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Substituted tetrahydroquinolines as potent allosteric inhibitors of reverse transcriptase and its key mutants

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ABSTRACT

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are key elements of multidrug regimens, called HAART (Highly Active Antiretroviral Therapy), that are used to treat HIV-1 infections. Elucidation of the structure–activity relationships of the thiocarbamate moiety of the previous published lead compound **2** provided a series of novel tetrahydroquinoline derivatives as potent inhibitors of HIV-1 RT with nanomolar intrinsic activity on the WT and key mutant enzymes and potent antiviral activity in infected cells. The SAR optimization, mutation profiles, preparation of compounds, and pharmacokinetic profile of compounds are described.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are key elements of multidrug regimens, called HAART (Highly Active Antiretroviral Therapy), that are used to treat HIV-1 infections. HAART consists of combinations of nucleoside HIV reverse transcriptase inhibitors (NRTIs), NNRTIs, and protease inhibitors (PIs). However, due to the propensity of HIV to rapidly mutate, the efficacy and durability of HAART can be compromised. The most frequent HIV RT mutations observed in patients failing therapy with first generation NNRTIs are K103N and Y181C. Therefore, new agents with better activity profiles against both wild type (WT) HIV-1 and the RT K103N and Y181C strains are needed.

Tetrazole and triazole thioacetanilides, **1**, were reported as potent NNRTIs against WT and K103N mutant (Scheme 1).[1] It is also known that the tetrazole and triazole moiety in **1** can be replaced by tertiary thiocarbamates (**2**), amides, and carbamates.[1c] This Letter reports the discovery and SAR of a structurally novel class of compounds related to compound **2** that exhibit good antiviral efficacy against the WT enzyme and the clinically relevant mutants K103N and Y181C.

Compound **2** exhibited desirable intrinsic potency against the WT and key mutant K103N and was about 10-fold less potent against Y181C (IC_{50}: 8 nM (WT), 9 nM (K103N), 80 nM (Y181C)).[2] In a cell based antiviral assay (Spread assay),[3] compound **2** also demonstrated good activities and had a minimal shift between 10% fetal bovine serum (FBS) and 50% normal human serum (NHS) (CIC95: WT, 31 nM (10% FBS), 62 nM (50% NHS)). Conformational analysis of **2** suggested that the *N*-methyl group of the thiocarbamate and the *ortho*-methyl group of the mesitylene could be in close proximity. Therefore it appeared reasonable to form a bond between the two methyl groups, to generate a tetrahydroquinoline motif which led to compound **3** (Scheme 1 and Table 1). Compound **3** showed improved potency against the WT and K103N enzymes. However, this modification decreased potency against the Y181C mutant enzyme compared to the unconstrained analogue **2**. Compound **3** also displayed a five-fold decrease in potency in the presence of 50% NHS in the Spread assay presumably due to its high

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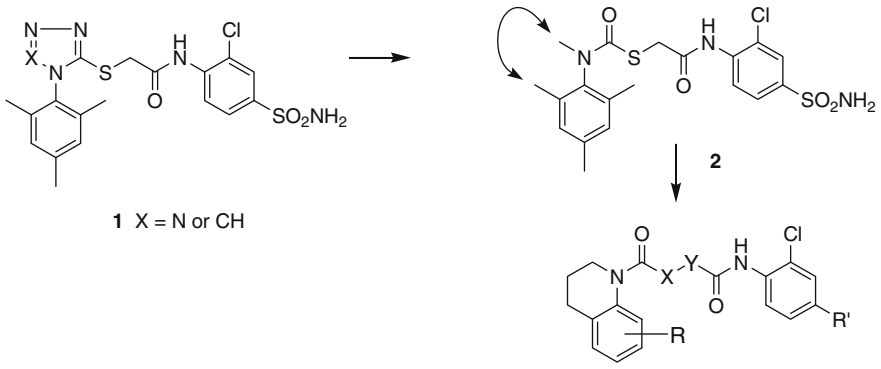
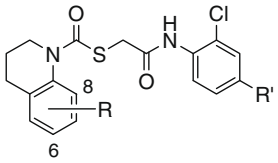


Scheme 1.

