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ACCELERATED COMMUNICATION

Cardiotonic Agents. 3. A Topographical Model of the Cardiac cAMP Phosphodiesterase Receptor

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SUMMARY

Based on the pharmacophoric relationship heterocycle-phenyl-imidazole (H-P-I) and upon consideration of several potent inhibitors of cardiac cAMP phosphodiesterase, a topographical model of this receptor is proposed. The model consists of two binding sites which interact with H, two steric features, preferential rotation of P away from coplanarity with H, and a binding site for an electron-rich system (I). It is supported by molecular modeling studies and accommodates a variety of inhibitors. It also encompasses the active site of the enzyme and can distinguish cAMP from cGMP as substrates.

In pursuit of cardiotonic drugs (1) which act through selective (2), competitive (3) inhibition of cardiac cAMP PDE III,[1] we became interested in the structural details of this receptor. Based upon the SARs for the pyridazinone series **1**, it recently has been suggested (6) that this receptor has a "generally flat topography" which contains binding sites for a "dipolar moiety," an "adjacent acidic proton," and a "hydrogen bonding region." In addition, because **1b** exhibits greater inhibitory potency than either **1a** (imazodan) or **1c** (7), a "small lipophilic space" was proposed to reside above the general plane and opposite the site for the acidic proton (Scheme 1). It is apparent, however, that this model cannot explain SARs for the inhibitors represented by **2** where increased potency is similarly observed for **2b** (milrinone) compared to **2a** and **2c** (8), even though the alkyl group *R* now resides on the wrong side for interaction with the purported lipophilic space.

Alternatively, we have proposed[2] that the small alkyl groups influence the conformation between rings I and II (Fig. 1) such that their importance at either *R* or *R'* is to rotate the rings away from coplanarity. It follows that, in our model, the preferred pharmacophoric relationship reflects a nonplanar receptor topography. This possibility has also been implied by Wells *et al.* (9) after considering xanthines and, more recently, by Leclerc *et al.* (10, 11), who considered quinolone derivatives. The latter suggest that the pharmacophoric relationship for rings I and II in **1a** is about 12° away from coplanarity (12).

We have reported (13, 14) that certain heterocycle-phenyl-imidazoles (H-P-Is) exhibit inotropic activity through inhibition of PDE. One compound, **3**, is an extremely potent inhibitor (14) and represents an ideal ligand to model this receptor. We now describe theoretical and molecular modeling studies which have led to a topographical model of the cardiac PDE III receptor and its active site.

Experimental Procedures

Theoretical studies. Calculations were preformed on fragments **4** and **5** as models for compound **3** (Scheme 2). The semiempirical MNDO and AM1 methods of Dewar and co-workers (15, 16) were implemented in a version of MOPAC[3] modified by J. McKelvey,[4] and Version 1.00 of AMPAC,[5] respectively. Except as noted in the text, the atoms of, and bonded to, the imidazolone ring were constrained to coplanarity while all other geometric parameters were relaxed. *Ab initio* Hartree-Fock calculations via GAUSSIAN 82[6] programs with the STO-3G (17) basis set were performed for the optimized geometries of the

[1] Also referred to as low K_m or high affinity cAMP PDE, FIII PDE (4), or type IV PDE (5).

[2] Presented in part by P. W. Erhardt at the IXth International Symposium on Medicinal Chemistry, September 1986, in West Berlin, FRG. Abstract number 111.

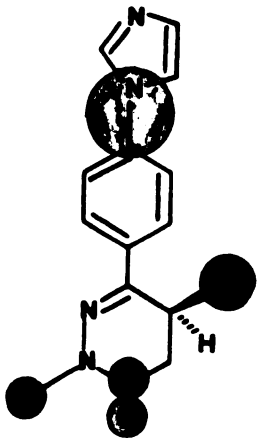
[3] Dewar Group, University of Texas at Austin.

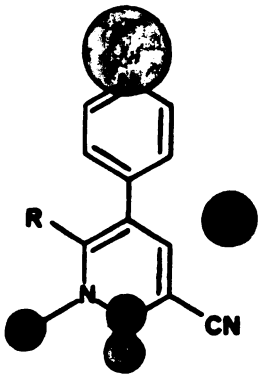
[4] Personal communications with J. McKelvey, Eastman Kodak, Rochester, NY.

[5] Quantum Chemistry Program Exchange, Program 506, Indiana University, Chemistry Department.

[6] J. S. Binkley, M. J. Frisch, D. J. DeFrees, K. Raghavachari, R. A. Whiteside, H. B. Schlegel, E. M. Fluder, and J. A. Pople, Carnegie-Mellon University.

ABBREVIATIONS: PDE, phosphodiesterase; SAR, structure-activity relationship; MNDO, modified neglect of diatomic overlap; STO-3G, Slater-type atomic orbitals represented by expansions in terms of 3 gaussian functions; AM1, Austin model 1.





1a R' = H
b = CH_3
c = CH_2CH_3

2a R = H
b = CH_3
c = CH_2CH_3
d = $CH_2CH_2CH_3$

Scheme 1

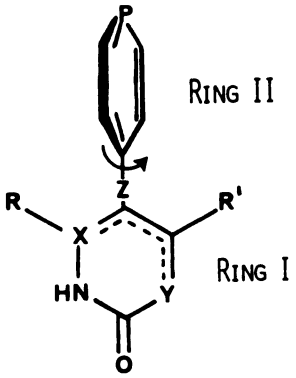
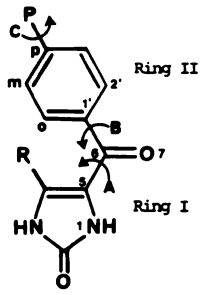


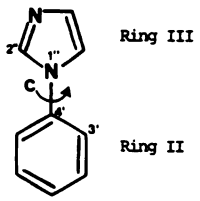
Fig. 1. Nonplanar conformation resulting from the presence of alkyl groups at *R* or *R'*. *X*, *Y*, *Z*, *P*, and unsaturation can be adjusted to accommodate structures such as 1–3 and many of the other new cardiotonic drugs thought to inhibit cAMP PDE.

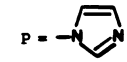
semiempirical schemes. The notation *A*/ /*B* designates an energy calculation with method A using the structure obtained from method B.

Molecular modeling. Matching studies were performed as follows. Structures **7–9** were entered into CHEMLAB-II[7] and their geometries were optimized by molecular mechanics. Compounds **7** and **8** were matched with the indicated (**a*) atoms essentially overlapped. The ethyl group in **7** was then rotated at 45° intervals through 360° while the methyl carbon atom in **8** was allowed to search for potential overlap (**b*) at each interval via appropriate bond rotations. The methyl carbons of structures **8** and **9** were similarly matched with rotations at 45° intervals of the ethyl group of **9**. The energies of the conformations resulting from successful matches (average deviations ≤ 0.5 Å) were again calculated using molecular mechanics. In all cases these energies

[7] Revision 8.0; Molecular Design Limited, San Leandro, CA.





3 P =  (Ring III)
R = Et

4 P = H
R = Et

6 P = SCH_3
R = see Table 3

5

Scheme 2

were found to compare favorably with the initial energies (differences <1 kcal/mol).

Results

Theoretical studies. At the MNDO/ /MNDO level, the lowest energy conformation of **4** (Table 1, row [1]) has both the imidazolone (I) and phenyl (II) groups rotated ~60° away from the carbonyl group. Forcing the imidazolone and ketone portions to lie in one plane during optimization results in a conformation (Table 1, row [2]) that is 1.5 kcal/mol less stable than the lowest energy conformation and causes the phenyl ring to become nearly perpendicular to the plane of the imidazolone ring. When the benzoyl portion is constrained planar (Table 1, row [3]), the imidazolone ring is nearly perpendicular to that plane and unavailable for hydrogen bonding such that the energy is 2.2 kcal/mol higher than that of the lowest energy conformation. The STO-3G calculations using these structures (STO-3G/ /MNDO) suggest that **4** prefers the imidazolone and ketone portion to be coplanar, allowing a hydrogen bond length of 2.58 Å. Such stabilization due to hydrogen bonding at this level of calculation is expected. Within the AM1 scheme, the lowest energy conformation has rings I and II rotated 29° and 37° away from the carbonyl group, and the energies for the relaxed and constrained conformations (Table 1, rows [1] to [3]) span 0.9 kcal/mol. At the STO-3G/ /AM1 level, the planar-benzoyl conformation is the most stable of the AM1 structures (~1.4 kcal/mol lower in energy than the one in which the ketone is in the plane of ring 1). Again, it can be noted that rings I and II are still significantly rotated from coplanarity. Most important, with all methods, the fully conjugated conformation (Table 1, row [4]), where rings I and II would be coplanar, is predicted to be unstable by a range of 9.2–19.8 kcal/mol. While all calculations performed on fragment **5** (Table 2) suggest that the imidazole ring (III) prefers to rotate away from ring II, the energy barrier to its rotation tends to be small, such that a generalized statement about the conformational relationship between rings II and III is not appropriate.

The theoretical calculations are in line with published (14) X-ray and NMR data for **3**. In the crystalline state, angles *A*,

TABLE 1
Theoretical study of *4*

Optimization constraints	MNDO optimized geometries				AM1 optimized geometries			
	Dihedral angles[a]		Relative energy		Dihedral angles		Relative energy	
	A	*B*	MNDO/ /MNDO	STO-3G/ /MNDO	*A*	*B*	AM1/ /AM1	STO-3G/ /AM1
	degrees		*kcal/mol*		*degrees*		*kcal/mol*	
[1]	−57	−63	0.0	2.9	−29	−37	0.0	0.3
[2] A = 0°	0	−88	1.5	0.0	0	−87	0.3[b]	1.4[b]
[3] B = 0°	−90	0	2.2	1.4	−130	0	0.9	0.0
[4] A = B = 0°	0	0	19.8	8.6	0	0	11.4	9.2

[a] $A = \sphericalangle N_1C_5C_6O_7$ and $B = \sphericalangle C_2'C_1'C_6O_7$.
[b] In this optimization, the value of the dihedral angle that the ethyl group makes with C_4C_6 is fixed at −135° (i.e., the result of the AM1 optimizations [1] and [3]); without this additional constraint, the value of this dihedral angle is 180° and the AM1/ /AM1 and STO-3G/ /AM1 relative energies are 0.0 and 2.6 kcal/mol, respectively.

