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N-{[1-(2-Phenylethyl)pyrrolidin-2-yl]methyl}cyclohexanecarboxamides as Selective 5-HT_{1A} Receptor Agonists

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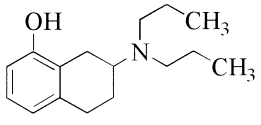
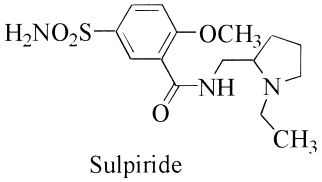
Abstract—A series of benzamides was synthesized as selective agonists for the 5-HT_{1A} receptor. It was found that (*S*)-*N*-{[1-(2-phenylethyl)pyrrolidin-2-yl]methyl}cyclohexanecarboxamide(**7-(*S*)**) has potent and selective agonistic activity for the 5-HT_{1A} receptor (5-HT_{1A}; $K_i = 0.49$ nmol/L, D_2; $IC_{50} = >1000$ nmol/L, 5-HT_2; $K_i = 240$ nmol/L).

The 5-HT_{1A} receptor, one of the subtypes of the 5-HT receptor, is distributed in the limbic area of the brain and has been linked to emotional response. The variety of possible therapeutic uses for 5-HT_{1A} agonists and antagonists has prompted intensive research, and several potent and selective 5-HT_{1A} ligands have been reported over the past decade.[1] Recently, it has been suggested that 5-HT_{1A} agonists reduce the occurrence of the extrapyramidal side effects (EPS) induced by typical neuroleptics.[2,3] However, considerable research efforts over the years have afforded only a handful of compounds which act as selective full agonists at 5-HT_{1A} receptors, for example, 8-OH-DPAT.[4] We attempted to find a new class of selective 5-HT_{1A} agonists.



8-OH-DPAT



Sulpiride

Several substituted benzamides, as represented by sulpiride, have been shown to be selective and potent dopamine D_2 receptor antagonists.[5,6] Meanwhile, we previously reported that several substituted benzamides, which were *N*-(2-pyrrolidinylmethyl)-2,3-dihydro-1-benzofuran-6-carboxamide derivatives or *N*-(2-pyrrolidinylmethyl)-2-methoxy-5-sulfamoylbenzamide derivatives (**1-(*S*)**, **(*R*)–2-(*S*)**, **(*R*)**), possess high affinity for the 5-HT_{1A} receptor.[7,8] The chemical structure of these compounds is different from those of the well known 5-HT_{1A} ligands. However, these benzamide derivatives also possess high affinity for the dopamine D_2 receptor. The task is, therefore, to heighten their selectivity for the 5-HT_{1A} receptor by reducing D_2 receptor affinity. In order to increase selectivity for the 5-HT_{1A} receptor, we attempted to modify the substituents on the benzene ring of the benzamides and the substituent at the 1-position of the pyrrolidine ring. In this communication, we report that the deletion of the alkoxy substituent on the benzene ring gave selectivity toward the 5-HT_{1A} receptor, and that the replacement of the benzene ring with a cycloalkyl ring increased 5-HT_{1A} receptor affinity.

Compounds **3-(*S*)**, **(*R*)–6-(*S*)**, and **(*R*)** were synthesized by coupling of the corresponding carboxylic acids **8a–d** with the enantiomer of [1-(2-phenylethyl)pyrrolidin-2-yl]methylamine **9**[8] via mixed anhydrides or acid chlorides, as shown in Scheme 1. The affinity of compounds **3-(*S*)**, **(*R*)–6-(*S*)**, and **(*R*)** for 5-HT_{1A}, dopamine D_2, and 5-HT_2 receptors is shown in Table 1, together with that of **1-(*S*)**, **(*R*)–2-(*S*)** and **(*R*)**.[8] Affinity for the serotonergic

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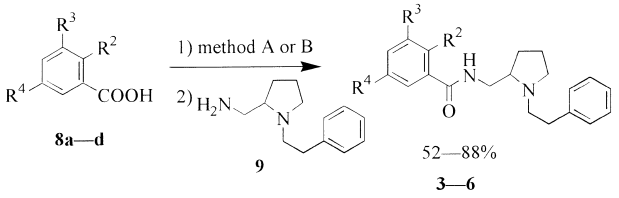


Scheme 1. 8a: R^2–R^3 = -OCH_2CH_2-, $R^4 = SCH_3$; **8b**: $R^2 = OCH_3$, $R^3 = H$, $R^4 = SCH_3$; **8c**: $R^2 = H$, $R^3 = H$, $R^4 = SCH_3$; **8d**: $R^2 = H$, $R^3 = H$, $R^4 = H$. Method A: iso-BuOCOCl, Et_3N; method B: $SOCl_2$.

