Phenylpiperazinylalkylamino Substituted Pyridazinones as Potent α_1 Adrenoceptor Antagonists

Daniela Barlocco,*,§ Giorgio Cignarella,§ Vittorio Dal Piaz,† M. Paola Giovannoni,† Pier G. De Benedetti,*,‡ Francesca Fanelli,‡ Federica Montesano,# Elena Poggesi,# and Amedeo Leonardi#

Istituto Chimico Farmaceutico e Tossicologico, Viale Abruzzi 42, 20131 Milano, Italy, Dipartimento di Scienze Farmaceutiche, Via G. Capponi 9, 50121 Firenze, Italy, Dipartimento di Chimica, Via Campi 183, 41100 Modena, Italy, and Pharmaceutical R & D Division, Recordati s.p.a., Via Civitali 1, 20148 Milano, Italy

Received February 28, 2000

QSAR models have been used for designing a series of compounds characterized by a *N*-phenylpiperazinylalkylamino moiety linked to substituted pyridazinones, which have been synthesized. Measurements of the binding affinities of the new compounds toward the α_{1a}-, α_{1b}-, and α_{1d}-AR cloned subtypes as well as the 5-HT_{1A} receptor have been done validating, at least in part, the estimations of the theoretical models. This study provides insight into the structure activity relationships of the α_1-ARs ligands and their α_1-AR/5-HT_{1A} selectivity.

Introduction

α_1-Adrenergic receptors (α_1-AR) are members of the superfamily of G protein coupled receptors (GPCR) that transduce signals across the cell membrane. The α_1-ARs mediate the functional effects of catecholamines such as epinephrine and norepinephrine by coupling to the Gαq that induces the activation of phospholipase C, culminating into the phosphoinositide (PI) hydrolysis.

Molecular biology techniques allowed the identification of cDNAs encoding three α_1-ARs (α_{1a}, α_{1b}, and α_{1d}).[1−5] The three recombinant α_1-ARs correlate closely with the three α_1-AR subtypes that were identified in native tissues and which mediate their functional responses (α_{1A}, α_{1B}, and α_{1D}).[6−9] The presence of these different α_1-AR subtypes in blood vessels and other smooth muscles points to the importance of developing selective drugs for receptor classification and characterization as well as for therapeutic effectiveness.

A relevant aspect of chemical research is to predict the behavior of new molecules from their structure, prior to synthesis. It is generally assumed that noncovalent forces control ligand−receptor interactions and that these forces can be described in terms of electrostatic and steric effects, which depend on molecular size and shape. Recently, quantitative structure activity relationship (QSAR) analysis based on ad hoc defined supermolecules and the corresponding size-shape descriptors has been employed for deciphering the molecular features responsible for the affinity and selectivity in a wide ranging series of noncongeneric antagonists of the cloned bovine α_{1a}-, hamster α_{1b}-, and rat α_{1d}-ARs.[10] The QSAR models previously obtained have been herein used to design and estimate the binding affinities of a new molecular series of potential α_1-AR ligands.

This work describes the design and the synthesis of a series of phenylpiperazine derivatives (**1a**−**d,k,i,m,o**−**r**) obtained from the previously reported[11] 2-methyl-4-nitro-5-acetyl-6-substituted-3(2H)-pyridazinones **Ia,b,** by nucleophilic substitution at position 4. Several different substituents at position 5 (compounds **1e**−**h,j,l,n**) have been also considered. The binding affinity assays of the new compounds toward the human cloned α_{1a}-, α_{1b}-, and α_{1d}-AR subtypes as well as the human cloned 5-HT_{1A} receptor have been also carried out.

The results of this study provide insight into the structure activity relationships (SAR) of α_1-ARs ligands and their α_1-AR/5-HT_{1A} selectivity.

Chemistry

Compounds **1a**−**d,k,i,m,o**−**q** were obtained in good yields by nucleophilic displacement of the 4-nitro group of the known pyridazinones **Ia,b**,[11] by stirring for 30 min at room temperature an ethanolic solution of the appropriate **I** with an excess of the required *N*,*ω*-aminoalkyl-N^1-phenylpiperazine. The latter were in turn prepared by reduction of the corresponding cyano-derivatives or by deprotection of the appropriate phthalimide, following standard procedures. (See Scheme 1). Compound **1r** (see Table 1) was obtained in the same way by directly condensing **Ia** and *N*-(2-methoxyphenyl)piperazine. The homologues **1e,f** were prepared according to the same scheme starting from the previously reported 4-nitro-5-propanonyl- (**Ic**)[11] and 4-nitro-5-butanonyl (**Id**)[12] analogues. The 5-unsubstituted **1g,h,j,l,n** (see Table 1) were obtained from the appropriate 4-chloropyridazinones,[13,14] by heating at 140 °C with an excess of the required *N*,*ω*-aminoalkyl-N^1-phenylpiperazine.

Results and Discussion

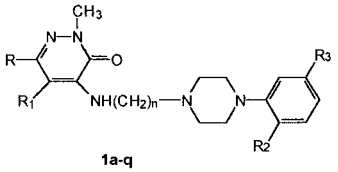
In this work, a theoretical QSAR approach based on size and shape descriptors has been employed to design novel α_1-AR ligands, taking also advantage of the information inferred from a previous study on monocyclic and bicyclic substituted pyridazinones.[15]

A typical α_1-blocker contains an unsaturated or aromatic ring on each side of a protonated nitrogen atom, one of these two moieties being closer to the

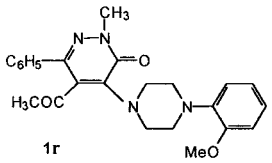
* To whom correspondence should be addressed. Tel: 39.02.29502223. Fax: 39.02.29514197. E-mail: daniela.barlocco@unimi.it.
§ Istituto Chimico Farmaceutico e Tossicologico.
† Dipartimento di Scienze Farmaceutiche.
‡ Dipartimento di Scienze Chimiche.
# Pharmaceutical R & D Division, Recordati s.p.a.

