Supporting Information
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Chemical Constituents, Antimicrobial and Cytotoxic Activities of *Hypericum riparium* (Guttiferae)

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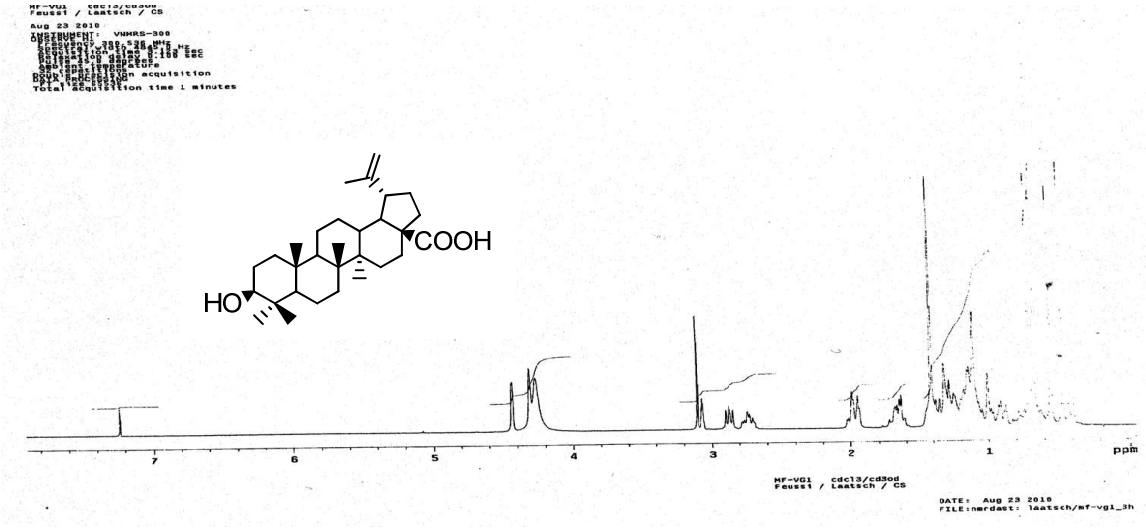
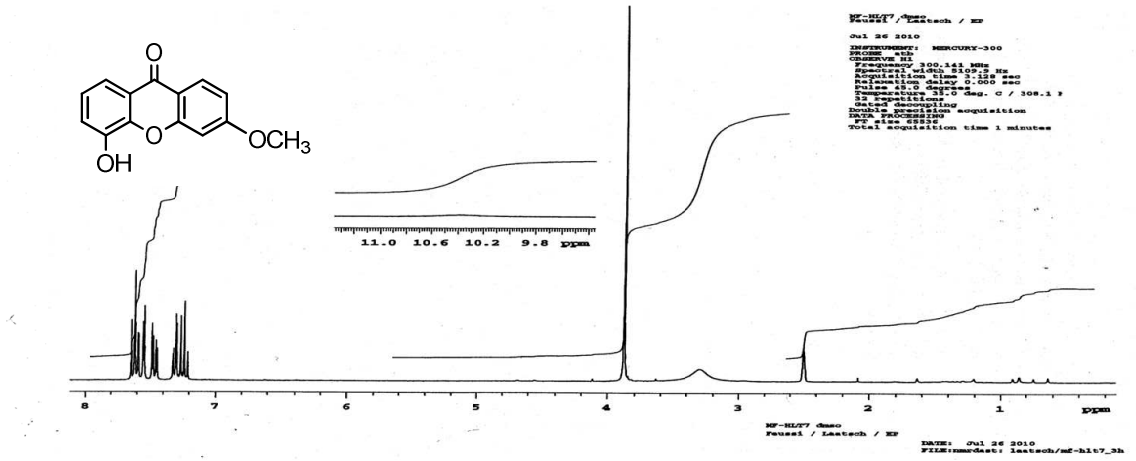
Table of Contents **Page**

Experimental

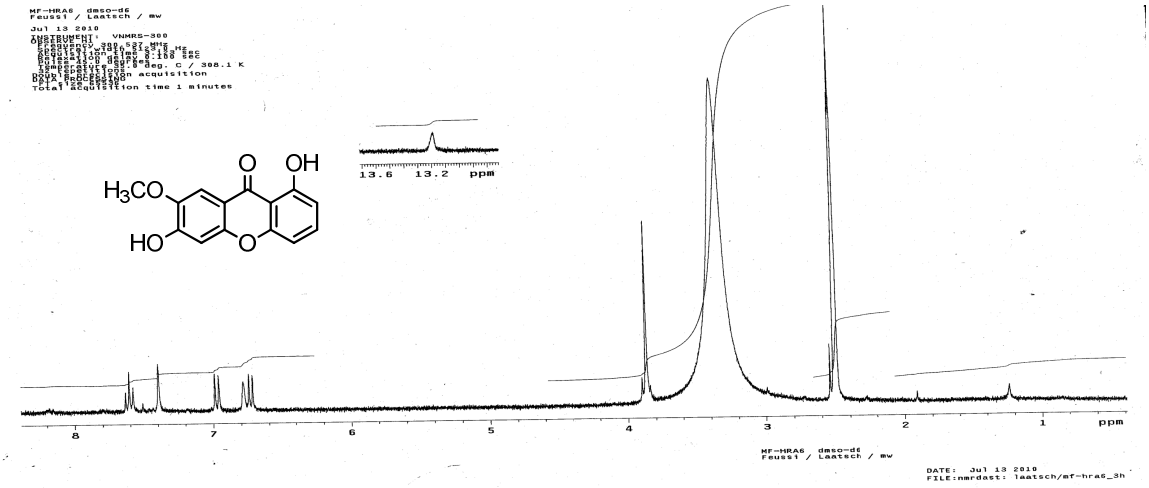
The roots of *Hypericum riparium* were cut, air-dried and ground. The fine powder (2.5 Kg) was extracted with the mixture CH_2Cl_2-MeOH (1:1) (3 x 15 L, 72 H). Evaporation under reduced pressure at room temperature afforded a crude extract (150 g). The obtained CH_2Cl_2-MeOH (1:1) extract was extracted with EtOAc to yield an EtOAc-soluble fraction (60 g) and an EtOAc-insoluble residue (84 g). Preliminary antibacterial and antifungal activities were carried out. A Portion of the EtOAc-soluble fraction (46 g) was subjected to column chromatography on silica gel using a stepwise gradient of *n*-hexane-EtOAc. The collected fractions were pooled into 6 major fractions (A1-F1). Fractions A1 and C1 contained mostly fatty material and were not further investigated. Fraction B1 was chromatographed over silica gel using a gradient of *n*-hexane-EtOAc (10:0, 9.5:0.5, 9:1, 8.5:1.5 and 8:2) to give betulinic acid (**1**, 1.7 g). Fraction D1 was chromatographed over silica gel using a gradient of *n*-hexane-EtOAc (9:1, 8,5:1.5, 8:2, 7.5:2.5, 7:3, 6:4 and 1:1) to afford three main sub-fractions. Subfraction D1-A was purified on Sephadex LH-20 (CH_2Cl_2-MeOH 1:9) to yield 5-hydroxy-3-methoxyxanthone (**2**, 15 mg). Subfraction D1-B was purified over silica gel using *n*-hexane-EtOAc (9:1) as solvent system to afford 1,6-dihydroxy-7-methoxyxanthone (**3**, 4 mg). Subfraction D1-C was separated by preparative TLC using CH_2Cl_2-Me_2CO-MeOH (11:0.2:0.2) to yield hypercalin C (**6**, 25 mg). Fraction F1 was purified on Sephadex LH-20 with MeOH as solvent to give cadensin D (**8**, 8.6 mg). 80 g of the EtOAc-insoluble residue was subjected to column chromatography on silica gel using a stepwise gradient of CH_2Cl_2-MeOH. The collected fractions were pooled into five main fractions (A2-E2). Daucosterol (**4**, 12.3 mg) crystallized overnight from fraction A2. Fraction B2 was passed through a Sephadex LH-20 column eluting with MeOH as solvent to afford bijaponicaxanthone C (**5**, 7 mg). Fraction C2 was chromatographed over silica gel using CH_2Cl_2-Me_2CO-MeOH (11:0.5:0.5) as isocratic system to afford 1-hydroxy-6,7-dimethoxyxanthone (**7**, 2.6 mg). 5-Hydroxy-1,3-dimethoxyxanthone (**9**, 4 mg) was obtained from chromatography of fraction E2 over silica gel, eluting with (CH_2Cl_2-Me_2CO-MeOH 11:1:1).



