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SYNTHESIS AND PHARMACOLOGICAL CHARACTERIZATION OF A-80426: A PUTATIVE NOVEL ANTIDEPRESSANT COMBINING α-2 ANTAGONISM WITH 5-HT UPTAKE INHIBITION[1]

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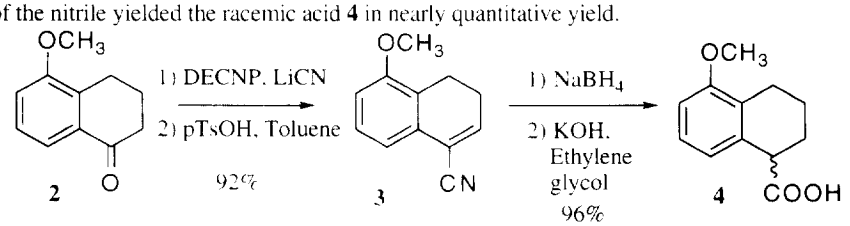
Abstract: A-80426, (N-[2-(Benzofuran-6-yl)ethyl]-N-[(R)-5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl-methyl]-N-methylamine) represents the first example of a new structural class of agents combining potent α-2 antagonist activity with equivalent 5-HT uptake inhibitory activity. This profile of combined activities may have utility in the treatment of depression.

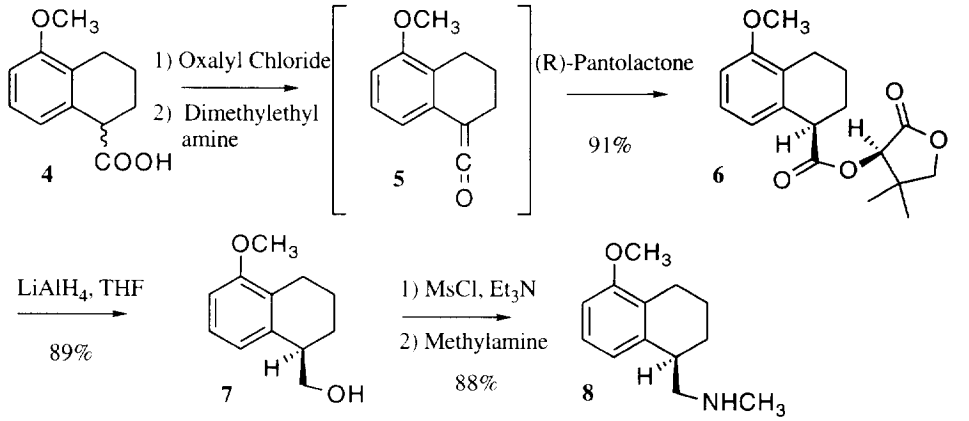
Modulation of biogenic amines, particularly serotonin and norepinephrine, is the principal pharmacological mechanism of action of all therapeutic agents currently in use for the treatment of depressive disorders.[2] Inhibitors of norepinephrine (NE) or serotonin (5-HT) reuptake are currently among the most efficacious pharmacological treatments of depression. However, neurotransmitter release is under the control of inhibitory presynaptic auto and heteroreceptors.[3-6] Several studies have indicated that there may be a correlation between desensitization of inhibitory presynaptic α-2 receptors and onset of antidepressant action.[7-9] The presence of presynaptic α-2 heteroreceptors on 5-HT neurons, and their inhibitory effect on 5-HT release, suggest that antagonism of these sites may act to increase synaptic concentrations of 5-HT. Thus, a combined profile of α-2 antagonism and 5-HT uptake inhibition may more efficaciously increase synaptic 5-HT availability than either pharmacological action alone, and consequently result in an improved antidepressant profile. Herein, we describe the synthesis and pharmacological characterization of a novel agent, A-80426 (**1**) which combines high affinity at α-2 receptors with potent and selective inhibitory activity for the reuptake of 5-HT, and present preliminary evidence of activity in an animal model predictive of antidepressant efficacy.[10-11]

