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Total synthesis and antibacterial screening of (±)-7-butyl-6,8-dihydroxy-3-pentyl-3,4-dihydroisochromen-1-one

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A new total synthesis of (±)-7-butyl-6,8-dihydroxy-3-pentyl-3,4-dihydroisochromen-1-one, isolated as R-enantiomer from *Geotrichum* sp., has been described. Reaction of 4-butyl-3,5-dimethoxyhomophthalic anhydride with hexanoyl chloride in the presence of 1,1,3,3-tetramethyl guanidine and triethyl amine afforded 7-butyl-6,8-dimethoxy-3-pentylisochromen-1-one, which was converted into corresponding 3,4-dihydroisochromen-1-one by successive ring opening and reduction. Final demethylation to furnish the natural product was achieved using anhydrous aluminum chloride in ethanethiol. The target compound and the intermediates were subjected to antibacterial evaluation against 10 bacterial strains using levofloxacin as standard.

Keywords: isochromen-1-one; *Geotrichum* sp; 3,5-dimethoxy-4-butylhomophthalic acid; antibacterial

1. Introduction

More than 300 isocoumarins (1*H*-2-benzopyran-1-ones) have been isolated from a wide range of natural sources (microbes, plants, and insects) [1]. These compounds have also been detected in pheromones of termites and ants [2]. Several biological activities have been reported for this class, including cytotoxic, antimicrobial, antiallergic, algicidal, gastroprotective, protease inhibitors, antifungal, and plant growth regulators [3–6]. In addition, nephratoxic, immunomodulatory, antiallergic, and antimalarial activities have also been ascribed to this class of natural products [7–11]. Isocoumarins are isomeric to coumarins with an inverted lactone ring, and most of the natural isocoumarins possess a 3-alkyl (C_1–C_{17}) or a 3-(un)substituted phenyl ring and 6,8-dioxygenation due to their typical biosynthetic origin [12,13].

Kongsaeree and coworkers reported the isolation of three novel dihydroisochromen-1-ones derivatives, with antimalarial, antituberculous, antifungal, and cytotoxic activities by bioassay-guided fractionation from an endophytic fungus, *Geotrichum* sp., collected from *Crassocephalum crepidioides* [14]. The structures were established as 7-butyl-6,8-dihydroxy-3(*R*)-pent-11-enyl-3,4-dihydroisochromen-1-one, 7-but-15-enyl-6,8-dihydroxy-3(*R*)-pent-11-enyl-3,4-dihydroiso chromen-1-one, and 7-butyl-6,8-dihydroxy-3(*R*)-pentyl-3,4-dihydroisochromen-1-one respectively, using spectroscopic techniques (Figure 1).

An elegant total synthesis of 7-butyl-6,8-dihydroxy-3(*R*)-pentyl-3,4-dihydroisocoumarin has been reported in 19% overall yield [15]. Herein, we report a total synthesis of title compound as a racemate. The important feature of present synthesis includes the accessibility of not only 7-butyl-6,8-dihydroxy-3-pentyl-3,4-dihydroisocoumarin but also the corresponding 7-butyl-6,8-dimethoxy-3-pentylisocou-

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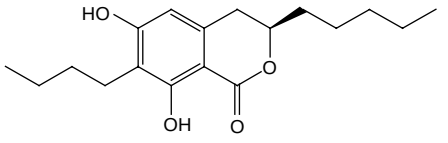
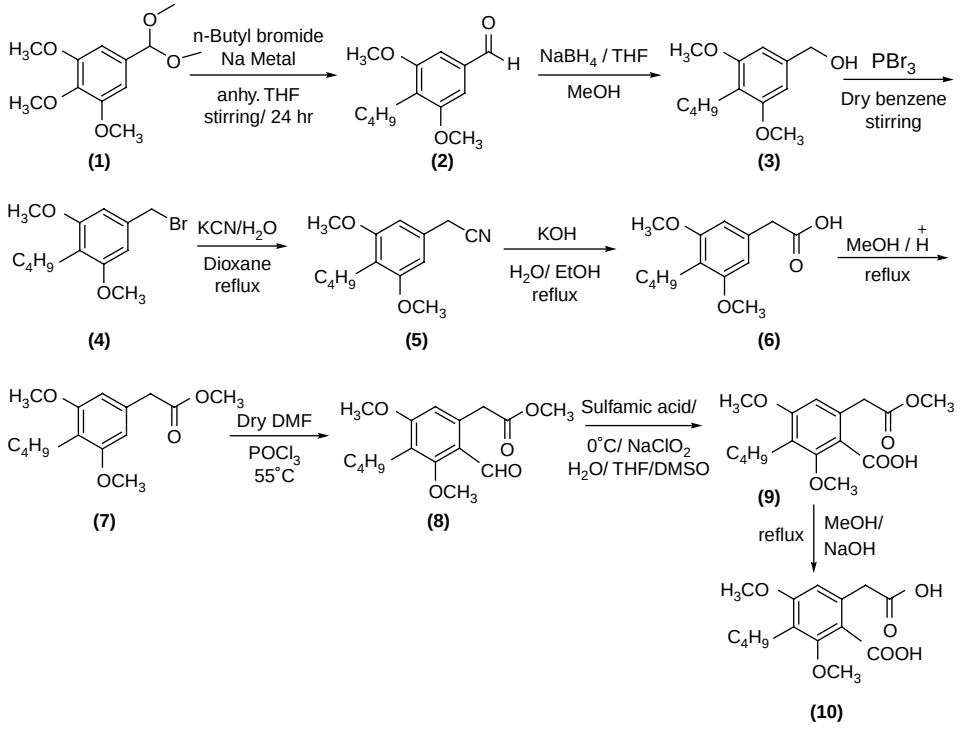


Figure 1. The structure of 7-butyl-6,8-dihydroxy-3(*R*)-pent-11-enyl-3,4-dihydroisocoumarin from *Geotrichum* sp.