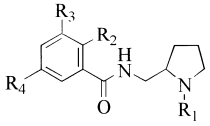
5-HT_{1A} and 5-HT_2 receptors was measured in terms of the ability of the compounds to displace [^{3}H]8-OH-DPAT and [^{3}H]ketanserin, respectively, from 5-HT_{1A} and 5-HT_2 receptors isolated from the striata of male Wistar rats. Affinity for dopamine D_2 receptor was determined by displacement of [^{3}H]spiperone.

Of the benzoxazine-8-carboxamide derivatives, *n*-butyl-substituted **1-(*S*)** possessed high affinities for both 5-HT_{1A} and D_2 receptors (K_i = 34 and 12 nmol/L, respectively). Replacement of the *n*-butyl substituent with a 2-phenylethyl substituent reduced the affinity for D_2 receptors and slightly potentiated that for 5-HT_{1A} receptors (**1-(*S*)** and **2-(*S*)**). (*S*)-Enantiomers had higher affinity for 5-HT_{1A} receptors than the (*R*)-enantiomers (**1-(*S*)**, **(*R*)** and **2-(*S*)**, **(*R*)**). The benzofuran derivatives **3-(*S*)** and **3-(*R*)** had similar affinities of the benzoxazine derivatives **2-(*S*)** and **2-(*R*)** for 5-HT_{1A} receptors. Thus, (*S*)-enantiomers of the benzofuran derivatives and benzoxazine derivatives had higher affinity for 5-HT_{1A} receptors than the (*R*)-enantiomers. Conversion of the furan ring to a methoxy substituent reduced affinity for D_2 receptors (K_i = 120 and 460 nmol/L, respectively) without significant influence on 5-HT_{1A} receptor affinity (**3-(*S*)** and **4-(*S*)**, K_i = 21 and 24 nmol/L, respectively). It has already been reported that, in substituted benzamides, the hydrogen bond between the oxygen atom of the methoxy substituent on the benzene ring and the amide proton potentiates affinity for D_2 receptors.[9–15] We removed this methoxy substituent, which not only reduced affinity for D_2 receptors (K_i = 460 nmol/L to IC_{50} = >1000 nmol/L, respectively), but also potentiated that for 5-HT_{1A} receptors, and produced substantially increased selectivity for 5-HT_{1A} receptors compared to benzofuran derivatives or methoxy derivatives (**5-(*S*)**, **6-(*S*)** and **3-(*S*)**). There was no significant difference in affinity for either 5-HT_{1A} or D_2 receptors (K_i = 4.3 nmol/L, >1000 nmol/L and 4.6 nmol/L, >1000 nmol/L) between compounds **5-(*S*)** and **6-(*S*)**; **6-(*S*)** had slightly better selectivity for 5-HT_{1A} versus 5-HT_2 receptors than **5-(*S*)**. Interestingly, **6-(*R*)**, the counterpart of **6-(*S*)**, had no affinity for the 5-HT_{1A} receptor (IC_{50} = >1000 nmol/L).

Next, we replaced the benzene ring of the benzamides with a cyclohexane ring to investigate the importance of the aromatic ring. Compounds **7-(*S*)**[16,17] and **(*R*)**[16] were synthesized by coupling of cyclohexanecarbonyl chloride with the enantiomer of [1-(2-phenylethyl)pyrrolidin-2-yl]methylamine **9**[8] as shown in Scheme 1. The replacement of the benzene ring with a cyclohexane ring (**6-(*S*)** and **7-(*S*)**) increased affinity for 5-HT_{1A} receptors (K_i = 0.49 nmol/L) as shown in Table 2. This result suggests that the aromatic ring is not important for 5-HT_{1A} receptor affinity.

