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Styrylquinazoline Derivatives as HIV-1 Integrase Inhibitors

Styrylquinazoline derivatives were prepared by Perkin condensation and evaluated for inhibitory activity against HIV-1 integrase. Among them, compound **5 c** containing a free catechol ring was the most potent (IC_{50} = 20.8 ± 1.9 μM) and showed 6-fold more potency than the corresponding styrylquinoline compound (IC_{50} = 130.7 ± 8.6 μM).

Keywords: HIV; Integrase inhibitor; Styrylquinazoline; Perkin condensation; AIDS

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Introduction

The alarming spread of the acquired immune deficiency syndrome (AIDS) epidemic has stimulated the discovery of therapeutic agents to inhibit the replication of the causative virus, human immunodeficiency virus (HIV) [1]. The advanced understanding of the viral cell cycle has made it possible to define the targets to interrupt the life cycle of the virus. Among them, one such target is the viral integrase which is responsible for the integration of proviral DNA into the host cell DNA. It catalyzes two distinct reactions: terminal cleavage at each 3′ end of the proviral DNA removing a pair of bases and strand transfer which results in the joining of each 3′ end to 5′-phosphates in the target DNA [2]. As these reactions are essential for the life cycle of the virus, integrase represents an attractive target for treatment of HIV infections. While a large number of compounds that inhibit integrase have already been identified [3], only a handful displayed antiviral activity in cell culture. To date, only G-rich oligonucleotide (Zentivir), which was shown to inhibit integrase activity both *in vitro* and *in vivo*, has been undergoing phase I/II clinical studies in AIDS patients [4]. However, the clinical use of such an oligonucleotide has a limit because there is a problem of the capacity to produce amounts of the compound compatible with therapeutic exigencies [5].

Recently, it was found that the styrylquinoline derivatives (**I**) inhibited HIV-1 integrase *in vitro* and blocked HIV-1 replication in CEM cells at nontoxic concentrations as shown in Figure 1 [6].

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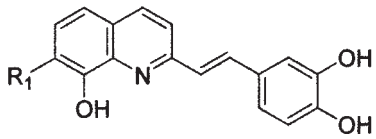
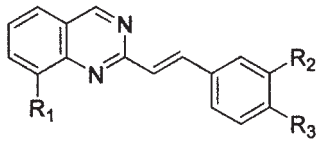


Styrylquinolines (I)

a: R_1 = H (IC_{50} = 7.4 μM)[6]

b: R_1 = CO_2H (IC_{50} = 2.4 μM)[6]



Styrylquinazolines (II)

R_1 = H, OH, OCH_3

R_2, R_3 = H, OH, OCH_3, OAc,

Figure 1

Based upon this result and the various biological activities of the quinazoline ring system [7], we set out to synthesize styrylquinazoline derivatives (**II**) as isosters of styrylquinolines (**I**), with a view to investigating the effect of the quinazoline subunit on the inhibitory activity against HIV integrase and develop structurally simple inhibitors of HIV integrase (Figure 1). In this paper, we will describe the synthesis of new styrylquinazoline derivatives (**II**) and the inhibitory activity against HIV-1 integrase in 3′-processing reaction.

Full Paper

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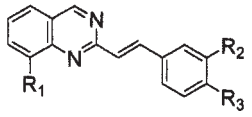
Results and discussion

