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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

Katsuhide Kamei,[a,*] Noriko Maeda,[a] Ryoko Katsuragi-Ogino,[a] Makoto Koyama,[a] Mika Nakajima,[a] Toshio Tatsuoka,[b] Tomochika Ohno[a] and Teruyoshi Inoue[b]

[a]*Daiichi Suntory Biomedical Research Co., Ltd, 1-1-1, Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618-8513, Japan*
[b]*Daiichi Suntory Pharma Co., Ltd, Mori Bldg. No. 31, 5-7-2, Kojimachi, Chiyoda-ku, Tokyo 102-8530, Japan*

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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 $\alpha_1$-adrenergic receptors and remarkable neuroprotective activity in a transient middle cerebral artery occlusion (t-MCAO) model.


## 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 $\alpha_1$-adrenergic receptor is very important because $\alpha_1$-adrenergic receptor antagonists cause hypotensive effects and worsen clinical states.

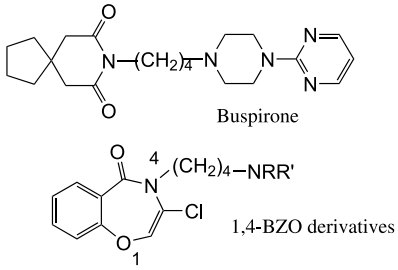


**Figure 1.** Buspirone and 4-(4-aminobutyl)-3-chloro-1,4-benzoxazepin-5(4*H*)-one derivatives.

The alignment of primary sequences of 5-HT$_{1A}$, dopamine D$_2$, and $\alpha_1$-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

* Corresponding author. Tel.: +81 75 962 8194; fax: +81 75 962 6448; e-mail: Katsuhide_Kamei@dsup.co.jp

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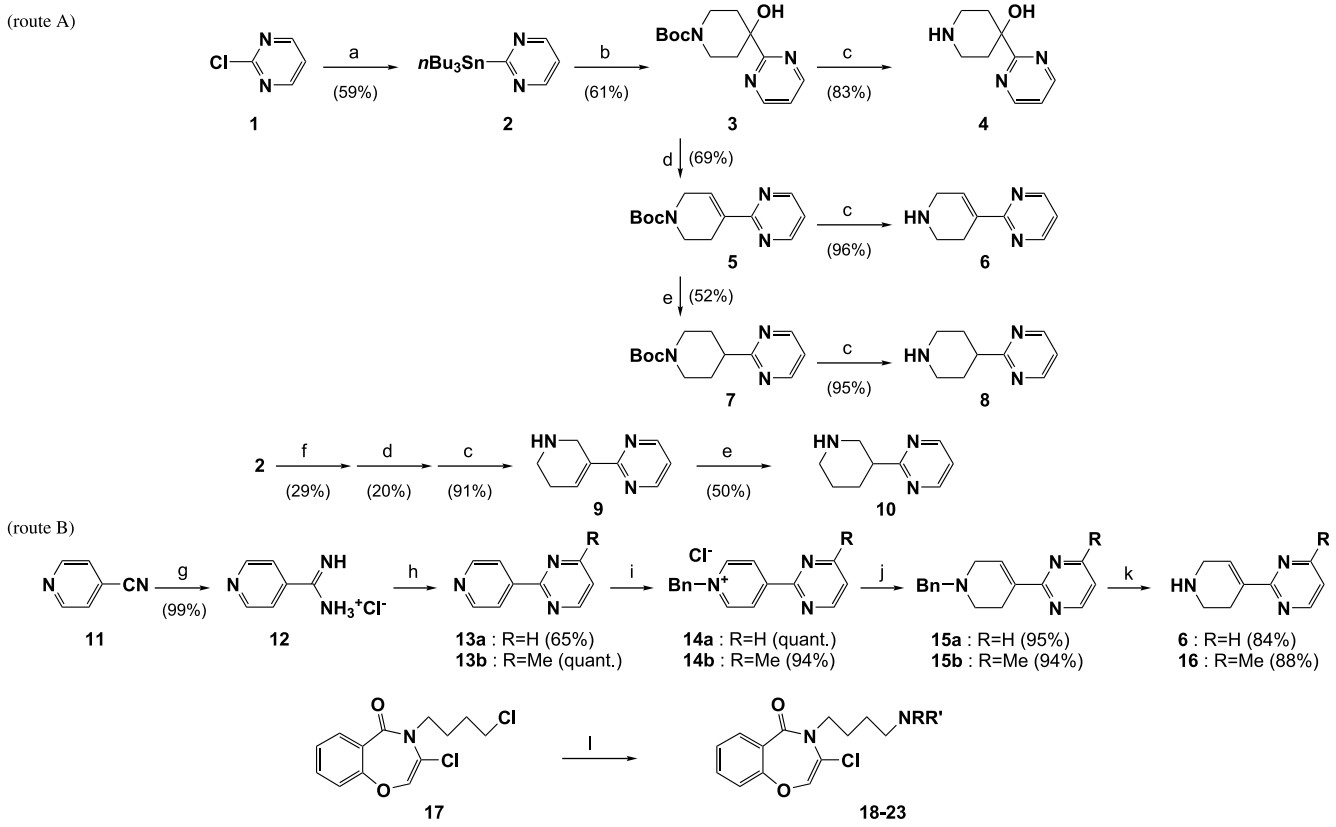
selective 5-$HT_{1A}$ receptor agonist over dopamine $D_2$ and $\alpha_1$-adrenergic receptors and many researchers around the world have been working on this issue.[12–14] We have also reported 1,4-benzoxazepine (1,4-BZO) derivatives which are selective 5-$HT_{1A}$ receptor agonists with potent anti-ischemic 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

