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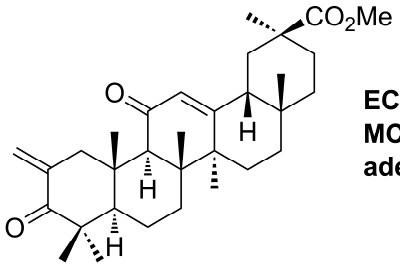

EC_{50} = 0.74 μM
MCF-7, human breast adenocarcinoma cells)

Incorporation of a Michael acceptor enhances the antitumor activity of triterpenoic acids

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Graphical abstract

Incorporation of a Michael acceptor enhances the antitumor activity of triterpenoic acids

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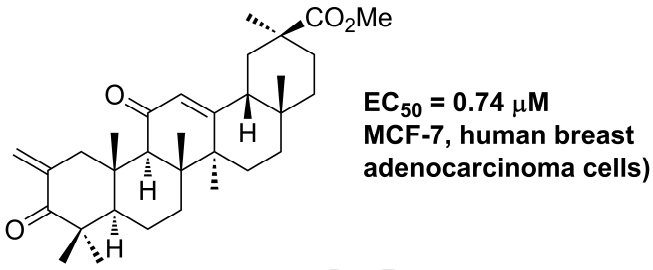

Abstract

Finding and developing drugs for the treatment of cancer has been challenging scientists for many decades, and using compounds of natural origin represents one of several strategies. Triterpenoic acids are a very promising class of secondary metabolites being able to induce apoptosis while their cytotoxicity is low. Therefore, derivatizations have to be conducted to improve cytotoxicity while retaining their ability to induce programmed cell death. The incorporation of a Michael acceptor into molecules resulted very often in drugs of improved cytotoxicity. Thus, in this study we synthesized and evaluated several Michael acceptor substituted compounds derived from glycyrrhetinic, ursolic, oleanolic and platanic acid. The influence of the presence of such a functional group onto the cytotoxicity was investigated in colorimetric sulforhodamine B assays employing several human cancer cell lines. EC_{50} values in the single-digit micromolar range were measured. Thus, the incorporation of a Michael acceptor unit into triterpenoic acids enhances the cytotoxicity of these compounds significantly.

1. Introduction

The search for antitumor drugs has been a task for generations of chemists, physicians and pharmacists. One of these strategies starts from biological active substances found in nature and tries to optimize their activity by derivatization. Triterpenoic acids represent a promising class of antitumor compounds easily to be obtained from several natural sources. Betulinic acid,[1-4] oleanolic acid [5-7] or glycyrrhetinic acid [8-12] have already been part of numerous investigations. In a few cases rather potent cytotoxic compounds were obtained showing activity in micromolar or even nanomolar concentrations. Furthermore, the ability to trigger apoptosis could be shown for several of them, comprising the triterpenoic acids [9, 10, 13] as well as analogs derived from the parent pentacyclic triterpenoic acids (Fig. 1).[8, 13]

Insert Fig. 1

Derivatization of natural occurring compounds is usually performed by functional group

modifications. Thus, altering the molecular scaffold is an effective strategy for optimizing biological activities. As far as the antitumor activity is concerned, the incorporation of a Michael-acceptor into a given molecular structure has already been shown to enhance the cytotoxic properties of secondary natural products.[14, 15] Therefore, we decided to synthesize a series of different triterpenoic acid bearing a Michael acceptor moiety and to evaluate their cytotoxic potential in sulforhodamine B assays employing different human cancer celllines.

2. Results and discussion

2.1. Chemistry

Starting from the methyl esters of ursolic (**1**), 11-oxo ursolic (**2**), oleanolic (**3**), 11-oxo oleanolic (**4**), glycyrrhetinic (**5**) or platanic acid (**6**), a Michael acceptor group was introduced in a two-step synthesis (Scheme 1). First, these esters were oxidized in position 3 by Jones oxidation using chromium(VI)oxide and sulphuric acid in acetone (**7**-**11**, **22**)[16]. The Michael acceptor moiety was created by the reaction of the 3-oxo-esters **7**-**11** (Scheme 1) or **22** (Scheme 2) with paraformaldehyde/finely grounded potassium carbonate in DMF, and α-methylenated compounds **12**-**16** [6, 17] were obtained in 44-67 % isolated yield.

In order to investigate the influence of the Michael acceptor, three types of modifications were performed: First, the Michael-acceptor moiety was reduced. The reaction of **16** with sodium borohydride in the presence of cerium(III) chloride gave an 81 % yield of the 3-β-hydroxy-2-methylene derivative **21**. Furthermore, compounds **7**, **8** and **10** were allowed to react with bromine in glacial acetic acid and converted into their corresponding 2,2-dibromo-3-oxo derivatives **17**-**19**. Finally, a 2β-methyl-3-oxo derivative **20** was synthesized starting from **7** by its reaction with diisopropylamine and *n*-butyllithium in THF at -78 °C followed by the addition of iodomethane [18]. From NOESY-NMR spectra a 2β configuration of the methyl group was deduced.

Inspection of the ^{1}H NMR spectra of compounds **12**-**16** showed the chemical shift for their methylene protons between δ = 5.90 and 6.02 ppm as well as between δ = 5.06 and 5.23 ppm, while the chemical shift for the alkenic carbons were detected in the ^{13}C NMR spectra between δ = 123.6 and 124.1 ppm and δ = 141.8 and 142.0 ppm, respectively. For

dibrominated compounds **17-19** in their ^{13}C NMR spectra for C-2 a chemical shift of about δ = 63 ppm was observed. Furthermore, for these compounds the typical isotopic pattern $[M^{79}Br+1]^+$:$[M^{81}Br+1]^+$ was observed in their ESI-MS spectra.

Insert Scheme 1

Methyl 2-oxo-platanoate (**22**, scheme 2), however, could not be transformed into the corresponding bis alkene: As expected, the Michael acceptor was created in ring A (scheme 2), but also the methyl ketone attached to ring E was transformed, and products **23** (71 %) and **24** (2 %) were formed. The formation of an 8-membered ring in these reactions, however, is not unprecedented.[19, 20]

Insert Scheme 2

2.2. **Biology**

Cytotoxicity of the compounds was determined using the photometric sulforhodamine B assay [21-23] employing five different human cancer cell lines. The results from these assays are compiled in the Table. Compounds **23** and **24** could not be measured due to their poor solubility under the conditions of the assay.

As compared to their parent 3-oxo compounds **7-11** the cytotoxicity is considerably increased for all analogues **12-16** bearing Michael acceptor groups. While the EC_{50} values for compounds **7-11** were greater than 30 µM (cut-off of the assay), the incorporation of a Michael acceptor group into ring A significantly improved the cytotoxicity of the compounds, and EC_{50} values in the single-digit micromolar range were observed. The highest activities could be determined for methyl glcycyrrhetinoate derived **16** showing an EC_{50} as low as 0.74 µM for MCF-7 breast cancer cells. Interestingly, the oleanolic acid derived compounds **14** and **15** are less active than methyl ursoate derived compounds **12** and **13**, respectively. Nevertheless, all four compounds gave EC_{50} values between 1.2 and 5.4 µM.

Insert Table

When the Michael-acceptor unit was destroyed by reduction of either the methylene group (→

20) or the carbonyl group (→ **21**) the cytotoxicity was considerably decreased, while the introduction of two bromo-substituents in position C-3 (→ **17**-**19**) decreased cytotoxicity only slightly or not at all. (**20**, **21**).

3. Conclusions

A series of 18 derivatives – derived from glycyrrhetinic, oleanolic, ursolic and platanic acid – were synthesized and tested for their cytotoxic activity employing five different human malignant cell lines. The incorporation of a Michael acceptor unit into ring A of the triterpenoid skeleton considerably enhanced the cytotoxic potential as compared to their parent compounds and derivatives devoid of the this group. Being the highlight of our investigations, compound **16**, an derivative derived from glycyrrhetinic acid, gave EC_{50} values as low as 0.74 µM with MCF-7 cells. Although 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO), a common known and comparable oleanolic acid analogue, is still a little more active (here EC_{50} values of 0.16 µM [24] have been reported) the enhancing potential [6] of the Michael acceptor moiety was clearly demonstrated.

Acknowledgements

We like to thank Dr. R. Kluge for measuring the ESI-MS spectra, Dr. D. Ströhl for NMR measurements, and Miss J. Wiese for IR spectra and optical rotations. Many thanks are due to Dr. Th. Müller from the Dep. of Haematology/Oncology for providing the cells, and to the "Gründerwerkstatt-Biowissenschaften" for support.

4. Experimental section

4.1. General

Melting points are uncorrected (Leica hot stage microscope), NMR spectra were recorded using the Varian spectrometers Gemini 2000 or Unity 500 (δ given in ppm, J in Hz, internal Me_4Si), IR spectra (film or KBr pellet) on a Perkin Elmer FT-IR spectrometer Spectrum 1000, MS spectra were taken on a Finnigan MAT LCQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. The optical rotation was measured on a Perkin Elmer polarimeter at

20 °C; TLC was performed on silica gel (Merck 5554); elemental analyses were performed on a Vario EL (CHNS). The solvents were dried according to usual procedures. The purity of the compounds was determined by HPLC and found to be > 98 %.

Oleanolic, ursolic and platanic acid were obtained from Betulinines (Stribrna Skalice, Czech Republic), glycyrrhetinic acid was bought from Orgentis (Gatersleben, Germany), and the methyl esters as well as the 3-oxo derivatives were synthesized according to literature. [22, 23] The synthesis of **11** has already been described.[25]

4.2. General procedure for oxidation at position C(3) (method A)

The methyl triterpenoate (1 equiv.) was dissolved in acetone (10 mL per 0.1 g starting material) and cooled to 0 °C. CrO_3 (2.5 equiv.) in sulphuric acid (1 M, 1 mL per 0.1 mmol CrO_3) was added, and the mixture was stirred at room temperature for 1 hour. Ethanol (half the amount of acetone) was added, the mixture filtered, and the filtrate was evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexane/EtOAc, 4:1) to yield the methyl 3-oxo-triterpenoate.

4.3. General procedure for the incorporation of the Michael acceptor moiety (method B)

The methyl 3-oxo-triterpenoate (1 equiv.) was dissolved in dry DMF (10 mL per 200 mg starting material), and powdered potassium carbonate (4 equiv.) as well as paraformaldehyde (5 equiv.) were added. The mixture was heated to 90 °C and stirred for 1 h. After cooling to 25 °C, water was added, the reaction mixture was neutralized by adding aqueous hydrochloric acid (2 M), and the aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine (25 mL), dried (Na_2SO_4), filtered and evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexane-EtOAc, 9:1) to yield the product.

4.4. General procedure for the bromination reaction (method C)

The methyl 3-oxo-triterpenoate derivative (1 equiv.) was dissolved in acetic glacial acetic acid (1 mL per 10 mg of starting material), and bromine (4.5 equiv. dissolved in 1 mL of glacial

acetic acid per 30 mg bromine) was slowly added. Stirring was continued for 15 min, water (10 mL), an aqueous solution of potassium bisulphite solution (satd., 10 mL) and of sodium hydrogen carbonate (satd., 20 mL) were added. The mixture was extracted with DCM (3 × 30 mL), the combined organic layers were washed (NaCl), dried (Na_2SO_4), filtered and evaporated to dryness. The residue was subjected to chromatographic purification (silica gel, hexane-EtOAc, 4:1) to yield the dibromo compound.

