Pyrrolobenzodiazepines and Related Systems. 2. Synthesis and Biological Properties of Isonoraptazepine Derivatives

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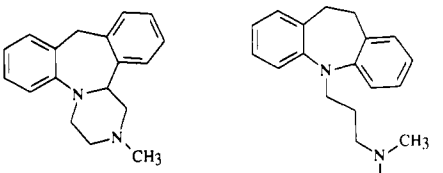
The synthesis of some derivatives and analogues of 12,13,14,14a-tetrahydro-9*H*,11*H*-pyrazino-[2,1-*c*]pyrrolo[1,2-*a*][1,4]benzodiazepine (isonoraptazepine) is reported. The new derivatives have been subjected to pharmacological tests for evaluation of antidepressant effects. Neurobehavioral assays were also carried out to acquire data on neurotoxicity and sedative action. Isonoraptazepine analogues and derivatives lacked the pharmacological activity of mianserin and aptazepine and showed properties similar to imipramine. Molecular modeling studies revealed structural similarities between isonoraptazepine derivatives and imipramine, thus explaining the similar pharmacological profile found in some of the tests employed. Based on pharmacological data the title compounds cannot be regarded as α_2 presynaptic adrenoceptors antagonists. In vitro studies for receptor binding gave support to this observation. The above studies lead us to conclude that isonoraptazepine derivatives are conformationally restricted analogues of imipramine, but their antidepressant activity cannot be correlated to inhibition of 5HT uptake. Among the derivatives tested, **7b** and **8e** show some affinity for the *d*-fenfluramine receptor site, a serotonin presynaptic site connected with anorectic activity.

Mianserin (1), a clinically active atypical antidepressant agent, has a lower incidence of anticholinergic side effects than the typical tricyclic antidepressant drugs, such as imipramine (2) and amitriptyline (3).[1,2] A comparative evaluation of "atypical" versus "typical" antidepressant drugs has stimulated new searches for novel CNS agents with a therapeutic profile similar to that of mianserin.[3-5] Studies on tri- and tetracyclic ring systems containing a fused pyrrole moiety led to the discovery of 1,3,4,14b-tetrahydro-2-methyl-2*H*,10*H*-pyrazino[1,2-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine (4, aptazepine).[6-8] Like mianserin, which it structurally resembles, aptazepine is a potent inhibitor of the binding of the α_2-adrenoceptor ligand, [^{3}H]clonidine, but it is less active in displacing the α_1-adrenoceptor ligand [^{3}H]prazosin from its binding site. The potent α_2-adrenoceptor antagonistic activity of aptazepine has been confirmed in vivo, and clinical investigation of this drug or its analogs would be of interest to further assess the involvement of α_2-adrenoceptors in depressive diseases.[1]



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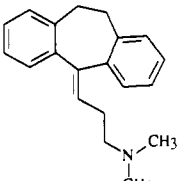

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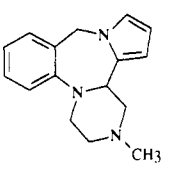

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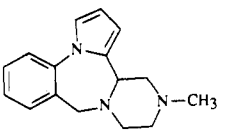

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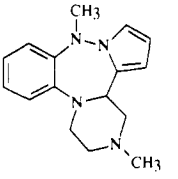

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Our laboratories have been recently engaged in the synthesis of new aptazepine isomers and isosters, in particular compounds 5 (isoaptazepine)[9,10] and 6 (methylazaaptazepine).[11] In the present work we are reporting

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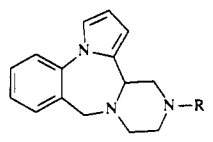
the synthesis of several derivatives 7–10 related to compound 5. The new derivatives have been tested as antidepressive agents by Nomura's test and the phenylquinone-induced writhing test. Also, their neurobehavioral effects as well as the binding affinity of some of them versus various receptors have been evaluated.

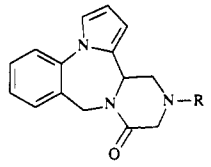
Chemistry

The synthesis of derivatives **7a**, **7b**, **8a**, **8b**, and **9** has been reported in previous works.[9,10] Alkylation of 12,13,14,14a-tetrahydro-11-oxo-9*H*,11*H*-pyrazino[2,1-*c*]-pyrrolo[1,2-*a*][1,4]benzodiazepine (**8a**) at position 13 has been performed by the action of the proper halogen derivative in the presence of anhydrous potassium carbonate (method A) to give **8c**, **8e**, **8f**, and **8h**. Acylation of **8a** was achieved by reacting this compound with acetyl chloride or 4-chlorobenzoyl chloride in the presence of triethylamine to afford **8d** and **8g**, respectively (method B). Lithium aluminum hydride–sulfuric acid (2:1) reduction of **8d** gave **7c**. Method A was used for the preparation of **7d** and **7g** starting from 12,13,14,14a-tetrahydro-9*H*,11*H*-pyrazino[2,1-*c*]pyrrolo[1,2-*a*][1,4]-benzodiazepine (**7a**), while acylation of the latter compound by method B furnished **7e** and **7f**.



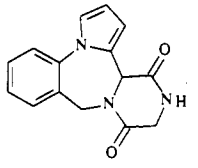
7

compd.	R
7a	H
7b	CH_3
7c	CH_2CH_3
7d	CH_2-CH=CH_2
7e	$COCH_3$
7f	COC_6H_4-p-Cl
7g	$CH_2C_6H_4$-p-Cl



8

8a	H
8b	CH_3
8c	CH_2CH_3
8d	$COCH_3$
8e	$CH_2C_6H_5$
8f	$CH_2C_6H_4$-p-F
8g	COC_6H_4-p-Cl
8h	$CH_2C_6H_4$-p-Cl



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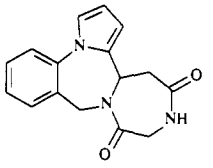

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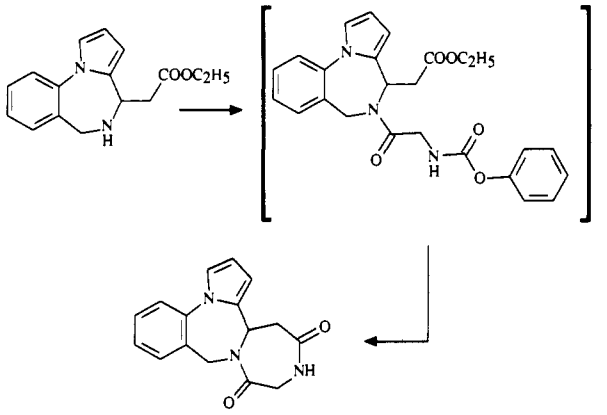
11,14-Dioxo-12,13,14,14a-tetrahydro-9*H*-[1,4]diazepino-[7,1-*c*]pyrrolo[1,2-*a*][1,4]benzodiazepine (**10**), the homocyclic analogue of **9**, has been obtained by starting from ethyl 5,6-dihydro-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepine-4-acetate[12] by reaction with (benzyloxycarbonyl)glycine in the presence of *N*′-ethyl-*N*-[3-(dimethylamino)propyl]-carbodiimide followed by transfer catalytic hydrogenation (sodium hypophosphite and 10% Pd/C) of the glycinamido intermediate (Scheme I).

