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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 2201–2205

HIV-1 replication inhibitors of the styrylquinoline class: introduction of an additional carboxyl group at the C-5 position of the quinoline

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Received 3 January 2005; revised 3 February 2005; accepted 7 February 2005

Abstract—Novel variants of HIV-1 replication inhibitors of the styrylquinoline class, bearing an additional acid group or a propenoic acid moiety at the C-5 position of the quinoline have been synthesized. Key steps included Heck reaction and palladium catalyzed carbonylation reaction of 5-haloquinaldine derivatives. These compounds exhibited reinforced anti-integrase potency and significant antiviral activities.

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] The availability of in vitro assays using recombinant integrase has allowed the discovery of numerous classes of compounds with inhibitory activity.[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 unique class of integrase inhibitors, distinct from the diketoacid group (illustrated by L 731,988, **2**), which affect viral DNA integration.[3a] Within the carbon framework of SQLs, we have identified the salicylic acid moiety at C-7, C-8 of the quinoline ring, and the 4′-OH on the ancillary aromatic nucleus as critical pharmacophores for antiviral activity.

Since it was recognized that carboxylic acid groups are beneficial to both integrase inhibition potency and cytotoxicity, we envisioned to elaborate new structural variants possessing an additional carboxyl group at C-5 while keeping the salicylic acid system at C-7/C-8. Herein, we report the synthesis and the evaluation of the biological activity of new styrylquinoline derivatives **3a**,**b** bearing a carboxyl group either directly bound to the quinoline ring or through an ethylenic spacer.

From the outset of the work we planned to incorporate the requisite function at an early stage of the synthesis, via a palladium-catalyzed coupling reaction of the 5-bromoquinaldine **6**, before to elaborate the arylethenyl

Keywords: Antivirals; Enzyme inhibitors; Heck reactions; Carbonylation; Quinolines.
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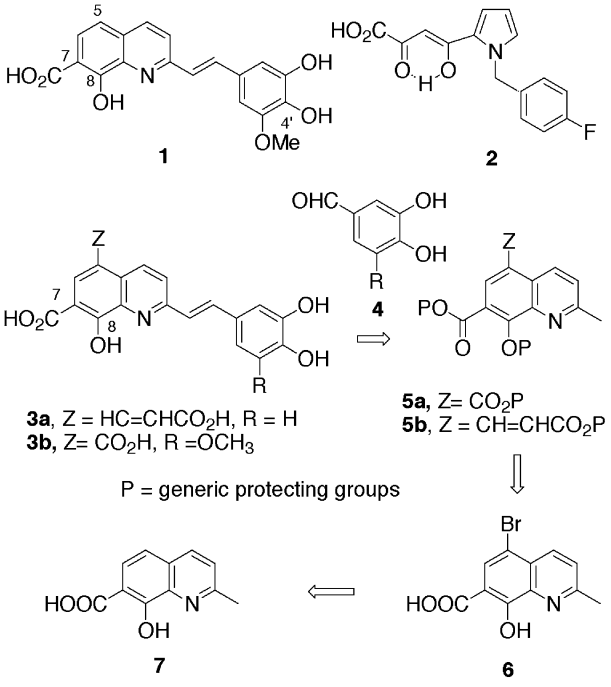
doi:10.1016/j.tetlet.2005.02.033

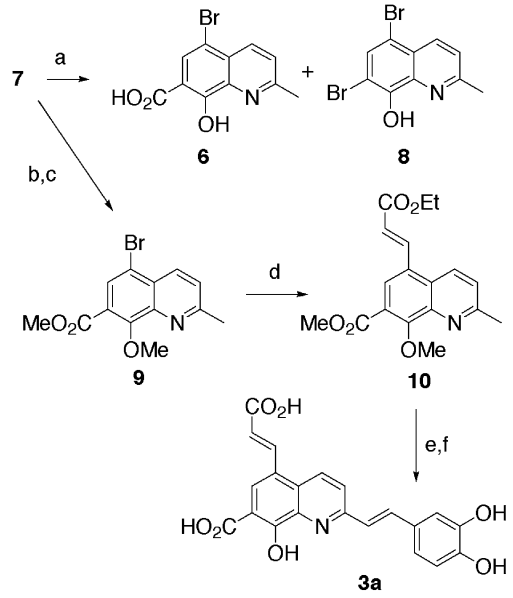
system through a Perkin-type condensation with aromatic aldehydes **4**. Our starting point for the synthesis of compound **3a** was the 8-hydroxy-2-methyl-quinoline-7-carboxylic acid **7** available, although in moderate yield, by Kolbe–Schmidt carboxylation of 8-hydroxyquinaldine (Scheme 1).[6]

