Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 7, pp. 913-918, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00143-6

CRYSTAL RECEPTOR MODELS IN MEDICINAL CHEMISTRY: APPLICATION TO THE GENERATION OF HIGHLY POTENT POTASSIUM CHANNEL OPENERS

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Abstract: The cavity produced by removing one molecule of the potassium channel opener (PCO) cromakalim from its crystal lattice, contains shape and electrostatic information that has been exploited as a tool in drug design. Copyright © 1996 Elsevier Science Ltd

Given the challenge of designing a receptor antagonist, enzyme inhibitor, or ion channel modulator, the medicinal chemist has a number of alternative approaches at his/her disposal. In most cases, a three-dimensional structure of the target protein is not available, although this situation is changing rapidly. Even when presented with a detailed structural image of the receptor binding site it is by no means a trivial exercise to design more potent agents or control the degree of agonist/antagonist activity present in potential ligands.[1]

Our aim was to develop a surrogate receptor binding site for a particular ligand of interest. We further required that the site contained shape and electrostatic information similar to that in the natural protein binding site. This line of thought led us to consider that *the crystal lattice is a 'receptor' for one of its molecular components.* Put another way, the vacancy remaining after the removal of one molecule from the lattice is, in essence, a negative three - dimensional image of the lattice molecule. It contains shape information and electrostatic information which can be exploited by the medicinal chemist in the same way as the structural properties of the biological agent are used. The information content within a crystal lattice has been recognized as being valuable in a medicinal chemistry context by others.[2-8] We have developed a novel approach to using this information that we have called the crystal receptor model concept to compare the shape and electrostatics of a number of structurally distinct potassium channel openers (PCOs). This approach has generated a number of ideas for the design of structurally novel PCOs and importantly, has given us a method of prioritizing our selection of targets to investigate. Here we describe the design of a series of novel fluoroalkyl benzoxazine PCOs using this approach. The limitations of this method will also be discussed.

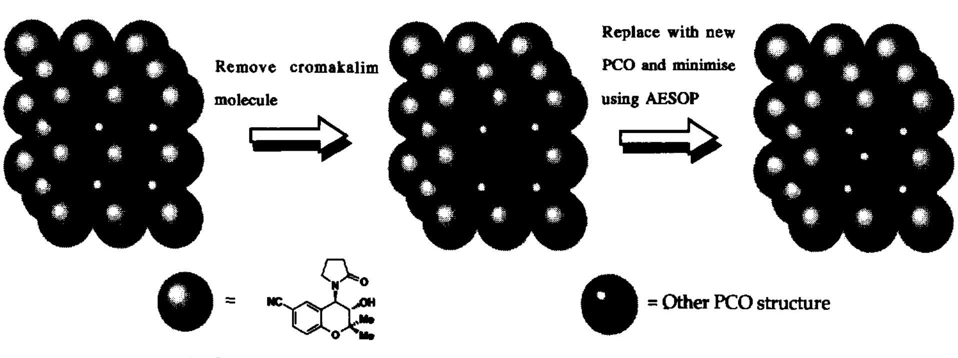
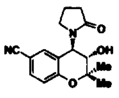


Fig. 1. Application of the crystal receptor model concept using the cromakalim crystal lattice.

The method described here uses a cromakalim crystal lattice[9] to illustrate the general approach. Cromakalim is a potassium channel opener at the K_{ATP} channel. It is thought to act via a receptor site on the channel protein to increase the open probability of the channel.[10] The cromakalim crystal lattice is also a "receptor" for cromakalim. If we remove one molecule of cromakalim from the lattice we are left with a cavity which contains shape and electrostatic information relevant to the binding of cromakalim. Our assumption is that we can use this crystal receptor model as a means of comparing molecular shape and electrostatic information and thus generate ideas for the synthesis of novel PCO structures . The general procedure used is illustrated in Figure 1.



