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Discovery and SAR of a series of 4,6-diamino-1,3,5-triazin-2-ol as novel non-nucleoside reverse transcriptase inhibitors of HIV-1

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ABSTRACT

The discovery and SAR study of a series of 4,6-diamino-1,3,5-triazin-2-ol compounds as novel HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) are reported. The lead compounds in this series showed excellent activity against wild-type and drug-resistant RT enzymes and viral strains. In addition, compounds from this series demonstrated favorable pharmacokinetic profile in rat. A preliminary modeling study was conducted to understand the binding mode of this series of compounds.

Human immunodeficiency virus (HIV) reverse transcriptase (RT) is a DNA polymerase enzyme that converts single stranded viral genomic RNA into double stranded DNA. HIV RT is one of three key enzymes in the HIV life cycle and the primary target of numerous anti-viral drug discovery efforts.[1] There are two major classes of HIV RT inhibitors: nucleoside reverse transcriptase inhibitors (NRTIs) that bind to the substrate binding site, and non-nucleoside reverse transcriptase inhibitors (NNRTIs) that bind to the allosteric site, distinct from the catalytic binding site. Highly active antiretroviral therapy (HAART) combination regimens, which typically include both NRTIs and NNRTIs as essential components, have dramatically decreased the morbidity and mortality among patients with HIV infections. In general, non-nucleoside reverse transcriptase inhibitors are highly specific to HIV-1 RT and relatively less toxic compared with nucleoside reverse transcriptase inhibitors (NRTIs).[2]

NNRTIs bind in the hydrophobic pocket of HIV-1 RT approximately 10 Å from the catalytic site. Upon binding, NNRTIs inhibit RT catalytic function by causing a large conformational change in the protein and a distortion of the geometry of the substrate binding site.[3] This binding pocket, which is induced by binding of the NNRTIs, is quite flexible and can accommodate many structurally diverse inhibitors.[4]

The first generation NNRTIs which include Efavirenz (EFZ), Nevirapine (NVP), and Delavirdine (DLV) have suffered from a relatively low genetic barrier to resistance mutations, and consequently the therapeutic effectiveness of these agents was significantly limited over time.[5] Efforts to develop the next-generation NNRTIs have focused on the design of compounds with an improved drug resistance profile. Etravirine (TMC 125), an NNRTI that was approved recently by the U.S. Food and Drug Administration, demonstrated activity against HIV-1 viral strains that developed resistance to the first generation NNRTI drugs. Etravirine belongs to the diaminopyrimidine (DAPY) class.[6] A closely related structure, diaryltriazine (DATA), also has been identified as highly potent NNRTIs against wild-type HIV-1 RT (Fig. 1).[7] Recently, a series of pyridinone derivatives has been reported as potent HIV-1 NNRTIs.[8]

In the process of our anti-viral drug discovery program, we discovered that 4,6-diamino-1,3,5-triazin-2-ol compound **1** (Fig. 2) possessed low micromolar potency in HIV cytoprotection assay. Subsequent mechanism of action studies, in which compound **1** was tested against the various anti-HIV targets including protease,

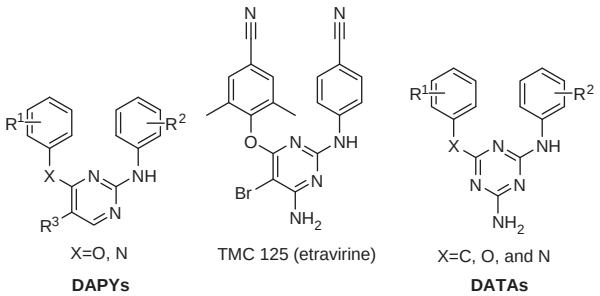

Figure 1. Chemical structures of DATA and DAPY as NNRTIs.