Table 1
SAR results



Compounds	R	R′	RT_Pol[a] IC_{50} (nM)			Antiviral Activity[b] CIC_{95} (nM)	
			WT	K103N	Y181C	10% FBS	50% NHS
3	6-Me	SO_2NH_2	3	3	1300	78	396
4	8-Me	SO_2NH_2	6	4	61	47	219
5	6-Cl	SO_2NH_2	8	—	—	141	1250
6	8-Cl	SO_2NH_2	2	4	31	23	47
7	6,8-Cl	SO_2NH_2	1	1	4	13/18[c]	40/33[c]
8	6,8-Cl	H	4	—	175	125	406
9	6,8-Cl	CN	7	14	95	156	1250
10	6,8-Cl	CONHBn	2	2	4	63[c]	307[c]
11	6,8-Cl	SO_2CH_3	4	3	11	5.6[c]	5.7[c]
12	6,8-Cl	$CONH(CH_2)_3NH_2$	2	2	7	97[c]	34[c]

[a] Compounds were evaluated in a standard SPA assay. The values are the geometric mean of at least two determinations. All individual values are within 25% of the mean.
[b] CIC95 (cell culture inhibitory concentration) is defined as the concentration at which the spread of virus is inhibited by >95% in MT-4 human T-lymphoid cells maintained in RPMI 1640 medium containing either 10% fetal bovine serum (FBS) or 50% normal human serum (NHS). The antiviral activity of compound against wild-type (H9IIIB) virus was measured. The values are the geometric mean of at least two determinations. All individual values are within 25% of the mean.
[c] The antiviral activity of compound against wild-type (R8) virus was measured.

protein binding (99% in human plasma). Introducing a methyl group at the 8-position (**4**) maintained WT and K103N potency, and regained activity versus the Y181C mutant. It is known that the mesitylene group can be replaced with a mono- or di-chloro substituted phenyl ring.[1] Therefore, a chlorine atom was introduced into this series at the 6 and 8 position (**5** and **6**). A similar trend was observed in this series of chlorine analogues, where the 8-chloro compound (**6**) was significantly more potent than the 6-chloro isomer (**5**). The 8-Chloro analogue was also more potent than the corresponding methyl analogue (**6** vs **4**). Combining the 6- and 8-chlorine substitution on the aryl ring provided compound **7** with a further increase in potency. Compound **7** exhibited good potency against WT enzyme, K103N, and in particular Y181C mutants. These improvements translated into excellent antiviral activities in cell culture.

Co-crystallization[4] of compounds **3** and **7** with WT RT revealed that the 6-substitutent (methyl group in **3** and chlorine atom in **7**) of the phenyl group interacts with Trp-229 and the anilide side chain is accommodated within the solvent channel lying under Pro-236 (Fig. 1). The anilide carbonyl group in both **3** and **7** is positioned to interact with Lys-103 backbone NH via a hydrogen bond, and this interaction is considered to contribute to the excellent potency of both **3** and **7** against the K103N mutant. The thiocarbamate carbonyl group in **7** is orientated differently compared to the one in **3** presumably to minimize its interaction with the 8-chlorine atom.

Since the 4-position of the sulfonamide is solvent exposed, we further explored the SAR of the anilide moiety using a library-based approach to replace the primary sulfonamide. Selected results are listed in Table 1. Deletion of the sulfonamide led to compound **8** and reduced potency against the Y181C enzyme and activity in cell based assays. Replacement of the sulfonamide with a small cyano group (**9**) or a large benzyl amide (**10**) was tolerated. Replacement of the sulfonamide with methyl sulfone yielded compound **11** with improved antiviral activities compared to **7** and no shift in potency in the presence of 50% human serum in the Spread assay. Incorporation of an amide side chain with a basic amine group (**12**) was tolerated and its antiviral activity was maintained. This result is consistent with the observation from the co-crystal structures that the *para* position of the anilide is solvent exposed and therefore tolerates a variety of substituents (Fig. 1).

