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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of new potential anticancer agents based on 4-thiazolidinone and oleanane scaffolds

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Abstract The synthesis and evaluation of the anticancer activity of new acylated oximes derivatives of oleanolic acid with 4-thiazolidinone-3(5)-carboxylic acid moieties were described. Newly synthesized compounds were elucidated on the basis of elemental analyses and spectral data (IR, ^{1}H, and ^{13}C NMR). Anticancer activity of the tested compounds has been evaluated in vitro at National Cancer Institute (NCI) in which some structure activity relationships (SARs) were discussed. Among the tested compounds, 3-[(2,4-thiazolidinedione-5-ylidene)-carboxyimino] olean-12-en-28-oic acid methyl ester (**IVm**) was superior to other related compounds with mean values of pGI_{50} = 5.51/5.57, pTGI = 5.09/5.13, and pLC_{50} = 4.62/4.64, low toxicity and moderate activity level in vivo hollow fiber assay.

Keywords 4-Thiazolidinone-3(5)-carboxylic acids · 3-Hydroxyimino-oleananes · Anticancer activity

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Introduction

4-Azolidinone-3(5)-carboxylic acids represent one of the most promising group among 4-azolidinone and related derivatives with wide spectrum of pharmacological properties including anticancer effect (Lesyk and Zimenkovsky, 2004; Kaminskyy *et al.*, 2009; Kaminskyy and Lesyk, 2010) which was realized via necroptosis inhibition (**1**) (Teng *et al.*, 2005), inhibition of Bcl-BH3 proteins interaction (**2**) (Degterev *et al.*, 2001), JSP-1 (**3**) (Cutshall *et al.*, 2005) and farnesyl tranferase (**4**) (Lee *et al.*, 2006) activities, etc. On the other hand, 4-azolidinone-3(5)-carboxylic acids are attractive molecular fragments for different scaffolds modification including linking with natural compounds. Among the above-mentioned compounds, β-amyrin triterpenoids, especially oleanolic acid (OA) derivatives compose one of the most perspective group as new lead-compound sources with anti-inflammatory (**5**) (Honda *et al.*, 2000a, b), antiviral (**6**) (Cichewicz and Kouzi, 2004), antibacterial and anticancer (**7**) activities (Honda *et al.*, 2000a; Liby *et al.*, 2007; Liu 2005; Couch *et al.*, 2005) (Fig. 1). Also, background for their utilization is ability to activate the transdermal transport (Konoki and Tachibana, 1996; Han *et al.*, 1997). This aspect probably can be used as an approach to design delivery systems where triterpene fragment possesses not only specific influence but also plays role of biologically active 4-thiazolidinone fragment transporter. Attempts to combine two above-mentioned fragments into one molecule, as part of "double-drugs" creation, can be considered as hybrid pharmacophore approach in drug-like substances design. Its usage can increase the molecular fragments activities level or the appearance of new type of activity and/or improve the drug-like molecules characteristics. It is known that modification of steroids anti-androgens A-ring

by thia(oxa)zolidinone moiety led to identification of thiazole and oxazole derivatives as novel anti-androgens agents (Mallarno *et al.*, 1992). A unique class of 3-spirofused heterocyclic corticosteroids was developed as "soft drugs" by the usage of the chemical delivery system approach (Bodor 1984). Cholesterylchloroformate was used for the alkylation of potassium salt of 5-arylidene-2,4-thiazolidinediones in the design of anticancer agents (Popov-Pergal *et al.*, 2006) as well as modification of OA leads to increasing of derivatives activity levels (Liby *et al.*, 2007; Dinkova-Kostova *et al.*, 2005; Finlay *et al.*, 2002).

Hence, in this article we aimed to synthesize new OA derivatives with 4-thiazolidinone-carboxylic acids fragments as new potential anticancer agents (Fig. 1).

Results and discussion

The design of the target compounds is outlined in Fig. 1 and consists of modification of OA A-ring-linking group by heterocyclic acids, with the usage of oxime function as a link. The preliminary synthetic efforts were focused on C-3 and C-28 positions of OA modifications due to the following points. OA derivatives with additional moiety in position C-3 possess higher activity level that can be related with structure of natural triterpenoids saponines where sugar fragment is combined with C-3 atom. The critical influence of double bond between C-3 and exocyclic nitrogen atom for anticancer activity achievement is disclosed by following results of correlation between structure of OA derivatives and their anticancer activities. The presence of carboxylic group in C rings of triterpenes increases biological activity as well (Zaprutko *et al.*, 2004; Ma *et al.*, 2000; Chen *et al.*, 2006).

Chemistry

Starting oximes (**IIa–d**) (Scheme 1) were synthesized by reacting hydroxylamine hydrochloride with 3-oxooleanolic acid (**Ia**), its methyl ester (**Ib**), morpholide (**Ic**), or 12-bromolactone (**Id**) in ethanol in the presence of anhydrous sodium acetate (Ma *et al.*, 2000; Yasue *et al.*, 1974). The mentioned 3-oxooleananes (**Ia–d**) were obtained from OA extracted from *Viscum album L.*

The synthesis of heterocyclic acids as starting compounds was performed in several stages and is outlined in

