

Abstract

Background: K103N is the most abundant mutation in the allosteric pocket of the HIV reverse transcriptase (RT) of patients treated with efavirenz or nevirapine. The transmission of this mutation to newly infected patients is a serious issue. In fact, when this mutation is present in a treatment-naive patient, it precludes the selection of any of the currently approved non-nucleoside reverse transcriptase inhibitors (NNRTIs) for first-line therapy. This limits the treatment options to the use of a protease inhibitor (PI) and a cocktail of nucleoside analogues (NRTIs), therefore eliminating an entire class of inhibitors. These limitations motivated our research team to focus our development program on a new molecule that would have the potential to be used in the event that the K103N mutation was present in newly infected patients.

Methods: We started from a high throughput screening hit with micromolar potency, and we synthesized several hundred analogues and derivatives of this hit. A rational study of the structure-activity relationship (SAR) led us to several nanomolar development candidates, from which RDEA806 was selected for further clinical studies based on a favorable resistance profile, desirable pharmacokinetic properties (12 hour $T_{1/2}$ in dogs), and excellent water-solubility (240 mg/mL).

Results: RDEA806 is the potassium salt of a benzoic acid derivative. Its IC_{50} against the purified wild-type RT enzyme is 3.1 nM. In a cell based assay, it exhibits EC_{50} s against WT, K103N and L100I-K103N of 3.0 nM, 1.4 nM and 1.0 nM, respectively. It can be synthesized in 6 steps from 2 starting materials. This straight-forward synthesis scheme allowed us to explore several areas of the molecule, including its triazole core structure, the benzoic acid moiety and its 3 substituents.

Conclusion: RDEA806 is a novel NNRTI, with the potential to be used in both treatment-experienced and naive patients, including those harboring the K103N mutation. It has favorable physical and chemical properties and can be readily synthesized.

Introduction

- > K103N is the most abundant mutation in the allosteric pocket of the HIV reverse transcriptase (RT) of patients treated with efavirenz or nevirapine.
- > Transmission of this mutation to newly infected patients is a serious issue because it precludes the choice of efavirenz or any of the currently approved NNRTIs for first-line therapy.
- > This limitation motivated our research team to design a new molecule that would have the potential to be used in the event that the K103N mutation or other common mutations associated with previous NNRTI use were present.

Design Strategy

- > Compound 0387902 (**Table 1**) was discovered by screening a library of 87,000 compounds using a cell-based assay (Abstract #1662). This compound showed moderate activity against viruses carrying wild-type or K103N-Y181C mutations in RT.
- > Medicinal chemists synthesized over 1,000 analogues of 0387902 in a structure-activity relationship (SAR) effort to optimize potency and pharmacokinetic properties.
- > Compounds were evaluated for their ability to inhibit WT and mutant HIV RT.
- > SAR was guided by antiviral activity against some of the most prevalent mutant viruses after NNRTI treatment failure (K103N, Y181C, K103N-Y181C, K103N-L100I).
- > SAR was also guided by microsomal stability, plasma stability and rat PK profile.

Results

- > Co-crystal structure was obtained with the early lead 0413660 (**Figure 1**).
- > Presence of interactions between the substituted lower phenyl group and Y181, Y188 and W229.
- > Presence of interactions between the extended chlorophenyl group and P236, F227 and Y106.
- > Analysis of these interactions pointed to several different SAR vectors:
 - extension of the bottom methyl group to optimize interactions with W229
 - filling-up the space between Y181 and Y188 by substituting the phenyl ring with a naphthalene ring
 - taking advantage of solvent access near the top of chlorophenyl group

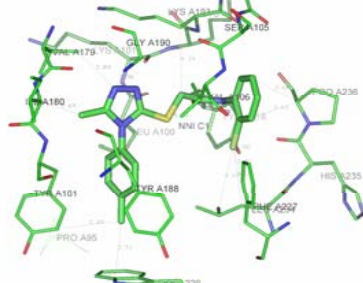


Figure 1. Interactions of 0413660 with WT HIV RT

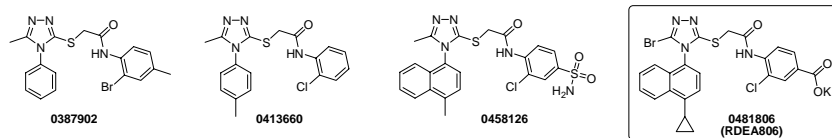


Table 1. Evolution of the NNRTI Triazole Series

Cell-based activity (EC_{50} , nM)	0387902	0413660	0458126	RDEA806
WT	100	1	0.2	3.0
K103N	320	7.6	0.6	1.4
Y181C	560	20	4.3	9.7
Y188L	>20,000	>20,000	20	19
K103N-Y181C	1,000	150	31	9.0
K103N-L100I	170	18	0.7	1.0

