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In Vivo Phenotypic Drug Discovery: Applying a Behavioral Assay to the Discovery and Optimization of Novel Antipsychotic Agents

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I. Synthetic procedures and characterization data

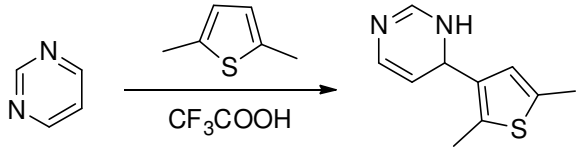
1. General Procedures for Compound Synthesis

Unless otherwise indicated, all temperatures are set forth in degrees Celsius and all parts and percentages are by weight. Reagents were either purchased from commercial suppliers and used without further purification unless otherwise indicated or prepared following standard literature procedures. The reactions set forth below were done generally at room temperature, unless otherwise indicated. Unless otherwise specified, generally the reaction flasks were fitted with rubber septa for introduction of substrates and reagents via syringe. Analytical thin layer chromatography (TLC) was performed using glass-backed silica gel pre-coated plates and eluted with appropriate solvent ratios (v/v). Reactions were assayed by TLC or liquid chromatography/ mass spectroscopy (LCMS) and terminated as judged by the consumption of starting material. Visualization of the TLC plates was done with UV light (254 wavelength) or with an appropriate TLC visualizing solvent, such as basic aqueous $KMnO_4$ solution activated with heat. Flash column chromatography[1] was performed using, silica gel 60 or various medium pressure liquid chromatography (MPLC) systems (e.g., Biotage® or ISCO® separation systems).

The compound structures in the examples below were confirmed by one or more of the following methods: proton nuclear magnetic resonance spectroscopy (^{1}H NMR) or mass spectroscopy (MS). ^{1}H NMR spectra were determined using a NMR spectrometer operating at certain field strength. Chemical shifts are reported in parts per million (ppm, δ) downfield from an internal standard, such as TMS. Alternatively, ^{1}H NMR spectra were referenced to signals from residual protons in deuterated solvents, for example, as follows: $CDCl_3$ = 7.25 ppm; DMSO-d_6 = 2.49 ppm; C_6D_6 = 7.16 ppm; CD_3OD = 3.30 ppm. Peak multiplicities are designated, for example, as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; dt, doublet of triplets; q, quartet; br, broadened; and m, multiplet. Coupling constants are given in Hertz (Hz). MS data were obtained using a mass spectrometer with APCI or ESI ionization.

2. Experimental Procedures

A. General Method A for the formation of thiophenyl-1,6-dihydropyrimidines under ultrasonic conditions with direct isolation. Synthesis of the HCl salt of 6-(2,5-dimethylthiophen-3-yl)-1,6-dihydropyrimidine (1).

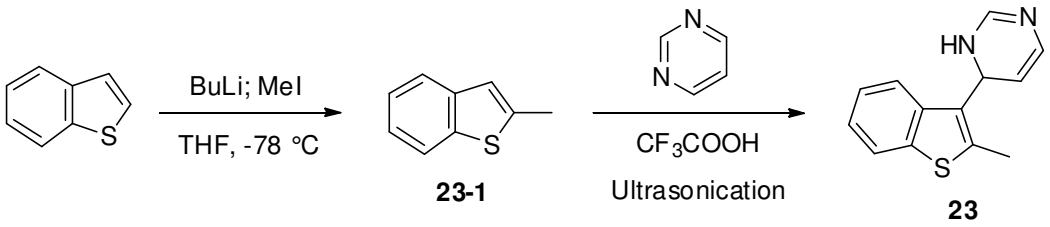


1

A solution of pyrimidine (800 mg, 9.99 mmol) in TFA (5 mL) was treated with 2,5-dimethylthiophene (1.35 g, 11.99 mmol). The reaction mixture was sonicated for 30 min at room temperature in an ultrasonic bath. Saturated aqueous $NaHCO_3$ (50 mL) and EtOAc (50 mL) were added, and the resulting biphasic mixture was transferred to a separatory funnel. The aqueous phase was extracted with EtOAc (2×30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting oil was crystallized using hexane/ether to provide 6-(2,5-dimethylthiophen-3-yl)-1,6-dihydropyrimidine **1** (1.6 g, 83%) as an off-white solid,

which was converted to the HCl salt by dissolution in a small amount of MeOH and addition of a large excess of a solution of HCl in ether. MS ESI(+): *m/z* 193 [M+H]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07 (s, 1H), 6.78 (s, 1H), 6.37 (d, *J* = 8.4 Hz, 1H), 5.52 (dd, *J* = 3.6, 1.5 Hz, 1H), 5.04 (dd, *J* = 8.1, 3.3 Hz, 1H), 2.41 (s, 6H).

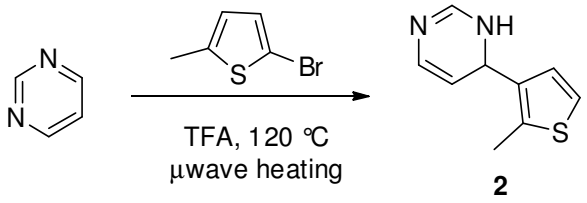
HCl salt of 6-(2-Methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine (23).



A solution of benzo[b]thiophene (3 g, 22.35 mmol) in anhydrous THF (50 mL) under N_2 atmosphere was cooled to -70°C in a dry ice/acetone bath and treated with *n*-BuLi (10.73 mL, 2.5 M in hexanes) in a drop-wise manner over 20 min. The reaction mixture was warmed to -40°C and stirred for 30 min. After cooling back to -70°C, iodomethane (4.76 g, 33.53 mmol) was added dropwise. After the addition was completed, the reaction mixture was warmed to room temperature and stirred for 1 h. Excess lithium reagent was quenched by the addition of saturated aqueous NH_4Cl (40 mL). The mixture was washed with ether (3×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to provide 2-methylbenzo[b]thiophene **23-1** (3 g, 90%) as a brown solid that was used without further purification.

6-(2-Methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine **(23)** was synthesized from intermediate **23-1** as described above in the General Method A. MS (ESI+): *m/z* 229 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): *δ* 8.21 (s, 1H), 7.93-7.85 (dd, *J* = 16.9, 7.5 Hz, 2H), 7.45-7.34 (m, 2H), 6.46 (d, *J* = 8.2 Hz, 1H), 6.13 (t, *J* = 2.4 Hz, 1H), 5.28 (dd, *J* = 8.3, 2.9 Hz, 1H), 2.64 (s, 3H).

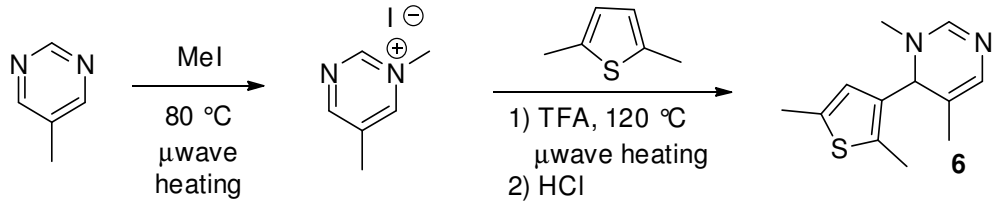
B. General Method B for the formation of thiophenyl-1,6-dihydropyrimdines under microwave conditions with direct isolation. Synthesis of the HCl salt of 6-(2-methylthiophen-3-yl)-1,6-dihydropyrimidine (2).



A 10 mL microwave reaction tube was charged with TFA (0.4 mL), 2-bromo-5-methylthiophene (200 mg, 1.12 mmol), and pyrimidine (180 mg, 2.25 mmol). The reaction tube was sealed and heated at 120°C under microwave irradiation for 10 min. After cooling to room temperature, the reaction mixture was poured into saturated aqueous Na_2CO_3 and extracted with EtOAc (3×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration and concentration under reduced pressure, purification by silica gel column chromatography afforded **2**, which was dissolved in ether and cooled in an ice bath. Gaseous HCl was bubbled through a solution at 0ºC for 10

min. The resulting solid precipitate was collected by filtration and dried under vacuum to give the HCl salt of 6-(2-methylthiophen-3-yl)-1,6-dihydropyrimidine **2** (20 mg, 10%). MS (ESI+): *m/z* 179 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.09 (s, 1H), 6.95 (d, J = 3.5 Hz, 1H), 6.75 (d, J = 1.2 Hz, 1H), 6.44 (d, J = 8.1 Hz, 1H), 5.70 (d, J = 3.5 Hz, 1H), 5.42 (dd, J = 8.1, 0.8 Hz, 1H), 2.50 (s, 3H).

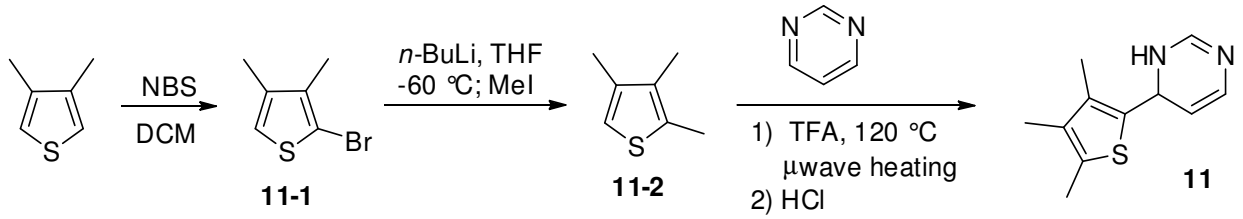
HCl salt of 6-(2,5-Dimethylthiophen-3-yl)-1,5-dimethyl-1,6-dihydropyrimidine (6).



