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Synthesis of Dihydrofuroaporphine Derivatives: Identification of a Potent and Selective Serotonin 5-HT$_{1A}$ Receptor Agonist

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A series of new aporphine analogues were synthesized and pharmacologically evaluated. 11-Allyloxy-(**17**), 11-propargyloxy-(**20**), and dihydrofuro-(**19**) aporphines displayed the highest affinity at the 5-HT$_{1A}$ receptor with K_i values of 12.0, 14.0, and 6.7 nM, respectively. The high binding potential of the diastereomeric mixture of aporphine **19** was found residing in the *cis*-diastereomer (*cis*-**19**). [^{35}S]GTPγS function assays on 5-HT$_{1A}$ receptor indicated that aporphines **17** and **20** were partial agonists, while *trans*-**19** behaved as a high efficacy full antagonist and *cis*-**19** was a full agonist. The agonistic property of *cis*-**19** at the 5-HT$_{1A}$ receptor was further confirmed in vitro and in vivo. This compound may be useful as a potential treatment for anxiety.

Introduction

The serotonin 5-HT$_{1A}$ receptor is one of the most studied subtypes among the large family of serotonin receptors. It was first discovered in 1981 and then successfully cloned and sequenced at the end of the same century.[1,2] Accumulating evidence has indicated that alteration of 5-HT$_{1A}$ receptor function is associated with a number of neuropsychological disorders, including anxiety and depression,[3,4] pain,[5] neuroprotection,[6,7] schizophrenia,[8,9] Parkinson's disease,[10−12] and Alzheimer disease.[13] During the past two decades, hundreds of compounds have been reported with agonistic or antagonistic activities at the 5-HT$_{1A}$ receptor.[14,15] Among these compounds, aminotetralins and arylpiperazines are the major structural skeletons for binding to this receptor and are represented by the well demonstrated and widely used agonistic tool drug, 8-hydroxy-*N*,*N*-dipropylaminotetralin (8-OH-DPAT[a], **1**)[16] and antagonist, (*S*)-*N*-*tert*-butyl-3-(4-(2-methoxyphenyl) piperazin-1-yl)-2-phenyl propanamide (WAY-100635, **2**),[17] respectively (Figure 1). Recently, several compounds originated from these two structural classes or others (e.g., indolylalkylamines, ergolines) have been approached to clinical trials, but most of them still suffer from either poor selectivity over other neurotransmitter receptors or unpromising pharmacokinetic profiles.[15,18] Therefore, new compounds with high potency and selectivity at the 5-HT$_{1A}$ receptor are still needed either as probes to explore the molecular mechanisms in which the 5-HT$_{1A}$ receptor is involved or as leads for the development of therapeutic drugs.

Aporphine analogues, represented by the antiparkinsonian drug, *R*-(−)-apomorphine (**3**, Figure 1), belong to one of the earliest class of dopamine (DA) D$_2$ receptor agonists.[19,20] The catecholic hydroxyl groups, especially the C11-hydroxy, the C6a-*R* configuration, as well as the *N*-substituent are the crucial pharmacophoric portions for interactions with the D$_2$ receptor. Meanwhile, a number of research groups including ours reported that this tetracyclic scaffold can also be employed to pursue serotonin 5-HT$_{1A}$ receptor agents with appropriate structural modifications.[20−26] For example, we have reported that 11-hydroxy-*N*-propylnoraporphine (**4a**) and its *n*-butyryl ester (**5**) exhibited potent dual binding affinity at both D$_2$ and 5-HT$_{1A}$ receptors with slight preference for the latter receptor.[21] In the early 1990s, Cannon et al.[22,23] reported that replacing the 10-hydroxyl moiety in compound **3** with a methyl group resulted in compound **6** possessing high affinity for the 5-HT$_{1A}$ receptor and almost complete loss of affinity for the D$_2$ receptor. Since then, a C10-"methyl pocket" was proposed as the 5-HT$_{1A}$ receptor binding site in aporphine analogues. Hedberg and co-workers[24−26] further explored this hypothesis and found that other

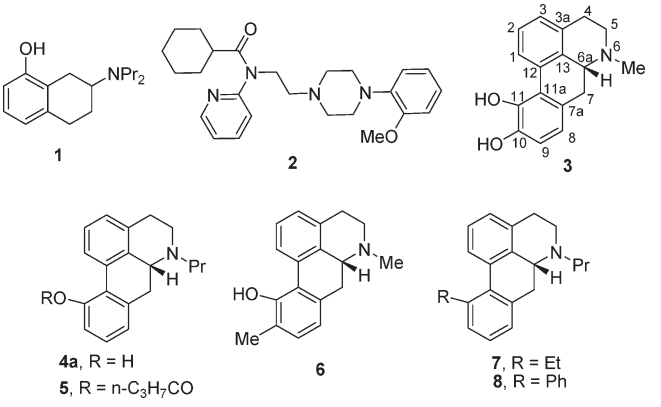

Figure 1. Representative compounds for 5-HT1$_A$ or D$_2$ receptors.

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[a] Abbreviations: DA, dopamine; VTA, ventral tegmental area; EPM, elevated plus maze; 8-OH-DPAT, 8-hydroxy-*N*,*N*-dipropylaminotetralin; 5-HT$_{1A}$, serotonin-1A; Apomorphine, (6a*R*)-5,6,6a,7-tetrahydro-6-methyl-4*H*-dibenzo[*de*,*g*] quinolin-10,11-diol; raclopride, 3,5-dichloro-*N*-{[(2*S*)-1-ethylpyrrolidin-2-yl]methyl}-2-hydroxy-6-methoxybenzamide; GTPγS, guanosine 5′-*O*-[γ-thio]triphosphate.

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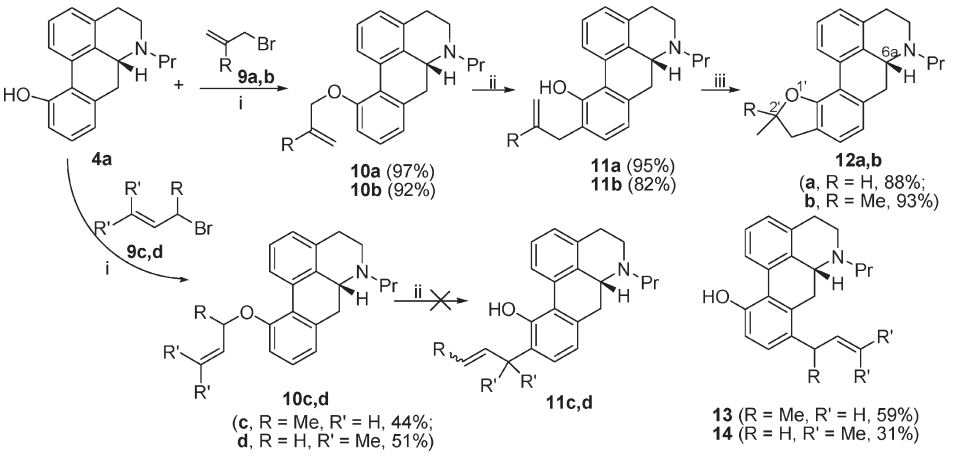
Scheme 1[a]



[a] Reagents and conditions: (i) K_2CO_3, KI, DMF, 0 °C−rt; (ii) $PhNEt_2$, reflux; (iii) HBr, HOAc, reflux.

