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New 5-HT_{1A} Receptor Agonists Possessing 1,4-Benzoxazepine Scaffold Exhibit Highly Potent Anti-Ischemic Effects

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Abstract—A series of new 3-substituted-4-(4-aminobutyl)-1,4-benzoxazepin-5(4*H*)-one derivatives (**1**–**5**) which showed a very high affinity for 5-HT_{1A} receptor with good selectivity over dopamine D_2 receptor was synthesized. Among these compounds, 3-chloro-4-[4-[4-(2-pyridinyl)-1,2,3,6-tetrahydropyridin-1-yl]butyl]-1,4-benzoxazepin-5(4*H*)-one (**5**: SUN N4057) exhibited remarkable neuroprotective activity in a transient middle cerebral artery occlusion (t-MCAO) model.

Acute cerebral ischemia is one of major causes of death and the pharmacological treatments have been focused on limiting neuronal damage, so called neuroprotection.[1] Serotonin (5-HT) and its receptors are known to play important roles in various physiological and pathophysiological processes.[2,3] Of these receptors, the 5-HT_{1A} receptor subtype is generally accepted to be involved in psychiatric disorders such as depression,[4] anxiety,[5] and psychosis.[6] It has been reported that 5-HT_{1A} receptor agonists have protective effects on the brain in cerebral ischemic conditions,[7–12] due to hyperpolarization of cell membrane[13] and glutamate release inhibition.[14]

Buspirone (Fig. 1) has developed as a 5-HT_{1A} agonist and it has been useful in the treatment of anxiety and depression.[15,16] This compound, however, is not optimal in terms of selectivity versus dopamine D_2 receptor. It has been said that dopamine D_2 antagonists might cause undesirable side effects such as prolactin stimulation[17] and extrapyramidal symptoms.[18] In order to obtain the high affinity and selectivity for the 5-HT_{1A} receptor, we have attempted to modify the liposoluble unit and the amine part of Buspirone. In this paper, we describe the synthesis of novel 1,4-benzoxazepine (BZO) compounds (**1**–**5**) which bind to 5-HT_{1A} receptor with good selectivity over dopamine D_2 receptor. The neuroprotective effect in an in vivo t-MCAO model is also presented.

Chemistry

BZO derivatives **1**–**5**[19] were prepared by the pathway shown in Scheme 1. 3-H-BZO **1** was prepared from salicylamide **6**. Compound **6** was selectively *O*-alkylated with 2-bromomethyl-1,3-dioxolane in the presence of K_2CO_3 and followed by cyclization with 10% HCl and dehydration with methanesulfonyl chloride and triethylamine, giving **7**. Compound **1** was obtained by the alkylation of **7** with 1-bromo-4-chlorobutane and

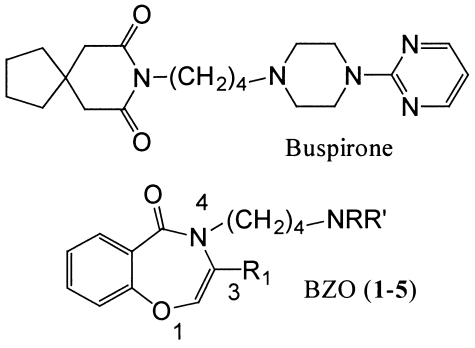

Figure 1. Buspirone and 3-substituted-4-(4-aminobutyl)-1,4-benzoxazepine-5(4*H*)-one derivatives (**1**–**5**).