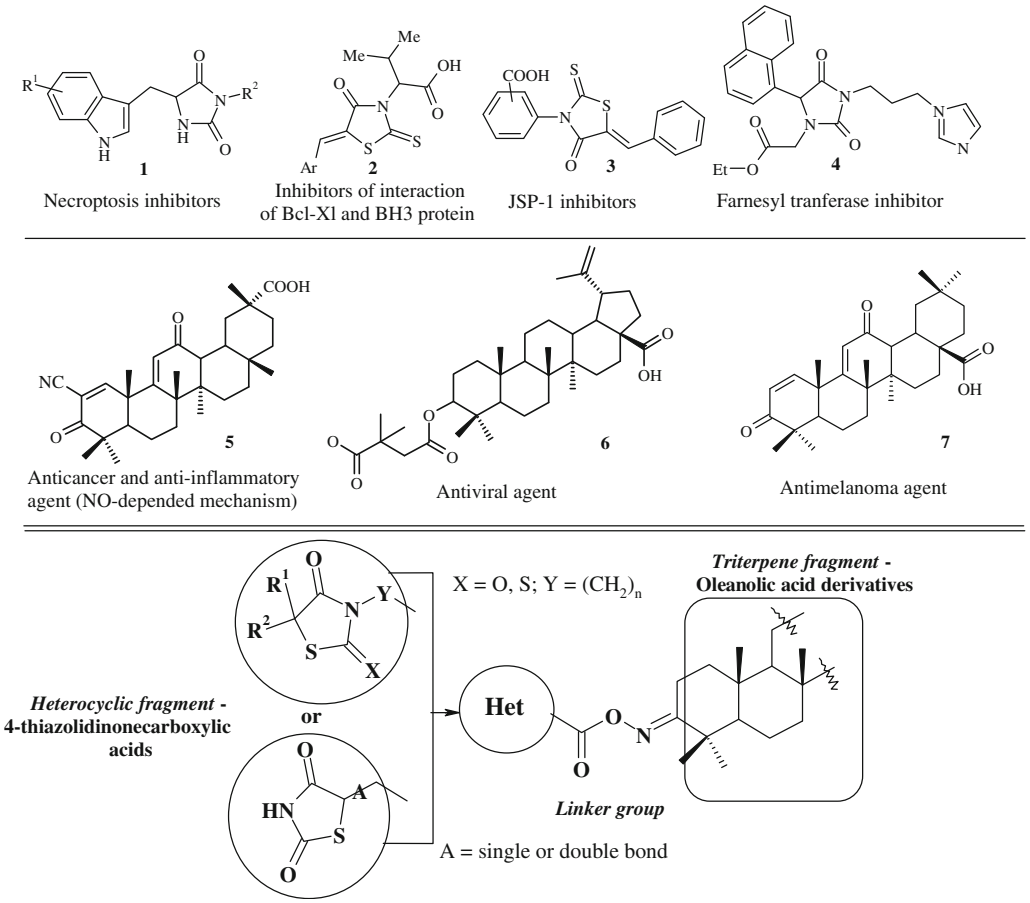
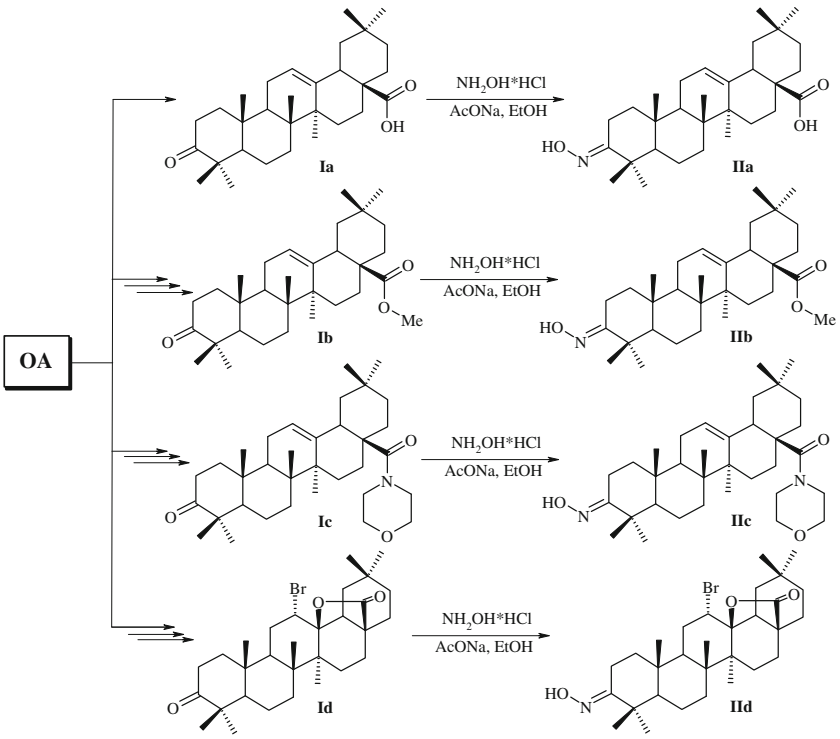


Fig. 1 Structure of lead compounds belongs to 4-azolidinone and OA derivatives and background for target compounds synthesis

Scheme 1 Synthesis of OA 3-hydroxyiminoderivatives with different fragment at C-28



Scheme 2. 5-Unsubstituted as well as 5-ethylrhodanine-3-carboxylic acids (**IIIa–d**) were obtained via [2 + 3]-cyclocondensation of dithiocarbaminates which were prepared in situ and α-chlorocarboxylic acid (Yakubych and Fedirko, 1983). The modification of core ring at C-5 position was achieved through Knoevenagel condensation with aromatic aldehydes. 2,4-Thiazoliodinedione-5-carboxylic acids (**IIIi**, **j**) were synthesized based on maleic anhydride in one phase method. It consisted in condensation reaction with further acid hydrolysis and bromination in case of 2,4-dioxo-thiazolidin-5-ylidene-acetic acid (**IIIj**) (Zimenkovsky *et al.*, 2006; Deghengni and Daneault, 1960). 2,4-Thiazolidinedione-3-acetic acid (**IIIg**) was synthesized by alkylation of 2,4-thiazolidinedione (Lesyk *et al.*, 2002).

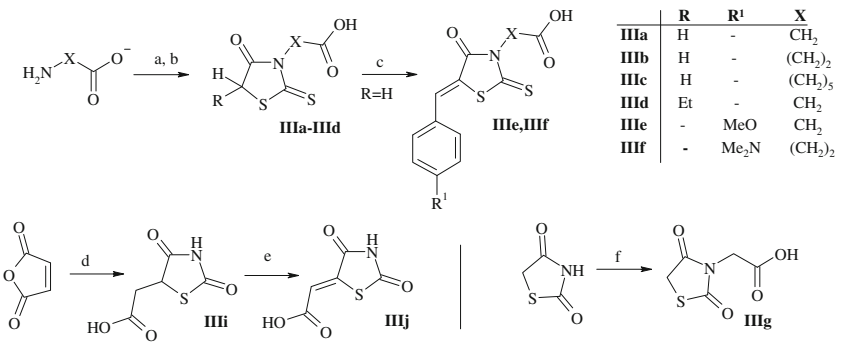
For the synthesis of target 3-*O*-acyloleanolic acid derivatives (**IVa–q**) the oximes **IIa–d** were acylated by heterocyclic acids (**IIIa–g**) in the presence of *N,N′*-dicyclohexylcarbodiimide (DCC) (Scheme 3) in anhydrous dioxane or THF at room temperature. The reactions were monitored by TLC (benzene:AcOEt). Some of the obtained compounds were purified by column chromatography. The spectral data of newly synthesized compounds are presented in "Experimental" section. Analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR, MS) confirmed the structure of the synthesized compounds. Usage of simple aliphatic acids in this reaction is one of the triterpenes structure optimization successful methods in drug discovery (Ma *et al.*, 2000, 2002).

