Development of a Three-Dimensional *CysLT*$_1$ (LTD$_4$) Antagonist Model with an Incorporated Amino Acid Residue from the Receptor

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This paper describes the molecular modeling of leukotriene *CysLT*$_1$ (or LTD$_4$) receptor antagonists. Several different structural classes of *CysLT*$_1$ antagonists were superimposed onto the new and highly rigid *CysLT*$_1$ antagonist 8-carboxy-3′-[2-(2-quinolinyl)ethenyl]flavone (**1**, VUF 5017) to generate a common pharmacophoric arrangement. On the basis of known structure−activity relationships of *CysLT*$_1$ antagonists, the quinoline nitrogen (or a bioisosteric equivalent thereof) and an acidic function were taken as the matching points. In order to optimize the fitting of acidic moieties of all antagonists, an arginine residue from the receptor was proposed as the interaction site for the acidic moieties. Incorporation of this amino acid residue into the model revealed additional interactions between the guanidine group and the nitrogen atoms of quinoline-containing *CysLT*$_1$ antagonists. In some cases, the arginine may even interact with π-clouds of phenyl residues of *CysLT*$_1$ antagonists. The alignment of Montelukast (MK-476) suggests the presence of an additional pocket in the binding site for *CysLT*$_1$ antagonists. The derived model should be useful for a better understanding of the molecular recognition of the leukotriene *CysLT*$_1$ receptor.

Introduction

During the last 2 decades cysteinyl leukotrienes (LTC$_4$, LTD$_4$, and LTE$_4$) have been revealed as important mediators in asthma.[1−5] These arachidonic acid metabolites produce their effects by stimulation of the *CysLT*$_1$ receptor, which belongs to the G-protein-coupled superfamily of receptors.[6] *CysLT*$_1$ receptor activation leads to bronchoconstriction, mucus secretion, and increased bronchial hyperresponsiveness, all characteristics of asthma. *CysLT*$_1$ receptor antagonists have been demonstrated to be clinically effective in the treatment of asthma and are considered as the most promising new antiasthmatic drugs.[7−14]

Several classes of highly potent *CysLT*$_1$ antagonists are known (Chart 1).[14,15] Despite their very different molecular structures, qualitative structure−activity relationship (SAR) studies have revealed several common features among these ligands. The similar structure−activity relationships, found for both agonists and antagonists, especially LTD$_4$ analogues, have led to the suggestion that they might have similar interactions with the *CysLT*$_1$ receptor. This was supported by the finding that minor structural changes of *CysLT*$_1$ agonists resulted in compounds with antagonistic activities.[16−21] Furthermore, several antagonists share identical structural elements with the agonists,[15] and hence a common binding site for agonists and antagonists is suggested.[22]

Essential structural requirements for *CysLT*$_1$ receptor ligands are (1) a lipophilic anchor which fits into the lipophilic pocket of the *CysLT*$_1$ receptor; (2) a central lipophilic and flat unit, which is believed to mimic the triene system of the natural agonist LTD$_4$; (3) one or two acidic groups, as potential mimics of the peptide unit and/or the C1-carboxylic acid of LTD$_4$; and (4) spacers to connect and preorganize these elements. Ligands lacking any of these interacting groups may compensate by stronger interactions in other regions of the receptor.[23] Since many ligands contain only one acidic moiety, this moiety may mimic the C1-carboxylic acid or the peptide carboxylate. It was suggested that the acidic moieties of most antagonists mimic the peptide carboxylate and that the C1-carboxyl of LTD$_4$ is involved in receptor activation rather than receptor binding.[19,24]

Several attempts for constructing a pharmacophoric model of *CysLT*$_1$ receptor antagonists have been reported.[22,24−26] Young[22,27] was the first to compare the molecular structures of *CysLT*$_1$ antagonists with that of LTD$_4$ to propose a conceptual model of the *CysLT*$_1$ antagonists (Chart 2). Terada and co-workers[24] have recently proposed a 3D QSAR model based on the comparison of **7** (Pranlukast, ONO-1078) and some derivatives with LTE$_4$. Although in this model the 3D alignment of the structural elements and even the activation site of the receptor was proposed, other classes of *CysLT*$_1$ antagonists were not included.

