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Eastern extension of azoles as non-nucleoside inhibitors of HIV-1 reverse transcriptase; cyano group alternatives

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ABSTRACT

Design of non-nucleoside inhibitors of HIV-1 reverse transcriptase is being pursued with the assistance of free energy perturbation (FEP) calculations to predict relative free energies of binding. Extension of azole-containing inhibitors into an 'eastern' channel between Phe227 and Pro236 has led to the discovery of potent and structurally novel derivatives.

Non-nucleoside inhibitors of HIV-1 reverse transcriptase (NNRTIs) are an important component of highly active anti-retroviral therapy (HAART) for the treatment of HIV infection.[1] NNRTIs disrupt the activity of HIV-RT via binding to an allosteric pocket in the vicinity of the polymerase active site.[2–4] However, the therapeutic effectiveness of NNRTIs is challenged by rapid emergence of drug-resistant, variant strains of the virus.[5] Mutations such as V106A, Y181C, and K103N occur in the NNRTI binding pocket. While the search for an effective vaccine continues, the need for new anti-retroviral drugs with improved pharmacological properties and resistance profiles is apparent.

Recent reports have described the discovery and free energy perturbation (FEP)-guided optimization of an U-5Het-NH-Ar scaffold, where U is an unsaturated hydrophobic group, and 5Het is an azole.[6,7] Though potent NNRTIs were identified with EC_{50} values as low as 10–20 nM, search for novel NNRTIs that provide activity against a broader spectrum of variants continues. In particular, we have sought to reduce the dependence of the NNRTI binding on the interaction between the U group and Tyr181 by extending the Ar group into the distal region of the binding site, which is lined by Val106, Glu224, Pro225, Pro226, Phe227, and Pro236. The present report summarizes FEP-guided results for these 'eastern extensions' in the oxazole and oxadiazole series.

The syntheses of oxadiazole **1** and oxazole **3** derivatives were performed as shown in Schemes 1 and 2.[6] The isothiocyanates **2** required for the synthesis of oxazole derivatives **3** were prepared from substituted benzyl alcohols, phenols, or halonitrobenzenes.[8,9] As illustrated for **1e**, extended oxadiazole derivatives were synthesized by Cu-catalyzed coupling of 2-amino-oxadiazole **4** with aryl iodides, for example, **5**.[10,11] **4** was synthesized from 2-(2,6-dichlorophenyl)acetohydrazide and cyanogen bromide.[12,13] Coupling partner **5** was prepared by alkylation of 4-iodo-benzyl alcohol with the requisite bromomethyl-pyridine.

Activities against the IIIB and variant strains of HIV-1 were measured using MT-2 human T-cells; EC_{50} values are obtained as the dose required to achieve 50% protection of the infected cells by the MTT colorimetric method. CC_{50} values for inhibition of MT-2 cell growth by 50% are obtained simultaneously.[6,14,15]

Monte Carlo/FEP (MC/FEP) calculations were executed to compute relative free energies of binding using standard protocols.[16] Coordinates of HIV-RT complexes were constructed from the 1s9e PDB file[17] using the *BOMB* program.[7] Desired analogs were grown from ammonia, which was positioned to hydrogen-bond with the C=O of Lys101 in the NNRTI binding site.[6,17] The model included the 178 amino acid residues nearest the ligand. Short conjugate-gradient minimizations were carried out on the initial structures for all complexes to relieve any unfavorable contacts. Coordinates for the free ligands were obtained by extraction from the complexes.

