Electronic Supplementary Information (ESI)

Synthesis of a novel series of 2,3,4-trisubstituted oxazolidines designed by isosteric replacement or rigidification of the structure and cytotoxic evaluation

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S. Experimental procedures

S.1. General

Melting points were determined on Microquímica MQAPF 301 apparatus. The ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance DPX-200 or DRX-400 spectrometer. The proton and carbon chemical shifts (δ) are given with respect to TMS. Optical rotations were measured on an ADP220 Bellinghan + Stanley Ltd polarimeter. IR spectra were recorded on a Spectrum One, Perkin-Elmer ATR system. Reactants were obtained from commercial suppliers and used without further purification. Column chromatography was performed on silica gel 60 0.063-0.200 mm/70-230 mesh Merck. Flash column chromatography was performed on silica gel 60 0.040-0.063 mm/230-400 mesh Merck. Preparative TLC was performed on Fluka Kieselgel GF254 plates. THF and toluene were dried over Na/benzophenone and distilled prior to use. CH_2Cl_2 was dried over 4 Å molecular sieves and distilled prior to use. Oxalyl chloride was freshly distilled before using. For reactions at -78 or -15 ºC an EtOAc-dry ice or acetone-salt-ice baths were used, respectively. UPLC-MS analyses were carried out using an ACQUITY Ultra Performance LC system (Waters, Milford, MA, USA) linked simultaneously to both PDA 2996 photo diode array detector (Waters, Milford, MA, USA) and an ACQUITY TQ Detector (Waters MS Technologies, Manchester, UK), equipped with a Z-spray electrospray ionization (ESI) source operating in positive mode. MassLynx software (version 4.1, Waters, Milford, MA, USA) was used to control the instruments, as well as for data acquisition and processing. Sample solutions (2 µl; 1.0 mg mL^{-1}) were injected into a reversed phase column (BEH_{C18}, 1.7 µm, 1 mm × 50 mm, Waters, Milford, MA), which was maintained at 40°C. The mobile phase consisted of solvent A (H_2O/0.1 HCOOH) and solvent B (acetonitrile/0.1 HCOOH) at a flow rate of 300 µL min^{-1}:

T=0 min., 5% B; T=10 min., 95% B; T= 11 min., 5% B; T= 13 min., 5% B. The effluent was introduced into a PDA detector (scanning range 210–400 nm, resolution 1.2 nm) and subsequently into an electrospray source (source block temperature 120°C, desolvation temperature 350°C, capillary voltage 3.0 kV, cone voltage 30 V). Nitrogen was used as desolvation gas (500 L h^{-1}).

S.2. Synthesis of 1,1-dimethylethyl (*R*)-4-iodomethyl-2,2-dimethyl-3-oxazolidinecarboxylate (16)

To a solution of **15** (0.200 g, 0.866 mmol) in anhydrous toluene (15 mL) was added imidazole (0.130 g, 1.91 mmol), iodine (0.528 g, 2.08 mmol) and PPh_3 (0.454 g, 1.73 mmol) under vigorous stirring at room temperature. After 5 h, 10% (w/v) aqueous $Na_2S_2O_3$ (6 mL), saturated NH_4Cl solution (3 mL) and EtOAc (35 mL) was added. The organic layer was washed with 10% (w/v) aqueous $Na_2S_2O_3$ (20 mL) and water (2 X 20 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (hexanes/EtOAc 975:25) to give **16** as colorless oil (0.180 g, 61% yield): $[\alpha]_D^{21}$ -5.4 (c 1.86, $CHCl_3$), lit $[\alpha]_D^{25}$ -5.5 (c 1.86, $CHCl_3$);[1] IR *ν* 2978, 2935, 2878, 1691, 1478, 1455, 1421, 1374, 1364 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm): 4.18- 3.97 (m, 3H, CHN, CH_2O), 3.50-3.05 (m, 2H, CH_2I), 1.58, 1.54, 1.45 (s, 15H, CH_3, $C(CH_3)_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) major rotamer: 152.0 (C=O); 94.8 (C); 80.7 ($\underline{C}(CH_3)_3$), 67.2 (CH_2O) 59.0 (CHN), 28.4 ($C(\underline{C}H_3)_3$), 27.8, 24.3 (CH_3), 6.8 (CH_2I).

S.3. Synthesis of 1,1-dimethylethyl (*R*)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate (10)

To a cold (-78 ºC) stirred solution of oxalyl chloride (0.45 mL, 5.19 mmol) in anhydrous CH_2Cl_2 (4 mL) was added a solution of anhydrous DMSO (0.74 mL, 10.4 mmol) in anhydrous CH_2Cl_2 (3 mL) under nitrogen atmosphere. The reaction mixture was allowed to warm to -60 ºC over 25 min and a solution of **15** (0.40 g, 1.73 mmol) in anhydrous CH_2Cl_2 (3 mL) was slowly added. The reaction mixture was warmed to -45 ºC upon 35 min and after 5 min, triethylamine (2.90 mL, 20.8 mmol) was slowly added. After a further 5 min of stirring, the cooling bath was removed and the resulting solution was stirred for 10 min. The mixture was diluted with CH_2Cl_2 (60 mL). The organic layer was washed with 1M aqueous HCl (40 mL) and water (2 X 40 mL), dried over Na_2SO_4, filtered and concentrated to give **10** as colorless oil (0.4 g, 100% yield): $[\alpha]_D^{21}$ +85 (c 1.34, $CHCl_3$), lit: $[\alpha]_D$ +91,7 (c 1.34, $CHCl_3$);[2] IR *ν*: 2980, 2936, 2886, 2812, 2708, 1737, 1689, 1478, 1457, 1376, 1365 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm): 9.53 (d, 1H, CHO, J = 9.6 Hz); 4.29-4.02 (m, 3H, CHN, CH_2O); 1.60-1.39 (s, 15H, CH_3, $C(CH_3)_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) major rotamer: 199.4 (CHO), 151.2 (C=O), 95.0 (C), 81.0 ($\underline{C}(CH_3)_3$), 64.6, 63.8 (CH_2O, CHN), 28.2 ($C(\underline{C}H_3)_3$), 25.7, 23.7 (CH_3).

