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Optimization of diarylazines as anti-HIV agents with dramatically enhanced solubility

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Abstract

Non-nucleoside inhibitors of HIV-1 reverse transcriptase are reported that have ca. 100-fold greater solubility than the structurally related drugs etravirine and rilpivirine, while retaining high anti-viral activity. The solubility enhancements come from strategic placement of a morpholinylalkoxy substituent in the entrance channel of the NNRTI binding site. Compound **4d** shows lownanomolar activity similar to etravirine towards wild-type HIV-1 and key viral variants.

The use of non-nucleoside inhibitors of HIV-1 reverse transcriptase (C) is commonplace for treatment of HIV infection.[1,2] Among the five FDA-approved drugs in the class, the most recent introductions have been etravirine and rilpivirine. These diarylpyrimidines provide much improved performance in cell assays against variant forms of HIV-1 that incorporate mutations in the vicinity of the NNRTI binding site.[3,4] The earliest approved NNRTIs, nevirapine and delavirdine, are debilitated by most common mutations. Though the second-generation compound, efavirenz, performs well against variants bearing the clinically prevalent Tyr181Cys mutation, resistance arises from other common variants such as those including Lys103Asn.[2-4] The clinical significance of efavirenz and rilpivirine is particularly great since they are incorporated into the once-a-day combination therapies Atripla and Complera, respectively.[5] The other two active components of the pills are the same, the nucleosides emtricitabine and tenofovir. Though the performance in cell-based assays is far better for rilpivirine than for efavirenz, surprisingly more virologic failure is observed for patients under treatment with Complera than Atripla.[5-7] Thus, from this observation and the desire to further diminish dosages and side effects, improvements are still possible for the NNRTI class.

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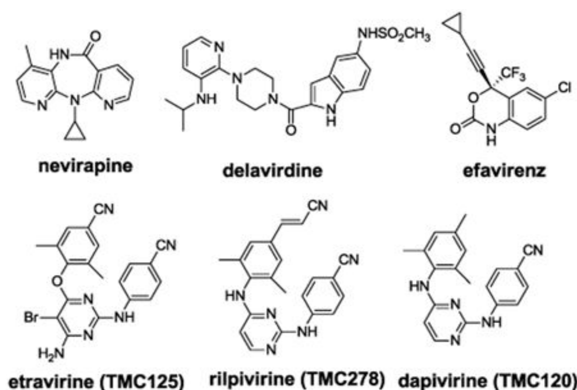
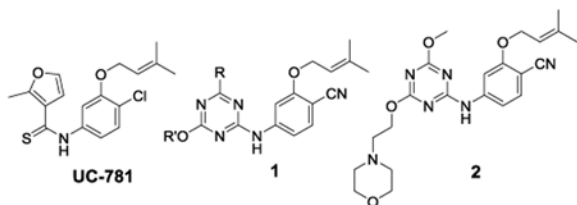


A particular issue with aminoazine NNRTIs has been poor solubility, which often has undesirable ramifications including low bioavailability, difficulties in formulation, and accumulation in fatty tissues.[8,9] Most oral drugs have an aqueous solubility (S) in the range 10^{-5} to 10^{-2} M, which for a drug with a molecular weight of 400, corresponds to 4 to 4,000 μg/mL. It is very rare for an FDA-approved oral drug to have a solubility near neutral pH below 10^{-6} M.[9] However, rilpivirine "is practically insoluble in water (20 ng/mL at pH 7.0) ",[4] which translates to an S of 5×10^{-8} M. It appears to have an unusual absorption mechanism involving aggregates.[10] For etravirine, the solubility is also "≪1 μg/mL", and extensive formulation work was needed to bring the daily dosage to 0.4 g per day.[11] Furthermore, in view of its low solubility, dapivirine is being evaluated as a vaginal microbicide.[12] This was also the fate of UC-781, an earlier, NNRTI with poor solubility (<30 ng/mL).[13] Interestingly, the daily dosage for nevirapine, like etravirine, is 0.4 g despite the fact that its potency towards WT HIV-1 is ca. 100-fold less than for etravirine. An important factor is undoubtedly that the observed aqueous solubility of nevirapine is 167 μg/mL ($10^{-3.2}$ M).[14] Nevirapine demonstrates that it is possible to have a viable NNRTI that has an EC_{50} of ca. 100 nM in cell assays, if the compound has good solubility and bioavailability.



