYX-7158 (**8**).[13]

Our group, in collaboration with the Eskitis Institute, recently reported the whole-organism high throughput screening (HTS)-lead discovery of several classes of drug-like compounds that potently inhibited *T. b. brucei*.[14] *T. b. brucei* is a subspecies of *T. brucei*, is nonpathogenic to humans, and is used as a model subspecies in drug discovery. We have recently described the discovery of the pyridoxazole anilides[15] and pyridyl benzamides[16] as potent in vitro inhibitors of *T. b. brucei*. Herein, we reveal our SAR investigation into another of these HTS hits (**9**, Figure 2) that contains a 2-amino-5-aryl-3-aminopyrazine carboxamide core presenting very attractive

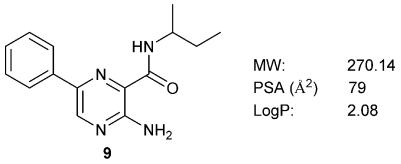

Figure 2. Structure and physicochemical properties of the recently identified HTS hit (**9**).

physicochemical properties. We have discovered several novel and drug-like analogues with EC_{50} values against *T. b. brucei* and *T. b. rhodesiense* of less than 50 nM. One of these compounds (**65**) displayed a selectivity index to the parasite against L6 cells of greater than 1000, with a molecular weight below 300 g·mol^{-1}.

RESULTS AND DISCUSSION

Synthetic entry to **9** and its analogues was readily undertaken in a two-step sequence starting with the commercially available methyl 3-amino-6-bromopyrazine-2-carboxylate, which could be selectively converted to the corresponding amide under basic conditions or microwave irradiation followed by installation of a 6-aryl group via a Suzuki coupling reaction (Scheme 1).

In the first instance, the amide was probed using a suite of commercially available amines and the results are shown in Table 1. Here it can be seen that shortening the alkylamide from *sec*-butyl (**9**, EC_{50} 0.49 μM) to methyl (**13**) leads to a loss of activity. Even shortening by just one methylene to the isopropyl (**10**, EC_{50} 2.6 μM) leads to a 5-fold loss in activity, indicating that hydrophobicity is an important parameter at this position. Conversely, increasing hydrophobicity is favorable and the cyclopentylamide (**11**, EC_{50} 0.26 μM) as well as the 3-pentyl analogue (**12**, EC_{50} 0.32 μM) show a 2-fold improvement in activity. Importantly, this hydrophobic effect on potency has a ceiling and the bulkier amides such as **21** lose activity (EC_{50} 2.6 μM) or, in the case of **19**, become inactive at the highest concentrations tested. This is supportive of the notion of binding to a hydrophobic pocket where a size limit on

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Scheme 1. Synthetic Route to Pyrazine-Based SAR Probes

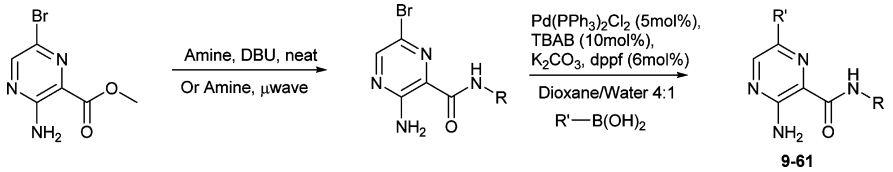
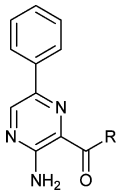


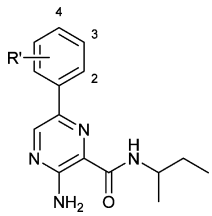
Table 1. Amide Side Chain SAR against *T. b. brucei*



no.	amine (R)	EC_{50} (μM)	SI[a]	no.	amine (R)	EC_{50} (μM)	SI[a]
9	$NHCH(CH_3)CH_2CH_3$	0.49 ± 0.08	>263	**18**	$NHCH_2CH_2$-pyrrolidin-1-yl	>10	
10	$NHCH(CH_3)_2$	2.6 ± 0.4	>33	**19**	$NHCH_2CH_2Ph$	>10	
11	NH-cyclopentyl	0.26 ± 0.01	>368	**20**	$NHCH_2CH_2$-morpholin-1-yl	>10	
12	$NHCH(CH_2CH_3)_2$	0.32 ± 0.09	>263	**21**	$NHCH_2Ph$	2.6 ± 0.5	32
13	$NHCH_3$	>10		**22**	$NHC(CH_3)_3$	3.0 ± 0.4	>14
14	$NHCH_2(CH_2)_3CH_3$	1.7 ± 0.2	50	**23**	NHPh	>10	
15	$NHCH(CH_3)CH_2CH_2CH_3$	0.53 ± 0.05	>156	**24**	pyrrolidin-1-yl	6.1 ± 1.5	>14
16	$NHCH_2(CH_2)_3OH$	7.3 ± 0.5	>11	**25**	piperidin-1-yl	4.6 ± 1.2	>18
17	$NHCH_2CH_2OH$	>10		**26**	morpholin-1-yl	>10	

[a]SI = Selectivity index relative to HEK293 cells for selected compounds.

Table 2. SAR around the 6-Aryl Group against *T. b. brucei*



no.	aromatic substitution (R′)	EC_{50} (μM)	SI[a]	no.	aromatic substitution (R′)	EC_{50} (μM)	SI[a]
9	none	0.49 ± 0.08	>263	**40**	3-NO_2	0.45 ± 0.03	185
27	2-F	0.75 ± 0.34	111	**41**	3-NH_2	1.1 ± 0.1	>77.8
28	2-Cl	1.1 ± 0.4	76	**42**	3-$COCH_3$	>10	
29	2-Br	>10		**43**	3-CH_3	0.17 ± 0.01	>495
30	2-OCH_3	5.4 ± 0.3	>15.4	**44**	3-CN	2.7 ± 0.57	>30.5
31	2-NO_2	0.89 ± 0.23	94	**45**	4-F	0.40 ± 0.04	>206
32	2-NH_2	>10		**46**	4-Cl	0.68 ± 0.05	122
33	2-$COCH_3$	10 ± 1	7.9	**47**	4-Br	2.2 ± 0.6	38
34	2-CH_3	1.2 ± 0.1	>71	**48**	4-OCH_3	1.3 ± 0.1	>63.9
35	2-CN	0.35 ± 0.14	>239	**49**	4-NO_2	4.5 ± 2.1	>18.5
36	3-F	0.82 ± 0.19	102	**50**	4-NH_2	2.9 ± 0.1	>28.4
37	3-Cl	0.48 ± 0.04	175	**51**	4-$COCH_3$	4.7 ± 1.0	8.8
38	3-Br	0.57 ± 0.02	147	**52**	4-CH_3	3.74 ± 1.1	22
39	3-OCH_3	0.41 ± 0.03	>201	**53**	4-CN	2.7 ± 1.0	>30.4

[a]SI = Selectivity index relative to HEK293 cells for selected compounds.

the amide side chain is imposed. The anilide (**23**) was also inactive at the highest concentration tested and likewise suggests that a planar aromatic group provides steric hindrance resulting in a loss of activity. Further branching to a *tert*-butylamide is not particularly favorable (**22**, EC_{50} 3.0 μM). Within the pentylamide series, the longer *sec*-pentyl (**15**, EC_{50} 0.53 μM) has a comparable activity to the initial hit, whereas the linear *n*-pentyl (**14**, EC_{50} 1.7 μM) displays a reduction in activity. These results suggest that having a branched aliphatic group is highly favored. All attempts to introduce solubilizing groups in this region led to significant losses in activity (**16**–**18**, **20**). Tertiary amides **24**–**26** all displayed large losses of activity, suggesting that the NH may contribute to hydrogen bonding to the biological target. We have interpreted the amide SAR in the

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Table 3. Combination of Favorable Groups to Look for Additive SAR against *T. b. brucei*

no.	amine (R)[a]	aromatic substitution (R′)	EC_{50} (μM)	SI[b]	no.	amine (R)[a]	aromatic substitution (R′)	EC_{50} (μM)	SI[b]
54	A	2-CN	0.17 ± 0.00	488	**64**	B	2-CN	0.20 ± 0.04	416
55	A	3-CH_3	0.11 ± 0.01	774	**65**	B	3-CH_3	0.035 ± 0.008	2380
56	A	3-CH_3 and 5-CH_3	0.48 ± 0.21	173	**66**	B	4-F	0.071 ± 0.006	590
57	A	4-F	0.08 ± 0.03	1024	**67**	B	3-CH_3 and 4-F	0.024 ± 0.001	3540
58	A	3-CH_3 and 4-F	0.025 ± 0.01	423	**68**	B	2-CN and 4-F	0.057 ± 0.003	1470
59	A	2-CN and 4-F	0.10 ± 0.00	850	**69**	B	3-CF_3	0.15 ± 0.00	560
60	A	3-CF_3	0.26 ± 0.03	80	**70**	B	3-Cl	0.24 ± 0.06	340
61	A	3-Cl	0.36 ± 0.05	110	**71**	B	3-Cl and 5-Me	0.50 ± 0.19	170
62	A	3-Cl and 5-Me	0.77 ± 0.31	50	**72**	B	3-Cl and 4-F	0.11 ± 0.01	190
63	A	3-Cl and 4-F	0.22 ± 0.11	190					

[a]A = $NHCH(CH_2CH_3)_2$, B = NH-cyclopentyl. [b]SI = Selectivity index relative to HEK293 cells.

context of binding to a specific biological target. It cannot be ruled out that the less hydrophobic amides are less active because of poorer membrane permeability. However, we believe this is a relatively minor effect based on the observations that the bulkiest amides are less active even if they are more hydrophobic, and the tertiary amides, for which membrane permeability should be enhanced and are about the same size as our most potent amides, are also less active.

To understand the SAR around the northern aromatic ring, a range of singly substituted phenyl rings were introduced probing different properties (including lipophilicity and hydrophilicity) at each position (Table 2). When considering the *ortho* position, the introduction of a small halogen atom (**27** and **28**, EC_{50} 0.75 and 1.1 μM), a nitro (**31**, EC_{50} 0.89 μM), or a methyl (**34**, EC_{50} 1.2 μM) were all tolerated but did show a minor decrease in activity compared with the activity of **9**. When a larger halogen atom such as bromine (**29**) was introduced, the resulting compound was inactive. The presence of more hydrophilic substituents led to either a major decrease in activity as in the case of a methoxy (**30**, EC_{50} 5.4 μM) or a total loss in activity as in the case of an amino or acetyl group (**32** and **33**). The cyano group (**35**, EC_{50} 0.35 μM) was the only *ortho*-substituent leading to a boost in activity. When looking at the *meta*-position, polar groups were not beneficial with an unchanged activity for a nitro substituent (**40**, EC_{50} 0.45 μM), a moderate decrease for the amino and cyano groups (**41** and **44**, EC_{50} 1.1 and 2.7 μM), and a total loss in activity for the acetyl (**42**). Unlike polar groups, the small lipophilic substituent fluorine (**36**, EC_{50} 0.82 μM) showed a slight decrease in activity while larger substituents like chlorine (**37**, EC_{50} 0.48 μM), bromine (**38**, EC_{50} 0.57 μM), and methoxy (**39**, EC_{50} 0.41 μM) were well tolerated, exhibiting potency similar to the initial hit (**9**). The introduction of a methyl group (**43**, EC_{50} 0.17 μM) led to a distinct boost with a 3-fold improvement in activity. Finally, at the *para*-position, a similar activity was observed for substitution with small halogens like fluorine (**45**, EC_{50} 0.40 μM) and chlorine (**46**, EC_{50} 0.68 μM) while any other substituent (bromo **47**, methoxy **48**, nitro **49**, amino **50**, acetyl **51**, methyl **52**, cyano **53**) tended to be slightly less active. One should be wary of overinterpreting this SAR for several reasons. First, the readout is from a cell-based assay and each substituent may have subtly different effects on important factors such as serum protein binding and permeability in addition to target(s) activity. Also, there is the potential for different SAR streams for the two possible *ortho* positions (2 and 6) and similarly for the *meta* positions (3 and 5). It does appear, however, that the preferred drug-like substitutions (common in known drugs and also not too hydrophobic) for the best potency were 2-cyano, 3-methyl, and 4-fluoro.

