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NEW 2-[(5-METHOXY-1-METHYLINDOLYL)]-ALKYLAMINE DERIVATIVES: THE EFFECT OF BRANCHING AND ELONGATION OF THE SIDE CHAIN ON MAO INHIBITION

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A novel series of previously synthesised 2-[(5-methoxy-1-methylindolyl)]alkylamine derivatives were irreversible and time-dependent mechanism-based inhibitors of MAO. The effect of branching and elongation of the side chain was evaluated on the inhibitory potency towards MAO-A and MAO-B activities. The K_I of the reversible step and the k_{inact} of the irreversible one were determined in each case. The results obtained lead to the conclusion that neither the elongation nor the branching of the side chain improve the potency of the compounds as MAO inhibitors.

Keywords: Monoamine oxidase; Inhibition; Indolalkylamine derivatives; Kinetics

INTRODUCTION

Monoamine oxidase [MAO; monoamine oxidoreductase; EC 1.4.3.4] is a FAD-dependent enzyme responsible for the deamination of some biogenic amines neurotransmitters and other xenobiotic amines. The existence of two isoforms of the enzyme according to pharmacological criterion[1] was later confirmed by protein sequence studies.[2] These two isoforms present 70% homology in their primary sequence and are coded by two different genes

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located on the X chromosome.[3] MAO-A is responsible for the selective deamination of norepinephrine and serotonin (5-HT) and is sensitive to inhibition by a nanomolar concentration of clorgyline and MAO-B deaminates β-phenylethylamine (PEA) and benzylamine, being sensitive to inhibition by a nanomolar concentration of 1-deprenyl. Some amines, such as tyramine and dopamine, are mixed substrates for both MAO isoforms. Selective MAO-A inhibitors exhibit antidepressant effect.[4] Although apparently devoid of any antidepressant effects, MAO-B inhibitors have been shown to be of value in the treatment of Parkinson disease.[5] Hence, the search for new compounds with greater potency and selectivity towards each MAO form has been greatly increased. Despite the large number of MAO inhibitors that have been described,[6–8] the structural features that determine inhibitory potency and selectivity towards MAO-A and MAO-B have not yet been elucidated. We have reported previously[9–13] that N-acetylenic and N-allenic derivatives of tryptamine act as effective and selective MAO inhibitors. In this context, we have studied the branching and elongation effect on the side chain of a series of 2-[(5-methoxy-1-methylindol-2-yl)] alkylamine derivatives (see Table I) as MAO-A and MAO-B inhibitors as part of a project aimed at determining the structural requirements necessary for inhibitory potency and selectivity.

MATERIAL AND METHODS

Chemicals

5-hydroxy [side chain – 2-^{14}C]tryptamine creatinine sulphate (55 mCi/mmol 50 μCi/mL) was purchased from Amersham (Amersham, UK). [Ethyl-1-^{14}C] phenylethylamine HCl (50 mCi/mmol 0.1 mCi/mL) was purchased from New England Nuclear (Stevenage, UK). Kynuramine dihydrobromide and benzylamine HCl were obtained from the Sigma Chemical Co. (Poole, UK) and deprenyl from Research Biochemical International (R.B.I., USA).

The series of 5-OCH_3-indolalkylamine derivatives were synthetized by Fernández *et al.*[14–16]

Preparation of Rat Liver Mitochondria

Rat liver were recovered from male Sprague-Dawley rats (weighing 200–250 g) which had been fasted for 12 h. The liver homogenates were prepared in 10 vol. (w/v) of a 50 mM potassium phosphate buffer (pH 7.2) by use of a Dounce homogenizer. The mitochondrial fraction was prepared by a

standard differential centrifugation method.[17] The pellets were resuspended in the same buffer and frozen as small aliquots at −20°C until required.

Protein concentration was determined by the Hartree method[18] with bovine serum albumin as a standard.

Radiochemical Assays

MAO activity was determined radiochemically at 37°C by the method of Fowler and Tipton.[19] PEA (20 μM) [2.5 mCi/mmol] and 5-HT (100 μM) [0.5 mCi/mmol] were used as substrates for MAO-B and MAO-A, respectively. The reaction was carried out in a final volume of 225 μL in a 50 mM potassium phosphate buffer (pH 7.2) containing 200–400 μg of protein, and was stopped by the addition of 100 μL of 2 M citric acid. The products were extracted into toluene/ethyl acetate 1 : 1 (v/v) containing 0.6% (w/v) 2,5-diphenyloxazole and radioactivity was measured in a scintillation counter (Wallac, L.K.B.).

