# Articles

## Modeling of β-Adrenoceptors Based on Molecular Electrostatic Potential Studies of Agonists and Antagonists

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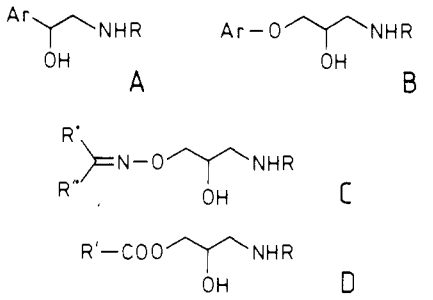
The molecular electrostatic potential (MEP) of 32 β-adrenoceptor ligands, mainly antagonists, was calculated by the STO-3G ab initio quantum mechanical method. The MEP of phenylethanolamines (PEAs) features a negative minimum in the meta region (designated M1) which is topographically equivalent to a minimum (designated M2) found in the vicinity of the aromatic ring in all (aryloxy)propanolamines (AOPAs). In these compounds, a second negative zone located beyond the meta position and designated M3 is found in all $\beta_1$-selective antagonists and in some nonselective and $\beta_2$-selective antagonists. The $\beta_1$-selective antagonists feature in the para position an additional zone which is positive (P4) in the full antagonists and negative (M4) in the antagonists displaying intrinsic sympathomimetic activity (ISA). The MEP-based pharmacophoric models of PEAs, AOPAs, and oxime ethers show common elements and lead to a proposed general model for β-adrenoceptor ligands.

β-Adrenoceptors are known to play an important role in the regulation of the autonomic nervous system. β-Adrenoceptor blockers have been shown to be useful therapeutic agents when abnormal function (hyperactivity) of these receptors results in a number of diseases and syndromes such as angina pectoris, hypertension, arrythmia, migraine, and tremors. On the basis of specificities and activities of various β-adrenoceptor ligands, Lands et al.[1] have subdivided this receptor into $\beta_1$ and $\beta_2$ subtypes. Many experimental studies[2-5] have added massive support to this proposal, and the $\beta_1/\beta_2$ profile of many agonists and antagonists has now been established.

Dichloroisoproterenol has been the first recognized nonselective β-adrenoceptor antagonist. Subsequently, many potent β-adrenoceptor antagonists were synthesized which belong to two general classes, namely phenylethanolamines (PEAs, Chart IA) and (aryloxy)propranolamines (AOPAs, Chart IB). The two classes have many structural features in common and differ only by a $OCH_2$ unit in their side chain. Several hypotheses have been proposed in order to explain the similar pharmacological behavior of the two classes of drugs. From a structural comparison of PEAs and AOPAs,[6-8] it was concluded that the specific electronic distribution necessary for interaction with the β-adrenergic receptor must not necessarily be generated by an aromatic ring. Two further classes of β-adrenolytic drugs have recently been developed, namely oxime ether derivatives[9-11] (Chart IC) and aliphatic 3-(acyloxy)propranolamines[12] (Chart ID). Despite the lack of an aromatic system, a number of drugs in these two classes exhibit marked competitive β-blocking activity.

Chart I. General Structures of Four Classes of β-Adrenolytic Agents[a]




[a] A, phenylethanolamines (PEAs); B, (aryloxy)propanolamines (AOPAs); C, oxime ethers; D, 3-(acyloxy)propanolamines.

The assessment of the structural features governing the specificity of interactions between a ligand and its receptor sites is certainly a major problem in medicinal chemistry. The structural conditions necessary to improve β-adrenoceptor affinity and selectivity have been investigated in several studies.[13-15] Most structural modifications have

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involved aromatic ring substituents and, more recently, substituents on the basic amino group. While the nature and position of aromatic substituents has been shown to dramatically influence the pharmacological profile, little is known yet regarding the stereoelectronic features of β-adrenolytic drugs dictating their $\beta_1/\beta_2$ selectivity.

In recent years, molecular electrostatic potentials (MEPs) have proven a powerful tool for characterizing the essential electronic features of drugs and their stereoelectronic complementarity with receptor sites.[16-20] Although it seem obvious and straightforward that negative potentials are found near electronegative groups or atoms (e.g. N, O, Cl) and positive potentials near aliphatic groups, this is not always the case due to electron delocalization. Thus, previous studies[20] have shown that a methoxy group may lie in a positive area despite the influence of the oxygen atom. Because receptors recognize stereoelectronic effects and not atoms per se, studies of two- or three-dimensional MEPs have become a powerful tool for characterizing pharmacologically active molecules from an electronic point of view. Thus, MEPs have been computed for a limited number of β-adrenolytic drugs in the search of molecular determinants of receptor recognition.[7,8,21-23] Martinelli et al.[21] have suggested a correlation between the biological activity of some β-adrenolytic drugs and their ring nucleophilicity which indicates that an electrophilic group in the receptor should interact with the ring portion of β-blockers. A reactivity index derived from the MEP of PEA derivatives was chosen by Solmajer et al.[22,23] as a parameter to be correlated with ligand–receptor dissociation constants.

In the present study, MEPs were calculated for a large series of β-adrenolytic and β-adrenergic agents belonging to three general classes, namely PEAs, AOPAs, and oxime ethers. The objective of this work was to identify some of the stereoelectronic features responsible for the $\beta_1/\beta_2$ selectivity of β-adrenolytic drugs. Our attention has focussed essentially on the aromatic region of the 32 investigated compounds, and the role of substituents on the basic amino group was not considered. Indeed, $\beta_1$ selectivity is under the influence of proper substitution not only in the aromatic region but also on the side-chain amino group.[24,25] However, the selectivities due to both types of substituents are not additive and probably result from different binding modes.[25] Since electronic properties cannot be completely separated from lipophilic properties, some attention was also paid to the connection between selectivity and lipophilicity.

## Conformational Rationalization

An important step in the calculation of MEPs is the choice of conformation(s) to be used as input data, the active conformer not being necessarily the most stable one. As a consequence, a careful comparison between published conformational studies of β-adrenoceptor ligands was needed before carrying out our investigations. Data obtained from $^1$H NMR studies[26] of protonated (aryloxy)propanolamines suggested a stable "rigid" conformation of the side chain with two intramolecular hydrogen bonds forming a bicyclic structure. In this study, the influence of the anion as a factor competing with the intramolecular hydrogen bonds was neglected, leading Zaagsma[27] to postulate a seven-membered ring structure. It should be noted that these studies were performed mostly in aprotic media favoring intramolecular H bonds.

