Synthesis and HIV-1 Integrase Inhibition Activity of some *N*-Arylindoles

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Eight simple *N*-arylindoles were designed, synthesized and evaluated as human immunodeficiency virus (HIV)-1 integrase inhibitors *in vitro* for the first time. Among these compounds, 3b, 3e and 3g demonstrated significant anti-HIV-1 integrase activity. Especially 3b showed the highest anti-HIV-1 integrase activity with EC_{50} value of 7.88 μg/ml and TI value of 24.61. Meantime, some structure–activity relationships were also observed and will provide a new lead for design and discovery of more potent *N*-arylindoles as HIV-1 integrase inhibitors.

Key words *N*-arylindole; anti-human immunodeficiency virus type 1; integrase inhibitor; synthesis

In the past two decades, a worldwide search has been made for new chemotherapeutic agents targeting the human immunodeficiency virus (HIV). However, many drugs have only limited or transient clinical benefit due to their side effects and the development of virus–drug resistance.[1)] Therefore, the development of new, selective and safe HIV-1 integrase inhibitors still remains a high priority for medical research. *N*-Arylindoles are central structural scaffolds in many pharmacologically important and bioactive molecules, which display antiestrogen,[2)] analgesic,[3)] neuroleptic,[4)] antiallergy,[5)] 5-HT_6 receptor antagonists,[6)] and FTase inhibitors (FTIs) activity.[7)] Although Merino *et al.* reported that a set of pyrimido[5,4-*b*]indole derivatives possess anti-HIV-1 activity,[8)] to the best of our knowledge, little attention has been paid to the anti-HIV-1 integrase activity of the single *N*-arylindoles, with low-molecular weight. As part of our program aimed at the discovery and development of bioactive molecules,[9—11)] herein we report the synthesis and anti-HIV-1 integrase activity of some single *N*-arylindoles with various functional groups.

Results and Discussion

Eight simple *N*-arylindoles **3a**—**h** (Fig. 1) were prepared successfully by cross-coupling various indoles with activated fluoroarenes *via* nucleophilic aromatic substitution (S_NAr) reactions as shown in Chart 1, and were characterized by ^{1}H-NMR (400 MHz), HR-MS or elemental analysis, EI-MS and melting point. Subsequently, the *N*-arylindoles **3a**—**h** were tested *in vitro* for their anti-HIV-1 integrase activity (EC_{50}) and cytotoxicity (CC_{50}) in cell-based assays against HIV-1 integrase replication in acutely infected C8166 cells and C8166 cells, respectively. In addition, the therapeutic index (TI) was also calculated as shown in Table 1. 3′-Azido-3′-deoxythymidine (AZT) was used as a positive control.

As indicated in Table 1, among these compounds, **3b**, **3e** and **3g** showed the more potent anti-HIV-1 integrase activity with EC_{50} values of 7.88, 11.24 and 19.22 μg/ml, and TI values of 24.61, 9.48 and 8.26, respectively. Especially **3b** exhibited the most potent and promising anti-HIV-1 integrase activity (TI=24.61). On the contrary, compounds **3d** and **3h** showed lower TI values (1.90 for **3d**, and 1.28 for **3h**) and higher cytotoxicity (5.40 μg/ml for **3d**, and 14.41 μg/ml for **3h**) when compared with the others.

From the comparative study, it is possible to draw some structure–activity relationships as shown in Table 1. The cytotoxicity (CC_{50}), anti-HIV-1 integrase activity (EC_{50}) and TI values of **3a** and **3b** were 91.44/191.9 μg/ml, 35.82/7.88

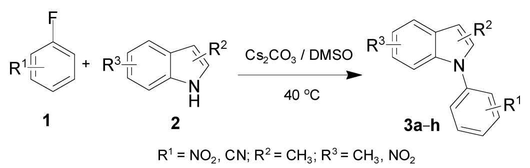

Chart 1. The Synthetic Route of *N*-Arylindoles **3a**—**h**

Table 1. Anti-HIV-1 Integrase Activity of *N*-Arylindoles (**3a**—**h**) *in Vitro*

