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Original article

Cytotoxic, antioxidant activities and structure activity relationship of some newly synthesized terpenoidal oxaliplatin analogs

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Abstract

The terpenoidal oxaliplatin derivatives (**6**) and (**12**) were newly synthesized using 2β,3α-dihydroxy-11-oxo-18β-olean-12-ene-30-oic acid (**1**) and 2α,2β-dihydroxy-18β-ursan-12-ene-28-oic acid (**7**) as starting materials. The synthesized compounds were evaluated for their cytotoxicity and antioxidant activities and were compared to Oxaliplatin® and vitamin C as positive controls. Some of the compounds exhibited better cytotoxicity and antioxidant activities than the reference controls. The detailed synthesis, spectroscopic data, toxicity (LD_{50}) and pharmacological screening for the synthesized compounds were reported.

Keywords: Terpenoid diol; Oxaliplatin derivatives; Cytotoxic; Antioxidant activity

1. Introduction

The 2-benzylidene derivatives of glycyrrhetinic acid and the C-30 esters and amides of glycyrrhetinic acid were prepared [1,2]. The benzylidene derivatives showed potent anti-ulcer activities, while both the ester and amide derivatives showed potent analgesic activities [1,2]. Further chemical alterations on glycyrrhetinic acid and oleanolic acid lead to the synthesis of their 3-menthyl carbonyl derivatives that exhibited potent anti-ulcer activities [3], also synthesis of the ureides derivatives of both glycyrrhetinic acids and oleanolic acid of moderate anti-inflammatory potency has been reported [4]. El-Gamal et al. [5,6] described the synthesis of 2β,3α-glycol derivatives of glycyrrhetic acid but they didnot investigate its anticancer activity. Recently Chao-Mei et al. [7] described the isolation of 2α-hydroxyl-ursolic acid (**7**) from the ethyl acetate extract of the peel of apples (*Malus pumila* Mill). In view of these reports and in continuation of our previous works in chemistry of natural products [8–12], we have synthesized some new compounds containing a terpenoid ring system for biological evaluation against colorectal carcinoma in comparison to Oxaliplatin as the reference drug. Also the antioxidant activities of the newly synthesized compound were estimated and compared to that of vitamin C as positive control. The chemical structures of glycyrrhetinic acid and Oxaliplatin are given in Fig. 1.

2. Results and discussion

2.1. Chemistry

2β,3α-Dihydroxy-11-oxo-18β-olean-12-ene-30-oic acid (**1**) and 2α,2β-dihydroxy-18β-ursan-12-ene-28-oic acid (**7**) were synthesized and isolated according to the literature [5–7] and were used as starting materials. The carboxylic acid derivatives **1** and **7** were esterified with diazomethane in chloroform to give the corresponding methyl ester derivatives **2** and **8** this reaction was completed according to the method given by Dean et al. [13] for the protection of the carboxylic group. The methyl esters **2** and **8** were reacted with sodium azide in the presence of methane sulfonyl chloride and triethylamine to give the corresponding diazide derivatives **3** and **9** according to the literature method [14–17]. The terpenoidal diamine

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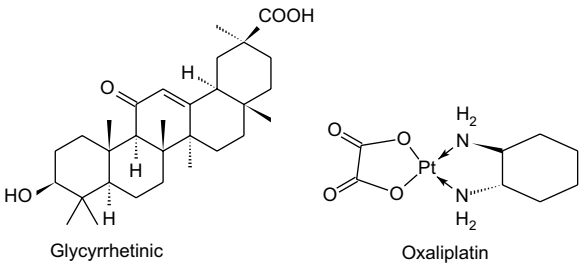


Fig. 1.

ligands **4** and **10** were isolated after catalytic hydrogenation of diazide derivatives **3** and **9** over palladium charcoal (Pd/C) in formic acid [18,19] (Schemes 1 and 2).