We therefore made a selection of compounds bearing these substituents using the favorable cyclopentylamide and 3-pentylamide groups identified previously, an added attraction being the loss of chirality in these two side chains. The results are reported in Table 3. Considering the 3-pentylamide series first, introduction of 2-cyano (**54**, EC_{50} 0.17 μM) resulted in a 3-fold increase in activity in comparison to **34**, and the 4-fluoro (**57**, EC_{50} 0.08 μM) followed a similar trend with a 5-fold boost in activity. Surprisingly, the combination of the preferred amide side chains with the favored 3-methyl group gave a similarly active compound, this being **55**, with an EC_{50} of 0.11 μM. As a potential homologue of a methyl group, the 3-trifluoromethyl analogue (**60**, EC_{50} 0.26 μM) was also made and tested as part of this second-generation SAR effort but showed a slight decrease in activity compared with **55**. Relative to its methyl counterpart, this weaker activity could be a result of excessive hydrophobicity or electronegativity in the trifluoromethyl group. A similar result was obtained with a 3-chloro group and **61** returned an EC_{50} 0.36 μM. We then investigated some analogues containing two substituents, in particular with a view to discovery of additive SAR. For example, the 3,5-dimethyl compound (**56**, EC_{50} 0.48 μM) was made and tested but resulted in a loss of activity, confirming that the two *meta* positions are not equally favorable. A similar result was obtained for the 3-choro, 5-methyl analogue **62** with an EC_{50} of 0.77 μM. However, the 3-methyl, 4-fluoro analogue **58** displayed a noticeable improvement in activity with an EC_{50} of 0.025 μM, corresponding to a 4- and 3-fold improvement in potency compared with **55** and **57**, respectively. The combination of 2-cyano and 4-fluoro (**59**, EC_{50} 0.10 μM) did not exhibit any improvement compared with **54** and **57**.

A similar set of analogues was then prepared based on the cyclopentyl analogue to compare the properties of each side chain. As previously described for the 3-pentylamide series, the 3-chloro analogues **70**, **71**, and **72** were the least active analogues of this set. It can be seen that the cyclopentylamide with a nitrile in the *ortho* position (**64**, EC_{50} 0.20 μM) gave a compound similarly active to the *sec*-butyl derivative (**34**). Finally, when combining the cyclopentylamide with a *meta*-methyl substituent, some additive SAR was observed, leading to 20-fold improvement in potency (**65**, EC_{50} 0.035 μM). Attracted by this major improvement, analogues having a 3-Me substituent or an analogue were tested. Similarly to the 3-pentylamide, the 3-trifluoromethyl-substituted analogue (**69**, EC_{50} 0.15 μM) showed a 4-fold drop in activity while the 4-fluoro counterpart **66** exhibited additive SAR with an EC_{50} of

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Table 4. Activity of Selected Compounds against a Panel of Parasites[a]

	EC_{50} (μM)						
no.	*T. b. brucei*	*T. b. rhodesiense*[c]	*T. cruzi*[d]	*L. donovani* Axe[e]	*P. falciparum* K1[f]	cytotox L6[g]	SI[h]
9	0.49 ± 0.08	0.97 ± 0.45[a]	19 ± 5[a]	>10	11 ± 5[a]	18 ± 3[a]	19
11	0.26 ± 0.01	0.14 ± 0.08[a]	13 ± 7[a]	>10	6.1 ± 2.6[a]	20 ± 7[a]	140
35	0.35 ± 0.14	0.64 ± 0.50[b]	11 ± 2[a]	>10	3.6 ± 0.1[a]	18 ± 2[a]	7
37	0.48 ± 0.04	2.02 ± 0.9[a]	25 ± 11[a]	>10	1.1 ± 0.5[a]	39 ± 12[a]	20
43	0.17 ± 0.01	0.13 ± 0.07[a]	25 ± 12[a]	>10	19 ± 8[a]	43 ± 11[a]	340
45	0.40 ± 0.04	0.22 ± 0.05[b]	17 ± 4[a]	>10	9 ± 4.5[a]	17 ± 3[a]	61
54	0.17 ± 0.00	0.13 ± 0.10[b]	15 ± 1[a]	>10	4.8 ± 0.8[a]	10 ± 1[a]	80
55	0.11 ± 0.01	0.14 ± 0.06[a]	20 ± 4[a]	>10	4.1 ± 0.4[a]	20 ± 4[a]	150
57	0.08 ± 0.03	0.053 ± 0.032[a]	15 ± 3[a]	>10	7.6 ± 0.1[a]	28 ± 3[a]	540
58	0.025 ± 0.01	0.038 ± 0.018[a]	13 ± 1[a]	>10	1.8 ± 0.5[a]	24 ± 10[a]	630
59	0.10 ± 0.00	0.089 ± 0.061[b]	19 ± 4[a]	>10	2.9 ± 0.2[a]	20 ± 1[a]	230
64	0.20 ± 0.04	0.57 ± 0.05[a]	5.2 ± 0.7[a]	>10	2.5 ± 1.2[a]	11 ± 1[a]	19
65	0.035 ± 0.008	0.024 ± 0.095[a]	17 ± 4[a]	>10	4.9 ± 1.8[a]	42 ± 9[a]	1790

[a]Values are means of two experiments. [b]Values are means of three experiments. [c]*T. b. rhodesiense* strain STIB 900, bloodstream form (trypomastigotes). [d]*T. cruzi* Tulahaen C4 strain, amastigote stage. [e]*L. donovani* MHOM-ET-67/L82 strain, amastigote stage. [f]*P. falciparum* K1 strain, erythrocytic stage. [g]Rat skeletal myoblast cell L-6 strain. [h]SI = Selectivity index relative to L6 cells over *T. b. rhodesiense* for selected compounds.

Table 5. Key Physicochemical Parameters and in Vitro Metabolic Stability of Selected Compounds

				solubility (μg/mL)[c]					
no.	MW	PSA (Å^2)[a]	log D[b] pH 7.4	pH 2	pH 6.5	cPPB[d] (%)	degradation half-life (min)	in vitro CL_{int} (μL/min/mg protein)[e]	microsome-predicted E_H[e]
11	282	81	4.1	6.3−12.5	25−50	95.8	9	198	0.88
12	284	80.9	4.3	6.3−12.5	3.1−6.3	95	13	133	0.84
22	290	81	4.3	<1.6	<1.6	98.5	7	246	0.91
35	295	105	3.6	1.6−3.1	1.6−3.1	95.0	14	122	0.82
37	305	81	4.4	1.6−3.1	1.6−3.1	97.2	12	147	0.85
45	288	81	4.0	6.3−12.5	50−100	94.2	15	119	0.82
46	305	81	4.5	3.1−6.3	1.6−3.1	97.0	36	48	0.65
55	298	80.9	4.7	1.6−3.1	1.6−3.1	96.4	9	194	0.88
57	302	80.9	4.4	3.1−6.3	3.1−6.3	96.4	21	84	0.77
58	316	80.9	4.8	<1.6	<1.6	97.8	8	212	0.89
59	327	104.7	4.2	<1.6	1.6−3.1	97.1	26	66	0.72
64	307	105	3.9	3.1−6.3	<1.6	96.2	10	165	0.86
65	296	81	4.6	3.1−6.3	3.1−6.3	97.7	7	266	0.91

[a]Calculated using ACD/Laboratories software, version 9. [b]Measured chromatographically. [c]Kinetic solubility determined by nephelometry. [d]Human plasma protein binding estimated using a chromatographic method. [e]In vitro intrinsic clearance determined in human liver microsomes and predicted hepatic extraction ratio calculated from in vitro data.

0.071 μM. To see if the additive SAR could be pushed further, disubstituted analogues have been synthesized such as the 3-Me and 4-F (**67**, EC_{50} 0.024 μM), giving the most active compound of this study. The same effect is also obtained with **68** having a 2-cyano and 4-fluoro (EC_{50} 0.057 μM).

In Table 4, it can be seen that potent *T. b. brucei* activity corresponds to similarly potent *T. b. rhodesiense* activity for most compounds in this assay while **9**, **35**, **37**, and **64** are less potent than expected on this basis against *T. b. rhodesiense*. All compounds are highly selective for *T. brucei* over the other protozoan parasites tested including other kinetoplastids. Compounds **57** and **59** were standouts with respective EC_{50} values against *T. b. rhodesiense* of 53 and 89 nM and with selectivity indices relative to mammalian L6 cells of 540-fold and 230-fold, respectively. Once again, excellent results were obtained with compounds **58** and **65** showing EC_{50} values of 24 and 38 nM, respectively, with excellent selectivity toward other parasites (from 250-fold to >1500-fold) and mammalian cells (630-fold and 1790-fold, respectively).

We then assessed the drug-likeness of our lead compounds by measuring physicochemical and metabolic parameters, outlined in Table 5. All compounds are attractively small, with most having a molecular weight under or around 300 g·mol^{-1} and polar surface area (PSA) values are between 81 and 105 Å^2. While these values fall outside of the generally accepted range for optimal CNS penetration (<85 Å^2),[17] which is crucial in order to treat the second stage of HAT, it is important to remember that PSA is a relatively crude and simplistic measure for such purposes. Intramolecular electrostatic interactions between the 3-amino and 2-acetyl and between the 1-pyridinyl and 2-carboxamide are likely to result in a significantly diminished effective PSA.[18] Furthermore, Pfizer has shown CNS penetration relies on a combination of factors and favorable properties in some characteristics can lead to tolerance of outlying values for other properties.[19] On the basis of certain physicochemical properties such as lipophilicity and molecular weight among others, a CNS multiparameter score (CNS MPO) can be calculated for a compound, with optimal CNS penetration predicted for compounds having a

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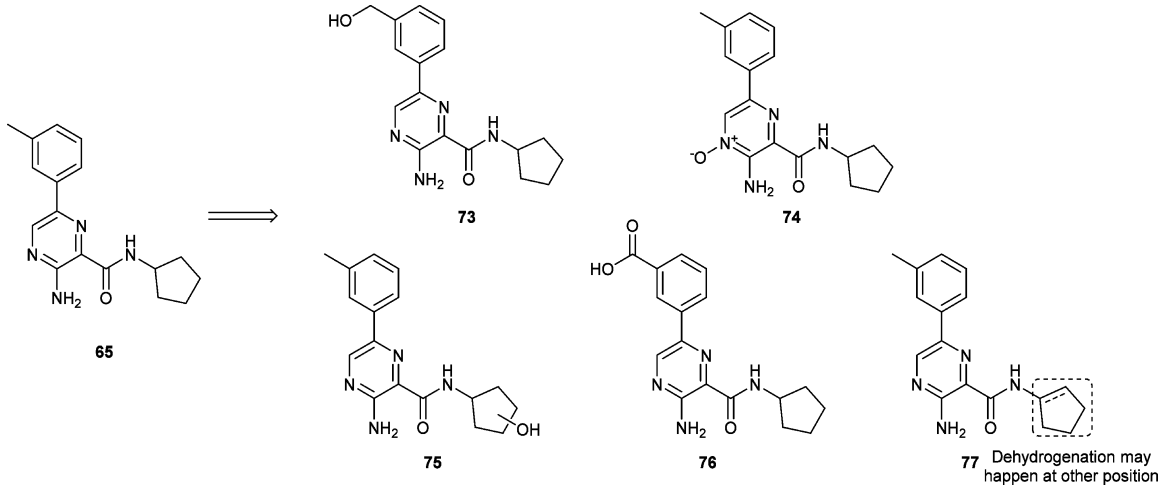

Figure 3. Putative structures of identified metabolites of **65**.

CNS MPO score higher than 4.[19] Compound **65** has a CNS MPO score of 4.25, suggesting it has the potential to cross the BBB. The distribution coefficients lie within an acceptable range of 3.6−4.5 at physiological pH and some, such as **45**, have good solubility (50−100 μg/mL at pH 6.5), although others are less soluble. Plasma protein binding[20] is well below the preferred conventional limits of 99.5% for most of the compounds.[21] Microsomal stability is more limiting in this series, the most stable compound being **46** with a degradation half-life of 36 min.