IC_{50} values (the inhibitor concentration necessary to give 50% inhibition) of the 5-OCH_3-indolalkylamine derivatives were determined with and without pre-incubation of the inhibitor with the enzyme for 30 min at 37°C and at inhibitor concentrations ranging for 10^{-3}–10^{-10} M. The MAO-A and MAO-B activities remaining were measured radiochemically with 5-HT and PEA, respectively, as substrates and expressed as percentages of the control value.

The time-dependence of the inhibitory process was determined at an inhibitor concentration that gave essentially complete inhibition after 30 min of incubation. After different periods of pre-incubation of the inhibitor with the enzyme, samples of the mixture were assayed for MAO-A and MAO-B activities by the radiochemical method described previously.

To determine whether reversible or irreversible inhibition was produced by these compounds, samples of the mitochondrial fraction were incubated for 30 min at 37°C with the inhibitor at an inhibitor concentration near its IC_{50} value and then the remaining activity was determined. The mixture was centrifuged and the pellet was washed by resuspension in the same volume of 50 mM potassium phosphate buffer (pH 7.2) and centrifuged three times. The recovered activity was determined after each washing. Controls in which the inhibitor was replaced by an identical volume of distilled water were run through the same procedure. The reversibility of the inhibition was also assessed by dialysis. The enzyme was diluted in 50 mM potassium phosphate buffer (pH 7.2) and incubated at 37°C for 30 min with inhibitor at a concentration that gave approximately 50% inhibition. The mixture

was then dialysed overnight against 2000 volumes of the same buffer at 37°C and the enzyme activity determined. Control samples where the inhibitor was replaced by an identical volume of distilled water were treated in the same way.

Spectrophotometric Method

Spectrophotometric assays for MAO-B activity were performed at 37°C using benzylamine as a substrate by measuring the appearance of the product at 250 nm by a modification[11] of the Tabor method,[20] which allowed the sequential determination of six samples each with the appropriate blank. MAO-A activity was determined spectrophotometrically with kynuramine as a substrate[21] by measuring the appearance of the product at 324 nm.[11] Since kynuramine is a common substrate of both MAO forms, it was necessary beforehand to inhibit MAO-B activity with $3 \cdot 10^{-7}$ M deprenyl to ensure that only MAO-A activity was present.

The mechanism-based inhibition reaction with these compounds was quantified by a modification of the Walker and Elmore method[22] previously reported.[11] The kinetic parameters were determined by direct analysis of the progress curves of the reaction between the enzyme and a fixed amount of substrate in the presence of varying amounts of inhibitor.

The inhibition progress curves were fitted to a first-order rate equation by non-linear regression analysis using the computer program ENZFITTER (Elsevier-Biosoft) to obtain the apparent first-order rate constants K_{app}.

The K_m values of MAO-A and MAO-B for kynuramine and benzylamine as substrates were 35 and 300 μM, respectively, under the conditions used in these experiments. The kinetic parameter K_I which defines the affinity of the reversible step and k_{inact}, the velocity constant corresponding to the covalent step of the total inhibitory process, were determined using the following equation by non-linear regression analysis of K_{app} versus [I]. The catalytic efficacy of the inhibitory process was defined as the k_{inact}/K_I ratio.

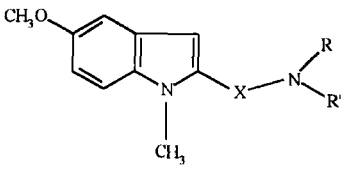
$$E + I \overset{K_I}{\rightleftharpoons} E \cdot I \overset{k_{inact}}{\longrightarrow} E\text{–}I$$

RESULTS

The structural formulae of the 5-OCH_3-indolalkylamine derivatives are shown in Table I. These N-acetylenic and N-allenic derivatives of 2-[(5-methoxy-1-methylindol-2-yl)] alkylamine derivatives were synthesised

by Fernández *et al.* [14–16] These compounds have in common a methoxy group at the 5-position and a methyl on the N of the indole ring. They differ in the groups present at R and R′ and also in the length (and branching) of the carbon side chain, in position X (see Table I). Compounds from Series I have α-methyl branching at the carbon side chain (X) (FAC 6, 12, 13, 14, 17 and 18) and compounds from Series II present this structural feature as well as an elongation of the side chain in a $-CH_2$ group (FAC 20, 21, 22, 26 and 28). Series III have β-methyl branching at the carbon chain (X) (FAC 68, 69 and 71). The effect of elongation of the side chain ($X = -CH_2-CH_2-CH_2-$) present in Series IV (FAC 34, 38, 35, 36, 37, 39, 40 and 41), was also studied on the potency and selectivity of MAO-A and MAO-B inhibition.

