Novel synthetic luteolin analogue-caused sensitization of tumor necrosis factor-α-induced apoptosis in human tumor cells

Lili Cheng,[a] Huiling Tan,[b] Xiaoyu Wu,[a] Ruijun Hu,[a] Carlin Aw,[a] Min Zhao,[b] Han-Ming Shen*[b] and Yixin Lu*[a,c]

[a]*Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore, 117543*

[b]*Department of Community, Occupational and Family Medicine, Yong Loo Lin School of Medicine National University of Singapore, 16 Medical Drive, Singapore, 117597*

[c]*Medicinal Chemistry Program, Life Sciences Institute, National University of Singapore*

Email: chmlyx@nus.edu.sg or cofshm@nus.edu.sg

Supporting Information

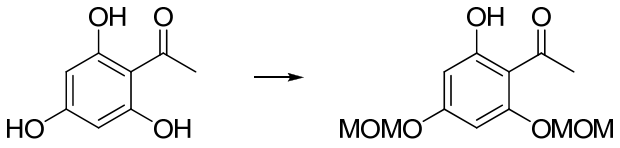
Experimental Procedures and Characterization of Novel Luteolin analogues

A. General information

Chemicals and solvents were purchased from commercial suppliers and used as received. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACF300 or DPX300 (300 MHz) or AMX500 (500 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: proton (chloroform δ 7.26), carbon (chloroform δ 77.0). Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), br s (broad singlet). Coupling constants were reported in Hertz (Hz). Low resolution mass spectra were obtained on a Finnigan/MAT LCQ spectrometer in ESI mode, and a Finnigan/MAT 95XL-T mass spectrometer in FAB mode. All high resolution mass spectra were obtained on a Finnigan/MAT 95XL-T spectrometer. For thin-layer chromatography (TLC), Merck pre-coated TLC plates (Merck 60 F_{254}) was used, and compounds were visualized with a UV light at 254 nm. Further visualization was achieved by staining with iodine, or ceric ammonium molybdate followed by heating on a hot plate. Flash chromatography separations were performed on Merck 60 (0.040 - 0.063 mm) mesh silica gel. Microwave irradiation was performed using a CEM Discover Synthesis Unit with a 0.5-2 microwave reaction vial.

B. Experimental procedures

1-(2-Hydroxy-4,6-bis(methoxymethoxy)phenyl)ethanone (**1**)[1]

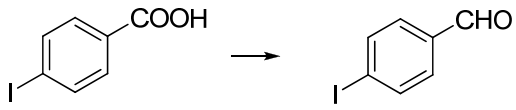


1

To a solution of 2',4',6'-trihydroxyacetophenone (4 g, 23.8 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C was added diisopropylethyl amine (DIPEA) (8.5 mL, 50 mmol) under an atmosphere of argon with stirring. After stirring at 0 ^oC for 30 minutes, chloromethyl methyl ether (MOMCl) (2.86 mL, 50 mmol) was added dropwise to the mixture at this temperature. The reaction mixture was stirred for an additional 30 minutes before it was allowed to warm up to room temperature. When TLC analysis indicated the disappearance of the starting material, ice water (30 mL) was added to quench the reaction. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were washed with water (20 mL), brine (20 mL) and dried over Na_2SO_4. Solvents were evaporated under reduced pressure to give the crude product, which was purified by flash chromatography over silica gel (hexanes/EtOAc = 7/1) to afford **1** as a white solid (2.9 g, 46%).

^1H NMR (300 MHz, $CDCl_3$) δ 2.65 (s, 3H), 3.47 (s, 3H), 3.52 (s, 3H), 5.17 (s, 2H), 5.25 (s, 2H), 6.24 (d, J = 2.4 Hz, 1H), 6.26 (d, J = 2.4 Hz, 1H), 13.71 (s, 1H); MS (EI) m/z 256.2 (M).

4-Iodobenzaldehyde

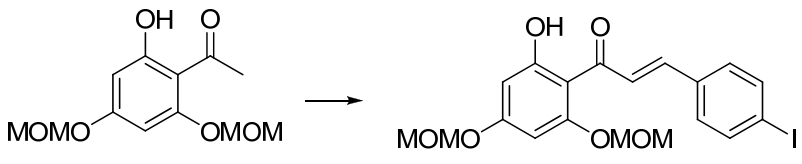


To a vacuum-dried two-necked round bottom flask was added 4-iodobenzoic acid (1.98 g, 8 mmol), and then the flask was immersed in an ice-water bath. 1 M BH_3/THF complex (15 mL, 15 mmol) was added dropwise into the flask under argon. Upon the completion of the addition, the reaction mixture was allowed to stir vigorously at room temperature for 2 hours.

When the white suspension turned into a colorless solution, a suspension of PCC (4 g, 18 mmol) in anhydrous CH_2Cl_2 (10 mL) was added to the reaction mixture at 0 ^oC. The reaction mixture was stirred at 0 °C for 30 minutes before the ice bath was removed, and the reaction was allowed to continue stirring at room temperature for 3 hours. The reaction mixture was filtered through a celite pad and the filter cake was washed with diethyl ether. The combined organic washes were dried over Na_2SO_4. Solvents were removed under reduced pressure to give the crude aldehyde as a brown solid (1.90 g, quantitative), which was used directly in the next step.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8.4 Hz, 2H), 7.90 (d, *J* = 8.4 Hz, 2H), 9.95 (s, 1H).

(*E*)-1-(2-Hydroxy-4,6-bis(methoxymethoxy)phenyl)-3-(4-iodophenyl)prop-2-en-1-one (**2**)



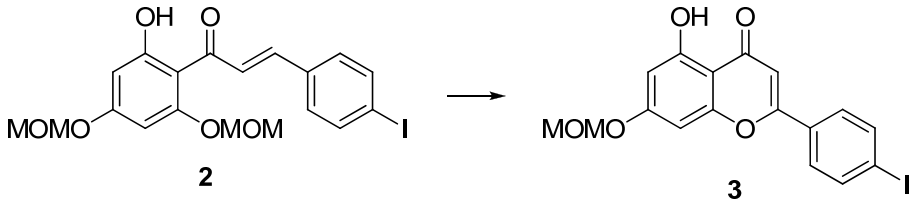
2

Ketone (**1**) (707 mg, 2.76 mmol) and 4-iodobenzaldehyde (705 mg, 3 mmol) were dissolved in dioxane (17 mL) and the mixture was stirred vigorously. 1 N aqueous NaOH (13.8

mL, 13.8 mmol) was then added dropwise into the mixture. The reaction was stirred vigorously under ambient temperature for 18 hours. Upon the completion of the reaction, dioxane was removed under reduced pressure, and the residue was extracted with EtOAc and washed with water. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography (hexanes/EtOAc = 7/1) to give the desired **2** as an orange yellow solid **2** (1.09 g, 84%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.48 (s, 3H), 3.52 (s, 3H), 5.19 (s, 2H), 5.28 (s, 2H), 6.24 (s, 1H), 6.32 (s, 1H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 15.7 Hz, 1H), 7.91 (d, *J* = 15.6 Hz, 1H); MS (EI) m/z 470.0 (M).

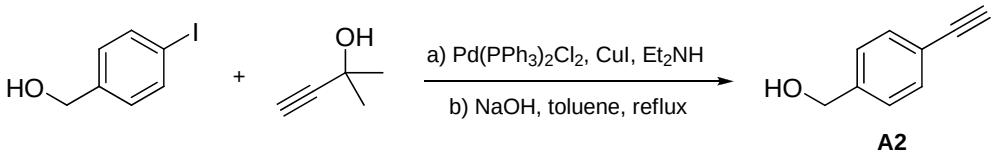
5,7-Dihydroxy-2-(4-iodophenyl)-4H-chromen-4-one (**3**)



To an oven-dried 50 mL round bottom flask equipped with a magnetic stirring bar and refluxing condenser cooled under a stream of dry argon was added chalcone **2** (235 mg, 0.5 mmol) and DDQ (340 mg, 1.5 mmol). Anhydrous dioxane (20 mL) was added *via* a syringe, and the reaction mixture was brought to reflux and stirred vigorously for 36 hours. Upon completion of the reaction, the mixture was cooled down to room temperature, and dioxane was removed under reduced pressure. The crude mixture was then dissolved in EtOAc and washed with distilled water. The organic extracts were dried over Na_2SO_4 and filtered. Solvents were removed under reduced pressure to give the crude product which was purified by flash chromatography (hexanes/EtOAc = 7.5/1) to afford **3** as a pale yellow solid (109 mg, 51%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.51 (s, 3H), 5.25 (s, 2H), 6.50 (d, *J* = 1.4 Hz, 1H), 6.67 (m, 2H), 7.58-7.63 (d, *J* = 8.7 Hz, 2H), 7.80-7.90 (d, *J* = 8.7 Hz, 2H), 12.59 (s, 1H); MS (EI) for $C_{17}H_{13}IO_5$, found 424.0.

Representative procedure for the preparation of alkynes

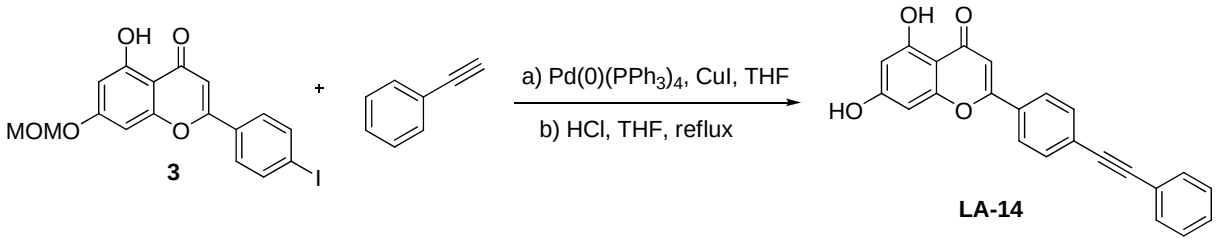


A2

To an oven-dried 10 mL microwave glass vessel equipped with a magnetic stirring bar under a stream of dry argon, 4-iodobenzyl alcohol (233 mg, 1 mmol) and 2-methyl-3-butyn-2-ol (0.2 mL, 2 mmol) were added, followed by diethyl amine (3.0 mL). Finally, catalytic amount of $Pd(PPh_3)_2Cl_2$ (7 mg, 0.01 mmol) and CuI (4 mg, 0.02 mmol) were added to the mixture. The mixture was then irradiated under microwave (temperature = 100 °C, time = 20 min, holding time = 10 min). After cooling down to room temperature, the reaction mixture was poured into H_2O (10 mL) and extracted with EtOAc (15 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated, and the crude was purified by flash chromatography (hexanes/EA = 4/1 to 1/1) to afford **A2** as a yellow solid, which was used directly in the next step.

