Synthesis and biochemical evaluation of (*R*)-5-acyloxymethyl- and (*S*)-5-acylaminomethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinones as new anti-monoamine oxidase (anti-MAO) agents

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Abstract

(*R*)-5-Acyloxymethyl- and (*S*)-5-acylaminomethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinones **2a-s** were synthesized as pyrrole analogues of toloxatone (*Humoryl*®), an anti-MAO agent used in clinical therapy because of its antidepressant properties. Their ability to inhibit the enzymatic isoforms MAO-A and MAO-B was evaluated. From the data most **2a-s** showed high reversibility and selective MAO-A inhibitory activity. They exhibited an inhibitory potency (K_{iMAO-A}) of 0.16-0.90 μM comparable to that found for toloxatone (K_{iMAO-A} = 0.38 μM), the A isoform being 11-fold more selective. The results indicate that **2a-s** show promise as new antidepressant agents.

Keywords: MAO-A, MAO inhibitors (MAOIs), 2-oxazolidinones, 3-(1*H*-pyrrol-1-yl)-2-oxazolidinones, antidepressant agents

Introduction

Monoamine oxidase (MAO, EC 1.4.3.4) catalyzes the oxidative deamination of biogenic amines both from exogenous and endogenous sources, such in the central nervous system and in peripheral tissues. This enzyme is a flavoprotein tightly bound to the mitochondrial outer membranes of neuronal, glial and other cells.[1] On the basis of their different amino acid sequences, substrate and inhibitor specificities two subtypes of MAO, termed MAO-A and MAO-B, have been described. Isoform A preferentially oxidases serotonin, adrenaline and norepinephrine, and is selectively inhibited by clorgyline, whereas isoform B mainly catalyses the oxidative deamination of β-phenylethylamine and benzylamine, and is selectively inhibited by L-deprenyl.[2] Both isoenzymes appear to consist of two subunits[3,4] coded by different genes, and they have a 70% amino acid identity.[5] Each isoenzyme has an FAD moiety covalently linked to a cysteine residue, Cys404 (MAO-A) and Cys397 (MAO-B), through a

8α–cysteinyl-riboflavin.[6-9] Although the peptide sequences of the two forms present certain differences, the FAD cofactor is linked to the same pentapeptide (Ser-Gly-Gly-Cys-Tyr) whatever the form (A or B) of MAO found in different mammalian tissues.[5]

Due to the key role played by the two MAO forms in metabolism of monoamine neurotransmitters and exogenous aryl-alkylamines, these oxidases have long been a pharmacological focus because both reversible and irreversible MAO inhibitors (MAOIs) of both isoforms A and B have been administered clinically in the treatment of several neurological diseases, such as depression, social phobias, obsessive-compulsive disorders, schizophrenia, Parkinson's Disease (PD). In particular, selective MAO-A inhibitors (e.g., clorgyline)[10] are used as antidepressant and antianxiety drugs, and they are claimed to protect neuronal cells against apoptosis,[11] while selective MAO-B inhibitors (e.g., L-deprenil)[12] can be used either alone or in combination with L-DOPA, in the treatment of PD.[13] Elevated levels of the isoform B have also been assayed in plaque-associated astrocytes of brains from Alzheimer's patients,[14] and MAO-B inhibitors are currently in clinical trials for the treatment of this disease.

On one hand, such isoform-selective MAOIs present a favourable tolerability profile, being effective agents in many neuropsychiatric and effective disorders. On the other hand, they suffer from many important limitations to the use (loss of selectivity at high dosages, wide range of MAOI-drug and MAOI-food interactions) as they inhibit the MAO forms in an irreversible manner.[15,16]

During the 1980s new selective, reversible MAOIs appeared, showing higher selectivity over a wide dose range and during chronic use, and inducing minimal adverse side reactions. Among them, 2-oxazolidinone derivatives including toloxatone (*Humoryl*®) and befloxatone are used as new antidepressant agents (Figure 1).[17-23]