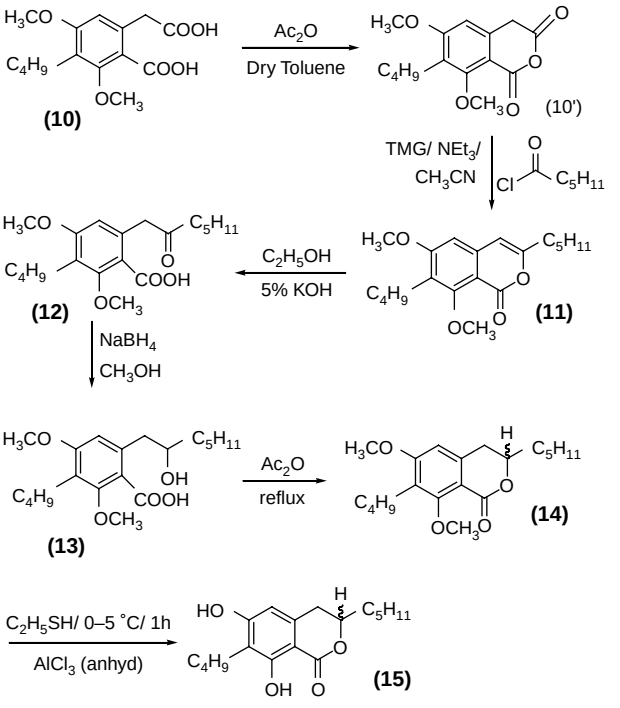
marin, together with the keto- and hydroxy acid derivatives for comprehensive and comparative bioevaluation studies. This work is a continuation of our focusing on the synthesis, characterization, crystal structure, and biological evaluation of this important class of secondary metabolites [16–18].

2. Results and discussion

4-Butyl-3,5-dimethoxy-homophthalic acid was synthesized starting from 3,4,5-trimethoxybenzaldehyde dimethyl acetal (**1**) according to the route indicated in Scheme 1. Thus, the regioselective reductive electrophilic alkylation of dimethyl acetal (**1**) using *n*-butyl bromide in dry tetrahydrofuran (THF) at 0°C, followed by hydrolysis, afforded 4-butyl-3,5-dimethoxybenzaldehyde (**2**) [19], which was reduced to benzyl alcohol (**3**), followed by conversion into corresponding bromide (**4**) and nitrile (**5**). Hydrolysis afforded 4-butyl-3,5-dimethoxyphenylacetic acid (**6**). Methyl ester (**7**) of the latter acid was subjected to Vilsmeier Haack formylation to furnish methyl 4-butyl-2-formyl-3,5-dimethoxyphenyl acetate (**8**). The aldehyde exhibited peak for —CHO at δ_H 10.41 and at δ_C 201.8 in ^{1}H NMR and ^{13}C NMR spectra, respectively. Oxidation of the aldehydic function was achieved using sulfamic acid and sodium chlorite in a mixture of water, THF, and dimethyl sulfoxide (DMSO) to afford the corresponding carboxyl ester (**9**). In IR



Scheme 1. Synthesis of 3,5-dimethoxy-4-butylhomophthalic acid.



Scheme 2. Synthesis of (±)-7-butyl-6,8-dihydroxy-3-pentyl-3,4-dihydroisocoumarin.

spectrum, the carbonyls of ester and carboxyl groups were found at 1731 and 1716 cm^{-1}, respectively. ^1H NMR spectrum indicated the singlets for ester —OCH_3 at δ 3.61 and benzylic-CH_2 at δ 4.32, respectively. The carbonyls of carboxylic and ester appeared at δ 175.2 and δ 167.6 in ^{13}C NMR spectrum. Alkaline hydrolysis of the latter afforded 4-butyl-3,5-dimethoxyhomophthalic acid (**10**) in good yield. In IR spectrum, the carboxylic acid carbons (C═O) were found at 1737 and 1718 cm^{-1}, respectively, and the base peak observed at *m/z* 147 in the mass spectrum. ^1H NMR showed two characteristic singlets for acidic —OH protons at δ 11.02 and δ 10.91 ppm, whereas the carbonyl carbons appeared at δ 175.6 and δ 168.5 ppm in ^{13}C NMR.

Cyclocondensation of 4-butyl-3,5-dimethoxyhomophthalic acid (**10**) with an excess of hexanoyl chloride in the presence of 1,1,3,3-tetramethyl guanidine (TMG) and triethyl amine furnished the 7-butyl-6,8-dimethoxy-3-pentylisochromen-1-one (**11**) in 74% yield [20]. The lactonic carbonyl stretching in IR spectrum appeared at 1725 cm^{-1} and in mass spectrum base peak was observed at *m/z* 234. The isochromen-1-one derivative (**11**) exhibited the characteristic singlets in ^1H NMR spectrum for H-4 and H-5 at δ 6.17 and δ 6.64. In ^{13}C NMR spectrum, the carbonyl carbon (C═O) signal was observed at δ 162.6 and C-3 appeared at δ 158.5 (Scheme 2).

Alkaline hydrolysis of isochromen-1-one (**11**) furnished the keto-acid (**12**). In IR spectrum, the characteristic acidic and ketonic carbonyl peak absorption of keto-acid appeared at 1734 and 1712 cm^{-1}, respectively. In ^1H NMR spectrum, the singlet for benzylic protons appeared at δ 2.83, and in ^{13}C NMR spectrum, ketonic and carboxylic carbonyls were observed at

δ 207.3 and δ 168.5, respectively. The keto-acid (**12**) was reduced to corresponding hydroxy acid (**13**) using sodium borohydride, followed by cyclodehydration with acetic anhydride to yield the corresponding 3,4-dihydroisochromen-1-one (**14**). In IR spectrum, the lactonic carbonyl absorptions appeared at 1721 cm^{-1}, and ^{1}H NMR spectrum showed two double doublets of H-4 protons at δ 3.31 and δ 3.12. In ^{13}C NMR spectrum, the carbonyl and C-3 were observed at δ 168.4 and 78.5.

