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Simplified Catechin-Gallate Inhibitors of HIV-1 Reverse Transcriptase

L. M. V. Tillekeratne,[a] A. Sherette,[a] P. Grossman,[a] L. Hupe,[b] D. Hupe[b] and R. A. Hudson[a,c,*]

[a]*Department of Medicinal and Biological Chemistry, College of Pharmacy, University of Toledo, Toledo, OH 43606, USA*
[b]*Pfizer Global Research and Development, 2800 Plymouth Rd, Ann Arbor, MI 48105, USA*
[c]*Departments of Biology and Chemistry, College of Arts and Sciences, University of Toledo, Toledo, OH 43606, USA*

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Abstract—Systematic simplification of the molecular structures of epicatechin gallate and epigallocatechin gallate to determine the minimum structural characteristics necessary for HIV-1 reverse transcriptase inhibition in vitro resulted in several compounds that strongly inhibited the native as well as the A17 double mutant (K103N Y181C) enzyme, which is normally insensitive to most known nonnucleoside inhibitors.

The life cycle of the human immunodeficiency virus type-1 (HIV-1), the primary causative agent of AIDS, provides a number of targets for potential chemotherapeutic intervention.[1] Most of the chemotherapeutic agents that are currently in use for HIV treatment target at reverse transcriptase (HIV-1-RT), a key enzyme that catalyzes the reverse transcription of viral RNA into a double-stranded DNA.[2,3] More recently introduced protease inhibitors also have become important components in current anti-HIV drug regimens.[4]

Since the discovery of azidothymidine (AZT), other nucleoside inhibitors of HIV-1-RT have been developed for clinical use. These include oxathiolanylcytosine (3TC), dideoxyinosine (ddI), dideoxycytidine (ddC), and didehydro-dideoxythymidine (d4T).[5] These drugs, after cellular conversion to a 5′-triphosphate, compete with deoxynucleotide triphosphate (dNTP) binding to HIV-1-RT and also act as competitive substrates that lead to chain termination of the DNA. More recently, nonnucleoside inhibitors of reverse transcriptase (NNRTIs), that act noncompetitively with dNTP substrates, have come to play an increasingly important role in anti-HIV therapy.[6,7] NNRTIs constitute a group of compounds of very diverse structure. However, they have been shown to interact with a specific binding site on HIV through a common mode of binding. Their binding site is closely associated with, but distant from the substrate binding site.[8] Several NNRTIs (nevirapine, delaviridine, and efavirenz) have been approved for clinical use while a number of others (e.g., tivirapine, loviride, MKC-422, and troviridine) remain in clinical trials.[8]

A major impediment to currently available anti-HIV-RT and other therapies is the emergence of drug-resistant viral variants due to the rapid rate of replication of HIV and to its inherent genetic variability. NNRTIs in particular, are known to induce drug-resistant viral variants, hence compromising clinical effectiveness.[9–11] Combined use of NRTIs, NNRTIs, and protease inhibitors have been shown to confer additive/synergistic anti-HIV activity. The emergence of resistant viral strains can be prolonged if the NNRTIs, NRTIs, and protease inhibitors are used in combination.[8] However, current treatment regimens can only suppress viral replication and control plasma viremia, but do not eradicate viral reservoirs. Thus, the discovery of new agents that act at different sites on HIV-RT, or at other key viral proteins or enzymes, adds to the anti-HIV drug armamentarium for effective control of HIV infection.

Nakane and Ono reported that (−)-epicatechin gallate and (−)-epigallocatechin gallate (Fig. 1), two natural products isolated from tea (*Camellia sinensis*), differentially inhibited reverse transcriptase and cellular DNA

