Notes

New Analogues of N-(2-Aminoethyl)-4-chlorobenzamide (Ro 16-6491). Some of the Most Potent Monoamine Oxidase-B Inactivators

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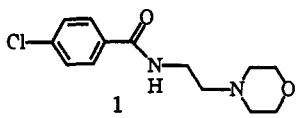
A series of halo- and nitro-substituted analogues of N-(2-aminoethyl)benzamide has been synthesized. All of the compounds are competitive, time-dependent inhibitors of monoamine oxidase-B (MAO-B), but upon dialysis complete return of enzyme activity is observed for all compounds. Therefore, these are mechanism-based reversible inhibitors of MAO-B. The relative potencies of the compounds are rationalized in terms of steric and hydrophobic effects.

Monoamine oxidase (MAO; EC 1.4.3.4) is a flavoenzyme that oxidatively deaminates a variety of biogenic amines and, therefore, plays an important role in the regulation of the intracellular concentrations of these monoamine neurotransmitters.[1] Two different isozymes of MAO, known as MAO-A and MAO-B, have been identified; MAO-A selectively oxidizes norepinephrine and serotonin and MAO-B oxidizes phenylethylamine and benzylamine.[2] Compounds that selectively inhibit MAO-A exhibit antidepressant activity,[3] whereas selective inhibitors of MAO-B are used in the treatment of Parkinson's disease.[4] Moclobemide (tradename Aurorix, 1) was the first non-



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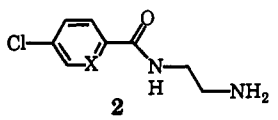
hydrazine, reversible MAO-A selective inhibitor[5] approved for use as an antidepressant drug.[6] Although it is a potent MAO-A inhibitor in vivo, it is only a weak inhibitor in vitro. This suggested that a metabolite of moclobemide is the actual inhibitor. A search was made for an active metabolite, but none was found that was as potent as moclobemide.[7] One of the metabolites that was isolated, N-(2-aminoethyl)-4-chlorobenzamide hydrochloride (Ro 16-6491; 2, X = CH), however, was found to be a very



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potent and selective time-dependent, reversible inhibitor of MAO-B.[7] Using 2 (X = CH) as a lead for the design of selective MAO-B inhibitors, a variety of structure modifications were made on the side chain and the aromatic ring. It was found that the potency was strongly dependent upon the integrity of the 2-aminoethyl side chain, but large variations in potency were observed with variances in the aromatic ring. The most potent analogue prepared was the corresponding pyridine analogue, 2 (X = N) (Ro 19-6327; lazabemide). Other substituted benzamides also were reported.[8] We wanted to see the effect of simple aromatic substitution on the potency of the benzamide analogues; consequently, we have synthesized a series of halo- and nitro-substituted N-(2-aminoethyl)-benzamides and tested their potency as in vitro inactivators of MAO-B. The results of that study are reported here.

Results

Chemistry. The percent yields, melting points, ^{1}H NMR spectral data, and elemental analysis results for all of the analogues are summarized in Table I.

Enzymology. Every compound was a time-dependent inhibitor of MAO-B; dialysis or gel filtration, however, led to complete return of enzyme activity. The IC_{50} values for each of the compounds were determined from the amount of enzyme activity remaining after a 20-min incubation at various concentrations of the inhibitors. These data are summarized in Table II.

Discussion

All of the compounds were relatively potent inhibitors of MAO-B, but there is a variation of over three orders of magnitude between the *o*-iodo analogue (least potent) and the *m*-iodo analogue (most potent). Although the structure of the active site is unknown, certain inferences can be made on the basis of these results. In the case of the *ortho*-substituted analogues, steric effects appear to be important; the smaller the substituent, the more potent the inhibitor. It is not clear if this steric effect results from interference with groups on the enzyme at the active site or intramolecular steric hindrance in which the substituent interferes with the optimum conformation of the side chain.

The *meta*-substituent series exhibits an opposite effect to that of the *ortho* series. In this case the larger the substituent, the more potent the inhibitor. Apparently, there is a cavity at the active site that can accommodate a substituent at the *meta* position. The potency of the halo series also correlates with the hydrophobicity of the substituent as well as the size. Possibly, there is a positive hydrophobic effect that stabilizes the enzyme–inhibitor complex. If that is the case, then the nitro analogue must be involved in a different type of interaction, possibly electrostatic. The *m*-iodo analogue is slightly more potent than Ro 16-6491 and almost as potent as Ro 19-6327.