A 5 mL microwave reaction tube was charged with iodomethane (2 mL) and 5-methylpyrimidine (320 mg, 4 mmol). The resulting mixture was heated at 80°C under microwave irradiation for 20 minutes. After cooling to room temperature, the resulting precipitate was collected by filtration, washed with ether, and dried under vacuum to give 1, 3-dimethylpyrimidin-1-ium bromide (226 mg, 30%) as a brown solid, which was used without further purification.

The HCl salt of 6-(2,5-Dimethylthiophen-3-yl)-1,5-dimethyl-1,6-dihydropyrimidine (**6**) was prepared as described above in General Method B. ^{1}H NMR (400 MHz, CD_3OD): δ 8.11 (s, 1H), 6.67 (s, 1H), 6.21 (s, 1H), 5.33 (s, 1H), 3.04 (s, 3H), 2.44 (s, 3H), 2.41 (s, 3H), 1.53 (s, 3H).

HCl salt of 6-(3,4,5-Trimethylthiophen-2-yl)-1,6-dihydropyrimidine (11).

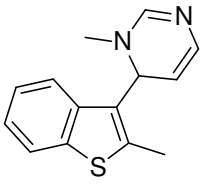


A solution of 3,4-dimethylthiophene (2 g, 17.8 mmol) in DCM (100 mL) was treated with N-bromosuccinimide (3.2 g, 17.8 mmol) and the mixture was stirred overnight at room temperature. After concentration, the crude product was purified by silica gel column chromatography to give 2-bromo-3,4-dimethylthiophene **11-1**.[2]

A solution of **11-1** (1 g, 5.2 mmol) in THF (50 mL) under N_2 was cooled to -60°C and then treated in a dropwise manner with *n*-BuLi (2.1 mL, 5.2 mmol, 2.5 M in hexanes) dropwise. After stirring at -78°C for 1 h, the reaction mixture was allowed to warm to -20°C and stirred for 0.5 h. The reaction mixture was then cooled to -78°C and iodomethane (1.5 g, 10 mmol) was added. The reaction mixture was then allowed to warm gradually to room temperature while stirring overnight. The mixture was poured into water, extracted with ether (3×100 mL), and the combined organic layers were dried over anhydrous Na_2SO_4. After concentration, the crude product was purified by silica gel column chromatography to give 2,3,4-trimethylthiophene **11-2** (590 mg, 90%).

The HCl salt of compound **11** was prepared as described above in General Method B. MS (ESI+): *m/z* 207 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.05 (s, 1H), 6.41 (d, J = 8.2 Hz, 1H), 5.82 (dd, J = 3.6 Hz, 1.50 Hz, 1H), 5.36-5.32 (m, 1H), 2.35 (s, 3H), 2.14 (s, 3H), 2.00 (s, 3H).

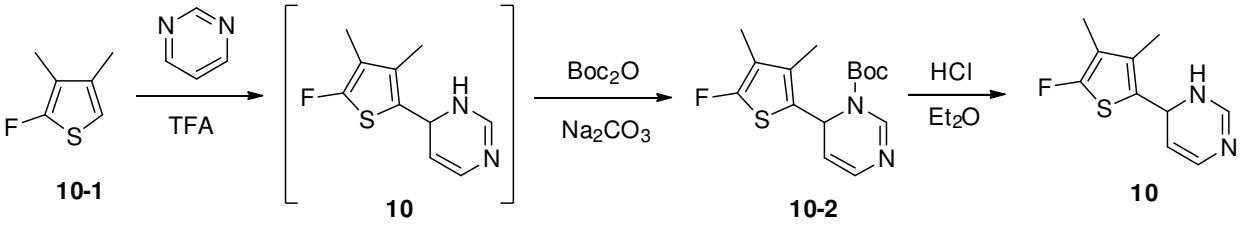
HCl salt of 1-Methyl-6-(2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine (35).



35

The HCl salt of compound **35** was prepared as described above in General Method B. ^{1}H NMR (400 MHz, CD_3OD): δ 8.53 (s, 1H), 8.19 (s, 1H), 7.85 (t, J = 8.0 Hz, 2H), 7.43-7.33 (m, 2H), 6.45 (d, J = 8.0 Hz, 1H), 6.05 (br s, 1H), 5.23 (dd, J = 8.0, 3.2 Hz, 1H), 2.93 (s, 3H), 2.2.64 (s, 3H).

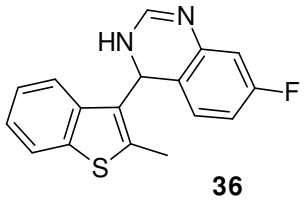
C. General Method C for the formation of thiophenyl-1,6-dihydropyrimdines using Boc group for isolation. Synthesis of the HCl salt of 6-(5-fluoro-3,4-dimethylthiophen-2-yl)-1,6-dihydropyrimidine (10).



10-1 **10** **10-2** **10**

A solution of pyrimidine (150 mg, 1.87 mmol) in TFA (1.5 mL) was treated with 2-fluoro-3,4-dimethylthiophene **10-1** (200 mg, 1.54 mmol), and the reaction mixture was stirred at room temperature for 1 h. The mixture was made basic by the addition of 10 mL of saturated aqueous Na_2CO_3. $(Boc)_2O$ (616 mg, 3.08 mmol) and EtOAc (10 mL) were then added, and the resulting biphasic mixture was stirred at room temperature overnight. The aqueous phase was extracted with EtOAc (3×50 mL), washed with brine (3×50 mL), and dried over anhydrous Na_2SO_4. After filtration and concentration under reduced pressure, the crude product was purified by column chromatography to give *tert*-butyl 6-(5-fluoro-3,4-dimethylthiophen-2-yl)pyrimidine-1(6H)-carboxylate **10.2**. A solution of **10-2** in ether was treated with gaseous HCl at 0ºC for 10 min. The resulting precipitate was collected by filtration and dried under vacuum to give 6-(5-fluoro-3,4-dimethylthiophen-2-yl)-1,6-dihydropyrimidine **10** as the HCl salt (54 mg, 17%). MS (ESI+): *m/z* 211 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.07 (s, 1H), 6.46 (d, J = 8.1 Hz, 1H), 5.90-5.87 (m, 1H), 5.37 (dd, J = 7.5, 3.0 Hz, 1H), 2.19 (s, 3H), 2.03 (m, 3H).

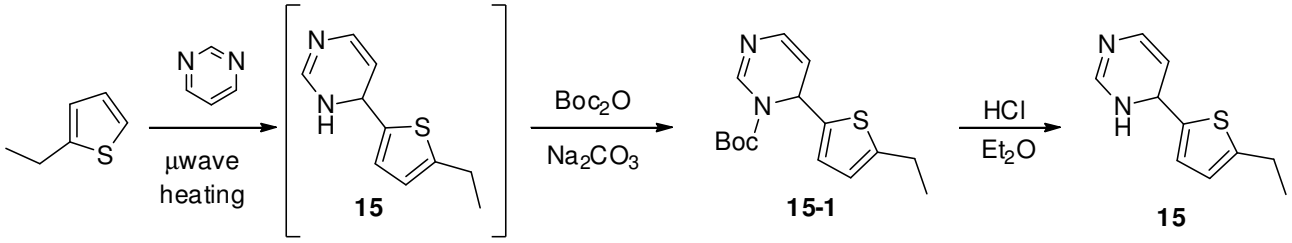
HCl salt of 7-Fluoro-4-(2-methylbenzo[b]thiophen-3-yl)-3,4-dihydroquinazoline (36)



36

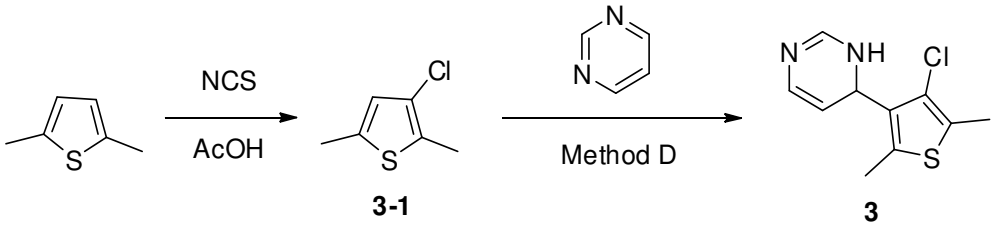
The HCl salt of **36** was prepared as described above in General Method C from 2-methylbenzo[b]thiophene **23-1** and 7-fluoroquinazoline. MS (ESI+): *m/z* 297 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.43 (s, 1H), 7.87-7.84 (m, 1H), 7.52-7.50 (m, 1H), 7.34-7.29 (m, 2H), 6.99-6.84 (m, 3H), 6.70 (s, 1H), 2.67 (s, 3H).

D. General Method D for the formation of thiophenyl-1,6-dihydropyrimdines under microwave conditions using Boc group for isolation. Synthesis of the HCl salt of 6-(5-ethylthiophen-2-yl)-1,6-dihydropyrimidine (15).



A 10 mL microwave reaction tube was charged with TFA (1 mL) and 2-ethylthiophene (200 mg, 1.78 mmol) and pyrimidine (255 mg, 3.18 mmol) were added. The reaction was heated at 120^{o}C under microwave irradiation for 12 minutes. After cooling to room temperature, the reaction mixture was made basic by the addition of a saturated aqueous Na_2CO_3 solution, and then a solution of Boc_2O (1.5 equiv) in EtOAc (20 mL) was added dropwise. After the starting material was consumed completely by TLC, the reaction mixture was extracted with EtOAc (3×20mL), washed with brine (3×50 mL) and dried over anhydrous Na_2SO_4. After filtration and concentration, the crude product was purified by column chromatography to give *tert*-butyl 6-(5-ethylthiophen-2-yl)pyrimidine-1(6H)-carboxylate **15-1**, which was dissolved in ether and cooled in an ice bath. A stream of gaseous HCl was bubbled through the solution at 0ºC for 10 min. The solid precipitate that formed was collected by filtration and dried under vacuum to give the HCl salt of **15** (103 mg, 30%) as a tan powder. MS (ESI+): *m/z* 193 [M+H]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51-7.25 (m, 1H), 6.87-6.81 (m, 1H), 6.64-6.62 (m, 1H), 6.26-6.23 (m, 1H), 5.43-5.36 (m, 1H), 4.96-4.93 (m, 1H), 2.84-2.76 (m, 2H), 1.30-1.26 (m, 3H).

