**Supporting Information**

for

**A Potent, *in vivo* Active Antimalarial Series Based on a Triazolopyrazine Core: Communal Lead Optimization in an Open Source Malaria Series**

Edwin G. Tse,1,2 Alice Motion,2 Maryam Alobaidly,3 Jenya Antonova,4 Nkengafeh Asong,3 Jake Baum,5 Sue Charman,6 Kelechukwu Chukwu,3 Darren J. Creek,7 Michael J. Delves,5 Adelaide S. M. Dennis,8 Vy Duong,3 Korina Eribez,4 Fernando Galvan,3 Mark Gardner,9 Irene Hallyburton,10 Jessica Hauger,3 Daisy J. Kim,3 Kiaran Kirk,8 Dana M. Klug,1 Marat Korsik,2 Adele M. Lehane,8 Kenneth Lowe,3 Kimberly Lowe,3 Tracy T. Ly,3 Thomas S. C. MacDonald,2 María Santos Martínez-Martínez,11 Stephan Meister,4 Ho Leung Ng,12 R. Scott Obach,13 Sabine Ottilie,4 Melanie C. Ridgway,8 Peter J. Rutledge,2 Anthony Sama,14 Christian Scheurer,15,16 Ben F. Sedzro,3 Raman Sharma,13 David G. Smith,17 Chase C. Smith,3 Christopher Southan,18 Anubhav Srivastava,7 Chris Swain,19 Fernando Sánchez-Román Terán,5 Joanna Ubels,2 Sara Viera,11 Gregory S. Walker,13 David Waterson,20 Paul A. Willis,20 Elizabeth A. Winzeler,4 Sergio Wittlin,15,16 Michael Witty20 and Matthew H. Todd1\*

1. School of Pharmacy, University College London, London WC1N 1AX, U.K.
2. School of Chemistry, The University of Sydney, NSW 2006, Sydney, Australia
3. School of Pharmacy-Worcester/Manchester, Massachusetts College of Pharmacy and Health Sciences, 19 Foster Street, Worcester, MA 01608, United States
4. Department of Pediatrics, University of California, San Diego, School of Medicine, La Jolla, California 92093, United States
5. Department of Life Sciences, Imperial College London, Exhibition Road, South Kensington, London, SW72AZ, U.K.
6. Centre for Drug Candidate Optimisation, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia
7. Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Melbourne, Victoria 3052, Australia
8. Research School of Biology, Australian National University, Canberra, ACT 2601, Australia
9. AMG Consultants, Discovery Park, Ramsgate Rd, Sandwich, Kent, CT13 9ND, U.K.
10. Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dundee DD1 5EH, U.K.
11. GlaxoSmithKline R&D, C/ Severo Ochoa, 2, 28760 Tres Cantos, Spain
12. Department of Biochemistry & Molecular Biophysics, Kansas State University, 1711 Claflin Rd., 141 Chalmers Hall. Manhattan, KS 66506, United States
13. Pfizer Inc., Groton, CT 06340, United States
14. [Email: asamawsfl@protonmail.com](mailto:asamawsfl@protonmail.com)
15. Swiss Tropical and Public Health Institute, Socinstrasse 57, 4002 Basel, Switzerland
16. University of Basel, 4002 Basel, Switzerland
17. School of Health and Life Sciences, Federation University, Gippsland Campus, Churchill, VIC 3842, Australia
18. Deanery of Biomedical Sciences, University of Edinburgh, Edinburgh, EH8 9XD, U.K.
19. Cambridge MedChem Consulting, 8 Mangers Lane, Duxford, Cambridge CB22 4RN, U.K.
20. Medicines for Malaria Venture, PO Box 1826, 20 rte de Pre-Bois, 1215 Geneva 15, Switzerland

\*[matthew.todd@ucl.ac.uk](mailto:matthew.todd@ucl.ac.uk)

Table of Contents

[General Chemical Procedures 3](#_Toc88046915)

[General Synthetic Procedures 4](#_Toc88046916)

[General Procedure A: Hydrazinylpyrazine synthesis 4](#_Toc88046917)

[General Procedure B: Condensation reaction 4](#_Toc88046918)

[General Procedure C: Improved condensation reaction 4](#_Toc88046919)

[General Procedure D: Oxidative cyclisation 5](#_Toc88046920)

[General Procedure E: Nucleophilic aromatic substitution 5](#_Toc88046921)

[General Procedure F: Amide Coupling1 5](#_Toc88046922)

[General Procedure G: Suzuki Coupling 5](#_Toc88046923)

[General Procedure H: Buchwald-Hartwig Coupling 6](#_Toc88046924)

[Synthesis and Characterization of Intermediate Compounds 6](#_Toc88046925)

[Synthesis and Characterization of Final Compounds 30](#_Toc88046926)

[Table S1. hERG Activity of Representative Series 4 Compounds. 72](#_Toc88046927)

[Table S2. Cytotoxicity of Representative Series 4 Compounds. 73](#_Toc88046928)

[Table S3. Gametocyte Assay Results of Representative Series 4 Compounds. 73](#_Toc88046929)

[Figure S1. The effect of OSM compounds at 1 µM on Plasmodium falciparum intracellular Na+ concentration. 75](#_Toc88046930)

[Figure S2. The effect of OSM compounds at 5 µM on Plasmodium falciparum intracellular Na+ concentration. 76](#_Toc88046931)

[Figure S3. The effect of OSM compounds at either 1 µM or 5 µM on Plasmodium falciparum intracellular H+ concentration. 77](#_Toc88046932)

[Figure S4. PfATP4 mutant parasites are less susceptible than their parents to a selection of Series 4 compounds 78](#_Toc88046933)

[General Biological Procedures 79](#_Toc88046934)

[Parasite Strains 79](#_Toc88046935)

[Parasite Growth Assays (ANU) 79](#_Toc88046936)

[OSM-S-218/MMV669844 Assay against Drug-Resistant Strains 79](#_Toc88046937)

[Liver Stage Potency Assays 79](#_Toc88046938)

[Parasite cytosolic [Na+] and pH Assays 79](#_Toc88046939)

[hERG Patch Clamp Assays 79](#_Toc88046940)

[Metabolomics Analysis 80](#_Toc88046941)

[Late-Stage Biofunctionalization 81](#_Toc88046942)

[SCID Mouse Model Evaluations 82](#_Toc88046943)

[References 82](#_Toc88046944)

# General Chemical Procedures

All commercially available reagents and solvents were purchased and used as received from Sigma-Aldrich or Alfa-Aesar. Drying of glassware at 115 ˚C overnight and activation of molecular sieves in a microwave was performed when anhydrous conditions were required. Dichloromethane was distilled over calcium hydride. Reflux reactions were performed with a paraffin oil bath. Flash column chromatography was performed with Grace Silica Gel 60 (40 – 63 μm, 230 – 400 mesh), with solvent ratios as specified. All novel compounds listed below are italicised.

Melting points were obtained on an Optimelt Automated Melting Point System and reported in degrees Celsius. Optical rotation was recorded on a Perkin Elmer 341 polarimeter with Na lamp (589 nm).

1H and 13C nuclear magnetic resonance spectroscopy was conducted on a Bruker Avance III 500 (1H at 500.1 MHz, 13C at 125.8 MHz, 19Fat 470.6 MHz), a Bruker Avance III 400 (1H at 400.1 MHz, 13C at 100.6 MHz, 19Fat 376.5 MHz), a Bruker Avance 300 (1H at 300.1 MHz, 13C at 75.5 Hz, 19Fat 282.4 MHz) or a Bruker Avance 200 (1H at 200.1 MHz) with deuterated solvents (CDCl3,DMSO-d*6*, MeOD) used without further purification. Signals are reported in the order chemical shift (ppm downfield with respect to the solvent residual), integration, multiplicity, coupling constants *J* (in Hertz) and assignments.

Low-resolution mass spectrometry was performed on a Finnigan LCQ mass spectrometer, with either electrospray ionisation (ESI) mode or atmospheric-pressure chemical ionisation (APCI) under positive mode. High-resonance mass spectrometry was performed on a Bruker 7T Fourier Transform Ion Cyclotron Resonance mass spectrometer, with either electrospray ionisation (ESI) mode or atmospheric-pressure chemical ionisation (APCI) under positive mode.

Infrared spectroscopy was performed on a Bruker Alpha FT-IR spectrometer under transmission mode, with absorbances reported as wave numbers.

Each experimental entry contains a publicly accessible hyperlink to the representative example from the Open Source Malaria electronic lab notebook (ELN, http://malaria.ourexperiment.org) reported in this experimental section and also to a page where all attempts at the reaction are collated. Raw and processed data is available on the ELN.

# General Synthetic Procedures

## General Procedure A: Hydrazinylpyrazine synthesis

Chloropyrazine (1 equiv.) was stirred in EtOH (0.35 M) and hydrazine monohydrate (2 equiv.) was added. The reaction was stirred at 80 °C until completion as indicated by TLC. The solvent was removed under reduced pressure, and the residue diluted with H2O and EtOAc. The organic layer was separated and the aqueous layer extracted with EtOAc (3 ×) and the combined organic layers washed with brine, dried (Na2SO4), filtered and concentrated under reduced pressure to give the corresponding hydrazinylpyrazine that was used without further purification unless otherwise stated.

## General Procedure B: Condensation reaction

The product from General Procedure A (1 equiv.) was dissolved in MeCN (0.60 M) and glacial acetic acid (1 equiv.) and the appropriate aldehyde (1 equiv.) were added. The reaction mixture was stirred at rt until completion as indicated by TLC. The reaction mixture was concentrated under reduced pressure to give the crude condensation product that was used without further purification unless otherwise stated.

## General Procedure C: Improved condensation reaction

The product from General Procedure A (1 equiv.) was suspended in EtOH (0.1 M) and the appropriate aldehyde (1 equiv.) was added. The reaction mixture was stirred at rt until completion as indicated by TLC. The reaction mixture was concentrated under reduced pressure to give the crude condensation product that was used without further purification unless otherwise stated.

## General Procedure D: Oxidative cyclisation

The product from General Procedure B or C (1 equiv.) was dissolved in CH­2Cl2 (0.1 M) and (diacetoxyiodo)benzene (1 equiv.) was added. The reaction mixture was stirred at rt until completion as indicated by TLC, then quenched by the addition of a sat. aq. solution of NaHCO3. The aqueous layer was separated and extracted with CH­2Cl2 (3 ×) and the combined organic layers washed with brine, dried (MgSO4), filtered and concentrated under reduced pressure to give the crude material that was purified according to the stated method to give the corresponding cyclisation product.

General Procedure E: Nucleophilic aromatic substitution

The product from General Procedure D (1.0 equiv.) was suspended in anhydrous PhMe (0.17 M). Powdered KOH (3.0 equiv.) and 18-crown-6 (~0.1 equiv.) were added and the reaction mixture was stirred at rt under Ar. The appropriate nucleophile (1.0 equiv.) was added and the reaction mixture was stirred at the stated temperature until completion as indicated by TLC. The reaction mixture was quenched by the addition of H2O and diluted with EtOAc. Organic layer was separated and the aqueous layer extracted with EtOAc (3 ×). The combined organic layers were washed with water until the aqueous layer became neutral, followed by brine, dried (MgSO­4), filtered and concentrated under reduced pressure to give the crude material that was purified according to the stated method to give the corresponding substitution product.

General Procedure F: Amide Coupling1

6-Chloropyrazine-2-carboxlic acid (1.0 equiv.), the appropriate amine (1.0 equiv.) and DIPEA (1.5 equiv.) were dissolved in DMF (~1.0 M) and the reaction mixture cooled to 0 °C. T3P (50% in EtOAc, 1.5 equiv.) was added dropwise with stirring and the reaction mixture stirred at rt for ~18 h. The reaction mixture was diluted with EtOAc and washed with a sat. aq. solution of NaHCO3 (3 ×). The combined organic layers were washed with H2O, brine, dried (MgSO­4), filtered and concentrated under reduced pressure to give the crude material that was purified according to the stated method to give the corresponding amide product.

## General Procedure G: Suzuki Coupling

To a mixture of halogenated triazolopyrazine (0.3 mmol, 1.0 equiv.), Na2CO3 (6.0 equiv.), Pd(PPh3)4 (0.1 equiv.) and the appropriate boronic acid/ester (1.3 equiv.) were successively added H2O (1 mL), EtOH (2 mL) and 1,4-dioxane (4 mL). The reaction mixture was heated at 90 °C under N2 for 8 h. The reaction was cooled to rt, silica was added and the solvent removed under reduced pressure to give the crude material that was purified by flash chromatography on silica to give the corresponding Suzuki product.

## General Procedure H: Buchwald-Hartwig Coupling

A flask was charged with halogenated triazolopyrazine (1.00 mmol, 1.0 equiv.), the appropriate amine (1.3 equiv.), Pd(dba)2 (0.2 equiv.), JohnPhos (0.4 equiv.) and NaO*t*-Bu (1.4 equiv.) then backfilled with N2 (3 ×). PhMe (10 mL) was added and the reaction mixture was heated at 100 °C overnight under N2. The reaction was cooled to rt, diluted with H2O (30 mL) and extracted with CH2Cl2 (3 × 30 mL). The combined organic layers were dried (Na2SO4), filtered and concentrated under reduced to give the crude material that was that was purified by flash chromatography on silica to give the corresponding Buchwald-Hartwig product.

# Synthesis and Characterization of Intermediate Compounds

**5-Chloro-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (S1)**

****

Compound **S1** was previously synthesized and characterized according to literature procedures.2

*ClC1=CN=CC2=NN=C(C3=CC=C(OC(F)F)C=C3)N21*

*InChI=1S/C12H7ClF2N4O/c13-9-5-16-6-10-17-18-11(19(9)10)7-1-3-8(4-2-7)20-12(14)15/h1-6,12H*

**2-(Benzylsulfonyl)-6-chloropyrazine (S2)**

****

NaH (60% dispersion in mineral oil, 1.60 g, 67.1 mmol) was added to benzyl mercaptan (8.00 mL, 67.1 mmol) in PhMe (64 mL). The mixture was heated at reﬂux for 1 h, then cooled to rt and a solution of 2,6-dichloropyrazine (10.0 g, 67.1 mmol) in PhMe (64 mL) was added. The mixture was heated at reﬂux for 24 h, cooled to rt, then washed with H2O (80 mL). The organic layer was separated, dried (Na2SO4), ﬁltered and concentrated under reduced pressure to give the crude sulﬁde as a yellow liquid (16.9 g). The crude sulﬁde (4.91 g, 20.7 mmol) in glacial AcOH (50 mL) was added to a solution of KMnO4 (3.50 g) in H2O (28 mL) and the mixture was stirred at rt for 1 h. The mixture was adjusted to pH 7 with sat. aq. NH4OH solution (40 mL) then ﬁltered, extracted with CHCl3 (3 × 100 mL), dried (Na2SO4), ﬁltered and concentrated under reduced pressure to give a cloudy yellow liquid (3.14 g); puriﬁed by automated ﬂash chromatography on silica (6–75% EtOAc in hexanes) to give **S2** as large white crystals (1.31 g, 24%); **m.p.** 84–92 °C (lit.3 86–87 °C); **1H NMR** (300 MHz, CDCl3) δ: 8.85 (s, 1H), 8.79 (s, 1H), 7.71–6.67 (m, 5H), 4.65 (s, 2H); **13C NMR** (75 MHz, CDCl3) δ: 151.2, 149.3, 148.7, 141.4, 131.2, 129.4, 129.1, 126.4, 59.0; ***m/z*** (ESI+) 291 ([M+Na]+, 100%), 559 ([2M+Na]+, 74%); **HRMS** (ESI+) found 290.9969 ([M+Na]+), C11H9ClN2O2SNa+ requires 290.9965. Compound reported in the literature not fully characterized.3

*ClC1=CN=CC(S(CC2=CC=CC=C2)(=O)=O)=N1*

*InChI=1S/C11H9ClN2O2S/c12-10-6-13-7-11(14-10)17(15,16)8-9-4-2-1-3-5-9/h1-7H,8H2*

**2-(Benzylsulfonyl)-6-hydrazinylpyrazine (S3)**

**

Prepared according to General Procedure A from: **S2** (503 mg, 1.86 mmol) to give **S3** as a yellow powder (483 mg); **1H NMR** (400 MHz, DMSO-d*6*) δ: 8.86 (s, 1H), 8.31 (s, 1H), 7.92 (s, 1H), 7.41–7.10 (m, 3H), 7.26–7.19 (m, 2H), 4.74 (s, 2H), 4.55 (s, 2H); **13C NMR** (101 MHz, DMSO-d*6*) δ: 156.1, 148.4, 131.1, 128.4, 128.26, 128.34, 128.0, 57.1 (1 obscured signal).

*NNC1=CN=CC(S(CC2=CC=CC=C2)(=O)=O)=N1  
InChI=1S/C11H12N4O2S/c12-15-10-6-13-7-11(14-10)18(16,17)8-9-4-2-1-3-5-9/h1-7H,8,12H2,(H,14,15)*

**(*E*)-2-(Benzylsulfonyl)-6-(2-(4-(difluoromethoxy)benzylidene)hydrazinyl)pyrazine (S4)**



Prepared according to General Procedure C from: **S3** (100 mg, 0.38 mmol) and 4-(difluoromethoxy)benzaldehyde (50.0 μL, 0.38 mmol); purified by trituration with CH2Cl2 to give **S4** as a light brown powder (140 mg, 88%); **m.p.** 238–242 °C; **1H NMR** (500 MHz, DMSO-d*6*) δ: 11.99 (br s, 1H), 8.93 (s, 1H), 8.24 (s, 1H), 8.14 (s, 1H), 7.85 (d, *J* = 8.7 Hz, 2H), 7.37–7.32 (m, 3H), 7.31 (t, *J* = 73.9 Hz, 1H), 7.27–7.21 (m, 4H), 4.74 (s, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 152.0, 151.8, 148.5, 142.3, 135.7, 131.9, 131.4, 131.2, 128.6, 128.5, 127.6, 118.9, 118.3, 116.2, 58.0; ***m/z*** (ESI+) 441 ([M+Na]+, 100%); **HRMS** (ESI+) found 441.0807 ([M+Na]+), C19H16F2N4O3SNa+ requires 441.0803.

*FC(F)OC(C=C1)=CC=C1/C=N/NC2=CN=CC(S(CC3=CC=CC=C3)(=O)=O)=N2*

*InChI=1S/C19H16F2N4O3S/c20-19(21)28-16-8-6-14(7-9-16)10-23-25-17-11-22-12-18(24-17)29(26,27)13-15-4-2-1-3-5-15/h1-12,19H,13H2,(H,24,25)/b23-10+*

**2-Chloro-6-(phenethylsulfonyl)pyrazine (S5)**



NaH (60% dispersion in mineral oil, 403 mg, 16.8 mmol) was added to phenylethyl mercaptan (2.25 mL, 16.8 mmol) in PhMe (16 mL). The mixture was heated at reﬂux for 1 h, then cooled to rt and a solution of 2,6-dichloropyrazine (2.50 g, 16.8 mmol) in PhMe (16 mL) was added. The mixture was heated at reﬂux for 24 h, cooled to rt, then washed with H2O (30 mL). The organic layer was separated, dried (Na2SO4), ﬁltered and concentrated under reduced pressure to give the crude sulﬁde as a yellow liquid (3.92 g). The crude sulﬁde (3.92 g, 15.6 mmol) in glacial AcOH (40 mL) was added to a solution of KMnO4 (2.5 g) in H2O (20 mL) and the mixture was stirred at rt for 1 h. The mixture was adjusted to pH 7 with sat. NH4OH solution (40 mL) then ﬁltered, extracted with CHCl3 (3 × 100 mL), dried (Na2SO4), ﬁltered and concentrated under reduced pressure to give an orange semi-solid (1.23 g); puriﬁed by automated ﬂash chromatography on silica (6–50% EtOAc in hexanes) to give **S5** as large white crystals (547 mg, 12%); **m.p.** 92–100 °C; **1H NMR** (200 MHz, CDCl3) δ: 9.04 (s, 1H), 8.72 (s, 1H), 7.98–6.81 (m, 5H), 3.90–3.59 (m, 2H), 3.28–3.04 (m, 2H).

*ClC1=CN=CC(S(CCC2=CC=CC=C2)(=O)=O)=N1*

*InChI=1S/C12H11ClN2O2S/c13-11-8-14-9-12(15-11)18(16,17)7-6-10-4-2-1-3-5-10/h1-5,8-9H,6-7H2*

**(*E*)-2-(2-(4-(Difluoromethoxy)benzylidene)hydrazinyl)-6-(phenethylsulfonyl)pyrazine (S6)**



Prepared according to General Procedure A from: **S5** (444 mg, 1.57 mmol) to give 2-hydrazinyl-6-(phenethylsulfonyl)pyrazine as a yellow powder (400 mg); followed by General Procedure C from: the hydrazine product (400 mg, 1.44 mmol) and 4-(difluoromethoxy)benzaldehyde (190 μL, 1.44 mmol); purified by flash chromatography on silica (12–100% EtOAc in hexanes) to give **S6** as a yellow powder (275 mg, 44%); **1H NMR** (300 MHz; CDCl3) δ: 8.97 (s, 1H), 8.67 (s, 1H), 8.42 (s, 1H), 7.80 (s, 1H), 7.72 (d, *J* = 8.7 Hz, 2H), 7.42–7.05 (m, 7H), 6.57 (t, *J* = 73.4 Hz, 1H), 3.40–3.69 (m, 2H), 3.33–3.01 (m, 2H).

*FC(F)OC(C=C1)=CC=C1/C=N/NC2=CN=CC(S(CCC3=CC=CC=C3)(=O)=O)=N2*

*InChI=1S/C20H18F2N4O3S/c21-20(22)29-17-8-6-16(7-9-17)12-24-26-18-13-23-14-19(25-18)30(27,28)11-10-15-4-2-1-3-5-15/h1-9,12-14,20H,10-11H2,(H,25,26)/b24-12+*

**6-Chloro-*N*-(3-chlorophenyl)pyrazine-2-carboxamide (S7)**

****

Prepared according to General Procedure F from: 6-chloropyrazine-2-carboxylic acid (1.50 g, 9.46 mmol) and 3-chloroaniline (1 mL, 9.46 mmol); purified by flash chromatography on silica (10–50% EtOAc in hexanes) to give **S7** as a light brown powder (1.90 g, 75%); **m.p.** 101–102 °C (lit.4 107–108 °C); **1H NMR** (400 MHz, DMSO-d*6*) 10.82 (s, 1H), 9.24 (s, 1H), 9.07 (s, 1H), 8.04 (t, *J* = 1.9 Hz, 1H), 7.83 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.41 (t, *J* = 8.1 Hz, 1H), 7.22 (dd, *J* = 7.9, 1.3 Hz, 1H); **13C NMR** (101 MHz, DMSO-d*6*) 160.9, 147.6, 146.9, 144.8, 142.4, 139.4, 132.9, 130.3, 124.2, 120.3, 119.2; ***m/z*** (EI+) 267 ([M]+, 100%). Compound reported in the literature but not fully characterized.4,5

*ClC1=CN=CC(C(NC2=CC=CC(Cl)=C2)=O)=N1*

*InChI=1S/C11H7Cl2N3O/c12-7-2-1-3-8(4-7)15-11(17)9-5-14-6-10(13)16-9/h1-6H,(H,15,17)*

***N*-(3-Chlorophenyl)-6-hydrazinylpyrazine-2-carboxamide (S8)**



Prepared according to General Procedure A from: **S7** (1.80 g, 6.70 mmol) to give **S8** as a yellow powder (1.46 g, 82%); **m.p.** 187 °C; **1H NMR** (400 MHz, DMSO-d*6*) δ: 10.47 (s, 1H), 8.52 (s, 1H), 8.32 (s, 1H), 8.19 (s, 1H), 8.00 (t, *J* = 2.0 Hz, 1H), 7.81 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.42 (t, *J* = 8.1 Hz, 1H), 7.20 (dd, *J* = 7.9, 1.9 Hz, 1H), 4.64 (s, 2H); **13C NMR** (101 MHz, DMSO-d*6*) δ: 162.6, 155.0, 140.8, 139.6, 135.7, 133.0, 130.4, 129.7, 123.8, 119.7, 118.7; ***m/z*** (ESI+) 264 ([M+H]+, 100%).

*NNC1=CN=CC(C(NC2=CC=CC(Cl)=C2)=O)=N1*

*InChI=1S/C11H10ClN5O/c12-7-2-1-3-8(4-7)15-11(18)9-5-14-6-10(16-9)17-13/h1-6H,13H2,(H,15,18)(H,16,17)*

**(*E*)-*N*-(3-Chlorophenyl)-6-(2-(4-(difluoromethoxy)benzylidene)hydrazinyl)pyrazine-2-carboxamide (S9)**



Prepared according to General Procedure B from: **S8** (300 mg, 1.14 mmol) and 4-(difluoromethoxy)benzaldehyde (0.15 mL, 1.14 mmol); purified by flash chromatography on silica (5–100% EtOAc in hexanes) to give **S9** as a pale yellow powder (217 mg, 46%); **m.p.** 229–230 °C; **1H NMR** (400 MHz, DMSO-d*6*) 11.48 (s, 1H), 10.43 (s, 1H), 8.89 (s, 1H), 8.59 (s, 1H), 8.16 (s, 1H), 8.02 (t, *J* = 2.0 Hz, 1H), 7.84 (dapp, *J* = 8.8 Hz, 2H), 7.71 (ddd, *J* = 8.1, 1.7, 0.6 Hz, 1H), 7.42 (t, *J* = 8.1 Hz, 1H), 7.31 (t, *J* = 73.9 Hz, 1H), 7.24 (dapp, *J* = 8.6 Hz, 2H), 7.21 (ddd, *J* = 8.0, 2.0, 0.8 Hz, 1H); **13C NMR** (101 MHz, DMSO-d*6*) 162.6,151.6 (t, *J* = 3.2 Hz), 151.2, 142.3, 141.1, 139.6, 134.0, 133.4, 133.1, 131.6, 130.5, 128.3 (2C), 123.8, 119.5, 118.8 (2C), 118.4, 116.2 (t, *J* = 257.8 Hz); ***m/z*** (APCI+) 418 ([M+H]+, 100%); **HRMS** (APCI+) found 418.0877 ([M+H]+), C19H15ClF2N5O2+ requires 418.0877.

*FC(F)OC(C=C1)=CC=C1/C=N/NC2=CN=CC(C(NC3=CC=CC(Cl)=C3)=O)=N2*

*InChI=1S/C19H14ClF2N5O2/c20-13-2-1-3-14(8-13)25-18(28)16-10-23-11-17(26-16)27-24-9-12-4-6-15(7-5-12)29-19(21)22/h1-11,19H,(H,25,28)(H,26,27)/b24-9+*

**6-Chloro-*N*-(3-chloro-2-methylphenyl)pyrazine-2-carboxamide (S10)**



Prepared according to General Procedure F from: 6-chloropyrazine-2-carboxylic acid (530 mg, 3.35 mmol) and 3-chloro-2-methylaniline (0.40 mL, 3.34 mmol); purified by flash chromatography on silica (10–50% EtOAc in hexanes) to give **S10** as a crystalline beige solid (580 mg, 62%); **m.p.** 155–156 °C; **1H NMR** (400 MHz, CDCl3) δ: 9.44 (s, 1H), 9.40 (s, 1H), 8.84 (s, 1H), 7.98 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.27 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.21 (t, *J* = 8.0 Hz, 1 H), 2.44 (s, 3H); **13C NMR** (101 MHz, CDCl3) δ: 159.7, 147.9, 147.7,144.1, 142.4, 136.2, 135.2, 127.9, 127.4, 126.8, 121.3, 14.6; ***m/z*** (APCI+) 282 ([M+H]+, 100%), (EI+) 281 ([M]+, 100%).

