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Comparative Molecular Field Analysis of Some Pyridazinone-Containing α_1-Antagonists

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Abstract—Diverse series of piperazines linked at N1 to 4, 5, or 6 positions of 3-(2*H*)-pyridazinone ring and at N4, by a suitable alkyl spacer, to some classical α_1-adrenoceptor pharmacophore moieties, were tested in vitro for their α_1-adrenoceptor antagonist activity. The modeling of their biological activity (pK_b) by comparative molecular field analysis led to the development of a statistically significant partial least squares (PLS) model able to detect at 3-D level the main physicochemical interactions responsible for α_1-adrenoceptor antagonist activity.

Introduction

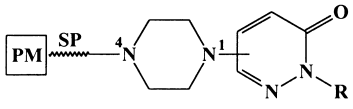
In recent years, the search for new and selective α_1-adrenoceptor antagonists has increased due to their therapeutical potential in the treatment of hypertension[1] and benign prostatic hypertrophy.[2]

Alpha1-adrenoceptors (α_1-AR) are members of the super family of seven transmembrane G protein coupled receptors (GPCR)[3] and regulate several important physiological processes.[4]

Pharmacological evidence and recent molecular cloning studies have demonstrated that α_1-AR are not a homogeneous population and so far three α_1-AR subtypes have been characterized by functional, radioligand binding and molecular biology studies.

They are usually referred to as α_{1A} (α_{1a}), α_{1B} (α_{1b}), α_{1D} (α_{1d}) adrenoceptors where the lower and upper case letters are used for recombinant and native receptors, respectively.[5]

Some of us have long been studying piperazine derivatives of general structure **I**, for their interesting α_1-adrenoceptor antagonist activity.[6–9]



I

In this class of compounds the N1 piperazine nitrogen has been directly linked to 4, 5, or 6 positions of the pyridazinone ring, and the N4 nitrogen, by a suitable spacer (SP), to α_1-adrenoceptor pharmacophore moieties (PM) such as 1,4-benzodioxanyl, 2-methoxyphenoxyethyl or phenoxyethyl groups. Moreover, to better define the SAR of this class of α_1-adrenoceptor antagonists, the acid hydrogen at N2 of the pyridazinone ring has also been substituted by phenyl (Table 2) or methyl groups (Tables 2 and 3). The biological activity, expressed as p*K*b, of a set of α_1-adrenoceptor antagonists of general structure **I** was modeled by comparative molecular field analysis and the results are reported in the present paper.

Very recently, synthesis, modeling and binding studies of 3-(2*H*)-pyridazinones structurally related to those already prepared by us,[6–9] have been reported.[10]

Results and Discussion

Previous qualitative SAR studies[6–9] of compounds reported in Tables 1–3 have shown that the presence of the benzodioxanyl nucleus or its 2-methoxyphenoxyethyl

Key words: 3-D-QSAR study; piperazine-pyridazinones; α_1-adrenoceptor antagonists.
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open analogue is necessary for a strong α_1-adrenoceptor antagonist activity. The phenyl ring of these pharmacophore moieties must be at a correct distance from the protonated piperazine nitrogen atom to achieve high activities. Moreover, an oxygen atom, present in the 2-methoxyphenoxyethyl or benzoxanyl groups, reinforces receptor binding due to a likely formation of a hydrogen bond with the receptor. No substantial modification of biological activity has been observed for 4- and 5-(1-piperazinyl) pyridazinone isomers (Tables 3 and 2), while a lower activity generally resulted from the 6 substituted isomers (Table 1). In the 5-(1-piperazinyl) isomers the activity decreased when the acid hydrogen at N2 was substituted either by a methyl or a phenyl group (Table 2). Conversely, the introduction of a methyl group at N2 in 4-(1-piperazinyl) isomers produced a higher activity (Table 3).