*4.5. Methyl 3-oxo-urs-12-en-28-oate (**7**)*

Compound **7** (2.33 g, 88 %) was obtained as colorless crystals from methyl (3β) 3-hydroxy-urs-12-en-28-oate (**1**) using method A; mp 193-195 °C (lit.:[26] 192-193 °C,[27] 191-194 °C [28]); R_f = 0.67 (hexane/EtOAc, 8:2). $[\alpha]_D$ = +90° (*c* = 5.90, $CHCl_3$) (lit.: [27] +85° (*c* = 1.23, $CHCl_3$)); UV-vis (MeOH): λ_{max} = 222 nm (log ε = 3.80); IR (KBr): ν = 2970*s*, 2947*s*, 2857*m*, 1731*s*, 1698*s*, 1461*m*, 1446*m*, 1430*w*, 1385*w*, 1378*w*, 1366*w*, 1308*w*, 1273*w*, 1226*m*, 1202*m*, 1169*m*, 1144*m*, 1113*w*, 1102*w*, 1080*w*, 1030*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.20 (m, 1H, H-12), 3.54 (s, 3H, OMe), 2.47 (ddd, *J* = 15.9, 11.0, 7.3 Hz, 1H, H-2), 2.30 (ddd, *J* = 15.9, 6.9, 3.8 Hz, 1H, H-2'), 2.18 (d, *J* = 11.2 Hz, 1H, H-18), 1.94 (ddd, *J* = 13.4, 13.3, 4.6 Hz, 1H, H-16), 1.91-1.87 (m, *J* = 8.7, 3.4 Hz, 2H, H-11, H-11'), 1.85 (ddd, *J* = 13.2, 7.3, 3.8 Hz, 1H, H-1), 1.71 (ddd, *J* = 13.7, 13.6, 4.5 Hz, 1H, H-15), 1.64-1.58 (m, 2H, H-22, H-16'), 1.55-1.49 (m, 2H, H-9, H-22'), 1.46-1.34 (m, 5H, H-6, H-6', H-21, H-7, H-1'), 1.32-1.20 (m, 4H, H-19, H-5, H-7', H-21'), 1.05-1.00 (m, 1H, H-15'), 1.02 (s, 3H, H-23), 1.01 (s, 3H, H-27), 0.98 (s, 3H, H-25), 0.97 (s, 3H, H-24), 0.95-0.91 (m, 1H, H-20), 0.87 (d, *J* = 6.3 Hz, 3H, H-30), 0.79 (d, *J* = 6.5 Hz, 3H, H-29), 0.73 (s, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 217.7 (C-3), 177.9 (C-28), 138.2 (C-13), 125.3 (C-12), 55.3 (C-5), 52.9 (C-18), 51.4 (OMe), 48.1 (C-17), 47.4 (C-4), 46.7 (C-9), 42.1 (C-14), 39.4 (C-8), 39.1 (C-1), 39.0 (C-19), 38.8 (C-20), 36.7 (C-10), 36.6 (C-22), 34.1 (C-2), 32.5 (C-7), 30.6 (C-21), 28.0 (C-15), 26.5 (C-23), 24.2 (C-16), 23.5 (C-27), 23.4 (C-11), 21.5 (C-24), 21.1 (C-30), 19.6 (C-6), 17.0 (C-29), 16.8 (C-26), 15.2 (C-25) ppm; ESI-MS (MeOH): *m/z* (%) = 469.3 ($[M + H]^+$, 75), 523.0 ($[M + Na + MeOH]^+$, 100); elemental anal. calcd for $C_{31}H_{48}O_3$ (468.71) C 79.44, H 10.32, found: C 79.31, H 10.47.

*4.6. Methyl 3,11-dioxo-urs-12-en-28-oate (**8**)*

Compound **8** (1.70 g, 88 %) was obtained as an amorphous solid from methyl (3β) 3-hydroxy-11-oxo-urs-12-en-28-oate (**2**) using method A; R_f = 0.33 (hexane/EtOAc, 8:2); $[\alpha]_D$ = +108° (*c* = 4.80, $CHCl_3$); UV/vis (MeOH): λ_{max} = 268 (log ε = 4.09) nm; IR (KBr): ν = 2950*s*,

2871*m*, 1728*s*, 1705*s*, 1661*s*, 1619*w*, 1458*m*, 1386*w*, 1321*w*, 1273*w*, 1245*w*, 1226*w*, 1200*m*, 1144*w*, 1110*w*, 1084*w*, 1001*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.62 (s, 1H, H-12), 3.60 (s, 3H, OMe), 2.94 (ddd, *J* = 13.5, 7.1, 4.1 Hz, 1H, H-1), 2.61 (ddd, *J* = 15.9, 11.2, 7.2 Hz, 1H, H-2), 2.42 (d, *J* = 11.0 Hz, 1H, H-18), 2.38 (s, 1H, H-9), 2.31 (ddd, *J* = 15.8, 10.6, 6.6 Hz, 1H, H-2'), 2.08 (ddd, *J* = 14.6, 14.6, 4.8 Hz, 1H, H-16), 1.83-1.73 (m, 3H, H-16', H-22, H-15), 1.64-1.46 (m, 5H, H-7, H-22', H-6, H-6', H-21), 1.43-1.34 (m, 3H, H-19, H-7', H-1'), 1.32-1.21 (m, 3H, H-5, H-15', H-21'), 1.29 (s, 3H, H-27), 1.23 (s, 3H, H-25), 1.09-1.02 (m, 1H, H-20), 1.08 (s, 3H, H-23), 1.03 (s, 3H, H-24), 0.95 (d, *J* = 6.6 Hz, 3H, H-30), 0.93 (s, 3H, H-26), 0.85 (d, *J* = 6.6 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 217.0 (C-3), 199.0 (C-11), 177.1 (C-28), 163.2 (C-13), 130.6 (C-12), 60.7 (C-9), 55.4 (C-5), 52.7 (C-18), 51.8 (OMe), 47.7 (C-4), 47.6 (C-17), 44.4 (C-8), 43.8 (C-14), 39.7 (C-1), 38.6 (C-19), 38.6 (C-20), 36.7 (C-10), 35.9 (C-22), 34.2 (C-2), 32.4 (C-7), 30.3 (C-21), 28.4 (C-15), 26.4 (C-23), 23.9 (C-16), 21.4 (C-24), 21.0 (C-27), 20.9 (C-30), 18.7 (C-6), 18.7 (C-26), 17.1 (C-29), 15.5 (C-25) ppm; ESI-MS (MeOH): *m/z* (%) = 483.5 ($[M + H]^+$, 100); elemental anal. calcd for $C_{31}H_{46}O_4$ (482.69): C 77.14; H9.61; found: 76.95; H 9.82.

*4.7. Methyl 3-oxo-olean-12-en-28-oate (**9**)*

Compound **9** (2.65 g, 83 %) was obtained from methyl (3β) 3-hydroxy-olean-12-en-28-oate (**3**) using method A; colorless crystals; mp 183-186 °C (lit.:[29] 184 °C); R_f = 0.66 (hexane/EtOAc, 8:2); $[\alpha]_D$ = +86° (*c* = 3.60, $CHCl_3$) (lit.:[29] +90° (*c* = 1.20, $CHCl_3$)); UV/vis (MeOH) λ_{max} = 229 (log ε = 3.70) nm; IR (KBr): ν = 2941*s*, 2863*m*, 1726*s*, 1703*s*, 1458*m*, 1382*w*, 1364*w*, 1304*w*, 1264*w*, 1230*w*, 1205*m*, 1178*w*, 1163*m*, 1125*w*, 1094*w*, 1040*w*, 1016*w*, 991*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.29 (m, 1H, H-12), 3.61 (s, 3H, OMe), 2.86 (dd, *J* = 13.7, 4.3 Hz, 1H, H-18), 2.53 (ddd, *J* = 16.0, 11.2, 7.3 Hz, 1H, H-2), 2.34 (ddd, *J* = 15.9, 6.7, 3.6 Hz, 1H, H-2'), 1.99-1.-83 (m, 4H, H-1, H-16, H-11, H-11'), 1.67 (ddd, *J* = 14.0, 13.9, 4.6 Hz, 1H, H-22), 1.64-1.56 (m, 4H, H-9, H-15, H-19, H-16'), 1.53-1.44 (m, 4H, H-22', H-7, H-6, H-6'), 1.42-1.26 (m, 4H, H-1', H-21, H-7', H-5), 1.20-1.11 (m, 2H, H-19', H-21'), 1.12 (s, 3H, H-27), 1.09-1.04 (m, 1H, H-15'), 1.06 (s, 3H, H-23), 1.02 (s, 3H, H-24), 1.02 (s, 3H, H-25), 0.91 (s, 3H, H-30), 0.87 (s, 3H, H-29), 0.76 (s, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 217.6 (C-3), 178.2 (C-28), 143.8 (C-13), 122.1 (C-12), 55.3 (C-5), 51.5 (OMe), 47.4 (C-9), 46.9 (C-4), 46.7 (C-17), 45.8 (C-19), 41.7 (C-14), 41.4 (C-18), 39.2 (C-8), 39.1 (C-1), 36.7 (C-10), 34.1 (C-2), 33.8 (C-21), 33.1 (C-29), 32.3 (C-7), 32.2 (C-22), 30.7 (C-20), 27.7 (C-15), 26.4 (C-23), 25.8 (C-27), 23.6 (C-30), 23.5 (C-11), 23.0 (C-16), 21.4 (C-24), 19.6 (C-6), 16.7 (C-26), 15.0 (C-25) ppm; ESI-MS (MeOH): *m/z* (%) = 469.4

([M + Na]$^+$, 90), 523.0 ([M + Na + MeOH]$^+$, 100); elemental anal. calcd for $C_{31}H_{48}O_3$ (468.71): C 79.44, H 10.32, found: C 79.28, H 10.52.

*4.8. Methyl 3,11-dioxo-olean-12-en-28-oate (**10**)*

Compound **10** (1.12 g, 75 %) was obtained as an amorphous solid from methyl (3β) 3-hydroxy-11-oxo-olean-12-en-28-oate (**4**) using method A; R_f = 0.46 (hexane/EtOAc, 8:2); $[\alpha]_D$ = +119° (*c* = 3.30, $CHCl_3$); UV/vis (MeOH): λ_{max} = 269 (log ε = 4.08) nm; IR (KBr): ν = 3432*br*, 2950*s*, 2866*m*, 1718*s*, 1704*s*, 1654*s*, 1460*m*, 1386*m*, 1365*w*, 1307*w*, 1261*m*, 1234*w*, 1210*m*, 1169*m*, 1125*w*, 1083*w*, 1012*w*, 997*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.66 (s, 1H, H-12), 3.63 (s, 3H, OMe), 3.05-2.95 (m, 2H, H-18, H-1), 2.64-2.56 (m, 1H, H-2), 2.41 (s, 1H, H-9), 2.39-2.32 (m, 1H, H-2'), 2.05 (ddd, *J* = 13.4, 13.0, 3.8 Hz, 1H, H-16), 1.78-1.57 (m, 6H, H-19, H-16', H-7, H-15, H-22, H-22'), 1.54-1.32 (m, 5H, H-1', H-7', H-21, H-6, H-6'), 1.35 (s, 3H, H-27), 1.31-1.18 (m, 4H, H-5, H-19', H-21', H-15'), 1.21 (s, 3H, H-25), 1.08 (s, 3H, H-23), 1.04 (s, 3H, H-24), 0.94 (s, 3H, H-26), 0.93 (s, 3H, H-30), 0.92 (s, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 217.1 (C-3), 199.5 (C-11), 177.4 (C-28), 169.1 (C-13), 127.8 (C-12), 61.0 (C-9), 55.4 (C-5), 51.9 (OMe), 47.7 (C-4), 46.2 (C-17), 44.8 (C-14), 44.3 (C-19), 43.6 (C-8), 41.6 (C-18), 39.7 (C-1), 36.8 (C-10), 34.2 (C-2), 33.7 (C-21), 32.8 (C-29), 32.2 (C-7), 31.5 (C-22), 30.6 (C-20), 27.8 (C-15), 26.5 (C-23), 23.5 (C-27), 23.4 (C-30), 22.9 (C-16), 21.3 (C-24), 18.8 (C-26), 18.7 (C-6), 15.5 (C-25) ppm; ESI-MS (MeOH): *m/z* (%) = 483.5 ([M + H]$^+$, 100); elemental anal. calcd for $C_{31}H_{46}O_4$ (482.69): C 77.14, H 9.61; found: C 77.02, H 9.79.

