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Identification of a pharmacophore of SKCa channel blockers

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Abstract

Small conductance calcium-activated potassium channels (SK) are widely expressed throughout the central nervous system (CNS) and the periphery. Three subtypes of SK channels have so far been identified in different parts of the brain. Activation of the SK channels by a rise in intracellular calcium leads to the hyperpolarisation of the membrane, reducing cell excitability. Blocking the SK channels might be beneficial in the treatment of depression, Parkinson's disease and cognitive disorders. However, few blockers of SK channels have been characterized. In this study, a pharmacophoric model of SK channels blockers is presented. It is based on a series of nonpeptidic compounds and apamin, a peptidic blocker. To create the pharmacophore model, the conformational space of nonpeptidic blockers was investigated to generate a series of distance constraints applied to a simulated annealing study of apamin. The resulting conformation was superimposed with the nonpeptidic blockers to give a pharmacophore.

Keywords: *SK channels, blockers, apamin, dequalinium, pharmacophore, model*

Introduction

There are numerous ways to modulate neuronal activity, such as transporters, neurotransmitters and receptors, which have been thoroughly employed to develop new drugs. However, ion channels open a new opportunity in this domain, and have not yet been extensively exploited.

Among the many channels present in the central nervous system (CNS), Ca^{2+}-activated potassium channels seem to be very promising. This family is diverse and its members differentially expressed in the CNS. These channels have also been identified in peripheral tissues. Whatever their location, their activation by an increase in the cytoplasmic calcium concentration leads to the hyperpolarisation of the membrane, thus inhibiting cell excitability.

Three families of Ca^{2+}-activated potassium channels have been so far identified, which can be separated on both biophysical and pharmacological characteristics. They are called BK, IK, and SK channels, standing for Big, Intermediate and Small conductance K^+ channels, respectively [1,2]. This study will only focus on the SK channels.

Small conductance Ca^{2+}-activated K^+ channels (SK or SK_{Ca}) have a conductance between 2 and 20 picosiemens [3]. They were first discovered in skeletal muscle [4], then in other tissues, such as neurons or smooth muscle [5]. Moreover, structural and pharmacological studies have suggested the existence of

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subtypes of SK channels, which have been differentiated by DNA cloning, giving rise to SK1, SK2, and SK3 subtypes [6].

Structurally speaking, they are homo- or hetero-tetramers of subunits composed of six transmembrane domains [2] and their activation by calcium is mediated by a calmodulin unit bound to each intracellular C-terminal extremity [7,8].

Few blockers are known. Venoms of arthropods or insects contain natural peptides such as apamin and scyllatoxin, while a small number of nonpeptidic blockers have been discovered, including tubocurarine, dequalinium and some compounds derived from a structure-activity relationship study of the latter and more recently *N*-methyl laudanosine [9,10].

Blockers of SK channels are of great interest in therapeutics. For example, gastro-intestinal motility can be raised by SK channels blockers [11]; they may also improve memory [13] or mood [9] and abnormalities in the expression of SK-channels are involved in the pathogenesis of myotonic muscular dystrophy [12].

The aim of this study is to define a pharmacophore model from different peptidic and nonpeptidic compounds for blocking SK channels. This pharmacophore could then be used to design new molecules which fulfill the conditions found in our study.

Materials and methods

All the calculations were performed using the SYBYL 6.9.1 molecular modelling package [14] on Silicon Graphics Octane 2 workstations.

Selection of the blockers

Apamin was selected as the primary peptidic blocker. (Figure 1) This compound extracted from honey bee (*Apis mellifica*) venom, is an octadecapeptide structurally held rigid by two disulfide bridges and displays a subnanomolar affinity for SK2 and SK3 subtypes [9]. The activity of apamin is mediated by two arginines in positions-13 and-14 maintained in an active conformation by the whole structure [15]. It is noteworthy that the arginine residues are protonated *in vivo* to give the active guanidinium moieties.