TABLE 2
Theoretical study of *5*

Method of calculation	Minimum energy dihedral angle *C*[a]	Barrier to rotation *C* = 0°	Barrier to rotation *C* = 90°
	degrees	*kcal/mol*	
MNDO/ /MNDO	90	3.8	0.0
AM1/ /AM1	26	0.3	1.6
STO-3G/ /MNDO[b]	35	0.9	2.3
STO-3G/ /AM1[b]	35	1.0	2.4

[a] $C = \sphericalangle C_2''N_1''C_4'C_3'$.
[b] The STO-3G values were derived from a least squares fit of the results of several optimizations, where *C* is constrained at selected values, to a cosine Fourier expansion truncated to three terms.

TABLE 3
SARs for alkyl substituents *R* or *R'*

Compound	*R* or *R'*	Relative potency (*dp/dt*) *in vivo*, canine[a]
1a	H	0.3
1b	CH_3	1.0[b]
1c	CH_2CH_3	0.01
2a	H	0.01
2b	CH_3	1.0[c]
2c	CH_2CH_3	0.3
2d	$CH_2CH_2CH_3$	0.05
6a	H	0.1
6b[d]	CH_3	0.6
6c	CH_2CH_3	1.0[e]
6d	$CH_2CH_2CH_3$	0.06

[a] As determined from data contained in Refs. 7, 8, and 18. Tests in our laboratories have shown that the same trends in potency are observed for cAMP PDE inhibition.
[b] Most potent compound in series **1**.
[c] Most potent compound in series **2**.
[d] Enoximone.
[e] Most potent compound in series **6**.

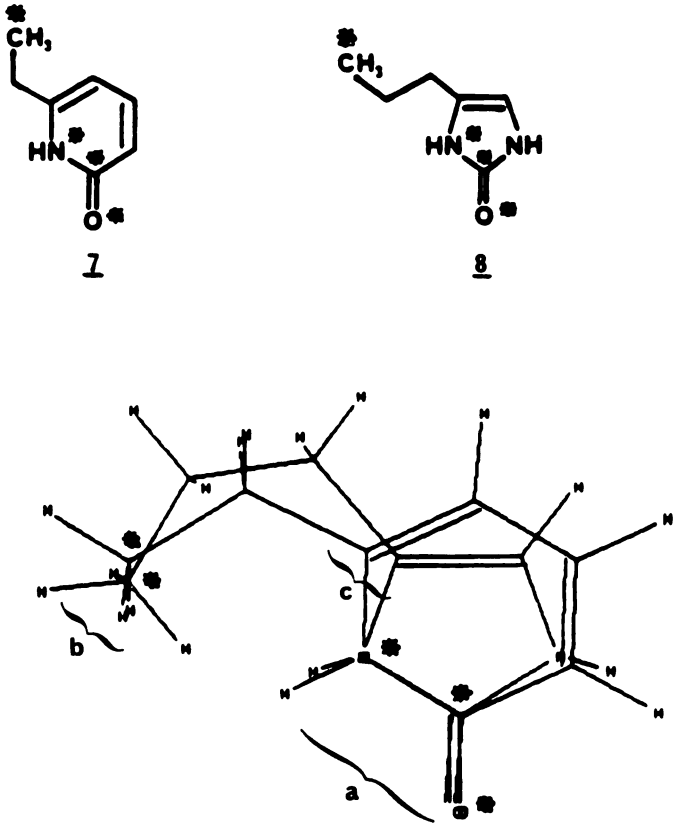


Fig. 2. Steric hypothesis and probe of receptor surface on the same side as the acidic proton. Matching of model structures **7** with **8** (atoms matched indicated by •) is shown. Average deviations for matched atoms were: 0.03 Å (*a*) and 0.33 Å (*b*). The distance of the match point (*b*) from the plane of heterocycles is 0.11 Å. Also note the closeness of electron density at region *c*.

B, and *C* were previously found to be −20°, −33°, and 31°, respectively, which agrees most closely with the AM1/ /AM1 calculations. For solutions of **3** and **4** in deuterated dimethyl sulfoxide, the NMR chemical shifts of the ring II ortho and meta/para protons were within 0.06 ppm, suggesting that ring II is rotated from, and is not conjugated with, the carbonyl moiety since conjugation would tend to separate these resonances (e.g., 0.5 ppm separations are observed for acetophenone). It should be noted that, although the magnitude of rotation for ring II can be estimated to be in the range 15–45°, the sense of rotation (clockwise or counterclockwise) relative to the plane established by ring I cannot be specified from any of these studies.

Molecular modeling studies. The SARs associated with the alkyl group *R* in **1** and **2**, and for analogous substitution in **3** and the related imidazolones **6** (18), provide additional insight about receptor topography. As shown in Table 3, although a small *R* group such as methyl in **1** and **2** or ethyl in **6** improves potency, activity then decreases sharply when the alkyl is further lengthened by one carbon in each of these systems. It seems reasonable that these abrupt decreases in potency result from collision with common steric boundaries on the receptor surface. The simplest model for such interactions would be for the terminal methyl group present in **1c** and the terminal methyl of **6d** to collide with a similar boundary located on the same side as the acidic proton and, because it is

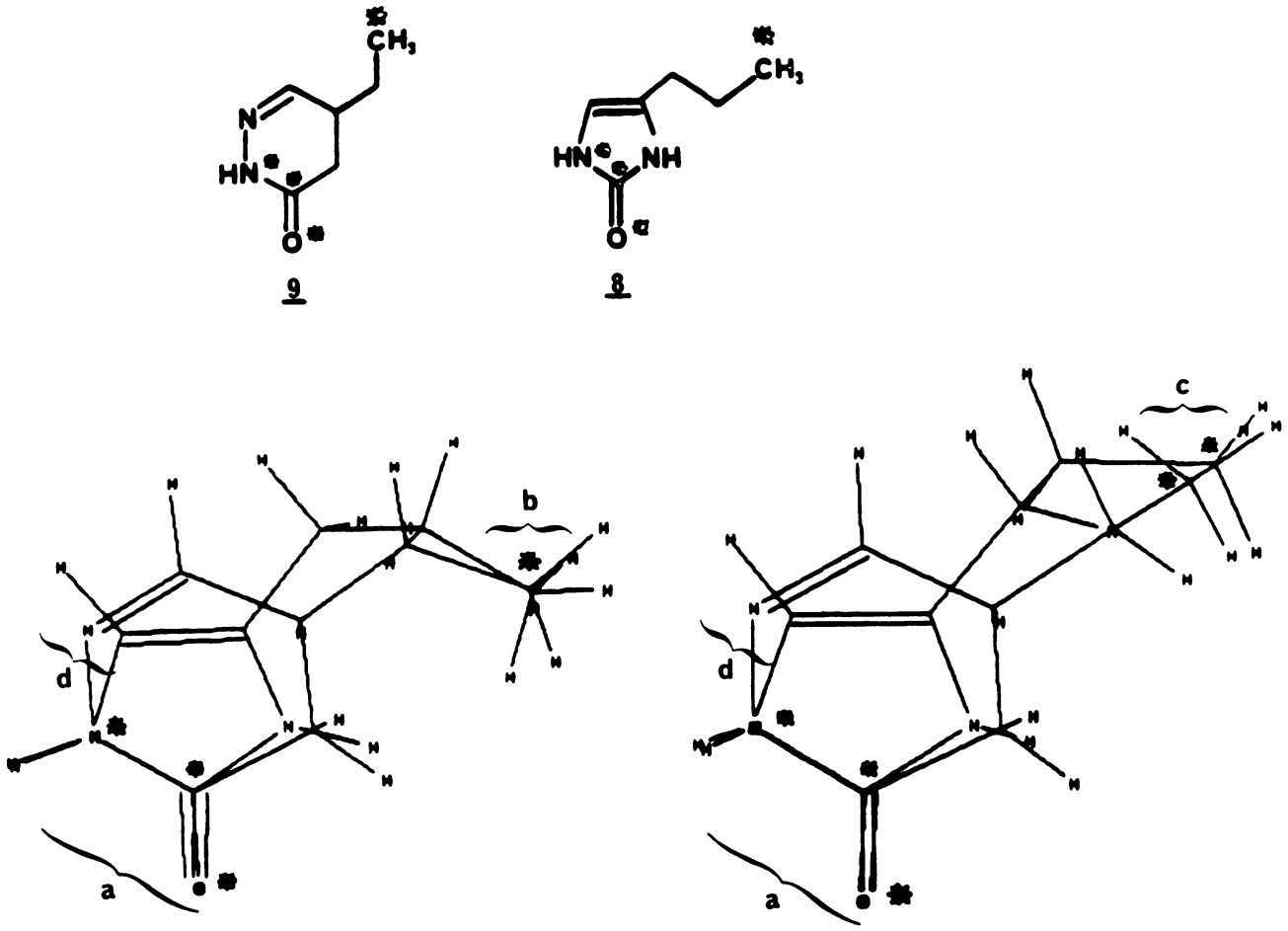


Fig. 3. Steric hypothesis and probe of receptor surface on the side opposite to the acidic proton. Two matches of model structures **8** with **9** were found (atoms matched indicated by *). Average deviations for matched atoms are: 0.03 Å (a), 0.35 Å (*b*), and 0.48 Å (*c*). Distances of match points *b* and *c* from the plane of heterocycles are 0.67 Å and 1.57 Å, respectively. Also note closeness of electron density at region *d*.

