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Quinazolin-4-piperidin-4-methyl sulfamide PC-1 inhibitors: Alleviating hERG interactions through structure based design

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ABSTRACT

PC-1 (NPP-1) inhibitors may be useful as therapeutics for the treatment of CDDP (calcium pyrophosphate dehydrate) deposition disease and osteoarthritis. We have identified a series of potent quinazolin-4-piperidin-4-ethyl sulfamide PC-1 inhibitors. The series, however, suffers from high affinity binding to hERG potassium channels, which can cause drug-induced QT prolongation. We used a hERG homology model to identify potential key interactions between our compounds and hERG, and the information gained was used to design and prepare a series of quinazolin-4-piperidin-4-methyl sulfamides that retain PC-1 activity but lack binding affinity for hERG.

PC-1 (Plasma Cell Membrane Protein-1), also known as NPP-1, belongs to a family of enzymes with nucleoside triphosphate pyrophophohydrolase (NTPPPH) activity; PC-1 catalyzes the hydrolysis of ATP to ePPi (extracellular inorganic pyrophosphate) and AMP.[1] PC-1 is a class II transmembrane glycoprotein that is highly expressed in articular chondrocytes, and is the major source of the elevated PPi levels in chondrocytes and fibroblasts of patients with familial CPPD (calcium pyrophosphate dehydrate) deposition disease.[2,3] CPPD crystals may be a hallmark of the pathology of osteoarthritis.[4] This suggests that inhibitors of PC-1 may block PPi production by chondrocytes which may result in the reduction of CPPD crystals, thereby having utility in the treatment of chondrocalcinosis and osteoarthritis. Further, PC-1 interferes with insulin receptor signaling, and epidemiological data suggests a link between mutations in PC-1 and insulin resistance.[5–8] PC-1 inhibitors may therefore also have use in treating diabetic patients. Since both osteoarthritis and diabetes require chronic treatment, a PC-1 inhibitor drug requires an excellent safety profile. Our PC-1 inhibitor lead compound **1** is a potent ligand for the human 'ether-a-go-go' (hERG) channel, a strong cardiovascular safety liability, so understanding and alleviating this liability is a major goal in the project.

Compounds with a high affinity for the hERG potassium channel, which plays a key role in regulating cardiac rhythm,[9] have the propensity to cause undesirable effects on cardiac repolarization in man; in particular they can prolong the QT interval in the electrocardiogram.[10] This QT prolongation can, in rare cases, lead to drug-induced ventricular fibrillation and sudden death, Torsades de Pointes (TdP).[11] Affinity for the hERG potassium channel is commonly tested using a whole cell voltage clamp assay, which is a low throughput and technically challenging technique.[10] We instead employed a predictive, high-throughput competitive binding assay that measures the ability of a test compound to displace [^{3}H]-dofetilide from the hERG channel stably expressed in HEK-293 cells.[12] Dofetilide is an antiarrhythmic agent, the structure of which is shown in Figure 1. While a binding assay such as this does not provide functional information on drug effects, the tritiated dofetilide binding assay is often used in early drug discovery projects as a high throughput surrogate for the patch clamp assay.

Our lead compound **1** is a potent inhibitor of PC-1 (36 nM, Table 1), as measured by an in vitro Kinase-Glo assay (Promega) monitoring the consumption of ATP by the PC-1 enzyme. However, the compound is also a potent ligand for the hERG channel, measuring 601 nM in the tritiated dofetilide binding assay. Therefore we sought out the structural requirements for hERG binding, hoping to find divergent SAR between hERG binding and PC-1 inhibition.

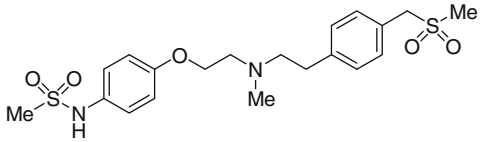

Figure 1. Structure of dofetilide.

