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Bioorganic & Medicinal Chemistry Letters 16 (2006) 2105–2108
Bioorganic & Medicinal Chemistry Letters

Design, synthesis and biological evaluations of novel oxindoles as HIV-1 non-nucleoside reverse transcriptase inhibitors. Part I

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Received 29 November 2005; revised 17 January 2006; accepted 17 January 2006
Available online 9 February 2006

Abstract—A novel oxindole was discovered as an HIV non-nucleoside reverse transcriptase inhibitor via HTS using a cell-based assay. Systematic structural modifications were carried out to establish its SAR. These modifications led to the identification of oxindoles with low nanomolar potency for inhibiting HIV replication. These novel and potent oxindoles could serve as advanced leads for further optimizations.

HIV-1/AIDS is a global health threat and the leading cause of deaths due to infectious disease. More than 20 million people have died of AIDS since the first reported cases in 1981, with 3.1 million deaths in 2004 alone (UNAIDS/WHO). Rates of new HIV infections in developing countries continue to climb at an alarming rate, largely due to the high cost of the current drug regimen. For HIV-infected patients undergoing highly active antiretroviral therapy (HAART), mortality rates have significantly declined. However, treatment failures remain high due to side effect-driven compliance issues that in turn drive the evolution of drug-resistant viruses and regimen failure.[1,2] Non-nucleoside reverse transcriptase inhibitors (NNRTI) are among the most commonly prescribed drugs for antiviral therapy and are often used as first line therapy. To date, three NNRTIs have been approved for clinical use including nevirapine (Viramune®),[3] delavirdine (Rescriptor®),[4] and efavirenz (Sustiva®).[5] However, these and all other approved drugs that target viral gene products induce drug-resistant variants of HIV-1.[6,7] It is clear that more effective, safer, and economical drugs targeting drug-resistant virus are needed in order to control the spread of the disease.

Using a cell-based HIV reporter infection assay, a screening hit, oxindole **1** (Figure 1), was identified with ~75 nM EC_{50}. Compound **1** is an oxindole with a unique spiral cyclopropane moiety. Since the relative stereochemistry around the cyclopropane moiety was not known, we first carried out resynthesis to establish its structure and confirm the activity. The synthesis of **1** is shown in Scheme 1.

Treatment of isatin **2** with Wittig reagent **3** leads to the formation of two stereoisomers **4** and **5** in a 5:1 ratio with a combined yield of 75%. Olefin **4** was first treated with diazomethane, and the resulting crude mixture was then brought to reflux in toluene to give oxindole **1** in 67% yield.[8] Similarly, isomer **6** was prepared from olefin **5** in a comparable yield. The stereochemistry of compounds **4**–**6** and **1** was established based on NOE studies. While **1** exhibited a 50 nM EC_{50} in the cell-based assay, its diastereoisomer **6** was completely inactive, affirming that the antiviral activity of **1** is due to its specific interactions

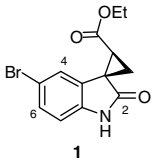

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Figure 1.

Keywords: AIDS; HIV; Reverse transcriptase; Inhibitor; Oxindole; SAR.
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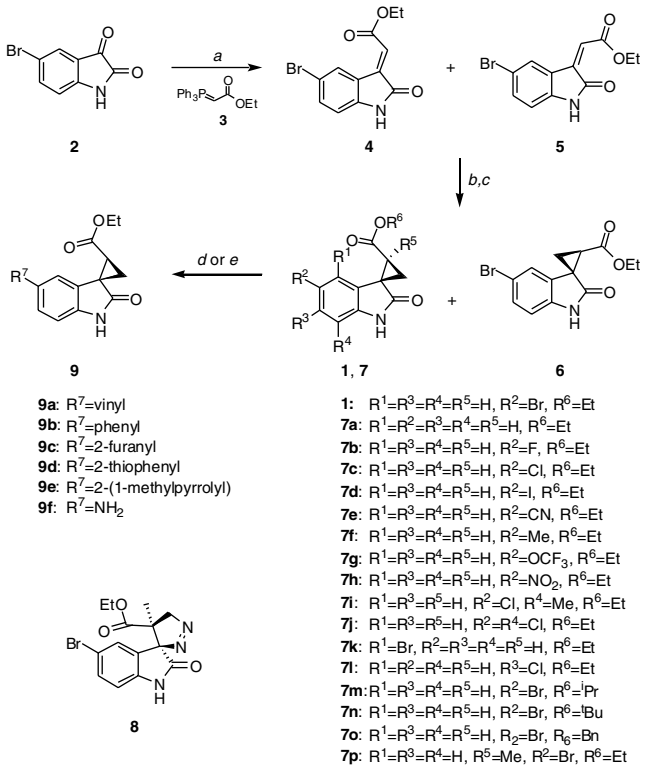
doi:10.1016/j.bmcl.2006.01.073