TABLE I Structural formulae for 5-OCH_3-indolalkylamine derivatives



Compound	X	R	R′
Series I			
FAC 6	$-CH(CH_3)-$	H–	H–
FAC 12	$-CH(CH_3)-$	CH_3-	H–
FAC 13	$-CH(CH_3)-$	H–	$CH\equiv C-CH_2-$
FAC 14	$-CH(CH_3)-$	H–	$CH_2=C=CH-CH_2-$
FAC 17	$-CH(CH_3)-$	CH_3-	$CH_2=C=CH-CH_2-$
FAC 18	$-CH(CH_3)-$	CH_3-	$CH_3-C\equiv C-CH_2-$
Series II			
FAC 20	$-CH_2-CH(CH_3)$	H–	H–
FAC 21	$-CH_2-CH(CH_3)-$	H–	$CH\equiv C-CH_2-$
FAC 22	$-CH_2-CH(CH_3)-$	H–	$CH_2=C=CH-CH_2-$
FAC 26	$-CH_2-CH(CH_3)-$	CH_3-	$CH\equiv C-CH_2-$
FAC 28	$-CH_2-CH(CH_3)-$	CH_3-	$CH_3-C\equiv C-CH_2-$
Series III			
FAC 68	$-CH(CH_3)-CH_2-$	H–	H–
FAC 69	$-CH(CH_3)-CH_2-$	CH_3-	H–
FAC 71	$-CH(CH_3)-CH_2-$	CH_3-	$CH_2=C=CH-CH_2-$
Series IV			
FAC 34	$CH_2-CH_2-CH_2-$	H–	H–
FAC 38	$-CH_2-CH_2-CH_2-$	CH_3-	H–
FAC 35	$-CH_2-CH_2-CH_2-$	H–	$CH\equiv C-CH_2-$
FAC 36	$-CH_2-CH_2-CH_2-$	H–	$CH_2=C=CH-CH_2-$
FAC 37	$-CH_2-CH_2-CH_2-$	$CH_3-C\equiv C-CH_2-$	$CH_3-C\equiv C-CH_2-$
FAC 39	$CH_2-CH_2-CH_2-$	CH_3-	$CH\equiv C-CH_2-$
FAC 40	$-CH_2-CH_2-CH_2-$	CH_3-	$CH_2=C=CH-CH_2-$
FAC 41	$-CH_2-CH_2-CH_2-$	CH_3-	$CH_3-C\equiv C-CH_2-$