The structural overlapping of different classes of *CysLT*$_1$ antagonists was first studied by Palomer et al.[28] The model described was obtained by fitting the acidic residues of *CysLT*$_1$ antagonists and subsequently optimizing the lipophilic regions. It thus describes the alignment of the lipophilic residues and the acidic moieties of *CysLT*$_1$ antagonists but does not cover the putative "third pocket" of the *CysLT*$_1$ receptor. Fur-

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Chart 1. *CysLT*$_1$ Antagonists Included in the Building of a *CysLT*$_1$ Antagonist Model

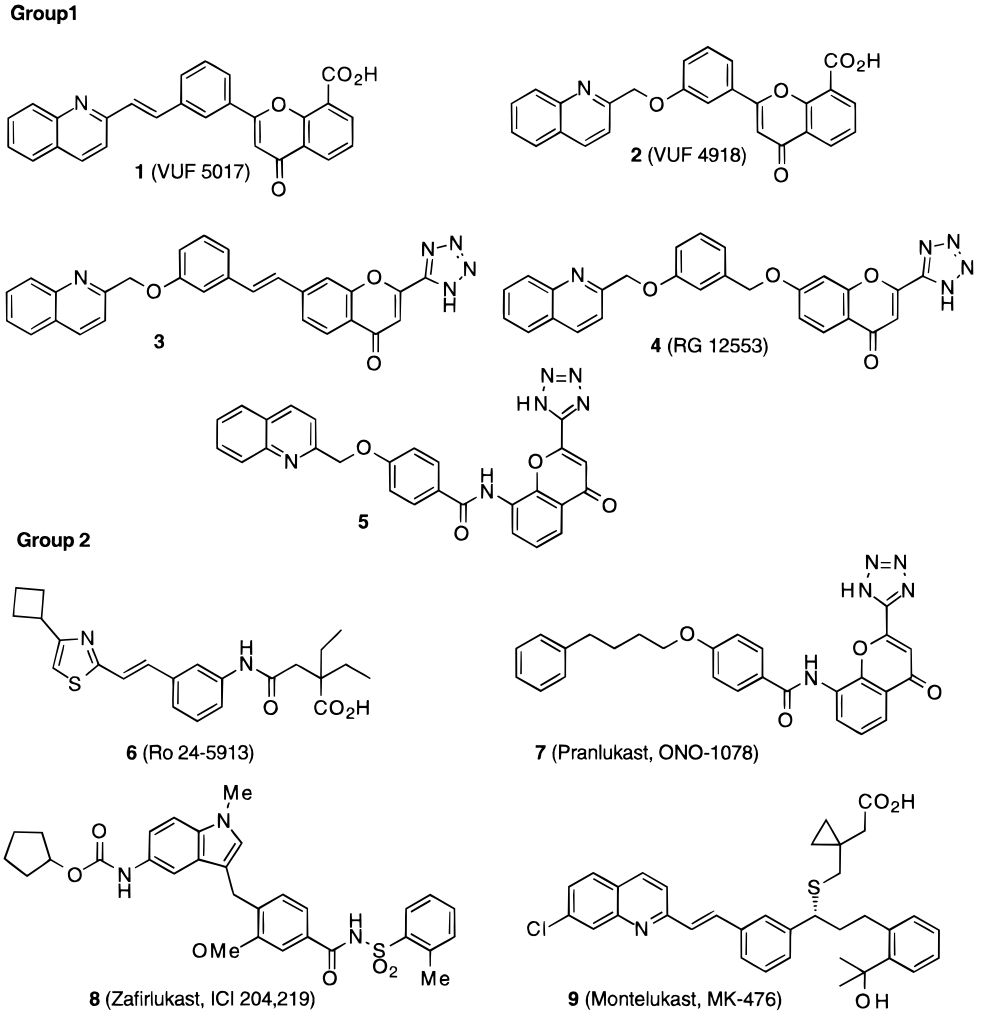
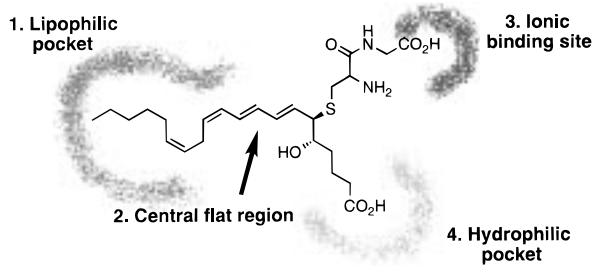