10.1021/jm0009336 CCC: $20.00
Published on Web 06/16/2001

Table 1. Physicochemical Data of Compounds **1a**−**r**



1a-q

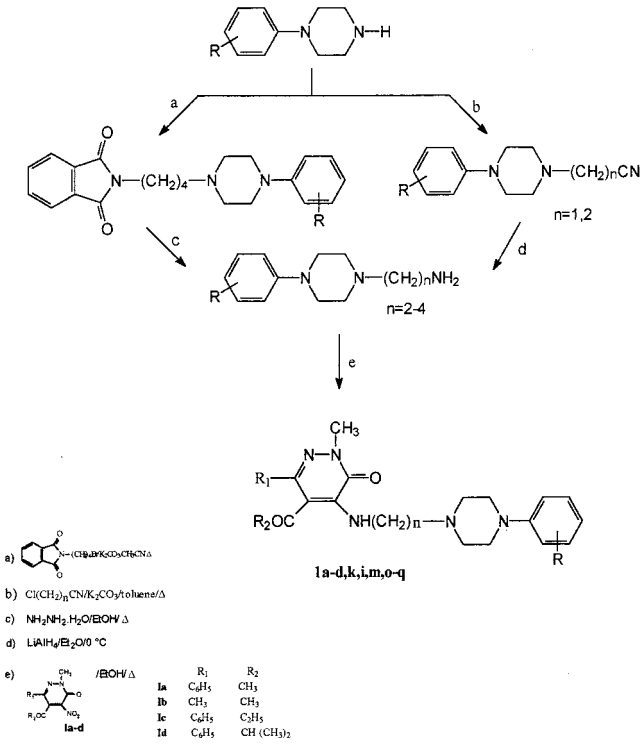


1r

compd	*n*	R	R_1	R_2	R_3	% yield	mp (°C)	formula[a]
1a	2	C_6H_5	$COCH_3$	OCH_3	H	84	oil	$C_{26}H_{30}N_4O_3$
1b	2	C_6H_5	$COCH_3$	F	H	91	147−148	$C_{25}H_{28}FN_5O_2$
1c	2	C_6H_5	$COCH_3$	Cl	H	98	162−163	$C_{25}H_{28}ClN_5O_2$
1d	2	C_6H_5	$COCH_3$	OCH_3	Cl	83	oil	$C_{26}H_{30}ClN_5O_3$
1e	2	C_6H_5	COC_2H_5	OCH_3	H	76	oil	$C_{27}H_{33}N_5O_3$
1f	2	C_6H_5	$COCH(CH_3)_2$	OCH_3	H	92	oil	$C_{28}H_{35}N_5O_3$
1g	2	C_6H_5	H	OCH_3	H	53	133−134	$C_{24}H_{29}N_5O_2$
1h	2	C_6H_5	H	OCH_3	Cl	48	oil	$C_{24}H_{28}ClN_5O_2$
1k	3	C_6H_5	$COCH_3$	OCH_3	H	95	oil	$C_{27}H_{33}N_4O_3$
1i	3	C_6H_5	$COCH_3$	OCH_3	Cl	72	128−129	$C_{27}H_{32}ClN_5O_3$
1j	3	C_6H_5	H	OCH_3	H	23	130−131	$C_{25}H_{31}N_5O_2$
1l	3	C_6H_5	H	OCH_3	Cl	41	oil	$C_{25}H_{30}ClN_5O_2$
1m	2	CH_3	$COCH_3$	OCH_3	H	79	133−134	$C_{21}H_{29}N_5O_3$
1n	2	CH_3	H	OCH_3	H	22	177−178	$C_{19}H_{27}N_5O_2$
1o	3	CH_3	$COCH_3$	OCH_3	H	94	83−84	$C_{22}H_{31}N_5O_3$
1p	3	CH_3	$COCH_3$	OCH_3	Cl	79	101−102	$C_{22}H_{30}ClN_5O_2$
1q	4	CH_3	$COCH_3$	OCH_3	Cl	79	101−102	$C_{22}H_{30}ClN_5O_3$
1r						95	173−175	$C_{24}H_{26}N_4O_3$

[a] Elemental analyses for C,H,N were within ± 0.4% of the calculated data.

Scheme 1



protonated nitrogen atom than the other. The protonated nitrogen atom of the ligands is thought to interact with the conserved aspartate on helix 3 of the receptor according to a precise geometry dictated by a directional charge reinforced hydrogen bonding interaction.[15,16] The protonated nitrogen atom of the α_1-AR ligands may be found as part of aliphatic chains, of conjugated systems (i.e. quinazoline, isoquinoline, imidazoline) as well as of aliphatic rings (i.e. piperazine and piperidine). While the protonated nitrogen atom is an essential pharmacophoric element for the long-range electrostatic recognition and productive interaction with the aspartate of the α_1-AR binding site, its contribution to the binding energy is almost constant.[16] On the contrary, short-range forces (repulsive, dispersive, inductive, etc.) modulate both ligand affinity and selectivity. Thus, the modulation of the binding affinity by a wide noncongeneric series of α_1-AR ligands can be described and explained by the variation of the ligand size-shape features that are related to the short range acting forces. These constitute the foundations of the supermolecule-based QSAR approach previously proposed.[10,17,18]

According to the ligand based three-dimensional (3D) pharmacophore, the supermolecule approach assumes that the volume obtained by superimposing the most structurally different ligands that show the highest affinities for the same receptor (supermolecule) might reflect the overall shape and conformational flexibility of the high-affinity receptor binding site. Therefore, size and shape descriptors can be defined ad hoc (that is, on a specific molecular series and in connection with a specific bioactivity) with respect to the supermolecule.

The supermolecule for the α_{1a}-AR subtype (Figure 1a) is based on six structurally different compounds (**1**−**6**, Chart 1). Fewer compounds yielded the supermolecules for the α_{1b}- and the α_{1d}-AR subtypes (**7**−**10** for the α_{1b}-AR and **7**, **8**, **10**−**12** for the α_{1d}-AR; Chart 1, Figure 1b,c); these two supermolecules share three ligands: compounds **7**, **8**, and **10** (Chart 1).

The selected size and shape descriptors of the compounds considered in this study are listed in Table 2. In particular, the table reports the inner (V_{in1a}, V_{in1b}, and V_{in1d}), the outer (V_{out1a}, V_{out1b}, and V_{out1d}), and the difference (V_{dif1a}, V_{dif1b}, and V_{dif1d}) van der Waals (vdw) volumes relative to the reference volume of ad hoc defined supermolecules, one for each subtype (Figure 1). According to the definition of these molecular

