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THE EFFECT OF SIDE CHAIN SUBSTITUTION AT POSITIONS 2 AND 3 OF THE HETEROCYCLIC RING OF *N*-ACETYLENIC ANALOGUES OF TRYPTAMINE AS MONOAMINE OXIDASE INHIBITORS

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Abstract—*N*-Acetylenic analogues of tryptamine in which the side chain is located at position 2 of the indole ring are compared with those in which the side chain is located at position 3, in terms of their actions as inhibitors of monoamine oxidases A and B. IC_{50} values at 0 and 30 min of pre-incubation were determined. Time-dependence and irreversible inhibition confirmed that all of them behave as mechanism-based inhibitors. The kinetic constants of each inhibition step were determined for both monoamine oxidase forms and compared between them. In all cases the first-order rate constants for the covalent adduct formation were similar to inhibitor selectivity which is derived solely from differences in affinities for non-covalent binding to the A and B enzymes. Those compounds where the acetylenic side chain was substituted at position 2 of the heterocyclic ring and selective inhibitors of monoamine oxidase A were more potent than those with the side chain in position 3.

The mitochondrial enzyme monoamine oxidase (MAO§) [monoamine; O_2 oxidoreductase (deaminating) (flavin containing) EC 1.4.3.4], is responsible for the deamination of biogenic amines, some of them with a neurotransmitter function. The importance of inhibitors of MAO was recognized when the mood-elevating actions of iproniazid were observed while testing this drug as a potential tuberculostatic agent. The existence of two MAO forms, first reported by Johnston [1], led to the research of new compounds which were more selective towards one or the other form of MAO and more potent than iproniazid [2].

In the terminology of Johnston [1], monoamine oxidase A (MAO A) is sensitive to inhibition by clorgyline and has 5-hydroxytryptamine (5-HT) as its preferred substrate, whereas monoamine oxidase B (MAO B) is sensitive to inhibition by l-deprenyl and has benzylamine and 2-phenylethylamine (PEA) as preferred substrates. Inhibitors selective towards MAO A have been shown to be effective antidepressants. Although apparently devoid of antidepressant action, MAO B inhibitors have been shown to be of value in the treatment of Parkinson's disease [3].

Despite the large number of MAO inhibitors described, and the structure–activity relationship studies [4–6], the structural features responsible for a greater potency and selectivity towards each MAO form, are not completely clear.

N-Acetylenic analogues of tryptamine, in which the side chain is located at the 2 and 3 positions of the indole ring have been shown to be selective inhibitors of MAO A [7, 8].

As part of a project aimed at determining the structural features that lead to inhibitor potency and selectivity, we have studied the effects of side-chain substitution at position 2 of the indole ring in some *N*-acetylenic analogues of tryptamine and compared them with those in which the side chain is substituted at position 3.

MATERIALS AND METHODS

Chemicals. 5-Hydroxy [side chain-2-^{14}C]tryptamine creatinine sulphate 55 mCi/mmol 50 μCi/mL was purchased from Amersham (Amersham, U.K.). HCl [*ethyl*-1-^{14}C]phenylethylamine 50 mCi/mmol 0.1 mCi/mL was purchased from New England Nuclear (Stevenage, U.K.). Kynuramine dihydrobromide and benzylamine HCl were obtained from the Sigma Chemical Co. (Poole, U.K.). Deprenyl was a generous gift from Prof. Knoll of Semmelweis University (Hungary).

The series of *N*-acetylenic analogues of tryptamine, in which the side chain is substituted at position 2 (FA 6, FA 27, FA 43) and position 3 (FA 17, FA 22, FA 37) of the indole ring, were synthetized by Cruces *et al.* [9].

Tissue homogenates and MAO preparations. Rat liver homogenates were prepared from male Sprague–Dawley rats (weighing 200–250 g) which had previously fasted overnight, in 10 vol. (w/v) of a 50 mM potassium phosphate buffer (pH 7.2) by use of a Dounce homogenizer. Mitochondria were prepared by a standard differential centrifugation method [10]. The pellets were suspended in the same

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§ Abbreviations: MAO, monoamine oxidase; MAOI, monoamine oxidase inhibitors; MAO A, monoamine oxidase A form; MAO B, monoamine oxidase B form; 5-HT, 5-hydroxytryptamine; PEA, 2-phenylethylamine.

buffer and frozen as small aliquots at −20° until required.

