A New Quinoline Family of HIV-1 Integrase Inhibitors Acting on HIV-1 Mutants Selected by Integrase Strand Transfer Inhibitors

J-118

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Abstract

Background: Integrase strand transfer inhibitors (INSTI) entered clinical use recently. Nevertheless, the development of new anti-integrase inhibitors with a different mechanism of action remains of particular interest to overcome the resistance that has emerged due to mutations in integrase selected by INSTIs. This new Quinoline family is an Integrase Binding Inhibitors (INBIs) compound acting as inhibitors of 3' processing step. The activity of quinoline compounds was studied on wild-type (WT) virus and viruses carrying mutations in the Reverse Transcriptase (RT) or Integrase (IN) genes.

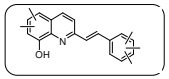
Methods: Quinoline compounds were tested for their specific anti-integrase and antiviral activities. Oligonucleotide-based IN assays were used to characterize the 3' processing and strand transfert steps. Selective inhibitors of HIV-1 replication in cell culture were identified by the MT-4 and Hela P4 cells plus viability assay. To determine the efficiency of quinolines on RTI or INSTI's mutations, viruses were constructed by site directed mutagenesis and their replication was assayed in presence of quinoline derivatives.

Results: Quinoline family inhibitors showed activity on the 3'processing step (100<IC50<1000nM) and consequently, on the strand transfer step too. Antiviral activity against HIV-1 virus was evaluated demonstrating an inhibition of HIV-1 replication in MT-4 and HeLa P4 cells (500<IC50<2000nM). 1-[8-Hydroxy-2-[2-(4-hydroxy-3-methoxy-5-nitro-phenyl)-vinyl]-quinoline-5-yl]-ethanone (QNL111) compound was chosen as a potential lead. QNL111 inhibits the 3'processing with an IC50=700nM and HIV-1 virus replication at an IC50=800nM. The cytotoxicity/antiviral activity ratio is over 100. QNL111 displays a comparable level of efficiency against viruses resistant to RT inhibitors. More interestingly, QNL111 is also active against the main resistance mutations selected by Raltegravir and Elvitegravir, acting as INSTI's inhibitors (Fold Change : 1.5±0.3, and 1.2±0.2 for E92Q/N155H and G140S/Q148H respectively).

Conclusions: Quinoline compounds display a potent activity against IN enzyme and HIV-1 virus replication. The RTI's and INSTI's mutations don't impact antiviral quinoline activity, suggesting a new mechanism of action compared to well described INSTI's inhibitors.

Background

- AIDS morbidity and mortality have led to major efforts to identify effective inhibitors of the replication of HIV-1 virus. Over the past few years the treatment of HIV has been extensively developed to limit the virus replication at a low or undectable level using a cocktail of drugs acting on different targets.
- New strategies involved:- antiretroviral combination to reduce pills burden,
  - new classes of antiretroviral, such as integrase inhibitors.
- Integrase Strand Transfer inhibitors (INSTI) entered clinical used recently. Nevertheless, the emergence of resistant viral strains and cross-resistance mutations between INSTI's bring out the needs to develop new anti-integrase compounds.
- Quinolines, with QNL111 lead, (structure shown below) are a new family of integrase inhibitors. These compounds act at the 3'processing step, and subsequently at the strand transfer step.QNL111 was discovered and characterized by a strategy of structure-activity relationship optimization.



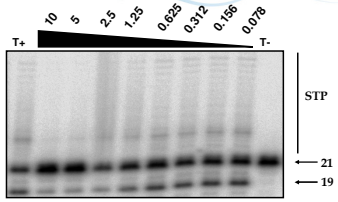
Quinolines characterization strategy:

- To select and characterize quinolines as new specific integrase inhibitors, the following tests were done :
  - **Oligonucleotide-based Integrase assays,** to characterize the 3' processing and strand transfert steps, radiolabeled substrate and EMSA (Electrophoresis Mobility Shift Assay) migration.
  - **Cellular Antiviral Activity and cytoxicity**, and IC50, CCIC50, SI were determined in HeLa-CD4 cells & MT4.
  - **Activity against multi-resistant viruses**, with Quinolines antiviral activity on NRTI'S & NNRTI's viral strains
  - **Activity against INSTI's resistant viruses**, with Quinolines antiviral activity on INSTI's viral strains

Antiviral activity Methods

- Characterization of quinolines antiviral activity was determined on a panel of drug-resistant HIV-1 virus compared to the antiviral activity on the wild type pNL43 virus (IC50 resistant virus/ IC50 NL4.3 reference virus)
- drug-resistant HIV-1 viruses were obtained by site-directed mutagenesis in a pNL4.3 backbone:
  - NNRTI resistant viruses: K103N,Y181C, 190A, 108/151, K103N/190A, K103N/Y181C:
  - NRTI resistant viruses:178L, 41/215/K103N, 41/215/Y181C, 41/215/M184V
  - INSTI's resistant viruses: E92Q, Q148H, N155H, E92Q/N155H, G140S/Q148H