possible for the imidazolone ring in **3** or **6** to offer either NH to the receptor, for the terminal methyl in **6d** and the terminal methyl of **2c** to collide with a second boundary located on the side opposite to the acidic proton. As a test of this proposal, and to define the three-dimensional space potentially occupied by such protrusions, pairs of model compounds **7** and **8**, and **9** and **8** were compared by molecular modeling techniques. The results from these comparisons are shown in Figs. 2 and 3. First, since significant overlaps were found, these studies support the notion that at least two steric protrusions may be present at this receptor and that they act to diminish the inhibitory potency observed for structures such as **1–6**. Second, the distance between the matched methyl carbons and the carbonyl carbons is approximately 4.0 Å for **7** with **8**, and approximately 4.6 and 5.0 Å for the two matches of **9** with **8**. The distances of these match points from the plane of the heterocyclic ring were approximately 0.11 Å, 0.67 Å, and 1.57 Å, respectively. Thus, the relative three-dimensional relationships between the two purported steric protrusions and the heterocyclic ring I of **1**, **2**, and **3** have been defined. While the actual size of the protrusions cannot be determined, it is apparent that no protrusions can extend closer to the acidic proton-binding site unless they then form a pocket which interacts favorably with appropriate alkyl ligands (e.g., with **1b**, **2b**, and **6c**). Since the alkyl overlaps were found to occur near the plane established by ring I, it follows that ligands which are fully unsaturated in ring I, and thus rigidly hold their substituents in this plane, should experience greater constraint than their saturated analogs when they possess steric bulk at *R* or *R'*. Previously reported (7, 19) SARs support the latter possibility in that this type of unfavorable interaction appears to negate the potentially beneficial conformational effect resulting from such substitution in fully unsaturated versions of **1** (e.g., various pyridazinone derivatives). Finally, there is a similar electron-rich center in each of the molecules as shown in Figs. 2 and 3. Based on this observation and on results from our laboratory, where fully saturated versions of ring I (e.g., pyrrolidinones) were found to be inactive as cardiac PDE inhibitors,[8] we propose that this electronic similarity also reflects an important receptor-binding region for ring I.

While the steric protrusions are something to be avoided, they could extend toward the region of ring I and in that location form a pocket which binds appropriate alkyl groups at *R* or *R'* in a manner similar to that originally proposed by Bristol *et al.* (6). Similarly, they could extend toward the region of ring II and in that location form the pocket which binds ring II. In this regard, the SARs for compounds related to **3**, in which ring II has been replaced by alkyl chains of varying lengths connected to a terminal imidazole, suggest that the

[8] Details concerning the chemical synthesis and biological evaluation of these compounds are in preparation for publication elsewhere.

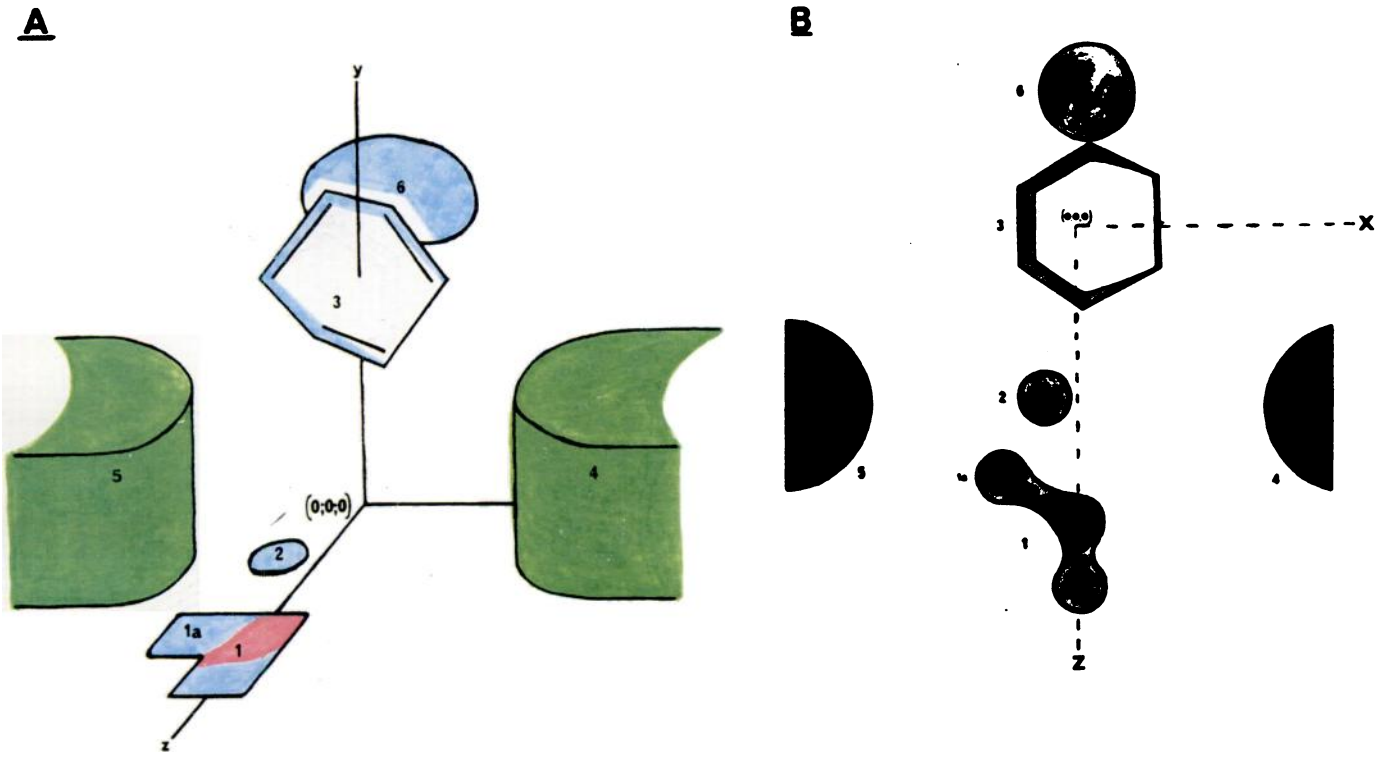


Fig. 4. Topographical model of the cardiac cAMP PDE receptor shown in three-dimensional (A) and overhead (B) views. Features (whose centers have the indicated *x*, *y*, *z* coordinates in Å) include: **1** (0, 0, 5.4 for center of negative region), dipole-binding site; **1a** (−1.4, 0, 4.6), location of other heteroatom of the resonance hybrid; **2** (−0.6, 0, 3.2), binding site for electron-rich center present in antagonist molecules; **3** (−1.0, ~2.0–3.0, 0), binding site for aromatic ring (note that a benzene ring has been used to depict this site in order to show the ~15–20° angle of the π-electron cloud that is thought to be optimal for its interaction with ligands); **4** (4.0, 0.7–1.6, 3.2), steric protrusion; **5** (−4.4, 0.1, 3.2), steric protrusion; **6** (0, ~0.5–1.5, *ca.* −2.5 to −4.0), binding site for electron-rich system such as imidazole. The rigid representations of this model are not meant to imply that a more dynamic relationship, such as mutual molding, is not also operative during drug-receptor interaction.

presence of an aryl ring, or possibly just unsaturation, may be requisite since the alkyl chain compounds are devoid of PDE-inhibitory activity (see Footnote 8).

The final binding site necessary to accommodate the pharmacophoric relationship H-P-I is that which interacts with the imidazole ring (III) or some related electron-rich functionality. Since the quaternized imidazolium analogue of **3** is completely inactive (14), ring III is probably unprotonated when interacting with this site. The X-ray structure for **3** shows that, relative to the phenyl ring, the imidazole group is rotated back toward the plane of ring I (14). A similar preferred conformation between rings II and III was obtained from theoretical studies on compound **5**, although barriers to rotation were found to be small. The analog having a methylene group between rings II and III shows reasonable activity (14), suggesting that this final site is less restrictive in its spatial accommodation of an electron-rich pharmacophore. Alternatively, substitution with small alkyl groups on the phenyl ring ortho to the II-III bond or at any position on the imidazole ring tends to decrease potency (14) such that the binding site in this region may, indeed, be sensitive to steric or spatial constraints.

The topographical model. Assembly of the receptor sites affords the topographical model depicted in three-dimensional fashion and as an overhead view in Fig. 4. The Cartesian coordinates are specified in Å. The overhead view is obtained by looking directly down the *y* axis toward the *xz* plane. Fig. 5 depicts the interaction of molecules **1b**, **2b**, and **6c** with the receptor model (overhead view). Alternatively, it is apparent that for each of these cases, adding one more methyl group to the alkyl substituent (to give the less active compounds **1c**, **2c**, and **6d**) would present steric problems with protrusion site 4 or 5.

