3-(1*H*-Pyrrol-1-yl)-2-oxazolidinones as Reversible, Highly Potent, and Selective Inhibitors of Monoamine Oxidase Type A

Antonello Mai,[§] Marino Artico,*,[§] Monica Esposito,[§] Gianluca Sbardella,[‡] Silvio Massa,[#] Olivia Befani,[†] Paola Turini,[†] Valentina Giovannini,[†] and Bruno Mondovì*,[†]

Dipartimento di Studi Farmaceutici, Università degli Studi di Roma "La Sapienza", P.le A. Moro 5, 00185 Roma, Italy, Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, via Ponte Don Melillo, 84084 Fisciano (SA), Italy, Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, via Aldo Moro, 53100 Siena, Italy, and Dipartimento di Scienze Biochimiche "A. Rossi Fanelli" e Centro di Biologia Molecolare del CNR, Università degli Studi di Roma "La Sapienza", P.le A. Moro 5, 00185 Roma, Italy

*Received October 16, 2001*

Abstract: 3-(1*H*-Pyrrol-1-yl)-2-oxazolidinones **1a−i** have been synthesized as pyrrole analogues of toloxatone (Humoryl), an antidepressant agent belonging to the 3-phenyl-2-oxazolidinone class, and their monoamine oxidase (MAO) type A and B inhibitory activities have been evaluated. The majority of **1a−i** showed inhibitory activity against the A isoform of the enzyme higher than that exerted against the MAO-B, the sole exception being the (*S*)-5-aminomethylderivative **1d**. (*R*)-5-Methoxymethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinone **1b**, the most potent among test derivatives, was **78**-fold more potent than toloxatone.

Introduction. Monoamine oxidase (EC 1.4.3.4; MAO) is a FAD-containing enzyme[1,2] located in the outer membrane of mitochondria.[3] Two MAO isoforms, called MAO-A and MAO-B, have been distinguished till now, depending on different specificity for substrates, inhibitor selectivity, and tissue distribution.[4−6] The two forms catalyze oxidative deamination of endogenous monoamines in the brain and the peripheral tissues, MAO-A preferentially deaminating serotonin, epinephrine, and nor-epinephrine and MAO-B mainly acting on dopamine, β-phenylethylamine, and benzylamine.[7]

Due to the key role played by the two MAO forms in the metabolism of monoamine neurotransmitters, MAO inhibitors (MAOIs) can represent an useful tool for treatment of several neurological diseases. In particular, selective MAO-A inhibitors (e.g., clorgyline[8]) are used as antidepressant and antianxiety drugs and are claimed to protect neuronal cells against apoptosis,[9] and selective MAO-B inhibitors (e.g., L-deprenyl[10]) can be used in the treatment of Parkinson's disease either alone or in combination with L-DOPA.[11] In comparison with older, nonselective MAOIs such as phenelzine, tranyl-

Chart 1. Irreversible (I) and Reversible (R) MAO Inhibitors
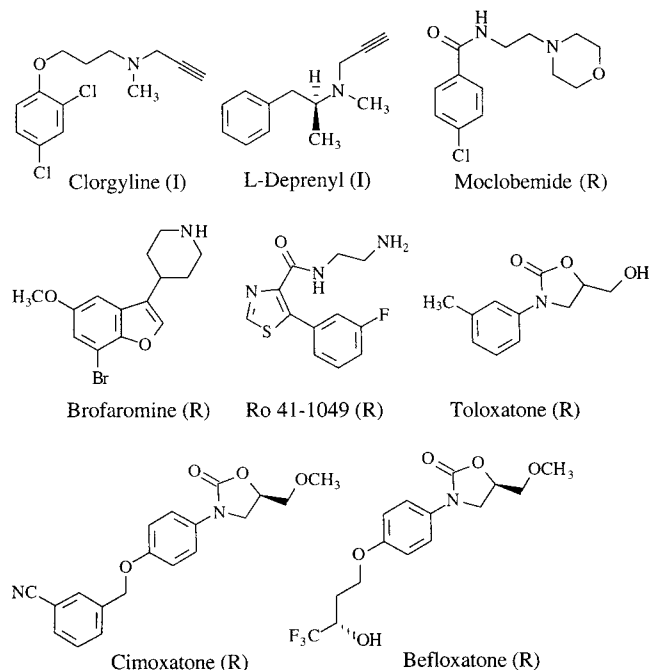
cypromine, and isocarboxazid, these MAOIs of second generation present a more favorable tolerability profile, and they have resulted in clinically effective agents in many neuropsychiatric and affective disorders. Unfortunately, they inhibit the MAO forms in an irreversible manner, forming stable covalent adducts between the enzyme and the drug or its reactive intermediate. This irreversible way of action produces many important limitations to the use of these compounds in therapy, such as the loss of selectivity at high dosage and the wide range of MAOI−drug and MAOI−food interactions, mainly with sympathomimetic drugs or tyramine-containing foods, that result in severe hypertensive reactions. Other undesired side effects connected with the use of irreversible MAOIs are central nervous effects (insomnia, irritability, agitation, hypomania, suppression of REM sleep), cardiovascular disfunctions (orthostatic hypotension), and sexual disturbances.[12,13]