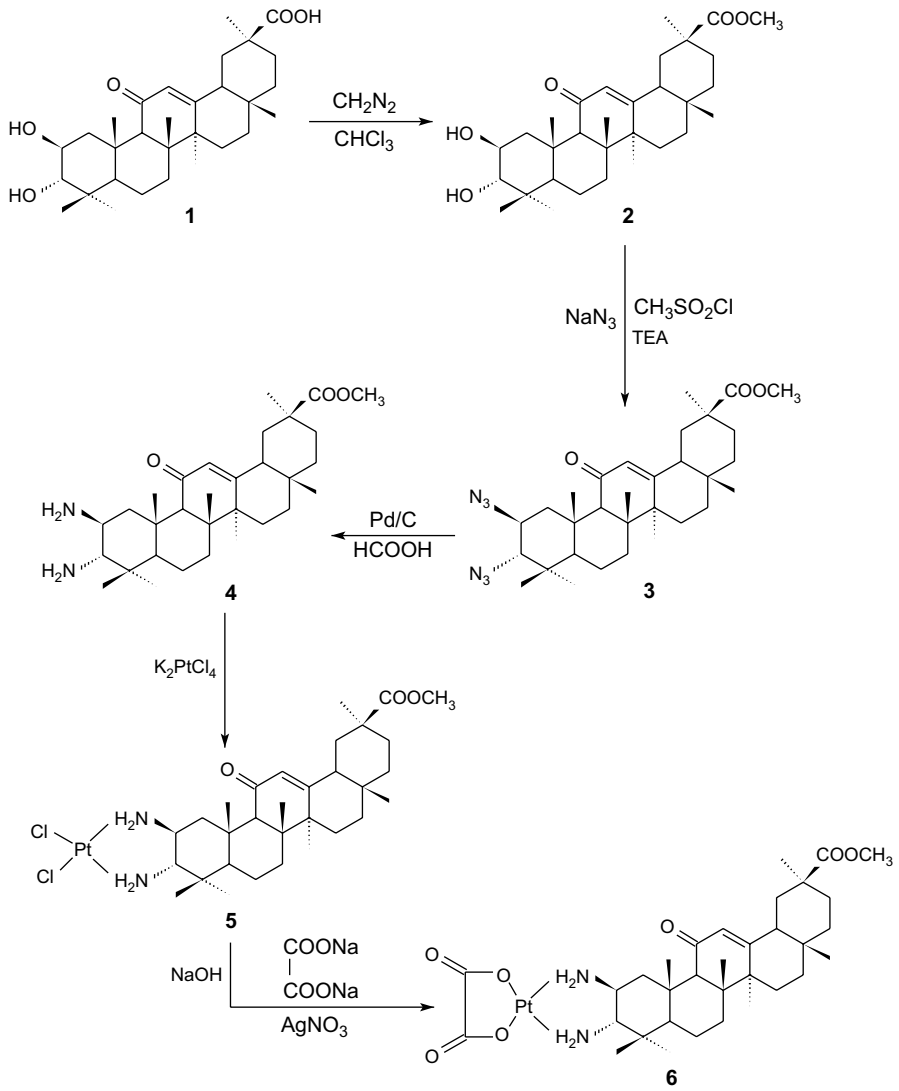
The diaminodichloroplatinum complexes **5** and **11** were synthesized via the reaction of potassium tetrachloroplatinate (II) (K_2PtCl_4) with diamine ligands **4** and **10**. The reaction of sodium oxalate with diaminodichloroplatinum complexes **5** and **11** in water in the presence of silver nitrate ($AgNO_3$) as a precipitating agent for chloride and in the presence of sodium hydroxide, gave the final oxalate complexes **6** and **12** in good yields Schemes 1 and 2.

2.2. Pharmacological screening

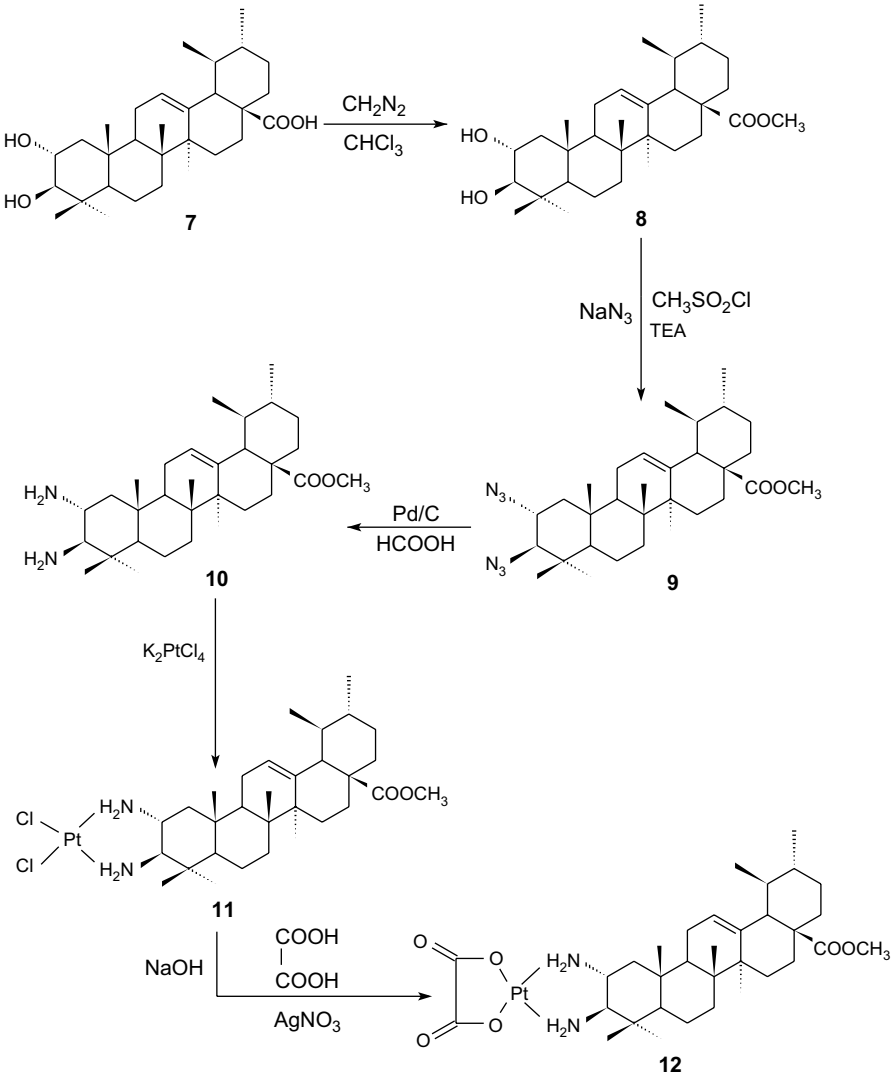
Initially, the acute toxicity of the compounds was assayed via the determination of their LD_{50}. All compounds were interestingly less toxic than the reference control (Table 1). The 12 compounds **1**–**12** were further studied for their cytotoxic and antioxidant activities.