We then initiated an effort to replace the thiocarbamate and selected results are summarized in Table 2. Replacement of the thiocarbamate with carbamate (**13**), amide (**14**) and urea (**15**) linkers showed modest activities in the enzyme assay, and all had significant potency shifts in the Spread assay in the presence of 50% NHS. A cyclopropane ring has been used successfully as a

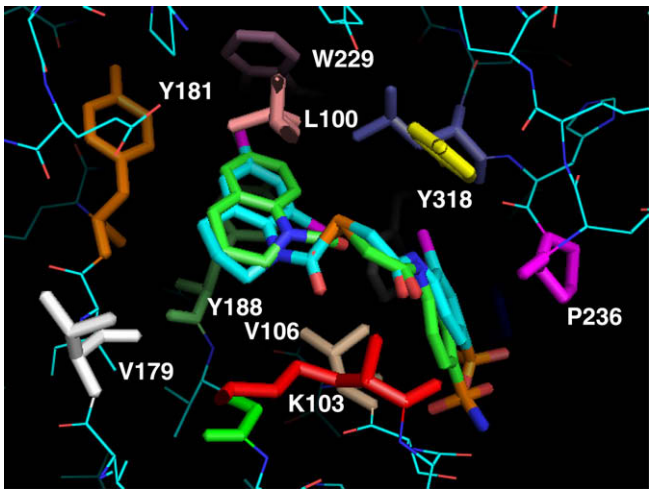


Figure 1. Crystal structures of compounds **3** and **7**. Compound **3** is colored in green and **7** in cyan with nitrogen atom in blue, oxygen atom in salmon, sulfur atom in orange, and chlorine atom in magenta.[4]

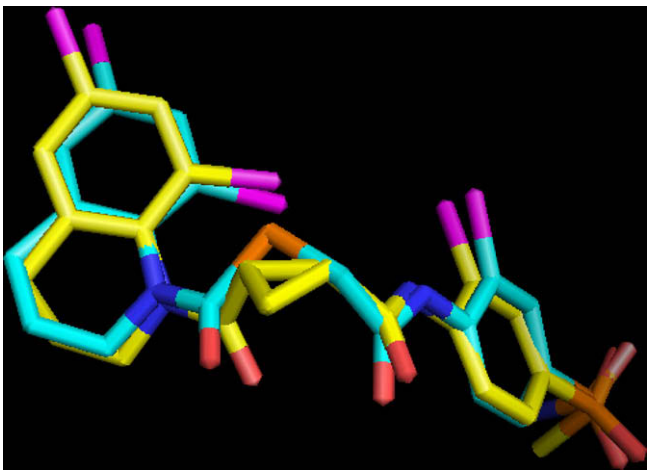


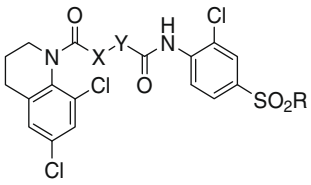
Figure 2. Modeling study results. Compound **7** is colored in cyan and *cis*-cyclopropyl analogue in yellow with nitrogen atom in blue, oxygen atom in salmon, sulfur atom in orange, and chlorine atom in magenta. Details are provided in Ref. 6.

linker in a series of structurally related analogues.[5] We speculated that a cyclopropyl ring could be used as a surrogate for the sulfur-methylene linker in this series. A modeling study suggested that the conformation of a *cis*-cyclopropyl amide could mimic the thiocarbamate (Fig. 2).[6] Based on these observations, the sulfur methylene linker was replaced with a *cis*-cyclopropyl ring. Reverse phase chiral chromatography of the racemic *cis*-cyclopropyl derivative delivered the two enantiomers, the first eluted enantiomer, **16**, and **17**, the second. As anticipated, the antiviral activity and the configuration of the asymmetric centers were linked, with enzyme inhibition residing predominantly in **16**. Compound **16** is about 3–5-fold more potent in the Spread assay than its enantiomer **17** with a small shift from 10% FBS to 50% NHS. Based on the molecular modeling, we speculated that the more active enantiomer **16** has the absolute configuration as shown in Figure 2. Interestingly the related other ring systems, such as, *cis*-1-3-cyclopentane, 1,3-cyclohexane, 2-5-tetrahydrofuran, 1,3-phenyl, and 2,5-thiophene, showed lower activity (data not shown).

