Bioorganic & Medicinal Chemistry 17 (2009) 4873–4880

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

'Click' D_1 receptor agonists with a 5-HT_{1A} receptor pharmacophore producing D_2 receptor activity ☆

Jing Zhang [a,†], Hai Zhang [b,†], Wenxian Cai [b], Leiping Yu [b], Xuechu Zhen [b,*], Ao Zhang [a,*]

[a] Synthetic Organic and Medicinal Chemistry Laboratory (SOMCL), Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences, Shanghai 201203, China
[b] Neuropharmacological Laboratory, Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 27 May 2009
Revised 10 June 2009
Accepted 11 June 2009
Available online 16 June 2009

Keywords:
Arylbenzazepine
Arylpiperazine
Click reaction
Dopamine receptor
Serotonin 5-HT_{1A} receptor

ABSTRACT

A series of new 1-aryl-3-benzazepine derivatives containing an arylpiperazinyl function as the N3 substituent were synthesized by combining a D_1 receptor agonistic pharmacophore and a 5-HT_{1A} receptor pharmacophore through Click reaction. Interestingly, these compounds generally do not have good binding affinity at the D_1 receptor, but most compounds are potent at both D_2 and 5-HT_{1A} receptors. Compound **8h**, containing 1-*m*-tolyl-benzazepine scaffold and 2-methoxyphenylpiperazine core, displayed good affinity at all tested receptors, with K_i values of 144, 80, and 133 nM, for the D_1, D_2, and 5-HT_{1A} receptors, respectively. Compound **13** with the triazole moiety formed differently from that in **8h** showed the highest affinity at the D_2 receptor with K_i value of 19 nM. This compound also showed moderate affinity at the 5-HT_{1A} (K_i, 105 nM), and D_1 (K_i, 551 nM) receptors. Functional assays indicated that both compounds **13** and **8h** are antagonists at D_1 and D_2 receptors, whereas full agonistic activity at the 5-HT_{1A} receptor was observed. In agreement with the binding affinity, compound **13** is a high efficacy D_2 antagonist and 5-HT_{1A} agonist.

1. Introduction

Dopamine (DA) D_1 and D_2 receptors represent the most abundant DA receptors in the mammalian brains, and are implicated in the pathophysiology of several neurobehavioral disorders, such as Parkinson's disease, and a number of other movement and hyperactivity disorders, including schizophrenia, mania, depression, substance abuse, and eating disorders.[1,2] Indeed, D_1 and D_2 receptors are the most studied therapeutic targets for these neurological and psychiatric diseases, and compounds targeting these two receptors generally have utilities in the treatment of Parkinson's disease, depression, and schizophrenia.[3]

Recently, accumulating evidences have indicated that serotonin 5-HT_{1A} receptor is also implicated in many CNS disorders, including anxiety,[4] depression,[5] neuroprotection,[6,7] schizophrenia,[8,9] Parkinson's disease,[10–12] and Alzheimer disease[13] by acting alone or together with other neurotransmitter receptors, especially DA receptor. Therefore, it has been proposed that 5-HT_{1A} agonists combined with DA receptor agonism or antagonism may be an optimal option for treating these disorders.[14–17] In fact, sarizotan, a compound possessing high binding at both D_2 and 5-HT_{1A} receptors, and acting as a 5-HT_{1A} agonist and D_2 receptor partial agonist/antagonist, has been found in effectively improving DA-induced motor complications by reducing striatal serotoninergic nerve impulse activity without altering L-dopa efficacy. These compounds have been in clinical trial as an innovative bisfunctional drug for treating dyskinesias associated with L-dopa therapy in PD.[18]

1-Aryl-3-benzazepines, including SKF-38393 (**1**) and SKF-83959 (**2**) (Fig. 1), represent the prototypical structural scaffold possessing D_1 receptor activities.[19–22] These compounds generally display high binding affinity and selectivity at the D_1 receptor, and are useful tool drugs for the study of the receptor and its therapeutic indications. However, poor intrinsic activity, low metabolic stability, and several unwanted side effects are generally associated with these compounds.[19] We recently found that compounds (e.g., **3**) with a larger lipophilic substituent at the C6 position displayed equi-potent or even higher binding affinity than the C6 non-substituted prototypes.[20,21] As a continuation of this work toward a full understanding on the SAR of this scaffold, we decided to explore the relatively not-well-explored N3 position of the benzazepine template where only a few N-substituents including H, Me, allyl, propargyl, and few alkylamino groups have been reported.[19]

Our original objective is to incorporate a 5-HT_{1A} agonistic pharmacophore as the N3 side chain in the 1-aryl-3-benzazepine scaffold to achieve compounds possessing both D_1 and 5-HT_{1A} agonistic properties.[21] Since 1-arylpiperazin-4-yl functionality is

☆ This work has been partially presented at the 237th American Chemical Society National Meeting at Salt Lake City, UT 2009, MEDI 0311.

* Corresponding authors. Tel./fax: +86 21 50806750 (X.Z.); tel.: +86 21 50806035; fax: +86 21 50806600 (A.Z.).
E-mail addresses: xzhen@mail.shcnc.ac.cn (X. Zhen), aozhang@mail.shcnc.ac.cn (A. Zhang).
† These two authors contributed equally to this work.

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.06.019

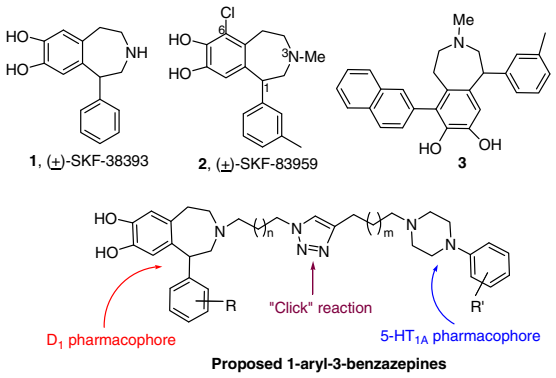


Figure 1. Representative and proposed 1-aryl-3-benzazepines.

a well-established pharmacophore for the 5-HT_{1A} receptor,[23–25] it was selected to attach to the N3 position of 1-aryl-3-benzazepines through a linker by a Click reaction (Fig. 1). To our surprise, all these new compounds significantly lost binding affinity at the D_1 receptor, and instead high affinity at the D_2 receptor, along with good 5-HT_{1A} binding, was observed. Herein, in this report, we describe the details of the synthesis and pharmacological investigations of these compounds.