Complete demethylation of 3,4-dihydroisochromen-1-one (**14**) was achieved using ethanethiol and anhydrous aluminum chloride [21]. In IR spectrum, the characteristic (O—H) stretching appeared at 3421 cm^{-1} and in mass spectrum, the base peak was observed at *m/z* 206. In ^{1}H NMR spectrum, besides the disappearance of C-6 and C-8 methoxy protons, a broad singlet for —OH proton appeared at δ 11.43. The multiplet for H-3 was noted at δ 4.46–4.49 and double doublets for H-4 at δ 3.56 and δ 3.25. In ^{13}C NMR spectrum, the carbonyl carbon (C═O) was observed at δ 169.5 and C-3 appeared at δ 79.3. Two downfield carbon signals at δ 161.7 and δ 160.1 were assigned to C-6 and C-8, respectively. The spectroscopic data (^{1}H and ^{13}C NMR, mass spectrum) of the synthetic compound were in close agreement with those of the natural product [14,15].

In vitro evaluation of antibacterial activity of the key precursors homophthalic acid (**10**), 7-butyl-6,8-dimethoxy-3-pentylisochromen-1-one (**11**), keto-acid (**12**), hydroxy acid (**13**) dihydroisocoumarin (**14**), and the title 6,8-dihydroxy-3,4-dihydroisochromen-1-one (**15**) was performed under aseptic conditions against 10 different bacterial strains [22]. These include the six Gram-negative strains *viz. Pseudomonas aeroginosa*, *Escherichia coli*, *Salmonella typhi*, *Shigella specie*, *Salmonella paratyphi*, and *Proteus mirabilis* and four were Gram-positive strains *viz Bacillus subtilus, Micrococcus aureus, Staphylococcus aureus,* and *Streptococcus specie.* Antibacterial assay was determined by agar well diffusion assay technique by using the Mueller Hinton Agar. The fresh inoculums of these bacteria were prepared and diluted by sterilized normal saline. The turbidity of these cultures was adjusted by using 0.5 McFarland. A homogeneous bacterial lawn was developed by sterile cotton swabs. The inoculated plates were bored by 6-mm-sized borer to make the wells. The sample dilutions were prepared by dissolving each sample (5.0 mg) in 1.0 ml of DMSO used as negative control in this bioassay. The equimolar concentration of levofloxacin (5.0 mg/ml), a broad-spectrum antibiotic (positive control), was prepared. These plates were incubated at 37°C for 24 h. Antibacterial activity of the synthesized compounds (**10**–**15**) was determined by measuring the diameter of zone of inhibition (mm, ± standard deviation) and presented by subtracting the activity of the negative control. The experiments were repeated thrice to minimize the errors; only the mean values are reported in Table 1.

7-Butyl-6,8-dimethoxyhomophthalic acid (**10**) showed antibacterial activity against six different strains of gram-positive and gram-negative bacteria, though it was more effective against *Micrococcus luteus.* 7-Butyl-6,8-dimethoxy-3-pentylisocoumarin (**12**) displayed significant activity against seven different bacterial strains but remarkable activity more than the standard drug against *Shigella specie* and *S. paratyphi*.

Keto-acid (**13**) exhibited minute activity against seven strains of bacteria. The hydroxy acid (**14**) showed good activity comparable to the standard drug against *S. paratyphi* and *S. typhi*, besides the rest of six bacterial strains. 3,4-Dihydroisocoumarin (**15**) was effective against *E. coli* only, and the target 7-butyl-6,8-dihydoxy-dihydroisocoumarin (**16**)

Table 1. Antibacterial bioassay of compounds **10**–**15**.

Compound	*P. aeroginosa*	*E. coli*	*S. typhi*	*P. mirabilis*	*B. subtilus*	*M. luteus*	*S. aureus*	*Sh. specie*	*S. para typhi*	*St. specie*
10	–	9	13	–	16	17	13	–	–	4
11	–	–	18	**20**	–	15	21	**16**	**27**	15
12	9	–	11	14	11	12	12	–	–	10
13	11	4	**19**	15	16	15	–	–	**19**	8
14	–	8	–	–	–	–	–	–	–	–
15	–	2	9	11	–	10	–	–	13	7
Levofloxacin	25	33	24	29	34	38	32	14	21	24

Notes: Gram-positive bacteria: *B. subtilus*, *M. aureus*, *S. aureus*, *St. specie, P. mirabilis*.
Gram-negative bacteria: *P. aeroginosa*, *E. coli*, *S. typhi*, *Sh. specie*, *S. paratyphi*.
Key: –, no activity concentration = 5.0 mg/ml; the bold values indicate good activity compared with the standard.

showed little antibacterial activity against six different bacterial strains (Table 1).

In conclusion, an efficient total synthesis of the natural dihydroisochromen-1-one (±)-7-butyl-6,8-dihydroxy-3-pentyl-3,4-dihydroisochromen-1-one (**15**) has been successfully achieved using 7-butyl-6,8-dimethoxyhomophthalic acid as a key precursor. 7-Butyl-6,8-dimethoxy-3-pentylisochromen-1-one (**11**) and the hydroxy acid (**13**) showed significant antibacterial activity against different bacterial strains.

3. Experimental

3.1 General experimental procedures

Melting points were recorded using a digital Gallenkamp (SANYO) model MPD BM 3.5 apparatus (Loughborough, UK) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were determined in $CDCl_3$ at 300 and 75 MHz, respectively, using Bruker AM-300 spectrophotometer (Billerica, Middlesex, MA, USA). FT IR spectra were recorded using Bio-Rad Excalibur FTS 3000 MX spectrophotometer (Madison, WI, USA); Mass spectra (EI, 70 eV) on a GC-MS instrument (Agilent Technologies 1200 series Santa Clara, CA, USA) and elemental analyses with a LECO-183 CHNS analyzer (LECO Corporation, St Joseph, MI, USA). All compounds were purified by thin layer chromatography (TLC) using silica gel from Merck (Darmstadt, Germany).