11-hydroxy-10-alkyl substitution patterns were also tolerated. Furthermore, it was found that without any hydroxyl group at either C10 or C11, a single alkyl group at C11 was sufficient for good binding and selectivity for the 5-HT_{1A} receptor, exemplified by compounds **7**[24] and **8**[24] with K_i values of 4.5 and 1.8 nM, respectively. These results indicated that a relatively larger lipophilic pocket might exist in the 5-HT_{1A} receptor binding site for interaction with C10 and/or C11 substituted aporphines. To find more potent and selective 5-HT_{1A} ligands, and in view of the likely better pharmacokinetic profile of these aporphines which lack the oxidation-susceptible phenolic hydroxyl group, we decided to synthesize a series of novel aporphines by allylation or propargylation of 11-hydroxy-*N*-alkylnoraporphines, followed by Cope rearrangement and cyclization. Herein, we report the synthesis of these novel aporphine compounds and pharmacological evaluation at dopamine D_1, D_2, and serotonin 5-HT_{1A} receptors.

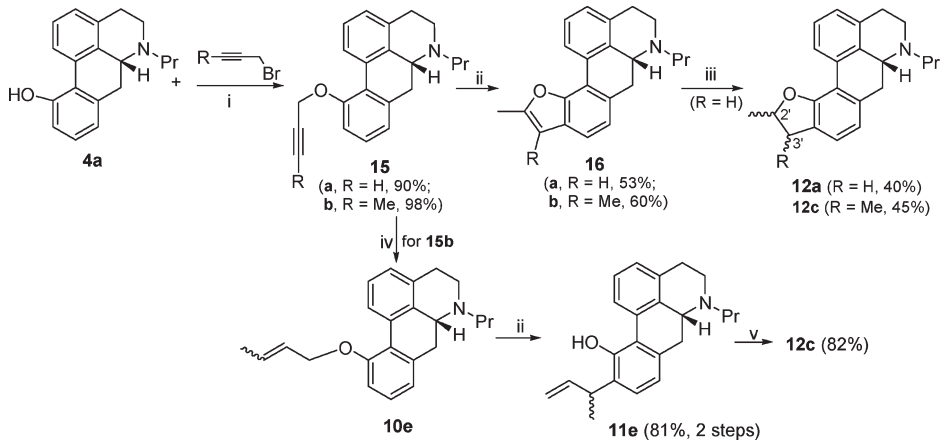
Results

Synthesis. First, *R*-(−)-11-hydroxy-*N*-propylnoraporphine **4a** was prepared from morphine using a procedure we reported previously.[21,27,28] Alkylation of **4a** with substituted or nonsubstituted allyl bromides **9a**, **9b**, **9c** (racemic), and **9d** gave 11-allyloxy-*N*-propyl-noraporphines **10a**,**b** in 97% and 92% yields and **10c**,**d** in 44% and 51% yields, respectively (Scheme 1). The reaction of the more sterically hindered allyl bromides **9c**,**d** with aporphine **4a** occurred very sluggishly, affording the products in lower yields. Cope rearrangement[29−31] of ethers **10a**−**d** was conducted in $PhNEt_2$ under reflux (200 °C). Allyl ether **10a** and 2-methylallyl ether **10b** were converted to the corresponding 11-hydroxy-10-allylaporphines **11a** and **11b** smoothly in 95% and 82% yields, respectively. However, methylallyl- and dimethylallyl-ethers **10c** and **10d** did not yield the expected 11-hydroxy-10-allyl-aporphines **11c** and **11d**, instead double rearranged products **13** (mixture of two diastereomers) and **14** were obtained in 59% and 31% yields, respectively, together with a large amount of *N*-quarternized salts. Cyclization[32] of 11-hydroxy-10-allyl-aporphines **11a**,**b** was facilitated in refluxing HOAc/HBr, yielding the corresponding dihydrofuroaporphines **12a** and **12b** in 88% and 93% yields, respectively. Although some stereoselectivity induced by the C6a-*R* configuration for C2′ in the 2′,3′-dihydrofuryl portion of compound **12a** was expected, this compound turned out to be a diastereomeric mixture of cis (related to the H-6a) and trans isomers, evidenced by the existence of two sets of signals for both the 2′-H and 2′-Me with ratios of 1:1, respectively, in the ^1H NMR spectra.

Similarly, phenol **4a** was treated with substituted or nonsubstituted propargyl bromides and K_2CO_3/KI, affording 11-propargyloxyaporphines **15a** and **15b** in 90% and 98% yields, respectively (Scheme 2). Cyclization[33] was succeeded by reacting aporphines **15a**,**b** with $PhNEt_2$ under reflux yielding furoaporphines **16a** and **16b** in moderate yields (53% and 60% yield, respectively). Hydrogenation[34] of these two furans with Pd/C under H_2 atmosphere went through very sluggishly, and the corresponding dihydrofuroaporphines **12a** and **12c** were obtained as diastereomeric mixtures after two days in 40% and 45% yields, respectively. Alternatively, the propargyl group of aporphine **15b** was hydrogenated to **10e** (*E*/*Z*) with Lindar's catalyst (Pd/$BaSO_4$)/quinoline/H_2, following Cope rearrangement provided 11-hydroxy-10-allyl-(2-butenyl)-*N*-propyl-noraporphine **11e** in 81% overall yield. Treating aporphine **11e** with HOAc/HBr under reflux yielded dihydrofuroaporphine **12c** in 82% yield. Similar to compound **12a**, compound **12c** was also an inseparable mixture of all diastereomers.

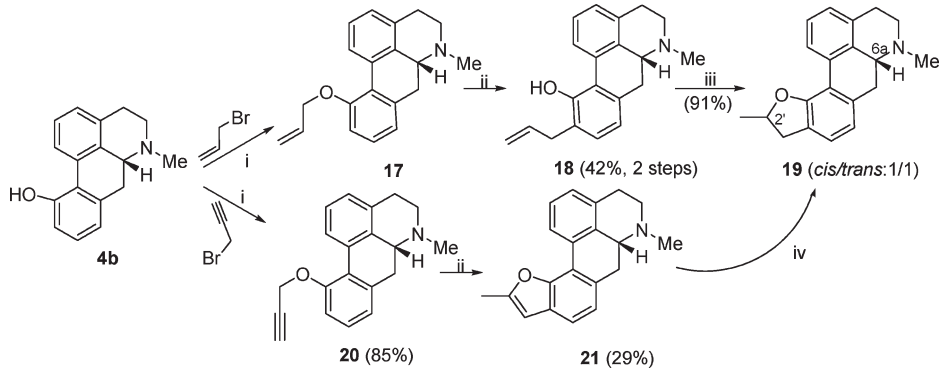
The *N*-substituent in aporphine analogues is one of the determinants for the DA (D_1, D_2) receptor binding and selectivity,[20] however, it is not clear if this is also the case for the 5-HT_{1A} receptor, especially in the new synthetic aporphines. To this end, *N*-methyl analogues **17**−**21** were prepared (Scheme 3). First, *R*-(−)-11-hydroxy- aporphine **4b**[28,35] was prepared using a previously reported procedure from morphine. Treating **4b** with allyl bromide under K_2CO_3/KI followed by Cope rearrangement in refluxing $PhNEt_2$ yielded 11-hydroxy-10-allyl-aporphine (**18**) in 42% overall yield. Cyclization of **18** with HBr/HOAc under reflux afforded a diastereomeric mixture of dihydrofuroaporphine **19** (*cis*/*trans*-**19**) in 91% yield. Similarly, treating phenol **4b** with propargyl bromide followed by cyclization offered furoaporphine **21** in 25% overall yield. The low yield was caused by the sluggish cyclization process (29%, single step). Aporphine **21** could be also hydrogenated to the diastereomeric mixture of dihydrofuroaporphine **19** under Pd/C and H_2 atmosphere, but the process was very slow probably due to the difficulty in breaking the aromatic system of benzofuran component in aporphine **21**. Again, both the 2′-H and 2′-Me in the dihydrofuran portion of aporphine **19** displayed