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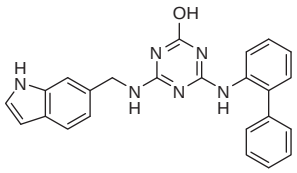


Compound 1

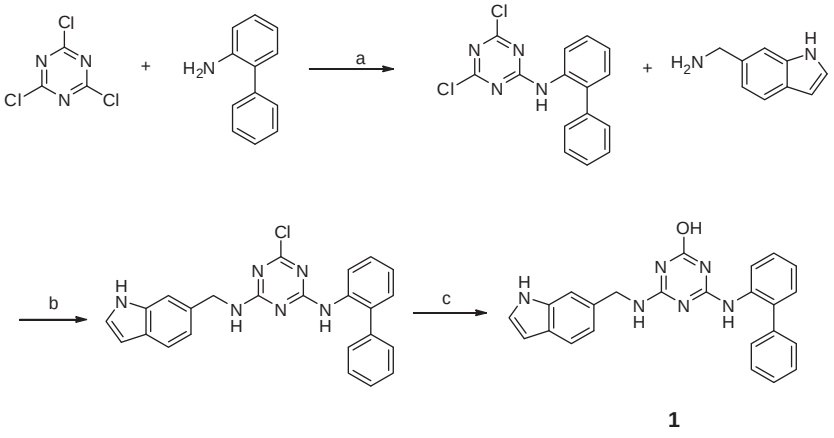
Figure 2. Structure of compound **1**.

fusion process, integrase, and RT, indicated that RT was the primary target for this compound with an IC_{50} of 1.3 μM.[9]

A medicinal chemistry optimization was carried out in order to improve the potency of this initial hit compound. The SAR study ultimately resulted in a series of 4,6-diamino-1,3,5-triazin-2-ol compounds with low nanomolar activity against wild-type reverse transcriptase and highly potent anti-viral activity. This series of compounds also demonstrated good preliminary pharmacokinetics including favorable bioavailability. In addition, these 4,6-diamino-1,3,5-triazin-2-ol inhibitors demonstrated a SAR pattern different from the DATA NNRTIs,[7] suggesting a possible different binding mode. In this Letter, we describe structure–activity relationship studies, initial in vivo PK properties and the putative binding mode of the 4,6-diamino-1,3,5-triazin-2-ol compounds with reverse transcriptase. This series of compounds could be considered as lead molecules for the discovery of novel NNRTIs with diversified structures.

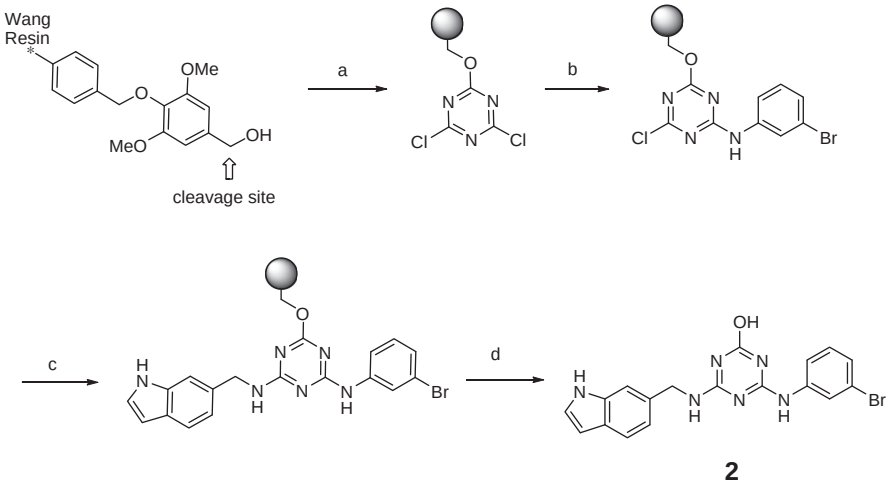
The synthesis of 4,6-diamino-1,3,5-triazin-2-ol compounds has been reported previously.[10] In order to expedite the SAR study, both solution phase synthesis and solid phase synthesis were utilized. A general solution phase synthetic route is illustrated with the preparation of compound **1** in Scheme 1. To this end, cyanuric chloride was first treated with the biphenyl-2-amine to afford the intermediate *N*-(biphenyl-2-yl)-4,6-dichloro-1,3,5-triazin-2-amine, which was treated subsequently with the more nucleophilic primary amine, (1*H*-indol-6-yl)methanamine. Finally, hydrolysis of the remaining chlorine was accomplished by heating with aqueous NaOH and H_2O_2 to yield the desired 1,3,5-triazin-2-ol analog in relatively good yield. Previously, we reported an efficient synthesis of the biaryl building blocks which are analogs of biphenyl-2-amine.[11] These building blocks were used in the synthesis of compounds described herein.

