Electronic Supplementary Material (ESI) for MedChemComm.

Supporting Information

Spirooxadiazoline oxindoles with promising *in vitro* antitumor activities

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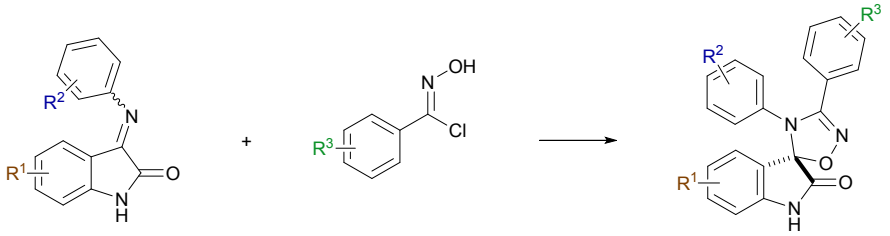
1. EXPERIMENTAL SECTION: CHEMISTRY.

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Melting points were determined using a Kofler camera Bock monoscope M and are uncorrected. The infrared spectra were collected on a Shimadzu FTIR Affinity-1 spectrophotometer. Elemental analysis (C, H, and N) were performed in a Flash 2000 CHNS-O analyzer (ThermoScientific, UK) at Liquid Chromatography and Mass Spectrometry Laboratory, Faculty of Pharmacy of Lisbon University. Merck Silica Gel 60 F254 plates were used for analytical TLC; flash column chromatography was performed on Merck Silica Gel (200–400 mesh) and Combi-Flash Rf from Teledyne ISCO (columns RediSep Rf, silica). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 Ultra-Shield at 400 MHz (1H NMR) and 100 MHz (13C NMR). Data are reported as follows: chemical shift (d), multiplicity (s: singlet, d: doublet, dd: doublet of doublet; t: triplet, m: multiplet, br: broad), coupling constants (*J*) in Hertz and integration. ^{1}H and ^{13}C chemical shifts are expressed in ppm using the solvent as internal reference. LRMS were performed on Micromass® Quattro Micro triple quadrupole (Waters®, Ireland) with an electrospray in positive ion mode (ESI+), ion source at 120 ºC, capillary voltage of 3.0 kV and source voltage of 30V, at the Liquid Chromatography and Mass Spectrometry Laboratory, Faculty of Pharmacy of Lisbon University.

GENERAL PROCEDURE FOR THE SYNTHESIS OF SPIRO[INDOLINE-3,5'-[1,2,4]OXADIAZOLINE]-2-ONES.

Triethylamine (2.0 equiv) was added dropwise to a mixture of 3-imino-indolin-2-one derivative (**3**, 1.0 equiv), and hydroximoyl chloride derivative (**2**, 2.0 equiv) in dry CH_2Cl_2 (1mL/0.1mmol of 3-imino-indolin-2-one) under nitrogen atmosphere. The reaction was stirred at room temperature for 5-12 h. The mixture was then washed with brine (2x) and the aqueous phase extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was removed under reduce pressure. The residue was purified by flash chromatography on silica gel using as eluent a gradient from *n*-hexane/EtOAc (95:5) to *n*-hexane/EtOAc (60:40) and recrystallized from diethyl ether/*n*-hexane or just washed with diethyl ether, to afford the final product.



2 3 1

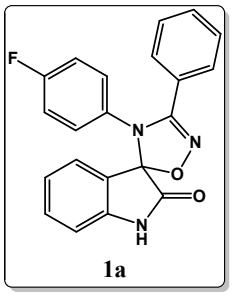
Compd	R^1	R^2
2a	H	4-F
2b	H	3-Cl
2c	H	4-Cl
2d	5-Cl	H
2e	5-Cl	3-Cl
2f	5-Cl	4-Cl
2g	6-Cl	H
2h	6-Cl	3-Cl
2i	6-Cl	4-Cl
2j	5-Br	H
2k	5-Br	4-Cl
2l	5-Br	3-Cl
2m	5-Br	3-Br
2n	5-Br	3-Cl,4-F
2o	5-Br	2-F,3-Cl

Compd	R^3
3a	H
3b	3-Cl
3c	4-Cl
3d	4-Me
3e	3-Br
3f	2-F,3-Cl

Compd	R^1	R^2	R^3
1a	H	4-F	H
1b	H	3-Cl	H
1c	H	4-Cl	H
1d	H	3-Cl	3-Cl
1e	H	3-Cl	4-Cl
1f	H	4-Cl	3-Cl
1g	H	4-Cl	4-Cl
1h	5-Cl	H	H
1i	5-Cl	3-Cl	H
1j	5-Cl	3-Cl	3-Cl
1k	5-Cl	3-Cl	4-Cl
1l	5-Cl	4-Cl	H
1m	5-Cl	4-Cl	3-Cl
1n	5-Cl	4-Cl	4-Cl
1o	6-Cl	H	H
1p	6-Cl	H	4-Me
1q	6-Cl	H	4-Cl
1r	6-Cl	3-Cl	3-Cl
1s	6-Cl	3-Cl	4-Cl
1t	6-Cl	4-Cl	H
1u	6-Cl	4-Cl	3-Cl
1v	6-Cl	4-Cl	4-Cl
1w	5-Br	H	H
1x	5-Br	4-Cl	H
1y	5-Br	3-Cl	H
1z	5-Br	3-Cl	4-Cl
1aa	5-Br	3-Cl	3-Cl
1ab	5-Br	3-Br	3-Br
1ac	5-Br	3-Cl,4-F	3-Cl
1ad	5-Br	2-F,3-Cl	3-Cl
1ae	5-Br	3-Cl	2-F,3-Cl

4'-(4-fluorophenyl)-3'-phenyl-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1a).

Synthesized according to the general procedure, and starting with **2a** and **3a**, this compound was obtained as a white solid (119.7 mg, 80 % yield).



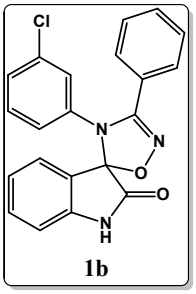
1a

Mp: 168-169 ºC; **IR** (KBr, selected peaks): 3211, 1726, 1626, 1506, 1473, 1386, 1208, 842, 749 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.47 (br s, 1H), 7.65 (d, J = 7.5 Hz, 1H), 7.52 – 7.42 (m, 3H), 7.42 – 7.32 (m, 3H), 7.12 (t, J = 7.5 Hz, 1H), 7.00 – 6.93 (m, 4H), 6.91 (d, J = 7.8 Hz, 1H); **^{13}C NMR** (100 MHz,

Acetone-d_6) δ (ppm): 172.88, 161.91 (d, J_{FC} = 244.0 Hz), 156.03, 143.81, 134.18 (d, J_{FCCCC} = 3.0 Hz), 133.05, 131.41, 130.30 (d, J_{FCCC} = 9.0 Hz, 2CH), 129.48 (2CH), 129.04 (2CH), 127.44, 125.67, 125.46, 123.92, 116.69 (d, J_{FCC} = 22.0 Hz, 2CH), 111.73, 99.02 (Cspiro). Anal. Calcd for $C_{21}H_{14}FN_3O_2 \cdot 0.2H_2O$: C 69.49, H 4.01, N 11.58, found: C 69.22, H 3.79, N 11.38.

4'-(3-chlorophenyl)-3'-phenyl-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1b).

Synthesized according to the general procedure, and starting with **2b** and **3a** this compound was obtained as a white solid (120.0 mg, 82 % yield).

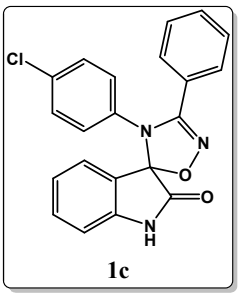


1b

Mp: 186-187 ºC; **IR** (KBr, selected peaks): 3242, 1729, 1624, 1587, 1470, 1385, 1206, 847 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.56 (br s, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.55 – 7.46 (m, 3H), 7.45 – 7.35 (m, 3H), 7.22 – 7.15 (m, 2H), 7.12 (t, J = 7.6 Hz, 1H), 6.96 (d, J = 7.6 Hz, 1H), 6.86 (s, 1H), 6.81 (d, J = 7.2 Hz, 1H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.60, 155.54, 143.78, 139.58, 134.68, 133.23, 131.61, 131.29, 129.61 (2CH), 129.06 (2CH), 127.60, 127.40, 127.08, 125.85, 125.58, 125.24, 124.04, 111.86, 98.84 (Cspiro). Anal. Calcd for $C_{21}H_{14}ClN_3O_2 \cdot 0.2H_2O$: C 66.47, H 3.83, N 11.08, found: C 66.11, H 3.62, N 10.94.