To address the metabolic instability of this series, the in vitro metabolism of the most active analogue in this study (**65**) was analyzed in greater depth (Figure 3). When incubated in NADPH-supplemented human liver microsomes, 92% of **65** was consumed over 60 min. Five putative mono-oxygenated metabolites were detected with a mass of $[M + 16]^+$, suggesting three different oxidation sites: the methyl on the northern aromatic ring with the formation of a hydroxymethyl moiety (**73**), the tolyl−pyrazine scaffold with the speculated formation of the *N*-oxide (**74**), and the amide moiety with the hydroxylation of the cyclopentane (**75**). The putative carboxylic acid $[M + 30]^+$ (**76**) results from the further oxidation of the hydroxymethyl (**73**), while a metabolite having a mass of $[M - 2]^+$ is indicative of the dehydrogenation of the cyclopentane (**77**). Several secondary metabolites resulting from the combination of the previously mentioned modifications were also detected. Five putative compounds having a mass of $[M + 32]^+$ corresponding to bis-oxygenation and four other metabolites with a molecular ion of $[M + 14]^+$ resulting from both an oxygenation and a dehydrogenation could be detected, but their structure could not be confirmed due to the weakness of the MS/MS signal. The incubation of **65** in the presence of NADPH and UDP−glucuronic acid (UDPGA) did not reveal any substantial difference in the extent of metabolism compared with NADPH alone, although the formation of several secondary glucuronide metabolites was observed and confirmed by MS/MS, suggesting a potential contribution of phase II metabolic pathway. Finally, in microsomal samples devoid of cofactors, despite observing only approximately 6% loss of parent over 60 min, more putative *N*-oxide (**74**) was detected (based on relative peak area in each incubation) than in NADPH supplemented incubation samples. In addition, this $[M + 16]^+$ peak area decreased over time in NADPH-supplemented incubations, suggesting that this metabolite likely undergoes further metabolic degradation. Although it appears that cofactor independent enzymes play a major role in the formation of the putative *N*-oxide (**74**), overall it seems that NADPH-dependent metabolism by cytochrome P450 enzymes plays a key role in the degradation of this series of compounds. We have not proven that the *N*-oxide is the $[M + 16]^+$ metabolite and the 6-hydroxypyrazine cannot be ruled out. However, formation of this metabolite is usually catalyzed by cytosolic aldehyde oxidases which are usually not present in the liver microsomes.

The metabolite analysis points to deployment of fluorinated aliphatics and perhaps nitrile replacement of heterocyclic nitrogen[22] as future potential solutions to rendering this class more stable to metabolic degradation.

The importance of a CNS-penetrating drug to treat the second stage of HAT is such that in addition to calculating PSA, we selected two representative compounds from the previous study for in vivo assessment of brain penetration based on their activity and physicochemical properties (Table 6). The brain-to-plasma partitioning ratios for compounds **11** and **45** were determined 5 min postdose IV administration using male Swiss outbred mice. As shown, the average brain-to-plasma ratios of compounds **11** and **45** were very high (5.7 and 5.1, respectively), revealing a high brain uptake for both compounds.[23]

Table 6. Measured in Vivo CNS Exposure of Selected Compounds

no.	plasma concentration (ng/mL)[a]	brain concentration (ng/g)[a]	in vivo B:P ratio
11	205.1 ± 40.5	1192.3 ± 444.5	5.7 ± 1.2
45	219.5 ± 64.9	1069.5 ± 161.1	5.1 ± 1.2

[a]Plasma and brain concentrations have been normalized to a dose of 1 mg/kg. Data are the mean and standard deviation from $n = 3$ mice.

Despite its unoptimized metabolic profile, **57** was subjected to in vivo testing based on its degradation half-life (third highest) and EC_{50} against *T. b. brucei* (0.08 μM). The results obtained are supportive of the predicted data with an apparent half-life of 9.1 h. Compound **57** exhibited a high blood volume of distribution (14.7 L/kg) and moderate blood clearance (69 mL/min/kg). The blood clearance corresponds to approximately 60% of the nominal hepatic blood flow in a mouse (120

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mL/min/kg) and is lower than the predicted blood clearance based on the in vitro metabolic stability observed in mouse liver microsomes. This apparent overprediction of in vivo clearance by the in vitro test system is most likely due to the role of protein binding (both in plasma and microsomes) which was not taken into account in the calculation of the microsome-predicted hepatic extraction ratio. While insufficient to justify expensive and demanding in vivo efficacy studies, we believe these results are promising for the potential of this compound class to be advanced to oral pharmacokinetics and efficacy studies.

In terms of possible modes-of-action of these compounds, the 3-amino-6-aryl-2-carboxamide substructure is reported in a number of different kinase inhibitors.[24,25] Similar structures such as diaminopyrimidines have been identified as inhibitors of *T. b. brucei* and the related protozoan *Leishmania* spp. through the inhibition of mitogen-activated protein kinases (MAPKs) and cdc2-related kinases (CRKs).[26] An extensive library of kinase inhibitors has recently been screened against *T. b. brucei* in a tour de force demonstration of the susceptibility of this parasite to such compounds.[27,28] It is therefore possible that a parasite kinase or indeed multiple kinases are involved in the mechanism of action of these compounds[28] but this remains to be determined.

CONCLUSION

We have identified 3-amino-6-arylpyrazine-2-carboxamides as a novel class of selective *T. brucei* inhibitors and describe investigation of the SAR of the 2-amide and 6-aryl groups. This led to the discovery of new compounds with improved potency from that of the parent compound, having up to a 40-fold increase in activity. Compound **65** inhibited *T. b. rhodesiense* viability, the human pathogenic subspecies of *T. brucei*, with an EC_{50} of 24 nM and was 1790 times less active against the L6 mammalian cell line. This class of compound has many attractive drug-like physicochemical attributes such as low molecular weight, low PSA, and is CNS penetrant. Although its microsome stability is not optimal, we believe that it may not be a limiting factor to progress these because compound **57** showed a good half-life in vivo. Published studies around related heterocycles suggest that a parasite kinase or kinases may be involved in the mechanism of action of these compounds, however, this remains to be determined.

EXPERIMENTAL SECTION

General Chemistry Experimental. Analytical thin-layer chromatography was performed on silica gel 60 F254 precoated aluminum sheets (0.25 mm, Merck) and visualized at 254 nm or by chemical staining with a solution ceric sulfate/phosphomolybdic acid followed by heating. Flash column chromatography was carried out using Merck silica gel 60, 0.63−0.20 mm (70−230 mesh). The purity of all compounds for biological testing was >95% in all cases, the method being either Exp1 or Exp2 as described below.

Experimental Conditions (Exp1). All nonaqueous reactions were performed under an atmosphere of dry nitrogen unless otherwise specified. Melting points were recorded on an "OptiMelt" automated melting point system and are uncorrected. The solvent for recrystallizations for melting point is noted in brackets. Automated flash chromatography was performed on the Biotage Flash Master II, while manual flash chromatography was carried out with silica gel supplied by Merck Chemicals. NMR spectra were recorded on a Bruker UltraShield 300 with the solvents indicated (^{1}H NMR at 300 MHz; ^{13}C NMR at 75 MHz). Chemical shifts are recorded as δ values in parts per million (ppm) values, referenced to the appropriate solvent peak (i.e., $CHCl_3$ = 7.26 and 77.16, MeOH = 3.31 and 49.00, DMSO = 2.50 and 39.52) and coupling constants (J) were recorded in Hz. Infrared spectra were obtained on a Bruker Tensor 27 FT-IR spectrometer at a resolution of 4 cm^{-1}, and absorptions are given in wavenumbers (cm^{-1}). Liquid chromatography−mass spectrometry (LCMS) was performed on two different instruments, a Finnigan LCQ Advantage MAX carried out on a Phenomenex column (Gemini, 3 μm, 110 Å, 20 mm × 4 mm), and a Waters Auto Purification System 3100 carried out with a Waters column (XBridge, 4 μm, 100 Å, 4.6 mm × 100 mm). High performance liquid chromatography (HPLC) was also carried out on two different instruments, the Waters Auto Purification System 3100 with a Waters column (XBridgePrep C18, 5 μm, OBD, 19 mm × 100 mm) and the Waters Alliance HT 2795 with a Phenomenex column (Luna, 5 μm, C18, 100 Å, 150 mm × 10 mm). Compounds purified via this method are noted. Some compounds occasionally gave a shoulder, and this is denoted by an asterisk (*). This can be attributed to overloading but has not definitively identified to be the cause.

Experimental Conditions (Exp2). Microwave reactions were performed on a CEM discovery fitted with an intellivent explorer unit. The temperature range of the unit is −80 to 300 °C, a pressure range of 0−27 bar, power range of 0−300 W, and no prestirring was required. Analytical reverse-phase HPLC was carried out on a Waters Millennium 2690 system, fitted with a Phenomenex Luna C8 100 Å, 5 μm (150 mm × 4.6 mm I.D.) column. A binary solvent system was used (solvent A, 0.1% aqueous TFA; solvent B, 0.1% TFA/19.9% H_2O/80% ACN), with UV detection at 214 nm. A gradient of 0−80% buffer B over 10 min for a 20 min run time was used, with a flow rate of 1 mL/min. ^{1}H and ^{13}C NMR spectra were recorded at 400.13 and 100.62 MHz, respectively, on a Bruker Avance III Nanobay spectrometer with BACS 60 sample changer, using solvents from Cambridge Isotope Laboratories. Chemical shifts (δ, ppm) are reported relative to the solvent peak ($CDCl_3$, 7.26 [^{1}H] or 77.16 [^{13}C]; DMSO-d_6, 2.50 [^{1}H] or 39.52 [^{13}C]). Proton resonances are annotated as chemical shift (δ), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), coupling constant (J, Hz), and number of protons. Low resolution mass spectrometry was performed on an Agilent 6100 series single quad LC/MS coupled with an Agilent 1200 series HPLC, G1311A quaternary pump, G1329A thermostated autosampler, and G1314B variable wavelength detector (214 and 254 nm). LC conditions: Phenomenex Luna C8(2) column (100 Å, 5 mm, 50 mm × 4.6 mm), 30 °C; sample (5 mL) was eluted using a binary gradient (solvent A. 0.1% aq HCO_2H; solvent B.0.1% HCO_2H in CH_3CN; 5−100% B [10 min], 100% B [10 min]; 0.5 mL/min). MS conditions: quadrupole ion source with multimode-ESI; drying gas temperature, 300 °C; vaporizer temperature, 200 °C; capillary voltage, 2000 V (positive mode) or 4000 V (negative mode); scan range, 100−1000 m/z; step size, 0.1 s over 10 min. High resolution MS was performed on an Agilent 6224 TOF LC/MS coupled to an Agilent 1290 Infinity LC. All data were acquired and reference mass corrected via a dual-spray electrospray ionization (ESI) source. Each scan or data point on the total ion chromatogram (TIC) is an average of 13700 transients, producing a spectrum every second. Mass spectra were created by averaging the scans across each peak and subtracting the background from first 10 s of the TIC. Acquisition was performed using the Agilent Mass Hunter Data Acquisition software version B.05.00 Build 5.0.5042.2, and analysis was performed using Mass Hunter Qualitative Analysis version B.05.00 Build 5.0.519.13. Acquisition parameters: mode, ESI; drying gas flow, 11 L/min; nebulizer pressure, 45 psi; drying gas temperature, 325 °C; voltages: capillary, 4000 V; fragmentor, 160 V; skimmer, 65 V; octapole RF, 750 V; scan range, 100−1500 m/z; positive ion mode internal reference ions, m/z 121.050873 and 922.009798. LC conditions: Agilent Zorbax SB-C18 Rapid Resolution HT (2.1 mm × 50 mm, 1.8 mm column), 30 °C; sample (5 mL) was eluted using a binary gradient (solvent A, 0.1% aq HCO_2H; solvent B, 0.1% HCO_2H in CH_3CN; 5 to 100% B [3.5 min], 0.5 mL/min).

General Chemistry Procedures. *General Procedure A.* Methyl 3-amino-6-bromopyrazine-2-carboxylate was dissolved with the relevant amine in a microwave reactor vessel. The reaction mixture

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was then irradiated at 180 °C for 4 h. The solvent was removed to give the crude product, which was purified by column chromatography, eluting with a mixture of ethyl acetate/petroleum benzene to give the desired product.