3.2 4-Butyl-3,5-dimethoxybenzaldehyde (2)

Compound **2** was prepared according to the synthetic procedure reported in the literature [15]. Yield 82%; R_f 0.5; amorphous solid; mp 47–48°C; IR (KBr) ν_{max}: 2936 (C═C—H), 1734 (C═O ester), 1568 (C═C), 1056 (C—O) cm^{-1}; ^{1}H NMR ($CDCl_3$, δ ppm): 9.83 (1H, s, —CHO), 6.97 (2H, s, Ar–H), 3.81 (6H, s, —OCH_3), 2.63 (2H, t, J = 6.3 Hz, H-1′), 1.33–1.36 (2H, m, H-2′), 1.27–1.31 (2H, m, H-3′), 0.89 (3H, t, J = 6.6 Hz, H-4′); ^{13}C NMR ($CDCl_3$, δ ppm): 201.5 (aldehydic C═O), 158.4 (C-3,C-5), 135.1 (C-2, C-6), 127.2 (C-4), 104.6 (C-1), 55.6 (2 × —OCH_3), 38.7 (C-1′), 23.1 (C-2′), 22.6 (C-3′), 13.8 (C-4′); Elemental analysis: Found: C, 70.17%; H, 8.04%; calcd for $C_{13}H_{18}O_3$: C, 70.23%, H, 8.15%.

3.3 4-Butyl-3,5-dimethoxybenzyl alcohol (3)

4-Butyl-3,5-dimethoxybenzaldehyde (**2**) (4.3 g, 0.0193 mmol) and sodium borohydride (4.18 g, 116 mmol) were suspended in freshly distilled THF (120 ml). The reaction mixture was stirred for 15–20 min at 65°C and then added methanol (120 ml) dropwise

during 30 min. The mixture was refluxed for 4 h, then cooled to room temperature and treated with saturated ammonium chloride solution (100 ml). Stirring was continued for 1 h, and then the mixture was acidified with dilute hydrochloric acid and extracted with ethyl acetate (3 × 25 ml). The extract was dried and evaporated, to yield 4-butyl-3,5-dimethoxybenzyl alcohol (**3**); yield 74%; R_f 0.2; amorphous solid; mp 180–182°C; IR (KBr) ν_{max}: 3342 (O—H), 2933 (C═C—H), 1575 (C═C), 1034 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 6.36 (2H, s, Ar–H), 4.34 (2H, s, —CH_2), 4.07 (1H, s, —OH), 3.80 (6H, s, —OCH_3), 2.64 (2H, t, $J = 6.2$ Hz, H-1′), 1.33–1.36 (2H, m, H-2′), -1.28-1.32-134 (2H, m, H-3′), 0.89 (3H, t, $J = 6.5$ Hz, H-4′); ^{13}C NMR ($CDCl_3$, δ ppm): 158.2 (C-3,C-5), 137.1 (C-2,C-6), 117.8 (C-4), 104.7 (C-1), 55.6 (—OCH_3), 41.3 (—CH_2), 38.9 (C-1′), 23.4 (C-2′), 22.7 (C-3′), 14.1 (C-4′); Elemental analysis: found: C, 69.47%; H, 8.84%; calcd for $C_{13}H_{20}O_3$: C, 69.60%, H, 8.97%.

3.4 4-Butyl-3,5-dimethoxybenzyl bromide (4)

4-Butyl-3,5-dimethoxybenzyl alcohol (**3**) (4.1 g, 180 mmol) was dissolved in dry benzene (30–35 ml). The solution was treated with phosphorous tribromide (6.7 ml, 360 mmol) and the resulting mixture was stirred for 4 h. Then, the reaction mixture was poured on to ice cold water, and the organic layer was separated and evaporated to afford crude 4-butyl-3,5-dimethoxybenzyl bromide (**4**); yield: 70%; R_f 0.6; oil; IR (KBr): 2931 (C═C—H), 1581 (C═C), 1026 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 6.32 (2H, s, Ar–H), 4.57 (2H, s, —CH_2), 3.76 (6H, s, —OCH_3), 2.61 (2H, t, $J = 5.8$ Hz, H-1′), 1.28–1.34 (4H, m, H-2′,H-3′), 0.94 (3H, t, $J = 6.1$ Hz, H-4′); ^{13}C NMR ($CDCl_3$, δ ppm): 158.1 (C-3,C-5), 138.4 (C-2,C-6), 117.5 (C-4), 134.5 (C-1), 55.4 (—OCH_3), 39.5 (—CH_2), 23.1 (C-2′), 22.4 (C-3′), 16.1 (C-1′), 13.8 (C-4′); Elemental analysis: Found: C, 54.27%; H, 6.56%; calcd for $C_{13}H_{19}BrO_2$: C, 54.36%; H, 6.65%.

3.5 2-(4-Butyl-3,5-dimethoxyphenyl) acetonitrile (5)

4-Butyl-3,5-dimethoxybenzyl bromide (**4**) (4.0 g, 1.34 mmol) was dissolved in a mixture of ethyl alcohol (100 ml) and water (100 ml). Potassium cyanide (1.3 g, 1.91 mmol) was then added to reaction flask and the resulting mixture was refluxed for 4 h. Reaction mixture was poured onto ice cold water and extracted with ethyl acetate (3 × 20 ml). The extract was dried over anhydrous Na_2SO_4, evaporated to afford the 2-(4-butyl-3,5-dimethoxyphenyl) acetonitrile (**5**); yield 68%; R_f 0.25; amorphous solid; mp 64–66°C; IR (KBr) ν_{max}: 2933 (C═C—H), 2268 (—CN), 1576 (C═C), 1028 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 6.27 (2H, s, Ar–H), 4.32 (6H, s, —OCH_3), 2.57 (2H, t, $J = 7.1$ Hz, H-1′), 3.68 (2H, s, —CH_2), 1.32–1.62 (4H, m, H-2′,H-3′), 0.93 (3H, t, $J = 6.8$ Hz, H-4′); ^{13}C NMR (75 MHz, δ $CDCl_3$): 158.6 (C-3,C-5), 130.5 (C-1), 118.6 (—C≡N), 108.7 (C-4), 105.3 (C-2,C-6), 56.2 (—OCH_3), 38.7 (—CH_2), 34.3 (C-1′), 23.5 (C-2′), 22.4 (C-3′), 14.1 (C-4′); Elemental analysis: Found: C, 71.97%; H, 8.13%; N, 5.91%; calcd for $C_{14}H_{19}NO_2$: C, 72.06%; H, 8.20%; N, 5.98%.

