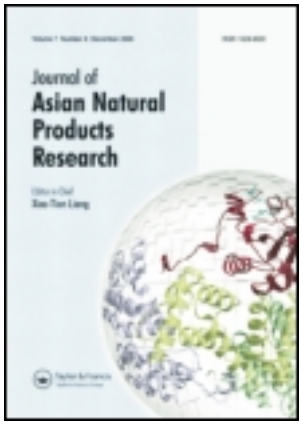

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Total synthesis and cytotoxic activity of stellatin

Aamer Saeed [a], Zaman Ashraf [a] & Hummera Rafique [a]

[a] Department of Chemistry, Quaid-I-Azam University, Islamabad, 45320, Pakistan

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Total synthesis and cytotoxic activity of stellatin

Aamer Saeed*, Zaman Ashraf and Hummera Rafique

Department of Chemistry, Quaid-I-Azam University, Islamabad 45320, Pakistan

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Stellatin (3,4-dihydro-8-hydroxy-7-hydroxymethyl-6-methoxyisocoumarin) (**8**), an extrolite of fungal genera *Emericella* and *Aspergillus*, was synthesized. Thus, Vilsmeier–Haack formylation of methyl ester of 3,5-dimethoxy-4-methylphenylacetic acid (**1**) to afford the formyl ester (**2**) followed sulfamic acid–sodium chlorite oxidation of the aldehydic function to yield the carboxy ester (**3**). Chemoselective reduction of ester function in the latter using $NaBH_4$/THF/MeOH furnished the corresponding hydroxy acid (**4**) that on cyclodehydration afforded the 3,4-dihydro-6,8-dimethoxy-7-methylisocoumarin (**5**). Benzylic bromination of the C-7 methyl in **5** using NBS/benzoyl peroxide to give the 7-bromomethyldihydroisocoumarin (**6**) followed the nucleophilic substitution using aqueous acetone to provide 7-hydroxymethyl-dihydroisocoumarin (**7**). Finally, the regioselective demethylation of 8-methoxyl group using anhydrous magnesium iodide furnished the stellatin (**8**). The dihydroisocoumarins (**5**–**8**) were screened for cytotoxic activity against human keratinocyte cell line and were found to exhibit moderate to good activity.

Keywords: dihydroisocoumarin; stellatin; *Aspergillus variecolor*; cytotoxic activity

1. Introduction

Isocoumarins and dihydroisocoumarins are the extrolites (secondary metabolites) of a wide variety of fungi, bacteria, plants, marine organisms, and also among insect venoms and pheromones, exhibiting a wide variety of structural diversity and biological activities [1–3]. Nearly, 300 isocoumarins derivatives have been isolated from various natural sources, exhibiting a wide spectrum of biological activities such as antiallergic, antimicrobial [4], immunomodulatory [5], cytotoxic [6], antifungal, anti-inflammatory [7], antiangiogenic [8], anticalmodulin-sensitive cGMP phosphodiesterase effects [9], antileukemic [10], and anti-HIV activities [11].

Majority of the natural isocoumarins being of polyketide origin are derived biogenetically from acetate-polymalonate pathway; hence, most of them possess a C-3 alkyl/aryl substituent and C-8 oxygenation as in phyllodulcin and hydrangenol, the low-calorie sweetening agents, or a C-6/C-8 dioxygenation [1,3]. Common examples of the latter include 6-methoxymellein, diaporthin, fusamarin, asperentin derivatives, agrimonolide, canescins A&B, sclerotinins A&B, feralolide, achlisocoumarin, *Ononis natrix* dihydroisocoumarins, scorzocreticin, hiburipyranone [12–16], and cerocphorins A-C, antifungal and cytotoxic metabolites from *Cercophora areolata* [6]. Stellatin (Figure 1) is a novel phenolic dihydroisocoumarin metabolite first isolated from the mycelium of *Aspergillus variecolor* (syn. *Aspergillus stellatus*) and its structure was determined by the chemical and modern NMR spectroscopic techniques as

*Corresponding author. Email: aamersaeed@yahoo.com

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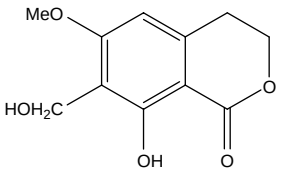


Figure 1. Structure of stellatin (3,4-dihydro-8-hydroxy-7-hydroxymethyl-6-methoxyisocoumarin, **8**).