The coupling product was dissolved in toluene (2 mL), and powdered NaOH (10 mg) was added. Then the mixture was brought to reflux for 12 hours. After cooling down to room temperature, the solution was filtered through a short column of silica gel, washed with toluene and concentrated. The crude product could be used directly in the subsequent Sonogashira coupling reactions.

Representative procedure for the Sonogashira coupling between the alkyne and the iodide



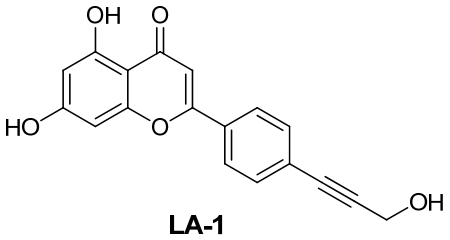
Sonogashira coupling: To an oven-dried 5 mL microwave glass vessel equipped with a magnetic stirring bar under a stream of dry argon, 4'-iodoflavone (45 mg, 0.106 mmol) and phenylacetylene (116 μl, 1.06 mmol) were added. Anhydrous THF (4.0 mL) was then added, followed by the addition of anhydrous Et_3N (3 mL). Finally, catalytic amount of tetrakis-(triphenylphosphine) palladium (0) [Pd (0)$(PPh_3)_4$] (10 mg, 0.01 mmol) and copper iodide (CuI, 4 mg, 0.02 mmol) were added to the mixture. The mixture was then irradiated under microwave (temperature = 80 °C, time = 10 min, holding time = 10 min). Upon completion of the reaction, the solvents were removed. The residue was then extracted with EtOAc and washed with distilled water. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. Purification by flash chromatography (hexanes/EtOAc = 6/1) afforded the desired coupling product as a yellow solid.

Deprotection: The coupling product from previous step was dissolved in THF (3 mL), and one drop of concentrated HCl was added into the solution. The mixture was brought to reflux under an atmosphere of argon. After 4 hours, the reaction mixture was cooled down to room temperature. Saturated aqueous $NaHCO_3$ solution was added to neutralize the mixture. THF was removed under reduced pressure, and the residue was extracted with EtOAc and washed with deionized water. The combined organic extracts were dried over Na_2SO_4, filtered, and

concentrated. The crude product was purified by flash chromatography (hexanes/EtOAc = 3/1 to 1/1) to afford **LA-14** as a brown solid (35 mg, 93%).

C. Characterization of synthetic luteolin analogues

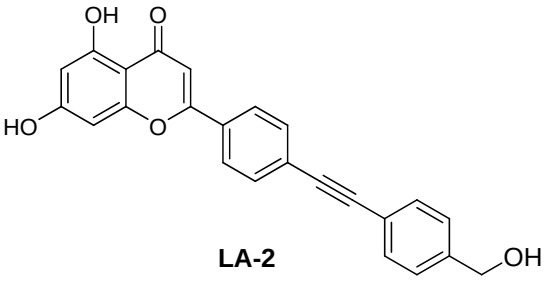
5,7-Dihydroxy-2-(4-(3-hydroxyprop-1-ynyl)phenyl)-4H-chromen-4-one (**LA-1**)



LA-1

A yellow solid; ^{1}H NMR (300 MHz, DMSO-D_6) δ 4.35 (s, 2H), 5.40 (s, 1H), 6.23 (s, 1H), 6.53 (s, 1H), 7.02 (s, 1H), 7.60 (d, *J* = 4.5 Hz, 2H), 8.08 (d, *J* = 4.5 Hz, 2H), 10.93 (br s, 1H), 12.79 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ 49.7, 83.4, 93.4, 94.5, 99.5, 104.3, 106.0, 126.2, 127.0, 130.8, 132.2, 157.8, 161.8, 162.6, 165.0, 182.1; HRMS (ESI) *m/z* calcd for $C_{18}H_{11}O_5$, 307.0601, found 307.0598.

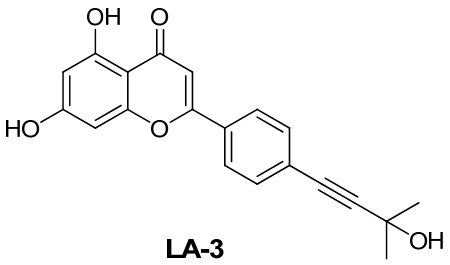
5,7-Dihydroxy-2-(4-((4-(hydroxymethyl)phenyl)ethynyl)phenyl)-4H-chromen-4-one (**LA-2**)



LA-2

A yellow solid; ^{1}H NMR (300 MHz, DMSO-D_6) δ 4.54 (d, 2H), 5.27-5.33 (br s, 1H), 6.23 (s, 1H), 6.54 (s, 1H), 7.05 (s, 1H), 7.38-7.41 (d, *J* = 8.2 Hz, 1H), 7.54-7.59 (d, *J* = 8.2 Hz, 2H), 7.70-7.75 (d, *J* = 8.5 Hz, 2H), 8.11-8.16 (d, *J* = 8.5 Hz, 2H), 10.94 (s, 1H), 12.80 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ 62.8, 88.7, 92.9, 94.5, 99.4, 104.4, 106.1, 120.3, 126.2, 127.0, 130.8, 131.7, 132.3, 157.8, 161.8, 162.5, 164.9, 182.2; HRMS (ESI) *m/z* calcd for $C_{24}H_{15}O_5$ 383.0914, found 383.0910.

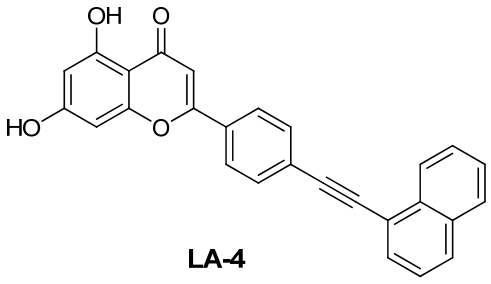
5,7-Dihydroxy-2-(4-(3-hydroxy-3-methylbut-1-ynyl)phenyl)-4H-chromen-4-one (**LA-3**)



LA-3

A yellow solid; ^{1}H NMR (300 MHz, DMSO-D_6) δ 1.48 (s, 6H), 5.55 (s, 1H), 6.22 (s, 1H), 6.52 (s, 1H), 7.00 (s, 1H), 7.50-7.60 (d, *J* = 8.4 Hz, 2H), 8.00-8.10 (d, *J* = 8.4 Hz, 2H), 10.93 (s, 1H), 12.79 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6) δ 31.4, 31.8, 64.0, 80.2, 86.1, 94.5, 99.4, 104.4, 105.9, 126.4, 127.0, 130.5, 132.4, 157.8, 161.8, 162.6, 164.9, 182.1; HRMS (ESI) *m/z* calcd for $C_{20}H_{15}O_5$ 335.0914, found 335.0923.

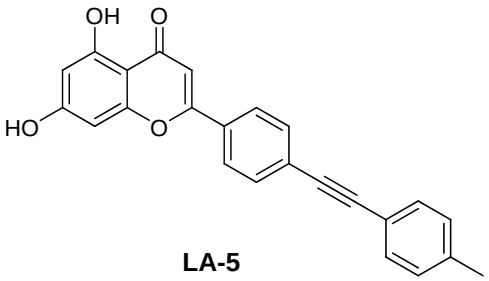
5,7-Dihydroxy-2-(4-(naphthalen-1-ylethynyl)phenyl)-4H-chromen-4-one (**LA-4**)



LA-4

A yellow solid; ^{1}H NMR (300 MHz, DMSO-D_6) δ 6.23 (s, 1H), 6.56 (s, 1H), 7.08 (s, 1H), 7.50-7.80 (m, 3H), 7.90-8.00 (m, 2H), 8.05-8.10 (m, 2H), 8.20-8.30 (m, 2H), 8.40-8.43 (d, *J* = 8.4 Hz, 1H), 10.5-11.5 (br s, 1H), 12.81 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ 90.5, 94.0, 94.6, 99.5, 104.4, 106.1, 119.6, 125.8, 126.0, 126.1, 127.1, 127.3, 127.9, 129.0, 130.1, 131.1, 131.3, 132.5, 132.8, 133.2, 157.8, 161.8, 162.5, 164.9; HRMS (ESI) *m/z* calcd for $C_{27}H_{15}O_4$ 403.0965, found 403.0970.

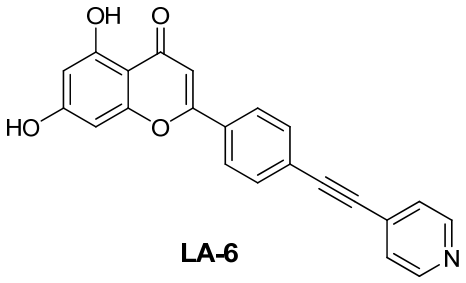
5,7-Dihydroxy-2-(4-(p-tolylethynyl)phenyl)-4H-chromen-4-one (**LA-5**)



LA-5

A yellow solid; ^{1}H NMR (300 MHz, DMSO-D_6) δ 2.35 (s, 3H), 6.23 (s, 1H), 6.53 (s, 1H), 7.01 (s, 1H), 7.24-7.28 (d, *J* = 7.5 Hz, 2H), 7.47-7.50 (d, *J* = 7.5 Hz, 2H), 7.68-7.72 (d, *J* = 8.4 Hz, 2H), 8.00-8.13 (d, *J* = 8.4 Hz, 2H), 10.90-11.10 (br s, 1H), 12.70-12.80 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ 21.4, 88.5, 92.9, 94.5, 99.4, 104.4, 106.0, 119.1, 126.3, 127.0, 129.8, 130.7, 131.8, 132.2, 139.5, 157.8, 161.8, 162.6, 164.9, 182.1; HRMS (ESI) *m/z* calcd for $C_{24}H_{15}O_4$ 367.0965, found 367.0973.

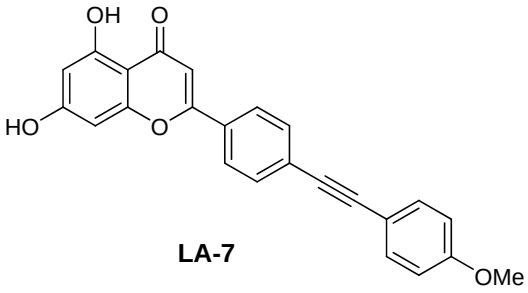
5,7-Dihydroxy-2-(4-(pyridin-4-ylethynyl)phenyl)-4H-chromen-4-one (**LA-6**)



LA-6

A yellow solid; ^{1}H NMR (300 MHz, DMSO-D_6) δ 6.24 (s, 1H), 6.56 (s, 1H), 7.06 (s, 1H), 7.80-7.90 (m, 4H), 8.15-8.25 (d, *J* = 8.4 Hz, 2H), 8.78-8.82 (m, 2H); ^{13}C NMR (75 MHz, DMSO-D_6): δ 88.9, 94.6, 96.2, 99.5, 104.4, 106.5, 124.2, 127.2, 127.4, 132.3, 133.0, 146.8, 157.8, 161.8, 162.2, 165.0, 182.1; HRMS (ESI) *m/z* calcd for $C_{22}H_{12}NO_4$ 354.0734, found 354.0767.