Molecular modeling and CoMFA study of 3-(2*H*)-pyridazinone derivatives

A set of twenty-eight 3-(2*H*)-pyridazinone derivatives, reported in Tables 1–3, were selected from our previous papers[6–9] and used as a training set for a 3-D-QSAR study based on comparative molecular field analysis. CoMFA[11] is a widely used approach for studying quantitative structure–activity relationships (QSAR) at a three dimensional (3-D) level.[12]

CoMFA relates the biological activity of a series of molecules with their steric and electrostatic fields sampled at several grid points around the molecule. CoMFA molecular descriptors commonly consist of steric and electrostatic potentials computed for each molecule, at each grid point, by means of a suitable probe, usually, an sp^3 carbon atom with a charge of +1. Partial least squares (PLS)[13] is used as the regression method to develop the relationship between steric and electrostatic potentials and biological activity. PLS analysis produces model equations which explain the variance in the biological property in terms of molecular potentials.[14] The optimum number of components (latent variables) is determined by cross validation and the model predictive ability is assessed by cross validated r^2 (r^2_{cv}, q^2).[15] The graphic representation of CoMFA, in the form of coefficient isocontour maps, efficiently located the regions where the variation in steric and electrostatic properties of different molecules in a data set is correlated with the variation of biological activity. CoMFA isocontour maps may furnish useful indications to prioritize future synthesis and to develop sound working hypotheses on the nature of putative ligand–receptor interactions.

One of the known limitations of CoMFA is the composition of the set being studied which should not include derivatives with unique structural features. For this reason, only twenty-eight 3(2*H*)-pyridazinone derivatives, namely compounds **1**–**28**, have been selected from all the synthetized compounds.[6–9] Moreover, for some low activity congeners, a truncated pK_b value of 4 was used, to avoid the loss of relevant information for the CoMFA study.

Three-dimensional models were constructed starting from standard bond length and angles of the fragment library of Sybyl ver. 6.4[16] and their geometry optimized

Table 1. Chemical structures and α_1-adrenoceptor antagonist activity of compounds **1**–**10** (see Scheme 1)

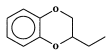
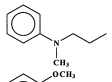
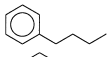
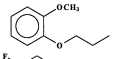
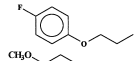
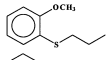
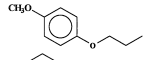
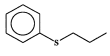
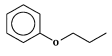
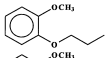
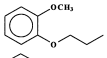
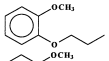
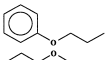
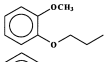
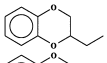
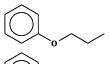
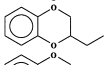
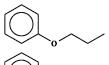
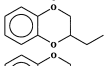
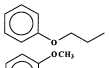
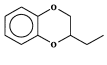
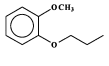
Compds	R	pK_b	Compds	R	pK_b
1		6.05	**6**		4.83
2		5.38	**7**		6.09
3		4.00	**8**		4.89
4		4.00	**9**		4.00
5		4.00	**10**		5.74

Table 3. Chemical structures and α_1-adrenoceptor antagonist activity of compounds **24**–**28** (see Scheme 3)

Compds	R	X	R_1	pK_b
24	OCH_3	Cl	H	7.15
25	OCH_3	Cl	CH_3	7.19
26	H	Cl	H	6.73
27	H	Cl	CH_3	7.04
28	OCH_3	Br	H	6.88

Table 2. Chemical structures and α_1-adrenoceptor antagonist activity of compounds **11**–**23** (see Scheme 2)

Compds	R	X	R_1	pK_b	Compds	R	X	R_1	pK_b
11		Cl	H	7.29	**18**		H	CH_3	5.50
12		Cl	CH_3	6.89	**19**		H	CH_3	5.60
13		Cl	C_6H_5	6.96	**20**		Cl	H	6.57
14		Cl	H	7.10	**21**		Cl	CH_3	6.41
15		Cl	CH_3	6.31	**22**		Cl	C_6H_5	6.55
16		Cl	C_6H_5	6.61	**23**		H	CH_3	6.10
17		H	H	6.24					

first by molecular mechanics. In these calculations two different nitrogen types were selected for the piperazine ring: the first, most basic N4 nitrogen, bearing the alkyl spacer as substituent, was considered as fully tetrahedric (N3), whereas the N1 nitrogen, less basic and directly attached to the pyridazinone ring, was defined as trigonal-planar (N.pl3). This selection is supported not only by a plausible strong conjugation effect of the N1 with the pyridazinone ring but also by X-ray experimental data reported for other similar *N*-aryl-piperazines on the Cambridge Structural Database (CSD).[17] Moreover, the same selection has been made in another CoMFA study of some piperazine derivatives acting on $5HT_{1A}$ receptor as antagonists.[18]

