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BIARYL ACIDS: NOVEL NON-NUCLEOSIDE INHIBITORS OF HIV REVERSE TRANSCRIPTASE TYPES 1 AND 2.

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Abstract: A series of biaryl acids has been found to show micromolar inhibition of the HIV reverse transcriptase (RT) from types 1 and 2 with IC_{50}s in the micromolar range. The series was discovered by consideration of the polymerase active site and sub-structure searching of the company compound collection. Synthesis of analogues to investigate the SAR is described. Two of these compounds have shown inhibition of HIV-2 RT only.

Reverse transcriptase is a key enzyme in the replicative cycle of the human immunodeficiency virus (HIV). It is a multifunctional enzyme having RNA- and DNA- dependent DNA polymerase activity, as well as RNase H activity. It catalyses the formation of double stranded proviral DNA from the single stranded RNA genome. Its central role in viral replication thus makes RT a prime target for anti-HIV therapy.

Two main categories of HIV RT inhibitors have been discovered to date. The first of these are the nucleoside analogues (e.g. AZT, 3TC, ddI, ddC)[1] which, when anabolised to triphosphates and following incorporation into the growing DNA strand by the RT enzyme act as DNA chain terminators. The second category of inhibitors are the non-nucleosides. These are chemically highly diverse,[2] but all possess the common features of generally being highly selective for HIV-1 RT relative to HIV-2 RT. They are non-competitive inhibitors and interact with the enzyme at a hydrophobic site close to, but distinct from, the catalytic site of the enzyme. This hydrophobic pocket expands to accommodate the inhibitors leading in turn to a conformational change in the polymerase active site which attenuates activity.[3]

The development of non-nucleoside inhibitors showing inhibition of both type 1 and 2 HIV RT would be a significant improvement on current HIV-1RT selective antiretroviral agents.

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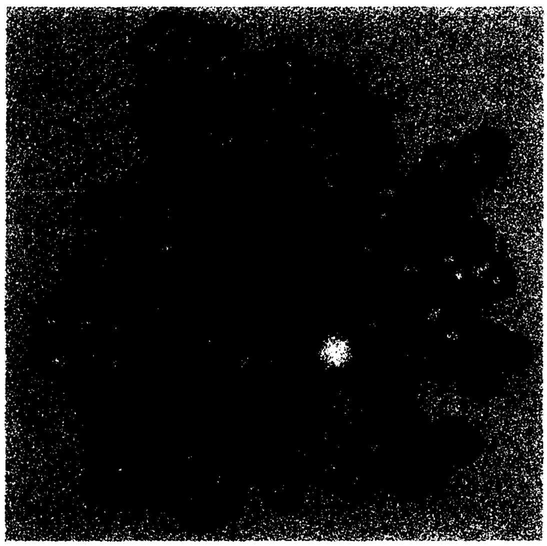
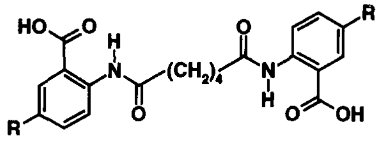
Such novel inhibitors will likely possess a unique resistance profile and potential synergy with established drug treatments. In this paper we describe the discovery, synthesis and structural modification of some biaryl acids, a new series of non-nucleoside inhibitors active against both HIV-1 and 2 RT.

The search for a new series of RT inhibitor began with the identification of a number of catalytic site amino acid residues highly conserved in RT-1 and 2 protein sequences.[4] The residues identified were Asp110, Asp185, Asp186 (catalytic triad with associated Mg^{2+}), Tyr115, Gln151, Gln91, Leu92 and Ile94. The published HIV-1 RT crystal structure[5] was then used to obtain a topological map of the catalytic site.[6]



A focused search of the company compound collection was performed to identify molecules containing a potential magnesium chelating group which also would fit the topology of the active site (nucleosides were excluded). Compounds with these properties were tested initially at 100μM against HIV-1 and HIV-2 RT using rC.dG and [^{3}H]-dGTP as substrates[7]. This led to the identification of two biaryl acids (**1**, **2**) active in the micromolar range against both enzymes.