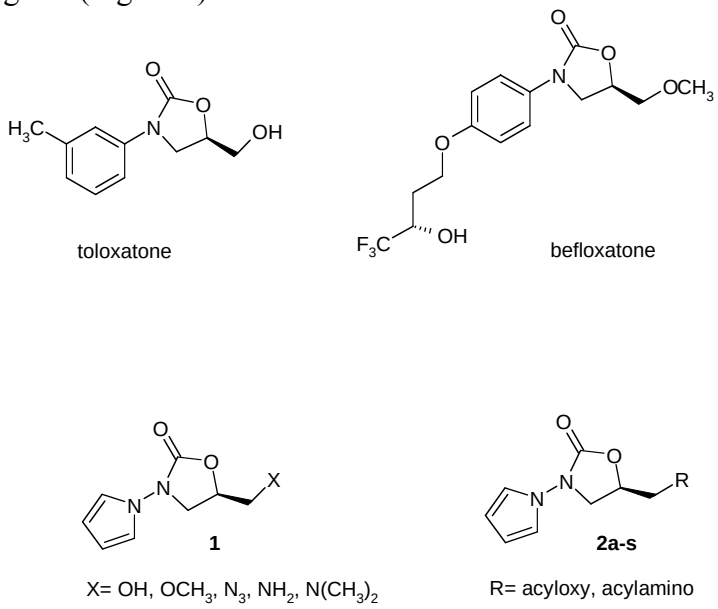


Figure 1. 3-Phenyl- and 3-(1*H*-pyrrol-1-yl)-2-oxazolidinones.

Recently we disclosed a new series of 3-(1*H*-pyrrol-1-yl)-2-oxazolidinones **1** bearing a hydroxymethyl, alkoxymethyl, (un)substituted-aminomethyl, or azidomethyl side chain at the C_5

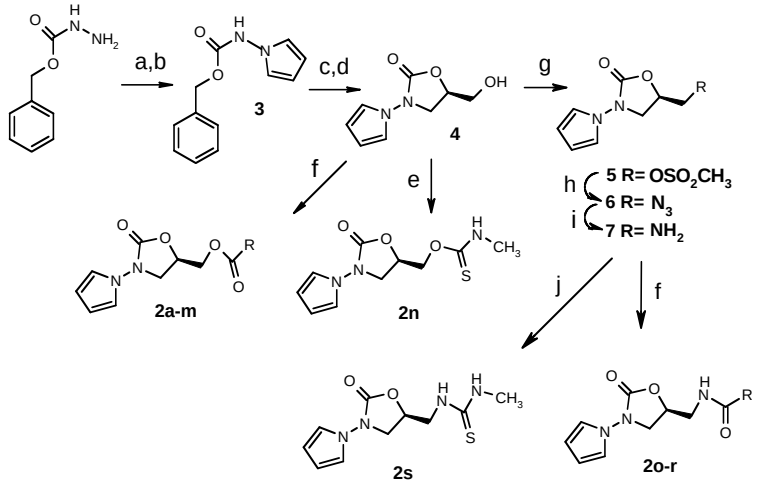
position of the oxazolidinone ring (Figure 1).[24] Among them, *R*-hydroxymethyl, *R*-alkoxymethyl, *R*-azidomethyl derivatives were endowed with reversible, high potent and selective anti-MAO-A activity (K_i values = 0.2-0.0049 M; A-selectivity = 100-10,200).[24]
Pursuing the study in this field, we describe in the present paper the synthesis and the biochemical characterization of novel 3-(1*H*-pyrrol-1-yl)-2-oxazolidinones **2** carrying as substitutions at C_5 position different acyloxymethyl or acylaminomethyl moieties (Figure 1).

Results and Discussion

Chemistry

1-(Phenylmethoxycarbonylamino)-1*H*-pyrrole **3**[25] was prepared from benzyl carbazate and 2,5-dimethoxytetrahydrofuran in ethanol/acetic acid medium as the starting material for the synthesis of title derivatives. After treatment with *n*-butylithium in hexanes at –78 °C , the lithiated intermediate reacted with (*R*)-glycidyl butyrate to furnish directly, after spontaneous hydrolysis of the butyrate function, the (*R*)-5-hydroxymethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinone **4**. The alcohol **4** was converted into the corresponding esters **2a-m** by adding the appropriate acyl chlorides and triethylamine to the **4** dichloromethane solution. Treatment of **4** with *N*-methylisothiocyanate in tetrahydrofuran (THF) gave the thiourethane derivative **2n**.

Reaction of **4** with methanesulfonyl chloride and triethylamine afforded the (*R*)-5-methanesulfonyloxymethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinone **5**, which was converted into the corresponding (*R*)-5-azidomethylderivative **6**. The (*S*)-5-aminomethylpyrrole **7**, obtained by catalytic reduction of **6**, provided the amide derivatives **2o-q** by reacting with the appropriate acyl chlorides and triethylamine. Treatment of **7** with methyl chloroformate and *N*-methylisothiocyanate afforded the methoxycarbonylamino (**2r**) and thioureido (**2s**) derivatives, respectively (Scheme 1).



Scheme 1. (a) 2,5-dimethoxytetrahydrofuran, AcOH, EtOH, Δ. (b) AcOH, Δ. (c) 2.5 M *n*-BuLi in hexanes, THF, -78 °C. (d) (1) *R*-Glycidyl butyrate, (2) NH_4Cl, rt. (e) $SCNCH_3$, NaH, THF. (f) Et_3N, ClCOR, CH_2Cl_2, rt. (g) CH_3SO_2Cl, Et_3N, CH_2Cl_2, rt. (h) NaN_3, DMF, 70°C. (i) H_2, Pd/C, rt. (j): $SCNCH_3$, THF.