S.4. General procedure for the synthesis of substituted benzyl iodides 19 and 24

To a cold (-15 ºC) stirred solution of appropriate benzaldehyde (3.31 mmol) in anhydrous THF (7.5 mL) was added $NaBH_4$ (0.50 g, 13.2 mmol). After 15 min, MeOH (3.3 mL) was slowly added and the reaction mixture was allowed to warm to room temperature. After 1.5 h, the mixture was cooled in a ice-bath and water (2 mL) was added dropwise. The mixture was concentrated under reduced pressure and water

(60 mL) and EtOAc (60 mL) was added. The organic layer was washed with water (60 mL), dried over Na_2SO_4, filtered and concentrated to give the benzyl alcohols **18** and **23**, which were used without further purification (87-88% yield).

To a vigorously stirred solution of appropriate benzyl alcohol (1.96 mmol) in anhydrous toluene (25 mL) was added imidazole (0.30 g, 4.33 mmol), iodine (1.20 g, 4.71 mmol) and PPh_3 (1.03 g, 3.92 mmol) at room temperature. After 1 h, 10% w/v aqueous $Na_2S_2O_3$ (15 mL), saturated aqueous NH_4Cl (6 mL) and EtOAc (60 mL) were added to the mixture. The organic layer was washed with 10% w/v aqueous $Na_2S_2O_3$ (2 X 60 mL) and water (2 X 60 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography eluting with hexanes/EtOAc (9:1).

S.4.1. 4-(Iodomethyl)nitrobenzene (19)

Yellow solid. 78% yield: mp 120-122 ºC, lit: mp 124 ºC;[3] IR *v* 3103, 3076, 1604, 1594, 1515, 1417, 1389, 1340 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm): 8.14 (d, 2H, Ar, *J* = 8.5 Hz), 7.51 (d, 2H, Ar, *J* = 8.5 Hz), 4.47 (s, 2H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 146.7 (Ar), 129.6 (Ar), 124.1 (Ar), 2.1 (CH_2).

S.4.2. Methyl 4-(iodomethyl)benzoate (24)

Yellow solid. 70% Yield: mp 60-62 ºC; lit: mp 67 ºC;[4] IR *v* 3024, 2957, 2841, 1716, 1606, 1574, 1505, 1431, 1414 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.93 (d, 2H, Ar, *J* = 8.2 Hz), 7.40 (d, 2H, Ar, *J* = 8.2 Hz), 4.43 (s, 2H, CH_2), 3.88 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 166.5 (C=O), 144.3 (Ar), 130.0 (Ar), 129.5 (Ar), 128.7 (Ar), 52.1 (OCH_3), 3.9 (CH_2).

S.5. General procedure for the synthesis of substituted benzyltriphenylphosphonium iodides 11 and 25

To a stirred solution of appropriate benzyl iodide **19** or **24** (0.68 mmol) in anhydrous toluene (3 mL) was added PPh_3 (0.198 g, 0.75 mmol) at room temperature. After 16 h, the resulting suspension was filtered and washed with toluene (3 mL). The phosphonium salts **11** and **25** were used immediately without further purification.

S.6. General procedure for the synthesis of olefins 3, 4 and 21

To a stirred suspension of appropriate phosphonium salt (0.37 mmol) in anhydrous THF (2 mL) was added a solution of BuLi 1.6 M (0.273 mL, 0.437 mmol) in hexanes under nitrogen atmosphere. After 20 min, a solution of garner's aldehyde **10** (0.077 g, 0.336 mmol) in THF (2 mL) was added. After 16 h, water (20 mL) and EtOAc (40 mL) were added to the mixture. The organic layer was washed with water (20 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography eluting with hexanes/EtOAc (9:1). (*E*/*Z*)-isomers mixture was obtained in 68-70% yield. The isomers **3-4** were separated using preparative TLC (hexanes/EtOAc 85:15). Mixture **21** was used in the next step without isomers separation.