General Procedure B. Relevant carboxamide (1.0 equiv), potassium carbonate (4.0 equiv), phenylboronic acid (1.2 equiv), TBAB (0.1 equiv), $PdCl_2$ (0.05 equiv), and dppf (0.055 equiv) were combined in a microwave reactor vessel with 4:1 dioxane and water (0.5 M final concentration) added. The reaction mixture was irradiated in a CEM microwave at 130 °C for the time specified. The reaction was diluted with ethyl acetate and filtered through Celite and the filtrate collected. The solvent was removed to give the crude product, which was purified by column chromatography, eluting with 15% ethyl acetate/petroleum benzene to give the desired product.

Compound Characterization. *3-Amino-6-bromo-N-(sec-butyl)-pyrazine-2-carboxamide (Exp2).* Methyl 3-amino-6-bromopyrazine-2-carboxylate (2.15 mmol) was combined with *sec*-butylamine (21.50 mmol) in a microwave reactor vessel and irradiated in the CEM microwave for 6 h at 100 °C. The reaction mixture was transferred to a round-bottom flask and the solvent removed under reduced pressure to give the crude material which was purified by column chromatography, eluting with 10% ethyl acetate/petroleum benzene. The carboxamide was isolated as a yellow solid (87%). HPLC: rt 9.22 min >96% purity at 254 nm. LRMS $[M + H]^+$ 273.1 m/z (^{79}Br), 275.1 m/z (^{81}Br). HRMS calcd for $C_9H_{14}BrN_4O$ $[M + H]^+$ 273.0346 m/z, found 273.0346 m/z (^{79}Br), 275.0326 m/z, found 275.0326 m/z (^{81}Br). ^1H NMR (400 MHz, DMSO) δ 8.33 (s, 1H), 8.18 (d, J = 8.8 Hz, 1H), 7.70 (s, 2H), 3.89 (dt, J = 21.7, 7.2 Hz, 1H), 1.67−1.39 (m, 2H), 1.14 (d, J = 6.6 Hz, 3H), 0.84 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 164.13, 154.20, 148.51, 125.70, 121.46, 46.21, 28.56, 19.96, 10.76.

3-Amino-6-bromo-N-isopropylpyrazine-2-carboxamide (Exp1). Title compound prepared according to general procedure A (76%). LCMS (Finnigan): rt 7.15 min >99% purity at 254 nm. LRMS $[M + H]^+$ 259.1 m/z (^{79}Br), 261.1 m/z (^{81}Br). ^1H NMR (400 MHz, MeOD) δ 8.20 (s, 1H), 7.61 (s, 1H), 4.30−4.23 (m, 1H), 1.38 (d, J = 6.8 Hz, 6H).

3-Amino-6-bromo-N-cyclopentylpyrazine-2-carboxamide (Exp1). Title compound prepared according to general procedure A (93%). LCMS (Finnigan): rt 9.32 min >99% purity at 254 nm. LRMS $[M + H]^+$ 285.1 m/z (^{79}Br), 287.1 m/z (^{81}Br). ^1H NMR (400 MHz, MeOD) δ 8.22 (s, 1H), 4.26 (s, 1H), 2.05−1.86 (m, 2H), 1.80−1.54 (m, 6H).

3-Amino-6-bromo-N-pentylpyrazine-2-carboxamide (Exp2). Methyl 3-amino-6-bromopyrazine-2-carboxylate (2.15 mmol) was combined with 1-ethylpropylamine (21.50 mmol) in a microwave reactor vessel and irradiated in the CEM microwave for 4 h at 115 °C. The reaction mixture was transferred to a round-bottom flask and the solvent removed under reduced pressure to give the crude material which was recrystallized from acetic acid and water to give the carboxamide as a pale-yellow solid (72%). HPLC: rt 11.59 min >99% purity at 254 nm. LRMS $[M + H]^+$ 287 m/z (^{79}Br), 289 m/z (^{81}Br). HRMS calcd for $C_{10}H_{16}BrN_4O$ $[M + H]^+$ 287.0502 m/z, found 287.0502 m/z (^{79}Br), 289.0482 m/z, found 289.0483 m/z (^{81}Br). ^1H NMR (400 MHz, DMSO) δ 8.34 (s, 1H), 8.10 (d, J = 9.3 Hz, 1H), 7.70 (s, 2H), 3.82−3.65 (m, 1H), 1.65−1.37 (m, 4H), 0.83 (t, J = 7.4 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 164.7, 154.2, 148.5, 125.7, 121.5, 52.0, 26.8 (2C), 10.7 (2C).

3-Amino-6-bromo-N-methylpyrazine-2-carboxamide (Exp2). Title compound prepared according to general procedure A (53%). HPLC: rt 7.86 min >99% purity at 254 nm. LRMS $[M + H]^+$ 231.0 m/z (^{79}Br), 233.0 m/z (^{81}Br). HRMS calcd for $C_6H_8BrN_4O$ $[M + H]^+$ 229.9803 m/z, found 229.9810 m/z (^{79}Br), 231.9783 m/z, found 231.9789 m/z (^{81}Br). ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J = 33.1 Hz, 1H), 7.87−7.51 (m, 1H), 2.97 (d, J = 5.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.61, 153.88, 150.37, 148.89, 126.17, 123.13, 26.08.

3-Amino-6-bromo-N-pentylpyrazine-2-carboxamide (Exp1). Title compound prepared according to general procedure A (53%). LCMS (Finnigan): rt 9.56 min >99% purity at 254 nm; $[M + H]^+$ 287.1 m/z (^{79}Br), 289.1 m/z (^{81}Br). ^1H NMR (300 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.78 (s, 1H), 3.50−3.43 (m, 2H), 1.63−1.56 (m, 2H), 1.48−1.27 (m, 4H), 0.90 (t, J = 7.3 Hz, 3H).

3-Amino-6-bromo-N-(pentan-2-yl)pyrazine-2-carboxamide (Exp1). Title compound prepared according to general procedure A (66%). LCMS (Finnigan): rt 9.54 min >99% purity at 254 nm; $[M + H]^+$ 287.1 m/z (^{79}Br), 289.1 m/z (^{81}Br). ^1H NMR (300 MHz, $CDCl_3$) δ δ 8.61 (s, 1H), 7.40−7.36 (s, 1H), 4.20−4.10 (m, 1H), 1.72−1.59 (m, 2H), 1.49−1.37 (m, 2H), 1.12 (d, J = 6.4 Hz, 4H), 0.93 (t, J = 7.0 Hz, 3H).

3-Amino-6-bromo-N-(4-hydroxybutyl)pyrazine-2-carboxamide (Exp1). Title compound prepared according to general procedure B (36%). LCMS (Finnigan): rt 5.53 min >99% purity at 254 nm; $[M + H]^+$ 289.1 m/z (^{79}Br), 291.1 m/z (^{81}Br). ^1H NMR (300 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.15 (s, 1H), 7.89−7.79 (m, 2H), 7.52−7.43 (m, 2H), 7.43−7.33 (m, 1H), 3.72 (br t, J = 5.2 Hz, 2H), 3.51 (q, J = 6.4 Hz, 2H), 1.89 (s, 1H), 1.83−1.59 (m, 4H).

3-Amino-N-(2-hydroxyethyl)-6-phenylpyrazine-2-carboxamide (Exp1). Title compound prepared according to general procedure B (91%). LCMS (Finnigan): rt 5.40 min >95% purity at 254 nm; $[M + H]^+$ 261.0 m/z (^{79}Br), 263.0 m/z (^{81}Br). ^1H NMR (300 MHz, $CDCl_3$) δ 8.76 (s, 1H), 7.38 (s, 1H), 3.51−3.39 (m, 2H), 3.36−3.29 (m, 2H).

3-Amino-6-bromo-N-(2-(piperidin-1-yl)ethyl)pyrazine-2-carboxamide (Exp1). Title compound prepared according to general procedure B (65%). LCMS (Finnigan): rt 5.29 min >99% purity at 254 nm; $[M + H]^+$ 328.0 m/z (^{79}Br), 330.0 m/z (^{81}Br). ^1H NMR (300 MHz, $CDCl_3$) δ 8.81 (br s, 1H), 7.39 (s, 1H), 3.46 (d, J = 6.1 Hz, 2H), 2.48 (t, J = 6.2 Hz, 2H), 2.43−2.36 (m, 4H), 1.78−1.66 (m, 4H), 1.44−1.37 (m, 2H).

3-Amino-6-bromo-N-phenethylpyrazine-2-carboxamide (Exp1). Title compound prepared according to general procedure B (77%). LCMS (Finnigan): rt 8.23 min >95% purity at 254 nm; $[M + H]^+$ 321.0 m/z (^{79}Br), 323.0 m/z (^{81}Br). ^1H NMR (300 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.49 (s, 1H), 3.71 (m, 2H), 2.93 (t, J = 7 Hz, 2H).

3-Amino-6-bromo-N-(2-morpholinoethyl)pyrazine-2-carboxamide (Exp1). Title compound prepared according to general procedure B (78%). LCMS (Finnigan): rt 5.02 min >99% purity at 254 nm; $[M + H]^+$ 330.0 m/z (^{79}Br), 332.0 m/z (^{81}Br). ^1H NMR (300 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.32 (s, 1H), 3.72 (br d, J = 4 Hz, 4H), 3.51 (m, 2H), 2.76−2.52 (m, 6H).

3-Amino-6-bromo-N-benzylpyrazine-2-carboxamide (Exp1). Title compound prepared according to general procedure B (92%). LCMS (Finnigan): rt 7.17 min >99% purity at 254 nm; $[M + H]^+$ 307.0 m/z (^{79}Br), 309.0 m/z (^{81}Br). ^1H NMR (300 MHz, $CDCl_3$) δ 8.72 (s, 1H), 7.81−7.73 (m, 2H), 7.51−7.46 (m, 4H), 3.90 (s, 2H).

3-Amino-6-bromo-N-(tert-butyl)pyrazine-2-carboxamide (Exp2). Title compound prepared according to general procedure A (26%). HPLC: rt 8.63 min >99% purity at 254 nm. LRMS $[M + H]^+$ 273.1 m/z (^{79}Br), 275.1 m/z (^{81}Br). ^1H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 5.2 Hz, 1H), 7.75−7.39 (m, 1H), 1.46 (s, 9H).

3-Amino-6-bromo-N-phenylpyrazine-2-carboxamide (Exp2). Title compound prepared according to general procedure B (98%). HPLC: rt 7.69 min 96% purity at 254 nm. LRMS $[M + H]^+$ 293.1 m/z (^{79}Br), 295.1 m/z (^{81}Br). ^1H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 8.05−7.90 (m, 2H), 7.50−7.30 (m, 3H).

(3-Amino-6-bromopyrazin-2-yl) (pyrrolidin-1-yl)methanone (Exp2). Title compound prepared according to general procedure B (89%). HPLC: rt 6.15 min >99% purity at 254 nm. LRMS $[M + H]^+$ 271.1 m/z (^{79}Br), 273.1 m/z (^{81}Br). ^1H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.38−7.31 (s, 1H), 5.79 (s, 2H), 3.80−3.70 (m, 4H), 1.84−1.74 (m, 6H).

(3-Amino-6-bromopyrazin-2-yl) (piperidin-1-yl)methanone (Exp2). Title compound prepared according to general procedure A (26%). HPLC: rt 9.35 min >99% purity at 254 nm. LRMS $[M + H]^+$ 285.0 m/z (^{79}Br), 287.0 m/z (^{81}Br). ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 5.96−5.54 (m, 2H), 3.84−3.47 (m, 4H), 1.79−1.57 (m, 6H).

(3-Amino-6-bromopyrazin-2-yl) (morpholino)methanone (Exp2). Title compound prepared according to general procedure A (45%). HPLC: rt 7.58 min >99% purity at 254 nm. LRMS $[M + H]^+$ 287.0 $m/$

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z (^{79}Br), 289.0 m/z (^{81}Br). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 5.92 (s, 2H), 3.89−3.63 (m, 9H).

3-Amino-N-(sec-butyl)-6-phenylpyrazine-2-carboxamide ***(9)*** *(Exp1).* Title compound prepared according to general procedure B (85%). LCMS (Finnigan): rt 7.58 min >99% purity at 254 nm; $[M + H]^+$ 271.2. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.95−7.75 (m, 2H), 7.54−7.44 (m, 2H), 7.44−7.36 (m, 1H), 4.17−3.96 (m, 1H), 1.69−1.57 (m, 3H), 1.27 (d, J = 6.6 Hz, 3H), 0.99 (t, J = 7.4 Hz, 3H).