2.2.1. Cytotoxic activity

From Table 2, all the newly synthesized tested compounds showed potent cytotoxic activities against HT-29 cell line (colorectal carcinoma). The cytotoxic activities increase as the doses increase, therefore the 150 μg/ml doses induced more cell death than the 100 μg/ml doses and the latters induced more cell death than 50 μg/ml doses. Also the cytotoxic activities increase by increasing the time of contact between cell line and cytotoxic agents at any given dose level. So the cell



Scheme 1.



Scheme 2.

death after 48 h is greater than that after 24 h and the cell death after 24 h is higher than that after 12 h. The compounds arranged in descending order of cytotoxic activity are **12**, **6**, **11**, **5**, **10**, **4**, **9**, **3**, **8** and **2**.

Table 1
Acute toxicity (LD_{50}) of the synthesized compounds

Compound	LD_{50} (mg/kg)
1	656 ± 0.62
2	793 ± 0.78
3	898 ± 0.78
4	893 ± 0.88
5	864 ± 0.89
6	871 ± 0.85
7	816 ± 0.88
8	818 ± 0.82
9	825 ± 0.86
10	836 ± 0.85
11	846 ± 0.89
12	822 ± 0.99
Oxaliplatin	223 ± 0.28

2.2.2. Antioxidant activities

The antioxidant activities of the newly synthesized compounds were obtained by measuring the scavenging effects of the synthesized compounds on the superoxide produced by HX/XO system. We measure the scavenging activities of the tested compounds at a dose level of 50 μg/ml.

From the results in Table 3, it was found that all compounds showed antioxidant activities via inhibition of NBT reduction in the following descending order **12**, **6**, **11**, **5**, **10**, **4**, **9**, **3**, **8** and **2**.

2.2.3. Structure–activity relationship (SAR)

- The triterpenoid function is essential for the cytotoxic and antioxidant activities, the ursane one induces higher activities than the olean one.
- The oxaloplatin moiety sharply increases the cytotoxic and antioxidant activities.
- The ring A 2,3-disubstituents play an important role in regulating the cytotoxic and antioxidant activities. In

Table 2
Cytotoxic effect on HT-29 at a concentration of 50, 100, 150 μg/ml after time in hours

Compound	Cytotoxic effect at different concentrations							
	50 μg/ml			100 μg/ml			150 μg/ml	
	12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h
2	19	21	33	39	41	42	40	52
3	29	31	36	42	46	52	55	60
4	40	43	45	48	50	58	65	70
5	50	52	55	58	63	65	73	76
6	65	70	73	75	77	79	81	88
8	22	29	35	40	44	50	53	58
9	33	35	40	44	48	55	61	66
10	44	47	50	56	60	62	70	75
11	52	54	57	60	65	70	75	78
12	70	75	77	80	83	86	88	89
Oxaliplatin	20	23	33	40	40	44	50	50

a descending order of activities, we found the oxaliplatinium, chloroplatinum, diamino, diazido and, lastly the diol functional groups.

3. Experimental

3.1. Chemistry

Melting points were determined on open glass capillaries using a Electrothermal IA 9000 digital melting point apparatus and are uncorrected. Elemental analyses were performed on Elementar, Vario EL, Microanalytical Unit, Cairo University, Egypt and were found within ±0.4% of the theoretical values. Infrared (IR) spectra were recorded on Carlzeise Spectrophotometer model ''UR 10'' spectrophotometer using the KBr disc technique. The NMR spectra were recorded on Varian Gemini 270 MHz spectrometer (DMSO-d_6) and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. The mass spectra (MS) were measured using a Finnigan SSQ 7000 mass spectrometer.