Scheme 1. Synthesis of oxindoles **1**, **4**–**9**. Reagents and conditions: (a) **3** (1.2 equiv), benzene, 80 °C, 4 h, 76%; (b) CH_2N_2, Et_2O, 0–25 °C, 5 h; (c) toluene, reflux, 8 h, 67% over 2 steps; (d) for **9a**–**9e**: **1** (1.0 equiv), $^nBu_3SnR^7$ (1.4 equiv), $Pd(PPh_3)_2Cl_2$ (cat), DMF, 25 °C, 16 h, 63–88%; (e) for **9f**: **7h** (1.0 equiv), H_2, 10% Pd/C (cat), 25 °C, 2 h, 78%.

with the reverse transcriptase. Oxindole **1** could be effectively docked into the HIV-1 NNRTI site as shown in Figure 2. The key binding features of the molecule **1** are the hydrogen bond between NH of the oxindole ring and the backbone oxygen of K101, and a placement of the ester into the hydrophobic pocket outlined by residues P95, L100, Y181, Y188, W229, and L234.

With the stereochemistry established and activity confirmed, we set out to conduct systematic structural modifications to establish its SAR. Using the established chemistry, analogs with different substituents on the aromatic ring were synthesized (Scheme 1, **7a**–**7l**, **9a**–**9f**).

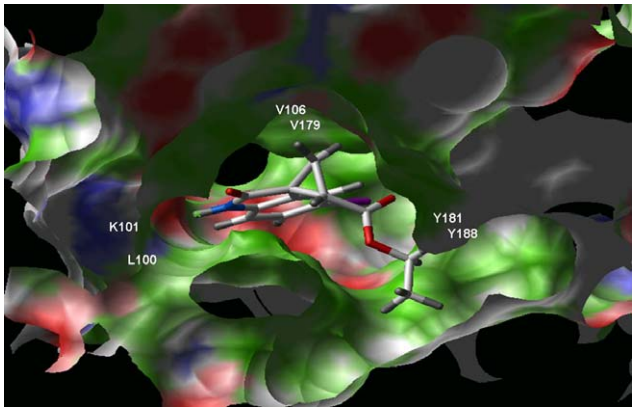


Figure 2. Docking of oxindole **1** into the HIV-1 NNRTI site.[9]

Analogs with different ester moieties (**7m**–**7o**) were also synthesized in a similar fashion. Similar chemistry was also utilized to synthesize the tetra-substituted cyclopropane analog **7p**. Interestingly, a diazo intermediate **8** could be isolated after the initial reaction with diazomethane, which subsequently lost N_2 to yield the cyclopropane. To further explore the SAR around the 5 position of the oxindole, compound **1** was allowed to couple with several stannanes under the Stille conditions, giving rise to the corresponding coupled products **9a**–**9e**. Additionally, nitro analog **7h** was hydrogenated to give the corresponding anilino analog **9f**.