Poor solubility has also been an issue with aminoazine-containing NNRTIs (**1**) from our laboratory. In view of the structural overlap of **1**, the TMC compounds, and UC-781, it is not surprising that the measured solubility of **1** (R = H, R′ = Me) is 0.1 μg/mL.[15] In order to improve the solubility of such aminoazines, extensive modeling, synthesis, assaying and crystallography were undertaken that demonstrated that it was possible to attach a solubilizing substituent as OR′ that would extend into the entrance channel of the NNRTI binding site.[15,16] Specifically, **2** has S = 42 μg/mL, while retaining an EC_{50} of 92 nM in a standard assay using MT-2 cells infected with WT HIV-1.[15]

In the present communication, results are presented for applying this strategy to improve the solubility of diarylpyrimidines and triazines by investigating **3** and **4** (Scheme 1). Synthesis of the compounds proceeded as indicated via three S_NAr reactions.[17] The final intermediate **7** was also reduced to obtain the corresponding analogs **8** and **9** lacking the morpholinoalkoxy group. The identities of all assayed compounds were confirmed by ^1H

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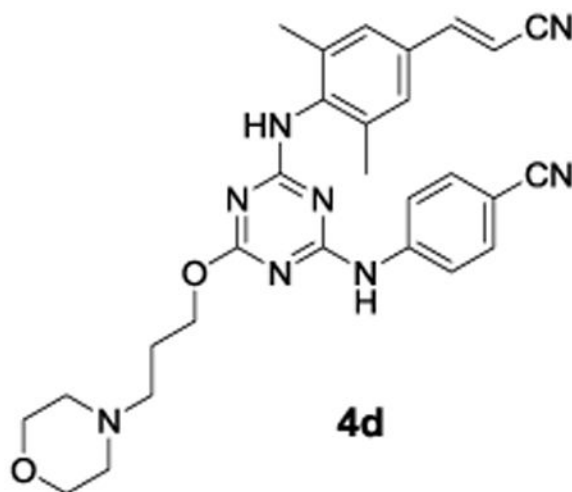
and ^{13}C NMR and high-resolution mass spectrometry; purity was >95% as judged by high-performance liquid chromatography.

Activities against the IIIB and variant strains of HIV-1 were measured using MT-2 human T-cells, as previously described.[16,18] 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. Solubility measurements used a shake-flask protocol with triplicate samples.[19] The compounds were dissolved in Britton-Robinson buffer and stirred in vials for 48 hours at 25 °C. The pH of the buffer solutions was measured by a Corning General Purpose pH Combination probe (4136L21). The solution containing excess solid was filtered using a Whatman Mini-UniPrep syringeless filter device with a 0.45 μm pore size, and the supernatant was analyzed by UV-vis spectrophotometry (Agilent 8453). Piroxicam was used as a reference compound; our solubility result of 7.2 μg/mL compares well with the prior report of 6.4 μg/mL.[19]

The results of the anti-viral assays are presented in Table 1. Our synthesized TMC120 (**8a**) yielded 0.7 nM potency in the WT assay and 39 nM results for both the Y181C and K103N/Y181C variants. This can be compared with previous results of 1.2, 7, and 54 nM using MT-4 cells.[4] The other compound that was previously reported, the triazine **9a**, was found here to have 2.3, 47, and 90 nM EC_{50} values, while the MT-4 assays yielded 0.3, 8, and 50 nM.[20] Thus, the accord is reasonable except that it appears that the Y181C containing strain used here is more challenging.



