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Syntheses and optimization of new GS39783 analogues as positive allosteric modulators of $GABA_B$ receptors

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Abstract—The optimization of GS39783 into potent, selective, and safe positive allosteric modulators of $GABA_B$ receptors is presented.

The receptors for the major inhibitory neurotransmitter in the central nervous system, GABA, are subdivided into ionotropic $GABA_A$ and $GABA_C$ receptors and metabotropic $GABA_B$ receptors. Whereas $GABA_A$ and $GABA_C$ receptors form chloride-permeable ion channels, $GABA_B$ receptors are G-protein coupled receptors (GPCRs). These receptors were discovered in 1980 by Norman G. Bowery[1] and act post- and pre-synaptically to inhibit neuronal excitability and neurotransmitter release, respectively. A possible role of $GABA_B$ receptors in a large number of CNS disorders such as cognition deficits, anxiety, depression, epilepsy, pain, and drug addiction has been discussed.[2] Some of these diseases like anxiety, pain, and drug addiction could potentially be treated by activation of $GABA_B$ receptors, which can be achieved by administration of either agonists or positive allosteric modulators. Whereas benzodiazepines are well-known positive allosteric modulators of $GABA_A$ receptors, the first examples of allosteric enhancers for $GABA_B$ receptors have been described only recently.[3] One of the most interesting compounds found was GS39783 (Fig. 1).[3b] However, despite an interesting in vitro and in vivo profile,[3b,4] GS39783 was found to be genotoxic probably because of its aromatic nitro group (Fig. 1).[5]

This communication describes our efforts toward the identification of a novel, drug-like class of compounds acting as positive allosteric modulators for $GABA_B$ receptors.

In order to introduce molecular diversity in position 5 of the pyrimidine ring, a four steps procedure depicted in Scheme 1 was optimized to obtain compounds with a chlorine or with a hydrogen in position 6. 4,6-Dichloro-2-methylpyrimidine was first substituted by cyclopentylamine and then iodinated to lead to compound **6**. This scaffold was then used in a Suzuki cross coupling[6] to give very efficiently a small focused library of substituted 4-amino-6-chloro-5-phenylpyrimidines (compounds **7a**–**15a**) which were then hydrogenated under standard conditions to give the desired 4-amino-5-phenylpyrimidines (compounds **7b**–**15b**). As a means to introduce molecular diversity at the last step in position 4 of the pyrimidine ring, a versatile way of synthesis was designed (Scheme 2). Starting from the commercially

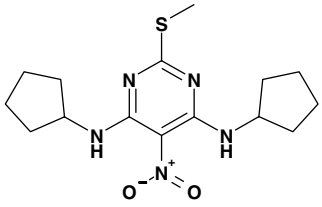

Figure 1. Structure of GS39783.

