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Novel indole-3-sulfonamides as potent HIV non-nucleoside reverse transcriptase inhibitors (NNRTIs)

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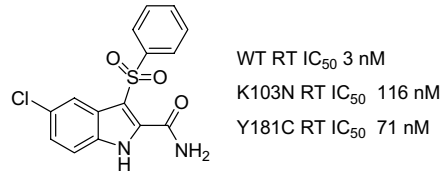
Abstract—This Letter describes the design, synthesis, and biological evaluation of novel 3-indole sulfonamides as potent non-nucleoside reverse transcriptase inhibitors (NNRTIs) with balanced profiles against common HIV RT mutants K103N and Y181C.

HIV reverse transcriptase (HIV-RT) plays an essential role in the life cycle of HIV replication. Three non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been introduced as a key class of drugs for the treatment of HIV infection (Viramune®, Rescriptor®, and Sustiva®) in highly active antiretroviral therapy (HAART).[1] However, the emergence of drug-resistant mutations in HIV-RT from NNRTI treatment raises an urgent need to discover and develop new agents with improved resistance profiles.[2] In this letter, we wish to report our efforts in the discovery of novel NNRTIs based on an indole core through an iterative analogue library approach.

In 1993, Williams and co-workers reported that 5-chloro-3-(phenylsulfonyl)indole-2-carboxamide (**1**, Fig. 1) was a potent NNRTI against wild-type enzyme.[3] Compound **1** also displays sub-micromolar activity against K103N and Y181C, two HIV-RT mutants observed clinically with high frequency. It has been demonstrated that the two oxygen atoms on the sulfone moiety are critical to maintain both the enzymatic and cellular activity of **1**. In recent years, other groups have reported the discovery of indole derivatives as NNRTIs.[4,5] In light of these encouraging results, we undertook an effort to identify NNRTIs from this series with an improved mutant profile.



1

Figure 1. Structure and HIV-RT inhibitory activity of **1**.

Our initial attempt was to replace the sulfone group in **1** with a sulfonamide group to generate compounds of general structure **7**. This change was motivated by docking studies of **1** and HIV-RT in which the indole 3-phenylsulfonyl group occupies a hydrophobic pocket formed by Y181, Y188, and W229;[5] we hypothesized that due to its close contact with Y181, the indole 3-substituent may affect activity versus the Y181C mutant. Earlier results demonstrated the necessity for substitution at the indole 5-position for optimal potency;[3] because of this requirement, and to provide a handle for further derivatization, 5-bromoindole analogues **7** were prepared in parallel.

Keywords: HIV; Antiviral; Indole; Sulfonamide.
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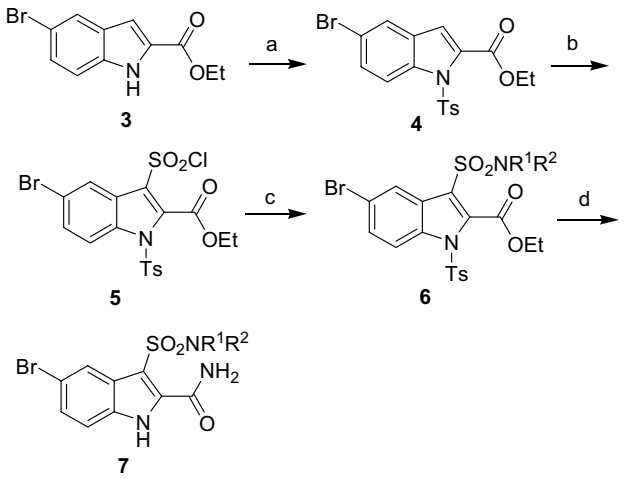


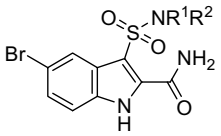
Scheme 1. Synthesis of indole-sulfonamide-amide **7** series. Reagents and conditions: (a) i—NaH, THF, rt, ii—TsCl, 70–80%; (b) SO_2Cl_2, DCM, rt, 90%; (c) NHR^1R^2, DIEA, DCM; (d) NH_3, MeOH, 110 °C, overnight, 60–90%. All compounds were purified by mass-directed HPLC.[7]

