Synthesis and Evaluation of Cyclic Diamino Benzamide Based D3 Receptor Ligands

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Research Article

Keywords: Dopamine, D3 dopamine receptor, D2 dopamine receptor

Posted Date: November 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1025538/v1

Version of Record: A version of this preprint was published at Medicinal Chemistry Research on February 2nd, 2022. See the published version at https://doi.org/10.1007/s00044-021-02845-z.

Synthesis and evaluation of cyclic diamino benzamide based D_3 receptor ligands

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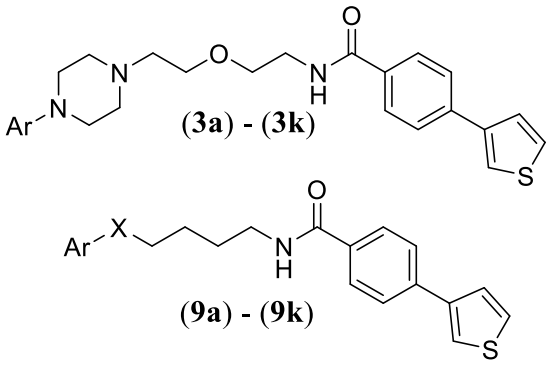
Abstract: Dopamine (**1**) is a key neurotransmitter whose impact on pharmacological processes is mediated by a family of dopamine receptors designated D_1, D_2, D_3, D_4, and D_5. Various diseases and conditions such as schizophrenia, drug abuse, depression, restless leg syndrome, Parkinson's disease (PD), and inflammatory diseases have been linked to aberrant D_3 activity. Herein, we report a series of novel D_3 ligands with improved solubility over our previous lead compound, MC25-41 (**2**).

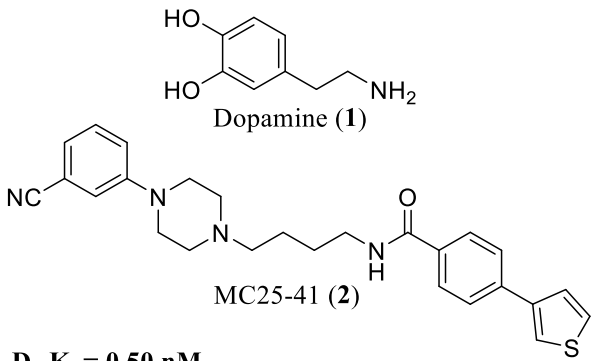
Graphical Abstract:



Keywords: Dopamine, D_3 dopamine receptor, D_2 dopamine receptor

Introduction: The key neurotransmitter known as dopamine (**1**) was prepared synthetically for the first time in 1910 by George Barger and James Ewens [1] long before its pharmacological role was recognized. Over 45 years later, Katharine Montagu determined that dopamine was present in the human brain,[2] and in 1958 Arvid Carlsson and Nils-Åke Hillarp demonstrated that this chemical acts as a neurotransmitter.[3] Over the next several decades, the pharmacological function and the means through which dopamine exerts its impact on biological systems has been elucidated. It is known, for example, that dopamine (**1**) is synthesized in the brain and the periphery, and it has been conclusively linked to a wide range of physiological functions. These functions include, vasodilation, modulation of renal sodium excretion, altering urine output, learning, movement, and behavioral motivations.[4]

At the cellular level, dopamine signaling is mediated by a family of G-protein coupled receptors (GPCRs) that are designated as D_1, D_2, D_3, D_4, and D_5. The D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) sub-families are based on the genetic organization, amino acid homology and pharmacological properties of the individual family members.[5] The D_3 receptor has been the subject of intense interest as a potential therapeutic target as it has been linked to various disease states and conditions such as schizophrenia, drug abuse, depression, restless leg syndrome [6], Parkinson's disease (PD) [7], and various inflammatory diseases.[8] We recently described our effort to identify novel, selective D_3 ligands with potential utility for the treatment of cocaine use disorder. These studies led to the identification of MC25-41 (**2,** figure 1) as a potent D_3 ligand that 1) possesses a high level of selectivity for D_3 over other dopamine receptors, as well as a range of other key CNS targets, 2) has a pharmacokinetic profile suitable for *in vivo* studies,[9] and 3) attenuates motivation for cocaine in Sprague-Dawley rats.[10] While MC25-41 (**2**) has proven to be an effective tool molecule, its limited solubility (2 μM) may be lead to future problems in formulation and dosing. As part of an effort to address this issue, we have developed a new series of novel, selective D_3 ligands whose solubility is significantly improved over our original lead compound (**2**).



D_3 K_i = 0.50 nM
D_1 K_i = 10,000 nM
D_2 K_i = 743 nM
D_4 K_i = 806 nM
D_5 K_i = 10,000 nM

RLM $T_{1/2}$ >60 min
HLM $T_{1/2}$ >60 min
Sol. < 2 mmol
SD rat IV 1 mg/kg $T_{1/2}$ = 10.9 h,

MW: 444
cLogP: 4.9
TPSA: 87.6

Figure 1: Structures of Dopamine (**1**) and

Results and Discussion: Our effort to improve the solubility of our lead series began with the incorporation of an oxygen atom in the central linker region (**3a**-**3k**). This addition adds an additional hydrogen bond acceptor, which could improve the solubility of our compounds, but also increases the length of the linker, which could alter D_3 binding affinity and selectivity. Target compounds were prepared as outlined in scheme 1. DMT-MM mediated coupling of biaryl acid (**4**) with amino-alcohol (**5**) provided the corresponding amide, which was converted to the corresponding bromide (**6**) with carbon tetrabromide and triphenylphosphine. The bromine was then displaced with an aryl piperazine (**7**) to provide the target compounds (**3a**-**3k**).

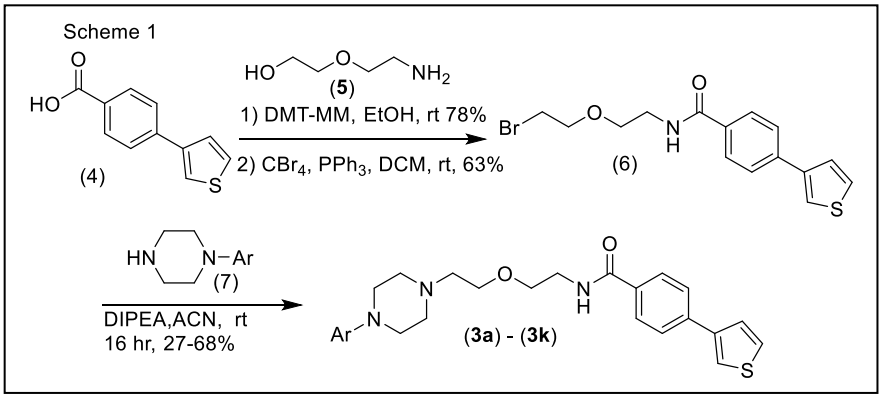


Table 1 includes the *in vitro* binding (K_i at D_3 and D_2) as well as the physicochemical properties (MW, TPSA, LogP) of target compounds (**3a**-**3k**). Table 2 provides a comparison of the solubility of the target compounds (**3a**-**3k**) with the corresponding analogs of MC25-41 (**8a**-**8k**) The compounds prepared and tested have MW, TPSA, and cLogP values that are consistent with drug like properties, but some examples have cLogP and TPSA values that are outside of the range suggested of BBB penetration. It is noteworthy, however, that we have previously demonstrated that MC25-41 (**2**) is efficacious in mouse models of cocaine addiction [11] and a marmoset model of Parkinson's disease.[12] This indicates that MC25-41 (**2**) is able to penetrate the BBB despite the fact that its clogP (4.9) and TPSA (87.6) values are outside of the range suggested of BBB penetration. It

further suggests that related compounds may also be able to penetrate the BBB despite out-of-range values for cLogP and TPSA.

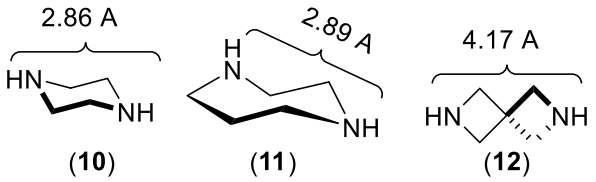
The structure-activity relationship analysis began with the 3-cyano analog (**3a**), which provided a direct comparison with our previous lead compound MC25-41 (**2**). A decrease in both D_3 binding affinity (K_i = 128 nM) and selectivity over D_2 (13-fold) were observed. Similar D_3 potency was observed when the 3-CN (**3a**) was replaced with a 3-CF_3 (**3b,** D_3 K_i = 175 nM), but selectivity decreased (3.2-fold). Relocation of the cyano substituent to the 2-position (**3c**) once again produced a compound with similar D_3 potency (K_i = 140 nM) and selectivity (3.5-fold). Replacing the 2-CN (**3c**) with either a 2-Cl (**3d**) or 2-CF_3 (**3e**) lead an increase in both D_3 potency (K_i = 27 nM and 37 nM respectively) and selectivity (11-fold and 17-fold respectively versus D_2). Addition of a second chlorine atom as seen in either the 3-position (**3f**) or the 4-position (**3g**), caused a drop in D_3 potency (K_i = 127 nM and 1653 nM respectively), selectivity (3.6-fold and 1.3-fold respectively versus D_2). D_3 binding affinity was restored when the 2 chlorine atoms were relocated to the 3- and 5-positions (**3h**, D_3 K_i = 22 nM), selectivity over D_2 improved (D_2 K_i = 332 nM, 14.9-fold). Incorporation of a methoxy group produced mixed results depending on the positioning of the substituent. The 2-methoxy analog (**3i**) is a potent D_3 ligand (K_i = 24 nM), but selectivity was marginal (3.1-fold). The 4-methoxy analog (**3j**), on the other hand, showed little binding affinity for both D_3 (K_i = 15265 nM) and D_2 (K_i = 25435 nM). Finally, the 1-napthyl analog (**3k**) had moderate D_3 binding affinity (K_i = 79 nM), low D_2 selectivity (4-fold).

