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Synthesis and HIV-1 integrase inhibitory activities of caffeic acid dimers derived from *Salvia officinalis*

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Abstract—The synthesis of two caffeoyl-coumarin conjugates, derived from sagecoumarin, has been accomplished, starting from ferulic acid, isoferulic acid and sesamol. Both compounds exhibited potent inhibitory activities at micromolar concentrations against HIV-1 integrase in 3′-end processing reaction but were less effective against HIV-1 replication in a single-round infection assay of HeLa-β-gal-CD4+ cells.

Sage (*Salvia officinalis*) is a popular herb which is widely cultivated in various parts of the world and used in flavouring and folk medicines. In various studies, sage has been shown to be the most potent natural antioxidant of the common spices.[1] Its antioxidant effect has been attributed to the main phenolic compounds, rosmarinic acid, a caffeic acid dimer and carnosic acid.[2] Sage also contains an impressive array of biologically active caffeic acid oligomers, ranging from trimers, tetramers and higher oligomers, such as salvianolic acids, lithospermic acids and yunnaneic acids.[3] Along with salvianolic acid K (a caffeic acid trimer), sagerinic acid (a tetramer) and rosmarinic acid, Lu et al. also characterized three further caffeic acid trimers, melitric acid A, methyl melitrate A and sagecoumarin.[4] This last compound was particularly interesting as it was the first caffeic acid oligomer with a coumarin structure. The full assignment of the ^{1}H and ^{13}C NMR resonances of sagecoumarin showed that it is a 6,7-dihydroxycoumarin linked via an ether bond on its C-3 position to a rosmarinic acid, as shown in Figure 1. It exhibited strong antioxidant activities.[5]

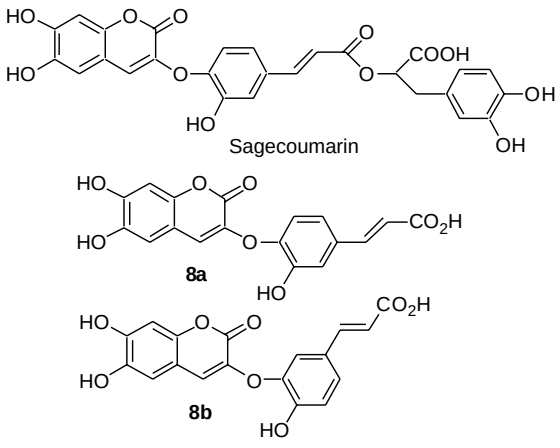

Figure 1. Structures of sagecoumarin and caffeoyl-coumarin conjugates.

Amongst the several varied biological responses elicited by the caffeic acid derivatives, the inhibition of human immunodeficiency virus type 1 (HIV-1), integrase appeared recently as their most promising biological activity. Apart from reverse transcriptase and protease, the two enzymes targeted by clinically used anti-HIV drugs, integrase has recently emerged as an attractive and alternative target. This led to the elaboration of a great number of HIV-1 integrase inhibitors.[6–9] The

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main classes of inhibitors included mononucleotides,[10] dinucleotides,[11,12] oligonucleotides,[13,14] peptides,[15] flavonoids,[16] natural polyaromatics,[17] geometrically restrained bis-catechols,[18] salicyl and thiosalicylhydrazines,[19,20] naphthalenesulfonic acid derivatives,[21] diketo derivatives[22,23] and diketo acids.[24] Caffeic acid derivatives represented another widely studied class of natural and synthetic HIV-1 integrase inhibitors. Dicaffeoyltartaric acids and dicaffeoylquinic acids,[25] rosmarinic acid,[22] lithospermic acid B and lithospermic acid,[26] L-chicoric acid,[25a] were found to be potent inhibitors of 3′-processing and strand transfer reactions of HIV-1 integrase. Furthermore, lithospermic acid was weakly cytotoxic.

