Supporting information

Combined acylselenourea-diselenide structures: new potent and selective antitumoral agents as autophagy activators.

Pablo Garnica [1,2], Ignacio Encío [3], Daniel Plano [1,2], Juan A. Palop [1,2], Carmen Sanmartín [1,2*]

[1] University of Navarra, Faculty of Pharmacy and Nutrition, Department of Organic and Pharmaceutical Chemistry, Irunlarrea 1, E-31008 Pamplona, Spain. [2] Instituto de Investigación Sanitaria de Navarra (IdiSNA), Irunlarrea 3, E-31008 Pamplona, Spain. [3] Department of Health Sciences, Public University of Navarra, Avda. Barañain s/n, E-31008 Pamplona, Spain

1. Chemistry

1.1. Material and methods

Proton (^{1}H), carbon (^{13}C) and selenium (^{77}Se) NMR spectra were recorded on a Bruker 400 Ultrashield™ spectrometer (Rheinstetten, Germany) using DMSO-d_6 or $CDCl_3$ as solvent. IR spectra were recorded on a Thermo Nicolet FT-IR Nexus spectrophotometer using KBr pellets for solid samples. Elemental analysis was performed on a LECO CHN-900 Elemental Analyser. Purity of all final compounds was 95% or higher. Chemicals were purchased from E. Merck (Darmstadt, Germany), Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma-Aldrich Química, S.A. (Alcobendas, Madrid, Spain) and Acros Organics (Janssen Pharmaceuticalaan, Geel, Belgium).

1.2. General procedure for the synthesis of compounds **1**–**16**

Potassium selenocyanate (2.5 mmol) was solubilized in 30 mL of dry acetone under N_2 flux, and the corresponding acyl chloride (2.5 mmol) was added dropwise through the septum cover with a syringe. The mixture was stirred at room temperature for 1 h. Bis(4-aminophenyl)diselenide (1.25 mmol) is then added solubilized in 5 mL of dry acetone and stirred for a variable time of 1 h up to 12 h at room temperature. Then reaction was quenched with cold water, and stirred for 3 h. This resulted in the solubilisation of interfering KCl and the subsequent precipitation of a solid. This resulting powder was then filtered and purified by washing or extraction.

In order to assign the chemical shifts in NMR spectroscopy the following assignment has been done: central rings A and A', external rings B and B' (**Figure S1**).

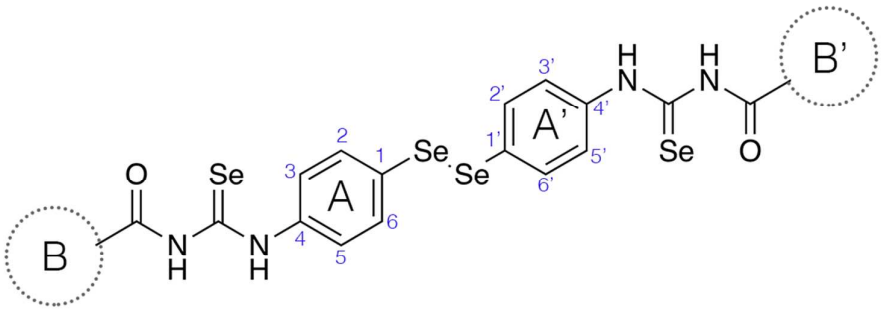


Figure S1. General structure and nomenclature used to locate the position of the substituted groups in the compounds presented herein.

1.3. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] dibenzamide (**1**)

From benzoyl chloride. Conditions: 2 h at room temperature. The product was washed with ethyl ether twice (2 × 25 mL). A yellow powder was obtained. Yield: 70%. Mp: 134–135ºC. IR (KBr) cm^{-1}: 1669 (C=O), 822 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6): δ: 12.95 (bs, 2H, NH), 11.86 (bs, 2H, NH), 7.97 (d, 4H, B+B', $J_{2\text{-}3}$=$J_{6\text{-}5}$= 7.9 Hz, H_2+H_6), 7.73–7.64 (m, 10H, B+B', H_4, A+A', H_2+H_3+H_5+H_6), 7.55 (t, 4H, B + B', $J_{3\text{-}2}$=$J_{3\text{-}4}$=$J_{5\text{-}6}$=$J_{5\text{-}4}$= 7.9 Hz, H_3+H_5). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.95 (C=Se), 167.82 (C=O), 151.52 (B+B', C_4), 138.88 (A+A', C_4), 133.33 (A+A', C_2+C_6), 131.18 (A+A', C_1), 129.73 (B+B', C_1), 129.27 (B+B', C_3+C_5), 128.91 (B+B', C_2+C_6), 127.91 (A+A', C_3+C_5). ^{77}Se NMR ($CDCl_3$, 76 MHz) δ: 472.31 (C=Se), 421.01 (Se-Se). MS [*m/z* (% abundance)]: 77 (63), 105 (100), 121 (7). Elemental analysis calculated (%) for $C_{28}H_{22}N_4O_2Se_4$ · ½ H_2O: C: 43.58, H: 3.01, N: 7.26; found: C: 43.43, H: 2.71, N: 6.97.

1.4. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-4-methylbenzamide (**2**)

From 4-methylbenzoyl chloride. Conditions: 1 h at room temperature. The product was washed with ethyl ether twice (2 × 25 mL). A yellow powder was obtained. Yield: 93%. Mp: 155–156ºC. IR (KBr) cm^{-1}: 3411 and 3375 (N-H), 2971 (C-H, CH_3), 1665 (C=O), 821 (Se-Se). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.14 (bs, 2H, NH), 9.40 (bs, 2H, NH), 7.81 (d, 4H, B+B', $J_{2\text{-}3}$=$J_{6\text{-}5}$= 8.2 Hz, H_2+H_6), 7.74–7.66 (m, 8H, A+A', H_2+H_3+H_5+H_6), 7.36 (d, 4H, B+B', $J_{3\text{-}2}$=$J_{5\text{-}6}$= 8.2 Hz, H_3+H_5), 2.47 (s, 6H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.45 (C=Se), 167.81 (C=O), 143.77 (B+B', C_4), 138.80 (A+A', C_4), 132.92 (A+A', C_2+C_6), 131.32 (A+A', C_1), 128.99 (B+B', C_1), 128.87 (B+B', C_3+C_5), 128.00 (B+B', C_2+C_6), 126.00 (A+A', C_3+C_5), 21.16 (CH_3). ^{77}Se NMR ($CDCl_3$, 76 MHz) δ: 472.30 (C=Se), 414.08 (Se-Se). MS [*m/z* (% abundance)]: 91 (55), 119 (100), 121 (7). Elemental analysis calculated (%) for $C_{30}H_{26}N_4O_2Se_4$ · HCl: C: 43.58, H: 3.29, N: 6.78; found: C: 43.30, H: 3.21, N: 6.72.

1.5. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-4-nitrobenzamide (**3**)

From 4-nitrobenzoyl chloride. Conditions: 2 h at room temperature. The product was washed with ethyl ether twice (2 × 25 mL). A yellow powder was obtained. Yield: 75%. Mp: 163–164ºC. IR (KBr) cm^{-1}: 1657 (C=O), 1343 and 1259 (NO_2 st), 819 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.49 (s, 1H, NH), 12.24 (s, 1H, NH), 11.38 (s, 1H, NH), 10.69 (s, 1H, 1H), 8.33 (d, 4H, B +B', $J_{2\text{-}3}$=$J_{6\text{-}5}$= 8.8 Hz, H_2+H_6), 8.18 (d, 4H, B+B', $J_{3\text{-}2}$=$J_{5\text{-}6}$= 8.8 Hz, H_3+H_5), 7.80–7.49 (m, 8H, A+A', H_2+H_3+H_5+H_6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.71 (C=Se), 166.70 (C=O), 150.69 (B+B', C_4), 137.21 (A+A', C_4), 132.23 (A+A', C_2+C_6), 131.58 (A+A', C_1), 131.19 (B+B', C_1), 130.71 (B+B', C_2+C_6), 124.60 (A+A', C_3+C_5) 124.21 (B+B', C_3+C_5). MS [*m/z* (% abundance)]: 76 (33), 104 (37), 122 (7) 150 (100). Elemental analysis calculated (%) for $C_{28}H_{20}N_6O_6Se_4$ · ½ HCl: C: 38.60, H: 2.37, N: 9.65; found: C: 38.45, H: 2.17, N: 9.95.

