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Linker-modified quinoline derivatives targeting HIV-1 integrase: synthesis and biological activity

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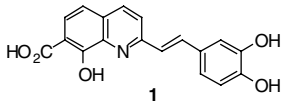
Abstract—A novel series of HIV-1 integrase inhibitors was synthesized and tested in both in vitro and ex vivo assays. These inhibitors are featured by the presence of a quinoline subunit and an ancillary aromatic ring linked by functionalized spacers such as amide, hydrazide, urea and 1-hydroxyprop-1-en-3-one moiety. Amide derivatives are the most promising ones and could serve as leads for further developments.

1. Introduction

AIDS is essentially a viral disease and should be treated with antiretroviral agents. Although the advent of combination therapy with reverse transcriptase and protease inhibitors has made it possible to suppress the replication of HIV-1 in infected persons to such an extent that it becomes almost undetectable in the plasma for more than two years, the virus persists in reservoirs such as peripheral blood mononuclear cells or resting T-lymphocytes. This means that AIDS can be temporarily controlled, but not eradicated with current treatments.[1–3] It is therefore important to identify new agents targeting HIV-1 at a step of its replicative cycle, which is not yet affected by the above combination therapy. In this respect, we have recently reported that polyhydroxylated styrylquinolines, exemplified by **1**, are micromolar inhibitors of the third essential enzyme of HIV-1: integrase (IN), block the replication of the virus in cell culture and are devoid of cytotoxicity (Fig. 1).[4]

Drugs of the styrylquinoline family are featured by the presence of a quinoline moiety connected to an ancillary aromatic nucleus by means of an ethylenic linker. To date, most of the synthetic efforts in the area have been directed towards modification of the two aromatic/heteroaromatic subunits.[4a,b,g,h]



1

Figure 1. Example of polyhydroxylated styrylquinoline.

Herein, we report the preliminary results of our expanded SAR investigation directed towards the replacement of the central ethylenic linker by functionalized spacers (amide, hydrazide, urea, 1-hydroxyprop-1-en-3-one moieties).

2. Preparation of the quinoline subunits

Although styrylquinolines of type **1** were efficiently elaborated through Perkin condensation between the known 8-hydroxyquinaldine-7-carboxylic acid **2a** and an aromatic aldehyde,[4a] it appeared that a synthetic sequence directed to the introduction of functionalized spacers should require from the outset the protection of

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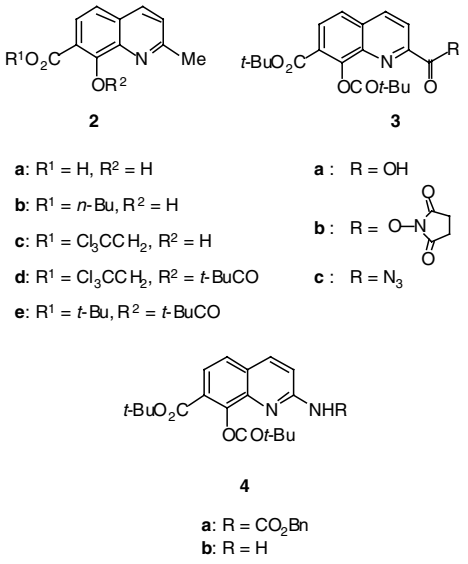


Figure 2. Quinoline subunits.

