Anti Human Immunodeficiency Virus-1 (HIV-1) Agents 1. Discovery of Benzyl Phenyl Ethers as New HIV-1 Inhibitors *in Vitro*

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Ten single benzyl phenyl ethers were synthesized and evaluated as human immunodeficiency virus-1 (HIV-1) inhibitors *in vitro* for the first time. Among these compounds, especially 4-nitrobenzyl phenyl ether (3h) exhibited the highest anti-HIV-1 activity with EC_{50} (concentration of drug that reduces syncytia formation by 50%) value of 5.96 μg/ml and therapeutic index value of 18.32. The preliminary structure–activity relationships of these benzyl phenyl ethers were also described.

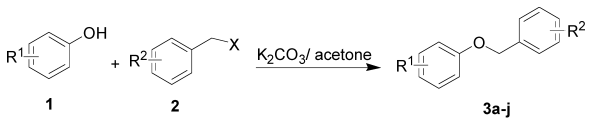
Key words benzyl phenyl ether; human immunodeficiency virus; inhibitor

Acquired immune-deficiency syndrome (AIDS) is a recently identified syndrome in which a person's immune system after functioning normally ceases to function adequately. In the past twenty years, the standard of care for the treatment of the human immunodeficiency virus (HIV) infection has evolved from the use of single-drug therapy, to dual-nucleoside analogue therapy to the current standard of care, 3-drug therapy with 2 nucleoside analogues in combination with a non-nucleoside reverse transcriptase inhibitor or a protease inhibitor. However, many drugs have only limited or transient clinical benefit due to their major side effects (*e.g.*, toxicity problems) and the rapidly development of virus–drug resistance.[1)] Based on the AIDS crisis, therefore, the discovery and development of new, selective, efficacy and safe HIV-1 inhibitors still remains a high priority and challenge for medical research.[2—5)] Benzyl aryl ethers (BAEs) are found many applications as starting materials in organic synthesis and in analytical chemistry, such as the synthesis of diboronic acids, dendrimers and dendritic polymers, and *ortho* lithiation of BAEs to react with electrophiles.[6—8)] To the best of our knowledge, the anti-HIV-1 activity of the single benzyl phenyl ethers has not been previously studied. In previous paper, we found that some single *N*-arylindoles demonstrated significant anti-HIV-1 activity *in vitro*.[9)] These encouraging results prompted us to study the anti-HIV-1 activity of some other single compounds. Meanwhile, according to our program aimed at the discovery and development of bioactive molecules,[9—14)] in this paper we want to report the synthesis and anti-HIV-1 activity of some single benzyl phenyl ethers for the first time.

Results and Discussion

As shown in Chart 1, firstly, ten single benzyl phenyl ethers **3a**—**j** (Fig. 1) were prepared successfully by the reaction of the appropriate phenols with benzyl chloride or substituted benzyl methanesulfonates in the presence of K_2CO_3, and were characterized by ^{1}H-NMR (400 MHz), EI-MS and melting point. Then the benzyl phenyl ethers **3a**—**j** were tested *in vitro* for their anti-HIV-1 activity and 3′-azido-3′-deoxythymidine (AZT) was used as a positive control as shown in Table 1.

Among these tested benzyl phenyl ethers, fortunately,



R^1 = H, Cl, NO_2, Me, Bu(*t*); R^2 = H, NO_2, OMe; X = Cl or OMs

Chart 1. The Synthetic Route of Benzyl Phenyl Ethers **3a**—**j**

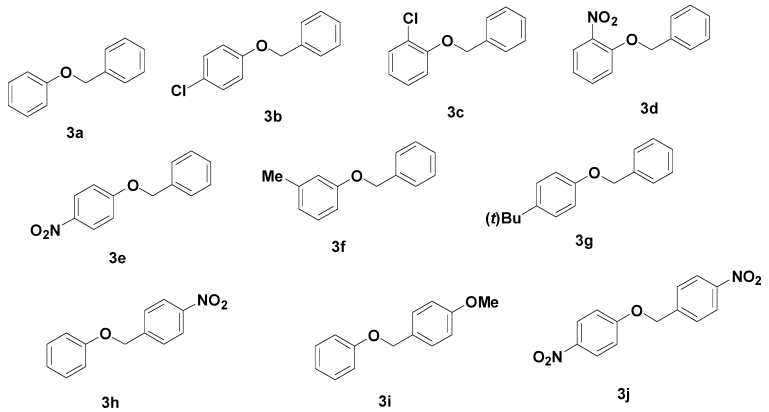


Fig. 1. Structures of Different Benzyl Phenyl Ethers **3a**—**j**

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Table 1. Anti-HIV-1 Activity of Benzyl Phenyl Ethers (**3a**—**j**) *in Vitro*[a)]