3,4-dihydro-8-hydroxy-7-hydroxymethyl-6-methoxyisocoumarin. It is also an extrolite of *Emericella venezuelensis*, *Emericella variecolor*, and *Emericella heterothallica* [17].

Stellatin is unique in being unsubstituted at both C-3 and C-4 and in having a carbon substituent at C-7. The only structural relatives known to date are the 8-hydroxyisocoumarin and 3,4-dihydro-8-hydroxyisocoumarin found in the defensive secretion of the tenebrionid beetle, *Apsena pubescens* [18], and 5-formyl-3,4-dihydroisocoumarin isolated from a medicinal plant *Centaurium erythraea* [19]. Those having a C-7 carbon substituent include stoloniferol A from the sea squirt-derived fungus, *Penicillium stoloniferum* [20], and the antimalarial dihydroisocoumarins from endophytic fungus *Geotrichum* sp. [21]. A closely related isochroman mycotoxin 3,7-dimethyl-8-hydroxy-6-methoxyisochroman and its derivatives have been isolated from *Penicillium corylophilum*, which significantly inhibit the etiolated wheat coleoptile growth and also act as auxin polar transport inhibitors [22]. A novel chromane derivative related to stellatin was isolated along with stellatin from *E. heterothallica* [23].

The study of biosynthesis of stellatin is also very interesting. The overall structure is consistent with a polyketide origin. However, if stellatin is a tetraketide, then C-3 must be derived by the introduction of a methyl group from C_1-pool on the methyl carbon of the chain-initiating unit. Alternatively, if it is of pentaketide origin, the methyl carbon of the chain-initiating unit must be lost. Both of these processes are unprecedented in polyketide biosynthesis. Biosynthetic studies involving the incorporation of ^{13}C-labeled acetates and methionine supported the polyketide biosynthetic pathway for stellatin, as a bis-*C*-methylated tetraketide [24].

Taking into consideration the unique structure, interesting biosynthesis and for making it available for bioevaluation, an efficient synthesis of stellatin was desired.

2. Results and discussion

We envisaged the synthesis of **8** using 3,5-dimethoxy-4-methylphenylacetic acid (**1**) as the key starting material, itself prepared from the commercial *p*-toluic acid. Thus, esterification of *p*-toluic acid followed the nuclear bromination using swamping catalyst effect involving bromine and an excess of anhydrous $AlCl_3$ to afford the 3,5-dibromo-4-methylbenzoic acid. Copper (I) chloride-catalyzed nucleophilic substitution of the latter with methoxide provided 3,5-dimethoxy-4-methylbenzoic acid [25] which was then converted into 3,5-dimethoxy-4-methylphenylacetic acid (**1**) by the standard homologation sequence. Methyl ester of the acid (**1**) was subjected to Vilsmeier–Haack formylation to furnish methyl 2-formyl-3,5-dimethoxyphenyl acetate (**2**). The peaks for CHO at δ_H 9.75 and δ_C 179.3 were observed in ^{1}H NMR and ^{13}C NMR spectra, respectively. Oxidation of the aldehydic function was achieved using sulfamic acid and sodium chlorite at 0°C in 79% yield to afford carboxy ester (**3**). The carbonyl absorption in the IR spectrum shifted from 1690 to 1715 cm^{-1} in addition to broad absorption at 3265 cm^{-1} for carboxylic hydroxyl. In the ^{1}H NMR spectrum, the singlet for carboxylic hydrogen at δ 8.19 and downfield shift from δ 179.3 to δ 197.7 for carboxylic carbon were noted in the ^{13}C