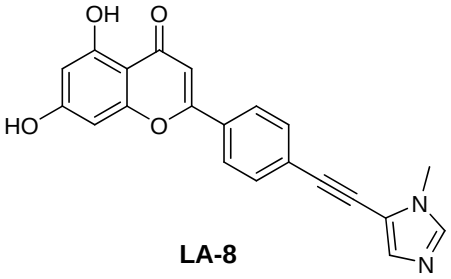
5,7-Dihydroxy-2-(4-((4-methoxyphenyl)ethynyl)phenyl)-4H-chromen-4-one (**LA-7**)



LA-7

A yellow solid; ^{1}H NMR (300 MHz, DMSO-D_6) δ 3.81 (s, 3H), 6.23 (s, 1H), 6.55 (s, 1H), 7.00-7.03 (d, *J* = 9 Hz, 2H), 7.03 (s, 1H), 7.53-7.56 (d, *J* = 9 Hz, 2H), 7.68-7.71 (d, *J* = 8.7 Hz, 2H), 8.10-8.13 (d, *J* = 8.7 Hz, 2H), 10.95 (s, 1H), 12.80 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ 55.7, 87.9, 93.0, 94.5, 99.4, 104.4, 105.9, 114.0, 114.9, 126.6, 127.0, 130.5, 132.1, 133.6, 157.8, 160.3, 161.8, 162.6, 164.9, 182.1; HRMS (ESI) *m/z* calcd for $C_{24}H_{15}O_5$ 383.0914, found 383.0912.

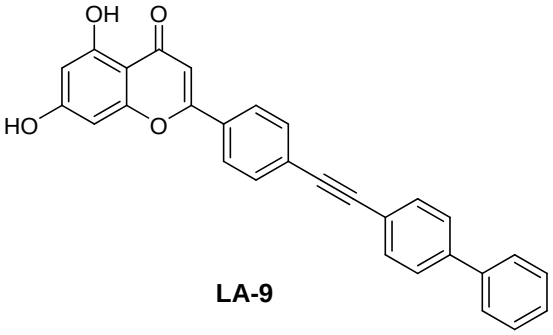
5,7-Dihydroxy-2-(4-((1-methyl-1H-imidazol-5-yl)ethynyl)phenyl)-4H-chromen-4-one (**LA-8**)



LA-8

A yellow solid; ^{1}H NMR (300 MHz, DMSO-D_6) δ 3.91 (s, 3H), 6.25 (s, 1H), 6.56 (s, 1H), 7.08 (s, 1H), 7.81-7.84 (d, *J* = 8.4 Hz, 2H), 8.05 (s, 1H), 8.16-8.20 (d, *J* = 8.4 Hz, 2H), 9.00 (s, 1H), 11.00-11.05 (br s, 1H), 12.73-12.78 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6) δ 34.1, 77.5, 94.6, 97.1, 99.5, 104.4, 106.5, 116.8, 124.1, 126.4, 127.2, 132.0, 132.4, 138.1, 157.8, 161.8, 162.2, 165.0, 182.1; HRMS (ESI) *m/z* calcd for $C_{21}H_{13}N_2O_4$ 357.0910, found 357.0878.

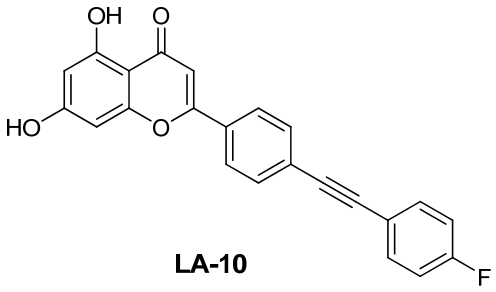
2-(4-(Biphenyl-4-ylethynyl)phenyl)-5,7-dihydroxy-4H-chromen-4-one (**LA-9**)



LA-9

A yellow solid; ^{1}H NMR (300 MHz, DMSO-D_6) δ 6.23 (s, 1H), 6.55 (s, 1H), 7.05 (s, 1H), 7.30-7.60 (m, 4H), 7.65-7.90 (m, 8H), 8.11-8.16 (d, *J* = 8.4 Hz, 2H), 10.95 (s, 1H), 12.80 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ 89.5, 92.2, 94.2, 99.1, 104.0, 105.7, 125.7, 126.7, 126.8, 127.0, 127.1, 128.0, 129.1, 130.6, 132.0, 132.2, 133.0, 157.4, 161.5, 162.1, 164.5; HRMS (ESI) *m/z* calcd for $C_{29}H_{17}O_4$ 429.1121, found 429.1128.

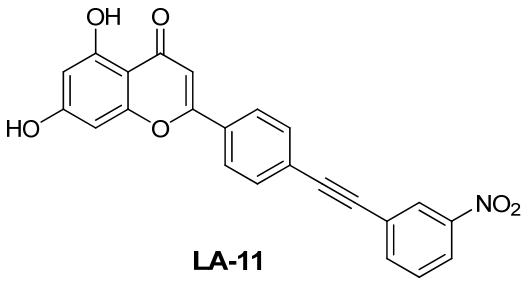
2-(4-((4-Fluorophenyl)ethynyl)phenyl)-5,7-dihydroxy-4H-chromen-4-one (**LA-10**)



LA-10

A yellow solid; ^{1}H NMR (300 MHz, DMSO-D_6) δ 6.23 (s, 1H), 6.53 (s, 1H), 7.03 (s, 1H), 7.28-7.31 (m, 2H), 7.65-7.80 (m, 4H), 8.11 (d, *J* = 4.5 Hz, 2H), 10.90-11.20 (br s, 1H), 12.79 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ 88.8, 91.5, 94.5, 99.5, 104.40 106.1, 116.4, 116.6, 125.9, 127.1, 131.0, 132.3, 134.3, 134.4, 157.8, 161.8, 162.5, 164.9; HRMS (ESI) *m/z* calcd for $C_{23}H_{12}FO_4$ 371.0703, found 371.0714.

5,7-Dihydroxy-2-(4-((3-nitrophenyl)ethynyl)phenyl)-4H-chromen-4-one (**LA-11**)

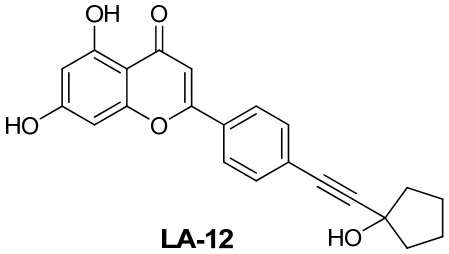


LA-11

A yellow solid; ^{1}H NMR (300 MHz, DMSO-D_6) δ 6.23 (s, 1H), 6.55 (s, 1H), 7.05 (s, 1H), 7.70-7.85 (m, 3H), 8.04-8.10 (d, *J* = 7.8 Hz, 2H), 8.15-8.22 (d, *J* = 8.4 Hz, 2H), 8.27-8.31 (m, 1H), 8.42 (s, 1H), 10.97 (s, 1H), 12.79 (s, 1H); ^{13}C NMR (75 MHz, DMSO-D_6): δ 91.0, 94.6, 104.4,

106.3 123.8, 124.3, 126.5, 127.1, 130.9, 131.5, 132.6, 138.0, 157.8, 161.8, 162.4, 164.9, 182.2; HRMS (ESI) *m/z* calcd for $C_{23}H_{12}NO_6$ 398.0686, found 398.0666.

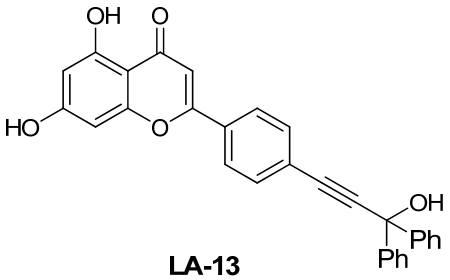
5,7-Dihydroxy-2-(4-((1-hydroxycyclopentyl)ethynyl)phenyl)-4H-chromen-4-one (**LA-12**)



LA-12

A pale yellow solid; mp 215.2-217.0 ^{o}C; ^{1}H NMR (300 MHz, acetone-D_6) δ 12.3 (s, 1H), 8.04-8.07 (d, *J* = 8.82 Hz, 2H), 7.57-7.60 (d, *J* = 8.82 Hz, 2H), 6.81 (s, 1H), 6.58 (s, 1H), 6.28 (s, 1H), 2.04-1.99 (m, 4H), 1.75-1.89 (m, 4H); HRMS (ESI) *m/z* calcd for $C_{22}H_{17}O_5$ 361.1071, found 361.1069.

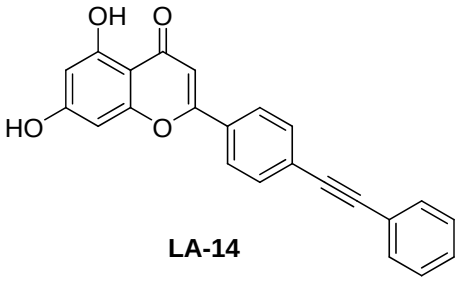
5,7-Dihydroxy-2-(4-(3-hydroxy-3,3-diphenylprop-1-ynyl)phenyl)-4H-chromen-4-one (**LA-13**)



LA-13

A yellow solid; ^{1}H NMR (300 MHz, acetone-D_6) δ 6.28 (s, 1H), 6.58 (s, 1H), 6.82 (d, *J* = 4.4 Hz, 1H), 7.27-8.08 (m, 14 H); ^{13}C NMR (75 MHz, acetone-D_6): δ 69.0, 95.7, 100.7, 100.8, 107.3, 108.1, 125.7, 128.0, 129.5, 129.8, 130.3, 130.9, 132.7, 134.2, 138.0, 159.6, 164.0, 166.1, 168.7, 183.6; HRMS (ESI) *m/z* calcd for $C_{30}H_{20}O_5$ $[M-H]^-$ 459.1238, found 459.1232.

