3-(1*H*-Pyrrol-2-yl)-2-oxazolidinones as Novel Monoamine Oxidase Type A Inhibitors

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Abstract: A novel series of 5-substituted-3-(1*H*-pyrrol-2-yl)-2-oxazolidinones **2a-s** has been described as pyrrole analogues of toloxatone and befloxatone, two phenyl-oxazolidinones active as anti-MAO agents and used in antidepressant therapy. Tested against MAO-A and MAO-B enzymes, the majority of **2a-s** show highly potent inhibitory effect against the A isoform of the enzyme, with K_i values in the range 0.52-0.004 μM, whilst their anti-MAO-B activity is considerably lower (K_i = >100-0.5 μM). Structurally, **2a-s** differs for the substituent inserted at the C5 position of the 2-oxazolidinone ring (hydroxymethyl (**2a-d**), methoxymethyl (**2e-h**), azidomethyl (**2i-l**), methylaminomethyl (**2m-p**), and aminomethyl (**2q-s**)), and the size of the alkyl chain at the pyrrole N1 position (methyl, ethyl, allyl, or benzyl). As a rule, apart from the C5 substitution, the bulkier is the alkyl group at the pyrrole-N1, the lower is the anti-MAO-A activity of the compounds, being the N1-methyl derivatives **2a**, **2e**, **2i**, and **2q** among the most potent (K_{iMAO-A} = 0.087-0.004 μM) and A-selective (A-selectivity ratio: >11,111-41) compounds in this series. Exceptions are represented by the N1-benzyl derivative **2d** (K_{iMAO-A} = 0.009 μM) and the N1-allylpyrrole **2o** (K_{iMAO-A} = 0.04 μM). In comparison with the reference drugs, these highly active derivatives are more potent than toloxatone, slightly less potent than befloxatone, and several times more A-selective than both the references. Such results indicate that **2a-s** may represent a new promising series of antidepressant agents.

Key Words: MAO-A, MAO inhibitors, 2-oxazolidinones, 3-(1*H*-pyrrol-2-yl)-2-oxazolidinones, antidepressant agents.

INTRODUCTION

Monoamine oxidases (MAOs, EC 1.4.3.4) are flavin-containing enzymes (FAD or FMN) that catalyze the oxidative deamination of structurally different amines including the neurotransmitters dopamine, norepinephrine, serotonin (5-HT), tyramine, 2-phenylethylamine (PEA), and also exogenous amines [1-5]. MAO represents an integral protein of the mitochondrial outer membranes of neuronal, glial, and other cells. In mammals, there are two major isoforms, MAO-A and MAO-B, distinguishable by their differences in substrate specificity, amino acid sequences [6,7] and inhibitor selectivities [6-10]. MAO-A preferentially catalyses the oxidation of 5-HT and norepinephrine and it is selectively inhibited by clorgyline [6], whereas MAO-B catalyses the oxidation of PEA and benzylamine and is selectively inhibited by (*R*)-deprenyl [10,11]. Tyramine, dopamine, and tryptamine appear to be substrates for both subtypes. In humans, most tissues, with the exception of blood platelets and myocardium that are particularly rich in MAO-B, express both isozymes [12]. High levels of MAO-A are detected in the human placenta, lung and small intestine [13-15].

Isoenzyme A occurs in catecholaminergic neurons, whereas isoenzyme B is mainly present in the human brain and in other different cell types. The different localization suggests that the two subtypes have different physiological functions. In fact, MAO-A and B are probably related to psychiatric and neurological disorders such as depression and Parkinson's disease, respectively. MAO inhibitors (MAOIs) represent a useful tool in the treatment of depression, but, unfortunately, their administration is associated to severe side effects [16]. Selective and reversible inhibitors of MAO-A and MAO-B are useful therapeutic agents in the treatment of depression and anxiety, respectively [6]; moreover, they are claimed to protect neuronal cells against apoptosis [17] and are considered coadjuvant agents in the treatment of Parkinson's disease [11,18] and maybe also Alzheimer's disease [19]. MAOIs are classified into irreversible and reversible inhibitors. Compounds belonging to the first generation of irreversible MAOIs, like iproniazid and tranylcypromine, were mechanism-based inactivators capable of modifying proteins, inactivating the P450 enzyme therefore causing hepatotoxic side effects [20-22] and inducing of the so-called

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1573-4064/05 $50.00+.00

"cheese effect" that lead to hypertensive crises [23,24]. These reasons led to a decline of irreversible MAOIs and to the synthesis of reversible and subtype-selective inhibitors.