A conformational search on some representative structures (classes a, b, c, d and e in Chart 1) was carried out by means of the semi-empirical quantomechanical method AM1.[19] In order to decrease the *CPU* time and the number of conformations to be analyzed, only rotation around the τ_1 dihedral angle was explored with a stepsize of 30°, leaving the rest of the alkyl spacer in an extended conformation as observed in several X-ray crystallographic studies on similar compounds reported on CSD.[17] Energetic profiles showed a global minimum with τ_1 angles ranging from 120 to 180° for class a, with a τ_1 angle nearly 180° for class b, whereas classes c, d and e share two common minima with τ_1 angles close to 150 and 60°. These data compared well with the corresponding dihedral angles reported for some piperazine derivatives bearing a pyrimidine, phenyl or other aromatic ring showing a quasi-coplanar orientation ($\tau_1 = 0 \pm 30°$). Thus, derivatives belonging to class a and b were aligned in the CoMFA model with a τ_1 angle of 180°, whereas derivatives belonging to the other classes either with τ_1 angle of 150 or 60°.

A similar conformational analysis was carried out on other representative structures (f, g and h) in order to detect the energetically permitted conformations of the phenoxy or benzodioxane moieties linked to the alkyl spacer. Classes f and g showed a minimum with a τ_2 angle near 60°, whereas for class h the minimum rotamer has a τ_3 of 90°.

In order to enhance the molecular overlapping of putative pharmacophoric elements in the CoMFA study, a manual selection of a suitable rotamer for class f, g and h was performed, within an energy window up to 2 kcal/mol over the detected global minimum.

All the conformations of the 3-(2*H*)-pyridazinone selected for the 3-D–QSAR study were aligned on the lowest energy conformer of the most active ligand (compound **11**), chosen as the template, allowing an energy penalty up to 5 kcal/mol to reach good superposition of the selected fitting points. As fitting elements, the two piperazine nitrogen atoms and the centroid of the aromatic ring of the pharmacophore moieties, connected through the spacer to the piperazine ring, were used.

Since the piperazine nitrogen linked to the alkyl spacer could be protonated at the physiological pH, both the protonated and neutral forms were taken into account.

The possible τ_1/τ_2 and τ_1/τ_3 combinations for both neutral (models N and N′, respectively) and protonated (models P and P′, respectively) molecules as well as some different topological overlays for classes a, b, c, d and e were tested. Table 4 summarizes the statistical results of the CoMFA study based on the most representative examined combinations and from AM1 calculations.[19] Slightly poorer statistical results (not shown) were obtained from PM3 calculations.[19] Since PLS models pertaining to the molecules in the neutral state, (N1–N3 and N′1–N′3), gave only slightly improved statistics than the corresponding protonated models (P1–P3 and P′1–P′3), we decided to develop the isocontour maps for the two-fields model P′1 based on protonated molecules which actually should be the bioactive form of the ligands. It is worth noting that good statistical figures were also obtained when the electrostatic field was taken into account alone (see models P3 and P′3).

The good fitting capability of the P′1 model can be appreciated in the plot of the observed versus predicted biological data (Fig. 1).

