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New piperidinyl- and 1,2,3,6-tetrahydropyridinyl-pyrimidine derivatives as selective 5-HT$_{1A}$ receptor agonists with highly potent anti-ischemic effects

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Abstract—A series of new piperidinyl- and 1,2,3,6-tetrahydropyridinyl-pyrimidine derivatives were synthesized. Among these compounds, 4-methyl-2-(1,2,3,6-tetrahydropyridin-4-yl)pyrimidine derivative **23** (**SUN N5147**) exhibited sub-nanomolar affinity for 5-HT$_{1A}$ receptor with 1000-fold selectivity over both dopamine D$_2$ and α_1-adrenergic receptors and remarkable neuroprotective activity in a transient middle cerebral artery occlusion (t-MCAO) model.
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1. Introduction

Acute ischemic stroke is one of the major causes of death. Many pharmacological treatments by means of so-called neuroprotective drugs such as glutamate receptor antagonists and Ca and/or Na channel blockers have been tried; however, they have not been successful in improving clinical outcome in patients.[1] Recently, cerebral serotonergic neuron has been suggested to be involved in cerebral ischemic conditions. Serotonin (5-HT) and its receptors are known to play important roles in various physiological and pathophysiological processes.[2] The 5-HT$_{1A}$ receptor, which is one of numerous 5-HT receptor subtypes, has been generally accepted to have a role in psychiatric disorders such as depression, anxiety, and psychosis.[3] It has also been reported that 5-HT$_{1A}$ receptor agonists have protective effects in cerebral ischemic conditions,[4–6] due to hyperpolarization of cell membrane and glutamate release inhibition.[7]

Buspirone (Fig. 1) is a well-known 5-HT$_{1A}$ receptor agonist and it has been useful in the treatment of anxiety and depression.[8] This compound, however, shows a poor selectivity for 5-HT$_{1A}$ receptor versus dopamine D$_2$ receptor and causes undesirable side effects such as prolactin stimulation and extrapyramidal symptoms.[9] In the treatment of cerebral ischemia, it is also considered that a selective affinity for the 5-HT$_{1A}$ receptor over α_1-adrenergic receptor is very important because α_1-adrenergic receptor antagonists cause hypotensive effects and worsen clinical states.

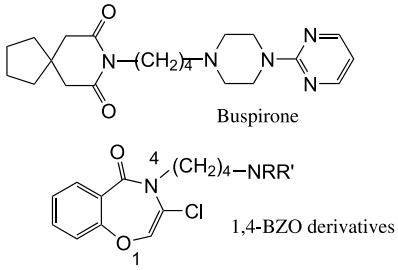

Figure 1. Buspirone and 4-(4-aminobutyl)-3-chloro-1,4-benzoxazepin-5(4*H*)-one derivatives.

The alignment of primary sequences of 5-HT$_{1A}$, dopamine D$_2$, and α_1-adrenergic receptors show a high degree of homology in the transmembrane regions of G-protein coupled receptors (GPCRs).[10] Biogenic amine receptors, which are the rhodopsin family of GPCRs, share a comparable transmembrane structure formed by a highly organized heptahelical transmembrane bundle.[11] As a consequence, it is difficult to discover a

