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Synthesis, Biological Evaluation and Quantitative Structure Activity Relationship Analysis of Nuclear-substituted Pargylines as Competitive Inhibitors of MAO-A and MAO-B

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Abstract—A series of nuclear substituted derivatives of pargyline has been prepared and tested (under controlled conditions designed to measure the competitive component of the inhibition) as competitive inhibitors of MAO-A and -B. Adequate correlation of the biological data with the physicochemical constants of substituent groups was obtained only when the *m*- and *p*-substituted derivatives were considered separately. Due to the narrow range of activity displayed by the *p*-substituted derivates when inhibiting MAO-B, meaningful correlations were not found. However, the inhibition of MAO-B by the *m*-substituted derivatives required the inclusion of the Verloop L parameter for adequate correlation, suggesting that the inhibitor binding site of MAO-B is present within a cavity of more limited lateral dimensions than that present on the MAO-A surface. Inhibition of both MAO-A and -B demonstrated a parabolic relationship between inhibitory activity and π. Whereas this parabolic relationship showed a maximal value for inhibition of MAO-A (mean $\pi_o = 0{\cdot}86$), inhibition of MAO-B demonstrated a minimal value of π ($\pi_{min} = -0{\cdot}5$) i.e. the optimal value of π for inhibition of MAO-B has not been achieved for this series of compounds but such would be greater than that demonstrated for MAO-A. The Hammett σ function was important or significant only in the inhibition of MAO-A by the *p*-substituted derivatives.

The differentiation of the different forms of the enzyme monoamine oxidase (MAO) [monoamine: O_2 oxido-reductase (deaminating) (flavin containing) EC 1.4.3.4.] within a tissue or enzyme preparation is accomplished by the use of selective substrates and inhibitors. Thus, MAO-A in the presence of 5-hydroxytryptamine (5-HT) (MAO-A specific substrate) is selectively inhibited by clorgyline in the nM concentration range whereas MAO-B in the presence of 2-phenylethylamine (PEA) or benzylamine (MAO-B specific substrates) is selectively inhibited in the nM concentration range by selegiline ((−)-deprenyl) (Yang & Neff 1973; Houslay et al 1976). Other endogenous amine substrates, for example tyramine and tryptamine are metabolized by both MAO-A and -B (Fowler & Tipton 1984).

Although clorgyline and selegiline are selective towards different forms of MAO, both compounds, being derivatives of propargylamine, are identical, from a chemical mechanistic viewpoint, in their inhibition. Both compounds *N*-alkylate the reduced flavin prosthetic group of the enzyme (Singer & Salach 1981) possibly via a mechanism involving an intermediate radical cation species (Silverman et al 1980; Richards & Burger 1986). Thus, such compounds are suicide inhibitors and show time-dependent non-competitive irreversible kinetics (formation of EI*) which is preceded by a competitive reversible phase (formation of EI complex) and represented as:

$$[E]+[I]\underset{k_{-1}}{\overset{k_1}{}}[EI]\overset{k_2}{}[EI^*] \qquad \frac{k_{-1}}{k_1}=K_i$$

It has been suggested that the selectivity of a suicide-type inhibitor towards the different forms of MAO may be dependent upon the differences in affinity of the inhibitor for MAO-A and -B as measured by the competitive K_i value; i.e. selectivity is dictated by the reversible phase of the inhibition (Tipton & Mantle 1981). Kinetic studies of the inhibition of MAO-A and -B by selective and non-selective propargylamine-type suicide inhibitors lend some support to this view (Tipton & Mantle 1981; Fowler et al 1982; Cruces et al 1990).

Although the two forms of MAO are associated with different and separable proteins (Callingham & Parkinson 1979; Denny et al 1982; Pearce & Roth 1984), the primary structures of which are known (Bach et al 1988), the structural features of inhibitors leading to high affinity (and possible selectivity towards a particular form) at the respective active sites are unclear. Although several previous QSAR studies of MAO using a variety of different structural types of inhibitors have been reported (for early review see Fujita 1973), many of the compounds studied were capable of non-competitive inhibition and precautions were not taken to limit the studies to the initial competitive phase of the inhibition (Finkelstein et al 1965; Martin et al 1975). In other instances, the substrate employed has been capable of metabolism by both forms of the enzyme and the results preclude any consideration of the influence of the different forms of the enzyme (Lien et al 1970; Green 1962). The present studies therefore report the preparation and testing of a series of nuclear-substituted pargyline derivatives as inhibitors of MAO-A and -B (using 5-HT and PEA, respectively, as substrates). Although such derivatives, being alkylated propargylamine derivatives, are capable of irreversible non-competitive inhibition on prolonged incubation of the inhibitor with the enzyme, the conditions of testing were controlled (initiation of the enzyme-catalysed reaction by addition of enzyme to the mixture of substrate and inhibitor and allowing the enzyme-catalysed reaction to proceed for

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only a short period of time) such that only the competitive phase of the inhibition was measured (Tipton & Mantle 1981; Fowler et al 1982; Robinson 1985). The competitive K_i values thus obtained reflect the affinity of inhibitors for the substrate binding site and allow a QSAR analysis of the two forms of the enzyme with the objective of defining the optimal physicochemical properties of inhibitors leading to high affinity and selective competitive inhibition.