The synthesis of library **7** is illustrated in Scheme 1. Commercially available indole **3** was *N*-protected and converted smoothly to sulfonyl chloride **5** via treatment with SO_2Cl_2. Sulfonyl chloride **5** was then reacted with 48 amines in a parallel fashion to generate 48 sulfonamides represented by general structure **6**. Finally, concomitant deprotection of indole N1 and aminolysis of 2-carbonyl ester were executed in one pot to provide the sulfonamide library **7**. Selected compounds and their HIV-RT inhibition data are presented in Table 1.[6]

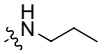
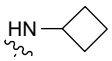
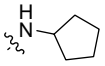
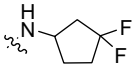
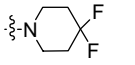
As shown in Table 1, both secondary and tertiary indole sulfonamides exhibit potent inhibition of wild-type HIV-RT ($IC_{50} < 55$ nM). Greater variation was observed for activity in a cell-based assay versus wild-type HIV (SPREAD) where cell culture inhibitor concentrations (CIC_{95}) are defined as those which inhibited 95% of the spread of HIV infection in susceptible cell cultures. Only the tertiary cyclic sulfonamides **7e** and **7f** displayed a small shift (<3) between enzyme inhibition activity (WT RT) and cell based activity (SPREAD, 10% FBS). Indeed, sulfonamide **7e** exhibited antiviral activity versus wild-type HIV equivalent to sulfone **1** indicating that the 3-pyrrolidine sulfonamide is an acceptable isosteric replacement for the 3-phenylsulfonyl group in **1**.

Keeping the indole-3-pyrrolidinosulfonamide constant, investigation of the indole 5-substituent revealed that Br, Cl, and CN were the preferred groups (Table 2); compounds in Table 2 were prepared via the route described in Scheme 1 (comparable yields were obtained) or via cross-coupling reactions of **7e**. Consistent with previous work,[3] 5-Cl (**8a**) is well tolerated and provides potent enzymatic and cellular activity versus wild type RT and virus. The nitrile analogue **8b** also exhibits high activity versus wild type RT; however, other substitutions are less well tolerated (e.g., **8c**–**8g**) including small non-polar groups (see **8c** and **8d**).

Table 1. Anti-HIV activities of indole sulfonamide amide library **7**



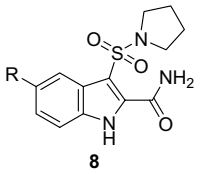
7

Compound	–NR^1R^2	WT RT IC_{50}[a] (nM)	SPREAD CIC_{95}[b] (nM)
7a		19	160
7b		0.5	125
7c		18	125
7d		14	125
7e		3.6	<7.8
7f		5.7	16
7g		0.2	140
7h		4.5	410

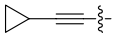
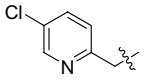
[a] IC_{50} values versus isolated RT enzyme.
[b] The values of CIC_{95} are defined as those which inhibited by 95% the spread of HIV infection in susceptible cell cultures in the presence of 10% FBS (fetal bovine serum).

Despite potent activity versus wild-type enzyme and virus compounds **7e**, **8a**, and **8b** displayed only weak inhibition against the mutant RT K103N (>2000 nM) regardless of variation at the sulfonamide moiety or indole 5-position. Analogues **7e** and **8b** suffer from comparatively weak Y181C RT activity as well. As a result, attention was turned to the indole 2-carboxamide to improve the mutant profile; simple secondary amide replacements have been reported to improve activity versus the Y181C and the Y181C/K103N double mutant.[4,5] Again the optimized indole-3-pyrrolidine sulfonamide **7e** was selected for further modification, and the synthesis of these analogues is illustrated in Scheme 2. Sulfonyl chloride **5** was converted to pyrrolidine sulfonamide **9**, before being subjected to parallel aminolysis of the ester and tosyl groups in **9** with 48 primary amines generating a secondary amide library **10**. Consistent with prior observations,[4,5] balanced inhibition of wild-type and mutant K103N and Y181C RT could be achieved by extending the 2-carboxamide substituent (Table 3). In particular, benzamide analogues bearing *ortho* substituents (e.g., **10b** and**10c**) exhibit <5-fold shifts in their wild-type and mutant enzyme potencies.