To assess the antiviral activities of compounds in this series against a broad panel of clinically relevant mutants, compound **7** and **11** were selected for a PhenoScreen assay performed by Monogram Bioscience.[7] Both **7** and **11** have a similar profile of activity against the panel of clinically isolated mutant viruses (Table 3). These results further suggest that the cyclopropane function is an isostere of the thiomethylene region of the thiocarbamate moiety.

In terms of pharmacokinetics, compounds in this series exhibited extra-hepatic clearance in rats (e.g., for **7**, clearance: 109 mL/min/kg and the half-life: 0.4 h) following iv administration.[8] This may be attributed to the amide hydrolysis of compound. However compound **7** had reasonable oral bioavailability (30%), distribution volume (5 L/kg), and half-life (4.9 h) in dogs.[8] Replacement of pri-

Table 2
SAR results: replacement of thiocarbamate



Compounds	X–Y	R	RT_Pol[a] IC_{50} (nM)			Antiviral activity[b] CIC_{95} (nM)	
			WT	K103 N	Y181C	10% FBS	50% NHS
13	OCH_2	NH_2	19	21	116	187	2500
14	CH_2CH_2	NH_2	194	—	—	406	5000
15	$NHCH_2$	NH_2	336	—	—	562	>10,000
16	 *cis*, 1st eluted	CH_3	18	18	99	176	304
17	 *cis*, 2st eluted	CH_3	210	430	6000	922	922

[a] Compounds were evaluated in a standard SPA assay. The values are the geometric mean of at least two determinations. All individual values are within 25% of the mean.
[b] CIC95 (cell culture inhibitory concentration) is defined as the concentration at which the spread of virus is inhibited by >95% in MT-4 human T-lymphoid cells maintained in RPMI 1640 medium containing either 10% fetal bovine serum (FBS) or 50% normal human serum (NHS). The antiviral activity of compound against wild-type (H9IIIB) virus was measured. The values are the geometric mean of at least two determinations. All individual values are within 25% of the mean.

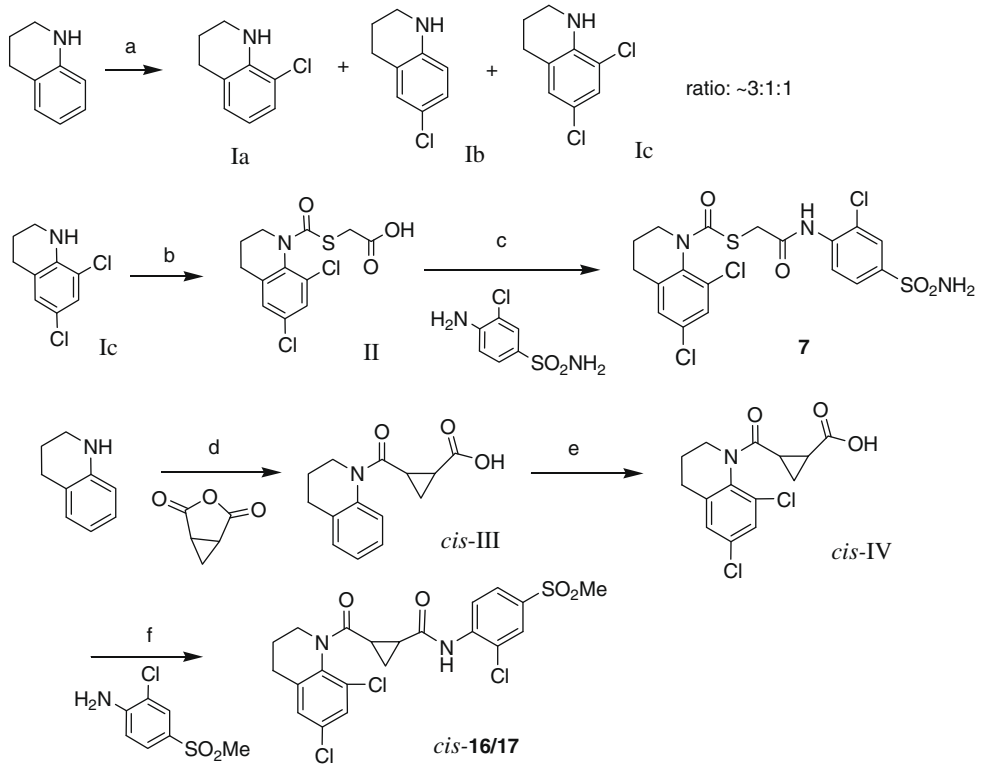
Table 3
Antiviral potency of compounds **7** and **11** against clinically isolated mutant viruses (NL4-3 isolates)[a]

