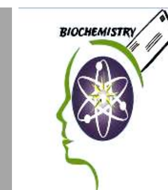
Biochemistry Letters, 11(20) 2016, pages 259-274

Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:



Interactions of Monoamine Oxidase from Human And Rat Liver Mitochondria with (S)-2-[4-(3-Flouro-Benzyloxy)-Benzylamino]-Propionamide

Zakia Ben Ramadan*[1] , Keith Tipton[2] , Safaa khater[3]

1-Department of Biochemistry, Faculty of Medicine University of Tripoli-Libya.
2-Department of Biochemistry, Trinity College, Dublin 2, Ireland .
3- Department of Biochemistry, Zagazig University –Egypt.

ARTICLE INFO

Article history:
Received :
Accepted :
Available online :

Keywords: *Monoamine oxidase-A and-B (MAO-A& MAO -B), kinetics of inhibition, (S)-2-[4-(3-flourobenzyloxy)-benzylamino] propionamide, (FCE 26743), Inhibition Studies.*

ABSTRACT

Background: The interactions of the anticonvulsant (S)-2-[4-(3-Flouro-Benzyloxy) Benzylamino]- Propionamide (FCE 26743) with MAO-A and MAO -B from human and rat liver mitochondria has been studied. **Objective:** This compound is an alanine derivative that differs from the parent anticonvulsant compound, 2-n-pentylamino acetamide in that alaninamide residue replaces the glycinamide moiety, and in the presence of a phenyl ring substituted in the *para*-position by a 3-flouro-benzyloxy group. **Results:** The results indicated that, The Ki values for rat and human MAO-A were > 1000 times higher than their corresponding values for rat and human MAO-Consistent with the IC_{50}values that showed this compound to be > 1000 times more potent as an inhibitor of MAO-B than of MAO-A of the same species. There was an increase in the strength of inhibition when MAO-B was incubated with this compound. This was confirmed by the extended time-courses studies which showed a rather rapid increase in the degree of inhibition of MAO-B, despite incubation being continued for a total of 4h. This is consistent with FCE 26743 being a reversible inhibitor for MAO-A and a slow- binding reversible inhibitor of MAO-B.FCE 26743 was found to be a time-independent weak inhibitor of MAO-A from both rat and human preparations. However, there was a significant difference in the inhibitory potency of FCE 26743 with MAO-B from the two species, the Ki value for rat MAO-B was about two times higher than that for human MAOB. **Conclusion:** The FCE 26743 was not metabolized (*in vitro*) by either human or rat liver mitochondrial MAO-A or -B. Without enzyme-inhibitor preincubation FCE 26743 was found to be a competitive inhibitor of both MAO-A and MAO-B from rat and human liver mitochondria.

© 2016 Publisher All rights reserved.

INTRODUCTION

Monoamine oxidase inhibitors, hydrazines that used clinically include phenelzine, phenylhydrazine, nialamide and isocarboxazid, act as enzyme activated irreversible inhibitors where the active inhibitory species is formed through the action of MAO itself, Monoamine oxidase (MAO) inhibitors, although not used as first line antidepressants, due to risk of the drug's interaction with diet or other drugs, continue to have a niche in the treatment of

Corresponding author:

psychiatric and neurological disorders. Interest in these drugs has also increased markedly in recent years following numerous reports of their neuroprotective actions *in vivo* and *in vitro* (3).The potent anticonvulsant compound FCE 26743, The alanine derivative (S)-2-[4-(flourobenzyloxy)-benzylamino] propionamide belongs to a series of analogues of milacemide (2-(n-pentylamino) acetamide) (4). a glycine derivative with atypicalanti-epileptic and potential psychotropic properties and differs from milacemide in that alaninamide residue replaces the glycinamide moiety, and in the presence of a phenyl ring substituted in the *para*-position by a 3-flouro-benzyloxy group. It has been suggested in the literature that the anticonvulsant compound milacemide (2-n-pentylaminoacetamide) acts as a pro-drug delivering glycine into the central nervous system (5,6) and this is a prerequisite for milacemide's anticonvulsant activity. It has been shown that milacemide functions as a suicide substrate and being a better substrate for the enzyme MAO-B than as a mechanism based inhibitor for all the species studied (7). Moreover from our previous studies, the compound 2-(benzylamino)acetamide, another glycine derivative acts as substrates for MAO-B and as inhibitors for MAO-A & MAO-B and displaying lower anticonvulsant activity (8). It seems apparent that, the 'delivery of glycine to the brain' as aprerequisite for anticonvulsant activity remains an imperfect hypothesis. Since the amino acid alanine,unlike glycine, does not possess an anticonvulsant activity, an investigation of the mechanism of action of this compound with MAO may help in understanding the anticonvulsant behavior of this group of compounds. Introduction of a benzyloxy group in the *para* position of a phenyl ring in MAO substrates and inhibitors has been shown to transform the substrates into inhibitors (9) and to increase the inhibitory potency of the inhibitors (10,11). FCE 26743 showed potent protective properties in a number of models of epilepsy in rodents, such as bicuculline-induced convulsions and lethality, maximal electroshock and kainic acid-induced seizures and status epilepticus (8,12).

In vitro studies with rat brain homogenates (13) showed FCE 26743 to be a potent inhibitor of MAO-B [IC_{50} = 0.17 ± 0.01 μM, mean ± SD, after 2min of preincubation] and to weakly inhibit MAO-A [IC_{50} = 0.37 ± 0.035 mM]. These workers also showed that the results of *in vitro* preincubation studies and of experiments involving the *in vitro* and "*ex vivo*" dilution of brain homogenates experiments were consistent with FCE 26743 acting as an irreversible MAO-B inhibitor. In contrast, the time-course of "*ex vivo*" inhibition of rat brain MAO-B with this compound was a typical of short acting inhibitor. Additional experiments are needed to establish whether FCE 26743 acts as a slow-binding inhibitor, or is metabolized to some degree by MAO-B to form an adduct with the enzyme.