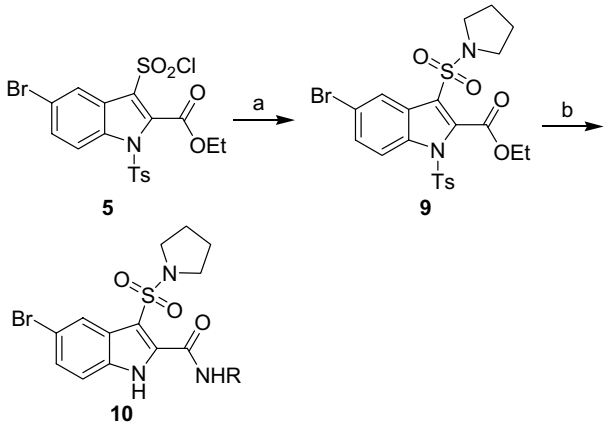
Table 2. Anti-HIV activities of pyrrolidine sulfonamides **8**



8

Compound	R	WT RT IC_{50}[a] (nM)	SPREAD CIC_{95}[b] (nM)	K103N RT IC_{50}[a] (nM)	Y181C RT IC_{50}[a] (nM)
8a	Cl	3.9	<7.8	1963	48
8b	CN	9.1	16	2300	>10000
8c		940	160	nd	nd
8d		430	630	nd	nd
8e	Ph	550	1300	nd	nd
8f		2200	310	nd	nd
8g		59	nd	8200	>10000

[a] IC_{50} values versus isolated RT enzyme.
[b] The values of CIC_{95} are defined as those which inhibited by 95% the spread of HIV infection in susceptible cell cultures in the presence of 10% FBS (fetal bovine serum).



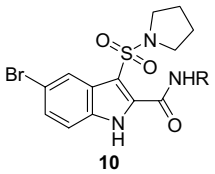
Scheme 2. Synthesis of indole-sulfonamide-amide **10** series. Reagents and conditions: (a) pyrrolidine, DCM, (b) RNH_2, *i*-PrOH, 110 °C, overnight, 60–90%. All compounds were purified by mass-directed HPLC.[7]

As they retain potent cellular activity, **10b** and **10c** represent the best balance among cellular, wild-type, and mutant activity within the amide series.

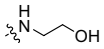
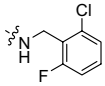
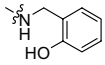
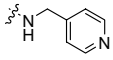
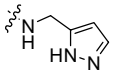
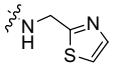
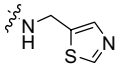
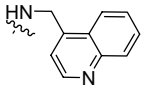
Despite this balanced profile, **10b** and **10c** exhibit less than optimal physical properties and high clearance in animal pharmacokinetic studies. In an effort to improve these properties, the 2-carboxamide moiety in library **10** was replaced with an amide surrogate, an approach previously reported by Young and co-workers within the indole sulfone series.[8] This work demonstrated that replacing the carboxamide in **1** with an imidazole ring could not only improve physical properties but also boost inhibition of K103N RT (Fig. 2). Synthesis of a library of 2-(imidazol-2-yl)-indoles **13** is shown in Scheme 3.