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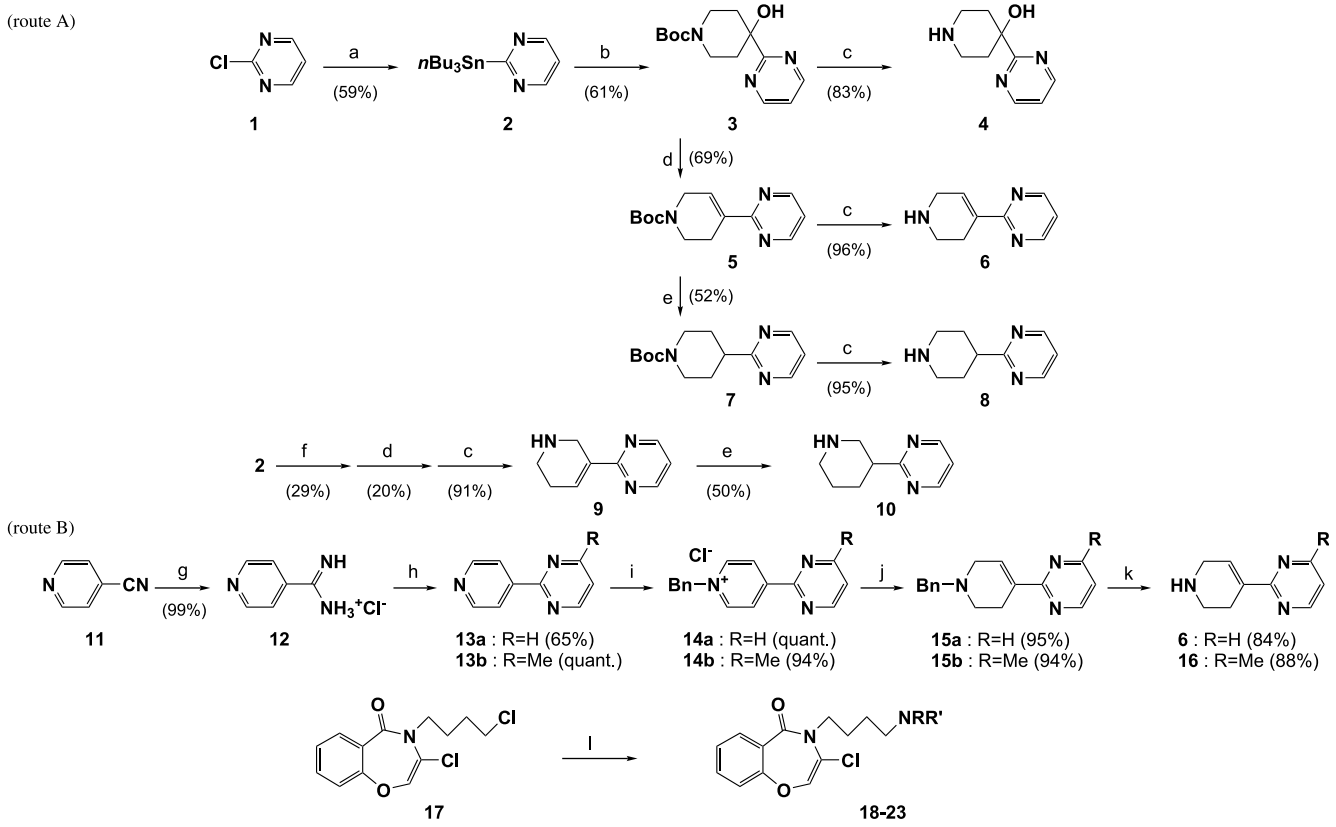
selective 5-HT_{1A} receptor agonist over dopamine D_2 and α_1-adrenergic receptors and many researchers around the world have been working on this issue.[12–14] We have also reported 1,4-benzoxazepine (1,4-BZO) derivatives which are selective 5-HT_{1A} receptor agonists with potent anti-ischemic effects.[15] In order to improve affinity and selectivity for the 5-HT_{1A} receptor, we have attempted to modify amine moiety of 1,4-BZO derivatives. In the present report, we describe the synthesis of novel piperidinyl- and 1,2,3,6-tetrahydropyridinyl-pyrimidine derivatives and their favorable binding profiles. Their neuroprotective effects in an in vivo transient middle cerebral artery occlusion (t-MCAO) model are also presented.

2. Chemistry

Pyrimidine derivatives were prepared by the pathway shown in Scheme 1. Tin–lithium exchange at the 2-position of pyrimidine at −78 °C resulted in 2-lithiopyrimidine,[16] and subsequent alkylation with *N*-Boc-4-piperidone gave *N*-Boc-4-hydroxy-4-(2-pyrimidinyl)piperidine **3** in moderate yield. 4-Hydroxy-4-(2-pyrimidinyl)piperidine **4** was obtained by the deprotection of **3** with TFA. 4-(2-Pyrimidinyl)-1,2,3,6-tetrahydropyridine **6** was synthesized by dehydration of **3** with phosphorus oxychloride and the subsequent deprotection with TFA. A piperidine compound **8** was derived by hydrogenation in the presence of Pd/C from **5** and deprotection with TFA. 3-(2-Pyrimidinyl)-1,2,5,6-tetrahydropyridine **9** and 3-(2-pyrimidinyl)piperidine **10**, which are regio-isomers of **6** and **8**, were synthesized as the same way above from 3-piperidone instead of 4-piperidone. Reaction yields of alkylation of 3-piperidone with 2-stannylpyrimidine **2** and dehydration were lower than expected.