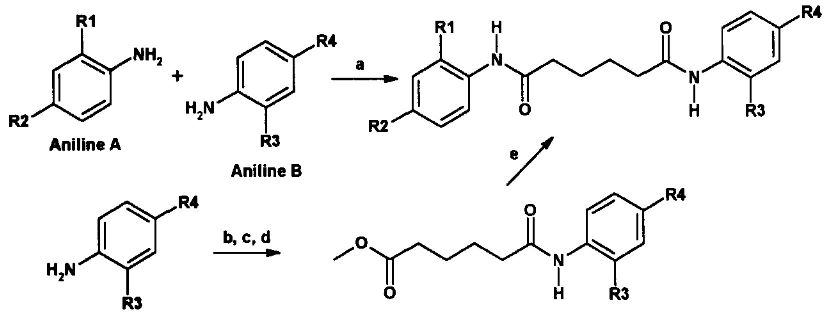


1, R = Br, IC50 RT1 = 3.1μM, RT2 = 1.7μM

2. R = H, IC50 RT1 = 21μM, RT2 = 9μM

The SAR of these novel RT inhibitors was investigated by preparing a series of analogues in which the aryl carboxylate and bromo groups were sequentially removed in order to establish the importance of each in the molecule's activity. Compounds (**1**, **2**, **4**) were conveniently prepared by reaction of the appropriate anilines (**A** and **B**) with adipoyl chloride in acetone / Et_3N.[8] Compounds (**3**, **5-8**) were prepared by the reaction of aniline **B** with methyl adipyl chloride, followed by saponification and coupling with aniline **A**. HPLC purification yielded the pure materials as white solids in 30 - 80% yields (scheme 1). Biological results are shown in Table 1.[9]

Scheme 1



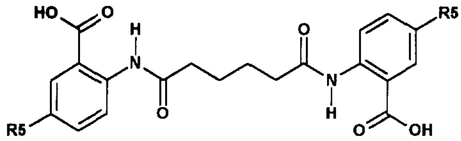
Reagents: a) Adipoyl chloride, acetone, Et_3N, 50°C, 24h.
b) Methyl adipyl chloride, pyridine, CH_2Cl_2. c) NaOH, THF.
d) $(COCl)_2$, CH_2Cl_2, DMF (2-3 drops). e) **Aniline A**, pyridine.

Cpd.No.	R1	R2	R3	R4	IC_{50} RT1	IC_{50} RT2
1	CO_2H	Br	CO_2H	Br	3.1μM	1.7μM
3	CO_2H	Br	H	Br	15μM	32μM
4	CO_2H	Br	CO_2H	H		
2	CO_2H	H	CO_2H	H	21μM	9μM
5	CO_2H	Br	H	H	Inactive[a]	Inactive[a]
6	CO_2H	H	H	Br	Inactive[a]	Inactive[a]
7	CO_2H	H	H	H	Inactive[a]	Inactive[a]
8	H	H	H	Br	Inactive[a]	Inactive[a]

a Inactive means <50% inhibition @100μg/ml concentration.

Table 1.

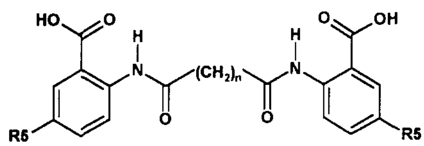
These results confirmed that a carboxyl group in one ring was essential (**3**, **5**, **6**, 7, **8**), but that the role of the bromo groups was far less important (**2**, **4**). For optimal inhibition of RT it appeared that both bromines and carboxyls were required (**1**). The replacement of the aryl bromines was then examined (Table 2).



Cpd.No.	R5	IC_{50} RT1	IC_{50} RT2
2	H	21μM	9μM
9	F	>120μM	>120μM
10	Cl	8.7μM	1.8μM
1	Br	3.1μM	1.7μM
11	I	8.5μM	4.1μM
12	Me	14μM	9.2μM
13	MeO	10.6μM	4.3μM
14	OH	120μM	6.7μM
15	NHCOMe	>100μM	>100μM
16	SMe	19.0μM	6.9μM
17	CF_3	12.0μM	8.3μM
18	CN	>100μM	>100μM

Table 2.