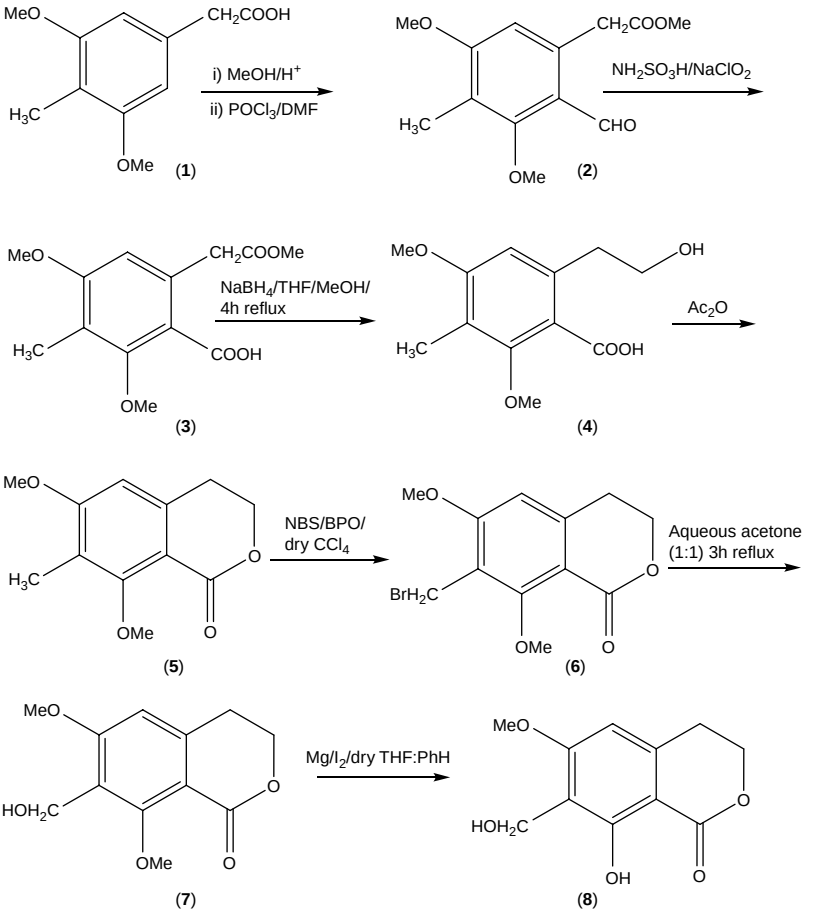
NMR spectrum. Chemoselective reduction of ester function in **3** leaving acid group intact was achieved using $NaBH_4$/THF/MeOH to give the hydroxy acid (**4**) which on cyclodehydration in refluxing acetic anhydride afforded 3,4-dihydro-6,8-dimethoxy-7-methylisocoumarin (**5**). In the IR spectrum of the dihydroisocoumarin, the lactonic carbonyl absorption was observed at 1725 cm^{-1}. The protons of C-4 methylene group were shown as a triplet at δ 2.56 ($J = 3.6$ Hz) and that for C-3 methylene were shown slightly downfield at δ 4.25 ($J = 3.6$ Hz) due to vicinity of oxygen. Benzylic bromination of the 7-methyl group using NBS/benzoyl peroxide was carried out using dry CCl_4 to yield the bromide (**6**). In the ^{1}H NMR spectrum, the singlet for 7-methyl protons shifted downfield from δ 2.66 in **5** to δ 4.85 in **6**. Due to the presence of the base-sensitive lactone ring, a mild method for nucleophilic substitution of 7-bromomethyl group to 7-hydroxymethyl was required. It was achieved by refluxing the bromide (**6**) in aqueous acetone (1:2) for 2 h to provide **7**. The IR spectrum showed a broad band at 3467 cm^{-1} for hydroxyl group, while in the ^{1}H NMR spectrum a singlet appeared at δ 2.36.

Regioselective demethylation of **7** was finally accomplished using Mg/I_2 refluxed in THF/benzene to afford 8-hydroxy-7-(hydroxymethyl)-6-methoxy-3,4-dihydro-1*H*-isochromen-1-one (*stellatin*) (**8**, Scheme 1). In the ^{1}H NMR spectrum, a singlet at δ 10.90 appeared for 8-hydroxy, while that for 7-hydroxy proton at δ 2.29. The presence of phenolic hydroxyl was confirmed by a purple ferric chloride test and solubility in dilute aqueous sodium hydroxide. In the IR spectrum, the lactonic carbonyl absorption was lowered from 1714 to 1695 cm^{-1} due to chelation with 8-hydroxyl, which appeared at 3559 cm^{-1}. The spectroscopic data (^{1}H and ^{13}C NMR, mass spectrum) of the synthetic compound was identical with that of the natural product [17]; however, no physico-chemical data were reported in the original paper nor was a specimen available for comparison purposes.