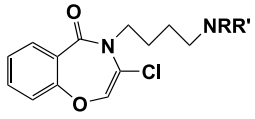
Compounds **18**–**23** were evaluated for their binding affinity to 5-$HT_{1A}$, dopamine $D_2$ and $\alpha_1$-adrenergic receptor by radioligand binding assays (Table 1). The specific ligands and tissue sources used were as follows: (a) 5-$HT_{1A}$ serotonergic receptor: [$^3$H]8-OH-DPAT (8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin), rat hippocampus membranes; (b) dopamine $D_2$ receptor: [$^3$H] raclopride, rat striatum membranes; (c) $\alpha_1$-adrenergic receptor: [$^3$H]prazosin, rat cerebral cortex membranes.

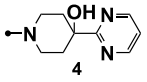
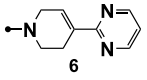
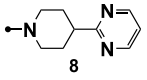
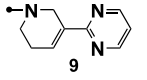
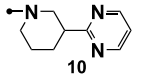
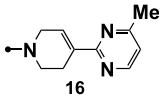
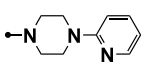
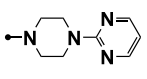
While alcohol derivative **18** showed a very weak affinity for 5-$HT_{1A}$ receptor, tetrahydropyridine derivative **19** and its piperidine derivative **20** displayed higher 5-$HT_{1A}$ receptor affinity and selectivity over both dopamine $D_2$ and $\alpha_1$-adrenergic receptors. $IC_{50}$ ratios 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 $\alpha_1$-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-$HT_{1A}$ | $D_2$ | $\alpha_1$ | $D_2$/5-$HT_{1A}$ | $\alpha_1$/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.

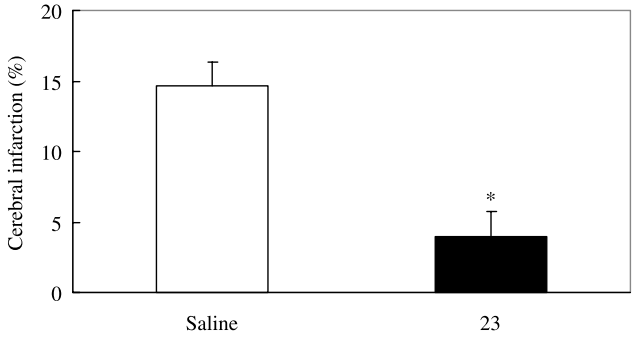



**Figure 2.** Effect of **23** on cerebral infarction in rats subjected to transient focal ischemia (60 min middle cerebral artery occlusion). Compound **23** (1 mg/kg) was administered sc immediately after occlusion of the MCA. Each datum represents mean ± SEM. $n = 5$; $^{*}p < 0.05$, versus saline (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 $\alpha_1$-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 $\alpha_1$-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-$d_6$): $\delta$ 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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