Scheme 2[a]



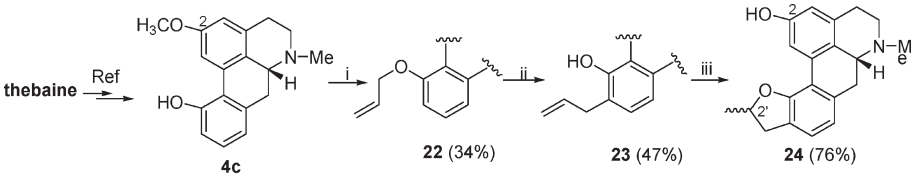
[a] Reagents and conditions: (i) K_2CO_3, KI, DMF, 0 °C−rt; (ii) $PhNEt_2$, reflux; (iii) H_2, Pd/C; (iv) Lindar catalyst, quinoline, H_2, EtOH; (v) HOAC, HBr, reflux.

Scheme 3[a]



[a] Reagents and conditions: (i) K_2CO_3, KI, DMF, 0 °C−rt; (ii) $PhNEt_2$, reflux; (iii) HBr, HOAc, reflux; (iv) H_2, Pd/C.

Scheme 4[a]



[a] Reagents and conditions: (i) K_2CO_3, KI, DMF, 0 °C−rt; (ii) $PhNEt_2$, reflux; (iii) HBr, HOAc, reflux.

two sets of signals with ratios of 1:1 in the 300 MHz ^{1}H NMR spectra, indicating compound **19** is a mixture of *cis* and *trans* diastereomers (*cis*/*trans*-**19**).

During the investigation of aporphine analogues as DA receptor ligands,[20] a C2-methoxy or hydroxy substituent in the aporphine skeleton is generally beneficial to the interaction between these compounds and DA receptor targets. To evaluate the influence of such C2-methoxy/hydroxy substituent on the interaction of the newly synthesized aporphines with the 5-HT_{1A} receptor, 2-methoxy/hydroxy-aporphines **22**−**24** were prepared (Scheme 4). First, 2-methoxy-11-hydroxyaporphine **4c**[35] was prepared from thebaine according to a reported procedure. Allylation of **4c** provided ether **22** in 34% yield. Cope rearrangement of ether **22** in $PhNEt_2$ yielded phenol **23** in 47% yield. Cyclization of phenol **23** in acidic condition provided 2-hydroxy-dihydrofuroaporphine **24** as a diastereomeric mixture in 76% yield.

Compounds **10c**, **10e**, **11e**, **12a**, **12c**, **13**, **19**, and **24** are all mixtures of corresponding cis and trans diastereomers and are inseparable through silica gel chromatography or regular HPLC analysis. Fortunately, an exhaustive investigation revealed that aporphine **19** was separable on chiral OD column with hexane/isopropanol/diethylamine as the mobile phase. Therefore, an analytical amount of each isomer (faster-moving vs slower-moving) was obtained in optically pure (>99% *de*, see Supporting Information) with optical rotation ($[\alpha]^{20}_D$) of −30° (*c* 0.071, EtOH) for the faster-moving isomer and −109° (*c* 0.075, EtOH) for the slower-moving isomer. Further, an X-ray crystal of the slower-moving isomer was obtained by careful recrystallization from $CHCl_3$ and its absolute configuration was ascertained to be *cis*-**19** (6a*R*,2′*R*) (Figure 2). Therefore, the faster-moving isomer was deduced as *trans*-**19** with 6a*R*,2′*S* -configuration.

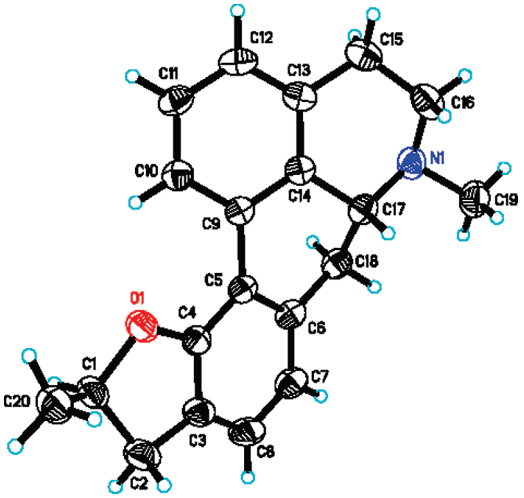


Figure 2. X-ray crystal analysis of *cis*-19 (6a*R*,2′*R*).

Biological Activity

DA (D_1, D_2) and 5-HT_{1A} Receptor Binding Assays. All the new aporphines were subjected to the competitive binding assays for DA (D_1, D_2) and serotonin (5-HT_{1A}) receptors, respectively, using membrane preparation obtained from stable transfected HEK293 (for D_1, D_2) and CHO (for 5-HT_{1A}) cells. These procedures are similar to those reported previously by us.[21,27,36] [^{3}H]-8-Chloro-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-ol (SCH23390),[36] [^{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. The results were summarized in Table 1.

Similar to the results we reported recently,[20,21,27,28] 11-hydroxy-aporphines **4a**−**c** displayed moderate affinity at the D_1 and/or D_2 (see footnote [b] in Table 1), as well as 5-HT_{1A} receptors. The *N*-substituent is a determinant for the selectivity between the two DA receptors. The smaller *N*-methyl group (**4b**) leads to rather modest binding selectivity for the D_1 receptor, whereas the larger *N*-propyl substituent (**4a**) prefers binding at the D_2 receptor. However, no significant difference was observed for the binding at the 5-HT_{1A} receptor; both **4a** and **4b** gave similar affinity at this receptor.

All the new synthetic aporphine analogues, either lacking the 11-hydroxyl group or retaining the 11-hydroxyl but with a 10-alkyl substituent larger than methyl, lost their affinity completely at both D_1 and D_2 receptors. However, these new compounds displayed a widely different binding profile at the 5-HT_{1A} receptor. In comparison to *N*-propyl-11-hydroxynoraporphine **4a**, 11-allyloxy-aporphines **10a**,**b**,**d** retained moderate affinity (3- to 8-fold less potent) at this receptor. The number and location of methyl substituent in the allyl moiety elicited relatively minor influence on the interaction between the compounds and the 5-HT_{1A} receptor. The Cope rearranged products, 11-hydroxy-10-allyl-aporphines **11a**,**b** showed significantly reduced affinity at the 5-HT_{1A} receptor in comparison to the phenolic precursor **4a**. Remarkable reduction in binding affinity was also observed for the double rearranged product **14**, which was inactive at this receptor. The dihydrofuroaporphine **12b** displayed moderate affinity (K_i, 630 nM) at the 5-HT_{1A} receptor.