3-Amino-N-isopropyl-6-phenylpyrazine-2-carboxamide ***(10)*** *(Exp1).* Title compound prepared according to general procedure B (78%). LCMS (Finnigan): rt 5.47 min >99% purity at 254 nm; $[M + H]^+$ 257.1. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.91−1.79 (m, 3H), 7.53−7.49 (m, 2H), 7.49−7.38 (m, 1H), 4.26 (hept, J = 6.9 Hz, 1H), 1.37 (d, J = 6.9 Hz, 6H).

3-Amino-N-cyclopentyl-6-phenylpyrazine-2-carboxamide ***(11)*** *(Exp1).* Title compound prepared according to general procedure B (82%). LCMS (Finnigan): rt 7.87 min >99% purity at 254 nm; $[M + H]^+$ 283.3. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.95 (br d, J = 8.2 Hz, 1H), 7.89−7.80 (m, 2H), 7.49 (ddd, J = 6.7, 2.0, 1.0 Hz, 2H), 7.44−7.35 (m, 1H), 4.45−4.27 (m, 1H), 2.22−2.01 (m, 2H), 1.87−1.50 (m, 6H).

3-Amino-N-(pentan-3-yl)-6-phenylpyrazine-2-carboxamide ***(12)*** *(Exp1).* Title compound prepared according to general procedure B (93%). LCMS (Finnigan): rt 7.98 min 97% purity at 254 nm; $[M + H]^+$ 285.3. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.89−7.81 (m, 2H), 7.54−7.45 (m, 2H), 7.44−7.36 (m, 1H), 4.04−3.85 (m, 1H), 1.76−1.62 (m, 2H), 1.60−1.49 (m, 2H), 0.98 (t, J = 7.4 Hz, 6H).

3-Amino-N-methyl-6-phenylpyrazine-2-carboxamide ***(13)*** *(Exp1).* Title compound prepared according to general procedure B (75%). LCMS (Finnigan): rt 6.12 min >99% purity at 254 nm; $[M + H]^+$ 229.2. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.00 (s, 1H), 7.90−7.82 (m, 2H), 7.52−7.43 (m, 2H), 7.43−7.35 (m, 1H), 3.04 (d, J = 5.1 Hz, 3H).

3-Amino-N-pentyl-6-phenylpyrazine-2-carboxamide ***(14)*** *(Exp1).* Title compound prepared according to general procedure B (81%). LCMS (Finnigan): rt 8.37 min >99% purity at 254 nm; $[M + H]^+$ 285.2. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.04 (br s, 1H), 7.90−7.80 (m, 2H), 7.53−7.44 (m, 2H), 7.44−7.35 (m, 1H), 3.45 (dd, J = 13.9, 6.6 Hz, 2H), 1.73−1.56 (m, 2H), 1.48−1.27 (m, 4H), 0.93 (t, J = 7.1 Hz, 3H).

3-Amino-N-(pentan-2-yl)-6-phenylpyrazine-2-carboxamide ***(15)*** *(Exp1).* Title compound prepared according to general procedure B (76%). LCMS (Finnigan): rt 8.10 min >95% purity at 254 nm; $[M + H]^+$ 285.3. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.84 (ddd, J = 10.2, 5.5, 3.3 Hz, 3H), 7.53−7.44 (m, 2H), 7.44−7.37 (m, 1H), 4.15 (tt, J = 15.3, 6.6 Hz, 1H), 1.64−1.52 (m, 2H), 1.49−1.37 (m, 2H), 1.27 (d, J = 6.6 Hz, 4H), 0.96 (t, J = 7.2 Hz, 3H).

3-Amino-N-(4-hydroxybutyl)-6-phenylpyrazine-2-carboxamide ***(16)*** *(Exp1).* Title compound prepared according to general procedure B (79%). LCMS (Finnigan): rt 5.73 min >99% purity at 254 nm; $[M + H]^+$ 287.2. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.15 (s, 1H), 7.89−7.79 (m, 2H), 7.52−7.43 (m, 2H), 7.43−7.33 (m, 1H), 3.72 (br t, J = 5.2 Hz, 2H), 3.51 (q, J = 6.4 Hz, 2H), 1.89 (s, 1H), 1.83−1.59 (m, 4H).

3-Amino-N-(2-hydroxyethyl)-6-phenylpyrazine-2-carboxamide ***(17)*** *(Exp1).* Title compound prepared according to general procedure B (91%). LCMS (Finnigan): rt 5.40 min >99% purity at 254 nm; $[M + H]^+$ 259.3. ^{1}H NMR (300 MHz, DMSO) δ 8.84 (s, 1H), 8.75 (s, 1H), 8.12 (d, J = 7.2 Hz, 2H), 7.63 (br s, 2H), 7.47 (t, J = 7.5 Hz, 2H), 7.38 (t, J = 7.2 Hz, 1H), 4.80 (t, J = 5.2 Hz, 1H), 3.61−3.50 (m, 2H), 3.41 (dd, J = 11.7, 5.7 Hz, 2H).

3-Amino-6-phenyl-N-(2-(piperidin-1-yl)ethyl)pyrazine-2-carboxamide ***(18)*** *(Exp1).* Title compound prepared according to general procedure B (53%). LCMS (Finnigan): rt 5.00 min >99% purity at 254 nm; $[M + H]^+$ 326.3. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (br s, 1H), 8.65 (s, 1H), 8.01−8.15 (m, 2H), 7.46 (t, J = 7.2 Hz, 2H), 7.39 (t, J = 7.2 Hz, 1H), 3.53 (dd, J = 11.4, 6.1 Hz, 2H), 2.59 (t, J = 6.2 Hz, 2H), 2.53−2.41 (m, 4H), 1.74−1.60 (m, 4H), 1.54−1.44 (m, 2H).

3-Amino-N-phenethyl-6-phenylpyrazine-2-carboxamide ***(19)*** *(Exp1).* Title compound prepared according to general procedure B (77%). LCMS (Finnigan): rt 7.73 min >95% purity at 254 nm; $[M + H]^+$ 319.2. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.09 (s, 1H), 7.76 (dd, J = 5.4, 2.4 Hz, 2H), 7.51−7.18 (m, 3H), 3.73 (q, J = 6.6 Hz, 2H), 2.95 (td, J = 6.9, 3.2 Hz, 2H).

3-Amino-N-(2-morpholinoethyl)-6-phenylpyrazine-2-carboxamide ***(20)*** *(Exp1).* Title compound prepared according to general procedure B (78%). LCMS (Finnigan): rt 4.75 min >99% purity at 254 nm; $[M + H]^+$ 328.3. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (m, 2H), 8.03−7.82 (m, 2H), 7.58−7.31 (m, 3H), 3.79 (br d, J = 3.8 Hz, 4H), 3.56 (m, 2H), 2.72−2.48 (m, 6H).

3-Amino-N-benzyl-6-phenylpyrazine-2-carboxamide ***(21)*** *(Exp1).* Title compound prepared according to general procedure B (92%). LCMS (Finnigan): rt 7.17 min >99% purity at 254 nm; $[M + H]^+$ 305.4. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.36 (s, 1H), 7.83 (dd, J = 8.3, 1.4 Hz, 2H), 7.45 (dd, J = 8.1, 6.4 Hz, 2H), 7.41−7.28 (m, 6H), 4.68 (d, J = 6.2 Hz, 2H), 3.88 (s, 2H).

3-Amino-N-(tert-butyl)-6-phenylpyrazine-2-carboxamide ***(22)*** *(Exp2).* Title compound prepared according to general procedure B (87%). HPLC: rt min 8.13 min >99% purity at 254 nm. LRMS $[M + H]^+$ 271.2. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.00 (s, 1H), 7.88−7.80 (m, 2H), 7.51−7.45 (m, 2H), 7.42−7.36 (m, 1H), 1.50 (s, 9H).

3-Amino-N,6-diphenylpyrazine-2-carboxamide ***(23)*** *(Exp2).* Title compound prepared according to general procedure B (98%). HPLC: rt 7.54 min >95% purity at 254 nm. LRMS $[M + H]^+$ 291.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.93 (s, 1H), 8.68 (s, 1H), 8.00−7.81 (m, 2H), 7.81−7.64 (m, 2H), 7.57−7.47 (m, 2H), 7.47−7.31 (m, 3H), 7.21−7.08 (m, 1H).

(3-Amino-6-phenylpyrazin-2-yl) (Pyrrolidin-1-yl)methanone ***(24)*** *(Exp2).* Title compound prepared according to general procedure B (89%). HPLC: rt 6.15 min >99% purity at 254 nm; LRMS $[M + H]^+$ 269.2. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.90−7.76 (m, 2H), 7.49−7.40 (m, 2H), 7.40−7.36 (m, 1H), 5.776 (s, 2H), 3.80 (m, 4H), 1.80−171 (m, 6H).

(3-Amino-6-phenylpyrazin-2-yl) (Piperidin-1-yl)methanone ***(25)*** *(Exp2).* Title compound prepared according to general procedure B (84%). HPLC: rt 6.39 min 98% purity at 254 nm. LRMS $[M + H]^+$ 283.2. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H), 7.88 (dd, J = 5.2, 3.3 Hz, 2H), 7.49−7.41 (m, 2H), 7.40−7.34 (m, 1H), 5.74 (s, 2H), 3.74 (br d, J = 21.0 Hz, 4H), 1.70 (br s, 6H).

(3-Amino-6-phenylpyrazin-2-yl) (morpholino)methanone ***(26)*** *(Exp2).* Title compound prepared according to general procedure B (73%). HPLC: rt 5.34 min 99% purity at 254 nm. LRMS $[M + H]^+$ 285.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 7.89−7.80 (m, 2H), 7.51−7.42 (m, 2H), 7.42−7.33 (m, 1H), 5.88 (br s, 2H), 4.02−3.69 (m, 8H).

3-Amino-N-(sec-butyl)-6-(2-fluorophenyl)pyrazine-2-carboxamide ***(27)*** *(Exp1).* Title compound prepared according to general procedure B (75%). LCMS (Finnigan): rt 8.00 min >99% purity at 254 nm; $[M + H]^+$ 289.3. HRMS calcd for $C_{15}H_{18}FN_4O$ $[M + H]^+$ 289.1459 m/z, found 289.1461 m/z. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (d, J = 2.4 Hz, 1H), 7.79 (td, J = 7.8, 2.0 Hz, 1H), 7.79 (br s, 1H), 7.41−7.32 (m, 1H), 7.27 (td, J = 7.5, 1.4 Hz, 2H), 7.18 (ddd, J = 11.3, 8.1, 1.3 Hz, 1H), 4.16−3.96 (m, 1H), 1.69−1.52 (m, 2H), 1.26 (d, J = 6.6 Hz, 3H), 0.98 (t, J = 7.4 Hz, 3H). ^{1}H NMR (400 MHz, DMSO) δ 8.60 (d, J = 2.6 Hz, 1H), 8.28 (d, J = 8.8 Hz, 1H), 8.07 (td, J = 8.2, 1.9 Hz, 1H), 7.73 (s, 2H), 7.51−7.38 (m, 1H), 7.38−7.25 (m, 2H), 3.94 (dt, J = 21.7, 7.1 Hz, 1H), 1.71−1.41 (m, 2H), 1.18 (d, J = 6.6 Hz, 3H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.1, 159.5 (J_{C-F} = 246 Hz), 153.9, 146.5 (J_{C-F} = 12 Hz), 134.4 (J_{C-F} = 3 Hz), 130.3 (J_{C-F} = 3 Hz), 130.1 (J_{C-F} = 9 Hz), 125.3, 124.9 (J_{C-F} = 3 Hz), 124.0 (J_{C-F} = 12 Hz), 116.1 (J_{C-F} = 22 Hz), 46.1, 28.7, 20.1, 10.8.

3-Amino-N-(sec-butyl)-6-(2-chlorophenyl)pyrazine-2-carboxamide ***(28)*** *(Exp1).* Title compound prepared according to general procedure B (69%). LCMS (Finnigan): rt 11.53 min >99% purity at 254 nm; $[M + H]^+$ 305.2. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.79 (br d, J = 7.2 Hz, 1H), 7.57−7.47 (m, 2H), 7.44−7.30 (m, 2H), 4.17−3.94 (m, 1H), 1.69−1.45 (m, 2H), 1.24 (d, J = 6.6 Hz, 3H), 0.97 (t, J = 7.4 Hz, 3H).