*ClC1=CN=CC(C(NC2=CC=CC(Cl)=C2C)=O)=N1*

*InChI=1S/C12H9Cl2N3O/c1-7-8(13)3-2-4-9(7)17-12(18)10-5-15-6-11(14)16-10/h2-6H,1H3,(H,17,18)*

***N*-(3-Chloro-2-methylphenyl)-6-hydrazinylpyrazine-2-carboxamide (S11)**



Prepared according to General Procedure A from: **S10** (525 mg, 1.86 mmol) to give **S11** as a yellow solid (525 mg, >100%: residual hydrazine); **1H NMR** (400 MHz, DMSO-d*6*) δ: 10.15 (s, 1H), 9.83 (s, 1H), 8.65 (s, 1H), 8.54 (s, 1H), 7.69 (d, *J* = 7.0 Hz, 1H), 7.34 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.27 (t, *J* = 7.8 Hz, 1H), 4.37 (s, 2H), 2.33 (s, 3H); ***m/z*** (ESI+) 278 ([M+H]+, 100%).

*O=C(NC1=CC=CC(Cl)=C1C)C2=NC(NN)=CN=C2*

*InChI=1S/C12H12ClN5O/c1-7-8(13)3-2-4-9(7)17-12(19)10-5-15-6-11(16-10)18-14/h2-6H,14H2,1H3,(H,16,18)(H,17,19)*

**(*E*)-*N*-(3-Chloro-2-methylphenyl)-6-(2-(4-(difluoromethoxy)benzylidene)hydrazinyl)pyrazine-2-carboxamide (S12)**



Prepared according to General Procedure B from: **S11** (300 mg, 1.08 mmol) and 4-(difluoromethoxy)benzaldehyde (0.14 mL, 1.08 mmol); purified by flash chromatography on silica (5–100% EtOAc in hexanes) to give **S12** as bright yellow ﬂakes (265 mg, 56%); **m.p.** 209 °C; **1H NMR** (400 MHz, DMSO-d*6*) δ: 11.48 (s, 1H), 10.06 (s, 1H), 8.87 (s, 1H), 8.62 (s, 1H), 8.17 (s, 1H), 7.84 (dapp, *J* = 8.8 Hz, 2H), 7.79 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.34 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.32 (t, *J* = 73.9 Hz, 1H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.24 (dapp, *J* = 8.7 Hz, 2H), 2.37 (s, 3H); **13C NMR** (101 MHz, DMSO-d*6*) 161.9, 151.6 (t, *J* = 3.1 Hz), 151.0, 141.6, 141.3, 137.3, 134.5, 133.8, 133.2, 131.7, 129.0, 128.3 (2C), 127.3, 126.1, 122.5, 118.8 (2C), 116.2 (t, *J* = 257.8 Hz); ***m/z*** (APCI+) 432 ([M+H]+, 100%); **HRMS** (APCI+) found 432.1033 ([M+H]+), C20H17ClF2N5O2+ requires 432.1033.

*O=C(NC1=CC=CC(Cl)=C1C)C2=NC(N/N=C/C3=CC=C(OC(F)F)C=C3)=CN=C2*

*InChI=1S/C20H16ClF2N5O2/c1-12-15(21)3-2-4-16(12)27-19(29)17-10-24-11-18(26-17)28-25-9-13-5-7-14(8-6-13)30-20(22)23/h2-11,20H,1H3,(H,26,28)(H,27,29)/b25-9+*

**5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-387)**

****

**OSM-S-592**6 (2.14 g, 13.5 mmol, 1.00 equiv.), 2-(3,4-difluorophenyl)ethan-1-ol (2.09 g, 13.5 mmol, 1.00 equiv.) and 18-crown-6 (0.29 g, 1.08 mmol, 0.08 equiv.) were dissolved in PhMe (100 mL) and cooled in an ice bath. KOH (2.28 g, 40.6 mmol, 3.00 equiv.) was added portionwise within 1 h while stirring and the mixture was allowed to warm to rt and stirred for 3 h. After completion of the reaction as indicated by TLC, H2O (100 mL) was added and the resulting suspension filtered through a sintered funnel, washed with H2O (3 x 100 mL) then acetone (3 x 50 mL) and dried *in vacuo* to give the crude product as a pale violet solid (1.87 g); purified by recrystallisation from MeOH (1 g of crude per 100 mL of MeOH) to give **OSM-S-387** as yellow needles (1.51 g, 40%); **m.p.** 205–207 °C; **1H NMR** (400 MHz, DMSO-d*6*) δ: 9.43 (s, 1H), 9.04 (s, 1H), 7.64 (s, 1H), 7.54 (ddd, *J* = 11.9, 7.9, 2.2 Hz, 1H), 7.42–7.31 (m, 1H), 7.31–7.23 (m, 1H), 4.64 (t, *J* = 6.5 Hz, 2H), 3.20 (d, *J* = 6.4 Hz, 2H); **13C NMR** (101 MHz, DMSO-d*6*) δ: 150.0 (dd, *J* = 85.5, 12.7 Hz), 147.6 (dd, *J* = 84.7, 12.6 Hz), 145.8, 142.3, 135.3 (dd, *J* = 6.0, 3.7 Hz), 134.5, 133.1, 126.1 (dd, *J* = 6.3, 3.4 Hz), 118.2 (d, *J* = 16.9 Hz), 117.2 (d, *J* = 16.8 Hz), 108.3, 70.8, 33.5; ***m/z*** (APCI+) 277 ([M+H]+, 100%); **HRMS** (ESI+) found 277.0892 ([M+H]+), C13H11F2N4O+ requires 277.0895.

*FC(C=C1)=C(F)C=C1CCOC2=CN=CC3=NN=CN32*

*InChI=1S/C13H10F2N4O/c14-10-2-1-9(5-11(10)15)3-4-20-13-7-16-6-12-18-17-8-19(12)13/h1-2,5-8H,3-4H2*

**3-Bromo-5-(3,4-difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-432, MMV1576796)**

****

*N*-Bromosuccinimide (1.08 g, 6.07 mmol, 1.5 equiv.) and **OSM-S-387** (1.12 g, 4.05 mmol, 1.0 equiv.) were dissolved in a mixture of CH2Cl2 (147 mL) and THF (3 mL) and heated at refluxed for 6 h. The reaction mixture was cooled to rt, washed with conc. Na2S2O3 solution (20 mL), H2O (2 x 30 mL) and brine (20 mL), then concentrated under reduced pressure and purified by flash chromatography on silica (20–100% EtOAc in hexanes) to give **OSM-S-432** as a pale brown solid (1.10 g, 77%); **m.p.** 171–172 °C (decomp.); **1H NMR** (400 MHz, CDCl3) δ: 8.93 (s, 1H), 7.28 (s, 1H), 7.21–7.08 (m, 2H), 7.05 (ddd, *J* = 8.4, 4.0, 1.9 Hz, 1H), 4.52 (t, *J* = 6.5 Hz, 2H), 3.26 (t, *J* = 6.5 Hz, 2H); **13C NMR** (101 MHz, CDCl3) δ: 151.4 (dd, *J* = 78.1, 12.6 Hz), 148.9 (dd, *J* = 78.1, 12.6 Hz), 148.7, 143.6, 136.5, 133.5 (dd, *J* = 5.7, 4.0 Hz), 125.0 (dd, *J* = 6.3, 3.6 Hz), 118.3–117.5 (m, 3C), 108.8, 71.5, 34.3; ***m/z*** (ESI+) 377 ([M+Na]+, 100%); **HRMS** (ESI+) found 356.9977 ([M+H]+), C13H10BrF2N4O+ requires 356.9980.

*FC(C=C1)=C(F)C=C1CCOC2=CN=CC3=NN=C(Br)N32*

*InChI=1S/C13H9BrF2N4O/c14-13-19-18-11-6-17-7-12(20(11)13)21-4-3-8-1-2-9(15)10(16)5-8/h1-2,5-7H,3-4H2*

**3-(Benzo[*d*][1,3]dioxol-4-yl)-5-chloro-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-661)**



Adapted from the literature procedures.7 To a stirred suspension of 2-chloro-6-hydrazinylpyrazine (308 mg, 2.13 mmol, 1.0 equiv.) in EtOH (10 mL) was added benzo[*d*][1,3]dioxole-4-carbaldehyde (320 mg, 2.13 mmol, 1.0 equiv.) and the mixture heated at reflux overnight. After consumption of starting material as indicated by TLC, the reaction was cooled in an ice bath and chloramine T trihydrate (780 mg, 2.77 mmol, 1.3 equiv.) was added portionwise while stirring over 1 h. After consumption of the intermediate was confirmed by TLC, cold H2O (100 mL) was added. The solution was stirred for 10 min, then filtered through a sintered glass funnel (P3 porosity), washed with H2O (3 x 30 mL) then Et2O (30 mL) and dried *in vacuo* to give **OSM-S-661** as a pale brown solid (320 mg, 1.17 mmol, 55%); **m.p.** 164–167 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.50 (s, 1H), 8.13 (s, 1H), 7.18 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.13 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.02 (t, *J* = 7.9 Hz, 1H), 6.08 (s, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 147.07, 147.06, 142.9, 142.4, 129.5, 124.1, 121.7, 121.4, 110.8, 108.9, 101.7 (one carbon signal is overlapping or obscured); ***m/z*** (ESI+) 297 ([M+Na]+, 100%); **HRMS** (ESI+) found 297.0150 ([M+Na]+), C12H7ClN4O2Na+ requires 297.0150.

*ClC1=CN=CC2=NN=C(C3=CC=CC4=C3OCO4)N21*

*InChI=1S/C12H7ClN4O2/c13-9-4-14-5-10-15-16-12(17(9)10)7-2-1-3-8-11(7)19-6-18-8/h1-5H,6H2*

**(*E*)-4-((2-(6-Chloropyrazin-2-yl)hydrazinylidene)methyl)benzonitrile (S13)**

****

Prepared according to General Procedure C from: 2-chloro-6-hydrazinylpyrazine (717 mg, 4.96 mmol) and 4-formylbenzonitrile (650 mg, 4.96 mmol); triturated with EtOH to give **S13** as an orange powder (1.16 g, 91%); **1H NMR** (500 MHz, DMSO-d*6*) δ: 11.85 (s, 1H), 8.65 (s, 1H), 8.12 (s, 1H), 8.10 (s, 1H), 7.93 (d, *J* = 8.4 Hz, 2H), 7.87 (d, *J* = 8.5 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 152.1, 145.5, 140.5, 139.0, 133.2, 132.7, 129.1, 127.2, 118.8, 111.1.

*ClC1=NC(N/N=C/C2=CC=C(C#N)C=C2)=CN=C1*

*InChI=1S/C12H8ClN5/c13-11-7-15-8-12(17-11)18-16-6-10-3-1-9(5-14)2-4-10/h1-4,6-8H,(H,17,18)*

**4-(5-Chloro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)benzonitrile (S14)**

****

Prepared according to General Procedure D from: **S13** (750 mg, 2.91 mmol); purified by flash chromatography on silica (12–100% EtOAc in hexanes) to give **S14** as an orange powder (359 mg, 48%); **1H NMR** (500 MHz, CDCl3) δ: 9.39 (s, 1H), 7.95 (s, 1H), 7.84 (d, *J* = 8.4 Hz, 2H), 7.80 (d, *J* = 8.5 Hz, 2H); **13C NMR** (126 MHz, CDCl3) δ: 147.5, 146.6, 143.3, 132.2, 131.8, 131.3, 130.3, 121.7, 118.0, 114.9.

*ClC1=CN=CC2=NN=C(C3=CC=C(C#N)C=C3)N21*

*InChI=1S/C12H6ClN5/c13-10-6-15-7-11-16-17-12(18(10)11)9-3-1-8(5-14)2-4-9/h1-4,6-7H*

**(*E*)-2-Chloro-6-(2-(4-chlorobenzylidene)hydrazinyl)pyrazine (S15)**

****

Prepared according to General Procedure C from: 2-chloro-6-hydrazinylpyrazine (1.00 g, 6.92 mmol) and 4-chlorobenzaldehyde (972 mg, 6.92 mmol); triturated with EtOH to give **S15** as a yellow powder (1.53 g, 82%); **1H NMR** (500 MHz, DMSO-d*6*) δ: 11.64 (s, 1H), 8.58 (s, 1H), 8.07 (s, 1H), 8.05 (s, 1H), 7.77 (d, *J* = 8.5 Hz, 2H), 7.48 (d, *J* = 8.5 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 152.2, 145.5, 141.3, 133.8, 133.4, 132.6, 128.9, 128.3.

*ClC(C=C1)=CC=C1/C=N/NC2=CN=CC(Cl)=N2*

*InChI=1S/C11H8Cl2N4/c12-9-3-1-8(2-4-9)5-15-17-11-7-14-6-10(13)16-11/h1-7H,(H,16,17)/b15-5+*

**5-Chloro-3-(4-chlorophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (S16)**

****

Prepared according to General Procedure D from: **S15** (750 mg, 2.81 mmol); purified by flash chromatography on silica (25–100% EtOAc in hexanes) to give **S16** as an orange powder (634 g, 85%); **1H NMR** (500 MHz, CDCl3) δ: 9.29 (s, 1H), 7.86 (s, 1H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.48 (d, *J* = 8.5 Hz, 2H); **13C NMR** (126 MHz, CDCl3) δ: 147.2, 142.9, 137.2, 132.6, 129.8, 128.3, 125.1, 121.8.

*ClC1=CN=CC2=NN=C(C3=CC=C(Cl)C=C3)N21*

*InChI=1S/C11H6Cl2N4/c12-8-3-1-7(2-4-8)11-16-15-10-6-14-5-9(13)17(10)11/h1-6H*

**(*E*)-2-Chloro-6-(2-(4-(methylsulfonyl)benzylidene)hydrazinyl)pyrazine (S17)**

****

Prepared according to General Procedure B from: 2-chloro-6-hydrazinylpyrazine (306 mg, 2.11 mmol) and 4-chlorobenzaldehyde (390 mg, 2.11 mmol); filtered and washed with anhydrous MeCN (10 mL) to give **S17** (846 mg); used without further purification; **1H NMR** (500 MHz, CDCI3) δ: 8.68 (s, 1H), 8.41 (s, 1H), 8.13 (d, *J* = 0.6 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 2H), 7.87 (d, *J* = 8.3 Hz, 2H), 7.82 (d, *J* = 1.0 Hz, 1H), 3.09 (s, 3H).

*ClC1=NC(N/N=C/C2=CC=C(S(C)(=O)=O)C=C2)=CN=C1*

*InChI=1S/C12H11ClN4O2S/c1-20(18,19)10-4-2-9(3-5-10)6-15-17-12-8-14-7-11(13)16-12/h2-8H,1H3,(H,16,17)/b15-6+*

**5-Chloro-3-(4-(methylsulfonyl)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (S18)**

****

Prepared according to General Procedure D from: **S17** (658 mg, 2.11 mmol); purified by flash chromatography on silica (50–100% EtOAc in hexanes) to give **S18** (606 mg, 93%); **1H NMR** (500 MHz, CDCl3) δ: 9.40 (d, *J* = 0.5 Hz, 1H), 8.13 (d, *J* = 8.6 Hz, 2H), 7.96 (d, *J* = 0.5 Hz, 1H), 7.89 (d, *J* = 8.6 Hz, 2H), 3.15 (s, 3H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 147.2, 146.1, 142.7, 142.3, 132.4, 132.3, 129.4, 126.2, 121.9, 43.4.

*ClC1=CN=CC2=NN=C(C3=CC=C(S(C)(=O)=O)C=C3)N21*

*InChI=1S/C12H9ClN4O2S/c1-20(18,19)9-4-2-8(3-5-9)12-16-15-11-7-14-6-10(13)17(11)12/h2-7H,1H3*

**5-Chloro-3-phenyl-[1,2,4]triazolo[4,3-*a*]pyrazine (S19)**

****

Prepared according to General Procedure C from: 2-chloro-6-hydrazinylpyrazine (250 mg, 1.73 mmol) and benzaldehyde (0.18 mL, 1.73 mmol); ﬁltered and triturated with Et2O to give the condensation product as a yellow powder (253 mg, 63%); carried forward without puriﬁcation or complete characterization; **m.p.** 210–217 °C; **1H NMR** (400 MHz, DMSO-d*6*) δ: 11.58 (s, 1H), 8.57 (s, 1H), 8.08 (s, 1H), 8.05 (s, 1H), 7.73 (d, *J* = 6.9 Hz, 2H), 7.49–7.33 (m, 3H); **13C NMR** (101 MHz, DMSO-d*6*) δ: 152.3, 145.5, 142.7, 134.4, 132.3, 129.4, 128.8, 126.6 (1 obscured signal). Compound reported in the literature but no NMR characterization data were provided.8 The condensation product (220 mg, 0.95 mmol) was subjected to General Procedure Dto give **S19** as an orange powder (189 mg, 87%) that was carried forward without further purification or characterization.

*ClC1=CN=CC2=NN=C(C3=CC=CC=C3)N21*

*InChI=1S/C11H7ClN4/c12-9-6-13-7-10-14-15-11(16(9)10)8-4-2-1-3-5-8/h1-7H*

**1-(3,4-difluorophenyl)ethan-1-one (S20)**

****

1-(3,4-Diﬂuorophenyl)ethan-1-ol (1.50 g, 9.48 mmol, 1.0 equiv.) was dissolved in CH2Cl2 (15 mL). HIO3 (1.84 g, 10.4 mmol, 1.1 equiv.) and TEMPO (74.1 mg, 0.47 mmol, 5 mol%) were added and the reaction stirred under Ar at rt for 19 h. The reaction was poured into an aqueous solution of Na2S2O3 (50 mL) and extracted with a 1:1 mixture of Et2O:hexane (3 × 50 mL). The combined organic layers were washed with brine, dried (Na2SO4), ﬁltered and concentrated under reduced pressure to give the crude product as an orange liquid (1.39 g); puriﬁed by ﬂash chromatography on silica (6–50% EtOAc in hexanes) to give **S20** as a clear red liquid (1.04 g, 70%); **1H NMR** (200 MHz, CDCl3) δ: 7.98–7.61 (m, 2H), 7.28 (q, *J* = 9.4, 9.0 Hz, 1H), 2.62 (s, 3H). Spectroscopic data matched those in the literature.9

*CC(C1=CC(F)=C(F)C=C1)=O*

*InChI=1S/C8H6F2O/c1-5(11)6-2-3-7(9)8(10)4-6/h2-4H,1H3*

**2-(3,4-Difluorophenyl)-2,2-dimethoxyethan-1-ol (S21)**

****

To a solution of KOH (2.70 g, 48.0 mmol, 10 equiv.) in MeOH (30 mL) was added **S20** (750 mg, 4.80 mmol, 1.0 equiv.) dropwise over 15 min at 0 °C. PhI(OAc)2 (3.09 g, 9.61 mmol, 2.0 equiv.) was added in small portions over 20 min and the resulting solution stirred at rt for 23 h. The solvent was removed and the residue dissolved and H2O and extracted with EtOAc (3 ×). The combined organic layers were washed with brine, dried (Na2SO4), ﬁltered and concentrated under reduced pressure to give **S21** as a brown liquid (1.41 g); carried forward without further puriﬁcation or complete characterization; **1H NMR** (200 MHz, CDCl3) δ: 7.53–6.85 (m, 3H), 3.78 (s, 2H), 3.25 (s, 6H) (alcohol OH signal not seen). Compound reported in the literature but no characterization data were provided.10

*OCC(OC)(OC)C1=CC(F)=C(F)C=C1*

*InChI=1S/C10H12F2O3/c1-14-10(6-13,15-2)7-3-4-8(11)9(12)5-7/h3-5,13H,6H2,1-2H3*

**3-(4-(Difluoromethoxy)phenyl)-5-(2-(3,4-difluorophenyl)-2,2-dimethoxyethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-393; MMV1557933)**

****

Prepared according to General Procedure E from: **S1** (227 mg, 0.77 mmol) and **S21** (167 mg, 0.77 mmol) to give the crude product as a brown solid (321 mg); puriﬁed by ﬂash chromatography on silica (25–100% EtOAc in hexanes) to give **OSM-S-393** as a light brown powder (221 mg, 60%); **m.p.** 148–153 °C; **1H NMR** (300 MHz, CDCl3) δ: 8.97 (s, 1H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 8.5 Hz, 2H), 7.25 (br s, 1H), 7.03–6.82 (m, 1H), 6.69 (t, *J* = 73.3 Hz, 1H), 6.75–6.58 (m, 2H), 4.32 (s, 2H), 3.18 (s, 6H); **13C NMR** (75 MHz, CDCl3) δ: 152.5, 147.8, 146.2, 143.6, 137.1, 134.7, 132.3, 124.8, 122.9 (dd, *J* 6.2, 3.5 Hz), 119.2, 117.1 (d, *J* = 17.5 Hz), 116.6 (d, *J* = 18.9 Hz), 115.7 (t, *J* = 261.7 Hz), 108.6, 99.5, 71.2, 49.1 (two phenyl C–F signals expected between 155 and 148 ppm; observed for compound **OSM-S-392**; too weak to be seen); ***m/z*** (ESI+) 501 ([M+Na]+, 100%); **HRMS** (ESI+) found 501.1160 ([M+Na]+), C22H18F4N4O4Na+ requires 501.1156.

*FC(F)OC(C=C1)=CC=C1C2=NN=C3C=NC=C(OCC(OC)(OC)C4=CC(F)=C(F)C=C4)N32*

*InChI=1S/C22H18F4N4O4/c1-31-22(32-2,14-5-8-16(23)17(24)9-14)12-33-19-11-27-10-18-28-29-20(30(18)19)13-3-6-15(7-4-13)34-21(25)26/h3-11,21H,12H2,1-2H3*

**Ethyl 2-(3,4-difluorophenyl)acetate (S22)**

**

3,4-Difluorophenylacetic acid (5.00 g, 29.1 mmol 1 equiv.) and *p*-TsOH (100 mg, 0.58 mmol, 0.02 equiv.) were dissolved in EtOH (20 mL, 1.45 M) and the reaction heated at reflux overnight. The reaction was cooled to rt and the solvent removed. EtOAc was added to the residue and the organic layer washed with H2O, followed by a sat. aq. NaHCO3 solution, brine, dried (MgSO4), filtered and concentrated under reduced pressure to give **S22** as a pale yellow oil (5.95 g, quant.); carried forward without further purification; **1H NMR** (200 MHz, CDCl3) δ: 7.20–6.87 (m, 3H), 4.16 (q, *J* = 7.1 Hz, 2H), 3.56 (s, 2H), 1.25 (t, *J* = 7.1 Hz, 3H); **13C NMR** (75 MHz, CDCl3) δ: 171.0, 150.2 (dd, *J* = 248.0, 12.7 Hz), 149.7 (dd, *J* = 247.3, 12.6 Hz), 133.1–128.9 (m), 125.4 (dd, *J* = 5.9, 3.7 Hz), 118.3 (d, *J* = 17.6 Hz), 117.2 (d, *J* = 17.3 Hz), 61.2, 40.4, 14.1. Spectroscopic data matched those in the literature.11

*FC1=CC=C(CC(OCC)=O)C=C1F*

*InChI=1S/C10H10F2O2/c1-2-14-10(13)6-7-3-4-8(11)9(12)5-7/h3-5H,2,6H2,1H3*

**Ethyl 2-bromo-2-(3,4-difluorophenyl)acetate (S23)**

****

Compound **S22** (2.00 g, 10.0 mmol, 1.00 equiv.) was dissolved in α,α,α-trifluorotoluene (10 mL). NBS (1.83 g, 10.3 mmol, 1.03 equiv.) and HBr (48% aq., 4 drops) were added and the reaction heated at 80 °C for 19 h. The reaction was cooled to rt, filtered and concentrated under reduced pressure to give **S23** as a yellow liquid (2.13 g, 76%); carried forward without further purification or characterization; **1H NMR** (200 MHz, CDCl3) δ: 7.55–7.36 (m, 1H), 7.34–7.03 (m, 2H), 5.27 (s, 1H), 4.26 (qd, *J* = 7.1, 2.0 Hz, 2H), 1.30 (t, *J* = 7.1 Hz, 3H). Spectroscopic data matched those in the literature.11

*FC1=CC=C(C(Br)C(OCC)=O)C=C1F*

*InChI=1S/C10H9BrF2O2/c1-2-15-10(14)9(11)6-3-4-7(12)8(13)5-6/h3-5,9H,2H2,1H3*

**Ethyl 2-(3,4-difluorophenyl)-2-(dimethylamino)acetate (S24)**

****

Compound **S23** (500 mg, 1.79 mmol, 1 equiv.)was dissolved in DMF (5 mL). Dimethylamine solution (33% in alcohol, 0.32 mL, 1.79 mmol, 1 equiv.) and K2CO3 (743 mg, 5.37 mmol, 3 equiv.) were added and the reaction stirred at rt for 2 h. The mixture was filtered and the solvent removed. The residue was partitioned between EtOAc and H2O. The aqueous layer was extracted with EtOAc and the combined organic layers washed with brine, dried (Na2SO4), filtered and concentrated under reduced pressure to give **S24** as a yellow oil (410 mg, 94%); carried forward without further purification or characterization; **1H NMR** (200 MHz, CDCl3) δ: 7.41–7.15 (m, 1H), 7.15–6.90 (m, 2H), 4.29–3.99 (m, 2H), 3.74 (s, 1H), 2.17 (s, 6H), 1.16 (t, *J* = 7.1 Hz, 3H).

*FC1=CC=C(C(C(OCC)=O)N(C)C)C=C1F*

*InChI=1S/C12H15F2NO2/c1-4-17-12(16)11(15(2)3)8-5-6-9(13)10(14)7-8/h5-7,11H,4H2,1-3H3*

**2-(3,4-Difluorophenyl)-2-(dimethylamino)ethan-1-ol (S25)**

****

Compound **S24** (450 mg, 1.85 mmol, 1.00 equiv.) was dissolved in anhydrous THF (3.27 mL, 566 mM) and cooled to 0 °C. LiAlH4 (1 M in THF, 1.18 mL, 1.18 mmol, 0.64 equiv.) was added dropwise and the reaction mixture stirred for 10 min at 0 °C, then at rt overnight. The reaction was cooled in an ice bath and excess LAH was quenched with EtOAc dropwise, followed by sat. aq. Rochelle’s salt solution. The mixture was stirred at 0 °C, the at rt until two distinct layers were seen. The organic layer was separated and the aqueous layer extracted with EtOAc (3 ×). The combined organic layers were dried (MgSO4), filtered and concentrated under reduced pressure to give **S25** as a yellow oil (245 mg, 66%); carried forward without further purification or characterization; **1H NMR** (200 MHz, CDCl3) δ: 7.23–6.79 (m, 3H), 3.91–3.76 (m, 1H), 3.67 (dd, *J* = 10.8, 5.1 Hz, 1H), 3.48 (dd, *J* = 8.2, 5.2 Hz, 1H), 2.47 (s, 1H), 2.20 (s, 6H).

*FC1=CC=C(C(CO)N(C)C)C=C1F*

*InChI=1S/C10H13F2NO/c1-13(2)10(6-14)7-3-4-8(11)9(12)5-7/h3-5,10,14H,6H2,1-2H3*

**Ethyl 2-(diethylamino)-2-(3,4-difluorophenyl)acetate (S26)**

****

Compound **S23** (600 mg, 2.15 mmol, 1 equiv.)was dissolved in DMF (6 mL). Diethylamine (0.22 mL, 2.15 mmol, 1 equiv.) and K2CO3 (891 mg, 6.45 mmol, 3 equiv.) were added and the reaction stirred at rt until completion as indicated by TLC. The mixture was filtered and the solvent removed. The residue was partitioned between EtOAc and H2O. The aqueous layer was extracted with EtOAc and the combined organic layers washed with brine, dried (Na2SO4), filtered and concentrated under reduced pressure to give **S26** as a dark yellow oil (481 mg, 82%); carried forward without further purification or characterization; **1H NMR** (200 MHz, CDCl3) δ: 7.41–7.23 (m, 1H), 7.22–7.04 (m, 2H), 4.40 (s, 1H), 4.18 (dtt, *J* = 10.8, 7.2, 3.7 Hz, 2H), 2.59 (q, *J* = 7.1 Hz, 4H), 1.24 (t, *J* = 7.1 Hz, 3H), 0.99 (t, *J* = 7.1 Hz, 6H).