*4.9. Methyl 2-methylene-3-oxo-urs-12-en-28-oate (**12**)*

Compound **12** (640 mg, 45 %) was obtained as an amorphous solid from **7** using method B; R_f = 0.75 (hexane/EtOAc, 8:2); $[\alpha]_D$ = +100° (*c* = 3.20, $CHCl_3$); UV-vis (MeOH). λ_{max} = 221 (log ε = 3.82), 254 (log ε = 3.53) nm; IR (KBr): ν = 2948*s*, 1726*s*, 1688*m*, 1456*m*, 1382*m*, 1272*w*, 1229*m*, 1200*m*, 1166*w*, 1145*m*, 1113*w*, 1057*w*, 1033*w*, 1002*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.93 (m, 1H, methylene), 5.23 (m, 1H, H-12), 5.07 (m, 1H, methylene), 3.55 (s, 3H, OMe), 2.60 (d, *J* = 15.2 Hz, 1H, H-1), 2.19 (d, *J* = 11.4 Hz, 1H, H-18), 2.09 (d, *J* = 15.0 Hz, 1H, H-1'), 1.97-1.90 (m, 3H, H-11, H-11', H-16), 1.71 (ddd, *J* = 13.6, 13.2, 4.6 Hz, 1H, H-15), 1.65-1.18 (m, 12H, H-19, H-5, H-9, H-6, H-6', H-21, H-21', H-7, H-7', H-22, H-22', H-16'), 1.08-1.02 (m, 1H, H-15'), 1.06 (s, 3H, H-23), 1.04 (s, 3H, H-27), 1.00 (s, 3H, H-24), 0.97-0.92 (m, 1H, H-20), 0.88 (d, *J* = 6.3 Hz, 3H, H-30), 0.86 (s, 3H, H-25), 0.80 (d, *J* = 6.2 Hz, 3H, H-29), 0.73 (s, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 207.3 (C-3),

177.9 (C-28), 141.9 (C-2), 138.2 (C-13), 125.3 (C-12), 123.6 (C-31), 54.2 (C-5), 53.0 (C-18), 51.4 (OMe), 48.1 (C-17), 47.0 (C-1), 45.8 (C-4), 45.2 (C-9), 42.2 (C-14), 39.4 (C-8), 39.1 (C-19), 38.8 (C-20), 36.6 (C-10), 36.6 (C-22), 32.3 (C-7), 30.6 (C-21), 28.3 (C-23), 28.0 (C-15), 24.2 (C-16), 23.4 (C-27), 23.4 (C-11), 22.7 (C-24), 21.1 (C-30), 20.1 (C-6), 17.0 (C-29), 16.8 (C-26), 15.1 (C-25) ppm; ESI-MS (MeOH): *m/z* (%) = 481.1 ($[M + H]^+$, 100); elemental anal. calcd for $C_{32}H_{48}O_3$ (480.72): C 79.95, H 10.06; found: C 79.93, H 10.19.

*4.10. Methyl 2-methylene-3,11-dioxo-urs-12-en-28-oate (**13**)*

Compound **13** (435 mg, 44 %) was obtained from **8** using method B; colorless crystals; mp 219-222 °C; R_f = 0.5 (hexane/EtOAc, 8:2); $[\alpha]_D$ = +126° (*c* = 4.40, $CHCl_3$), UV/vis (MeOH): λ_{max} = 266 (log ε = 4.13) nm; IR KBr): ν = 2950*s*, 2870*m*, 1728*s*, 1687*m*, 1661*s*, 1618*w*, 1458*m*, 1384*m*, 1320*w*, 1273*w*, 1225*w*, 1200*m*, 1147*w*, 1113*w*, 1085*w*, 1057*w*, 1004*w* cm-1; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.95 (m, 1H, methylene), 5.59 (s, 1H, H-12), 5.18 (m, 1H, methylene), 3.69 (d, *J* = 15.6 Hz, 1H, H-1), 3.56 (s, 3H, OMe), 2.39 (d, *J* = 11.4 Hz, 1H, H-18), 2.35 (s, 1H, H-9), 2.10 (d, *J* = 15.3 Hz, 1H, H-1'), 2.03 (ddd, *J* = 14.7, 14.4, 4.7 Hz, 1H, H-16), 1.78-1.68 (m, 3H, H-16', H-22, H-15), 1.61-1.46 (m, 3H, H-7, H-22', H-21), 1.44-1.20 (m, 7H, H-19, H-7', H-6, H-6', H-5, H-15', H-21'), 1.25 (s, 3H, H-27), 1.08 (s, 3H, H-25), 1.06 (s, 3H, H-23), 1.04-1.02 (m, 1H, H-20), 1.01 (s, 3H, H-24), 0.91 (d, *J* = 6.4 Hz, 3H, H-30), 0.89 (s, 3H, H-26), 0.81 (d, *J* = 6.4 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.6 (C-3), 198.9 (C-11), 177.1 (C-28), 163.3 (C-13), 141.8 (C-2), 130.6 (C-12), 124.1 (methylene), 58.9 (C-9), 54.4 (C-5), 52.8 (C-18), 51.8 (OMe), 47.6 (C-17), 47.2 (C-1), 46.0 (C-4), 44.3 (C-8), 43.8 (C-14), 38.7 (C-19), 38.6 (C-20), 36.7 (C-10), 35.9 (C-22), 32.1 (C-7), 30.3 (C-21), 28.4 (C-15), 28.3 (C-23), 23.9 (C-16), 22.7 (C-24), 20.9 (C-27), 20.9 (C-30), 19.3 (C-6), 18.5 (C-26), 17.0 (C-29), 15.0 (C-25) ppm; ESI-MS (MeOH): *m/z* (%) = 495.5 ($[M + H]^+$, 100); elemental anal. calcd for $C_{32}H_{46}O_4$ (494.71): C 77.69, H 9.37; found C 77.50, H 9.49.

*4.11. Methyl 2-methylene-3-oxo-olean-12-en-28-oate (**14**)*

Compound **14** (720 mg, 56 %) was obtained as an amorphous solid from **9** using method B; R_f = 0.76 (hexane/EtOAc, 8:2); $[\alpha]_D$ = +102° (*c* = 3.60, $CHCl_3$); UV/vis (MeOH): λ_{max} = 217 (log ε = 4.06) nm; IR (KBr): ν = 2949*s*, 1729*s*, 1669*w*, 1461*m*, 1385*m*, 1364*w*, 1303*w*, 1262*m*, 1231*w*, 1191*m*, 1163*m*, 1124*w*, 1077*w*, 1060*w*, 1035*w*, 1016*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.92 (m, 1H, methylene), 5.26 (m, 1H, H-12), 5.07 (m, 1H, methylene), 3.56 (s, 3H, OMe), 2.82 (dd, *J* = 13.9, 4.4 Hz, 1H, H-18), 2.57 (d, *J* = 15.0 Hz, 1H, H-1), 2.06

(ddd, *J* = 15.0 Hz, 1H, H-1'), 1.94-1.87 (m, 3H, H-16, H-11, H-11'), 1.66-1.51 (m, 5H, H-22, H-9, H-15, H-19, H-16'), 1.49-1.24 (m, 7H, H-21, H-22', H-7, H-7', H-6, H-6', H-5), 1.15-1.02 (m, 3H, H-19', H-21', H-15'), 1.09 (s, 3H, H-27), 1.06 (s, 3H, H-23), 0.99 (s, 3H, H-24), 0.87 (s, 3H, H-25), 0.86 (s, 3H, H-30), 0.84 (s, 3H, H-29), 0.72 (s, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 207.3 (C-3), 178.2 (C-28), 143.8 (C-13), 142.0 (C-2), 123.6 (C-31), 122.1 (C-12), 54.1 (C-5), 51.5 (OMe), 46.7 (C-1), 46.0 (C-4), 45.9 (C-17), 45.8 (C-19), 45.4 (C-9), 41.8 (C-14), 41.4 (C-18), 39.1 (C-8), 36.8 (C-10), 33.8 (C-21), 33.1 (C-29), 32.3 (C-22), 32.0 (C-7), 30.7 (C-20), 28.3 (C-23), 27.7 (C-15), 25.7 (C-27), 23.6 (C-30), 23.4 (C-11), 23.0 (C-16), 22.7 (C-24), 20.1 (C-6), 16.6 (C-26), 14.9 (C-25) ppm; ESI-MS (MeOH): *m/z* (%) = 481.3 ($[M + H]^+$, 80), 535.0 ($[M + Na + MeOH]^+$, 100); elemental anal. calcd for $C_{32}H_{48}O_3$ (468.71): C 79.95, H 10.06; found: C 79.83, H 10.25.

*4.12. Methyl 2-methylene-3,11-dioxo-olean-12-en-28-oate (**15**)*

Compound **15** (295 mg, 67 %) was obtained from **10** using method B; colorless crystals; mp 210-212 °C; R_f = 0.52 (hexane/EtOAc, 8:2); $[\alpha]_D$ = +129° (*c* = 4.30, $CHCl_3$); UV/vis (MeOH): λ_{max} = 266 (log ε = 4.14) nm; IR (KBr): ν = 3432*br*, 2949*s*, 2867*m*, 1728*s*, 1687*m*, 1659*s*, 1622*w*, 1463*m*, 1385*m*, 1363*w*, 1328*w*, 1263*w*, 1190*m*, 1167*m*, 1126*w*, 1081*w*, 1059*w*, 1014*w*, 978*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.99 (m, 1H, methylene), 5.67 (s, 1H, H-12), 5.23 (m, 1H, methylene), 3.78 (d, *J* = 15.8 Hz, 1H, H-1), 3.62 (s, 3H, OMe), 3.02 (dd, *J* = 13.8, 4.0 Hz, 1H, H-18), 2.43 (s, 1H, H-9), 2.13 (d, *J* = 15.6 Hz, 1H, H-1'), 2.04 (ddd, *J* = 14.0, 13.8, 4.0 Hz, 1H, H-16), 1.78-1.57 (m, 6H, H-19, H-16', H-7, H-15, H-22, H-22'), 1.51-1.44 (ddd, *J* = 8.6, 7.4, 3.1 Hz, 1H, H-6), 1.41-1.30 (m, 4H, H-5, H-7, H-21, H-6'), 1.36 (s, 3H, H-27), 1.28-1.17 (m, 3H, H-19', H-21', H-15'), 1.11 (s, 3H, H-25), 1.10 (s, 3H, H-23), 1.06 (s, 3H, H-24), 0.93 (s, 3H, H-26), 0.92 (s, 3H, H-30), 0.91 (s, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.7 (C-3), 199.4 (C-11), 177.4 (C-28), 169.2 (C-13), 141.8 (C-2), 127.8 (C-12), 124.0 (methylene), 59.3 (C-9), 54.4 (C-5), 51.9 (OMe), 47.1 (C-1), 46.2 (C-17), 46.1 (C-4), 44.6 (C-14), 44.3 (C-19), 43.6 (C-8), 41.6 (C-18), 36.8 (C-10), 33.7 (C-21), 32.8 (C-29), 31.9 (C-7), 31.5 (C-22), 30.7 (C-20), 28.3 (C-23), 27.8 (C-15), 23.4 (C-27), 23.4 (C-30), 22.9 (C-16), 22.6 (C-24), 19.3 (C-6), 18.5 (C-26), 15.0 (C-25) ppm; ESI-MS (MeOH): *m/z* (%) = 495.5 ($[M + H]^+$, 100); elemental anal. calcd for $C_{32}H_{46}O_4$ (494.71): C 77.69, H 9.37; found: C 77.49, H 9.50.