Seven nonpeptidic blockers with a nanomolar activity on the SK2/3 subtypes were also selected. (Figure 2) These compounds are dequalinium, [10] the first nonpeptidic compound discovered, and three bisquinolinic analogues, [16] named BQ1-3 for convenience, as well as three bisquinoliniums in cyclophane structures, namely UCL 1684, UCL 1848, and UCL 1530 [17–20]. They are more or less rigid and possess two protonatable or protonated

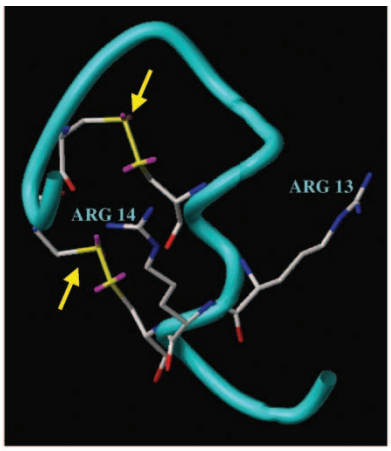

Figure 1. Structure and sequence of apamin (the two disulfide bridges are indicated by yellow arrows).

quinoline moieties, thus mimicking the arginine residues of apamin. The spacers linking the quinoline rings are thought to play a role in the positioning of the quinolinium groups in their bioactive conformation [21,22].

Conformational analysis

As the structure of the SK channel is currently unknown, the conformational space of each compound was explored in order to create a pool containing their putative bioactive conformation. This study has been conducted in two separated steps.

Nonpeptidic blockers were considered first in order to build a heuristic pharmacophore model. Their conformational space was generated using the method CONFEX [23] based on the distance geometry program DGEOM [24]. Distance geometry is a general method for converting a set of distance constraints into a set of three-dimensional coordinates consistent with the constraints. The distance constraint matrix describes completely the conformational space of a molecule by including the maximum possible distance between each atom pair and the minimum possible distance. DGEOM produces a rapid and efficient sampling of conformational space by selecting random distances within these two extreme values and cannot guarantee to cover all of conformational space. To palliate this disadvantage, the program CONFEX was developed to generate with DGEOM a conformational space at a defined resolution threshold up to the selected convergence criteria. CONFEX runs in a two-step procedure. An initial set of 200 conformers was created. All conformations presenting an energy higher than a cutoff value of 1000 Kcal/mol were eliminated. A