The modern search in the anti-MAO field is now directed toward the discovery of new reversible, third-generation MAOIs that are as selective as possible to one isoform of the enzyme. Reported reversible MAOIs belong to the morpholino (moclobemide),[14] piperidino (brofaromine),[15] 2-aminoethylcarboxamide (Ro 41-1049),[16] and 2-oxazolidinone series[17] (toloxatone, cimoxatone, befloxatone) (Chart 1). Toloxatone (Humoryl), a new antidepressant agent marketed in France in 1985, is the prototype of 3-phenyl-2-oxazolidinones, a class of MAOIs highly active mainly against the A isoform of the enzyme.[18−21] Chemical modifications performed on toloxatone have led to cimoxatone and, more recently, to befloxatone, an anti-MAO agent active at nanomolar concentrations and more A-selective than toloxatone (Chart 1).[22,23] However, such chemical manipulations have regarded the substituent(s) located on the phenyl ring at the N3 position or at the C5 methylene group of

* To whom correspondence should be sent. M.A.: tel and fax, +39 06 446 2731; e-mail, marino.artico@uniroma1.it. B.M.: tel, +39 06 445 0298; fax, +39 06 444 0062; e-mail, bruno.mondovi@uniroma1.it.

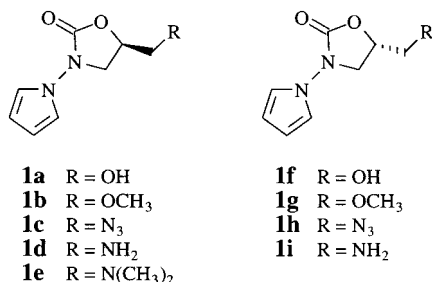§ Dipartimento di Studi Farmaceutici, Università degli Studi di Roma "La Sapienza".

‡ Università degli Studi di Salerno.

Università degli Studi di Siena.

† Dipartimento di Scienze Biochimiche "A. Rossi Fanelli" e Centro di Biologia Molecolare del CNR, Università degli Studi di Roma "La Sapienza".

the 2-oxazolidinone ring, while little or no attention has been devoted to the replacement of phenyl with heteroaromatic rings.[24]

Pursuing our searches on synthesis and biological evaluation of pyrrole-containing compounds active on central nervous system,[25–28] we planned the preparation of 3-(1*H*-pyrrol-1-yl)-2-oxazolidinones **1a**−**i** to be tested as new anti-MAO agents. Derivatives **1a**−**i** are char-

1a	R = OH	**1f**	R = OH
1b	R = OCH$_3$	**1g**	R = OCH$_3$
1c	R = N$_3$	**1h**	R = N$_3$
1d	R = NH$_2$	**1i**	R = NH$_2$
1e	R = N(CH$_3$)$_2$		

acterized by the pyrrole moiety linked to the 2-oxazolidinone ring through a N−N linkage, and bear different substituents (hydroxy, methoxy, azido, amino, and dimethylamino) at the C5-methyl of oxazolidinone portion. Due to the presence of a chiral center at the C5 position of oxazolidinone moiety, both the enantiomerically pure (*R* and *S*) series of compounds were synthesized and tested.

