(S)-N-tert-Butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide [(S)-WAY-100135]: A Selective Antagonist at Presynaptic and Postsynaptic 5-HT$_{1A}$ Receptors

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The increasing number of receptor subtypes for the neurotransmitter serotonin (5-HT) has attracted the attention of many pharmaceutical companies. In particular, the discovery of high-affinity binding to the 5-HT$_{1A}$ receptor by the non-benzodiazepine anxiolytic agent buspirone has encouraged the development of selective 5-HT$_{1A}$ receptor ligands as potential drug candidates. Facilitation of 5-HT neurotransmission in general has an anxiogenic action in animal models, and buspirone, which acts as a partial agonist at the 5-HT$_{1A}$ receptor, may produce its anxiolytic effect by means of either an agonist action at the presynaptic somatodendritic 5-HT$_{1A}$ receptor or an antagonist action at the postsynaptic 5-HT$_{1A}$ receptor. A number of selective 5-HT$_{1A}$ receptor agonists have now been developed but few substantiated reports of selective 5-HT$_{1A}$ receptor antagonists have been made. Such compounds would be of immense value as pharmacological research tools.[1]

Several compounds previously thought to act as 5-HT$_{1A}$ receptor antagonists have now been shown to inhibit raphe cell firing and decrease 5-HT release *via* agonist actions at the somatodendritic 5-HT$_{1A}$ receptor. These compounds are now best classified as 5-HT$_{1A}$ receptor partial agonists.[1] Examples of such agents are the (aminomethyl)-benzodioxan binospirone (MDL-73005EF),[2] the β-adrenoreceptor antagonists, *e.g.* (−)-pindolol,[3] and the arylpiperazines BMY-7378[4] and NAN-190.[5] The Sandoz compound SDZ 216-525 is claimed[6] to be a potent, selective, and "silent" 5-HT$_{1A}$ receptor antagonist, but supporting pharmacological evidence regarding the action of this compound at the presynaptic 5-HT$_{1A}$ receptor has not been presented. To the best of our knowledge, (S)-UH-301[7] is the only compound which acts as an antagonist at both the presynaptic and postsynaptic 5-HT$_{1A}$ receptor, but (S)-UH-301 shows only an 8-fold selectivity for 5-HT$_{1A}$ binding sites compared to D$_2$ sites.[8]

We now report initial studies from our laboratory which show that racemic *N-tert*-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide dihydrochloride (WAY-100135, 4) is a highly selective and potent antagonist at both the presynaptic and postsynaptic 5-HT$_{1A}$ receptor. This compound also displays no 5-HT$_{1A}$ agonist activity.[9] We also show that the pharmacological effects of 4 reside mainly in the (S)-enantiomer 5.

The synthesis of 4 and 5 is shown in Scheme I.[10] Michael reaction of 1-(2-methoxyphenyl)piperazine (1) and atropic acid (2)[11] gives the amino acid 3. Reaction of 3 with *tert*-butylamine using 1,1′-carbonyldiimidazole (CDI) as the coupling reagent produces the free base of 4, which is resolved by multiple recrystallizations of (−)-di-*p*-toluoyl-L-tartaric acid salts in acetonitrile.[12] It should be noted that the optical rotation of 5 in the free base form has a positive sign of rotation, whereas the dihydrochloride salt has a negative sign of rotation. The absolute configuration of the (−)-di-*p*-toluoyl-L-tartaric acid salt of 5 was determined by X-ray crystallography to be *S*.[13]

Scheme I

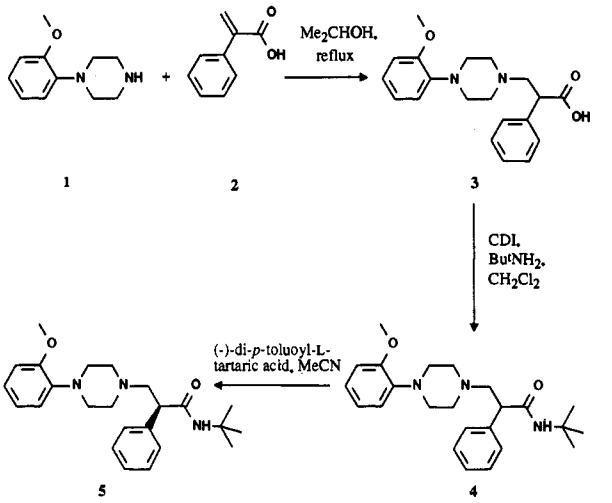

Table I. In Vitro Receptor Binding Profile of 4, 5, (R)-4, and Buspirone

	IC$_{50}$ ± SEM (nM) or [% inhibition at 10^{-6} M ± SEM][a]			
binding site	4	5	(R)-4	buspirone
5-HT$_{1A}$	33.9 ± 3.3	15.5 ± 4.6	437 ± 128	24[b]
5-HT$_{1B}$	[27 ± 6]	[22 ± 10]	[21 ± 10]	nt[c]
5-HT$_{1C}$	[37 ± 8]	[50 ± 6]	[14 ± 7]	1000 ± 300
5-HT$_2$	[37 ± 12]	1393 ± 400	[13 ± 4]	2150 ± 442
D$_1$	[20 ± 4]	[22 ± 6]	[2 ± 2]	nt[c]
D$_2$	[14 ± 3]	[20 ± 3]	[4 ± 4]	265 ± 35[b]
α-1	1491 ± 404	1878 ± 808	2781 ± 651	>1000[b]
α-2	[10 ± 4]	[11 ± 6]	[7 ± 6]	>1000[b]
β	[20 ± 7]	[12 ± 8]	[7 ± 6]	>1000[b]

[a] Reference 17. [b] Data from ref 18. [c] Not tested.