It has been suggested that molecules that, in addition to their anticonvulsant properties, possess potent MAO-inhibitory activities might decrease the oxidative stress associated with epilepsy as a consequence of hydrogen peroxide formation impairment (8,14). In this respect, it is worth noting that, a significantly higher MAO-B activity was found in hippocampi from epileptic patients as compared with non-epileptic control subjects, after correction for age-related changes (15). Higher brain monoamine levels have been shown to reduce seizure susceptibility (16).It has been reported that the synthesis and release of monoamines, dopamine in particular, is increased in seizure foci compared with no focus regions in the human brain (17). This may represent defense response to seizure susceptibility. Administration of the MAO-B inhibitor selegiline to Parkinson's disease patients was shown to enhance brain dopamine concentrations (18). Therefore, it is possible that, The MAO-B inhibitory property of FCE 26743 might contribute to its anti-epileptic activity by increasing brain dopamine levels during long-term treatment. In the present work the behavior of FCE 26743 as a MAO-A and –B substrate and inhibitor was examined in human and rat liver mitochondria. Furthermore the kinetic parameters of inhibition of rat liver mitochondrial MAO-A and -B, and human liver mitochondrial MAO-A and MAO-B by FCE 26743 were determined *in vitro*.

2. Experimental procedures

2.1. *Materials*.

5-Hydroxytryptamine (5-HT) [side chain-2-^{14}C] creatine sulphate and phenylethylamine -(PEA)(ethyl-1^{14}C) hydrochloride were obtained from Amersham International or New England Nuclear. Benzylamine HCL and 5-Hydroxytryptamine creatine sulphate (5-HT) were obtained from Sigma Co. FCE 26743 and its aldehyde derivative were kindly given by Pharmacia-Farmitalia Carlo Erba. All other chemicals were standard laboratory chemicals and were of analytical reagent grade whenever possible.

2.2. Methods.

Rat liver mitochondria and human liver mitochondria were prepared by the method of (19). The mitochondrial pellet obtained was suspended in a small volume of 0.1M potassium phosphate buffer, pH 7.2 and stored at -20°C until use for MAO-B or MAO-A activity. Human liver was obtained within 12 hours of death and transferred to the laboratory on ice.

The aldehyde dehydrogenase (ALDH) was partly purified from ox liver by a modification (20) of the method of (21) and IU of activity is defined as the amount that catalyzes the formation of 1μ mol product / min at 37°C in the presence of 500 μM NAD^{+}and 3mM acetaldehyde. The molar extinction coefficient (ε) of NADH at 340 nm was taken to be 6.22x10^{3} M^{-1}.cm^{-1} (22).

All enzyme assays were performed at 37°C and pH 7.2.(7). MAO-B activity was determined spectrophotometrically by directly monitoring the formation of benzaldehyde from benzylamine by following the increase in absorbance at 250 nm (23). The molar extinction coefficient (ε) of benzaldehyde at 250 nm was taken to be 13.8 x 10^{3} M^{-1}.cm^{-1} (24). The activity of MAO-A was examined, using the substrate 5-HT, by the coupled assay in which the formation of NADH is followed continuously at 340 nm as the aldehyde product is further oxidized by ALDH(20).

The absorbance spectra of FCE 26743 and its corresponding aldehyde, which would be expected tobe produced by MAO if FCE 26743 is a substrate, were scanned at 200 - 500 nm (in 0.1 M potassium phosphate buffer, pH 7.2) and the molar extinction coefficients were determined. FCE 26743 was examined as a substrate of MAO by the direct spectrophotometric assay at 283 nm. The effects of FCE 26743 on the activity of MAO-A and -B were determined using the radiochemical assay by the method of (25) as modified by (26) using 5-Hydroxytryptamine(5-HT) and phenylethylamine(PEA), as the selective substrates for MAO-A and -B, respectively. IC_{50}values (concentration of the inhibitor giving 50% inhibition) were determined at zero-time and after 30 min preincubation of the enzyme and inhibitor using the computer program Kaleidagraph .

The kinetic behavior of FCE 26743 on MAO-A and-B from both species were determined (a) spectrophotometrically, by coupled or direct assays by varying the substrate concentration (5-HT and benzylamine, respectively), in the presence of several fixed concentrations of the inhibitor. (b) Radiochemically, using 5-HT and PEA as substrates in the presence of several fixed concentrations of the inhibitor. The K_i values were calculated by fitting the data of the apparent K_m/V_{max}values (slopes)versus the inhibitor concentrations using the computer program Mac-CurveFit. Extended time courses of inhibition of human and rat liver mitochondrial MAO-A and -B were determined spectrophotometrically by monitoring the product formation by either coupled (at 340 nm, using 500μM5-HT as a substrate) or direct (at 250 nm with 250 μM benzylamine as substrate) assays in the presence of the indicated concentrations of FCE 26743. Control experiments in which enzymes were incubated under identical conditions but in the absence of FCE 26743 were included. The kinetic parameters K_i and k_{+2}were determined by analyzing the graphs of the time courses of inhibition of each form of the enzyme according to the equations: (27).

$$\ln (P_{\infty} - P_t) = \ln P_{\infty} - k't \quad (1)$$

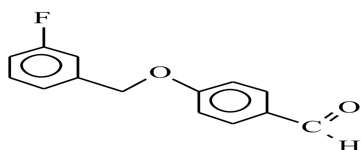
$$k' = \frac{k_{+2}}{1 + (K_i / i)[1 + (s / K_m)]} \quad (2)$$

Where P_t is the product concentration at any time t, and P_∞ is the final product concentration when the reaction has ceased, s represents the substrate concentration, i represents the inhibitor concentration and K_i is the dissociation constant for the non-covalent complex .Thus a graph of 1/k' against 1/i will give a straight line with a slope of $(1 + s / K_m) K_i / k_{+2}$ that intersect with the y axis at a point corresponding to $1/k_{+2}$. However, the computer program (Mac-Curve Fit) was used to determine k' and P_∞ values in the present work. The double reciprocal plots are used only for illustrative purposes.

3. Results:

3.1. The Extinction coefficient of the Aldehyde derivative of FCE 26743:

The oxidation of FCE 26743 by MAO would be expected to yield the corresponding aldehyde, 4-(3-flourobenzyloxy)-benzaldehyde:



This aldehyde shows an absorbance peak at 282 - 284 nm that differs from its corresponding amine FCE 26743, which shows smaller double-peaks at about 260 nm, Figs 1(a - c). The molar extinction coefficient was calculated to be 4.358×10^3 ($l\ mol^{-1}.cm^{-1}$). This allows the use of a direct spectrophotometric assay at 283 nm to determine whether FCE 26743 is a substrate of MAO.

3.2. Oxidation studies of FCE 26743 by MAO-A and MAO -B

In this study, the behavior of FCE 26743 as a substrate of MAO was assessed *in vitro* by measuring the change in absorbance at 283 nm using direct spectrophotometric assay. Rat and human liver mitochondrial MAO-A and MAO-B were assayed with FCE 26743, but no change in the absorbance could be detected .Thus FCE 26743 was not metabolized at any significant rate by MAO in any of these preparations.