3.1.1. Synthesis of methyl 2β,3α-dihydroxy-11-oxo-18β-olean-12-ene-30-oate (2)

To a solution of acid derivative **1** (2.1 mmol) in dry chloroform (25 ml), ethereal solution of diazomethane (25 ml) was added with stirring at room temperature. The reaction mixture was left overnight at the same temperature. The solvent was evaporated under reduced pressure to dryness. The residue was crystallized from methanol/chloroform to give the corresponding methyl ester **2**. Yield 96%, mp 106 °C, $[\alpha]_D^{25}$ +91 (*c* 1, MeOH); IR (KBr, cm^{-1}): 1746, 1710 (2C=O); ^{1}H NMR (DMSO-d_6): δ 0.78, 0.82 (2s, 6H, $2CH_3$), 0.86 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.20–1.23 (m, 4H, $2CH_2$), 1.26 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.42–1.50 (m, 4H, $2CH_2$), 1.60–1.70 (m, 6H, $3CH_2$), 1.75 (m, 1H, CH), 1.95 (m, 1H, CH), 2.41 (m, 1H, CH), 2.61 (m, 1H, CH), 3.61 (m, 1H, CH), 3.75–3.78 (m, 2H, CH_2), 4.42 (s, 3H, $COOCH_3$), 4.50, 4.55 (2br s, 2H, 2OH, exchangeable with D_2O), 5.78 (s, 1H, CH=C); ^{13}C NMR (DMSO-d_6): δ 39.11, 89.09, 91.12, 47.61, 55.12, 18.91, 37.0, 37.21, 45.16, 40.13, 191.18, 128.61, 170.28, 48.55, 26.71, 23.41, 32.81, 53.17, 41.56, 43.31, 32.71, 31.27, 30.19, 18.91, 17.51, 23.27, 24.16, 30.81, 31.91, 108.7 (C1–C30), 62.17 (OCH_3); MS (EI): *m/z* 500 (25%) [M^+]. Anal. Calcd for $C_{31}H_{48}O_5$: C, 74.36; H, 9.66. Found: C, 74.30; H, 9.60.

3.1.2. Synthesis of methyl 2β,3α-diazedo-11-oxo-18β-olean-12-ene-30-oate (3)

To a mixture of ester (**2**) (20 mmol) and methane sulfonyl chloride (3.6 ml) in benzene (60 ml), triethylamine (1.8 ml) was slowly added with stirring at room temperature for 1 h and then filtered off. The filtrate was evaporated under reduced pressure, the residue was dissolved in ethanol (75 ml) and then sodium azide (2.4 g) was added. The reaction mixture was refluxed for 4 h followed by periodic additions of water, stirred at room temperature overnight, diluted with water and extracted with dichloromethane. The extracted part was washed with water, dried over anhydrous calcium chloride, and evaporated under reduced pressure. The residue was suspended with a solution of sodium hydroxide (32 g/100 ml water), stirred at 45 °C for 0.5 h, and extracted with dichloromethane. The aqueous fraction was acidified with concentrated hydrochloric acid and extracted with dichloromethane, dried over anhydrous calcium chloride, and then evaporated under reduced pressure. The residue was solidified with ethanol, filtered off, dried and crystallized from ethanol to give the corresponding diazide derivatives (**3**). Yield 81%, mp 321 °C, $[\alpha]_D^{25}$ +67 (*c* 1, MeOH); IR (KBr, cm^{-1}): 2231 (N≡N), 1742, 1715 (2C=O); ^{1}H NMR (DMSO-d_6): δ 0.79, 0.82 (2s, 6H, $2CH_3$), 0.88 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.20–1.24 (m, 4H, $2CH_2$), 1.25 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.43–1.51 (m, 4H, $2CH_2$), 1.60–1.68 (m, 6H, $3CH_2$), 1.76 (m, 1H, CH), 1.92 (m, 1H, CH), 2.43 (m, 1H, CH), 2.57 (m, 1H, CH), 3.66 (m, 1H, CH), 3.80–3.82 (m, 2H, CH_2), 4.52 (s, 3H, $COOCH_3$), 5.82 (s, 1H, CH=C); ^{13}C NMR (DMSO-d_6): δ 39.11, 48.71, 47.6, 37.71, 55.11, 18.91, 37.0, 37.19, 45.5, 40.16, 191.18, 128.41, 170.16, 48.33, 26.51, 23.4, 32.8, 55.16, 41.41, 43.31, 32.7, 31.17, 30.0, 18.9, 17.5, 23.21, 24.16, 30.83, 31.9, 168.7 (C1–C30), 62.16 (OCH_3); MS (EI): *m/z* 550 (12%) [M^+]. Anal. Calcd for $C_{31}H_{46}N_6O_3$: C, 67.61; H, 8.42; N, 15.26. Found: C, 67.55; H, 8.36; N, 15.20.