A comparison of the solubility of the ether linked compounds (**3a**-**3k**) with the corresponding MC25-41 analogs (**8a**-**8k**) indicated that this change produced mixed results. A 10-fold increase in solubility, for example, was observed with the 2-OMe analog (**3i** versus **8i**),

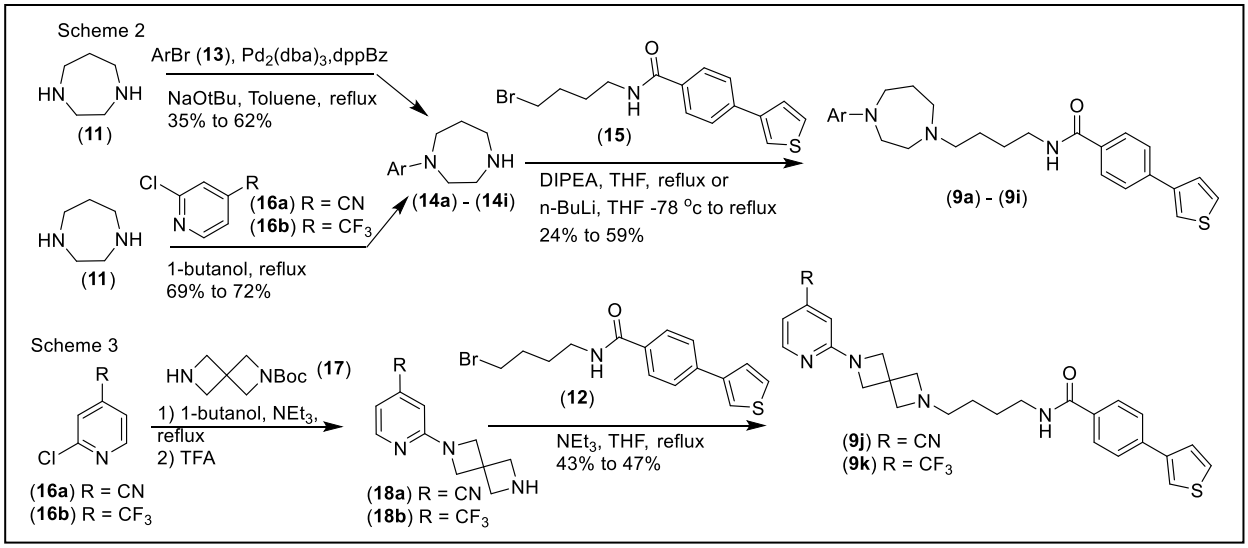
but when the methoxy substituent is in the 4-position (**3j** versus **8j**), solubility did not increase. Significant increases in solubility over the MC25-41 analogs were also observed for the 3-CN (**3a**, 29-fold), 2-Cl (**3d**, 19-fold) and 2,4-di-Cl (**3g**, 10-fold) analogs, but the impact on solubility was smaller in the 2-CF_3 (**3e**, 6-fold), 2,3-di-Cl (**3f**, 5-fold), 2-CN (**3c**, 3-fold), and 3-CF_3 (**3b**, 2-fold) analogs. Interestingly, no improvement in solubility was observed for the 3,5-di-Cl (**3h**) and 1-napthyl (**3k**) analogs, both of which were poorly soluble (sol <10 μM).

We next turned our attention to replacing the piperazine ring of our lead compound MC25-41 (**2**) with either a homopiperazine (**9a**-**9i**) or a 2,6-diazaspiro[3.3]heptane (**9j**, **9k**). Both of these moieties have been effectively used as piperazine bioisosteres,[13] but there are likely differences that could impact D_3 binding and selectivity (Figure 2). While the distance between the nitrogen atoms is similar in a piperazine (**10**) and homopiperazine (**11**) is similar (2.86 A versus 2.89 A), this is not the case for 2,6-diazaspiro[3.3]heptane (**12**). The distance between nitrogen atoms is substantially larger (4.17 A). In addition, the 3-dimensional shapes of the three rings systems are not the same. The shape of the piperazine ring (**10**) and homopiperazine (**11**) are similar in that the first exists in a standard chair confirmation, while the second exists in pseudo-chair conformation. The presence of an addition carbon atom, however, makes the homopiperazine (**11**) larger than the piperazine ring system. The difference in shape is more dramatic with the 2,6-diazaspiro[3.3]heptane (**12**), as the two rings that comprise this system are perpendicular to each other.

Figure 2:



The homopiperazine (**9a**-**8i**) and the 2,6-diazaspiro[3.3]heptane (**9j**, **9k**) analogs were prepared by the methods described in schemes 2 and 3. Buchwald coupling of homopiperazine (**11**) with an aryl bromide (**13**) provided the requisite aryl homopiperzines (**14a**-**14i**), which were reacted with alkyl bromide (**15**) under basic conditions to provide target compounds (**9a**-**9g**). The synthesis of pyridine analogs (**9h**) and (**9i**), on the other hand, began with a displacement reaction of homopiperazine (**11**) and the corresponding chloropyridine (**16a** or **16b**) to provide



aryl homopiperzines (**14h**) and (**14i**), This was followed by reaction with alkyl bromide (**15**) under basic conditions to provide target compounds (**9j**) and (**9k**). In a similar manner, the synthesis of 2,6-diazaspiro[3.3]heptane (**9j**) and (**9k**) began with the reaction of Boc-2,6-diazaspiro[3.3]heptane (**17**) with a chloropyridine (**16a** or **16b**) followed by TFA mediated deprotection to provide (**18a**) and (**18b**). Reaction with alkyl bromide (**15**) under basic conditions to provide target compounds (**9j**) and (**9k**).

Table 3 includes the *in vitro* binding (K_i at D_3 and D_2) as well as the physicochemical properties (MW, TPSA, LogP) of target compounds (**9a**-**9k**). Table 4 provides a comparison of the solubility of the target compounds (**9a**-**9k**) with the corresponding analogs of MC25-41 (**19a**-

19i) The majority compounds prepared and tested have MW and TPSA values that are consistent with drug like properties. The cyano-pyridine analogs (**9h**) and (**9j**) are notable exceptions, as their TPSA are 101. cLogP values of the majority of compounds are above the range suggested for orally delivered compounds. Notable exceptions include (**9h**), (**9j**), and (**9k**). Overall, replacing the piperazine ring with a homopiperazine produced better results than those observed with 2,6-diazaspiro[3.3]heptane with respect to D_3 binding and selectivity over D_2. Incorporation of a cyano substituents in either the 2-position (**9a**) or 3- positions (**9c**) produced compounds that were highly potent (K_i = 5.7 nM ad 6.3 nM) and highly selective (139-fold and 114-fold versus D_2). Replacing the cyano substituents with a CF_3 group in either the 2-postion (**9b**) or 3-position (**9d**) lead to a small decrease in D_3 binding potency (K_i = 30.2 nM and 18.6 nM), but selectivity over D_2 was substantially diminished (43-fold and 47-fold). The 2,4-di-chloro (**9e**) and 3,5-di-chloro (**9f**) analogs were also less potent D_3 ligands (K_i = 16.7 nM ad 33.1 nM) and less selective over D_2 (80-fold and 27-fold) than the 3-CN analog (**9a**). Replacing the benzene rings of (**9a**) and (**9b**) with a pyridine ring led to a further reduction of D_3 binding potency (**9h** D_3 K_i = 64.7 nM, **9i** K_i = 70.2 nM), but selectivity over D_2 increased (197-fold and 131-fold). Lastly. incorporation of the 2,6-diazaspiro[3.3]heptane ring system (**9j** and **9k**) led to a significant decrease in D_3 binding affinity (K_i = 1093 nM ad 464 nM respectively).

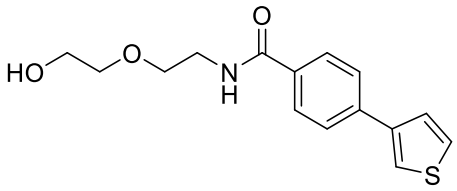
A comparison of the solubility of the homopiperazine (**9a**-**9i**) and the 2,6-diazaspiro[3.3]heptane based compounds with the corresponding MC25-41 analogs (**19a**-**19i**) (Table 4) demonstrated improvements in all but one of the homopiperazine analogs (**9f**, sol = 2 μM). Solubility improved by a 2- to 13-fold in all of the remaining examples. The 2,6-diazaspiro[3.3]heptane analogs (**9j** and **9k**) had the highest aqueous solubility (sol = 148 μM and 109 μM), but as noted above, these compounds also demonstrated low affinity for D_3 (K_i = 1093

nM ad 464 nM respectively). The 2-CN homopiperazine analog (**9c**) is notable as it is highly soluble (sol = 103 μM), has high affinity for D_3 (K_i = 6.3 nM) and is highly selective for D_3 over D_2 (114-fold). Follow-up studies have demonstrated that this compound is highly selective for D_3 over D_4 (D_4 K_i = 1077 nM). Additional studies on this compound (**9c**) to assess its affinity for D_1 and D_5, as well as full *in vitro* ADME profiling (e.g. Cyp450 inhibition, mouse and human liver microsome stability, permeability) are on-going.

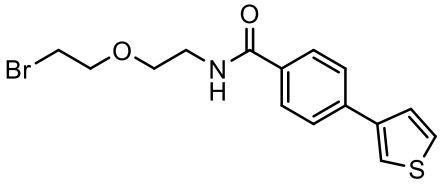
Conclusion: In summary, we have identified analogs of our initial lead compound MC25-41 (**2**) that have high affinity for D_3, are selective for this receptor over D_2, and whose aqueous solubility is improved over the corresponding MC25-41 analogs (**8a**-**8k** and **19a**-**19i**). In addition, we have identified (**9c**) as a potential next generation lead compound, given it high D_3 affinity, selectivity over D_2 and D_4, and its improved solubility. Future efforts will include follow-up studies on (**9c**) and close analogs thereof, as well as assessment of our current collection of compounds at the remaining members of the dopamine receptor family (D_1, D_4, and D_5) and in battery of *in vitro* ADME screens (e.g. Cyp450 inhibition, mouse and human liver microsome stability, permeability).

Experimental methods and materials: Reagents were purchased from Fisher Scientific, VWR International, Sigma Aldrich, and Combi-Blocks, Inc. Chromatographic purification of compounds (normal phase and reverse phase) was carried out on a Teledyne Isco Combiflash RF system. H-NMR spectra were obtained on a Bruker 400-MHz NMR. Chemical shift values (δ values) were reported in ppm relative to TMS. For multiplicity, s = singlet, d = doublet, t = triplet, m = multiplet. Purity (%) and mass spectral data were determined with a Waters Agilent 1200 HPLC/MS (Zorbax SB-C18, 2.1 x 30 mm, 3.5 μm, 100% water/0.1% formic acid to 100%

acetonitrile/0.1% formic acid over 4.0 minutes, 1.0 mL/min.) with a diode array detector from 210-400 nm and Agilent 6130 quadrupole MS. All compounds were purified to 95% purity or greater as determined by HPLC/MS and 1H-NMR. Melting points were recorded on a capillary melting point apparatus.