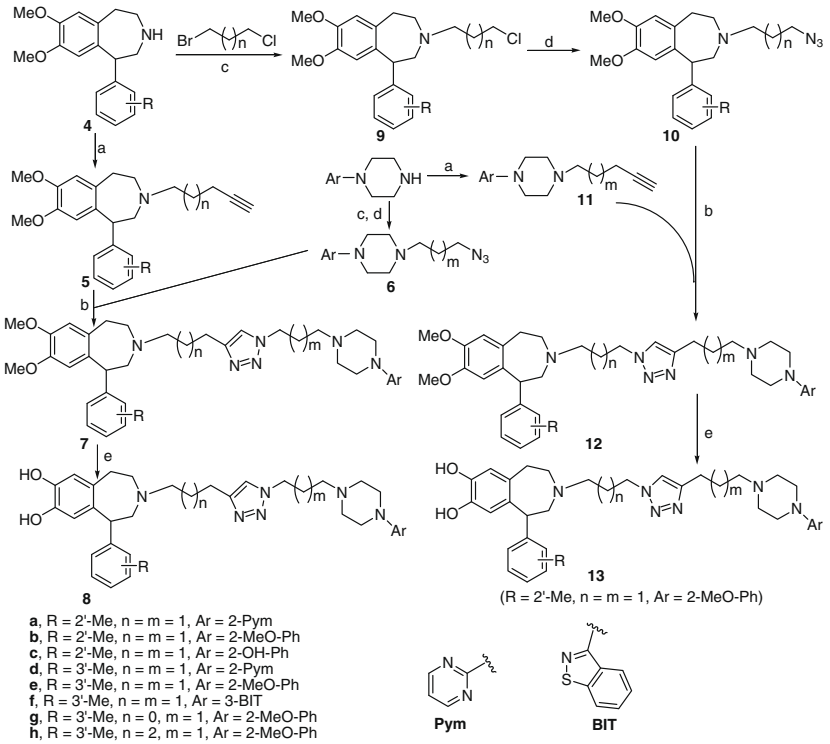
2. Chemistry

2′-Methyl or 3′-methyl substituted benzazepines **4** were prepared from 3,4-dimethoxyphenylethylamine and corresponding styrene oxide by using a literature procedure.[20,26,27] Treating **4**[27] with pentyn-4-yl 4-methylbenzenesulfonate which was prepared by tosylation of the corresponding alcohol provided N-alkylated benzazepine **5** in 60–80% yield. Azide **6** was prepared from the corresponding arylpiperazine and chloroalkyl bromide followed by azidation with NaN_3 in 70–75% overall yield. Treating **5** and **6** by Click reaction[28] under CuI/DIPEA provided triazole **7** in 82–99% yields. Compounds **7** were O-demethylated using BBr_3 (1 M, in CH_2Cl_2) at −78 °C yielding the final compounds **8a–h** in 17–70% yields. The general low yields were due to the incomplete demethylation during this reaction which led to monohydroxy products. Similarly, benzazepine **4** (R = 2′-Me) was treated with chloropropyl bromide to yield the corresponding chloride **9** in 80% yield (Scheme 1). Azidation of chloride **9** with NaN_3 provided azide **10** in 90% yield. Reaction of the corresponding arylpiperazine with pent-4-ynyl 4-methylbenzenesulfonate afforded compound **11** in 76% yield. Click reaction of azide **10** and alkyne **11** yielded the dimethoxybenzazepine **12** in 97% yield, which was then treated with BBr_3 (1 M, in CH_2Cl_2) at −78 °C providing the final compound **13** (R = 2′-Me, $n = m = 1$, Ar = 2-MeO-Ph). However, this compound was not stable at storage, and a side compound with very similar polarity on TLC was observed after two days. After several trials on chromatography and preparative TLC, we were unable to isolate the side compound in pure form, therefore no other analogues were made in this series.

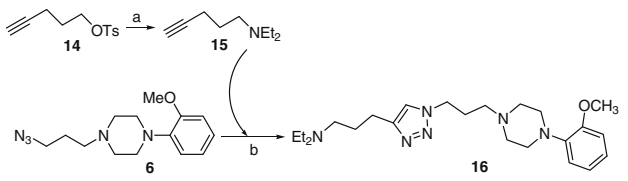
The control compound **16** was prepared in a similar manner. Treatment of diethylamine with tosylate **14** which was prepared from pent-4-yn-1-ol provided *N,N*-diethylpent-4-yn-1-amine **15** in quantitative yield. Click reaction of **15** with azide **6** in the presence of CuI/DIEPA in THF at RT provided triazole **16** as a colorless liquid in 90% yield (Scheme 2).

3. Results and discussion

All the new compounds (**8a–h**, and **13**, **16**) were racemic, and were converted to their HBr or TFA salts for the bioassay. The binding affinity of these compounds was assayed at D_1, D_2, and 5-HT_{1A} receptors using membrane preparation obtained from stable transfected HEK293 or CHO cells (Table 1). This procedure is similar to those reported by us previously.[15,19,20] [^{3}H]SCH23390,



Scheme 1. Synthesis of compounds **8a–h**, and **13** via Click reaction as the key step. Reagents and conditions: (a) alkynyl tosylate, K_2CO_3, MeCN, reflux; (b) CuI, DIPEA, THF, rt, overnight; (c) chloroalkyl bromide, K_2CO_3, MeCN, 50 °C; (d) DMSO, NaN_3, 100 °C; (e) BBr_3, CH_2Cl_2.



Scheme 2. Synthesis of control compound **16**. Reagents and conditions: (a) diethylamine, K_2CO_3, MeCN, reflux; (b) CuI, DIPEA, THF, rt, overnight.

Table 1
In vitro binding assays of new compounds **8a–h**, **13,** and **16** at D_1, D_2, and 5-HT_{1A} receptors in HEK293 or CHO cells[a]

Compound[a]	K_i (nM)		
	D_1 ([^{3}H]SCH23390)	D_2 ([^{3}H]Spiperone)	5-HT_{1A} ([^{3}H]8-OH-DPAT)
1, (±)-SKF-38393	393 ± 5 (190[b])	NA (720,000[b])	NA
2 (±)-SKF-83959	1.93 ± 0.47 (1.18[c])	NA	>1000
8a	NA	NA	799 ± 37
8b	501 ± 74	39.1 ± 10.5	85 ± 15
8c	NA	NA	98 ± 11
8d	963 ± 171	NA	648 ± 83
8e	655 ± 0.4	115 ± 20	133 ± 5.4
8f	221 ± 29	200 ± 35	235 ± 78
8g	492 ± 165	NA	457 ± 87
8h	144 ± 50	80.0 ± 10.3	133 ± 6.2
13	551 ± 0.1	19.1 ± 1.3	105 ± 28
16	NA	NA	381 ± 92

[a] Values are means of three to five experiments, and all compounds were tested as HBr or THF salts in racemates, NA = not active (less than 80% of inhibition in radioligand binding at 10 μM), NT = not tested.
[b] Data from Ref. 26.
[c] Data from Ref. 22.

[^{3}H]Spiperone, and [^{3}H]8-OH-DPAT were used as the standard radioligands for DA D_1, D_2, and serotonin 5-HT_{1A} receptors, respectively. Racemic (±)-SKF-38393 (**1**) and (±)-SKF-83959 (**2**) were also tested in our assays for comparison.

Compound **8a** which contains an *ortho*-tolyl as the C1 substituent in the benzazepine core and pyrimidin-2-yl (Pym) as the aryl group did not show binding affinity at both D_1 and D_2 receptors; however, some affinity (K_i, 800 nM) was observed at the 5-HT_{1A} receptor. Modification of the aryl fragment in the arylpiperazine core provided compounds **8b,c**. Compound **8b** containing 2-MeO-Ph as the aryl group displayed significant binding affinity at both D_2 and 5-HT_{1A} receptors with K_i values of 39 and 85 nM, while moderate affinity (K_i, 501 nM) was obtained at the D_1 receptor. This compound can be viewed as a bisfunctional compound for D_2 and 5-HT_{1A} receptors, with a twofold binding selectivity for the D_2 receptor. Removal of the methyl group in the 2-MeO-Ph fragment yielded compound **8c** which was inactive at both D_1 and D_2 receptors. Interestingly, compound **8c** retained good affinity at the 5-HT_{1A} receptor (K_i, 98 nM). This result indicated that the aryl group in the arylpiperazine function is not only a determinant factor for the 5-HT_{1A} receptor, but also a crucial factor for both D_1 and D_2 receptors.

