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New HIV-1 replication inhibitors of the styryquinoline class bearing aroyl/acyl groups at the C-7 position: Synthesis and biological activity

Marie Normand-Bayle,[a] Christophe Bénard,[a] Fatima Zouhiri,[a,b] Jean-François Mouscadet,[c] Hervé Leh,[b] Claire-Marie Thomas,[b] Gladys Mbemba,[c] Didier Desmaële[a,*] and Jean d'Angelo[a,*]

[a]*Unité Associée au CNRS, UMR 8076, Faculté de Pharmacie, 5, rue J.-B. Clément, 92290 Châtenay-Malabry, France*
[b]*BioAlliance Pharma SA, 59 bd du Général Martial Valin, 75015 Paris, France*
[c]*Unité Associée au CNRS, UMR 8113, Ecole Normale Supérieure de Cachan, 94235 Cachan, France*

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Abstract—Novel variants of HIV-1 replication inhibitors of the styrylquinoline class harboring aroyl/acyl group at the C-7 position have been synthesized. In sharp contrast with styrylquinolines bearing a carboxylic acid group at C-7, these compounds proved to be inactive toward HIV-1 integrase in in vitro assays.

1. Introduction

The substantial incidence of resistance observed in therapy-experienced patients and newly acquired HIV-1 infections underscores the need for new antiretroviral agents. All oral agents licensed to treat HIV-1 diseases target two of the three essential, virally encoded enzymes, reverse transcriptase and protease.[1] The third enzyme, integrase, inserts the viral DNA into the cellular genome through a multi-step process that includes two catalytic reactions, 3′-endonucleolytic processing of the viral DNA ends, and joining of the viral and cellular DNAs (strand transfer).[2] Although numerous classes of HIV-1 integrase inhibitors have been identified, only a few of them proved to be active against HIV-1 replication.[3]

We have reported that polyhydroxylated styrylquinolines (SQLs), exemplified by **1**, are potent HIV-1 integrase inhibitors in in vitro experiments, block the replication of HIV-1 in cell culture, and are devoid of cytotoxicity.[4]

Although the exact mechanism by which drug **1** and analogs exert their inhibitory potency remains unknown, it has been recently proposed that such drugs might act prior to integration by preventing viral DNA integrase binding.[5] Thus, SQLs constitute a unique class of integrase inhibitors, distinct from diketo acids (illustrated by L 731,988, **2**), which affect viral DNA integration.[3a] Within the framework of SQLs, we have identified the salicylic acid moiety at C-7 and C-8 of the quinoline ring, and the 4′-OH of the ancillary aromatic nucleus as critical pharmacophores for antiviral activity.[4b] Here, we report the preliminary results of our expanded SAR investigation of SQLs directed toward the replacement of the carboxylic group at C-7 by a variety of aroyl/acyl moieties as in **3**. The new designed SQLs were found to be inactive against HIV-1 integrase but some of them inhibited the HIV-1 replication in cell culture. Note that naphthyridine ketones **4**, a recent class of HIV-1 replication inhibitors and SQLs of type 3, belong to the same chemical family (Fig. 1).[6]

2. Chemistry

It appeared that a sequence directed to the synthesis of SQLs substituted at C-7 by aroyl/acyl moieties should require at the outset the elaboration of 7-bromo-8-

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* Corresponding authors. Tel.: +33 1 46 48 57 53; fax: +33 0 146 83 5752; e-mail addresses: didier.desmaele@cep.u-psud.fr; jean.dangelo@cep.u-psud.fr

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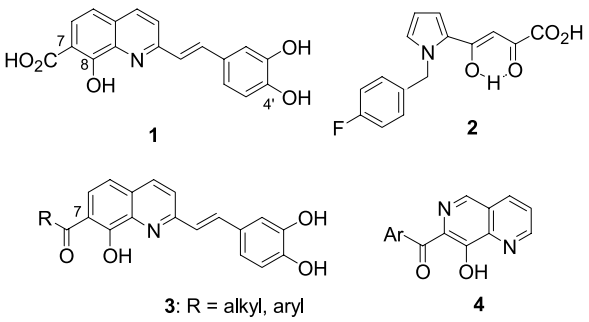


Figure 1.

hydroxyquinaldine **6**, starting from readily available 8-hydroxyquinaldine **5**.