3.6 2-(4-Butyl-3,5-dimethoxyphenyl) acetic acid (6)

2-(4-Butyl-3,5-dimethoxyphenyl) acetonitrile (**5**) (3.8 g, 19.8 mmol) was dissolved in a mixture of water (15 ml) and dioxane (15 ml). Then, potassium hydroxide (12.81 g in 12 ml of H_2O, 228 mmol) was added, and the mixture was refluxed for 11–12 h. The reaction mixture was poured onto ice cold water and extracted with ethyl acetate (25 ml). The extract was discarded and the aqueous layer was acidified with dilute HCl. Precipitates were filtered out to

afford 2-(4-butyl-3,5-dimethoxyphenyl) acetic acid (**6**); yield 73%; R_f 0.4; amorphous solid; mp 123–125°C; IR (KBr) ν_{max}: 3284 (O—H), 2924 (C═C—H), 1711 (C═O acidic), 1575 (C═C), 1046 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 11.13 (1H, s, —COOH), 6.26 (2H, s, Ar), 4.26 (2H, s, —CH_2), 3.73 (6H, s, —OCH_3), 2.64 (2H, t, $J = 7.1$ Hz, H-1′), 1.30–1.61 (4H, m, H-2′,H-3′), 0.92 (3H, t, $J = 6.8$ Hz, H-4′); ^{13}C NMR ($CDCl_3$, δ ppm): 176.3 (carboxylic C═O), 159.2 (C-3,C-5), 134.4 (C-1), 108.5 (C-4), 106.3 (C-2,C-6), 56.5 (—OCH_3), 48.7 (—CH_2), 34.6 (C-1′), 23.7 (C-2′), 22.3 (C-3′), 14.1 (C-4′); Elemental analysis: found: C, 66.56%; H, 7.86%; calcd for $C_{14}H_{20}O_4$: C, 66.64%; H, 7.97%.

3.7 Methyl 2-(4-Butyl-3,5-dimethoxyphenyl) acetate (7)

A stirred solution of 2-(4-butyl-3,5-dimethoxyphenyl) acetic acid (**6**) (3.5 g, 13.8 mmol) in dry methanol (20 ml) was treated dropwise with conc. H_2SO_4 (3 ml). The mixture was refluxed for 7–8 h. The reaction was monitored by TLC. After the completion of reaction, the mixture was concentrated to 55 ml and extracted with ethyl acetate (3 × 30 ml). The extract was washed with saturated brine, dried, and concentrated to give crude oil which was distilled to afford methyl 2-(4-butyl-3,5-dimethoxyphenyl) acetate (**7**); yield 81%; R_f 0.5; oil; IR (KBr) ν_{max}: 2926 (C═C—H), 1732 (C═O ester), 1587 (C═C), 1053 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 6.36 (2H, s, Ar–H), 4.28 (2H, s, —CH_2), 3.73 (6H, s, —OCH_3), 3.63 (3H, s, ester —OCH_3). 2.65 (2H, t, $J = 7.1$ Hz, H-1′), 1.29–1.63 (4H, m, H-2′,H-3′), 0.93 (3H, t, $J = 6.8$ Hz, H-4′); ^{13}C NMR ($CDCl_3$, δ ppm): 168.6 (ester C═O), 158.7 (C-3,C-5), 134.2 (C-1), 108.7 (C-4), 106.6 (C-2,C-6), 56.4 (—OCH_3), 52.7 (ester —OCH_3), 46.5 (—CH_2), 34.7 (C-1′), 23.3 (C-2′), 22.4 (C-3′), 14.1 (C-4′); Elemental analysis: found: C, 67.57%; H, 7.96%; calcd for $C_{15}H_{22}O_4$: C, 67.64%; H, 8.32%.

3.8 Methyl 4-butyl-2-formyl-3,5-dimethoxyphenyl acetate (8)

Phosphorous oxychloride (1.24 ml, 11.3 mmol) was added dropwise into a stirred solution of methyl 2-(4-butyl-3,5-dimethoxyphenyl) acetate (**7**) (3.0 g, 11.3 mmol) in dry DMF (15 ml) at 55°C. The reaction mixture was heated at about 100°C for 2 h and stirred overnight at room temperature. Then, the reaction mixture was poured into aqueous solution of sodium acetate (10%, 10 ml) and shaken vigorously. Methyl 4-butyl-2-formyl-3,5-dimethoxyphenyl acetate (**8**) was precipitated out; yield 75%; R_f 0.3; semi-solid; IR (KBr) ν_{max}: 2932 (C═C—H), 1724 (ester C═O), 1686 (aldehyde C═O), 1577 (C═C), 1052 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 10.41 (1H, s, —CHO), 6.37 (1H, s, Ar–H), 4.31 (2H, s, —CH_2), 3.73 (3H, s, —OCH_3), 3.71 (3H, s, —OCH_3), 3.26 (3H, s, ester —OCH_3), 2.68 (2H, t, $J = 7.1$ Hz, H-1′), 1.26—1.61 (4H, m, H-2′,H-3′), 0.92 (3H, t, $J = 6.8$ Hz, H-4′); ^{13}C NMR ($CDCl_3$, δ ppm): 201.8 (aldehyde C═O), 169.4 (ester C═O), 163.4 (C-5), 161.3 (C-3), 135.8 (C-2), 125.2 (C-6), 121.4 (C-4), 116.5 (C-1), 56.3 (Ar–OCH_3), 52.4 (ester —OCH_3), 42.2 (—CH_2), 34.3 (C-1′), 23.4 (C-2′), 22.5 (C-3′), 14.1 (C-4?); Elemental analysis: found: C, 65.17%; H, 7.46%; calcd for $C_{16}H_{22}O_5$: C, 65.28%; H, 7.52%.