Synthesis

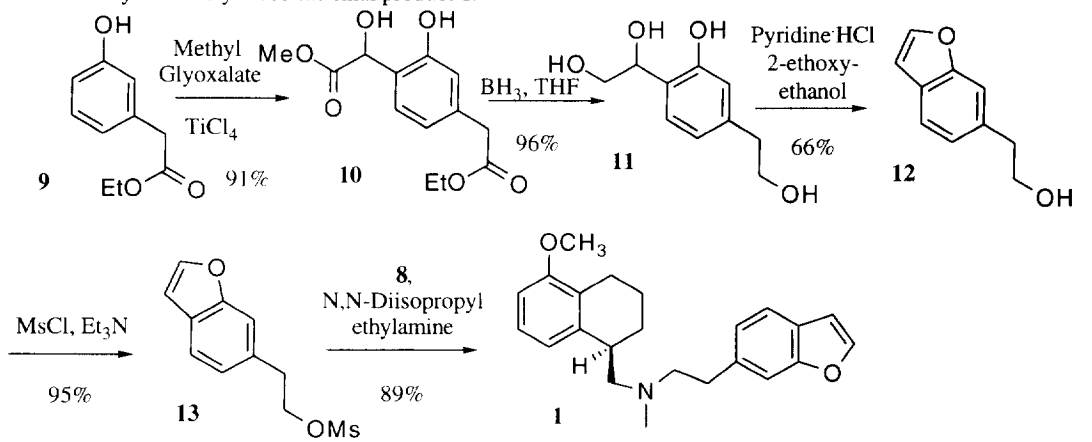
The title compound was prepared in enantiomerically pure form in eight steps from the commercially available 5-methoxytetralone. LiCN catalyzed condensation of the ketone **2** with diethylcyanophosphonate followed by acid catalyzed elimination yielded the unsaturated nitrile **3** in 92%. Conjugate reduction followed by hydrolysis of the nitrile yielded the racemic acid **4** in nearly quantitative yield.



In the key step in the synthetic sequence, the acid was converted to the ketene intermediate **5** which was then condensed with (R)-pantolactone to yield as the only detectable product the 1-(R) ester **6** in >98% *de.*[12] $LiAlH_4$ reduction proceeded without racemization to yield the alcohol **7**, which was then activated for displacement as its mesylate ester and then displaced with anhydrous methylamine (solvent) to yield the secondary amine **8**.



The benzofuran fragment was prepared from the ester of commercially available 3-hydroxyphenylacetic acid **9**. $TiCl_4$ mediated condensation of **9** with methyl glyoxalate regioselectively yielded the 1,2,4 substituted intermediate **10.**[13,14] Diborane reduction to the tetraol intermediate, followed by acid catalyzed elimination of water yielded the benzofuran-alcohol **12**. Activation of the alcohol by mesylation followed by displacement by the secondary amine **8** yielded the final product **1**.



The (S)-(-) enantiomer of **1** was prepared similarly, substituting (S)-ethyl lactate for (R)-pantolactone in the ketene addition step. The *de* in this addition was lower (92%) than that observed in the (R)-pantolactone addition. Several recrystallizations of the hydrochloride salt of the (S) enantiomer of **8** resulted in a product of identical, but opposite rotation to the product obtained from the (R)-pantolactone route.

Pharmacology

Compounds were characterized for affinity at α-1 (rat liver)[15] and α-2 (rat cortex) receptors in radioligand binding assays using [^{3}H]-prazosin and [^{3}H]-rauwolscine as radioligands, respectively. Affinity for the 5-HT uptake site was determined using [^{3}H]-paroxetine.[16] The functional biogenic amine reuptake inhibitory activity of these compounds was assessed in synaptosomal rat brain preparations.[13] Results are summarized in Table 1. Compound **1** exhibits high affinity for the α-2 binding site comparable to the standard α-2 antagonist rauwolscine, and demonstrates 50 fold selectivity vs. α-1 receptors. Affinity comparable to fluoxetine is observed at the paroxetine binding site. In functional assays of biogenic amine uptake inhibitory activity, **1** exhibits approximately 20 fold greater activity at the 5-HT uptake site than fluoxetine and approximately 2 fold lesser activity than paroxetine. Excellent selectivity for the 5-HT uptake site vs. NE and DA uptake sites is observed for **1**, exhibiting 66 and 224 fold selectivities respectively. The (S) enantiomer of **1** shows significantly weaker activity at the α-2 and paroxetine binding sites, as well as significantly decreased activity as an inhibitor of 5-HT uptake.

