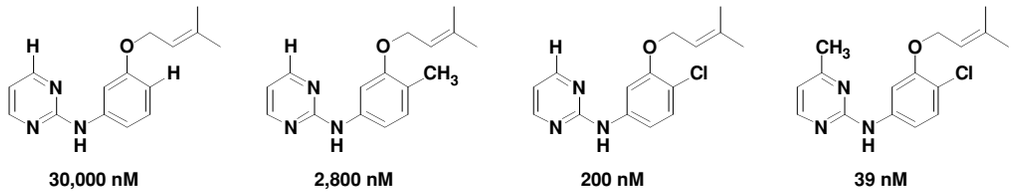


Figure 1.14. The power of chlorine and methyl scans; experimental EC_{50} values for anti-HIV activity in an MT-2 cell assay.

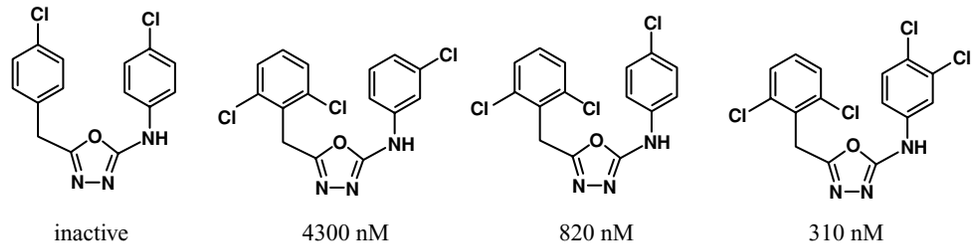
which were both predicted and found to be inactive (Figure 1.13). It is noted that the MT-2 assay is run to a maximum concentration of 100 μM; the thiadiazole showed no activity or cytotoxicity up to this concentration, whereas the thiazole has a CC_{50} of 24 μM and no anti-HIV activity to this point. Overall, this provides another example of the sensitivity of activity to structure and the desirability of rigorous computational guidance. Graphical display of modeled complexes is inadequate to gauge relative potency. In retrospect, the results indicate that the longer C-S bonds in the 2,5-disubstituted sulfur-containing heterocycles cause crowding of the dichlorobenzyl group and Tyr181, and the nitrogen in the 3-position has an electrostatically unfavorable interaction with Glu138.

An interesting aside is that in the original publication, it was thought that the 2,5-disubstituted thiazole in Figure 1.13 showed weak activity with an EC_{50} of 3.1 μM, which was out of line with the FEP results.[24] It was subsequently found that instead of the 2,5-isomer, the 2,4-isomer (S and N interchanged in the structure in Figure 1.13) was the actual compound that had been synthesized and assayed, as confirmed by a crystal structure. The two isomers are not unequivocally distinguishable by NMR. An alternative synthetic route was then pursued to yield the 2,5-isomer, which is indeed inactive, as predicted by the FEP calculations.

Small group scans

In addition to the heterocycle scans, small group scans are highly informative. These are performed routinely with BOMB to build the structures and provide initial scoring, followed by refinement with FEP calculations. A standard protocol with BOMB is to replace each hydrogen of a core, especially aryl hydrogens, with ten small groups that have been selected for difference in size, electronic character, and hydrogen-bonding patterns: Cl, CH_3, NH_2, OH, CH_2NH_2, CH_2OH, CHO, CN, $NHCH_3$, and OCH_3. This is generally adequate to define likely places for beneficial substitution of hydrogen by the least polar groups, Cl, CH_3, and OCH_3. The situation with the polar groups is less clear because of the competition for the ligand between hydrogen bonding in the complex versus unbound in water. As long as some hydrogens appear viable for substitution, a chlorine and/or methyl scan using FEP calculations is then desirable to obtain quantitatively reliable predictions. The potential value of using both a chlorine and methyl scan is well illustrated by the results in Figure 1.14; knowing the optimal position for a methyl group and a chlorine provides an activity boost from 30 μM to 39 nM in this case.[11–13]

A chlorine scan was also particularly helpful in evolving the inactive oxadiazole **3** in Figure 1.4 into potent anti-HIV agents. **3** had emerged in third place after the docking exercise and embedded among known, potent NNRTIs. The docking pose and the structure of the complex as built by BOMB also looked reasonable, although the score from BOMB was modest because of poor accommodation of the methoxy groups in the vicinity of Tyr181 and Tyr188. Assuming that the tricyclic core might be viable, the substituents were removed and a chlorine scan was performed using MC/FEP simulations.[5,24] The predicted changes in free energy of binding for replacing each hydrogen by chlorine are summarized in Figure 1.15; again formally equivalent positions become nonequivalent in the complexes. The scan indicated that the most favorable positions for introduction of chlorines were at C3 and C4 in the phenyl ring and at C2 and C6 in the benzyl ring. A series of polychloro analogs were then synthesized and the activities were found to closely parallel the predictions (Scheme 3). The core and, for example, the 4,4´-dichloro analog were inactive; however, the illustrated



Scheme 3.