3.9 Methyl 4-butyl 2-carboxy-3,5-dimethoxyphenyl acetate (9)

Methyl 4-butyl-2-formyl-3,5-dimethoxyphenyl acetate (**8**) (2 g, 6.802 mmol) and sulfamic acid (2.24 g, 23.13 mmol) in 30 ml H_2O:THF:DMSO (20:10:1) at 0°C were treated with $NaClO_2$ (1.99 g, 22.01 mmol) in 5 ml H_2O. The reaction mixture was stirred for 20 min at 0°C and then diluted with ethyl acetate (100 ml), washed with saturated aqueous ammonium

chloride (2 × 30 ml) and saturated aqueous sodium chloride (30 ml). Organic layer was dried over anhydrous sodium sulfate and evaporated to afford methyl 4-butyl 2-carboxy-3,5-dimethoxyphenyl acetate (**9**); yield 67%; R_f 0.35; oil; IR (KBr) ν_{max}: 3276 (O—H), 2935 (C═C—H), 1731 (ester C═O), 1716 (carboxylic C═O), 1564 (C═C), 1046 (C-O) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 8.76 (1H, s, —COOH), 7.56 (1H, s, H-6), 4.32 (2H, s, $—CH_2$), 3.72 (3H, s, $—OCH_3$), 3.69 (3H, s, $—OCH_3$), 3.61 (3H, s, ester $—OCH_3$), 2.63 (2H, t, $J = 7.1$ Hz, H-1′), 1.31–1.63 (4H, m, H-2′, H-3′), 0.90 (3H, t, $J = 6.8$ Hz, H-4′); ^{13}C NMR ($CDCl_3$, δ ppm): 175.2 (carboxylic C═O), 167.6 (ester C═O), 161.2 (C-3), 160.4 (C-5), 136.8 (C-2), 127.3 (C-6), 121.6 (C-4), 117.4 (C-1), 62.4 (ester $—OCH_3$), 55.7 ($—OCH_3$), 55.4 ($—OCH_3$), 42.6 ($—CH_2$), 34.5 (C-1′), 23.5 (C-2′), 22.6 (C-3′), 14.1 (C-4′); Elemental analysis: Found: C, 61.83%; H, 7.07%; calcd for $C_{16}H_{22}O_6$: C, 61.91%; H, 7.13%.

3.10 4-Butyl-3,5-dimethoxyhomophthalic acid (10)

Methyl 4-butyl-2-carboxy-3,5-dimethoxyphenyl acetate (**9**) (1.5 g, 4.84 mmol) was dissolved in ethanol (15 ml) and treated with KOH (5%, 10 ml). The reaction mixture was refluxed for 1–2 h and the ethanol was rotary evaporated. The aqueous layer was acidified with dilute hydrochloric acid to afford 4-butyl-3,5-dimethoxyhomophthalic acid (**10**); yield 77%; R_f 0.3; semi-solid; mp 123–125°C; IR (KBr) ν_{max}: 3226 (O—H), 2937 (C═C—H), 1737 (carboxylic C═O), 1718 (carboxylic CH_2—C═O), 1586 (C═C), 1044 (C—O) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 11.02 (1H, s, —COOH), 10.91 (1H, s, $—CH_2—$COOH), 6.34 (1H, s, H-6), 3.73 (3H, s, $—OCH_3$), 3.71 (3H, s, $—OCH_3$), 2.87 (2H, s, $—CH_2$), 2.64 (2H, t, $J = 7.1$ Hz, H-1′), 1.32—1.67 (4H, m, H-2′,H-3′), 0.91 (3H, t, $J = 6.8$ Hz, H-4′); ^{13}C NMR ($CDCl_3$, δ ppm): 175.6 (carboxylic —C═O), 168.5 (carboxylic $—CH_2—$C═O), 161.7 (C-5), 160.5 (C-3), 136.2 (C-2), 124.3 (C-1), 118.4 (C-4), 105.7 (C-6), 55.8 ($—OCH_3$), 55.6 ($—OCH_3$), 42.5 ($—CH_2$), 34.7 (C-1′), 23.4 (C-2′), 22.5 (C-3′), 14.1 (C-4′); MS (70 eV) *m/z* (%): 296 $[M]^{+\cdot}$ (57), 251 (34), 177 (16), 147 (100%), 91 (28); Elemental analysis: Found: C, 60.63%; H, 6.65%; calcd for $C_{15}H_{20}O_6$: C, 60.80%, H, 6.78%.

3.11 7-Butyl-6,8-dimethoxy-3-pentylisochromen-1-one (11)

4-Butyl-3,5-dimethoxyhomophthalic acid (**10**) was converted into corresponding anhydride (**10′**) by refluxing it with acetic anhydride in the presence of dry toluene as solvent and used as such in next step. The anhydride (**10′**) (0.5 g, 1.96 mmol) was condensed with hexanoyl chloride (1.14 ml, 8.29 mmol) in the presence of TMG and triethyl amine afforded the 7-butyl-6,8-dimethoxy-3-pentylisochromen-1-one. After completion, the reaction mixture was concentrated and then extracted with ethyl acetate. The two phases were separated, and the organic layer was washed with saturated sodium chloride solution and then dried over anhydrous (Na_2SO_4) to get 7-butyl-6,8-dimethoxy-3-pentylisochromen-1-one (**11**), which was then purified by preparative TLC using (petroleum ether and ethyl acetate, 7:3) as eluent; yield 74%; R_f 0.7; amorphous solid; mp 195–196°C; IR (KBr) ν_{max}: 3027 (C═C—H), 2956 (C—H), 1725 (C═O lactonic), 1577 (C═C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 6.64 (1H, s, H-5), 6.17 (1H, s, H-4), 3.76 (3H, s, $—OCH_3$), 3.72 (3H, s, $—OCH_3$), 2.63 (2H, t, $J = 7.4$ Hz, H-1′), 2.53–2.56 (2H, m, H-1″), 1.34–1.85 (10H, m, H-2′,H-3′,H-4′,H-2″,H-3″), 0.93 (3H, t, $J = 7.2$ Hz, H-4″), 0.89 (3H, t, $J = 6.8$ Hz, H-5′); ^{13}C NMR ($CDCl_3$, δ ppm): 162.6 (C═O), 161.4 (C-6), 160.7 (C-8), 158.5 (C-3), 138.4 (C-10), 127.8 (C-4), 118.5 (C-7), 106.3 (C-5), 103.6 (C-9), 56.7 ($—OCH_3$), 56.4