Chemistry

Although for the purposes of the above analysis, compounds capable only of competitive inhibition of the different forms of the enzyme are preferable, the use of nuclear-substituted pargyline derivatives was chosen for the following reasons: (a) the ease of synthesis from readily available starting materials (substituted benzaldehydes); (b) the absence of a chiral centre so removing any possible stereochemical influences upon the biological results and (c) the potential use of the same compounds for later QSAR studies at the non-competitive irreversible phase of the inhibition of MAO-A and -B.

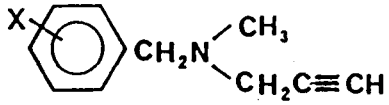
The individual substituents employed in the present work were chosen to provide a reasonable range of π and σ values (both +ve and −ve). Substituents capable of ionization at physiological pH, e.g. $-NH_2$, $-COOH$, etc. were not selected because of the further problems such substitutents introduce into the analysis. Accordingly, nineteen compounds (9 substituent groups, *m*- and *p*-derivatives plus pargyline) have been prepared and tested as competitive inhibitors of MAO-A and -B.

The commercial availability of several nuclear-substituted benzaldehydes allowed the preparation of the corresponding substituted pargyline derivatives (compounds 1–7; 11–16; Table 1) by a reductive amination reaction employing sodium cyanoborohydride as reducing agent (Scheme Ia) (Borch et al 1971). This reaction, carried out in methanol solution, requires the prior formation in-situ of the imine, the formation of which is optimal in the pH range 6–8. At this pH, reduction by cyanoborohydride of unreacted aldehyde is extremely slow. Because of the acidity of *N*-methylpropargylamine ($pK_a \simeq 8$), a pH in the 6–8 range was attained by addition of methanolic HCl to a mixture of aldehyde and amine resulting in an amine to amine hydrochloride ratio of approximately 8:1. Under such conditions formation of the imine and reduction to the desired substituted pargyline proceeded smoothly. Use of an amine to amine hydrochloride ratio of 2:1 (commonly employed conditions for this reaction) resulted in the isolation of the dimethyl acetal of the starting aldehyde in high yield.

Compounds 8–10, 17–19 (X = —COOR) were prepared from the appropriate benzyl chloride by alkylation of *N*-methylpropargylamine (Scheme Ib).

The physical characteristics of the products (which were in all instances consistent with the proposed structure) are given in Table 1, and chemical analyses in Table 2.

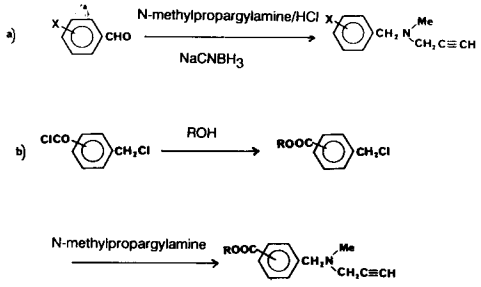
Table 1. Physical properties of nuclear substituted *N*-methyl-*N*-propargylbenzylamines.



Compound X=	bp (base) (°C)/mm Hg	mp (HCl) (°C)	Mol. formula
1 H	88–90/4 (lit[a] bp 96–97/11)	156–157 (lit[a] 154–155)	$C_{11}H_{14}NCl$
2 4-CH_3	80–84/4 72–76/0·4	186–187·5 (d) (lit[b] 199–200)	$C_{12}H_{16}NCl$
3 4-OCH_3	68–71/0·2	155–157 (lit[b] 152)	$C_{12}H_{16}NOCl$
4 4-NO_2	96–100/0·2	191–193	$C_{11}H_{13}N_2O_2Cl$
5 4-CN	85–88/0·15	223–224	$C_{12}H_{13}N_2Cl$
6 4-Cl	48–50/0·05 80–82/2	209–210 (d)	$C_{11}H_{13}NCl_2$
7 4-Br	62–64/0·15	208–208·5 (lit[b] 210–212)	$C_{11}H_{13}NBrCl$
8 4-$COOCH_3$	88–90/0·2	180–181	$C_{13}H_{16}NO_2Cl$
9 4-$COOC_2H_5$	90/0·2	179–181	$C_{14}H_{18}NO_2Cl$
10 4-$COOCH_2C_6H_5$	152–155/0·2	165–167	$C_{19}H_{20}NO_2Cl$
11 3-CH_3	48–50/0·05	148–149 (d)	$C_{12}H_{16}NCl$
12 3-OCH_3	64–69/0·2	160–162	$C_{12}H_{16}NOCl$
13 3-NO_2	91–96/0·2	168–170	$C_{11}H_{13}N_2O_2Cl$
14 3-CN	76–78/0·2	137·5–138·5	$C_{12}H_{13}N_2Cl$
15 3-Cl	74–76/1 48–50/0·05	168–169 (d)	$C_{11}H_{13}NCl_2$
16 3-Br	62–64/0·15	143·5–145 (d)	$C_{11}H_{13}NBrCl$
17 3-$COOCH_3$	92/0·15	135–136	$C_{13}H_{16}NO_2Cl$
18 3-$COOC_2H_5$	90/0·2	110–111	$C_{14}H_{18}NO_2Cl$
19 3-$COOCH_2C_6H_5$	122–125/0·2	79–80	$C_{19}H_{20}NO_2Cl$