The ^{1}H NMR spectra display characteristic singlets of triterpenes methyl groups at about 0.60–1.20 ppm and signals of oxime group for compounds **IIa–d** in weak magnetic field at about 8.0–10.0 ppm. Compounds **IVa–q** were characterized by signals of oleanane and thiazole fragments. Signals of exocyclic CH_2 groups of 4-thiazolidinone-3-carboxylic moieties (**IVa–g**) and aromatic protons (**IVi**, **j**) are presented in the classical manner. Chemical shifts of methylidene group of 5-arylidene derivatives (**IVi**, **j**) are shifted in weak magnetic field at about 8.0 ppm. Also the CH group of 5-carboxymethylidene fragment (**IVm**, **o**, **q**) showed a characteristic singlet at about 8.00 ppm. Compounds with 4-thiazolidinone-5-acetic (**IVk**, **l**, **n**, **p**) and 5-ethylrodanine fragments (**IVb**, **g**) showed characteristic patterns of an AMX system of CH_2CH fragment as well as in case of simple 4-thiazolidinone analogues (Kaminskyy *et al.*, 2009).

Biological activity

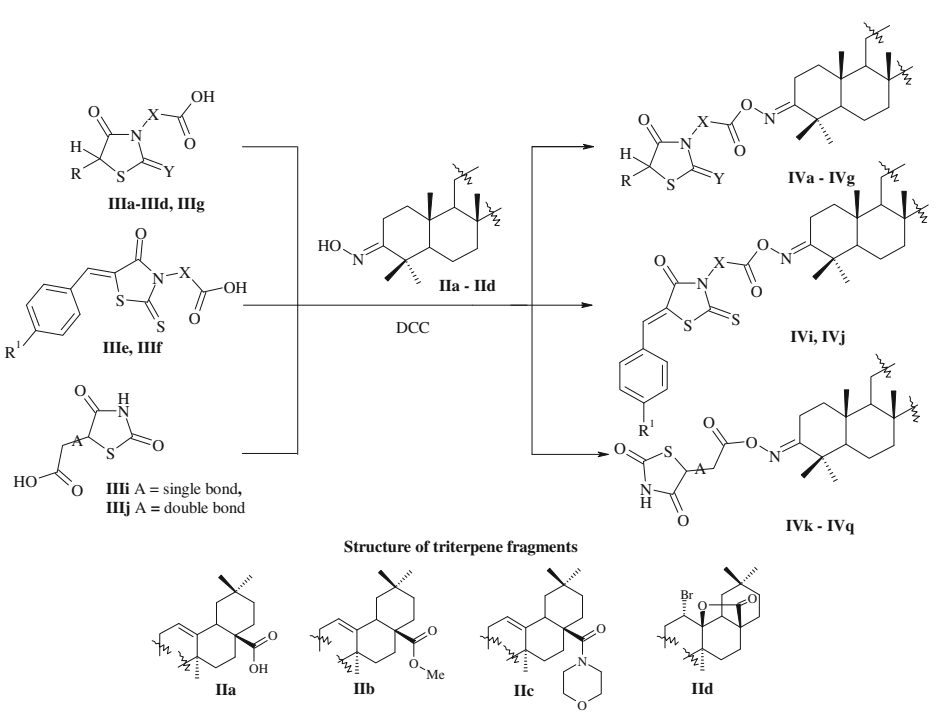
In vitro anticancer activity

The newly synthesized compounds as well as 3-oxoolean-12-en-28-oic acid methyl ester (**Ib**) and 12α-bromo-3-oxoolean-28$\beta \rightarrow 13\beta$ olide (**Id**) were selected by National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for in vitro cell line screening to investigate their anticancer activity. Anticancer assays were performed according to US NCI protocol, which was



	R	R¹	X
IIIa	H	-	CH_2
IIIb	H	-	$(CH_2)_2$
IIIc	H	-	$(CH_2)_5$
IIId	Et	-	CH_2
IIIe	-	MeO	CH_2
IIIf	-	Me_2N	$(CH_2)_2$

Scheme 2 Synthesis of starting heterocyclic carboxylic acids. Reagents and condition: *a* amino acid (1.0 equiv), CS_2 (1.0 equiv), KOH (2.0 equiv), H_2O, stirring, rt, 2 h; *b* 1 ClCHRCOOK (1.0 equiv), H_2O, stirring, rt, 24 h; 2 concentration HCl (2.5 equiv), heating, 90°C, 2 h; *c* appropriate **IIIa–d** (1.0 equiv), ArCHO (1.0 equiv), AcONa (1.0 equiv), AcOH, reflux, 5 h; *d* maleic anhydride (1.0 equiv), thiourea (1.0 equiv), HCl, reflux, 3 h; *e* **IIIi** (1.0 equiv), Br_2 (1.1 equiv), AcOH, reflux, 3 h; *f* 1 2,4-thiazolidinedione (1.0 equiv), KOH (1.0 equiv), $ClCH_2COEt$ (1.0 equiv), EtOH, reflux, 3 h; 2 concentartion HCl, reflux, 3 h



	triterpene fragment			
IVa	IIa	$X = CH_2$,	Y = S,	R = H
IVb	IIa	$X = CH_2$,	Y = S,	R = Et
IVc	IIb	$X = CH_2$,	Y = S,	R = H
IVd	IIb	$X = CH_2$,	Y = O,	R = H
IVe	IIb	$X = (CH_2)_2$,	Y = S,	R = H
IVf	IIb	$X = (CH_2)_5$,	Y = S,	R = H
IVg	IIb	$X = CH_2$,	Y = S,	R = Et
IVi	IIb	$X = CH_2$,	$R^1 = OMe$	
IVj	IIb	$X = (CH_2)_2$,	$R^1 = NMe_2$	
IVk	IIa	A = single bond		
IVl	IIb	A = single bond		
IVm	IIb	A = double bond		
IVn	IIc	A = single bond		
IVo	IIc	A = double bond		
IVp	IId	A = single bond		
IVq	IId	A = double bond		

Scheme 3 Synthesis of target 3-N*O*-acyl-oleanolic acid derivatives

described elsewhere (Monks *et al.*, 1991; Boyd and Paull, 1995; Boyd, 1997; Shoemaker, 2006). The tested compounds were first evaluated at one dose primary anticancer assay towards approximately 60 cell lines (concentration 10^{-5} M). The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers. In the screening protocol, each cell line was inoculated and pre-incubated for 24–48 h on a microtiter plate. Test agents were then added using single concentrations and the culture was incubated for a further 48 h. The end point determinations were made with a protein binding dye, sulforhodamine B (SRB). The results for each test agent were reported as the percent growth of the treated cells compared with the untreated control cells. The preliminary screening results are shown in Table 1.