Kynuramine is a substrate for both MAOs. In order to use this substrate for the determination of MAO A activity, the MAO B was first inhibited by preincubating with 0.3 μM *l*-deprenyl at 37° for 60 min. After this incubation, the free inhibitor was removed by centrifugation at 4° and 20,000 *g* for 15 min. The pellet was washed by resuspension and centrifugation three times before it was finally resuspended in a 50 mM potassium phosphate buffer (pH 7.2) and aliquots were frozen at −20° until used.

Radiochemical assays. MAO activity was determined radiochemically at 37° by the method of Fowler *et al.* [11]. PEA (22.2 μM) and 5-HT (100 μM) were used as substrates. The reaction was carried out in a final volume of 225 μL of a 50 mM potassium phosphate buffer (pH 7.2) containing 200–400 μg of protein, and was stopped by the addition of 100 μL, 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.

Protein assay. Protein concentration was determined by the Hartree method [12] with bovine serum albumin as standard.

Reversibility test. In order to test whether reversible or irreversible inhibition was produced by these indolealkylamine derivatives, 200 μL of mitochondria (8.5 mg/mL) from rat liver was preincubated for 30 min at 37° with 100 μL of the inhibitor at a concentration near its IC_{50} value (the inhibitor concentration necessary to give 50% inhibition under the incubation and assay conditions used), in a final volume of 1 mL of a 50 mM potassium phosphate buffer (pH 7.2) and after this the remaining activities were determined. The mixture was centrifuged and the pellet was washed by resuspension in the same volume of buffer and centrifuged three times. Regaining the activity was determined after each washing. Controls in which the inhibitor was replaced by an identical volume of a buffer were run through the same procedure.

Inhibitor sensitivity. IC_{50} values were determined with and without preincubation of the inhibitor with the enzyme for 30 min at 37°. Concentration of the inhibitors was in the range of 10^{-12}–10^{-2} M). The remaining MAO A and MAO B activities were measured radiochemically with 5-HT and PEA, respectively as substrates and expressed as percentages of the control.

Time-dependence. The time-dependence of the inhibitory process was determined at an inhibitor concentration that gives total inhibition after 60 min of incubation. After different periods of time for the preincubation of the inhibitor (400 μL), with 800 μL (8.5 mg/mL) of the enzyme in a total volume of 2 mL of a 50 mM potassium phosphate buffer (pH 7.2) 50 μL samples of the mixture were assayed for MAO A and MAO B by the radiochemical method as described before.

Spectrophotometric method. Spectrophotometric assays for MAO B activity were performed at 37° using benzylamine as the substrate by measuring the appearance of the product at 250 nm [13]. A Perkin–Elmer Lambda 2 spectrophotometer equipped with a six-cell autochanger, which allowed the sequential determination of six samples each with the appropriate blanks, was used. The enzyme (200 μg) was incubated with 333 μM benzylamine HCl in a total volume of 3 mL of a 50 mM potassium phosphate buffer. An extinction coefficient of 13,800 $\text{M}^{-1}\,\text{cm}^{-1}$ for benzaldehyde was used to calculate the rates of product formation in molar terms. MAO A activity was determined spectrophotometrically with kynuramine as the substrate. Considerable interference was found when measuring the decrease of absorbance at 340 nm as recommended by Weisbach *et al.* [14], due to the crude enzyme used. To avoid this, we measured the appearance of the product at 324 nm. The activity was assayed using kynuramine dihydrobromide (40 μM) as the substrate in a total volume of 2 mL of a 50 mM potassium phosphate buffer (pH 7.2) containing 500 μg of the mitochondrial preparation. The extinction coefficient at this wavelength was calculated to be 20,000 $\text{M}^{-1}\,\text{cm}^{-1}$. Since kynuramine is a common substrate of both MAO forms, it was necessary to inhibit MAO B with *l*-deprenyl to ensure that only MAO A activity was present.

