

# New HIV-1 reverse transcriptase inhibitors based on a tricyclic benzothiophene scaffold: Synthesis, resolution, and inhibitory activity

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**Abstract**—We synthesized, separated into enantiomers, and tested for the HIV-1 reverse transcriptase inhibitory activity a group of analogs of dimethyl-1-(1-piperidinyl)cyclobuta[*b*][1]benzothiophene-2,2a(7*bH*)-dicarboxylate (NSC-380292). Absolute configurations of the enantiomers were determined based on absolute X-ray structures and analysis of CD spectra. Within pairs of enantiomers the (*R,R*)-enantiomer was always much more potent HIV-1 reverse transcriptase inhibitor.

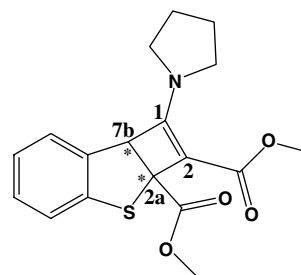
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The HIV-1 reverse transcriptase (RT) is one of the enzymes crucial for the HIV virus replication cycle. Upon entering a host cell, RT converts the single-stranded viral RNA into double-stranded DNA, which is then inserted into the genome of the host cell. RT has a DNA polymerase domain that can copy either RNA or DNA, and an RNase H domain that cleaves RNA. RNase H is required for the degradation of the RNA template after it has been copied into DNA, which then permits the synthesis of the second DNA strand by DNA polymerase. These enzymatic activities are essential for viral replication, therefore RT is an important target for anti-HIV drug development and its inhibitors are widely used as anti-HIV drugs.<sup>1</sup> Two major classes of HIV-1 RT inhibitors are currently used as anti-viral drugs. Nucleoside RT inhibitors (NRTIs) terminate DNA synthesis after their incorporation into the growing DNA chain. Non-nucleoside RT inhibitors (NNRTIs) bind to RT and inhibit its enzymatic activity.<sup>2–4</sup> Despite the presence of many RT inhibitors, a search for more suitable ones is still very important, because

of the appearance of a large number of drug-resistant mutants of HIV virus.<sup>5</sup>

During the screening of compounds from the NCI chemical repository for anti-HIV activity, we (Y. Zhang and V. Pathak) found that compound NSC-380292 (dimethyl-1-(1-pyrrolidinyl)cyclobuta[*b*][1]benzothiophene-2,2a(7*bH*)-dicarboxylate, Fig. 1) is a potent inhibitor of HIV-1 RT with an IC<sub>50</sub> = 1.24 μM and therapeutic index of over 800 (CC<sub>50</sub> > 1 mM).

To explore the relationship between the lead compound structure and the inhibitory activity, we synthesized



**Figure 1.** NSC-380292 (**1**) is a mixture of 2a*S*,7*bS*- and 2a*R*,7*bR*-enantiomers of dimethyl-1-(1-pyrrolidinyl)cyclobuta[*b*][1]benzothiophene-2,2a(7*bH*)-dicarboxylate.

**Keywords:** Reverse transcriptase inhibitors; Synthesis; Chiral separation; CD spectra; X-ray structures; RT inhibitory assay.

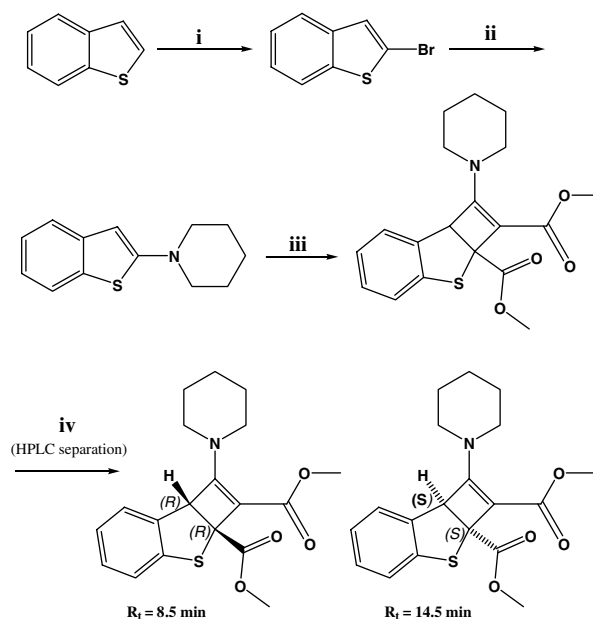
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several, closely related structural analogs of this compound (see Fig. 2).