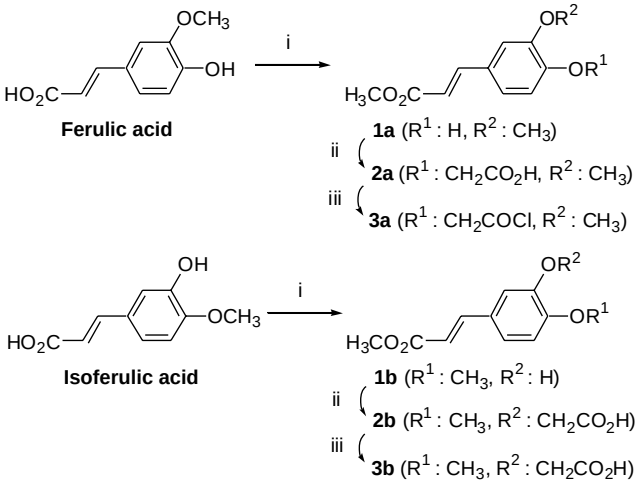
In our laboratory, we are interested in the design, synthesis and pharmacological evaluation of polyhydroxylated lead molecules with antioxidant potencies aimed at inhibiting HIV-1 integrase.[27] In this paper, we report a simple and efficient synthesis of two caffeoyl-coumarin conjugates, derived from sagecoumarin (Fig. 1). The newly synthesized compounds were tested in vitro for their HIV-1 integrase inhibitory activities and antiviral effect.

We first attempted to synthesize the coumarin derivatives by simple one-step routes.

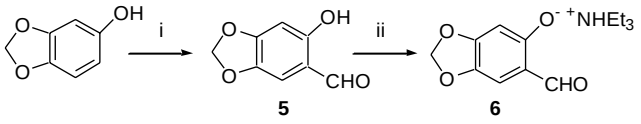
The complex of phosphorus oxychloride and *N*,*N*-diethylphenoxyacetamides on reaction with substituted salicylaldehydes was shown to yield various 3-substituted coumarins.[28] Unfortunately, only reactants were recovered by this method. The literature also reported another similar method based on the condensation of salicylaldehydes and phenoxyacetic acids using triethylamine, acetic anhydride and DMF or benzene as solvent.[29] Here again, we were not able to isolate the products in satisfactory yields. Then we turned to another phosphorus activating agent, phenyldichlorophosphate.[30] Surprisingly, heating under reflux of a solution containing salicylaldehyde **5**, phenoxyacetic acids **2a**,**b**, triethylamine and phenyldichlorophosphate did not give the desired coumarins but the esters **4a**,**b** formed by coupling of the two main precursors in 17–25% yield. These novel compounds were expected to be excellent precursors of the coumarins by cyclisation under basic conditions.

The final sequence was based on the preliminary isolation of the esters **4a**,**b** as key intermediates. These esters were obtained from two precursors, namely the phenoxyacetic acid chlorides **3a**,**b** (Scheme 1) and the salicylaldehyde derivative **5** (Scheme 2). Synthesis began from 4-hydroxy-3-methoxycinnamic acid (ferulic acid) and its isomer, 3-hydroxy-4-methoxycinnamic acid (isoferulic acid). In a first step, they were converted to their methyl ester, respectively, **1a** and **b**, by heating under reflux with a methanolic solution of thionyl chloride. Then the phenolic groups of **1a**,**b** were reacted with bromoacetic acid in the presence of sodium hydride to give the phenoxyacetic acids **2a**,**b** in 60% and 77% yield.

For the elaboration of the other precursor **5**, we turned to a simple one-step sequence starting from the commercially available sesamol (Scheme 2). Duff formylation[31]



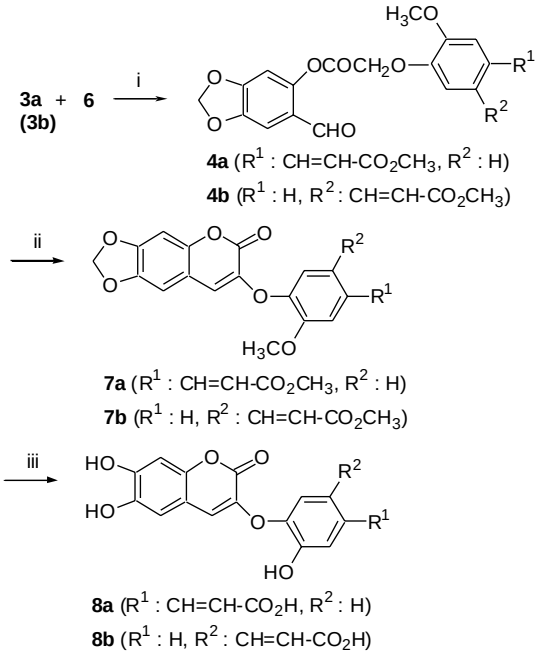
Scheme 1. Reagents and conditions: (i) $SOCl_2$, MeOH, 0 °C then reflux, **1a** 95%, **1b** 95%; (ii) $BrCH_2CO_2H$, NaH, THF, rt then reflux, **2a** 60%, **2b** 77%; (iii) $SOCl_2$, AcOEt, **3a** 97%, **3b** 98%.