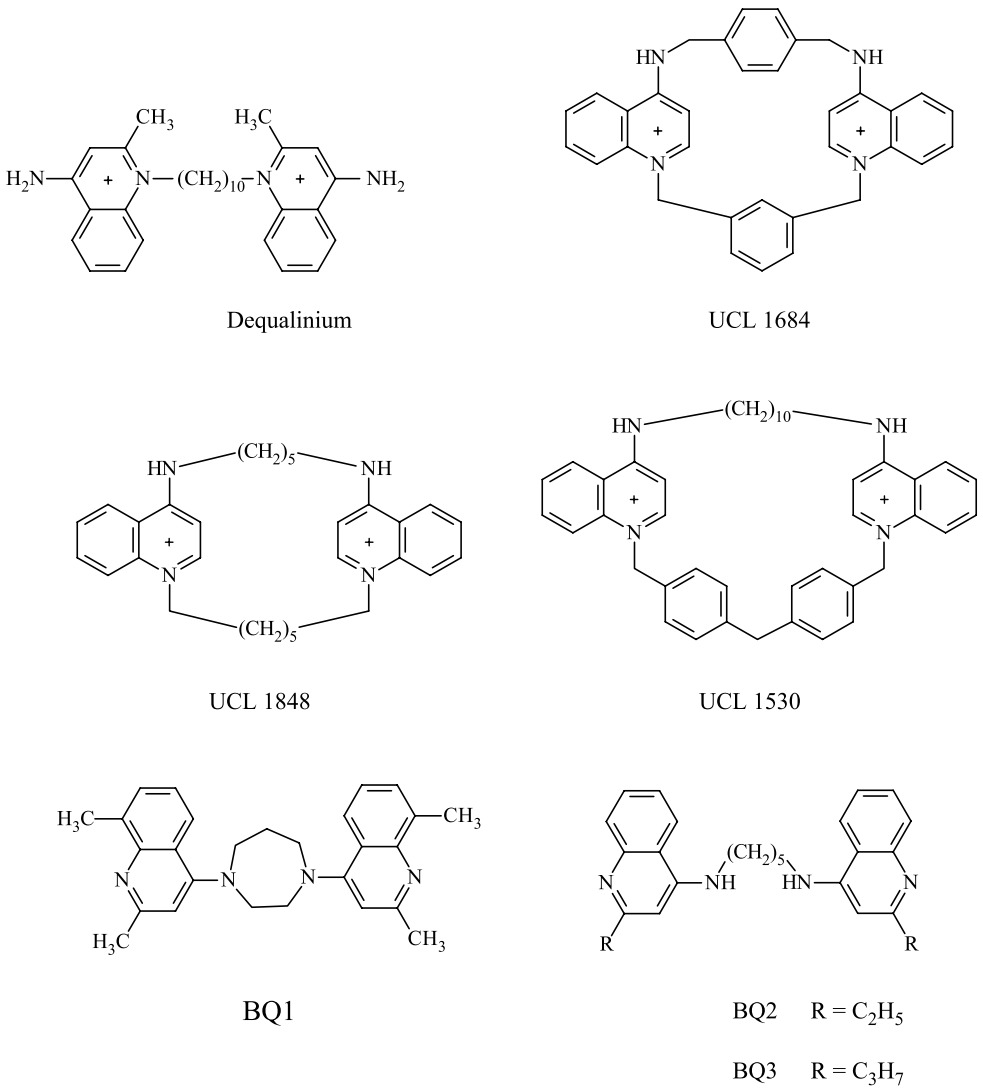

Figure 2. Structure of the nonpeptidic blockers.

topological cutoff, based on the Root Mean Square (RMS) value between the heavy atoms of the molecule was also employed to keep the most different conformations. The resulting database was then iteratively enlarged by the generation of new conformational sets, with the rejection of newly generated conformers based on their energy and their topological comparison with the conformers already in the database. This enrichment process was stopped after five cycles had produced no new conformation. The whole conformational set was subsequently optimized using the Tripos force field [25] including the electrostatic term calculated from Gasteiger and Hückel atomic charges. The method of Powell available in the Maximin2 procedure was used for energy minimization until the gradient value was smaller than 0.001 Kcal/mol Å.

Secondly, the conformational space of apamin was investigated. Its great flexibility is somewhat restricted by the two disulfide bridges, but nonetheless excludes the possibility of an exhaustive enumeration of its possible conformers. Moreover, such an enumeration would have been pointless when balancing the results required to carry on the study and the time consumed by the calculations. This problem was treated by generating a limited conformational space allowing only a gross coverage of the whole space but in the meantime focused toward the putative bioactive conformation of apamin. In order to achieve this targeted conformational analysis, apamin was submitted to a constrained simulated annealing. This peptide was heated to 1000 K for 1 picosecond, then cooled to 300 K for another 1 picosecond interval, following an exponential cooling model. 200 annealing cycles were completed under a set of distance constraints extracted from the nonpeptidic pharmacophore model.

Generation of the pharmacophoric model

Two different methods, namely DISCO [26] implemented in SYBYL, and the Align-list module of SURFLEX [27] were used. In fact, the former uses

a full conformer enumeration and a one-on-one superimposition attempt of all conformers for each compound involved, the best model corresponding to the lowest Root Mean Square (RMS) value and a given set of superimposed conformers. The latter is based on a similarity score derived from an alignment of multiple compounds on a reference molecule. In both cases, a number of atoms have to be marked as the key features for the alignment attempt. Recent works [28,29] strongly suggested the inclusion in the model of the charged or protonable nitrogens and the centres of both aromatic groups bearing them, as the charge must be delocalised on the whole aromatic structure. This results in a total of four pharmacophoric points.

Results and discussion

The number of conformations found for each molecule (Table I) is in direct relation to their flexibility. This fact has led us to turn to the generation of a nonpeptidic model of pharmacophore with a good chance of finding the probable bioactive conformation of the studied compounds in the conformational space generated without having to cycle again through the conformational analysis process. However, such models were thought to be of low interest, as they do not include the reference blocker, apamin. Their goal is to provide meaningful constraints for the exploration of the conformational space of apamin.

Nonpeptidic pharmacophoric models

In order to generate at least a model with insight, we used two pharmacophore researches with DISCO, using respectively UCL 1684 and UCL 1848 as references, as these compounds are the most rigid available in the set and display a high affinity [17–20] The retained elements for the pharmacophore generation are the positively charged nitrogens and the aromatic rings of both quinolines. UCL 1684 yielded 30 models, while UCL 1848 gave only 17 models. This result is in keeping with the lower flexibility of UCL1848 compared to UCL1684 in the energetic window allowed by the minimisation of the sets of conformers. To summarise, more conformations of UCL1848 were closely related to the same energy minima than those for UCL1684. The selection of the best model was supported by the lowest possible RMS value and the comparison of the number of pharmacophoric elements conserved by the superimposition proposed by the program (Tables II and III). It eventually reduced to a single comparison between UCL 1684 model 9 (RMS 1.030 Å) and UCL 1848 model 11 (RMS 1.145 Å). Both are very similar in the resulting superimposition of the molecules, so we chose the model showing the lowest RMS value, UCL 1684 model 9. It is interesting to note that a number of models have been created with only three of the four pharmacophoric elements thought to be important for the activity, giving them, in fact, a worse superposition of these elements, but an overall better fit of the heavy atoms of the molecules and thus a lower RMS value (UCL 1684 model 11 with an RMS of 0.831 Å, for example) (Tables II and III).