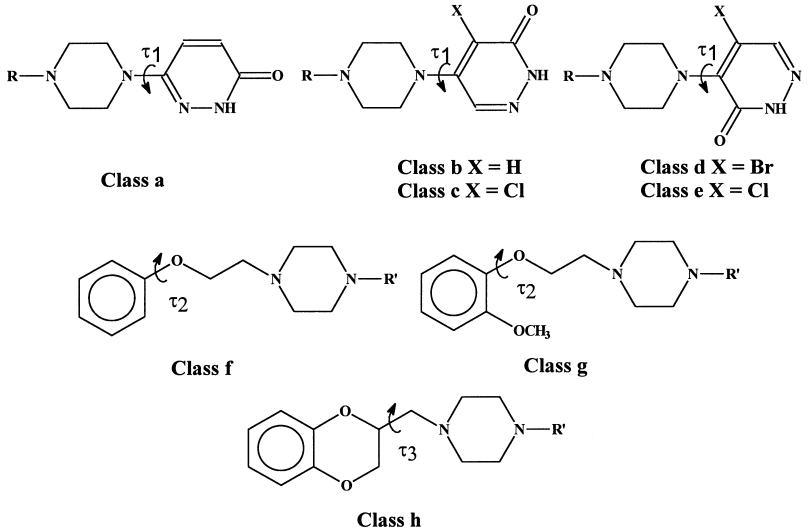


Chart 1. Torsion angles screened in the conformational analyses of the indicated classes of pryidazinone derivatives.

It can be observed in Table 4 that all the retained final CoMFA models had q^2 values well above 0.30, which corresponds to a low probability of chance correlation ($p < 0.05$, that is $< 5\%$).[20] However, to reduce the potential risk of chance correlations, the biological data were reassigned randomly to the compounds and the PLS analyses were repeated. In no case were good predictive PLS models obtained (q^2 always < 0.2).

Table 4. CoMFA statistical results on 3-(2*H*)-pyridazinone derivatives **1**–**28**

Model[a]	τ_1	Field(s)[b]	q^2	ONC	r^2	$s_{e/v}$	s_{fin}
N1	180/150	Ste + Ele	0.707	5	0.952	0.638	0.258
N2	180/150	Ste	0.612	5	0.931	0.734	0.310
N3	180/150	Ele	0.748	5	0.915	0.592	0.343
N′1	180/60	Ste + Ele	0.814	5	0.966	0.508	0.218
N′2	180/60	Ste	0.595	2	0.762	0.703	0.539
N′3	180/60	Ele	0.839	5	0.959	0.472	0.237
P1	180/150	Ste + Ele	0.722	5	0.952	0.636	0.258
P2	180/150	Ste	0.660	5	0.937	0.687	0.297
P3	180/150	Ele	0.739	4	0.917	0.588	0.331
P′1	180/60	Ste + Ele	0.768	5	0.968	0.567	0.209
P′2	180/60	Ste	0.586	2	0.752	0.711	0.551
P′3	180/60	Ele	0.798	5	0.953	0.529	0.256

[a] N and P stand for neutral and protonated molecules respectivley, whereas the prime sign indicates the different τ_1 torsion angle value combinations for classes a, b, c, d and e.
[b] Fields(s): steric (Ste) and electrostatic (Ele) fields; q^2: cross validated correlation coefficient; ONC: optimal number of components used for the final analysis; r^2 correlation coefficient, s_{cv} and s_{fin} standard deviation of the cross validated and non-cross validated final models, respectively.

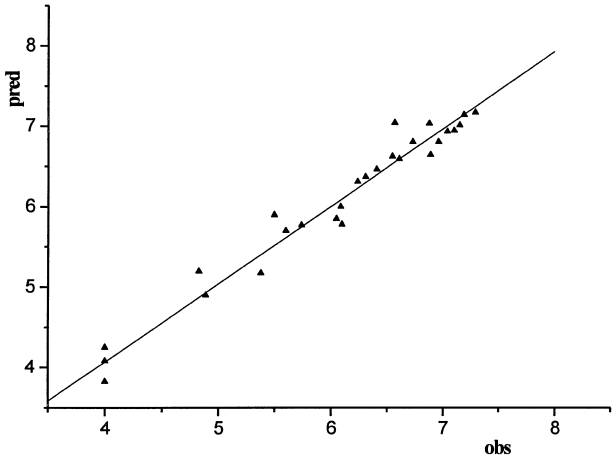
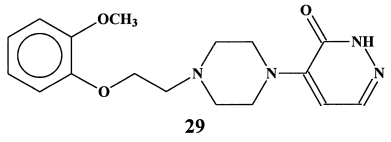


Figure 1. Plot of observed versus predicted pK_b values from model P′1.