1.6. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-4-methylthiobenzamide (**4**)

From 4-methylthiobenzoyl chloride. Conditions: 3 h at room temperature. The product was washed with ethyl ether twice (2 × 25 mL). A yellow powder was obtained. Yield: 76%. Mp: 140–141ºC. IR (KBr) cm^{-1}: 1664 (C=O), 817 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.99 (bs, 2H, NH), 11.79 (bs, 2H, NH), 7.93 (d, 4H, B+B', $J_{2\text{-}3}$= $J_{4\text{-}5}$= 8.2 Hz, H_2+H_6), 7.76–7.59 (m, 8H, A+A', H_2+H_3+H_5+H_6), 7.38 (d, 4H, $J_{3\text{-}2}$= $J_{5\text{-}6}$= 8.2 Hz, H_3+H_5), 2.55 (s, 6H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.48 (C=Se), 167.43 (C=O), 145.85 (B+B', C_4), 138.85 (A+A', C_4), 133.02 (A+A', C_2+C_6), 131.36 (A+A', C_1), 129.33 (B+B', C_1), 126.08 (A+A', C_3+C_5), 124.87 (B+B', C_3+C_5), 124.61 (B+B', C_2+C_6), 13.95 (SCH_3). ^{77}Se NMR ($CDCl_3$, 76 MHz) δ: 472.23 (C=Se), 415.24 (Se-Se). MS [*m/z* (% abundance)]: 123 (11), 151 (100). Elemental analysis calculated (%) for $C_{30}H_{26}N_4O_2S_2Se_4 \cdot H_2O \cdot HCl$: C: 39.64, H: 3.22, N: 6.16; found: C: 39.35, H: 3.01, N: 6.09.

1.7. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-4-chlorobenzamide (**5**)

From 4-chlorobenzoyl chloride. Conditions: 2 h at room temperature. The product was kept under stirring for 24 h with 100 mL of ethyl ether then filtered to obtain final product. A yellow powder was obtained. Yield: 69%. Mp: 157ºC. IR (KBr) cm^{-1}: 1651 (C=O), 1090 (C-Cl), 815 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.85 (bs, 2H, NH), 11.97 (bs, 2H, NH), 7.98 (d, 4H, B+B', $J_{2\text{-}3}$= $J_{6\text{-}5}$= 8.6 Hz, H_2+H_6), 7.74–7.54 (m, 12H, A+A', H_2+H_3+H_5+H_6, B+B', H_3+H_5). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 182.80 (C=Se), 167.31 (C=O), 141.37 (B+B', C_4,), 138.65 (A+A', C_4), 132.00 (A+A', C_2+C_6), 131.66 (A+A', C_1), 130.25 (B+B', C_1), 129.60 (B+B', C_2+C_6), 129.15 (B+B', C_3+C_5), 126.98 (A+A', C_3+C_5). MS [*m/z* (% abundance)]: 111 (44), 139 (100). Elemental analysis calculated (%) for $C_{28}H_{20}Cl_2N_4O_2Se_4 \cdot HCl$: C: 38.63, H: 2.41, N: 6.43; found: C: 38.35, H: 2.06, N: 6.15.

1.8. *N,N'*- [(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-4-methoxybenzamide (**6**)

From 4-methoxybenzoyl chloride. Conditions: 12 h at room temperature. The product was kept under stirring for 24 h with 100 mL of ethyl ether then filtered to obtain final product. A yellow powder was obtained. Yield: 46%. Mp: 134–135ºC IR (KBr) cm^{-1}:1655 (C=O), 1254 (C-O), 819 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.89 (bs, 2H, NH), 11.63 (bs, 2H, NH), 7.96 (d, 4H, B+B', $J_{2\text{-}3}$= $J_{4\text{-}5}$= 8.5 Hz, H_2+H_6), 7.80–7.54 (m, 8H, A+A', H_2+H_3+H_5+H_6), 7.07 (d, 4H, $J_{3\text{-}2}$= $J_{5\text{-}6}$= 8.5 Hz, H_3+H_5), 3.84 (s, 6H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.73 (C=Se), 165.44 (C=O), 162.47 (B+B', C_4,), 140.16 (A+A', C_4), 133.40 (A+A', C_2+C_6), 131.80 (A+A', C_1), 130.12 (B+B', C_1), 124.35 (A+A', C_3+C_5), 121.47 (B+B', C_3+C_5), 114.09 (B+B', C_2+C_6), 55.91 (OCH_3). MS [*m/z* (% abundance)]: 107 (64), 135 (100). Elemental analysis calculated (%) for $C_{30}H_{26}N_4O_4Se_4 \cdot HCl$: C: 41.95, H: 3.17, N: 6.52; found: C: 42.23, H: 3.22, N: 6.15.

1.9. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-3,5-dimethoxybenzamide (**7**)

From 3,5-dimethoxybenzoyl chloride. Conditions: 3 h at room temperature. The product was kept under stirring for 24 h with 100 mL of ethyl ether then filtered to obtain final product. A yellow powder was obtained. Yield: 65%. Mp: 150–151ºC. IR (KBr) cm^{-1}: 1670 (C=O), 1244 (C-O), 830 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.94 (bs, 2H, NH), 11.84 (bs, 2H, NH), 7.60–7.74 (m, 8H, A+A', H_2+H_3+H_5+H_6), 7.14 (s, 4H, B+B', H_2+H_6), 6.77 (s, 2H, B+B', H_4), 3.84 (s, 12H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.35 (C=Se), 167.32 (C=O), 160.90 (B+B', C_3+C_5), 138.70 (A+A', C_4), 136.23 (B+B', C_1), 133.60 (A+A', C_2+C_6), 131.22 (A+A', C_1), 125.94 (A+A', C_3+C_5), 106.30 (B+B', C_2+C_6), 105.37 (B+B', C_4), 55.49 (OCH_3). ^{77}Se NMR (DMSO-d_6, 76 MHz) δ: 456.75 (C=Se), 429.05 (Se-Se). MS [*m/z* (% abundance)]: 137 (34), 165 (100). Elemental analysis calculated (%) for $C_{32}H_{30}N_4O_6Se_4$: C: 43.55, H: 3.43, N: 6.35; found: C: 43.18, H: 3.52, N: 5.97.

1.10. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-3,4,5-trimethoxybenzamide (**8**)

From 3,4,5-trimethoxybenzoyl chloride. Conditions: 2 h at room temperature. The product was washed with ethyl ether twice (2 × 25 mL). A yellow powder was obtained. Yield: 25%. Mp: 105–106ºC. IR (KBr) cm^{-1}: 1664 (C=O), 827 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.06 (bs, 2H, NH), 11.84 (bs, 2H, NH), 7.68 (bs, 8H, A+A', H_2+H_3+H_5+H_6), 7.38 (s, 4H, B+B', H_2+H_6), 3.90–3.65 (m, 18H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.23 (C=Se), 167.88 (C=O), 153.00 (B+B', C_3+C_5), 148.59 (B+B', C_4), 139.30 (A+A', C_4), 133.63 (A+A', C_2+C_6), 131.88 (A+A', C_1),

126.53 (A+A', C_3+C_5), 121.96 (B+B', C_1), 106.95 (B+B', C_2+C_6), 60.63 (OCH_3) 56.50 (OCH_3). MS [*m/z* (% abundance)]: 195 (100). Elemental analysis calculated (%) for $C_{34}H_{34}N_4O_8Se_4 \cdot HCl$: C: 41.71, H: 3.60, N: 5.72; found: C: 42.05, H: 3.91, N: 5.77.

1.11. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-2-furanamide (**9**)

From 2-furoyl chloride. Conditions: 12 h at room temperature. The product was kept under stirring with alkaline water (pH= 8–9) for 24 h, then filtered and washed with ethyl ether (2 × 25 mL). A yellow powder was obtained. Yield: 46%. Mp: 120–121ºC. IR (KBr) cm^{-1}: 3387 (N-H), 1674 (C=O), 823 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.72 (bs, 2H, NH), 11.54 (bs, 2H, NH), 8.09 (bs, 2H, B+B', H_3), 7.87 (d, 2H, B+B', $J_{5\text{-}4}$= 1.7 Hz, H_5), 7.68 (d, 4H, A+A', $J_{2\text{-}3}$= $J_{6\text{-}5}$ = 7.5 Hz, H_2+H_6), 7.63 (d, 4H, A+A', $J_{3\text{-}2}$= $J_{5\text{-}6}$ = 7.5 Hz, H_3+H_5), 6.76 (t, 2H, B+B', $J_{4\text{-}5}$= 1.7 Hz, H_4). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 179.95 (C=Se), 157.16 (C=O), 148.64 (B+B', C_1), 144.44 (B+B', C_4), 138.82 (A+A', C_4), 132.96 (A+A', C_2+C_6), 131.36 (A+A', C_1), 126.10 (A+A', C_3+C_5), 119.12 (B+B', C_2), 112.80 (B+B', C_3). MS [*m/z* (% abundance)]: 43 (100), 95 (84), 111 (22). Elemental analysis calculated (%) for $C_{24}H_{18}N_4O_4Se_4 \cdot HCl$: C: 37.02, H: 2.46, N: 7.19; found: C: 36.84, H: 2.73, N: 7.36.