3.3. Inhibition Studies:

Studies on the effects of FCE 26743 on the activities of human and rat liver mitochondrial MAO-A and MAO-B(as shown in Fig 2 and is taken as a representative of the other enzyme forms used) shown that, In the absence of preincubation FCE 26743 was found to inhibit human liver mitochondrial MAO-A and MAO-B with IC_{50} values of > 100 μM and 0.18 ± 0.017 μM, respectively and inhibit rat liver mitochondrial MAO-A and MAO-B, with IC_{50} values of approx.100 μM and 1.34 ± 0.13 μM, respectively. Preincubation of FCE 26743 with the enzyme for 30 min at 37°C was found to induce a slight time-dependent inactivation of human and rat liver MAO-B whereas no such time-dependent inhibition was observed with MAO-A. The IC_{50} values for the inhibition of 5-HT and PEA oxidation after preincubation were approximately 100 μM and 0.079 ± 0.01μM, for rat liver mitochondrial MAO-A and MAO-B, respectively and IC_{50} values of 100 μM and 0.052 ± 0.008 μM, for human liver MAO-A and MAO-B, respectively.(Table 2).

(Figs 3, 4) show the kinetics of inhibition of rat liver mitochondrial MAO-A and MAO-B, by FCE 26743, respectively and is taken as being representative of the other enzyme forms used. FCE 26743 was shown to be linearly competitive towards the amine substrate for, rat liver mitochondrial MAO-A and-B, and human liver mitochondrial MAO-A and MAO-B, with FCE 26743 being over 1000 times more potent as an inhibitor of MAO-B than of MAO-A of the same species.

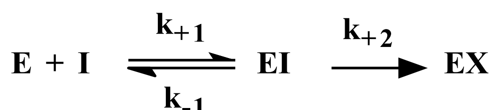
(Figs5,6) represent the inhibition of human liver mitochondrial MAO-A and MAO-B respectively, by FCE 26743 using the radiochemical method for assaying and they are taken as being representative of the other enzyme forms used .The K_i values for the inhibition of the different forms of the enzyme were determined and shown in (Table 1).

3.4. Time courses of inhibition and determination of kinetic parameters

Fig 7a represents Extended time-courses of the inhibition of rat liver mitochondrial MAO-A by FCE 26743 in the presence of the

appropriate substrate, determined spectrophotometrically by monitoring the product formation in the presence of FCE 26743 and is taken as being representative of the other enzyme forms used. Control experiments in which the enzyme was incubated under identical conditions but in the absence of FCE 26743 were included. The graphs would be consistent with FCE 26743 behaving as a short-acting irreversible inhibitor that initially interacts competitively with respect to the amine substrate for MAO-B from rat and human liver mitochondria and it is a much weaker and a time-independent inhibitor of MAO-A from both species and that FCE 26743 is a competitive inhibitor of this enzyme form. The inhibition of rat liver mitochondrial MAO-A required FCE 26743 concentrations in the range (40 -300µM) which is close to that used for human liver mitochondrial MAO-A (40 - 200 µM) and much higher than the ones used for rat liver mitochondrial MAO-B (0.1 -0.7 µM) and human liver mitochondrial MAO-B (0.02 - 0.08 µM).The k_{+2}values for MAO-B from rat and human were similarly very small and differed from their values for MAO-A from both species which were essentially zero.

The low K_i and k_{+2} values, for rat and human liver mitochondrial MAO-B with $K_i$values of 0.0084 and 0.0038 µM respectively and k_{+2} values of 0.0041 and 0.0044 min^{-1} respectively, obtained by Analyzing the progress curves as described above and shown in Fig 7baccording to the simple mechanism:



Show that such analysis is not applicable for these types of reactions in which an initial rapid phase of time-dependent but partial inhibition appears to be superimposed on an inhibitor-independent slower inactivation of the enzyme.

4. Discussion

If the anticonvulsant compound FCE 26743 acted as a substrate for rat or human liver mitochondria *in vitro* this result would support the proposed mechanism for the interaction of MAO with FCE 26743 (13)and shown below

(I) FCE 26743, (E) the Enzyme, (EI) a non-covalent compound, (EI^*) an activated intermediate, (P) aldehyde product,

Where an imine intermediate is formed, which subsequently can be hydrolysed to yield the corresponding aldehyde. *In vivo the* aldehyde formed might be rapidly cleared, favoring the reversibility of the reaction from EI* back to the imine, with FCE 26743 behaving as a short acting *ex- vivo.* However when the activities of human and rat liver mitochondria were assayed towards this compound, no product formation could be detected since there were no change in the absorbance. Thus FCE 26743 was not metabolized at any significant rate by MAO in any of these preparations. Though, the very rapid conversion of the aldehyde into another form that did not absorb at the same wavelength by another component of that system cannot be excluded.

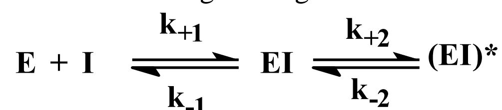
The lack of any direct oxidation of FCE 26743 by either form of MAO is in agreement with the *in vivo* results of. (14), who could detect no urinary excretion of alaninamide in mice after administration of 0.38 $mmol.kg^{-1}$ FCE 26743. These results would rule out the possibility that accumulation of the aldehyde product is involved in the inhibition of MAO by FCE 26743, suggesting that the inhibition is due to the molecule itself.

The results of the present inhibition studies indicate that FCE 26743 is far more potent inhibitor of the B-form of monoamine oxidase than the A-form in both species used. In the *in vitro* studies with rat liver mitochondria the selectivity of FCE 26743 towards MAO-B, as measured by the ratio IC_{50} MAO-A / IC_{50} MAO-B, was greater than 74 and 1200 after 0 and 30 min incubation, respectively. Whereas the degree of inhibition of MAO-A did not change after 30 min enzyme-inhibitor preincubation, the degree of inhibition of MAO-B increased significantly, see (Table 2). Comparison of the reported IC_{50} values of FCE 26743 with MAO-A and-B from rat brain mitochondria (14) with the values obtained from the present study from rat liver mitochondria (Table 2) shows FCE 26743 to

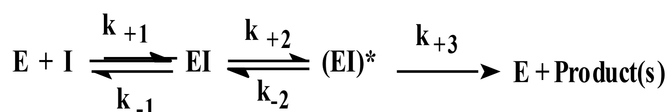
be about 7-times more potent inhibitor of MAO-B and about 3-times less potent inhibitor of MAO-A from rat brain mitochondria. This may result from differences in the mitochondrial preparations procedures, assay conditions or strain of rats used in the different studies.

