8-(Heteroaryl)phenalkyl-1-Phenyl-1,3,8-triazaspiro[4.5]decan-4-ones as Opioid Receptor Modulators

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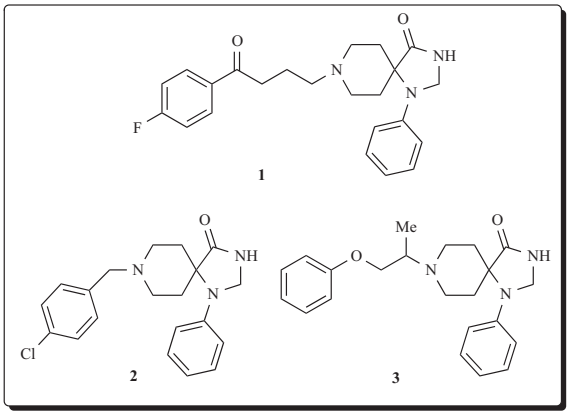
Abstract: A series of *N*-biarylalkyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-ones were prepared and evaluated for biological activity at opioid (μ, δ, κ) and opioid receptor like-1 (ORL-1) G-protein coupled receptors. Substitution on the biaryl moiety produced enhanced affinity for the μ-opioid receptor.

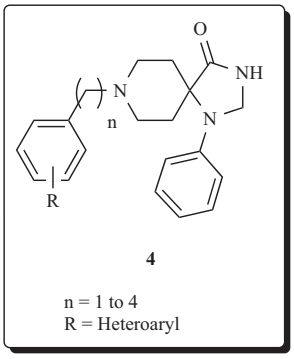
Key Words: Mu receptor, delta receptor, kappa receptor, nociceptin receptor, pain, 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, heteroarylphenylalkyl, SAR, [^{35}S]GTPγS binding assay, Suzuki reaction, reductive amination.

INTRODUCTION

The μ, δ and κ opioid receptors (MOP, DOP, and KOP) have been the focus of intense research in drug discovery since the early 1970s [1]. The closely related nociceptin/ orphanin FQ (N/OFQ) receptor (NOP receptor, previously named ORL-1) was identified in 1994 [2]. Recently, NOP receptor has generated much interest as a biological target [3]. Opioid receptors are involved in modulating pain transmission in the CNS and spinal cord. In particular, many agonists for the μ receptor have superior efficacy for the treatment of moderate to severe pain. One of the Challenges of μ opioid research is the identification of ligands possessing analgesic effects without respiratory depression, nausea and the potential for abuse or dependency. In our opioid SAR program, we have used the versatile 1-phenyl-1,3,8-triazaspiro [4.5]decan-4-one scaffold [4] found in the neuroleptic spiperone (**1**), to obtain libraries of compounds that display varying degrees of selectivity for one or multiple sites of action. In this paper we describe a series of *N*-biarylalkyl derivatives that are relatively selective for the μ opioid receptor.

Screening of the JNJ corporate compound library revealed that **2** and **3** had interesting opioid receptor properties that we wished to investigate in more detail. We choose **2** as our starting point for SAR development because it lacked chirality, which simplifies the design of our synthetic plan. Hydrophobic substitution on the piperidine nitrogen was modified to incorporate biaryl functionality, which has been identified as a privileged substructure in medicinal chemistry. In particular, (heteroaryl)phenyl was examined in analogs such as shown in **4** to both ensure structural novelty as well as moderate the hydrophobic nature of simple biphenyl substitution alone. The effect of carbon chain length between the piperidine ring and biaryl moieties was also investigated.





CHEMISTRY

The synthesis of the target compounds commenced with the preparation of **5** (Table **1**) as described in Scheme **1**. Suzuki coupling [5] of 2-bromobenzaldehyde and thiophene-

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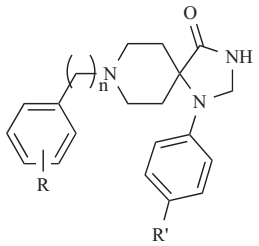
Table 1. Opioid Receptor Subtype Binding, K_i's (nM) [95% CL]



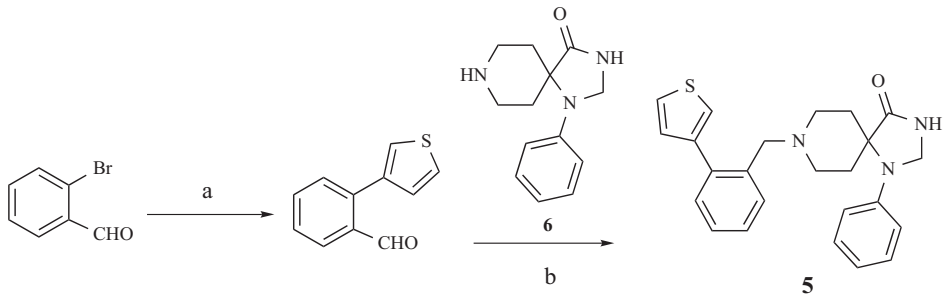
Cmp #	n	R	R`	μ	δ	κ	NOP
2	-	-	-	3.66	3979	239.6	72.3
3	-	-	-	0.34	112.4	74.0	151.7
5	1	2-(3-thienyl)	H	0.22 [0.16,0.32]	120.2 [71.8,201.3]	0.90 [0.73,1.11]	3.4 [2.2,5.2]
7	1	3-(3-thienyl)	H	5.4 [3.9,7.6]	302.5 [206.9,442.4]	85.81 [69.09,106.6]	944 [545,1635]
8	1	4-(3-thienyl)	H	32.5 [24.9,42.4]	510.5 [195.1,1336]	391.2 [296.1,516.9]	5000 -
10	1	2-(2-thienyl)	H	0.21 [0.15,0.30]	19.53 [13.45,28.37]	0.81 [0.62,1.05]	4.9 [3.6,6.7]
11	2	2-(2-thienyl)	H	0.53 [0.38,0.75]	173.8 [138.2,218.5]	5.57 [4.11,7.55]	8.9 [5.6,14.0]
12	3	2-(2-thienyl)	H	5.43 [3.9,7.6]	302.5 [206.9,442.4]	32.52 [24.64,42.92]	5.7 [3.8,8.5]
19	4	2-(2-thienyl)	H	2.46 [1.78,3.40]	296.3 [188.9,464.9]	133.6 [99.17,180.0]	79.5 [65.1,97.1]
22	1	2-(2-thienyl)	F	0.30 [0.22,0.42]	49.87 [34.34,72.43]	1.11 [0.95,1.31]	11.5 [8.1,16.3]
23	1	2-(2-furanyl)	H	0.25 [0.18,0.37]	150.5 [93.78,241.6]	2.76 [2.21,3.44]	3.6 [2.5,5.3]
24	1	2-(2-thiazolyl)	H	3.02 [2.09,4.39]	1314 [627.5,2751]	20.96 [17.00,25.85]	108 [79.6,147.1]

3-boronic acid in the presence of $(PPh_3)_4Pd$ and aqueous sodium carbonate gave 2-(3-thienyl)benzaldehyde. This compound was reacted with 1-phenyl-1,3,8-triazaspiro[4.5] decan-4-one **6** [4] and sodium triacetoxyborohydride in methylene chloride to give **5**. Likewise, **7** was prepared using the same approach starting from 3-bromobenzaldehyde. Compound **8** was prepared by reductive amination reaction [6] of 4-bromobenzaldehyde and **6** with sodium triacetoxyborohydride in 1,2-dichloroethane to furnish aryl bromide **9** which was subsequently coupled under Suzuki conditions with 3-thiophene boronic acid in refluxing 1,2-dimethoxyethane (DME) (Scheme **2**).