These analogs were tested in the aforementioned cell-based HIV infection assay, and their antiviral activities are shown in Table 1. Removal of Br, or its replacement with a smaller F (**7a** and **7b**), led to significantly reduced antiviral activities. On the other hand, replacement of Br with electron-withdrawing Cl and CN gave analogs **7c** and **7e** with similar antiviral activities. The vinyl analog **9a** showed slightly reduced activity (EC_{50} = 126 nM). Any other substitutions examined, whether larger or smaller, electron-donating or -withdrawing, gave analogs (**7d**, **7f**, **7g**, **7h**, and **9c**–**9f**) with significantly reduced activities. These data suggest that there is a limited space for substitution at position 5, and a small hydrophobic moiety (Cl, Br, CN or vinyl) is required for optimal interactions. Several analogs with substitutions at other positions of the aromatic ring (**7i**–**7l**) were also examined, and all of these analogs were inactive, suggesting that there is a very limited space for substitution at positions 4, 6, and 7.

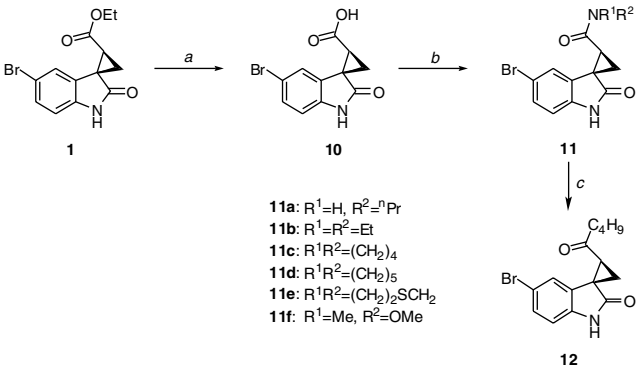
While the isopropyl ester analog **7m** showed comparable antiviral activity, the bulkier *tert*-butyl ester analog **7n** exhibited slightly improved activity. However, the extended benzyl ester analog **7o** showed only weak activity, suggesting that the target has a somewhat defined hydrophobic pocket to accommodate the changes in this region. Free acid **10** showed only micromolar inhibition for HIV reporter virus. These results are consistent with the molecular modeling studies which suggest that the reverse transcriptase has a medium-size hydrophobic pocket for this region of the molecule (Fig. 2).

Continuing the exploration of the ester region in **1**, a series of amides (**11a**–**11f**) were prepared from acid **10** and various amines (Scheme 2). Among these analogs, the pyrrolidinyl and thiazolidinyl amides (**11c** and **11e**) showed the highest antiviral activities with low submicromolar potency. The primary amide **11a** was completely inactive, which was presumably due to the presence of an additional amide NH. The diethyl amino analog **11b** exhibited significantly reduced antiviral activity compared to **1**, which is likely due to increased steric bulk. The smaller but more polar methoxymethyl amino analog **11f** also displayed submicromolar potency (Table 1). Treatment of **11f** gave rise to ketone **12**, which had activity similar to that of **11f**. The decrease in antiviral activities was likely due to their increased polarity in this region which would lead to unfavorable interactions with the reverse transcriptase as suggested by the molecular modeling studies (Fig. 2).

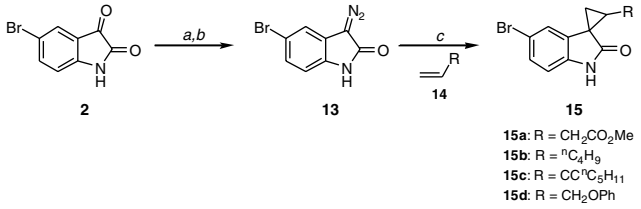
Table 1. Anti-HIV activities of oxindoles[13,14]

Compound	EC_{50} (μM)	CC_{50} (μM)
Efavirenz	0.0005	~10
Nevirapine	0.050	>10
1	0.066	>10
4	2.276	>10
5	>10	>10
6	>10	>10
7a	0.279	>10
7b	0.289	>10
7c	0.059	>10
7d	0.486	>10
7e	0.066	>10
7f	1.095	>10
7g	2.234	>10
7h	1.031	>10
7i	2.252	>10
7j	7.363	>10
7k	>10	>10
7l	>10	>10
7m	0.059	>10
7n	0.040	>10
7o	5.578	>10
7p	33.296	>10
9a	>10	>10
9b	0.126	>10
9c	0.608	2.194
9d	2.651	1.938
9e	0.358	8.062
9f	2.702	1.990
10	7.629	>10
11a	>10	>10
11b	1.421	>10
11c	0.191	>10
11d	0.586	>10
11e	0.180	>10
11f	0.656	>10
12	0.620	>10
15a	>10	>10
15b	0.197	>10
15c	0.463	6.625
15d	5.624	>10
16	>10	>10
17	>10	>10
20	>10	>10
22a	0.015	>10
22b	0.030	>10
22c	1.210	>10
22d	>10	>10
22e	1.459	>10