S.6.1. 1,1-Dimethylethyl (*S*)-2,2-dimethyl-4-[(*Z*)-2-(4-nitrophenyl)ethenyl]-3-oxazolidinecarboxylate (3)

White solid: mp 115-118 ºC; $[\alpha]_D^{21}$ -125 (c 0.34, CH_2Cl_2); IR *v* 3108, 3079, 2979, 2935, 2875, 1698, 1656, 1596, 1508, 1478, 1458, 1376, 1364, 1340 cm^{-1}; ^1H NMR

(400 MHz, $CDCl_3$, 45 ºC) δ (ppm): 8.18 (d, 2H, Ar, *J* = 8.8 Hz), 7.49-7.47 (m, 2H, Ar), 6.54 (d, 1H, CH_{alk}, *J* = 11.6 Hz), 5.87 (dd, 1H, CH_{alk}, *J* = 11.6, 9.6 Hz), 4.80 (bs, 1H, CHN), 4.11, 3.78 (bs, 2H, CH_2O), 1.63, 1.51, 1.38 (s, 15H, CH_3, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$, 45 ºC) δ (ppm): 152.1 (C=O), 146.8 (Ar), 143.2 (Ar), 136.5 (CH_{alk}), 129.5 (Ar), 127.9 (CH_{alk}), 123.6 (Ar), 94.5 (C), 80.3 ($\underline{C}(CH_3)_3$), 68.7 (CH_2O), 55.1 (CHN), 28.4 ($C(\underline{C}H_3)_3$), 27.2, 24.9 (CH_3); MS calcd for $C_{18}H_{25}N_2O_5$ [M + H^+] 349.2, found 349.5.

S.6.2. 1,1-Dimethylethyl (*S*)-2,2-dimethyl-4-[(*E*)-2-(4-nitrophenyl)ethenyl]-3-oxazolidinecarboxylate (4)

Colorless oil: $[\alpha]_D^{21}$ +57 (c 0.38, CH_2Cl_2); IR *v* 3108, 3073, 2979, 2935, 2875, 1693, 1656, 1596, 1516, 1478, 1455, 1376, 1365, 1340 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 45 °C) δ (ppm): 8.17 (d, 2H, Ar, *J* = 8.8 Hz), 7.49 (d, 2H, Ar, *J* = 8.8 Hz), 6.60 (d, 1H, CH_{alk}, *J* = 16.0 Hz), 6.34 (dd, 1H, CH_{alk}, *J* = 16.0, 7.6 Hz), 4.53 (bs, 1H, CHN), 4.14 (dd, 1H, CH_2O, *J* = 9.0, 6.2 Hz), 3.85 (dd, 1H, CH_2O, *J* = 9.0, 2.4 Hz), 1.66, 1.56, 1.46 (s, 15H, CH_3, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$, 45 °C) δ (ppm): 152.0 (C=O), 147.2 (Ar), 143.3 (Ar), 133.8 (CH_{alk}), 129.6 (Ar), 127.0 (CH_{alk}), 124.0 (Ar), 94.3 (C), 80.4 ($\underline{C}(CH_3)_3$), 68.0 (CH_2O), 59.3 (CHN), 28.5 ($C(\underline{C}H_3)_3$), 28.4, 28.3 (CH_3); MS calcd for $C_{18}H_{25}N_2O_5$ [M + H^+] 349.2, found 349.3.

S.7. Synthesis of 1,1-dimethylethyl (*S*)-2,2-dimethyl-4-[[2-(4-methoxycarbonyl)phenyl]ethyl]-3-oxazolidinecarboxylate (6)

To a solution of **21** (0.040 g, 0.11 mmol) in THF (10 mL) was added Pd/C (10% w/w, 0.040 g). The mixture was stirred under hydrogen atmosphere at 55 ºC and 55 atm. After 18 h, the catalyst was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC eluting with hexanes/EtOAc (85:15) to give **6** as colorless oil (0.021 g, 50% yield): IR *v* 2978, 2934, 2870, 1720, 1691, 1610, 1455, 1435, 1386, 1375, 1364 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 45 °C) δ (ppm): 7.95 (d, 2H, Ar, *J* = 7.8 Hz), 7.25 (d, 2H, Ar, *J* = 7.8 Hz), 3.96-3.45 (m, 6H, CHN, CH_2O, OCH_3), 2.68 (bs, 2H, CH_2Ar), 2.16-1.85 (m, 2H, CH_2), 1.58-1.16 (m, 15H, CH_3, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$, 45 °C) δ (ppm): 167.1 ($\underline{C}OOCH_3$), 147.1 (Ar), 129.9 (Ar), 128.4 (Ar), 93.8 (C), 66.9 (CH_2O), 51.9 (CHN), 32.7 (CH_2, CH_2Ar), 28.5 ($C(\underline{C}H_3)_3$), 27.5 (CH_3); MS calcd for $C_{20}H_{29}NNa$ [M + Na^+] 386.2, found 386.3.

S.8. Synthesis of 1,1-dimethylethyl (*R*)-2,2-dimethyl-4-[[(4-nitrophenyl)amino]carbonyl]-3-oxazolidinecarboxylate (5)

To a stirred solution of **14** (1.0 g, 3.86 mmol) in a mixture of acetone (7.5 mL) and water (3.5 mL) was added LiOH (0.259 g, 6.18 mmol) at room temperature. After 5 h, the mixture was concentrated under reduced pressure and water (50 mL) was added. The aqueous layer was washed with ether (50 mL) and acidified by slow addition of 1 M aqueous HCl. The resulting mixture was extracted with EtOAc (3 X 50 mL). The combined organic layers were washed with water (50 mL), dried over Na_2SO_4, filtered and concentrated to give **20** as yellow oil (0.9 g, 95% yield): $[\alpha]_D^{21}$ +59 (c 1.1, $CHCl_3$), lit: $[\alpha]_D^{24}$ +63 (c 1.1, $CHCl_3$) [29].

To a stirred solution of **20** (0.050 g, 0.204 mmol) in CH_2Cl_2 (5 mL) was added triethylamine (0.028 mL, 0.204 mmol) and benzyl chloroformate (0.029 mL, 0.204 mmol) at -15 ºC. After 20 min, 4-nitroaniline (0.0287 g, 0.208 mmol) was added.

