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Bioorganic & Medicinal Chemistry Letters 16 (2006) 668–671
Bioorganic & Medicinal Chemistry Letters

Optimization of diarylamines as non-nucleoside inhibitors of HIV-1 reverse transcriptase

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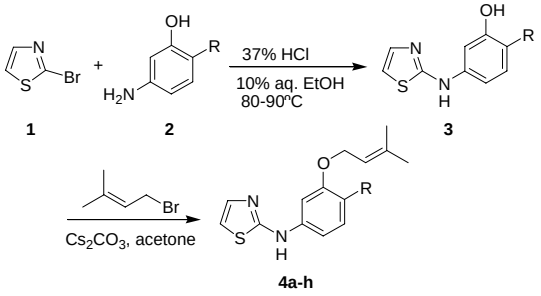
Received 23 September 2005; accepted 11 October 2005
Available online 17 November 2005

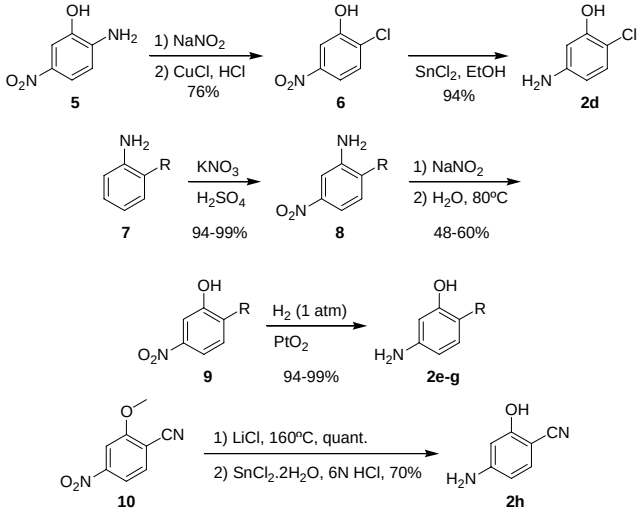
Abstract—Following computational analyses, potential non-nucleoside inhibitors of HIV-1 reverse transcriptase have been pursued through synthesis and assaying for anti-viral activity. The general class Het–NH–Ph–U has been considered, where Het is an aromatic heterocycle and U is an unsaturated, hydrophobic group. Results for compounds with Het = 2-thiazoyl and 2-pyrimidinyl are the focus of this report.

Delineation of general design principles and computational modeling has led to pursuit of diarylamines as non-nucleoside inhibitors of HIV reverse transcriptase (NNRTIs).[1] The Het–NH–Ph–U class has been considered in depth, where Het is an aromatic heterocycle and U is an unsaturated, hydrophobic group. Lead optimization has focused to date on Het = 2-thiazoyl and 2-pyrimidinyl derivatives, as described here.

Thiazoles. Variations of the R-group in the 4-position of the phenyl ring were explored for the thiazole series **4** with U as *O*-3,3-dimethylallyl (ODMA) via the synthetic route in Scheme 1. The requisite aminophenols were obtained commercially or they were prepared in two or three steps as in Scheme 2. 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% cytopathic protection of the HIV-infected cells using the MTT colorimetric method.[2,3] As listed in Table 1, the parent compound **4a** turned out to be a 10 μM inhibitor. Addition of a methyl, methoxy, or ethyl group provides a modest boost, while propyl and isopropyl are deactivating. However, substitution with



Scheme 1.



Scheme 2.

Keywords: NNRTI; Computer-aided drug design; Diarylamine; Anti-HIV drug; Thiazoylamine; Pyrimidinylamine.
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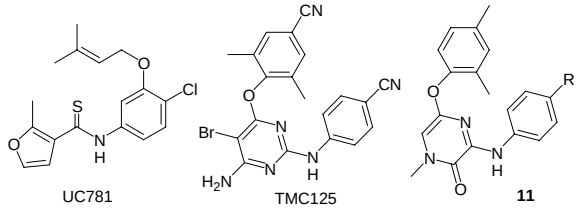
doi:10.1016/j.bmcl.2005.10.037

Table 1. Anti-HIV-1 activity (EC_{50}) and cytotoxicity (CC_{50}), μM, for thiazoles **4** and reference compounds[a]

Compound	R	EC_{50}	CC_{50}
4a	H	10	23
4b	CH_3	3.0	17
4c	OCH_3	3.8	42
4d	Cl	0.30	26
4e	CH_2CH_3	5.0	31
4f	$CH_2CH_2CH_3$	23	23
4g	$CH(CH_3)_2$	NA	31
4h	CN	0.21	0.75
4i	COOH	NA	17
d4T		3 ± 1	>100
Nevirapine		0.12	>10
UC781		0.002	>100
TMC125		<0.002	>1

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



chlorine or a cyano group enhances activity to the 0.3 and 0.2 μM levels. These qualitative trends agree well with the MC/FEP predictions,[1] especially the preference for R = Cl and CN in **4**.