The intrinsic activity of compound **7-(*S*)** for the 5-HT_{1A} receptor was evaluated by electrophysiological measurements of the 5-HT_{1A} receptor-mediated response in acutely dissociated dorsal raphe neurones. Methods are

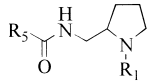
Table 1. Affinities of benzamides for 5-HT_{1A}, D_2 and 5-HT_2 receptors



Compound no.[a]	R_1	R_2	R_3	R_4	Configuration	Binding affinity K_i (nmol/L) 5-HT_{1A}[b]	D_2[c]	5-HT_2[d]
1-(*S*)[e]	*n*-Bu	-$OCH_2CH_2N(CH_3)$-		Cl	*S*	34	12	300
1-(*R*)[e]	*n*-Bu	-$OCH_2CH_2N(CH_3)$-		Cl	*R*	430	630	86
2-(*S*)[e]	CH_2CH_2Ph	-$OCH_2CH_2N(CH_3)$-		Cl	*S*	10	800	280
2-(*R*)[e]	CH_2CH_2Ph	-$OCH_2CH_2N(CH_3)$-		Cl	*R*	35	57	>1000[f]
3-(*S*)	CH_2CH_2Ph	-OCH_2CH_2-		OCH_3	*S*	21	120	660
3-(*R*)	CH_2CH_2Ph	-OCH_2CH_2-		SCH_3	*R*	100	6.7	430
4-(*S*)	CH_2CH_2Ph	OCH_3	H	SCH_3	*S*	24	460	170
5-(*S*)	CH_2CH_2Ph	H	H	SCH_3	*S*	4.3	>1000[f]	160
5-(*R*)	CH_2CH_2Ph	H	H	SCH_3	*R*	110	>1000[f]	>1000[f]
6-(*S*)	CH_2CH_2Ph	H	H	H	*S*	4.6	>1000[f]	430
6-(*R*)	CH_2CH_2Ph	H	H	H	*R*	>1000[f]	>1000[f]	>1000[f]

[a]All compounds gave satisfactory IR, ^{1}H NMR, MS and elemental analysis. The enantiomeric purities of the enantiomers were confirmed to be >98% ee by HPLC (column: Chiralpac OD (DAICEL Chemical Industries, Ltd.)).
[b][^{3}H]8-OH-DPAT binding.
[c][^{3}H]spiperone binding.
[d][^{3}H]ketanserin binding.
[e]5-HT_{1A}, D_2 and 5-HT_2 receptor affinities of compounds 1-**(*S*)**, **(*R*)**–2-**(*S*)**, **(*R*)** have previously been reported.[8]
[f]IC_{50} value.

Table 2. Affinities of benzamides for 5-HT_{1A}, D_2, and 5-HT_2 receptors



Compound no.[a]	R_1	R_5	Configuration	Binding affinity K_i (nmol/L) 5-HT_{1A}[b]	D_2[c]	5-HT_2[d]
6-(*S*)	CH_2CH_2Ph		*S*	4.6	>1000[e]	430
6-(*R*)	CH_2CH_2Ph	phenyl	*R*	>1000[e]	>1000[e]	>1000[e]
7-(*S*)	CH_2CH_2Ph		*S*	0.49	>1000[e]	240
7-(*R*)	CH_2CH_2Ph	cyclohexyl	*R*	190	>1000[e]	>1000[e]
8-OH-DPAT				0.47	>1000[e]	>1000[e]
Haloperidol				>1000[e]	1.5	43
Ketanserin				>1000[e]	240	0.66

[a]All compounds gave satisfactory IR, ^1H NMR, MS and elemental analysic. The enantiomeric purities of the enantiomers were confirmed to be >98% ee by HPLC (column Chiralpac OD (DAICEL Chemical Industries, Ltd.)).
[b]$[^3H]$8-OH-DPAT binding.
[c]$[^3H]$spiperone binding.
[d]$[^3H]$ketanserin binding.
[e]IC_{50} value.

described in detail in a previous report.[18] Compound **7-(*S*)** (10^{-7} mol/L) induced an inward current with peak amplitude of 70.0 ± 28.1 pA, and after 30-s treatment with WAY-100635 (10^{-6} mol/L), a selective 5-HT_{1A} antagonist, the **7-(*S*)**-induced inward current declined to 1.7 ± 1.5 pA (Fig. 1A). Figure 1B shows the dose–response curve of the inward current induced by compound **7-(*S*)** and 8-OH-DPAT. Maximum response of compound **7-(*S*)** was about 90% of the response by 10^{-7} M 8-OH-DPAT. EC_{50} values for compound **7-(*S*)** and 8-OH-DPAT were 4.2 and 14.0 nmol/L, respectively. These results indicate that compound **7-(*S*)** is a potent 5-HT_{1A} agonist with high intrinsic activity.