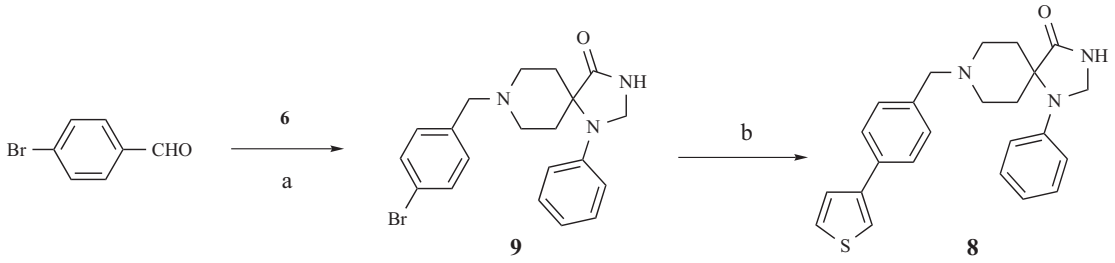
2-Thienyl derivative **10** was prepared in the same way as for **5** using thiophene-2-boronic acid.

Phenethyl derivative **11** was prepared starting with reaction of 2-bromophenethyl alcohol with thiophene-2-boronic acid, $(PPh_3)_4Pd$ in 1,2-DME to give (2-thienyl) phenethyl alcohol **13** (Scheme **3**). This alcohol was treated with methanesulfonyl chloride in methylene chloride to give mesylate **14**, which was reacted with **6** and diisopropylethylamine in 1-methyl-2-pyrrolidinone to yield target **11**.

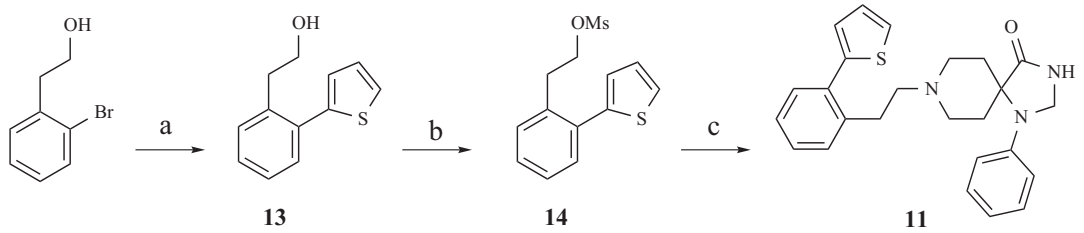
For the synthesis of three methylene homolog **12**, 3-(2-bromophenyl)propionic acid was converted to corresponding methyl ester **15** using trimethylsilyldiazomethane (Scheme **4**). Suzuki reaction of **15** and thiophene-2-boronic acid gave methyl 2-(2-thienyl)phenylpropionate **16**. Reduction of **16** with sodium borohydride and lithium chloride provided 2-(2-thienyl)phenpropyl alcohol **17**. This alcohol was converted



Scheme 1. (a) Thiophene-3-boronic acid, $(PPh_3)_4Pd$, 2 M aq. Na_2CO_3, toluene, reflux (100%); (b) sodium triacetoxyborohydride, AcOH, CH_2Cl_2 (97%).



Scheme 2. (a) Sodium triacetoxyborohydride, AcOH, $Cl(CH_2)_2Cl$ (94%); (b) thiophene-3-boronic acid, $(PPh_3)_4Pd$, 1 M aq. $NaHCO_3$, 1,2-DME , reflux (52%).



Scheme 3. (a) Thiophene-2-boronic acid, $Pd(PPh_3)_4$, 1 N aq. $NaHCO_3$, 1,2-DME (95%); (b) CH_3SO_2Cl, Et_3N, CH_2Cl_2 (100%); (c) **6**, DIEA, 1-methyl-2-pyrrolidinone, 65 ^oC (63%).

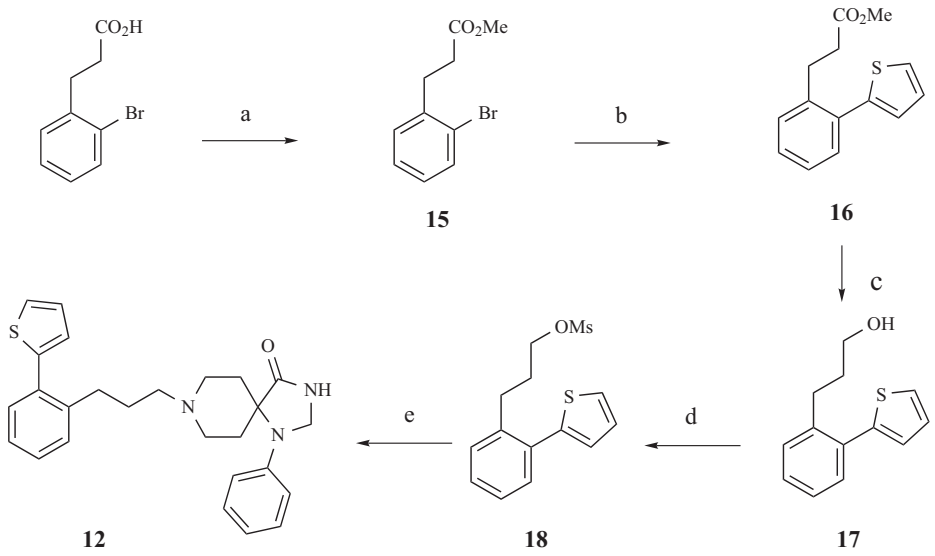
to mesylate **18** and subsequently reacted with **6** to give target **12**.

Four carbon chain analog **19** was prepared starting from 2-bromobenzyl bromide and alkylation with allyl magnesium bromide to yield butenyl benzene **20** (Scheme **5**). This alkene was hydroborated with 9-BBN and subsequently oxidized with hydrogen peroxide and sodium hydroxide to generated desired alcohol **21** [7] which was subjected to the same reaction sequence as for **11** to give **19**.

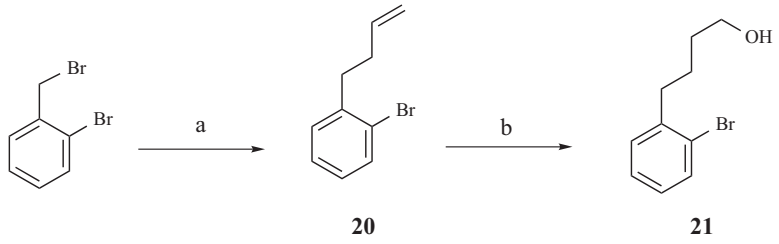
4-Fluorophenyl derivative **22** was synthesized by incorporating the 4-fluorophenyl variant of **6** into the same reaction sequence as used to prepare **10**. Furanyl analog **23** was prepared in a similar fashion starting from furan-2-boronic acid. Thiazole derivative **24** was prepared starting with reaction of 2-bromothiazole, 2-formylbenzene boronic acid and $(PPh_3)_4Pd$ in refluxing 1,2-DME to provide 2-(2-thiazolyl)benzaldehyde **25**. This aldehyde was treated with **6** under the standard reductive amination conditions to furnish target **24**. Compound **24** was treated with HCl in Et_2O to provide the mono hydrochloride salt.

RESULTS AND DISCUSSION

The 8-(heteroaryl)phenalkyl 1-phenyl-1,3,8-triazaspiro [4.5]decan-4-one derivatives were evaluated for their binding affinity at all four opioid receptors (Table **1**). For comparison purposes, opioid binding affinity data for HTS lead compound **2** and **3** are also included. In general, the new compounds show greater potency at the MOP receptor versus the DOP, KOP and NOP receptors. Four of the compounds displayed subnanomolar affinities against MOP and the other analogs possessed activity in the 2-32 nM Ki range. The position of substitution of the thienyl group on the phenyl ring resulted in an effect on MOP with 2-(3-thienyl) **5** being the most potent (0.22 nM MOP Ki) and the 3-(3-thienyl) **7** and 4-(3-thienyl) **8** derivatives being less potent with 5.4 and 32.5 nM Ki's, respectively. The enhanced affinity of **5** could be attributed to the 2(3-thienyl)benzyl group's ability to access



Scheme 4. (a) $TMSCHN_2$, MeOH, benzene (100%); (b) thiophene-2-boronic acid, $Pd(PPh_3)_4$, 1 N aq. $NaHCO_3$, 1,2-DME, reflux (49%); (c) LiCl, $NaBH_4$, EtOH, THF (95%); (d) CH_3SO_2Cl, Et_3N, CH_2Cl_2(98%) ; (e) **6**, DIEA, 1-methyl-2-pyrrolidinone, 65 ^oC (43%).