Scheme 2. Reagents and conditions: (i) $C_6H_{12}N_4$, TFA, CH_2Cl_2, 0 °C then reflux, 65%; (ii) NEt_3, CH_2Cl_2, rt.

using hexamethylenetetramine and trifluoroacetic acid under reflux afforded the salicylaldehyde derivative **5** in 64% yield.

Scheme 3 shows the final sequence adopted for the synthesis of the coumarin derivatives.



Scheme 3. Reagents and conditions: (i) 0 °C then rt, **4a** 82%, **4b** 65%; (ii) DBU, toluene, reflux, **7a** 25%, **7b** 20%; (iii) a—BBr_3, CH_2Cl_2, rt then reflux; b—H_2O, rt then reflux, **8a** 90%, **8b** 68%.

Table 1. HIV-1 integrase inhibitory potencies (IC_{50}) and antiviral activities (EC_{50})

Entry	IC_{50} (μM)	EC_{50} (μM)
3,6,7-Trihydroxycoumarin	2.0	12.0
8a	2.0	37.0
8b	1.0	31.0

For the condensation of the phenoxyacetic acid chlorides **3a**,**b** and the salicylaldehyde **5**, **5** was converted to its phenolate **6** and then mixed with **3a**,**b** to give **4a** (82%) and **4b** (65%). Cyclisation of **4a**,**b** to the corresponding coumarins **7a**,**b** was ineffective with sodium methylate or sodium hydride. But refluxing with DBU (1,8-diazabicyclo-[5.4.0]-7-undecene; ca. 1.6 mol equiv) in toluene as previously described[32] afforded the coumarins **7a**,**b** in modest but reproducible yields (25% and 20%). Final deprotection of **7a**,**b** to their corresponding polyhydroxylated counterparts **8a**,**b** was accomplished, using boron tribromide. Standard conditions with an excess of BBr_3 at −78 °C then room temperature did not give the fully deprotected coumarins **8a**,**b**. More drastic conditions were necessary with the reflux of the solution after the addition of boron tribromide and hydrolysis. The coumarins **8a**,**b** were obtained in 90% and 68% yield, respectively. Intermediates and final compounds of both series were fully characterized by IR, ^1H NMR, ^{13}C NMR and MS.[33]

Each new compound was screened for inhibitory activity against HIV-1 integrase in Mg^{2+}-dependent 3′-end processing reaction. 3,6,7-Trihydroxycoumarin[27b] was also tested for comparative studies. Table 1 shows that micromolar activities (IC_{50}) were obtained for the three compounds.

They were also evaluated for their antiviral activities against the HIV-1 early replicative steps in HeLa-β-gal-CD4+ (P4) reporter cells. P4 cells were infected with HIV-1 for 2 h and subsequently treated with increasing drug concentrations. Antiviral effect was evaluated 72 h post-infection by quantifying β-galactosidase activity in cellular extracts. In parallel, cytotoxicity on P4 cells was evaluated by a standard MTT assay. No cytotoxicity was observed up to 50 μM, the highest concentration tested in the antiviral assay. Results are listed in Table 1. Both caffeoyl derivatives exhibited poor antiviral activities with EC_{50} values around 30 μM and were three times less potent than 3,6,7-trihydroxycoumarin. The fact that the compounds active against integrase showed an antiviral activity in cultured cell lines at concentrations significantly higher than those required for enzyme inhibition raised the possibility that they acted non-specifically. In fact, the abilities of the compounds to inhibit binding of HIV-1 gp120 to CD4, HIV-1 reverse transcriptase and cellular RNA polymerase II should be evaluated to estimate their degree of selectivity and determine their possible primary cellular target. This is a tremendous work, which requires numerous pharmacological assays, as demonstrated for the study of the mechanism of inhibition of HIV-1 replication by L-chicoric acid.[25b,34–36]

In summary, a concise and rapid synthesis of **8a**,**b** was achieved in six steps from ferulic and isoferulic acids with 10% and 6.3% overall yields, respectively. The present synthetic route could be used for the elaboration of novel oligomeric caffeic acid derivatives. Although the compounds were effective in inhibiting in vitro HIV-1 integrase and presented low cytotoxicities, they were modest inhibitors of HIV-1 replication in HeLa-β-gal-CD4+ cells.