Chemistry

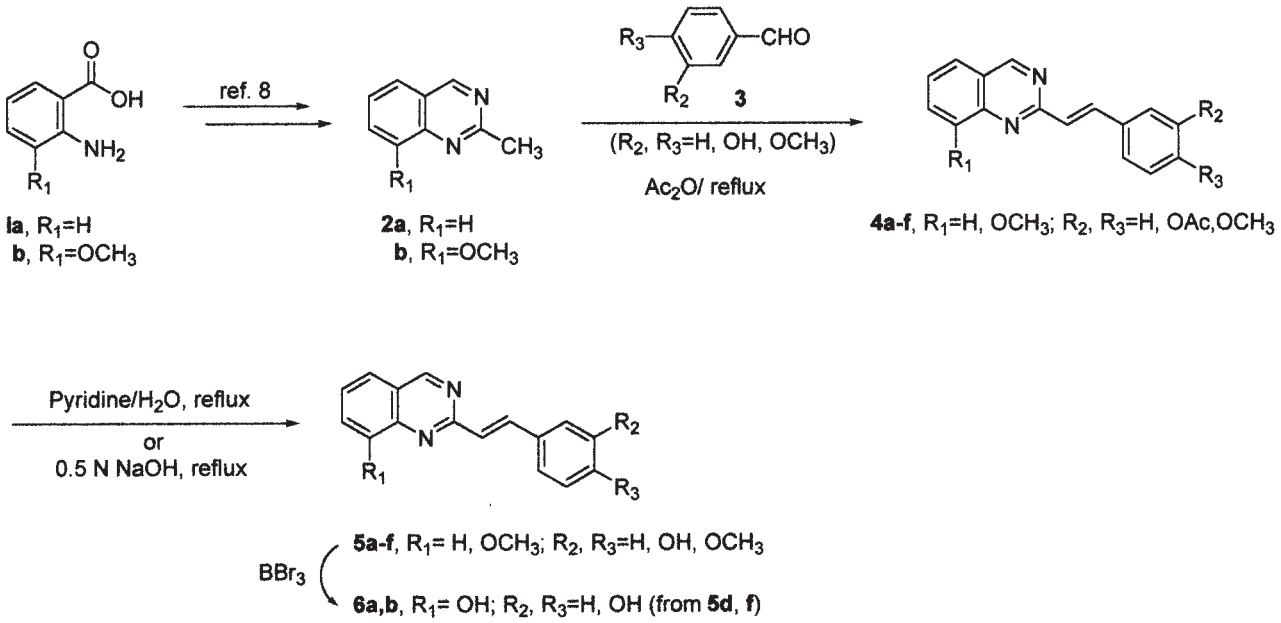
Two 2-methylquinazolines (**2 a**: R_1 = H, **2 b**: R_1 = OCH_3) as the starting materials were easily prepared from anthranilic acids (**1 a**: R_1 = H, **1 b**: R_1 = OCH_3), respectively, by known procedures in order to investigate the effect of the C-8 substituent on the quinazoline ring as well as the quinazoline scaffold on the inhibitory activity (Scheme 1) [8]. The Perkin condensation between 2-methylquinazoline (**2 a** or **2 b**) and various aromatic aldehydes (**3**) gave styrylquinazolines (**4 a–f**) with pure *E* geometry under reflux condition of acetic anhydride in 27–47 % yield, and all hydroxy groups of compounds **4 a–f** were concomitantly acetylated during this reaction [9]. In the meanwhile, it is well known to date that the most potent HIV integrase inhibitors such as L-chicoric acid and 4,5-dicaffeoylquinic acid (4,5-DCQA) generally contain a free

Table 1. Structures and yields of styrylquinazoline derivatives (**4 a–f**, **5 a–f**, **6 a**, and **6 b**).



No	R_1	R_2	R_3	Yield (%)[a]	No	R_1	R_2	R_3	Yield (%)[b]	No	R_1	R_2	R_3	Yield (%)
4a	H	H	OAc	27	**5a**	H	H	OH	56	**6a**	OH	H	OH	21[c]
4b	H	OAc	OCH_3	45	**5b**	H	OH	OCH_3	89	**6b**	OH	OH	OH	24[d]
4c	H	OAc	OAc	31	**5c**	H	OH	OH	45					
4d	OCH_3	H	OAc	47	**5d**	OCH_3	H	OH	17					
4e	OCH_3	OAc	OCH_3	47	**5e**	OCH_3	OH	OH	89					
4f	OCH_3	OAc	OAc	30	**5f**	OCH_3	OH	OH	63					

[a] Yield from **2a** or **2b**; [b] Yield from **4a-f**; [c] Yield from **5d**; [d] Yield from **5f**



Scheme 1

catechol-type aromatic moiety [10]. Therefore, the hydrolysis of acetoxy groups of styrylquinazolines (**4 a–f**) was accomplished with pyridine/water or 0.5 N NaOH (aq) to afford free hydroxystyrylquinazolines (**5 a–f**) in 21–87 % yield. With a view to testing the effect of another "free" hydroxy group of the quinazoline ring on the inhibitory activity against HIV-1 integrase, we also accomplished BBr_3-induced demethylation of C-8 methoxy group of compounds **5 d** and **5 f** to provide 8-hydroxyquinazolines (**6 a** and **6 b**) in 21 and 24 % yield, respectively, as shown in Scheme 1 [11]. The yields of all reactions were summarized in Table 1.