Compounds	CC_{50}[a)] (μg/ml)	EC_{50}[b)] (μg/ml)	TI[c)]
3a	91.44	35.82	2.55
3b	191.9	7.88	24.61
3c	>200	82.28	>2.66
3d	5.40	2.93	1.90
3e	99.61	11.24	9.48
3f	>200	37.76	>6.63
3g	157.14	19.22	8.26
3h	14.41	12.09	1.28
AZT[d)]	1288.24	0.007	184034.28

a) CC_{50} (50% cytotoxic concentration), concentration of drug that causes 50% reduction in total C8166 cell number, Drugs with CC_{50} values >200 μg/ml cannot be tested at higher concentrations for a more exact CC_{50} value due to the effect of the solvent DMSO; *b*) EC_{50} (50% effective concentration), concentration of drug that reduces syncytia formation by 50%; *c*) therapeutic index (TI) is a ratio of the CC_{50} value/EC_{50} value; *d*) AZT was used as a positive control.

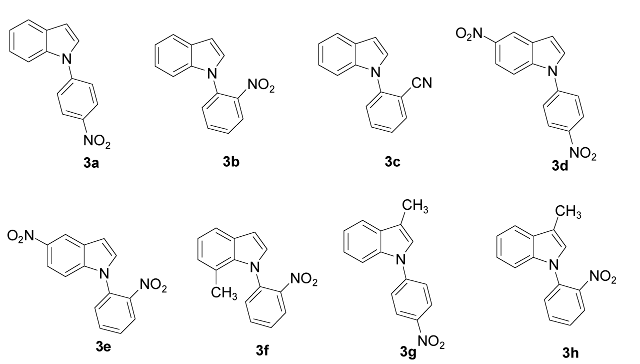

Fig. 1. Structures of Different *N*-Arylindoles **3a**—**h**

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μg/ml, and 2.55/24.61, respectively. Obviously, the TI value of **3b** was almost 10 times of that of **3a**, while the cytotoxicity of **3b** was decreased 2 times compared with **3a**. Meanwhile, the CC_{50}, EC_{50} and TI values of **3d** and **3e** were 5.40/99.61 μg/ml, 2.93/11.24 μg/ml, and 1.90/9.48, respectively. Accordingly, the TI value of **3e** was almost 6 times of that of **3d**, while the cytotoxicity of **3e** was significantly decreased 19 times compared with **3d**. That is, introducing *ortho*-nitro group on the *N*-phenyl ring of indoles, would lead to give compounds possessing more potent anti-HIV-1 integrase activity than those having *para*-nitro group on the *N*-phenyl ring of indoles; Moreover, the cytotoxicity of the compounds having *ortho*-nitro group on the *N*-phenyl ring of indoles, were significantly decreased when compared with those having *para*-nitro group on the *N*-phenyl ring of indoles (**3b** *vs.* **3a**, **3e** *vs.* **3d**). However, when introducing *para*-nitro group on the *N*-phenyl ring of 3-methylindole, the corresponding compound showed the more potent anti-HIV-1 integrase activity than the one having *ortho*-nitro group on the *N*-phenyl ring of 3-methylindole (**3g** *vs.* **3h**). For example, the CC_{50}, EC_{50} and TI values of **3g** and **3h** were 157.14/14.41 μg/ml, 19.22/12.09 μg/ml, and 8.26/1.28, respectively. Consequently, the TI value of **3g** was more than 6 times of that of **3h**, while the cytotoxicity of **3g** was almost decreased 11 times compared with **3h**. In addition, the EC_{50} and TI values of **3b**, **3e**, **3f** and **3h** were 7.88, 11.24, 37.76 and 12.09 μg/ml, and 24.61, 9.48, >6.63 and 1.28, respectively; Therefore, whether introducing electron-withdrawing (nitro group) or electron-donating group (methyl group) on the indole's ring of *N*-(2-nitrophenyl)indole (**3b**) will give less active compounds than **3b**.