Mutantion	Compound **7**		Compound **11**	
	IC_{50}[b] (mM)	IC_{50} fold shift	IC_{50}[b] (mM)	IC_{50} fold shift
WT	0.0012	1	0.0045	1
L100I	0.0055	4.6	0.0505	11
K103N	0.0009	0.8	0.0020	0.4
Y181C	0.0168	14	0.0866	19
Y188L	>1.000	>833	>0.25	>56
G190A	0.0121	10.1	0.1568	34.8
G190S	0.0619	51.6	>0.25	>56
K103N/V179E	0.0022	1.8	0.0075	1.7
K103N/Y181C	0.0642	53.5	01493	33.2
K103N/G190A	0.0082	6.8	0.0572	21.3
Y181C/G190A	0.327	272.5	>0.25	>56
K103N/P225H	0.0032	2.7	0.0093	2.1
K101E/G190A	0.1131	94.3	>0.25	>56
K101E/Y181C/G190A	>1.000	>833	>0.25	>56
K103N/Y181C/G190A	>1.000	>833	>0.25	>56
V106A/G190A/F227L	>1.000	>833	>0.25	>56

[a] Phenoscreen assay, Monogram Bioscience, in presence of 40% NHS. For details, see Ref. 7.
[b] The IC_{50} is defined as the concentration of compound in cell culture required to inhibit 50% of viral replication.

mary sulfonamide with methyl sulfone (**11**) improved the clearance (84 mL/min/kg) and the half-life (1.7 h) in rats slightly. Introduction of *cis*-cyclopropane (**16**) lowered clearance in both rats and dogs (22 mL/min/kg and 14 mL/min/kg, respectively), but no improvement in the half-life (0.5 h and 1.3 h in rats and dogs, respectively). Further study of **7** revealed that it underwent oxidation of tetrahydroquinoline ring in human liver microsomes in the presence of NADPH. The oxidative metabolism was mediated by CYP3A4 and could be inhibited significantly by ketoconazole, a known inhibitor specific for human CYP3A4. These oxidative metabolites were also found in rat liver microsomes, rat hepatocytes, rat bile, rat plasma, and human hepatocytes.

Compounds described in this Letter were prepared according to the route depicted in Scheme 2. Chlorination of commercially available tetrahydroquinoline with NCS provided a mixture of mono (Ia and Ib) and bis (Ic) chlorinated derivatives (in a ratio of ~3:1:1) which can be easily separated by silica gel chromatography. Treatment of the 6,8-dichloro tetrahydroquinoline (Ic) with phosgene, coupling with methyl thioglycolate, followed by hydrolysis of the methyl ester afforded the thiocarbamate acid (II). The final com-



Scheme 2. Reagents and conditions: (a) NCS, DCM, (b) (i) phosgene, DCM, pyridine, 0 °C, 40%; (ii) methyl thioglycolate, pyridine, microwave, 90 °C, 30 min, (iii) 5 N NaOH, 73%; (c) oxalyl chloride, DMF (cat.), THF, 0 °C, 23%; (d) ^{t}BuOK, THF, 72%; (e) NCS, microwave, 60 °C, 2 h, 13–51%; (f) $(COCl)_2$, DMF (cat), DCM, 0 °C, 30 min, then aniline, pyridine, microwave, 60 °C, 1 h, 35%.