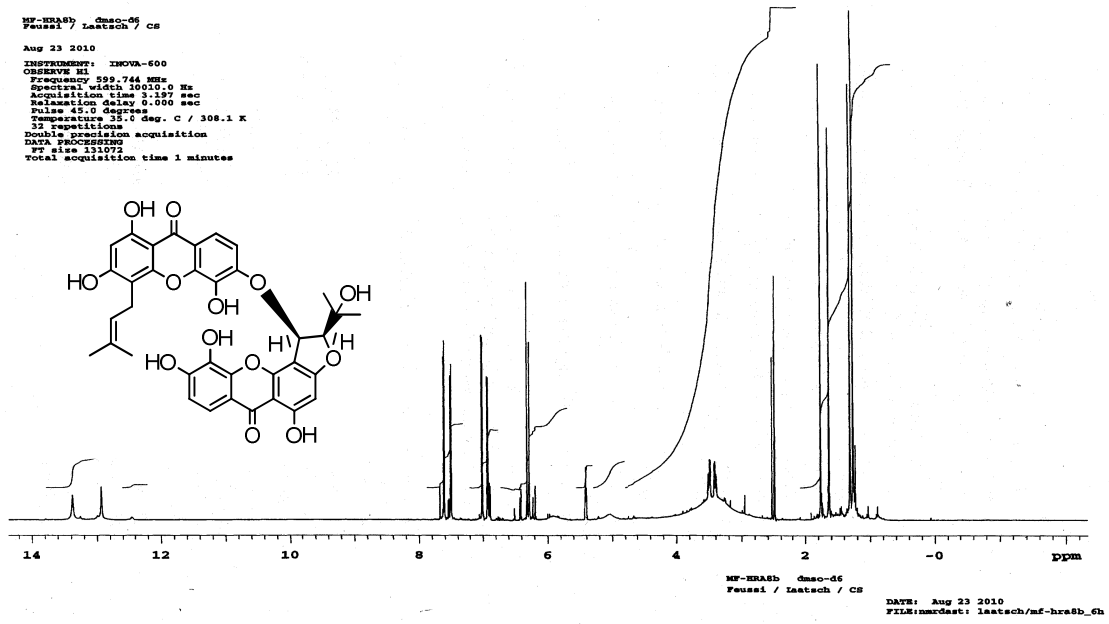
S1: ^{1}H NMR spectrum of betulinic acid (1)



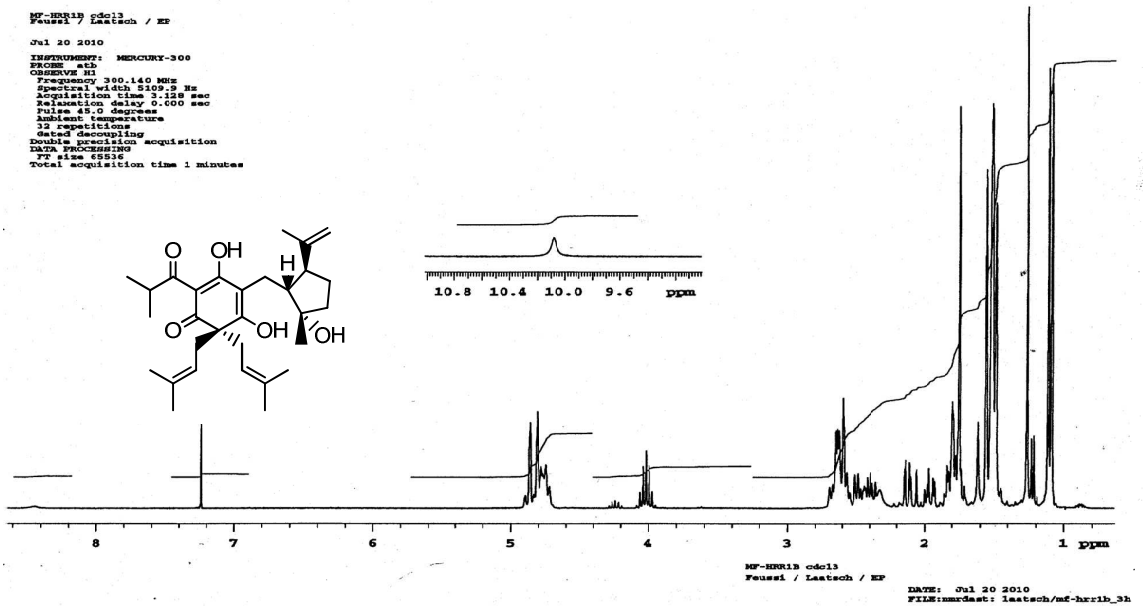
S2: ^{1}H NMR spectrum of 5-hydroxy-3-methoxyxanthone (2)



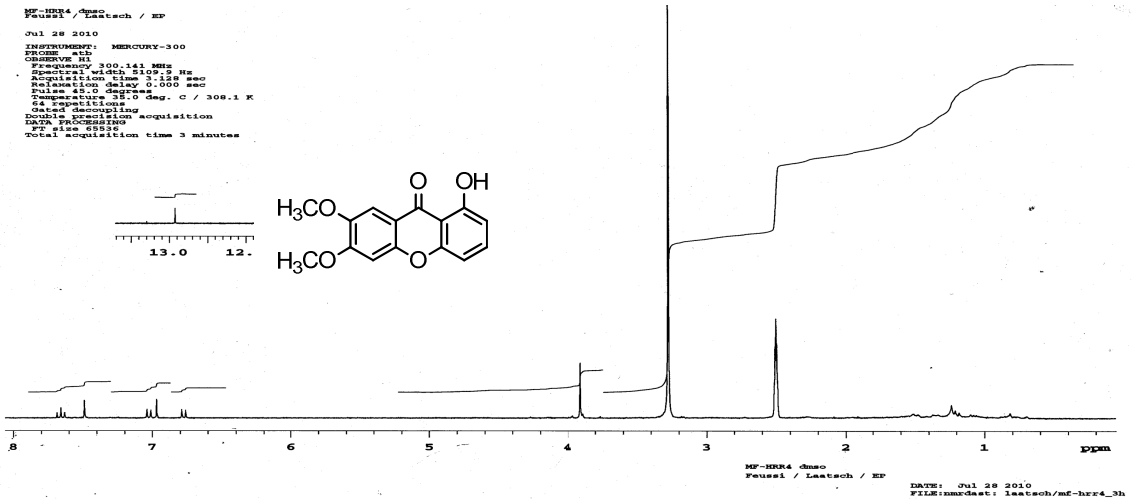
S3: ^{1}H NMR spectrum of 1,6-dihydroxy-6-methoxyxanthone (3)