(—OCH_3), 34.7 (C-1′), 34.1 (C-1″), 31.5 (C-2′), 24.8 (C-2″), 24.3 (C-3′), 22.9 (C-4′), 22.4 (C-3″), 14.2 (C-4″); 14.1 (C-5′); MS (70eV) *m/z* (%): 332 $[M]^{+\cdot}$ (37), 261 (62), 234 (100), 157 (26); Elemental analysis: Found: C, 72.14%; H, 8.36%; calcd for $C_{20}H_{28}O_4$: C, 72.28%; H, 8.43%.

3.12 3-Butyl-2,4-dimethoxy-6-(2-oxoheptyl) benzoic acid (12)

A stirred solution of 7-butyl-6,8-dimethoxy-3-pentylisochromen-1-one (**11**) (0.35 g, 1.05 mmol) in ethanol (7–8 ml) was treated with 5% KOH (15 ml), and the mixture was refluxed for 4 h. After cooling the reaction mixture, most of the ethanol was evaporated under reduced pressure. Cold water (20 ml) was added and the mixture was acidified with dilute hydrochloric acid when solid was precipitated. Filtration followed by drying under vacuum afforded 3-butyl-2,4-dimethoxy-6-(2-oxoheptyl) benzoic acid (**12**); yield 72%; R_f 0.45; sticky solid; IR (KBr) ν_{max}: 3342 (O—H), 3023 (C═C—H), 2952 (C—H), 1734 (carboxylic C═O), 1712 (ketonic C═O), 1584 (C═C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 10.7 (1H, s, —COOH), 6.36 (1H, s, H-5), 3.77 (3H, s, —OCH_3), 3.74 (3H, s, —OCH_3), 2.83 (2H, s, —$CH_{2benzylic}$), 2.64 (2H, t, $J = 7.2$ Hz, H-1″), 2.54–2.57 (2H, m, H-1′), 1.33–1.83 (10H, m, H-2′,H-3′,H-4′,H-2″,H-3″), 0.92 (3H, t, $J = 7.2$ Hz, H-4″), 0.89 (3H, t, $J = 6.7$ Hz, H-5′); ^{13}C NMR ($CDCl_3$, δ ppm): 207.3 (ketonic C = O), 168.5 (carboxylic C═O), 162.4 (C-5), 160.8 (C-3), 136.4 (C-1), 108.5 (C-4), 105.6 (C-2), 102.7 (C-6), 56.5 (—OCH_3), 56.3 (—OCH_3), 46.7 (C-1′), 43.5 (C-3′), 34.4 (Ar–CH_3), 31.3 (C-1″), 24.7 (C-4′), 23.6 (C-2″), 23.1 (C-5′), 22.7 (C-3″), 22.3 (C-6′), 14.2 (C-4″), 14.0 (C-7′); MS (70 eV) *m/z* (%): 350 $[M]^{+\cdot}$ (31), 279 (42), 251 (56), 191 (100), 91 (25); Elemental analysis: Found: C, 68.47%; H, 8.41%; calcd for $C_{20}H_{30}O_5$: C, 68.57%; H, 8.57%.

3.13 3-Butyl-6-(2-hydroxyheptyl)-2,4-dimethoxybenzoic acid (13)

Sodium borohydride (0.13 g, 3.43 mmol) was added portionwise to a stirred solution of 3-butyl-2,4-dimethoxy-6-(2-oxoheptyl) benzoic acid (**12**) (0.2 g, 0.573 mmol) in absolute ethanol (15 ml). The reaction mixture was stirred for 2 h at room temperature, diluted with water (50 ml), acidified with conc. HCl, and stirred for a further 2 h. It was then saturated with ammonium sulfate, and extracted with ethyl acetate (3 × 30 ml). The organic layer was dried over anhydrous magnesium sulfate and concentrated to afford 3-butyl-6-(2-hydroxyheptyl)-2,4-dimethoxy benzoic acid (**13**); yield 76%; R_f 0.4; semi-solid; IR (KBr) ν_{max}: 3453 (O—H), 3026 (C═C—H), 2914 (C—H), 1726 (carboxylic C═O), 1581 (C═C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 10.9 (1H, s, —COOH), 6.42 (1H, s, H-5), 4.43 (1H, s, —OH), 3.78 (3H, s, —OCH_3), 3.76 (3H, s, —OCH_3), 2.81–2.84 (2H, m, —CH_2), 2.62 (2H, t, $J = 7.4$ Hz, H-1′), 2.55–2.58 (2H, m, H-1″), 1.34–1.85 (10H, m, H-2′,H-3′,H-4′,H-2″,H-3″), 0.94 (3H, t, $J = 7.1$ Hz, H-4″), 0.89 (3H, t, $J = 6.7$ Hz, H-5′); ^{13}C NMR ($CDCl_3$, δ ppm): 207.3 (ketonic C═O), 168.7 (carboxylic C═O), 162.8 (C-5), 160.7 (C-3), 136.7 (C-1), 118.5 (C-4), 105.6 (C-2), 102.7 (C-6), 78.5 (C-2′), 56.7 (—OCH_3), 56.4 (—OCH_3), 40.7 (C-1′), 37.5 (C-3′), 34.6 (Ar–CH_3), 31.5 (C-1″), 24.6 (C-4′), 23.8 (C-2″), 22.9 (C-5′), 22.5 (C-3″), 22.1 (C-6′), 14.2 (C-4″), 14.1 (C-7′); MS (70eV) *m/z* (%): 352 $[M]^{+\cdot}$ (25), 307 (32), 291 (100), 251 (22), 193 (13); Elemental analysis: Found: C, 68.10%; H, 9.08%; calcd for $C_{20}H_{32}O_5$: C, 68.15%; H, 9.16%.