4'-(4-chlorophenyl)-3'-phenyl-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1c).

Synthesized according to the general procedure, and starting with **2c** and **3a** this compound was obtained as a white solid (127.4 mg, 87 % yield).

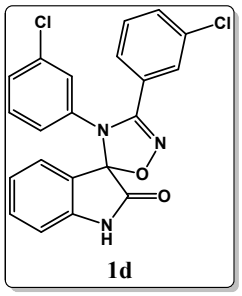


1c

Mp: 161-163 ºC; **IR** (KBr, selected peaks): 3216, 1725, 1622, 1490, 1471, 1381, 1209, 588 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.51 (br s, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.53 – 7.45 (m, 3H), 7.44 – 7.33 (m, 3H), 7.20 (d, J = 8.6 Hz, 2H), 7.11 (t, J = 7.6 Hz, 1H), 6.93 (d, J = 7.8 Hz, 1H), 6.87 (d, J = 8.6 Hz, 2H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.67, 155.74, 143.81, 137.04, 133.15, 132.69, 131.53, 129.98 (2CH), 129.58 (2CH), 129.16 (2CH), 129.05 (2CH), 127.41, 125.65, 125.35, 123.98, 111.82, 98.90 (Cspiro). Anal. Calcd for $C_{21}H_{14}ClN_3O_2 \cdot 0.2H_2O$: C 66.47, H 3.83, N 11.08, found: C 66.11, H 3.64, N 10.87.

3',4'-bis(3-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1d).

Synthesized according to the general procedure, and starting with **2b** and **3b**, this compound was obtained as a white solid (135.1 mg, 85 % yield).

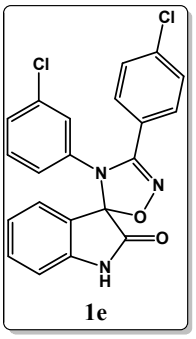


1d

Mp: 143-145 ºC; **IR** (KBr, selected peaks): 3215, 1726, 1622, 1558, 1471, 1384, 1207, 788 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.56 (br s, 1H), 7.66 (d, J = 7.4 Hz, 1H), 7.57 (s, 1H), 7.54 (d, J = 7.5 Hz, 1H), 7.48 – 7.41 (m, 2H), 7.39 (t, J = 7.7 Hz, 1H), 7.26 – 7.16 (m, 2H), 7.12 (t, J = 7.6 Hz, 1H), 6.96 (d, J = 7.8 Hz, 1H), 6.91 (s, 1H), 6.86 (d, J = 7.3 Hz, 1H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.44, 154.57, 143.83, 139.20, 134.96, 134.84, 133.37, 131.62, 131.46, 131.43, 128.81, 127.96, 127.59, 127.58, 127.54, 127.19, 126.05, 124.95, 124.07, 111.90, 99.12 (Cspiro). Anal. Calcd for $C_{21}H_{13}Cl_2N_3O_2 \cdot 0.1H_2O$: C 61.21, H 3.24, N 10.20, found: C 60.88, H 3.22, N 9.90.

3'-(4-chlorophenyl)-4'-(3-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4] oxadiazol] -2-one (1e).

Synthesized according to the general procedure, and starting with **2b** and **3c**, this compound was obtained as a white solid (127.9 mg, 80 % yield).



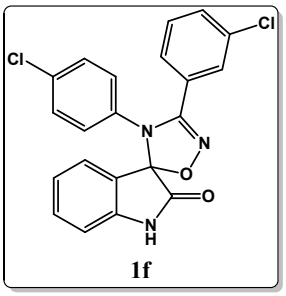
1e

MP: 173-174 ºC; **IR** (KBr, selected peaks): 3228, 1727, 1624, 1472, 1379, 1206, 825, 761, 692 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.56 (br s, 1H), 7.63 (d, J = 7.4 Hz, 1H), 7.53 (d, J = 8.6 Hz, 2H), 7.48 (d, J = 8.6 Hz, 2H), 7.39 (t, J = 7.7 Hz, 1H), 7.25 – 7.15 (m, 2H), 7.12 (t, J = 7.6 Hz, 1H), 6.95 (d, J = 7.8 Hz, 1H), 6.88 (s, 1H), 6.83 (d, J = 7.3 Hz, 1H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm) 172.50, 154.78, 143.83, 139.33, 137.10, 134.82, 133.34, 131.44, 130.74 (2CH), 129.87 (2CH), 127.87, 127.48, 127.14, 126.04, 125.03, 124.40, 124.06, 111.89, 99.03 (Cspiro). Anal. Calcd for $C_{21}H_{13}Cl_2N_3O_2 \cdot 0.15H_2O$: C 61.07, H 3.25, N 10.18, found: C 60.71, H 3.22, N 9.92.

3'-(3-chlorophenyl)-4'-(4-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4] oxadiazol]-2-one (1f).

Synthesized according to the general procedure, and starting with **2c** and **3b**, this compound was obtained as a white solid (134.2 mg, 84 % yield).

Mp: 176-178 ºC; **IR** (KBr, selected peaks): 3190, 1725, 1624, 1554, 1491, 1473, 1377, 1211, 749 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.53 (br s, 1H), 7.64 (d, J = 7.5 Hz, 1H), 7.55 (s, 1H), 7.54 – 7.50 (m, 1H), 7.43 (t, J = 7.7 Hz, 1H), 7.41 – 7.35 (m, 2H), 7.23 (d, J = 8.7 Hz, 2H), 7.11 (t, J = 7.5 Hz, 1H), 6.96 – 6.88 (m, 3H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.51, 154.77, 143.83, 136.63, 134.93, 133.28, 133.03, 131.54, 131.40, 130.14 (2CH), 129.25 (2CH), 128.77, 127.63, 127.54, 127.52, 125.03, 124.02, 111.86, 99.15 (Cspiro). Anal. Calcd for $C_{21}H_{13}Cl_2N_3O_2 \cdot 0.45H_2O$: C 60.29, H 3.36, N 10.05, found: C 59.97, H 3.06, N 9.95.

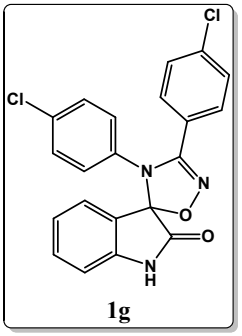


1f

3',4'-bis(4-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1g).

Synthesized according to the general procedure, and starting with **2c** and **3c**, this compound was obtained as a white solid (135.8 mg, 85 % yield).

Mp: 104-106 ºC; **IR** (KBr, selected peaks): 3251, 1735, 1621, 1492, 1473, 1364, 1203, 1093, 828 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.53 (br s, 1H), 7.61 (d, J = 7.4 Hz, 1H), 7.54 – 7.43 (m, 4H), 7.38 (t, J = 7.7 Hz, 1H), 7.23 (d, J = 8.6 Hz, 2H), 7.11 (t, J = 7.5 Hz, 1H), 6.93 (d, J = 7.8 Hz, 1H), 6.88 (d, J = 8.6 Hz, 2H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.62, 154.98, 143.88, 137.02, 136.74, 133.24, 132.92, 130.71 (2CH), 130.11 (2CH), 129.83 (2CH), 129.21 (2CH), 127.46, 125.11, 124.42, 124.00, 111.87, 99.07 (Cspiro). Anal. Calcd for $C_{21}H_{13}Cl_2N_3O_2 \cdot 0.6H_2O$: C 59.90, H 3.41, N 9.99, found: C 60.28, H 3.54, N 9.61.

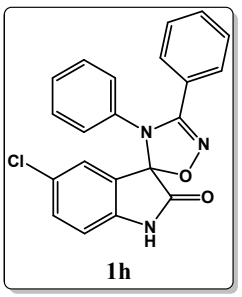


1g

5-chloro-3',4'-diphenyl-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazoline]-2-one (1h).

Synthesized according to the general procedure, and starting with **2d** and **3a**, this compound was obtained as a white solid (111.0 mg, 76 % yield).

