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FEP-Guided Selection of Bicyclic Heterocycles in Lead Optimization for Non-Nucleoside Inhibitors of HIV-1 Reverse Transcriptase

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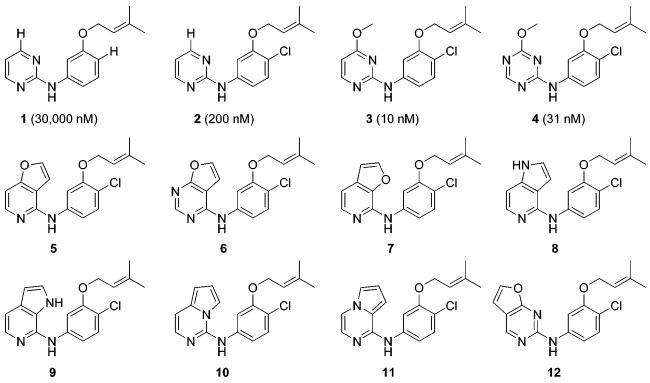
In the course of development of anti-HIV agents, Monte Carlo (MC) simulations using free energy perturbation (FEP) theory have guided rapid optimization of micromolar leads to nanomolar non-nucleoside inhibitors of HIV-1 reverse transcriptase (NNRTIs).[1−3] For example, **1**, which has an EC_{50} of 30 μM for protection of human MT-2 cells from cytopathogenicity by HIV-1, was advanced to 200 nM (**2**) and 10 nM (**3**) by addition of only a chlorine and methoxy group.[1,2] Triazenes were also predicted and found to be potent, for example, **4** at 31 nM, and the cyano replacing chlorine analogues have even 3- to 10-fold lower EC_{50} values.[3] As reported here, the application of FEP calculations has been extended to the optimization of diverse bicyclic heterocycles. The study was motivated by prior MC/FEP results that showed strong preference for the methoxy group to be oriented as indicated in **3** and **4** in complexes with HIV-RT,[3] which made incorporation into a furan ring as in **5** and **6** intriguing. The exploration was expanded with MC/FEP calculations to the isosteres **5**−**11** by perturbing from **9**.[4−6] These are straightforward FEP calculations as the underlying six−five-fused-ring core is invariant. The computed relative free energies of binding, $\Delta\Delta G_b$, are listed in Table 1, and a typical structure for the complex with **6**, is shown in Figure 1. The most potent compounds are predicted to be the azabenzofurans **5** and **6** and the pyrrolopyrazine **11**.



The synthetic challenge for **5**−**11** was undertaken, and **12**, an isomer of **6**, was also prepared, though it was anticipated to be less potent as the furanyl fragment is in an analogous orientation as for the disfavored methoxy rotamer of **3**.[3] Compound **8** was also predicted to have low potency (Table 1) and was not pursued. The synthetic approach is shown in Scheme 1. The required heteroaryl chlorides **13** underwent nucleophilic substitution with amino phenols **14** in acidic media to produce diarylamines **15**, which reacted with dimethylallyl bromide to give the desired compounds.[7] The syntheses of the heteroaryl chlorides are described in the Supporting Information.

The correlation of the predicted and observed activities turned out well with **9** as the weakest inhibitor and **5**, **6**, and **11** with EC_{50} values all below 20 nM. The cyano analogues are even more potent,

Table 1. Computed Relative Free Energies of Binding ($\Delta\Delta G_b$, kcal/mol), Anti-HIV-1 Activity (EC_{50}), and Cytotoxicity (CC_{50})

compound	$\Delta\Delta G_b$[a]	EC_{50} (μM)[b]	CC_{50} (μM)[b]
5	−5.3	0.006	25.0
6	−7.1	0.005	17.0
7	−4.2	0.080	21.0
8	−0.9		
9	0.0	0.900	9.2
10	−4.2	0.130	17.0
11	−6.1	0.019	20.0
12		0.130	20.0
5-CN[c]		0.002	0.320
7-CN[c]		0.011	0.091
11-CN[c]		0.012	0.150
nevirapine		0.110	>10
efavirenz		0.002	>0.1
TMC125		0.002	>1.0

[a] Uncertainty 1σ is ± 0.3 kcal/mol. [b] Reference 8; anti-HIV and anti-RT activities correlate well for NNRTIs (ref 9). [c] CN replacing Cl analog.