For solid phase synthesis, a modified Wang resin was utilized following literature precedent.[12] Scheme 2 shows a typical solid phase synthesis we applied for the preparation of analog **2**. Briefly, the modified Wang resin was treated with an excess of cyanuric chloride in anhydrous THF in the presence of DIEA to obtain the resin-bound dichlorotriazine. The resin-bound dichlorotriazine intermediate was treated with 3-bromoaniline. The resin was washed



1

Scheme 1. Reagents and conditions: (a) DIEA, dioxane, 0 °C; (b) DIEA, dioxane, room temperature; (c) NaOH, H_2O_2, MeOH, dioxane, 80 °C.



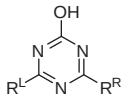
2

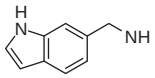
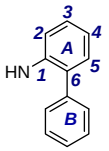
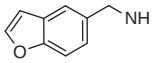
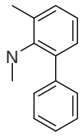
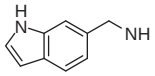
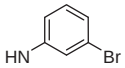
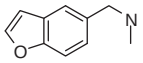
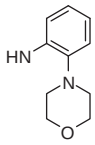
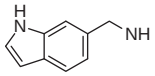
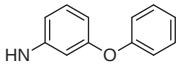
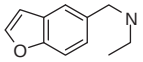
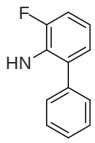
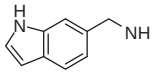
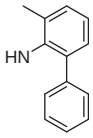
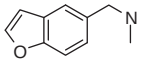
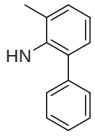
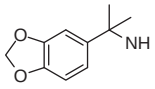
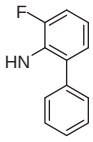
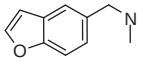
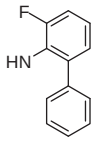
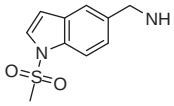
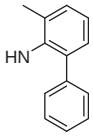
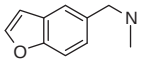
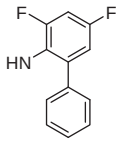
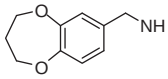
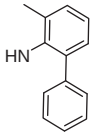
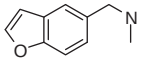
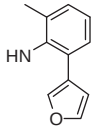
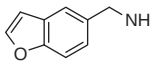
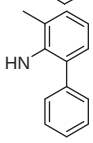
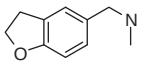
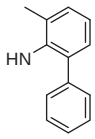
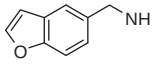
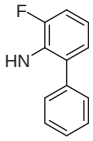
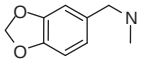
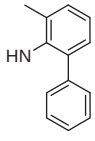
Scheme 2. Reagents and conditions: (a) Cyanuric chloride, DIEA, THF, 0 °C; (b) 3-bromoaniline, DIEA, THF, room temperature; (c) (1*H*-indol-6-yl)methanamine, DIEA, THF, 50 °C; (d) TFA/DMS, DCM.

with THF and DCM, which was subsequently reacted with (1*H*-indol-6-yl)methanamine before it was treated with 5% TFA and 5% DMS in DCM. The current 1,3,5-triazin-2-ol structures appear to exist as a mixture of enol and amide forms based on NMR spectrum analysis.

