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Synthesis of styrylbenzofuran derivatives as styrylquinoline analogues for HIV-1 integrase inhibitor

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Abstract

A series of styrylbenzofuran derivatives (**8a**–**i**) as styrylquinoline isosters were efficiently prepared by Wittig reaction and evaluated for inhibitory activity against HIV-1 integrase. In this series, compounds **8g**, **8h** and **8i** containing a free catechol ring showed moderate inhibitory activities ($IC_{50} = \sim 40$ μM) against HIV-1 integrase, while less than the corresponding styrylquinoline compound (**I**).

Keywords: AIDS; HIV; Integrase inhibitor; Styrylbenzofuran; Wittig reaction

1. Introduction

AIDS is essentially a viral disease and should be treated by antiretroviral agents [1,2]. From this standpoint, HIV DNA integration into genomic DNA of the host cell, a crucial step in the life cycle of the virus, constitutes a particularly attractive target for AIDS chemotherapeutics, including potential synergy with currently available HIV reverse transcriptase and protease inhibitors [3,4]. HIV-1 IN is currently recognized as an attractive and safe target against AIDS [5], particularly because it is essential in the replication of HIV-1 and there are no similar enzymes involved in human cellular function.

Therefore, a large number of IN inhibitors have been described for the past 13 years and many inhibitors exhibited potent inhibition of integrase in extracellular assays. However, most of them failed to provide antiviral efficacy in HIV-infected cells except the recent 5CITEP [6] and L-708,906 [7], which enter trial in human volunteers. It was additionally reported that the styrylquinoline derivative (I) as shown in Fig. 1 is a potent HIV-1 integrase inhibitor in in vitro experiments, blocks the replication of HIV-1 in cell culture, and is devoid of cytotoxicity [8].

In our continuous efforts to discover new HIV-1 integrase inhibitor [9], we decided to modify the basic scaffold of styrylquinoline compound as styrylbenzofuran compound in order to investigate the effect of its core structure on the inhibitory activity against the HIV-1 integrase. The present paper describes the synthesis and evaluation of styrylbenzofuran derivatives as styrylquinoline isosters.

2. Results and discussion

2.1. Chemistry

As shown in Scheme 1, 7-methoxy-2-methylbenzofuran (**2**) was prepared by cyclization reaction of 6-allyl-2-methoxyphenol (**1**) with iodine in the presence of DBU as a base [10]. After demethylation of compound **2** with pyridine hydrochloride [11], the obtained compound **3** was subjected to Kolbe–Schmit condition (K_2CO_3, CO_2 (gas), 285 °C) to afford 7-hydroxy-2-

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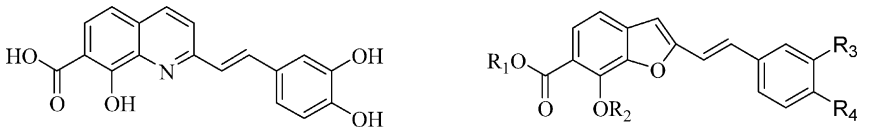


Styrylquinoline (I)

IC_{50} = 2.4 μM [8, 9]

Styrylbenzofuran (8a-i)

R_1, R_2 = H, CH_3
R_3, R_4 = OH, OCH_3

Fig. 1. Styrylquinoline (**I**) and styrylbenzofuran derivatives (**8a**–**i**).

methyl-benzofuran-6-carboxylic acid (**4**) in 54% yield [12].

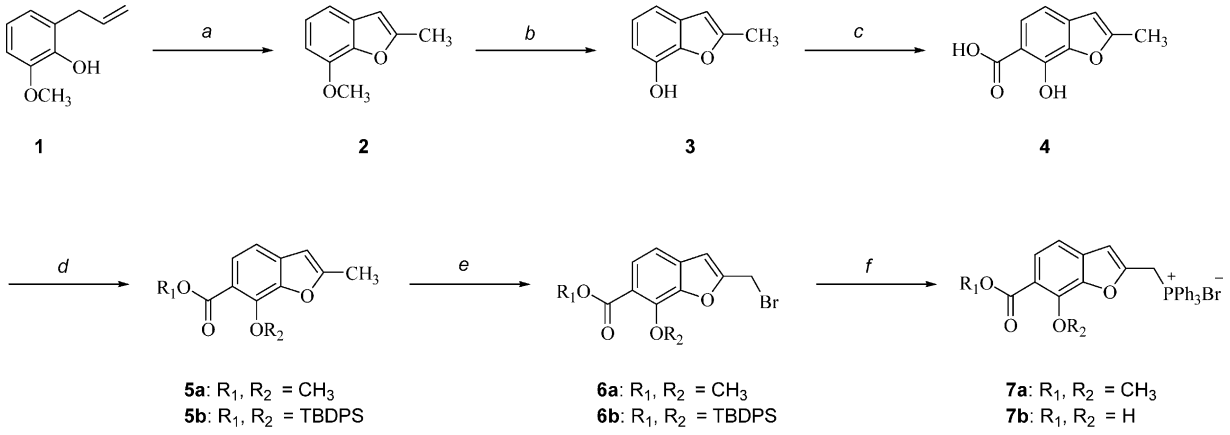
In order to test the inhibitory effect of substituents of benzofuran ring against HIV-integrase, both functional groups of compound **4** were protected by two different procedures: CH_3I, K_2CO_3 and TBDPSCl, imidazole, respectively [13,14]. Allylic positions of compound **5a** and **5b** were halogenated to afford compounds **6a** and **6b**, respectively in 75 and 91% yields by treating them with *N*-bromosuccinimde (NBS) and benzoyl peroxide [15]. The phosphonium salt **7a** for the Wittig reaction was prepared from the reaction of **6a** with triphenylphosphine in acetonitrile [16]. For compound **7b** containing free salicylic moiety, on the other hand, the desilylation of **6b** with TBAF was followed by the subsequent treatment of Ph_3P to provide the compound **7b** in 50% yield [17] (Scheme 1).