Without enzyme-inhibitor preincubation FCE 26743 was found to be a competitive inhibitor of both MAO-A and MAO-B from rat liver mitochondria (K_i = 59.7 ± 15 μM and 0.037 ± 0.016 μM, respectively) consistent with the IC_{50} values that showed this compound to be > 1200 times more potent as an inhibitor of MAO-B than of MAO-A. Although the studies on the time-dependence of inhibition reported above indicated that the inhibition of MAO-A was unaffected by preincubation with FCE 26743, there was an increase in the strength of inhibition when MAO-B was incubated with this compound. This was confirmed by the extended time-courses studies which showed a rather rapid increase in the degree of inhibition of MAO-B, which appeared to be complete after about 15 min after which time there was no significant further time-dependent effect, despite incubation being continued for a total of 4h.

In the present *in vitro* studies with human liver mitochondria, the selectivity of FCE 26743 towards MAO-B as measured by the ratio IC_{50} MAO-A / IC_{50} MAO-B was greater than 500 and 2000 after 0 and 30 min incubation, respectively. However while the degree of inhibition of MAO-A did not change after 30 min enzyme-inhibitor preincubation, there was an increase in the strength of inhibition when MAO-B was incubated with this compound, which is consistent with FCE 26743 being a reversible inhibitor for MAO-A and a slow- binding reversible inhibitor of MAO-B. These findings from the IC_{50} values towards MAO-A and-B from human tissues, were substantiated by the determination of the kinetic parameters for the inhibition of the two forms of MAO from human liver mitochondria by FCE 26743, as summarised in Table 1.The time dependence of the inhibition of these preparations was also similar to that observed with the rat enzymes. Time-dependent inhibition can however occur with irreversible inhibitors and with competitive, slow and tight-binding reversible inhibitors (11). The time-dependent behavior observed in the present work would be inconsistent with FCE 26743 acting as an irreversible inhibitor but could describe either slow binding reversible inhibition according to the general mechanism:



Where the slowly-formed species (EI*) is a tighter complex than EI.The kinetic behavior of FCE 26743 as an inhibitor of rat and human liver mitochondrial MAO-B as shown in these reactions would be consistent with such a mechanism, with an initial competitive interaction, with respect to the amine substrate with $K_i$values of 0.0084 and 0.0038 μM, respectively followed by a slower step described by k_{+2}values of 0.0041 and 0.0044 min^{-1}, respectively. This mechanism does not preclude the possibility of some transformation of FCE 26743 by the action of MAO, such that the inhibitor is chemically changed in the (EI*) species. Neither is it possible at this stage to exclude the possibility that FCE 26743 acts as a very poor substrate according to a mechanism of the type:



The failure to detect product formation might argue against such a mechanism, but so far the only product examined has been 2-(4-(3-fluorobenzyloxy)benzaldehyde and the possibility of alternative products being formed cannot be excluded.

Acknowledgements

I am grateful to the staff in the biochemistry laboratory one for their cooperation during my work at Trinity College Dublin as a research fellow.

References

1-Ben Ramadan Z, Tipton KF and Maria L. 2007. (N) - -Species Differences in the Selective Inhibition of Monoamine Oxidase (1-methyl-2-phenylethyl) hydrazine and its Potentiation by Cyanide. *Neurochem. Res.*, DOI 10.1007/s11064-007-9309-x.

2- Shulman KI, HerrmannN and Walker ES. 2013.Current Place of Monoamine Oxidase Inhibitors in the Treatment of Depression. CNS Drugs., 27: 789-797.

3- Baker GB, DmitriyMatveychuk, MacKenzie EM, Dursun SM and Mousseau D D. 2012. Monoamine Oxidase Inhibitors and Neuroprotective Mechanisms. Bulletin of Clinical Psychopharmacology, 22 (4).

4- Dostert P,Perarello P, Heidempergher F, Varasi M, Bonsignori A and Roncucci R. 1990. Preparation of a-(phenylalkylamino) carboxamides as drugs. Eur. Pat. Appl., Ep. 400, 495.

5- Janssens de Varebeke P, Cavalier R, David-Remacle M and Youdim MBH. 1988. Formation of the neurotransmitter glycine from the anticonvulsant milacemide is mediated by brain monoamine oxidase B. J. Neurochem., 50: 1011-1016.

6- Janssens de Varebeke P, Pauwells G, Buyse C, David-Remacle M, De Mey J, Roba J and Youdim MBH. 1989. The novel neuropsychotropic agent milacemide is a specific enzyme activated inhibitor of brain monoamine oxidase-B. J. Neurochem., 53: 1109-1116.

7- Ben Ramadan Z and Tipton KF. 2012. Suicide Inhibition Of Monoamine Oxidase From Different Species By Milacemide. *Jordan Journal of Biological Sciences,* 5: 269-278.

8- Dostert P, Strolin Benedetti M and Tipton KF. 1991. New anticonvulsants with selective MAO-B inhibitoty activity. Eur. J. Neuropsychopharmacol, 1: 317 – 319.

9- Sullivan J P and Tipton K F. 1990. The interactions of monoamine oxidase with some derivatives of 1-methyl-4-phenyl - 1.2.3.6-tetra-hydropyridine (MPTP). J. Neural Transm. 29(Suppl.): 269 – 277.

10- Dostert P, Strolin Benedetti M and Tipton KF. 1989. Interactions of monoamine oxidase with substrares and inhibitors. *Med. Res. Rev*., 9: 45 – 89.

11- Mazouz F, Gueddari S, Burstein C, Manusy D and Milecent R. 1993. 5-[4-(Benzyloxy)phenyl]-1.3.4-oxadiazol-2(3H)-one derivatives and related analogues: new reversible, highly potent and selective monoamine oxidase type B inhibitors. J. Med.Chem., 36: 1157 – 1167.

12- Maj R, Antongiovanni V, Bonsignori A, Breda M, Dostert P, Farriello RG, Mcarthur, RA, Varasi M and Bianchetti A. 1993. Anticonvulsant profile of benzylaminopropanamidedervatives in mice and rats. Abstract presented at the focus on Epilepsy 11. International Conference. Whistler (Canada), September 11 – 14.