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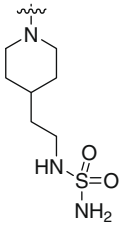
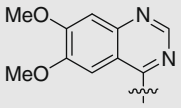
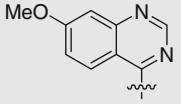
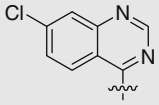
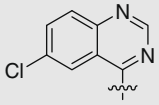
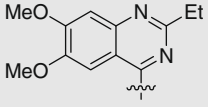
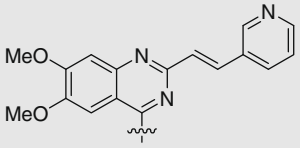
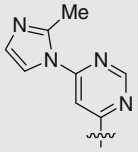
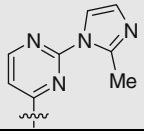
Table 1
Modifications to the quinazoline region of compound **1**



	Structure	PC-1 (μM)	Dofetilide (μM)
1		0.0362	0.601
2		0.187	0.1202
3		>10	3.4
4		5.98	5.42
5		0.214	0.0174
6		0.0353	0.276
7		0.6	>10
8		2.45	6.867

There have been several ligand-based quantitative structure–activity relationship (QSAR) and pharmacophore models built using structurally diverse compounds known to bind to and block hERG channels.[13–16] Similar models built with large amounts of diverse internal compound data were poor predictors of hERG binding liability for this series. Therefore we explored the use of a structure-based hERG homology model for guidance in determining the most important functional group interactions facilitating the hERG binding affinity of our lead compound. Similarly to several reported hERG homology models,[15–19] the models for both the open and closed conformations of the hERG channel were generated using crystal structures of the MthK[20] and KcsA[21] potassium channels, respectively. The homology model employed contains four subunits spanning Gly546 to Ile662, which comprise the highly symmetrical homo-tetrameric pore domain. Docking of compound **1** into both conformations of this model (Fig. 2) reveals two areas of important interactions: stacking of the quinazoline with the Phe656 residues (Fig. 3), and extensive hydrogen bonding of the sulfamide to Ser624 residues in a tight pocket (Fig. 4). The nitrogen in the 3-position of the quinazoline may also be in a position to participate in cation–π interactions with a Tyr652 residue (Fig. 3); this nitrogen has a predicted pK_a of 9.7 and would therefore be the first site of protonation, based on calculations using ACD/pK_a DB software (v. 8.07, Advanced Chemistry Development, Inc., Toronto, Canada). All of these interactions are in keeping with the known key residues necessary for the binding of many potent hERG channel blockers.[19,22]

Modifications to the quinazoline region of compound **1** are outlined in Table 1. As suggested by observation of the docking model, small modifications to the quinazoline ring do not have a large effect on the compounds' ability to displace tritiated dofetilide, as illustrated by 7-OMe quinazoline compound **2**, which is equipotent in PC-1 and dofetilide assays. However, an electron withdrawing group on the quinazoline, such as 7-Cl or 6-Cl quinazoline derivatives **3** and **4**, results in a reduction in dofetilide potency; unfortunately it also significantly interferes with PC-1 potency as well. Substitution of the quinazoline in the 2-position enhances activity in the dofetilide assay, as evidenced by 2-Et compound **5**, which has dofetilide activity of 17 nM. Compound **6**, also substituted in the 2-position of the quinazoline, also displays submicromolar activity in the dofetilide assay. Both 2-substituted examples maintain PC-1 potency. Two examples are shown that break up the quinazoline, and in both cases the dofetilide binding efficiency is reduced: 6-methylimidizole substituted pyrimidine **7** has no detectable dofetilide binding activity, but retains submicromolar PC-1 activity, and the 2-methylimidizole substituted pyrimidine **8** shows a dofetilide binding potency of 6.8 μM, but has PC-1 inhibitory activity of only 2.5 μM. While replacement of the quinazoline with a substituted pyrimidine initially seemed promising as a path forward for separating the SAR between dofetilide binding and PC-1 activity, we were unable to identify a compound with PC-1 activity better than the 600 nM potency of compound **7** using this strategy. Compounds were synthesized according to Scheme 1.