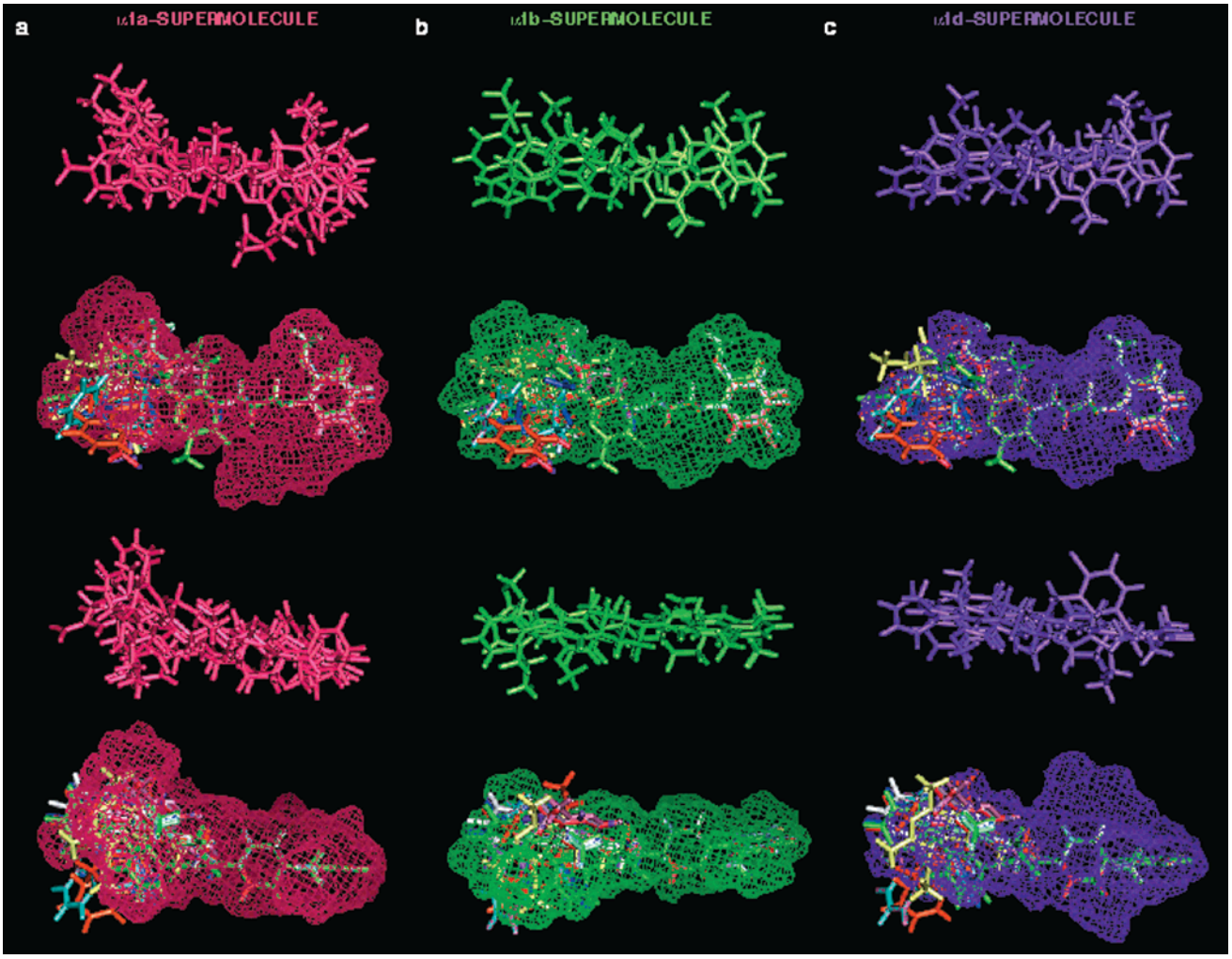


Figure 1. (a) Frontal (top) and side (bottom) views of the supermolecule obtained by superimposing the most active and structurally different antagonists for the cloned bovine α_{1a}-AR. The same side views have been employed for representing the volume of the a_{1a} supermolecule embedding all the ligands considered in this study superimposed on the a_{1a} supermolecule. The same description of point (a) is valid for points (b) and (c) with the difference that they refer, respectively, to the a_{1b} and a_{1d} supermolecules. The van der Waals volumes of the a_{1a}, a_{1b}, and a_{1d} supermolecules are 1016.00 Å^3, 680.75 Å^3, and 754.13 Å^3, respectively.

descriptors, higher affinities are realized by maximizing V_{in} and minimizing V_{out}. For its formulation V_{dif} [$V_{dif} = (V_{in}-V_{out})/V_{sup}$] is a size and shape descriptor that takes into account the information content codified by both V_{in} and V_{out} normalized by the volume of the supermolecule (V_{sup}). By definition, the ligands constituting the supermolecules show the following singularities: $V_{mol} = V_{in}$, $V_{out} = 0$, and $V_{in}/V_{mol} = 1$, where V_{mol} is the vdw molecular volume. The van der Waals volumes of the α_{1a}, α_{1b}, and α_{1d} supermolecules are 1016.00 Å^3, 680.75 Å^3, and 754.13 Å^3, respectively. In general, a V_{dif} value close to that of one of the supermolecule components corresponds to high binding affinity value.

As for the α_{1a}-AR subtype, theoretical size-shape descriptors predict that a two carbon atom linker is compatible with the structural variability of the substituent in position 5 of the pyridazinone ring. In other words, changing the size-shape of the substituent in position 5 of the pyridazinone ring invariantly produces high V_{dif}. In fact, **1a, 1e,** and **1f** mainly realize relatively high V_{dif}, by means of high V_{in} and intermediate V_{out}, whereas compound **1g** realizes high V_{dif} through low V_{in} and V_{out}.

Reducing the size-shape of the substituent in position 6 (**1m**) or in both positions 5 and 6 (**1n**) slightly lowers V_{dif} by reducing V_{in} more than V_{out}, as compared to compound **1a**.

The fluoro or chloro substitutions for the methoxy group in position 2′ of the phenyl ring (**1b** and **1c**) as well as the introduction of a chlorine in position 5′ of the phenyl ring (i.e. **1d**) do not induce relevant changes in V_{dif}, as compared to compound **1a**.

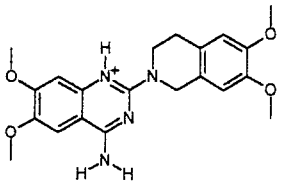
Prolonging the two carbon atom spacer of **1a** to three carbon atoms (**1k**, **1i**), while leaving the phenyl substituent in position 6, increases the outer volume, lowering V_{dif}. Thus, the computed indices predict that size-shape variability in position 5 of the pyridazinone is well tolerated, whereas increasing the length of the spacer from 2 to 3 methylenic units or substituting the phenyl ring in position 6 of the pyridazinone with a methyl group slightly lowers the α_{1a}-AR binding affinity.

As for the α_{1b}-AR, the different substitutions in position 5 of the pyridazinone ring, combined with an ethylenic spacer (**1a**, **1e**, and **1f**), produce relatively high V_{dif}. Halogen substitutions in positions 2′ or 5′ of the phenyl ring (**1b**−**d**) produce V_{dif} comparable with those of the 2′-methoxy derivative (**1a**). Replacing the phenyl ring in position 6 by a methyl group (**1m**) or reducing the size in both positions 5 and 6 (**1n**) lowers the inner volume more than the outer volume, lowering V_{dif}.