*4.13. Methyl 2-methylene-3,11-dioxo-olean-12-en-30-oate (**16**)*

Compound **16** (450 mg, 59 %) was obtained from methyl 3,11-dioxo-olean-12-en-30-oate

(**11**) using method B; colorless crystals; mp 186-190 °C; R_f = 0.67 (hexane/EtOAc, 8:2); $[\alpha]_D$ = +160° (*c* = 0.33, $CHCl_3$); UV/vis (MeOH): λ_{max} = 247 (log ε = 4.10) nm; IR (KBr) ν = 3427*br*, 2926*s*, 1724*s*, 1686*m*, 1651*s*, 1468*m*, 1385*m*, 1324*w*, 1279*w*, 1247*w*, 1221*m*, 1160*m*, 1090*w*, 1050*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 6.02 (dd, *J* = 2.2 Hz, 1H, methylene), 5.72 (s, 1H, H-12), 5.26 (dd, *J* = 2.2 Hz, 1H, methylene), 3.76 (d, *J* = 15.3 Hz, 1H, H-1), 3.70 (s, 3H, OMe), 2.47 (s, 1H, H-9), 2.14 (m, 1H, H-1'), 2.14 (m, 1H, H-18), 2.04 (ddd, *J* = 13.7, 13.7, *J* = 4.4 Hz, 1H, H-15), 2.00 (m, 1H, H-21), 1.93 (ddd, *J* = 13.6, 3.7, 2.8 Hz, 1H, H-19), 1.85 (ddd, *J* = 13.7, 13.7, 4.6 Hz, 1H, H-16), 1.71 (m, 1H, H-7), 1.62 (dd, *J* = 13.5, 13.5 Hz, 1H, H-19'), 1.54 (m, 1H, H-6), 1.53 (m, 1H, H-6'), 1.48 (ddd, *J* = 12.8, 3.1, 3.1 Hz, 1H, H-7'), 1.41 (m, 1H, H-5), 1.40 (m, 1H, H-22), 1.39 (s, 3H, H-27), 1.32 (m, 2H, H-22', H-21'), 1.23 (m, 1H, H-16'), 1.17 (s, 3H, H-26), 1.17 (s, 3H, H-25), 1.15 (s, 3H, H-29), 1.15 (s, 3H, H-23), 1.09 (s, 3H, H-24), 1.04 (m, 1H, H-15'), 0.83 (s, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.7 (C-3), 199.3 (C-11), 176.9 (C-30), 169.7 (C-13), 141.9 (C-2), 128.5 (C-12), 124.1 (methylene), 59.3 (C-9), 54.4 (C-5), 51.8 (OMe), 48.4 (C-18), 47.2 (C-1), 46.1 (C-4), 45.0 (C-8), 44.0 (C-20), 43.3 (C-14), 41.2 (C-19), 37.7 (C-22), 36.7 (C-10), 31.8 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-28), 28.3 (C-29), 28.2 (C-23), 26.5 (C-16), 26.4 (C-15), 23.3 (C-27), 22.7 (C-24), 19.4 (C-6), 18.2 (C-26), 15.1 (C-25) ppm; ESI-MS (MeOH): *m/z* (%) = 495.5 ($[M + H]^+$, 100), 517.4 ($[M + Na]^+$, 7), 548.9 ($[M + Na + MeOH]^+$, 61), 989.1 ($[2M + H]^+$, 16), 1011.2 ($[2M + Na]^+$, 18); elemental anal. calcd for $C_{32}H_{46}O_4$ (494.71): C 77.69, H 9.37; found: 77.45, H 9.52.

*4.14. Methyl 2,2-dibromo-3-oxo-urs-12-en-28-oate (**17**)*

Compound **17** (195 mg, 80 %) was obtained as an amorphous solid from **7** using method C; R_f = 0.57 (hexane/EtOAc, 9:1); $[\alpha]_D$ = +43° (*c* = 5.70, $CHCl_3$); UV/vis (MeOH): λ_{max} = 217 (log ε = 3.98) nm; IR (KBr): ν = 3424*br*, 2952*s*, 1725*s*, 1637*w*, 1457*m*, 1387*w*, 1203*m*, 1112*w* cm-1; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.28 (m, 1H, H-12), 3.60 (d, *J* = 15.9 Hz, 1H, H-1), 3.59 (s, 3H, OMe), 3.17 (d, *J* = 16.2 Hz, 1H, H-1'), 2.25 (d, *J* = 11.3 Hz, 1H, H-18), 2.02-1.90 (m, 3H, H-16, H-11, H-11'), 1.78-1.73 (m, 2H, H-5, H-15), 1.70-1.63 (m, 3H, H-22, H-22', H-16'), 1.57-1.45 (m, 4H, H-9, H-6, H-21, H-7), 1.51 (s, 3H, H-23), 1.42-1.35 (m, 3H, H-19, H-7', H-6'), 1.30-1.25 (m, 1H, H-21'), 1.23 (s, 3H, H-24), 1.13-1.09 (m, 1H, H-15'), 1.10 (s, 3H, H-27), 1.03 (s, 1H, H-20), 1.00 (s, 3H, H-25), 0.94 (d, *J* = 6.1 Hz, 3H, H-30), 0.86 (d, *J* = 6.4 Hz, 3H, H-29), 0.78 (s, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 204.4 (C-3), 177.9 (C-28), 138.4 (C-13), 124.9 (C-12), 65.7 (C-1), 63.2 (C-2), 53.0 (C-18), 52.8 (C-5), 51.5 (OMe), 48.1 (C-17), 47.9 (C-4), 45.0 (C-9), 42.3 (C-14), 39.3 (C-8), 39.1 (C-19), 38.8 (C-20),

38.7 (C-10), 36.5 (C-22), 33.2 (C-23), 31.9 (C-7), 30.6 (C-21), 27.9 (C-15), 24.2 (C-24), 24.1 (C-16), 23.5 (C-11), 23.4 (C-27), 21.1 (C-30), 20.0 (C-6), 17.0 (C-29), 16.7 (C-26), 15.5 (C-25) ppm; ESI-MS (MeOH): *m/z* (%) = 647.1 ([M(2×^{79}Br) + Na]$^+$, 56), 649.2 ([M(^{79}Br, ^{81}Br) + Na]$^+$, 100), 651.2 ([M(2×^{81}Br) + Na]$^+$, 46); elemental anal. calcd for $C_{31}H_{46}Br_2O_3$ (626.50): C 59.43, H 7.40; found: C 59.11, H. 7.53.

*4.15. Methyl 2,2-dibromo-3,11-dioxo-urs-12-en-28-oate (**18**)*

Compound **18** (123 mg, 93 %)was obtained from **8** using method C; slightly yellowish crystals; mp 225-227 °C; R_f = 0.5 (hexane/EtOAc, 8:2); $[\alpha]_D$ = +48° (*c* = 5.90, $CHCl_3$); UV/vis (MeOH): λ_{max} = 269 (log ε = 4.11); IR (KBr): ν = 3436*br*, 2951*s*, 1728*s*, 1684*w*, 1655*s*, 1612*w*, 1458*m*, 1387*m*, 1318*w*, 1287*w*, 1273*w*, 1248*w*, 1223*m*, 1204*m*, 1167*w*, 1150*w*, 1137*w*, 1110*w*, 1080*w*, 1036*w*, 1012*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.66 (s, 1H, H-12), 4.51 (d, *J* = 16.8 Hz, 1H, H-1), 3.60 (s, 3H, OMe), 3.20 (d, *J* = 16.5Hz, 1H, H-1'), 2.44 (d, *J* = 11.4 Hz, 1H, H-18), 2.37 (s, 1H, H-9), 2.09 (ddd, *J* = 13.8, 13.4, 4.7 Hz, 1H, H-16), 1.89 (dd, *J* = 11.8, 3.0 Hz, 1H, H-5), 1.84-1.73 (m, 3H, H-16', H-22, H-15), 1.65-1.47 (m, 5H, H-7, H-22', H-6, H-21), 1.53 (s, 3H, H-23), 1.43-1.35 (m, 3H, H-19, H-7', H-6'), 1.33-1.26 (m, 2H, H-15', H-21'), 1.31 (s, 3H, H-27), 1.24 (s, 3H, H-24), 1.21 (s, 3H, H-25), 1.09-1.01 (m, 1H, H-20), 0.96 (d, *J* = 6.4 Hz, 3H, H-30), 0.90 (s, 3H, H-26), 0.87 (d, *J* = 6.4 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 203.5 (C-3), 197.7 (C-11), 177.0 (C-28), 163.8 (C-13), 130.3 (C-12), 65.1 (C-1), 63.0 (C-2), 59.2 (C-9), 52.8 (C-18), 51.9 (C-5), 51.8 (OMe), 47.7 (C-4), 46.8 (C-17), 44.2 (C-8), 44.0 (C-14), 38.7 (C-19), 38.6 (C-20), 38.5 (C-10), 35.9 (C-22), 33.2 (C-23), 31.6 (C-7), 30.3 (C-21), 28.5 (C-15), 24.1 (C-24), 23.9 (C-16), 21.1 (C-27), 21.0 (C-30), 19.2 (C-6), 18.5 (C-26), 17.1 (C-29), 16.1 (C-25) ppm; ESI-MS (MeOH): *m/z* (%) = 639.3 ([M(2×^{79}Br) + H]$^+$, 23), 641.3 ([M(^{79}Br, ^{81}Br) + H]$^+$, 51), 643.3 ([M(2×^{81}Br) + H]$^+$, 27), 661.1 ([M(2×^{79}Br) + Na]$^+$, 46), 663.1 ([M(^{79}Br, ^{81}Br) + Na]$^+$, 100), 665.0 ([M(2×^{81}Br) + Na]$^+$, 52); elemental anal. calcd for $C_{31}H_{44}Br_2O_4$ (624.49): C 58.13, H 6.92; found: C57.84, H 7.14.

*4.16. Methyl 2,2-dibromo-3,11-dioxo-olean-12-en-28-oate (**19**)*

Compound **19** (95 mg, 71 %) was obtained from **10** using method C; slightly yellowish crystals; mp 223-226 °C; R_f = 0.55 (hexane/EtOAc, 8:2); $[\alpha]_D$ = +82° (*c* = 3.40, $CHCl_3$), UV/vis (MeOH): λ_{max} = 269 (log ε = 4.18) nm; IR (KBr): ν = 3434*br*, 2948*s*, 1727*s*, 1684*w*, 1658*s*, 1464*m*, 1387*m*, 1364*w*, 1330*w*, 1264*w*, 1199*m*, 1162*m*, 1138*w*, 1083*w*, 1039*w*, 1014*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.70 (s, 1H, H-12), 4.55 (d, *J* = 16.5 Hz, 1H, H-1),

3.63 (s, 3H, OMe), 3.18 (d, *J* = 16.8 Hz, 1H, H-1'), 3.04 (dd, *J* = 13.7, 4.8 Hz, 1H, H-18), 2.40 (s, 1H, H-9), 2.06 (ddd, *J* = 13.7, 13.7, 4.0 Hz, 1H, H-16), 1.91 (dd, *J* = 11.6, 3.4 Hz, 1H, H-5), 1.78-1.68 (m, 2H, H-16', H-22), 1.66-1.57 (m, 4H, H-7, H-19, H-22', H-15), 1.55-1.47 (m, 2H, H-6, H-6'), 1.53 (s, 3H, H-23), 1.42-1.33 (m, 2H, H-21, H-7'), 1.38 (s, 3H, H-27), 1.30-1.19 (m, 3H, H-19', H-21', H-15'), 1.25 (s, 3H, H-24), 1.20 (s, 3H, H-25), 0.94 (s, 3H, H-29), 0.94 (s, 3H, H-30), 0.91 (s, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 203.7 (C-3), 198.4 (C-11), 177.4 (C-28), 169.9 (C-13), 127.5 (C-12), 65.0 (C-1), 63.0 (C-2), 59.5 (C-9), 51.9 (C-5), 51.9 (OMe), 46.8 (C-4), 46.1 (C-17), 44.6 (C-14), 44.3 (C-19), 43.8 (C-8), 41.7 (C-18), 38.5 (C-10), 33.7 (C-21), 33.2 (C-23), 32.8 (C-29), 31.5 (C-22), 31.4 (C-7), 30.7 (C-20), 27.8 (C-15), 24.0 (C-24), 23.5 (C-27), 23.4 (C-30), 22.8 (C-16), 19.1 (C-6), 18.5 (C-26), 16.0 (C-25) ppm; ESI-MS (MeOH): *m/z* (%) = 639.3 ([M(2×^{79}Br) + H]$^+$, 30), 641.3 ([M(^{79}Br, ^{81}Br) + H]$^+$, 61), 643.2 ([M(2×^{81}Br) + H]$^+$, 35), 661.1 ([M(2×^{79}Br) + Na]$^+$, 49), 663.1 ([M(^{79}Br, ^{81}Br) + Na]$^+$, 100), 665.1 ([M(2×^{81}Br) + Na]$^+$, 50); elemental anal. calcd for $C_{31}H_{44}Br_2O_4$ (640.49): C 58.13, H 6.92; found: C 57.81, H 6.99.