The Neutralrot-Test was carried out for cytotoxic activity of compounds **5**–**8** according to the protocol of the National Institutes of Health. In the vital dye, neutral red is taken up by living cells and then protonated. The cell acts as an ion trap, allowing the dye not to diffuse out of it. By destroying the cells, the neutral is released again and can be determined photometrically. The absorption is a measure of cell viability, and a lower absorption indicates the less living cells. For the tests, the immortalized human keratinocyte cell line was used. Incubation with the test substances was carried out for 3 days and was conducted in two independent experiments with a number of parallels. The stock solutions of substances were prepared in DMSO. Each of the tested concentration range was between 1.56 and 100 μM. The concentrations of the solvent DMSO were all tested in concentrations of 0.1%. Etoposide as positive control was carried in a concentration of 10 μM. Etoposide showed an IC_{50} value of 0.8 μM (Figure 2).

The results showed that the cytotoxic activity increases with an increase in the concentration of compounds. Stellatin exhibits higher activity than its precursors. While compounds **5** and **6** showed moderate to low cytotoxic activity at the highest concentration (100 μM). However, when 7-methyl group in compound **5** is converted into 7-hydroxymethyl group in **7**, the cytotoxic activity is increased with a decrease in viability of up to 45%. Selective demethylation of the 8-methoxy group in stellatin **8** resulted in further increase in cytotoxic activity. Thus, the presence of the 7-hydroxymethyl group and the free 8-hydroxy group play a vital role in cytotoxic activity of stellatin.

In summary, the first total synthesis of stellatin, a natural bioactive dihydro-



Scheme 1. Synthesis route to stellatin (**8**).

isocoumarin and its cytotoxic activity has been reported.

3. Experimental

3.1 General experimental procedures

The solvents were purified and dried according to the standard procedures before using. The dried solvents were stored under molecular sieves (4A). Standard procedures were employed for the purification and drying of solvents. Melting points were recorded using a digital Gallenkamp (SANYO) model MPD BM 3.5 apparatus and are uncorrected. FT-IR spectra were recorded using an FTS 3000 MX spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were determined in $CDCl_3$ solutions at 300 MHz on a Bruker AM-300 spectrophotometer. Mass spectra (EI, 70 eV) were performed on a GCMS instrument, and elemental analyses with a LECO-183 CHNS analyzer. All the compounds were purified by thin layer chromatography using silica gel HF-254 from Merck (Darmstadt, Germany).

3.2 Methyl (3,5-dimethoxy-4-methyl phenyl) acetate

A stirred solution of 3,5-dimethoxy-4-methylphenylacetic acid (**1**) (5.0 g,

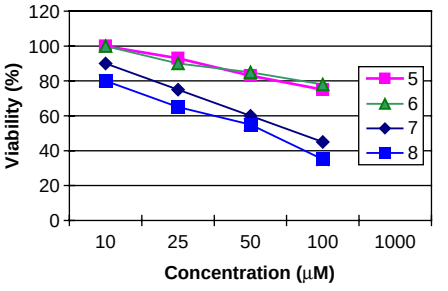


Figure 2. Cytotoxic activities of compounds **5**–**8**.

23.8 mmol) in dry methanol (30 ml) was treated dropwise with conc. H_2SO_4 (5 ml). The mixture was refluxed for 8–9 h. The reaction was monitored by TLC. After the completion of the reaction, the mixture was concentrated to 55 ml and extracted with ethyl acetate (3× 50 ml). The extract was washed with saturated brine, dried, and concentrated to give crude oil that was distilled to afford methyl ester (4.7 g, yield 88.18%), R_f^*: 0.7, mp 38–40°C, IR (KBr): ν_{max} 3023 (C—H), 1734 (C═O), 1573 (C═C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): δ 7.45 (2H, s, H-2, H-6), 3.96 (6H, s, 2× OCH_3), 3.54 (2H, s, Ar—CH_2), 3.47 (3H, s, $COOCH_3$), 2.55 (3H, s, Ar—CH_3); ^{13}C NMR ($CDCl_3$, δ ppm): 168.2 (C═O), 132.5 (C3, C5), 128.3 (C2, C6), 119.4 (C4), 112.2 (C1), 68.5 (ester OCH_3), 55.3 (Ar—OCH_3), 36.9 (CH_2), 28.6 (Ar—CH_3); MS (70 eV): *m/z* (%) 224$[M]^{+\cdot}$ (46), 193 (43), 165 (100), 59 (12); Elemental analysis: Found: C, 64.28%, H, 7.14%; calcd for $C_{12}H_{16}O_4$: C, 64.02%, H, 6.96%.