Kinetic techniques. The acetylenic indolealkylamine derivatives have been shown to act as mechanism-based inhibitors of MAO, according to the general mechanism [15]:

$$\mathrm{E} + \mathrm{I} \overset{K_i}{\rightleftharpoons} \mathrm{EI} \xrightarrow{k_i} \mathrm{EI}^*$$

where E and I represent the enzyme and the inhibitor, respectively. EI represents the reversible complex formed and EI* the covalent adduct obtained from the EI complex. Walker and Elmore [16] have shown that in the experiments in which irreversible inhibition and transformation of the substrate proceeded concurrently, the concentration of substrate [S] and inhibitor [I] must be chosen so that no more than 1% of substrate was used before the enzyme is completely inhibited and, consequently, [S] remains effectively constant. The product formed [P] in the presence of the substrate at any time is given by the expression:

$$(\mathrm{P}) = \frac{K_{\mathrm{cat}}\, k_i\, [\mathrm{S}]\, [\mathrm{Eo}]}{k_i\, K_m\, [\mathrm{I}]} \times ((1 - \exp(-\mathrm{A}\, k_i\, K_m\, [\mathrm{I}]\, (t)/K_i\, [\mathrm{S}])) \quad (1)$$

$$\text{where } \mathrm{A} = 1/((K_m/[\mathrm{S}]) + 1 + (K_m\, [\mathrm{I}]/K_i\, [\mathrm{S}])). \quad (2)$$

This equation represents the kinetics of a first-order process in which K_m and K_{cat} are the Michaelis–Menten parameters for the oxidation of the substrate. K_i is the dissociation constant for the non-covalent enzyme–inhibitor complex and k_i the kinetic constant that governs the covalent adduct formation. The apparent first-order rate constant is:

$$k_{\mathrm{app}} = \mathrm{A}\, k_i\, K_m\, [\mathrm{I}]/K_i\, [\mathrm{S}]. \quad (3)$$

We have investigated the parameters determining inhibition by direct analysis of the progress curves of the reaction between an enzyme and a fixed

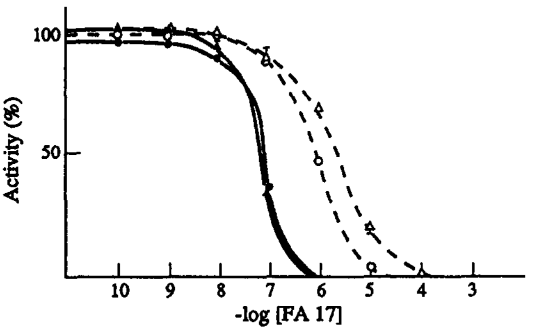


Fig. 1. MAO inhibition by different concentrations of compound FA 17, measuring the remaining activity towards 5-HT and PEA as substrates, after 0 and 30 min of preincubation. (●) MAO A 30 min, (○) MAO A 0 min, (▲) MAO B 30 min and (△) MAO B 0 min.

amount of substrate in the presence of varying amounts of an inhibitor [8, 17–19].

The inhibition progress curves were fitted to a first-order rate equation with the aid of the non-linear regression analysis computer program ENZFITTER (Elsevier–Biosoft) in order to get the apparent first-order rate constant 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 parameters K_i and k_i of the inhibitory process were determined by non-linear regression analysis of the k_{app} versus [I].

RESULTS

Inhibitory potencies

Figure 1 shows the concentration dependence of MAO inhibition by compound FA 17 taken as being representative of the rest of the compounds. The remaining activity towards 5-HT and PEA as substrates was determined after 0- and 30-min preincubation with the enzyme.

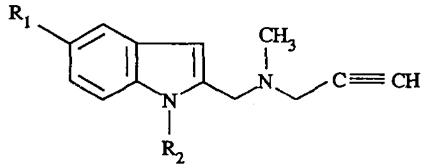
Tables 1 and 2 show the IC_{50} values after 0- and 30-min preincubations, determined from such curves with each of the inhibitors. The data for the *N*-acetylenic analogues of tryptamine in which the side chain is attached at position 2 of the indole ring, are shown in Table 1. The most potent compound towards MAO A in this series is FA 43 (IC_{50} for MAO A = 0.0003 μM), in which a methoxy group is attached at position 5 of indole ring and a methyl group is located at the R_2 position. This compound also gives the highest selectivity towards MAO A (333) after 30-min preincubation. The selectivity is expressed by IC_{50} for MAO B/IC_{50} for MAO A. When the methoxy group is lacking at the R_1 position, (FA 27), compared with FA 43 it has diminished potency towards MAO A (0.003 μM), but it has a similar potency towards MAO B (0.1 μM). These changes result in the selectivity being decreased approx. 10 times.

When H atoms are attached at both the R_1 and R_2 positions of the heterocyclic ring (FA 6), the potency towards MAO A has the lowest value of the series (0.1 μM), but that towards MAO B remains practically unchanged (0.2 μM). This compound has the lowest selectivity towards MAO A.

