Synthesis and Structure−Activity Relationships of a New Model of Arylpiperazines. 5.[1] Study of the Physicochemical Influence of the Pharmacophore on 5-HT_{1A}/α_1-Adrenergic Receptor Affinity: Synthesis of a New Derivative with Mixed 5-HT_{1A}/D_2 Antagonist Properties[†]

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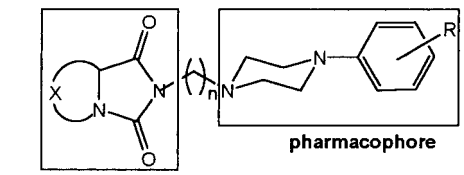
In this paper we have designed and synthesized a test series of 32 amide arylpiperazine derivatives **VI** in order to gain insight into the physicochemical influence of the pharmacophores of 5-HT_{1A} and α_1-adrenergic receptors. The training set was designed applying a fractional factorial design using six physicochemical descriptors. The amide moiety is a bicyclohydantoin or a diketopiperazine (X = $-(CH_2)_3-$, $-(CH_2)_4-$; $m = 0, 1$), the spacer length is 3 or 4 methylene units, which are the optimum values for both receptors, and the aromatic substituent R occupies the *ortho*- or *meta*-position and has been selected from a database of 387 substituents using the EDISFAR program. The 5-HT_{1A} and α_1-adrenergic receptor binding affinities of synthesized compounds **VI (1−32)** have been determined. This data set has been used to derive classical quantitative structure−activity relationships (QSAR) and neural networks models for both receptors (following paper). A comparison of these models gives information for the design of the new ligand EF-7412 **(46)** (5-HT_{1A}: $K_i = 27$ nM; α_1: $K_i > 1000$ nM). This derivative displays affinity for the dopamine D_2 receptor ($K_i = 22$ nM) and is selective versus all other receptors examined (5-HT_{2A}, 5-HT_3, 5-HT_4 and Bz; $K_i > 1000$ nM). EF-7412 **(46)** acts as an antagonist in vivo in pre- and postsynaptic 5-HT_{1A} receptor sites and as an antagonist in the dopamine D_2 receptor. Thus, EF-7412 **(46)** is a derivative with mixed 5-HT_{1A}/D_2 antagonist properties and this derivative could be useful as a pharmacological tool.

Introduction

Receptors interacting with serotonin (5-HT)[2] can be classified into at least 14 different subtypes on the basis of operational pharmacology, sequence analysis, and transduction mechanisms. During the past decade, 5-HT_{1A} receptors have been a major target for neurobiological research and drug development.[3] Many compounds, belonging to different chemical classes, diplay high affinity for 5-HT_{1A} receptors. Most of these ligands are agonists or partial agonists. Full antagonists, devoid of any agonistic activity at presynaptic or postsynaptic receptors, are still scarce. Agonists and partial agonists have been proven to be effective in anxiety and depression.[4] In addition to therapeutic applications in the field of psychiatry, more recent preclinical studies have suggested that 5-HT_{1A} receptor agonists have also pronounced neuroprotective properties.[5] Potential therapeutic applications for 5-HT_{1A} receptor antagonists are evaluated, for example, in cognition disorders.[6]

The 5-HT_{1A} receptor belongs to the class of G-protein coupled receptors (GPCRs), and the members of this class have a number of characteristic amino acid patterns in common. In particular, the transmembrane amino acid sequence of the 5-HT_{1A} subtype is noteworthy for its high degree of homology to α_1-adrenergic receptor (approximately 45%).[7] So, a great number of ligands show high 5-HT_{1A} affinity and poor selectivity. In this way, we have synthesized[8] a series of bicyclohydantoin arylpiperazines **I**, which showed affinity for



I

5-HT_{1A} and α_1-adrenergic receptors. Structure−activity relationship (SAR and 3D-QSAR) studies[9] showed that the length of the alkyl chain is of great importance for 5-HT_{1A} and α_1-adrenergic receptor affinities. As a general trend, maximum affinity for 5-HT_{1A} as well as α_1-adrenergic receptors is reached with $n = 3$ or 4, and reduction of the hydrocarbon chain by 2 and 1 carbon atoms causes a decrease in affinity in both receptors. Regarding the aromatic ring substitution it seems that *ortho*- and *meta*-positions are favorable for the affinity at both receptors, while *para*-substituted derivatives are

[†] Dedicated to Professor José Luis Soto on the occasion of his 70th birthday.

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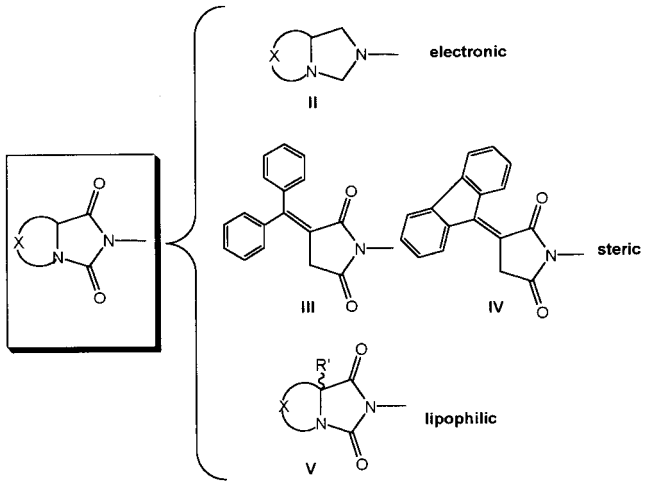
practically inactive. As part of our ongoing program to develop new selective compounds, we were interested in a systematic research based on the no-pharmacophoric and pharmacophoric sites of both receptors, to gain insight into the structural factors that are responsible for 5-HT_{1A}/α_1 selectivity.

With respect to the no-pharmacophoric sites, in recent works[1,10,11] we have described a series of derivatives **II**−**IV** in order to determine the influence of electronic and steric factors on the stabilization of receptor−ligand complex. SAR studies[10] on compounds **II**, which are devoid of the terminal amide fragment present in related 5-HT_{1A} ligands but which preserve the steric requeriments of this moiety, suggest that there is influence of electronic factors on the no-pharmacophoric part of the α_1-adrenergic receptor site; however, they have no influence on the stabilization of the 5-HT_{1A} receptor−ligand complex. In the series[1,11] of arylpiperazines **III** and **IV**, in which we have explored some steric requirements modifying the size and the shape of the amide portion, with respect to the bicyclohydantoin **I**, we could suggest that the no-pharmacophoric pocket in the 5-HT_{1A} receptor would have less restriction than the corresponding pocket in the α_1-adrenergic receptor. These studies allow us to suggest some differences between the no-pharmacophoric sites of both 5-HT_{1A} and α_1-adrenergic receptors. Regarding the influence of lipophilic factors, we have synthesized a new series of compounds **V**, but no data has been published yet.



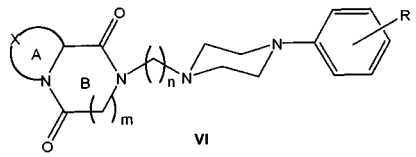
To gain insight into the physicochemical influence of 5-HT_{1A} and α_1 pharmacophores, we have designed a training set of 32 compounds of general structure **VI**. The amide moiety is a bicyclohydantoin or a diketopiperazine (X = $-(CH_2)_3-$, $-(CH_2)_4-$; m = 0, 1), the spacer length is 3 or 4 methylene units, which are the optimum values for both receptors, and the aromatic substituent R occupies the *ortho*- or *meta*-position and has been selected from a database of 387 substituents using the EDISFAR program.[12] In the present paper we report the design, synthesis and binding profile on 5-HT_{1A} and α_1-adrenergic receptors of compounds **VI**. This data set will be used to derive classical QSAR and neural networks models for both receptors (following paper), which will be useful in the design of the new ligand EF-7412 **(46)** (5-HT_{1A}: K_i = 27 nM; α_1: K_i > 1000 nM). We also report here the synthesis, binding assays, and preliminary pharmacological characterization of EF-7412 **(46)**, a derivative with mixed 5-HT_{1A}/D_2 antagonist properties.

Table 1. Values of the Parameters I_A, I_B, and I_n

parameter	−	+
I_A	$-(CH_2)_3-$	$-(CH_2)_4-$
I_B	$m = 0$	$m = 1$
I_n	$n = 3$	$n = 4$

Table 2. Marker Point Values Calculated for the Parameter Space Using Eqs 1−3

parameter	−	0	+
π	−0.85	0.25	1.34
$\sigma_{o,m}$	−0.19	0.18	0.54
MR	8.54	16.63	24.71



X = $-(CH_2)_3-$, $-(CH_2)_4-$
m = 0, 1; n = 3, 4
R = *ortho* and *meta* positions

Training Set Design

The structural field of compounds **VI** is defined with six parameters: three indicator variables (I_A, I_B and I_n) which determine the size of the A and B rings and the length of the alkyl chain, and the lipophilic (π), electronic (σ_{o-} or σ_{m-}), and steric (MR) parameters, which determine the position and nature of the aromatic substituent R. Indicator variable I_A = 0 or 1 for X = $-(CH_2)_3-$ or $-(CH_2)_4-$, I_B = 0 or 1 for m = 0 (hydantoin) or m = 1 (diketopiperazine), and I_n = 0 or 1 for n = 3 or 4.

The application of a factorial experimental design 2^n would imply the synthesis of 64 compounds (2^6). However, we have reduced the number of compounds applying a fractional factorial design: $2^{n-1} = 2^{6-1} = 32$ compounds. In this case, the last variable (MR) is a cross-term of the other five. To apply this technique all the variables were represented in a binary form (±), and each experiment was designed so each level combination occurs only once in all the series. The meaning of the parameters I_A, I_B and I_n is illustrated in Table 1. The aromatic substituent R was selected using the database (387 substituents) of the EDISFAR program.[12] This method considers three levels (+, 0, −) to each parameter, the *marker points* (Table 2). The program calculated the values of the marker points of each parameter using eqs 1−3:

$$P_0 = 1/K \cdot \sum P_i \quad (1)$$

$$P_- = P_0 - \mathrm{SD} \quad (2)$$

$$P_+ = P_0 + \mathrm{SD} \quad (3)$$

where K is the total number of selected substituents in the parameter space and SD is the standard deviation for the corresponding parameter. EDISFAR looked for the substituents which are nearest, in terms of the