Styrylbenzofuran derivatives (**8a**–**8c**, **8f**, **8h**–**8i**) with *E* geometry were prepared through Wittig reaction between triphenylphosphonium salts (**7a** or **7b**) and various benzaldehydes under the basic condition, as shown in Scheme 2 [16]. Compounds **8e** and **8g** were also prepared by demethylation from **8b** and **8h**, respectively, with 1 M BBr_3 [18]. Finally, **8d** was prepared through the hydrolysis of **8b** by LiOH [19] for the deprotection of only methyl ester group of compound **8b**. The results of all reactions were summarized in Table 1.

2.2. Biological activity

The prepared styrylbenzofuran derivatives (**8a**–**i**) were in vitro screened for inhibitory activity against HIV-1 IN in 3′-processing reaction and the resultant anti-HIV integrase activity was summarized in Table 1. For the comparison of biological activity, styrylquinoline compound (**I**) as a standard compound was synthesized and tested for its inhibitory activity under our assay conditions, and its data ($IC_{50} = 18.9 \pm 10.8$ μM) was inserted in Table 1.

A shown in Table 1, at first glance, these compounds can be roughly classified as inactive (**8a**–**f**) and active (**8g**–**i**). As a structural characteristic based on the inhibitory activity, all compounds (**8g**–**i**) bearing a free cathecol ring again have moderate effects on anti-HIV-1 IN activity with IC_{50} values ranging from 42.9 to 44.1 μM, though two fold less than the standard compound (**I**, $IC_{50} = 18.9 \pm 10.8$ μM). However, other compounds containing monohydroxy- (**8d**–**f**), monomethoxy- (**8a**)



1 **2** **3** **4**

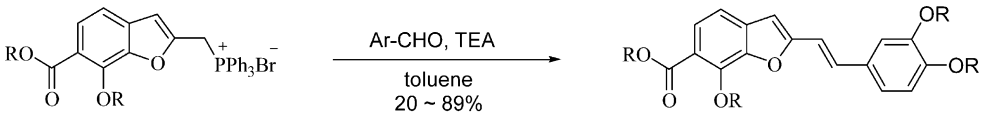
5a: R_1, R_2 = CH_3
5b: R_1, R_2 = TBDPS

6a: R_1, R_2 = CH_3
6b: R_1, R_2 = TBDPS

7a: R_1, R_2 = CH_3
7b: R_1, R_2 = H

[a] iodine, acetonitrile; then DBU, CH_2Cl_2, 57%; [b] pyridine hydrochloride, 210 °C, 86% [c] K_2CO_3, CO_2 gas, 285 °C, 54%; [d] for **5a**) K_2CO_3, CH_3I, acetone, 99% or for **5b**) TBDPSCl, imidazole, DMF, 72%; [e] NBS, benzoylperoxide, PhH, (**6a** = 75%, **6b** = 91%); [f] for **7a**) PPh_3, CH_3CN, 83% or for **7b**) Bu_4NF, THF, then PPh_3, CH_3CN, 50%

Scheme 1. Synthesis of intermediates (**7a** and **7b**) for the Wittig reaction.

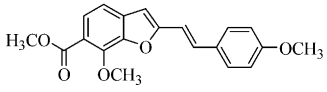
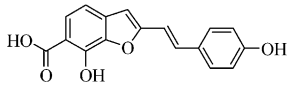
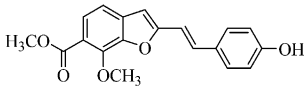
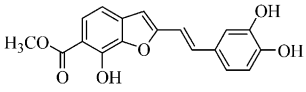
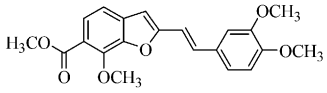
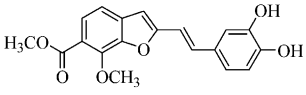
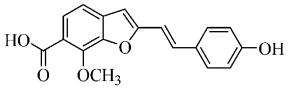
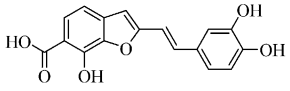
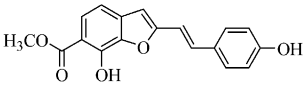
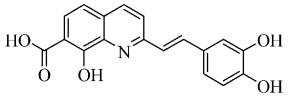


7a: R =CH_3
7b: R =H

8a-i: R =CH_3 or H

Scheme 2. Synthesis of styrylbenzofurans (**8a**–**i**).

