Eur J Med Chem (1994) 29, 967–973
© Elsevier, Paris

Short communication

3-(Methyleneaminoxy)methylpiperidine derivatives as uptake inhibitors of biogenic amines in the brain synaptosomal fraction *

A Balsamo[1], A Lapucci[1], A Lucacchini[2], M Macchia[1], C Martini[2], C Nardini[1], S Nencetti[1]

[1]*Dipartimento di Scienze Farmaceutiche, Università di Pisa;*
[2]*Istituto Policattedra di Discipline Biologiche, Università di Pisa, 56126 Pisa, Italy*

(Received 13 January 1994; accepted 12 August 1994)

Summary — A series of 3-(methyleneaminoxy)methylpiperidines (**5a–h**) and their corresponding *N*-methyl derivatives (**6a–h**) with a variety of substituents on the imino carbon were synthesized and tested for their potential antidepressant properties; their capacity to inhibit the re-uptake of biogenic amines (NA, 5-HT and DA) in rabbit brain synaptosomal fractions was also evaluated. The biological results obtained for the piperidine derivatives **5a–h** and **6a–h** and viloxazine **1**, the reference drug, on the 3 re-uptake systems revealed that compounds **5** and **6** are generally able to inhibit biogenic amine uptake. The IC_{50} values for **5** and **6** were often lower than that of viloxazine **1**, in particular for the serotonin- and/or dopamine-uptake systems. A higher activity was found for compounds substituted with at least one phenyl ring on the imino carbon with respect to completely aliphatic systems, and for *N*-unsubstituted compounds with respect to *N*-methyl-substituted compounds.

3-(methyleneaminoxy)methylpiperidine derivative / NA uptake inhibitor / 5-HT uptake inhibitor / DA uptake inhibitor / antidepressant drug

Introduction

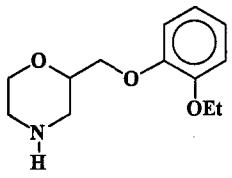
Depression is a central nervous system (CNS) disease believed to be associated with a perturbation of central monoamine transmission. Most of the drugs employed for clinical purposes in the treatment of depression are either tricyclic antidepressants or monoamineoxidase inhibitors (MAO-I). These drugs induce a potentiation of the monoaminergic transmission obtained by an increase in the intersynaptic concentration of neurotransmittors, which, for tricyclic antidepressants, is due to a more or less selective inhibition of the re-uptake systems of the mediators themselves. For MAO-I, this is due to a slowing down of their catabolic processes through the inhibition of monoaminoxidases [2].

The tricyclic drugs possess good antidepressant activity, but those available at present have substantial undesirable secondary effects [3], partly due to the lack of specificity of their pharmacological action [4]. As regards MAO-I, their use is limited by the development of cardiovascular effects [3] and by the risk of serious hypertensive crises [3, 5] as a result of an interaction with sympathomimetic amines. For both these types of antidepressants, the therapeutic response to administration is observed after about 2 weeks' latency [6].

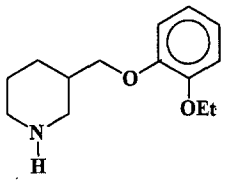
As a result, research in the field of antidepressant drugs has received increasing attention in recent years. This research is aimed at developing new compounds that possess a different chemical structure and pharmacological profile from the antidepressants in use at present, together with more limited side effects, faster pharmacological action, and lower risks in the case of overdosing [4, 7].

Viloxazine **1** has been the leading compound for targeting new molecules with a potential antidepressant activity belonging to a different class of non-tricyclic antidepressants with an aryloxyalkylaminic structure [8–12]. Even if these drugs do not exhibit a very high degree of activity and show, in some cases, a certain sympathomimetic activity, they are devoid of some of the secondary effects of imipramine-like and MAO-I drugs [3]. For these drugs too, the mechanism of action can be traced to a more or less selective interaction with biogenic monoamine uptake mechanisms which, for the most interesting drugs from an application point of view, appears to concern mainly the serotoninergic system [13].

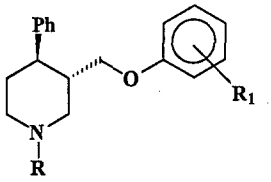
*Part of this work has been reported previously [1].