Crystal structures of several β-adrenolytic drugs have been determined by X-ray diffraction methods.[28-32] Crystallographic investigations on both the phenylethanolamines and the (aryloxy)propranolamines have shown that the side chain is consistently found in an extended conformation, which is therefore considered as the

**Table I.** β-Adrenolytic and β-Adrenergic Drugs in This Study

| no. | compound name or code | selectivity | | ISA[a] | ref |
|---|---|---|---|---|---|
| | Phenylethanolamines | | | | |
| 1 | norepinephrine | $\beta_1$ | $\beta_2$ | ++ | 13 |
| 2 | salbutamol | | $\beta_2$ | ++ | 13 |
| 3 | dichloroisoproterenol | $\beta_1$ | $\beta_2$ | + | 39 |
| 4 | sotalol | $\beta_1$ | $\beta_2$ | - | 13 |
| | (Aryloxy)propanolamines | | | | |
| 5 | 6f | $\beta_1$ | | ++ | 40 |
| 6 | alprenolol | $\beta_1$ | $\beta_2$ | + | 13 |
| 7 | bunitrolol | $\beta_1$ | $\beta_2$ | + | 13 |
| 8 | carteolol | $\beta_1$ | $\beta_2$ | + | 13 |
| 9 | mepindolol | $\beta_1$ | $\beta_2$ | + | 13 |
| 10 | pindolol | $\beta_1$ | $\beta_2$ | + | 13 |
| 11 | bunolol | $\beta_1$ | $\beta_2$ | - | 15 |
| 12 | bupranolol | $\beta_1$ | $\beta_2$ | - | 15 |
| 13 | nadolol | $\beta_1$ | $\beta_2$ | - | 15 |
| 14 | propranolol | $\beta_1$ | $\beta_2$ | - | 13 |
| 15 | tertatolol | $\beta_1$ | $\beta_2$ | - | 15 |
| 16 | acebutolol | $\beta_1$ | | + | 15 |
| 17 | celiprolol | $\beta_1$ | | + | 15 |
| 18 | practolol | $\beta_1$ | | + | 15 |
| 19 | H 87/07 | $\beta_1$ | | + | 15 |
| 20 | (*S*)-38 | $\beta_1$ | | + | 41 |
| 21 | atenolol | $\beta_1$ | | - | 15 |
| 22 | betaxolol | $\beta_1$ | | - | 15 |
| 23 | bisoprolol | $\beta_1$ | | - | 15 |
| 24 | metoprolol | $\beta_1$ | | - | 15 |
| 25 | (*S*)-51 | $\beta_1$ | | - | 41 |
| 26 | spirendolol | | $\beta_2$ | - | 15 |
| 27 | ICI 118551 | | $\beta_2$ | - | 15 |
| | Oxime Ethers | | | | |
| 28 | IPS 339 | | $\beta_2$ | - | 15 |
| | Miscellaneous | | | | |
| 29 | bufuralol | $\beta_1$ | $\beta_2$ | + | 15 |
| 30 | tazolol | $\beta_1$ | $\beta_2$ | + | 15 |
| 31 | timolol | $\beta_1$ | $\beta_2$ | - | 15 |
| 32 | VII | | $\beta_2$ | ++ | 42 |

[a] ISA is the intrinsic sympathomimetic activity (++ means agonist, + means antagonist with partial agonistic properties, - means pure antagonist).

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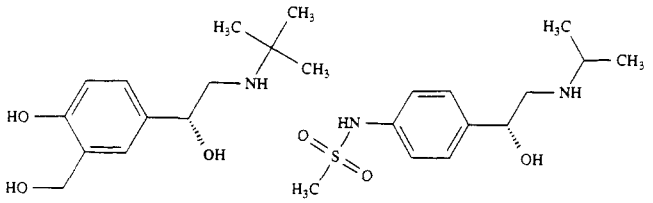
Chart II. Molecular Structure of Salbutamol (2) and Sotalol (4)



(2) (4)

preferred conformation of β-adrenoceptor ligands in the solid state. Theoretical quantum chemical studies[33-35] have also concluded that fully extended side-chain conformations are the preferred ones. A conformational study based on the distance geometry method revealed two useful models in which the side chain also appears as almost fully extended.[36,37] A crystallographic and theoretical confromational analysis of a number of β-adrenoceptor ligands has led to a unified model where the side chain of PEAs and AOPAs is essentially an extended one.[38]

At this point, it appears that the problem of the active conformation of β-adrenoceptor ligands is rather complex and still contradictory. In fact, all conformational analyses were performed under experimental conditions quite different from the biological situation. The least unsatisfactory technique appears to be NMR spectroscopy, but solvent effects are of such significance that any interpretation must be viewed with great care.

On the basis of the results of crystallographic and theoretical conformational studies, it was decided to place the side chain for all molecules in an extended position and in the plane of the aromatic ring system. The torsion angles C-C-C-N and O-C-C-O were fixed at 180° and -60°, respectively. Studies indicate that the orientation of the side chain has only a negligible influence on the electronic properties of the aromatic region (results not shown). The compounds investigated are listed in Table I. The molecules were set in an extended conformation as explained above, and the absolute configuration considered was always the active one, i.e. *S* for AOPAs and *R* for PEAs.

## Results and Discussion

**Phenylethanolamines (PEAs).** Petrongolo et al.[43] and Macchia et al.[7] compared the MEPs of the β-adrenoceptor agonists isoproterenol and tazolol (an AOPA) with those of two antagonists, INPEA (*N*-isopropyl(*p*-nitrophenyl)-ethanolamine) and doberol (an AOPA). Agonists were found to have a negative potential in the aromatic region and antagonists a positive one, leading these workers to hypothesize a different mode of binding of the agonists and antagonists with the β-adrenoceptor. The MEP patterns of noradrenaline (1) and dichloroisoproterenol (3) (supplementary material) calculated in the present study are in agreement with this rationalization. However, the MEPs of salbutamol (2) and sotalol (4) (Chart II) are not consistent with this model. Indeed, the aromatic ring in the agonist salbutamol is surrounded by a positive zone above the molecular plane and a negative zone below it (Figure 1). As for the antagonist sotalol, its aromatic ring is entirely surrounded by a negative zone (Figure 2).

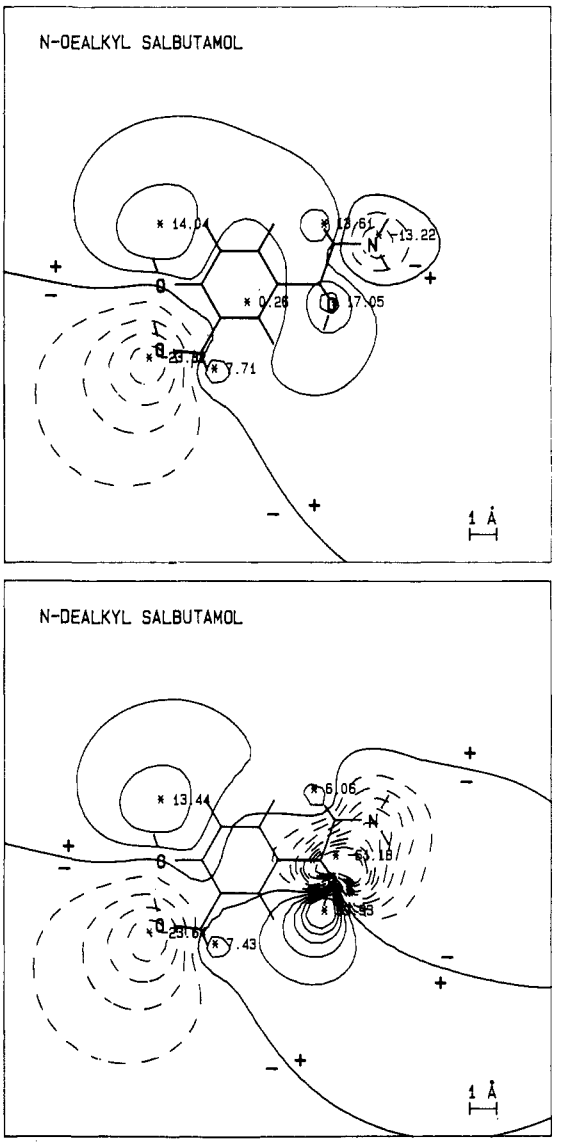



**Figure 1.** MEP (calculated by the STO-3G ab initio method) of the *N*-dealkyl derivative of the agonist salbutamol (2). The isoenergy contours (in kcal/mol) are in planes 2 Å above (A) and 2 Å below (B) the plane of the aromatic ring.