1.12. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] diisoxazole-5-amide (**10**)

From isoxazole-5-carbonyl chloride. Conditions: 12 h at room temperature. Product was extracted with dichloromethane and solvent was removed under vacuum by rotatory evaporation. In the next step the product is washed with ethyl ether (2 × 25 mL). A yellow poder was obtained. Yield: 16.5%. Mp: 138–139ºC. IR (KBr) cm^{-1}: 3163 (N-H st), 1685 (C=O), 819 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.45 (bs, 1H, NH), 12.12 (bs, 1H, NH), 11.40 (s, 0.5H, NH), 10.86 (s, 0.5H, NH), 10.32 (s, 0.5H), 8.87 (s, 2H, B+B', H_4+H_5), 7.77–7.53 (m, 10H, A+A', H_2+H_3+H_5+H_6, B+B', H_5). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.14 (C=Se), 168.29 (C=O), 156.90 (B+B', C_1), 152.38 (B+B', C_4), 138.28 (A+A', C_4), 133.35 (A+A', C_2+C_6), 131.07 (A+A', C_1), 126.81 (A+A', C_3+C_5), 108.96 (B+B', C_5). MS [*m/z* (% abundance)]: 43 (97). Elemental analysis calculated (%) for $C_{22}H_{16}N_6O_4Se_4$: C: 35.50, H: 2.17, N: 11.29; found: C: 35.60, H: 2.55, N: 10.92.

1.13. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-2-thiophenamide (**11**)

From 2-thiophencarbonyl chloride. Conditions: 2 h at room temperature. The product was washed with ethyl ether twice (2 × 25 mL). An orange powder was obtained. Yield: 100%. Mp: 100–101ºC. IR (KBr) cm^{-1}: 1653 (C=O), 827 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.81 (bs, 2H, NH), 11.88 (bs, 2H, NH), 8.42 (d, 2H, B+B', $J_{5\text{-}4}$= 3.5, H_5), 8.08 (d, 2H, B+B', $J_{3\text{-}4}$= 4.7, H_3), 7.77–7.61 (m, 8H, A+A', H_2+H_3+H_5+H_6), 7.27 (t, 2H, B+B', $J_{4\text{-}3}$= 4.7, $J_{4\text{-}5}$= 3.5 Hz, H_4). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.37 (C=Se), 163.38 (C=O), 140.79 (A+A', C_4), 135. 10 (B+B', C_1), 133.67 (A+A', C_2+C_6), 131.42 (A+A', C_1), 129.11 (B+B', C_3), 128.69 (B+B', C_5), 128.34 (B+B', C_4), 126.57 (A+A', C_3+C_5). MS [*m/z* (% abundance)]: 43 (100), 111 (85), 83 (34). Elemental analysis calculated (%) for $C_{24}H_{18}N_4O_2S_2Se_4 \cdot HCl$: C: 35.55, H: 2.36, N: 6.91; found: C: 35.38, H: 2.60, N: 6.60.

1.14. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-3-chlorothiophen-2-amide (**12**)

Chlorination of 3-chlorothiophene-2-carboxylic acid with thionyl chloride was needed to obtain the corresponding acyl chloride. Conditions: 12 h at room temperature. The product was washed with ethyl ether twice (2 × 25 mL). An orange powder was obtained. Yield: 83%. Mp: 133–134ºC. IR (KBr) cm^{-1}: 1643 (C=O), 1017 (C-Cl), 824 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.80 (bs, 2H, NH), 11.87 (bs, 2H, NH), 8.48–7.46 (m, 10H, A+A', H_2+H_3+H_5+H_6, B+B', H_3), 7.47–7.11 (m, 2H, B+B', H_4). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.67 (C=Se), 163.33 (C=O), 140.79 (A+A', C_4), 133.73 (A+A', C_2+C_6), 135.11 (B+B', C_1), 131.42 (A+A', C_1), 129.11 (B+B', C_3), 128.69 (B+B', C_5), 128.34 (B+B', C_4), 121.15 (A+A', C_3+C_5). MS [*m/z* (% abundance)]: 111 (100). Elemental analysis calculated (%) for $C_{24}H_{16}Cl_2N_4O_2S_2Se_4 \cdot H_2O$: C: 33.47, H: 2.11, N: 6.51; found: C: 33.56, H: 2.34, N: 6.49.

1.15. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-benzo[b]thiophene-2-amide (**13**)

Chlorination of 1-benzothiophene-2-carboxylic acid with thionyl chloride was needed to obtain the corresponding acyl chloride. Conditions: 12 h at room temperature. The product was washed with ethyl ether twice (2 × 25 mL). A yellow powder was obtained. Yield: 95%. Mp: 150–152ºC. IR (KBr) cm^{-1}: 1654 (C=O), 824 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.73 (bs, 2H, NH), 12.13 (bs, 2H, NH), 8.80 (s, 2H, H_9), 8.10 (d, 2H, B+B', $J_{4\text{-}5}$= 8.2 Hz, H_4), 8.04 (d, 2H, B+B', $J_{7\text{-}6}$=7.81, H_7), 7.74–7.63 (m, 8H, A+A', H_2+H_3+H_5+H_6), 7.60–7.48 (m, 4H, B+B', H_5+H_6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.57 (C=Se), 167.23 (C=O), 146.95 (B+B', C_1), 141.79 (B+B', C_3), 139.34 (B+B', C_8), 138.69 (A+A', C_4), 132.98 (A+A', C_2+C_6), 131.32 (A+A', C_1), 130.64 (B+B', C_9), 128.86 (B+B', C_5), 127.36 (B+B', C_7), 126.44 (B+B', C_6), 125.95 (A+A', C_3+C_5), 124.11 (B+B', C_4). MS [*m/z* (% abundance)]: 161 (100), 211 (80). Elemental analysis calculated (%) for $C_{32}H_{22}N_4O_2S_2Se_4 \cdot$ ½ HCl: C: 43.01, H: 2.52, N: 6.27; found: C: 42.96, H: 2.86, N: 6.07.

1.16. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-1,3-benzodioxole-5-amide (**14**)

Chlorination of 1,3-benzodioxole-5-carboxylic acid with thionyl chloride was needed to obtain the corresponding acyl chloride. Conditions: 2 h at room temperature. The product was washed with ethyl ether twice (2 × 25 mL). A yellow powder was obtained. Yield: 80%. Mp: 142–144ºC. IR (KBr) cm^{-1}: 1662 (C=O), 821 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.97 (bs, 2H, NH), 11.63 (bs, 2H, NH), 7.72–7.60 (m, 10H, A+A', H_2+H_3+H_5+H_6, B+B', H_9), 7.54 (s, 2H, B+B', H_2), 7.07 (d, 2H, B+B', $J_{8\text{-}7}$ = 8.2 Hz, H_8), 6.17 (s, 4H, B+B', H_5). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.87 (C=Se), 167.11 (C=O), 152.57 (B+B', C_7), 152.11 (B+B', C_3), 147.93 (B+B', C_1), 139.27 (A+A', C_4), 133.45 (A+A', C_2+C_6), 131.86 (A+A', C_1), 126.48 (A+A', C_3+C_5), 125.52 (B+B', C_9), 122.95 (B+B', C_8), 108.96 (B+B', C_2), 102.71 (B+B', C_5). MS [*m/z* (% abundance)]: 43 (100), 76 (85), 147 (65). Elemental analysis calculated (%) for $C_{30}H_{22}N_4O_6Se_4$: C: 42.37, H: 2.61, N: 6.59; found: C: 42.42, H: 2.83, N: 6.48.

1.17. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-3-quinolineamide (**15**)

Chlorination of 3-quinolinecarboxylic acid with thionyl chloride was needed to obtain the corresponding acyl chloride. Conditions: 5 h at room temperature. The product was washed with ethyl ether twice (2 × 25 mL). A yellow powder was obtained. Yield: 82%. Mp: 155–156ºC. IR (KBr) cm^{-1}: 1669 (C=O), 821 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.92 (bs, 2H, NH), 12.33 (bs, 2H, NH), 9.26 (s, 2H, B+B', H_{10}), 9.05 (s, 2H, B+B', H_2), 8.24–8.05 (m, 4H, B+B', H_5+H_8), 7.94 (bs, 2H, B+B', H_7), 7.70 (bs, 10H, A+A', H_2+H_3+H_5+H_6, B+B', H_6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.05 (C=Se), 166.74 (C=O), 150.26 (B+B', C_4), 149.34 (B+B', C_2), 139.06 (B+B', C_1), 138.69 (A+A', C_4), 132.44 (A+A', C_2+C_6), 130.04 (A+A', C_1), 129.23 (B+B', C_9), 128.18 (B+B', C_6), 127.97 (B+B', C_8), 126.54 (B+B', C_7), 126.43 (A+A', C_3+C_5), 125.84 (B+B', C_{10}), 121.23 (B+B', C_5). MS [*m/z* (% abundance)]: 43 (100), 77 (55), 128 (70). Elemental analysis calculated (%) for $C_{34}H_{24}N_6O_2Se_4 \cdot$ HCl: C: 45.33, H: 2.80, N: 9.33; found: C: 45.65, H: 3.13, N: 9.11.