In comparison to parent compound **4a**, 11-propargyloxy-aporphines **15a**,**b** showed good affinity at the 5-HT_{1A} receptor. The former compound (**15a**) has a comparable affinity to that of **4a**, with K_i value of 55 nM, while the latter compound **15b**, with a methyl substituent, was 2-fold less potent than **15a** at this receptor. Furoaporphines **16a**,**b** showed lower potency at the 5-HT_{1A} receptor, indicating that extension of the aromaticity of the phenyl function (D-ring) does not afford positive contribution to the 5-HT_{1A} receptor binding. Compounds **16a**,**b** were 7- to 11-fold less potent than compound **4a** and were also 4- to 6-fold less potent than their precursors **15a**,**b**.

Different from the *N*-propylnoraporphine series, all the new compounds **17**−**21** derived from *N*-methylaporphine **4b** displayed enhanced affinity at the 5-HT_{1A} receptor, indicating that the *N*-methyl group is beneficial to the binding at this receptor. 11-Allyloxy-(**17**) and 11-propargyloxy-(**20**) aporphines showed similar high binding affinity at this receptor with K_i values of 12.0 and 14.0 nM, respectively. They are 4-fold more potent than their parent compound **4b**. A 10-fold reduction in binding was observed for the Cope rearranged aporphine **18.** Compared to precursor **20**, the cyclized product, furoaporphine **21** showed a 2-fold decrease in binding, whereas the diastereomeric mixture of dihydrofuroaporphine **19** (*cis*/*trans*-**19**) displayed a 2-fold enhancement in binding to the 5-HT_{1A} receptor with K_i value of 6.7 nM. Further, the separated *cis*-isomer of **19** (*cis*-**19**, 6a*R*,2′*R*) showed a K_i value of 3.1 nM and was 2-fold more potent than the diastereomeric mixture (*cis*/*trans*-**19**), whereas *trans*-**19** (6a*R*,2′*S*) was 7-fold less potent with a K_i value of 45.3 nM. Therefore, *cis*-**19** is 15-fold more potent than *trans*-**19**.

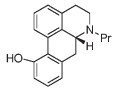
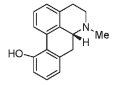
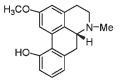
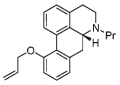
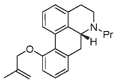
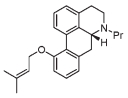
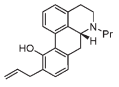
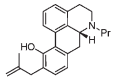
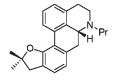
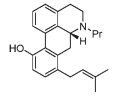
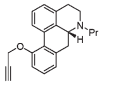
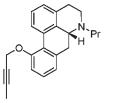
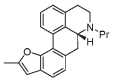
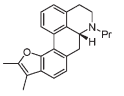
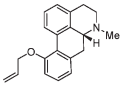
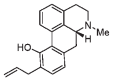
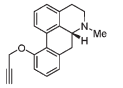
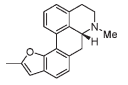
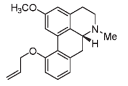
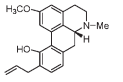
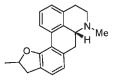
Compared to the corresponding C2 nonsubstituted aporphines **17** and **18**, the C2-methoxy-substituted analogues **22** and **23** also displayed good binding affinity at the 5-HT_{1A} receptor but they were generally less potent than aporphines **17** and **18**, indicating that a 2-methoxy substituent does not contribute positively to the binding of the compounds to the 5-HT_{1A} receptor, different from the observation on the DA receptors.[20]

Diastereomeric mixtures of **10c**, **10e**, **11e**, **12a**, **12c**, **13**, and **24** were also evaluated under the same bioassay conditions. Some of them (compounds **10e**, **12c**, and **24**) displayed moderate to good binding affinity at the 5-HT_{1A} receptor (see Supporting Information). These compounds were not further evaluated due to the failure to separate their corresponding diastereomers.

[^{35}S]GTPγS Assays on Aporphines 17, 20, and 19. Among the newly synthesized final compounds (furoaporphines and dihydrofuroaporphines) and the intermediates, 11-allyloxy-(**17**), 11-propargyloxy-(**20**), and dihydrofuro-(**19**, especially the *cis*-isomer) aporphines displayed the highest affinity at the 5-HT_{1A} receptor with K_i values of 12.0, 14.0, and 6.7 nM, respectively, and no appreciable affinity at both D_1 and D_2 receptors was observed. To explore agonistic or antagonistic properties of the three most potent compounds at the 5-HT_{1A} receptor, compounds **17** and **20**, as well as the separated diastereomers of **19**, *trans*-**19** (6a*R*,2′*S*), and *cis*-**19** (6a*R*,2′*R*) were subjected to [^{35}S]GTPγS function assays. The results were summarized in Table 2.

With serotonin (5-HT) itself as the reference agonist compound, aporphines **17** and **20** showed partial agonistic activity with E_{max} values of 61% and 73%, respectively. Although both compounds were equally potent in the receptor binding assay, aporphine **20** was 3-fold less potent in activation of 5-HT_{1A} receptor than aporphine **17** in the [^{35}S]GTPγS function assay. *cis*/*trans*-**19** and its two

Table 1. Binding Affinity of Aporphines for the 5-HT_{1A} Receptor from CHO Cells[a]

Compound	Structure	$K_i \pm$ SEM (nM)[b]
buspirone	-	20.0 (24[c])
1	-	1.2 (3.1[d])
4a		45±28
4b		49±20
4c		258±18
10a		279±52
10b		373±43
10d		149±11
11a		793±43
11b		NA
12b		630±160
14		NA
15a		55.0±6.5
15b		125±19
16a		320±26
16b		510±55
17		12.0±5.0
18		135±39
20		14.0±3.0
21		29.0±3.0
22		66±22
23		144±18
cis/trans-**19**		6.7±0.05
***trans*-19**	*6aR,2'S*	45.3±7.8
***cis*-19**	*6aR,2'R*	3.1±0.25

[a] Values are means of 5−6 experiments; NA = not active ($K_i > 10\,\mu$M); dashed lines denote that no experiment was conducted. [b] All the aporphine analogues listed herein from **4a** to *cis*-**19** had K_i values $> 10\,\mu$M at both D_1 and D_2 receptors except the parent phenolic compounds **4a**[21] (D_1, $K_i > 10\,\mu$M; D_2, K_i = 114 nM), **4b**[28] (D_1, K_i = 61 nM; D_2, K_i = 208 nM), and **4c**[35] (D_1, K_i = 46 nM; D_2, K_i = 235 nM). [c] Data from ref 17. [d] Data from ref 14.

Table 2. [^{35}S]GTPγS Studies of Novel Aporphine Derivatives[a]

	[^{35}S]GTPγS studies			
	compd alone		compd + 5-HT	
compd	E_{max}(%)	EC_{50} (nM)	I_{max}(%)	IC_{50} (nM)
17	61	56	100	323 ± 96
20	73	180		
19	–	–	100	323 ± 96
trans-**19**	–	–	100	18.0 ± 8.8
cis-**19**	127	86 ± 31	–	–
5-HT	100	147 ± 20	–	–

[a] Values are means of five experiments. Dash lines indicate no significant values were detected.

diastereomers showed significant differences in the functional assays. *cis*/*trans*-**19** and its *trans*-isomer (*trans*-**19**) did not produce notable stimulation, indicating that they lack agonistic activity. However, these two compounds showed a full inhibition (I_{max} = 100%) to the stimulation produced by serotonin (5-HT), suggesting that *cis*/*trans*-**19** and *trans*-**19** are 5-HT_{1A} receptor full antagonists. The *trans*-isomer showed 18-fold higher potency (IC_{50}, 18 nM) than *cis*/*trans*-**19** (IC_{50}, 323 nM). In sharp contrast to the results observed above, *cis*-**19** displayed a full agonistic activity with E_{max} of 127%, and no antagonizing effect to the 5-HT-stimulated [^{35}S]GTPγS binding was observed. Therefore, *cis*-**19** is a 5-HT_{1A} receptor full agonist and was selected for further evaluation in vivo.