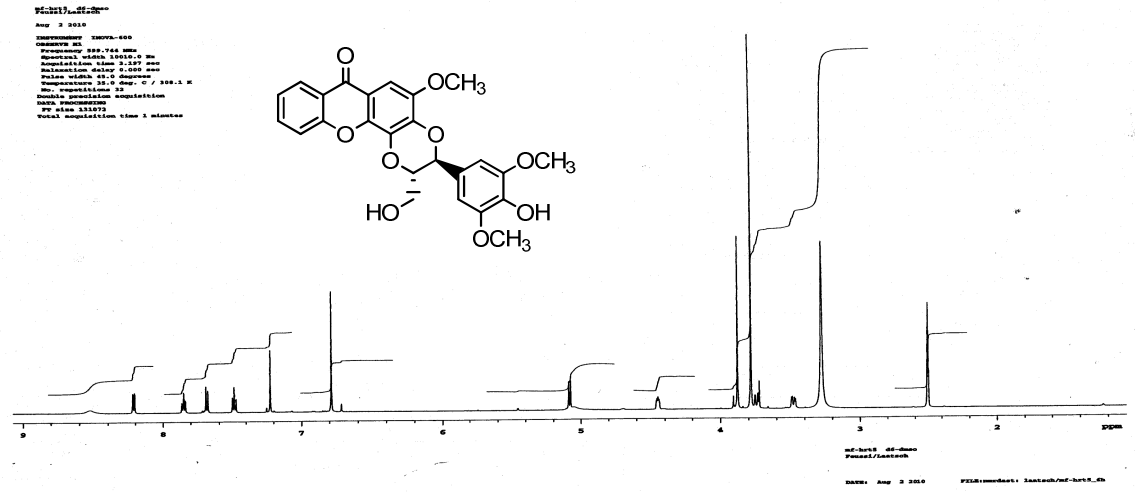
S4: ^{1}H NMR spectrum of bijaponicaxanthone C (5)



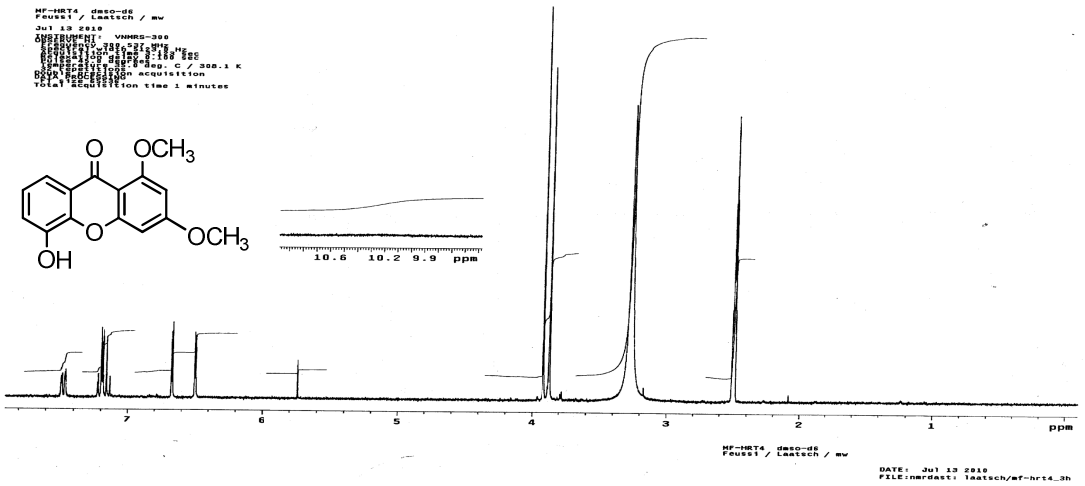
S5: ^{1}H NMR spectrum of hypercalin C (6)



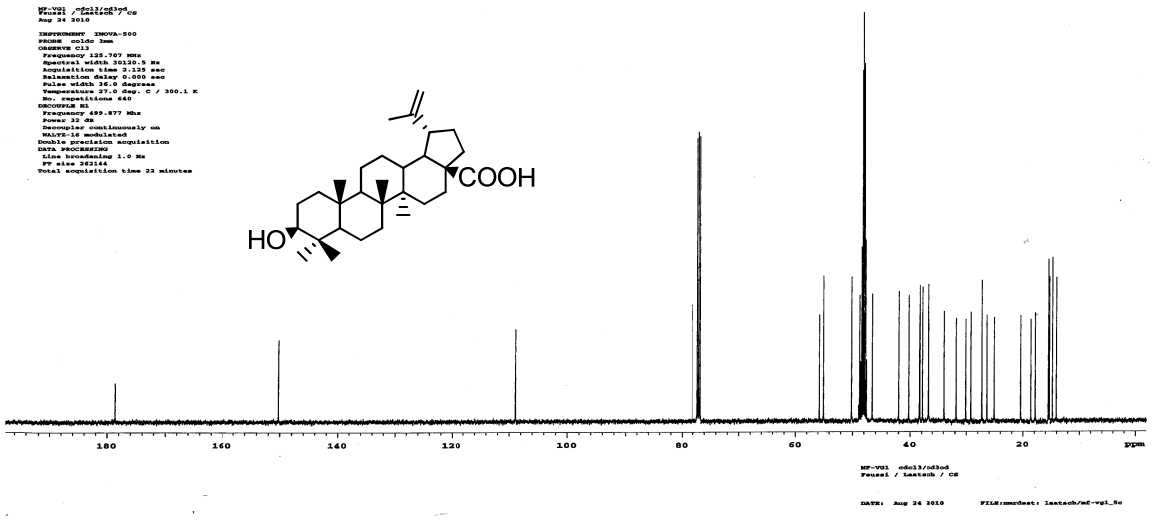
S6: ^{1}H NMR spectrum of 1-hydroxy-6,7-dimethoxyxanthone (7)