*FC1=CC=C(C(C(OCC)=O)N(CC)CC)C=C1F*

*InChI=1S/C14H19F2NO2/c1-4-17(5-2)13(14(18)19-6-3)10-7-8-11(15)12(16)9-10/h7-9,13H,4-6H2,1-3H3*

**2-(Diethylamino)-2-(3,4-difluorophenyl)ethan-1-ol (S27)**

**

Compound **S26** (350 mg, 1.29 mmol, 1.00 equiv.) was dissolved in anhydrous THF (2.28 mL, 566 mM) and cooled to 0 °C. LiAlH4 (1 M in THF, 0.83 mL, 0.83 mmol, 0.64 equiv.) was added dropwise and the reaction mixture stirred for 10 min at 0 °C, then at rt overnight. The reaction was cooled in an ice bath and excess LAH was quenched with EtOAc dropwise, followed by sat. aq. Rochelle’s salt solution. The mixture was stirred at 0 °C, the at rt until two distinct layers were seen. The organic layer was separated and the aqueous layer extracted with EtOAc (3 ×). The combined organic layers were dried (MgSO4), filtered and concentrated under reduced pressure to give **S27** as an orange oil (291 mg, 98%); carried forward without further purification or characterization; **1H NMR** (200 MHz, CDCl3) δ: 7.25–6.82 (m, 3H), 4.02–3.51 (m, 3H), 2.98–2.10 (m, 4H), 1.08 (t, *J* = 7.1 Hz, 6H) (alcohol OH signal not seen).

*FC1=CC=C(C(CO)N(CC)CC)C=C1F*

*InChI=1S/C12H17F2NO/c1-3-15(4-2)12(8-16)9-5-6-10(13)11(14)7-9/h5-7,12,16H,3-4,8H2,1-2H3*

**Ethyl 2-hydroxy-2-phenylacetate (S28)**

****

DL-Mandelic acid (10.0 g, 65.7 mmol, 1 equiv.) and *p*-TsOH (226 mg, 1.31 mmol, 0.02 equiv.) were dissolved in EtOH (45 mL, 1.45 M) and the reaction heated at reflux overnight. The reaction was cooled to rt and the solvent removed. EtOAc was added to the residue and the organic layer washed with H2O, followed by a sat. aq. NaHCO3 solution, brine, dried (MgSO4), filtered and concentrated under reduced pressure to give **S28** as a clear yellow oil (12.3 g, quant.); carried forward without further purification; **1H NMR** (400 MHz, CDCl3) δ: 7.46–7.40 (m, 2H), 7.40–7.29 (m, 3H), 5.16 (s, 1H), 4.34–4.09 (m, 2H), 3.64 (br s, 1H), 1.22 (t, *J* = 7.1 Hz, 3H); **13C NMR** (101 MHz, CDCl3) δ: 173.7, 138.6, 128.6, 128.4, 126.6, 73.0, 62.2, 14.1. Spectroscopic data matched those in the literature.12

*OC(C1=CC=CC=C1)C(OCC)=O*

*InChI=1S/C10H12O3/c1-2-13-10(12)9(11)8-6-4-3-5-7-8/h3-7,9,11H,2H2,1H3*

**Ethyl 2-phenyl-2-((tetrahydro-2*H*-pyran-2-yl)oxy)acetate (S29)**

****

Compound **S28** (10.0 g, 5.55 mmol, 1.0 equiv.) was dissolved in CH2Cl2 (200 mL). *p*-TsOH (1.91 g, 1.11 mmol, 0.2 equiv.) and 3,4-dihydro-2*H*-pyran (5.57 mL, 61.0 mmol, 1.1 equiv.) were added and the reaction stirred at rt. The reaction was quenched with ice cold H2O (80 mL) and the organic layer separated. The aqueous layer was extracted with CH2Cl2 (3 × 50 mL) and the combined organic layers washed with H2O (50 mL), brine (40 mL), dried (MgSO4), ﬁltered and concentrated under reduced pressure to give **S29** as a black oil (14.1 g, 96%); carried forward without further puriﬁcation or characterisation; **1H NMR** (400 MHz, CDCl3) δ: 7.53–7.42 (m, 2H), 7.41–7.27 (m, 3H), 5.26 (d, *J* = 41.6 Hz, 1H), 4.74 (dt, *J* = 120.7, 3.3 Hz, 1H), 4.16 (ddtd, *J* = 18.0, 14.5, 7.6, 3.7 Hz, 2H), 3.71 (ddd, *J* 11.2, 9.9, 3.2 Hz, 1H), 3.60–3.42 (m, 1H), 2.01–1.38 (m, 6H), 1.20 (t, *J* = 7.1 Hz, 3H). Compound reported in the literature but no characterisation data were provided.13

*O=C(C(OC1OCCCC1)C2=CC=CC=C2)OCC*

*InChI=1S/C15H20O4/c1-2-17-15(16)14(12-8-4-3-5-9-12)19-13-10-6-7-11-18-13/h3-5,8-9,13-14H,2,6-7,10-11H2,1H3*

**2-Phenyl-2-((tetrahydro-2*H*-pyran-2-yl)oxy)ethan-1-ol (S30)**

****

Compound **S29** (12.0 g, 45.4 mmol, 1.00 equiv.) was dissolved in anhydrous THF (80.2 mL, 566 mM) and cooled to 0 °C. LiAlH4 (1 M in THF, 29.1 mL, 29.1 mmol, 0.64 equiv.) was added dropwise and the reaction mixture stirred for 10 min at 0 °C, then at rt overnight. The reaction was cooled in an ice bath and excess LAH was quenched with EtOAc dropwise, followed by sat. aq. Rochelle’s salt solution. The mixture was stirred at 0 °C, the at rt until two distinct layers were seen. The organic layer was separated and the aqueous layer extracted with EtOAc (3 ×). The combined organic layers were dried (MgSO4), filtered and concentrated under reduced pressure to give a dark red oil (11.3 g); purified by flash chromatography on silica (25–100% EtOAc in hexanes) to give **S30** as a dark red oil (3.24 g, 32%); **1H NMR** (400 MHz, CDCl3, present as a mixture of diastereomers) δ: 7.47–7.06 (m, 10H), 4.91 (t, *J* = 3.6 Hz, 1H), 4.82 (dd, *J* = 8.3, 3.7 Hz, 1H), 4.73 (dd, *J* = 6.9, 4.9 Hz, 1H), 4.52 (dd, *J* = 5.7, 2.8 Hz, 1H), 4.22–3.20 (m, 8H), 3.04 (dapp, *J* = 10.8 Hz, 1H), 2.25 (br s, 1H), 2.13–1.32 (m, 12H); **13C NMR** (101 MHz, CDCl3, present as a mixture of diastereomers) δ: 140.0, 138.9, 128.6, 128.4, 128.1, 127.7, 126.9, 126.8, 99.2, 98.0, 80.8, 79.9, 67.7, 66.8, 63.8, 62.7, 31.1, 30.7, 25.4, 25.36, 20.4, 19.5. Spectroscopic data matched those in the literature.14,15

*OCC(OC1OCCCC1)C2=CC=CC=C2*

*InChI=1S/C13H18O3/c14-10-12(11-6-2-1-3-7-11)16-13-8-4-5-9-15-13/h1-3,6-7,12-14H,4-5,8-10H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(2-phenyl-2-((tetrahydro-2*H*-pyran-2-yl)oxy)ethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (S31)**

****

Prepared according to General Procedure E from: **S1** (275 mg, 0.93 mmol) and **S30** (206 mg, 0.93 mmol) to give a black sludge (477 mg); purified by flash chromatography on silica (25–100% EtOAc in hexanes) to give **S31** as a brown powder (201 mg, 45%); carried forward without characterization.

*FC(F)OC(C=C1)=CC=C1C2=NN=C3C=NC=C(OCC(OC4OCCCC4)C5=CC=CC=C5)N32*

*InChI=1S/C25H24F2N4O4/c26-25(27)34-19-11-9-18(10-12-19)24-30-29-21-14-28-15-22(31(21)24)33-16-20(17-6-2-1-3-7-17)35-23-8-4-5-13-32-23/h1-3,6-7,9-12,14-15,20,23,25H,4-5,8,13,16H2*

**Ethyl 3-hydroxy-2-phenylpropanoate (S32)**

****

Tropic acid (500 mg, 3.01 mmol, 1 equiv.) and *p*-TsOH ( mg, 1.31 mmol, 0.02 equiv.) were dissolved in EtOH (2.1 mL, 1.45 M) and the reaction heated at reflux overnight. The reaction was cooled to rt and the solvent removed. EtOAc was added to the residue and the organic layer washed with H2O, followed by a sat. aq. NaHCO3 solution, brine, dried (MgSO4), filtered and concentrated under reduced pressure to give **S32** as a clear oil (604 mg); carried forward without further purification; **1H NMR** (200 MHz, CDCl3) δ: 7.58–7.08 (m, 5H), 4.37–3.98 (m, 3H), 3.91–3.71 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 3H) (alcohol OH signal not seen). Spectroscopic data matched those in the literature.16

*OCC(C1=CC=CC=C1)C(OCC)=O*

*InChI=1S/C11H14O3/c1-2-14-11(13)10(8-12)9-6-4-3-5-7-9/h3-7,10,12H,2,8H2,1H3*

**Ethyl 2-phenyl-3-((tetrahydro-2*H*-pyran-2-yl)oxy)propanoate (S33)**

****

Compound **S32** (4.78 g, 24.6 mmol, 1.0 equiv.) was dissolved in CH2Cl2 (123 mL). *p*-TsOH (848 mg, 4.92 mmol, 0.2 equiv.) and 3,4-dihydro-2*H*-pyran (2.47 mL, 27.1 mmol, 1.1 equiv.) were added and the reaction stirred at rt. The reaction was quenched with ice cold H2O (80 mL) and the organic layer separated. The aqueous layer was extracted with CH2Cl2 (3 × 50 mL) and the combined organic layers washed with H2O (50 mL), brine (40 mL), dried (MgSO4), ﬁltered and concentrated under reduced pressure to give **S33** as a brown oil (14.1 g); carried forward without further puriﬁcation or characterisation.

*O=C(C(COC1OCCCC1)C2=CC=CC=C2)OCC*

*InChI=1S/C16H22O4/c1-2-18-16(17)14(13-8-4-3-5-9-13)12-20-15-10-6-7-11-19-15/h3-5,8-9,14-15H,2,6-7,10-12H2,1H3*

**2-Phenyl-3-((tetrahydro-2*H*-pyran-2-yl)oxy)propan-1-ol (S34)**

****

Compound **S33** (6.00 g, 21.6 mmol, 1.00 equiv.) was dissolved in anhydrous THF (38.1 mL, 566 mM) and cooled to 0 °C. LiAlH4 (1 M in THF, 13.8 mL, 13.8 mmol, 0.64 equiv.) was added dropwise and the reaction mixture stirred for 10 min at 0 °C, then at rt overnight. The reaction was cooled in an ice bath and excess LAH was quenched with EtOAc dropwise, followed by sat. aq. Rochelle’s salt solution. The mixture was stirred at 0 °C, the at rt until two distinct layers were seen. The organic layer was separated and the aqueous layer extracted with EtOAc (3 ×). The combined organic layers were dried (MgSO4), filtered and concentrated under reduced pressure to give an orange oil (4.98 g); purified by flash chromatography on silica (25–100% EtOAc in hexanes) to give **S34** as a clear pale yellow oil (1.11 g, 22%); **1H NMR** (200 MHz, CDCl3) δ: 7.75–6.58 (m, 5H), 4.61 (br s, 1H), 4.35–3.62 (m, 6H), 3.63–3.42 (m, 1H), 3.37–3.04 (m, 1H), 2.75–2.49 (m, 1H), 1.87–1.54 (m, 4H) (alcohol OH signal not seen). Spectroscopic data matched those in the literature.17

*OCC(COC1OCCCC1)C2=CC=CC=C2*

*InChI=1S/C14H20O3/c15-10-13(12-6-2-1-3-7-12)11-17-14-8-4-5-9-16-14/h1-3,6-7,13-15H,4-5,8-11H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(2-phenyl-3-((tetrahydro-2*H*-pyran-2-yl)oxy)propoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (S35)**

****

Prepared according to General Procedure Efrom: **S1** (1.23 g, 4.16 mmol) and **S34** (983 mg, 4.16 mmol) to give a black sludge (2.13 g); purified by flash chromatography on silica (25–100% EtOAc in hexanes) to give **S35** as a sticky brown solid (1.56 g, 75%); carried forward without characterization.

*FC(OC(C=C1)=CC=C1C2=NN=C3C=NC=C(OCC(COC4OCCCC4)C5=CC=CC=C5)N32)F*

*InChI=1S/C26H26F2N4O4/c27-26(28)36-21-11-9-19(10-12-21)25-31-30-22-14-29-15-23(32(22)25)34-16-20(18-6-2-1-3-7-18)17-35-24-8-4-5-13-33-24/h1-3,6-7,9-12,14-15,20,24,26H,4-5,8,13,16-17H2*

**2-(Hydroxymethyl)-2-phenylpropane-1,3-diol (OSM-S-665)**

****

Prepared according to literature procedures.18 Phenylacetaldehyde (4.20 g, 35.0 mmol) and Ca(OH)2 (10.4 g, 140 mmol) were added to a suspension of paraformaldehyde (4.19 g, 140 mmol) in THF (50 mL). The reaction mixture was stirred at 60–65 °C for 4 days. After cooling to rt, the reaction mixture was filtered through celite and the celite was rinsed with CH2Cl2 (2 x 10 mL). The combined filtrates were concentrated under reduced pressure and the residue purified by flash chromatography on silica (0–10% MeOH in CH2Cl2) to give **OSM-S-665** as a white solid (3.96 g, 62%); **m.p.** 84–86 °C (lit.19 80–81 °C); **1H NMR** (500 MHz, DMSO-d*6*) δ: 7.42 (dd, *J* = 8.3, 1.5 Hz, 2H), 7.26 (t, *J* = 7.6 Hz, 2H), 7.19–7.12 (m, 1H), 4.40 (t, *J* = 5.2 Hz, 3H), 3.73 (d, *J* = 5.2 Hz, 6H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 143.0, 127.7, 127.5, 125.5, 63.5, 49.1; ***m/z*** (ESI+) 205 ([M+Na]+, 100%). Spectroscopic data matched those in the literature.18,19

*OCC(CO)(CO)C1=CC=CC=C1*

*InChI=1S/C10H14O3/c11-6-10(7-12,8-13)9-4-2-1-3-5-9/h1-5,11-13H,6-8H2*

**(2,2-Dimethyl-5-phenyl-1,3-dioxan-5-yl)methanol (OSM-S-705)**

****

**OSM-S-665** (288 mg, 1.58 mmol) was dissolved in acetone (5 mL) and few drops of HCl (10% in H2O) were added and the resulting solution stirred overnight at rt. The solvent was removed and the residue purified by flash chromatography on silica (0–10% MeOH in CH2Cl2) to give **OSM-S-705** as a clear oil (300 mg, 85%); **1H NMR** (500 MHz, DMSO-d*6*) δ: 7.31 (d, *J* = 4.3 Hz, 4H), 7.22 (h, *J* = 4.2 Hz, 1H), 4.65 (t, *J* = 5.1 Hz, 1H), 4.05 (d, *J* = 11.6 Hz, 2H), 3.94 (d, *J* = 11.6 Hz, 2H), 3.77 (d, *J* = 5.1 Hz, 2H), 1.36 (s, 3H), 1.34 (s, 3H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 142.1, 128.0, 126.5, 126.2, 97.2, 64.0, 63.3, 41.0, 26.8, 20.8; ***m/z*** (ESI+) 245 ([M+Na]+, 100%); **HRMS** (ESI+) found 245.1148 ([M+Na]+), C13H18O3Na+ requires 245.1148.

*OCC1(COC(C)(C)OC1)C2=CC=CC=C2*

*InChI=1S/C13H18O3/c1-12(2)15-9-13(8-14,10-16-12)11-6-4-3-5-7-11/h3-7,14H,8-10H2,1-2H3*

**3-(4-(Difluoromethoxy)phenyl)-5-((2,2-dimethyl-5-phenyl-1,3-dioxan-5-yl)methoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-651; MMV1794873)**

****

Prepared according to General Procedure E from: **S1** (235 mg, 0.79 mmol) and **OSM-S-705** (176 mg, 0.79 mmol); the solvent was removed and the residue directly puriﬁed by ﬂash chromatography on silica (20–100% EtOAc in hexanes) to give **OSM-S-651** as a white solid (267 mg, 70%); **m.p.** 176–179 °C; **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.05 (s, 1H), 7.69 (s, 1H), 7.52–7.46 (m, 2H), 7.32–7.24 (m, 3H), 7.23 (t, *J* = 73.7 Hz, 1H), 7.16–7.09 (m, 2H), 6.92–6.85 (m, 2H), 4.83 (s, 2H), 3.87 (d, *J* = 12.0 Hz, 2H), 3.50–3.44 (m, 2H), 1.41 (s, 3H), 1.31 (s, 3H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 151.7 (t, *J* = 3.2 Hz), 147.3, 145.1, 144.2, 138.8, 135.3, 132.2, 128.2, 127.0, 126.4, 124.7, 117.6, 116.1 (t, *J* = 258.4 Hz), 108.9, 97.8, 71.6, 63.8, 40.6, 28.1, 19.3; ***m/z*** (ESI+) 505 ([M+Na]+, 100%); **HRMS** (ESI+) found 483.1837 ([M+H]+), C25H25F2N4O4+ requires 483.1838.

*CC(OC1)(C)OCC1(COC2=CN=CC3=NN=C(C4=CC=C(OC(F)F)C=C4)N32)C5=CC=CC=C5*

*InChI=1S/C25H24F2N4O4/c1-24(2)33-15-25(16-34-24,18-6-4-3-5-7-18)14-32-21-13-28-12-20-29-30-22(31(20)21)17-8-10-19(11-9-17)35-23(26)27/h3-13,23H,14-16H2,1-2H3*

**6-Chloro-*N*-(4-chlorophenyl)pyrazine-2-carboxamide (S36)**

****

Prepared according to General Procedure F from: 6-chloropyrazine-2-carboxylic acid (1.00 g, 6.31 mmol) and 4-chloroaniline (805 mg, 6.31 mmol) to give a pale brown solid (1.21 g); purified by flash chromatography on silica (12–100% EtOAc in hexanes) to give **S36** as pale brown crystals (853 mg, 40%); **m.p.** 148–151 °C (lit.5 145–146 °C); **1H NMR** (300 MHz, CDCl3) δ: 9.38 (br s, 2H), 8.82 (s, 1H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.36 (d, *J* = 8.8 Hz, 2H); ***m/z*** (ESI+) 290 ([M+Na]+, 67%), 555 ([2M+Na]+, 100%); **HRMS** (ESI+) found 289.9859 ([M+Na]+), C11H7Cl2N3ONa+ requires 289.9858. Spectroscopic data matched those in the literature.5

*O=C(NC1=CC=C(Cl)C=C1)C2=NC(Cl)=CN=C2*

*InChI=1S/C11H7Cl2N3O/c12-7-1-3-8(4-2-7)15-11(17)9-5-14-6-10(13)16-9/h1-6H,(H,15,17)*

***N*-(4-Chlorophenyl)-6-hydrazinylpyrazine-2-carboxamide (S37)**

****

Prepared according to General Procedure A from: **S36** (4.00 g, 14.9 mmol) to give **S37** as a yellow solid (3.45 g); carried forward without further purification; **1H NMR** (400 MHz, DMSO-d*6*) δ: 10.46 (s, 1H), 8.50 (s, 1H), 8.31 (s, 1H), 8.19 (s, 1H), 7.87 (d, *J* = 8.9 Hz, 2H), 7.45 (d, *J* = 8.9 Hz, 2H), 4.63 (br s, 2H); **13C NMR** (101 MHz, DMSO-d*6*) δ: 162.5, 155.0, 141.0, 137.1, 135.6, 129.8, 128.7, 127.8, 121.9.

*O=C(NC1=CC=C(Cl)C=C1)C2=NC(NN)=CN=C2*

*InChI=1S/C11H10ClN5O/c12-7-1-3-8(4-2-7)15-11(18)9-5-14-6-10(16-9)17-13/h1-6H,13H2,(H,15,18)(H,16,17)*

**(*E*)-*N*-(4-Chlorophenyl)-6-(2-(4-(difluoromethoxy)benzylidene)hydrazinyl)pyrazine-2-carboxamide (S38)**

****

Prepared according to General Procedure C from: **S37** (150 mg, 0.57 mmol) and 4-(difluoromethoxy)benzaldehyde (75.2 μL, 0.57 mmol) to give **S38** as a yellow powder (253 mg); carried forward without further purification; **1H NMR** (400 MHz, DMSO-d*6*) δ: 11.41 (s, 1H), 10.46 (s, 1H), 8.83 (s, 1H), 8.59 (s, 1H), 8.13 (s, 1H), 7.82 (dd, *J* 8.9, 2.9 Hz, 4H), 7.44 (d, *J* = 8.9 Hz, 2H), 7.26 (t, *J* = 73.8 Hz, 1H), 7.23 (d, *J* = 8.6 Hz, 2H); **13C NMR** (101 MHz, DMSO-d*6*) δ: 162.5, 151.8, 151.2, 142.3, 141.6, 137.2, 134.3, 133.6, 131.7, 129.0, 128.5, 128.2, 122.0, 119.1, 116.4 (t, *J* = 258.0 Hz).

*O=C(NC1=CC=C(Cl)C=C1)C2=NC(N/N=C/C3=CC=C(OC(F)F)C=C3)=CN=C2*

*InChI=1S/C19H14ClF2N5O2/c20-13-3-5-14(6-4-13)25-18(28)16-10-23-11-17(26-16)27-24-9-12-1-7-15(8-2-12)29-19(21)22/h1-11,19H,(H,25,28)(H,26,27)/b24-9+*

**(*E*)-*N*-(3-Chloro-4-fluorophenyl)-6-(2-(4-(difluoromethoxy)benzylidene)hydrazinyl)pyrazine-2-carboxamide (S39)**

****

Prepared according to General Procedure F from: 6-chloropyrazine-2-carboxylic acid (500 mg, 3.15 mmol) and 3-chloro-4-fluoroaniline (459 mg, 3.15 mmol) to give the amide product as a light brown solid (1.04 g); **1H NMR** (200 MHz, DMSO-d*6*) δ: 10.86 (s, 1H), 9.23 (s, 1H), 9.07 (s, 1H), 8.15 (dd, *J* = 6.8, 2.4 Hz, 1H), 7.94–7.77 (m, 1H), 7.44 (t, *J* = 9.1 Hz, 1H); the amide product (976 mg, 3.41 mmol) was subjected to General Procedure A to give the corresponding hydrazine as a yellow solid (644 mg); carried forward without further purification or characterization; the hydrazine product (325 mg, 1.15 mmol) was subjected to General Procedure C with 4-(difluoromethoxy)benzaldehyde (0.15 mL, 1.15 mmol) to give **S39** as a bright yellow solid (567 mg); carried forward without further purification or characterization.

*O=C(NC1=CC=C(F)C(Cl)=C1)C2=NC(N/N=C/C3=CC=C(OC(F)F)C=C3)=CN=C2*

*InChI=1S/C19H13ClF3N5O2/c20-14-7-12(3-6-15(14)21)26-18(29)16-9-24-10-17(27-16)28-25-8-11-1-4-13(5-2-11)30-19(22)23/h1-10,19H,(H,26,29)(H,27,28)/b25-8+*

**5-(2-Phenyl-2-((tetrahydro-2*H*-pyran-2-yl)oxy)ethoxy)-3-(6-(trifluoromethyl)pyridin-3-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (S40)**

****

Prepared according to General ProcedureE from: 5-chloro-3-(6-(trifluoromethyl)pyridin-3-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (404 mg, 1.35 mmol) and **S30** (300 mg, 1.35 mmol) to give a dark olive green sludge (706 mg); purified by flash chromatography on silica (25–100% EtOAc in hexanes) to give **S40** as a dark olive green powder (363 mg, 55%); carried forward without characterization.

*FC(C(C=C1)=NC=C1C2=NN=C3C=NC=C(OCC(OC4OCCCC4)C5=CC=CC=C5)N32)(F)F*

*InChI=1S/C24H22F3N5O3/c25-24(26,27)19-10-9-17(12-29-19)23-31-30-20-13-28-14-21(32(20)23)34-15-18(16-6-2-1-3-7-16)35-22-8-4-5-11-33-22/h1-3,6-7,9-10,12-14,18,22H,4-5,8,11,15H2*

# Synthesis and Characterization of Final Compounds

**(*R*)-4-(5-(2-(3,4-difluorophenyl)-2-methoxyethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)benzonitrile (OSM-S-218; MMV669844)**

****

Compound **OSM-S-218** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 9.07 (s, 1H), 7.93 (d, *J* = 8.3 Hz, 2H), 7.82 (d, *J* = 8.2 Hz, 2H), 7.34 (s, 1H), 7.14 (q, *J* = 8.6 Hz, 1H), 6.93–6.86 (m, 2H), 4.26 (quin, *J* = 7.7 Hz, 2H), 4.19 (d, *J* = 7.6 Hz, 1H), 3.12 (s, 3H); ***m/z*** (ESI+) 408 ([M+H]+, 100%).

*FC1=C(F)C=CC([C@@H](OC)COC2=CN=CC3=NN=C(C4=CC=C(C#N)C=C4)N32)=C1*

*InChI=1S/C21H15F2N5O2/c1-29-18(15-6-7-16(22)17(23)8-15)12-30-20-11-25-10-19-26-27-21(28(19)20)14-4-2-13(9-24)3-5-14/h2-8,10-11,18H,12H2,1H3/t18-/m0/s1*

**(*R*)-4-(5-(2-(difluoromethoxy)-2-(3,4-difluorophenyl)ethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)benzonitrile (OSM-S-377; MMV670652)**

****

Compound **OSM-S-377** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 9.11 (s, 1H), 7.93 (d, *J* = 8.3 Hz, 2H), 7.82 (d, *J* = 8.4 Hz, 2H), 7.39 (s, 1H), 7.14 (q, *J* = 8.7 Hz, 1H), 6.87–6.82 (m, 2H), 6.10 (t, *J* = 73.3 Hz), 5.19–5.16 (m, 1H), 4.45 (dd, *J* = 10.5, 6.5 Hz, 1H), 4.38 (dd, *J* = 10.5, 3.8 Hz, 1H); ***m/z*** (ESI+) 444 ([M+H]+, 100%).

*FC1=C(F)C=CC([C@@H](OC(F)F)COC2=CN=CC3=NN=C(C4=CC=C(C#N)C=C4)N32)=C1*

*InChI=1S/C21H13F4N5O2/c22-15-6-5-14(7-16(15)23)17(32-21(24)25)11-31-19-10-27-9-18-28-29-20(30(18)19)13-3-1-12(8-26)2-4-13/h1-7,9-10,17,21H,11H2/t17-/m0/s1*

**3-(4-Chlorophenyl)-5-(3,4-difluorophenethoxy)imidazo[1,5-*a*]pyrazine (OSM-S-274; MMV670250)**

****

Compound **OSM-S-274** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, DMSO-d*6*) δ: 8.76 (s, 1H), 7.92 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.23 (s, 1H), 7.21–7.16 (m, 1H), 6.89–6.83 (m, 1H), 6.72–6.71 (m, 1H), 4.45 (t, *J* = 6.2 Hz, 2H), 2.88 (t, *J* = 6.2 Hz, 2H); ***m/z*** (ESI+) 386 ([M+H]+, 100%).