The tested compounds showed moderate potency of anticancer activity but, have weak average values of anticancer activity, possessed the significant specific influence on some cancer cell lines even with stimulation influence on cells growth (value of growth more than 100%). This activity pattern appeared probably due to distinctive molecular mechanisms of action for the mentioned substances and feature of some cancer cell lines (www.dtp.nci.nih.gov;

Table 1 Cytotoxic activity of the tested compounds in concentration 10^{-5} M against 60 cancer cell lines

Test compounds	Mean Growth, %	Range of Growth, %	Most sensitive cell Line growth, %
Ib	92.20	63.87–119.15	63.87 (SR/L); 85.25 (PC-3/PC); 75.65 (T-47D/BC)
Id	120.31	78.11–335.31	78.11 (K-562/L); 270.75 (SF-295/CNSC); 335.31(SK-OV-3/OC); 302.68 (COLO 205/CC)
IIb	60.08	−41.06–117.85	–41.06 (SR/L); (A549/ATTC/NSCLC); 44.14 (NCI-H460/NSCLC); 23.74 (NCI-H522/NSCLC); 3.39 (HCT-116/CC); 33.89 (U251/CNSC); 11.30 (OVCAR-8/OC); 26,08 (MCF-7/BC); 38.63 (PC-3/PC)
IId	75.84	−62.66–258.88	38.33 (RPMI-8226/L); 21.17 (K-562/L); 20,29 (MOLT-4/L); –62.66 (SR/L); 38.45 (PC-3/PC); 19.14 (SF-295/CNSC); 150.51 (A498/RC); 31.05 (TK-10/RC); 258.88 (HS 578T);
IVc	101.79	56.34–154.78	56.34 (CCRF-CEM/L);
IVf	102.75	42.83–178.01	76.91 (T-47D/BC); 42.83 (SR/L)
IVi	101.77	67.6–142.08	67.36 (BT-549/BC); 72.97 (SF-295/CNSC)
IVj	103.94	82.21–161.85	82.21 (IGROV1/OC)
IVk	87.70	37.86–183.59	54.55 (K562/L); 37.86 (RPMI-8226/L); 54.18 (SR/L);
IVm	73.35	−46.91–135.17	9.18 (T-47D/BC); –24.80 (CCRF-CEM/L); –46.91 (HL-60(TB)/L); 26.64 (K562/L); 2.28 (MOLT-4/L); 16.33 (RPMI-8226/L); –22.69 (SR/L);
IVn	82.02	120.34–21.46	29.41 (T-47D/BC); 42.47 (HL-60(TB)/L); 21.46 (RPMI-8226/L); 48.74 (SR/L);
IVq	81.40	24.85–150.58	24.85 (T-47D/BC); 26.14 (HL-60(TB)/L); 27.12 (K562/L); 26.82 (RPMI-8226/L); 29.41 (SR/L);

ColC colon cancer, *M* melanoma, *NSCLC* non-small cell lung cancer, *RC* renal cancer, *CNSC* CNS cancer, *L* leukemia, *BC* breast cancer, *PC* prostate cancer, *OV* ovarian cancer

http://www.lgcpromochem-atcc.com). Comparison of different cancer cell lines sensitivity to the tested compounds showed that leukemia panel is the most sensitive in all cases except of compounds **IVi** and **j**. The influence on this panel provided not only to increase of cell growth, but even to cell death (growth percent with negative value) and this fact can be used for purposeful synthesis of antileukemic agents. Compound **IId** possessed the most pronounced influence within this panel. Among renal cancer panel the *UO-31* line was the most sensitive. Compounds **IVm** and **n** specifically restrained the growth of this cancer lines (54.58 and 56.30 growth percent, correspondingly) as well as PC-3 line to **IId** (38.63%).

SARs features

Oximes **IIb** and **d** possessed higher activity level in comparison with appropriate 3-oxo-oleanane derivatives, which had no anticancer activity and stimulated some cancer cell line growth (Table 1). 4-Thiazolidinone derivatives possessed significant influence on non-small lung cancer (NSLC) panel (Lesyk and Zimenkovsky, 2004; Kaminskyy *et al.*, 2009; Lesyk *et al.*, 2006), but in our data only *NCI-H522* cell growth suppression was observed in comparison with other lines of NSLC panel. However, compound **IIb** visibly inhibited *NCI-H522* and *NCI-H460* cell lines growth. Also, the *T-47D* (breast cancer panel) cell growth was significantly suppressed by tested compound action in comparison to another breast cancer cell lines.

The presence of an ylidene moiety in position C-5 of thiazolidine ring played crucial role for anticancer activity achievement of 4-thiazolidinone derivatives (Lesyk and Zimenkovsky, 2004; Kaminskyy *et al.*, 2009; Kaminskyy and Lesyk, 2010). Comparison of **IVc** and **i** activity levels which differed in the presence of the mentioned fragment did not allowed us to determine significant difference as well as in case of comparison of **IVc** and **f** activity which differs in the amount of CH_2 groups in carbon-chain of heterocyclic fragments. Compounds with 2,4-dioxothiazolidin-5-ylideneacetic acid fragment (**IVm** and **q**) and different triterpene moieties possessed the most prominent level of anticancer activity and **IVm** possessed the strongest antimitotic effect with significant influence on leukemia cell lines.

In vitro full panel 60-cell line assay

Thus compounds **IIb**, **d**, and **IVm** were selected for advanced assay and screened towards about 60 cell lines of above-mentioned types of human cancers at five different concentrations (10^{-4}–10^{-8} M). A 48-h continuous drug exposure protocol was used with SRB protein assay to estimate cell viability and growth. Results (Table 2) are expressed as log GI_{50}, log TGI, log LC_{50} (GI_{50}—molar concentration of the compound that inhibits 50% netcell growth; TGI—molar concentration of the compound leading to total inhibition of cell growth; LC_{50}—molar concentration of the compound leading to 50% net cell death).