A second strategy suggested by the model is to shorten the distance between the quinazoline, which is locked into place through the π-stacking with Phe656 residues and a cation–π interaction with a Tyr652 residue, and the sulfamide, which is also in a tight pocket with extensive hydrogen bonding interactions to Ser624 residues. Figure 5 shows the docking of lead compound **1** to the model compared to the docking of compound **9**, in which the linker from the piperidine to the sulfamide is shortened from two carbons to one. The model suggests that compound **9** does not form the network of hydrogen bonds to Ser624 side chains that occurs in **1** and would therefore bind with lower affinity.

Experimentally, when the linker from the piperidine to the sulfamide is shortened from two carbons to one, we find that in most cases tested, compounds have no detectable dofetilide binding activity. More importantly, we also find that PC-1 activity is preserved in the quinazoline analogs. Table 2 shows four comparisons of quinazoline (or pyrimidine) head groups with differing linker lengths, along with PC-1 enzymatic data, dofetilide binding data, and the ratio of the two assay readouts. The dofetilide/PC-1 ratio can be interpreted as a safety window.

Analogs with the 6,7-dimethoxyquinazoline head group are represented by compounds **1**, **9**, and **10**, which have an ethylene, methylene, and no spacer, respectively. PC-1 inhibitory activity is maintained for compounds **1** and **9**, but there is no detectable PC-1 inhibition noted for compound **10**. However, compounds **9**

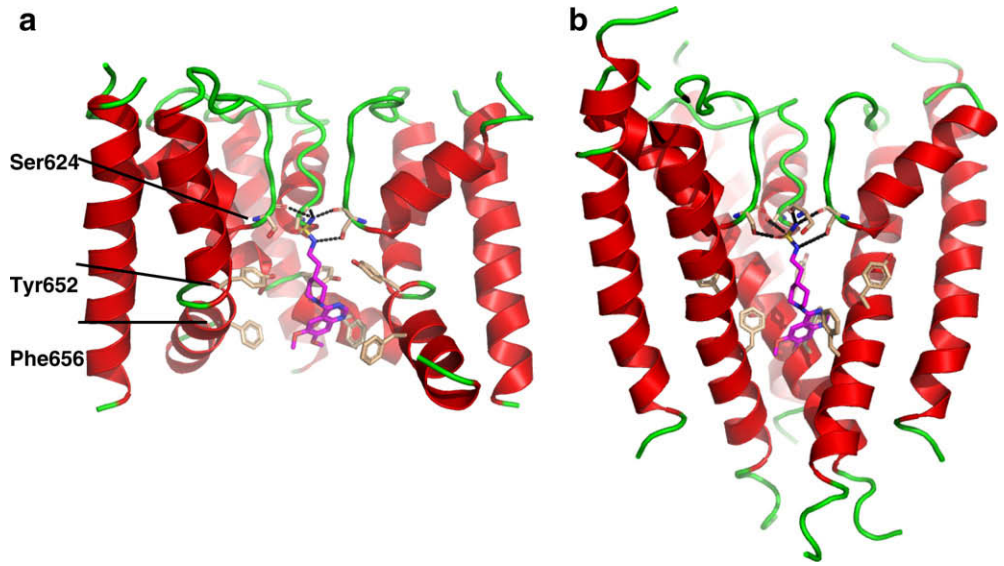


Figure 2. Compound **1** (purple) modeled binding mode in homology models of the open (a) and closed (b) conformations of the hERG channel, with key residues shown in stick format. One monomer of the tetrameric channel is not shown to improve clarity.