3.14 (±)-7-Butyl-6,8-dimethoxy-3-pentyl-3,4-dihydroisochromen-1-one (14)

3-Butyl-6-(2-hydroxyheptyl)-2,4-dimethoxybenzoic acid (**13**) (0.14 g, 0.41 mmol) was dissolved in acetic anhydride (4 ml) and refluxed for 3 h. The reaction mixture was then poured into chilled water, and it was

extracted with ethyl acetate (2 × 20 ml). Organic layer was washed with 1% $NaHCO_3$ and water. The organic layer was dried with Na_2SO_4 and concentrated to get (±)-7-butyl-6,8-dimethoxy-3-pentyl-3,4-dihydroisochromen-1-one (**14**); yield 66%; R_f 0.65; yellow oil; IR (KBr) ν_{max}: 3037 (C═C—H), 2943 (C—H), 1721 (C═O), 1586 (C═C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 6.31 (1H, s, H-5), 4.42–4.46 (1H, m, H-3), 3.84 (3H, s, —OCH_3), 3.81 (3H, s, —OCH_3), 3.51 (1H, dd, $J_{gem} = 12.4$ Hz, $J_{trans} = 14.2$ Hz, H-4), 3.22 (1H, dd, $J_{gem} = 12.4$ Hz, $J_{cis} = 6.2$ -Hz, H-4), 2.77–2.81 (2H, m, —CH_2), 2.61 (2H, t, $J = 7.4$ Hz, H-1′), 2.52–2.56 (2H, m, H-1″), 1.35–1.87 (10H, m, H-2′,H-3′, H-4′,H-2″,H-3″), 0.93 (3H, t, $J = 7.2$ Hz, H-4″), 0.89 (3H, t, $J = 6.7$ Hz, H-5′); ^{13}C NMR ($CDCl_3$, δ ppm): 168.4 (C═O), 161.7 (C-6), 158.8 (C-8), 138.4 (C-10), 114.2 (C-7), 106.3 (C-5), 102.6 (C-9), 79.1 (C-3), 56.7 (—OCH_3), 56.2 (—OCH_3), 41.8 (C-4), 34.3 (C-1′), 33.2 (C-1″), 31.2 (C-2′), 30.7 (C-2″), 24.4 (C-3′), 22.7 (C-4′), 22.2 (C-3″), 14.1 (C-4″), 14.0 (C-5′); MS (70eV) *m/z* (%): 334 $[M]^{+\cdot}$ (37), 306 (53), 263 (28), 234 (100), 206 (36), 165 (21); Elemental analysis: Found: C, 71.73%; H, 8.97%; calcd for $C_{20}H_{30}O_4$: C, 71.85%; H, 9.04%.

3.15 (±)-7-Butyl-6,8-dihydroxy-3-pentyl-3,4-dihydroisochromen-1-one (15)

(±)-7-Butyl-6,8-dimethoxy-3-pentyl-3,4-dihydroisochromen-1-one (**14**) (1.25 mmol) was dissolved in ethanethiol (3.5 ml) and the solution was cooled on ice. Aluminium chloride (3.8 mmol) was added as three portions with an interval of 30 min. After all the aluminium chloride was added, the reaction mixture was stirred on ice for 1 h. The reaction was quenched with water, alkalinized (10% $NaHCO_3$), and extracted with ethyl acetate. Sodium chloride was added to enhance layer separation. The combined organic layers were washed once with brine, dried over sodium sulfate, and concentrated to give (±)-7-butyl-6,8-dihydroxy-3-pentyl-3,4-dihydroisochromen-1-one (**15**); yield 62%; R_f 0.3; oil; IR (KBr) ν_{max}: 3421 (O—H), 3046 (C═C—H), 2954 (C—H), 1724 (C═O), 1587 (C═C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): 11.43 (2H, s, —OH), 6.20 (1H, s, H-5), 4.46–4.49 (1H, m, H-3), 3.56 (1H, dd, $J_{gem} = 12.4$ Hz, $J_{trans} = 14.2$ Hz, H-4), 3.25 (1H, dd, $J_{gem} = 12.4$ Hz, $J_{cis} = 6.2$ Hz, H-4), 2.80–2.83 (2H, m, —CH_2), 2.64 (2H, t, $J = 7.5$ Hz, H-1′), 2.51–2.54 (2H, m, H-1″), 1.34–1.87 (10H, m, H-2′,H-3′,H-4′,H-2″,H-3″), 0.94 (3H, t, $J = 7.2$ Hz, H-4″), 0.91 (3H, t, $J = 6.8$ Hz, H-5′); ^{13}C NMR ($CDCl_3$, δ ppm): 169.5 (C═O), 161.7 (C-8), 160.1 (C-6), 138.4 (C-10), 114.7 (C-7), 106.1 (C-5), 101.8 (C-9), 79.3 (C-3), 34.6 (C-1′), 33.1 (C-4), 32.8 (C-1″), 31.4 (C-2′), 24.5 (C-2″), 22.8 (C-3′), 22.5 (C-4′), 22.1 (C-3″), 14.1 (C-4″), 14.0 (C-5′); MS (70 eV) *m/z* (%): 306 $[M]^{+\cdot}$ (47), 278 (35), 235 (67), 206 (100), 189 (43), 146 (34); Elemental analysis: Found: C, 70.43%; H, 8.47%; calcd for $C_{18}H_{26}O_4$: C, 70.55%; H, 8.54% (Petroleum ether: ethyl acetate, 8:2).

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