[a] Brit. Pat. 906245; [b] Japan Patent 15674 via Chemical Abstracts 62,6431 (1965).

All bases showed IR (liquid film) (alkyne C-H stretch $\simeq$ 3300 cm^{-1}) and NMR (CCl_4 solution) (C≡C—H, t; —CH_2C≡C, d; aromatic resonances) spectra appropriate to the desired structure.



SCHEME I. Synthetic routes to nuclear substituted pargylines.

Biological Studies

A mitochondrial enzyme preparation of MAO derived from rat liver was employed in the inhibition studies. Such an enzyme preparation has been shown to contain both MAO-A and -B (Fowler et al 1978). Enzyme preparations containing a single active form of the enzyme were prepared from the above by incubation with either selegiline (MAO-B selective inhibitor) or clorgyline (MAO-A selective inhibitor) followed by washing to remove the excess inhibitor. Enzyme activity in such preparations was determined using the appropriate specific substrate (5-HT or PEA).

In all inhibition studies, in order to prevent the appearance of any time-dependent non-competitive inhibition by covalent adduct (EI*) formation, the enzyme-catalysed reaction was initiated by addition of enzyme to a solution of substrate and inhibitor; i.e. preincubation of the enzyme with inhibitor was omitted, and the enzyme-catalysed reaction was allowed to proceed for only 3 min. Employing such conditions, all plots of resulting data were indicative of competitive inhibition kinetics. The computed values of K_i are given in Table 3.

Materials and Methods

Melting points were determined using a Thomas Hoover Unimelt capillary melting point apparatus and are uncorrected. IR spectra were obtained from a Perkin-Elmer 1330 Infra-red spectrophotometer and NMR spectra (in CCl_4 solution) from a Varian T60 spectrometer using tetramethyl silane as external standard. Determination of Cl^- was by non-aqueous titration in glacial acetic acid in the presence of mercuric acetate and using perchloric acid in glacial acetic acid as titrant.

N-Methyl-N-propargyl-4-chlorobenzylamine hydrochloride (compound 6)

4-Chlorobenzaldehyde (2·6 g, 0·018 mol) and *N*-methylpropargylamine (5 g, 6 mL, 0·072 mol) were dissolved in methanol (25 mL), methanolic HCl (1·5 mL, 5 M, 0·0075 mol) added and the solution stirred for 15 min. Sodium cyanoborohydride (0·9 g, 0·014 mol) was added and the solution stirred for 24 h. The mixture was evaporated under reduced pressure, water (20 mL) added, the solution made alkaline with ammonia and extracted with chloroform (3 × 20 mL). The combined extracts were dried (Na_2SO_4), filtered and evaporated. The residue was fractionally distilled, the fraction bp 48–50°C at 0·05 mm being collected. Upon redistillation, the fraction bp 80–82°C at 2 mm Hg was collected, yield 0·8 g. IR (liquid film) 3300 cm^{-1} (C≡C), NMR (CCl_4) δ 2·2 (t, J = 2 cycles s^{-1}, 1 H, C≡CH); 2·35 (s, 3H, N—CH_3); 3·3 (d, J = 2 cycles s^{-1}, 2H, N—CH_2C≡C); 3·55 (s, 2H, benzylic—CH2—); 7·25 (s, 4H aromatic, *o*- and *m*-protons not resolved).

The product was dissolved in dry ether and ether saturated with dry HCl gas added. The precipitate was filtered, dried and recrystallized from absolute ethanol; mp 209–210°C (d); 0·6 g.

Compounds 1–7, 11–16 were similarly prepared starting with the appropriate aldehyde. The physical characteristics of these products are given in Table 1, and chemical analyses in Table 2.

Benzyl 4-chloromethylbenzoate

Benzyl alcohol (10 mL, 10·5 g, 0·1 mol) was added to 4-chloromethylbenzoyl chloride (10 g, 0·05 mol) and the

Table 2. Analytical data (Cl^-% and N%) for nuclear substituted pargyline derivatives.

