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Optimization of pyrimidinyl- and triazinyl-amines as non-nucleoside inhibitors of HIV-1 reverse transcriptase

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Abstract—Non-nucleoside inhibitors of HIV-1 reverse transcriptase are being pursued through synthesis and assaying for anti-viral activity. Following computational analyses, the focus has been on the motif Het–NH–Ph–U, where Het is an aromatic heterocycle and U is an unsaturated, hydrophobic group. Previous investigations with Het = 2-thiazoyl and 2-pyrimidinyl are extended here to triazinyl derivatives. The result is several NNRTIs in the 2–20 nM range with negligible cytotoxicity and auspicious predicted pharmacological properties.
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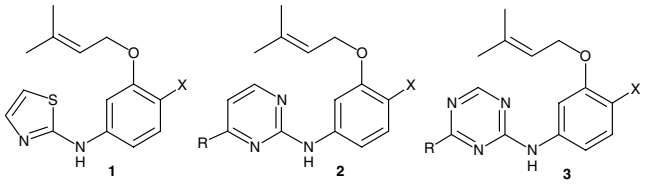
The AIDS crisis and the need for more effective therapeutic agents to combat HIV infection continue.[1] In recent papers, results were presented on our initial efforts to design non-nucleoside inhibitors of HIV reverse transcriptase (NNRTIs).[2,3] In particular, diarylamines have been pursued in a motif, Het–NH–Ph–U, where Het is a heterocycle and U is an unsaturated, hydrophobic group. Computational analyses featuring free energy perturbation (FEP) calculations directed us toward Het = 2-thiazolyl and 2-pyrimidinyl derivatives. The parent compounds **1** and **2** (X = H, R = H) turned out to be 10–30 μM inhibitors in an MT-2 cell protection assay; modest lead optimization provided the chloro, methoxy derivative of **2** (X = Cl, R = OMe) as a 10-nM NNRTI.[2,3] Subsequent FEP computations indicated that triazines **3** should also have promising activity, and further motivation for their pursuit was provided by possible avoidance of the cytotoxicity that plagued some of the more active pyrimidines, particularly with X = CN.[3] Low solubility is also a common issue with NNRTIs, and the triazines could be expected to show benefits in that regard.[4] Key findings from the resultant investigations are summarized here.



Chemistry and biology. Syntheses of the 2-thiazole and 2-pyrimidine derivatives were described previously and followed the general route in Scheme 1.[3] For the triazene analogs, dichloro and trichlorotriazine were used as starting materials. They were typically added to the preformed dimethylallyl (DMA)-derivatized aminophenol, which was prepared by $SnCl_2$ reduction of the corresponding nitrophenol with subsequent substitution of chlorines on the triazine. The routes for a di- and a tri-substituted triazine are shown in Scheme 2. The parent triazine **3** (R = H) was not easily available owing to instability of chlorotriazine. Monochloro triazines such as **5** (R = Cl) could also be converted to the amino derivatives in near quantitative yield by treatment with the amine in methanol. The thiomethoxy analog of **5** (R = H) was prepared in 77% yield using NaSMe in THF rather than NaOMe.

A variety of alternatives for the U group was also tested in the 2-thiazole series, and ODMA emerged as preferred for potency.[3] Further investigations were made