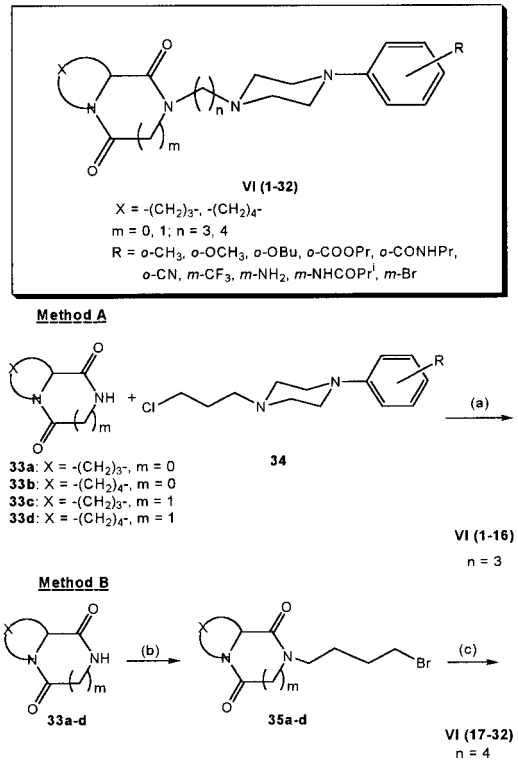
Table 3. Selected Substituents for Each Marker Point

marker points	R	marker points	R
− − −	*o*-OCH_3 or *m*-NH_2	− − +	*o*-CN
+ − −	*o*-CH_3	+ − +	*m*-Br or *m*-CF_3
− + −	*m*-$NHCOPr^i$	− + +	*o*-CONHPr
+ + −	*o*-OBu	+ + +	*o*-COOPr

Table 4. Quality Tests for the Chosen Series: Correlation Matrix, Its Determinant and Eigen Values for Orthogonality, and Relative Variance Means for Dissimilarity

	π	MR	σ	Eigen	% var	% cum
π	1			1.375	45.83	45.83
MR	0.3517	1		1.0318	34.39	80.23
σ	−0.168	0.0411	1	0.5931	19.77	100
corr matrix det = 0.8415 (opt: 1.000)						
corr coef mean = 0.1869 (opt: 0.000)						
rel var mean = 1.2219 (should be >1.000)						

Scheme 1[a]



[a] Reagents: (a) NaH, DMF, N_2, 60 °C, 1 h, then **34**, 110 °C, 1−3 h; (b) NaH, DMF, N_2, 60 °C, 1 h, then $Br(CH_2)_4Br$, 110 °C, 1−3 h; (c) 1-arylpiperazine, NEt_3, CH_3CN, 60 °C, 20−24 h.

normalized euclidean distance (NED), to the calculated marker points. So, we selected in each group the substituent nearest to the marker point or the most adequate in terms of synthetic feasibility and stability (Table 3). Once the substituents were selected, the program tested their quality evaluating the orthogonality and the dissimilarity of the chosen series, expressed as the correlation matrix and the relative variances mean, respectively (Table 4).

Chemistry

The synthesis of derivatives **VI (1−32)** listed in Table 5 is outlined in Scheme 1. Compounds **1−16** (n = 3) were obtained by reaction of the hydantoins[13,14] or diketopiperazines[15,16] **33** with the corresponding 1-aryl-4-(3-chloropropyl)piperazines **34** in the presence of sodium hydride and *N*,*N*-dimethylformamide (DMF) (method A). Treatment of 1-arylpiperazines with 1-bromo-3-chloropropane, in the presence of potassium carbonate and DMF, gave the corresponding intermediates **34**. Derivatives **17−32** (n = 4) were synthesized by treatment of intermediates **35** with the corresponding 1-arylpiperazines in the presence of triethylamine and in acetonitrile as solvent (method B). The reaction of compounds **33** with 1,4-dibromobutane gave the key intermediates **35**. When R = *m*-NH_2 or *m*-$NHCOPr^i$, compounds **VI** were prepared by catalytic hydrogenation of the corresponding nitro compounds and a subsequent reaction of the amino derivatives with isobutyryl chloride in pyridine (Scheme 2). Respective hydrochloride salts were prepared as samples for biological assays. All new compounds (Table 5) were characterized by IR and ^1H and ^{13}C NMR spectroscopy and gave satisfactory combustion analyses (C, H, N).

Biochemistry

The 5-HT_{1A} and α_1-adrenergic receptor binding affinities of synthesized compounds **VI (1−32)** were determined by measurement of the displacement of [^{3}H]-8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT) and [^{3}H]prazosin binding, respectively, in rat cerebral cortex membranes. All the compounds were used in form of hydrochloride salts and were water-soluble. The inhibition constant K_i was defined from the IC_{50} by the Cheng−Prusoff equation.[17] The results of these assays are illustrated in Table 5.

Results and Discussion

From the binding data, most of compounds **VI** demonstrated moderate to high affinity for 5-HT_{1A} and α_1-adrenergic receptor binding sites. The results, shown in Table 5, lead to a series of considerations with regard to the SAR for compounds of general structure **VI**. The wide range of receptor affinities for these compounds, which is due to a rational choice of substituents, makes this material suitable for a QSAR investigation. The results using Hansch analysis and neural networks will be presented in a subsequent article. However, some interesting SAR considerations can be made in advance.

(i) Regarding the length of the alkyl chain, although most of compounds **1−32** show affinity at both receptors, it seems that ligands **17−32** with a 4-carbon chain in the spacer display higher affinities at 5-HT_{1A} receptors than comparably substituted compounds with a 3-carbon chain spacer (e.g., **28**: K_i(5-HT_{1A}) = 3.6 nM vs **16**: K_i(5-HT_{1A}) = 214 nM; **18**: K_i(5-HT_{1A}) = 3.9 nM vs **6**: K_i(5-HT_{1A}) = 60 nM). With respect to the α_1-adrenergic receptor both series (n = 3 or 4) show a similar trend (e.g., **7**: $K_i(\alpha_1)$ = 13 nM and **19**: $K_i(\alpha_1)$ = 8.0 nM; **11**: $K_i(\alpha_1)$ = 28 nM and **31**: $K_i(\alpha_1)$ = 27 nM). This fact is in agreement with the results previously reported by us[1] for compounds **III** and **IV,** where the optimum length of the alkyl chain for 5-HT_{1A} affinity is 4 carbon atoms and for α_1-adrenergic receptors is 3−4 methylene units.

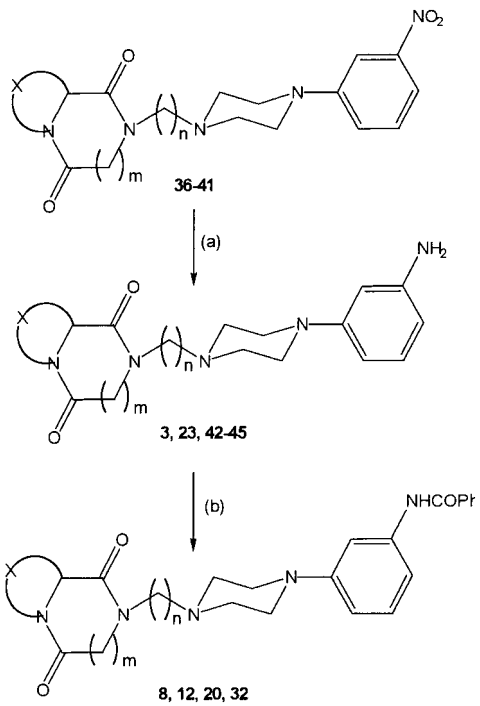
(ii) In general, the size of the amide portion in compounds **1−32** seems to have no influence on α_1-adrenergic receptor affinity (e.g., **7, 11, 19** and **31**: $K_i(\alpha_1)$ = 13, 28, 8.0 and 27 nM, respectively). Regarding the 5-HT_{1A} receptor, there is a little difference in the affinity between the two series (n = 3 or 4). For example,

Table 5. Physical Properties and in Vitro Binding Data[a]

							$K_i \pm$ SEM (nM)	
compd	X	*m*	*n*	R	mp (°C)	formula	5-HT_{1A} [^{3}H]-8-OH-DPAT	α_1 [^{3}H]prazosin
1	$-(CH_2)_3-$	0	3	*o*-OBu	187−190	$C_{23}H_{34}N_4O_3 \cdot 2HCl$	11 ± 2	30 ± 4
2	$-(CH_2)_3-$	0	3	*o*-CONHPr	181−183	$C_{23}H_{33}N_5O_3 \cdot 2HCl$	>1000	>1000
3	$-(CH_2)_3-$	0	3	*m*-NH_2	151−153	$C_{19}H_{27}N_5O_2 \cdot 2HCl \cdot 2H_2O$	>1000	>1000
4	$-(CH_2)_3-$	0	3	*m*-Br	205−207	$C_{19}H_{25}BrN_4O_2 \cdot 2HCl$	33 ± 9	29 ± 2
5	$-(CH_2)_4-$	0	3	*o*-CH_3	180−182	$C_{21}H_{30}N_4O_2 \cdot 2HCl$	134 ± 2	5.3 ± 0.2
6	$-(CH_2)_4-$	0	3	*o*-COOPr	185−186	$C_{24}H_{34}N_4O_4 \cdot HCl \cdot H_2O$	60 ± 5	41 ± 1
7	$-(CH_2)_4-$	0	3	*o*-CN	55−57	$C_{21}H_{27}N_5O_2 \cdot 2HCl \cdot {}^1/_2H_2O$	33 ± 12	13 ± 1
8	$-(CH_2)_4-$	0	3	*m*-NHCOPr^i	198−201	$C_{24}H_{35}N_5O_3 \cdot 2HCl \cdot {}^1/_2H_2O$	>1000	>10000
9	$-(CH_2)_3-$	1	3	*o*-CH_3	259−261	$C_{21}H_{30}N_4O_2 \cdot 2HCl$	27 ± 1	23 ± 1
10	$-(CH_2)_3-$	1	3	*o*-COOPr	69−70	$C_{24}H_{34}N_4O_4 \cdot HCl \cdot H_2O$	44 ± 3	26 ± 7
11	$-(CH_2)_3-$	1	3	*o*-CN	214−215	$C_{21}H_{27}N_5O_2 \cdot 2HCl$	31 ± 5	28 ± 3
12	$-(CH_2)_3-$	1	3	*m*-NHCOPr^i	193−195	$C_{24}H_{35}N_5O_3 \cdot 2HCl \cdot {}^5/_2H_2O$	326 ± 72	>1000
13	$-(CH_2)_4-$	1	3	*o*-OCH_3	149−151	$C_{22}H_{32}N_4O_3 \cdot 2HCl \cdot 2H_2O$	126 ± 8	16 ± 1
14	$-(CH_2)_4-$	1	3	*o*-OBu	187−188	$C_{25}H_{38}N_4O_3 \cdot 2HCl \cdot H_2O$	110 ± 15	79 ± 15
15	$-(CH_2)_4-$	1	3	*o*-CONHPr	104−107	$C_{25}H_{37}N_5O_3 \cdot HCl \cdot H_2O$	>10000	>1000
16	$-(CH_2)_4-$	1	3	*m*-CF_3	276−278	$C_{22}H_{29}F_3N_4O_2 \cdot 2HCl \cdot H_2O$	214 ± 21	252 ± 3
17	$-(CH_2)_3-$	0	4	*o*-CH_3	231−233	$C_{21}H_{30}N_4O_2 \cdot HCl \cdot {}^3/_2H_2O$	16 ± 2	10 ± 2
18	$-(CH_2)_3-$	0	4	*o*-COOPr	69−70	$C_{24}H_{34}N_4O_4 \cdot HCl \cdot {}^3/_2H_2O$	3.9 ± 1.4	51 ± 4
19	$-(CH_2)_3-$	0	4	*o*-CN	185−186	$C_{21}H_{27}N_5O_2 \cdot HCl$	3.7 ± 0.8	8.0 ± 1.4
20	$-(CH_2)_3-$	0	4	*m*-NHCOPr^i	189−192	$C_{24}H_{35}N_5O_3 \cdot 2HCl \cdot H_2O$	102 ± 8	>10000
21	$-(CH_2)_4-$	0	4	*o*-OBu	214−216	$C_{25}H_{38}N_4O_3 \cdot 2HCl$	1.2 ± 0.1	0.4 ± 0.3
22	$-(CH_2)_4-$	0	4	*o*-CONHPr	85−87	$C_{25}H_{37}N_5O_3 \cdot HCl \cdot {}^3/_2H_2O$	341 ± 106	>1000
23	$-(CH_2)_4-$	0	4	*m*-NH_2	167−169	$C_{21}H_{31}N_5O_2 \cdot 3HCl$	80 ± 18	215 ± 6
24	$-(CH_2)_4-$	0	4	*m*-Br	175−176	$C_{21}H_{29}BrN_4O_2 \cdot HCl \cdot H_2O$	4.0 ± 1.1	5.3 ± 0.3
25	$-(CH_2)_3-$	1	4	*o*-OCH_3	204−206	$C_{22}H_{32}N_4O_3 \cdot 2HCl \cdot H_2O$	8.6 ± 0.7	28 ± 1
26	$-(CH_2)_3-$	1	4	*o*-OBu	188−190	$C_{25}H_{38}N_4O_3 \cdot 2HCl \cdot 2H_2O$	6.5 ± 1.1	18 ± 8
27	$-(CH_2)_3-$	1	4	*o*-CONHPr	88−90	$C_{25}H_{37}N_5O_3 \cdot 2HCl \cdot H_2O$	>1000	>1000
28	$-(CH_2)_3-$	1	4	*m*-CF_3	182−183	$C_{22}H_{29}F_3N_4O_2 \cdot HCl \cdot H_2O$	3.6 ± 0.6	95 ± 11
29	$-(CH_2)_4-$	1	4	*o*-CH_3	266−267	$C_{23}H_{34}N_4O_2 \cdot HCl$	27 ± 8	29 ± 2
30	$-(CH_2)_4-$	1	4	*o*-COOPr	77−78	$C_{26}H_{38}N_4O_4 \cdot 2HCl \cdot 2H_2O$	24 ± 5	561 ± 33
31	$-(CH_2)_4-$	1	4	*o*-CN	98−100	$C_{23}H_{31}N_5O_2 \cdot HCl \cdot H_2O$	14 ± 1	27 ± 5
32	$-(CH_2)_4-$	1	4	*m*-NHCOPr^i	216−218	$C_{26}H_{39}N_5O_3 \cdot 2HCl \cdot {}^1/_2H_2O$	169 ± 64	>10000
46	$-(CH_2)_3-$	0	4	*m*-$NHSO_2Et$	187−190	$C_{22}H_{33}N_5O_4S \cdot 2HCl \cdot {}^1/_2H_2O$	27 ± 6	>10000