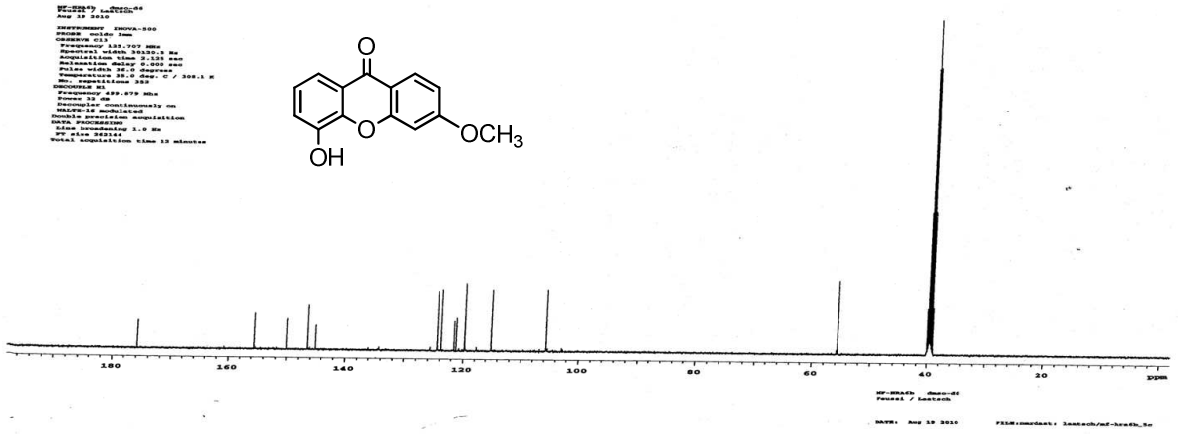
S7: ^{1}H NMR spectrum of cadensin D (8)



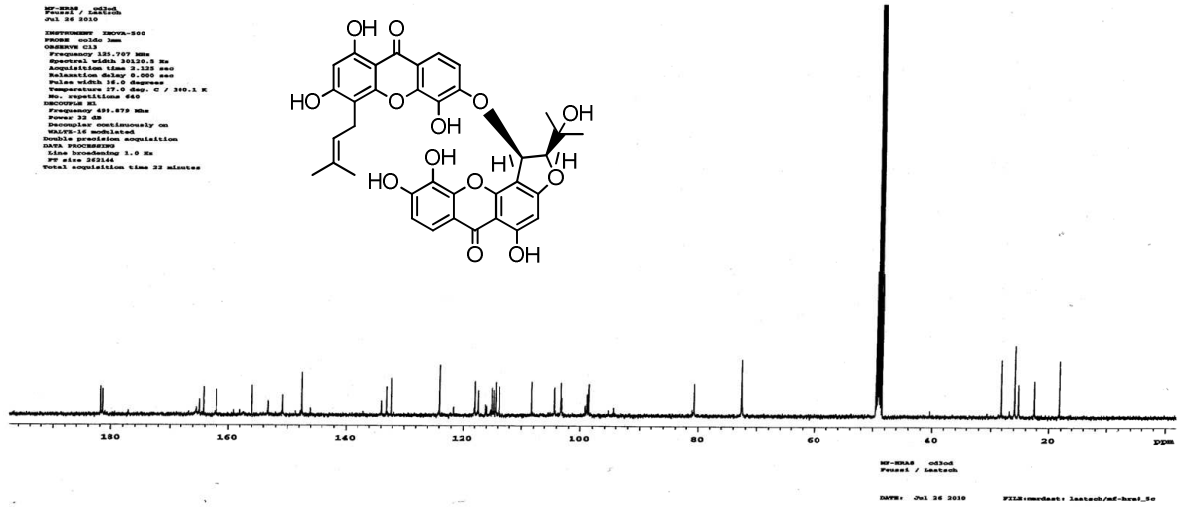
S8: ^{1}H NMR spectrum of 5-hydroxy-1,3-dimethoxyxanthone (9)



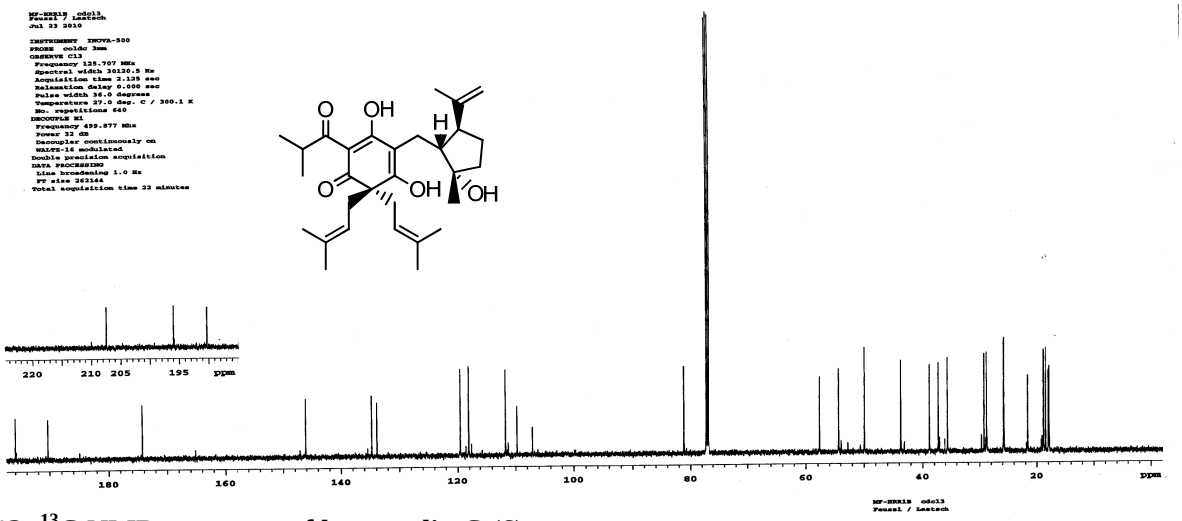
S9: ^{13}C NMR spectrum of betulinic acid (1)



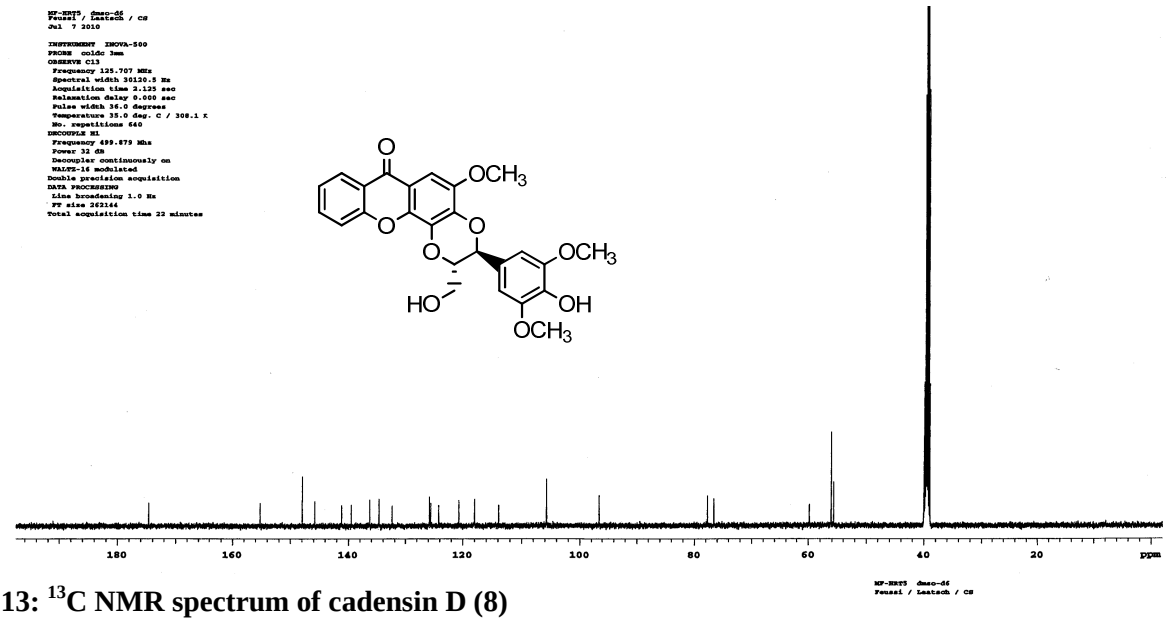
S10: ^{13}C NMR spectrum of 5-hydroxy-3-methoxyxanthone (2)



