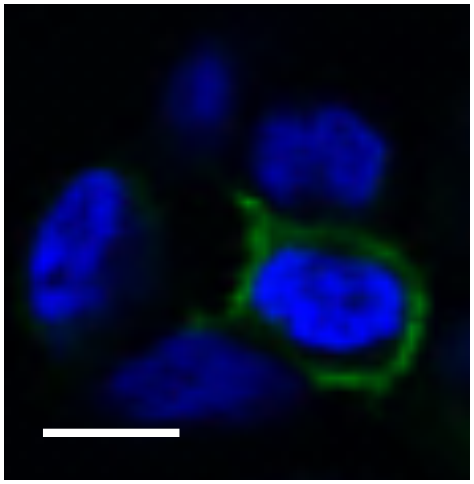
Supplemental Data

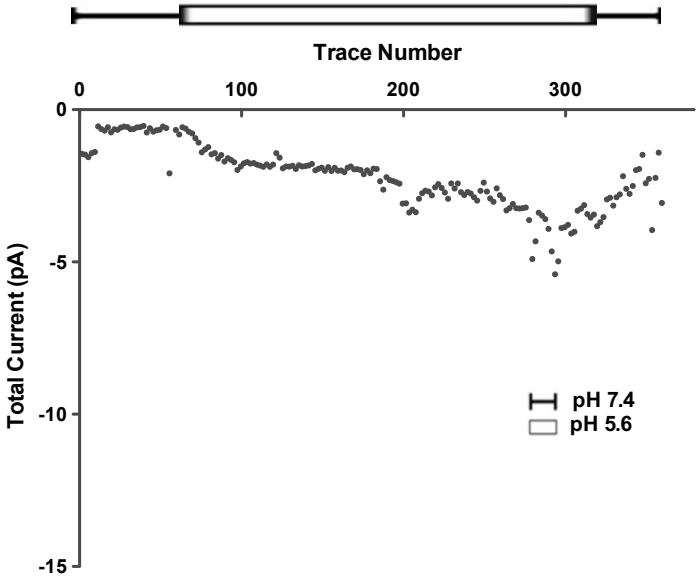
Mechanisms of action of novel influenza A/M2 viroporin inhibitors derived from hexamethylene amiloride

Pouria H. Jalily, Jodene Eldstrom, Scott C. Miller, Daniel C. Kwan, S.-H. Sheldon Tai, Doug Chou, Masahiro Niikura, Ian Tietjen, David Fedida

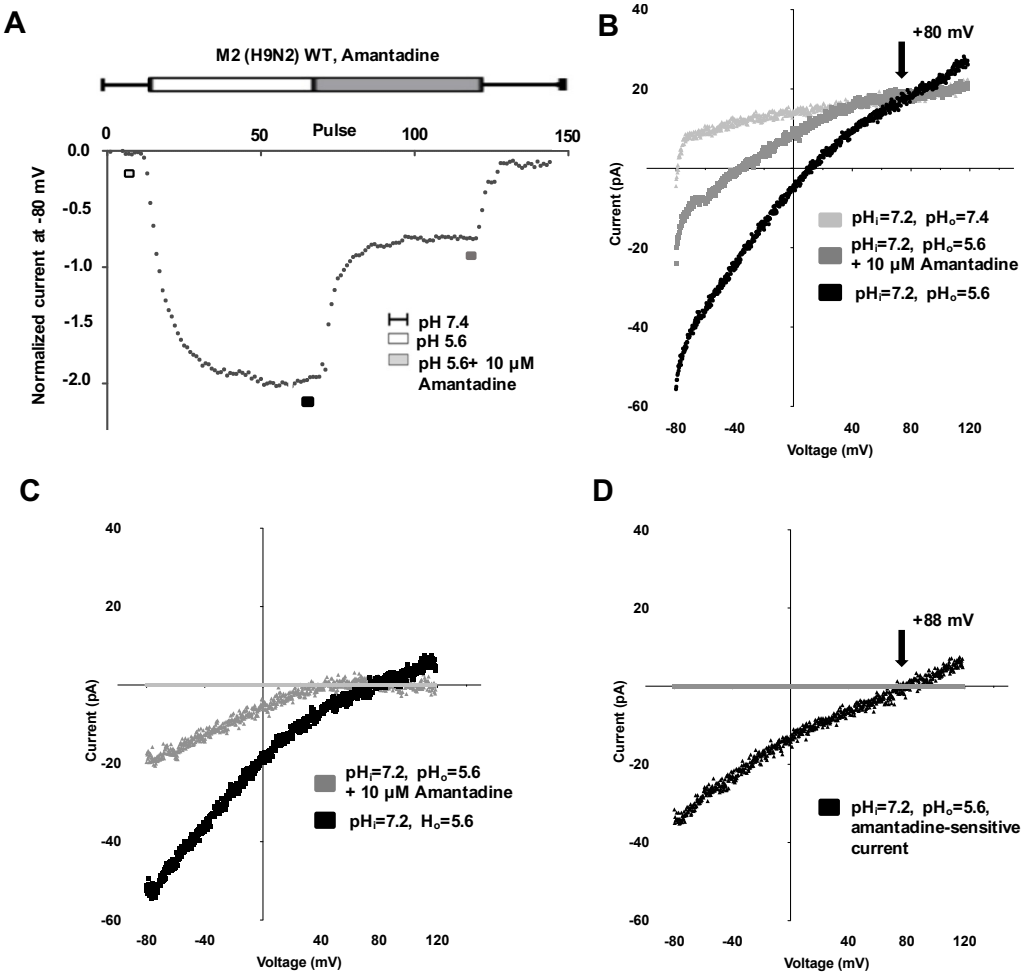
Molecular Pharmacology



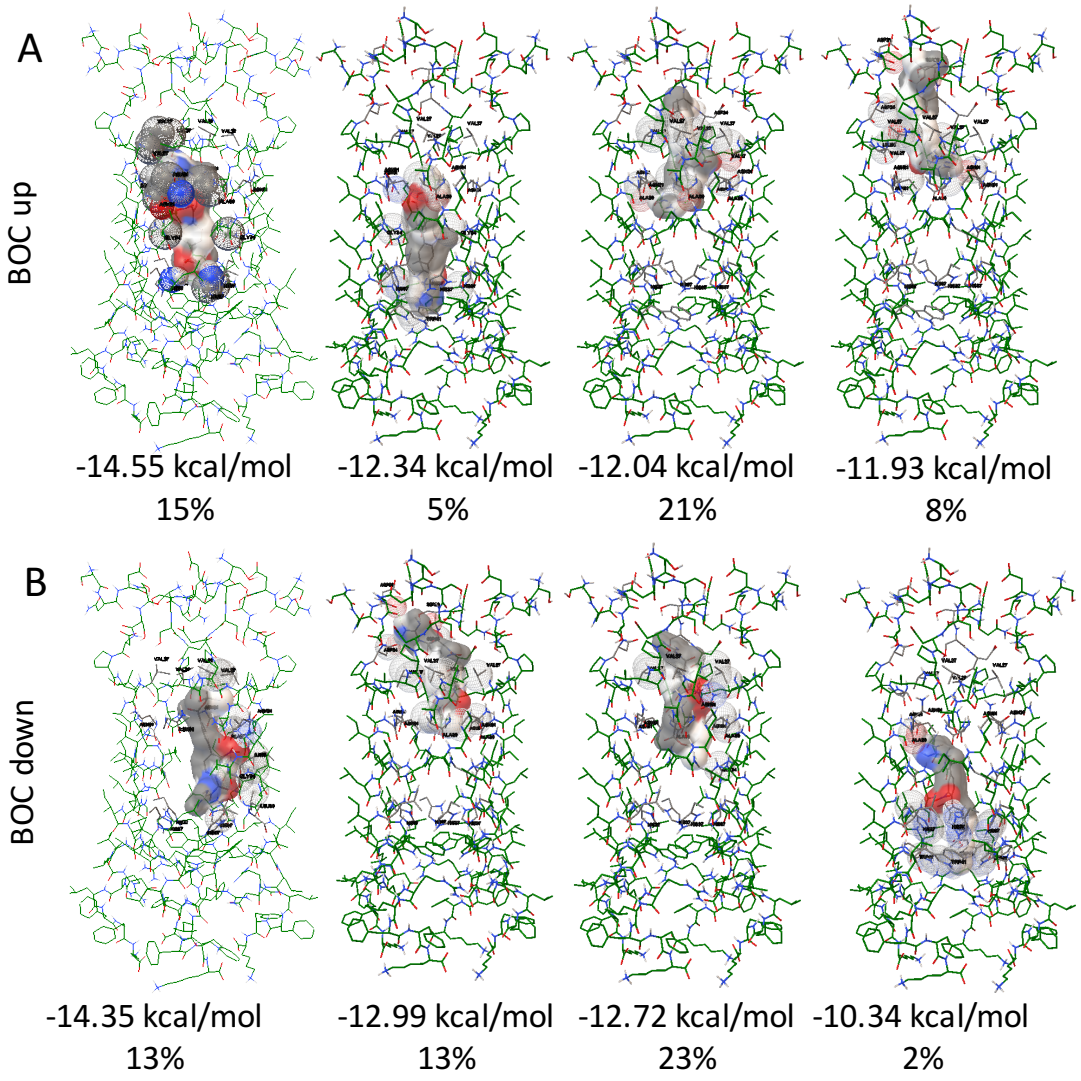
Supplemental Figure 1. Immunocytochemistry of cell-surface. FLAG-M2(WT) expression in non-permeabilized HEK 293T cells. Cells transfected with M2 were assessed for M2 expression using the reagents and indirect immunofluorescent cytochemical staining protocol described in the product information brochure for the Monoclonal ANTI-FLAG M2, clone M2 antibody (Sigma-Aldrich, St. Louis, MO; Catalog Number F1804). Scale bar, 20 μm.



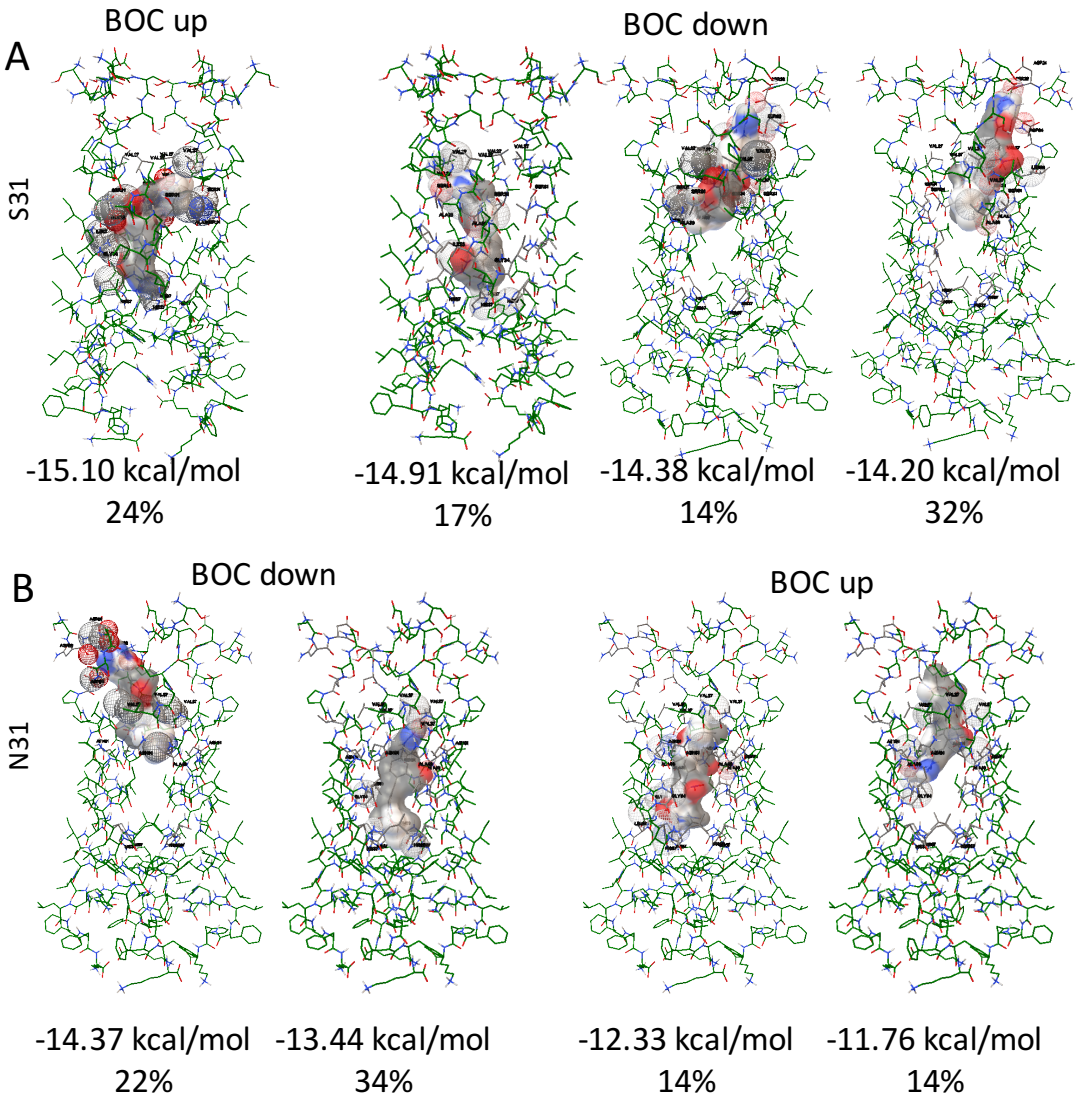
Supplemental Figure 2. Diary plot of total current against trace number of a non-transfected cell. Total current activated at pH 5.6 approximates to ~3 pA which we attribute to the presence of endogenous channels. As for Figure 2, this is a diary plot of currents measured at -80 mV every 4 s.



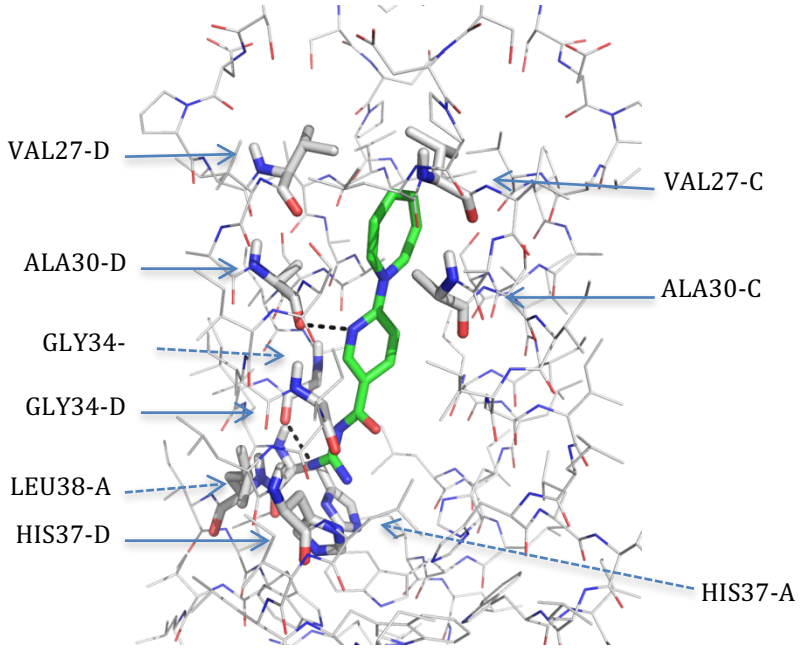
Supplemental Figure 3. Current-voltage relations of H^+-activated current and block by amantadine. Diary plot of M2 (WT) current against protocol pulse number. Cells were pulsed every 6 s from a holding potential of -40 mV, first to -80 mV for 200 ms and then ramped for 600 ms to +120 mV, a 400 ms repolarization to 0 mV, and then back to -40 mV. A current reading was taken at -80 mV and plotted against pulse number. Cells were exposed to pH_o 7.4 solution twice as denoted by the thin black bar above the graph, to pH_o 5.6 during the periods denoted by the thick bars, and also to pH_o 5.6 + 10 µM amantadine **(**grey thick bar**)**. Current voltage relations obtained under the three conditions at the times indicated in (A) by the symbols are plotted in panel **B**. All three relationships show mild inward rectification, but cross at ~+80 mV. **C**, Current activated at pH_o 5.6, before and in the presence of 10 µM amantadine, obtained by subtracting current at pH_o 7.4. **D**, Amantadine-sensitive current only, obtained by subtracting relationships in pH_o 5.6, before and after amantadine exposure, E_{rev}=+88 mV.