*Corresponding author. Fax: +1-419-530-7946; e-mail: rhudson@uoft02.utoledo.edu

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and RNA polymerases, the strongest inhibition being observed with HIV-RT.[12] They suggested that these compounds bind directly to the enzyme and interfere with template-primer binding. Further, the hydrolysis products [(−)-epicatechin, (−)-epigallocatechin, and gallic acid] had virtually no reverse transcriptase inhibitory activity in the concentration ranges tested. However, the natural products tested were both observed to be cytotoxic in cell culture studies at concentrations at which they did not inhibit HIV-1-induced cytopathogenicity. It was suggested that these natural products may not penetrate the cell membrane. Here, we describe efforts to systematically simplify the molecular structures of epicatechin and epigallocatechin gallates to determine the minimum structural characteristics necessary for reverse transcriptase inhibition with a view to developing active inhibitors with enhanced pharmacokinetic properties and reduced toxicity. We investigated the action of inhibitors on both wild-type and the A17 mutant enzyme, against which NNRTIs are inactive.

Initially, we focused on the two general classes of agents A and B (Fig. 1) to determine whether catechins can be significantly simplified with retention of reverse transcriptase inhibition. The gallate ester moiety was retained as its removal has been shown to result in loss of activity. The two classes A and B consist of two hydroxylated aromatic ring systems joined together in tacitly *transoid* and *cisoid* arrangements, held together by a five-bond tether. Assuming that the role of the cyclic component of the tether is merely to hold the two aromatic rings in their respective orientations, classes A and B may be further simplified to types A1 and B1, where *transoid* and *cisoid* differentiation is untenable in the context of a conformationally flexible tether. By deleting ring B and changing the number and the nature of the hydroxyl substituents, a series of compounds **1**–**9** (Fig. 2) were synthesized and their RT inhibitory activities evaluated.

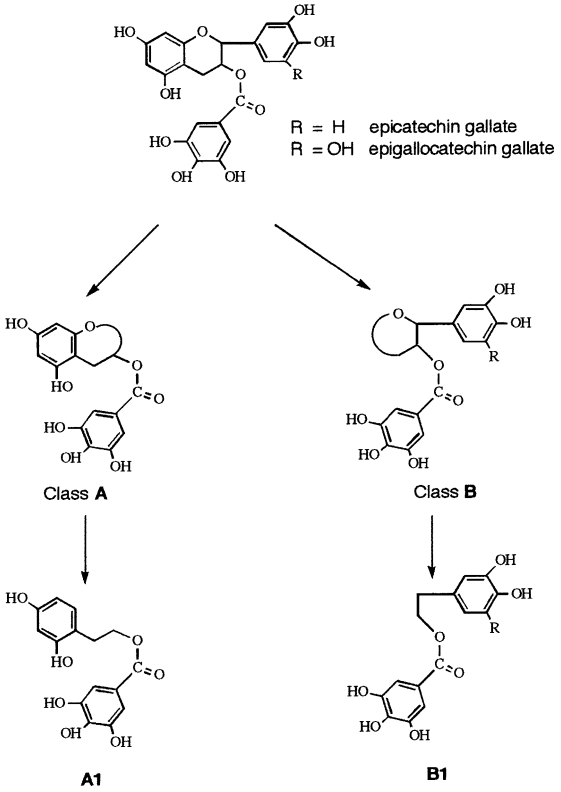


Figure 1. Simplification of catechin structures.

The synthesis of the compounds **1**–**8** is shown in Scheme 1. 3,4-Dihydro-2*H*-1-benzopyran-3-ols **14** were synthesized by diborane reduction of the corresponding 4*H*-1-benzopyran-4-ones (**13**).[13,14] Condensation of *o*-hydroxy-acetophenone and its derivatives **11** with ethyl formate in the presence of sodium powder gave the hemi-acetal **12**, which was easily converted to the corresponding 4*H*-1-benzopyran-4-one **13** by acid-catalyzed dehydration.[15] For the synthesis of the target molecules **1** and **5**–**8**, 4′,6′-dibenzyloxy-2′-hydroxyacetophenone **11** ($R^3 = R^4 = -OCH_2C_6H_5$) was obtained by selective *O*-benzylation of 2′,4′,6′-trihydroxyacetophenone with 2.2 mol equiv of benzyl chloride in the presence of anhy-