*4.17. Methyl (2β) 2-methyl-3-oxo-urs-12-en-28-oate (**20**)*

To a solution of diisopropylamine (85 mg, 0.84 mmol) in dry THF (10 mL) at -78 °C, *n*-butyllithium (1.6 M in hexane, 0.60 mL, 0.96 mmol) was added, and the mixture was stirred for 15 min. A solution of **7** (300 mg, 0.64 mmol) in dry THF (3 mL) iodomethane (200 mg, 1.41 mmol, dissolved in dry THF (3 mL)) were added dropwise. The solution was allowed to warm to room temperature and water (30 mL) was added. The mixture was extracted with DCM (3 × 20 mL), the combined organic layers were dried (Na_2SO_4), filtered and evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexane/EtOAc, 9:1) to yield **20** (180 mg, 58 %); amorphous solid; R_f = 0.4 (hexane/EtOAc, 9:1); $[\alpha]_D$ = +49° (*c* = 2.00, $CHCl_3$); UV/vis: λ_{max} (MeOH): 216 (log ε = 3.80) nm; IR (KBr): ν = 3440*br*, 2928*s*, 1726*m*, 1704*m*, 1653*w*, 1456*w*, 1387*w*, 1229*w*, 1199*w*, 1145*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.22 (m, 1H, H-12), 3.59 (s, 3H, OMe), 2.78-2.70 (m, 1H, H-2), 2.21 (d, *J* = 11.3 Hz, 1H, H-18), 2.02-1.89 (m, 4H, H-11, H-11', H-16, H-1), 1.75 (ddd, *J* = 13.8, 13.8, 4.6 Hz, 1H, H-15), 1.69-1.62 (m, 2H, H-22, H-16'), 1.56 (ddd, *J* = 13.4, 13.4, 4.0 Hz, 1H, H-22'), 1.52-1.41 (m, 5H, H-6, H-6', H-21, H-7, H-9), 1.35-1.22 (m, 3H, H-19, H-7', H-21'), 1.19 (s, 3H, H-31), 1.12 (d, *J* = 12.2 Hz, 1H, H-5), 1.08-1.01 (m, 2H, H-15', H-1'), 1.05 (s, 3H, H-23), 1.04 (s, 3H, H-27), 1.03 (s, 3H, H-24), 1.00-0.95 (m, 1H, H-20), 0.99 (s, 3H, H-25), 0.91 (d, *J* = 6.1 Hz, 3H, H-30), 0.83 (d, *J* = 6.4 Hz, 3H, H-29), 0.78 (s, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 218.0 (C-3), 177.9 (C-28), 138.3 (C-13), 125.2 (C-12), 57.2 (C-5),

52.8 (C-18), 51.4 (OMe), 49.5 (C-1), 48.0 (C-17), 48.0 (C-4), 47.0 (C-9), 42.0 (C-14), 39.5 (C-8), 38.9 (C-19), 38.8 (C-20), 37.2 (C-10), 36.6 (C-22), 36.4 (C-2), 32.7 (C-7), 30.6 (C-21), 28.0 (C-15), 25.4 (C-23), 24.1 (C-16), 23.5 (C-27), 23.4 (C-11), 22.0 (C-24), 21.1 (C-30), 19.3 (C-6), 17.0 (C-29), 16.9 (C-26), 15.5 (C-25), 15.5 (C-31) ppm; ESI-MS (MeOH): *m/z* (%) = 483.3 ($[M + H]^+$, 65), 537.0 ($[M + Na + MeOH]^+$, 100); elemental anal. calcd for $C_{32}H_{50}O_3$ (482.74): C 79.62, H 10.44; found: 79.45, H 10.61.

*4.18. Methyl (3β) 3-hydroxy-2-methylene-11-oxo-olean-12-en-30-oate (**21**)*

Compound **16** (390 mg, 0.79 mmol) was dissolved in 100 mL dry methanol and cerium trichloride heptahydrate (630 mg, 1.69 mmol) was added. Sodium borohydride (50 mg, 1.32 mmol) was added, and the mixture was stirred at room temperature for 48 hours. The solution was concentrated to half volume and poured into water. The aqueous layer was extracted with DCM (3 x 20 mL), the combined organic layers were washed with brine (25 mL), dried (Na_2SO_4), filtered and evaporated to dryness. Chromatographic purification (silica gel, hexane/EtOAc, 4:1) yielded **21** (320 mg, 81 %); colorless crystals; mp 111-114 °C; R_f = 0.52 (hexane/EtOAc, 8:2); $[\alpha]_D$ = +121° (*c* = 0.51, $CHCl_3$); UV/vis (MeOH): λ_{max} = 249 (log ε = 4.09) nm; IR (KBr): ν = 3529*br*, 2950*s*, 2868*s*, 1731*s*, 1653*s*, 1621*m*, 1455*s*, 1388*s*, 1365*m*, 1315*m*, 1280*m*, 1249*m*, 1219*s*, 1190*s*, 1155*s*, 1112*m*, 1081*m*, 1055*s*, 1030*m*, 977*m*, 965*m*, 897*m*, 733*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (s, 1H, H-12), 5.04 (m, 1H, methylene), 4.97 (m, 1H, methylene), 3.74 (m, 1H, H-3), 3.70 (s, 3H, OMe), 3.44 (d, *J* = 12.9 Hz, 1H, H-1), 2.44 (s, 1H, H-9), 2.10 (dd, *J* = 13.9, 3.8 Hz, 1H, H-18), 2.03 (ddd, *J* = 13.5, 13.5, 4.6 Hz, 1H, H-15), 2.00 (m, 1H, H-21), 1.93 (ddd, *J* = 13.4, 4.2, 2.7 Hz, 1H, H-19), 1.84 (ddd, *J* = 13.5, 13.5, 4.5 Hz, 1H, H-16), 1.70 (m, 1H, H-7), 1.68 (m, 1H, H-1'), 1.62 (dd, *J* = 13.5, 13.5 Hz, 1H, H-19'), 1.57 (m, 1H, H-6), 1.54 (m, 1H, H-6'), 1.45 (m, 1H, H-7'), 1.42 (m, 1H, H-22), 1.39 (s, 3H, H-27), 1.35 (m, 2H, H-22'), 1.32 (m, 1H, H-21'), 1.20 (m, 1H, H-16'), 1.15 (s, 3H, H-29), 1.13 (s, 3H, H-26), 1.11 (s, 3H, H-23), 1.06 (s, 3H, H-25), 1.02 (m, 1H, H-15'), 0.96 (m, 1H, H-5), 0.81 (s, 3H, H-28), 0.70 (s, 3H, H-24) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.8 (C-11), 176.9 (C-30), 169.3 (C-13), 146.9 (C-2), 128.6 (C-12), 108.4 (methylene), 80.3 (C-3), 61.1 (C-9), 55.0 (C-5), 51.8 (OMe), 49.2 (C-1), 48.4 (C-18), 45.5 (C-8), 44.0 (C-20), 43.2 (C-14), 41.4 (C-4), 41.1 (C-19), 39.2 (C-10), 37.8 (C-22), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.2 (C-23), 26.5 (C-16), 26.4 (C-15), 23.4 (C-27), 18.6 (C-26), 17.9 (C-6), 16.1 (C-25), 15.4 (C-24) ppm; ESI-MS (MeOH): *m/z* (%) = 497.6 ($[M + H]^+$, 16), 519.5 ($[M + Na]^+$, 6), 537.3 ($[M + Na + H_2O]^+$, 5), 551.1 ($[M + Na + MeOH]^+$, 100), 993.2 ($[2M + H]^+$, 18), 1015.3 ($[2M + Na]^+$, 74); elemental anal. calcd

for $C_{32}H_{48}O_4$ (496.72): C 77.38, H 9.74; found C 77.19, H 9.81.

*4.19. Methyl 3,20-dioxo-30-norlupan-28-oate (**22**)*

Compound **22** (3.4 g, 97 %) was obtained from methyl (3β) 3-hydroxy-20-oxo-30-norlupan-28-oate (**6**) using method A; colorless crystals; mp 155-157 °C; R_f = 0.38 (toluene/EtOAc/formic acid/heptane, 80:26:5:10); $[\alpha]_D$ = + 3° (*c* = 0.55, $CHCl_3$), IR (KBr): ν = 3446*w*, 2941*s*, 2866*s*, 2362*w*, 1733*s*, 1703*s*, 1455*s*, 1430*m*, 1385*m*, 1352*m*, 1319*m*, 1274*m*, 1241*m*, 1188*s*, 1161*s*, 1129*s*, 1079*m*, 1046*w*, 1005*m*, 978*m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 3.61 (s, 3H, H-30), 3.19 (dt, *J* = 11.0, 4.4 Hz, 1H, H-19), 2.46-2.29 (m, 2H, H-2), 2.19 (dt, *J* = 11.9, 3.0 Hz, 1H, H-16), 2.11 (s, 3H, H-29), 2.04 (dd, *J* = 21.4, 10.6 Hz, 1H, H-18), 1.99-1.89 (m, 2H, H-13, H-21), 1.86-1.76 (m, 2H, H-1, H-22), 1.51-1.20 (m, 13H, H-1', H-5, H-6, H-7, H-9, H-11, H-15, H-16', H-21', H-22'), 1.16-1.09 (m, 1H, H-15'), 1.06-1.01 (m, 2H, H-12), 1.00 (s, 3H, H-23), 0.95 (s, 3H, H-24), 0.94 (s, 3H, H-27), 0.86 (s, 3H, H-26), 0.84 (s, 3H, H-25) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 218.0 (C-3), 212.1 (C-20), 176.4 (C-28), 56.3 (C-17), 54.8 (C-5), 51.4 (C-30), 51.2 (C-19), 49.7 (C-9), 49.2 (C-18), 47.2 (C-4), 42.2 (C-14), 40.5 (C-8), 39.5 (C-1), 37.4 (C-13), 36.9 (C-10), 36.5 (C-22), 34.0 (C-2), 33.5 (C-7), 31.6 (C-16), 30.1 (C-29), 29.7 (C-15), 28.2 (C-21), 27.2 (C-12), 26.7(C-23), 21.4 (C-11), 21.0 (C-24), 19.6 (C-6), 15.9 (C-25), 15.6 (C-26), 14.6 (C-27) ppm; ESI-MS (MeOH): *m*/*z* (%) = 471.3 ($[M + H]^+$, 24), 488.3 ($[M + NH_4]^+$, 12), 493.5 ($[M + Na]^+$, 5), 728.8 ($[3M + 2Na]^{2+}$, 100), 963.3 ($[2M + Na]^+$, 64); elemental anal. calcd for $C_{30}H_{46}O_4$ (494.71): C 76.55, H 9.85; found: C 76.41, H 9,97.

*4.20. Methyl 19-(hydroxymethylacryloyl)-2-methylene-3-oxo-20,29,30-trinorlupan-28-oate (**23**) and methyl 29-hydroxymethyl-2-methylene-3,20-oxo-29-(1,3,5-trioxocane)-20,29,34-trinorlupan-28-oate (**24**)*

Compounds **23** (1.45 g, 71 %) and **24** (30 mg, 2 %) were obtained from **22** using method B.