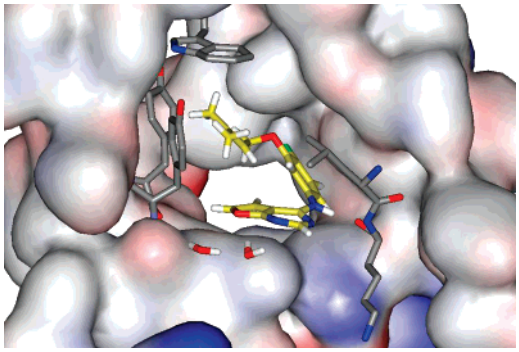
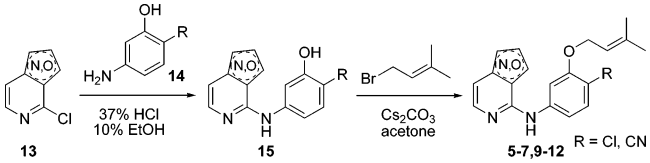


Figure 1. Typical snapshot of **6** bound to HIV-RT from a MC simulation: carbon atoms of **6** are gold; from the left, Tyr181, Tyr188, Leu100, Lys101; Trp229 at the top; H-bonds with Lys101 O on right. Some residues in front including Glu138 have been removed for clarity. The water on N7 is also H-bonded to a carboxylate O of Glu138, and the second water forms a bridge to the O of Ile180. The furanyl O does not participate in H-bonds.

Scheme 1. Synthesis of NNRTIs



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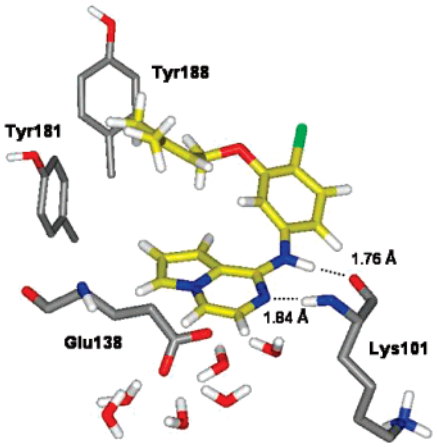
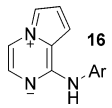
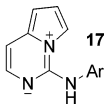


Figure 2. Last configuration from a MC simulation of **11** bound to HIV-RT. Carbon atoms of **11** are gold; H-bonds with Lys101 are highlighted. Six water molecules are H-bonded to Glu138. Ca. 6500 atoms are hidden.

though they are relatively cytotoxic (Table 1). Compounds **5** and **6** are equipotent; the added nitrogen in **6** is well-accommodated in the bound structure (Figure 1), but it is also fully solvent-exposed unbound. They are far more potent than nevirapine (Viramune) and similar in potency to efavirenz (Sustiva). Compounds **5** and **6** are also more potent than their progenitors **3** and **4**. This outcome was not obvious owing to the following: (1) narrowing of the activity funnel, that is, **3** and **4** are already potent; (2) the conversions effectively embody a 2-pyrimidine to 2-pyridine change, which is unfavorable on the basis of prior results for **2** (0.2 μM) and its pyridine analog (3.2 μM);[1] (3) **5** and **6** with QPlogS values[1] of −4.9 and −4.7 are predicted to be 0.2 log unit more soluble in water than **3** and **4**.