Scheme I



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Pharmacological Results and Discussion

Derivatives **7–10** were submitted to pharmacological tests in mice to explore their neuropsychopharmacological effects (Table I) and to evaluate their antidepressant activity (Tables II and III). All derivatives showed a minimal lethal dose (MLD) = 125 mg/kg (ip administration), with the exception of compound **8a**, having a MLD = 75 mg/kg.

It is evident from the data of Table I that all derivatives, with exception of **8c**, **9**, and imipramine, were found to exert sedation at the test dose, with particular reference to aptazepine and derivatives **7a**, **7b**, **7g**, **8b**, and **8d**. Manifest neurotoxic effects were shown by aptazepine and derivatives **7a**, **7b**, **7g**, and **8b** in the Rota-Rod test (loss of motor coordination). Also, a strong abatement of reflexes was shown in decreasing order by derivatives **8b**, aptazepine (75 mg/kg), **7b**, and **7c** (see myorelaxant action). The compounds assayed (aptazepine, imipramine, and derivatives **7–10**) did not show any protection in the electroshock test for anticonvulsant activity, with the exception of imipramine. A decrease of exploratory activity (hole–board test) was observed for a large number of compounds. This behavior could be due to depressant effects on both motor reflexes and spontaneous motility.

Antidepressant activity (Tables II and III) was assayed by Nomura's test and the phenylquinone-induced writhing procedure. Only aptazepine and derivatives **9** and **8h** showed statistically significant antidepressant activity in the phenylquinone test, whereas most of the compounds were found active as antidepressants in Nomura's test. Compounds **7a–e**, **8a**, **8b**, **8d**, **8e**, **9**, and **10**, together with imipramine, displayed a significant antidepressant activity, **8d**, **8e**, and **10** being the most active. These experimental data clearly demonstrate that aptazepine is highly active in the phenylquinone test, but inactive in Nomura's procedure, while the reverse is true for imipramine. These discrepancies are difficult to explain due to the lack of correlation between Nomura's test and the central biochemical mechanisms.

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Table I. Neurobehavioral Effects of Selected Derivatives 7–10[a]

compd or controls	dose, mg/kg ip	spontaneous motor activity[b]	exploratory activity[c]	myorelaxant action (traction test)[d]	Rota–Rod test[e]
TC		319 ± 105	17.7 ± 11.3	1.1 ± 0.4	0.9 ± 0.6
aptazepine	10	168 ± 67*	8.8 ± 7.7	2.5 ± 1.9*	5.0 ± 4.2
	75	59 ± 39*	0.5 ± 0.9*	8.1 ± 8.9* (25%)	6.5 ± 5.8* (12.5%)
imipramine	37.5	183 ± 126	2.2 ± 2.6	5.7 ± 4.3	4.8 ± 6.4
7a	75	111 ± 35*	5.5 ± 2.5*	4.9 ± 4.2*	3.0 ± 1.5* (37.5%)
7b	75	96 ± 67*	3.8 ± 5.2*	4.4 ± 3.7* (12.5%)	5.0 ± 3.4*
7c	75	188 ± 84*	7.6 ± 6.39*	3.1 ± 1.9* (12.5%)	1.1 ± 1.3
7g	75	142 ± 55*	9.3 ± 10.6	2.0 ± 1.2*	2.1 ± 1.0*
8b	75	90 ± 65*	13.0 ± 10.5	1.0 ± 1.4* (62.5%)	8.7 ± 6.0*
8c	75	285 ± 102	9.8 ± 5.9	2.1 ± 0.6*	0.4 ± 0.5
8d	75	121 ± 80*	10.7 ± 7.9	3.5 ± 2.4*	1.0 ± 1.2
8f	75	178 ± 112*	6.0 ± 5.5*	4.2 ± 2.6*	1.8 ± 2.4
9	75	247 ± 74	14.7 ± 13.4	5.5 ± 4.2*	2.5 ± 3.2
10	75	173 ± 119*	17.2 ± 6.6	2.3 ± 2.8	0.6 ± 0.9

[a] All values represent the mean ± standard error of the mean for each group of animals. Drugs were administered ip to groups of 10 mice (5 male and 5 female); a group of mice was treated only with vehicle (treated controls, TC). Statistical significance (*t* test): (*) $p < 0.05$; only derivatives which reached statistically significant values are reported. [b] Total number of movements in 5 min. [c] Total number of explored holes in 5 min. [d] Time in seconds on the horizontal wire with forelegs (% of animals without writhing reflexes on wire is reported in the brackets. [e] Total number of falls in 100 s (% of animals which fall constantly is reported in the brackets).

Table II. Antidepressant Effects (Nomura's test) of Derivatives 7–10[a]

compd or controls	dose, mg/kg	rotation number (mean ± SD)[b] 1st determ after 9 min	2nd determ after 12 min	3rd determ after 15 min
TC		30 ± 15	20 ± 10	4.2 ± 2.9
aptazepine	2.5	12 ± 7*	3 ± 2*	2 ± 2
	5	5 ± 6*	8 ± 7*	2 ± 2
	10	8 ± 3*	5 ± 2*	2 ± 2
imipramine	10	48 ± 11°	40 ± 10°	36 ± 7°
7a	10	17 ± 13	10 ± 9	12 ± 7°
7b	10	21 ± 21	16 ± 11	11 ± 6°
7c	10	30 ± 27	27 ± 18	24 ± 20°
7d	10	16 ± 22	16 ± 16	14 ± 8°
7e	10	21 ± 16	23 ± 16	17 ± 10°
7f	10	7 ± 5*	6 ± 8*	7 ± 6
7g	10	22 ± 20	14 ± 11*	11 ± 9
8a	10	15 ± 8*	17 ± 13	10 ± 6°
8b	10	17 ± 11*	14 ± 10	10 ± 6°
8c	10	22 ± 12	17 ± 9	22 ± 10°
8d	10	54 ± 8°	57 ± 11°	49 ± 13°
8e	10	45 ± 3°	41 ± 18°	31 ± 27°
9	10	18 ± 10*	15 ± 10	11 ± 7°
10	10	35 ± 16	27 ± 15	27 ± 20°

[a] Only derivatives which reached statistically significant values are reported. Significance versus controls has been evaluated as follows: (* or °) $p < 0.05$; circlets refer to antidepressive effect significance versus controls and asterisks to sedative effect significance versus controls. [b] SD = standard deviation.