Substituent	Mol. formula	Calc. Cl^-%	Found Cl^-%		Calc. N%	Found N%.	
			m	*p*		*m*	*p*
H	$C_{11}H_{14}NCl$ (195·68)	18·12	18·15	—		nd	
CH_3	$C_{12}H_{16}NCl$ (209·71)	16·90	16·97	16·92	6·68	6·63	nd
OCH_3	$C_{12}H_{16}NOCl$ (225·71)	15·70	15·6	15·88	6·20	6·10	nd
NO_2	$C_{11}H_{13}N_2O_2Cl$ (240·68)	14·73	14·85	15·03	11·63	11·67	11·36
CN	$C_{12}H_{13}N_2Cl$ (220·69)	16·06	16·24	16·30	12·69	12·5	12·74
Cl	$C_{11}H_{13}NCl_2$ (230·12)	15·41	15·42	15·37	6·08	5·82	5·98
Br	$C_{11}H_{13}NClBr$ (274·68)	12·91	13·18	12·65	5·10	5·09	nd
$COOCH_3$	$C_{13}H_{16}NO_2Cl$ (253·72)	13·97	14·12	13·95	5·52	5·35	5·48
$COOC_2H_5$	$C_{14}H_{18}NO_2Cl$ (267·74)	13·24	13·07	13·33	5·23	5·27	5·19
$COOCH_2C_6H_5$	$C_{19}H_{20}NO_2Cl$ (329·81)	10·74	10·92	10·66	4·25	4·25	4·50

nd = not determined.

Table 3. Competitive K_i values of nuclear substituted pargylines when inhibiting MAO-A and MAO-B.

	MAO-A inhibition				MAO-B inhibition			
Compound	K_i (μM) (found) (+ s.e.)	pK_i (found)	pK_i (calc.)	ΔpK_i	K_i (μM) (found) (±s.e.)	pK_i (found)	pK_i (cal.)	ΔpK_i
1	14·74 (1·21)	4·83	4·83[a]	0·00	0·40 (0·03)	6·40	6·74[c]	−0·34
			4·74[b]	0·09				
2	5·51 (0·24)	5·26	5·46[a]	−0·2	1·45 (0·09)	5·84	—	—
3	6·88 (0·27)	5·16	5·03[a]	0·13	0·63 (0·03)	6·20	—	—
4	216·0 (3·2)	3·67	3·83[a]	−0·16	0·40 (0·004)	6·40	—	—
5	292·0 (20·1)	3·54	3·45[a]	0·09	1·02 (0·03)	6·00	—	—
6	6·76 (0·12)	5·17	5·19[a]	−0·02	0·21 (0·03)	6·69	—	—
7	6·43 (0·24)	5·19	5·24[a]	−0·05	0·27 (0·03)	6·57	—	—
8	46·4 (2·8)	4·33	4·45[a]	−0·12	0·35 (0·004)	6·45	—	—
9	5·88 (0·35)	5·23	4·91[a]	0·32	0·57 (0·01)	6·25	—	—
10	17·60 (0·52)	4·75	4·75[a]	0·00	0·55 (0·02)	6·26	—	—
11	7·46 (0·14)	5·13	5·22[b]	−0·09	0·22 (0·01)	6·65	6·80[c]	−0·15
12	6·30 (0·20)	5·20	5·20[b]	0·00	0·70 (0·01)	6·15	5·47[c]	0·68
13	15·04 (0·75)	4·82	4·74[b]	0·08	0·95 (0·02)	6·02	5·68[c]	0·34
14	51·12 (0·86)	4·30	4·43[b]	−0·13	14·77 (0·66)	4·83	5·14[c]	−0·31
15	4·90 (0·01)	5·31	5·32[b]	−0·01	0·20 (0·01)	6·70	6·72[c]	−0·02
16	5·15 (0·24)	5·29	5·32[b]	−0·03	0·11 (0·004)	6·96	6·82[c]	0·14
17	2·88 (0·16)	5·54	5·42[b]	0·12	15·04 (1·56)	4·82	4·92[c]	−0·10
18	1·28 (0·15)	5·90	5·96[b]	−0·06	32·24 (0·40)	4·49	4·79[c]	−0·30
19	7·51 (0·13)	5·12	5·10[b]	0·02	0·60 (0·01)	6·22	6·19[c]	0·03

[a]Calculated using equation xv; [b]Calculated using equuation xiii; [c]Calculated using equation iv. For equations see Table 5

solution warmed (45°C) and stirred with exclusion of moisture during 4 h. Water (25 mL) was added and the mixture extracted with chloroform (3 × 30 mL). The extracts were dried ($MgSO_4$), filtered, evaporated under reduced pressure and the residue fractionally distilled, the fraction bp 130° C at 0·05 mm Hg being collected. The distillate slowly solidified and was recrystallized from hexane; mp 51–53°C (7·2 g). NMR. (CCl_4) δ 4·6 (s,2H,benzylic—CH_2Cl); 5·36 (s, 2H, benzylic—CH_2—O—); 7·43 (s, 5H, phenyl); 7·43, 8·06 (d of d, J = 8 cycles s^{-1}, 4H aromatic).