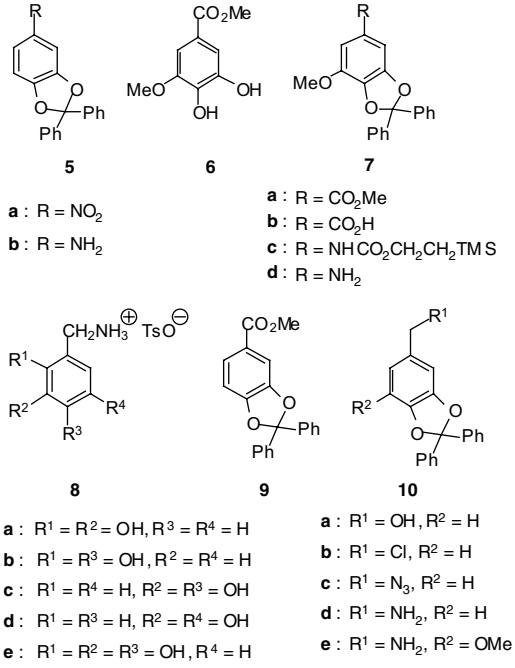


Figure 3. Ancillary aromatic subunits.

these phenolic and carboxylic acid functions. Esterification of the carboxylic acid function of **2a** was investigated first. Treatment of the latter compound with *n*-butanol at 100 °C in the presence of polyphosphoric acid (PPA)[5] gave the *n*-butyl ester **2b** with a 80% yield. However, the use of the strongly basic conditions required for the cleavage of this ester at the final stage having resulted in the destruction of target molecules, this protecting group was not suited to the task.

Alternatively, the 2,2,2-trichloroethyl ester **2c** was prepared by condensing **2a** with 2,2,2-trichloroethanol at 100 °C in the presence of PPA (60% yield). However, the target molecules were found to be again deteriorated under the conditions required for the cleavage of this ester function (Zn, AcOH[6] or Se, $NaBH_4$).[7]

The group that was ultimately adopted for protecting the carboxylic acid of **2a** was the *tert*-butyl ester. For that purpose, the hydroxyl of **2c** was first protected as the pivaloyl derivative **2d** (*t*-BuCOCl, pyridine, 85% yield), which was next transesterified into **2e** (*t*-BuONa, 20 °C, THF, 60% yield). Benzylic oxidation of **2c** (SeO_2, refluxing pyridine)[8] afforded with a 80% yield the acid **3a**, which was condensed with *N*-hydroxysuccinimide in the presence of DCC to give **3b** (80% yield). Condensation of NaN_3 with **3b** (DMF) led quantitatively to azide **3c**, which was converted into carbamate **4a** (BnOH, refluxing toluene, 57% yield). Finally, hydrogenolysis of **4a** (4 bar of H_2, Pd/C, EtOH) gave quantitatively the primary amine **4b** (Fig. 2).

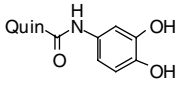
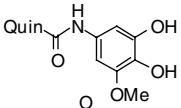
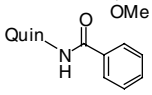
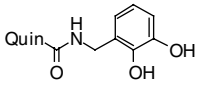
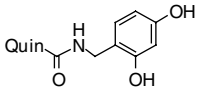
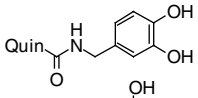
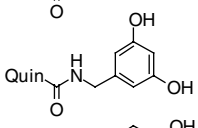
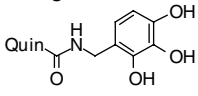
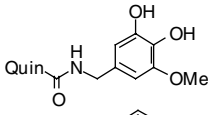
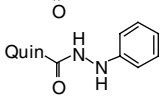
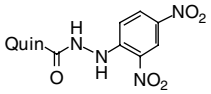
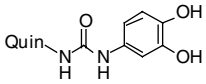
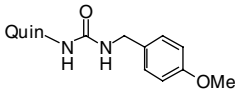
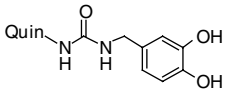
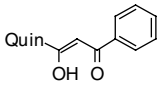
3. Preparation of the ancillary aromatic subunits