The *in vitro* receptor binding profile (Table I) indicates that 4 is a highly selective ligand, having an IC$_{50}$ value of 34 nM at the 5-HT$_{1A}$ binding site and IC$_{50}$ values of >1000 nM for a range of other 5-HT, dopamine D$_2$, and noradrenergic binding sites. The (S)-enantiomer 5 has higher affinity than the (R)-enantiomer and like the racemate 4 shows excellent selectivity. The binding profile of buspirone is included in Table I for the purposes of comparison.

Postsynaptic 5-HT$_{1A}$ receptor function was assessed using the 5-HT$_{1A}$ agonist-induced behavioral syndrome in the rat (extended flat body posture, forepaw treading, and hyperlocomotion).[14] Compound 4 and its enantiomers at doses up to 10 mg/kg iv induce no behavioral effects related to 5-HT$_{1A}$ agonist activity. Pretreatment of rats with 4 30 min prior to the intravenous administration of the standard 5-HT$_{1A}$ receptor agonist 8-OH-DPAT results in an increase in the dose necessary for 8-OH-DPAT to induce the behavioral syndrome. The minimum effective dose (MED) of 4 to block the effect of 8-OH-DPAT is 3 mg/kg sc. This antagonist property of 4 appears to be stereoselective. The (S)-enantiomer 5 has an MED value

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of 1 mg/kg sc whereas the (*R*)-enantiomer is inactive up to a dose of 10 mg/kg sc.

The action of **4** at the presynaptic 5-HT_{1A} receptor was measured by *in vivo* microdialysis experiments in conscious freely-moving rats. Up to a dose of 10 mg/kg sc, **4** and its enantiomers have no significant effects on extracellular levels of 5-HT in the rat hippocampus within 2.5 h of drug administration. In contrast, BMY-7378 (5 mg/kg sc), buspirone (1 mg/kg sc), and 8-OH-DPAT (0.1 mg/kg sc) significantly decrease hippocampal levels of 5-HT to 37.6 ± 6.2, 39.9 ± 15.0, and 19.2 ± 9.9% of preinjection control levels, respectively. The antagonist activities of **4** and the (*S*)-enantiomer **5** at the presynaptic 5-HT_{1A} receptor were shown by their abilities to block the 8-OH-DPAT-induced decrease of 5-HT release. Pretreatment with either **4** or **5** at 10 mg/kg sc completely blocks the effects of 8-OH-DPAT (0.1 mg/kg sc). In contrast, the (*R*)-enantiomer has no significant effect on the 8-OH-DPAT response up to a dose of 10 mg/kg sc. These results support previous electrophysiological findings in anaesthetised rats which show **4** to have little or no intrinsic effect on raphe cell firing *in vivo* but to block the inhibition of firing caused by 8-OH-DPAT.[15,16]

In summary, **4** is a highly selective and potent antagonist at both presynaptic and postsynaptic 5-HT_{1A} receptors with activity residing mainly in the (*S*)-enantiomer **5**. Reports on anxiolytic activity in animal models, further pharmacological characterisation, and structure–activity relationships will be forthcoming.

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(10) All compounds exhibited ^{1}H NMR and IR spectra in accordance with their assigned structures. Elemental analyses of new compounds were within ±0.3% of the theoretical values for C, H, and N. Compound **3**: mp 160–163 °C [Anal. ($C_{20}H_{24}N_2O_3 \cdot 0.5H_2O$) C, H, N]. Compound **4**: mp 230–231 °C [Anal. ($C_{24}H_{33}N_3O_2 \cdot 2HCl$) C, H, N]. **5** dihydrochloride salt: mp 207–213 °C; $[\alpha]^{26}_D$ −48.8° (*c* 1.0, MeOH) [Anal. ($C_{24}H_{33}N_3O_2 \cdot 2HCl$) C, H, N]; free base is an oil $[\alpha]^{26}_D$ +8° (*c* 0.5, $CHCl_3$). (*R*)-**4** dihydrochloride salt: mp 204–214 °C; $[\alpha]^{26}_D$ +49.7° (*c* 1.0, MeOH) [Anal. ($C_{24}H_{33}N_3O_2 \cdot 2HCl$) C, H, N]; free base is an oil $[\alpha]^{24}_D$ −7.4° (*c* 0.7, $CHCl_3$).

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