cromakalim

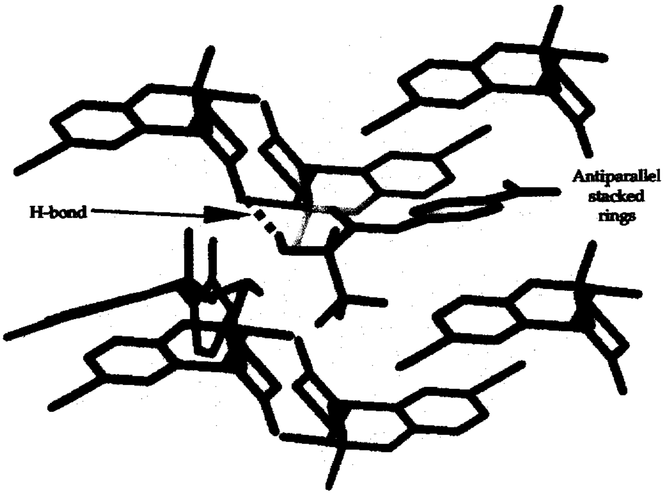
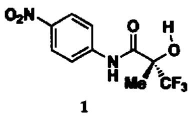


Fig. 2. An example of a docked geometry for the tertiary carbinol 1 in the cromakalim crystal lattice. Several molecules of the surrounding cromakalim have been removed for clarity. In addition to the features highlighted in the diagram is the presence of a snug hydrophobic binding pocket for the trifluoromethylmethyl unit in 1.

We have recently described a structurally novel series of potassium channel openers, the tertiary carbinols.[11] We were interested to explore how a member from this series would fit into the cromakalim crystal lattice. The nitrophenyl analog 1 was used in all docking studies rather than molecules containing larger electron withdrawing substituents such as phenylsulfone, 2 because of the limited volume available at this position in the cromakalim crystal receptor model.[12] Docking experiments were performed to try to suggest ideas as to how the tertiary carbinol PCOs could interact with the same K_{ATP} binding site as cromakalim. Several starting geometries were tried and the bound molecule was minimized using AESOP.[13] The cromakalim lattice was fixed in position during the minimization. Many of the starting points converged to the same final structure. Figure 2 illustrates such a docked model. If we compare the bound tertiarycarbinol geometry to that of the original resident cromakalim molecule we see that the tertiary carbinol aligns it's dipolar ring with that of cromakalim although the two rings are displaced relative to one another (shown in Fig. 3). The hydroxyl occupies the same region of space in both molecules and forms a hydrogen bond to the pyrrolidinone carbonyl



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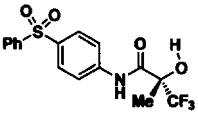

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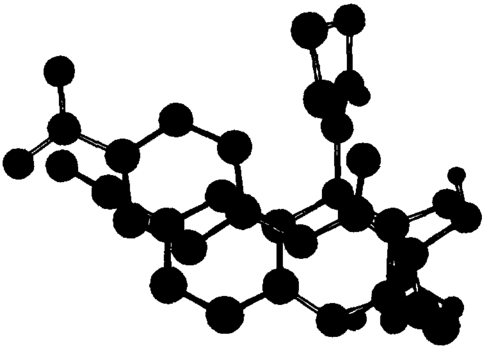
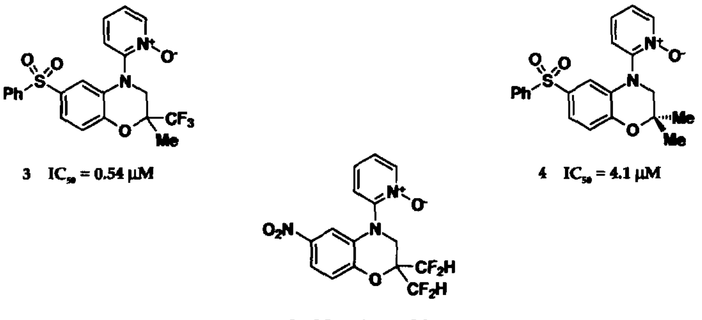