Table 1
HIV-1 Inhibitory activities of styrylbenzofurans (**8a**–**i**)

Entry	Structure	IC_{50} (μM)[a,b]	Entry	Structure	IC_{50} (μM)[a,b]
8a		>> 100	**8f**		>> 100
8b		>> 100	**8g**		42.90 ± 14.1
8c		>> 100	**8h**		43.80 ± 9.9
8d		>> 100	**8i**		44.01 ± 8.3
8e		>> 100	**I**[c]		18.90 ± 10.8

[a] Inhibitory activity against 3'-end-processing; [b] Assays were performed in three separate experiments; [c] Styrylquinoline compound **(I)** was prepared by the known method for comparison [8, 9].

or dimethoxy (**8c**)-substituented phenyl ring showed the complete lack of inhibitory potency ($IC_{50} = \gg 100$ μM), irrespective of the presence of a free salicylic moiety (*ortho* hydroxyl and carboxyl pattern of the parent benzofuran ring). This result is very remarkable in that in the case of standard compound, styrylquinoline (I), both of a salicylic pattern in the quinoline and a cathecol in the subunit are required for the inhibitory activity against anti-HIV-1 IN [8].

When compared the activities of compound **8g** and **8h** with that of **8i**, it seems that the free cathcol moiety in subunit of benzofuran derivatives plays more important role than its salicylic moiety for 3′-processing inhibition against HIV-1 IN. This result implies that in the case of styrylbenzofuran derivatives, the presence of free salicylic moiety is not always required for the biological activity.

In conclusion, a series of styrylbenzofuran derivatives (**8a**–**i**) were prepared and evaluated for the HIV-1 IN inhibitory activities as styrylquinoline isosters. Among them, styrylbenzofuran derivatives (**8g**–**i**) bearing free cathechol ring showed comparable inhibitory activities to styrylquinoline (**I**) against HIV-1 IN in 3′-processing reaction. Also, it were concluded that the salicylic moiety in benzofuran derivatives don't have any important role in binding mode for the inhibitory activity against HIV-1 IN in 3′-processing reaction, but the free catechol moiety at phenyl ring is required for the inhibitory activity. This results would show the possibility that styrylbenzofuran derivatives have different

binding modes from that of styrylquinoline in the active site of the HIV-1 IN [8].

3. Experimental

3.1. Chemistry: general

^{1}H- and ^{13}C NMR spectra were recorded on a Gemini Varian-300 (300 and 75 MHz, respectively). Mass spectra (EI) were determined on HP GC 5972 and HP MS 5988A system at 70 eV. Melting points (m.p.) were determined on a Thomas–Hoover capillary melting apparatus and are uncorrected. Infrared (IR) spectra were recorded on Perkin Elmer 16F-PC FT-IR and MIDAC 101025 using a potassium bromide pellet. Analytical thin layer chromatographies (TLC) were carried out by precoated silica gel (E. Merck Kiesegel $60F_{254}$ layer thickness 0.25 mm). Flash column chromatographies were performed with Merck Kiesegel 60 Art 9385 (230–400 mesh). All solvents used were purified according to standard procedures.

3.1.1. 7-Methoxy 2-methylbenzofuran (**2**)

To a solution of 6-allyl-2-methoxyphenol (**1**, 15 g, 91.4 mmol) in 100 ml of acetonitrile was added iodine (23.2 g, 182.8 mmol) in the dark. The mixture was stirred for 27 h at room temperature (r.t.). The reaction mixture was diluted with 100 ml of CH_2Cl_2 and treated with DBU (68.0 ml, 457.0 mmol). After being stirred for 20 h at r.t., the reaction mixture was extracted with $CHCl_3$. The organic layer was washed with aqueous $Na_2S_2O_3$, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:20) to give **2** (8.4 g, 57%) as an oil: IR (KBr) 2950, 1614, 1492, 1272, 1096 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.00–6.98 (2H, m, benzofuran–*H4*,*H6*), 6.66–6.63 (1H, m, benzofuran–*H5*), 6.27 (1H, s, benzofuran–*H3*), 3.83 (3H, s, benzofuran–OCH_3), 2.31 (3H, s, benzofuran–CH_3); ^{13}C NMR (CD_3OD) δ 159.4, 149.1, 148.0, 135.1, 127.1, 116.5, 109.7, 106.8, 59.2, 16.8.

3.1.2. 7-Hydroxy-2-methylbenzofuran (**3**)

The mixture of compound **2** (3.2 g, 19.7 mmol) and pyridine hydrochloride (6.8 g, 59.7 mmol) was heated under reflux for 2 h at 210 °C. After cooling to r.t., the reaction mixture was neutralized with 3 N HCl and extracted with diethyl ether. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:3) to give **3** (2.5 g, 86%) as an oil: IR (KBr) 3394, 2924, 1608, 1452, 1196, 1046 cm^{-1}; ^{1}H NMR (CD_3OD) δ 6.94–6.91 (2H, m, benzofuran–*H4*,*H6*), 6.63–6.61 (1H, m, benzofuran–*H5*), 6.35 (1H, s, benzofuran–*H3*), 2.42 (3H, s, benzofuran–CH_3); ^{13}C NMR (CD_3OD) δ 156.2, 144.6, 142.8, 132.3, 124.0, 112.4, 110.5, 103.8, 14.3.