HCl salt of 6-(4-Chloro-2,5-dimethylthiophen-3-yl)-1,6-dihydropyrimidine (3)

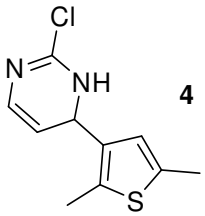


3-1 **3**

A solution of 2,5-dimethylthiophene (3.36 g, 30 mmol) in AcOH (50 mL) at 0°C was treated with N-chlorosuccinimide (4.42 g, 33 mmol) in one portion. The reaction mixture was warmed gradually to room temperature and stirred overnight. The mixture was then treated with Na_2SO_3 and water, followed by extraction with EtOAc. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. After filtration and concentration, the crude product was purified by column chromatography to give 3-chloro-2,5-dimethylthiophene **3-1** (2.2 g, 50%).

The HCl salt of compound **3** was prepared as described above for General Method D. MS (ESI+): *m/z* 226 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.12 (s, 1H), 6.36 (d, J = 8.4 Hz, 1H), 5.82-5.80 (m, 1H), 5.15 (dd, J = 7.5, 2.3 Hz, 1H), 2.46 (s, 3H), 2.36 (s, 3H).

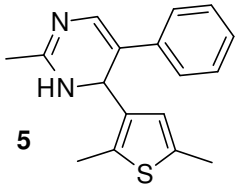
HCl salt of 2-Chloro-6-(2,5-dimethylthiophen-3-yl)-1,6-dihydropyrimidine (4)



4

The HCl salt of compound **4** was prepared as described above for General Method D using 2,5-dimethylthiophene and 2-chloropyrimidine. MS (ESI+): *m/z* 227 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.22 (s, 1H), 6.77 (s, 1H), 6.75 (s, 1H), 5.66 (s, 1H), 2.44 (s, 3H), 2.42 (s, 3H).

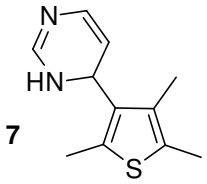
HCl salt of 6-(2,5-Dimethylthiophen-3-yl)-2-methyl-5-phenyl-1,6-dihydropyrimidine (5)



5

The HCl salt of compound **5** was prepared as described above for General Method D using 2,5-dimethylthiophene and 2-methyl-5-phenylpyrimidine. MS (ESI+): *m/z* 283 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 7.28-7.33 (m, 5H), 6.81 (s, 1H), 6.71 (s, 1H), 5.95 (s, 1H), 2.45 (s, 3H), 2.33 (s, 3H), 2.32 (s, 3H).

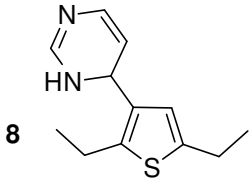
HCl salt of 6-(2,4,5-Trimethylthiophen-3-yl)-1,6-dihydropyrimidine (7)



7

The HCl salt of compound **7** was prepared as described above for General Method D using 2,3,5-trimethylthiophene. MS (ESI+): *m/z* 206 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.09 (s, 1H), 6.33 (d, *J* = 8.3 Hz, 1H), 5.73 (t, *J* = 2.4 Hz, 1H), 5.15 (dd, *J* = 8.3, 2.9 Hz, 1H), 2.41 (s, 3H), 2.29 (s, 3H), 2.18 (s, 3H).

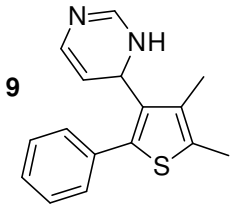
HCl salt of 6-(2,5-Diethylthiophen-3-yl)-1,6-dihydropyrimidine (8)



8

The HCl salt of compound **8** was prepared as described above for General Method D using 2,5-diethylthiophene and pyrimidine. MS (ESI+): *m/z* 221 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.06 (s, 1H), 6.82 (s, 1H), 6.36 (d, *J* = 8.2 Hz, 1H), 5.55 (dd, *J* = 3.2, 1.4 Hz, 1H), 5.24 (dd, *J* = 8.2, 3.3 Hz, 1H), 2.87-2.76 (m, 4H), 1.31-1.25 (m, 6H).

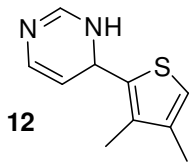
HCl salt of 6-(4,5-Dimethyl-2-phenylthiophen-3-yl)-1,6-dihydropyrimidine (9)



9

The HCl salt of compound **9** was prepared as described above for General Method D using 2,3-dimethyl-5-phenylthiophene and pyrimidine. MS (ESI+): *m/z* 269 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 7.96 (s, 1H), 7.49-7.36 (m, 5H), 6.25 (d, *J* = 8.2 Hz, 1H), 5.70 (t, *J* = 2.4 Hz, 1H), 5.23 (dd, *J* = 8.2, 2.8 Hz, 1H), 2.41 (s, 3H), 2.32 (s, 3H).

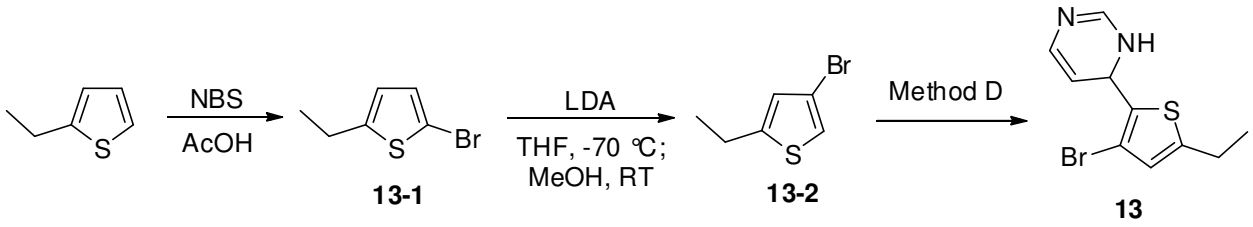
HCl salt of 6-(3,4-Dimethylthiophen-2-yl)-1,6-dihydropyrimidine (12)



12

The HCl salt of compound **12** was prepared as described above for General Method D using 3,4-dimethylthiophene and pyrimidine. MS (ESI+): *m/z* 193 [M+H]; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.38 (s, 1H), 10.95 (s, 1H), 8.23 (s, 1H), 7.21 (s, 1H), 6.49 (d, J = 8.0 Hz, 1H), 5.80 (d, J = 3.3 Hz, 1H), 5.32-5.28 (m, 1H), 2.11 (s, 6H).

HCl salt of 6-(3-Bromo-5-ethylthiophen-2-yl)-1,6-dihydropyrimidine (13)

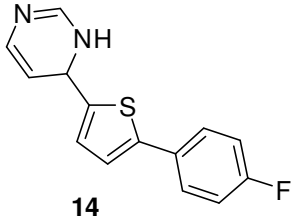


A solution of 2-ethylthiophene (5 g, 44.6 mmol) in 30 mL of $CHCl_3$ and 30 mL of AcOH was cooled in an ice bath to 0°C, and treated with N-bromosuccinimide (8.7 g, 49.6 mmol). The resulting mixture was stirred at 0°C for 3 h. After the starting material was consumed completely as shown by TLC, the reaction was quenched with saturated Na_2CO_3 and extracted with $CHCl_3$. The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$. After filtration and concentration under reduced pressure, the crude product was purified by column chromatography to give 2-bromo-5-ethylthiophene **13-1** (7.67 g, 90%).

A solution of **13-1** (4 g, 21.05 mmol) in anhydrous THF (50 mL) under a N_2 atmosphere was cooled in a dry ice/acetone bath to -70°C. LDA (42.1 mmol) was added dropwise, and the resulting mixture was stirred at -70°C for 2 h. MeOH (120 mL) was then added and stirring was continued for another 1 h. The cold bath was removed and the reaction mixture was then allowed to warm to room temperature where it was quenched with aqueous saturated Na_2CO_3 and extracted with EtOAc (3×200 mL). The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$. After filtration and concentration under reduced pressure, purification by column chromatography afforded 3-bromo-5-ethylthiophene **13-2** (3.7 g, 92%).

The HCl salt of compound **13** was prepared as described above for General Method D. MS (ESI+): *m/z* 272 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.14 (s, 1H), 6.84 (s, 1H), 6.48 (d, J = 8.4 Hz, 1H), 5.86 (dd, J = 3.6, 1.2 Hz, 1H), 5.40-5.37 (m, 1H), 2.91-2.83 (m, 2H), 1.31 (t, J = 7.5 Hz, 3H).

HCl salt of 6-(5-(4-Fluorophenyl)thiophen-2-yl)-1,6-dihydropyrimidine (14)

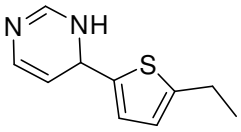


14

The HCl salt of compound **14** was prepared as described above for General Method D using 2-(4-fluorophenyl)thiophene. MS (ESI+): *m/z* 259 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.14 (s, 1H),

7.69-7.64 (m, 2H), 7.32 (d, *J* = 3.7 Hz, 1H), 7.19-7.14 (m, 3H), 6.51 (d, *J* = 8.1 Hz, 1H), 5.80-5.78 (m, 1H), 5.52-5.48 (m, 1H).

