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Evolution of Anti-HIV Drug Candidates Part 2: Diaryltriazine (DATA) Analogues

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Abstract—A synthesis program directed toward improving the stability of imidoyl thiourea based non-nucleoside reverse transcriptase inhibitors (NNRTIs) led to the discovery of diaryltriazines (DATAs), a new class of potent NNRTIs. The synthesis and anti-HIV structure–activity relationship (SAR) studies of a series of DATA derivatives are described.

In the preceding paper, the imidoyl thioureas (ITUs) were introduced as a new class of potent non-nucleoside reverse transcriptase inhibitors (NNRTIs) of HIV-1.[1] During the course of this work, however, it was quickly established that ITUs such as **1** ($IC_{50} = 2.5$ nM vs the LAI strain of HIV-1) were prone to both hydrolytic lability and oxidative instability. The latter was shown by the facile conversion to thiadiazole **2**, a compound that was completely devoid of HIV inhibitory activity.

This suggested to us that compounds in the ITU series were able to achieve favorable conformations for binding to HIV-1 reverse transcriptase (RT) that are not available to the more rigid thiadiazole system and prompted us to pursue a strategy of attempting to stabilize the ITUs while maintaining their conformational flexibility. In this context, an attempt was made to prepare compound **5**, which incorporated a cyanoguanidine moiety, a well-known thiourea bioisostere.[2] The reaction of amidine **3** with *N*-cyano-*O*-phenyl isourea **4** resulted in clean conversion to a single product that was revealed by spectral analysis to be triazine **6**, and not the desired product **5**. Presumably, compound **5** had formed but cyclized spontaneously under the reaction conditions (Fig. 1). Considering the inactivity of **2**, we were surprised to find that **6** was a very potent NNRTI of HIV-1_{LAI} ($IC_{50} = 6.3$ nM). Indeed, it was nearly as active as **1**, the most potent compound in the ITU series. More importantly, **6** was also highly active against a battery of HIV-1 mutant strains (Table 1). The combination of potent biological activity and greatly enhanced structural stability relative to the ITUs encouraged us to embark on a program to identify and optimize a second generation of NNRTIs based on the diaryltriazine (DATA) compound **6**. Selection of some of the modifications made, particularly on the Ar_1 ring and the C-4 position of the triazine ring, was guided by molecular modeling (3D-SAR) based on the crystal structure of HIV-1 RT bound with the prototypic DATA compound **6**.[3] The molecular modeling showed the Ar_1 ring was positioned in the important Y181, Y188, W229 region of the enzyme, while the C-4

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position was located at the opening of the binding pocket. This led us to believe modifications to the Ar_1 ring should have an important influence on binding affinity and the mutant profile, while the C-4 position should be able to accommodate a large range of structural changes.

The chemistry that led to the formation of **6** was readily adapted to the preparation of triazine analogues **11** in which both Ar_1 and Ar_2 could be varied as shown in Scheme 1. The requisite amidines **8** were readily prepared from the corresponding phenylacetonitriles **7** by the procedure of Garigipati,[4] while the isourea partners **10** were obtained in modest yields by the reaction of diphenyl cyanocarbonimidate **9**[5] with the appropriate aniline.

A limitation of this approach was that ring formation resulted in placement of an NH_2 group at the C-4 position of the triazine. The NH_2 group was derivatized by a variety of acylating reagents under standard conditions; however, in order to further probe the SAR of the C-4 position a second synthetic route was required. The 2,6-dichlorophenyl moiety, which was associated with very good HIV inhibitory activity, was chosen as Ar_1. Use of this Ar_1 group was compatible with the synthetic route shown in Scheme 2. The readily formed Grignard reagent of 2,6-dichlorobenzyl bromide **12**, when reacted with one equivalent of cyanuric chloride (**15**), afforded a dichloro intermediate,[6] which could be treated with 4-aminobenzonitrile to form **13** in an efficient one-pot procedure. Chlorotriazine **13** was a useful, stable intermediate that reacted readily with a wide variety of

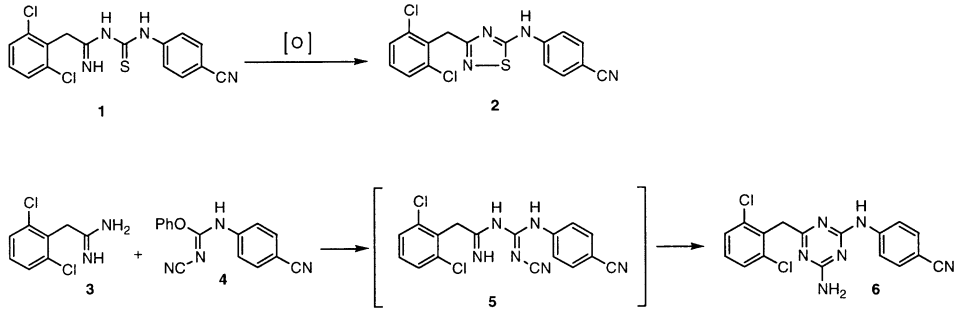
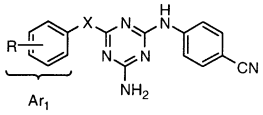


Figure 1.