Mp: 232-234 ºC; **IR** (KBr, selected peaks): 3242, 1743, 1620, 1498, 1474, 1394, 1213, 828, 752 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.59 (br s, 1H), 7.70 (d, J = 1.6 Hz, 1H), 7.51 – 7.43 (m, 3H), 7.40 – 7.34 (m, 3H), 7.22 – 7.11 (m, 3H), 6.96 – 6.90 (m, 3H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.69, 155.96, 142.49, 137.74, 132.86, 131.42, 130.02 (2CH), 129.41 (2CH), 129.13 (2CH),

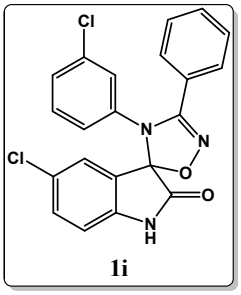


1h

128.59, 127.94, 127.78 (2CH), 127.64, 127.47, 125.69, 113.21, 98.73 (Cspiro). Anal. Calcd for $C_{21}H_{14}ClN_3O_2 \cdot 0.6H_2O$: C 65.23, H 3.97, N 10.87, found: C 64.88, H 3.71, N 10.66.

5-chloro-4'-(3-chlorophenyl)-3'-phenyl-4'*H*-spiro[indoline-3,5'-[1,2,4] oxadiazol]-2-one (1i).

Synthesized according to the general procedure, and starting with **2e** and **3a**, this compound was obtained as a white solid (78.6 mg, 56 % yield).

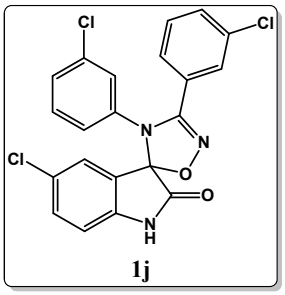


1i

Mp: 186-188 ºC; **IR** (KBr, selected peaks): 3213, 1743, 1620, 1589, 1476, 1385, 1266, 760, 692 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.67 (br s, 1H), 7.75 (s, 1H), 7.52 (d, J = 7.4 Hz, 2H), 7.49 (d, J = 7.2 Hz, 1H), 7.46 – 7.37 (m, 3H), 7.26 – 7.15 (m, 2H), 6.99 (d, J = 8.3 Hz, 1H), 6.93 (s, 1H), 6.87 (d, J = 7.4 Hz, 1H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.33, 155.57, 142.43, 139.24, 134.79, 133.13, 131.69, 131.43, 129.59 (2CH), 129.17 (2CH), 128.79, 127.90, 127.50, 127.28, 127.18, 125.95, 125.29, 113.39, 98.61 (Cspiro). Anal. Calcd for $C_{21}H_{13}Cl_2N_3O_2 \cdot 0.15H_2O$: C 61.07, H 3.25, N 10.18, found: C 60.78, H 3.15, N 10.06.

5-chloro-3',4'-bis(3-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1j).

Synthesized according to the general procedure, and starting with **2e** and **3b**, this compound was obtained as a white solid (82.2 mg, 54 % yield).

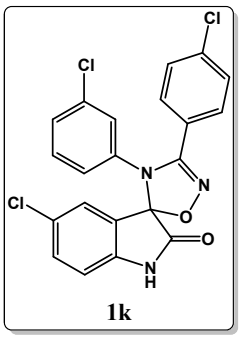


1j

Mp: 215-217 ºC; **IR** (KBr, selected peaks): 3211, 1735, 1617, 1593, 1560, 1476, 1373, 854, 819 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.69 (br s, 1H), 7.78 (d, J = 1.6 Hz, 1H), 7.58 (d, J = 0.8 Hz, 1H), 7.56 – 7.50 (m, 1H), 7.47 – 7.40 (m, 3H), 7.28 – 7.19 (m, 2H), 7.01 – 6.96 (m, 2H), 6.92 (d, J = 7.6 Hz, 1H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.18, 154.59, 142.47, 138.84, 134.94 (2Cq), 133.26, 131.70, 131.60, 131.40, 128.95, 128.83, 128.26, 127.69, 127.65, 127.38, 127.30, 126.93, 126.16, 113.44, 98.87 (Cspiro). Anal. Calcd for $C_{21}H_{12}Cl_3N_3O_2 \cdot 0.15H_2O$: C 56.37, H 2.78, N 9.39, found: C 56.00, H 2.70, N 9.15.

5-chloro-4'-(3-chlorophenyl)-3'-(4-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1k).

Synthesized according to the general procedure, and starting with **2e** and **3c**, this compound was obtained as a white solid (88.4 mg, 58 % yield).

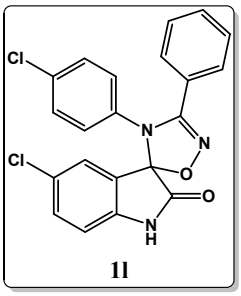


1k

Mp: 241-243 ºC; **IR** (KBr, selected peaks): 3178, 1744, 1616, 1590, 1475, 1384, 1197, 760, 690 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.69 (br s, 1H), 7.75 (d, J = 1.7 Hz, 1H), 7.54 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 8.5 Hz, 2H), 7.42 (dd, J = 8.3, 1.7 Hz, 1H), 7.27 – 7.19 (m, 2H), 6.99 (d, J = 8.3 Hz, 1H), 6.95 (s, 1H), 6.89 (d, J = 7.5 Hz, 1H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.24, 154.81, 142.50, 138.99, 137.20, 134.93, 133.23, 131.59, 130.87 (2CH), 129.86 (2CH), 128.82, 128.17, 127.59, 127.33, 127.01, 126.16, 124.13, 113.44, 98.79 (Cspiro). Anal. Calcd for $C_{21}H_{12}Cl_3N_3O_2 \cdot 0.15H_2O$: C 56.37, H 2.78, N 9.39, found: C 56.00, H 2.83, N 9.04.

5-chloro-4'-(4-chlorophenyl)-3'-phenyl-4'*H*-spiro[indoline-3,5'-[1,2,4] oxadiazol]-2-one (1l).

Synthesized according to the general procedure, and starting with **2f** and **3a**, this compound was obtained as a white solid (97.5 mg, 69% yield).



1l

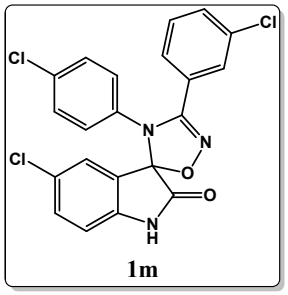
Mp: 274-276 ºC; **IR** (KBr, selected peaks): 3176, 1748, 1617, 1491, 1385, 1248, 1198, 1089, 696 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.61 (br s, 1H), 7.73 (d, J = 1.7 Hz, 1H), 7.53 – 7.45 (m, 3H), 7.44 – 7.37 (m, 3H), 7.24 (d, J = 8.6 Hz, 2H), 6.97 (d, J = 8.4 Hz, 1H), 6.93 (d, J = 8.6 Hz, 2H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.38, 155.78, 142.49, 136.69, 133.07, 133.01, 131.62, 130.12 (2CH), 129.56 (2CH), 129.34 (2CH), 129.16 (2CH), 128.75, 127.48, 127.36, 125.36, 113.36, 98.69 (Cspiro). Anal. Calcd for $C_{21}H_{13}Cl_2N_3O_2 \cdot 0.15H_2O$: C 61.07, H 3.25, N 10.18, Found: C 60.75, H 3.22, N 10.25.

5-chloro-3'-(3-chlorophenyl)-4'-(4-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1m).

Synthesized according to the general procedure, and starting with **2f** and **3b**, this compound was obtained as a white solid (100.9 mg, 66 % yield).

Mp: 206-208 ºC; **IR** υ_{max} (KBr, selected peaks): 3176, 1747, 1620, 1493, 1478, 1385, 1198, 1096, 828 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.65 (br s, 1H), 7.77

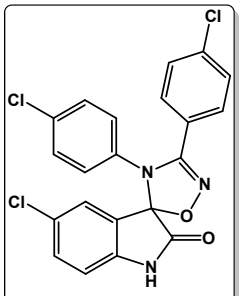
(s, 1H), 7.56 (s, 1H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.46 – 7.38 (m, 3H), 7.27 (d, *J* = 8.6 Hz, 2H), 7.00 – 6.95 (m, 3H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.20, 154.80, 142.48, 136.28, 134.91, 133.33, 133.19, 131.62, 131.39, 130.28 (2CH), 129.43 (2CH), 128.92, 128.79, 127.66, 127.61, 127.36, 127.07, 113.41, 98.92 (Cspiro). Anal. Calcd for $C_{21}H_{12}Cl_3N_3O_2 \cdot 0.7H_2O$: C 55.14, H 2.96, N 9.19, found: C 54.82, H 2.63, N 8.99.



