Imidazo[1,2-*a*]pyridines as HIV NNRTIs

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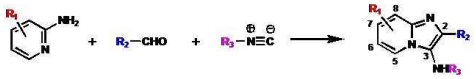
Introduction

The implementation of highly active antiretroviral therapy (HAART) has dramatically altered both the life expectancy and quality of life of HIV-AIDS sufferers. Most first-line regimen drug cocktails are made up of a combination of nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). These drugs inhibit reverse transcriptase (RT) by binding to a lipophilic, non-substrate binding pocket located about 10Å from the substrate binding site. Screening of a small in-house library of compounds for activity against HIV-1 RT led to identification of imidazo[1,2-*a*]pyridines as potential NNRTIs. A broader synthesis and testing campaign was carried out based on this result.

Results and Discussion

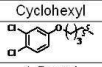
Compound **1**, an imidazo[1,2-*a*]pyridine, was identified in the preliminary screen and served as the hit compound for this study. The Groebke multi-component reaction serves as a versatile method for the rapid assembly of imidazo[1,2-*a*]pyridines (Scheme 1). Several small libraries were generated by varying functionality on the aldehyde, isocyanide and 2-aminopyridine components. All compounds were tested against HIV-1 RT and selected compounds were tested in a cell-based anti-HIV assay (PBMC or MAGI).



Scheme 1. Groebke reaction

A set of first-generation analogues incorporating maximum structural diversity in the isocyanide and aldehyde components was prepared and a selection of these is shown in Table 1.

Table 1. Selected first generation analogues (R_1 = H)

No.	R_2	R_3	Biological Activity		
			% Residual Enzyme Activity†	IC_{50} (μM) Enzymatic Assay	IC_{50} (μM) PBMC§/MAGI¶ Assay
1	*iso*-Propyl	Cyclohexyl	40	29	40§
2	2-Chlorophenyl	2-Pentyl	44	ND#	33¶
3		Adamantyl	78	ND	ND
4	2-Furyl	Cyclohexyl	71	ND	95§
5	2-Furyl		36	ND	44¶
6	2-Chlorophenyl	1-Pentyl	55	ND	>200¶
7	4-Methoxyphenyl	2-Naphthyl	100	ND	ND
8	2-Hydroxyphenyl	Cyclohexyl	87	ND	32§
9	*iso*-Propyl	2-Pentyl	55	ND	>200¶
10	2-Chlorophenyl	Cyclohexyl	7	4	0.16§ and 0.41¶
11	4-Methoxyphenyl		83	ND	ND
12	Phenyl	Cyclohexyl	75	ND	ND
13	*iso*-Propyl	1-Pentyl	84	ND	>200¶
14		Cyclohexyl	25	14	102¶
15		Cyclohexyl	92	ND	57§
16	Nevirapine (standard)		1	0.8	0.033§/0.10¶

†The percentage RT activity remaining after addition of the inhibitor
§ PBMC (peripheral blood mononuclear cell) assay uses human PBMCs infected with HIV to test compounds for the ability to inhibit infection
¶ MAGI (multinuclear activation of a galactosidase indicator) assay uses HIV-infected modified HeLa cells to test compounds for the ability to inhibit infection. Antiviral activity is determined as a reduction in virus-induced β-galactosidase enzyme expression using a chemiluminescence detection method.
# Not determined

Only one compound (**10**, Table 1) out of the approximately 65 first generation analogues prepared showed significant activity both in the enzymatic and the whole cell assay. Table 1 shows that the specific combination of 2-chlorobenzaldehyde and cyclohexyl isocyanide is required for potency, as other isocyanide combinations with this aldehyde gave poor inhibition results (cf. **10** with **2** and **6**). Compound **10** acted as the lead compound for preparation of second-generation analogues, in which the isocyanide was kept constant and variously halogenated benzaldehydes were tested (Table 2).

From the enzyme inhibition results (Table 2), there is a clear preference for the 2-halo substituent in all cases of monosubstituted systems (**10**, **17** – **23**). The size of the halogen has a strong influence when placed at position 4 (**21** – **23**).