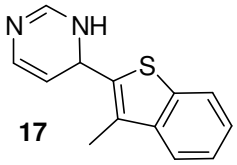
HCl salt of 6-(5-Ethylthiophen-2-yl)-1,6-dihydropyrimidine (15)



15

The HCl salt of compound **15** was prepared as described above for General Method D using 2-ethylthiophene. MS (ESI+): *m/z* 193 [M+H]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51-7.25 (m, 1H), 6.87-6.81 (m, 1H), 6.64-6.62 (m, 1H), 6.26-6.23 (m, 1H), 5.43-5.36 (m, 1H), 4.96-4.93 (m, 1H), 2.84-2.76 (m, 2H), 1.30-1.26 (m, 3H).

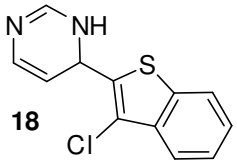
HCl salt of 6-(3-Methylbenzo[b]thiophen-2-yl)-1,6-dihydropyrimidine (17)



17

The HCl salt of compound **17** was prepared as described above for General Method D using 3-methylbenzo[b]thiophene. MS (ESI+): *m/z* 229 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.17 (s, 1H), 7.89 (d, *J* = 7.1 Hz, 1H), 7.0.83 (d, *J* = 7.7 Hz, 1H), 7.48-7.42 (m, 2H), 6.50 (d, *J* = 8.0 Hz, 1H), 6.13 (d, *J* = 3.2 Hz, 1H), 5.46 (dd, *J* = 8.0 Hz, *J* = 3.4 Hz, 1H), 2.48 (s, 3H).

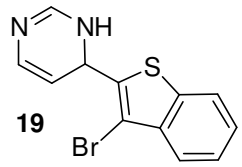
HCl salt of 6-(3-Chlorobenzo[b]thiophen-2-yl)-1,6-dihydropyrimidine (18)



18

The HCl salt of compound **18** was prepared as described above for General Method D using 3-chlorobenzo[b]thiophene. MS (ESI+): *m/z* 249 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.25 (s, 1H), 7.89-7.99 (m, 2H), 7.51-7.59 (m, 2H), 6.56 (d, *J* = 8.1 Hz, 1H), 6.17 (dd, *J* = 3.6, 1.3 Hz, 1H), 5.49 (dd, *J* = 7.5, 3.6 Hz, 1H).

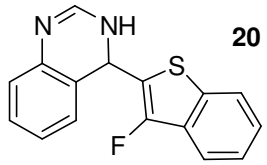
HCl salt of 6-(3-Bromobenzo[b]thiophen-2-yl)-1,6-dihydropyrimidine (19)



19

The HCl salt of compound **19** was prepared as described above for General Method D using 3-bromobenzo[b]thiophene. MS (ESI+): *m/z* 293 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.25 (s, 1H), 8.00-7.97 (m, 1H), 7.91-7.88 (m, 1H), 7.60-7.50 (m, 2H), 6.56 (d, J = 8.1 Hz, 1H), 6.18-6.17 (m, 1H), 5.50 (dd, J = 7.6, 3.1 Hz, 1H).

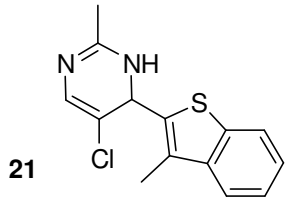
HCl salt of 4-(3-Fluorobenzo[b]thiophen-2-yl)-3,4-dihydroquinazoline (20)



20

The HCl salt of compound **20** was prepared as described above for General Method D using 3-fluorobenzo[b]thiophene[3]and quinazoline. MS (ESI+): *m/z* 283 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.44 (s, 1H), 7.84-7.87 (m, 2H), 7.60-7.63 (m, 1H), 7.39-7.43 (m, 3H), 7.20-7.26 (m, 2H), 7.03 (d, J = 7.6 Hz, 1H), 6.73 (s, 1H).

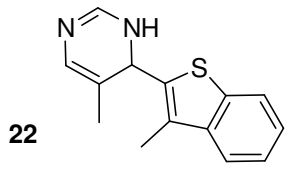
HCl salt of 5-Chloro-2-methyl-6-(3-methylbenzo[b]thiophen-2-yl)-1,6-dihydropyrimidine (21)



21

The HCl salt of compound **21** was prepared as described above for General Method D using 3-methylbenzo[b]thiophene and 5-chloro-2-methylpyrimidine. MS (ESI+): *m/z* 277 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 7.82-7.91 (m, 2H), 7.42-7.50 (m, 2H), 6.74 (s, 1H), 6.21 (s, 1H), 2.52 (s, 3H), 2.33 (s, 3H).

HCl salt of 5-Methyl-6-(3-methylbenzo[b]thiophen-2-yl)-1,6-dihydropyrimidine (22)

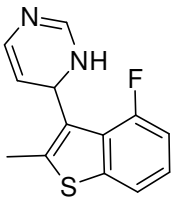


22

The HCl salt of compound **22** was prepared as described above for General Method D using 3-methylbenzo[b]thiophene and 5-methylpyrimidine. MS (ESI+): *m/z* 243 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.13 (s, 1H), 7.81-7.89 (m, 2H), 7.39-7.49 (m, 2H), 6.33 (s, 1H), 6.01 (s, 1H), 2.52 (s, 3H), 1.67 (s, 3H).

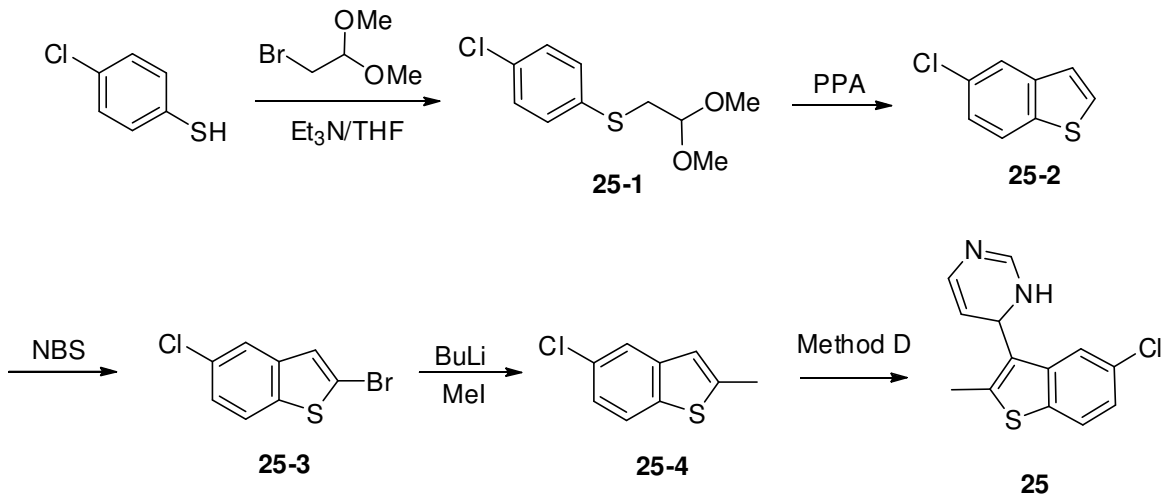
HCl salt of 6-(4-Fluoro-2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine (24)

24



The HCl salt of compound **24** was prepared as described above for General Method D using 4-fluoro-2-methylbenzo[b]thiophene. MS (ESI+): *m/z* 247 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.21 (s, 1H), 7.93-7.88 (m, 1H), 7.64 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.23 (td, *J* = 9.0, 2.5 Hz, 1H), 6.47 (dd, *J* = 8.2, 0.6 Hz, 1H), 6.12-6.10 (m, 1H), 5.29-5.26 (m, 1H), 2.63 (s, 3H).

HCl salt of 6-(4-Fluoro-2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine (25)



A solution of 4-chlorothiophenol (14.5g, 100 mmol) and K_2CO_3 (13.8 g, 100 mmol) in 100 mL of DMF was cooled in an ice bath to 0ºC, and treated with 2-bromo-1,1-dimethoxyethane (18.7 g, 110 mmol). After the thiophenol was consumed completely as shown by TLC, water (200 mL) was added and the mixture was extracted with EtOAc (3×100 mL). The combined organic layers were washed with brine (3×50 mL) and dried over anhydrous Na_2SO_4. After filtration and concentration under reduced pressure, purification by column chromatography provided (4-chlorophenyl) (2,2-dimethoxyethyl)sulfane **25-1** (19.5 g, 84%).

Intermediate **25-1** (22.1 g, 95 mmol) was dissolved in 50 mL of chlorobenzene. The solution was added dropwise to a boiling mixture of polyphosphoric acid (200 g) in chlorobenzene (100 mL) over 10 minutes. The reaction mixture was poured into ice water (500 mL) and made basic by the addition of solid Na_2CO_3 until the pH 8. The aqueous layer was then extracted with EtOAc (3×100

mL), and the combined organic layers were washed with brine (3×50 mL) and dried over anhydrous Na_2SO_4. After filtration and concentration under reduced pressure, purification by column chromatography afforded 5-chlorobenzo[b]thiophene **25-2** (7.69 g, 48%).