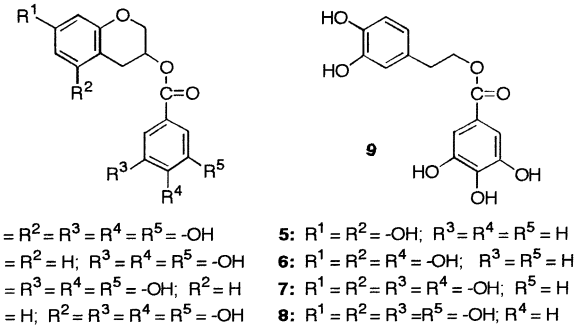
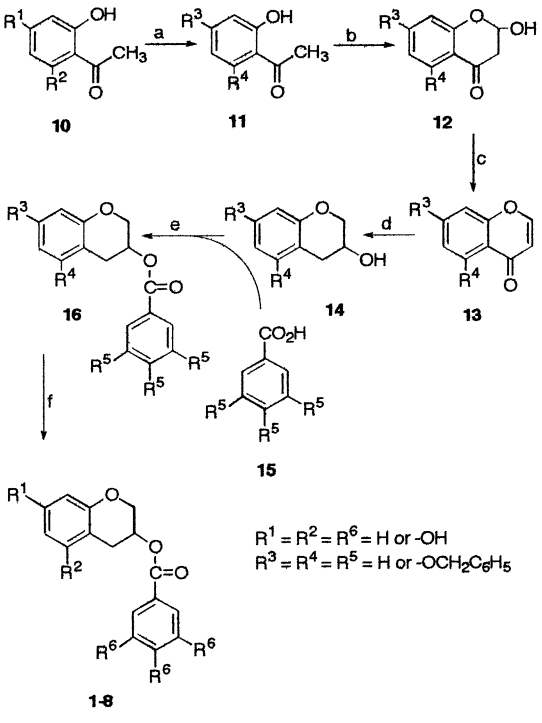


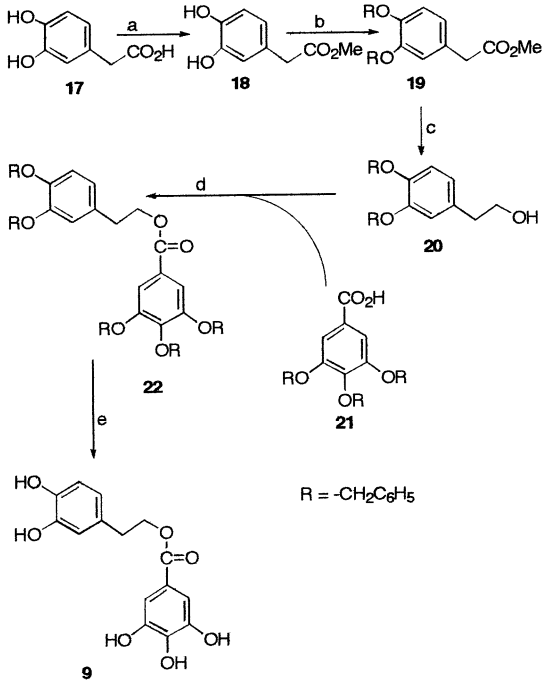
Figure 2. Structures of HIV-1-RT inhibitors synthesized.



Scheme 1. Synthesis of compounds **1**–**8**. Reagents: (a) benzyl chloride, K_2CO_3, DMF; (b) HCO_2Et, Na; (c) H^+ ($-H_2O$); (d) BH_3, NaOH/H_2O; (e) DCC, DMAP, CH_2Cl_2; (f) H_2/Pd–C.