Acknowledgments

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33. Compound **4a**: beige solid; mp 163–167 °C; IR ν_{max} (KBr/cm^{-1}) 1794, 1701, 1670, 1638, 1623, 1518, 1478, 1438, 1309, 1268, 1202, 1186, 1167, 1137, 1078; ^{1}H NMR ($CDCl_3$): 3.80 (s, 3H), 3.93 (s, 3H), 5.05 (s, 2H), 6.09 (s, 2H), 6.34 (d, 1H, ^3J = 15.9 Hz), 6.67 (s, 1H), 7.02 (d, 1H, ^3J = 8.3 Hz), 7.05 (m, 2H), 7.07 (m, 2H), 7.25 (d, 1H, ^3J = 1.9 Hz), 7.63 (d, 1H, ^3J = 15.9 Hz), 9.87 (s, 1H); ^{13}C NMR ($CDCl_3$): 51.7, 56.0, 66.2, 102.9, 104.3, 108.3, 110.8, 114.4, 116.5, 122.0, 122.1, 129.4, 144.3, 146.4, 147.9, 148.9, 149.8, 153.2, 167.2, 167.5, 186.7; IEMS (70 eV): 414 ($M^{+\cdot}$, 12%), 207 (11%), 206 (26%), 180 (12%), 179 (100%), 175 (8%), 166 (11%), 165 (13%), 145 (7%). Compound **4b**: beige solid; mp 180–184 °C; IR ν_{max} (KBr/cm^{-1}) 1785, 1721, 1707, 1676, 1609, 1505, 1491, 1436, 1274, 1175, 1143, 1034; ^{1}H NMR (DMSO-d_6): 3.72 (s, 3H), 3.83 (s, 3H), 5.23 (s, 2H), 6.22 (s, 2H), 6.62 (d, 1H, ^3J = 16.0 Hz), 6.91 (d, 1H, ^3J = 8.3 Hz), 6.93 (s, 1H), 7.34 (m, 2H), 7.53 (s, 1H), 7.60 (d, 1H, ^3J = 16.0 Hz), 9.95 (s, 1H); ^{13}C NMR (DMSO-d_6): 45.1, 49.5, 58.8, 97.0, 98.3, 100.4, 105.9, 106.0, 109.4, 115.7, 117.7, 120.6, 138.2, 139.9, 140.8, 141.9, 144.8, 146.8, 160.7, 161.4, 181.7; IEMS (70 eV): 414 ($M^{+\cdot}$, 12%), 221 (10%), 208 (10%), 207 (8%), 206 (40%), 189 (12%), 180 (11%), 179 (100%), 175 (9%), 166 (13%), 165 (14%), 161 (11%), 145 (7%). Compound **7a**: white solid; mp 234 °C; IR ν_{max} (KBr/cm^{-1}) 1722, 1640, 1600, 1505, 1487, 1455, 1430, 1352, 1306, 1261, 1196, 1175, 1156, 1133, 1070; ^{1}H NMR ($CDCl_3$): 3.79 (s, 3H), 3.87 (s, 3H), 6.02 (s, 2H), 6.38 (d, 1H, ^3J = 16.0 Hz), 6.68 (s, 1H), 6.84 (s, 1H), 6.86 (s, 1H), 7.03 (d, 1H, ^3J = 8.0 Hz), 7.10–7.13 (m, 2H), 7.65 (d, 1H, ^3J = 16.0 Hz); ^{13}C NMR ($CDCl_3$): 51.8, 56.1, 98.2, 102.2, 104.4, 111.9, 112.5, 117.7, 120.4, 121.7, 122.2, 132.2, 140.2, 144.0, 145.2, 145.4, 147.3, 149.6, 150.8, 157.2, 167.2; IEMS (70 eV): 397 ($M^{+\cdot}$+H, 11%), 396 ($M^{+\cdot}$, 45%), 365 (7%), 309 (10%), 204 (27%), 203 (100%), 177 (25%), 175 (14%). Compound **7b**: beige solid; mp 199–203 °C; IR