(2*S*,3*S*,5*R*)-2-(3,5-Difluorophenyl)-3,5-dimethyl-2-morpholinol: A Novel Antidepressant Agent and Selective Inhibitor of Norepinephrine Uptake

James L. Kelley,*,† David L. Musso,† G. Evan Boswell,† Francis E. Soroko,‡ and Barrett R. Cooper*,‡

Divisions of Organic Chemistry and Pharmacology, Burroughs Wellcome Co., Research Triangle Park, North Carolina 27709

Received August 25, 1995

Introduction. Clinical depression is a common neurological disorder with a 1-year prevalence of 5−8% in clinical and community populations.[1,2] Until recently the tricyclic antidepressants and monoamine oxidase (MAO) inhibitors were the principal drugs in the psychiatrist's armamentarium. Because both classes of drugs suffer from side effects and other problems associated with their use, new antidepressant agents with greater efficacy and safety are needed.[3,4] Within the past 2 decades, a second generation of antidepressant agents has emerged. These new agents include bupropion (**1**, Wellbutrin), an amino ketone with a mechanism of action not explained by biochemical mechanisms common to other antidepressants,[5−9] and the serotonin-selective drugs such as fluoxetine and sertraline.[10] Efforts to develop new antidepressants that are selective for the noradrenergic system have been less successful. Nomifensine, a norepinephrine uptake inhibitor with promising clinical effects, was withdrawn from the market because of bone marrow suppression associated with its use.[11] Imipramine and related tricyclic drugs are norepinephrine-selective but have anticholinergic effects and the potential to cause cardiac depression.[12]

Bupropion (**1**) is a clinically efficacious, antidepressant agent that was first marketed in the United States in 1989 for the treatment of depressed patients unresponsive to, or intolerant of, other antidepressant therapy.[13−16] The potential of bupropion as an antidepressant agent was initially established through its activity in the anti-tetrabenazine test model in mice.[5] It is metabolized to several acidic and basic products that vary according to the species used to study metabolism. Welch et al.[17] reported that the rat metabolizes bupropion primarily by side-chain cleavage to acidic metabolites, whereas mice, dogs, and humans primarily form the basic, side-chain-hydroxylated product BW 306U. The structure of BW 306U was initially reported to be that of **2**, a noncyclic hydroxy ketone,[18,19] but a subsequent report revealed that BW 306U is the 2-phenylmorpholinol **3**.[17,20] Although the role of **3** in the clinical profile of bupropion is unresolved,[17,21,22] recent reports suggest that **3** may contribute to the antidepressant profile of bupropion in depressed patients.[23,24] Consequently, we initiated a program to study the structure−activity relationships of 2-phenylmorpholinols in animal models that are predictive of antidepressant activity in humans.

† Division of Organic Chemistry.
‡ Division of Pharmacology.

Research on phenylmorpholinols has led to the discovery of (3,5-difluorophenyl)morpholinol **4** (BW 1555U88), a potent, selective inhibitor of norepinephrine uptake. The compound is active in the anti-tetrabenazine animal behavioral model that responds to clinically effective antidepressant drugs, at doses that are free of other significant pharmacological activities. Compound **4** represents a novel, potent, selective inhibitor of norepinephrine uptake with antidepressant properties and, as such, may provide a therapeutic alternative to the serotonin uptake inhibitors currently used in clinical practice.

Chemistry. Compound **4** was prepared in three steps from 3,5-difluorobenzonitrile (**5**) (Scheme 1). The nitrile **5** was reacted with ethylmagnesium bromide in diethyl ether to give propiophenone **6** in high yield as an oil. Ketone **6** was brominated with dioxane dibromide[25] in dioxane to provide racemic bromide **7** as an oil in quantitative yield. An analytical sample of **7** was prepared by Kugelrohr distillation, bp 60−62 °C (0.3 mmHg). The crude bromopropiophenone **7** was reacted with (*R*)-(−)-2-amino-1-propanol in acetonitrile in the presence of 2,6-lutidine to give amino ketone **8**, which cyclized *in situ* to provide the cyclic ketal **4** as the hydrobromide salt, mp 240-241 °C dec. The hydrobromide salt was converted to the free base of **4** with aqueous sodium hydroxide in high yield, mp 113−115 °C. The free base of **4** was converted to the hydrochloride salt with ethereal hydrogen chloride to give (2*S*,3*S*,5*R*)-2-(3,5-difluorophenyl)-3,5-dimethyl-2-morpholinol hydrochloride (**4**) as a white solid, mp 255−257 °C.[26,27]

Pharmacology. The effects of **4** and three clinical antidepressant drugs on inhibition of biogenic amine uptake[28] are tabulated in Table 1. In a synaptosomal preparation of rat brain, **4** competitively inhibited biogenic amine uptake, with an IC_{50} of 0.07 μM for norepinephrine (NE) and 1.0 μM for dopamine (DA), and caused no significant inhibition of serotonin (5HT) uptake at 10 μM. Compound **4** was 30-fold more active than bupropion as an inhibitor of NE uptake and displayed inhibitory activity comparable to nomifensine and imipramine. The profile for inhibition of DA uptake (IC_{50} of 1.0 μM) with no effect on 5HT uptake is similar to that of nomifensine and bupropion. These data indicate that morpholinol **4** appears to be a potent inhibitor of NE uptake with weak affinity for DA uptake and no effect on 5HT uptake.

