Synthesis, Screening, and Molecular Modeling of New Potent and Selective Antagonists at the α_{1d} Adrenergic Receptor

Amedeo Leonardi,*,† Daniela Barlocco,§ Federica Montesano,& Giorgio Cignarella,§ Gianni Motta,† Rodolfo Testa,† Elena Poggesi,† Michele Seeber,# Pier G. De Benedetti,# and Francesca Fanelli‡

Pharmaceutical R & D Division, Recordati s.p.a., Via Civitali 1, 20148 Milano, Italy, Pharmaco-Chemistry and Toxicology Institute, University of Milano, V.le Abruzzi 42, 20131 Milano, Italy, Department of Chemistry, University of Modena and Reggio Emilia, Via Campi 183, 41100 Modena, Italy, and Dulbecco Telethon Institute (DTI) and Department of Chemistry, University of Modena and Reggio Emilia, Via Campi 183, 41100 Modena, Italy

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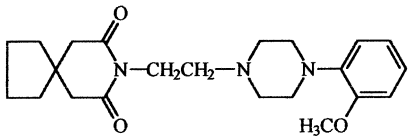
In the present study, more than 75 compounds structurally related to BMY 7378 have been designed and synthesized. Structural variations of each part of the reference molecule have been introduced, obtaining highly selective ligands for the α_{1d} adrenergic receptor. The molecular determinants for selectivity at this receptor are essentially held by the phenyl substituent in the phenylpiperazine moiety. The integration of an extensive SAR analysis with docking simulations using the rhodopsin-based models of the three α_1-AR subtypes and of the 5-HT_{1A} receptor provides significant insights into the characterization of the receptor binding sites as well as into the molecular determinants of ligand selectivity at the α_{1d}-AR and the 5-HT_{1A} receptors. The results of multiple copies simultaneous search (MCSS) on the substituted phenylpiperazines together with those of manual docking of compounds BMY 7378 and **69** into the putative binding sites of the α_{1a}-AR, α_{1b}-AR, α_{1d}-AR, and the 5-HT_{1A} receptors suggest that the phenylpiperazine moiety would dock into a site formed by amino acids in helices 3, 4, 5, 6 and extracellular loop 2 (E2), whereas the spirocyclic ring of the ligand docks into a site formed by amino acids of helices 1, 2, 3, and 7. This docking mode is consistent with the SAR data produced in this work. Furthermore, the binding site of the imide moiety does not allow for the simultaneous involvement of the two carbonyl oxygen atoms in H-bonding interactions, consistent with the SAR data, in particular with the results obtained with the lactam derivative **128**. The results of docking simulations also suggest that the second and third extracellular loops may act as selectivity filters for the substituted phenylpiperazines. The most potent and selective compounds for α_{1d} adrenergic receptor, i.e., **69** (Rec 26D/038) and **128** (Rec 26D/073), are characterized by the presence of the 2,5-dichlorophenylpiperazine moiety.

Introduction

Within recent years, the heterogeneity of the α_1 adrenergic receptor (AR) has been realized on both a molecular and pharmacological level. At the molecular level, three subtypes of the human α_1 receptor have been identified and cloned: α_{1a}, α_{1b}, and α_{1d}. These receptors correlate with the pharmacologically defined receptors α_{1A}, α_{1B}, and α_{1D}.[1,2] Current evidence indicates that the α_{1A} subtype is mainly present in rat submaxillary gland, human liver, and various tissues such as prostatic vas deferens, rabbit prostate, and prostatic urethra.[3−6] Rat liver and spleen are considered α_{1B} adrenoceptor preparations, while the α_{1D} subtype mediates the contractility of rat aorta.[7,8] Interestingly, α_{1d} mRNA has been shown to be the dominant α_1 subtype present in the human bladder detrusor.[9] Moreover, the functional involvement of the α_{1D} adrenergic receptor in detrusor instability secondary to bladder outlet obstruction, as well as in mediating constriction of rat skeletal muscles arterioles and protein synthesis by arterial smooth muscles, has been reported.[10−12] Therefore, the availability of selective α_{1D} antagonists could be very useful in the treatment of diseases such as urinary incontinence, vasoconstriction, and atherosclerosis, with no effects on blood pressure. In this respect, some *N*,*ω*-aminoalkyl cyclic imides are reported in the scientific literature. Among others, BMY 7378 (see Chart 1), first described in 1983,[13] was later shown to be a selective α_{1D} antagonist.[14] However, its selectivity profile is also limited by high affinity for the 5-HT_{1A} serotonergic receptor.

Chart 1



BMY 7378

On these bases, we have recently undertaken a study on compounds structurally related to BMY 7378 aimed at identifying highly selective antagonists at the α_{1d} receptor. The following design approaches have been done: (a) variation of the type and number of the substituent on the phenyl ring; (b) replacement of the piperazine ring by either different cyclic moieties or by

* To whom corresponcence should be addressed. Phone: 39-02-48787408. Fax: 39-02-48709017. E-mail: Leonardi.A@recordati.it.
† Recordati s.p.a.
§ University of Milano.
# Department of Chemistry, University of Modena and Reggio Emilia.
‡ Dulbecco Telethon Institute (DTI) and Department of Chemistry, University of Modena and Reggio Emilia.

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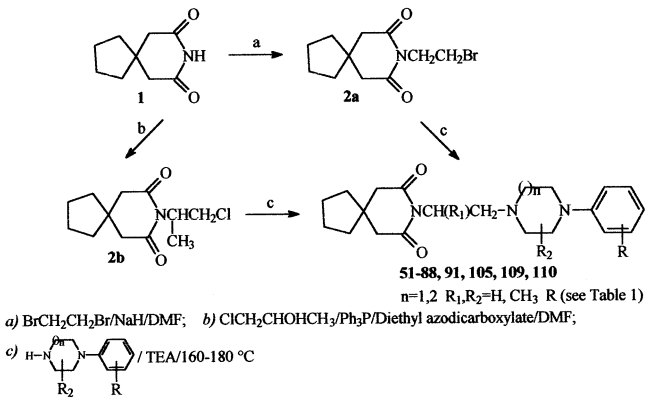
Scheme 1



acyclic analogues; (c) combination of approaches a and b. In addition, the following structural changes have also been considered: (d) homologation or suppression of the cyclopentyl ring as well as its formal opening to linear alkyl chains; (e) modification of the imido moiety. All compounds have been tested in radioreceptor binding assays. The most significant compounds were further investigated for functional activity at the three α_1 adrenoceptor subtypes.

Finally, docking simulations employing molecular models of the three α_1-AR subtypes and of the 5-HT_{1A} receptor, built by comparative modeling using rhodopsin structure as a template,[15] have also been done in order to gain insight into the putative ligand-binding domains of these receptors and into the molecular determinants of ligand specificity at the α_{1d}-AR.

Chemistry

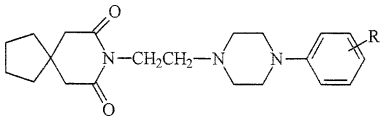
BMY 7378 analogues having different substituents on the phenyl ring (**51**−**88**) were prepared by nucleophilic substitution on the known 8-(2-bromoethyl)-8-azaspiro-[4.5]decane-7,9-dione (**2a**)[16] with the appropriate substituted phenylpiperazine at 160−180 °C for 30 min in the presence of triethylamine. Following the same procedure but using 3,5-dimethyl-1-(2-nitrophenyl)piperazine, 3-methyl-1-(2-nitrophenyl)piperazine, and 1-(2-chlorophenyl)-1,4-diazepane, compounds **109**, **110**, and **105**, respectively, were obtained (Scheme 1 and Tables 1 and 3). It should be noted that compounds **51**, **54**, and **55** have already been described.[17]

The required piperazines, when not commercial, were in turn prepared from the appropriate aniline derivatives by treatment with bis(2-chloroethyl)amine hydrochloride, potassium carbonate, and potassium iodide in refluxing *n*-butyl alcohol according to standard procedures.[18] In the case of **88**, the required 2-Cl-,5-I-phenylpiperazine was obtained from the corresponding 2-Cl analogue by treatment with ICl in acetic acid.[19] Compound **91**, having a branched spacer instead of the ethylene chain typical of this series, was prepared from 3,3-tetramethyleneglutarimide, which was reacted with 1-chloro-2-propyl alcohol, triphenylphosphine, and diethyl azodicarboxylate in dimethylformamide. The so-obtained intermediate **2b** (Scheme 1) was then reacted with 2,5-dichlorophenylpiperazine, as above-reported (Scheme 1 and Table 2).

Compounds **89** and **90**, having longer linear spacers, were synthesized by condensation, in toluene and in the presence of *p*-toluenesulfonic acid, of the 8-oxaspiro[4.5]-decane-7,9-dione (**48**) with 3-[4-(2,5-dichlorophenyl)-piperazino]-1-propanamine and 4-[4-(2,5-dichlorophenyl)-piperazino]-1-butanamine, respectively. These, in turn, were prepared by already known standard procedures[20] (Scheme 2 and Table 2).

Compounds having linear or branched aminoalkyl chains instead of the piperazine ring were prepared

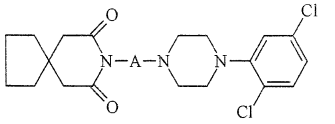
Table 1. Physical Properties of Spiroethyl Phenyl(substituted)piperazine Compounds (**51**−**88**)[a]



compd	R	% yield	mp, °C	formula[b]
51	H	98	92−93	$C_{21}H_{29}N_3O_2$
52	4-OCH_3	30	oil	$C_{22}H_{31}N_3O_3$
53	3-OCH_3	65	oil	$C_{22}H_{31}N_3O_3$
54	2-Cl	30	oil	$C_{21}H_{28}ClN_3O_2$
55	3-Cl	18	oil	$C_{21}H_{28}ClN_3O_2$
56	4-Cl	63	134−135	$C_{21}H_{28}ClN_3O_2$
57	4-F	18	oil	$C_{21}H_{28}FN_3O_2$
58	2-$OCH(CH_3)_2$	80	oil	$C_{24}H_{35}N_3O_3$
59	2-OH	96	108−109	$C_{21}H_{29}N_3O_3$
60	2-F	74	78−79	$C_{21}H_{28}FN_3O_2$
61	2,3-Cl_2	97	133−134	$C_{21}H_{27}Cl_2N_3O_2$
62	2,4-CH_3	84	114−115	$C_{23}H_{33}N_3O_2$
63	2-CH_3	74	oil	$C_{22}H_{31}N_3O_2$
64	3-CF_3	78	68−69	$C_{22}H_{28}F_3N_3O_2$
65	2-NO_2	41	117−118	$C_{21}H_{28}N_4O_4$
66	2-CN	29	114−115	$C_{22}H_{28}N_4O_2$
67	2-$COOC_2H_5$	25	oil	$C_{24}H_{33}N_3O_4$
68	2-Br	56	oil	$C_{21}H_{28}BrN_3O_2$
69	2,5-Cl_2	51	149−150	$C_{21}H_{27}Cl_2N_3O_2$
70	2,4-Cl_2	77	93−94	$C_{21}H_{27}Cl_2N_3O_2$
71	3,4-Cl_2	91	110−111	$C_{21}H_{27}Cl_2N_3O_2$
72	2-CF_3	23	125−126	$C_{22}H_{28}F_3N_3O_2$
73	2-cyclopropyl	41	oil	$C_{24}H_{33}N_3O_2$
74	4-$CH(CH_3)_2$	63	108−109	$C_{24}H_{35}N_3O_2$
75	2,6-Cl_2	48	94−95	$C_{21}H_{27}Cl_2N_3O_2$
76	2,5-F_2	30	112−113	$C_{21}H_{27}F_2N_3O_2$
77	2-Cl, 5-CF_3	60	90−91	$C_{22}H_{27}ClF_3N_3O_2$
78	2-Cl, 5-CH_3	73	139−140	$C_{22}H_{30}ClN_3O_2$
79	2-F, 5-CH_3	59	103−104	$C_{22}H_{30}FN_3O_2$
80	2,5-$(CH_3)_2$	76	128−129	$C_{23}H_{33}N_3O_2$
81	2-F, 5-CF_3	81	85−86	$C_{22}H_{27}F_4N_3O_2$
82	2-F, 5-NO_2	66	121−122	$C_{21}H_{27}FN_4O_4$
83	2-Cl, 5-NO_2	31	116−117	$C_{21}H_{27}ClN_4O_4$
84	2-CH_3, 5-Cl	86	132−133	$C_{22}H_{30}ClN_3O_2$
85	2,5-Br_2	55	159−160	$C_{21}H_{27}Br_2N_3O_2$
86	2-CN, 5-Cl	23	oil	$C_{22}H_{27}ClN_4O_2$
87	2-Cl, 5-F	74	139−140	$C_{21}H_{27}ClFN_3O_2$
88	2-Cl, 5-I	73	109−110	$C_{21}H_{27}ClIN_3O_2$

[a] All compounds were synthesized according to Scheme 1. [b] C, H, N (Cl) analysis within ±0.4%.

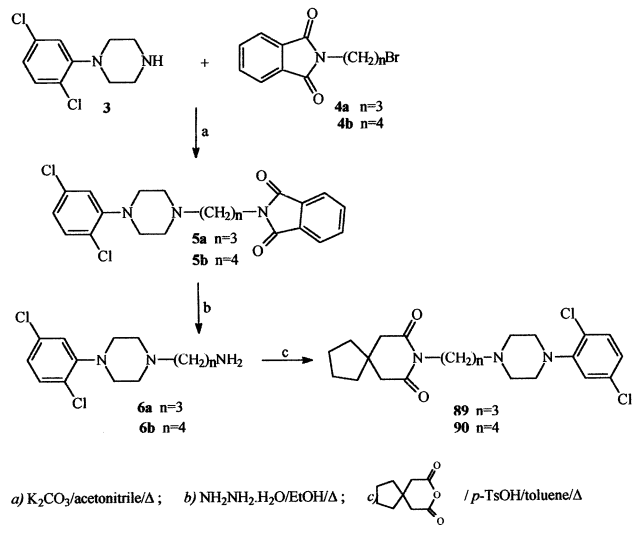
Table 2. Physical Properties of Spiroalkyl 2,5-Dichlorophenylpiperazine Compounds (**89**−**91**)[a]



compd	A	% yield	formula[b]	scheme no.
89	$(CH_2)_3$	51	$C_{22}H_{29}Cl_2N_3O_2$	2
90	$(CH_2)_4$	85	$C_{23}H_{31}Cl_2N_3O_2$	2
91	$CH(CH_3)CH_2$	21	$C_{22}H_{29}Cl_2N_3O_2$	1

[a] The compounds were all isolated as oily substances. [b] C, H, N, Cl analysis within ±0.4%.

Scheme 2



according to different methods, depending on their structures. In particular, the terms having the linear alkanediamino moiety *N*1,*N*2-dimethyl-1,2-ethanediamine (**92**) and *N*1,*N*3-dimethyl-1,3-propanediamine (**93**) were obtained from 2,5-dichloroaniline, which was condensed with the appropriate chloroalkanol in the presence of triethylamine at 150 °C and subsequently methylated by refluxing with 37% formaldehyde and formic acid. Substitution of the hydroxy group by chlorine using thionyl chloride and condensation with methanolic methylamine in an autoclave at 80 °C gave the required intermediate, which was heated in the presence of triethylamine with **2a** at 160 °C to obtain compounds **92** and **93**. According to the same method, but omitting methylation of the amino alcohol, compound **94** was prepared (Scheme 3, path a and Table 3). Similarly, the amido derivatives (**95**−**97**) were prepared from 2,5-dichloroaniline by condensing with bromoacetyl bromide in dichloromethane at 40 °C. Methylation of the amide nitrogen with iodomethane in the presence of potassium carbonate in dimethylformamide and subsequent reaction with methanolic ammonia or methylamine in autoclave at 100 °C gave the required amine intermediates, which were alkylated as usual with **2a** to give **96** and **97**. Analogously, compound **95** was prepared by omitting methylation of the intermediate amide (Scheme 3, path b and Table 3).