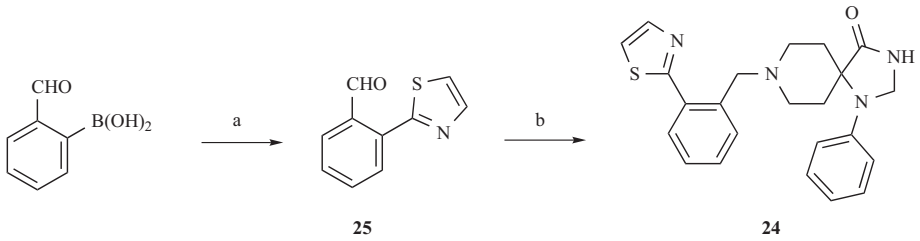
Scheme 5. (a) CH_2=$CHCH_2MgBr$, THF, reflux (95%); (b) (i) 9-BBN in hexane, THF, (ii) H_2O_2, 6 N aq. NaOH, reflux (56%).

a more bioactive conformation of the MOP receptor than compounds **7** and **8**. Cometta-Morini, *et al.*, [8] using computation studies, suggested that there are four structural elements necessary for mu receptor recognition to spiropiperidine ligands related to **5**. The proposed pharmacophore model includes a protonated nitrogen center, a proton accepting center (carbonyl group), a π-π stacking center (ring B) and a lipophilic ring A appended off the piperidine ring. It is possible that the 2-(3-thienyl) ring occupies the same region of conformation space as ring A in the model. Further, **5**, **7**, and **8** are fairly selective *vs*. DOP and NOP, but less so relative to KOP. For example, the ratio of KOP/MOP is only 4X for **5**. 2-(2-Thienyl)benzyl derivative **10** displayed equivalent activity as 2-(3-thienyl)benzyl analog **5**, except at the DOP receptor where **10** was 6X more potent than **5**. The length of the tether between the (heteroaryl) phenyl moiety and the 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one core was then varied by the addition of 1 (**11**), 2 (**12**), and 3 (**19**) methylenes. Of this group, the rank order of activity at the MOP receptor was **5** > **11** > **19** > **12**. MOP was still the most potent opioid receptor interaction compared to DOP, KOP, and NOP, although it is noteworthy that the MOP and NOP activities were nearly equivalent for **12**. Compound **22** with a 4-fluorophenyl group had very similar binding affinity as for **5.** Similarly, 2-furanyl deri-vative **23** had very similar activity when compared with the corresponding 2-thienyl congener **12**. 2-Thiazole analog **24** was 4-13X less potent at all of the opioid receptors when compared to 2-thienyl **12**.

The highest affinity compounds were tested for functional activity at the MOP receptor by measuring the stimulation of [^{35}S]GTP-γS binding (Table **2**). This data revealed that **5** was only a weak MOP agonist, and possibly functioning as an antagonist whereas related derivatives **7**, **10**, and **23** were more potent compounds. Both **10** and **23** were near full MOP agonists in terms of efficacy (0.88-0.87 of DAMGO).

CONCLUSIONS

The thienyl analog **10** and furanyl analog **23** were equipotent in the mu receptor binding assay. However, the mu binding affinity for the thiazolyl analog **24** was decreased by ten fold. The 2-(2-thienyl)benzyl compound (**10**) was found to be more potent than the two, three and four carbon extended analogs **11**, **12**, and **19** respectively. In the GTP-γS functional assay, analogs **10** and **23** exhibited the highest efficacy at the mu receptor displayed with full agonist activity.



25 **24**

Scheme 6. (a) 2-Bromothiazole, $Pd(PPh_3)_4$, 1 N aq. $NaHCO_3$, 1,2-DME, reflux (87%); (b) i. **6**, sodium triacetoxyborohydride, $Cl(CH_2)_2Cl$, ii HCl in Et_2O (67%).

Table 2. Mu Opioid Receptor Functional Data for Selected Compounds

Cmp #	EC_{50} (μM)	Efficacy	10 μM cmpd Plus 1 μM DAMGO % Inhibition
2	4.17	0.61	47
3	0.75	0.91	20
5	10.0	NA	55
7	0.61	0.54	44
10	1.39	0.88	23
23	1.34	0.87	29

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EXPERIMENTAL SECTION

General Methods

^1H and ^{13}C NMR spectra were recorded on an NMR spectrometer at 300 and 75.5 MHz, respectively. Chemical shifts were reported as ppm (δ values) in Hertz (Hz) downfield from tetramethylsilane as the internal reference in DMSO-d_6. Mass spectra were obtained with a single quadrapole and fitted with an electrospray source. Elemental analyses were within 0.4% of the theoretical value. Purifications were conducted by preparative thin layer chromatography (TLC) on silica gel (230-400 mesh). The progress of reactions was monitored by TLC on 250-μM precoated silica gel plates. Most reagents and solvents were purchased and used without further purification.

1-PHENYL-8- [2-(3-THIENYL)BENZYL]-1,3,8-TRIAZA-SPIRO[4.5]DECAN-4-ONE (5)

To a mixture of 2-bromobenzaldehyde (0.32 mL, 2.70 mmol) and 3 mL of 2 M aqueous sodium carbonate in 15 mL of toluene was added thiophene-3-boronic acid (384 mg, 3.00 mmol) in ethanol (3 mL). The mixture was stirred and then treated with tetrakis(triphenylphosphine)palladium (0) (93.0 mg, 3 mole %) and heated to reflux under nitrogen atmosphere for 4.5 h. The resulting solution was cooled to room temperature; the dark brown reaction mixture was diluted with ethyl acetate (75 mL) and washed with water (2 X 75 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield 2-(3-thienyl) benzaldehyde as a brown oil. The reaction was repeated on a 50 mmol scale with 2-bromobenzaldehyde (5.8 mL), thiophene-3-boronic acid (7.03 g, 0.0555 mol) in ethanol (55 mL), $Pd(PPh_3)_4$ (1.7 g, 3 mol %), 2 M aqueous Na_2CO_3 (55 mL) in toluene (275 mL) to yield crude 2-(3-thienyl) benzaldehyde as an oil. Both batches of the crude 2-(3-thienyl)benzaldehyde were combined and carried onto the next step without purification. MS (loop pos.) MH^+ = 189. ^1H NMR (300 MHz, DMSO-d_6) δ 7.30-7.80 (m, 7H), 10.0 (s, 1H).

To a mixture of 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (416 mg, 1.80 mmol) and crude 2-(3-thienyl) benzaldehyde (340 mg) and acetic acid (0.1 mL, 1.80 mmol) in CH_2Cl_2 (14 mL) was added sodium triacetoxyborohydride (763 mg, 3.60 mmol). The resulting mixture was stirred at room temperature for 20 h. The reaction mixture was quenched with 1 N aqueous NaOH and extracted with CH_2Cl_2 (2X). The combined extracts were washed with 1N aqueous NaOH, dried over K_2CO_3, filtered, and concentrated *in vacuo* to yield the crude product **5** as an oil.