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=CN=C(C4=CC=C(C=C4)Cl)N23*

*InChI=1S/C20H14ClF2N3O/c21-15-4-2-14(3-5-15)20-25-11-16-10-24-12-19(26(16)20)27-8-7-13-1-6-17(22)18(23)9-13/h1-6,9-12H,7-8H2*

**3-(4-Chlorophenyl)-5-(3,4-difluorophenethoxy)imidazo[1,2-*a*]pyrazine (OSM-S-273; MMV669846)**

**

Compound **OSM-S-273** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 8.80 (s, 1H), 7.63 (s, 1H), 7.37 (sapp, 4H), 7.34 (s, 1H), 6.98–6.93 (m, 1H), 6.51–6.47 (m, 2H), 4.34 (t, *J* = 6.0 Hz, 2H), 2.83 (t, *J* = 6.0 Hz, 2H); ***m/z*** (ESI+) 386 ([M+H]+, 100%).

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NC=C(C4=CC=C(C=C4)Cl)N23*

*InChI=1S/C20H14ClF2N3O/c21-15-4-2-14(3-5-15)18-10-25-19-11-24-12-20(26(18)19)27-8-7-13-1-6-16(22)17(23)9-13/h1-6,9-12H,7-8H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(3,4-difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-260; MMV675960)**

****

Prepared according to General Procedure E from: **S1** (159 mg, 0.54 mmol, 1.0 equiv.) and 2-(3,4-difluorophenyl)ethan-1-ol (98.0 mg, 0.62 mmol, 1.1 equiv.); purified by flash chromatography on silica (20% EtOH in hexanes) followed by recrystallization from EtOAc to give **OSM-S-260** as white needles (43.0 mg, 19%); **m.p.** 111–112 °C; **1H NMR** (200 MHz; DMSO-d*6*) δ: 9.05 (s, 1H), 7.79–7.73 (m, 2H), 7.60 (s, 1H), 7.36 (t, *J* = 73.6 Hz, 1H), 7.30–6.69 (m, 5H), 4.51 (t, *J* = 6.2, 2H), 2.90 (t, *J* = 6.2, 6.0 Hz, 2H); **13C NMR** (75 MHz; DMSO-d*6*) δ: 151.9, 147.4, 146.4, 145.4, 143.8, 135.1, 132.5, 125.3 (2C), 124.7, 117.5, 117.3, 117.0, 116.9, 116.1 (t, *J* = 256.7 Hz), 108.8, 70.6, 32.8; ***m/z*** (APCI+) 419 ([M+H]+, 100%); **HRMS** (APCI+) found 419.1122 ([M+H]+), C20H15F4N4O+ requires 419.1126.

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCCC4=CC=C(C(F)=C4)F)=CN=C3)F*

*InChI=1S/C20H14F4N4O2/c21-15-6-1-12(9-16(15)22)7-8-29-18-11-25-10-17-26-27-19(28(17)18)13-2-4-14(5-3-13)30-20(23)24/h1-6,9-11,20H,7-8H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(3,4-difluorophenethoxy)-8-methyl-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-X-063; MMV669541)**



Compound **OSM-X-063** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 7.66 (d, *J* = 8.6 Hz, 2H), 7.22 (d, *J* = 8.6 Hz, 2H), 7.14 (s, 1H),6.98–6.92 (m, 1H), 6.58 (t, *J* = 73.2 Hz, 1H), 6.51–6.47 (m, 2H), 4.33 (t, *J* = 6.2 Hz, 2H), 2.89 (s, 3H), 2.85 (t, *J* = 6.2 Hz, 2H); ***m/z*** (ESI+) 433 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCCC4=CC=C(C(F)=C4)F)=CN=C3C)F*

*InChI=1S/C21H16F4N4O2/c1-12-19-27-28-20(14-3-5-15(6-4-14)31-21(24)25)29(19)18(11-26-12)30-9-8-13-2-7-16(22)17(23)10-13/h2-7,10-11,21H,8-9H2,1H3*

**3-(4-(Difluoromethoxy)phenyl)-6-(3,4-difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-X-001; MMV670945)**



Compound **OSM-X-001** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 9.03 (s, 1H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.29 (s, 1H), 7.00–6.92 (m, 1H), 6.59 (t, *J* = 73.6 Hz, 1H), 6.51–6.46 (m, 2H), 4.40–4.38 (m, 2H), 2.89–2.88 (m, 2H) (*p*-phenyl CH2 obscured by solvent peak); ***m/z*** (ESI+) 419 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C=C(N=C3)OCCC4=CC=C(C(F)=C4)F)F*

*InChI=1S/C20H14F4N4O2/c21-15-6-1-12(9-16(15)22)7-8-29-18-11-28-17(10-25-18)26-27-19(28)13-2-4-14(5-3-13)30-20(23)24/h1-6,9-11,20H,7-8H2*

**3-(4-(Difluoromethoxy)phenyl)-6-(3,4-difluorophenethoxy)-[1,2,4]triazolo[4,3-*b*]pyridazine (OSM-X-061; MMV672939)**



Compound **OSM-X-061** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 8.46 (d, *J* = 8.8 Hz, 2H), 8.03 (d, *J* = 9.7 Hz, 1H), 7.28 (d, *J* = 8.8 Hz, 2H), 7.15–7.08 (m, 2H), 7.03–7.00 (m, 1H), 6.80 (d, *J* = 9.7 Hz, 1H), 6.61 (t, *J* = 73.3 Hz, 1H), 4.59 (t, *J* = 6.6 Hz, 2H), 3.13 (t, *J* = 6.6 Hz, 2H); ***m/z*** (ESI+) 419 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2N=C(C=C3)OCCC4=CC=C(C(F)=C4)F)F*

*InChI=1S/C20H14F4N4O2/c21-15-6-1-12(11-16(15)22)9-10-29-18-8-7-17-25-26-19(28(17)27-18)13-2-4-14(5-3-13)30-20(23)24/h1-8,11,20H,9-10H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(3,4-difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-8(7*H*)-one (OSM-X-055; MMV669025)**



Compound **OSM-X-055** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 7.70 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 8.4 Hz, 2H), 6.98–6.94 (m, 1H), 6.59 (t, *J* = 73.1 Hz, 1H), 6.54–6.52 (m, 2H), 6.25 (s, 1H), 4.01 (t, *J* = 6.4 Hz, 2H), 2.72 (t, *J* = 6.4 Hz, 2H); ***m/z*** (ESI+) 435 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCCC4=CC=C(C(F)=C4)F)=CNC3=O)F*

*InChI=1S/C20H14F4N4O3/c21-14-6-1-11(9-15(14)22)7-8-30-16-10-25-19(29)18-27-26-17(28(16)18)12-2-4-13(5-3-12)31-20(23)24/h1-6,9-10,20H,7-8H2,(H,25,29)*

**2-Amino-1-(3-(4-(difluoromethoxy)phenyl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl)ethan-1-one (OSM-X-077; MMV668962)**



Compound **OSM-X-077** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, DMSO-d*6*) δ: 8.17 (br s, 2H), 7.81 (t, *J* = 8.7 Hz, 2H), 7.38 (t, *J* = 8.7 Hz, 2H), 7.37 (t, *J* = 73.7 Hz, 1H), 4.93 (d, *J* = 11.7 Hz, 2H), 4.29–3.82 (m, 6H); ***m/z*** (ESI+) 324 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2CCN(C3)C(CN)=O)F*

*InChI=1S/C14H15F2N5O2/c15-14(16)23-10-3-1-9(2-4-10)13-19-18-11-8-20(12(22)7-17)5-6-21(11)13/h1-4,14H,5-8,17H2*

**3-(4-(Difluoromethoxy)phenyl)-5-phenoxy-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-579; MMV1581345)**

****

**OSM-S-579** was previously synthesized and characterized according to literature procedures.2

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OC4=CC=CC=C4)=CN=C3)F*

*InChI=1S/C18H12F2N4O2/c19-18(20)26-14-8-6-12(7-9-14)17-23-22-15-10-21-11-16(24(15)17)25-13-4-2-1-3-5-13/h1-11,18H*

**5-(Benzyloxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-368; MMV897697)**

****

**OSM-S-368** was previously synthesized and characterized according to literature procedures.2

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCC4=CC=CC=C4)=CN=C3)F*

*InChI=1S/C19H14F2N4O2/c20-19(21)27-15-8-6-14(7-9-15)18-24-23-16-10-22-11-17(25(16)18)26-12-13-4-2-1-3-5-13/h1-11,19H,12H2*

**3-(4-(Difluoromethoxy)phenyl)-5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-369; MMV897698)**

****

**OSM-S-369** was previously synthesized and characterized according to literature procedures.2

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCCC4=CC=CC=C4)=CN=C3)F*

*InChI=1S/C20H16F2N4O2/c21-20(22)28-16-8-6-15(7-9-16)19-25-24-17-12-23-13-18(26(17)19)27-11-10-14-4-2-1-3-5-14/h1-9,12-13,20H,10-11H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(3-phenylpropoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-578; MMV1581344)**



**OSM-S-578** was previously synthesized and characterized according to literature procedures.2

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCCCC4=CC=CC=C4)=CN=C3)F*

*InChI=1S/C21H18F2N4O2/c22-21(23)29-17-10-8-16(9-11-17)20-26-25-18-13-24-14-19(27(18)20)28-12-4-7-15-5-2-1-3-6-15/h1-3,5-6,8-11,13-14,21H,4,7,12H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(3-phenylpropyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-X-011; MMV669304)**



Compound **OSM-X-011** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 9.27 (s, 1H), 7.64 (s, 1H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.29–7.14 (m, 5H), 6.88 (d, *J* = 7.0 Hz, 2H), 6.63 (t, *J* = 72.8 Hz, 1H), 2.58 (t, *J* = 7.8 Hz, 2H), 2.32 (t, *J* = 7.2 Hz, 2H), 1.72–1.65 (m, 2H); ***m/z*** (ESI+) 381 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(CCCC4=CC=CC=C4)=CN=C3)F*

*InChI=1S/C21H18F2N4O/c22-21(23)28-18-11-9-16(10-12-18)20-26-25-19-14-24-13-17(27(19)20)8-4-7-15-5-2-1-3-6-15/h1-3,5-6,9-14,21H,4,7-8H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(phenethylthio)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-571; MMV1581336)**

****

**OSM-S-571** was previously synthesized and characterized according to literature procedures.6

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(SCCC4=CC=CC=C4)=CN=C3)F*

*InChI=1S/C20H16F2N4OS/c21-20(22)27-16-8-6-15(7-9-16)19-25-24-17-12-23-13-18(26(17)19)28-11-10-14-4-2-1-3-5-14/h1-9,12-13,20H,10-11H2*

**5-(Benzylsulfonyl)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-361; MMV693163)**

**

Prepared according to General Procedure D from: **S4** (100 mg, 0.24 mmol); purified by flash chromatography on silica (12–100% EtOAc in hexanes) to give **OSM-S-361** as a pale yellow powder (24.9 mg, 25%); **m.p.** 222–224 °C; **1H NMR** (300 MHz, CDCl3) δ: 9.56 (s, 1H), 8.41 (s, 1H), 7.76 (d, *J* = 8.6 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.44–7.18 (m, 3H), 6.88 (d, *J* = 7.4 Hz, 2H), 6.69 (t, *J* = 72.7 Hz, 1H), 4.02 (s, 2H); ***m/z*** (ESI–) 415 ([M–H]–, 100%); **HRMS** (ESI+) found 417.0831 ([M+H]+), C19H15F2N4O3S+ requires 417.0828.

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(S(CC4=CC=CC=C4)(=O)=O)=CN=C3)F*

*InChI=1S/C19H14F2N4O3S/c20-19(21)28-15-8-6-14(7-9-15)18-24-23-16-10-22-11-17(25(16)18)29(26,27)12-13-4-2-1-3-5-13/h1-11,19H,12H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(phenethylsulfonyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-365; MMV693166)**

**

Prepared according to General Procedure D from: **S6** (275 mg, 0.64 mmol); purified by flash chromatography on silica (12–100% EtOAc in hexanes) to give **OSM-S-365** as a white powder (179 mg, 65%); **m.p.** 142–143 °C; **1H NMR** (300 MHz, CDCl3) δ: 9.50 (s, 1H), 8.62 (s, 1H), 7.65 (d, *J* = 8.7 Hz, 2H), 7.39–7.11 (m, 6H), 6.89 (m, 1H), 6.61 (t, *J* = 72.8 Hz, 1H), 3.03 (ddd, *J* = 8.4, 6.1, 1.8 Hz, 2H), 2.88 (ddd, *J* = 8.3, 6.1, 1.8 Hz, 2H); **13C NMR** (75 MHz, CDCl3) δ: 153.4, 150.4, 147.3, 144.4, 141.5, 136.5, 130.2, 129.3, 128.8, 128.3, 126.8, 120.8, 118.9, 115.5 (t, *J* = 262.6 Hz), 54.6, 28.9; ***m/z*** (ESI+) 453 ([M+Na]+, 100%); **HRMS** (ESI+) found 431.0986 ([M+H]+), C20H17F2N4O3S+ requires 431.0984.

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(S(CCC4=CC=CC=C4)(=O)=O)=CN=C3)F*

*InChI=1S/C20H16F2N4O3S/c21-20(22)29-16-8-6-15(7-9-16)19-25-24-17-12-23-13-18(26(17)19)30(27,28)11-10-14-4-2-1-3-5-14/h1-9,12-13,20H,10-11H2*

***N*-(3-Chlorophenyl)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine-5-carboxamide (OSM-S-202; MMV669542)**



Prepared according to General Procedure D from: **S9** (150 mg, 0.36 mmol); purified by flash chromatography on silica (20–100% EtOAc in hexanes) to give **OSM-S-202** as a pearlescent white powder (60.0 mg, 39%); **m.p.** 257–258 °C; **1H NMR** (400 MHz, DMSO-d*6*) δ: 10.88 (br s, 1H), 9.66 (s, 1H), 8.30 (s, 1H), 7.63 (dapp, *J* = 8.7 Hz, 2H), 7.40 (t, *J* = 1.9 Hz, 1H), 7.29 (t, *J* = 8.1 Hz, 1H) 7.17 (d, *J* = 7.9 Hz, 1H), 7.15 (d, *J* = 7.9 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 2H), 7.11 (t, *J* = 73.6 Hz, 1H); **13C NMR** (101 MHz, DMSO-d*6*) δ: 157.4, 152.11 (t, *J* = 3.2 Hz), 146.8, 146.1, 145.7, 138.6, 132.8, 130.31, 130.25, 130.2, 124.33, 124.28, 124.0, 119.2, 118.2, 118.1, 115.9 (t, *J* = 258 Hz); ***m/z*** (ESI+) 438 ([M+Na]+, 100%); **HRMS** (APCI+) found 416.0718 ([M+H]+), C19H13ClF2N5O2+ requires 416.0720.

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(C(NC4=CC=CC(Cl)=C4)=O)=CN=C3)F*

*InChI=1S/C19H12ClF2N5O2/c20-12-2-1-3-13(8-12)24-18(28)15-9-23-10-16-25-26-17(27(15)16)11-4-6-14(7-5-11)29-19(21)22/h1-10,19H,(H,24,28)*

***N*-(3-Chloro-2-methylphenyl)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine-5-carboxamide (OSM-S-201; MMV675718)**



Prepared according to General Procedure D from: **S12** (200 mg, 0.46 mmol); purified by flash chromatography on silica (20–100% EtOAc in hexanes) to give **OSM-S-201** as a fine pearly white powder (140 mg, 71%); **m.p.** 211–212 °C; **1H NMR** (500 MHz, DMSO-d*6*) δ: 10.63 (br s, 1H), 9.66 (s, 1H), 8.38 (s, 1H), 7.70 (dapp, *J* = 8.7 Hz, 2H), 7.32 (dd, *J* = 1.0, 8.0 Hz, 1H), 7.32 (t, *J* = 73.6 Hz, 1H), 7.30 (dapp, *J* = 8.6 Hz, 2H), 7.14 (t, *J* = 8.0 Hz, 1H), 6.97 (dd, *J* = 1.2, 8.3 Hz, 1H), 2.22 (s, 3H); **13C NMR** (101 MHz, DMSO-d*6*) δ: 158.0, 152.1 (t, *J* = 3.5 Hz), 146.8, 146.1, 145.9, 136.1, 133.8, 130.50, 130.47, 130.3 (2C), 126.9, 126.7, 124.36, 124.31, 123.9, 118.5 (2C), 115.9 (t, *J* = 256.9 Hz), 14.9; ***m/z*** (ESI−) 428 ([M−H]−, 100%); **HRMS** (APCI+) found 430.0872 ([M+H]+), C20H15ClF2N5O2+ requires 430.0877.

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(C(NC4=CC=CC(Cl)=C4C)=O)=CN=C3)F*

*InChI=1S/C20H14ClF2N5O2/c1-11-14(21)3-2-4-15(11)25-19(29)16-9-24-10-17-26-27-18(28(16)17)12-5-7-13(8-6-12)30-20(22)23/h2-10,20H,1H3,(H,25,29)*

**3-(4-(Difluoromethoxy)phenyl)-*N*-(3,4-difluorophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine-5-carboxamide (OSM-X-031; MMV669850)**

**

Compound **OSM-X-031** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, DMSO-d*6*) δ: 10.92 (s, 1H), 9.66 (s, 1H), 8.29 (s, 1H), 7.62 (d, *J* = 8.6 Hz, 2H), 7.39–7.29 (m, 2H), 7.17–7.14 (m, 2H), 7.02–6.99 (m, 1H) (CHF2 triplet obscured under peaks between 7.39 and 6.99 ppm); ***m/z*** (ESI+) 418 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(C(NC4=CC(F)=C(C=C4)F)=O)=CN=C3)F*

*InChI=1S/C19H11F4N5O2/c20-13-6-3-11(7-14(13)21)25-18(29)15-8-24-9-16-26-27-17(28(15)16)10-1-4-12(5-2-10)30-19(22)23/h1-9,19H,(H,25,29)*

***N*-(3-Chlorobenzyl)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine-5-carboxamide (OSM-S-176, MMV668958)**



Compound **OSM-S-176** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, DMSO-d*6*) δ: 9.59 (s, 1H), 9.45 (t, *J* = 5.7 Hz, 1H), 8.19 (s, 1H), 7.62 (d, *J* = 8.6 Hz, 2H), 7.52–7.15 (m, 7H), 4.09 (d, *J* = 5.7 Hz, 2H); ***m/z*** (ESI+) 430 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(C(NCC4=CC=CC(Cl)=C4)=O)=CN=C3)F*

*InChI=1S/C20H14ClF2N5O2/c21-14-3-1-2-12(8-14)9-25-19(29)16-10-24-11-17-26-27-18(28(16)17)13-4-6-15(7-5-13)30-20(22)23/h1-8,10-11,20H,9H2,(H,25,29)*

***N*-(3,4-Difluorobenzyl)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine-5-carboxamide (OSM-X-012; MMV669543)**



Compound **OSM-X-012** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 9.40 (s, 1H), 8.06 (s, 1H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.19 (d, *J* = 8.6 Hz, 2H), 7.17–7.10 (m, 1H), 7.02–6.97 (m, 1H), 6.92–6.89 (m, 1H), 6.61 (t, *J* = 72.9 Hz, 1H), 6.59–6.58 (m, 1H), 4.23 (d, *J* = 5.8 Hz, 2H); ***m/z*** (ESI+) 432 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(C(NCC4=CC=C(C(F)=C4)F)=O)=CN=C3)F*

*InChI=1S/C20H13F4N5O2/c21-14-6-1-11(7-15(14)22)8-26-19(30)16-9-25-10-17-27-28-18(29(16)17)12-2-4-13(5-3-12)31-20(23)24/h1-7,9-10,20H,8H2,(H,26,30)*

**3-(4-(Difluoromethoxy)phenyl)-*N*-phenethyl-[1,2,4]triazolo[4,3-*a*]pyrazine-5-carboxamide (OSM-X-051; MMV669027)**

****

Compound **OSM-X-051** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 9.42 (s, 1H), 7.87 (s, 1H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.37–7.22 (m, 5H), 7.16 (d, *J* = 7.2 Hz, 2H), 6.59 (t, *J* = 73.2 Hz, 1H), 6.03–6.02 (m, 1H), 3.44 (q, *J* = 6.5 Hz, 2H), 2.78 (t, *J* = 6.8 Hz, 2H); ***m/z*** (ESI) 410 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(C(NCCC4=CC=CC=C4)=O)=CN=C3)F*

*InChI=1S/C21H17F2N5O2/c22-21(23)30-16-8-6-15(7-9-16)19-27-26-18-13-24-12-17(28(18)19)20(29)25-11-10-14-4-2-1-3-5-14/h1-9,12-13,21H,10-11H2,(H,25,29)*

***N*-(3-Chlorobenzyl)-3-(4-(difluoromethoxy)phenyl)-*N*-methyl-[1,2,4]triazolo[4,3-*a*]pyrazine-5-carboxamide (OSM-X-036; MMV669105)**



Compound **OSM-X-036** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3, mixture of amide rotamers) δ: 9.44, 9.41 (s, 1H), 7.94, 7.91 (s, 1H), 7.66–7.62 (m, 2H), 7.36–7.25 (m, 4H), 7.14–7.09 (m, 1H), 7.00, 6.98 (s, 1H), 6.82–6.44 (m, 1H), 4.28 (br s, 1H), 3.75 (br s, 1H), 2.55, 2.52 (s, 3H); ***m/z*** (ESI) 444 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(C(N(C)CC4=CC=CC(Cl)=C4)=O)=CN=C3)F*

*InChI=1S/C21H16ClF2N5O2/c1-28(12-13-3-2-4-15(22)9-13)20(30)17-10-25-11-18-26-27-19(29(17)18)14-5-7-16(8-6-14)31-21(23)24/h2-11,21H,12H2,1H3*

***N*-(3-(4-Cyanophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)-2-(3,4-difluorophenyl)acetamide (OSM-W-6)**



A flask was charged with **S14** (100 mg, 0.39 mmol, 1.0 equiv.), 2-(3,4-difluorophenyl)acetamide (80.4 mg, 0.47 mmol, 1.2 equiv.), Pd2(dba)3 (72.0 mg, 0.08 mmol, 0.2 equiv.), Xantphos (46.0 mg, 0.08 mmol, 0.2 equiv.) and Cs2CO3 (381 mg, 1.17 mmol, 3.0 equiv.) then backfilled with N2. Anhydrous 1,4-dioxane (10 mL) was added and the mixture heated at 105 °C until completion as indicated by TLC. The reaction was cooled to rt, the solvent was removed and the residue directly purified by flash chromatography on silica (10% methanol in dichloromethane) to give **OSM-W-6** (15.7 mg, 10%); **1H NMR** (500 MHz, CDCl3) δ 9.36 (s, 1H), 8.03 (s, 1H), 7.88–7.81 (m, 2H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.20–7.11 (m, 2H), 6.80 (ddd, *J* = 10.1, 7.3, 2.2 Hz, 1H), 6.69 (d, *J* = 8.4 Hz, 1H), 3.25 (s, 2H); **13C NMR** (126 MHz, CDCI3) δ: 170.1, 148.5 (dd, *J* = 245.2, 12.8 Hz), 147.3 (dd, *J* = 244.9, 12.7 Hz), 147.1, 145.9, 142.1, 131.6, 131.3, 131.0, 126.9 (dd, *J* = 6.5, 3.4 Hz), 126.8, 125.4, 118.1, 118.0 (d, *J* = 17.3 Hz), 116.8 (d, *J* = 17.0 Hz), 112.4, 39.5; ***m/z*** (ESI+) 391 ([M+H]+, 100%).

*O=C(CC1=CC=C(C(F)=C1)F)NC2=CN=CC3=NN=C(C(C=C4)=CC=C4C#N)N32*

*InChI=1S/C20H12F2N6O/c21-15-6-3-13(7-16(15)22)8-19(29)25-17-10-24-11-18-26-27-20(28(17)18)14-4-1-12(9-23)2-5-14/h1-7,10-11H,8H2,(H,25,29)*

**3-(4-Chlorophenyl)-5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-293; MMV663915)**

**

Prepared according to General Procedure E from: **S16** (300 mg, 1.13 mmol) and 2-phenylethanol (136 mL, 1.13 mmol); puriﬁed by ﬂash chromatography on silica (12–100% EtOAc in hexanes) to give **OSM-S-293** as a yellow powder (279 mg, 70%); **m.p.** 128–130 °C; **1H NMR** (300 MHz, CDCl3) δ: 8.97 (s, 1H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.38 (d, *J* = 8.5 Hz, 2H), 7.30 (s, 1H), 7.25–7.16 (m, 3H), 7.01–6.75 (m, 2H), 4.44 (t, *J* = 6.5 Hz, 2H), 2.95 (t, *J* = 6.5 Hz, 2H); **13C NMR** (75 MHz, DMSO-d*6*) δ: 147.4, 145.3, 143.8, 137.2, 135.0, 134.7, 132.4, 128.6, 128.2, 127.6, 126.7, 126.4, 108.9, 71.2, 33.8; ***m/z*** (ESI+) 373 ([M+Na]+, 100%), 723 ([2M+Na]+, 89%); **HRMS** (ESI+) found 373.0825 ([M+Na]+), C19H15ClN4ONa+ requires 373.0826.

*ClC(C=C1)=CC=C1C2=NN=C3N2C(OCCC4=CC=CC=C4)=CN=C3*

*InChI=1S/C19H15ClN4O/c20-16-8-6-15(7-9-16)19-23-22-17-12-21-13-18(24(17)19)25-11-10-14-4-2-1-3-5-14/h1-9,12-13H,10-11H2*

**3-(4-Chlorophenyl)-5-(4-fluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-W-10)**

**

Prepared according to General Procedure E from: **S16** (265 mg, 1.00 mmol, 1.0 equiv.) and 2-(4-fluorophenyl)ethan-1-ol (0.15 mL, 1.20 mmol, 1.2 equiv.); purified by flash chromatography on silica (25–100% ethyl acetate in hexanes) to give **OSM-W-10** (186 mg, 50%); **m.p.** 128–130 °C; **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.05 (s, 1H), 7.72 (d, *J* = 8.7 Hz, 2H), 7.61 (s, 1H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.00 (t, *J* = 8.9 Hz, 2H), 6.93 (dd, *J* = 8.8, 5.7 Hz, 2H), 4.50 (t, *J* = 6.4 Hz, 2H), 2.89 (t, *J* = 6.3 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 160.4, 147.9, 145.8, 144.3, 135.5, 135.2, 133.9, 132.9, 130.9 (d, *J* = 7.9 Hz), 128.1, 115.3 (d, *J* = 20.9 Hz), 109.4, 71.5, 33.4.

*FC(C=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=C(C=C4)Cl)N23*

*InChI=1S/C19H14ClFN4O/c20-15-5-3-14(4-6-15)19-24-23-17-11-22-12-18(25(17)19)26-10-9-13-1-7-16(21)8-2-13/h1-8,11-12H,9-10H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(3-methoxyphenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-383; MMV897711)**



Prepared according to General Procedure E from: **S1** (250 mg, 0.84 mmol) and 3-methoxyphenethyl alcohol (0.12 mL, 0.84 mmol); the solvent was removed and the residue directly puriﬁed by ﬂash chromatography on silica (25–100% EtOAc in hexanes) to give **OSM-S-383** as an orange powder (130 mg, 37%); **m.p.** 107–112 °C; **1H NMR** (300 MHz, CDCl3) δ: 9.00 (s, 1H), 7.63 (d, *J* = 8.8 Hz, 2H), 7.31 (s, 1H), 7.15 (dapp, *J* = 8.5 Hz, 3H), 6.79–6.70 (m, 1H), 6.58 (t, *J* = 73.4 Hz, 1H), 6.51–6.45 (m, 2H), 4.44 (t, *J* = 6.5 Hz, 2H), 3.75 (s, 3H), 2.92 (t, *J* = 6.5 Hz, 2H); **13C NMR** (75 MHz, CDCl3) δ: 160.0, 152.6, 147.9, 146.4, 144.0, 137.8, 136.5, 132.5, 129.8, 124.9, 120.8, 118.6, 115.8 (t, *J* = 260.8 Hz), 114.9, 111.9, 108.5, 71.1, 55.3, 34.5; ***m/z*** (ESI+) 435 ([M+Na]+, 100%); **HRMS** (ESI+) found 435.1241 ([M+Na]+), C21H18F2N4O3Na+ requires 435.1239.