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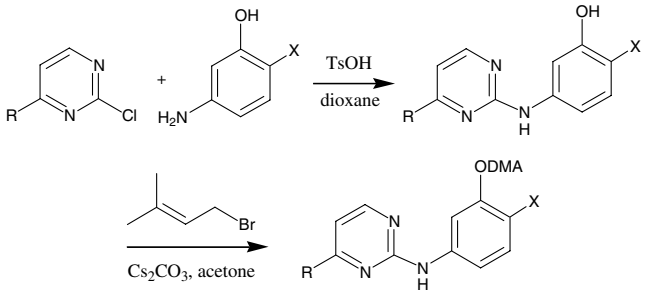
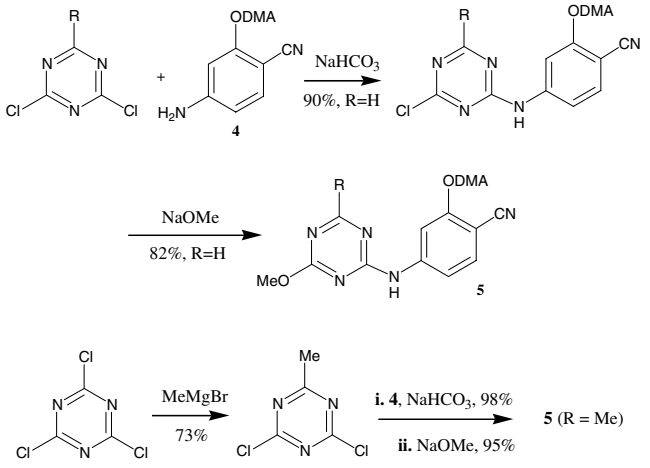
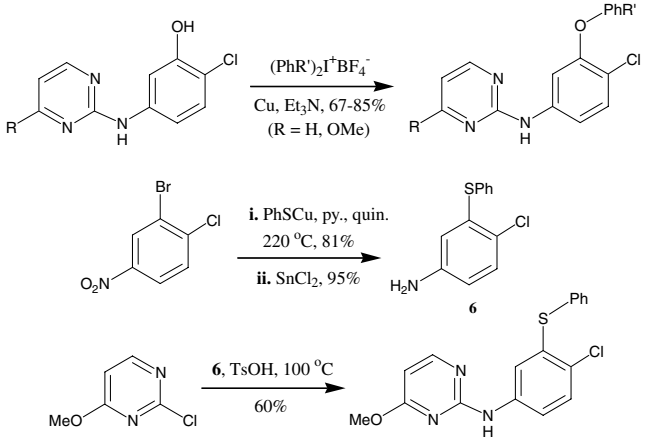


Scheme 1.



Scheme 2.

for the 2-pyrimidines. Attachment was normally effected by base-catalyzed substitution (Scheme 1) or by alcohol coupling under Mitsunobu conditions (Ph_3P, DEAD).[3] However, phenyl and tolyl ether analogs, Het–NH–PhX–OPhR′, were prepared by coupling $(R'Ph)_2I^+$ salts and the phenol derivative, while preparation of a thioether analog utilized PhSCu to yield selectively the desired meta relationship with the amino group in **6** (Scheme 3).[5]

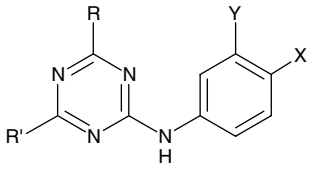


Scheme 3.

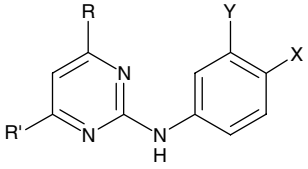
Activities against the IIIB strain of HIV-1 were determined using MT-2 human T-cells; the EC_{50} values are the dose required to achieve 50% protection of the infected cells using the MTT colorimetric method. The CC_{50} for inhibition of MT-2 cell growth by 50% was obtained simultaneously.[6,7]

Activities and cytotoxicities. Results are listed in Table 1 for the triazene and pyrimidine derivatives **7**–**10**. Some general patterns for equivalently substituted systems are: (1) the triazenes are less active than the pyrimidines by a factor of 3–5; (2) the cyano analogs (X = CN) are more active than the chloro analogs (X = Cl) by a factor

Table 1. Anti-HIV-1 activity (EC_{50}) and cytotoxicity (CC_{50}), μM, for triazene and pyrimidine derivatives



7 (X=Cl, Y=ODMA)
8 (X=CN, Y=ODMA)



9 (X=Cl, Y=ODMA)
10 (X=CN, Y=ODMA)
11 (X=Cl, R'=H)