The results obtained in the phenylquinone test led us to assume that, in general, derivatives **7–10**, as well as imipramine, cannot be considered α_2 presynaptic adrenoceptor antagonists. Due to their structural resemblance with aptazepine, we expected for isonoraptazepine derivatives a similar pharmacological profile, in particular with regard to the antidepressant activity. Contrary to our expectation, on the basis of the pharmacological results in Nomura's test, compounds **7–10** seem to have an imipramine-like, rather than an aptazepine-like, antidepressant activity. A support to this observation has been obtained through molecular modeling studies, using as basic models some isonoraptazepine derivatives, aptazepine, and imipramine.

Table III. Antagonism of Clonidine-Induced Analgesia by Derivatives 8h and 9[a]

compd or controls	writhing number (mean ± SE)[b]	% inhibition of abdominal costrict.
controls + phenylquinone	20 ± 3°[c]	
controls + phenylquinone + clonidine	0.3 ± 0.2	98
imipramine	0	100
aptazepine	18 ± 3°	14
8h	8 ± 3°	63
9	12 ± 5°	43

[a] Dose aptazepine, imipramine, and derivatives **8h** and **9**: 10 mg/Kg ip; dose clonidine: 0.1 mg/Kg po; dose phenylquinone: 0.125% of 5% alcoholic solution. [b] SE = standard error. [c] Significance versus clonidine controls: (°) $p < 0.05$. Derivatives **7a–g**, **8a–g**, and **10** did not reach statistically significant values.

Structure–Activity Relationships

Some structure–activity relationships can be derived from the data of Nomura's test (Table II). Replacement of hydrogen with the benzyl group at the piperazinyl NH of **8a** led to **8e** with increased antidepressant activity and decreased neurotoxicity. The presence of halogens (compounds **8f** and **8h**) in the para position of the benzyl group dramatically abated the antidepressant activity with concomitant increase of neurotoxicity (**8f**). Elongation of the alkyl chain led to more neurotoxic derivatives, without affecting the antidepressant activity as observed with the derivatives **7a–c** and **8a–c**. The acetyl derivatives **7e** and **8d** showed good and excellent activity respectively, whereas the aroyl derivatives **7f** and **8g** were inactive. Dioxo derivatives **9** and **10** were found to be less toxic than the monooxo derivative **8a** (MLD = 75 mg/kg) and both showed a significant antidepressant activity (compound **9** also in the phenylquinone-induced writhing test).

Molecular Modeling

Compounds **7b**, **8c**, **8d**, and **10**, representative examples of the compounds studied, were chosen as reference compounds for computational studies on the basis of their pharmacological properties. Available X-ray crystal coordinates were used as input geometry for imipramine.[13]

Due to the lack of X-ray crystal coordinates for the selected compounds and aptazepine, their input geometry was generated and initially minimized by using the program MODEL (version KS 2.95).[14]

First a thorough conformational analysis was carried out for the above derivatives and aptazepine (all conformationally rigid molecules) in order to evaluate the putative global minimum energy conformations using the grid option of MODEL. Although two conformers with an energy difference of less then 3 kcal/mol were found in the case of **8c** and **8d**, only the minimum energy conformers were used for further studies. A further minimization was carried out, as far as convergence was reached, for the low-energy conformations of all derivatives, aptazepine, and the imipramine X-ray structure with the program MMX (version 89),[15] which uses the MM2 '77 force field (Allinger-QCPE 395) with MMPI Pi subroutines (Allinger-QCPE 318) and is particularly indicated to treat aromatic systems. The structures so obtained were then transferred to the program SYBYL.[16]

To test the reliability of the data obtained by MMX minimizations we also transferred the minimum energy conformers, obtained by the systematic conformational analysis, from MODEL to SYBYL and minimized them using the MAXIMIN2 package (Amber force field)inside this program. Comparison of the energy-minimized conformer of each compound found using MMX and MAXIMIN2 was performed and showed a very good agreement (0.1 Å ≤ RMS ≤ 0.7 Å).

Superimpositions of compounds **7b**, **8c**, **8d**, and **10** on imipramine and aptazepine were performed with the FIT procedure within SYBYL. This method is particularly helpful to evaluate a possible three-dimensional arrangement of significant molecular structural features in comparison to the reference molecule. The structural features taken into account for the matching processes were the center of the benzene ring, the normal to the plane of the benzene ring and the remote nitrogen for each compound. The fitting experiments between imipramine and our compounds showed a good superimposition of the chosen features (0.3 Å ≤ RMS ≤ 1 Å), while a poor superimposition (RMS ≫ 1 Å) was found when our derivatives were matched with aptazepine.

A constrained conformational analysis was performed on the (dimethylamino)propyl side chain of imipramine in order to determine whether the proposed pharmacophoric atoms of this molecule can adopt, in energetically stable conformers, the spatial relationships observed in the most active isonoraptazepine derivatives. This was done by using the SEARCH procedure within SYBYL and by allowing the four rotatable chain bonds of this molecule to rotate with a 10-deg stepwise increment of the dihedral angles, until the conformation was found that best overlapped the chosen molecular features with the corresponding groups of the selected compounds. A very good superimposition was observed after these experiments between the fitted molecules [RMS = 0.075 Å (**8c**), RMS = 0.032 Å (**8d**)]. An example of these fittings is illustrated in Figure 1.

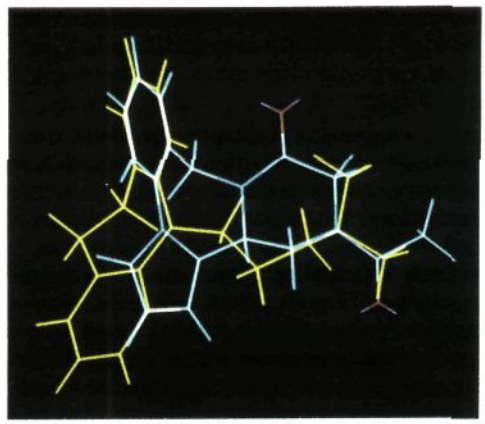

Figure 1. Superimposition of **8d** (blue) and imipramine (yellow).

Table IV. Comparison of the Distances between the Remote Nitrogen and Selected Structural Elements

distance, Å	compound					
	7b	**8c**	**8d**	**10**	imipramine	aptazepine
center/N[a]	6.34	5.79	5.87	6.52	7.15	5.54
normal/N[a]	7.66	5.42	4.65	5.78	6.00	6.71
plane/N[a]	*0.32*	*2.56*	*3.61*	*3.15*	*3.88*	*0.20*

[a] For a graphical representation see Figure 2.