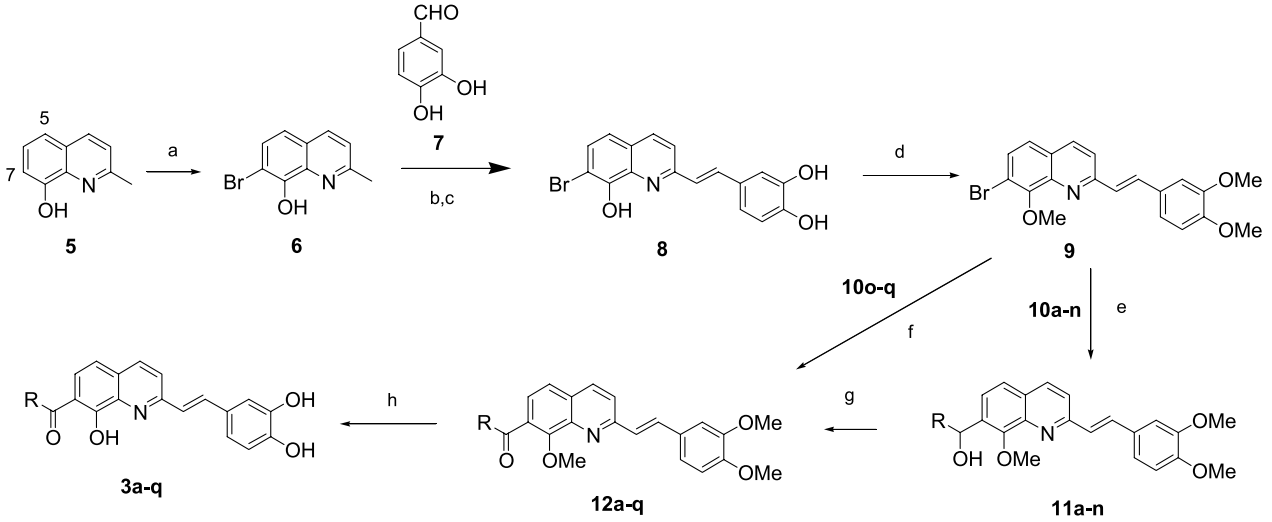
However, bromination of **5** under standard conditions proved to be non-regioselective, giving mixtures of 5-bromo-, 7-bromo-, and 5,7-dibromo-8-hydroxyquinaldines. In contrast, when the experimental protocol developed by Pearson (Br_2, *t*-$BuNH_2$, −78 °C)[7] was applied, the desired 7-bromo derivative **6** was obtained as a single product in 74% yield. Perkin-type condensation of **6** with 3,4-dihydroxybenzaldehyde **7** (refluxing Ac_2O, then H_2O, pyridine, 100 °C) next furnished styrylquinoline **8** (66% yield) that was converted into trimethoxy derivative **9** (K_2CO_3, MeI, DMF, 40 °C, 62% yield). At this juncture, we stood ready to proceed to the introduction of the desired aroyl/acyl group at C-7. For that purpose, **9** was submitted to Br/Li exchange according to the Quéguiner procedure (PhLi, Et_2O, −78 °C),[8] and the resulting lithio derivative was condensed with electrophiles **10a**–**q**[9] giving either alcohols **11a**–**n** (45–80% yield) or ketones **12o**–**q** (20–25% yield). Alcohols **11a**–**n** were further oxidized (MnO_2) into corresponding ketones **12a**–**n** with a 50–90% yield. Finally, treatment of **12a**–**n** with BBr_3 in CH_2Cl_2 at 20 °C or **12o**–**q** with 48% aqueous HBr at reflux delivered our goals **3a**–**q** (35–60% yield) (Scheme 1).[10]

3. Biological activity of the target molecules

Cellular antiviral IC_{50} was determined as the drug concentration that inhibits 50% of viral particles infectivity in standard MAGI assay.[5b] TC_{50} was the drug concentration that corresponds to 50% of cell survival as determined by a standard MTT assay.[4b] The results are illustrated in Table 1. At first glance, the present molecules can be categorized as inactive, cytotoxic, and active. Within the active compound series, five of them exhibit IC_{50} values ranging from 2 to 6 μM, with TC values in the range of 60–100 μM: 3,4-difluorophenyl, *para*-hydroxyphenyl, *ortho*-nitrophenyl, *para*-nitrophenyl, and 3-pyridyl derivatives **3b**, **3d**, **3e, 3g**, and **3j**, respectively. Although the great structural diversity of the above molecules rules out the identification of a common definitive pharmacophore, some comments can be made, based on the present SAR investigation.