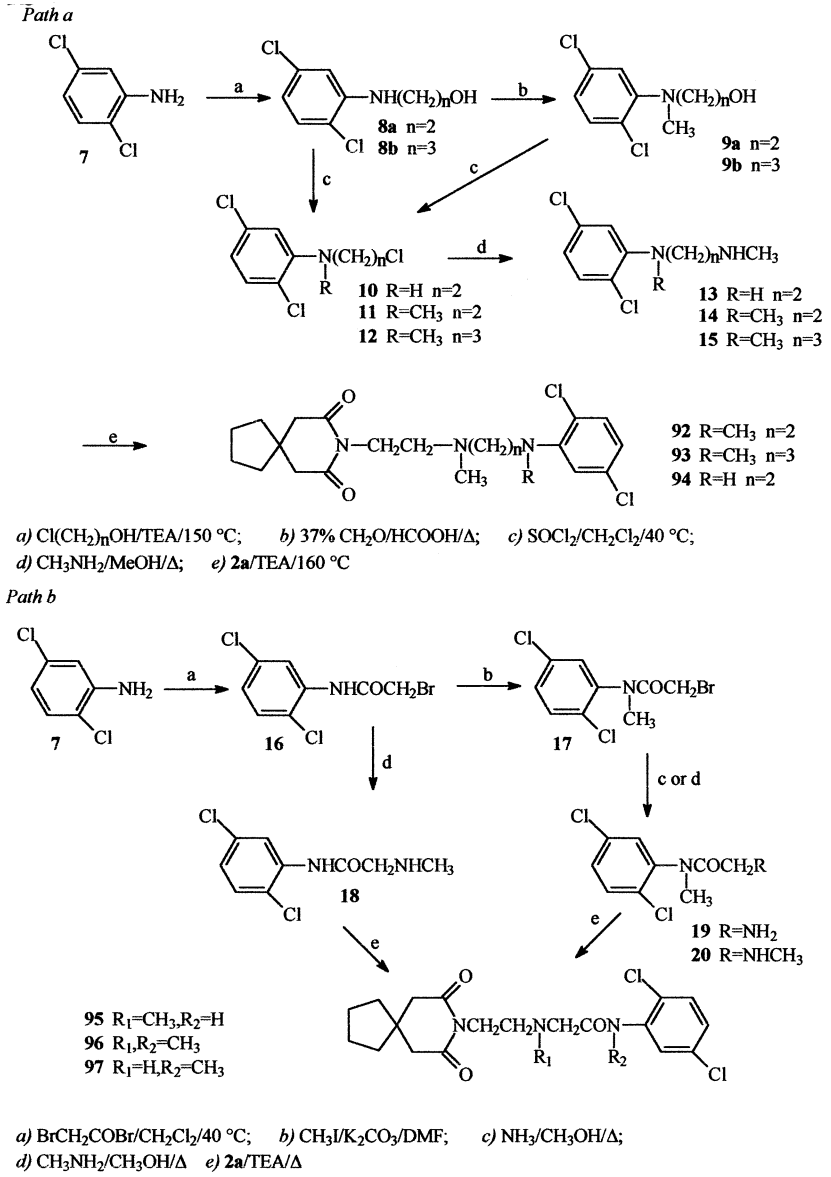
The aminoalkylphenoxy derivatives (compounds **98**−**104**) were prepared by reaction of 2-chloro-, 2,5-dichloro-, or 2-methoxyphenol with 1,2-dibromoethane and 2,5-dichlorophenol with 1,3-dibromopropane in diluted sodium hydroxide, followed by treatment of the so-obtained intermediate by potassium phthalimide and hydrazine hydrate in sequence or by methylamine, and final alkylation with **2a** (Scheme 4 and Table 3). Finally, compounds having the piperidine ring instead of the piperazine (**106**−**108**) were prepared by reacting 8-oxaspiro[4.5]decane-7,9-dione with 2-(4-phenylpiperidino)-1-ethanamine (**38a**) or the relative 2-chloro or 2,5-dichlorophenyl derivatives (**38b**,**c**)**.** These intermediates were synthesized starting from benzaldehyde, 2-chlorobenzaldehyde, or 2,5-dichorobenzaldehyde (**33 a**−**c**), which were condensed with diethyl malonate in two steps (**35a**−**c**). Decarboxylation of the bismalonate intermediates was then promoted by heating with hydrobromic acid, and cyclization of the so-obtained dicarboxylic acids (**36a**−**c**) was performed with aminoacetonitrile to give **37a**−**c**. Reduction of **37a**−**c** with lithium aluminum hydride gave the required amines (**38a**−**c**), which were condensed to the desired final products (**106**−**108**) (Scheme 5 and Table 3).

Compounds **112**, **113**, and **118**, modified at the spirocyclopentyl ring with a cyclohexyl moiety or dimethyl group, were prepared from the appropriate cyclic imide (**39**, **40**) by treating with 1,2-dibromoethane in the presence of sodium hydride. The so-obtained bromoethylimide was then condensed with the 1-(2-chlorophenyl)piperazine or 1-(2,5-dichlorophenyl)piperazine by heating at 180 °C in the presence of triethylamine (Scheme 6, path a and Table 4). On the other hand, compounds **117** and **119**−**121** were prepared by refluxing in toluene and in the presence of *p*-toluenesulfonic acid and the appropriate cyclic anhydride (**43**−**46**) with 2-[4-(2,5-dichlorophenyl)-piperazino]-1-ethanamine (**6c**)[21] (Scheme 6, path b and Table 4). The spiropiperidyl derivatives (**114**−**116**), were prepared from diethyl 1-benzyl-4,4-piperidinediacetate (**47**), which was cyclized in an autoclave with 2-[4-(2-methoxyphenyl)-piperazino]-1-ethanamine in toluene at 140−150 °C to give **114**. Following catalytic debenzylation gave **115**, which was acetylated by standard procedure to **116** (Scheme 7 and Table 4). Analogously, the 7-[2-[4-(2,5-dichlorophenyl)-1-piperazinyl]ethyl]-7-azaspiro[3.5]-nonane-6,8-dione (**111**) was synthesized starting from cyclobutane-1,1-diacetic acid[22] and cyclizing with 2-[4-(2,5-dichlorophenyl)piperazino]-1-ethanamine (**6c**) in the presence of dicyclohexylcarbodiimide and DMF (Table 4).

Compounds substituted by cyclic amide (**122**, **123**, **125**−**128**) were prepared from 8-oxaspiro[4.5]decane-7,9-dione (**48**) by heating in methanol to give the mono methyl ester (**49**). It was transformed into the corresponding acyl chloride by oxalyl chloride and then catalytically reduced to the aldehyde (**50**) in acetone in the presence of 10% Pd−C and (*N*,*N*)-diisopropylamine. Condensation of **50** with the required 2-[4-(substituted phenyl)piperazino]-1-ethanamine (**6c**−**e**) in the presence of *p*-toluenesulfonic acid in refluxing toluene gave the unsaturated compounds **122**, **125**, and **127**. The saturated derivatives **123**, **128**, and **126**, instead, were synthesized by direct condensation, at room temperature, of intermediate aldehyde **50** with the required 2-[4-(substituted phenyl)piperazino]-1-ethanamine (**6c**−**e**) in methanol, in the presence of sodium borohydride (Scheme 8 and Table 5). Any attempt to selectively reduce the imide ring to the corresponding amide was unfruitful because complex mixtures were always obtained and only the fully reduced amine could be isolated. The case of compound **124** is demonstrative (Table 5). On the other hand, compound **124** was directly obtained in better yield by reduction of **54**.

Compound **129**, bearing a contracted imido moiety, was synthesized by reaction of the 1-ethoxycarbonylmethylcyclopentanecarboxylic acid ethyl ester, prepared as already described,[21] with 2-[4-(2,5-dichlorophenyl)-1-piperazinyl]ethylamine in an autoclave at 160 °C (Table 5).

Scheme 3



All the final compounds were isolated as free bases with the exception of **115**, which was obtained as the hydrochloride salt.

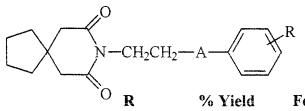
Biology

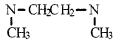
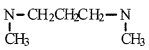
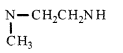
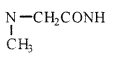
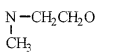
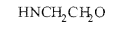
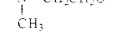
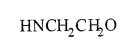
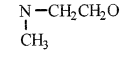
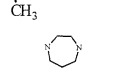
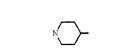
All compounds were evaluated in binding studies for their affinity at the α_{1a}, α_{1b}, α_{1d} adrenergic receptors as well as at the 5-HT_{1A} serotoninergic receptor (Table 6). BMY 7378 and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) were used as standard compounds. Moreover, in-depth studies on the most significant derivatives were performed to assess their functional activity to the three α_1-adrenenoreceptor subtypes: α_{1A} (rat vas deferens), α_{1B} (guinea pig spleen), and α_{1D} (rat aorta).[8] The potency of the compounds was expressed as pK_B values (Table 7; see Experimental Section for details).

Results and Discussion

SAR Analysis. Among the BMY 7378 analogues monosubstituted on the phenyl ring, the most potent toward α_{1d} was the 2-Cl (**54**), which displayed elevated affinity and good selectivity for the α_{1d} over both the α_{1a} and α_{1b} subtypes but significantly bound also 5-HT_{1A}, though being in this respect more selective than the reference compound. Shifting the 2-Cl to the meta position (**55**) enhanced affinity for 5-HT_{1A}. In contrast, the para analogue (**56**) was much less active toward both receptors. Substitution of the 2-Cl with 2-F (**60**) almost paralleled the binding profile of **54**, with a better selectivity for the α_{1d} receptor over the 5-HT_{1A} being achieved. Similarly to **54**, the shifting of the fluoro group in the para position (**57**) reduced the activity at both receptors. An opposite effect was obtained by the 2-Br (**68**) substitution. In fact, in this case, selectivity over the 5-HT_{1A} receptor was lost, the compound showing almost the same potency versus α_{1d} and 5-HT_{1A} (K_i = 0.67 and 1.29 nM, respectively). Demethylation of the 2-OMe of the model to give **59** reduced affinity toward α_{1d}, still retaining high potency for the serotonergic receptor. Some potency for the target subtype was recovered in the 2-isopropoxy analogue (**58**), but also affinity toward all considered receptors was enhanced. The 2-CN derivative (**66**) was very similar to the 2-Cl,

Table 3. Physical Properties of Non Piperazine/Substituted Piperazine Compounds (**92**−**110**)[a]

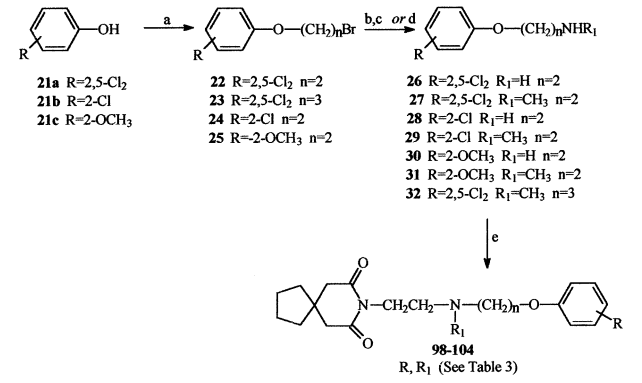


Compd	A	R	% Yield	Formula[b]	Scheme
92		2,5-Cl_2	18	$C_{21}H_{29}Cl_2N_3O_2$	3 path a
93		2,5-Cl_2	56	$C_{22}H_{31}Cl_2N_3O_2$	3 path a
94		2,5-Cl_2	69	$C_{20}H_{27}Cl_2N_3O_2$	3 path a
95		2,5-Cl_2	55	$C_{20}H_{25}Cl_2N_3O_3$	3 path b
96		2,5-Cl_2	25	$C_{21}H_{27}Cl_2N_3O_3$	3 path b
97		2,5-Cl_2	9	$C_{20}H_{25}Cl_2N_3O_3$	3 path b
98		2-Cl	78	$C_{20}H_{27}ClN_2O_3$	4
99		2-Cl	10	$C_{19}H_{25}ClN_2O_3$	4
100		2,5-Cl_2	28	$C_{20}H_{26}Cl_2N_2O_3$	4
101		2,5-Cl_2	47	$C_{19}H_{24}Cl_2N_2O_3$	4
102		2-OMe	33	$C_{20}H_{28}N_2O_4$	4
103		2-OMe	38	$C_{21}H_{30}N_2O_4$	4
104		2,5-Cl_2	13	$C_{21}H_{28}Cl_2N_2O_3$	4
105		2-Cl	82	$C_{22}H_{30}ClN_3O_2$	1
106		2,5-Cl_2	27	$C_{22}H_{28}Cl_2N_2O_2$	5
107		2-Cl	14	$C_{22}H_{29}ClN_2O_2$	5
108		H	93	$C_{22}H_{30}N_2O_2$	5
109		2-NO_2	19	$C_{23}H_{32}N_4O_4$	1
110		2-NO_2	66	$C_{22}H_{30}N_4O_4$	1

[a] All compounds were obtained as oily free bases with the exception of **109**, which was isolated in solid form (mp = 104−105 °C). [b] C, H, N (Cl) analysis within ±0.4%.

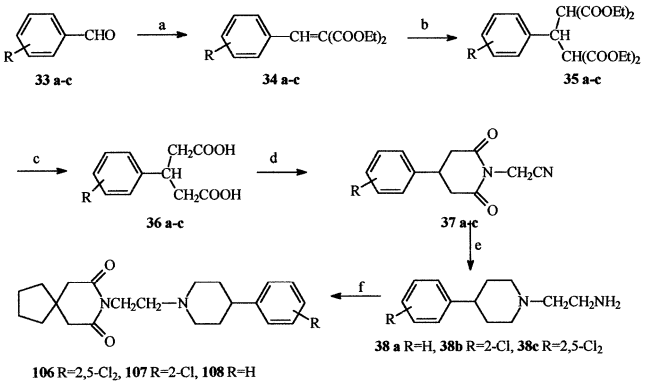
while the 2-NO_2 (**65**) showed lower potency. The introduction of one or two methyl groups on the piperazine ring of **65** led to compounds **109** and **110** with highly reduced affinity. Any other attempt made on the monosubstituted analogues gave detrimental results, compounds losing selectivity and/or affinity toward α_{1d} receptor (Table 6). Insertion of a second substituent in the phenyl ring of **54** led to different results, depending on both the position and the nature of the added substituent. In particular, when a second chlorine was considered, the most interesting compound was obtained

Scheme 4



a) $Br(CH_2)_nBr/NaOH/H_2O/\Delta$; *b)* Potassium phthalimide/DMF/Δ; *c)* $NH_2NH_2.H_2O/EtOH/\Delta$; *d)* $CH_3NH_2/MeOH/\Delta$; *e)* **2a**/Toluene/Δ

Scheme 5



a) Diethyl malonate/$AlCl_3$/rt; *b)* Diethyl malonate/$AlCl_3$/EDP/60 °C; *c)* 48% HBr / Δ; *d)* $CNCH_2NH_2$/DCC/DMF/AcONa/Ac_2O; *e)* $LiAlH_4/Et_2O$; *f)*  /*p*-TsOH/toluene/Δ

with the 2,5-Cl_2 (**69**), which was 6-fold more potent toward α_{1d} and about 50-fold more selective with respect to the 5-HT_{1A} receptor than the monosubstituted analogue. However, the compound was less selective over the α_{1b} subtype. Several other substituents at the 5-position of **54** (e.g., F, I, NO_2, Me, CF_3) as well as different combinations (e.g., Me/X, Br/Br, CN/Cl, Me/Me, X/NO_2) and positions (2,3-Cl_2, 2,4-Cl_2, 3,4-Cl_2, 2,6-Cl_2) were explored. As shown in Table 6, none of the compounds displayed higher affinity toward α_{1d} than **69**. It should be noted that substitution of both chlorine atoms of **69** with fluorine (**76**) gave a compound with almost the same affinity for the α_{1d} subtype (K_i = 0.14 and 0.13 nM) and better selectivity over the 5-HT_{1A} (276- vs 436-fold, respectively). Unfortunately, **76** showed reduced selectivity with respect to the α_{1b} receptor.

Finally, elongation of the ethylene spacer of **69** by one (**89**) or two (**90**) methylene groups retained significant affinity and selectivity for the α_{1d} subtype with respect to the 5-HT_{1A} but dramatically lost selectivity over the α_{1a} and α_{1b} subtypes. However, the branched analogue of **89** (compound **91**) showed an improved selectivity profile (Tables 2 and 6).