Supplemental Figure 4. Mechanisms of binding by **26**. Models of **26** interacting with the transmembrane pore of M2(N31) (PDB entry 2LY0; residues 19-49 of M2 of A/Chiba/5/71(H3N2)) (Wang et al., 2013). (**A**),(**B**) Conformations are shown left to right with increasing binding energies where the lowest energy of the cluster and the percentage of runs falling into the cluster are shown below the representative conformation. Additionally, the conformations are broken down by whether the acyl guanidine group was pointing towards the cytoplasmic domain (**A**), or towards the extracellular domain (**B**). The far left conformations in **A** and **B** are depicted in more detail in Figure 6 D and E. residues in contact with **26** are shown in mesh-spheres. Labelled residues are either in contact with **26** or were flexible during the docking. (Val27, Ser31/Asn31, His37). **26** is depicted in molecular surface form.



Supplemental Figure 5. Mechanisms of binding by **27**. Models of **27** interacting with the transmembrane pore (**A**) M2(WT), or (**B**) M2(N31) (PDB entry 2LY0; residues 19-49 of M2 of A/Chiba/5/71(H3N2)) (Wang et al., 2013). (**A**),(**B**) Conformations are shown left to right with increasing binding energies, the lowest energy of the cluster and the percentage of runs falling into the cluster are shown below the representative conformation. Indicated above the conformations are whether the acyl guanidine group was pointing towards the cytoplasmic domain (BOC Up), or towards the extracellular domain (BOC down). Closer views of the lowest energy binding conformations for each orientation in **A** and **B** are shown in Figure 7A. Figure details are otherwise as per Supplemental Figure 5.



Supplemental Figure 6. Alternate orientation of M2 block by 9. Models of 9 interacting with the transmembrane pore of M2(WT) (PDB entry 2LY0; residues 19-49 of M2 of A/Chiba/5/71(H3N2)) (Wang et al., 2013). Predicted binding energies was -14.83 kcal/mol. Interacting residues of M2 are shown in stick format while the rest of M2 is in line format. Only interacting residues are labeled. Hydrogens are hidden for clarity. Predicted H-bonds with a bond distance of 3.2 angstroms or less are shown by black dotted lines. Figure was created using PyMOL with the PyMOL Autodock Plugin (Seeliger & De Groot, 2010).

Supplemental Table 1. Primer sequences used to generate viruses for cytopathic assay.

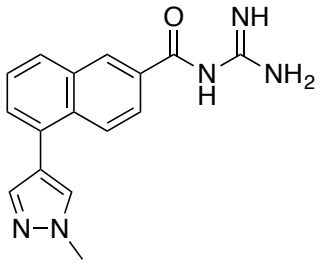
Primer	Sequence (5' – 3')
TSH284	GGGACACTTTCGGACATCTGGTCGA
TSH285	GACAGGTGTCCGTGTCCGTGTCG
TSH289	tct*cgtctc*aGGGAGCAAAAGCAGGTAGATATTGAAAGATGAGTCTTC
TSH290	GCAAGATCCCAATGAT**AC**TTGCGGCAAT**AG****C**GAGAGGATCACTTGAACCG
TSH291	ACGGTTCAAGTGATCCTCTC**G****C**TATTGCCGCA**A****G**TATCATTGGGATCTTG
TSH292	tct*cgtctc*gTATTAGTAGAAACAAGGTAGTTTTTTACTCCAGCTC
TSH293	TGCGGCAATA**AC**GAGAGGATCACTTGAACCGTTGCA
TSH294	AATGGGGGTGCAGATGCAACGGTTCAAGTGATCCTCTC**GT**TATTGCCGCA

Bold, mutated bases; underlined, mutated codons; italic, *Bsm*BI site; lower case, bases removed after *Bsm*BI digestion.

Detailed Materials and Methods

Following the purification of each compound by column chromatography, and thorough characterization by NMR, a pharmaceutically-accepted salt of each analogue was made. Hydrochloride, acetate, and fumarate are the salts employed in this study to enhance aqueous solubility and chemical stability. The respective salt was chosen based on the relative solubility of the corresponding formed salt in the crystallization solvent. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Ascend spectrometer at 400 and 100 MHz, respectively. Proton NMR data were reported as multiplicities: *s* for singlet, *d* for doublet, *dd* for double of doublets, *t* for triplet, *q* for quartet, *br s* for broad singlet, and *m* for multiplet. Chemical shifts were reported in parts per million (ppm) and coupling constants in hertz (Hz). For ^{1}H NMR spectra, $CDCl_3$, or DMSO-d_6 was used as solvent and served as the internal standard at δ 7.26, or 2.54, respectively. For ^{13}C NMR spectra, multiplicities were established by DEPT experiments and $CDCl_3$ or DMSO-d_6 was used as solvent, which served as the internal standard at δ 77.16 ($CDCl_3$), or 39.52 (DMSO-d_6). The purity of each analogue was confirmed by HPLC analysis and using a Finnigan Surveyor HPLC system (Thermo Scientific) consisting of a solvent degasser, a quaternary pump and a Surveyor Auto-sampler Plus module. Chromatographic separation was achieved using gradient elution system consisting of 10-60% acetonitrile in distilled water containing either 5mm ammonium acetate or 0.1% acetic acid. Solvent was pumped through a C18 analytical column (HALO C18 2.7 µM, 4.6 X 50 mm) and delivered at 1.2-2.0 mL/min over 5 min. The column was maintained at room temperature using a column oven. All compounds were dissolved in methanol and detected using a variable wavelength UV detector at 254 nm. The purity was calculated from an integral of peaks and described in terms of percentage (%). Low-resolution mass spectra were obtained using Waters® ZQ instrument equipped with ESCi ion source with in-like Waters® 2695 HPLC system controlled by MassLynx® 4.1 software.

N-carbamimidoyl-5-(1-methyl-1*H*-pyrazol-4-yl)-2-naphthamide (**BIT-225**) was synthesized as previously reported[1].

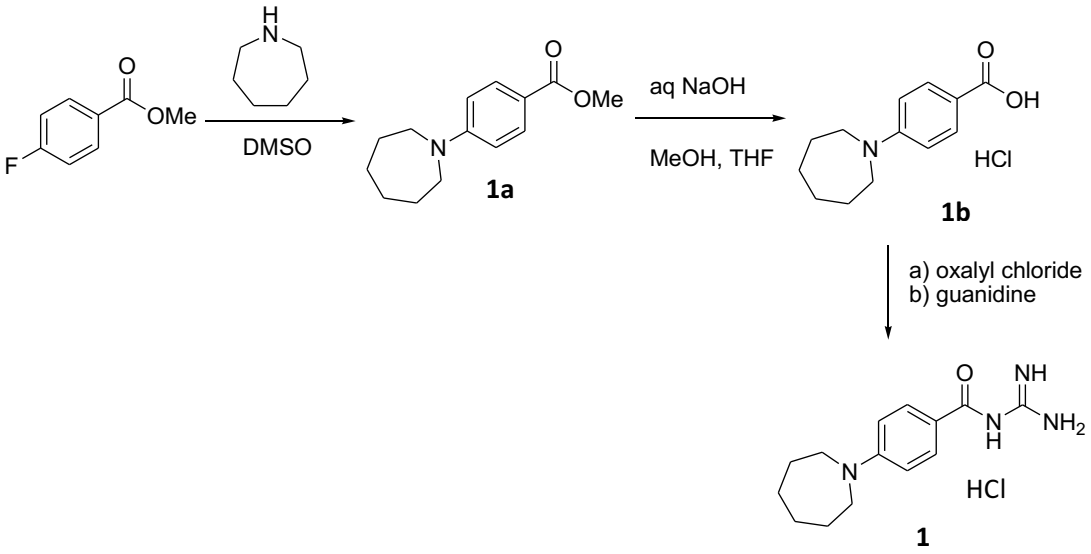


^{1}H NMR (400 MHz, $CDCl_3$): δ 1.9 (s, 2H), 3.9 (s, 3H), 7.7 (dd, J = 1.6 Hz, J = 8.5 Hz, 1H), 7.8 (d, J = 8.5 Hz, 1H), 7.9 (d, J = 8.5 Hz, 1H), 8.0 (s, 1H), 8.1 (s, 1H), 8.1 (dd, J = 1.6 Hz, J = 8.5 Hz, 1H), 8.2 (s, 1H), 8.5 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 21.5, 122.2, 122.4, 124.9, 126.6, 127.1, 128.8, 128.9, 130.0, 131.2, 131.7, 135.2, 136.1, 136.8, 163.2, 172.5, 175.9. MS (ESI) m/z: $[M+H]^+$ Calculated for $C_{16}H_{15}N_5O$: 294.13; Found: 294.4. Purity: >99%.

N-((5-phenylisoxazol-3-yl)methyl)adamantan-1-amine (**M2WJ352**) was synthesized as previously reported[2]. A fumarate salt was made by adding methanolic solution of fumaric acid to a solution of the free amine in MeOH. The mixture was vigorously stirred at RT for 15 min. The precipitated fumarate salt was filtered off and washed with cold MeOH, and dried *in vacuo* to yield **t**he final product as brown crystalline solid in very high yield.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.85-7.9 (m, 2H), 7.5(m, 3H), 7.1 (s, 1H), 6.5 (s, 2H), 4.6 (br s), 4.0 (s, 2H), 2.05-2.1 (m, 3H), 1.75 (s, 6H), 1.6 (m, 6H). MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{20}H_{24}N_2O$: 309.1; Found: 309.4. Purity: 98.6%.

Synthetic Procedures for Compound (1)



Synthesis of methyl 4-(azepan-1-yl)benzoate (1a)

A solution of methyl 4-fluorobenzoate (4.0 g, 25.5 mmol) and hexamethyleneimine (6.0 mL, 51.0 mmol) in dimethylsulfoxide (30 mL) was heated at 60 °C for 12 hours. The mixture was diluted with water (300 mL) which resulted in the formation of a precipitate. The solid was filtered off and washed with water. The solid was then dissolved in dichloromethane (500 mL) and ethyl acetate (500 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The resultant solid was suspended in ethyl acetate and filtered to give **1a** as a solid (3.05 g, 51%).

Synthesis of 4-(azepan-1-yl)benzoic acid (1b)

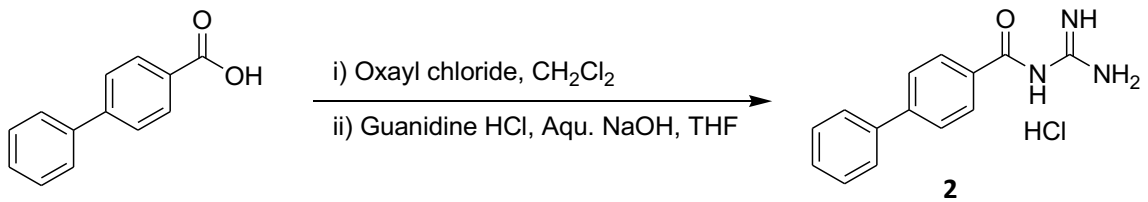
A solution of compound **1a** (3.05 g, 13.1 mmol) in methanol (50 mL) and 0.8 *N* aqueous NaOH (50 mL) was heated at 60 °C for 6 hours. The mixture was concentrated *in vacuo* to completely remove the methanol. The resultant aqueous solution was extracted with dichloromethane (discarded), acidified with 1.0 N HCl, and extracted with ethyl acetate. The ethyl acetate extract was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give a solid (quantitative yield). This material was used crude in the following step.

Synthesis of 4-(azepan-1-yl)-*N*-carbamimidoylbenzamide hydrochloride (1)

To a suspension of compound **1b** (918 mg, 4.19 mmol) in dichloromethane (20 mL) containing 4 drops of *N,N*-dimethylformamide was added oxalyl chloride (0.38 mL, 4.40 mmol). After being stirred for 1 hour, the mixture was concentrated *in vacuo* and then dissolved in tetrahydrofuran (50 mL). In a separate vessel, NaOH (8.0 g, 0.2 mol) was dissolved in water (70 mL) followed by the addition of guanidine HCl (19.1 g, 0.2 mol). The THF solution was then pipetted into the aqueous guanidine solution and stirred overnight. The volatile organics were removed *in vacuo* and the resulting aqueous mixture was partitioned between dichloromethane and water. The separated dichloromethane layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude material was chromatographed (silica gel, Silica Flash®-Silicycle) using a gradient elution, 5% - 20% methanol in dichloromethane. This material was dissolved in 1.25 M HCl and concentrated *in vacuo* to give a solid, which was suspended in ethyl acetate and filtered. This solid was then suspended in acetonitrile and filtered. The solid was suspended in acetonitrile for a second time and filtered off to give **1** as an off-white solid (70 mg, 6%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.4 – 1.5 (m, 4H), 1.6 - 1.7 (br s, 4H), 3.5 (t, *J* = 6.0 Hz, 4H), 6.7 (d, *J* = 9.2 Hz, 1H), 7.9 (d, *J* = 9.2 Hz, 1H), 8.3 (br s, 2H), 8.7 (br s, 2H), 11.5 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.5, 26.9, 49.4, 110.8, 116.2, 131.1, 152.9, 156.4, 167.1. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{14}H_{21}N_4O$: 261.34; Found: 261.5. Purity: 96.8%.

