Poster # 80

A Novel NNRTI Class with Potent Anti-HIV Activity Against NNRTI-Resistant Viruses

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Background

Current anti-retroviral therapy (ART) strategies for HIV infection utilize combinations of at least two classes of antiretroviral agents. Although ART has proven to be effective, its benefits can be compromised by the development of drug resistance. For the non-nucleoside reverse transcriptase inhibitors (NNRTIs), many mutations such as K103N cause cross-resistance, rendering this class unavailable for combination therapies in subjects infected with these resistant viruses. Newer NNRTIs, active against the NNRTI-resistant viruses, are urgently needed to expand the NNRTI armamentarium. The characterization of activities against a panel of NNRTI-resistant HIV-1 viruses suggests this NNRTI series of pyrrolopyrimidine compounds has the potential to overcome the most prevalent of these resistant strains.

Methods

Antiviral activities of the NNRTIs were determined using either VSV-g pseudotyped HIV-1 containing wild-type (wt) and NNRTI-resistant sequences or clinical HIV-1 isolates containing NNRTI-resistant mutations. Cytotoxicity was evaluated in cell lines by ATP measurement. Non-linear regression analysis was used to calculate IC_{50} values. The resistance profiling using clinical isolates was performed by Monogram Biosciences using the PhenoSense™ HIV Assay.

Results and Discussion

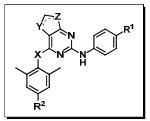
The NNRTI series presented comprises potent inhibitors of wild-type (wt) HIV-1 with EC_{50} values of approximately 1 nM and CC_{50} values of ≥ 10 μM. The fold-changes (FCs) in EC_{50} against the major NNRTI-resistant viruses found in patients failing efavirenz therapy are significantly lower than those of efavirenz. For instance, FCs for compounds in the series against K103N are < 1, versus > 10 for efavirenz. The FC in activity over wild-type virus in a broad panel of NNRTI-resistant mutant viruses is superior to efavirenz and superior, or similar, to etravirine and TMC278. These compounds are stable in human plasma and have favorable pharmacokinetic properties.

This novel series of compounds was first tested against a limited panel of prevalent NNRTI-resistant mutant viruses, including Y181C, Y188L and the double mutant L100I-K103N. The activity against wild-type as well as against these mutants directed our Structure Activity Relationship (SAR) effort toward the bicyclic compounds shown in Table 1. Atoms X, Y and Z as well as groups R^1 and R^2 were varied and optimized with the goal of reaching equipotency across our virus panel. After evaluation of several substituents R^1 was fixed as a CN group, which showed a greater potency than all other substitutions evaluated. The R^2 group was also varied between a methyl and a cyano, but the cyano group consistently showed a better activity against the panel of mutant viruses tested. Several atom substitutions were attempted at the X position, but the oxygen atom provided the best overall activity. It is noteworthy that results may be different based on a different resistance panel, especially between the O and the NH which proved to be equally potent on wild-type and Y181C viruses, with the NH being slightly less potent against L100I-K103N and several-fold worse against the Y188L mutant.

Several 5-membered fused rings were synthesized and compared, and to our surprise, they proved almost evenly matched in terms of activity. The two best compounds with a fused pyrrole ring (pyrrolo[2,3-*d*]scaffold and pyrrolo[3,2-*d*]scaffold, Figure 1) were evaluated side-by-side against an extended panel of mutant viruses, and showed only minor variation in terms of potency (Table 2).

Results

Table 1. Effect of Substitutions on Activity Against Four Viruses



Compounds	R^1	R^2	X	Y	Z	WT EC_{50}, nM	Y181C EC_{50}, nM	Y188L EC_{50}, nM	L100I-K103N EC_{50}, nM
1	CN	Me	O	CH	NH	1.0	11	14	34
2	CN	Me	S	CH	NH	3.0	24	45	>600
3	CN	Me	NH	CH	NH	0.60	10	81	67
4	CN	Me	O	N	NH	0.20	1.2	2.8	7.6
5	CN	Me	NH	N	NH	0.31	1.0	9.5	16
6	CN	Me	O	NH	CH	0.68	2.8	4.0	5.2
7	CN	Me	S	NH	CH	1.6	3.8	4.4	35
8	CN	CN	O	CH	NH	0.16	4.5	4.0	7.8
9	CN	CN	O	N	NH	0.20	1.2	1.4	11
10	CN	CN	O	NH	CH	0.25	1.4	1.1	3.8
11	SO_2NH_2	Me	O	N	NH	68	630	2,500	950
12	CO_2Me	Me	O	N	NH	1.9	220	320	>2,500
13	$CONH_2$	Me	O	N	NH	16	>600	>2,500	>2,500
14	CO_2H	Me	O	N	NH	>150	>600	>2,500	>2,500
15	F	Me	O	N	NH	0.15	5.6	17	33
16	OMe	Me	O	N	NH	0.46	44	240	310
17	cPr	Me	O	N	NH	21	580	1,300	2,200
18	CF_3	Me	O	N	NH	25	580	900	2,300
Efavirenz	na	na	na	na	na	0.37	0.59	48	640