What the MEPs of compounds 1-4 consistently show, however, is a negative minimum (designated M1) located in the vicinity of the meta substituent, as discussed later.

**(Aryloxy)propanolamines (AOPAs).** In order to explore the possible role of electrostatic interactions in the $\beta_1$-selectivity of β-blockers of the AOPA class (Chart III), we have compared the MEPs of $\beta_1$-selective (compounds 21 and 23, Figure 3), $\beta_2$-selective (compounds 26 and 27, Figure 4), and nonselective antagonists (compounds 10 and 14, Figure 5). All compounds display a local negative minimum in the vicinity of the ether oxygen atom. This

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(43) Petrongolo, C.; Macchia, B.; Macchia, F.; Martinelli, A. *J. Med. Chem.* **1977**, *20*, 1645.

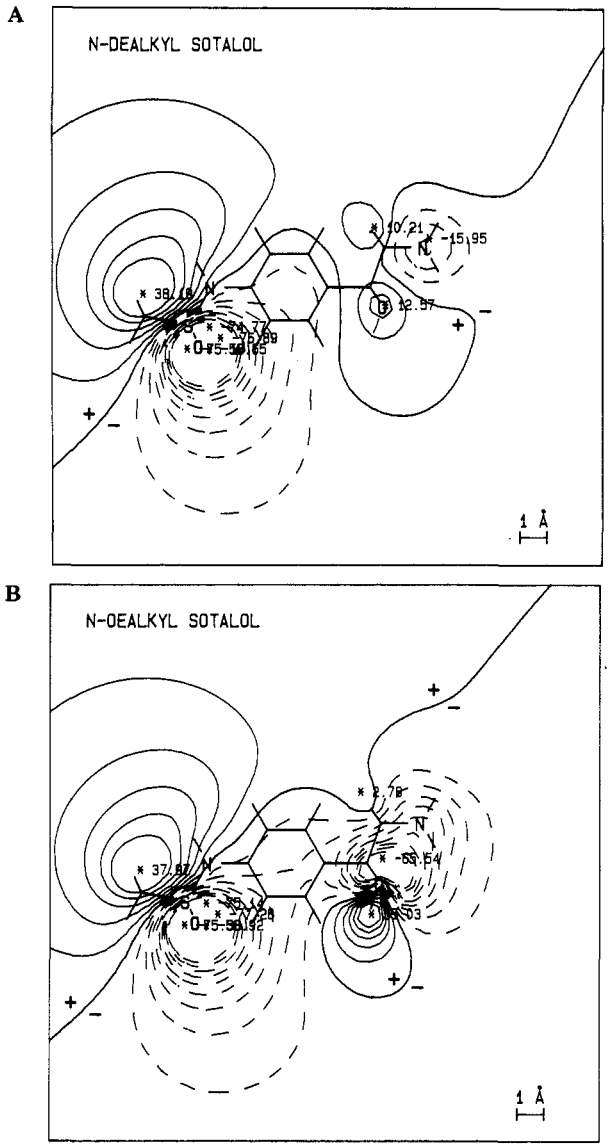



**Figure 2.** MEP (calculated by the STO-3G ab initio method) of the *N*-dealkyl derivative of the antagonist sotalol (**4**). The isoenergy contours (in kcal/mol) are in planes 2 Å above (A) and 2 Å below (B) the plane of the aromatic ring.

minimum is visible above the plane of the aromatic ring but is overshadowed below this plane by the strong negative influence of the aliphatic hydroxyl group (see Figures 3B, 4C, and 5C). In addition, all compounds generate above and below their aromatic ring a more or less diffuse negative zone (designated M2) centered close to the ortho carbon. More precisely, M2 is centered above and below the aromatic ring or approximately beyond the ortho position of the ring. The $\beta_2$-selective antagonist spirendolol (**26**, Figure 4A) displays an additional negative minimum (designated M3) beyond the meta position of the aromatic ring. Such a feature in not found in the MEP of the $\beta_2$-selective compound **27** (Figure 4B,C) and of the nonselective antagonists pindolol (Figure 5A) and propranolol (Figures 5B,C). Yet M3 can be seen in the MEP of some (**8**, **11**, and **13**) but not other (**6**, **7**, **9**, **12**, and **15**) nonselective antagonists (see supplementary material). We also note that most $\beta_2$-selective and nonselective antagonists have an additional ring fused to the aromatic ring, or at least a lipophilic group attached to it. Lipophilicity must therefore also be of importance, as discussed later.

Interestingly, the $\beta_1$-selective AOPAs appear more homogeneous and display some distinctive features. Indeed, these compounds consistently generate the negative min-

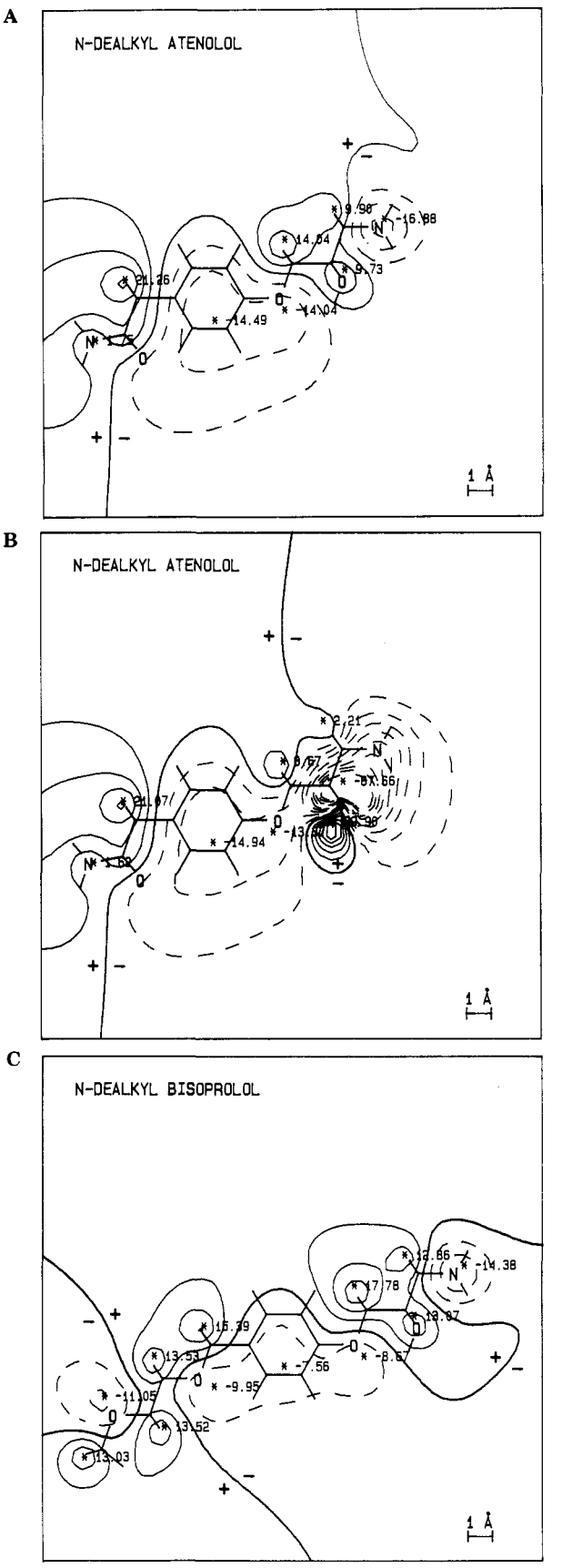



**Figure 3.** MEP (calculated by the STO-3G ab initio method) of the *N*-dealkyl derivatives of the $\beta_1$-selective antagonists atenolol (**21**, A and B) and bisoprolol (**23**, C). The isoenergy contours (in kcal/mol) are in planes 2 Å above (A and C) and 2 Å below (B) the plane of the aromatic ring.