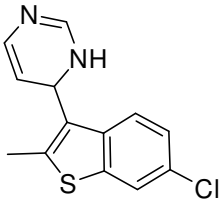
N-Bromosuccinimide (5.34 g, 30 mmol) was added in a single portion to a solution of **25-2** (5.06 g, 30 mmol) in AcOH (10 mL) and CH_2Cl_2 (50 mL) at room temperature. After stirring overnight, the reaction mixture was treated with Na_2SO_3 and water, followed by extraction with EtOAc. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. After filtration and concentration under reduced pressure, purification by column chromatography gave 2-bromo-5-chlorobenzo[b]thiophene **25-3** (6.68 g, 90%).

A solution of **25-3** (6.19 g, 25 mmol) in anhydrous THF (100 mL) under nitrogen was cooled in a dry ice/acetone bath to -78ºC and then treated with n-butyl lithium (10 mL, 2.5 M in hexane) in a dropwise manner. The mixture was stirred at -78ºC for 30 min, and then warmed to -20ºC and treated with iodomethane (7.1 g, 50 mmol). After stirring at -20ºC for 30 min, the reaction mixture was poured into water and extracted with ether (3×50 mL). The combined organic layers were washed with brine (3×50 mL) and dried over anhydrous Na_2SO_4. After concentration under reduced pressure, purification by column chromatography afforded 5-chloro-2-methylbenzo[b]thiophene **25-4** (4.18 g, 90%).

The HCl salt of compound **25** was prepared as described above for General Method D. MS (ESI+): *m/z* 263 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.26 (s, 1H), 7.90 (s, 1H), 7.87-7.84 (d, J = 8.6 Hz, 1H), 7.36 (dd, J = 8.6, 1.9 Hz, 1H), 6.50 (d, J = 7.8 Hz, 1H), 6.12 (t, J = 2.4 Hz, 1H), 5.29 (dd, J = 8.2, 2.5 Hz, 1H), 2.66 (s, 3H).

HCl salt of 6-(6-Chloro-2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine (27)

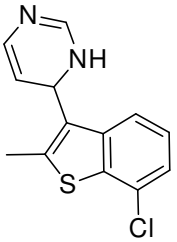
27



The HCl salt of compound **27** was prepared as described above for General Method D using 6-chloro-2-methylbenzo[b]thiophene. MS (ESI+): *m/z* 263.5 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.22 (s, 1H), 7.92-7.86 (m, 2H), 7.44-7.40 (m, 1H), 6.47 (d, J = 8.5 Hz, 1H), 6.11 (m, 1H), 5.28-5.26 (m, 1H), 2.64 (s, 3H).

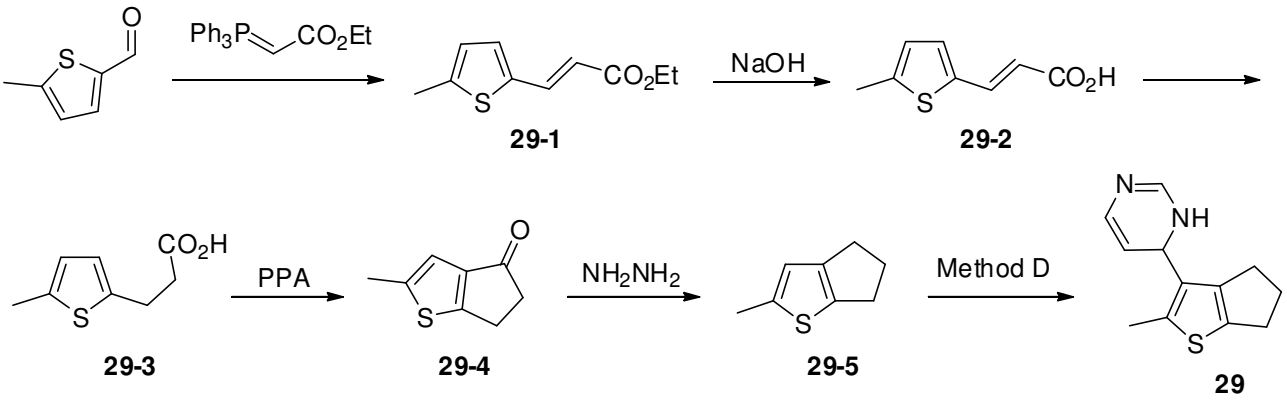
HCl salt of 6-(7-Chloro-2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine (28)

28



The HCl salt of compound **28** was prepared as described above for General Method D using 7-chloro-2-methylbenzo[b]thiophene. MS (ESI+): *m/z* 263 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.23 (s, 1H), 7.89 (dd, J = 7.4, 1.4 Hz, 1H), 7.48-7.40 (m, 2H), 6.48 (d, J = 8.0 Hz, 1H), 5.29 (dd, J = 8.1, 2.4 Hz, 1H), 2.65 (s, 3H).

HCl salt of 6-(2-Methyl-5,6-dihydro-4H-cyclopenta[b]thiophen-3-yl)-1,6-dihydropyrimidine (29)



(Carbethoxymethylene)triphenylphosphorane (191.4 g, 0.55 mol) was added to a solution of 5-methylthiophene-2-carbaldehyde (63 g, 0.5 mol) in CH_2Cl_2 (200 mL) at room temperature and the reaction mixture was stirred overnight. After concentration under reduced pressure, purification by column chromatography gave ethyl 3-(5-methylthiophen-2-yl)acrylate **29-1** (98 g, 97%).

A solution of NaOH (130 g, 3.25 mol) in 2L of H_2O was added to a solution of **29-1** (80 g, 408 mmol) in 2L of methanol at room temperature. The mixture was heated to 50°C and stirred for 0.5 h. After the starting material was consumed completely as shown by TLC, the reaction mixture was concentrated under reduced pressure. The crude 3-(5-methylthiophen-2-yl)acrylic acid **29-2** was used directly without further purification.

A mixture of **29-2** (20 g, 119 mmol) and a catalytic amount of Pd/C in 1 L of H_2O was stirred under an atmosphere of H_2 at room temperature. After the reduction was complete by TLC, the suspension was filtered through a Celite® pad, rinsing with H_2O. The filtrate was acidified to pH 2 and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give 3-(5-methylthiophen-2-yl) propanoic acid **29-3**, which was used without further purification in the following reaction.

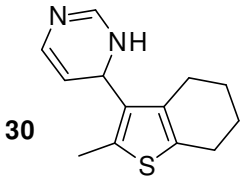
The crude **29-3** was dissolved in 200 mL of 1,2-dichloroethane and added to 300 g of polyphosporic acid (PPA). The resulting mixture was refluxed overnight. After cooling to room temperature, volatiles were removed by concentration under reduced pressure. The resulting mixture was made basic by the addition of solid $NaCO_3$. H_2O was added to dissolve the inorganic solids, and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After filtration and concentration under reduced pressure, purification by column chromatography afforded 2-methyl-5,6-dihydro-4H-cyclopenta[b]thiophen-4-one **29-4** (7.4 g, 41% over 2 steps).

A solution of **29-4** (5 g, 33 mmol) in ethylene glycol (10 mL) and water (2 mL) was treated with KOH (7.4 g, 132 mmol) and hydrazine hydrate(50 mL). The reaction mixture was heated to reflux and stirred for 48 h. After cooling to room temperature, the mixture was diluted with H_2O and

extracted with EtOAc. The organic layer was then washed with brine and dried over anhydrous Na_2SO_4. After filtration and concentration under reduced pressure, purification by column chromatography provided 2-methyl-5,6-dihydro-4H-cyclopenta[b]thiophene **29-5** (2.8 g, 62%).

The HCl salt of compound **29** was prepared as described above for General Method D. MS (ESI+): *m/z* 219 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.09 (s, 1H), 6.34 (d, J = 8.2 Hz, 1H), 5.64-5.63 (m, 1H), 5.16 (dd, J = 7.5, 2.5 Hz, 1H), 2.86-2.71 (m, 4H), 2.52-2.47 (m, 2H), 2.43 (s, 3H).

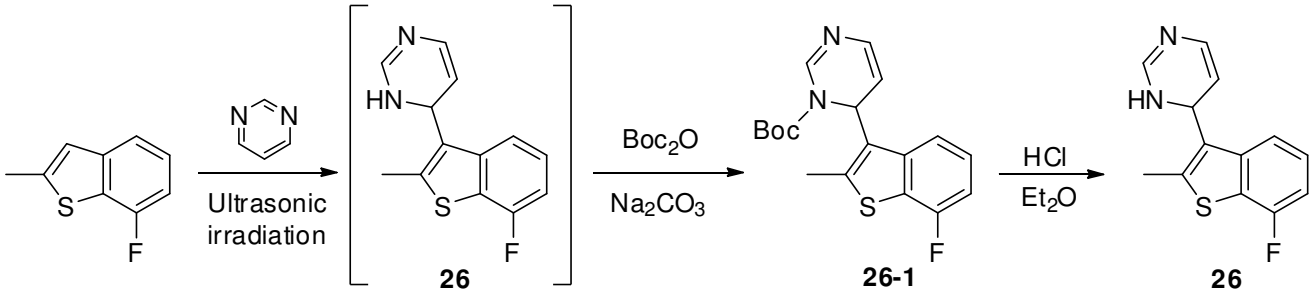
HCl salt of 6-(7-Chloro-2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine (30)



30

The HCl salt of compound **30** was prepared as described above for General Method D using 2-methyl-4,5,6,7-tetrahydrobenzo[b]thiophene (synthesized from 5-methyl-2-thiophenebutanoic acid as described for compound **29**. MS (ESI+): *m/z* 233 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.07 (s, 1H), 6.32 (d, J = 8.2 Hz, 1H), 5.69 (t, J = 2.3 Hz, 1H), 5.15 (dd, J = 7.7, 2.3 Hz, 1H), 2.75-2.55 (m, 4H), 2.42 (s, 3H), 1.84-1.83 (m, 4H).