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCCC4=CC=CC(OC)=C4)=CN=C3)F*

*InChI=1S/C21H18F2N4O3/c1-28-17-4-2-3-14(11-17)9-10-29-19-13-24-12-18-25-26-20(27(18)19)15-5-7-16(8-6-15)30-21(22)23/h2-8,11-13,21H,9-10H2,1H3*

**3-(4-(Difluoromethoxy)phenyl)-5-(4-methoxyphenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-384; MMV897712)**



Prepared according to General Procedure E from: **S1** (250 mg, 0.84 mmol) and 4-methoxyphenethyl alcohol (128 mg, 0.84 mmol); the solvent was removed and the residue directly puriﬁed by ﬂash chromatography on silica (25–100% EtOAc in hexanes) to give **OSM-S-384** as an orange powder (138 mg, 40%); **m.p.** 134–138 °C; **1H NMR** (300 MHz, CDCl3) δ: 9.00 (s, 1H), 7.66 (d, *J* = 8.7 Hz, 2H), 7.30 (s, 1H), 7.20 (d, *J* = 8.6 Hz, 2H), 6.81–6.69 (m, 4H), 6.58 (t, *J* = 73.3 Hz, 1H), 4.39 (t, *J* = 6.5 Hz, 2H), 3.76 (s, 3H), 2.88 (t, *J* = 6.5 Hz, 2H); **13C NMR** (75 MHz, CDCl3) δ: 158.8, 152.5, 147.9, 146.4, 144.0, 136.5, 132.6, 129.6, 128.2, 125.0, 118.7, 115.7 (t, *J* = 261.1 Hz), 114.2, 108.4, 71.6, 55.4, 33.8; ***m/z*** (ESI+) 435 ([M+Na]+, 100%); **HRMS** (ESI+) found 435.1241 ([M+Na]+), C21H18F2N4O3Na+ requires 435.1239.

*COC(C=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=C(OC(F)F)C=C4)N23*

*InChI=1S/C21H18F2N4O3/c1-28-16-6-2-14(3-7-16)10-11-29-19-13-24-12-18-25-26-20(27(18)19)15-4-8-17(9-5-15)30-21(22)23/h2-9,12-13,21H,10-11H2,1H3*

**3-(4-(Difluoromethoxy)phenyl)-5-(2-(thiophen-2-yl)ethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-608; MMV1634428)**

****

Prepared according to General Procedure E from: **S1** (155 mg, 0.522 mmol) and 2-(thiophen-2-yl)ethan-1-ol (67.0 mg, 0.522 mmol); the solvent was removed and the residue directly puriﬁed by ﬂash chromatography on silica (20–100% EtOAc in hexanes) to give **OSM-S-608** as a white solid (72.1 mg, 35%); **m.p.** 129–132 °C; **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.04 (s, 1H), 7.80–7.74 (m, 2H), 7.61 (s, 1H), 7.38 (dd, *J* = 5.0, 3.0 Hz, 1H), 7.33 (t, *J* = 73.7 Hz, 1H), 7.26 (d, *J* = 8.6 Hz, 2H), 6.95 (dd, *J* = 2.9, 1.3 Hz, 1H), 6.77 (dd, *J* = 5.0, 1.2 Hz, 1H), 4.50 (t, *J* = 6.5 Hz, 2H), 2.91 (t, *J* = 6.5 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 151.9 (t, *J* = 3.3 Hz), 147.4, 145.5, 143.9, 137.4, 135.0, 132.6, 128.3, 125.8, 124.7, 121.8, 117.5, 116.1 (t, *J* = 258.2 Hz), 108.8, 70.6, 28.5; ***m/z*** (ESI+) 411 ([M+Na]+, 100%); **HRMS** (ESI+) found 411.0698 ([M+Na]+), C18H14F2N4O2SNa+ requires 411.0698.

*FC(F)OC(C=C1)=CC=C1C2=NN=C3C=NC=C(OCCC4=CC=CS4)N32*

*InChI=1S/C18H14F2N4O2S/c19-18(20)26-13-5-3-12(4-6-13)17-23-22-15-10-21-11-16(24(15)17)25-8-7-14-2-1-9-27-14/h1-6,9-11,18H,7-8H2*

**5-(2-(Cuban-1-yl)ethoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-371; MMV897700)**



**OSM-S-371** was previously synthesized and characterized according to literature procedures.2

*FC(F)OC(C=C1)=CC=C1C2=NN=C3C=NC=C(OCCC45C6C7C4C8C5C6C87)N32*

*InChI=1S/C22H18F2N4O2/c23-21(24)30-10-3-1-9(2-4-10)20-27-26-11-7-25-8-12(28(11)20)29-6-5-22-17-14-13-15(17)19(22)16(13)18(14)22/h1-4,7-8,13-19,21H,5-6H2*

**4-(2-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)ethyl)morpholine (OSM-S-498; MMV1577575)**



**OSM-S-498** was previously synthesized and characterized according to literature procedures.2

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCCN4CCOCC4)=CN=C3)F*

*InChI=1S/C18H19F2N5O3/c19-18(20)28-14-3-1-13(2-4-14)17-23-22-15-11-21-12-16(25(15)17)27-10-7-24-5-8-26-9-6-24/h1-4,11-12,18H,5-10H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(2-(piperidin-1-yl)ethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-499; MMV1577576)**



**OSM-S-499** was previously synthesized and characterized according to literature procedures.2

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCCN4CCCCC4)=CN=C3)F*

*InChI=1S/C19H21F2N5O2/c20-19(21)28-15-6-4-14(5-7-15)18-24-23-16-12-22-13-17(26(16)18)27-11-10-25-8-2-1-3-9-25/h4-7,12-13,19H,1-3,8-11H2*

**5-(3,4-Difluorophenethoxy)-3-phenyl-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-415; MMV1557949)**

**

Prepared according to General Procedure E from: **S19** (150 mg, 0.65 mmol) and 2-(3,4-diﬂuorophenyl)ethan-1-ol (103 mg, 0.65 mmol); puriﬁed by ﬂash chromatography on silica (25–100% EtOAc in hexanes) to give **OSM-S-415** as a yellow powder (16.4 mg, 7%); **m.p.** 100–110 °C; **1H NMR** (500 MHz, CDCl3) δ: 9.03 (s, 1H), 7.70 (ddd, *J* = 7.5, 4.2, 1.6 Hz, 2H), 7.66–7.38 (m, 3H), 7.27 (s, 1H), 7.06–6.82 (m, 1H), 6.41 (ddd, *J* = 13.3, 6.8, 4.6 Hz, 2H), 4.37 (t, *J* = 6.0 Hz, 2H), 2.86 (t, *J* = 6.0 Hz, 2H); **13C NMR** (126 MHz, CDCl3) δ: 150.2 (dd, *J* = 248.7, 12.7 Hz), 149.5 (dd, *J* = 247.9, 12.5 Hz), 147.8, 147.2, 144.0, 136.8, 133.6 (dd, *J* = 5.4, 4.1 Hz), 131.0, 130.9, 130.3, 128.0, 124.6 (dd, *J* = 5.9, 3.6 Hz), 117.8 (d, *J* = 17.2 Hz), 117.4 (d, *J* = 17.1 Hz), 108.2, 71.1, 34.0; ***m/z*** (ESI+) 375 ([M+Na]+, 100%); **HRMS** (ESI+) found 375.1031 ([M+Na]+), C19H14F2N4ONa+ requires 375.1028.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=CC=C4)N23*

*InChI=1S/C19H14F2N4O/c20-15-7-6-13(10-16(15)21)8-9-26-18-12-22-11-17-23-24-19(25(17)18)14-4-2-1-3-5-14/h1-7,10-12H,8-9H2*

**2-Chloro-5-(5-(3,4-difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)aniline (OSM-S-548; MMV1580433)**



Prepared according to General Procedure G from: **OSM-S-432** (107 mg, 0.30 mmol) and (3-amino-4-chlorophenyl)boronic acid (67.0 mg, 0.39 mmol) to give **OSM-S-548** as an off-white solid (110 mg, 91%); **m.p.** 165–168 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.02 (s, 1H), 7.55 (s, 1H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.18 (dt, *J* = 10.8, 8.5 Hz, 1H), 7.14 (d, *J* = 2.1 Hz, 1H), 6.96 (ddd, *J* = 12.0, 7.9, 2.1 Hz, 1H), 6.85 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.74–6.68 (m, 1H), 5.60 (s, 2H), 4.49 (t, *J* = 6.2 Hz, 2H), 2.95 (t, *J* = 6.2 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 149.6 (dd, *J* = 109.1, 12.4 Hz), 147.6 (dd, *J* = 107.7, 12.5 Hz), 147.3, 146.0, 144.0, 143.7, 135.3–135.2 (m), 135.1, 128.1, 127.1, 125.5 (dd, *J* = 6.3, 3.3 Hz), 119.1, 118.5, 117.51, 117.51 (d, *J* = 17.0 Hz), 116.8 (d, *J* = 16.7 Hz), 108.7, 70.7, 33.0; ***m/z*** (ESI+) 424 ([M+Na]+, 100%); **HRMS** (ESI+) found 402.0926 ([M+H]+), C19H15ClF2N5O+ requires 402.0928.

*ClC1=CC=C(C=C1N)C2=NN=C3N2C(OCCC4=CC=C(C(F)=C4)F)=CN=C3 InChI=1S/C19H14ClF2N5O/c20-13-3-2-12(8-16(13)23)19-26-25-17-9-24-10-18(27(17)19)28-6-5-11-1-4-14(21)15(22)7-11/h1-4,7-10H,5-6,23H2*

**5-(5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-2-fluoroaniline (OSM-S-585; MMV1581334)**



Prepared according to General Procedure G from: **OSM-S-432** (83.0 mg, 0.23 mmol) and 3-amino-4-fluorophenylboronic acid (47.0 mg, 0.31 mmol) to give **OSM-S-585** as an off-white solid (27.0 mg, 30%, contains 0.8% DMA); **m.p.** 161–164 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.01 (s, 1H), 7.54 (s, 1H), 7.20 (dt, *J* = 10.7, 8.5 Hz, 1H), 7.15–7.09 (m, 1H), 7.13–7.05 (m, 1H), 6.97 (ddd, *J* = 11.9, 7.9, 2.1 Hz, 1H), 6.84 (ddd, *J* = 8.3, 4.4, 2.2 Hz, 1H), 6.73 (ddd, *J* = 9.1, 4.2, 2.1 Hz, 1H), 5.39 (s, 2H), 4.47 (t, *J* = 6.2 Hz, 2H), 2.94 (t, *J* = 6.2 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 152.3, 150.4, 149.6 (dd, *J* = 104.8, 12.6 Hz), 148.4–146.9 (m), 147.2, 146.1, 143.7, 135.7 (d, *J* = 13.5 Hz), 135.23 (dd, *J* = 5.9, 3.6 Hz), 135.17, 125.5 (dd, *J* = 6.4, 3.3 Hz), 124.1 (d, *J* = 3.1 Hz), 118.8–118.6 (m), 117.6 (d, *J* = 17.0 Hz), 116.9 (d, *J* = 16.7 Hz), 114.1 (d, *J* = 19.2 Hz), 108.6, 70.7, 33.0; ***m/z*** (ESI+) 408 ([M+Na]+, 100%); **HRMS** (ESI+) found 386.1217 ([M+H]+), C19H15F3N5O+ requires 386.1223.

*FC1=CC=C(C=C1N)C2=NN=C3N2C(OCCC4=CC=C(C(F)=C4)F)=CN=C3*

*InChI=1S/C19H14F3N5O/c20-13-3-1-11(7-15(13)22)5-6-28-18-10-24-9-17-25-26-19(27(17)18)12-2-4-14(21)16(23)8-12/h1-4,7-10H,5-6,23H2*

**3-(5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)aniline (OSM-S-549; MMV1580434)**



Prepared according to General Procedure G from: **OSM-S-432** (89.0 mg, 0.25 mmol) and (3-aminophenyl)boronic acid (45.0 mg, 0.33 mmol) to give **OSM-S-549** as an off-white solid (92 mg, 93%); **m.p.** 150–152 °C; **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.00 (s, 1H), 7.51 (s, 1H), 7.23–7.12 (m, 2H), 6.97–6.89 (m, 2H), 6.85–6.80 (m, 1H), 6.76 (dd, *J* = 7.9, 2.3 Hz, 1H), 6.69–6.62 (m, 1H), 5.33 (s, 2H), 4.45 (t, *J* = 6.1 Hz, 2H), 2.91 (t, *J* = 6.1 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 149.5 (dd, *J* = 99.2, 12.5 Hz), 148.0, 147.6 (dd, *J* = 98.0, 12.6 Hz), 147.2, 146.9, 143.7, 135.3 (dd, *J* = 6.0, 3.7 Hz), 135.2, 128.3, 128.0, 125.7 (dd, *J* = 6.3, 3.3 Hz), 118.3, 117.7 (d, *J* = 16.8 Hz), 116.9 (d, *J* = 16.7 Hz), 116.2, 115.0, 108.6, 70.9, 33.1; ***m/z*** (ESI+) 390 ([M+Na]+, 100%); **HRMS** (ESI+) found 368.1313 ([M+H]+), C19H16F2N5O+ requires 368.1317.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=CC(N)=C4)N23*

*InChI=1S/C19H15F2N5O/c20-15-5-4-12(8-16(15)21)6-7-27-18-11-23-10-17-24-25-19(26(17)18)13-2-1-3-14(22)9-13/h1-5,8-11H,6-7,22H2*

**4-(5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)aniline (OSM-S-526; MMV1579342)**

**

Prepared according to General Procedure G from: **OSM-S-432** (105 mg, 0.30 mmol) and 4-aminophenylboronic acid hydrochloride (66.7 mg, 0.39 mmol) to give **OSM-S-526** as an off-white foam (63.0 mg, 58%); **m.p.** 161–164 °C; **1H NMR** (500 MHz, DMSO-d*6*) δ: 8.95 (s, 1H), 7.46 (s, 1H), 7.40–7.34 (m, 2H), 7.19 (dt, *J* = 10.8, 8.5 Hz, 1H), 6.95 (ddd, *J* = 11.9, 7.9, 2.1 Hz, 1H), 6.81–6.72 (m, 1H), 6.70–6.64 (m, 2H), 5.54 (s, 2H), 4.46 (t, *J* = 6.2 Hz, 2H), 2.93 (t, *J* = 6.2 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 149.6 (dd, *J* = 100.3, 12.5 Hz), 147.6 (dd, *J* = 99.3, 12.6 Hz), 147.3, 147.1, 143.9, 135.3 (dd, *J* = 6.0, 3.7 Hz), 135.2, 131.7, 125.7 (dd, *J* = 6.2, 3.3 Hz), 117.7 (d, *J* = 16.9 Hz), 116.9 (d, *J* = 16.6 Hz), 114.3, 112.4, 108.3, 70.8, 33.1; ***m/z*** (ESI+) 390 ([M+Na]+, 100%); **HRMS** (ESI+) found 368.1313 ([M+H]+), C19H16F2N5O+ requires 368.1317.

*NC(C=C1)=CC=C1C2=NN=C3N2C(OCCC4=CC=C(C(F)=C4)F)=CN=C3*

*InChI=1S/C19H15F2N5O/c20-15-6-1-12(9-16(15)21)7-8-27-18-11-23-10-17-24-25-19(26(17)18)13-2-4-14(22)5-3-13/h1-6,9-11H,7-8,22H2*

**3-(5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-*N*,*N*-dimethylaniline (OSM-S-545; MMV1580430)**



Prepared according to General Procedure G from: **OSM-S-432** (101 mg, 0.28 mmol) and 3-(dimethylamino)phenylboronic acid (60.9 mg, 0.37 mmol) to give **OSM-S-545** as a grey solid (108 mg, 96%); **m.p.** 137–140 °C; **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.02 (s, 1H), 7.53 (s, 1H), 7.30 (t, *J* = 7.9 Hz, 1H), 7.13 (dt, *J* = 11.0, 8.5 Hz, 1H), 7.04 (t, *J* = 2.0 Hz, 1H), 6.93 (ddd, *J* = 23.8, 8.1, 2.1 Hz, 3H), 6.73 (ddd, *J* = 11.8, 7.9, 2.2 Hz, 1H), 6.67–6.60 (m, 1H), 4.46 (t, *J* = 6.0 Hz, 2H), 2.96 (s, 6H), 2.83 (t, *J* = 5.9 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 149.6, 149.5 (dd, *J* = 99.1, 12.6 Hz), 148.0–147.0 (m), 147.2, 146.9, 143.8, 135.3 (dd, *J* = 6.0, 3.7 Hz), 135.0, 128.5, 128.0, 125.4 (dd, *J* = 6.5, 3.3 Hz), 118.6, 117.6 (d, *J* = 16.8 Hz), 116.8 (d, *J* = 16.7 Hz), 114.6, 113.4, 108.7, 70.7, 40.1, 33.1; ***m/z*** (ESI+) 418 ([M+Na]+, 100%); **HRMS** (ESI+) found 396.1627 ([M+H]+), C21H20F2N5O+ requires 396.1630.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=CC(N(C)C)=C4)N23*

*InChI=1S/C21H19F2N5O/c1-27(2)16-5-3-4-15(11-16)21-26-25-19-12-24-13-20(28(19)21)29-9-8-14-6-7-17(22)18(23)10-14/h3-7,10-13H,8-9H2,1-2H3*

**4-(5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-*N*,*N*-dimethylaniline (OSM-S-531; MMV1579351)**



Prepared according to General Procedure G from: **OSM-S-432** (98.0 mg, 0.28 mmol) and 4-(dimethylamino)phenylboronic acid hydrochloride (74.0 mg, 0.36 mmol) to give **OSM-S-531** as an off-white solid (85.0 mg, 77%); **m.p.** 177–179 °C; **1H NMR** (300 MHz, CDCl3) δ: 8.97 (s, 1H), 7.59–7.49 (m, 2H), 7.21 (s, 1H), 6.93 (dt, *J* = 10.4, 8.1 Hz, 1H), 6.82–6.71 (m, 2H), 6.56–6.44 (m, 2H), 4.37 (t, *J* = 6.0 Hz, 2H), 3.04 (s, 6H), 2.94 (t, *J* = 6.0 Hz, 2H); **13C NMR** (126 MHz, CDCl3) δ: 151.4, 150.8 (dd, *J* = 90.4, 12.6 Hz), 148.8 (dd, *J* = 89.4, 12.6 Hz), 148.1, 147.8, 144.2, 136.9, 133.9 (dd, *J* = 5.7, 4.0 Hz), 131.9, 124.7 (dd, *J* = 6.2, 3.6 Hz), 117.9 (d, *J* = 17.1 Hz), 117.3 (d, *J* = 17.0 Hz), 111.3, 108.0, 70.9, 40.6, 34.0 (one carbon signal is overlapping or obscured); ***m/z*** (ESI+) 418 ([M+Na]+, 100%); **HRMS** (ESI+) found 396.16240 ([M+H]+), C21H20F2N5O+ requires 396.1630.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=C(C=C4)N(C)C)N23*

*InChI=1S/C21H19F2N5O/c1-27(2)16-6-4-15(5-7-16)21-26-25-19-12-24-13-20(28(19)21)29-10-9-14-3-8-17(22)18(23)11-14/h3-8,11-13H,9-10H2,1-2H3*

**3-(5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)benzoic acid (OSM-S-552; MMV1580437)**



Prepared according to General Procedure G from: **OSM-S-432** (88.0 mg, 0.25 mmol) and 3-boronobenzoic acid (54.0 mg, 0.32 mmol); purified by flash chromatography on silica (0–5% MeOH in EtOAc, then 0.2% formic acid in 5% MeOH in EtOAc) to give **OSM-S-552** as an off-white solid (67.0 mg, 68%, contains 1.2% EtOAc); **m.p.** 223–225 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*) δ: 13.16 (s, 1H), 9.06 (s, 1H), 8.30 (t, *J* = 1.7 Hz, 1H), 8.08 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.96 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.65–7.58 (m, 2H), 7.14 (dt, *J* = 10.8, 8.5 Hz, 1H), 6.78 (ddd, *J* = 11.9, 7.9, 2.2 Hz, 1H), 6.69 (ddd, *J* = 8.8, 4.3, 1.9 Hz, 1H), 4.51 (t, *J* = 6.3 Hz, 2H), 2.87 (t, *J* = 6.3 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 166.8, 149.5 (dd, *J* = 104.0, 12.4 Hz), 147.6 (dd, *J* = 103.1, 12.4 Hz), 147.5, 145.6, 143.8, 135.1, 134.9 (dd, *J* = 6.1, 3.6 Hz), 134.8, 131.5, 130.33, 130.31, 128.3, 128.0, 125.2 (dd, *J* = 6.4, 3.3 Hz), 117.4 (d, *J* = 16.9 Hz), 116.9 (d, *J* = 16.7 Hz), 109.0, 70.6, 32.7; ***m/z*** (ESI+) 419 ([M+Na]+, 100%); **HRMS** (ESI+) found 397.1100 ([M+H]+), C20H15F2N4O3+ requires 397.1107.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=CC(C(O)=O)=C4)N23*

*InChI=1S/C20H14F2N4O3/c21-15-5-4-12(8-16(15)22)6-7-29-18-11-23-10-17-24-25-19(26(17)18)13-2-1-3-14(9-13)20(27)28/h1-5,8-11H,6-7H2,(H,27,28)*

**4-(5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)benzoic acid (OSM-S-551; MMV1580436)**

**

Prepared according to General Procedure G from: **OSM-S-432** (83.0 mg, 0.23 mmol) and 4-boronobenzoic acid (50.0 mg, 0.30 mmol); purified by flash chromatography on silica (0–5% MeOH in EtOAc, then 0.2% formic acid in 5% MeOH in EtOAc) to give **OSM-S-551** as an off-white solid (63.0 mg, 68%); **m.p.** 223–225 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*) δ: 13.19 (s, 1H), 9.08 (s, 1H), 8.00 (d, *J* = 7.9 Hz, 2H), 7.81 (d, *J* = 7.9 Hz, 2H), 7.63 (s, 1H), 7.15 (q, *J* = 9.2 Hz, 1H), 6.88 (t, *J* = 10.0 Hz, 1H), 6.72 (s, 1H), 4.53 (t, *J* = 6.2 Hz, 2H), 2.89 (t, *J* = 6.2 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 166.9, 149.5 (dd, *J* = 109.2, 12.5 Hz), 147.6 (dd, *J* = 108.2, 12.5 Hz), 147.5, 145.6, 143.7, 135.04, 134.99 (d, *J* = 3.7 Hz), 131.8, 131.7, 130.8, 128.3, 125.2 (dd, *J* = 6.3, 3.3 Hz), 117.4 (d, *J* = 17.0 Hz), 116.9 (d, *J* = 16.7 Hz), 109.1, 70.6, 32.7; ***m/z*** (ESI–) 396 ([M–H]–, 100%); **HRMS** (ESI+) found 419.0929 ([M+Na]+), C20H14F2N4O3Na+ requires 419.0929.

*O=C(C(C=C1)=CC=C1C2=NN=C3N2C(OCCC4=CC=C(C(F)=C4)F)=CN=C3)O*

*InChI=1S/C20H14F2N4O3/c21-15-6-1-12(9-16(15)22)7-8-29-18-11-23-10-17-24-25-19(26(17)18)13-2-4-14(5-3-13)20(27)28/h1-6,9-11H,7-8H2,(H,27,28)*

**3-(5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)benzamide (OSM-S-495; MMV1576794)**



Prepared according to General Procedure G from: **OSM-S-432** (101 mg, 0.28 mmol) and (3-carbamoylphenyl)boronic acid (61.0 mg, 0.37 mmol) to give **OSM-S-495** as an off-white solid (81.0 mg, 72%); **m.p.** 186–188 °C; **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.06 (s, 1H), 8.29 (t, *J* = 1.8 Hz, 1H), 8.11 (s, 1H), 8.07 (dt, *J* = 7.9, 1.5 Hz, 1H), 7.88 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.66–7.56 (m, 2H), 7.52 (s, 1H), 7.14 (dt, *J* = 10.9, 8.5 Hz, 1H), 6.81 (ddd, *J* = 11.9, 7.8, 2.2 Hz, 1H), 6.68–6.57 (m, 1H), 4.49 (t, *J* = 6.3 Hz, 2H), 2.86 (t, *J* = 6.3 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 167.1, 149.5 (dd, *J* = 103.2, 12.6 Hz), 147.6 (dd, *J* = 102.0, 12.9 Hz), 147.4, 145.8, 143.8, 135.1, 135.0–134.9 (m), 133.5, 133.2, 130.0, 128.6, 127.9, 127.6, 125.4 (dd, *J* = 6.2, 3.3 Hz), 117.4 (d, *J* = 16.8 Hz), 116.9 (d, *J* = 16.8 Hz), 108.9, 70.7, 32.8; ***m/z*** (ESI+) 418 ([M+Na]+, 100%); **HRMS** (ESI+) found 396.1263 ([M+H]+), C20H16F2N5O2+ requires 396.1267.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=CC(C(N)=O)=C4)N23*

*InChI=1S/C20H15F2N5O2/c21-15-5-4-12(8-16(15)22)6-7-29-18-11-24-10-17-25-26-20(27(17)18)14-3-1-2-13(9-14)19(23)28/h1-5,8-11H,6-7H2,(H2,23,28)*

**4-(5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)benzamide (OSM-S-494; MMV1576793)**



Prepared according to General Procedure G from: **OSM-S-432** (100 mg, 0.28 mmol) and (4-carbamoylphenyl)boronic acid (60.5 mg, 0.37 mmol) to give **OSM-S-494** as an off-white solid (67.0 mg, 60%); **m.p.** 217–220 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.06 (s, 1H), 8.11 (s, 1H), 8.02–7.97 (m, 2H), 7.86–7.77 (m, 2H), 7.61 (s, 1H), 7.50 (s, 1H), 7.15 (dt, *J* = 10.9, 8.5 Hz, 1H), 6.88 (ddd, *J* = 12.0, 7.9, 2.2 Hz, 1H), 6.68–6.61 (m, 1H), 4.51 (t, *J* = 6.2 Hz, 2H), 2.88 (t, *J* = 6.2 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 167.3, 149.5 (dd, *J* = 106.3, 12.5 Hz), 147.6 (dd, *J* = 105.2, 12.6 Hz), 147.5, 145.7, 143.8, 135.13, 135.06, 135.0–134.9 (m), 130.6, 130.4, 126.6, 125.4 (dd, *J* = 6.3, 3.4 Hz), 117.5 (d, *J* = 16.9 Hz), 116.9 (d, *J* = 16.7 Hz), 109.0, 70.8, 32.9; ***m/z*** (ESI+) 418 ([M+Na]+, 100%); **HRMS** (ESI+) found 396.1263 ([M+H]+), requires C20H16F2N5O2+ 396.1272.