Deprotection of **9** generated the free indole ester **11** which was reduced ($LiAlH_4$) and reoxidized (MnO_2) to provide indole-2-carboxaldehyde **12**. Conversion of **12** to imidazoles **13** was achieved under microwave heating conditions in the presence of NH_4OH and various 2-ketoaldehydes or phenyldiamines. Imidazoles bearing various substituents were prepared and in general minimal effect on wild-type RT enzyme inhibition was observed (Table 4). However, significant effects on mutant enzyme inhibition were found. In accord with results in the indole sulfone series, >20-fold improvement in K103N enzyme inhibition was observed (**7e** versus **13a**). A more modest 5-fold improvement was observed in Y181C RT potency (**7e** versus **13a**), although Y181C potency was strongly dependent on substitution on the imidazole ring with a trend toward decreasing activity with increasing steric bulk of the substituent (compare

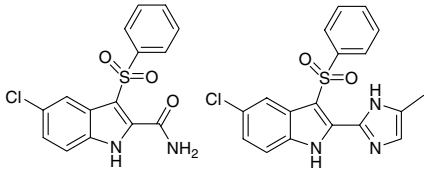
Table 3. Antiviral activities of indole sulfonamide amide library **10**



10

Compound	–NHR	WT RT IC_{50}[a] (nM)	SPREAD CIC_{95}[b] (nM)	K103N RT IC_{50}[a] (nM)	Y181C RT IC_{50}[a] (nM)
7e		3.6	<7.8	10000	120
10a		15	625	2800	6.9
10b		3.8	25	20	16
10c		2.4	23	31	9.4
10d		14	<7.8	170	6.3
10e		2.5	<7.8	260	829
10f		2.8	<7.8	280	200
10g		6.2	16	93	6.0
10h		3.1	82	25	63

[a] IC_{50} values versus isolated RT enzyme.
[b] The values of CIC_{95} are defined as those which inhibited by 95% the spread of HIV infection in susceptible cell cultures in the presence of 10% FBS (fetal bovine serum).

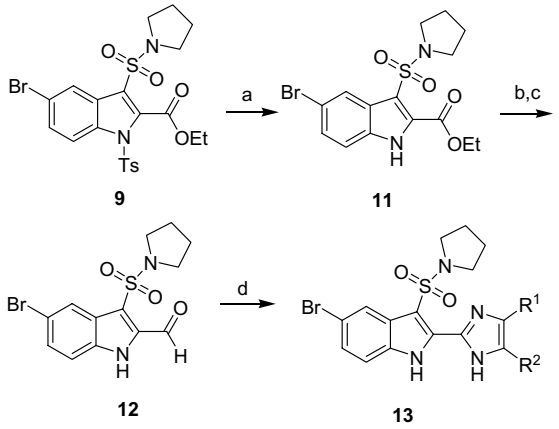


Compound	**1**	**2**
WT RT IC_{50} (nM)	3	5
SPREAD CIC_{95} (nM)	3	12
K103N RT IC_{50} (nM)	116	9

Figure 2. Structure and antiviral activity of **1** and **2**.

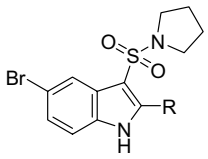
13a with **13b** and **13d**). Comparison of **13f** and **13h** suggests there may be an electronic component to this substituent effect as well.

An X-ray co-crystal structure of wild type RT-bound **7e** was obtained at 2.4 Å resolution which provides insight

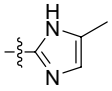
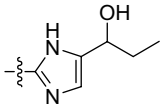
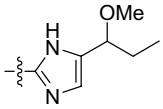
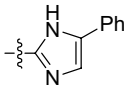
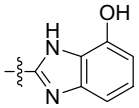
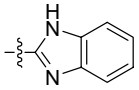
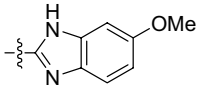
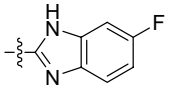
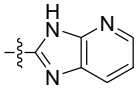


Scheme 3. Synthesis of imidazole library **13**. Reagents and conditions: (a) PS-trisamine, DCM, 45 °C 70–80%; (b) $LiAlH_4$, THF, 0 °C, 80%; (c) MnO_2, THF, rt, 70–85%; (d) RCOCHO or phenyldiamine, NH_4OH, EtOH, microwave, 70 °C, 20–65%. All compounds were purified by mass-directed HPLC.[7]