3.1.3. 7-Hydroxy-2-methylbenzofuran-6-carboxylic acid (**4**)

The mixture of compound **3** (5 g, 33.7 mmol), potassium carbonate (9.3 g, 67.5 mmol) and CO_2 gas (10 atm) in the autoclave was heated to 285 °C (15 atm). This condition was kept for 6 h. After cooling to r.t., the reaction mixture was diluted with water and washed out with EtOAc. The aqueous layer was acidified by 3 N HCl to pH 3 and extracted with diethyl ether. The organic layer was dried over $MgSO_4$, filtered and concentrated to give **4** (3.5 g, 54%) as a yellow solid: m.p. 225–227 °C; IR (KBr) 2986, 2572, 1662, 1438, 1298 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.64 (1H, d, $J = 9.0$ Hz, benzofuran–*H5*), 6.95 (1H, d, $J = 9.0$ Hz, benzofuran–*H4*), 6.44 (1H, s, benzofuran–*H3*), 2.46 (3H, s, benzofuran–CH_3); ^{13}C NMR (CD_3OD) δ 174.4, 160.3, 149.2, 143.7, 137.7, 125.3, 111.5, 108.4, 104.6, 14.0.

3.1.4. Methyl 7-methoxy-2-methylbenzofuran-6-carboxylate (**5a**)

To a solution of compound **4** (280 mg, 1.4 mmol) in 30 ml of acetone was added potassium carbonate (667 mg, 4.9 mmol) and iodomethane (0.2 ml, 3.5 mmol). The reaction mixture was heated under reflux for 2 h. The mixture was concentrated and washed with CH_2Cl_2. The organic layer was concentrated and purified by flash column chromatography (EtOAc:*n*-hexane = 1:6) to give **5a** (250 mg, 99%) as an oil; IR (KBr) 2922, 1726, 1602, 1486, 1238, 1080 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.56 (1H, d, $J = 8.4$ Hz, benzofuran–*H5*), 7.02 (1H, d, $J = 8.4$ Hz, benzofuran–*H4*), 6.25 (1H, s, benzofuran–*H3*), 4.12 (3H, s, benzofuran–CO_2CH_3), 3.81 (3H, s, benzofuran–OCH_3), 2.35 (3H, s, benzofuran–CH_3); ^{13}C NMR ($CDCl_3$) δ 167.0, 158.4, 146.2, 145.5, 135.6, 125.8, 117.3, 114.0, 103.5, 61.5, 52.0, 14.2.

3.1.5. Methyl 2-bromomethyl-7-methoxybenzofuran-6-carboxylate (**6a**)

To a solution of compound **5a** (100 mg, 0.5 mmol) in 5 ml of boiling benzene was added benzoyl peroxide (50 mg, 0.3 mmol) and NBS (120 mg, 0.7 mmol). The reaction mixture was heated under reflux for 2 h. After cooling to r.t., the mixture was concentrated and purified by flash column chromatography (EtOAc:*n*-hexane = 1:8) to give **6a** (39 mg, 75%) as an oil; IR (KBr) 2924, 1722, 1614, 1456, 1292, 1236 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.71 (1H, d, $J = 8.1$ Hz, benzofuran–*H5*), 7.24 (1H, d, $J = 8.1$ Hz, benzofuran–*H4*), 6.79 (1H, s, benzofuran–*H3*), 4.61 (2H, s, benzofuran–CH_2Br), 4.27 (3H, s, benzofuran–CO_2CH_3), 3.95 (3H, s, benzofuran–OCH_3); ^{13}C NMR ($CDCl_3$) δ 167.7, 159.6, 146.9, 127.1, 126.8, 117.5, 101.8, 60.9, 51.8, 22.0.

*3.1.6. Methyl 7-methoxy-2-methylbenzofuran-6-carboxylate triphenylphosphonium bromide salt (**7a**)*

To a solution of compound **6a** (250 mg, 0.8 mmol) in 15 ml of acetonitrile was added triphenylphosphine (438 mg, 1.7 mmol). The reaction mixture was heated under reflux for 2 h, cooled to r.t., and concentrated. The resultant residue was solidified with diethyl ether to give **7a** (390 mg, 83%) as a solid: m.p. 201–203 °C; IR (KBr) 2920, 1718, 1614, 1444, 1288, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.88–7.60 (16H, m, PPh_3–*H*), 7.57 (1H, d, $J = 8.1$ Hz, benzofuran–*H5*), 7.22 (1H, s, benzofuran–*H3*), 7.06 (1H, d, $J = 8.1$ Hz, benzofuran–*H4*), 6.13 (2H, d, $J = 14.4$ Hz, benzofuran–CH_2PPh_3), 3.88 (3H, s, benzofuran–CO_2CH_3) 3.72 (3H, s, benzofuran–OCH_3); ^{13}C NMR ($CDCl_3$) δ 167.9, 149.3, 149.2, 147.1, 146.3, 136.6, 135.2, 133.7, 133.0, 132.9, 131.5, 130.0, 129.9, 127.2, 119.7, 119.4, 118.3, 115.8, 111.1, 111.0, 61.7, 52.7.