To possibly obtain more selective derivatives, modifications of the piperazine ring were also considered and combined with the most promising substrates in the previous series. Substitution of the piperazine with diaminoalkyl (**92**−**94**), amido (**95**−**97**), and phenoxyalkylamino chains (**98**−**104**) always resulted in a dramatic loss of affinity. In contrast, the homopiperazine

Scheme 6

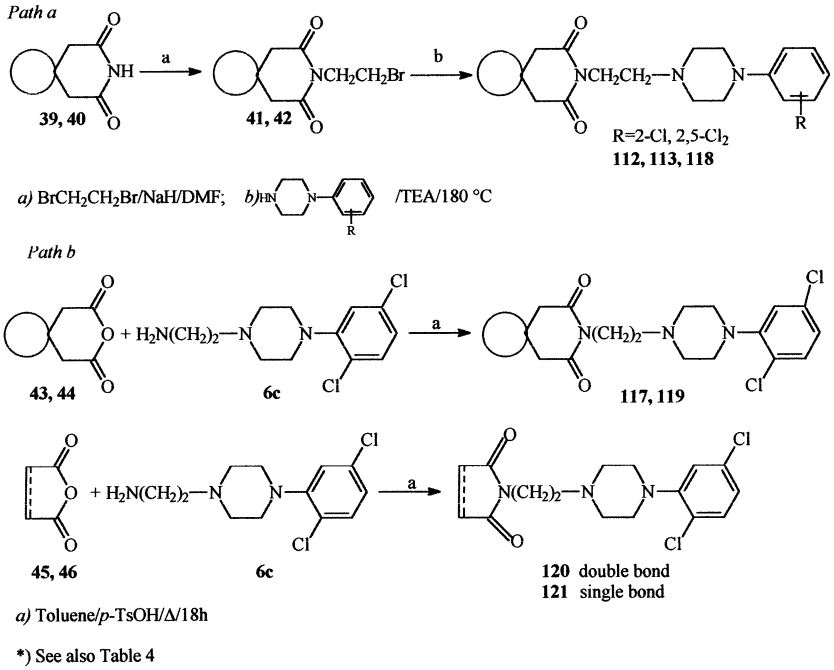
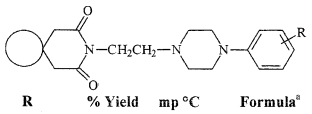
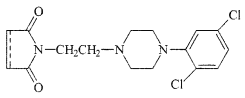


Table 4. Physical Properties of Imido Derivatives (**111**−**121**)



Compd	R	% Yield	mp °C	Formula[a]	Scheme
111	2,5-Cl_2	29	126-127	$C_{20}H_{25}Cl_2N_3O_2$	
112	2,5-Cl_2	66	oil	$C_{22}H_{29}Cl_2N_3O_2$	6 path a
113	2-Cl	57	88-89	$C_{22}H_{30}ClN_3O_2$	6 path a
114	2-OMe	23	oil	$C_{29}H_{38}N_4O_3$	7
115[b]	2-OMe	67	255-256	$C_{22}H_{32}N_4O_3.HCl$	7
116	2-OMe	68	oil	$C_{24}H_{34}N_4O_4$	7
117	2,5-Cl_2	59	98-99	$C_{20}H_{27}Cl_2N_3O_2$	6 path b
118	2-Cl	34	126-127	$C_{19}H_{26}ClN_3O_2$	6 path a
119	2,5-Cl_2	25	oil	$C_{17}H_{21}Cl_2N_3O_2$	6 path b

a) C, H, N, (Cl) analysis within ± 0.4%
b) As its hydrochloride



Compd	3,4 Bond	% Yield	mp °C	Formula[a]
120	Double	54	oil	$C_{16}H_{17}Cl_2N_3O_2$
121	Single	65	122-123	$C_{16}H_{19}Cl_2N_3O_2$

[a] C, H, N, Cl analysis within ±0.4%.

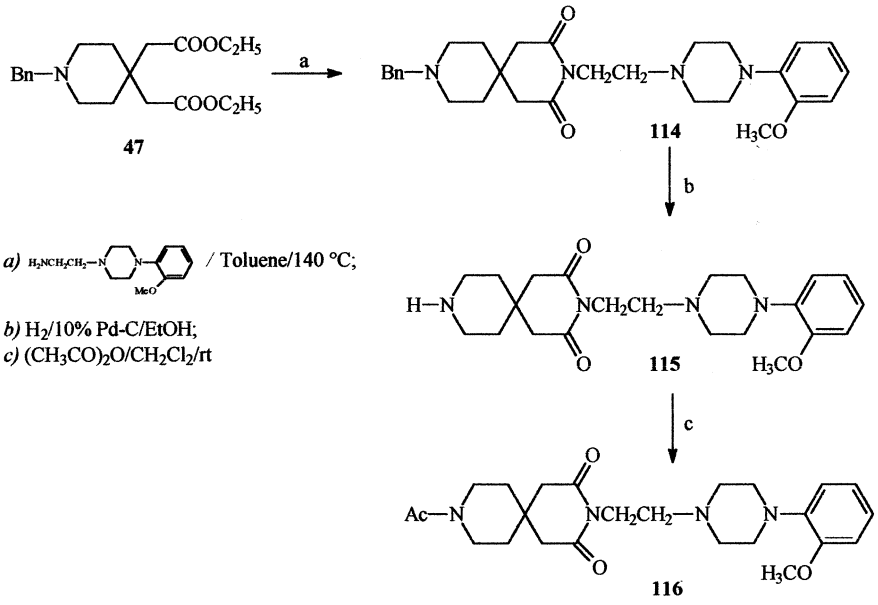
derivative (**105**) retained high affinity toward α_{1d} but completely lost selectivity over the serotoninergic receptor. Interesting compounds were obtained when a piperidine ring replaced the piperazine moiety (**106**−**108**). Once more, the best substrate was the 2,5-Cl_2 substituted **106**, which, besides high affinity toward α_{1d}, was also provided with significant selectivity.

Compounds modified in the spiro moiety also gave interesting results. As shown in Table 6, contraction (**111**) as well as extension (**112**) of the cyclopentyl spiro moiety of **69** gave less active compounds at the α_{1d} receptor, though significantly selective, particularly over 5-HT_{1A}. However, when the 2,5-Cl_2 of **112** was substituted by 2-Cl (**113**), this selectivity was completely lost. Formal opening of the cyclopentyl ring of **69** (compound **117**) as well as its elimination (**119**) still retained activity. In contrast, the 3,3-dimethyl derivative, bearing a 2-Cl phenyl substituent (**118**), was less active. Similarly, substitution of the five-membered carbon ring with a piperidine nucleus, either unsubstituted or N-substituted by an acetyl or a benzyl group (**114**−**116**), gave inactive compounds (see Table 6).

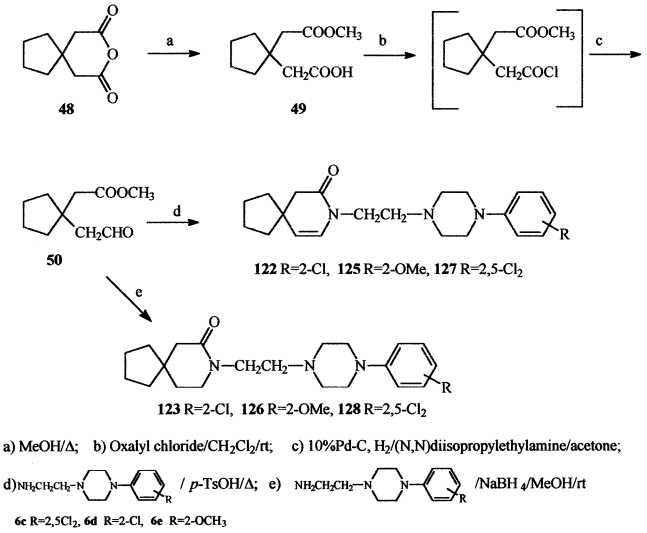
Modifications of the imide moiety were initially directed to the formal reduction of one or both the carbonyl groups. While the latter proved to be detrimental for activity, the presence of an amide function, also in the unsaturated form, led to very active derivatives. Similarly to **69**, in this case the best substitution in the phenyl ring was the 2,5-Cl_2. In particular, compound **128** was found to be very potent toward the α_{1d} receptor and significantly selective with respect to both the other α_1 subtypes and the 5-HT_{1A} receptor. The corresponding 2-Cl substituted **123** was both less potent and selective. The same holds for the 2-OMe derivative **126**, which gained selectivity with respect to the α_{1b} but not over the 5-HT_{1A} receptor. In all the cases, the presence of a double bond in the amide ring (**122**, **125**, **127**) gave less potent compounds at the α_{1d} receptor, though with different magnitude. Finally, when the importance of the imide ring size was considered, the contracted homologue **129** displayed lower α_{1d} affinity compared to the corresponding higher homologue **69** but displayed significant selectivity with respect to 5-HT_{1A} (see Table 6).

In summary, SAR analysis indicates that both modifications concerning the piperazine moiety and the

Scheme 7



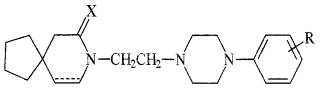
Scheme 8



substituent on the phenyl ring are responsible for modulating the ligand α_1-ARs binding affinity and α_1-AR/5-HT_{1A} selectivity. In this respect, the importance of the 2,5-dichlorophenylpiperazine moiety should be considered in order to confer preferential affinity for the α_{1d} receptor and selectivity over the 5-HT_{1A} receptor. This is a relevant result if one considers that the reference compound BMY 7378 binds to the 5-HT_{1A} receptor with nanomolar affinity.

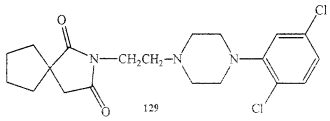
It should be highlighted that selectivity of **69** and **128** for the cloned human α_{1d} receptor over α_{1a} and α_{1b} in the binding assay was in good agreement with that observed in the functional assay. In fact, both compounds were much more potent in inhibiting rat aorta (α_{1D}) contractions than both rat vas deferens (α_{1A}) and guinea pig spleen (α_{1B}) contractions (Table 7). In these tissues expressing the three receptor subtypes, **69** was shown to be more potent and much more selective than BMY 7378. In addition, SAR analysis indicated that modifications concerning the cyclopentyl and the imide ring could also modulate the ligand α_1-AR binding affinities and α_1-AR/5-HT_{1A} selectivities. In this respect, the importance of a cyclic amido, instead of the imide moiety, in the presence of a 2,5-dichlorophenylpiperazine as a basic moiety should be highlighted.

Table 5. Physical Properties of Non-Imido Spiro Derivatives (**122**−**129**)[a]



Compd	X	R	% Yield	mp °C	Formula[b]
122	O	2-Cl	21	oil	$C_{21}H_{28}ClN_3O$
123	O	2-Cl	26	oil	$C_{21}H_{30}ClN_3O$
124	H,H	2-Cl	28	oil	$C_{21}H_{32}ClN_3$
125	O	2-OMe	50	oil	$C_{22}H_{31}N_3O_2$
126	O	2-OMe	43	oil	$C_{22}H_{33}N_3O_2$
127	O	2,5-Cl_2	51	oil	$C_{21}H_{27}Cl_2N_3O$
128	O	2,5-Cl_2	68	88-89	$C_{21}H_{29}Cl_2N_3O$

a) All compounds were prepared according to Scheme 8, with the exception of **124**
b) C, H, N, (Cl) analysis within ± 0.4%



129

Compd	% Yield	mp °C	Formula[a]
129	15	oil	$C_{20}H_{25}Cl_2N_3O_2$

[a] C, H, N, Cl analysis within ±0.4%.

Automatic Docking of Phenylpiperazine Fragments. One of the most striking features inferred from

Table 6. Affinity Constants (K_i, nM) of Compounds **51**−**129** toward Cloned α_1 Adrenoceptor Subtypes and 5-HT_{1A} Receptor[a]

	K_i (nM)[b]					K_i (nM)[b]			
compd	α-**1a**	α-**1b**	α-**1d**	5-HT_{1A}	compd	α-**1a**	α-**1b**	α-**1d**	5-HT_{1A}
51	3355.00	264.47	4.32	35.71	**92**	786.30	915.40	16.38	432.70
52	NT	NT	37.81	54.59	**93**	NT	NT	110.41	>1000
53	>1000	>1000	54.30	13.44	**94**	NT	NT	673.67	717.00
54	551.61	86.96	0.83	4.24	**95**	NT	NT	>1000	>10000
55	>1000	220.52	2.02	1.09	**96**	NT	NT	>1000	>1000
56	NT	NT	35.87	599.02	**97**	NT	NT	>1000	>1000
57	NT	NT	24.06	153.00	**98**	NT	NT	14.79	125.30
58	3.54	4.97	2.90	0.54	**99**	NT	NT	>1000	>1000
59	NT	NT	16.08	1.36	**100**	414.10	127.80	19.08	>1000
60	>1000	62.62	0.91	32.57	**101**	NT	NT	207.20	>1000
61	NT	NT	12.66	3.37	**102**	NT	NT	>1000	525.60
62	NT	NT	73.29	2363.10	**103**	>1000	8.21	18.22	259.34
63	584.16	73.53	2.16	25.35	**104**	NT	NT	110.41	451.60
64	NT	NT	152.54	8.22	**105**	295.10	11.50	1.38	0.34
65	NT	NT	15.34	11.27	**106**	118.74	37.57	1.50	248.19
66	213.60	46.18	0.93	5.37	**107**	2.01	7.76	1.11	5.25
67	NT	NT	28.99	4.07	**108**	>1000	274.38	3.66	25.90
68	376.41	122.03	1.60	1.29	**109**	NT	NT	>1000	399.20
69	128.00	9.90	0.13	36.00	**110**	NT	NT	596.93	86.25
70	NT	NT	3.89	12.08	**111**	119.60	79.62	2.65	>1000
71	NT	NT	5.38	19.82	**112**	330.51	52.89	2.21	127.84
72	NT	NT	5.25	26.27	**113**	421.60	57.65	2.88	19.97
73	10.89	35.56	1.34	1.20	**114**	294.40	262.76	35.11	>1000
74	NT	NT	>1000	>1000	**115**	630.84	>1000	71.67	>1000
75	NT	NT	29.85	234.40	**116**	1518.70	>1000	429.40	>10.00
76	135.12	5.88	0.14	61.00	**117**	59.82	43.35	1.23	104.10
77	NT	790.88	39.11	>1000	**118**	NT	NT	23.35	65.80
78	125.55	193.29	2.91	69.55	**119**	77.88	54.08	3.25	258.10
79	>1000	119.18	2.38	52.31	**120**	NT	NT	125.96	>1000
80	119.28	168.28	10.14	275.44	**121**	91.96	86.38	7.78	>1000
81	>1000	>1000	7.56	160.30	**122**	53.93	20.25	0.84	9.38
82	NT	NT	46.26	>1000	**123**	87.55	35.94	0.54	1.06
83	43.96	812.58	18.19	>1000	**124**	>1000	84.08	8.74	85.40
84	190.25	26.60	0.37	134.84	**125**	29.25	131.95	3.15	3.84
85	114.06	47.54	3.72	62.22	**126**	54.43	54.99	0.37	2.33
86	503.20	17.01	0.20	29.64	**127**	27.21	15.84	1.43	91.86
87	107.70	10.05	0.18	12.07	**128**	42.41	7.95	0.11	17.43
88	479.50	447.86	15.29	803.57	**129**	68.26	83.61	3.11	>1000
89	2.85	13.61	1.72	400.91	BMY 7378	494.70	168.20	8.60	0.80
90	1.81	5.38	0.67	26.15	8-OH-DPAT	2082.00	18863.00	5421.00	3.80
91	186.68	13.11	0.41	206.96					

[a] BMY 7378 and 8-OH-DPAT are included as reference. [b] Equilibrium dissociation constants (K_i) were derived from IC_{50} using the Cheng−Prusoff equation.[36] The affinities estimated were derived from displacement of [^{3}H]prazosin binding for α_1 adrenoceptors and [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetraline ([^{3}H-8-OH-DPAT) for the 5-HT_{1A} receptor. Each experiment was performed in triplicate. The K_i values are the mean of different data, with the individual datum differing less than 20% from the mean. NT = not tested.