13- Strolin Benedetti P, Tocchetti M, Rocchetti M, Martignoni P, MarrariPoggesi I and Dostert P. 1995. Enantioselective recognition of two anticonvulsants, FCE 26743 and FCE 28073, by MAO, and relationship between MAO-B inhibition and FCE 26743 concentrations in rat brain. Progress in brain research, Vol.106. Elsevier Science BV.

14- Strolin Benedetti M, Marrari P, Colombo M, Castelli MG, Arand M, Oesch F and Dostert P. 1994. The new anticonvulsant FCE 26743 is a selective and short acting MAO-B inhibitor devoid of inducing properties towards cytochrome p-450-dependent testosterone hydroxylation. J. Pharm. pharmacol., 46: 814 – 819.

15- Kumlien E, Sherif F, Ge L and Oreland, L. 1995. Platelet and brain GABA-transaminase and monoamine oxidase activities in complex partial epilepsy. Epilepsy Res., 20: 161 -170.

16- Mishra PK, Kahle EH, Bettendorf AF, Dailey JW and Jobe PC. 1993. Anticonvulsant effects of intracerebroventricularly adminstered norepinephrine are potentiated in the presence of monoamine oxidase inhibition in severe seizure genetically epilepsy-prone rats (GEPR-9s). Life Sci., 52:1435 – 1441.

17- Pintor M, Mefford IN, Huttler I, Pocotte SL, Wyler AR and Nadi NS. 1990. Levels of biogenic amines, their metabolites. and tyrosine hydroxylase activity in the human epileptic temporal cortex. Synapse, 5: 152 – 156.

18- Riederer P, Lachenmayer L and Laux G. 2004. Clinical applications of MAO-inhibitors. Current Medicinal Chemistry, 11: 2033–2043.

19- Kearney EB, Salach JI, Walker WH, Seng RL, Kenney W, Zeszotek E and Singer TP. 1971. The covalently bound flavin of hepatic monoamine oxidase. I. Isolation and sequence of a flavin peptide and evidence for binding at the 8a position. Eur. J. Biochem., 24: 321-327.

20- Houslay MD and Tipton KF. 1973. The nature of the electrophoretically-separable multiple forms of rat liver monoamine oxidase. Biochem J., 135: 173-186.

21-Deitrich RA, Hellerman L and Wein J. 1962. Diphosphopyridine nucleotide-linked aldehyde dehydrogenase. I. Specificity and sigma-rhofunction JBiol Chem.,237: 560-564.

22- Dawson RMC, Elliott DC, Elliott WH and Jones KM. 1986. Vitamins and Coenzymes in: Data for Biochemical Research, 3rd edition: 131-134. Oxford Science Publications.

23- Tabor CW, Tabor H and Rosenthal SM. 1954. Purification of amine oxidase from beef plasma. J. Biol.Chem., 208: 645-661.

24- Tipton KFand Youdim MBH. 1983. The assay of monoamine oxidase activity in: Methods in Biogenic Amine Research (Parvez S, Nagatsu T, Nagatsu I and Parvez, H., eds.), pp. 441-465, Elsevier Press, Amsterdam, New York and Oxford.

25- Otsuka S and Kobayashi Y. 1964. A radioisotopic assay for monoamine oxidase determinations in human plasma. Biochem. Pharmacol.,13: 995-1006.

26- Fowler C J and Tipton K F. 1981. Concentration dependence of the oxidation of tyramine by the two forms of rat liver mitochondrial monoamine oxidase. *Biochem. Pharmacol.,* 30: 3329-3332.

27- Tipton KF, Balsa D and Unzeta M. 1993. "Newer approaches to the study of monoamine oxidase inhibition", (Eds.Yasbwa , H.Paves, S.H., Oguchi, K.Sandler, M.andNagastu,T.) pp.1-13 ,VSP BV, Utrecht.The Netherlands.

Table 1. K_i Values for the Inhibition of MAO from Human and Rat Liver Mitochondria by FCE 26743

Enzyme preparation	Substrate	K_i (μM)
Rat liver mitochondria		
MAO-A	5-HT (b)	59.7 ± 15
	5-HT (c)	51.0 ± 6.0
MAO-B	Benzylamine (a)	0.037 ± 0.016
	PEA (c)	0.021 ± 0.003
Human liver mitochondria		
MAO-A	5-HT (b)	69.3 ± 12.3
	5-HT (c)	38.0 ± 14
MAO-B	Benzylamine (a)	0.022 ± 0.01
	PEA (c)	0.014 ± 0.004

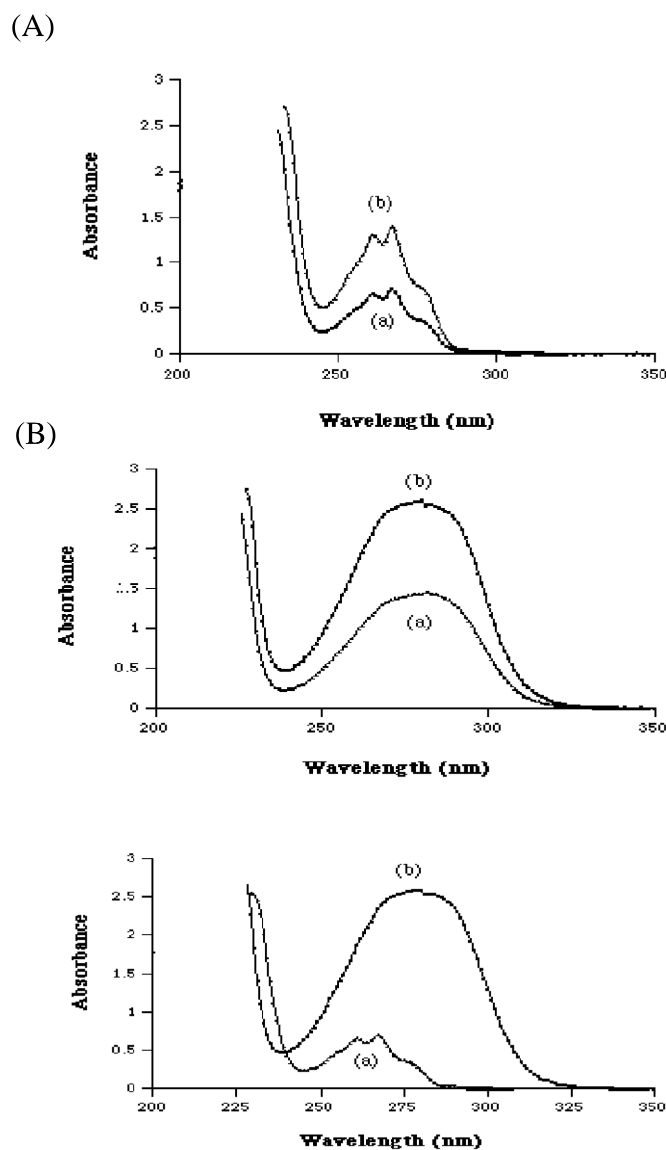
The Ki values were calculated as described above using the computer program Mac-CurveFit. The assay methods used were: (a) direct spectrophotometric assay at 250 nm. (b) Coupled spectrophotometric assay at 340 nm. (c) Radiochemical assay. The values are the mean ± S.E.M. of three determinations.