Table 3
The % scavenging effect of compounds on superoxide at a dose 50 μg/ml

Compound	% Scavenging at a 50 μg/ml	Relative potency to vitamin C
2	65.12	1.447
3	68.12	1.513
4	70.12	1.558
5	75.12	1.669
6	86.66	1.925
8	67.98	1.510
9	69.14	1.536
10	74.15	1.647
11	80.14	1.780
12	91.24	2.027
Vitamin C	45.00	1.000

*3.1.3. Synthesis of methyl 2β,3α-diamino-11-oxo-18β-olean-12-ene-30-oate (**4**)*

A mixture of the azide derivative **3** (10 mmol) and formic acid (20 mmol) in ethanol (50 ml) was warmed, and then palladium (10% on carbon) was added slowly until evolution of nitrogen gas. The mixture was filtered though Celite and the excess of ethanol was removed under reduced pressure. The residue was dissolved in water, neutralized with sodium carbonate (1 N) to pH ~7, the product was extracted with methylene chloride, and dried over calcium chloride anhydrous. The solvent was evaporated under reduced pressure to dryness to afford the crude diamino terpenoidal derivative (**4**). Yield 69%, mp 216 °C, $[\alpha]_D^{25}$ +128 (*c* 1, MeOH); IR (KBr, cm^{-1}): 3338–3324 ($2NH_2$), 1747, 1712 (2C=O); ^1H NMR (DMSO-d_6): δ 0.78, 0.82 (2s, 6H, $2CH_3$), 0.84 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 1.19–1.23 (m, 4H, $2CH_2$), 1.27 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.41–1.51 (m, 4H, $2CH_2$), 1.58–1.67 (m, 6H, $3CH_2$), 1.78 (m, 1H, CH), 1.96 (m, 1H, CH), 2.40 (m, 1H, CH), 2.61 (m, 1H, CH), 3.18, 3.25 (2br s, 4H, $2NH_2$, exchangeable with D_2O), 3.84–3.86 (m, 2H, CH_2), 3.71 (m, 1H, CH), 4.34 (s, 3H, $COOCH_3$), 5.76 (s, 1H, CH=C); ^{13}C NMR (DMSO-d_6): δ 34.41, 48.71, 47.91, 37.91, 55.81, 18.83, 37.11, 37.39, 45.51, 40.17, 191.28, 128.53, 171.16, 48.56, 26.41, 23.39, 32.83, 55.56, 41.39, 43.21, 32.67, 31.61, 30.19, 18.79, 17.43, 23.19, 24.26, 30.93, 31.91, 169.12 (C1–C30), 62.63 (OCH_3); MS (EI): *m/z* 498 (5%) [M^+]. Anal. Calcd for $C_{31}H_{50}N_2O_3$: C, 74.65; H, 10.10; N, 5.62. Found: C, 74.60; H, 10.05; N, 5.56.

*3.1.4. Synthesis of dichloro (methyl 2β,3α-diamino-11-oxo-18β-olean-12-ene-30-oate) platinum (**5**)*

A solution of **4** (7.22 mmol) and potassium tetrachloroplatinate(II) (3.00 g, 7.23 mmol) in 160 ml of water was stirred at room temperature. A yellow solid was formed, which was filtered off and dried under reduced pressure over P_2O_5 to give the corresponding dichloro compound (**5**). Yield 66%, mp 271 °C dec., $[\alpha]_D^{25}$ +39 (*c* 1, MeOH); IR (KBr, cm^{-1}): 3341 (NH_2), 1741, 1710 (2C=O); ^1H NMR (DMSO-d_6): δ 0.79, 0.82 (2s, 6H, $2CH_3$), 0.86 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.22–1.25 (m, 4H, $2CH_2$), 1.28 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.41–1.50 (m, 4H, $2CH_2$), 1.58–1.62 (m, 6H, $3CH_2$), 1.81 (m, 1H, CH), 1.92 (m, 1H, CH), 2.39 (m, 1H, CH), 2.58 (m, 1H, CH), 3.14, 3.28 (2br s, 4H, $2NH_2$, exchangeable with D_2O), 3.68 (m, 1H, CH), 3.78–3.80 (m, 2H, CH_2), 4.51 (s, 3H, $COOCH_3$), 5.84 (s, 1H, CH=C); ^{13}C NMR (DMSO-d_6): δ 39.58, 48.83, 48.11, 37.83, 55.73, 18.91, 37.12, 37.41, 45.61, 40.17, 191.28, 128.61, 171.17, 48.53, 26.36, 23.41, 32.91, 55.73, 41.41, 43.21, 32.67, 31.61, 30.15, 18.73, 17.41, 23.24, 24.16, 30.91, 31.16, 169.17 (C1–C30), 62.63 (OCH_3); MS (EI): *m/z* 764 (15%) [M^+]. Anal. Calcd for $C_{31}H_{50}Cl_2N_2O_3Pt$: C, 48.69; H, 6.59; Cl, 9.27; N, 3.66. Found: C, 48.62; H, 6.55; Cl, 9.22; N, 3.60.