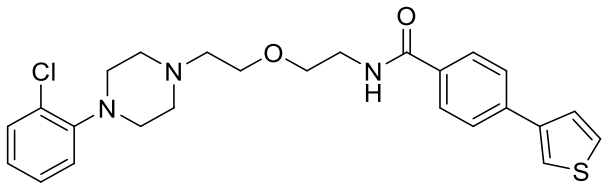


Preparation of N-(2-(2-hydroxyethoxy)ethyl)-4-(thiophen-3-yl)benzamide: A solution of 4-(thiophen-3-yl)benzoic acid (6 g, 29.27 mmol) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (8.88 g, 32.09 mmol) in ethanol (200 ml) was stirred at room temperature for 1 hour. After 1 hour, 2-(2-aminoethoxy)-ethanol (3.66 ml, 32.2 mmol) was added into the reaction. The reaction was further stirred for 2 days. After 2 days, the reaction was filtered to give a filtrate. The filtrate was purified by reverse phase chromatography (50% acetonitrile/0.1% formic acid in water) to provide N-(2-(2-hydroxyethoxy)ethyl)-4-(thiophen-3-yl)benzamide (6.68, 78% yield) : ^{1}H NMR (400 MHz, MeOD-*d*4) δ 7.90-7.88 (d, J = 8.52 Hz, 2H), 7.79-7.77 (m, 3H), 7.53 (d, J = 2.16 Hz, 2H), 3.72-3.67 (quint, J = 4.84, 4.68, 4.28, 1.92, 1.68, 1.4, 0.72 Hz, 4H), 3.63-3.59 (quart, 4H); MS (LC/MS, M+H^{+}): 292.75.

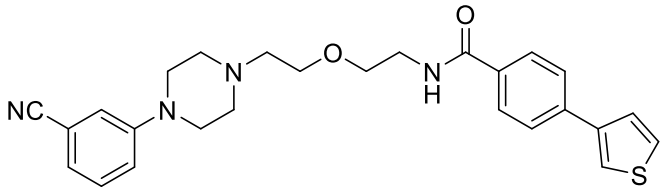


Preparation of N-(2-(2-bromoethoxy)ethyl)-4-(thiophen-3-yl)benzamide (**6**): N-(2-(2-hydroxyethoxy)ethyl)-4-(thiophen-3-yl)benzamide (1.67 g, 5.71 mmol) and carbon tetrabromide (2.84 g, 8.58 mmol) were dissolved in dichloromethane. Triphenylphosphine (2.27 g, 8.58 mmol,

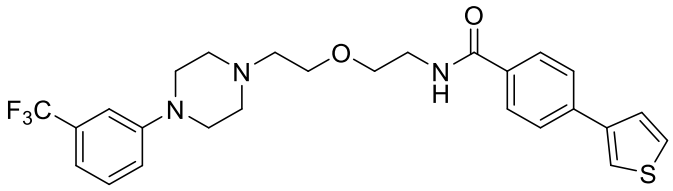
1.5 eq) was slowly added into the reaction in an ice bath. The reaction was warmed up to room temperature and was further stirred for 48 hours. After 48 hours, the reaction mixture was concentrated to provide a solid-oil residue. The resulting material was purified by normal phase chromatography (Ethyl acetate/hexane 0 to 100% gradient) to provide N-(2-(2-bromoethoxy)ethyl)-4-(thiophen-3-yl)benzamide (1.28 g, 63% yield. ^{1}H NMR (400 MHz, MeOD-*d*4) δ 7.92-7.90 (d, J = 8.43 Hz, 2H), 7.75-7.71 (m, 3H), 7.56 (d, J = 2.26 Hz, 2H), 3.75 (m, 4H), 3.57 (m, 2H), 3.48 (m, 2H), MS (LC/MS, M+H$^+$): 354.70.



Preparation of N-(2-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (**3d**): A solution of N-(2-(2-bromoethoxy)-ethyl)-4-(thiophen-3-yl)benzamide (0.14 g, 0.3 mmol), 1-(2-chlorophenyl)piperazine (0.06 g, 0.3 mmol) and *N*,*N*-diisopropylethylamine (0.12 ml, 1.2 mmol, 4.0 eq) in anhydrous acetonitrile (6 ml) at room temperature for 48 hours. After 48 hours, the reaction was purified by normal phase chromatography (100% dichloromethane to 10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (10% acetonitrile/0.1% formic acid in water to 40% acetonitrile/0.1% formic acid in water) to provide N-(2-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl) benzamide (38.2 mg, 48% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 8.36 Hz, 2H), 7.63 (d, J = 8.8 Hz, 2H), 7.47-7.46 (m, 1H), 7.40-7.35 (m, 2H), 7.28-7.25 (m, 1H), 6.93-6.87 (m, 2H), 6.74 (m, 1H), 3.85 (t, J = 4.84 Hz, 2H), 3.73-3.68 (m, 4H), 3.17 (broad s, 4H), 3.13-3.09 (broad s, 6H); MS (LC/MS, M+H$^+$): 470.60.

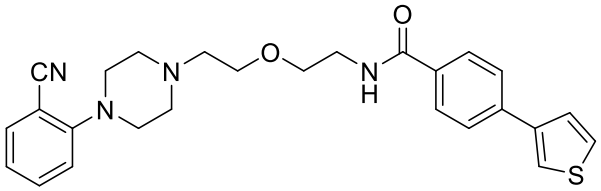


Preparation of N-(2-(2-(4-(3-cyanophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (**3a**): The title compound was prepared according to the procedure for N-(2-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide, except 3-(piperazin-1-yl)benzonitrile was substituted for 1-(2-chlorophenyl)piperazine. The reaction was purified by normal phase chromatography (100% dichloromethane to 10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (10% acetonitrile/0.1% formic acid in water to 40% acetonitrile/0.1% formic acid in water) to provide N-(2-(2-(4-(3-cyanophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (32% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8 Hz, 2H), 7.63 (d, J = 8 Hz, 2H), 7.58 (s, 1H), 7.49 (s, 1H), 7.48 (s, 1H), 7.42 (s, 1H), 7.13-6.89 (m, 3H), 6.87 (s, 1H), 3.79 (broad s, 2H), 3.70 (broad s, 4H), 3.22 (broad s, 4H), 2.98 (broad s, 6H); MS (LC/MS, $M+H^+$): 461.65.



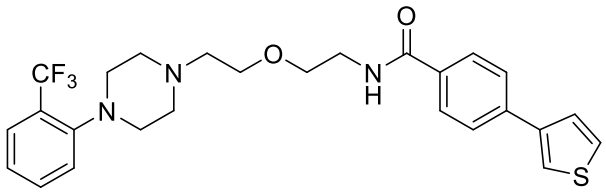
Preparation of 4-(thiophen-3-yl)-N-(2-(2-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethoxy) ethyl)benzamide (**3b**): The title compound was prepared according to the procedure for N-(2-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide, except 1-(3-(trifluoromethyl)phenyl)-piperazine was substituted for 1-(2-chlorophenyl)piperazine. The reaction was purified by normal phase chromatography (100% dichloromethane to 10%

methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (10% acetonitrile/0.1% formic acid in water to 40% acetonitrile/0.1% formic acid in water) to provide 4-(thiophen-3-yl)-N-(2-(2-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethoxy)ethyl)benzamide (61% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.28 Hz, 2H), 7.65 (d, J = 8.32 Hz, 2H), 7.49-7.48 (m, 2H), 7.42-7.7.40 (m, 1H), 7.38-7.28 (m, 1H), 7.17 (t, J = 8, 7.84 Hz, 1H), 7.09-7.05 (m, 2H), 6.85 (d, J = 8.08 Hz, 1H), 3.81 (t, J = 4.84 Hz, 2H), 3.72 (s, 4H), 3.27 (t, J = 4.76, 4.36 Hz, 4H), 3.00 (t, J = 4.6 Hz, 4H), 2.95 (t, J = 4.84 Hz, 2H); MS (LC/MS, M+H$^+$): 504.60.

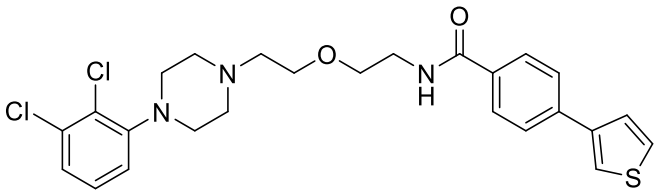


Preparation of N-(2-(2-(4-(2-cyanophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (**3c**): The title compound was prepared according to the procedure for N-(2-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide, except 2-(piperazin-1-yl)benzonitrile was substituted for 1-(2-chlorophenyl)piperazine. The reaction was purified by normal phase chromatography (100% dichloromethane to 10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (10% acetonitrile/0.1% formic acid in water to 40% acetonitrile/0.1% formic acid in water) to provide N-(2-(2-(4-(2-cyanophenyl)-piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (27% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 8.07 (d, J = 8.32 Hz, 2H), 7.61 (d, J = 8.32 Hz, 2H), 7.44-7.43 (t, J = 2.28, 1.64 Hz, 1H), 7.41-7.39 (dd, J = 6.16, 1.44, 1.36 Hz, 1H), 7.35-7.32 (m, 2H), 7.00-6.96 (t, J = 6.32 Hz, 1H), 6.93-6.89 (t, J = 7.32 Hz, 1H),

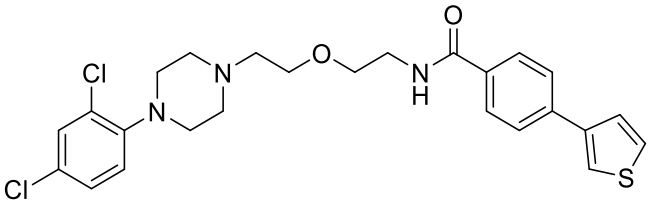
6.59 (d, J = 8.08 Hz, 1H), 3.91 (t, J = 4.76 Hz, 2H), 3.74 (t, J = 4.72, 4.56 Hz, 2H), 3.69-3.65 (m, 2H), 3.30 (broad s, 7H), 3.19 (m, 3H); MS (LC/MS, $M+H^+$): 461.70.



Preparation of 4-(thiophen-3-yl)-N-(2-(2-(4-(2-(trifluoromethyl)phenyl)piperazin-1-yl)ethoxy) ethyl)benzamide (**3e**): The title compound was prepared according to the procedure for N-(2-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide, except 1-(2-(trifluoromethyl)phenyl)piperazine was substituted for 1-(2-chlorophenyl)piperazine. The reaction was purified by normal phase chromatography (100% dichloromethane to 10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (10% acetonitrile/0.1% formic acid in water to 40% acetonitrile/0.1% formic acid in water) to provide 4-(thiophen-3-yl)-N-(2-(2-(4-(2-(trifluoromethyl)phenyl)piperazin-1-yl)ethoxy)ethyl)benzamide (57% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.24 Hz, 2H), 7.65 (d, J = 8.24 Hz, 2H), 7.61 (broad s, 1H), 7.49 (broad s, 1H), 7.49-7.41 (m, 1H), 7.38-7.37 (m, 1H), 7.15 (t, J = 7.96, 7.84 Hz, 1H), 7.08 (d, J = 7.72 Hz, 1H), 7.04 (broad s, 1H), 6.83 (d, J = 7.88 Hz, 1H), 3.81 (t, J = 4.68, 4.6 Hz, 2H), 3.72 (broad s, 4H), 3.25 (t, J = 4.68 Hz, 4H), 3.05 (t, J = 4.44 Hz, 4H), 2.99 (t, J = 4.64, 4.56 Hz, 2H); MS (LC/MS, $M+H^+$): 504.60.