1.18. *N,N'*-[(diselanediylbis(benzene-4,1-diyl)selenecarbamoyl)] di-2-phenyl-4-quinolineamide (**16**)

Chlorination of 2-phenyl-4-quinolinecarboxylic acid with thionyl chloride was needed to obtain the corresponding acyl chloride. Conditions: 8 h at room temperature. The product was washed with ethyl ether twice (2 × 25 mL). A yellow powder was obtained. Yield: 42%. Mp: 138–139ºC. IR (KBr) cm^{-1}: 1679 (C=O), 818 (Se-Se). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.87 (bs, 2H, NH), 12.61 (bs, 2H, NH), 8.40 (s, 2H, B+B', H_2), 8.38–3.29 (bs, 4H, B+B', H_7+H_8), 8.29–8.12 (bs, 4H, B+B', H_6+H_9), 7.92–7.83 (m, 2H, C+C', H_4), 7.83–7.67 (m, 8H, A+A', H_2+H_3+H_5+H_6), 7.67–7.50 (m, 8H, C+C', H_2+H_3+H_5+H_6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.67 (C=Se), 167.43 (C=O), 155.97 (B+B', C_5), 148.28 (B+B', C_3), 138.51 (A+A', C_4), 135.38 (C+C', C_1), 134.61 (B+B', C_1), 133.43 (A+A', C_2+C_6), 130.85 (A+A', C_1), 130.52 (B+B', C_{10}), 130.52 (B+B', C_7), 129.40 (C+C', C_3+C_5), 127.81 (C+C', C_2+C_6), 127.52 (C+C', C_4), 125.24 (A+A', C_3+C_5), 123.14 (B+B', C_8), 121.26 (B+B', C_2), 118.49 (B+B', C_6), 114.98 (B+B', C_9). MS [*m/z* (% abundance)]: 204 (55), 232 (27),

170 (60) Elemental analysis calculated (%) for $C_{46}H_{32}N_6O_2Se_4 \cdot HCl$: C: 52.46, H: 3.16, N: 7.98; found: C: 52.28, H: 3.43, N: 7.62.