1m

5-chloro-3',4'-bis(4-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1n).

Synthesized according to the general procedure, and starting with **2f** and **3c**, this compound was obtained as a white solid (98.2 mg, 64 % yield).

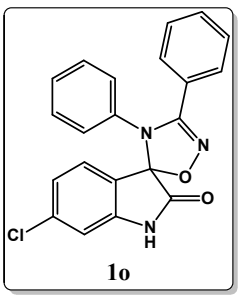


1n

Mp: 218-220 ºC; **IR** (KBr, selected peaks): 3179, 1747, 1616, 1492, 1414, 1388, 1194, 1090, 829 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.62 (br s, 1H), 7.73 (d, *J* = 1.7 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 2H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.41 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.26 (d, *J* = 8.6 Hz, 2H), 6.97 (d, *J* = 8.3 Hz, 1H), 6.95 (d, *J* = 8.6 Hz, 2H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.27, 155.00, 142.51, 137.13, 136.41, 133.24, 133.16, 130.82 (2CH), 130.26 (2CH), 129.82 (2CH), 129.37 (2CH), 128.77, 127.55, 127.16, 124.16, 113.41, 98.85 (Cspiro). Anal. Calcd for $C_{21}H_{12}Cl_3N_3O_2$: C 56.71, H 2.73, N 9.45, found: C 56.97, H 2.75, N 9.31.

6-chloro-3',4'-diphenyl-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1o).

Synthesized according to the general procedure, and starting with **2g** and **3a**, this compound was obtained as a white solid (118.1 mg, 81 % yield).

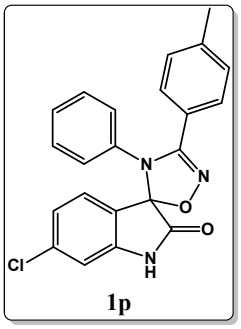


1o

Mp: 245-247 ºC; **IR** (KBr, selected peaks): 3201, 1748, 1621, 1595, 1494, 1385, 1213, 756, 690 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.62 (br s, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.50 – 7.42 (m, 3H), 7.41 – 7.34 (m, 2H), 7.21 – 7.13 (m, 3H), 7.11 (d, *J* = 8.0 Hz, 1.6 Hz, 1H), 6.95 (d, *J* = 1.6 Hz, 1H), 6.92 – 6.87 (m, 2H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.84, 155.99, 145.14, 137.98, 137.87, 131.44, 130.03 (2CH), 129.45 (2CH), 129.06 (2CH), 128.88, 127.91, 127.79 (2CH), 125.78, 124.36, 123.78, 112.08, 98.47 (Cspiro). Anal. Calcd for $C_{21}H_{14}ClN_3O_2 \cdot 0.6H_2O$: C 65.23, H 3.97, N 10.87, found: C 64.88 H 3.71, N 10.66.

6-chloro-3'-(4-methylphenyl)-4'-phenyl-4'*H*-spiro[indoline-3,5'-[1,2,4] oxadiazol]-2-one (1p).

Synthesized according to the general procedure, and starting with **2g** and **3d**, this compound was obtained as a white solid (106.3 mg, 70 % yield).

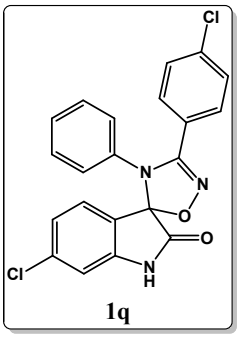


1p

Mp: 269-271 ºC; **IR** (KBr, selected peaks): 3277, 1737, 1614, 1385, 1316, 1203, 1143, 812, 751 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.59 (br s, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.34 (d, J = 7.9 Hz, 2H), 7.21 – 7.13 (m, 5H), 7.11 (d, J = 8.0 Hz, 1H), 6.94 (s, 1H), 6.89 (d, J = 7.6 Hz, 2H), 2.32 (s, 3H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.95, 155.95, 145.20, 141.70, 138.02, 137.93, 130.06 (2CH), 130.01 (2CH), 129.00 (2CH), 128.86, 127.86, 127.83 (2CH), 124.49, 123.76, 122.87, 112.07, 98.39 (Cspiro) 21.34. Anal. Calcd for $C_{22}H_{16}ClN_3O_2 \cdot 0.3H_2O$: C 66.85, H 4.24, N 10.63, found: C 66.51, H 4.17, N 10.41.

6-chloro-3'-(4-chlorophenyl)-4'-phenyl-4'*H*-spiro[indoline-3,5'-[1,2,4] oxadiazol]-2-one (1q).

Synthesized according to the general procedure, and starting with **2g** and **3c**, this compound was obtained as a white solid (131.1 mg, 82 % yield).



1q

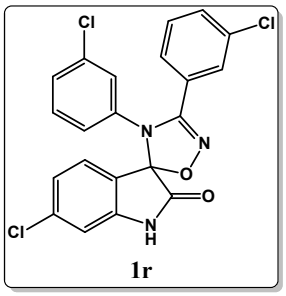
Mp: 125-127 ºC; **IR** (KBr, selected peaks): 3234, 1735, 1616, 1446, 1406, 1384, 1207, 1142, 701 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.60 (br s, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.49 (d, J = 8.6 Hz, 2H), 7.45 (d, J = 8.6 Hz, 2H), 7.26 – 7.17 (m, 3H), 7.14 (d, J = 8.0 Hz, 1H), 6.97 (s, 1H), 6.92 (d, J = 7.6 Hz, 2H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.76, 155.21, 145.23, 138.08, 137.59, 136.93, 130.70 (2CH), 130.17 (2CH), 129.71 (2CH), 128.94, 128.13, 127.83 (2CH), 124.58, 124.15, 123.81, 112.15, 98.63 (Cspiro). Anal. Calcd for $C_{21}H_{13}Cl_2N_3O_2 \cdot 0.55H_2O$: C 60.03, H 3.39, N 10.00, found: C 59.71, H 3.27, N 9.62.

6-chloro-3',4'-bis(3-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazoline]-2-one (1r).

Synthesized according to the general procedure, and starting with **2h** and **3b**, this compound was obtained as a white solid (114.3 mg, 75 % yield).

Mp: 209-211 ºC; **IR** (KBr, selected peaks): 3186, 1731, 1617, 1484, 1384, 1267, 1205, 821 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.74 (br s, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.56 (s, 1H), 7.54 (d, J = 7.7 Hz, 1H), 7.48 – 7.39 (m, 2H), 7.28 – 7.19 (m,

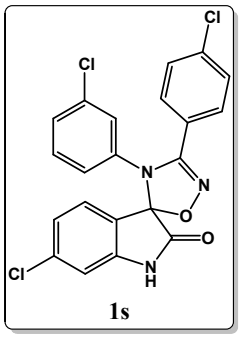
2H), 7.15 (d, *J* = 8.0 Hz, 1H), 7.00 (s, 1H), 6.94 (s, 1H), 6.89 (d, *J* = 7.4 Hz, 1H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.39, 154.63, 145.21, 138.97, 138.41, 134.97, 134.95, 131.71, 131.63, 131.45, 129.02, 128.84, 128.23, 127.61, 127.39, 127.37, 126.14, 124.01, 123.61, 112.35, 98.62 (Cspiro). Anal. Calcd for $C_{21}H_{12}Cl_3N_3O_2$: C 56.71, H 2.73, N 9.45, found: C 56.42, H 2.70, N 9.27.



1r

6-chloro-4'-(3-chlorophenyl)-3'-(4-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazoline]-2-one (1s).

Synthesized according to the general procedure, and starting with **2h** and **3c**, this compound was obtained as a white solid (119.0 mg, 78 % yield).