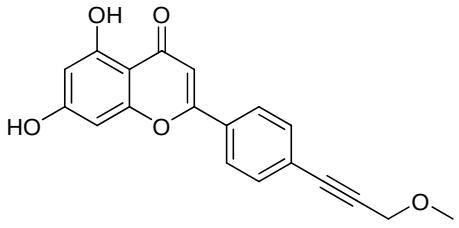
5,7-Dihydroxy-2-(4-(phenylethynyl)phenyl)-4H-chromen-4-one (**LA-14**)



LA-14

A brown solid; ^{1}H NMR (300 MHz, acetone-D_6) δ 6.29 (s, 1H), 6.60 (s, 1H), 6.85 (s, 1H), 7.45 (m, 3H), 7.60 (m, 2H), 7.74 (m, 2H), 8.12 (m, 2H); ^{13}C NMR (75 MHz, acetone-D_6): δ 66.9, 67.2, 95.6, 100.7, 107.3, 124.2, 128.1, 130.3, 131.1, 132.7, 133.2, 133.6, 134.1, 166.1, 168.7, 183.7; HRMS (ESI) *m/z* calcd for $C_{23}H_{14}O_4$ $[M-H]^-$ 353.0819, found 353.0812.

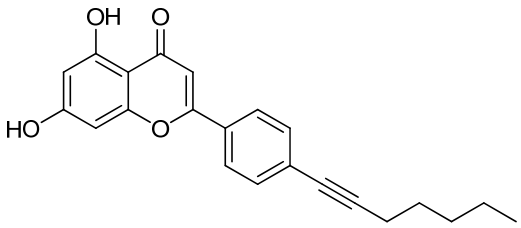
5,7-dihydroxy-2-(4-(3-methoxyprop-1-ynyl)phenyl)-4H-chromen-4-one (**LA-15**)



LA-15

A yellow solid; ^{1}H NMR (300 MHz, acetone-D_6) δ 3.41 (s, 3H), 4.36 (s, 2H), 6.28 (d, J = 2.0 Hz, 1H), 6.58 (d, J = 2.4 Hz, 1H), 6.82 (s, 1H), 7.65 (d, J = 8.9 Hz, 2H), 8.08 (d, J = 8.9 Hz, 2H); ^{13}C NMR (75 MHz, acetone-D_6): δ 58.3, 61.2, 86.5, 90.1, 95.6, 100.6, 106.3, 107.3, 127.7, 128.1, 132.8, 133.7, 159.6, 164.1, 164.4, 166.0, 183.7; HRMS (ESI) *m/z* calcd for $C_{19}H_{14}O_5$ $[M-H]^-$ 321.0768, found 321.0753.

2-(4-(hept-1-ynyl)phenyl)-5,7-dihydroxy-4H-chromen-4-one (**LA-16**)



LA-16

A pale yellow solid; ^{1}H NMR (300 MHz, acetone-D_6) δ 0.93 (m, 3H), 1.50 (m, 6H), 2.47 (m, 2H), 6.27 (s, 1H), 6.57 (s, 1H), 6.78 (s, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 8.02 (d, *J* = 8.4 Hz, 2H), 12.3 (br s, 1H); ^{13}C NMR (75 MHz, acetone-D_6): δ 14.9, 20.5, 23.5, 31.3, 32.5, 81.5, 95.3, 95.6, 100.6, 107.0, 127.9, 129.2, 131.9, 133.5, 159.6, 164.1, 164.5, 166.1, 183.7; HRMS (ESI) *m/z* calcd for $C_{22}H_{20}O_4$ $[M-H]^-$ 347.1289, found 347.1293.

Cytotoxicity Studies and Sensitization Experiments of Synthetic Luteolin Analogues

A. General information

Reagents

3-[4, 5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO), cycloheximide (CHX) were purchased from Sigma-Aldrich (St. Louis, USA). Human recombinant tumor necrosis factor-α (TNF-α) was purchased from Calbiochem (San Diego, CA).

Cell culture and treatment

Human cervical carcinoma HeLa cells and human hepatoma HepG2 cells were maintained in Dulbecco's modified Eagle's medium (Sigma) containing 10% fetal bovine serum

(HyClone) and 1% penicillin-streptomycin (Invitrogen) in a 5% CO_2 atmosphere at 37 °C. Luteolin and its analogues (dissolved in DMSO) were prepared as stock solutions just before treatment.

Detection of cell death and cell viability

The cell viability was measured by MTT test as described previously.[2] Briefly, cells were seeded into a 96 well plate and incubated (37 °C, 5% CO_2) overnight, followed by designated treatments with luteolin or its analogues for 24 hours. At the end of the treatment, 25 μL of MTT (5 mg/mL) was added to each well and incubated for another 2 hours (37 °C, 5% CO_2). Finally, 100 μL of lysing buffer (50% DMF and 20% SDS, pH 4.6) was added to each well and incubated for additional 2 hours (37 °C, 5% CO_2). The plate was read using a microplate reader at the wavelength of 595 nm (Tecan SPECTRAFLUOR PLUS). The results were presented as percentage of cell viability as comparing to the untreated control group.

For cell death detection, HeLa and HepG2 cells were treated as indicated and the morphological changes of cells were examined and captured using an inverted phase-contrast microscope (Nikon ECLIPSE TE2000-S). Dead cells were identified by their morphology (rounded and detached).

Sensitization effect of luteolin analogues on TNF-α-induced apoptosis

Among the 16 compounds, **LA-4**, **LA-11**, **LA-13**, **LA-14** and **LA-15** demonstrated similar cytotoxicity as their parent compound, luteolin. **LA-12** and **LA-16** appeared to be more cytotoxic than luteolin (Figure 6). Next, we found that **LA-12** was capable of significantly promoting TNF-α-induced cell death in both HeLa and $HepG_2$ cells (Figure 3). Finally, we compared the

sensitization effect of **LA-12** and that of luteolin and found that **LA-12** was more effective than luteolin to sensitize TNF-α-induced cell death (Figure 4).

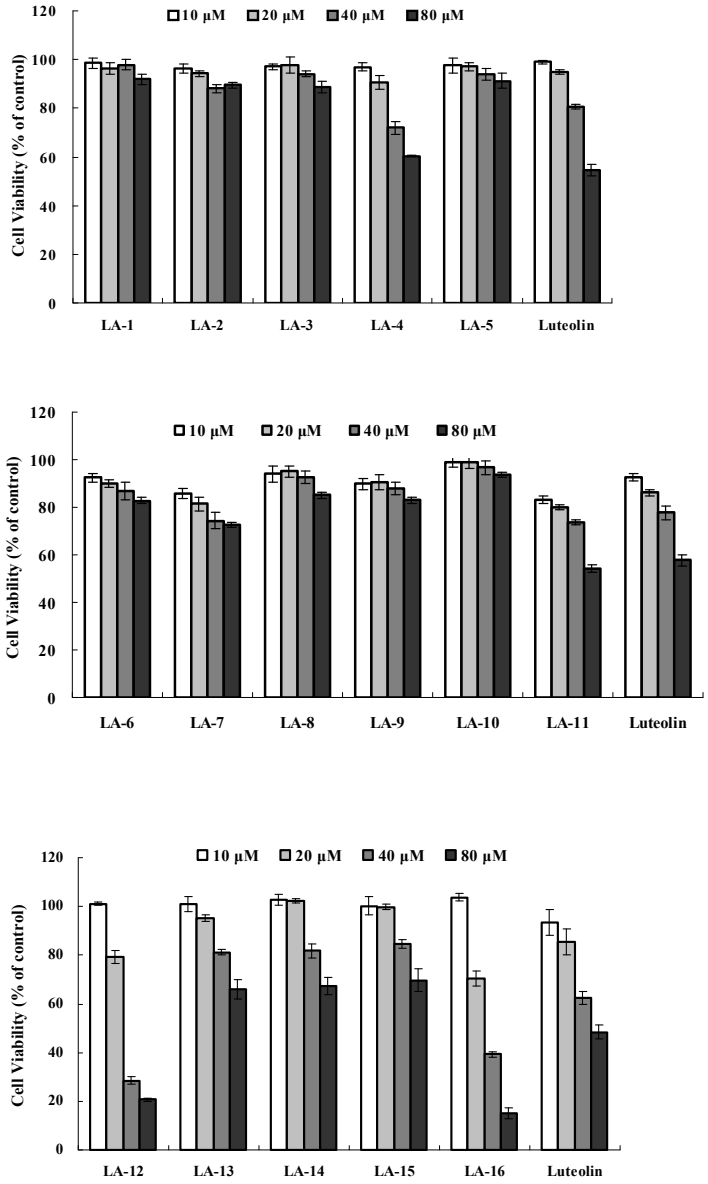
B. Figure 6 and full legends of Figures 3-6

Figure 3. Luteolin analogues sensitize TNF-α-induced apoptosis : HeLa and $HepG_2$ cells were treated with **LA-4**, **LA-11**, **LA-12** and **LA-16**, or CHX (10 μg/ml) in the presence or absence of TNF-α (40 ng/ml) for 24 hours. Cell viability was determined by MTT test. Data were presented as mean ± SD of three independent experiments. (**: $p<0.01$, *t*-test, comparing with the group without TNF-α).

Figure 4. The sensitization effect of LA-12 on TNF-α-induced cell death determined by MTT. HeLa and $HepG_2$ cells were treated with **LA-12** (80 μM) and luteolin (80 μM) in the presence or absence of TNF-α (40 ng/ml) for 24 hours. Cell viability was determined by MTT test. Data were presented as mean ± SD of three independent experiments. (*: $p<0.05$, **: $p<0.01$, *t*-test, comparing with the group without TNF-α).

Figure 5. The sensitization effect of LA-12 on TNF-α-induced cell death determined by morphological changes. HeLa and $HepG_2$ cells were treated with **LA-12** (80 μM) and luteolin (80 μM) in the presence or absence of TNF-α (40 ng/ml) for 24 hours. Representative images were taken by a phase-contrast microscope (×200). Dead cells were identified by their morphology (rounded and detached).