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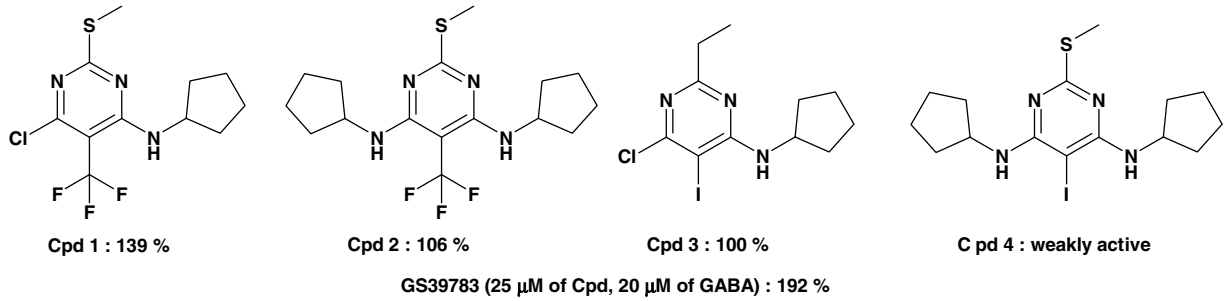
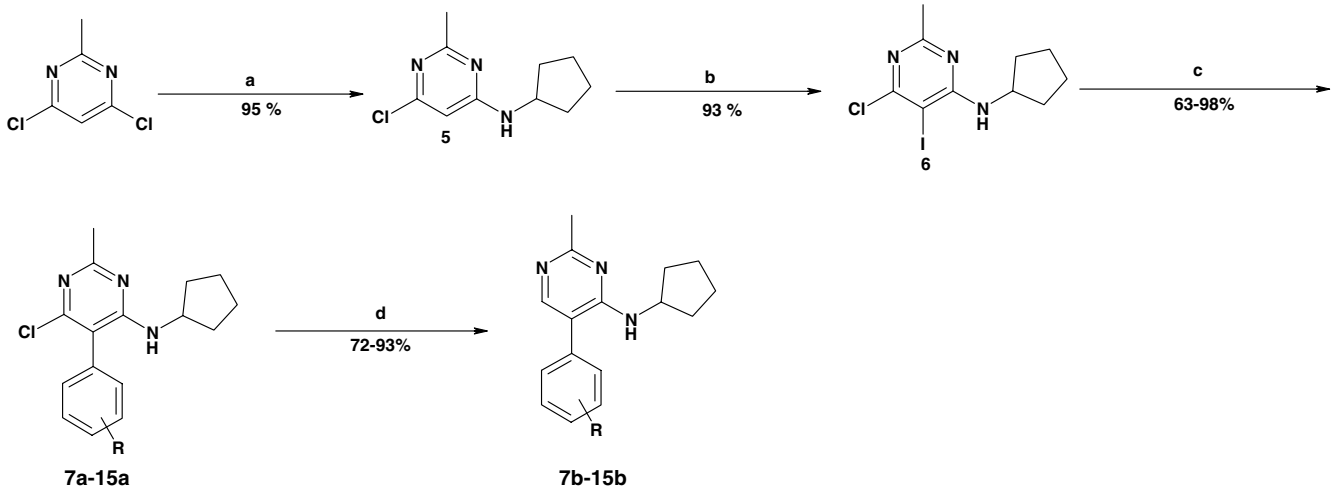
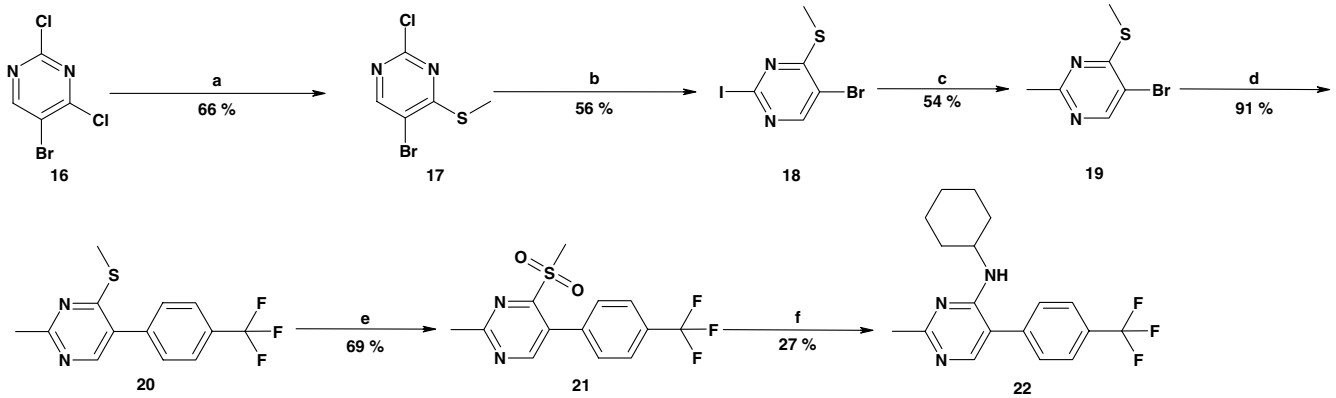


Figure 2. Biological activity of some GS39783 derivatives bearing nitro-mimetics in position 5. The potentiation of the GABA-induced stimulation of GTP(γ)^{35}S binding was measured using membranes from $GABA_{B(1b/2)}$ expressing CHO-K1 cells as described.[3a] The activities of compounds (25 µM) were determined in the presence of 20 µM GABA. The data are normalized to the maximal effect (100%) obtained with a saturating concentration of GABA (1 mM). Twenty micromolars GABA, when applied alone, stimulates to approximately EC_{80} levels (80%).