I. In vitro activity Results



QNL111

Fig 1: A representative gel of *In Vitro* integrase activity: 3'processing & Stand Transfer. Radiolabeled oligonucleotide derived from U5 LTR (21pb) was used with recombinant integrase. The 3'Processing activity is characterized by the releasing of GT dinucleotide (19pb). The strand transfer reaction generates a series of products (STP). The 21 and 19 mers were separated by electrophoresis

Anti-Integrase activity

	3' processing (µM)	Strand transfer (µM)	Antiviral Activity HeLa-CD4 cells (µM)	Antiviral Activity MT4 cells (µM)	Cytotoxicity (µM)	Selective Index
Compound 1	0.72	0.25	0.5	5	7	1.4-14
Compound 2	0.15	0.18	6	7	35	5-6
Compound 3	0.225	0.225	12	0.35	12	1-34
Compound 4	0.53	0.53	1	0.5	15	15-30
Compound 5	0.56	0.56	1	0.5	15	15-30
Compound 6 (=QNL111)	0.7	0.7	0.8	0.8	100	125-125

Table 1: *In Vitro* antiviral activity compounds summary

II. Activity of Quinolines on RTI's mutants Results

Compounds	NRTI's & NNRTI's Mutants viruses Fold Change (IC50 mutant virus/ IC50 NL4.3 reference virus)										
	K103N	178L	Y181C	190A	108/151	103N/190A	103/188	103/181	41/215/103	41/215/181	41/215/184
Compound 1	0.6	0.9	1.1	0.4	1.1	1.2	1.3	1.4	0.9	1.4	0.7
Compound 4	1.0	0.7	1.3	1.3	1.2	0.9	0.8	1.2	0.6	1.0	0.8
Compound 6	0.7	0.8	0.7	1.4	1.1	0.9	1.0	1.0	0.7	0.7	0.7
AZT	0.9	0.4	0.8	1.4	2.8	1	1.1	0.8	3.1	6.0	1.8
3TC	0.8	42.9	1.2	1.1	0.7	0.6	1.2	0.7	0.5	2.2	42.9
EFV	70.4	0.9	55.6	14.8	0.3	>370.4	>370.4	203.7	20.0	3.2	0.2
NVP	62.5	1.3	196.4	119.6	3.5	>446.4	>446.4	>446.4	24.1	262.5	0.3

Table 2. Activity of Quinolines, included QNL111, on RTI's mutants viruses.
The color subdivide the resistance fold-change into three levels as follows: green, <3-fold; light orange, >3 & <50-fold; red>50-fold. Data represent the mean of 3 independent experiments.

III. Activity of Quinolines on INSTI's mutants Results

	INSTI's Mutants viruses Fold Change (IC50 mutant virus/ IC50 NL4.3 reference virus)					
	WT	E92Q	Q148H	N155H	E92Q/ N155H	G140S/ Q148H
RGV	1	2	12	>36	>36	>36
Compound 1	1	2.3	2.1	2.1	2	1.7
Compound 2	1	1	1.2	0.6	0.8	0.8
Compound 5	1	0.7	0.8	0.7	1.1	1.1
Compound 6 (=QNL 111)	1	1.1	0.8	1.1	1.2	1.5

Table 3. Activity of Quinolines, included QNL111, on INSTI's mutants viruses.
The color subdivide the resistance fold-change into three levels as follows: green, <3-fold, light brown >10-fold; red, >30-fold; Data for Raltegravir (RGV) & quinolines compounds represent the mean of 3 independent experiments.

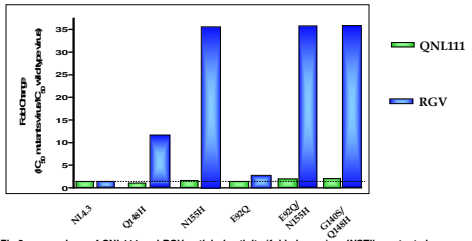


Fig 2. comparison of QNL111 and RGV antiviral activity (fold change) on INSTI's mutant viruses.

Conclusion

- Quinolines family and, QNL111 as a lead, are new potent HIV integrase inhibitors.
- *In Vitro* assays demonstrate a specific activity of Quinolines during the 3' processing step of integration and, consequently an inhibition of the strand transfer step.
- The data highlight that NRTI's and NNRTI's mutations do not impact on Quinolines antiviral activity
- Furthermore, Quinolines remain still active against INSTI's mutants suggesting a different mechanism of action.