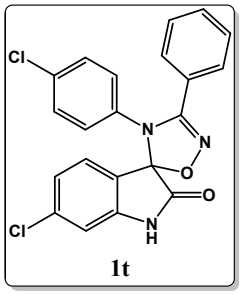


1s

Mp: 229-231 C; **IR** (KBr, selected peaks): 3267, 1736, 1617, 1405, 1384, 1142, 1099, 693 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.65 (br s, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.29 – 7.18 (m, 2H), 7.14 (d, *J* = 8.0 Hz, 1H), 7.00 (s, 1H), 6.91 (s, 1H), 6.86 (d, *J* = 7.1 Hz, 1H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.44, 154.84, 145.21, 139.10, 138.37, 137.20, 134.92, 131.60, 130.77 (2CH), 129.89 (2CH), 128.96, 128.14, 127.34, 126.13, 124.18, 124.00, 123.68, 112.33, 98.54 (Cspiro). Anal. Calcd for $C_{21}H_{12}Cl_3N_3O_2 \cdot 0.9H_2O$: C 54.72, H 3.02, N 9.12, found: C 54.78 H 2.65, N 8.72.

6-chloro-4'-(4-chlorophenyl)-3'-phenyl-4'*H*-spiro[indoline-3,5'-[1,2,4] oxadiazol]-2-one (1t).

Synthesized according to the general procedure, and starting with **2i** and **3a**, this compound was obtained as a white solid (112.2 mg, 80 % yield).

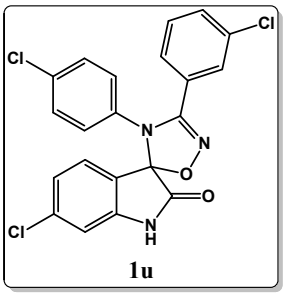


1t

Mp: 226-228 ºC; **IR** (KBr, selected peaks): 3231, 1731, 1617, 1384, 1211, 1141, 821, 692 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.62 (br s, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.52 – 7.45 (m, 3H), 7.44 – 7.38 (m, 2H), 7.23 (d, *J* = 8.6 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 1H), 6.98 (s, 1H), 6.89 (d, *J* = 8.6 Hz, 2H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.57, 155.81, 145.21, 138.20, 136.84, 132.96, 131.64, 130.14 (2CH), 129.61 (2CH), 129.30 (2CH), 129.10 (2CH), 128.90, 125.46, 124.08, 123.95, 112.27, 98.43 (Cspiro). Anal. Calcd for $C_{21}H_{13}Cl_2N_3O_2 \cdot 0.55H_2O$: C 60.03, H 3.39, N 10.00, Found: C 59.71 H 3.07, N 9.82.

6-chloro-3'-(3-chlorophenyl)-4'-(4-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1u).

Synthesized according to the general procedure, and starting with **2i** and **3b**, this compound was obtained as a white solid (122.5 mg, 80 % yield).

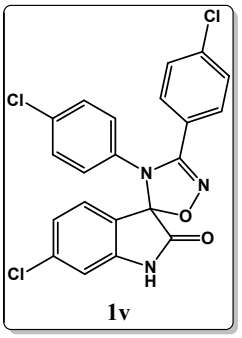


1u

Mp: 214-216 ºC; **IR** (KBr, selected peaks): 3261, 1733, 1617, 1492, 1431, 1384, 830, 749 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.71 (br s, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.56 – 7.50 (m, 2H), 7.44 (t, J = 7.6 Hz, 1H), 7.39 (d, J = 7.6 Hz, 1H), 7.26 (d, J = 8.7 Hz, 2H), 7.15 (dd, J = 8.0, 1.7 Hz, 1H), 6.98 (d, J = 1.6 Hz, 1H), 6.94 (d, J = 8.7 Hz, 2H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.42, 154.83, 145.25, 138.33, 136.42, 134.95, 133.28, 131.64, 131.44, 130.30 (2CH), 129.38 (2CH), 129.01, 128.82, 127.59, 127.45, 123.98, 123.78, 112.33, 98.67 (Cspiro) ppm. Anal. Calcd for $C_{21}H_{12}Cl_3N_3O_2 \cdot 0.10H_2O$: C 56.49, H 2.76, N 9.41, found: C 56.11, H 2.83, N 9.20.

6-chloro-3',4'-bis(4-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1v).

Synthesized according to the general procedure, and starting with **2i** and **3c**, this compound was obtained as a white solid (120.7 mg, 79 % yield).



1v

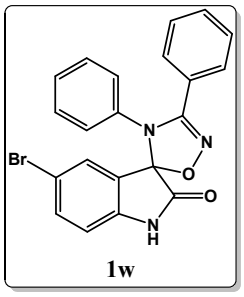
Mp: 222-224 ºC; **IR** (KBr, selected peaks): 3242, 1736, 1616, 1496, 1383, 1207, 1139, 830 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.62 (br s, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.50 (d, J = 8.6 Hz, 2H), 7.46 (d, J = 8.6 Hz, 2H), 7.25 (d, J = 8.6 Hz, 2H), 7.14 (d, J = 8.0 Hz, 1H), 6.98 (s, 1H), 6.91 (d, J = 8.6 Hz, 2H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.45, 155.02, 145.22, 138.29, 137.13, 136.53, 133.17, 130.73 (2CH), 130.26 (2CH), 129.86 (2CH), 129.31 (2CH), 128.95, 124.22, 123.97, 123.83, 112.30, 98.57 (Cspiro). Anal. Calcd for $C_{21}H_{12}Cl_3N_3O_2 \cdot 0.5H_2O$: C 55.59, H 2.89, N 9.26, found: C 55.48 H 2.81, N 8.88.

5-bromo-3',4'-diphenyl-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1w).

Synthesized according to the general procedure, and starting with **2j** and **3a**, this compound was obtained as a white solid (101.9 mg, 73 % yield).

Mp: 246-248 ºC; **IR** (KBr, selected peaks): 3250, 1735, 1617, 1444, 1385, 1142, 1068, 823 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.61 (br s, 1H), 7.82 (d, J = 1.7 Hz, 1H), 7.53 (dd, J = 8.3, 1.8 Hz, 1H), 7.51 – 7.43 (m, 3H), 7.41 – 7.34 (m, 2H), 7.22 –

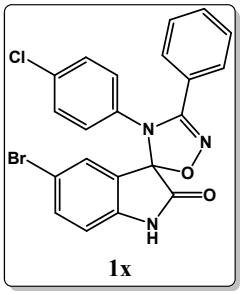
7.11 (m, 3H), 6.96 – 6.86 (m, 3H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.51, 155.97, 142.96, 137.76, 135.78, 131.43, 130.29, 130.04 (2CH), 129.42 (2CH), 129.15 (2CH), 128.02, 127.96, 127.80 (2CH), 125.71, 115.72, 113.67, 98.68 (Cspiro). Anal. Calcd for $C_{21}H_{14}BrN_3O_2$: C 60.01, H 3.36, N 10.00, found: C 60.09, H 3.18, N 10.28.



1w

5-bromo-4'-(4-chlorophenyl)-3'-phenyl-4'*H*-spiro[indoline-3,5'-[1,2,4] oxadiazol]-2-one (1x).

Synthesized according to the general procedure, and starting with **2k** and **3a**, this compound was obtained as a white solid (92.1 mg, 68 % yield).

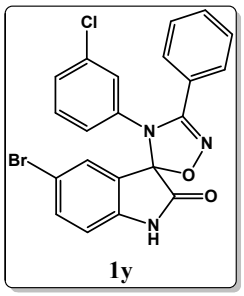


1x

Mp: 290-292 ºC; **IR** (KBr, selected peaks): 3174, 1748, 1619, 1491, 1473, 1392, 1253, 1197, 694 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 10.00 (br s, 1H), 7.82 (s, 1H), 7.54 (d, J = 7.9 Hz, 1H), 7.51 – 7.44 (m, 3H), 7.43 – 7.36 (m, 2H), 7.22 (d, J = 8.0 Hz, 2H), 6.95 – 6.88 (m, 3H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.21, 155.79, 142.94, 136.70, 135.98, 133.02, 131.63, 130.30, 130.13 (2CH), 129.57 (2CH), 129.34 (2CH), 129.18 (2CH), 127.72, 125.37, 115.87, 113.81, 98.63 (Cspiro). Anal. Calcd for $C_{21}H_{13}BrClN_3O_2 \cdot 0.25H_2O$: C 54.92, H 2.97, N 9.15, found: C 54.54, H 2.87, N 8.88.

5-bromo-4'-(3-chlorophenyl)-3'-phenyl-4'*H*-spiro[indoline-3,5'-[1,2,4] oxadiazol]-2-one (1y).

Synthesized according to the general procedure, and starting with **2l** and **3a**, this compound was obtained as a white solid (84.0 mg, 62 % yield).