Bromination of acid **7** using bromine or NBS provided the desired bromo acid **6** in 30–40% yield, along a substantial amount of dibromoquinoline **8**.[7] Since these procedures turned out to be quite erratic, we turned to a more reliable method. Thus methylation of **7** produced the corresponding dimethylated derivative, which uneventfully gave bromoester **9** in 72% yield upon treatment with bromine. To our delight, Heck reaction of **9** with ethyl acrylate delivered the desired unsaturated ester **10** in 66% yield. Finally, Perkin-type condensation of quinaldine **9** with 3,4-dihydroxybenzaldehyde (**4**, R=H), followed by deprotection afforded the desired diacid **3a** in 49% overall yield from **10** (Scheme 2).[8]

We next turned our attention to the synthesis of diacid **3b**, bearing the carboxyl group directly bound to the quinoline nucleus. Introduction of a nitrile function as carboxyl group surrogate was first investigated. Thus, when the *n*-butyl ester **11**, easily available from acid **7**,[4f] was reacted with sodium iodide in the presence of chloramine-T,[9] the desired 5-iodoquinaldine derivative **12** was obtained in 80% yield. Protection of the phenol group as a pivalate, followed by palladium(0) and copper(I) co-catalyzed cyanation reaction[10] delivered the carbonitrile **13** in 62% overall yield. However the final hydrolysis was disappointing. For example, hydrolysis of **13** with concentrated hydrochloric acid in refluxing acetic acid gave mainly the hydroxy-acid **14** in which the nitrile group was unaffected. On the other hand,



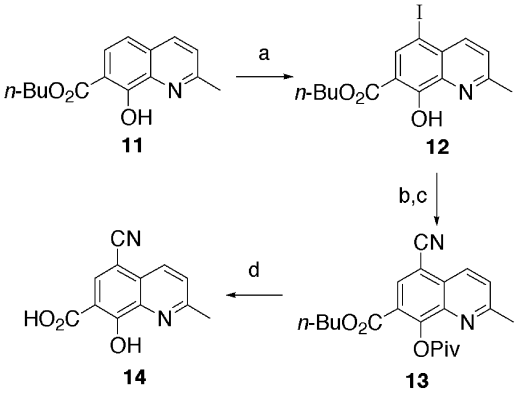
Scheme 1.



Scheme 2. Reagents and conditions: (a) NBS, DMF, 20 °C, 1 h, **6**: 40%; (b) 20 equiv MeI, 20 equiv K_2CO_3, DMF–acetone, 12 h at 50 °C, 95%; (c) Br_2, DMF, CH_2Cl_2, 20 °C, 72%; (d) 5 mol % $Pd(OAc)_2$, 20 mol % PPh_3, 5 equiv $H_2C{=}CHCO_2Et$, 5 equiv Et_3N, 110 °C, 2.5 h, 66%; (e) (i) 3,4-dihydroxybenzaldehyde (**4**, R = H) 4 equiv, Ac_2O, 140 °C, 12 h; (f) HBr 48%, AcOH, 100 °C, 30 h, 49%.

the use of more drastic conditions (e.g., 50% H_2SO_4, 100 °C) led to complete decomposition (Scheme 3).