Chart 2. Conceptualized *CysLT*$_1$ Receptor According to Young[22]



thermore, a poor correlation for the more rigid ligands, such as LM 1368 (5-[4-[6-(2-quinolinylmethoxy)benzamidomethylnaphth-2-yl]phenyl]tetrazole),[28] indicates a suboptimal alignment of the ligands. Recently, a 3D QSAR model based on the comparison of biophoric patterns of the different *CysLT*$_1$ antagonists, especially the hydroxyacetophenones, was published.[29] The alignment of the ligands employed in this model is however in conflict with the generally accepted alignment of *CysLT*$_1$ antagonists based on observed SAR. For example, the tetrazole residue of the hydroxyacetophenone derivative LY 163443 (1-[2-hydroxy-3-propyl-4-[[4-(1*H*-tetrazol-5-ylmethyl)phenol]methyl]phenyl]ethanone) and the sulfonylamide moiety of Zafirlukast (ICI 204,219) (**8**) are known to be important because of their acidic character.[30−33] In the study of Hariprasad et al., however, these residues were assigned as the pharmacophoric equivalents of the quinoline found in MK-571 (3-[α-[3-[2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-α-[[3-(dimethylamino)-3-oxopropyl]thio]methylthio]propanoic acid) or thiazole present in Ro 24-5913 (**6**) [(*E*)-4-[[3-[2-(4-cyclobutyl-2-thiazolyl)ethenyl]phenyl]amino]-2,2-diethyl-4-oxobutanoic acid), which are normally designated as lipophilic regions. Although this model stimulates a critical evaluation of the generally agreed ideas, we feel there is too little experimental support for the proposed alignment.

The main difficulty in the development of a reliable 3D pharmacophoric model for *CysLT*$_1$ antagonists has been the flexibility of most antagonists. To gain a better understanding of the bioactive conformation of *CysLT*$_1$ antagonists, we have therefore developed a series of carboxyflavones as conformationally restrained *CysLT*$_1$ receptor antagonists.[34] Among these flavones are the most rigid *CysLT*$_1$ receptor antagonists known, having receptor affinities approaching the nanomolar range. In this paper we wish to describe the development of a 3D *CysLT*$_1$ antagonist model, using one of these potent and highly rigid flavones, **1** (VUF 5017, 8-carboxy-3′-[2-(2-quinolinyl)ethenyl]flavone), as the template.

Methods

We selected a number of *CysLT*$_1$ antagonists from the different structural classes, having both high potency and

Table 1. Data on Selected *CysLT$_1$* Antagonists

compound	*CysLT$_1$* affinities K_i or IC_{50} (nM)[a]	no. of conformations (no. of solutions remaining after fitting)	ΔE (kcal/mol)[b]
	Group 1		
1 (VUF 5017)	17	48	0.51
2 (VUF 4918)	17	104 (*11*)	6.13
3	2.5	147 (*3*)	8.80
4 (RG 12553)	3.0	157 (*1*)	8.11
5	1.1	211 (*1*)	8.53
	Group 2		
6 (Ro 24-5913)	6.4		
7 (Pranlukast)	1.0		
8 (Zafirlukast)	0.3		
9 (Montelukast)	0.5		

[a] Displacement of [^{3}H]LTD$_4$ binding, data are taken from ref 14 except for **1**, **2**, and **7** whose affinity data are taken from ref 34. [b] Energy difference of the lowest-energy conformation and the energy of the fitted conformation, as calculated in MACROMODEL using the Amber force field.

maximum rigidity (Chart 1). These compounds were divided into two groups. The first group (group 1: compounds **1**−**5**) contains the ligands having a relatively high rigidity. This group will provide the basis of the antagonist model. Group 1 ligands all contain a quinoline moiety, which we designated to be a pharmacophoric group and hence provided a fitting point when comparing the ligands. The second group (group 2: compounds **6**−**9**) contain ostensibly dissimilar structures. Group 2 ligands are mostly well-known and highly potent antagonists, including the clinical candidates Pranlukast (**7**), Zafirlukast (**8**), and Montelukast (**9**).