Data for compound **23**: colorless crystals; mp 204-206 °C; R_f = 0.19 (toluene/EtOAc/formic acid/heptane, 80:26:5:10); $[\alpha]_D$ = +10° (*c* = 0.57, $CHCl_3$); IR (KBr): ν = 3470*m*, 2947*s*, 1728*s*, 1690*s*, 1456*m*, 1379*m*, 1270*w*, 1138*s*, 1049*m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.10 (br t, *J* = 4.3 Hz, 1H, methylene-31), 5.95 (br t, *J* = 4.3 Hz, 1H, methylene-31), 5.90 (t, *J* = 2.1 Hz, 1H, methylene-34), 5.06 (t, *J* = 2.1 Hz, 1H, methylene-34), 4.78-4.68 (m, 2H, H-30), 3.62 (s, 3H, H-32), 3.73 (dt, 1H, *J* = 11.2, 4.7 Hz, H-19), 2.63 (d, *J* = 15.3 Hz, 1H, H-1), 2.33-2.18 (m, 1H, H-18), 2.21 (dt, *J* = 12.5, 3.0 Hz, 1H, H-16), 2.02-1.96 (m, 2H, H-1', H-13), 1.96-1.88 (m, 1H, H-21), 1.88-1.78 (m, 1H, H-22), 1.51-1.11 (m, 13H, H-5, H-6, H-7, H-9, H-

11, H-15, H-16', H-21', H-22'), 1.10-1.00 (m, 1H, H-12), 1.00-0.91 (m, 1H, H-12'), 1.04 (s, 3H, H-23), 0.97 (s, 3H, H-24), 0.96 (s, 3H, H-27), 0.87 (s, 3H, H-26), 0.76 (s, 3H, H-25) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 207.6 (C-3), 205.8 (C-20), 176.6 (C-28), 146.8 (C-31), 142.2 (C-33), 125.3 (C-29), 123.7 (C-2), 63.0 (C-30), 56.3 (C-17), 53.9 (C-5), 51.5 (OMe), 49.5 (C-9), 48.3 (C-18), 47.0 (C-1), 45.8 (C-4), 45.0 (C-19), 42.3 (C-14), 40.5 (C-8), 37.4 (C-13), 37.0 (C-10), 36.8 (C-22), 33.3 (C-7), 31.4 (C-16), 29.7 (C-15), 29.8 (C-21), 28.1 (C-23), 27.4 (C-12), 22.4 (C-24), 21.5 (C-11), 19.7 (C-6), 15.5 (C-26), 15.1 (C-25), 14.6 (C-27) ppm; ESI-MS (MeOH): *m/z* (%) = 525.5 ($[M + H]^+$, 21), 547.4 ($[M + Na]^+$, 7); elemental anal. calcd for $C_{33}H_{48}O_5$ (524.73): C 75.53, H 9.22; found: C 75.38, H 9.37.

Data for compound **24**: colorless crystals; mp 190-194 °C; R_f = 0.20 (toluene/EtOAc/formic acid/heptane, 80:26:5:10); $[\alpha]_D$ = +5° (*c* = 0.53, $CHCl_3$); IR (KBr): ν = 3473*s*, 2948*s*, 1728*s*, 1690*s*, 1445*m*, 1377*m*, 1260*w*, 1168*s*, 1138*s*, 1047*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.90 (t, *J* = 2.2 Hz, 1H, methylene), 5.06 (t, *J* = 2.2 Hz, 1H, methylene), 4.76-4.69 (m, 4H, H-31, H-32), 4.10 (d, *J* = 11.9 Hz, 1H, H-30), 3.99 (d, *J* = 11.9 Hz, 1H, H-33), 3.88-3.84 (m, 1H, H-30'), 3.83-3.79 (m, 1H, H-33'), 3.71 (d, *J* = 10.9 Hz, 1H, H-34), 3.67 (d, *J* = 10.9 Hz, 1H, H-34'), 3.62 (s, 3H, H-35), 3.53 (dt, *J* = 11.3, 4.9 Hz, 1H, H-19), 2.62 (d, *J* = 15.3 Hz, 1H, H-1), 2.27 (t, *J* = 11.2 Hz, 1H, H-18), 2.21 (dt, *J* = 12.5, 3.0 Hz, 1H, H-16), 2.02-1.97 (m, 1H, H-1'), 1.97 (s, 1H, H-13), 1.96-1.88 (m, 1H, H-21), 1.85-1.79 (m, 1H, H-22), 1.51-1.11 (m, 13H, H-5, H-6, H-7, H-9, H-11, H-15, H-16', H-21', H-22'), 1.10-1.00 (m, 1H, H-12), 1.00-0.91 (m, 1H, H-12'), 1.04 (s, 3H, H-23), 0.97 (s, 3H, H-24), 0.96 (s, 3H, H-27), 0.87 (s, 3H, H-26), 0.76 (s, 3H, H-25) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 215.8 (C-20), 207.6 (C-3), 176.4 (C-28), 142.2 (C-2), 123.6 (methylene), 95.5 (C-31 and C-32), 68.1 (C-33), 67.6 (C-30), 64.3 (C-34), 57.7 (C-29), 56.0 (C-17), 53.9 (C-5), 51.5 (C-36), 48.6 (C-18), 48.2 (C-9), 47.8 (C-19), 47.0 (C-1), 45.8 (C-4), 42.3 (C-14)), 40.4 (C-8), 37.4 (C-13), 36.9 (C-10), 36.6 (C-22), 33.3 (C-7), 31.4 (C-16), 29.6 (C-15), 28.1 (C-23), 28.0 (C-21), 27.2 (C-12), 22.4 (C-24), 21.5 (C-11), 20.1 (C-6), 15.5 (C-26), 15.4 (C-25), 14.6 (C-27) ppm; ESI-MS (MeOH): *m/z* (%) = 615.4 ($[M + H]^+$, 49), 637.7 ($[M + Na]^+$, 17), 1251.3 ($[2M + Na]^+$, 100); elemental anal. calcd for $C_{36}H_{54}O_8$ (614.81): C 70.33, H 8.85; found: C 70.17, H 8.98.

4.21. Cytotoxicity studies

4.21.1. Cell lines and culture conditions

The human cell lines 518A2 (melanoma), 8505C (thyroid carcinoma), A549 (alveolar basal epithelial adenocarcinoma), HT29 (colorectal adenocarcinoma), MCF-7 (breast adenocarcinoma) were included in this study. Cultures were maintained as monolayer in

RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10 % heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin / streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5 % CO_2/ 95 % air.

4.21.2. Cytotoxicity assay [22, 30, 31]

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the compounds (0-100 μM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5 %, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96 well plates was discarded, and the cells were fixed with 10 % TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a strip washer. The washing was done four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 μl of 0.4 % SRB (sulforhodamine B) for about 20 min. After dying, the plates were washed with 1 % acetic acid to remove the excess of the dye and allowed to air dry overnight. 100 μl of 10 mM Tris base solution were added to each well, and absorbance was measured at λ = 570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). The EC_{50} value was determined from three independent measurements (each in triplicate) applying the two-parametric Hill slope equation.

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Figure Captions

Figure 1. Structure of triterpenoic acids: ursolic acid (**UA**), oleanolic acid (**OA**), glycyrrhetinic acid (**GA**), and platanic acid (**PA**).

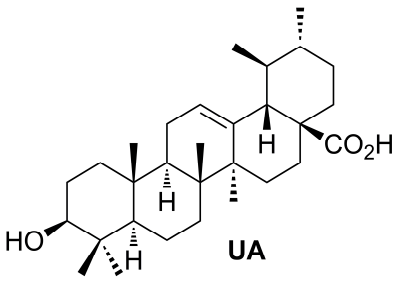
Scheme 1. Synthesis of the 2-methylene-3-oxo (**12-16**) and the 2,2-dibromo-3-oxo (**17-19**) derivatives starting from the corresponding 3-oxo compounds as well as of **20** and **21**: Reagents and conditions: a) CrO_3, acetone, H_2SO_4, r.t., 1 hour; b) K_2CO_3, paraformaldehyde, DMF, 90 °C, 1 hour; c) Br_2, CH_3COOH, r.t., 15 min; d) diisopropylamine, *n*-BuLi, THF, -78 °C, 30 min; then: iodomethane, THF, -78 °C → r.t.; e) $CeCl_3$, $NaBH_4$, MeOH, r.t., 48 hours.

Scheme 2. Formation of **23** (71 %) and **24** (2 %) from methyl platanoate (**22**): Reagents and conditions: a) K_2CO_3, paraformaldehyde, DMF, 90 °C, 1 hour.

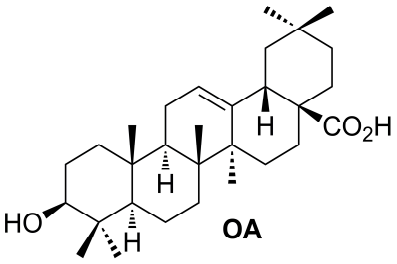
Table 1. Cytotoxicity (EC_{50} in μM, 96 hours of treatment, from SRB assay) of compounds **1-16** as well as of parent compounds glycyrrhetinic acid (**GA**), ursolic acid (**UA**), oleanolic acid (**OA**) and platanic acid (**PA**). EC_{50} values represent mean values of three independent measurements (each performed in triplicate) and were calculated applying the two-parametric Hill slope equation (confidence interval CI = 95 %). The cell lines are human cancer cell lines: 518A2 (melanoma), 8505C (thyroid carcinoma), A549 (alveolar basal epithelial adenocarcinoma), HT29 (colorectal adenocarcinoma), MCF-7 (breast adenocarcinoma); n.d. = not detected.

EC_{50}	**518A2**	**8505C**	**A549**	**HT-29**	**MCF-7**
GA	83.92 ± 4.20	86.50 ± 4.33	82.76 ± 4.14	80.09 ± 4.05	84.70 ± 4.36
5	>30	>30	>30	>30	>30
10	1.19 ± 0.06	1.58 ± 0.08	1.23 ± 0.06	1.34 ± 0.13	0.74 ± 0.08
15	16.78 ± 0.84	15.45 ± 0.77	17.90 ± 0.90	n.d.[a]	n.d.
UA	14.7 ± 0.13	13.5 ± 1.54	15.5 ± 1.72	10.6 ± 0.71	12.7 ± 0.13
1	>30	n.d.	n.d.	>30	>30
2	>30	n.d.	n.d.	>30	>30
6	1.50 ± 0.15	1.57 ± 0.16	1.54 ± 0.15	1.69 ± 0.17	1.55 ± 0.16
7	1.24 ± 0.12	1.56 ± 0.16	1.64 ± 0.16	1.15 ± 0.12	1.30 ± 0.13
11	>30	20.5 ± 0.82	26.1 ± 3.04	26.7 ± 5.42	16.7 ± 1.03
12	2.16 ± 0.22	6.10 ± 0.61	2.93 ± 0.29	1.35 ± 0.14	4.43 ± 0.44
14	>30	>30	>30	>30	>30
OA	>30	>30	>30	>30	>30
3	>30	n.d.	n.d.	>30	>30
4	>30	n.d.	n.d.	27.62	28.69
8	3.71 ± 0.21	4.32 ± 0.24	3.83 ± 0.20	3.71 ± 0.21	4.24 ± 0.23
9	4.11 ± 0.41	n.d.	n.d.	4.47 ± 0.45	5.44 ± 0.54
13	1.51 ± 0.15	1.62 ± 0.16	1.57 ± 0.15	3.16 ± 0.31	4.68 ± 0.46

PA	>30	>30	>30	>30	>30
16	>30	>30	>30	>30	24.11 ± 2.12



UA



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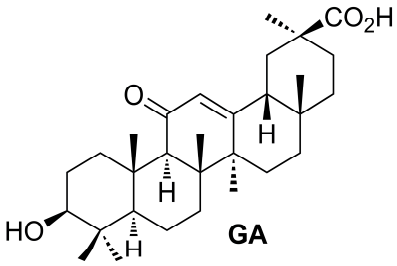
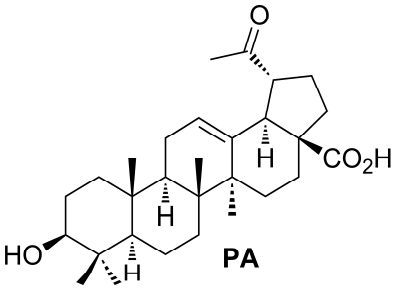
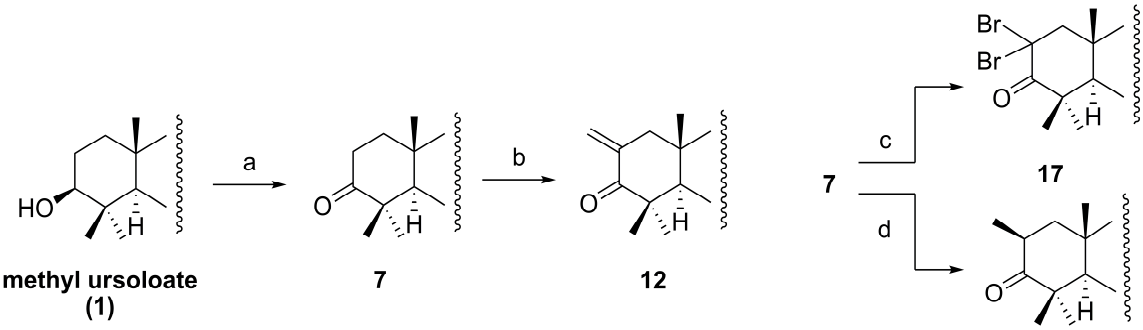