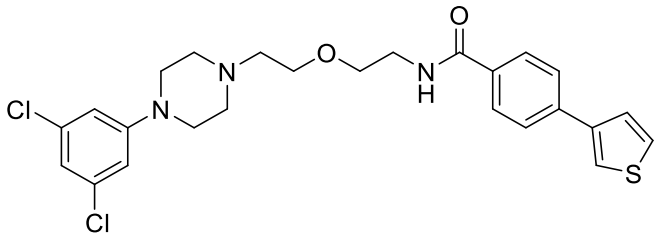


Preparation of N-(2-(2-(4-(2,3-dichlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (**3f**): The title compound was prepared according to the procedure for N-(2-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide, except 1-(2,3-dichlorophenyl)piperazine was substituted for 1-(2-chlorophenyl)piperazine. The reaction was purified by normal phase chromatography (100% dichloromethane to 10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (10% acetonitrile/0.1% formic acid in water to 40% acetonitrile/0.1% formic acid in water) to provide N-(2-(2-(4-(2,3-dichlorophenyl)-piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (37% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 8.36 Hz, 2H), 7.66 (d, J = 8.44 Hz, 2H), 7.53-7.52 (m, 1H), 7.44-7.40 (m, 2H), 7.15-7.13 (dd, J = 1.4 Hz, 1H), 7.01 (t, J = 8.04 Hz, 2H), 6.82 (dd, J = 6.8, 1.32,1.25 Hz, 1H), 3.71 (broad s, 6H), 3.03 (broad s, 4H), 2.75 (m, 6H); MS (LC/MS, M+H$^+$): 506.10.

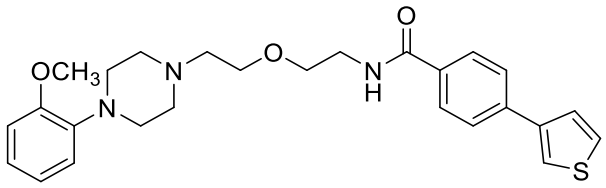


Preparation of N-(2-(2-(4-(2,4-dichlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benz-amide (**3g**): The title compound was prepared according to the procedure for N-(2-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide, except 1-(2,4-dichlorophenyl)piperazine was substituted for 1-(2-chlorophenyl)piperazine. The reaction was purified by normal phase chromatography (100% dichloromethane to 10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (10% acetonitrile/0.1% formic acid in water to 40% acetonitrile/0.1% formic acid in water) to provide N-(2-(2-(4-(2,4-dichlorophenyl)piperazin-1-

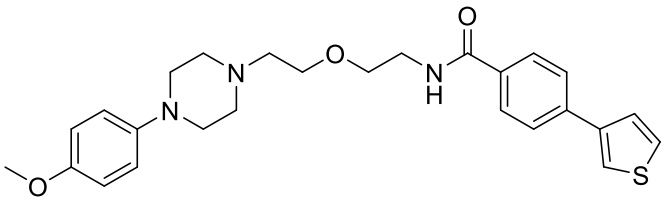
yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (45% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 8.28 Hz, 2H), 7.35 (s, 1H), 7.28-7.26 (m, 1H), 7.23 (m, 1H), 7.19 (s, 1H), 6.92-6.90 (dd, J = 6.32, 2.32, 2.24 Hz, 1H), 6.68 (d, J = 8.64 Hz, 1H), 3.53 (m, 6H), 2.83 (broad s, 4H), 2.54 (broad s, 6H); MS (LC/MS, $M+H^+$): 505.50.



Preparation of N-(2-(2-(4-(3,5-dichlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (**3h**): The title compound was prepared according to the procedure for N-(2-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide, except 1-(3,5-dichlorophenyl)piperazine was substituted for 1-(2-chlorophenyl)piperazine. The reaction was purified by normal phase chromatography (100% dichloromethane to 10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (10% acetonitrile/0.1% formic acid in water to 40% acetonitrile/0.1% formic acid in water) to provide N-(2-(2-(4-(3,5-dichlorophenyl)-piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (49% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.28 Hz, 2H), 7.64 (d, J = 8.24 Hz, 2H), 7.50 (s, 2H), 7.41-7.37 (m, 2H), 6.80 (s, 1H), 6.64 (s, 2H), 3.77 (t, J = 4.8, 4.72 Hz, 2H), 3.70 (broad s, 4H), 3.21 (t, J = 4.8, 4.32 Hz, 4H), 2.97 (t, J = 4.2, 4.68 Hz, 4H), 2.93 (t, J = 4.88, 4.72 Hz, 2H); MS (LC/MS, $M+H^+$): 504.50.

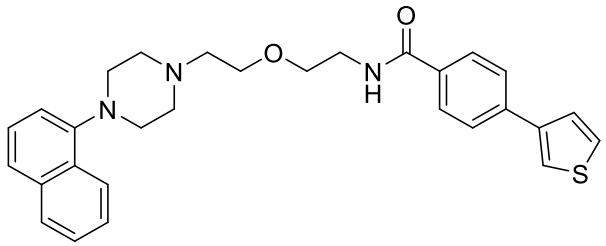


Preparation of N-(2-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (**3i**): The title compound was prepared according to the procedure for N-(2-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide, except 1-(2-methoxyphenyl)piperazine was substituted for 1-(2-chlorophenyl)piperazine. The reaction was purified by normal phase chromatography (100% dichloromethane to 10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (10% acetonitrile /0.1% formic acid in water to 40% acetonitrile/0.1% formic acid in water) to provide N-(2-(2-(4-(2-methoxyphenyl)-piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (55% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (broad s, 1H), 8.00-7.97 (d, J = 9.72 Hz, 2H), 7.59-7.57 (d, J = 8.28 Hz, 2H), 7.40-7.39 (m, 1H), 7.34-7.30 (m, 2H), 6.91-6.86 (m, 1H), 6.72-6.70 (d, J = 8.04 Hz, 1H), 6.58-6.56 (m, 2H), 3.82-3.78 (m, 2H), 3.70 (s, 3H), 3.65-3.61 (m, 4H), 3.15 (broad s, 4H), 3.04 (broad s, 6H); MS (LC/MS, M+H$^+$): 466.70.

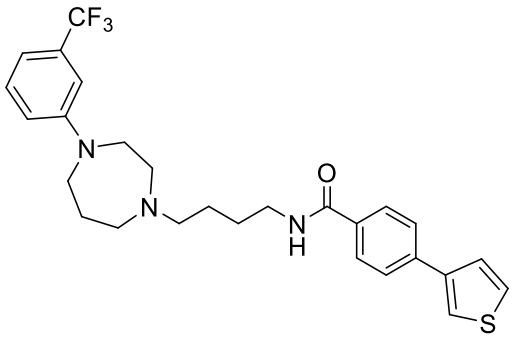


Preparation of N-(2-(2-(4-(4-methoxyphenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (**3j**): The title compound was prepared according to the procedure for N-(2-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide, except 1-(4-methoxyphenyl)piperazine was substituted for 1-(2-chlorophenyl)piperazine. The reaction was purified by normal phase chromatography (100% dichloromethane to 10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (10% acetonitrile/0.1% formic acid in water to 40%

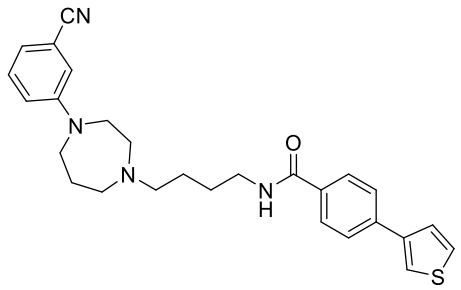
acetonitrile/0.1% formic acid in water) to provide N-(2-(2-(4-(4-methoxyphenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (68% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 8.4 Hz, 2H), 7.49-7.48 (m, 1H), 7.43-7.41 (m, 1H), 7.39-7.28 (m, 1H), 6.97 (broad s, 1H), 6.83 (d, J = 9.2 Hz, 2H), 6.78 (d, J = 9.16 Hz, 2H), 3.77 (s, 3H), 3.71-3.68 (m, 6H), 3.06 (t, J = 5.12, 4.68 Hz, 4H), 2.71-2.68 (m, 6H); MS (LC/MS, M+H$^+$): 466.60.



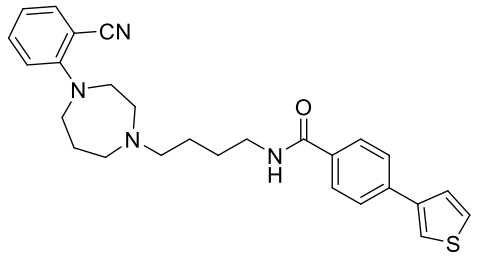
Preparation of N-(2-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (**3k**): The title compound was prepared according to the procedure for N-(2-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide, except 1-(naphthalen-1-yl)piperazine was substituted for 1-(2-chlorophenyl)piperazine. The reaction was purified by normal phase chromatography (100% dichloromethane to 10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (10% acetonitrile/0.1% formic acid in water to 40% acetonitrile/0.1% formic acid in water) to provide N-(2-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethoxy)ethyl)-4-(thiophen-3-yl)benzamide (34% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21-8.17 (m, 1H), 7.88 (d, J = 1.6 Hz, 2H), 7.87-7.82 (m, 1H), 7.63 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.2 Hz, 1H), 7.48-7.45 (m, 3H), 7.42-7.40 (m, 1H), 7.37-7.32 (m, 1H), 7.30-7.28 (d, J = 8.28 Hz, 1H), 7.01 (d, J = 6.88 Hz, 1H), 6.96 (broad s, 1H), 3.74 (m, 6H), 3.13 (broad s, 4H), 2.84 (broad s, 3H), 2.78 (t, J = 5.52, 5.48 Hz, 2H), 2.07 (broad s, 2H); MS (LC/MS, M+H$^+$): 487.15.