Table 7. Functional Activity of α_1-Antagonists **69**, **128**, and BMY 7378 as Reference for the Different Subtypes of the α_1 Adrenoceptor, Expressed as pK_B

compd	rat aorta α_{1D}	rat vas deferens α_{1A}	guinea pig spleen α_{1B}
69	8.99 ± 0.12^a	5.43 ± 0.14^b	5.55 ± 0.12^b
128	8.89 ± 0.19^a	7.00 ± 0.29^b	6.42 ± 0.09^b
BMY 7378	8.32 ± 0.09^a	$6.67 \pm 0.15^{a,c}$	$6.55 \pm 0.18^{a,c}$

[a] In the in vitro functional studies, the dissociation constant (K_B) was estimated by the technique of Arunlakshana and Schild[39] from a Schild plot with the slope constrained to unity, where the intercept represents the negative logarithm$_{10}$ of the K_B (pK_B). [b] When only one or two concentrations of the tested compounds were used, the K_B value was calculated with the formula K_B = [B]/(dose ratio − 1), where [B] is the antagonist concentration. [c] Reference 44.

SAR analysis is that the phenylpiperazine moiety carries not only the primary ligand recognition site, i.e., the protonated nitrogen atom (N1), but also the key structural determinants for ligand selectivity at the α_{1d} receptor versus the remaining three receptors considered in this study. In fact, the 2,5-Cl_2 substituent at the phenyl ring confers distinguished α_{1d}-AR selectivity to the new compounds.

To investigate the putative interaction sites for the phenyl-substituted phenylpiperazine moiety, we have employed the multiple copies simultaneous search (MCSS) method.[23] MCSS determines energetically favorable positions and orientations (local minima of the potential energy) of functional groups on the surface or in the binding site of a protein subjected to simultaneous minimization. In this case, the 2-OCH_3- and the 2,5-dichlorophenylpiperazine moieties have been used as functional groups. For each receptor, 10 clusters of the different functional groups have been finally selected. These clusters represent local minima of the interaction energy between each functional group and the receptor. For each multiple-copy complex, the fraction of clusters that are involved in the salt bridge with Asp3:11 has been computed (the amino acid numbering is arbitrary; the digits before the colon indicate the receptor domain, whereas the two digits after the colon indicate the position of the amino acid in that domain; see the alignment in Figure 1), the amino acid that was demonstrated to be the receptor key recognition point for cationic ligands[24] (F1, Table 8). Interestingly, this fraction is correlated with the selectivity profile shown

Figure 1. Pairwise alignments between each of the target receptors and a modified rhodopsin template (i.e., rhodchim). Bold sequences on rhodchim indicate that the amino acids are extracted from the ab initio model of the α_{1b}-AR in its input arrangement and substituted to rhodopsin's segments. In the 5-HT_{1A} receptor sequence, the 218−227 and 336−243 amino acid stretches, which have been subjected to α-helical restraints, are in lower-case italics.

by BMY 7378 and **69** (Tables 6 and 8), i.e., those compounds that carry the substituted phenylpiperazines considered in the MCSS approach.

The extracellular loops 2 and 3 (E2 and E3, respectively) are suggested to drive the cluster distribution within the putative receptor binding sites. In particular, in the α_{1a}-AR, the presence of a glutamine at position 11 of E2 (i.e., GlnE2:11), which is directed toward the putative ligand binding pocket, exerts a steric hindrance and prevents most of the copies from interacting with Asp3:11. As a consequence, the protonated nitrogen atom of the two fragments shows preferences for interacting with either GluE2:7 or, better, GluE3:9. In fact, the cluster that realizes the lowest interaction energy

Table 8. Binding Selectivity of BMY 7378 and **69** for the Four Receptors and Indexes Computed from the Outputs of MCSS

	BMY 7378	**69**
$S_{\alpha 1a}$[a]	618.37	984.61
$S_{\alpha 1b}$[a]	210.25	76.15
$S_{\alpha 1d}$[a]	10.75	1.00
S_{5-HT1A}[a]	1.0	276.92
$F1_{\alpha 1a}$[b]	0.00	0.10
$F1_{\alpha 1b}$[b]	0.10	0.10
$F1_{\alpha 1d}$[b]	0.30	0.40
$F1_{5-HT1A}$[b]	0.30	0.10

[a] Selectivity for the four receptors. For each of the two compounds, this index is the ratio of the affinity (K_i, nM) for each of four receptors to the highest affinity (K_i, nM). [b] Fraction of substituted phenylpiperazines involved in salt bridge interactions with Asp3:11 of the receptor, as inferred from MCSS.

(i.e., red molecule in Figure 2) is characterized by a salt bridge between the positively charged nitrogen atom of the ligand and GluE3:9 of the receptor.

For the α_{1b}-AR, the tendency of the two phenylpiperazines to cluster in the outer part of the receptor instead of approaching Asp3:11 is almost the same as in the α_{1a}-AR. In fact, despite the presence of a glycine instead of a glutamine at position 11 of E2 that would favor the ligand entrance into the binding site, negatively charged amino acids in E2 and E3, i.e., AspE2:7, GluE2:9, and AspE3:9, form an attractive surface for the positively charged nitrogen atom of the ligands. Indeed, the lowest interaction energy is realized by the copies stored in the extracellular domains (red copies, Figure 2).

For the α_{1d}-AR, it appears clear the better abilities of the copies to penetrate into the receptor binding sites, i.e., to approach Asp3:11 (Figure 2). In fact, for the two different functional groups, the lowest interaction energy (i.e., highest complex stability) is realized by copies involved in charge-reinforced H-bonds with Asp3:11 (red molecules, Figure 2). The different behavior of the α_{1d}-AR compared to the other two AR subtypes may be due, at least in part, to the synergistic effects of a phenylalanine and a glycine at positions 9 and 11, respectively, of E2. In fact, the lack of a negatively charged amino acid at position E2:09 and of a bulky side chain at position E2:11 would favor the entrance of the copies into the receptor binding site.

Finally, similarly to the α_{1d}-AR, also in the 5-HT_{1A} receptor, the presence of an alanine immediately before the cysteine in E2 contributes to expose to the ligand a hydrophobic surface. Moreover, E3 lacks the negatively

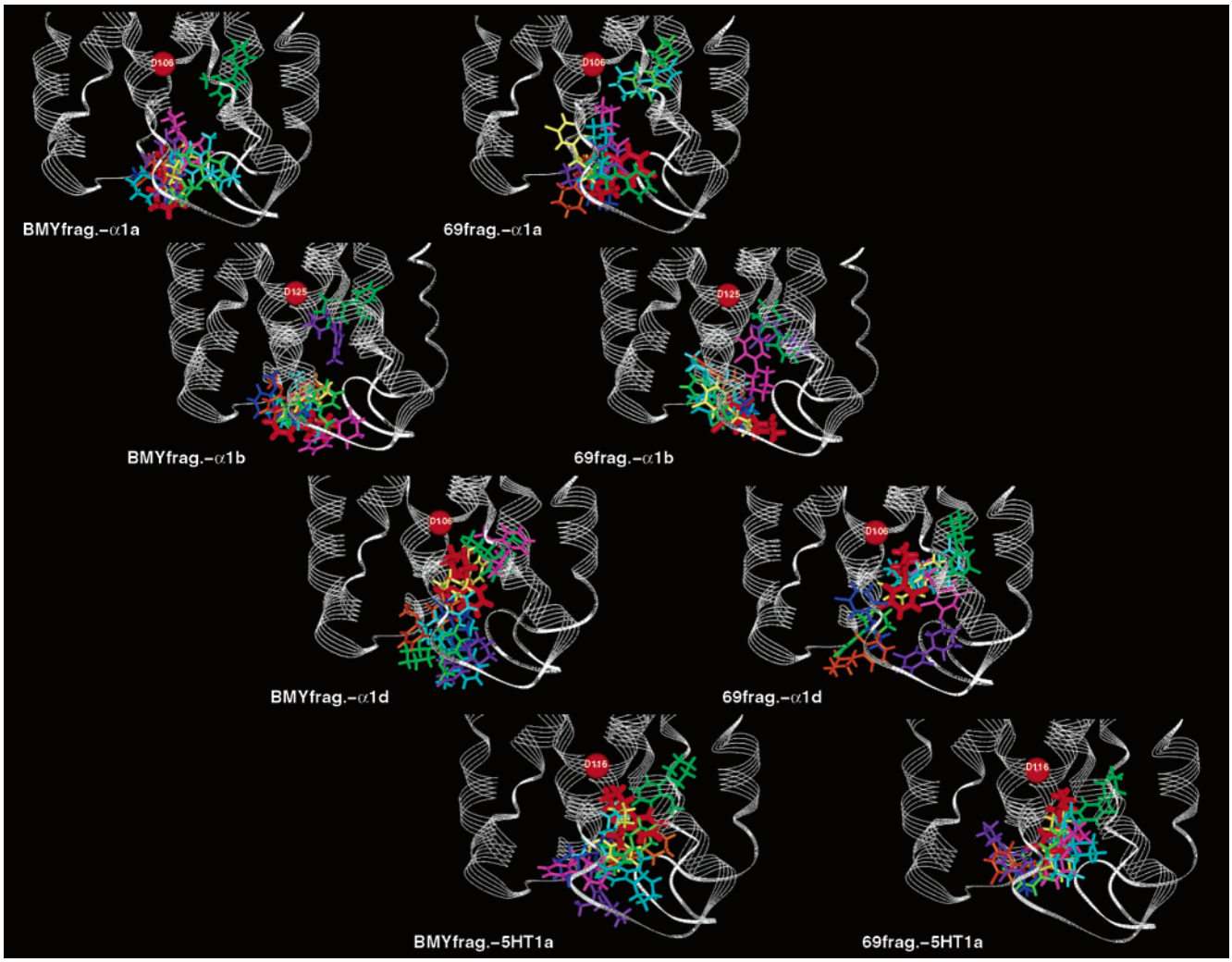


Figure 2. Side views (direction parallel to the membrane plane) of **69** and BMY 7378 fragments docked into the binding site of the α_1-adrenergic and 5-HT_{1A} receptors. Only the extracellular halves are shown. For each receptor, 10 copies of the selected fragments have been chosen that correspond to local minima of the interaction energy, the lowest interaction energy value being realized by the red copy. The putative binding site aspartate is indicated by a red sphere centered on the α-carbon atom.

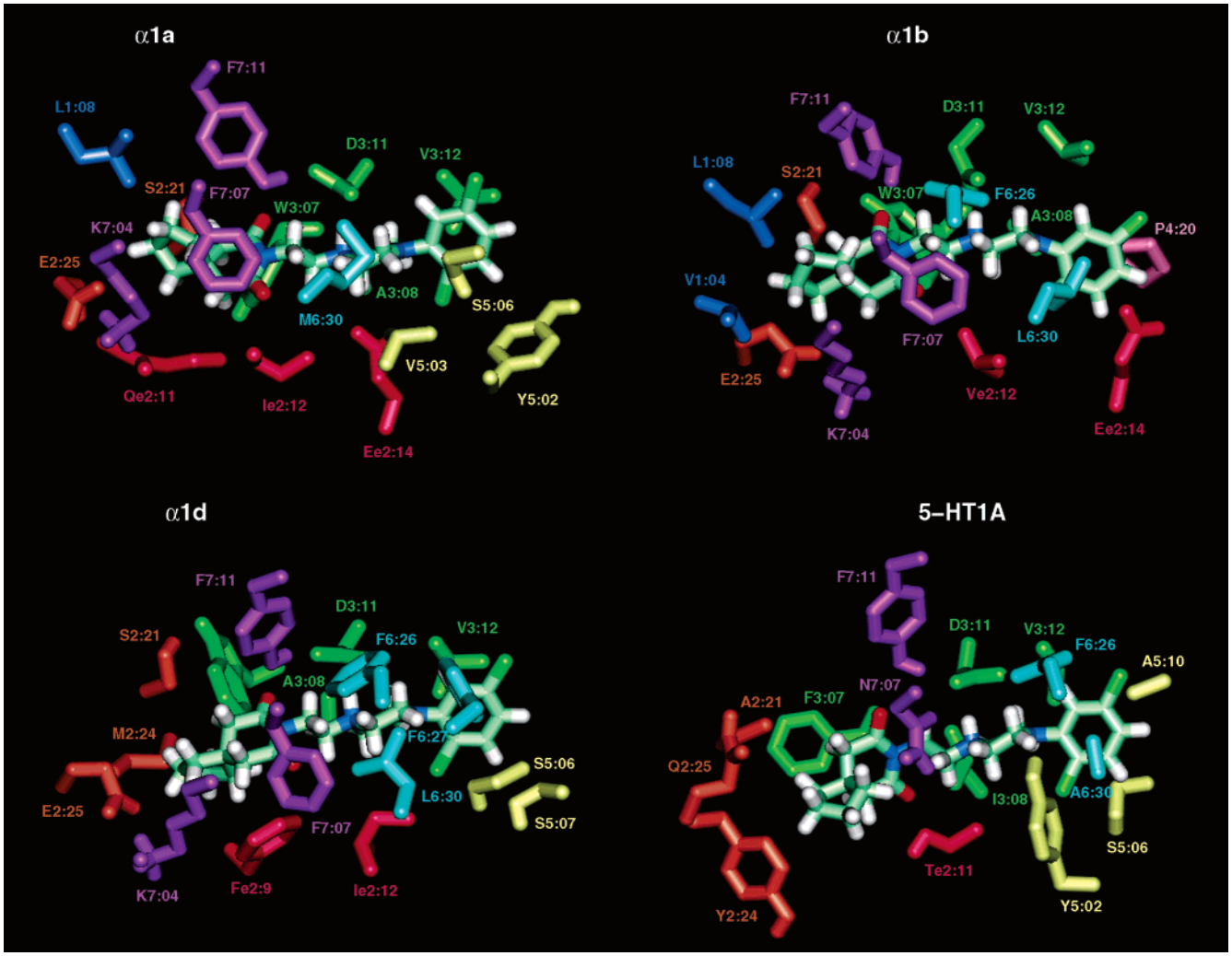


Figure 3. Amino acids that interact with **69** and BMY 7378 in their average minimized complexes with the three α_1 adrenergic and the 5-HT_{1A} receptor models. The amino acid side chains are colored according to their locations, i.e., helices 1, 2, 3, 4, 5, 6, and 7 are, respectively, in blue, orange, green, pink, yellow, cyan, and violet, whereas the second intracellular loop is in red.

charged amino acid at position 9 (Figure 1). These features of the 5-HT_{1A} receptor contribute to make Asp3:11 the preferential recognition site for the two different probes; i.e., the lowest interaction energies are realized through the interaction between the protonated nitrogen atom of the ligand and Asp3:11. The differences between the amino acids that lie at positions E2:11 of the α_{1d}-AR and the corresponding E2:12 of the 5-HT_{1A} (Figure 1) might contribute to the preferential entrance of the 2,5-Cl_2 substituted phenylpiperazine into the α_{1d}-AR binding site compared to the 5-HT_{1A}.

In summary, the results of MCSS analysis suggest that the second and third extracellular loops may act as selectivity filters for the substituted phenylpiperazines. In particular, the two positions adjacent to the cysteine in the second extracellular loop influence the distribution of the functional groups into the extracellular halves of the four receptors.

Molecular Dynamics Simulations of the Ligand−Receptor Complexes. The results of automatic docking have suggested that the formation of a salt bridge between the protonated nitrogen atom of the ligand and Asp3:11 of the receptor is associated with the docking of the substituted phenylpiperazine moiety into a site formed by amino acids of helices 3, 4, 5, and 6 as well as of E2. These results agree with the main orientation of the whole compounds BMY 7378 and **69** as achieved by manual docking.

The amino acid positions involved in the interaction with the ligands are almost the same in all the eight selected average minimized complexes (Figure 3). The majority of these positions are occupied by the same amino acids in the four receptors.

In general, in all the ligand−receptor complexes, the substituted phenyl ring of the phenylpiperazine mainly interacts with (a) Val3:12, (b) amino acids at positions 2, 3, 6, and 10 of helix 5, and/or (c) amino acids at position 27 and 30 of helix 6. In contrast, the piperazine ring, the ethylenic spacer, and the imidic ring interact with amino acids at positions (a) 12 and/or 14 of E2 and (b) 3:03, 6:26, 7:07, and 7:11 (Figure 3). Finally, the cyclopentyl ring mainly interacts with amino acids in helices 1, 2, and 7, i.e., positions 1:04, 1:08, 2:21, 2:24, 2:25, and 7:04.