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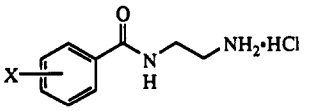
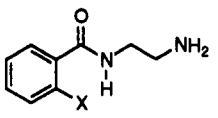
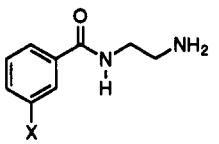
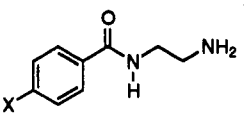
Table I. Chemical Data for Benzamide Analogues



X	% yield	mp (°C)	^{1}H NMR[a] (δ)	anal.[b]
4-F	49	215–216	7.86 (m, 2H), 7.27 (dt, 2H), 3.73 (t, 2H), 3.28 (t, 2H)	CHClN
4-Cl	75	209–210	7.79 (d, 2H), 7.56 (d, 2H), 3.74 (t, 2H), 3.30 (t, 2H)	CHN
4-Br	43	234–235	7.73 (s, 4H), 3.71 (t, 2H), 3.27 (t, 2H)	CHN[c]
4-I	41	280–281	7.88 (d, 2H), 7.49 (d, 2H), 3.79 (t, 2H), 3.22 (t, 2H)	CHN[d]
4-NO_2	45	237–238	8.31 (d, 2H), 7.93 (d, 2H), 3.56 (t, 2H), 2.98 (t, 2H)	CHN[e]
3-F	54	183–184	7.58 (t, 1H), 7.52 (d, 2H), 7.35 (t, 1H), 3.70 (t, 2H), 3.27 (t, 2H)	CHN[f]
3-Cl	68	207–208	7.78 (s, 1H), 7.66 (d, 1H), 7.49 (d, 1H), 7.45 (t, 1H), 3.68 (t, 2H), 3.23 (t, 2H)	CHN[g]
3-Br	55	219–220	7.95 (s, 1H), 7.75 (t, 2H), 7.42 (t, 1H), 3.70 (t, 2H), 3.24 (t, 2H)	CHClN[h]
3-I	61	250–251	7.99 (s, 1H), 7.82 (d, 1H), 7.62 (d, 1H), 7.12 (t, 1H), 3.68 (t, 2H), 3.22 (t, 2H)	CHN
3-NO_2	50	198–199	8.59 (s, 1H), 8.49 (d, 1H), 8.13 (d, 1H), 7.71 (t, 1H), 3.73 (t, 2H), 3.28 (t, 2H)	CHN[i]
2-F	51	160–161	7.79 (t, 1H), 7.64 (m, 1H), 7.38 (t, 1H), 7.29 (t, 1H), 3.78 (t, 2H), 3.31 (t, 1H)	CHClN
2-Cl	56	164–165	7.51 (m, 2H), 7.46 (d, 1H), 7.42 (d, 1H), 7.45 (t, 1H), 3.72 (t, 2H), 3.27 (t, 2H)	CHN[j]
2-Br	43	184–185	7.76 (t, 1H), 7.53 (t, 1H), 7.48 (m, 2H), 3.76 (t, 2H), 3.32 (t, 2H)	CHN
2-I	47	207–208	8.28 (t, 1H), 7.78 (d, 1H), 7.74 (d, 1H), 7.55 (t, 1H), 4.01 (t, 2H), 3.60 (t, 2H)	CHClN
2-NO_2	49	217–218	8.19 (d, 1H), 7.83 (t, 1H), 7.73 (t, 1H), 7.62 (d, 1H), 3.72 (t, 2H), 3.28 (t, 2H)	CHClN
H	43	166–167	7.79 (d, 2H), 7.71 (t, 1H), 7.52 (t, 2H), 3.71 (t, 2H), 3.24 (t, 2H)	CHN[k]

[a] Spectra were recorded in D_2O relative to DSS as standard on either a Varian XL-400 400-MHz spectrometer or a Varian Gemini 300-MHz spectrometer. [b] All elements shown were within ±0.4% of the calculated percentages. [c] HRMS: calcd for $C_9H_{12}{}^{81}BrN_2O$ (m + 1) 245.0113, found 245.0114. [d] HRMS: calcd for $C_9H_{12}IN_2O$ (m + 1) 290.9994, found 290.9992. [e] HRMS: calcd for $C_9H_{12}N_3O_3$ (m + 1) 210.0879, found 210.0894. [f] HRMS: calcd for $C_9H_{12}FN_2O$ (m + 1) 183.0934, found 183.0934. [g] HRMS: calcd for $C_9H_{12}ClN_2O$ (m + 1) 199.0638, found 199.0640. [h] HRMS: calcd for $C_9H_{12}{}^{81}BrN_2O$ (m + 1) 245.0113, found 245.0103. [i] HRMS: calcd for $C_9H_{12}N_3O_3$ (m + 1) 210.0879, found 210.0882. [j] HRMS: calcd for $C_9H_{12}ClN_2O$ (m + 1) 199.0638, found 199.0632. [k] HRMS: calcd for $C_9H_{13}N_2O$ (m + 1) 165.1028, found 165.1035.