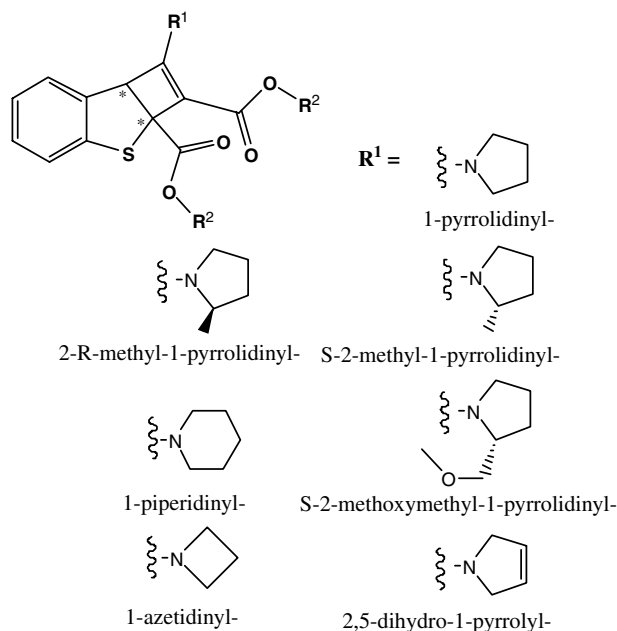
There are two chiral centers in this molecule, but because of the compound structure, only one pair of enantiomers can exist. The tested repository sample was a mixture of both enantiomers, so we separated them using a chiral HPLC column and tested for HIV-1 RT inhibitory activity.

Most synthesized analogs were also separated into enantiomers and tested in homochiral forms. The absolute configurations of all compounds were determined using X-ray crystallography and CD spectroscopy.

**Synthesis and chiral separation.** The synthesis of compound NSC-380292 and analogs is presented in Scheme 1 (except compound 10, see Scheme 2). The commercially available thionaphthene (benzo[*b*]thiophene) was used as a starting material. The selective bromination at the position 2 was performed by treatment of thionaphthene with *n*-BuLi followed by addition of Br<sub>2</sub>.<sup>6,7</sup>



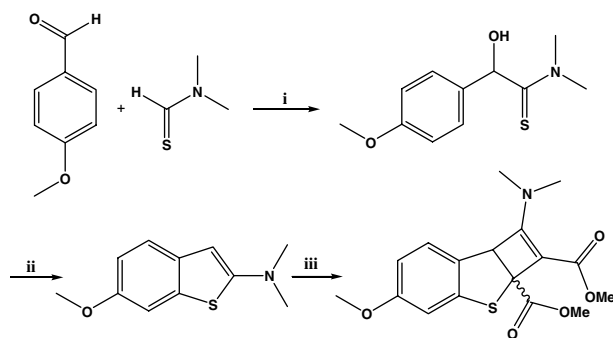
**Scheme 1.** The synthesis of NSC-380292 analogs (compound 7 as an example). Reagents and conditions: (i) 1—*n*-BuLi in hexane/Et<sub>2</sub>O, 2—Br<sub>2</sub>, (66%); (ii) piperidine, Pd<sub>2</sub>(dba)<sub>3</sub>, (±)BINAP, *t*-BuONa in toluene, under argon (26 h, 80 °C, 41%); (iii) dimethyl acetylenedicarboxylate in acetonitrile (22 h, rt).



Compounds		
No	R <sup>1</sup>	R <sup>2</sup>
1	1-pyrrolidinyl-	Me
2	1-pyrrolidinyl-	Et
3	1-pyrrolidinyl-	tBu
4	R-2-methyl-1-pyrrolidinyl-	Me
5	S-2-methyl-1-pyrrolidinyl-	Me
6	S-2-methoxymethyl-1-pyrrolidinyl-	Me
7	1-piperidinyl-	Me
8	1-azetidiny-	Me
9	2,5-dihydro-1-pyrrolyl-	Me
10	dimethylamino-*	Me

\* 5-methoxy-, see Scheme 2

**Figure 2.** Modifications of R<sup>1</sup> and R<sup>2</sup> groups in the synthesized analogs of NSC-380292.



**Scheme 2.** The synthesis of compound 10. Reagents and conditions: (i) *i*-Pr<sub>2</sub>NH, *n*-BuLi in THF/hexane (−78 °C → 0 °C, 2.5 h, 30%); (ii) MsOH in DCM (2.5 h, rt 65%); (iii) dimethyl acetylenedicarboxylate in acetonitrile (22 h, rt).

