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Synthesis of poinsettifolin A

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ABSTRACT

A synthesis of poinsettifolin A (**1**), a prenylated flavonol isolated from *Dorstenia poinsettifolia*, is described. Two routes starting from quercetin were explored, and **1** could be prepared if a prenyl group first was incorporated at C-6 of the protected quercetin followed by a condensation with citral at C-8. The key synthetic steps are a Mitsunobu reaction, an europium (III)-catalysed Claisen rearrangement coupled with cross-metathesis, and a benzopyran-forming geranylation. The two geranylated 3,5,3′,4′-tetrahydroxyflavonols prepared, **1** and **3**, were assayed for antileishmanial activity against *Leishmania amazonensis* and *Leishmania braziliensis*, and found to be active. Compound **3** showed cytotoxic activity against leukaemia and lung cancer cells while **1** lacked cytotoxicity.

1. Introduction

All plants produce flavonoids, and more than 9000 individual compounds have been described in the scientific literature. They are secondary metabolites that have been proposed to have different functions in the plant, for example defending it, protecting it from UV radiation, and giving the petals beautiful colours that attract insects and birds.[1] Some chemical properties and biological activities of the flavonoids are considered to be beneficial for human health,[2] which is highly relevant as we consume significant amounts daily. Studies have shown that they, in general, are antioxidants and free-radical scavengers,[3] and possess anti-inflammatory,[4] antiviral[5] and anticancer properties.[6] In recent years prenylated flavonoids have attracted attention because the prenyl groups increase the lipophilicity and thereby the ability to penetrate biological membranes. Prenylflavonoids display a series of interesting biological activities such as leishmanicidal activity.[7–9]

A previous study of the extracts of the herb *Dorstenia poinsettifolia*, examined in a search for new antiparasitic agents, yielded small amounts of the new geranylated flavonol poinsettifolin A (**1**).[10] The possibility to investigate different biological properties of **1**, such as the antileishmania activity, which is considered important for the *Dorstenia* genus,[11] requires a synthetic procedure, that besides yielding larger amounts of **1** also can produce derivatives and analogues for subsequent SAR studies.

2. Results and discussion

2.1. Retrosynthesis

Even if different synthetic approaches based on de novo synthesis of **1** were considered, we decided to start from the closely related and ready available flavonol quercetin (**2**). The two principal routes examined, both depending on the selective reactivity of ring A, are shown in Scheme 1.

Route **a** starts with the selective condensation of **2** with citral to produce the pyrano flavonol intermediate **3**. A prenylation of this intermediate at C-6 would yield the target compound **1**. Route **b** is based on the selective insertion of a prenyl group at C-6 of **2** to produce the intermediate **4**, prior to its condensation with citral to give **1**.

2.2. Route a, direct C-prenylation via benzopyran 3

It is known that selective aldol-type condensations of polyhydroxy aromatic compounds can yield the corresponding benzopyran systems.[12,13] Even if this has not been previously carried out with flavonoids, several successful examples with skeletons as diverse as dihydroxyphtalides, dihydroxybenzoates and xanthones

