Supplementary Information

Combretastatin A-4: The Antitubulin Agent that Inspired the Design and Synthesis of Styrene and Spiroisatin Hybrids as Promising Cytotoxic, Antifungal and Antiviral Compounds

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General information (chemistry)

Infrared (FTIR) spectra were recorded on a Lumex Infralum FT-02 spectrometer, ν_{max} in cm^{-1}. Bands are characterized according to the functional group. ^1H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance-400 (400 MHz) spectrometer. Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CDCl_3$: δ 7.26 ppm; DMSO-d_6: δ 2.50 ppm). Data were reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, br = broad, m = multiplet), coupling constants (Hz) and integration. ^{13}C NMR spectra were recorded on a Bruker Avance-400 (400 MHz) spectrometer with complete proton decoupling. Chemical shifts are reported in ppm with solvent resonance as the internal standard ($CDCl_3$: δ 77.00 ppm; DMSO-d_6: δ 40.45 ppm). On DEPT-135 spectra, the signals of CH_3 and CH carbons are shown as positive (+) and CH_2 carbons are shown negative (−). Quaternary carbons are not shown. A Hewlett Packard 5890a Series II Gas Chromatograph interfaced to an HP 5972 Mass Selective Detector (MSD) with an HP MS ChemStation Data system was used for MS identification at 70 eV using a 60 m capillary column coated with HP-5 [5%-phenylpoly (dimethylsiloxane)]. Accurate mass data were obtained on Micromass Q-TOF by electrospray ionisation (ESI). Melting points were measured on a Fisher Johns melting point apparatus and are uncorrected.

Unless otherwise noted, all reactions have been carried out with distilled and dried solvents and under atmosphere pressure. All work-up and purification procedures were carried out with reagent grade solvents (purchased from Sigma Aldrich (Saint Louis, USA) and Merck, (Darmstadt, Germany)) in air. Thin-layer chromatography (TLC) was performed using Merck silica gel 60 F254 precoated plates (0.25 mm). Column chromatography was performed using silicagel 60 (0.063-0.200 mm) 70-230 mesh. The synthesis of spiroisatin-dihydroquinoline hybrids **14a**-**14c** and spiroisatin-nitropyrrolizidine hybrids **20a**-**20k** were carried out according to our previous published protocols.[1,2]

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Characterization data of synthesized β-nitrostyrenes **10a**-**10c**

3,4-(Methylenedioxy)-β-nitrostyrene (**10a**)

Yellow solid (0.95 g, 4.95 mmol, 99%), retention factor (Rf) [hexane-EtOAc 2:1] = 0.68; mp 156-158 °C; IR (KBr) ν / cm^{-1} 3116 $\nu_{(=CH_{Ar})}$, 2915 $\nu_{(OCH_2O)}$, 1635 $\nu_{(C_{Ar}-C_{Ar})}$, 1558 $\nu_{(C-NO_2)}$, 1326 $\nu_{(C-NO_2)}$, 1265 $\nu_{(C-O-C)}$; GC retention time (t_R) = 14.50 min, MS (EI), *m/z*, 193 ($M^{+\cdot}$, 193), 146 (100), 145 (44), 89 (83), 63 (58).[3]

3,4-Dimethoxy-β-nitrostyrene (**10b**)

Yellow solid (1.03 g, 4.95 mmol, 99%), retention factor (Rf) [hexane-EtOAc 2:1] = 0.78; mp 138-140 °C; IR (KBr) ν / cm^{-1} 3116 $\nu_{(=CH_{Ar})}$, 2931 $\nu_{(OCH_3)}$, 1589 $\nu_{(C-NO_2)}$, 1496 $\nu_{(C_{Ar}-C_{Ar})}$, 1342 $\nu_{(C-NO_2)}$, 1265 $\nu_{(C-O-C)}$; GC t_R = 16.13 min, MS (EI), *m/z*, 209 ($M^{+\cdot}$, 100), 162 (72), 147 (27), 91 (31), 77 (29).[4]

3,4,5-Trimethoxy-β-nitrostyrene (**10c**)

Yellow solid (1.18 g, 4.95 mmol, 99%), retention factor (Rf) [hexane-EtOAc 2:1] = 0.76; mp 123-125 °C; IR (KBr) ν / cm^{-1} 3116 $\nu_{(=CH_{Ar})}$, 2931 $\nu_{(OCH_3)}$, 1635 $\nu_{(C_{Ar}-C_{Ar})}$, 1589 $\nu_{(C-NO_2)}$, 1326 $\nu_{(C-NO_2)}$, 1265 $\nu_{(C-O-C)}$; GC t_R = 21.88 min, MS (EI), *m/z*, 239 ($M^{+\cdot}$, 100), 192 (32), 177 (32), 149 (23), 77 (17).[5]

Characterization data of synthesized spiroisatin-thiazolidinone hybrids **17a**-**17c**

(*S*,*Z*)-3′-(4-Chlorophenyl)-5′-(3,4,5-trimethoxybenzylidene)spiro[indoline-3,2′-thiazolidine]-2,4′-dione (**17a**)

