Scientific and technical report 2019

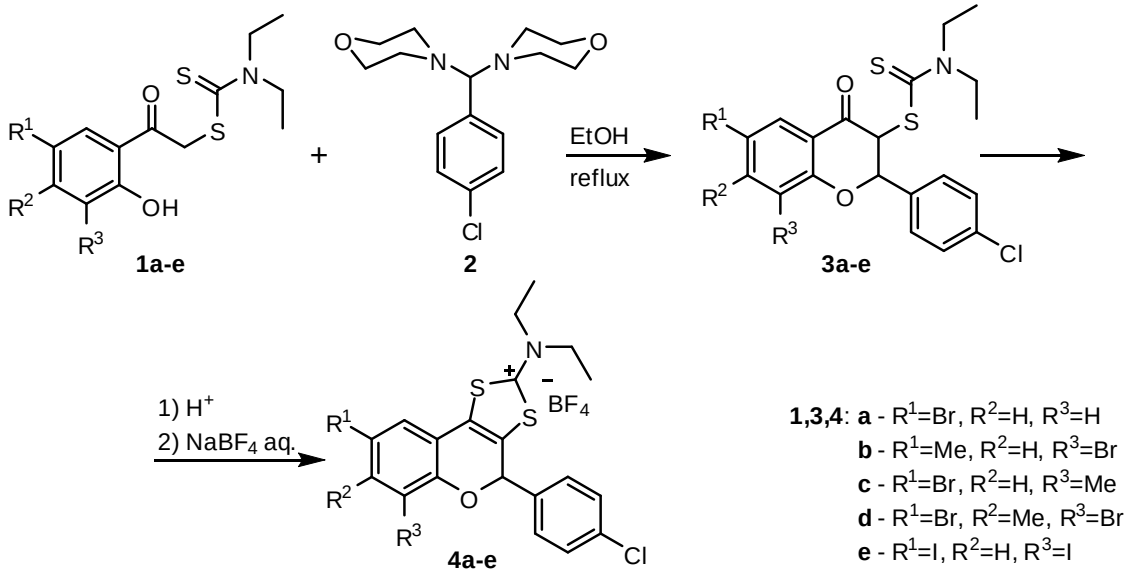
Project title: The synthesis and evaluation of some tribenzotriquinacene-flavonoids tripodal antibacterial agents

Project code: PN-III-P1-1.1-PD-2016-1117

Current stage objectives: Synthesis, antibacterial and cytotoxic properties, structure optimization and result dissemination.

Synthesis and cytotoxicity studies

A cytotoxicity study was performed on a series of tricyclic flavonoids which includes previously reported derivatives, as well as newly synthesized ones. The latter were obtained according to Scheme 1:



Scheme 1 – The synthesis of **4a-e**, which were used for the cytotoxicity study.

*Synthesis of **3a-e***: To a solution of dithiocarbamate **1a-e** (2 mmol) in ethanol (30 mL), aminal **2** (2 mmol) is added. The resulting solution is refluxed for 2 hours, cooled down to room temperature, filtered and the solid, recrystallized from ethanol.

3b: 0.82 g (83%) colorless crystals. P.t. 149-150 °C. IR (ATR, cm^{-1}) 2889, 1692, 1492, 1271, 1239, 803, 649, 548. ^{1}H NMR (DMSO-*d*6, major isomer) δ 7.81 (d, *J* = 1.4 Hz, 1H), 7.62 (d, *J* = 1.4 Hz, 1H), 7.50 (m, 4H), 6.17 (d, *J* = 10.4 Hz, 1H), 5.85 (d, *J* = 10.4 Hz, 1H), 3.83 (m, 4H), 2.31 (s, 3H), 1.10 (m, 6H). ^{13}C NMR (DMSO-*d*6, major isomer) δ 189.8, 187.1, 154.8, 140.7, 135.0, 134.0, 133.3, 130.1, 128.8, 128.5, 126.8, 111.2, 80.0, 57.7, 50.1, 47.6, 20.1, 12.7, 11.5. MS (EI) *m/z*: 497.1 (M^+, 7%).