Similarly prepared using the appropriate alcohol as solvent and reactant were methyl 4-chloromethylbenzoate (bp 75°C at 7 mm Hg), ethyl 4-chloromethylbenzoate (bp 156–160°C at 15 mm Hg), methyl 3-chloromethylbenzoate (bp 168–170°C at 15 mm Hg), ethyl 3-chloromethylbenzoate (bp 172–174°C at 15 mm Hg) and benzyl 3-chloromethylbenzoate (bp 159–160° C at 0·2 mm Hg).

N-Methyl-N-propargyl-(4-benzyloxycarbonyl)-benzylamine hydrochloride (10)

N-Methylpropargylamine (2·5 mL, 2 g; 0·03 mol) was added to a solution of benzyl 4-chloromethylbenzoate (5 g, 0·02 mol) in hexane (20 mL) and the mixture heated in a water bath (45°C) with stirring for 72 h. The cooled reaction mixture was extracted with dilute HCl (50 mL), the extract made alkaline with ammonia and then extracted with chloroform (6 × 20 mL). The organic extracts were dried (Na_2SO_4), filtered and evaporated under reduced pressure and the residue fractionally distilled, the fraction bp 152–155°C at 0·2 mm Hg being collected. IR (liquid film) 3280 cm^{-1} (C≡CH) and 1720 cm^{-1} (C=O), NMR. (CCl_4) δ 2·35 (s, with underlying triplet, 4H, N—CH_3, C≡CH), 3·3 (d, J = 2 cycles s^{-1}, 2H, —$CH_2C{\equiv}C$), 3·6 (s, 2H, —CH_2 N, benzylic protons), 5·3 (s, 2H, —CH_2—O—), 7·26 (s) 7·3 and 7·93 (d of d, J = 8 cycles s^{-1}. 9H, phenyl and *p*-substituted aromatic ring).

The product was dissolved in dry ether and ether saturated with dry HCl gas added. The precipitate was filtered, dried and recrystallized from absolute ethanol mp 165–167°C.

Compounds 8, 9, 17–19 were similarly prepared. The physical characteristics of these products are given in Table 1.

Enzyme preparation

Two male Sprague-Dawley rats, 250–300 g, were killed using

CO_2 and the livers removed. All subsequent steps were performed at 0 to 4°C. The livers (30 g) were homogenized in five vol of 0·25 M sucrose in phosphate buffer (10 mM, pH 7·4) using a Waring blendor (4 min). The homogenate was centrifuged (600 *g*, 10 min), the supernatant decanted and further centrifuged (15 000 *g*, 10 min) and the mitochondrial pellet collected. The pellet was suspended in fresh sucrose-phosphate buffer solution (25 mL) and either MAO-A or -B activity selectively inhibited using clorgyline or selegiline, respectively.

MAO-B mitochondrial enzyme preparation. To a freshly prepared liver mitochondrial enzyme preparation (25 mL) was added clorgyline solution (100 mL; $4{\cdot}55 \times 10^{-8}$ M) in phosphate buffer (10 mM, pH 7·4) (final clorgyline concentration $3{\cdot}64 \times 10^{-8}$ M) and the mixture incubated at 35°C with shaking for 1 h. The mixture was centrifuged (15 000 *g*, 10 min) and the resulting mitochondrial pellet was suspended in 0·25 M sucrose in phosphate buffer (10 mM, pH 7·4) solution, centrifuged (15 000 *g*, 10 min) and the supernatent discarded. The above washing process was repeated a further two times. The final washed mitochondrial pellet was suspended in fresh sucrose buffer solution (50 mL), distributed into ampoules (2 mL per ampoule) and stored at −20°C until required.

MAO-A mitochondrial enzyme preparation. This was prepared as for MAO-B except the clorgyline solution was replaced by a solution of selegiline (100 mL, $3{\cdot}93 \times 10^{-7}$ M) in phosphate buffer (10 mM, pH 7·4) (final selegiline concentration $3{\cdot}15 \times 10^{-7}$ M in the incubation mixture).

Enzyme assays

Enzyme activity was determined by radiochemical methods (Wurtman & Axelrod 1963; Squires 1972). [^{14}C]2-Phenylethylamine hydrochloride (50 mCi mmol^{-1}) and [^{14}C]5-hydroxytryptamine binoxalate (50 mCi mmol^{-1}) were purchased from New England Nuclear, Boston, MA.

Immediately before use, the MAO-B enzyme preparation (5–7 mg prot. mL^{-1}) was diluted 1 to 10 with phosphate buffer (10 mM, pH 7·4). The MAO-A enzyme preparation was used undiluted. Within these studies, the inhibitor (50 μL) was preincubated at 35°C for three min in the presence of substrate in phosphate buffer (10 mM, pH 7·4) and the enzyme-catalysed reaction initiated by the addition of enzyme preparation (50 μL). Final volume of incubation mixture was 300 μL. The reaction was allowed to proceed for 3 min and was stopped by the addition of HCl (200 μL, 2 M). A tenfold range of substrate concentration was employed and the enzyme activity was determined in the presence and absence of threefold concentrations of inhibitor, the concentration range of which had been determined from preliminary studies. All determinations were carried out in duplicate.