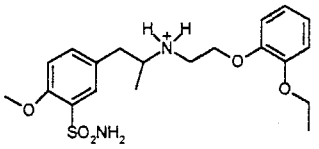
Chart 1

Ligands constituting the α1a-SUPERMOLECULE

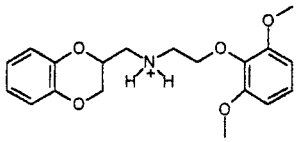
1



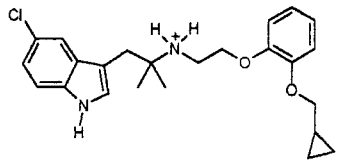
2



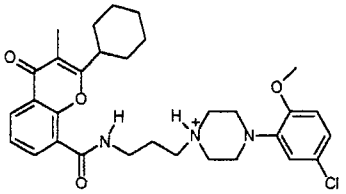
3



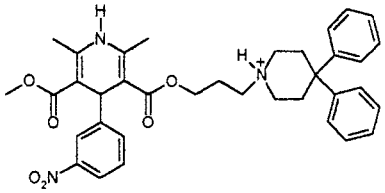
4



5

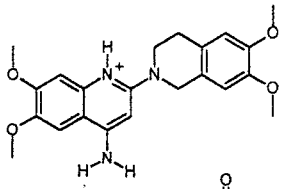


6

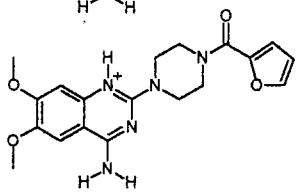


Ligands constituting the α1b-SUPERMOLECULE

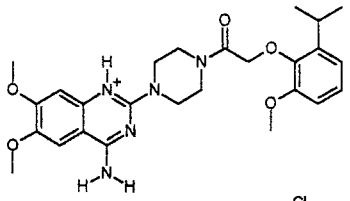
7



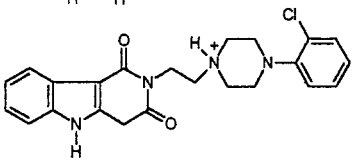
8



9

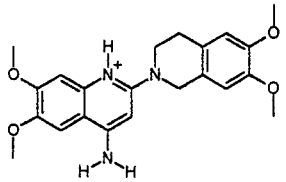


10

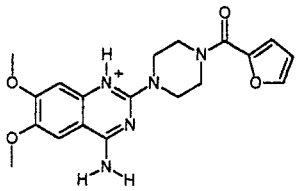


Ligands constituting the α1d-SUPERMOLECULE

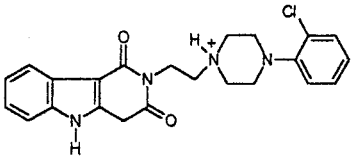
7



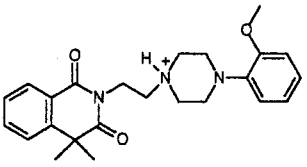
8



10



11



12

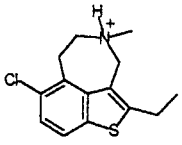


Table 2. Ad Hoc Defined Size and Shape Descriptors (V_{in}, V_{out}, V_{in}/V_{mol}, and V_{dif}) of the Set of Molecules Considered in This Study

compd	V_{in1a} (Å^3)	V_{out1a} (Å^3)	V_{dif1a}	V_{in1b} (Å^3)	V_{out1b} (Å^3)	V_{dif1b}	V_{in1d} (Å^3)	V_{out1d} (Å^3)	V_{dif1d}
1a	351.75	45.00	0.3158	350.13	47.62	0.4641	347.75	50.25	0.4124
1b	335.00	43.75	0.2998	334.13	48.50	0.4382	329.00	53.50	0.3819
1c	341.38	45.37	0.3047	343.75	43.88	0.4600	340.63	47.12	0.4069
1d	365.88	46.87	0.3284	358.75	53.13	0.4688	358.00	53.88	0.4216
1e	355.50	56.75	0.3076	349.38	59.87	0.4441	342.38	66.87	0.3819
1f	358.75	66.38	0.3010	356.75	68.25	0.4426	365.50	59.63	0.4240
1g	332.25	31.63	0.3095	327.00	37.13	0.4447	318.00	46.38	0.3765
1h	348.63	33.37	0.3245	336.00	45.88	0.4451	326.38	55.50	0.3755
1k	336.50	73.88	0.2704	307.00	105.00	0.3099	325.38	86.50	0.3311
1i	351.38	74.00	0.2856	314.00	111.75	0.3103	332.38	93.25	0.3315
1j	307.38	69.50	0.2449	296.63	82.87	0.3279	313.38	66.12	0.3428
1l	320.00	73.38	0.2539	297.75	95.13	0.3108	316.00	77.00	0.3313
1m	317.25	26.88	0.2989	309.25	35.63	0.4197	318.88	26.00	0.4060
1n	295.88	14.00	0.2902	283.88	27.75	0.3929	287.25	24.38	0.3644
1o	333.63	25.50	0.3172	306.63	55.87	0.3847	322.13	40.25	0.3908
1p	347.13	27.87	0.3287	324.13	51.62	0.4180	333.75	41.75	0.4048
1q	358.00	29.88	0.3378	317.63	70.87	0.3785	293.13	95.00	0.2747
1r	302.88	52.25	0.2580	296.75	60.88	0.3618	304.75	52.63	0.3495

Table 3. Calculated by V_{dif} Based QSAR Models ($pK_{1a\ calc}$, $pK_{1b\ calc}$, $pK_{1d\ calc}$) and Experimental ($pK_{1a\ exp}$, $pK_{1b\ exp}$, $pK_{1d\ exp}$) Binding Affinities