drous K_2CO_3 in DMF. Also, selective *O*-benzylation of 2′,6′-dihydroxy and 2′,4′-dihydroxy-acetophenones under similar conditions with 1.2 mol equiv of benzyl chloride gave 6′-benzyloxy-2′-hydroxyacetophenone **11** (R^3 = H, R^4 = –$OCH_2C_6H_5$) and 4′-benzyloxy-2′-hydroxyacetophenone **11** (R^3 = –$OCH_2C_6H_5$, R^4 = H), respectively. The 3,4-dihydro-4*H*-benzopyran-3-ols **14** were esterified with appropriately substituted benzoic acid derivatives **15** using DCC to obtain the ester **16**.[16] Hydrogenolysis of the benzyl protecting groups gave the target compounds **1**–**8**. Esterification and subsequent *O*-benzylation of 3,4-dihydroxyphenylacetic acid **17** gave methyl 3,4-dibenzyloxyphenylacetate **19** that was reduced with $LiBH_4$ to 2-(3,4-dibenzyloxy-phenyl)ethyl alcohol **20**. The alcohol **20** was esterified with 3,4,5-tribenzyloxybenzoic acid using DCC to give the ester **22** (Scheme 2). The benzyl groups were removed by hydrogenolysis to obtain **9**. All compounds were characterized by ^{1}H NMR and ^{13}C NMR spectroscopy and by elemental analysis.[17]

Inhibition of HIV-1-RT polymerization was carried out using a template primer of poly rC/dG_{12-18} and [^{3}H] dGTP.[18,19] The purified, wild-type and A17 mutant HIV-1-RT enzymes used were p66/p51 heterodimers and were prepared as described elsewhere.[20] Reactions were initiated by the addition of the enzyme (90 nM final concentration) to the reaction mixture (final total volume 50 μL) containing 10 mM Tris–HCl (pH 7.6), 1 mM dithiothreitol, 80 mM KCl, 12 mM $MgCl_2$, 0.005% Triton X-100, 60 μg/mL bovine serum albumin, 20 μM [^{3}H]dGTP (5 μCi per assay mixture), 20 μg/mL poly(rC)/dG_{12-18} (Pharmacia), and the test compounds over a range of concentrations in DMSO (1.5 μL, 3% final DMSO concentration). After 15 min incubation at 37 °C, the reaction mixture was treated with 13% TCA/10 mM sodium pyrophosphate and allowed to stand on ice for 30 min. The acid-precipitable material was collected on filters, rinsed with 1 M HCl acid/10 mM sodium pyrophosphate and radioactivity was determined by liquid scintillation counting. Reduction of initial polymerization rates as a function of inhibitor concentrations was also determined in this manner and used to estimate IC_{50} for polymerization inhibition.



Scheme 2. Synthesis of compound **9**. Reagents: (a) $SOCl_2$/MeOH; (b) benzyl chloride, K_2CO_3, DMF; (c) $LiBH_4$, THF; (d) DCC, DMAP, CH_2Cl_2; (e) Pd–C, H_2.

Table 1 records the observed IC_{50}'s for polymerization inhibition in both wild-type and A17 mutant enzymes for the reported compounds. Some agents were inhibitors of wild-type HIV-1-RT and, in most cases, comparable inhibition was observed in the A17 mutant enzyme. Compound **1** was the most potent inhibitor whereas compound **2** showed no activity, indicating that one or both of the hydroxyl groups on the aromatic ring of the chromanol moiety were necessary for inhibitory activity. Compounds **3** and **4** showed significant but reduced inhibition when compared to **1**, suggesting a cumulative effect of the 5- and 7-hydroxyl groups of the chromanol moiety on activity. Compound **5** was also inactive, suggesting one or more of the hydroxyl groups on the gallate ring were required. Thus, compounds **6**–**8** were also prepared and examined. These studies showed that the 3,4,5-trihydroxy substitution of this ring was important for inhibition. Compound **9** showed activity comparable to that of **1**, indicating that the tether between the two aromatic rings may be acyclic and that the central portion of the molecule is not intimately involved in binding to the enzyme. The lead natural product molecules (−)-epicatechin gallate and (−)-epigallocatechin gallate were also tested under the assay conditions used in this study and were found to be more active than any of the simplified analogues synthesized (Table 1). The hydrolysis products (−)-epicatechin and gallic acid did not show any inhibitory activity at concentrations below 100 μM.