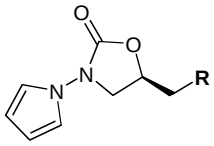
Biological Evaluation

(*R*)-5-Acyloxymethyl- and (*S*)-5-acylaminomethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinones **2a-s** were evaluated for their ability to inhibit MAO-A and MAO-B, in comparison with toloxatone as reference drug.

Bovine brain mitochondria were used as the enzyme source and were isolated according to Basford.[26] Activities of MAO-A and MAO-B were determined by a fluorometric method with kynuramine as a substrate, at several different final concentrations. The K_i values (μM) against the two isoenzymatic MAO forms and the A-selectivity (expressed as $K_{iMAO\text{-}B}/K_{iMAO\text{-}A}$ ratio) are reported in Table 1.

All compound showed inhibitory activity against the A isoform of the MAO enzyme higher than that exerted against MAO-B. Furthermore, all derivatives displayed a reversible mode of action since dialysis for 24 h in a cold room, at 4 °C, against 0.1 M potassium phosphate buffer (pH 7.2) was able to restore from 90 to 100% activity of the native enzyme.

Table 1. Monoamine oxidase inhibitory activity of compounds **2**[a]



Compd	R	K_i values (μM)		A-selectivity
		MAO-A	MAO-B	
2a	$OCOCH_3$	0.40	2.0	5
2b	$OCOCH_2CH_3$	0.90	400	444.4
2c	$OCOCH(CH_3)_2$	0.16	3.2	20
2d	$OCOCH_2CH_2CH_3$	0.64	40	62.5
2e	$OCOC(CH_3)_3$	0.73	14.2	19.4
2f	OCOPh	1.08	12.2	11.3
2g	OCOPhCl (*o*)	0.44	80	181.8
2h	OCOPhCl (*m*)	0.31	100	322.6
2i	OCOPhCl (*p*)	0.64	23.2	36
2j	$OCOPhCH_3$ (*o*)	0.52	100	192.3
2k	$OCOPhCH_3$ (*m*)	0.40	100	250
2l	$OCOPhCH_3$ (*p*)	1.00	17	17
2m	$OCOCH_2Ph$	0.62	70	112
2n	$OCSNHCH_3$	0.48	150	312.5
2o	$NHCOCH_3$	0.92	100	108.7
2p	NHCOPh	0.44	160	363.6
2q	$NHCOCH_2Ph$	0.89	2.3	2.6
2r	$NHCOOCH_3$	4.6	100	21.7
2s	$NHCSNHCH_3$	1.32	100	75.7
toloxatone		0.38	15	39.5

[a] Data represent mean values of least three separate experiments.

Among test derivatives, the majority of them (**2a,d,g-k,m,n,p**) showed similar MAO-A inhibiting activity to that of toloxatone, they being often (**2d,g,h,j,k,m,n,p**) more A-selective. Derivative **2c** was 2-fold more powerful than toloxatone in our enzyme assay, but 2-fold less selective. With the sole exception of derivative **2c**, among ester derivatives **2a-m** elongation of the 5-acyloxymethyl chain (**2a-e,m**) or introduction on phenyl ring of both electron-attractor and -withdrawing groups (**2f-l**) did not increase the inhibitory activity of compounds. Interestingly, **2b** with an aliphatic chain longer by one carbon atom with respect to that of **2a** was 88-fold more A-selective than **2a**.

No amide derivatives (**2o-s**) showed higher inhibiting activity than toloxatone; nevertheless, compounds **2o,p,s** were more A-selective, with **2p** reaching the selectivity value of 364. Replacement of (*S*)-5-acetylaminomethyl with (*S*)-5-benzoylaminomethyl substitution gave an improvement of activity as well as A-selectivity, while introduction of phenylacetylaminomethyl moiety at 2-oxazolidinone C_5 position (**2q**) led to a drastic decrease of selectivity.

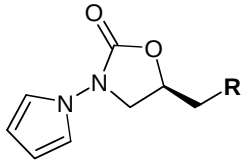
Conclusions

A series of novel (*R*)-5-acyloxymethyl- and (*S*)-5-acylaminomethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinones **2a-s** have been described as MAO inhibitory agents. From biochemical evaluation data many of them emerged as reversible, selective MAO-A inhibitors, with the same inhibitory potency ($K_{iMAO\text{-}A}$ = 0.16-0.90 μM) of toloxatone (*Humoryl*®) ($K_{iMAO\text{-}A}$ = 0.38 μM), in clinical use as antidepressant drug, but being up to 11-fold more selective for the A isoform of the enzyme. Such results indicate that these compounds could show promise as new antidepressant agents.