compd	$pK_{1a\ calc}$	$pK_{1a\ exp}$	Δ	$pK_{1b\ calc}$	$pK_{1b\ exp}$	Δ	$pK_{1d\ calc}$	$pK_{1d\ exp}$	Δ
1a	8.82	9.46	−0.64	8.98	8.48	0.50	9.19	9.89	−0.70
1b	8.74	9.49	−0.75	8.83	8.42	0.41	8.89	9.31	−0.42
1c	8.77	9.52	−0.75	8.96	9.12	−0.16	9.13	9.60	−0.47
1d	8.89	9.55	−0.66	9.01	8.40	0.61	9.28	9.39	−0.11
1e	8.78	9.72	−0.94	8.87	8.84	0.03	8.89	9.60	−0.71
1f	8.75	9.74	−0.99	8.86	9.19	−0.33	9.30	9.37	−0.07
1g	8.79	9.66	−0.87	8.87	8.70	0.17	8.84	7.93	0.91
1h	8.87	9.00	−0.13	8.87	8.75	0.12	8.83	8.97	−0.14
1k	8.59	8.82	−0.23	8.08	7.69	0.39	8.39	8.37	0.02
1i	8.67	8.64	0.03	8.08	8.85	−0.77	8.40	8.41	−0.01
1j	8.46	8.59	−0.13	8.18	8.26	−0.08	8.51	7.93	0.58
1l	8.50	8.70	−0.20	8.04	8.70	−0.66	8.40	8.53	−0.13
1m	8.74	8.64	0.10	8.72	7.30	1.42	9.12	8.50	0.62
1n	8.69	7.73	0.96	8.57	7.48	1.09	8.72	7.75	0.97
1o	8.83	9.09	−0.26	8.52	7.27	1.25	8.98	7.46	1.52
1p	8.89	9.29	−0.40	8.71	8.10	0.61	9.11	8.27	0.84
1q	8.94	8.52	0.42	8.48	7.39	1.09	7.84	8.30	−0.46
1r	8.53	6.79	1.74	8.38	6.59	1.79	8.57	5.00	3.57

[a] pK_{1a} values have been calculated according to the following regression equation: $pK_{1a} = 5.18\ (\pm 0.83)\ V_{dif} + 7.19\ (\pm 0.27)$, $n = 34$, $r = 0.74$, $s = 0.53$, and $F = 39.01$, where n is the number of compounds, r is the correlation coefficient, s is the saturated deviation, and F is the Fisher ratio. [b] pK_{1b} values have been calculated according to the following regression equation: $pK_{1b} = 5.87\ (\pm 0.72)\ V_{dif} + 6.26\ (\pm 0.22)$, $n = 30$, $r = 0.83$, $s = 0.51$, and $F = 66.08$. [c] pK_{1d} values have been calculated according to the following regression equation: $pK_{1d} = 0.74(\pm 1.14)\ V_{dif} + 5.17(\pm 0.33)$, $n = 31$, $r = 0.84$, $s = 0.57$, and $F = 72.86$.

Moreover, the elongation of the ethylenic spacer of **1a** to three carbon atoms (**1k**) reduces V_{in} and increases V_{out}, lowering V_{dif}. In general, compounds with a three or four carbon atom spacer show lower V_{dif} than the corresponding two carbon atom spacer compounds (Tables 1−4).

Thus, for the α_{1b}-AR, size-shape indices predict that, similarly to the α_{1a}-AR, structural variability in position 5 of the pyridazinone is better tolerated than that in position 6. Moreover, the elongation of the alkyl spacer is predicted to lower the α_{1b}-AR binding affinity.

As for the α_{1d}-AR, size and shape descriptors show a behavior almost similar to that of the α_{1a}-AR.

The linear regression equations previously obtained (Table 3)[10] have been used for estimating the binding affinities of the new compounds. On the basis of both the predicted binding affinities data values (nanomolar range) and the smaller variance of the estimated α_{1a}-AR binding affinities (about 0.5 in a logarithmic scale) with respect to the other two subtypes (about 1.0 and 1.5, respectively), we decided to synthesize the designed compounds.

The predicted and experimental binding affinities for the three α_1-AR subtypes, together with their differences (Δ), are reported in Table 3. Recalling that the simple approach used is mainly devoted to ligand design purposes and is based on QSAR models generated by a highly noncongeneric molecular series,[10] the agreement between the predicted and measured binding affinity data values can be considered satisfactory. In fact, we consider these QSAR models as useful ligand design and decision making tools. In this case, these tools showed low predictive resolution (about 0.5, 1, and 1.5 respectively, in a logarithmic scale). Nevertheless, they allowed the selection among the many hypothetical ligands designed by estimating their affinities for the α_1-ARs according to three levels: (a) low affinity, pK_i 7−8; (b) high affinity, pK_i 8−9; and (c) very high affinity, pK_i > 9. On these bases, a few wrong estimations (Δ >1.1) have been done (pK_{1a}: compound **1r**; pK_{1b}: compounds **1m**, **1o**, **1r**; pK_{1d}: compounds **1o** and **1r**). The molecular descriptor V_{dif} completely failed in estimating the affinities of **1r** for all the three α_1-AR subtypes, suggesting that the low binding affinity of **1r** does not depend on

Table 4. Affinity Constants (K_i, nM) of Compounds **1a**−**r**; Prazosin, **BMY7378**, and **8-OH-DPAT** toward Human Cloned α_1 Adrenoceptor Subtypes and 5-HT_{1A} Receptor[a]

	K_i (nM)[a]				
compd	α-1a	α-1b	α-1d	5-HT_{1A}	binding[b] [^{35}S] GTPγS
1a	0.63	3.29	0.13	2.47	n.a
1b	0.32	3.77	0.49	24.12	
1c	0.30	0.75	0.25	11.60	
1d	0.28	3.95	0.41	76.23	
1e	0.19	1.45	0.25	4.06	pEC_{50} = 8.6; 19%
1f	0.18	0.64	0.43	10.20	n.a.
1g	0.22	2.01	11.78	13.98	
1h	1.0	1.76	1.07	322.1	
1k	1.51	20.28	4.32	134.50	
1i	0.44	1.44	3.92	1000.00	
1j	2.59	5.51	11.79	126.72	
1l	2.0	2.01	2.92	>1000	
1m	2.29	49.84	3.20	13.00	
1n	18.56	33.13	17.69	32.10	
1o	0.89	53.39	34.45	1.48	pEC_{50} = 8.8; 17%
1p	0.53	8.04	5.34	11.24	
1q	2.99	49.08	5.96	1.39	
1r	162.80	258.92	>10.000	>1000	
Prazosin	0.61	0.42	0.23	>10.000	
BMY7378	381.05	68.97	1.43	0.93	pEC_{50} = 9.27; 26%
8-OH-DPAT	1757.20	5975.00	>1000	3.44	pEC_{50} = 76; 100%

[a] Equilibrium dissociation constants (K_i) were derived from IC_{50} using the Cheng-Prusoff equation.[23] 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 for 5-HT_{1A} receptor. Each experiment was performed in triplicate. K_i values were from 2 to 3 experiments which agreed within 20%. [b] n.a. = not active, indicative of neutral antagonism. For partial agonists the pEC_{50} and percent of maximum effect are given. Values are from 2 to 3 experiments which agreed within 20%.

the ligand size-shape features. In fact, the very low affinity of this compound for all the three α_1-ARs is probably due to the fact that no nitrogen atom suitable for protonation, at physiological pH, is present in this compound, thus preventing the ligand to perform the proper salt-bridge interaction with the aspartate of the receptor binding site. The overestimation of the α_{1b}-AR binding affinity of **1m** is due to the fact that reducing the size of the pyridazinone moiety, with respect to **1a**, lowers both the inner and outer volumes of this compound with respect to the reference α_{1b}-AR supermolecule but not enough for producing a good estimation of the binding affinity of this compound. The overestimation of the α_{1b}- and the α_{1d}-AR binding affinity of **1o** is due to the fact that reducing the size of the pyridazinone moiety increases V_{in} and reduces V_{out} with respect to **1l**, thus producing a V_{dif} too high for predicting correctly the binding affinity of this compound. As a consequence, QSAR models fail in estimating the α_{1a}-AR selectivity of **1o**.