Compound **4** was active in the anti-tetrabenazine test, an animal model that is predictive for antidepressant activity in humans (Table 1). The compound prevented tetrabenazine-induced sedation[29] in rats and mice: oral ED_{50}s were 1.4 and 6.2 mg/kg, respectively. In rats **4** was more potent than the clinical antidepressant drugs nomifensine, imipramine, and bupropion, whereas in mice **4** was more potent than imipramine or bupropion and one-half as active as nomifensine. Compound **4** shows comparable potency to nomifensine in an animal model that is predictive for antidepressant activity in humans.

Compound **4** was evaluated in numerous receptor-binding, enzyme inhibition, and biogenic amine release test systems. Other than in the norepinephrine transport system, **4** exhibited no action that could explain its pharmacological effects in the anti-tetrabenazine

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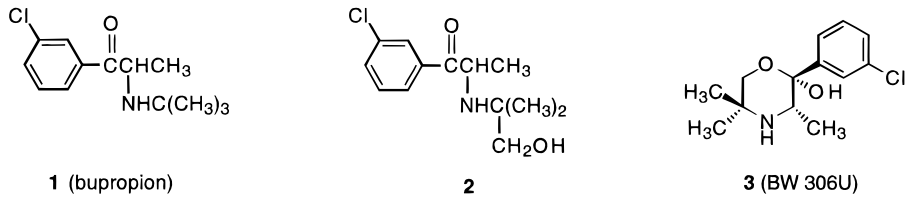
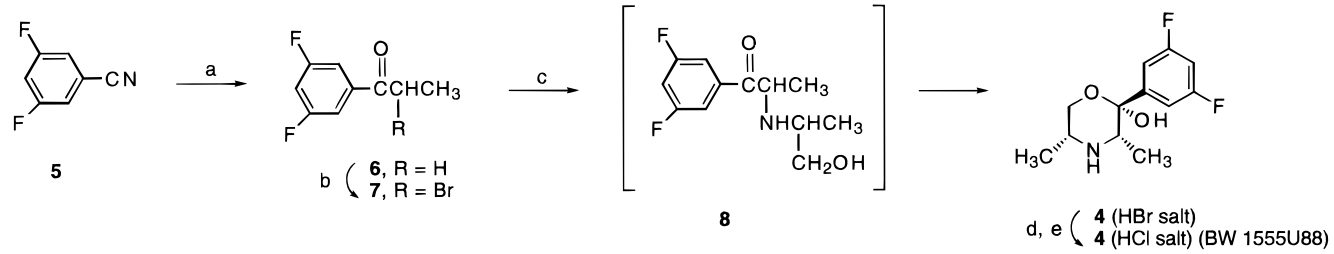


Figure 1.

Scheme 1[a]



[a] (a) EtMgBr, Et_2O; (b) dioxane·Br_2, dioxane; (c) (*R*)-(−)-2-amino-1-propanol, 2,6-lutidine, CH_3CN; (d) 40% aqueous NaOH, Et_2O; (e) Et_2O·HCl.

Table 1. Inhibition of Biogenic Amine Uptake and Anti-tetrabenazine Activity of **4** and Three Clinical Antidepressant Drugs

	in vitro inhibition of biogenic amine uptake (IC_{50}, μM)[a–c]			inhibition of tetrabenazine-induced sedation (po, ED_{50}, mg/kg)[d]	
compd	NE	DA	5HT	rat	mouse
4	0.07 ± 0.02	1.0 ± 0.4	>10	1.4 ± 0.2	6.2 ± 0.3
nomifensine	0.05 ± 0.02	0.5 ± 0.1	>10	3.3 ± 0.6	3.3 ± 0.4
imipramine	0.04 ± 0.01	>10	0.5 ± 0.1	13.4 ± 0.2	9.0 ± 0.6
bupropion	2.4 ± 1.4	0.9 ± 0.3	>10	*e*	32 ± 6