3c: 0.46 g (80%), colorless crystals. P.t. 178-179 °C. IR (ATR, cm^{-1}) 2877, 1696, 1490, 1419, 1260, 1200, 811, 652, 539. ^{1}H NMR (DMSO-*d*6, major isomer) δ 7.70 (m, 2H),

7.50 (m, 4H), 6.13 (d, *J* = 10.7 Hz, 1H), 5.85 (d, *J* = 10.4 Hz, 1H), 3.82 (m, 4H), 2.19 (s, 3H), 1.10 (m, 6H). ^{13}C NMR (DMSO-*d*6, major isomer) δ 189.9, 186.9, 157.9, 139.6, 135.1, 133.3, 130.8, 130.1, 128.7, 126.8, 121.2, 113.7, 79.5, 57.7, 51.0, 47.6, 15.5, 12.9, 11.5. MS (EI) *m/z*: 496.0 (M^+, 4%).

3d: 0.83 g (72%), colorless crystals. P.t. 164-165 °C. IR (ATR, cm^{-1}) 2888, 1697, 1490, 1417, 1337, 1199, 1011, 802, 642, 528. ^{1}H NMR (DMSO-*d*6, major isomer) δ 7.94 (m, 2H), 7.52 (m, 4H), 6.22 (d, *J* = 9.5 Hz, 1H), 5.89 (d, *J* = 9.5 Hz, 1H), 3.80 (m, 4H), 2.58 (s, 3H), 1.08 (m, 6H). ^{13}C NMR (DMSO-*d*6, major isomer) δ 190.7, 186.0, 158.3, 145.7, 135.5, 134.2, 130.3, 128.8, 128.7, 121.1, 117.1, 115.2, 82.5, 57.3, 50.7, 47.7, 24.9, 12.7, 11.4. MS (EI) *m/z*: 574.9 (M^+, 10%).

*Synthesis of **4a-e***: To a mixture of acetic acid (1.5 mL) and sulfuric acid (0.5 mL), flavanone **3a-e** (1 mmol) is added. The reulting solution is heated to 80 °C for 20 minutes, after which it is cooled down to room temperature. An aqueous solution of sodium tetrafluoroborate (250 mg) is then added and the resulting solid is filtered and recrystallized from ethanol.

4b: 0.5 g (88%), colorless crystals. P.t. 232–233 °C. IR (ATR, cm-1) 1538, 1441, 1235, 1041, 774, 734, 460, 451. ^{1}H NMR (DMSO-*d*6) δ 7.52 (m, 4H), 7.48 (d, J = 1 Hz, 1H), 7.31 (d, J = 1 Hz,) 6.92 (s, 1H), 3.94 (m, 4H), 2.27 (s, 3H), 1.41 (t, J = 7.1 Hz, 3H), 1.34 (t, J = 7.1 Hz, 3H). ^{13}C NMR (DMSO-*d*6) δ 185.1, 145.7, 135.9, 135.6, 135.0, 134.6, 129.7, 129.6, 128.3, 127.9, 125.1, 117.9, 110.8, 75.1, 54.7, 54.6, 20.1, 10.8, 10.6. MS (EI) *m/z*: 480.1 (M^+-BF4, 7%).

4c: 0.48 g (84%), colorless crystals. P.t. 225-226 °C. IR (ATR, cm^{-1}) 1549, 1454, 1210, 1036, 789, 741, 467, 448. ^{1}H NMR (DMSO-*d*6) δ 7.54 (m, 6H), 6.87 (s, 1H), 3.90 (m, 4H), 2.21 (s, 3H), 1.40 (t, *J* = 7.2 Hz, 3H), 1.33 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (DMSO-*d*6) δ 185.1, 148.4, 136.2, 135.7, 135.0, 129.7, 129.6, 129.5, 128.2, 127.8, 125.0, 118.3, 114.3, 74.7, 54.7, 54.6, 15.6, 10.8, 10.6. MS (EI) *m/z*: 480.1 (M^+-BF_4, 11%).