2. Representative spectra (^1H and ^{13}C) of final products.

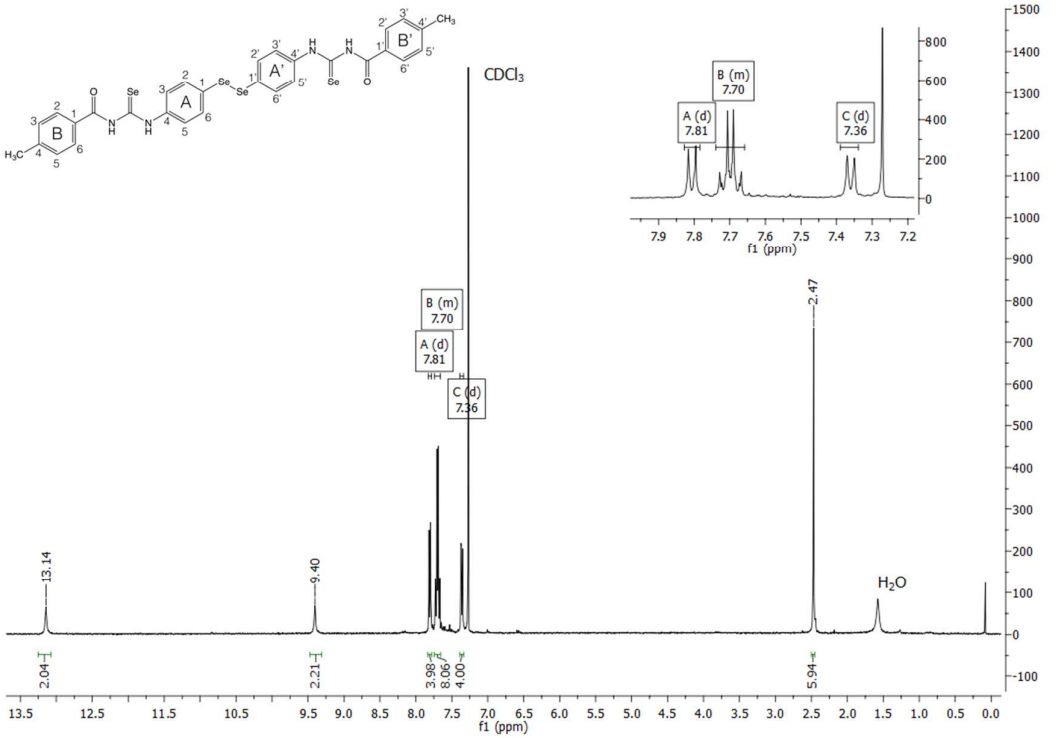


Figure S2. 1H NMR of compound **2**

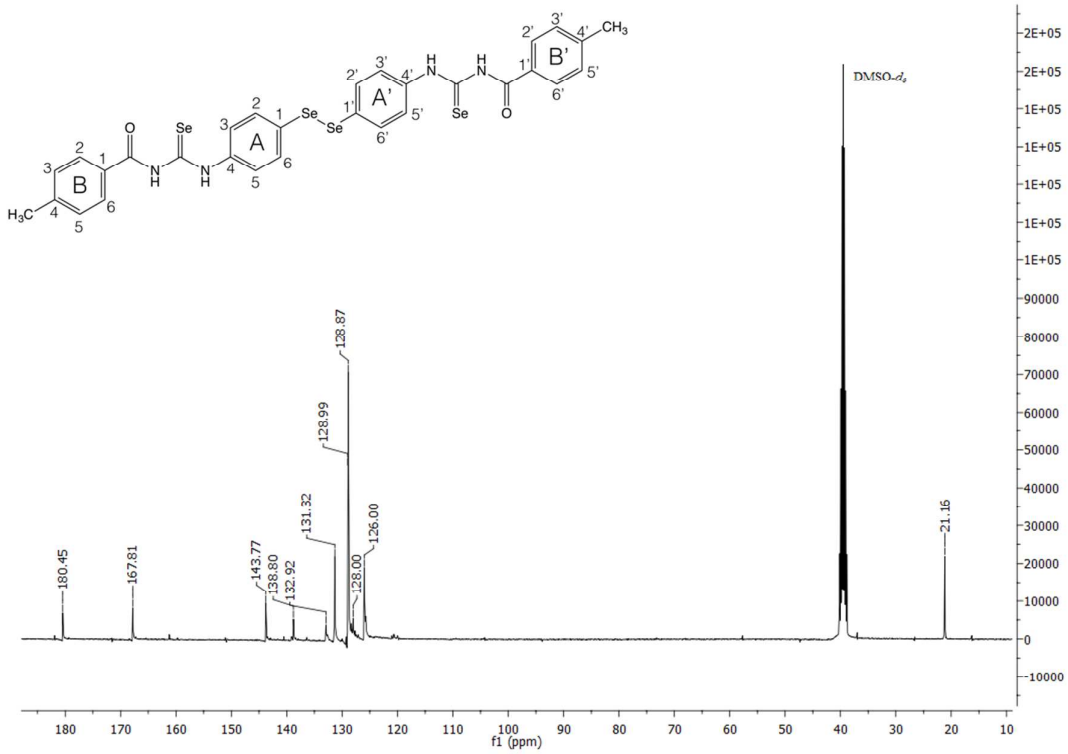


Figure S3. ^{13}C NMR of compound **2**

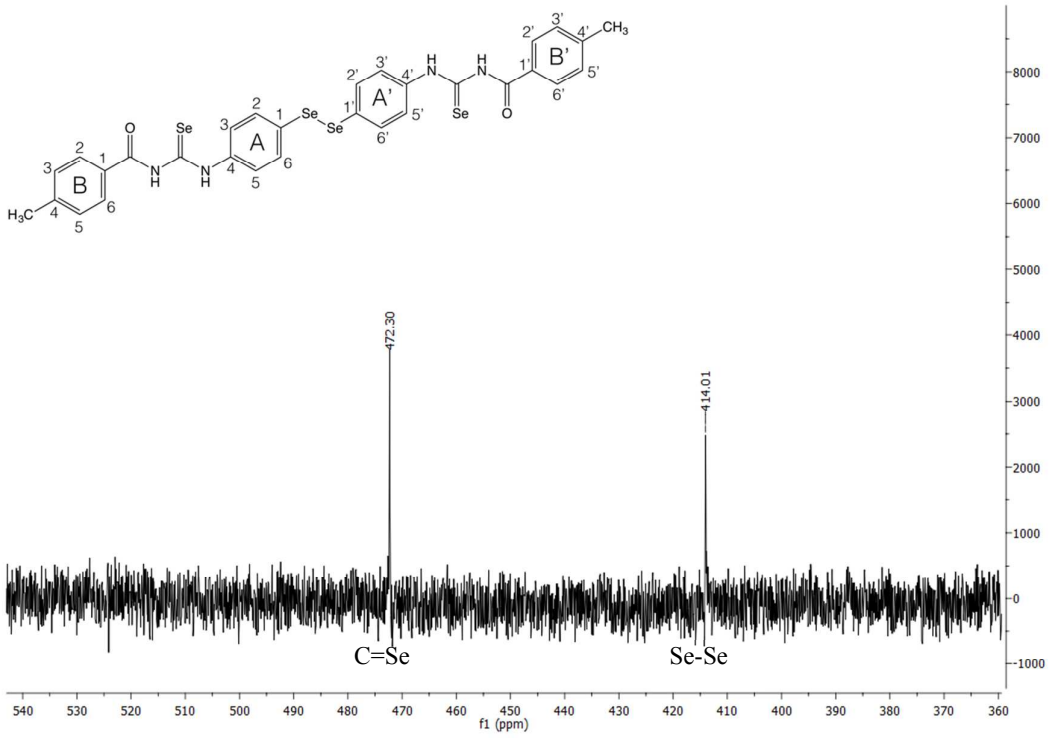


Figure S4.^{77}Se NMR of compound **2**

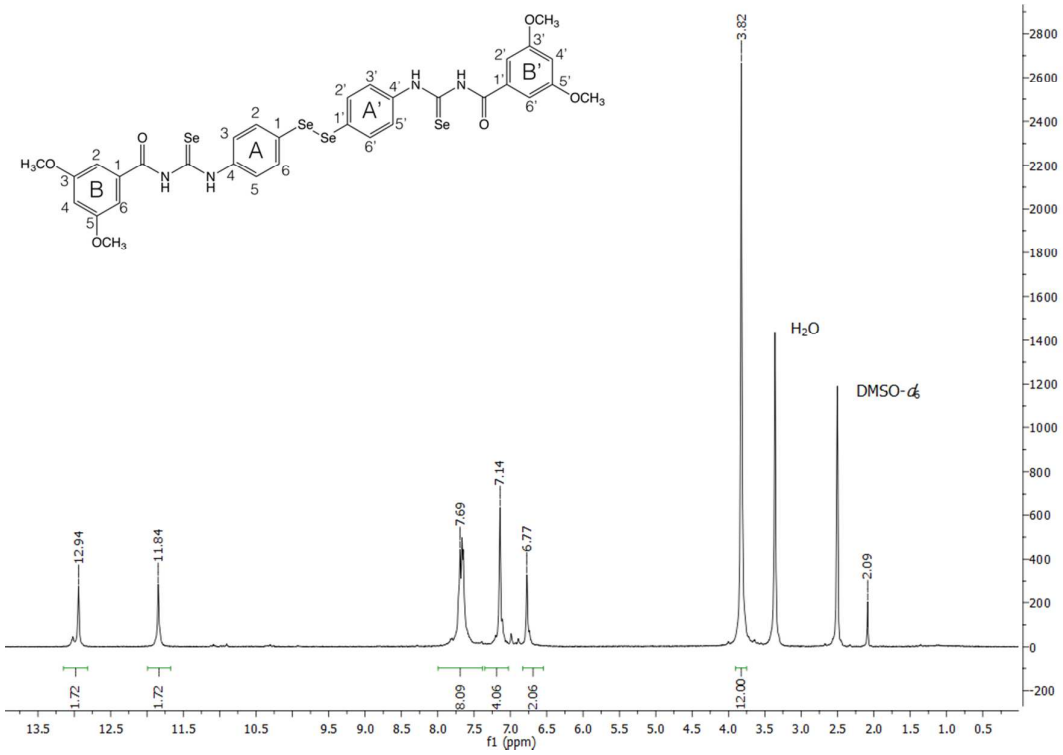


Figure S5. ^{1}H NMR of compound **7**

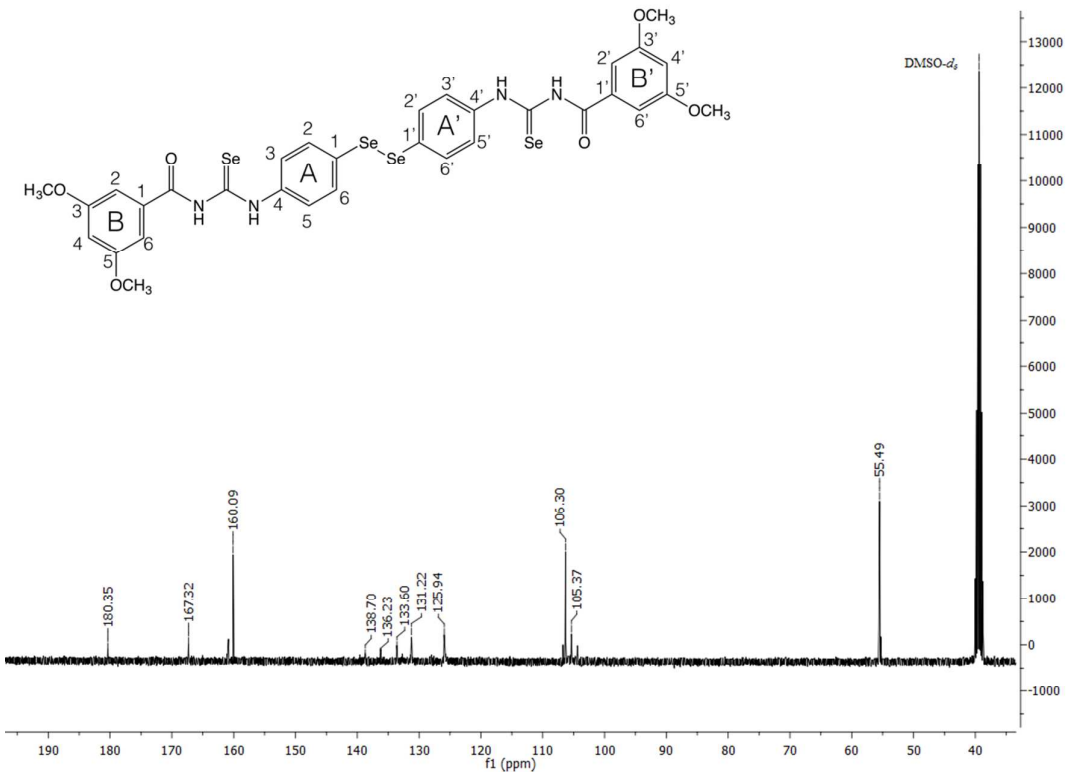


Figure S6. ^{13}C NMR of compound **7**

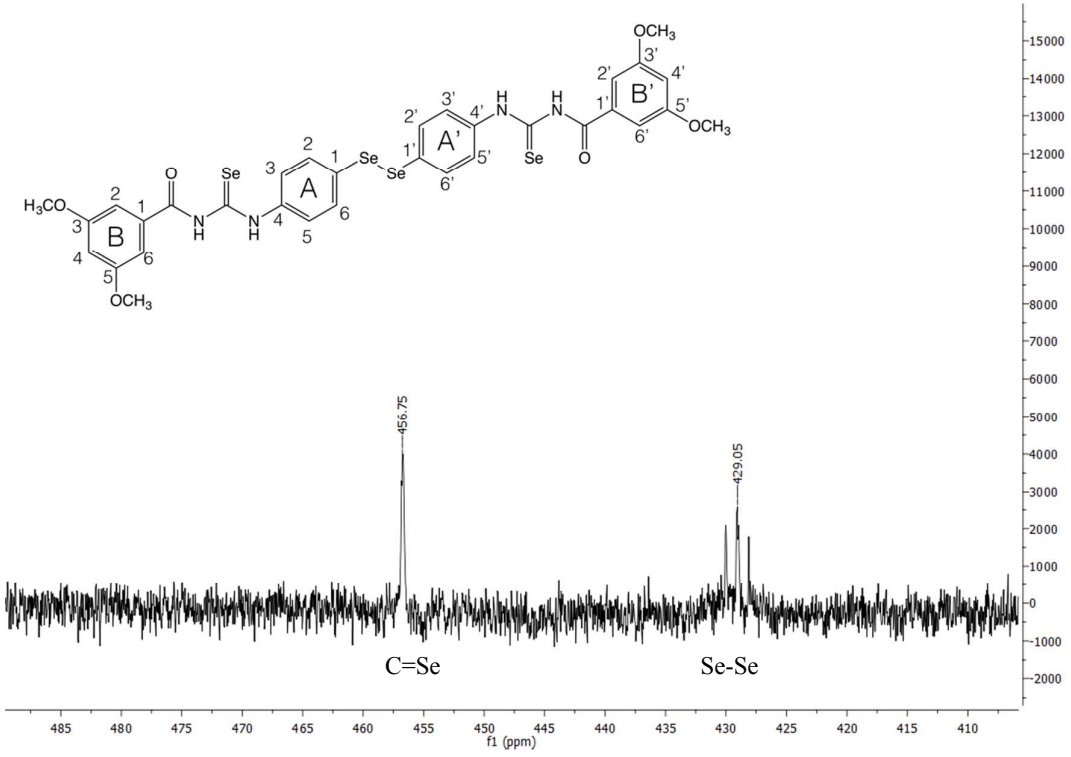


Figure S7. ^{77}Se NMR of compound **7**

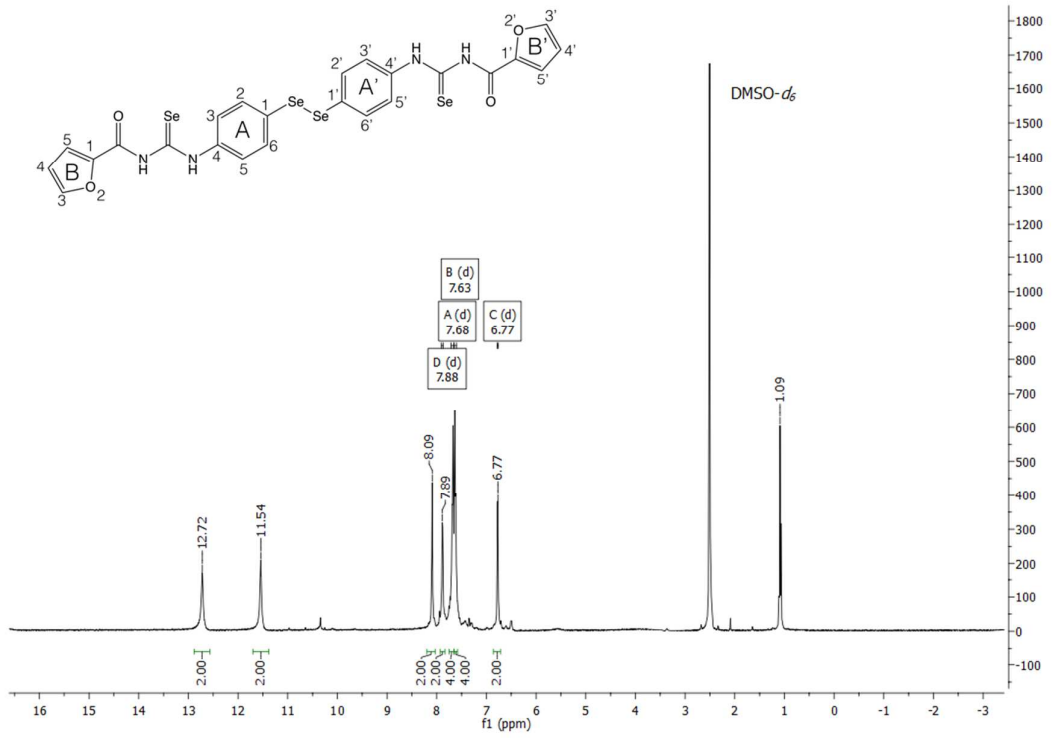


Figure S8. ^{1}H NMR of compound **9**

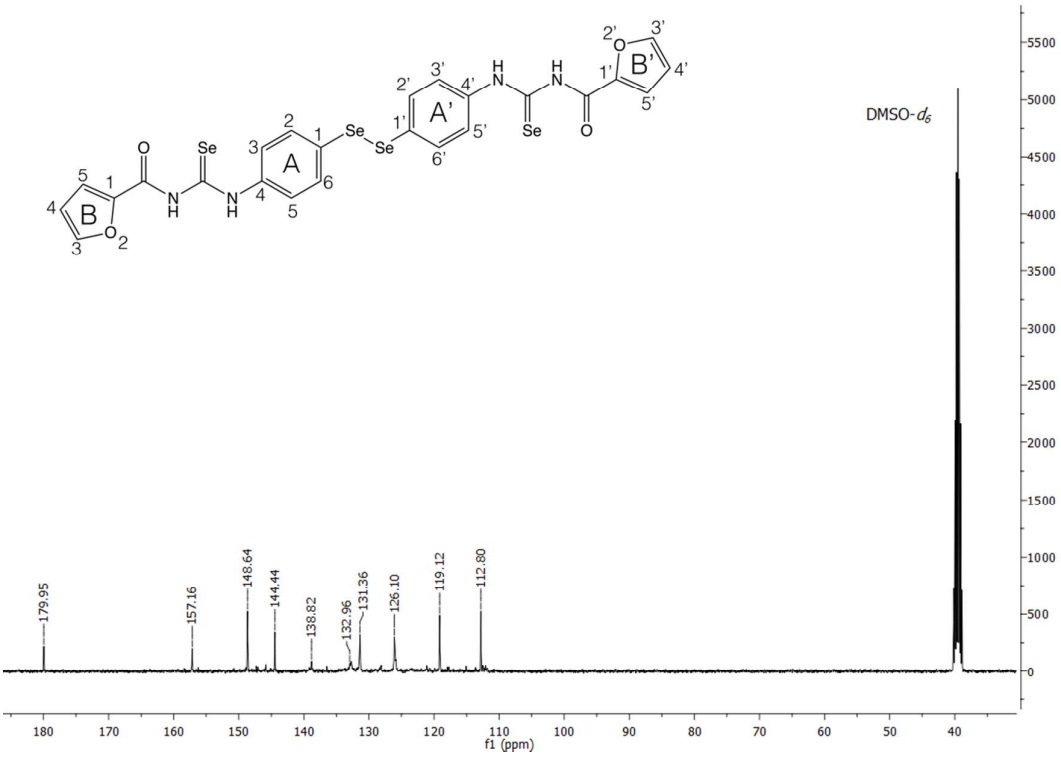


Figure S9. ^{13}C NMR of compound **9**

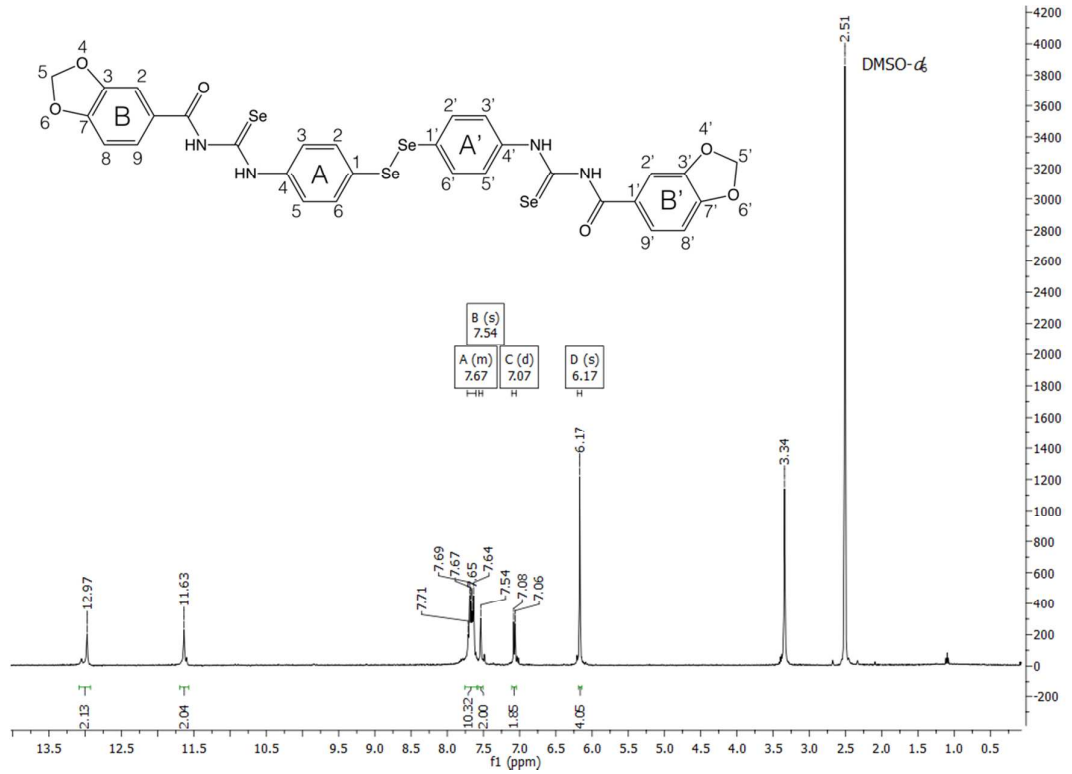


Figure S10. ^{1}H NMR of compound **14**

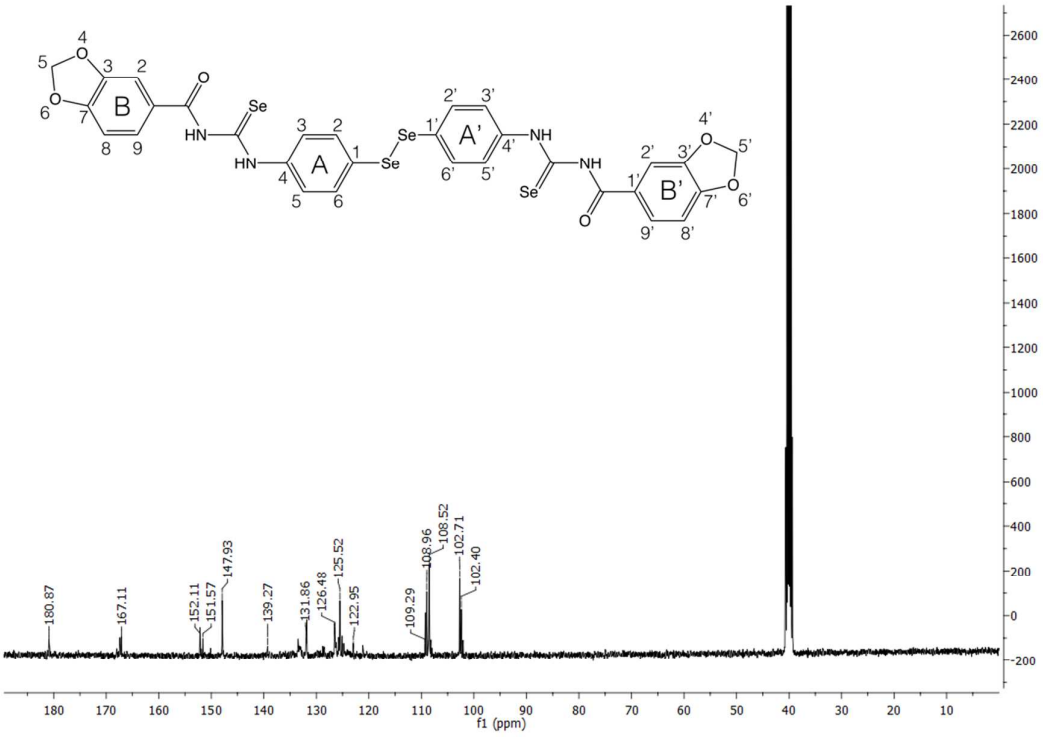


Figure S11. ^{13}C NMR of compound **14**

3. Biological evaluation

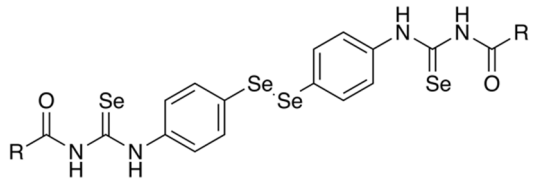
3.1. Cell cultures

Cell lines were purchased from the American Type Culture Collection (ATCC). MCF-7, CCRF-CEM, HT-29, HTB-54, K-562 and PC-3 cell lines were grown in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 units/mL penicillin and 100 mg/mL streptomycin (Gibco). BEAS-2B cell line (normal epithelial lung) was cultured in DMEM (Gibco), 10% FBS, 100 units/mL penicillin and 100 µg/mL streptomycin. 184B5 cells were grown in DMEM/F12 medium supplemented with 5% FBS, 1 ITS (Lonza), 100 nM hydrocortisone (Aldich), 2 mM sodium pyruvate (Lonza), 20 ng/mL EGF (Sigma- Aldrich), 0.3 nM *trans*-retinoic acid (Sigma-Aldrich), 100 units/mL penicillin and 100 mg/mL streptomycin. Cells were maintained at 37ºC and 5% CO_2.

3.2. Cytotoxic and antiproliferative activities