In conclusion, we found (*S*)-*N*-{[1-(2-phenylethyl)pyrrolidin-2-yl]methyl}cyclohexanecarboxamide **7-(*S*)** to be a selective full 5-HT_{1A} agonist. **7-(*S*)** possessed high affinity and good selectivity for 5-HT_{1A} receptors. Further biochemical and pharmacological studies are in now progress on the compound **7-(*S*)**.

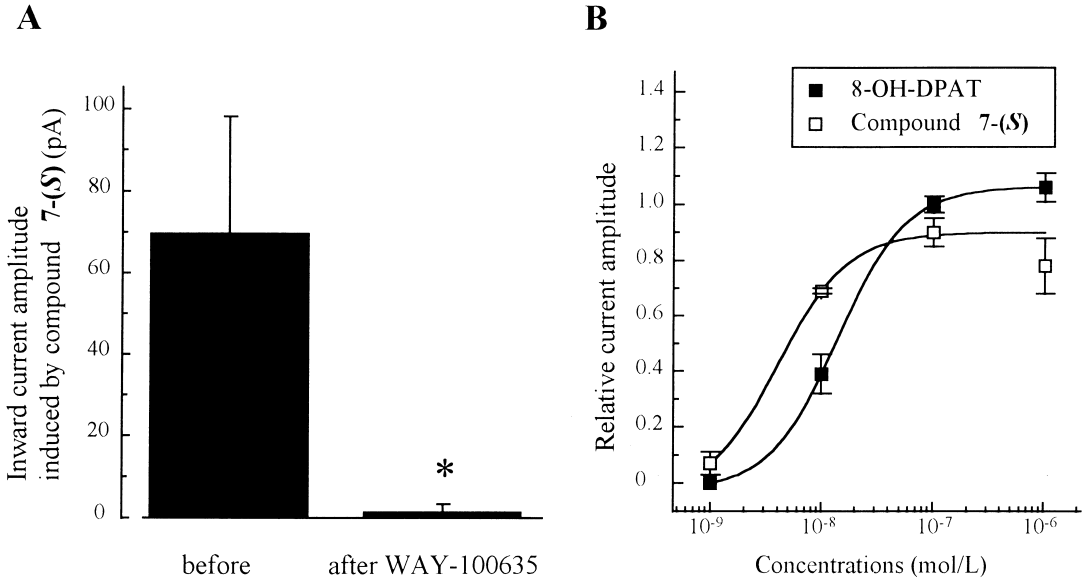


Figure 1. Compound **7-(*S*)** acts as a potent 5-HT_{1A} agonist. A: Amplitudes of the inward current induced by compound **7-(*S*)** (10^{-7} mol/L) before and after treatment with WAY-100635. WAY-100635 (10^{-6} mol/L) was pretreated for 30 s followed by simultaneous application with compound **7-(*S*)**. *Significantly different from before WAY-100635 treatment (paired *t*-test, $P < 0.05$). B: Concentration–response relationships of inward current induced by compound **7-(*S*)** and 8-OH-DPAT. Non-linear regression was used for curve fitting and EC_{50} estimation. Values (A and B) expressed as the mean ± SEM of 3 neurones.

Acknowledgements

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16. Optical rotations were measured in methanol at 25 °C for compounds **7-(*S*)** (−83.2°, c = 1.0) and **7-(*R*)** (+82.0°, *c* = 1.0).
17. Data of **7-(*S*)**: colourless crystals, mp 89–91 °C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.14–1.33 (5H, m), 1.41–1.47 (1H, m), 1.58–1.83 (9H, m), 2.20–2.25 (1H, m), 2.46–2.55 (2H, m), 2.70–2.97 (4H, m), 3.27–3.31 (1H, m), 3.44–3.50 (1H, m), 5.50 (1H, brs), 7.19–7.31 (5H, m).
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