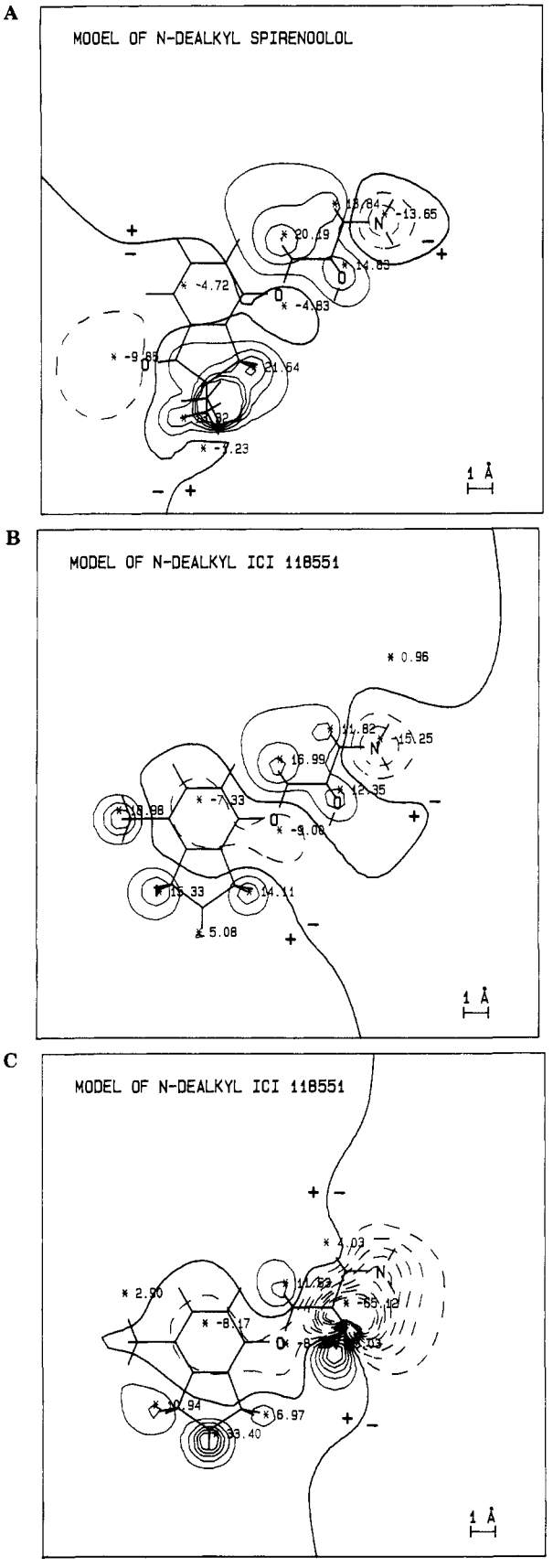



**Figure 4.** MEP (calculated by the STO-3G ab initio method) of model compounds of the *N*-dealkyl derivatives of the $\beta_2$-selective antagonists spirendolol (**26**, A, cyclohexyl ring replaced by two methyl groups) and ICI 118551 (**27**, B and C, methyl group in side chain removed). The isoenergy contours (in kcal/mol) are in planes 2 Å above (A and B) and 2 Å below (C) the plane of the aromatic ring.

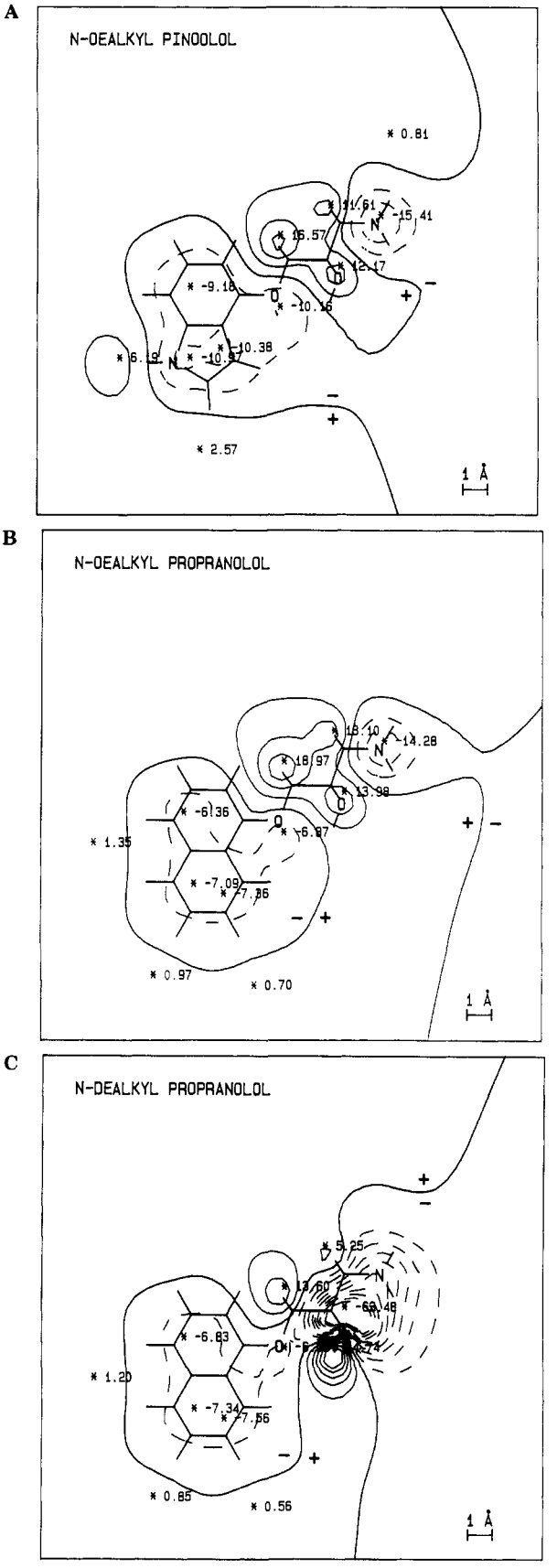
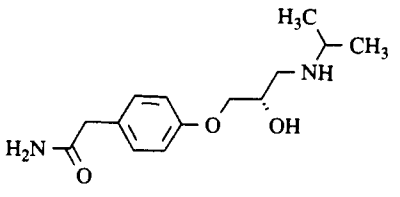



**Figure 5.** MEP (calculated by the STO-3G ab initio method) of the *N*-dealkyl derivatives of the nonselective $\beta$-antagonists pindolol (**10**, A) and propranolol (14, B and C). The isoenergy contours (in kcal/mol) are in planes 2 Å above (A and B) and 2 Å below (C) the plane of the aromatic ring.