Table 2 shows the IC_{50} values of different *N*-acetylenic analogues of tryptamine in which the side chain is attached at position 3 of the heterocyclic ring. The potencies of these compounds as inhibitors of each form of MAO before and after 30-min preincubation with the enzyme were expressed as the drug concentration necessary to inhibit 50% of the total activity.

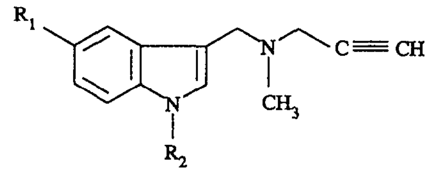
When no substituting groups are located at R_1 and R_2 positions of the indole ring, the resulting compound (FA 17) acts potently but without selectivity against both enzymes. It presents the highest and similar potencies towards MAO A (0.09) and MAO B (0.08), and a slightly higher selectivity towards MAO B. When a methyl group is attached at the R_2 position (FA 22), its potency towards both MAO forms decreased about 10 times as compared with FA 17, and no selectivity was apparent. When a methoxy group is attached at the R_1 position of the indole ring, and a methyl group is located at the

Table 1. Structural formulae and IC_{50} values for MAO inhibition by *N*-acetylenic indolalkylamine derivatives with the side chain located at position 2



Compound	R_1	R_2	IC_{50} (μM) (0 min) MAO A	MAO B	IC_{50} (μM) (30 min) MAO A	MAO B	(B)/(A)
FA 6	H	H	0.8	10.0	0.1	0.2	2
FA 27	H	CH_3	0.03	0.3	0.003	0.1	33
FA 43	CH_3O	CH_3	0.003	1.0	0.0003	0.1	333

Table 2. Structural formulae and IC_{50} values for MAO inhibition by *N*-acetylenic indolalkylamine derivatives with the side-chain located at position 3



			IC_{50} (µM) (0 min)		IC_{50} (µM) (30 min)		
Compound	R_1	R_2	MAO A	MAO B	MAO A	MAO B	(B)/(A)
FA 17	H	H	1.0	2.5	0.09	0.08	0.8
FA 22	H	CH_3	1.0	1.0	0.1	0.1	1.0
FA 37	CH_3O	CH_3	30.0	316.0	3.0	30.0	10.0

R_2 position (FA 37), the potency towards MAO A and MAO B decreased 33 and 375 times, respectively in comparison with FA 17. In this case the selectivity towards MAO A was increased about 10 times.

Comparing the results shown in both tables it can be concluded that the substitution of R_2 by a CH_3 group (FA 22), when the side chain is located at position 3, decreases the potency and selectivity towards MAO A and MAO B. When the side chain is located at position 2, the introduction of this group (FA 27) results in an adverse effect and the potency and the selectivity towards MAO A is increased. When a methoxy group substitutes at position R_1, a large increase of selectivity and potency towards MAO A is observed when the side chain is at position 2. In contrast there was only a moderate increase of selectivity towards MAO A and a decrease of potency towards MAO A and MAO B when the side chain was attached at position 3.

Time-dependence

The time-dependence of inhibition was confirmed in all cases by measuring MAO A and MAO B activities after preincubation of an enzyme and an inhibitor for different periods of time. All the inhibitors showed a time-dependence behaviour. Figure 2 shows the time-dependence inhibition towards each form of MAO by compound FA 17, at a concentration of 10^{-6} M. In both cases the inhibition increased with preincubation time, becoming essentially complete at the end of the preincubation procedure (15 min for MAO B and 20 min for MAO A).

Reversibility test

To determine whether inhibition was an irreversible process, mitochondria were incubated with an inhibitor concentration that gave 60–70% inhibition after 30 min. Samples were then washed by repeated centrifugation and resuspension in a 50 mM potassium phosphate buffer (pH 7.2). By this procedure no significant recuperation of MAO A and MAO B was obtained in any case, after three successive washings and resuspensions. Figure 3 shows the representative results for compound