Table 2 Anticancer activity of compounds **IIb**, **d**, and **IVm**

	Cancer cell line	Dose-dependent parameters							
		pGI_{50}			pTGI			pLC_{50}	
		IVm*	**IIb**	**IId**	**IVm***	**IIb**	**IId**	**IVm***	**IIb**
Lukemia	CCRF-CEM	5.76/5.61	4.55	4.76	5.43/5.11	4.09	<4.00	5.10/4.43	<4.00
	HL-60(TB)	5.82/5.69	4.23	<4.00	5.46/5.27	<4.00	<4.00	5.10/4.58	<4.00
	K-562	5.74/5.56	4.43	4.25	5.37/5.09	<4.00	<4.00	4.96/4.52	<4.00
	MOLT-4	5.73/5.68	4.43	4.85	5.37/5.13	<4.00	<4.00	5.00/4.43	<4.00
	RPMI-8226	5.73/5.63	4.40	<4.00	5.34/5.22	<4.00	<4.00	4.69/<4.00	<4.00
	SR	5.77	4.57	5.67	5.41	4.26	5.10	5.05	<4.00
Non-small cell lung cancer	A549/ATCC	5.34/5.46	4.54	4.75	4.75/4.84	<4.00	<4.00	4.21/4.39	<4.00
	EKVX	5.17/5.52	4.55	4.04	4.65/5.03	4.14	<4.00	4.25/4.31	<4.00
	HOP-62	4.70/4.97	4.67	4.39	4.44/4.64	4.35	<4.00	4.19/4.31	4.03
	HOP-92	5.73/5.81	4.72	5.36	5.33/5.39	4.40	4.33	<4.00/4.94	4.08
	NCI-H226	4.91/5.71	4.56	4.55	4.51/5.34	4.18	<4.00	4.10/<4.00	<4.00
	NCI-H23	5.55/5.47	4.70	4.42	5.07/4.91	4.42	<4.00	4.38/4.40	4.13
	NCI-H322 M	4.74/4.89	4.14	4.70	4.49/4.58	<4.00	<4.00	4.23/4.27	<4.00
	NCI-H460	5.54/5.69	4.66	4.72	5.02/5.21	4.29	<4.00	4.39/4.77	<4.00
	NCI-H522	5.74/5.69	4.58	–	5.38/5.25	4.18	–	5.03/4.59	<4.00
Colon cancer	COLO 205	5.58/5.75	4.65	4.04	5.27/5.45	4.24	<4.00	4.80/5.15	<4.00
	HCC-2998	5.46	4.64	4.09	4.91/5.52	4.37	<4.00	4.37/5.21	4.10
	HCT-116	5.75/5.78	4.53	4.72	5.47/5.50	4.25	<4.00	5.19/4.21	<4.00
	HCT-15	5.59/5.56	4.62	4.51	4.96/4.96	4.26	<4.00	4.19/4.41	<4.00
	HT-29	5.65/5.66	4.64	4.75	5.33/5.29	4.10	<4.00	5.01/4.80	<4.00
	KM12	5.55/5.46	4.41	5.05	5.12/4.90	<4.00	<4.00	4.53/4.44	<4.00
	SW-620	5.70/5.77	4.70	5.17	5.41/5.48	4.39	<4.00	5.12/5.18	4.08
CNS cancer	SF-268	5.50/5.67	4.62	4.56	4.97/5.27	4.25	<4.00	4.38/4.73	<4.00
	SF-295	5.36/5.31	4.75	4.85	4.72/4.68	4.43	4.44	4.30/4.26	4.11
	SF-539	5.78/5.75	4.68	4.70	5.50/5.44	4.38	<4.00	5.22/5.12	4.09
	SNB-19	4.91/4.98	4.59	4.27	4.58/4.66	4.16	<4.00	4.25/4.33	<4.00
	SNB-75	4.97/5.51	4.74	4.69	4.64/4.85	4.41	4.22	4.31/4.42	4.07
	U251	5.77/5.75	4.72	4.65	5.47/5.47	4.47	<4.00	5.17/5.19	4.21
Melanoma	LOX IMVI	5.84/5.83	4.71	5.00	5.51/5.53	4.40	<4.00	5.18/5.23	4.08
	MALME-3 M	5.64/5.73	–	5.52	5.11/5.41	–	4.63	4.33/5.10	<4.00
	M-14	5.64/5.69	4.63	5.04	5.29/5.36	4.24	<4.00	4.70/−5.03	<4.00
	MDA-MB-435	5.55/5.62	4.40	–	5.04/5.16	<4.00	–	4.34/4.31	<4.00
	SK-MEL-2	5.66/5.34	4.19	<4.0	5.36/4.75	<4.00	<4.00	5.05/4.29	<4.00
	SK-MEL-28	5.69/5.73	4.61	4.36	5.42/5.45	4.18	<4.00	5.15/5.18	<4.00
	SK-MEL-5	5.28/5.39	4.67	4.58	4.75/4.81	4.20	<4.00	4.36/4.40	<4.00
	UACC-257	5.22/4.94	4.48	4.83	4.74/4.61	<4.00	<4.00	4.35/4.29	<4.00
	UACC-62	5.72/5.65	4.65	4.73	4.99/5.01	4.25	<4.00	4.44/4.47	<4.00
Ovarian cancer	IGROV1	–/5.72	4.47	–	–/5.38	<4.00	–	–/5.05	<4.00
	OVCAR-3	5.72/5.78	4.73	4.94	5.46/5.51	4.45	4.31	5.20/5.24	4.17
	OVCAR-4	5.60/5.63	4.52	4.94	5.22/5.16	<4.00	<4.00	4.62/4.48	<4.00
	OVCAR-5	4.73/4.78	4.61	<4.00	4.47/4.52	4.26	<4.00	4.22/4.25	<4.00
	OVCAR-8	5.66/5.45	4.46	5.07	5.31/4.82	<4.00	4.27	4.77/4.22	<4.00
	NCI/ADR-RES	5.12/5.36	4.43	4.57	4.64/4.82	<4.00	<4.00	4.21/4.39	<4.00
	SK-OV-3	4.70/4.87	4.39	4.51	4.46/4.58	<4.00	<4.00	4.21/4.29	<4.00

Table 2 continued

	Cancer cell line	Dose-dependent parameters							
		pGI_{50}			pTGI			pLC_{50}	
		IVm*	**IIb**	**IId**	**IVm***	**IIb**	**IId**	**IVm***	**IIb**
Renal cancer	786-0	5.45/5.68	4.75	4.31	4.94/5.32	4.46	<4.00	4.44/4.90	4.18
	A498	5.59/5.72	4.55	4.31	5.24/5.33	4.20	<4.00	4.75/4.85	<4.00
	ACHN	5.76/5.77	4.68	4.27	5.47/5.49	4.38	<4.00	5.18/5.21	4.08
	CAKI-1	5.77/5.61	4.71	4.55	5.45/4.79	4.32	<4.00	5.13/4.26	<4.00
	RXF 393	5.72/5.76	4.74	–	5.45/5.48	4.44	–	5.18/5.19	4.13
	SN12C	5.44/5.58	4.68	4.43	4.73/5.05	4.37	<4.00	4.10/4.24	4.06
	TK-10	5.55/5.74	4.47	4.73	5.09/5.32	4.01	4.28	4.40/4.76	<4.00
	UO-31	5.83/5.83	4.65	4.39	5.53/5.53	4.34	<4.00	5.24/5.24	4.02
PC	PC-3	5.51/5.50	4.69	4.39	4.95/4.85	4.35	<4.00	4.33/4.43	4.01
	DU-145	5.29/5.68	4.69	4.85	4.77/5.34	4.37	<4.00	4.37/4.98	4.05
Breast cancer	MCF7	5.61/5.75	4.69	4.93	4.97/5.37	4.39	4.22	4.35/4.95	4.09
	MDA-MB-231/ATCC	5.68/5.67	4.69	4.75	5.12/5.16	4.39	4.32	4.38/4.41	4.09
	HS 578T	5.60/5.81	4.56	5.33	5.17/5.20	4.18	4.61	4.39/4.38	<4.00
	BT-549	5.63/5.6o	4.19	5.03	5.22/5.04	<4.00	4.16	4.63/4.34	<4.00
	T-47D	5.37/5.57	4.60	4.76	4.73/4.85	4.14	4.13	4.15/4.33	<4.00
	MDA-MB-468	5.70/5.66	–	–	5.35/5.18	–	–	4.99/4.56	–
	MID	5.51/5.57	4.57	4.65	5.09/5.13	4.21	4.09	4.62/4.64	4.03