In almost all the ligand−receptor complexes, the substituted phenylpiperazine docks in such a way that the protonated nitrogen atom performs a charge-reinforced H-bond with Asp3:11 of the receptor. This interaction constitutes a constraint that significantly reduces the possible docking modes of the considered

ligands into the binding site of the four receptors. In particular, in almost all the complexes, the para position of the phenyl ring interacts with amino acids in helix 5 or, less frequently, helix 4. According to this interaction mode, the dimensions of the cavity, which accommodates the phenylpiperazine moiety, are compatible with a para substitution equal to or only slightly bigger than hydrogen. These features are consistent with structure−affinity relationships indicating that increasing the size of the substituent in the para position of the phenyl ring reduces the ligand-binding affinity toward either the α_{1d}-AR and 5-HT_{1A} receptors (Table 6). On the basis of other experimental data, almost the same trend is expected for the α_{1a} and α_{1b}-ARs (results not shown). In agreement with an experimental and computational study previously done,[25] we have found that the three α_1-AR subtypes have lower tolerance to big substituents in meta position of the phenyl ring, compared to the 5-HT_{1A} receptor. This effect could be explained, at least in part, on the basis of both the conformational behavior of the meta-substituted phenylpiperazine and the features of the receptor binding pockets. Conformational analysis on substituted phenylpiperazines suggested that the meta substitution increases the number of allowed orientations of the phenyl ring, compared to the ortho substitution.[26] This conformational freedom is expected to increase following the formation of a salt bridge between the protonated nitrogen atom of the piperazine and Asp3:11 of the receptor, since quantum chemical calculations suggested that the neutral forms of phenylpiperazines show higher conformational freedom with respect to the corresponding protonated forms.[26] Thus, whereas in the ortho-substituted phenylpiperazines the phenyl ring prefers to stay almost coplanar with respect to both the lone pair of the N4 piperazine nitrogen atom and the piperazine $N1^+$−H bond, in the meta-substituted phenylpiperazine the phenyl ring can stay not only coplanar (absolute minimum energy) but also almost perpendicular to the N4 lone pair.[26] In the latter case, the 5-HT_{1A} receptor would tolerate the increase in size of the meta substituent better than the three α_1-ARs, since it carries two alanines in the two possible binding sites of this substituent, whereas the three α_1-ARs hold bigger amino acids such as a serine (i.e., Ser5:10) or a methionine/leucine (i.e., Met6:30 in the α_{1a} and Leu6:30 in the α_{1b} and α_{1d}). In summary, for all four receptors, structure−activity relationships are consistent with the models of the ligand−receptor complexes showing that amino acids from helices 3, 4, 5, and 6 and of E2 constitute the environment of the phenylpiperazine.

The docking mode of the phenylpiperazines described above is concurrent with the ethylenic spacer and the spiro-cyclic ring docked into a receptor site formed by amino acids of helices 1, 2, 3, and 7. In particular, Phe7:07 (that in the 5-HT_{1A} receptor is substituted by an asparagine) (Figure 1) performs hydrophobic interactions either with the ethylenic spacer or with the imidic ring of the ligand. This interaction model is consistent with the experimental findings suggesting that in the α_{1a}-AR Phe7:07 performs hydrophobic rather than aromatic interactions with BMY 7378.[27] In fact, both alanine or leucine substitutions for Phe7:07, which can still make van der Waals attractive interactions while losing the aromaticity, do not lower the affinity of BMY 7378 for the α_{1a}-AR.[27]

The analysis of the selected ligand−receptor complexes suggests that essentially two positions of E2 are involved in the interaction with the ligands. These are the positions that follow the disulfide-bridged cysteine of E2, i.e., positions E2:11 and E2:12 (corresponding, respectively, to positions E2:12 and E2:13 in the 5-HT_{1A} receptor, Figure 1). These results are consistent with those of experiments indicating that GlnE2:11 and IleE2:12 in the α_{1a}-AR are involved in the α_{1a} versus α_{1b} selectivity issues.[28]

MD simulations suggested that the two carbonyl oxygen atoms of the imide moiety are never simultaneously involved in H-bonding interactions with the receptors. In particular, in the complexes which involve the α_{1b}-AR subtype, one H-bonding interaction is found between a carbonyl oxygen atom of the ligand and either Trp3:07 or Tyr7:11 of the receptor. These results are consistent with structure−affinity relationships that suggest that eliminating one of these two oxygen atoms does not lower receptor affinity (see **128**, Table 6).

In the majority of the complexes between the ligands and the three α_1-AR subtypes, the side chains of Glu2:25 and Lys7:04 contribute to perform van der Waals attractive interactions with the cyclopentane ring of the ligand. Interestingly these two charged amino acids perform also an intramolecular salt bridge both in the ligand-free and ligand-bound forms of the three α_1-ARs. The structural relationship between these amino acids is supported by their tendency to mutate in a correlated manner. In fact, in the 5-HT_{1A} receptor, the glutamate at position 2:25 and the lysine at position 7:04 are respectively replaced by a glutamine and an alanine (Figures 1−3). The structural link between positions 2:25 and 7:04 disagrees with the hypothesized presence of a link between Lys7:04 and the binding site aspartate Asp3:11 of the α_{1b}-AR.[29] On the same line, the rhodopsin-based models of the three α_1-AR subtypes do not support the hypothesis that a salt bridge between Lys7:04 and Asp3:11 would stabilize the inactive state of the α_{1b}-AR.

In general, each ligand gives different interaction patterns in the four different receptors. Focusing on **69**, the results of MD simulations suggest that it realizes a very good complementarity with the α_{1d}-AR. In fact, the 2,5-dichlorophenyl ring interacts with Phe6:27, whereas aromatic intermolecular interactions do not occur between the same ligand and neither one of the other three subtypes (Figure 3). Furthermore, a large number of hydrophobic amino acids complement the hydrophobic moieties of the ligand. These amino acids include PheE2:09 and Met2:24, which constitute a peculiarity of the α_{1d}-AR compared to the other two α_1-AR-subtypes and the 5-HT_{1A} receptor (Figure 3). It is worth noting that the amino acid at position 2:24 has been previously indicated as one of the main responsible for the α_{1a} versus the α_{1d} selectivity of dihydropiridine antagonists.[30]

Conclusions

In this work, we have discovered selective ligands for the α_{1d}-AR over the other α_1-AR subtypes and the 5-HT_{1A} receptor. The two ligands most selective for the

α_{1d} subtype, **69** (Rec 26D/038) and **128** (Rec 26D/073), have been shown to be endowed with potent antagonistic activity.

The integration of the results of the extensive SAR analysis with those of two different docking approaches provides significant insight into the characterization of the receptor binding sites as well as into the molecular determinants of ligand selectivity at the α_{1d}-AR and the 5-HT_{1A} receptor.

The results of MCSS on the substituted phenylpiperazines together with those of manual docking of compounds BMY 7378 and **69** into the putative binding sites of the α_{1a}-AR, α_{1b}-AR, α_{1d}-AR, and the 5-HT_{1A} receptors suggest that the phenylpiperazine moiety would dock into a site formed by amino acids in helices 3, 4, 5, 6, and E2, whereas the spiro-cyclic ring of the ligand docks into a site formed by amino acids of helices 1, 2, 3, and 7. This docking mode is consistent with the SAR data produced in this work. In particular, the low tolerance of all four receptors to para substitutions in the phenylpiperazine moiety is suggested to be due to the limited distance between the α-carbon atom of Asp3:11 and the α-carbon atoms of amino acids in helices 4 and 5, found at the same depth as the crucial aspartate. Moreover, the higher tolerance of the 5-HT_{1A} receptor to increasing the size of the meta-substituent on the phenyl ring is suggested to be due to the smaller amino acid side chains at positions 5:10 or 6:30 of this receptor compared to the other three α_1-ARs.

Furthermore, the binding site of the imide moiety does not allow for the simultaneous involvement of the two carbonyl oxygen atoms in H-bonding interactions, consistent with the SAR data.

The results of docking simulations also suggest that the second and third extracellular loops may act as selectivity filters for the substituted phenylpiperazines. In particular, the positions in E2 adjacent to the disulfide-bridged cysteine are suggested to contribute to selective binding of the phenylpiperazine derivatives, in particular, those that hold the 2,5-dichloro substitution on the phenyl ring.

Extensive chemical modification of BMY 7378 structure allowed us to obtain selectivity for the α_{1d}-AR with respect to the 5-HT_{1A} receptor by introducing, in particular, the 2,5-dichlorophenylpiperazine as new basic moiety. The BMY 7378 skeleton has been confirmed as optimal for interaction with the α_{1d} receptor, with the new finding of the imide−amide bioequivalence in this series.

The two most interesting compounds from this series, **69** and **128**, are being submitted to a deeper pharmacological characterization.

Experimental Section

Chemistry. Melting points were determined on a Büchi 510 capillary melting points apparatus and are uncorrected. Microanalyses indicated by the symbols were within ±0.4 of the theoretical values. ^{1}H NMR spectra were recorded on a Bruker AC200 spectrometer; chemical shifts are reported as δ (ppm) relative to tetramethylsilane. TLC on silica gel plates was used to check product purity. Silica gel 60 (Merck, 230−400 mesh) was used for flash chromatography. The following intermediates **4a**, **4b**, **25**, **28**, **30**, **36a**, ethyl 1-cyclopentanecarboxylate, and 3,3-tetramethyleneglutaric anhydride are commercially available.

8-[2-(4-(Substituted phenyl)-1-piperazinyl)ethyl]-8-azaspiro[4.5]decane-7,9-diones. General Method. A mixture of 8-(2-bromoethyl)-8-azaspiro[4.5]decane-7,9-dione[16] (**2a**, 1 mmol), the appropriate (substituted phenyl)piperazine (1.5 mmol), and triethylamine (1.5 mmol) was heated at 160−180 °C for 30 min. After cooling, the crude product was purified by flash chromatography (toluene/acetone 9:1) to give the desired derivatives (see Scheme 1 and Tables 1 and 2).

Following the same method but using 1-(2-chlorophenyl)-homopiperazine, compound **105** was prepared (see Scheme 1 and Table 3).

Some representative ^{1}H NMR spectra are reported.

For **54**. ^{1}H NMR ($CDCl_3$, δ): 1.52−1.60 (m, 4H), 1.63−1.79 (m, 4H), 2.56 (t, 2H, J = 7.01 Hz), 2.60 (s, 4H), 2.63−2.76 (m, 4H), 2.98−3.12 (m, 4H), 3.97 (t, 2H J = 7.01 Hz), 6.92−7.05 (m, 2H), 7.14−7.27 (m, 2H). Anal. ($C_{21}H_{28}ClN_3O_2$) C, H, Cl, N.

For **69**. ^{1}H NMR ($CDCl_3$, δ): 1.43−1.59 (m, 4H), 1.61−1.78 (m, 4H), 2.60 (s, 4H), 2.78−2.98 (m, 10H), 4.10 (t, 2H, J = 7.54 Hz), 6.87−6.97 (m, 2H), 7.18−7.24 (m, 1H). Anal. ($C_{21}H_{27}Cl_2N_3O_2$) C, H, Cl, N.

For **105**. ^{1}H NMR ($CDCl_3$, δ): 1.46−1.58 (m, 4H), 1.74−1.80 (m, 4H), 1.83−1.98 (m, 2H), 2.60 (s, 4H), 2.75 (t, 2H, J = 7.4 Hz), 2.81−2.97 (m, 4H), 3.19−3.35 (m, 4H), 3.90 (t, 2H, J = 7.4 Hz), 6.80−6.90 (m, 1H), 7.00−7.19 (m, 2H), 7.28−7.35 (m, 1H). Anal. ($C_{22}H_{30}ClN_3O_2$) C, H, Cl, N.

Synthesis of 8-[(4-(2,5-Dichlorophenyl)-1-piperazinyl)-*n*-propyl]-8-azaspiro[4.5]decane-7,9-dione (89) and 8-[(4-(2,5-Dichlorophenyl)-1-piperazinyl)-*n*-butyl]-8-azaspiro-[4.5]decane-7,9-dione (90). The synthesis of compound **90** is reported as representative.

(a) *N*-[4-[4-(2,5-Dichlorophenyl)-1-piperazinyl]butyl]-phthalimide (5b). A mixture of *N*-(4-bromobutyl)phthalimide (0.29 g, 1.05 mmol), 1-(2,5-dichlorophenyl)piperazine (0.25 g, 1.08 mmol), and K_2CO_3 (0.36 g, 2.64 mmol) in CH_3CN (30 mL) was refluxed for 18 h under N_2 atmosphere. After cooling to room temperature, the crude was purified by flash chromatography (petroleum ether/ethyl acetate 4:6) to give 0.35 g (76%) of **5b**. ^{1}H NMR ($CDCl_3$, δ): 1.49−1.85 (m, 4H), 2.42 (t, 2H, J = 7.54 Hz), 2.54−2.74 (m, 4H), 2.97−3.16 (m, 4H), 3.76 (t, 2H J = 7.54 Hz), 6.96 (dd, 2H, J = 3.77 and 9.43 Hz), 7.24 (d, 1H, J = 9.43 Hz), 7.67−7.79 (m, 2H), 7.81−7.92 (m, 2H).

(b) 4-[4-(2,5-Dichlorophenyl)-1-piperazinyl]butylamine (6b). A solution of **5b** (0.35 g, 0.81 mmol) and $NH_2NH_2 \cdot H_2O$ (0.12 mL, 2.42 mmol) in EtOH (20 mL) was refluxed for 3 h. After cooling to room temperature, the precipitate was filtered off, washed with iced EtOH (3 × 10 mL), then with Et_2O (3 × 10 mL). The reunited solution was alkalanized with 2 N NaOH (pH 8), the organic solvents were evaporated under vacuum, and water was extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried, Na_2SO_4 was filtered off, and the solvent was evaporated to dryness to give 0.24 g (100%) of **6b**. ^{1}H NMR ($CDCl_3$, δ): 0.99−1.17 (bs, 2H), 1.39−1.67 (m, 4H), 2.43 (t, 2H, J = 7.4 Hz), 2.57−2.69 (m, 4H), 2.74 (t, 2H, J = 7.4 Hz), 2.99−3.17 (m, 4H), 6.90 (d, 1H, J = 3.07 Hz), 6.98 (dd, 1H, J = 9.25 and 3.07 Hz), 7.23 (d, 1H, J = 9.25 Hz).

(c) 8-[(4-(2,5-Dichlorophenyl)-1-piperazinyl)-*n*-butyl]-8-azaspiro[4.5]decane-7,9-dione (90). A mixture of (3,3)-tetramethyleneglutaric anhydride (0.06 g, 0.36 mmol), the above amine **6b** (0.12 g, 0.4 mmol), and *p*-toluenesulfonic acid (0.02 g) in toluene (10 mL) was refluxed for 18 h. After cooling to room temperature, the solvent was evaporated at reduced pressure and the crude was purified by flash chromatography (toluene/MeOH 94:6) to give 0.14 g of **90**. ^{1}H NMR ($CDCl_3$, δ): 1.41−1.61 (m, 8H), 1.62−1.77 (m, 4H), 2.37−2.50 (m, 2H), 2.51−2.68 (m, 8H), 3.01−3.17 (m, 4H), 3.72−3.83 (m, 2H), 6.88−6.90 (m, 2H), 7.21−7.31 (m, 1H). Anal. ($C_{23}H_{31}Cl_2N_3O_2$) C, H, Cl, N.

Synthesis of Diamino Compounds (92−94). The preparation of compound **92** is given as representative.

(a) *N*-(2,5-Dichlorophenyl)-2-aminoethanol (8a). Compound **8a** was prepared following a reported method.[21] Accordingly, a mixture of 2,5-dichloroaniline (**7**) (0.16 g, 1 mmol), 2-chloroethanol (0.8 g, 10 mmol), and triethylamine (0.15 g, 1.5 mmol) was stirred at 150 °C for 16 h. The product was

adsorbed on silica gel and purified by flash chromatography (cyclohexane/ethyl acetate 7:3) to give 0.09 g (45%) of **8a**. ^{1}H NMR ($CDCl_3$, δ): 1.59−1.88 (m, 1H, exchanged with D_2O), 3.36 (t, 2H, J = 5.6 Hz), 3.93 (t, 2H, J = 5.6 Hz), 4.57−4.84 (m, 1H,), 6.63 (dd, 2H, J = 3,7, and 7.5 Hz), 7.17 (d, 1H, J = 7.5 Hz).