Appendage of the morpholinoethoxy substituent to **1** (R = H, R′ = Me) to yield **2** causes a 9-fold reduction in WT potency,[15] while the modification of pyrimidine **8a** to yield **3a** and **3b** results in 10- to 20-fold declines. Though the compounds are still potent NNRTIs towards WT virus, the EC_{50} results of 700 and 480 nM for the Y181C-bearing variant are more problematic. However, the impressive performance of **3b** towards the challenging double mutant is notable. The corresponding triazine **4b** fares even better with 8, 310, and 31 nM EC_{50} values. Thus, these results encouraged further study of triazine analogs. Replacement of the 4-Me substituent of the mesityl group by cyano and cyanovinyl was explored and provided the remarkably potent **4d** with EC_{50} results of 1, 12, and 1 nM for the WT and mutant HIV-1 strains. **4d** may be viewed as a triazine relative of rilpivirine with a strategically added morpholinopropoxy group. Its activity results are very similar to those obtained here for etravirine. 2,6-difluorophenyl alternatives **4e** - **4i** with various 4-R groups were also considered, but did not surpass the overall performance of **4d**. However, the 4-

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methyl analog **4e** was strikingly potent, 190 pM, in the WT assay. Only one NNRTI with greater anti-HIV activity has been previously reported.[18]

The solubility results are summarized in Table 2. Consistent with the results for **1** and **2**,[15] the addition of the morpholinoalkoxy groups has profound effects with 83- and 182-fold increases in S in going from TMC120 (**8a**) to **3a** and **3b**. The aqueous solubilty of the corresponding triazine **9a** is also very low (0.2 μg/mL); large enhancements are again delivered by the morpholinoalkoxy analogs. Notably, **4d** has a solubility of 14.2 μg/mL, which is 100-fold greater than for dapivirine (TMC120, **8a**) and 59- and 710-fold greater than the prior reports for rilpivirine.

As demonstrated crystallographically for **2**, it is expected that the morpholinoalkoxy side chains for the present compounds in complex with HIV-1 reverse transcriptase extend past Glu138 into the entrance channel of the NNRTI binding site.[15,16] An illustration for **4b** is provided in Figure 1, as created by modeling with the *BOMB* and *MCPRO* programs[22,23] using the OPLS/CM1A force field[24] starting with the 1S9E crystal structure, which has an anilinyltriazine as the ligand.[25] Consistent with the modeling and crystallography for **2**,[15] the contacts in the NNRTI binding site are normal and the morpholinopropoxy side chain extends past Glu138 towards Glu28. A salt bridge, which is sometimes observed between Glu138 and Lys101, cannot be present to allow the passage. With the $(CH_2)_3$ spacer a salt-bridge between Gu138 and the protonated morpholine is unlikely. However, if Glu28 reoriented, it could be in close contact with the morpholine terminus. This suggested possible benefit of replacing the ether oxygen with a positively charged group. Thus, the piperidine analogs **10a** and **10b** were synthesized (Scheme 2), but they turned out to be 2-3-fold less potent than the corresponding morpholine analogs **4a** and **4b** (Table 1). The intended salt-bridge is largely solvent-exposed and in competition with the Glu28-Lys32 interaction.

In summary, structural analyses suggested the possibility of appending solubilizing groups to the diarylazine class of NNRTIs at the 6-position in the azine ring. The strategy was successful and delivered promising, new NNRTIs, whose formulation should be facilitated. Notably, compound **4d** has similar potency as etravirine in infected T-cell assays using WT HIV-1 (IIIB) as well as viral variants that incorporate the two most commonly found resistance mutations in the RT enzyme, Tyr181Cys and Lys103Asn.[2] However, the solubility of **4d** is ca. 100-fold greater than for the diarylpyrimidines dapivirine (**8a**), etravirine, and rilpivirine. Further fine-tuning of properties and activities can be envisioned now that the benefits of exploration of the NNRTI entrance channel are clear.