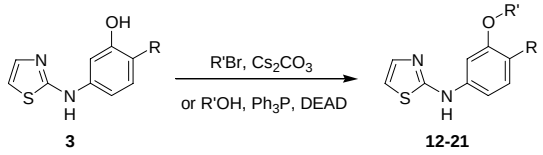
The position of the chlorine in the computed structure for the complex of RT with **4d** is similar to those found in computed and X-ray structures for RT-complexes with other NNRTIs including efavirenz, PNU142721, UC781, and 8-chloro-TIBO.[1] In addition, the cyano alternative is precedented in TMC120, TMC125, and rilpivirine. Computation of the electric field of the protein at the position of the R group in **4** in the complexes supports the favorability of the interaction of the protein with the C–Cl and C–CN dipoles. Prior SAR results on this substituent effect are notable. For analogues of UC781, comparative data were only reported for R = Cl, CN, and OCH_3.[4] These activities show negligible variation, while the methoxy analogue is 10- to 20-fold less active here. More data are available for pyrazinone **11** and 8-R-TIBO from MT-4 cell-based assays.[5,6] The results are again quite different with activities of 0.25, >100, 0.50, 0.025, and <0.001 μM for **11** with R = H, Me, CF_3, Cl, and CN, that is, the relative gain is much greater for R = CN and the inactivity of the methyl compound is striking. For the TIBOs, the activities are 0.044, 0.014, 0.004, 0.034, and 0.056 μM for 8-R = H, Me, Cl, OMe, and CN; here, CN provides negligible boost over the parent. Thus, SAR patterns at analogous sites in NNRTIs are erratic; the rest of the structure matters.

Attention was next turned to alternatives to ODMA for the unsaturated group. The analyses with the *BOMB* program had suggested several promising possibilities including various heteroarylmethoxyl groups.[1] So far, only other ether derivatives have been considered (Table 2); synthesis proceeded by base-catalyzed O-alkylation, as for **4**, or via a Mitsunobu reaction (Scheme 3).

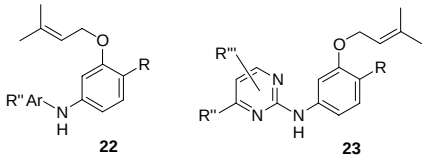
Table 2. Anti-HIV-1 activity (EC_{50}) and cytotoxicity (CC_{50}), μM, for thiazoles **12**–**21**[a]

Compound	R	R′O	EC_{50}	CC_{50}
12d	Cl	2-Furanylmethoxy	4.0	18
12h	CN	2-Furanylmethoxy	1.2	18
13d	Cl	3-Furanylmethoxy	7.0	26
13h	CN	3-Furanylmethoxy	NA	4.0
14d	Cl	2-Thienylmethoxy	10	38
14h	CN	2-Thienylmethoxy	NA	4.1
15d	Cl	3-Thienylmethoxy	5.5	38
15h	CN	3-Thienylmethoxy	NA	1.1
16d	Cl	2-Thiazoylmethoxy	NA	20
16h	CN	2-Thiazoylmethoxy	4.5	20
17d	Cl	Benzyloxy	6.0	17
18d	Cl	(*E*/*Z*)-3-Ethyl,3-methylallyloxy	1.0	18
19d	Cl	Cyclopent-1-enylmethoxy	5.0	15
19h	CN	Cyclopent-1-enylmethoxy	NA	1.7
20d	Cl	2-Methyl-cyclopent-1-enylmethoxy	NA	11
21d	Cl	Cyclohexylmethoxy	NA	15

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



Scheme 3.



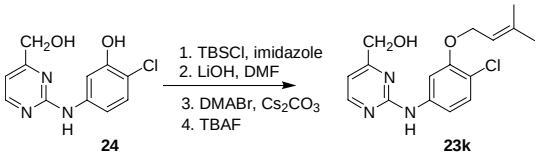
In the computational analysis,[1] the furanylmethoxy, thienylmethoxy, and cyclopent-1-enylmethoxy alternatives ranked similarly to ODMA for the U group. Indeed, activity is found in these cases, for example, **12d**, **13d**, **14d**, **15d**, and **19d**, though it was surprising that none surpassed the activity of the ODMA analogue **4d**. In all cases, the computations address binding differences, which are related to, but are not the same as, the biological readout from the cell-based assays. Additional factors come into play in the assays including permeability, intracellular metabolism, and binding to alternative cellular components. In vitro binding assays with recombinant protein are currently underway to gain further insights in this regard.