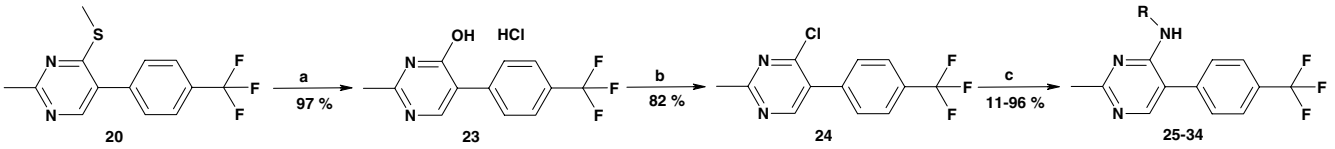
available 5-bromo-2,4-dichloropyrimidine, a regioselective nucleophilic substitution was performed in position 4 exclusively to give the 4-methylthio derivative **17**[7] which was then involved in a halogen exchange[8] to give 2-iododerivative **18**. The 2-methyl group and the 5-[(4-trifluoromethyl)phenyl] substituents were introduced by a Negishi cross coupling[9] and a Suzuki cross coupling,[10] respectively, to afford **20**. Our first attempt was to oxidize the methylsulfide moiety of **20** to the corresponding methylsulfonyl (**21**) by treatment with



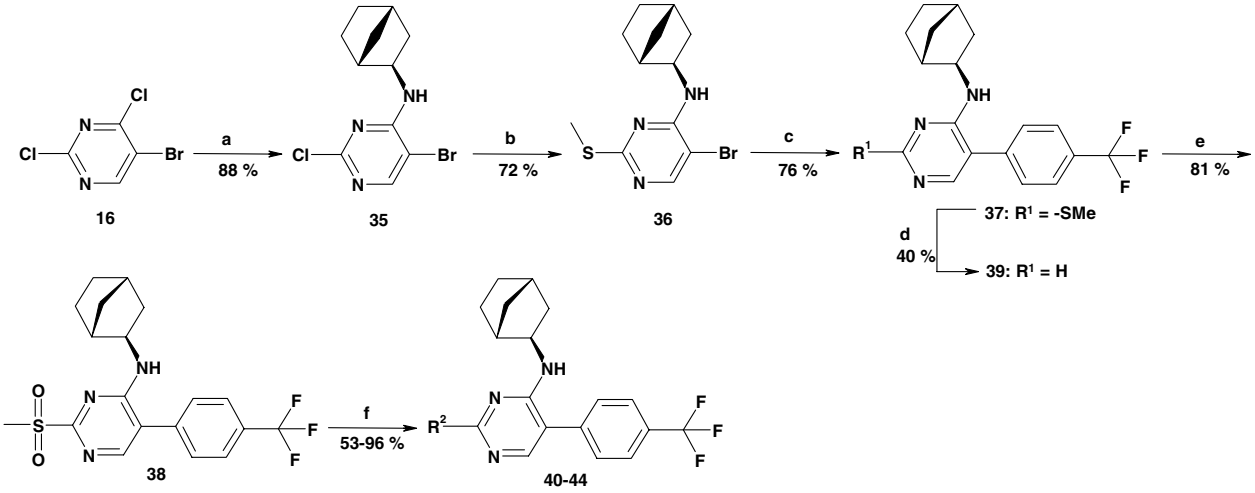
Scheme 1. Reagents and conditions: (a) cyclopentylamine (4.0 equiv), dioxane, 0 °C–rt, 24 h; (b) NIS, DMF, 80 °C, 24 h; (c) arylboronic acid (1.05 equiv), $Pd(OAc)_2$ (0.02 equiv), dppf (0.03 equiv), K_3PO_4 (2.0 equiv), DME, water, 85 °C; (d) Pd/C 10%, AcONa (1.1 equiv), EtOH, H_2, rt.



Scheme 2. Reagents and conditions: (a) MeSNa, THF, rt; (b) HI 57%, rt; (c) MeZnCl, $Pd(PPh_3)_4$, THF, rt–60 °C; (d) 4-trifluoromethylbenzeneboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, EtOH, toluene, water, 110 °C; (e) *m*-CPBA, DCM, rt; (f) cyclohexylamine, DMF, 150 °C, microwaves, 20 min.