Table 1. Inhibition of HIV-1 (IC_{50}, μM)[9]



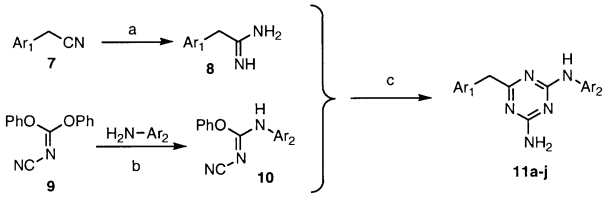
Compd	R	X	LAI	100I	103N	181C	188L
6	2,6-diCl	CH_2	0.0063	0.398	0.040	0.200	0.316
11a	H	CH_2	0.285	8.07	8.47	>100	nd
11b	2-Cl	CH_2	0.130	2.65	0.400	2.57	7.37
11c	2,4-diCl	CH_2	0.014	2.10	0.274	0.791	2.32
11d	2,3,6-triCl	CH_2	0.0030	0.416	0.063	0.286	0.265
11e	2,4,6-triCl	CH_2	0.0014	0.221	0.016	0.062	0.161
11f	2,6-diMe	CH_2	0.0012	1.46	0.042	0.225	0.105
11g	2,5-diMe	CH_2	0.0031	5.86	0.273	1.57	1.03
11h	3,5-diMe	CH_2	0.091	77.9	5.08	6.37	>100
11i	2,3,5,6-tetraMe	CH_2	0.0046	6.70	0.085	1.19	0.367
11j	2,4,6-triMe	CH_2	0.0008	0.082	0.0030	0.015	0.045
21a	2,4,6-triMe	NH	0.0010	0.037	0.0020	0.011	0.124
21b	2,4,6-triMe	S	0.0029	0.064	0.0060	0.019	0.036
21c	2,4.6-triMe	O	0.0010	0.075	0.0020	0.013	0.182
21d	2,6-diMe	O	0.0031	0.851	0.017	0.333	0.327
21e	2,6-diMeO	O	0.031	nd	0.526	1.56	nd
21f	2,6-diCl	O	0.0031	0.384	0.082	0.279	4.57
21g	2,4,6-triCl	O	0.0024	0.087	0.012	0.062	0.502
21h	2,4,6-triBr	O	0.0054	0.131	0.017	0.061	0.619
21i	2,4,6-triF	O	0.051	0.216	0.087	3.65	8.95
21j	2,6-diCl-4-F	O	0.0073	0.706	0.065	0.259	1.02
21k	2Cl,4Br,6-Me	O	0.0021	0.059	0.0060	0.035	0.103
21l	2,6-diBr-4-Me	O	0.0020	0.019	0.0050	0.017	0.415
21m	2,6-diMe-4-Br	O	0.0015	0.133	0.0080	0.042	0.056
21n	2,6-diMe-4-Cl	O	0.0035	0.171	0.011	0.055	0.121
2o	2,6-diMe-4-I	O	0.030	0.204	0.010	0.068	0.075
21p	2,6-diMe-4-NO_2	O	0.0020	0.330	0.0080	0.057	0.123
21q	2,6-diMe-4-NH_2	O	7.83	nd	>100	>100	nd

nd: not determined.

nucleophiles to form numerous analogues of **6** with different substituents at the triazine C-4 position. Moreover, the DATA lead, compound **6**, could be prepared by this route in high yield by the treatment of **13** with a solution of ammonia in dioxane–isopropanol at elevated temperature in a sealed tube. Compound **14f** was prepared in two steps by the reaction of intermediate **13** with *O*-(trimethylsilyl)hydroxylamine and subsequent hydrolysis of the TMS group under aqueous base conditions. This prevented the formation of hydroxylamine linked bis-adducts.