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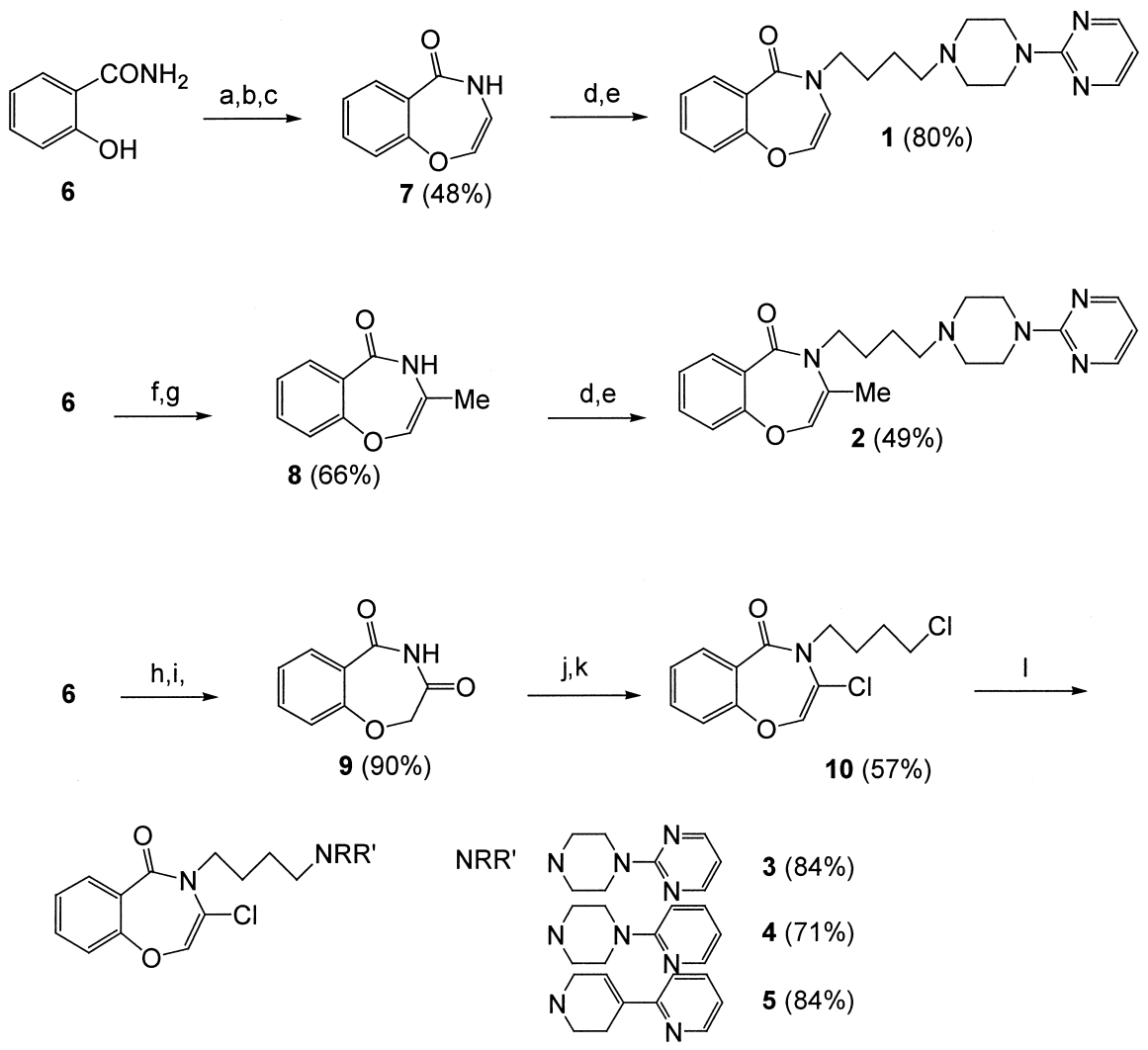


Scheme 1. Reagents: (a) K_2CO_3, 2-bromomethyl-1,3-dioxolane; (b) 10% HCl; (c) MsCl, Et_3N; (d) NaH, $Br(CH_2)_4Cl$; (e) NaI, Et_3N, 1-(2-pyrimidinyl)piperazine; (f) K_2CO_3, $BrCH_2COMe$; (g) *p*-TsOH; (h) K_2CO_3, $BrCH_2CO_2Et$; (i) NaOEt; (j) K_2CO_3, $Br(CH_2)_4Cl$; (k) $POCl_3$, $PhNEt_2$; (l) NaI, Et_3N, HNRR′.

the subsequent amination with 1-(2-pyrimidinyl)piperazine. 3-Me-BZO **2** was prepared in the same way as 3-H-BZO **1**, by employing bromoacetone instead of 2-bromomethyl-1,3-dioxolane.