* Data of repeat assay; $pGI_{50} = -\log GI_{50}$; pTGI = −log TGI; $pLC_{50} = -\log LC_{50}$; pLC_{50} values for **IId** < 4.0

The values in Table 2 were calculated for each of these parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value was expressed as more or less than the maximum or minimum concentration tested. Furthermore, mean graph midpoints (MG_MID) were calculated for each of the parameters, giving an average activity parameter over all cell lines for each compound. For the calculation of the MG_MID, insensitive cell lines were included with the highest concentration tested. The dose-dependent 60-cell line assay is summarized in Table 2. The presented data showed that compound **IVm** was characterized by the strong effect on all cancer cell lines with insignificant selectivity to leukemia cell lines (Fig. 2). Unsubstituted oximes (**IIb**, **d**) showed weak levels of dose-depended parameters.

In vivo anticancer activity: hollow fiber assay

Compound **IVm** was found to have reproducible activity in the in vitro anticancer drug screening which was evaluated by NCI using hollow fiber assay. This assay provided quantitative indications of the compound's drug efficacy (http://dtp.nci.nih.gov/branches/btb/hfa.html) (Plowman *et al.*, 1997; Hollingshead *et al.*, 1995). In the hollow fiber model, polyvinylidene fluoride fibers containing various human cancer cell cultures were implanted intraperitoneally (ip) and subcutaneously (sc) into athymic nude mice and compounds were administrated by ip route. The effects of the compounds on reduction of viable cancer cell mass compared with those of controls were determined. To simplify evaluation, the NCI protocol adopts a point system that allows rapid viewing of the activity of a given compound. For this, a value of 2 is assigned for each compound dose that results in a 50% or greater reduction in viable cell mass. Compounds with a combined ip + sc score ≥ 20, a sc score ≥ 8, or a net cell kill of one or more cell lines were considered significantly active. Compound **IVm** exhibited ip score—8, sc score—16 and total score (ip + sc score) 16, with low cytotoxicity (no cell death).

Non-tumored animal toxicity assay for **IVm** was performed according to NCI protocol (http://dtp.nci.nih.gov/branches/btb/pdf/acutetox.pdf). The result shown the maximum tolerated dose is more than 400 mg/kg. It can be used to calculate the amount of material given to experimental mice during further in vivo antitumor testing.

COMPARE analysis

NCI web-resources allows to compare selectivity patterns (mean graph fingerprints) of tested compounds with standard anticancer agents, NCI active synthetic compounds and natural extracts, which are present in public available data bases. Such analysis is based on the comparing the patterns of differential growth inhibition for cultured cell

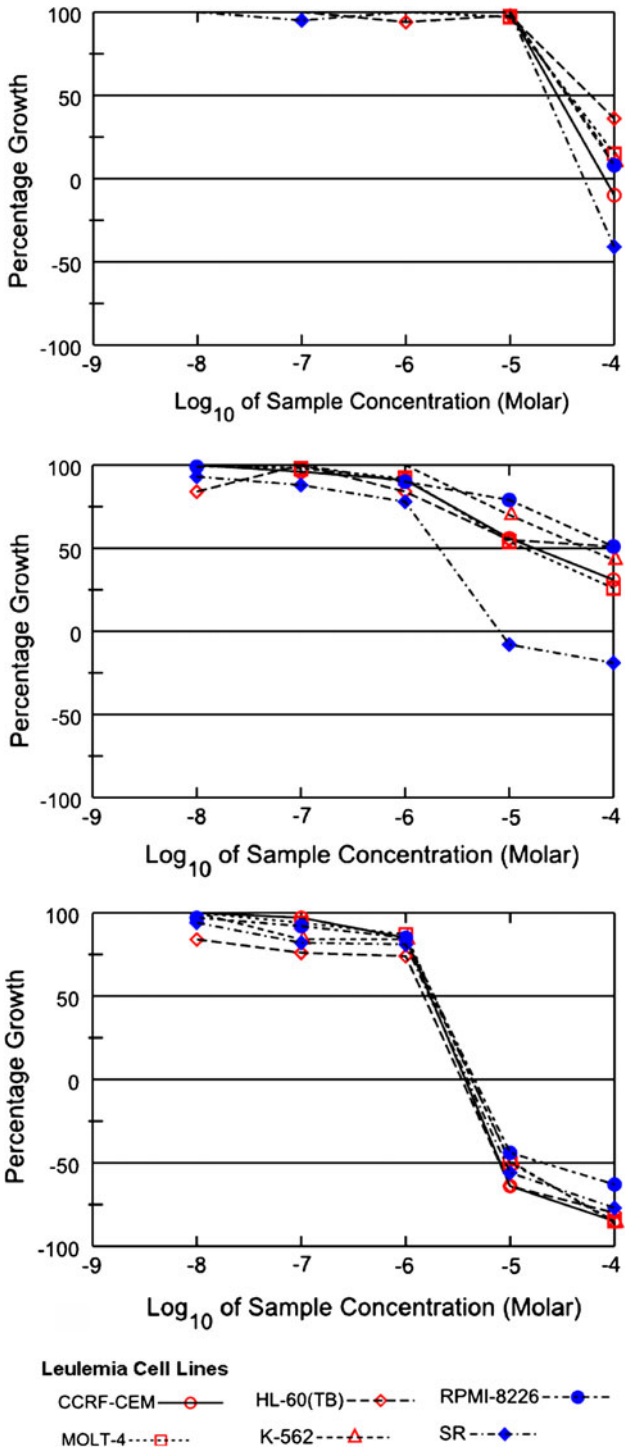


Fig. 2 Influence of **IIb**, **d**, and **IVm** on Leukemia panel cell lines, corresponding

lines and can potentially gain insight into the mechanism of the cytotoxic action. If the data pattern correlates well with that of the compounds belonging to a standard agent database (Pearson's correlation coefficient (PCC) > 0.6), the compound of interest may have the same mechanism of action. On the other hand, if the activity pattern does not correlate with any standard agent, it is possible that the compound has a novel mechanism of action. Standard COMPARE analyses (http://dtp.nci.nih.gov/docs/compare/compare.html) (Zaharevitz *et al.*, 2002) were performed at GI_{50} level, obtained correlation coefficients (r) did not allow to distinguish cytotoxicity mechanism of tested compounds with high probability. Nevertheless moderate correlations with fluorodopan (Nair *et al.*, 1980) (NSC73754, alkylating agent, $r = 0.737$), as well as with S-trityl-L-cysteine (NSC83265 $r = 0.702$)—aminoacyl-tRNA synthetases inhibitor with antiproliferative effect against leukemia (Brier *et al.*, 2004) were detected. Interesting, that other 4-azolidinone derivatives also have significant value of correlation coefficients to the above-mentioned substance (Havrylyuk *et al.*, 2009; Subtel'na *et al.*, 2010).