Then, to find a correlation between pharmacological activities and structural features of our compounds, we measured the distances of the remote nitrogen atom from the center of the benzene ring, from the plane of the same ring and from the dummy atom placed at 4.0 Å away from the center of the benzene ring on the line normal to it. The values obtained for **7b**, **8c**, **8d**, and **10** are reported in Table IV, while a representation of the relative arrangement of the pharmacophoric groups is shown in Figure 2. It appears from Table IV that the distance of the remote nitrogen from the plane of the benzene ring is the most significant parameter to correlate molecular structure and antidepressant activity based on Nomura's test.

As a further attempt to explain the results in Nomura's test with the structural similarities among our most active compounds and imipramine, we considered the electrostatic potential maps of **8d**, **8e**, **10**, and **2**.[17] First we calculated the negative isopotential map of these compounds as minimum energy conformers using the ISOPOTENTIAL MAP procedure within SYBYL, the atomic point charges being computed by the program MOPAC.[18] The most negative areas of **8d**, **8e**, and **10** were found on the carbonyl oxygen, while for imipramine and aptazepine were localized on the remote nitrogen atoms. A good matching among derivatives **8d**, **8e**, and **10** was observed when their lactam carbonyl oxygens (common negative areas) were overlapped, keeping the benzene ring superimposed (Figure 3). When the negative surface of imipramine was constrained to overlap with that of our compounds after superimposition of the benzene rings, a good matching was also obtained, as illustrated in Figure 4 for imipramine and **8e**. It is interesting to note that the energy difference between this conformation of imipramine

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(14) Steliou, K. MODEL (Version KS 2.95) University of Montreal, Quebec Canada.

(15) QCPE Program 3965, Quantum Chemistry Program Exchange, Bloomington, IN.

(16) Tripos Associates, St. Louis, MO 63117.

(17) We thank one of the referees for useful suggestions on this subject.

(18) MOPAC version 5.0 (QCPE program no. 455) distributed with SYBYL.

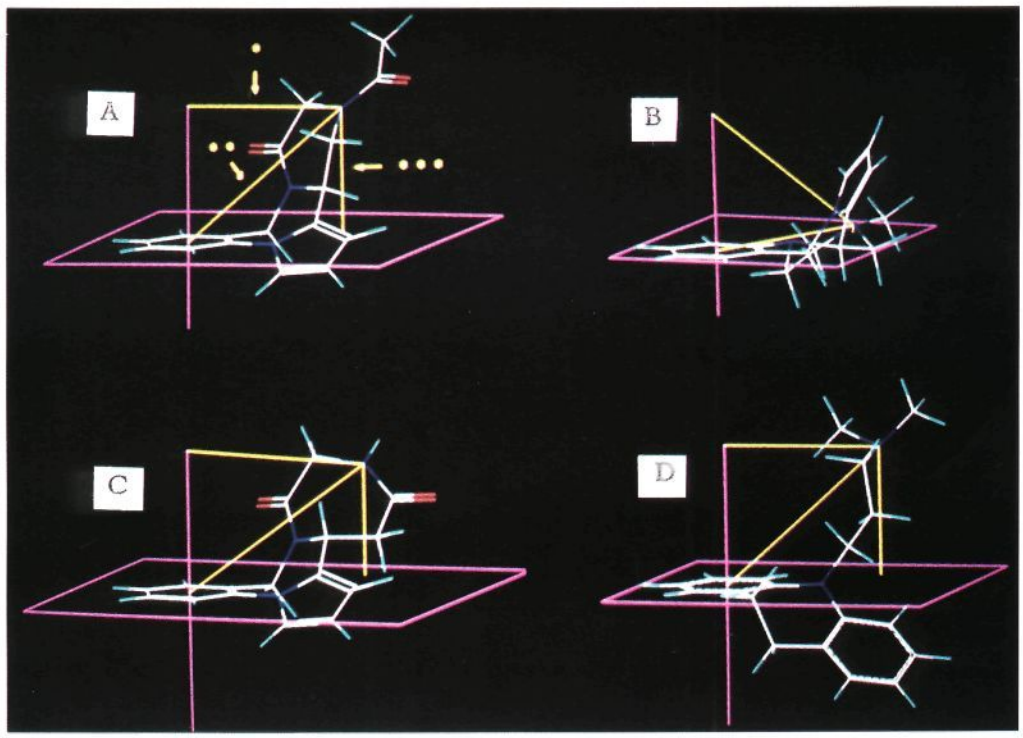

Figure 2. Energy-minimized structures of **8d** (A), aptazepine (B), **10** (C), and imipramine (D). Yellow lines represent the distances between the remote nitrogen of each compound and (°) the center of the benzene ring, (°°) the dummy atom placed at 4.0 Å away from the center of the benzene ring on the normal to it, (°°°) the plane of the benzene ring.

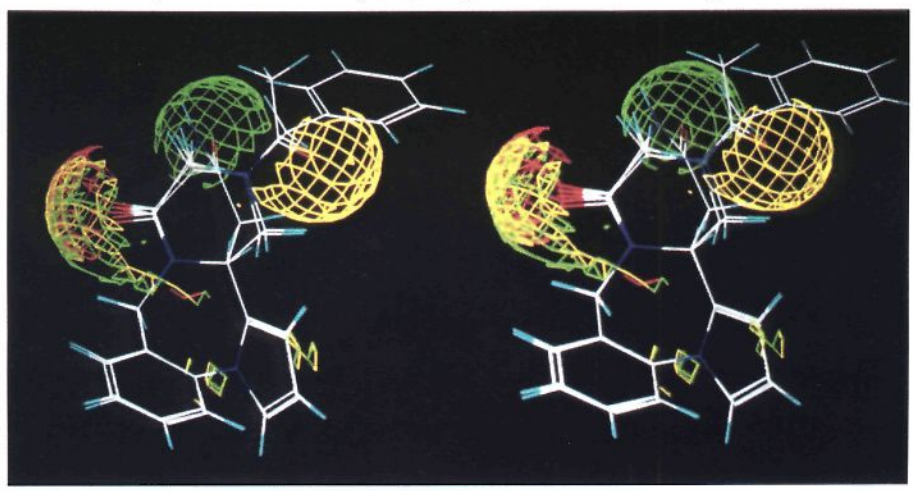

Figure 3. Stereoview of the superposition of the one level electrostatic isopotential surfaces of compounds **8d** (yellow), **8e** (red), and **10** (green). The value of potential energy is −30.0 kcal/mol.

and its X-ray conformation is only 3.5 kcal/mol. This ΔE can be easily filled by binding interactions with the receptor. In the case of aptazepine a similar overlapping with our compounds is not possible, owing to the rigidity of its structure.

In view of the results obtained by the molecular modeling studies we are allowed to conclude that both approaches point to a high structural similarity between imipramine and our tetracyclic derivatives.