Compound *cis*-19 Elicited 5-HT_{1A} Receptor Agonistic Activity in Vivo. To further determine 5-HT_{1A} receptor agonistic property of (6a*R*,2′*R*)-dihydrofuroaporphine *cis*-**19**, we studied the effect of this compound on the ventral tegmental area (VTA) DA neuron firing by employing an in vivo recording system as reported previously.[37−44] Because it is known that exciting effects of 5-HT_{1A} agonists on VTA

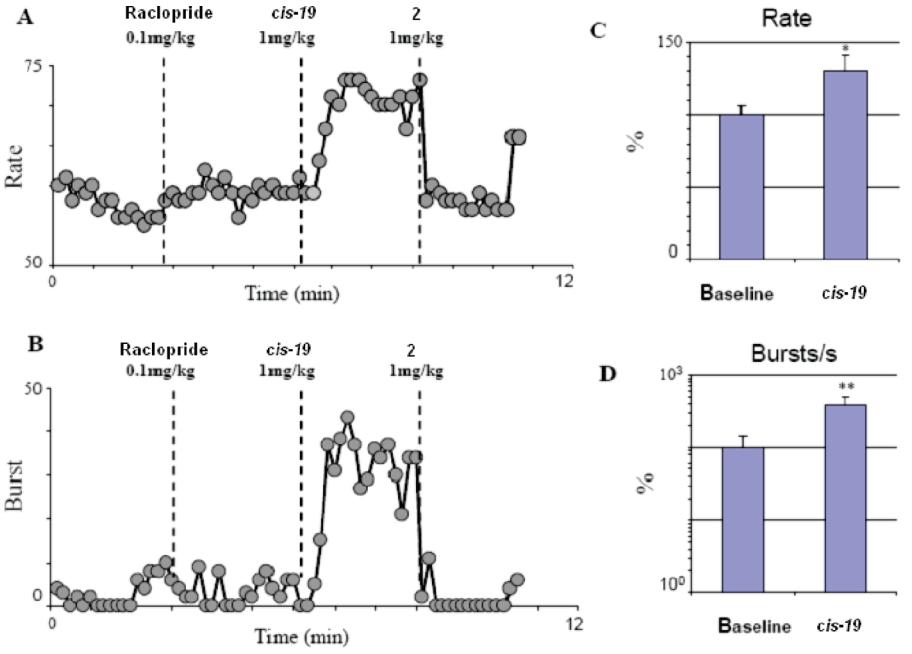


Figure 3. Baseline of the firing rate and bursting levels after raclopride administration. (A) Typical rate scatter diagram showing the excitatory effect by *cis*-**19** (1.0 mg/kg), suggesting that *cis*-**19** can increase the firing rate, which can be reversed by antagonist **2**. (B) Typical burst scatter diagram showing the excitatory effect by *cis*-**19** (1.0 mg/kg), suggesting that *cis*-**19** can increase the burst, which also can be reversed by antagonist **2**. (C) Summary bar graph showing that firing rates were all increased after *cis*-**19** injection compared to the baseline. (D) Summary bar graph showing that bursting was all increased after *cis*-**19** injection compared to the baseline. All values were expressed as percent of baseline. $*p < 0.05$, $**p < 0.01$ compared to baseline.

DA neurons are masked by D_2 receptor activation, the 5-HT_{1A}-excited VTA DA neurons can only be observed while the D_2 action is blocked by a D_2 receptor selective antagonist.[44] Therefore, rats were administrated with raclopride, a D_2-like receptor antagonist, before *cis*-**19** treatment. Consistent with literature reports,[40,44] raclopride (0.1 mg/kg) alone produced only a small effect on DA neuron activities ($n = 6$, data not shown). Subsequent injection of *cis*-**19** (1 mg/kg) significantly increased firing rate of DA neurons in all cells tested ($n = 6$, Figure 3). In comparison to the results prior to raclopride administration (paired t test, $p < 0.05$), the firing rate of DA neurons was significantly increased to $129.7 \pm 11.3\%$ during the test after *cis*-**19** treatment. Compound *cis*-**19** also increased the bursts/s (burst per second) of DA neurons compared to the result from preadministration of *cis*-**19** (paired t test, $p < 0.01$). To confirm the role of 5-HT_{1A} receptor activation in *cis*-**19**-enhanced firing of VTA DA neurons, compound **2** (1.0 mg/kg), a selective 5-HT_{1A} standard antagonist, was administered following *cis*-**19** injection. Both DA neuron firing rate and bursting level which were excited by *cis*-**19** were reversed to baseline, indicating that *cis*-**19**-elicited increase was completely blocked by the 5-HT_{1A} standard antagonist **2** (n = 2, Figure 3).

To further confirm the excitation of *cis*-**19** via 5-HT_{1A} receptor, rats were injected with 5-HT_{1A} antagonist **2** prior to *cis*-**19** administration. Antagonist **2** alone produced a slight decrease on DA neuron activities ($n = 4$, $p > 0.05$, data not shown). However, in antagonist **2**-pretreated rats, the enhanced firing and bursting of *cis*-**19** on DA neurons were completely abolished (Figure 4). Taken together, these results demonstrated that *cis*-**19** acts as a 5-HT_{1A} receptor agonist to excite VTA DA neurons in vivo.

Anxiolytic Effects of Dihydrofuroaporphines *cis*-19. It is well documented that serotonin system plays an important role in the regulation of anxiety behavior.[45−49] Increased anxiety has been observed in 5-HT_{1A} receptor knockout mice in the elevated plus maze (EPM) in which more entries and time spending in open arms are associated with axiolytic effects.[50] Recent studies also revealed that a reduced 5-HT_{1A} receptor binding was observed in the brains of anxiety patients.[51,52] In fact, several 5-HT_{1A} receptor agonists such as buspirone have been used as anxiolytic treatment.[44] Because dihydrofuroaporphine *cis*-**19** acts as a 5-HT_{1A} agonist in vitro and in vivo, we decided to evaluate its potential anxiolytic effects in the EPM studies. The 5-HT_{1A} antagonist, *trans*-**19**, was also investigated under same conditions as a comparison.

Rats were injected (ip) with either saline or *trans*-**19** or *cis*-**19**, respectively, for 1 h before EPM test. As shown in Figure 5, the number of entering and the time spending at open arm were significantly increased in rats treated with *cis*-**19** (3.0 mg/kg), which was abolished by pretreatment of 5-HT_{1A} receptor selective antagonist **2**. In contrast, *trans*-**19** did not have any anxiolytic effects at both doses. This clearly indicated that *cis*-**19** exerted its anxiolytic effect via its 5-HT_{1A} receptor agonistic activity.