Experimental Section

General Procedures. Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra (KBr) were recorded on a Perkin-Elmer Spectrum One instrument. ^{1}H NMR spectra were recorded at 200 MHz on a Bruker AC 200 spectrometer; chemical shifts are reported in δ (ppm) units relative to the internal reference tetramethylsilane (Me_4Si). All compounds were routinely checked by TLC and ^{1}H NMR. TLC was performed on aluminum-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F_{254}) with spots visualized by UV light. All solvents were reagent grade and, when necessary, were purified and dried by standards methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of *ca.* 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Analytical results are within ±0.40% of the theoretical values. All chemicals were purchased from Aldrich Chimica, Milan (Italy) or Lancaster Synthesis GmbH, Milan (Italy) and were of the highest purity. Chemical and physical properties of compounds **2,4-7** are reported in Table 2.

Table 2. Chemical and physical properties of compounds **2,4-7**



Compd	R	Mp, °C	Recrystallization solvent	Yield %	Rotatory power $[\alpha]_D^{25}$ ($CHCl_3$)
2a	$OCOCH_3$	113-114	cyclohexane/benzene	90	-0.40
2b	$OCOCH_2CH_3$	69-70	cyclohexane/benzene	88	-0.22
2c	$OCOCH(CH_3)_2$	97-99	cyclohexane/benzene	82	-0.13
2d	$OCOCH_2CH_2CH_3$	54-56	diethyl ether	91	-0.25
2e	$OCOC(CH_3)_3$	132-133	benzene	72	-0.12
2f	OCOPh	84-85	cyclohexane/benzene	92	-0.51
2g	OCOPhCl (*o*)	98-100	cyclohexane/benzene	86	-0.10
2h	OCOPhCl (*m*)	110-112	cyclohexane/benzene	84	-0.15
2i	OCOPhCl (*p*)	133-134	cyclohexane/benzene	89	-0.54
2j	$OCOPhCH_3$ (*o*)	85-86	cyclohexane/benzene	79	-0.70
2k	$OCOPhCH_3$ (*m*)	78-80	cyclohexane/benzene	73	-0.20
2l	$OCOPhCH_3$ (*p*)	86-87	diethyl ether	85	-1.05
2m	$OCOCH_2Ph$	123-124	benzene	88	-0.38
2n	$OCSNHCH_3$	94-95	cyclohexane/benzene	64	-0.26
2o	$NHCOCH_3$	135-136	benzene/acetonitrile	80	-0.50
2p	NHCOPh	155-157	benzene/acetonitrile	78	-0.17
2q	$NHCOCH_2Ph$	130-132	benzene	74	-0.65
2r	$NHCOOCH_3$	oil	-	82	-0.04
2s	$NHCSNHCH_3$	oil	-	58	-0.26
4	OH	105-107	benzene	54	-0.67
5	OSO_2CH_3	oil	-	62	-0.20
6	N_3	oil	-	80	-0.48
7	NH_2	oil	-	77	-0.36

Synthesis of (*R*)-5-hydroxymethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinone (4). To a solution of compound **3**[25] (1.0 g, 4.63 mmol) in dry THF (20 mL) at –78 °C under nitrogen atmosphere *n*-butyllithium (1.6 M in hexanes, 2.3 mL, 3.69 mmol) was added dropwise over a period of 5 min. The reaction mixture was stirred at –78 °C for 1 h, followed by addition of (*R*)-glycidyl butyrate (0.53 mL, 3.69 mmol). The reaction mixture was stirred initially at –78 °C for 1 h and then at room temperature for 5 h. After, the reaction mixture was quenched by addition of saturated NH_4Cl solution (100 mL) and then extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL), and dried. The residue obtained upon evaporation of solvent was chromatographed over silica gel and eluted with ethyl acetate to give the alcohol **4** as a white solid. IR (KBr) 3400 (OH), 1760 (NCOO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.72 (s, 1H, OH exchangeable with D_2O), 3.70-3.77 (m, 1H, NCH_2),

3.96-4.04 (m, 3H, NCH_2 and CH_2OH), 4.72-4.83 (m, 1H, OCH), 6.19-6.21 (m, 2H, pyrrole β-proton), 6.75-6.77 (m, 2H, pyrrole α-proton).