*3.1.7. t-Butyldiphenylsilyl 7-t-butyldiphenylsilyloxy-2-methylbenzofuran-6-carboxylate (**5b**)*

To a solution of compound **4** (500 mg, 2.6 mmol) in 10 ml of DMF was added TBDPSCl (2.0 ml, 7.8 mmol) and imidazole (780 mg, 10.4 mmol). The reaction mixture was stirred for 24 hr at r.t., diluted with a mixture of 100 ml of EtOAc and 100 ml of water, and extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was solidified with diethyl ether to give **5b** (1.3 g, 72%) as a solid: m.p. 146–147 °C; IR (KBr) 2936, 1708, 1606, 1476, 1282, 1118 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.99–7.84 (9H, m, benzofuran–*H5*, TBDPS–*phenyl*), 7.52–7.32 (12H, m, TBDPS–*phenyl*), 7.11 (1H, d, $J = 8.2$ Hz benzofuran–*H4*), 6.18 (1H, s, benzofuran–*H3*), 1.89 (3H, s, benzofuran–CH_3), 1.33 (9H, s, TBDPS–CH_3) 1.15 (9H, s, TBDPS–CH_3); ^{13}C NMR ($CDCl_3$) δ 164.2, 157.7, 135.9, 135.6, 135.1, 134.9, 134.5, 132.8, 130.1, 129.5, 127.9, 127.4, 126.2, 112.2, 103.0, 27.5, 26.9, 20.3, 19.7, 13.7.

*3.1.8. t-Butyldiphenylsilyl 7-t-butyldiphenylsilyloxy-2-bromomethylbenzofuran-6-carboxylate (**6b**)*

To a solution of compound **5b** (1.1 g, 1.7 mmol) in 150 ml of boiling benzene was added benzoyl peroxide (205.9 mg, 0.8 mmol) and NBS (450.3 mg, 2.5 mmol). The reaction mixture was heated under reflux for 2 h and cooled to r.t. The mixture was concentrated and the resultant residue was solidified with *n*-hexane to give **6b** (1.2 g, 91%) as a solid: m.p. 154–155 °C; IR (KBr) 2936, 1712, 1610, 1476, 1278, 1124 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.93 (1H, d, $J = 8.1$ Hz, benzofuran–*H5*), 7.87–7.78 (8H, m, TBDPS–*phenyl*), 7.48–7.28 (12H, m, TBDPS–*phenyl*), 7.13 (1H, d, $J = 8.1$ Hz, benzofuran–*H4*), 6.52 (1H, s, benzofuran–*H3*), 3.90 (2H, s, benzofuran–CH_2), 1.25 (9H, s, TBDPS–CH_3), 1.07 (9H, s, TBDPS–CH_3); ^{13}C NMR ($CDCl_3$) δ 162.8, 157.0, 135.8, 135.7, 135.6, 134.1, 133.4, 132.6, 130.1, 129.6, 127.9, 127.5, 126.6, 113.2, 106.3, 27.5, 26.8, 22.1, 20.2, 19.7.

*3.1.9. 7-Hydroxy-2-methylbenzofuran-6-carboxylic acid triphenylphosphonium bromide salt (**7b**)*

To a solution of compound **6b** (500 mg, 0.7 mmol) in 10 ml of THF was added a solution of TBAF (1M solution, 2.0 ml) in THF at 0 °C. The reaction mixture was stirred for 40 min at 0 °C and concentrated in vacuo. The resulting oily residue was diluted in 20 ml of acetonitrile and treated with triphenylphosphine (702 mg, 2.6 mmol) at r.t. The reaction mixture was heated under reflux for 5 h, treated with 10 ml of water, and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was solidified with *n*-hexane to give **7b** (180 mg, 50%) as a solid: m.p. 223–225 °C; IR (KBr) 3414, 1660, 1444, 1292, 1024 cm^{-1}; ^1H NMR (CD_3OD) δ 7.8–7.69 (16H, m, benzofuran–*H5*, triphenylphosphine–*15H*), 6.81 (1H, d, $J = 8.4$ Hz, benzofuran–*H4*), 6.68 (1H, s, benzofuran–*H3*), 5.37 (2H, d, $J = 14.4$ Hz, benzofuran–CH_2–PPh_3); ^{13}C NMR (CD_3OD) δ 176.0, 153.9, 149.3, 148.2, 148.0, 144.8, 136.6, 135.2, 135.0, 134.2, 133.0, 131.5, 131.4, 130.0, 126.7, 119.6, 118.5, 114.4, 111.1, 111.0, 110.8, 53.8.

*3.2. General procedure A for Wittig reaction between **7a** or **7b** and the various aromatic aldehydes*

To a solution of **7a** or **7b** in toluene was added triethylamine (2.5 equiv.) and the various aromatic aldehydes (2.0 equiv.). The reaction mixture was heated under reflux for 2–4 h. The mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:1–1:7) to give various styrylbenzofurans (**8a**–**8c**, **8f**, **8h**, **8i**)

*3.2.1. Methyl (E)-7-methoxy-2-[2-(4-methoxyphenyl)ethenyl]-6-benzofurancarboxylate (**8a**)*

Procedure A using **7a** (200 mg, 0.36 mmol), triethylamine (0.12 ml, 0.89 mmol) and 4-methoxybenzaldehyde (92 mg, 0.72 mmol) in toluene (5 ml) afforded **8a** (63 mg, 52%) as a solid: m.p. 75–77 °C; IR (KBr) 2940, 1720, 1602, 1418, 1260 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.69 (1H, d, $J = 8.4$ Hz, benzofuran–*H5*), 7.49 (2H, d, $J =$ 6.9 Hz, phneyl–*H2*, *H6*), 7.34 (1H, d, $J = 16.5$ Hz, –CH=CH–phenyl), 7.19 (1H, d, $J = 8.4$ Hz, benzofuran–*H4*), 6.93 (2H, d, $J = 6.9$ Hz, phneyl–*H3*, *H5*), 6.88 (1H, d, $J = 16.5$ Hz, –CH=CH–phenyl), 6.60 (1H, s, benzofuran–*H3*), 4.31 (3H, s, CO_2CH_3), 3.93 (3H, s, benzofuran–OCH_3), 3.83 (3H, s, phenyl–OCH_3); ^{13}C NMR (DMSO) δ 166.0,159.8, 157.7, 145.3, 144.5, 134.9,

131.5, 128.6, 128.5, 125.5, 117.9, 114.8, 114.3, 113.7, 104.6, 61.4, 55.2, 52.0.