3.3 Methyl (2-formyl-3,5-dimethoxy-4-methyl phenyl) acetate (2)

Phosphorous oxychloride (1.61 g, 10.0 mmol) was added dropwise into a stirred solution of methyl (3,5-dimethoxy-4-methyl phenyl) acetate (2.0 g, 8.9 mmol) in freshly distilled DMF (10 ml) at 55°C. Reaction mixture was heated at about 100°C for 2 h and stirred overnight at room temperature. Then, the reaction mixture was poured into the aqueous solution of sodium acetate (10%, 10 ml) and shaked vigorously. Methyl (2-formyl-3,5-dimethoxy-4-methylphenyl) acetate (**2**) was precipitated out as yellowish precipitates (1.9 g, yield 84%), R_f^*: 0.55, mp 51–53°C, IR (KBr): ν_{max} 3029 (C—H), 1722 (C═O), 1690 (CHO), 1545 (C═C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): δ 9.75 (1H, s CHO), 7.96 (1H, s, H-6), 3.42 (3H, s, 3-OCH_3), 3.25 (3H, s, 5-OCH_3), 3.11 (3H, s, CO_2CH_3), 2.92 (2H, s, Ar—CH_2), 2.80 (3H, s, Ar—CH_3); ^{13}C NMR ($CDCl_3$, δ ppm): δ 179.3 (aldehyde C═O), 162.4 (ester C═O), 136.7 (C3, C5), 131.9 (C2), 126.2 (C6), 121.3 (C4), 117.5 (C1), 61.6 (ester OCH_3), 57.3 (Ar—OCH_3), 39.1 (Ar—CH_2), 32.0 (Ar—CH_3); MS (70 eV): *m/z* (%) 252$[M]^{+\cdot}$ (25), 251 (65), 224 (49), 223 (34), 165 (100), 29 (31); Elemental analysis: Found: C, 61.67%, H, 6.16%; calcd for $C_{13}H_{16}O_5$: C, 61.90%, H, 6.34%.

3.4 2,4-Dimethoxy-6-(2-methoxy-2-oxoethyl)-3-methylbenzoic acid (3)

Formyl ester (**2**) (6.3 g, 25.0 mmol) and sulfamic acid (8.3 g, 86.0 mmol) in 150 ml H_2O:THF:DMSO (20:0:1) at 0°C were treated with $NaClO_2$ (7.24 g, 80.0 mmol) in 20 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× 13 ml), and saturated aqueous sodium chloride (130 ml). Organic layer was dried over anhydrous sodium sulfate and evaporated to afford the keto acid (**3**) (6.6 g, yield 79%), R_f^*: 0.4, mp 164–166°C, IR (KBr): ν_{max} 3265 (O—H), 3037 (C—H), 1734 (C═O), 1715 (COOH), 1562 (C═C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): δ 8.19 (1H, s, COOH), 7.66 (1H, s, H-6), 3.82 (3H, s, 3-OCH_3), 3.67 (3H, s, 5-OCH_3), 3.63 (3H, s, CO_2CH_3), 2.54 (2H, s, Ar—CH_2), 2.25 (3H, s, Ar—CH_3); ^{13}C NMR ($CDCl_3$, δ ppm): δ 197.8 (carboxylic

C═O), 168.5 (ester C═O), 139.3 (C2, C4), 134.3 (C6), 127.1 (C5), 120.6 (C3), 114.1 (C1), 66.0 (ester OCH_3), 55.4 (Ar—OCH_3), 35.0 (Ar—CH_2), 29.8 (Ar—CH_3); MS (70 eV): *m/z* (%) 268 $[M]^{+\cdot}$ (32), 251 (51), 224 (65), 165 (100), 45 (25); Elemental analysis: Found: C, 58.04%, H, 5.76%; calcd for $C_{13}H_{16}O_6$: C, 58.20%, H, 5.97%.

3.5 2,4-Dimethoxy-6-(2-hydroxyethyl)-3-methylbenzoic acid (4)