Pyrimidines and other heterocycles. The MC/FEP calculations favored 2-thiazole, 2-oxazole, and 2-pyrimidine over furans, pyridines, and pyrazine for the heterocycle in the Het–NH–Ph–U motif.[1] The predictions have been tested so far through synthesis and assaying for the derivatives of **22** and **23** that are listed in Table 3.

Table 3. Anti-HIV-1 activity (EC_{50}) and cytotoxicity (CC_{50}), μM[a]

Compound	R	Ar	R″	R‴	EC_{50}	CC_{50}
4a	H	2-Thiazoyl	—	—	10	23
4d	Cl	2-Thiazoyl	—	—	0.30	26
22a	OMe	2-Thiazoyl	4-CH_2OH	—	9	80
22b	Me	2-Pyridinyl	H	—	NA	35
22c	Cl	2-Pyridinyl	H	—	3.2	15
22d	CN	2-Pyridinyl	H	—	NA	0.29
22e	H	Pyrazinyl	H	—	NA	23
22f	Cl	Pyrazinyl	H	—	NA	15
23a	H	2-Pyrimidinyl	H	H	30	>100
23b	Me	2-Pyrimidinyl	H	H	2.8	78
23c	CN	2-Pyrimidinyl	H	H	NA	<0.2
23d	Cl	2-Pyrimidinyl	H	H	0.20	2.5
23e	Cl	2-Pyrimidinyl	Me	H	NA	<0.2
23f	Cl	2-Pyrimidinyl	CF_3	H	0.065	2.5
23g	Cl	2-Pyrimidinyl	SMe	H	0.018	2.8
23h	Cl	2-Pyrimidinyl	OMe	H	0.010	9.0
23i	Me	2-Pyrimidinyl	OMe	H	0.32	41
23j	Cl	2-Pyrimidinyl	NH_2	H	0.075	0.5
23k	Cl	2-Pyrimidinyl	CH_2OH	H	NA	0.22
23l	Cl	2-Pyrimidinyl	CH_2OMe	H	NA	>25
23m	Cl	2-Pyrimidinyl	OMe	6-OMe	0.25	28
23n	Cl	2-Pyrimidinyl	NH_2	6-Me	NA	<0.004
23o	Cl	2-Pyrimidinyl	NH_2	6-Cl	NA	0.014
23p	Cl	2-Pyrimidinyl	NH_2	6-OMe	3.0	19
23q	Cl	2-Pyrimidinyl	NH_2	5-Br	0.70	12

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



Scheme 4.

Synthesis of the heteroaryl compounds was generally achieved as for **4** with replacement of the bromothiazole **1** with an appropriate heteroaryl halide, for example, 1-bromopyridine or 2-chloro,4-R″-pyrimidine. The coupling for the 2-chloropyrimidines occurred in ca. 70% yield using TsOH in dioxane.[7] For **23k**, the presence of the two hydroxyl groups in the intermediate **24** required inclusion of a selective deprotection step[8] as part of the sequence in Scheme 4, which proceeded in 91% overall yield. Compound **23k** was then methylated (MeI and Cs_2CO_3) to provide **23l** in 72% yield.

As predicted,[1] the thiazoles and pyrimidines, for example, **4d** and **23d**, were much more active than the corresponding 2-pyridines and pyrazines, for example, **22c** and **f**. This encouraged further optimization for R″ and R‴ in the pyrimidine series **23**. Models were constructed and scored with *BOMB*, which supported the addition of small groups including OCH_3, NH_2, and CH_2OH. Subsequent synthesis readily provided several potent compounds, for example, **23f**–**h** and **j**. Addition of a 5-Br substituent, as in TMC125, to **23j** to yield **23q** was not productive. Furthermore, the lack of activity for **23k** was surprising. As for the amino group in TMC125 and **23j**, the hydroxymethyl groups in **23k** and **22a** are predicted to form a hydrogen bond with Glu138 of HIV-RT.[1,9] Though there is an associated, greater desolvation penalty in these cases, additional cellular factors, as mentioned above, may be relevant. The differences between **23e** and **f** are also curious and warrant further exploration with recombinant protein. In any event, several compounds have been identified, which are significantly more active than nevirapine (Table 1) or NRTIs,[2] so their future depends on their pharmacological properties and resistance profiles.