For studies of MAO-B activity and inhibition (PEA as substrate), deaminated products were extracted by addition of toluene (6 mL); the mixture was extracted by shaking in a vortex mixer and then centrifuged. A portion (4 mL) of the toluene layer was removed and added to scintillation fluid (5 mL) (prepared from Liquifluor concentrate, (New England Nuclear)), and containing 0·4% w/v of PPO (2,5-diphenyloxazole) and 0·005% w/v of POPOP (1,4-bis-[2-(4-methyl-5-phenyl-oxazolyl]-benzene in the final toluene solution) and counted in a Beckman LS 7500 liquid scintillation counter. All counts were corrected for quenching (counting efficiency 96%).

For studies of MAO-A activity and inhibition (5-HT as substrate), deaminated products were extracted into benzene/ethyl acetate (1:1) (6 mL) and after vortex mixing and centrifuging, the mixture was refrigerated for 4 h at −10°C. A sample (4 mL) of the organic layer was added to scintillation fluid (5 mL) and counted as described above.

The data obtained were plotted as Lineweaver-Burk double reciprocal plots, the best straight line fitting the experimental points being determined by a least squares regression analysis and the data used to calculate K_m and K_i values. Under the above conditions K_m (MAO-A) was 48·2 μM (±7·82) (n = 13; 5-HT as substrate) and K_m (MAO-B) was 3·54 μM (±0·33) (n = 11; PEA as substrate). Values of K_i were also obtained from a replot of the gradient of the Lineweaver-Burk plot against inhibitor concentration. Such plots were linear, i.e. absence of detectable non-competitive kinetics, and allowed the determination of K_i from the values of the slope and intercept (y-axis) or the x-axis intercept of the extrapolated line.

Results and Discussion

The competitive K_i values for each of the inhibitors when interacting with MAO-A and -B are given in Table 2.

All the compounds tested were inhibitory towards both forms of the enzyme although the extent of the selectivity towards a particular form of the enzyme varied considerably. The parent compound pargyline (compound 1) appears approximately 35 times more potent as a competitive inhibitor of MAO-B than -A, consistent with a previous report (Fowler et al 1982). Only two of the compounds tested (*m*-$COOCH_3$, 17 and *m*-$COOC_2H_5$, 18) were more potent inhibitors of MAO-A than -B, the latter compound being about 25 times more inhibitory in this respect. These compounds were also the most potent inhibitors of MAO-A of all the compounds tested.

Although the remaining compounds were more inhibitory towards MAO-B, considerable variation in the selectivity towards MAO-B is shown in the data. Thus, the least selective inhibitor is the *m*-CN derivative (14) ($K_{i,MAO\text{-}A}/K_{i,MAO\text{-}B} = 3{\cdot}5$) whereas the *p*-CN (5) and *p*-NO_2 (4) derivatives are the most selective (ratios of 285:1 and 535:1, respectively), although not the most potent, inhibitors of MAO-B.

For the purposes of the quantitative structure activity studies, multiple regression analysis of the biological data was performed using a variety of substituent constants as independent variables. However, the most important correlation equations were obtained using hydrophobic (π), electronic (σ) (Hansch & Leo 1979) and steric (L) parameters (Verloop et al 1976), the individual values of which are given in Table 4 along with the cross-correlation matrix of these variables. The use of modified electronic substituent constants to account separately for the inductive and resonance effects (Unger 1980) and the use of the width components (B_1–B_4) within the Verloop STERIMOL programme (Verloop et al 1976) failed to improve the correlations and so are

Table 4. Values of substituent constants employed in QSAR studies (A) and squared cross-correlation matrix of parameters (B).

A Substituent	π^a	σ_m^a	σ_p^a	L^b
H	0·00	0·00	0·00	2·06
CH_3	0·56	−0·07	−0·17	3·00
OCH_3	−0·02	0·12	−0·27	3·98
NO_2	−0·28	0·71	0·78	3·44
CN	−0·57	0·56	0·66	4·23
Cl	0·71	0·37	0·23	3·52
Br	0·86	0·39	0·23	3·83
$COOCH_3$	−0·01	0·37	0·45	4·85
$COOCH_2H_5$	0·51	0·37	0·45	5·96
$COOCH_2C_6H_5$	1·84	0·37	0·45	9·10*

B	π	σ_m	σ_p	L
π	1·0	0·03	0·02	0·42
σ_m		1·0	0·84	0·08
σ_p			1·0	0·13
L				1·0

*By analogy with other substituents: $OCOC_6H_5$ (L = 8·15); $OCH_2C_6H_5$ (L = 8·20); CH_2- (L = approx. 0·95).
[a]Data from Hansch & Leo (1979); [b]Data from Verloop et al (1976).

not reported. In Table 5 are presented regression equations important to the analysis and discussion.