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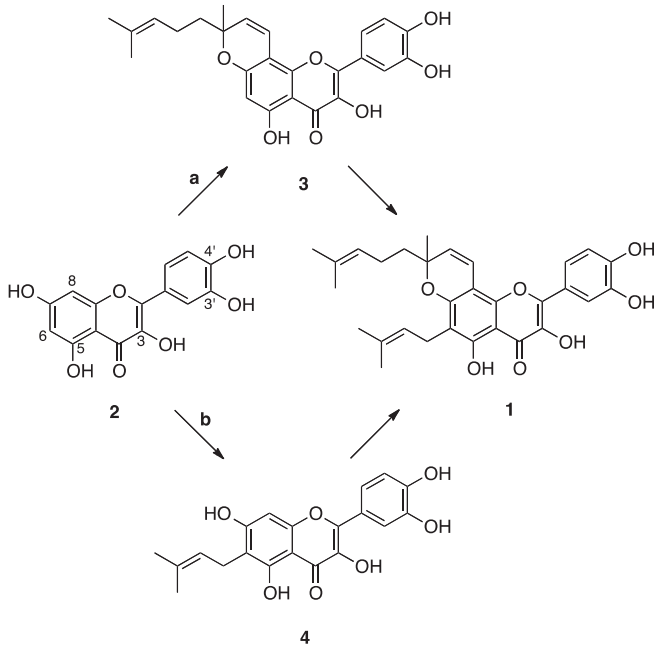
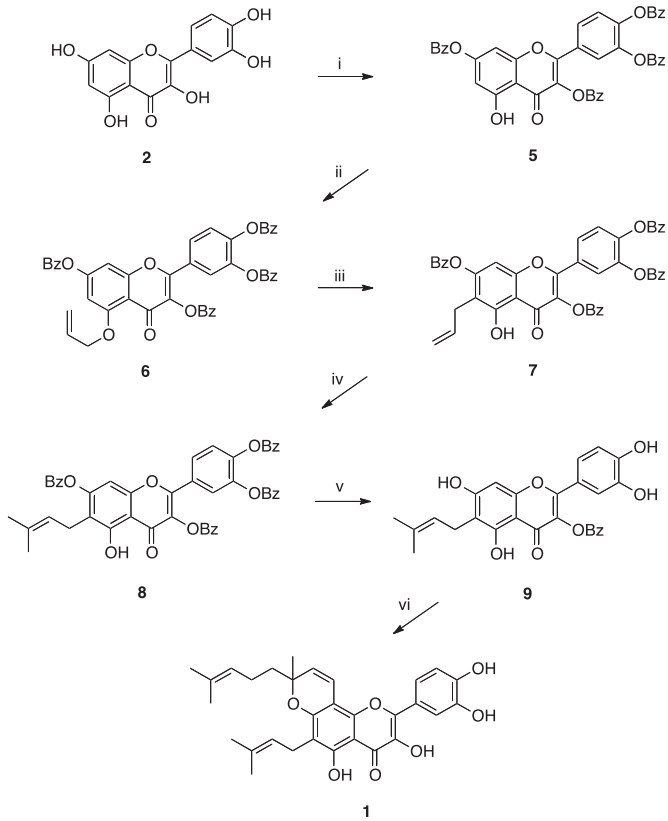


Scheme 1. The two principal synthetic routes from quercetin (**2**) to poinsettifolin A (**1**) investigated. Route **a** was an unsuccessful attempt to obtain **1** by C-prenylation of the benzopyran **3** (see text for details), while route **b** is shown in Scheme 2.



Scheme 2. Synthesis of poinsettifolin A. Reagents and conditions: (i) DCM, Bz_2O, DMAP, Et_3N, reflux, 4 h, 60%; (ii) THF, allyl alcohol, DIAD, PPh_3, 0 °C–20 °C, overnight, 90%; (iii) chloroform, $Eu(fod)_3$, rt, 15 h, 73%; (iv) DCM, Grubbs 2nd, 2-methyl-2-butene, rt, 48 h, 88%; (v) Et_3N, H_2O, MeOH, 50 °C, 2 h, 75%; (vi) citral, 140 °C, 5 h, 70%.

can be found. The condensation with citral was first attempted under thermal conditions and in the absence of a catalyst. Prolonged heating in an oil bath led to the formation of **3**, a compound that so far has not been reported as a natural product, in moderate yields. However, the use of microwave irradiation improved the yield and decreased the reaction time, and **3** was obtained in 80% yield from **2** and citral under such conditions. Only **3** was obtained, the corresponding C-6 condensation product was not observed in spite of the apparent similarity of the two positions. However, due to the presence of 3-OH in flavonols the electron density of C-8 can be expected to be higher than that of C-6, explaining the higher reactivity of that position.

With the geranylated intermediate **3** at hand, we attempted the introduction of the prenyl moiety at C-6. The direct prenylation of electron-rich phenolic compounds has been reported, although it is known to be a challenge to obtain selective prenylation at only one position in a compound like **2**. The use of prenyl halides in the presence of a base, e.g. DBU,[14] tetramethylammonium hydroxide,[15,16] potassium hydroxide[17,18] or sodium hydride,[19,20] failed as C-prenylated products were obtained together with O-prenylated as well as degradation products of **3** (**2** is not stable in basic conditions). Next, Lewis acid catalysed condensation of 2-methyl-3-buten-2-ol was attempted. This procedure is known to show an improved C/O-prenylation ratio,[21] and is not hampered by the stability problems of **3** in basic conditions. With boron trifluoride etherate C-prenylation of **3** was observed, but at several positions yielding inseparable mixtures. Attempts to overcome this problem by selective deactivation of the B ring with electron withdrawing protective groups failed. The cyclic carbonate in ring B involving the two adjacent hydroxyl groups turned out to be unstable to the conditions used for C-prenylation with boric trifluoride etherate, while the 3,3′,4′-tritoluenesulfonic ester derivative only gave O-prenylation at 5-O of **3**.