To further explore the SAR around the ester region, several analogs lacking the carbonyl moiety were prepared according to Scheme 3. Isatin **2** was first reacted with tosyl hydrazine to the corresponding tosyl hydrazone and then was treated with sodium hydroxide to give the diazo lactam **13**.[10] Under rhodium catalysis, diazo **13** reacted with olefin **14** to furnish the corresponding cyclopropane analog **15** in moderate yields.[11,12] Compounds **15a**–**15d** were typically obtained as inseparable mixtures of two diastereoisomers (in a ratio of roughly 4:1), which were directly assayed for their antiviral activity. The homolog **15a** and the bulky phenoxy analog **15d** exhibited diminished activities. The straight-chain analogs **15b** and **15c** also displayed substantially reduced



Scheme 2. Synthesis of oxindoles **11a**–**11f** and **12**. Reagents and conditions: (a) NaOH (1.0 M), MeOH, 25 °C, 8 h, 93%; (b) EDCI (1.2 equiv), RR′NH (1.2 equiv), CH_2Cl_2, 25 °C, 16 h; (c) from **11f**: ^{n}BuMgCl (2.5 equiv), −78 to 25 °C, 4 h, 74%.

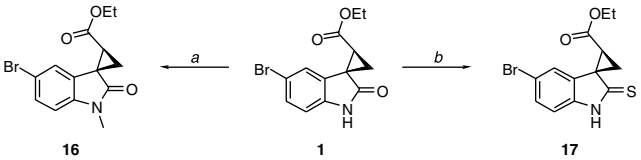


Scheme 3. Synthesis of oxindoles **15a**–**15d**. Reagents and conditions: (a) $TsNHNH_2$ (1.1 equiv), MeOH, reflux, 4 h, 95%; (b) NaOH (4.0 M), H_2O, 25 °C, 6 h, 90%; (c) **14** (5.0 equiv), $Rh(OAc)_2$ (cat), CH_2Cl_2, 25 °C, 16 h, 30–50%.

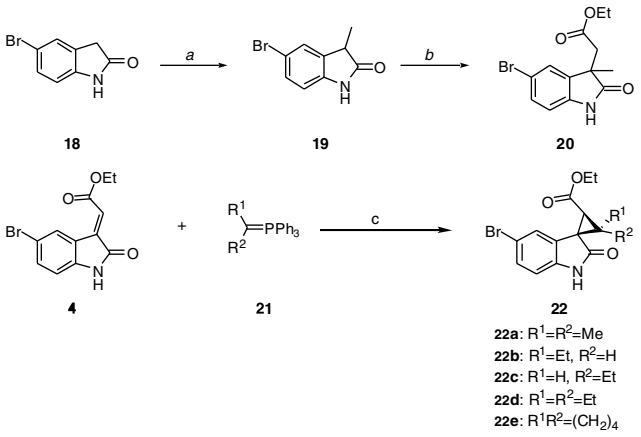
activity. These results suggested that the carbonyl moiety is important in anchoring the molecule for effective interactions with the target as predicted by the modeling studies.

To address the role of the lactam moiety, the *N*-methyl analog **16** and the thiolactam **17** were synthesized from **1** via methylation and treatment with Lawesson's reagent (Scheme 4). As expected, both compounds were inactive against the HIV reporter virus, suggesting that both the NH and the carbonyl oxygen moieties are critical for the antiviral activities. These results are in agreement with the modeling studies, which indicate that the lactam carbonyl forms a critical hydrogen bond with K101 of the reverse transcriptase, and there is no space for substitution on the lactam nitrogen (Fig. 2).