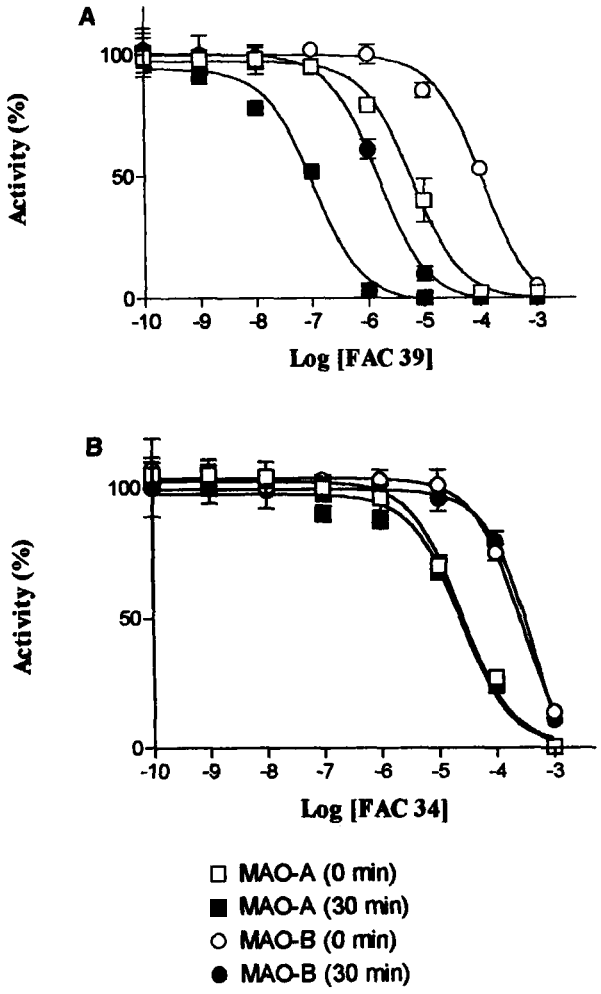


FIGURE 1 MAO inhibition by different concentrations of FAC 39 (A) and FAC 34 (B) by measuring the remaining activity towards 100 μM ^{14}C-5HT (for MAO-A) and 20 μM ^{14}C-PEA (for MAO-B) after 0 and 30 min pre-incubation. Percentage activity was calculated with respect to samples treated in the same way but in the absence of the compound.

MAO-A and MAO-B activities from a rat liver mitochondrial fraction were assayed after 0 and 30 min preincubation at 37°C in the presence of different concentrations of the 5-OCH_3-indolalkylamine derivatives. The IC_{50} values determined from the corresponding inhibition curves indicated that the inhibition was time-dependent in all cases with the exception of the parent primary amines (FAC 6, 12, 20, 68, 69, 34 and 38). Figure 1 shows the inhibition curves for FAC 39 (A) and FAC 34 (B) as representatives of

all compounds tested. In this respect, even though the IC_{50} values of some of the inhibitors determined showed some decrease after preincubating for 30 min (compared to the IC_{50} values obtained without preincubation) it is not possible to conclude from such relatively small changes that a time-dependent inhibition is involved. However, the presence of a time-dependent inhibition was confirmed by running the appropriate time-dependent inhibition curves.

The IC_{50} values (see Table II) calculated after 30 min pre-incubation for the compounds of Series I, all having in common the α-methyl branching ($X = -CH(CH_3)-$) and differing only in the R and R′ groups, show that when R′ was a 2-propynyl group (FAC 13) or a 2,3-butadienyl (FAC 14 and 17) or a 2-butynyl (FAC 18) they become potent MAO-A and MAO-B inhibitors. The most potent compound was found to be FAC 18, and with

TABLE II IC_{50} values for MAO inhibition by 5-OCH_3-indolalkylamine derivatives

Compound	IC_{50} (μM)		
	MAO-A	*MAO-B*	*B/A*
Series I			
FAC 6	40.8 ± 6.3	276 ± 30	6.8
FAC 12	175 ± 10	>1000	
FAC 13	1.24 ± 0.15	1.01 ± 0.07	0.8
FAC 14	0.053 ± 0.005	0.039 ± 0.003	0.7
FAC 17	0.016 ± 0.001	0.17 ± 0.03	10.3
FAC 18	0.014 ± 0.002	0.035 ± 0.001	2.5
Series II			
FAC 20	22.9 ± 3.5	279 ± 40	15.6
FAC 21	15.5 ± 1.9	21.1 ± 4.1	1.3
FAC 22	0.66 ± 0.05	22.7 ± 2.1	34.3
FAC 26	1.62 ± 0.32	32.3 ± 6.6	19.9
FAC 28	2.89 ± 0.55	54.7 ± 5.7	18.9
Series III			
FAC 68	54.8 ± 9.3	304 ± 16	5.5
FAC 69	171 ± 19.4	1000 ± 35	5.8
FAC 71	0.3 ± 0.04	79.1 ± 8.6	263.6
Series IV			
FAC 34	31.9 ± 5.6	773 ± 30	24.2
FAC 38	3.46 ± 0.36	172 ± 14.3	49.7
FAC 35	3.69 ± 0.74	7.84 ± 0.74	2.1
FAC 36	6.79 ± 1.29	0.94 ± 0.08	0.14
FAC 37	54.1 ± 5.6	>1000	
FAC 39	0.11 ± 0.01	1.48 ± 0.25	13.4
FAC 40	17.8 ± 2.9	13.8 ± 2.3	0.77
FAC 41	2.8 ± 0.51	2.32 ± 0.93	0.81

Each value represents mean ± SEM of at least two separate determinations. IC_{50} values were determined from the inhibition curves by preincubating for 30 min at 37°C rat mitochondrial fractions with different inhibitor concentrations and measuring the remaining activity towards 100 μM ^{14}C-5-HT (for MAO-A) and 20 μM ^{14}C-PEA (for MAO-B).

the exception of FAC 17 that increased its selectivity towards MAO-A ($IC_{50}B/IC_{50}A = 10.3$), (the presence of a methyl group diminished the affinity towards MAO-B), the rest of compounds appear to be less selective. The amines of this series (FAC 6 and 12) were poor MAO-A and B inhibitors and it would be interesting to study their possible behaviour as MAO-A and MAO-B substrates.