2.3. Route b, Claisen/metathesis via 6-isoprenylquercetin 4[22]

As the direct prenylation at C-6 proved considerably more difficult than we had anticipated, we instead turned our attention to the procedure for selective C-6 prenylation of flavonoids developed by Tischer and Metz.[23] The synthetic sequence comprises the selective acetylation of all hydroxyl groups except 5-OH, a Mitsunobu reaction to create the 5 allyl ether, an europium(III)-catalysed Claisen rearrangement to give the 6-allyl derivative, followed by a cross-metathesis reaction yielding the corresponding 6-prenylated compound followed by a transesterification to deprotect the acetoxy groups. Following this approach, which by necessity has to be carried out prior to the geranylation at C-8, the first step is a partial protection of quercetin. This is achieved in acceptable yields as the intramolecular hydrogen bond between 5-OH and the carbonyl oxygen makes this hydroxyl group less reactive. However, acetyl groups, used originally, are not suitable for quercetin (**2**) as it partially degrades during the final deprotection conditions. Instead, the 3,7,3′,4′,-tetrahydroxyl groups were protected as the corresponding benzoic acid esters, compound **5**, as the benzoyl group should be possible to remove using milder conditions. The subsequent Mitsunobu reaction of **5** with allyl alcohol provided the allylated intermediate **6**, while the Claisen rearrangement of **6** using $Eu(fod)_3$ provided compound **7** as the sole product in 73% yield. It should be noted that, apart from the critical role of the catalyst for achieving this selectivity, the amount of catalyst and the concentration of the starting materials play critical roles. Thus, increasing the concentration of **6** from 50 mM to 80 mM resulted in the formation of the isomeric compound allylated at C-8. The metathesis reaction was carried out using 2-methyl-2-butene to get compound **8** in 88% yield. The problem of the formation of the corresponding 6-(2-butenyl) product as an inseparable by-product when using the more practical 2-methyl-2-butene instead of isobutylene was overcome by using a 100-fold excess of 2-methyl-2-butene. Different conditions for the cleavage of the benzoyl groups under basic conditions were tested, but complete deprotection could not be achieved. The benzoic acid

ester **9** was the main product in most of the cases, and using a mixture of sodium methoxide and triethylamine gave **9** in 75% yield. An increase in temperature and/or pH to force complete deprotection resulted in decomposition, but 3-OBz was transformed to 3-OH during the geranylation of **9** to form the target **1**. This thermal condensation with citral was carried out essentially as discussed above, and poinsettifolin A (**1**) was obtained from intermediate **9** in 65% yield.

The use of other protective groups than benzoyl was also investigated. The MEM derivative corresponding to **5** was actually obtained in a higher yield (92%), and during the Claisen rearrangement of the MEM derivative corresponding to **6** prenylation at C-8 was not observed, independently of the concentration of starting material or the relative concentration of Eu(fod)$_3$. However, the deprotection of the MEM derivative corresponding to **8** could not be achieved without suffering from a 6-*endo-trig* cyclization involving the prenyl group and 5-OH.

2.4. Biological activities

2.4.1. Leishmanicidal activity. Compounds **1** and **3** were assayed for antileishmanial activity against the promastigote forms of the parasites *Leishmania amazonensis* and *Leishmania braziliensis*. Both compounds exhibit good leishmanicidal activity against both the tested strains, **3** being slightly more potent. It is possible that the leishmanicidal activity is favoured by an unsubstituted position 6, but the difference may also depend on the higher lipophilicty of **1** compared to **3** (Table 1).

Table 1
In vitro leishmanicidal activity of compounds **1** and **3** with Amphotericin B as positive control (IC_{50}, μg/mL)

Compound	*L. amazonensis*	*L. braziliensis*
1	22.6	4.5
3	4.9	2.5
Amphotericin B	0.3	0.2

2.4.2. Cytotoxicity activity. Compound **1** lacked cytotoxic activity against leukaemia (Jurkat) and lung cancer cells (A549) cells. In contrast, **3** was a potent cytotoxic compound initially suggesting that isoprenylation at C-6 was detrimental for the cytotoxic activity of **3** (Table 2). Interestingly, Jurkat cells that grow in suspension are more sensitive to the action of compound **3** than A549 cells, which are adherent cancer epithelial cells.