Table 2. The IC_{50} Values for the Inhibition of MAO from Human and Rat Liver Mitochondria by FCE 26743

Enzyme preparation	IC_{50} (μM) Without preincubation	IC_{50} (μM) 30 min preincubation
Rat liver mitochondria		
MAO-A	approx 100	approx. 100
MAO-B	1.34 ± 0.13	0.079± 0.010
Human liver mitochondria		
MAO-A	> 100	> 100
MAO-B	0.18 ± 0.017	0.052 ± 0.008

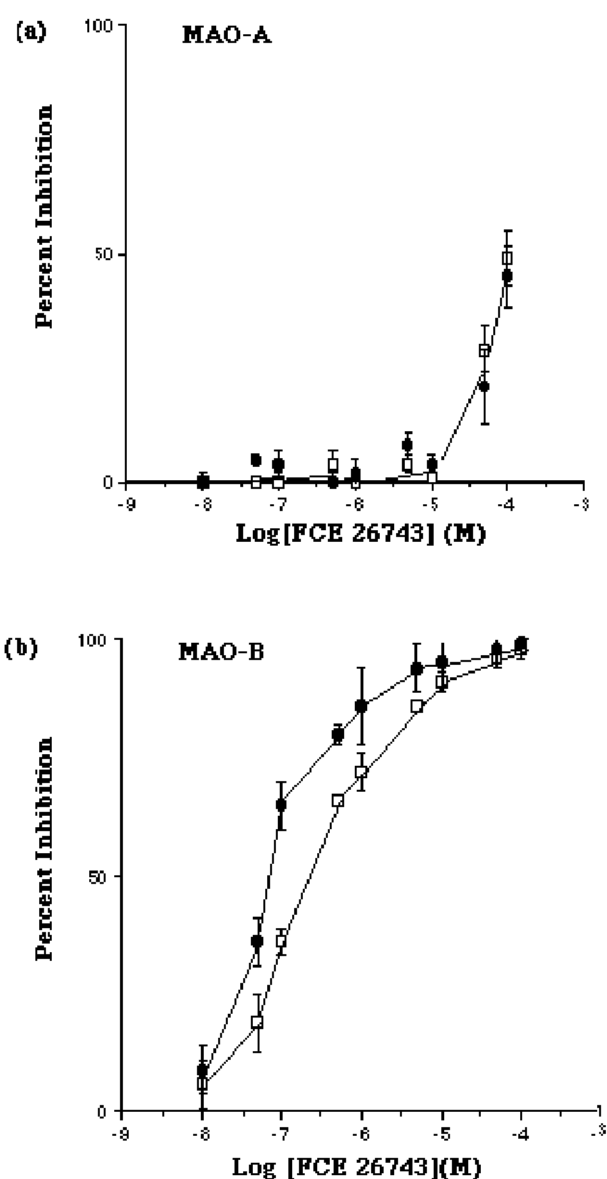
MAO activities were determined radiochemically at 37oC with 20 μM 2-phenylethylamine or 100 μM 5-hydroxytryptamine, as substrates for MAO-B and-A, respectively.

Figure1.Absorbance Spectra of the Compound FCE 26743 and its Corresponding Aldehyde.



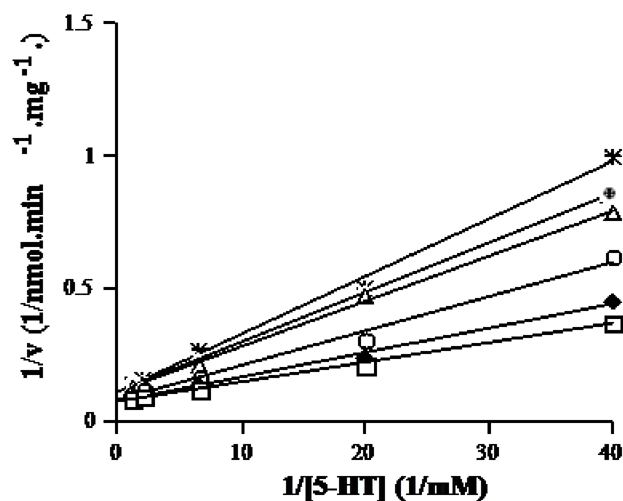
Spectra of: (A) 0.5 mM (a) and 1mM (b) of the compound FCE 26743.
(B) 0.25 mM (a) and 0.5 mM (b) of the corresponding aldehyde.
(C) 0.5 mM (a) of FCE 26743 and 0.5 mM (b) of its aldehyde form
in the presence of 0.1 M potassium phosphate buffer at pH 7.2

Figure 2. The Effects of FCE 26743 Concentrations on the Activities of Human Liver Mitochondrial MAO-A and -B



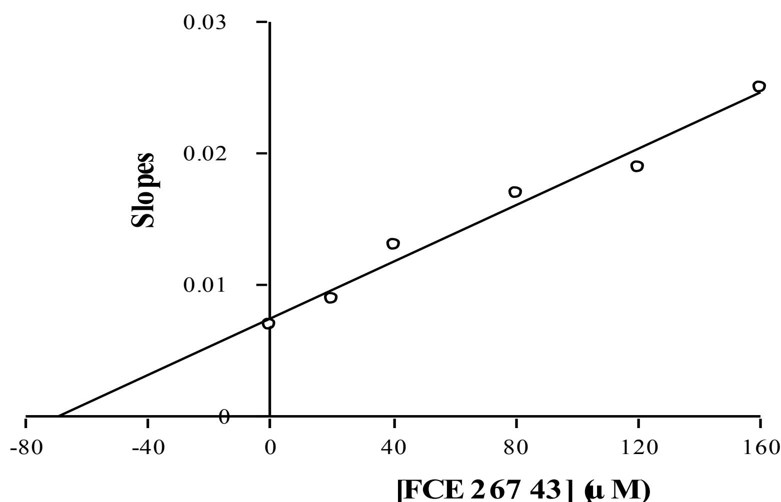
The enzyme preparation (0.2 mg/ml) was incubated with the indicated concentration of FCE 26743 for (□)0 time or (●)30 min before activity was determined radiochemically towards (a) 100µM 5-HT or (b) 20 µM PEA. Percentage inhibition was calculated with respect to samples preincubated for the same period in the absence of inhibitor. Each point is the mean ± Standard Error of the ratio from triplicate determinations in a single experiment.