The structural template for phenyl-2-oxazolidinone derivatives (Fig. (**1**)) was obtained from MD780236, a mechanism-based MAO-B inactivator [25-28]. Replacement of the amine function with related bioisostere groups abolished the MD780236 irreversible inactivation property and generated slow, tight-binding reversible MAOIs. The success of this strategy is highlighted by the discovery of potent and selective MAO-A inhibitors such as cimoxatone and toloxatone (Fig. (**1**)), having K_i values ranging from 0.03 to 2 μM [29-31]. Toloxatone is the first reversible and selective MAO-A inhibitor introduced in the clinical practice as antidepressant agent. Modifications of its structure led to the discovery of befloxatone (Fig. (**1**)), currently administered in phase III clinical trials for the treatment of depression [32].

Chemical manipulations performed on the phenyl-2-oxazolidinone structure have regarded the substituent(s) on the phenyl ring at the N3 position or the insertion of different chemical functions at the C5 methylene group of the 2-oxazolidinone ring, whilst little attention has been devoted to the replacement of the phenyl ring with other (hetero) aromatic moieties, the only examples being some benzothiazole and naphthalene derivatives [33-35].

Recently, we reported a new series of 3-(1*H*-pyrrol-1-yl)-2-oxazolidinones **1** active as reversible, potent and MAO-A-selective inhibitors [36-38] (Fig. (**2**)). Chemical feature of these compounds is the presence of a 2-oxazolidinone moiety carrying diverse substituents at the C5 position, linked to the N1 position of the pyrrole ring through a hydrazine linkage. Pursuing our searches in this field, we planned the synthesis of 3-(1*H*-pyrrol-2-yl)-2-oxazolidinone derivatives **2**, isomer of **1**, to test as new anti-MAO agents (Fig. (**2**)). In compounds **2**, the 2-oxazolidinone nucleus has been inserted at the C2 position of the pyrrole, leaving the pyrrole in N1 position free to accept various alkyl chains.

CHEMISTRY

1-Alkyl-1*H*-pyrrole-2-carboxylic acids **3a-d** were commercially available (**3a**) or were prepared as previously reported [39-41]. After treatment with diphenylphosphorylazide, triethylamine, and benzyl alcohol in benzene at 80 °C, **3a-d** afforded the 1-alkyl-2-benzyloxycarbonylamino-1*H*-pyrroles **4a-d** through Curtius rearrangement of the intermediate acylazides. Further reaction of **4a-d** with *n*-butyllithium in hexane at –78 °C followed by addition of (*R*)-glycidyl butyrate to the lithiated species furnished directly, after spontaneous hydrolysis of the butyrate function, the (*R*)-5-hydroxymethyl-3-(1-alkyl-1*H*-pyrrol-2-yl)-2-oxazolidinones **2a-d**. The alcohols **2a-d** were then converted into the corresponding (*R*)-5-methanesulfonyl-