Compounds	CC_{50}[b)] (μg/ml)	EC_{50}[c)] (μg/ml)	TI[d)]
3a	197.83	57.99	3.41
3b	106.04	11.53	9.19
3c	108.71	29.36	3.70
3d	117.14	12.53	9.34
3e	182.79	53.26	3.43
3f	134.75	44.87	3.00
3g	117.64	15.48	7.59
3h	109.20	5.96	18.32
3i	151.65	18.14	8.36
3j	169.36	58.20	2.91
AZT[e)]	1307.39	0.0024	544745.83

a) Values are means of two separate experiments; *b*) CC_{50} (50% cytotoxic concentration), concentration of drug that causes 50% reduction in total C8166 cell number; *c*) EC_{50} (50% effective concentration), concentration of drug that reduces syncytia formation by 50%; *d*) therapeutic index (TI) is a ratio of the CC_{50} value/EC_{50} value; *e*) AZT was used as a positive control.

compounds **3b**, **3d**, **3g**, **3h** and **3i** showed the more potent anti-HIV-1 activity with 50% effective concentration (EC_{50}) values of 11.53, 12.53, 15.48, 5.96 and 18.14 μg/ml, and therapeutic index (TI) values of 9.19, 9.34, 7.59, 18.32 and 8.36, respectively. Especially compound **3h** exhibited the most potent and promising anti-HIV-1 activity with EC_{50} value of 5.96 μg/ml and TI value of 18.32, which was as potent as *N*-(2-nitrophenyl)indole with EC_{50} value of 7.88 μg/ml and TI value of 24.61.[9)]

From the comparative study, it is possible to draw some preliminary structure–activity relationships on these benzyl phenyl ethers. As indicated in Table 1, the EC_{50} and TI values of **3a**, **3b** and **3c** were 57.99/11.53/29.36 μg/ml, and 3.41/9.19/3.70, respectively. Obviously, introducing chloro group on the *para* position on the phenyl ring of compound **3a**, would lead to give more potent compound than that having *ortho*-chloro group on the phenyl ring (**3b** *vs.* **3c**). On the contrary, when nitro group was introduced on the *ortho* or *para* position on the phenyl ring of compound **3a**, the EC_{50} and TI values of the corresponding compounds **3d** and **3e** were 12.53/53.26 μg/ml, and 9.34/3.43, respectively. The EC_{50} and TI values of compounds **3f**, having *meso*-methyl group on the phenyl ring, and **3g**, having *para*-*tert*-butyl group on the phenyl ring, were 44.87/15.48 μg/ml, and 3.00/7.59, respectively. Consequently, introducing electron-donating group on the *para* position on the phenyl ring of **3a**, would give more potent compound than that having electron-donating group on *meso* position on the phenyl ring. Meantime, when electron-donating group (*tert*-butyl group) was introduced on the *para* position on the phenyl ring of **3a**, it will give more potent compound than that having electron-withdrawing group (nitro group) on the *para* position on the phenyl ring (**3g** *vs.* **3e**). The EC_{50} and TI values of **3a**, **3e** and **3h** were 57.99/53.26/5.96 μg/ml, and 3.41/3.43/18.32, respectively. That is, the TI value of **3h** was more than 5 times of those of **3a** and **3e**, and the EC_{50} of **3h** was almost decreased 9 times compared with **3a** and **3e**. Therefore, introducing nitro group on the *para* position on the benzyl ring of **3a** will give more active compound than that having *para*-nitro group on the phenyl ring (**3h** *vs.* **3e**).

Once the nitro group (electron-withdrawing group) on the *para* position on the benzyl ring of compound **3h** was substituted by the methoxy group (electron-donating group) to give compound **3i**, however, the anti-HIV-1 activity of which was decreased more than twice compared with **3h**. For example, the EC_{50} and TI values of **3h** and **3i** were 5.96/18.14 μg/ml, and 18.32/8.36, respectively.

Interestingly, when the nitro group was introduced on the *para* position on the phenyl ring of **3h** to give **3j**, the anti-HIV-1 activity of which was sharply decreased compared with **3h**. For example, The EC_{50} and TI values of **3h** and **3j** were 5.96/58.20 μg/ml, and 18.32/2.91, respectively. The anti-HIV-1 activity of **3h** was nearly 10 times of that of **3j**. Based upon the above investigation, the nitro group on the *para* position on the benzyl ring certainly is an important functional group for **3h** being good HIV-1 inhibitory activity. In addition, the mechanisms of the anti-HIV-1 function of these compounds, of course, need to be studied further.

Conclusion

In summary, ten single benzyl phenyl ethers were synthesized and evaluated *in vitro* as HIV-1 inhibitors. Compounds **3b**, **3d**, **3g**, **3h** and **3i** demonstrated significant anti-HIV-1 activity as displayed in Table 1; especially compound **3h** showed the most promising and best activity against HIV-1, which will pave the way for further optimal structural modifications of the benzyl phenyl ethers to discover the more potent compounds possessing anti-HIV-1 activity.

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. EI-MS was carried out with Thermo DSQ GC/MS instrument.