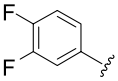
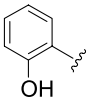
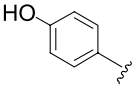
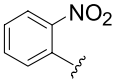
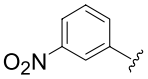
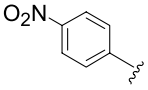
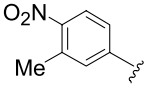
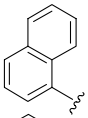
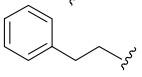
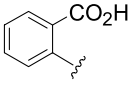
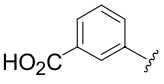
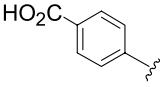
In this respect, there existed a striking geometrical fit requirement for antiviral activity versus cytotoxicity. By way of illustration, replacement of the *para*-hydroxyphenyl moiety of **3d** by an *ortho*-substituted counterpart (**3c**) results in dramatic cytotoxicity. Likewise, *meta*-nitrophenyl derivative **3f** proved to be much more cytotoxic than the *ortho*- and *para*- substituted analogs **3e** and **3g**. In addition, while 3-pyridyl derivative **3j** exhibits no significant cytotoxicity, the corresponding *ortho* and *para* isomers (**3i** and **3k**, respectively) were found to be moderately to highly cytotoxic. A last remark can be made, regarding the three isomeric compounds **3o**–**q**, in which a carboxylic acid is directly linked to the phenyl moiety: in contrast with the "unsubstituted" derivatives **3a** and **3l**, they proved to be almost non-cytotoxic. However, no significant antiviral activity was observed with these compounds.

To precise the nature of ex vivo interacting target(s) of the above 'active' molecules, their in vitro anti-HIV-1 integrase potency was evaluated next. Surprisingly, in



Scheme 1. Synthesis of compounds **3a**–**q**, reagents and conditions: (a) Br_2, *t*-$BuNH_2$, toluene, −78 to 20 °C, 74%; (b) 2 equiv **7**, Ac_2O, 140 °C, 18 h; (c) H_2O, pyridine, 100 °C, 2 h, 66%; (d) K_2CO_3, 10 equiv CH_3I, acetone, DMF, 70 °C, 12 h, 62%; (e) i—2.5 equiv PhLi, cyclohexane, Et_2O, −78 °C, ii—1.25 equiv **10a**–**n**, THF, HMPA, −78 to 20 °C; (f) i—2.5 equiv PhLi, cyclohexane, Et_2O, −78 °C, ii—1.25 equiv **10o**–**q**, THF, HMPA, −78 to 20 °C; (g) 10 equiv MnO_2, $ClCH_2CH_2Cl$, 70 °C, 8 h; (h) 48% aqueous HBr, 120 °C, 12 h for **12o**–**q** or BBr_3, CH_2Cl_2, −78 to 20 °C, 8 h for **12a**–**n**.

Table 1. Antiviral activity and toxicity of C-7-modified styrylquinolines (μM)

Compound	R	IC_{50}	TC_{50}
1		1	>100
3a		NR[a]	3
3b		4	63
3c		NR	1
3d		6	87
3e		4	70
3f		0,5	1,5
3g		2	60
3h		60	90
3i		NR	15
3j		2	>100
3k		NR	4
3l		NR	1
3m		2	7
3n	*n*-C_8H_{17}-	5	10
3o		35	>100
3p		63	>100
3q		40	80

[a] NR, IC_{50} not reached.

sharp contrast with SQLs of type **1** harboring a carboxylic acid group at C-7, compounds **3b**, **3d**, **3e**, **3g**, and **3j** proved to be inactive in both 3′-processing and strand transfer assays, ruling out integration as the step target in ex vivo experiments (Table 2). The mode of action of this new class of antiretroviral agents is currently under investigation.

Table 2. In vitro activity of C-7-modified styrylquinolines (μM)[a]

Compound	Biological activity IC_{50}	
	3′-processing	Strand transfer
1	2	1
3b	>100	>100
3d	>100	>100
3e	>100	70
3g	100	>100
3j	>100	>100

[a] Drug concentration that inhibits 50% of the recombinant integrase activity in standard 3′-processing and strand transfer assays.

