Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 3267–3272

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

BLOCKERS OF HUMAN T CELL KV1.3 POTASSIUM CHANNELS USING DE NOVO LIGAND DESIGN AND SOLID-PHASE PARALLEL COMBINATORIAL CHEMISTRY

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Received 23 August 1999; accepted 14 September 1999

Abstract: Blockers of the human Kv1.3 potassium channel were designed using Biosym/MSI's ligand design program LUDI. Parallel combinatorial synthesis of the resultant substituted phenyl-stilbenes on solid phase, followed by ^{125}I Charybdotoxin (^{125}I ChTx) screening, yielded 12 Kv1.3 channel blockers with modest activity.

Introduction

One of the early events in T cell activation is an increase in intracellular Ca^{+2} concentration, ultimately resulting in cytokine production and cellular proliferation.[1] The modulation of intracellular [Ca^{2+}] in turn depends upon the activation of Kv1.3 channels,[2–5] which are voltage gated potassium ion channels found predominantly in T cell membranes.[6,7] Since blocking Kv1.3 channels prevents increases in intracellular [Ca^{2+}] that are necessary for T cell proliferation,[8] these channels are an attractive target in the search for new immunosuppressive agents.

Since the discovery of Kv1.3 channels in T cells, several groups[9–11] have attempted to identify specific and potent Kv1.3 channel blockers that might cause fewer side effects than the immunosuppressants currently in use. Although a crystal structure for the Kv1.3 channel does not exist, Chandy and Ayiar have identified nine pairs of toxin-channel interactions in a model[15,16] of the Kv1.3 vestibule based on mutant cycle analyses,[12,13] electrostatic compliance,[14] and mutagenesis of the picomolar affinity scorpion toxins, kaliotoxin (KTx), margatoxin (MgTx), charybdotoxin (ChTx), and noxiustoxin (NTx) bound to the Kv1.3 channel. Our approach in designing potential immunosuppressants employs ligand design based on this model of the Kv1.3 channel, followed by solid-phase combinatorial synthesis of structural families of small molecules identified by the modeling.[17]

Results and Discussion

The general structures of Kv1.3 channel blocker candidates were chosen using Biosym/MSI's ligand design program LUDI.[18,19] This program chooses small molecules from a library containing over 1000 random molecular fragments, searching and fitting into the active site of the Kv1.3 channel based on each fragment's ability to form favorable hydrogen bonding and hydrophobic interactions with the channel. Thus by filling hydrophobic pockets with hydrophobic groups and placing molecular fragments that hydrogen bonds with residues lining the inner vestible of the channel, LUDI provided lead molecules that are predicted to block Kv1.3 channels.

The Kv1.3 channel is comprised of four identical subunits, each with six transmembrane segments (S1 to S6). Tetramerization of the amino acids between S5 and S6 forms a C_4 symmetric funnel with a central conduction pore for potassium ions. Calculations using LUDI focused on the amino acids His404, Gly380, and

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Asp386 in each subunit of the Kv1.3 channel because the positions of these amino acids were well known through scorpion toxin binding studies.[12,13] In addition, particular attention was given to His404 because the residue is unique to Kv1.3, and modulators interacting with His404 may allow for some selectivity among the many types of K^+ channels that are expressed. Since Asp402 and Tyr400 are highly conserved among K^+ channels, we designed blockers also intended to interact with these two amino acids in order to attain specificity for K^+ rather than Na^+ or Ca^{+2} channels. Standard LUDI protocols were therefore employed to generate molecules predicted to interact with Tyr 400, Asp 402, His404, Gly380 and Asp386.

A Control Experiment: Does LUDI Identify Known Blockers of the Kv1.3 Channel?

To check the internal consistency of the postulated model of the Kv1.3 channel and the LUDI program, a LUDI library of energetically favorable conformers of known bockers was generated: CP-339,818 (1-benzyl-4-pentylimino-1,4-dihydroquinoline) and CP-394,322 (1-naphthyl-4-pentylimino-1,4-dihydroquinoline), both potent and selective nonpeptide Kv1.3 channel blockers with IC_{50} values of ~0.20 μM[10] LUDI calculations on the postulated Kv1.3 channel model mapped these CP drugs to the outer vestible of Kv1.3, suggesting an aromatic interaction between the quinoline and His 404 (Figure 1). In contrast, LUDI calculations with a small library of several nonselective blockers (4-aminopyridine, cromakalim, and several others) of the Kv1.3 channel failed to map these compounds near His 404. Since LUDI was able to map known blockers of the Kv1.3 channel to the postulated model, but unable to map inactive compounds, we were reassured that the model was self-consistent and that LUDI was competent in suggesting molecular binding interactions.