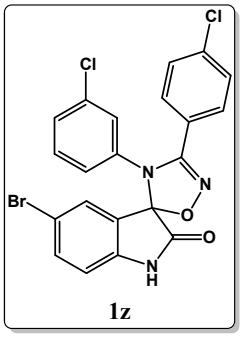


1y

Mp: 208-210 ºC; **IR** (KBr, selected peaks): 3132, 1749, 1617, 1590, 1475, 1384, 1195, 687 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.66 (br s, 1H), 7.87 (d, J = 1.6 Hz, 1H), 7.56 (dd, J = 8.3 Hz, 1.6 Hz, 1H) 7.54 – 7.46 (m, 3H), 7.45 – 7.38 (m, 2H), 7.25 – 7.15 (m, 2H), 6.94 (d, J = 8.4 Hz, 1H), 6.93 (s, 1H), 6.87 (d, J = 7.6 Hz, 1H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.15, 155.57, 142.86, 139.24, 136.04, 134.79, 131.69, 131.43, 130.31, 129.59 (2CH), 129.18 (2CH), 127.91, 127.54, 127.29, 125.95, 125.29, 115.92, 113.82, 98.55 (Cspiro). Anal. Calcd for $C_{21}H_{13}BrClN_3O_2$: C 55.47, H 2.89, N 9.24, found: C 55.52, H 2.91, N 9.07.

5-bromo-3'-(4-chlorophenyl)-4'-(3-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1z).

Synthesized according to the general procedure, and starting with **2l** and **3c**, this compound was obtained as a white solid (87.5 mg, 60 % yield).

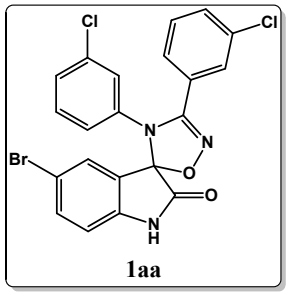


1z

Mp: 256-258 ºC; **IR** (KBr, selected peaks): 3173, 1745, 1617, 1590, 1474, 1384, 1197, 760, 690 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.70 (s, 1H), 7.87 (s, 1H), 7.59 – 7.55 (m, 1H), 7.54 (d, J = 8.7 Hz, 2H), 7.47 (d, J = 8.7 Hz, 2H), 7.28 – 7.17 (m, 2H), 6.96 – 6.92 (m, 2H), 6.89 (d, J = 7.6 Hz, 1H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.07, 154.81, 142.94, 138.99, 137.20, 136.14, 134.93, 131.59, 130.87 (2CH), 130.40, 129.85 (2CH), 128.17, 127.36, 127.33, 126.14, 124.12, 115.93, 113.87, 98.73 (Cspiro). Anal. Calcd for $C_{21}H_{12}BrCl_2N_3O_2 \cdot 0.4H_2O$: C 50.81, H 2.60, N 8.47, found: C 50.43, H 2.48, N 8.23.

5-bromo-3',4'-bis(3-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1aa).

Synthesized according to the general procedure, and starting with **2l** and **3b**, this compound was obtained as a white solid (88.9 mg, 61 % yield).



1aa

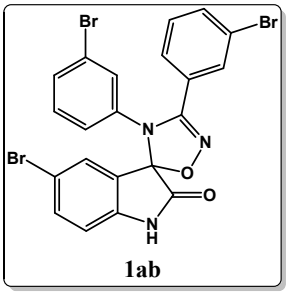
Mp: 246-248 ºC; **IR** (KBr, selected peaks): 3210, 1734, 1617, 1592, 1474, 1437, 1375, 818 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.74 (br s, 1H), 7.90 (s, 1H), 7.60 – 7.50 (m, 2H), 7.58 (s, 1H), 7.48 – 7.41 (m, 2H), 7.29 – 7.19 (m, 2H), 6.99 – 6.89 (m, 3H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.06, 154.60, 142.96, 138.84, 136.17, 134.94, 134.93, 131.70, 131.61, 131.41, 130.45, 128.96, 128.27, 127.71, 127.40, 127.30, 127.28, 126.15, 115.94, 113.89, 98.81 (Cspiro). MS (ESI) *m/z* calcd for $C_{21}H_{12}BrCl_2N_3O_2$, 487; found 488 $[M + H]^+$. Anal. Calcd for $C_{21}H_{12}BrCl_2N_3O_2 \cdot 0.2H_2O$: C 51.18, H 2.54, N 8.53, found: C 50.81, H 2.48, N 8.31.

5-bromo-3',4'-bis(3-bromophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1ab).

Synthesized according to the general procedure, and starting with **2m** and **3e**, this compound was obtained as a white solid (102.2 mg, 67 % yield).

Mp: 207-209 ºC; **IR** (KBr, selected peaks): 3230, 1734, 1616, 1589, 1473, 1438, 1372, 1199, 819 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.75 (br s, 1H), 7.91 (d, J =

1.5 Hz, 1H), 7.74 (s, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.57 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.47 (d, *J* = 7.8 Hz, 1H), 7.41 – 7.34 (m, 2H), 7.19 (t, *J* = 8.1 Hz, 1H), 7.13 (s, 1H), 6.99 – 6.92 (m, 2H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.03, 154.44, 142.92, 138.92, 136.17, 134.65, 131.87, 131.83, 131.59, 131.20, 130.48, 130.31, 128.10, 127.49, 127.26, 126.56, 122.87, 122.81, 115.95, 113.87, 98.82 (Cspiro). MS (ESI) *m/z* calcd for $C_{21}H_{12}Br_3N_3O_2$, 575; found 576 $[M + H]^+$. Anal. Calcd for $C_{21}H_{12}Br_3N_3O_2 \cdot 0.05H_2O$: C 43.56, H 2.11, N 7.26, found: C 43.16, H 1.89, N 7.04.

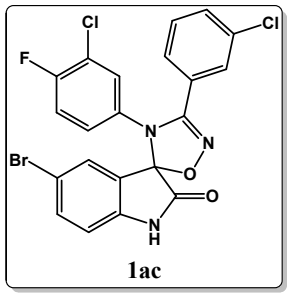


1ab

5-bromo-4'-(3-chloro-4-fluorophenyl)-3'-(3-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1ac).

Synthesized according to the general procedure, and starting with **2n** and **3b**, this compound was obtained as a white solid (78.8 mg, 55 % yield).

Mp: 214-216 ºC; **IR** (KBr, selected peaks): 3174, 1745, 1617, 1500, 1473, 1384, 1193, 825, 760 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.70 (br s, 1H), 7.94 (d, *J* = 1.6 Hz, 1H), 7.59 (s, 1H), 7.57 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.56 – 7.50 (m, 1H) 7.49 – 7.39 (m, 2H), 7.22 (t, *J* = 8.9 Hz, 1H), 7.17 (dd, *J* = 6.5, 2.4 Hz, 1H), 7.06 – 7.00 (m, 1H), 6.94 (d, *J* = 8.3 Hz, 1H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 172.02, 157.62 (d, J_{FC} = 247.0 Hz), 154.74, 142.98, 136.23, 134.96, 134.36 (d, J_{FCCCC} = 4.0 Hz), 131.75, 131.44, 130.51, 130.37, 129.02, 128.99 (d, J_{FCCC} = 7.0 Hz), 127.75, 127.13, 127.06, 121.57 (d, J_{FCC} = 19.0 Hz), 118.24 (d, J_{FCC} = 22.0 Hz), 115.99, 113.92, 98.92 (Cspiro). MS (ESI) *m/z* calcd for $C_{21}H_{11}BrCl_2FN_3O_2$, 505; found 506 $[M + H]^+$. Anal. Calcd for $C_{21}H_{11}BrCl_2FN_3O_2$: C 49.73, H 2.19, N 8.29, found: C 50.03, H 2.49, N 8.11.

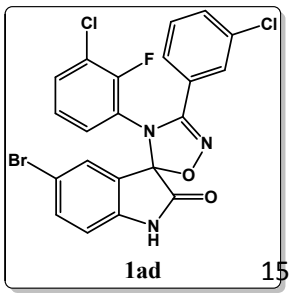


1ac

5-bromo-4'-(3-chloro-2-fluorophenyl)-3'-(3-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1ad).

Synthesized according to the general procedure, and starting with **2o** and **3b**, this compound was obtained as a white solid (81.6 mg, 57 % yield).