White solid (0.90 g, 1.77 mmol, 71%), retention factor (Rf) [hexane-EtOAc 3:1] = 0.48; mp 215-217 °C; IR (KBr) ν / cm^{-1} 3270 $\nu_{(NH)}$, 2993 $\nu_{(=CH_{Ar})}$, 2931 $\nu_{(OCH_3)}$, 1743 $\nu_{(C=O\text{-}isatin)}$, 1697 $\nu_{(C=O\text{-}thiazolidinone)}$, 1619 $\nu_{(C_{Ar}=C_{Ar})}$, 1326 $\nu_{(OCH_3)}$, 1234 $\nu_{(C-N)}$, 817 $\nu_{(C-Cl)}$, 678 $\nu_{(C-S)}$; ^{1}H NMR (400 MHz, DMSO) δ 10.53 (1H, s, N*H*), 7.71 (1H, d, *J* 7.0 Hz, 4-H_{Ar}), 7.62 (1H, s, 6'-H (=C*H*)), 7.46-7.42 (2H, m, 15' and 17'-H_{Ar}), 7.29 (1H, td, *J* 7.8, 1.1 Hz, 6-H_{Ar}), 7.15-7.12 (2H, m, 14' and 18'-H_{Ar}), 7.07 (1H, td, *J* 7.6, 0.7 Hz, 5-H_{Ar}), 6.88 (2H, s, 8' and 12'-H_{Ar}), 6.83 (1H, d, *J* 7.8 Hz, 7-H_{Ar}), 3.79 (6H, s, 2 × OCH_3), 3.70 (3H, s, OCH_3); ^{13}C NMR (101 MHz, DMSO) δ 174.0, 166.3, 153.2 (2C), 141.9, 138.1, 134.7, 133.4, 131.8 (+), 130.4 (+, 2C), 129.9, 129.6 (+, 2C), 127.1 (+), 126.9 (+), 123.7, 123.2, 123.1 (+), 111.1 (+), 106.8 (+, 2C), 69.3, 60.2 (+), 56.0 (+, 2C).

(*S*,*Z*)-3′-(4-Methylphenyl)-5′-(3,4,5-trimethoxybenzylidene)spiro[indoline-3,2′-thiazolidine]-2,4′-dione (**17b**)

White solid (0.94 g, 1.92 mmol, 77%), retention factor (Rf) [hexane-EtOAc 3:1] = 0.48; mp 258-260 °C; IR (KBr) ν / cm^{-1} 3239 $\nu_{(NH)}$, 3070 $\nu_{(=CH_{Ar})}$, 2931 $\nu_{(CH_3)}$, 1743 $\nu_{(C=O\text{-}isatin)}$, 1681 $\nu_{(C=O\text{-}thiazolidinone)}$, 1604 $\nu_{(C_{Ar}=C_{Ar})}$, 1465 $\nu_{(CH_3)}$, 1218 $\nu_{(C-N)}$, 678 $\nu_{(C-S)}$; ^{1}H NMR (400 MHz, DMSO) δ 8.87 (1H, s, NH), 7.67 (1H, s, 6'-H (=CH)), 7.51 (1H, d, *J* 7.2 Hz, 4-H_{Ar}), 7.19 (1H, td, *J* 7.7, 1.1 Hz, 6-H_{Ar}), 7.10 (1H, td, *J* 7.6, 0.7 Hz, 5-H_{Ar}), 6.99 (4H, q, *J* 8.5 Hz, 14', 15', 17', 18'-H_{Ar}), 6.75 (1H, s, 7-H_{Ar}), 6.73 (2H, s, 8' and 12'-H_{Ar}), 3.86 (3H, s, OCH_3), 3.84 (6H, s, 2 × OCH_3), 2.21 (3H, s, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 175.0, 167.4, 153.3 (2C), 140.8, 138.9, 138.4, 132.8, 131.5 (+), 130.4, 130.2 (+, 2C), 128.6 (+, 2C), 128.2 (+), 126.5 (+), 125.1, 123.8 (+), 122.8, 111.4 (+, 2C), 106.8, 70.0, 61.0 (+), 56.1 (+, 2C), 21.2 (+).

(*S*,*Z*)-3'-(4-Methoxylphenyl)-5'-(3,4,5-trimethoxybenzylidene)spiro[indoline-3,2'-thiazolidine]-2,4'-dione (**17c**)

White solid (0.98 g, 1.95 mmol, 78%), retention factor (Rf) [hexane-EtOAc 3:1] = 0.46; mp 236-237 °C; IR (KBr) ν / cm^{-1} 3225 $\nu_{(NH)}$, 3070 $\nu_{(=CH_{Ar})}$, 2931 $\nu_{(OCH_3)}$, 1727 $\nu_{(C=O\text{-}isatin)}$, 1666 $\nu_{(C=O\text{-}thiazolidinone)}$, 1604 $\nu_{(C_{Ar}=C_{Ar})}$, 1326 $\nu_{(OCH_3)}$, 1234 $\nu_{(C-N)}$, 617 $\nu_{(C-S)}$; ^{1}H NMR (400 MHz, DMSO) δ 10.96 (1H, s, NH), 7.71 (1H, d, *J* 7.5 Hz, 4-H_{Ar}), 7.60 (1H, s, 6'-H (=CH)), 7.28 (1H, td, *J* 7.8, 0.9 Hz, 6-H_{Ar}), 7.07 (1H, t, *J* 7.5 Hz, 5-H_{Ar}), 7.02 (2H, d, *J* 8.9 Hz, 15' and 17'-H_{Ar}), 6.88 (2H, s, 8' and 12'-H_{Ar}), 6.88 (2H, d, *J* 8.9 Hz, 14' and 18'-H_{Ar}), 6.80 (1H, d, *J* 7.8 Hz, 7-H_{Ar}), 3.79 (6H, s, 2 × OCH_3), 3.70 (3H, s, OCH_3), 3.68 (3H, s, OCH_3); ^{13}C NMR (101 MHz, DMSO) δ 174.1, 166.4, 159.1, 153.1 (2C), 141.9, 138.0, 131.6 (+), 130.2 (+, 2C), 130.0, 128.0 (+), 126.9 (+), 126.7 (+), 124.2, 123.4 (+), 123.1, 114.6 (+, 2C), 111.0 (+), 106.7 (+, 2C), 69.5, 60.2 (+), 55.9 (+, 2C), 55.3 (+).