The reaction was repeated on a 6.6 mmol scale with spiropiperidine (1.53 g), crude 2-(3-thienyl)benzaldehyde (1.24 g), AcOH (0.38 mL) and $Na(OAc)_3BH$ (2.80 g, 0.0132 mol) in CH_2Cl_2 (50 mL) reacted to yield crude product as an oil. The total combined, crude product from both experiments described above was purified by flash chromatography on silica gel (2% MeOH in CH_2Cl_2) to yield the title compound **5** as a free base. The free base (2.15 g) was dissolved in isopropyl alcohol and acidified with 1N HCl in diethyl ether to yield the title product as a monohydrochloride salt (1.79 g, 61%). MS (loop pos.) MH^+ = 404.1 (100%). ^1H NMR (300 MHz, DMSO d_6) δ 1.50-1.60 (m, 2H), 2.75-2.90 (m, 2H), 3.20-3.45 (m, 4H), 4.40 (s, 2H), 4.55 (s, 2H), 6.75-6.80 (m, 1H), 6.95-7.00 (m, 2H), 7.15-7.25 (m, 3H), 7.40-7.45(m, 1H), 7.50-7.55 (m, 2H), 7.65 (m, 1H), 7.70-7.75 (m, 1H), 7.90-7.95 (m, 1H), 8.9 (s, 1H), 10.40 (br s, 1H exchangeable). Anal. Calcd for $C_{24}H_{25}N_3OS{\cdot}HCl{\cdot}0.1H_2O$: C, 65.25; H, 5.98; N, 9.51; S, 7.26; Cl, 8.02; H_2O, 0.41. Found: C, 65.08; H, 6.30; N, 9.30; S, 6.93; Cl, 8.50; H_2O, 0.12.

1-PHENYL-8-[3-(3-THIENYL)BENZYL]-1,3,8-TRIAZA-SPIRO[4.5]DECAN-4-ONE (7)

To a mixture of 3-bromobenzaldehyde (0.60 mL, 5.14 mmol) and tetrakis(triphenylphosphine) palladium (0) (179

mg, 0.155 mmol) in 1,2-dimethoxyethane (30 mL) was added 3-thiopheneboronic acid (779 mg, 6.09 mmol) and 1N aqueous $NaHCO_3$ (20 mL). The resultant mixture was heated at reflux for 20 h. The reaction mixture was diluted with water and extracted with EtOAc (2 X 50 mL). The organic solution was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by gradient flash chromatography on the ISCO (40 g silica gel column; 5% to 15% EtOAc in hexane) to yield 736 mg of 3-(3-thienyl) benzaldehyde as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.30-7.80 (m, 7H), 10.0 (s, 1H).

To a mixture of 3-(3-thienyl)benzaldehyde (713 mg, 3.79 mmol) and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (866 mg, 3.75 mmol) in 1,2-dichloroethane (30 mL) was added sodium triacetoxyborohydride (1.1362 g, 5.36 mmol). The resultant mixture was stirred at room temperature for 18 h, quenched with aqueous $NaHCO_3$ (50 mL) and extracted with $CHCl_3$ (2 x 50 mL). The organic solution was dried over Na_2SO_4, filtered and concentrated. The crude free base was purified by flash chromatography on silica gel (4% MeOH in CH_2Cl_2) to yield 700 mg (46%) of **7** as a white amorphous powder. MS (loop pos): MH^+ = 404.3 (100%). ^{1}H NMR (300 MHz, DMSO d_6) δ 1.50-1.60 (m, 2H), 2.75-2.90 (m, 2H), 2.74-2.77 (m, 4H), 3.58 (s, 2H), 4.57 (s, 2H), 6.76 (t, *J* = 7.26, 7.24Hz, 1H), 6.88 (d, *J* = 8.24 Hz, 2H), 7.23-7.30 (m, 3H), 7.39 (t, *J*= 7.55, 7.52 Hz, 1H), 7.55-7.68 (m, 4H), 7.87-7.88 (m, 1H), 8.62 (s, 1H). Anal. Calcd for $C_{24}H_{25}N_3OS{\cdot}0.1H_2O$: C, 71.11; H, 6.27; N, 10.36; S, 7.91; H_2O, 0.44. Found: C, 70.91; H, 6.21; N, 10.23; S, 7.74; Cl, 8.50; H_2O, 0.41.

1-PHENYL-8-[4-(3-THIENYL)BENZYL]-1,3,8-TRIAZA-SPIRO[4.5]DECAN-4-ONE (8)

To a mixture of 4-bromobenzaldehyde (816.1 mg, 4.41 mmol) and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (925.2 mg, 4.00 mmol) in 1,2-dichloroethane (60 mL) was sodium triacetoxyborohydride (1.4810 g, 6.99 mmol). The resultant mixture was stirred at room temperature for 18 h, quenched with aqueous $NaHCO_3$ (50 mL) and extracted with $CHCl_3$ (2 x 100 mL). The organic solution was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude free base was purified by flash chromatography on silica gel (4% MeOH in CH_2Cl_2) to yield 1.5034 g (94%) of 1-phenyl-8-[4-bromobenzyl]-1,3,8- triazaspiro[4.5]decan-4-one **9** as a white amorphous powder. MS (loop pos.) MH^+ = 400 and 402. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.65-1.75 (m, 2H), 2.60-2.80 (m, 6H), 3.55 (s, 2H), 4.75 (s, 2H), 6.75-6.90 (m, 4H), 7.25-7.35 (m, 4H), 7.40-7.45 (m, 2H).

To a mixture of 1-phenyl-8-[4-bromobenzyl]-1,3,8-triazaspiro[4.5]decan-4-one (401.5 mg, 1.00 mmol) and tetrakis(triphenylphosphine) palladium (0) (35.0 mg, 0.030 mmol) in 1,2-dimethoxyethane (25 mL) was added 3-thiopheneboronic acid (177.2 mg, 1.38 mmol) and 1N aqueous $NaHCO_3$ (10 mL). The resultant mixture was heated at reflux for 3 h. The reaction mixture was diluted with water and extracted with EtOAc (2 X 50 mL). The organic solution was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by tapered preparative thin layer chromatography (four silica gel plates; 6% MeOH in $CHCl_3$) to yield 290.4 mg (72%) of the titled product **8** as a tan solid. MS (loop pos): MH^+ = 404.1 (100%). ^{1}H NMR (300 MHz, DMSO d_6) δ 1.56-1.60 (m, 2H), 2.50-2.60 (m, 2H), 2.72-2.75 (m, 4H), 3.54 (s, 2H), 4.57 (s, 2H), 6.76 (t, J = 7.26, 7.24Hz, 1H), 6.88 (d, J = 8.24 Hz, 2H), 7.26 (t, J = 7.96, 7.92 Hz, 2H), 7.38 (d, J= 7.99 Hz, 2H), 7.55-7.70 (m, 4H), 7.84-7.85 (m, 1H), 8.63 (s, 1H). Anal. Calcd for $C_{24}H_{25}N_3OS{\cdot}0.15H_2O$: C, 70.96; H, 6.28; N, 10.34; S, 7.89; H_2O, 0.66. Found: C, 70.90; H, 6.15; N, 10.21; S, 7.84; H_2O, 0.33.

1-PHENYL)-8-[[2-(2-THIENYL)PHENYL]METHYL]-1,3,8-TRIAZASPIRO[4.5]DECAN-4-ONE (10)

To a mixture of 2-bromobenzaldehyde (0.60 mL, 5.14 mmol) and tetrakis(triphenylphosphine) palladium (0) (179 mg, 0.155 mmol) in 1,2-dimethoxyethane (40 mL) was added 2-thiopheneboronic acid (779 mg, 6.09 mmol) and 1N aqueous $NaHCO_3$ (20 mL). The resultant mixture was heated at reflux for 20 hrs. The reaction mixture was diluted with water and extracted with EtOAc (2 X 50 mL). The organic solution was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by gradient flash chromatography on the ISCO (40 g silica gel column; 5% to 15% EtOAc in hexane) to yield 756 mg of 2-(2-thienyl) benzaldehyde as a yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.00-7.10 (m, 1H), 7.11-7.20 (m, 1H), 7.40-7.65 (m, 4H), 7.95-8.00 (m, 1H), 10.1 (s, 1H).