The computed/experimental comparison is particularly good for the isomers **9**, **10**, and **11**, which provide a striking example of the sensitivity of activity to structure. Compound **9** is a weak inhibitor because all hydrogen bonding with the pyrrolyl NH is lost upon binding. From the ΔG results for the unbound and bound legs of the FEP calculations, the greater potency for **11** than **10** stems from better interactions with the protein rather than poorer hydration unbound. The average energy components confirm that the interaction with HIV-RT is more favorable for **11** than **10** by 6.0 kcal/mol, while the interaction in water is only more favorable by 3.3 kcal/mol. The variation comes entirely from the Coulombic energy components, and the average dipole moment for bound **11** is 3.8 D, while it is 2.0 D for **10**. At least part of the difference could be interpreted to reflect greater contribution from resonance structure **16** for **11** than from **17** for **10**. CM1A charges[10] scaled by 1.14 have been used here for the inhibitors;[5] for the aza N and bridgehead N they are −0.44 and +0.01 e for **11** and −0.38 and −0.22 e for **10**. The greater negative charge on the aza N of **11** strengthens the hydrogen bond with the backbone NH of Lys101 (Figure 2). The hydrogens on C5 and C6 in **11** are also more positive than the corresponding ones in **10**, which makes interaction with Glu138 more favorable. In the absence of the computational results, prediction of the significantly greater activity of **11** would be elusive, particularly since pyrimidine **2** is far more active than the corresponding pyridine and pyrazine.[1]





In summary, selection of heterocycles to optimize potency is a central activity in the pursuit of therapeutic agents. The present study clearly demonstrates the utility of FEP calculations for guiding these efforts in the context of structure-based drug design and for providing insights into the origins of variations in activity. Diverse sets of candidate heterocycles can be screened in parallel in a few days before committing to synthesis. The current application led to compounds **5**, **6**, and **11**, which are novel, highly potent inhibitors of HIV-RT with greater than 1000-fold safety margins (CC_{50}/EC_{50}).

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Supporting Information Available: ^{1}H, ^{13}C spectral data of NNRTIs in Table 1, and synthetic details for the heteroaryl chlorides **13**. This material is available free of charge via the Internet at http://pubs.acs.org.)

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(4) Initial structures for the inhibitors and complexes were built with the *BOMB* program and included 159 residues of HIV-RT. A total of 1250 and 2000 TIP4P water molecules were added in 25 Å caps for the complexes and unbound ligands, respectively. Each FEP utilized 20 free energy increments and typically covered 20×10^6 (20 M) configurations for equilibration and 40 M configurations for averaging. The OPLS-AA force field was used for the protein and OPLS/CM1A for the inhibitors, and the MC/FEP calculations were performed with *MCPRO 2.0*. Details on the *BOMB* and FEP calculations can be found in ref 1.
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(7) Representative procedure (**5**): 4-Chloro-furo[3,2-*c*]pyridine (169 mg, 1.1 mmol), 5-amino-2-chlorophenol (149 mg, 1.04 mmol), and 37% HCl (108 μL, 1.09 mmol) in 10% aqueous EtOH solution were stirred at 90 °C for 12 h. The reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ solution and brine. The organic phase was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash silica gel chromatography with 20% EtOAc in hexanes to give 2-chloro-5-(furo[3,2-*c*]pyridine-4-ylamino)phenol (237 mg, 83% yield). This material (237 mg, 0.91 mmol) and Cs_2CO_3 (356 mg, 1.09 mmol) in acetone (5 mL) were treated with 3,3-dimethylallyl bromide (117 μL, 1.0 mmol) at room temperature. After 2 h, the reaction mixture was diluted with EtOAc and washed with aqueous NH_4Cl solution and brine. The organic phase was separated, dried, and concentrated as above. Purification by flash silica gel chromatography with 10% EtOAc in hexanes gave **5** (281 mg, 94% yield).
(8) Activities against the IIIB strain of HIV-1 were determined using MT-2 human T-cells; the EC_{50} dose yields 50% protection of infected cells using the MTT method. The CC_{50} for inhibition of MT-2 cell growth by 50% was obtained simultaneously: Ray, A. S.; Yang, Z.; Chu, C. K.; Anderson, K. S. *Antimicrob. Agents Chemother.* **2002**, *46*, 887−891.
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