4d: 0.51 g (79%), colorless crystals. P.t. 241-242 °C. IR (ATR, cm^{-1})1561, 1450, 1228, 1154, 1041, 717, 489. ^{1}H NMR (DMSO-*d*6) δ 7.81 (s, 1H), 7.51 (m, 4H), 6.99 (s, 1H), 3.92 (m, 4H), 2.19 (s, 3H), 1.41 (t, *J* = 7.2 Hz, 3H), 1.34 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (DMSO-*d*6) δ 185.1, 147.5, 140.7, 135.7, 135.2, 129.8, 129.6, 128.3, 127.2, 127.1, 117.4, 117.1, 114.8, 75.5, 54.8, 54.6, 24.3, 10.8, 10.6. MS (EI) *m/z*: 557.9 (M^{+}-BF_4, 8%).

Derivatives **4a** and **4e** were previously published,[1] while **4b-d** were reported during this study for the first time.

The cell lines used in the study were NHDF (normal cells), HOS (cancerous cells) and MCF7 (cancerous cells).

The first test preformed during the cytotoxicity study was a Wound Scratch Assay, meant to determine to what extent a tested compound inhibits the proliferation of tumor cells. This test was performed on the HOS and MCF7 cell lines. It consists in scratching the cell cultures and monitoring the evolution of the induced "wound" in untreated vs. treated cell cultures. The obtained results indicate that the investigated compounds **4a-e** inhibit the growth of tumor cells, with a slightly more pronounced effect against the HOS cell line (Figure 1).

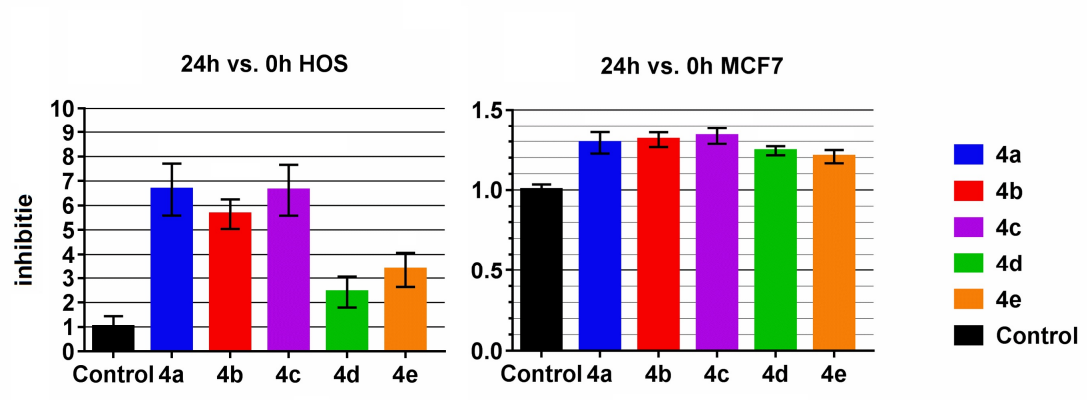


Figure 1 – The results obtained for the Wound scratch assay; published data.[2]

Next, the cell viability was evaluated using the MTS assay. This method is based on the ability of living cells to metabolize the reagents used by the assays, leading to products which can be quantified by analytical methods. Tricyclic flavonoid **4a** was selected as a

model compound and the results are presented in Figure 2. It was found that **4a** was slighlty more toxic when tested on the cancerous cell lines. Thus, at a concentration of 5 µg/mL, the NHDF cells viability was 62%, whereas for the MCF7 and HOS cells, the viability was only 31% and 49% respectively.