Mp: 242-244 ºC; **IR** (KBr, selected peaks): 3289, 1742, 1618, 1560, 1484, 1474, 1436, 1369, 1096, 747 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.74 (br s, 1H), 7.65 (s, 1H), 7.61 (s, 1H), 7.58 – 7.51 (m, 2H), 7.48 – 7.43 (m, 2H), 7.43 – 7.37 (m, 1H), 7.19 – 7.09 (m, 2H), 6.94 (d, *J* = 8.3 Hz, 1H); **^{13}C**

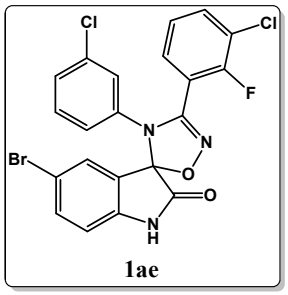


1ad

NMR (100 MHz, Acetone-d_6) δ (ppm): 172.03, 154.79, 154.58 (d, J_{FC} = 249.0 Hz), 143.25, 136.27, 135.06, 131.96, 131.58, 131.04, 130.28, 128.75, 128.38, 127.27, 127,25 (d, J_{FCC} = 12 Hz), 127.13, 126.21 (d, J_{FCCC} = 5 Hz), 126.09, 122.34 (d, J_{FCC} = 16 Hz), 115.53, 113.83, 98.34 (Cspiro). MS (ESI) *m/z* calcd for $C_{21}H_{11}BrCl_2FN_3O_2$, 505; found 506 $[M + H]^+$. Anal. Calcd for $C_{21}H_{11}BrCl_2FN_3O_2 \cdot 0.2H_2O$: C 49.38, H 2.25, N 8.23, found: C 48.99, H 1.87, N 8.12.

5-bromo-3'-(3-chloro-2-fluorophenyl)-4'-(3-chlorophenyl)-4'*H*-spiro[indoline-3,5'-[1,2,4]oxadiazol]-2-one (1ae).

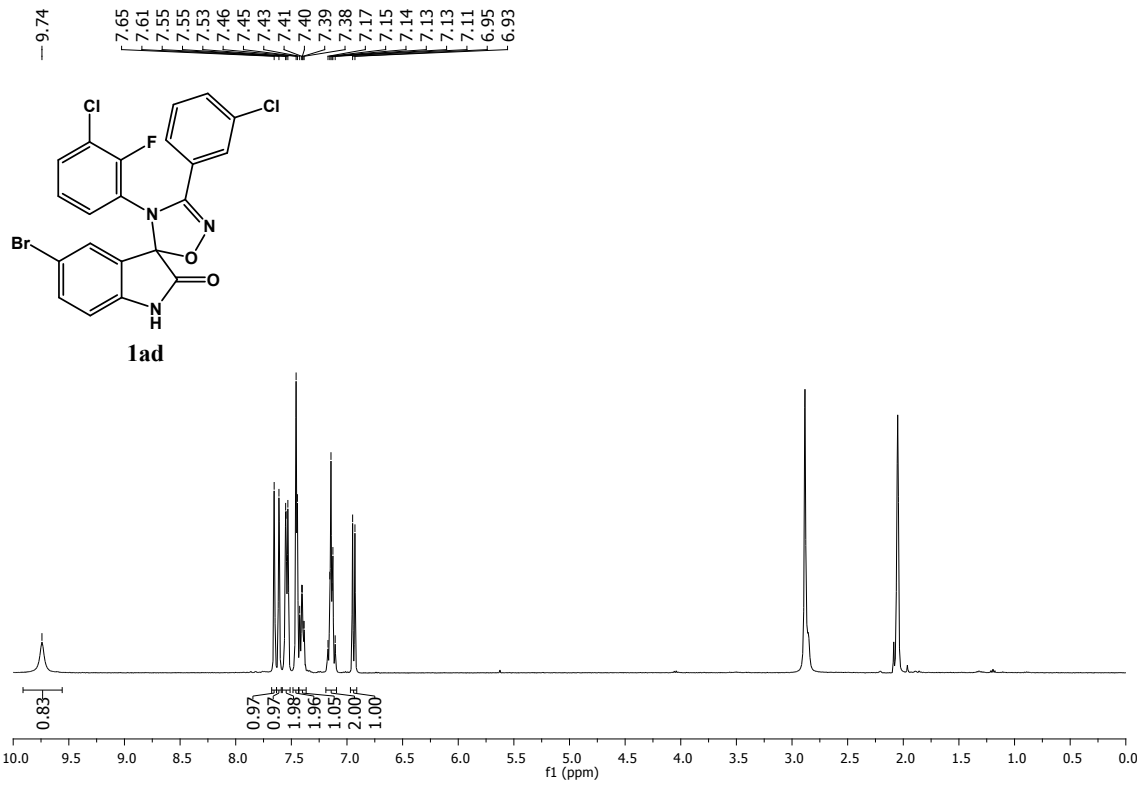
Synthesized according to the general procedure, and starting with **2l** and **3f**, this compound was obtained as a white solid (87.1 mg, 61 % yield).



1ae

Mp: 242-244 ºC; **IR** (KBr, selected peaks): 3171, 1748, 1619, 1476, 1455, 1395, 1194, 826, 687 cm^{-1}; **^{1}H NMR** (400 MHz, Acetone-d_6) δ (ppm): 9.77 (br s. 1H), 7.90 (d, J = 1.6 Hz, 1H), 7.75 – 7.68 (m, 2H), 7.58 (dd, J = 8.3, 1.6 Hz, 1H) 7.37 (t, J = 7.9 Hz, 1H), 7.26 – 7.15 (m, 2H), 6.96 (d, J = 8.3 Hz, 1H), 6.94 (s, 1H), 6.90 (d, J = 7.5 Hz, 1H); **^{13}C NMR** (100 MHz, Acetone-d_6) δ (ppm): 171.80, 156.15 (d, J_{FC} = 227.0 Hz), 151.35, 142.91, 138.29, 136.32, 134.94, 134.49, 131.68, 130.91 (d, J_{FCCC} = 1 Hz), 130.20, 128.19, 127.10, 126.69, 126.25, 125.04, 122.34 (d, J_{FCC} = 17 Hz), 116.03, 115.26 (d, J_{FCC} = 14 Hz), 114.02, 98.54 (Cspiro). MS (ESI) *m/z* calcd for $C_{21}H_{11}BrCl_2FN_3O_2$, 505; found 506 $[M + H]^+$. Anal. Calcd for $C_{21}H_{11}BrCl_2FN_3O_2$: C 49.73, H 2.19, N 8.29, found: C 49.63, H 1.99, N 8.01.

^{1}H-NMR SPECTRUM OF DERIVATIVE 1AD



1ad

2. EXPERIMENTAL SECTION: BIOLOGY.

HCT116 cells were grown in McCoy's 5A supplemented with 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) and 1% penicillin/streptomycin solution (Sigma-Aldrich, St Louis, MO, USA). SW620 cells were grown in DMEM (Gibco) supplemented with 10% FBS and 1% antibiotic/antimycotic solution (Sigma-Aldrich). HepG2 human hepatoma cells were grown in DMEM (Gibco) supplemented with 10% FBS, 1% non-essential amino acids and 1% antibiotic/antimycotic solution (Sigma-Aldrich). Cells were maintained at 37 ºC in a humidified atmosphere of 5% CO_2. For cell viability assays cells were seeded on 96-well plates at 1 x 10^5 cells/mL, and for Western blot and flow cytometry analysis cells were seeded on 100mm and 35mm plates, respectively, at 3 × 10^5 cells/mL. HCT116 human colorectal carcinoma cells rendered p53-null by somatic knockout [1] were a kind gift from Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD).

2.1. *In vitro* anti-proliferative assay.

The cellular growth inhibitory activity was evaluated in four cell lines: human hepatocellular carcinoma cell line [HepG2 (wild-type p53)], an isogenic matched pair of wild type p53 and deleted human colorectal cancer cell lines [HCT116 $p53^{(+/+)}$ and $p53^{(-/-)}$], and human colorectal adenocarcinoma cell lines [SW620 (mut p53)]. Cells were incubated with vehicle or the compounds approximately 24 h after plating. Compounds were dissolved in DMSO and diluted in culture medium to a range of concentrations from 0.5 to 200 μM (at least twelve different concentrations were used). The final concentration of DMSO in culture medium during treatment did not exceed 0.8% (v/v) and the same concentration of DMSO was added to the control. Each compound concentration and DMSO was tested in duplicate in a single experiment which was repeated at least 3 times. Cells were incubated at 37 ºC in humidified 5% CO_2 atmosphere. Cell viability was assessed 72 h after compound incubation by using the CellTiter96® AQueous Non-Radioactive Cell Proliferation Assay (Promega Corporation, Madison, WI, USA), according to the manufacturer's protocol. The method is based on the reduction of MTS tetrazolium compound by viable cells to generate a colored formazan product that is soluble in cell culture media. The absorbance was measured at 490 nm using Bio-Rad microplate reader Model 680 (Bio-Rad, Hercules, CA, USA). Nutlin-3a was used as positive control. The concentrations of the compounds that inhibited cell growth by 50% (GI_{50}) were determined by non-linear regression using GraphPad PRISM software.