Among the C1 *meta*-tolyl substituted benzazepine derivatives **8d–h**, the Pym containing compound **8d** showed moderate affinity at both D_1 and 5-HT_{1A} receptors, and is inactive at the D_2 receptor. Again, replacing the Pym function with 2-MeO-Ph yielded compound **8e** showing a significant enhancement in binding to the D_2 receptor, together with a moderate increase in binding to the D_1 and 5-HT_{1A} receptors. This compound is equipotent at both D_2 and 5-HT_{1A} (K_i, 115 and 133 nM, respectively), and is fivefold more potent against the D_1 receptor. Further changing the 2-MeO-Ph to the benzo[*d*]isothiazol-3-yl moiety (BIT) provided compound **8f** which is equi-potent at all the three tested receptors (D_1, D_2, 5-HT_{1A}) with K_i values of 221, 200, and 235 nM, respectively. The length of the linker between the benzazepine core and the triazole moiety played a remarkable influence on the binding at the D_2 receptor, but only a minor influence on binding affinity at the D_1 and 5-HT_{1A} receptors. Compared to **8e**, compound **8g** with a shorter linkage lost binding affinity at the D_2 receptor; however, compound **8h** containing a longer chain displayed an enhancement for both D_1 and D_2 receptors, and the affinity at the 5-HT_{1A} receptor was well retained. Therefore, compound **8h** has good affinity at all the tested receptors (D_1, D_2, 5-HT_{1A}) with K_i values of 144, 80, and 133 nM, respectively. Interestingly, compound **13** with the triazole moiety formed differently from that in **8a–h** showed the highest affinity at the D_2 receptor with K_i value of 19 nM. This compound also showed moderate affinity at the 5-HT_{1A} receptor (K_i, 105 nM), along with moderate affinity at the D_1 receptor (K_i, 551 nM). Therefore, compound **13** is D_2-selective binder and is 5.5- and 29-fold more potent at this receptor against the 5-HT_{1A} and D_1 receptor, respectively.

Since all the final compounds (**8a–h**, **13**) only contain pharmacophores for the D_1 (benzazepine core) and 5-HT_{1A} (arylpiperazine core) receptors, it is intriguing that where the extra D_2 binding affinity observed from most of these compounds is originated. To examine the possible role of the triazole-containing linker, compound **16** which does not contain the benzazepine core was prepared as a control and was evaluated in the same conditions. To our surprise, this compound is indeed inactive at both D_1 and D_2 receptors, except its moderate 5-HT_{1A} receptor binding (K_i, 381 nM). Therefore, it is likely that substitution on the N3 of benzazepine scaffold produced the observed D_2 receptor affinity.

Compound **13**, which is the most potent at the D_2 receptor, and compound **8h,** which has good affinity at all the three tested receptors, were further evaluated in the [^{35}S]GTPγS binding assays to determine their agonistic and antagonistic activities. The results are summarized in Figure 2 and Table 2.

As shown in Figure 2 and Table 2, both compounds **8h** and **13** did not show appreciable stimulation at both D_1 and D_2 receptors (Fig. 2 a and b) in the [^{35}S]GTPγS binding assays indicating that they lack agonistic activities at the two DA receptors. However, co-administration of these compounds with their corresponding standard full agonists (SKF 38393 for the D_1, and quinpirole for the D_2 receptors), these two compounds displayed a strong inhibition to the binding of the standard agonists at the corresponding receptors. Therefore, compounds **8h** and **13** are antagonists at both D_1 and D_2 receptors. In relevance to their binding affinity, compound **13** has stronger antagonistic activity than compound **8h**, especially at the D_2 receptor with an IC_{50} value of 480 nM (1.57 μM for **8h**). Different from the functional profiles at the D_1 and D_2 receptors, compounds **8h** and **13** displayed strong agonistic activity (E_{max} >100%) at the 5-HT_{1A} receptor (Table 2, Fig. 2 c), which is even higher than the standard agonist, 5-HT (E_{max} = 100%). These compounds are full 5-HT_{1A} agonist with EC_{50} values of 200 and 330 nM, respectively.

4. Conclusion

In summary, a series of new benzazepine derivatives containing an arylpiperazinyl function at the N3 side chain were synthesized by using a Click reaction as the key step. Although these compounds were prepared by combining the D_1 and 5-HT_{1A} receptor agonistic pharmacophores, their binding potency at the D_1 receptor is generally lower, and most compounds showed good to moderate affinity at both D_2 and 5-HT_{1A} receptors. Compound **8h**, containing 1-*m*-tolyl-benzazepine

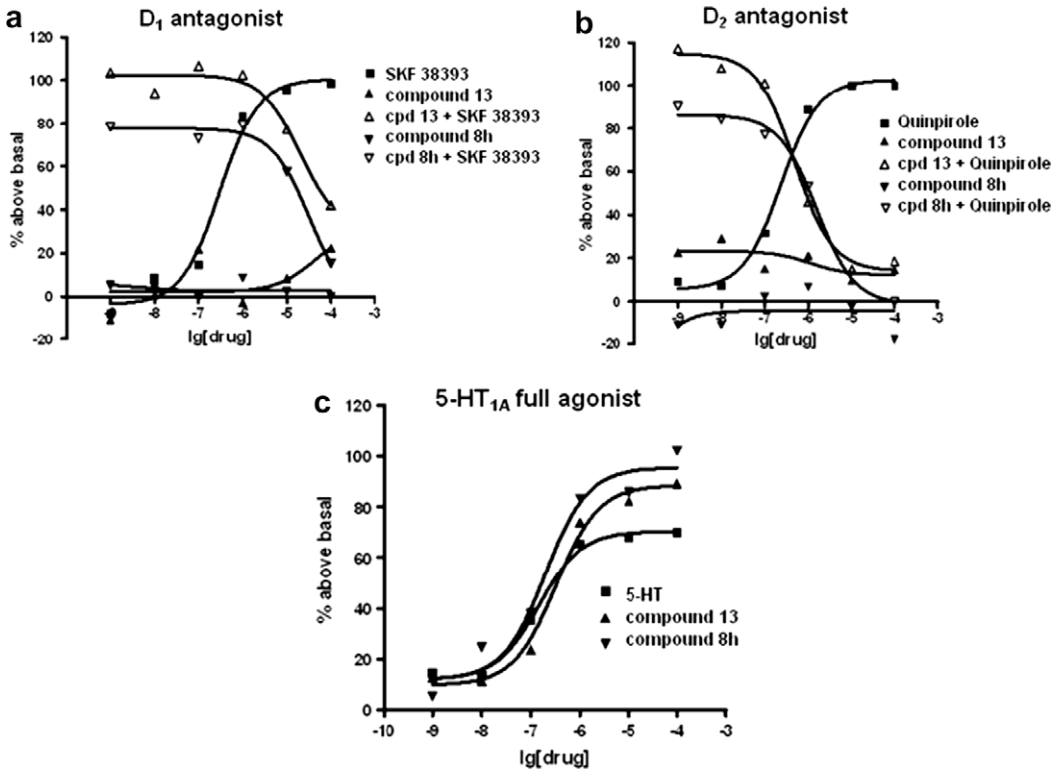


Figure 2. [^{35}S]GTPγS binding assays of compounds **8h** and **13** at D_1, D_2, and 5-HT_{1A} receptors.

Table 2
D_1, D_2, and 5-HT_{1A} receptor binding and [^{35}S]GTPγS studies of compounds **8h** and **13**[a]

Compound	D_1		D_2		5-HT_{1A}
	Cpd alone E_{max} (%)/EC_{50}	Cpd + SKF38393 I_{max} (%)/IC_{50}	Cpd alone E_{max} (%)/EC_{50}	Cpd + Quinpirole I_{max} (%)/IC_{50}	Cpd alone E_{max} (%)/EC_{50}
8h	NA	100%/32.7 μM	NA	100%/1.57 μM	136%/200 nM
13	NA	70%/19.8 μM	NA	100%/480 nM	125%/330 nM
SKF38393	100%/300 nM	—	—	—	—
Quinpirole	—	—	100%/240 nM	—	—
5-HT	—	—	—	—	100%/150 nM

[a] Values are means of three to five experiments, and NA indicates no significant stimulation was detected; Dash lines indicate not applicable.

scaffold and 2-methoxyphenylpiperazine core, displayed good affinity at all tested receptors, with K_i values of 144, 80, and 133 nM, for the D_1, D_2, and 5-HT_{1A} receptors, respectively. Compound **13** with the triazole moiety formed differently from that in **8h** showed the highest affinity at the D_2 receptor with K_i value of 19 nM. This compound also showed good affinity at the 5-HT_{1A} receptor (K_i, 105 nM), and moderate affinity at the D_1 receptor (K_i, 551 nM). Functional assays indicated that both compounds **13** and **8h** are antagonists at D_1 and D_2 receptors, whereas full agonistic activity at the 5-HT_{1A} receptor was observed. In agreement with the binding affinity, compound **13** is a high efficacy D_2 antagonist and 5-HT_{1A} agonist.