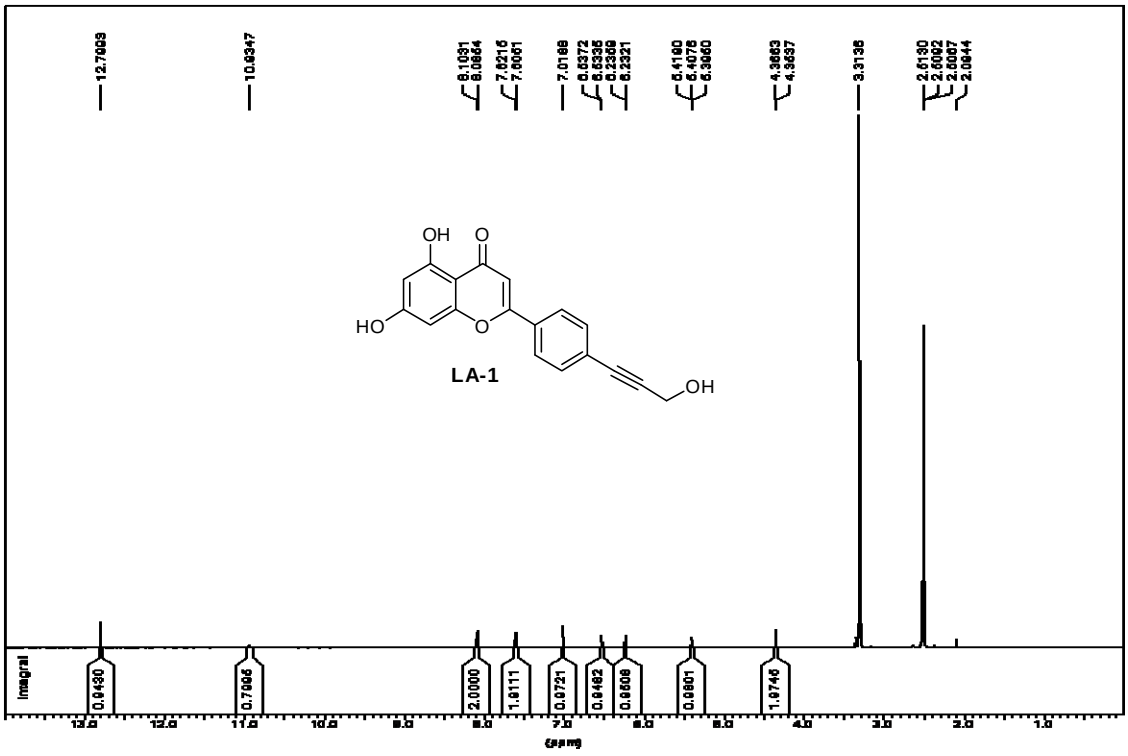
Figure 6. The cytotoxicity of luteolin analogues in HeLa cells: HeLa cells were treated with luteolin or its analogues as indicated for 24 hours. Cell viability was measured by MTT. Results represent mean ± SD of three independent experiments.

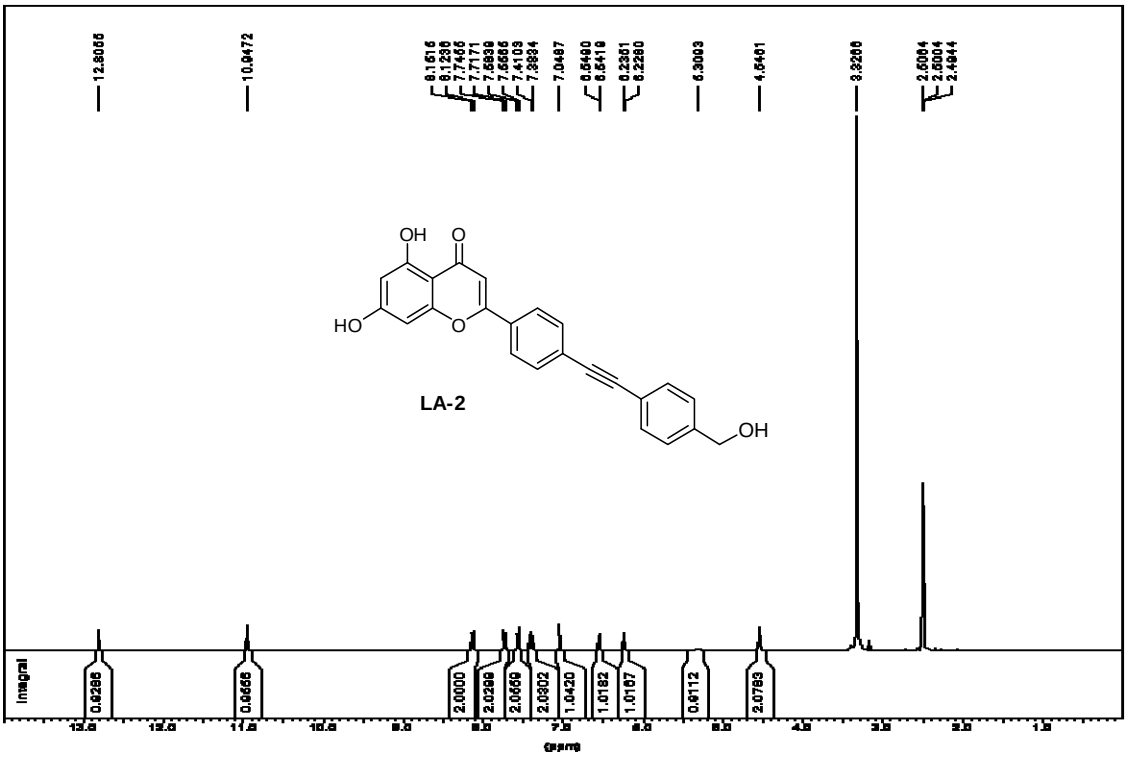


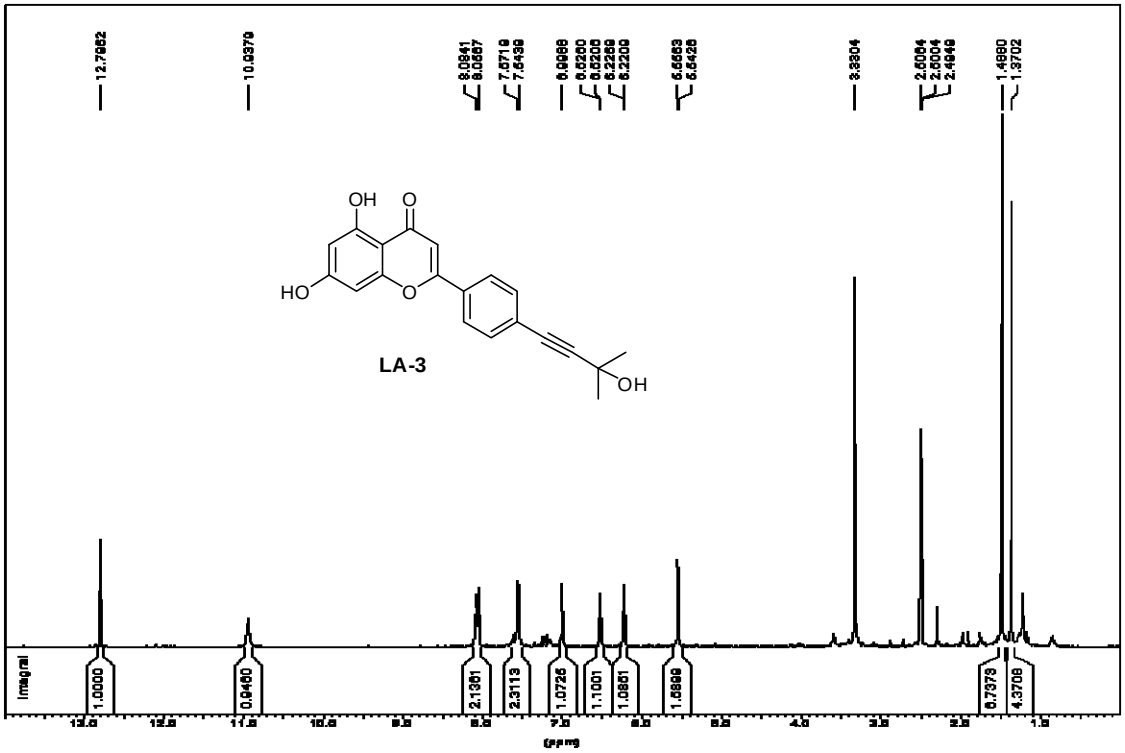
References:

1. Y. F. Li, Y. Luo, W. G. Huang, J. L. Wang, W. Lu, *Tetrahedron Lett.*, 2006, **47**, 4153
2. C. F. Yang, H.-M. Shen, C. N. Ong, *Biochem. Pharmacol.*, 1999, **57**, 273.