3.2.2. Methyl (*E*)-7-methoxy-2-[2-(4-hydroxyphenyl)ethenyl]-6-benzofurancarboxylate (**8b**)

Procedure A using **7a** (200 mg, 0.36 mmol), triethylamine (0.12 ml, 0.89 mmol) and 4-hydroxybenzaldehyde (88 mg, 0.72 mmol) in toluene (5 ml) afforded **8b** (100 mg, 86%) as a solid: m.p. 185–186 °C; IR (KBr) 3356, 2948, 1694, 1432, 1272 cm^{-1}; ^1H NMR (CD_3OD) δ 7.63 (1H, d, $J = 8.1$ Hz, benzofuran–*H5*), 7.48 (2H, d, $J = 6.9$ Hz, phneyl–*H2*, *H6*), 7.34 (1H, d, $J = 15.9$ Hz, –C*H*=CH–phenyl), 7.24 (1H, d, $J = 8.1$ Hz, benzofuran–*H4*), 6.99 (1H, d, $J = 15.9$ Hz, –CH=C*H*–phenyl), 6.83 (2H, d, $J = 6.9$ Hz, phneyl–*H3*, *H5*), 6.74 (1H, s, benzofuran–*H3*), 4.26 (3H, s, CO_2CH_3), 3.91 (3H, s, benzofuran–OCH_3); ^{13}C NMR (DMSO) δ 166.7, 159.1, 158.7, 146.0, 145.2, 135.8, 132.7, 129.4, 127.6, 126.3, 118.6, 116.5, 115.4, 113.4, 104.8, 62.1, 52.7.

3.2.3. Methyl (*E*)-7-methoxy-2-[2-(3,4-dimethoxyphenyl)ethenyl]-6-benzofurancarboxylate (**8c**)

Procedure A using **7a** (200 mg, 0.36 mmol), triethylamine (0.12 ml, 0.89 mmol) and 3,4-dimethoxybenzaldehyde (119.6 mg, 0.72 mmol) in toluene (7 ml) afforded **8c** (100 mg, 75%) as a solid: m.p. 117–118 °C; IR (KBr) 2950, 1716, 1456, 1268, 1006 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65 (1H, d, $J = 8.1$ Hz, benzofuran–*H5*), 7.27 (1H, d, $J = 16.2$ Hz, –C*H*=CH–phenyl), 7.14 (1H, d, $J = 8.4$ Hz, phneyl–*H5*), 7.06 (1H, d, $J = 8.4$ Hz, phneyl–*H6*), 7.03 (1H, s, phneyl–*H2*), 6.84 (1H, d, $J = 16.2$ Hz, –CH=C*H*–phenyl), 6.83 (1H, d, $J = 8.1$ Hz, benzofuran–*H4*), 6.57 (1H, s, benzofuran–*H3*), 4.27 (3H, s, CO_2CH_3), 3.91 (3H, s, benzofuran–OCH_3), 3.89 (3H, s, phenyl–OCH_3), 3.87 (3H, s, phenyl–OCH_3); ^{13}C NMR (DMSO) δ 166.7, 158.5, 150.4, 149.7, 146.1, 145.2, 135.7, 132.7, 129.5, 126.3, 121.7, 118.7, 115.5, 114.6, 112.4, 110.3, 105.2, 62.1, 56.2, 56.1, 52.7.

3.2.4. (*E*)-7-Hydroxy-2-[2-(4-hydroxyphenyl)ethenyl]-6-benzofurancarboxylic acid (**8f**)

Procedure A using **7b** (85 mg, 0.16 mmol), triethylamine (0.04 ml, 0.32 mmol) and 4-hydroxybenzaldehyde (39 mg, 0.32 mmol) in toluene (3 ml) afforded **8f** (25 mg, 53%) as a solid: m.p. 227 °C (dec.); IR (KBr) 3482, 3298, 1704, 1604, 1438, 1276 cm^{-1}; ^1H NMR (CD_3OD) δ 7.72 (1H, d, $J = 8.4$ Hz, benzofuran–*H5*), 7.47 (2H, d, $J = 8.7$ Hz, phneyl–*H2*, *H6*), 7.36 (1H, d, $J = 15.9$ Hz, –C*H*=CH–phenyl), 6.98 (1H, d, $J = 15.9$ Hz, –CH=C*H*–phenyl), 6.93 (1H, d, $J = 8.4$ Hz, benzofuran–*H4*), 6.83 (2H, d, $J = 8.7$ Hz, phneyl–*H3*, *H5*), 6.66 (1H, s, benzofuran–*H3*); ^{13}C NMR (DMSO) δ 177.8, 163.4, 161.3, 156.7, 148.9, 137.8,135.1, 133.7, 132.8, 130.3, 121.2, 120.5, 119.1, 111.7, 110.3.