Table 2
In vitro cytotoxic activity of compounds **1** and **3** towards Jurkat and A549 cells (IC_{50} as μg/mL)

Compound	Jurkat	A549
1	>40	>40
3	2.1	9.7

3. Conclusion

In summary, the first total synthesis route to poinsettifolin A (**1**) has been accomplished in six steps and with an acceptable overall yield, starting from commercially available materials. The selectivity of the condensation of **2** with citral is remarkable, and potentially useful for the synthesis of other geranylated flavonols. Although **3** is more potent than **1** in the assays reported here, the biological characterization of **1** will continue as it shows a better selectivity. While appearing to be essentially non-toxic to mammalian cells it has a significant anti-leishmanial activity especially towards *L. braziliensis*. It should be noted that the *L. braziliensis* strain used here is associated with the muco-cutaneous manifestation of the disease, for which the treatments available today are more aggressive and not always effective.[24] The synthesis described here includes transformations, for example the metathesis, that facilitate a systematic variation of the structure of **1** and the preparation of libraries of analogues to be used in SAR studies.

The potent cytotoxicity of compound **3** should also be studied in further detail. Interestingly, 3′-O as well as 4′-O prenylated side products obtained during the attempts to transform **3** to **1** also lacked cytotoxic activity, so the cytotoxicity of **3** does in fact not depend on an unsubstituted position 6. Again, analogues of **3** would be readily available for a SAR study.

4. Experimental

4.1. General information

Reagents was purchased from commercial suppliers and used without further purification unless otherwise noted. THF was distilled from sodium under N_2, while dichloromethane and chloroform were distilled from CaH_2 under N_2. All reactions were carried out in standard dry glassware and atmospheric surroundings unless otherwise stated. Thin layer chromatography (TLC) was carried out on Merck pre-coated silica gel aluminium sheets (60 F254), detected under UV light and visualized with sulfuric acid or ferric chloride. Column chromatography was performed on SiO_2 (Matrex LC-gel: 60 Å, 35-70 MY, Grace). Melting points were obtained using SMP10 melting point apparatus. ^1H and ^{13}C NMR spectra were recorded using a Bruker DR400 at room temperature. Chemical shifts are given in ppm relative to TMS using the residual solvent signal as internal standard (7.27 and 77.23, respectively for $CDCl_3$, 3.31 and 49.05, respectively for CD_3OD, 2.50 and 39.51, respectively for $(CD_3)_2SO$). Mass spectra (HRESI) were recorded with a Micromass Q-TOF Micro.

4.2. 2-(3,4-Dihydroxyphenyl)-3,5-dihydroxy-8-methyl-8-(4-methyl-pent-3-en-1-yl)pyrano[2,3-*f*]chromen-4(8*H*)-one (3)

Quercetin (3 g, 9.92 mmol), citral (17 mL, 99.2 mmol) and methanol (3 mL) was added to a microwave vessel and mixed in order to obtain a homogenous solution. The reaction mixture was irradiate at 120 °C for 30 min the mixture was then cooled to room temperature and the residual solvent was removed using a Kugelrohr apparatus. The product was purified by chromatography (SiO_2, CH_2Cl_2:MeOH 8:1) to give the *title compound* **3** as greenish solid (3.2 g, 80%). Mp 220–222 °C; ν_{max} (neat) 3500, 1650, 1592, 1533, 1357, 1279, 1160, 1001, 901, 821, 770 cm^{-1}; δ_H (400 MHz $(CD_3)_2SO$) 12.61 (1H, s, 5-OH), 7.72 (1H, d, *J* 2.2 Hz, 2′-H), 7.61 (1H, dd, *J* 2.2, 8.5 Hz, 6′-H), 6.90 (1H, d, *J* 8.5 Hz, 5′-H), 6.86 (1H, d, *J* 10.1 Hz, 1″-H), 6.20 (1H, d, *J* 0.3 Hz, 6-H), 5.75 (1H, d, *J* 10.1 Hz, 2″-H), 5.08 (1H, tqq, *J* 7.1, 1.3, 1.3 Hz, 6″-H), 2.04 (2H, ddd, *J* 7.1, 8, 8 Hz, 5″-H_2), 1.70 (2H, m, 4″-H_2), 1.60 (3H, s, 8″-H_3), 1.51 (3H, s, 10″-H_3), 1.40 (3H, s, 9″-H_3); δ_C (101 MHz $(CD_3)_2SO$) 176.1, 160.2, 158.7, 150.0, 147.9, 146.9, 145.2, 136.1, 131.1, 126.6, 123.8, 122.0, 120.2, 115.8, 114.8, 114.7, 103.8, 100.5, 98.4, 80.6, 40.8, 26.5, 25.4, 22.2, 17.5; HRMS (ES): $M+H^+$, found 437.1590. $C_{25}H_{25}O_7$ requires 437.1600.