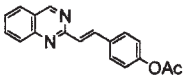
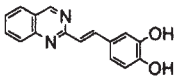
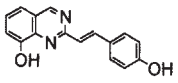
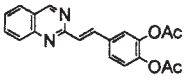
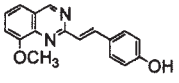
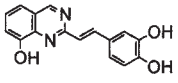
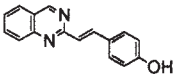
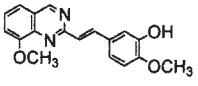
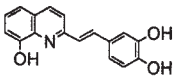
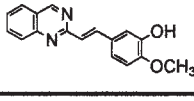
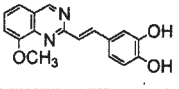
HIV-1 integrase inhibitory activity

Styrylquinazoline derivatives (**5 a–f**, **6 a**, **b**) were evaluated for inhibitory activity against 3′-processing of HIV-1 integrase [12] and the results are summarized in Table 2. To compare the data with those of standards, styrylquinoline compound (**I a**) in Figure 1 was prepared and its inhibitory activities under our assay conditions were inserted into the data set. In particular, the IC_{50} value (130.7 ± 8.6 μM) of styrylquinoline compound (**I a**) which we obtained is significant higher than the value found by previous workers (literature, IC_{50} = 7.4 μM) [6]. It is thought that the difference is at least partly attributable to the nature of the enzyme and substrate as well as the overall assay conditions. As shown in Table 2, most of compounds were largely divided into two classes according to the biological activity. For inactive compounds, in which the catechol moiety is *O*-acylated (**4 c** and **4 f**) and/or *O*-methylated (**5 b** and **5 e**), a complete lack of inhibitory activity ($IC_{50} \geqslant 200$ μM) was observed, and the compounds containing monoacetoxy (**4 a**) or monohydroxy group (**5 a**, **5 d**, and **6 a**) were also inactive irrespective of C-8 substituents of quinazoline ring.

The active compounds (**5 c**, **5 f**, and **6 b**), in which a free catechol ring is present, showed good inhibitory activities with IC_{50} values of 20.08 ± 1.9, 31.6 ± 6.1, and 57.1 ± 9.3 μM, respectively. This observation means that the free catechol moiety of styrylquinazoline compounds is required for the inhibitory activity against HIV-1 integrase. Among them, compound **5 c** bearing no substituent at C-8 was the most potent (IC_{50} = 20.08 ± 1.9 μM) and showed 6-fold more potency than the styrylquinoline compound (**I a**, IC_{50} = 130.7 ± 8.6 μM) under our assay condition. Compound **5 f** and **6 b**, however, bearing methoxy group and free hydroxy group, respectively, at the C-8 position of the quinazoline ring, displayed lower inhibitory activity (IC_{50} = 31.6 ± 6.1 and 57.1 ± 9.3 μM, respectively) than the parent compound (**5 c**). Nonetheless, compound **5 f** and **6 b** were more potent than the corresponding stryrylquinoline compound (**I a**). Unlike styrylquinoline compounds (**I**), in which the hydroxy group at C-8 was important for the inhibitory activity against HIV-1 integrase as well as the carboxyl group at C-7 of the quinoline ring [6], the introduction of a hydroxy group at C-8 in styrylquinazoline derivatives (**II**) resulted in the reduction of the inhibitory activity. Therefore, it seems that the binding mode of strylquinazoline derivatives to HIV-1 integrase would be different from that of styrylquinoline derivatives [6].

In conclusion, the new HIV-1 integrase inhibitors, which have a quinazoline ring as a scaffold, were synthesized and evaluated for integrase inhibitory activity. Among them, the compound (**5 c**) bearing no substituent at C-8 of the quinazoline subunit was the most potent and showed 6-fold greater potency than the corresponding

Table 2. HIV-1 integrase inhibitory activities of styrylquinazolines.

Entry	Structure	IC_{50} (μM)[a,b]	Entry	Structure	IC_{50} (μM)[a,b]	Entry	Structure	IC_{50} (μM)[a,b]
4a		>>200	5c		20.8±1.9	6a		>>200
4c		>>200	5d		>>200	6b		57.1±9.3
5a		>>200	5e		>>200	Ia[c]		130.7±8.6
5b		>>200	5f		31.6±6.1			

[a] Inhibitory activity against 3'-processing; [b] Assays were performed in three separate experiments; [c] Quinoline compound (**Ia**) was prepared by a known method [6] for comparison.

styrylquinoline compound (**I**). On the other hand, styrylquinazoline (**6 b**) bearing a free hydroxy group at C-8 of the quinazoline ring showed less potent activity than the parent compounds (**5 c** and **5 f**). Therefore, it is necessary to study further the binding mode of styrylquinazoline derivatives to HIV-1 integrase for the development of novel antiviral agents based upon quinazoline ring as the scaffold. For more extensive QSAR and the development of more potent compound, we are undertaking the synthesis of styrylquinazolines containing a carboxyl group at the C-7 position of the quinazoline ring.