(b) *N*-(2,5-Dichlorophenyl)-2-methylaminoethanol (9a). A mixture of **8a**[21] (0.21 g, 1 mmol), 37% HCHO (0.08 mL, 3 mmol), and formic acid (0.14 g, 3 mmol) was refluxed for 18 h. After the mixture was cooled, 12 N HCl was added until pH 1, the solvent was evaporated to dryness, and the residue was made alkaline by 2 N NaOH and extracted with CH_2Cl_2 (3 × 10 mL). After drying (Na_2SO_4), the solvent was evaporated under vacuum and the residue was purified by flash chromatography (petroleum ether/ethyl acetate 8:2) to give 0.05 g (23%) of **9a**. ^{1}H NMR ($CDCl_3$, δ): 2.49−2.63 (m, 1H exchanged with D_2O), 2.77 (s, 3H), 3.17 (t, 2H, J = 5.8 Hz), 3.74 (t, 2H, J = 5.8 Hz), 6.96 (dd, 2H, J = 2.9 and 8.8 Hz), 7.06 (d, 1H, J = 2.9 Hz), 7.27 (d, 1H, J = 8.8 Hz).

(c) *N*-(2,5-Dichlorophenyl)-*N*-methyl-2-chloroethylamine (11). To an ice-cooled solution of **9a** (0.22 g, 1 mmol) in CH_2Cl_2 (10 mL) containing three drops of DMF, thionyl chloride (0.14 g, 1.2 mmol) was added and the mixture was stirred at room temperature for 0.5 h and then refluxed for 3 h. The solvent was evaporated, and the crude was suspended in CH_2Cl_2 (10 mL) and re-evaporated. The residue was made alkaline by 2 N NaOH and extracted with CH_2Cl_2 (3 × 10 mL). After drying (Na_2SO_4) and evaporation of the solvent under vacuum, **11** was obtained (0.19 g, 83%), which was used without further purification. ^{1}H NMR ($CDCl_3$, δ): 2.85 (s, 3H), 3.40 (t, 2H, J = 7.3 Hz), 3.68 (t, 2H, J = 7.3 Hz), 6.97 (dd, 1H, J = 3.6 and 9.1 Hz), 7.06 (d, 1H, J = 3.6 Hz), 7.24 (d, 1H, J = 9.1 Hz).

(d) *N*,*N*′-Dimethyl-*N*-(2,5-dichlorophenyl)ethylenediamine (14). A mixture of **11** (0.24 g, 1 mmol) and 2 N methanolic methylamine (4.32 mL, 8.65 mmol) was heated at 60−80 °C for 20 h in an autoclave. After cooling to room temperature and evaporation of the solvent under vacuum, the residue was purified by flash chromatography (dichloromethane/2 N methanolic ammonia 96:4) to give 0.067 g (29%) of **14**. ^{1}H NMR ($CDCl_3$, δ): 1.93−2.02 (m, 1H, exch with D_2O), 2.46 (s, 3H), 2.77 (m, 5H), 3.17 (t, 2H, J = 6.4 Hz), 6.97 (dd, 1H, J = 3.2 and 11.8 Hz), 7.06 (d, 1H, J = 3.2 Hz), 7.24 (d, 1H, J = 11.8 Hz).

(e) 8-[2-[*N*-[*N*-(2,5-Dichlorophenyl)methylamino]ethyl]methylaminoethyl]-8-azaspiro[4.5]decane-7,9-dione (92). Compound **14** was condensed with **2a** by heating at 160 °C for 0.5 h in the presence of TEA. The crude was purified by flash chromatography (toluene/methanol 95:5) to give **92**. The compound was then transformed into its hydrochloride salt to further purify it. The free base was eventually reobtained (see Scheme 3, path a and Table 3). ^{1}H NMR ($CDCl_3$, δ): 1.43−1.59 (m, 4H), 1.61−1.79 (m, 4H), 2.36 (s, 3H), 2.44−2.73 (m, 8H), 2.82 (s, 3H), 3.09−3.18 (m, 2H), 3.88 (t, 2H, J = 7.69 Hz), 6.90 (dd, 1H, J = 11.53 and 3.84 Hz), 7.01 (d, 1H, J = 3.84 Hz), 7.24 (d, 1H, J = 11.53 Hz). Anal. ($C_{21}H_{29}Cl_2N_3O_2$) C, H, Cl, N.

Synthesis of Amido Compounds (95−97). The synthesis of compound **97** is reported as representative.

(a) 2-Bromo-*N*-(2,5-dichlorophenyl)acetamide (16). To a solution of 2,5-dichloroaniline (2 g, 12.3 mmol) in CH_2Cl_2 (20 mL), cooled at 0 °C, bromoacetyl bromide (1.29 mL, 14.8 mmol) was added, and the resulting mixture was heated at 40 °C for 5 h and then left at room temperature overnight. The precipitate was filtered off and triturated with petroleum ether/Et_2O (1:3) to give by filtration 1.88 g (54%) of **16**. ^{1}H NMR ($CDCl_3$, δ): 4.07 (s, 2H), 7.08 (dd, 1H), 7.31 (d, 1H), 8.43 (d, 1H), 8.72−8.91 (br, 1H).

(b) 2-Bromo-*N*-(2,5-dichlorophenyl)-*N*-methylacetamide (17). To a suspension of **16** (0.2 g, 0.71 mmol) and K_2CO_3 (0.12 g, 0.85 mmol) in DMF (2.5 mL) a solution of CH_3I (0.5 g, 3.53 mmol) in DMF (2.5 mL) was added, and the resulting mixture was heated at 40 °C for 4 h. After the mixture was cooled to room temperature, H_2O (50 mL) was added and the solution was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were washed with H_2O, dried (Na_2SO_4), filtered, and evaporated to dryness at reduced pressure. The crude was purified by flash chromatography (cyclohexane/ethyl acetate 9:1) to give 0.08 g (38%) of **17**. ^{1}H NMR ($CDCl_3$, δ): 3.19 (s, 3H), 3.37 (d, 1H), 3.64 (d, 1H), 7.26 (d, 1H), 7.37 (dd, 1H), 7.45 (d, 1H).

(c) 2-Amino-*N*-(2,5-dichlorophenyl)-*N*-methylacetamide (19). A mixture of **17** (0.24 g, 0.81 mmol) and 2 N methanolic ammonia (10 mL, 20 mmol) was heated at 100 °C in an autoclave for 18 h. After the mixture was cooled to room temperature, the solvent was evaporated at reduced pressure and the crude was purified by flash chromatography (CH_2Cl_2/2 N methanolic NH_3 97:3) to give 0.10 g (53%) of **19**. ^{1}H NMR ($CDCl_3$, δ): 1.47−2.06 (br, 2H), 2.94 (d, 1H), 3.11 (d, 1H), 3.19 (s, 3H), 7.22−7.48 (m, 3H).

(d) 8-[2-[(*N*-Methyl-2,5-dichlorophenylaminocarbonylmethyl)amino]ethyl]-8-azaspiro[4.5]decane-7,9-dione (97). A solution of **2a** (0.28 g, 1.03 mmol), **19** (0.24 g, 1.03 mmol), and TEA (0.31 g, 3.1 mmol) in toluene (10 mL) was refluxed for 18 h. After cooling to room temperature, the solvent was evaporated in vacuo and the residue was purified by flash chromatography to give 0.04 g of **97** after three purification steps (toluene/MeOH 96:4, toluene/acetone 8:2, CH_2Cl_2/acetone 8:2) (see Scheme 3, path b and Table 3). ^{1}H NMR ($CDCl_3$, δ): 1.38−1.56 (m, 4H), 1.55−1.77 (m, 4H), 2.36−2.42 (m, 2H), 2.49 (s, 2H), 3.20 (s, 3H), 3.66−3.78 (m, 2H), 3.79−3.91 (m, 2H), 4.09−4.21 (m, 2H), 7.31−7.39 (m, 2H), 7.43−7.54 (m, 1H). The NH signal was not detectable.

Synthesis of Phenoxyalkylamino Compounds (98−104). The synthesis of compound **100** is reported as representative.

(a) 1-Bromo-2-(2,5-dichlorophenoxy)ethane (22). Compound **22** was prepared as indicated in the literature.[31] Accordingly, to a mixture of 2,5-dichlorophenol (3 g, 18.4 mmol) and 1,2-dibromoethane (6.91 g, 36.8 mmol) heated at 100 °C a solution of NaOH (0.73 g, 18.4 mmol) in H_2O (10 mL) was added dropwise, and the resulting mixture was stirred at 100 °C for 7 h. After 12 h of resting, CH_2Cl_2 (20 mL) was added, the organic layer was washed with 2 N NaOH (3 × 15 mL) and brine (1 × 15 mL), dried over Na_2SO_4, filtered and evaporated at reduced pressure. The crude was purified by flash chromatography (petroleum ether/ethyl acetate 95:5) to give 2.61 g (52%) of **22** as oil. ^{1}H NMR ($CDCl_3$ δ): 3.67 (t, 2H, J = 6.67 Hz), 4.32 (t, 2H, J = 6.67 Hz), 6.91 (dd, 2H, J = 10 and 3.34 Hz), 7.29 (d, 1H, J = 3.34 Hz).

(b) *N*-Methyl-2-(2,5-dichlorophenoxy)ethylamine (27). A solution of **22** (0.56 g, 2.07 mmol) and 2 N CH_3NH_2 in MeOH (9 mL, 18 mmol) was heated at 100 °C in autoclave for 12 h. After cooling to room temperature, the crude obtained by evaporation was purified by flash chromatography (CH_2Cl_2/2 N methanolic ammonia gradient from 98:2 to 95:5) to afford 0.4 g (75%) of **27**. ^{1}H NMR ($CDCl_3$ δ): 1.94−2.05 (bs, 1H, exchanged with D_2O), 2.53 (s, 3H), 3.03 (t, 2H, J = 5.1 Hz), 4.12 (t, 2H, J = 5.1 Hz), 6.88 (dd, 2H, J = 10.1 and 3.4 Hz), 7.29 (d, 1H, J = 3.4 Hz).

(c) 8-[2-[*N*-2-(2,5-Dichlorophenoxy)ethyl]-*N*-methylamino]ethyl]-8-azaspiro[4.5]decane-7,9-dione (100). A solution of **2a** (0.141 g, 0.51 mmol) and **27** (0.34 g, 1.54 mmol) in toluene was refluxed for 40 h. After cooling to room temperature, the solvent was removed at reduced pressure and the crude was purified by flash chromatography (toluene/MeOH 95:5), affording 0.06 g of **100**. ^{1}H NMR ($CDCl_3$ δ): 1.42−1.57 (m, 4H), 1.62−1.77 (m, 4H), 2.46 (s, 3H), 2.58 (s, 4H), 2.59 (t, 2H, J = 6.77 Hz), 2.95 (t, 2H, J = 5.76 Hz), 3.96 (t, 2H, J = 6.77 Hz), 4.03 (t, 2H, J = 5.76 Hz), 6.81−6.97 (m, 2H), 7.20−7.29 (m, 1H). Anal. ($C_{20}H_{26}Cl_2N_2O_3$) C, H, Cl, N.

Synthesis of 8-[2-[4-(Substituted phenyl)-1-piperidinyl]ethyl]-8-azaspiro[4.5]decane-7,9-diones (106−108). The synthesis of compound **106** is reported as representative.

(a) Diethyl 2,5-Dichlorobenzylidenemalonate (34c). A mixture of 2,5-dichlorobenzaldehyde (0.17 g, 1 mmol), diethyl malonate (0.32 g, 2 mmol), and $AlCl_3$ (0.66 g, 5 mmol) was stirred at room temperature for 4 h, then poured into ice−

water (30 mL). A 37% HCl solution was added until all the salts were dissolved, and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). After drying (Na_2SO_4), the solvent was evaporated under vacuum and the residue was purified by flash chromatography (petroleum ether/ethyl acetate 9:1) to give 0.16 g (51%) of **34c**. ^{1}H NMR ($CDCl_3$, δ): 1.24 (t, 3H, J = 8 Hz), 1.37 (t, 3H, J = 8 Hz), 4.31 (q, 4H, J = 8 Hz), 7.24 (dd, 2H, J = 4 and 8 Hz), 7.41 (d, 1H, J = 4 Hz), 7.94 (s, 1H).

(b) Diethyl 3-(2,5-Dichlorophenyl)-2,4-diethoxycarbonylglutarate (35c). A mixture of **34c** (0.31 g, 1 mmol), diethyl malonate (0.16 g, 1 mmol), and $AlCl_3$ (0.4 g, 3 mmol) was heated at 60 °C for 6 h. Isolation of the crude product and its purification was carried out as above-reported for the diethyl 2,5-dichlorobenzylidenemalonate to give 0.16 g (34%) of **35c**. ^{1}H NMR ($CDCl_3$, δ): 1.07 (t, 6H, J = 1.07 Hz), 1.22 (t, 6H, J = 7.7 Hz), 4.01 (q, 4H, J = 7.7 Hz), 4.12 (q, 6H, J = 7.7 Hz), 4.68−4.86 (m, 1H), 7.10 (dd, 1H, J = 3.8 and 9.6 Hz), 7.22 (d, 1H, J = 9.6 Hz), 7.46 (d, 1H, J = 3.8 Hz).

(c) 3-(2,5-Dichlorophenyl)glutaric Acid (36c). A mixture of **35c** (0.48 g, 1 mmol) and 47% HBr (5 mL) was refluxed for 48 h. After the mixture was cooled to room temperature, a precipitate was formed, which was collected by suction to give 0.2 g (73%) of **36c**. ^{1}H NMR (DMSO-d_6, δ): 2.40 (t, 4H, J = 12.1 Hz), 3.96 (q, 1H, J = 12.1), 7.31 (dd, 1H, J = 5.6 and 11.8 Hz), 7.49 (d, 1H, J = 11.8 Hz), 7.57 (d, 1H, J = 5.6 Hz), 11.98−12.69 (br s, 2H, exchanged with D_2O).

(d) 1-Cyanomethyl-4-(2,5-dichlorophenyl)piperidine-2,6-dione (37c). A solution of **36c** (0.28 g, 1 mmol) and *N,N′*-dicyclohexylcarbodiimide (0.23 g, 1.1 mmol) in anhydrous DMF was stirred at room temperature for 1.5 h, then aminoacetonitrile (0.22 g, 1.05 mmol) and triethylamine (0.11 g, 1.05 mmol) were added. The mixture was stirred at room temperature for 18 h, the so-formed precipitate was filtered off, and the solvent was evaporated under vacuum. To the residue, sodium acetate (0.25 g, 3 mmol) and acetic anhydride (5 mL) were added, and the mixture was heated at 100 °C for 2 h. The cooled suspension was poured into ice−water (10 mL), stirred for 30 min, made alkaline by 2.5 N NaOH, and extracted with CH_2Cl_2 (3 × 20 mL). After drying (Na_2SO_4), the solvent was evaporated under vacuum and the residue was purified by flash chromatography (petroleum ether/ethyl acetate 85:15) to give 0.14 g (47%) of **37c**. ^{1}H NMR ($CDCl_3$, δ): 2.73−2.97 (m, 2H), 3.02−3.19 (m, 2H), 3.74−3.94 (m, 1H), 4.71 (s, 2H), 7.14−7.42 (m, 3H).

(e) 2-[4-(2,5-Dichlorophenyl)-1-piperidinyl]ethylamine (38c). To a suspension of $LiAlH_4$ (0.15 g, 4 mmol) in anhydrous Et_2O (5 mL) a solution of **37c** (0.3 g, 1 mmol) in anhydrous Et_2O (5 mL) was added at such a rate to maintain a gentle reflux. The mixture was further refluxed for 2 h and then left overnight at room temperature. Excess $LiAlH_4$ was destroyed by 20% NaOH addition under cooling. The inorganic salts were filtered off and washed with CH_2Cl_2. After drying (Na_2SO_4) and evaporation of the solvent under vacuum, the residue was purified by flash chromatography (CH_2Cl_2/2 N methanolic ammonia 98:2) to give 0.1 g (37%) of **38c**. ^{1}H NMR ($CDCl_3$, δ): 1.77−1.96 (m, 4H), 1.97−2.28 (m, 2H), 2.42−2.56 (m, 2H), 2.77−2.94 (m, 5H), 2.96−3.21 (m, 2H), 7.08−7.37 (m, 3H).