Copies of ^{1}H NMR, ^{13}C NMR and DEPT-135 charts of the synthesized spiroisatin-thiazolidinone hybrids **17a**-**17c**

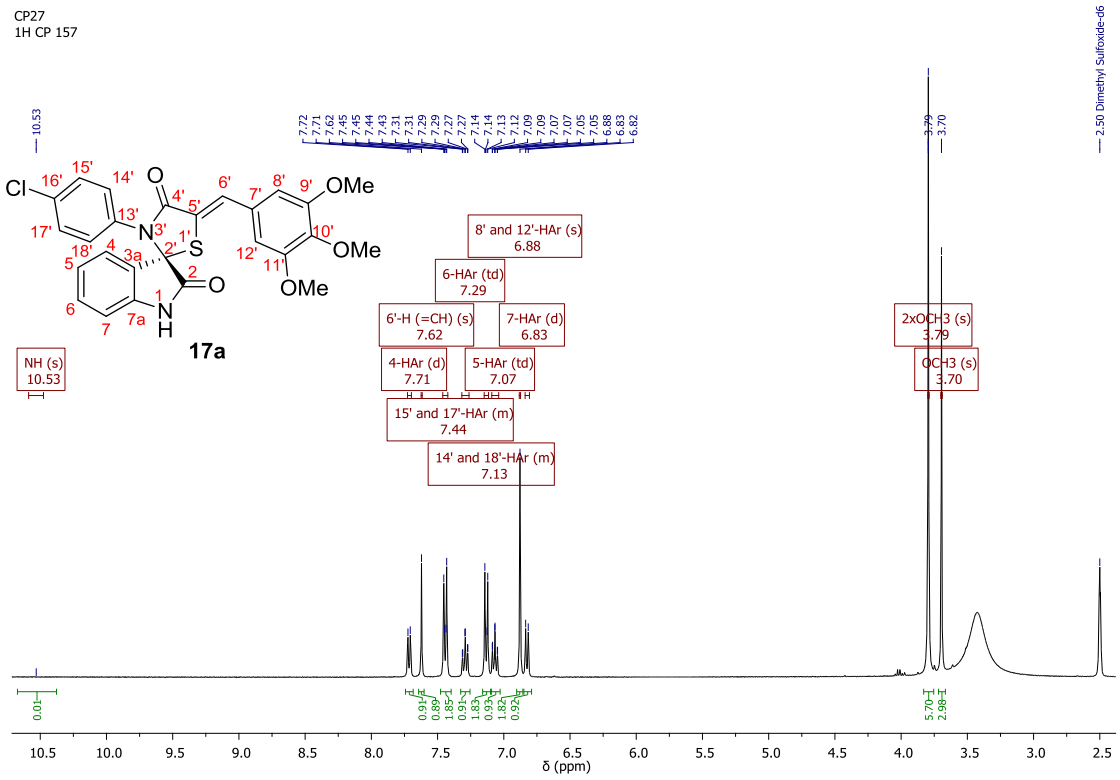

Figure S1. ^{1}H NMR (400 MHz, DMSO) spectrum of (*S*,*Z*)-3'-(4-chlorophenyl)-5'-(3,4,5-trimethoxybenzylidene) spiro[indoline-3,2'-thiazolidine]-2,4'-dione (**17a**).