**Chart III.** Molecular Structure of Atenolol (**21**), Bisoprolol (**23**), Spirendolol (**26**), ICI 118551 (**27**), Pindolol (**10**), and Propanolol (**14**)



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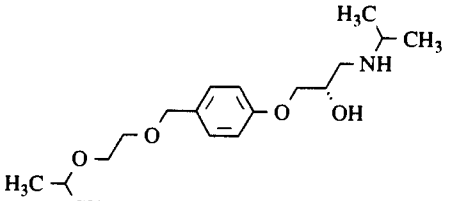

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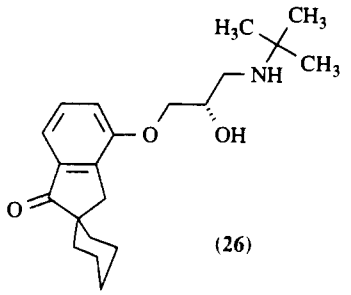

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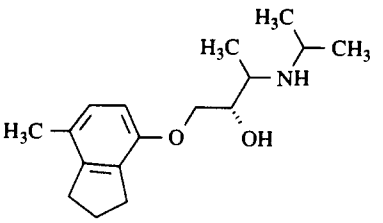

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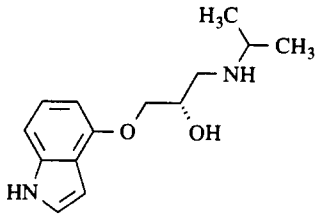

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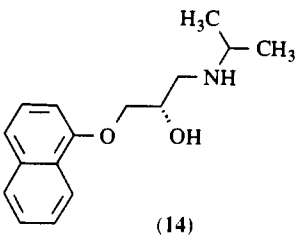

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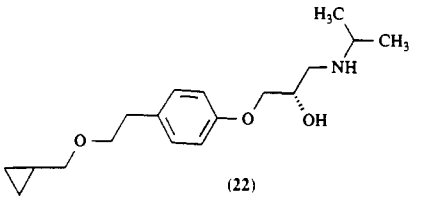
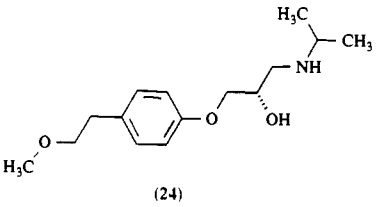
**Chart IV.** Molecular Structure of Betaxolol (**22**), Metoprolol (**24**), Celiprolol (**17**), and H 87/07 (**19**)



(22)



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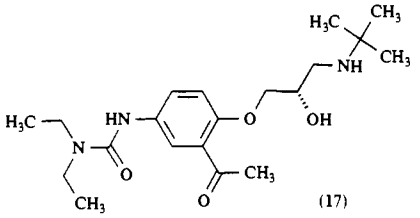

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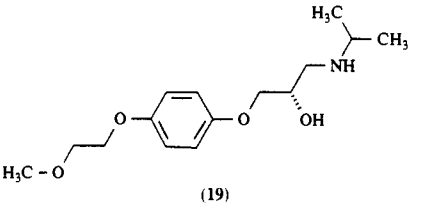

(19)

imum previously designated as M3 and centered 2–3 Å or more away from the meta position and a positive maximum (designated P4) in the para position.

Another interesting feature is observed when comparing $\beta_1$-selective full antagonists (compounds **22** and **24**) with $\beta_1$-selective partial antagonists possessing intrinsic sympathomimetic activity (ISA) (compounds **17** and **19**) (Chart IV). Their MEPs (Figures 6 and 7, respectively) show that the positive potential (P4) observed in the para position of all $\beta_1$-selective antagonists is replaced in the $\beta_1$-selective partial antagonists by a negative zone or minimum designated M4). This negative potential expresses the electronegativity of an heteroatom in the para substituent. On the basis of this observation, it can be suggested that the agonist-like activity of the $\beta_1$-selective partial antagonists is induced by an electrostatic interaction between this zone of negative potential and a complementary area in the $\beta_1$-adrenoceptor. In addition, a change in the para substituent from a negative potential to a positive one, or its displacement in an unfavorable location, would eliminate the ISA while retaining the $\beta_1$ selectivity of these drugs. As a representative example among a number of highly $\beta_1$-selective partial antagonists,[44] Baldwin et al.[41] found that the most effective way of reducing or eliminating the ISA, while retaining the $\beta_1$ selectivity, was the insertion of a methylene group (spacer) between the imidazolyl para substituent and the benzene ring in compound **20**, affording compound **25** (Chart V). In contrast ISA could not be eliminated by replacing the NH moiety with S, O, or $NCH_3$. This also invalidates an older theory suggesting that H-bond donation by the para substituent is responsible for ISA.

To explain the lack of ISA in the spacer-containing drugs, a conformational hypothesis was proposed[41]

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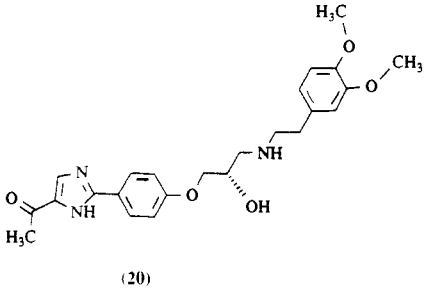
Chart V. Molecular Structure of Compounds (*S*)-38 (**20**) and (*S*)-51 (**25**)



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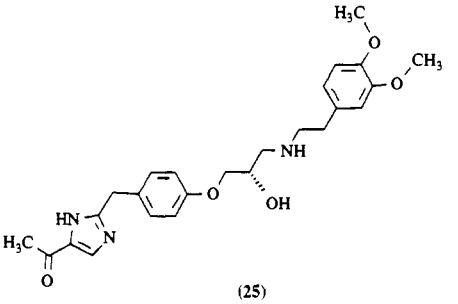


(25)

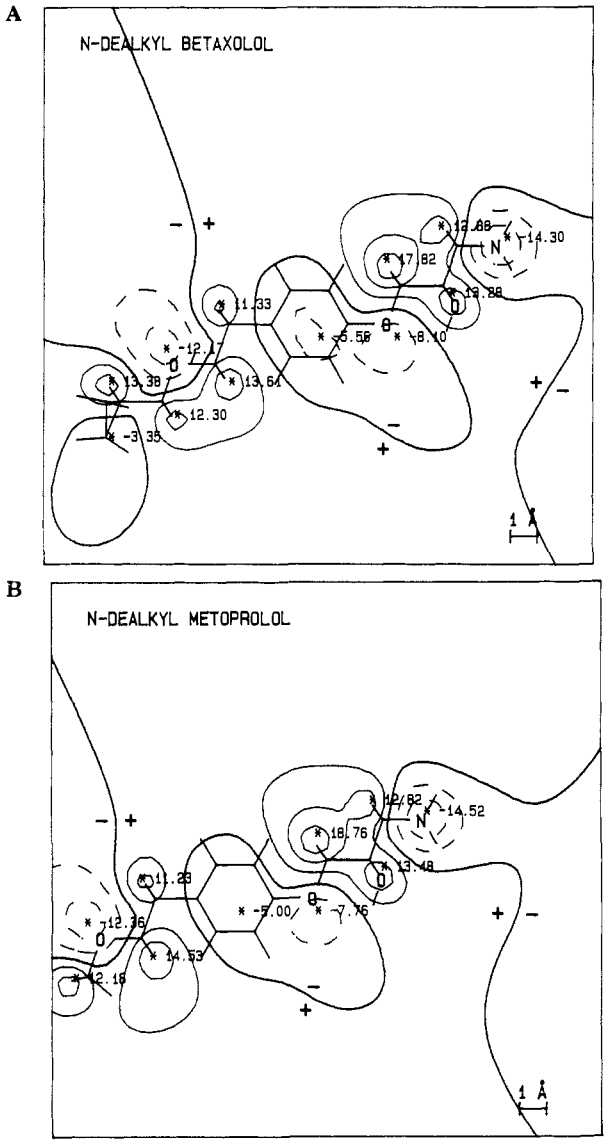



**Figure 6.** MEP (calculated by the STO-3G ab initio method) of the *N*-dealkyl derivatives of the $\beta_1$-selective full antagonists betaxolol (**22**, A) and metoprolol (**24**, B). The isoenergy contours (in kcal/mol) are in a plane 2 Å above the plane of the aromatic ring.