2.2. Western blot analysis.

Total protein extracts from HCT116 *p53*$^{(+/+)}$ cells incubated with vehicle, compound **1aa**, and nutlin-3a at 5 and 10 μM for 24 h, were prepared following standard protocols.[2] Protein concentrations were determined using the Bio-Rad protein assay kit, according to the manufacturer's specifications. 60 μg of total protein extracts were separated on 8 and 14% (w/v) sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis. After electrophoretic transfer onto nitrocellulose membranes, and blocking with a 5% (w/v) non-fat dry milk solution, membranes were incubated overnight at 4-8 ºC with primary mouse monoclonal antibodies reactive to p53 (DO-1; sc-126; 1:200), Mdm2 (SMP14; sc-965; 1:200), and rabbit polyclonal antibody reactive to PARP-1/2 (H-250; sc-7150; 1:1000), and p-p53 (Ser 15) (sc-101762; 1:200) (Santa Cruz Biotechnology, Santa Cruz, CA). Finally, a secondary goat anti-mouse or anti-rabbit IgG antibody conjugated with horseradish peroxidase (BioRad Laboratories, Hercules, CA, USA) was added for 3 h at room temperature. The membranes were processed for protein detection using the Immobilon Western Chemiluminescent HRP substrate (Millipore Corporation, Billerica, MA, USA). β-Actin (AC-15; Sigma-Aldrich; 1:8000) was used as a loading control. The relative intensities of protein bands were analyzed using the ImageLab 5.1 densitometric analysis program (Bio-Rad) and normalized to the corresponding loading control.

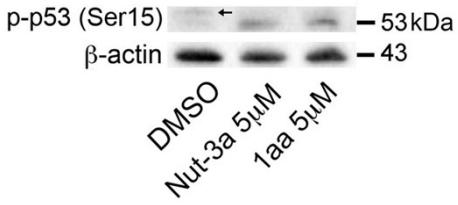


Figure. Compound **1aa** does not induce p53 Ser^{15} phosphorylation. Representative immunoblots of phospho-Ser15-p53 phosphorylation status. HCT116 *p53*$^{(+/+)}$ cells were incubated with vehicle, Nutlin-3a or compound **1aa** at 5 μM for 24 h and total proteins were processed for immunoblot analysis. Blots were normalized to endogenous *β*-actin.

2.3. Luciferase reporter assay

p53 transcriptional activation was assessed based on luciferase reporter constructs harboring the *p21/WAF1* (WWP-Luc; #16451) or the *PUMA* (PUMA Frag1-Luc; #16591) promoter (Addgene, Cambridge, MA), both comprising p53 responsive elements. The empty pBV-Luc vector was used as negative control (plasmid #16539; Addgene). Renilla luciferase activity was measured for transfection efficiency

normalization by co-transfecting cells with the pRL-SV40 vector (Promega). Briefly, HCT116 *p53*$^{(+/+)}$ cells were seeded at 3 x 10^5 cells/well on 12-well plates, and co-transfected with 250 ng of luciferase reporter constructs and 5 ng of pRL-SV40 vector, using Lipofectamine 3000 (Invitrogen). Cells were treated 24 h after transfection with 10 μM nutlin-3a and compound **1aa**. Reporter assays were performed 24 h post-treatment using the Dual-Luciferase Reporter Assay System (Promega).

2.4. Evaluation of caspase-3/7 activity

Caspase-3 and -7 activities were measured using the Caspase-Glo 3/7 Assay (Promega). This assay is based on the cleavage of a pro luminescent substrate containing the specific DEVD sequence recognized by caspase-3 and -7 to release aminoluciferin in cell lysates. The subsequent luciferase cleavage of the unconjugated aminoluciferin generates a luminescent signal directly proportional to the amount of caspase activity present in the sample. Equal volumes of total protein extracts and caspase-Glo 3/7 reagent were incubated on a 96-well plate and mixed by orbital shaking for 30 s, as previously described.[3, 4] Subsequently, the mixture was incubated at room temperature for 30 min, leading to stabilization of substrate cleavage by caspases, and accumulation of luminescent signal. The resulting luminescence was measured using the GloMax-Multi$^+$ Detection System (Promega).

2.5. Bimolecular Fluorescence Complementation (BiFC) assay.

HCT116 p53$^{(-/-)}$ cells were co-transfected using 1 μg of each BiFC pair plasmid and Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific). 4–6 h after transfection, the medium was replaced with fresh medium, and derivatives **1aa**, and nutlin-3a to a final concentration of 5, 10 and 20 μM. The same concentrations of DMSO were tested as control. Cells were washed twice with Ca^{2+}- and Mg^{2+}-free phosphate buffered saline (PBS) (Invitrogen), treated with StemPro Accutase (Gibco) and harvested in culture medium. Cell suspensions were centrifuged, supernatants discarded, and cell pellets resuspended in PBS[5]. Fluorescence was measured using Guava® easyCyte™ 5HT Flow Cytometer.

3. EXPERIMENTAL SECTION: STABILITY.

3.1. HPLC analysis.

High-performance liquid chromatography (HPLC) measurements were carried out using a VWR HITACHI assembly equipped with a UV detector L-2400, a column oven L-2300, and a pump L-2130. An injection valve equipped with 20 µL sample loop was used. The separation was performed on a LichroCART® RP-18 (5 µm, 250-4 mm) analytical column (Merck). Methanol:H_2O with 1% HCOOH was used as eluent system for compound **1ad** (82.5:17.5). Elution was performed at a solvent flow rate of 1 mL/min. Chromatograms were monitored by UV detection at 268 nm. All analyses were performed at 25 ºC. Acquisition and treatment of data were done using Ezchrom Elite software.

3.2. Stability in pH 7.4 phosphate buffer.

12.5 µL of a 10^{-3} M stock solution of compound **1ad** in DMSO were added to 2.5 mL of potassium phosphate buffer solution (pH 7.4, 0.5M) at 37ºC. At appropriate intervals, samples (100 µL) were removed and added to acetonitrile (200 µL), and analyzed by HPLC using the methodology previously described. The stability was assessed for a period of 72 h.

3.3. Stability in human plasma.

Human plasma was obtained from the pooled, heparinized blood of healthy donors, and was frozen and stored at -20 ºC prior to use. 12.5 µL of a 10^{-3} M stock solution of compound **1ad** in DMSO were incubated at 37 °C in 2.5 mL of human plasma diluted to 80% (v/v) with potassium phosphate buffer (pH 7.4, 0.5M). At appropriate intervals, aliquots (100 µL) were removed and added to acetonitrile (200 µL) to quench the reaction and precipitate plasma proteins. These samples were vortexed, centrifuged and the supernatant was analyzed by HPLC using the methodology previously described. The stability was assessed for a period of 72 h.

3.4. Stability in rat microsomes.

Male Rat Pooled Liver Microsomes (Sprague-Dawley) BD Gentest™ were used. A mixture of 570 µL purified water, 160 µL potassium phosphate (pH 7.4, 0.5 M), 40 µL NADPH Regenerating System Solution A (BD Biosciences Cat. No. 451220), 8 µL

NADPH Regenerating System Solution B (BD Biosciences Cat. No. 451200), and 20 μL of microsomes was incubated 5 minutes at 37 ºC in a water bath before addition of 8 μL of a 10^{-3} M stock solution of compound **1ad** in DMSO. At appropriate intervals, aliquots (50 μL) were removed and added to acetonitrile (50 μL). These samples were mixed, centrifuged and the supernatant was analyzed by HPLC using the methodology previously described. The half-life was determined applying the pseudo-first-order reaction equation: $t_{1/2}$= ln(2)/k. The viability of the rat microsomes was verified by evaluating their CYP2E1-catalyzed *p*-nitrophenol hydroxylation capacity.[6]

4. REFERENCES.

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