[a] All values are means ± SEM of 2−4 experiments performed in triplicate.

Scheme 2[a]



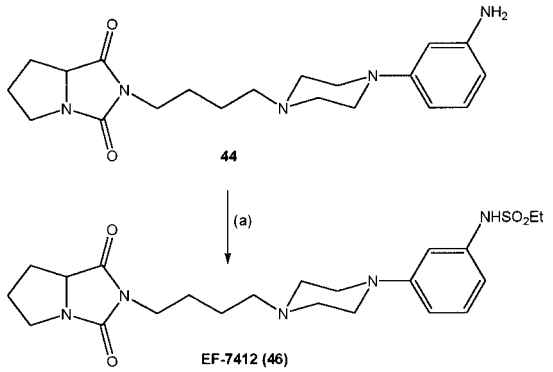
[a] Reagents: (a) H_2, Pd(C), MeOH, rt, 1−3 h; (b) $(CH_3)_2CHCOCl$, pyridine, 0 °C, 1−2 h.

comparing compound **1** (X = $-(CH_2)_3-$, *m* = 0, *n* = 3, R = *o*-OBu: K_i = 11 nM) with **14** (X = $-(CH_2)_4-$, *m* = 1, *n* = 3, R = *o*-OBu: K_i = 110 nM) there is a 10-fold decrease in 5-HT_{1A} affinity. However, the same change in compounds with *n* = 4 has less effect (e.g., ligands **17** (X = $-(CH_2)_3-$, *m* = 0, *n* = 4, R = *o*-CH_3: K_i = 16 nM) and **29** (*X* = $-(CH_2)_4-$, *m* = 1, *n* = 4, R = *o*-CH_3: K_i = 27 nM)). This fact can be explained considering that the optimum length of the alkyl chain for 5-HT_{1A} affinity is 4 carbon atoms. So, ligands with *n* = 3 and with a no-pharmacophoric part with a bigger volume (X = $-(CH_2)_4-$, *m* = 1) have higher restrictions to reach the active site of the receptor, while the bicyclohydantoin derivatives (X = $-(CH_2)_3-$, *m* = 0) allow the ligand−receptor interaction, probably because part of the hydantoin portion acts as a spacer. This hypothesis is in agreement with our previous reports.[1]

(iii) With respect to the phenylpiperazine substitution, we observe only a small influence on 5-HT_{1A}/α_1 selectivity. Regarding the *ortho*-position, electron-donating substituents (*o*-CH_3, *o*-OCH_3, *o*-OBu) as well as electron-withdrawing substituents (*o*-COOPr, *o*-CN) give the highest affinity values at both receptors. An exception is represented by *o*-CONHPr, which leads to totally inactive derivatives. Taking into account that this group has a similar van der Waals volume as *o*-COOPr and that it would not be possible for an unfavorable steric interaction, we can explain the inactivity of these derivatives by the smaller lipophilicity of this group ($\pi_{o-CONHPr} = -0.09$ vs $\pi_{o-COOPr} = 1.17$). Thus, the 5-HT_{1A}/α_1 selectivity seems to be difficult to obtain with aromatic substituents at this position.

With regard to the *meta*-position we can observe that the increase of volume in this position could lead to 5-HT_{1A}-selective compounds. So, a *m*-bromo or *m*-amino group (V_W(Br) = 17.2 Å^3; $V_W(NH_2)$ = 11.4 Å^3) leads to

Scheme 3[a]



[a] Reagents: (a) $EtSO_2Cl$, pyridine, acetone, N_2, 24 h.

compounds with the same affinity at both receptors (e.g., derivatives **23** and **24**). A trifluoromethyl group (V_W = 24.2 Å^3) at this position leads to an increase in the 5-HT_{1A} selectivity (compound **28**: 26-fold). But the best selectivity ratio is reached with the most voluminous group, a *m*-$NHCOPr^i$ (V_W = 70.8 Å^3) which leads to the most selective compounds **20** and **32** (98- and 59-fold, respectively).

With these previous data, our aim is to gain deeper insight into the physicochemical factors for 5-HT_{1A}/α_1 selectivity. Using classical QSAR (Hansch analysis) and artificial neural networks (ANNs) studies in both receptors, we have designed the new ligand EF-7412 **(46)** (X = $-(CH_2)_3-$, m = 0, n = 4, R = *m*-$NHSO_2Et$) (subsequent paper). On this basis, EF-7412 **(46)** was synthesized by reaction of the amino derivative **44** with ethylsulfonyl chloride in the presence of pyridine in anhydrous acetone as solvent (Scheme 3) and the hydrochloride salt was prepared as a sample for biological assays. This compound bound at 5-HT_{1A} sites (K_i = 27 nM) and showed no significant affinity for the α_1-adrenergic receptor (K_i > 1000 nM) (Table 5). Due to the high 5-HT_{1A} affinity and selectivity over the α_1-adrenergic receptor, compound EF-7412 **(46)** was selected for further binding and pharmacological studies. Binding affinities show that compound EF-7412 **(46)** exhibits an appreciable affinity for the D_2 receptor subtype (K_i = 22 nM) and is selective versus all other receptors examined (5-HT_{2A}, 5-HT_3, 5-HT_4, and benzodiazepine (Bz); K_i > 1000 nM).

Pharmacological Results. Pharmacological evaluation of the activity of EF-7412 **(46)** on 5-HT_{1A} receptor function was assessed by measuring mouse rectal temperature, 5-HT_{1A} agonist-induced behavioral syndrome in the rat (flat body posture (FBP) and lower lip retraction (LLR)), corticosterone plasma levels in the rat, and 5-hydroxyindoleacetic acid (5-HIAA)/5-HT in mice hypothalamus. Dopamine receptor function was assesed in mice by measuring 3,4-dihydroxyphenylacetic acid (DOPAC)/dopamine (DA) ratio in hypothalamus, and spontaneous locomotor activity in the open field.

Presynaptic 5-HT_{1A} receptor function was assessed by measuring mouse rectal temperature. Doses ranging from 5 to 20 mg/kg of EF-7412 **(46)** did not alter rectal temperature in mice (Figure 1) but blocked hypothermia induced by the administration of 8-OH-DPAT (0.5 mg/kg) (Figure 2). The action of EF-7412 **(46)** at postsynaptic sites was evaluated by measuring the intensity of the 5-HT_{1A} agonist-induced behavioral syndrome in the

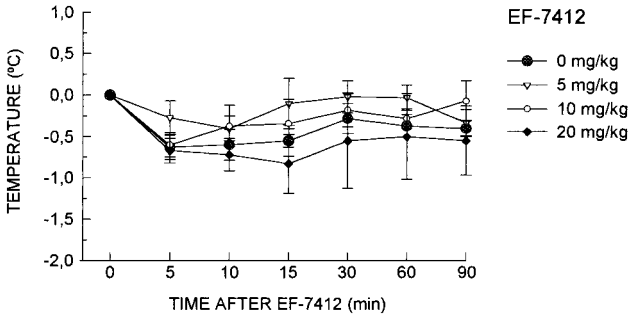


Figure 1. Dose−response and time-course effects of EF-7412 **(46)** on rectal temperature. Mouse rectal temperature was measured 0, 5, 10, 15, 30, 60, and 90 min after sc administration of either vehicle (water, 4 mL/kg) or doses of EF-7412 **(46)** (0, 5, 10, and 20 mg/kg). Values represent the means ± SEM of rectal temperature in 6−8 mice. A hypothermic response was considered when decrease of rectal temperature was more than 1.1 °C.