Discussion

It is important at this point to challenge the receptor model with structurally varied PDE inhibitors which might not, at first glance, be accommodated. A tabulation of the newer cardiac cAMP PDE inhibitors has appeared recently (1). Inspection of these structures reveals striking similarities in that most conform to the general structure depicted in Fig. 1. Particularly important is that both an acidic proton and a dipole moiety are in the specific relationship required by this recognition site on the receptor model and that most of the structures possess an electron-rich region that can interact with site 2. Such compounds are reasonable candidates for interaction with the receptor model in a manner analogous to that depicted for **1**, **2**, and **6**. The most challenging of these structures would be compounds in which the rings analogous to I and II are connected in bicyclic or tricyclic arrangement and, therefore, could be constrained in a relationship that may not be compatible with the model. As a representative of this type of PDE inhibitor, compound **10** (OPC-8212) (20) was constructed (Dreiding model) and placed on an overhead view of the receptor (1 cm = 0.4 Å). This result is recorded in Fig. 6 and shows a reasonably good fit for **10**. Ring II resides at an angle approximately 20° from the plane established by the ring I dipole and electron-

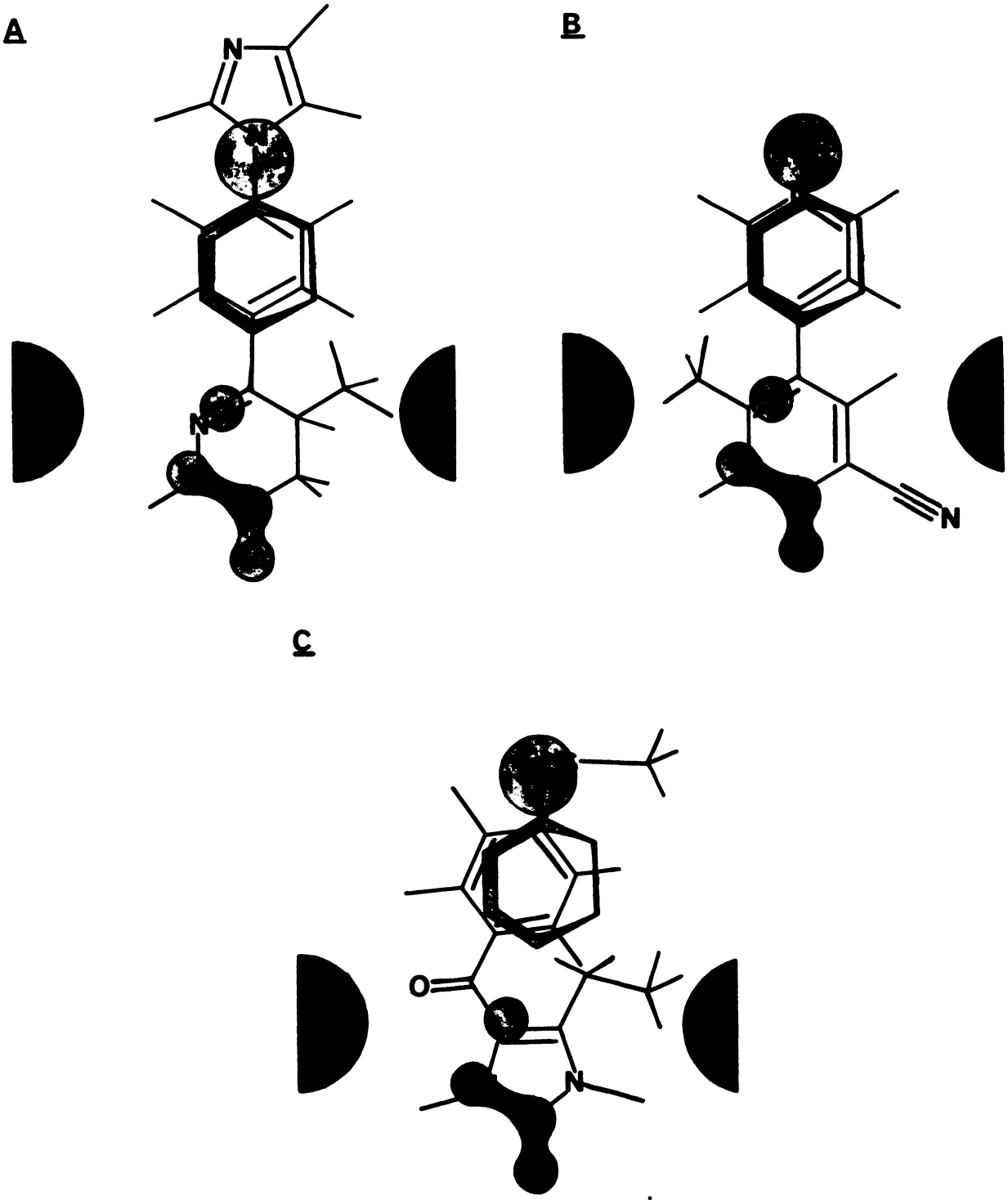


Fig. 5. Pictures showing the favorable interaction between Dreiding structures (1 cm = 0.4 Å) of **1b** (A), **2b** (B), and **6c** (C) on the overhead view of the receptor model. Note that interaction with site 3 (and perhaps site 6) actually occurs from the top side of the Dreiding structure rather than from below, as for sites 1 and 2 (this pertains to all following figures as well).

rich sites (side view[9] in Fig. 6B). As a test of this mode of binding for **10**, we prepared compound **11**, which should retain activity even though the elaborate substituent group has been drastically changed (Scheme 3). Compound **11** was found to be nearly as potent as **10** as an inhibitor of cAMP PDE and slightly more potent in producing positive inotropy *in vitro* (see Footnote 8). The quinazolinone **12** (ORF 16600) (21) and the more linear system **13a** (anagrelide) (22) represent rigidly planar versions of rings I and II. However, as shown in Fig. 7, when these compounds are fitted on the receptor model, the dimethoxy or dichloro substituents actually play the role of ring II and interact with site 3. Presumably, the methoxy oxygens or chlorine atoms are oriented so that their lone pairs

[9] "Side views" of various structures, as they pertain to interaction with the receptor model, result from the observer looking essentially along the *z* axis toward the *xy* plane. The terms "behind," "above," and to the "right" also relate to this view of the model.

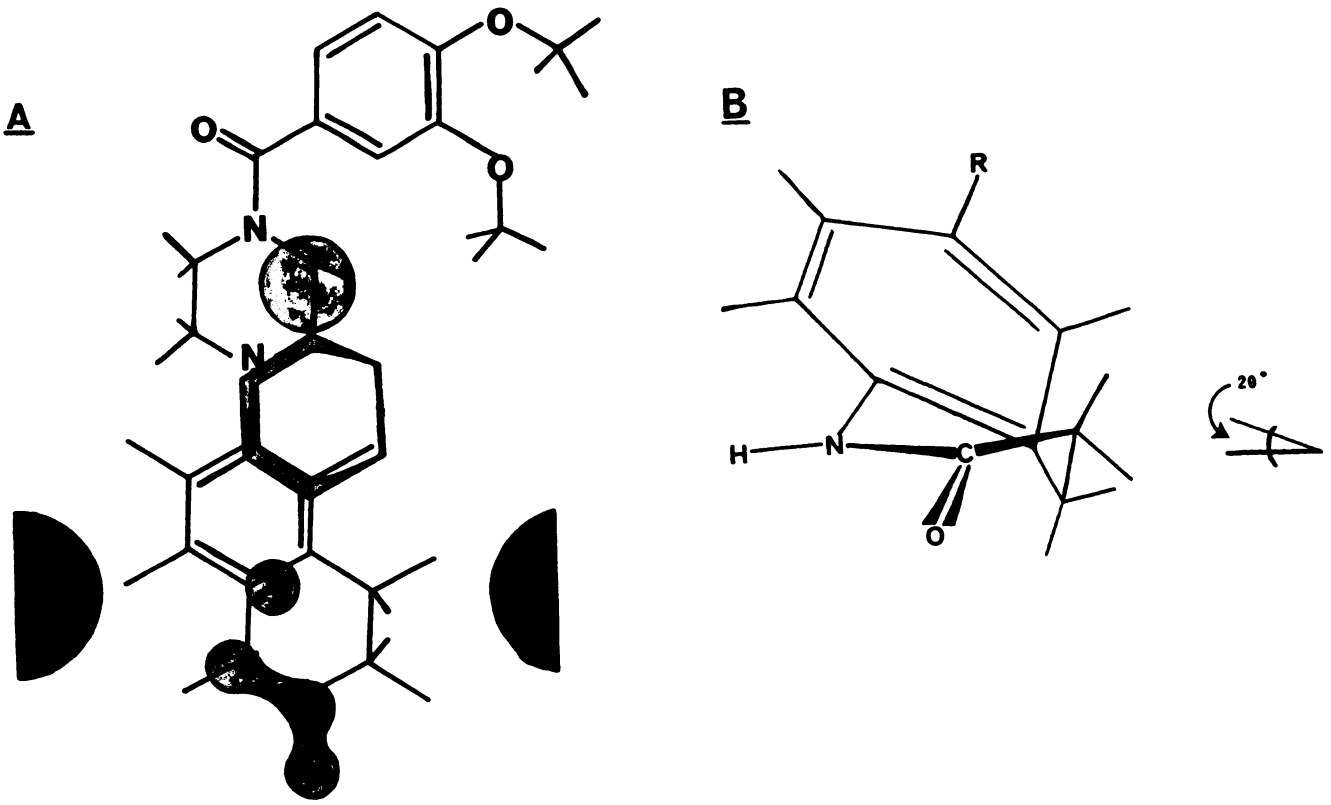


Fig. 6. Picture showing the favorable interaction between a Dreiding structure of compound **10** on the receptor model (A). Also presented (B) is a side view of **10** that shows an approximately 20° deviation between the plane of the amide and the plane of the benzene ring.