ν_{max} (KBr/cm^{-1}) 1716, 1637, 1599, 1519, 1488, 1451, 1329, 1263, 1223, 1176, 1164, 1132, 1023; ^{1}H NMR ($CDCl_3$): 3.75 (s, 3H), 3.82 (s, 3H), 6.02 (s, 2H), 6.26 (d, 1H, ^3J = 16.0 Hz), 6.66 (s, 1H), 6.76 (s, 1H), 6.82 (s, 1H), 6.99 (d, 1H, ^3J = 8.6 Hz), 7.22 (s, 1H), 7.35 (d, 1H, ^3J = 8.6 Hz), 7.60 (d, 1H, ^3J = 16.0 Hz); ^{13}C NMR ($CDCl_3$): 45.4, 50.0, 92.0, 96.0, 98.2, 106.4, 106.8, 110.4, 113.4, 115.1, 120.5, 121.7, 134.4, 137.3, 137.5, 139.0, 141.0, 143.3, 146.3, 151.0, 161.1; IEMS (70 eV): 397 ($M^{+\cdot}$+H, 11%), 396 ($M^{+\cdot}$, 49%), 365 (10%), 204 (21%), 203 (100%), 177 (24%), 175 (16%). Compound **8a**: brown solid; mp 248 °C (dec); IR ν_{max} (KBr/cm^{-1}) 1670, 1605, 1523, 1444, 1406, 1346, 1278, 1262, 1228, 1217, 1136, 1100; ^{1}H NMR (acetone-d_6): 6.42 (d, 1H, ^3J = 15.9 Hz), 6.84 (s, 1H), 7.01 (s, 1H), 7.09 (d, 1H, ^3J = 8.4 Hz), 7.16 (dd, 1H, ^3J = 8.4 Hz, ^4J = 1.8 Hz), 7.28 (s, 1H), 7.30 (d, 1H, ^4J = 1.8 Hz), 7.09 (d, 1H, ^3J = 15.9 Hz); ^{13}C NMR (acetone-d_6): 103.3, 112.1, 112.5, 117.1, 118.5, 120.6, 121.5, 124.8, 132.7, 140.0, 143.7, 144.9, 145.8, 146.8, 148.8, 149.2, 158.0, 167.8; IEMS (70 eV): 357 ($M^{+\cdot}$+H, 16%), 356 ($M^{+\cdot}$, 80%), 339 (7%), 338 (20%), 253 (8%), 192 (8%), 191 (100%), 178 (46%), 177 (94%), 166 (13%), 165 (26%), 163 (7%), 162 (27%), 150 (46%), 137 (10%), 121 (11%), 67 (11%). Compound **8b**: black solid; mp 280 °C (dec); IR ν_{max} (KBr/cm^{-1}) 1666, 1620, 1572, 1513, 1456, 1435, 1411, 1284, 1215, 1184; ^{1}H NMR (acetone-d_6): 6.35 (d, 1H, ^3J = 16.0 Hz), 6.81 (s, 1H), 6.88 (s, 1H), 7.03 (d, 1H, ^3J = 8.4 Hz), 7.28 (s, 1H), 7.41 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.0 Hz), 7.49 (d, 1H, ^4J = 2.0 Hz), 7.57 (d, 1H, ^3J = 16.0 Hz), 8.38 (br s, 1H), 8.91 (br s, 1H), 9.00 (br s, 1H); ^{13}C NMR (acetone-d_6): 97.2, 106.0, 106.3, 110.7, 112.2, 114.2, 118.1, 120.9, 121.8, 134.3, 137.5, 138.0, 138.6, 140.5, 142.6, 145.1, 152.0, 161.8; IEMS (70 eV): 357 ($M^{+\cdot}$+H, 11%), 356 ($M^{+\cdot}$, 54%), 339 (8%), 338 (33%), 328 (7%), 312 (8%), 191 (45%), 178 (63%), 177 (90%), 165 (23%), 162 (61%), 150 (71%), 137 (9%).
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