Table 2. Selected second generation analogues (R_1 = H, R_3 = cyclohexyl)

No.	R_2	% Residual Enzyme Activity	IC_{50} (μM) MAGI Assay	No.	R_2	% Residual Enzyme Activity
17	2-Fluorophenyl	14	18	**23**	4-Bromophenyl	100
18	2-Bromophenyl	30	2	**24**	2,6-Difluorophenyl	18
19	3-Chlorophenyl	76	ND	**25**	2,6-Dichlorophenyl	56
20	3-Bromophenyl	84	ND	**26**	2,4-Dichlorophenyl	40
21	4-Fluorophenyl	45	ND	**27**	3-Chloro-2-fluorophenyl	50
22	4-Chlorophenyl	78	ND	**28**	2,4,5-Trifluorophenyl	19

The addition of a second halogen to the 2-halo system at the 3-, 4- or 6-position is deleterious for any halogen except fluorine (cf. **24** and **28** with **25** – **27**). The whole-cell results show unexpectedly good results for 2-bromophenyl (**18**), but there is still clear preference for chlorine (**10**) over bromine. Hence, refinement using the preferred 2-chlorophenyl group at R_2 was undertaken, varying the cycloalkyl group at R_3 and introducing substituents on the 2-aminopyridine-derived component.

Table 3. Lead optimization

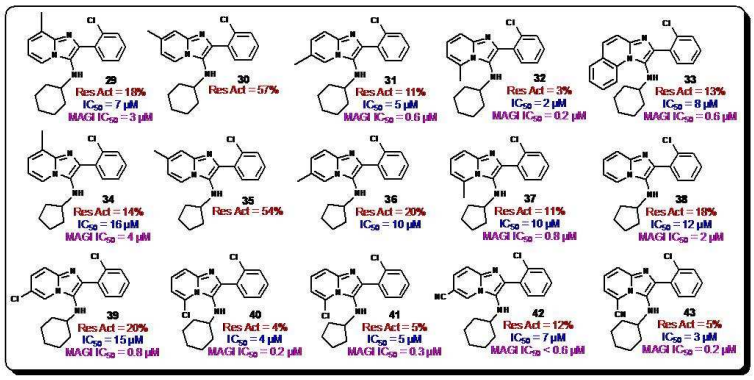
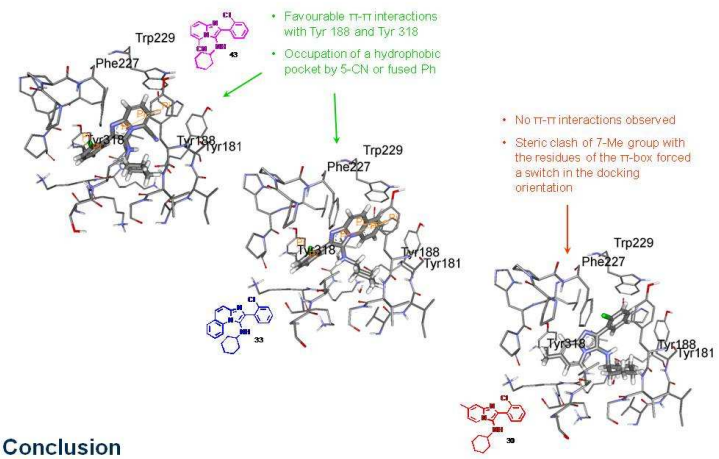


Table 3 shows that substitution at position 5 or 6 is highly favoured (**31**, **32**, **36**, **37**, **39**, **40**-**43**), while that at 8 is tolerated (**29** and **34**). However, substitution at position 7 results in a dramatic loss in potency (**30** and **35**). Interestingly, the imidazo[1,2-*a*]quinoline (**33**) with ring fusion at positions 5 and 6 shows good activity, while the isoquinoline analogue with ring fusion at position 7 and 8 is completely inactive. A possible molecular explanation for this substitution preference is illustrated below. Cyclopentyl and cyclohexyl derivatives gave very similar results. Compounds substituted with 5-chloro, -cyano or –methyl groups showed whole-cell anti-HIV activity comparable to that of nevirapine (**16**, Table 1).



Conclusion

A large number of imidazo[1,2-*a*]pyridines were synthesised using the Groebke reaction and tested for anti-HIV activity. Compounds prepared from 2-chlorobenzaldehyde, cycloalkyl isocyanide and 6-substituted 2-aminopyridines showed excellent inhibitory activity.

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