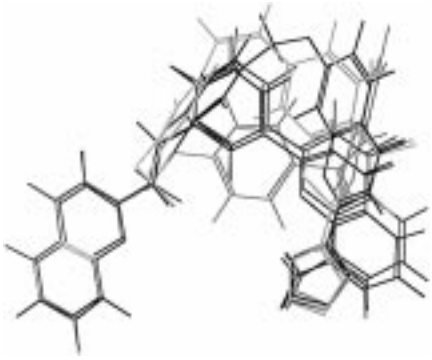
Our approach to the development of a *CysLT$_1$* antagonist model was to first establish a preliminary antagonist model by a quantitative comparison of the conformations of group 1 antagonists. Subsequently, this basic model could be verified and extended by the introduction of group 2 antagonists, using the acidic and lipophilic residues as the pharmacophoric points.

All minimum energy conformations of group 1 antagonists were calculated by a conformational analysis of the neutral species to prevent the strong electrostatic repulsion between the otherwise anionic group and the basic nitrogen of the quinoline moieties. All possible conformations were generated in MACROMODEL by starting from the (Newman) staggered minimum and rotating bonds by 120° (for sp^3-sp^3 bonds), 60° (sp^2-sp^3 bonds), or 180° (sp^2-sp^2 bonds). These conformations were minimized using the AMBER force field. All conformations within 10 kcal from the lowest-energy conformation located were selected. This 10 kcal/mol criterion was chosen to account for the increase in energy when the lowest-energy conformation was found to be distorted due in many cases to an intramolecular H-bond. Therefore, local minima having no H-bond lie artificially about 5 kcal higher in energy with respect to the lowest-energy conformation located.[35] The number of conformations obtained for group 1 antagonists is shown in Table 1.

All local minima within 10 kcal/mol of the lowest-energy conformation located were combined in a database. Compound **1** (VUF 5017), the most rigid antagonist, was taken as the template onto which other antagonists of group 1 were fitted. In this cross-fitting procedure, all conformations of a second ligand were rigidly fitted on all conformations of **1**. Subsequently, all solutions were taken, and onto these solutions a set of low-energy conformations of a third ligand were fitted, and so on. This procedure was repeated until all five ligands of group 1 were introduced into the model.

The quinoline heterocycle and the acidic moieties were selected as fitting points. For the quinoline, N1, C4, C5, and C8 were selected to obtain a complete overlap of this heterocyclic moiety (restraint values 50). For a fit of the acidic residues, initially the carbon atoms of the bond connecting the acid to the heterocycle were selected as fitting atoms (restraints 100). Although in the solutions the quinoline moieties could be fitted on top of each other very nicely, we did not succeed in this way to gain a satisfactory overlap of the acidic residues. We realized however that for the tetrazole moieties the negative charge could be delocalized over all tetrazole nitrogen atoms. Therefore, the carbon atoms connecting the acidic groups and the heterocycles (i.e., chromones or benzopyrans) do not necessarily overlap and are still capable of interacting with the same amino acid residue.

Chart 3. Cross-Fitting of Group 1 Antagonists



Comparison of the chromone and flavone structures shows that both groups contain a ketone carbonyl group. Although the chromone residue has not been subjected to extensive SAR studies, in the development of Pranlukast (**7**) it was observed that replacement of the chromone by other bicyclic systems devoid of a ketone carbonyl group (e.g., benzodioxans, quinolines, benzofurans, or naphthalenes) resulted in significant decrease of activity.[36] Since several other *CysLT$_1$* antagonists also contain ketone carbonyl groups in this region,[37−39] this moiety may be prone to specific interactions with the receptor. We therefore selected this carbonyl (restraint value 2) and the carbon atoms of the acidic moieties (restraint 100) as fitting points. This yielded fits with orientations of the acidic residues set much better for forming hydrogen bonds to a basic amino acid residue.