To a mixture of 2-(2-thienyl)benzaldehyde (742 mg, 3.95 mmol) and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (900 mg, 3.89 mmol) in 1,2-dichloroethane (35 mL) was added sodium triacetoxyborohydride (1.1736 g, 5.36 mmol). The resultant mixture was stirred at room temperature for 18 h, quenched with aqueous $NaHCO_3$ (50 mL) and extracted with $CHCl_3$ (2 x 50 mL). The organic solution was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography on the ISCO (120g packed silica gel column; 0% to 8% MeOH in CH_2Cl_2) to yield 1.0403 g (60%) of the title compound as a free base. A sample of the free base (84.4 mg) was dissolved in $CHCl_3$ (5 mL), and treated with 0.2 mL of 1N HCl in Et_2O. The HCl salt was precipitated by addition of Et_2O, collected by filtration and dried in the vacuum oven at 55 °C for 20 h to yield 77.9 mg of **10** as a white amorphous solid. MS (loop pos): MH^+ = 404.3 (100%). ^{1}H NMR (300 MHz, DMSO d_6) δ 1.77-1.82 (m, 2H), 2.80-2.90 (m, 2H), 3.27-3.48 (m, 4H), 4.47-4.48 (m, 2H), 4.58 (s, 2H), 4.57 (s, 2H), 6.76 (t, J = 7.26, 7.24Hz, 1H), 6.88 (d, J = 8.24 Hz, 2H), 7.20-7.24 (m, 4H), 7.49-7.58 (m, 3H), 7.72-7.75 (m, 1H), 7.97-8.00 (m, 1H), 8.94 (s, 1H) Anal. Calcd for $C_{27}H_{31}N_3OS{\cdot}1.3HCl{\cdot}0.1H_2O$: C, 63.67; H, 5.90; N, 9.28; S, 7.08; Cl, 10.18; H_2O, 0.40. Found: C, 63.54; H, 5.88; N, 9.10; S, 7.02; Cl, 9.97; H_2O, <0.10.

1-PHENYL-8-[2-[2-(2-THIENYL)PHENYL]ETHYL]-1,3,8-TRIAZASPIRO[4.5]DECAN-4-ONE (11)

To a stirring mixture of 2-bromophenethyl alcohol (0.72 mL, 5.31 mmol) and tetrakis (triphenylphosphine) palladium (0) (620 mg, 10 mol%) in 1,2-dimethoxyethane (45 mL) was added thiophene-2-boronic acid (2.0391 g, 0.0159 mol) and 1 N aqueous $NaHCO_3$ (15 mL). The resultant reaction mixture was heated at reflux for 66 h under argon atmosphere. The dark reaction mixture was diluted with H_2O

(20 mL) and extracted with EtOAc (2 x 75 mL). The organic solution was dried over Na_2SO_4, filtered and concentrated. The dark residue was purified by flash chromatography on silica gel (30% EtOAc in hexane) to yield 2-(2-thienyl) phenethyl alcohol **13** as a light yellow oil. ^{1}H NMR (300 MHz, DMSO d_6) δ 2.87 (t, J=7.44, 7.43 Hz), 2H), 3.52-3.58 (m, 2H), 4.68 (t, J =5.17, 5.18 Hz, 1H (exchangeable)), 7.14-7.15 (m, 2H), 7.20-7.38 (m, 4H), 7.59-7.61 (m, 1H).

To a cold (0°°C) solution of **13** (206 mg, 1.01 mmol) and triethylamine (170 µL, 1.22 mmol) in anhydrous CH_2Cl_2 (10 mL) was added methanesulfonyl chloride (94 µL, 1.21 mmol). Upon complete addition of the methanesulfonyl chloride, the reaction was stirred at room temperature under argon atmosphere for 1 h. The reaction mixture was then diluted with CH_2Cl_2 (50 mL), washed with H_2O (1 x 25 mL), aq $NaHCO_3$ (2 x 25 mL), dried over Na_2SO_4 , filtered and concentrated to yield the mesylate compound **14** as a yellow oil, which was taken into the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.81 (s, 3H), 3.22 (t, J= 7.12, 7.13 Hz, 2H), 4.30 (t, J= 7.13, 7.12 Hz), 7.02-7.04 (m, 1H), 7.08-7.12 (m, 1H), 7.27-7.41 (m, 5H).

Crude mesylate compound **14** (270 mg, ca 1.0 mmol), 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (197 mg , 0.852 mmol) and diisopropylethylamine (0.20 mL, 1.15 mmol) in 1-methyl-2-pyrrolidinone (5 mL) were stirred in an preheated oil bath (60°C) for 18 h and at 85 °C for 6 h. The reaction mixture was diluted with aq NaCl and extracted with $CHCl_3$ (2x25 mL). The organic solution with H_2O (6x 50 mL), dried over Na_2SO_4, filtered and concentrated. The crude oil was purified by flash chromatography (4% CH_3OH in $CHCl_3$) to yield an oil (256 mg) which contained N-methyl-2-pyrrolidinone. The crude product was dissolved in EtOAc (25 mL), and treated with 1 mL of 1 N HCl in Et_2O. The HCl salt was precipitated by addition of Et_2O, collected by filtration and dried in the vacuum oven at 70°C for 1 day to yield 62.6 mg (16%) of product **11** as an amorphous off-white solid. MS (loop pos): MH^+ = 418.1 (100%). ^{1}H NMR (300 MHz, DMSO d_6) δ 1.50-1.60 (m, 2H), 2.89-2.93 (m, 2H), 3.23-3.27 (m, 4H), 3.40-3.50 (m, 4H), 4.62 (s, 2H), 6.78-6.82 (m, 1H), 7.01-7.05 (m, 2H), 7.18-7.29 (m, 4H), 7.33-7.43 (m, 3H), 7.47-7.50 (m, 1H), 7.68-7.70 (m, 1H), 9.02 (s, 1H), 10.46 (br s, 1H exchangeable). Anal. Calcd for $C_{25}H_{27}N_3OS{\cdot}HCl{\cdot}0.25H_2O$: C, 65.48; H, 6.26; N, 9.20; S, 6.99; Cl, 7.73; H_2O, 0.98. Found: C, 65.36; H, 5.89; N, 9.14; S, 6.64; Cl, 7.52; H_2O, 0.53.

8-[3-(2-THIOPHEN-2-YLPHENYL)PROPYL-1-PHENYL-1,3,8-TRIAZASPIRO[4.5]DECAN-4-ONE (12)

Trimethylsilyldiazomethane (2M in hexanes, 5.0 mL, 10.0 mmol) was added to a solution of 3-(2-bromophenyl) propionic acid (1.376 g, 6.00 mmol) in anhydrous benzene (28 mL) and anhydrous methanol (8 mL). The reaction mixture was stirred at room temperature for 2 h, and then the volatiles were removed *in vacuo*, to yield crude methyl 3-(2-bromophenyl)propionate **15** which was carried forward without further purification. ^{1}H NMR (300 MHz, DMSO d_6) δ 2.39 (t, 7.55, 7.55 Hz, 2H), 2.72 (t = 7.55, 7.55Hz, 2H), 3.36 (s, 3H), 6.89-6.96 (m, 1H), 7.05-7.14 (m, 2H), 7.34-7.37 (m, 1H).