Heteroatom linkages connecting Ar_1 to the central triazine ring could be incorporated as shown in Scheme 3. Starting with commercially available cyanuric chloride (**15**), the preferable route was to pass through intermediates **17**; however, weaker nucleophiles of type Ar_1XH, especially anilines (X = NH) with strong electron withdrawing substituents, occasionally necessitated following the route through intermediates **18**. The chlorotriazines **19** could then be reacted with either ammonia or a variety of nucleophiles, as was described previously in the case of **13**, to provide DATA analogues **21**. This approach was amenable to another structural variation of interest in that dichlorotriazine **16**, prepared by the method of Harris,[7] could be used for entry into analogues **20** in which Z is hydrogen.[8]

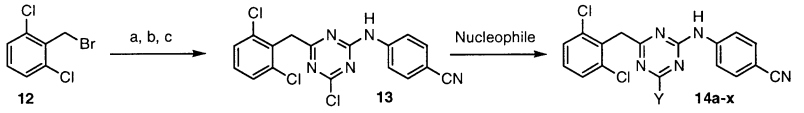
The primary screen for evaluating the DATA compounds was potency in inhibiting replication of wild-type HIV-1 (LAI strain). Also, a 'safety index' (or therapeutic index, expressed as a ratio of toxic concentration to inhibitory concentration) was determined and used as a guide to avoid selecting compounds that exhibited a general cytotoxic effect.[9] Promising compounds were subsequently tested against a number of known mutant strains, of which some are clinically significant due to resistance to NNRTIs that have previously been approved for the treatment of AIDS.



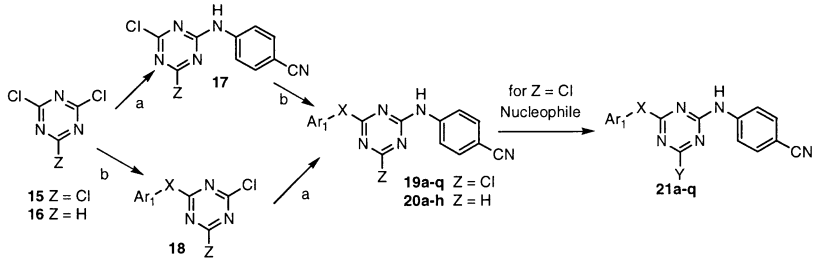
Scheme 1. (a) NH_4Cl, $AlMe_3$/PhMe, 0 °C then 80 °C; (b) 1,4-dioxane/DMF, 70–80 °C; (c) DMF, 70–80 °C.

A preference for the NH linkage between Ar_2 and the triazine ring was rapidly established since an *O*-linked analogue of **6** was found to be completely inactive. The nature of the Ar_2 portion of the DATA series was then investigated by preparing a small series of Ar_2 analogues of **6**. For example, the 3-cyano and the 4-chloro analogues had activities (IC_{50}, μM) against HIV-1_{LAI} of 0.251 and 0.016, respectively. These results led us to conclude that for Ar_2, the SAR of the ITU series[1] was applicable and that a 4-cyanoanilino substituent was optimal. Thus, the 4-cyanoanilino moiety was maintained for the remainder of our DATA analogue investigations.

A SAR profile was initially established for Ar_1 by exploration of DATA compounds **11**, which possess both a methylene linkage to the triazine and an NH_2 group at the C-4 position. Later in the project, DATA compounds **21** (Y = NH_2), in which Ar_1 is linked to the triazine by a heteroatom, were in hand and the results reinforced the earlier observations for the SAR of Ar_1. Furthermore, the linkage between Ar_1 and the triazine did not have a significant effect on the potency of DATA compounds as can be seen from **11j**, **21a**, **21b**, and **21c**, all of which contain 2,4,6-trimethylphenyl as the Ar_1 group. Numerous compounds were prepared; however, the entries in Table 1 illustrate the trends that were observed. In general, halogens and methyl substituents on Ar_1 provided similar activity in inhibiting HIV-1_{LAI}, with the methyl containing derivatives being slightly more effective. At least one *ortho* substituent appears to be required for a significant level of potency while 2,6-substitution is generally the most active of the disubstituted Ar_1 patterns (**6** > **11c** ≫ **11b** > **11a** and **11f** > **11g** ≫ **11h**). As long as a halogen or methyl 2,6-disubstitution pattern is present, additional halogens or methyls in the C-3 and/or C-5 positions tend to be close in potency towards HIV-1_{LAI}. The range in potency for Ar_1 with these multiple substituent patterns is broader among the mutant strains but without clear trends (i.e.,



Scheme 2. (a) Mg, ether; (b) cyanuric chloride, benzene; (c) 4-aminobenzonitrile, DIEA, 1,4-dioxane.