The quality of the fits was judged both by eyeball on the overall overlap of the backbones and by small distances between the fitting points (<1 Å for the quinoline parts and the acidic groups when they are carboxylic acids and <2 Å for the acidic groups when one of them is a tetrazole). The decrease of the solutions from the database fitting after inclusion of an additional ligand is shown in Table 1. After fitting of group 1 antagonists, one unique solution was obtained, which is depicted in Chart 3. To test whether this solution was dependent on the sequence of the fitted ligands, we repeated the database fitting using another ranking order. The same fit was found, and we therefore took the fit as shown in Chart 3 as the basis for further refinement.

All energy differences between the fitted conformations and the corresponding lowest-energy conformations (Table 1) lie within 9 kcal/mol. Especially the energy difference of **1** is relatively low. The energy differences for compounds **2**−**5** are somewhat higher, but their lowest-energy conformations are artificial due to the formation of intramolecular hydrogen bonds. Taking this into account, the energies of the fitted conformations are satisfactory.

Results

The importance of an acidic residue suggests that this part of *CysLT$_1$* antagonists may interact with a positively charged amino acid residue of the receptor. The amino acid residues with a potential positive charge are lysine, arginine, and histidine. It is known in the literature that one or more arginine residues are important for binding and/or activation of several G-protein-coupled receptors, e.g., muscarinic receptors,[40,41] C5a receptor,[42] thyrotropin-releasing hormone receptor,[43] human growth hormone receptor,[44] and glycine

receptor.[45] Most interestingly, an arginine residue in the seventh transmembrane domain of the prostaglandin E receptor EP3D was found to interact with the negatively charged α-carboxylic acid of PGE_2 and is important for the activation of the receptor by PGE_2 but not by other nonacidic agonists.[46,47] Giving the fact that PGE_2 is also a carboxyl-containing lipid metabolite of arachidonic acid, it is not unreasonable to suggest that an arginine residue in the *CysLT*$_1$ receptor is also important for binding to its ligands. Evaluation of the molecular electrostatic potential of the conformations of antagonists **1**−**5** that resulted from the fit procedure, but now with an anionic acidic group, shows a high complementarity with the molecular electrostatic potential of arginine, rather than lysine or histidine. Furthermore, since the positive charge of an arginine residue is delocalized over the whole guanidine system and this residue is able to form two hydrogen bonds with, for example, a carboxylate, this residue is more likely to have strong interactions with acidic moieties than a lysine or histidine residue which can only be involved in one hydrogen bond. Therefore we incorporated an arginine residue as an interaction site for the acidic moieties into the preliminary model.

Intermolecular interactions between delocalized systems are considered not to be adequately taken into account by classical molecular mechanical approaches. Therefore we used a quantum chemical approach to calculate the interactions between acidic moieties occurring in *CysLT*$_1$ antagonists, e.g., carboxylic acid, tetrazole, and sulfonylamide, and an arginine residue. To this end we applied a density functional approach and used the ADF program package (version 2.0.3a).[48,49] The underlying theory of the ADF program is the Kohn−Sham approach to density functional theory. This implies a one-electron approach to a many-electron system yet yields, in principle, exact properties such as the electron density and the total energy. Triple ζ basis functions plus polarization have been applied. The cores of C, N, and O were frozen up to 1s; the core of S was frozen up to 2p. For the local part of the density functional (also called the exchange and correlation functional) we selected the Vosko−Wilk−Nusail approximation, and for the nonlocal part (also called gradient corrections) we used the combined Becke/Perdew approach. Becke's gradient correction is usually considered as a correction to the exchange part of the potential, whereas Perdew aims at correcting the correlation part.[50−52] For the necessity of using gradient correction (especially for the resulting geometries) when calculating intermolecular interactions (with emphasis on H-bonds), the reader is referred to Sim et al.[53] The optimal interactions obtained from these calculations were fixed, which enabled the positioning of the guanidine system of the arginine residue with respect to the antagonist. The arginine Cα*C*β was assumed to always take the same position, as this is the site at which the flexible residue is connected to the receptor. Initially, the arginine chain was set in the energetically most favorable all-trans conformation. After introduction of the arginine residue, the conformations of the ligands in the fit were minimized in ChemX using the van der Waals force field and the C*γ*-N*ϵ* chain as well. The overlap of the resulting conformations, which strongly resemble the conformations obtained without the arginine residue, is shown in Chart 4. Besides the interactions between the arginine and the acidic residue, a hydrogen bond between the arginine and the quinoline nitrogen atom was observed, as exemplified for VUF 5017 (**1**) in Figure 1. This may very well explain the importance of the quinoline nitrogen, which was found for all quinoline-containing *CysLT*$_1$ antagonists.