Receptor Binding and 5HT Uptake Assay

Taking into account the pharmacological effects of derivatives **7–10**, we assumed that these compounds cannot be regarded as α_2 presynaptic adrenoceptor antagonists. To confirm our assumption by further assays we undertook in vitro (Table V) receptor binding studies. Four compounds, namely **7b**, **8d**, **8e**, and **10**, representative examples of the chemical groups **7–10**, were chosen for these studies. The IC_{50} values of compounds **7b**, **8d**, **8e**, and **10** were found considerably higher ($>10^{-5}$ M) than those of the reference compounds, which are in the micromolar range. Thus, it seems possible to conclude that the tested compounds have no affinity for a number of receptors (5HT-1A, 5HT-1B, 5HT-1C, 5HT-2, 5HT-3, DA-1, DA-2, α-1, α-2, and β-1) for neurotransmitters and modulators in the CNS. They did not affect the serotonin uptake, measured both as binding of [^{3}H]paroxetine (IC_{50} were 278, 2646, and 3337 nM for imipramine, mianserin, and aptazepine, respectively; derivatives **7b**, **8d**, **8e**, and **10** were not active at 10^{-5} concentrations) and inhibition of [^{3}H]5HT uptake by rat brain synaptosomes (IC_{50} were 1.62×10^{-7}, 2.72×10^{-5}, and $>10^{-5}$ M for imipramine,

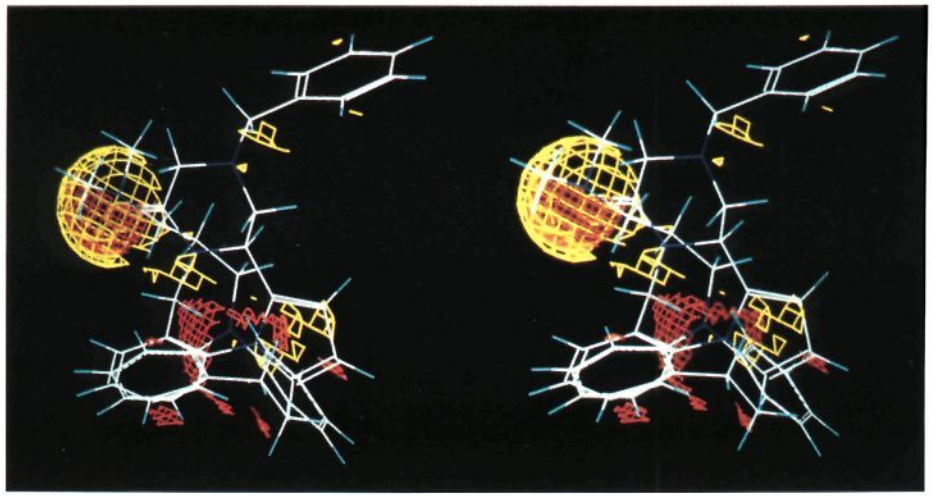

Figure 4. Stereoview of the superposition of the one level electrostatic isopotential surfaces of **8d** (yellow) and imipramine (red). The values of potential energy are −30.0 kcal/mol for **8d** and −20.0 kcal/mol for imipramine.

Table V. Methods for Binding Assays[a]

receptor	H ligand	[nM]	receptor source	preparation	incubation buffer[c]	nonspecific binding	final vol, mL	incubation
5HT-1A	[^{3}H]-8OH-PAT	1	hippocampus	cmp,[b] 100 vols	D+E+F+G	serotonin, 1 μM	0.5	30 min, 25 °C
5HT-1B	[^{3}H]serotonin	2	striatum	cmp, 100 vols	D+E+F+G	serotonin, 10 μM	1	30 min, 25 °C
5HT-1C	[^{3}H]mesulergine	1	cortex	cmp, 50 vols	D+E+F+G+C	mesulergine, 10 μM	1	30 min, 37 °C
5HT-2	[^{3}H]ketanserin	0.7	(pref.) cortex	cmp, 200 vols	D	methysergid, 1 μM	1	15 min, 37 °C
5HT-3	[^{3}H]GR65630	0.4	cortex	cmp, 50 vols	H+E	ICS 205930, 1 μM	2	30 min, 37 °C
serotonin uptake-site	[^{3}H]paroxetine	0.1	cortex	cmp, 1000 vols	B+E+F+I	serotonin, 100 μM	2	60 min, 22 °C
alpha-1 Na	[^{3}H]prazosin	0.2	total brain minus cerebellum	cmp, 100 vols	D+E+F	phentolamine, 3 μM	1	30 min, 25 °C
alpha-2 Na	[^{3}H]-*p*-aminoclonidine	1	total brain minus cerebellum	cmp, 100 vols	D+E+F	(−)-noradrenaline, 100 μM	1	20 min, 25 °C
beta-1	[^{3}H]DHA	1	cortex	cmp, 100 vols	D+E+F+J	(−)-isoprenaline, 100 μM	1	20 min, 25 °C
DA-1	[^{3}H]SCH 23390	0.4	striatum	cmp, 200 vols	L+E+F+I+K	(−)-*cis*-flupentixol, 10 μM	0.5	15 min, 37 °C
DA-2	[^{3}H]spiperone	0.2	striatum	cmp, 400 vols	L+E+F+I+K	(−)-sulpiride, 100 μM	1	15 min, 37 °C
anorectic	[^{3}H]-*d*-fenfluramine	10	total brain minus cerebellum	cmp, 100 vols	A	*d*-fenfluramine, 10 μM	1	90 min, 37 °C

[a] Membrane separation has been performed by filtration on GF/B filters. In the 5HT-3 assay filters were soaked in 0.1% polyethylene. [b] Crude membrane preparation. [c] (A) Phosphate 50 mM, pH 7.4. (B) Tris-HCl, 50 mM, pH 7.4. (C) Spiperone 0.1 μM. (D) Tris-HCl, 50 mM, pH 7.7. (E) Pargyline 10 μM. (F) Ascorbic acid 0.1%. (G) 4 mM $CaCl_2$. (H) HEPES, 50 mM, pH 7.4. (I) 120 mM NaCl + 5 mM KCl. (J) 1 μM serotonin. (K) 2 mM $CaCl_2$ + 1 mM $MgCl_2$. (L) Tris-HCl, 50 mM, pH 7.1.

mianserin, and aptazepine, respectively; derivatives **7b**, **8d**, **8e**, and **10** showed $IC_{50} > 10^{-5}$ M). In these tests we found imipramine highly active, and mianserin and aptazepine scarcely active, with a potency order imipramine ≫ mianserin > aptazepine in both tests. Unexpectedly, derivatives **7b** and **8e** were found to be active (two and four times less potent than imipramine, respectively) as inhibitors of *d*-fenfluramine receptor binding at a serotonin presynaptic site connected with anorectic activity.[19,20] IC_{50} (in nanomoles) values of drug affinities on *d*-fenfluramine receptor were 17×10^3, 11×10^3, 719, 1350, and 2290 for aptazepine, mianserin, imipramine, **7b**, and **8e**, respectively; compounds **8d** and **10** were not active at 10^{-5} M concentrations. In this test isonoraptazepine derivatives **7b** and **8e** resemble imipramine, the most active among the drugs used as references.