The cyclopropane-ring-opened analog **20** was prepared from lactam **18** via sequential alkylations with methyl iodide and ethyl bromoacetate (Scheme 5). Oxindole **20** was found to be completely inactive in the antiviral



Scheme 4. Synthesis of oxindoles **16** and **17**. Reagents and conditions: (a) NaH (2.0 equiv), MeI (2.0 equiv), DMF, 25 °C, 1 h, 94%; (b) Lawesson's reagent (3.0 equiv), toluene, reflux, 6 h, 85%.



Scheme 5. Synthesis of oxindoles **20**, **22a**–**22e**. Reagents and conditions: (a) ^{n}BuLi (2.0 equiv), TMEDA (2.0 equiv), MeI (1.0 equiv), 25 °C, 8 h, 65%; (b) ^{n}BuLi (2.0 equiv), TMEDA (2.0 equiv), $BrCH_2CO_2Et$ (1.0 equiv), 25 °C, 8 h, 60%; (c) **21** (2.0 equiv), THF, −78 to 25 °C, 16 h, 45–68%.

assay. Additionally, neither the *E* nor the *Z* olefin analog had any antiviral activity. These data demonstrated a critical role of the cyclopropane moieties in optimizing the molecules for effective interactions. Various substituents were placed on the cyclopropane in order to further explore the SAR around the cyclopropane region. The synthesis of these analogs (**22a**–**22e**) is shown in Scheme 5, which involves the cyclopropanation of olefin **4** with the carbene precursor **21**. Among these analogs, the gem-dimethyl analog **22a** exhibited significantly improved potency with an EC_{50} of 15 nM. While one of the ethyl analogs, **22b**, showed a 30 nM EC_{50} in the HIV infection assay, its diastereoisomer, **22c**, had significantly diminished potency (EC_{50} = 1.2 μM). The cyclcopentane analog **22e** also showed similar activity to **22c**. However, the bulkier analog, **22d**, completely lost antiviral activity, indicating a limited hydrophobic pocket to accommodate this part of the molecule.

While the gem-dimethyl analog **22a** has significantly improved potency, analog **7p**, which has a methyl substitution on the other carbon of the cyclopropane moiety, was completely inactive. These results suggested that the cyclopropane region is sensitive to small perturbation.

In summary, a series of novel oxindole was discovered as a potent non-nucleoside reverse transcriptase inhibitor using HTS. Modeling was utilized to aid the design of new analogs and the detailed understanding of ligand–target interactions. Systematic modifications of the lead molecule not only defined its SAR, but also led to the identification of increased antiviral activity targeting the early stages in HIV infection.

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14. *Antiviral Efficacy Assay:* Compounds were serially diluted in DMSO and added to adherent HEK293T target cells prior to addition of VSV-g pseudotyped HIV-1 luciferase reporter virus (HIV-1 pseudovirions) harvested from 293T producer cells following a triple transient transfection (CaP, Clontech) of the three plasmid HIV-1 lentiviral vector system comprised of the VSV-g envelope expression plasmid, packaging construct (delta psi), and the HIV-1 LTR:Luc plasmid. The VSV-g envelope expression plasmid generates the pseudovirus receptor that permits a broad tropism and mediates entry into the 293T target cells. The delta psi packaging construct supplies all of the structural and regulatory gene products needed to generate the pseudovirus. The viral vector RNA synthesized from the HIV-1 LTR:Luc plasmid possesses the *cis* RNA packaging signal (psi sequence) in addition to the luciferase reporter gene and the HIV-1 LTR. The supernatants of transfected producer cells contain HIV-1 pseudovirions carrying only the luciferase gene in the viral genome. Upon transduction of the target 293T cells, the viral genomic RNA will undergo reverse transcription, nuclear translocation, integration, and transcription of the integrated luciferase gene driven by the PGK (phosphoglycerate kinase promoter). Luciferase activity using Bright-Glo reagent (Promega) substrate was measured 48 h post-infection using a CLIPR plate reader (Molecular Devices) to determine EC_{50} values.