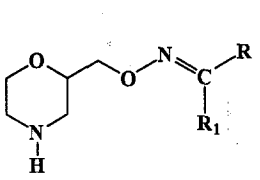
1, Viloxazine



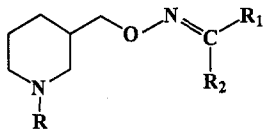
2



3a, R=Me, R_1=*p*-OMe Femoxetine
3b, R=H, R_1=3,4-O-CH_2-O- Paroxetine



4



5, R = H
6, R = Me

Our research in the field of antidepressants has been concentrated for some time on the study of the effects produced by various structural modifications of the viloxazine molecule **1** on its pharmacological properties.

A previous paper [14] reported that the substitution of the morpholinic ring of viloxazine **1** with a piperidinic ring leads to compound **2**, which possesses an antidepressant activity together with a profile of the inhibitory properties of the various monoamine-uptake systems comparable with that of viloxazine itself **1**. Furthermore femoxetine **3a** and paroxetine **3b** present a piperidinic ring in their structure, even if it is substituted with a phenyl group on the ring. Unlike viloxazine **1**, which is not particulary specific [2], these compounds appear to be selective for the serotonin-uptake system [2, 4, 13, 15].

A subsequent study [16] aimed to verify the possibility of the existence of a bioisosterism between the $ArOCH_2$ group and the (methyleneaminoxy)methyl moiety (MAOMM) in the field of antidepressant drugs. Morpholinic derivatives of type **4** were synthesized and described as analogues of viloxazine **1** with an MAOMM in the place of the $ArOCH_2$ group of viloxazine **1**, *ie* the *o*-ethoxyphenoxymethylic group. Some compounds of type **4** were tested *in vivo* for their antagonism to reserpine-induced hypothermy in the mouse and were found to possess a pharmacological profile similar to that of the model compound **1**.

Compounds of types **5** and **6** include both the piperidinic ring (which is unsubstituted on the nitrogen of compound **2** and paroxetine **3b**, or *N*-methyl-substituted in femoxetine **3a**) and an MAOMM from morpholinic derivatives of type **4**. The combination of these different molecular portions in single structures like those of **5** and **6** might lead to compounds which interact with biogenic amine-uptake systems like compounds **1**–**4** and also possess the selectivity for the serotonin-uptake system, which is typical of the more interesting antidepressants, such as **3a** and **3b**.

This paper describes the synthesis of a series of 3-(methyleneaminoxy)methylpiperidines of types **5** and **6** (see table I), together with the results obtained for these compounds in screening tests for their potential antidepressant properties, based on their capacity to inhibit the re-uptake of biogenic amines in rabbit brain synaptosomal fractions. The choice of the substituents R_1 and R_2 of compounds **5** and **6** was made in such a way as to have various combinations of the possible substituents on the iminic carbon, such as a hydrogen atom and aliphatic, cycloaliphatic, aryl and heteroaromatic groups.

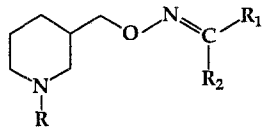
Chemistry

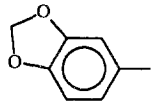
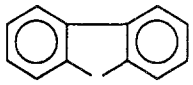
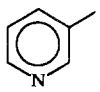
The general procedure for the synthesis of 3-(methyleneaminoxy)methylpiperidines **5a–h** and **6a–h** is described in scheme 1. The 3-chloromethylpiperidine **9** and the 3-chloromethyl-1-methylpiperidine **10** were obtained by treatment of the corresponding 3-hydroxymethylpiperidine **7** and **8**, respectively, with thionyl chloride and anhydrous hydrogen chloride [17].

Reaction of **9** and **10** with the appropriate oximes **11** and potassium hydroxide in DMSO or *t*-BuOH in the presence of 18-crown-6, led to the corresponding crude 3-(methyleneaminoxy)methylpiperidines **5** and **6**, which were chromatographed using a silica-gel column and then transformed into the corresponding oxalate salts.

The structures of compounds **5** and **6** were confirmed by their spectral data. In particular, the chemical shift differences of the signal of the protons of the methylenic group linked to the piperidine ring for the 3-hydroxymethyl derivatives **7**, **8**, the 3-chloromethyl derivatives **9**, **10** and the oxime ethers **5**, **6** are in accordance with the different paramagnetic effects of the hydroxylic or amino ethereal oxygen of **7**, **8** and **5**, **6**, respectively, or the chlorine atom of **9**, **10** linked to the same methylenic group. An upfield shift of the signal was observed on passing from **7**, **8** to **9**, **10** and

Table I. Chemical data of 3-(methyleneaminoxy)methylpiperidines **5a–g** and 1-methyl-3-(methyleneaminoxy)methylpiperidines **6a–g**.