Table II. IC_{50} Values[a] for *N*-(Aminoethyl)benzamide Analogues with MAO-B

	IC_{50} (μM)		
X[b]			
I	61.5	0.037	0.17
Br	1.20	0.052	0.042
Cl	0.49	0.12	0.045[c]
F	0.16	0.20	0.056
NO_2	2.17	0.06	48.2
H	0.25	0.25	0.25

[a] See the Experimental Section for procedure; duplicate runs varied by 0–5%. [b] The IC_{50} value for Ro 19–6327 was 0.030 μM under these conditions. [c] Ro 16–6491.

The *para*-substituted analogues seem to exhibit a combination of effects (i.e., no clear trend), but the much higher IC_{50} value for the nitro analogue suggests either a steric hindrance or electron-repulsive effect. Excluding the nitro analogue there appears to be both a stabilizing inductive effect and a destabilizing steric effect.

Given the potency of the *meta*-substituted series, which correlates with increasing hydrophobicity and size, it would be interesting to test a series of *m*-alkyl-substituted analogues to determine the limits of these effects.

Experimental Section

General Procedures. Chemistry. Melting points were determined with a Fisher-Johns melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini 300-MHz or Varian XL-400 400-MHz spectrometer; chemical shifts are reported as δ values in parts per million (ppm) relative to TMS as internal standard in D_2O. Elemental analyses were performed either by Searle Laboratory (Skokie, IL) or by Oneida Research Services (Whitesboro, NY). Thin-layer chromatography was run on alumina 60 F_{254}, neutral (type E) by Merck and developed with ninhydrin. High-resolution mass spectra (HRMS) were recorded on a VG Instruments (VG70-250SE high-resolution spectrometer. All reagents were purchased from Aldrich Chemical Co. and were used without further purification.

General Procedure for the Preparation of the Benzamides. Ethylenediamine (0.7 mL, 12 mmol) was added all at once to a vigorously stirred solution of the halo- or nitro-substituted benzoyl chloride (10 mmol) in methanol (50 mL) cooled to 0–5 °C in an ice–water bath. Stirring was continued for 20–60 s, then the reaction was quenched with concentrated HCl (5 mL). The heterogeneous mixture was stirred at room temperature for 5 min and then filtered. The white solid was washed with water, and the combined filtrates were evaporated to dryness by rotary evaporation. The residue was triturated with water, and the solid was removed by filtration. The filtrate was evaporated, and the residue was column chromatographed (alumina, water/ethanol; 1:2). The fractions containing the benzamides were collected, the solvent was evaporated, and the product was recrystallized several times from ethanol. Analytical data for the compounds is given in Table I.

N-(2-Aminoethyl)-3-iodobenzamide Hydrochloride. 3-Iodobenzoic acid (1.24 g, 5 mmol) was refluxed in excess $SOCl_2$ (3 mL) for 1 h. The excess $SOCl_2$ was removed by distillation, dry hexane was added to the residue, and the hexane was distilled. The resulting acid chloride was added to a stirred mixture of ethylenediamine (0.07 mL, 1 mmol) in methanol (25 mL) cooled to 0–5 °C in an ice–water bath. The reaction was quenched after 30 s with concentrated HCl (5 mL). The white solid was removed by filtration, the filtrate was evaporated to dryness, and the residue was column chromatographed (alumina:water/ethanol, 1:2).

Enzymes and Assays. Mitochondrial MAO-B was purified from bovine liver and assayed as described previously.[9]

Determination of the IC_{50} Values. Various concentrations of inactivators were incubated with MAO-B for 20 min. Aliquots (10 μL) of the incubation mixtures were assayed for remaining activity in 490 μL of benzylamine hydrochloride (1 mM substrate in 50 mM Tris-HCl, pH 9.0). The IC_{50}s were extrapolated from plots of the percent enzyme activity remaining versus inhibitor concentration.

Reversibility of the Inactivations. MAO inactivated by each of the inactivators was dialyzed against 50 mM potassium phosphate, pH 7.4, or applied to Sephadex G-50 by the method of Penefsky,[10] and then the activity was assayed.

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