Synthetic Procedure for Compound (2)



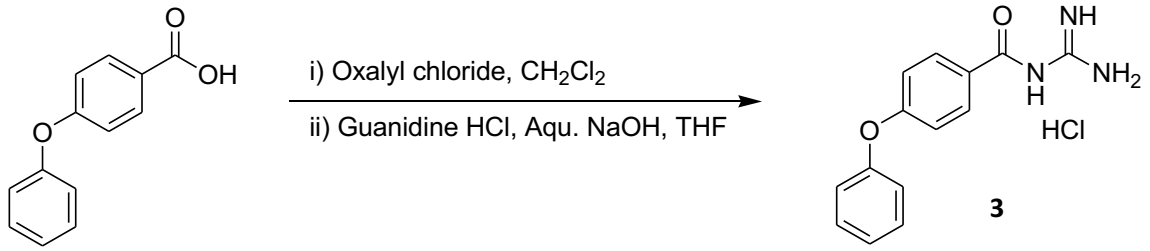
2

Synthesis of *N*-carbamimidoyl- [1,1'-biphenyl]- 4carboxamide hydrochloride (2)

To a suspension of biphenyl-2-carboxylic acid (1.0 g, 5.04 mmol) in DCM (15 mL) containing a drop of DMF was added oxalyl chloride (0.52 mL, 6.05 mmol). The mixture was stirred for 4 hours at room temperature and then concentrated *in vacuo* to give an oily residue. The residue was then dissolved in THF (15 mL). A stock solution of free-base guanidine was prepared as follows. Guanidine HCl (15.9 g, 0.17 mol) was added to a 2 M aq. NaOH solution (8 g NaOH, 0.2 mol, dissolved in 100 mL of H_2O). The THF solution of the acid chloride was then added to the free-base guanidine solution (15 mL) and stirred overnight at room temperature. The mixture was then concentrated *in vacuo* and the resulting aqueous mixture was extracted with EtOAc. The organic extract was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography (silica gel, Silica Flash®-Silicycle) using 5% MeOH in DCM followed by 5% (7 *N* NH_3 in MeOH) in DCM. The purified material was dissolved in MeOH followed by the addition of 1.25 M HCl in MeOH (4 mL). The solution was concentrated *in vacuo* to give an off-white solid. The solid was suspended in acetonitrile to give a white crystalline solid which was collected via filtration to give **2** as a white solid (800 mg, 58%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.4-7.4 (M, 1H), 7.4-7.5 (m, 2H), 7.7-7.8 (m, 2H), 7.9 (d, *J* = 8.4 Hz, 2H), 8.2 (d, *J* = 8.8 Hz, 2H), 8.6 (br s, 2H), 8.8 (br s, 2H), 12.1 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 127.4, 127.5, 129.0, 129.5, 129.6, 130.2, 138.9, 145.7, 156.2, 167.5. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{14}H_{14}N_3O$: 240.28; Found: 240.4. Purity: 98.4%.

Synthetic Procedure for Compound (3)

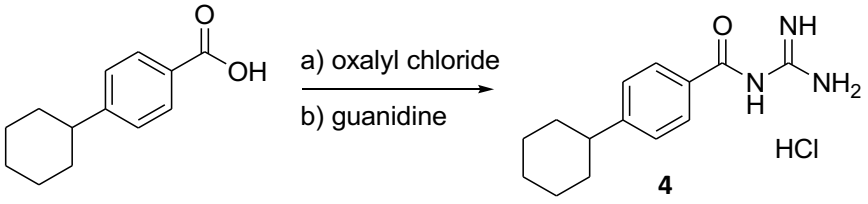


Synthesis of *N*-carbamimidoyl-4-phenoxybenzamide hydrochloride (3)

To a suspension of 4-phenoxybenzoic acid (2.0 g, 9.34 mmol) in DCM (30 mL) containing a drop of DMF was added oxalyl chloride (0.96 mL, 11.2 mmol). The mixture was stirred for 4 hours at room temperature and then concentrated *in vacuo* to give an oily residue. The residue was then dissolved in THF (15 mL). A stock solution of free-base guanidine was prepared as follows: Guanidine HCl (19.1 g, 0.2 mol) was added to a 2 M aq. NaOH solution (8 g NaOH (0.2 mol) dissolved in 100 mL H_2O). The THF solution of the acid chloride was then added to the free-base guanidine solution (15 mL) and left to stir at room temperature overnight. After stirring overnight, the mixture was concentrated *in vacuo* and the resulting material was partitioned between EtOAc and water. The separated EtOAc layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude material was dissolved in MeOH which resulted in the formation of a white solid, then 1.25 M HCl in MeOH was added but the solid did not dissolve. The solid was filtered (704 mg) and NMR spectrum is consistent with the structure. The filtrate was concentrated *in vacuo* to give a thick syrup. The syrup was dissolved in acetonitrile which resulted in the formation of a white solid. After being stirred overnight at room temperature, the solid was collected by filtration to give **3** as a white solid (1.16 g, 43%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.0 - 7.1 (m, 2H), 7.1 - 7.1 (m, 2H), 7.2 - 7.3 (m, 1H), 7.4 – 7.5 (m, 2H), 8.1 - 8.2 (m, 2H), 8.5 (br s, 2H), 8.7 (br s, 2H), 12.0 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.5, 120.6, 125.4, 125.6, 130.8, 131.5, 155.0, 156.2, 162.4, 167.0. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{14}H_{14}N_3O_2$: 256.28; Found: 256.4. Purity: 97.2%.

Synthetic Procedure for Compound (4)



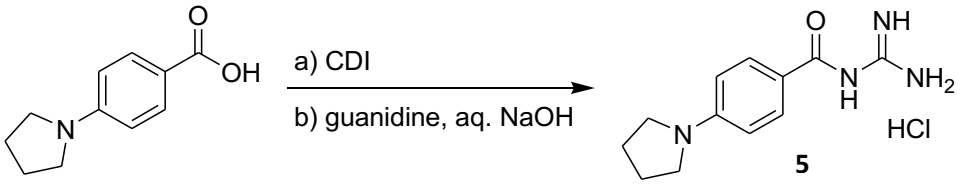
4

Synthesis of *N*-carbamimidoyl-4-cyclohexylbenzamide hydrochloride (4)

To a suspension of 4-cyclohexylbenzoic acid (4.00 g, 19.6 mmol) in dichloromethane (80 mL) containing oxalyl chloride (1.85 mL, 21.5 mmol) was added dimethylformamide (3 drops). After being stirred for 4 hours, the mixture was concentrated *in vacuo* and the residue dissolved in tetrahydrofuran (50 mL). In a separate vessel, NaOH (8.0 g, 0.2 mol) was dissolved in water (70 mL) followed by the addition of guanidine HCl (19.1 g, 0.2 mol). The tetrahydrofuran solution was then pipetted into the aqueous guanidine solution and stirred for 2 hours. The volatile organics were removed *in vacuo*, resulting in the formation of a white precipitate. The aqueous mixture was extracted with dichloromethane, but the white precipitate did not dissolve. The solid was filtered off and washed with dichloromethane. The solid was then suspended in methanol followed by the addition of 1.25 M HCl in methanol. The solution required heating to get complete dissolution. Upon cooling to ambient temperature, a white precipitate formed and was filtered off to give the title compound **4** as a white solid (800 mg, 14%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.1 - 1.3 (m, 1H), 1.3 -1.5 (m, 4 H), 1.6 - 1.7 (m, 1H), 1.7 - 1.8 (m, 4H), 2.5 - 2.6 (m, 1H), 7.4 (d, *J* = 8.4 Hz, 2H), 8.0 (d, *J* = 8.4 Hz, 2H), 8.5 (br s, 2H), 8.7 (br s, 2H), 11.9 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.9, 26.6, 31.1, 33.9, 44.2, 127.6, 129.1, 154.5, 156.2, 167.8. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{14}H_{20}N_3O$: 246.33; Found: 246.4. Purity: >99.0%.

Synthetic Procedure for Compound (5)



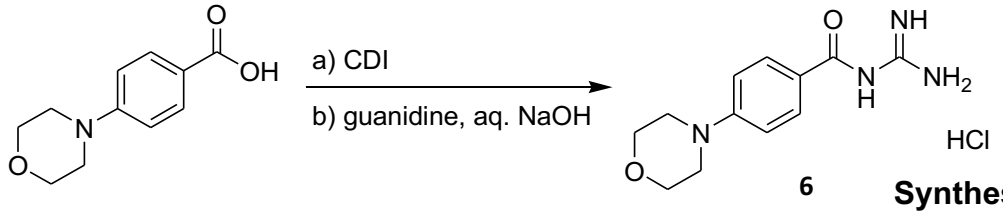
Synthesis of *N*-carbamimidoyl-4-(pyrrolidin-1-yl)benzamide hydrochloride (5)

1',1'-Carbonyldiimidazole (2.02 g, 12.6 mmol) was added in a single portion to a suspension of 4-(4-pyrrolidinyl)benzoic acid (2.0 g, 10.5 mmol) in THF (20 mL).

After 15 minutes, no visible reaction (I.e., bubbling of CO_2) was apparent. DMF (4 mL) was added as a co-solvent and bubbles started to form. A stock solution of free-base guanidine was prepared as follows. Guanidine HCl (19.1 g, 0.2 mol) was added to an aqueous NaOH solution (8 g NaOH (0.2 mol) dissolved in 70 mL H_2O). The THF solution of the acyl imidazole was then added to the free-base guanidine solution (70 mL) and stirred overnight to give a white precipitate. The precipitate was filtered off, washed with water, and air-dried. The solid was suspended in MeOH followed by the addition of 1.25 M HCl in MeOH (10 mL) to give a light-yellow precipitate which was collected by filtration to give **5** as a light yellow solid (1.44 g, 51%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.9 – 2.0 (m, 4H), 3.2 – 3.4 (m, 4H), 6.6 (d, *J* = 9.2 Hz, 2H), 8.0 (d, *J* = 8.8 Hz, 2H), 8.3 (br s, 2H), 8.7 (br s, 2H), 11.6 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.3, 47.7, 111.4, 116.6, 130.9, 151.5, 156.7, 167.6. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{12}H_{17}N_4O$: 233.29; Found: 233.4. Purity: >99.0%.

Synthetic Procedure for Compound (6)



6

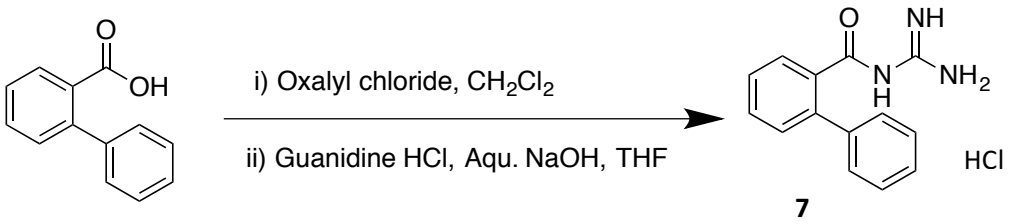
Synthesis of *N*-carbamimidoyl-4-morpholinobenzamide hydrochloride (6)

1,1′-Carbonyldiimidazole (1.88 g, 11.6 mmol) was added in a single portion to a suspension of 4-(4-morpholinyl)benzoic acid (2.0 g, 9.65 mmol) in THF (20 mL). After 15 minutes, no visible reaction (I.e., bubbling of CO_2) was apparent. DMF (4 mL) was added as a co-solvent and bubbles started to form. A stock solution of free-base guanidine was prepared as follows. Guanidine HCl (19.1 g, 0.2 mol) was added to an aqueous NaOH solution (8 g NaOH (0.2 mol) dissolved in 70 mL H_2O). The THF solution of the acyl imidazole was then added to the free-

base guanidine solution (70 mL) and stirred overnight. The mixture was concentrated *in vacuo* resulting in the formation of a white precipitate. The precipitate was filtered off and washed with water. The solid was briefly air-dried and then suspended in MeOH (solid would not dissolve). 1.25 M HCl in MeOH (12 mL) was then added and the suspension was stirred at room temperature for 30 minutes. The solid was collected via filtration to give **6** as a white solid (1.40 g, 51%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 3.0 - 3.3 (m, 4H), 3.6 - 3.7 (m, 4H), 7.0 (d, *J* = 9.2 Hz, 2H), 8.0 (d, *J* = 9.2 Hz, 2H), 8.4 (br s, 2H), 8.8 (br s, 2H), 11.7 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.9, 66.2, 113.4, 119.5, 130.7, 154.9, 156.4, 167.1. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{12}H_{17}N_4O_2$: 249.29; Found: 249.4. Purity: >99.0%.

Synthesis Procedure for Compound (7)



7

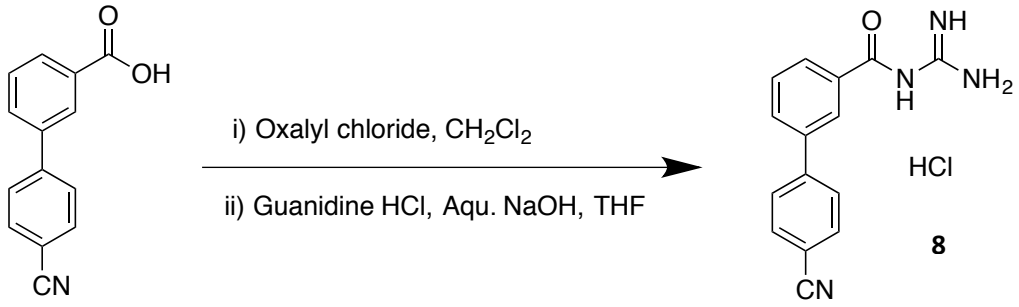
Synthesis of *N*-carbamimidoyl-[1,1'-biphenyl]-2-carboxamide hydrochloride (7)

To a suspension of [1,1'-biphenyl]-2-carboxylic acid (1.0 g, 5 mmol) in dichloromethane (20 mL) containing a drop of DMF was added oxalyl chloride (0.45 mL, 5 mmol). The mixture was stirred for 3 hours at room temperature and then concentrated *in vacuo* to give an oily residue which was dissolved in THF (30 mL). A stock solution of free-base guanidine was prepared as follows. Guanidine HCl (18.5 g, 0.19 mol) was added to a 2 M aq. NaOH solution (8 g NaOH (0.2 mol) dissolved in 100 mL H_2O). The THF solution of the acid chloride was then added to the free-base guanidine solution (50 mL) and stirred for 3 hours at room temperature. The mixture was concentrated *in vacuo* without heat,

which resulted in the formation of a thick white solid. Upon sitting overnight, most of the solid had dissolved (the thick solid was apparently the result of the cooling). This material was partitioned between EtOAc and saturated brine solution. The separated EtOAc layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give an off-white solid. The solid was dissolved in MeOH followed by the addition of 1 *N* aqueous HCl (aqueous acid used to avoid potential chloro hydrolysis with anhydrous acid) and then concentrated *in vacuo*. After the MeOH had evaporated off, a white crystalline solid precipitated from the aqueous solution. The solid was collected by filtration to give **7** as a white solid (200 mg).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.9 – 7.8 (m, 1H), 7.6 – 7.6 (m, 2H), 7.5 (s, 1H), 7.5 – 7.4 (m, 2H), 7.5 – 7.4 (m, 1H), 7.4 (s, 3H), 7.4 – 7.3 (m, 2H), 7.3 – 7.2 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.4, 160.5, 137.3, 137.1, 135.8, 131.4, 129.6, 128.8, 128.6, 127.4. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{14}H_{13}N_3O$ 240.28; Found 240.9.