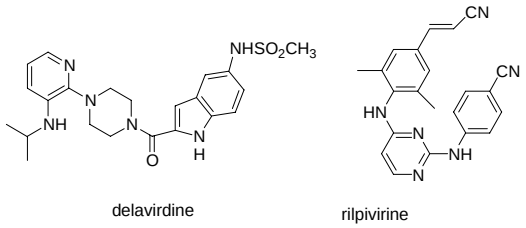
It should be noted that the cytotoxicity (CC_{50}) of all nitrile derivatives, for example, **4h**, **22d**, **23c**, and of the amino- and methyl-pyrimidines, for example, **23e**,**j**,**n**, and **o**, has been high, so their further investigation as NNRTIs is less promising. The origin of cytotoxicity is unknown, though similar substructures are not problematic in TMC125 (Table 1), rilpivirine, or PNU142721. The cytotoxicity of **23n** and **o** was disappointing as these disubstituted pyrimidine derivatives score particularly well in the binding calculations. Additional analogues are being pursued along with testing of the more potent compounds against common mutant forms of HIV.

Predicted properties. Some predicted properties from *QikProp* for a selection of the current compounds and known NNRTIs are summarized in Table 4; the overall rms errors for *QikProp* predictions of log *P*, log *S*, and log *P*Caco are 0.5–0.6 log unit.[10,11] For further reference, we have run *QikProp* for 119 oral drugs with reported experimental values for percentage human absorption (%*F*). For the 112 compounds with %*F* > 10%, only four have MW > 500, three have QPlog *P* > 5, and three have QPlog *S* < −5.5. For a larger set of 575 drugs, 90% have MW < 470, QPlog *P* < 4.7, QPlog *S* > −5.8, and QPPCaco > 12. The MW and log *P* limits are similar to those in Lipinski's rule-of-five, that is, MW < 500 and log *P* < 5.[13]

Table 4. Predicted properties for selected NNRTIs

Compound	MW[a]	QPlog *P*[b]	QPlog *S*[c]	QP PCaco[d]
Nevirapine	266.3	2.5	−3.6[e]	2090
Efavirenz	315.7	3.5	−5.0	1586
Delavirdine	456.6	2.8	−5.6	285
UC781	335.8	5.1	−5.8	7570
TMC120	329.4	3.9	−6.3	779
TMC125	435.3	2.5	−6.5	62
Rilpivirine	366.4	3.4	−6.4	150
4a	260.4	3.5	−3.8	3775
4d	294.8	4.1	−4.6	3750
23a	255.3	3.7	−4.1	3733
23d	289.8	4.1	−4.6	3942
23e	303.8	4.5	−4.9	4303
23f	357.8	5.0	−5.8	4006
23g	335.9	4.8	−5.2	6542
23h	319.8	4.3	−4.7	5668
23j	304.8	3.0	−3.9	1211
23k	319.8	3.1	−4.0	1721

[a] Molecular weight.
[b] Predicted octanol/water log *P* from *QikProp*, v 2.3.
[c] Predicted aqueous solubility from *QikProp*, v 2.3; *S* in mol/l.
[d] Predicted Caco-2 cell permeability in nm/s from *QikProp*, v 2.3.
[e] An experimental value of −3.2 has been reported in Ref. 12.



The most potent compounds reported here, **23g** and **h**, compare favorably with all of these limits, so optimism can be expressed for acceptable oral bioavailability.

In general, NNRTIs are relatively hydrophobic, so solubility is expected to be more problematic than cell permeability.[1,14] This is reflected in Table 4, and the compounds whose oral bioavailabilities are known to be low, delavirdine, UC781, and TMC125, have QP log *S* values of −5.5 or less. Many factors can limit bioavailability, but poor aqueous solubility is not a desirable starting point.[14] Rilpivirine is also predicted and reported[15] to have very low aqueous solubility and high serum–protein binding, though the oral bioavailability in rats and dogs is 31–32%.[15] It has been proposed that rilpivirine gains bioavailability by formation of aggregates in a specific size range, which are absorbed and transported through the lymphatic system.[15,16] Rational design for such a feature is currently elusive, so the maxim remains that oral formulation of compounds with log *S* below ca. −5.5 is likely to be difficult.[11]

In conclusion, a joint computational and experimental study has been carried out to develop new NNRTIs which combine good efficacy, desirable pharmacological properties, and ease of synthesis. This general goal for drug discovery has been pursued here with the aid of a computational approach featuring lead generation with the ligand-growing program *BOMB*, lead-optimization with free-energy perturbation calculations, and prediction of pharmacological properties with *QikProp*. In concert with synthetic forethought in proposing lead templates, the present approach facilitated identification of 10–30 μM lead compounds, which could rapidly be refined to a 10 nM NNRTI with predicted properties that are auspicious for oral formulation.

Acknowledgment

Gratitude is expressed to the National Institutes of Health (AI44616, GM35208, and GM49551) for support.

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