This problem combined with the modest yield encountered in the Kolbe–Schmidt carboxylation to access the acid **7**, prompted us to explore an alternative strategy based on the regioisomeric quinaldine **16**.[11] Since this material was originally prepared according to a lengthy sequence, we decided to explore the Doebner and Miller condensation of commercially available 3-amino-4-hydroxybenzoic acid **15**. In the event treatment **15** with crotonaldehyde in refluxing 6 N hydrochloric acid provides quinoline **16** in 52% yield. Iodination of **16** with the KI/I_2 system as reported[12] provided iodo phenol **17**. Benzylation of **17** afforded ester **18**, which,



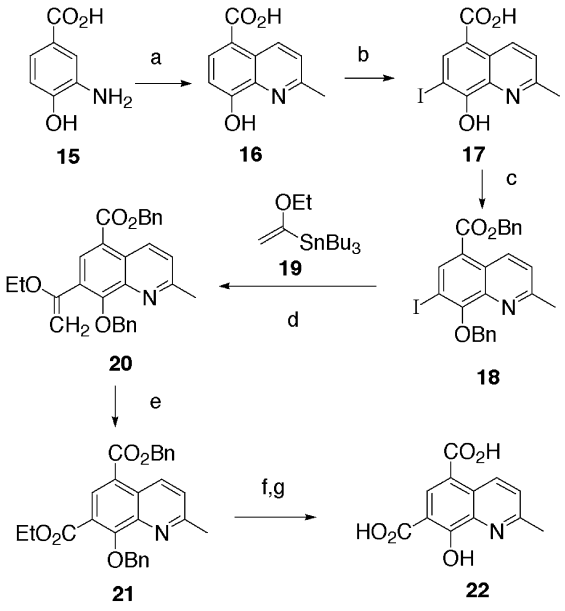
Scheme 3. Reagents and conditions: (a) 1.5 equiv NaI, 1.5 equiv *p*-TolN(Na)Cl, DMF, 20 °C, 1 h, 80%; (b) *t*-BuCOCl, py, DMF, 18 h, 20 °C, 82%; (c) 5 mol % $Pd(PPh_3)_4$, 10 mol % CuI, 2 equiv KCN, refluxing THF, 2 h, 76% (d) HCl concd, AcOH, 110 °C, 16 h, 54%.

upon Stille reaction with tributyl-(1-ethoxy-vinyl)-stannane **19**[13] gave the enol ether **20** (82% yield). The later product was ozonolyzed to give rise to diester **21**, but with a disappointing low yield.[14] Notwithstanding this drawback we continued our synthesis. Thus saponification of the ethyl ester followed by conventional hydrogenolysis of the two benzyl groups furnished the expected diacid **22** in 38% overall yield from **21** (Scheme 4).

Although this route was successful, it suffered from the tedious procedure for the introduction of the C-7 carboxylic group. Clearly a direct hydroxycarbonylation reaction would be a more suitable method. In the event, treatment iodoquinaldine **18** with palladium acetate and dppp in wet DMSO under 1 atm of CO directly afforded the expected acid **23** in 41% yield.[15]

To our surprise, the debenzylation of **23** turned out to be ineffective probably due to the presence in this polar material of phosphine traces poisoning the palladium catalyst. We thus have recourse to a variant of the carbonylation reaction using benzyl alcohol as nucleophilic partner.

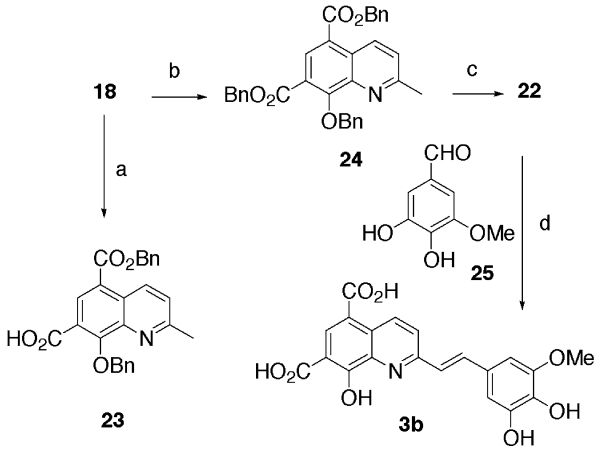
Heating a DMSO solution of **18** in the presence of benzyl alcohol and palladium acetate under CO pressure led to diester **24** with a 55% yield after chromatographic purification. By contrast with the reaction of **23**, deprotection of this material now proceeded smoothly to give diacid **22** in 70% yield. With an efficient route to this material, we next proceeded to the implementation of the arylethenyl appendage. Thus condensation of **22** with 3,4-dihydroxy-5-methoxybenzaldehyde **25**, followed by hydrolysis of the intermediate acetylated compound produced the targeted diacid **3b** in 73% overall yield from **22** (Scheme 5).[16]