^{1}H NMR Spectra of Products



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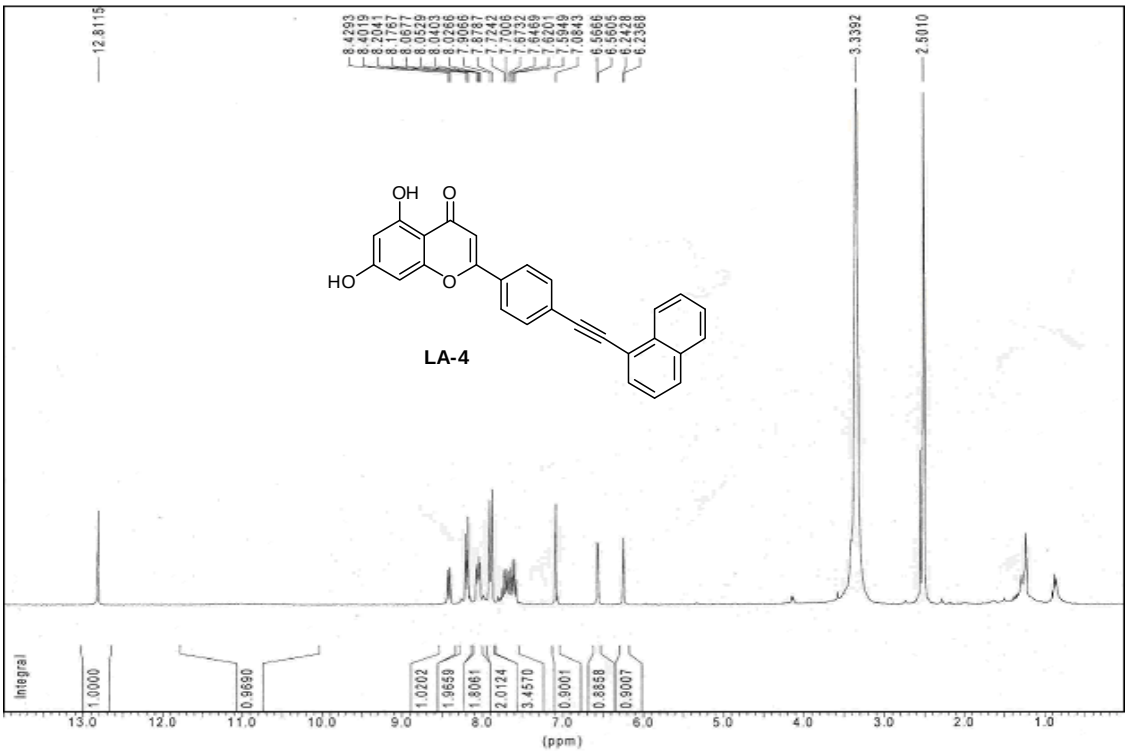
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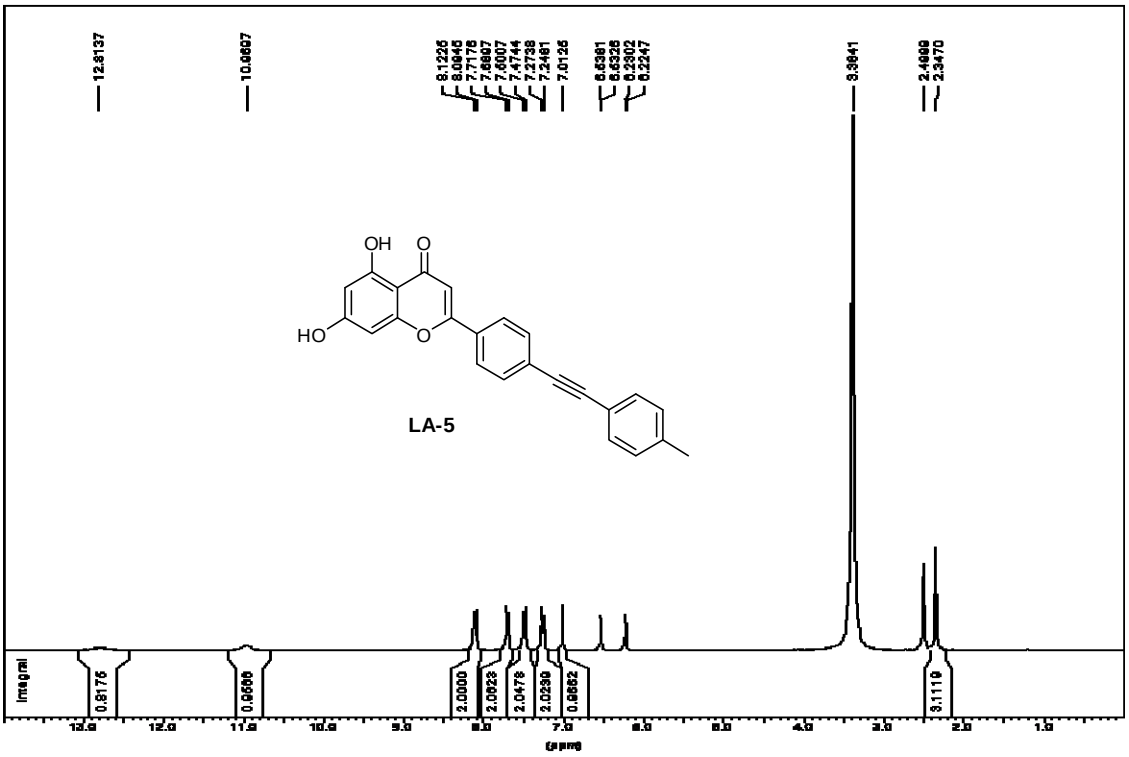
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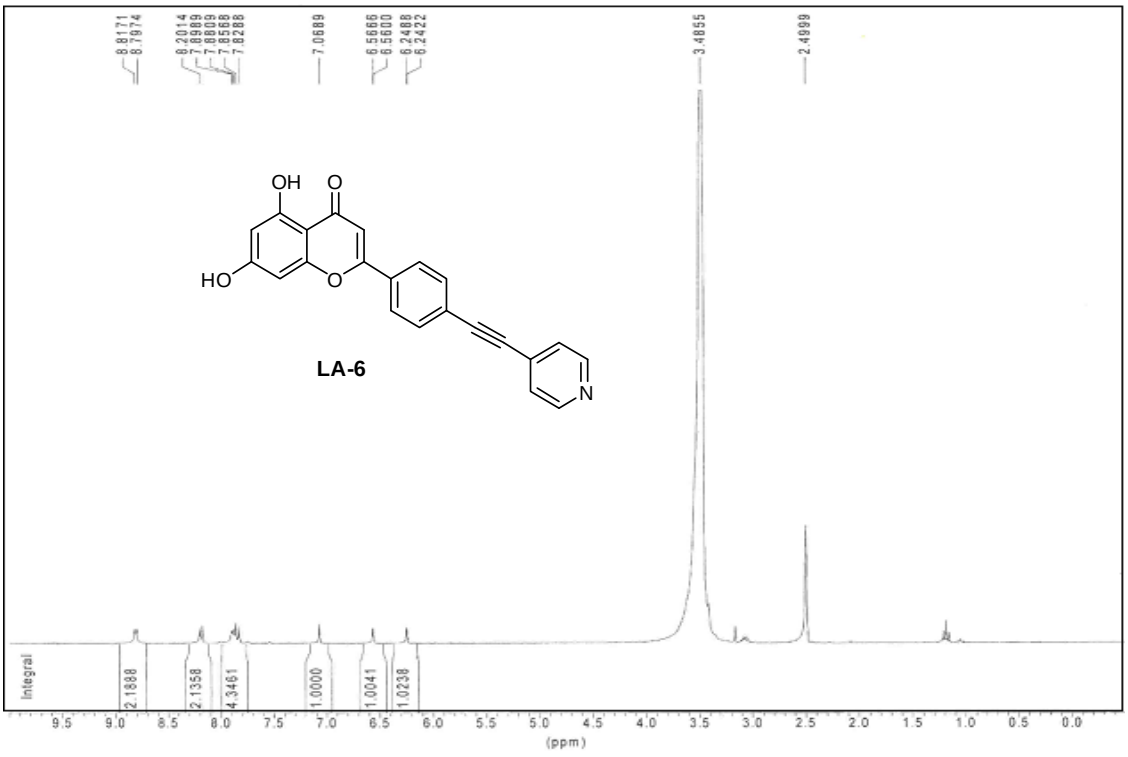
LA-5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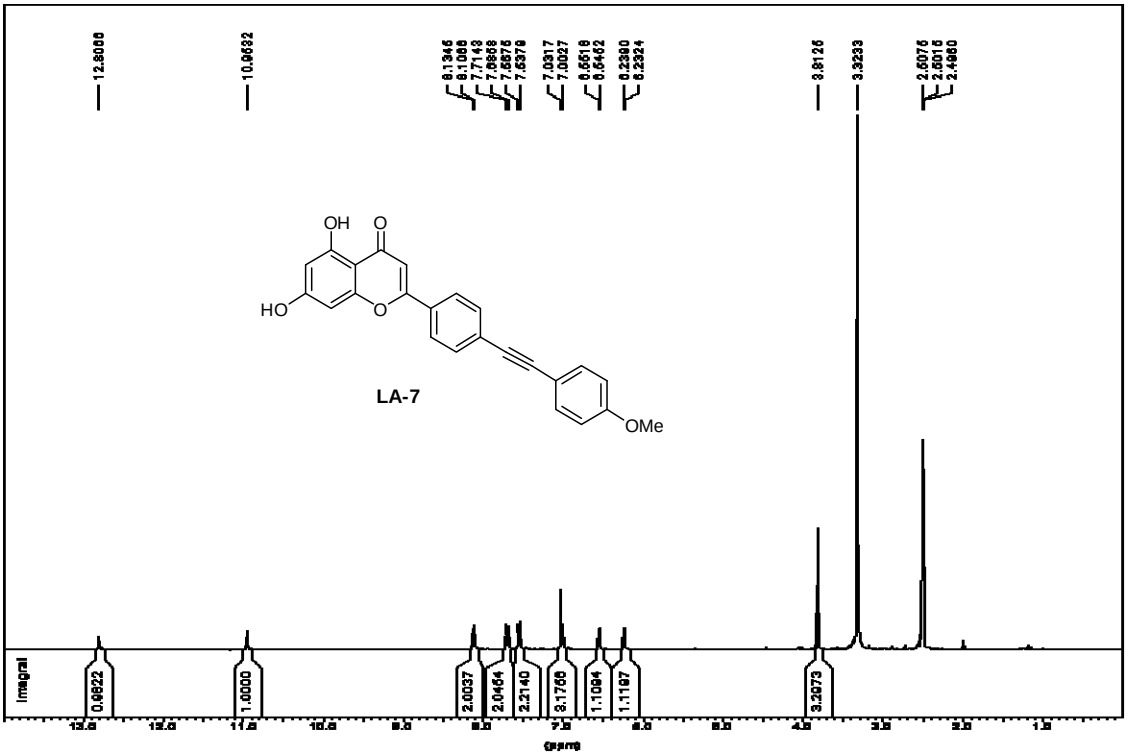
LA-6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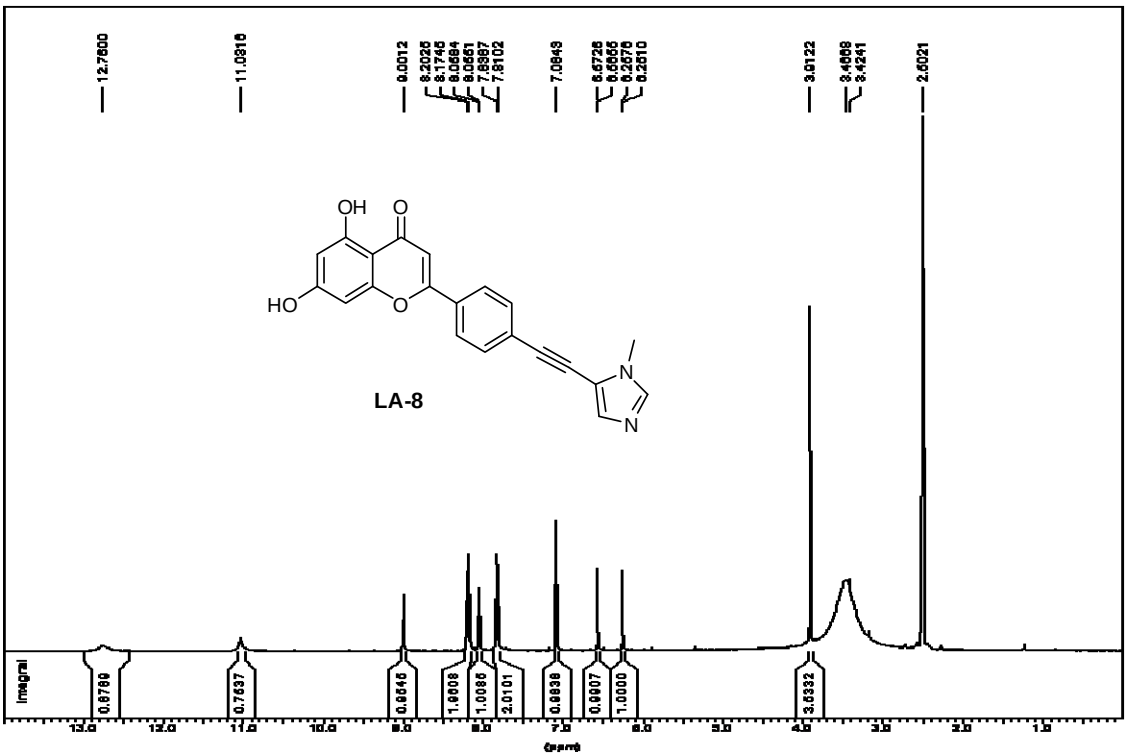