The MC/FEP calculations were performed with *MCPRO*.[18] The unbound ligands and complexes were solvated in 25 Å caps with

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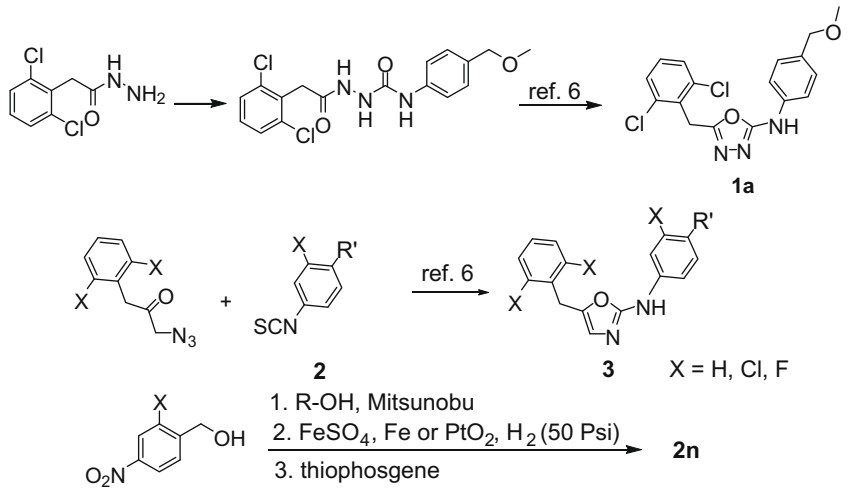
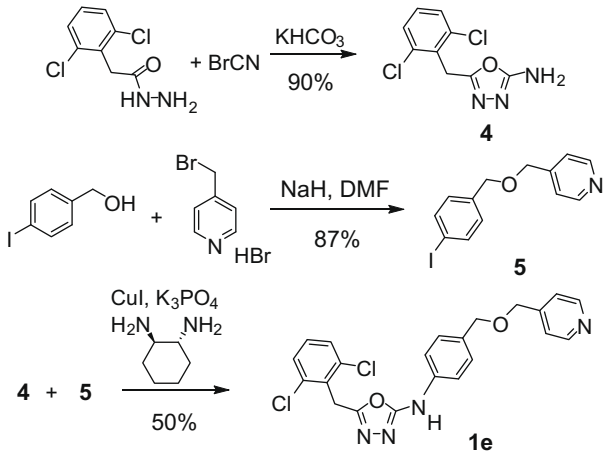


Scheme 1.



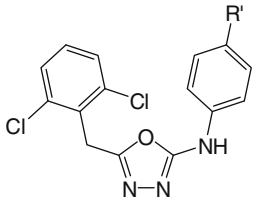
Scheme 2.

2000 and 1250 TIP4P water molecules. The FEP calculations utilized 11 windows of simple overlap sampling.[19] Each window covered 10–15 million (M) configurations of equilibration and 20–30 M configurations of averaging. The ligand and side chains within ca. 10 Å of the ligand were fully flexible, while the protein backbone was kept fixed during the MC runs. The energetics were evaluated with the OPLS-AA force field for the protein,[20,21] OPLS/CM1A[21] for the ligands, and TIP4P for water.[22]

Model building with *BOMB* indicated that extension from the *para* position of the anilinyl ring with a linker-Het motif was feasible. In view of our report of **1a**,[6b] a MOM linker was considered first. An FEP pyridine scan[7] provided the results in Table 1. The 2- and 6-pyridinyl and 3- and 5-pyridinyl structures for the complexes do not interconvert during the MC simulations, so they are separately considered. The FEP results predicted little difference in activity for the pyridinyl isomers. The oxadiazole derivatives were then synthesized and assayed (Table 2). Indeed, though **1c**–**e** are active, the EC_{50} values are similar with little change from **1a**. Furanyl and thienyl analogs did not fare better. Based on prior experience,[6,7] the differences in Table 1 are too small to lead to any clear preference in the cell-based assay. The inactivity of the phenyl analog **1b** reflects poorer electrostatic interaction with the proximal protonated His235.