Acknowledgements

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Experimental

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 (mp) 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 chromatography (TLC) was carried out on precoated silica gel (E. Merck Kiesegel 60F_{254} layer thickness 0.25 mm). Flash column chromatography was performed with Merck Kiesegel 60 Art 9385 (230–400 mesh). All solvents used were purified according to standard procedures.

Perkin condensation between quinazoline and various aromatic aldehydes

(E)-2-[2-(4-Acetoxyphenyl)ethenyl]quinazoline (**4 a**)

To a solution of 2-methylquinazoline (**2 a**) (100 mg, 0.7 mmol) in 3 mL of acetic anhydride was added 4-hydroxybenzaldehyde (341 mg, 2.1 mmol). The reaction mixture was heated under reflux for 3 days. After cooling to rt, the mixture was poured into a mixture of saturated $NaHCO_3$ solution and CH_2Cl_2. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts was dried over $MgSO_4$, concentrated, and purified by flash column chromatography (EtOAc : CH_2Cl_2 = 1 : 5) to give **4 a** (54 mg, 27 %) as a yellow solid: mp 108–112 °C; IR (KBr) 2934, 2552, 1756, 1686, 1426, 1372, 1198, 914, 758, 504 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.42 (1 H, s, quinazoline-*H*4), 8.18–8.12 (3 H, m, C–*CH*=CH-phenyl, phenyl-*H*2′6′), 8.06 (1 H, d, *J* = 8.8 Hz, quinazoline-*H*8), 7.92–7.88 (1 H, m, quinazoline-*H*7), 7.71 (1 H, d, *J* = 8.6 Hz, quinazoline-*H*5), 7.62–7.57 (1 H, m, quinazoline-*H*6), 7.45 (1 H, d, *J* = 16.0 Hz, C–CH=*CH*-phenyl), 7.23–7.19 (2 H, d, *J* = 8.7 Hz, phenyl-*H*3′,5′), 2.33 (3 H, s, –O*Ac*); ^{13}C NMR ($CDCl_3$) δ 170.0, 160.9, 160.6, 138.2, 135.0, 134.8, 132.1, 129.3, 129.0, 128.1, 127.6, 122.8, 122.0, 121.8, 21.6.

(E)-2-[2-(3-Acetoxy-4-methoxyphenyl)ethenyl]quinazoline (**4 b**)

A similar procedure to that for the preparation of **4 a** using 2-methylquinazoline (**2 a**) (100 mg, 0.7 mmol) and 3-hydroxy-4-methoxybenzaldehyde (135 mg, 2.1 mmol) afforded **4 b** (94 mg, 31 %) as a yellow solid: mp 110–112 °C; IR (KBr) 3008, 1764, 1600, 1506, 1376, 1214, 1018, 750, 470 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.30 (1 H, s, quinazoline-*H*4), 8.06 (1 H, d, *J* = 15.8 Hz, C–*CH*=CH-phenyl), 7.95 (1 H, d, *J* = 8.4 Hz, quinazoline-*H*8), 7.84–7.81 (2 H, m, quinazoline-*H*5,7), 7.51–7.45 (2 H, m, quinazoline-*H*6 and phenyl-*H*6′), 7.36 (1 H, s, phenyl-*H*2′), 7.26 (1 H, d, *J* = 15.8 Hz, C-CH=*CH*-phenyl), 6.96 (1 H, d, *J* = 8.6 Hz, phenyl-*H*5′), 3.83 (3 H, s, –OCH_3), 2.33 (3 H, s, –O*Ac*); ^{13}C NMR ($CDCl_3$) δ 168.9, 161.2, 160.3, 160.1, 151.8, 150.5, 140.0, 137.4, 134.2, 129.5, 128.0, 126.7, 123.3, 121.6, 112.4, 103.0, 55.9, 20.5.