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9. **10a**: benzaldehyde; **10b**: 3,4-difluorobenzaldehyde; **10c**: 2-methoxybenzaldehyde; **10d**: 4-methoxybenzaldehyde; **10e**: 2-nitrobenzaldehyde; **10f**: 3-nitrobenzaldehyde; **10g**: 4-nitrobenzaldehyde; **10h**: 4-methyl-3-nitrobenzaldehyde; **10i**: 2-pyridine-carboxaldehyde; **10j**: 3-pyridinecarboxaldehyde; **10k**: 4-pyridinecarboxaldehyde; **10l**: 1-naphthaldehyde; **10m**: dihydrocinnamaldehyde; **10n**: nonanal; **10o**: phthalic anhydride; **10p**: 3-cyano-*N*-methoxy-*N*-methyl-benzamide (prepared through condensation of 3-cyanobenzoyl chloride with *N*,*O*-dimethyl-hydroxylamine in the presence of pyridine); **10q**: 4-cyano-*N*-methoxy-*N*-methyl-benzamide (prepared by analogy with **10p**, starting from 4-cyanobenzoyl chloride).
10. Characterization data for compounds **3b**, **3d**, **3e**, **3g** and **3j**. **3b**: greenish solid, mp 178 °C; IR (neat, cm^{-1}) *v* 3600–2500, 1617, 1588, 1544; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.345 (d, J = 8.8 Hz, 1H), 8.08 (d, J = 16.2 Hz, 1H), 7.95-7.80 (m, 2H), 7.75–7.42 (m, 4H), 7.21 (d, J = 16.2 Hz, 1H), 7.17 (br s, 1H), 7.05 (br d, J = 7.9 Hz, 1H), 6.85 (d, J = 7.9 Hz, 1H); ^{19}F NMR ($CDCl_3$, 188 MHz) δ −130.2 (m, 1F), −136.1 (m, 1F), ^{13}C NMR (DMSO-d_6, 50 MHz) δ 194.7 (CO), 156.1 (C), 153.3 (dd, J_{CF} = 253 Hz, 13 Hz, CF), 153.2 (C), 150.3 (dd, J_{CF} = 235 Hz, 14 Hz, CF), 147.8 (C), 146.5 (C), 139.1 (C), 137.4 (CH), 136.9 (CH), 136.5 (dd, J = 4.9 Hz, 3.7 Hz, C), 129.9 (CH), 128.9 (C), 128.1 (dd, J_{CF} = 7.8 Hz, 2.9 Hz, CH), 126.8 (CH), 124.8 (C), 123.7 (CH), 121.0 (C); 120.8 (CH), 119.3–118.5 (3CH) 116.8 (CH), 115.0 (CH): Anal. Calcd. for $C_{24}H_{15}F_2NO_4.H_2O$: C, 65.90; H, 3.92; N, 3.20. Found C, 65.75; H, 4.10; N, 3.41. **3d**: red solid, mp 245 °C; IR (neat, cm^{-1}) *v* 3600–2500, 1580, 1509; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.60 (d, J = 8.7 Hz, 1H), 8.14 (d, J = 8.7 Hz, 1H), 8.13 (d, J = 16.4 Hz, 1H), 7.75 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.7 Hz, 1H), 7.51 (d, J = 8.6 Hz, 1H), 7.45 (d, J = 16.4 Hz, 1H), 7.18 (d, J = 1.5 Hz, 1H), 7.09 (dd, J = 8.0 Hz, 1.5 Hz, 1H), 6.92 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.0 Hz, 1H). **3e:** brown solid, mp 188 °C; IR (neat, cm^{-1}) *v* 3500–2500, 1617, 1598, 1522; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.35 (d, J = 8.6 Hz, 1H), 8.33 (d, J = 8.0 Hz, 1H), 8.11 (d, J = 16.1 Hz, 1H), 8.00–7.80 (m, 4H), 7.68 (d, J = 7.3 Hz, 1H), 7.47 (d, J = 8.7 Hz, 1H), 7.16 (d, J = 16.1 Hz, 1H), 7.12 (br s, 1H), 7.01 (d, J = 8.1 Hz, 1H), 6.81 (d, J = 8.1 Hz, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 192.9 (CO), 156.5 (C), 155.9 (C), 147.8 (C), 146.6 (C), 146.4 (C), 139.8 (C), 139.3 (C), 136.7 (CH), 136.5 (CH), 135.7 (CH), 131.2 (CH), 130.9 (C), 128.9 (CH), 128.8 (C), 126.1 (CH), 124.9 (CH), 124.7 (CH), 124.1 (CH), 120.8 (CH), 118.6 (C),118.4 (CH), 116.7 (CH), 114.8 (CH). **3g**: red solid, mp 153 °C; IR (neat, cm^{-1}) *v* 3500–2500, 1612, 1592, 1512; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.45-8.35 (m, 3H), 8.15-8.04 (m, 3H), 7.91 (d, J = 8.6 Hz, 1H), 7.60 (d, J = 8.9 Hz, 1H), 7.49 (d, J = 8.9 Hz, 1H), 7.21 (d, J = 16.0 Hz, 1H), 7.15 (br s, 1H), 7.03 (br d, J = 8.2 Hz, 1H), 6.83 (d, J = 8.2 Hz, 1H). **3j**: brown solid, mp 241 °C; IR (neat, cm^{-1}) *v* 3200–2200, 1605, 1588, 1509, 1416; ^1H NMR (DMSO-d_6, 200 MHz) δ 9.29 (br s, 1OH), 9.08 (br s, 1OH), 8.96 (s, 1H), 8.84 (d, J = 3.7 Hz, 1H), 8.36 (d, J = 8.6 Hz, 1H), 8.20 (d, J = 8.1 Hz, 1H), 8.07 (d, J = 16.1 Hz, 1H), 7.88 (d, J = 8.6 Hz, 1H), 7.62–7.45 (m, 3H), 7.20 (d, J = 16.1 Hz, 1H), 7.10 (br s, 1H), 6.99 (br d, J = 8.5 Hz, 1H), 6.79 (d, J = 8.5 Hz, 1H).