All compounds of Series II had an elongation of the side chain before the α-methyl branching (the term before used here is meant to indicate that the elongation of the side chain is on the side chain of the indole ring) but still differed in the groups in the R and R′ positions. When R and R′ are H, the primary amine (FAC 20) was 2 times more potent and selective than the corresponding amine without the elongation of the side chain (FAC 6), for comparative purposes. When R′ is a 2-propynyl group (FAC 21), the potency towards MAO-B increased 13-fold compared with the parent amine (FAC 20) after 30 min pre-incubation, but did not change with respect to MAO-A. Therefore the presence of a 2-propynyl group, when combined with the elongation of the side chain, increased the potency towards MAO-B with apparent loss of selectivity for MAO-A ($IC_{50}B/IC_{50}A = 1.3$).

The presence of a 2,3-butadienyl group at the R′ position (FAC 22) increased the potency 23-fold towards MAO-A, without affecting the potency for MAO-B when compared to FAC 21 which contains a 2-propynyl group. In this case, the selectivity towards MAO-A increased significantly (26-fold). The introduction of a methyl group at the R position and a 2-propynyl group at the R′ position (FAC 26) increased the potency 10 times towards MAO-A when compared with FAC 21 and slightly decreased the potency towards MAO-B, so that the selectivity towards MAO-A increased by a factor of 15. The introduction of a 2-butynyl group at the R′ position (FAC 28) did not change significantly either the affinity or the selectivity towards both MAO forms when compared with FAC 26 which contains a 2-propynyl group at the R′ position.

All compounds from Series III, had in common the feature that the –X– has been elongated after the α-methyl branching (the term after used here is meant to indicate that the elongation of the side chain is on the amine side), and differed via the R and R′ groups. The parent amines FAC 68 and 69 acted as very poor MAO inhibitors, however when a 2,3-butadienyl group was present at the R′ position (FAC 71), the potency towards MAO-A and MAO-B increased 570 and 13 times respectively, so that the selectivity of the inhibition towards MAO-A increased 44-fold when comparing with the parent amine FAC69.

The elongation effect of the side chain ($X = (-CH_2-CH_2-CH_2-)$ without branching was studied in the compounds of Series IV. The corresponding

parent amines (FAC 34 and 38) behaved as poor MAO inhibitors as in previous cases, nevertheless they showed greater selectivity towards MAO-A. The introduction at the R′ position of a 2-propynyl or 2,3-butadienyl group (FAC 35 and 36) respectively, increased the potency towards MAO-B, in particular for compound FAC 36 when compared with the parent amine. (FAC 34).

The introduction of a second 2-butynyl group at the R position (FAC 37) had a negative effect on the affinity, in particular towards MAO-B when comparing with FAC 36. Between FAC 39, 40 and 41, with a methyl group at the R position and differing in the group located at the R′ position, the compound that behaved as the most potent and selective MAO-A inhibitor was FAC 39, which had a 2-propynyl group at the R′ position.

Overall, for all the compounds studied it can be concluded that the compounds of Series I, with the exception of the corresponding parent amines, showed the highest potency towards both MAO forms.

In order to study the time-course of the inhibition of MAO by these compounds, aliquots of rat liver mitochondrial fraction were preincubated with the different compounds and assayed at concentrations that resulted in complete inhibition after 30 min pre-incubation. All the compounds assayed showed a time-dependent increase in MAO inhibition. Figure 2 shows the time course of the inhibition of both MAO forms by compound FAC 39 as representative of all the compounds tested. The concentrations of FAC 39 used in the assay were 1 μM for MAO-A and 10 μM for MAO-B and in both cases the inhibition increased with time of pre-incubation reaching a maximum after 20 min.