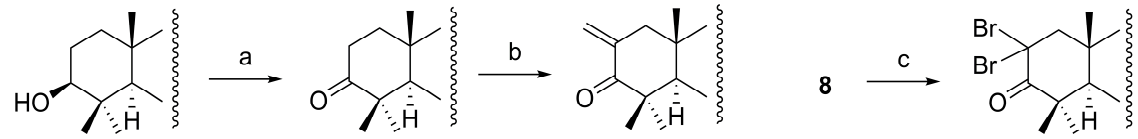
GA



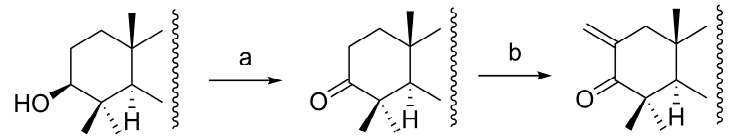
PA



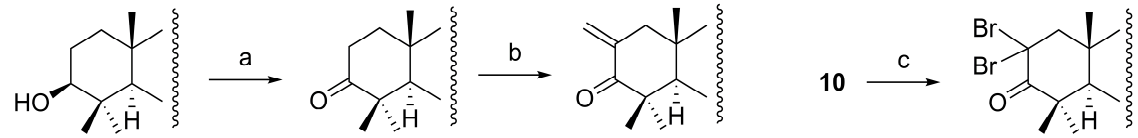
methyl ursoloate (1) **7** **12**



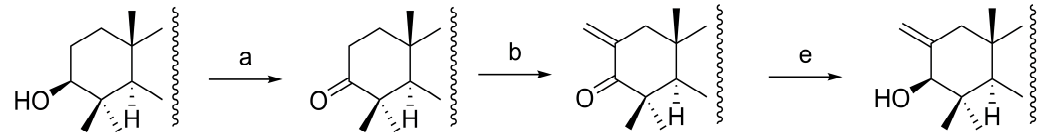
methyl 11-oxo-ursoloate (2) **8** **13** **18**



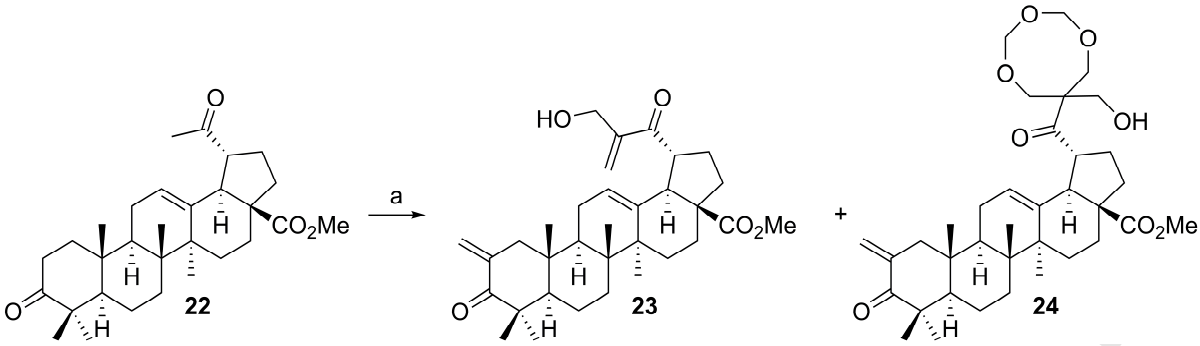
methyl oleanoloate (3) **9** **14**



methyl 11-oxo-oleanoloate (4) **10** **15** **19**



methyl glycyrrhetinoate (5) **11** **16**



- We investigated of derivatives of Michael acceptor substituted methyl triterpenoates
- The compounds showed antitumor activity on different human cancer cell lines
- Many of the compounds are cytotoxic even in a low micro mol concentration
- Highest cytotoxicity was found for a glycyrrhetinic acid derived compound
- EC_{50} values as low as 0.74 μM were found employing MCF-7 breast carcinoma cells

SUPPLEMENTARY MATERIAL

Incorporation of a Michael acceptor enhances the antitumor activity of triterpenoic acids

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Typical NMR spectra:

^{1}H NMR

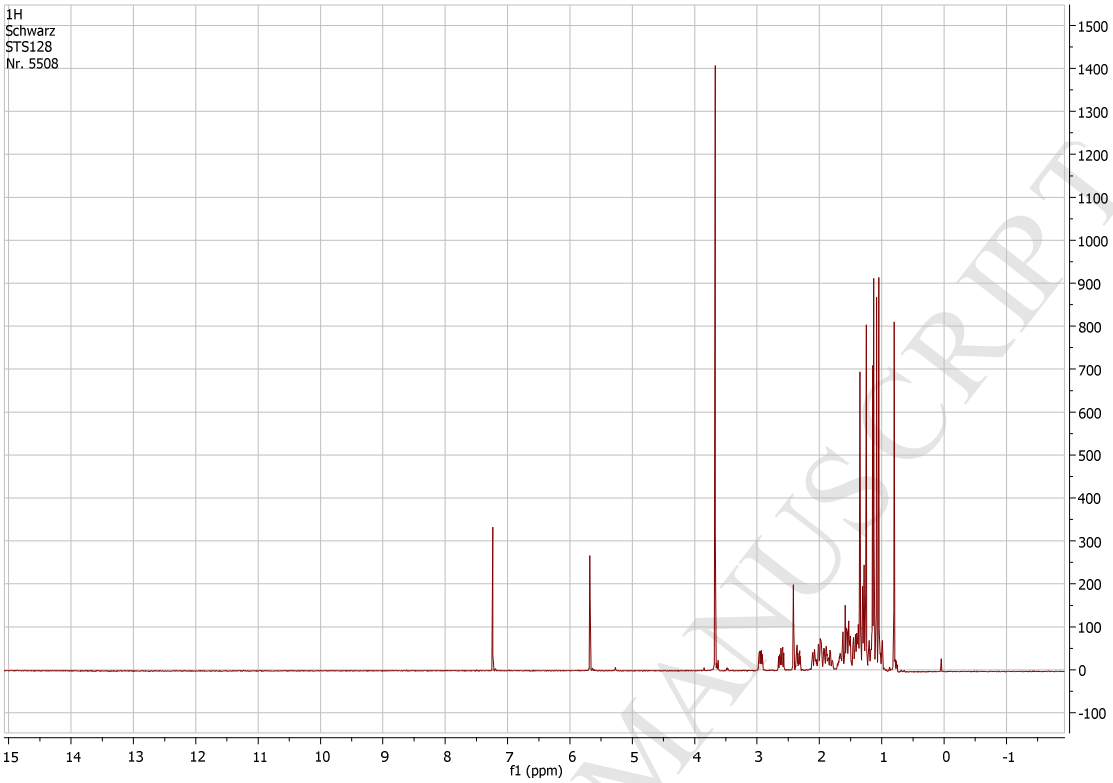
Device: Varian Unity 400/Inova; Observe frequency: 399.9613289 MHz;Temperature: 27 °C; Acquisition time:5.112 sec; Frequency width: 6410.3 Hz; Pulse sequence: s2pul; Pulse: 45 °; Relaxation delay 1.000 sec; No of scans: 16; Line broadening: 0.1 Hz; FT-size: 131072; Processing software: Genuine Varian.

^{13}C NMR

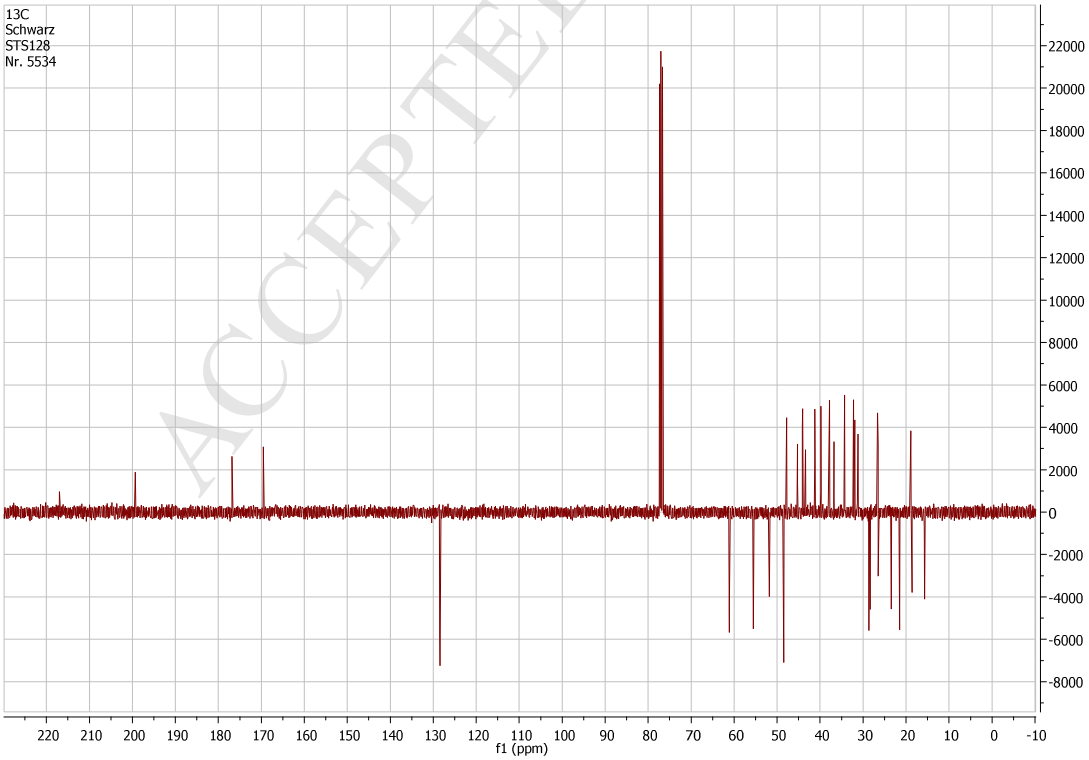
Device: Varian Unity400/Inova; Observe frequency 100.5703561 MHz; Temperature: 27 °C; Acquisition time: 1.311 sec; Relaxation delay: 2.000 sec; 1st pulse: 90 °; 2nd pulse: 45 °; Pulse width: 25000.0 Hz; Pulse sequence: s2pul; Decoupling power: 39 dB, continuously; Modulation: WALTZ-16; Processing: Fourier transformation; FT-Size: 65536; Line broadening 0.5 Hz; Processing software: Genuine Varian.