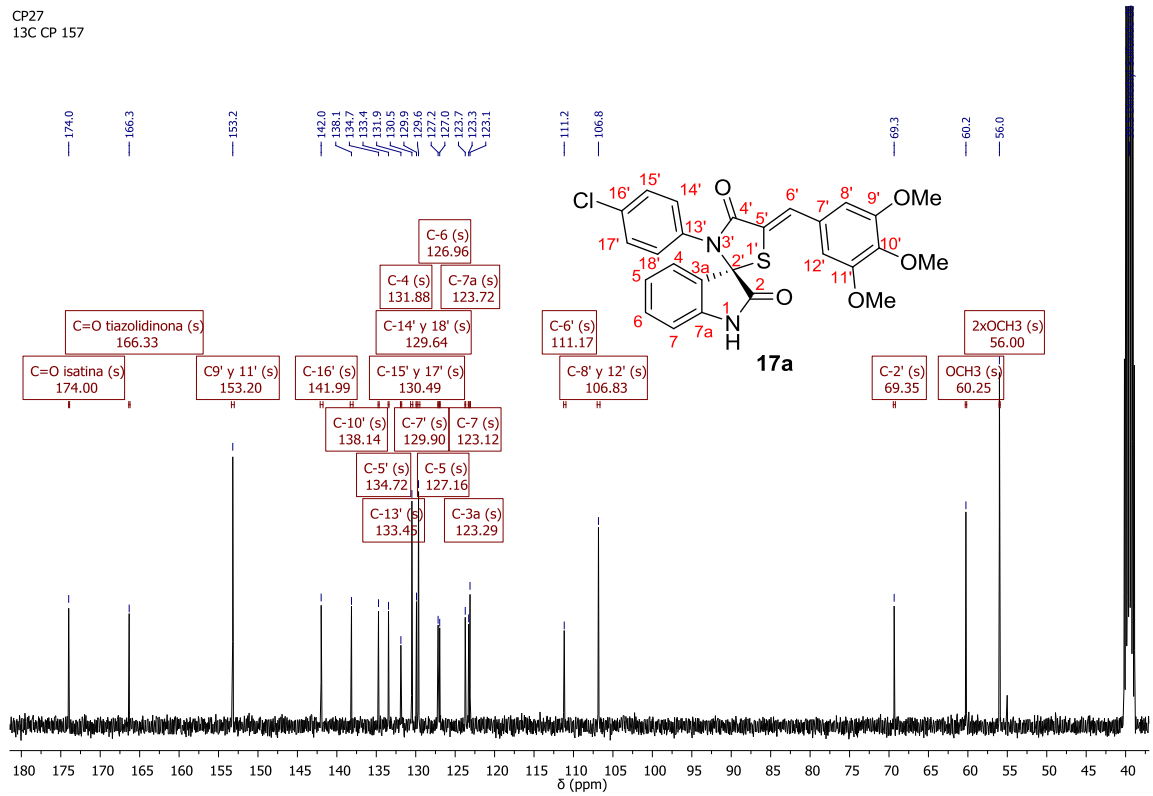

Figure S2. ^{13}C NMR (101 MHz, DMSO) spectrum of (*S*,*Z*)-3'-(4-chlorophenyl)-5'-(3,4,5-trimethoxybenzylidene) spiro[indoline-3,2'-thiazolidine]-2,4'-dione (**17a**).

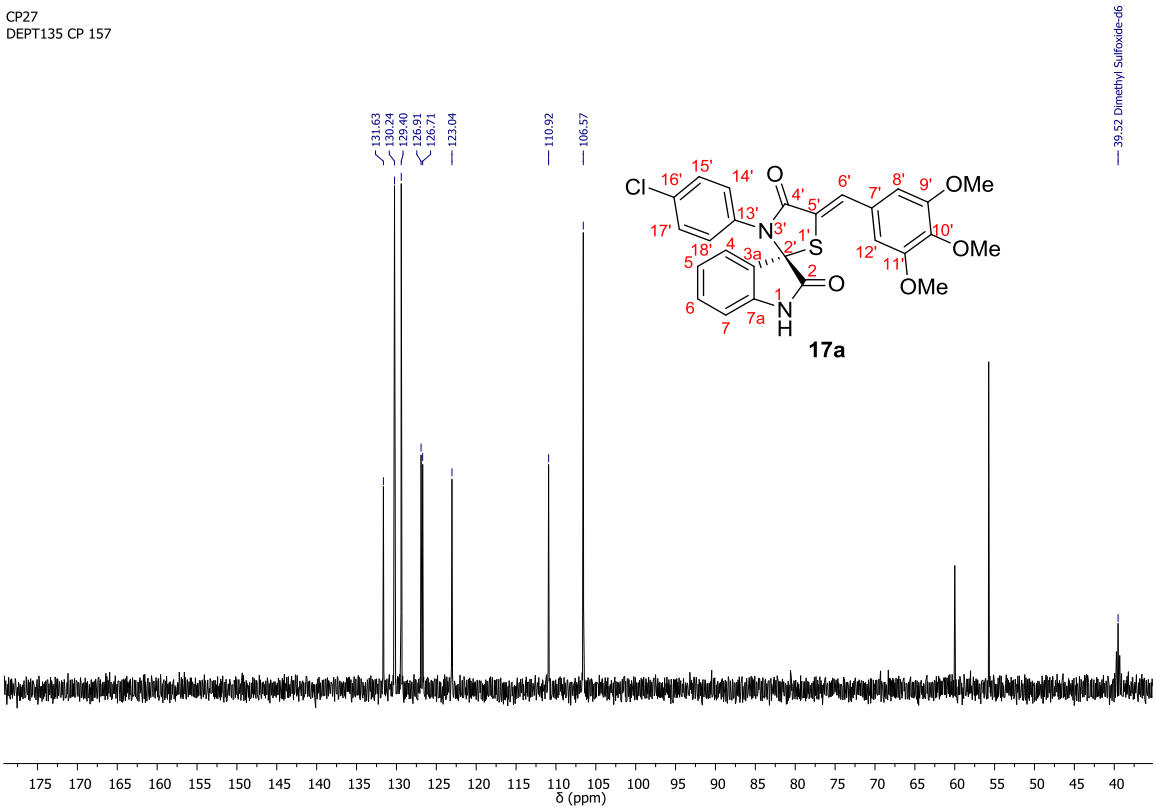

Figure S3. DEPT-135 (101 MHz, DMSO) spectrum of (*S*,*Z*)-3'-(4-chlorophenyl)-5'-(3,4,5-trimethoxybenzylidene) spiro[indoline-3,2'-thiazolidine]-2,4'-dione (**17a**).