Fig. 3. Shape overlay of cromakalim and 1, created using the crystal receptor model. Both molecules are fixed in the exact geometry and position they adopt in the cromakalim lattice.

moiety. The tertiary carbinol does not contain a significant H-bond acceptor system as present in cromakalim. There is a close correspondence between the gem dimethyl unit in cromakalim and the trifluoromethyl methyl unit in the tertiary carbinol compound. Indeed this region seems to function as an anchor unit for the rest of the molecule because of the tight shape complementarity between this unit and the crystal cavity. This immediately suggested that one of the methyl groups in cromakalim could be replaced with a trifluoromethyl group since it was known that the trifluoromethyl moiety is important for obtaining good potassium channel activity in the tertiarycarbinol series. In practice this idea was tested on a series of benzoxazine pyridine-N-oxides,[14] that are structurally related to cromakalim, and was validated by comparison of the trifluoromethyl methyl benzoxazine 3 to the dimethyl compound 4. Fluoroalkylbenzoxazine 3 was found to be 8 times more potent than the non-fluorinated compound 4. This same line of thought also led us to synthesize the bis(difluoromethyl)benzoxazine 5 which is one of the most potent known potassium channel openers on guinea pig detrusor. The synthesis of a related bis(trifluoromethyl) analog of cromakalim has also recently been reported.[15] The model actually predicts



3 $IC_{50} = 0.54\ \mu M$

4 $IC_{50} = 4.1\ \mu M$

5 $IC_{50} = 0.007\ \mu M$

Fig. 4. Incorporation of fluorine substituents into our lead structure 4, as suggested by docking the tertiary carbinol 1 into the cromakalim crystal receptor model, leads to the generation of highly potent potassium channel openers 3, and 5.

that the R enantiomer of 3 should be the best fit to the cromakalim lattice. In practice the two enantiomers of 3 are both significantly active as potassium channel openers, with the S enantiomer being the most potent (R enantiomer, IC_{50} = 0.68 μM; S enantiomer, IC_{50} = 0.28 μM). The difference in IC_{50}s corresponds to an energy difference of only 0.5 kcal. This probably indicates a slightly different binding geometry for the two systems.

Traditionally a key component of drug design and other molecular recognition studies has been the use of atom-atom overlays of molecules thought to bind at the same site. The choice of how molecules overlay is often guided by the recognition of common pharmacophoric groups such as hydrogen bonding moieties in the individual molecules, although the choice of such 'anchor points' is sometimes arbitrary since their contribution to binding is not known. This is however often a very fruitful way of generating new ideas and hypotheses which necessitate the synthesis of additional molecules. In many cases this approach has led to the generation of higher affinity agents, often with unique structures.

The concept of using crystal receptor models presented in this paper is a novel alternative approach that allows us to compare molecules on the basis of shape and electrostatic properties with a lead compound. The value of this type of comparison is that it is these properties that are actually recognized by the protein receptor and not the positions of the individual atoms themselves. In the current case it is not clear what anchor points should be used to compare cromakalim and the tertiarycarbinol structure using an atom-atom overlay. If the atoms of the corresponding dipolar benzene rings are overlayed then the dimethyl unit in cromakalim does not overlay well with the trifluoromethylmethyl unit in the tertiarycarbinol structures. Many other overlay option can be tried without clear justification as to which one is best. The novel approach described in this paper uses steric and