Acknowledgments

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17. General procedure: 5 (0.34 g, 1.2 mmol) was dissolved in anhydrous THF (10 mL), following by addition of the corresponding aniline 6 (1.2 mmol) and DIPEA (0.18 g, 1.44 mmol). The reaction mixture was stirred at room temperature or refluxed overnight. THF was removed under pressure; the crude was purified on silica gel to afford the corresponding compounds 7. Then, to a solution of the hydroxyalkylmorpholine (12.8 mmol) in anhydrous THF, NaH (95 %, 7.1 mmol) was added in portions at 0 oC After 30 min, a solution of 7 in dioxane was added dropwise and stirred at room temperature or at 80 oC overnight. After this period, the solvent was removed under pressure; the crude was purified on silica gel to give the target compounds. For compound 4d: 1H NMR (500 MHz, $CDCl_3$) δ 7.81 - 7.58 (m, 2H), 7.47-7.32 (m, 4H), 7.17 (s, 1H), 6.73 (s, 1H), 5.91 (d, J = 16.8 Hz, 1H), 4.42 (s, 2H), 4.22 (s, 1H), 3.72 (s, 4H), 2.46 (t, J = 33.9 Hz, 6H), 2.29 (d, J = 9.2 Hz, 6H), 1.98 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 173.77, 166.10, 150.29, 143.64, 136.48, 132.60, 127.27, 119.45, 118.47, 66.17, 64.45, 55.21, 53.33, 48.58, 40.12, 39.91, 39.70, 39.49, 38.86, 18.24. HR-MS (ES) calcd for $C_{28}H_{3}0\ N_8O_2$ $[M+1]^+$ 511.0009, found 511.0011.

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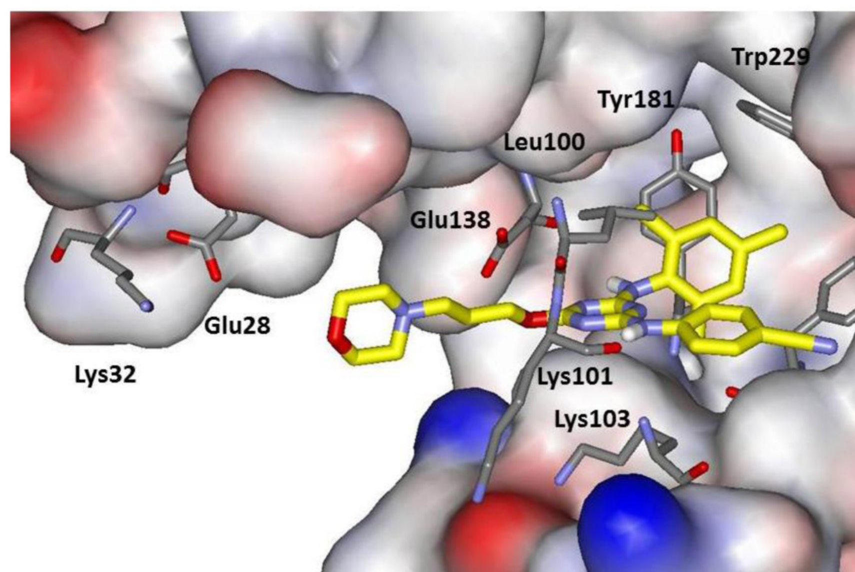

Figure 1.
Computed structure for the complex of **4b** with WT HIV-1 reverse transcriptase. Carbon atoms of **4b** in yellow. Some residues removed for clarity.

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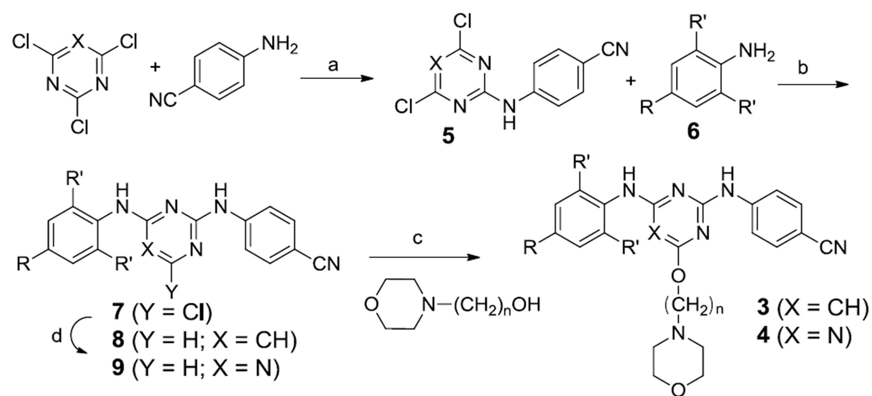

Scheme 1.