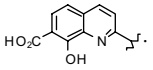
Aniline **5b** was synthesized from the known nitro compound **5a**[9] (3 bar of H_2, Raney Ni, EtOH, 85%). Aniline **7d** was prepared from the known ester **6**[10] (**6** → **7a**: Ph_2CCl_2, neat, 170 °C, 94%;[11] **7a** → **7b**: 3 N NaOH, MeOH, 96%; **7b** → **7c**: $(PhO)_2P(O)N_3$, TMS–CH_2–CH_2OH, Et_3N, 80 °C, toluene, 89%;[12] **7c** → **7d**: 1 N *n*-Bu_4NF in THF, 50 °C, CH_3CN, 80%). Benzylamines **8a**–**e** (as their tosylate salts) were prepared according to known procedures.[13] Amine **10d** was synthesized from the known ester **9**[14] (**9** → **10a**: LAH, 88%; **10a** → **10b**: MsCl, cat. DMAP, 86%; **10b** → **10c**: NaN_3, DMF, quantitative; **10c** → **10d**: PPh_3, THF–H_2O, 54%). Amine **10e** was prepared in a similar fashion from ester **7a** (Fig. 3).

4. Synthesis and biological activity of the target molecules

The synthesis of the target molecules was realized by coupling first the quinoline subunits with the ancillary aromatic moieties under the operating conditions reported below. Subsequent removal of the protecting groups was performed in all cases by treating the resulting adducts with TFA at 20 °C in CH_2Cl_2 in the presence of guaiacol.[15] Amides **11** and **12** were prepared in 75% yield by condensing **3b** with **5b** and **7d**, respectively, at 20 °C in dry pyridine. Amide **13** was prepared from **4b** (PhCOCl, pyridine, cat. DMAP, 70%). Amides **14**–**19** were synthesized from **3b** and the corresponding benzylamines **8a**–**e** and **10e** (pyridine, 20 °C, 60–90%). Hydrazides **20** and **21** were obtained by condensing **3b** with phenylhydrazine and 2,4-dinitrophenylhydrazine, respectively (pyridine, 20 °C, 75–95%). Ureas **22**–**24** were prepared by heating azide **3c** in refluxing toluene until complete nitrogen evolution (2–5 min), followed by all at once addition of the requisite amines: **5b**, *p*-methoxybenzylamine and **10d**, respectively (25–40%). Ketoenol **25** was synthesized by condensing the lithium

Table 1. Structure–activity relationships for linker-modified quinoline derivatives[16]

	Compound[a]	Biological activity		
		IC_{50}[b]	IC_{50}[c]	TC_{50}[d]
11		0.9	30	>100
12		6.5	2	100
13		>100	>100	>100
14		5	40	>100
15		5	10	>100
16		5	2	>100
17		>100	>100	>100
18		1.5	4	>100
19		6.5	25	100
20		>100	45	>100
21		3	>100	>100
22		>100	>100	70
23		>100	>100	>100
24		>100	55	>100
25		30	>100	>100

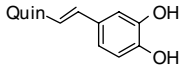
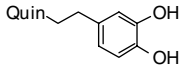
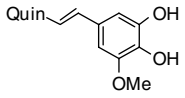
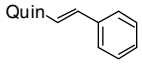
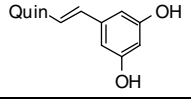
[a] Quin = 

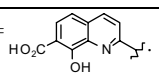
[b] In vitro activity on the 3′-end-processing assay (μM).
[c] Ex vivo (μM).
[d] Toxicity.

enolate of benzophenone (preformed by means of LDA in THF at −78 °C) with **3b** (THF, −78 °C, 70%).