Using the competitive inhibition data for all 19 compounds, the resultant equations show a poor correlation although the results for inhibition of MAO-A (see eqns vii–x) were slightly superior to those derived from the MAO-B inhibition data (see eqns i–iii). More meaningful information, together with a rationale for the above poor correlations, derives from the separate consideration of the *m*- and *p*-substituted derivatives.

In considering the data from inhibition of MAO-B, reasonably meaningful correlations were obtained only in the case of inhibition by the *m*-substituted derivatives (eqns iii, iv). The extremely low correlation coefficient of the best equation fitting the data for the *p*-substituted derivatives (eqn vi) is due, not to an inability to identify the parameters having a major contribution towards the activity of these compounds but rather to the narrow range of inhibitory activity displayed by the compounds (see Table 3) (pK_i range, 5·84–6·68; mean pK_i 6·30).

It is noted that, for inhibition of MAO-B by *m*-substituted derivatives, both equations iii and iv contain a negative coefficient for the Verloop length parameter (L), as also do equations i and ii; i.e. bulky *m*-substituent groups are not tolerated by MAO-B. Further, the positive coefficients associated with the π and π^2 terms (eqn iv) indicate the inhibitory activity passes through a minimum with respect to π ($\pi_{min} \simeq 0{\cdot}5$) (see also eqn i).

Separate consideration of the *m*- and *p*-substituted derivatives as competitive inhibitors of MAO-A generates well-

Table 5. Important regression equations for the inhibition of MAO-A and MAO-B by nuclear substituted pargyline derivatives. (Numbers in parentheses are s.e.)

	n	r^2	s	F
Inhibition of MAO-B; *m*- and *p*-substituted derivatives				
(i) $pK_{i-B} = 0{\cdot}485\pi + 0{\cdot}354\pi^2 - 0{\cdot}343\ L + 7{\cdot}259$ (0·355) (0·331) (0·131)	19	0·414	0·568	3·525; $P < 0{\cdot}05$
(ii) $pK_{i-B} = 1{\cdot}071\pi + 1{\cdot}126\sigma - 0{\cdot}365L + 6{\cdot}957$ (0·311) (0·672) (0·114)	19	0·468	0·541	4·406; $P < 0{\cdot}025$
Inhibition of MAO-B; *m*-substituted derivatives only				
(iii) $pK_{i-B} = 1{\cdot}352\pi - 0{\cdot}45\ L + 7{\cdot}417$ (0·327) (0·116)	10	0·737	0·575	9·797; $P < 0{\cdot}01$
(iv) $pK_{i-B} = 0{\cdot}767\pi + 0{\cdot}777\pi^2 - 0{\cdot}652\ L + 8{\cdot}079$ (0·347) (0·319) (0·121)	10	0·868	0·394	13·13; $P < 0{\cdot}005$
Inhibition of MAO-B; *p*-substituted derivatives only				
(v) $pK_{i-B} = 0{\cdot}222\pi - 0{\cdot}127\pi^2 + 6{\cdot}294$ (0·237) (0·161)	10	0·111	0·272	0·438
(vi) $pK_{i-B} = 0{\cdot}391\pi - 0{\cdot}244\pi^2 + 0{\cdot}366\sigma + 6{\cdot}197$ (0·269) (0·184) (0·303)	10	0·285	0·263	0·797
(vii) $pK_{i-B} = 0{\cdot}217\pi - 0{\cdot}102\pi^2 - 0{\cdot}014\ L + 6{\cdot}343$ (0·258) (0·237) (0·09)	10	0·115	0·293	0·259
Inhibition of MAO-A; *m*- and *p*-substituted derivatives				
(viii) $pK_{i-A} = 1{\cdot}203\pi - 0{\cdot}649\pi^2 + 4{\cdot}861$ (0·251) (0·17)	19	0·591	0·406	11·55; $P < 0{\cdot}001$
(ix) $pK_{i-A} = 1{\cdot}063\pi - 0{\cdot}564\pi^2 - 0{\cdot}353\sigma + 4{\cdot}985$ (0·319) (0·208) (0·484)	19	0·605	0·413	7·655; $P < 0{\cdot}005$
(x) $pK_{i-A} = 1{\cdot}257\pi - 0{\cdot}888\pi^2 + 0{\cdot}136\ L + 4{\cdot}369$ (0·244) (0·228) (0·09)	19	0·644	0·391	9·064; $P < 0{\cdot}005$
Inhibition of MAO-A; *m*-substituted derivatives only				
(xi) $pK_{i-A} = 0{\cdot}869\pi - 0{\cdot}489\pi^2 + 5{\cdot}104$ (0·277) (0·187)	10	0·585	0·317	4·933; $P < 0{\cdot}05$
(xii) $pK_{i-A} = 0{\cdot}998\pi - 0{\cdot}573\pi^2 + 0{\cdot}438\sigma + 4{\cdot}966$ (0·32) (0·215) (0·512)	10	0·63	0·323	3·406; $P < 0{\cdot}01$
(xiii) $pK_{i-A} = 0{\cdot}961\pi - 0{\cdot}932\pi^2 + 0{\cdot}247\ L + 4{\cdot}235$ (0·089) (0·082) (0·031)	10	0·964	0·101	53·17; $P < 0{\cdot}001$
Inhibition of MAO-A; *p*-substituted derivatives only				
(xiv) $pK_{i-A} = 1{\cdot}538\pi - 0{\cdot}808\pi^2 + 4{\cdot}613$ (0·277) (0·188)	10	0·816	0·317	15·56; $P < 0{\cdot}005$
(xv) $pK_{i-A} = 1{\cdot}162\pi - 0{\cdot}546\pi^2 - 0{\cdot}816\sigma + 4{\cdot}831$ (0·188) (0·128) (0·212)	10	0·947	0·184	35·76; $P < 0{\cdot}001$