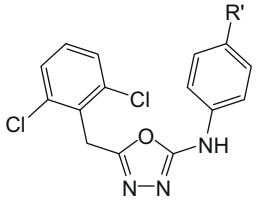
A computed structure for the 4-pyridinyl analog is shown in Figure 1. The benzyl and anilinyl rings of **1e** form π–π interactions

Table 1
Relative free energies of binding from MC/FEP calculations



R′	ΔΔG (kcal/mol)
CH_2OCH_2-Phenyl	0.0
CH_2OCH_2-2-Pyridinyl	−0.25 ± 0.28
CH_2OCH_2-3-Pyridinyl	−0.89 ± 0.20
CH_2OCH_2-4-Pyridinyl	−0.36 ± 0.27
CH_2OCH_2-5-Pyridinyl	0.51 ± 0.22
CH_2OCH_2-6-Pyridinyl	0.24 ± 0.22

Table 2
Anti-HIV-1 activity (EC_{50}) and cytotoxicity (CC_{50}), μM



Compound	R′	EC_{50}[a]	CC_{50}
1a	CH_2OCH_3	4.3	100
1b	CH_2OCH_2-Phenyl	NA	84
1c	CH_2OCH_2-2-Pyridinyl	5.0	>100
1d	CH_2OCH_2-3-Pyridinyl	4.6	81
1e	CH_2OCH_2-4-Pyridinyl	1.6	81
1f	CH_2OCH_2-2-Furanyl	2.8	12.0
1g	CH_2OCH_2-3-Furanyl	NA	21.0
1h	CH_2OCH_2-3-Thienyl	NA	>100

[a] NA indicates that $EC_{50} > CC_{50}$.

with Tyr181, Tyr188, Trp229, and Tyr318 (not shown), and there is an NH–O hydrogen bond with the oxygen atom of Lys101. The MOM linker directs the pyridinyl ring into the eastern channel. The pyridinyl nitrogen has a favorable interaction with the NH of

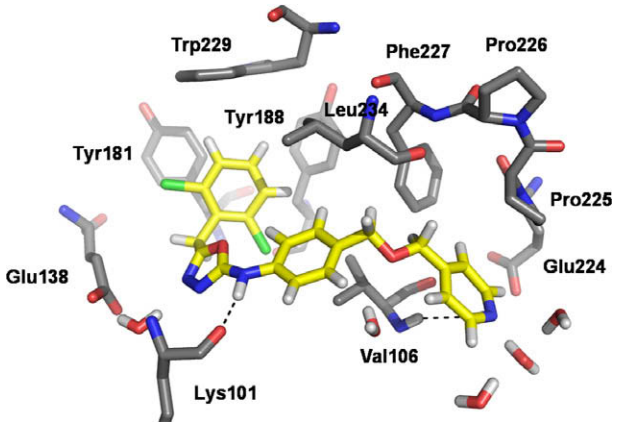


Figure 1. Snapshot of **1e** bound to HIV-RT from a MC/FEP simulation. Carbon atoms of **1e** are in yellow. Some residues including His235, Pro236 and Tyr318 are omitted for clarity.

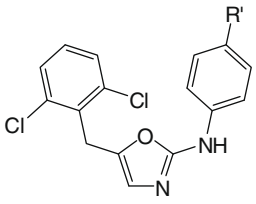
Val106 and also accepts a hydrogen bond from a water molecule. The 2-pyridinyl analog (not shown) has the nitrogen towards the viewer in Figure 1. Its computed binding affinity is sensitive to the presence or absence of a water molecule that can bridge between the nitrogen and MOM oxygen atoms. If a water molecule starts there in the MC simulations, it does not diffuse away. However, a JAWS analysis[23] found that the water molecule has an unfavorable binding free energy and it was removed.

Alternatives to the MOM linker were considered for the oxazoles **3**. MC/FEP calculations were performed to interconvert five XYZ possibilities (Table 3). Only the thio modifications were predicted to be more favorable than MOM. Indeed, a small gain was observed for the parent **3d** versus **3a** (Table 4); however, enthusiasm for pursuit of additional thio analogs was limited by likely metabolic liabilities. Reduction of the linker length to reduce potential conformational penalties on binding was also considered by *BOMB*-building/energy minimizations. This indicated that CH_2O, O, and no linker should all be viable and likely better than MOM. The corresponding FEP modifications are not straightforward and were not pursued. However, synthesis and assaying of **3j** and **3k** did provide activity boosts to 0.40 and 0.19 μM.