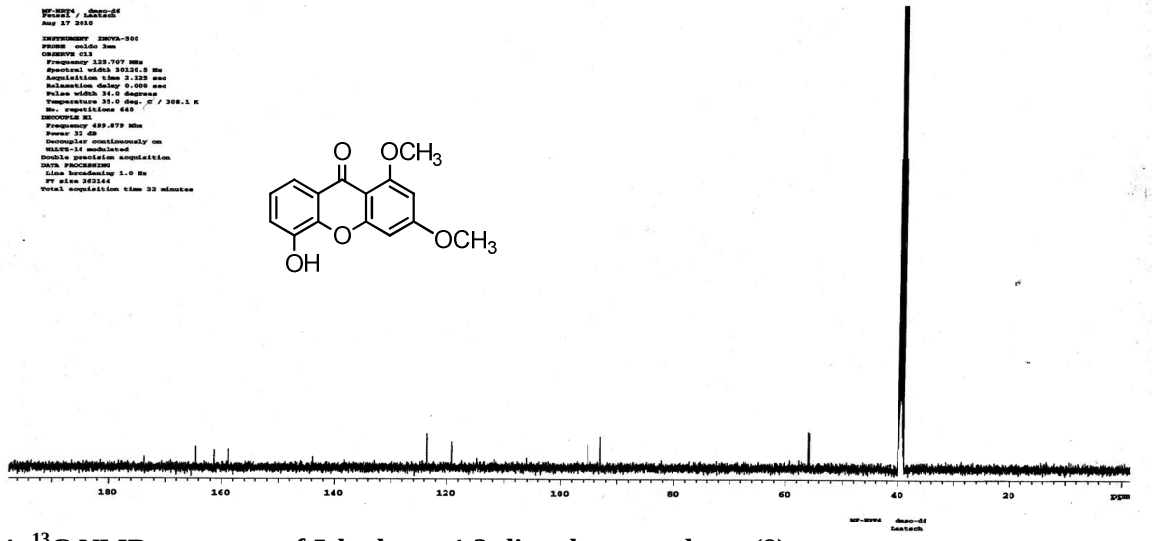
S11: ^{13}C NMR spectrum of bijaponicaxanthone C (5)



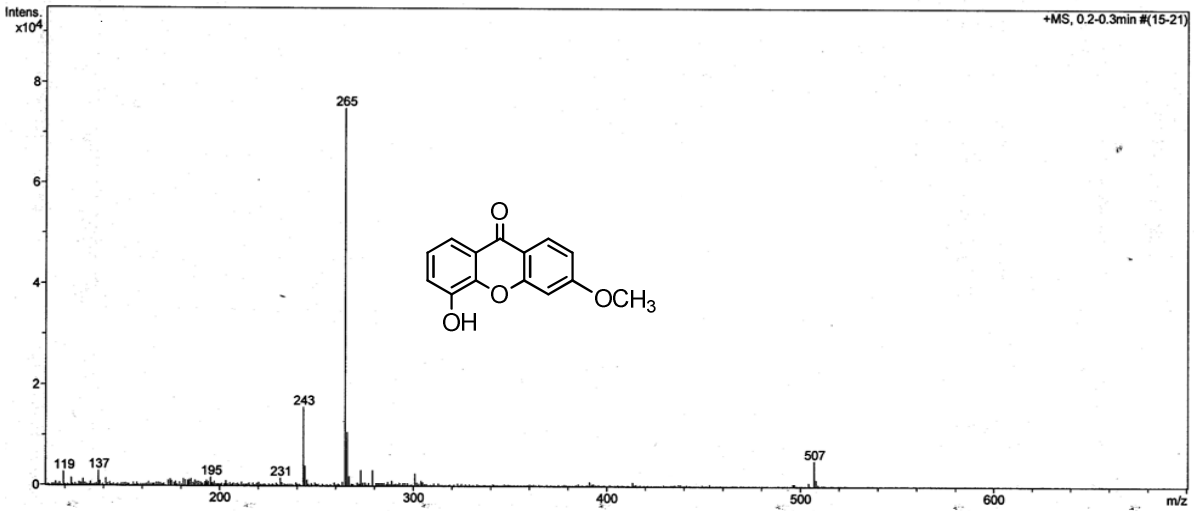
S12: ^{13}C NMR spectrum of hypercalin C (6)



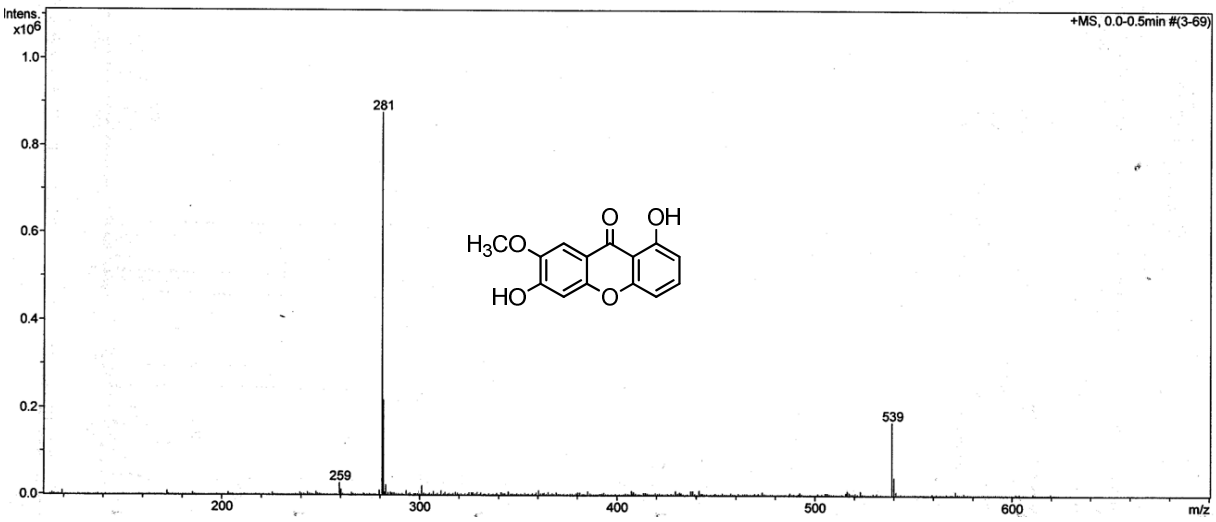
S13: ^{13}C NMR spectrum of cadensin D (8)