*O=C(C1=CC=C(C=C1)C2=NN=C3N2C(OCCC4=CC=C(C(F)=C4)F)=CN=C3)N*

*InChI=1S/C20H15F2N5O2/c21-15-6-1-12(9-16(15)22)7-8-29-18-11-24-10-17-25-26-20(27(17)18)14-4-2-13(3-5-14)19(23)28/h1-6,9-11H,7-8H2,(H2,23,28)*

**4-(5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)benzenesulfonamide (OSM-S-506; MMV1577569)**



Prepared according to General Procedure G from: **OSM-S-432** (94.9 mg, 0.27 mmol) and (4-sulfamoylphenyl)boronic acid (69.8 mg, 0.35 mmol) to give **OSM-S-506** as an off-white solid (58.0 mg, 50%); **m.p.** 166–167 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.08 (s, 1H), 7.95 (d, *J* = 8.4 Hz, 2H), 7.91 (d, *J* = 8.4 Hz, 2H), 7.64 (s, 1H), 7.53 (s, 2H), 7.19 (dt, *J* = 10.8, 8.5 Hz, 1H), 7.01 (ddd, *J* = 11.8, 7.8, 2.1 Hz, 1H), 6.67 (ddd, *J* = 9.2, 4.0, 2.2 Hz, 1H), 4.52 (t, *J* = 6.4 Hz, 2H), 2.88 (t, *J* = 6.4 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 149.6 (dd, *J* = 113.8, 12.4 Hz), 147.6 (dd, *J* = 112.5, 12.4 Hz), 147.5, 145.2, 145.0, 143.7, 135.1, 134.9 (dd, *J* = 5.9, 3.7 Hz), 131.2, 131.0, 125.5 (dd, *J* = 6.5, 3.3 Hz), 124.8, 117.4 (d, *J* = 16.8 Hz), 117.0 (d, *J* = 16.9 Hz), 109.1, 70.9, 32.9; ***m/z*** (ESI+) 454 ([M+Na]+, 100%); **HRMS** (ESI+) found 432.0934 ([M+H]+), C19H16F2N5O3S+ requires 432.0936.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=C(C=C4)S(N)(=O)=O)N23*

*InChI=1S/C19H15F2N5O3S/c20-15-6-1-12(9-16(15)21)7-8-29-18-11-23-10-17-24-25-19(26(17)18)13-2-4-14(5-3-13)30(22,27)28/h1-6,9-11H,7-8H2,(H2,22,27,28)*

**5-(3,4-Difluorophenethoxy)-3-(4-(methylsulfonyl)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-W-5)**



Prepared according to General Procedure E from: **S18** (130 mg, 0.42 mmol, 1.0 equiv.) and 2-(3,4-difluorophenyl)ethan-1-ol (0.015 mL, 0.50 mmol, 1.2 equiv.); purified by flash chromatography on silica (25–100% ethyl acetate in hexanes) to give **OSM-W-5** (87.5 mg, 48%); **m.p.** 67–69 °C; **1H NMR** (500 MHz, CDCl3) δ: 9.08 (s, 1H), 8.07 (d, *J* = 8.5 Hz, 2H), 7.90 (d, *J* = 8.6 Hz, 2H), 7.39 (s, 1H), 7.01 (dt, *J* = 10.2, 8.4 Hz, 1H), 6.57–6.47 (m, 2H), 4.45 (t, *J* = 6.3 Hz, 2H), 3.11 (s, 3H), 2.91 (t, *J* = 6.2 Hz, 2H); **13C NMR** (126 MHz, CDCl3) δ: 148.1, 145.4, 143.7, 142.1, 136.9, 133.3, 131.8, 127.0, 124.7 (dd, *J* = 6.1, 3.7 Hz), 117.8 (d, *J* = 17.3 Hz), 117.1 (d, *J* = 17.3 Hz), 108.9, 71.1, 44.6, 33.8; ***m/z*** (ESI+) 431 ([M+H]+, 100%).

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=C(C=C4)S(C)(=O)=O)N23*

*InChI=1S/C20H16F2N4O3S/c1-30(27,28)15-5-3-14(4-6-15)20-25-24-18-11-23-12-19(26(18)20)29-9-8-13-2-7-16(21)17(22)10-13/h2-7,10-12H,8-9H2,1H3*

**4-(5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)benzonitrile (OSM-W-9)**



Prepared according to General Procedure E from: **S14** (255 mg, 1.00 mmol, 1.0 equiv.) and 2-(3,4-difluorophenyl)ethan-1-ol (0.02 mL, 1.20 mmol, 1.2 equiv.); purified by flash chromatography on silica (25–100% ethyl acetate in hexanes) to give **OSM-W-9** (118 mg, 31%); **m.p.** 164–166 °C;**1H NMR** (500 MHz, DMSO-d*6*) δ: 9.10 (s, 1H), 7.92 (d, *J* = 8.6 Hz, 2H), 7.89 (d, *J* = 8.6 Hz, 2H), 7.67 (s, 1H), 7.26–7.17 (m, 1H), 6.98–6.89 (m, 1H), 6.76 (s, 1H), 4.55 (t, *J* = 6.3 Hz, 2H), 2.92 (t, *J* = 6.2 Hz, 2H); ***m/z*** (ESI+) 378 ([M+H]+, 100%).

*FC1=CC=C(C=C1F)CCOC2=CN=CC3=NN=C(C(C=C4)=CC=C4C#N)N23*

*InChI=1S/C20H13F2N5O/c21-16-6-3-13(9-17(16)22)7-8-28-19-12-24-11-18-25-26-20(27(18)19)15-4-1-14(10-23)2-5-15/h1-6,9,11-12H,7-8H2*

**5-(3,4-Difluorophenethoxy)-3-(4-ethylphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-550; MMV1580435)**

**

Prepared according to General Procedure G from: **OSM-S-432** (104 mg, 0.29 mmol) and (4-ethylphenyl)boronic acid (57.2 mg, 0.38 mmol) to give **OSM-S-550** as a brown solid (97.8 mg, 88%); **m.p.** 130–132 °C; **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.02 (s, 1H), 7.67–7.59 (m, 2H), 7.55 (s, 1H), 7.33 (d, *J* = 7.9 Hz, 2H), 7.17 (dt, *J* = 10.8, 8.5 Hz, 1H), 6.77 (ddd, *J* = 11.9, 7.9, 2.1 Hz, 1H), 6.65 (ddd, *J* = 9.0, 4.4, 2.2 Hz, 1H), 4.49 (t, *J* = 6.0 Hz, 2H), 2.88 (t, *J* = 5.9 Hz, 2H), 2.69 (q, *J* = 7.6 Hz, 2H), 1.20 (d, *J* = 7.6 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 149.5 (dd, *J* = 103.5, 12.6 Hz), 147.6 (dd, *J* = 102.4, 12.5 Hz), 147.3, 146.3, 145.6, 143.8, 135.3 (dd, *J* = 6.1, 3.6 Hz), 135.1, 130.7, 126.9, 125.4 (dd, *J* = 6.3, 3.3 Hz), 125.3, 117.5 (d, *J* = 16.8 Hz), 116.9 (d, *J* = 16.7 Hz), 108.6, 70.7, 32.9, 28.0, 15.4; ***m/z*** (ESI+) 403 ([M+Na]+, 100%); **HRMS** (ESI+) found 381.1519 ([M+H]+), C21H19F2N4O+ requires 381.1521.

*CCC1=CC=C(C=C1)C2=NN=C3N2C(OCCC4=CC=C(C(F)=C4)F)=CN=C3*

*InChI=1S/C21H18F2N4O/c1-2-14-3-6-16(7-4-14)21-26-25-19-12-24-13-20(27(19)21)28-10-9-15-5-8-17(22)18(23)11-15/h3-8,11-13H,2,9-10H2,1H3*

**5-(3,4-Difluorophenethoxy)-3-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-553; MMV1580438)**



Prepared according to General Procedure G from: **OSM-S-432** (98.0 mg, 0.28 mmol) and (4-nitrophenyl)boronic acid (60.0 mg, 0.36 mmol) to give **OSM-S-553** as a yellow solid (82.0 mg, 75%, contains 0.5% Et2O); **m.p.** 173–177 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.11 (s, 1H), 8.28–8.22 (m, 2H), 7.99–7.92 (m, 2H), 7.70 (s, 1H), 7.15 (dt, *J* = 10.9, 8.5 Hz, 1H), 6.95 (ddd, *J* = 12.0, 7.9, 2.2 Hz, 1H), 6.83–6.76 (m, 1H), 4.59 (t, *J* = 6.2 Hz, 2H), 2.94 (t, *J* = 6.2 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 149.4 (dd, *J* = 119.0, 12.6 Hz), 147.8, 147.6, 147.5 (dd, *J* = 118.1, 12.5 Hz), 144.6, 143.8, 135.0–134.9 (m), 134.9, 134.0, 131.9, 125.1 (dd, *J* = 6.3, 3.4 Hz), 122.5, 117.2 (d, *J* = 17.0 Hz), 116.9 (d, *J* = 16.8 Hz), 109.4, 70.2, 32.5; ***m/z*** (APCI+) ([M+H]+) 398; **HRMS** (ESI+) found 398.1056 ([M+H]+), C19H14F2N5O3+ requires 398.1059.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=C(C=C4)[N+]([O-])=O)N23*

*InChI=1S/C19H13F2N5O3/c20-15-6-1-12(9-16(15)21)7-8-29-18-11-22-10-17-23-24-19(25(17)18)13-2-4-14(5-3-13)26(27)28/h1-6,9-11H,7-8H2*

**5-(3,4-Difluorophenethoxy)-3-(1*H*-indol-6-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-525; MMV1579341)**



Prepared according to General Procedure G from: **OSM-S-432** (80.0 mg, 0.23 mmol) and indole-6-boronic acid (47.0 mg, 0.29 mmol) to give **OSM-S-525** as an off-white solid (51.0 mg, 58%, contains 0.8% EtOAc); **m.p.** 210–213 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*) δ: 11.42 (s, 1H), 9.02 (s, 1H), 7.79 (dt, *J* = 1.6, 0.8 Hz, 1H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.57–7.47 (m, 2H), 7.33 (dd, *J* = 8.2, 1.5 Hz, 1H), 6.92 (dt, *J* = 10.9, 8.5 Hz, 1H), 6.60 (ddd, *J* = 11.9, 7.9, 2.2 Hz, 1H), 6.54 (ddd, *J* = 3.0, 1.9, 0.9 Hz, 1H), 6.36 (dq, *J* = 6.5, 2.6, 2.0 Hz, 1H), 4.44 (t, *J* = 6.2 Hz, 2H), 2.79 (t, *J* = 6.1 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 149.4 (dd, *J* = 100.6, 12.5 Hz), 147.7, 147.4 (dd, *J* = 99.2, 12.5 Hz), 147.3, 144.0, 135.2, 135.0 (dd, *J* = 6.0, 3.8 Hz), 134.9, 128.5, 127.4, 125.3 (dd, *J* = 6.3, 3.3 Hz), 121.7, 120.0, 118.8, 117.4 (d, *J* = 16.9 Hz), 116.5 (d, *J* = 16.6 Hz), 114.4, 108.5, 101.2, 70.7, 33.0; ***m/z*** (ESI+) 392 ([M+H]+); **HRMS** (ESI+) found 392.1311 ([M+H]+), C21H16F2N5O+ requires 392.1317.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=C(C=CN5)C5=C4)N23*

*InChI=1S/C21H15F2N5O/c22-16-4-1-13(9-17(16)23)6-8-29-20-12-24-11-19-26-27-21(28(19)20)15-3-2-14-5-7-25-18(14)10-15/h1-5,7,9-12,25H,6,8H2*

**5-(3,4-Difluorophenethoxy)-3-(1*H*-indol-4-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-547; MMV1580432)**



Prepared according to General Procedure G from: **OSM-S-432** (101 mg, 0.28 mmol) and (1*H*-indol-4-yl)boronic acid (59.5 mg, 0.37 mmol) to give **OSM-S-547** as an off-white solid (72.0 mg, 65%); **m.p.** 199–201 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*) δ: 11.37 (s, 1H), 9.06 (s, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.51–7.44 (m, 2H), 7.31–7.25 (m, 1H), 7.24 (d, *J* = 7.4 Hz, 1H), 6.94 (dt, *J* = 10.9, 8.5 Hz, 1H), 6.48 (ddd, *J* = 11.8, 7.9, 2.1 Hz, 1H), 6.27–6.19 (m, 2H), 4.27 (t, *J* = 5.9 Hz, 2H), 2.35 (t, *J* = 5.9 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 149.4 (dd, *J* = 93.6, 12.5 Hz), 147.4 (dd, *J* = 92.3, 12.6 Hz), 147.2, 145.7, 143.8, 135.6, 135.3, 135.1 (dd, *J* = 6.0, 3.9 Hz), 128.3, 126.5, 125.4 (dd, *J* = 6.4, 3.3 Hz), 122.1, 120.1, 119.4, 117.4 (d, *J* = 16.9 Hz), 116.6 (d, *J* = 16.7 Hz), 113.2, 108.7, 100.7, 70.8, 32.8; ***m/z*** (ESI+) 414 ([M+Na]+, 100%); **HRMS** (ESI+) found 392.1313 ([M+H]+), C21H16F2N5O+ requires 392.1317.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=CC5=C4C=CN5)N23*

*InChI=1S/C21H15F2N5O/c22-16-5-4-13(10-17(16)23)7-9-29-20-12-24-11-19-26-27-21(28(19)20)15-2-1-3-18-14(15)6-8-25-18/h1-6,8,10-12,25H,7,9H2*

**5-(3,4-Difluorophenethoxy)-3-(1-methyl-1*H*-indol-6-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-582; MMV1581331)**



Prepared according to General Procedure G from: **OSM-S-432** (84.0 mg, 0.24 mmol) and 1-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indole (79.0 mg, 0.31 mmol) to give **OSM-S-582** as an off-white solid (51.0 mg, 53%); **m.p.** 172–175 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*)δ: 9.03 (s, 1H), 7.83 (s, 1H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.53 (s, 1H), 7.49 (d, *J* = 3.1 Hz, 1H), 7.35 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.92 (dt, *J* = 10.7, 8.5 Hz, 1H), 6.53 (d, *J* = 3.1 Hz, 1H), 6.48 (ddd, *J* = 11.9, 7.9, 2.1 Hz, 1H), 6.42–6.35 (m, 1H), 4.45 (t, *J* = 6.0 Hz, 2H), 3.87 (s, 3H), 2.74 (t, *J* = 6.0 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 149.3 (dd, *J* = 100.1, 12.5 Hz), 147.6, 147.4 (dd, *J* = 98.9, 12.6 Hz), 147.3, 144.0, 135.4, 135.2, 135.0 (dd, *J* = 5.8, 4.0 Hz), 131.6, 128.8, 125.2 (dd, *J* = 6.4, 3.4 Hz), 121.7, 120.3, 119.1, 117.3 (d, *J* = 16.8 Hz), 116.5 (d, *J* = 16.7 Hz), 112.6, 108.5, 100.5, 70.6, 33.0, 32.6; ***m/z*** (ESI+) 428 ([M+Na]+, 100%); **HRMS** (ESI+) found 406.1472 ([M+H]+), C22H18F2N5O+ requires 406.14740.

*CN1C=CC2=CC=C(C=C21)C3=NN=C4N3C(OCCC5=CC=C(C(F)=C5)F)=CN=C4*

*InChI=1S/C22H17F2N5O/c1-28-8-6-15-3-4-16(11-19(15)28)22-27-26-20-12-25-13-21(29(20)22)30-9-7-14-2-5-17(23)18(24)10-14/h2-6,8,10-13H,7,9H2,1H3*

**3-(Benzo[*d*][1,3]dioxol-4-yl)-5-(3,4-difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-662; MMV1794874)**



Prepared according to General Procedure E from: **OSM-S-661** (99.0 mg, 0.36 mmol) and 2-(3,4-difluorophenyl)ethanol (57.0 mg, 0.36 mmol) to give **OSM-S-662** as a yellow solid (79.0 mg, 55%); **m.p.** 162–165 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.06 (s, 1H), 7.60 (s, 1H), 7.21 (dt, *J* = 10.9, 8.5 Hz, 1H), 7.10 (ddd, *J* = 13.9, 7.9, 1.2 Hz, 2H), 6.98 (t, *J* = 7.9 Hz, 1H), 6.88 (ddd, *J* = 12.0, 7.9, 2.2 Hz, 1H), 6.71 (ddt, *J* = 8.4, 3.9, 1.7 Hz, 1H), 6.06 (s, 2H), 4.49 (t, *J* = 6.1 Hz, 2H), 2.83 (t, *J* = 6.1 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 149.6 (dd, *J* = 101.9, 12.5 Hz), 147.6 (dd, *J* = 100.8, 12.5 Hz), 147.2, 147.0, 146.5, 143.6, 140.9, 135.1, 135.0 (dd, *J* = 5.9, 3.8 Hz), 125.3 (dd, *J* = 6.5, 3.4 Hz), 123.6, 121.1, 117.5 (d, *J* = 17.1 Hz), 117.0 (d, *J* = 16.8 Hz), 110.1, 110.0, 109.1, 101.5, 70.7, 33.1; ***m/z*** (ESI+) 419 ([M+Na]+, 100%); **HRMS** (ESI+) found 419.0926 ([M+Na]+), C20H14F2N4O3Na+ requires 419.0926.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=CC5=C4OCO5)N23*

*InChI=1S/C20H14F2N4O3/c21-14-5-4-12(8-15(14)22)6-7-27-18-10-23-9-17-24-25-20(26(17)18)13-2-1-3-16-19(13)29-11-28-16/h1-5,8-10H,6-7,11H2*

**5-(3,4-Difluorophenethoxy)-3-(2*H*-indazol-6-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-546; MMV1580431)**



Prepared according to General Procedure G from: **OSM-S-432** (95.0 mg, 0.27 mmol) and (1*H*-indazol-6-yl)boronic acid (56.0 mg, 0.35 mmol) to give **OSM-S-546** as an off-white solid (24.0 mg, 23%, contains 0.5% CH2Cl2); **m.p.** 184–188 °C (decomp.); **1H NMR** (500 MHz, DMSO-d*6*) δ: 13.36 (s, 1H), 9.06 (s, 1H), 8.19 (s, 1H), 7.92 (s, 1H), 7.85 (d, *J* = 8.4 Hz, 1H), 7.57 (s, 1H), 7.44 (dd, *J* = 8.4, 1.4 Hz, 1H), 6.95 (dt, *J* = 10.9, 8.5 Hz, 1H), 6.63 (ddd, *J* = 12.0, 7.9, 2.2 Hz, 1H), 6.44 (ddt, *J* = 7.1, 5.0, 2.3 Hz, 1H), 4.47 (t, *J* = 6.2 Hz, 2H), 2.79 (t, *J* = 6.2 Hz, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 149.9 (dd, *J* = 103.9, 12.6 Hz), 147.91, 147.90 (dd, *J* = 102.8, 12.5 Hz), 147.1, 144.4, 139.4, 135.6, 135.4 (dd, *J* = 6.1, 3.6 Hz), 134.1, 125.7, 125.6 (dd, *J* = 6.3, 3.3 Hz), 123.6, 123.4, 119.9, 117.7 (d, *J* = 16.9 Hz), 117.1 (d, *J* = 16.8 Hz), 113.4, 109.3, 71.1, 33.4; ***m/z*** (ESI+) 415 ([M+Na]+, 100%); **HRMS** (ESI+) found 393.1267 ([M+H]+), C20H15F2N6O+ requires 393.1270.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=C5C=NNC5=C4)N23*

*InChI=1S/C20H14F2N6O/c21-15-4-1-12(7-16(15)22)5-6-29-19-11-23-10-18-26-27-20(28(18)19)13-2-3-14-9-24-25-17(14)8-13/h1-4,7-11H,5-6H2,(H,24,25)*

**5-(3,4-Difluorophenethoxy)-3-(piperidin-1-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-X-049; MMV669009)**



Compound **OSM-X-049** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, DMSO-d*6*) δ: 8.73 (s, 1H), 7.56–7.52 (m, 1H), 7.41–7.35 (m, 2H), 7.25 (m, 1H), 4.55 (t, *J* = 6.8 Hz, 2H), 3.21–3.14 (m, 4H), 1.57 (br s, 6H) (methylene CH2 obscured by solvent peak); ***m/z*** (ESI) 360 ([M+H]+, 100%).

*FC1=CC=C(C=C1F)CCOC2=CN=CC3=NN=C(N4CCCCC4)N23*

*InChI=1S/C18H19F2N5O/c19-14-5-4-13(10-15(14)20)6-9-26-17-12-21-11-16-22-23-18(25(16)17)24-7-2-1-3-8-24/h4-5,10-12H,1-3,6-9H2*

**5-(3,4-Difluorophenethoxy)-3-(4,4-difluoropiperidin-1-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-518; MMV1579347)**



Prepared according to General Procedure H from: **OSM-S-432** (305 mg, 0.86 mmol) and 4,4-difluoropiperidine hydrochloride (176 mg, 1.12 mmol) to give **OSM-S-518** as a yellow solid (8.10 mg, 2%); **1H NMR** (500 MHz, CDCl3) δ: 8.80 (s, 1H), 7.21–7.09 (m, 3H), 7.01 (ddd, *J* = 8.5, 4.1, 1.9 Hz, 1H), 4.47 (t, *J* = 6.8 Hz, 2H), 3.53–3.47 (m, 4H), 3.22 (t, *J* = 6.7 Hz, 2H), 2.13 (tt, *J* = 13.8, 5.9 Hz, 4H); **13C NMR** (126 MHz, CDCl3) δ: 151.2 (dd, *J* = 98.1, 12.5 Hz), 149.2 (dd, *J* = 97.2, 12.6 Hz), 144.0, 137.5, 133.1 (dd, *J* = 5.9, 4.0 Hz), 130.4 (d, *J* = 8.6 Hz), 129.0 (d, *J* = 9.9 Hz), 124.9 (dd, *J* = 6.2, 3.6 Hz), 121.3 (t, *J* = 242.0 Hz), 118.0 (d, *J* = 2.3 Hz), 117.8 (d, *J* = 2.5 Hz), 107.8, 70.9, 49.3 (t, *J* = 5.3 Hz), 34.3, 33.4 (t, *J* = 23.3 Hz); ***m/z*** (ESI+) 418 ([M+Na]+, 100%); **HRMS** (ESI+) found 396.1435 ([M+H]+), C18H18F4N5O+ requires 396.1442.

*FC1(CCN(CC1)C2=NN=C3N2C(OCCC4=CC=C(C(F)=C4)F)=CN=C3)F*

*InChI=1S/C18H17F4N5O/c19-13-2-1-12(9-14(13)20)3-8-28-16-11-23-10-15-24-25-17(27(15)16)26-6-4-18(21,22)5-7-26/h1-2,9-11H,3-8H2*

**4-(5-(3,4-Difluorophenethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)morpholine (OSM-S-517; MMV1579337)**



Prepared according to General Procedure H from: **OSM-S-432** (101 mg, 0.28 mmol) and morpholine (34.0 mg, 0.37 mmol) to give **OSM-S-517** as a yellow solid (5.00 mg, 5%); **m.p.** 137–140 °C (decomp.); **1H NMR** (500 MHz, CDCl3) δ: 8.78 (s, 1H), 7.22 (ddd, *J* = 11.1, 7.4, 2.2 Hz, 1H), 7.18–7.09 (m, 2H), 7.03 (ddt, *J* = 6.5, 4.3, 1.9 Hz, 1H), 4.47 (t, *J* = 6.7 Hz, 2H), 3.83–3.78 (m, 4H), 3.41–3.35 (m, 4H), 3.21 (t, *J* = 6.8 Hz, 2H); **13C NMR** (126 MHz, CDCl3) δ: 152.5, 151.2 (dd, *J* = 100.5, 12.4 Hz), 149.2 (dd, *J* = 99.8, 12.6 Hz), 146.6, 143.9, 137.6, 133.2 (dd, *J* = 5.6, 4.1 Hz), 124.9 (dd, *J* = 6.2, 3.6 Hz), 117.9 (d, *J* = 3.7 Hz), 117.8 (d, *J* = 3.5 Hz), 107.8, 70.8, 66.3, 52.4, 34.2; ***m/z*** (ESI+) 384 ([M+Na]+, 100%); **HRMS** (ESI+) found 362.1420 ([M+H]+), C17H18F2N5O2+ requires 362.1423.

*FC(C(F)=C1)=CC=C1CCOC2=CN=CC3=NN=C(C4=CC=C(C=C4)N5CCOCC5)N23*

*InChI=1S/C23H21F2N5O2/c24-19-6-1-16(13-20(19)25)7-10-32-22-15-26-14-21-27-28-23(30(21)22)17-2-4-18(5-3-17)29-8-11-31-12-9-29/h1-6,13-15H,7-12H2*

**1-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)-2-(3,4-difluorophenyl)propan-2-ol (OSM-X-004; MMV672723)**



Compound **OSM-X-004** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 9.05 (s, 1H), 7.62 (d, *J* = 8.6 Hz, 2H), 7.31 (s, 1H), 7.23 (d, *J* = 8.6 Hz, 2H), 7.11–7.02 (m, 2H), 6.92–6.88 (m, 1H), 6.62 (t, *J* = 73.5 Hz, 1H), 4.27 (d, *J* = 9.2 Hz, 1H), 4.15 (d, *J* = 9.2 Hz, 1H), 1.27 (s, 3H); ***m/z*** (ESI) 449 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCC(C4=CC=C(C(F)=C4)F)(C)O)=CN=C3)F*

*InChI=1S/C21H16F4N4O3/c1-21(30,13-4-7-15(22)16(23)8-13)11-31-18-10-26-9-17-27-28-19(29(17)18)12-2-5-14(6-3-12)32-20(24)25/h2-10,20,30H,11H2,1H3*

**2-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)-1-(3,4-difluorophenyl)ethan-1-one (OSM-S-392; MMV1557932)**



**OSM-S-393** (100 mg, 0.21 mmol, 1 equiv.) was dissolved in 3:1 THF:H2O (10 mL) and *p*-TsOH (72.0 mg, 0.42 mmol, 2 equiv.) was added. The mixture was stirred at reﬂux. The condenser was removed and the THF allowed to boil off to give a mixture of black sludge and H2O. The reaction was quenched with H2O and extracted with EtOAc (3 ×). The combined organic layers were washed with brine, dried (Na2SO4), ﬁltered and concentrated under reduced pressure to give the crude product as a brown solid (94.1 mg); puriﬁed by ﬂash chromatography on silica (25–100% EtOAc in hexanes) to give a light brown powder (50.4 mg, 56%); repuriﬁed by preparative TLC (5% MeOH in CH2Cl2) to give **OSM-S-392** as an off-white powder (24.0 mg, 27%); **m.p.** 192–200 °C; **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.11 (s, 1H), 8.06–7.98 (m, 1H), 7.90–7.78 (m, 1H), 7.83 (d, *J* = 8.8 Hz, 2H), 7.71 (s, 1H), 7.63 (dt, *J* = 10.1, 8.4 Hz, 1H), 7.34 (t, *J* = 73.7 Hz, 1H), 7.23 (d, *J* = 8.7 Hz, 2H), 5.85 (s, 2H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 190.2, 153.1 (dd, *J* = 254.9, 12.8 Hz), 152.0 (t, *J*

= 2.9 Hz), 149.4 (dd, *J* = 247.9, 13.0 Hz), 147.5, 145.8, 143.5, 135.7, 132.5, 131.3–130.5 (m), 126.2 (dd, *J* = 7.9, 3.2 Hz), 124.4, 118.2 (d, *J* = 17.9 Hz), 117.7 (d, *J* = 18.1 Hz), 117.4, 115.1 (d, *J* = 257.8 Hz), 109.9, 72.3; ***m/z*** (ESI+) 455 ([M+Na]+, 100%); **HRMS** (ESI+) found 455.0730 ([M+Na]+), C20H12F4N4O3Na+ requires 455.0738.

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCC(C(C=C4F)=CC=C4F)=O)=CN=C3)F*

*InChI=1S/C20H12F4N4O3/c21-14-6-3-12(7-15(14)22)16(29)10-30-18-9-25-8-17-26-27-19(28(17)18)11-1-4-13(5-2-11)31-20(23)24/h1-9,20H,10H2*

**3-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)-2-(3,4-difluorophenyl)propan-1-ol (OSM-S-381; MMV670947)**



Compound **OSM-S-381** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz,DMSO-d*6*) δ: 9.04 (s, 1H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.61 (s, 1H), 7.35 (t, *J* = 73.5 Hz, 1H), 7.23–7.16 (m, 3H), 7.09–7.04 (m, 1H), 6.82–6.80 (m, 1H), 4.83 (t, *J* = 5.3 Hz, 1H), 4.60–4.49 (m, 2H), 3.39–3.35 (m, 2H), 3.05–3.01 (m, 1H); ***m/z*** (ESI) 449 ([M+H]+, 100%).