Preparation of 4-(thiophen-3-yl)-N-(4-(4-(3-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)butyl) benzamide (**9b**): A solution of 1-(3-(trifluoromethyl)-phenyl)-1,4-diazepane (0.11 g, 0.466 mmol), *N*-(4-bromobutyl)-4-(thiophen-3-yl)-benzamide (0.16 g, 0.466 mmol) and *N*,*N*-diisopropylethylamine (0.25 ml, 1.4 mmol, 3.0 eq) in dry tetrahydrofuran (6 ml) was refluxed at 66 ^{o}C for 48 hours. After 48 hours, the mixture was cooled and filtered to obtain a filtrate. The filtrate was purified by normal phase chromatography (10% methanol in dichloromethane) to provide 4-(thiophen-3-yl)-N-(4-(4-(3-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)butyl) benzamide (132.8 mg, 56% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.36 Hz, 2H), 7.57 (d, J = 8.36 Hz, 2H), 7.45 (t, J= 2.16 Hz, 1H), 1.59-1.52 (m, 4H), 1.90 (pent, J = 5.16 Hz, 2H), 2.48 (t, J = 7 Hz, 2H), 2.57 (t, J = 5.52 Hz, 2H), 7.33 (d, J = 2.16 Hz, 2H), 7.19 (t, J = 8.16 Hz, 2H), 6.80 (d, J = 7.64 Hz, 1H), 6.77 (broad s, 1H), 6.72 (dd, J = 8.4, 2.48 Hz, 2H), 6.47 (broad s, 1H), 3.48 (t, J = 3.88 Hz, 2H), 3.37-3.43 (m, 4H), 2.72 (t, J = 4.16 Hz, 2H); MS (LC/MS, $M+H^+$): 502.60.

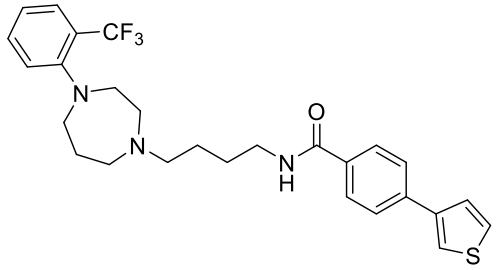


Preparation of N-(4-(4-(3-cyanophenyl)-1,4-diazepan-1-yl)butyl)-4-(thiophen-3-yl)benzamide (**9a**): The title compound was prepared according to the procedure for 4-(thiophen-3-yl)-N-(4-(4-(3-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)butyl)benzamide, except 3-(1,4-diazepan-1-yl)benzonitrile was substituted for 1-(3-(trifluoromethyl)phenyl)-1,4-diazepane. The reaction mixture was first purified by normal phase chromatography (10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (50% acetonitrile/0.1% formic acid in water) to provide N-(4-(4-(3-cyanophenyl)-1,4-diazepan-1-yl)butyl)-4-(thiophen-3-yl)benzamide (48% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72-7.7.70 (d, J = 8.38 Hz, 2H), 7.56-7.54 (d, J = 8.36 Hz, 2H), 7.44-7.43 (t, J = 2.24, 2 Hz, 1H), 7.32 (d, J = 1.88 Hz, 1H), 7.16-7.12 (m, 1H), 6.81-6.79 (d, J = 7.56 Hz, 1H), 6.76-675 (m, 2H), 6.57-6.54 (t, J = 5.32, 5.28 Hz, 1H), 3.42-3.38 (m, 2H), 3.37-3.34 (m, 4H), 2.67-2.65 (t, J = 4.92, 4.88 Hz, 2H), 2.52-2.49 (t, J = 5.56, 5.4 Hz, 2H), 2.44-2.41 (t, J = 6.96, 6.84 Hz, 2H), 1.96-1.82 (m, 2H). 1.59-1.45 (m, 4H); MS (LC/MS, $M+H^+$): 459.

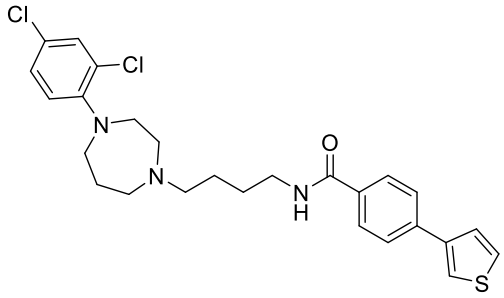


Preparation of N-(4-(4-(2-cyanophenyl)-1,4-diazepan-1-yl)butyl)-4-(thiophen-3-yl)benzamide (**9c**): The title compound was prepared according to the procedure for 4-(thiophen-3-yl)-N-(4-(4-(3-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)butyl)benzamide, except 2-(1,4-diazepan-1-yl)benzonitrile was substituted for 1-(3-(trifluoromethyl)-phenyl)-1,4-diazepane to provide N-(4-(4-(2-cyanophenyl)-1,4-diazepan-1-yl)butyl)-4-(thiophen-3-yl)benzamide (33% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02-8.00 (m, 2H), 7.64-7.63 (m, 3H), 7.55-7.45 (m, 3H), 7.38 (broad s,

2H), 6.99-6.96 (m, 2H), 3.85 (broad s, 2H), 3.52-3.42 (m, 8H), 3.20 (broad s, 2H), 2.54 (broad s, 2H), 2.04 (broad s, 2H), 1.76 (broad s, 2H); MS (LC/MS, $M+H^+$): 459.

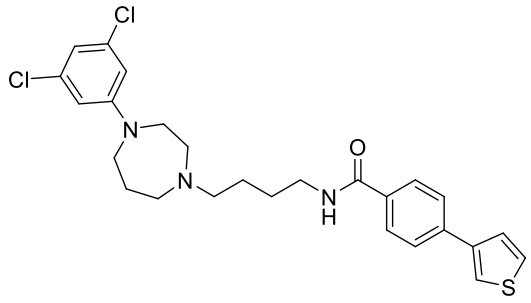


Preparation of 4-(thiophen-3-yl)-N-(4-(4-(2-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)butyl)-benzamide (**9d**): The title compound was prepared according to the procedure for 4-(thiophen-3-yl)-N-(4-(4-(3-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)butyl)benzamide, except 1-(2-(trifluoromethyl)phenyl)-1,4-diazepane was substituted for 1-(3-(trifluoromethyl)-phenyl)-1,4-diazepane to provide 4-(thiophen-3-yl)-N-(4-(4-(2-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)butyl)benzamide (59% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91-7.90 (d, J = 7.52 Hz, 3H), 7.60-7.59 (d, J = 4.92 Hz, 3H), 7.52-7.48 (t, J = 7.64, 6.88 Hz, 2H), 7.35-7.23 (m, 4H), 3.47 (broad s, 2H), 3.40 (broad s, 2H), 3.31 (broad s, 2H), 3.23 (broad s, 2H) 3.06 (broad s, 4H), 3.17-2.13 (m, 2H), 1.85 (broad s, 2H), 1.67 (broad s, 2H); MS (LC/MS, $M+H^+$): 502.

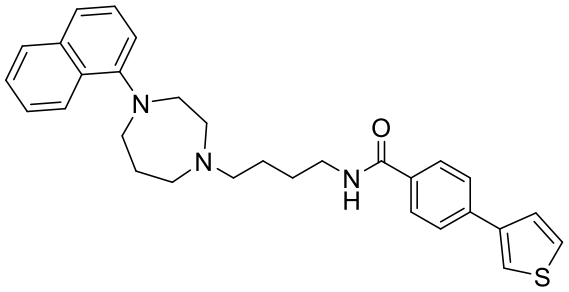


Preparation of N-(4-(4-(2,4-dichlorophenyl)-1,4-diazepan-1-yl)butyl)-4-(thiophen-3-yl) benzamide (**9e**): The title compound was prepared according to the procedure for 4-(thiophen-3-yl)-N-(4-(4-(3-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)butyl)benzamide, except 1-(2,4-dichlorophenyl)-1,4-diazepane was substituted for 1-(3-(trifluoromethyl)-phenyl)-1,4-diazepane

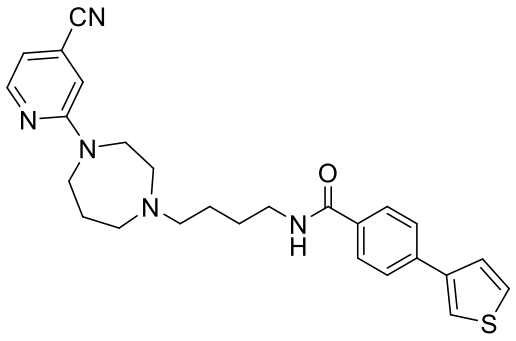
to provide N-(4-(4-(2,4-dichlorophenyl)-1,4-diazepan-1-yl)butyl)-4-(thiophen-3-yl)benzamide (24% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97-7.95 (d, J = 7.92 Hz, 2H), 7.67-7.65 (d, J = 7.88 Hz, 2H), 7.54-7.53 (t, J = 2, 1.96 Hz, 2H), 7.42 (t J = 1.84 Hz, 2H), 7.38-7.37 (d, J = 2.4 Hz, 1H), 7.20-7.17 (dd, J = 6.24, 2.4, 2.36 Hz, 2H), 7.02-7.00 (d, J = 8.64 Hz, 2H), 3.54 (broad s, 2H), 3.43 (broad s, 2H), 3.36 (broad s, 4H), 3.24 (broad s, 2H), 3.09 (broad s, 2H), 2.29 (broad s, 2H), 1.93 (broad s, 2H), 1.75 (broad s, 2H); MS (LC/MS, M+H$^+$): 503.



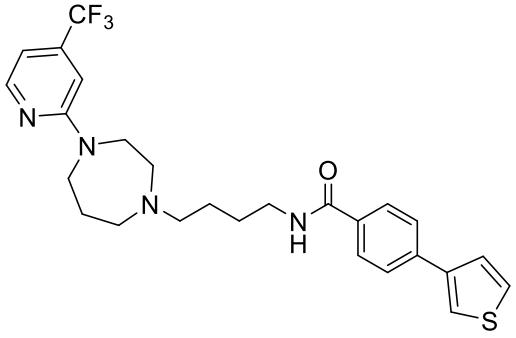
Preparation of N-(4-(4-(3,5-dichlorophenyl)-1,4-diazepan-1-yl)butyl)-4-(thiophen-3-yl) benzamide (**9f**): The title compound was prepared according to the procedure for 4-(thiophen-3-yl)-N-(4-(4-(3-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)butyl)benzamide, except 1-(3,5-dichlorophenyl)-1,4-diazepane was substituted for 1-(3-(trifluoromethyl)-phenyl)-1,4-diazepane to provide N-(4-(4-(3,5-dichlorophenyl)-1,4-diazepan-1-yl)butyl)-4-(thiophen-3-yl)benzamide (35% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90-7.89 (d, J = 7.72 Hz, 2H), 7.62-7.60 (d, J = 7.84 Hz, 2H), 7.50 (broad s, 1H), 7.38 (broad s, 2H), 6.70 (broad s, 2H), 6.48 (s, 2H), 3.69 (broad s, 2H), 3.43 (broad s, 4H), 3.12 (broad s, 2H), 3.04 (broad s, 2H), 2.93 (broad s, 2H), 2.28 (broad s, 2H), 1.81 (broad s, 2H), 1.65 (broad s, 2H); MS (LC/MS, M+H$^+$): 503.