Chemistry. 1-(Phenylmethoxycarbonylamino)-1*H*-pyrrole **2**[29] 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*-butyllithium in hexanes at −78 °C, the lithiated intermediate reacted with enantiomerically pure glycidyl butyrate to furnish directly, after spontaneous hydrolysis of the butyrate function, the (*R*)- and (*S*)-5-hydroxymethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinones **1a** and **1f**, depending on the *R*- or *S*-glycidyl butyrate used, respectively. The alcohols **1a**,**f** were converted into the corresponding methanesulfonates **3a**,**b** with methanesulfonyl chloride and triethylamine, and these compounds were subjected to nucleophilic displacement by sodium methoxide and sodium azide to give (*R*)- and (*S*)-5-methoxymethyl and

(*R*)- and (*S*)-5-azidomethyl derivatives **1b**,**g** and **1c**,**h**, respectively. 5-Aminomethyl- and 5-dimethylaminomethyl-3-(1*H*-pyrrol-1-yl)-2-oxazolidinones **1d**,**i** and **1e** were obtained by catalytic reduction of the appropriate 5-azidomethyl counterparts **1c**,**h** with hydrogen (aminomethyl) or with hydrogen and formaldehyde in excess (dimethylaminomethyl compounds). A sample of toloxatone was synthesized and used as reference drug in biological tests of derivatives **1a**−**i**.

Results and Discussion. 5-Substituted-3-(1*H*-pyrrol-1-yl)-2-oxazolidinones **1a**−**i** were evaluated for their ability to inhibit MAO-A and MAO-B, in comparison with toloxatone as reference drug. Inhibitory data of befloxatone, a new toloxatone analogue actually in phase III clinical trials, were also reported.[22,23] Bovine brain mitochondria were used as the enzyme source and were isolated according to the Basford method.[30] Activities of MAO-A and MAO-B were determined by a fluorimetric method with kynuramine as substrate.[31] The K_i values against the two isoenzymatic MAO forms and the A-selectivity (expressed as $K_{i\ MAO–B}/K_{i\ MAO–A}$ ratio) are summarized in Table 1.

Test compounds have been divided into two groups (A and B), according to their enantiomeric structure (Table 1). All compounds except **1d** showed inhibitory activity against the A isoform of MAO enzyme higher than that exerted against the MAO-B. Furthermore, 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.
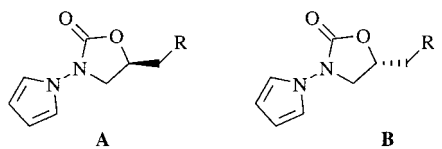
Among compounds belonging to the A group, derivatives **1a**−**c** were the most active with concentration values of MAO-A inhibitory activity in the nanomolar range. In particular, in our experiments we found that (*R*/*S*)-toloxatone and the related pyrrole analogue (racemic mixture of **1a** and **1f**) were equipotent as MAO-A inhibitors, the latter being 6-fold less MAO-A selective than the former. Furthermore, the assays performed on the *R* (**1a**) and *S* (**1f**) enantiomers showed **1a** to possess the best activity and selectivity.

Replacement of OH with other hydrophilic groups (amino, azido, and dimethylamino) gave derivatives

Scheme 1[a]
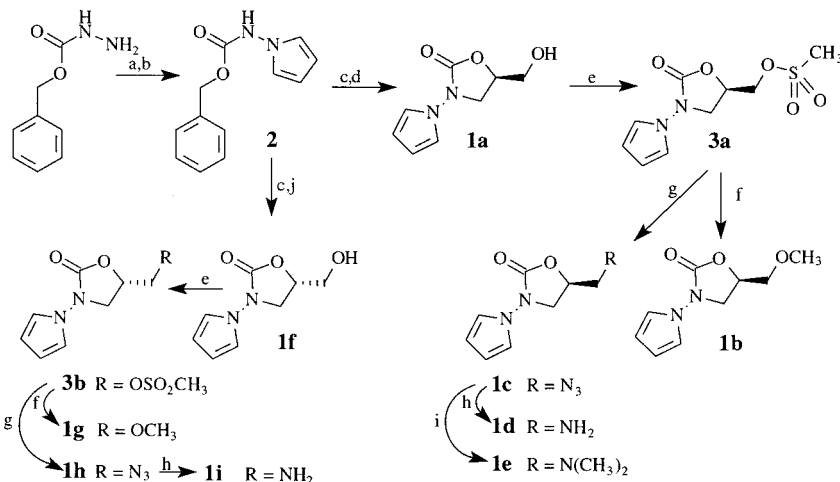
[a] a: 2,5-Dimethoxytetrahydrofuran, AcOH, EtOH, Δ. b: AcOH, Δ. c: 2.5 M *n*-BuLi in hexanes, THF, −78 °C. d: (1) *R*-Glycidyl butyrate, (2) NH$_4$Cl, rt. e: CH$_3$SO$_2$Cl, Et$_3$N, CH$_2$Cl$_2$, rt. f: CH$_3$ONa, CH$_3$OH, rt. g: NaN$_3$, DMF, 70 °C. h: H$_2$, Pd/C, rt. i: H$_2$, CH$_2$O, Pd/C, rt. j: (1) *S*-Glycidyl butyrate, (2) NH$_4$Cl, rt.