Scheme 3. Reagents and conditions: (a) HCl 37%, MeOH, reflux; (b) $POCl_3$, one drop of DMF, 80 °C; (c) R–NH_2, solvent, base, 80 °C.



Scheme 4. Reagents and conditions: (a) *exo*-2-aminonorbornane, THF, 0 °C–rt; (b) MeSNa, THF, 0 °C–rt; (c) 4-trifluoromethylbenzeneboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, EtOH, water, 110 °C; (d) Ni-Raney, EtOH, 60 °C; (e) *m*-CPBA, DCM, rt, 1.5 h; (f) compound **40**: KCN, DMSO, 80 °C. Compound **41**: sodium methoxide, THF, rt. Compound **42**: methylamine, EtOH, rt. Compound **43**: dimethylamine, THF, 80 °C. Compound **44**: *N*-methylpiperazine, THF, 80 °C.

m-CPBA[11] and then to use **21** as a template. Unfortunately, only few amines reacted cleanly with **21** probably because of the steric hindrance near the leaving group. Our second attempt was to hydrolyze the methylsulfide moiety of **20** with hydrochloric acid[12] to give **23** (Scheme 3). This compound was then reacted with $POCl_3$ to give the corresponding 4-chloropyrimidine **24**. This useful intermediate was submitted to nucleophilic substitutions with a collection of amines to give compounds **25**–**34**. Finally, the third library with molecular diversity in position 2 was synthesized starting from compound **16**, which was substituted first with *exo*-2-aminonorbornane[13] and then with MeSNa to give **36** (Scheme 4). The phenyl moiety was introduced by a Suzuki cross coupling to afford **37**. On one hand, a desulfurization by treatment with Ni-Raney in EtOH[14] leads to **39** and, on the other hand, **37** can also be oxidized into the corresponding 2-methylsulfonylpyrimidine **38** which was then reacted with various nucleophiles.

Thanks to a preliminary work with nitro-mimetics (Fig. 2), we found that to replace the nitro group, we should have a lipophilic substituent (see compound **3**) with an electron-withdrawing effect (see compounds **1** and **2**). One way to combine both of these effects is to substitute the position 5 of the pyrimidine ring by a substituted phenyl. Moreover, substantial efficacy in the biological assay[15] was observed for compounds with only one cyclopentylamine substituent in position 4 of the pyrimidine ring (compare **1** with **2** and **3** with **4**). Furthermore, it was also shown earlier that the replacement of the 2-methylthio substituent by a 2-methyl group is not detrimental for the activity.[3b] After screening for $GABA_B$ receptor positive modulatory activity of the first library of compounds (Table 1), we found that the 6-chloro substituent was detrimental to the efficacy at the receptor (compare, for example, **8a** with **8b** at 25 μM of compound, **12a** with **12b** and **15a** with **15b**). Moreover, the introduction of a second cyclopentylamino substituent in position 6 led to only weakly active or inactive compounds (data not shown), confirming our hypothesis that the space in the receptor is very limited and that a proton is the best substituent for the position 6 of the pyrimidine ring. On the other hand, as postulated before, electron-withdrawing groups on the phenyl ring such as a 4-trifluoromethyl or a 4-trifluoromethoxy showed the best results (compare **9b** with **12b** and **10b** with **15b**). Indeed, the replacement of a 4-methylphenyl (**9b**) or 4-methoxyphenyl (**10b**) by a 4-trifluoromethylphenyl (**12b**) or a 4-trifluoromethoxyphenyl (**15b**) led to more efficacious positive modulators (Table 1). Other electron-withdrawing substituents were used on the phenyl ring but none of them had increased activity at the receptor compared to the trifluoromethyl or trifluoromethoxy groups (data not shown). The introduction of a substituent in position 3 of the phenyl ring led to a decrease of activity (compare **14b** with **15b**). To conclude, the