The new compounds presented here represent an attempt to define a limiting structure necessary for binding to the catechin site in HIV-1-RT. Compounds

Table 1. Inhibition of wild-type and double mutant HIV-1 reverse transcriptase

Compound	IC_{50} (μM) polymerization inhibition	
	Wild-type	A17 double mutant
1	3.83±0.41	2.36±1.21
2	>100	>100
3	36.29±7.72	30.84±8.35
4	45.50±9.65	64.67±15.01
5	>100	>100
6	>100	ND
7	37.24±10.80	>100
8	96.30±25.95	ND
9	10.74±1.66	6.33±1.53
(−)-Epicatechin gallate	0.76±0.44	ND
(−)-Epigallocatechin gallate	0.73±0.30	ND
(−)-Epicatechin	>100	ND
Gallic acid	>100	ND

ND, not determined.

1, **3**, **4**, and **9** were distinct in their ability to inhibit both wild-type and A17 double mutant HIV-1-RT. In preliminary cell culture studies with compound **1** antiviral activity was not observed. Although this compound is considerably less hydrophilic compared to the lead catechins, it may still be unable to penetrate the cell membrane. We have focused here on substitution patterns in the two phenyl groups and retaining the structure of the tether which joins the catechin and gallate segments. These are significant structural changes that allow retention of much of the HIV-RT inhibitory character of the catechins.

Acknowledgements

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17. Physical data for the compounds **1**–**9**:

3,4-Dihydro-5,7-dihydroxy-2*H*-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate (1). Overall yield 3%; mp 242–243 °C, ^{1}H NMR (300 MHz, acetone-d_6) 2.79 (dd, 1H, J = 17.1, 2.63 Hz), 2.97 (dd, 1H, J = 17.1, 5.2 Hz), 4.14 (d, 1H, J = 11.34 Hz), 4.22 (dd, 1H, J = 11.34, 4.34 Hz), 5.36 (m, 1H), 5.88 (d, 1H, J = 2.2 Hz), 6.04 (d, 1H, J = 2.2 Hz), 7.06 (s, 2H); ^{13}C NMR (75.4 MHz, acetone-d_6) 25.46 (C-4), 66.57 (C-3), 67.25 (C-2), 95.66, 96.37 (ArCH), 99.18 (ArC), 109.87 (ArCH), 121.71, 138.89, 145.98, 156.42, 157.35, 157.66 (ArC), 166.25 (C=O). Anal. calcd for $C_{16}H_{14}O_8$: C, 57.49; H 4.22. Found: C, 57.03; H 4.52.

3,4-Dihydro-2*H*-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate (2). Overall yield 11%; mp 221–223 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 2.96 (d, 1H, J = 16.9 Hz), 3.27 (dd, 1H, J = 16.9, 4.63 Hz), 4.24 (m, 1H), 4.31 (m, 1H), 5.41 (brm, 1H), 6.84 (m, 2H), 7.08 (s, 2H), 7.11 (d, 2H, J = 6.87 Hz); ^{13}C NMR (75.4 MHz, acetone-d_6) δ 30.62 (C-4), 66.6 (C-3), 67.55 (C-2), 109.9, 117.12 (ArCH), 120.32 (ArC), 121.56 (ArCH), 121.64 (ArC), 128.21, 130.93 (ArCH), 138.94, 146.01, 154.99 (ArC), 166.22 (C=O). Anal. calcd for $C_{16}H_{14}O_6$: C, 63.56; H, 4.67. Found: C, 63.06; H, 4.60.