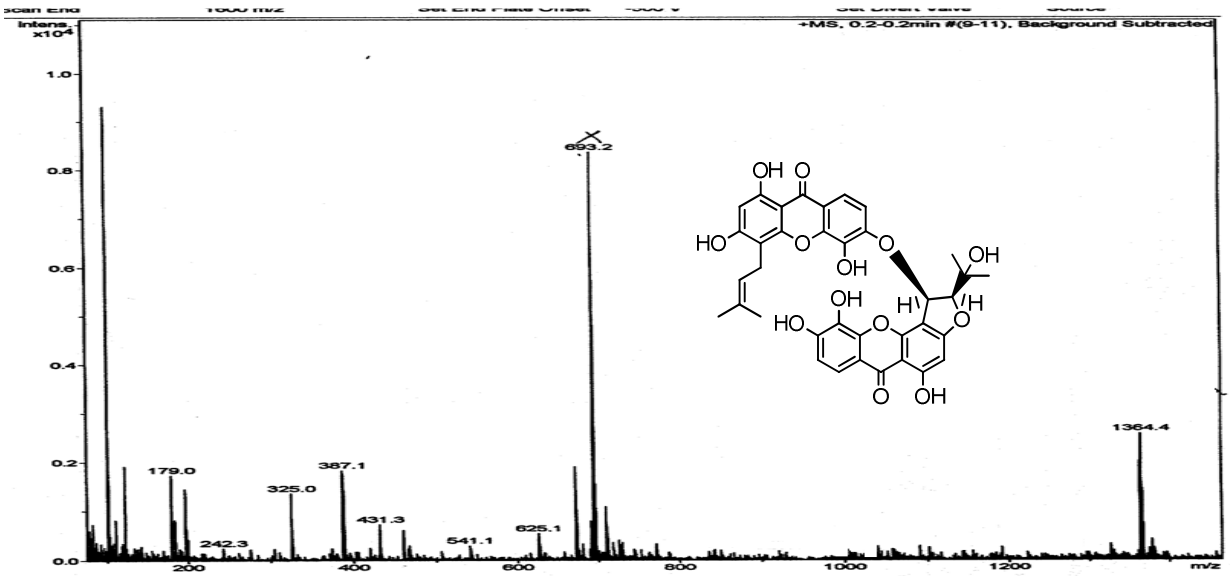
S14: ^{13}C NMR spectrum of 5-hydroxy-1,3-dimethoxyxanthone (9)



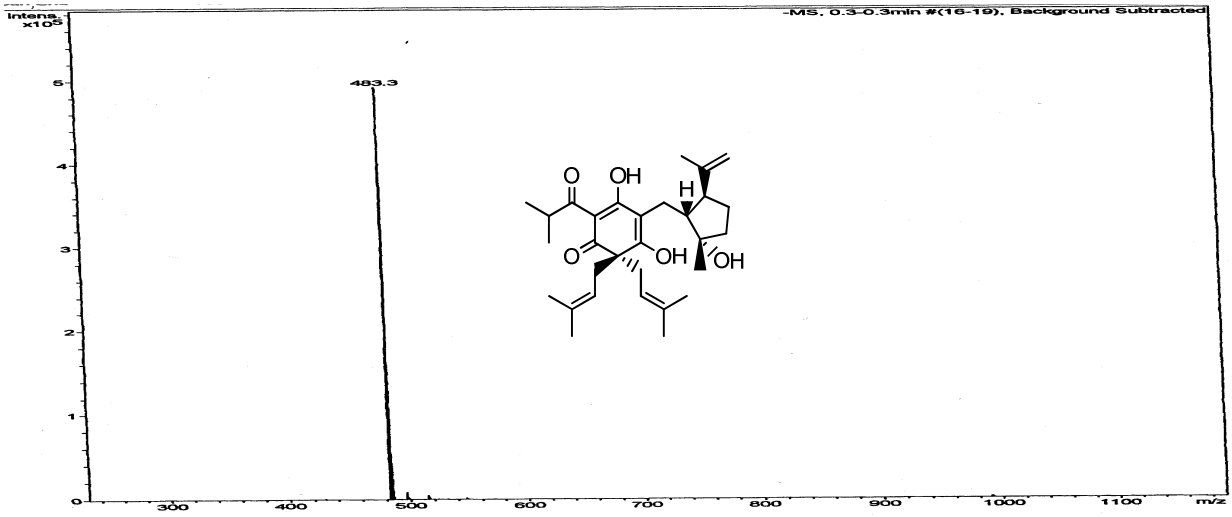
S15: ESI-MS (positive mode) of 5-hydroxy-3-methoxyxanthone (2)



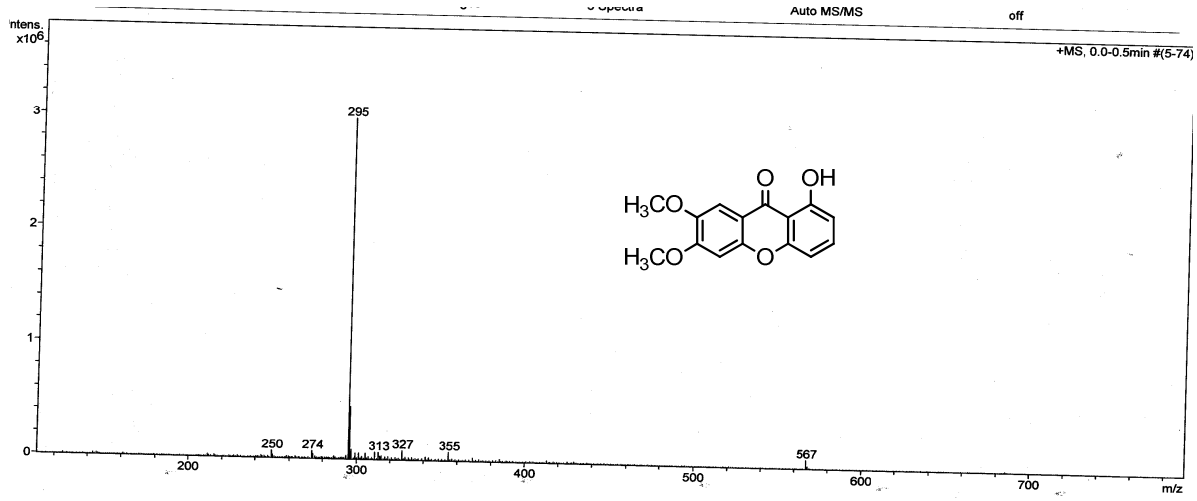
S16: ESI-MS (positive mode) of 1,6-dihydroxy-7-methoxyxanthone (3)