CoMFA steric isocontour maps (Fig. 2) show sterically unfavorable regions (red) close to the carbonyl groups of low activity 6-substituted congeners (i.e. compound **9** in the map) and to the methyl substituents in the same topological position (i.e. compound **19**). Sterically favorable regions (green) can be reached by methyl, carbonyl and halogen groups of the pyridazinone ring (i.e. compounds **25** and **11**).

The isocontour maps associated with the electrostatic field are depicted in Figure 3. Regions where an increased electronic density corresponds to higher or lower biological activity are represented by magenta and white colors, respectively. The oxygen of the oxyethylene bridge of compound **11** is located on the magenta region while a white zone is unfavorably contacted by electron rich carbonyls of low activity 6-substituted congeners and by electronegative substituents at the *para* position of the phenyl ring (i.e. F and OCH_3 of compounds **8** and **9**, respectively).

To judge the predictive capability of our PLS model, the P′1 model was used for the prediction of the α_1-adrenoceptor antagonist activity of a recently synthesized and tested molecule (compound **29**). This compound, in the lowest energy conformation, has a predicted pK_b of 6.56 slightly higher than the observed pK_b of 6.18.



29

Conclusions

An informative CoMFA model with good statistical figures was developed for a set of pyridazinone-piperazines endowed with interesting α_1-adrenoceptor antagonist activity.

CoMFA isocontour maps pointed out, at the 3-D level, some key molecular determinants for a high α_1-adrenoceptor antagonist activity of this class of ligands and

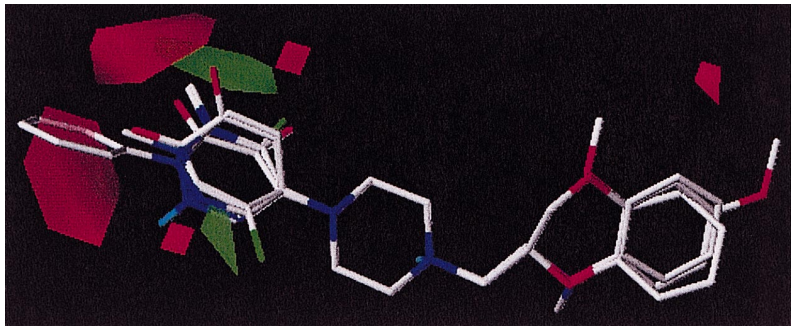


Figure 2. Steric contour plot from model P′1 expressed as STDEV×COEFF (contour level: −0.035 red; 0.027 green); compounds **9** (pK_b = 4.00), **11** (pK_b = 7.29), **19** (pK_b = 5.60), **25** (pK_b = 7.19) are displayed to aid interpretation.

may be helpful for the design of more potent α_1-adrenoceptor antagonists.

Experimental

Chemistry

The synthesis of the examined compounds outlined in Schemes 1–3 has already been described.[6–9] Compound **29** was prepared by dechlorination of compound **24**, using 10% Pd/C at room temperature and atmospheric pressure, in a methanolic solution of KOH. Compound **29** hydrochloride (mp 175–179°C) had analytical and spectral data consistent with the proposed structure.

Pharmacology

The pharmacological activity of compounds listed in Tables 1–3, as α_1 and α_2-adrenoceptor antagonists, has been already described.[8,9] The α_1-adrenoceptor blocking activity of compound **29** was assessed on isolated rat vas deferens tissues by antagonism of (–)-noradrenaline induced contractions of the epididymal portion, and expressed as pK_b according to Drew.[21]

Molecular modeling and CoMFA study

Molecular models of the neutral and N4-protonated ligands were constructed with standard bond distances and angles using SYBYL version. 6.4 running on a Silicon Graphics Indigo2 R4400 workstation. Full geometry optimization was performed first by molecular mechanics (Tripos FF) and then by AM1 and PM3 Hamiltonians using the parameter set reported in the MOPAC suite of programs.[19] The partial Mulliken atomic charges from AM1 and PM3 calculations were used in the CoMFA study.