Cell viability was determined using the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) method at five different doses ranging from 0.01 to 100 µM, for some compounds lower doses where needed in order to reach 50% cell growth. Depending on cell size, 8,000 to 40,000 cells were seeded per well in 96-well plates, and incubated with the compounds for 72 h. Cells were then incubated with 50 µL of MTT (2 mg/mL stock) for 4 h, medium was removed by aspiration and formazan crystals dissolved in 150 µL of DMSO. The absorbance was measured at 550 nm in a microplate reader (Sunrise reader, Tecan). At least three independent experiments performed in quadruplicate were analysed. Results are summarized in **Table S**1 and **S2** expressed as GI_{50}, the concentration that reduces by 50% the growth of treated cells with respect to untreated controls, TGI, the concentration that completely inhibits cell growth, and LC_{50}, the concentration that kills 50% of the cells.

Table S1. Average values of GI_{50}, TGI and LD_{50} (μM).



Code	R/compound	MCF-7			PC-3			HTB-54			HT-29			K-562			CCRF-CEM		
		GI_{50} [a]	TGI[b]	LC_{50}[c]	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	phenyl	9.76×10^{-3}	19.29	65.23	3.96×10^{-2}	2.78	44.43	24.78	51.11	77.44	9.12	37.03	66.90	25.47	58.48	91.48	0.26	33.71	69.55
2	4-methylphenyl	1.30×10^{-3}	35.01	>100	5.87×10^{-2}	5.23	47.41	7.74	30.78	73.19	6.61	31.98	66.87	25.56	59.86	94.16	6.79	44.62	83.87
3	4-nitrophenyl	3.24×10^{-2}	28.99	72.28	0.27	15.77	58.14	28.61	55.58	82.54	9.02	37.70	68.92	32.25	>100	>100	0.96	33.34	71.61
4	4-methylthiophenyl	5.60×10^{-3}	15.50	56.59	4.46	18.73	59.11	30.84	>100	>100	5.27	30.95	63.65	>100	>100	>100	6.29×10^{-2}	31.86	72.03
5	4-chlorophenyl	4.95×10^{-3}	8.42	>100	4.11	32.23	72.47	13.16	46.98	80.80	8.95	47.15	89.38	25.38	75.97	>100	2.59×10^{-2}	36.61	82.90
7	3,5-dimethoxyphenyl	1.51×10^{-4}	10.42	24.92	7.85×10^{-2}	8.97	52.92	20.22	45.73	71.25	17.09	73.20	>100	>100	>100	>100	9.35×10^{-2}	28.46	72.17