Discussion

The absence of DA (D_1, D_2) receptor affinity of all the new synthetic aporphines further confirms that the 11-hydroxy group is an essential requirement for DA receptor interaction.[20] The abolished DA receptor binding affinity for 10-allyloxy-11-hydroxyaporphines (**11a**,**b**, **18**, **23**) suggested that a larger alkyl substituent than methyl at C10 may hamper the interaction of these compounds with DA receptors. In comparison to the parent phenols **4a**−**c**, the high binding affinity of 11-allyloxy/propargyloxy-aporphines **15a**, **17**, and **20** (K_i: 12−55 nM) at the 5-HT_{1A} receptor indicated that a lipophilic pocket larger than the earlier-proposed "methyl pocket"[20,22] in

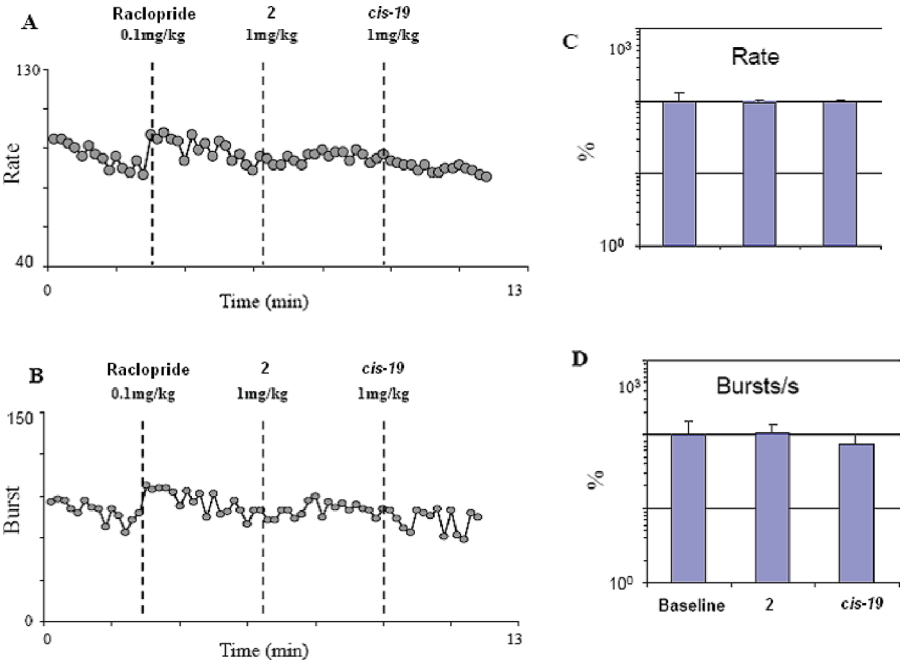


Figure 4. *cis*-**19** fails to excite VTA DA neurons in antagonist **2**-pretreated rats. Baseline of the firing rate and bursting levels after raclopride administration. (A) Typical rate scatter diagram showing that 5-HT_{1A} antagonist **2** completely blocks the firing rate induced by *cis*-**19** injection. (B) Typical rate scatter diagram showing that 5-HT_{1A} antagonist **2** also completely blocks the bursting induced by *cis*-**19** injection. (C) Summary bar graph showing that firing rate had no significantly change after *cis*-**19** injection compared to the baseline. (D) Summary bar graph showing that bursting had no significantly change after *cis*-**19** injection compared to the baseline. All values are expressed as percent of baseline. $*p < 0.05$, $**p < 0.01$ compared to baseline.

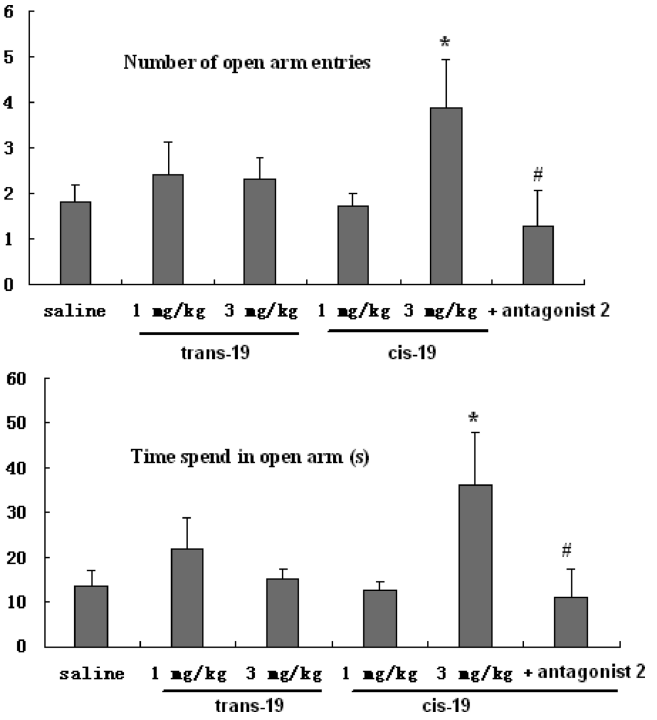


Figure 5. Number of open arm entries and time spending in rats. Rats received ip injection of saline, *trans*-**19** (1.0 or 3.0 mg/kg), or *cis*-**19** (1.0 or 3.0 mg/kg), respectively, for 1 h prior to EPM test. To block the effect, a selective 5-HT_{1A} receptor antagonist **2** (0.3 mg/kg) was administrated (sc) 5 min prior to *cis*-**19** (3.0 mg/kg) treatment for 1 h. EPM test was conducted as described in Experimental Section using a video tracking system. The number of open arm entries and time spending were recorded and data were expressed as mean ± SE $n = 7-9$. $*p < 0.05$, compared with saline treated. $\#p < 0.05$, compared with *cis*-**19** (3.0 mg/kg) alone.

the receptor binding site may exist for interaction with these compounds, and the 11-hydroxyl group is not required for this interaction. The generally lower affinity of 11-hydroxy-10-allylaporphines **11a**,**b**, **18**, **23**, and 11-hydroxy-8-allylaporphine (**14**) suggested that larger alkyl groups at the C10 or C8 positions are not tolerated for interaction with the 5-HT_{1A} receptor.

Aromatic furoaporphines **16a**,**b**, and **21** showed remarkable difference in binding to the 5-HT_{1A} receptor, indicative of the importance of *N*-methyl substitution on the receptor binding. This was further confirmed by the high binding affinity of *N*-methylaporphines **17** and **20**. High binding was also observed in the diastereomeric mixture **19**. Further evaluation of its trans and cis isomers indicated that the high binding potential resided in the cis isomer (K_i, 3.1 nM).

Among all the synthetic aporphines, 11-allyloxy-(**17**), 11-propargyloxy-(**20**), and dihydrofuro-(**19**, especially the *cis*-isomer) aporphines displayed the highest affinity at the 5-HT_{1A} receptor with K_i values of 12.0, 14.0, and 6.7 nM, respectively. [^{35}S]GTPγS function assays revealed that aporphines **17** and **20** were partial agonists at this receptor. Diastereomeric mixture **19** (*cis*/*trans*-**19**) and its trans isomer (*trans*-**19**) acted as full antagonists, whereas *cis*-**19** behaved as a full agonist at the 5-HT_{1A} receptor. These results indicated that the stereochemistry in the dihydrofuran portion of aporphine **19** not only played a role on the 5-HT_{1A} receptor binding but also generated a sharp difference in the receptor functionality. The agonistic activity of *cis*-**19** was further confirmed by the 5-HT_{1A} receptor-involved exciting effects on VTA DA neurons and by the 5-HT_{1A}-blockade experiments. Further, *cis*-**19** exerted anxiolytic effect via 5-HT_{1A} receptor activation in the EPM test, indicating that *cis*-**19** may be useful as potential treatment for anxiety.