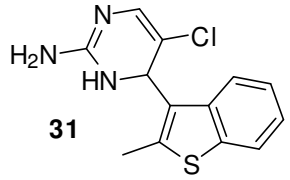
E. General Method E for the formation of thiophenyl-1,6-dihydropyrimidines under ultrasonic conditions using Boc group for isolation. Synthesis of the HCl salt of 6-(7-fluoro-2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine (26).



A solution of pyrimidine (97 mg, 1.21 mmol) in TFA (5 mL) was treated with 7-fluoro-2-methylbenzo[b]thiophene (200 mg, 1.20 mmol). The reaction was sonicated for 1 h at room temperature in an ultrasonic bath. After the starting material was consumed completely, the reaction mixture was quenched with saturated aqueous Na_2CO_3 solution and a solution of Boc_2O (1.5 equiv) in EtOAc (20 mL) was added dropwise. After stirring overnight, the reaction mixture was extracted with EtOAc (3×20 mL). The combined organics were washed with brine (3×50 mL) and dried over anhydrous Na_2SO_4. After filtration and concentration under reduced pressure, purification by column chromatography gave *tert*-butyl 6-(7-fluoro-2-methylbenzo[b]thiophen-3-yl)pyrimidine-1(6H)-carboxylate **26-1**, which was dissolved in ether. The solution was cooled in an ice bath and gaseous HCl was bubbled through for 10 min. The precipitated solid was collected by vacuum filtration and dried under vacuum to provide 6-(7-fluoro-2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine HCl salt **26** (121 mg, 41%). MS (ESI+): *m/z* 247 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.21 (s, 1H), 7.74

(d, *J* = 8.1 Hz, 1H), 7.48-7.41 (m, 1H), 7.17 (dd, *J* = 9.6, 8.3 Hz, 1H), 6.47 (d, *J* = 8.2 Hz, 1H), 6.13 (t, *J* = 2.4 Hz, 1H), 5.29 (dd, *J* = 8.2, 2.8 Hz, 1H), 2.67 (s, 3H).

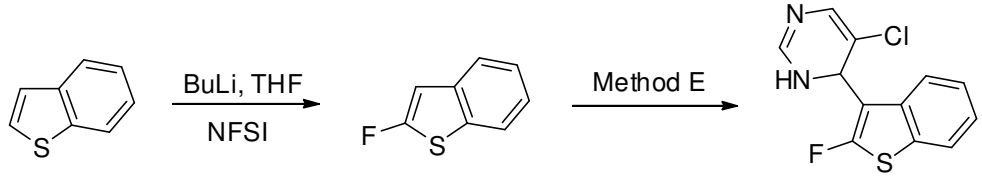
HCl salt of 5-Chloro-6-(2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidin-2-amine (31)



31

The HCl salt of compound **31** was prepared as described above for General Method E using 2-methylbenzo[b]thiophene **23-1** and 2-amino-5-chloropyrimidine. MS (ESI+): *m/z* 278 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 7.85-7.83 (m, 1H), 7.76-7.73 (m, 1H), 7.41-7.32 (m, 2H), 6.66 (s, 1H), 5.91 (s, 1H), 2.64 (s, 3H).

HCl salt of 5-Chloro-6-(2-fluorobenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine (32)

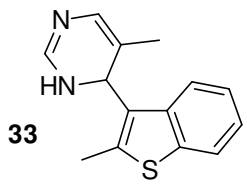


32-1 **32**

A solution of benzo[b]thiophene (1.34 g, 10 mmol) in anhydrous THF (100 mL) was chilled in a dry ice/acetone bath and treated by the dropwise addition of *n*-butyl lithium (4.4 mL, 11 mmol, 2.5 M in hexane). After stirring at -78ºC for 30 min, N-fluorobenzenesulfonimide (NFSI, 6.3 g, 20 mmol) was added. The reaction mixture was stirred at -78ºC for 30 min. The mixture was warmed gradually to room temperature and stirred for two days. Excess base was carefully quenched with water and the mixture was extracted with ether (3×50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give a crude product that was purified by column chromatography to provide 2-fluorobenzo[b]thiophene **32-1** (807 mg, 53%).

The HCl salt of compound **32** was prepared as described above for General Method E using 5-chloropyrimidine. MS (ESI+): *m/z* 267 ($M+H^+$); ^{1}H NMR (300 MHz, CD_3OD): δ 6.78 (s, 1H), 6.61-6.59 (d, *J* = 6.3 Hz, 1H), 5.78 (s, 1H), 2.42 (s, 3H), 2.27 (s, 3H)

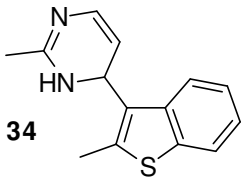
HCl salt of 5-Methyl-6-(2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine (33)



33

The HCl salt of compound **33** was prepared as described above for General Method E using 2-methylbenzo[b]thiophene **23-1** and 5-methylpyrimidine. MS (ESI+): *m/z* 243 [M+H]; ^{1}H NMR (400 MHz, CD_3OD): δ 8.11 (s, 1H), 7.85 (t, *J* = 6.5 Hz, 2H), 7.41-7.32 (m, 2H), 6.28 (s, 1H), 6.02 (br s, 1H), 2.63 (s, 3H), 1.47 (s, 3H).

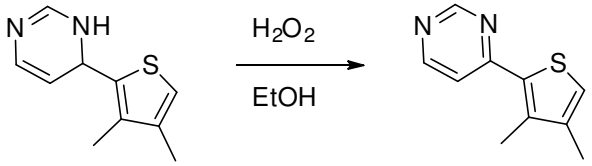
HCl salt of 2-Methyl-6-(2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine (34)



34

The HCl salt of compound **34** was prepared as described above for General Method E using 2-methylbenzo[b]thiophene **23-1** and 2-methylpyrimidine. MS (ESI+): *m/z* 243 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 7.89-7.84 (m, 2H), 7.44-7.33 (m, 2H), 6.44 (dd, *J* = 8.1, 1.8 Hz, 1H), 6.09-6.07 (m, 1H), 5.27-5.23 (dd, *J* = 8.1, 3.0 Hz, 1H), 2.63 (s, 3H), 2.28 (s, 3H).

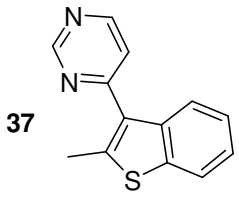
F. Oxidative synthesis of 4-(3,4-dimethylthiophen-2-yl)pyrimidine (16)



12 **16**

A solution of compound **12** (600 mg, 3.12 mmol) in ethanol (40 mL) was treated with hydrogen peroxide (3.53 mL, 31.2 mmol, 30% in H_2O), and heated to 70^{o}C for 1 h. Water (10 mL) and EtOAc (10 mL) were added to the reaction vessel and the resulting biphasic mixture was transferred to a separatory funnel. The layers were separated and the aqueous phase was extracted with EtOAc (2×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting oil was purified by flash column chromatography (SiO_2, gradient elution from 5% EtOAc/hexanes to 15% EtOAc/hexanes) to afford 4-(3,4-dimethylthiophen-2-yl)pyrimidine **16** (134 mg, 23%) as a brown solid. MS (ESI+): *m/z* 193 [M+H]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.18 (d, *J* = 1.1 Hz, 1H), 8.70 (d, *J* = 5.5 Hz, 1H), 7.52 (dd, *J* = 5.46, 1.4 Hz, 1H), 7.14 (s, 1H), 2.50 (s, 3H), 2.24 (s, 3H).

6-(2-Methylbenzo[b]thiophen-3-yl)-pyrimidine (37)

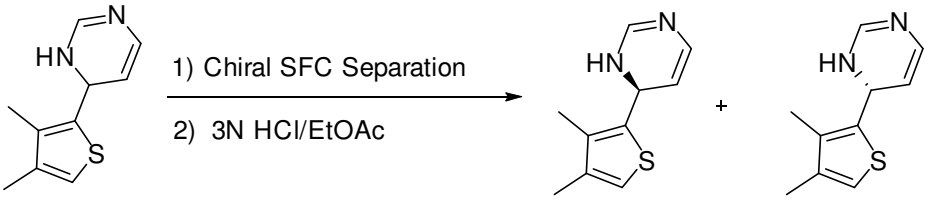


37

Compound **37** was prepared by oxidation of 6-(2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine **23** as described above. MS (ESI+): *m/z* 227 [M+H]; ^{1}HNMR (300 MHz, CDCl3) δ

9.40 (d, *J* = 1.2 Hz, 1H), 8.87 (d, *J* = 5.1 Hz, 1H), 7.88-7.81 (m, 2H), 7.53 (dd, *J* = 5.1, 1.5 Hz, 1H), 7.41-7.33 (m, 2H), 2.72 (s, 3H).