*3.1.5. Synthesis of (methyl 2β,3α-diamino-11-oxo-18β-olean-12-ene-30-oate)oxalo platinum (**6**)*

To a suspension of **5** (2.84 mmol) in water (60 ml), silver nitrate (920 mg, 5.40 mmol) was added in one portion. The mixture was stirred for a period of one day at room temperature. The precipitated silver chloride was filtered off, and then a mixture of oxalic acid (240 mg, 2.70 mmol) in NaOH solution (5.4 ml) was added with stirring. The reaction mixture was stirred overnight at room temperature, the precipitate was filtered off and dried under reduced pressure over P_2O_5 to give the corresponding oxalo platinum complex (**6**). Yield 56%, mp 328 °C dec., $[\alpha]_D^{25}$ +71 (*c* 1, MeOH); IR (KBr, cm^{-1}): 3341 (NH_2), 1748, 1723, 1712 (4C=O); ^1H NMR (DMSO-d_6): δ 0.79, 0.83 (2s, 6H, CH_3), 0.88 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.21–1.26 (m, 4H, $2CH_2$), 1.32 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.38–1.48 (m, 4H, $2CH_2$), 1.54–1.61 (m, 6H, $3CH_2$), 1.79 (m, 1H, CH), 1.88 (m, 1H, CH), 2.41 (m, 1H, CH), 2.48 (m, 1H, CH), 2.61 (m, 1H, CH), 3.11, 3.31 (2br s, 4H, $2NH_2$, exchangeable with D_2O), 3.82 (m, 2H, CH_2), 4.48 (s, 3H, $COOCH_3$), 5.86 (s, 1H, CH=C); ^{13}C NMR (DMSO-d_6): δ 39.61, 48.91, 48.12, 37.91, 55.75, 18.20, 37.13, 37.51, 45.73, 40.27, 191.18, 128.58, 171.28, 48.63, 26.41, 23.55, 32.88, 55.91, 41.38, 43.19, 32.71, 31.68, 30.15, 18.73, 17.39, 23.41, 24.72, 30.87, 31.26, 168.89 (C1–C30), 62.13 (OCH_3), 172.85, 173.60 (2CO); MS (EI): *m/z* 781 (8%) [M^+]. Anal. Calcd for $C_{33}H_{50}N_2O_7Pt$: C, 50.70; H, 6.45; N, 3.58. Found: C, 50.65; H, 6.42; N, 3.52.

*3.1.6. Synthesis of methyl-2α,3β-dihydroxy-18β-ursan-12-ene-28-oate (**8**)*

The same procedure for the preparation of compound **2** was used with compound **7** as the starting material. Yield 71%, mp 183 °C, $[\alpha]_D^{25}$ +22 (*c* 1, MeOH); IR (KBr, cm^{-1}): 3340–3335 (2OH), 1742, 1705 (2C=O); ^1H NMR (DMSO-d_6): δ 0.82, 0.86 (2s, 6H, $2CH_3$), 0.91 (s, 3H, CH_3), 1.05 (m, 1H, CH), 1.11 (s, 3H, CH_3), 1.18–1.24 (m, 4H, $2CH_2$), 1.28 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.43–1.55 (m, 4H, $2CH_2$), 1.60–1.71 (m, 4H, $2CH_2$), 1.75 (m, 1H, CH), 1.81 (m, 1H, CH), 1.95 (m, 1H, CH), 2.24–2.28 (m, 4H, $2CH_2$), 2.41 (m, 1H, CH), 3.78 (m, 1H, CH), 3.92 (m, 1H, CH), 4.48 (s, 3H, $COOCH_3$), 4.50, 4.55 (2br s, 2H, 2OH, exchangeable with D_2O), 5.60 (m, 1H, CH=C); ^{13}C NMR (DMSO-d_6): δ 39.12, 89.19, 91.16, 47.18, 55.71, 18.33, 37.16, 37.41, 45.19, 40.41, 40.41, 39.16, 128.36, 136.31, 48.61, 26.73, 23.61, 32.71, 53.76, 41.12, 44.17, 32.71, 32.16, 31.12, 19.83, 17.56, 23.67, 24.18, 169.17, 31.56, 30.18 (C1–C30), 62.63 (OCH_3); MS (EI): *m/z* 486 (5%) [M^+]. Anal. Calcd for $C_{31}H_{50}O_4$: C, 76.50; H, 10.35. Found: C 76.44; H, 10.28.

*3.1.7. Synthesis of methyl-2α,3β-diazido-18β-olean-12-ene-28-oate (**9**)*

The same procedure for the preparation of compound **3** was used with compound **8** as the starting material. Yield 79%, mp 152 °C, $[\alpha]_D^{25}$ +117 (*c* 1, MeOH); IR (KBr, cm^{-1}): 2231 (N≡N), 1738, 1712 (2C=O); ^1H NMR (DMSO-d_6): δ 0.84, 0.89 (2s, 6H, $2CH_3$), 0.92 (s, 3H, CH_3), 1.03 (m, 1H, CH), 1.13 (s, 3H, CH_3), 1.17–1.25 (m, 4H, $2CH_2$), 1.26 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.48–1.55 (m, 4H, $2CH_2$), 1.60–1.75 (m, 4H, $2CH_2$), 1.78 (m, 1H, CH), 1.81 (m, 1H, CH), 1.96 (m, 1H, CH), 2.25–2.30 (m, 4H, $2CH_2$), 2.40 (m, 1H, CH), 3.77 (m, 1H, CH), 3.91 (m, 1H,