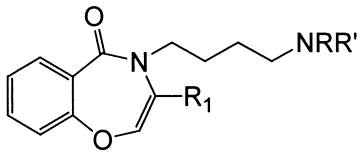
3-Cl-BZO **3**–**5** were synthesized through the intermediate 1,4-benzoxazepine-3,5-dione **9**. Compound **9** was prepared by *O*-alkylation of **6** with ethyl bromoacetate and subsequent cyclization by treatment with sodium ethoxide. Compounds **3**–**5** were prepared by *N*-alkylation of **9** with 1-bromo-4-chlorobutane and followed by chlorination with $POCl_3$ in the presence of *N,N*-diethylaniline and the subsequent amination with the corresponding amine.[20]

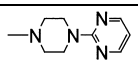
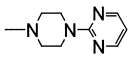
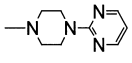
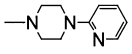
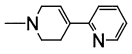
Results and Discussion

Compounds **1**–**5** in Table 1 were evaluated for their binding affinity to 5-HT_{1A} and dopamine D_2 receptor by radioligand binding assays. The specific ligands and tissue sources were used as follows: (a) 5-HT_{1A} serotonergic receptor:[21] [^{3}H]8-OH-DPAT (8-hydroxy-2-(*N,N*-di-*n*-propylamino)tetralin), rat hippocampus membranes; (b) dopamine D_2 receptor:[22] [^{3}H]Raclopride, rat striatum membranes.

We investigated the effects of various groups at the 3-position of the BZO derivatives (see **1**–**3**). The methylene chain length at the 4-position of the BZO and the amino moiety were the same as Buspirone. As regards the 5-HT_{1A} receptor, 3-Cl-BZO **3** showed higher binding affinity than 3-H-BZO **1** and 3-Me-BZO **2**. 3-Cl-BZO **3** showed stronger affinity and more selectivity for 5-HT_{1A} receptor than Buspirone. The amine moiety of the side chain was of great importance for both 5-HT_{1A} receptor binding affinity and selectivity (see **3**–**5**). Replacement to 1-(2-pyridinyl)piperazinyl group **4** from 1-(2-pyrimidinyl)piperazinyl group **3** showed more potent binding to 5-HT_{1A} receptor but poorer selectivity over dopamine D_2 receptor. The 4-(2-pyridinyl)-1,2,3,6-tetrahydropyridinyl derivative **5**, however, exhibited the highest affinity and selectivity for 5-HT_{1A} receptor.[23]

Table 1. Structures and their receptor binding data of 3-substituted-4(4-aminobutyl)-1,4-benzoxazepin-5(4*H*)-one derivatives (**1**–**5**)



Compd[a]	R_1	NRR′	IC_{50} (nM) 5-HT_{1A}	IC_{50} (nM) D_2	IC_{50} ratio D_2/5-HT_{1A}
1[b]	H		18.0	NT	—
2[b]	Me		10.2	1100	108
3[b]	Cl		1.59	199	125
4[b]	Cl		0.77	51	66
5[c]	Cl		0.47	84	179
Buspirone			11.0	55	5

[a]All compounds had analytical results within 0.4% of the theoretical values.
[b]Fumarate.
[c]$2HCl{\cdot}2H_2O$.

Table 2. Neuronal protective effects of compounds against ischemic brain damage in rat t-MCAO model[a]

Compd	% inhibition
5 (SN4057)	63%**
Buspirone	53%**
Dimethylaminoantipyrine	21%

[a]See ref 27 for details. $^{**}p < 0.01$ versus vehicle (one-way ANOVA followed by Dunnett's multiple comparison test).