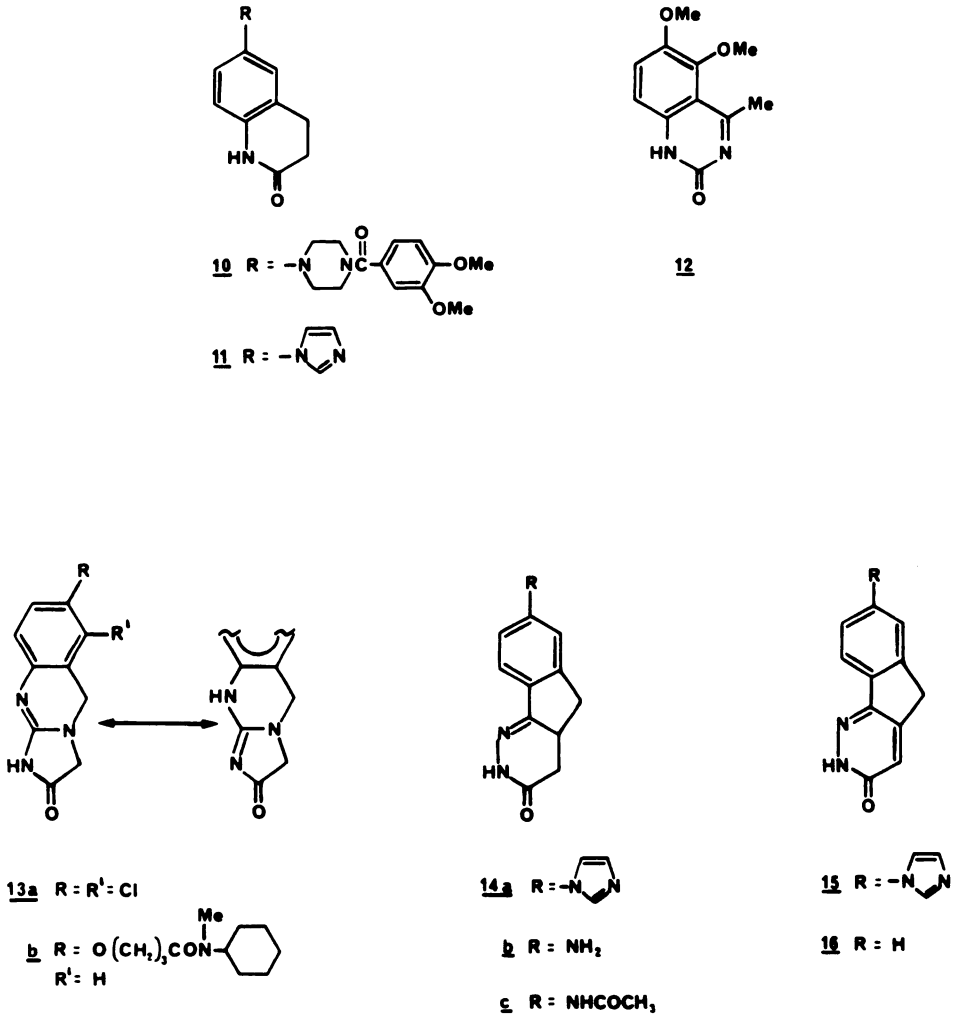
can interact favorably with site 3. In line with this analysis, the recent papers by Venuti and co-workers (23, 24) suggest that, while the parent heterocycle (three-ring nucleus of **13**) is unimpressive as a cAMP PDE inhibitor, the oxybutyramide side chain (seemingly capable of enchancing interaction with sites 3 and 6) as in **13b** (RS-82856) provides an "unambiguous advantage" in potency.

Sircar *et al.* (25), in a recently published extension of their earlier work (6, 7), have also examined rigid, planar analogs of **1.** They report that compounds **14a** and **15** have activity (*in vivo* canine data) comparable to that of **1a** and again conclude that a "generally planar ring structure is desirable for maximum positive inotropic activity." Alternatively, Cignarella *et al.* (26), for the same ring system (e.g., **14b** and **c**), conclude that "embodying the freely rotating phenyl ring into rigid quasi-planar structures" appears to result in "substantial differences in drug-receptor interactions" (*in vivo* rat data). Fig. 8 shows how our model can accommodate structures **14** and **15.** Similar to compound **10,** the "quasi-planar" relationship between rings I and II in **14** deviates ~20° from coplanarity (Fig. 8B, side view of **14a**). For **15,** however, its Dreiding model is rigidly planar and it is necessary to perform theoretical calculations to determine its conformational flexibility. For this purpose, structure **16** was employed as a model of **15** and was subjected to molecular mechanics calculations. The costs in energy to bring the pyridazinone ring approximately 10° and 20° away from coplanarity with the phenyl ring in **16** are 1.6 and 6.5 kcal/mol, respectively. Such energies are probably obtainable at physiological temperature (~1 kcal/mol) during interaction with a receptor when other energetically favorable processes also occur (~5 kcal/mol). Therefore, these structures would be predicted to be active, although they should be weaker than compounds that have preferred conformations rotated approximately 15–20° from coplanarity. Interestingly, compounds in which the preferred conformations are rotated significantly higher than ~20° (e.g., **3**) may also have to pay a modest energy toll to interact with site 3, although their enhanced rotational flexibility would allow such costs to be substantially less than for the rigid systems.

A different challenge for the receptor model is posed by the xanthines **17.** For the latter, SARs (9) reveal that, even when all nitrogen atoms, which might have otherwise provided an acidic hydrogen, are substituted as in **17b,** PDE inhibitory potency is maintained.[10] Therefore, these heterocycles probably do not bind like ring I of structures **1–3.** Instead of using receptor sites 1 and 2, the xanthines may interact with sites 3 and 6 as well as with other sites which are as yet unmapped in our receptor model. A possible binding mode is shown in Fig. 9. In this arrangement the orientation of the dipole for the xanthine system (27) is directed so as to lie nearly along the bisector of the x and negative z axes and is similar to that for the adenine nucleus (28) as discussed later. As a test for this mode of binding, an interpretation of the general SARs (9) for xanthines was made through inspection of our model (Scheme 4). At xanthine positions 1 and 3, a lipophilic substituent enhances activity (unobstructed areas on the model), whereas substitution at position 8 dramatically reduces activity (protrusion 5)[11], with electron-withdrawing substituents being the

[10] In addition, the in-plane electrostatic potential map for the xanthine system, as represented by caffeine, does not resemble those depicted in Fig. 11A (as discussed later).

[11] It should be noted that in this analysis, protrusion 5 becomes similar to the "sterically hindered site" in the receptor model published by Wells *et al.* (9).



Scheme 3

worst offenders (binding site 3 prefers an electron-rich system).

It is also important to ask whether the receptor model, even though it is derived from inhibitors, can accommodate the endogenous substrate cAMP (**18**) and, if so, whether it distinguishes cAMP from cGMP. Fig. 10 addresses these considerations. First, in order to align the phosphate for interaction with the resonance dipole-binding site 1, cAMP must turn its sugar and phosphate rings perpendicular to the plane established by this site. Since this positions these rings so as to offer their most narrow width in this region, it supports the notion that there may be a groove or pocket for binding sites 1 and 2 which is situated between two walls or protruding steric features (sites 4 and 5). The "anti" conformation shown in **18** (Scheme 5), which is thought (29) to be the preferred conformation for cAMP, then allows the imidazole ring portion of its adenine to interact with the electron-rich binding site 3. Furthermore, as shown in the side view of cAMP (Fig. 10B), the imidazole portion of the adenosine ring is approximately 5.0 Å behind (see Footnote 9) the phosphate oxygen of the acidic OH group[12] and lies at about a 15–20° angle and 1.5–2.0 Å above (see Footnote 9) the plane established by the phosphate phosphonyl and acidic OH group (see Footnote 12). This relationship correlates remarkably well with the notion that preferred binding of the phenyl groups (ring II) in the various inhibitors occurs when the phenyl rings are able to rotate so as to raise one edge slightly above the plane established by their heterocyclic rings (I) and, perhaps even more importantly, to turn their π-electron cloud at about a 15–20° angle from the perpendicular to this plane (see Fig. 10). Finally, the remainder of the adenine nucleus lies about 1.0 Å above the plane and near site 6 which correlates with the suggestion that binding site 6, when compared to 3, is closer to the plane of sites 1 and 2. Binding site 2 is not utilized by the substrate and, therefore, appears to be associated only with inhibitors. Alternatively, it is likely that there are other binding sites utilized by the substrate, for example, a specific site for recognizing the amino substituent of cAMP. Such an amino substituent site would be located near the axis of the dipole and a few Å behind or further away than site 6. From this analysis, the sense of rotation as shown in Fig. 4 appears to be the correct one. In addition, it seems reasonable to speculate that if binding site 3 (and perhaps site 6) protrudes from above as opposed to originating from the same plane as sites 1 and 2, then cAMP and the various inhibitors can be thought of as trying to reach up to this site as they interact with site 1. This, then, explains why both

[12] These distances and angles are similar to those specified in the receptor model published by Leclerc *et al.* (10) where: a distance of 5 Å is specified between the nitrogen atom of the acidic amide NH and the center of the electron-rich phenyl system; the angles between the two planes vary from about 15° to about 45° for various compounds, and the distance between the two planes varies from about 0.5 to 1 Å.