(E)-2-[2-(3,4-Diacetoxyphenyl)ethenyl]quinazoline (**4 c**)

A similar procedure to that for the preparation of **4 a** using 2-methylquinazoline (**2 a**) (70 mg, 0.4 mmol) and 3,4-dihydroxybenzaldehyde (221 mg, 1.6 mmol) afforded **4 c** (157 mg, 45 %) as a yellow solid: mp 120–123 °C; IR (KBr) 2930, 2674, 1758, 1376, 1216, 1108, 1014, 754, 582 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.37 (1 H, s, quinazoline-*H*4), 8.12 (1 H, d, *J* = 15.9 Hz, C-*CH*=CH-phenyl), 8.01 (1 H, d, *J* = 8.0 Hz, quinazoline-*H*8), 7.92–7.87 (2 H, m, quinazoline-*H*5,7), 7.63–7.60 (1 H, m, quinazoline-*H*6), 7.58–7.56 (1 H, dd, *J* = 8.4, 2.0 Hz, phenyl-*H*2′), 7.50 (1 H, d, *J* = 2.0 Hz, phenyl-*H*6′), 7.39 (1 H, d, *J* = 15.9 Hz, C-CH=*CH*-phenyl), 7.25 (1 H, d, *J* = 8.4 Hz, phenyl-*H*5′), 2.33 (3 H, s, -O*Ac*), 2.31 (3 H, s, -O*Ac*); ^{13}C NMR ($CDCl_3$) δ 168.6, 161.3, 160.7, 150.9, 142.9, 142.8, 137.0, 135.6, 134.7, 129.5, 128.6, 127.8, 127.6, 126.2, 124.2, 123.8, 122.7, 21.1.

(E)-8-Methoxy-2-[2-(4-acetoxyphenyl)ethenyl]quinazoline (**4 d**)

A similar procedure to that for the preparation of **4 a** using 2-methyl-8-methoxyquinazoline (**2 b**) (120 mg, 0.7 mmol) and 4-hydroxybenzaldehyde (337 mg, 2.8 mmol) afforded **4 d** (77 mg, 47 %) as a yellow solid: mp 149–152 °C; IR (KBr) 3450, 2932, 2364, 1760, 1558 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.35 (1 H, s, quinazoline-*H*4), 8.15 (1 H, d, *J* = 16.0 Hz, C-*CH*=CH-phenyl), 7.69 (2 H, d, *J* = 8.6 Hz, phenyl-*H*2′,6′), 7.55–7.46 (3 H, m, quinazoline-*H*5,7 and C-CH=*CH*-phenyl), 7.25 (1 H, dd, *J* = 7.3 Hz, 1.5 Hz, quinazoline-*H*6), 7.15 (1 H, d, *J* = 8.6 Hz, phenyl-*H*3′,5′), 4.12 (3 H, s, -OCH_3), 2.32 (3 H, s, -O*Ac*); ^{13}C NMR ($CDCl_3$) δ 169.7, 161.0, 160.4, 155.0, 151.5, 137.5, 134.5, 129.1, 128.9, 127.8, 124.6, 122.3, 119.1, 112.4, 56.6, 21.5.

(E)-8-Methoxy-2-[2-(3-acetoxy-4-methoxyphenyl)ethenyl]-quinazoline (**4 e**)

The similar procedure for the preparation of **4 a** using 2-methyl-8-methoxyquinazoline (**2 b**) (250 mg, 1.4 mmol) and 3-hydroxy-4-methoxybenzaldehyde (656 mg, 4.3 mmol) afforded **4 e** (372 mg, 73 %) as a bright yellow amorphous solid: mp 60–65 °C; IR (KBr) 2930, 2366, 1766, 1610, 1512, 1268, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.20 (1 H, s, quinazoline-*H*4), 7.97 (1 H, d, *J* = 15.9 Hz, C-*CH*=CH-phenyl), 7.41–7.32 (5 H, m, quinazoline-*H*5,6,7, phenyl-*H*2′ and C-CH=*CH*-phenyl), 7.09 (1 H, d, *J* = 8.4 Hz, phenyl-*H*6′), 6.89 (1 H, d, *J* = 8.4 Hz, phenyl-*H*5′), 3.98 (3 H, s, -OCH_3), 3.75 (3 H, s, -OCH_3), 2.23 (3 H, s, -O*Ac*); ^{13}C NMR ($CDCl_3$) δ 169.4, 160.9, 160.3, 154.8, 152.1, 142.9, 140.3, 137.4, 129.9, 127.7, 127.4, 127.1, 124.4, 122.1, 119.1, 112.8, 112.3, 56.6, 56.3, 21.1.