Table 1. Biological activities obtained for compounds **7a**–**15b**

Compound	R	2.5 μM of compound, 1 μM of GABA Effect (%) ± SEM	25 μM of compound, 1 μM of GABA Effect (%) ± SEM	1 μM of GABA	
				pEC_{50} ± SEM	E_{max} (%) ± SEM
GS39783	—	132 ± 4	153 ± 2	6.13 ± 0.06	146 ± 5
7a	4-^{n}Butyl	27 ± 1	39 ± 1	—	—
7b		25 ± 1	18 ± 3	—	—
8a	4-Ethyl	23 ± 2	39 ± 2	—	—
8b		29 ± 1	61 ± 1	—	—
9a	4-Methyl	23 ± 2	34 ± 1	—	—
9b		23 ± 2	41 ± 2	—	—
10a	4-Methoxy	21 ± 1	29 ± 1	—	—
10b		21 ± 1	38 ± 2	—	—
11a	3-Methyl	25 ± 1	36 ± 1	—	—
11b		21 ± 0	28 ± 2	—	—
12a	4-CF_3	17 ± 1	26 ± 1	—	—
12b		62 ± 3	114 ± 2	5.30 ± 0.03	127 ± 3
13a	4-COOEt	14 ± 1	5 ± 3	—	—
13b		26 ± 0	31 ± 0	—	—
14a	3-OCF_3	20 ± 1	33 ± 1	—	—
14b		23 ± 1	25 ± 1	—	—
15a	4-OCF_3	19 ± 1	35 ± 1	—	—
15b		48 ± 3	100 ± 3	5.34 ± 0.07	117 ± 7

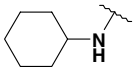
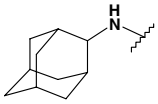
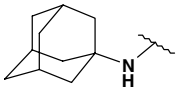
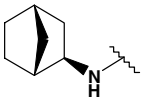
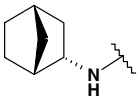
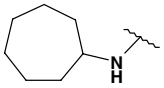
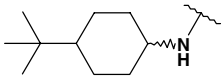
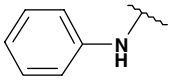
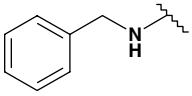
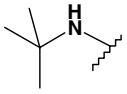
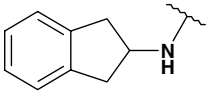
The compounds were assayed as described.[3,15] When applied alone, 1 μM GABA stimulates to approximately EC_{20} levels (20% stimulation). The effects (%) are normalized to the maximal effect of a saturating concentration of GABA (1 mM). Values were calculated from triplicate measurements. pEC_{50} and E_{max} values were calculated from concentration–response curves (eight concentrations) in the presence of 1 μM of GABA.

best nitro-mimetic group identified in this series was a 4-(trifluoromethyl)phenyl substituent. Then we focused our attention on position 4 of the pyrimidine ring (Schemes 2 and 3).

This collection of compounds shows interesting structure–activity relationships (Table 2). For this series it became obvious that an increased size of the cycloalkyl led to increased efficacy. This trend was first observed with compound **29** in which the cyclopentyl substituent was replaced by a cycloheptyl (compare **12b** and **22** with **29** at 2.5 μM). Interestingly, the introduction of an additional bulky substituent on the cyclohexyl ring (**30**) led to a less active product (compare **30** with **22**). Moreover, the introduction of an aromatic ring onto the amino group was not tolerated by the receptor (compare **31**, **32**, **34** with **22**); therefore, we thought that a hydrophobic interaction with a spatially extended substituent was necessary at this position. Surprisingly, despite its 4-*tert*-butylamino substituent, **33** was found to be a weak positive modulator. The needs of bulky, cyclic aliphatic side chain was then confirmed when cycloalkyl chains was used such as a norbornyl (**27**), an adamantyl (**25** and **26**), or a cycloheptyl (**29**). A substantial increase in activity was observed when comparing **29** to **12b** and to **22**. Despite its good efficacy, **29** was not investigated any further because of significant binding activities to other GPCRs (data not shown). We were then interested in compounds **25** and **26** bearing an adamantyl substituent on the amino group. Both compounds were potent positive allosteric modulators (Table 2) which led to the hypothesis that the substituents in position 4 of the pyrimidine ring bind into a large lipophilic pocket in the receptor. However, again these products were not considered for further evaluation despite an increased selectivity profile because of their high log *P*s ($\log P > 5.9$) and low water solubilities (<10 mg L^{-1}). Finally, we focused our attention on **27** which bears a norbornyl group at its amino function. Both the *exo* isomer (**27**) and the *endo* isomer (**28**) were evaluated (Table 2). Compound **27** was more efficacious, and was of similar potency and efficacy compared to GS39783. Genotoxicity[16] and mutagenicity[17] assays were performed and **27** was found to be safe both in the micronucleus test and in the Ames test. For that reason **27** was considered for further in vitro and in vivo evaluations and the *exo*-2-norbornyl substituent was kept for the optimization of the position 2.

