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Atractyligenine chemistry, part VI[1]: Synthesis and biological activities of atractyligenine derivatives

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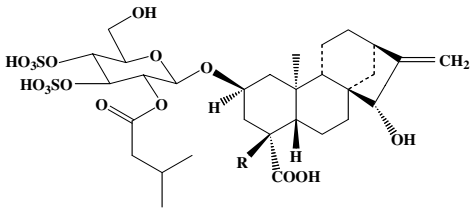
Derivatives of atractyligenine, the aglycone of atractyloside, were tested *in vitro* for their antimicrobial and antiproliferative activity against K-562 (human chronic myelogeneous leukemia), HL-60 (human leukemia) and MCF-7 (human breast adenocarcinoma) cell lines. The most active compound showed IC_{50} values in the range 0.8–6.9 μM.

1. Introduction

Atractyloside (**1**) and 4-carboxyatractyloside (**2**) are two highly toxic constituents of rhizomes of *Atractylis gummifera* L. (Compositae), a plant which grows in the Mediterranean area [2–5].

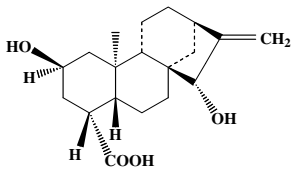
Both **1** and **2**, and at a lesser extent, the aglycone atractyligenine **3**, show interesting biological activities, as they impair the transfer of adenine nucleotide into mytochondria [6, 7]. It has also been reported that atractyloside (**1**) induces partial inhibition of the growth of murine melanoma metastatic cells [8].

This paper reports the synthesis of some derivatives of atractyligenine and the evaluation of their properties *in vitro*.



1 R = H

2 R = CO_2H



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2. Investigations, results and discussion

2.1. Synthesis

The derivatives of atractyligenine **4**–**12** were obtained by different routes. Compounds **4**–**9** and **12** were synthesized by previously described methods [9–11]. Compounds **10**–**11** were prepared by treating compoud **9** with semicarbazide and thiosemicarbazide, respectively.

2.2. Biological results and discussion

Compounds **3**–**12** (Table 1) were tested *in vitro* for their antimicrobial activity against Gram positive and Gram negative bacteria, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923.

The compounds resulted to be inactive at a maximum tested concentration of 100 μg/ml, except for the compounds **6** and **7** which showed a weak activity against *S. aureus*, with MIC values of 50 and 25 μg/ml.

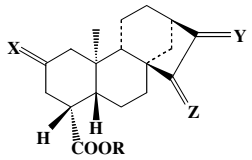
The antifungal tests were done by an *in vitro* method using the strains *Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 13803. Compounds **3**–**12** were found not to be active against *C. albicans* and *C. tropicalis* at a concentration of 100 μg/ml.

Compounds **3**–**6** and **8**–**11** were tested for their *in vitro* antiproliferative activity against K-562 (human chronic myelogenous leukemia) and HL-60 (human leukemia) cell lines.

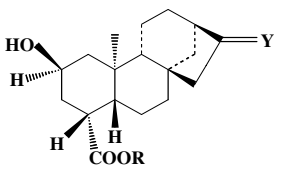
The antiproliferative effects of the compounds were estimated in terms of growth inhibition percentage. The activity of those compounds which showed a value of growth inhibition percentage greater than 15%, at a concentration of 100 μM or 10 μM, is reported in Table 2.

Methotrexate (**MTX**), whose activity as antileukemic is well known, was used as a reference compound and tested at 0.1 μM on K-562 or 0.01 μM on HL-60.

Table 1: Structures of atractyligenine derivatives 3–12



Compd.	R	X	Y	Z
4	H	α-H, β-OH	α-OH, β-CH_2OH	β-H, α-OH
5	CH_3	α-H, β-OH	H_2	β-H, α-OH
6	CH_3	O	H_2	O
7	CH_3	α-H, β-OH	H_2	O
8	CH_3	O	α-OH, β-CH_2OH	O
12	H	α-H, β-OH	α-H, β-CH_3	O



Compd.	R	Y
9	H	α-CHO, β-H
10	CH_3	α-CH=NNHCONH_2 , β-H
11	CH_3	α-CH=NNHCSNH_2 , β-H