G. Chiral separation of Compound 12 into enantiomers 12a and 12b.



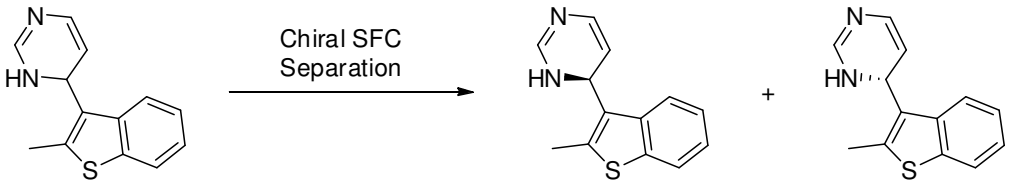
12 **12a** **12b**

Compound **12** (1.8 g, 7.9 mmol) was separated into its enantiomers (*S*)-6-(3,4-dimethylthiophen-2-yl)-1,6- dihydropyrimidine **12a** and (*R*)-6-(3,4-dimethylthiophen-2-yl)-1,6-dihydropyrimidine **12b** by preparative supercritical fluid chromatography (SFC) using an SFC-200 instrument (Thar, Waters) and a Whelk-O1 50×250 mm 5um (Daicel) column (column temperature: 40ºC; mobile phase A: CO_2; B: 1:1 hexanes/isopropanol (1% isopropylamine), A:B = 50:50). The flow rate was 120 g/min, back pressure was 100 bar and cycle time of stack injections was 15 min. The two isomers were separately concentrated under reduced pressure with a bath temperature below 30°C. The residue was dissolved in 10 mL of H_2O and lyophilized to give the freebase as a powder. The powder was dissolved in EtOAc (5 mL) and cooled in an ice bath. 3N HCl/EtOAc (1.5 e.q.) was added, and the mixture was stirred for 5 min and concentrated to dryness under reduced pressure to afford the HCl salts of **12a** (650 mg, 36%; retention time: 4.46 min; 98.6% ee) as a yellow solid and **12b** (570 mg, 33%, retention time 6.35 min, 95.9% ee) as a yellow solid.

HCl salt of Compound 12a. MS (ESI+): m/z 193 [M+H]; ^{1}H NMR (300 MHz, DMSO-*d6*): δ 11.38 (s, 1H), 10.95 (s, 1H), 8.23 (s, 1H), 7.21 (s, 1H), 6.50-6.47 (d, *J* = 8.0 Hz, 1H), 5.80 (d, *J* = 3.3 Hz, 1H), 5.32-5.28 (m, 1H), 2.11 (s, 6H). [α] = + 189 º (MeOH, c = 0.1).

HCl salt of Compound 12b. MS (ESI+): m/z 193 [M+H]; ^{1}H NMR (300 MHz, DMSO-*d6*): δ 11.38 (s, 1H), 10.95 (s, 1H), 8.23 (s, 1H), 7.21 (s, 1H), 6.50-6.47 (d, *J* = 8.0 Hz, 1H), 5.80 (d, *J* = 3.3 Hz, 1H), 5.32-5.28 (m, 1H), 2.11 (s, 6H). [α] =-161 º for **12b** (MeOH, c = 0.2).

H. Chiral separation of Compound 23 into enantiomers 23a and 23b



23 **23a** **23b**

Compound **23** (1.15 g, 5.0 mmol) was separated into its enantiomers (S)-6-(2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine **23a** and (*R*)-6-(2-methylbenzo[b]thiophen-3-yl)-1,6-dihydropyrimidine **23b** by preparative supercritical fluid chromatography (SFC) using an SFC-200

instrument (Thar, Waters) and a ChiralPak OD-H 30×250 mm 5um (Chiral Technologies) column (column temperature: 25ºC; mobile phase A: CO_2; B: 1:1 Methanol/1% isopropylamine, A:B = 75:25). The flow rate was 80 mL/min, back pressure was 125 bar. Following separation, the two enantiomers were individually concentrated under reduced pressure at room temperature to afford **23a** (418 mg, 36%; retention time: 2.09 min; 99.4% ee) and **23b** (431 mg, 37%, retention time 3.39 min, 99.6% ee).

½ L-tartrate salt of **23a** was obtained by treating **23a** free base with ½ L-tartrate in MeOH. ([α] = + 31 º (MeOH, c = 0.3); ½ D- tartrate salt of **23b** was obtained by treating **23b** free base with ½ D-tartrate in MeOH. ([α] = -32 º (MeOH, c = 0.4).

The enantiomer freebases were also converted to the HCl salt as described above.

HCl salt of Compound 23a. MS (ESI+): *m/z* 229 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.21 (s, 1H), 7.89 (dd, *J* = 16.9, 7.5 Hz, 2H), 7.45-7.34 (m, 2H), 6.46 (d, *J* = 8.2 Hz, 1H), 6.13 (t, *J* = 2.4 Hz, 1H), 5.28 (dd, *J* = 8.3, 2.9 Hz, 1H), 2.64 (s, 3H).

HCl salt of Compound 23b. MS (ESI+): *m/z* 229 [M+H]; ^{1}H NMR (300 MHz, CD_3OD): δ 8.21 (s, 1H), 7.89 (dd, *J* = 16.9, 7.5 Hz, 2H), 7.45-7.34 (m, 2H), 6.47-6.45 (d, *J* = 8.2 Hz, 1H), 6.13 (t, *J* = 2.4 Hz, 1H), 5.28 (dd, *J* = 8.3, 2.9 Hz, 1H), 2.64 (s, 3H).

II. Chemical stability studies of compounds 12a and 23a

Compounds **12a** and **23a** were separately dissolved in either a 0.01 M HCl solution (pH 2) or a 20 mM NH_4Ac buffer solution (pH 7.1) and placed in sealed vials. The solutions were kept at room temperature and monitored periodically by HPLC over the course of 96 h to measure the peak area percent remaining of parent compound.. At pH 2, the area percent remaining for both **12a** and **23a** remained at or above 98.9% over the time monitored, indicating that both were stable under these conditions (Figure 1 and Table 2). In contrast, at pH 7, compound **23a** was stable while compound **12a** decomposed gradually over the course of 96 h (Figure 1 and Table 2).

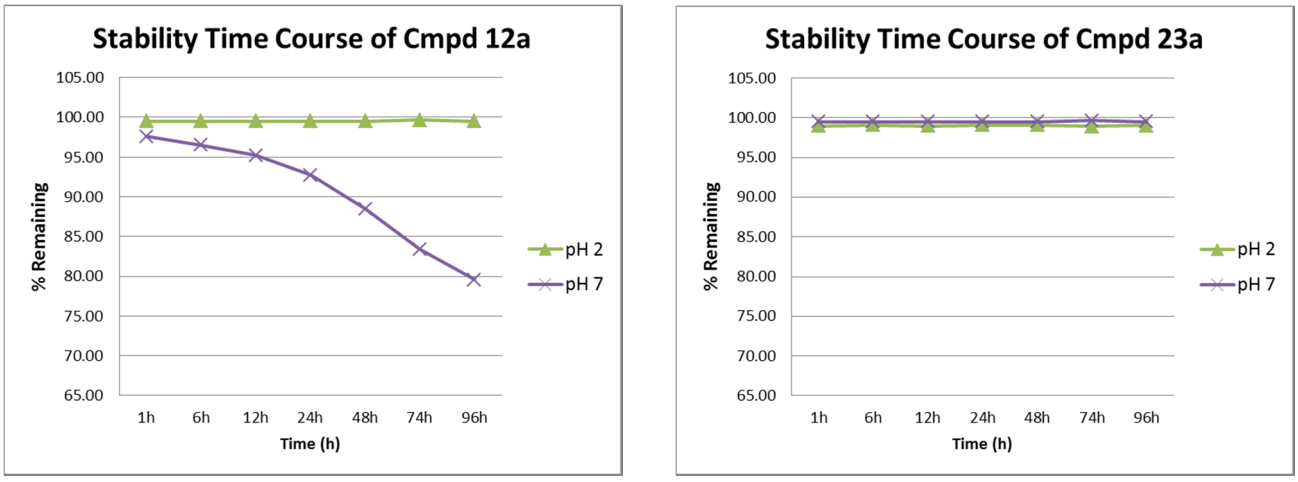


Figure 1. Time Course of Chemical Stability of **12a** and **23a** in pH 2 (0.01M HCl) and 7.1 (20 mM NH_4Ac) buffer solution)

Compounds	23a		12a	
Duration (h)	Peak% at pH 2	Peak% at pH 7	Peak% at pH 2	Peak% at pH7
1	99.003	99.49	99.49	97.59
6	99.092	99.49	99.49	96.48
12	98.967	99.49	99.49	95.20
24	99.086	99.51	99.51	92.7
48	99.076	99.49	99.49	88.4
74	98.946	99.67	99.67	83.41
96	99.057	99.50	99.50	79.58

Table 1. Stability testing of **12a** and **23a** at pH 2 (0.01 M HCl) and pH 7.1 (20 mM NH_4Ac) buffer solution)

III. *In vivo* Behavioral Pharmacological Assays

Antipsychotic-like activity of the compounds was evaluated in mice using the PCP hyperactivity models of schizophrenia.

Animals: Male C57Bl/6J mice from Jackson Laboratories (Bar Harbor, Maine) were used. Mice were received at approximately 6-7 weeks of age. Upon receipt, mice were assigned unique identification numbers (tail marked) and were group housed with 4 mice/cage in OptiMICE ventilated cages. All animals remained housed in groups of four during the remainder of the study. All mice were acclimated to the colony room for at least one week prior to testing. During the period of acclimation, mice were examined on a regular basis, handled, and weighed to assure adequate health and suitability. Animals were maintained on a 12/12 light/dark cycle. The room temperature was maintained between 20 and 23°C with a relative humidity maintained between 30% and 70%. Chow and water were provided *ad libitum* for the duration of the study. In each test, animals were randomly assigned across treatment groups. All testing was performed during the animal's light cycle phase. The behavioral test was conducted according to established protocols approved by the IACUC committee and PGI Standard Operation Procedures (SOP).

Phencyclidine-induced hyperactivity in the open field test (OF) is used as a model of psychosis.[4-7] The OF chambers are Plexiglas square chambers (27.3 x 27.3 x 20.3 cm; Med Associates Inc., St Albans, VT) surrounded by infrared photobeams (16 x 16 x 16) to measure horizontal and vertical activity. Distance traveled was measured from horizontal beam breaks as the mouse moved.