4.3. 4-(3,7-bis(Benzoyloxy)-5-hydroxy-4-oxo-4*H*-chromen-2-yl)-1,2-phenylene dibenzoate (5)

Triethylamine (5.8 mL, 40.86 mmol) was added to a solution of quercetine (**2**) (4.00 g, 13.23 mmol), 4-dimethylaminopyridine

(DMAP, 0.17 g, 1.39 mmol) and benzoic anhydride (12.90 g, 57.02 mmol) in anhydrous CH_2Cl_2 (40 mL). The reaction was heated to reflux for 4 h, allowed to cool to room temperature, quenched with water and extracted with EtOAc (90 mL). The organic phase was dried and evaporated in vacuo to give the crude product, which was purified by chromatography (SiO_2, ethyl acetate:toluene 1:50) to obtain the pure *title compound* **5** as yellowish crystals (6.00 g, 60%). Mp 197–199 °C; ν_{max} (neat) 1741, 1655, 1600, 1450, 1346, 1233, 1194, 1172, 1145, 1042, 1019, 917, 862, 794, 697 cm^{-1}; δ_H (400 MHz $CDC1_3$) 12.17 (1H, s, 5-OH), 8.26–8.20 (4H, m, Bz), 8.05–8.01 (5H, m, Bz), 7.95 (1H, dd, *J* 8.6, 2.2 Hz, 6′-H) 7.69–7.64 (2H, m, Bz), 7.58–7.50 (7H, m, Bz, 2′-H and 5′-H), 7.40–7.35 (4H, m, Bz), 7.07 (1H, d, *J* 2.0 Hz, 8-H), 6.77 (1H, d, *J* 2.0 Hz, 6-H); δ_C (100.6 MHz, $CDCl_3$) 176.3, 164.0, 163.8, 163.6, 163.6, 161.8, 156.7, 156.0, 155.5, 145.3, 142.9, 134.2, 134.1, 134.0, 133.9, 132.6, 130.7 (2C), 130.3 (2C), 130.2 (4C), 128.7 (2C), 128.7 (2C), 128.6, 128.5 (2C), 128.5 (2C), 128.2, 128.2, 127.9, 127.7, 126.8, 124.1, 123.9, 108.9, 105.7, 101.4; HRMS (ES): M+Na^+, found 741.1373. $C_{43}H_{26}O_{11}Na$ requires 741.1351.

4.4. 5-(Allyloxy)-2-(3,4-bis(benzoyloxy)phenyl)-4-oxo-4*H*-chro-mene-3,7-diyl dibenzoate (6)