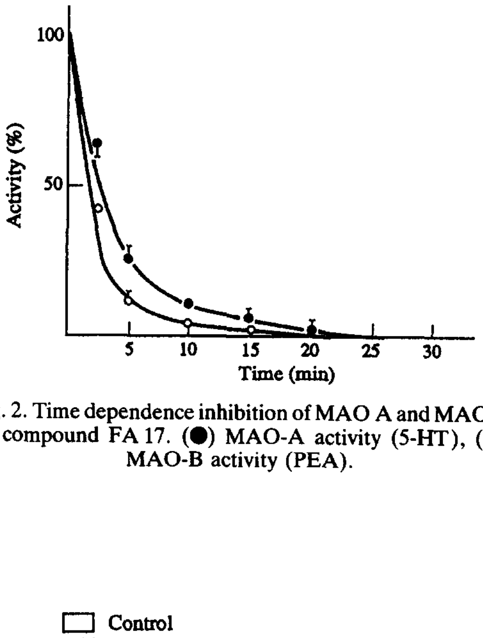


Fig. 2. Time dependence inhibition of MAO A and MAO B by compound FA 17. (●) MAO-A activity (5-HT), (O) MAO-B activity (PEA).

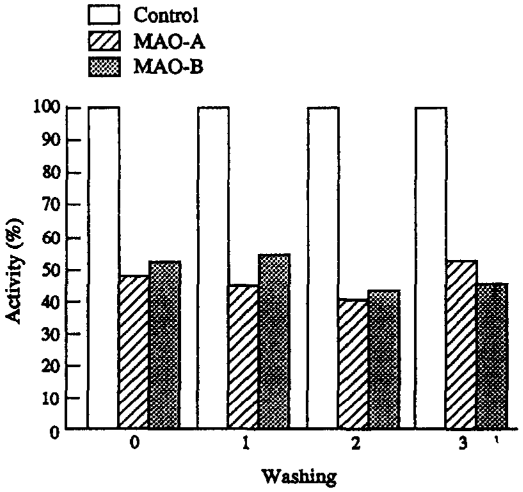


Fig. 3. Reversibility test of MAO A and MAO B inhibition by compound FA 17.

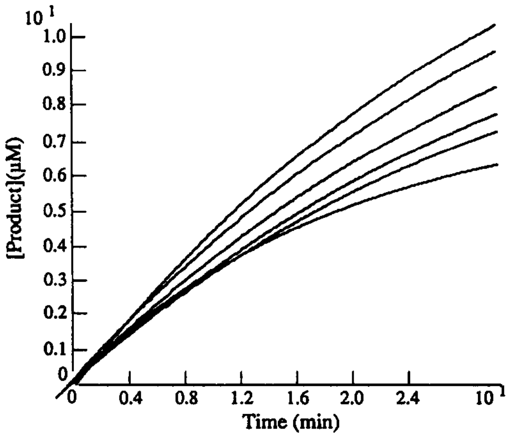


Fig. 4. Effect of increasing concentration of compound FA 17 on time-course of the oxidation of benzylamine (333 μM) by MAO B.

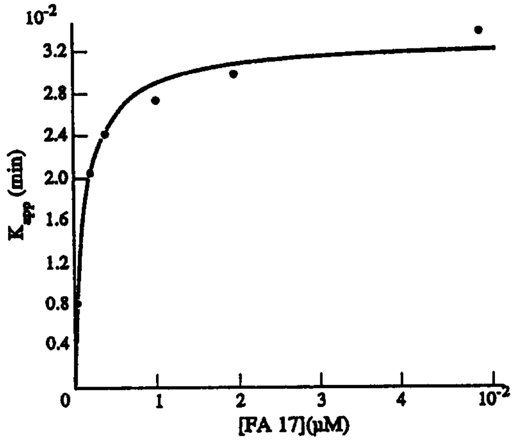


Fig. 5. Graphical representation of K_{app} values versus inhibitor concentration (FA 17), for MAO B.

FA 17. To exclude the possibility of pseudo-irreversible inhibition, dialysis at 4° and 37° was performed. The results indicated that no tight-binding (slow-dissociating) process occurred (data not shown).

Inhibition kinetics

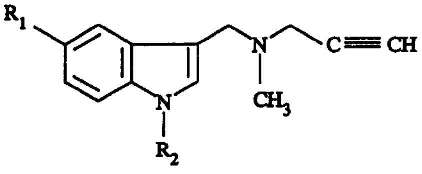
Figure 4 shows representative curves for the MAO B inhibition by compound FA 17, towards benzylamine as the substrate. Similar data were obtained with the other compounds (data not shown). The time-dependent inhibition results in an increasing curvature of the reaction progress curves when the inhibitor concentration was increased. Fitting these data to Eqn 1 allows the first-order rate-constant k_{app} to be calculated at each inhibitor concentration (Fig. 5), and the corresponding kinetic parameters K_i and k_i were then determined by non linear-regression analysis of k_{app} versus inhibitor concentration.