Compound	X	R	R′	EC_{50}[a]	CC_{50}[b]
7a	Cl	Cl	H	NA	45.0
7b	Cl	OMe	H	0.031	18.0
7c	Cl	NH_2	H	0.390	48.0
7d	Cl	NHMe	H	0.031	3.1
7e	Cl	Me	Cl	12.0	40.0
7f	Cl	OMe	Cl	NA	26.0
7g	Cl	OMe	Me	0.100	12.0
7h	Cl	OMe	OMe	0.290	20.0
8a	CN	Cl	H	0.310	>100
8b	CN	OMe	H	0.011	42.0
8c	CN	NH_2	H	0.015	0.20
8h	CN	OMe	OMe	0.022	>100
8i	CN	SMe	H	0.005	8.4
8j	CN	OMe	NH_2	0.009	0.11
9b	Cl	OMe	H	0.010	9.0
9c	Cl	NH_2	H	0.075	0.50
9d	Cl	NHMe	H	0.006	0.69
9h	Cl	OMe	OMe	0.250	28.0
9i	Cl	SMe	H	0.018	2.8
9j	Cl	OMe	NH_2	3.0	19.0
9k	Cl	OEt	H	0.041	19.0
9l	Cl	CH_2OMe	H	NA	>25
9m	Cl	CH_2OH	H	NA	0.22
9n	Cl	H	H	0.200	2.5
9o	Cl	Me	H	0.039	0.15
9p	Cl	Et	H	0.016	0.33
10b	CN	OMe	H	0.002	0.23
10c	CN	NH_2	H	NA	0.041
10d	CN	NHMe	H	0.005	0.022
10h	CN	OMe	OMe	0.160	0.810
10n	CN	H	H	0.017	0.036
Nevirapine				0.110	>10
Efavirenz				0.002	>0.10
TMC125				0.002	>1

[a] For 50% protection in MT-2 cells; antiviral curves used triplicate samples at each concentration. NA for $EC_{50} > CC_{50}$.

[b] For 50% inhibition of MT-2 cell growth; toxicity curves also used triplicate samples.

of 3–10; (3) compounds with a single OMe, SMe, or NHMe substituent on the heterocycle are particularly potent, that is, **7**–**10b**, **10d**, **10i**; (4) the triazenes show little cytotoxicity with the exception of the amino, cyano compounds **8c** and **8j**; (5) the cyano pyrimidines are both potent and cytotoxic, though **10b** has a safety margin (CC_{50}/EC_{50}) > 100; and (6) many of the compounds are highly potent with EC_{50} values below 20 nM, and three of the potent triazines (**8b**, **8h**, **8i**) also have safety margins >1000. Understanding of the origins and variations of the cytotoxicity is lacking. Since it was more pronounced for the 2-pyrimidines than the 2-thiazoles, a recognition element associated with the heterocycle in the present NNRTI series appeared to be operative. Indeed, this notion is further supported by the favorable results for the triazenes.

Results are also included in Table 1 for three reference NNRTIs, nevirapine (Viramune®), efavirenz (Sustiva®), and TMC125 (etravirine). The present compounds are considerably more effective against WT HIV-1 than nevirapine, and the most active ones are in the low nM-range like efavirenz and TMC125. Of course, pharmacologically important properties of the NNRTIs are also relevant,[2,3] as discussed further below.

The results for the alternative choices for the U group in the pyrimidine series are listed in Table 2. As with the 2-thiazoles for which various heteroarylmethoxy options for U were tried,[3] no choice has emerged superior to ODMA. Removal of the *Z*-methyl group of ODMA to yield the *E*-buten-2-yl ethers, **11a** and **11c**, reduces potency ca. 10-fold. However, the structural analyses below, including Figure 1, suggest that this change could be advantageous for increased resilience to the Y181C variant of HIV-RT. The phenyl ethers **11b** and **11d** are ca. 100-fold less potent than the DMA ethers, and only the *m*-tolyl analog **11f** showed somewhat improved performance. Among the aryl ethers, the thioether **11h** was the most potent, though its EC_{50} is still 32-fold higher than that for the DMA ether **9b**.