Table 1. Monoamine Oxidase Inhibitory Activity of Compounds **1**[a]

compd	formula	(R or S)	R	K_i values (μM)		A selectivity
				MAO-A	MAO-B	
1a	A	(R)	OH	0.09	9	100
1b	A	(R)	OCH$_3$	0.0049	50	10,200
1c	A	(R)	N$_3$	0.2	200	1,000
1d	A	(S)	NH$_2$	53	4.4	0.08
1e	A	(S)	N(CH$_3$)$_2$	40	140	3.5
1f	B	(S)	OH	1.2	2	1.7
1g	B	(S)	OCH$_3$	1	23	23
1h	B	(S)	N$_3$	2.5	24	9.6
1i	B	(R)	NH$_2$	1.2	34	28
1a, **1f**		(R/S)	OH	0.27	2	7
(R/S) toloxatone				0.38	15	39.5
(R,R) befloxatone[b]				0.0025	0.22	88

[a] Data represent mean values of at least three separate experiments. [b] Reference 22.

1c−**e** less potent and sometimes less selective than **1a**. On the contrary, O-methylation of this compound afforded (R)-5-methoxymethyl-3-(1H-pyrrol-1-yl)-2-oxazolidinone (**1b**), an MAO-A inhibitor endowed with very high potency and A selectivity. In fact, compound **1b** ($K_{i\ MAO-A}$ = 4.9 nM) is equipotent to befloxatone (R,R form) ($K_{i\ MAO-A}$ = 2.5 nM), but it is characterized by very high selectivity toward the MAO-A isoenzyme (A selectivity = 10 200, about 116-fold greater than that of befloxatone).

The high anti-MAO-A activity displayed by derivative **1b** leads us to argue that the biological activity strongly correlates with the lipophilic nature of pyrrolyloxazolidinone C5 side chain, and this assumption is confirmed by the low activity of derivatives **1a**,**c**−**e** replacing methoxyl with hydrophilic groups. To confirm this hypothesis and to explore the nature of interactions of ligands at the receptor site, we have in mind as a future approach the synthesis of various **1b** analogues with different sized alkyloxymethyl chains at position 5 of the oxazolidinone ring.

A preliminary SAR evaluation showed compounds of group B (**1f**−**i**) to be less potent than the group A counterparts (**1a**−**d**), with the sole exception of (R)-5-aminomethyl derivative **1i**. Interestingly, the 3-(1H-pyrrol-1yl)-2-oxazolidinones **1d** and **1i** containing the aminomethyl side chain showed different biochemical behaviors toward MAO-A and MAO-B depending on the stereochemistry of the examined compound. Contrary to other pyrrolyloxazolidinones of A series, **1d** (S enantiomer) showed inhibitory activity against the MAO-B isoform (A selectivity = 0.08), whereas **1i** (R enantiomer) was more A-selective (A selectivity = 28) according to the trend of both the A and B series.

In conclusion, we have designed novel toloxatone-like oxazolidinones characterized by the presence of pyrrole ring. Among test derivatives we selected **1b** as a potent anti-MAO agent showing a very high selectivity toward the MAO-A isoform. Such result can be regarded as a very important goal in the search for novel selective anti-MAO agents to be used as drugs.