To a mixture of the crude methyl 3-(2-bromophenyl) propionate (1.59 g, ca 0.006 mol) and tetrakis(triphenyl-phosphine) palladium (0) (695 mg, 0.601 mmol) in 1,2-dimethoxyethane (45 mL) were added thiophene-2-boronic acid (2.304 g, 0.018 mol) and 1N aqueous $NaHCO_3$ (15 mL). The resulting mixture was heated at reflux under nitrogen atmosphere for 66 h. The dark reaction mixture was then diluted with water (100 mL) and extracted with EtOAc (2 X 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and then concentrated. The resulting crude product was purified by flash chromatography (5% EtOAc in hexane) to yield methyl 2-(2-thienyl)phenylpropionate **16** as a light green oil. ^{1}H NMR (300 MHz, DMSO d_6) δ 2.50-2.56 (m, 2H), 2.95-2.98 (m, 2H), 3.55 (s, 3H), 7.14-7.17 (m, 2H), 7.27-7.35 (m, 4H), 7.61-7.63 (m, 1H).

To a cold (0 °C) solution of methyl 2-(2-thienyl) phenylpropionate (387 mg, 1.57 mmol) and anhydrous lithium chloride (353 mg, 8.32 mmol) in an EtOH/THF mixture (4:3; 28 mL) was added sodium borohydride (315 mg, 8.32 mmol). The reaction mixture was then stirred at room temperature for 20 h. Aqueous NH_4Cl (50 mL) was added and the crude product was extracted with EtOAc (2 x 50 mL). The organic layer was separated, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by flash chromatography (5% EtOAc in hexane) to yield 2-(2-thienyl)phenpropyl alcohol **17** as a light yelllow oil. ^{1}H NMR (300 MHz, DMSO d_6) δ 1.59-1.68 (m, 2H), 2.69-2.74 (m, 2H), 3.34-3.38 (t, J = 6.6, 6.6 Hz, 2H), 4.01-4.06 (br s, 1H), 7.12-7.34 (m, 6H), 7.59-7.61 (m, 1H).

To a cold (0°C) solution of 2-(2-thienyl)phenpropyl alcohol (312 mg, 1.43 mmol) and triethylamine (250µL, 1.79 mmol) in anhydrous CH_2Cl_2 (10 mL) was added methane-sulfonyl chloride (120 µL, 1.55 mmol). Upon complete addition of the methanesulfonyl chloride, the reaction was stirred at room temperature under argon atmosphere for 1 h. The reaction mixture was then diluted with CH_2Cl_2 (75 mL), washed with H_2O (2x50 mL), aq $NaHCO_3$ (2x25 mL), dried over Na_2SO_4 , filtered and concentrated to yield 3-(2-thien-2-yl-phenyl)-propyl ester methanesulfonic acid **18** as a yellow oil, which was taken into the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.90-1.99 (m, 2H), 2.84-2.90 (m, 2H), 2.93 (s, 3H), 4.15 (t, J= 6.37, 6.37 Hz, 2H), 7.00-7.02 (m, 1H), 7.07-7.10 (m, 1H), 7.21-7.38. (m, 5H).

The crude mesylate prepared in previous step (397 mg, 1.34 mmol), 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (295 mg , 1.28 mmol) and diisopropylethylamine (200 µL, 1.55 mmol) in 1-methyl-2-pyrrolidinone (4 mL) were stirred in an preheated oil bath (65 °C) for 18 h. The reaction mixture was diluted with aq NaCl and extracted with EtOAc (2 x 40 mL). The organic solution was washed with H_2O (4x 50 mL), dried over Na_2SO_4, filtered and concentrated. The resulting crude product was purified by tapered preparative TLC (4% MeOH in $CHCl_3$) to furnish 239 mg of a beige solid. The free base was dissolved in $CHCl_3$ (25 mL), and then treated with 1 mL of 1N HCl in Et_2O. The HCl salt was precipitated by addition of Et_2O, collected by filtration and dried in the vacuum oven at 60 °C for 20 h to yield 202.3 mg (33%) of the title product **12** as an amorphous beige solid. MS (loop

pos): MH^+ = 432.1 (100%). ^{1}H NMR (300 MHz, DMSO d_6) δ 1.75-1.85 (m, 2H), 1.90-2.05 (m, 2H), 2.75-2.80 (m, 2H), 2.85–2.95 (m, 2H), 2.95-3.10 (m, 2H), 3.40-3.60 (m, 4H), 4.62 (s, 2H), 6.78-6.82 (m, 1H), 7.01-7.05 (m, 2H), 7.18-7.43 (m, 8H), 7.60-7.70 (m, 1H), 9.02 (s, 1H), 10.46 (br s, 1H exchangeable). Anal. Calcd for $C_{24}H_{24}FN_3OS{\cdot}1.2HCl{\cdot}0.25H_2O$: C, 65.08; H, 6.42; N, 8.76; S, 6.68; Cl, 8.87; H_2O, 0.94. Found: C, 64.89; H, 6.10; N, 8.60; S, 6.48; Cl, 8.83; H_2O, 0.84.

8-[4-(2-THIOPHEN-2-YL-PHENYL)-BUTYL]-1-PHENYL-1,3,8-TRIAZASPIRO[4.5]DECAN-4-ONE (19)

To an ice cold solution of 2-bromobenzyl bromide (5.00 g, 0.020 mol) in THF (25 mL) was added 1M allyl magnesium bromide (100 mL, 0.100 mol) slowly *via* cannula. The reaction mixture was stirred at reflux for 1.5 h, cooled in an ice bath and quenched with 50 mL of aqueous 2M H_2SO_4. Water (50 mL) was added to dissolve any remaining solid and the layers were separated. The aqueous layer was extracted with Et_2O (2 x 150 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated to yield 1-bromo-2-but-3-enyl-benzene **20** as a light yellow oil. The isolated crude product was carried forward without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33-2.40 (m, 2H), 2.80-2.85 (m, 2H), 4.98-5.17 (m, 2H), 5.81-5.94 (m, 1H), 7.02-7.08 (m, 1H), 7.19-7.27 (m, 2H), 7.51-7.54 (m, 1H).

To a solution of 0.4 M 9-BBN in hexane (72mL, 28.8 mmol) was added 4-bromophenyl-1-butene (3.99 g, 18.9 mmol) at room temperature. The resulting mixture was stirred at room temperature for 20 h. The mixture was treated sequentially with 3.3 mL of 6N aqueous NaOH (19.8 mmol), THF (7 mL), and 30% H_2O_2 in H_2O (7 mL), then refluxed for 2h. The reaction mixture was then cooled to room temperature. The organic layer was washed with aqueous sodium sulfite (40 mL), H_2O (20 mL), and brine (20 mL). The aqueous extracts were combined, saturated with solid K_2CO_3 and extracted with Et_2O (3x50 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. The resulting crude product was purified by flash chromatography twice (33% EtOAc in hexane and 20% EtOAc in hexane) to yield 4-(o-bromophenyl)butanol) **21**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41-1.64 (m, 4H), 2.69 (t, = 7.28, 7.61 Hz, 2H), 3.42 (t, J = 6.40, 6.41 Hz, 2H), 4.40 (br s, 1H), 7.10-7.16 (m, 1H), 7.30-7.33 (m, 2H), 7.55-7.57 (m, 1H).

To a solution of 4-(*o*-bromophenyl)-1-butanol (1.222 g, ca 0.0053 mol) and tetrakis(triphenylphosphine) palladium (0) (650 mg, 0.562 mmol) in 1,2-dimethoxyethane (55 mL) was added thiophene-2-boronic acid (2.057 g, 0.016 mol) and 1N aqueous $NaHCO_3$ (15 mL). The resulting mixture was heated at reflux under nitrogen atmosphere for 3 days. The dark reaction mixture was diluted with water (50 mL) and extracted with EtOAc (100 mL). The organic layer was dried over Na_2SO_4, filtered through a bed of Celite and concentrated to yield a crude which was purified by flash chromatography (30% EtOAc in hexane) to yield 4-(2-thien-2-yl-phenyl)-butan-1-ol as a light brown oil. ^{1}H NMR (300 MHz, DMSO d_6) δ 1.37-1.56 (m, 4H), 2.66-2.71 (m, 2H), 3.31-3.35 (m, 2H), 4.33 (br s, 1H), 7.10-7.15 (m, 2H), 7.21-7.26 (m, 1H), 7.31-7.34 (m, 3H), 7.59-7.61 (m, 1H).