Synthesis of (*R*)-5-methanesulfonyloxymethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinone (5). To a solution of the alcohol **4** (510 mg, 3 mmol) in dry dichloromethane (15 mL) at 0 °C under nitrogen atmosphere triethylamine (1.04 mL, 7.5 mmol) was added, followed by addition of methanesulfonyl chloride (0.27 mL, 2.1 mmol) over a period of 2 min. The reaction mixture was stirred at 0 °C for 1 h, and then was worked up by adding water (100 mL) followed by extraction with dichloromethane (3 x 50 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL), and dried. The solvent was evaporated to give the desired methanesulfonate **5** as a gum, which was taken up for the next step without any purification. IR 1760 (NCOO), 1710 (OSO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.86-3.93 (m, 1H, NCH_2), 4.07-4.15 (m, 1H, NCH_2), 4.45-4.58 (m, 2H, CH_2O), 4.88-5.03 (m, 1H, OCH), 6.17-6.22 (m, 2H, pyrrole β -proton), 6.71-6.77 (m, 2H, pyrrole α -proton).

Synthesis of (*R*)-5-azidomethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinone (6). To a solution of **5** (600 mg, 2.42 mmol) in dry *N*,*N*-dimethylformamide (DMF, 10 mL) under nitrogen atmosphere was added sodium azide (315 mg, 4.84 mmol), and the resulting mixture was stirred at 80 °C for 2 h. The reaction mixture was allowed to cool to room temperature and worked up by addition water (100 mL) followed by extraction with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL), and dried. The residue obtained upon evaporation of solvent was chromatographed over silica gel and eluted with ethyl acetate:chloroform 1:5 to give the azide **6** as an oil. IR 2080 (N_3), 1760 (NCOO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.44-3.68 (m, 1H, CH_2N_3), 3.70-3.81 (m, 2H, $CH_2N_3$and NCH_2), 3.92-4.04 (m, 1H, NCH_2), 4.73-4.89 (m, 1H, OCH), 6.17-6.23 (m, 2H, pyrrole β-proton), 6.72-6.78 (m, 2H, pyrrole α -proton).

Synthesis of (*S*)-5-aminomethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinone (7). To a solution of **7** (0.96 g, 4.64 mmol) in methanol (80 mL) placed in Parr apparatus palladium on 10% carbon was added, then the mixture was hydrogenated at 50 psi and 25 °C for 1 h. At last, palladium was filtered and methanol was evaporated to afford an oily residue that was chromatographed on silica gel and eluted with chloroform:methanol 9:1 to provide the amine derivative **7**. IR 3350-3280 (NH_2), 1760 (NCOO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.26 (s, 2H, NH_2 exchangeable with D_2O), 2.91-2.98 (q, 1H, CH_2NH_2), 3.13-3.25 (q, 1H, CH_2NH_2), 3.80-3.88 (t, 1H, NCH_2), 3.95-4.03 (t, 1H, NCH_2), 4.65-4.77 (m, 1H, OCH), 6.19-6.23 (m, 2H, pyrrole β -proton), 6.76-6.80 (m, 2H, pyrrole α -proton).

General procedure for the synthesis of (*R*)-5-acyloxymethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinones 2a-m

To a solution of the alcohol **4** (1 eq) in dry dichloromethane (50 mL) at 0 °C under nitrogen triethylamine (2.5 eq) was added, followed by addition of the appropriate acyl chloride (1.2 eq) dropwise. The mixture was stirred at room temperature for 1-6 h (TLC control), then it was diluted with dichloromethane (50 mL) and washed with water (2 x 100 mL) followed by brine (100 mL). The organic extract was dried, evaporated, and passed through a column over silica gel eluting with ethyl acetate:chloroform 1:10 to afford the acylated product as a solid.

(2a). IR (KBr) 1780 (NCOO), 1740 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.10 (s, 3H, CH_3),

3.68-3.75 (m, 1H, NCH_2), 3.98-4.05 (m, 1H, NCH_2), 4.25-4.45 (m, 2H, CH_2O), 4.75-4.85 (m, 1H, OCH), 6.14-6.17 (m, 2H, pyrrole β-proton), 6.68-6.70 (m, 2H, pyrrole α-proton).

(2b). IR (KBr) 1784 (NCOO), 1741 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.17-1.25 (t, 3H, CH_2CH_3), 2.44-2.47 (q, 2H, CH_2CH_3), 3.75-3.85 (m, 1H, NCH_2), 4.05-4.12 (m, 1H, NCH_2), 4.30-4.45 (m, 2H, CH_2O), 4.85-4.95 (m, 1H, OCH), 6.22-6.24 (m, 2H, pyrrole β-proton), 6.74-6.77 (m, 2H, pyrrole α-proton).

(2c). IR (KBr) 1782 (NCOO), 1740 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.25-1.28 (d, 6H, $CH(CH_3)_2$), 2.65-2.75 (q, 1H, $CH(CH_3)_2$), 3.82-3.90 (m, 1H, NCH_2), 4.05-4.15 (m, 1H, NCH_2), 4.35-4.48 (m, 2H, CH_2O), 4.90-4.95 (m, 1H, OCH), 6.24-6.26 (m, 2H, pyrrole β-proton), 6.77-6.79 (m, 2H, pyrrole α-proton).