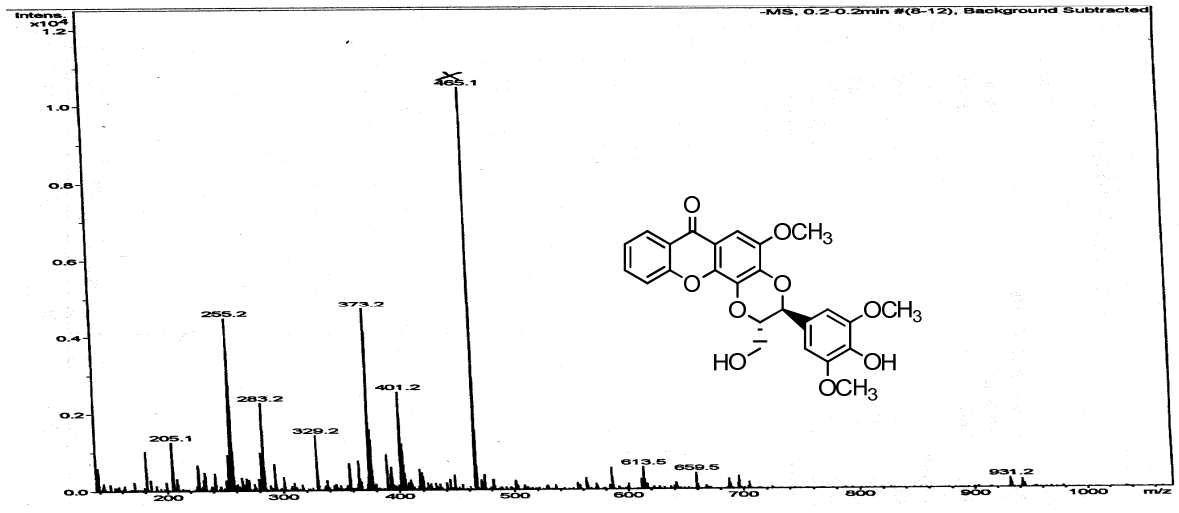
S17: ESI-MS (positive mode) of bijaponicaxanthone C (5)



S18: ESI-MS (positive mode) of hypercalin C (6)



S19: ESI-MS (positive mode) of 1-hydroxy-6,7-dimethoxyxanthone (7)



S20: ESI-MS (negative mode) of cadensin D (8)

Spectroscopic data of compounds 1-3 and 5-9

Betulinic acid (1) : White needles ; m.p. 319-320 ^{o}C; IR ν_{max} (KBr): 3456, 3076, 1685, 1641, 885; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 178.6 (C-28), 150.2 (C-20),108.9 (C-29), 78.2 (C-3), 55.8 (C-17), 55.0 (C-5), 50.4 (C-9), 48.8 (C-18), 47.6 (C-19), 41.9 (C-14), 40.2 (C-8), 38.4 (C-4), 38.3 (C-1), 37.9 (C-13), 36.7 (C-10), 34.6 (C-22), 33.9 (C-7), 30.1 (C-16), 29.2 (C-21), 27.4 (C-23), 27.3 (C-2), 27.0 (C-15), 25.1 (C-12), 20.8 (C-11), 20.4 (C-30), 18.6 (C-6), 17.1 (C-26), 15.5 (C-25), 15.3 (C-24), 14.7 (C-27); EIMS m/z (rel. int.%): 456 ($[M]^{+\cdot}$ (24), 438 (4), 423(13), 207 (52), 189 (80), 95 (100), 81 (93), 135 (82), 121 (72)

*5-**hydroxy-3-methoxyxanthone (2)***: Yellow powder; m.p. 166-168 °C; UV (MeOH): λ_{max} nm (log ε): 368 (3.40), 257 (4.63), 202 (4.30); IR (KBr): ν_{max} cm^{-1}: 3326, 1641, 1579, 1120, 864; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.65 (1H, d, J = 9.2 Hz, H-1), 7.60 (1H, dd, J = 1.5, 9.2 Hz, H-8), 7.55 (1H, d, J = 3.0 Hz, H-4), 7.46 (1H, dd, J = 3.0, 9.2 Hz, H-2), 7.32 (1H, dd, J = 1.5, 9.2 Hz, H-6), 7.25 (1H, t, J = 8.7 Hz, H-7), 3.95 (3H, s, OMe); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 175.3 (C-9), 155.5 (C-3), 150.0 (C-4a), 146.5 (C-5), 145.1 (C-10a), 124.4 (C-2), 123.7 (C-7), 121.5 (C-8a), 121.2 (C-9a), 119.8 (C-1), 119.7 (C-6), 115.0 (C-8), 105.6 (C-4), 55.6 (3-OMe); m/z (rel. int.%): 242 ($[M]^{+}$, 100), 241 (61), 227 (52), 228 (15), 213 (30), 212 (34), 199 (20), 171 (42), 115 (28); HREIMS: m/z 242.0583 (Calcd. for $C_{14}H_{10}O_4$: 242.0579).

***1,6-dihydroxy-7-methoxyxanthone (3)*:** Yellow amorphous solid; IR (KBr): ν_{max} cm^{-1}: 3347, 1647, 1580, 1126; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 13.00 (1H, s, 1-OH), 7.61 (1H, t, J = 8.5 Hz, H-3), 7.42 (1H, s, H-8), 6.98 (1H, dd, J = 1.0, 8.5 Hz, H-4), 6.90 (1H, s, H-5), 6.70 (1H, dd, J = 1.0, 8.5 Hz, H-2), 3.90 (3H, s, 6-OMe); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 180.2 (C-9), 160.7 (C-1), 155.7 (C-4a), 155.3 (C-6), 150.0 (C-5a), 146.0 (C-7), 136.2 (C-3), 111.6 (C-8a), 109,8 (C-2), 107.6 (C-10a), 106.7 (C-4), 104.7 (C-5), 102.7 (C-8), 56.0 (6-OMe); EIMS m/z (rel. int.%): 258 ($[M]^{+}$, 100), 243 (42), 224 (10), 215 (30), 212 (34), 187 (18).

***Bijaponicaxanthone C (5)* :** Yellow powder, m.p. 242-246 ºC; IR (KBr), ν_{max} (cm^{-1}): 3425; 1645; 1608; 1510; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 13.40 (1H, s, 1"-OH), 12.90 (1H,s, 1-OH), 7.63 (1H, d, J = 9.1 Hz, H-8), 7.51 (1H, d, J = 1.7 Hz, H-8"), 7.02 (1H, d, J = 9.1 Hz, H-7), 6.94 (1H, d, J = 9.1 Hz, H-7"), 6.29 (1H, s, H-2"), 6.25 (1H, s, H-2), 5.92 (1H, br, H-2"'), 5.40 (1H, t, J = 7.0 Hz, H-3'), 5.01 (1H, br, H-3"'), 3.36 (2H, d, J = 7.0 Hz, H-2'), 1.31 (3H, s, H-5"'), 1.26 (3H, s, H-6"'); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 179.9 (C-9"), 179.6 (C-9), 162.8 (C-3), 162.7 (C-3"), 160.4 (C-1), 157.3 (C-1"), 154.1 (C-4a), 151.9 (C-6"), 150.2 (C-4"a), 149.5 (C-6), 145.9 (C-10a), 145.7 (C-10"a), 132.5 (C-3), 131.6 (C-5), 116.7 (C-8), 115.7 (C-8"), 132.5 (C-4), 131.0 (C-4'), 122.4 (C-3'), 113.9 (C-9a), 113.5 (C-8a), 113.3 (C-7), 113.0 (C-7"), 112.9 (C-8"a), 106.4 (C-4), 103.1 (C-9"a), 101.4 (C-9a), 101.7 (C-4"), 97.7 (C-2 and C-2"), 79.1 (C-2"'), 71.0 (C-3"'), 70.2 (C-4"'), 28.4 (C-5"'), 25.5 (C-6"'), 21.2 (C-2') ; HRESIMS: m/z 671.1768 (Calcd. for $C_{36}H_{31}O_{13}$, 671.1765); ESIMS m/z (rel. int.%): 693 ($[M + Na]^{+}$, 92) ; $[M + H]^{+}$ 671 (21) ; 625 (7) ; 431 (9) ; 387 (21) ; 325 (18) ; 179 (20) ; 100 (100).