3.2.5. Methyl (*E*)-7-methoxy-2-[2-(3,4-dihydroxyphenyl)ethenyl]-6-benzofurancarboxylate (**8h**)

Procedure A using **7a** (200 mg, 0.36 mmol), triethylamine (0.12 ml, 0.89 mmol) and 3,4-dihydroxybenzaldehyde (99 mg, 0.72 mmol) in toluene (7 ml) afforded **8h** (90 mg, 73%) as a solid: m.p. 193–194 °C; IR (KBr) 3396, 1588, 1442, 1230 cm^{-1}; ^1H NMR (CD_3OD) δ 7.67 (1H, d, $J = 8.2$ Hz, benzofuran–*H5*), 7.30 (1H, d, $J = 16.3$ Hz, –C*H*=CH–phenyl), 7.27 (1H, d, $J = 8.1$ Hz, phneyl–*H5*), 7.11 (1H, s, phneyl–*H2*), 7.00 (1H, d, $J = 8.1$ Hz, phneyl–*H6*), 6.97 (1H, d, $J = 16.3$ Hz, –CH=C*H*–phenyl), 6.85 (1H, d, $J = 8.2$ Hz, benzofuran–*H4*), 6.77 (1H, s, benzofuran–*H3*), 4.28 (3H, s, CO_2CH_3), 3.94 (3H, s, benzofuran–OCH_3); ^{13}C NMR (DMSO) δ 166.0, 158.0, 146.8, 145.6, 145.3, 144.5, 135.1, 132.4, 127.4, 125.6, 119.7, 117.8, 115.8, 114.7, 113.7, 112.6, 104.1, 61.4, 52.0.

3.2.6. (*E*)-7-Hydroxy-2-[2-(3,4-dihydroxyphenyl)ethenyl]-6-benzofurancarboxylic acid (**8i**)

Procedure A using **7b** (80 mg, 0.15 mmol), triethylamine (0.04 ml, 0.30 mmol) and 3,4-dihydroxybenzaldehyde (41 mg, 0.30 mmol) in toluene (4 ml) afforded **8i** (10 mg, 20%) as a solid: m.p. 250 °C (dec.); IR (KBr) 3376, 1620, 1590, 1444, 1298 cm^{-1}; ^1H NMR (CD_3OD) δ 7.62 (1H, d, $J = 8.1$ Hz, benzofuran–*H5*), 7.15 (1H, d, $J = 16.5$ Hz, –C*H*=CH–phenyl), 6.98 (1H, s, phneyl–*H2*), 6.87 (3H, m, benzofuran–*H4*, phenyl–*H6*, –CH=C*H*–phenyl), 6.72 (1H, d, $J = 8.1$ Hz, benzofuran–*H4*), 6.62 (1H, s, benzofuran–*H3*); ^{13}C NMR (CD_3OD, DMSO) δ 174.7, 156.7, 145.8, 145.0, 142.4, 133.4, 130.6, 128.0, 124.7, 119.1, 115.2, 113.9, 112.9, 112.7, 108.4, 104.0.

3.3. General procedure B for demethylation

To a solution of **8b** or **8h** in CH_2Cl_2 was slowly added 1 M BBr_3 at −78 °C. The reaction mixture was stirred for 5 h at r.t. The mixture was quenched by an addition of MeOH, concentrated and purified by flash column chromatography (EtOAc:*n*-hexane = 1:5 and 2:1) to give styrylbenzofurans (**8e**, **8g**).

3.3.1. Methyl (*E*)-7-hydroxy-2-[2-(4-hydroxyphenyl)ethenyl]-6-benzofurancarboxylate (**8e**)

Procedure B using **8b** (100 mg, 0.31 mmol) and 1 M BBr_3 (0.6 ml) in CH_2Cl_2 (10 ml) afforded **8e** (50 mg, 52%) as a solid: m.p. 235–236 °C; IR (KBr) 3382, 1668, 1436, 1296 cm^{-1}; ^1H NMR (CD_3OD) δ 7.70 (1H, d, $J = 8.1$ Hz, benzofuran–*H5*), 7.49 (2H, d, $J = 8.7$ Hz, phneyl–*H2*, *H6*), 7.35 (1H, d, $J = 15.9$ Hz, –C*H*=CH–phenyl), 7.26 (1H, d, $J = 8.1$ Hz, benzofuran–*H4*), 7.01 (1H, d, $J = 15.9$ Hz, –CH=C*H*–phenyl), 6.84 (2H, d, $J = 8.7$ Hz, phneyl–*H3*, *H5*), 6.76 (1H, s, benzofuran–*H3*), 3.97 (3H, s, benzofuran–CO_2CH_3); ^{13}C NMR

(DMSO) δ 170.9, 159.4, 159.2, 147.2, 142.6, 136.7, 133.0, 129.6, 127.8, 125.0, 116.6, 113.6, 112.4, 108.6, 105.4, 53.3.