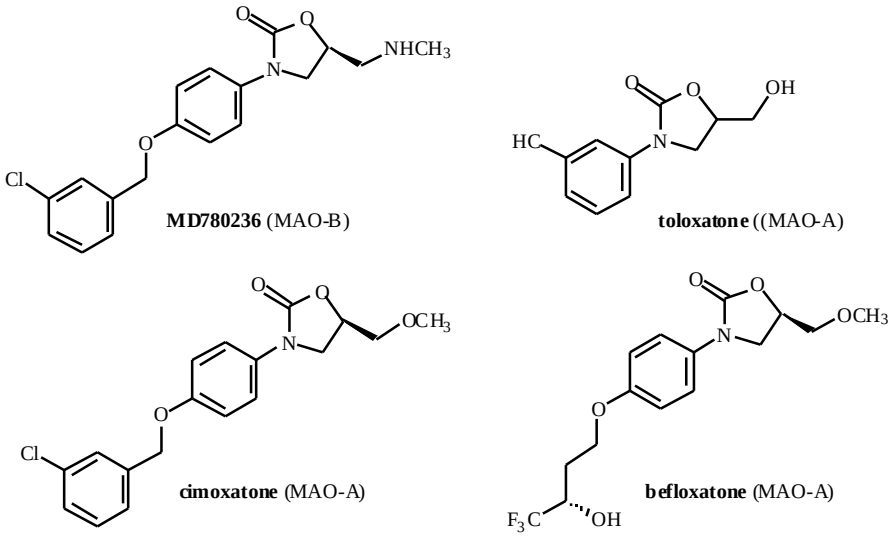


Fig. (1). Known 3-phenyl-2-oxazolidinones.

Fig. (2). 1*H*-Pyrrolyl-2-oxazolidinones.

oxymethyl derivatives **5a-d** with methanesulfonyl chloride and triethylamine, and the mesylates **5a-d** were subjected to nucleophilic displacements with sodium methoxide in methanol (r.t.), or with sodium azide in dimethylformamide (65 °C), or methylamine in methanol (65 °C) to afford the (*R*)-5-methoxymethyl-, or the (*R*)-5-azidomethyl-, or the (*S*)-5-aminomethyl-3-(1-alkyl-1*H*-pyrrol-2-yl)-2-oxazolidinones **2e-h**, or **2i-l**, or **2m-p**, respectively. From the (*R*)-5-azidomethyl derivatives **2i,j,l** the corresponding aminomethyl compounds **2q-s** have been obtained by catalytic reduction (Scheme **1**).

Chemical and physical data of compounds **2-5** are listed in Table **1**.

RESULTS AND DISCUSSION

3-(1*H*-Pyrrol-2-yl)-2-oxazolidinones **2a-s** have been tested against MAO-A and MAO-B enzymes, in comparison with toloxatone as reference drug. Bovine brain mitochondria have been used as the enzyme source and were isolated according to Basford [42]. Activities of MAO-A and MAO-B have been determined by a fluorometric method with kynuramine as substrate at several different concentrations. The K_i values against the two MAO isozymes and the A-selectivity (expressed as $K_{i\text{-MAO-B}}/K_{i\text{-MAO-A}}$ ratio) are reported in Table **2**. Inhibitory data of befloxatone have been also added for comparison.

All tested compounds showed higher anti MAO-A activity than anti B.

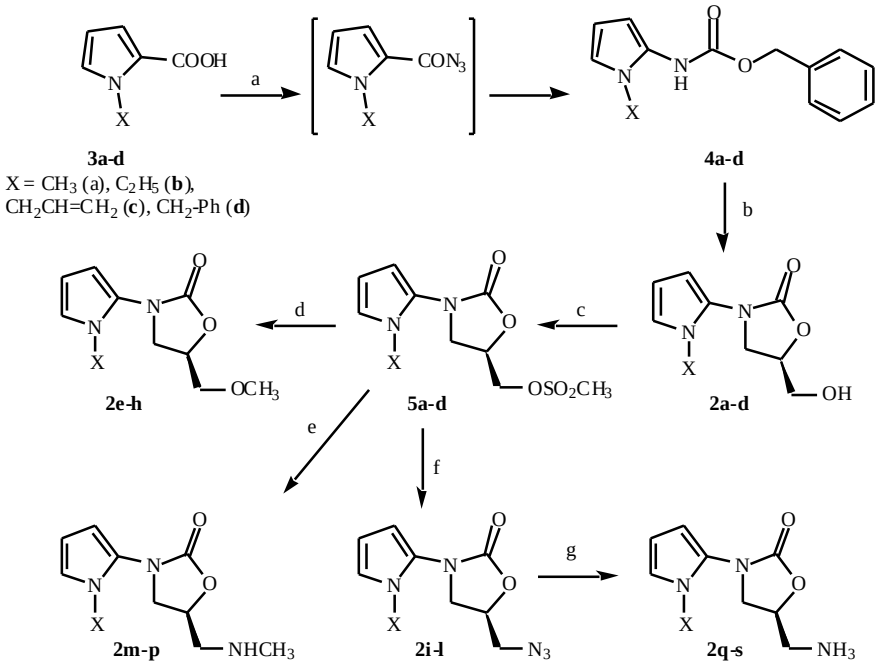
Moreover, all derivatives displayed a reversible mode of action, since dialysis for 24 h in a cold room against 0.1 M potassium phosphate buffer (pH 7.2) was able to restore 90-100% of the enzyme activity.