Conclusion

In a search for new atypical antidepressant agents related to both mianserin and aptazepine, we synthesized various isonoraptazepine derivatives. Owing to the structural relationship between these derivatives and aptazepine, we expected that they would act as atypical agents. On the contrary, pharmacological data and molecular modeling studies suggest that the isonoraptazepine derivatives can be correlated to classical antidepressant imipramine rather than to atypical aptazepine.

It is of interest to consider that the significant similarities, both in the molecular structural features and in the antidepressant activity (Nomura's test), that these compounds share with imipramine were not in full accordance with the in vitro activities on serotonin uptake and adrenoceptor binding. We can argue that the in vivo activity elicited by the isonoraptazepine derivatives could be indirect, i.e. due to the formation of active metabolites or to the activation of neurotransmitter mechanisms different from those studied in the present work. Also the supposition that stimulant effects of the compounds examined could have led to false positive results in the

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(20) Mennini, T.; Gobbi, M.; Taddei, C.; Garattini, S. Characterization of High-affinity and Stereospecific [^{3}H]*d*-Fenfluramine Binding to Rat Brain. *Neurochem. Int.* **1988**, *13*, 345–351.

Nomura's "water wheel" antidepressant test is not in accordance with the data registered in the locomotor activity studies.

With regard to in vitro binding studies, qualitative similarities were restricted to the sole activity on the *d*-fenfluramine receptor, which has been characterized as a serotonin presynaptic site[19,20] involved in anorectic activity. Therefore, isonoraptazepine derivatives **7b** and **8e** could be regarded as conformationally restricted analogues of imipramine with some affinity for the *d*-fenfluramine receptor site, but their antidepressant activity seems to be unrelated to the inhibition of 5HT uptake.

Experimental Section

Chemistry. Melting points were taken on a Büchi 530 apparatus and are uncorrected. IR spectra (Nujol mulls) were run on a Perkin-Elmer spectrophotometer. ^{1}H NMR spectra (200 MHz, TMS as an internal standard) were recorded on a Varian XL-200 instrument. Merck silica gel 60 and alumina 90 were used for chromatographic purifications. All compounds were routinely checked by TLC and ^{1}H NMR. Thin-layer chromatography (TLC) was performed by using aluminum-baked silica gel plates (Carlo Erba Stratocrom SIF-254). Developed plates were visualized by UV light. Solvents were reagent grade and when necessary were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved use of a rotary evaporator operating at reduced pressure of approximately 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Elemental analyses were performed by Prof. A. Pietrogrande, Padova, Italy. Analyses for C, H, N, and, where present, Cl and F, were within ±0.4% of the calculated values.

13-Ethyl-11-oxo-12,13,14,14a-tetrahydro-9*H*,11*H*-pyrazino[2,1-*c*]pyrrolo[1,2-*a*][1,4]benzodiazepine (8c). Method A. Typical Procedure. Ethyl iodide (1.16 g, 7.4 mmol) and finely powdered anhydrous potassium carbonate (1.70 g, 12.3 mmol) were added to a well-stirred solution of 11-oxo-12,13,14,14a-tetrahydro-9*H*,11*H*-pyrazino[2,1-*c*]pyrrolo[1,2-*a*][1,4]benzodiazepine (**8a**)[10] (1.55 g, 6.1 mmol) in freshly distilled dry DMF (10 mL). The mixture was heated at 90 °C for 3 h. After cooling the suspension was diluted with water (200 mL) and extracted with EtOAc (3 × 50 mL). The organic layers were collected, washed with brine (5 × 100 mL), dried, and evaporated in vacuo to furnish a crude oil, which was chromatographed on silica gel eluting with EtOAc to afford 1.4 g (79%) of **8c** as a pale yellowish oil: IR 1645 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3 H), 2.60 (q, 2 H), 2.91 (t, *J* = 11 Hz, 1 H), 3.02 (d, *J* = 16.6 Hz, 1 H), 3.33 (dd, *J* = 11 Hz, *J* = 3.8 Hz, 1 H), 3.64 (d, *J* = 16.6 Hz, 1 H), 3.68 (d, *J* = 13.7 Hz, 1 H), 4.51 (dd, *J* = 11 Hz, *J* = 4 Hz, 1 H), 5.41 (d, *J* = 13.7 Hz, 1 H), 6.22 (m, 1 H), 6.30 (m, 1 H), 7.02 (m, 1 H), 7.2–7.5 (m, 4 H).

By a similar procedure **7d**, **7g**, **8e**, **8f**, and **8h** were prepared, while **7a**, **7b**, **8a** and **8b** have been described in previous works.[9,10]

Chemical and physical data of new derivatives are reported as follows:

7d, oil from Al_2O_3/$CHCl_3$, 60% yield; **7g**, oil from SiO_2/EtOAc–$CHCl_3$ (1:1), 70% yield; **8e**, oil from SiO_2/$CHCl_3$, 73% yield; **8f**, oil from SiO_2/$CHCl_3$, 77% yield; **8h**, from Al_2O_3/$CHCl_3$–C_6H_6 (1:1), mp 170–171 °C (ethanol), 80% yield.

13-Acetyl-11-oxo-12,13,14,14a-tetrahydro-9*H*,11*H*-pyrazino[2,1-*c*]pyrrolo[1,2-*a*][1,4]benzodiazepine (8d). Method B. Typical Procedure. A solution of acetyl chloride (0.71 g, 9.1 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a stirred solution of **8a** (2.1 g, 8.3 mmol) and triethylamine (1.68 g, 16.6 mmol) in CH_2Cl_2 (100 mL). The mixture was stirred at room temperature for 2 h, then extracted with 1 N HCl (1 × 100 mL), washed with saturated Na_2CO_3 aqueous solution (1 × 100 mL) and brine (1 × 100 mL), and dried. After evaporation the oily residue was purified by column chromatography on silica gel. First eluates with $CHCl_3$ were discarded, and subsequent elution with EtOAc afforded 2.01 g (82%) of **8d** as viscous oil: IR 1635 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$) δ 2.09 (s, 3 H), 3.40 (dd, *J* = 13.2 Hz, *J* = 11 Hz, 1 H), 3.78 (d, *J* = 14 Hz, 1 H), 4.13 (d, *J* = 17 Hz, 1 H), 4.25–4.35 (superimposed signals, dd, *J* = 13.2 Hz, *J* = 4 Hz, 1 H, and d, *J* = 17 Hz, 1 H), 4.96 (dd, *J* = 11 Hz, *J* = 4 Hz, 1 H), 5.40 (d, *J* = 14 Hz, 1 H), 6.32 (m, 2 H), 7.05 (m, 1 H), 7.30–7.55 (m, 4 H).