3-Amino-N-(sec-butyl)-6-(2-bromophenyl)pyrazine-2-carboxamide ***(29)*** *(Exp1).* Title compound prepared according to general procedure B (53%). LCMS (Finnigan): rt 8.58 min >99% purity at

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254 nm; $[M + H]^+$ 349.2. ^1H NMR (300 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.70 (dd, J = 8.0, 1.0 Hz, 1H), 7.50 (dd, J = 7.7, 1.7 Hz, 1H), 7.42 (td, J = 7.5, 1.2 Hz, 1H), 7.27 (ddd, J = 8.0, 7.3, 1.9 Hz, 1H), 4.14−3.93 (m, 1H), 1.64−1.51 (m, 2H), 1.23 (d, J = 6.6 Hz, 3H), 0.95 (t, J = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(2-methoxyphenyl)pyrazine-2-carboxamide (**30**) (Exp1).* Title compound prepared according to general procedure B (59%). LCMS (Finnigan): rt 9.25 min >99% purity at 254 nm; $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.73 (s, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.68 (dd, J = 7.6, 1.7 Hz, 1H), 7.38 (ddd, J = 8.3, 7.4, 1.8 Hz, 1H), 7.10 (td, J = 7.5, 1.1 Hz, 1H), 7.02 (d, J = 8.3 Hz, 1H), 4.16−3.97 (m, 1H), 3.88 (s, 3H), 1.68−1.51 (m, 2H), 1.24 (d, J = 6.6 Hz, 3H), 0.97 (t, J = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(2-nitrophenyl)pyrazine-2-carboxamide (**31**) (Exp1).* Title compound prepared according to general procedure B (48%). LCMS (Finnigan): rt 9.17 min >99% purity at 254 nm; $[M + H]^+$ 301.2. ^1H NMR (300 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.72 (dd, J = 7.9, 0.7 Hz, 1H), 7.69−7.59 (m, 2H), 7.51 (ddd, J = 7.9, 6.8, 2.1 Hz, 1H), 7.44 (d, J = 7.4 Hz, 1H), 4.00 (ddd, J = 13.2, 8.7, 6.6 Hz, 1H), 1.66−1.53 (m, 2H), 1.24 (d, J = 6.6 Hz, 3H), 0.97 (t, J = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(2-aminophenyl)pyrazine-2-carboxamide (**32**) (Exp1).* Title compound prepared according to general procedure B (49%). LCMS (Finnigan): rt 8.00 min >99% purity at 254 nm; $[M + H]^+$ 286.2. ^1H NMR (300 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.57 (s, 1H), 7.36 (d, J = 7.6 Hz, 1H), 7.21 (dd, J = 8.4, 6.9 Hz, 1H), 6.95−6.68 (m, 2H), 4.63 (s, 2H), 4.07 (dd, J = 15.3, 6.7 Hz, 1H), 1.66−1.51 (m, 2H), 1.24 (dd, J = 4.3, 3.6 Hz, 3H), 0.96 (t, J = 7.4 Hz, 3H).

*6-(2-Acetylphenyl)-3-amino-N-(sec-butyl)pyrazine-2-carboxamide (**33**) (Exp1).* Title compound prepared according to general procedure B (35%). LCMS (Finnigan): rt 8.98 min >99% purity at 254 nm; $[M + H]^+$ 313.3. ^1H NMR (300 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.67 (d, J = 7.1 Hz, 1H), 7.59−7.35 (m, 4H), 4.12−3.91 (m, 1H), 2.15 (s, 3H), 1.72−1.56 (m, 2H), 1.27 (d, J = 6.6 Hz, 3H), 0.98 (t, J = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(o-tolyl)pyrazine-2-carboxamide (**34**) (Exp1).* Title compound prepared according to general procedure B (87%). LCMS (Finnigan): rt 5.76 min >99% purity at 254 nm; $[M + H]^+$ 285.3. ^1H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.76 (br d, J = 7.8 Hz, 1H), 7.43−7.36 (m, 1H), 7.36−7.28 (m, 3H), 4.13−3.96 (m, 1H), 2.40 (s, 3H), 1.64−1.49 (m, 2H), 1.22 (d, J = 6.6 Hz, 3H), 0.95 (t, J = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(2-cyanophenyl)pyrazine-2-carboxamide (**35**) (Exp1).* Title compound prepared according to general procedure B (25%). LCMS (Finnigan): rt 7.65 min >99% purity at 254 nm; $[M + H]^+$ 296.3. ^1H NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.14 (s, 1H), 7.92−7.73 (m, 2H), 7.73−7.60 (m, 1H), 7.46 (t, J = 7.6 Hz, 1H), 4.07 (dt, J = 21.9, 6.8 Hz, 1H), 1.76−1.49 (m, 2H), 1.27 (d, J = 6.6 Hz, 3H), 0.98 (t, J = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(3-fluorophenyl)pyrazine-2-carboxamide (**36**) (Exp1).* Title compound prepared according to general procedure B (87%). LCMS (Finnigan): rt 5.70 min >99% purity at 254 nm; $[M + H]^+$ 289.1. ^1H NMR (300 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.95−7.84 (m, 1H), 7.81−7.70 (m, 1H), 7.45−7.33 (m, 1H), 4.15−4.00 (m, 1H), 1.71−1.60 (m, 2H), 1.26 (d, J = 6.6 Hz, 3H), 0.98 (t, J = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(3-chlorophenyl)pyrazine-2-carboxamide (**37**) (Exp1).* Title compound prepared according to general procedure B (75%). LCMS (Finnigan): rt 11.75 min >99% purity at 254 nm; $[M + H]^+$ 305.3 m/z. HRMS calcd for $C_{15}H_{18}ClN_4O$ $[M + H]^+$ 305.1164 m/z, found 305p.1163 m/z. ^1H NMR (300 MHz, $CDCl_3$) δ 8.57 (s, 1H), 7.86−7.79 (m, 1H), 7.73−7.65 (m, 1H), 7.39−7.33 (m, 1H), 4.19−3.98 (m, 1H), 1.71−1.54 (m, 2H), 1.28 (d, J = 6.6 Hz, 3H), 0.99 (t, J = 7.4 Hz, 3H). ^1H NMR (400 MHz, DMSO) δ 8.87 (s, 1H), 8.43 (d, J = 8.8 Hz, 1H), 8.19 (t, J = 1.8 Hz, 1H), 8.14−8.05 (m, 1H), 7.73 (s, 2H), 7.49 (t, J = 7.9 Hz, 1H), 7.42 (ddd, J = 8.0, 2.1, 1.1 Hz, 1H), 3.95 (dt, J = 21.7, 7.2 Hz, 1H), 1.73−1.45 (m, 2H), 1.21 (d, J = 6.6 Hz, 3H), 0.88 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.7, 154.8, 144.6, 138.6, 137.1, 134.2, 131.0, 128.2, 125.5, 125.1, 124.5, 16.7, 29.1, 20.6, 11.3.

*3-Amino-N-(sec-butyl)-6-(3-bromophenyl)pyrazine-2-carboxamide (**38**) (Exp1).* Title compound prepared according to general procedure B (62%). LCMS (Finnigan): rt 9.55 min >99% purity at 254 nm; $[M + H]^+$ 349.2. ^1H NMR (300 MHz, $CDCl_3$) δ 8.57 (s, 1H), 7.98 (s, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.52 (dd, J = 5.0, 3.9 Hz, 1H), 7.33 (dd, J = 17.1, 9.3 Hz, 1H), 4.22−3.94 (m, 1H), 1.74−1.53 (m, 2H), 1.28 (d, J = 6.6 Hz, 3H), 1.00 (t, J = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(3-methoxyphenyl)pyrazine-2-carboxamide (**39**) (Exp1).* Title compound prepared according to general procedure B (65%). LCMS (Finnigan): rt 9.58 min >99% purity at 254 nm; $[M + H]^+$ 301.13. HRMS calcd for $C_{16}H_{21}N_4O_2$ $[M + H]^+$ 301.1659 m/z, found 301.1660 m/z. ^1H NMR (300 MHz, $CDCl_3$) δ 8.59 (s, 1H), 7.82 (d, J = 8.2 Hz, 1H), 7.47−7.35 (m, 3H), 6.99−6.90 (m, 1H), 4.07 (tt, J = 15.4, 6.7 Hz, 1H), 3.89 (s, 3H), 1.70−1.54 (m, 2H), 1.27 (d, J = 6.6 Hz, 3H), 0.99 (t, J = 7.4 Hz, 3H). ^1H NMR (400 MHz, DMSO) δ 8.83 (s, 1H), 8.35 (d, J = 8.8 Hz, 1H), 7.78−7.53 (m, 2H), 7.38 (t, J = 8.0 Hz, 1H), 6.95 (ddd, J = 8.2, 2.6, 0.8 Hz, 1H), 4.06−3.87 (m, 1H), 3.84 (s, 3H), 1.70−1.45 (m, 2H), 1.20 (d, J = 6.6 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.3, 159.8, 154.1, 144.0, 138.0, 137.4, 129.9, 124.4, 117.8, 113.5, 111.0, 55.1, 46.1, 28.7, 20.1, 10.8. Note: the amine peak is visible as a broad stretch within the aromatic region of the proton NMR, as such it was not characterized.

*3-Amino-N-(sec-butyl)-6-(3-nitrophenyl)pyrazine-2-carboxamide (**40**) (Exp1).* Title compound prepared according to general procedure B (65%). LCMS (Finnigan): rt 11.90 min >99% purity at 254 nm; $[M + H]^+$ 316.2. ^1H NMR (300 MHz, $CDCl_3$) δ 8.78−8.64 (m, 2H), 8.24 (d, J = 6.0 Hz, 1H), 8.17 (d, J = 7.8 Hz, 1H), 7.75 (s, 1H), 7.66 (t, J = 8.0 Hz, 1H), 4.31−3.96 (m, 1H), 1.74−1.58 (m, 2H), 1.30 (d, J = 6.6 Hz, 3H), 1.01 (t, J = 7.3 Hz, 3H).

*3-Amino-6-(3-aminophenyl)-N-(sec-butyl)pyrazine-2-carboxamide (**41**) (Exp1).* Title compound prepared according to general procedure B (56%). LCMS (Finnigan): rt 6.47 min >99% purity at 254 nm; $[M + H]^+$ 286.3. ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (s, 1H), 7.80 (s, 1H), 7.31−7.19 (m, 1H), 7.16 (br s, 1H), 6.76−6.69 (m, 1H), 4.15−3.99 (m, 1H), 3.80 (s, 2H), 1.68−1.56 (m, 2H), 1.27 (d, J = 6.6 Hz, 3H), 0.99 (t, J = 7.4 Hz, 3H).