The conformational analysis was carried out with the SYSTEMATIC SEARCH option of SYBYL, screening the conformational space of each torsion angle at 30° increments.

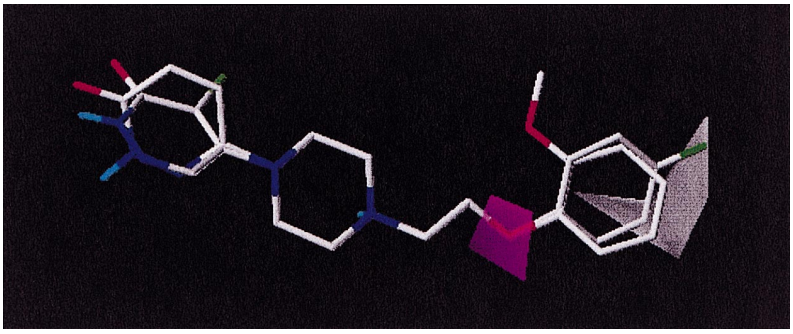
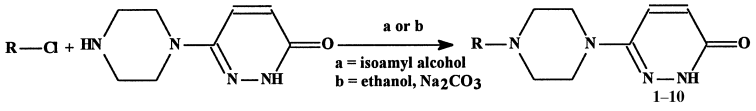
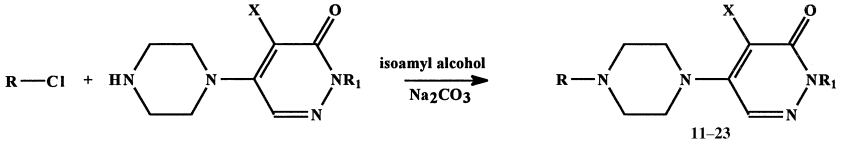


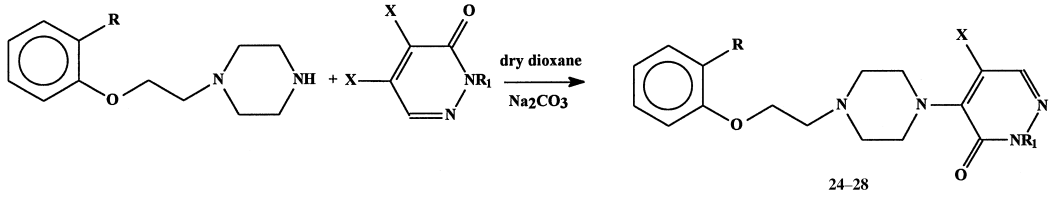
Figure 3. Electrostatic contour plot from model P′1 expressed as STDEV×COEFF (contour level: −0.130 magenta; 0.030 white); compounds **8** ($pK_b = 4.89$), **9** ($pK_b = 4.00$), **11** ($pK_b = 7.29$) are displayed to aid interpretation.



Scheme 1. Synthesis of 6-(1-piperazinyl)-3-(2*H*)-pyridazinones.[6–9]



Scheme 2. Synthesis of 4-substituted-5-(1-piperazinyl)-3-(2*H*)-pyridazinones.[6–9]



Scheme 3. Synthesis of 2,5-disubstituted-4-(1-piperazinyl)-3-(2*H*)-pyridazinones.[6–9]

In the CoMFA study a 3-D cubic lattice, with a 2 Å grid spacing, was generated around the aligned compounds based on the molecular volume of the structures (grid beyond the molecules extended by 4.0 Å in all directions). Steric and electrostatic potentials were generated by a sp^3 carbon atom probe with a charge of +1. The option 'drop electrostatic' was set to NO.

The CoMFA models were derived by the partial least squares (PLS) method, implemented in SYBYL, with cross validation (leave-one-out procedure).