Since it has been well documented[19,20,22] that a chloro-substituent at the C7 of the benzazepine core is a determinant for D_1 receptor antagonistic activity, the antagonism of compounds **8h** and **13** at the D_1 receptor suggested that an additional antagonistic binding site for the D_1 receptor may exist at the N3 side chain. Further, our current results also indicated that a lipophilic binding site for the D_2 receptor is existed at the N3 side chain in the D_1 receptor agonistic scaffold, 1-aryl-3-benzazepine skeleton.

5. Experimental

5.1. Chemistry

Melting points were determined on a Thomas–Hoover capillary tube apparatus and are reported uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AC300 spectrometer using tetramethylsilane as an internal reference. Element analyses, performed by the Analytic Lab, SIMM, were within ±0.4% of theoretical values. Analytical thin-layer chromatography (TLC) was carried out on 0.2-mm Kieselgel 60F 254 silica gel plastic sheets (EM Science, Newark). The column output was monitored with TLC. Yields of all the reactions were not optimized. Compounds **4** (2′-tolyl or 3′-tolyl) were prepared using a reported procedure.[27]

5.1.1. General procedure for the preparation of N3-substituted benzazepines 5 and 11

Alkynol tosylate (1.1 equiv) was added to a solution of benzazepine **4** (1.0 equiv) and K_2CO_3 (2.0 equiv) in acetonitrile. The

mixture was stirred at 90 °C overnight. After cooling to rt, the mixture was filtered and concentrated. The crude material was purified by silica gel chromatography to give the corresponding benzazepines **5** and **11**.

5.1.1.1. 7,8-Dimethoxy-3-(pentyn-4-yl)-1-o-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (5, R = 2′-Me, *n* = 1). Yield 61%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.21 (m, 4H), 6.68 (s, 1H), 5.92 (s, 1H), 4.46 (d, *J* = 9.0 Hz, 1H), 3.85 (s, 3H), 3.47 (s, 3H), 3.29 (m, 2H), 3.14 (dd, *J* = 6.6, 12.0 Hz, 1H), 2.82 (m, 4H), 2.41 (m, 3H), 2.16 (s, 3H), 1.96 (t, *J* = 2.4 Hz, 1H), 1.73 ppm (m, 2H).

5.1.1.2. 7,8-Dimethoxy-3-(pent-4-ynyl)-1-m-tolyl-2,3,4,5-tetrahydro-1*H*-benzo [*d*]azepine (5, R = 3′-Me, *n* = 1). Yield 75%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.23 (m, 1H), 7.06 (d, *J* = 7.5 Hz, 1H), 6.98 (m, 2H), 6.67 (s, 1H), 6.30 (s, 1H), 4.23 (dd, *J* = 3.0, 6.0 Hz, 1H), 3.89 (s, 3H), 3.72 (s,3H), 3.00 (m, 3H), 2.81 (m, 2H), 2.56 (m, 3H), 2.34 (s, 3H), 2.20 (m, 2H), 1.94 (t, *J* = 2.7 Hz, 1H), 1.72 ppm (m, 2H).

5.1.1.3. 3-(But-3-ynyl)-7,8-dimethoxy-1-*m*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (5, R = 3′-Me, *n* = 0). Yield 74%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.24 (m, 1H), 7.03 (m, 3H), 6.68 (s, 1H), 6.30 (s, 1H), 4.24 (m, 1H), 3.87 (s, 3H), 3.64 (s, 3H), 3.11 (m, 2H), 2.90 (m, 5H), 2.62 (t, *J* = 10.2 Hz, 1H), 2.38 (m, 5H), 1.97 ppm (t, *J* = 2.4 Hz, 1H).

5.1.1.4. 3-(Hex-5-ynyl)-7,8-dimethoxy-1-*m*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (5, R = 3′-Me, *n* = 2). Yield 79%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.22 (m, 1H), 7.06 (d, *J* = 7.8 Hz, 1H), 6.99 (m, 2H), 6.68 (s, 1H), 6.28 (s, 1H), 4.24 (dd, *J* = 2.4, 6.6 Hz, 1H), 3.86 (s, 3H), 3.63 (s, 3H), 3.01 (m, 3H), 2.83 (m, 2H), 2.50 (m, 3H), 2.34 (s, 3H), 2.19 (dt, *J* = 2.7, 6.9 Hz, 2H), 1.93 (t, *J* = 2.7 Hz, 1H), 1.57 ppm (m, 4H).

5.1.1.5. 1-(2-Methoxyphenyl)-4-(pent-4-ynyl)piperazine (11, Ar = 2-MeO-Ph, *m* = 1). Yield 76%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.91 (m, 4H), 3.84 (s, 3H), 3.08 (br s, 4H), 2.64 (br s, 4H), 2.49 (t, *J* = 7.2 Hz, 2H), 2.24 (dt, *J* = 2.7, 7.2 Hz, 2H), 1.95 (t, *J* = 2.7 Hz, 1H), 1.74 ppm (m, 2H).

5.1.2. General procedure for the preparation of azides 6 and 10

The corresponding arylpiperazines or benzazepine **4** (1.0 equiv), chloroalkyl bromide (10.0 equiv), and K_2CO_3 (2.0 equiv) were stirred at 50 °C for 2 h. Then the solution was filtered and concentrated. The crude material was chromatographed to give the corresponding chloride derivatives that were used directly for the next step.

The mixture of the chlorides obtained above (1.0 equiv) and NaN_3 (2.0 equiv) was dissolved in DMSO (5 mL) and stirred at 100 °C for 2 h. After cooling to rt, the reaction mixture was diluted with $CHCl_3$, and washed with water, brine, and dried. The solution was concentrated and chromatographed to give the corresponding azides **6** and **10**.

5.1.2.1. 2-(4-(3-Azidopropyl)piperazin-1-yl)pyrimidine (6, Ar = 2-Pym, *m* = 1). Yield 75% (for two steps); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.26 (d, *J* = 4.5 Hz, 2H), 6.43 (t, *J* = 4.8 Hz, 1H), 3.79 (t, *J* = 4.8 Hz, 4H), 3.33 (t, *J* = 6.9 Hz, 2H), 2.43 (m, 6H), 1.76 ppm (m, 2H).

5.1.2.2. 1-(3-Azidopropyl)-4-(2-methoxyphenyl)piperazine (6, Ar = 2-MeO-Ph, *m* = 1). Yield 70% (for two steps); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.95 (m, 3H), 6.86 (m, 1H), 3.86 (s, 3H), 3.36 (t, *J* = 6.9 Hz, 2H), 3.09 (br s, 4H), 2.65 (br s, 4H), 2.49 (t, *J* = 6.9 Hz, 2H), 1.81 ppm (m, 2H).

5.1.2.3. 3-(4-(3-Azidopropyl)piperazin-1-yl)benzo[*d*]isothiazole (6, Ar = 3-BIT, *m* = 1). Yield 73% (for two steps); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.68 (dd, *J* = 0.9, 8.1 Hz, 1H), 7.62 (dd, *J* = 1.2, 7.5 Hz, 1H), 7.55 (dt, *J* = 1.5, 7.5 Hz, 1H), 7.27 (m, 1H), 3.20 (t, *J* = 6.6 Hz, 2H), 3.09 (t, *J* = 4.8 Hz, 4H), 2.52 (t, *J* = 5.1 Hz, 4H), 2.41 (t, *J* = 6.9 Hz, 2H), 1.73 ppm (m, 2H).