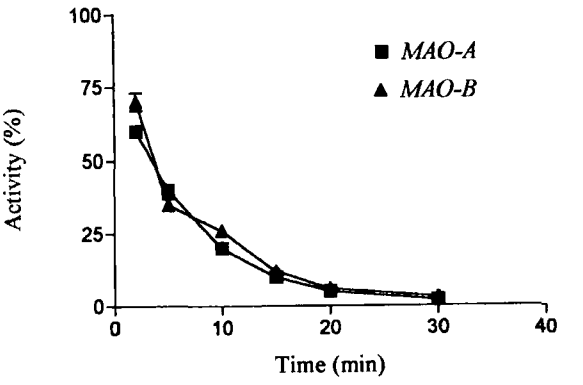


FIGURE 2 Time-course of the inhibition of MAO-A and MAO-B by FAC 39. Concentrations of FAC 39 used were 1 μM for MAO-A and 10 μM for MAO-B. Remaining activities were measured radiochemically to using 100 μM ^{14}C-5HT (for MAO-A) and 20 μM ^{14}C-PEA (for MAO-B). Percentage activity was calculated with respect to samples treated in the same way but in the absence of the compound.

In order to check the reversibility of the inhibition, the different compounds were incubated with the enzyme at concentrations that gave approximately 50% inhibition. After that, samples were washed 3 times by repeated centrifugation and resuspension as described previously in the Material and Methods section. No significant recovery of MAO-A and MAO-B activities were obtained in the case of N-allenic and N-acetylenic derivatives, indicating an irreversible inhibition process. Figure 3 shows the reversibility test for compound FAC 39, as representative of all the compounds tested. However all the parent amines of each series, showed a recovery of both MAO-A and B activities after the three centrifugation and resuspension cycles, indicating that these compounds behaved as reversible inhibitors (data not shown). The irreversible inhibitor behaviour was confirmed by dialysis studies. For this purpose, aliquots of mitochondrial fraction were incubated 30 min at 37°C in the presence of the compounds at a concentration near their IC_{50} values and the activities remaining were measured for each MAO form. After that, the mixture was dialysed overnight as described in Materials and Methods. No recovery of MAO-A or MAO-B activity was observed in any case (data not shown).

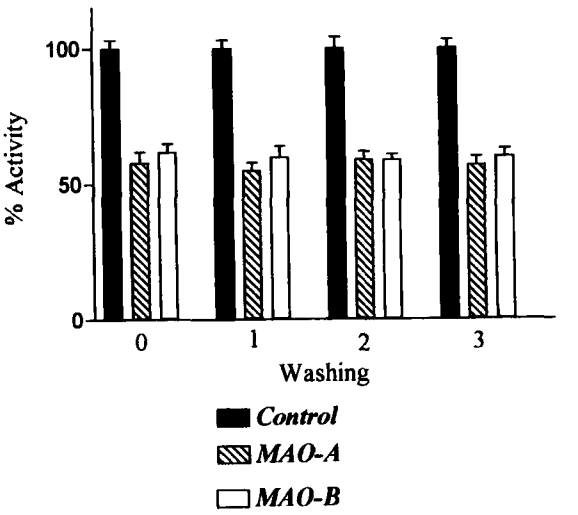


FIGURE 3 Assesment of the nature of the inhibition of MAO-A and MAO-B by FAC 39. Concentrations of FAC 39 used were 0.1 μM for MAO-A and 1 μM for MAO-B. Activities were assayed radiochemically using 100 μM ^{14}C-5HT (for MAO-A) and 20 μM ^{14}C-PEA (for MAO-B) immediately (0) and after each of three centrifugation–resuspension cycles. Percentage activity was calculated with respect to samples treated in the same way but in absence of the compound.

The time-dependence inhibition curves in which the activity was measured towards benzylamine as MAO-B substrate and kynuramine as MAO-A substrate resulted in an increasing curvature of the reaction progress curves as the inhibitor concentration was increased (data not shown). The first-order rate constant K_{app} was calculated at each inhibitor concentration as described previously.[11] Kinetic parameters, K_I and k_{inact} were then determined by non-linear regression analysis of K_{app} versus inhibitor concentration. Figure 4 shows the graphical representation of K_{app} versus concentration for compound FAC 39. Table III presents the kinetic parameters towards each MAO form of the 2-[(5-methoxy-1-methylindol-2-yl)]alkylamine derivatives with the exception of the parent amines.

For compounds of Series I, the introduction of a methyl group at the R position (FAC 17) and the presence of a 2,3-butadienyl group at R′ when compared with FAC 14, enhanced the affinity (K_I) and the potency, expressed as the catalytic efficiency (k_{inact}/K_I), towards MAO-A without altering the velocity constant. Similar kinetic behaviour was observed when a methyl group was introduced at the R position and a 2-butynyl group was attached at the R′ position (see FAC 18).