In general, α_{1a}-AR binding affinities are slightly underestimated (Table 3) whereas, the binding affinities for the other two α_1-AR subtypes, in particular the α_{1b}-AR, are rather overestimated (Table 3) by the theoretical model. For these reasons, the theoretical model does not estimate properly the α_{1a}/α_{1b} selectivity of compounds **1m**, **1o**, **1p**, and **1q** and the α_{1a}/α_{1d} selectivity of compounds **1g**, **1o**, and **1p**.

SAR analysis shows that three compounds, i.e., **1g**, **1o**, and **1p,** are selective for the α_{1a}-AR as compared to the other two α_1-AR subtypes. Selectivity of **1g** is mainly due to the lack of substitution in position 5 of the pyridazinone that primarily lowers the affinity for the α_{1d}-AR and, by a lower extent, that for the α_{1b}-AR. The α_{1a}-AR selectivity of compound **1o** is mainly due to the replacement of the phenyl ring in position 6 of the pyridazinone with a methyl group, on one hand, that lowers the affinity for the α_{1b}-AR, and to the elongation of the ethylenic spacer to three carbon atoms, on the other one, that lowers the affinity for the α_{1d}-AR. However, the presence of a phenyl ring in position 6 increases the affinity of this compound for the 5-HT_{1A} receptor. This drawback is overcome by introducing a chlorine in position 5′ of the phenyl ring (i.e. **1p**), that lowers the affinity for the serotoninergic receptor while also retaining lower affinities for the α_{1b}- and α_{1d}-AR subtypes, than for the α_{1a}-AR.

Interestingly, the majority of the compounds proposed in this work show low affinities for the 5-HT_{1A} receptor. This is mainly due to the presence of a chlorine substituent in position 5′ of the phenyl ring and/or to the elongation of the alkyl spacer. However, the latter needs the presence of a phenyl ring in position 6 of the pyridazinone to produce a low affinity for the 5-HT_{1A} receptor.

As for functional studies, compound **1i** was tested for its functional activity on rat aorta contractions induced by (−)-noradrenaline and, as expected, proved to be an antagonist with pK_b of 8.21.

Furthemore, compounds showing a relevant affinity for the 5-HT_{1A} receptor were tested for their ability to influence the binding of [^{35}S]GTPγS to HeLa cell membranes expressing the human 5-HT_{1A} receptor. Compounds **1a**, **1f**, and **1q** behaved as neutral antagonists, whereas compounds **1e** and **1o** proved to be partial agonists with a pEC_{50} of 8.6 and 8.8 and a percent maximum effect of 19 and 17%, respectively. (See Table 4.)

In conclusion, the QSAR models previously obtained on a very heterogeneous series of protonated α_1-AR antagonists have been challenged in their capability to design and estimate the α_1-AR binding affinities of a set of *N*-arylpiperazines with satisfactory results.

This study indicates that substitutions in positions 5 and/or 6 of the pyridazinone ring as well as in positions 2′ and 5′ of the phenyl ring together with the length of

the linker are responsible for modulating the ligand α_1-AR binding affinities and α_1-AR/5-HT_{1A} selectivities.

Experimental Section

Chemistry. Melting points were determined on a Büchi 510 capillary melting points apparatus and are uncorrected. Analyses 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.

2-Methyl-4-[(4-arylpiperazin-1-yl)aminoalkyl]-5-acetyl-6-phenyl(methyl)-3-(2*H*)-pyridazinones 1a−d,k,i,m,o−q. A solution of the required **Ia,b**[11] (1 mmol) and the appropriate *N*,*ω*-aminoalkyl-*N*^{1}-phenylpiperazine[15] (2.5 mmol) in EtOH (6 mL) was stirred at room temperature for 30 min. Water (5 mL) was added, the solvent was evaporated under vacuum, and the residue was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, the solid was filtered off, and the solvent was evaporated under vacuum. The residue was purified by flash chromatography eluting with cyclohexane/ethyl acetate 4/6 to give as the first run the desired compounds **1**. (See Table 1 for data.)

For 1a: ^{1}H NMR ($CDCl_3$) δ: 1.9.(s, 3H); 2.7−2.8 (m, 6H); 3.1−3.2 (m, 4H); 3.3−3.4 (m, 2H); 3.8 (s, 3H); 3.9 (s, 3H); 6.8−7.0 (m, 4H); 7.4 (s, 5H); 8.0 (br. S, 1H, exch. with D_2O).

The higher homologues **1e,f** were prepared according to the same method starting from 2-methyl-4-nitro-5-propanonyl-(**Ic**)[11] or isobutanonyl- (**Id**),[12] respectively.

For 1e: ^{1}H NMR ($CDCl_3$) δ: 0.8 (t, 3H, J = 8 Hz); 2.1 (q, 2H; J = 8 Hz); 2.6−2.8 (m, 6H); 3.0−3.2 (m, 6H); 3.8 (s, 3H); 3.9 (s, 3H); 6.8−7.0 (m, 4H); 7.3−7.5 (m, 6H)

For 1f: ^{1}H NMR ($CDCl_3$) δ: 0.8 (2s, 6H); 2.2−2.3 (m 1H); 2.6−2.7 (m, 6H); 3.0−3.1 (m, 4H); 3.2−3.6 (m, 2H); 3.8 (s, 3H); 3.9 (s, 3H); 6.8−7.1 (m, 4H); 7.3−7.5 (m, 6H).

Compound **1r** was obtained by condensation in the same conditions of **Ia** and *N*-(2-methoxyphenyl)piperazine. ^{1}H NMR ($CDCl_3$) δ: 2.1 (s, 3H); 3.1−3.2 (m, 4H); 3.5−3.6 (m, 4H); 3.9 (2s, 6H); 6.9−7.0 (m, 4H); 7.4 (app.s, 5H). (See Table 1 for data.)