Interestingly, once the nitro group of *N*-(2-nitrophenyl)indole (**3b**) was substituted by cyano group to give *N*-(2-cyanophenyl)indole (**3c**), the anti-HIV-1 integrase activity of which was decreased sharply. For example, the EC_{50} and TI values of **3b** and **3c** were 7.88/82.28 μg/ml, and 24.61/>2.66, respectively. The anti-HIV-1 integrase activity of **3b** was nearly 10 times of that of **3c**. Based upon the above investigation, the nitro group certainly is an important functional group for **3b** being good HIV-1 integrase inhibitory activity. Furthermore, efforts to explain the reason why **3b** showed the most potent anti-HIV-1 integrase activity are ongoing in our laboratory.

Conclusion

In conclusion, eight simple *N*-arylindoles were designed, synthesized and evaluated as HIV-1 integrase inhibitors *in vitro*. Three compounds **3b**, **3e** and **3g** demonstrated significant anti-HIV-1 integrase activity as displayed in Table 1. Especially **3b** showed the most promising and best activity against HIV-1 integrase. In order to decrease cytotoxicity and increase anti-HIV-1 integrase activity, further structural modifications of *N*-arylindoles will be conducted in our research group.

Experimental

All the solvents were of analytical grade and the reagents were used as purchased. Thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed with silica gel plates using silica gel 60 GF_{254} (Qingdao Haiyang Chemical Co., Ltd.). Melting points were determined on a digital melting-point apparatus and were uncorrected. ^{1}H-NMR spectra were recorded on a Bruker Avance DMX 400 MHz instrument using TMS as internal standard and $CDCl_3$ as solvent. HR-MS and EI-MS were carried out with APEX II Bruker 4.7T AS and Thermo DSQ GC/MS instruments, respectively. Elemental analysis was executed on Carlo-Erba 1106 CHN microanalyzer.

General Procedure for the Synthesis of *N*-Arylindoles 3a—h The mixture of the appropriate activated fluoroarenes (**1**, 1.0 mmol), the indoles (**2**, 1.2 mmol), and anhydrous Cs_2CO_3 (2.0 mmol) in DMSO (2 ml) in 25 ml rockered flask was stirred at 40 °C in an air atmosphere until complete consumption of the starting material checked by TLC. Then ice water (40 ml) was added to the above mixture, and the latter was extracted by EtOAc (60 ml×3). Subsequently, the combined organic phase was washed by brine (40 ml), dried over anhydrous Na_2SO_4, concentrated *in vacuo* and purified by preparative TLC to give the pure *N*-arylation indoles, which were characterized by ^{1}H-NMR (400 MHz), HR-MS or elemental analysis, EI-MS and mp.

3a: Yield: 86%, yellow solid, mp 109—109.5 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.77 (1H, d, J=3.2 Hz), 7.21 (2H, m), 7.37 (1H, d, J=3.6 Hz), 7.64 (4H, m), 8.39 (2H, d, J=8.8 Hz); EI-MS *m/z*: 238 (M^+, 100); HR-MS *m/z*: 239.0818 $[M+H]^+$, Calcd 239.0815.

3b: Yield: 94%, orange solid, mp 69—70 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.72 (1H, d, J=3.2 Hz), 7.11 (4H, m), 7.53 (2H, m), 7.68 (2H, m), 8.01 (1H, d, J=8.4 Hz); EI-MS *m/z*: 238 (M^+, 100); HR-MS *m/z*: 239.0818 $[M+H]^+$, Calcd 239.0815.

3c: Yield: 76%, white solid, mp 96—96.5 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.76 (1H, d, J=3.6 Hz), 7.18 (2H, m), 7.33 (1H, d, J=8.4 Hz), 7.40 (1H, d, J=3.2 Hz), 7.46 (1H, m), 7.60 (1H, d, J=8.4 Hz), 7.69 (2H, m), 7.83 (1H, d, J=7.6 Hz); EI-MS *m/z*: 218 (M^+, 100); HR-MS *m/z*: 219.0919 $[M+H]^+$, Calcd 219.0917.

3d: Yield: 67%, yellow solid, mp 220—221 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.95 (1H, d, J=3.6 Hz), 7.53 (1H, d, J=3.2 Hz), 7.61 (1H, d, J=8.8 Hz), 7.70 (2H, d, J=8.4 Hz), 8.18 (1H, dd, J=8.8 Hz, 2.0 Hz), 8.46 (2H, d, J=8.8 Hz), 8.66 (1H, d, J=2.0 Hz); EI-MS *m/z*: 283 (M^+, 28).