3.3.2. Methyl (*E*)-7-hydroxy-2-[2-(3,4-dihydroxyphenyl)ethenyl]-6-benzofurancarboxylate (**8g**)

Procedure B using **8d** (110 mg, 0.32 mmol) and 1M BBr_3 (0.65 ml) in CH_2Cl_2 (10 ml) afforded **8g** (50 mg, 42%) as a solid: m.p. 203–205 °C; IR (KBr) 3404, 2952, 1672, 1438, 1298 cm^{-1}; ^1H NMR (CD_3OD) δ 7.60 (1H, d, $J = 8.4$ Hz, benzofuran–*H5*), 7.26 (1H, d, $J = 16.2$ Hz, –C*H*=CH–phenyl), 7.04 (1H, s, phneyl–*H2*), 6.99 (1H, d, $J = 8.4$ Hz, phneyl–*H5*), 6.92 (1H, d, $J = 8.4$ Hz, phneyl–*H6*), 6.84 (1H, d, $J = 16.2$ Hz, –CH=C*H*–phenyl), 6.78 (1H, d, $J = 8.4$ Hz, benzofuran–*H4*), 6.62 (1H, s, benzofuran–*H3*), 3.93 (3H, s, benzofuran–CO_2CH_3); ^{13}C NMR (DMSO) δ 170.8, 159.3, 147.6, 147.0, 146.3, 142.4, 136.6, 133.2, 128.1, 124.9, 120.5, 116.5, 114.4, 113.3, 112.2, 108.5, 105.2, 53.2.

3.3.3. (*E*)-7-Methoxy-2-[2-(4-hydroxyphenyl)ethenyl]-6-benzofurancarboxylic acid (**8d**)

To a solution of **8b** (100 mg, 0.31 mmol) in mixed solvent (THF:H_2O = 7 ml:7 ml) was added LiOH (129 mg, 3.08 mmol). The reaction mixture was heated for 12 h to 40 °C. After cooling to r.t., the mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was solidified by diethyl ether–*n*-hexane to give **8d** (85 mg, 89%) as a solid: m.p. 236–237 °C; IR (KBr) 3172, 1700, 1600, 1430, 1286 cm^{-1}; ^1H NMR (CD_3OD) δ 7.70 (1H, d, $J = 8.1$ Hz, benzofuran–*H5*), 7.49 (2H, d, $J = 8.7$ Hz, phneyl–*H2*, *H6*), 7.35 (1H, d, $J = 15.9$ Hz, –C*H*=CH–phenyl), 7.26 (1H, d, $J = 8.1$ Hz, benzofuran–*H4*), 7.01 (1H, d, $J = 15.9$ Hz, –CH=C*H*–phenyl), 6.84 (2H, d, $J = 8.7$ Hz, phneyl–*H3*, *H5*), 6.76 (1H, s, benzofuran–*H3*), 3.97 (3H, s, benzofuran–CO_2CH_3); ^{13}C NMR (CD_3OD) δ 168.4, 158.8, 158.7, 145.9, 145.5, 136.3, 132.4, 128.1, 126.4, 118.2, 115.9, 114.6, 112.9, 104.1, 61.2.

3.4. Biological test

3.4.1. HIV-1 integrase [20]

Recombinant human immunodeficiency virus type 1 (HIV-1) integrase was expressed in *Escherichia coli* and purified using nickel-chelated column in an one-step manner. Aliquots of HIV-1 integrase of 0.5 mg/ml as stock solutions were stored at −70 °C until used.

3.4.2. Oligonucleotide substrates

Two 20-mer oligonucleotides whose sequences resemble the end of U5-LTR were obtained from Korea Biotech. Inc.: K16 (U5-LTR, +strand), 5′-TGTGGAAAATCTCTAGCAGT-3′; K17 (U5-LTR, -strand), 5′-ACTGCTAGAGATTTTCCACA-3′. The oligonucleotides were purified by 20% polyacrylamide gel before use. In order to construct oligonucleotide substrate, oligonucleotide K16 of 30 pmol was labeled at the 5′ end using [-^{32}P]-ATP of 250 Ci (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham) and T4 polynucleotide kinase (T4 PNK, New England Biolabs) of 10 U in 40 l of reaction buffer (70 mM Tris–HCl [pH 7.6], 10 mM $MgCl_2$, 5 mM dithiothreitol) at 37 °C for 15 min. The labeling reaction was subjected to 10 mM EDTA, and heated to 85 °C for 15 min to inactivate T4 PNK. After addition of complementary oligonucleotide K17 of 30 pmol, the reaction mixture was boiled for 3 min and cooled down slowly. Labeled substrate was separated from unincorporated nucleotide by passage through a Biospin 6 (Bio-Rad).

3.4.3. HIV-1 integrase reaction

A standard reaction assay of the endonucleolytic activity was carried out in the presence of potential inhibitor containing 0.1 pmol of duplex oligonucleotide substrate and 15 pmol of HIV-1 integrase in 15 mM Tris–HCl (pH 7.4), 100 mM NaCl, 1 mM $MnCl_2$, 2 mM 2-mercaptoethanol, 2.5 mM CHAPS, 0.1 mM EDTA, 0.1 mM PMSF, 1% glycerol, and 10 mM imidazole in a total volume of 10 μl. Inhibitors or drugs were dissolved in 100% DMSO and added to the reaction to be 5% DMSO in the final volume. Reaction mixtures were incubated at 33 °C for 90 min and stopped by addition of 4 μl of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF. The reactions were heated to 90 °C for 3 min and electrophoresed on a 20% denaturing polyacrylamide gel. Reaction products were visualized by autoradiography of the wet gel. IC_{50} was calculated by scanning bands on Kodak-5 film (Image Master VDS, Pharmacia Biotech.).

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