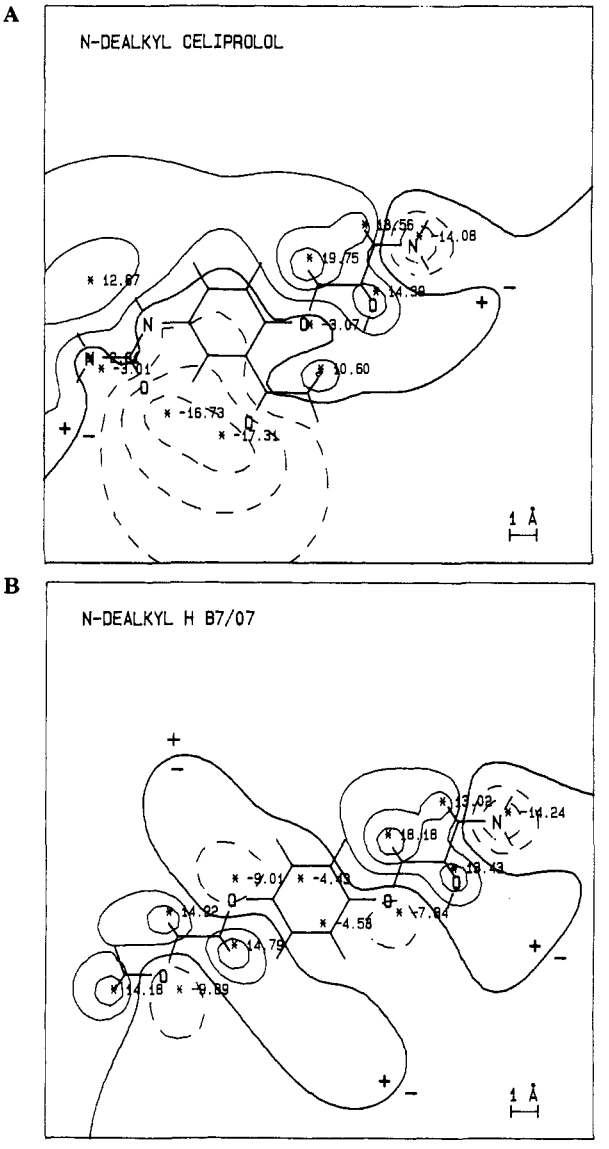



**Figure 7.** MEP (calculated by the STO-3G ab initio method) of the *N*-dealkyl derivatives of the $\beta_1$-selective partial antagonists celiprolol (**17**, A) and H 87/07 (**19**, B). The isoenergy contours (in kcal/mol) are in a plane 2 Å above the plane of the aromatic ring.

whereby the spacer breaks the near coplanarity of the imidazole and benzene rings and decreases their steric interactions. In our opinion, this ad hoc hypothesis remains unproven. A second hypothesis is the suggestion that a different binding mode exists for compounds displaying ISA. But again such idea remains unproven without having X-ray structures of the ligand–receptor complex. An alternative explanation becomes evident when comparing the MEPs of compounds **20** and **25** (Figure 8). Indeed, the negative minimum (M4) observed in the MEP plot of the $\beta_1$-selective partial antagonist **20** (Figure 8A) is in compound **25** (Figure 8B) shifted away from its activating position and replaced by a positive maximum, in full agreement with our hypothesis presented above. In fact, the insertion of an alkylene bridge into the para substituent of other partial antagonists has also been responsible for the development of $\beta_1$-selective antagonists devoid of ISA.[24]

As an approximate rule to be discussed in greater details below, it can be concluded from this Section that in

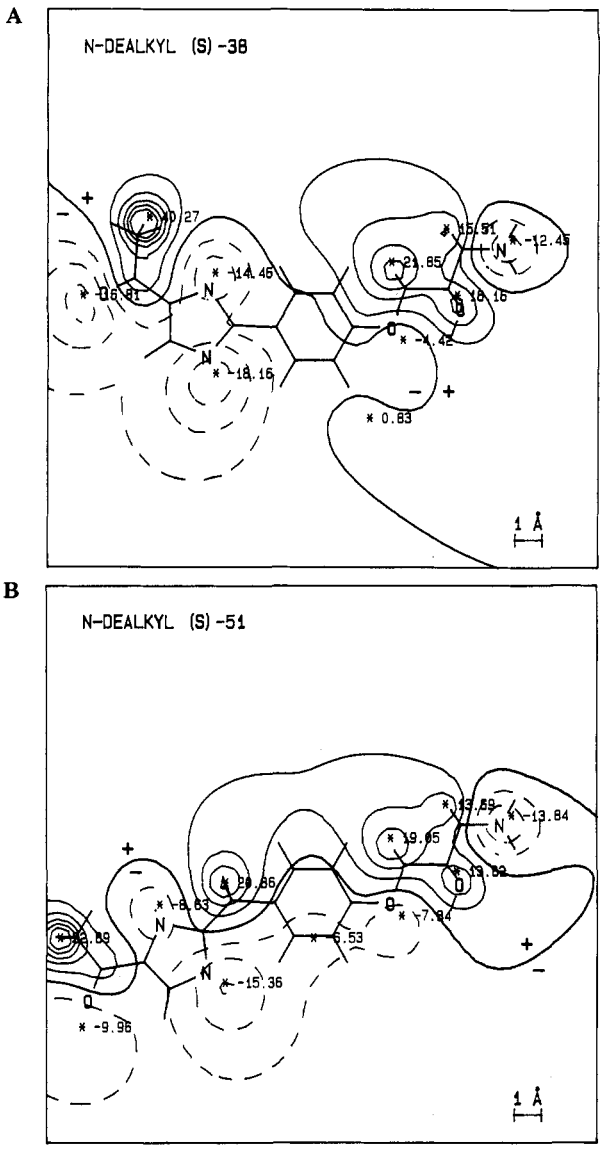
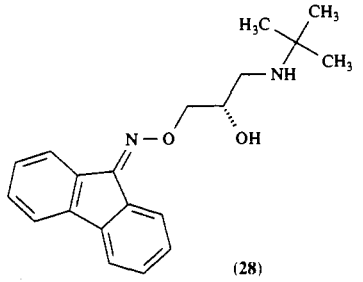



**Figure 8.** MEP (calculated by the STO-3G ab initio method) of the *N*-dealkyl derivatives of the $\beta_1$-selective antagonist (*S*)-38 (**20**, A) and (*S*)-51 (**25**, B) displaying and lacking ISA, respectively. The isoenergy contours (in kcal/mol) are in a plane 2 Å above the plane of the aromatic ring.

**Chart VI.** Molecular Structure of Compound IPS 339 (**28**)



(28)

para-substituted AOPAs $\beta_1$ selectivity arises from the simultaneous presence of a negative minimum M3 (located away from the meta position) and a minimum (M4) or maximum (P4) in the para position. The positive or negative sign of the latter electrostatic feature is postulated to condition the absence or presence, respectively, of an ISA.