(2d). IR (KBr) 1780 (NCOO), 1746 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.99-1.06 (t, 3H, $CH_2CH_2CH_3$), 1.72-1.76 (q, 2H, $CH_2CH_2CH_3$), 2.39-2.43 (t, 2H, $CH_2CH_2CH_3$), 3.80-3.90 (m, 1H, NCH_2), 4.08-4.18 (m, 1H, NCH_2), 4.35-4.48 (m, 2H, CH_2O), 4.88-4.95 (m, 1H, OCH), 6.25-6.27 (m, 2H, pyrrole β-proton), 6.77-6.79 (m, 2H, pyrrole α-proton).

(2e). IR (KBr) 1786 (NCOO), 1744 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.30 (s, 9H, CH_3), 3.85-3.92 (m, 1H, NCH_2), 4.08-4.18 (m, 1H, NCH_2), 4.32-4.48 (m, 2H, CH_2O), 4.90-4.98 (m, 1H, OCH), 6.25-6.27 (m, 2H, pyrrole β-proton), 6.77-6.79 (m, 2H, pyrrole α-proton).

(2f). IR (KBr) 1785 (NCOO), 1743 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.80-3.88 (m, 1H, NCH_2), 4.05-4.14 (m, 1H, NCH_2), 4.58-4.60 (d, 2H, CH_2O), 4.95-5.05 (m, 1H, OCH), 6.14-6.16 (m, 2H, pyrrole β-proton), 6.65-6.67 (m, 2H, pyrrole α-proton), 7.41-7.48 (m, 2H, benzene H-3,5), 7.55-7.58 (m, 1H, benzene H-4), 8.00-8.05 (m, 2H, benzene H-2,6).

(2g). IR (KBr) 1786 (NCOO), 1745 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.96-3.99 (m, 1H, NCH_2), 4.10-4.18 (m, 1H, NCH_2), 4.59-4.68 (m, 2H, CH_2O), 4.99-5.08 (m, 1H, OCH), 6.19-6.21 (m, 2H, pyrrole β-proton), 6.72-6.74 (m, 2H, pyrrole α-proton), 7.35-7.45 (m, 1H, benzene H-5), 7.49-7.52 (m, 2H, benzene H-3,4), 7.88-7.92 (m, 2H, benzene H-6).

(2h). IR (KBr) 1782 (NCOO), 1743 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.90-3.98 (m, 1H, NCH_2), 4.15-4.22 (m, 1H, NCH_2), 4.65-4.68 (m, 2H, CH_2O), 5.02-5.12 (m, 1H, OCH), 6.24-6.26 (m, 2H, pyrrole β-proton), 6.76-6.78 (m, 2H, pyrrole α-proton), 7.45-7.50 (m, 1H, benzene H-5), 7.60-7.70 (m, 1H, benzene H-4), 7.95-8.00 (m, 1H, benzene H-6), 8.08-8.11 (m, 1H, benzene H-2).

(2i). IR (KBr) 1782 (NCOO), 1721 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.88-3.92 (m, 1H, NCH_2), 4.13-4.17 (m, 1H, NCH_2), 4.62-4.64 (m, 2H, CH_2O), 5.02-5.08 (m, 1H, OCH), 6.19-6.22 (m, 2H, pyrrole β-proton), 6.70-6.73 (m, 2H, pyrrole α-proton), 7.28-7.32 (d, 2H, benzene H-3,5), 7.95-7.99 (d, 2H, benzene H-2,6).

(2j). IR (KBr) 1780 (NCOO), 1740 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.67 (s, 3H, CH_3), 3.91-3.98 (m, 1H, NCH_2), 4.12-4.20 (m, 1H, NCH_2), 4.62-4.70 (m, 2H, CH_2O), 5.02-5.10 (m, 1H, OCH), 6.24-6.26 (m, 2H, pyrrole β-proton), 6.76-6.78 (m, 2H, pyrrole α-proton), 7.30-7.40 (m, 2H, benzene H-3,5), 7.48-7.55 (m, 1H, benzene H-4), 7.95-8.05 (m, 1H, benzene H-6).

(2k). IR (KBr) 1785 (NCOO), 1744 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.46 (s, 3H, CH_3), 3.95-4.02 (m, 1H, NCH_2), 4.16-4.22 (m, 1H, NCH_2), 4.65-4.72 (m, 2H, CH_2O), 5.02-5.08 (m, 1H, OCH), 6.20-6.24 (m, 2H, pyrrole β-proton), 6.75-6.80 (m, 2H, pyrrole α-proton), 7.40-7.48 (m, 2H, benzene H-4,5), 7.85-7.96 (m, 2H, benzene H-2,6).