Kinetic parameters towards MAO A and MAO B corresponding to the series FA 17, FA 22 and FA 37, when the side chain is attached at position 3, are shown in Table 3. The values represent means ± SEM of three experiments.

These results show that all of these compounds present similar k_i values and thus in this series the different substituting groups have no effects on the rate constant for covalent adduct formation. In contrast large differences in affinity constants for the non-covalent complex formation were observed.

When hydrogens are located at both the R_1 and R_2 positions (FA 17), the dissociation constant K_i is greater for MAO B than for MAO A, and the catalytic efficacy expressed by k_i/K_i presents the highest value of this series for MAO B. This result is in agreement with high selectivity towards MAO B, shown in Table 2. When a methyl group is substituted at the R_2 position of the indole ring (FA 22), the affinity towards MAO B decreases 35 times and the affinity towards MAO A is similar to that of

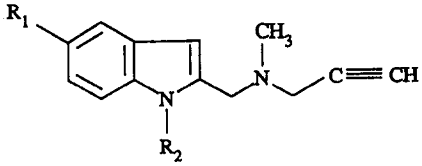
Table 3. Kinetic parameters of MAO inhibition by *N*-acetylenic indol-alkylamine derivatives with the side chain located at position 3



Compound	R_1	R_2	K_i (nM) MAO A	 MAO B	k_i (min^{-1}) MAO A	 MAO B	k_i/K_i (10^{-3}) MAO A	 MAO B
FA 17	H	H	8.6	0.625	0.052	0.037	5.9	59.7
FA 22	H	CH_3	9.8	21.4	0.025	0.076	2.5	3.5
FA 37	CH_3O	CH_3	5187.0	2050.0	0.044	0.054	0.8	0.02

Each value is the mean of three separate experiments. In all cases the SEM is less than 5%.

Table 4. Kinetic parameters of MAO inhibition by *N*-acetylenic indol-alkylamine derivatives with the side chain located at position 2



Compound	R_1	R_2	K_i (nM) MAO A	K_i (nM) MAO B	k_i (min^{-1}) MAO A	k_i (min^{-1}) MAO B	k_i/K_i MAO A	k_i/K_i MAO B
FA 6	H	H	11.9	6.3	0.069	0.053	5.84	8.43
FA 27	H	CH_3	0.36	3.0	0.033	0.040	90.0	13.00
FA 43	CH_3O	CH_3	1.50	30.0	0.057	0.059	38.0	2.00

Each value is the mean of three separate experiments. In all cases the SEM is less than 5%.

compound FA 17. The catalytic efficacy is similar to both MAO forms, and consequently no selectivity is observed. This result is in agreement with that in Table 2. It can thus be concluded that the introduction of a methyl group at position R_2 is responsible for the loss of selectivity.

When a methoxy group is attached at the R_1 position, and a methyl group is attached at the R_2 position (compound FA 37), the affinity towards MAO A and MAO B greatly diminishes (527 times for MAO A and 97 times for MAO B), compared with FA 22, and consequently the catalytic efficacy shows the lowest value of this series of compounds towards both MAO forms. We can conclude that the introduction of a methoxy group at the R_1 position decreases the affinity towards both MAO forms and also consequently decreases the selectivity.

Table 4 shows the catalytic parameters of the indole compounds in which the side chain is attached at position 2. The rate constants for covalent adduct formation are similar in all cases, thus the catalytic efficacy expressed by k_i/K_i depends on the affinity of the reversible complex formation.

When there are no substitutions at the R_1 and R_2 positions of the indole ring (FA 6), similar affinities towards MAO A and MAO B are observed and consequently similar catalytic efficacy is obtained. This corresponds with the lack of selectivity observed in Table 1 for the same compound.

When a hydrogen is substituted by a methyl group at the R_2 position (FA 27), the affinity towards MAO A increases 33 times and the affinity towards MAO B increases twice. Consequently, the catalytic efficacy increases 15 times for MAO A as compared with FA 6, nevertheless, in the case of MAO B the catalytic efficacy increases only 1.5 times.