The predictive ability of the model was quantitated in terms of q^2 which is defined as:

$$q^2 = (\mathrm{SD} - \mathrm{PRESS})/\mathrm{SD} \text{ where } \mathrm{PRESS} = \Sigma(\mathrm{Ypred} - \mathrm{Yactual})^2$$

and

$$SD = \Sigma(\mathrm{Yactual} - \mathrm{Ymean})^2$$

SD is the sum of squares of deviations of the observed values from their mean and PRESS is the prediction error sum of squares. The final models were developed by a conventional (non-cross validated) regression analysis with the optimum number of components equal to that yielding the highest q^2 value. Since the final equations are not very useful to represent efficiently the CoMFA models, 3-D graphics or isocontour maps were developed. They represent areas in the space where steric and electrostatic interactions are responsible for the observed variations of the biological activity.

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References and Notes

1. Kasztreiner, E.; Màtyus, P.; Rabloczky, G.; Jaszlits, L. *Drugs Future* **1989**, *14*, 622.
2. Beduschi, R.; Beduachi, M. C.; Oesterling *J. Geriatrics* **1998**, *53*, 24.
3. Gunderman, T.; Nurnberg, B.; Schultz, G. *J. Mol Med.* **1995**, *73*, 51.
4. Hieble, J. P.; Bondinell, W. E.; Ruffolo, R. R. *J. Med. Chem.* **1995**, *38*, 3415.
5. Hieble, J. P.; Bylund, D. B.; Clerke, D. E.; Eikenberg, D. C.; Langer, S. Z.; Lefkowitz, R. T.; Minneman, K. P.; Ruffolo, R. R. *Pharmacol Rev.* **1995**, *47*, 267.
6. Corsano, S.; Strappaghetti, G.; Codagnone, A. *Arch. Pharm.* **1989**, *322*, 833.
7. Corsano, S.; Strappaghetti, G.; Leonardi, A.; Rhazri, K.; Barbaro, R. *Eur. J. Med. Chem.* **1997**, *32*, 339.
8. Corsano, S.; Scapicchi, R.; Strappaghetti, G.; Marucci, G.; Paparelli, F. *Eur. J. Med. Chem.* **1993**, *28*, 647.
9. Corsano, S.; Scapicchi, R.; Strappaghetti, G.; Marucci, G.; Paparelli, F. *Eur. J. Med. Chem.* **1995**, *30*, 71.
10. Montesano, F.; Barlocco, D.; Del Piaz, V.; Leonardi, A.; Poggesi, E.; Fanelli, F.; De Benedetti, P. G. *Bioorg. Med. Chem.* **1998**, *6*, 925.
11. Cramer, R. D.; Patterson, D. E.; Bunce, J. D. *J. Am. Chem. Soc.* **1988**, *110*, 5959.
12. Cramer, R. D.; De Prierst, S. A.; Patterson, D. E.; Hect, P. In *3D-QSAR in Drug design: theory, methods and applications*; Kubinyi, H., Ed.; Escom: Leiden, 1993, pp 443–485.
13. Dunn, W. J. III.; Wold, S.; Edlund, U.; Helberg, S. *Quant. Struct. -Act. Relat.* **1984**, *3*, 131.
14. Wold, S.; Johansson, E.; Cocchi, M. in ref 13, pp 523–549.
15. Cramer, R. D. III.; Bunce, J. D.; Patterson, D. E. *Quant. Struct. -Act. Relat.* **1988**, *7*, 18.
16. Tripos Assoc. St. Louis, MO, USA
17. Allen, F. H.; Kennard, O. *Chem. Des. News* **1993**, *1*, 31.
18. Gaillard, P.; Carrupt, P. A.; Testa, B.; Schambel, P. *J. Med. Chem.* **1996**, *39*, 126.
19. AM1 and PM3 quantomechanical semiempirical methods can be found on the package MOPAC 6.0 which is available from QCPE (506).
20. Clark, M.; Cramer, R. D. III.; Jones, D. M.; Patterson, D. E.; Simeroth, P. *Tetrahedron Comput. Methodol.* **1990**, *3*, 47.
21. Drew, G. M. *Eur. J. Pharmacol.* **1977**, *27*, 123.