8	3,4,5-trimethoxyphenyl	9.10	16.22	65.23	8.16	55.35	>100	16.03	45.05	74.07	21.97	51.91	81.86	9.24	61.93	>100	6.64	47.89	>100
9	furan-2-yl	7.93	40.80	81.81	5.51	71.22	>100	6.35	20.72	60.65	3.21	9.12	56.65	84.87	>100	>100	0.62	4.18	57.80
10	isoxazol-2-yl	3.81	6.58	9.34	4.33	8.02	46.31	5.86	25.42	62.67	6.66	34.06	66.83	9.02	42.93	81.10	2.33	5.75	9.17
11	thiophene-2-yl	15.85	30.41	75.93	73.92	>100	>100	60.75	>100	>100	6.58	33.63	67.48	10.20	49.86	89.53	6.40	33.54	77.32
12	3-chloro-thiophene-2-yl	13.60	26.93	80.03	9.74	37.84	66.33	48.23	>100	>100	5.90	33.08	63.89	15.13	53.92	92.71	26.71	59.10	91.49
13	benzo[*b*]thiophene-2-yl	27.85	59.05	90.26	17.64	>100	>100	22.58	50.45	78.31	3.78	30.13	66.14	25.13	68.71	>100	8.71	50.53	98.02
14	1,3-benzodioxole-5-yl	25.33	49.16	72.99	66.03	>100	>100	60.62	>100	>100	6.82	34.25	69.67	26.81	81.30	>100	6.39	22.61	96.29
15	quinoline-3-yl	15.10	45.16	75.22	36.42	>100	>100	9.11	37.36	66.00	7.71	35.60	67.90	25.36	58.94	92.51	4.25	7.16	21.81
16	2-phenylquinoline-4-yl	14.72	41.16	67.59	10.65	26.50	42.34	>100	>100	>100	11.45	43.37	75.28	17.88	51.50	85.12	12.09	48.14	84.18
	Reference drugs																		
	doxorubicin	2.00×10^{-3}	0.27	7.83	1.00×10^{-2}	1.16	6.57	$<1.00 \times 10^{-2}$	1.25	3.45	0.10	3.98	25.12	2.00×10^{-2}	0.15	0.28	3.30×10^{-2}	7.1×10^{-2}	0.29
	cisplatin	3.16	>100	>100	5.01	50.12	>100	9.64	32.73	50	7.94	>100	>100	5.01	>100	>100	1	79.63	>100
	etoposide	19.95	>100	>100	0.63	3.98	79.43	n.d[f]	n.d.	n.d.	31.62	>100	>100	12.59	>100	>100	1.26	50.12	89.9
	MSA	1.28	3.43	>100	2.45	8.39	37.14	3.54	6.85	19.87	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.08	5.31	9.54

[a] GI_{50}, concentration that reduces growth by 50% compared to control. [b] TGI, concentration that completely inhibits cell growth. [c] LC_{50}, concentration that kills 50% of cells. [d] Not determined.

Table S2. Average values of GI_{50}, TGI and LD_{50} (μM) and calculated SI.