Keto acid (**3**) (0.5 g, 1.86 mmol) and sodium borohydride (0.84 g, 22.32 mmol) were suspended in freshly distilled THF (10 ml). The reaction mixture was stirred for 15 min at 65°C and then methanol (10 ml) was added dropwise for 30 min. The mixture was refluxed for 4 h, then cooled to room temperature, and treated with saturated ammonium chloride solution (10 ml). Stirring was continued for 1 h, then acidified with dilute hydrochloric acid, and extracted with ethyl acetate (3 × 20 ml). The extract was dried, evaporated to afford hydroxyl acid (**4**) (0.35 g, yield 78%), R_f^*: 0.3, mp 72–74°C, IR (KBr): ν_{max} 3481 (O—H), 3009 (C—H), 1710 (C═O), 1574 (C═C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): δ 8.22 (1H, s, COOH), 7.48 (1H, s, H-5), 4.21 (2H, t, $J = 3.8$ Hz, H-1′), 3.90 (3H, s, 2-OCH_3), 3.75 (3H, s, 4-OCH_3), 2.65 (2H, t, $J = 3.8$ Hz, H-2′), 2.51 (3H, s, Ar—CH_3); ^{13}C NMR ($CDCl_3$, δ ppm): δ 190.5 (COOH), 166.2 (C2), 162.4 (C4), 141.3 (C6), 108.1 (C3), 105.6 (C1), 101.2 (C5), 63.2 (C2′), 56.4 (2-OCH_3, 4-OCH_3), 32.3 (C1′), 28.8 (Ar—CH_3); MS (70 eV): *m/z* (%) 240 $[M]^{+\cdot}$ (32), 223 (24), 196 (43), 165 (100), 45 (19), 31 (37); Elemental analysis: Found: C, 59.87%, H, 6.57%; calcd for $C_{12}H_{16}O_5$: C, 60.00%, H, 6.66%.

3.6 6,8-Dimethoxy-7-methyl-3,4-dihydro-1H-isochromen-1-one (5)

Hydroxy acid (**4**) (1.0 g, 4.16 mmol) was dissolved in acetic anhydride (5 ml) and refluxed for 1 h. Then, the reaction mixture was poured into ice-cold water and extracted with ethyl acetate (3 × 20 ml). The combined ethyl acetate extract was washed with 1% $NaHCO_3$ and then with water. Ethyl acetate was evaporated under reduced pressure to afford 6,8-dimethoxy-7-methyl-3,4-dihydro-1*H*-isochromen-1-one (**5**). Yield 78%, R_f^*: 0.6, mp 145–147°C, IR (KBr): ν_{max} 3010 (C—H), 1702 (C═O), 1591 (C═C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): δ 7.48 (1H, s, H-5), 4.25 (2H, t, $J = 3.6$ Hz, H-3), 3.90 (6H, s, 6-OCH_3, 8-OCH_3), 2.66 (3H, s, Ar—CH_3), 2.56 (2H, t, $J = 3.6$ Hz, H-4); ^{13}C NMR ($CDCl_3$, δ ppm) δ 163.9 (C1), 152.3 (C6, C8), 140.8 (C4a), 134.6 (C8a), 108.9 (C7), 103.7 (C5), 65.9 (C3), 56.4 (6-OCH_3, 8-OCH_3), 27.4 (C4), 26.2 (Ar—CH_3); MS (70 eV): *m/z* (%) 222 $[M]^{+\cdot}$ (52), 194 (45), 192 (100), 164 (31), 30 (21); Elemental analysis: Found: C, 64.75%, H, 6.19%; calcd for $C_{12}H_{14}O_4$: C, 64.86%, H, 6.30%.

3.7 6,8-Dimethoxy-7-(bromomethyl)-3,4-dihydro-1H-isochromen-1-one (6)

To a stirred solution of dihydroisocoumarin (**5**) (0.5 g, 2.25 mmol) in dry carbon tetrachloride (10 ml), *N*-bromosuccinimide (0.6 g, 3.37 mmol) and benzoyl peroxide (7.5 mg) were added. The reaction mixture was refluxed for 6 h, then cooled, filtered, and washed with a little carbon tetrachloride. The solvent was rotary evaporated to leave 7-bromo dihydroisocoumarin (**6**). Yield 81%, R_f^*: 0.6, mp 91–93°C, IR (KBr): ν_{max} 3013 (C—H), 2926 (Ar—C—H), 1718 (C═O), 1582 (C═C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): δ 7.50 (1H, s, H-5), 4.85 (2H, s, CH_2—Br), 4.24 (2H, t, $J = 3.6$ Hz, H-3), 3.90 (6H, s, 6-OCH_3, 8-OCH_3), 2.58 (2H, t, $J = 3.6$ Hz, H-4); ^{13}C NMR ($CDCl_3$, δ ppm): δ 169.2 (C1), 155.4 (C6, C8), 144.1 (C4a), 110.7 (C7), 106.2 (C8a), 104.6 (C5), 67.4 (C3), 53.7 (6-OCH_3, 8-OCH_3), 39.5 (CH_2—Br), 26.4 (C4); MS (70 eV): *m/z* (%) 200 $[M]^{+\cdot}$ (24), 302 [M + 2] (24), 272 (39), 221 (19),

191 (100), 30 (28); Elemental analysis: Found: C, 47.72%, H, 4.23%; calcd for $C_{12}H_{13}O_4Br$: C, 47.84%, H%, 4.31%.