In vitro IC_{50} was determined as the drug concentration that inhibits 50% of the recombinant integrase activity in a standard 3′-processing assay.[4a] Ex vivo IC_{50} was determined as the drug concentration that inhibits 50% of viral particles production in de novo infection assay of CEM cells. TC_{50} was the drug concentration that corresponds to 50% of cells survival as determined by a standard MTT assay.[4b] Results are illustrated in Table 1. Remarkably, all these compounds exhibit no significant cytotoxicity; in contrast, the styrylquinoline **28** in which the ancillary phenyl ring is unsubstituted and compound **26**, equivalent to **1**, in which the ethylenic linker has been hydrogenated, proved to be notably cytotoxic (Table 2).[4a,b] Among amides **11**–**19**, compound **13**, in which the nitrogen atom is attached to the quinoline subunit, and amide **17** in which the ancillary phenyl nucleus possesses two hydroxyls at C-3′ and C-5′ display no in vitro activity. In contrast the corresponding styryquinolines **28** and **29** show a significant in vitro activity (Table 2). Other amides where the phenyl half is substituted by at least a hydroxyl group at C-4′ show good activities in both in vitro and ex vivo assays. It is worthy of note that a nearly micromolar antiviral activity was observed with amides **12**, **16** and **18**, comparable to that of reference styryquinolines **1** and **27** (Table 2). Hydrazides **20**, **21** and ureas **22**–**24** are devoid of biological activity, with the exception of the 2,4-dinitrophenylhydrazide **21**, for which a substantial anti-integrase activity was restored. Since the ketoenol linker has been recognized as a potential pharmacophore in the design of HIV-IN inhibitors,[3] we have also evaluated the activity of **25**. However, no antiviral activity was observed with this compound, possibly because of the absence of hydroxyl groups on the ancillary phenyl ring. In conclusion, among the various linker-modified

Table 2. Biological activity for reference styrylquinoline derivatives[4a,b]

	Compound[a]	Biological activity		
		IC_{50}[b]	IC_{50}[c]	TC_{50}[d]
1		2.4	1	>100
26		2.3	NR	61
27		0.7	12	>100
28		5.3	NR[e]	31
29		3.2	>100	>100

[a] Quin = 

[b] In vitroactivity on the 3′-end-processing assay (μM).
[c] Ex vivo (μM).
[d] Toxicity.
[e] NR: not reached.

quinoline derivatives synthesized within this paper, amides are the most promising ones and could serve as leads for further developments.