3,4-Dihydro-7-hydroxy-2*H*-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate (3). Overall yield 5%; mp 234–235 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 2.85 (dd, 1H, J = 16.85, 2.38 Hz), 3.15 (dd, 1H, J = 16.85, 4.87 Hz), 4.18 (dd, 1H, J = 11.54, 1.90 Hz), 4.26 (m, 1H), 5.37 (brm, 1H), 6.30 (d, 1H, J = 2.41 Hz), 6.39 (dd, 1H, J = 8.23, 2.41 Hz), 6.89 (d, 1H, J = 8.23 Hz), 7.04 (s, 2H); ^{13}C NMR (75.4 MHz, MeOH-d_4) δ 30.57 (C-4), 67.56 (C-3), 67.98 (C-2), 104.02, 109.89, 110.23 (ArCH), 111.39, 121.51 (ArC), 131.58 (ArCH), 140.0, 146.54, 156.01, 158.01 (ArC), 167.83 (C=O). Anal. calcd for $C_{16}H_{14}O_7$ (+0.5 H_2O): C, 58.72; H, 4.62. Found: C, 58.81; H, 4.26.

3,4-Dihydro-5-hydroxy-2*H*-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate (4). Overall yield 8%; mp 260–261 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 2.87 (dd, 1H, J = 17.67, 1.85 Hz), 3.03 (dd, 1H, J = 17.67, 5.21 Hz), 4.18 (dd, 1H, J = 11.43, 0.74 Hz), 4.26 (m, 1H), 5.41 (m, 1H), 6.34 (d, 1H, J = 8.1 Hz), 6.43 (d, 1H, J = 8.1 Hz), 6.92 (t, 1H, J = 8.1 Hz), 7.06 (s, 2H); ^{13}C NMR (75.4 MHz, acetone-d_6) δ 25.8 (C-4), 66.42 (C-3), 67.14 (C-2), 107.73 (ArCH), 108.02 (ArC), 108.54, 109.87 (ArCH), 121.70 (ArC), 127.85 (ArCH), 138.87, 145.97, 156.08, 156.87 (ArC), 166.23 (C=O). Anal. calcd for $C_{16}H_{14}O_7$ (+0.5 H_2O): C, 58.72; H, 4.62. Found: C, 59.00; H, 4.43.

3,4-Dihydro-5,7-dihydroxy-2*H*-1-benzopyran-3-yl benzoate (5). Overall yield 3%; mp 193–194 °C; ^{1}H NMR (300 MHz, $CDCl_3$-acetone-d_6) δ 2.88 (dd, 1H, J = 17.06, 2.87 Hz), 3.03 (dd, 1H, J = 17.06, 5.35 Hz), 4.18 (d, 1H, J = 11.32 Hz), 4.29 (m, 1H), 5.51 (m, 1H), 6.04 (s, 2H), 7.41 (t, 2H, J = 8.0 Hz), 7.54 (t, 1H, J = 8.0 Hz), 8.0 (d, 2H, J = 8.0 Hz); ^{13}C NMR (75.4 MHz, $CDCl_3$-acetone-d_6) δ 24.73 (C-4), 65.97 (C-3), 66.70 (C-2), 95.95 (ArCH), 99.19 (ArC), 128.34, 129.77 (ArCH), 129.87 (ArC), 133.17 (ArCH), 155.38, 155.43, 155.51 (ArC), 166.22 (C=O). Anal. calcd for $C_{16}H_{14}O_5$ (+0.5 H_2O): C, 65.08; H, 5.12. Found: C, 65.62; H, 5.22.

3,4-Dihydro-5,7-dihydroxy-2*H*-1-benzopyran-3-yl 4-hydroxybenzoate (6). Overall yield 7%; mp 236–237 °C; ^{1}H NMR (300 MHz, acetone-d_6–MeOH-d_4) δ 2.79 (dd, 1H, J = 17.03, 4.24 Hz), 2.96 (dd, 1H, J = 17.03, 5.00 Hz), 4.15 (dd, 1H, J = 11.39, 2.21 Hz), 4.18 (dd, 1H, J = 11.39, 1.66 Hz), 5.39 (brm, 1H), 5.87 (d, 1H, J = 2.32 Hz), 6.0 (d, 1H, J = 2.32 Hz), 6.86 (dd, 2H, J = 9.5, 2.7 Hz), 7.84 (dd, 2H, J = 9.5, 2.7 Hz); ^{13}C NMR (75.4 MHz, acetone-d_6) 29.8 (C-4), 70.87 (C-3), 71.52 (C-2), 99.88, 100.60 (ArCH), 103.41 (ArC), 120.25 (ArCH), 126.65 (ArC), 136.75 (ArCH), 160.66, 161.59, 161.94, 166.98 (ArC), 170.32 (C=O), Anal. calcd for $C_{16}H_{14}O_6$ (+0.25 H_2O): C, 62.64; H, 4.76. Found: C, 62.95; H 4.74.