Supporting Information Available: Chemical and physical data for compounds **1a**−**i** are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Mondovì, B. *Structure and function of amine oxidases;* CRC Press: Boca Raton, FL, 1985.
(2) Kearney, E. B.; Salach, J. I.; Walker, W. H.; Seng, R. L.; Kenney, W.; Zeszotek, E.; Singer, T. The covalently bound flavin of hepatic monoamine oxidase. Isolation and sequence of a flavin peptide and evidence of a binding at the 8-a position. *Eur. J. Biochem.* **1971**, *24*, 321−327.
(3) Greenawalt, J. W. Localization of monoamine oxidase in rat liver mitochondria. *Adv. Biochem. Psycopharmacol.* **1972**, *5*, 207−226.
(4) Geha, R. M.; Rebrin, I.; Chen, K.; Shih, J. C. Substrate and inhibitor specificities for human monoamine oxidase A and B are influenced by a single amino acid. *J. Biol. Chem.* **2001**, *276*, 9877−9882.
(5) Kalgutkar, A. S.; Castagnoli, N., Jr.; Testa, B. Selective inhibitors of monoamine oxidase (MAO-A and MAO-B) as probes of its catalytic site and mechanism. *Med. Res. Rev.* **1995**, *15*, 325−388.
(6) Westlund, R. N.; Denney, R. M.; Kochersperger, L. M.; Rose, R. M.; Abell, C. W.; Distinct monoamine oxidase A and B populations in primate brain. *Science* **1985**, *230*, 181−183.
(7) Grimsby, J.; Lan, N. C.; Neve, R.; Chen, K.; Shih, J. C. Tissue distribution of human monoamine oxidase A and B mRNA. *J. Neurochem.* **1990**, *55*, 1166−1169.
(8) Johnston, J. P. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmacol.* **1968**, *17*, 1285−1297.
(9) Malorni, W.; Gianmmorioli, A. M.; Matarrese, P.; Pietrangeli, P.; Agostinelli, E.; Ciaccio, A.; Grassili, E.; Mondovì, B. Protection against apoptosis by monoamine oxidase A inhibitors. *FEBS Lett.* **1998**, *426*, 155−159.
(10) Knoll, J.; Ecsery, Z.; Kelemen, K.; Nievel, J.; Knoll, B. Phenyl-isopropylmethyl-propinylamine (E-250), a new psychic energizer. *Arch. Int. Pharmachodyn. Ther.* **1965**, *155*, 154−164.
(11) Tetrud, J. W.; Langston, J. W. The effect of deprenyl (selegiline) on the natural history of Parkinson's disease. *Science* **1989**, *245*, 519−522.
(12) Cesura, A. M.; Pletscher, A. The new generation of monoamine oxidase inhibitors. *Prog. Drug Res.* **1992**, *38*, 171−257.
(13) Strolin-Benedetti, M.; Dostert, P. Monoamine oxidase: from physiology and pathophysiology to the design and clinical application of reversible inhibitors. *Adv. Drug Res.* **1992**, *23*, 65−125.
(14) Da Prada, M.; Kettler, R.; Keller, H. H.; Burkard, W. P.; Muggli-Maniglio, D.; Haefely, W. E. Neurochemical profile of moclobemide, a short-acting and reversible inhibitor of monoamine oxidase type A. *J. Pharmacol. Exp. Ther.* **1989**, *248*, 400−414.
(15) Waldmeier, P. C.; Felner, A. E.; Tipton, K. F. The monoamine oxidase inhibiting properties of CGP 11305 A. *Eur. J. Pharmacol.* **1983**, *94*, 73−83.
(16) Da Prada, M.; Kettler, R.; Keller, H. H.; Cesura, A. M.; Richards, J. G.; Saura Marti, J.; Muggli-Maniglio, D.; Wyss, P.-C.; Kyburz, E.; Imhof, R. From moclobemide to Ro 19-6327 and Ro 41-1049: the development of a new class of reversible, selective MAO-A and MAO-B inhibitors. *J. Neural Transm. [Suppl.]* **1990**, *29*, 279−292.
(17) Dostert, P.; Strolin-Benedetti, M.; Tipton, K. F. Interactions of monoamine oxidase with substrates and inhibitors. *Med. Res. Rev.* **1989**, *9*, 45−89.
(18) Kan, J.-P.; Pujol, J.-F.; Malnoe, A.; Strolin-Benedetti, M.; Gouret, C.; Raynaud, G. Effects of a new antidepressant (3-methyl)-3-phenyl-5-hydroxymethyl-2-oxazolidinone (toloxatone) upon 5-hydroxytryptamine pathways. *Eur. J. Med. Chem. − Chim. Ther.* **1977**, *12*, 13−16.
(19) Moureau, F.; Wouters, J.; Vercauteren, D. P.; Collin, S.; Evrard, G.; Durant, F.; Ducrey, F.; Koenig, J. J.; Jarreau, F. X. A reversible monoamine oxidase inhibitor, toloxatone: structural and electronic properties. *Eur. J. Med. Chem.* **1992**, *27*, 939−948.
(20) Moureau, F.; Wouters, J.; Vercauteren, D. P.; Collin, S.; Evrard, G.; Durant, F.; Ducrey, F.; Koenig, J. J.; Jarreau, F. X. A reversible monoamine oxidase inhibitor, Toloxatone: spectrophotometric and molecular orbital studies of the interaction with flavin adenine dinucleotide (FAD). *Eur. J. Med. Chem.* **1994**, *29*, 269−277.