Lastly, the compounds were superimposed using the Align-list module of SURFLEX. Even if it is not really a pharmacophore-oriented alignment, since Align-list only fits a series of molecules on a reference, it has nonetheless permitted the achievement of a superimposition fairly like the models generated by DISCO. However, the impossibility of weighting the atom pairs in the alignment has led to some larger discrepancies in the position of the nitrogens relative to their counterpart in the reference compound when comparing this superimposition with the DISCO models.

The DISCO-generated UCL 1684 model 9 (Figure 3) was selected to continue the study. This model is in extremely good agreement with recent works [28,29] pointing toward a distance of 5.80 Å between the centres of the pyridinium rings and heralding the importance of the polarizability of a compound to bind to SK channels. These two points are taken into account in the chosen pharmacophore, with a distance of 6.20 Å between the pyridinium rings and the inclusion of the charged nitrogens which are separated by a distance of 5.62 Å.

This study has also led to the perception of some of the limitations and advantages of the two softwares tested. DISCO proved to be more adaptatable than Align-list but somewhat more cumbersome to use, while Align-list appeared to be highly efficient in aligning compounds, but with the major drawback that the user cannot alter the weighting of the atom pairs and thus target a specific group alignment rather than a global structural superimposition. The major limitation of DISCO is its restricted set of features used to design the pharmacophoric elements. The aromatic groups involved in the delocalisation of the charge due to the charged nitrogen, which seems to play a major

Table I. Number of conformers found for each nonpeptidic blocker.

Compounds	Dequalinium	UCL1684	UCL1848	UCL1530	BQ1	BQ2	BQ3
n	47	21	11	30	42	24	31

Table II. Models generated from UCL 1684 (⋆ indicates a model built on 3 of the 4 pharmacophoric elements chosen). RMS scores are given in Ångströms.

Model	RMS score
1	1.261
2	1.248
3	1.174
4⋆	1.199
5⋆	0.908
6⋆	1.271
7⋆	0.889
8	1.188
9	1.030
10⋆	1.044
11⋆	0.831
12⋆	1.200
13⋆	0.927
14	1.169
15	1.170
16	1.218
17	1.055
18⋆	1.201
19⋆	0.905
20⋆	1.268
21⋆	0.891
22	1.136
23	1.315
24	1.173
25	1.092
26	1.230
27	1.256
28	1.149
29	1.208
30	1.297

part in the binding of the blockers, are treated as hydrophobic centres, with no consideration of their real role in the biological interaction scheme. Similarly, nitrogen can only bear a 0 or +1 charge, with no possible delocalisation.

Table III. Models generated from UCL 1848 (⋆ indicates a model built on 3 of the 4 pharmacophoric elements chosen). RMS scores are given in Ångströms.

Model	RMS score
1⋆	1.165
2	1.316
3	1.199
4	1.185
5⋆	1.034
6	1.236
7⋆	1.138
8⋆	1.368
9	1.160
10	1.181
11	1.145
12	1.425
13⋆	1.202
14	1.232
15	1.163
16	1.207
17⋆	0.894