In the alcohol series (compounds **2a-d**), the MAO-A inhibitory activity depends on the size of the alkyl substituent at the pyrrole N1 position (N-methyl **2a** > N-ethyl **2b** > N-allyl **2c**), with the exception of the N-benzyl derivative **2d**, which is one of the most potent and A-selective ($K_{i\text{-MAO-A}}$ = 9 nM; $K_{i\text{-MAO-B}}$ = >100 μM; A-selectivity ratio >11,111) compound among all the synthesized 3-(1*H*-pyrrol-2-yl)-2-oxazolidinones.

The 5-methoxymethyl derivatives **2e-h** showed an anti MAO-A activity in the range of 50-0.020 μM, being that again the little size (methyl) of the pyrrole N1 substituent is the determinant for high inhibitory activity.

Among the azides **2i-l**, the 5-azidomethyl-3-(1-methyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (**2i**) is active at 4 nM as MAO-A inhibitor, whilst its MAO-B inhibitory concentration is 1000-fold higher. In this series, the introduction at the pyrrole N1 position of substituents bulkier than methyl led to 50- (**2j**), 87- (**2k**), and 7,500-fold (**2l**) less active compounds.

The 5-methylaminomethyl compounds (**2m-p**) show an inversion of the structure-activity relationships: an increase in the size of the N1-substituent produces more active derivatives (with the little exception of **2p**, that is just 3-times less potent than **2o**). Noteworthy, the 5-methylaminomethyl-3-(1-benzyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (**2p**) shows an interesting inhibitory activity also against the B isoform of the enzyme ($K_{i\text{-MAO-B}}$ = 0.5 μM).



a: $(PhO)_2PON_3$, Et_3N, $PhCH_2OH$, benzene, 80 °C. b: (1) *n*-BuLi, THF, N_2, -78 °C; (2) (*R*)-glycidyl butyrate, N_2, from –78 °C to rt. c: CH_3SO_2Cl, Et_3N, CH_2Cl_2. d: CH_3ONa, MeOH, N_2, rt. e: CH_3NH_2, MeOH, 60 °C. f: NaN_3, DMF, N_2, 65 °C. g: H_2, Pd/C, rt.

Scheme 1.

Table 1. Chemical and Physical Properties of Compounds 2,4,5.

compd	R	X	mp, °C	recrystall. solvent	yield %	rotat. power $[\alpha]_D^{25}$ ($CHCl_3$)
2a	OH	CH_3	125-127	C_6H_6	40	-0.58
2b	OH	CH_2CH_3	oil	-	35	-0.63
2c	OH	$CH_2CH=CH_2$	oil	-	39	-0.25
2d	OH	CH_2-Ph	140-141	C_6H_6/MeCN	47	-0.61
2e	OCH_3	CH_3	oil	-	40	-0.28
2f	OCH_3	CH_2CH_3	39-40	*n*-C_6H_{14}	49	-0.50
2g	OCH_3	$CH_2CH=CH_2$	oil	-	53	-0.18
2h	OCH_3	CH_2-Ph	oil	-	52	-0.24
2i	N_3	CH_3	oil	-	64	-1.26
2j	N_3	CH_2CH_3	69-70	*n*-C_6H_{14}	74	-1.16
2k	N_3	$CH_2CH=CH_2$	oil	-	61	-1.50
2l	N_3	CH_2-Ph	oil	-	66	-1.18
2m	$NHCH_3$	CH_3	oil	-	56	-0.20
2n	$NHCH_3$	CH_2CH_3	oil	-	48	-0.10
2o	$NHCH_3$	$CH_2CH=CH_2$	oil	-	43	-0.27
2p	$NHCH_3$	CH_2-Ph	oil	-	52	-0.80
2q	NH_2	CH_3	224-225	MeOH/EtOH	55	-0.30
2r	NH_2	CH_2CH_3	oil	-	57	-0.37
2s	NH_2	CH_2-Ph	206-207	MeCN	58	-0.35
4a		CH_3	oil	-	72	-
4b		CH_2CH_3	oil	-	62	-
4c		$CH_2CH=CH_2$	oil	-	74	-
4d		CH_2-Ph	oil	-	68	-
5a		CH_3	oil	-	63	-0.67
5b		CH_2CH_3	oil	-	82	-0.53
5c		$CH_2CH=CH_2$	oil	-	69	-0.49
5d		CH_2-Ph	84-85	C_6H_6	58	-0.36