Table 1 summarizes the SAR of the left side R^L and the right side R^R modifications while maintaining the central 1,3,5-triazin-2-ol core. In a wild-type (WT) RT enzymatic assay, a few notable SAR trends were observed. First, introduction of a methyl group in the 2-position of phenyl ring **A** (see entry 1 for ring and atom

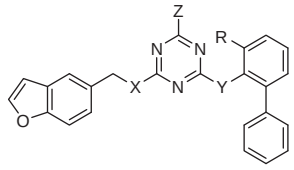
Table 1
SAR of left side and right side of the inhibitor



Compd	Left side R^L	Right side R^R	WT RT IC_{50}[a]	Anti HIV EC_{50}[a,b]	Compd	Left side R^L	Right side R^R	WT RT IC_{50}[a]	Anti HIV EC_{50}[a,b]
1			3.5	7.2	**10**			5.1	1.5
2			40	>10	**11**			4.1	nd
3			>100	>10	**12**			>100	nd
4			0.5	0.9	**13**			0.083	0.12
5			>100	nd	**14**			0.008	0.005
6			>100	>10	**15**			0.07	0.01
7			4.4	2.8	**16**			0.08	0.007
8			0.07	0.2	**17**			0.055	0.003
9			0.09	0.4	**18**			0.09	0.13

[a] IC_{50} and EC_{50} are in μM.
[b] Test derivatives were evaluated for inhibitory effect versus the wild-type RT enzyme and anti-retroviral activity against HIV-1 WT strain. The efficacy of test compounds against HIV-1 WT strain was determined at EC_{50} which is the 50% inhibitory concentration for inhibition of the cytopathic effect of HIV-1RF in CEM-SS cells or HIV-1IIIB in MT-4 cells.

Table 2
SAR study of hydroxyl and amino linker replacements



Compd	Structures				WT RT Anti-HIV-1	
	X	Y	Z	R	IC_{50}[a]	EC_{50}[a]
8	NH	NH	OH	Me	0.07	0.2
21	NH	O	OH	Me	9.7	12.6
22	O	NH	OH	Me	23.6	4
23	NH	O	NH_2	Me	>100	>50
24	NH	NH	Cl	F	>100	ND
25	NH	NH	OMe	Me	2.57	3.0

[a] IC_{50} and EC_{50} are in μM.

2numbering) in compound **1** resulted in sevenfold increase of potency (**4** vs **1**). Secondly, replacement of (3*H*-indol-6-yl)methanamine with benzofuran-5-ylmethanamine led to another sevenfold increase in activity (**8** vs **4**). However, both substituted indole (**6**) and (3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)methanamine (**7**) resulted in loss of the activity in enzymatic assay. Fluoride substitution at the 2-position of phenyl ring **A** offered improved potency similar to methyl substitution (**9** vs **8**). Interestingly, methylation of the left side NH linker displayed a 10-fold increase of potency (**14** vs **9**) in one case, while equal potency was observed in another case (**13** vs **8**). On the other hand, ethylation of the same NH linker resulted in almost complete loss of the potency (**12**). Taken together, the SAR suggested that either a tight binding pocket or a favorable conformational change induced by the NH methyl group caused this 'methyl effect.' Conversely, N-methylation on the right side NH resulted in significant loss of potency (**10** vs **8**) suggesting the potential involvement of the NH group in binding with RT. Introduction of a second fluorine on ring A resulted in a 10-fold loss of activity (**15** vs **14**). In addition, replacement of ring B by other aromatic rings such as furan provided similar potency (**16** vs **13**). Two analogs with substitutions at the 5-position on ring A (**2** and **3**) displayed diminished activity. Finally, the SAR suggested the replacement of benzofuran in compound **13** with (2,3-dihydrobenzofuran-5-yl)methanamine (**17**) or benzo[*d*][1,3]dioxol-5-ylmethanamine (**18**) resulted in comparable activity in RT inhibition.