***Hypercalin C (6)*:** White needles, m.p. 153-155 ºC; IR (KBr), ν_{max} (cm^{-1}): 3410, 2960, 2860, 1635; 1562; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.85 (1H, brs, H-14a), 4.81 (1H, brs, H-14b), 4.78 (1H, brs, H-18), 4.75 (1H, brs, H-23), 4.00 (1H, sept, J = 6.7 Hz, H-28), 2.63 (1H, m, H-17b), 2.61 (1H, d, J = 7., H-7a), 2.58 (1H, m, H-17a), 2.47 (1H, m, H-22a), 2.43 (1H, m, H-22b), 2.40 (1H, dd, J = 12.0, 7.0 Hz, H-12), 2.07 (1H, dd, J = 14.5, 13.0 Hz, H-7b), 1.97 (1H, m, H-10a), 1.77 (1H, m, H-11a), 1.74 (3H, s, H-15), 1.72 (1H, m, H-10b), 1.66 (3H, s, H-25), 1.55 (3H, s, H-26), 1.52 (3H, s, H-20), 1.49 (3H, s, H-21), 1.40 (1H, m, H-11b), 1.25 (3H, s, H-16), 1.10 (3H, s, H-30), 1.09 (3H, s, H-29); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 207.5 (C-27), 195.9 (C-3), 190.3 (C-1), 174.3 (C-5), 146.1 (C-13), 134.8 (C-24), 133.9 (C-19), 119.6 (C-18), 118.1 (C-23), 111.7 (C-14), 109.7 (C-6), 107.1 (C-2), 81.1 (C-9), 57.7 (C-4), 54.4 (C-12), 49.9 (C-8), 43.7 (C-10), 38.8 (C-22), 37.3 (C-17), 35.6 (C-28), 29.2 (C-16), 28.8 (C-11), 25.9 (C-25), 25.8 (C-21), 21.6 (C-7), 18.9 (C-30), 18.8 (C-29), 18.5 (C-15), 18.0 (C-26), 17.9 (C-20); HRESIMS: m/z 485.3135 (Calcd. for $C_{30}H_{44}O_5$, 385.3189); ESIMS m/z (rel. int.%): $[2M + Na]^{+}$ 991 (100) ; $[M + Na]^{+}$ 507 (75) ; $[M + H]^{+}$ 485 (29).

***1-hydroxy-6,7-dimethoxyxanthone (7)*:** Pale yellow needles; m.p. 187-188 °C; IR (KBr): ν_{max} cm^{-1}: 3000, 2970, 1640, 1575; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.90 (1H, s, 1-OH), 7.63 (1H, t, J = 9.0 Hz, H-3), 7.44 (1H, s, H-8), 7.08 (1H, d, J = 9.0 Hz, H-4), 6.96 (1H, s, H-5), 6.85 (1H, d, J = 9.0 Hz, H-2), 3.30 (6H, s, 6-OMe and 7-OMe); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 179.6 (C-9), 162.0 (C-1), 156.8 (C-4a), 153.1 (C-6), 148.9 (C-5a), 143.0 (C-7), 136.5 (C-3), 114.1 (C-8a), 110,8 (C-2), 109.7 (C-10a), 108.9 (C-4), 106.0 (C-8), 97.2 (C-5), 56.1 (6-OMe and 7-OMe); EIMS m/z (rel. int.%): 272 ($[M]^{+\cdot}$, 33), 257 (100), 242 (3), 230 (18).

***Cadensin D (8)*:** Yellow powder, m.p. 243-245 ºC; IR (KBr), ν_{max} (cm^{-1}): 3485; 1645; 1600; 1517; 1191; 1110; 757; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.21 (1H, dd, J = 8.1, 1.8 Hz, H-8), 7.84 (1H, dd, J = 7.7, 1.1 Hz, H-6), 7.66 (1H, dd, J = 8.1, 0.5 Hz, H-5), 7.43 (1H, td, J = 7.7, 0.5 Hz, H-7), 7.21 (1H, s, H-1), 6.79 (2H, s, H-2" and H-6"), 5.05 (1H, d, J = 8.1 Hz, H-6'), 4.43 (1H, m, H-5'), 3.90 (3H, s, 2-OMe), 3.80 (6H, s, 3"-OMe and 5"-OMe); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 174.6 (C-9), 155.2 (C-10a), 148.0 (C-5' and C-3'), 145.8 (C-2), 141.2 (C-4a), 139.5 (C-3), 136.3 (C-4'), 134.7 (C-6), 132.5 (C-4), 125.8 (C-8), 125.6 (C-1'), 124.2 (C-7), 120.4 (C-8a), 118.0 (C-5),), 113.9 (C-9a), 105.7 (C-2' and C-6'), 96.5 (C-1), 77.9 (C-8'), 76.6 (C-7'), 59.4 (C-9'), 56.1 (3'-OMe and 5'-OMe), 55.7 (2-OMe); HRESIMS ($[M-H]^-$): m/z = 465.1178 (calcd. for $C_{25}H_{21}O_9$, 465.1186); ESIMS (negative mode) m/z (rel. int.%): $[M - H]^-$ 465 (100) ; 401 (24) ; 373 (45) ; 329 (14) ; 283 (22) ; 255 (43) ; 205 (13).

***5-hydroxy-1,3-dimethoxyxanthone (9)* :** Yellow powder; m.p. 261-263 °C; IR (KBr): ν_{max} cm^{-1}: 3230, 1640, 1581, 1519, 1120, 864; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.60 (1H, dd, J = 2.0, 8,7 Hz, H-8), 7.22 (1H, dd, J = 2.0 Hz, H-6), 7.15 (1H, t, J = 8.7 Hz, H-7), 6.63 (1H, d, J = 2.5 Hz, H-4), 6.32 (1H, d, J = 2.5 Hz, H-2), 3.85 (3H, s, 1-OMe), 3.90 (3H, s, 3-OMe); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 173.6 (C-9), 164.5 (C-3), 161.4 (C-1), 158.8 (C-4a), 143.8 (C-5), 123.5 (C-7), 119.1 (C-6), 114.5 (C-8a), 106.1 (C-10a), 95.2 (C-4), 93.1 (C-2), 56.1 (1-OMe); 55.8 (3-OMe) ; EIMS m/z (rel. int.%): 272 ($[M]^+$, 50), 257 (17) 241 (l00), 227 (65), 212 (16), 199 (25), 171 (22)**.**