Synthesis Procedure for Compound (8)



8

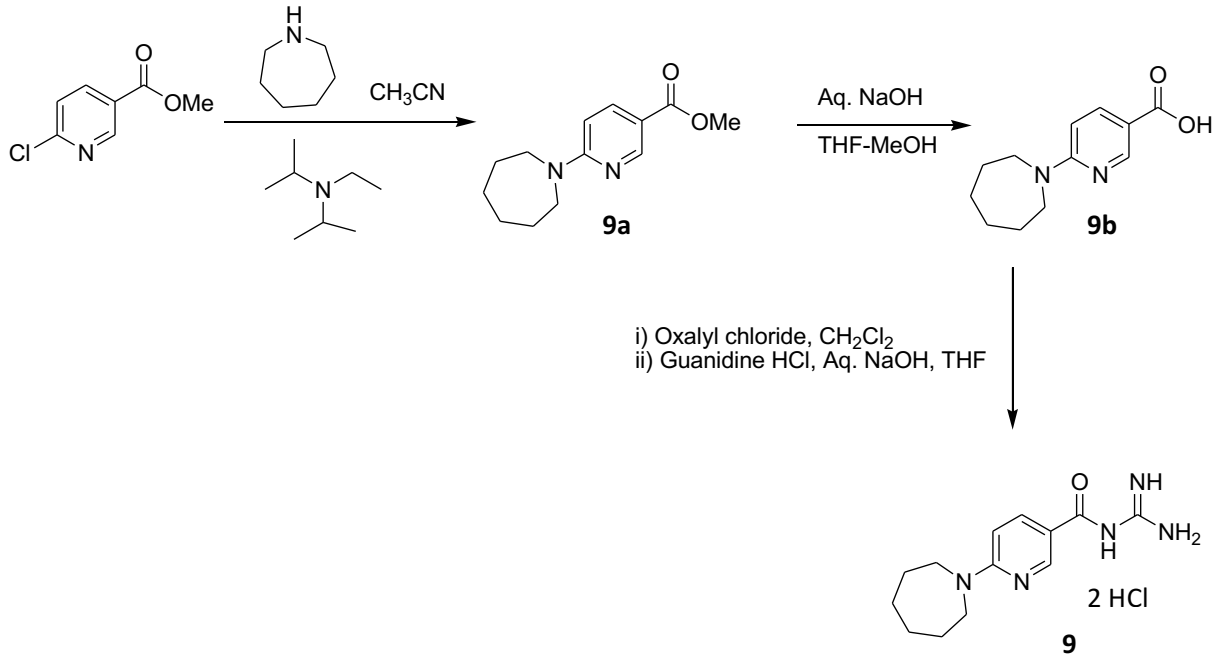
Synthesis of *N*-carbamimidoyl-4'-cyano-[1,1'-biphenyl]-3-carboxamide hydrochloride (8)

To a suspension of 4'-cyano-[1,1'-biphenyl]-3-carboxylic acid (3.0 g, 13.5 mmol) in dichloromethane (50 mL) containing a drop of DMF was added oxalyl chloride (1.2 mL, 14 mmol). The mixture was stirred for 3 hours at room temperature and then concentrated *in vacuo* to give an oily residue which was dissolved in THF

(30 mL). A stock solution of free-base guanidine was prepared as follows. Guanidine HCl (18.5 g, 0.19 mol) was added to a 2 M aq. NaOH solution (8 g NaOH (0.2 mol) dissolved in 100 mL H_2O). The THF solution of the acid chloride was then added to the free-base guanidine solution (50 mL) and stirred for 3 hours at room temperature. The mixture was concentrated *in vacuo*, which resulted in the formation of a thick white solid. Upon sitting overnight, most of the solid had dissolved (the thick solid was apparently the result of the cooling). This material was partitioned between EtOAc and saturated brine solution. The separated EtOAc layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give an off-white solid. The solid was dissolved in MeOH followed by the addition of 1 *N* aqueous HCl and then concentrated *in vacuo*. After the MeOH had evaporated off, a white crystalline solid precipitated from the aqueous solution. The solid was collected by filtration to give **8** as a white solid (780 mg).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.3 – 8.2 (m, 1H), 8.0 – 7.9 (m, 3H), 7.9 – 7.8 (m, 2H), 7.7 – 7.7 (m, 2H), 3.8 (s, 2H), 2.0 (s, 1H) ^{13}C NMR (100 MHz, DMSO-d_6): 110.8, 117.5, 127.1, 128.2, 129.6, 130.7, 132.5, 133.3, 134.0, 141.7, 142.4, 160.4, 167.0. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{15}H_{12}N_4O$ 261.10; Found 261.9. Purity: >99.0%.

Synthetic Procedures for Compound (9)



Synthesis of methyl 6-(azepan-1-yl)nicotinate (9a)

A solution of methyl 6-chloronicotinate (3.12 g, 18.2 mmol), hexamethyleneimine (1.98 g, 20.0 mmol), and *N,N*-diisopropylethylamine (4.70 g, 36.4 mmol) in acetonitrile (5 mL) was stirred at ambient temperature for 3 days. The mixture was concentrated *in vacuo* and the crude material was partitioned between dichloromethane and 0.1 M NaOH. The separated dichloromethane layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude material was chromatographed (SiO_2) using dichloromethane followed by dichloromethane:diethyl ether (8:1) as eluent to give **9a** as an oil (3.08 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.4 – 1.6 (m, 4H), 1.7 – 1.8 (m, 4H), 3.6 – 3.8 (m, 4H), 3.8 (s, 3H), 6.4 (d, *J* = 9.2 Hz, 1H), 7.9 (dd, *J* = 2.4 Hz, *J* = 9.2 Hz, 1H), 8.7 (d, *J* = 2.4 Hz, 1H).

Synthesis of 6-(azepan-1-yl)nicotinic acid hydrochloride (9b)

A solution of compound **9a** (3.08 g, 13.1 mmol) in tetrahydrofuran (7 mL), methanol (7 mL), and NaOH (1.05 g, 26.3 mmol) dissolved in water (7 mL) was stirred at ambient temperature for 1 hour. The mixture was heated at 60 °C for 1.5 hours and concentrated *in vacuo* to remove the volatile organics. The resulting aqueous solution was acidified with 5 M HCl (10.5 mL) and then concentrated to dryness. The solid was concentrated from toluene to remove remaining moisture. The material was then scraped off the walls of the flask and concentrated from acetonitrile. This was repeated a second time to give a powder (contaminated with NaCl). This material was used crude in the following step.

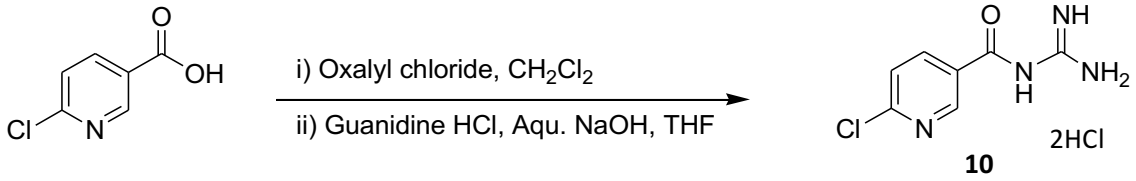
Synthesis of 6-(azepan-1-yl)-*N*-carbamimidoylnicotinamide hydrochloride (9)

To a suspension of the compound **9b** (3.36 g, 13.1 mmol) in dichloromethane (100 mL) containing 4 drops of *N,N*-dimethylformamide was added oxalyl chloride (1.35 mL, 15.7 mmol). After being stirred for 1 hour, the mixture was

concentrated *in vacuo* and then dissolved in THF (50 mL). In a separate vessel, NaOH (8.0 g, 0.2 mol) was dissolved in water (70 mL) followed by the addition of guanidine HCl (19.1 g, 0.2 mol). The THF solution was then pipetted into the aqueous guanidine solution and stirred overnight. The volatile organics were removed *in vacuo* and the resulting aqueous mixture was partitioned between dichloromethane and water. A solid slowly began to crystallize from the dichloromethane phase, and within a few minutes, a significant amount of solid had formed. The separated dichloromethane layer was filtered and the collected solid was washed with water and then dichloromethane. After being air-dried, 1.36 g of a white solid was obtained. The solid was dissolved in methanol followed by the addition of 1.25 M HCl in methanol (5 mL). Upon concentration of this solution, a crystalline white solid formed. This material was suspended in acetonitrile, and filtered to give **9** as a white crystalline solid (970 mg, 25%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.3 - 1.5 (m, 4H), 1.7 (br s, 4H), 3.6 (br s, 4H), 6.7 (d, *J* = 9.2 Hz, 2H), 8.1 (dd, *J* = 2.8 Hz, *J* = 9.2 Hz, 1H), 7.8 - 8.8 (m, 4H), 8.8 (d, *J* = 2.4 Hz, 1H), 11.7 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.7, 27.2, 47.8, 105.0, 115.2, 137.5, 150.9, 157.3, 160.0, 168.0. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{13}H_{20}N_5O$: 262.33; Found: 262.5. Purity: >99.0%.

Synthetic Procedures for Compound (10)



10

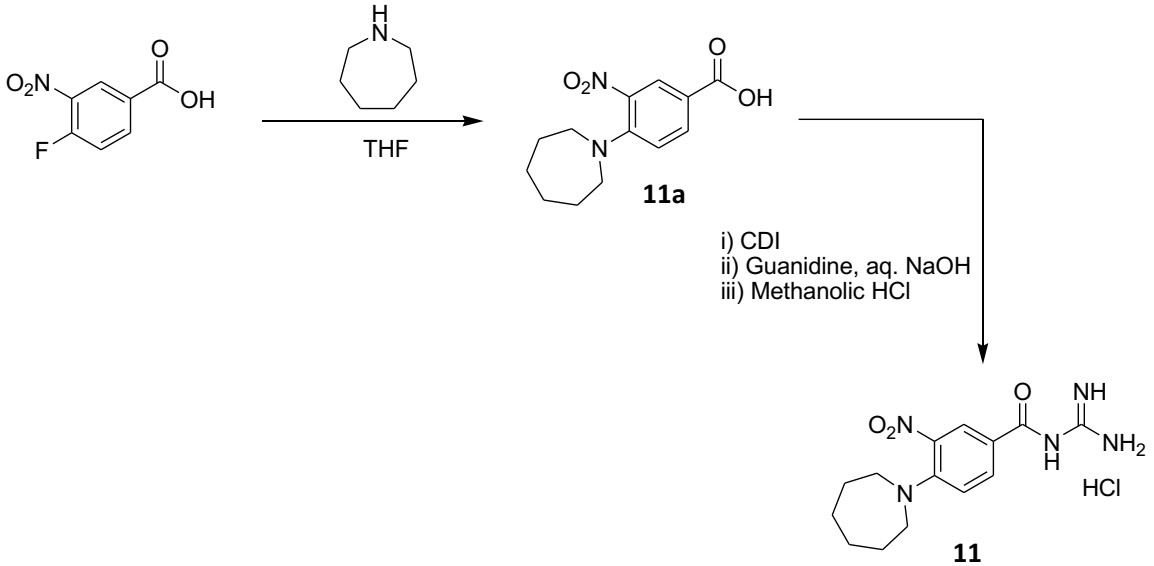
Synthesis of *N*-(6-Chloro-pyridine-3-carbonyl)-guanidine hydrochloride (10)

To a suspension of 6-chloronicotinic acid (2.0 g, 12.7 mmol) in dichloromethane (50 mL) containing a drop of DMF was added oxalyl chloride (1.2 mL, 14.0 mmol). The mixture was stirred for 3 hours at room temperature and then concentrated *in vacuo* to give an oily residue which was dissolved in THF (30

mL).A stock solution of free-base guanidine was prepared as follows. Guanidine HCl (18.5 g, 0.19 mol) was added to a 2 M aq. NaOH solution (8 g NaOH (0.2 mol) dissolved in 100 mL H_2O). The THF solution of the acid chloride was then added to the free-base guanidine solution (50 mL) and stirred for 3 hours at room temperature. The mixture was concentrated *in vacuo* without heat, which resulted in the formation of a thick white solid. Upon sitting overnight, most of the solid had dissolved (the thick solid was apparently the result of the cooling). This material was partitioned between EtOAc and saturated brine solution. The separated EtOAc layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give an off-white solid. The solid was dissolved in MeOH followed by the addition of 1 *N* aqueous HCl (aqueous acid used to avoid potential chloro hydrolysis with anhydrous acid) and then concentrated *in vacuo*. After the MeOH had evaporated off, a white crystalline solid precipitated from the aqueous solution. The solid was collected by filtration to give **10** as a white solid (490 mg, 20%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.7 (d, *J* = 8.4 Hz, 1H), 8.5 (dd, *J* = 2.4 Hz, *J* = 8.4 Hz, 1H), 8.6 - 8.7 (m, 4 H), 9.1 (d, *J* = 2.4 Hz, 1H), 12.4 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 124.9, 127.2, 140.0, 150.6, 155.1, 155.8, 165.8. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_7H_8ClN_4O$: 199.62; Found: 199.4. Purity: 96.5%.

Synthetic Procedures for Compound (11)



Synthesis of 4-Azepan-1-yl-3-nitro-benzoic acid (11a)

Hexamethyleneimine (2.2 g, 21.6 mmol) was slowly added to a solution of 4-fluoro-3-nitrobenzoic acid (2.0 g, 10.8 mmol) in THF (10 mL). The reaction mixture was left to stir at room temperature for 1 hour. The mixture was then concentrated *in vacuo* and the crude material was partitioned between DCM (50 mL) and 0.1 N HCl (40 mL). The separated DCM layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography (silica gel, Silica Flash®-Silicycle) using DCM followed by DCM:Et_2O (8:1) to give **11a** as an oil (3.08 g, quantitative yield) which was used directly for the coupling step.

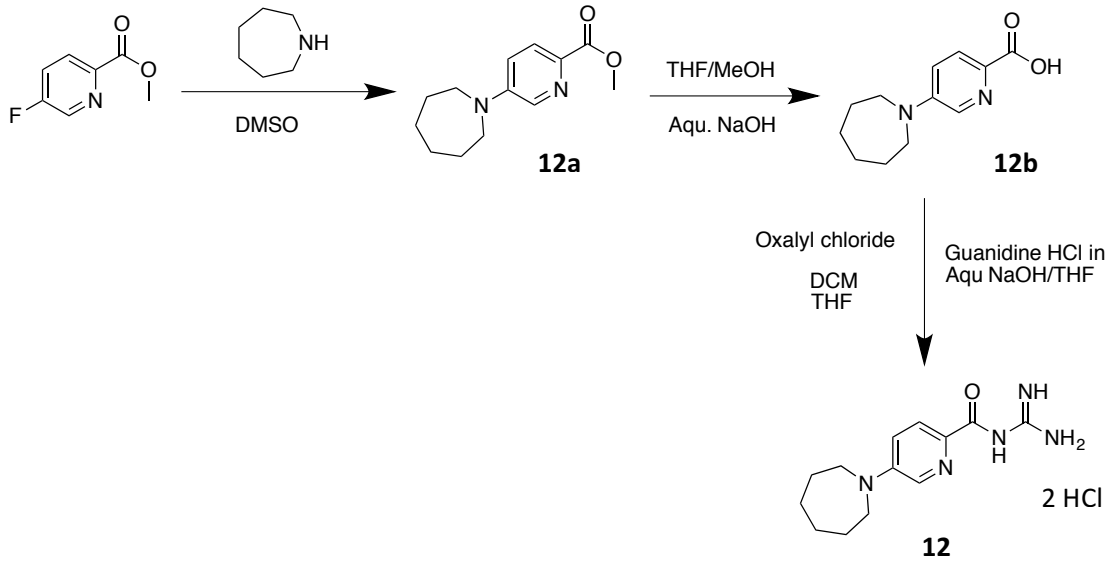
Synthesis of *N*-(4-Azepan-1-yl-3-nitro-benzoyl)-guanidine hydrochloride (11)

1,1'-Carbonyldiimidazole (1.23 g, 7.58 mmol) was added in a single portion to a suspension of compound **11a** (1.67 g, 6.32 mmol) in tetrahydrofuran (20 mL) and stirred for 2 hours. In a separate vessel, NaOH (8.0 g, 0.2 mol) was dissolved in water (70 mL) followed by the addition of guanidine HCl (19.1 g, 0.2 mol). The tetrahydrofuran solution was then pipetted into the aqueous guanidine solution and stirred for 2 hours. The volatile organics were removed *in vacuo* resulting in the formation of a yellow gum. The aqueous mixture was then partitioned between dichloromethane and water. The separated organic layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude material was chromatographed (silica gel) using dichloromethane followed by 10% MeOH in dichloromethane as eluent. This material was dissolved in methanol followed by the addition of 1.25 M HCl in methanol. The solution was concentrated *in vacuo* and then dissolved in acetonitrile, resulting in the formation of a crystalline precipitate which was filtered to give **11** as a yellow solid (630 mg, 29%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.4 - 1.5 (m, 4H), 1.7 - 1.8 (m, 4H), 3.2 - 3.3(m, 4H), 7.3 (d, *J* = 9.2 Hz, 1H), 8.2 (dd, *J* = 2.4 Hz, *J* = 9.2 Hz, 1H), 8.4 (br s, 2H), 8.5 (d, *J* = 2.4 Hz, 1H), 8.6 (d, 1H), 11.9 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-

d_6): δ 27.4, 27.5, 51.4, 117.8, 128.6, 132.5, 136.6, 147.9, 156.3, 166.0. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{14}H_{20}N_5O_3$: 306.34; Found: 306.4. Purity: >99.0%.