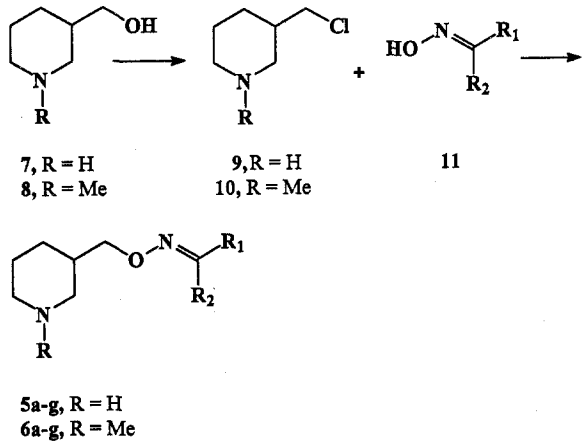


compd	*R*	R_1	R_2	*mp* °C	*recrystn solvent*[a]	*% yied*[b]	*formula*[c]
5a.$H_2C_2O_4$	H	*i*-Pr	*i*-Pr	102-105	B	23	$C_{15}H_{28}N_2O_5$
6a·$H_2C_2O_4$	Me	*i*-Pr	*i*-Pr	132-140	A	43	$C_{16}H_{30}N_2O_5$
5b.$H_2C_2O_4$	H			152-155	A	27	$C_{15}H_{24}N_2O_5$
6b·$H_2C_2O_4$	Me			172-178	A	66	$C_{16}H_{26}N_2O_5$
5c.$H_2C_2O_4$	H	Ph	H	110-114	A	31	$C_{15}H_{20}N_2O_5$
6c·$H_2C_2O_4$	Me	Ph	H	148-150	A	66	$C_{16}H_{22}N_2O_5$
5d.·$H_2C_2O_4$	H		H	113-120	A	62	$C_{16}H_{20}N_2O_7$
6d·$H_2C_2O_4$	Me			212-215	A	27	$C_{17}H_{22}N_2O_7$
5e·$H_2C_2O_4$	H	Ph	Me	118-123	A	42	$C_{16}H_{22}N_2O_5$
6e·$H_2C_2O_4$	Me	Ph	Me	152-154	A	64	$C_{17}H_{24}N_2O_5$
5f·$H_2C_2O_4$	H	Ph	Ph	158-163	A	57	$C_{21}H_{24}N_2O_5$
6f·$H_2C_2O_4$	Me	Ph	Ph	150-151	A	51	$C_{22}H_{26}N_2O_5$
5g·$H_2C_2O_4$	H			162-164	A	52	$C_{21}H_{22}N_2O_5$
6g·$H_2C_2O_4$	Me			178-179	A	75	$C_{22}H_{24}N_2O_5$
5h·$H_2C_2O_4$	H		H	125-127	B	30	$C_{14}H_{19}N_3O_5$
6h·$H_2C_2O_4$	Me			149-150	A	24	$C_{15}H_{21}N_3O_5$

[a]A = *i*-PrOH, B = MeOH/Et_2O; [b]no effort was made to optimize yields; [c]products purified by column chromatography on silica gel: toluene/AcOEt/MeOH 7:3:1.

a downfield shift of the signal on passing from **9**, **10** to **5**, **6**.

The geometry around the double bond of the methyleneaminoxy group of compounds **5**, **6**, in the cases where a *cis–trans* isomerism is possible (**c–e**, **h**), was deduced on the basis of the configuration of the starting oximes **11c–e**, **h**, bearing in mind that the latter have been proved to be configurationally stable under the reaction conditions that lead from **9**, **10** to **5**, **6**, respectively.



Scheme 1.

Biochemistry

Compounds **5**, **6** were submitted to *in vitro* screening in order to evaluate their ability to inhibit the re-uptake of the biogenic amines norepinephrine (NE), serotonin (5-HT) and dopamine (DA) in synaptosomal fractions from the rabbit occipital and frontal cerebral cortex and the striatum nucleus, respectively. [^{3}H]NE, [^{3}H]DA, and [^{3}H]5-HT were used as specific tritiated ligands. The results obtained for the compounds tested, together with those obtained in the same tests for the reference drug, viloxazine **1**, are shown in table II.