Table 4. Antiviral activities of indole sulfonamideimidazole **13**



13

Compound	R	WT RT IC_{50}[a] (nM)	SPREAD CIC_{95}[b] (nM)	K103N RT IC_{50}[a] (nM)	Y181C RT IC_{50}[a] (nM)
13a		3.1	<7.8	42	25
13b		8.8	50	62	2600
13c		16	100	110	240
13d		11	67	150	>9100
13e		10	82	48	810
13f		6.9	78	280	38
13g		45	400	150	5100
13h		15	280	220	5300
13i		40	82	370	5000

[a] IC_{50} values versus isolated RT enzyme.
[b] The values of CIC_{95} are defined as those which inhibited by 95% the spread of HIV infection in susceptible cell cultures in the presence of 10% FBS (fetal bovine serum).

into the binding interactions of the inhibitor (Fig. 3).[9] The overall structure of the enzyme is consistent with previously described NNRTI/HIV-RT complexes, where a conformational change results in the formation of the characteristic NNRTI binding pocket occupied by **7e**. The newly formed binding pocket consists of aromatic residues (Y181, Y188, F227, Y318), aliphatic residues (L100, L234), and two polar interactions between **1**) the indole nitrogen and the carbonyl of K101 and **2**) the amide carbonyl oxygen and the nitrogen backbone of K101. An intramolecular H-bond is also observed between the indole 2-carboxamide NH and an oxygen of the sulfonamide in **7e**. This arrangement provides a structural basis for the necessity for a sulfone in inhibitors within this series: in addition to stabilizing a butterfly-like conformation characteristic of NNRTIs,[10] the sulfone increases the acidity of the indole NH permitting the formation of a hydrogen bond with K101. Additionally, a hydrogen bond between K101 and E138 is observed. X-ray crystal structures of related inhibitors (**1** and **2**) reveal a nearly identical binding pocket, position of inhibitor, and orientation of binding site side chains.

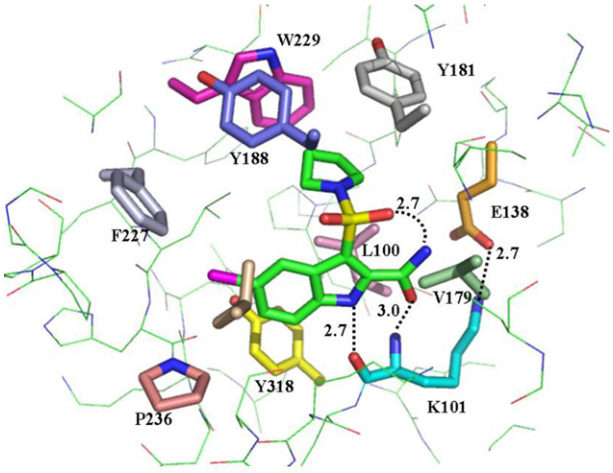


Figure 3. X-ray co-crystal structure of **7e** bound to wild-type RT.

These insights notwithstanding, the highly flexible nature of the NNRTI binding site[11] makes rationalization of inhibitor activity versus mutant RTs challenging. As such, optimization of mutant RT inhibitors (e.g., in the cases of **10b**, **10c**, and **13a** versus **7e**) was empirically driven.

In summary, a new series of NNRTIs bearing an indole-3-sulfonamide has been described. Introduction of a pyrrolidine sulfonamide at the 3-position of the indole ring generated analogues with excellent wild-type HIV-RT potency and comparatively weak K103N and Y181C RT activities. Variation in the indole 2-substituent (to provide analogues **10b**, **10c**, and **13a**) improved mutant activities and resulted in optimized inhibitors with balanced wild-type, K103N, and Y181C RT inhibition which retain potent cellular activity. Further results in this series will be reported in due course.

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