*6-(3-Acetylphenyl)-3-amino-N-(sec-butyl)pyrazine-2-carboxamide (**42**) (Exp1).* Title compound prepared according to general procedure B (76%). LCMS (Finnigan): rt 8.52 min >99% purity at 254 nm; $[M + H]^+$ 313.3. ^1H NMR (300 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.44 (t, J = 1.8 Hz, 1H), 8.09−8.02 (m, 1H), 7.97 (dd, J = 6.4, 1.4 Hz, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.59 (t, J = 7.8 Hz, 1H), 4.17−4.00 (m, 1H), 2.67 (s, J = 0.4 Hz, 3H), 1.73−1.54 (m, 2H), 1.28 (d, J = 6.6 Hz, 3H), 1.00 (t, J = 7.5 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(m-tolyl)pyrazine-2-carboxamide (**43**) (Exp1).* Title compound prepared according to general procedure B (98%). LCMS (Finnigan): rt 8.38 min >99% purity at 254 nm; $[M + H]^+$ 285.3. ^1H NMR (300 MHz, $CDCl_3$) δ 8.59 (d, J = 0.9 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.70−7.58 (m, 2H), 7.37 (t, J = 8.0 Hz, 1H), 7.22 (dd, J = 4.2, 3.7 Hz, 1H), 4.16−3.97 (m, 1H), 2.45 (s, 3H), 1.69−1.55 (m, 2H), 1.28 (d, J = 6.6 Hz, 3H), 0.99 (t, J = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(3-cyanophenyl)pyrazine-2-carboxamide (**44**) (Exp1).* Title compound prepared according to general procedure B (52%). LCMS (Finnigan): rt 8.82 min >99% purity at 254 nm; $[M + H]^+$ 296.2. HRMS calcd for $C_{16}H_{18}N_5O$ $[M + H]^+$ 296.1506 m/z, found 296.1505 m/z. ^1H NMR (300 MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.14 (dd, J = 2.3, 1.1 Hz, 1H), 8.05 (dt, J = 7.8, 1.3 Hz, 1H), 7.75−7.64 (m, 2H), 7.59 (t, J = 7.8 Hz, 1H), 4.18−3.99 (m, 1H), 1.72−1.58 (m, 2H), 1.30 (d, J = 6.6 Hz, 3H), 1.00 (t, J = 7.4 Hz, 3H). ^1H NMR (400 MHz, DMSO) δ 8.89 (s, 1H), 8.16 (d, J = 8.1 Hz, 2H), 7.97 (dd, J = 7.8, 1.1 Hz, 1H), 7.80 (td, J = 7.8, 1.4 Hz, 1H), 7.57 (td, J = 7.6, 1.4 Hz, 1H), 3.93 (ddd, J = 13.4, 8.6, 6.7 Hz, 1H), 1.54 (p, J = 7.3 Hz, 2H), 1.16 (d, J = 6.6 Hz, 3H), 0.88 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.0, 154.4, 145.5, 138.3, 135.5, 135.4, 133.6, 128.6, 127.5, 123.5, 120.5, 107.8, 46.2, 28.9, 20.2, 10.5. Note: the amine peak is visible as a broad stretch within the aromatic region of the proton NMR, as such it was not characterized.

*3-Amino-N-(sec-butyl)-6-(4-fluorophenyl)pyrazine-2-carboxamide (**45**) (Exp1).* Title compound prepared according to general procedure B (83%). LCMS (Finnigan): rt 9.15 min >99% purity at

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254 nm; $[M + H]^+$ 289.2. HRMS calcd for $C_{15}H_{18}FN_4O$ $[M + H]^+$ 289.1459 *m/z*, found 289.1459 *m/z*. ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (s, 1H), 7.88−7.73 (m, 3H), 7.22−7.09 (m, 2H), 4.15−4.00 (m, 1H) 1.70−1.54 (m, 2H), 1.26 (t, *J* = 5.6 Hz, 3H), 0.99 (t, *J* = 7.4 Hz, 3H). ^1H NMR (400 MHz, DMSO) δ 8.81 (s, 1H), 8.36 (d, *J* = 8.8 Hz, 1H), 8.17 (dd, *J* = 8.8, 5.5 Hz, 2H), 7.63 (bs, 2H), 7.29 (t, *J* = 8.9 Hz, 2H), 4.06−3.84 (m, 1H), 1.71−1.43 (m, 2H), 1.20 (d, *J* = 6.6 Hz, 3H), 0.88 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.3, 162.2 (J_{C-F} = 245 Hz), 154.0, 143.6, 137.4, 132.5 (J_{C-F} = 3 Hz), 127.6 (J_{C-F} = 9 Hz, 2C), 124.4, 115.5 (J_{C-F} = 22 Hz, 2C), 46.1, 28.7, 20.1, 10.8.

*3-Amino-N-(sec-butyl)-6-(4-chlorophenyl)pyrazine-2-carboxamide (**46**) (Exp1).* Title compound prepared according to general procedure B (81%). LCMS (Finnigan): rt 9.35 min >99% purity at 254 nm; $[M + H]^+$ 305.3. HRMS calcd for $C_{15}H_{18}ClN_4O$ $[M + H]^+$ 305.1164 *m/z*, found 305.1164 *m/z*. ^1H NMR (300 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.82−7.73 (m, 3H), 7.48−7.39 (m, 2H), 4.16−3.93 (m, 1H), 1.71−1.53 (m, 2H), 1.27 (d, *J* = 6.6 Hz, 3H), 0.99 (t, *J* = 7.5 Hz, 3H). ^1H NMR (400 MHz, DMSO) δ 8.84 (s, 1H), 8.43 (d, *J* = 8.8 Hz, 1H), 8.39 (d, *J* = 8.9 Hz, 1H), 8.17 (d, *J* = 8.8 Hz, 2H), 7.71 (s, 21H), 7.51 (d, *J* = 8.7 Hz, 2H), 3.95 (ddd, *J* = 14.7, 7.7, 6.6 Hz, 1H), 1.71−1.45 (m, 2H), 1.20 (d, *J* = 6.6 Hz, 3H), 0.88 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.2, 154.2, 143.8, 137.0, 134.9, 132.7, 128.7 (2C), 127.1 (2C), 124.5, 46.2, 28.7, 20.1, 10.9.

*3-Amino-N-(sec-butyl)-6-(4-bromophenyl)pyrazine-2-carboxamide (**47**) (Exp1).* Title compound prepared according to general procedure B (64%). LCMS (Finnigan): rt 8.68 min >99% purity at 254 nm; $[M + H]^+$ 349.2. ^1H NMR (300 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.82−7.65 (m, 3H), 7.65−7.52 (m, 2H), 4.18−3.93 (m, 1H), 1.71−1.54 (m, 2H), 1.27 (d, *J* = 6.6 Hz, 3H), 0.99 (t, *J* = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(4-methoxyphenyl)pyrazine-2-carboxamide (**48**) (Exp1).* Title compound prepared according to general procedure B (89%). LCMS (Finnigan): rt 9.72 min >99% purity at 254 nm; $[M + H]^+$ 301.3. ^1H NMR (300 MHz, $CDCl_3$) δ 8.55 (s, 1H), 7.88−7.72 (m, 3H), 7.06−6.93 (m, 2H), 4.14−3.98 (m, 1H), 3.87 (s, 3H), 1.70−1.55 (m, 2H), 1.27 (d, *J* = 6.6 Hz, 3H), 0.99 (t, *J* = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(4-nitrophenyl)pyrazine-2-carboxamide (**49**) (Exp1).* Title compound prepared according to general procedure B (68%). LCMS (Finnigan): rt 7.90 min >99% purity at 254 nm; $[M + H]^+$ 316.2. ^1H NMR (300 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.38−8.27 (m, 2H), 8.06−7.97 (m, 2H), 7.72 (s, 1H), 4.16−4.03 (m, 1H), 1.72−1.59 (m, 2H), 1.29 (d, *J* = 6.6 Hz, 3H), 1.00 (t, *J* = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(4-aminophenyl)pyrazine-2-carboxamide (**50**) (Exp1).* Title compound prepared according to general procedure B (79%). LCMS (Finnigan): rt 4.54 min >99% purity at 254 nm; $[M + H]^+$ 286.1. ^1H NMR (300 MHz, $CDCl_3$) δ 8.53 (s, *J* = 0.8 Hz, 1H), 7.84 (d, *J* = 10.4 Hz, 1H), 7.72−7.60 (m, 2H), 6.83−6.72 (m, 2H), 4.17−3.96 (m, 1H), 3.82 (s, 2H), 1.62 (dt, *J* = 14.2, 7.2 Hz, 2H), 1.27 (d, *J* = 6.6 Hz, 3H), 0.99 (t, *J* = 7.4 Hz, 3H).

*6-(4-Acetylphenyl)-3-amino-N-(sec-butyl)pyrazine-2-carboxamide (**51**) (Exp1).* Title compound prepared according to general procedure B (65%). LCMS (Finnigan): rt 8.86 min >99% purity at 254 nm; $[M + H]^+$ 313.3. ^1H NMR (300 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.10−8.03 (m, 2H), 7.99−7.91 (m, 2H), 7.77 (d, *J* = 8.8 Hz, 1H), 4.16−4.01 (m, 1H), 2.64 (s, 3H), 1.71−1.52 (m, 2H), 1.28 (d, *J* = 6.6 Hz, 3H), 1.00 (t, *J* = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(p-tolyl)pyrazine-2-carboxamide (**52**) (Exp1).* Title compound prepared according to general procedure B (91%). LCMS (Finnigan): rt 5.80 min >99% purity at 254 nm; $[M + H]^+$ 85.1. ^1H NMR (300 MHz, $CDCl_3$) δ 8.58 (d, *J* = 0.8 Hz, 1H), 7.84 (d, *J* = 8.6 Hz, 1H), 7.74 (d, *J* = 8.0 Hz, 2H), 7.29 (dd, *J* = 7.9, 0.6 Hz, 2H), 4.08 (dt, *J* = 15.5, 6.8 Hz, 1H), 2.41 (s, 3H), 1.71−1.55 (m, 3H), 1.27 (d, *J* = 6.6 Hz, 3H), 0.99 (t, *J* = 7.4 Hz, 3H).

*3-Amino-N-(sec-butyl)-6-(4-cyanophenyl)pyrazine-2-carboxamide (**53**) (Exp1).* Title compound prepared according to general procedure B (68%). LCMS (Finnigan): rt 8.59 min >99% purity at 254 nm; $[M + H]^+$ 296.3. ^1H NMR (300 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.00−7.92 (m, 2H), 7.81−7.73 (m, 2H), 7.71 (s, 1H), 4.08 (ddd, *J* = 13.3, 8.8, 6.6 Hz, 1H), 1.70−1.58 (m, 2H), 1.29 (d, *J* = 6.6 Hz, 3H), 1.00 (t, *J* = 7.4 Hz, 3H).

*3-Amino-6-(2-cyanophenyl)-N-(pentan-3-yl)pyrazine-2-carboxamide (**54**) (Exp2).* The title compound was prepared according to general procedure 3-A1 and was irradiated for 2 h and 30 min. Title compound was isolated as a pale-yellow solid (20%). HPLC: rt 8.38 min >97% purity at 254 nm. LRMS $[M + H]^+$ 310.3 *m/z*. HRMS calcd for $C_{17}H_{20}N_5O$ $[M + H]^+$ 310.1662 *m/z*, found 310.1664 *m/z*. ^1H NMR (400 MHz, DMSO) δ 8.89 (s, 1H), 8.15 (d, *J* = 7.6 Hz, 1H), 8.07 (d, *J* = 9.3 Hz, 1H), 7.96 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.79 (td, *J* = 7.8, 1.4 Hz, 1H), 7.57 (td, *J* = 7.6, 1.1 Hz, 1H), 3.90−3.63 (m, 1H), 1.73−1.34 (m, 4H), 0.86 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 165.5, 154.4, 145.5, 138.3, 135.5, 135.4, 133.5, 128.6, 127.5, 123.5, 120.5, 107.8, 51.8, 26.9 (2C), 10.4 (2C). Note: The amine peak appears as a broad stretch under the aromatic protons, as such it has not been reported.

*3-Amino-N-(pentan-3-yl)-6-(m-tolyl)pyrazine-2-carboxamide (**55**) (Exp2).* The title compound was prepared according to general procedure 3-A1 and was irradiated for 30 min. Title compound was isolated as a pale-yellow solid (99%). HPLC: rt 11.28 min >99% purity at 254 nm. LRMS $[M + H]^+$ 299.3 *m/z*. HRMS calcd for $C_{17}H_{23}N_4O$ $[M + H]^+$ 299.1866 *m/z*, found 299.1868 *m/z*. ^1H NMR (400 MHz, DMSO) δ 8.81 (s, 1H), 8.22 (d, *J* = 9.3 Hz, 1H), 7.97−7.82 (m, 2H), 7.62 (s, 2H), 7.37 (t, *J* = 7.6 Hz, 1H), 7.20 (d, *J* = 7.5 Hz, 1H), 3.91−3.68 (m, 1H), 2.40 (s, 3H), 1.71−1.47 (m, 4H), 0.89 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 165.8, 154.0, 143.8, 138.4, 137.8, 135.9, 128.7, 128.7, 125.8, 124.4, 122.7, 51.8, 26.9 (2C), 21.2, 10.6 (2C).

*3-Amino-6-(3,5-dimethylphenyl)-N-(pentan-3-yl)pyrazine-2-carboxamide (**56**) (Exp2).* The title compound was prepared according to general procedure B. Title compound was isolated as a yellow solid (61%). HPLC: rt 8.92 min >99% purity at 254 nm. LRMS $[M + H]^+$ 313.2 *m/z*. ^1H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H), 7.82 (d, *J* = 9.2 Hz, 1H), 7.43 (s, 2H), 7.02 (d, *J* = 22.2 Hz, 1H), 3.94 (dtt, *J* = 10.6, 7.8, 5.4 Hz, 1H), 2.39 (d, *J* = 14.6 Hz, 6H), 1.79−1.43 (m, 5H), 0.98 (t, *J* = 7.4 Hz, 6H).