(E)-8-Methoxy-2-[2-(3,4-diacetoxyphenyl)ethenyl]quinazoline (**4 f**)

A similar procedure to that for the preparation of **4 a** using 2-methyl-8-methoxyquinazoline (**2 b**) (70 mg, 0.4 mmol) and 3,4-dihydroxybenzaldehyde (221 mg, 1.6 mmol) afforded **4 f**

(103 mg, 47 %) as a yellow solid: mp 138–141 °C; IR (KBr) 2947, 1760, 1560, 1378, 1258, 1214, 1012, 898, 762 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.34 (1 H, s, quinazoline-*H*4), 8.10 (1 H, d, *J* = 16.0 Hz, C-*CH*=CH-phenyl), 7.56–7.44 (5 H, m, quinazoline-*H*5,6,7 and phenyl-*H*2′, C-CH=*CH*-phenyl), 7.24 (2 H, m, phenyl-*H*5′,6′), 4.11 (3 H, s, -OCH_3), 2.33 (3 H, s, -O*Ac*), 2.31 (3 H, s, -O*Ac*); ^{13}C NMR ($CDCl_3$) δ 168.5, 160.7, 160.4, 155.0, 142.8, 136.7, 135.8, 129.8, 127.9, 126.1, 124.6, 124.1, 122.7, 119.1, 112.4, 56.6, 21.0.

*General procedure for deacetylation of acetylated compounds (***4 a**, **4 b**, and **4 e**)

(E)-2-[2-(4-Hydroxyphenyl)ethenyl]quinazoline (**5 a**)

(E)-2-[2-(4-Acetoxyphenyl)ethenyl]quinazoline (**4 a**) (46 mg, 0.2 mmol) was dissolved in 2 mL of 0.5 N NaOH, and the resulting solution was heated under reflux for 10 min. After cooling to rt, the reaction mixture was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and concentrated to give **5 a** (22 mg, 56 %) as a white solid: mp 227–230 °C (dec); IR (KBr) 3012, 2362, 1598, 1454, 1402, 1254, 1172, 974, 828, 762, 642 cm^{-1}; ^1H NMR (CD_3OD) δ 9.42 (1 H, s, quinazoline-*H*4), 8.11 (1 H, d, *J* = 15.9 Hz, C-*CH*=CH-phe2nyl), 8.03 (1 H, d, *J* = 8.1 Hz, quinazoline-*H*8), 7.94–7.92 (2 H, m, quinazoline-*H*5,7), 7.66–7.61 (1 H, m, quinazoline-*H*6), 7.56 (2 H, d, *J* = 8.6 Hz, phenyl-*H*2′,6′), 7.19 (1 H, d, *J* = 15.9 Hz, C-CH=*CH*-phenyl), 6.86 (2 H, d, *J* = 8.6 Hz, phenyl-*H*3′,5′); ^{13}C NMR (CD_3OD) δ 149.7, 149.3, 148.8, 138.4, 127.8, 123.1, 117.8, 117.4, 115.8, 115.3, 111.8, 111.5, 104.0, 103.3.

(E)-2-[2-(3-Hydroxy-4-methoxyphenyl)ethenyl]quinazoline (**5 b**)

A similar procedure to that for the preparation of **5 a** using compound **4 b** (50 mg, 0.2 mmol) afforded **5 b** (42 mg, 89 %) as a yellow solid: mp 107–110 °C; IR (KBr) 3142, 1608, 1506, 1398, 1272, 1030, 978, 758 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.35 (1 H, s, quinazoline-*H*4), 8.09 (1 H, d, *J* = 15.9 Hz, C-*CH*=CH-phenyl), 8.00 (1 H, d, *J* = 8.8 Hz, quinazoline-*H*8), 7.90–7.85 (2 H, m, quinazoline-*H*5,7), 7.59–7.55 (1 H, m, quinazoline-*H*6), 7.29 (1 H, s, phenyl-*H*2′), 7.24–7.16 (1 H, m, C-CH=*CH*-phenyl and phenyl-*H*6′), 6.89 (1 H, d, *J* = 8.3 Hz, phenyl-*H*5′), 3.91 (3 H, s, -OCH_3); ^{13}C NMR ($CDCl_3$) δ 161.8, 159.4, 139.8, 137.7, 135.6, 133.5, 129.5, 128.4, 127.4, 126.3, 125.5, 122.3, 120.2, 114.4, 112.1, 110.0, 55.4.