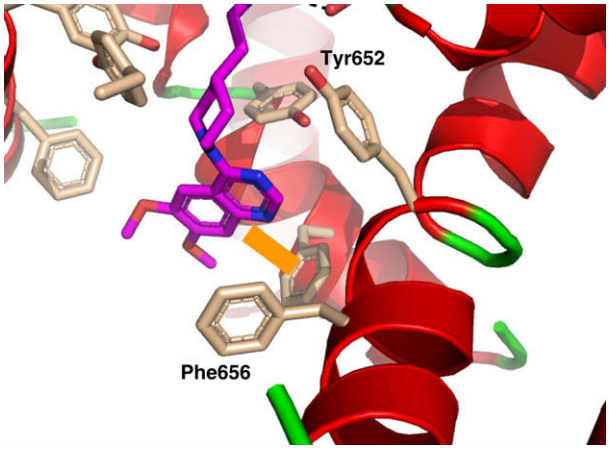


Figure 3. Detail of compound **1** modeled into the open conformation of the hERG channel; the quinazoline t-stacks with at least one of the Phe656 residues of the tetrameric channel, as indicated by the orange bar. Quinazoline N3 may also participate in a cation–pi interaction with Tyr652.

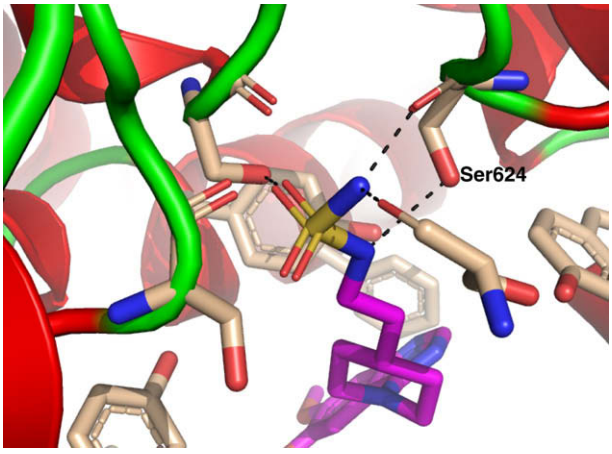
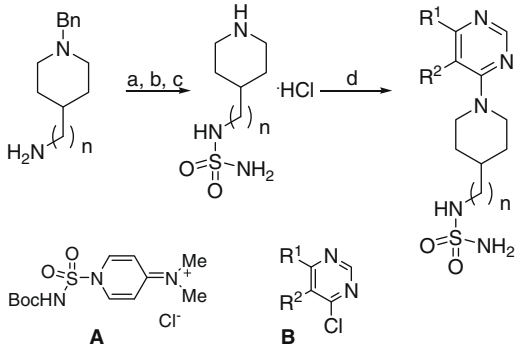


Figure 4. Detail of compound **1** modeled into the open conformation of the hERG channel; the sulfamide is in a tight binding pocket with the Ser624 residues from the four symmetrical subunits. Potential hydrogen bonds are indicated with black dashed lines.

and **10** are both >10 μM in the dofetilide binding assay, as suggested by the dockings shown in Figure 5. Thus compound **9**, with the methylene spacer, has the optimal ratio of PC-1 inhibition to dofetilide binding, at >160-fold. This trend is mirrored in the 7-OMe headgroup series, compounds **2** and **11**, in that the methylene spacer **11** is equipotent in PC-1 activity to the ethylene spacer compound **2**, but has no detectable dofetilide binding activity, resulting in a much greater safety window (>60-fold for **11** vs 1-fold for **2**). The 2-substituted quinazoline example is slightly different, in that the methylene spacer compound **12** still has dofetilide binding activity, although an order of magnitude less potent than ethylene spacer compound **6** (3.3 μM and 0.28 μM, respectively); however the trend is in the same direction and the safety window is enhanced by having the shorter linker (52-fold for **12** vs 9-fold for **6**). Interestingly, for the non-quinazoline example shown, both the ethyl and methyl spacer compounds (**7** and **13**) lack detectable dofetilide binding activity, but only ethylene spacer compound **7** has PC-1 inhibitory activity.



Scheme 1. Synthetic route to analogs in Tables 1 and 2. Reagents and conditions: (a) DIPEA, DCM, **A**,[26] 16 h (79–91%); (b) 15%Pd/C, EtOH; (c) HCl (g), DCM (89–97%, two steps); (d) TEA, DCE, 50 °C, **B**, 16 h (68–97%).