Synthetic Procedures for Compound (12)



Synthesis of methyl 5-(azepan-1-yl)picolinate (12a)

A solution of 5-Fluoro-pyridine-2-carboxylic acid methyl ester (2g, 12.9 mmol), hexamethyleneimine (2.18 mL) ad potassium carbonate (2.67 g, 19.3 mmol) in DMSO (10 mL) was heated at 90 °C for 4 hours. The reaction mixture was quenched with water and extracted with EtOAc (3 X 20 mL). The combined organic layers were washed with brine (30 mL) and dried over Na_2SO_4 filtered and concentrated under reduced pressure to give a thick pale yellow syrup. The syrup was triturated with hexane, resulting in an off-white solid. The solid was suspended and excess solvent was decanted and finally dried under vacuum and used with no further purification.

Synthesis of 5-(azepan-1-yl)picolinic acid (12b)

A mixture of **12a** (2g, 8.54 mmol) in THF (10 mL) and MeOH (10 mL) was stirred at room temperature until a clear solution was obtained, then NaOH (0.68 g) pre-dissolved in water (10 mL) was added. The mixture was stirred at 50 °C. the

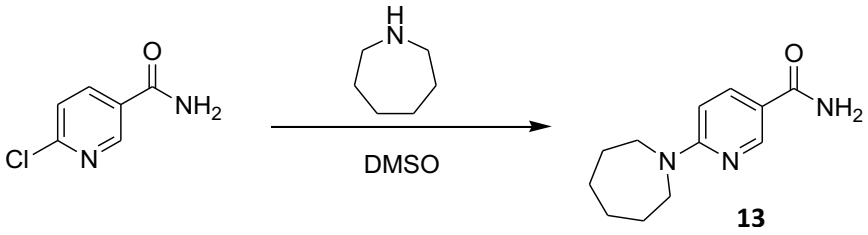
reaction mixture was concentrated under reduced pressure to remove volatile organic solvent. The resulting aqueous solution was acidified with 5M HCl (Aq.) until pH 3-4 is achieved and the carboxylic acid precipitates out of solution. The solid was filtered and recrystallized in acetonitrile to give an off-white powder that was used in the following step wit no further purification.

Synthesis of 5-(azepan-1-yl)-*N*-carbamimidoylpicolinamide (12)

1,1'-Carbonyldiimidazole (1.23 g, 7.58 mmol) was added in a single portion to a suspension of the solution of **12b** (1.54 g, 7 mmol) in THF (10 mL) & DMF (5 mL). The mixture was stirred for 3 hours. A stock solution of free-base guanidine was prepared as follows. Guanidine HCl (6.08 g, 63.6 mmol) was added to an aqueous NaOH solution (2.54 g NaOH (63.6 mmol) dissolved in 10 mL H_2O). The THF solution of the acyl imidazole was then added to the free-base guanidine solution (10 mL) and stirred for 3.5 hours. The reaction mixture was left to stir at room temperature overnight. The mixture was concentrated *in vacuo* resulting in the formation of a yellow-red oil. The aqueous mixture was diluted with NaOH (0.1 M, 20 mL), and the aqueous was extracted with EtOAc (3 x 25 mL). During the extraction, a precipitate began to form from the EtOAc layer and more EtOAc was added but it did not dissolve the ppt. The precipitate was separately collected and the organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. This material was dissolved in methanol followed by the addition of 1.25 M HCl in methanol. The solution was concentrated *in vacuo* and then dissolved in acetonitrile, resulting in the formation of a crystalline precipitate, which was filtered to give **12** as a yellow solid. (750 mg, 32%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.4 (s, 1H), 8.8 (s, 4H), 8.1 (d, J = 2.8 Hz, 1H), 7.9 (d, J = 8.9 Hz, 1H), 7.2 (dd, J = 2.8, 9.0 Hz, 1H), 3.6 (t, J = 6.0 Hz, 4H), 1.7 (t, J = 5.5 Hz, 4H), 1.5 (q, J = 3.2, 3.6 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.5, 155.8, 148.0, 132.8, 132.5, 125.5, 117.7, 49.3, 26.6, 26.5. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{13}H_{19}N_5O$: 261.16; Found: 261.8. Purity: >99.0%.

Synthetic Procedures for Compound (13)



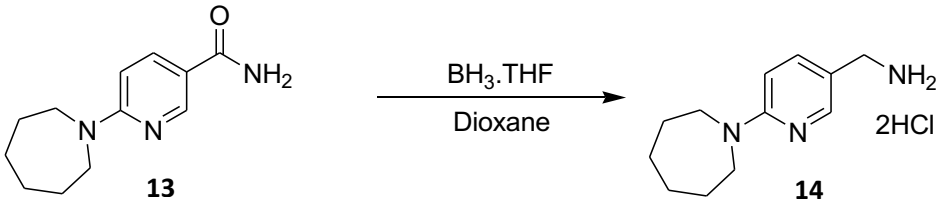
13

Synthesis of 6-Azepan-1-yl-nicotinamide (13)

A solution of 6-chloronicotinamide (15 g, 95.8 mmol) and hexamethyleneimine (11.4 g, 115 mmol) in DMSO (20 mL) was heated at 60 ^{o}C for 12 hours after which TLC indicate complete consumption of the starting material. The mixture was diluted with water which resulted in the formation of a thick precipitate and required further dilution to give a free-flowing mixture to filter (300 mL water). The solid was filtered off and washed with water (50 mL). The solid was dissolved in DCM (500 mL) and EtOAc (500 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The resulting solid was suspended in EtOAc and filtered to give **13** as a beige crystalline solid (12 g, 57%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.4 - 1.5 (m, 4H), 1.6 – 1.7 (m, 4H), 3.5 – 3.7 (m, 4H), 6.6 (d, *J* = 9.2 Hz, 1H), 7.0 (br s, 1H), 7.6 (br s, 1H), 7.9 (dd, *J* = 2.4 Hz, *J* = 9.2 Hz, 1H), 8.5 (d, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.8, 27.4, 47.6, 104.5, 117.2, 137.0, 149.0, 159.3, 167.5. MS (ESI) m/z: $[M - H]^+$ Calculated for $C_{12}H_{16}N_3O$: 218.27; Found: 218.4. Purity: >99.0%.

Synthetic Procedures for Compound (14)



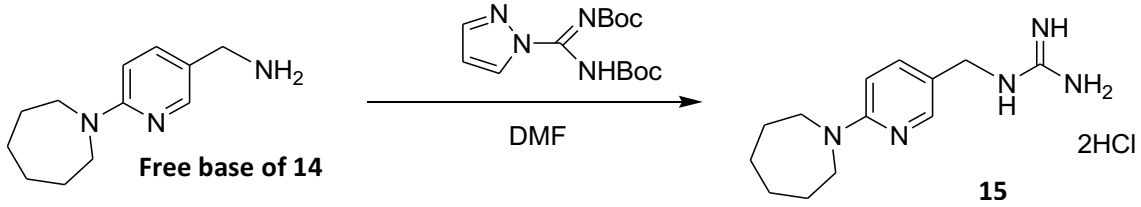
13 **14**

Synthesis of (6-Azepan-1-yl-pyridin-3-yl)-methanamine (14)

BH_3.THF (75 mL, 74.3 mmol) was slowly added to a solution of compound **13** (5.82 g, 26.5 mmol) in dioxane (10 mL) over a period of 3 hours at room temperature. Upon complete addition, the mixture was heated at 55 °C for 1 hour. The mixture was quenched slowly by adding to 5 M HCl (150 mL) via pipette (i.e., inverse addition). Upon complete addition, the mixture was stirred at room temperature for 1 hour and then at 60 °C for 20 minutes. After cooling the mixture to room temperature, the mixture was concentrated under vacuum to remove the dioxane. The remaining acidic aqueous solution was basified with aqueous sodium hydroxide (30 g in 100 mL water) and extracted with DCM (2 x 50 mL). The DCM extracts were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give **an** off-white solid. The resulting solid was dissolved in MeOH followed by the addition of 1.25 M HCl in MeOH (48 mL). The mixture was concentrated *in vacuo* and suspended in acetonitrile to give a crystalline precipitate. The solid was collected via filtration to give **14** as an off-white solid (500 mg, 7 %).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.4 - 1.5 (m, 4H), 1.7 - 1.8 (m, 4H), 3.6 - 3.9 (m, 6H), 7.2 (d, *J* = 9.2 Hz, 1H), 7.5 (br s, 1H), 8.1 - 8.3 (m, 3H), 8.4 (d, *J* = 2 Hz, 1H), 8.6 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ26.2, 26.5, 49.0, 50.0, 111.5, 118.3, 141.3, 152.5, 164.4. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{12}H_{20}N_3$: 206.31; Found: 206.5. Purity: 96.1%.

Synthetic Procedures for Compound (15)

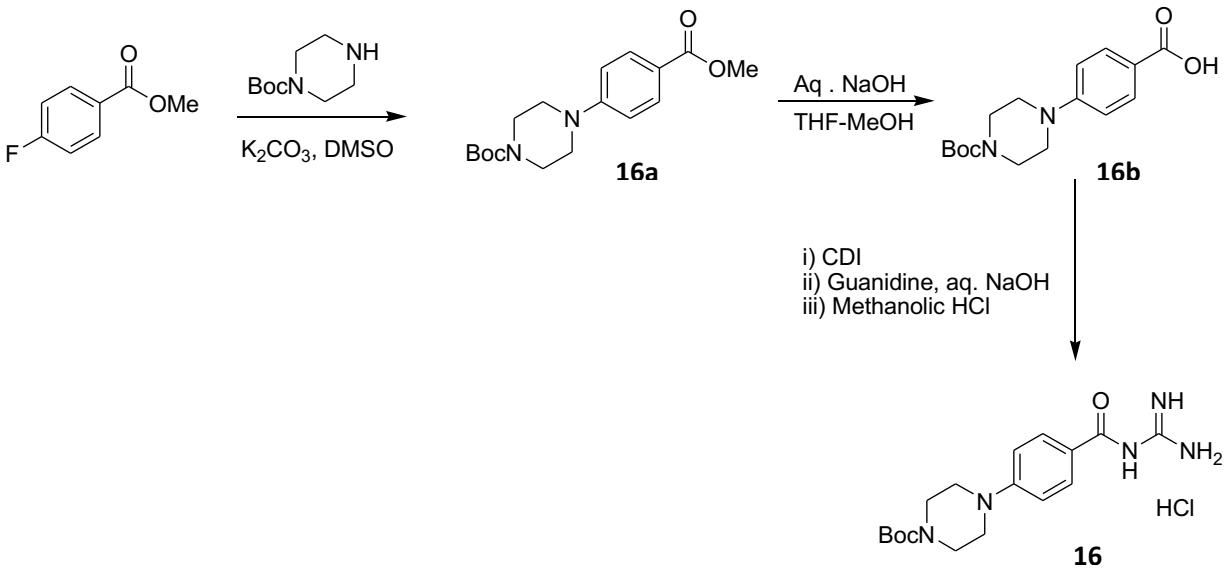


Synthesis of 1-(6-Azepan-1-yl-pyridin-3-ylmethyl)guanidine dihydrochloride (15)

A solution of the free base **14** (720 mg, 3.5 mmol) and N,N′-Di-Boc-1H-pyrazole-1-carboxamidine (1.2 g, 3.86 mmol) in DMF (3 mL) was stirred at room temperature for 16 hours. The mixture was diluted with water until the product oiled out of solution. The oil was rinsed with water 2 more times. The resulting oil was dissolved in DCM, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude material was chromatographed (silica gel, Silia Flash®-Silicycle) using 10% Et_2O in DCM as eluent. The purified fractions were concentrated, dissolved in DCM, and TFA (3 mL) was added. After being stirred for 12 h, the mixture was basified with dilute aq. NaOH. The separated DCM layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude material was dissolved in 1.25 M HCl in MeOH and concentrated *in vacuo*. The solid was suspended in isopropyl alcohol and filtered to give **15** as an off-white solid (160 mg, 14%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.4 – 1.5 (m, 4H), 1.7 – 1.8 (m, 4H), 3.7 – 3.8 (m, 4H), 4.3 (d, *J* = 6.4 Hz, 2H), 7.3 (d, *J* = 9.2 Hz, 1H), 7.3 – 7.8 (br s, 4H), 7.9 (d, *J* = 9.2 Hz, 1H), 8.0 (br s, 1H), 8.4 (dt, *J* = 6, *J* = 12 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.9, 26.3, 26.6, 49.5, 120.3, 121.3, 130.6, 137.0, 157.3, 159.8. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{13}H_{22}N_5$: 248.35; Found: 248.5. Purity: >99.0%.

Synthetic Procedures for Compounds (16)



Synthesis of 4-(4-Methoxycarbonyl-phenyl)-piperazine-1-carboxylic acid *tert*-butyl ester (16a)

A mixture of *N*-Boc-piperazine (6.04 g, 32 mmol), methyl 4-fluorobenzoate (5 g, 32) and potassium carbonate (6.63 g, 48 mmol) in DMSO (33 mL) was stirred at 120 °C for 24 hrs. The reaction mixture was cooled to room temperature and then poured into a vessel containing water (300 mL) resulting in the formation of an off-white precipitate. The mixture was left to stir at room temperature for 30 minutes and the solid was collected via filtration, washed with water (20 mL) and air dried overnight to give **16a** as an off-white solid (4.2 g, 40%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.49 (s, 9H), 3.28 - 3.36 (m, 4H), 3.59 – 3.67 (m, 4H), 3.88 (s, 3H), 6.95 (dd, *J* = 8.4 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 2H).

Synthesis of 4-(4-Carboxy-phenyl)-piperazine-1-carboxylic acid *tert*-butyl ester (16b)

A mixture of **16a** (4.0 g, 12.5 mmol), in THF (125 mL) and MeOH (31 mL) was stirred room temperature until a clear solution was obtained, followed by the addition of 1 *N* NaOH (125 mL) was added. The mixture was stirred at 50 °C for 4 hours after which TLC indicated completed consumption of **16a**. The reaction mixture was cooled to room temperature and then poured into an aqueous solution of HCl (1 M, 200 mL) resulting in the formation of a white precipitate. The mixture was left to stir at room temperature for 30 minutes and the precipitate was collected via filtration, washed with water (15 mL), and air dried for 24 hours to give **16b** as a white solid (3.17 g, 83%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.42 (s, 9H), 3.24 – 3.36 (m, 4H), 3.39 – 3.51 (m, 4H), 6.96 (d, *J* = 8.8 Hz, 1H), 7.78 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 28.65, 47.23, 79.67, 114.22, 120.33, 131.45, 154.11, 154.46, 167.81.