[^{3}H]NE uptake inhibition

All the compounds examined (**5** and **6**), with the exception of **5a**, **h** and **6e**, proved to be capable of inhibiting the uptake of NE. The greatest affinity was exhibited by derivatives unsubstituted on the nitrogen, **5c**, **d**, **f**, and by the *N*-methyl-substituted derivatives **6c**, **f**. In particular, compound **5f** revealed a greater capacity to inhibit NE re-uptake than the reference drug **1**, with an IC_{50} value of 0.10, compared with 0.22.

[^{3}H]5-HT uptake inhibition

The results obtained for compounds **5** and **6** on the serotonin uptake system (see table II) indicate that most of the compounds examined exhibit a good inhibition capacity. The compounds unsubstituted on the nitrogen are those which exhibit the lowest IC_{50} values, which, in the cases of **5c** and **5g**, are more than one order of magnitude lower than that of the reference drug **1** (0.65 and 1.10, respectively, compared with 20.4). Among the *N*-methyl-substituted piperidinic derivatives, the greatest affinity was shown by compound **6g**, with an IC_{50} value almost one order of magnitude lower than that of **1** (2.30 compared with 20.4).

[^{3}H]DA uptake inhibition

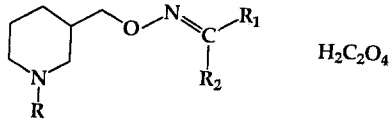
The IC_{50} values shown in table II indicate that most of the compounds **5** and **6** exhibit a good capacity to inhibit DA uptake in striatal synaptosomal fractions. Both the compounds unsubstituted on the nitrogen, **5c–f**, and the *N*-methyl-substituted derivatives, **6b**, **f**, **h**, exhibit a greater capacity to inhibit DA uptake than the reference drug, viloxazine **1**. Furthermore for this uptake system, the compound unsubstituted on the nitrogen, **5f**, exhibited the lowest IC_{50} value (0.15 μM), which is considerably lower than the findings for viloxazine **1** (38.2 μM). The derivatives **5a**, **b**, **h** and **6e**, **g** proved to be devoid of any capacity to inhibit DA uptake.

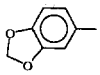
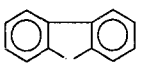
Discussion

The data shown in table II indicate that all the piperidinic derivatives, both *N*-unsubstituted **5** and *N*-methyl-substituted **6**, interact significantly with at least one of the biogenic amine (NE, 5-HT, DA) uptake systems examined. In general, the compounds that contain at least one aromatic nucleus in their structure are more active than those that are completely aliphatic, even if the norbornilidene derivative **6b** possesses a lower IC_{50} value for the DA system than that of viloxazine **1**; this value is comparable to that of the aryl-substituted compounds (**5d**, **5e** and **5f**) which present a higher inhibition capacity. The aromatic compounds that exhibit the highest affinity proved to be **5c** and **5f**, in which the imino carbon is substituted by a phenyl and a hydrogen or by 2 phenyls, respectively. In particular, compound **5f**, in which the imino carbon is linked with 2 phenyl groups, presents much lower IC_{50} values than viloxazine **1** for all 3 uptake systems. With the exception of the couples of completely aliphatic compounds **5a**, **6a** and **5b**, **6b** and the pyridinic derivatives **5h** and **6h** for the NE and DA re-uptake systems, all the compounds unsubstituted on the nitrogen **5** possess a greater capacity to interact with all 3 uptake systems considered than the corresponding *N*-methyl-substituted derivatives **6**.

An examination of the results obtained for **5**, **6** and viloxazine **1** for the single uptake systems (see table II) shows that the affinity of compounds of types **5** and **6** improves, with respect to viloxazine **1**, on passing from the NE uptake system to those of DA and 5-HT. For the NE uptake system, only compound **5f** shows

Table II. *In vitro* re-uptake inhibition in rabbit occipital (NE), frontal (5-HT) and striatal (DA) synaptosomes by piperidine derivatives **5a–g** and **6a–g** and viloxazine **1**.