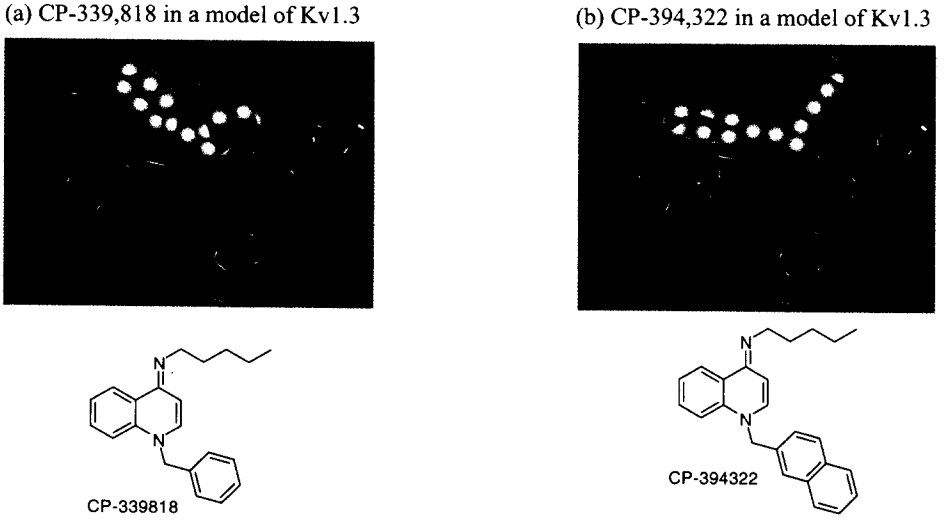
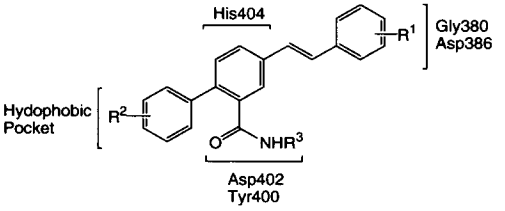


Figure 1. Side view of the calculated binding of known blockers to the outer vestibule of the Kv1.3 channel. Blockers are represented in CPK mode and color coded by atom type: carbon, green; nitrogen, blue; oxygen, red; hydrogen, white. The four His404's are highlighted in blue and represented in stick mode. (a) Binding of CP-339,818 in the Kv1.3 channel. The imine nitrogen hydrogen bonds to Asp402 at a distance of 2.51 Å. π-Stacking interactions between His404 and the aromatic ring of CP-339,818 is 1.88–2.63 Å. (b) Binding of CP-394,322 in the Kv1.3 channel. Both nitrogens of CP-394,322 hydrogen bond with one of the four Asp402s in the channel; one at a distance of 1.98 Å and another at 2.0 Å. π-Stacking interaction with His404 and the central aromatic ring occurs at a distance of 1.04–1.93 Å.

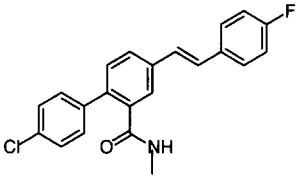
LUDI Calculations for the Postulated Model of the Kv1.3 Channel.

LUDI was next directed to suggest molecular fragments that interact with the key amino acids Tyr 400, Asp 402, His 404, Asp 386, and Gly 380. These fragments were then connected with suitable spacer fragments to form single molecules. Modifications of these molecules to restrict gross conformational rotations followed by further dynamic studies to minimize the energy of the molecules led to the phenyl-stilbene scaffold shown in Figure 2a. Further LUDI calculations with a library[20] of low energy conformers of the scaffold found six different binding conformations. In all six conformers, R^1 contacts Gly 380 and Asp 386, R^2 fits into the Val 406 hydrophobic pocket, R^3 contacts Asp 402 or Tyr 400, and the central aromatic ring in the core scaffold aligns parallel to the aromatic ring of His 404, which is predicted to result in a favorable π-stacking electrostatic interaction.

(a) The phenyl-stilbene scaffold



(b) A representative of the scaffold in Kv1.3



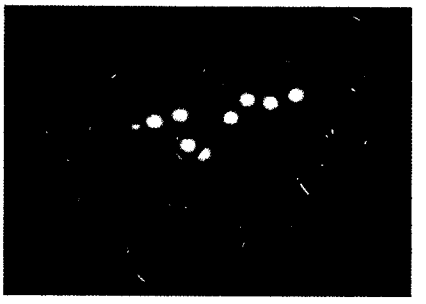


Figure 2. (a) The scaffold for blockers of the Kv1.3 channel with the interactions between key amino acids shown in brackets. (b) Side view of a representative of the phenyl-stilbene scaffold binding to the Kv1.3 channel. The scaffold is represented in CPK mode and color coded as in Figure 1 except chlorine, yellow-green; fluorine, dark green. The four His404's of the Kv1.3 channel are highlighted in blue, and the four Asp402's are highlighted in orange. All eight amino acids are represented in stick mode. The amide nitrogen is hydrogen bonded to Asp402 at 1.95 Å.