To a cold (0 °C) solution of 2-(2-thienyl)phenylbutanol (1.149 g, 0.00495 mol) and triethylamine (0.87 mL, 6.24 mmol) in anhydrous CH_2Cl_2 (40 mL) was added methanesulfonyl chloride (0.48 mL, 6.20 mmol). Upon complete addition of the methanesulfonyl chloride, the reaction was stirred at room temperature under argon atmosphere for 1.5 h. The reaction mixture was then diluted with CH_2Cl_2 (50 mL), washed with H_2O (3x50 mL), dried over Na_2SO_4, filtered and concentrated to yield crude 4-(2-then-2-yl-phenyl)-butyl ester methane sulfonic acid as a brown oil, which was taken into the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.66-1.76 (m, 4H), 2.77 (t, J = 7.1, 7.4 Hz, 2H), 2.94 (s, 3H), 4.15 (t, J= 6.08, 6.08 Hz), 7.00-7.02 (m, 1H), 7.07-7.10 (m, 1H), 7.25-7.35. (m, 5H).

The crude mesylate (390 mg, 1.24 mmol), 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (231 mg, 1.00 mmol) and diisopropylethylamine (210 µL, 1.20 mmol) in 1-methyl-2-pyrrolidinone (2.5 mL) were stirred in an preheated oil bath (70 °C) for 20 h. The reaction mixture was diluted with aq NaCl (25 mL) and extracted with EtOAc (2 x 20 mL). The organic layer was washed with H_2O (4 x 50 mL), dried over Na_2SO_4, filtered and concentrated to yield a crude oil, which was purified by flash chromatography (5% CH_3OH in $CHCl_3$) to yield the title compound as a free base, as an oil (218 mg). The free base was dissolved in $CHCl_3$ (15 mL), and treated with 1 mL of 1N HCl in Et_2O. The HCl salt was precipitated by addition of Et_2O, collected by filtration and dried in the vacuum oven at 50 °C for 20 h to yield 155.8 mg (32%) of the title product **19** as an amorphous beige solid. MS (loop pos): MH^+ = 446.1 (100%). ^{1}H NMR (300 MHz, DMSO d_6) δ 1.75-1.85 (m, 2H), 1.90-2.05 (m, 2H), 2.75-2.80 (m, 2H), 2.85 –2.95 (m, 2H), 2.95-3.10 (m, 2H), 3.40-3.60 (m, 4H), 4.62 (s, 2H), 6.80-6.90 (m, 1H), 7.01-7.05 (m, 2H), 7.18-7.43 (m, 8H), 7.60-7.65 (m, 1H), 9.00 (s, 1H), 10.60 (br s, 1H exchangeable). Anal. Calcd for $C_{27}H_{31}N_3OS{\cdot}1.1HCl{\cdot}0.25H_2O$: C, 66.15; H, 6.72; N, 8.57; S, 6.54. Cl, 7.95; H_2O, 0.91. Found: C, 65.81; H, 6.63; N, 8.34; S, 6.21; Cl, 7.76; H_2O, 0.30.

1-(4-FLUOROPHENYL)-8-[[2-(2-THIENYL)PHENYL] METHYL]-1,3,8-TRIAZASPIRO[4.5]DECAN-4-ONE (22)

To a mixture of 2-bromobenzaldehyde (1.17 mL, 10.0 mmol) and 2.0 M aqueous sodium carbonate (75 mL) in DME (225 mL) were added thiophene-2-boronic acid (1.53 g, 0.012 mol) and tetrakis(triphenylphosphine)palladium[0] (578 mg, 0.5 mmol). The mixture was heated to reflux under nitrogen for 16 h. The resulting solution was cooled, and the crude product was extracted from aqueous solution with ethyl acetate. The organic layer was dried over $MgSO_4$, and the solvents were removed under vacuum. The crude product was purified on flash column with 25% dichloromethane in hexane to yield 2-(2-thienyl)benzaldehyde.

To a mixture of 2-(2-thienyl)benzaldehyde (151 mg, 0.807 mmol) and 1-(4-fluorophenyl)-1,3,8-triazaspiro[4,5]

decan-4-one (168 mg, 0.673 mmol) in anhydrous 1,2-dichloroethane (30 mL) and DMF (0.5 mL) was added acetic acid (75 µL). The reaction was stirred for 2 h. Sodium tri(acetoxy)borohydride (285 mg, 1.34 mmol) was then added, and the reaction was stirred for 16 h. The reaction was quenched with 1.0 M of sodium hydroxide aqueous solution and the crude product was extracted from the aqueous layer with dichloromethane. The organic solvent were then removed under vacuum. The crude product was purified by gradient column chromatography on the ISCO (silica gel; 50% EtOAc to 80% EtOAc in hexane. The free base was dissolved in $CHCl_3$ and treated with 1 mL of 1N HCl in Et_2O. The HCl salt was precipitated by addition of Et_2O, collected by filtration and dried in the vacuum oven at 50 ^oC for 20 h to yield 192 mg (61%) of the title product **22** as an amorphous solid. MS (loop pos): MH^+ = 422.1 (100%). ^1H NMR (300 MHz, DMSO d_6) δ 1.89 (m, 2H), 2.80 (m, 2H), 3.54 (m, 2H), 3.69 (m, 2H), 3.93 (s, 3H), 4.47(s, 2H), 4.60 (s, 2H), 6.81(m, 1H), 6.95(d, J=5Hz, 2H), 7.26-7.13 (m, 4H), 7.55 (d, J=2 Hz, 1H), 8.14 (s, 1H), 9.00 (s, 1H). Anal. Calcd for $C_{24}H_{24}FN_3OS{\cdot}1.2HCl{\cdot}0.25H_2O$: C, 61.39; H, 5.53; N, 8.68; Cl, 9.19; H_2O, 0.28. Found: C, 61.36; H, 5.51; N, 8.94; Cl, 9.06; H_2O, 0.96.

1-(PHENYL)-8-[[2-(2-FURANYL)PHENYL]METHYL]-1,3,8-TRIAZASPIRO[4.5]DECAN-4-ONE (23)

To a mixture of 2-bromobenzaldehyde (0.60 mL, 5.14 mmol) and tetrakis(triphenylphosphine) palladium (0) (178.5 mg, 0.154 mmol) in 1,2-dimethoxyethane (40 mL) was added furan-2-boronic acid (690.7 mg, 5.98 mmol) and 1N aqueous $NaHCO_3$ (25 mL). The resultant mixture was heated at reflux for 1.5 h. The reaction mixture was diluted with water and extracted with EtOAc (150 mL). The organic solution was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography (silica gel; 5% EtOAc in hexane) to yield 757.5 mg of 2-(2-furanyl)benzaldehyde as a yellow oil. ^1H NMR (300 MHz, DMSO-d_6) δ 6.50-6.55 (m, 1H), 6.60-6.65 (m, 1H), 7.40-7.50 (m, 1H), 7.60-7.80 (m, 4H), 7.95-8.00 (m, 1H), 10.4 (s, 1H).