CH), 4.46 (s, 3H, $COOCH_3$), 5.60 (m, 1H, CH=C); ^{13}C NMR (DMSO-d_6): δ 39.12, 48.63, 47.51, 47.18, 55.83, 18.41, 37.66, 37.93, 45.29, 40.41, 39.17, 128.46, 136.51, 48.66, 26.81, 23.57, 32.61, 53.17, 41.12, 44.17, 32.61, 32.17, 31.16, 19.81, 17.56, 23.77, 24.19, 169.17, 31.56, 30.19 (C1–C30), 62.61 (OCH_3); MS (EI): *m/z* 536 (2%) [M^+]. Anal. Calcd for $C_{31}H_{48}N_6O_2$: C, 69.37; H, 9.01; N, 15.66. Found: C, 69.32; H, 8.95; N, 15.60.

3.1.8. Synthesis of methyl-2α,3β-diamino-18β-ursan-12-ene-28-oate (**10**)

The same procedure for the preparation of compound **4** was used with compound **9** as the starting material. Yield 56%, mp 275 °C, $[\alpha]_D^{25}$ +167 (*c* 1, MeOH); IR (KBr, cm^{-1}): 3363–3362 ($2NH_2$), 1743, 1714 (2C=O); ^1H NMR (DMSO-d_6): δ 0.80, 0.87 (2s, 6H, CH_3), 0.90 (s, 3H, CH_3), 1.05 (m, 1H, CH), 1.12 (s, 3H, CH_3), 1.19–1.23 (m, 4H, $2CH_2$), 1.31 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.44–1.52 (m, 4H, $2CH_2$), 1.58–1.65 (m, 4H, $2CH_2$), 1.78 (m, 1H, CH), 1.86 (m, 1H, CH), 1.99 (m, 1H, CH), 2.22–2.28 (m, 4H, $2CH_2$), 2.45 (m, 1H, CH), 3.21, 3.41 (2br s, 4H, $2NH_2$, exchangeable with D_2O), 3.79 (m, 1H, CH), 3.97 (m, 1H, CH), 4.48 (s, 3H, $COOCH_3$), 5.62 (m, 1H, CH=C); ^{13}C NMR (DMSO-d_6): δ 39.12, 48.61, 47.78, 47.16, 55.51, 18.63, 37.66, 37.51, 45.20, 40.39, 39.31, 128.46, 136.70, 48.53, 26.81, 23.60, 32.63, 53.66, 41.13, 44.13, 32.65, 32.17, 31.15, 19.71, 17.55, 23.66, 24.17, 169.16, 31.55, 30.18 (C1–C30), 62.61 (OCH_3); MS (EI): *m/z* 484 (6%) [M^+]. Anal. Calcd for $C_{31}H_{52}N_2O_2$: C, 76.81; H, 10.81; N, 5.78. Found: C, 76.76; H, 10.78; N, 5.72.

3.1.9. Synthesis of dichloro (methyl-2α,3β-diamino-18β-ursan-12-ene-28-oate) platinum (**11**)

The same procedure for the preparation of compound **5** was used with compound **10** as the starting material. Yield 49%, mp 211 °C dec., $[\alpha]_D^{25}$ +36 (*c* 1, MeOH); IR (KBr, cm^{-1}): 3348–3330 (NH_2), 1742, 1711 (2C=O); ^1H NMR (DMSO-d_6): δ 0.84, 0.88 (2s, 6H, $2CH_3$), 0.93 (s, 3H, CH_3), 1.04 (m, 1H, CH), 1.14 (s, 3H, CH_3), 1.16–1.22 (m, 4H, $2CH_2$), 1.31 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.47–1.53 (m, 4H, $2CH_2$), 1.58–1.63 (m, 4H, $2CH_2$), 1.76 (m, 1H, CH), 1.84 (m, 1H, CH), 1.94 (m, 1H, CH), 2.26–2.32 (m, 4H, $2CH_2$), 2.41 (m, 1H, CH), 3.28, 3.38 (2br s, 4H, $2NH_2$, exchangeable with D_2O), 3.81 (m, 1H, CH), 3.94 (m, 1H, CH), 4.50 (s, 3H, $COOCH_3$), 5.61 (m, 1H, CH=C); ^{13}C NMR (DMSO-d_6): δ 39.17, 48.60, 47.76, 47.14, 55.48, 18.59, 37.56, 37.60, 45.19, 40.30, 39.20, 128.40, 136.69, 48.15, 26.79, 23.51, 32.59, 33.60, 41.11, 44.10, 32.59, 32.11, 31.16, 19.69, 17.48, 23.57, 24.11, 169.11, 31.48, 30.11 (C1–C30), 62.11 (OCH_3); MS (EI): *m/z* 750 (14%) [M^+]. Anal. Calcd for $C_{31}H_{52}Cl_2N_2O_2Pt$: C, 49.60; H, 6.98; Cl, 9.44; N, 3.73. Found: C, 49.55; H, 6.92; Cl, 9.40; N, 3.68.