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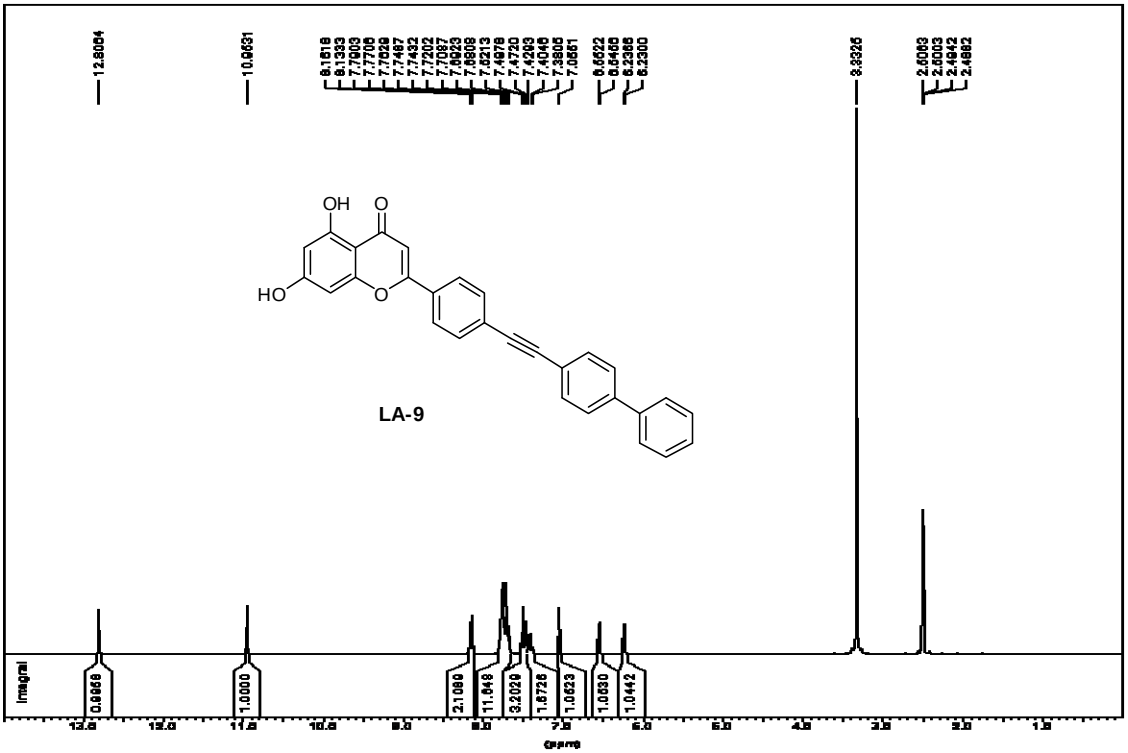
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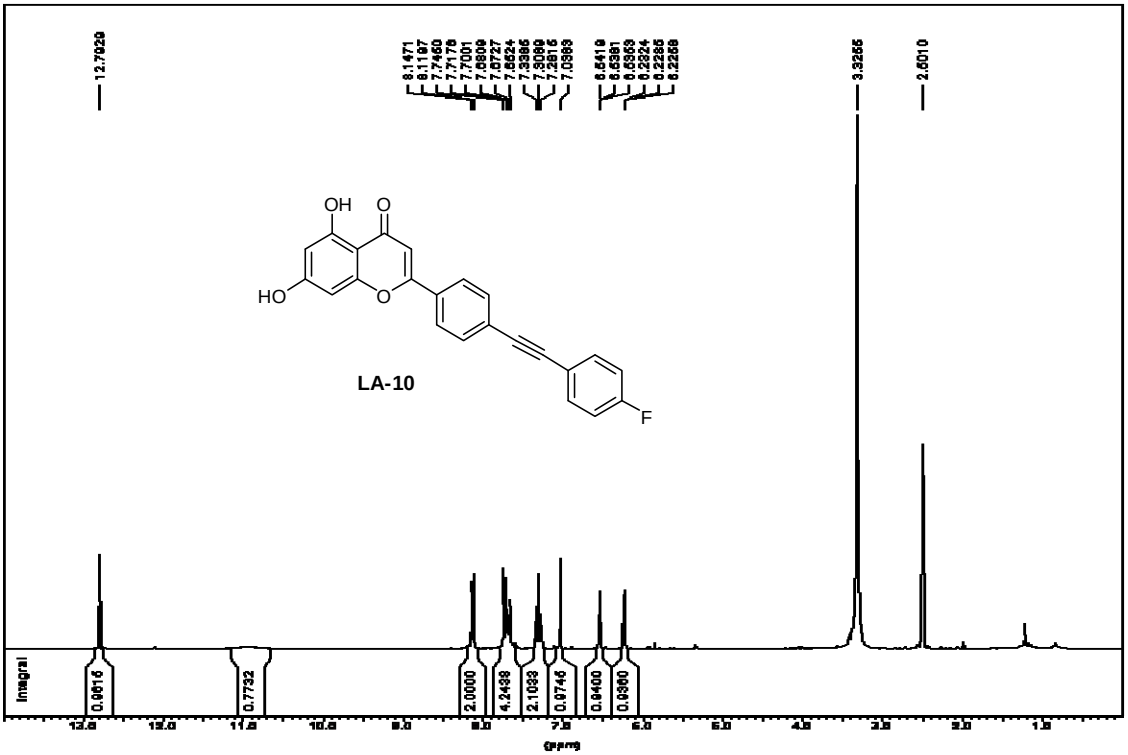
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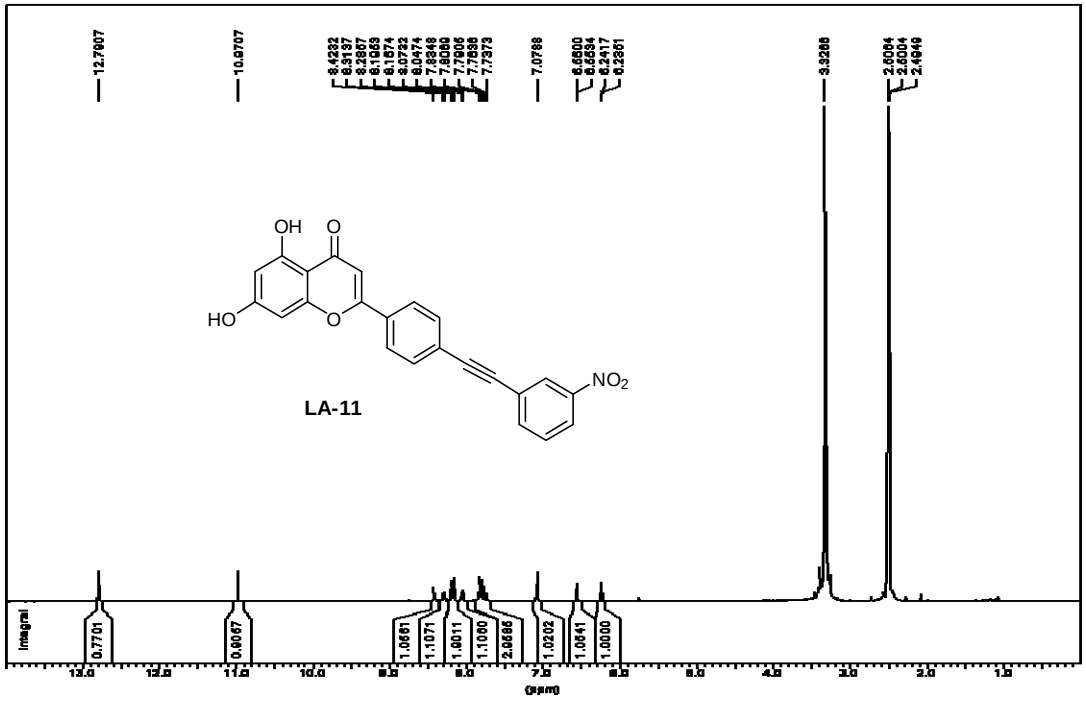
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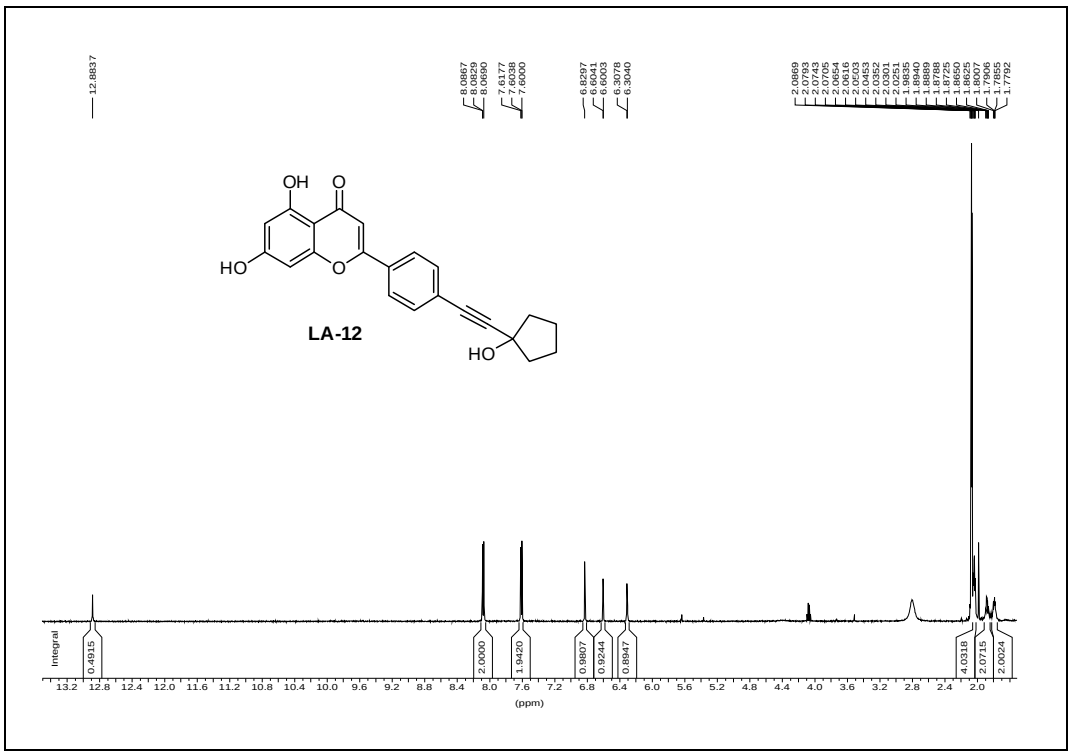
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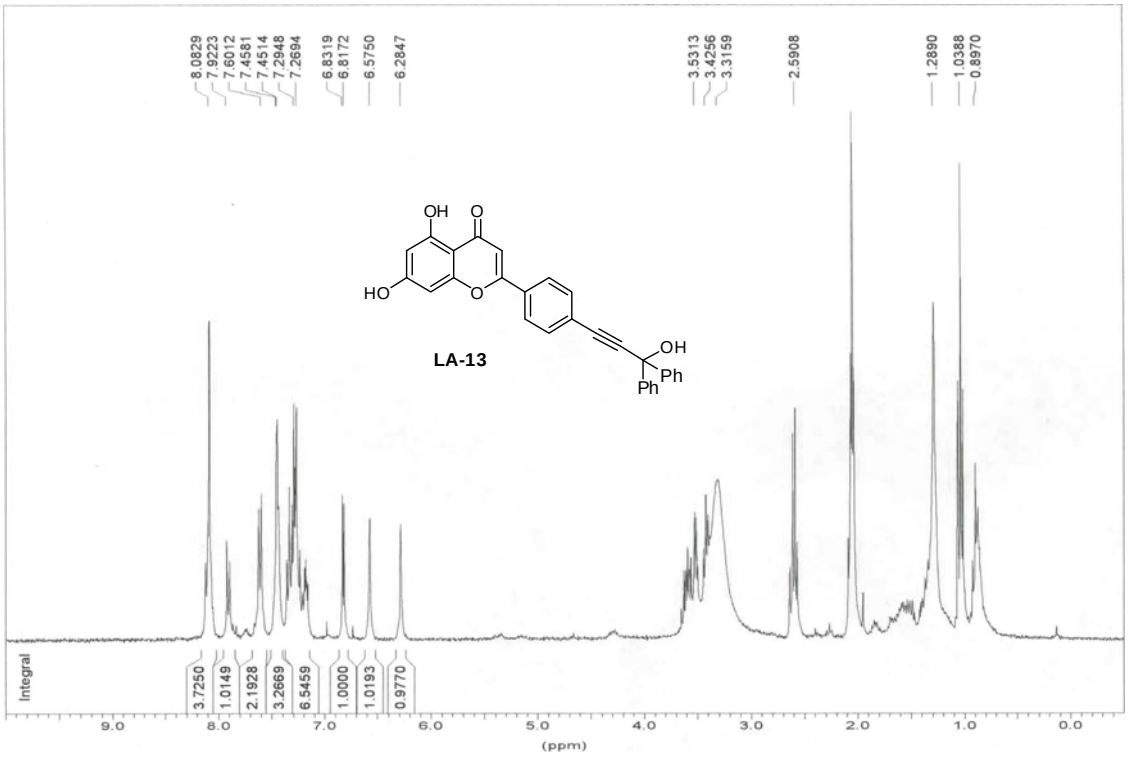