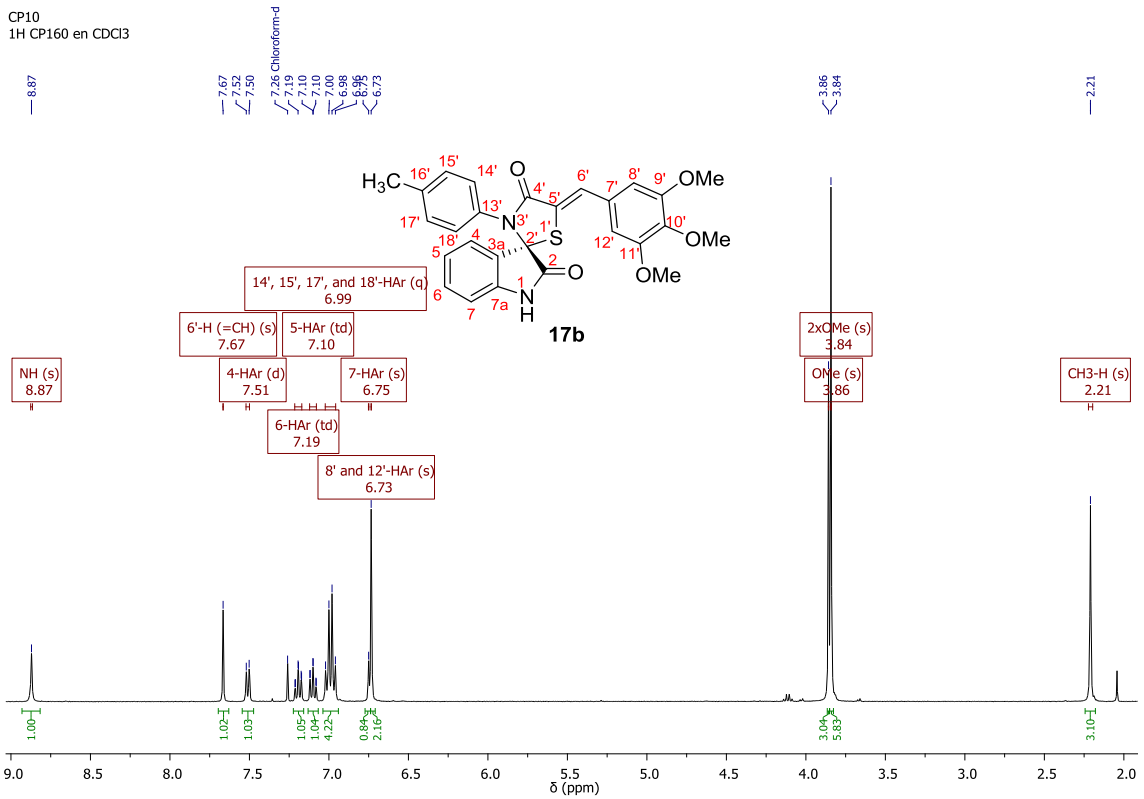

Figure S4. ^{1}H NMR (400 MHz, DMSO) spectrum of (*S*,*Z*)-3'-(4-methylphenyl)-5'-(3,4,5-trimethoxybenzylidene) spiro[indoline-3,2'-thiazolidine]-2,4'-dione (**17b**).

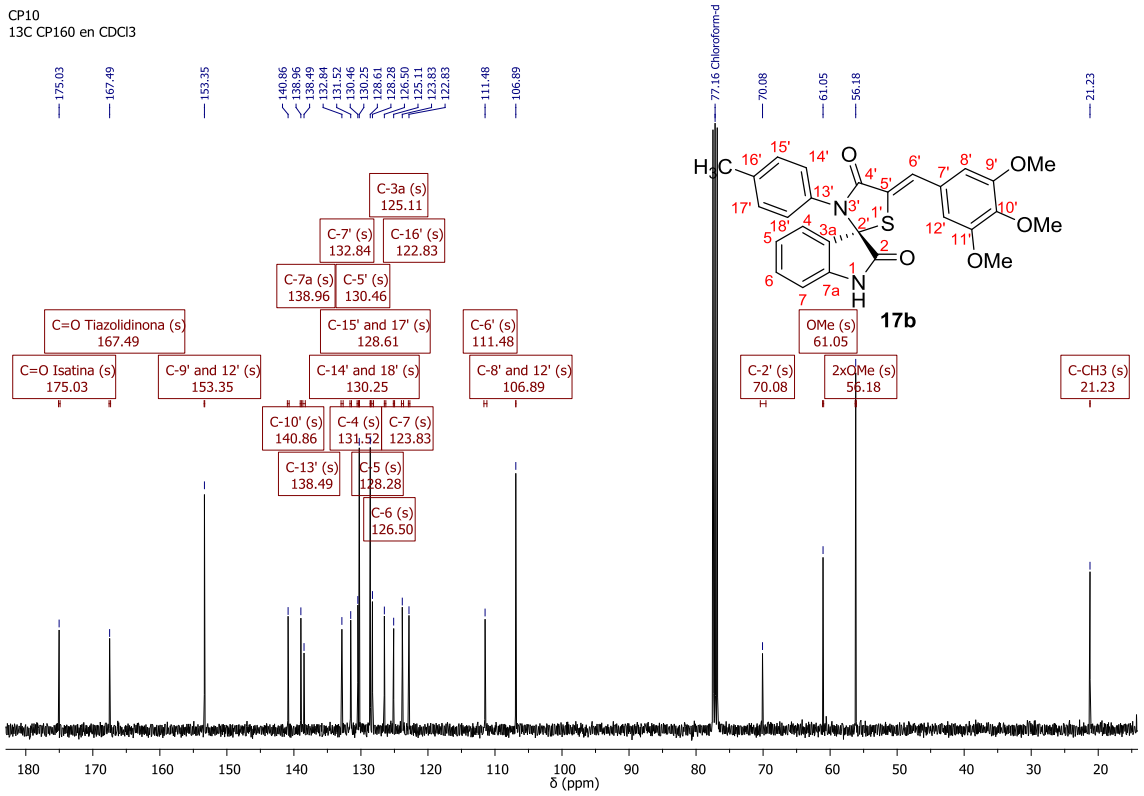

Figure S5. ^{13}C NMR (101 MHz, DMSO) spectrum of (*S*,*Z*)-3'-(4-methylphenyl)-5'-(3,4,5-trimethoxybenzylidene) spiro[indoline-3,2'-thiazolidine]-2,4'-dione (**17b**).