3e: Yield: 91%, orange solid, mp 104.5—106 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.90 (1H, d, J=3.2 Hz), 7.10 (1H, d, J=9.2 Hz), 7.32 (1H, d, J=3.2 Hz), 7.59 (1H, dd, J=8.0 Hz, 0.8 Hz), 7.68 (1H, m), 7.81 (1H, m), 8.08 (2H, m), 8.63 (1H, d, J=1.6 Hz); EI-MS *m/z*: 283 (M^+, 100); HR-MS *m/z*: 284.0592 $[M+H]^+$, Calcd 284.0588.

3f: Yield: 37%, orange solid, mp 96.5—97 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.94 (3H, s), 6.67 (1H, d, J=3.2 Hz), 6.92 (1H, d, J=6.8 Hz), 7.05 (2H, m), 7.49 (2H, m), 7.66 (2H, m), 7.97 (1H, dd, J=8.0 Hz, 1.2 Hz); EI-MS *m/z*: 252 (M^+, 95); HR-MS *m/z*: 253.0973 $[M+H]^+$, Calcd 253.0972.

3g: Yield: 90%, yellow solid, mp 137—139 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.39 (3H, s), 7.18 (1H, s), 7.24 (2H, m), 7.63 (2H, d, J=8.4 Hz), 7.64 (2H, d, J=8.8 Hz), 8.36 (2H, d, J=8.8 Hz); EI-MS *m/z*: 252 (M^+, 100); *Anal.* Calcd for $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.76; N, 11.11. Found: C, 71.54; H, 4.52; N, 10.98.

3h: Yield: 98%, red liquid; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.35 (3H, s), 6.90 (1H, s), 7.11 (3H, m), 7.43 (2H, m), 7.61 (2H, m), 7.94 (1H, dd, J=8.0 Hz, 1.2 Hz); EI-MS *m/z*: 252 (M^+, 80); HR-MS *m/z*: 253.0971 $[M+H]^+$, Calcd 253.0972.

Anti-HIV-1 Integrase Activity Assay. Cells and Virus Cell line (C8166) and the laboratory-derived virus (HIV-1_{IIIB}) were obtained from MRC, AIDS Reagent Project, UK. C8166 was maintained in RPMI-1640 supplemented with 10% heat-inactivated newborn calf serum (Gibco). The cells used in all experiments were in log-phase growth. The 50% HIV-1_{IIIB} tissue culture infectious dose ($TCID_{50}$) in C8166 cells was determined and calculated by the Reed and Muench method. Virus stocks were stored in small aliquots at −70 °C.[12]

MTT-Based Cytotoxicity Assay Cellular toxicity of compounds **3a**—**h** on C8166 cells was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method as described previously.[13] Briefly, cells were seeded on 96-well microtiter plate in the absence or presence of various concentrations of compounds in triplicate and incubated at 37 °C in a humid atmosphere of 5% CO_2 for 3 d. The supernatants were discarded and MTT reagent (5 mg/ml in PBS) was added to each well, then incubated for 4 h, 100 μl of 50% *N*,*N*-dimethylformanide (DMF)–20% sodiumdodecyl sulfate (SDS) was added. After the formazan was dissolved completely, the plates were read on a Bio-Tek Elx 800 ELISA reader at 595/630 nm. The cytotoxic concentration that caused the reduction of viable C8166 cells by 50% (CC_{50}) was determined from dose–response curve.

Syncytia Assay In the presence of 100 μl various concentrations of compounds, C8166 cells (4×10^5/ml) were infected with virus HIV-1_{IIIB} at a multiplicity of infection (M.O.I) of 0.06. The final volume per well was 200 μl. Control assays were performed without the testing compounds in

HIV-1_{IIIB} infected and uninfected cultures. After 3 d of culture, the cytopathic effect (CPE) was measured by counting the number of syncytia. Percentage inhibition of syncytia formation was calculated and 50% effective concentration (EC_{50}) was calculated. AZT (Sigma) was used as a positive control. Therapeutic index (TI)=CC_{50}/EC_{50}.[14)]

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