Figure 3a. Kinetics of the Inhibition of Rat Liver Mitochondrial MAO-A by FCE26743



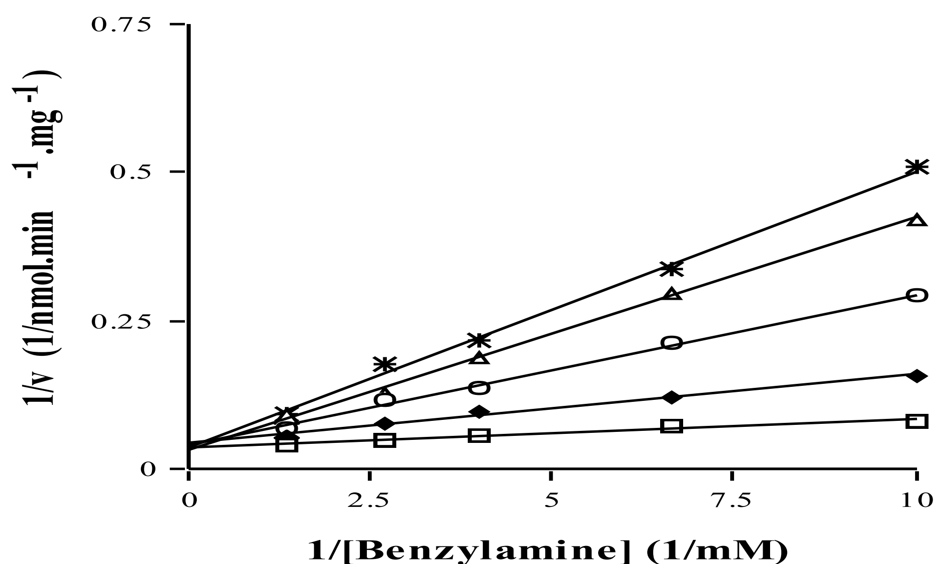
Initial rates were measured spectrophotometrically at 340 nm in the presence of the indicated concentrations of 5HT and in the presence of (□)0, (◆)20,(○) 25, (△) 35, (●) 45,(✱) 55 µM FCE 26743. Each point is themean of three separate experiments. The error bars have been omitted for clarity.

Figure 3b-Determination of the Ki Values of FCE 26743 towards Rat Liver Mitochondrial MAO-A



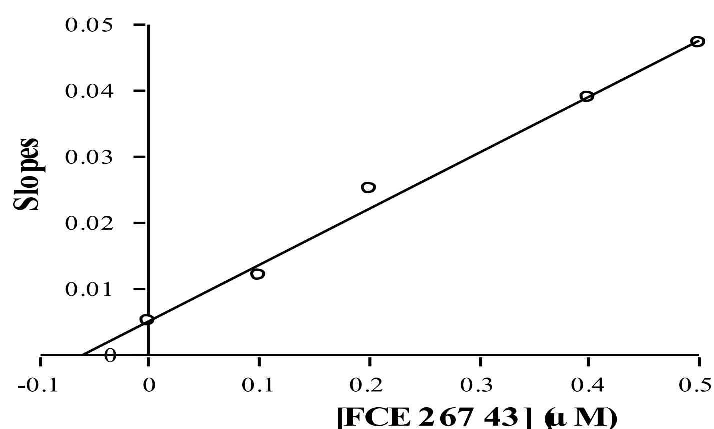
The dependence of the slopes obtained from the double reciprocal plots shown above of rat liver mitochondrial MAO-A on FCE 26743 concentration. The intercept of the extrapolated line on the FCE 26743 concentration axis gives a value of Ki.

Figure 4- Kinetics of the Inhibition of Rat Liver Mitochondrial MAO-B by FCE26743



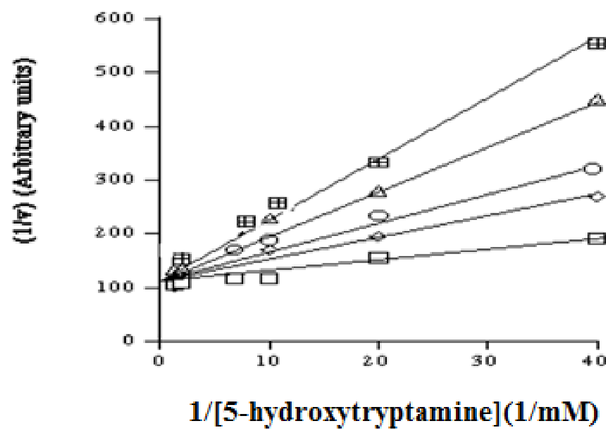
a. The Inhibition of Rat Liver Mitochondrial MAO -B by FCE26743
Initial rates were measured spectrophotometrically at 250 nm in the presence of the indicated concentrations of Benzylamine and in the presence of (□) 0, (◆) 0.1, (○) 0.2, (△) 0.4, (✱) 0.5 µM FCE 26743. Each point is the mean of three separate experiments. The error bars have been omitted for clarity.

b. Determination of the Ki Values of FCE 26743 towards Rat Liver Mitochondrial MAO-B



The dependence of the slopes obtained from the double reciprocal plots shown above of rat liver mitochondrial MAO-B on FCE 26743 concentration. The intercept of the extrapolated line on the FCE 26743 concentration axis gives a value of Ki .