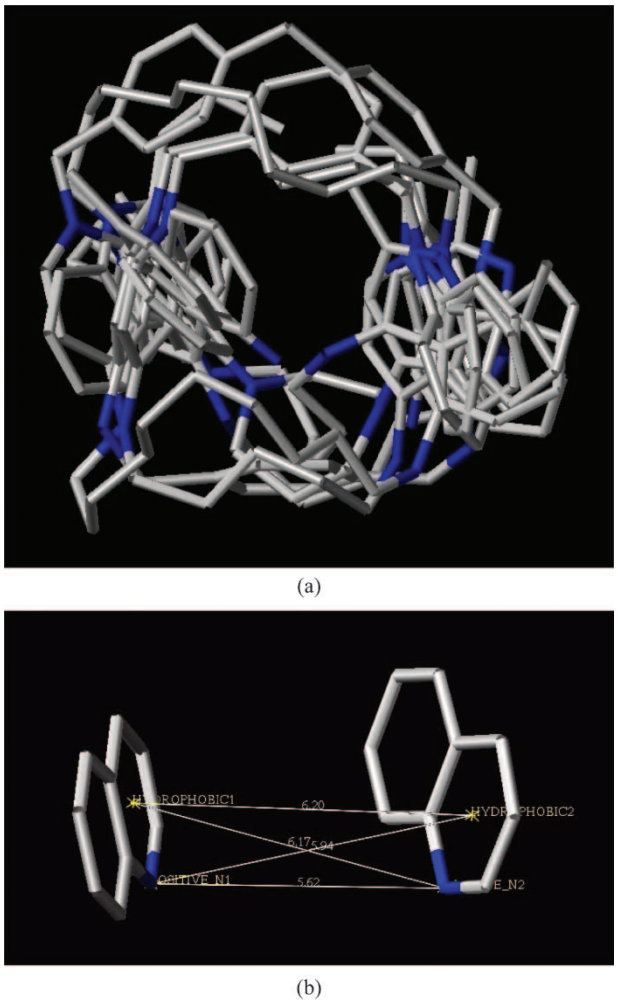

Figure 3. UCL 1684 model 9; (a) superimposition of the nonpeptidic blockers; (b) 4-point pharmacophoric model showing the distances between the pharmacophore elements.

Conformational analysis of apamin and construction of the final model

As a means to restrict the conformational space of apamin, a distance constraint between the guanidines of arginine-13 and-14 was applied. This constraint has been extracted from the nonpeptidic model, which shows a 5.62 Å distance between the charged nitrogens. The conformational space generated by simulated annealing amounted to 200 conformers, which were subsequently minimised. However, this minimisation had the predictable effect of dragging several conformers toward the same energy minimum. The duplicated conformations were removed while being sure to conserve the lowest energy ones. To achieve this, a simple topological comparison was realised, leading to the reduction of the initial 200-conformation space to a mere 11-topological group space. The lowest energy conformation of each group was selected as representative of the whole group, practically working with 11 different

conformations only, compared to the flexibility of a 18-residue peptide. Lastly, each of these conformers was aligned manually with the nonpeptidic model in order to identify the best superposition of apamin with the putative pharmacophore. It appeared that the best fit (Figure 4) was achieved with a conformation having an energy 7 Kcal/mol higher than the lowest energy conformer. The selected conformation of apamin appears as a reasonable hypothesis for its bioactive conformation.

Conclusion

The goal of this study was to establish a pharmacophoric model of SK channel blockers. As no 3D structure of these channels is currently known, a peptidic reference blocker, apamin, and a series of nonpeptidic molecules from the literature were selected. The flexibility of apamin required to proceed in two steps. The first step was the generation of a heuristic model from the nonpeptidic compounds, based on their conformational analysis. This first model led to distance constraints being derived between the two arginines of apamin known to be important for its blocking activity. These constraints permitted exploration of the conformational space of apamin accurately and as thoroughly as we could while balancing this time-consuming operation with the limited nature of the information required to carry on the study. Apamin was found to fit well with the previous pharmacophore, which is in very good agreement with the data available in the literature, thus assessing the validity of the pharmacophore. It offers important structural insight into designing novel SKCa channel blockers prior to their synthesis and permits us to perform a similarity search into available databases to find new leads.

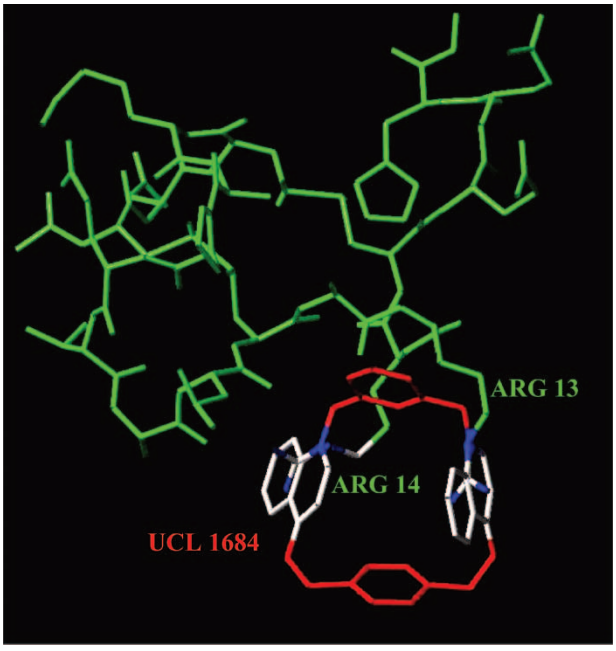

Figure 4. Superimposition of apamin (in green) and UCL 1684 from model 9 (in red); pharmacophoric elements are shown in colour by atom type.