correlated but different equations and equations which perhaps point to some important differences between the two forms of the enzyme. All equations demonstrate a high dependance upon the partition coefficient of the inhibitor (eqns viii-xv) and from the sign of the coefficients, suggest an optimal partition coefficient for maximal activity (mean π_o, calculated from equations xi–xv, = 0·86; range 0·52–1·06). The consistent demonstration of a parabolic relationship (with a maximum) between MAO-A inhibition and hydrophobicity (π) probably reflects a potentially important contributory factor in the design of potent and selective inhibitors of the different forms of MAO. That the *m*-substituted derivatives, when inhibiting MAO-B, showed a parabolic relationship with a trough suggests that the optimal partition coefficient for maximal inhibitory activity of MAO-B is higher than that shown by MAO-A and may even be outside the range of π values employed in this study.

The separate consideration of the *m*- and *p*-substituted derivatives of pargyline as inhibitors of MAO-A further demonstrate the different influences of the various physicochemical parameters upon activity. Thus, high inhibitory activity towards MAO-A by the *p*-substituted derivatives is greatly influenced by the magnitude of the Hammett σ constant (eqn xv); i.e. a strongly electron-donating group is desirable. Such a finding, however, is consistent with the base form of inhibitors (and substrates) being the active species in such interactions (McEwen et al 1968, 1969; Williams 1974). In contrast, inhibition of MAO-A by *m*-substituted derivatives is influenced more by steric factors as measured by the Verloop L parameter (compare eqns xi and xiii).

However, since all values of L are positive, this steric factor will always increase activity, in contrast to the corresponding equation generated for MAO-B inhibition (eqn iv) where a large negative coefficient for L is demonstrated. The above data would suggest that, since the steric parameter makes a significant contribution or influences activity predominantly among the *m*-substituted derivatives, the inhibitor binding site on the enzyme active surface lies within a groove the lateral dimensions of which vary greatly between the two forms of the enzyme, those for MAO-B being far more restrictive than for MAO-A. Consistent with these conclusions would be the low inhibitory activity towards MAO-B shown by *m*-CN (14); *m*-$COOCH_3$ (17) and *m*-$COOC_2H_5$ (18). Although the *m*-CN substituted pargyline (14) displays low MAO-B inhibitory activity consistent with the presence of a bulky substituent (L = 4·23) it should also be noted that minimal MAO-B activity is predicted with hydrophilic substituents having π values close to $-0{\cdot}5$ (eqn iv) ($\pi_{CN} = -0{\cdot}57$). The benzyl ester (*m*-$COOCH_2C_6H_5$; 18), however, has considerably more activity towards MAO-B than anticipated on the basis of steric factors. This can only be rationalized by assuming additional interactions of the extremely bulky substituent with areas outside but close to the inhibitor binding site which contribute favourably to the inhibitory activity; i.e. that hydrophobic influences overcome steric factors.

Although each of the nuclear substituted pargyline derivatives were found to have affinity for both MAO-A and -B (as measured by the competitive K_i value), the inability to derive significant correlation equations for all derivatives appears to reside in the different steric influences upon *m*- and *p*-substituted derivatives. Thus, the need to include a substituent length parameter (L) into correlation equations for inhibition of MAO-B by *m*-substituted derivatives (negative coefficient, reduction of affinity) contrasts with the inhibition of MAO-A by the same compounds. Here a bulky substituent group has a small but positive influence upon affinity. Inhibition of MAO-A by *p*-substituted derivatives does not appear to depend upon bulk (length) of the substituent; the situation regarding MAO-B is more difficult to determine with the *p*-substituted derivatives, since all the compounds were of similar inhibitory potency. However, such data suggest that the active site of MAO-B lies within a groove upon the enzyme surface or alternatively that the lateral dimensions of the groove are more restrictive for MAO-B than for MAO-A.

The correlation equations further suggest that differences exist in the optimal lipophilicity requirements for competitive inhibitors of MAO-A and -B. Whereas the compounds studied span the optimal lipophilicity for MAO-A inhibition, the maximal (optimal) value for MAO-B has not been attained, although an apparent minimal value has been identified.

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