(2l). IR (KBr) 1781 (NCOO), 1740 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.45 (s, 3H, CH_3),

3.88-3.92 (m, 1H, NCH_2), 4.12-4.20 (m, 1H, NCH_2), 4.62-4.64 (m, 2H, CH_2O), 5.02-5.08 (m, 1H, OCH), 6.20-6.22 (m, 2H, pyrrole β-proton), 6.71-6.73 (m, 2H, pyrrole α-proton), 7.28-7.32 (m, 2H, benzene H-3,5), 7.95-7.99 (m, 2H, benzene H-2,6).

(2m). IR (KBr) 1785 (NCOO), 1743 (COO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.68-3.77 (m, 1H, NCH_2), 3.74 (s, 2H, $COCH_2$ overlapped signal), 3.98-4.05 (m, 1H, NCH_2), 4.35-4.50 (m, 2H, CH_2O), 4.85-4.92 (m, 1H, OCH), 6.21-6.23 (m, 2H, pyrrole β-proton), 6.65-6.69 (m, 2H, pyrrole α-proton), 7.27-7.28 (m, 2H, benzene H-2,6), 7.33-7.35 (m, 3H, benzene H-3,4,5).

Synthesis of (*R*)-5-*N*-methylaminothiocarbonyloxymethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinone (2n). To a solution of **4** (0.5 g, 2.75 mmol) in dry tetrahydrofuran (20 mL) sodium hydride (82.5 mg, 2.75 mmol) in mineral oil (80%) was added, and the reaction was stirred for 15 min. Then *N*-methylisothiocyanate (0.23 mL, 3.3 mmol) was added, and the resulting mixture was stirred while cooling (ice bath). At the complete consumption of starting material (TLC control, 2 days) the reaction was worked. The residue obtained was passed through a column of silica gel eluting with ethyl acetate:chloroform 1:1 to afford the product **2n** as a solid. IR (KBr) 1780 (CO), 1760 (CS) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.14 (s, 3H, CH_3), 3.38-3.46 (m, 2H, NCH_2), 3.92-4.00 (m, 1H, OCH), 4.30-4.38 (m, 1H, CH_2O), 4.50-4.60 (m, 1H, CH_2O), 5.05 (s, 1H, NH exchangeable with D_2O), 6.11-6.13 (m, 2H, pyrrole β-proton), 6.73-6.77 (m, 2H, pyrrole α-proton).

General procedure for the synthesis of (*S*)-5-acylaminomethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinones 2o-r

To a solution of **7** (1 eq) in dry dichloromethane (50 mL) at 0 °C under nitrogen triethylamine (2.5 eq) was added, followed by addition of the appropriate acyl chloride (1.2 eq) dropwise. The resulting mixture was stirred at room temperature for 1-6 h (TLC control), then it was diluted with dichloromethane (50 mL) and washed with water (2 x 100 mL) followed by brine (100 mL). The organic extract was dried, evaporated, and chromatographed on silica gel eluting with chloroform:methanol 9:1 to afford the product as a solid. **2o**: IR (KBr) 3100 (NH), 1780 (NCOO), 1730 (NCO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.00 (s, 3H, CH_3), 3.45-3.62 (m, 2H, C*H*$_2$NHCO), 3.62-3.76 (m, 1H, NCH_2 overlapped signal), 3.89-4.03 (m, 1H, NCH_2), 4.74-4.86 (m, 1H, OCH), 6.07-6.19 (m, 2H, pyrrole β-proton), 6.69-6.74 (m, 2H, pyrrole α-proton), 6.81 (s, 1H, NH exchangeable with D_2O). **2p**: IR (KBr) 3120 (NH), 1785 (NCOO), 1726 (NCO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.80-3.95 (m, 3H, NCH_2 (1H) and C*H*$_2$NHCO (2H)), 4.06-4.14 (m, 1H, NCH_2), 4.92-4.98 (m, 1H, OCH), 6.19-6.21 (m, 2H, pyrrole β-proton), 6.69-6.71 (m, 2H, pyrrole α-proton), 7.00 (s, 1H, NH exchangeable with D_2O), 7.48-7.55 (m, 3H, benzene H-3,4,5), 7.83-7.86 (m, 2H, benzene H-2,6). **2q**: IR (KBr) 3100 (NH), 1784 (NCOO), 1725 (NCO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.60-3.70 (m, 5H, NCH_2 (1H), C*H*$_2$NHCO (2H), and CH_2Ph (2H)), 3.95-4.05 (m, 1H, NCH_2), 4.78-4.82 (m, 1H, OCH), 6.18-6.22 (m, 2H, pyrrole β-proton), 6.66-6.69 (m, 2H, pyrrole α-proton), 7.35-7.45 (m, 5H, benzene H). **2r**: IR 3130 (NH), 1780 (NCOO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.65-3.88 (m, 3H, NCH_2 (1H) and C*H*$_2$NHCO (2H)), 3.76 (s, 3H, CH_3 overlapped signal), 4.02-4.13 (m, 1H, NCH_2), 4.75-4.88 (m, 1H, OCH), 6.20-6.24 (m, 2H, pyrrole β-proton), 6.74-6.78 (m, 2H, pyrrole α-proton).