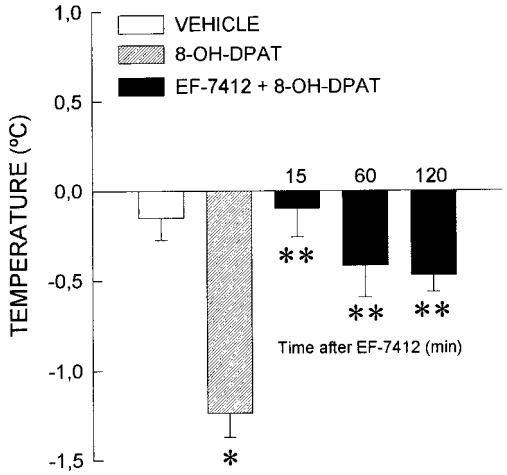


Figure 2. Time-course effects of EF-7412 **(46)** on 8-OH-DPAT-induced hypothermia. Mouse rectal temperature was measured before the administration of vehicle (water, 4 mL/kg) or EF-7412 **(46)** (20 mg/kg). Vehicle or EF-7412 **(46)** was given 15, 60, and 120 min before 8-OH-DPAT. Rectal temperature was measured 30 min after 8-OH-DPAT (0.5 mg/kg). Each bar represents the means ± SEM of rectal temperature in 6−8 mice. *Values from the 8-OH-DPAT group (line bar) that are significantly different from the vehicle control group (white bar). **Values from the EF-7412 **(46)** groups (black bars) that are significantly different (P < 0.05) from the 8-OH-DPAT control group.

rat (FBP and LLR) and the increase of corticosterone secretion in the rat. The administration of EF-7412 **(46)** (10 mg/kg) 60 min before behavioral evaluation did not alter FBP or LLR, but pretreatment with EF-7412 **(46)** (1−10 mg) 30 min before the administration of 8-OH-DPAT (0.5 mg/kg) completely blocked both effects (Figure 3). Similarly, doses of EF-7412 **(46)** in the range 0.3−10 mg/kg did not alter corticosterone plasma levels in the rat, but pretreatment with EF-7412 **(46)** (10 mg/kg) 30 min before the administration of 8-OH-DPAT (1 mg/kg) significantly attenuated the enhancement of corticosterone secretion induced by 8-OH-DPAT (Figure 4). The administration of EF-7412 **(46)** induced an increase in 5-HIAA/5-HT and DOPAC/dopamine ratios in whole hypothalamus of mice. The administration of 8-OH-DPAT blocks the increase in 5-HT neuronal activity induced by EF-7412 **(46)** but was without effect on dopamine neuronal activity (Figure 5A,B). Further

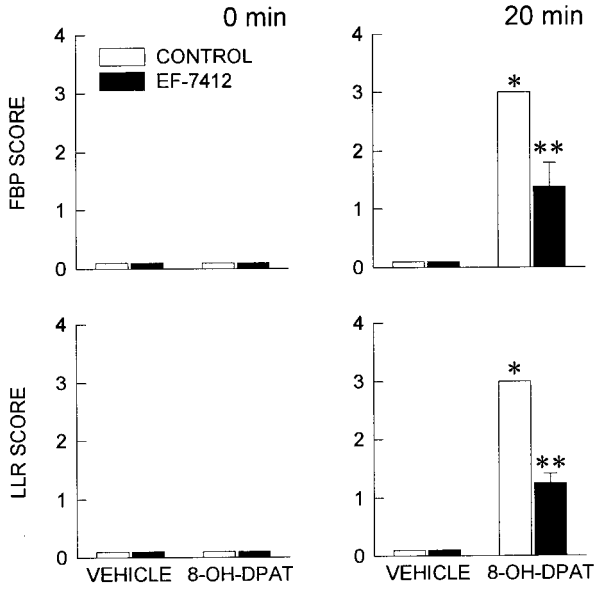


Figure 3. Time-course effects of EF-7412 **(46)** on FBP and LLR induced by 8-OH-DPAT. According to an arbitrary scale going from 0 (no effect) to 3 (maximum effect), FBP and LLR were evaluated before and 20 min after sc administration of vehicle (water, 4 mL/kg) or 8-OH-DPAT (1 mg/kg). Vehicle or EF-7412 **(46)** (10 mg/kg) was given 30 min before 8-OH-DPAT. Each bar represents the means ± SEM of a FBP or LLR score of 8 rats. *Values from the 8-OH-DPAT control group that are significantly different ($P < 0.05$) from the vehicle control group. **Values from the EF-7412 **(46)**/8-OH-DPAT group that are significantly different ($P < 0.05$) from the 8-OH-DPAT control group. Means score of vehicle-treated rats was 0.

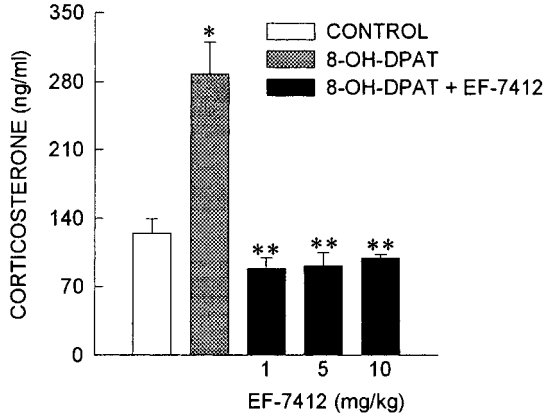


Figure 4. EF-7412 **(46)** effects on plasma corticosterone increase induced by 8-OH-DPAT in rats. Vehicle (water, 4 mL/kg) or 8-OH-DPAT (1 mg/kg) was administered 30 min after administration of vehicle or EF-7412 **(46)** (10 mg/kg). Values represent the mean ± SEM of plasma corticosterone levels in 6−8 rats. *Values from the 8-OH-DPAT group that are significantly different ($P < 0.05$) from the vehicle control group. **Values from the EF-7412 **(46)**/8-OH-DPAT group that are significantly different ($P < 0.05$) from the 8-OH-DPAT control group.

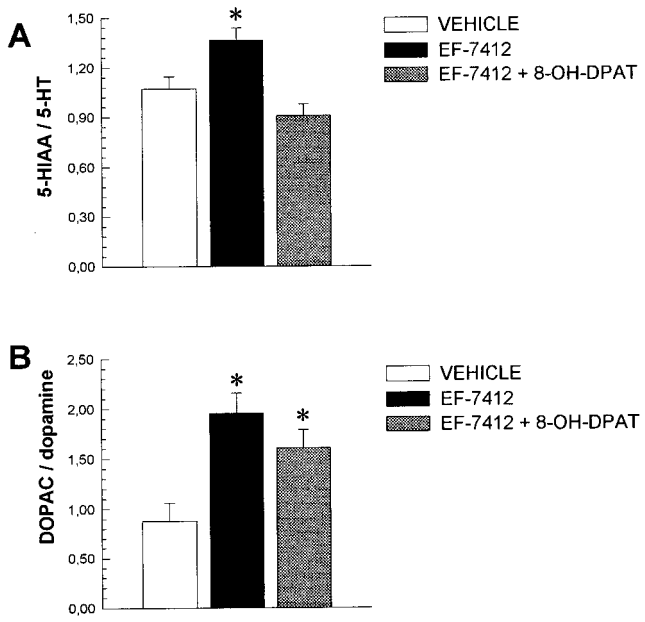


Figure 5. Effects of EF-7412 **(46)** on 5-HIAA/5-HT (A) and DOPAC/dopamine (B) ratios in mouse whole hypothalamus. 5-HIAA, 5-HT, DOPAC, and dopamine concentrations were measured 60 min after administration of vehicle (water, 4 mL/kg sc) or EF-7412 **(46)** (10 mg/kg). 30 min after EF-7412 **(46)**, mice received 8-OH-DPAT (0.3 mg/kg). Values represent the means ± SEM of 5-HIAA/5-HT and DOPAC/dopamine ratios in vehicle- or drug-treated mice. *Values from EF-7412 **(46)** or EF-7412 **(46)**/8-OH-DPAT-treated mice that are significantly different ($P < 0.05$) from their respective vehicle-treated mouse control group.

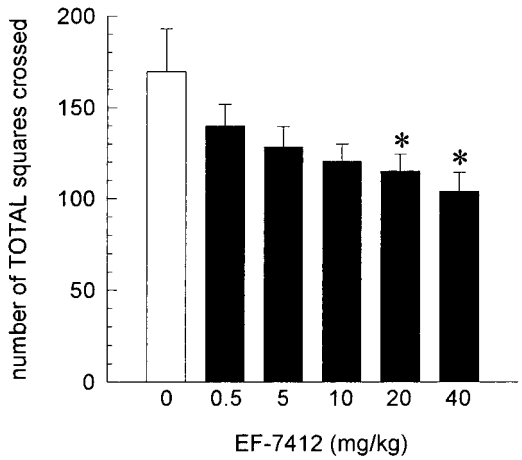


Figure 6. Dose−response effects of EF-7412 **(46)** on locomotor activity in mice. The total squares crossed by a mouse were counted, 60 min after administration of either vehicle (water, 4 mL/kg sc) or doses of EF-7412 **(46)** (0.5, 2.5, 10, 20, and 40 mg/kg). Values represent the means ± SEM of total squares crossed during 5 min test in vehicle- and drug-treated mice. *Values from 8-OH-DPAT-treated mice that are significantly different ($P < 0.05$) from their respective vehicle-treated group.

evaluation of EF-7412 **(46)** at the D_2 dopamine receptor was carried out by examining the effects of this compound on spontaneous locomotor activity in the open field. The results showed that EF-7412 **(46)** produces a slight decrease in spontaneous locomotor activity at the highest dose used (Figure 6). Taken together, these results suggest that EF-7412 **(46)** may be acting as an antagonist at both 5-HT_{1A} and D_2 dopamine receptors. Thus, a compound with dual action such as EF-7412 **(46)** may contribute to better understanding the role of simultaneous blockade of both 5-HT_{1A} and D_2 receptors upon serotoninergic and dopaminergic transmission. Further pharmacological studies are being carried out in our laboratory in order to determine the potential antagonist properties of EF-7412 **(46)** at the dopamine D_2 receptors. The goal of these studies is to examine the ability of EF-7412 **(46)** to block behaviors mediated by D_2 receptors such as climbing behavior induced by

apomorphine, stereotypy and increased locomotor activity induced by amphetamine, and the presence or lack of catalepsy induced by higher doses of this compound. These results will be included in a pharmacological manuscript.

Conclusions

We report on the design and synthesis of a training set of 32 arylpiperazines of general structure **VI** with affinity for 5-HT_{1A} and α_1-adrenergic receptors. To gain insight into the physicochemical influence of the pharmacophore on 5-HT_{1A}/α_1 selectivity, classical QSAR and neural networks models for both receptors are described in the following paper. A comparison of these models gives information for the design and synthesis of a new ligand EF-7412 **(46)** (5-HT_{1A}: $K_i = 27$ nM; α_1: $K_i > 1000$ nM). This compound displays affinity for the dopamine D_2 receptor ($K_i = 22$ nM) and is selective versus all other receptors examined (5-HT_{2A}, 5-HT_3, 5-HT_4 and Bz). Pharmacological evaluation of the activity of EF-7412 **(46)** suggests that it acts as an antagonist in vivo at pre- and postsynaptic 5-HT_{1A} receptor sites and as an antagonist at the dopamine D_2 receptor.

In this study we describe a derivative (EF-7412, **46**) with mixed 5-HT_{1A}/D_2 antagonist properties, and this derivative could be useful as a pharmacological tool.

Experimental Section

Chemistry. Melting points (uncorrected) were determined on a Gallenkamp electrothermal apparatus. Infrared (IR) spectra were obtained on a Perkin-Elmer 781 infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300S or Bruker 250-AM instrument. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane; coupling constants (J) are in hertz. Elemental analyses (C, H, N) were determined within 0.4% of the theoretical values. Thin-layer chromatography (TLC) was run on Merck silica gel 60 F-254 plates. For normal pressure and flash chromatography, Merck silica gel type 60 (size 70−230 and 230−400 mesh, respectively) was used. Unless stated otherwise, starting materials used were high-grade commercial products.