3.1.10. Synthesis of (methyl-2α,3β-diamino-18β-ursan-12-ene-28-oate) oxalo platinum (**12**)

The same procedure for the preparation of compound **6** was used with compound **11** as the starting material. Yield 53%, mp 347 °C dec., $[\alpha]_D^{25}$ +116 (*c* 1, MeOH); IR (KBr, cm^{-1}): 3356–3326 (NH_2), 1740, 1728, 1712 (3C=O); ^1H NMR (DMSO-d_6): δ 0.85, 0.89 (2s, 6H, $2CH_3$), 0.94 (s, 3H, CH_3), 1.05 (m, 1H, CH), 1.17 (s, 3H, CH_3), 1.20–1.24 (m, 4H, $2CH_2$), 1.30 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.49–1.54 (m, 4H, $2CH_2$), 1.59–1.64 (m, 4H, $2CH_2$), 1.679 (m, 1H, CH), 1.85 (m, 1H, CH), 1.97 (m, 1H, CH), 2.24–2.30 (m, 4H, $2CH_2$), 2.38 (m, 1H, CH), 3.31, 3.47 (2br s, 4H, $2NH_2$, exchangeable with D_2O), 3.88 (m, 1H, CH), 3.97 (m, 1H, CH), 4.51 (s, 3H, $COOCH_3$), 5.67 (m, 1H, CH=C); ^{13}C NMR (DMSO-d_6): δ 39.18, 48.56, 47.66, 47.14, 55.46, 18.69, 37.66, 37.00, 45.17, 40.30, 39.16, 128.41, 136.71, 48.17, 26.78, 23.49, 32.61, 33.56, 41.13, 44.13, 35.60, 32.17, 31.17, 19.71, 17.51, 23.61, 24.13, 169.22, 31.48, 30.15 (C1–C30), 63.11 (OCH_3), 172.9, 173.2 (2CO, oxalate); MS (EI): *m/z* 767 (22%) [M^+]. Anal. Calcd for $C_{33}H_{52}N_2O_6Pt$: C, 51.62; H, 6.83; N, 3.65. Found: C, 51.56; H, 6.78; N, 3.60.

3.2. Biological methodology

3.2.1. Determination of acute toxicity (LD_{50})

The LD_{50} were determined by injection of increasing doses of the tested compounds to adult male albino rats, and then the dose causing 50% animals' death was calculated according to Austen et al. [20].

3.2.2. Determination of the cytotoxicity in HT-29 cells

3.2.2.1. Cell cultures. HT-29 cells were obtained from the Korean cell line Bank (KCLB, Seoul, Korea) and cultured in DMEM supplemented 10% FBS, 100 μg/ml penicillin and 100 μg/ml streptomycin at 37 °C under 5% CO_2 atmosphere. Cells (10^6 ml^{-1}) were grown in 35 mm culture dishes and in 96-well plates (Becton Dickinson Lab ware). The final volumes of culture media were 2 ml for the 35 mm culture dish and 100 μl for each well on the 96-well plate.

3.2.2.2. Neutral red assay. All experiments were performed on the cultured HT-29 cells. Briefly, cells (10^6 ml^{-1}) were exposed to 100 mU/ml GO in culture media containing 0.5% D-glucose without fetal bovine serum and then incubated in the presence of various concentrations of heterocyclic compounds of thiazoldinone derivatives. After 2 days of growth, a neutral red uptake assay was performed according to the method of Wadsworth and Koop (1999) [21]. Cells (10^6 ml^{-1}) were cultured in a 96-well plate for 12 and 24 h. Following the various treatments, the medium was removed and the cells were incubated in 100 μl of new medium containing 10 μg/ml neutral red for 90 min at 37 °C. After neutral red treatment, the medium was removed and the wells were washed three times with 100 μl PBS. One hundred microliters of 50% ethanol containing 50 mM sodium citrate (pH 4.2) was added into each well on the plate. After 20 min, the absorbance was measured at 510 nm using

a Spectra Count TM (Packard Instrument Co., Downers Grove, USA) ELISA reader.

3.2.3. Determination of antioxidant activity

Superoxide radicals were generated by xanthine/xanthine oxidase (XO) and measured by the Nitroblue Tetrazolium (NBT) reduction method. A test sample was mixed in a 100 mM phosphate buffer solution (pH ~7.0) containing XO (1.65×10^{-2} units/ml) and NBT (133 μM) at 25 °C in 96-well flat-bottomed microassay plates. The measurement was started by adding xanthine (164 μM). Production of superoxide radical was followed spectrophotometerically at 560 nm at 25 °C for 10 min. The superoxide scavenging activity was calculated according to the following formula:

$$\text{Superoxide scavenging activity}(\%) = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

where $\text{Absorbance}_{\text{control}}$ and $\text{Absorbance}_{\text{sample}}$ represent the increased absorbance in the absence and presence of samples, respectively.

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