Acknowledgement

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15. Guaiacol was used as *tert*-butyl cation scavenger to prevent the formation of by-products issuing from the recapture of this cation. In this respect, see: Yajima, H.; Takeyama, M.; Kanaki, J.; Nishimura, O.; Fujino, M. *Chem. Pharm. Bull.* **1978**, *26*, 3752–3757.
16. Data for **11**: yellow solid, mp >350 °C (dec); IR (neat, cm^{-1}) ν 3600–2500, 1645, 1601; ^1H NMR (200 MHz, DMSO-d_6) δ 10.34 (br s, 1H), 8.38 (d, $J = 8.6$ Hz, 1H), 8.30 (br s, 1H), 8.17 (d, $J = 8.6$ Hz, 1H), 7.95 (d, $J = 8.3$ Hz, 1H), 7.44 (d, $J = 1.5$ Hz, 1H), 7.03 (m, 2H), 6.77 (d, $J = 8.3$ Hz, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 171.1 (C), 165.3 (C), 162.3 (C), 147.1 (C), 145.2 (C), 142.2 (C), 140.2 (C), 137.6 (CH), 132.5 (C), 130.4 (C), 129.6 (CH), 119.7 (CH), 115.4 (CH), 114.4 (C), 111.0 (2CH), 109.0 (CH). Data for **13**: beige solid, mp 224–226 °C; IR (neat, cm^{-1}) ν 3400–2900, 1676, 1616, 1572; ^1H NMR (200 MHz, DMSO-d_6) δ 11.32 (br s, 1H), 8.42 (d, $J = 9.1$ Hz, 1H), 8.37 (d, $J = 9.1$ Hz, 1H), 8.09 (d, $J = 8.1$ Hz, 2H), 7.79 (d, $J = 8.6$ Hz, 1H), 7.57 (m, 3H), 7.38 (d, $J = 8.6$ Hz, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 172.2 (C), 166.9 (C), 158.5 (C), 150.9 (C), 138.8 (CH), 136.2 (C), 133.5 (C), 132.2 (CH), 129.5 (C), 128.2 (4CH), 124.7 (CH), 118.0 (CH), 116.7 (CH), 110.5 (C). Data for **16**: brown solid, mp 231–233 °C; IR (neat, cm^{-1}) ν 3600–2500, 1675, 1626; ^1H NMR (400 MHz, DMSO-d_6) δ 9.73 (t, $J = 5.9$ Hz, 1H), 8.86 (br s, 1H), 8.73 (br s, 1H), 8.54 (d, $J = 8.5$ Hz, 1H), 8.28 (d, $J = 8.5$ Hz, 1H), 7.92 (d, $J = 8.7$ Hz, 1H), 7.48 (d, $J = 8.7$ Hz, 1H), 6.79 (d, $J = 1.3$ Hz, 1H), 6.71 (d, $J = 8.1$ Hz, 1H), 6.64 (dd, $J = 8.1$, 1.3 Hz, 1H), 4.46 (d, $J = 5.9$ Hz, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 168.5 (C), 163.3 (C), 156.4 (C), 148.5 (C), 145.2 (C), 144.3 (C), 137.8 (CH), 137.2 (C), 131.6 (C), 130.1 (C), 128.9 (CH), 120.9 (CH), 118.4 (CH), 116.8 (CH), 115.4 (CH), 114.9 (CH), 112.9 (C), 42.0 (CH_2). Data for **20**: brown solid, mp 160–162 °C; IR (neat, cm^{-1}) ν 3600–2900, 1666, 1602, 1559; ^1H NMR (200 MHz, DMSO-d_6) δ 11.25 (br s, 1H), 8.57 (d, $J = 8.5$ Hz, 1H), 8.23 (d, $J = 8.5$ Hz, 1H), 7.95 (d, $J = 8.7$ Hz, 1H), 7.51 (d, $J = 8.7$ Hz, 1H), 7.18 (t, $J = 7.7$ Hz, 2H), 6.84 (d, $J = 7.7$ Hz, 2H), 6.75 (t, $J = 7.7$ Hz, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 167.6 (C), 163.1 (C), 155.5 (C), 149.1 (C), 147.6 (C), 137.8 (CH), 137.1 (C), 131.4 (C), 129.3 (CH), 128.7 (2CH), 120.6 (CH), 118.7 (CH), 116.7 (CH), 113.5 (C), 112.3 (2CH). Data for **24**: yellow solid, mp 241–243 °C (dec); IR (neat, cm^{-1}) ν 3600–2500, 1697, 1633, 1583; ^1H NMR (200 MHz, DMSO-d_6) δ 9.96 (br s, 1H), 9.82 (br s, 1H), 8.78 (m, 2H), 8.22 (d, $J = 8.8$ Hz, 1H), 7.68 (d, $J = 8.6$ Hz, 1H), 7.41 (d, $J = 8.8$ Hz, 1H), 7.27 (d, $J = 8.6$ Hz, 1H), 6.79 (s, 1H), 6.67 (m, 2H), 4.31 (d, $J = 5.4$ Hz, 2H). Data for **25**: yellow solid, mp 194–196 °C; IR (neat, cm^{-1}) ν 3600–2500, 1678, 1598; ^1H NMR (200 MHz, DMSO-d_6) δ 8.51 (d, $J = 8.6$ Hz, 1H), 8.29 (d, $J = 8.6$ Hz, 1H), 8.10 (m, $J = 7.4$ Hz, 2H), 7.94 (d, $J = 8.6$ Hz, 1H), 7.84 (s, 1H), 7.60 (m, 3H), 7.42 (d, $J = 8.6$ Hz, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 184.2 (C), 170.6 (C), 165.2 (C), 161.0 (C), 150.1 (C), 138.6 (C), 137.7 (CH), 134.2 (C), 133.2 (CH), 132.6 (C), 129.0 (2CH), 128.7 (CH), 128.6 (CH), 127.2 (2CH), 120.5 (CH), 111.8 (C), 93.9 (CH).