The inhibitory trend of the described derivatives is restored in the 5-aminomethyl series (compounds **2q-s**), being that the N1-methyl derivative **2q** is the most active ($K_{i\text{-}MAOA}$ = 10 nM) and shows a high A-selectivity ratio (2,500).

CONCLUSIONS

A novel series of 5-substituted-3-(1-alkyl-1*H*-pyrrol-2-yl)-2-oxazolidinones (**2a-s**) have been described as potent and selective anti-MAO-A agents. In particular, **2d** ($K_{i\text{-}MAO\text{-}A}$ = 9 nM), **2i** ($K_{i\text{-}MAO\text{-}A}$ = 4 nM), and **2q** ($K_{i\text{-}MAO\text{-}A}$ = 10 nM) showed an inhibitory activity 42-, 95-, and 38-fold higher than that of toloxatone ($K_{i\text{-}MAO\text{-}A}$ = 380 nM), respectively. In comparison with befloxatone, **2d**, **2i**, and **2q** were slightly less active against MAO-A, but showed an increase in A-selectivity ratio of >126, 11, and 28 times respectively. Such results suggest that these compounds may be active as new promising antidepressant agents.

EXPERIMENTAL SECTION

Chemistry

Melting points were determined on a Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra

Table 2. Monoamine Oxidase Inhibitory Activity of Compounds 2a-s.[a].

compd	R	X	K_i, μM		A-selectivity
			MAO-A	MAO-B	
2a	OH	CH_3	0.087	3.6	41
2b	OH	CH_2CH_3	0.1	50	500
2c	OH	$CH_2CH=CH_2$	0.43	5	12
2d	OH	CH_2-Ph	0.009	>100	>11,111
2e	OCH_3	CH_3	0.020	1	50
2f	OCH_3	CH_2CH_3	0.6	5.9	10
2g	OCH_3	$CH_2CH=CH_2$	0.52	6.5	13
2h	OCH_3	CH_2-Ph	50	70	1.4
2i	N_3	CH_3	0.004	4	1,000
2j	N_3	CH_2CH_3	0.2	4	20
2k	N_3	$CH_2CH=CH_2$	0.35	5	14
2l	N_3	CH_2-Ph	30	77	2.6
2m	$NHCH_3$	CH_3	0.7	40	57
2n	$NHCH_3$	CH_2CH_3	0.36	20	57
2o	$NHCH_3$	$CH_2CH=CH_2$	0.04	5.5	138
2p	$NHCH_3$	CH_2-Ph	0.14	0.5	3.6
2q	NH_2	CH_3	0.01	25	2,500
2r	NH_2	CH_2CH_3	0.2	45	225
2s	NH_2	CH_2-Ph	50	80	1.6
toloxatone			0.38	15	40
befloxatone[b]			0.0025	0.22	88

[a]Data represent mean values of at least three separate experiments. [b]Reference 32.

(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 extraction 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 and +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.

General Procedure for the Synthesis of 1-alkyl-2-benzyloxycarbonylamino-1*H*-pyrroles 4a-d. Example: Synthesis of 1-ethyl-2-benzyloxycarbonylamino-1*H*-pyrrole (4b)

To a solution of 1-ethyl-1*H*-pyrrole-2-carboxylic acid (**3b**) (3.0 g, 23.9 mmol) in benzene (50 mL) triethylamine (3.7 mL, 26.3 mmol), diphenylphosphorylazide (7.7 mL, 35.8 mmol) and benzyl alcohol (3.0 mL, 28.7 mmol) were added. The resulting mixture was stirred at 80 °C overnight. After, the solution was evaporated and the residue was chromatographed over silica gel by eluting with ethyl acetate:chloroform 1:10 to provide a pure oily residue (**4b**). ^{1}H NMR ($CDCl_3$) δ 1.25-1.32 (t, 3H, CH_2CH_3), 3.73-3.77 (q, 2H, CH_2CH_3), 5.14 (s, 2H, OCH_2), 5.95 (s, NH exchangeable with D_2O), 6.04-6.07 (m, 2H, pyrrole β-proton), 6.53-6.54 (m, 1H, pyrrole α-proton), 7.22-7.32 (m, 5H, Ph).