Molecular modeling. In the previous study,[2] structures were built for complexes of the 2-thiazoyl and 2-pyrimidinyl NNRTIs, and they were validated by the good accord between FEP-computed and observed relative activities for variation of Het and Y in Het–NH–PhY–ODMA inhibitors. The heterocycles were unsubstituted

Table 2. Anti-HIV-1 activity (EC_{50}) and cytotoxicity (CC_{50}), μM, for derivatives of pyrimidine **11**[a]

Compound	R	Y	EC_{50}	CC_{50}
9n	H	ODMA	0.200	2.5
11a	H	(*E*)-$OCH_2CH{=}CHCH_3$	2.3	31.0
11b	H	OPh	13.0	30.0
9b	OMe	ODMA	0.010	9.0
11c	OMe	(*E*)-$OCH_2CH{=}CHCH_3$	0.089	17.0
11d	OMe	OPh	2.5	38.0
11e	OMe	O–*o*-MePh	NA	13.0
11f	OMe	O–*m*-MePh	0.540	18.0
11g	OMe	O–*p*-MePh	10.0	>100
11h	OMe	SPh	0.320	21.0

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

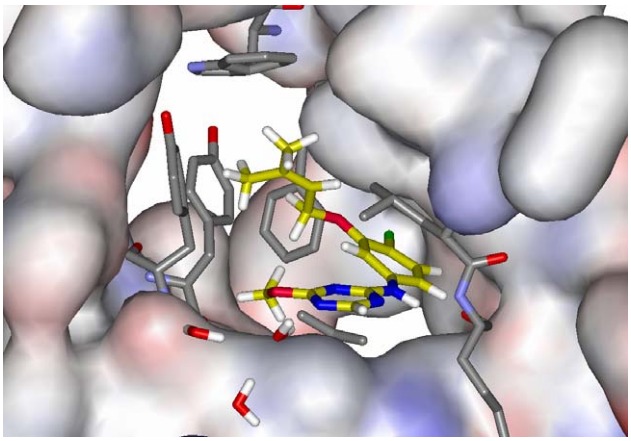


Figure 1. Typical snapshot of **7b** bound to HIV-RT from an MC simulation. Carbon atoms of **7b** are gold; from the left, Tyr181, Tyr188, Phe227, Leu100, Lys101; Trp229 at the top, Val106 at the bottom. H-bond with Lys101 O on right. Some residues in front including Glu138 have been removed for clarity. The water on N5 is also H-bonded to a carboxylate O of Glu138.

in the FEP calculations, so the positioning of a substituent, for example, whether it would correspond to R or R′ in **9** in a complex with RT, was not addressed in detail. In fact, by visualization, energy minimizations, and even Monte Carlo (MC) simulations in explicit water it was ambiguous which position would be preferred for the methoxy group in **9b**. MC/FEP calculations were subsequently performed at 25 °C to address this issue; they followed standard protocols[2,8] including the use of 178 residues of RT, 1250 (complex) and 2000 (unbound) TIP4P water molecules, the OPLS/CM1A force field,[9] and 14 free-energy windows for each perturbation.

The preferred orientation of a methyl group was addressed first. To assist in visualization, Figure 1 can be consulted. The bound inhibitors adopt a right-handed helical form. The substituent on the heterocycle can either be directed 'in' toward Tyr181 and Tyr188, as illustrated for **7b**, or 'out' toward Lys101. During the simulations, interconversion between 'in' and 'out' never occurred. MC/FEP calculations were performed for the closed cycle: Me_2-**9** → Me_{in}-**9** → H_2-**9** → Me_{out}-**9** → Me_2-**9**. The hysteresis for the free energies of binding using this cycle was 0.40 ± 0.25 kcal/mol. Then, additional MC/FEP calculations were performed to convert OMe, SMe, and other small substituents to the methyl analogs, which yielded the relative free energies of binding in Table 3.

There is a very strong preference, 6–10 kcal/mol, for the Me, OMe, SMe, and OEt groups to be 'in.' They project into the pocket near C_α and C_β of Tyr181 and Tyr188 (Fig. 1). The pocket cannot accommodate substituents larger than OEt, and there is some reduction in activity in going from OMe to OEt. Water is not observed in this region during the MC simulations. Thus, N3 of the polyazines does not participate in hydrogen bonding in the complexes. Also, the pocket is formed for the unsubstituted cases, so there is no penalty for further cavity

Table 3. MC/FEP results for relative free energies of binding for analogs of **9** with HIV-RT