Table 2 summarizes the SAR study which explored the role of the central 1,3,5-triazin-2-ol core substituent (Z) and different linkers (X and Y). Analogs **21–25** were synthesized using similar chemistry (Scheme 3). The SAR suggested that replacement of the NH linker with an oxygen linker on either side of the 1,3,5-triazin-2-ol core resulted in reduced potency (**21, 22** vs **8**). The SAR clearly indicated that replacement of the hydroxyl group in compound **8** with an amino group (**23**) or chloride (**24**) was detrimental and resulted in significant loss of activity, implying a possible loss of a favorable interaction. This SAR observation is opposite to the reported SAR of the DATA series, where replacement of the amino group with hydroxyl group resulted in much less active derivatives.[7] Replacement of the hydroxyl group with methoxy diminished the activity by about 30-fold (**25** vs **8**). Taken together, the SAR demonstrated the importance of the 1,3,5-triazin-2-ol core and amino linkers.

Analysis of data generated in HIV viral cytoprotection assay indicated that the potency rank of tested compounds tends to correlate well with enzymatic RT activity. Notably, although compounds **16** and **17** displayed inhibitory activity against WT RT in the medium to high double digit nanomolar range, both compounds showed excellent cellular potency. Overall, compounds **14** and **17** demonstrated the best potency in anti-HIV cellular assay.[13]

Compounds **14** and **17** were selected for testing against three key NNRTI resistance RT mutants (L100I, V106A, and Y188L) in enzymatic assay, and the results are shown in Table 3. Both compounds showed good activity against L100I RT mutant, but were not effective against V106A and Y188L RT mutants. Next, the activity of compounds **14** and **17** against mutant HIV viruses was also

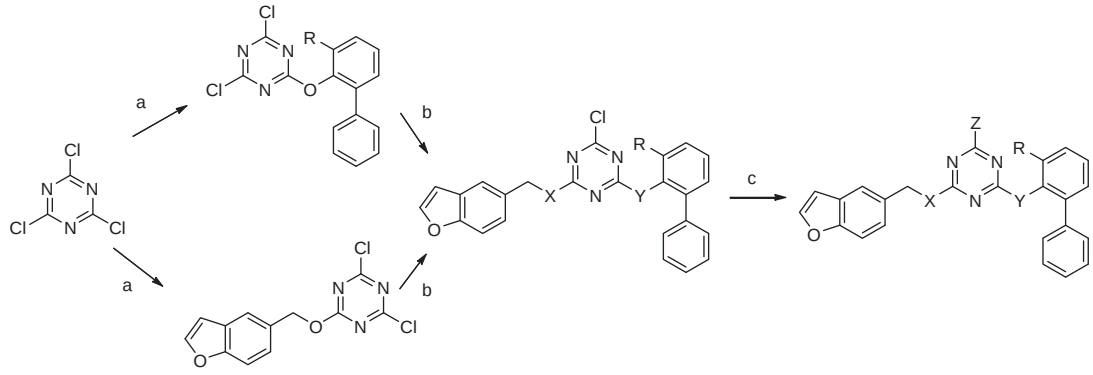
Table 3
Compound mutation resistance in RT enzymatic assay[a]

Compd	WT	L100I	V106A	Y188L
14	0.008	0.047	9.1	6.1
17	0.055	0.070	36.7	9.5

[a] IC_{50} in μM.

Table 4
Compound mutation resistance in Anti-HIV cytoprotection evaluation

Compd	CeMSS/NL4-3 EC_{50} (μm)	CEMSS TC_{50} (μm)	CeMSS/NL4-3 K103N EC_{50} (μm)	CeMSS/NL4-3 Y181C EC_{50} (μm)
NVP	0.2	>1	>1.0	>1.0
EFZ	0.004	>1	>1.0	0.005
14	0.006	>1	>1.0	0.1
17	0.050	>1	>1.0	0.2



Scheme 3. Reagents and conditions: (a) benzofuran-5-ylmethanol or biphenyl-2-ol, DIEA, THF, room temperature; (b) benzofuran-5-ylmethanamine or biphenyl-2-amine derivatives, DIEA, THF, 50 °C; (c) NaOH, H_2O_2, MeOH, dioxane, or MeONa, MeOH, or NH_3, MeOH, 80 °C.