Preparation of N-(4-(4-(naphthalen-1-yl)-1,4-diazepan-1-yl)butyl)-4-(thiophen-3-yl)benzamide (**9g**): The title compound was prepared according to the procedure for 4-(thiophen-3-yl)-N-(4-(4-(3-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)butyl)benzamide, except 1-(naphthalen-1-yl)-1,4-diazepane was substituted for 1-(3-(trifluoromethyl)phenyl)-1,4-diazepane. The reaction mixture was first purified by normal phase chromatography (10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by normal amine phase (RediSep Rf Gold○,R Amine #: 69-2203-507) chromatography (100% isopropanol) to provide N-(4-(4-(naphthalen-1-yl)-1,4-diazepan-1-yl)butyl)-4-(thiophen-3-yl)benzamide (57% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77-7.72 (m, 3H), 7.57 (d, J = 8 Hz, 2H), 7.45-7.43 (m, 1H), 7.39-7.36 (m, 2H), 7.33 (d, J = 1.88 Hz, 2H), 7.28 (t, J = 7.64 Hz, 1H), 7.03 (dd, J = 6.68, 0.72, 0.64 Hz, 1H), 6.82 (broad s, 1H), 3.46 (quart, J = 6.16, 6.12, 5.68 Hz, 2H), 3.30 (m, 2H), 3.26 (t, J = 5.92 Hz, 2H), 2.91 (m, 4H), 2.63 (m, 2H), 1.98 (pent, 2H), 1.66 (m, 4H); MS (LC/MS, $M+H^+$): 484.65.

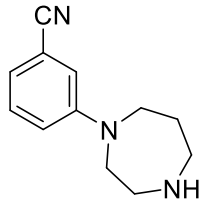


Preparation of N-(4-(4-(4-cyanopyridin-2-yl)-1,4-diazepan-1-yl)butyl)-4-(thiophen-3-yl) benzamide (**9h**): The title compound was prepared according to the procedure for 4-(thiophen-3-yl)-N-(4-(4-(4-(trifluoromethyl)pyridin-2-yl)-1,4-diazepan-1-yl)butyl)benzamide, except 2-(1,4-diazepan-1-yl)isonicotinonitrile was substituted for 1-(4-(trifluoromethyl)pyridin-2-yl)-1,4-diazepane. The reaction mixture was first purified by normal phase chromatography (10% methanol in dichloromethane) to give a partial pure desired product. The impure product was further purified by normal amine phase (RediSep Rf Gold ○,R Amine #: 69-2203-507) chromatography (100% isopropanol) to provide N-(4-(4-(4-cyanopyridin-2-yl)-1,4-diazepan-1-yl)butyl)-4-(thiophen-3-yl)benzamide (34% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (dd, J = 4.44, 0.56, 0.48 Hz, 1H), 7.78 (d, J = 8.36 Hz, 2H) 7.57 (d, J = 8.44 Hz, 2H), 7.45 (t, J = 2.16 Hz, 1H), 7.33 (d, J = 2.12 Hz, 2H), 6.77 (broad s, 1H), 6.62 (dd, J = 4, 1.04, 1 Hz, 1H), 6.57 (s, 1H), 3.82 (broad s, 2H), 3.51 (t, J = 6.2 Hz, 2H), 3.41 (quart, J = 6.24, 5.92 Hz, 2H), 2.84 (t, J = 4.28 Hz, 2H), 2.73 (broad s, 2H), 2.62 (t, J = 6.6 Hz, 2H), 2.06 (broad s, 2H), 1.62 (broad s, 4H), 1.42 (d, J = 6.6 Hz, 2H); MS (LC/MS, M+H$^+$): 460.70.

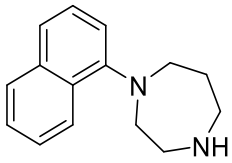


Preparation of 4-(thiophen-3-yl)-N-(4-(4-(4-(trifluoromethyl)pyridin-2-yl)-1,4-diazepan-1-yl) butyl)benzamide (**9i**): A solution of n-butyllithium, 1.6 M in hexane (0.43 ml, 0.69 mmol) was added dropwise into a solution of 1-(4-(trifluoromethyl)pyridin-2-yl)-1,4-diazepane (0.17 g, 0.69 mmol) in anhydrous tetrahydrofuran (6 ml) at -78 ^{o}C for 1 hour. After 1 hour, N-(4-bromobutyl)-

4-(thiophen-3-yl)benzamide (0.23 g, 0.69 mmol) was added into the reaction and was stirred at 0°C for another 1 hour. The reaction mixture was further refluxed at 66°C for 48 hours. After 48 hours, the reaction mixture was cooled and purified by normal phase chromatography (100% ethyl acetate to 10% methanol in dichloromethane) to give a partial pure desired product. The impure product was further purified by reverse phase chromatography (100% acetonitrile with 0.1% formic acid) to provide 4-(thiophen-3-yl)-N-(4-(4-(4-(trifluoromethyl)pyridine-2-yl)-1,4-diazepan-1-yl)butyl)benzamide (279 mg, 41%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 3.36 Hz, 1H), 7.82 (d, J = 8.32 Hz, 2H), 7.58 (d, J = 8.32 Hz, 2H), 7.46 (t, J = 2.44 Hz, 1H), 7.34 (m, 2H), 7.16 (s, 1H), 6.72 (d, J = 5.12 Hz, 1H), 6.58 (s, 1H), 4.02 (broad s, 2H), 3.54 (t, J = 6.28 Hz, 2H), 3.44 (t, J = 1 Hz, 2H), 3.08 (broad s, 2H), 2.98 (broad s, 2H), 2.86 (t, J = 7.4 Hz, 2H), 2.29-2.25 (m, 2H), 1.79-1.75 (m, 2H), 1.64-1.61 (m, 2H); MS (LC/MS, M+H$^+$): 503.60.

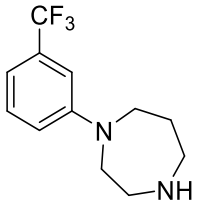


1-(3-cyanophenyl)-1,4-diazepane (**14a**) was prepared as described in WO2018089493.[14]

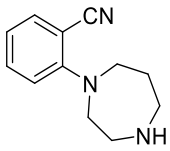


Preparation of 1-(naphthalen-1-yl)-1,4-diazepane (**14g**): 1-Bromonaphthalene (2.89 ml, 20.65 mmol) was stirred with homopiperazine (2.08 g, 20.65 mmol) in the presence of tris(dibenzylideneacetone)dipalladium(0) (0.05g), sodium tert-butoxide (2.77 g, 28.92 mmol, 1.4 eq), and (±)-2,2′-Bis(diphenylphosphino)-1,1′-binaphthalene (0.05 g) in anhydrous toluene (100 ml). The reaction mixture was refluxed at 110 °C for 48 hours. After 48 hours, the reaction

mixture was cooled and filtered with a celite pad to obtain a filtrate. The filtrate was purified by normal phase chromatography (20% methanol in ethyl acetate) to provide 1-(naphthalen-1-yl)-1,4-diazepane (2.91 g, 62% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23-8.25 (d, J = 8Hz, 1H), 7.88-7.85 (d, J = 7.76 Hz, 1H), 7.66-7.64 (d, J = 8.2 Hz, 1H), 7.58-7.50 (m, 2H), 7.45-7.41 (t, J = 8.08, 7.52 Hz, 1H), 7.24-7.22 (dd, J = 6.56, 0.88, 0.84 Hz, 1H), 3.68-3.64 (m, 4H), 3.59-3.57 (m, 2H), 3.41-3.38 (t, J = 6, 5.92 Hz, 2H), 2..44-2.39 (quint, J = 5.88, 5.72, 5.68 Hz, 2H); MS (LC/MS, $M+H^+$): 227.90.

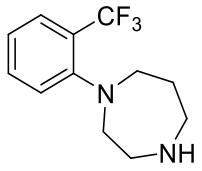


Preparation of 1-(3-(trifluoromethyl)phenyl)-1,4-diazepane (**14b**): The title compound was prepared according to the procedure for 1-(naphthalen-1-yl)-1,4-diazepane, except 1-bromo-3-(trifluoromethyl)benzene was substituted for 1-bromonaphthalene. The reaction was purified by normal phase chromatography (20% methanol in ethyl acetate) to provide 1-(3-(trifluoromethyl)phenyl)-1,4-diazepane (53% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (td, J = 7.96, 0.68, 0.64, 0.44 Hz, 1H), 6.75 (m, 3H), 3.47 (t, J = 6.12 Hz, 2H), 3.43 (t, J = 5.48 Hz, 2H), 2.91 (t, J = 5.44 Hz, 2H), 2.71 (t, J = 5.68 Hz, 2H), 1.78 (m, 3H); MS (LC/MS, $M+H^+$): 245.85.

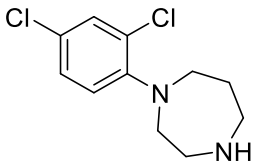


Preparation of 2-(1,4-diazepan-1-yl)benzonitrile (**14c**): The title compound was prepared according to the procedure for 1-(naphthalen-1-yl)-1,4-diazepane, except 2-bromobenzonitrile was substituted for 1-bromonaphthalene. The reaction was purified by normal phase chromatography (10% methanol in dichloromethane) to provide 2-(1,4-diazepan-1-

yl)benzonitrile (47% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (dd, J = 6.08, 1.68 Hz, 1H), 7.37 (tdtd, J = 7.04, 1.76, 1.6 Hz, 1H), 6.90 (d, J = 8.6 Hz, 1H), 6.78 (tdd, J = 7.28, 0.88 Hz, 1H), 3.66 (m, 4H), 3.15 (m, 2H), 2.99 (t, J = 5.64 Hz, 2H), 2.01 (quint 2H); MS (LC/MS, M+H$^+$): 202.90

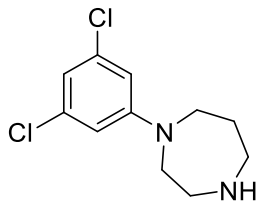


Preparation of 1-(2-(trifluoromethyl)phenyl)-1,4-diazepane (**14d**): The title compound was prepared according to the procedure for 1-(naphthalen-1-yl)-1,4-diazepane, except 1-bromo-2-(trifluoromethyl)benzene was substituted for 1-bromonaphthalene. The reaction was purified by normal phase chromatography (20% methanol in ethyl acetate) to provide 1-(2-(trifluoromethyl)phenyl)-1,4-diazepane (61% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (broad s, 1H), 7.55-7.53 (d, J = 6.96 Hz, 1H), 7.50-7.46 (t, J = 7.76, 7.6 Hz, 1H), 7.33-7.31 (d, J = 7.96 Hz, 1H), 7.22-7.18 (t, J = 7.64 Hz, 1H), 3.46-3.43 (t, J = 5.68, 5.56 Hz, 2H), 3.39-3.35 (m, 2H), 3.30-3.27 (m, 2H), 3.15-3.12 (t, J = 6.36 Hz, 2H), 2.21-2.15 (quint, J = 6.32, 6.24, 5.72, 5.64 Hz, 2H); MS (LC/MS, M+H$^+$): 246.10

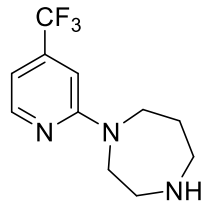


Preparation of 1-(2,4-dichlorophenyl)-1,4-diazepane (**14e**): The title compound was prepared according to the procedure for 1-(naphthalen-1-yl)-1,4-diazepane, except 1-bromo-2,4-dichlorobenzene was substituted for 1-bromonaphthalene. The reaction was purified by normal phase chromatography (20% methanol in ethyl acetate) to provide 1-(2,4-dichlorophenyl)-1,4-diazepane (38% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (broad s, 1H), 7.14-7.11 (m, 1H),

7.02-7.00 (d, J = 7.8 Hz, 1H), 3.27-3.22 (quart, J = 6.44, 6 Hz, 4H), 3.09-3.05 (quart, J = 6.24, 5.56 Hz, 4H), 1.98-1.92 (quint, J = 6, 5.84, 5.76 Hz, 2H); MS (LC/MS, $M+H^+$): 245.90.