When the side chain has been elongated before the α-methyl branching (Series II), the affinity expressed as K_I diminished for both MAO forms, being more pronounced in the case of MAO-B. FAC 21 diminished the affinity 1.5-fold towards MAO-A and 6-fold towards MAO-B with respect to FAC 13 from Series I, in which the side chain had not been elongated.

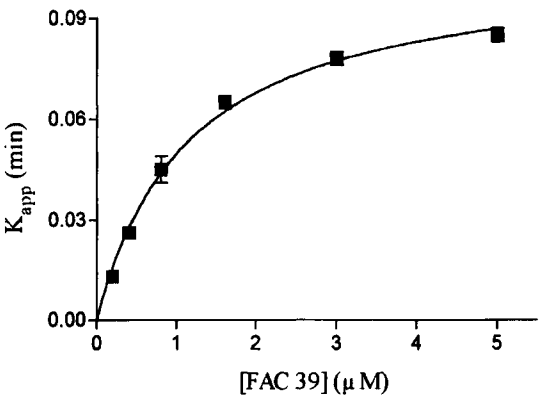


FIGURE 4 Graphical representation of K_{app} values versus inhibitor concentration (FAC 39) for MAO-B. Concentrations of FAC 39 used were 0.2, 0.4, 0.8, 1.6, 3 and 5 μM. Activity was measured spectrophotometrically using 333 μM benzylamine. K_{app} values were obtained from the inhibition progress curves.

TABLE III Kinetic parameters of MAO inhibition by 5-OCH_3-indoalkylamine

Compound	K_I (μM)			k_{inact} (min^{-1})		k_{inact}/K_I	
	MAO-A	*MAO-B*	$K_I(B/A)$	*MAO-A*	*MAO-B*	*MAO-A*	*MAO-B*
Series I							
FAC 13	2.1	0.36	0.17	0.038	0.058	0.018	0.16
FAC 14	0.023	0.007	0.30	0.023	0.037	0.13	5.29
FAC 17	0.0067	0.074	11.04	0.033	0.063	4.93	0.85
FAC 18	0.0056	0.016	2.8	0.033	0.064	5.89	4
Series II							
FAC 21	3.14	2.14	0.68	0.037	0.09	0.012	0.042
FAC 22	0.63	21.61	34.3	0.071	0.082	0.11	0.0034
FAC 26	1.96	4.27	2.17	0.086	0.042	0.044	0.0098
FAC 28	2.01	2.55	1.26	0.039	0.037	0.019	0.014
Series III							
FAC 71	0.51	4.47	8.76	0.054	0.036	0.11	0.008
Series IV							
FAC 35	1.5	3.96	2.64	0.02	0.081	0.013	0.04
FAC 36	2.73	1.32	0.48	0.024	0.08	0.0088	0.061
FAC 39	0.19	2.3	12.1	0.043	0.053	0.22	0.023
FAC 40	9.71	0.98	0.1	0.026	0.028	0.0027	0.028
FAC 41	1.27	1.72	1.35	0.059	0.097	0.046	0.056
Clorgyline	0.03	74.32	2477.3	0.11	0.12	3.67	0.0016
Deprenyl	0.37	0.0168	0.045	0.0108	0.049	0.029	2.92

Each value is the mean of three separate experiments. In all cases the SEM is less than 5%. MAO activities, in presence of different inhibitor concentrations, were measured spectrophotometrically with 40 μM kynuramine, as MAO-A substrate, and with 333 μM benzylamine, as MAO-B substrate. The kinetic parameters were obtained from the inhibition progress curves.

The velocity constant k_{inact} was similar for all of them and did not alter when compared with compounds of Series I. It can be concluded that elongation by one carbon before α-methyl branching in the side chain, did not alter the catalytic constant, and consequently the catalytic efficacy as MAO inhibitors diminished in this series. These results are in agreement with those shown in Table II.

When the side chain has been elongated after the α-methyl branching (FAC 71) the affinity (K_I) decreased 76-fold towards MAO-A and 60-fold towards MAO-B when compared with compound FAC 17 from Series I. The velocity constant (k_{inact}) did not vary with respect to the other Series, therefore the inhibitory potency (k_{inact}/K_I) in this case decreased 45-fold for MAO-A and 106-fold for MAO-B.