Code	R/compound	184B5			SI[a]	BEAS-2B			SI[b]
		GI_{50}	TGI	LD_{50}		GI_{50}	TGI	LD_{50}	
1	**phenyl**	9.79	40.24	71.07	1,003.20	20.01	49.73	79.45	0.81
2	**4-methylphenyl**	9.09	73.44	>100	7,005.30	4.17	46.25	95.56	0.54
3	**4-nitrophenyl**	8.21	40.39	76.32	253.18	4.01	33.49	70.63	0.14
4	**4-methylthiophenyl**	12.68	45.87	79.07	2,264.07	7.44	37.17	77.44	0.24
5	**4-chlorophenyl**	8.20	84.45	>100	1,660.78	2.51	>100	>100	0.19
7	**3,5-dimethoxyphenyl**	10.49	40.48	70.47	69,673.53	9.72	43.07	76.99	0.48
8	**3,4,5-trimethoxyphenyl**	6.41	27.05	62.86	0.70	9.49	40.54	73.02	0.59
9	**furan-2-yl**	4.58	12.50	70.61	0.58	5.45	19.50	63.02	0.86
10	**isoxazol-2-yl**	2.80	5.04	7.28	0.73	0.80	8.31	51.26	0.14
11	**thiophene-2-yl**	6.96	33.06	66.45	0.44	7.64	34.66	68.97	0.13
12	**3-chloro-thiophene-2-yl**	8.05	33.41	62.58	0.59	7.13	35.11	69.89	0.15
13	**benzo[b]thiophene-2-yl**	70.83	>100	>100	2.54	13.59	>100	>100	0.60
14	**1,3-benzodioxole-5-yl**	20.44	75.75	>100	0.81	>100	>100	>100	n.d.[c]
15	**quinoline-3-yl**	9.66	75.42	>100	0.64	3.48	21.94	65.14	0.38
16	**2-phenylquinoline-4-yl**	8.64	34.63	63.78	0.59	>100	>100	>100	n.d.[c]
doxorubicin		1.15	4.60	8.06	574	0.13	0.45	0.78	>13
MSA		1.79	3.84	>100	1.40	3.66	6.18	8.69	1.03

[a] Selectivity index (SI) calculated as GI_{50} (184B5)/GI_{50} (MCF-7). [b] SI calculated as GI_{50} (BEAS-2B)/GI_{50}(HTB-54). [c] Not determined.

3.3. Evaluation of cell cycle progression and cell death

A fixed population of MCF-7 cells were seeded in 25 cm^2 flasks and incubated overnight. Cultures were treated with the corresponding concentration of compounds **2**, **7**, DMSO (control) or 6 μM camptothecin (positive control). Seeded population was dependent on studied time point: 3×10^6 cells/flask for 24 h or shorter treatment, 2×10^6 cells/flasks for 48 h treatment and finally 1×10^6 cells/flask for 72 h experiments. Apo-Direct kit (BD Pharmigen) was used to determine cell cycle distribution and cell death percentage. Cells were fixed in a 1% paraformaldehyde solution in PBS for 30–40 min at 0ºC, washed with PBS twice and incubated for 30 min with 70% ethanol on ice. Staining was performed following manufacturer's protocol and samples were analysed by flow cytometry using a Counter Epics XL cytometer (Beckman Counter). **Figure S12** shows a stacked cell cycle histogram corresponding to compound **7** effects at different time-course experiments.

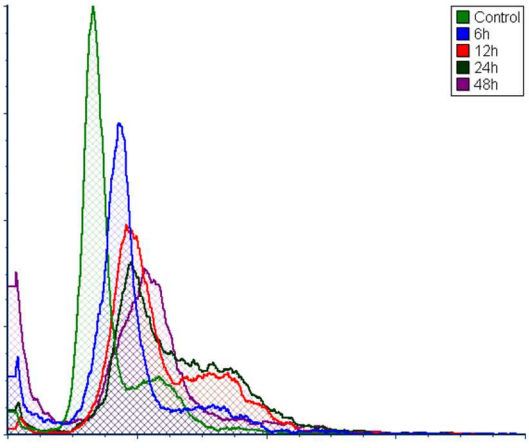


Figure S12. Cell cycle analysis after treatment with compound **7** for different periods of time.

In order to perform the inhibition assays, cells were pre-treated with 50 μM of the pan-caspase inhibitor Z-VAD-FMK (BD Pharmigen) or 100 nM of the autophagy inhibitor wortmannin (Santa Cruz) for 1 h. The cells were treated with 10 μM of **2** or **7**, DMSO was added to the control cells. Samples were processed following the same methodology stated above. **Figure S13** corresponds to representative cell death analysis for the inhibitor assays.

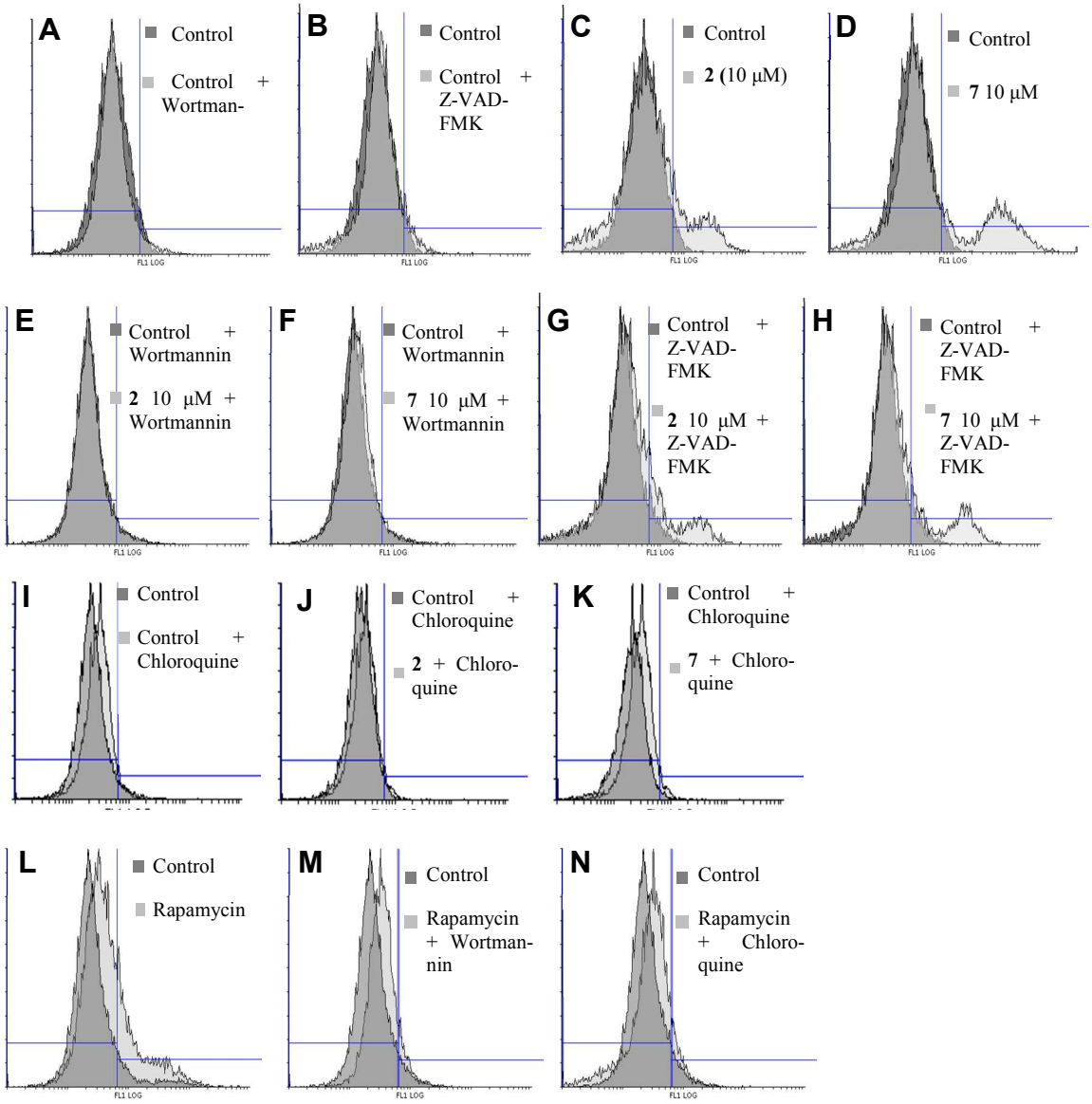


Figure S13. Cell death induced by compounds **2** and **7** is blocked by wortmannin but not by caspase inhibitor Z-VAD-FMK. Figures **A-H** show representative experiments stacked with the control graphs for each experiment.

3.4. Statistical analysis

Statistical data represent the mean ± SEM of at least three independent experiments performed in duplicate. Mann-Whitney U-test was used to stablish statistical significance of differences between control and treatment groups. GraphPad Prism version 7 was used, significant differences were considered at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

3.5. Protein analysis

Proteins were detected by Western blot. Specific antibodies LC3B (Cell Signaling), Beclin-1 (D40C5, Cell Signaling) and Actin (H-300) (Santa Cruz Biotechnology). Anti-rabbit IgG, conjugated with peroxidase was used as secondary anti-body from Cell Signaling.