Conclusion

In the present article, 16 new acylated oximes of OA derivatives with 4-thiazolidinone-3(5)-carboxylic acids fragments were described. In vitro anticancer activity for synthesized compounds as well as some chemical precursors was evaluated. Obtained results confirmed availability of combining oleanane and thiazolidinone scaffolds as direction of anticancer agent design. Among the tested compounds, 3-[(2,4-thiazolidinedione-5-ylidene)-carboxyimino] olean-12-en-28-oic acid methyl ester (**IVm**) was identified as the most active substance with following values of dose-depended parameters: $pGI_{50} = 5.51/5.57$, $pTGI = 5.09/5.13$, and $pLC_{50} = 4.62/4.64$, low toxicity and moderate activity level in vivo hollow fiber assay.

Experimental

Chemistry

The starting (2,4-dioxothiazolidin-5-yl)-acetic acid (**IIIi**) (Zimenkovsky *et al.*, 2006), 2,4-dioxothiazolidin-5-ylidene-acetic acid (**IIIj**) (Deghengni and Daneault, 1960), rhodanine-3-carboxylic acids (**IIIa–f**) (Yakubych and Fedirko, 1983), 2,4-dioxothiazolidin-3-acetic acid (**IIIg**) (Lesyk *et al.*, 2002) were obtained according to methods described previously, as well as the 3-oxooleanolic acid (**Ia**) (Shirane *et al.*, 1996) and its metyl ester (**Ib**) (Ma *et al.*, 2000; Simonsen and Rossk, 1957), morpholide (**Ic**) (Yasue *et al.*, 1974) and 28β → 13β lactone of 12α-bromo-3-oxoolean-28-oic acid (**Id**) (Lewis and Tucker, 1983).

Melting points were measured in open capillary tubes on a BUCHI B-545 melting point apparatus and are uncorrected. The elemental analyses (C, H, N) were performed

using the Perkin–Elmer 2400 CHN analyzer and were within ±0.4% of the theoretical values. The IR spectra were recorded using Specord IR–75 spectrophotometer, for 0.5% mixtures of tested compounds and KBr. The ^{1}H- and ^{13}C-NMR spectra were recorded on Varian Gemini 400 MHz or Bruker 125 MHz for frequencies, respectively, 400 or 300 MHz and 100 or 75 MHz, in pyridine, $CDCl_3$, or DMSO-d_6 using tetramethylsilane as internal standard. The chemical shifts are reported in ppm with the usage of δ scale. Mass spectra were recorded using AMD 402 spectrophotometer with electroionisation.

*General procedure for synthesis of oleanolic acids derivatives oximes (**IIa–d**)*

Hydroxylamine hydrochloride (3.47 g, 50 mmol) was added to appropriate 3-oxoolean derivative (**I**) (10 mmol) in ethanol (50 ml), then anhydrous sodium acetate (6.56 g, 80 mmol) was added and the resulted mixture was refluxed for 20–60 min (TLC control). After cooling reaction mixture was diluted with water (300 ml) slightly acidified with concentration HCl. The formed solid was filtered, washed with water, and crystallized from ethanol to give **IIa–d**.

*3-Hydroxyiminoolean-12-en-28-oic acid (**IIa**)* Yield 90%, mp 297–299°C (lit. 291°C (Simonsen and Rossk, 1957)). IR (ν, cm^{-1}): 3350 (OH, COOH), 3240 (OH, N–OH), 1680 (C=O, COOH). ^{1}H NMR (Ma *et al.*, 2000). ^{13}C NMR (75 MHz, pirydine) δ: 180.1 (C-28), 164.1 (C-3), 144.8 (C-13), 122.4 (C-12), 46.6 (C-17). DEPT: 7 × CH_3, 10 × CH_2, 4 × CH, 30 × C at. EI-MS (*m/z*): 469.7 (5.6%) $M^{+\bullet}$. Anal. calcd. for $C_{30}H_{47}NO_3$: C, 76.71; H, 10.09; N, 2.98; Found: C, 77.00; H, 10.20; N, 3.10%.

*3-Hydroxyiminoolean-12-en-28-oic acid methyl ester (**IIb**)* Yield 93%, mp 247–249°C (lit. 254–255°C (Simonsen and Rossk, 1957). IR (ν, cm^{-1}): 3245 (OH, N–OH), 1720 (C=O, $COOCH_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.76 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 2.90 (dd, 1H, J_1 = 4.0, 13.6 Hz; C_{18}–H_β), 3.63 (s, 3H, $COO\underline{CH_3}$), 5.29 (t, 1H, J = 3.4 Hz; C_{12}–H), 9.37 (s, 1H, N–OH). ^{13}C NMR (75 MH, $CDCl_3$) δ: 178.3 (C-28), 166.9 (C-3), 143.8 (C-13), 122.2 (C-12), 51.5 (C-31), 46.6 (C-17). DEPT: 8 × CH_3, 10 × CH_2, 4 × CH, 31 × C at. EI-MS (*m/z*): 483.0 (6.0%) $M^{+\bullet}$. Anal. calcd. for $C_{31}H_{49}NO_3$: C, 76.97; H, 10.21; N, 2.90; Found: C, 77.10; H, 10.10; N, 2.80%.

*3-Hydroxyiminoolean-12-en-28-oic acid morpholide (**IIc**)* Yield 87%, mp 206–211°C (lit. 192–195°C (Yasue *et al.*, 1974). IR (ν, cm^{-1}): 3250 (OH, N–OH), 1625 (C=O, amide), 995 (C–O, morph.). ^{1}H NMR (Yasue *et al.*, 1974). ^{13}C NMR (Yasue *et al.*, 1974). Anal. calcd. for $C_{34}H_{54}N_2O_3$: C, 75.79; H, 10.10; N, 5.20; Found: C, 76.00; H, 10.00; N, 5.35%.