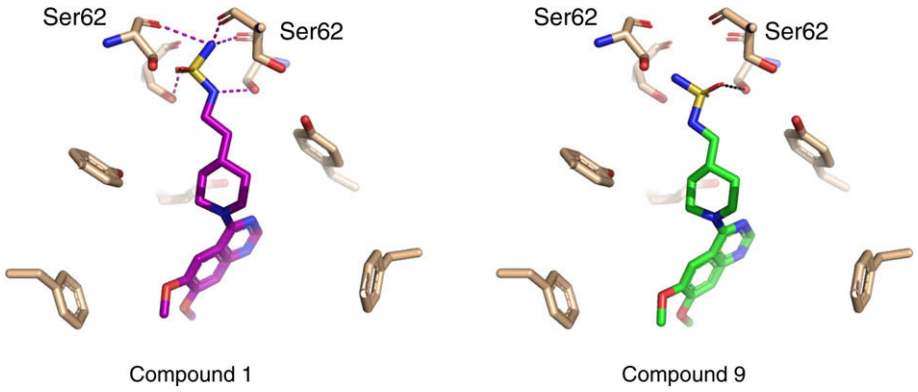
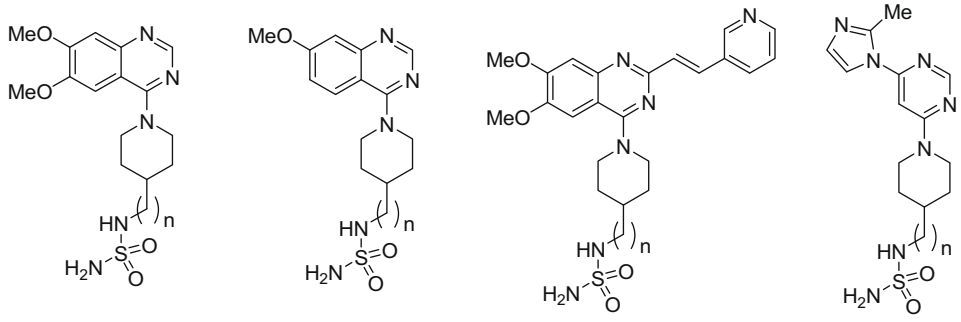


Figure 5. Modeling suggests that **9** does not form the network of hydrogen bonds to Ser624 side chain and backbone that occurs with **1**.

Table 2
Comparison of compounds with differing linker lengths between the sulfamide and the piperidine



n = 2	**1**	**2**	**6**	**7**
PC-1 (μM)	0.036	0.187	0.035	0.6
dof (μM)	0.601	0.12	0.276	>10
Ratio dof/PC-1	17	1	8	>16
n = 1	**9**	**11**	**12**	**13**
PC-1 (μM)	0.061	0.166	0.063	>10
dof (μM)	>10	>10	3.296	>10
Ratio dof/PC-1	>160	>60	52	—
n = 0	**10**			
PC-1 (μM)	>10	NA	NA	NA
dof (μM)	>10	NA	NA	NA

In conclusion, the hERG homology model enabled the PC-1 program to identify the cause of the unwanted hERG activity in the lead compound and to rapidly design potent PC-1 inhibitors with high selectivity over binding to the hERG ion channel. It is interesting to note that the usual pharmacophore for hERG blockage is thought to be through π-stacking interactions with the Phe656 and cation–π interactions with Tyr652; however, in this series, we found that the polar group interaction with the Ser624, often thought to be of lesser importance,[13] seems to be the determining factor in eliminating hERG binding. Consistent with our observations, published mutagenesis work suggests that Ser624 is a significant contributor to hERG inhibitor binding of droperidol, risperidone, haloperidol,[23] cisapride,[24] and dofetilide.[25] Understanding the hERG liability and designing away from it at an early stage in the project is imperative, and utilizing structure-based drug design through a hERG homology model is a potent approach.

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