After 1 h, the mixture was warmed to room temperature and stirred for 18 h. The solvent was evaporated under reduced pressure and EtOAc (50 mL) was added. The organic layer was washed with water (20 mL), 20% (w/v) aqueous citric acid (20 mL), water (20 mL), saturated $NaHCO_3$ solution (20 mL) and water (20 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash silica gel column chromatography (toluene/EtOAc 9:1) to give **5** as a yellow solid (0.042 g, 57% yield): mp 214-217 ºC; $[\alpha]_D^{21}$ +89 (c 0.85, CH_2Cl_2); IR *v* 3298, 3097, 2983, 2938, 2877, 1718, 1674, 1613, 1595, 1558, 1504, 1404, 1379, 1365, 1338 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm): 9.79 (s, 1H, NH), 8.09 (d, 2H, Ar, *J* = 8.8 Hz), 7.61 (d, 2H, Ar, *J* = 8.8 Hz), 4.56-4.07 (m, 3H, CHN, CH_2O), 1.64, 1.56, 1.51 (s, 15H, CH_3, $C(CH_3)_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 168.9 (NHC=O), 154.4 (C=O), 143.8 (Ar), 143.4 (Ar), 124.9 (Ar), 119.0 (Ar), 95.1 (C), 82.4 ($\underline{C}(CH_3)_3$), 64.7 (CH_2O), 60.7 (CHN), 28.3 ($C(\underline{C}H_3)_3$), 26.6 (CH_3); MS calcd for $C_{17}H_{23}N_3NaO_6$ [M + Na^+] 388.2, found 388.3.

S.9. Synthesis of 1,1-dimethylethyl (*R*)-2,2-dimethyl-4-[(4-nitrothiophenoxy)methyl]-3-oxazolidinecarboxylate (7)

To a stirred solution of **15** (0.100 g, 0.433 mmol), 4-nitrothiophenol (0.101 g, 0.65 mmol) and PPh_3 (0.182 g, 0.693 mmol) in anhydrous toluene (5 mL) was added DIAD (0.14 mL, 0.693 mmol) at 80 ºC. After 3 h, EtOAc (40 mL) was added to the resulting solution. The organic layer was washed with 0.5 M aqueous NaOH (40 mL) and water (2 X 40 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash silica gel column chromatography eluting with Hexanes/EtOAc (95:5) to give **7** as a brown oil (0.049 g, 31% yield): $[\alpha]_D^{21}$ -37 (c 0.54, CH_2Cl_2); IR *v* 3098, 2979, 2935, 2881, 1682, 1595, 1579, 1514, 1480, 1386, 1376, 1365, 1335 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) major rotamer: 8.14 (d, 2H, Ar, *J* = 8.4 Hz), 7.57 (d, 2H, Ar, *J* = 8.4 Hz), 4.10-3.89 (m, 3H, CHN, CH_2O), 3.64-3.41 (m, 1H, CH_2S), 2.95-2.77 (m, 1H, CH_2S), 1.63-1.45 (m, 15H, CH_3, $C(CH_3)_3$); ^{13}C NMR (200 MHz, $CDCl_3$) δ (ppm) major rotamer: 152.2 (C=O), 146.3 (Ar), 144.9 (Ar), 126.3 (Ar), 124.0 (Ar), 94.0 (C), 80.7 ($\underline{C}(CH_3)_3$), 65.9 (CH_2O), 56.1 (CHN), 32.2 (CH_2S), 27.7 ($C(\underline{C}H_3)_3$), 26.9, 24.1 (CH_3); MS calcd for $C_{17}H_{25}N_2O_5S$ [M + H^+] 369.2, found 369.3.

S.10. HL60 and JURKAT

HL60 cells (human promyelocytic leukemia) and JURKAT (T cell leukemia) were cultivated in the logarithmic phase of growth in RPMI 1640 Sigma-Aldrich (St. Louis, MO) supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin (GIBCO BRL, Grand Island, NY), enriched with 2 mM of L-glutamine and 10% of fetal bovine serum.

S.11. MDA-MB231, LNCaP and VERO

MDA-MB-231 (human adenocarcinoma mammary gland), LNCaP (human carcinoma prostate – CRL-1740, ATCC – Manassas, VA, EUA) and VERO cells (normal monkey kidney) were cultivated in DMEM medium (Gibco-BRL Grand Island/NY, MDA-MB-231/VERO) or RPMI-1640 Medium (ATCC - Manassas, VA, EUA, LNCaP) supplemented with 100 U/mL penicillin and 100 ug/mL streptomycin

(Sigma-Aldrich – St Louis, MO) and enriched with 10% of fetal bovine serum (Sigma-Aldrich – St Louis, MO).

S.12. PBMC

PBMC were prepared using the protocol previously described.[5,6] Briefly, PBMC samples were obtained through agreement with Minas Gerais Hematology and Hemotherapy Center Foundation – HEMOMINAS (protocol nº 105/2004). PBMC were obtained from healthy adult volunteers of both sexes by centrifugation of heparinized venous blood over Ficoll cushion (Sigma-Aldrich, St. Louis, MO). Mononuclear cells were collected from the interphase after Ficoll separation and washed three times in RPMI-1640 before further processing. All cultures were carried out in RPMI-1640 medium Sigma-Aldrich (St. Louis, MO), supplemented with 5% (v/v) heat-inactivated, pooled AB (GIBCO/BRL, Grand Island, NY) sera and 2 mM L-glutamine. An antibiotic/antimicotic solution containing 1000 U/mL penicillin, 1000 µg/mL streptomycin and 25 µg/mL fungisone (GIBCO/BRL, Grand Island, NY) was added to control fungal and bacterial contamination.