Synthesis of (*S*)-5-*N'*-methylthioureidomethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinone (2s). To a solution of **7** (0.5 g, 2.76 mmol) in dry tetrahydrofuran (20 mL) *N*-methylisothiocyanate

(0.23 mL, 3.3 mmol) was added, and the reaction was stirred while cooling (ice bath) overnight. After, water was added (100 mL) to the reaction mixture, followed by extraction with ethyl acetate (3 x 50 mL). The obtained residue upon evaporation of the solvent was passed through a column of silica gel eluting with ethyl acetate to afford the product as a oil. IR 3150 (NH), 1780 (CO), 1760 (CS) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.90-2.93 (d, 3H, CH_3), 3.80-4.15 (m, 4H, NCH_2 and CH_2NH), 4.90-5.00 (m, 1H, OCH), 6.22-6.24 (m, 2H, pyrrole β-proton), 6.66-6.68 (m, 2H, pyrrole α-proton).

Biochemical assay. Monoamine oxidase activity was determined using kinuramine as a substrate, at four different final concentrations ranging from 5 mM to 0.1 mM, by a sensitive fluorometric assay according to Matsumoto *et al*.[27] In all assays the incubation mixtures contained: potassium phosphate buffer pH 7.4, mitochondria (6 mg/mL), drug solutions in dimethyl-sulfoxide (DMSO), added to the reaction mixture from 0 to 10^{-3} mM. Solutions were preincubated for 30 min before adding the substrate and then incubated for others 30 min. The inhibitory activities of both MAO A and B separately, were determined after incubation of the mitochondrial fractions for 30 min at 38 °C, in the presence of the specific inhibitor (L-deprenyl 1 mM to estimate the MAO A activity, and clorgyline 1 mM to assay the isoform B), taking in account that MAO-A is irreversibly inhibited by low concentration of clorgyline, but is unaffected by low concentration of L-deprenyl, utilized contrary in the form MAO-B. The addition of perchloric acid ended the reaction. Then the samples were centrifuged at 10,000 g for 5 min and the supernatant was added to 2.7 mL 1N NaOH. Fluorometric measurements were recorded using a Perkin-Elmer LS 50B spectrofluorimeter, at λ_{exc} 317 nm and λ_{em} 393 nm. The protein concentration was determined according to Goa.[28] Dixon plot were used to estimate the inhibition constant (K_i) of the inhibitors. Data are the means of three or more experiments each performed in duplicate.

Mitochondria preparation. Mitochondria were prepared according to Basford.[26] The following reagents were used: medium A contains 0.4 M sucrose, 0.001 EDTA, 0.02% PES or heparin and pH value to 6.8-7.0 is adjusted by addition of KOH; while, medium F made up of the medium A to which Ficoll is added to a final concentration of 8%. Calf or beef brains are removed from the animals within 5-10 min after their death. The brains are immediately placed in cold Medium A and then stored on ice, to be transported to the laboratory. In cold room, at 5 °C, the cerebral hemispheres are removed from the brains and the meninges are taken up with forceps. The gray matter is scraped from the cortices using a dull spatula. Two brains yield corresponds to about 100 g of wet tissue, and is homogenized in 2 mL of Medium A/g of wet tissue. The homogenate is kept at pH 7.0 adding some drops of TRIS-buffer 2M, 1 mg of ε-aminocaproic acid/g of tissue and then the mixture is stirred at 0-4 °C for 15 min. The suspension is diluted with Medium A (20 mL/g of the original tissue) and centrifuged twice, first at 184 g for 20 min and then, without transferring of the supernatant, at 1153 g for others 20 min. The residue R_1 is discarded while the supernatant S_1 is centrifuged at 12,000 g for 15 min, to yield a crude mitochondria pellet R_2, and the supernatant S_2, which is discarded. The fraction R_2 is dissolved in Medium F (6 mL/g of original tissue), gently homogenized and centrifuged at 12,000 g for 30 min. The resulting mitochondria fraction R_3, is washed using 4 mL of Medium A/g of original tissue and again centrifuged at 12,000 g for 15 min, to yield the final mitochondria fraction R_4, which is

homogenized in 0.25 M potassium phosphate buffer, pH 7.4. The yield of mitochondria protein obtained is between 100 and 140 mg per 50 g wet weight of the original tissue.

Appendix

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Acknowledgments

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