General Procedure for the Synthesis of (*R*)-5-hydroxymethyl-3-(1-alkyl-1*H*-pyrrol-2-yl)-2-oxazolidinones (2a-d). Example: Synthesis of (*R*)-5-hydroxymethyl-3-(1-methyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (2a)

To a solution of 1-methyl-2-benzyloxycarbonylamino-1*H*-pyrrole (**4a**) (5.5 g, 18 mmol) in dry THF (50 mL) *n*-butyllithium (2.5 M in hexane, 10.8 ml, 27 mmol) was added dropwise, over a period of 5 min, under nitrogen atmosphere at –78 °C. The reaction mixture was stirred at –78 °C for 1h, then it was followed by addition of (*R*)-glycidyl butyrate (2.8 mL, 19.8 mmol). The resulting mixture was stirred initially at –78 °C for 1h, and then it was kept at room temperature overnight. After, the reaction was quenched by addition of saturated NH_4Cl solution (100 mL) and 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 by eluting with ethyl acetate to give the alcohol (**2a**) as a white solid that was recrystallized by benzene. ^{1}H NMR ($CDCl_3$) δ 2.73 (bs, 1H, OH exchangeable with D_2O), 3.54 (s, 3H, CH_3), 3.70-3.75 (m, 1H, NCH_2), 3.86-3.93 (m, 2H, C*H*$_2$OH), 3.97-4.06 (m, 1H, NCH_2), 4.75-4.78 (m, 1H, OCH), 6.06-6.13 (m, 2H, pyrrole β-proton), 6.55-6.58 (m, 1H, pyrrole α-proton).

General Procedure for the Synthesis of (*R*)-5-methanesulfonyloxymethyl-3-(1-alkyl-1*H*-pyrrol-2-yl)-2-oxazolidinones (5a-d). Example: Synthesis of (*R*)-5-methanesulfonyloxymethyl-3-(1-allyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (5c)

To a solution of (*R*)-5-hydroxymethyl-3-(1-allyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (**2c**) (2.1 g, 9.4 mmol) in dry dichloromethane (30 mL), triethylamine (2.6 mL, 18.8 mmol) and methanesulfonyl chloride (1.1 mL, 14 mmol) were added at 0 °C under N_2 atmosphere. The reaction mixture was stirred at 0 °C for 1h, 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 **5c** as pure oil, which was taken up for the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 2.98 (s, 3H, CH_3), 3.55-3.68 (m, 1H, C*H*$_2$CH=CH_2), 3.80-3.90 (m, 1H, C*H*$_2$CH=CH_2), 4.18-4.26 (m, 1H, NC*H*$_2$CHO), 4.31-4.38 (m, 3H, NC*H*$_2$CHO and CH_2OSO_2), 4.70-4.80 (m, 1H, NCH_2C*H*O), 4.85-4.95 (m, 1H, CH_2CH=C*H*$_2$), 5.05-5.12 (m, 1H, CH_2CH=C*H*$_2$), 5.75-5.88 (m, 1H, CH_2C*H*=CH_2), 6.00-6.03 (m, 2H, pyrrole β-proton), 6.48-6.49 (m, 1H, pyrrole α-proton).