*General procedure for deacetylation of acetylated compounds (***4 c**, **4 d**, *and* **4 f**)

(E)-2-[2-(3,4-Dihydroxyphenyl)ethenyl]quinazoline (**5 c**)

(E)-2-[2-(3,4-Diacetoxyphenyl)ethenyl]quinazoline **4** c (254 mg, 0.7 mmol) was dissolved in 1.5 mL of pyridine, and the resulting solution was heated under reflux for 4 h. 1 mL of water was then added to the mixture and the reaction mixture was further heated under reflux for 1 h. After cooling to rt, the reaction mixture was extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and concentrated to leave a solid, which was recrystallized from EtOAc and ether to provide **5 c** (209 mg, 60 %) as a yellow solid: mp 204–208 °C (dec.); IR (KBr) 3545, 3066, 2378, 1608, 1406 cm^{-1}; ^1H NMR (acetone-d_6) δ 9.56 (1 H, s, quinazoline-*H*4), 8.17 (1 H, d, *J* = 15.9 Hz, C-*CH*=CH-phenyl), 8.16 (1 H, d, *J* = 8.0 Hz, quinazoline-*H*8), 8.06–7.99 (2 H, m, quinazoline-*H*5,7), 7.75–7.70 (1 H, m, quinazoline-*H*6), 7.36 (1 H, d, *J* = 2.0 Hz, phenyl-*H*2′), 7.28 (1 H, d, *J* = 15.9 Hz, C-CH=*CH*-phenyl), 7.23 (1 H, dd, *J* = 8.2 Hz, 2.0 Hz, phenyl-*H*6′), 7.00 (1 H, d, *J* = 8.2 Hz, phenyl-*H*5′); ^{13}C NMR (acetone-d_6) δ 162.9, 161.6, 151.9, 148.1, 146.7, 139.7, 135.4, 129.9, 129.1, 128.8, 128.1, 126.5, 124.6, 122.1, 116.8, 115.1.

(E)-8-Methoxy-2-[2-(4-hydroxyphenyl)ethenyl]quinazoline (**5 d**)

A similar procedure to that for the preparation of **5 c** using compound **4 d** (254 mg, 0.7 mmol) afforded **5 d** (37 mg, 17 %) as a yellow solid: mp 170–174 °C; IR (KBr) 2928, 1604, 1560, 1514 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.28 (1 H, s, quinazoline-*H*4), 8.02 (1 H, d, *J* = 16.0 Hz, C-*CH*=CH-phenyl), 7.57–7.34 (4 H, m, quinazoline-*H*5,6,7 and C-CH=*CH*-phenyl), 7.28–7.17 (2 H, m, phenyl-*H*2′,6′), 6.82 (2 H, d, *J* = 8.5 Hz, phenyl-*H*3′, 5′), 4.05 (3 H, s, -OCH_3); ^{13}C NMR ($CDCl_3$) δ 163.9, 163.2, 161.6, 157.3, 145.0, 142.0, 133.4, 132.4, 130.9, 130.6, 127.0, 121.9, 118.8, 115.6, 58.9.

(E)-8-Methoxy-2-[2-(3-hydroxy-4-methoxyphenyl)ethenyl]-quinazoline (**5 e**)

A similar procedure to that for the preparation of **5 a** using compound **4 e** (50 mg, 0.2 mmol) afforded **5 e** (30 mg, 65 %) as a bright yellow solid: mp 197–200 °C (dec.); IR (KBr) 3456, 1624, 1572, 1466, 1344, 986, 762,474 cm^{-1}; ^1H NMR (CD_3OD) δ 9.30 (1 H, s, quinazoline-*H*4), 8.04 (1 H, d, *J* = 15.9 Hz, C-*CH*=CH-phenyl), 7.54–7.45 (2 H, m, quinazoline-*H*5,7), 7.32–7.25 (2 H, m, C-CH=*CH*-phenyl and quinazoline-*H*6), 7.11 (1 H, d, *J* = 1.6 Hz, phenyl-*H*2′), 6.89 (1 H, dd, *J* = 8.1, 1.6 Hz, phenyl-*H*6′), 6.81 (1 H, d, *J* = 8.2 Hz, phenyl-*H*5′), 4.30 (3 H, s, -OCH_3), 4.10 (3 H, s, -OCH_3); ^{13}C NMR (CD_3OD) δ 162.9, 161.8, 158.0, 155.7, 154.9, 143.7, 142.7, 131.2, 128.8, 125.5, 124.3, 120.4, 117.7, 117.1, 114.3, 112.0, 57.0, 56.3.

(E)-8-Methoxy-2-[2-(3,4-dihydroxyphenyl)ethenyl]quinazoline (**5 f**)

A similar procedure to that for the preparation of **5 c** using compound **4 f** (80 mg, 0.3 mmol) afforded **5 f** (80 mg, 63 %) as a yellow solid: mp 204–208 °C; IR (KBr) 3198, 2930, 1562, 1518 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.47 (1 H, s, quinazoline-*H*4), 7.93 (1 H, d, *J* = 15.9 Hz, C-*CH*=CH-phenyl), 7.61–7.56 (2 H, m, quinazoline-*H*5,7), 7.41 (1 H, d, *J* = 7.3 Hz, quinazoline-*H*6), 7.13–7.04 (3 H, m, phenyl-*H*2′,6′, C-CH=*CH*-phenyl), 6.81 (1 H, d, *J* = 8.1 Hz, phenyl-*H*5′), 4.11 (3 H, s, -OCH_3); ^{13}C NMR (DMSO-d_6) δ 160.6, 160.4, 154.5, 147.5, 145.9, 138.4, 127.7, 124.9, 124.0, 120.7, 119.1, 116.2, 114.5, 113.3, 55.2.