By this procedure **7e**, **7f**, and **8g** were also prepared. Chemical and physical data of these derivatives are reported as follows: **7e**, from SiO_2/EtOAc, mp 97–99 °C (cyclohexane), 72% yield; **7f**, oil from SiO_2/EtOAc–$CHCl_3$ (1:1), 66% yield; **8g**, from SiO_2/EtOAc–$CHCl_3$ (1:1), mp 176–178 °C (ethanol), 78% yield.

13-Ethyl-12,13,14,14a-tetrahydro-9*H*,11*H*-pyrazino[2,1-*c*]pyrrolo[1,2-*a*][1,4]benzodiazepine (7c). Concentrated sulfuric acid (2.3 mL, 42.9 mmol) was carefully added over 20 min to an ice-cooled stirred suspension of $LiAlH_4$ (3.26 g, 85.8 mmol) in dry THF (100 mL). The mixture was stirred at 0 °C for 30 min, and then a solution of **8c** (2.1 g, 7.15 mmol) in dry THF (20 mL) was added dropwise and stirring was maintained for 2 h at room temperature. The reaction mixture was cautiously quenched by dropwise addition of 2 N NaOH, concentrated, and extracted with Et_2O (3 × 50 mL). The ethereal extracts were combined, washed with brine (3 × 50 mL), dried, and evaporated to give 1.2 g (67%) of **7c** as a yellowish oil: ^{1}H NMR ($CDCl_3$) δ 1.07 (t, 3 H), 2.45 (m, 2 H), 2.5–3.1 (m, 6 H), 3.17 (m, 1 H), 3.45 (d, *J* = 14 Hz, 1 H), 3.78 (d, *J* = 14 Hz, 1 H), 6.27 (m, 2 H), 6.96 (m, 1 H), 7.2–7.5 (m, 4 H).

11,14-Dioxo-11,12,13,14,15,15a-hexahydro-9*H*-[1,4]diazepino[7,1-*c*]pyrrolo[1,2-*a*][1,4]benzodiazepine (10). To an ice-cooled solution of 5,6-dihydro-4*H*-pyrrolo[1,2-*a*][1,4]-benzodiazepine-4-acetic acid ethyl ester[12] (1.0 g, 3.9 mmol) in CH_2Cl_2 (100 mL) were added in one portion (benzyloxycarbonyl)-glycine (1.0 g, 4.8 mmol) and *N*-ethyl-*N'*-[3-(dimethylamino)-propyl]carbodiimide hydrochloride (0.92 g, 4.8 mmol) as carboxyl group activator. After the mixture was stirred at room temperature for 2 h, the solution was washed with 2 N HCl (1 × 100 mL), with saturated Na_2CO_3 aqueous solution (1 × 100 mL), and then with brine (1 × 100 mL). The solution was dried and evaporated to give a residue, homogeneous on TLC (silica gel, EtOAc). The residue (1.74 g) was dissolved in glacial acetic acid (50 mL) containing 10% Pd/C (50 mg). A solution of $NaH_2PO_3 \cdot H_2O$ (0.83 g, 7.8 mmol) in water (70 mL) was added, and the mixture was refluxed for 1 h. After filtration the reaction mixture was diluted with water (100 mL), basified with concentrated NH_4OH and extracted with EtOAc (3 × 50 mL). The combined extracts were washed with brine (1 × 100 mL), dried, and evaporated to yield 1.2 g of a pale yellow oil, pure by TLC (silica gel, 1% triethylamine/1% isopropyl alcohol/$CHCl_3$). This residue was dissolved in toluene (50 mL) and heated at 110 °C for 8 h. On cooling a white precipitate formed which was collected and recrystallized from EtOH to give pure **10**: 1.27 g (58%); mp 222–224 °C; IR 1620 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6 + D_2O) δ 2.35 (m, 2 H), 3.55 (d, *J* = 15.2 Hz, 1 H), 3.96 (d, *J* = 13.8 Hz, 1 H), 4.38 (d, *J* = 15.2 Hz, 1 H), 4.83 (d, *J* = 13.8 Hz, 1 H), 5.53 (m, 1 H), 6.24 (m, 1 H), 6.35 (m, 1 H), 7.19 (m, 1 H), 7.3–7.5 (m, 4 H).

Derivative **9** was described previously by us.[10]

Pharmacological Test Procedures. Materials and Methods. Male and female Swiss inbred mice (Charles River Italia) weighing 25 ± 3 g were used. Animals were housed in standardized environmental conditions: temperature 22 ± 1 °C, humidity 60–65%, light period 5 a.m.–9 p.m. Every single dose of test compound, dissolved in 1% Tween 80, was administered ip to each group of 10 mice (5 male and 5 female). For neurobehavioral effects mice were treated only with vehicle (treated controls, TC) and other groups with aptazepine (75 and 10 mg/kg ip) and imipramine (37.5 mg/kg ip), respectively. For antidepressant activity (Nomura and phenylquinone-induced writhing tests) the doses were 2.5, 5, and 10 mg/kg ip for aptazepine and 10 mg/kg for imipramine and derivatives **7–10**. Results of experiments were statistically analyzed by the "Dunnett *t* test"[21] and percent controls were calculated using the "Fisher exact probability test".

In all the method's statistical significance against controls was estimated as follows: (* or °) $p < 0.05$. Comparison was performed according to the two-tailed Student's *t* test.

(21) Dunnett, C. W. A Multiple Comparison Procedure for Comparing Several Treatments with a Control. *J. Am. Stat. Assoc.* **1955**, *50*, 1096–1121.

Neuropsychopharmacological Effects. Tests were performed on each animal according to the following schedule:[22] time 0 min = application of substance; time 60 min = spontaneous activity in an open field; time 65 min = exploratory activity (hole-board); time 70 min = myorelaxant action (traction test); time 80 min = motor coordination in Rota-Rod test; time 90 min = maximal electroshock seizure.

Spontaneous Motor Activity. Mice were placed individually in standard polypropylene mouse cages (19 × 25 cm). Each cage was placed on the top of a Small Animal Activities Monitor Platform (Coulbourn Instruments, Lehigh Valley).

The sensitivity setting on each platform was adjusted to a level (10.00) producing a significant number of counts in absence of any toxin or drug, increased counts after low doses of *d*-amphetamine and decreased counts after high doses of *d*-amphetamine. The activity of each mouse was continuously monitored throughout 5 min.

Exploratory Activity on the Hole Board. This test was performed using Boissier and Simon's technique.[23] The number of explorations was recorded automatically by an infrared device placed below the four hole lines. The total number of holes explored by each animal during 5 min was recorded.