Figure 1. Structures of Compounds 8 and 10

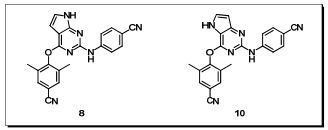


Table 2. Comparison of Compounds 8 and 10 with Other NNRTIs Against Prevalent NNRTI-resistant Viruses

Mutations	Prevalence[a,b]		Fold Change (wt EC_{50})				
	EFV-resistant	NNRTI-resistant	Efavirenz (0.28 nM)	Etravirine (0.54 nM)	TMC278 (0.53 nM)	8 (0.8 nM)	10 (0.66 nM)
K103N	88.5	16.6	19	0.6	1.1	0.3	0.3
K103N-P225H	32.7	1.6	285	1.5	1.4	3.4	1.4
K103N-V108I	28.8	1.9	36	0.3	0.7	0.4	0.4
K101Q-K103N	16.3	0.5	159	1.3	1.4	2.5	1.4
L100I-K103N	10.6	4.3	1269	1.9	11	6.2	9.5
G190S	10.6	0.4	129	0.3	0.2	1.0	0.5
K103N-V108I-P225H	9.6	0.1	116	0.8	0.8	0.4	0.7
Y188L	6.7	2.1	78	2.2	5.9	2.9	2.8
K101E	4.8	0.8	8.0	1.5	3.0	3.9	3.0
K103N-F227L	4.8	0.1	18	0.4	0.6	0.6	0.3
V106I-Y188L	4.8	0.5	1418	6.1	11	30	13
G190A	3.8	1.4	3.4	0.6	0.8	0.5	0.6
K103N-G190A	3.8	1.2	87	0.5	0.6	0.2	0.3
K101Q-K103N-P225H	3.8	0.1	163	1.4	1.3	0.6	1.0
A98G-K103N-V108I	3.8	0.3	126	1.2	2.4	0.7	1.3
K103N-Y188L	3.8	0.5	1627	5.0	102	nd[c]	19
A98G	2.9	2.3	5.0	1.6	2.4	3.6	2.1
K103N-Y181C	2.9	4.7	35	1.4	1.1	11	3.8
V106I	1.9	2.7	0.7	1.0	1.2	0.8	0.7
Y181C	1.9	4.1	2.3	3.2	1.7	8.0	3.9

[a]Prevalence of mutations from patients failing efavirenz therapy, from Bacheler et al. 2000[1]
[b]Prevalence of mutations in all NNRTI-resistant sequences in Stanford database[2]
[c]nd No data

- ***Compounds 8 and 10 show low levels of cross-resistance to most NNRTI-resistant mutations***
- ***Compounds 8 and 10 have comparable or better activity against most of these NNRTI-resistant viruses than efavirenz, nevirapine , etravirine and TMC278***

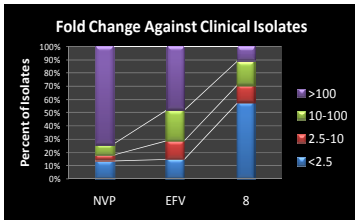
Table 3. Selectivity Index

Compounds	EC_{50}, nM	CC_{50}, μM	SI[a]
8	0.80	31	>38,000
10	0.66	9	>13,000
Etravirine	0.54	>100	>185,000
TMC278	0.53	10	>18,000

[a]Selectivity Index (CC_{50}/EC_{50}) in JC53 HeLa cells

- ***Compounds 8 and 10 show low cellular cytotoxicity***
- ***Other compounds in this series have CC_{50} values greater than 50,000 nM and a selectivity index of at least 50,000***

Figure 2. Cpd 8 is More Active Against a Panel of Clinical Isolates Containing NNRTI Mutations than Nevirapine (NVP) and Efavirenz (EFV)



Resistance profiling was performed by Monogram Biosciences using the PhenoSense™ HIV Assay

- ***Compound 8 exhibited less than 2.5-fold change in activity against 57% of 94 clinical isolates containing NNRTI-resistant mutations***
- ***Compound 8 had an EC_{50} of less than 10 nM in 68% of clinical isolates, compared with 28% for EFV***
- ***The fold-change profile of compound 8 is far superior to those of EFV and NVP***

Conclusions

- A clear SAR in this series guided the optimization of the two pyrrolopyrimidine scaffold to highly active NNRTIs.
- Compounds in this series are potent against both wild-type and prevalent NNRTI-resistant viruses, with similar or better activity than efavirenz, etravirine and TMC278.
- These compounds are clearly superior to efavirenz and nevirapine against a broad panel of NNRTI-resistant clinical isolates.
- The in vitro characterization of these novel NNRTIs shows strong potential for improved performance over current NNRTIs and warrants further evaluation.

References

1. Bacheler LT, ED Anton, P Kudish et al. 2000 *Antimicrob. Agents Chemother*. 44:2475-2484
2. www.hivdb.stanford.edu