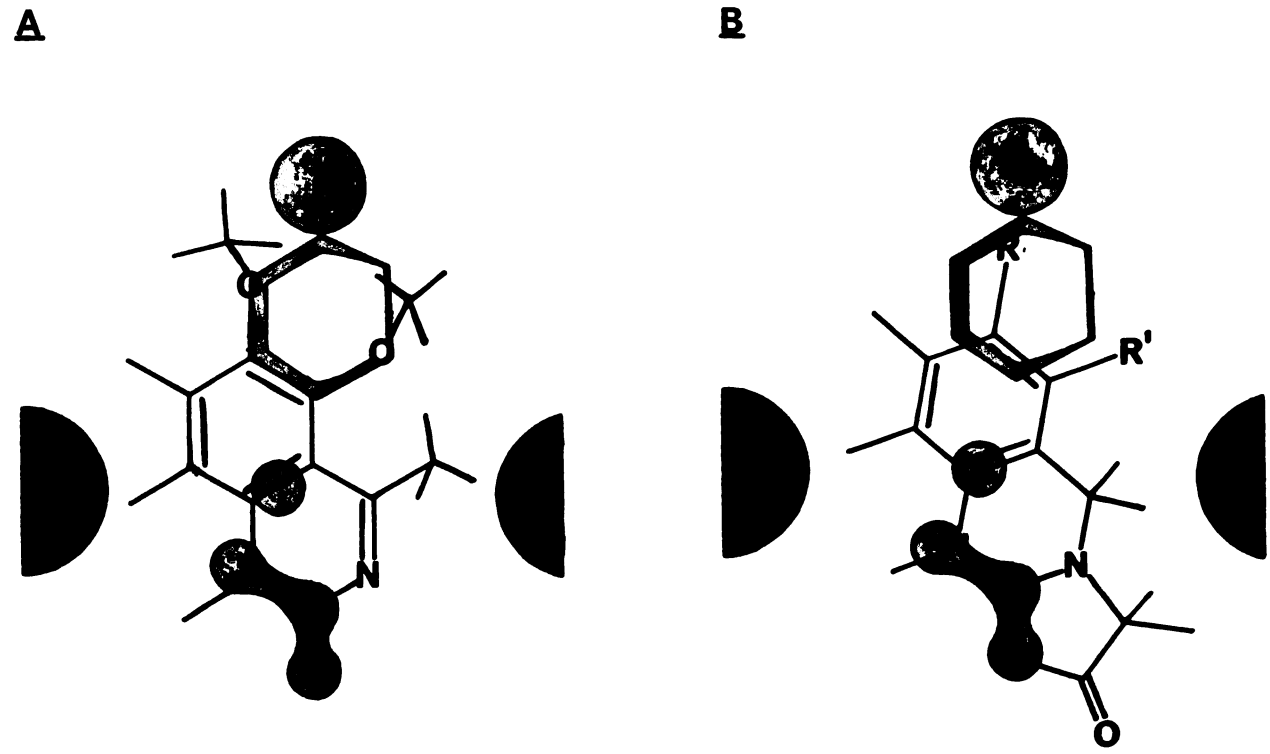


Fig. 7. A. Picture showing the favorable interaction between a Dreiding structure of **12** and the receptor model. Note that the aromatic-oxygen bonds can rotate so as to place the oxygen lone electron pairs in an approximately 20° angle from the perpendicular to the general plane of the molecule. B. Similar picture showing the favorable interactions with structures **13a** and **b**.

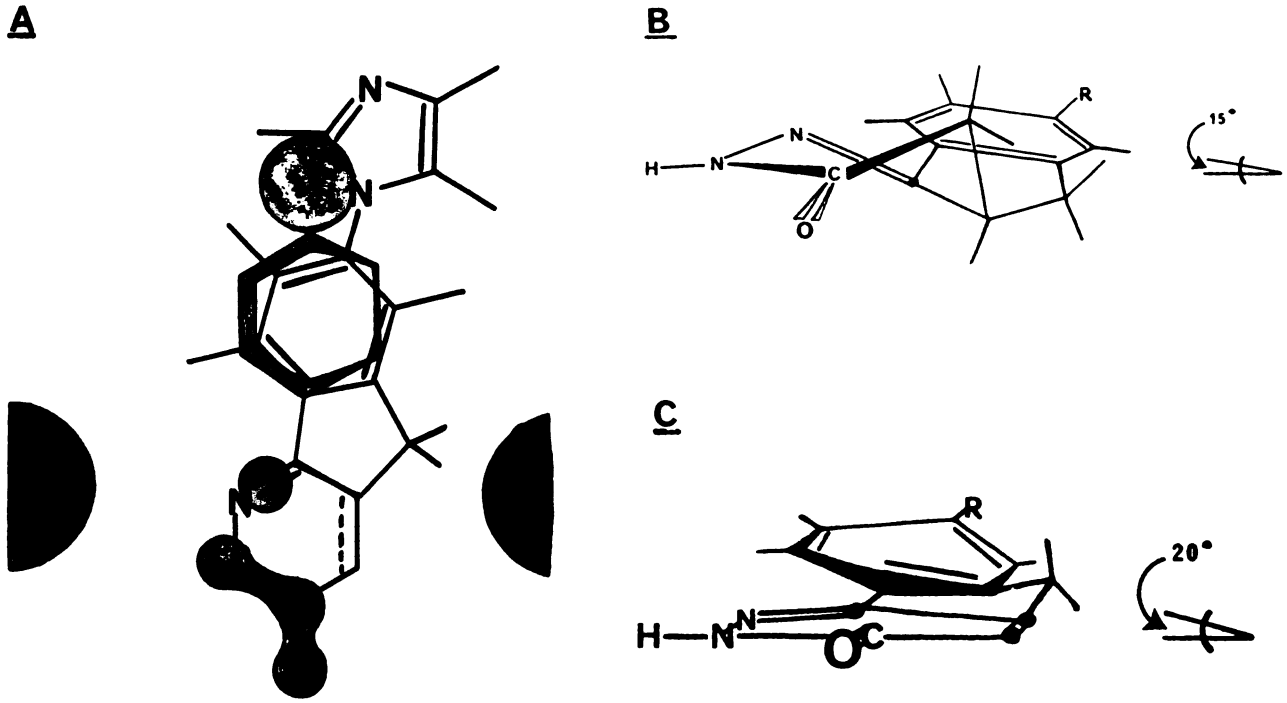


Fig. 8. Pictures showing the interactions between Dreiding structures of **14a** and **15** with the receptor model (A), a side view of **14a** (B), and a side view of **15** (C) for its twisted conformation that is ~6 kcal/mol higher in energy than its planar conformation.

conformationally flexible systems and semi-rigid systems can show inhibitory activity, i.e., it is not requisite for these structures to be flexible in order to avoid and adjust for a binding protrusion originating from the same plane as sites 1 and 2.

At this point a brief discussion concerning the relative acidities of the various heterocyclic systems (ring I) versus the cyclic phosphate present in the substrate is necessary. The pK_a for the imidazolone in **3** is approximately 12 and this ring is

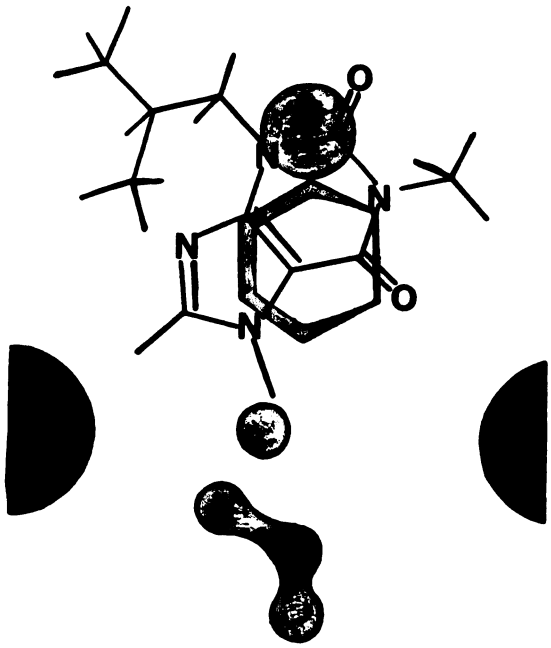
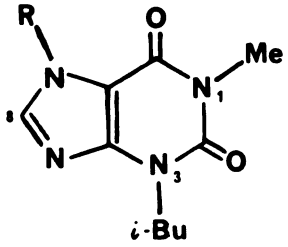


Fig. 9. Picture showing the favorable interaction between a Dreiding structure of **17a** and the receptor model.



17a R = H

b R = Me

Scheme 4

largely nonionized at physiological pH, whereas the pK_a for phosphate is approximately 2 and this group is essentially completely ionized at pH 7.4. While one can argue that the actual receptor site is microscopic and does not need to obey the laws of bulk solution (i.e., the phosphate may be nonionized) or that site 1 contains a group that has substantial amounts of both protonated and unprotonated equilibrium forms at physiological pH (e.g., an imidazole residue), the receptor must be able to locate and attract its specific substrate in the biological milieu of the cell and, therefore, it is most appealing to consider that site 1 bears a positive charge (or partial positive charge) in order to assist in this initial recognition and attraction process for an anionic phosphate group. Nevertheless, since the nonionized heterocycles (I) and ionized phosphate form strikingly similar resonance dipole systems, as shown in Fig. 11A, it is this analogy which is utilized in our topographical model to depict recognition site 1. In this regard, however, we do not mean to imply that more complicated interactions should be ruled out. For example, the alternate binding schemes depicted in Fig. 11, B and C, seem reasonable, and one of these could, instead, be operative. The involvement of Mg^{2+} in these schemes derives from the requirement (30, 31) for this ion to be present during measurement of PDE hydrolytic activity.

The proposed alignment of cAMP has some literature precedent. During their analysis of buquineran, **19a** (Scheme 6), Campbell *et al.* (32) also considered various binding schemes at the active site of PDE and similarly concluded that the carbonyl function in **19a** may act "as a surrogate electrophilic center in place of the natural phosphate moiety." Furthermore, theoretical calculations for **19b** "revealed that the piperidine ring and the quinazoline nucleus lie at an angle of *ca.* 20°" away from coplanarity.

For cGMP, which prefers a "syn" conformation (9, 29), it is obvious that if the cyclic phosphate is aligned near site 1, site 6 cannot be utilized for binding. Even when cGMP is placed in an "anti" conformation (Fig. 10C) similar to cAMP, its guanine amino group is located too far to the right (see Footnote 9) to be associated with either site 6 or the nearby binding site specific for the amino group on cAMP. When the alternate "anti" conformation is adopted (Fig. 10D), so as to place its amino group close to site 6, the electron-rich imidazole area is now too far to the right to interact with site 3, and there is an apparent collision with protrusion 4. Therefore, our model of the cardiac cAMP PDE receptor does not accommodate cGMP as a substrate, and this finding is in accord with the report (3) that cGMP is poorly hydrolyzed by this enzyme. It should be noted that in this analysis the requirements for binding and hydrolysis of the phosphate systems in cAMP and cGMP have been treated in a similar fashion. That the corresponding PDEs have conserved these features associated with their identical functional activities and rely on binding differences of the two purines for their substrate specificity seems reasonable. Alternatively, if cGMP were to give up its interaction and potential hydrolysis at the functional site 1 and, instead, adjust solely for favorable interactions with sites 3 and 6 (Fig. 10E, "syn" conformation) in a manner similar to the xanthines, its binding could then result in competitive inhibition of cAMP. This, again, is in accord with the literature which indicates that cGMP, although not a substrate, is a competitive inhibitor of this enzyme (3, 33).