pound could then be prepared by converting the acid to the acid chloride with oxalyl chloride and reaction with the requisite aniline. The carbamate, urea, and amide analogues were synthesized in an analogous fashion. The cyclopropane derivatives **16** and **17** were synthesized from commercially available 3-oxabicycl-[3.1.0]-hexane-2,4-dione. The coupling of the chlorinated tetrahydroquinoline Ic with 3-oxabicycl-[3.1.0]-hexane-2,4-dione was not successful under numerous conditions presumably due to the steric hindrance caused by the 8-chlorine atom. Therefore, the parent tetrahydroquinoline was deprotonated with KO^{t}Bu and acylated with 3-oxabicycl-[3.1.0]-hexane-2,4-dione to form the *cis*-cyclopropane carboxylic acid (III) and was then chlorinated. The chlorination with NCS under thermal conditions was unsuccessful; however, microwave heating condition yielded dichlorinated tetrahydroquinoline acid (IV) in modest yield. The anilide formation via the intermediate acid chloride afforded the racemate which was resolved into **16** and **17** by chiral HPLC.

In summary, we have designed and synthesized a series of novel tetrahydroquinoline derivatives as potent inhibitors of HIV-1 RT with nanomolar intrinsic activity on the WT and key mutant enzymes and potent antiviral activity in infected cells. Based on the modeling study results, tetrahydroquinoline amides have been identified as an effective isostere for the *N*-methyl mesitylene amide moiety, and the *cis*-cyclopropyl ring was used as a surrogate for the sulfur-methylene linker. Compounds in this series tended towards high plasma clearance in rats after iv administration. CYP3A4 mediated oxidation of the tetrahydroquinoline ring was identified as a significant metabolic pathway in vitro and in vivo. These compounds represent a novel and potent NNRTIs with activity against a number of clinically significant mutant viruses.

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4. Compounds **3** and **7** were soaked into a pre-existing crystal of HXB2 RT containing a weakly bound efavirenz analog. Electron density was mapped at a resolution of 3.0 Å (**3**) and 2.7 Å (**7**) and the structure solved by Fourier synthesis. The distances between carbonyl O and Lys103-N are 2.88 Å and 2.74 Å for compound **3** and **7**, respectively. The structures have been deposited in the PDB; PDB codes are 310R and 310S for compounds **3** and **7**, respectively.
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6. Approximately 100 conformations of each compound were generated using two algorithms: a distance geometry (see, (a) Crippen, C. M.; Havel, T. F. In Distance Geometry and Molecular Conformation; Bawden, E., Ed.; Research Studies Press: Wiley, New York, 1988; (b) Kuszewski, J.; Nilges, M.; Brunger, A. T. *J. Biomol. NMR* **1992**, *2*, 33) and a knowledge based method (see, Feuston, B. P.; Miller, M. D.; Culberson, J. C.; Nachbar, R. B.; Kearsley, S. L. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 754.). All conformations were then subjected to energy minimization using a distance dependent dielectric constant of compound and applying the MMFFs forcefield (Halgren, T. A. *J. Comput. Chem*. **1996**, *17*, 490, 520, 553, 616; Halgren, T. A.; Nachbar, R. B. *J. Comput. Chem*. **1996**, *17*, 587.).
7. Assays performed by Monogram Bioscience, San Francisco, CA. Values are the average of two determinations. Detailed assay protocols are available at: http://www.monogramhiv.com/assays/hcp/phenolHIVTechnology.aspx.
8. For rat singles, Sprague-Dawley rats ($n = 3$), po: 10 mg/kg, iv: 2 mg/kg. For dog singles, Beagle dogs ($n = 2$), po: 1 mg/kg, iv: 0.5 mg/kg. For rat iv cassette (co-administration with other analogues): 0.25 mg/kg, For dog iv cassette (co-administration with other analogues): 0.125 mg/kg. Interanimal variability was less than 20%.