A solution of diisopropylazodicarboxylate DIAD (1.92 mL, 9.51 mmol) in THF (10 mL) was added to a cooled (ice bath) solution of **5** (4.30 g, 5.95 mmol), allyl alcohol (0.6 mL, 8.92 mmol) and triphenylphosphine (2.03 g, 7.73 mmol) in anhydrous THF (100 mL). The resulting yellow solution was allowed to warm to room temperature and stirred overnight. The *title compound* **6** had then precipitated and was filtered off and washed with cold diethyl ether. After drying **6** was obtained as white crystals (4.08 g, 90%). Mp 220–222 °C; ν_{max} (neat) 1743, 1653, 1600, 1459, 1242, 1173, 1058, 1019, 700 cm^{-1}; δ_H (400 MHz $CDC1_3$) 8.24–8.20 (4H, m, Bz), 8.04–8.00 (5H, m, Bz), 7.94 (1H, dd, *J* 8.6, 2.2 Hz, 6′-H), 7.69 (1H, m, Bz), 7.63 (1H, m, Bz), 7.56–7.47 (7H, m, Bz, 2′-H and 5′-H), 7.40–7.35 (4H, m, Bz), 7.15 (1H, d, *J* 2.1 Hz, 8-H) 6.75 (1H, d, *J* 2.1 Hz, 6-H), 6.10 (1H, m, 2″-H), 5.59 (1H, dt, *J* 17.3, 1.7 Hz, 3″-Ha) 5.33 (1H, dt, *J* 10.6, 1.4 Hz, 3″-Hb), 4.72 (2H, ddd, *J* 4.9, 1.7, 1.4 Hz, 1″-H_2); δ_C (100.6 MHz, $CDCl_3$) 170.4, 164.1, 163.8, 163.8, 163.7, 160.1, 157.9, 155.2, 152.2, 144.7, 142.7, 134.9, 134.1, 133.9, 133.8, 133.8, 131.7, 130.7 (2C), 130.3 (2C), 130.2 (4C), 128.7 (2C), 128.6, 128.5 (2C), 128.5 (4C), 128.4, 128.4, 128.3, 128.2, 126.6, 123.9, 123.5, 118.5, 112.4, 103.4, 102.5, 70.2; HRMS (ES): M+Na^+, found 781.1681. $C_{46}H_{30}O_{11}Na$ requires 781.1671.

4.5. 6-Allyl-2-(3,4-bis(benzoyloxy)phenyl)-5-hydroxy-4-oxo-4*H*-chromene-3,7-diyl dibenzoate (7)

A solution of **6** (4 g, 5.28 mmol), Eu(fod)$_3$ (500 mg, 0.53 mmol) and anhydrous $CHCl_3$ (100 mL) was heated in a sealed tube to 75 °C for 15 h. The solvent was evaporated in to give the crude product, which was purified by chromatography (SiO_2, ethyl acetate:toluene 1:50) to give the *title compound* **7** as yellowish crystals (3.4 g, 80%). Mp 199–201 °C; ν_{max} (neat) 1737, 1599, 1450, 1243, 1154, 1018, 1047, 1018, 907, 795, 698 cm^{-1}; δ_H (400 MHz $CDC1_3$) 12.46 (1H, s, 5-OH), 8.26–8.21 (4H, m, Bz), 8.04–8.01 (5H, m, Bz), 7.95 (1H, dd, *J* 8.6, 2.2 Hz, 6′-H), 7.70–7.64 (2H, m, Bz), 7.58–7.50 (7H, m, Bz, 2′-H and 5′-H), 7.40–7.35 (4H, m, Bz), 7.07 (1H, s, 8-H), 5.93 (1H, m, 2″-H), 5.00 (1H, m, 3″-Ha) 4.97 (1H, m, 3″-Hb), 3.47 (2H, d, *J* 6.1 Hz, 1″-H_2); δ_C (100.6 MHz, $CDCl_3$) 176.5, 164.1, 163.8, 163.7, 163.6, 159.5, 155.4, 155.1, 154.1, 145.2, 142.9, 134.6, 134.2, 134.1, 134.0, 133.9, 132.5, 130.7 (2C), 130.3 (2C), 130.2 (4C), 128.8 (2C), 128.8 (2C), 128.7, 128.6 (2C), 128.5 (2C), 128.3, 128.3, 128.0, 127.9, 126.8, 124.1, 123.9, 116.1, 115.6, 108.7, 101.8, 27.4; HRMS (ES): M+Na^+, found 781.1686. $C_{46}H_{30}O_{11}Na$ requires 781.1671.

