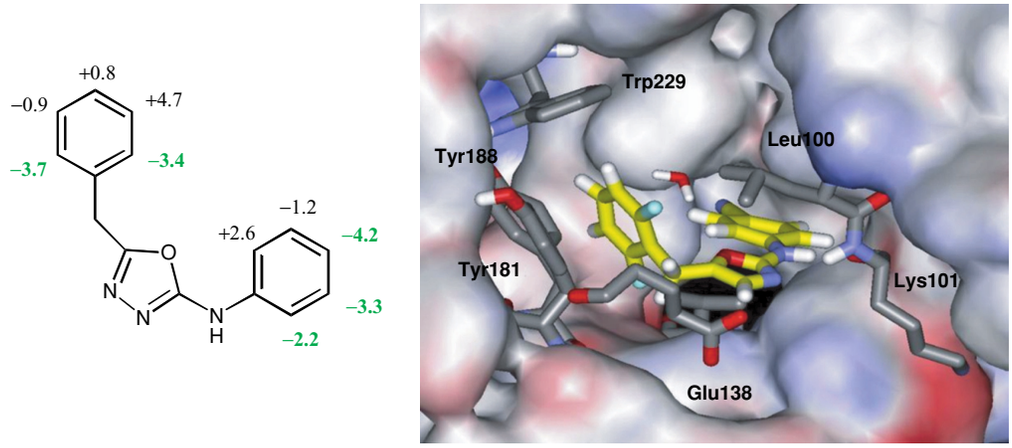
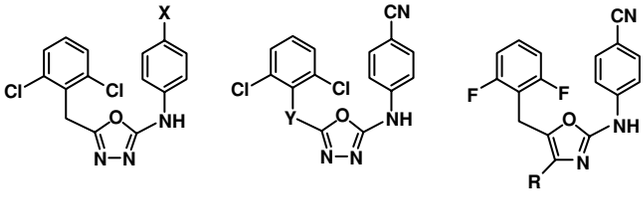


Figure 1.15. (a) FEP-computed changes in ΔG_b (kcal/mol) for replacement of the indicted hydrogens by chlorine. (b) Snapshot of the complex of the 13-nM NNRTI **4** bound to HIV-RT from the MC/FEP simulations.

trichloro and tetrachloro analogs followed the expectations from the FEP results and yielded sub-μM NNRTIs. Thus, with the aid of the FEP chlorine scan it was possible to evolve the false positive from the docking calculations into true positives.[5,24]

Small group and linker refinement

Given the positive outcome of chlorine and/or methyl scans, it is natural to consider further optimization at the replacement sites. This has been successfully guided by FEP results several times, for example, in the optimization of the substituent in the pyrimidine ring and at the 4-position in the phenyl ring for the Het-NH-3-Ph-U compounds in Figure 1.3.[11–13,24] More recent examples occurred with the azoles (Scheme 4). At C4 in the illustrated 2′,6′-dichlorobenzyloxadiazole, FEP calculations were performed and predicted relative ΔG_b values in kcal/mol for X = H (0.0), CH_2CH_3 (−0.3), CH_3 (−1.6), CH_2OCH_3 (−1.7), OCH_3 (−1.8), CF_3 (−2.2), F (−2.3), Cl (−4.0), and CN (−5.2). The X = CH_3, CH_2OCH_3, Cl, and CN analogs were synthesized and the assay results with EC_{50} values of 4, 4, 0.8, and 0.1 μM, respectively, conformed well to the expectations.[24]



Scheme 4.

FEP-guided optimization of the linker Y between the oxadiazole and dichlorophenyl rings was also pursued. The options considered were Y = CH_2, (*R*)-$CHCH_3$, (*S*)-$CHCH_3$, NH, NCH_3, O, and S. Though display of the corresponding complexes appears reasonable, the FEP predictions for modification of the methylene group were all unfavorable except for minor improvement for the methylamino (−1.6) and thio (−1.4) alternatives. The Y = NH and racemic $CHCH_3$ analogs were synthesized and indeed found to be less active than the methylene compound; the methylamino compound turned out to have similar activity (0.2 μM) as the methylene analog (0.1 μM) with X = CN, and the oxo and thio options were not pursued. Overall, combination of the FEP-guided heterocycle, small substituent, and linker optimizations delivered the 13-nM difluorobenzyloxazole derivative **4**, which is shown in Figures 1.4 and 1.15(b).[24]

As a last thrust, FEP calculations were performed for possible replacement of the oxazole C4 hydrogen by R = F, Et, Me, CF_3, and CH_2OH. The five analogs were predicted to be less well bound than the unsubstituted compound by 0.8, 1.5, 1.8, 2.2, and 3.9 kcal/mol, respectively. Visual inspections of modeled structures were, once more, ambiguous. The qualitative FEP result was confirmed experimentally for the C4-methyl derivative, which was found to be seven-fold less potent than the unsubstituted compound. The other options were not pursued.

Overall approach and logistics

The experiences with FEP-guided lead optimization have led to the scheme in Figure 1.1. De novo design or virtual screening can be expected to provide one or more lead compounds with low-μM activity. The substituents in the lead are likely not optimal, especially from virtual screening. Consequently, removal of any small substituents from the core followed by chlorine and methyl FEP scans are then desirable. Synthesis and assaying of the most promising di- or trisubstituted compounds from the scans can provide significant activity improvements, as in Figures 1.13 and 1.14, often with modest synthetic effort. FEP-guided refinement of the small substituents, linkers, and heterocycles is