*3-Amino-6-(4-fluorophenyl)-N-(pentan-3-yl)pyrazine-2-carboxamide (**57**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (75%). HPLC: rt 10.95 min >99% purity at 254 nm. LRMS $[M + H]^+$ 303.2 *m/z*. HRMS calcd for $C_{16}H_{20}FN_4O$ $[M + H]^+$ 303.1616 *m/z*, found 303.1622 *m/z*. ^1H NMR (400 MHz, DMSO) δ 8.82 (s, 1H), 8.26 (d, *J* = 9.3 Hz, 1H), 8.21−8.09 (m, 2H), 7.63 (s, 2H), 7.30 (t, *J* = 8.9 Hz, 2H), 3.90−3.69 (m, 1H), 1.70−1.43 (m, 4H), 0.87 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 165.8, 162.2 (J_{C-F} = 244 Hz), 153.9, 143.6, 137.4, 132.5 (J_{C-F} = 3 Hz), 127.5 (J_{C-F} = 8 Hz), 124.4, 115.5 (J_{C-F} = 21 Hz), 51.9, 26.9 (2C), 10.7 (2C).

*3-Amino-6-(4-fluoro-3-methylphenyl)-N-(pentan-3-yl)pyrazine-2-carboxamide (**58**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (83%). HPLC: rt 8.63 min >99% purity at 254 nm. LRMS $[M + H]^+$ 317.2 *m/z*. HRMS calcd for $C_{17}H_{22}FN_4O$ $[M + H]^+$ 317.1773 *m/z*, found 317.1775 *m/z*. ^1H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, *J* = 1.6 Hz, 1H), 7.75 (d, *J* = 8.7 Hz, 1H), 7.63 (d, *J* = 6.6 Hz, 2H), 7.08 (dd, *J* = 30.5, 21.5 Hz, 1H), 4.10−3.86 (m, 1H), 2.36 (s, 3H), 1.93−1.40 (m, 4H), 0.97 (td, *J* = 7.3, 1.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 160.6, 153.9, 143.8, 139.7, 132.4, 128.9, 128.9, 125.4, 124.9, 124.9, 115.7 (d, J_{C-F} = 22 Hz), 52.1, 27.7, 14.9, 10.5.

*3-Amino-6-(2-cyano-4-fluorophenyl)-N-(pentan-3-yl)pyrazine-2-carboxamide (**59**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 3 h. Title compound was isolated as a yellow solid (44%). HPLC: rt 9.21 min >99% purity at 254 nm. LRMS $[M + H]^+$ 328.2 *m/z*. HRMS calcd for $C_{17}H_{19}FN_5O$ $[M + H]^+$ 328.1568 *m/z*, found 328.1574 *m/z*. ^1H NMR (400 MHz, DMSO) δ 8.86 (s, 1H), 8.20 (dd, *J* = 9.0, 5.4 Hz, 1H), 8.04 (d, *J* = 9.3 Hz, 1H), 7.98 (dd, *J* = 8.7, 2.7 Hz, 1H), 7.82−7.64 (m, 1H), 3.91−3.67 (m, 1H), 1.66−1.40 (m, 4H), 0.87 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 165.4, 161.0 (*J* C−F = 248 Hz), 154.4, 145.5, 135.4 (J_{C-F} = 3 Hz), 134.8, 130.0 (J_{C-F} = 8 Hz), 123.5, 122.0 (J_{C-F} = 25 Hz), 121.1 (J_{C-F} = 21 Hz), 119.1 (J_{C-F} = 9 Hz), 109.5 (J_{C-F}

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= 10 Hz), 51.8, 26.9 (2C), 10.4 (2C). Note: amine peak is a broad peak under the aromatic protons, as such it has not been reported.

*3-Amino-N-(pentan-3-yl)-6-(3-(trifluoromethyl)phenyl)pyrazine-2-carboxamide (**60**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (65%). HPLC: rt 8.79 min >99% purity at 254 nm. LRMS $[M + H]^+$ 353.1 *m/z*. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.21−8.12 (m, 1H), 7.75−7.68 (m, 1H), 7.61−7.51 (m, 1H), 3.99−3.91 (m, 1H), 1.71−1.64 (m, 4H), 1.12 (d, *J* = 6.9 Hz, 6H).

*3-Amino-6-(3-chlorophenyl)-N-(pentan-3-yl)pyrazine-2-carboxamide (**61**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (79%). HPLC: rt 8.68 min >99% purity at 254 nm. LRMS $[M + H]^+$ 319.2 *m/z*. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (s, 1H), 7.78−7.72 (m, 1H), 7.70−7.68 (m, 1H), 7.36−7.30 (m, 1H), 4.05−3.98 (m, 1H), 1.68−1.50 (m, 4H), 1.30 (d, *J* = 6.5 Hz, 3H).

*3-Amino-6-(3-chloro-5-methylphenyl)-N-(pentan-3-yl)pyrazine-2-carboxamide (**62**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (82%). HPLC: rt 9.12 min >99% purity at 254 nm. LRMS $[M + H]^+$ 333.0 *m/z*. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.30−8.20 (m, 1H), 8.03−7.93 (m, 1H), 7.46−7.36 (m, 1H), 3.85−3.75 (m, 1H), 1.68−1.50 (m, 4H), 1.30 (d, *J* = 6.5 Hz, 3H), 1.03 (s, 3H).

*3-Amino-6-(3-chloro-4-fluorophenyl)-N-(pentan-3-yl)pyrazine-2-carboxamide (**63**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (63%). HPLC: rt 9.15 min >99% purity at 254 nm. LRMS $[M + H]^+$ 337.1 *m/z*. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (s, 1H), 7.95−7.85 (m, 1H), 7.71−7.61 (m, 2H), 7.21−7.1 (m, 1H), 4.10−3.86 (m, 1H), 1.86−1.47 (m, 4H).

*3-Amino-6-(2-cyanophenyl)-N-cyclopentylpyrazine-2-carboxamide (**64**) (Exp1).* The title compound was prepared according to general procedure B and was irradiated for 3 h. Title compound was isolated as a yellow solid (12%). HPLC: rt 7.55 min >99% purity at 254 nm. LRMS $[M + H]^+$ 308.2 *m/z*. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74 (s, 1H), 8.04−8.00 (m, 1H), 7.90−7.82 (m, 1H), 7.82−7.71 (m, 1H), 7.59−7.48 (m, 1H), 4.35−4.28 (m, 1H), 2.19−1.95 (m, 2H), 1.72−1.55 (m, 4H).

*3-Amino-N-cyclopentyl-6-(m-tolyl)pyrazine-2-carboxamide (**65**) (Exp2).* Title compound was isolated as a yellow solid (86%). HPLC: rt 9.32 min >99% purity at 254 nm. LRMS $[M + H]^+$ 297.2 *m/z*. HRMS calcd for $C_{17}H_{21}N_4O$ $[M + H]^+$ 297.1710 *m/z*, found 297.1716 *m/z*. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 7.96 (d, *J* = 7.3 Hz, 1H), 7.69−7.59 (m, 1H), 7.42−7.32 (m, 1H), 7.22 (dd, *J* = 7.8, 0.6 Hz, 1H), 4.43−4.30 (m, 1H), 2.45 (s, 1H), 2.16−2.02 (m, 1H), 1.86−1.63 (m, 2H), 1.64−1.50 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.8, 153.9, 144.1, 140.6, 138.7, 136.5, 129.4, 129.0, 126.5, 125.4, 123.0, 51.1, 33.3, 24.0, 21.8.

*3-Amino-N-cyclopentyl-6-(4-fluorophenyl)pyrazine-2-carboxamide (**66**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (91%). HPLC: rt 8.96 min >99% purity at 254 nm. LRMS $[M + H]^+$ 301.1 *m/z*. ^{1}H NMR (400 MHz, DMSO) δ 8.92 (s, 1H), 8.23 (d, *J* = 9.2 Hz, 1H), 8.17−8.05 (m, 2H), 7.59 (s, 2H), 7.23 (t, *J* = 8.6 Hz, 2H), 4.40−4.30 (m, 1H), 2.38 (s, 1H), 2.13−2.00 (m, 1H), 1.82−1.65 (m, 2H).

*3-Amino-N-cyclopentyl-6-(4-fluoro-3-methylphenyl)pyrazine-2-carboxamide (**67**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (86%). HPLC: rt 9.12 min >99% purity at 254 nm. LRMS $[M + H]^+$ 315.0 *m/z*. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 1.2 Hz, 1H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.60 (d, *J* = 6.3 Hz, 2H), 7.25−7.10 (m, 1H), 4.40−4.23 (m, 1H), 2.41 (s, 1H), 2.09−1.99 (m, 1H), 1.82−1.64 (m, 2H).

*3-Amino-6-(2-cyano-4-fluorophenyl)-N-cyclopentylpyrazine-2-carboxamide (**68**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (83%). HPLC: rt 7.95 min >99% purity at 254 nm. LRMS $[M + H]^+$ 326.1 *m/z*. ^{1}H NMR (400 MHz, DMSO) δ 8.83 (s, 1H), 8.59−8.47 (m, 1H), 8.13 (d, *J* = 9.1 Hz, 1H), 8.01 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.72−7.44 (m, 1H), 4.39−4.29 (m, 1H), 2.39 (s, 1H), 2.17−2.05 (m, 1H), 1.78−1.703 (m, 2H).

*3-Amino-N-cyclopentyl-6-(3-(trifluoromethyl)phenyl)pyrazine-2-carboxamide (**69**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (62%). HPLC: rt 9.02 min >99% purity at 254 nm. LRMS $[M + H]^+$ 351.2 *m/z*. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.20−8.13 (m, 1H), 7.81−7.74 (m, 1H), 7.59−7.50 (m, 1H), 4.45−4.32 (m, 1H), 2.50 (s, 1H), 2.17−2.02 (m, 1H), 1.83−1.60 (m, 2H).

*3-Amino-6-(3-chlorophenyl)-N-cyclopentylpyrazine-2-carboxamide (**70**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (76%). HPLC: rt 8.51 min >99% purity at 254 nm. LRMS $[M + H]^+$ 317.1 *m/z*. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (s, 1H), 7.81−7.76 (m, 1H), 7.70−7.63 (m, 1H), 7.34−7.28 (m, 1H), 4.35−4.21 (m, 1H), 2.34 (s, 1H), 2.13−2.00 (m, 1H), 1.81−1.63 (m, 2H).

*3-Amino-6-(3-chloro-5-methylphenyl)-N-cyclopentylpyrazine-2-carboxamide (**71**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (81%). HPLC: rt 9.12 min >99% purity at 254 nm. LRMS $[M + H]^+$ 331.1 *m/z*. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (s, 1H), 8.33−8.26 (m, 1H), 8.14−7.00 (m, 1H), 7.45−7.39 (m, 1H), 4.30−4.23 (m, 1H), 2.51 (s, 1H), 2.20−2.08 (m, 1H), 1.81−1.50 (m, 2H), 1.64−1.50 (s, 3H).

*3-Amino-6-(3-chloro-4-fluorophenyl)-N-cyclopentylpyrazine-2-carboxamide (**72**) (Exp2).* The title compound was prepared according to general procedure B and was irradiated for 30 min. Title compound was isolated as a yellow solid (79%). HPLC: rt 9.46 min >99% purity at 254 nm. LRMS $[M + H]^+$ 335.2 *m/z*. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.93−7.87 (m, 1H), 7.73−7.65 (m, 2H), 7.29−7.15 (m, 1H), 4.38−4.27 (m, 1H), 2.39 (s, 1H), 2.18−2.05 (m, 1H), 1.84−1.60 (m, 2H).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00438.

Experimental procedures for biological assays and metabolism assays (PDF)

Molecular formula strings (CSV)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

HAT, human African trypanosomiasis; *T. b. brucei*, *Trypanosoma brucei brucei*; *T. b. gambiense*, *Trypanosoma brucei gambiense*; *T. b. rhodesiense*, *Trypanosoma brucei rhodesiense*; MPO, multiparameter optimization

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