The hydantoins **33a,b** and the diketopiperazines **33c,d** were prepared following the procedure reported in refs 13−16. Noncommercial 1-arylpiperazines (R = *o*-OBu, *o*-COOPr, *o*-CONHPr, *m*-Br, *m*-NO_2) were prepared by reaction of the corresponding anilines with bis(2-chloroethyl)amine, as previously described.[18,19] 1-(*o*-Cyanophenyl)piperazine[18b] was obtained by reaction of *o*-bromobenzonitrile with piperazine. 1-(3-Chloropropyl)-4-(*o*-tolyl)piperazine[20] (**34a**), 1-(3-chloropropyl)-4-(*o*-methoxyphenyl)piperazine[21] (**34b**) and compounds **35a,b**[8c] were synthesized according to the literature.

1-Aryl-4-(3-chloropropyl)piperazines 34c−i. General Procedure. To a stirred suspension of anhydrous potassium carbonate (1.7 g, 15 mmol), the corresponding 1-arylpiperazine (12 mmol) in anhydrous *N*,*N*-dimethylformamide (12 mL), under argon at room temperature, was added dropwise 1-bromo-3-chloropropane (1.5 mL, 15 mmol). The reaction mixture was stirred at room temperature for 16−24 h and was filtered to eliminate the potassium carbonate. The solvent was evaporated under reduced pressure and the resultant oil was purified by column cromatography (eluent: hexane/ethyl acetate, relative proportions depending upon the compound).

4-(3-Chloropropyl)-1-(*o*-butoxyphenyl)piperazine (34c): yield 1.98 g (53%); oil; ^{1}H NMR ($CDCl_3$) δ 0.99 (t, 3H), 1.52 (sex, 2H), 1.83 (qt, 2H), 2.00 (qt, 2H), 2.57 (t, 2H), 2.64 (brs, 4H), 3.11 (brs, 4H), 3.63 (t, 2H), 3.99 (t, 2H), 6.84−6.98 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 13.8, 19.3, 29.7, 31.3, 43.1, 50.4, 53.4, 55.4, 67.4, 112.0, 117.9, 120.7, 122.5, 141.1, 151.5. Anal. ($C_{17}H_{27}ClN_2O$) C, H, N.

1-(3-Chloropropyl)-4-(*o*-(propoxycarbonyl)phenyl)piperazine (34d): yield 2.69 g (69%); oil. Anal. ($C_{17}H_{25}ClN_2O_2$) C, H, N.

1-(3-Chloropropyl)-4-(*o*-(propylcarbamoyl)phenyl)piperazine (34e): yield 2.76 g (71%); oil. Anal. ($C_{17}H_{26}ClN_3O$) C, H, N.

1-(3-Chloropropyl)-4-(*o*-cyanophenyl)piperazine (34f): yield 2.06 g (65%); oil. Anal. ($C_{14}H_{18}ClN_3$) C, H, N.

1-(3-Chloropropyl)-4-(*m*-(trifluoromethyl)phenyl)piperazine (34g): yield 2.61 g (71%); oil. Anal. ($C_{14}H_{18}ClF_3N_2$) C, H, N.

1-(3-Chloropropyl)-4-(*m*-nitrophenyl)piperazine (34h): yield 2.55 g (75%); oil. Anal. ($C_{13}H_{18}ClN_3O_2$) C, H, N.

4-(3-Chloropropyl)-1-(*m*-bromophenyl)piperazine (34i): yield 2.44 g (64%); oil. Anal. ($C_{13}H_{18}BrClN_2$) C, H, N.

General Procedure for the Synthesis of Compounds 35c,d. To a suspension of the corresponding diketopiperazines **33c,d** (26 mmol) in anhydrous *N*,*N*-dimethylformamide (30 mL) was added 60% NaH (1.0 g, 26 mmol). After stirring for 1 h at 60 °C under argon, a solution of 1,4-dibromobutane (11.2 g, 52 mmol) in anhydrous *N*,*N*-dimethylformamide (25 mL) was added dropwise. The mixture was refluxed under argon at 110 °C for 1−3 h. Then, the solvent was evaporated under reduced pressure, and the residue was resuspended in water (50 mL) and extracted with methylene chloride (3 × 50 mL). The combined organic layers were washed with water and dried over $MgSO_4$. After evaporation of the solvent, the crude oil was purified by column chromatography and then distillation.

2-(4-Bromobutyl)-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (35c): yield 4.14 g (55%); bp 200−220 °C (0.4 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.66−2.16 (m, 7H), 2.36−2.45 (m, 1H), 3.32−3.69 (m, 6H), 3.80 (d, 1H), 4.10 (t, 1H), 4.16 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 22.6, 25.6, 28.9, 29.5, 33.0, 45.1, 45.3, 51.6, 59.0, 163.1, 167.3. Anal. ($C_{11}H_{17}BrN_2O_2$) C, H, N.

2-(4-Bromobutyl)-1,4-dioxoperhydropyrido[1,2-*a*]pyrazine (35d): yield 4.10 g (52%); bp 145−155 °C (0.2 mmHg). Anal. ($C_{12}H_{19}BrN_2O_2$) C, H, N.

Method A. Preparation of Compounds 1, 2, 4−7, 9−11, 13−16, and 36−38. To a solution of the corresponding hydantoins or diketopiperazines **33a−d** (6 mmol) in anhydrous *N*,*N*-dimethylformamide (3.3 mL) was added slowly 60% NaH (0.25 g, 6 mmol), and the mixture was warmed under argon at 60 °C for 1 h. After stirring 1 h, a solution of the corresponding 1-aryl-4-(3-chloropropyl)piperazine **34** (6 mmol) in anhydrous *N*,*N*-dimethylformamide (3.3 mL) was added dropwise and the mixture was refluxed at 110 °C under argon for 1−2 h. Then, the solvent was evaporated under reduced pressure, the residue was resuspended in water (50 mL) and extracted with methylene chloride (3 × 50 mL). The combined organic layers were washed with water and dried over anhydrous $MgSO_4$. After evaporation of the solvent, the crude oil was purified by column chromatography (eluents: hexane/ethyl acetate, ethyl acetate/ethanol or chloroform/methanol, relative proportions depending upon the compound). Spectral data of title compounds refer to the free bases, and then hydrochloride salts were prepared.

2-[3-[4-(*o*-Butoxyphenyl)piperazin-1-yl]propyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (1): yield 2.19 g (75%); mp 187−190 °C (methanol/ethyl ether); ^{1}H NMR ($CDCl_3$) δ 0.99 (t, J = 7.5, 3H, CH_3), 1.51 (sex, J = 7.5, 2H, CH_3CH_2), 1.65−1.72 (m, 1H, H_7), 1.77−1.86 (m, 4H, CH_2, CH_2CH_2O), 2.03−2.10 (m, 2H, $2H_6$), 2.22−2.26 (m, 1H, H_7), 2.45 (t, J = 7.5, 2H, CH_2-Npip), 2.62 (brs, 4H, $2CH_2$-pip), 3.10 (brs, 4H, $2CH_2$-pip), 3.20−3.28 (m, 1H, H_5), 3.55 (td, J = 7.5, 0.9, 2H, NCH_2), 3.68 (dt, J = 11.1, 7.8, 1H, H_5), 3.98 (t, J = 6.6, 2H, OCH_2), 4.08 (dd, J = 9.0, 7.5, 1H, H_{7a}), 6.83−6.97 (m, 4H, ArH); ^{13}C NMR ($CDCl_3$) δ 13.8 (CH_3), 19.3 (CH_3CH_2), 25.0 (CH_2), 26.9 (C_6), 27.4 (C_7), 31.3 (CH_2CH_2O), 37.3 (NCH_2), 45.4 (C_5), 50.4 ($2CH_2$-pip), 53.3 ($2CH_2$-pip), 55.7 (CH_2-Npip), 63.2 (C_{7a}), 67.4 (OCH_2), 111.9 (C_6-phenyl), 117.9 (C_3-phenyl), 120.7 (C_4-phenyl), 122.5 (C_5-phenyl), 141.1 (C_1-phenyl), 151.5 (C_2-phenyl), 160.7 (C_3), 173.8 (C_1). Anal. ($C_{23}H_{34}N_4O_3 \cdot 2HCl$) C, H, N.

2-[3-[4-(*o*-(Propylcarbamoyl)phenyl)piperazin-1-yl]propyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2): yield 1.95 g (65%); mp 181−183 °C (methanol/ethyl ether). Anal. ($C_{23}H_{33}N_5O_3$·2HCl) C, H, N.

2-[3-[4-(*m*-Bromophenyl)piperazin-1-yl]propyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (4): yield 2.28 g (77%); mp 205−207 °C (methanol/ethyl ether). Anal. ($C_{19}H_{25}BrN_4O_2$·2HCl) C, H, N.

2-[3-[4-(*m*-Nitrophenyl)piperazin-1-yl]propyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (36): yield 1.93 g (76%); mp 223−226 °C (methanol/ethyl ether). Anal. ($C_{19}H_{25}N_5O_4$·HCl) C, H, N.

2-[3-[4-(*o*-Tolyl)piperazin-1-yl]propyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (5): yield 2.15 g (81%); mp 180−182 °C (methanol/ethyl ether); ^{1}H NMR ($CDCl_3$) δ 1.24−1.50 (m, 3H, H_{6ax}, H_{7ax}, H_{8ax}), 1.73 (d, J = 9.6, 1H, H_{6ec}), 1.83 (qt, J = 7.5, 2H, CH_2), 1.97 (d, J = 12.9, 1H, H_{7ec}), 2.20 (dd, J = 12.6, 3.0, 1H, H_{8ec}), 2.27 (s, 3H, CH_3), 2.44 (t, J = 7.5, 2H, CH_2-Npip), 2.57 (brs, 4H, 2CH_2-pip), 2.82 (td, J = 13.2, 3.3, 1H, H_{5ax}), 2.90 (t, J = 4.8, 4H, 2CH_2-pip), 3.57 (t, J = 7.5, 2H, NCH_2), 3.73 (dd, J = 11.7, 3.9, 1H, H_{8a}), 4.16 (dd, J = 12.9, 4.2, 1H, H_{5ec}), 6.92−7.01 (m, 2H, H_4- and H_6-phenyl), 7.12−7.16 (m, 2H, H_3- and H_5-phenyl); ^{13}C NMR ($CDCl_3$) δ 17.8 (CH_3), 22.7 (C_7), 24.9 (C_6), 25.3 (CH_2), 27.7 (C_8), 37.1 (NCH_2), 39.2 (C_5), 51.6 (2CH_2-pip), 53.6 (2CH_2-pip), 55.8 (CH_2-Npip), 57.3 (C_{8a}), 118.8 (C_6-phenyl), 122.9 (C_4-phenyl), 126.4 (C_5-phenyl), 130.9 (C_3-phenyl), 132.4 (C_2-phenyl), 151.4 (C_1-phenyl), 154.5 (C_3), 173.1 (C_1). Anal. ($C_{21}H_{30}N_4O_2$·2HCl) C, H, N.