2-Bromobenzo[*b*]thiophene was used in the next step, palladium-catalyzed substitution of bromine by cyclic secondary amines.<sup>8</sup>

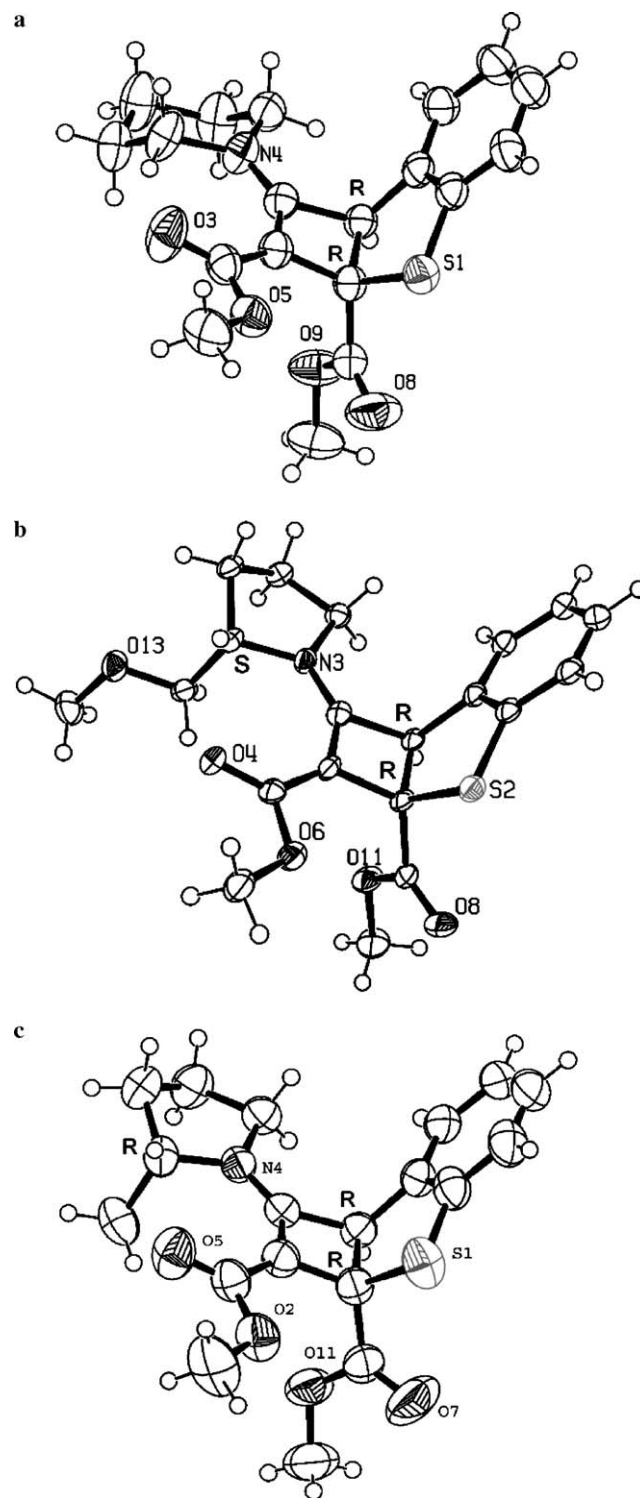
The last step of synthesis was the addition reaction between the benzo[*b*]thiophene derivative and dimethyl acetylenedicarboxylate, and a subsequent rearrangement.<sup>9</sup> This reaction was carried out in acetonitrile, at room temperature, for 22 h.

Compound 10 was synthesized starting from commercially available anisaldehyde and *N,N*-dimethylthioformamide.<sup>10</sup> The product of condensation of these compounds was converted into thionaphthene derivative by treatment with methanesulfonic acid in DCM.<sup>10</sup> The final step was the same as for the other compounds. The products were purified by a flash

chromatography. The products of the rearrangement were mixtures of two stereoisomers (with *S,S* and *R,R* absolute configurations at the chiral bridgehead carbon atoms). We used preparative HPLC on a chiral column<sup>11</sup> to obtain homochiral compounds. Fractions with shorter retention times were denoted **i** (e.g., **1i**), fractions with longer retention times **ii** (e.g., **1ii**). We were unable to separate mixture of **3**. The purity of final products was confirmed by <sup>1</sup>H NMR and MALDI-TOF-MS (Kratos Axima-CFR instrument, matrix:  $\alpha$ -cyano-4-hydroxycinnamic acid). The observed monoisotopic masses, measured for the mixtures of stereoisomers and for the homochiral compounds after chiral HPLC, were in good agreement with theoretical masses.