General procedure for demethylation of compounds (**5 d** *and* **5 f**)

(E)-8-Hydroxy-2-[2-(4-hydroxyphenyl)ethenyl]quinazoline (**6 a**)

To a mixture of (*E*)-8-methoxy-2-[2-(4-hydroxyphenyl)ethenyl]-quinazoline (**5 d**) (55 mg, 0.2 mmol) in 8 mL of CH_2Cl_2 was added BBr_3 (1 M solution in *n*-hexane, 1.7 mL, 1.7 mmol) under N_2 atmosphere. The reaction mixture was stirred at rt for 18 h and quenched with water. The organic layer was separated, washed with saturated $NaHCO_3$ solution and brine successively, and dried over $MgSO_4$. After concentration, the residue was purified by flash column chromatography (EtOAc : *n*-hexane = 3 : 2) to afford **6 a** (11 mg, 21 %) as a brown-yellow solid: mp 197–200 °C (dec.); IR (KBr) 3148, 2930, 1602, 1560 cm^{-1}; ^1H NMR (CD_3OD) δ 9.22 (1 H, s, quinazoline-*H*4), 8.01 (1 H, d, *J* = 16.0 Hz, C-*CH*=CH-phenyl), 7.46 (2 H, d, *J* = 8.5 Hz, phenyl-*H*2′,6′), 7.37 (2 H, m, quinazoline-*H*5,7), 7.21 (1 H, m, quiazoline-*H*6), 7.17 (1 H, d, *J* =16.0 Hz, C-CH=*CH*-phenyl), 6.76 (1 H, d, *J* = 8.5 Hz, phenyl-*H*3′,5′); ^{13}C NMR (CD_3OD) δ 161.7, 160.3, 154.0, 142.8, 140.2, 130.8, 129.6, 129.5, 125.5, 119.2, 117.6, 117.2, 116.2.

(E)-8-Hydroxy-2-[2-(3,4-dihydroxyphenyl)ethenyl]quinazoline (**6 b**)

A similar procedure to that for the preparation of **6 a** using compound **5 f** (117 mg, 0.3 mmol) afforded **6 b** (22 mg, 24 %) as a yellow solid: mp 160 °C (dec.); IR (KBr) 3390, 2364, 1562, 1470, 1392, 1278, 762 cm^{-1}; ^1H NMR (CD_3OD) δ 9.22 (1 H, s, quinazoline-*H*4), 7.95 (1 H, d, *J* = 15.9 Hz, C-*CH*=CH-phenyl), 7.36–7.34 (2 H, m, quinazoline-*H*5,7), 7.19 (1 H, t, *J* = 4.7 Hz, quinazoline-*H*6), 7.10 (1 H, d, *J* = 15.9 Hz, C-CH=*CH*-phenyl), 7.07 (1 H, d, *J* = 1.8 Hz, phenyl-*H*2′), 6.93 (1 H, dd, *J* = 8.2 Hz, *J* = 1.7 Hz, phenyl-*H*6′), 6.72 (1 H, d, *J* = 8.2 Hz, phenyl-*H*5′); ^{13}C NMR (CD_3OD) δ 160.3, 160.28, 152.7, 147.3, 145.7, 141.3, 139.2, 128.7, 128.0, 124.1, 123.9, 121.0, 117.7, 116.1, 115.5, 113.7.

Biological test

HIV-1 integrase

Recombinant human immunodeficiency virus type 1 (HIV-1) integrase was expressed in *Escherichia coli* and purified using a 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.

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′-ACTGCTAGA-GATTTTCCACA-3′. The oligonucleotides were purified with 20 % polyacrylamide gel before use. In order to construct oligonucleotide substrate, oligonucleotide K16 of 30 pmol was labeled at the 5′ end using [γ-^{32}P]-ATP of 250 μCi (3,000 Ci/mmol; 1 Ci = 37 GBq; Amersham) and T4 polynucleotide kinase (T4 PNK, New England Biolabs) of 10 units in 40 μL 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).

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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