5.1.2.4. 3-(3-Azidopropyl)-7,8-dimethoxy-1-o-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (10, R = 2′-Me, *n* = 1). Yield 71% (for two steps); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.22 (m, 4H), 6.68 (s, 1H), 5.93 (s, 1H), 4.46 (d, *J* = 9.0 Hz, 1H), 3.85 (s, 3H), 3.47 (s, 3H), 3.29 (m, 4H), 3.12 (dd, *J* = 6.3, 11.7 Hz, 1H), 2.77 (m, 2H), 2.62 (t, *J* = 6.6 Hz, 2H), 2.33 (t, *J* = 11.1 Hz, 1H), 2.17 (s, 3H), 1.78 ppm (m, 2H).

5.1.3. General procedure for Click reaction[28]

A mixture of alkyne (1.0 equiv), CuI (0.2 equiv), and azide (1.1 equiv) in THF (5 mL) was stirred for 5 min, and then *N,N*-diisopropylethylamine (DIPEA, 5.0 equiv) was added slowly, and the mixture was stirred at rt overnight. The solution was filtered and concentrated to give the crude material, which was chromatographed to afford the desired cyclization products **7**, **12,** and **16**.

5.1.3.1. 7,8-Dimethoxy-3-(3-(1-(3-(4-(pyrimidin-2-yl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)propyl)-1-o-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (7, R = 2′-Me, *n* = *m* = 1, Ar = 2-Pym). Yield 85%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.28 (d, *J* = 4.8 Hz, 2H), 7.21 (m, 5H), 6.66 (s, 1H), 6.46 (t, *J* = 4.8 Hz, 1H), 5.89 (s, 1H), 4.45 (d, *J* = 9.0 Hz, 1H), 4.38 (t, *J* = 6.9 Hz, 2H), 3.82 (m, 7H), 3.45 (s, 3H), 3.28 (m, 2H), 3. 13 (dd, *J* = 6.3, 12.3 Hz, 1H), 2.74 (m, 4H), 2.59 (t, *J* = 7.5 Hz, 2H), 2.44 (t, *J* = 4.8 Hz, 4H), 2.33 (m, 3H), 2.14 (s, 3H), 2.06 (m, 2H), 1.88 ppm (m, 2H).

5.1.3.2. 7,8-Dimethoxy-3-(3-(1-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)propyl)-1-*o*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (7, R = 2′-Me, *n* = *m* = 1, Ar = 2-MeO-Ph). Yield 82%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.21 (m, 4H), 6.93 (m, 4H), 6.67 (s, 1H), 5.90 (s, 1H), 4.46 (d, *J* = 9.3 Hz, 1H), 4.38 (t, *J* = 6.6 Hz, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.45 (s, 3H), 3.29 (m, 2H), 3.13 (m, 5H), 2.75 (m, 4H), 2.60 (m, 6H), 2.34 (m, 3H), 2.15 (s, 3H), 2.07 (m, 2H), 1.90 ppm (m, 2H).

5.1.3.3. 7,8-Dimethoxy-3-(3-(1-(3-(4-(pyrimidin-2-yl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)propyl)-1-*m*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (7, R = 3′-Me, *n* = *m* = 1, Ar = 2-Pym). Yield 88%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.28 (d, *J* = 4.8 Hz, 2H), 7.23 (m, 2H), 7.00 (m, 3H), 6.65 (s, 1H), 6.47 (t, *J* = 7.8 Hz, 1H), 6.24 (s, 1H), 4.83 (t, *J* = 6.9 Hz, 2H), 4.23 (d, *J* = 6.9 Hz, 1H), 3.82 (m, 7H), 3.60 (s, 3H), 2.86 (m, 6H), 2.45 (m, 10H), 2.31 (s, 3H), 2.07 (m, 2H), 1.88 ppm (m, 2H).

5.1.3.4. 7,8-Dimethoxy-3-(3-(1-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)propyl)-1-*m*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (7, R = 3′-Me, *n* = *m* = 1, Ar = 2-MeO-Ph). Yield 99%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.22 (m, 1H), 6.95 (m, 7H), 6.66 (s, 1H), 6.25 (s, 1H), 4.38 (t, *J* = 6.6 Hz, 2H), 4.25 (d, *J* = 7.5 Hz, 1H), 3.85 (s, 6H), 3.60 (s, 3H), 2.68 (m, 18H), 2.39 (t, *J* = 6.6 Hz, 2H), 2.32 (s, 3H), 2.05 (m, 2H), 1.90 ppm (m, 2H).

5.1.3.5. 3-(4-(3-(4-(3-(7,8-Dimethoxy-1-m-tolyl-4,5-dihydro-1*H*-benzo[*d*]azepin-(2*H*)-yl)propyl)-1*H*-1,2,3-triazol-1-yl)propyl)piperazin-1-yl)benzo[*d*]isothiazole (7, R = 3'-Me, *n* = *m* = 1, Ar = 3-BIT). Yield 84%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.67 (d, *J* = 7.5 Hz, 1H), 7.62 (d, *J* = 7.5 Hz, 1H), 7.55 (dt, *J* = 1.2, 7.5 Hz, 1H), 7.28 (m, 1H), 7.22 (m, 2H), 7.04 (d, *J* = 7.5 Hz, 1H), 6.97 (m, 2H), 6.66 (s, 1H), 6.24 (s, 1H), 4.33 (t, *J* = 6.9 Hz, 2H), 4.23 (d, *J* = 7.2 Hz, 1H),

3.85 (s, 3H), 3.60 (s, 3H), 3.03 (m, 7H), 2.83 (m, 2H), 2.69 (t, J = 7.2 Hz, 2H), 2.52 (m, 7H), 2.32 (m, 5H), 2.01 (m, 2H), 1.87 ppm (m, 2H).

5.1.3.6. 7,8-Dimethoxy-3-(2-(1-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)ethyl)-1-*m*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (7, R = 3′-Me, *n* = 0, *m* = 1, Ar = 2-MeO-Ph). Yield 96%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.20 (m, 1H), 7.13 (s, 1H), 6.96 (m, 6H), 6.85 (m, 1H), 6.67 (s, 1H), 6.33 (s, 1H), 4.31 (m, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.64 (s, 3H), 3.14 (m, 6H), 2.89 (m, 7H), 2.63 (m, 5H), 2.38 (t, J = 7.2 Hz, 2H), 2.27 (s, 3H), 2.04 ppm (m, 2H).

5.1.3.7. 7,8-Dimethoxy-3-(4-(1-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)butyl)-1-*m*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (7, R = 3′-Me, *n* = 2, *m* = 1, Ar = 2-MeO-Ph). Yield 91%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.27 (s, 1H), 7.22 (m, 1H), 6.95 (m, 7H), 6.66 (s, 1H), 6.24 (s, 1H), 4.39 (t, J = 7.2 Hz, 2H), 4.25 (d, J = 7.5 Hz, 1H), 3.85 (s, 6H), 3.60 (s, 3H), 6.90 (m, 10H), 2.63 (brs, 4H), 2.47 (m, 5H), 2.32 (s, 3H), 2.11 (m, 3H), 1.62 ppm (m, 4H).