*FC(F)OC(C=C1)=CC=C1C2=NN=C3C=NC=C(OCC(CO)C4=CC(F)=C(F)C=C4)N32*

*InChI=1S/C21H16F4N4O3/c22-16-6-3-13(7-17(16)23)14(10-30)11-31-19-9-26-8-18-27-28-20(29(18)19)12-1-4-15(5-2-12)32-21(24)25/h1-9,14,21,30H,10-11H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(2-(3,4-difluorophenyl)-2-fluoroethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-X-003; MMV672936)**



Compound **OSM-X-003** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 9.07 (s, 1H), 7.76 (d, *J* = 8.7 Hz, 2H), 7.28 (d, *J* = 8.7 Hz, 2H), 7.17–7.13 (m, 1H), 6.96–6.88 (m, 2H), 6.61 (t, *J* = 73.0 Hz, 1H), 5.54–5.41 (m, 1H), 4.46–4.35 (m, 2H) (core CH obscured by solvent peak); ***m/z*** (ESI) 473 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCC(C4=CC=C(C(F)=C4)F)F)=CN=C3)F*

*InChI=1S/C20H13F5N4O2/c21-14-6-3-12(7-15(14)22)16(23)10-30-18-9-26-8-17-27-28-19(29(17)18)11-1-4-13(5-2-11)31-20(24)25/h1-9,16,20H,10H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(2-(3,4-difluorophenyl)-2-fluoropropoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-X-006; MMV672727)**

**

Compound **OSM-X-006** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, CDCl3) δ: 9.06 (s, 1H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.29 (s, 1H), 7.22 (d, *J* = 8.4 Hz, 2H), 7.11–7.06 (m, 1H), 6.97–6.93 (m, 1H), 6.86–6.84 (m, 1H), 6.61 (t, *J* = 73.3 Hz, 1H), 4.39–4.21 (m, 2H), 1.37 (d, *J* = 22.0 Hz, 3H); ***m/z*** (ESI) 451 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCC(C4=CC=C(C(F)=C4)F)(C)F)=CN=C3)F*

*InChI=1S/C21H15F5N4O2/c1-21(26,13-4-7-15(22)16(23)8-13)11-31-18-10-27-9-17-28-29-19(30(17)18)12-2-5-14(6-3-12)32-20(24)25/h2-10,20H,11H2,1H3*

**(*R*)-2-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)-1-(3,4-difluorophenyl)ethan-1-amine (OSM-X-010; MMV671651)**



Compound **OSM-X-010** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, DMSO-d*6*) δ: 9.05 (s, 1H), 7.78 (d, *J* = 7.6 Hz, 2H), 7.60 (s, 1H), 7.36 (t, *J* = 73.8 Hz, 1H), 7.33–7.18 (m, 4H), 6.95 (s, 1H), 4.38–4.18 (m, 2H), 4.03 (br sapp, 1H) (amine NH2 signals not seen); ***m/z*** (ESI) 434 ([M+H]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OC[C@@H](C4=CC=C(C(F)=C4)F)N)=CN=C3)F*

*InChI=1S/C20H15F4N5O2/c21-14-6-3-12(7-15(14)22)16(25)10-30-18-9-26-8-17-27-28-19(29(17)18)11-1-4-13(5-2-11)31-20(23)24/h1-9,16,20H,10,25H2/t16-/m0/s1*

**2-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)-1-(3,4-difluorophenyl)-*N*,*N*-dimethylethan-1-amine (OSM-S-389; MMV897763)**



Prepared according to General Procedure E from: **S1** (295 mg, 0.99 mmol) and **S25** (200 mg, 0.99 mmol) to give the crude product as a dark brown solid (372 mg); puriﬁed by ﬂash chromatography on silica (25–100% EtOAc in hexanes) to give **OSM-S-389** a brown powder (169 mg, 37%); **m.p.** 132–135 °C; **1H NMR** (400 MHz, DMSO-d*6*) δ: 9.04 (s, 1H), 7.72 (d, *J* = 8.7 Hz, 2H), 7.65 (s, 1H), 7.37 (t, *J* = 73.7 Hz, 1H), 7.27 (d, *J* = 8.7 Hz, 2H), 7.31–7.20 (m, 1H), 7.03 (ddd, *J* = 11.7, 8.0, 1.8 Hz, 1H), 6.91–6.79 (m, 1H), 4.95–4.46 (m, 2H), 3.55 (t, *J* = 5.9 Hz, 1H), 2.00 (s, 6H); **13C NMR** (101 MHz, DMSO-d*6*) δ: 151.8 (t, *J* = 3.3 Hz), 147.4, 147.3, 145.5, 143.7, 134.9 (dt, *J* = 39.2, 4.5 Hz), 132.4, 125.0 (dd, *J* = 6.1, 3.3 Hz), 124.5, 117.6, 117.0 (d, *J* = 17.0 Hz), 116.6 (d, *J* = 17.0 Hz), 116.1 (t, *J* = 258.4 Hz), 109.0, 70.6, 65.7, 41.8 (two phenyl C–F signals expected between 155 and 148 ppm; observed for **OSM-S-392**; too weak to be seen); ***m/z*** (ESI+) 462 ([M+H]+, 100%); **HRMS** (ESI+) found 462.1560 ([M+H]+), C22H20F4N5O2+ requires 462.1548.

*FC(F)OC(C=C1)=CC=C1C2=NN=C3N2C(OCC(N(C)C)C4=CC(F)=C(F)C=C4)=CN=C3*

*InChI=1S/C22H19F4N5O2/c1-30(2)18(14-5-8-16(23)17(24)9-14)12-32-20-11-27-10-19-28-29-21(31(19)20)13-3-6-15(7-4-13)33-22(25)26/h3-11,18,22H,12H2,1-2H3*

**2-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)-1-(3,4-difluorophenyl)-*N*,*N*-diethylethan-1-amine (OSM-S-430; MMV1576791)**



Prepared according to General Procedure E from: **S1** (38.8 mg, 131 µmol) and **S27** (30.0 mg, 131 µmol) to give the crude product as a brown solid; puriﬁed by ﬂash chromatography on silica (25–100% EtOAc in hexanes) to give **OSM-S-430** as a yellow powder (40.1 mg, 63%); insuﬃcient material remaining for complete characterisation; **1H NMR** (200 MHz, CDCl3) δ: 9.04 (s, 1H), 7.57 (d, *J* = 8.6 Hz, 2H), 7.34 (s, 1H), 7.13 (d, *J* = 8.5 Hz, 2H), 7.08–6.88 (m, 1H), 6.83–6.62 (m, 2H), 6.41 (t, *J* = 73.1 Hz, 1H), 4.64–4.25 (m, 2H), 3.85 (t, *J* = 5.7 Hz, 1H), 2.68–2.19 (m, 4H), 0.95 (t, *J* = 7.1 Hz, 6H); ***m/z*** (ESI+) 512 ([M+Na]+, 100%); **HRMS** (ESI+) found 512.1677 ([M+Na]+), C24H23F4N5O2Na+ requires 512.1680.

*FC(F)OC(C=C1)=CC=C1C2=NN=C3N2C(OCC(N(CC)CC)C4=CC(F)=C(F)C=C4)=CN=C3*

*InChI=1S/C24H23F4N5O2/c1-3-32(4-2)20(16-7-10-18(25)19(26)11-16)14-34-22-13-29-12-21-30-31-23(33(21)22)15-5-8-17(9-6-15)35-24(27)28/h5-13,20,24H,3-4,14H2,1-2H3*

**3-(4-(Difluoromethoxy)phenyl)-5-((3-(3,4-difluorophenyl)oxetan-3-yl)methoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-X-022; MMV670438)**



Compound **OSM-X-022** was synthesized by TCG using analogous procedures and the following characterization data have been provided; **1H NMR** (400 MHz, DMSO-d*6*) δ: 9.05 (s, 1H), 7.71 (d, *J* = 8.6 Hz, 2H), 7.61 (s, 1H), 7.35 (t, *J* = 73.6 Hz, 1H), 7.26 (d, *J* = 8.6 Hz, 2H), 7.18–7.13 (m, 1H), 6.76–6.71 (m, 1H), 6.46–6.44 (m, 1H), 4.79 (s, 2H), 4.64 (d, *J* = 6.4 Hz, 2H), 4.59 (d, *J* = 6.4 Hz, 2H); ***m/z*** (ESI) 461 ([M+H]+, 100%).

*FC(F)OC(C=C1)=CC=C1C2=NN=C3N2C(OCC4(COC4)C5=CC(F)=C(F)C=C5)=CN=C3*

*InChI=1S/C22H16F4N4O3/c23-16-6-3-14(7-17(16)24)22(10-31-11-22)12-32-19-9-27-8-18-28-29-20(30(18)19)13-1-4-15(5-2-13)33-21(25)26/h1-9,21H,10-12H2*

**3-(4-(Difluoromethoxy)phenyl)-5-(2-phenylpropoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (OSM-S-607; MMV1634429)**

**

Prepared according to General Procedure E from: **S1** (163 mg, 0.55 mmol) and 2-phenylpropan-1-ol (74.8 mg, 0.55 mmol); the solvent was removed and the residue directly puriﬁed by ﬂash chromatography on silica (20–100% EtOAc in hexanes) to give **OSM-S-607** as an orange gum (163 mg, 75%); **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.03 (s, 1H), 7.74–7.68 (m, 2H), 7.60 (s, 1H), 7.53–7.21 (m, 1H), 7.31–7.26 (m, 2H), 7.27–7.11 (m, 4H), 7.07–7.01 (m, 2H), 4.41 (dd, *J* = 9.5, 6.8 Hz, 1H), 4.33 (dd, *J* = 9.4, 7.1 Hz, 1H), 3.01 (h, *J* = 7.0 Hz, 1H), 0.98 (d, *J* = 7.0 Hz, 3H); **13C NMR** (126 MHz, DMSO-d*6*) δ: 151.9 (t, *J* = 3.3 Hz), 147.3, 145.4, 143.9, 142.4, 135.0, 132.4, 128.3, 127.1, 126.5, 124.9, 117.7, 116.1 (t, *J* = 258.4 Hz), 108.8, 75.2, 38.1, 17.7; ***m/z*** (ESI+) 419 ([M+Na]+, 100%); **HRMS** (ESI+) found 419.1291 ([M+Na]+), C21H18F2N4O2Na+ requires 419.1290.

*FC(F)OC(C=C1)=CC=C1C2=NN=C3C=NC=C(OCC(C)C4=CC=CC=C4)N32*

*InChI=1S/C21H18F2N4O2/c1-14(15-5-3-2-4-6-15)13-28-19-12-24-11-18-25-26-20(27(18)19)16-7-9-17(10-8-16)29-21(22)23/h2-12,14,21H,13H2,1H3*

**2-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)-1-phenylethan-1-ol (OSM-S-279; MMV688896)**

**

Compound **S31** (100 mg, 0.21 mmol, 1 equiv.) was dissolved in EtOH (2 mL). CuCl2·2H2O (1.77 mg, 0.01 mmol, 5 mol%) was added and the mixture heated at reﬂux for 3 h. The solvent was removed and the residue dissolved in EtOAc, washed with H2O, brine, dried (MgSO4), ﬁltered and concentrated under reduced pressure to give the crude product as a dark brown liquid (90.0 mg); puriﬁed by ﬂash chromatography on silica (25–100% EtOAc in hexanes) to give **OSM-S-279** as a brown powder (65.2 mg, 79%); **m.p.** 85–90 °C; **1H NMR** (300 MHz, CDCl3) δ: 8.92 (br s, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.27 (dapp, *J* = 28.7 Hz, 8H), 6.62 (t, *J* = 73.3 Hz, 1H), 4.91 (br s, 1H), 4.31 (d, *J* = 5.0 Hz, 2H), 3.26 (br s, 1H); **13C NMR** (75 MHz, CDCl3) δ: 152.5, 147.7, 146.4, 144.0, 139.0, 136.5, 132.6, 128.9, 128.7, 126.2, 124.8, 118.8, 115.7 (t, *J* = 261.4 Hz), 108.8, 75.2, 71.5; ***m/z*** (ESI+) 399 ([M+H]+, 100%); **HRMS** (ESI+) 399.1260 ([M+H]+), C20H17F2N4O3+ requires 399.1263.

*FC(F)OC1=CC=C(C2=NN=C3N2C(OCC(O)C4=CC=CC=C4)=CN=C3)C=C1*

*InChI=1S/C20H16F2N4O3/c21-20(22)29-15-8-6-14(7-9-15)19-25-24-17-10-23-11-18(26(17)19)28-12-16(27)13-4-2-1-3-5-13/h1-11,16,20,27H,12H2*

**2-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)-1-phenylethan-1-one (OSM-S-400; MMV1557940)**



**OSM-S-279** (2.00 g, 5.02 mmol, 1.0 equiv.) was dissolved in anhydrous CH2Cl2 (30 mL) and MnO2 (21.8 g, 251 mmol, 50 equiv.) was added and the reaction stirred at reﬂux. The mixture was ﬁltered through celite and the celite washed with EtOAc. The ﬁltrate was concentrated under reduced pressure to give the crude product as a brown solid (1.65 g); puriﬁed by ﬂash chromatograph on silica (25–100% EtOAc in hexanes) to give **OSM-S-400** as a brown powder (741 mg, 37%); **m.p.** 120–128 °C; **1H NMR** (400 MHz, CDCl3) δ: 8.99 (s, 1H), 7.84 (ddapp, *J* = 8.3, 1.1 Hz, 2H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.65 (t, *J* = 7.5 Hz, 1H), 7.49 (t, *J* = 7.8 Hz, 2H), 7.22 (s, 1H), 7.14 (d, *J* = 8.7 Hz, 2H), 6.57 (t, *J* = 73.4 Hz, 1H), 5.49 (s, 2H); **13C NMR** (101 MHz, CDCl3) δ: 190.4, 152.5, 147.9, 146.6, 143.5, 137.1, 134.9, 133.4, 132.5, 129.3, 128.0, 124.7, 118.5, 115.7 (t, *J* = 260.6 Hz), 109.2, 71.3; ***m/z*** (ESI+) 419 ([M+Na]+, 100%), 815 ([2M+Na]+, 13%); **HRMS** (ESI+) found 419.0927 ([M+Na]+), C20H14F2N4O3Na+ requires 419.0926.

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCC(C4=CC=CC=C4)=O)=CN=C3)F*

*InChI=1S/C20H14F2N4O3/c21-20(22)29-15-8-6-14(7-9-15)19-25-24-17-10-23-11-18(26(17)19)28-12-16(27)13-4-2-1-3-5-13/h1-11,20H,12H2*

**3-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)-2-phenylpropan-1-ol (OSM-S-353; OSM-LO-13; MMV693155)**



Compound **S35** (1.50 g, 2.82 mmol, 1 equiv.) was dissolved in EtOH (30 mL). CuCl2·2H2O (24.0 mg, 0.14 mmol, 5 mol%) was added and the mixture heated at reﬂux for 1 h. The solvent was removed and CH2Cl2 was added to the residue. The solid was ﬁltered, washed with CH2Cl2 and dried *in vacuo* to give **OSM-S-353** as a light brown powder (683 mg, 54%); **m.p.** 154–160 °C; **1H NMR** (400 MHz, DMSO-d*6*) δ: 9.05 (br s, 1H), 7.66 (d, *J* = 8.8 Hz, 2H), 7.63 (br s, 1H), 7.35 (t, *J* = 73.7 Hz, 1H), 7.26–7.12 (m, 5H), 6.98 (dd, *J* = 7.4, 2.0 Hz, 2H), 4.80 (t, *J* = 5.3 Hz, 1H), 4.68–4.38 (m, 2H), 3.39 (t, *J* = 5.8 Hz, 2H), 2.99 (p, *J* = 6.3 Hz, 1H); **13C NMR** (101 MHz, DMSO-d*6*) δ: 151.9, 146.2, 145.2, 139.6, 135.0, 132.4, 128.1, 127.9, 126.6, 124.8, 117.7, 116.2 (t, *J* = 258.1 Hz), 108.8, 71.7, 61.8, 46.6; ***m/z*** (ESI+) 435 ([M+Na]+, 100%); **HRMS** (ESI+) found 435.1245 ([M+Na]+), C21H18F2N4O3Na+ requires 435.1239.

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCC(C4=CC=CC=C4)CO)=CN=C3)F*

*InChI=1S/C21H18F2N4O3/c22-21(23)30-17-8-6-15(7-9-17)20-26-25-18-10-24-11-19(27(18)20)29-13-16(12-28)14-4-2-1-3-5-14/h1-11,16,21,28H,12-13H2*

**3-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)-2-phenylpropane-1,2-diol (OSM-S-556; MMV1581295)**



Isolated from the late-stage biofunctionalization of **OSM-S-353**; **1H NMR** (400 MHz, DMSO-d*6*) δ: 9.04 (s, 1H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.65 (s, 1H), 7.29 (t, *J* = 73.8 Hz, 1H), 7.28 – 7.22 (m, 2H), 7.20 (dd, *J* = 5.4, 2.2 Hz, 3H), 7.15 (d, *J* = 8.7 Hz, 2H), 4.48 (q, *J* = 10.0 Hz, 2H), 3.22 (d, *J* = 2.8 Hz, 2H); **13C NMR** (101 MHz, DMSO-d*6*) δ: 151.8, 147.4, 145.6, 144.4, 142.4, 135.0, 132.4, 127.5, 126.8, 125.9, 124.5, 117.7, 116.2, 109.2, 74.7, 74.2, 65.7; ***m/z*** (ESI+) 451 ([M+Na]+, 100%).

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCC(C4=CC=CC=C4)(O)CO)=CN=C3)F*

*InChI=1S/C21H18F2N4O4/c22-20(23)31-16-8-6-14(7-9-16)19-26-25-17-10-24-11-18(27(17)19)30-13-21(29,12-28)15-4-2-1-3-5-15/h1-11,20,28-29H,12-13H2*

**2-(((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)methyl)-2-phenylpropane-1,3-diol (OSM-S-609; MMV1634435)**

****

**OSM-S-651** (203 mg, 0.42 mmol) was dissolved in MeOH (10 mL) and few drops of HCl (10% in H2O) were added and the mixture stirred at rt for 2 days. The reaction mixture was quenched with NaHCO3, concentrated under reduced pressure and purified by flash chromatography on silica (0–10% MeOH in CH2Cl2) to give **OSM-S-609** as a white solid (153 mg, 82%); **m.p.** 157–159 °C; **1H NMR** (500 MHz, DMSO-d*6*) δ: 9.06 (s, 1H), 7.69 (s, 1H), 7.55–7.49 (m, 2H), 7.31–7.19 (m, 5H), 7.15 (t, *J* = 73.7 Hz, 1H), 6.87–6.81 (m, 2H), 4.60 (s, 2H), 4.49 (t, *J* = 5.5 Hz, 2H), 3.38 (dd, *J* = 10.8, 5.2 Hz, 2H), 3.16 (dd, *J* = 10.8, 5.2 Hz, 2H) (alcohol OH signals not seen); **13C NMR** (126 MHz, DMSO-d*6*) δ: 151.6 (t, *J* = 3.2 Hz), 147.3, 145.5, 144.6, 141.0, 134.8, 132.1, 127.7, 127.1, 126.0, 124.7, 117.6, 116.1 (t, *J* = 258.1 Hz), 108.9, 71.0, 62.6, 48.6; ***m/z*** (ESI+) 465 ([M+Na]+, 100%); **HRMS** (ESI+) found 443.1523 ([M+H]+), C22H21F2N4O4+ requires 443.1525.

*FC(F)OC(C=C1)=CC=C1C2=NN=C3C=NC=C(OCC(CO)(CO)C4=CC=CC=C4)N32*

*InChI=1S/C22H20F2N4O4/c23-21(24)32-17-8-6-15(7-9-17)20-27-26-18-10-25-11-19(28(18)20)31-14-22(12-29,13-30)16-4-2-1-3-5-16/h1-11,21,29-30H,12-14H2*

**3-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)-2-phenylpropanoic acid (OSM-S-515; MMV1579336)**



**OSM-S-353** (100 mg, 0.24 mmol, 1 equiv.) was dissolved in acetone (4.33 mL) and cooled to 0 °C. Jones reagent (2.5 M, 194 µL, 0.48 mmol, 2 equiv.) was added in 3 portions (64.7 µL each) with 20 min intervals between additions. After stirring for 20 min, the reaction was quenched with excess isopropanol and stirred for a further 10 min. The mixture was diluted with H2O and the organic solvents removed. The aqueous phase was diluted with H2O and extracted with EtOAc (4 ×), washed with brine, dried (Na2SO4), ﬁltered and concentrated under reduced pressure to give the crude product as a brownish-yellow solid (97.7 mg); puriﬁed by flash chromatograph on silica (75–100% EtOAc in hexanes) to give **OSM-S-515** as an oﬀ-white powder (25.7 mg, 25%); **m.p.** 115–120 °C; **1H NMR** (400 MHz, DMSO-d*6*) δ: 9.06 (s, 1H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.69 (s, 1H), 7.33 (t, *J* = 73.6 Hz, 1H), 7.31–7.26 (m, 5H), 7.14–7.10 (m, 2H), 4.79 (t, *J* = 9.0 Hz, 1H), 4.64–4.36 (m, 1H), 3.80 (dd, *J* = 8.4, 5.6 Hz, 1H), 3.38 (ddapp , *J* = 14.1, 7.1 Hz, 1H); **13C NMR** (101 MHz, DMSO-d*6*) δ: 171.9, 152.0, 147.4, 145.5, 143.6, 135.4, 135.1, 132.3, 128.6, 128.0, 127.7, 124.5, 117.7, 116.3, 109.0, 71.1, 49.6; ***m/z*** (ESI+) 449 ([M+Na]+, 41%), 471 ([M-H+2Na]+, 100%); **HRMS** (ESI+) found 427.1216 ([M+H]+), C21H17F2N4O4+ requires 427.1212.

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(OCC(C(O)=O)C4=CC=CC=C4)=CN=C3)F*

*InChI=1S/C21H16F2N4O4/c22-21(23)31-15-8-6-14(7-9-15)19-26-25-17-10-24-11-18(27(17)19)30-12-16(20(28)29)13-4-2-1-3-5-13/h1-11,16,21H,12H2,(H,28,29)*

**5-((1,2-Dicarba-*closo*-decaborane-1-yl)ethoxy)-3-(4-(diﬂuoromethoxy)phenyl)-[1,2,4] triazolo[4,3-*a*]pyrazine (OSM-S-418; MMV1576784)**

**

**OSM-S-418** was previously synthesized and characterized according to literature procedures.2

*FC(F)OC(C=C1)=CC=C1C2=NN=C3C=NC=C(OCCC4567[BH]89%10[CH]%11%124[BH]8%13%14[BH]%11%15%16[BH]%13%17%18[BH]%149%19[BH]%105%20[BH]%21%226[BH]%17%15([BH]%22%12%167)[BH]%18%19%20%21)N32*

*InChI=1S/C16H22B10F2N4O2/c27-14(28)34-10-3-1-9(2-4-10)13-31-30-11-7-29-8-12(32(11)13)33-6-5-16-15-17(16)19(15)20(15)18(15,16)22(16)21(16,17)23(17,19)25(19,20)24(18,20,22)26(21,22,23)25/h1-4,7-8,14-15,17-26H,5-6H2*

***N*-(4-Chlorophenyl)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine-5-carboxamide (OSM-S-367; MMV670246)**



Prepared according to General Procedure D from: **S38** (111 mg, 0.27 mmol) to give the crude product as an orange solid (1.16 g); puriﬁed by trituration with MeOH to give **OSM-S-367** as a white powder (398 mg, 49%); **m.p.** >300 °C; **1H NMR** (300 MHz, DMSO-d*6*) δ: 10.85 (s, 1H), 9.65 (s, 1H), 8.30 (s, 1H), 7.63 (d, *J* = 7.9 Hz, 2H), 7.54–6.69 (m, 7H); **13C NMR** (75 MHz, DMSO-d*6*) δ: 157.2, 152.0, 146.8, 146.0, 145.7, 136.3, 130.3, 130.2, 128.4, 128.2, 124.5, 124.0, 121.1, 118.3, 115.9 (t, *J* = 258.3 Hz); ***m/z*** (ESI+) 438 ([M+Na]+, 100%); **HRMS** (ESI+) found 438.0540 ([M+Na]+), C19H12ClF2N5O2Na+ requires 438.0540.

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(C(NC(C=C4)=CC=C4Cl)=O)=CN=C3)F*

*InChI=1S/C19H12ClF2N5O2/c20-12-3-5-13(6-4-12)24-18(28)15-9-23-10-16-25-26-17(27(15)16)11-1-7-14(8-2-11)29-19(21)22/h1-10,19H,(H,24,28)*

***N*-(3-Chloro-4-fluorophenyl)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine-5-carboxamide (OSM-S-379; MMV670767)**



Prepared according to General Procedure D from: **S39** (500 mg, 1.15 mmol) to give the crude product as an orange solid (663 mg); puriﬁed by ﬂash chromatography on silica (25–100% EtOAc in hexanes) to give **OSM-S-379** as an oﬀ-white powder (191 mg, 38%); **m.p.** 266–269 °C; **1H NMR** (300 MHz, DMSO-d*6*) δ: 10.93 (s, 1H), 9.66 (s, 1H), 8.30 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.51 (d, *J* = 6.5 Hz, 1H), 7.31 (t, *J* = 9.0 Hz, 1H), 7.43–6.82 (m, 4H); **13C NMR** (75 MHz, DMSO-d*6*) δ: 157.3, 155.5, 152.1, 146.8, 146.2, 145.7, 134.4, 130.4, 130.3, 124.3, 124.0, 121.1, 120.0 (d, *J* = 6.9 Hz), 119.1 (d, *J* = 18.4 Hz), 118.2, 116.8 (d, *J* = 22.0 Hz), 115.8; ***m/z*** (ESI+) 434 ([M+H]+, 27%), 466 ([M+CH3OH+H]+, 100%); **HRMS** (ESI+) found 434.0631 ([M+H]+), C19H12ClF3N5O2+ requires 434.0626.

*FC(OC(C=C1)=CC=C1C2=NN=C3N2C(C(NC4=CC(Cl)=C(C=C4)F)=O)=CN=C3)F*

*InChI=1S/C19H11ClF3N5O2/c20-13-7-11(3-6-14(13)21)25-18(29)15-8-24-9-16-26-27-17(28(15)16)10-1-4-12(5-2-10)30-19(22)23/h1-9,19H,(H,25,29)*

**1-Phenyl-2-((3-(6-(trifluoromethyl)pyridin-3-yl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)ethan-1-ol (OSM-S-278; MMV688895)**



Compound **S40** (200 mg, 0.41 mmol, 1 equiv.) was dissolved in EtOH (5 mL). CuCl2·2H2O (3.51 mg, 0.02 mmol, 5 mol%) was added and the mixture heated at reﬂux for 2 h. The solvent was removed and the residue directly puriﬁed by ﬂash chromatography on silica (25–100% EtOAc in hexanes) to give **OSM-S-278** as an olive green powder (145 mg, 88%); **m.p.** 145–148 °C; **1H NMR** (300 MHz, CDCl3) δ: 9.13 (s, 1H), 8.98 (br s, 1H), 8.30 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.37 (s, 1H), 7.31 (br s, 5H), 4.92 (br s, 1H), 4.44–4.21 (m, 2H), 3.59 (s, 1H); **13C NMR** (75 MHz, CDCl3) δ: 151.3, 148.9, 148.5, 143.8, 143.5, 139.8, 139.0, 136.5, 129.0, 127.0, 126.1, 123.3, 119.8, 119.6, 109.3, 75.4, 71.6; ***m/z*** (ESI+) 402 ([M+H]+, 100%); **HRMS** (ESI+) found 402.1181 ([M+H]+), C19H15F3N5O2+ requires 402.1172.