Parallel Combinatorial Synthesis of LUDI Hits

In order to maximize binding affinity, R^1, R^2 and R^3 of the phenyl-stilbene scaffold above were varied through combinatorial methods (Figure 3). Attachment of the Wittig Salt **1** to a polymeric support was accomplished by coupling the HATU-activated ester of **1** to the modified Kenner's safety catch linker **2**, available in five steps following Ellman's protocol.[21,22] The substituted phenyl stilbenes were made through a Wittig coupling[23–28] with commercially available substituted aryl aldehydes to vary R^1, followed by a Suzuki coupling[21,29,30] with commercially available substituted aryl boronic acids to vary R^2, and cleavage with various acyclic amines, piperazine and morpholine to vary R^3.

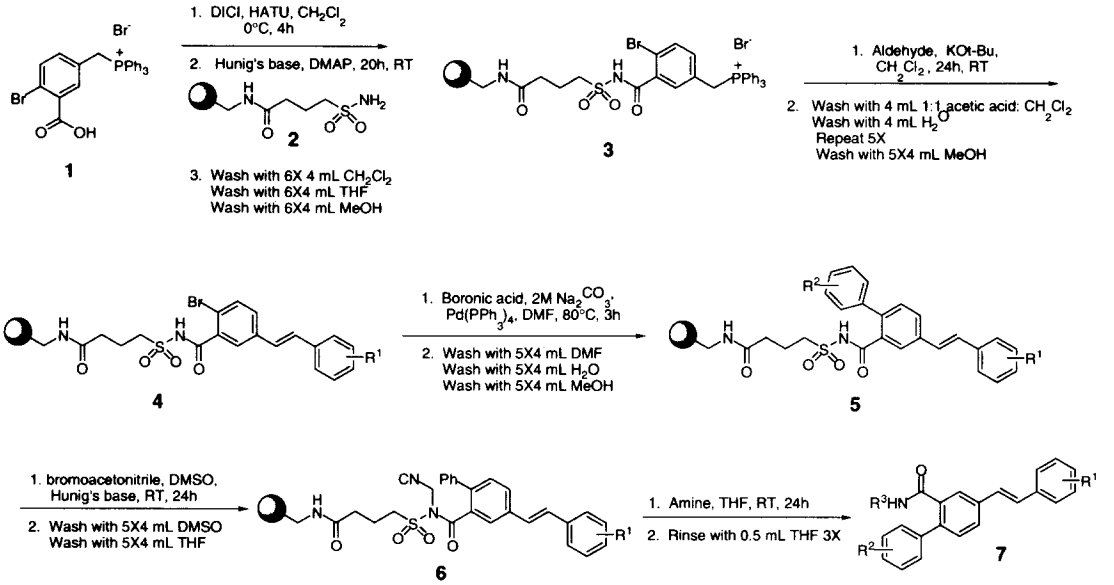


Figure 3. Combinatorial Synthesis of LUDI Hits.

The Wittig salt **1** was synthesized by an NBS bromination of 2-bromo-5-methyl benzoic acid (**8**) to give the monobrominated acid **9** (Figure 4). A small amount (5%) of dibromination occurred at the benzylic position, but subsequent treatment of **9** with triphenyl phosphine in reluxing dry acetone afforded the desired Wittig salt **7** as a white precipitate that was easily purified by simple filtration.

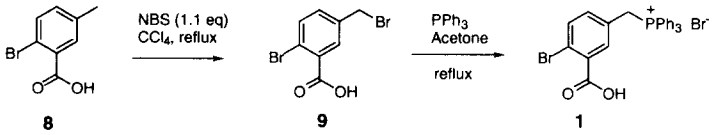


Figure 4. Synthesis of the Wittig Salt 1.

This strategy resulted in 400 compounds, synthesized in parallel on a semi-automated synthesizer (Advanced ChemTech's ReacTech) from the aryl aldehydes, aryl boronic acids and nucleophiles shown in Table 1. The first 40 compounds synthesized were purified and characterized by standard spectroscopic methods. Subsequent compounds were checked for purity by TLC. Compounds that were grossly impure were discarded; compounds that showed reasonable purity were passed through a plug of silica before submitting to Zeneca Pharmaceuticals for biological evaluation. ^{125}I-ChTx binding assays[31] identified several leads in this library; compounds with IC_{50} values lower than 10 μM are listed in Table 2.