When the side chain has been elongated by two carbons without branching (Series IV), the groups located at the R or R′ positions produced different effect on the inhibitory behaviour. The presence of a 2-propynyl (FAC 35) or a 2,3-butadienyl (FAC 36) group at the R′ position did not significantly alter the affinity (K_I) towards both MAO forms. However, the

introduction of a methyl group at position R (FAC 39) increased the affinity 8-fold towards MAO-A and did not alter the affinity towards MAO-B when compared to FAC 35 where the methyl group at the R position was absent. Furthermore, the selectivity towards MAO-A increased 5-fold. On the other hand, the introduction of a methyl group at the R position, when a 2,3-butadienyl group was located at R′ (FAC 40), reduced the affinity by 3.5-fold towards MAO-A, without altering the affinity towards MAO-B. As a consequence, the selectivity towards the MAO-A form decreased 5-fold, when compared with FAC 36. Also in Series IV, the catalytic constant showed similar values to those reported for the other series and the inhibitory potency (k_{inact}/K_I) was also very low.

DISCUSSION

With the aim to better understanding the structural features determining high potency and selectivity within Monoamine oxidase inhibitors, we have previously examined the behaviour of a novel series of acetylenic derivatives[9–12] and allenic derivatives[13] as MAO inhibitors.

In the present work the effect of the chain-length between the aromatic indole ring and the nitrogen atom, and the α- and β-branching of a novel series of 2-[(5-methoxy-1-methylindol-2-yl)]alkylamine derivatives on MAO inhibition was studied.

Within the series of compounds studied, the amine derivatives (FAC 6, 12, 20, 68, 69, 34 and 38) behaved as reversible MAO inhibitors. The N-acetylenic and N-allenic derivatives were time-dependent irreversible inhibitors of MAO-A and MAO-B and it is reasonable to assume that they acted as mechanism-based inhibitors.

All the series showed a similar velocity constant and higher selectivity towards MAO-A than MAO-B.

The effect of branching or elongation of the side chain affected only the affinity constant K_i of the first reversible step of the mechanism-based inhibition.The α-methyl branching at the side chain (compound FAC 17, Series I) significantly increased the affinity towards both MAO forms, when compared with compound FA 44,[13] (MAO-A, K_I: 0.033 μM, k_i: 0.502 min^{-1}; MAO-B, K_I: 0.225 μM, k_i: 0.127 min^{-1}) selected for comparative purposes, but the selectivity was not altered after 30 min pre-incubation with the enzyme. The β-methyl branching plus a $-CH_2-$ elongation (see FAC 71 Series III), has the opposite effect to that of α-methyl branching and the affinity was reduced especially towards MAO-B, and consequently the

selectivity towards MAO-A increased significantly (see FAC 17 , Series I for comparative purposes).

The α-methyl branching plus a $-CH_2-$ elongation (see FAC 28 Series II) had a similar effect to that observed in Series III. The elongation by a $-CH_2-CH_2-$ group at the carbon side chain (FAC 40, Series IV), with an allenic group, produced a significant reduction in the affinity constants towards MAO-A and a slight increase in the affinity towards MAO-B (see compound FA 44[13] for comparative purposes). The same effect was observed in FAC 41 and FAC 39 when comparing with FA 45[25] and FA 43[25] for comparative purposes. In these series of compounds the selectivity was lost. These results are in agreement with those observed with 1-deprenyl. It has been found[23] that the introduction into the 1-deprenyl molecule of one or two methylene units between the phenyl ring and the remainder of the molecule, reduces the inhibitory potency towards MAO. Opposite results[24] were reported on the role of the chain-length in a series of clorgyline analogues, where this structural effect was found to be important on the inhibitory potency but less important in conferring selectivity. The differences observed between these studies and the results presented here, could be explained by the differences between the nature of the aromatic ring moiety present and its substituents.

In this study the presence of an indole ring in all the compounds, probably avoids that the elongation chain effect would appear to be important on the inhibitory potency.

Overall the results presented here lead to the conclusion that the elongation and branching of the side chain of these new 2-(5-methoxy-1-methylindolyl)alkylamine derivatives, does not significantly improve their potency as MAO-A and MAO-B inhibitors. Further structure–activity studies need to be done with different MAO inhibitors in order to definitively establish the structural features necessary for high inhibitory potency and selectivity.

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