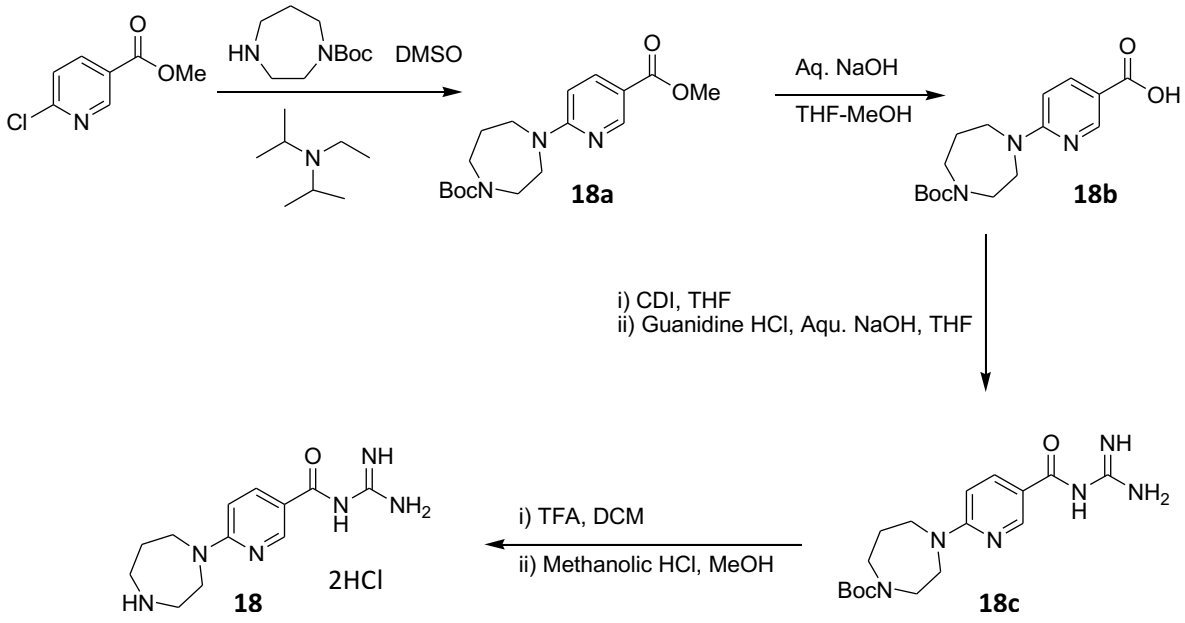
Synthesis of 4-(4-Guanidinocarbonyl-phenyl)-piperazine-1-carboxylic acid *tert*-butyl ester hydrochloride (16)

To a suspension of carboxylic acid **17b** (3.5 g, 11.4 mmol) in THF (15 mL) containing a drop of DMF was added 1'1**′**-carbonyldiimidazole (1.24 g, 13.7 mmol) in a single portion. The mixture was left to stir at room temperature for 18 hours. A stock solution of free-base guanidine was prepared as follows: Guanidine HCl (3.22 g, 34.2 mmol) was added to an aqueous NaOH solution (2 M, 10 mL). The THF solution of the acyl imidazole was then added to the free-base guanidine solution (10 mL) and stirred for 2 hours after which TLC indicated complete consumption of the acyl imidazole and a more polar spot was observed on TLC. Spot stayed on the TLC baseline when using a solvent system of 10% MeOH in DCM but move to an RF ~of 0.4 when a few drop of 7 *N* NH_3 in MeOH was added to the TLC solvent.

The mixture was concentrated *in vacuo* resulting in the formation of a pale yellow residue which was dissolved in EtOAc (35 mL). The organic layer was washed with water (3 x 25 mL), brine (1 x 30 mL), dried (Na_2SO_4), filtered, and concentrated under vacuo to give a pale yellow syrup. The crude product was further purified by flash column chromatography (silica gel, Silica Flash®-Silicycle) using an eluent of dichloromethane, 5% MeOH in dichloromethane, and 10% MeOH in dichloromethane to give an off-white syrup which was converted to the hydrochloride salt using 1.25 M HCl in methanol. The methanolic solution was concentrated under vacuo to give **16** as a light yellow solid (540 mg, 11%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.4 (s, 9H), 3.3 – 3.4 (m, 4H), 3.4 – 3.5 (m, 4H), 7.0 (d, *J* = 8.8 Hz, 1H), 8.0 (d, *J* = 9.2 Hz, 1H), 8.3 (br s, 2H), 8.7 (br s, 2H), 11.6 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 28.5, 43.2, 46.5, 79.5, 113.6, 119.3, 130.8, 154.2, 154.4, 156.5, 167.2. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{17}H_{26}N_5O_3$ 348.42; Found 348.5. Purity: >99.0%.

Synthetic Procedures for Compound (18)



Synthesis of 4-(5-methoxycarbonyl-pyridin-2-yl)-[1,4]diazepane-1-carboxylic acid *tert*-butyl ester (18a)

A solution of methyl 6-chloronicotinate (4.1 g, 23.8 mmol), 1-Boc-homopiperazine (5.0 g, 25.0 mmol), and *N,N*-diisopropylethylamine (6.2 g, 47.8 mmol) in DMSO (6 mL) was heated at 60 °C for 20 hours after which TLC indicate complete consumption of the starting material. The mixture was concentrated *in vacuo* and the crude material was partitioned between DCM and 0.1 N NaOH. The separated DCM layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography (silica gel, Silica Flash®-Silicycle) using dichloromethane followed by dichloromethane:diethyl ether ether (8:1-v/v) to give **18a** as an oil (3.08 g, 39%) which was used directly in the next step.

Synthesis of 4-(5-carboxy-pyridin-2-yl)-[1,4]diazepane-1-carboxylic acid *tert*-butyl ester (18b)

A solution of **18a** (2.93 g, 8.75 mmol) in THF (7 mL), MeOH (7 mL), and aq. NaOH (0.7 g dissolved in 7 mL of H_2O) was heated at 60 ^oC. After 1.5 hours, TLC indicated complete consumption of **18a**. The mixture was concentrated *in vacuo* to remove the THF and MeOH. The resulting aqueous solution was acidified with 1 *N* HCl (pH ~ 4-5) and extracted with DCM (2 x 20 mL). Very little product was obtained with most of the material in the aqueous phase, presumably as the protonated pyridine. The aqueous layer was saturated with solid NaCl and extracted with ethyl acetate (2 x 30 mL). The combined EtOAc extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to give **18b** which was directly in the next step without further purification.

Synthesis of 4-(5-guanidinocarbonyl-pyridin-2-yl)-[1,4]diazepane-1-carboxylic acid *tert*-butyl ester (18c)

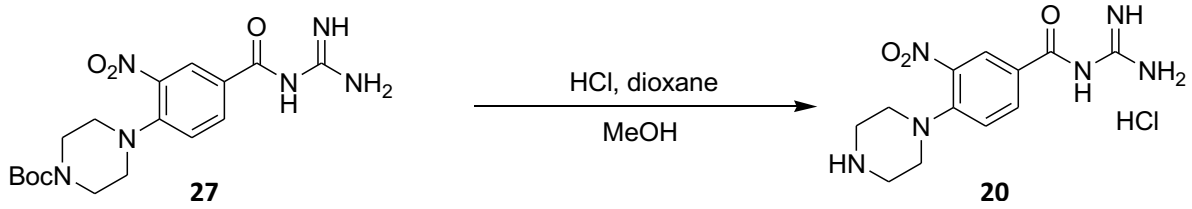
1'1**'-**Carbonyldiimidazole (1.43 g, 8.8 mmol) was added in a single portion to a suspension of **18b** (2.57 g, 8.0 mmol) in THF (20 mL). After 15 minutes, no visible reaction (ie., bubbling of CO_2) was apparent. DMF (10 mL) was added as a co-solvent and the mixture was stirred for 3 hours at room temperature. A stock solution of free-base guanidine was prepared as follows. Guanidine HCl (19.1 g, 0.2 mol) was added to an aqueous NaOH solution (8 g NaOH (0.2 mol) dissolved in 70 mL H_2O). The THF solution of the acyl imidazole was then added to the free-base guanidine solution (70 mL) and stirred overnight at room temperature. The mixture was concentrated *in vacuo* and the resulting material was partitioned between DCM and water. The separated DCM layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give crude **18c** which was purified by column chromatography (silica gel, Silica Flash®-Silicycle) using dichloromethane as eluent followed by 10% MeOH/dichloromethane. The purified fractions were combined to give **18c** as an oil (500 mg, 17%) which was used directly in the next step.

Synthesis of 4-(5-Guanidinocarbonyl-pyridin-2-yl)-[1,4]diazepane-1-carboxylic acid *tert*-butyl ester dihydrochloride (18)

A solution **18c** (0.5 g, 1.38 mmol) in DCM (15 mL) and TFA (3 mL) was stirred for 2 hours at room temperature. TLC indicated complete consumption of **18c** and the mixture was concentrated *in vacuo* and partitioned between DCM and dilute aqueous NaOH (0.1 M) but very little of the desired product was extracted into the DCM layer. The aqueous layer was then saturated with solid NaCl and extracted twice with ethyl acetate. The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give an oily residue. The resulting residue was dissolved in MeOH (5 mL) followed by the addition of 1.25 M HCl (3 mL). The resulting solution was concentrated to a light-yellow solid which was heated in EtOH. Upon heating, a fine white precipitate was formed and the solid was collected via filtration to give **18** as a white solid (300 mg, 65%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 2.0 – 2.1 (m, 2H), 3.1 – 3.1 (m, 2H), 3.2 – 3.2 (m, 2H), 3.7 – 3.8 (m, 2H), 4.0 – 4.1 (m, 2H), 6.9 (d, *J* = 9.2 Hz, 1H), 8.3 (dd, *J* = 2.4 Hz, *J* = 9.2 Hz, 1H), 8.6 (br s, 2H), 8.9 (br s, 2H), 8.9 (d, *J* = 2.4 Hz, 1H), 9.5 (br s, 2H),12.2 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.5, 31.1, 44.8, 45.1, 46.9, 107.9, 115.6, 138.7, 147.9, 156.3, 158.3, 165.8. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{12}H_{19}N_6O$: 263.32; Found: 263.5. Purity: 98.8%.

Synthetic Procedures for Compound (20)

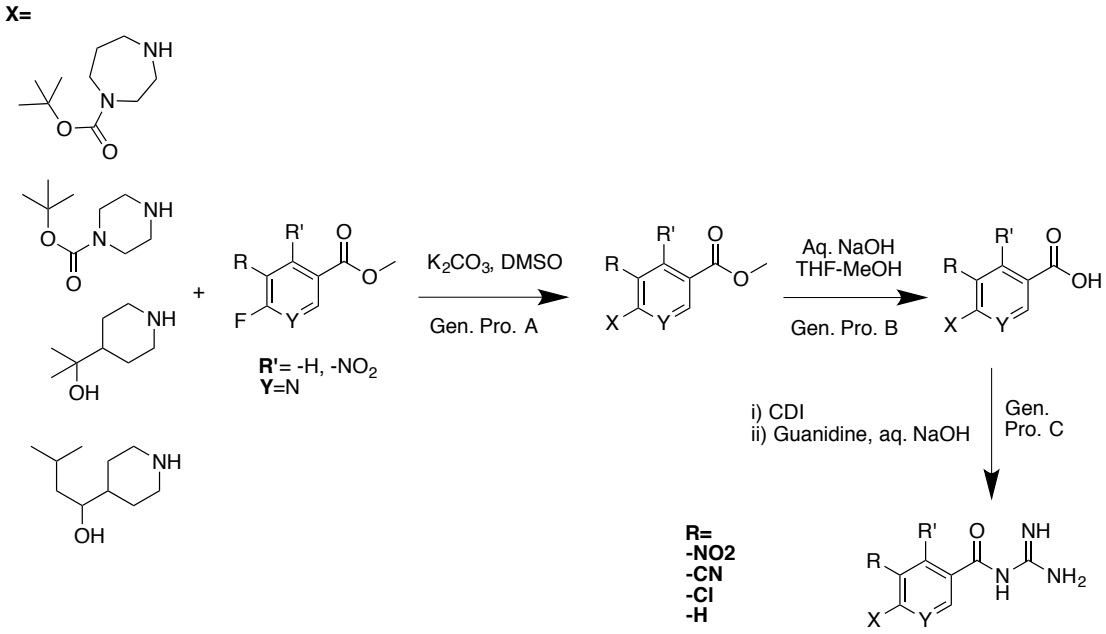


Synthesis of *N*-(3-Nitro-4-piperazin-1-yl-benzoyl)-guanidine hydrochloride (20)

A solution of HCl in dioxane (4M, 10 mL) was added to the free base of **27** (2.54 g, 7.23 mmol) in MeOH (4 mL). The reaction mixture was stirred at room temperature for 24 hours. TLC indicated complete consumption of **27** and the mixture was concentrated *in vacuo* and partitioned between DCM and dilute aqueous NaOH (0.1 M) but very little of the desired product was extracted into the DCM layer. The aqueous layer was then saturated with solid NaCl and extracted twice with ethyl acetate. The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give an oily residue. The resulting residue was dissolved in MeOH (5 mL) followed by the addition of 1.25 M HCL (3 mL). The resulting solution was concentrated under *vacuo* to give **20** as a yellow solid which was further fried under vacuum (300 mg, 11%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 3.1 – 3.2 (m, 4H), 3.4 – 3.5 (m, 4H), 7.5 (d, *J* = 8.8 Hz, 1H), 8.4 (dd, *J* = 2.0 Hz, 1H, *J* = 8.8 Hz, 1H), 8.6 (d, *J* = 2.4 Hz, 1H), 8.6 (br s, 2H), 8.8 (br s, 2H), 9.5 (br s, 2H), 12.4 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 42.8, 47.4, 121.3, 122.6, 128.1, 133.9, 139.53, 148.1, 156.1, 165.6. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{17}H_{26}N_5O_3$: 348.42; Found: 248.5. Purity: >99.0%.

Synthetic Procedures for Compounds 17, 19, 21, 22, 23, 24, 25, 33



General Procedure A

A mixture of substituted piperazine, homopiperazine or piperidine (X), methyl 4-fluorobenzoate and K_2CO_3 in DMSO was refluxed at 120 °C for 24 hrs resulting in the formation of an off-white precipitate. The reaction mixture was cooled to room temperature and then poured into a vessel containing water. The mixture was left to stir at room temperature for 30 minutes and the solid was collected via filtration, washed with water and air dried overnight to give the desired product.

General Procedure B

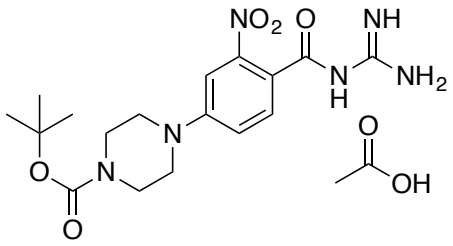
A mixture of the conjugated carboxylate in THF and MeOH was stirred at RT until a clear solution is obtained, Aq. NaOH (2M) was added and the mixture was refluxed at 120 °C for 30 min before TLC shows complete consumption of the starting material. Reaction mixture was cooled to RT and poured into acidified water (2M aq. HCl), and the aq. mixture was extracted using EtOAc. Combined organic layer was washed with brine, and dried over Na_2SO_4, filtered and

concentrated *in vacuo*. The carboxylic acid was further purified using flash column chromatography.