Synthesis of **3** (X = CH) and **4** (X = N). Reagents: (a) DIPEA THF, rt, overnight; (b) DIPEA, THF, 65 °C, 2 days; (c) NaH, THF, rt, overnight; (d) H_2/Pd-C.

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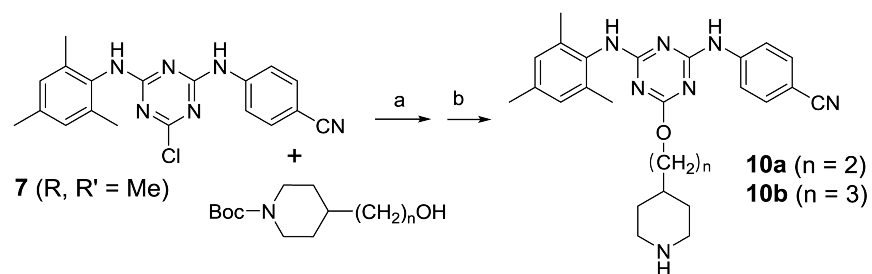

Scheme 2.

Synthesis of **10a** and **10b**. Reagents: (a) NaH, ACN, 70 °C, overnight; (b) TFA, DCM, 0 °C to rt, 15 min.

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Table 1

Anti-HIV-1 Activity (EC_{50}) and cytotoxicity (CC_{50}), μM[a]

Compnd	n	R′	R	EC_{50} WT	EC_{50} Y181C	EC_{50} K103N/Y181C	CC_{50}
8a[b]	-	Me	Me	0.0007	0.039	0.039	2.0
3a	2	Me	Me	0.019	0.700	0.060	9.0
3b	3	Me	Me	0.0086	0.480	0.038	3.1
9a	-	Me	Me	0.0023	0.047	0.090	7.0
4a	2	Me	Me	0.012	0.700	0.110	95
4b	3	Me	Me	0.0081	0.310	0.031	8.0
4c	3	Me	CN	0.0068	1.0	0.042	>100
4d	3	Me	CV[c]	0.0012	0.012	0.0013	4.5
4e	3	F	Me	0.00019	0.350	0.070	10.0
4f	3	F	Et	0.0016	0.500	0.050	13.0
4g	3	F	*i*-Pr	0.0022	0.270	0.030	1.5
4h	3	F	*c*-Pr	0.0028	1.100	0.150	2.2
4i	3	F	CE[d]	0.005	0.230	0.020	27.0
10a	2	Me	Me	0.024	1.000	0.100	4.2
10b	3	Me	Me	0.024	1.200	0.140	4.0
nevirapine				0.11	NA	NA	>100
efavirenz				0.002	0.010	0.030	15
etravirine				0.001	0.008	0.005	11
rilpivirine				0.00067	0.00065	0.002	8

[a]Results using human MT-2 cells. Antiviral and toxicity curves used triplicate samples at each concentration. NA = not active.

[b]**8a** = TMC120.

[c]CV = *E*-cyanovinyl.

[d]CE = 2-cyanoethyl.

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Table 2

Aqueous Solubility at pH 6.5 (S)

Compound	S, μg/mL	Compound	S, μg/mL
8a	0.15	**4d**	14.2
3a	12.5	**4e**	22.9
3b	27.3	**4i**	25.4
9a	0.20	nevirapine	167[a]
4a	4.42	efavirenz	68.0
4b	15.3	etravirine	≪ 1[b]
4c	13.3	rilpivirine	0.02,[c] 0.24[d]

[a]Ref. 14.

[b]Ref. 11.

[c]Ref. 4, pH 7.

[d]Ref. 21, pH 7.4.