Scheme 4. Reagents and conditions: (a) HCl 6 N, $CH_3CH{=}CHCHO$, 100 °C, 1 h, 52%; (b) I_2/KI, aq NaOH, 0.5 h, 15 °C, 70% (c) 3 equiv BnBr, 3 equiv K_2CO_3, DMF, 24 h, 50 °C, 80%; (d) 1.8 equiv **19**, 5 mol % $Pd_2dba_3{\cdot}CHCl_3$, DMF, 100 °C, 3 h, 82%; (e) (i) O_3, CH_2Cl_2, −78 °C, (ii) Me_2S, 20 °C, 8 h 20%; (f) 2 N NaOH, MeOH, THF, 18 h; (g) $Pd(OH)_2$, DMF, AcOH, 1 atm H_2, 20 °C, 8 h, 38% from **21**.

Diacids **3a**,**b** were assayed in vitro for inhibitory activity against HIV-1 integrase, and for antiviral activity against HIV-1 replication in cells. In vitro IC_{50} was determined as the drug concentration that inhibits 50% of the recombinant integrase activity in a standard 3′-processing and strand transfer assays. Cellular antiviral IC_{50} was determined as the drug concentration that inhibits 50% of viral particles production in de novo infection assay of HeLa P4 cells.[5b,17] TC_{50} was the drug concentration that corresponds to 50% of cells survival as determined by a standard MTT assay.[18,19] Results are listed in comparison with reference compound **1** in Table 1.[4b] Diacids **3a**,**b** were found to be highly potent inhibitors of integrase on both 3′-processing and strand transfer assays (Fig. 1). This result clearly established that carboxyl groups on the quinoline ring are beneficial to the inhibitor/integrase interaction as initially suspected. When compared to **1**, a marked decline in antiviral activity was observed for **3b**, on the other hand **3a** kept a good activity on cell culture, but with a slightly increased cytotoxicity.

In short we have devised efficient syntheses of two new styrylquinoline derivatives with additional carboxylic residues on the quinoline ring. Although improved anti-integrase activities were observed, no decisive



Scheme 5. Reagents and conditions: (a) 15 mol % $Pd(OAc)_2$, 20 mol % dppp, 10 equiv Et_3N, DMSO, H_2O, 2 atm CO, 70 °C, 20 °C 1 h then 70 °C 2 h, 41%; (b) 15 mol % $Pd(OAc)_2$, 20 mol % dppp, 10 equiv Et_3N, DMSO, 10 equiv BnOH, 2 atm CO, 70 °C, 20 °C 1 h then 70 °C 2 h, 55%; (c) $Pd(OH)_2$, 1 atm H_2, AcOEt, EtOH, AcOH, 20 °C, 24 h, 70%; (d) (i) 2.5 equiv **25**, Ac_2O, 4 d, (ii) py, H_2O, 110 °C, 3 h, 73%.

Table 1. Biological activities of **3a**,**b**

Compound	**1**	**3a**	**3b**
3′ Processing[a]	1.0	0.3	0.2
Integration[a]	1.7	0.06	0.07
Antiviral activity[a]	0.3	35	1.6
Cytotoxicity[b]	>100	>100	60

[a] IC_{50}, μM.
[b] TC_{50}, μM.