General Procedure for the Synthesis of (*R*)-5-methoxymethyl-3-(1-alkyl-1*H*-pyrrol-2-yl)-2-oxazolidinones (2e-h). Example: Synthesis of (*R*)-5-methoxymethyl-3-(1-ethyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (2f)

To a solution of sodium metal (0.034 g, 1.48 gram atom) in methanol (5 mL), (*R*)-5-methanesulfonyloxymethyl-3-(1-ethyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (**5b**) (0.11 g, 0.37 mmol) in methanol (2 mL) was added, and the resulting mixture was stirred under N_2 atmosphere at room temperature overnight. The reaction was quenched with water and 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 purified by column chromatography (silica gel, ethyl acetate:chloroform 1:1) to give the methoxymethyl derivative (**2f**) as a pure oil. ^{1}H NMR ($CDCl_3$) δ 1.37-1.44 (t, 3H, CH_2C*H*$_3$), 3.47 (s, 3H, OCH_3), 3.58-3.69 (m, 1H, NCH_2), 3.72-3.93 (m, 3H, NC*H*$_2$ and CH_2O), 4.73-4.80 (m, 1H, OCH), 6.04-6.07 (m, 1H, pyrrole β-proton), 6.12-6.15 (m, 1H, pyrrole β-proton), 6.62-6.64 (m, 1H, pyrrole α-proton).

General Procedure for the Synthesis of (*R*)-5-azidomethyl-3-(1-alkyl-1*H*-pyrrol-2-yl)-2-oxazolidinones (2i-l). Example: Synthesis of (*R*)-5-azidomethyl-3-(1-benzyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (2l)

Sodium azide (1.4 g, 20.8 mmol) was added under N_2 atmosphere to a solution of (*R*)-5-methanesulfonyloxymethyl-3-(1-benzyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (**5d**) (1.5 g, 4.2 mmol) in dry *N*,*N*-dimethylformamide (DMF, 20 mL), and the resulting mixture was stirred at 65 °C overnight. The reaction mixture was allowed to cool to room temperature and worked up by addition of 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 by eluting with ethyl acetate:chloroform 1:5 to give the azide derivative (**2l**) as a pure oil. ^{1}H NMR ($CDCl_3$) δ 3.05-3.13 (m, 1H, NCH_2), 3.18-3.20 (m, 2H, CH_2N_3), 3.28-3.37 (m, 1H, NCH_2), 4.32-4.38 (m, 1H, OCH), 4.99 (s, 2H, C*H*$_2$Ph), 6.02-6.04 (m, 1H, pyrrole β-proton), 6.07-6.11 (m, 1H, pyrrole β-proton), 6.59-6.61 (m, 1H, pyrrole α-proton), 7.00-7.04 (m, 2H, H-2,6 benzene protons), 7.21-7.28 (m, 3H, H-3,4,5 benzene protons).

General Procedure for the Synthesis of (*S*)-5-aminomethyl-3-(1-alkyl-1*H*-pyrrol-2-yl)-2-oxazolidinones (2q-s). Example: Synthesis of (*S*)-5-aminomethyl-3-(1-benzyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (2s)

To a solution of (*R*)-5-azidomethyl-3-(1-benzyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (**2l**) (0.8 g, 2.7 mmol) in methanol (80 mL) placed in Parr apparatus palladium on 10% carbon was added, and the mixture was hydrogenated at 50 psi and 25 °C for 1h. At last, palladium was filtered and methanol was evaporated to afford an oily residue that was chromatographed over silica gel by eluting with chloroform:methanol 9:1, to provide the amine derivative (**2s**) as a solid product that was recrystallized by acetonitrile/ethanol. ^{1}H NMR (DMSO-d_6) δ 2.98-3.00 (m, 1H, NCH_2), 3.23-3.29 (m, 2H, C*H*$_2$NH$_2$), 3.51-3.59 (m, 1H, NCH_2), 4.72-4.76 (m, 1H, OCH), 5.01 (s, 2H, C*H*$_2$Ph), 6.01-6.06 (m, 2H, pyrrole β-proton), 6.73-6.75 (m, 1H, pyrrole α-proton), 7.07-7.11 (m, 2H, H-2,6 benzene protons), 7.24-7.35 (m, 3H, H-3,4,5 benzene protons), 8.19 (bs, 2H, CH_2N*H*$_2$ exchangeable with D_2O).