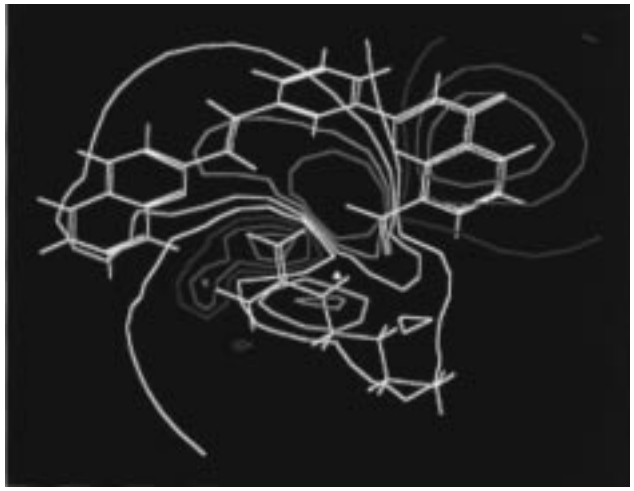
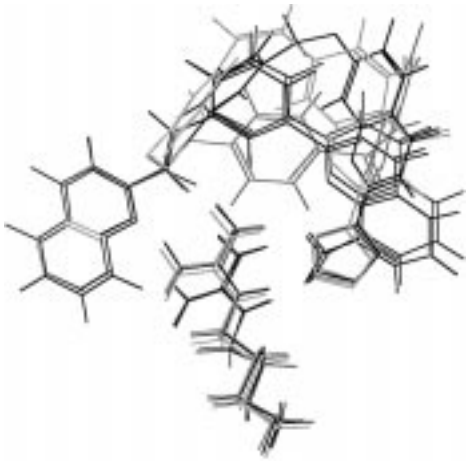


Figure 1. Electrostatic potential map of interactions between VUF 5017 (**1**) and an arginine residue (−100, red; −66, orange; −33, yellow; 0, white; 33, green; 66, blue; 100, magenta).

Chart 4. Interactions of Group 1 Antagonists and an Arginine Residue



The model obtained so far consists of an arginine residue having multiple interactions with the antagonists. For compound **5** an interaction between the *π*-cloud of the central pheny1 ring and the arginine was suggested. Due to a different relative alignment of the chromone and the central phenyl rings, this interaction was not found for other ligands.

Subsequently, we included group 2 antagonists **6**−**9** into the model. Only the lowest-energy conformations of this group of compounds were used, and they were obtained by a conformational analysis, starting from the staggered minima, performed with ChemX 95 using the same bond rotations as described above. The sulfonylamide part of **8** was taken from the Cambridge Structural Database (reference code CEKHIJ). The arginine residues were attached to the acidic moieties and fixed in their optimized alignments as determined with the

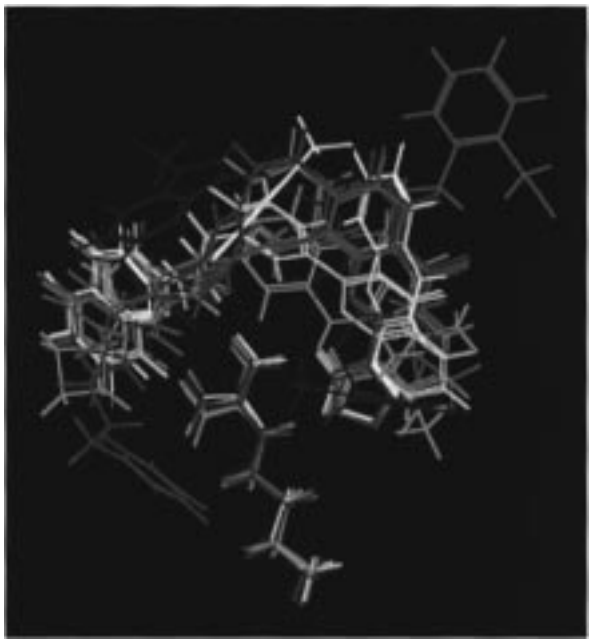


Figure 2. *CysLT$_1$* antagonist model: **1**, yellow; **2**, red; **3**, green; **4**, white; **5**, light blue; **6**, orange; **7**, light purple; **8**, blue; **9**, cyan.