S.13. Analysis of cell viability

HL60, JURKAT and PBMC were cultured in 96 wells plate at densities of 50,000 (HL60 and JURKAT) or 200,000 cells/well (PBMC) in a final volume of 200 µL/well. The plates were pre-incubated in a 5% CO_2/95% air-humidified atmosphere at 37 ºC for 24 h to allow adaptation of cells prior to the addition of the test compounds. All substances were dissolved in dimethyl sulfoxide (DMSO), prior to dilution. The 50% inhibitory concentration (IC_{50}) was determinated over a range of concentrations (10 nM-100 µM). All cell cultures were incubated in a 5% CO_2/95% air-humidified atmosphere at 37ºC for 48 hours. Cell viability was estimated measuring the rate of mitochondrial reduction of yellow tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma-Aldrich, St. Louis, MO) to insoluble purple formazan crystals.[7] After incubation with the test compounds, MTT solution (20 µL; 5 mg/mL) was added to each well and incubated for 4 hours. At the end of this incubation, the supernatant was removed and 200 µL of 0.04 M HCl in isopropyl alcohol were added to dissolve the formazan crystal. The optical densities (OD) were measured with a spectrophotometer at 590 nm. Results were normalized with DMSO control (0.05%) and expressed as percentage of cell viability inhibition. Interactions of compounds and media were estimated on the basis of the variations between drug-containing medium and drug-free medium to control for false-positive or false-negative results. The 50% inhibitory concentration (IC_{50}) values were obtained graphically from dose-effect curves using Prism 5.0 (GraphPad Software Inc.).

MDA-MB-231, LNCaP and VERO were cultured in 96 wells plate at densities of 2,500 cells/well (MDA-MB-231) or 10,000 cells/well (LNCaP and VERO) in a final volume of 100 µL/well. The plates were pre-incubated for 24 h to allow adaptation of cells. All substances were dissolved in dimethyl sulfoxide (DMSO), prior to dilution. The 50% inhibitory concentration (IC_{50}) was determinated over a range of concentrations (2-80 µM). All cell cultures were incubated in a 5% CO_2/95% air-humidified atmosphere at 37 ºC for 48 hours. Cell viability was estimated measuring the rate of mitochondrial reduction of yellow tetrazolium salt MTT to insoluble purple formazan crystals. After incubation with the test compounds, MTT solution (100 µL;

0,5 mg/mL) was added to each well and incubated for 2 hours. At the end of this incubation, the supernatant was removed and 100 µL of 0.01 M HCl in sodium dodecyl sulfate were added to dissolve the formazan crystal. The optical densities (OD) were measured with a spectrophotometer at 590 nm. Interactions of compounds and media were estimated on the basis of the variations between drug-containing medium and drug-free medium to control for false-positive or false-negative results. The 50% inhibitory concentration (IC_{50}) values were obtained graphically from dose-effect curves using Prism 5.0 (GraphPad Software Inc.).

S.14. DNA fragmentation assay

Cell cycle status and quantification of DNA fragmentation (hypodiploid DNA-content) were performed by propidium iodide staining.[8] HL60 and MDA-MB213 cells were treated with compounds **1**, **3** and **4** at the final concentration of 50 µM and incubated in a 5% CO_2/95% air-humidified atmosphere at 37 ºC for 24 hours. DMSO (0.5%) was used as negative control. Positive controls included cisplatin (cis, 50 µM) and doxorubicin (doxo, 50 µM). Afterwards, the cells were centrifuged, resuspended in a hypotonic fluorochrome solution - HFS (50 µg/mL PI in 0.1% sodium citrate plus 0.1% Triton X-100) and incubated at 4 ºC for 4 h. The PI fluorescence of 20,000 individual nuclei was measured using a FACScalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif.). Data were analyzed using FlowJo software (TreeStar Inc, CA). All results were expressed as the mean ± SD of two independent experiments carried out in triplicate. These data were analyzed using Student's t-test for paired comparisons, using Prism 5.0 (GraphPad Software Inc.). Statistical significance was determined as $P < 0.05$.