				IC_{50}[a] μ M		
compd	*R*	R_1	R_2	^{3}*H-NE*	^{3}*H-5HT*	^{3}*H-DA*
5a	H	*i*-Pr	*i*-Pr	b	30.2(27.4-32.7)	*b*
6a	Me	*i*-Pr	*i*-Pr	45.2(39.4-50.2)	*b*	42.3(39.0-45.0)
5b	H			43.2(39.5-4.8)	*b*	*b*
6b	Me			10.0(8.2-12.0)	*b*	9.20(8.0-10.5)
5c	H	Ph	H	1.10(0.7-1.5)	0.65(0.45-0.83)	5.50(4.5-6.0)
6c	Me	Ph	H	5.10(4.5-6.0)	*b*	48.3(44.0-52.0)
5d	H		H	2.40(1.8-3.2)	2.80(2.0-3.2)	10.2(8.8-11.6)
6d	Me			38.1(35.2-40.2)	18.4(16.6-20.1)	30.4(28.3-32.1)
5e	H	Ph	Me	13.2(11.3-14.8)	4.20(3.3-4.9)	8.20(6.9-9.5)
6e	Me	Ph	Me	*b*	20.1(18.1-22.0)	*b*
5f	H	Ph	Ph	0.10(0.09-0.11)	8.10(4.5-8.6)	0.15(0.12-0.18)
6f	Me	Ph	Ph	5.00(4.5-5.5)	30.2(28.3-32.0)	12.2(11.5-13.0)
5g	H			10.3(8.3-12.5)	1.10(0.9-1.2)	20.1(18.8-21.7)
6g	Me			25.1(23.1-27.1)	2.30(1.8-2.8)	*b*
5h	H		H	*b*	5.10(4.0-6.5)	*b*
6h	Me			41.1(38.3-44.2)	10.2(8.0-12.5)	24.3(22.0-27.0)
viloxazine				0.22(0.15-0.30)	20.4(13.6-27.2)	38.2(30.1-46.0)

[a]Concentrations necessary for 50% inhibition are geometric means of three separate determinations; confidence limits are shown in parentheses. [b]$IC_{50} > 50$ μM.

an IC_{50} value lower than that of viloxazine **1**; for the DA and 5-HT uptake systems, compounds **5c–e** and **6b, f**, and compounds **5c–h** and **6g**, respectively, exhibit IC_{50} values lower than those of **1**.

In contrast with the findings for viloxazine **1**, which is selective for NE uptake, the new compounds have a higher affinity (**5c, d, f, g**) and appear to possess a good degree of affinity for all 3 uptake systems. However, compounds **5c, d, g** are more selective for 5-HT, whereas **5f** possesses a greater affinity for the NE and DA uptake systems.

Conclusions

The aim of the present study was to verify whether the combination of the piperidinic ring of **2** and **3b** or **3a** and the bioisoster MAOMM in a single structure leads to compounds that are capable of inhibiting more or less selectively biogenic amine uptake, and are thus potentially active as antidepressants.

Compounds in which the imino carbon is linked with aliphatic groups and those in which the same carbon is linked with aromatic groups inhibit biogenic amine uptake, even if they do not generally possess a high selectivity for the serotoninergic system. The uptake inhibition capacity of the aryl-substituted derivatives is higher than that of the aliphatic compounds, which, in the case of **5c** and **5f**, clearly exhibit lower IC_{50} values than viloxazine for the DA and 5-HT uptake systems. Moreover, **5f** shows an IC_{50} value lower than that of **1**. In compounds of types **5** and **6**, therefore, the aromatic substituent linked to the imino carbon of the MAOMM appears to be capable of playing a role in the interaction with the biological structures governing biogenic amine reuptake. Analogous results were also found for type **4** compounds [16], which were evaluated for their potential antidepressant properties by means of the *in vivo* test of antagonism to reserpine-induced hypothermy in the mouse. Type **4** compounds substituted with an aromatic group on the imino carbon revealed a considerably higher activity than the analogues substituted only with aliphatic groups. On the basis of these results we hypothesized a possible participation of the aromatic systems in the bioisosterism between the MAOMM of **4** and the $ArOCH_2$ system of viloxazine [16].