3.8 7-(Hydroxymethyl)-6,8-dimethoxy-3,4-dihydro-1H-isochromen-1-one (7)

6,8-Dimethoxy-7-(bromomethyl)-3,4-dihydro-1*H*-isochromen-1-one (**6**) (1.0 g, 3.32 mmol) was dissolved in a mixture of water and acetone (10 ml, 1:1). The reaction mixture was refluxed for 1 h, then most of the acetone was rotary evaporated, and the residue was poured into ice-cold water and the solid was filtered, washed with water, and dried to afford 7-hydroxymethyl dihydroisocoumarin (**7**). Yield 77%, R_f: 0.4, mp 136–138°C, IR (KBr): ν_{max} 3467 (O—H), 3010 (C—H), 2919 (Ar—C—H), 1714 (C═O), 1587 (C—C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): δ 7.62 (1H, s, H-5), 4.88 (2H, s, CH_2—OH), 4.20 (2H, t, $J = 3.7$ Hz, H-3), 3.90 (6H, s, 6-OCH_3, 8-OCH_3), 2.56 (2H, t, $J = 3.7$ Hz, H-4), 2.36 (1H, s, O—H); ^{13}C NMR ($CDCl_3$, δ ppm): δ 168.6 (C1), 157.3 (C6, C8), 142.1 (C4a), 115.2 (C7), 105.2 (C8a), 102.6 (C5), 64.5 (C3), 55.7 (6-OCH_3, 8-OCH_3), 48.6 (CH_2—OH), 27.4 (C4); MS (70 eV): *m/z* (%) 238 $[M]^{+\cdot}$ (49), 221 (31), 210 (55), 208 (100), 30 (19); Elemental analysis: Found: C, 60.38%, H, 5.76%; calcd for $C_{12}H_{14}O_5$: C, 60.50%, H, 5.88%.

3.9 8-Hydroxy-7-(hydroxymethyl)-6-methoxy-3,4-dihydro-1H-isochromen-1-one (8)

7-(Hydroxymethyl)-6,8-dimethoxy-3,4-dihydro-1*H*-isochromen-1-one (**7**) (1.5 g, 6.3 mmol) was dissolved in dry THF (20 ml) and treated with magnesium (0.2 g, 7.58 mmol) and iodine (1.0 g, 8.38 mmol) in dry benzene (30 ml). The resulting mixture was refluxed for 30 min and then poured into water. The organic layer was separated and washed with water. Evaporation of the solvent under reduced pressure afforded stellatin (**8**). Yield 47%, R_f: 0.5, mp 127–128°C, IR (KBr): ν_{max} 3559 (O—H), 3001 (C—H), 2920 (Ar—C—H), 1695 (C═O), 1593 (C═C) cm^{-1}; ^1H NMR ($CDCl_3$, δ ppm): δ 10.90 (1H, s, 8-OH), 7.06 (1H, s, H-5), 4.54 (2H, s, CH_2OH), 4.12 (2H, t, $J = 3.7$ Hz, H-3), 3.90 (3H, s, 6-OCH_3), 2.86 (2H, t, $J = 3.7$ Hz, H-4), 2.29 (1H, s, CH_2OH); ^{13}C NMR ($CDCl_3$, δ ppm): δ 166.5 (C1), 158.7 (C8), 151.4 (C6), 143.1 (C4a), 116.9 (C7), 105.2 (C8a), 102.6 (C5), 63.9 (C3), 56.9 (6-OCH_3), 47.1 (CH_2—OH), 28.4 (C4); MS (70 eV): *m/z* (%) 224 $[M]^{+\cdot}$ (59), 222 (21), 207 (42), 194 (100), 30 (19); Elemental analysis: Found: C, 59.37%, H, 4.39%; calcd for $C_{11}H_{10}O_5$: C, 59.45%, H, 4.50%.

R_f^*: (petroleum ether: ethyl acetate, 4:1).

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