*Methyl 2-oxo-glycyrrhetinoate (**11**)*

^{1}H NMR ($CDCl_3$, 400 MHz):

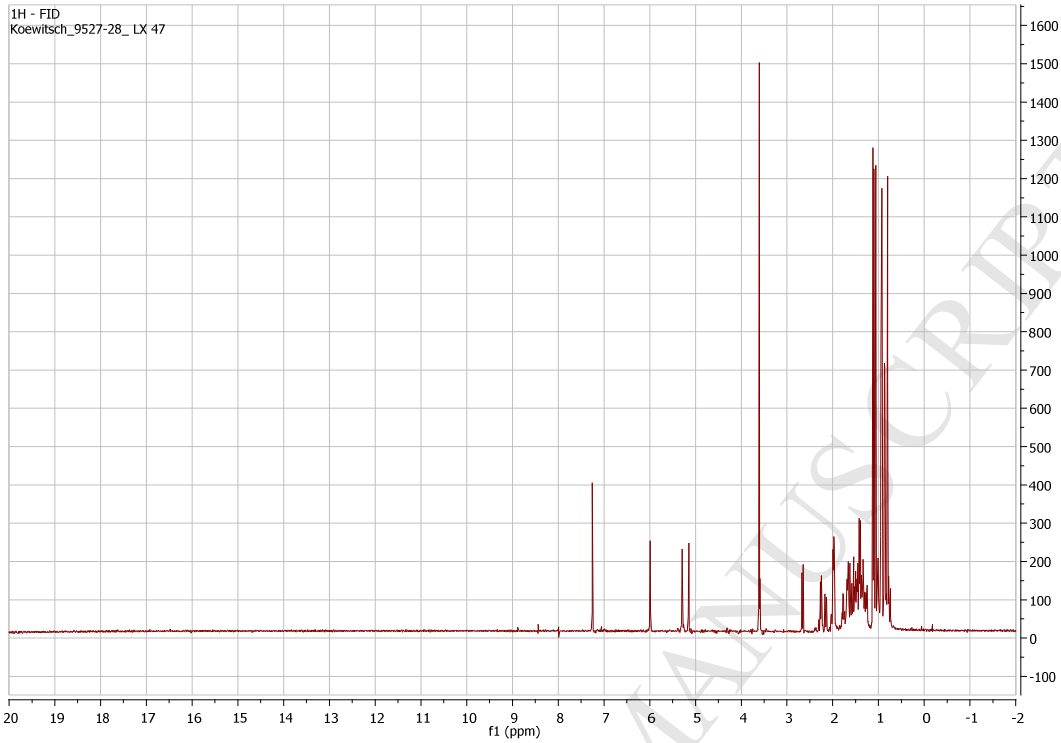


^{13}C NMR: ($CDCl_3$, 100 MHz):

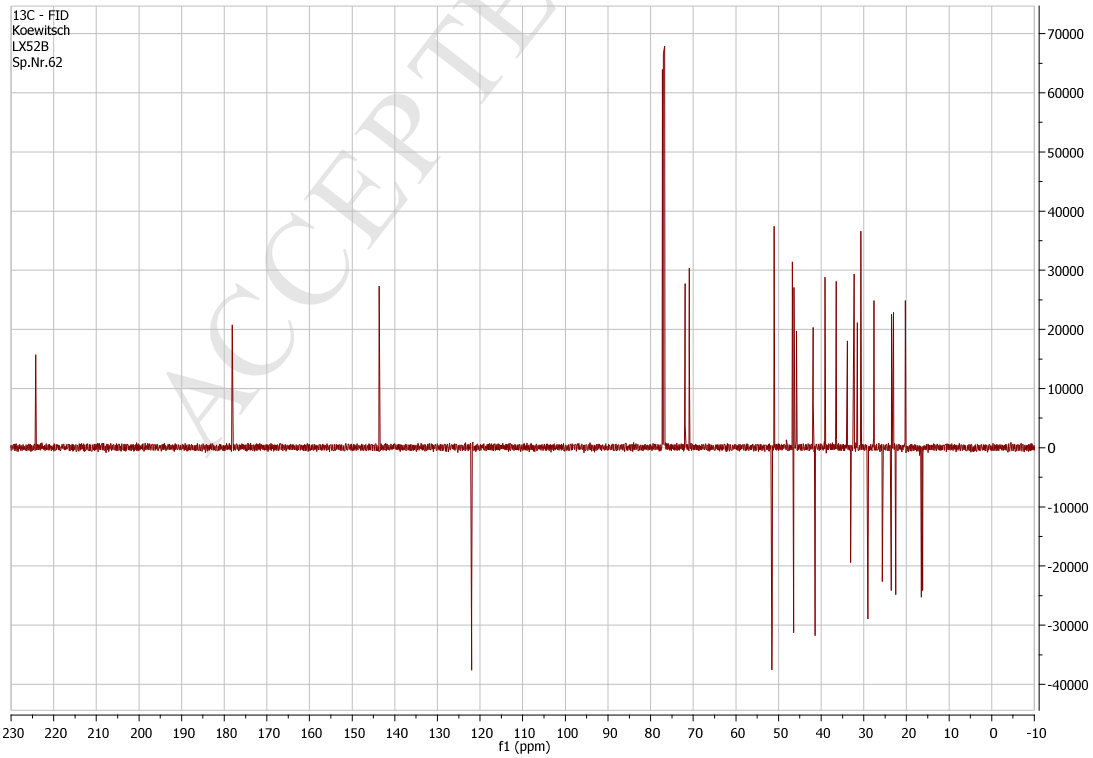


*Methyl 2-methylene-3-oxo-urs-12-en-28-oate (**12**)*

^{1}H NMR ($CDCl_3$, 400 MHz):

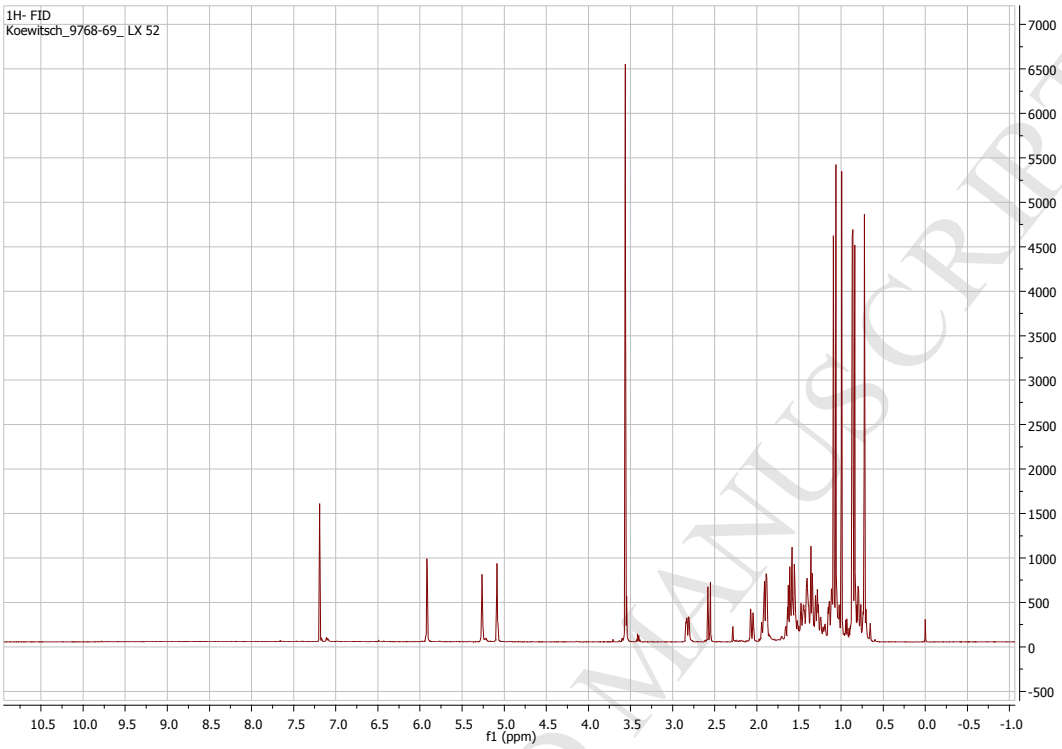


^{13}C NMR: ($CDCl_3$, 100 MHz):

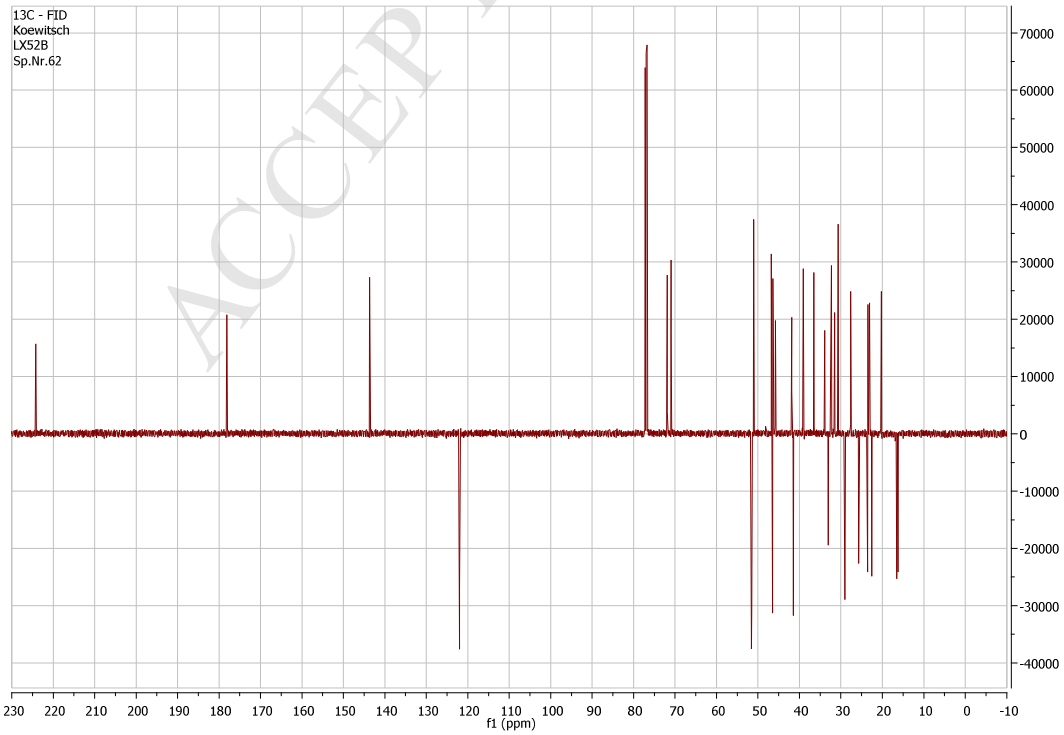


*Methyl 2-methylene-3-oxo-olean-12-en-28-oate (**14**)*

^{1}H NMR ($CDCl_3$, 400 MHz):



^{13}C NMR: ($CDCl_3$, 100 MHz):



*Methyl 2-methylene-3,11-dioxo-olean-12-en-30-oate (**16**)*

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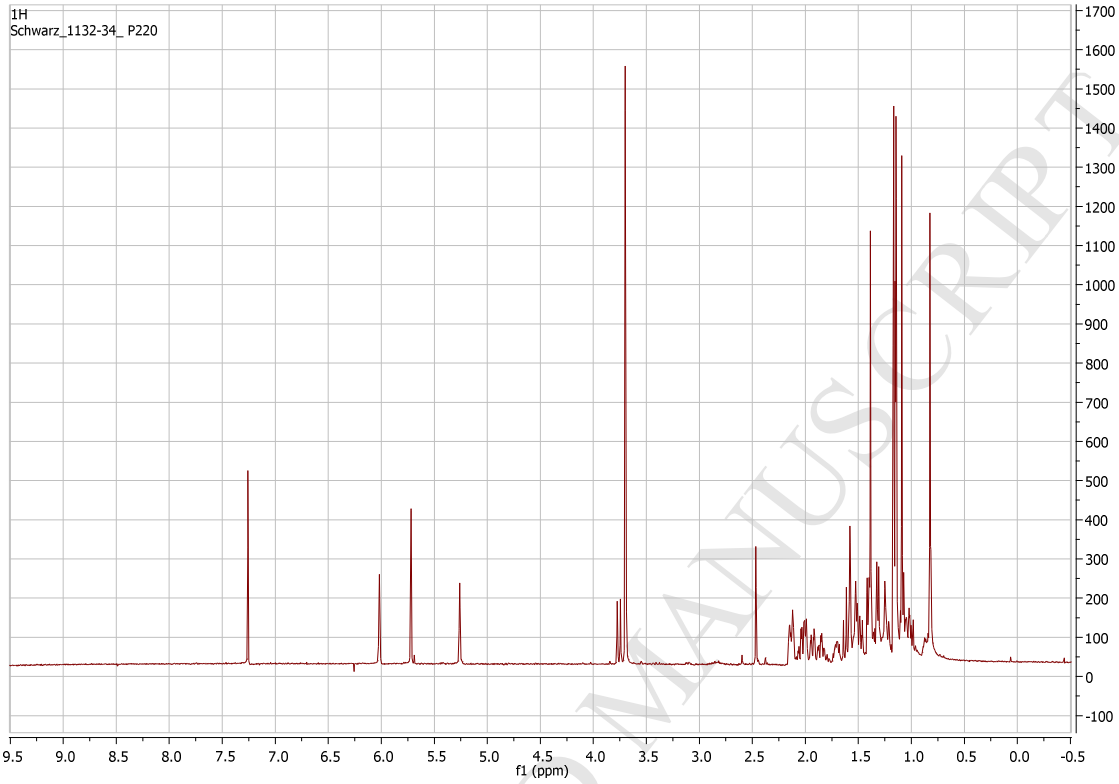

^{13}C NMR: ($CDCl_3$, 100 MHz):