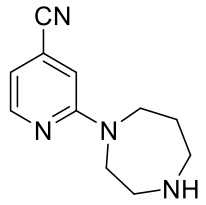


Preparation of 1-(3,5-dichlorophenyl)-1,4-diazepane (**14f**): The title compound was prepared according to the procedure for 1-(naphthalen-1-yl)-1,4-diazepane, except 1-bromo-3,5-dichlorobenzene was substituted for 1-bromonaphthalene. The reaction was purified by normal phase chromatography (20% methanol in ethyl acetate) to provide 1-(3,5-dichlorophenyl)-1,4-diazepane (35% yield): ^{1}H NMR (400 MHz, MeOD-*d*4) δ 6.68-6.66 (d, J = 6.92 Hz, 3H), 3.75 (broad s, 2H), 3.55-3.52 (t, J = 5.92 Hz, 2H), 3.33 (borad s, 2H), 3.22 (broad s, 2H), 2.21 (broad s, 2H); MS (LC/MS, $M+H^+$): 245.90

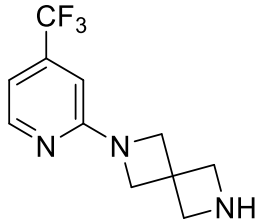


Preparation of 1-(4-(trifluoromethyl)pyridin-2-yl)-1,4-diazepane (**14i**): A solution of homopiperazine (0.1 g, 0.998 mmol, 1.1 eq) and 2-chloro-4-(trifluoro-methyl)pyridine (0.23 ml, 0.908 mmol) in 1-butanol was refluxed at 117^{o}C for 16 hours. After 16 hours, the reaction mixture was cooled and basified and was stirred with concentrated sodium hydroxide (20 ml) overnight. After that, the mixture was further purified by solid loading in normal phase chromatography (10% methanol with 1% ammonia hydroxide in dichloromethane) to give 1-(4-(trifluoromethyl)pyridin-2-yl)-1,4-diazepane (169 mg, 69%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 5.16 Hz, 1H), 6.63 (d, J = 5.12 Hz, 1H), 6.57 (s, 1H), 3.76 (t, J = 6.28 Hz, 2H), 3.66 (t, J =

6.16 Hz, 2H), 3.45 (s, 3H), 3.01 (t, 8 Hz, 2H), 2.84 (t, J = 5.6 Hz, 2H), 1.92 (pent, J = 6.12, 5.88, 5.84 Hz, 2H); MS (LC/MS, $M+H^+$): 245.85.

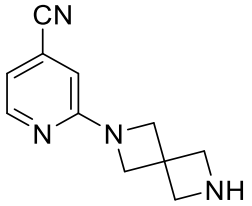


Preparation of 2-(1,4-diazepan-1-yl)isonicotinonitrile (**14h**): The title compound was prepared according to the procedure for 1-(4-(trifluoromethyl)pyridin-2-yl)-1,4-diazepane, except 2-bromoisonicotinonitrile was substituted for 2-chloro-4-(trifluoromethyl)pyridine. The mixture was purified by normal phase chromatography (10% methanol with 1% ammonia hydroxide in dichloromethane) to provide 2-(1,4-diazepan-1-yl)isonicotinonitrile (72% yield): ^{1}H NMR (400 MHz, MeOD-*d*4) δ 6.99 (d, J = 4 Hz, 1H), 5.78 (s, 1H), 5.58 (dd, J = 4.08, 0.96 Hz, 1H), 2.76 (t, J = 5.4 Hz, 2H), 2.47 (t, J = 6.16 Hz, 2H) 2.09-1.98 (m, 4H), 0.91 (pent, J = 5.68 Hz, 2H); MS (LC/MS, $M+H^+$): 203.

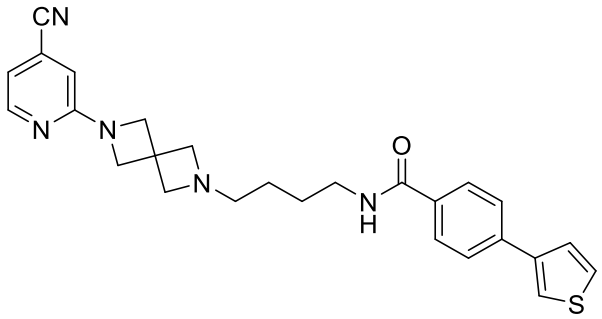


Preparation of 2-(4-(trifluoromethyl)pyridin-2-yl)-2,6-diazaspiro[3.3]heptane (**18b**): A solution of 2-chloro-4-(trifluoromethyl)pyridine (0.08 g. 0.411 mmol), tert-butyl 2,6-diazaspiro[3.3]heptane-2-carboxylate (0.1 g, 0.411 mmol) and triethylamine (0.1 ml) in 1-butanol (6 ml) was refluxed at 117 ^{o}C for 16 hours. After 16 hours, the reaction was cooled and purified by normal phase chromatography (10% methanol in dichloromethane) to provide an intermediate, tert-butyl 6-(4-(trifluoromethyl)pyridin-2-yl)-2,6-diazaspiro[3.3]heptane-2-

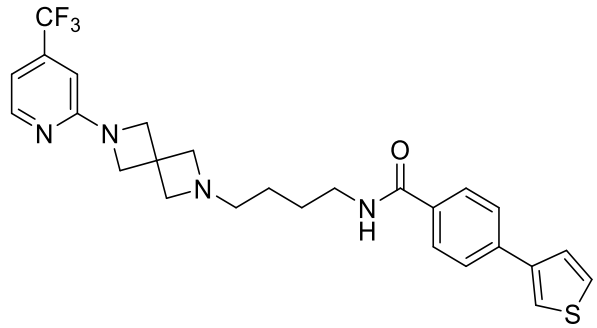
carboxylate: MS (LC/MS, M+H$^+$): 344.10. The intermediate was then dissolved in trifluoroacetic acid and stirred for 16 hours. After 16 hours, the reaction was concentrated to give an oil residue. The residue was dissolved in methanol (20ml) and stirred with Amberlite™ IRN-78 ion-exchange resin, OH-form at room temperature for 16 hours. The basified reaction was then filtered to remove the resin concentrated to give a product, 2-(4-(trifluoromethyl)pyridin-2-yl)-2,6-diazaspiro[3.3]heptane, used for the next step without chromatographic purification. (94.5 mg, 77% yield): ^{1}H NMR (400 MHz, MeOD) δ 8.18 (d, J = 5.16 Hz, 1H), 6.63 (d, J = 5.12 Hz, 1H), 6.57 (s, 1H), 3.62 (s, 4H), 3.35 (s, 4H). (LC/MS, M+H$^+$): 244.



Preparation of 2-(2,6-diazaspiro[3.3]heptan-2-yl)isonicotinonitrile (**18a**): The title compound was prepared according to the procedure for 2-(4-(trifluoromethyl)pyridin-2-yl)-2,6-diazaspiro[3.3]heptane, except 2-chloroisonicotinonitrile was substituted for 2-chloro-4-(trifluoromethyl)pyridine. *tert*-Butyl 6-(4-cyanopyridin-2-yl)-2,6-diazaspiro[3.3]heptane-2-carboxylate: MS (LC/MS, M+H$^+$): 302.10. The intermediate was then deprotected with trifluoroacetic acid and basified with Amberlite™ IRN-78 ion-exchange resin to give 2-(2,6-diazaspiro[3.3]heptan-2-yl)isonicotinonitrile (68% yield). ^{1}H NMR (400 MHz, MeOD) δ 8.12 (d, J = 5.04 Hz, 1H), 6.78 (d, J = 5.18 Hz, 1H), 6.49 (s, 1H), 3.72 (s, 4H), 3.41 (s, 4H), (LC/MS, M+H$^+$): 201.



Preparation of N-(4-(6-(4-cyanopyridin-2-yl)-2,6-diazaspiro[3.3]heptan-2-yl)butyl)-4-(thiophen-3-yl)benzamide (**9j**): The title compound was prepared according to the procedure for 4-(thiophen-3-yl)-N-(4-(6-(4-(trifluoro-methyl)pyridin-2-yl)-2,6-diazaspiro[3.3]heptan-2-yl)butyl) benzamide, except 2-(2,6-diazaspiro[3.3]heptan-2-yl)isonicotinonitrile was substituted for 2-(4-(trifluoro-methyl)pyridin-2-yl)-2,6-diazaspiro[3.3]heptane. The reaction mixture was first purified by normal phase chromatography (10% methanol in dichloromethane) and reverse phase chromatography (10% acetonitrile/0.1% formic acid in water) to provide N-(4-(6-(4-cyanopyridin-2-yl)-2,6-diazaspiro[3.3]heptan-2-yl)butyl)-4-(thiophen-3-yl)benzamide (47% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16-8.15 (dd, J = 4.48, 0.6 Hz, 1H), 7.81-7.79 (d, J = 8.32 Hz, 2H), 7.59-7.57 (d, J = 8.32 Hz, 2H), 7.47-7.46 (t, J = 2.12 Hz, 1H), 7.34 (d, J = 2.16 Hz, 2H), 7.09 (broad s, 1H), 6.72-6.71 (dd, J = 3.96, 1.2, 1.16 Hz, 1H), 6.37 (s, 1H), 4.12 (s, 4H), 3.96 (s, 4H), 3.40 (broad s, 2H), 2..93 (broad s, 2H), 1.62 (broad s, 4H); MS (LC/MS, $M+H^+$): 458.