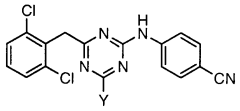
Scheme 3. (a) 4-Aminobenzonitrile, DIEA, 1,4-dioxane, 25 °C or reflux; (b) Ar_1NH_2, DIEA or Ar_1OH, NaH; 1,4-dioxane, 25 °C or reflux.

6 vs **11d** and **11f** vs **11i**). A variety of small substituents were tolerated at the C-4 position when combined with halogen or methyl in a 2,6-disubstituted Ar_1. With the exception of the inactive 4-amino **21q**, DATA derivatives with 2,4,6-trisubstitution were generally the most potent against HIV-1_{LAI} by a slight margin but were significantly more active against all of the HIV-1 mutant strains.

Investigation of substitutions at the triazine C-4 position of compounds **14** indicated that the original NH_2 group was indeed favorable for inhibitory potency whereas the chloride intermediate **13** was inactive (Table 2). The monomethylamine analogue **14a** was also quite potent while the dimethylamine analogue **14b** was considerably less active. Monoalkyl amines were active as long as the unfunctionalized alkyl group was not too bulky. Interestingly, a basic dimethylamine group (**14e** and **14r**) on the end of the chain did allow for good activity against HIV-1_{LAI} and some of the mutants. The size of the alkyl chain was also shown to be a factor in the hydroxylamine series **14f**–**14j** with the parent hydroxylamine being the most potent. A number of other small amine derivatives had good activity including acetamide **14t**, *N*,*N*-dimethylformamidine **14u**, ethylcarbamate **14v**, and methylamidine **14w**. The azido group **14k** provided good potency while the hydrazine **14l** had surprisingly low relative activity. Some non-amine functional groups were also prepared. The *S*-methyl **14m** and *O*-methyl **14o** were active while, unlike the amine and hydroxylamine, the corresponding thiol **14n** and hydroxyl **14p** derivatives were much less active. The sulfoxide **14q** was inactive, as was the fluoride **14x**. Thus, for activity against HIV-1_{LAI}, a variety of small functional groups were tolerated at this position on the triazine ring. However, as indicated previously, the activity against mutant strains was an important filter in evaluating the compounds. From this perspective, the hydroxylamine **14f** was of great interest, as it possessed a superior inhibition profile relative to **6** against a number of the mutant strains, especially 181C and 188L, which was a breakthrough. Unfortunately, in preclinical studies, **14f** was found to be very rapidly metabolized affording **6** and products resulting from hydroxylation of the methylene linkage and the C-4 position of Ar_1. Furthermore, **14f** and its metabolite **6** were readily glucuronidated at the hydroxylamine/amine functionality and excreted.

We reached a point in the program where potency was not the main SAR criterion for moving forward. We were confident that we had obtained compounds with adequate potency and needed to improve other features of the compounds such as solubility, bioavailability, and pharmacokinetics. The SAR of the DATA series to this point suggested that there might be some tolerance for making modifications with these goals in mind while maintaining highly active compounds. Consequently, DATA analogues with heteroatom Ar_1 triazine linkages and substituents at the C-4 position of Ar_1 were first synthesized. Indeed, compound **21a** (Table 1) possessed an excellent potency profile and was determined to have

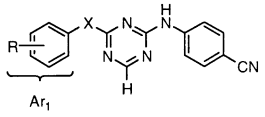
Table 2. Inhibition of HIV-1 (IC_{50}, μM)[9]



Compd	Y	LAI	100I	103N	181C	188L
6	NH_2	0.0063	0.398	0.040	0.200	0.316
13	Cl	1.08	nd	10.0	10.0	nd
14a	NHMe	0.0070	1.16	0.137	0.440	1.02
14b	NMe_2	0.166	32.2	4.69	13.4	nd
14c	NHnPropyl	0.849	nd	nd	nd	nd
14d	*N*-Morpholino	0.194	nd	nd	nd	nd
14e	$NHCH_2CH_2N(Me)_2$	0.0050	1.26	0.021	0.379	0.297
14f	NHOH	0.0024	0.107	0.013	0.038	0.026
14g	NHOMe	0.0062	1.38	0.082	0.628	0.804
14h	NHOEt	0.0246	nd	nd	nd	nd
14i	NHOPropyl	0.100	nd	nd	nd	nd
14j	NMeOMe	0.086	nd	nd	nd	nd
14k	N_3	0.014	nd	0.224	1.14	nd
14l	$NHNH_2$	0.610	5.83	1.74	5.83	7.32
14m	SMe	0.018	nd	0.336	2.35	nd
14n	SH	0.094	nd	0.723	1.61	nd
14o	OMe	0.012	1.15	0.231	1.45	1.55
14p	OH	0.160	nd	0.607	2.12	nd
14q	SOMe	1.55	nd	15.5	68.5	nd
14r	$OCH_2CH_2N(Me)_2$	0.0055	1.08	0.037	1.39	0.315
14s	NHCONHiPr	0.379	35.1	7.55	43.8	nd
14t	NHCOMe	0.016	100	0.040	100	0.158
14u	$N{=}CN(Me)_2$	0.0025	0.631	0.016	0.316	0.251
14v	$NHCO_2Et$	0.012	1.64	0.548	1.32	1.73
14w	NH(C=NH)Me	0.0027	0.611	0.040	0.210	0.358
14x	F	5.26	nd	52.6	100	nd