To a mixture of 2-(2-furanyl)benzaldehyde (173 mg, 1.00 mmol) and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (231 mg, 1.00 mmol) in 1,2-dichloroethane (15 mL) was added sodium triacetoxyborohydride (363 mg, 1.71 mmol). The resultant mixture was stirred at room temperature for 18 h, quenched with aqueous $NaHCO_3$ (50 mL) and extracted with $CHCl_3$ (2 x 50 mL). The organic solution was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (silica gel; 3% MeOH in $CHCl_3$) to yield 329 mg of the title compound as a free base. The free base was dissolved in $CHCl_3$ (20 mL), and treated with 1.2 mL of 1N HCl in Et_2O. The HCl salt was precipitated by addition of Et_2O, collected by filtration and dried in the vacuum oven at room temperature for 1 d to yield 316 mg (73%) of **23** as an amorphous solid. MS (loop pos): MH^+ = 388 (100%). ^1H NMR (300 MHz, DMSO d_6) δ 1.75-1.90 (m, 2H), 2.75-2.95 (m, 2H), 3.35-3.45 (m, 2H), 3.70-3.80 (m, 2H), 4.55-4.60 (m, 4H), 6.70-6.72 (m, 1H0, 6.75-6.80 (m, 1H), 6.90-7.05 (m, 3H), 7.15-7.25 (m, 2H), 7.40-7.60 (m, 2H), 7.75-7.80 (m, 1H), 8.00 (s, 1H), 9.00 (s, 1H). Anal. Calcd for $C_{24}H_{25}N_3O_2{\cdot}1.0HCl{\cdot}0.4H_2O$: C, 66.86; H, 6.26; N, 9.75; Cl, 8.22; H_2O, 1.67. Found : C, 66.53; H, 6.03; N, 9.62; Cl, 8.16; H_2O, 1.16.

1-PHENYL-8-[[2-(2-THIAZOLYL)PHENYL]METHYL]-1,3,8-TRIAZASPIRO[4.5]DECAN-4-ONE (24)

To a mixture of 2-bromothiazole (826 mg, 4.99 mmol) and tetrakis(triphenylphosphine) palladium (0) (175 mg, 0.151 mmol) in 1,2-dimethoxyethane (20 mL) was added 2-formylbenzeneboronic acid (0.9017 g, 6.01 mmol) and 1N aqueous $NaHCO_3$ (8 mL). The resultant mixture was heated at reflux for 6 h. The reaction mixture was diluted with water and extracted with EtOAc (2 X 50 mL). The organic solution was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by gradient flash chromatography (10% to 25% EtOAc in hexane) to yield 2-(2-thiazolyl) benzaldehyde **25** as a white solid. MS (loop pos) MH^+ = 190.1. ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (m, 1H), 7.55-7.60 (m, 1H), 7.65-7.70 (m, 1H), 7.75-7.80 (m, 1H), 7.95-7.97 (m, 1H), 8.00-8.05 (m, 1H), 10.5 (s, 1H).

To a mixture of 2-(2-thiazolyl)benzaldehyde (200 mg, 1.06 mmol) and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (.232 mg, 1.00 mmol) in 1,2-dichloroethane (20 mL) was added sodium triacetoxyborohydride (376 mg, 1.79 mmol). The resultant mixture was stirred at room temperature for 18 h, quenched with aqueous $NaHCO_3$ (50 mL) and extracted with $CHCl_3$ (2 x 50 mL). The organic solution was dried over Na_2SO_4, filtered and concentrated. The crude product was dissolved in 1:1 $CHCl_3$:CH_3OH (30 mL) and treated with 2 mL of 1 N HCl in Et_2O. The HCl salt was precipitated by addition of Et_2O, collected by filtration and dried in the vacuum oven at 60 ^oC for 18 h to yield 314 mg (67%) of product **24** as an amorphous solid. MS (loop pos): MH^+ = 405.1 (100%). ^1H NMR (300 MHz, DMSO d_6) δ 1.50-1.60 (m, 2H), 2.75-2.90 (m, 2H), 3.45-3.55 (m, 2H), 3.75-3.90 (m, 2H), 4.65 (s, 2H), 4.70 (s, 2H), 6.76-6.81 (m, 1H), 6.95-6.98 (m, 2H), 7.18-7.24 (m, 2H), 7.60-7.70 (m, 2H), 7.90-7.99 (m, 3H), 8.14-8.15 (m, 1H), 9.00 (s, 1H), 9.80 (br s, 1H exchangeable). Anal. Calcd for $C_{23}H_{24}N_4OS{\cdot}HCl{\cdot}0.4H_2O$: C, 61.64; H, 5.80; N, 12.55; S, 7.15; Cl, 7.90; H_2O, 1.61. Found: C, 61.56; H, 5.77; N, 12.23; S, 7.01; Cl, 8.27; H_2O, 1.24.

MOP, KOP, DOP AND NOP BINDING STUDIES ON HEK-293 CELLS

Filtration binding assays were performed with membranes isolated from HEK-293 cells transfected with the MOP, KOP, DOP and NOP receptors. Protocol for measuring the binding affinities for the ORL-1 receptor: HEK293 cell membranes were prepared as described [9] with the exception that the buffer used was a mixture of 50 mM Tris-Cl pH 7.8, 5 mM $MgCl_2$ and 1 mM EGTA. This suspension was added to PEI treated WGA FlashPlates (New England Nuclear) at 1 µg/well in binding buffer of 50 mM Tris- Cl pH 7.8, 5 mM $MgCl_2$ and 1 mM EGTA. ^{125}I-Tyr^{14}-nociceptin was added at a final concentration of 0.5 nM and the volume adjusted to 50 µl with binding buffer. The plate was incubated for 2 h at room temperature, the reactions were aspirated and the wells washed 2X with 200 µL binding buffer and then filled with 200 µL binding buffer. The plates

were then sealed and counted on a Packard Top Count to determine radioactivity bound to the membranes. Alternatively, binding reactions were performed as described in Costar 96-well round-bottom microplates. After 1 h, binding reactions were filtered onto Packard GF/C Filter-plates presoaked in 0.03% polyethyleneimine. The plates were then dried and 25 uL Microscint (Packard) was added to each filter well and bound radioactivity determined on a Packard TopCount. Both methods produced equivalent results. The concentration of competitor ligand needed to inhibit specific binding by 50% was used to calculate K_i values.

The binding studies for MOP, KOP and DOP opioid receptors were performed similiarly. Labeled agonists, [^{3}H]-DAMGO, [^{3}H] U69593 and [^{3}H] DPDPE specific for the opioid receptors, MOP , KOP and DOP receptors, respectively were used as competitors in these binding assays.

MOP [^{35}S]GTPγS FUNCTIONAL STUDIES

The [^{35}S]GTPγS binding assay in CHO cell membranes were adapted from an established procedure [10]. The functional activity of the four most selective mu ligands designed for this study and the lead compounds at 10 uM were normalized against the mu agonist DAMGO at 1 uM.

Preparation of Membranes

CHO-hd cell membranes were purchased from Receptor Biology, Inc. (Baltimore, MD). 10 mg/ml of membrane protein suspended in 10 mM TRIS-HC pH 7.2, 2 mM EDTA, 10% sucrose. Membranes were maintained at 4-8 °C. 1 ml of membranes was added into 15 ml cold binding assay buffer. The assay buffer contained 50 mM HEPES, pH = 7.6, 5 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT and 1 mM EDTA. The membrane suspension was homogenized with a Polytron for 2 times and centrifuged at 3000 rpm for 10 min. The supernatant was then centrifuged at 18,000 rpm for 20 min. The pellet was saved in a tube and 10 ml assay buffer was added into the tube. The pellet and buffer were mixed with a Polytron.

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Incubation Procedure

The pellet membranes (20 μg/ml) were preincubated with SPA (10 mg/ml) at 25 °C for 45 min in the assay buffer. The SPA (5 mg/ml) coupled with membranes (10 μg/ml) was then incubated with 0.5 nM [^{35}S] GTPγS in the same HEPES buffer containing 50 μM GDP in total volume of 200 μl. Increasing concentrations of receptor agonists were used to stimulate [^{35}S] GTPγS binding. The basal binding was tested in the absent agonists and no specific binding was tested in the present 10 μM unlabeled GTPγS. The data were analyzed on a Top counter. % of Basal = (stimulate - non specific)* 100/(basal - non specific). EC_{50} value values are calculated using a Prism program.

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