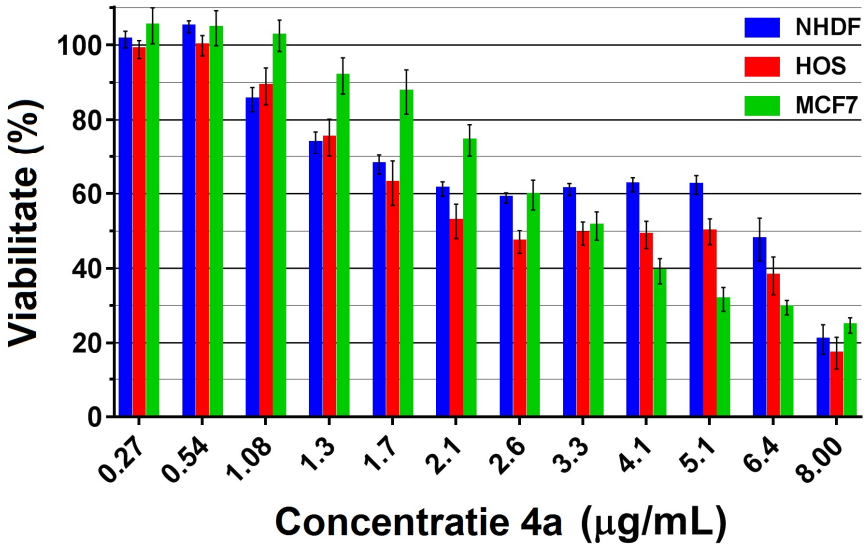


Figura 2 –MTS assay results; published data.[2]

The data collected during the MTS assay was confirmed by another test, the Live/dead staining, which is based on staining living cells with a dye and dead cells with a different one (Figure 3). Thus, after 48 hours, the tumor cells which were treated with 5 µg/mL of **4a** are visibly more affected than the normal NHDF cells.

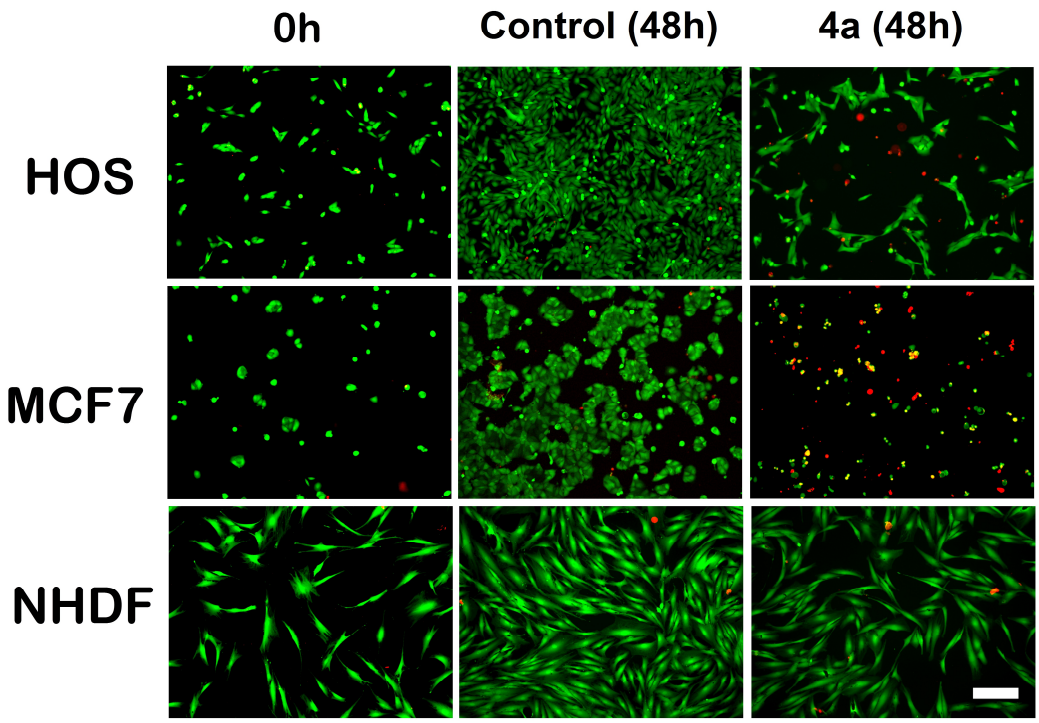


Figure 3 –Live/dead staining; published data.[2]

References

1. Bahrin, L. G., Jones, P. G., Hopf, H., *Beilstein J. Org. Chem.*, **2012**, *8*, 1999-2003.

2. Sarbu, L. G., Shova, S., Peptanariu, D., Sandu, I. A., Birsa, L. M., Bahrin, L. G., *Molecules*, **2019**, *24*, 2459.