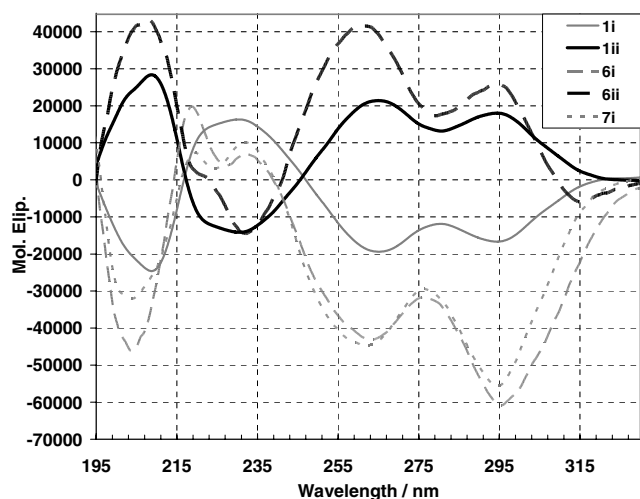
**Determination of absolute configuration.** For three of the homochiral products, the structures and the absolute configurations were determined by X-ray crystallography (see Fig. 3). All three compounds were found in the early eluting fractions (**4i**, **6i**, and **7i**) obtained after preparative HPLC of **4**, **6**, and **7**, on Chiralcel OD column. Monocrystals for the X-ray structure determination were obtained by a slow isothermal crystallization from cyclopentane/toluene (**4i**), toluene (**7i**), or Et<sub>2</sub>O/hexane (**6i**). The absolute configurations of **4i** and **7i** were determined using an anomalous dispersion, based on the presence of sulfur atom in these molecules. The absolute configurations on bridgehead carbon atoms in **6i** were assigned based on a known configuration of the third chiral center (from *S*-2-methoxymethylpyrrolidine). For all X-ray structures, the determined absolute configurations were *R* on both bridgehead carbons. Full crystallographic details have been deposited with Cambridge Crystallographic Data Center (deposition numbers: 286459 for **4i**, 286458 for **6i**, and 286457 for **7i**). A comparison of CD spectra (see Fig. 4) of **1i**, **6i**, and **7i** with spectra of **1ii**, **6ii**, and **7ii** showed that signs of four strong Cotton effects around 295, 260, 230, and 205 nm are determined by the configurations on the chiral bridgehead carbon atoms (**7ii** omitted for clarity). For the *R,R* configurations (**1i**, **6i**, and **7i**) the Cotton effect around 230 nm is positive and the other Cotton effects are negative. For the *S,S* configurations (**1ii**, **6ii**, and **7ii**) the signs of all these Cotton effects are opposite. The comparison of these data with CD spectra of other homochiral products allowed us to assign the absolute configuration for all isomers (for clarity we show only a superposition of five CD spectra). For all compounds, the first fractions from the chiral HPLC (**1i**, **4i**, **5i**, **6i**, **7i**, **8i**, **9i**, and **10i**) have *R,R* configurations on the chiral bridgehead carbon atoms. The third center of chirality present in **4** and **6** is responsible for the modulation of intensity of the Cotton effect around 260 nm and an additional Cotton effect at 220 nm.

**Inhibition of HIV-1 replication.** The ability of these compounds to inhibit HIV-1 replication was determined (Table 1) as previously described.<sup>12</sup> Briefly, an HIV-1 vector that expresses the firefly luciferase reporter gene and all HIV-1 proteins except for the Env and Nef was used. The vector was cotransfected into 293 T-cells with pHCMV-G, which expresses the G-glycoprotein of vesicular stomatitis virus. Virus harvested from the



**Figure 3.** X-ray structures of (a) **7i**-*R,R* enantiomer of **7**; (b) **6i**-*R,R*-stereoisomer of **6**; (c) **4i**-*R,R*-stereoisomer of **4**.

transfected cells was used to infect target cells in the presence or absence of the compounds tested. The compounds were added to target cell cultures for 4 h prior to infection and were present for the next 48–72 h, at which point the ability of the compounds to inhibit viral replication was determined by measuring the amount of luciferase activity in the infected cells.