5.1.3.8. 7,8-Dimethoxy-3-(3-(4-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-1-yl)propyl)-1-o-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (12, R = 2′-Me, *n* = *m* = 1, Ar = 2-MeO-Ph). Yield 97%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.21 (m, 5H), 6.93 (m, 4H), 6.68 (s, 1H), 5.95 (s, 1H), 4.39 (m, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.48 (s, 3H), 3.25 (m, 2H), 3.07 (m, 5H), 2.73 (m, 8H), 2.59 (m, 4H), 2.34 (t, J = 11.4 Hz, 1H), 2.18 (s, 3H), 2.07 (m, 2H), 1.90 ppm (m, 2H).

5.1.3.9. *N,N*-Diethyl-3-(1-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)propan-1-amine (16). Yield 90%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.30 (s, 1H), 6.90 (m, 4H), 4.39 (t, J = 6.9 Hz, 2H), 3.83 (s, 3H), 3.11 (br s, 4H), 2.70 (t, J = 7.5 Hz, 2H), 2.60 (br s, 4H), 2.50 (m, 6H), 2.38 (t, J = 6.9 Hz, 2H), 2.07 (m, 2H), 1.82 (m, 2H), 0.99 (t, J = 6.9 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.8, 147.4, 140.8, 122.6, 120.7, 120.6, 117.7, 110.8, 55.0, 54.4, 53.0, 51.8, 50.3, 47.6, 46.5, 27.1, 26.4, 23.3, 11.3 ppm; MS (EI) m/z 414 [M$^+$]; HRMS: m/z [M$^+$] calcd for $C_{23}H_{38}N_6O$: 414.3107, found: 414.3099.

5.1.4. General procedure for O-demethylation of compounds 7 and 12

A solution of **7** or **12** (1.0 equiv) in 9 mL dry CH_2Cl_2 was stirred at −78 °C under nitrogen for 30 min, then 1 M BBr_3 in CH_2Cl_2 (3.0 equiv) was added slowly. The mixture was stirred for additional 10 min at −78 °C and then at rt for 20 min. The reaction was quenched with MeOH at −78 °C for 1 h, and concentrated. The obtained residue was treated with MeOH again and concentrated. The crude material was purified with preparative TLC to give the final compounds **8** and **13**.

5.1.4.1. 3-(3-(1-(3-(4-(Pyrimidin-2-yl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl) propyl)-1-*o*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine-7,8-diol (8a). Yield 35%; ^{1}H NMR (300 MHz, CD_3OD): δ = 8.30 (d, J = 4.5 Hz, 2H), 7.82 (s, 1H), 7.20 (m, 4H), 6.66 (s, 1H), 6.58 (t, J = 4.8 Hz, 1H), 5.85 (s, 1H), 4.67 (dd, J = 3.6, 9.0 Hz, 1H), 4.43 (t, J = 6.6 Hz, 2H), 3.77 (m, 4H), 3.62 (m, 2 H), 3.34 (m, 1H), 3.24 (m, 1H), 3.04 (t, J = 7.8 Hz, 2H), 2.86 (dd, J = 6.0, 15.6 Hz, 1H), 2.75 (m, 3H), 2.47 (t, J = 5.1 Hz, 4H), 2.38 (t, J = 6.9 Hz, 2H), 2.14 (s, 3H), 2.03 ppm (m, 4H); ^{13}C NMR (100 MHz, CD_3OD): δ = 163.2, 159.6, 147.9, 145.4, 141.9, 137.9, 132.3, 128.9, 128.7, 128.2, 124.4, 118.5, 116.3, 111.8, 62.3, 59.8, 56.5, 54.4, 45.0, 43.0, 33.4, 28.5, 25.7, 24.1, 20.5, 20.1 ppm; MS (EI) m/z 582 [M$^+$]; Anal. Calcd for $C_{33}H_{42}N_8O_2{\cdot}1.2HBr{\cdot}1.0H_2O$: C, 56.80; H, 6.53; N, 16.06. Found: C, 57.25; H, 6.87; N, 15.45.

5.1.4.2. 3-(3-(1-(3-(4-(2-Methoxyphenyl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)propyl)-1-*o*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine-7,8-diol (8b). Yield 17%; ^{1}H NMR (300 MHz, $CD_{3OD\ +\ CDCl3}$): δ = 7.45 (s, 1H), 7.07 (m, 3H), 6.91 (m, 2H), 6.78 (m, 3H), 6.53 (s, 1H), 5.76 (s, 1H), 4.64 (d, J = 9.0 Hz, 1H), 4.27 (t, J = 6.9 Hz, 2H), 3.74 (s, 3H), 3.44 (m, 3H), 3.10 (m, 7H), 2.61 (m, 8H), 2.35 (t, J = 6.9 Hz, 2H), 2.00 ppm (m, 7H); ^{13}C NMR (100 MHz, CD_3OD + $CDCl_3$): δ = 152.0, 146.1, 142.7, 140.5, 136.2, 130.5, 126.9, 126.6, 126.1, 123.2, 121.7, 120.8, 118.0, 116.4, 114.4, 111.1, 60.4, 57.9, 55.0, 54.7, 54.6, 52.9, 50.0, 48.0, 41.3, 32.5, 26.7, 26.0, 24.2, 22.6, 19.1 ppm; MS (EI) m/z 610 [M$^+$]; Anal. Calcd for $C_{36}H_{46}N_6O_3{\cdot}1.5HBr{\cdot}0.5H_2O$: C, 58.34; H, 6.60; N, 11.34. Found: C, 58.60; H, 6.56; N, 11.04.

5.1.4.3. 3-(3-(1-(3-(4-(2-Hydroxyphenyl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)propyl)-1-*o*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine-7,8-diol (8c). Yield 32%; ^{1}H NMR (300 MHz, CD_3OD): δ = 7.86 (s, 1H), 7.18 (m, 4H), 6.92 (m, 2H), 6.82 (m, 2H), 6.68 (s, 1H), 5.86 (s, 1H), 4.71 (d, J = 9.3 Hz, 1H), 4.40 (t, J = 6.6 Hz, 2H), 3.62 (m, 2H), 3.40 (m, 1H), 3.28 (m, 1H), 3.01 (br s, 6H), 2.77 (m, 8H), 2.50 (t, J = 6.6 Hz, 2H), 2.12 ppm (m, 7H); ^{13}C NMR (100 MHz, CD_3OD): δ = 152.4, 147.9, 145.4, 145.3, 141.8, 140.8, 138.0, 134.9, 132.4, 131.8, 128.9, 128.8, 128.2, 126.2, 124.4, 121.6, 121.1, 118.5, 116.9, 116.3, 62.1, 59.7, 56.4, 56.3, 54.6, 51.9, 42.7, 33.3, 28.1, 25.6, 24.1, 20.1 ppm; MS (EI) m/z 596 [M$^+$]; Anal. Calcd for $C_{35}H_{44}N_6O_3{\cdot}2.25HBr$: C, 53.98; H, 5.99; N, 10.79. Found: C, 54.30; H, 6.00; N, 10.42.

5.1.4.4. 3-(3-(1-(3-(4-(Pyrimidin-2-yl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)propyl)-1-*m*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine-7,8-diol (8d). Yield 34%; ^{1}H NMR (300 MHz, CD_3OD): δ = 8.30 (d, J = 4.8 Hz, 2H), 7.77 (s, 1H), 7.23 (t, J = 7.5 Hz, 1H), 7.07 (d, J = 7.8 Hz, 1H), 6.97 (m, 2H), 6.59 (m, 2H), 6.09 (s, 1H), 4.42 (t, J = 6.9 Hz, 2H), 4.27 (d, J = 7.8 Hz, 1H), 3.77 (t, J = 4.8 Hz, 4H), 3.15 (m, 4H), 2.67 (m, 6H), 2.45 (t, J = 5.1 Hz, 4H), 2.35 (m, 5H), 2.09 (m, 2H), 1.95 ppm (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ = 163.3, 159.5, 148.4, 144.9, 144.8, 144.2, 140.1, 136.2, 132.6, 130.6, 130.3, 129.0, 127.0, 124.3, 118.6, 117.8, 111.8, 62.2, 59.7, 56.8, 56.6, 54.4, 48.9, 34.8, 28.6, 26.7, 24.4, 22.1 ppm; MS (EI) m/z 582 [M$^+$]; Anal. Calcd for $C_{33}H_{42}N_8O_2{\cdot}1.2HBr{\cdot}0.9H_2O$: C, 56.94; H, 6.52; N, 16.10. Found: C, 57.38; H, 6.51; N, 15.72.