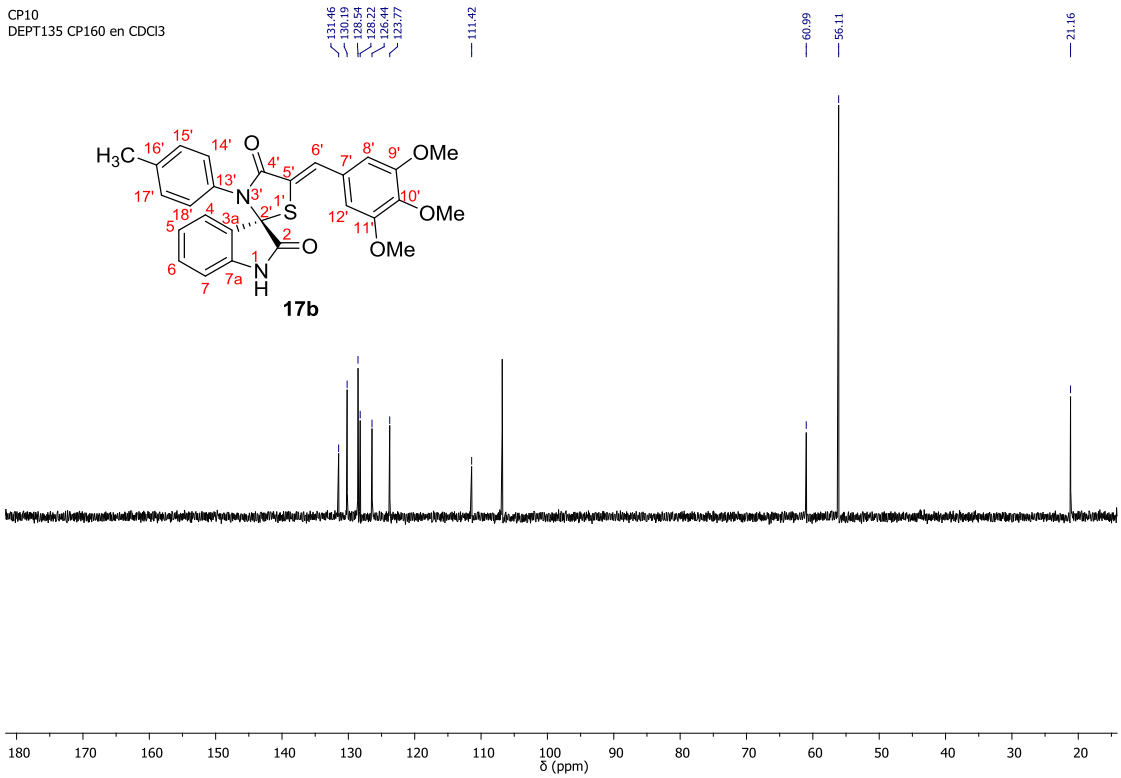

Figure S6. DEPT-135 (101 MHz, DMSO) spectrum of (*S*,*Z*)-3'-(4-methylphenyl)-5'-(3,4,5-trimethoxybenzylidene) spiro[indoline-3,2'-thiazolidine]-2,4'-dione (**17b**).