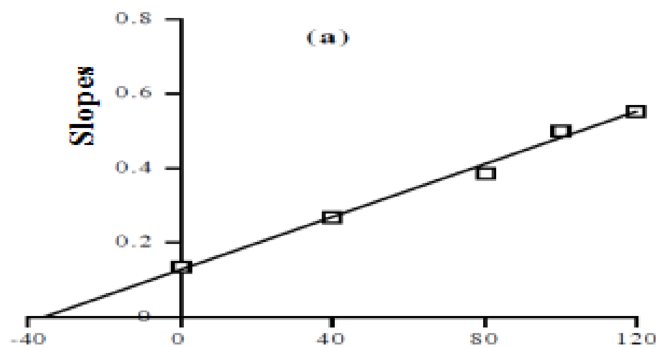
Figure 5.Inhibition of Human Liver Mitochondrial MAO-A by FCE 26743 without Enzyme-Inhibitor Pre-Incubation Using the Radiochemical Method for Assaying



a.Inhibition of Human Liver Mitochondrial MAO-A by FCE 26743

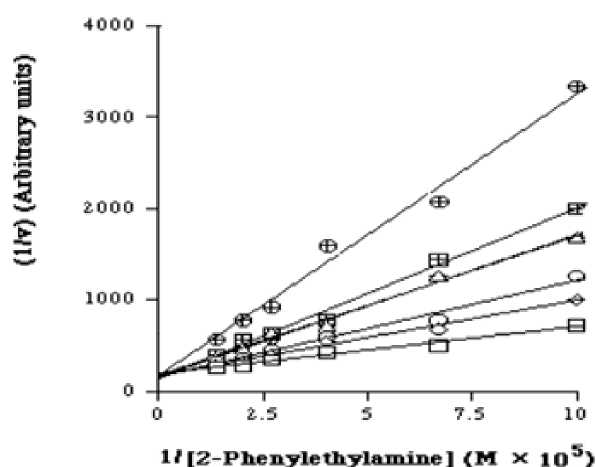
Initial rates were measured using radioactive substrates at the indicated concentrations of 5HT and in the presence of (□) 0, (◇) 40,(○) 80, (△)100, (⊞) 120 μM FCE 26743. Each point is the mean of three separate experiments. The error bars have been omitted for clarity.

b.Determination of the K_i Values of FCE 26743 towards Human Liver Mitochondrial MAO-A



The dependence of the slopes obtained from the double reciprocal plots shown above for Human liver mitochondrial MAO-A on FCE 26743 concentration. The intercept of the extrapolated line on the FCE26743 concentration axis gives a value of K_i.

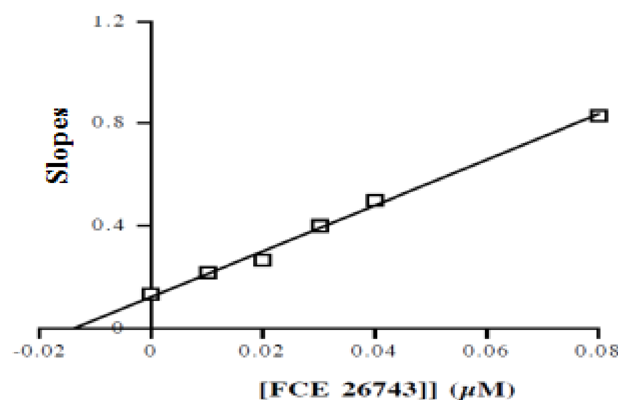
Figure 6. Inhibition of Human Liver Mitochondrial MAO-B by FCE 26743 without Enzyme-Inhibitor Pre-Incubation Using the Radiochemical Method for Assaying



a. Inhibition of Human Liver Mitochondrial MAO-B by FCE 26743

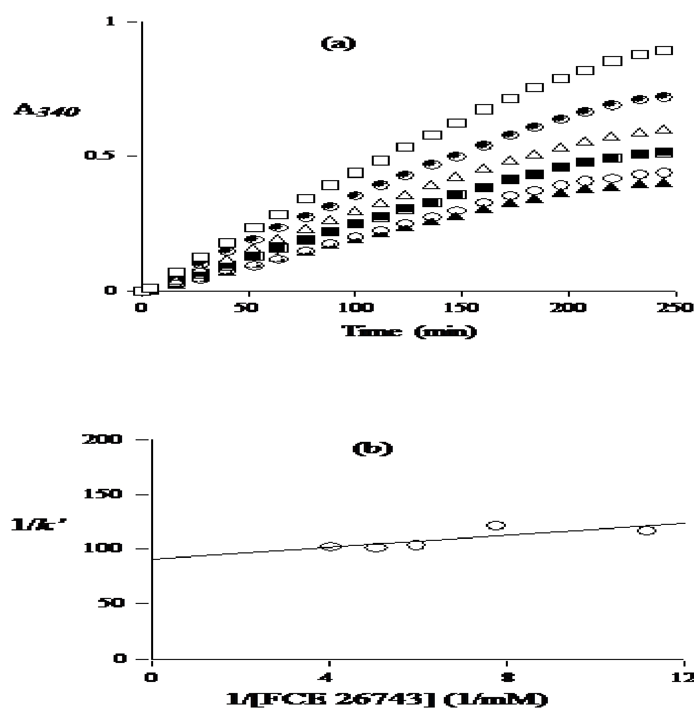
Initial rates were measured using radioactive substrates at the indicated concentrations of 2-Phenylethylamine and in the presence of (□) 0, (◇) 0.01, (○) 0.02, (△) 0.03, (⊞) 0.04, (⊕) 0.08 μM FCE 26743. Each point is the mean of three separate experiments. The error bars have been omitted for clarity.

b. Determination of the K_i Values of FCE 26743 towards Human Liver Mitochondrial MAO–B



The dependence of the slopes obtained from the double reciprocal plots shown above for Human liver mitochondrial MAO-B on FCE 26743 concentration. The intercept of the extrapolated line on the FCE26743 concentration axis gives a value of K_i.

Figure 7. Inhibition of Rat Liver Mitochondrial MAO-A by FCE26743 at a Series of Different Concentrations



a) **Time Courses of Inhibition of Rat Liver Mitochondrial MAO-A by FCE26743 at a Series of Different Concentrations**

The reactions of rat liver mitochondria (58 μg/ml) with 5HT at 37 ^{O}C and pH7.2 in the presence of, 0 (▲), 40(○), 100 (■), 160 (△), 240 (●) and 300 (□) μM FCE 26743 were monitored spectrophotometrically at 340nm. The points shown are the results from 6 representative experiments.

b) **Determination of the Kinetic Parameters K_i and k_{+2} for the Effects of FCE 26743 on Rat Liver Mitochondrial MAO-A**

A double reciprocal plot of the dependence of the apparent rate constants obtained from the time courses of inhibition as shown above on the inhibitor concentration. The slope and the intercept of this line give the values of K_i and k_{+2} respectively. Each point represents a single representative experiment.