When a methoxy group is attached at position R_1 and a methyl group is located at position R_2 of the heterocyclic ring (FA 43), this compound has diminished affinities towards MAO A and MAO B, of four and 10 times, respectively as compared with compound FA 27. The catalytic efficacy diminishes 2.3 times in the case of MAO A and six times with respect to MAO B. In this case, the selectivity towards MAO A was the highest value observed in this series, and these results agree with those shown in Table 1.

Comparing both series of compounds, those substituted at position 2 (FA 6, FA 27 and FA 43) with those substituted at position 3 (FA 17, FA 22 and FA 37), we can conclude that the compounds with the side chain at position 2 show greater catalytic efficacy and selectivity towards MAO A than those in which the alkyl chain is attached at position 3 of the indole ring.

DISCUSSION

The MAO inhibitory behaviour of different *N*-acetylenic analogues of tryptamine, in which the side chain is located at position 3 of the indole ring are compared with those in which the same side chain is located at position 2.

All the compounds behaved as irreversible and time-dependent inhibitors, a feature which is expected as a consequence of the presence of an acetylenic group in the side chain [15, 17].

The kinetic parameters were determined with accuracy by a continuous monitoring method in which the appearance of the product formed was continuously measured [8, 18, 19]. In all cases the progress curves of the enzyme in the absence of an inhibitor were linear and consequently the loss of linearity observed was due exclusively to the presence of different inhibitor concentrations. This method allowed us to measure the catalytic parameters of the mechanism-based inhibition, by this series of compounds, more accurately than with the discontinuous method.

A modification of the Weisbach method [14] standardized by us, allows one to measure the apparition of the product formed at 324 nm without the interferences observed at 340 nm as a result of the use of a crude enzyme preparation.

Examining the kinetic parameters of both series of compounds, it can be observed that the k_i values corresponding to the covalent adduct formation, were similar in all cases and similar to the k_i value obtained for clorgyline ($k_i = 0.02\ min^{-1}$). Thus the change in the selectivity was exclusively due to the alteration of the affinity for the non-covalent complex formation as a consequence of the different substitution at the R_1 and R_2 positions. In the case of the inhibition of MAO by pargyline the selectivity has also been shown to be derived solely from differences in the affinities for non covalent complex formation, whereas both K_i and k_i contribute to the selectivity of clorgyline and *l*-deprenyl [15].

The introduction of a methyl group at positions R_2 had different effects when the side chain was located at position 3 as compared with those at position 2. In the first case the affinity towards MAO B was significantly diminished, and selectivity was lost. In the second case the affinity was increased towards both MAO forms, but towards MAO A to a greater extent resulting in increased selectivity. The latter affinity value was similar to that of clorgyline for MAO A ($K_i = 0.36$ nM, $k_i = 0.02$), calculated by the same method. Furthermore, with the methyl group at the R_2 position, the substitution of a methoxy group in position 5 of the heterocyclic ring, in the first case (side chain substitution at position 3), the affinity was greatly diminished towards both MAO forms and the selectivity towards MAO A increased. Affinity was decreased when the side chain was in position 2, but to a lesser extent than in the former case. Again the selectivity was increased.

Comparing both series of compounds we can conclude that when the side chain is attached at position 2 of the indole ring the compounds are more potent MAO inhibitors, with a higher affinity for non-covalent complex formation, and higher selectivity values than those series in which the side chain was located at position 3. Some of them (FA 27 and FA 43) show similar K_i values to that found for clorgyline ($K_i = 0.3$ nM), presenting similar catalytic potencies. Since tryptamine is a substrate for both MAO forms [20], the rather small degree of selectivity shown by its acetylenic analogue is in accord with such behaviour. The dramatic effects of substitutions on the heterocyclic ring on inhibitory behaviour would make it of particular interest to study the behaviour of similarly substituted primary and secondary amine derivatives as substrates and also to determine the effects of the homologous series in which the side chain is substituted at position 2 of the indole ring.