Table 1. *In Vitro* Pharmacology[a]

	Radioligand Binding Ki (nM) (95% Confidence Limit)			Biogenic Amine Uptake IC_{50} (nM) (95% Confidence Limit)		
Compound	α-1	α-2	Paroxetine	NE	5-HT	DA
A-80426	110 (66.3, 184)	2.01 (1.07, 3.78)	3.77 (2.82, 5.05)	865 (485, 1540)	13.1 (8.10, 21.1)	2940 (708, 12200)
1-s Isomer	241 (210, 278)	110 (35.5, 342)	92.0 (39.8, 212)	476 (296, 766)	864 (245, 3050)	4750 (3610, 6250)
Fluoxetine	2620 (1810, 3780)	1920 (720, 5130)	3.17 (2.40, 4.18)	3280 (602, 17800)	308 (255, 371)	16,200 (13000, 70100)
Paroxetine	5320 (2200, 12900)	6310 (3850, 10300)	0.077 (0.065, 0.093)	160 (65.3, 393)	6.43 (2.29, 18.0)	7620 (2130, 27300)
Rauwolscine	450 (345, 585)	2.89 (2.10, 3.98)	n.d.	>10,000 (n=1)	>10,000 (n=1)	>10,000 (n=1)

[a]Unless otherwise noted, all Ki and IC_{50} values represent the mean of at least three determinations.

A-80426 was evaluated for affinity at a variety of structurally homologous G-protein linked receptor binding sites. Affinity was assessed at β-1, β-2, D-1, D-2, 5-HT_1, and 5-HT_2 binding sites, and the results are summarized in Table 2. Very low affinity (> 10 μM) was observed for D-3, D-4, D-5, 5-HT_{2C}, H-1, H-2, H-3, muscarinic, nicotinic, excitatory amino acid and central benzodiazepine receptors.

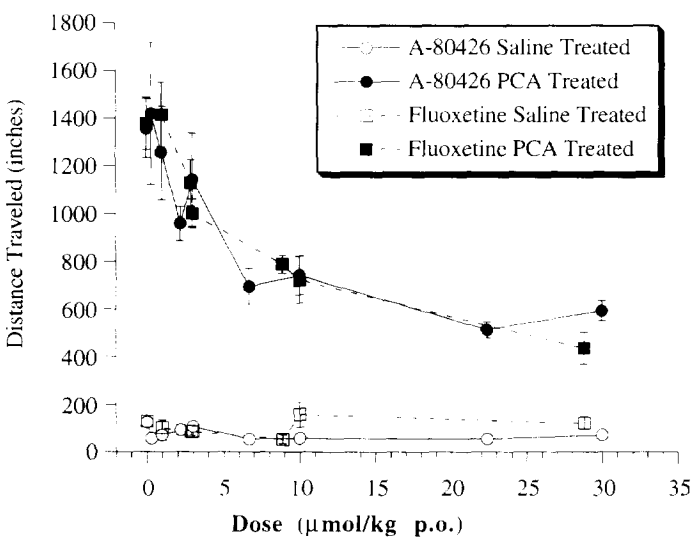
Table 2. Ancillary Radioligand Binding Affinities[a]

Receptor	Ki (nM)	n	95% Confidence Limits
β-1	836	6	(512, 1360)
β-2	1740	6	(1300, 2340)
D-1	744	9	(578, 958)
D-2	52	7	(31.9, 83.0)
5-HT_1	2970	3	(2010, 4380)
5-HT_2	144	3	(103, 202)

[a]The following radioligands and tissues were used for determination of Ki values. β-1: [^{3}H] DHA, rat ventricle; β-2: [^{3}H] DHA, rat lung; D-1: [^{3}H] SCH-23390, rat striatum; D-2: [^{3}H] spiroperidol, rat striatum; 5-HT_1: [^{3}H] 5-HT, rat cortex; 5-HT_2: [^{3}H] ketanserin, rat cortex.

To confirm the the *in vitro* 5-HT uptake inhibitory activity of **1**, the compound was examined for its ability to block p-chloroamphetamine (PCA) induced hyperactivity in rats.[17] PCA is a selective 5-HT neurotoxin which enters the serotonin neurons via the high affinity uptake pump, and elicits a variety of biochemical and behavioral activities, including hyperactivity. Prior administration of a 5-HT uptake inhibitor attenuates the PCA induced hyperactivity by preventing its uptake into the nerve terminals. An oral dose response study of **1** and fluoxetine, in which the compounds were administered 30 minutes before either saline or PCA, revealed equivalent PCA blocking activities for these two compounds. Results from this study are summarized in Figure 1.