[a] IC_{50} values ± SEM were calculated using data from at least five separate experiments. In each experiment, the IC_{50} value was determined from the concentration−effect curve using duplicate samples as described in ref 28. [b] Designation >10 means the IC_{50} value is >10 μM. [c] NE = norepinephrine, DA = dopamine, 5HT = serotonin. [d] The compounds were tested as described in ref 29. ED_{50} values ± SEM were calculated using data from at least eight determinations of the ED_{50} in separate experiments. In each experiment doses from 0.5 to 5.0 μM were evaluated in a minimum of 5 animals/dose. [e] At doses up to 100 mg/kg po, bupropion failed to produce a 50% antagonism of tetrabenazine-induced sedation in rats.

models. At a concentration of 10 μM, **4** had no significant effects on the following receptor-binding assays: α-1 and α-2 noradrenergic, β-noradrenergic, dopamine 1 and 2, serotonin 1A and 2, benzodiazepine, substance P, leukotriene D4, muscarinic M1 and M2, and cholecystokinin. The A and B forms of monoamine oxidase were not inhibited at 10 μM, although modest inhibition (15% and 8%, respectively) of these enzymes was observed in *ex vivo* studies. Compound **4** did not cause release of NE from hypothalamic synaptosomes or dopamine from striatal synaptosomes at concentrations up to 10 μM.

The general central nervous system (CNS) effects of **4** were studied over a large range of oral doses in both rats (0.5, 1.0, 5.0, 15, 40, 250, and 500 mg/kg) and mice (15, 40, 100, 250, and 500 mg/kg po). Other than activity in the antidepressant screen, the lower doses (≥5 mg/kg in rats, ≥15 mg/kg in mice) increased locomotion and produced dose-related stereotyped behaviors such as sniffing, licking, and biting in both species. Behavioral reflexes, nociception, body temperature, and weight gain were affected at doses of ≥250 mg/kg in both rats and mice. Doses of 250 mg/kg resulted in coarse tremors in both species. Deaths occurred at 500 mg/kg in mice (1/4) and at 250 mg/kg (1/4) and 500 mg/kg (1/4) in rats. A large separation exists between doses producing significant neurological effects and lethality in rodents and those showing activity in the antidepressant screening test.

Compound **4** produced only transient, dose-dependent changes in blood pressure at multiples of the active dose. Blood pressure responses to NE injection were potentiated, consistent with NE uptake inhibition. Unlike the tricyclic antidepressants or fluoxetine, **4** did not block electrical conduction in the cardiac Perkinje fiber system at concentrations up to 1 mM.

In summary, **4** is a potent, selective inhibitor of NE uptake and demonstrates much weaker effects on dopamine and serotonin uptake. The compound is active in behavioral tests that respond to clinically effective antidepressant drugs; this is true at doses that do not produce other significant pharmacological activities. Unlike the tricyclic antidepressants, **4** does not bind to M1 or M2 receptors, nor does it block electrical conduction in cardiac tissue at 1 mM. Cardiovascular studies in dogs indicate that **4** is unlikely to produce adverse effects at plasma levels associated with the therapeutic effect. Increased locomotion activity is the first observable CNS effect in rodents. This effect occurs at doses 16-fold above those that provide plasma levels equal to the IC_{50} for NE transport inhibition. These doses are expected to produce antidepressant activity during chronic treatment. The pharmacological profile of **4** suggests that it will display antidepressant activity in humans and that this activity will involve inhibition of the NE uptake mechanism but without the cholinergic and cardiac depression effects associated with tricyclic antidepressants.

Acknowledgment. We acknowledge the assistance of Ms. S. Jones-Humble and Mr. B. T. Kenney, who performed the biogenic amine uptake and anti-tetrabenazine experiments. The development of **4** was supported with the secondary studies of Dr. P. Morgan and Ms. F. Tang (receptor-binding assays), Dr. H. White (monoamine oxidase assays), Ms. S. Jones-Humble (effect on synaptosomes), Dr. C. Wang (Perkinje fiber system measurements), Mr. O. Beek (cardiovascular studies in dogs), and Ms. V. M. Boncek (locomotor activity studies). Mr. R. C. Crouch assigned the correct 2-phenylmorpholinol structure for **3**. We thank Ms. D. T. Staton for the art work, the Burroughs Wellcome Co. Research Document Center for assistance in preparation of the manuscript, and Laura Mansberg for proofreading the final draft. The support and encouragement of Dr. R. M. Ferris and Dr. R. W. Morrison are greatly appreciated.

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(26) Physical data for **4**: $[\alpha]^{20}_D = +42.1°$ ($c = 0.687$, 95% EtOH); ^{1}H NMR (DMSO-d_6) δ 0.97 (d, 3H, CH_3), 1.20 (d, 3H, CH_3), 3.49 (br m, 2H, CH), 3.83 (m, 2H, CH_2), 7.17−7.34 (m, 3H, ArH), 7.62 (s, 1H, OH), 8.75 and 10.10 (br s, 2H, NH, HCl). Anal. ($C_{12}H_{16}ClF_2NO_2$) C, H, N.
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JM950630P