Finally, in the case of femoxetine **3a** and paroxetine **3b** [13], the compound with an *N*-methyl-substituted piperidinic nucleus (**3a**) reveals a lower affinity, at least for the serotoninergic system, than the one with an *N*-unsubstituted piperidinic ring (**3b**). It is interesting to note that also for type **5** and **6** compounds, the introduction of a methyl group on the nitrogen of the piperidinic ring of **5** has negative effects, generally leading to compounds with a marked increase in the inhibition indices.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Boiling points refer to the air-bath temperature of bulb-to-bulb distillation using a Buchi GKR-51 apparatus. IR spectra for comparison of compounds were taken as paraffin oil mulls or as liquid films, on a Perkin–Elmer Model 1310 instrument. ^{1}H-NMR spectra were obtained with a Varian EM 360 instrument in ≈ 10% $CDCl_3$ (for the free bases Me_4Si) or D_2O (for the salts $Me_3SiCD_2CD_2CO_2Na$) solution. The proton magnetic resonance assignments were established on the basis of the expected chemical shifts and the multiplicity of the signals. Oximes of symmetrical ketones and *E*-oximes of aldehydes or asymmetrical ketones were prepared by the usual methods and their physical constants were in accordance with those reported in literature.

Analytical TLC was carried out on 0.25 mm layer silica-gel plates (Merck F_{254}) containing a fluorescent indicator; spots were detected under UV light (254 nm) (in the case of completely or partially aromatic compounds), or by spraying with 0.2 M $K_2Cr_2O_7$ in 40% aqueous sulphuric acid followed by gentle heating (in the case of completely aliphatic compounds). Column chromatographies were performed using 70–230 mesh silica gel. Magnesium sulphate was always used as the drying agent. Evaporations were performed *in vacuo* (rotary evaporator). Elemental analyses were carried out by our analytical laboratory and agreed with theoretical values to within ± 0.4%.

3-Chloromethylpiperidine hydrochloride ***9·HCl***
A solution of 3-hydroxymethylpiperidine **7** (10.0 g, 87 mmol) in anhydrous $CHCl_3$ (68 ml) was saturated with HCl (g) and then treated dropwise at reflux temperature with $SOCl_2$ (27.6 g; 0.23 mol). The resulting mixture was refluxed for 1.5 h and evaporated to yield a solid residue (15 g) which was crystallized from EtOH/Et_2O to yield **9·HCl** (13.3 g, 89%); mp 153–155°C. ^{1}H-NMR δ 3.42 (d, 2H, J = 6 Hz, CH_2Cl). Anal $C_6H_{12}NCl$ (C, H, N).

1-Methyl-3-chloromethylpiperidine hydrochloride ***10·HCl***
This compound was prepared from the 1-methyl-3-hydroxymethylpiperidine **8** (50 g, 0.38 mol) following the procedure described above for the preparation of compound **9·HCl** to yield **10·HCl** (58 g, 81%); mp 168–169°C (EtOH/Et_2O). ^{1}H-NMR δ 3.45 (d, 2H, J = 6 Hz, CH_2Cl) (lit [17] 169.1–170.2°C). Anal $C_7H_{14}NCl$ (C, H, N).

General procedure for the synthesis of the 3-(methyleneaminoxy)methylpiperidine derivatives ***5a–g·$H_2C_2O_4$*** *and 1-methyl-3-(methyleneaminoxy) methylpiperidine derivatives* ***6a–g·$H_2C_2O_4$***
The appropriate oxime (**11a–g**, 6 mmol) was added to a mixture of **9** or **10** (5.81 mmol) and KOH (21.38 mmol) in DMSO (5 ml) (in the case of **5b, c, e–g** and **6b, c, e–h**) or *t*-BuOH (10 ml) and 18-crown-6 (22 mmol) (in the case of **5a, d, h** and **6a, d**). The reaction mixture was stirred at 40°C for 4–6 d, then diluted with H_2O and extracted with $CHCl_3$. The chloroformic layer was washed (10% aqueous NaOH and H_2O), dried, filtered and evaporated to dryness to yield crude **5a–g** or **6a–g**, which was chromatographed through a silica-gel column, eluting with a mixture of toluene, AcOEt and MeOH

in the ratio 7:3:1. The oily products were dissolved in Et_2O and then treated with a solution of a molar equivalent of oxalic acid in a 2:8 mixture of $MeOH/Et_2O$. The crude products were filtered and crystallized from the proper solvent to give the pure oxalate salts of **5a–g** and **6a–g** (see table I).

In the ^{1}H-NMR spectra of **5** and **6** there is a multiplet attributed to the hydrogens of the methylenic group linked to the piperidine system (middle-point of the signals: 3.76–4.25 ppm). For other physical and microanalytical data, see table I.