density functional theory as described above. As fitting points we selected the arginine CαCβ and a fitting point in the lipophilic part. The selection of the latter fitting point was different for the separate ligands. For **6** (Ro 24-5913) the thiazole nitrogen was fitted flexibly to the quinoline nitrogen atoms and the rest of the thiazole was constrained in the plane of the quinoline ring. The quinoline moiety of **9** (Montelukast) was fitted in a flexible manner to the other quinoline moieties, while for **8** (Zafirlukast) the oxygen atom attached to the cyclopentyl was fitted to the quinoline nitrogen atom (restraint 10). The benzamide part of **7** (Pranlukast) was fitted onto the equal benzamide moiety of compound **5**.

Although the interaction of the acidic residues with the arginine as calculated with the ADF procedure provides a solid quantitative basis, we have fitted group 2 antagonists to the preliminary model using a qualitative approach. The main reason for this was the high flexibility of the latter compounds, which hampered a reliable conformational analysis. Despite large structural differences among the ligands, a satisfactory overlap of group 2 antagonists with the previously fitted antagonists was obtained, as shown in Figure 2.

Discussion

We have proposed a general *CysLT$_1$* antagonist model, based on analysis of representatives of several classes of *CysLT$_1$* receptor antagonists. (Figure 2). The model describes the position and interactions of the lipophilic moieties and the acidic residues, known as important structural features for *CysLT$_1$* receptor antagonists. An arginine residue of the receptor has been proposed as a basic interaction site for the acidic moieties. The antagonists may have multiple interactions with this arginine residue. Besides the hydrogen bonds between the acidic residues and the arginine guanidine moiety, additional interactions of the ligands with the guanidine group have been suggested. Quinoline-containing *CysLT$_1$* antagonists are shown to form an additional hydrogen bond between the quinoline nitrogen atom and a guanidine hydrogen atom, as shown in Figure 1. This interaction may be an explanation for the importance of the presence and position of the nitrogen atom, as is seen for most *CysLT$_1$* antagonists of the quinoline class. A qualitative correlation between pK_a's of the quinoline nitrogen and the *CysLT$_1$* receptor affinity has recently been reported.[54] The π-system of the terminal phenyl moiety of **7** and the central phenyl ring of compound **5** are also likely to interact with arginine hydrogen atoms.

The alignment of **9** (Montelukast) suggests the presence of an additional pocket in the binding site for *CysLT$_1$* antagonists. Whether this pocket also accommodates the third arm of LTD_4 remains to be investigated. It would be interesting to see compounds designed to occupy this pocket and one of the other two sites.

The selection of an arginine residue as a basic interaction site for the acidic residues of the ligands, in the *CysLT$_1$* antagonist model was guided by literature reports on the importance of this residue in several G-protein-coupled receptors in binding to their ligands, the complementarity of the electrostatic potentials of the antagonist conformations and arginine, and the potential formation of multiple hydrogen bonds. Preliminary experiments with an arginine-masking agent (phenylglyoxal) demonstrated that the potency of tested antagonists ICI 198,615 and **7** in displacing bound [^{3}H]-LTD_4 decreased over 5 log units after the blockade of arginine residues.[55] Since the affinity of LTD_4 itself was little affected, arginine residues were suggested to be important for the binding of antagonists but not for LTD_4. More experiments are necessary to establish the role of an arginine in interaction with *CysLT$_1$* antagonists. Taken together, our results indicate that a pharmacophoric model based on structural similarity of the agonist LTD_4 and various antagonists may not be valid. After all, antagonists do not necessarily bind to the same site or in the same manner as agonists.

The present model is the first 3D *CysLT$_1$* antagonist model based on structures of the most important classes of *CysLT$_1$* antagonists and includes an amino acid residue of the receptor. Consequently, this model contributes to a better understanding of the molecular recognition of the leukotriene *CysLT$_1$* receptor.

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