5.1.4.5. 3-(3-(1-(3-(4-(2-Methoxyphenyl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)propyl)-1-*m*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine-7,8-diol (8e). Yield 29%; ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD): δ = 7.45 (s, 1H), 7.13 (t, J = 7.2 Hz, 1H), 6.88 (m, 7H), 6.55 (s, 1H), 6.00 (s, 1H), 4.38 (d, J = 8.4 Hz, 1H), 4.28 (t, J = 7.8 Hz, 2H), 3.76 (s, 3H), 3.55 (m, 2H), 3.36 (m, 1H), 3.10 (m, 6H), 2.80 (m, 2H), 2.65 (m, 7H), 2.38 (t, J = 7.5 Hz, 2H), 2.21 (s, 3H), 2.00 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 152.0, 146.1, 142.6, 141.3, 140.4, 138.3, 134.1, 130.2, 128.8, 128.5, 127.5, 125.1, 123.2, 121.8, 120.8, 118.1, 116.7, 115.8, 111.0, 61.0, 59.8, 57.7, 55.1, 54.6, 52.9, 49.9, 48.0, 45.7, 32.2, 29.4, 26.6, 24.2, 22.5, 21.1 ppm; MS (EI) m/z 610 [M$^+$]; Anal. Calcd for $C_{36}H_{46}N_6O_3{\cdot}2.3HBr{\cdot}0.2H_2O$: C, 54.02; H, 6.13; N, 10.50. Found: C, 54.36; H, 6.11; N, 10.07.

5.1.4.6. 3-(3-(1-(3-(4-(Benzo[*d*]isothiazol-3-yl)piperazin-1-yl)-propyl)-1*H*-1,2,3-triazol-4-yl)propyl)-1-*m*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine-7,8-diol (8f). Yield 28%; ^{1}H NMR (300 MHz, CD_3OD): δ = 7.97 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 8.4 Hz, 1H), 7.76 (s, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.40 (t, J = 7.5 Hz, 1H), 7.20 (t, J = 8.1 Hz, 1H), 7.04 (d, J = 7.8 Hz, 1H), 6.92 (m, 2H), 6.60 (s, 1H), 6.09 (s, 1H), 4.43 (t, J = 7.2 Hz, 2H), 4.26 (d, J = 8.4 Hz, 1H), 3.57 (m, 4H), 3.25 (m, 1H), 3.08 (m, 3H), 2.65 (m, 10H), 2.41

(t, J = 6.9 Hz, 2H), 2.29 (s, 3H), 2.10 (m, 2H), 1.94 ppm (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ = 165.8, 154.3, 148.7, 144.7, 139.9, 136.7, 133.1, 130.7, 130.2, 129.6, 129.5, 128.8, 127.0, 125.9, 125.7, 124.2, 122.2, 118.5, 117.8, 62.7, 62.5, 59.8, 56.9, 56.6, 54.4, 51.4, 49.1, 35.3, 28.6, 27.0, 24.5, 22.1 ppm; MS (EI) m/z 637 [M^+]; Anal. Calcd for $C_{36}H_{43}N_7O_2S{\cdot}1.3HBr{\cdot}1.0H_2O$: C, 56.67; H, 6.38; N, 12.85. Found: C, 56.77; H, 6.09; N, 12.60.

5.1.4.7. 3-(2-(1-(3-(4-(2-Methoxyphenyl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)ethyl)-1-*m*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine-7,8-diol (8g). Yield 42%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.13 (t, J = 7.5 Hz, 1H), 7.00 (m, 3H), 6.86 (m, 5H), 6.70 (s, 1H), 6.13 (s, 1H), 4.21 (m, 3H), 3.84 (s, 3H), 2.97 (m, 12H), 2.64 (m, 6H), 2.39 (m, 2H), 2.25 (s, 3H), 2.00 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.1, 145.5, 143.1, 142.9, 142.4, 140.6, 138.1, 134.9, 131.5, 129.1, 128.4, 127.1, 125.3, 123.2, 122.2, 120.9, 118.2, 116.8, 116.5, 111.2, 59.7, 57.3, 55.3, 54.7, 54.2, 53.1, 49.8, 48.2, 48.1, 33.6, 26.8, 22.9, 21.4 ppm; MS (EI) m/z 597 [M+H]$^+$; Anal. Calcd for $C_{35}H_{44}N_6O_3{\cdot}0.65HBr$: C, 64.74; H, 6.93; N, 12.94. Found: C, 64.94; H, 7.06; N, 12.58.

5.1.4.8. 3-(4-(1-(3-(4-(2-Methoxyphenyl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-4-yl)butyl)-1-*m*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine-7,8-diol (8h). Yield 67%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.32 (s, 1H), 7.12 (m, 1H), 6.98 (m, 2H), 6.84 (m, 5H), 6.66 (s, 1H), 6.00 (s, 1H), 4.42 (m, 1H), 4.30 (m, 2H), 3.82 (s, 3H), 3.17 (m, 7H), 2.62 (m, 10H), 2.38 (m, 2H), 2.23 (s, 3H), 2.04 (m, 2H), 1.61 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.1, 147.0, 143.0, 142.8, 141.9, 140.8, 138.3, 134.5, 130.5, 129.1, 128.6, 127.6, 125.3, 123.0, 121.4, 120.9, 118.1, 117.1, 116.1, 111.1, 60.2, 58.1, 55.3, 54.7, 53.1, 50.0, 48.0, 45.7, 32.4, 29.6, 26.9, 26.6, 24.7, 24.2, 21.4 ppm; MS (EI) m/z 624 [M^+]; Anal. Calcd for $C_{37}H_{48}N_6O_3{\cdot}0.45HBr{\cdot}2.0H_2O$: C, 63.73; H, 7.58; N, 12.05. Found: C, 64.11; H, 7.39; N, 11.36.

5.1.4.9. 3-(3-(4-(3-(4-(2-Methoxyphenyl)piperazin-1-yl)propyl)-1*H*-1,2,3-triazol-1-yl)propyl)-1-*o*-tolyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine-7,8-diol (13). Yield 36%; ^{1}H NMR (300 MHz, CD_3OD): δ = 8.00 (s, 1H), 7.22 (m, 4H), 7.05 (m, 1H), 6.94 (m, 3H), 6.70 (s, 1H), 5.86 (s, 1H), 4.84 (d, J = 9.9 Hz, 1H), 4.51 (t, J = 6.9 Hz, 2H), 3.84 (s, 3H), 3.74 (m, 2H), 3.47 (m, 3H), 3.34 (m, 3H), 3.21 (m, 8H), 2.92 (m, 2H), 2.81 (t, J = 7.5 Hz, 2H), 2.44 (m, 2H), 2.16 ppm (m, 5H); ^{13}C NMR (100 MHz, CD_3OD): δ = 154.3, 147.9, 145.3, 145.2, 141.6, 141.2, 137.9, 134.8, 132.3, 131.7, 129.0, 128.8, 128.2, 125.8, 124.7, 122.7, 120.3, 118.5, 116.3, 113.4, 62.2, 57.9, 57.3, 56.6, 56.3, 54.1, 49.7, 49.0, 42.6, 33.1, 26.7, 25.4, 23.9, 20.2 ppm; MS (EI) m/z 610 [M^+]; Anal. Calcd for $C_{36}H_{46}N_6O_3{\cdot}1.2TFA{\cdot}1.0H_2O$: C, 60.24; H, 6.48; N, 10.98. Found: C, 60.49; H, 6.69; N, 11.02.