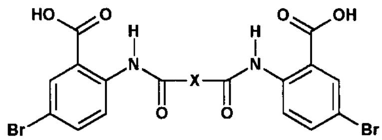
These results indicated that although a range of substituents were tolerated, bromo or chloro aryl acids were optimal. The activity found for compound **14** is particularly striking, and we believe it is the first non-nucleoside to show >10 fold selectivity for RT-2. The results of studies to examine the effect of varying the length of the linker between the two aryl groups are shown in Table 3.

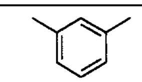
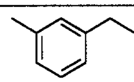
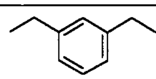
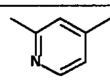
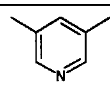
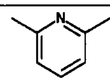
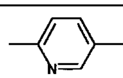
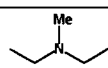
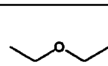


Cpd.No.	R5	n	IC_{50} RT1	IC_{50} RT2
19	Br	3	7.3μM	10μM
1	Br	4	3.1μM	1.7μM
20	Br	5	4.7μM	3.8μM
21	Br	6	18μM	7.4μM
22	Cl	3	8.7μM	9.6μM
10	Cl	4	8.7μM	1.8μM
23	Cl	5	9.0μM	2.6μM

Table 3.

In this series a linker length of n = 4 was optimal, although the activity did not fall off dramatically for homologues within one or even two methylene units. One possible explanation is that the linker is puckered when the inhibitor is bound and so a certain degree of flexibility in the linker length can be sustained. Finally, we prepared some compounds containing functionality or ring systems within the linker itself in order to probe for potential interactions with either of the two conserved amino acid residues Tyr115 (π-stacking) or Gln151 (H-bond with amide) (Table 4).



Cpd.No.	X	IC_{50} RT1	IC_{50} RT2
24		19μM	14μM
25		15μM	21μM
26		Inactive[a]	Inactive[a]
27		66μM	17μM
28		Inactive[a]	Inactive[a]
29		Inactive[a]	Inactive[a]
30		35μM	5.5μM
31		Inactive[a]	Inactive[a]
32		Inactive[a]	Inactive[a]

a Inactive means <50% inhibition @100μg/ml concentration.

Table 4.

Insertion of meta-substituted benzene into the linker resulted in two actives (**24**, **25**), but when an additional methylene was added (**26**) all activity was lost. A 2-pyridyl linker **27** was active, but the 3- and 4-pyridyl congeners (**28**, **29**) were inactive. Analogue **30**, containing a para-substituted 2-pyridyl group, was shown to be a second compound showing selectivity for RT-2.

These biaryl acids were tested against HIV-1 (strain HXB2) in MT4 cells at 50μM.[10] No antiviral activity was observed, presumably because the acids have poor solubility and poor cellular penetration. In conclusion, consideration of the active site of RT has allowed us to identify a novel series of inhibitors (biaryl acids) showing single micromolar activity against HIV RT types 1 and 2. Activity against HIV-2 RT is rare[11] for non-nucleoside inhibitors, and we have discovered two compounds that show selectivity for HIV-2 RT. It has not been possible to obtain crystals with these inhibitors bound into RT and further experiments will be needed to confirm the mode of action of this series.

Acknowledgements
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References and Notes

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6. Surface generated using InsightII, available from Molecular Simulations Inc., San Diego, CA.
7. RT assays were run essentially as described in Hart, G.J *et al, Antimicrobia Agents and Chemotherapy*, **1992**, 38, 1688 using rC.dG and [^{3}H]-dGTP as substrates.
8. In a typical preparation the appropriate aniline (2 equivs.), diacid chloride (1 equiv.), triethylamine (2 equivs.) in acetone were heated at 50°C for 24 hours. After this time the resultant white precipitate was filtered off, washed with warm 2M hydrochloric acid. The solid was again isolated, dried and in many cases needed no further purification. Where purification was necessary, HPLC proved to be the best method. All compounds gave satisfactory ^{1}H NMR, mass spectra, elemental analyses and / or accurate mass spectra. Yields were in the range of 30-80%. In cases where the diacid chloride was not available, it was made from the corresponding commercially available diacid by standard methods e.g. $SOCl_2$.
9. Nevirapine was used as standard in our biological assays, having an IC_{50} of 30nM against RT1.
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