Motivated by this improvement, further refinements were probed. An FEP chlorine scan was performed on the anilinyl ring of **3j** to ascertain if additional substitution was desirable.[7] The computed $\Delta\Delta G$ values in Table 5 indicated that chlorination at C5 should be uniquely beneficial. A computed structure for the 5-chloro analog bound to RT is shown in Figure 2. The chlorine fills a void in the hydrophobic cavity beneath Leu234. It may also make the *meta*-NH a better hydrogen-bond donor towards Lys101.[24]

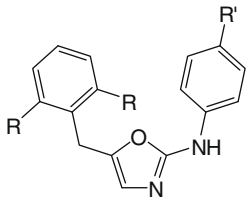
Table 6 contains related assay results. For the hydroxymethyl derivatives, **3i** and **3m**, addition of the chlorine enhances the WT

Table 3
MC/FEP results for optimization of the linker



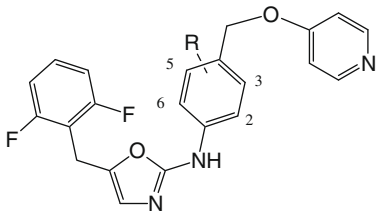
R′	ΔΔG (kcal/mol)
CH_2OCH_2-4-Pyridinyl	0.0
CH_2SCH_2-4-Pyridinyl	−2.3 ± 0.27
CH_2CH_2O-4-Pyridinyl	1.0 ± 0.27
CH_2CH_2S-4-Pyridinyl	−2.0 ± 0.26
$CH_2CH_2CH_2$-4-Pyridinyl	1.1 ± 0.21

Table 4
Anti-HIV-1 activity (EC_{50}) and cytotoxicity (CC_{50}), μM



Compound	R	R′	EC_{50}	CC_{50}
3a	F	CH_2OCH_3	2.1	32
3b	F	CH_2OCH_2-2-Furanyl	1.6	31
3c	Cl	CH_2OCH_3	1.2	8.1
3d	F	CH_2SCH_3	1.2	>100
3e	F	CH_2CH_2OH	2.7	35
3f	F	CH_2CH_2O-4-Pyridinyl	1.1	11
3g	F	OCH_2CH_2-1-Uracilyl	25	73
3h	F	CH_2CH_2S-2-Pyrimidinonyl	13	>100
3i	F	CH_2OH	3.8	>100
3j	F	CH_2O-4-Pyridinyl	0.40	>100
3k	F	*O*-4-Pyridinyl	0.19	21
3l	F	*O*-3-Pyridinyl	0.31	22

Table 5
MC/FEP results for replacements of hydrogen by chlorine



R	ΔΔG (kcal/mol)
H	0.0
2-Cl	−0.01 ± 0.14
3-Cl	0.12 ± 0.12
5-Cl	−2.87 ± 0.12
6-Cl	3.65 ± 0.17

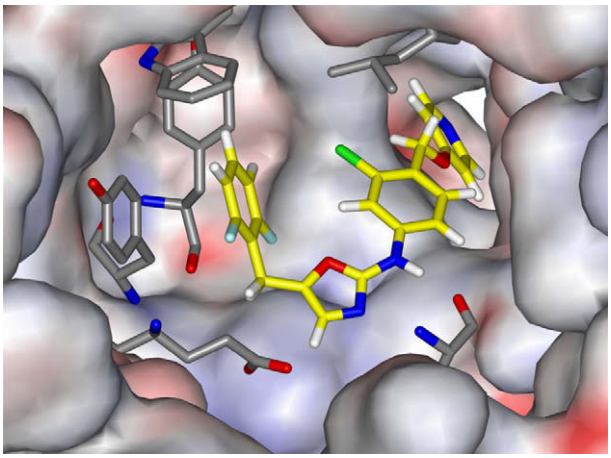
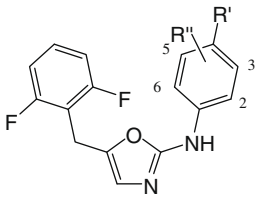


Figure 2. Complex for **3n** built with *BOMB* and energy-minimized.