Later during our study, we successfully made an improvement in the synthesis of 4-(2-pyrimidinyl)-1,2,3,6-tetrahydropyridine **6** via 2-(4-pyridinyl)pyrimidine **13a** (route B). Isonicotinonitrile **11** was converted to isonicotinamidine hydrochloride **12** by condensation with 1,1,3,3-tetramethoxypropane to give 2-(4-pyridinyl)pyrimidine **13a**.[17] Compound **13a** was regio-selectively alkylated with benzylchloride, giving quaternary ammonium derivative **14a** quantitatively. 1,2,3,6-Tetrahydro-4-(2-pyrimidinyl)piperidine **6** was obtained in high yield by the reduction of **14a** with $NaBH_4$ and the subsequent deprotection by 1-chloroethyl chloroformate. We were also able to synthesize of 4-(4-methylpyrimidin-2-yl)-1,2,3,6-tetrahydropyridine **16** in good yield in the same way. Compounds **18**–**23** were prepared from 3-chloro-4-(4-chlorobutyl)-1,4-benzoxazepin-5(4*H*)-one **17**[18] by amination with the corresponding amines.[19]



Scheme 1. Reagents and conditions: (a) *n*-Bu_3SnLi, THF, −78 °C to rt; (b) (i) *n*-BuLi, THF, −78 °C; (ii) *N*-Boc-4-piperidone, THF, −78 °C to rt; (c) (i) TFA, CH_2Cl_2; (ii) NaOH aq; (d) $POCl_3$, pyridine; (e) H_2, 10% Pd/C, EtOH; (f) (i) *n*-BuLi, THF, −78 °C; (ii) *N*-Boc-3-piperidone, THF, −78 °C to rt; (g) (i) cat. NaOMe, MeOH; (ii) NH_4Cl; (h) 1,1,3,3-tetramethoxypropane or acetylacetaldehyde dimethyl acetal, 1,4-dioxane, reflux; (i) BnCl, CH_3CN, reflux; (j) $NaBH_4$, EtOH; (k) (i) $ClCOOCH(Cl)CH_3$, 1,2-dichloroethane, reflux; (ii) MeOH, reflux; (l) amine (HNRR′), NaI, Et_3N, CH_3CN, reflux.

3. Results and discussion

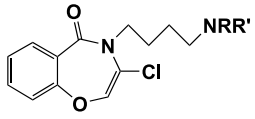
Compounds **18**–**23** were evaluated for their binding affinity to 5-HT_{1A}, dopamine D_2 and α_1-adrenergic receptor by radioligand binding assays (Table 1). The specific ligands and tissue sources used were as follows: (a) 5-HT_{1A} serotonergic receptor: [^{3}H]8-OH-DPAT (8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin), rat hippocampus membranes; (b) dopamine D_2 receptor: [^{3}H] raclopride, rat striatum membranes; (c) α_1-adrenergic receptor: [^{3}H]prazosin, rat cerebral cortex membranes.

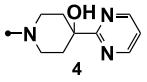
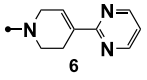
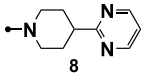
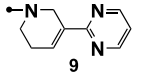
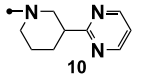
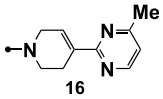
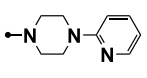
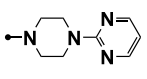
While alcohol derivative **18** showed a very weak affinity for 5-HT_{1A} receptor, tetrahydropyridine derivative **19** and its piperidine derivative **20** displayed higher 5-HT_{1A} receptor affinity and selectivity over both dopamine D_2 and α_1-adrenergic receptors. IC_{50} ratios of both **19** and **20** were improved from the previous reported[15] piperazine derivative **24** and **25**. Compounds **21** and **22**, which are regio-isomers of **19** and **20**, showed reduced 5-HT_{1A} receptor affinity. Compound **23** bearing methyl group at the 4-position of pyrimidine moiety of **19** exhibited the highest affinity (IC_{50} = 0.185 nM) and selectivity for 5-HT_{1A} receptor over dopamine D_2 (IC_{50} ratio = 4124) and α_1-adrenergic (IC_{50} ratio = 1324) receptors.

We investigated in vivo neuroprotective effects of **19**, **23**, and Buspirone in a rat model of transient focal cerebral ischemia. Using the intraluminal suture method of Koizumi et al.,[20] male Wistar rats were subjected to a t-MCAO. The evaluated compounds and vehicle (saline) were administered at a dose of 1 mg/kg sc immediately after the occlusion. Three days after reperfusion, seven coronary brain sections were prepared from rats subjected to ischemia. Coronal sections were stained with physiological saline including 0.2% 2,3,5-triphenyltetrazolium chloride. After staining, sections were analyzed for total area and cerebral infarction area and the cerebral infarction volume ratio was calculated.[21] Compound **23** exhibited a highly potent anti-ischemic effect (73% inhibition) (Fig. 2). Compound **19** and Buspirone also showed potent activities (65% and 53% inhibition, respectively).