3,4-Dihydro-5,7-dihydroxy-2*H*-1-benzopyran-3-yl 3,4-dihydroxybenzoate (7). Overall yield 4%; mp 218–219 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 2.80 (m, 1H), 2.97 (dd, 1H, J = 17.09, 5.21 Hz), 4.15 (dd, 1H, J = 11.36, 1.88 Hz), 4.22 (m, 1H), 5.38 (brm, 1H), 5.89 (d, 1H, J = 2.29 Hz), 6.04 (d, 1H, J = 2.29 Hz), 6.86 (d, 1H, J = 8.27 Hz), 7.40 (dd, 1H, J = 8.27, 2.05 Hz), 7.44 (d, 1H, J = 2.05 Hz); ^{13}C NMR (75.4 MHz, acetone-d_6) δ 25.52 (C-4), 66.62 (C-3), 67.27 (C-2), 95.64, 96.41 (ArCH), 99.16 (ArC), 115.74, 117.14 (ArCH), 122.74 (ArC), 123.47 (ArCH), 145.56, 150.98, 156.39, 157.39, 157.69 (ArC), 166.19 (C=O). Anal. calcd for $C_{16}H_{14}O_7$ (+0.5 H_2O): C, 58.72; H, 4.62. Found: C, 58.95; H, 4.52.

3,4-Dihydro-5,7-dihydroxy-2*H*-1-benzopyran-3-yl 3,5-dihydroxybenzoate (8). Overall yield 8%; mp 245–246 °C; ^{1}H NMR (300 MHz, MeOH-d_4) δ 2.77 (dd, 1H, J = 17.04, 2.69 Hz), 2.92 (dd, 1H, J = 17.04, 5.15 Hz), 4.11 (d, 1H,

$J = 11.09$ Hz), 4.21 (m, 1H), 5.37 (brm, 1H), 5.83 (d, 1H, $J = 2.24$ Hz), 5.93 (d, 1H, $J = 2.24$ Hz), 6.43 (t, 1H, $J = 2.24$ Hz), 6.89 (d, 2H, $J = 2.24$ Hz); ^{13}C NMR (75.4 MHz, MeOH-d_4) δ 25.75 (C-4), 67.52 (C-2), 67.72 (C-3), 95.71, 96.49 (ArCH), 99.52 (ArC), 108.38, 108.86 (ArCH), 133.08, 156.6, 157.79, 159.72 (ArC), 167.49 (C=O). Anal. calcd for $C_{16}H_{14}O_7$ (+1 H_2O): C, 57.14; H, 4.8. Found: C, 57.45; H, 4.65.

2-(3,4-dihydroxyphenyl)ethyl 3,4,5-trihydroxybenzoate (9). Overall yield 5%; mp 189–191 °C, ^{1}H NMR (300 MHz, acetone-d_6) δ 2.87 (t, 2H, $J = 6.92$ Hz), 4.33 (t, 2H, $J = 6.92$ Hz), 6.12 (dd, 1H, $J = 7.99$, 2.01 Hz), 6.74 (d, 1H, $J = 7.99$ Hz), 6.79 (d, 1H, $J = 2.01$ Hz), 7.10 (s, 2H); ^{13}C NMR (75.4 MHz, acetone-d_6) δ 35.2 (C-2), 66.03 (C-1), 109.78, 116.03, 116.75, 121.07 (ArCH), 121.97, 130.76, 138.65, 144.42, 145.8, 145.97 (ArC), 166.64 (C=O). Anal. calcd for $C_{15}H_{14}O_7$ (+0.5 H_2O): C, 57.14; H, 4.80. Found: C, 57.19; H, 4.60.

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