2-Methyl-4-[(4-arylpiperazin-1-yl)aminoalkyl]-6-phenyl-(methyl)-3-(2*H*)-pyridazinones 1g,h,j,l,n. A mixture of the required 4-chloro-3(2*H*)pyridazinone[13,14] and an excess of the appropriate phenylpiperazinalkylamine was heated at 140 °C for 12 h. The residue was first purified by flash chromatography eluting with CH_2Cl_2/MeOH 95/5 and then by gravimetric chromatography, eluting with toluene/methanol 9/1. (See Table 1 for data.)

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.[19] 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−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 and Schuell GF52 filters.

Radioligand Binding Assay at Human Cloned 5HT_{1A}-Serotoninergic Receptors. Genomic clone G-21 coding for the human 5HT_{1A}-serotoninergic receptor was stably transfected in a human cell line (HeLa).[20] 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 40 000 × g × 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.[21] 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 and Schuell GF52 filters.

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.[22] The IC_{50} value is converted to an affinity constant (K_i) by the equation of Cheng and Prusoff.[23]

Functional Studies. Evaluation of compounds for α_1-antagonism was performed as previously published.[24] 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 (1μM) to block β-receptors, and yohimbine (0.1 μM) to block α_2-receptors were added to the Krebs solution. Aortic strips (2 × 30 mm long) were mounted for isotonic tension recording in 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 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.[22] Schild-plot parameters were evaluated by linear-regression analysis according to Tallarida and Murray.[25]

Stimulation of [^{35}S]GTPγS Binding at Cloned 5-HT_{1A} Receptor. The effects of the compounds tested with [^{35}S]-GTPγS binding were evaluated according to the method of Stanton and Beer[26] with minor modifications. On the experimental day, cell membranes from HeLa cells transfected with human cloned 5-HT_{1A} receptors, prepared as above-described, were resuspended in buffer containing 20 mM HEPES, 3 mM $MgSO_4$, and 120 mM NaCl (pH 7.4). The membranes were incubated with 30 μM GDP and decreasing concentrations of test drugs (from 100 μM to 0.1 nM) or decreasing concentrations of 5-HT, from 100 μM to 0.1 nM (reference curve) for 20 min at 30 °C in a final volume of about 0.5 mL. Samples were then transferred to ice, added with [^{35}S]GTPγS (150−250 pM), and then incubated for a further 30 min at 30 °C. Nonspecific binding was determined in the presence of 10 μM GTPγS. The incubation was stopped by addition of ice-cold HEPES and rapid filtration on Schleicher and Schuell GF52 filters, using a Brandel cell harvester. The filters were washed three times with a total of 5 mL of the same buffer. Radioactivity was counted by liquid scintillation spectrometry with efficiency > 90.

Stimulation of [^{35}S]GTPγS binding induced by the compounds tested was expressed as percent increase in binding above basal value, being the maximal stimulation observed with 5-HT taken as 100%. The concentration−response curve of the agonistic activity was analyzed by nonlinear fitting program Allfit.[22] The maximal stimulation of [^{35}S]GTPγS

binding (E_{max}) achieved for each drug and the concentration required to obtain 50% of E_{max} (pEC_{50} value) were evaluated.

Molecular Modeling. The protonated structures of the ligands considered in this study were fully optimized by means of semiempirical molecular orbital calculations (AM1),[27] using the MOPAC 6.0 (QCPE 455) program.

The ad hoc modeling consisted of comparing the vdw volume of the minimized structure of each ligand (in its extended conformation) with the vdw volume of a supermolecule chosen as a template. Three different supermolecules were modeled for the three α_1-AR subtypes. In the series of structurally heterogeneous ligands considered in a previous study,[10] subsets of analogues can be identified. For each subset, the ligand showing the highest affinity for the specific α_1-AR subtype was chosen as a component of the respective reference supermolecule. Thus, the ligands used for the α_{1a} supermolecule are compounds **1**−**6**, the ligands for the α_{1b} supermolecule are compounds **7**−**10**, and the ligands for the α_{1d} supermolecule are **7, 8, 10**−**12** (Chart 1). The ligands chosen as components of each supermolecule were superimposed by a topologic rigid body fit procedure based on the following pharmacophoric criteria: (a) the hydrogen of the protonated nitrogen atom and (b) the aromatic rings closest to and farthest from the protonated nitrogen. All the other compounds were rigidly superimposed on the appropriate supermolecule with each ligand being superimposed on the analogue compound present in the supermolecule or on its structurally closest compound. Matching involved the moieties carrying the protonated nitrogen, i.e., the piperazinic ring.

QUANTA molecular modeling package (release 96; Molecular Simulation Inc., 200 Fifth Avenue, Waltham, MA 02154) was used for molecular comparison and computation of the vdw volumes. We considered the following size and shape descriptors: V_{in} and V_{out}, which are, respectively, the intersection and the outer van der Waals volume of the ligand considered with respect to the vdw volume of the reference supermolecule; and V_{dif}, which is computed according to the formula $V_{dif} = (V_{in} - V_{out})/V_{sup}$, where V_{sup} is the molecular volume of the reference supermolecule.

Acknowledgment. Financial support from CNR and technical support from CICAIA (University of Modena) are acknowledged.

References

(1) Cotecchia, S.; Schwinn, D. A.; Randall, L. L.; Lefkowitz, R. J.; Caron, M. G.; Kobilka, B. K. Molecular cloning and expression of the cDNA for the hamster α_1-adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7159−7163.

(2) Schwinn, D. A.; Lomasney, J. W.; Lorenz, W.; Szklut, P. J.; Fremeau, R. T.; Yang-Feng, T. L.; Caron, M. G.; Lefkowitz, R. J.; Cotecchia, S. Molecular cloning and expression of the cDNA for a novel α_1-adrenergic receptor subtype. *J. Biol. Chem.* **1990**, *265*, 8183−8189.

(3) Lomasney, J. W.; Cotecchia, S.; Lorenz, S.; Leung, W.; Schwinn, D. A.; Yang-Feng, T. L.; Brownstein, M.; Lefkowitz, R. J.; Caron, M. G. Molecular cloning and expression of the cDNA for the α_{1A}-adrenergic receptor. *J. Biol. Chem.* **1991**, *266*, 6365−6369.

(4) Perez, D. M.; Piascik, M. T.; Graham, R. M. Solution-phase library screening for the identification of rare clones: isolation of an α_{1D}-adrenergic receptor cDNA. *Mol. Pharmacol.* **1991**, *40*, 876−883.

(5) Schwinn, D. A.; Lomasney, J. W. Pharmacologic characterization of cloned α_1-adrenoceptor subtypes: selective antagonists suggest the existence of a fourth subtype. *Eur. J. Pharmacol.* **1992**, *227*, 433−436.