*OC(C1=CC=CC=C1)COC2=CN=CC3=NN=C(C4=CC=C(C(F)(F)F)N=C4)N23*

*InChI=1S/C19H14F3N5O2/c20-19(21,22)15-7-6-13(8-24-15)18-26-25-16-9-23-10-17(27(16)18)29-11-14(28)12-4-2-1-3-5-12/h1-10,14,28H,11H2*

***N*-(4-Chlorophenyl)-3-(4-(difluoromethoxy)phenyl)-8-oxo-7,8-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazine-5-carboxamide (OSM-S-410; MMV1557863)**

**

Isolated from the late-stage biofunctionalization of **OSM-S-367**; **1H NMR** (600 MHz, DMSO-d*6*) δ: 10.52 (s, 1H), 7.62–7.57 (m, 2H), 7.50 (s, 1H), 7.28 (d, *J* = 2.1 Hz, 4H), 7.18–7.14 (m, 2H); **HRMS** (ESI+) found 432.0667 ([M+H]+), C19H13ClF2N5O3+ requires 432.0669.

**4-(3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine-5-carboxamido)-2-(trifluoromethyl)pyridine 1-oxide (OSM-S-411; MMV1557864)**



Isolated from the late-stage biofunctionalization of **OSM-S-175**; **1H NMR** (600 MHz, DMSO-d*6*) δ: 9.69 (s, 1H), 8.36–8.26 (m, 2H), 8.18 (s, 1H), 7.80 (d, *J* = 3.1 Hz, 1H), 7.69–7.59 (m, 2H), 7.47 (dd, *J* = 7.2, 3.0 Hz, 1H), 7.19 (d, *J* = 9.0 Hz, 3H); **HRMS** (ESI+) found 467.0874 ([M+H]+), C19H12F5N6O3+ requires 467.0886.

# Table S1. hERG Activity of Representative Series 4 Compounds.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **hERG pIC50a** |  | **hERG pIC50b** |
| OSM-S-189; MMV639725 | <4.48 | OSM-S-369; MMV897698 | 5.13 |
| OSM-S-206; MMV675719 | 5.24 | OSM-S-371; MMV897700 | 5.37 |
| OSM-S-202; MMV669542 | 4.89 | OSM-S-418; MMV1576784 | 5.44 |
| OSM-S-201; MMV675718 | 5.12 | OSM-S-535; MMV1580423 | 5.12 |
| OSM-S-380; MMV669848 | <4.53 | OSM-S-515; MMV1579336 | <5.00 |
| OSM-S-366; MMV670936 | <4.48 | OSM-S-353; MMV693155 | 4.68 |
| OSM-S-218;  MMV669844 | 5.20 | OSM-S-525; MMV1579341 | 5.12 |
| OSM-S-175; MMV670944 | 5.60 |  |  |

aEvaluated according to the 2014 protocol. bEvaluated according to the 2018 protocol.

# Table S2. Cytotoxicity of Representative Series 4 Compounds.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **THP1 (μM)** |  | **HepG2 (μM)** |
| OSM-S-189;  MMV639725 | >50 | OSM-S-418;  MMV1576784 | >10 |
| OSM-S-206;  MMV675719 | >50 | OSM-S-419;  MMV1576785 | >10 |
| OSM-S-202;  MMV669542 | 45.19 | OSM-S-423;  MMV1576789 | >10 |
| OSM-S-201;  MMV675718 | 5.138 | OSM-S-424; MMV1576790 | >10 |
| OSM-S-380;  MMV669848 | 4.626 | OSM-S-496; MMV1576795 | >10 |
| OSM-S-366;  MMV670936 | >50 |  |  |

# Table S3. Gametocyte Assay Results of Representative Series 4 Compounds.

|  |  |  |
| --- | --- | --- |
|  | **Asexual EC50 (μM)** | **Male gamete formation EC50 (μM)** |
| OSM-S-379; MMV670767 | 0.295 | 12.5 |
| OSM-S-367; MMV670246 | 7.40 | >25 |
| OSM-S-279; MMV688896 | 0.364 | 6.89 |
| OSM-S-278; MMV688895 | 4.87 | >25 |



Figure S1. The effect of OSM compounds at 1 µM on *Plasmodium falciparum* intracellular Na+ concentration. Positive (cipargamin) and negative (DMSO) controls were used throughout.



Figure S2. The effect of OSM compounds at 5 µM on *Plasmodium falciparum* intracellular Na+ concentration. Positive (cipargamin) and negative (DMSO) controls were used throughout.



Figure S3. The effect of OSM compounds at either 1 µM or 5 µM on *Plasmodium falciparum* intracellular H+ concentration. Positive (cipargamin) and negative (DMSO) controls were used throughout.

\\franklin.anu.edu.au\home\u3356728\data\My_papers_updated\Series 4\Growth assay Fig OSM names.tif

Figure S4. PfATP4 mutant parasites are less susceptible than their parents to a selection of Series 4 compounds**.** The sensitivity of parasites with wild-type PfATP4 (Dd2 parent; black), or with a Q172K (green), A353E (blue) or T418N (red) mutation in PfATP4, was determined for the Series 4 compounds OSM-S-379 (**A**), OSM-X-013 (**B**) and OSM-S-175 (**C**). Included as controls were the PfATP4 inhibitor cipargamin (**D**), and the unrelated antimalarials chloroquine (**E**) and artemisinin (**F**). The data are the mean ± SEM from three independent experiments, each performed on different days.

# General Biological Procedures

## Parasite Strains

Unless specified otherwise, experiments with malaria parasites were carried out using the NF54, 3D7 or Dd2 strainsof the human malaria parasite, *Plasmodium falciparum*.

## Parasite Growth Assays (ANU)

Experiments investigating cross-resistance of *Pf*ATP4-mutant parasites were carried out using parasite lines generated from Dd2 parasites by exposure to the *Pf*ATP4-associated compounds MMV007275 (Dd2-PfATP4T418N)20,21 or MMV011567 (Dd2-PfATP4Q172K and *Pf*ATP4A353E)20 followed by limiting-dilution. Parasite survival in the presence of a range of concentrations of antiplasmodial compounds was assessed in 72 h growth assays commencing with *P. falciparum* cultures adjusted to a parasitaemia of 1% (consisting of predominantly ring-stage parasites) and a haematocrit of 1%. Parasite survival was measured using a fluorescent intercalating dye.22 The method has been described in detail previously.23

## OSM-S-218/MMV669844 Assay against Drug-Resistant Strains

OSM-S-218 was assayed according to previously published procedures.24

## Liver Stage Potency Assays

Original liver stage data evaluated using the 2014 Protocol.25

The 2018 UCSD liver stage data was evaluated using the 2018 Protocol.26

## Parasite cytosolic [Na+] and pH Assays

The cytosolic [Na+] and pH in mature asexual-stage parasites, isolated from their host erythrocytes by saponin-permeabilisation of the host-cell membrane, were measured using fluorescent indicators, as described elsewhere.27

## hERG Patch Clamp Assays

Original 2014 hERG assay data evaluated by AstraZeneca.28

The 2018 hERG assay protocol is as follows: Chinese Hamster Ovary (CHO) cells were used for electrophysiological patch clamp recordings due to their low levels of endogenous K+ current, which allows for more accurate characterisation of Kv11.1 channel current. Cells were cultured in Ham’s F12 Nutrient Mixture (Thermo Fisher Scientiﬁc Australia) supplemented with 5% foetal bovine serum (FBS), at 37 °C and 5% CO2. CHO cells stably expressing WT Kv11.1 were passaged using TrypLE TM Express (Thermo Fisher Scientiﬁc Australia). Cells were incubated for 48 hours at 37 °C before patch clamp experiments were performed.

CHO cells stably expressing WT Kv11.1 were detached from the culture ﬂask using Accumax (Sigma-Aldrich, USA), spun at 300 g for 5 mins, then resuspended in divalent free solution (in mM: NaCl 140, KCl 5, HEPES 10, D-Glucose 5; adjusted to pH 7.4 with NaOH), supplemented with 10% DMEM media. Cells were allowed to recover for 30 minutes at 10 °C while shaking on a rotating platform at 500 rpm before recording.

Automated patch clamp recordings were performed using the Syncropatch 384 PE platform (Nanion Technologies, Munich, Germany) at room temperature (∼25 °C). Single-hole 384-well recording chips with medium resistance (4–4.5 MΩ) were used and recordings were performed in the whole cell voltage clamp conﬁguration. External recording solution contained (in mM): NaCl 140, KCl 5, CaCl 2 2, MgCl 2 1, HEPES 10, D-Glucose 5; adjusted to pH 7.4 with NaOH. Cell sealing was aided using a modiﬁed external solution containing 10 mM CaCl2. The internal solution contained the following (in mM): KF 110, KCl 10, NaCl 10, HEPES 10, EGTA 10; adjusted to pH 7.2 with KOH. Automated patch clamp workﬂows were performed using Biomek software v4.1 (Beckman Coulter) and data were acquired at a sampling rate of 5 to 10 kHz, depending on the protocol, using PatchControl384 v1.5.6 software (Nanion Technologies).

Data analysis was performed using DataControl384 v1.5.2 software (Nanion Technologies, Munich, Germany). Stringent quality control criteria were used to determine the individual cell recordings to be included for ﬁnal data analysis: Seal resistance ≥ 0.5 GΩ; series resistance ≤ 20 MΩ; and cell capacitance between 5 to 50 pF. Results are expressed as mean ± SEM.

## Metabolomics Analysis

*Plasmodium falciparum* (3D7 strain) parasites were cultured *in vitro* according to the established method with minor modifications and incubated with test compounds as previously described.29,30 Briefly, parasites were brought to a tightly synchronous life-stage population (within 4 hours of the 48 hour life cycle) by treating with 5% (w/v) sorbitol twice at an interval of 14 hours, and incubated for a further 58 hours to bring all parasites to mid-trophozoite stage (27-31 hours post infection). In 96 well plates, 200 μL cultures at 7% parasitemia and 3% haematocrit were incubated with 1 µM of test compounds for a further 5 hours (32-36 hours post infection). Each compound was incubated in four replicates and untreated controls were treated with DMSO.

After incubation with the test compounds for 5 hours, all red blood cells were settled at the bottom of the culture wells. Culture medium was carefully removed and the metabolism of the cells was quenched by placing the plate on ice and adding ice-cold phosphate buffered saline (PBS) to the culture wells. All subsequent extraction steps were performed on ice. Cells were centrifuged for 5 min at 400g in a chilled centrifuge at 4°C. The supernatant was carefully removed and 135 µl of ice-cold methanol (containing internal standards TRIS, CHAPS, CAPS and PIPES) was added followed by rapid mixing of the cell suspension using the pipette three times. Spent media samples were also prepared by adding 10 µl of the culture supernatants to 140 µl of ice-cold methanol (containing internal standards). All samples were agitated on ice for 1 hour and then centrifuged for 10 min at 1000g in a chilled centrifuge at 4°C. The supernatant was transferred to glass vials and stored at -80°C until analysis. 5 µl of each sample was combined to generate a pooled biological quality control (QC) sample to control for sample stability and instrument induced variability.

Metabolite extracts were analysed using hydrophilic interaction (HILIC) liquid chromatography (LC) and high resolution mass spectrometry on an Orbitrap system as previously described.30 Eight mixtures of authentic standards containing a total of ~300 metabolites were analysed immediately before the samples to facilitate metabolite identification. Pooled QC samples were analysed at regular intervals throughout the LC-MS run and samples were randomised to avoid any impact of systematic instrument drift.

The raw LC-MS data was processed using IDEOM as previously described.30 Manual data filtering was performed to remove peaks which were of low quality or inconsistent across replicate groups. Metabolites which matched to authentic standards in accurate mass and retention time were given a score of 8-10 in IDEOM analysis (equivalent to level 1 confidence as per the metabolomics standards initiate). Other metabolite features were annotated based on accurate mass and predicted retention time in IDEOM with a score of 5-7 (equivalent to level 2 confidence as per the metabolomics standards initiate). A total of 811 and 626 robust mass features were detected in the data from the pellet and spent media samples respectively, out of which 471 putative metabolites were identified in the former and 246 putative metabolites were identified in the latter. Peak height was used as the determinant for metabolite abundance. Univariate statistical analyses were performed using Welch’s T-test (α = 0.05) and Pearson’s correlation (MS Excel). Multivariate statistics was based on sparse Partial Least Squares Discriminant Analysis (s-PLSDA) and was performed using Metaboanalyst.31,32 Briefly, data was auto-scaled (mean-centred and divided by the standard deviation of each variable) and sparseness parameters were set to five components with ten variables per component. This allowed production of a robust and easy-to-interpret model from the high-dimensional metabolomics data. All data is deposited at Metabolomics Workbench33 (In this repository, data for compounds treated with OSM-S-218 is labelled as OSM-S-313 due to an older naming convention).

## Late-Stage Biofunctionalization

Lead diversification of **OSM-S-367** (MMV670246), **OSM-S-175** (MMV670944), and **OSM-S-353** (MMV693155) through biosynthesis using liver microsomes followed this general procedure. Substrate (20-25 µM) was incubated with liver microsomes (2 mg/mL) and NADPH (1.3 mM) in 40 mL of 100 mM potassium phosphate (pH 7.5) containing 3.3 mM MgCl2. Incubations were carried out in 500 mL Erlenmeyer flasks at 37 °C in a shaking water bath to ensure adequate surface area to air. After 1-2 hrs, the incubations were terminated by addition of 40 mL MeCN and the mixtures spun in a centrifuge at 1800 x g. The supernatant was subjected to vacuum centrifugation to reduce the volume and remove the MeCN. To the resulting mixture was added 0.5 mL formic acid, 0.5 mL MeCN, and water to a volume of ~50 mL. This mixture was spun in a centrifuge at 40000 x g for 30 min. The clear supernatant was applied to a Phemonenex Luna C18 column (4.6 x 250 mm; 5µ) through a Jasco HPLC pump at 0.8 mL/min. The column was moved to an HPLC-UV-MS system comprised of a Waters Acquity pump and diode array UV/VIS detector in line with a Thermo LTQ Velos ion trap mass spectrometer and CTC Analytics fraction collector. A gradient was applied comprised of 0.1% formic acid in water (A) and MeCN (Β) at a flow rate of 0.8 mL/min. The gradient began at 98%A/2%B and was held for 5 min followed by a linear increase to 20% A/80% B at 90 min, an immediate increase to 5% A/95% B, held at this composition for 10 min, followed by a 20 min re-equilibration at initial conditions. The eluent passed through the UV/VIS detector (200-400 nm) and into a flow splitter set at approximately 10:1. The bulk of the flow went to the fraction collector with collection every 20 sec, and the remainder of the flow went to the source of the mass spectrometer. The mass spectrometer was operated in the positive ion mode with data-dependent scanning. Fractions containing potential products of interest were evaluated on a UHPLC-UV-HRMS instrument comprised of a Thermo Accela HPLC pump and diode array UV/VIS detector and Orbitrap Elite high resolution mass spectrometer. The column was a Phenomenex Kinetix C18 (2.1 x 50 mm; 1.7µ) in mobile phase comprised of 0.1% formic acid in water (A) and MeCN (B) at 0.4 mL/min. The mobile phase composition began at 10% B for 0.5 min, raised to 80% B over the next 6 min, washed at 95% B for 1 min, followed by a 1 min re-equilibration to initial conditions. Fractions of suitable purity were pooled and the solvent removed by vacuum centrifugation. Products were transferred to a glove box and reconstituted in 0.04 mL DMSO-d6 for NMR analysis.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **OSM-S-367;** MMV670246 | **OSM-S-175;** MMV670944 | **OSM-S-353;** MMV693155 |
| Substrate Concentration (µM) | 25 | 20 | 25 |
| Liver microsomes | Human | Dexamethasone-Induced Male Rat | Canine |
| Incubation Time (hr) | 2 | 1 | 1.2 |

Isolated products were dissolved in hexadeuterated dimethyl sulfoxide (DMSO-d6 “100%”). All samples were placed in a 1.7 mm NMR tube under a dry argon atmosphere. 1H and 13C spectra were referenced using residual DMSO-d6 (1H δ=2.50 ppm relative to TMS, δ = 0.00, 13C δ = 39.5 ppm relative to TMS, δ = 0.00). NMR spectra were recorded on a either a Bruker Neo 600 MHz or a Bruker Avance II 600 MHz (Bruker BioSpin Corporation, Billerica, MA) NMR spectrometer controlled by Topspin 4.0.2 or Topspin V3.2, respectively. Each spectrometer was equipped with a 1.7 mm TCI Cryo probe. 1D spectra were recorded using an approximate sweep width of 8400 Hz and a total recycle time of approximately 7 s. 2D data were recorded using the standard pulse sequences provided by Bruker. Post-acquisition data processing was performed with either Topspin V3.2 or MestReNova V12.0. (Quantitation of NMR isolates was performed by external calibration against the 1H NMR spectrum of a 5 mM benzoic acid standard using the quantitative functions within Topspin V3.2 or MestReNova)

## SCID Mouse Model Evaluations

For the SCID mouse model conducted by GSK (compound **OSM-S-272**; **MMV639565**) described in full in the SI, the human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol. All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EEC and the GSK Policy on the Care, Welfare and Treatment of Animals.

# References

1. Dunetz, J. R.; Xiang, Y.; Baldwin, A.; Ringling, J. General and Scalable Amide Bond Formation with Epimerization-Prone Substrates Using T3P and Pyridine. *Org. Lett.* **2011**, *13*, 5048-5051.
2. Tse, E. G.; Houston, S. D.; Williams, C. M.; Savage, G. P.; Rendina, L. M.; Hallyburton, I.; Anderson, M.; Sharma, R.; Walker, G. S.; Obach, R. S.; Todd, M. H. Nonclassical Phenyl Bioisosteres as Effective Replacements in a Series of Novel Open-Source Antimalarials. *J. Med. Chem.***2020**[,](https://sciwheel.com/work/bibliography/11567877) *63*, 11585–11601.
3. Cheeseman, G. W. H.; Godwin, R. A. Pyrazines. Part III. Some Nucleophilic Substitution Reactions of Chloropyrazines. *J. Chem. Soc. C* **1971**, 2873-2976.
4. Dolezal, M.; Zitko, J.; Osicka, Z.; Kunes, J.; Vejsova, M.; Buchta, V.; Dohnal, J.; Jampilek, J.; Kralova, K. Synthesis, Antimycobacterial, Antifungal and Photosynthesis-Inhibiting Activity of Chlorinated *N*-phenylpyrazine-2-carboxamides. *Molecules* **2010**, *15*, 8567-8581.
5. Dolezal, M.; Vicik, R.; Miletín, M.; Kralova, K. Synthesis and Antimycobacterial, Antifungal and Photosynthesis-Inhibiting Evaluation of some Anilides of Substituted Pyrazine-2-carboxylic Acids. *Chem. Pap.* **2000**, *54*, 245-248.
6. Korsik, M.; Tse, E. G.; Smith, D. G.; Lewis, W.; Rutledge, P. J.; Todd, M. H. *tele*-Substitution Reactions in the Synthesis of a Promising Class of 1,2,4-Triazolo[4,3-*a*]pyrazine-Based Antimalarials. *J. Org. Chem.* **2020**, *85*, 13438-13452.
7. Mal, S.; Prathap, K. J.; Smith, S. C.; Umarye, J. D. Facile One Pot Synthesis of 8-Chloro-[1,2,4]triazolo[4,3-*a*]pyrazines *via* Oxidative Cyclisation using Chloramine T. *Tetrahedron Lett.* **2015**, *56*, 2896-2901.
8. Bradac, J.; Furek, Z.; Janezic, D.; Molan, S.; Smerkolj, I.; Stanovnik, B.; Tisler, M.; Vercek, B. Heterocycles. 167. Telesubstitution and Other Transformations of Imidazo[1,2-*a*]- and s-Triazolo[4,3-*a*]pyrazines. *J. Org. Chem.* **1977**, *42*, 4197-4201.
9. Xu, S.; Yun, Z.; Feng, Y.; Tang, T.; Fang, Z.; Tang, T. Zeolite Y Nanoparticle Assemblies with High Activity in the Direct Hydration of Terminal Alkynes. *RSC Adv.* **2016**, *6*, 69822-69827.
10. Laug, B.; Murali Dhar, T. G.; Nagarathnam, D.; Jeon, Y. T.; Marzabadi, M. R.; Wong, W. C.; Gluchowski, C. Oxazolidinones as α1A Receptor Antagonists. **1997**, US6159990A.
11. Mattiello, L.; De Luca, C.; Rampazzo, L. Electrochemistry of some Ethyl α-bromo(dihalophenyl)acetates and Electrochemical Synthesis of Diastereomeric Diethyl 2,3-bis(dihalogenophenyl)succinates. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1041-1044.
12. Katritzky, A. R.; Singh, S. K.; Cai, C.; Bobrov, S. Direct Synthesis of Esters and Amides from Unprotected Hydroxyaromatic and -aliphatic Carboxylic Acids. *J. Org. Chem.* **2006**, *71*, 3364-3374.
13. Brouillette, W. J.; Smissman, E. E.; Grunewald, G. L. *N*-Acylcarbamates as Intermediates in Synthetic Approaches to a Bicyclic Trimethylene-Bridged 2,4-Oxazolidinedione and Hydantoin. *J. Org. Chem.* **1979**, *44*, 839-843.
14. Nieuwenhuis, S. A. M.; Vertegaal, L. B. J.; de Zoete, M. C.; van der Gen, A. Acid-catalyzed Solvolysis of Polyenol Ethers. III. Effect of the Alkoxy Moiety. *Tetrahedron* **1994**, *50*, 13207-13230.
15. Hamel, J.-D.; Paquin, J.-F. Au-catalyzed Intramolecular Hydroalkoxylation of *gem*-Difluorinated Alkynols. *J. Fluorine Chem.* **2018**, *216*, 11-23.
16. Soengas, R. G.; Estévez, A. M. Convenient Procedure for the Indium-Mediated Hydroxymethylation of Active Bromo Compounds: Transformation of Ketones into α-Hydroxymethyl Nitroalkanes. *Synlett* **2010**, *2010*, 2625-2627.\
17. Kouta, M.; Tomofumi, M.; Yoshiaki, H.; Teruaki, M. Cyanation of Alcohols with Diethyl Cyanophosphonate and 2,6-Dimethyl-1,4-benzoquinone by a New Type of Oxidation-Reduction Condensation. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1106-1117.
18. Keller, K.; Zalibera, M.; Qi, M.; Koch, V.; Wegner, J.; Hintz, H.; Godt, A.; Jeschke, G.; Savitsky, A.; Yulikov, M. EPR Characterization of Mn(II) Complexes for Distance Determination with Pulsed Dipolar Spectroscopy. *Phys. Chem. Chem. Phys.* **2016**, *18*, 25120-25135.
19. Viguier, R.; Serratrice, G.; Dupraz, A.; Dupuy, C. New Polypodal Polycarboxylic Ligands – Complexation of Rare-Earth Ions in Aqueous Solution. *Eur. J. Inorg. Chem.* **2001**, *2001*, 1789-1795.
20. Lehane, A. M.; Ridgway, M. C.; Baker, E.; Kirk, K. Diverse Chemotypes Disrupt Ion Homeostasis in the Malaria Parasite. *Mol. Microbiol.* **2014**, *94*, 327-339.
21. Rosling, J. E. O.; Ridgway, M. C.; Summers, R. L.; Kirk, K.; Lehane, A. M. Biochemical Characterization and Chemical Inhibition of *Pf*ATP4-Associated Na+-ATPase activity in *Plasmodium falciparum* Membranes. *J. Biol. Chem.* **2018**, *293*, 13327-13337.
22. Smilkstein M.; Sriwilaijaroen, N.; Kelly, J. X.; Wilairat, P.; Riscoe, M. Simple and Inexpensive Fluorescence-Based Technique for High-Throughput Antimalarial Drug Screening. *Antimicrob. Agents Chemother.* **2004**, *48*, 1803-1806.
23. Spry, C.; Macuamule, C.; Lin, Z.; Virga, K. G.; Lee, R. E.; Strauss, E.; Saliba, K. J. Pantothenamides are Potent, On-Target Inhibitors of *Plasmodium falciparum* Growth when Serum Pantetheinase is Inactivated. *PLoS One* **2013**, *8*, e54974.
24. Okaniwa, M.; Shibata, A.; Ochida, A.; Akao, Y.; White, K. L.; Shackleford, D. M.; Duffy, S.; Lucantoni, L.; Dey, S.; Striepen, J.; Yeo, T.; Mok, S.; Aguiar, A. C. C.; Sturm, A.; Crespo, B.; Sanz, L. M.; Churchyard, A.; Baum, J.; Pereira, D. B.; Guido, R. V. C.; Dechering, K. J.; Wittlin, S.; Uhlemann, A.-C.; Fidock, D. A.; Niles, J. C.; Avery, V. M.; Charman, S. A.; Laleu, B. Repositioning and Characterization of 1-(Pyridin-4-yl)pyrrolidin-2-one Derivatives as *Plasmodium* Cytoplasmic Prolyl-tRNA Synthetase Inhibitors. *ACS Infect. Dis.* **2021**, *7*, 1680-1689.
25. Swann, J.; Corey, V.; Scherer, C. A.; Kato, N.; Comer, E.; Maetani, M.; Antonova-Kock, Y.; Reimer, C.; Gagaring, K.; Ibanez, M.; Plouffe, D.; Zeeman, A.-M.; Kocken, C. H. M.; McNamara, C. W.; Schreiber, S. L.; Campo, B.; Winzeler, E. A.; Meister, S. High-Throughput Luciferaase-Based Assay for the Discovery of Therapeutics that Prevent Malaria. *ACS Infect. Dis.* **2016**, *2*, 281-293.
26. <https://github.com/OpenSourceMalaria/OSMSeries4Paper1/tree/master/Experimental/Protocols>
27. Spillman, N. J.; Allen, R. J. W.; McNamara, C. W.; Yeung, B. K. S.; Winzeler, E. A.; Diagana, T. T.; Kirk, K. Na+ Regulation in the Malaria Parasite *Plasmodium falciparum* Involves the Cation ATPase *Pf*ATP4 and is a Target of the Spiroindolone Antimalarials. *Cell Host Microbe.* **2013**, *13*, 227-237.
28. Bridgland-Taylor, M. H.; Hargreaves, A. C.; Easter, A.; Orme, A.; Henthorn, D. C.; Ding, M.; Davis, A. M.; Small, B. G.; Heapy, C. G.; Abi-Gerges, N.; Persson, F.; Jacobson, I.; Sullivan, M.; Albertson, N.; Hammond, T. G.; Sullivan, E.; Valentin, J.-P.; Pollard, C. E. Optimisation and Validation of a Medium-Throughput Electrophysiology-Based hERG Assay using IonWorksTM HT. *J. Pharmacol. Toxicol. Methods* **2006**, *54*, 189-199.
29. Trager, W.; Jensen, J. B. Human Malaria Parasites in Continuous Culture. *Science* **1976**, *193*, 673-675.
30. Creek, D. J.; Chua, H. H.; Cobbold, S. A.; Nijagal, B.; MacRae, J. I.; Dickerman, B. K.; Gilson, P. R.; Ralph, S. A.; McConville, M. J. Metabolomics-Based Screening of the Malaria Box Reveals both Novel and Established Mechanisms of Action. *Antimicrob. Agents Chemother.* **2016**, *60*, 6650-6663.
31. Lê Cao, K.-A.; Boitard, S.; Besse, P. Sparse PLS Discriminant Analysis: Biologically Relevant Feature Selection and Graphical Displays for Multiclass Problems. *BMC Bioinform.* **2011**, *12*, No. 253.
32. Xia, J.; Wishart, D. S. Using MetaboAnalyst 3.0 for Comprehensive Metabolomics Data Analysis. *Curr. Protoc. Bioinform.* **2016**, *55*, 14.10.1-14.10.91.
33. https://www.metabolomicsworkbench.org/data/DRCCMetadata.php?Mode=Project&ProjectID=PR000691