2-[3-[4-(*o*-(Propoxycarbonyl)phenyl)piperazin-1-yl]propyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (6): yield 2.21 g (74%); mp 185−186 °C (methanol/ethyl ether). Anal. ($C_{24}H_{34}N_4O_4$·HCl·H_2O) C, H, N.

2-[3-[4-(*o*-Cyanophenyl)piperazin-1-yl]propyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (7): yield 2.34 g (84%); mp 55−57 °C (methanol/ethyl ether). Anal. ($C_{21}H_{27}N_5O_2$·2HCl·$^1/_2H_2O$) C, H, N.

2-[3-[4-(*m*-Nitrophenyl)piperazin-1-yl]propyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (37): yield 1.60 g (61%); mp 228−231 °C (acetone). Anal. ($C_{20}H_{27}N_5O_4$·HCl) C, H, N.

2-[3-[4-(*o*-Tolyl)piperazin-1-yl]propyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (9): yield 2.26 g (85%); mp 259−261 °C (methanol/ethyl ether); ^{1}H NMR ($CDCl_3$) δ 1.79 (qt, J = 7.2, 2H, CH_2), 1.92−2.08 (m, 3H, 2H_7, H_8), 2.29 (s, 3H, CH_3), 2.35−2.39 (m, 1H, H_8), 2.43 (t, J = 6.9, 2H, CH_2-Npip), 2.59 (brs, 4H, 2CH_2-pip), 2.93 (t, J = 4.8, 4H, 2CH_2-pip), 3.39−3.46 (m, 1H, 1NCH_2), 3.52−3.64 (m, 3H, 1NCH_2, 2H_6), 3.87 (d, J = 16.5, 1H, H_{3b}), 4.08 (t, J = 7.2, 1H, H_{8a}), 4.19 (d, J = 16.5, 1H, H_{3a}), 6.97 (td, J = 6.6, 1.2, 1H, H_4-phenyl), 7.02 (d, J = 7.2, 1H, H_6-phenyl), 7.14−7.18 (m, 2H, H_3- and H_5-phenyl); ^{13}C NMR ($CDCl_3$) δ 17.7 (CH_3), 22.5 (C_7), 24.3 (CH_2), 28.6 (C_8), 44.7 (NCH_2), 45.0 (C_6), 51.5 (2CH_2-pip), 52.1 (C_3), 53.5 (2CH_2-pip), 55.3 (CH_2-Npip), 58.9 (C_{8a}), 118.7 (C_6-phenyl), 122.9 (C_4-phenyl), 126.3 (C_5-phenyl), 130.8 (C_3-phenyl), 132.3 (C_2-phenyl), 151.2 (C_1-phenyl), 163.2 (C_4), 167.1 (C_1). Anal. ($C_{21}H_{30}N_4O_2$·2HCl) C, H, N.

2-[3-[4-(*o*-(Propoxycarbonyl)phenyl)piperazin-1-yl]propyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (10): yield 1.94 g (65%); mp 69−70 °C (acetone). Anal. ($C_{24}H_{34}N_4O_4$·HCl·H_2O) C, H, N.

2-[3-[4-(*o*-Cyanophenyl)piperazin-1-yl]propyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (11): yield 2.45 g (90%); mp 214−215 °C (acetone). Anal. ($C_{21}H_{27}N_5O_2$·2HCl) C, H, N.

2-[3-[4-(*m*-Nitrophenyl)piperazin-1-yl]propyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (38): yield 0.97 g (37%); mp 247−249 °C (methanol/ethyl ether). Anal. ($C_{20}H_{27}N_5O_4$·HCl) C, H, N.

2-[3-[4-(*o*-Methoxyphenyl)piperazin-1-yl]propyl]-1,4-dioxoperhydropyrido[1,2-*a*]pyrazine (13): yield 1.80 g (59%); mp 149−151 °C (methanol/ethyl ether); ^{1}H NMR ($CDCl_3$) δ 1.39−1.61 (m, 3H, H_{7ax}, H_{8ax}, H_{9ax}), 1.70−1.76 (m, 1H, H_{7ec}), 1.82 (qt, J = 7.1, 2H, CH_2), 1.99 (d, J = 12.5, 1H, H_{8ec}), 2.36 (dm, J = 13.3, 1H, H_{9ec}), 2.44 (t, J = 6.9, 2H, CH_2-Npip), 2.52 (td, J = 13.0, 2.5, 1H, H_{6ax}), 2.64 (brs, 4H, 2CH_2-pip), 3.09 (brs, 4H, 2CH_2-pip), 3.44 (t, J = 7.2, 2H, NCH_2), 3.83 (d, J = 12.0, 1H, H_{9a}), 3.86 (s, 3H, OCH_3), 4.02 (s, 2H, 2H_3), 4.67 (dm, J = 13.2, 1H, H_{6ec}), 6.84−7.02 (m, 4H, ArH); ^{13}C NMR ($CDCl_3$) δ 23.6 (CH_2), 24.2, 24.4 (C_7,C_8), 31.2 (C_9), 42.3 (C_6), 44.5 (NCH_2), 49.4 (C_3), 50.5 (2CH_2-pip), 53.3 (2CH_2-pip), 55.2 (OCH_3), 55.4 (CH_2-Npip), 59.1 (C_{9a}), 111.0 (C_6-phenyl), 118.1 (C_3-phenyl), 120.8 (C_4-phenyl), 122.8 (C_5-phenyl), 141.1 (C_1-phenyl), 152.1 (C_2-phenyl), 161.6 (C_4), 165.1 (C_1). Anal. ($C_{22}H_{32}N_4O_3$·2HCl·2H_2O) C, H, N.

2-[3-[4-(*o*-Butoxyphenyl)piperazin-1-yl]propyl]-1,4-dioxoperhydropyrido[1,2-*a*]pyrazine (14): yield 1.66 g (52%); mp 187−188 °C (acetone). Anal. ($C_{25}H_{38}N_4O_3$·2HCl·H_2O) C, H, N.

2-[3-[4-(*o*-(Propylcarbamoyl)phenyl)piperazin-1-yl]propyl]-1,4-dioxoperhydropyrido[1,2-*a*]pyrazine (15): yield 2.11 g (69%); mp 104−107 °C (methanol/ethyl ether). Anal. ($C_{25}H_{37}N_5O_3$·HCl·H_2O) C, H, N.

2-[3-[4-(*m*-(Trifluoromethyl)phenyl)piperazin-1-yl]propyl]-1,4-dioxoperhydropyrido[1,2-*a*]pyrazine (16): yield 2.32 g (73%); mp 276−278 °C (methanol/ethyl ether). Anal. ($C_{22}H_{29}F_3N_4O_2$·2HCl·H_2O) C, H, N.

Method B. Preparation of Derivatives 17−19, 21, 22, 24−31, and 39−41. To a suspension of the corresponding derivatives **35a**−**d** (9 mmol) and the arylpiperazine (15 mmol) in acetonitrile (19 mL) was added 2.0 mL of triethylamine (1.5 g, 14.6 mmol). The mixture was refluxed for 20−24 h. Then, the solvent was evaporated under reduced pressure and the residue was resuspended in water and extracted with dichloromethane (3 × 100 mL). The combined organic layers were washed with water and dried over $MgSO_4$. After evaporation of the solvent the crude oil was purified by column chromatography (eluents: hexane/ethyl acetate, ethyl acetate/ethanol or chloroform/methanol, relative proportions depending upon the compound). Spectral data refer to the free base and then hydrochloride salts were prepared.

2-[4-[4-(*o*-Tolyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (17): yield 2.81 g (72%); mp 231−233 °C (methanol/ethyl ether); ^{1}H NMR ($CDCl_3$) δ 1.49−1.74 (m, 5H, -$(CH_2)_2$-, H_7), 1.98−2.11 (m, 2H, 2H_6), 2.19−2.23 (m, 1H, H_7), 2.29 (s, 3H, CH_3), 2.42 (t, J = 7.5, 2H, CH_2-Npip), 2.59 (brs, 4H, 2CH_2-pip), 2.93 (t, J = 5.1, 4H, 2CH_2-pip), 3.20−3.26 (m, 1H, H_5), 3.50 (t, J = 7.5, 2H, NCH_2), 3.67 (dt, J = 11.1, 6.9, 1H, H_5), 4.06 (dd, J = 9.3, 7.2, 1H, H_{7a}), 6.96 (td, J = 8.4, 1.2, 1H, H_4-phenyl), 7.01 (dd, J = 6.9, 1.5, 1H, H_6-phenyl), 7.13−7.17 (m, 2H, H_3- and H_5-phenyl); ^{13}C NMR ($CDCl_3$) δ 17.7 (CH_3), 23.9, 25.9 (-$(CH_2)_2$-), 26.8 (C_6), 27.4 (C_7), 38.6 (NCH_2), 45.3 (C_5), 51.5 (2CH_2-pip), 53.5 (2CH_2-pip), 57.9 (CH_2-Npip), 63.1 (C_{7a}), 118.7 (C_6-phenyl), 122.8 (C_4-phenyl), 126.3 (C_5-phenyl), 130.8 (C_3-phenyl), 132.3 (C_2-phenyl), 151.3 (C_1-phenyl), 160.6 (C_3), 173.8 (C_1). Anal. ($C_{21}H_{30}N_4O_2$·HCl·$^3/_2H_2O$) C, H, N.

2-[4-[4-(*o*-(Propoxycarbonyl)phenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (18): yield 2.28 g (50%); mp 69−70 °C (acetone). Anal. ($C_{24}H_{34}N_4O_4$·HCl·$^3/_2H_2O$) C, H, N.

2-[4-[4-(*o*-Cyanophenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (19): yield 2.41 g (64%); mp 185−186 °C (methanol/ethyl ether). Anal. ($C_{21}H_{27}N_5O_2$·HCl) C, H, N.

2-[4-[4-(*m*-Nitrophenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (39): yield 2.29 g (58%); mp 197−198 °C (methanol/ethyl ether). Anal. ($C_{20}H_{27}N_5O_4$·HCl) C, H, N.

2-[4-[4-(*o*-Butoxyphenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (21): yield 2.92 g (63%); mp 214−216 °C (methanol/ethyl ether). Anal. ($C_{25}H_{38}N_4O_3$·2HCl) C, H, N.

2-[4-[4-(*o*-(Propylcarbamoyl)phenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (22): yield 2.29 g (49%); mp 85−87 °C (methanol/ethyl ether). Anal. ($C_{25}H_{37}N_5O_3$·HCl·$^3/_2H_2O$) C, H, N.

2-[4-[4-(*m*-Bromophenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (24): yield 2.95 g

(65%); mp 175−176 °C (methanol/ethyl ether). Anal. ($C_{21}H_{29}BrN_4O_2 \cdot HCl \cdot H_2O$) C, H, N.

2-[4-[4-(*m*-Nitrophenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (40): yield 2.80 g (65%); mp 65−67 °C (methanol/ethyl ether). Anal. ($C_{21}H_{29}N_5O_4 \cdot HCl \cdot {}^3/_2H_2O$) C, H, N.

