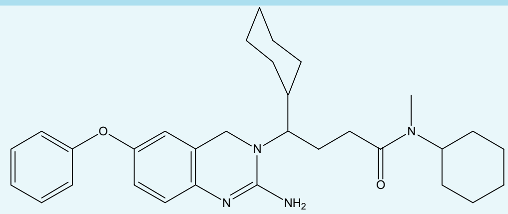
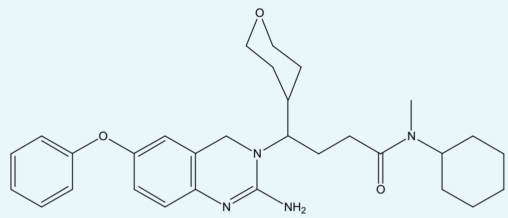
Table 12.4. Measured solubility of BACE ligands. Potencies for **12** and **14** are essentially equivalent at 10 and 6 nM, respectively

		Solubility mg/ml	
Compound	Structure	pH = 2	pH = 7.4
12		0.008	0.01
14		0.91	0.44
		114×	44×

or pharmacophore models for the ion channel. Although these may have some utility in drug discovery they are both relatively weak approaches for understanding how hERG activity might be mitigated in a particular series of interest. We reexamined the issue of a homology model but with one difference: Given the known flexibility of the channel, both open and closed structures of the bacterial ion channels have been observed experimentally, and we decided to include this flexibility explicitly in our model. We did this through the simple expedient of constructing multiple models where the channel is in different states with respect to channel opening. It has been thought that part of the reason hERG is so promiscuous is the fact that it can accommodate ligands of different shapes and sizes as the channel opens and closes. The first step is to align the hERG sequence with one or more of the available bacterial ion channel sequences (Figure 12.10). The homology in the filter region is very high and provides considerable insight into the alignment. There are also a few key residues in the S6 domain that are conserved. One residue of note is the conserved Gly in the S6 domain. Examination of homology models constructed from the closed KcsA[13] and open MthK[9,10] structures shows that the channel can be converted from open to closed by rotation of this hinge Gly.

To obtain potential intermediate states for the hERG channel, the conformation of the hinge Gly was rotated to mimic the open MthK channel. This structure was then rotated in small increments to close the channel until a structure more similar to KcsA (closed) was obtained (Figure 12.11). At each 1° increment molecular dynamics simulations were used to relax the side chains. The protocol at each increment was 0.4 ps of heating followed by 5 ps of equilibration using the CHARMM force field.[17,18] Two states were selected for study: the 10° partially open structure and the 19° fully open structure.

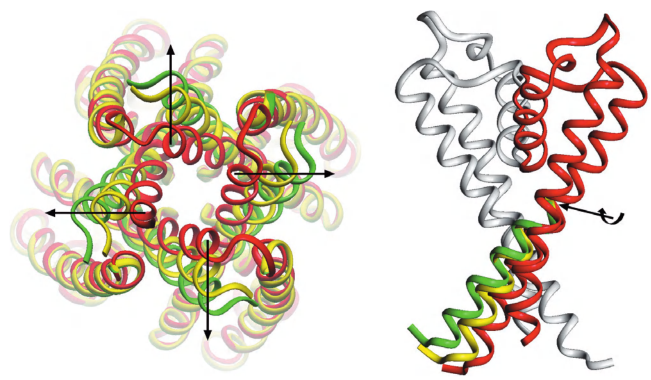


Figure 12.11. Rotation of S6 about the glycine hinge. The closed KcsA structure is shown in red, the partially open structure (10°) in yellow, and the open structure (19°) in green.

```
                 < Filter ><              S6 helix                >
hERG: ...YFTFSSLTSVGFGNVSPNTNSEKIFSICVMLIGSLMYASIFGNVSAII...
KcsA: ...WWSVETATTVGYGDLYPVTLWGRCVAVVVMVAGITSFGLVTAALATWF...
```

Figure 12.10. Sequence alignment.

These two states were then evaluated using a series of compounds with known hERG affinities from the literature.[75] In each case the ligand was docked to both states using GLIDE and then submitted for an LIE calculation of the ΔE_{vdw} and ΔE_{elec} values using OPLS and GB/SA water. Our first effort was to see if either state would provide a reasonable model for hERG activity. The compounds evaluated are listed in Table 12.5.