In conclusion, a series of new aporphine analogues were synthesized by allylation or propargylation of 11-hydroxy-aporphines **4a**−**c**, followed by Cope-rearrangement and cyclization. Such an approach leads to the identification of a highly potent and selective 5-HT_{1A} receptor ligand *cis*-**19** possessing a K_i value of 3.1 nM. This compound is a 5-HT_{1A} full agonist in vitro and in vivo and may be useful as potential treatment for anxiety.

Experimental Section

General Methods. ^{1}H NMR spectral data were recorded in $CDCl_3$ on Varian Mercury 300 NMR spectrometer, and ^{13}C NMR was recorded in $CDCl_3$ on Varian Mercury 400 NMR spectrometer. Low-resolution mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded at an ionizing voltage of 70 eV on a Finnigan/MAT95 spectrometer. Elemental analysis was performed on a CE 1106 elemental analyzer. Optical rotations were determined with a JASCO DCP-1000 digital polarimeter and were the average of three measurements. Column chromatography was carried out on silica gel (200−300 mesh). All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. Yields were of purified compounds and were not optimized. Compounds **4a**,[21,28] **4b**,[21,28] and **4c**[35] were prepared according to corresponding literature procedures. HPLC analysis was conducted for all new compounds listed in Table 1 on an Agilent 1100 series LC system (Agilent ChemStation Rev.A.10.02; ZORBAX Eclipse XDB-C8, 4.8 mm × 150 mm, 5 μM, 1.0 mL/min, uv 254 nM, rt) with two solvent systems (acetonitrile/water and methanol/water). All the assayed compounds displayed a purity of 95−99% in both solvent systems, and the diastereomeric mixtures of **10c**, **10e**, **11e**, **12a**, **12c**, **13**, **19**, and **24** were inseparable and displayed one peak with >95% purity in both systems.

General Procedure for the Preparation of 11-Alkyloxyaporphines. To a solution of 11-hydroxyaporphine **4a**, **4b**, or **4c** (1.0 mmol), K_2CO_3 (276 mg, 2.0 mmol), and a catalytic amount of KI (5 mg) in anhydrous DMF (10 mL) at 0 °C, an appropriate allyl or propargyl bromide (1.0 mmol) was dropped. After stirred for 1 h at 0 °C, the reaction mixture was warmed to rt and then diluted with Et_2O (30 mL) and H_2O (20 mL). The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by silica gel chromatography (petroleum:ethyl acetate = 3:1, 1% Et_3N) to give a pure oily product.

11-Allyloxy-*N*-methylaporphine (17). Light-brown oil (140 mg, 48%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, 1H, J = 7.8 Hz, H-1), 7.20 (m, 2H, H-2, H-3), 7.06 (d, 1H, J = 7.5 Hz, H-9), 6.91 (m, 2H, H-8, H-10), 6.10 (m, 1H, CH_2=C<u>H</u>CH_2O), 5.45 (dd, 1H, J = 17.4, 1.5 Hz, <u>CH_2</u>=CHCH_2O), 5.27 (dd, 1H, J = 10.8, 1.5 Hz, <u>CH_2</u>=CHCH_2O), 4.61 (m, 2H, CH_2=CH<u>CH_2</u>O), 3.13 (m, 4H, H-6a, H-7, H-5α), 2.76 (dd, 1H, J = 16.2, 2.4 Hz, H-5β), 2.55 (s, 3H, N−CH_3), 2.53 (m, 2H, H-4). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6 (C-11), 138.1 (C-7a), 134.6 (C-3a), 133.2 (C-10), 132.3 (C-13), 131.3 (C-12), 127.8 (C-9), 127.4 (C-2), 126.4 (C-3), 125.8 (C-1), 123.3 (C-11a), 121.0 (C-8), 117.1 (<u>CH_2</u>=CH−), 111.8 (CH_2=<u>C</u>H-), 69.3 (O<u>CH_2</u>), 62.0 (C-6a), 52.9 (C-5), 43.9 (N-<u>CH_3</u>), 35.1 (C-7), 29.1 (C-4). EI (MS) 291 (M^+). HRMS calcd for $C_{20}H_{21}NO_2$, 291.1623; found, 291.1620.

11-Propargyloxy-*N*-methylaporphine (20). Brown oil (248 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, 1H, J = 8.1 Hz), 7.22 (m, 2H), 7.06 (dd, 2H, J = 9.3, 8.4 Hz), 6.96 (d, 1H, J = 7.2 Hz), 4.74 (m, 2H), 3.13 (m, 4H), 2.28 (m, 2H), 2.58 (s, 3H), 2.52 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.5, 138.2, 134.7, 132.4, 130.9, 127.7, 127.5, 126.3, 125.9, 123.7, 121.8, 112.4, 78.6, 75.4, 61.9, 56.2, 52.9, 43.9, 35.0, 29.1. EI (MS) 289 (M^+). HRMS calcd for $C_{20}H_{19}NO_2$, 289.1467; found, 289.1448.

General Procedure for the Preparation of 11-Hydroxy-10-alkylaporphine Derivatives by Cope Rearrangement. A solution of 11-allyloxy or 11-propargyloxy- aporphine (1.0 mmol) in *N*,*N*-diethylaniline (10 mL) was refluxed for 1.5 h under N_2. After cooling to rt, the reaction mixture was purified by silica gel chromatography (petroleum:ethyl acetate = 2:1) to yield the rearranged products.

10-Allyl-11-hydroxy-*N*-methylaporphine (18). Pale solid (256 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, 1H, J = 7.5 Hz), 7.25 (m, 1H), 7.07 (d, 1H, J = 7.5 Hz), 7.00 (d, 1H, J = 7.8 Hz), 6.82 (d, 1H, J = 7.8 Hz), 6.05 (m, 1H), 5.17 (m, 2H), 3.46 (d, 2H, J = 6.0 Hz), 3.08 (m, 4H), 2.75 (d, 1H, J = 16.5 Hz), 2.57 (s, 3H), 2.52 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.1, 136.5, 136.3, 135.0, 133.3, 131.4, 129.0, 127.5, 126.4, 125.2, 124.4, 121.7, 120.3, 116.5, 62.2, 52.8, 43.9, 35.4, 34.8, 28.9. EI (MS) 291 (M^+). HRMS calcd for $C_{20}H_{21}NO_2$, 291.1623; found, 291.1607.

General Procedure for the Synthesis of Furo-/Dihydrofuroaporphines Derivatives. A solution of an appropriate 11-hydroxy-10-substituted-aporphine or 11-propargyloxyaporphines (0.10 mmol) in glacial acetic acid (6 mL) and concentrated HBr (2 mL) was refluxed for 3 h and then evaporated to dryness. The residue was cooled and treated with NH_4OH (5 mL). The solution was extracted with CH_2Cl_2 (4 × 20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by silica gel chromatography (petroleum:ethyl acetate = 2:1) or by preparative TLC with acetonitrile/trifluoroacetic acid (0.05%) as the eluents to give pure cyclized product.