Compound	R_{in}	R'_{out}	$\Delta\Delta G$[a]	EC_{50} (μM)
9n	H	H	0.0	0.200
	Me	Me	−0.78 ± 0.18	
	H	Me	3.00 ± 0.14	
9o	Me	H	−3.23 ± 0.41	0.039
9p	Et	H	−4.18 ± 0.15	0.016
9h	OMe	OMe	0.50 ± 0.40	0.250
	OMe	Me	0.06 ± 0.31	
9b	OMe	H	−2.41 ± 0.26	0.010
	H	OMe	4.99 ± 0.28	
9k	OEt	H	−1.39 ± 0.31	0.041
	H	OEt	7.49 ± 0.41	
9m	H	CH_2OH	0.05 ± 0.32	>0.220
9i	SMe	H	−2.68 ± 0.29	0.018
	H	SMe	7.21 ± 0.31	
	Me	NH_2	−2.51 ± 0.28	
	NH_2	Me	5.63 ± 0.26	
	Cl	NH_2	−1.89 ± 0.26	

[a] $\Delta\Delta G$ in kcal/mol, corrected by $RT\ln 2$ (+0.41 kcal/mol) for conformer loss on binding when $R \neq R'$.

growth to accommodate the small substituents. There is no serious steric problem for the 'out' orientation; its disfavoring appears more associated with interference with hydration of the side chains of Glu138, Lys101, and Lys103. The H-bond donating groups NH_2 and CH_2OH do favor being 'out' since their H-bonding needs are not satisfied if they are 'in;' in both cases they donate an H-bond to the COO^- of Glu138.

The preferences are expected to carry over to the triazines. Furthermore, as shown in Figure 1, a water molecule is found to donate an H-bond to N5. This water is also donating an H-bond to a carboxylate O of Glu138 and accepting H-bonds from two water molecules; the one on the left is also donating an H-bond to the O=C of Tyr181. Thus, N5 is well accommodated in the complexes, but it is also well accommodated in the unbound state, so there is no binding boost over the pyrimidines. Also, the 'in' alkoxy oxygen is rarely hydrogen-bonded to a water molecule in any of the MC runs for bound **7b**, **9b**, **9h**, and **9k**. Finally, it is noted that the trends in the free energy results correlate well with the observed activities (Table 3). The methyl and ethyl analogs are just predicted to be somewhat better bound than their activities reflect.

Predicted properties. Some predicted properties from *QikProp* for a selection of the current compounds and other NNRTIs are summarized in Table 4; rms errors for *QikProp* predictions are 0.5–0.6 log unit.[10] When *QikProp* is run on 1700 known oral drugs,[11] 90% have $M_W < 470$, QP log $P < 5.0$, QP log $S > -5.7$, and QP $P_{Caco} > 22$ nm/s. Many of the potent compounds reported here compare favorably with all these limits, so optimism can be expressed for acceptable oral bioavailability. Predicted solubilities, in particular, show significant improvement over rilpivirine and TMC125, which are in clinical trials.

Table 4. Predicted properties for selected NNRTIs

Compound	MW[a]	QP log P[b]	QP log S[c]	QP P_{Caco}[d]
Nevirapine	266.3	2.5	−3.2	2090
Eefavirenz	315.7	3.5	−5.0	1585
Delavirdine	456.6	2.6	−5.7	218
UC781	335.8	5.1	−5.7	6717
Rilpivirine	366.4	3.3	−6.5	150
TMC125	435.3	2.7	−6.7	75
7b	320.8	4.1	−4.8	2911
7d	319.8	3.7	−4.9	1745
8b	311.3	2.9	−5.1	739
8h	341.4	3.4	−5.5	1153
8i	327.4	3.4	−5.7	797
9b	319.8	4.6	−5.1	4512
9k	333.8	5.1	−5.2	4209
10b	310.4	3.5	−5.4	1062

[a] Molecular weight.
[b] Predicted octanol/water log P from QikProp, v 3.0.
[c] Predicted aqueous solubility from QikProp, v 3.0; S in mol/L.
[d] Predicted Caco-2 cell permeability in nm/s from QikProp, v 3.0.

In conclusion, lead optimization in the Het–NH–Ph–U motif with Het = 2-pyrimidinyl and triazinyl has led to the discovery of highly potent NNRTIs with low cytotoxicity and auspicious predicted properties. Development continues including crystallography and assaying against mutant HIV strains.

Acknowledgment

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