2-[4-[4-(*o*-Methoxyphenyl)piperazin-1-yl]butyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (25): yield 2.30 g (52%); mp 204−206 °C (methanol/ethyl ether). Anal. ($C_{22}H_{32}N_4O_3 \cdot 2HCl \cdot H_2O$) C, H, N.

2-[4-[4-(*o*-Butoxyphenyl)piperazin-1-yl]butyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (26): yield 2.43 g (49%); mp 188−190 °C (acetone). Anal. ($C_{25}H_{38}N_4O_3 \cdot 2HCl \cdot 2H_2O$) C, H, N.

2-[4-[4-(*o*-(Propylcarbamoyl)phenyl)piperazin-1-yl]butyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (27): yield 2.46 g (50%); mp 88−90 °C (acetone). Anal. ($C_{25}H_{37}N_5O_3 \cdot 2HCl \cdot H_2O$) C, H, N.

2-[4-[4-(*m*-(Trifluoromethyl)phenyl)piperazin-1-yl]butyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (28): yield 3.19 g (72%); mp 182−183 °C (acetone). Anal. ($C_{22}H_{29}F_3N_4O_2 \cdot HCl \cdot H_2O$) C, H, N.

2-[4-[4-(*o*-Tolyl)piperazin-1-yl]butyl]-1,4-dioxoperhydropyrido[1,2-*a*]pyrazine (29): yield 2.43 g (62%); mp 266−267 °C (acetone/ethyl ether). Anal. ($C_{23}H_{34}N_4O_2 \cdot HCl$) C, H, N.

2-[4-[4-(*o*-(Propoxycarbonyl)phenyl)piperazin-1-yl]butyl]-1,4-dioxoperhydropyrido[1,2-*a*]pyrazine (30): yield 2.45 g (47%); mp 77−78 °C (methanol/ethyl ether). Anal. ($C_{26}H_{38}N_4O_4 \cdot 2HCl \cdot 2H_2O$) C, H, N.

2-[4-[4-(*o*-Cyanophenyl)piperazin-1-yl]butyl]-1,4-dioxoperhydropyrido[1,2-*a*]pyrazine (31): yield 2.42 g (58%); mp 98−100 °C (methanol/ethyl ether). Anal. ($C_{23}H_{31}N_5O_2 \cdot HCl \cdot H_2O$) C, H, N.

2-[4-[4-(*m*-Nitrophenyl)piperazin-1-yl]butyl]-1,4-dioxoperhydropyrido[1,2-*a*]pyrazine (41): yield 2.62 g (59%); mp 192−193 °C (methanol/ethyl ether). Anal. ($C_{22}H_{31}N_5O_4 \cdot HCl \cdot {}^3/_2H_2O$) C, H, N.

General Procedure. Synthesis of Compounds 3, 23, and 42−45. To a solution of **36**−**41** (5 mmol) in methanol (18 mL) was added 0.1 g of 10% Pd(C). The mixture was hydrogenated (35 psi) at room temperature for 1−3 h. The reaction mixture was filtered over Celite and evaporated to dryness to afford the pure amines. Spectral data refer to the free bases, and then hydrochloride salts were prepared.

2-[3-[4-(*m*-Aminophenyl)piperazin-1-yl]propyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (3): yield 2.05 g (88%); mp 151−153 °C (methanol/ethyl ether). Anal. ($C_{19}H_{27}N_5O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N.

2-[3-[4-(*m*-Aminophenyl)piperazin-1-yl]propyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (42): yield 2.40 g (83%); mp 138−139 °C (methanol/ethyl ether). Anal. ($C_{20}H_{29}N_5O_2 \cdot 3HCl$) C, H, N.

2-[3-[4-(*m*-Aminophenyl)piperazin-1-yl]propyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (43): yield 2.64 g (97%); mp 165−167 °C (methanol/ethyl ether). Anal. ($C_{20}H_{29}N_5O_2 \cdot 3HCl \cdot {}^7/_2H_2O$) C, H, N.

2-[4-[4-(*m*-Aminophenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (44): yield 2.23 g (93%); mp 92−94 °C (methanol/ethyl ether). Anal. ($C_{20}H_{29}N_5O_2 \cdot 3HCl$) C, H, N.

2-[4-[4-(*m*-Aminophenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (23): yield 1.98 g (80%); mp 167−169 °C (methanol/ethyl ether). Anal. ($C_{21}H_{31}N_5O_2 \cdot 3HCl$) C, H, N.

2-[4-[4-(*m*-Aminophenyl)piperazin-1-yl]butyl]-1,4-dioxoperhydropyrido[1,2-*a*]pyrazine (45): yield 2.60 g (97%); mp 147−150 °C (d) (methanol/ethyl ether). Anal. ($C_{22}H_{33}N_5O_2 \cdot 3HCl \cdot {}^3/_2H_2O$) C, H, N.

General Procedure. Preparation of Derivatives 8, 12, 20, and 32. To a solution of **42**−**45** (4 mmol) in pyridine (50 mL) at 0 °C was added dropwise 0.5 mL of isobutyryl chloride (0.50 g, 4 mmol). After stirring at room temperature for 1.5 h, the mixture was washed with a saturated aqueous solution of $CuSO_4$, water and a saturated aqueous solution of NaCl (brine). The organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure to afford the desired compounds which were converted to their hydrochloride salts.

2-[3-[4-[*m*-(2-Methylpropanamido)phenyl]piperazin-1-yl]propyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (8): yield 1.44 g (69%); mp 198−201 °C (methanol/ethyl ether). Anal. ($C_{24}H_{35}N_5O_3 \cdot 2HCl \cdot {}^1/_2H_2O$) C, H, N.

2-[3-[4-[*m*-(2-Methylpropanamido)phenyl]piperazin-1-yl]propyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (12): yield 1.23 g (55%); mp 193−195 °C (methanol/ethyl ether). Anal. ($C_{24}H_{35}N_5O_3 \cdot 2HCl \cdot {}^5/_2H_2O$) C, H, N.

2-[4-[4-[*m*-(2-Methylpropanamido)phenyl]piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (20): yield 1.32 g (62%); mp 189−192 °C (methanol/ethyl ether). Anal. ($C_{24}H_{35}N_5O_3 \cdot 2HCl \cdot H_2O$) C, H, N.

2-[4-[4-[*m*-(2-Methylpropanamido)phenyl]piperazin-1-yl]butyl]-1,4-dioxoperhydropyrido[1,2-*a*]pyrazine (32): yield 0.90 g (41%); mp 216−218 °C (methanol/ethyl ether). Anal. ($C_{26}H_{39}N_5O_3 \cdot 2HCl \cdot {}^1/_2H_2O$) C, H, N.

2-[4-[4-(*m*-(Ethylsulfonamido)phenyl)piperazin-1-yl]-butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (EF-7412, 46). To a suspension of **44** (2.40 g, 6 mmol) and dry pyridine (0.8 mL) in anhydrous acetone (5.7 mL) at room temperature under argon, was added ethylsulfonyl chloride (0.6 mL, 0.8 g, 6 mmol). After stirring overnight at room temperature, the mixture was diluted with water and the solvents were evaporated under reduced pressure. The solid residue was purified by column chromatography (ethyl acetate/ethanol 9:1), to afford 2.28 g (82%) of EF-7412 **(46)**, which was converted to its hydrochloride salt: mp 187−190 °C (methanol/ethyl ether); ^{1}H NMR ($CDCl_3$) δ 1.35 (t, J = 7.5, 3H, CH_3), 1.50−1.72 (m, 5H, -$(CH_2)_2$-, H_7), 2.05−2.13 (m, 2H, $2H_6$), 2.23−2.27 (m, 1H, H_7), 2.41 (t, J = 7.5, 2H, CH_2-Npip), 2.57 (t, J = 4.8, 4H, $2CH_2$-pip), 3.12 (q, J = 7.5, 2H, SO_2CH_2), 3.20 (t, J = 5.4, 4H, $2CH_2$-pip), 3.23−3.29 (m, 1H, H_5), 3.50 (t, J = 7.2, 2H, NCH_2), 3.69 (dt, J = 11.1, 7.2, 1H, H_5), 4.09 (dd, J = 9.0, 7.5, 1H, H_{7a}), 6.67−6.71 (m, 2H, H_4- and H_6-phenyl), 6.82 (t, J = 2.1, 1H, H_2-phenyl), 7.18 (t, J = 8.4, 1H, H_5-phenyl); ^{13}C NMR ($CDCl_3$) δ 8.2 (CH_3), 23.6, 25.9 (-$(CH_2)_2$-), 26.9 (C_6), 27.5 (C_7), 38.6 (NCH_2), 45.4 (C_5, SO_2CH_2), 48.4 ($2CH_2$-pip), 52.9 ($2CH_2$-pip), 57.7 (CH_2-Npip), 63.3 (C_{7a}), 107.5 (C_2-phenyl), 111.0, 112.1 (C_4- and C_6-phenyl), 130.0 (C_5-phenyl), 137.9 (C_3-phenyl), 152.2 (C_1-phenyl), 160.8 (C_3), 174.0 (C_1). Anal. ($C_{22}H_{33}N_5O_4S \cdot 2HCl \cdot {}^1/_2H_2O$) C, H, N.

Radioligand Binding Assays. For all receptor-binding assays, male Sprague−Dawley rats (*Rattus norvegicus albinus*), weighing 180−200 g, were killed by decapitation and the brains rapidly removed and dissected.

5-HT$_{1A}$ Receptor. The receptor binding studies were performed by a modification of a previously described procedure.[22] The cerebral cortex was homogenized in 10 volumes of ice-cold Tris buffer (50 mM Tris-HCl, pH 7.7 at 25 °C) and centrifuged at 28000*g* for 15 min. The membrane pellet was washed twice by resuspension and centrifugation. After the second wash the resuspended pellet was incubated at 37 °C for 10 min. Membranes were then collected by centrifugation and the final pellet was resuspended in 50 mM Tris-HCl, 5 mM $MgSO_4$, and 0.5 mM EDTA buffer (pH 7.4 at 37 °C). Fractions of the final membrane suspension (about 1 mg of protein) were incubated at 37 °C for 15 min with 0.6 nM [^{3}H]-8-OH-DPAT (133 Ci/mmol), in the presence or absence of several concentrations of the competing drug, in a final volume of 1.1 mL of assay buffer (50 mM Tris-HCl, 10 nM clonidine, 30 nM prazosin, pH 7.4 at 37 °C). Nonspecific binding was determined with 10 μM 5-HT.

5-HT$_{2A}$ Receptor. The receptor binding assays were performed by a modification of a previously described procedure.[23] The frontal cortex was homogenized in 60 volumes of ice-cold buffer (50 mM Tris-HCl, 0.5 mM Na_2EDTA, 10 mM $MgSO_4$, pH 7.4 at 37 °C), and centrifuged at 30000*g* for 15 min. The membrane pellet was resuspended in buffer and incubated at 37 °C for 15 min. After centrifuging at 30000*g* for 15 min, the membranes were washed twice by resuspension and centrifugation, and the final pellet was resuspended in assay buffer

(50 mM Tris-HCl, 0.5 mM Na_2EDTA, 10 mM $MgSO_4$, 0.1% ascorbic acid, 10 μM pargyline, pH 7.4 at 37 °C). Fractions of the final membrane suspension (about 0.5 mg of protein) were incubated at 37 °C for 15 min with 0.4 nM [^{3}H]ketanserin (77 Ci/mmol), in the presence or absence of several concentrations of the competing drug, in a final volume of 2 mL of assay buffer. Nonspecific binding was determined with 1 μM cinanserin.