Table 6
Anti-HIV-1 activity (EC_{50}) and cytotoxicity (CC_{50}), μM



Compound	R′	R″	EC_{50}	CC_{50}
3m	CH_2OH	3-Cl	0.26	90
3n	CH_2O-4-Pyridinyl	3-Cl	0.030	3.8
3o	CH_2O-4-Pyridinyl-*N*-oxide	3-Cl	0.011	1.7
3p	CN	H	0.013	7.4
3q	CN	3-Cl	0.006	11
3r	*O*-4-Pyridinyl	3-Cl	0.12	21
3s	*O*-3-Pyridinyl	3-Cl	0.031	16
3t	*O*-3-Pyridinyl	3-Br	0.034	7.5
3u	4-Pryidinyl	3-Cl	0.14	9.3
3v	3-Pyridinyl	3-Cl	0.073	8.9

Table 7
Activity (EC_{50}) and cytotoxicity (CC_{50}) in μM for variant HIV-1 strains

Compound	WT		Y181C		K103 N/Y181C	
	EC_{50}	CC_{50}	EC_{50}	CC_{50}	EC_{50}	CC_{50}
3k	0.19	21	5.0	17	12	17
3o	0.011	1.7	NA	2.7	NA	2.1
3p	0.013	7.4	NA	8.0	ND	ND
3q	0.006	11	0.42	9.9	NA	8.9
3r	0.12	21	2.3	17	7.2	17
3s	0.031	16	3.2	16	4.5	15
3u	0.14	9.3	3.8	11	5.6	9.1
3v	0.073	8.9	6.0	15	NA	12.5
d4T	1.4	>100	0.7	>100	0.5	>100
Nevirapine	0.11	>100	NA	>100	NA	>100
Evafirenz	0.002	>0.1	0.010	>0.1	0.030	>0.1
Etravirine	0.001	>0.1	0.011	>0.1	0.005	>0.1

activity by a factor of 15. Indeed, a similar boost is found for the CH_2O-4-pyridinyl analogs, **3j** and **3n**, with **3n** at 30 nM. However, the predicted aqueous solubility of **3n** from *QikProp* was below 1 μM, which is undesirable.[7] Thus, the *N*-oxide **3o** was prepared and found to be a potent NNRTI with an EC_{50} of 11 nM, and with a predicted solubility near 10 μM. **3r** and **3s** also showed improvement over **3k** and **3l**. The structurally novel, linker-less **3u** and **3v** were also prepared and show good activity. The cyano analog **3p** was previously the most potent inhibitor in the oxazole series.[6b] Addition of the *meta*-chlorine brings the activity to 6 nM for **3q**.

The Y181C and K103N/Y181C variants have been particularly problematic for NNRTI therapy,[1,5] so several of the more potent NNRTIs were tested against corresponding strains of HIV-1 (Table 7). Low-micromolar activity towards the variants is obtained with most of the compounds, which is an improvement over **3p**.[6b] The addition of the chlorine in going from **3p** to **3q** also helps towards Y181C, but not with the double mutant. Though interesting alternatives to the cyano group in **3p** and **3q** were discovered, the 'eastern extensions' did not overcome well the deficiencies in performance on the variants. In comparing our computed structures to crystal structures for related NNRTIs including etravirine with azine[17] rather than azole cores, the former appear to feature better contact of the western substituted phenyl groups with Y188 or W229 and less dependence on interaction with Y181.

In conclusion, extension of azole-containing NNRTIs towards the eastern portion of the binding site has yielded new anti-HIV agents. The structural range of possibilities is striking arising from variation of the linker length in, for example, **3f**, **3j**, **3k**, and **3u**. MC/FEP calculations provided structural insights on the complexes and assisted in the discovery of inhibitors with ca. 10 nM activities towards wild-type HIV-1.

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