Based on these preliminary data, it appears that a para-Cl substituent at R^2 is required for activity, since F, OMe, H, or NH_2 substituents in the ortho, meta, or para positions resulted in analogs with IC_{50} values greater than 10 μM. In addition, bulky R^3 substituents such as cyclohexyl, piperazyl, and morpholine are not tolerated, which is consistent with the modeling prediction that R^3 protrudes into a pore that is only 5–6 Å wide. Even the relatively slender R^3 substitutions such as *n*-propyl or *n*-butyl are inactive if the associated R^1 substitutions are large; for example, only compound A (Table 2), with the relatively small methyl substitution at R^3 was active in compounds with the relatively large dibenzyloxy substitution at R^1. The relatively large pocket containing

Gly380 and Asp386 allows a wide range of substitutions at R^1; for example, substitutions ranging from the bulky dibenzyloxy to the slim F are tolerated.

Table 1. Components used in the Combinatorial Library

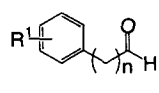
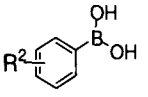
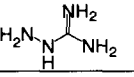
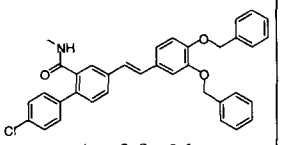
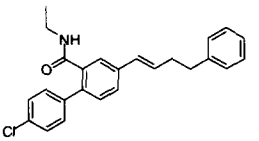
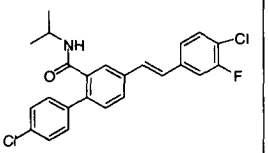
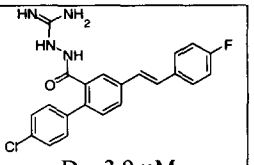
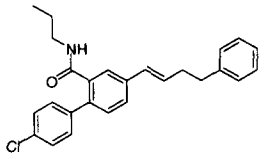
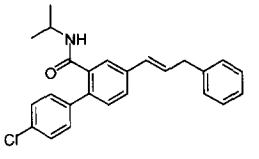
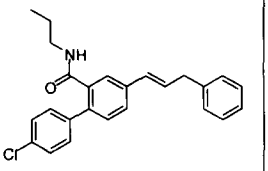
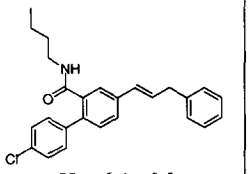
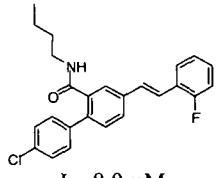
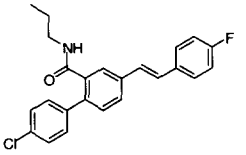
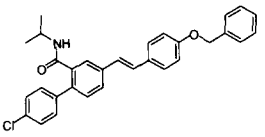
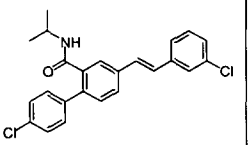
Aldehydes	Boronic Acids	Nucleophiles
 R' = H, Cl, F, OH, NMe_2, Phenyl, -OPh, and/or -OBn; n = 0,1, or 2	 R^2 = H, Cl, F, OMe, and/ or NH_2	R^3NH_2 R^3 = methyl, ethyl, propyl, isopropyl, butyl, cyclohexyl, piperazine, and/ or morphiline; NaOH; 

Table 2. Kv1.3 Channel Blockers

 A. 2.9 μM	 B. 3.2 μM	 C. 3.7 μM	 D. 3.9 μM
 E. 4.0 μM	 F. 5.6 μM	 G. 5.8 μM	 H. 6.1 μM
 I. 8.0 μM	 J. 9.7 μM	 K. 9.9 μM	 L. 10.1 μM

Previous approaches to identifying Kv1.3 channel blockers have relied on high-throughput random screening of very large chemical libraries [Win 17317-3 (IC_{50} = 0.083 μM)[9] and the UK-78282 series (IC_{50} = 1–10 μM)[11]], or through modifications of Win 17317-3, such as CP-339818 and analogs (IC_{50} = 0.20 μM).[10] Although our first generation phenyl-stilbene derivatives show only modest activity, they compare favorable to these other blockers and therefore serve as a reasonable starting point for further studies.

Acknowledgements: We are grateful to Zeneca Pharmaceuticals for providing financial support and to Dean Synder of Zeneca for determining IC_{50} values for the compounds synthesized. We thank George Gutman and George Chandy for providing Kv1.3 channel coordinates. We would like to thank Matthew Hart, Mike Klimas and Dean Phillips for helpful suggestions.

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