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REFERENCES

1. Johnston JP, Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem Pharmacol* **17**: 1285–1297, 1968.
2. Yang HYT and Neff NH, The monoamine oxidases of brain: selective inhibition with drugs and the consequences for the metabolism of biogenic amines. *J Pharmacol Exp Ther* **189**: 733–740, 1974.
3. Tipton KF, *Biochemical and Pharmacological Aspects of Depression* (Eds. Tipton KF and Youdim MBH), pp. 1–24. Taylor and Francis, London, 1989.
4. Knoll J, *Structure–Activity Relationships of the Selective Inhibitors of MAO B. Monoamine Oxidase: Structure, Function, and Altered Functions* (Eds. Singer TP, Von Korff RW and Murphy DL), pp. 431–446. Academic Press, 1979.
5. Kalir A, Sabbagh A and Youdim MBH, Selective acetylenic suicide and reversible inhibitors of monoamine oxidase type A and B. *Br J Pharmacol* **73**: 55–64, 1981.
6. Maycock AL, Abeles RH, Salach JI and Singer TP, The structure of the covalent adduct formed by interaction of 3-dimethylamino-1-propyne and the flavin of mitochondrial amine oxidase. *Biochemistry* **15**: 114–125, 1976.
7. Cruces MA, Elorriaga CE, Fernandez-Alvarez E and Nieto O, Synthesis and biochemical properties of *N*-acetylenic and *N*-allenic derivatives of 2-amine-methylindoles as selective inhibitors of monoamine oxidase. *Pharmacol Res Commun* **20**: 105–107, 1988.
8. Balsa MD, Fernandez-Alvarez E, Tipton KF and Unzeta M, Inhibition of MAO by substituted tryptamine analogues. *J Neural Transm* **[Suppl.] 32**: 103–105, 1990.
9. Cruces MA, Elorriaga C, Fernandez-Alvarez E and Nieto O, Acetylenic and allenic derivatives of 2-(5-methoxyindolyl) methylamine synthesis and evaluation as selective inhibitors of monoamine oxidases A and B. *Eur J Med Chem* **25**: 257–265, 1990.
10. Gomez N, Balsa MD and Unzeta M, A comparative study of some kinetic and molecular properties of microsomal and mitochondrial monoamine oxidase. *Biochem Pharmacol* **37**: 3407–3413, 1988.
11. Fowler CJ and Tipton KF, Concentration dependence of the oxidation of tyramine by the two forms of rat liver mitochondrial monoamine oxidase. *Biochem Pharmacol* **30**: 3329–3332, 1981.
12. Hartree EF, Determination of protein: a modification of the Lowry method that gives linear photometric response. *Anal Biochem* **48**: 422–427, 1972.
13. Tabor CW, Tabor H and Rosenthal SM, Purification of amine oxidase from beef plasma. *J Biol Chem* **208**: 644–661, 1954.
14. Weissbach H, Smith TE, Daly TW, Witkop B and Udenfriend S, A rapid spectrophotometric assay of monoamine oxidase based on the rate of disappearance of Kynuramine. *J Biol Chem* **235**: 1160–1163, 1960.
15. Fowler CJ, Mantle TJ and Tipton KF, The nature of the inhibition of rat liver monoamine oxidase types A and B by the ecetylenic inhibitors clorgyline, L-deprenyl and pargyline. *Biochem Pharmacol* **31**: 3555–3561, 1982.
16. Walker B and Elmore TD, The irreversible inhibition of urokinase kidney-cell plasminogen activator plasmin and B-trypsin by 1-(*N*-6-amino-*n*-hexyl)carbamoylimidazol. *Biochem J* **221**: 277–280, 1984.
17. Tipton KF and Mantle TJ, The inhibition of rat liver monoamine oxidase by clorgyline and deprenyl. In: *Monoamine Oxidase Inhibitors. The State of The Art* (Eds. Youdim MBH and Paykel ES), pp. 3–15. J Wiley and Sons Ltd, 1981.
18. Balsa D, Fernández-Alvarez E, Tipton KF and Unzeta M, Monoamine oxidase inhibitory potencies and selectivities of 2-(*N*-(2-propynyl)aminomethyl)-1-methylindole derivatives. *Biochem Soc Trans* **19**: 215–218, 1991.
19. Tipton KF, Balsa D and Unzeta M, Newer approaches to the study of monoamine oxidase inhibition. In: *Monoamine Oxidase: Basic and Clinical Aspects. A Tribute to Prof. Kamijo* (Eds. Yasuhara H, Parvez SH and Sandler M) VSP Press, Holland, 1992.
20. Sullivan JP, McDonell L, Hardeman OM, Farrell MA, Phillips JP and Tipton KF, Oxidation of tryptamine by the two forms of monoamine oxidase in human tissues. *Biochem Pharmacol* **35**: 3255–3260, 1986.