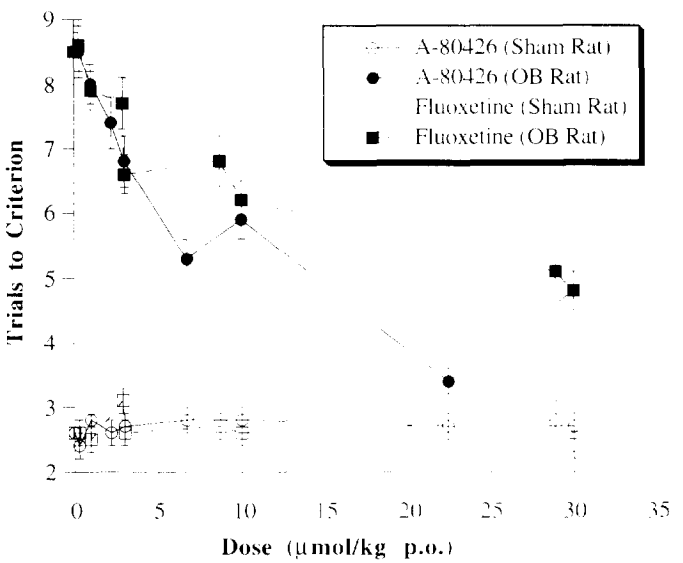
Figure 1. Reversal of PCA Induced Hyperactivity in Rats.



	ED_{50} (μmol/kg p.o.)	95% Conf. Limits
A-80426	13.3	(7.7 - 31.1)
Fluoxetine	11.7	(8.9 - 16.7)

To assess antidepressant potential of **1**, the olfactory bulbectomized (OB) rat model was used.[18] Removal of the olfactory bulbs from rats results in a variety of behavioral abnormalities, included among those a reduced ability to master a passive avoidance task. Of the observed abnormalities in OB rats, reversal of passive avoidance deficit correlates most highly with clinical efficacy of 5-HT uptake inhibitors. In this experimental paradigm, OB rats (ten per trial group) are placed on a platform above an electified grid. Stepping off the platform results in a mild foot shock. Whereas sham operated animals typically master the passive avoidance task in two to three trials, OB rats require greater than eight trials. If the animal fails to master the task after ten trials, the experiment is stopped. Administration of either **1** or fluoxetine 30 minutes before testing resulted in a dose-dependent reversal of the passive avoidance deficit, with **1** being perhaps slightly more efficacious than fluoxetine in this paradigm. Results are summarized in Figure 2.

Figure 2. Reversal of Passive Avoidance Deficit in Olfactory Bulbectomized Rats.



	ED_{70} (µmol/kg p.o.)	95% Conf. Limits
A-80426	7.1	(4.8 - 13.0)
Fluoxetine	12.1	(8.6 - 18.5)

Conclusion

A-80426 (**1**) represents a novel structural class which combines high affinity for the α-2 receptor with equivalent potency in the blockade of the reuptake of 5-HT. Both activities have been demonstrated to reside in a single enantiomer of the R configuration. Compound **1** exhibits comparable affinity for the α-2 receptor as the standard agent rauwolscine, and equivalent or greater binding and functional activity as an inhibitor of 5-HT uptake as fluoxetine. The *in vitro* uptake inhibitory activity can be demonstrated in an *in vivo* assay (inhibition of PCA induced hyperactivity) predictive of 5-HT uptake blockade, with equivalent oral activity to fluoxetine. Compound **1** has demonstrated potent, dose-dependent activity in an animal model predictive of clinical antidepressant efficacy, the olfactory bulbectomized rat model. Evidence exists that the slow onset of clinical efficacy for uptake inhibitor-based antidepressants correlates with adaptive changes in the α-2 receptor.[19,20]

Combined data from ten separate clinical investigations support an elevation in density of α-2 receptors in drug-free depressed patients and suggest a normalization of α-2 receptors following antidepressant drug treatment. Consequently, an agent which combines α-2 antagonism with 5-HT uptake inhibition may obviate the need for receptor desensitization to exhibit clinical efficacy, and therefore may exhibit a more rapid onset of antidepressant action.

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