Table 5
Rat pharmacokinetics[a]

Parameter	14	17
C_{max} (μM)	3.8	6.1
AUC (μM h)	12.5	15.6
$t_{1/2}$ (h)	1.45	1.40
V_{dss} (L/kg)	0.35	0.32
Cl (L/h/kg)	0.31	0.27
F (%)	38	44

[a] PO dose: 4 mg/kg. IV dose: 2 mg/kg.

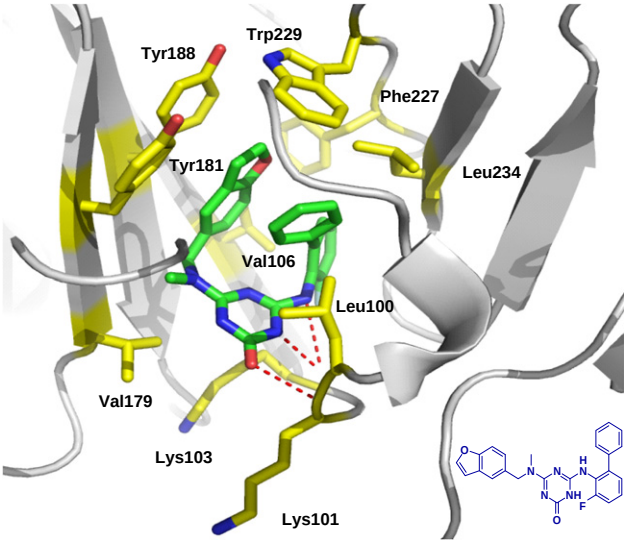


Figure 3. Proposed binding mode of compound **14** with wild-type RT.

evaluated (Table 4). Both compounds showed similar activity and trends as Nevirapine (NVP) and Efavirenz (EFZ).

In a pharmacokinetic study (Table 5), both compounds **14** and **17** are well absorbed when administered orally in rats (4 mg/kg), with a C_{max} of 3.8 μM and 6.1 μM, respectively. Following intravenous dosing (2 mg/kg), both compounds showed reasonable half-life (~1.4 h), and the apparent bioavailability was determined to be around 40% for both compounds.

A computational modeling study was carried out to understand the possible binding mode of this series of compounds and interactions with the reverse transcriptase. Briefly, the prediction of the binding mode was performed with a pharmacophore search followed by an energy minimization within the protein. The protein structure was obtained from the Protein Brookhaven Database (PDB code 1SUQ). A four point pharmacophore was defined based on 1SUQ ligand, which contains three aromatic features and one hydrogen bond donor interaction with Lys101 backbone carbonyl group. The shape of the binding site is defined with 190 exclusive volume points based on the 1SUQ protein structure. Ligand conformations were generated with a systematic search. The binding modes of the compounds were selected by visual inspection of the poses.[14] Figure 3 shows the predicted binding mode of compound **14**. It was suggested that the binding of this series of compounds can be better explained by the amide tautomer of the 1,3,5-triazin-2-ol (the structure shown in Fig. 3). The carbonyl oxygen and NH of the triazine ring form two hydrogen bonds with the Lys101 backbone NH and carbonyl, respectively, which is consistent with the SAR. The B ring of the biaryl moiety sits in the pocket formed by Leu100, Leu234, and Trp229. The benzofuran moiety of compound **14** sits between the lipophilic pocket formed by Trp229, Tyr188, Tyr181, Phe227, and Val106. The ligand also forms favorable π−π interaction with Tyr181 and Trp229. This proposed binding mode is different from the binding mode of the DATA class of NNRTIs,[15] presumably due to the substituents on the current 1,3,5-triazin-2-ol core which are bulkier than those in the DATA series.

In summary, a series of 4,6-diamino-1,3,5-triazin-2-ol compounds was discovered as novel NNRTIs, and the preliminary SAR of this series of compounds was established. This study led to the identification of inhibitors with single digit nanomolar potency both in enzymatic and cellular assays. Preliminary in vivo rat PK study showed that this class of compounds possesses favorable PK properties suitable for oral administration. Molecular modeling studies were employed to understand the binding mode between these inhibitors and the wild-type HIV reverse transcriptase. The current results indicate that this series of compounds could serve as potential leads to further develop novel and potent NNRTIs.

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