4.6. 4-(3,7-bis(Benzoyloxy)-5-hydroxy-6-(3-methylbut-2-en-1-yl)-4-oxo-4*H*-chromen-2-yl)-1,2-phenylene dibenzoate (8)

A mixture of **7** (3 g, 3.96 mmol) and Grubbs' second generation catalyst (100 mg, 0.12 mmol), was dissolved in anhydrous CH_2Cl_2 (70 mL) under a nitrogen atmosphere. After 30 min 2-methyl-2-butene (45 mL, 424 mmol) was added, and the solution was stirred for 48 h at room temperature. The solvent was evaporated in vacuo to give the crude product, which was purified by chromatography (SiO_2, ethyl acetate:toluene 1:50) to give the *title compound* **8** as a yellowish solid (2.75 g, 88%). Mp 207–208 °C; ν_{max} (neat) 1737, 1599, 1450, 1241, 1175, 1043, 1019, 798, 700 cm^{-1}; δ_H (400 MHz $CDC1_3$) 12.46 (1H, s, 5-OH), 8.26–8.21 (4H, m, Bz), 8.04–8.01 (5H, m, Bz), 7.94 (1H, dd, *J* 8.6, 2.2 Hz, 6′-H), 7.70–7.64 (2H, m, Bz), 7.58–7.50 (7H, m, Bz, 2′-H and 5′-H), 7.40–7.35 (4H, m, Bz), 7.02 (1H, s, 8-H), 5.18 (1H, m, 2″-H), 3.41 (2H, d, *J* 6.8 Hz, 1″-H_2), 1.61 (3H, s, 4″-H_3), 1.59 (3H, s, 5″-H_3); δ_C (100.6 MHz, $CDCl_3$) 176.5, 164.2, 163.9, 163.8, 163.7, 159.5, 155.3, 155.1, 153.9, 145.2, 142.9, 134.2, 134.1, 134.0, 133.9, 132.8, 132.5, 130.7 (2C), 130.3 (2C), 130.2 (4C), 128.9, 128.8 (2C), 128.7 (2C), 128.6 (2C), 128.6 (2C), 128.3, 128.3, 128.1, 127.9, 126.8, 124.1, 123.9, 120.9, 118.1, 108.7, 101.8, 25.7, 22.4, 17.9; HRMS (ES): M+Na^+, found 809.1999. $C_{48}H_{34}O_{11}Na$ requires 809.1992.

4.7. 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-6-(3-methylbut-2-en-1-yl)-4-oxo-4*H*-chromen-3-yl benzoate (9)

A solution of NaOMe in MeOH (7 mL, 28% *wt*) was added to a solution of **8** (2.7 g, 3.43 mmol) in 200 mL triethylamine/MeOH (1:1) and the mixture was heated to 60 °C for 1 h. The reaction mixture was then poured into 1 M HCl (250 mL) and extracted with EtOAc (400 mL). The organic phase was washed with brine, dried, and evaporated in vacuo to give the *title compound* **9** as a yellowish solid (1.2 g, 74%). Mp 150–152 °C; ν_{max} (neat) 3300, 1600, 1440, 1266, 1153, 1084, 789, 704 cm^{-1}; δ_H (400 MHz CD_3OD) 8.18 (2H, dd, *J* 8.4, 1.3 Hz, Bz), 7.71 (1H, m, Bz), 7.57 (2H, m, Bz), 7.37 (1H, d, *J* 2.2 Hz, 2′-H), 7.31 (1H, dd, *J* 8.4, 2.2 Hz, 6′-H), 6.80 (1H, d, *J* 8.4 Hz, 5′-H), 6.49 (1H, s, 8-H), 5.25 (1H, m, 2″-H), 3.33 (2H, m, 1″-H_2), 1.78 (3H, s, 4″-H_3), 1.67 (3H, s, 5″-H_3); δ_C (100.6 MHz, CD_3OD) 177.1, 165.5, 164.5, 159.8, 158.2, 156.6, 150.6, 146.8, 135.2, 132.2, 131.6, 131.5 (2C), 130.0, 129.9 (2C), 123.4, 122.1, 122.0, 116.5, 116.1, 113.4, 105.0, 94.4, 26.0, 22.3, 18.0; HRMS (ES): M+H^+, found 475.1323. $C_{27}H_{23}O_8$ requires 475.1299.