5-HT$_3$ Receptor. Binding assays were performed according to the procedure previously described in the literature.[24] The cerebral cortex was homogenized in 9 volumes of 0.32 M sucrose and centrifuged at 1000*g* for 10 min. The supernatant was centrifuged at 17000*g* for 20 min. The membrane pellet was washed twice by resuspension in 60 volumes of 50 mM Tris-HCl buffer (pH 7.4 at 25 °C) and centrifugation at 48000*g* for 10 min. After the second wash the resuspended pellet was incubated at 37 °C for 10 min, and centrifuged at 48000*g* for 10 min. Membranes were resuspended in 2.75 volumes of assay buffer (50 mM Tris-HCl, 10 μM pargyline, 0.6 mM ascorbic acid, and 5 mM $CaCl_2$, pH 7.4 at 25 °C). Fractions of 100 μL of the final membrane suspension (about 2 mg/mL of protein) were incubated at 25 °C for 30 min with 0.7 nM [^{3}H]LY 278584 (83 Ci/mmol), in the presence or absence of six concentrations of the competing drug, in a final volume of 2 mL of assay buffer. Nonspecific binding was determined with 10 μM 5-HT and represented less than 30% of the total binding.

5-HT$_4$ Receptor. Binding assays were performed according to the procedure previously described in the literature.[25] The striatum was homogenized in 15 volumes of ice-cold 50 mM HEPES buffer (pH 7.4 at 4 °C) and centrifuged at 48000*g* for 10 min. The pellet was resuspended in 4.5 mL of assay buffer (50 mM HEPES, pH 7.4 at 4 °C). Fractions of 100 μL of the final membrane suspension were incubated at 37 °C for 30 min with 0.1 nM [^{2}H]GR 113808 (85 Ci/mmol), in the presence or absence of six concentrations of the competing drug, in a final volume of 1 mL of assay buffer. Nonspecific binding was determined with 30 μM 5-HT and represented less than 40% of the total binding.

α_1-Adrenergic Receptor. The radioligand receptor binding studies were performed according to a previously described procedure.[26] The cerebral cortex was homogenized in 20 volumes of ice-cold buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4 at 25 °C) and centrifuged at 30000*g* for 15 min. Pellets were washed twice by resuspension and centrifugation. Final pellets were resuspended in the same buffer. Fractions of the final membrane suspension (about 250 μg of protein) were incubated at 25 °C for 30 min with 0.2 nM [^{3}H]prazosin (23 Ci/mmol) in the presence or absence of several concentrations of the competing drug, in a final volume of 2 mL of buffer. Nonspecific binding was determined with 10 μM phentolamine.

D$_2$-Dopaminergic Receptor. The receptor binding studies were performed according to a previously described procedure.[27] The *corpus striatum* was homogenized in 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) and centrifuged at 48000*g* for 10 min. The pellet was resuspended and centrifuged as before. The final pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.4 at 25 °C) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.1% ascorbic acid. Fractions of the final membrane suspension (125−150 μg of protein) were incubated at 25 °C for 60 min with 0.8 nM [^{3}H]raclopride (77 Ci/mmol), in the presence or absence of the competing drug, in a final volume of 1.1 mL of the assay buffer (pH 7.4 at 25 °C). Nonspecific binding was determined with 1 μM (+)-butaclamol.

Bz Receptor. The receptor binding assays were performed according to a previously described procedure.[28] The cerebral cortex was homogenized in 25 mM potassium phospate (KPi) buffer (pH 7.4). Homogenate fractions (about 100 μg of protein) were incubated at 0−4 °C for 90 min with 0.25 nM [^{3}H]-flunitrazepam (37 Ci/mmol), in the presence or absence of several concentrations of the competing drug, in a final volume of 1 mL of 25 mM KPi (pH 7.4). Nonspecific binding was determined with 2 μM diazepam.

For all binding assays, competing drug, nonspecific, total and radioligand bindings were defined in triplicate. Incubation was terminated by rapid vacuum filtration through Whatman GF/B filters, presoaked in 0.05% poly(ethylenimine), using a Brandel cell harvester. The filters were then washed with the assay buffer and dried. The filters were placed in poly-(ethylene) vials to which was added 4 mL of a scintillation cocktail (Aquasol), and the radioactivity bound to the filters was measured by liquid scintillation spectrometry. The data were analyzed by an iterative curve-fitting procedure (program Prism, Graph Pad), which provided IC_{50}, K_i, and r^2 values for test compounds, K_i values being calculated from the Cheng and Prusoff equation.[17] The protein concentrations of the rat cerebral cortex and the rat striatum were determined by the method of Lowry,[29] using bovine serum albumin as the standard.

Pharmacological Methods. Animals. Male Swiss albino mice (20−25 g, BW) and male Sprague−Dawley rats (200−250 g, BW) were obtained from Interfauna Ibérica (Sant Feliú de Codines, Barcelona, Spain) and maintained in a temperature and light (25 ± 1 °C, light on between 8.00 a.m. and 8.00 p.m.) controlled environment. Food and tab water were provided ad libitum. All experiments were performed between 9.00 a.m. and 1.00 p.m.

Hypothermia. After removal of mice from their home cages, basal rectal temperature was measured with a lubricated digital thermistoprobe which was inserted into the rectum 1.5 cm for 40 s. Rectal temperature was determined again after the appropriate treatments with 8-OH-DPAT, EF-7412 **(46)** or vehicle. The difference between the temperature measured before and after the administration represents an index of hypothermia. A decrease of more than 1.1 °C from basal rectal temperature was considered a hypothermic response.

Evaluation of 5-HT$_{1A}$ Receptor-Mediated Behavior in the Rat. Male Sprague−Dawley rats were acclimatized to the housing environment and handled every day for 1 week before testing. Rats were housed in groups of 3−4/cage. Lower lip retraction (LLR) and flat body posture (FBP) were used to evaluate the 5-HT$_{1A}$ receptor-mediated behavior. Both responses were measured 5, 10, 20 and 30 min after drug administration by using a 0−3 scale as previously described.[30]

Corticosterone Radioimmunoassay. Male Sprague−Dawley rats were acclimatized to the housing environment and handled every day for 1 week before testing. Rats were housed in groups of 3−4/cage. After the appropiate treatments with the vehicle, EF-7412 **(46)** or EF-7412 **(46)**/8-OH-DPAT, rats were decapited and trunk blood was collected. The samples were centrifugated and plasma samples were stored frozen at −80 °C before analysis. Corticosterone was extracted following a previously reported method.[31] Briefly, corticosterone binding protein transcortin was hydrolyzed by incubation with trypsin (Boehringuer, Mannheim, Madrid, Spain) for 60 min at room temperature. Trypsin was inactivated by trypsin inhibitor treatment (Boehringuer, Mannheim, Madrid, Spain) for 30 min at room temperature. Corticosterone (Sigma Chemicals Co., Madrid, Spain) was used as the assay standard. Radioimmunoassays were performed adding to this mixture corticosterone antiserum (Bioclin Clinical Services Ltd., Cardiff, U.K.) and [^{3}H]corticosterone (Amersham, Madrid, Spain) at specific activity of 88 Ci/mmol (8000 cpm/tube). After incubation at 4 °C for 16−24 h, the antigen−antibody complex was separated from unbound antigen with 1% charcoal and centrifugated at 4 °C, 3000 rpm, for 10 min. Finally, the supernatant was decanted and mixed with liquid scintillation cocktail before counting. The lower limit of sensitivity of assay was 2 ng/mL. The intra- and interassay variability was 4.76% and 5.84%, respectively.

Neurochemical Activity: 5-HIAA/5-HT and DOPAC/DA Ratios. Following appropriate treatments, mice were decapitated and brains were removed from the skull. Hyphotalami were dissected on ice and inmediately frozen over dry ice. Tissues samples were placed in 200 μL of 0.1 M phosphate-

citrate buffer (pH 2.5) containing 15% methanol and stored at −80 °C until assayed.

On the day of the assay tissue samples were thawed, sonicated for 3 s (Vibra Cell, model VC-501, Sonics and Materials Inc., Danbury, CT) and centrifuged for 60 s in a Microfuge (IEC, model Centra-MP4R, Needham, MA). 5-HIAA, 5-HT, DOPAC and DA concentrations in hypothalamus tissue extracts were measured by high-performance liquid chromatography (HPLC) with electrochemical detection. 20 μL of the supernatant were injected onto a C18 reverse-phase analytical column (5-μm spheres, 250 × 4.6 mm; Nucleosi, Scharlau, Barcelona, Spain) which was protected by a precolumn cartridge filter (5-μm spheres, 30 × 4.6 mm). The HPLC column was coupled to a single coulometric electrode conditioning cell in series with dual electrode analytical cells (Coulochem II, ESA, Bedford, MA). The conditioning electrode potential was set at 100 nA and the analytical electrodes were set at +0.12 and −0.31 V relative to internal Ag reference electrodes. The HPLC mobile phase consisted of 1.0 M phosphate/citrate buffer (pH 2.8) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.050% sodium octylsulfate and 25% methanol. 5-HIAA, 5-HT, DOPAC and dopamine contents of each sample were quantitated by comparing peak heights with those peaks of standards assayed the same day as determined by Shimadzu intregrator (Shimadzu, model C-R4AX-CHROMATOPAC, Kyoto, Japan). The lower limit of sensitivity of this assay for these compounds was 2−8 pg/sample. Tissue pellets were dissolved in 1.0 N NaOH and assayed for protein.[29]

Locomotor Activity. Open field locomotor activity was measured in male Swiss albino mice, after the appropriate treatments with EF-7412 **(46)** and 8-OH-DPAT, in an open field. The apparatus consisted in a square box (30 × 30 cm) with 10-cm high walls. The floor was a white plastic sheet divided into 25 (5 × 5) equally sized squares painted in fine black. Mice were maintained in the animal house at least 5 days before the experiment. On the day of the experiment drugs were dissolved in distilled water and sc injected. Mice were individually tested in 5-min sessions in the apparatus described above. The floor of each box was cleaned between sessions. Each mouse was tested by placing it in one corner of the square field. Its behavior was recorded on a videotape and locomotor activity analysis was subsequently performed from the recording. The number of total squares were counted during a 5-min test. A mouse whose four paws were in a new square was considered as having crossed a square.

Statistical Analyses. Statistical analyses were performed using analysis of varriance followed by the Student−Neeewman−Keul's test. Differences were considered significant if the probability of error was less than 5%.

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Supporting Information Available: Characterization data for new compounds **2**−**4**, **6**−**8**, **10**−**12**, **14**−**16**, **18**−**33**, **34d**−**i**, **35d**, and **36**−**45**. This information is available free of charge via the Internet at http://pubs.acs.org.

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