In conclusion, our topographical model of the cardiac cAMP PDE receptor can be described by the following features. 1) A general plane which contains a binding site for a resonance dipole moiety (site 1) similar to an acidic amidic or phosphate function and a binding site (2) for an electron-rich center similar to a double bond. The heterocyclic rings present in several of the cAMP PDE inhibitors tend to interact with these sites in a planar fashion. Alternatively, the general plane established by the phosphate ring and sugar ring in cAMP sits perpendicular to the plane of these binding sites, thus allowing the phosphate dipole portion to interact in an appropriately planar manner with site 1. Whereas site 2 is not utilized by the substrate, additional unmapped sites for the oxygen atoms present in the sugar ring are likely to be present in this region. 2) A binding area (site 3) which interacts with π-electrons when the latter are turned at an angle approximately 20° from the perpendicular to the plane established by sites 1 and 2. This site probably protrudes from the top of the receptor pocket such that conformationally flexible inhibitors tend to turn their aryl functions slightly clockwise (as viewed down the z axis or dipole function from negative to positive as it sits on site 1) for optimal interaction. Whereas the substrate is inherently poised

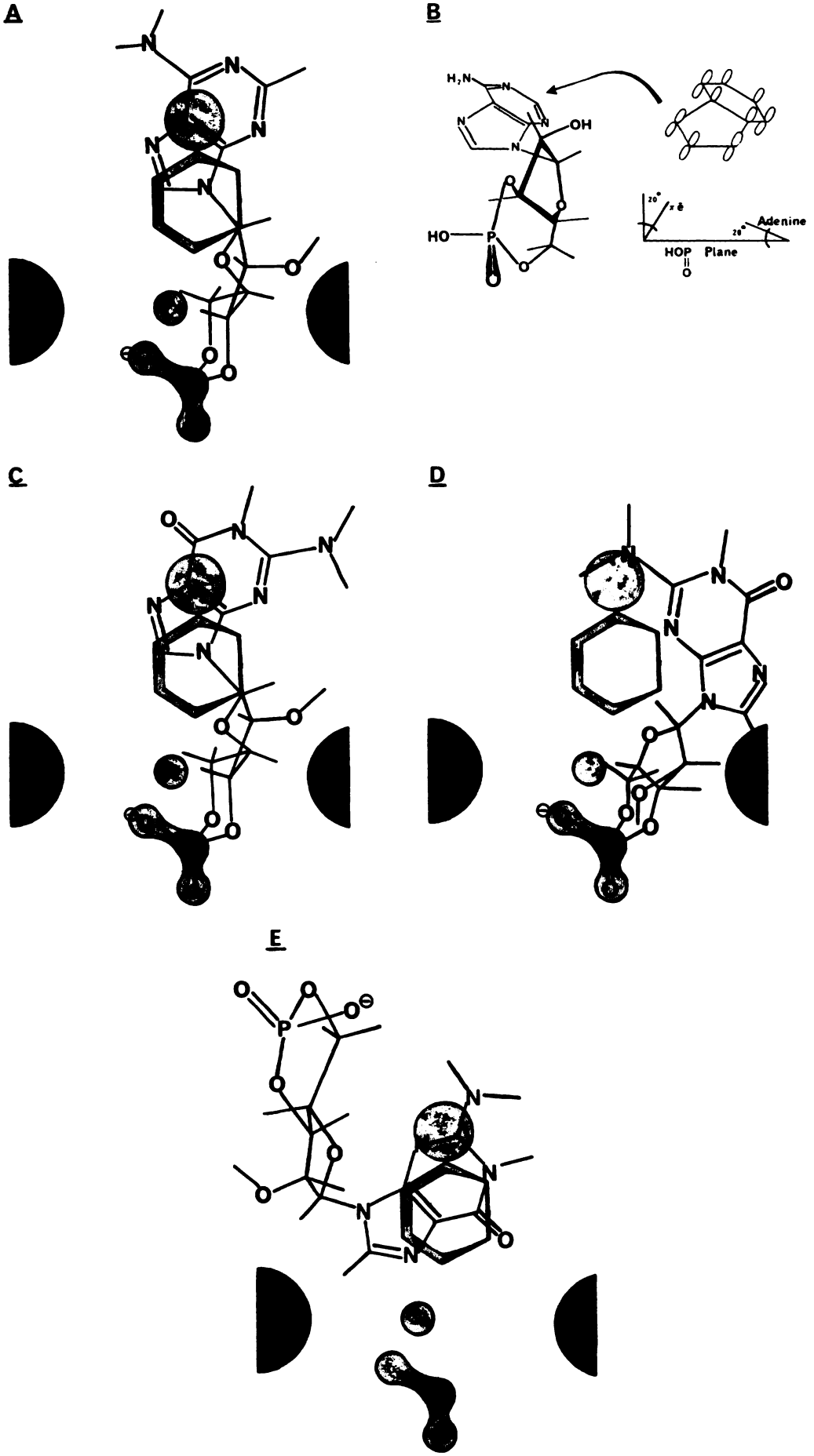
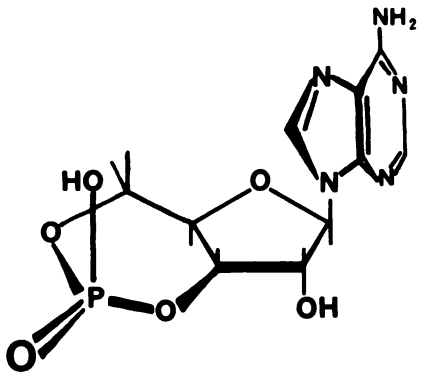


Fig. 10. Pictures showing: A, the favorable interaction of a Dreiding structure of cAMP with the receptor model; B, a side view of cAMP with the angle of its adenine π-electron cloud highlighted; C and D, unfavorable attempts to align cGMP with the model; and E, a possible inhibitory binding mode for cGMP.



18

Scheme 5

to interact with this site, an optimal interaction may not be requisite for inhibitors since certain rigid, nearly planar systems can demonstrate reasonable inhibitory activity. 3) Two steric boundaries (4 and 5), one on each side of the region containing sites 1 and 2, that limit the size of alkyl substituents which can be placed on the heterocyclic rings of various inhibitors and, also, probably serve to guide the phosphate and sugar rings of the substrate perpendicularly into its pocket for optimal interaction of the phosphate dipole with site 1. These boundaries may extend toward sites 1 and 2 and, thereby, form pockets which can bind favorably with small alkyl groups, or they may extend toward site 3 and thereby form part of that pocket. 4) A site (6) nearly in the plane of sites 1 and 2, that interacts with an electron-rich system similar to an imidazole or thiomethyl group as present in certain inhibitors, or with certain of the nitrogen atoms present in cAMP. The location of this site is probably critical in determining the selectivity exhibited

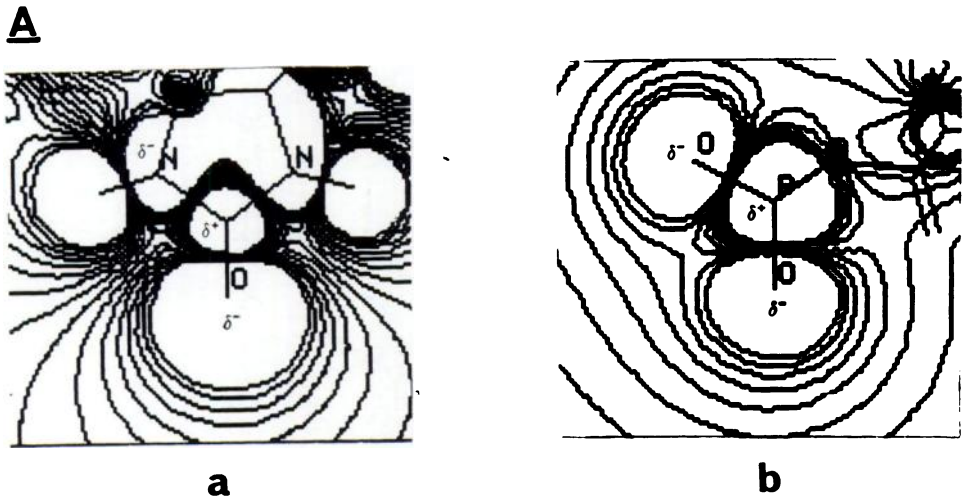
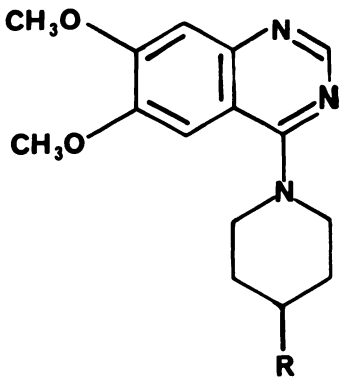


Fig. 11. A. AM1 in-plane molecular electrostatic potentials of the imidazolone (nonionized) portion of **3** (*a*) and the phosphate (ionized) portion of cAMP (*b*). B. Binding model showing receptor region 1 displacing the proton from the imidazolone directly (*a*) or via a Mg^{2+} ion (*b*). C. Binding model showing the proton from the imidazolone displacing the Mg^{2+} at the receptor.