5.2. Radioligand binding assays

The affinity of compounds to the D_1 and D_2 dopamine receptors, and the 5-HT_{1A} receptor was determined by competition binding assay. Membrane homogenates of 5-HT_{1A}-CHO cells, D_1- or D_2-HEK293 cells were prepared as described previously.[15,20] Duplicated tubes were incubated at 30 °C for 50 min with increasing concentrations (1 nM–100 μM) of the respective compound and with 0.7 nM [^{3}H]8-OH-DPAT (for 5-HT_{1A} receptor), [^{3}H]SCH23390 (for D_1 dopamine receptor), or [^{3}H]Spiperone (for dopamine D_2 receptor) in a final volume of 200 μL binding buffer containing 50 mM Tris, 4 mM $MgCl_2$, pH 7.4. Nonspecific binding was determined by parallel incubations with either 10 μM WAY100635 for 5-HT_{1A}, SCH23390 for D_1, or spiperone for D_2 dopamine receptors, respectively. The reaction was started by addition of membranes (15 μg/tube) and stopped by rapid filtration through Whatman GF/B glass fiber filter and subsequent washing with cold buffer (50 mM Tris, 5 mM EDTA, pH 7.4) using a Brandel 24-well cell harvester. Scintillation cocktail was added and the radioactivity was determined in a MicroBeta liquid scintillation counter. The IC_{50} and K_i values were calculated by nonlinear regression (PRISM, Graphpad, San Diego, CA) using a sigmoidal function.

5.3. [^{35}S]GTPγS binding

For detection of the agonism action of the compounds, the [^{35}S]GTPγS binding assay was performed at 30 °C for 30 min containing 10 μg membrane protein in a final volume of 100 μL with various concentrations of the drug. The antagonism effects of the compounds were tested in the presence of 10 μM SKF38393 for D_1 receptor or 10 μM quinpirole for D_2 receptor. The binding buffer contains 50 mM Tris, pH 7.5, 5 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl, 1 mM DTT, and 40 μM GDP. The reaction was initiated by adding [^{35}S]GTPγS (final concentration of 0.1 nM). Nonspecific binding was measured in the presence of 100 μM Gpp(NH)p. The reaction was terminated by the addition of 1 mL of ice-cold washing buffer (50 mM Tris, pH 7.5, 5 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl) and was rapidly filtered with GF/C glass fiber filters (Whatman) and washed three times. Radioactivity was determined by liquid scintillation counting.

Acknowledgments

This work was financially supported by grants from Chinese National Science Foundation (30772625, 30672517), Shanghai Commission of Science and Technology (07pj14104), and grant from Ministry of Science and Technology (2007AA022163). Support from Chinese Academy of Sciences, and Shanghai Institute of Materia Medica is also appreciated.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.06.019.

References and notes

1. Missale, C.; Nash, S. R.; Robinson, S. W.; Jaber, M.; Caron, M. G. *Physiol. Rev.* **1998**, *78*, 189.
2. Sidhu, A.; Niznik, H. B. *Int. J. Dev. Neurosci.* **2000**, *18*, 669.
3. Zhang, A.; Neumeyer, J. L.; Baldessarini, R. J. *Chem. Rev.* **2007**, *107*, 274.
4. Broekkamp, C. L. E.; Leysen, D.; Peeters, B. W. M. M.; Pinder, R. M. *J. Med. Chem.* **1995**, *38*, 4615.
5. Schechter, L. E.; Ring, R. H.; Beyer, C. E.; Hughes, Z. A.; Khawaja, X.; Malberg, E.; Rosenzweig-Lipson, S. *NeuroRx* **2005**, *2*, 590.
6. Alessandri, B.; Tsuchida, E.; Bullock, R. M. *Brain Res.* **1999**, *845*, 232.
7. Kamei, K.; Maeda, N.; Ogino, R.; Koyama, M.; Nakajima, M.; Tatsuoka, T.; Ohno, T.; Inoue, T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 595.
8. Newman-Tancredi, A.; Cussac, D.; Depoortere, R. *Curr. Opin. Invest. Drugs* **2007**, *8*, 539.
9. Wolf, W. A. *Curr. Opin. Invest. Drugs* **2003**, *4*, 878.
10. Bibbiani, F.; Oh, J. D.; Chase, T. N. *Neurology* **2001**, *57*, 1829.
11. Melamed, E.; Zoldan, J.; Friedberg, G.; Ziv, I.; Weizmann, A. *Adv. Neurol.* **1996**, *69*, 545.
12. Lucas, G.; Bonhomme, N.; De Deurwaerdere, P.; Le Moal, M.; Spampinato, U. *Psychopharmacology* **1997**, *131*, 57.
13. Nicholson, S. L.; Brotchie, J. M. *Eur. J. Neurol.* **2002**, *9*, 1.
14. Cavalli, A.; Bolognesi, A. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. *J. Med. Chem.* **2008**, *51*, 347.
15. Liu, Z.; Chen, X.; Sun, P.; Yu, L.; Zhen, X.; Zhang, A. *Bioorg. Med. Chem.* **2008**, *16*, 8335–8338.
16. Depoortère, R.; Barret-Grévoz, C.; Bardin, L.; Newman-Tancredi, A. *Eur. J. Pharmacol.* **2008**, *597*, 34.
17. de Almeida, J.; Palacios, J. M.; Mengod, G. *Prog. Brain Res.* **2008**, *172*, 101.
18. Mcintyre, J. A.; Castaner, J.; Bayes, M. *Drugs Future* **2006**, *31*, 314.
19. Zhang, J.; Xiong, B.; Zhen, X.; Zhang, A. *Med. Res. Rev.* **2009**, *29*, 272.
20. Zhang, J.; Chen, X.; Yu, L.; Zhen, X.; Zhang, A. *Bioorg. Med. Chem.* **2008**, *16*, 9425.
21. Liu, Z.; Zhang, J.; Zhang, A. *Curr. Pharm. Des.* **2009**, *15*, 682.

22. Neumeyer, J. L.; Kula, N. S.; Bergman, J.; Baldessarini, R. J. *Eur. J. Pharm.* **2003**, *474*, 137.
23. Caliendo, G.; Santagada, V.; Perissutti, E.; Fiorino, F. *Curr. Med. Chem.* **2005**, *12*, 1721.
24. Rasmussen, K.; Rocco, V. P. *Annu. Rep. Med. Chem.* **1995**, *30*, 1.
25. Lopez-Rodriguez, M. L.; Ayala, D.; Benhamu, B.; Morcillo, M. J.; Viso, A. *Curr. Med. Chem.* **2002**, *9*, 443.
26. Neumeyer, J. L.; Kula, N. S.; Baldessarini, R. J.; Baindur, N. *J. Med. Chem.* **1992**, *35*, 1466.
27. (a) Weinstock, J.; Hieble, J. P.; Wilson, J. W. *Drugs Future* **1985**, *10*, 645; (b) Weinstock, J.; Ladd, D. L.; Wilson, J. W.; Brush, C. K.; Yim, N. C.; Gallagher, G., Jr.; McCarthy, M. E.; Silvestri, J.; Sarau, H. M.; Flaim, K. E.; Ackerman, D. M.; Setler, P. E.; Tobia, A. J.; Hahn, R. A. *J. Med. Chem.* **1986**, *29*, 2315; (c) Shah, J. H.; Izenwasser, S.; Geter-Douglass, B.; Witkin, J. M.; Newman, A. H. *J. Med. Chem.* **1995**, *38*, 4284.
28. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *114*, 2708; . *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.