(6) Laz, T. M.; Forray, C.; Smith, K. E.; Bard, J. A.; Vaysse, P. J. J.; Branchek, T. A.; Weinshank, R. L. The rat homologue of the bovine α_{1c}-adrenergic receptor shows the pharmacological properties of the classical α_{1A} subtype. *Mol. Pharmacol.* **1994**, *46*, 414−422.

(7) Forray, C.; Bard, J. A.; Wetzel, J. M.; Chiu, G.; Shapiro, E.; Tang, R.; Lepor, H.; Hartig P. R.; Weinshank, R. L.; Branchek, T. A.; Gluchowski, C. The α_{1a} adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human α_{1c} subtype. *Mol. Pharmacol.* **1994**, *45*, 703−708.

(8) Forray, C.; Bard, J. A.; Laz, T. M.; Smith, K. E.; Vaysse, P. J.; Weinshank, R. L.; Gluchowski, C.; Branchek, T. A. *Faseb J.* **1994**, *8*, A353.

(9) Hieble, J. P.; Bylund, D. B.; Clarke, A. E.; Eikenberg, D. C.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Ruffolo, R. R. International Union of Pharmacology X. Recommendation for nomenclature of α_1-adrenoceptors: Consensus update. *Pharmacol. Rev.* **1995**, *47*, 267−270.

(10) Montorsi, M.; Menziani, M. C.; Cocchi, M.; Fanelli, F.; De Benedetti, P. G. Computer Modeling of Size and Shape Descriptors of α_1-Adrenergic Receptor Antagonists and Quantitative Structure-Affinity/Selectivity Relationships. *Methods Companion Methods Enzymol.* **1998**, *14*, 239−254.

(11) Dal Piaz, V.; Ciciani, G.; Turco, G. Oxidative cleavage of 7-oxo-6,7-dihydroisoxazolo[3,4-d]pyridazines by ceric(IV) ammonium nitrate: a synthetic approach to new 5-acyl-4-nitro-3-oxo-2,3-dihydropyridazines. *Synthesis* **1989**, 213−214.

(12) Dal Piaz, V.; Ciciani, G.; Giovannoni, M. P.; Franconi, F. Synthesis and evaluation as platelet aggregation inhibitors of 6-phenyl-2,4-substituted-3(2H)-pyridazinones and their rigid analogues benzo[*h*]cinnolin-3,5-diones. *Drug Des. Dis.* **1996**, *14*, 53−75.

(13) Nitta, Y.; Yoneda, F.; Ohtaka, T.; Kato, T. Pyridazin-derivate. V. Synthese der derivate des 6-phenyl-3(2H]pyridazinons. *Chem. Pharm. Bull.* **1964**, *12*, 69−73.

(14) Schonbeck, R.; Kloimstein, E. Uber Pyridazine mit kerngebundenem. *Chlor. Monatsh. Chem.* **1968**, *99*, 15−48.

(15) Montesano, F.; Barlocco, D.; Dal Piaz, V.; Leonardi, A.; Poggesi, E.; Fanelli, F.; De Benedetti, P. G. Isoxazolo-[3,4-d]-pyridazin-7-(6H)-ones and their corresponding 4,5-disubstituted-3-(2H) pyridazinone analogues as new substrates for α_1-adrenoceptor selective antagonists: synthesis, modeling and binding studies. *Bioorg Med. Chem.* **1998**, *6*, 925−935.

(16) Cavalli, A.; Fanelli, F.; Taddei, C.; De Benedetti, P. G.; Cotecchia, S. Amino acids of the α_{1B}-adrenergic receptor involved in agonist binding: further differences in docking catecholamines to receptor subtypes. *FEBS Lett.* **1996**, *399*, 9−13.

(17) De Benedetti, P. G.; Fanelli, F.; Menziani, M.; Cocchi, M.; Testa, R.; Leonardi, A. α_1-Adrenoceptor Subtype Selectivity: Molecular Modelling and Theoretical Quantitative Structure-Affinity Relationships. *Bioorg. Med. Chem.* **1997**, *5*, 809−816.

(18) De Benedetti, P. G.; Fanelli, F.; Menziani, M. C.; Cocchi, M. The ad hoc supermolecule approach to receptor ligand design. *J. Mol. Struct. (THEOCHEM)* **2000**, *503*, 1−16, and references therein.

(19) Testa, R.; Taddei, C.; Poggesi, E.; Destefani, C.; Cotecchia, S.; Hieble, J. P.; Sulpizio, A. C.; Naselsky, D.; Bergsma, D.; Ellis, S.; Swift, A.; Ganguly, S.; Ruffolo, R. R.; Leonardi, A. Rec 15/2739 (SB 216469): A novel prostate selective α_1-adrenoceptor antagonist. *Pharmacol. Commun.* **1995**, *6*, 79.

(20) Fargin, A.; Raymond, J. R.; Regan, J. W.; Cotecchia, S.; Lefkowitz, R. J.; Caron, M. G. Effector coupling mechanisms of the cloned $5HT_{1A}$ receptor. *J. Biol. Chem.* **1989**, *284*, 14848−14852.

(21) Fargin, A.; Raymond, J. R.; Lohse, M. J.; Kobilka, B. K.; Caron, M. G.; Lefkowitz, R. J. The genomic clone G-21 which resembles a β-adrenergic receptor sequence encodes the $5HT_{1A}$ receptor. *Nature* **1988**, *335*, 358−360.

(22) De Lean, A.; Munson, P. J.; Rodbard, D. Simultaneous Analysis of Families of Sigmoidal Curves: Application to Bioassay, Radioligand Assay, and Physiological Dose−Response Curves. *Am. J. Physiol.* **1978**, *235*, E97−E102.

(23) Cheng, Y. C.; Prusoff, W. H. Relationship Between the Inhibition Constant (Ki) and the Concentration of Inhibitor which Causes 50 Percent Inhibition (IC_{50}) of an Enzyme Reaction. *Biochem. Pharmacol.* **1973, *22***, 3099−3108.

(24) Testa, R.; Guarneri, L.; Poggesi, E.; Simonazzi, I.; Taddei C.; Leonardi, A. Mediation of noradrenaline-induced contractions of rat aorta by the α_{1B}-adrenoceptor subtype. *Br. J. Pharmacol.* **1995**, *14*, 745−750.

(25) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacologic Calculations with Computer Programs*, 2nd ed.; Springer-Verlag: Berlin, 1987.

(26) Stanton, J. A.; Beer, M. S. Characterization of a cloned human $5\text{-}HT_{1A}$ receptors cell line using [^{35}S]GTPγ binding. *Eur. J. Pharmacol.* **1997**, *320*, 267−275.

(27) Dewar, M. J. S.; Zoebisch, E. G.; Healey, E. F.; Stewart, J. J. P. AM1: a new general purpose quantum mecanical molecular model. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.

JM0009336