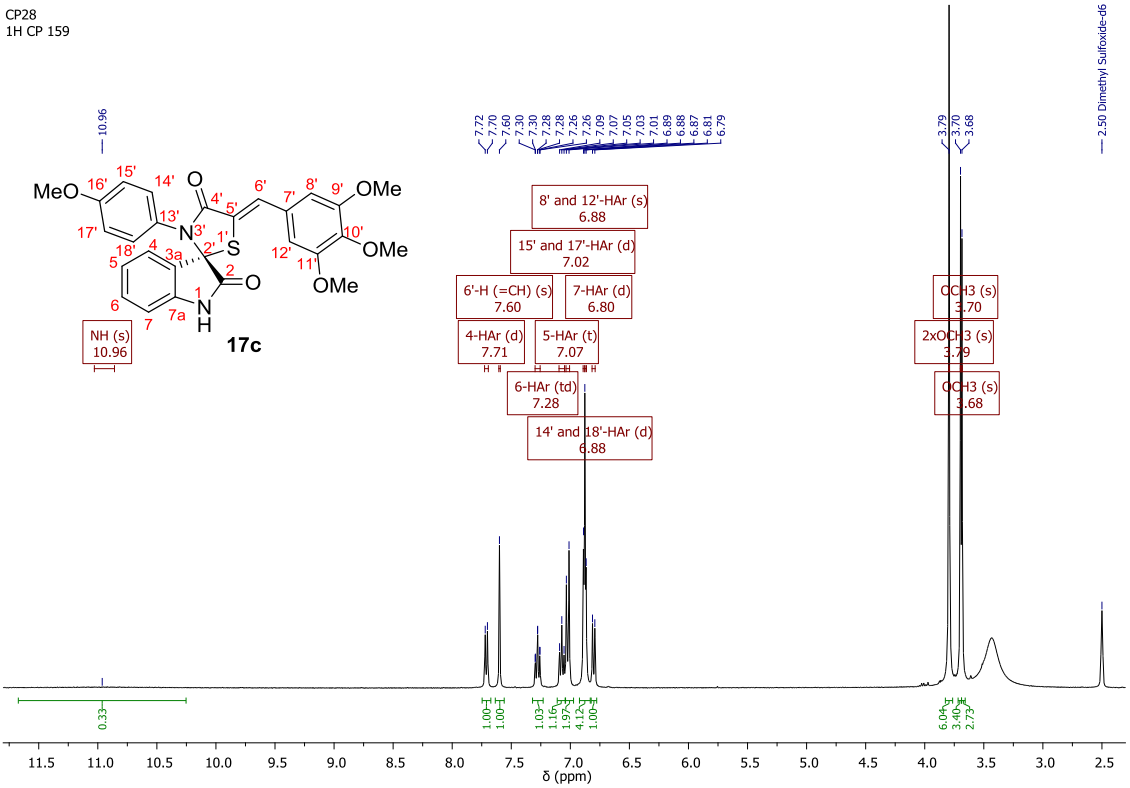

Figure S7. ^{1}H NMR (400 MHz, DMSO) spectrum of (*S*,*Z*)-3'-(4-methoxyphenyl)-5'-(3,4,5-trimethoxybenzylidene) spiro[indoline-3,2'-thiazolidine]-2,4'-dione (**17c**).

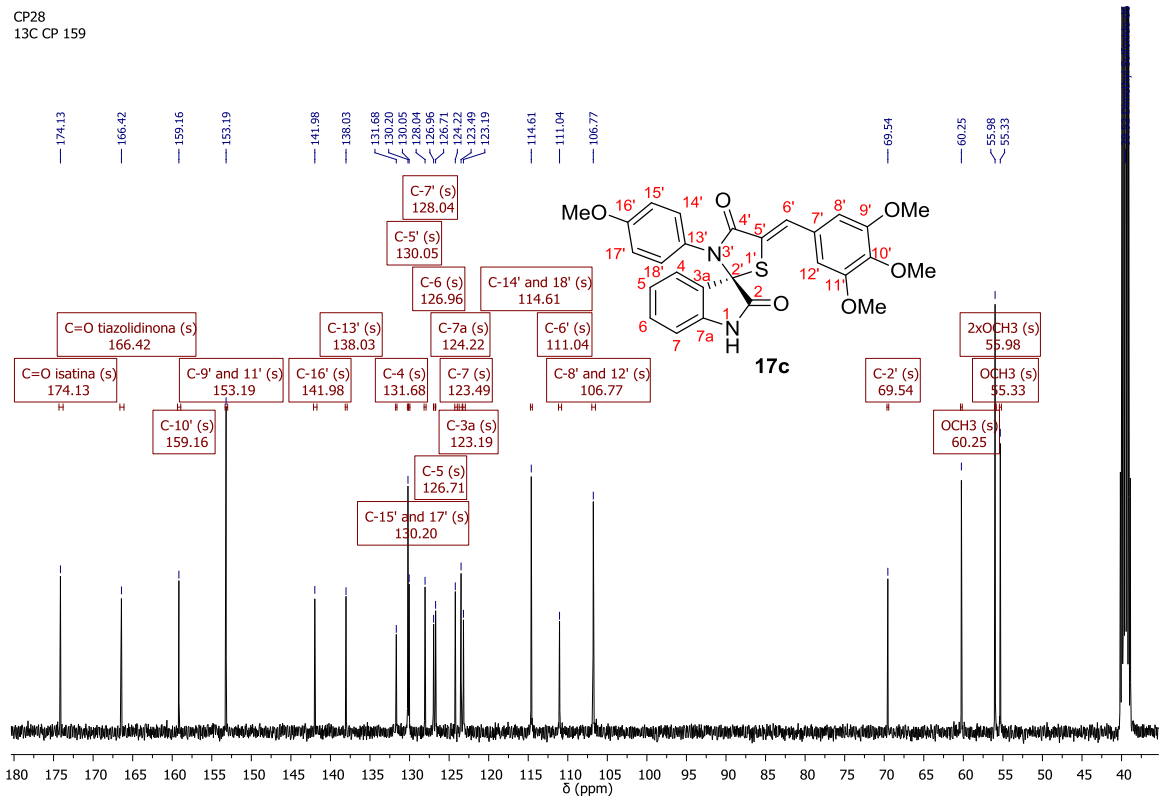

Figure S8. ^{13}C NMR (101 MHz, DMSO) spectrum of (*S*,*Z*)-3'-(4-methoxyphenyl)-5'-(3,4,5-trimethoxybenzylidene) spiro[indoline-3,2'-thiazolidine]-2,4'-dione (**17c**).