nd: not determined.

Table 3. Inhibition of HIV-1 (IC_{50}, μM)[9]



Cp	R	X	LAI	100I	103N	181C	188L	100I + 103N	103N + 181C
20a	2,4,6-triMe	NH	0.0003	0.013	0.003	0.0080	0.040	1.26	0.050
20b	2,6-diBr-4-Me	NH	0.0005	0.0030	0.0030	0.0030	0.079	>10.0	nd
20c	2,6-diMe-4-Br	NH	0.0006	0.079	0.0060	0.016	0.063	3.16	0.126
20d	2,6-diMe-4-CN	NH	0.0010	0.251	0.0080	0.050	0.050	2.51	0.126
20e	2,4,6-triMe	O	0.0006	0.020	0.0030	0.020	0.063	>10.0	nd
20f	2,6-di-Br-4-Me	O	0.0013	0.016	0.0060	0.040	0.398	nd	nd
20g	2,6-diMe-4-CN	O	0.0040	1.000	0.020	0.501	0.251	3.98	0.398
20h	2,4,6-triMe	S	0.0020	0.032	0.0030	0.013	0.020	>10.0	nd

increased metabolic stability versus **14f**, although a site for glucuronidation was still present in **21a**.

Consideration of these nonpotency issues led us to synthesize DATA compounds **20** with hydrogen at the C-4 position of the triazine. We were very pleased to see that compounds **20a–h** were very potent. In fact, **20a** was the most potent of all the DATA compounds against HIV-1_{LAI} and had a very good profile against all of the HIV-1 mutants that we had been testing up to that time (Table 3). Compound **20a** also had good relative metabolic stability in addition to not having a site for glucuronidation. However, new clinically relevant mutations were continuously being identified and we became aware of additional important HIV-1 strains with double mutations in the RT. Despite very good potency against the 100I and 103N single mutants, compounds **20** were effectively inactive against the double mutant 100I/103N.

Other promising DATA compounds such as **21a** were tested against the 100I/103N double mutant and were found to also be completely inactive. In the following paper in this series[10] we report how replacement of the triazine ring with a pyrimidine ring system allowed for the identification of a structural feature in which high potency in inhibiting even this double mutant could be achieved.

Starting from the active but unstable ITU series of HIV-1 NNRTIs, we discovered the new class of DATA compounds which maintained high potency and safety levels while solving the stability problem. A systematic SAR program, supplemented with 3D-SAR via molecular modeling and structural studies, identified features of the DATA series that led to subnanomolar potency versus the wild-type HIV-1 (LAI strain) and low nanomolar potency against a battery of clinically important HIV-1 mutants. As the project matured, nonpotency issues including solubility, bioavailability, and pharmacokinetics became the focus of the SAR development program. These efforts culminated in the identification of DATA compounds **20a** and **21a**, which met the goals of the project very well. However, HIV is a 'moving target' owing to its high mutation rate, and compounds in the DATA series had suboptimal activity against a newly characterized double mutant of HIV that was potentially important. These considerations led to the development of the diaminopyrimidine (DAPY) analogues, which are the subject of the next paper in this series.

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8. Attempts to prepare a methylene-linked analogue of **6** via a Grignard reaction with **16** were unsuccessful.
9. (a) All compounds were tested for potency (IC_{50}, μM) in terms of concentrations required to achieve 50% protection of MT-4 cells from HIV-1 cytopathicity as determined by the MTT method (Pauwels, R.; Balzarini, J.; Baba, M.; Snoek, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309). Unless noted otherwise, the LAI strain of HIV-1 was the infecting virus. Other infecting mutant strains of HIV-1 (with changes in the RT) are characterized in the tables by the mutated amino acid position and the one

letter codes. For instance, 181C refers to replacement of tyrosine at position 181 with cysteine. All determinations are the result of multiple tests. (b) nd, not determined.

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