Acknowledgements

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References

[1] Sah P, Trends Neurosci 1996;19:150–154.
[2] Vergara C, Latorre R, Marrion N, Adelman JP, Curr Opin Neurobiol 1998;8:321–329.
[3] Sah P, Faber ESL, Prog Neurobiol 66 2002;:345–353.
[4] Romey G, Lazdunsky M, Biochem Biophys Res Commun 1984;118:669–674.
[5] Kohler M, Hiscberg B, Bond CT, Kinzie JM, Marrion NV, Maylie JA, Adelman JP, Science 1996;273:1709–1714.
[6] Chandy KG, Fantino E, Wittekindt O, Kalman K, Tong LL, Ho TH, Gutman GA, Crocq MA, Ganguli R, Nimgaonkar V, Morris-Rosendahl DJ, Gargus JJ, Mol Psychiatry 1998;3:32–37.
[7] Keen JE, Khawaled R, Farrens DL, Neerlands T, Rivard A, Bond CT, Janowsky A, Fakler B, Adelman JP, Maylie J, J Neurosci 1999;19:8830–8888.
[8] Schumacher MA, Rivard AF, Bachinger HP, Adelman JP, Nature 2001;410:1120–1124.
[9] Liégeois J-F, Mercier F, Graulich A, Graulich-Lorge F, Scuvée-Moreau J, Seutin V, Curr Med Chem 2003;10: 625–647.
[10] Castle NA, Haylett DG, Morgan JM, Jenkinson DH, Eur J Pharmacol 1993;236:201–207.
[11] Gater PR, Haylett DG, Jenkinson DH, Br J Pharmacol 1985;86:861–868.
[12] Behrens MI, Jalil P, Serani A, Vergara F, Alvarez O, Muscle Nerve 1994;17:1264–1270.
[13] Belcadi-Abassi W, Destrade C, Neuroreport 1995;6: 1293–1296.
[14] SYBYL 6.9.1 - Molecular Modeling Software. Tripos Inc., 1699 South Hanley Road, St. Louis, MO 63144-2913, U.S.A. 2001.
[15] Demonchaux P, Ganellin CR, Dunn PM, Haylett DG, Jenkinson DH, Eur J Med Chem 1991;26:915–920.
[16] Coghlan MJ, Gopalakrishnan M, Carroll WA, Annu Rep Med Chem 2001;36:11–20.
[17] Campos Rosa J, Galanakis D, Ganellin CR, Dunn PM, J Med Chem 1996;39:4247–4254, UCL1530.
[18] Dunn PM, Benton DCH, Campos Rosa J, Ganellin CR, Jenkinson DH. 1996;117:35–42, UCL1530.
[19] Campos Rosa J, Galanakis D, Ganellin CR, Dunn PM, Jenkinson DH, J Med Chem 1998;41:2, UCL1684.
[20] UCL1848 is described in [10]
[21] Galanakis D, Davis CA, Del Rey Herrero B, Ganellin CR, Haylett DG, Jenkinson DH, J Med Chem 1995;26:595–606.
[22] Campos Rosa JC, Galanakis D, Ganellin CR, Dunn MD, J Med Chem 1996;39:4247–4254.
[23] Goldstein S, Neuwels M, Moureau F, Berkmans D, Lassoie MA, Differding E, Houssin R, Hénichart JP, LIPS 1995;2:125–134.

[24] Crippen GM. Distance Geometry and conformational calculations. New York: Research Study Press; 1981.
[25] Clark M, Cramer RD, Van Opdenbosch N, J Comp Chem 1989;10:982–1012.
[26] Martin Y, Bures M, Dahaner E, Delazzer J, Lico I, Parvlick P, J Comp-Aid Mol Des 1993;7:83–102.
[27] Jain AN, J Comp-Aid Mol Des 2000;14:199–213.
[28] Galanakis D, Ganellin CR, Chen JQ, Gunasekera D, Dunn PM, Bioorg Med Chem Lett 2004;14:4231–4235.
[29] Galanakis D, Ganellin CR, Bioorg Med Chem Lett 2004;14:4031–4035.