Table 2: Growth inhibition (%) recorded on K-562 cell line at 100 μM and on HL-60 at 10 μM concentrations

Compd.	K-562	HL-60
3	31.8	n.s.
4	n.s.	n.s.
5	22.6	n.s.
6	100	100
8	17.9	n.s.
9	22.6	n.s.
10	61.4	n.s.
11	45.4	n.s.
MTX*	88.2	38.8

MTX* = Metotrexate tested at 0.1 μM (K562) or 0.01 μM (HL-60); values are the average of at least three independent determinations; variation was less than 15%; n.s. = not significant

Table 3: IC_{50} (μM) of compounds 6 and 10 recorded on K-562, HL-60 and MCF-7 cell lines

Compd.	K-562	HL-60	MCF-7
6	0.8	0.9	6.9
10	80	–	–

We determined the IC_{50} values (drug concentration at which the cell proliferation was inhibited to 50% of the untreated growth control) for compounds **6** and **10**, which exhibited the best activities at screening concentration; moreover, compound **6** which was overall the most active derivative, was tested for growth inhibitory effects *in vitro* on MCF-7, human breast adenocarcinoma, and its IC_{50} was determined (Table 3).

Compounds **6** and **10** showed the best antiproliferative activity with an IC_{50} (μM) of 0.8 and 80 against K-562 respectively, only compound **6** exhibited good activity against HL-60 with a IC_{50} value of 0.9 μM, and a moderate activity on MCF-7, in fact its IC_{50} value was 6.9 μM.

3. Experimental

All melting points were determined on a Büchi-Tottoli apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer FT-IR 1720 spectrophotometer as nujol mull supported on NaCl disks. ^{1}H NMR spectra were obtained using a Bruker AC-E 250 MHz spectrometer. Chemical shifts are given in δ (ppm) relative to TMS and coupling costants, *J*, are quoted in Hz. MS were recorded on Magnet Orthogonal TOF Autospec Ultima Micromass (ionization beam energy: 70 eV, source temperature 200 °C, sample temperature 50 °C, acceleration voltage 8 kV, trap current 100 mA, resolution 1500).

3.1. Synthesis of the compounds

3.1.1. Atractyliretine methyl ester semicarbazone (**10**)

Atractyliretine methyl ester (**9**, 400 mg) was dissolved in methanol (20 ml); 15 ml of an aqueous solution containing 0.4 g of semicarbazide hydrochloride and 0.3 g of sodium acetate were added to the solution. The reaction mixture was refluxed for 4 h. After cooling methanol was evaporated under vacuum and the solution obtained was added with 10 ml of H_2O, extracted with EtOAc (2 × 25 ml) and dried over anh. Na_2SO_4. Evaporation of the solvent, flash CC of the residue on Sigel using dichloromethane-methyl alcohol (95 : 5) as the eluent, yielded compound **10** as a chromatographically pure glassy solid, m.p. 141–142 °C.

MS, m/z: 391(M^+), 373, 359, 314, 299, 281, 254, 199, 145. IR, ν_{max}: 1680 cm^{-1} (−$CONH_2$), 1725 cm^{-1} (−$COOCH_3$), 3200 cm^{-1} (−NH_2), 3320 cm^{-1} (−NH), 3470 cm^{-1} (−OH). ^{1}H NMR ($CDCl_3$) δ: 0.81 (3 H, s, −CH_3), 1.2–2.2 (aliph. −CH_2−.), 2.5 (1 H, m, H-4), 3.61 (3 H, s, −OCH_3), 4.12 (1 H, m, H-2), 5.78 (2 H, broad s, −NH_2), 7.03 (1 H, d, J = 6 Hz, H-17), 9.60 (1 H, broad s, −NH). Signals at 5.78 and 9.60 δ exchange with D_2O.

3.1.2. Atractyliretine methyl ester thiosemicarbazone (**11**)

Atractyliretine methyl ester (**9**, 400 mg) was dissolved in methanol (20 ml). 15 ml of a aqueous solution containing 0.4 g of thiosemicarbazide hydrochloride and 0.3 g of sodium acetate was added to the solution. The reaction mixture was refluxed for 4 h. After cooling methanol was evaporated under vacuum. To the solution obtained 10 ml of water were added, and the mixture was extracted with EtOAc (2 × 25 ml) and dried over anh. Na_2SO_4. Evaporation of the solvent, flash CC of the residue on Sigel using dichloromethane-methyl alcohol (98 : 2) as the eluent, yielded compound **11** as a chromatographically pure white solid, m.p. 143–144 °C.