General Procedure C

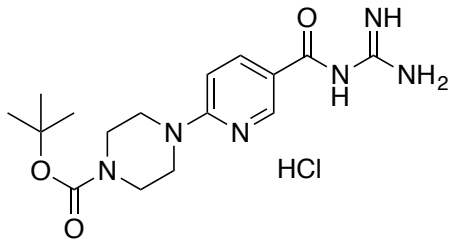
CDI was added in a single portion to a suspension of the carboxylic acid in THF containing a few drops of DMF. The mixture was stirred overnight at room temperature. A stock solution of free base guanidine was prepared as follows: Guanidine HCl was added to aq. NaOH (8 M). The THF solution of acyl imidazole was then added to the free-base guanidine solution and stirred for 3.5 hours after which TLC showed formation of a new spot. The mixture was concentrated *in vacuo* resulting in the formation of yellow-red oil. The aqueous mixture was diluted with aq. NaOH (2M), and extracted with EtOAc. Combined organic was dried, filtered and concentrated under vacuum to obtain a solid, which was further purified using flash column chromatography.

Compound 17



^{1}H NMR (400 MHz, DMSO-d_6) δ 9.2 (br s, 1H), 7.8 (d, J = 2.0 Hz, 1H), 7.6 (dd, J = 2.2, 7.3 Hz, 1H), 7.0 (d, J = 7.5 Hz, 1H), 3.6-3.7 (m, 4H), 3.1 – 3.2 (m, 4H), 2.6 (s, 3H), 2.1 (s, 2H), 1.5 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.1, 154.3, 152.3, 151.8, 132.2, 116.1, 108.0, 79.6, 47.4, 31.7, 28.5, 27.3, 24.7, 22.6, 21.2. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{17}H_{25}N_6O_5$: 393.42; Found: 393.1. Purity: >99.0%.

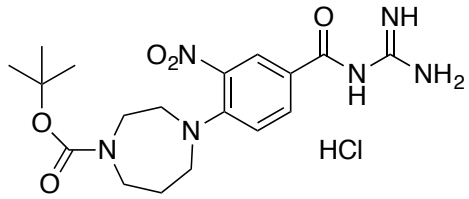
Compound 19



^{1}H NMR (400 MHz, DMSO-d_6) δ 11.4 (br s, 1H), 8.8 (d, *J* = 2.0 Hz, 1H), 8.1 (dd, *J* = 2.2, 7.3 Hz, 1H), 6.9 (d, *J* = 7.5 Hz, 1H), 3.6 – 3.7 (m, 4H), 3.3 – 3.4 (m, 4H), 1.5 (s, 9H).

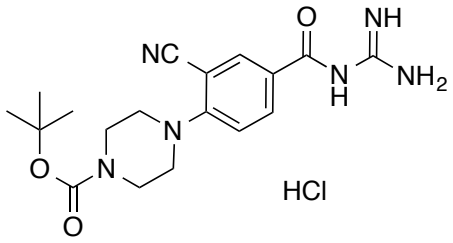
^{13}C NMR (100 MHz, DMSO-d_6) δ 178.1, 164.7, 161.6, 156.3, 151.6, 139.3, 124.2, 107.5, 81.6, 46.2, 28.8. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{16}H_{25}N_6O_3$: 349.41; Found: 349.1. Purity: 97.2 %.

Compound 21



^{1}H NMR (400 MHz, DMSO-d_6) δ 12.2 (br s, 1H), 8.7 (d, *J* = 2.3 Hz 1H), 8.5 (br s, 2H), 8.2 (dd, *J* = 7.3, 2.1 Hz, 1H), 7.3 (d, *J* = 7.1 Hz, 1H), 3.5 (m, 6H), 3.2 (m, 4H), 1.9 (br. s, 2H), 1.3 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 155.4, 153.9, 147.9, 136.5, 132.0, 127.3, 117.1, 78.3, 51.9, 49.3, 48.5, 46.1, 45.7, 28.3, 26.9, 25.2. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{18}H_{27}N_6O_5$: 407.44; Found: 407.2.

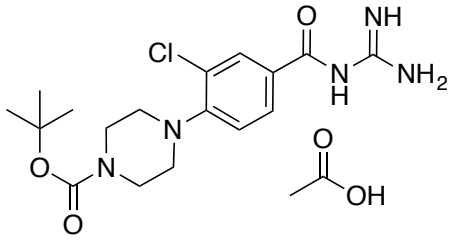
Compound 22



^{1}H NMR (400 MHz, DMSO-d_6) δ 12.3 (s, 1H), 8.7 (br s, 2H), 8.3 (d, *J* = 2.0 Hz, 1H), 8.1 (dd, *J* = 2.2, 7.3 Hz, 1H), 7.1 (d, *J* = 7.5 Hz, 1H), 3.8 (s, 4H), 3.6 (s, 4H), 2.0 (s, 2H), 1.4 (s, 9H).

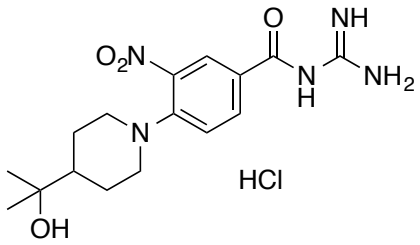
^{13}C NMR (100 MHz, DMSO-d_6) δ 165.0, 157.4, 155.8, 153.4, 135.4, 134.1, 122.9, 118.5, 117.5, 101.3, 79.2, 50.0, 28.3. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{18}H_{25}N_6O_3$: 373.43; Found: 372.1. Purity: 95.2%.

Compound 23



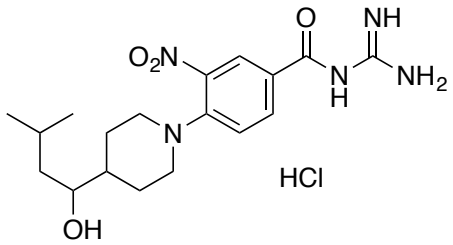
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.5 (br s, 1H), 8.1 (s, 1H), 8.0 (s, 2H), 7.9 (d, *J* = 2.0 Hz, 1H), 7.2 (d, *J* = 7.5 Hz, 1H), 3.6-3.7 (m, 4H), 3.1 – 3.0 (m, 4H), 2.6 (s, 3H), 1.5 (s, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ 180.2, 169.1, 167.5 166.9, 154.8, 149.1, 139.3, 130.5, 128.8, 126.0, 119.7, 79.3, 51.2, 35.2, 28.5. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{17}H_{25}ClN_5O_3$: 382.86; Found: 382.1.

Compound 24



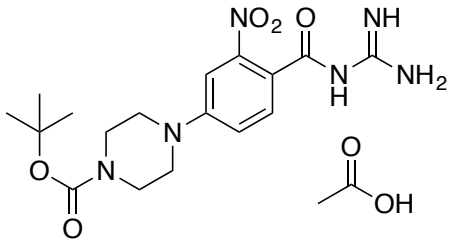
^{1}H NMR (400 MHz, DMSO-d_6) δ 14.7 (br s, 1H), 12.2 (s, 1H), 8.3 (d, *J* = 1.9 Hz, 1H), 8.0 (dd, *J* = 2.0, 7.5 Hz, 1H), 7.4 (d, *J* = 7.5 Hz, 1H), 6.0 (br s, 4H), 3.6 (t, *J* = 5.5, 2H), 3.0 (m, 2H), 1.8 (m, 2H), 1.4 (m, 3H), 1.1 (s, 6H).^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4, 155.9, 148.4, 138.9, 133.3, 127.8, 119.8, 119.6, 119.2, 70.4, 50.8, 45.3, 27.1, 26.8. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{16}H_{24}N_5O_4$: 350.39; Found: 350.1.

Compound 25



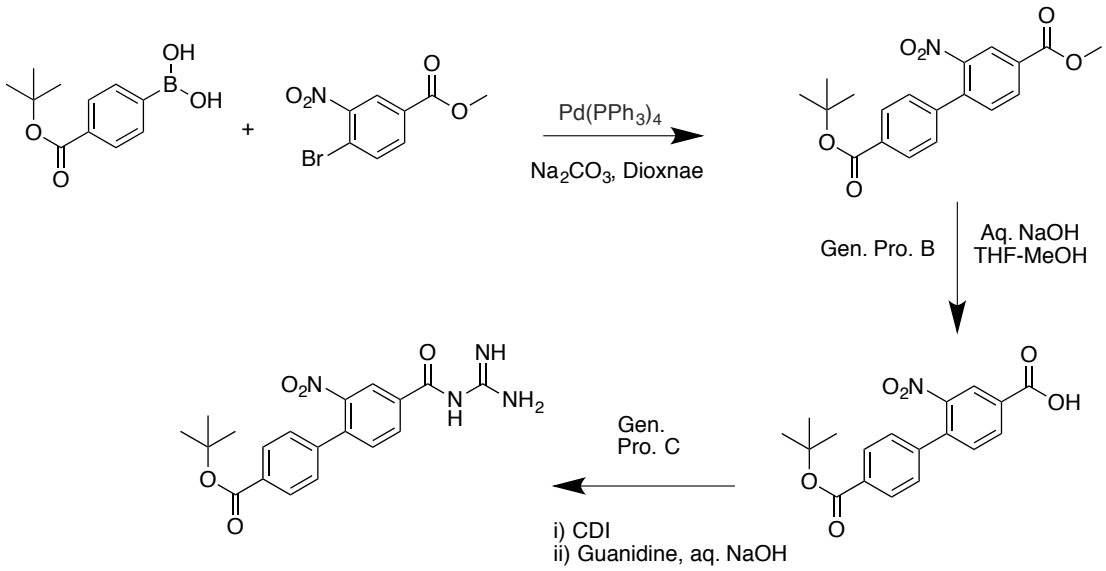
^{1}H NMR (400 MHz, DMSO-d_6) δ 14.6 (br s, 1H), 12.3 (br s, 1H), 8.8 (br s, 2H), 8.6 (d, *J* = 1.9 Hz, 1H), 8.3 (dd, *J* = 2.0, 7.5 Hz, 1H), 7.3 (d, *J* = 7.5 Hz, 1H), 4.6 (m, 4H), 3.8 (m, 4H), 3.6 (m, 1H), 3.3 (m, 1H), 2.7 (br s, 2H), 1.8 (m, 1H), ,1.2 – 1.1 (m, 2H), 1.0 – 0.8 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.0, 155.4, 148.5, 138.9, 130.3, 130.0, 128.8, 119.5, 73.9, 47.7, 41.6, 39.8, 27.6, 27.2, 22.8. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{18}H_{28}N_5O_4$: 378.45; Found: 378.2. Purity: >99.0%.

Compound 33



^{1}H NMR (400 MHz, DMSO-d_6) δ1.5 (s, 9H), 1.9 (s, 1H), 2.1 (s, 3H), 3.0 - 3.1 (m, 4H), 3.4 - 3.5 (m, 4H), 7.3 (dd, *J* = 8.7 Hz, 1H), 7.8 (br s, 1H), 7.9 (d, *J* = 2.2.0, 1H), 8.2 (d, *J* = 8.0 Hz, 1H), 9.5 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 27.3, 28.5, 47.4, 60.7, 79.5, 99.9, 108.02, 116.13, 132.1, 151.8, 152.3, 154.3, 157.2, 159.1, 168.1. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{17}H_{25}N_6O_5$: 393.42; Found: 393.1.

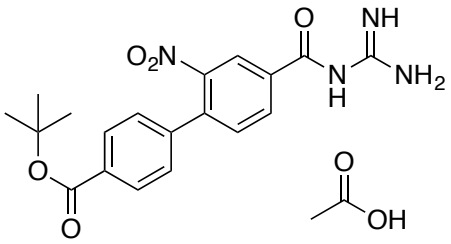
Synthetic Procedures for Compound 26



A mixture of the methyl ester (0.5 g, 1.9 mmol) in Dioxane, boronic acid (0.51 g, 2.3 mmol) and a 2M aqueous solution of Na_2CO_3 was added to a 50 mL RB flask and the flask was purged with N_2 for 15 min. $Pd(PPh_3)_4$ (0.10 g, 0.09 mmol) was added and the reaction mixture was slowly heated up to 100 °C and stirred for 30

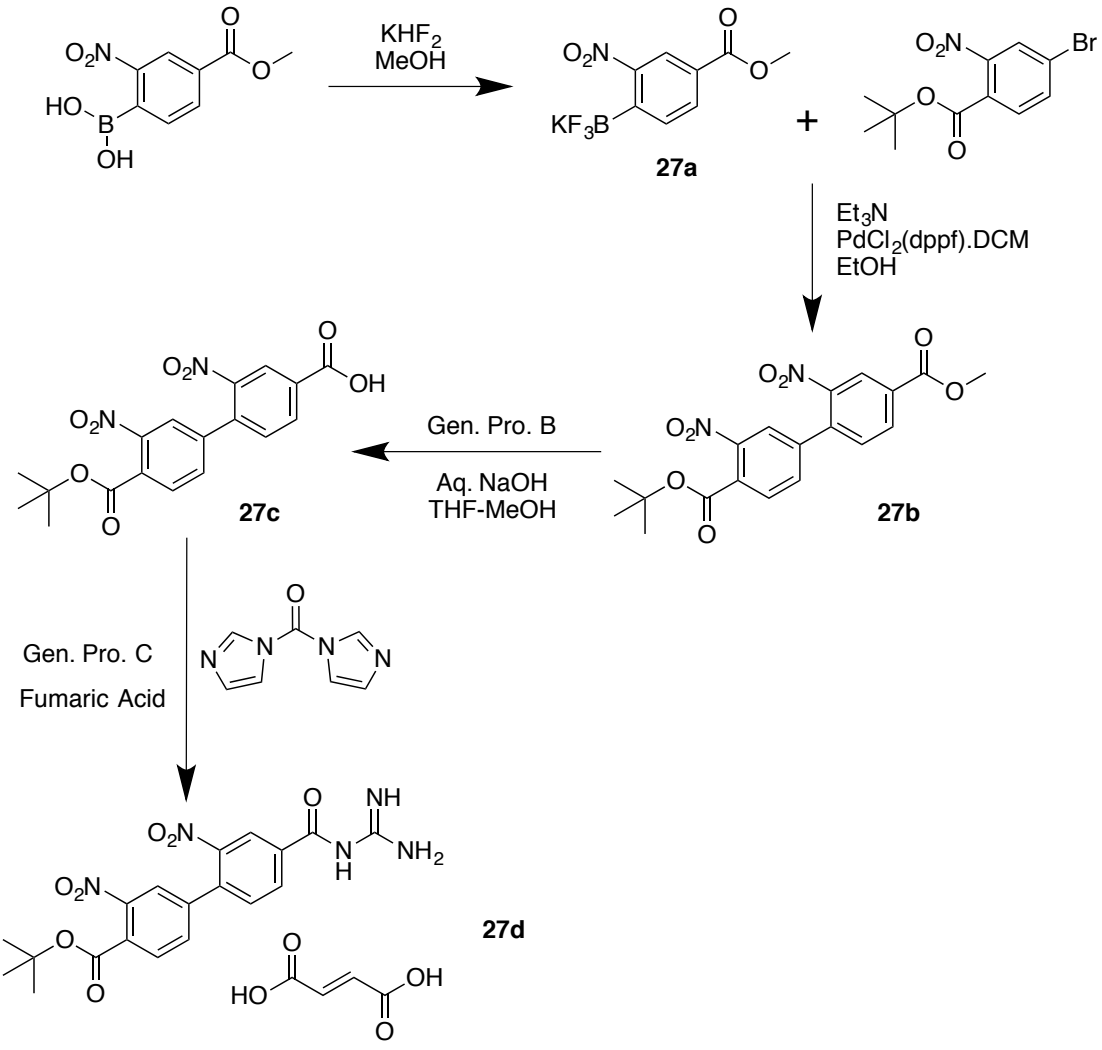
minutes. The reaction mixture was poured in ice water and extracted with EtOAc. The crude material was purified by flash column chromatography (silica gel, Silica Flash®-Silicycle) using EtOAc:Hexane (1:1) to give the product as an off-white solid which after characterization was used in the following steps General Procedures **B** and **C**.