**Oxime Ethers.** IPS 339 (compound **28** Chart VI) is a well-known representative of the class of oxime ethers β-blockers. This compound can only exist with an anti configuration around the N–O bond, the syn configuration

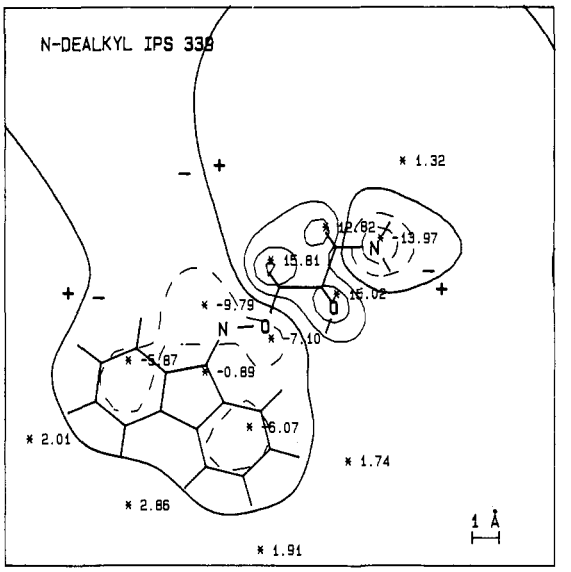



**Figure 9.** MEP (calculated by the STO-3G ab initio method) of the *N*-dealkyl derivative of the oxime ether IPS 339 (**28**). The isoenergy contours (in kcal/mol) are in a plane 2 Å above the plane of the aromatic ring.

being forbidden (results not shown). In the MEP of IPS 339 (Figure 9), a negative minimum corresponding to M2 is found above and below one ring at ca. 8 Å from the basic nitrogen, but additional negative minima are seen. One of them corresponds approximately to M3, while another one expresses the influence of the imino nitrogen lone pair.

Rakhit et al.[11] found oxime ethers to be generally more potent than their aryl ether analogues and explained this difference in terms of steric arguments. Figure 9 shows the complexity of the MEP of IPS 339 and suggests that, compared with PEAs and AOPAs, additional electrostatic interactions may be involved in the recognition and binding of oxime ethers to the β-adrenoceptor. Clearly our results for oxime ethers are preliminary, only one compound having been investigated, and further stereoelectronic studies of this interesting class of drugs are needed. Note in this context that the novel aliphatic 3-(acyloxy)-propranolamines[12] (Chart ID) are also suggested to be interesting objects of stereoelectronic studies. Here, a carbonyl group is expected to generate a marked negative potential corresponding to M2, suggesting an intriguing case of bioisosterism with PEAs, AOPAs, and oxime ethers.

**MEP-Based β-Adrenoceptor Model.** The MEPs of all 32 drugs studied here (Figures 1–9 and supplementary material), when compared at 2 Å above the plane of the aromatic ring and in congruent conformations, show a number of features common to all compounds within pharmacological subgroups. The same features are also found at −2 Å (i.e., on the side of the aliphatic OH group), but they are slightly less distinct in some cases due to the strong negative potential generated by the OH group. These features can be classified in two groups, namely those generated by the side chains (ethanolamino or oxypropanolamino) and those generated by the aromatic region of the molecules. The topographical relationships between these two sets of electronic features depend on the conformation of the side chain. The following rationalizations are based on the assumption that the active conformation is an extended one (see introduction). If other conformations are considered, they modify the topographical relationships between the two sets of electronic features without markedly affecting each separate set.

The electrostatic potential of the side chains is essentially influenced by the heteroatoms (i.e. the amino group,

the hydroxyl group, and the ether oxygen). These features are well-known and did not constitute the object of our study. In the aromatic region, a negative minimum is found in all compounds investigated and is designated as M1 in PEAs and M2 in AOPAs and oximes. In the conformation chosen, both M1 and M2 are located ca. 7.5–8.5 Å away from the basic nitrogen and the vector connecting them is always identically oriented. The M1 and M2 minima are thus topographically equivalent. We postulate that this negative minimum is an essential feature for recognition and binding to the β-adrenergic receptors.

$\beta_1$-Selective full and partial antagonists also display a negative minimum designated M3 and located in the meta position or somewhat beyond, at 1.5–2.5 Å from M2. This minimum is found not only in compounds with recognized $\beta_1$ selectivity but also in some but not all nonselective and $\beta_2$-selective antagonists. M3 alone thus cannot account for $\beta_1$ selectivity.

An additional pharmacophoric element is seen in the MEP of the $\beta_1$-selective antagonists, namely a negative minimum (M4) or a positive maximum (P4) located in the para position, at a distance of ca. 2 Å and 1.5–2 Å from M2 and M3, respectively. The P4 maximum characterizes the full antagonists; in contrast, the M4 minimum is found in the partial antagonists, i.e. those displaying ISA. Our model thus involves three pharmacophoric points required for affinity to β-adrenoceptors, namely N, OH and M1 or M2. Three other points, M3, M4, and P4, define $\beta_1/\beta_2$ selectivity. We postulate that $\beta_1$ selectivity results from the simultaneous presence of M3 and either M4 or P4. Compound 6f (**5**) is a $\beta_1$-selective full agonist, generating both M3 and M4, thus suggesting that this hypothesis might also hold for agonists.

As for the nonselective and $\beta_2$-selective antagonists, their MEPs do not reveal elements characteristic of these classes. Simple inspection of their fomulae however shows in most cases the presence of a second ring of marked lipophilicity fused to the aromatic ring (see below).

The pharmacophoric elements seen in the MEPs of the 32 compounds investigated here are summarized in a schematic way in Figure 10 where the various chemical and pharmacological classes of β-adrenoceptors antagonists are considered separately. A general pharmacophore for β-adrenoceptor antagonists is proposed in Figure 11.

**Lipophilicity and β-Andrenoceptor Selectivity.** The limitations of the above pharmacophoric models must be stressed, since they are based on electrostatic maxima and minima, i.e. features responsible for electrostatic components of drug–receptor interactions. Hydrophobic forces and van der Waals interactions are neglected in such an analysis, yet there is evidence that the pharmacological profile of β-adrenoceptors antagonists is also influenced by their lipophilic character.[45-47] Thus, the β-adrenoceptor subtypes selectivity of agonists and antagonists is partly influenced in vivo by selective tissue distribution,[48] a markedly lipophilicity-dependent phenomenon. Furthermore, we have demonstrated in a recent study that hydrophobic interactions play an important role in the β-adrenoceptor binding of nonselective and $\beta_2$-selective antagonists.[49] A good linear relationship has also been