Preparation of 4-(thiophen-3-yl)-N-(4-(6-(4-(trifluoromethyl)pyridin-2-yl)-2,6-diaza-spiro[3.3] heptan-2-yl)butyl)benzamide (**9k**): A solution of 2-(4-(trifluoromethyl)pyridin-2-yl)-2,6-

diazaspiro[3.3]heptane (0.1 g, 0.411 mmol), N-(4-bromobutyl)-4-(thiophen-3-yl)benzamide (0.14 g, 0.411 mmol) and triethylamine (0.3 ml, 2.12 mmol) in tetrahydrofuran (6 ml) was refluxed at 66^{o}C for 48 hours. After 48 hours, the reaction was cooled and purified by normal phase chromatography (10% methanol in dichloromethane) and reverse phase chromatography (40% acetonitrile/0.1% formic acid in water) to provide 4-(thiophen-3-yl)-N-(4-(6-(4-(trifluoromethyl)pyridin-2-yl)-2,6-diazaspiro[3.3]heptan-2-yl)butyl)benzamide (89.1 mg, 43% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, J = 5.16 Hz, J = 1H), 7.88 (d, J = 7.92 Hz, 2H), 7.64 (d, J = 8 Hz, 2H), 7.53 (s, 1H), 7.40-7.37 (s, 2H), 6.82-6.81 (d, J = 5.08 Hz, 1H), 6.44 (s, 1H), 4.21 (broad s, 4H), 4.07 (broad s, 4H), 3.47 (broad s, 2H), 3.03 (broad s, 2H), 1.68 (broad s, 4H); MS (LC/MS, M+H^{+}): 501.

Computational values: TPSA and cLogP values were calculated using the Dotmatics software suite (Dotmatics LLC The Old Monastery, Windhill Bishops, Stortford Herts, CW23 2ND UK).

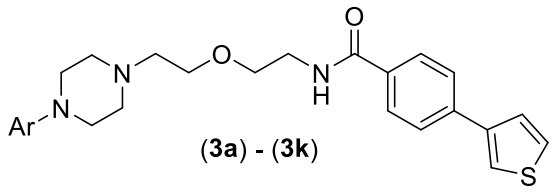
Competitive radioligand-binding studies. For competitive binding studies, transfected HEK293 cell homogenates were suspended in homogenization buffer and incubated with radioligand [^{125}I]IABN, in the presence or absence of inhibitor at 37 ^{o}C for 60 min with [^{125}I]IABN (total volume = 150 ul) as previously described.[15] Competitive radioligand studies were performed to determine the concentration of inhibitor that inhibits 50% of the specific binding of the radioligand (IC50 value). The final radioligand concentration was approximately equal to the Kd value for the binding of the radioligand. For each competition curve, triplicates were performed using two concentrations of inhibitor per decade over five orders of magnitude. Binding was terminated by the addition of cold wash buffer (10 mM Tris–HCl/150 mM NaCl,

pH = 7.5) and filtration over a glass-fiber filter (Pall A/B filters, #66198). A Packard Cobra Gamma Counter was used to measure the radioactivity of [^{125}I]IABN.

The competition curves were modeled for a single binding site using

$$\mathbf{Bs = Bo - ((Bo + L)/(IC50 + L))}$$

where Bs is the amount of ligand bound to receptor and Bo is the amount of ligand bound to receptor in the absence of competitive inhibitor. L is the concentration of the competitive inhibitor. The IC50 value is the concentration of competitive inhibitor that inhibits 50% of the total specific binding. IC50 values were determined using non-linear regression analysis with Table Curve 2D v 5.01 (Jandel, SYSTAT, Systat Software, Inc., San Jose, CA, USA). The values for Bns and Bo were constrained using experimentally derived values. The IC50 values were converted to equilibrium dissociation constants (K_i) using the Cheng and Prusoff (1973) correction. Mean K_i values are reported for at least three independent experiments.



(**3a**) - (**3k**)

Table 1: *In vitro* screening and physicochemical properties data for (**3a**) – (**3k**)

Entry	**Ar**	**MW**	**TPSA**	**cLogP**	**D_3**	**D_2**	**D_2/D_3**
					K_i (nM)		
3a	3-CN-Ph	461	97	3.9	128	1674	13.1
3b	3-CF_3-Ph	504	73	5.1	175	565	3.2
3c	2-CN-Ph	461	97	3.9	140	484	3.5
3d	2-Cl-Ph	470	73	4.8	27	300	10.9
3e	2-CF_3-Ph	504	73	5.1	37	627	16.7

3f	2,3-Di-Cl-Ph	505	73	5.4	127	456	3.6
3g	2,4-Di-Cl-Ph	505	73	5.4	1653	2062	1.3
3h	3,5-Di-Cl-Ph	505	73	5.4	22	332	14.9
3i	2-OMe-Ph	466	82	4.1	24	73.9	3.1
3j	4-OMe-Ph	466	82	4.1	15265	25435	1.7
3k	1-Napthyl	486	73	5.4	79	314	4.0

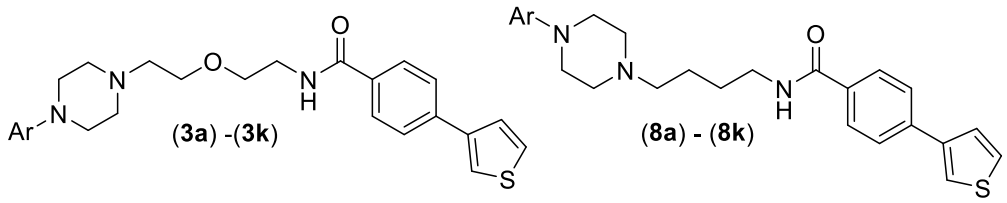
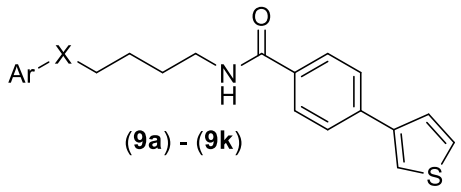


Table 2: Comparison of solubility of (**3a**) - (**3k**) and (**8a**) - (**8k**):

Entry	**Ar**	**Sol µM**	**Entry**	**Ar**	**Sol µM**	**Entry**	**Ar**	**Sol µM**
3a	3-CN-Ph	59	3e	2-CF_3-Ph	19	3i	2-OMe-Ph	180
8a	3-CN-Ph	2	8e	2-CF_3-Ph	3	8i	2-OMe-Ph	17
3b	3-CF_3-Ph	12	3f	2,3-Di-Cl-Ph	10	3j	4-OMe-Ph	2
8b	3-CF_3-Ph	6	8f	2,3-Di-Cl-Ph	2	8j	4-OMe-Ph	2
3c	2-CN-Ph	44	3g	2,4-Di-Cl-Ph	19	3k	1-Napthyl	2
8c	2-CN-Ph	14	8g	2,4-Di-Cl-Ph	2	8k	1-Napthyl	7
3d	2-Cl-Ph	58	3h	3,5-Di-Cl-Ph	2			
3a	3-CN-Ph	59	3e	2-CF_3-Ph	19			

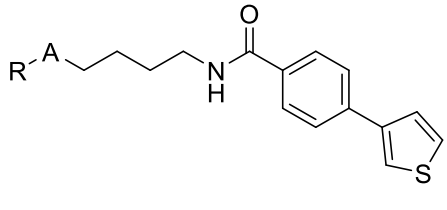


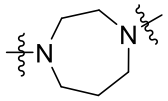
(**9a**) - (**9k**)

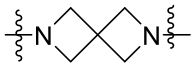
Table 3: *In vitro* screening and physicochemical properties data for (**9a**) – (**9k**)

Entry	**Ar**	**A**	**MW**	**TPSA**	**cLogP**	**D_3**	**D_2**	**D_2/D_3**
						K_i (nM)		
9a	3-CN-Ph	Homopip	459	88	5.3	5.7	794	139
9b	3-CF_3-Ph	Homopip	502	64	6.6	30.2	1311	43
9c	2-CN-Ph	Homopip	459	88	5.3	6.3	718	114
9d	2-CF_3-Ph	Homopip	502	64	6.6	18.6	868	47
9e	2,4-Di-Cl-Ph	Homopip	503	64	6.8	16.7	1331	80
9f	3,5-Di-Cl-Ph	Homopip	503	64	6.8	33.1	909	27
9g	1-Napthyl	Homopip	484	64	6.9	17.7	1252	71
9h	5-CN-2-Pyridyl	Homopip	460	101	4.4	64.7	12750	197
9i	5-CF_3-2-Pyridyl	Homopip	503	77	5.7	70.2	9218	131
9j	5-CN-2-Pyridyl		458	101	3.6	1093	31861	29
9k	5-CF_3-2-Pyridyl		501	77	4.9	464	20831	45

*Homopip = homopiperazine



(**9a**) - (**9i**) R = 

(**9j**) - (**9k**) R = 

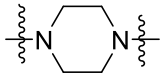
(**19a**) - (**19i**) R = 

Table 4: Comparison of solubility of (**9a**) - (**9k**) and (**19a**) - (**19i**):

Entry	Ar	R	Sol μM	Entry	Ar	R	Sol μM
9a	3-CN-Ph	Homopip	19	9g	1-Napthyl	Homopip	28
19a	3-CN-Ph	Piperazine	2	19g	1-Napthyl	Piperazine	7
9b	3-CF_3-Ph	Homopip	6	9h	5-CN-2-Pyridyl	Homopip	76
19b	3-CF_3-Ph	Piperazine	2	19h	5-CN-2-Pyridyl	Piperazine	34
9c	2-CN-Ph	Homopip	103	9i	5-CF_3-2-Pyridyl	Homopip	27
19c	2-CN-Ph	Piperazine	14	19i	5-CF_3-2-Pyridyl	Piperazine	10
9d	2-CF_3-Ph	Homopip	26	9j	5-CN-2-Pyridyl		148
19d	2-CF_3-Ph	Piperazine	3	9	5-CN-2-Pyridyl	Piperazine	34
9e	2,4-Di-Cl-Ph	Homopip	26	9k	5-CF_3-2-Pyridyl		109
19e	2,4-Di-Cl-Ph	Piperazine	2	19i	5-CF_3-2-Pyridyl	Piperazine	10
9f	3,5-Di-Cl-Ph	Homopip	2				
19f	3,5-Di-Cl-Ph	Piperazine	2				

*Homopip = homopiperazine

Acknowledgments: The research reported in this publication was supported by the National Institute on Drug Abuse (NIDA)/National Institutes of Health (NIH) under award numbers DA029840-06A1 and DA023957.

Declaration of interests: The authors have no financial interest to declare.

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