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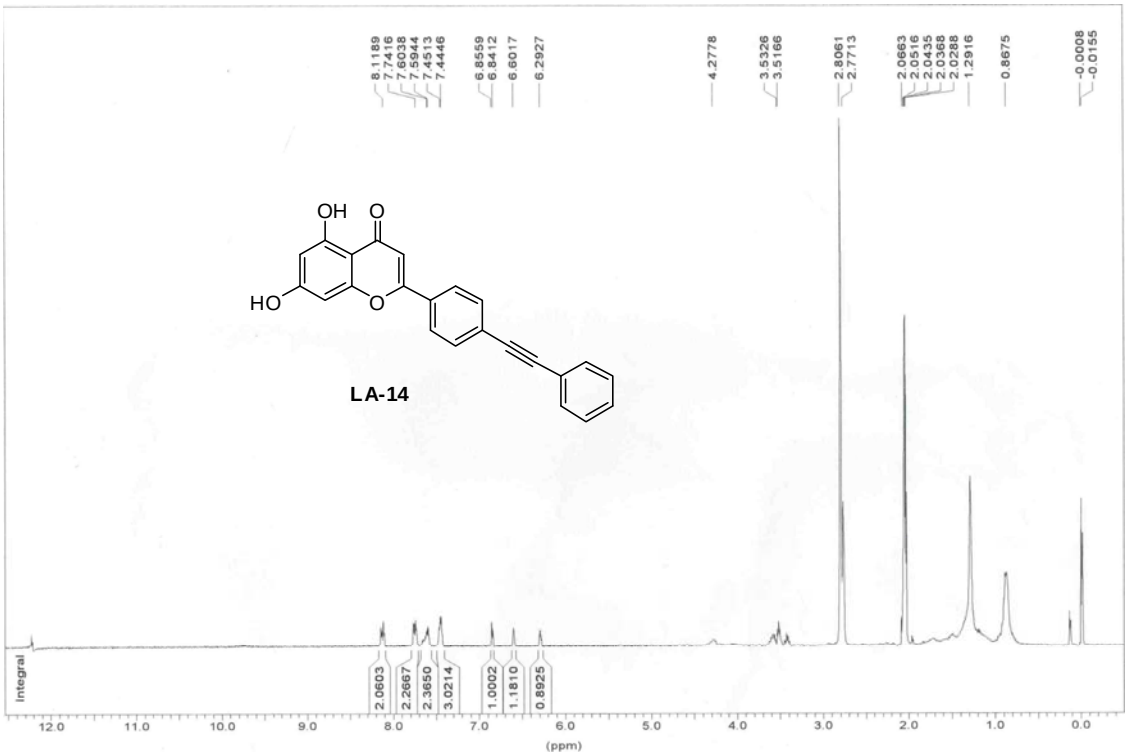
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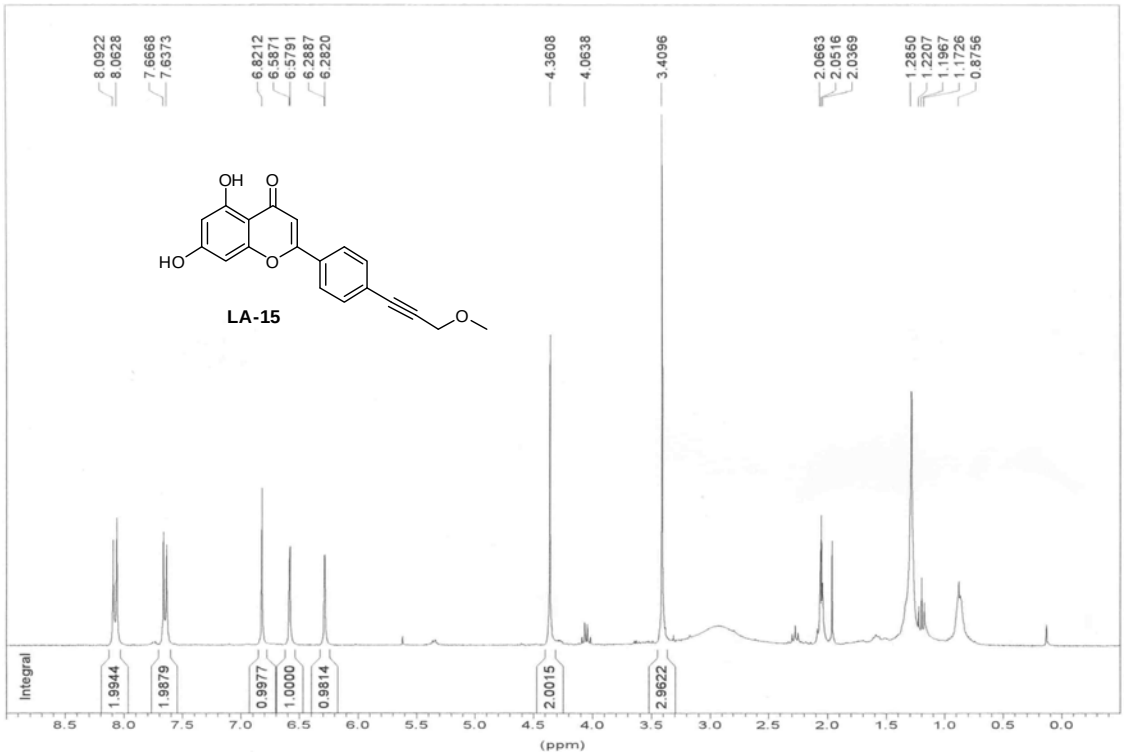
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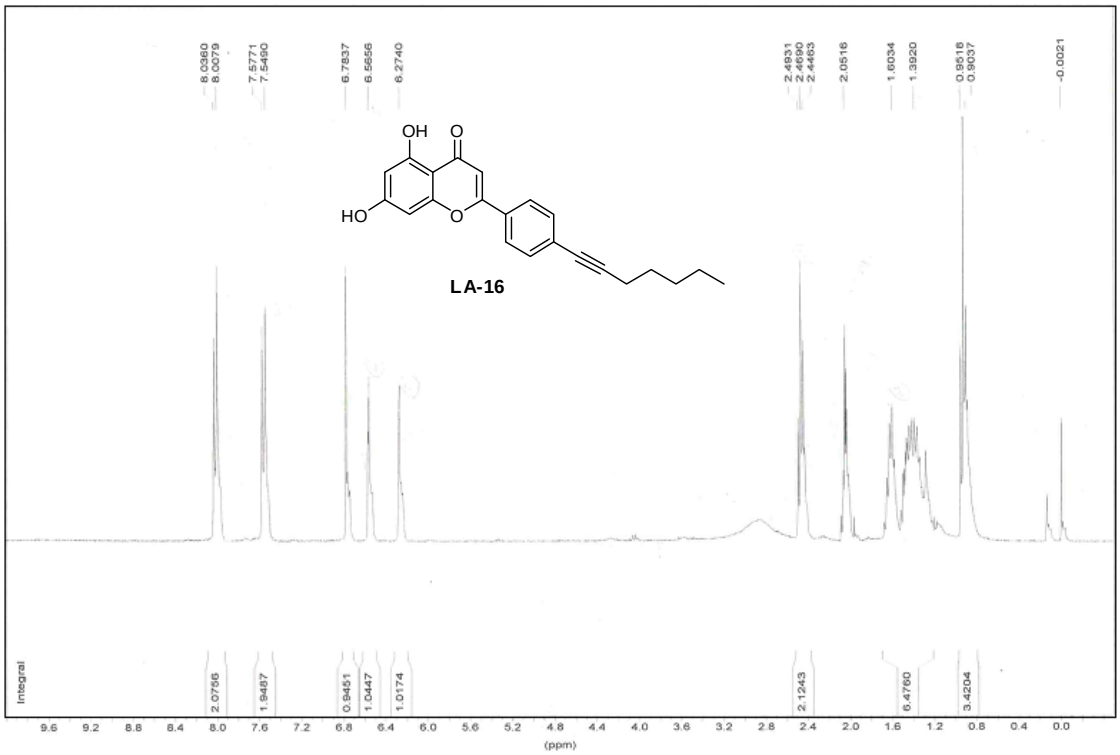
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0.9518
0.9037
-0.0021
OH
O
HO
O
LA-16
Integral
2.0756
1.9487
0.9451
1.0447
1.0174
2.1243
6.4760
3.4204
(ppm)