- > Addition of the naphthalene ring resulted in a boost in activity across all mutants, but most specifically on the Y188L mutant virus. This can be explained by the fact that as the tyrosine 188 mutates into a lysine, the strong interactions of the extra ring with tyrosine 181 compensates for the absence of Y188.

- > Various R groups can be used at positions R¹, R² and R³ (**Table 2**).
- > These groups have a strong influence on the EC_{50} against Y181C and K103N-Y181C.
- > Unlike other NNRTIs, this triazole scaffold afforded activity against the common K103N-L100I double mutant virus.

- > The presence of a sulfonamide group in 0458126 improves the solubility while improving the binding affinity to RT. This sulfonamide group was later replaced with a carboxylic acid group to allow the formation of a water soluble salt, a unique property in currently approved NNRTIs.
- > The exchange of the bottom methyl group with a cyclopropyl group allows a closer interaction with tryptophan 229, which is a conserved residue in the RT region.
- > The bromo atom on the triazole allows for a better filling of the space near the lysine 180 and may also have a favorable electronic effect on the triazole ring.
- > The carboxamide group in the linker portion of the scaffold makes a strong hydrogen bond with the backbone of the lysine 103. Several attempts to replace this group with a bioisostere did not lead to improved activity, especially on mutant viruses.

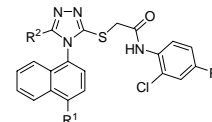
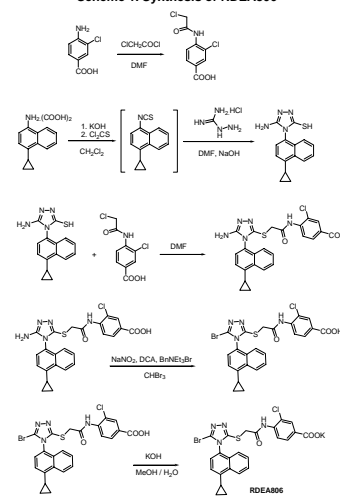


Table 2. SAR Data Within Triazole Series

Compounds	R ¹	R ²	R ³	WT EC_{50} , nM	Y181C EC_{50} , nM	K103N-Y181C EC_{50} , nM	K103N-L100I EC_{50} , nM
RDEA806	cPr	Br	COOK	3.0	10	9	1
0462068	H	H	Me	16	nd	nd	nd
0431002	H	Me	Me	4	28	54	6
0439642	H	Me	H	1	20	21	3.9
0439620	Me	Me	Me	1.1	8	15	1.1
0439643	Me	Me	COOMe	0.9	8	28	0.8
0448164	Et	Me	Me	1.5	6	32	1.7
0458126	Me	Me	SO ₂ NH ₂	0.2	4.3	31	0.7
0461890	N(Me) ₂	Me	SO ₂ NH ₂	0.5	5.6	29	0.4
0472651	H	CF ₃	SO ₂ NH ₂	0.5	4	nd	nd
0473978	Me	CF ₃	SO ₂ NH ₂	0.4	9	nd	nd
0474019	N(Me) ₂	CF ₃	SO ₂ NH ₂	1.9	4.1	nd	nd
0477482	H	Br	SO ₂ NH ₂	0.2	0.5	nd	nd
0481792	Et	Br	COOH	4	12	nd	nd

- > The synthesis of RDEA806 is presented in **Scheme 1**. The overall yield from the starting 4-cyclopropylamino naphthalene is 18-19% (average yield of 71% per reaction step)
- > The compound is isolated as a solid that exhibits an aqueous solubility of 240 mg/mL.

Scheme 1. Synthesis of RDEA806



Conclusions

- > RDEA806 is a novel NNRTI with the potential to be used in both treatment-experienced and naive patients, including those harboring the K103N mutation.
- > RDEA806 has favorable physical and chemical properties, including an excellent water solubility (240 mg/mL).
- > RDEA806 can be readily synthesized in 5 consecutive steps, with an overall yield of 18-19%.