General Procedure for the Synthesis of Benzyl Phenyl Ethers 3a—j The mixture of the appropriate phenols (**1**, 1.1 mmol), benzyl chloride or substituted benzyl methanesulfonates (**2**, 1.0 mmol), and anhydrous K_2CO_3 (3.6 mmol) in acetone (10 ml) was stirred under reflux in an air atmosphere (for benzyl chloride, 0.3 mmol KI was used as a catalyst). When the reaction was complete checked by TLC, the mixture was cooled to r.t., filtered, and purified by preparative TLC to give the pure benzyl phenyl ethers (**3a**—**j**), which were all known compounds and characterized by ^{1}H-NMR (400 MHz), EI-MS and mp.

3a: Yield: 87%, white solid, mp 38—39 °C (lit.,[15)] 37—38 °C); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.06 (2H, s), 6.93 (3H, m), 7.24 (3H, m), 7.35 (4H, m); EI-MS *m*/*z*: 184 (M^+, 18), 91 ($[C_6H_5CH_2]^+$, 100).

3b: Yield: 96%, light yellow solid, mp 71—72 °C (lit.,[15)] 70—71 °C); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.03 (2H, s), 6.87 (2H, m), 7.20 (2H, m), 7.30 (1H, m), 7.35 (4H, m); EI-MS *m*/*z*: 220 (M^+, 5), 218 (M^+, 16), 91 ($[C_6H_5CH_2]^+$, 100).

3c: Yield: 84%, light yellow liquid[16)]; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.14 (2H, s), 6.87 (1H, m), 6.95 (1H, d, J=8 Hz), 7.14 (1H, m), 7.29 (1H, m), 7.35 (3H, m), 7.45 (2H, d, J=7.6 Hz); EI-MS *m*/*z*: 220 (M^+, 3), 218 (M^+, 10), 91 ($[C_6H_5CH_2]^+$, 100).

3d: Yield: 89%, light yellow liquid[17)]; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.22 (2H, s), 6.99 (1H, m), 7.10 (1H, dd, J=8.4, 0.8 Hz), 7.29 (1H, m), 7.35 (2H, m), 7.43 (2H, m), 7.47 (1H, m), 7.83 (1H, dd, J=8.0, 1.6 Hz); EI-MS *m*/*z*: 229 (M^+, 1), 90.9 ($[C_6H_5CH_2]^+$, 100).

3e: Yield: 91%, white solid, mp 105—107 °C (lit.,[18)] 105—107 °C); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.15 (2H, s), 7.02 (2H, dd, J=7.2, 2.0 Hz), 7.36 (1H, m), 7.39 (4H, m), 8.19 (2H, dd, J=7.2, 2.0 Hz); EI-MS *m*/*z*: 229 (M^+, 7), 90.9 ($[C_6H_5CH_2]^+$, 100).

3f: Yield: 93%, white solid, mp 48—49 °C (lit.,[19)] 45—46 °C); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.32 (3H, s), 5.04 (2H, s), 6.76 (3H, m), 7.14 (1H, m), 7.31 (3H, m), 7.42 (2H, m); EI-MS *m*/*z*: 198 (M^+, 28), 90.9 ($[C_6H_5CH_2]^+$, 100).

4g Yield: 95%, white solid, mp 64—65 °C (lit.,[20)] 61—62 °C); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s), 5.03 (2H, s), 6.89 (1H, m), 7.28 (3H, m),

7.34 (2H, m), 7.41 (3H, m); EI-MS *m/z*: 240 (M^+, 29), 90.9 ($[C_6H_5CH_2]^+$, 100).

3h: Yield: 50%, light yellow solid, mp 85—87 °C (lit.,[18] 89—91 °C); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.17 (2H, s), 6.94 (3H, m), 7.30 (2H, m), 7.60 (2H, d, J=8.8 Hz), 8.23 (2H, d, J=8.8 Hz); EI-MS *m/z*: 229 (M^+, 70), 136 ($[(p)NO_2$–$C_6H_5CH_2]^+$, 100).

3i: Yield: 26%, white solid, mp 91—93 °C (lit.,[21] 93—93.5 °C); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.80 (3H, s), 4.98 (2H, s), 6.89 (5H, m), 7.25 (2H, m), 7.35 (2H, d, J=8.8 Hz); EI-MS *m/z*: 214 (M^+, 9), 121 ($[(p)CH_3O$–$C_6H_5CH_2]^+$, 100).

3j: Yield: 72%, light yellow solid, mp 190—192 °C (lit.,[22] 187.4 °C); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.27 (2H, s), 7.02 (2H, m), 7.61 (2H, d, J=8.4 Hz), 8.21 (2H, m), 8.26 (2H, d , J=8.8 Hz); EI-MS *m/z*: 274 (M^+, 3).

Anti-HIV-1 Activity Assay. Cells and Virus Cell line (C8166) and the laboratory-derived virus (HIV-1_{IIIB}) were obtained from MRC, AIDS Reagent Project, U.K. 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.[23]

MTT-Based Cytotoxicity Assay Cellular toxicity of compounds **3a**—**j** on C8166 cells was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method as described previously.[24] 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 wells, then incubated for 4 h, 100 μl of 50% *N*,*N*-dimethylformamide (DMF)–20% 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}.[25]

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