Ten animals were tested per treatment group. Mice were brought to the activity experimental room for at least 1 h acclimation to the experimental room conditions prior to testing. Mice were injected with vehicle, Compound **23a** dissolved in sterile injectable water (0.3, 1, 3, 10 and 30 mg/kg ; p.o.) or clozapine, dissolved in 10% DMSO (1 mg/kg, i.p.), and placed in the OF chambers for 30 min to measure baseline activity. Mice were then injected with either water or PCP (5 mg/kg, IP) and placed back in the OF chambers for a 60 min session. At the end of each OF test session, the OF chambers were thoroughly cleaned.

Pre-Pulse Inhibition (PPI; in mice) models of schizophrenia

Animals: Male C57Bl/6J mice from Jackson Laboratories (Bar Harbor, Maine) were used. Mice were received at approximately 6-7 weeks of age. Upon receipt, mice were assigned unique identification numbers (tail marked) and were group housed with 4 mice/cage in OptiMICE ventilated cages. All animals remained housed in groups of four during the remainder of the study. All mice were acclimated to the colony room for at least one week prior to testing. During the period of acclimation, mice were examined on a regular basis, handled, and weighed to assure adequate health and suitability. Animals were maintained on a 12/12 light/dark cycle. The room temperature was maintained between 20 and 23°C with a relative humidity maintained between 30% and 70%. Chow and water were provided *ad libitum* for the duration of the study. In each test, animals were randomly assigned across treatment groups. All testing was performed during the animal's light cycle phase. The behavioral test was conducted according to established protocols approved by the IACUC committee and PGI Standard Operation Procedures (SOP).

Prepulse Inhibition of Startle

The acoustic startle measures an unconditioned reflex response to external auditory stimulation. Prepulse Inhibition (PPI), consisting of an inhibited startle response (reduction in amplitude) to an auditory stimulation following the presentation of a weak auditory stimulus or prepulse, has been used as a tool for the assessment of deficiencies in sensory-motor gating, such as those seen in schizophrenia.[8-11] Mice were placed in the PPI chambers (Med Associates) for a 5 min session of white noise (70 dB) habituation. After the acclimation period, the test session was automatically started. The session started with a habituation block of 6 presentations of the startle stimulus alone, followed by 10 PPI blocks of 6 different types of trials. Trial types are: null (no stimuli), startle (120 dB), startle plus prepulse (4, 8 and 12 dB over background noise i.e. 74, 78 or 82 dB) and prepulse alone (82 dB). Trial types were presented at random within each block. Each trial started with a 50 ms null period during which baseline movements are recorded. There was a subsequent 20 ms period during which prepulse stimuli were presented and responses to the prepulse measured. After further 100 ms the startle stimuli were presented for 40 ms and responses recorded for 100 ms from startle onset. Responses were sampled every ms. The inter-trial interval was variable with an average of 15 s (range from 10 to 20 s). In startle alone trials the basic auditory startle was measured, and in prepulse plus startle trials the amount of inhibition of the normal startle was determined and expressed as a percentage of the basic startle response (from startle alone trials), excluding the startle response of the first habituation block. C57BL/6J mice administered vehicle, Haloperidol dissolved in 10% DMSO (1 mg/kg; IP), or Compound **23a** dissolved in sterile injectable water (1, 3 and 10 mg/kg ; PO) 30 minutes prior to testing. The PPI enclosures were cleaned following each test. All testing was performed during the animals light cycle.

IV. *In vitro* Pharmacology Profiling

1). Summary Results of compound **23a, 12a** and **12** in Cerep Profiling

	% Inhibition of Control Specific			
Compounds	**23a** (1μM)	**23a** (10μM)	**12a** (30μM)	**12** (30μM)
A_1 *(h)* (antagonist radioligand)	-23	-22	-4	16
A_{2A} *(h)* (agonist radioligand)	-6	-5	-7	12
A_3 *(h)* (agonist radioligand)	-35	-35	15	-10
α_1 (non-selective) (antagonist radioligand)	45	88	46	60
α_2 (non-selective) (antagonist radioligand)	72	97	81	90
β_1 *(h)* (agonist radioligand)	-10	4	31	16
β_2 *(h)* (agonist radioligand)	4	15	4	12
AT_1 *(h)* (antagonist radioligand)	-21	-29	-13	-3
BZD (central) (agonist radioligand)	-13	-19	3	-14
B_2 *(h)* (agonist radioligand)	-7	4	-4	-6
CB_1 *(h)* (agonist radioligand)	-15	-13	15	2
CCK_1 (CCKA) *(h)* (agonist radioligand)	-33	-9	-11	1
D_1 *(h)* (antagonist radioligand)	23	77	78	76
D_{2S} *(h)* (antagonist radioligand)	-4	-3	8	18
ET_A *(h)* (agonist radioligand)	1	-9	-12	-18
GABA (non-selective) (agonist radioligand)	6	2	5	-3
GAL_2 *(h)* (agonist radioligand)	-4	1	-8	-1
CXCR2 (IL-8B) *(h)* (agonist radioligand)	-1	-5	-14	-7
CCR1 *(h)* (agonist radioligand)	-2	7	2	-11
H_1 *(h)* (antagonist radioligand)	28	86	53	39
H_2 *(h)* (antagonist radioligand)	-6	38	13	30
MC_4 *(h)* (agonist radioligand)	-20	16	13	14
MT_1 (ML1A) *(h)* (agonist radioligand)	5	11	17	4
M_1 *(h)* (antagonist radioligand)	9	23	49	30
M_2 *(h)* (antagonist radioligand)	-4	6	33	34
M_3 *(h)* (antagonist radioligand)	-2	26	33	53
NK_2 *(h)* (agonist radioligand)	-7	-3	12	6
NK_3 *(h)* (antagonist radioligand)	9	2	-4	10
Y_1 *(h)* (agonist radioligand)	-8	3	-13	10
Y_2 *(h)* (agonist radioligand)	-3	-3	13	-7
NTS_1 (NT1) *(h)* (agonist radioligand)	-15	-13	-9	-8
δ_2 (DOP) *(h)* (agonist radioligand)	-3	-6	16	22
κ (KOP) (agonist radioligand)	-1	27	72	74
μ (MOP) *(h)* (agonist radioligand)	-1	18	34	65

NOP (ORL1) *(h)* (agonist radioligand)	-1	0	-3	-5
5-HT_{1A} *(h)* (agonist radioligand)	2	18	30	40
5-HT_{1B} (antagonist radioligand)	-3	4	18	37
5-HT_{2A} *(h)* (antagonist radioligand)	96	99	101	99
5-HT_{2B} *(h)* (agonist radioligand)	38	81	85	99
5-HT_3 *(h)* (antagonist radioligand)	11	65	29	95
5-HT_{5a} *(h)* (agonist radioligand)	8	47	21	22
5-HT_6 *(h)* (agonist radioligand)	15	61	13	-1
5-HT_7 *(h)* (agonist radioligand)	66	94	60	81
sst (non-selective) (agonist radioligand)	-8	-4	1	-3
$VPAC_1$ (VIP1) *(h)* (agonist radioligand)	0	-1	-3	-2
V_{1a} *(h)* (agonist radioligand)	7	7	-7	7
Ca^{2+} channel (L, verapamil site) (antagonist radioligand)	25	43	56	33
K_V channel (antagonist radioligand)	-2	-3	-5	-10
SK_{Ca} channel (antagonist radioligand)	-3	-1	5	0
Cl^- channel (GABA-gated) (antagonist radioligand)	3	-2	-8	-3
norepinephrine transporter *(h)* (antagonist radioligand)	12	51	36	54
dopamine transporter *(h)* (antagonist radioligand)	42	79	49	55
5-HT transporter (h) (antagonist radioligand)	59	94	31	54

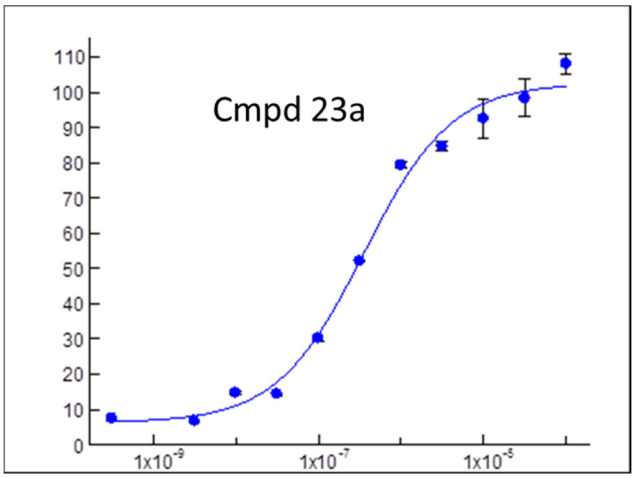
Table 2. Panel screen data of **12** and **12a** @30µM and **23a** (@ both 1µM and 10µM);

1) **Testing of 23a on 1 human recombinant G protein coupled receptor using IP One HTRF™ functional assay for 5-HT_{2a}**

5HT2A IP One *Dose-response test, antagonist mode*

concentration Antagonist (nM)	**% Inhibition 1**	**% Inhibition 2**	**% Inhibition average**	**Delta F 1**	**deltaF 2**	**HILL**
100000	106.06	110.11	108.08	1499	1542	0.82
31600	94.53	102.09	98.31	1378	1457	
10000	88.53	96.52	92.52	1315	1399	
3160	83.72	85.63	84.67	1264	1284	
1000	78.62	79.95	79.29	1210	1224	
316	52.14	52.07	52.11	931	931	
100	29.73	30.84	30.28	695	707	
32	14.10	14.65	14.38	530	536	
10	14.44	15.04	14.74	534	540	
3	6.33	7.18	6.75	449	458	

0.3	7.96	7.04	7.50	466	456	



IC50: 356 (nM)

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