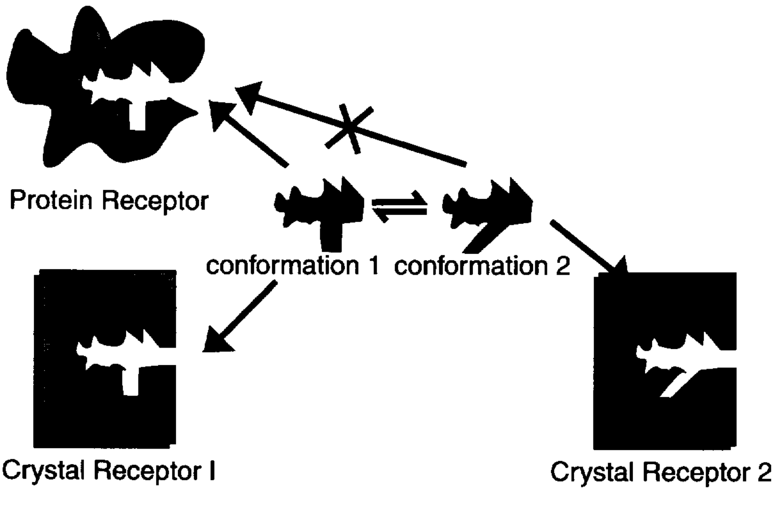


Fig. 5. Illustration of a general limitation of the crystal receptor model concept, that a ligand in the bioactive conformation binds to it's receptor and it's crystal receptor. If the ligand crystallizes in a different conformation from it's bioactive conformation the resulting crystal receptor model is of little value.

electrostatic shape as guides for overlay. Since these are clearly important parameters in determining the binding affinity of a ligand for its receptor, this approach may prove to be a valuable aid in the ligand design process in cases where a crystal structure of a lead compound is available.

It should be noted that application of the crystal receptor model concept is most appropriate in cases where the conformation of the crystallized molecule is thought to be relevant to the bioactivity of the molecule (see Figure 5). Another important limitation is the realization that the crystal receptor site merely provides an alternative view of the information encoded within the crystallized molecule itself. This system thus cannot provide information outside of the volume/shape occupied by the molecule. This is of course true of most QSAR and molecular modeling approaches, in the absence of the structure of the protein binding site.

The set of interactions provided by the crystal environment are different, and in some cases more limited than those provided by a protein environment although it is known that protein-like binding environments can be reproduced in some simple polymeric systems using the molecular imprinting technique.[16] This suggests that that there may be many ways to provide similar binding situations and that some degeneracy is present in the way proteins bind their ligands. The protein recognition site may or may not completely surround the bound ligand. In the crystal lattice most molecules are completely surrounded. In the current case a decision was made that one end of the crystal receptor model would be left open. We chose to remove atoms close to the left hand side (as drawn) of the central cromakalim molecule because we knew that much larger groups could be accommodated in this region than a simple cyano moiety as presented by cromakalim. At it's simplest level it can be viewed as a method of shape overlay to compliment the more usual atom based overlay techniques used by medicinal chemists.

As is the case in many modes of molecular comparison, we are also assuming that the ligands in question bind to the same protein binding site, in this case on the K_{ATP} potassium channel. We expect this technique could be of general utility in a number of areas of drug design.

Acknowledgments: I am indebted to Brian Masek for many helpful discussions and to James Empfield for discussions and for critically reading the manuscript.

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12. The most potent tertiarycarbinol analogs have large electron withdrawing phenylsulfone or 2-pyridylcarbonyl moieties. Since these large substituents have no counterpart in the cromakalim molecule, and lead to molecules with larger dimensions than cromakalim there was not enough room in the crystal receptor cavity to accommodate them. Our decision to use the nitro substituted analog stems from the similar molecular dimensions of this molecule to cromakalim. The IC_{50}s for **1** and **2** are 0.5 μM and 1.0 μM against potassium (15 mM) induced contractions of the guinea-pig detrusor smooth muscle. Cromakalim has an IC_{50} of 0.6 μM in this assay.

13. AESOP is an in-house molecular mechanics program, Brian B. Masek, ZENECA Inc., Wilmington, DE 19897, derived in part from QCPE 514: Nachbar, R.; Mislow, K. BIGSTRN-3: General-Purpose Empirical Force-Field Program. QCPE Bull. 1986, 6, 96-97. AESOP employs the MM2 force field parameters; see Allinger, N.L. QCPE #395, Indiana University, Chemistry Building 204, Bloomington, Indiana 47401.

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(*Received in USA* 5 *December* 1995; *accepted* 8 *March* 1996)