4.8. 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-6-(3-methylbut-2-en-1-yl)-4-oxo-4*H*-chromen-3-yl benzoate, poitsettifolin A (1)

A solution of **9** (580 mg, 1.22 mmol) in citral (2.5 mL, 12 mmol) was stirred at 140 °C for 5 h. Thereafter the excess citral was distilled of (kugelrohr) and the resulting crude product purified by chromatography (SiO_2, CH_2Cl_2:MeOH 35:1) to give the *title compound* **1** as a yellow oil. Solidification of the product was achieved by adding 50 mL of a 1:5 EtOAc:hexane mixture into the round bottom flask containing the compound. The solvent was then evaporated in vacuo to get **1** as yellowish powder (450 mg, 70%). Mp 199–201 °C; ν_{max} (neat) 3350, 1648, 1597, 1532, 1416, 1357, 1280, 1183, 820, 770 cm^{-1}; δ_H (400 MHz CD_3OD) 7.76 (1H, d, *J* 2.1 Hz, 2′-H), 7.66 (1H, dd, *J* 8.5, 2.2 Hz, 6′-H), 6.89 (1H, d, *J* 8.5 Hz, 5′-H), 6.88 (1H, d, *J* 10.2 Hz, 1″-H), 5.62 (1H, d, *J* 10.2 Hz, 2″-H), 5.19

(1H, m, 2‴-H), 5.10 (1H, m, 6″-H), 3.29 (2H, m, 1‴-H_2), 2.12 (2H, m, 5″-H_2), 1.79 (3H, s, 4‴-H_3), 1.73 (2H, m, 4″-H_2), 1.66 (3H, s, 5‴-H_3), 1.64 (3H, s, 9″-H_3), 1.55 (3H, s, 8″-H_3), 1.42 (3H, s, 10″-H_3); δ_C (100.6 MHz, CD_3OD) 177.5, 158.9, 158.6, 150.5, 149.0, 147.8, 146.4, 137.4, 132.7, 132.1, 126.9, 125.1, 124.3, 123.5, 121.7, 116.6, 116.4, 115.8, 112.4, 104.6, 101.8, 81.8, 42.8, 27.5, 26.0, 25.9, 24.0, 22.1, 18.2, 17.8; HRMS (ES): $M+Na^+$, found 527.2046. $C_{30}H_{32}O_7Na$ requires 527.2033.

4.9. Biological assays

4.9.1. Leishmanicidal test. The activity was measured on in vitro cultures of Leishmania parasite in promastigote forms of complex *L. amazonensis* (clon 1: Lma, MHOM/BR/76/LTB-012) and complex *L. braziliensis* (strand M2904 C192 RJA), cultivated at 26 °C in Schneider medium (pH 6.8) supplemented with inactivated (56 °C×30 min) calf bovine serum (10%). Parasites in logarithmic phase of growth, at a concentration of 1×10^6 parasites mL^{-1}, were distributed on a 96 micro well plates and different concentration of the substances (100; 50; 25; 12.5; 6.2; 3.1 and 1.5 μg mL^{-1}) were added. The micro well plates were incubated for 72 h at 26 °C. After incubation, a solution of XTT (1 mg mL^{-1}) in PBS (pH 7.0 at 37 °C) with PMS (Sigma–Aldrich, 0.06 mg mL^{-1}), was added (50 μl $well^{-1}$), and incubated again for 4 h at 26 °C. DMSO (1%) and Anphotericine B (0.5 mg mL^{-1}) were used as reference drugs during the evaluations, that were done by triplicate. Optical density of each well was obtained on a StatFax (Model 2100 series-Plate Reader) at 450 nm. The IC_{50} values calculated using Microsoft Excel 2000 program.[25]

4.9.2. Cytotoxicity. Jurkat cells (T cell leukaemia) and A549 cells (adenocarcinomic human alveolar basal epithelial cells) were maintained in RPMI and DMEM medium, respectively and supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 100 UI mL^{-1} penicillin, and 100 mg mL^{-1} streptomycin. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. The cytotoxic effect of the compounds was investigated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo-lium bromide) assay. Briefly, cells were cultivated at a density of 7×10^3 cells/well in 96-well plates, 100 μl cell suspension per well, and cultured in supplemented medium during 24 h. Cells were treated with increasing concentrations of the compounds for 24 h. After that, 50 μl of MTT (5 mg mL^{-1}) from a mixture solution of MTT: DMEM (1:2) were added to each well, and cells were incubated for 4 h at 37 °C in darkness. The reaction was stopped, supernatant was removed and 100 μl DMSO were added to each well for 10 min, in gentle shaking. Finally the absorbance was measured at 550 nm using a TriStar LB 941 (Berthold Technologies, GmbH & Co. KG). The IC_{50} values calculated using Microsoft Excel 2000 program.[25]

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Supplementary data

^1H and ^{13}C NMR data associated with this article. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2014.10.021.

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