(f) 8-[2-[4-(2,5-Dichlorophenyl)-1-piperidinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione (106). The compound was prepared by condensing **38c** (0.1 g, 3.7 mmol) and 3,3-tetramethyleneglutaric anhydride (0.6 g, 3.7 mmol) in refluxing toluene in the presence of *p*-toluenesulfonic acid for 20 h. After cooling to room temperature, the solvent was evaporated at reduced pressure and the crude was purified by flash chromatography (toluene/acetone 9:1) to give 0.03 g of **106**. ^{1}H NMR ($CDCl_3$, δ): 1.45−1.66 (m, 4H), 1.64−1.97 (m, 8H), 2.07−2.23 (m, 2H), 2.50 (t, 2H, J = 6.73 Hz), 2.64 (s, 4H), 2.83−3.16 (m, 3H), 4.38 (t, 2H, J = 6.73 Hz), 7.01−7.24 (m, 3H). Anal. ($C_{22}H_{28}Cl_2N_2O_2$) C, H, Cl, N (see Scheme 5 and Table 3).

Synthesis of Compounds Modified at the Spirocyclopentyl Ring. General Procedure for 112, 113, and 118 (Scheme 6, Path a). A suspension of the required azaspirodione (1 mmol) and 80% NaH (1.5 mmol) in DMF (2.0 mL) was stirred at 60 °C for 30 min. After cooling, it was poured into a solution of 1,2-dibromoethane (5 mmol) in DMF (6 mL) and the mixture was stirred for 5 h. The solvent was evaporated, and the residue was treated with H_2O (20 mL) and extracted with CH_2Cl_2 (3 × 8 mL). The organic layer was washed with H_2O, dried (Na_2SO_4), and then evaporated. The crude was purified by flash chromatography (cyclohexane/ethyl acetate 7:3) and condensed with the appropriate 1-(substituted phenyl)piperazine (molar ratio 1:1.5) by heating at 180 °C for 30 min in the presence of triethylamine (molar ratio 1:1.5). After cooling, the crude was purified by flash chromatography (petroleum ether/ethyl acetate 85:15) (see Scheme 6 and Table 4). For **112**, ^{1}H NMR ($CDCl_3$, δ): 1.37−1.58 (m, 10H), 2.56 (s + t, 6H, J = 7.14 Hz), 2.59−2.73 (m, 4H), 2.85−3.09 (m, 4H), 3.96 (t, 2H, J = 7.14 Hz), 6.87 (d, 1H, J = 3.57 Hz), 6.96 (dd, 1H, J = 3.57 and 8.92 Hz), 7.23 (d, 1H, J = 8.92 Hz). Anal. ($C_{22}H_{29}Cl_2N_3O_2$) C, H, Cl, N.

General Procedure for 117 and 119−121 (Scheme 6, Path b). A solution of the appropriate anhydride (1 mmol), 2-[4-(2,5-dichlorophenyl)-1-piperazinyl]ethylamine (**6c**, 0.3 g, 1.1 mmol), and *p*-toluenesulfonic acid (0.02 g) in toluene (10 mL) was refluxed under stirring for 20 h. After cooling, the solvent was evaporated and the residue was purified by flash chromatography (toluene/acetone 9:1) (see Scheme 6 and Table 4). For **117**, ^{1}H NMR ($CDCl_3$, δ): 0.86 (t, 3H, J = 7.69 Hz), 1.03 (s, 3H), 1.40 (q, 2H, J = 7.69 Hz), 2.47 (s, 4H), 2.56 (t, 2H, J = 5.76 Hz), 2.59−2.73 (m, 4H), 2.83−3.10 (m, 4H), 3.94 (t, 2H, J = 5.76 Hz), 6.88 (d, 1H, J = 3.8 Hz), 6.95 (dd, 1H, J = 3.8 and 11.5 Hz), 7.21 (d, 1H, J = 11.5 Hz). Anal. ($C_{20}H_{27}Cl_2N_3O_2$) C, H, Cl, N.

Synthesis of Ethyl 2-[1-Benzyl-4-(2-ethoxy-2-oxoethyl)-4-piperidyl]acetate Hydrochloride (47). (a) A solution of ethyl cyanoacetate (22.62 g, 0.2 mol) and 1-benzyl-4-piperidone (19.12 g, 0.1 mol) was added to 80 mL of 6.5 N NH_3 in dry EtOH, with stirring at −10/−1 °C. The solution was maintained for 4 days at 0−2 °C, and the precipitate (as ammonium salt) was collected by filtration, washed with cold EtOH, and dried. The salt was treated with 150 mL of hot H_2O, and the boiling suspension was acidified with 2 N HCl (50 mL). The resulting solution was cooled to room temperature to give a yellow precipitate, which was collected by filtration to afford 21 g (65.6%) of the 9-benzyl-2,4-dioxo-3,9-diazaspiro[5.5]-undecane-1,5-dicarbonitrile. ^{1}H NMR (DMSO-d_6, δ): 12.00−12.60 (bs, 1H, exchanged with D_2O), 10.30−10.80 (bs, 1H, exchanged with D_2O), 7.35−7.65 (m, 5H), 4.27 (s, 2H), 3.00−3.50 (m, 4H), 1.75−2.40 (m, 4H).

(b) A stirred mixture of the above prepared compound (19.2 g, 60 mmol) in 65% H_2SO_4 (39 mL) was refluxed for 2 h. Afterward, H_2O (24 mL) was added and reflux was continued for 6.5 h. The so-obtained solution was cooled to 10−15 °C, 50% NaOH (74 mL) was added dropwise, then 2 N HCl (100 mL) was added to the suspension at 10−15°C, and the solvent was removed by evaporation in vacuo. The residual water was removed by azeotropic distillation with toluene. The solid residue was treated with anhydrous EtOH (300 mL) and 98% H_2SO_4 (30 mL). The mixture was refluxed for 14 h, cooled to room temperature, diluted with Et_2O (300 mL), and neutralized with solid $NaHCO_3$ (130 g). The inorganic salts were filtered off and washed with Et_2O (2 × 300 mL). The organic layers were dried (Na_2SO_4) and evaporated to dryness at reduced pressure. The residue was dissolved in $CHCl_3$ (100 mL), and the solution was quickly washed with cold 10% $NaHCO_3$ (3 × 30 mL) and cold brine (3 × 20 mL) and then dried over Na_2SO_4. The solvent was evaporated at reduced pressure to give 17.84 g (84%) of **47** as a base.

The corresponding hydrochloride was obtained by dissolving the compound in Et_2O and acidifying with 3 N HCl in Et_2O. The solid was collected and crystallized from *i*-PrOAc. Mp 141−145°C. ^{1}H NMR (DMSO-d_6, δ): 1.08−1.18 (m, 6H), 1.70−2.05 (m, 4H), 2.41 (s, 2H), 2.71 (s, 2H,), 2.98−3.25 (m, 4H), 3.92−4.15 (m, 4H), 4.28 (d, 2H), 7.35−7.50 (m, 3H), 7.50−7.70 (m, 2H), 10.53 (bs, 1H, exchanged with D_2O).

Synthesis of 3,9-Diazaspiro[5.5]undecane-2,4-diones (114−116). 9-Benzyl-3-[2-[4-(2-methoxyphenyl)-1-piper-

azinyl]ethyl]-3,9-diazaspiro[5.5]undecane-2,4-dione (114). A mixture of **47** (0.305 g, 0.88 mmol) and 2-[4-(2-methoxyphenyl)-1-piperazinyl]ethylamine (1.24 g, 5.26 mmol) was heated in toluene at 140−150 °C for 48 h in an autoclave. After cooling to room temperature, the residue was purified by flash chromatography (CH_2Cl_2/MeOH 98:2) to give 0.103 g of **114**. ^{1}H NMR ($CDCl_3$ δ): 1.56−1.64 (m, 4H), 2.38−2.52 (m, 4H), 2.56 (t, 2H, J = 7.54 Hz), 2.58 (s, 4H), 2.63−2.77 (m, 4H), 2.93−3.16 (m, 4H), 3.54 (s, 2H), 3.90 (s, 3H), 3.97 (t, 2H, J = 7.54 Hz), 6.81−7.00 (m, 4H), 7.23 (s, 5H). Anal. ($C_{29}H_{38}N_4O_3$) C, H, N.

3-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-3,9-diazaspiro[5.5]undecane-2,4-dione Hydrochloride (115). To a solution of **114** (0.2 g, 0.41 mmol) in EtOH an excess of 2 N HCl in Et_2O was added. The hydrochloride salt was filtered and suspended in 95% EtOH (40 mL), 10% Pd−C (0.02 g) was added, and the mixture was stirred in H_2 atmosphere at room temperature for 20 h. The catalyst was filtered off, and the solvent was evaporated under vacuum. The residue was taken up in CH_2Cl_2 (15 mL), and 50% NaOH was added dropwise. The organic layer was dried on Na_2SO_4, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography. Elution with CH_2Cl_2/MeOH 85:15 afforded 0.11 g of an oily base that was transformed into the hydrochloride salt by HCl/Et_2O. The yield obtained was 0.12 g (67%) of **115**. ^{1}H NMR (DMSO-d_6 δ): 1.63−1.69 (m, 4H), 2.84 (s, 4H), 3.06−3.20 (m, 8H), 3.26−3.31 (m, 2H), 3.45−3.52 (m, 2H), 3.60−3.67 (m, 2H), 3.80 (s, 3H), 4.02−4.09 (m, 2H), 4.20−4.25 (m, 1H), 6.90−6.97 (m, 2H), 6.99−7.03 (m, 2H), 8.68 (br, 1H). Anal. ($C_{23}H_{33}ClN_4O_3$) C, H, Cl, N.

9-Acetyl-3-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-3,9-diazaspiro[5.5]undecane-2,4-dione (116). To an ice-cooled solution of **115** (0.08 g, 0.2 mmol) in CH_2Cl_2 (8 mL), acetic anhydride (0.028 mL, 0.3 mmol) was added, and the reaction mixture was stirred at room temperature for 2 h. The solvent was removed under vacuum, and the crude was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to give 0.06 g of **116**. ^{1}H NMR ($CDCl_3$ δ): 1.52−1.65 (m, 4H), 2.16 (s, 3H), 2.57 (t, 2H, J = 7.27 Hz), 2.61−2.76 (s + m, 4H + 4H), 2.93−3.13 (m, 4H), 3.38−3.48 (m, 2H), 3.57−3.63 (m, 2H), 3.80 (s, 3H), 3.95 (t, 2H, J = 7.27 Hz), 6.77−7.00 (m, 4H). Anal. ($C_{24}H_{34}N_4O_4$) C, H, N.

Synthesis of 8-[2-[4-(Substituted phenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7-ones (122, 123, 125−128). The preparation of compounds **127** and **128** is given as representative.

Synthesis of 8-[2-[4-(2,5-Dichlorophenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7-dione (127). To a solution of 1,1-cyclopentanediacetic acid monomethyl ester (**49**, 1 g, 5.5 mmol) in CH_2Cl_2 (20 mL), containing a few drops of DMF, oxalyl chloride (0.7 g, 5.5 mmol) was added dropwise at 10−15 °C. After stirring at room temperature for 2 h, the solvent was evaporated, the residue was dissolved in acetone (20 mL) and added to a mixture of diisopropylethylamine and 10% Pd−C (0.1 g) in anhydrous acetone (20 mL), previously stirred in a hydrogen atmosphere. The mixture was hydrogenated at normal pressure until 1 equiv of hydrogen was adsorbed, and then the catalyst was filtered off and the solvent was evaporated under vacuum. The residue was purified by flash chromatography (cyclohexane/ethyl acetate 9:1) to give 0.63 g (69%) of the methyl (1-formylmethyl-1-cyclopentyl)-acetate (**50**). ^{1}H NMR ($CDCl_3$, δ): 1.39−1.63 (m, 8H), 2.41 (s, 2H), 2.58 (s, 2H), 3.57 (s, 3H), 9.61−9.75 (m, 1H).

A mixture of **50** (0.08 g, 0.44 mmol), 2-(4-(2,5-dichlorophenyl)-1-piperazinyl)ethylamine (**6c**, 0.14 g, 0.51 mmol), and *p*-toluenesulfonic acid (0.02 g) in toluene (10 mL) was refluxed overnight, distilling off the azeotrope. After cooling to room temperature, the solvent was evaporated under vacuum and the residue was purified by flash chromatography (toluene/acetone 9:1) to give 0.09 g of **127**. ^{1}H NMR ($CDCl_3$, δ): 1.43−1.59 (m, 4H), 1.61−1.77 (m, 4H), 2.38 (s, 2H), 2.58 (t, 2H, J = 6.89 Hz), 2.60−2.98 (m, 4H), 2.96−3.15 (m, 4H), 3.61 (t, 2H, J = 6.89 Hz), 5.09 (d, 1H, J = 10.3 Hz), 5.96 (d, 1H, J = 10.3 Hz), 6.86−7.03 (m, 2H), 7.20−7.27 (m, 1H). Anal. ($C_{21}H_{27}Cl_2N_3O$) C, H, Cl, N (see Scheme 8 and Table 5)

Synthesis of 8-[2-[4-(2,5-Dichlorophenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]dec-9,10-en-7-one (128). To a stirred solution of **50** (0.092 g, 0.5 mmol) and 2-(4-(2,5-dichlorophenyl)-1-piperazinyl)ethylamine (**6c**, 0.16 g, 0.58 mmol) in MeOH (10 mL), $NaBH_4$ (0.009 g, 0.29 mmol) was added at 0 °C. After 1.5 h of stirring, the solvent was evaporated and the residue was treated with water (5 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried (Na_2SO_4) and evaporated under vacuum and the residue was purified by flash chromatography (toluene/acetone 75:25) to give 0.14 g of **128**. ^{1}H NMR ($CDCl_3$, δ): 1.19−1.58 (m, 4H), 1.59−1.80 (m + t, 4H + 2H, J = 6.67 Hz), 2.27 (s, 2H), 2.59 (t, 2H, J = 7.02 Hz), 2.62−2.78 (m, 4H), 2.97−3.17 (m, 4H), 3.21 (t, 2H, J = 6.67 Hz), 3.57 (t, 2H, J = 7.02 Hz), 6.88−7.02 (m, 2H), 7.22−7.32 (m, 1H). Anal. ($C_{21}H_{29}Cl_2N_3O$) C, H, Cl, N (see Scheme 8 and Table 5).

Synthesis of 2-[2-[4-(2,5-Dichlorophenyl)-1-piperazinyl]ethyl]-2-azaspiro[4.4]nonane-1,3-dione (129). To a solution of diisopropylamine (0.1 g, 1 mmol) in anhydrous THF (10 mL), stirred at −70 °C under N_2 atmosphere, 2.5 M *n*-butyllithium in THF (0.4 mL, 1 mmol) was added dropwise. After 30 min of stirring, a solution of ethyl 1-cyclopentanecarboxylate (0.14 g, 1 mmol) in anhydrous THF (5 mL) was added and stirring was continued for 1 h. A solution of ethyl bromoacetate (0.17 g, 1 mmol) in anhydrous THF (5 mL) was then added, and the mixture was stirred at −70 °C for 1 h, then at room temperature for 20 h. A solution of NH_4Cl (0.43 g) in H_2O (20 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 15 mL). The organic layer was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by flash chromatography (petroleum ether/ethyl acetate 95:5) to give 0.044 g (19%) of ethyl (1-ethoxycarbonyl-1-cyclopentyl)acetate. ^{1}H NMR ($CDCl_3$, δ): 1.21 (t, 6H, J = 7.54 Hz), 1.44−1.93 (m, 6H), 2.02−2.21 (m, 2H), 2.67 (s, 2H), 4.11 (q, 4H, J = 7.54 Hz).