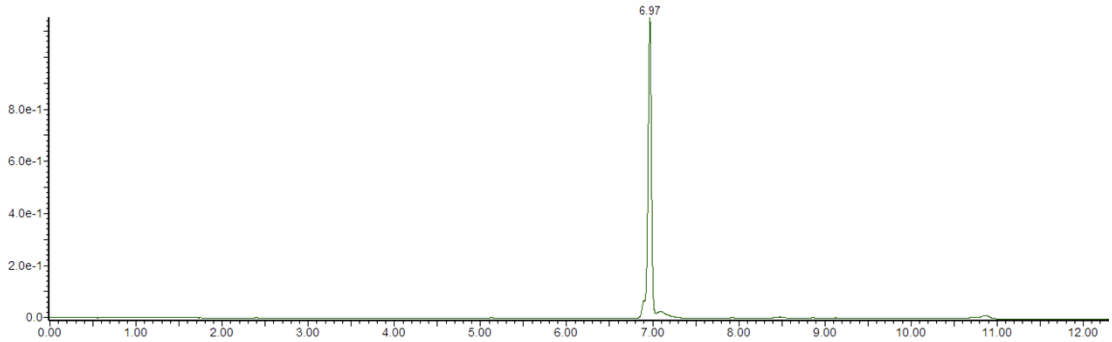

Fig. S1 UPLC chromatogram of compound **3.**

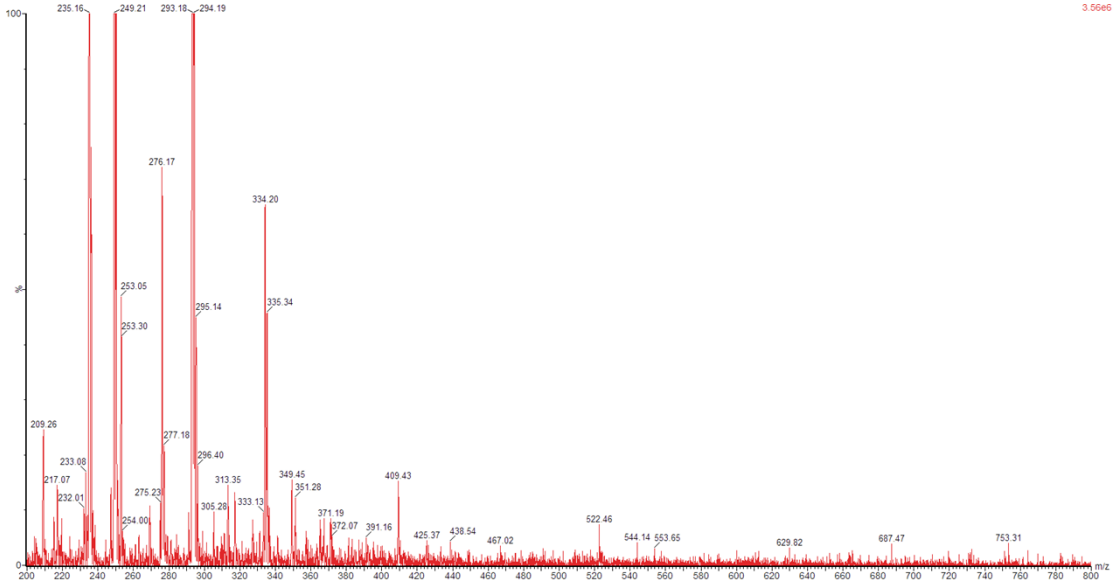

Fig. S2 compound **3**

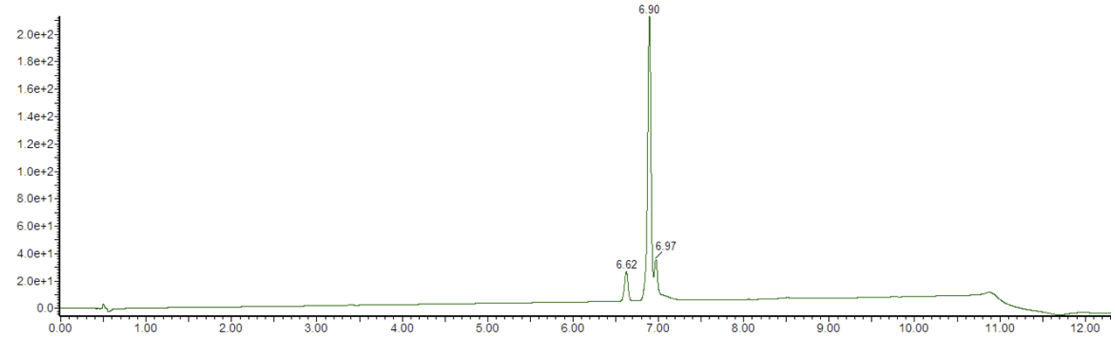

Fig. S3 UPLC chromatogram of compound **4**.