(21) Moureau, F.; Wouters, J.; Depas, M.; Vercauteren, D. P.; Durant, F.; Ducrey, F.; Koenig, J. J.; Jarreau, F. X. A reversible monoamine oxidase inhibitor, Toloxatone: comparison of its physicochemical properties with those of other inhibitors including Brofaromine, Harmine, R40519 and Moclobemide. *Eur. J. Med. Chem.* **1995**, *30*, 823−838.

(22) Rabasseda, X.; Sorbera, L. A.; Castaner, J. Befloxatone. *Drugs Future* **1999**, *24*, 1057−1067.

(23) Wouters, J.; Moureau, F.; Evrard, G.; Koenig, J. J.; Jegham, S.; George, P.; Durant, F. A reversible monoamine oxidase A inhibitor, Befloxatone: structural approach of its mechanism of action. *Bioorg. Med. Chem.* **1999**, *7*, 1683−1693.

(24) Dostert, P.; Douzon, C.; Bourgery, G.; Gouret, C.; Mocquet, G.; Coston, J. A. 3-Aryl-2-oxazolidinones (Delalande S. A., Fr.). Ger. Offen. DE 2708236, 1977.

(25) Massa, S.; Mai, A. Corelli, F. Synthesis of new tetracyclic system related to aptazapine (CGS 7525A) by one-pot double annelation. *Tetrahedron Lett.* **1988**, *29*, 6471−6474.

(26) Massa, S.; Mai, A.; Artico, M.; Corelli, F.; Botta, M. Synthesis of 3b,4,6,7-tetrahydro-5H,9H-pyrazino[2,1-c]pyrrolo[1,2-a][1,4]-benzodiazepine, a valuable precursor of potential central nervous system agents. *Tetrahedron* **1989**, *45*, 2763−2772.

(27) Massa, S.; Artico, M.; Mai, A.; Corelli, F.; Botta, M.; Tafi, A.; Pantaleoni, G. C.; Giorgi, R.; Coppolino, M. F.; Cagnotto, A.; Skorupska, M. Pyrrolobenzodiazepines and related systems. 2. Synthesis and biological properties of isonoraptazepine derivatives. *J. Med. Chem.* **1992**, *35*, 4533−4541.

(28) Mai, A.; Di Santo, R.; Massa, S.; Artico, M.; Pantaleoni, G. C.; Giorgi, R.; Coppolino, M. F.; Barracchini, A. Pyrrolobenzodiazepines with antinociceptive activity: synthesis and pharmacological activities. *Eur. J. Med. Chem.* **1995**, *30*, 593−601.

(29) Flitsch, W.; Kramer, U.; Zimmermann, H. Cyclische Verbindungen mit Heterobruckenatomen, V. Zur Chemie der 1-Aminopyrrole. *Chem. Ber.* **1969**, *102*, 3268−3276.

(30) Stahl, W. L.; Smith, J. C.; Napolitano, L. M.; Basford, R. E. Brain mitochondria. I. Isolation of bovine brain mitochondria. *J. Cell Biol.* **1963**, *19*, 293−307.

(31) Matsumoto, T.; Suzuki, O.; Furuta, T.; Asai, M.; Kurokawa, Y.; Nimura, Y.; Katsumata, Y.; Takahashi, I. A sensitive fluorimetric assay for serum monoamino oxidase with kynuramine as substrate. *Clin. Biochem.* **1985**, *18*, 126−129. Briefly, compounds **1a−i** and toloxatone were dissolved in DMSO, preincubated for 30 min before adding kynuramine, and then incubated for 30 min more. To study the inhibitory activities against MAO-A and MAO-B, the mitochondrial fractions were preincubated for 30 min at 38 °C with the proper inhibitor (1 μM L-deprenyl to estimate the MAO-A activity, and 1 μM clorgyline to assay MAO-B), and then the samples were processed as described above.