A mixture of ethyl (1-ethoxycarbonyl-1-cyclopentyl)acetate (0.11 g, 0.48 mmol) and 2-[4-(2,5-dichlorophenyl)-1-piperazinyl]-ethylamine (0.23 g, 0.84 mmol) was heated at 160 °C in an autoclave. After cooling to room temperature, the crude was purified by flash chromatography (toluene/acetone 9:1) to give 0.03 g of **129**. ^{1}H NMR ($CDCl_3$, δ): 1.60−1.82 (m, 4H), 1.83−2.05 (m, 2H), 2.06−2.24 (m, 2H), 2.57 (s, 2H), 2.62−2.75 (m + t, 6H, J = 7.27 Hz), 2.93−3.09 (m, 4H), 3.68 (t, 2H, J = 7.27 Hz), 6.96 (dd, 2H, J = 3.36 and 10.9 Hz), 7.23 (d, 1H, J = 10.9 Hz). Anal. ($C_{20}H_{25}Cl_2N_3O_2$) C, H, Cl, N.

Synthesis of 8-[2-[4-(2-Chlorophenyl)-1-piperazinyl]-ethyl]-8-azaspiro[4.5]decane (124). To a suspension of 8-[2-[4-(2-chlorophenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione (**54**, 0.2 g, 0.5 mmol) and $NaBH_4$ (0.065 g, 1.72 mmol) in THF (10 mL) stirred at 0°C under nitrogen atmosphere, was added boron trifluoride etherate (0.28 g, 1.97 mmol). The mixture was stirred at room temperature for 4 h, then 4 N HCl (4 mL) was added, the solvent was evaporated, and the residue was made alkaline by 2 N NaOH until pH 8 was attained. The mixture was extracted with CH_2Cl_2 (3 × 10 mL), the organic layer was dried (Na_2SO_4), and the solvent was evaporated under vacuum. The residue was purified by flash chromatography (CH_2Cl_2/2 N methanolic ammonia 96:4) to give 0.05 g of **124**. ^{1}H NMR ($CDCl_3$, δ): 1.35−1.44 (m, 4H), 1.45−1.56 (m, 4H), 1.57−1.77 (m, 4H), 2.36−2.49 (m, 4H), 2.51−2.62 (m, 4H), 2.63−2.81 (m, 4H), 2.99−3.18 (m, 4H), 6.84−7.08 (m, 2H), 7.24−7.39 (m, 1H). Anal. ($C_{21}H_{32}ClN_3$) C, H, Cl, N.

Synthesis of 7-[2-[4-(2,5-Dichlorophenyl)-1-piperazinyl]ethyl]-7-azaspiro[3.5]nonane-6,8-dione (111). A solution of cyclobutane-1,1-diacetic acid[22] (0.13 g, 0.75 mmol) and dicyclohexylcarbodiimide (0.17 g, 0.83 mmol) in anhydrous DMF (1 mL) was stirred at room temperature for 1.5 h. A solution of 2-(4-(2,5-dichlorophenyl)-1-piperazinyl)ethylamine (**6c**, 0.21 g, 0.79 mmol) in anhydrous DMF (1 mL) was added, and the mixture was stirred for 18 h. The so-formed precipitate was filtered off, and the solvent was evaporated. Sodium acetate (0.18 g, 2.26 mmol) and acetic anhydride (5 mL) were

added to the residue, and the mixture was stirred at 100 °C for 2 h. After cooling to room temperature, the suspension was poured into ice−water (10 mL), stirred for 30 min, made alkaline by 2.5 N NaOH, and extracted with CH_2Cl_2. The organic layer was washed with water, dried (Na_2SO_4), and evaporated under vacuum and the residue was purified by flash chromatography (toluene/acetone 8:2) to give 0.09 g of **111**. ^{1}H NMR ($CDCl_3$, δ): 1.84−2.06 (m, 6H), 2.51 (t, 2H, J = 7.4 Hz), 2.57−2.68 (m, 4H), 2.73 (s, 4H), 2.87−3.09 (m, 4H), 3.94 (t, 2H, J = 7.4 Hz), 6.88 (dd, 1H, J = 9.25 and 3.7 Hz), 6.96 (d, 1H, J = 3.7 Hz), 7.23 (d, 1H, J = 9.25 Hz). Anal. ($C_{20}H_{25}Cl_2N_3O_2$) C, H, Cl, N.

Synthesis of 8-(2-Chloro-1-methylethyl)-8-azaspiro-[4.5]decane-7,9-dione (2b). To a solution of 8-azaspiro[4.5]-decane-7,9-dione (**1**, 0.17 g, 1 mmol), 1-chloro-2-propanol (0.19, 2 mmol), and triphenylphosphine (0.26 g, 1 mmol) in DMF (3 mL) a solution of diethyl azodicarboxylate (0.17 g, 1 mmol) in DMF (1 mL) was added dropwise, and the mixture was stirred at 35 °C for 72 h. After the mixture cooled, water (20 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was washed with H_2O, dried (Na_2SO_4), and evaporated to dryness under vacuum and the residue was purified by flash chromatography (petroleum ether/ethyl acetate 9:1) to give 0.067 g (28%) of **2b**. ^{1}H NMR ($CDCl_3$, δ): 1.29−1.41 (m, 3H), 1.42−1.58 (m, 4H), 1.60−1.78 (m, 4H), 2.57 (s, 4H), 3.57−3.88 (m, 1H), 4.03−4.19 (m, 1H), 4.96−5.13 (m, 1H).

Synthesis of 1-(2-Chloro-5-iodophenyl)piperazine. To a solution of 1-(2-chlorophenyl)piperazine·2HCl (0.27 g, 1 mmol) in AcOH (5 mL) a solution of iodine monochloride (0.32 g, 2 mmol) in AcOH (5 mL) was added dropwise, and the mixture was stirred at room temperature for 2 h. Water (5 mL) was added, and the mixture was heated at 80 °C for 2 h under stirring. The solvents were evaporated, and the residue was dissolved in CH_2Cl_2 and washed in succession with $Na_2S_2O_3$ and 10% aqueous KOH. After drying (Na_2SO_4) and evaporation of the solvent under vacuum, the residue was purified by flash chromatography (CH_2Cl_2/2 N methanolic ammonia 96:4) to give 0.032 g (10%) of the title compound. ^{1}H NMR ($CDCl_3$, δ): 1.79 (s, 1H), 2.89−3.11 (m, 8H), 6.77 (d, 1H), 7.49 (dd, 1H), 7.63 (d, 1H).

Biology. Radioligand Binding Assay at Cloned α_1 Adrenoceptors. Binding to cloned human α_1 adrenoceptor subtypes was performed in membranes from CHO cells (Chinese hamster ovary cells) transfected by electroporation with DNA expressing the gene encoding each α_1 adrenoceptor subtype. Cloning and stable expression of the human α_1 adrenoceptor gene was performed as previously described.[32] CHO cell membranes (30 μg proteins) were incubated in 50 mM Tris-HCl, pH 7.4, with 0.1−0.4 nM [^{3}H]prazosin in a final volume of 1 mL for 30 min at 25°C in the absence or presence of competing drugs (1 pM to 10 μM). Nonspecific binding was determined in the presence of 10 μM phentolamine. The incubation was stopped by addition of ice-cold Tris-HCl buffer and rapid filtration through 0.2% polyethyleneimine pretreated Whatman GF/B or Schleicher & Schuell GF52 filters.

Radioligand Binding Assay at Human Cloned $5HT_{1A}$-Serotoninergic Receptors. Genomic clone G-21 coding for the human 5-HT_{1A}-serotoninergic receptor was stably transfected in a human cell line (HeLa).[33] HeLa cells were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum and gentamicin (100 μg/mL), 5% CO_2 at 37 °C. Cells were detached from the growth flask at 95% confluence by a cell scraper and were lised in ice-cold Tris 5 mM and EDTA 5 mM buffer (pH 7.4). Homogenates were centrifuged at 40000*g* for 20 min, and pellets were resuspended in a small volume of ice-cold Tris 5 mM and EDTA 5 mM buffer (pH 7.4) and immediately frozen and stored at −70 °C until use.

On the experimental day, cell membranes were resuspended in binding buffer 50 mM Tris (pH 7.4), 2.5 mM $MgCl_2$, 10 μM pargiline.[34] Membranes were incubated in a final volume of 1 mL for 30 min at 30 °C with 1.2 nM [^{3}H]8-OH-DPAT in the absence or presence of competing drugs; nonspecific binding was determined in the presence of 10 μM 5-HT. The incubation was stopped by addition of ice-cold Tris buffer and rapid filtration through 0.2% polyethyleneimine pretreated Schleicher & Schuell GF52 filters.

Data Analysis. The inhibition of specific binding of the radioligands by the tested drugs was analyzed to estimate the IC_{50} value by using the nonlinear curve-fitting program Allfit.[35] The IC_{50} value is converted to an affinity constant (K_i) by the equation of Cheng and Prusoff.[36]

Functional Studies. Evaluation of compounds for α_1 antagonism was performed as previously published.[37]

Rat Aorta (α_{1D}). Briefly, Sprague−Dawley rats (350−450 g body weight) were sacrificed by cervical dislocation. The aorta was isolated, freed of adhering connective tissue, and placed in Krebs solution containing NaCl (112 mM), glucose (11.1 mM), $NaHCO_3$ (25 mM), KCl (4.7 mM), $CaCl_2$ (2.5 mM), KH_2PO_4 (1.2 mM), and $MgSO_4$ (1.2 mM). Desmethylimipramine (0.1 μM) and corticosterone (1 μM) to block neuronal and extraneuronal uptake of noradrenaline, (±)-propranolol (1 μM) to block β-receptors, and yohimbine (0.1 μM) to block α_2 receptors were added to the Krebs solution. Aortic strips (2 mm × 30 mm long) were mounted for isotonic tension recording in a 20 mL organ bath containing Krebs buffer aerated constantly with 95% O_2/5% CO_2 and maintained at 37°C and loaded with a resting tension of 1.5 g. The strips were allowed to equilibrate for 60 min with washing every 20 min. After the equilibration period, tissues were primed twice (every 60 min) by addition of 10 μM noradrenaline. After another washing and equilibration period of 60 min, a noradrenaline concentration−response curve was constructed (basal curve). Following washout of noradrenaline, single concentrations of the compounds were incubated for 30 min before repeating the noradrenaline concentration−response curve. Responses were expressed as a percentage of the maximal contraction observed in the basal noradrenaline concentration−response curve and analyzed by nonlinear curve fitting according to the method reported by De Lean et al.[35] Schild plot parameters were evaluated by linear-regression analysis according to Tallarida and Murray.[38]

Guinea Pig Spleen (α_{1B}). Spleens were obtained from Hartley guinea pigs, cut in longitudinal strips, and placed in Krebs solution containing desmethylimipramine (0.1 μM), corticosterone (1 μM), propranol (1 μM), and yohimbine (0.1 μM). Strips were mounted for isometric tension recording in an organ bath containing Krebs buffer aerated constantly with 95% O_2/5% CO_2, maintained at 37°C and loaded with 1.0 g. After the equilibration period, strips were primed with 10 μM noradrenaline, then concentration−response curves for the agonist were constructed before (basal) and after 30 min of incubation with single-antagonist concentrations (response). Schild plot parameters were evaluated by linear-regression analysis, according to Tallarida and Murray.[38]

Epididymal Rat Vas Deferens "in Vitro" (α_{1A}). Male Sprague−Dawley rats between 300 and 400 g were killed by cervical dislocation. The vasa deferentia were removed, and associated blood vessels and mesentery were dissected away and were then bisected so that only the epididymal portion (15−20 mm length) was used. All the tissues were suspended in 10 mL tissue bath containing Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, and glucose 11, at 37 °C. They were bubbled with 95% O_2/5% CO_2. Cocaine and β-estradiol (both 10^{-5} M) were added in order to prevent agonist uptake. The vas deferens was placed under a 0.5 g resting tension and equilibrated for 1 h. Changes in isometric tension were measures with Grass FT.03 transducers.

In all tissues, a contraction to noradrenaline (10^{-5} M) was measured followed by a noncumulative concentration−effect curve to noradrenaline (10^{-8}−10^{-4} M, with a separation of 10 min between doses, basal curve). Antagonists were incubated for 30 min, and a second dose−response curve to noradrenaline was repeated (response curve).

The dose−response curves of the agonist were analyzed by nonlinear curve fitting of the logistic equation according to the method reported by De Lean et al.[35] with use of the ALLFIT

program (from NIH). The pA_2 values were calculated according to Arunlakshana and Schild.[39]

Computations. Comparative Modeling and MD Simulations of the α_1-AR Subtypes and of the 5HT$_{1A}$ Receptor. The model of the α_{1b}-AR was built by comparative modeling,[40] using the recently determined 2.8 Å X-ray structure of rhodopsin as a template.[14] Model building of the α_{1b}-AR has been described elsewhere.[41] The same chimeric rhodopsin template as that used for building the final model of the α_{1b}-AR[41] was employed for achieving the models of the human α_{1a}- and α_{1d}-AR subtypes. In this modified template, E2 and I3 were extracted from the input structure of the ab initio model of the α_{1b}-AR (sequences in bold in Figure 1). Furthermore, helices 5 and 6 were respectively elongated at the C-terminus and N-terminus, after deleting, respectively, the 226−235 and 240−248 rhodopsin segments (sequences in bold in Figure 1).

For the 5-HT$_{1A}$ receptor, a rhodopsin chimeric template was employed in which E2 (i.e., sequence 177−199) was replaced by the E2 extracted from the ab initio model of the α_{1b}-AR in its input arrangement.[41] Moreover, the template 226−235 and 240−248 segments, corresponding essentially to I3, were deleted. During the model building, α-helical restraints were imposed to the 5-HT$_{1A}$ receptor sequences 218−227 and 336−343 to prolong, respectively, the C-terminal and N-terminal ends of helices 5 and 6 (sequences in italics in Figure 1).[42] The huge I3 of the 5-HT$_{1A}$-receptor was not modeled. From each pairwise sequence alignment between rhodopsin and the α_{1a}-AR, α_{1d}-AR, and 5-HT$_{1A}$-receptor, 25 models were obtained. From each of the three sets of models, the one showing the lowest violation of restraints was selected.

The selected models of the four GPCRs were completed by the addition of the polar hydrogens and subjected to automatic and manual rotation of the side chain torsion angles when in nonallowed conformations, as well as to energy minimization and MD simulations according to the computational protocol recently described.[42] Different combinations of intrahelix distance constraints were also probed. For each receptor, one selected input structure was then used for docking simulations.

Different arrangements of the ligand−receptor complexes were built as inputs for MD simulations. Finally, for each of the three compounds, one complex with the α_{1a}-AR, α_{1b}-AR, α_{1d}-AR, and the 5-HT$_{1A}$ receptors, averaged over the structures collected during the last 100 ps and minimized, was finally considered for the analysis.

The protonated structures of compounds BMY 7378 and **69**, employed in docking simulations, were optimized using the semiempirical MO calculation (AM1) within the MOPAC 6.0 (QCPE 455) suite of programs.[43]

Multiple Copies Simultaneous Search. The receptor binding site analysis was performed by means of the multiple copies simultaneous search (MCSS) approach.[23] MCSS determines energetically favorable positions and orientations (local minima of the potential energy) of functional groups on the surface or the binding site of a protein subjected to simultaneous minimization. Each copy of the functional group interacts with the full force field of the target structure, but the group copies do not interact with each other. If two copies of a group minimize to the same position, then one of them is discharged. MCSS thus produces a collection of positioned and oriented functional groups that interact in some way with the binding site region. Some of these interactions will be more favorable than others. After the evaluation, only the most interesting replicas, i.e., the ones with the best interaction energies, are conserved and clustered on the basis of spatial distribution criteria for visual examination.

The binding site was defined as a sphere of radius 15 Å centered in such a way to include the whole extracellular half of the receptor. For each fragment, i.e., 2-OCH$_3$− and the 2,5-dichlorophenylpiperazines, the procedure was iterated 15 times, each time using 300 replicas.

The structures of the substituted phenylpiperazines in their protonated forms were optimized using the semiempirical MO calculation (AM1) within the MOPAC 6.0 (QCPE 455) suite of programs. The phenyl ring was oriented according to the geometries of the energy minima as inferred from conformational analysis.[43]

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Supporting Information Available: ^{1}H NMR data of the intermediate compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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