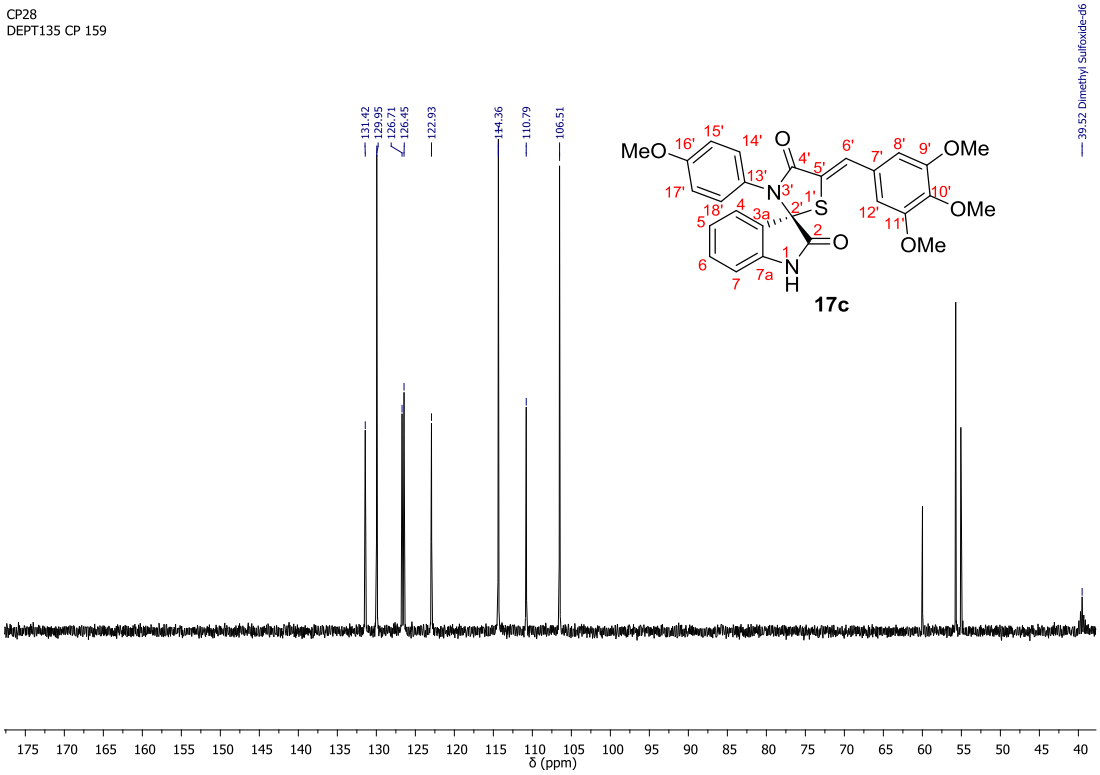

Figure S9. DEPT-135 (101 MHz, DMSO) spectrum of (*S*,*Z*)-3'-(4-methoxyphenyl)-5'-(3,4,5-trimethoxybenzylidene) spiro[indoline-3,2'-thiazolidine]-2,4'-dione (**17c**).

General information (biology)

Reagents and compounds

Dulbecco's modified Eagle's medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and Roswell Park Memorial Institute (RPMI)-1640 were obtained from Sigma-Aldrich Chemical Co. (Saint Louis, USA); fetal bovine serum (FBS), penicillin/streptomycin (PS) were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). Acyclovir was obtained from Biogen Laboratory (Cambridge, USA), ribavirin was purchased from Calbiochem (La Jolla, CA, USA). Dimethyl sulfoxide (DMSO) was acquired from Merck KGaA (Darmstadt, Germany). Paclitaxel, colchicine, doxorubicine, amphotericine B and itraconazole were purchased from Sigma-Aldrich Chemical Co. (Saint Louis, USA). Terbinafine was obtained from Recalcine Laboratories, Santiago de Chile (Chile). Stock solutions of compounds were prepared in DMSO and frozen at −70 °C. The concentration of DMSO in biological assays was of 0.05%. Cell controls with DMSO at 0.05% were used.

Cell culture and viruses

Human herpesvirus 1 (HHV-1 CDC Atlanta acyclovir-sensitive strain) was obtained from the Center for Disease Control (Atlanta, GA, USA, acyclovir-sensitive strain); human herpesvirus 2 (HHV-2 VR-734-G acyclovir-sensitive strain), obtained from the Center for Disease Control, were amplified in Vero cells. Virus stocks were titrated in Vero cells (African green monkey kidney, *Cercopithecus aethiops*, ATCC CCL-81 line) by plaque assay and expressed as plaque forming units (PFU mL^{-1}).

Dengue virus type 2 (DENV-2 New Guinea strain) was donated by Maria Elena Peñaranda and Eva Harris (Sustainable Sciences Institute and the University of California at Berkeley), which was amplified in C6/36HT of *Aedes albopictus* cells, from ATCC, and titrated in BHK-21 cells following our laboratory conditions.

Cells were maintained in DMEM supplemented with 5% FBS to Vero and BHK-21 cells, 100 units mL^{-1} of penicillin, 100 µg mL^{-1} of streptomycin, 100 µg mL^{-1} of L-glutamine, 0.14% $NAHCO_3$, and 1% of each nonessential amino acids and minimum essential medium vitamin solution (choline chloride, D-calcium pantothenate, folic acid, nicotinamide, pyridoxal hydrochloride, riboflavin, thiamine hydrochloride and i-inositol). Vero and BHK-21 cells were incubated at 37 °C in humidified 5% CO_2 atmosphere. For the cytotoxic activity, the cell lines of HeLa (human cervix epithelial carcinoma, ATCC CRL-1958), acute T cell leukemia (Jurkat, ATCC TIB-152) and human promonocytic cell line (U937, ATCC CRL-1593.2) were used, as well as, the non-tumor cell line Vero. Vero, BHK-21 and HeLa cells were grown in DMEM supplemented with 5% FBS. Jurkat and U937 cells were maintained in RPMI-1640 medium (supplemented with 10% FBS), 100 µg mL^{-1} of penicillin, 100 µg mL^{-1} of streptomycin, and 100 µg mL^{-1} of neomycin and maintained at 37 °C in humidified 5% CO_2 atmosphere.

Fungal strain

Fungal strains used in this research were: filamentous fungus *Fusarium oxysporum* (ATCC 48112), *Aspergillus fumigatus* (ATCC 204305), *Aspergillus flavus* (ATCC 204304), *Aspergillus terreus* (CDC 317) and dermatophytes, *Trichophyton rubrum* (ATCC 28188) and *Trichophyton mentagrophytes* (ATCC 24198), were used to evaluate antifungal activity at inoculum size of 0.2-2.5 × 10^5 CFU mL^{-1}.

References

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