***N*-Methyl-2′-methyl-2′,3′-dihydrofuro[*m*]aporphine (*cis*/*trans*-19).** Yellow solid (26.5 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (dd, 1H, J = 8.1, 9.0 Hz, H-1), 7.23 (t, 1H, J = 7.8 Hz, H-3), 7.02 (t, 2H, J = 7.5 Hz, H-2, H-9), 6.76 (d, 1H, J = 7.5 Hz, H-8), 5.09 and 4.96 (m, 1H, 1:1, H-2′), 3.20 (m, 5H, H-6a, H-7, H-3′), 2.78 (m, 4H, H-4, H-5), 2.51 (s, 3H, N−CH_3), 1.59 and 1.44 (d, 3H, J = 6.3 Hz, 1:1, 2′-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3 (2), 135.6, 133.6, 132.6, 131.3 (2), 127.3, 126.7 (2), 126.3, 125.1 (2), 123.3 (2), 119.9, 117.4, 80.0 (2), 62.2, 53.3, 43.9, 36.6 (2), 34.3, 29.0, 21.9 (2). EI (MS) 291 (M^+). Anal. ($C_{20}H_{21}NO\cdot0.4TFA\cdot1.5H_2O$) Calcd: C, 68.63; H, 6.76; N, 3.85. Found: C, 68.81; H, 7.04; N, 3.44.

Chiral HPLC Resolution of *N*-Methyl-2′-methyl-2′,3′-dihydrofuro[*m*]aporphine 19 (*cis*/*trans*-19). The chiral HPLC analysis of *cis*/*trans*-**19** was carried out on a Chiracel OD column (25 cm × 0.46 cm, Daicel, Japan) under the following conditions: flow rate 1.0 mL/ min, hexane/isopropanol/diethylamine (99/1/0.1) as the eluent. The retention times were 10.1 min for faster-moving trans isomer and 12.3 min for slower-moving cis isomer, respectively.

***trans*-19.** MS (EI) 291 (M^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, 1H, J = 7.8 Hz), 7.26 (t, 1H, J = 7.8 Hz), 7.04 (t, 2H, J = 7.8 Hz), 6.76 (dd, 1H, J = 7.5, 0.9 Hz), 4.97 (m, 1H), 3.20 (m, 5H), 2.80 (m, 1H), 2.64 (m, 3H), 2.58 (s, 3H), 1.62 (d, 3H, J = 6.3 Hz). $[\alpha]_D^{20}$ −30° (c 0.071, EtOH, 20 °C).

***cis*-19.** MS (EI) 291 (M^+). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, 1H, J = 7.5 Hz), 7.24 (t, 1H, J = 7.8 Hz), 7.02 (t, 2H, J = 7.8 Hz), 6.76 (dd, 1H, J = 7.5, 1.2 Hz), 5.09 (m, 1H), 3.38 (m, 1H), 3.14 (m, 4H), 2.78 (m, 3H), 2.58 (s, 3H), 2.56 (m, 1H), 1.44 (d, 3H, J = 6.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 135.6, 133.7, 132.6, 131.3, 127.3, 126.5, 126.3, 125.1, 123.4, 119.9, 117.4, 79.6, 62.2, 53.3, 44.0, 36.6, 34.4, 29.1, 22.0. $[\alpha]_D^{20}$ −109° (c 0.075, EtOH, 20 °C).

Binding Assay of New Compounds at the D_1, D_2 and 5-HT_{1A} Receptors. The affinity of new synthetic 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-HEK 293 cells were prepared as described previously.[21,27,36] Duplicated tubes were incubated at 30 °C for 50 min with increasing concentrations (1 nM to 100 μM) of respective compound and with 0.7 nM of [^{3}H]8-OH-DPAT (for 5-HT_{1A} receptor), [^{3}H]- 8-chloro-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-ol (SCH23390) (for dopamine D_1 receptor), or [^{3}H]spiperone (for dopamine D_2

receptor) in a final volume of 200 μL of binding buffer containing 50 mM Tris, 4 mM $MgCl_2$, pH 7.4. Nonspecific binding was determined by parallel incubations with either 10 μM of **2** for 5-HT_{1A}, 8-chloro-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1*H*-benzo-[*d*]azepin-7-ol for D_1, or spiperone for D_2 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.

[^{35}S]GTPγS Binding Assays for the Selected Compounds. 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 of membrane protein in a final volume of 100 μL with various concentration of the compound. The antagonism effects of the compounds were tested in the existence of 10 μM serotonin (5-HT) for the 5-HT_{1A} receptor. The binding buffer contains 50 mM Tris, pH 7.5, 5 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid (EDTA), 100 mM NaCl, 1 mM DL-dithiothreitol (DTT), and 40 μM guanosine triphosphate (GDP). The reaction was initiated by adding of [^{35}S]GTPγS (final concentration of 0.1nM). Nonspecific binding was measured in the presence of 100 μM 5′-guanylimidodiphosphate (Gpp(NH)p). The reaction was terminated by 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.

5-HT_{1A} Agonism-Involved DA Neuron Firing. Single-Unit Recordings in Vivo. Male Sprague−Dawley rats (Shanghai Experimental Animal Center, Chinese Academy of Sciences), weighing 250−300 g, were housed under standard laboratory conditions with constant temperature (22−23 °C) and humidity (50−60%). The animals were maintained in a 12 h light/dark cycle. All experiments were in compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Rats were anesthetized with chloral hydrate (400 mg/kg, ip, with supplemental doses administered via a lateral tail vein) and mounted in a stereotaxic instrument. Their body temperatures were maintained at 37 °C by means of a heating pad throughout the experiment. The skull was exposed, and the wound margins were infiltrated with a 0.3% solution of mepivacaine hydrochloride. A glass microelectrode (5−10 MΩ) filled with 2 M NaCl solution containing 2% pontamine sky-blue dye was lowered through a small burr hole drilled above the VTA (3.0 mm anterior to the lambda, 0.5−0.9 mm lateral to the midline, 6.5−8.5 mm ventral to the cortical surface). DA neurons were identified and recorded as described:[37−40] positive/negative action potentials of long duration (2−5 ms), firing rate (1−10 Hz) with slow irregular or burst firing pattern, low pitch sound produced on audio amplifier, duration of ≥1.1 ms from the start of the action potential to the negative trough.[41] Interspike intervals (ISI) and firing rates were collected online to a personal computer.

Data Analysis. All data analyses were performed using programs written in Visual Basic for Applications in Microsoft Excel.[42] Bursting was identified according to the "80/160 ms" definition proposed by Grace and Bunney.[37,38] Thus, the onset of a burst was identified as the concurrence of two spikes with an interspike interval less than 80 ms and the termination of a burst was defined as an interspike interval greater than 160 ms.[43] Data were analyzed off-line with a software package from BSPS Mathlib (Fudan University, Shanghai, China). The firing rate and bursting were analyzed every 10 s.[44] Effects of drugs were determined by comparing measures before and after drug injection using paired *t* test. All numerical data were expressed as mean ± SEM.

Elevated Plus Maze (EPM). The maze consists of two opposite open arms and two opposite closed arms which have 40 cm high walls. The arms were 50 cm long and 10 cm wide and are connected by a 10 cm × 10 cm central area. The maze is elevated to 50 cm height above the floor. The rat was placed on the central area with its head toward the closed arm. The animals were allowed to explore the maze for 5 min. Animal behavior was recorded by a video tracking system. Number of open arm entries and time spending in open arms were scored later. The maze was thoroughly cleaned with ethanol after each trial.

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Supporting Information Available: Experimental procedures, ^{1}H and ^{13}C spectra for all the final compounds and intermediates, X-ray data for *cis*-**19** and chiral HPLC conditions for resolution of *cis*/*trans*-**19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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