Finally, the screening of the collection of compounds with molecular diversity in position 2 gave surprising results (Table 3). The replacement of the methyl moiety of **27** by a hydrogen led to a less active compound which indicated that a substitution at this position is mandatory. The introduction of a 2-SMe group (**37**), a methylsulfonyl (**38**), or a methylamino (**42**) was detrimental for activity (compare **37**, **38** and **42** with **27**). In contrast, the replacement of a 2-methyl group by a cyano (**40**), a methoxy (**41**), or a dimethylamino (**43**) gave compounds with a similar potency than GS39783. Surprisingly, the introduction of a *N*-methylpiperazin-1-yl (**44**) reduced the biological activity suggesting that the space in the receptor is limited and that only small substituents are tolerated. Compounds **40**, **41**, and **43** are currently under evaluation for their physicochemical and pharmacokinetics properties as well as their full pharmacological characterization.

Table 2. Biological activities obtained for compounds **22**, **25**–**34**

Compound	R	2.5 μM of compound, 1 μM of GABA Effect (%) ± SEM	25 μM of compound, 1 μM of GABA Effect (%) ± SEM	1 μM of GABA	
				pEC_{50} ± SEM	E_{max} (%) ± SEM
22		80 ± 1	93 ± 5	6.06 ± 0.05	83 ± 4
25		90 ± 1	80 ± 2	5.56 ± 0.13	132 ± 14
26		125 ± 4	141 ± 6	5.46 ± 0.10	185 ± 15
27		122 ± 3	110 ± 0	5.78 ± 0.03	183 ± 4
28		82 ± 5	52 ± 4	—	—
29		128 ± 4	70 ± 4	5.78 ± 0.03	137 ± 3
30		28 ± 1	32 ± 2	—	—
31		36 ± 2	69 ± 6	—	—
32		29 ± 1	39 ± 2	—	—
33		40 ± 3	21 ± 7	—	—
34		58 ± 11	59 ± 4	—	—

The compounds were assayed as described in Table 1. Compounds **25**–**27**, **29**, and **34** have a relatively low water solubility due to their high lipophilicity. This is reflected in this assay by a similar or reduced activity at 25 μM versus 2.5 μM. Concentration-dependent increase in activity, however, was observed in full concentration–response curves, with maximal effects at 10 μM.

Positive allosteric modulators of $GABA_B$ receptors represent an interesting class of therapeutic agents for the treatment of anxiety and drug addiction. Despite its good in vitro and in vivo potency, GS39783 was only useful as a pharmacological tool because of its genotoxicity. We report herein a new class of positive allosteric modulators of $GABA_B$ receptors derived from GS39783 with an increased drug-likeness, a decreased toxicity, and an excellent selectivity profile. Some in vivo investigations are currently ongoing in anxiety and drug addiction models and the results of these studies will be reported in due course.

Table 3. Biological activities obtained for compounds **37**–**44**

Compound	R	2.5 μM of compound, 1 μM of GABA Effect (%) ± SEM	25 μM of compound, 1μM of GABA Effect (%) ± SEM	1 μM of GABA	
				pEC_{50} (μM) ± SEM	E_{max} (%) ± SEM
37	–SMe	82 ± 7	91 ± 4	—	—
38	Me–SO_2–	24 ± 7	30 ± 12	—	—
39	H	48 ± 3	63 ± 4	—	—
40	–CN	117 ± 3	127 ± 1	5.92 ± 0.11	198 ± 21
41	–OMe	115 ± 2	131 ± 6	5.69 ± 0.13	191 ± 21
42	–NHMe	20 ± 3	25 ± 8	—	—
43	–NMe_2	94 ± 4	126 ± 7	5.78 ± 0.08	167 ± 16
44	*N*-Methylpiperazine	26 ± 8	112 ± 7	—	—

The compounds were assayed as described in Table 1.

Acknowledgment

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