General Procedure for the Synthesis of (*S*)-5-methyl-aminomethyl-3-(1-alkyl-1*H*-pyrrol-2-yl)-2-oxazolidinones (2m-p). Example: Synthesis of (*S*)-5-methylaminomethyl-3-(1-methyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (2m)

Methylamine (2M solution in THF, 18.8 mL, 37.5 mmol) was added under N_2 atmosphere to a solution of (*R*)-5-methanesulfonyloxymethyl-3-(1-methyl-1*H*-pyrrol-2-yl)-2-oxazolidinone (**5a**) (2.1 g, 7.5 mmol) in methanol (20 mL), and the mixture was heated at 65 °C overnight. After, the solvent was evaporated, water (100 mL) was added to the residue and the product was extracted with ethyl acetate (3 x 50 mL). The residue obtained upon evaporation of solvent was chromatographed over silica gel by eluting with chloroform:methanol 9:1 to give the methylamino derivative (**2m**) as a pure oil. ^{1}H NMR ($CDCl_3$) δ 1.90 (bs, 1H, NH exchangeable with D_2O), 2.38 (s, 3H, NCH_3), 2.76-2.80 (m, 2H, CH_2NHCH_3), 3.40 (s, 3H, CH_2NHCH_3), 3.51-3.58 (m, 1H, NCH_2), 3.72-3.81 (m, 1H, NCH_2), 4.62-4.70 (m, 1H, OCH), 5.89-5.92 (m, 1H, pyrrole β-proton), 5.94-5.97 (m, 1H, pyrrole β-proton), 6.40-6.43 (m, 1H, pyrrole α-proton).

Mitochondria Preparation

Mitochondria were prepared according to Basford [42]. 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; medium F is made up of the medium A to which Ficoll is added to a final concentration of 8%. Calf or beef brains were removed from the animals 5-10 min after their death. The brains were immediately placed in cold Medium A and stored on ice to be transported to the laboratory. In cold room, at 5 °C, the cerebral hemispheres were removed from the brains and the meanings taken up with forceps. The gray matter is scraped from the cortices using a dull spatula. Two brains yielded about 100 g of wet tissue, which was homogenized in the ratio 2 mL medium A/g of wet tissue. The pH was buffered at 7.0 by adding some drops of TRIS-buffer 2M, 1 mg of ε-aminocaproic acid/g of tissue was added in order to inhibit proteases and then the mixture was stirred at 0-4 °C for 15 min. The suspension was 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 other 20 min. The residue R_1 was discarded and the supernatant S_1 centrifuged at 12,000 g for 15 min, to yield a crude mitochondria pellet R_2 and the supernatant S_2, which was discarded. The fraction R_2 was 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 was 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 mitochondrial 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.

Biochemical Assay

All chemicals were commercial reagents of analytical grade and were used without further purification. In all experiments, MAO activity of the beef brain mitochondria was determined by a sensitive fluorometric method according to Matsumoto *et al.* [43], using kynuramine as a substrate at four different final concentrations ranging from 5 μM to 0.1 μM. In all assays, the incubation mixtures contained: 0.1 mL of 0.25 M potassium phosphate buffer at pH 7.4, 30 μL of mitochondria (from an homogenate solution 6 mg/mL), and drug solutions. Drug derivatives were dissolved in dimethylsulfoxide (DMSO), and then added to the reaction mixture, at final concentrations ranging from 0 to 10^{-3} μM. The solutions were preincubated for 30 min at 38 °C before adding the substrate and then incubated for other 30 min at the same temperature. The inhibitory activities on both MAO A and B were separately determined after incubation of the mitochondrial fractions for 30 min at 38 °C in the presence of their specific inhibitors (1 μM L-deprenyl to estimate MAO A activity, and 1 μM clorgyline to assay the B isoform). Addition of perchloric acid ended the reaction. The samples were then centrifuged at 10,000 g for 5 min and the supernatant was added to 2.7 mL of NaOH 0.1 N. Fluorometric measurements were recorded with a Perkin-Elmer LS 50B Spectrofluorimeter, at λ_{exc} 317 nm and λ_{em} 393 nm. The protein concentration was determined according to Goa [44]. Dixon plots were used to estimate the inhibition constant value (K_i) of the inhibitors. Data represent the mean of three or more experiments each performed in duplicate.

ACKNOWLEDGEMENTS

This study was supported partially by MIUR funds PRIN 2003, by University of Rome "La Sapienza", by CNR Target Project on Biotechnology funds, and by Ministero della Salute (1% Fondo Sanitario Nazionale).

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Received: 17 September, 2004 Accepted: 11 October, 2004