Compound **5** is a 5-HT_{1A} receptor agonist because it inhibits forskolin-stimulated adenylate cyclase activity in plasma membrane prepared from the rat hippocampus ($IC_{50} = 2.67 \pm 0.74$ nM). Next, we investigated in vivo neuroprotective effect of 3-Cl-BZO **5** in a rat model of transient focal cerebral ischemia (Table 2). Male Wistar rats were subjected to t-MCAO using the intraluminal suture method of Koizumi et al.[24] The tested compounds and vehicle (saline) were subcutaneously administered immediately after the occlusion. The measurement of peripheral type benzodiazepine binding sites (PTBBS) in ipsilateral cortical and striatal homogenates was carried out as an index for quantification of neuronal damage 10 days after recirculation.[25,26] Compound **5** and Buspirone reduced the increase in PTBBS levels at a dose of 1 mg/kg sc.[27] Single administration of **5** at doses of 0.1, 0.3 and 1 mg/kg sc immediately after t-MCAO exerted a dose-dependent reduction of the increase in PTBBS levels by 21, 32* and 63%**, respectively ($^{*}p < 0.05$ vs vehicle, $^{**}p < 0.01$ vs vehicle).

In this model, rectal temperature was found to increase during ischemia to above 38.5 °C, but compound **5** reduced the ischemic hyperthermia at the neuroprotective doses. It has been reported that 5-HT_{1A} agonists possess a hypothermic effect.[28] In contrast, 4-dimethylaminoantipyrine, an antipyretic drug, at a dose of 200 mg/kg ip immediately after t-MCAO did not affect PTBBS levels by only 21% inhibition, although it caused hypothermia to the same degree as **5** (1 mg/kg sc). These results indicate that pharmacological effects in addition to the hypothermic effect are involved in the mechanism of neuroprotective effect of compound **5**.

In conclusion, we described the synthesis and biological evaluation of a novel class of 1,4-benzoxazepine derivatives **1**–**5** that show not only highly potent affinity for 5-HT_{1A} receptor but also low affinity for dopamine D_2 receptor. Since compound **5** is a potent and selective 5-HT_{1A} receptor agonist compared with Buspirone and has a desirable neuroprotective effect in vivo, it might be more suitable for a therapeutic agent for ischemic neuronal damage. The SARs of this series of compounds will be reported elsewhere. Compound **5** (SUN N4057) is currently being developed for treatment of acute phase of cerebral infarction.

Acknowledgements

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19. All compounds were fully characterized by spectral methods, and their purity checked by elemental analysis. Representative data of the most potent derivative: compound **5** ($2HCl{\cdot}2H_2O$); mp 133–134 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 1.70–1.76 (m, 2H), 1.84–1.86 (m, 2H), 2.91–2.93 (m, 2H), 3.21–3.25 (m, 3H), 3.64–3.67 (m, 1H), 3.84–3.87 (m, 3H), 4.03–4.08 (m, 1H), 6.74–6.76 (m, 1H), 7.14–7.17 (m, 2H), 7.34 (t, 1H, $J=8$ Hz), 7.42–7.44 (m, 1H), 7.60 (t, 1H, $J=8$ Hz), 7.70–7.72 (m, 1H), 7.79 (dd, 1H, $J=2$ and 8 Hz), 7.95–7.97 (m, 1H), 8.61–8.63 (m, 1H), 10.74 (br s, 1H); IR (KBr) cm^{-1}: 3320, 3015, 2600, 1644, 1612, 1513, 1455; FAB-MS *m/z*: 410 ($M^+ + 1$). Anal. calcd for $C_{23}H_{26}Cl_3N_3O_2\ 2H_2O$: C, 53.24; H, 5.83; N, 8.10. Found: C, 53.56; H, 6.04; N, 7.92.
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27. [^{3}H]PK 11195 was used as a radio-labeled ligand for PTBBS and its amount binding to the crude of cerebral cell membrane was measured. The PTBBS levels of rats subjected to t-MCAO were 4- to 6-fold higher than that of sham-operated group. Experimental data of **5**: [^{3}H]PK 11195 binding (pmol/g protein); saline treated ($n=14$): 402 ± 35; compound **5** treated ($n=11$): 197 ± 20; sham ($n=11$): 78 ± 7; Each data represents mean $\pm$ S.E.M. 63% inhibition.
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