MS, m/z: 407 (M^+), 372, 331, 299, 271, 254, 201, 132. IR, ν_{max}: 1722 cm^{-1} (−$COOCH_3$), 3156 cm^{-1} (−NH_2), 3320 cm^{-1} (−NH), 3420 cm^{-1} (−OH), 1523 and 1199 cm^{-1} (−C=S). ^{1}H NMR ($CDCl_3$) δ: 0.80 (3 H, s, −CH_3), 1.2 e 2.2 (aliph. −CH_2−), 2.54 (1 H, m, H-4), 3.61 (3 H, s, −OCH_3), 4.16 (1 H, m, H-2), 7.30 (1 H, d, J = 6 Hz, H-17), 7.01 and 7.05 (2 H, broad s, −NH_2), 10.16 (1 H, broad s, −NH). Signals at 7.01, 7.05 and 10.16 δ exchange with D_2O.

3.2. Antimicrobial assay in vitro

Compounds **3**–**12** were screened for their *in vitro* antimicrobial activity against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 13803.

The test media used were Iso-Sensitest Agar (Oxoid) for bacteria and Yeast Morphology Agar (Difco) for fungi. A suitable volume of each substance (in a solution of DMSO) was added to 20 ml of molten agar (at 50 °C) and the resulting mixture was poured onto plates and allowed to solidify. The plates were inoculated with a bacterial suspension containing ≈10^6 cfu/ml or a fungal suspension containing ≈10^5 cfu/ml, using a 1 μl calibrate loop. The microbial cultures were incubated at 37 °C for 24 h. The lowest concentration of substance which completely inhibited the growth of the test organism when compared with the growth of a drug-free control (containing the maximum DMSO concentration used) was considered the MIC.

3.3. Cytotoxicity studies in vitro

Compounds **3**–**6**, and **8**–**11** were tested *in vitro* for their antiproliferative activity against K-562 (human chronic myelogenous leukemia) and HL-60 (human leukemia) cell lines. These cell lines were grown at 37 °C in a humidified atmosphere containing 5% CO_2, in RPMI-1640 medium (Biochrom KG) supplemented with 10% fetal calf serum and antibiotics.

Methotrexate (MTX), whose activity as antileukemic is well known, was used as reference compound.

K-562 and HL-60 cells were suspended at a density of 1 × 10^5 or 2 × 10^5 respectively cells per ml in growth medium, transferred to 24-well plate (1 ml per well), cultured with or without screening concentration of compounds and incubated at 37 °C for 48 h. Numbers of viable cells were determined by counting in a hematocytometer after dye exclusion with trypan blue [12].

The antiproliferative activity against K-562 was also determined by the MTT assay [13], in this case cells were suspended at a density of 2 × 10^5 cells per ml in RPMI-1640 without phenol red, supplemented with 10% fetal calf serum, 0.0025% glutamine and antibiotics, transferred (50 μl per well) to a 96-well plate containing 50 μl of the medium described and cultured with or without test compounds at screening concentration and incubated at 37 °C for 4 days.

The antiproliferative effects of the compounds were estimated in terms of percent growth inhibition. The activity of those compounds which showed a growth inhibition value greater than 15% at a screening concentration of 100 μM are reported.

We determined IC_{50} values (test agent concentration at which the cell proliferation was inhibited to 50% of the untreated growth control) for compounds **6** and **10** which exhibited the best activities at screening concentration.

The inhibitory growth activity on MCF-7 was evaluated in a similar way. MCF-7 were suspended at a density of 2 × 10^5 cells per ml in RPMI-1640 supplemented with 10% fetal calf serum and antibiotics, transferred to 24 well plate (1 ml/well) and incubated at 37 °C for 48 h. After this incubation time the wells reached confluence the medium was removed and the cells were further incubated for 48 h at 37 °C with fresh medium added with several concentrations of compound 6. At the end of this incubation time, the cells were trypsinized, harvested in 1 ml of medium and counted by a hematocytometer after dye exclusion with trypan blue.

[1] Part V [1]

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