The configurational stability of the *E*-oximes of asymmetrical carbonyl compounds (**11c–e, h**) was tested by treating the appropriate oxime with KOH in DMSO under the above-described conditions. The usual work-up made it possible to recover the unaltered starting oximes.

[^{3}H]Norepinephrine, [^{3}H]dopamine and [^{3}H]serotonin uptake inhibition

Rabbit brains were rapidly dissected to remove the occipital and frontal cortex and the corpus striatum; synaptosomal fractions were then prepared as previously described [18]. The uptake of [^{3}H]NE (15 nM), [^{3}H]-5-HT (4 nM), and [^{3}H]DA (2 nM) into occipital and frontal cortex, and corpus striatum synaptosomes, respectively, was measured as previously described [18], in the presence of various concentrations of the compounds tested. Non-specific transport was determined by measuring the amount of uptake in the presence of desipramine (10 μM), chlorimipramine (10 μM), or benzotropine (100 μM), for NE, 5-HT, and DA transport, respectively. This non-specific transport was also evaluated by measuring the radioactivity of the synaptosomal preparations in the absence of drugs when the temperature of the incubation mixture was between 0 and 4°C. The concentrations of the compounds that inhibit specific monoamine uptake by 50% (IC_{50}) were determined by log-probit analysis with 4 concentrations of the displacers, each performed in triplicate.

The following labelled compounds were used: *dl*-[7-^{3}H(N)] norepinephrine (11.8 Ci/mmol) hydrochloride, [7-^{3}H(N)]dopamine (28 Ci/mmol) free base, and [1,2-^{3}H(N)]serotonin (26.2 Ci/mmol) bioxalate.

Acknowledgment

This work was partly supported by a grant from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica and the Consiglio Nazionale delle Ricerche.

References

1 Balsamo A, Croxatto M, Lapucci A *et al* (1986) *VI Convegno Nazionale Divisione di Chimica Farmaceutica of the Italian Chemical Society*, Alghero, Italy Abstr p 38
2 Kaiser C, Setler PE (1981) *In: Burger's Medicinal Chemistry. 4th Edition* (Wolf ME, ed) John Wiley & Sons, New York, USA, 997–1067
3 Borg S, Brodin K (1992) *In: Meyler's Side Effects of Drugs. 12th Edition* (Dukes MNG, ed) Elsevier, Amsterdam, The Netherlands, 30–78
4 Hollister LE, Claghorn JL (1993) *Annu Rev Pharmacol Toxicol* 32, 165–177
5 Blackwell B, Marley E, Price J, Taylor D (1967) *Br J Psychiat* 113, 349–365
6 Hollister LE (1981) *Drugs* 22, 129–152
7 Blackwell B (1987) *In: Psychopharmacology: The Third Generation of Progress* (Meltzer HY, ed) Raven Press, New York, USA, 1041–1049
8 Arya VP, David J, Grewal RS, Marathe SB, Patil SD, Shenoy SJ (1977) *Ind J Chem Sect B* 15B, 1125–1128
9 Pifferi G, Nicola M, Gaviraghi G, Pinza M, Banfi S (1983) *Eur J Med Chem* 18, 465–467
10 Carissimi M, Picciòla G, Ravenna F, Gentili P, Carenini G (1980) *Il Farmaco Ed Sci* 35, 504–526
11 Carissimi M, Picciòla G, Ravenna F, Carenini G, Gentili P (1980) *Il Farmaco Ed Sci* 35, 812–825
12 Melloni P, Carniel G, Della Torre A *et al* (1984) *Eur J Med Chem* 19, 235–242
13 Ives JL, Heym J (1989) *In: Annual Report in Medicinal Chemistry* Academic Press 24, 21–29
14 Balsamo A, Giorgi I, Lapucci A *et al* (1987) *J Med Chem* 30, 222–225
15 Thomas DR, Nelson DR, Johnson AM (1987) *Psychopharmacology* 93, 193–200
16 Balsamo A, Lapucci A, Macchia M *et al* (1994) *Il Farmaco* 49, 77–82
17 Feldkamp RF, Faust JA, Cushman AJ (1952) *J Am Chem Soc* 74, 3831–3833
18 Richelson E, Pfenning M (1984) *Eur J Pharmacol* 104, 277–286