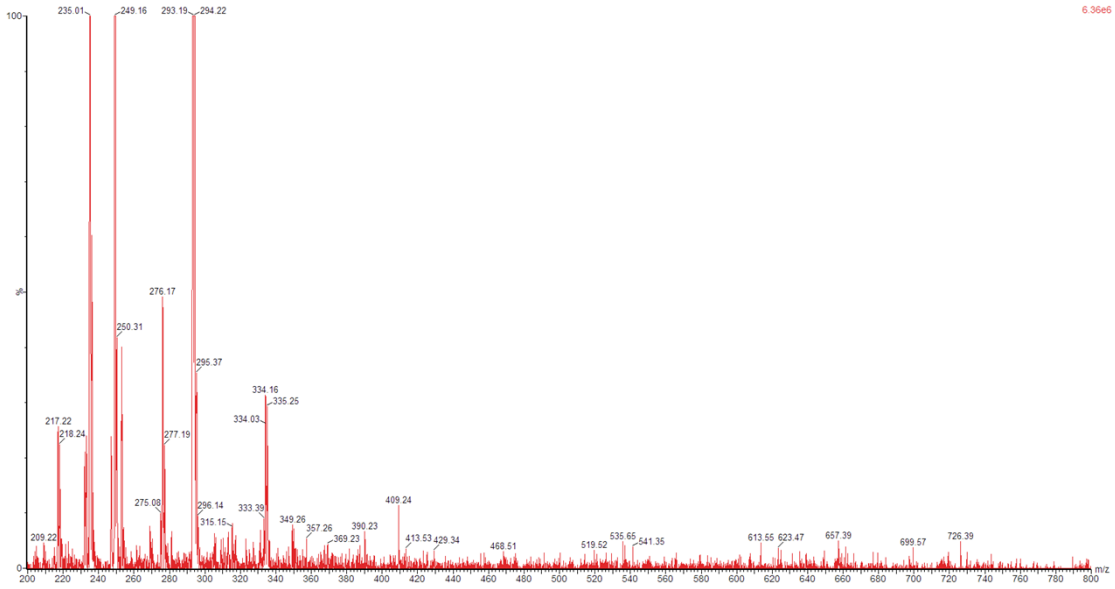

Fig. S4 compound **4**

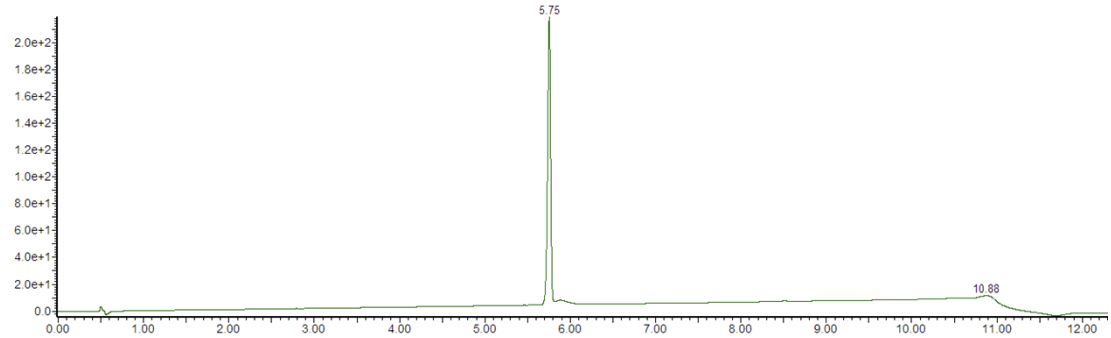

Fig. S5 UPLC chromatogram of compound **5**.

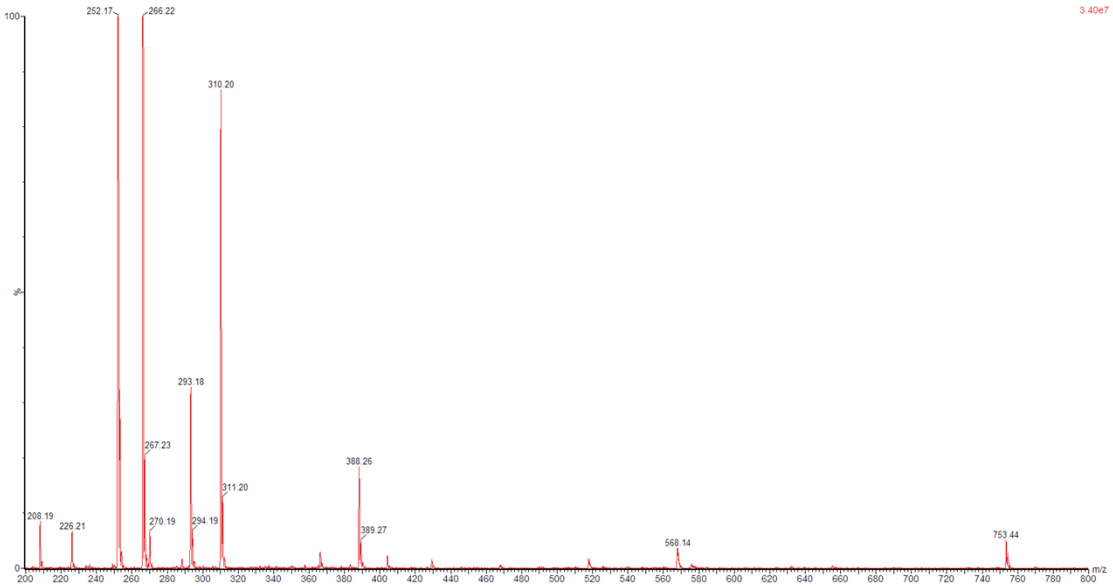

Fig. S6 compound **5**

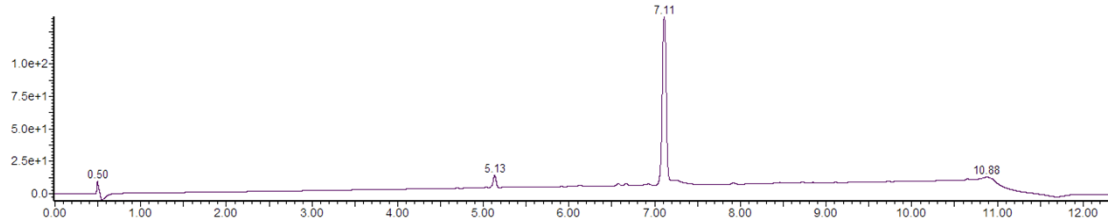

Fig. S7 UPLC chromatogram of compound **6**.