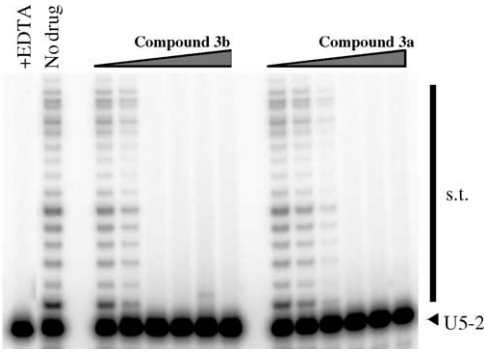


Figure 1. Phosphorimager picture of HIV IN integration activity in presence of increasing concentration of compounds **3a**,**b**. Integration assays were performed at 100 nM of IN, 12.5 nM of U5-2, and 7.5 mM of $MgCl_2$.

breakthrough resulted from this substitution pattern on the antiviral potency. Further studies aimed at exploring modulations of the ancillary rings of compounds **3a**,**b** are ongoing.

Acknowledgements

We thank Dr. M. Ourevitch for NMR spectral analysis and Mrs S. Mairesse-Lebrun for performing elemental analyses.

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8. Compound **3a**: Brick red solid; mp > 300 °C (dec); IR (neat, cm^{-1}) ν 3400–2300 (broad) δ 1677, 1626, 1587, 1518; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.04 (d, J = 9.1 Hz, 1H), 8.42 (d, J = 9.1 Hz, 1H), 8.41 (s, 1H), 8.27 (d, J = 15.7 Hz, 1H), 8.08 (d, J = 16.4 Hz, 1H), 7.73 (d, J = 16.4 Hz, 1H), 7.22 (broad s, 1H), 7.14 (d, J = 8.1 Hz, 1H), 6.90 (d, J = 8.1 Hz, 1H), 6.49 (d, J = 15.7 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.5 (C), 167.9 (C), 163.1 (C), 152.3 (C), 149.1 (C), 146.2 (C), 142.1 (CH), 139.0 (CH), 138.2 (CH), 134.3 (C), 128.8 (C), 127.9 (CH), 127.5 (C), 121.9 (CH), 121.4 (CH), 118.7 (CH), 118.3 (CH), 116.5 (CH), 115.8 (C), 114.9 (CH), 114.0 (C).
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16. Compound **3b**: Brick red solid; mp > 260 °C (dec); IR (neat, cm^{-1}) ν 3500–2500 (broad), 1693, 1690, 1584; ^1H NMR (DMSO-d_6, 200 MHz) δ 9.77 (d, J = 9.4 Hz, 1H), 8.73 (s, 1H), 8.47 (d, J = 9.4 Hz, 1H), 7.99 (d, J = 16.1 Hz, 1H), 7.78 (d, J = 16.1 Hz, 1H), 6.92 (s, 1H), 6.89 (s, 1H), 3.90 (s, 3H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 169.4 (C), 168.3 (C), 167.4 (C), 151.5 (C), 149.5 (C), 146.9 (C), 143.1 (CH), 141.6 (CH), 138.6 (C), 136.0 (CH), 135.0 (C), 130.4 (C), 126.7 (C), 122.2 (CH), 118.5 (CH), 113.4 (C), 110.7 (CH), 109.0 (C), 104.5 (CH), 56.8 (CH_3); Anal. Calcd for $C_{20}H_{15}NO_8 \cdot 2/3H_2O$: C, 58.68; H, 4.02; N, 3.42. Found: C, 58.53; H, 4.21; N, 3.36.
17. HeLa P4 cells were infected with viruses and grown in the presence of increasing concentrations of drugs. After 72 h, the infectivity of viruses was determined by measuring beta-galactosidase (β-Gal) production by the chlorophenol red-β-D-galactopyranoside (CPRG) assay.[18] The 50%

inhibitory concentration (IC_{50}) is the drug concentration at which 50% of β-Gal production was inhibited compared to that by untreated infected cells. Cell survival was also estimated by the use of a standard MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay.[19]

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