**Figure 4.** The CD spectra of compounds **1i** (solid gray line), **1ii** (solid black line), **6i** (dashed gray line), **6ii** (dashed black line) and **7i** (dotted gray line). The CD spectra first fractions from chiral HPLC (**1i**, **6i**, and **7i**) (gray lines) or *S,S* (black lines) reflect the absolute configurations at the bridgehead carbon atoms of the stereoisomers. Spectra were collected at 23 °C (0.2 mM in MeOH).

**Table 1.** Results of ex vivo HIV-1 RT inhibitory assay for compounds **1–10**, the *R,R* or *S,S* represent absolute configurations of chiral centers at 2a and 7b carbon atoms

No.	Compounds		IC <sub>50</sub> (μM)		
	R <sup>1</sup>	R <sup>2</sup>	<i>R,R/S,S</i>	<i>R,R</i>	<i>S,S</i>
1	1-Pyrrolidinyl-	Me	1.2	0.8	>20
2	1-Pyrrolidinyl-	Et	>20	>20	>20
3	1-Pyrrolidinyl-	<i>t</i> -Bu	>20	—	—
4	<i>R</i> -2-Methyl-1-pyrrolidinyl-	Me	—	0.5	11
5	<i>S</i> -2-Methyl-1-pyrrolidinyl-	Me	—	2.3	>25
6	<i>S</i> -2-Methoxymethyl-1-pyrrolidinyl-	Me	48	38	>50
7	1-Piperidinyl-	Me	8.9	7.7	>40
8	1-Azetidinyl-	Me	>10	—	—
9	2,5-Dihydro-1-pyrrolyl-	Me	5.1	—	—
10	Dimethylamino <sup>a</sup>	Me	50	—	—

<sup>a</sup> 5-Methoxy-, see Scheme 2.

*HIV-1 RT mutations that confer resistance to NNRTIs also confer resistance to NSC-380292.* The sensitivity of NSC-380292 to HIV-1 RT variants containing mutations associated with clinical resistance to NNRTIs was determined. Mutations K103N, Y181C, and Y188C are associated with high-level resistance in treated patients to NNRTIs nevirapine and efavirenz.<sup>13</sup> HIV-1 vectors that expressed the firefly luciferase gene and contained wild-type RT or mutant RTs containing the K103N, Y181C, and Y188C substitutions were cotransfected into 293 T-cells with pHCMV-G; viruses harvested from the transfected cells were used to infect target cells in the presence of increasing concentrations of nevirapine, efavirenz, or NSC-380292 and the IC<sub>50</sub> concentrations were determined 48–72 h after infection (Table 2).

It is clear from the study of the X-ray structures of the active reverse transcriptase inhibitors (**4**, **6**, and **7**), that

**Table 2.** Ex vivo sensitivity of wild-type and NNRTI-resistant HIV-1 RTs to nevirapine, efavirenz, and NSC-380292

No.	HIV-RT	IC <sub>50</sub> (μM)		
		Nevirapine	Efavirenz	NSC-380292
1	Wild-type	0.060	0.0008	1.2
2	K103N	5.750	0.0540	>30
3	Y181C	6.650	0.0060	>30
4	Y188C	0.860	0.0095	>30

these inhibitors take up a butterfly-like overall shape in their structure.<sup>14</sup> In this respect, this family of reverse transcriptase inhibitors resembles the other NNRTIs.<sup>15</sup> Our observations that mutations in HIV-1 RT associated with resistance to other clinically available NNRTIs strongly support the view that the NSC-380292 compound is an HIV-1 RT inhibitor and that it binds to the enzyme in a manner that is similar to other NNRTIs used to treat HIV-1 infected patients. The results of HIV-1 reverse transcriptase inhibitory assays for NSC-380292 (**1**) demonstrated that only one enantiomer (*R,R*; **1i**) is responsible for the sample inhibitory activity. This is also true for all analogues we tested in the homo-chiral form. The lack of activity of the analogs with R<sup>2</sup> other than Me (**2** and **3**) and a low tolerance for the changes of R<sup>1</sup> suggest that the lead compound (**1**) is a unique reverse transcriptase inhibitor. In this regard, it is found that a number of NNRTIs have been reported to directly inhibit the reverse transcriptase enzyme in an allosteric fashion by binding to a pocket near the polymerase active site.

The current structure/activity study provides an optimized structure, in compounds **1i** and **4i**, with *R* configurations at the chiral centers 2a and 7b.

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