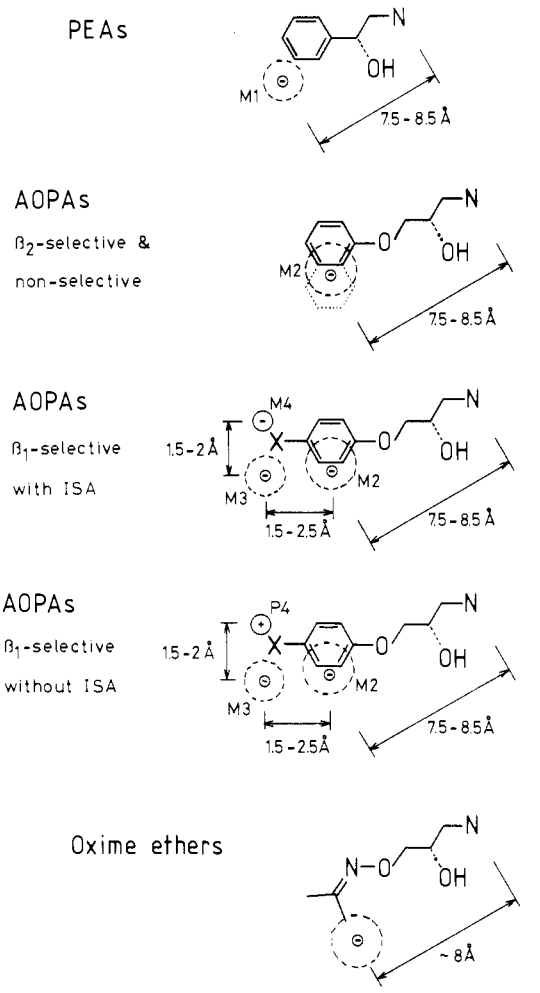



**Figure 10.** MEP-based pharmacophoric elements of various chemical and pharmacological classes of β-adrenoceptor ligands. The MEP of some but not all $\beta_2$-selective and nonselective AOPAs also displays M3, and the second ring present in most of these compounds is associated with marked lipophilicity. The MEP-based model of oxime ethers is based on one compound only and is merely indicative.

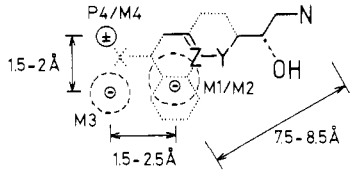



**Figure 11.** A general pharmacophoric model of β-adrenoceptor ligands derived from their MEPs. This model takes into consideration neither the lipophilic zone(s) believed to increase $\beta_2$ affinity nor the influence of N-substituents.

observed between lipophilicity and β-adrenoceptor selectivity of agonists and antagonists such that an increase in lipophilicity is associated with an increase in $\beta_2$-selectivity.[50] These findings may or may not be related to two structural features of β-adrenoceptor antagonists: (a) The simultaneous existence in the MEPs of $\beta_1$-selective antagonists only of two regions of marked potential (M3 and M4/P4) which add to the polarity and hence hydrophilicity of the compounds, and which may result in specific electrostatic interactions with the receptor. (b) The marked lipophilicity of the second ring and its substituents, which in nonselective and especially $\beta_2$-selective antagonists more

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than compensates the polarity of the M3 minimum.

Thus, if we postulate that $\beta_2$ affinity and selectivity are based on specific hydrophobic interactions, a validation of this hypothesis cannot be found in MEP patterns. Clearly a variety of approaches are necessary for a relevant pharmacophore to be obtained.

## Experimental Section

**Molecular Electrostatic Computations.** In order to reduce computing times the calculations were carried out on the primary amine analogues of all compounds. Although adrenoceptor ligands bind and act as in their protonated form, the MEP in thus study were calculated for the neutral molecules. This renders the MEP details much more visible while not altering the difference in electrostatic potential between local minima and maxima.[20]

Standard bond lengths and angles were used as input for the calculations. Wave functions and electronic densities were calculated with the ab initio Monstergauss 81 program as reported previously[20] and with the Gaussian 82 program,[51] using a minimal STO-3G basis set. MEPs were obtained with a slightly modified version of DENPOT (QCPE 360) and were generated in planes parallel to the aromatic ring, viewing compounds from above and below the aromatic ring plane for each compound. All calculations were performed on the CDC CYBER 170/855 and CRAY 1S computers of the Federal Institute of Technology in Lausanne and a Norsk Data ND 560 computer of the University of Lausanne.

(51) Pople, J. A.; Binkley, J. S.; Frisch, M. J.; DeFrees, D. J.; Raghavachari, K.; Whiteside, R. A.; Schelgel, H. B.; Fluder, E. M., University of Carnegie-Mellon, 1985.

**Acknowledgment.** We are indebted to the Swiss National Science Foundation for Grant 3.508-0.86.

**Registry No.** 1, 51-41-2; 2, 34391-04-3; 3, 20879-16-7; 4, 30236-31-8; 5, 116127-09-4; 6, 23846-71-1; 7, 59995-59-4; 8, 81102-77-4; 9, 26328-12-1; 10, 26328-11-0; 11, 47141-42-4; 12, 38104-34-6; 13, 42200-33-9; 14, 4199-09-1; 15, 116127-10-7; 16, 68107-82-4; 17, 95586-73-5; 18, 37936-65-5; 19, 91324-92-4; 20, 102151-02-0; 21, 93379-54-5; 22, 93221-48-8; 23, 99103-03-4; 24, 81024-42-2; 25, 102152-32-9; 26, 116076-61-0; 27, 72795-23-4; 28, 60979-28-4; 29, 54340-62-4; 30, 39832-48-9; 31, 26839-75-8; 32, 76596-57-1.

**Supplementary Material Available:** Plots of the MEP of compounds 1, 3, 5-9, 11-13, 15, 16, 18, 29-32 in a plane parallel to, and 2 Å above, the plane of the aromatic ring (9 pages). Ordering information is given on any current masthead page.

# Radioiodinated Benzodiazepines: Agents for Mapping Glial Tumors

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*Received March 3, 1988*

Two isomeric iodinated analogues of the peripheral benzodiazepine binding site (PBS) ligand Ro5-4864 have been synthesized and labeled in high specific activity with iodine-125. Competitive binding assays conducted with the unlabeled analogues indicate high affinity for PBS. Tissue biodistribution studies in rats with these $^{125}$I-labeled ligands indicate high uptake of radioactivity in the adrenals, heart, and kidney—tissues known to have high concentrations of PBS. Preadministration of the potent PBS antagonist PK 11195 blocked in vivo uptake in adrenal tissue by over 75%, but to a lesser degree in other normal tissues. In vivo binding autoradiography in brain conducted in $C_6$ glioma bearing rats showed dense, PBS-mediated accumulation of radioactivity in the tumor. Ligand **6** labeled with $^{123}$I may have potential for scintigraphic localization of intracranial glioma.

Since their discovery in the late 1950s, the 1,4-benzodiazepines have constituted a class of widely used anxiolytic and anticonvulsant drugs.[1] In the last several years, the presence of two pharmacologically distinct subclasses of benzodiazepine binding sites have been demonstrated. One class, the central benzodiazepine receptor, is localized in central neuronal tissue and represents the site at which benzodiazepine ligands exert their anxiolytic effects.[2,3] In addition, binding sites for benzodiazepines have been identified in peripheral tissues including the adrenal cortex, nasal epithelium, kidney, and heart; these sites were subsequently termed the peripheral benzodiazepine binding sites (PBS).[4,5] It has also been shown, however, that these sites though present mainly in the periphery are also located in the olfactory bulb as well as glial cells in brain.[6,7] The pharmacological distinction between these two types of binding sites can be demonstrated by the use of highly selective ligands. The clinically potent anticonvulsant clonazepam binds with nanomolar affinity to the central benzodiazepine receptor but with less than micromolar affinity to PBS.[8] Conversely Ro5-4864, which exhibits no anxiolytic activity, has nanomolar affinity for the peripheral binding site but a 1000-fold lower affinity

Table I. Radiosynthetic Data

| ligand | isolated radiochemical yield, % | sp. act., Ci/mmol | radiochemical purity, % |
|---|---|---|---|
| 5 | 18-31 ($N$ = 3) | 117-128 ($N$ = 3) | >98 |
| 6 | 21-43 ($N$ = 6) | 103-145 ($N$ = 3) | >98 |

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