Compound 26



^{1}H NMR (400 MHz, DMSO-d_6) δ 8.4 (d, *J* = 7 Hz, 1H), 8.2 (dd, *J* = 8.6 HZ, *J* = 2.0 Hz, 2H), 7.9 (d, *J* = 1.9 Hz, 1H), 7.7 (d, *J* = 8.2 Hz, 2H), 7.5 (d, *J* = 1.9 Hz, 1H), 3.9 (s, 3H), 1.5 (s, 9H) ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.3, 164.3, 164.1, 148.3, 140.1, 138.5, 133.1, 132.8, 131.5, 130.1, 129.6, 127.2, 124.7, 80.5, 52.3, 28.2, 26.2. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{19}H_{21}N_4O_5$: 385.39; Found: 385.1. Purity: >99.0%.

Synthetic Procedures for Compound 27



Synthesis of methyl 3-nitro-4-(trifluoroborate)benzoate potassium salt (27a)

To a solution of (4-(methoxycarbonyl)-2-nitrophenyl)boronic acid (0.2 g, 0.8 mmol) in MeOH was slowly added an aq. solution of potassium hydrogenfluoride (0.2, 2.4 mmol) with vigorous stirring. After 15 min, the precipitated product was collected and washed with cold MeOH. Recrystallization from minimal acetonitrile yielded white crystalline product, (0.21g, 92%) which was used in the next step with no further purification.

Synthesis of 4'-(*tert*-butyl) 4-methyl 2,3'-dinitro-[1,1'-biphenyl]-4,4'-dicarboxylate (27b)

To a mixture of **27a** (0.2 g, 0.7 mmol), tert-butyl 4-bromo-2-nitrobenzoate (0.21 g, 0.7 mmol), Et_3N (0.21g, 2.1mmol), and $PdCl_2(dppf).CH_2Cl_2$ (5.5 mg, 7×10^{-3} mmol) was added EtOH (5 mL). The reaction was heated at reflux with stirring in an open atmosphere for 12 hours, then cooled to RT. Upon addition of water, a precipitate was formed. The precipitate was filtered off and thoroughly washed with water, dissolved in dichloromethane and dried over $MgSO_4$. The solution was then filtered through a short pad of silica using a sequence of pentane and pentane/dichlormethane. The solvent was removed *in vacuo* and the crude product was purified by recrystallization with dichlromethane/hexane, to yield **27b** as a white solid (0.25 g, 88%)

^1H NMR (400 MHz, DMSO-d_6): δ 1.7 (s, 9H), 4.0 (s, 3H), 7.8 (dd, *J* = 8.2 Hz, *J* = 2.1 Hz, 2H), 7.9 (d, *J* = 8.3 Hz, 1H), 8.1 (m, 1H), 8.4 (d, *J* = 2.2 Hz, 1H), 8.6 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 28.3, 51.3, 81.6, 120.5, 123.5,124.8, 128.7, 131.8, 133.2, 140.8, 141.2, 145.3, 147.2, 149.3, 154.3, 165.5, 176.7. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{19}H_{18}N_2O_8$: 402.36; Found: 403.1. Purity: 98.1%.

Synthesis of 4'-(*tert*-butoxycarbonyl)-2,3'-dinitro-[1,1'-biphenyl]-4-carboxylic acid (27c)

To a mixture of the methyl ester (**27b**) (1.05 g, 2.6 mmol) in THF was added an aq. solution of LiOH (0.12 g, 2.9 mmol) and the reaction was stirred for 15 min at RT. The reaction mixture was concentrated and diluted with EtOAc (15 mL). The organic layer was extracted with 1 M NaOH (3 × 15 mL). The solution was acidified with 6 M HCl and extractyed with EtOAc (3 × 20 mL). The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*

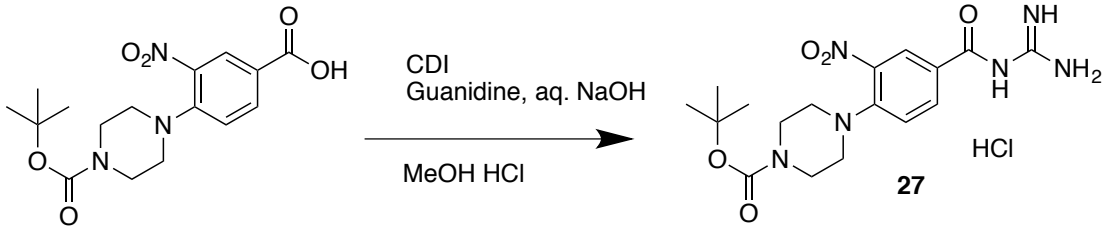
to yield **27c** as a white solid (0.95 g, 92%) which was used with no further purification.

Synthesis of *tert*-butyl 4'-(carbamimidoylcarbamoyl)-2',3-dinitro-[1,1'-biphenyl]-4-carboxylate fumarate (27d)

The acylguanidine **27d** was synthesized using the General Procedure C from **27c (**0.51 g, 47%**)**. To solution of the free amine (0.44 g, 1.14 mmol) in 5 mL MeOH was added methanolic solution of fumaric acid (0.15g, 1.4mmol). The mixture was vigorously stirred at RT for 15 min. The precipitated fumarate salt was filtered off and washed with cold MeOH, and dried *in vacuo* to yield **27d** as white crystalline solid. (0.4 g, 78%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.4 (d, *J* = 7 Hz, 1H), 8.2 (br s), 8.1 (m, 1 H), 7.95 (m, 1 H), 7.9 (d, *J* = 1.9 Hz, 1H) 7.8 (dd, *J* = 8.6 HZ, *J* = 2.0 Hz, 1H), 7.7 (d, *J* = 8.2 Hz, 1H), 6.6 (s, 2H), 1.5 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 167.5, 164.9, 163.4, 158.2, 149.6, 147.5, 143.2, 140.2, 134.4, 135.2, 133.1, 132.5, 130.9, 127.2, 124.6, 123.4, 83.3, 27.2. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{19}H_{19}N_5O_7$: 429.13; Found: 429.5. Purity: >99.0%.

Synthetic Procedures for Compound 28



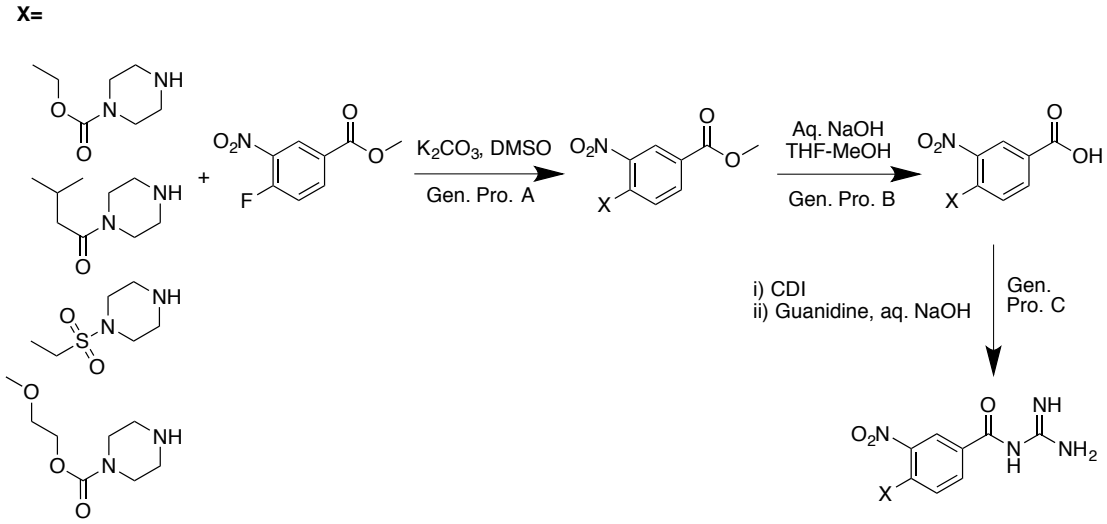
Synthesis of 4-(4-guanidinocarbonyl-2-nitro-phenyl)-piperazine-1-carboxylic acid *tert*-butyl ester hydrochloride (28)

1,1'-Carbonyldiimidazole (1.41 g, 8.68 mmol) was added in a single portion to a suspension of the carboxylic acid (2.54, 7.23 mmol) in THF (20 mL). The mixture was stirred for 2 hours, at which time bubbling had ceased. A stock solution of

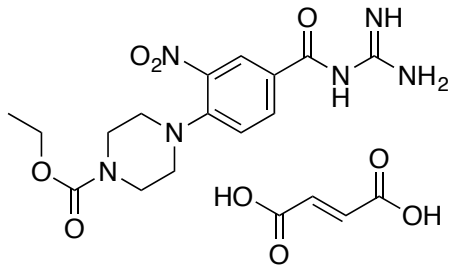
free-base guanidine was prepared as follows. Guanidine HCl (19.1 g, 0.2 mol) was added to an aq. NaOH solution (8 g NaOH (0.2 mol) dissolved in 70 mL H_2O). The THF solution of the acyl imidazole was then added to the free-base guanidine solution (70 mL) and stirred for 2 hours. The mixture was concentrated *in vacuo* resulting in the formation of a yellow-brown oil. The aqueous mixture was diluted with DCM, but the oil was very slow to dissolve. Upon shaking in the separatory funnel, an emulsion formed. The mixture was diluted with EtOAc which did not form an emulsion. The separated DCM/EtOAc layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography (silica gel, Silica Flash®-Silicycle) using DCM followed by 10% MeOH in DCM as eluent to give 2.0 grams of a thick yellow oil. The oil was dissolved in MeOH followed by the addition of a mixture of water (4 mL) and 1.25 M HCl in MeOH (5 mL). The solution was concentrated *in vacuo* to give a sticky semi-solid which was triturated in EtOAc to give **28** as a yellow solid (830 mg, 27%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.4 (s, 9H), 3.0 - 3.1 (m, 4H), 3.4 - 3.5 (m, 4H), 6.7 (br s, 2H), 7.2 (d, *J* = 8.7 Hz, 1H), 7.9 (br s, 2H), 8.1 (dd, *J* = 8.6 Hz, *J* = 2.0 Hz, 1H), 8.4 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 27.3, 28.5, 50.9, 79.6, 120.5, 126.7, 132.2, 134.0, 140.8, 147.2, 154.3, 163.3, 163.3, 176.7. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{17}H_{25}N_6O_5$: 393.42; Found: 393.5. Purity: 97.8%.

Synthetic Procedures for Compounds 29, 30, 31, 32

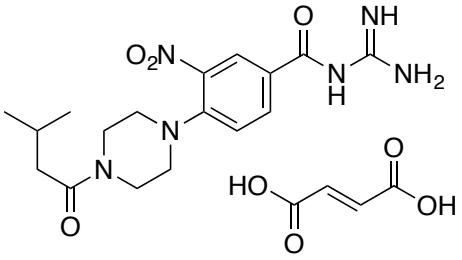


Compound 29



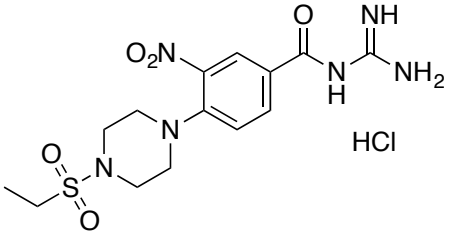
^{1}H NMR (400 MHz, DMSO-d_6): 8.5 (d, *J* = 2.1 Hz, 1H), 8.2 (dd, *J* = 8.4 Hz, *J* = 2.3 Hz, 1H), 8.1 (br s, 1H), 7.3 (d, *J* = 8.5 Hz, 1H), 6.9 (br s, 1H), 6.5 (s, 2H), 4.1 (q, *J* = 7.9 Hz, 2H), 3.4 - 3.5 (m, 4H), 3.0 - 3.1 (m, 4H), 1.2 (t, *J* = 7.6 Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6): δ 15.0, 43.6, 50.8, 61.3, 120.6, 126.8, 131.6, 134.0, 134.5, 140.7, 147.3, 155.1, 162.9, 166.6, 173.3. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{15}H_{21}N_6O_5$: 365.15; Found: 364.5. Purity: 98.2%.

Compound 30



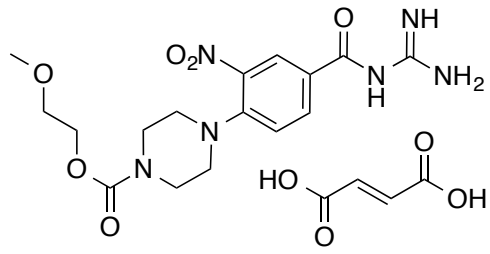
^{1}H NMR (400 MHz, DMSO-d_6): 8.6 (d, *J* = 2.3 Hz, 1H), 8.4 (br s, 1H), 8.3 (dd, *J* = 8.1 Hz, *J* = 2.5 Hz, 1H), 7.5 (br s, 1H), 7.4 (d, *J* = 8.1 Hz, 1H), 6.5 (s, 2H), 3.5 - 3.6 (m, 4H), 3.0 - 3.1 (m, 4H), 2.2 (s, 1H), 2.0 (m, 2H), 0.9 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.9, 25.5, 40.1, 45.1, 51.0, 120.3, 121.6, 127.1, 129.6, 134.9, 140.1, 147.3, 161.7, 167.4, 170.87 172.12. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{17}H_{25}N_6O_4$: 377.42; Found: 377.3.

Compound 31



^{1}H NMR (400 MHz, DMSO-d_6): 8.6 (d, *J* = 2.1 Hz, 1H), 8.3 (dd, *J* = 8.1 Hz, *J* = 2.5 Hz, 1H), 8.0 (br s, 1H), 7.3 (d, *J* = 8.0 Hz, 1H), 6.8 (br s, 1H), 5.8 (s, 1H), 3.5 (q, *J* = 8.1 Hz, 2H) 3.2 - 3.3 (m, 4H), 3.4 - 3.5 (m, 4H), 2.2 (br s, 1H), 1.3 (t, *J* = 8.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 8.2, 42.3, 45.6, 50.1, 121.3, 123.4, 128.9, 133.5, 139.6, 148.2, 157.3, 166.4.MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{14}H_{21}N_6O_5S$: 385.41; Found: 385.4. Purity: 97.1%.

Compound 32



^{1}H NMR (400 MHz, DMSO-d_6): 8.6 (d, *J* = 2.1 Hz, 1H), 8.4 (br s, 1H), 8.2 (dd, *J* = 8.1 Hz, *J* = 2.5 Hz, 1H), 7.2 (d, *J* = 8.1 Hz, 1H), 7.0 (br s, 1H), 6.5 (s, 2H), 4.2 (t, *J* = 8.0 Hz, 2H), 3.5 (m, 6H), 3.3 (s, 3H), 3.1 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 43.6, 50.7, 58.5, 64.6, 70.6, 120.6, 126.9, 131.2, 134.0, 134.7, 140.6, 147.3, 155.0, 162.6, 167.0, 173.0. MS (ESI) m/z: $[M + H]^+$ Calculated for $C_{16}H_{23}N_6O_6$: 395.39; Found: 395.16.

References

1. Ewart, G. D., Luscombe, C. A. & Miller, M. Hepatitis C antiviral compositions and methods. WO 2009018609 A1 (1996).
2. Wang Jun, Wu Y, Ma C, Fiorin G, Wang J, Pinto LH, Lamb RA, Klein ML, and Degrado WF (2013) Structure and inhibition of the drug-resistant S31N mutant of the M2 ion channel of influenza A virus. P Natl Acad Sci USA 110: 1315–1320.