19a R = $NHCONH(CH_2)_3CH_3$

b R = H

Scheme 6

toward both substrates and antagonists. Also important for selectivity, it is likely that a region specific for the amino group present in cAMP resides in nearly a direct line with the dipole portion of site 1 and a few Å further away than site 6.

The interaction of a ligand with our receptor model can be expressed as a summation of the interactions with each of the sites 1–6 where the contribution by each interaction is a function of both the site's relative importance and the correctness of the fit exhibited by the ligand for that site. In addition, some mutual molding is likely, especially for the functional end (site 1) where hydrolysis of the substrate occurs.

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References

1. Erhardt, P. W. In search of the digitalis replacement. *J. Med. Chem.* **30**:231–237 (1987).
2. Weishaar, R. E., M. H. Cain, and J. A. Bristol. A new generation of phosphodiesterase inhibitors: multiple molecular forms of phosphodiesterase and the potential for drug selectivity. *J. Med. Chem.* **28**:537–545 (1985).
3. Harrison, S. A., D. H. Reifsnyder, B. Gallis, G. G. Cadd, and J. A. Beavo. Isolation and characterization of bovine cardiac muscle cGMP inhibited phosphodiesterase: a receptor for new cardiotonic drugs. *Mol. Pharmacol.* **29**:506–514 (1986).
4. Hidaka, H., T. Tanaka, and H. Itoh. Selective inhibitors of three forms of cyclic nucleotide phosphodiesterases. *Trends Pharmacol. Sci.* 237–239 (1984).
5. Appleman, M. N., J. A. Beavo, G. Hardman, C. B. Klee, and W. J. Thompson. Nomenclature recommendations. *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* **16**:iv (1984).
6. Bristol, J. A., I. Sircar, W. H. Moos, D. B. Evans, and R. E. Weishaar. Cardiotonic agents. 1. 4,5-Dihydro-6-[4-(1H-imidazol-1-yl)phenyl]-3-(2H)-pyridazinones: novel positive inotropic agents for the treatment of congestive heart failure. *J. Med. Chem.* **27**:1099–1101 (1984).
7. Sircar, I., B. L. Duell, G. Bobowski, J. A. Bristol, and D. B. Evans. Cardiotonics agents. 2. Synthesis and structure-activity relationships of 4,5-dihydro-6-[4-(1H-imidazol-1-yl)phenyl]-3-(2H)-pyridazinones: a new class of positive inotropic agents. *J. Med. Chem.* **28**:1405–1413 (1985).
8. Robertson, D. W., E. E. Beedle, J. K. Swartzendruber, N. D. Jones, T. K. Elzey, R. F. Kauffman, H. Wilson, and J. S. Hayes. Bipyridine cardiotonics: the three-dimensional structures of amrinone and milrinone. *J. Med. Chem.* **29**:635–640 (1986).
9. Wells, J. N., J. E. Garst, and G. L. Kramer. Inhibition of separated forms of cyclic nucleotide phosphodiesterase from pig coronary arteries by 1,3-disubstituted and 1,3,8-trisubstituted xanthines. *J. Med. Chem.* **24**:954–958 (1981).
10. Leclerc, G., G. Marciniak, N. Decker, and J. Schwartz. Cardiotonic agents. 1. Synthesis and structure-activity relationships in a new class of 3-, 4-, and 5-pyridyl-2-(1H)-quinolone derivatives. *J. Med. Chem.* **29**:2427–2432 (1986).
11. Leclerc, G., G. Marciniak, N. Decker, and J. Schwartz. Cardiotonic agents. 2. Synthesis and structure-activity relationships in a new class of 6-, 7-, and 8-pyridyl-2-(1H)-quinolone derivatives. *J. Med. Chem.* **29**:2433–2438 (1986).
12. Rakhit, S., G. Marciniak, G. Leclerc, and J. Schwartz. Computer assisted pharmacophore search in a series of non-steroidal cardiotonics. *Eur. J. Med. Chem.* **21**:511–515 (1986).
13. Davey, D., P. W. Erhardt, W. C. Lumma, Jr., J. Wiggins, M. Sullivan, D. Pang, and E. Cantor. Cardiotonic agents. 1. Novel 8-aryl substituted imidazo[1,2a] and [1,5a]pyridines, and imidazo[1,5a]pyridinones as potential inotropic agents. *J. Med. Chem.* **30**:1337–1342 (1987).
14. Hagedorn III, A. A., P. W. Erhardt, W. C. Lumma, Jr., R. A. Wohl, E. Cantor, Y.-L. Chou, W. R. Ingebretsen, J. W. Lampe, D. Pang, C. A. Pease, and J. Wiggins. Cardiotonic agents. 2. (Imidazolyl)aroylimidazolones, highly potent and selective positive inotropic agents. *J. Med. Chem.* **30**:1342–1347 (1987).
15. Dewar, M. J., and W. Thiel. Ground states of molecules. 38. The MNDO method. Approximations and parameters. *J. Am. Chem. Soc.* **99**:4899–4907 (1977).
16. Dewar, M. J., E. G. Zoebisch, E. F. Healy, and J. J. Stewart. AM1: a new general purpose quantum mechanical molecular model. *J. Am. Chem. Soc.* **107**:3902–3909 (1985).
17. Hehre, W. J., R. F. Stewart, and J. A. Pople. Self-consistent molecular-orbital methods. I. Use of gaussian expansions of Slater-type atomic orbitals. *J. Chem. Phys.* **51**:2657–2664 (1969).
18. Schnettler, R. A., R. C. Dage, and J. M. Grisar. 4-Aroyl-1,3-dihydro-2H-imidazol-2-ones, a novel class of cardiotonic agents. *J. Med. Chem.* **25**:1477–1481 (1982).
19. Robertson, D. W., N. D. Jones, J. H. Krushinski, G. D. Pollock, J. K. Swartzendruber, and J. S. Hayes. Molecular structure of the dihydropyridazinone cardiotonic 1,3-dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2H-indol-2-one, a potent inhibitor of cyclic AMP phosphodiesterase. *J. Med. Chem.* **30**:623–627 (1987).
20. Mannhold, R. OPC-8212. *Drugs Future* **10**:28–31 (1985).
21. Falotico, R., J. B. Moore, E. L. Tolman, V. T. Bandurco, S. C. Bell, and A. J. Tobia. Cardiotonic activity of a series of substituted 4-alkyl-2(1H)-quinazolinones. *Pharmacologist* **27**:679 (1985).
22. Fleming, J. S., and J. P. Buyniski. Anagrelide, in *New Drugs Annual: Cardiovascular Drugs* (A. Scriabine, ed.). Raven Press, New York, 277 (1983).
23. Venuti, M. C., G. H. Jones, R. Alvarez, and J. J. Bruno. Inhibitors of cyclic AMP phosphodiesterase. 2. Structural variations of *N*-cyclohexyl-*N*-methyl-4-[(1,2,3,5-tetrahydro-2-oxoimidazo[2,1b-]quinazolin-7-yl)oxy]butyramide (RS-82856). *J. Med. Chem.* **30**:303–318 (1987).
24. Jones, G. H., M. C. Venuti, R. Alvarez, J. J. Bruno, A. H. Berks, and A. Prince. Inhibitors of cyclic AMP phosphodiesterase. 1. Analogues of cilostamide and anagrelide. *J. Med. Chem.* **30**:295–303 (1987).
25. Sircar, I., B. L. Duell, M. H. Cain, S. E. Burke, and J. A. Bristol. Cardiotonic agents. 4. Synthesis and biological evaluation of *N*-substituted 2,4,4a-5-tetrahydro-3H-indeno[1,2c]pyridazin-3-ones: rigid structures derived from CI-930 and analogues. *J. Med. Chem.* **29**:2142–2148 (1986).
26. Cignarella, G. D. Barlocco, G. A. Pinna, M. Loriga, O. Tofanetti, M. Germini, and F. Sala. Conformationally restricted congeners of hypotensive and platelet aggregation inhibitors: 6-aryl-5-methyl-4,5-dihydro-3(2H)-pyridazinones derived from 5H-indeno[1,2c]pyridazine. *J. Med. Chem.* **29**:2191–2194 (1986).
27. Weiler-Feilchenfeld, H., and Z. Neiman. Dipole moment and electronic structure of some xanthine and thioxanthine derivatives. *J. Chem. Soc. Sect. B* 596–598 (1970).
28. Berthod, H., and B. Pullman. A molecular orbital investigation of the dipole moments of the syn and anti conformers of purine and pyrimidine nucleosides. *Biochem. Biophys. Res. Commun.* **46**:125–129 (1972).
29. Yathindra, N., and M. Sundaralingam. Conformations of cyclic 3′,5′-nucleotides. Effects of the base on the syn/anti conformer distribution. *Biochem. Biophys. Res. Commun.* **56**:119–126 (1974).
30. Boudreau, R. J., and G. I. Drummond. A modified assay of 3′,5′-cyclic-AMP phosphodiesterase. *Anal. Biochem.* **63**:388–399 (1975).
31. Drummond, G. I., and S. Perrott-Yee. Enzymatic hydrolysis of adenosine 3′,5′-phosphoric acid. *J. Biol. Chem.* **236**:1126–1129 (1961).
32. Campbell, S. F., N. J. Cussans, J. C. Danilewicz, A. G. Evans, A. L. Ham, A. A. Jaxa-Chamiec, D. A. Roberts, and J. K. Stubbs. Phosphodiesterase inhibitors as cardiovascular agents, in *Second SCI-RSC Medicinal Chemistry Symposium*, Special Publication No. 50 (J. C. Emmett, ed.). Burlington House, London, 47–64 (1983).
33. Wells, J. N., Y. J. Wu, C. E. Baird, and J. G. Hardman. Phosphodiesterases from porcine coronary arteries: inhibition of separated forms by xanthines, papaverine, and cyclic nucleotides. *Mol. Pharmacol.* **11**:775–783 (1975).

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