Fig. S8 compound **6**

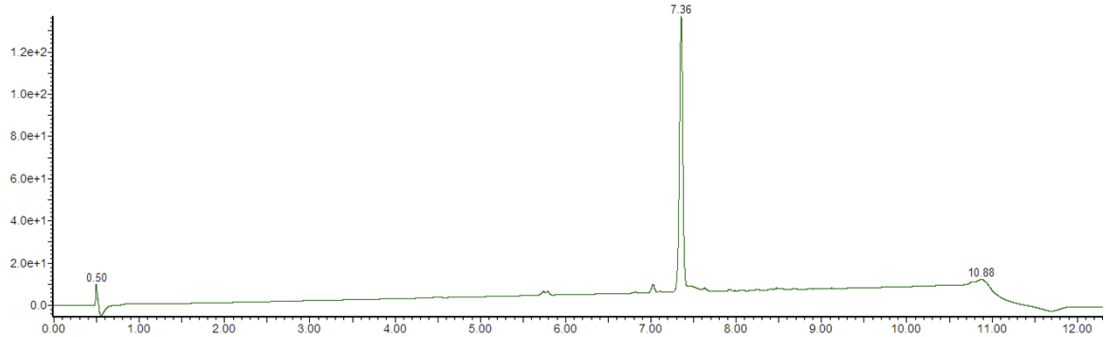

Fig. S9 UPLC chromatogram of compound **7**.

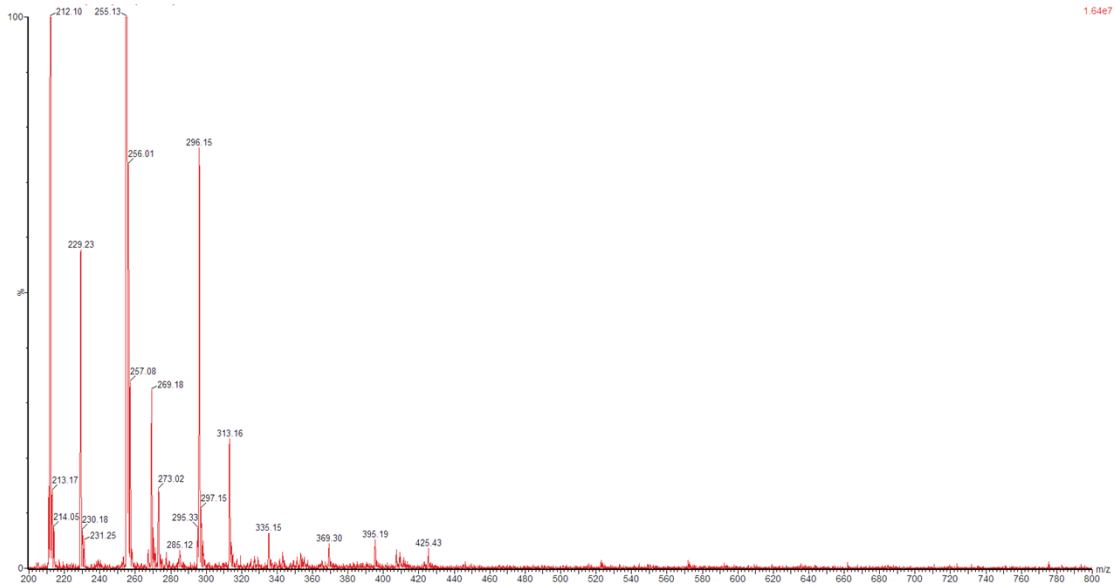

Fig. S10 compound **7**

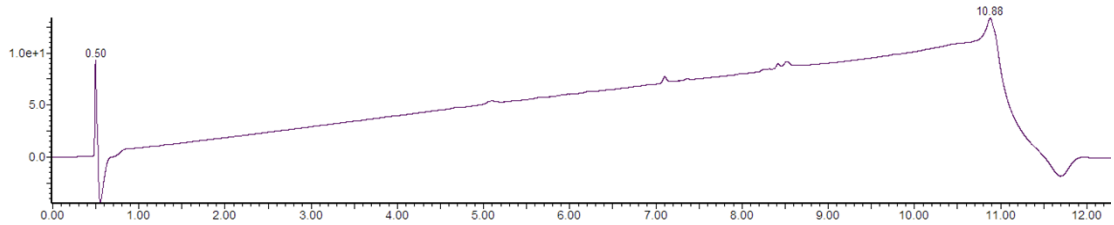

Fig. S11 UPLC chromatogram of blank.

References

1 A. Ollivier, M. Goubert, A. Tursun, I. Canet and M.-E. Sinibaldi, *ARKIVOC*, 2010, **9**, 108-126.
2 P. Garner and J. M. Park, *Org. Synth.*, 1992, **70**, 18-24.
3 L. Zervas and I. Dilaris, *J. Am. Chem. Soc.*, 1955, **77**, 5354-5357.
4 W.-Y. Liao, C.-L. Huang, D.-R. Huang, D.-Y. Chiang, A. T. Hu, H.-J. Lee, S.-J. Ye and Y.-J. Kao, US5958087, 1999, 28 Set.
5 M. C. X. Pinto, D. F. Dias, H. L. del Puerto, A. S. Martins, A. T.-Carvalho, O. A. Martins-Filho, B. Badet, P. Durand, R. J. Alves and E. M. Souza-Fagundes, *Life Sci.*, 2011, **89**, 786-794.
6 M. S. Lopes, R. C. C. S. Pietra, T. F. Borgati, C. F. D. Romeiro, P. A. S. Júnior, A. J. Romanha, R. J. Alves, E. M. Souza-Fagundes, A. P. S. M. Fernandes and R. B. Oliveira, *Eur. J. Med. Chem.*, 2011, **46**, 5443-5447.
7 T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55-63.
8 C. Riccardi and I. Nicoletti, *Nat. Protoc.*, 2006, **1**, 1458-1461.