All the tested compounds reduced the ischemic hyperthermia at the neuroprotective dose (about 2 °C). It is known that 5-HT_{1A} agonists possess a hypothermic

Table 1. Structures and the receptor binding data of pyrimidine derivatives



Compound	NRR′	IC_{50} (nM)			IC_{50} ratio	
		5-HT_{1A}	D_2	α_1	D_2/5-HT_{1A}	α_1/5-HT_{1A}
18[b]	 4	2650	NT[a]	9560	—	3.6
19[b]	 6	1.38	494	924	358	670
20[b]	 8	5.81	1800	2000	310	344
21[c]	 9	79.6	893	384	11	4.8
22[c]	 10	314	NT[a]	NT[a]	—	—
23[c]	 16	0.185	763	245	4124	1324
24[c,d]		0.770	51	43	66	56
25[c,d]		1.59	199	544	125	342
Buspirone		11.0	55	2920	5.0	265

[a] Not tested.
[b] Hydrochloride.
[c] Fumarate.
[d] Ref. 15.

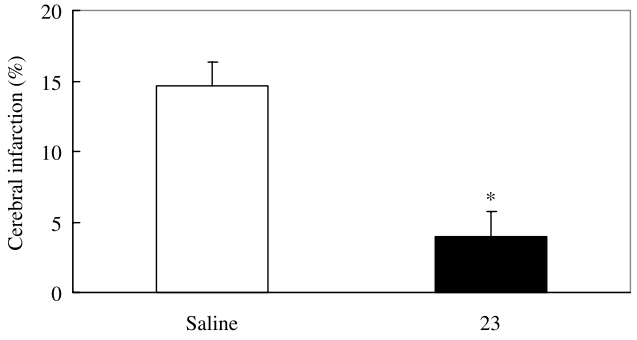


Figure 2. Effect of **23** on cerebral infarction in rats subjected to transient focal ischemia (60 min middle cerebral artery occlusion). Compound **23** (1 mg/kg) was administered sc immediately after occlusion of the MCA. Each datum represents mean ± SEM. $n = 5$; $^{*}p < 0.05$, versus saline (unpaired t-test).

effect.[22] In order to investigate a hypothermic effect in this model, we evaluated 4-dimethylaminoantipyrine, which is an antipyretic drug, at a dose of 200 mg/kg ip immediately after t-MCAO. Although it caused hypothermia to the same degree as **19** and **23**, it showed no anti-ischemic effect (21% inhibition). These results indicate that pharmacological effect for 5-HT_{1A} receptor agonist is involved in the mechanism of neuroprotective effect of **19** and **23**.

In conclusion, we presented the synthesis and biological evaluation of pyrimidine derivatives. 1,2,3,6-Tetrahydropyridinyl-pyrimidine derivatives **19** and **23** show not only highly potent affinity for 5-HT_{1A} receptor but good selectivity over dopamine D_2 and α_1-adrenergic receptors. Since 4-methyl-2-(1,2,3,6-tetrahydropyridin-4-yl)pyrimidine derivative **23** (**SUN N5147**) exhibited the highest 5-HT_{1A} receptor affinity with 1000-fold selectivity over both dopamine D_2 and α_1-adrenergic receptors and remarkable neuroprotective activity in a t-MCAO model, it might be more suitable as a therapeutic agent for ischemic neuronal damage. Further investigations of pharmacological profile of **23** are in progress.

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19. All compounds were fully characterized by spectral methods. Representative data of compound **23** (fumarate); colorless solid; mp 121–124 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.65–1.73 (m, 4H), 2.46 (s, 3H), 2.48–2.57 (m, 4H), 2.73–2.80 (m, 2H), 2.95–3.40 (m, 2H), 3.80–3.88 (m, 2H), 6.63 (s, 2H), 7.09 (s, 1H), 7.15–7.17 (m, 1H), 7.25 (d, 1H, $J = 5$ Hz), 7.34 (t, 1H, $J = 8$ Hz), 7.58–7.62 (m, 1H), 7.78–7.80 (m, 1H), 8.63 (d, 1H, $J = 5$ Hz); IR (KBr) cm^{-1}: 3424, 2940, 2582, 1655, 1578, 1454, 1372, 1330, 1249; FAB-MS *m/z*: 425 $[MH]^+$. Anal. Calcd for $C_{27}H_{29}ClN_4O_6$: C, 59.94; H, 5.40; N, 10.36. Found: C, 60.05; H, 5.28; N, 10.23.
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