Myorelaxant Action. This action was analyzed by the Boissier and Simon's "traction test",[24] determining the time employed by the animals to place their hind legs on an horizontal wire (wire reflex).

Action on Motor Coordination. This action was examined using the Rota-Rod test.[25] Fallings of the animals during an observation period of 100 s were determined.

Anticonvulsant Activity. This activity was tested determining the protection from seizures and from death caused by the electroshock intensity produced by the U. Basile ECT-Unit 7800 apparatus adjusted as follows: 200 frequency pulses/s; 60-mA current intensity; 0.4-s shock duration; 0.6-ms pulse width. The animals were considered "protected" when they did not show seizures.

Antidepressant Activity. Two methods were used to assay this activity: the Nomura test[26] and the phenylquinone test according to Fielding.[5,8,27]

Nomura Test. A plexiglass water tank with a water wheel in its center was used in this experiment. The water wheel was made of a shaft on which paddles were attached at constant intervals. Water was put into the tank with the paddle just resting on the surface of the water. When mice were dropped into the apparatus, they tried to climb onto the wheel in order to escape from the water but could not climb sufficiently due to the rotation of the water wheel. As a result, they continued to turn the wheel. The number of rotations of the water wheel were counted by a photointerrupter attached on the shaft. The animal was placed in the apparatus 1 h after a single administration, and the turning number was registered after 9, 12, and 15 min, respectively.

Nomura has found that antidepressant agents were increasing the number of rotations and that tranquillizers, anticholinergics and antihistaminics were not effective. He suggested that this test was more appropriate as screening test for antidepressants than Porsolt's test[28] with regard to both objectivity and specificity.

Phenylquinone-Induced Writhing. This test was used to assay the binding capability of the test compounds to the central α_2-adrenoceptors. Blockade of suppressant action (antinociceptic effect) exerted by clonidine on phenylquinone-induced writhing was estimated.

After 30 min from ip administration of test substance the animals were treated per os with clonidine (0.1 mg/kg). Twenty minutes later phenylquinone (3.75 mg/kg ip in 5% aqueous ethanol) was injected, and after 5 min the animals were placed in plexiglass observation cages and an experienced observer recorded the number of animals that exhibited characteristic writhing during the next 10 min. If an animal failed to exhibit phenylquinone-induced writhing during the observation period, the animal was considered to have been protected by clonidine against the nociceptive effect of phenylquinone.

In Vitro Activities for Receptor Binding and 5HT Uptake. The in vitro activities of derivatives **7b**, **8e**, **9**, and **10** for receptor binding have been studied. The following [^{3}H] ligands (receptors) were used: [^{3}H]-*d*-fenfluramine (anorectic), [^{3}H]-8OH-DPAT (5HT-1A), [^{3}H]serotonin (5HT-1B), [^{3}H]mesulergine (5HT-1C), [^{3}H]ketanserin (5HT-2), [^{3}H]GR65630(5HT-3), [^{3}H]paroxetine (5HT uptake site), [^{3}H]prazosin (Alpha-1 Na), [^{3}H]-*p*-aminoclonidine (Alpha-2 Na), [^{3}H]DHA(Beta-2), [^{3}H]SCH 23390 (DA-1), and [^{3}H]spiperone (DA-2). The methods for binding assays we used are summarized in Table V and are reported in details elsewhere.[29-31]

For all binding assays incubations were stopped by rapid filtration under vacuum through GF/B filters which were then washed with 12 mL of cold 50 mM Tris HCl buffer, pH 7.4, and counted in 8 mL of Filter Count (Packard) in a Beckman liquid scintillation spectrometer model LS 7500 with a counting efficiency about 50%. Dose inhibition curves were analyzed by the Allfit program[32] to obtain the concentration on unlabeled drugs that caused 50% inhibition of ligands binding. [^{3}H]-5HT uptake was studied on rat brain synaptosomes, as previously described,[33] by incubating 0.1 μM [^{3}H]-5HT for 5 min at 30 °C (or 4 °C to assess passive diffusion) in presence or absence of different inhibitor concentrations.

Molecular Modeling. Materials and Methods. Molecular mechanic calculations were performed on the synthesized molecules and on imipramine and aptazepine with use of the molecular modeling programs MODEL[14] (version KS 2.95) and MMX[15] on a Digital VAXSTATION II/GPX computer and SYBYL[16] (version 5.30) on an Evans and Sutherland PS 390 color graphics terminal coupled to a Digital MicroVAX 3000 computer.

X-ray crystal coordinates[13] were used as input geometry for the imipramine molecule. All the remaining structures were generated by the DRAW option in MODEL program and initially energy minimized by the MM2/M routine of the same program until convergence.

Systematic conformational analyses were performed by using the MODEL grid method selecting the default options. Only the conformers with conformational energy within the energy window of 3 kcal/mol over the lowest minimum were kept. Such structures were further minimized by MMX program.

The final conformationally characterized MMX-minimized structures were transferred to the program SYBYL via the TTY/DATA option of MODEL program.

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Least-square matchings were performed in SYBYL by employing the MATCH routine within the COMPARISON menu.

To undertake pharmacophore mapping dummy atoms were added at the center of the benzene ring of each molecule (centroid) and along the line perpendicular to the plane of the benzene ring passing through the centroid at distances of 4.0 Å away from the centroid (normals).

Least-square fittings of the atoms used in the distance comparison (i.e. centroid, normals, and remote nitrogen) were carried out by employing the FIT option of the COMPARISON menu in SYBYL.

Constrained conformational analyses were performed on the (dimethylamino)propyl side chain of imipramine by using the systematic SEARCH function of SYBYL. The distance of the remote nitrogen from the centroid and from the normal nearest to the nitrogen, observed in the tetracyclic compounds, were imposed as constrains on imipramine and the four rotatable side-chain bonds of the molecule were allowed to rotate with a 10-deg stepwise increment of the dihedral angles.

Electrostatic isopotential maps were computed by using the POTENTIAL command in SYBYL. One level surfaces of **8d**, **8e**, and **10** were contoured at a potential energy value of −30.0 kcal/mol. In the case of imipramine a value of −20.0 kcal/mol was chosen.

Atomic point changes used to calculate the isopotential maps were obtained by the MNDO method of MOPAC through a Mulliken electron population analysis. The geometry of imipramine used in the calculation of the isopotential map was manually built up by rotating the (dimethylamino)propyl side-chain bonds in order to superimpose the remote nitrogen of imipramine to the carbonyl oxygen common to **8d**, **8e**, and **10**. This geometry was then minimized until convergence by MMX program before calculating the map.

Least-square fittings of atoms used in the isopotential maps superimposition (centroid, normals, and carbonyl oxygen for **8d**, **8e**, and **10**; centroid, normals, and remote nitrogen for imipramine) were carried out by employing the FIT option in SYBYL.

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