*12α-Bromo-3-hydroxyiminoolean-28β → 13β olide (**IId**)* Yield 91%, mp 267–269°C (lit. 251–252°C (Simonsen and Rossk, 1957). IR (ν, cm^{-1}): 3245 (OH, N–OH), 1770 (C=O, lacton). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 2.36 (t, 1H, J = 4.5 Hz, C_{18}–H_β), 4.30 (t, 1H, J = 1.9 Hz, C_{12}–H), 8.50 (br.s, 1H, N–OH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 178.8 (C-28), 166.8 (C-3), 91.6 (C-13), 56.2 (C-12), 45.5 (C-17). DEPT: 7 × CH_3, 10 × CH_2, 4 × CH, 30 × C at. EI-MS (*m/z*): 548.4 (9.8%) $M^{+\bullet}$. Anal. calcd. for $C_{30}H_{46}BrNO_3$: C, 65.68; H, 8.45; N, 2.55; Found: C, 65.90; H, 8.60; N, 2.60%.

*General procedure for synthesis of 4-thiazolidinone substituted OA derivatives (**IVa–q**)*

A mixture of appropriate 3-hydroxyiminoolean-derivative (**IIa–d**) (2 mmol) and 4-thiazolidinone-carboxylic acid (**IIIa–g**) (2.4 mmol) in dry dioxane was stirred at room temperature to dissolution. DCC (0.619 g, 3 mmol) was added to the reaction mixture, the stirring in room temperature was continued until the triterpenic substrate consumption (0.5–1.5 h, TLC control). The solid formed was filtered and the filtrate was poured into of water (200 ml) slightly acidified with concentration HCl. The formed solid was filtered, washed with water and dried. The residue was purified by column chromatography if necessary (Kisielgel 60 (0.063–0.200 mm/70–230 mesh, eluent–benzene:AcOEt) and crystallized or precipitated with water from ethanol.

*3-(Rhodanine-3-acetyloxyimino)-olean-12-en-28-oic acid (**IVa**)* Yield 86%, mp 144–146°C. IR (ν, cm^{-1}): 3400 (O–H, COOH), 1705 (C=O, thiazole), 1670 (C=O, COOH), 1625 (C=O, COON), 1505 (C=S, thiazole). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.77 (s, 3H, CH_3), 0.88 (s, 6H, 2 × CH_3), 0.98 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 2.74–2.84 (m, 1H, C_{18}–H), 4.46 (s, 2H, CH_2-thiazole), 4.85 (s, 2H, NCH_2CO), 5.18 (br.s, 1H, C_{12}–H), 11.96 (br.s, 1H, COOH), ^{13}C NMR (100 MHz, DMSO-d_6) δ: 203.1 (C=S), 179.0 (C-28), 176.7 (C=O, thiazole), 174.1 (C=O, COON), 165.3 (C-3), 144.4 (C-13), 121.9 (C-12), 46.3 (C-17). DEPT: 7 × CH_3, 12 × CH_2, 4 × CH, 35 × C at. Anal. calcd. for $C_{35}H_{50}N_2O_5S_2$: C, 68.39; H, 7.84; N, 4.36; Found: C, 68.50; H, 7.90; N, 4.50%.

*3-(5-Ethylrhodanine-3-acetyloxyimino)-olean-12-en-28-oic acid (**IVb**)* Yield 95%, mp 102–109°C. IR (ν, cm^{-1}): 3405 (O–H, COOH), 1700 (C=O, thiazole), 1675 (C=O,

COOH), 1620 (C=O, COON), 1500 (C=S, thiazole). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.76 (s, 3H, CH_3), 0.88 (s, 6H, 2 × CH_3), 0.96 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 1.08 (s, 6H, 2 × CH_3), 1.10 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 2.00–2.08 (m, 2H, $\underline{CH_2}$CH-thiazole), 2.74–2.86 (m, 1H, C_{18}–H), 4.81–4.88 (m, 3H, $\underline{CH_2}$COO, Et–CH-thiazole), 5.18 (br.s, 1H, C_{12}–H), 12.06 (br.s 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 202.0 (C=S), 179.0 (C-28), 176.7 (C=O, thiazole), 176.3 (C=O, COON), 165.3 (C-3), 144.4 (C-13), 121.8 (C-12), 46.9 (C-17). DEPT: 8 × CH_3, 12 × CH_2, 5 × CH, 37 × C at. Anal. calcd. for $C_{37}H_{54}N_2O_5S_2$: C, 66.23; H, 8.11; N, 4.17; Found: C, 66.39; H, 8.22; N, 4.30%.

*3-(Rhodanine-5-acetyloxyimino)-olean-12-en-28-oic acid methyl ester (**IVc**)* Yield 90%, mp 128–130°C. IR (ν, cm^{-1}): 1720 (C=O, $COOCH_3$), 1705 (C=O, thiazole), 1635 (C=O, COON), 1505 (C=O, thiazole). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.76 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.13 (s, 6H, 2 × CH_3), 1.24 (s, 3H, CH_3), 2.88 (dd, J_1 = 4.2, 13.8 Hz, 1H, C_{18}–H), 3.63 (s, 3H, $COOCH_3$), 4.09 (s, 2H, CH_2COON), 4.96 (s, 2H, CH_2-thiazole), 5.29 (t, 1H, J = 3.6 Hz, C_{12}–H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 200.1 (C=S), 178.0 (C-28), 175.9 (C=O, thiazole), 172.9 (C=O, COON), 165.5 (C-3), 143.8 (C-13), 121.9 (C-12), 51.6 ($COO\underline{C}H_3$), 46.7 (C-17). DEPT: 8 × CH_3, 12 × CH_2, 4 × CH, 36 × C at. Anal. calcd. for $C_{36}H_{52}N_2O_5S_2$: C, 65.82; H, 7.98; N, 4.26; Found: C, 66.05; H, 8.15; N, 4.14%.

*3-(2,4-Thiazolidinedione-3-acetyloxyimino)-olean-12-en-28-oic acid methyl ester (**IVd**)* Yield 91%, mp 192–194°C. IR (ν, cm^{-1}): 1725 (C=O, $COOCH_3$), 1705 (C=O, thiazole), 1680 (C=O, thiazole), 1630 (C=O, COON). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.70 (s, 3H, CH_3), 0.89 (s, 6H, 2 × CH_3), 0.99 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 2.77–2.88 (m, 1H, C_{18}–H), 3.55 (s, 3H, $COOCH_3$), 4.37 (s, 2H, CH_2COON), 4.51 (s, 2H, CH_2-thiazole), 5.19–5.23 (m, 1H, C_{12}–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 177.8 (C-28), 176.6 (C=O, NH$\underline{C}$OCH-thiazole), 172.4 (C=O, S$\underline{C}$ONH-thiazole), 172.0, (C=O, COON), 166.3 (C-3), 144.2 (C-13), 122.3 (C-12), 52.1 ($COO\underline{C}H_3$), 46.7 (C-17); DEPT: 8 × CH_3, 12 × CH_2, 4 × CH, 36 × C at. Anal. calcd. for $C_{36}H_{52}N_2O_6S$: C, 67.47; H, 8.18; N, 4.37; Found: C, 67.57; H, 8.30; N, 4.51%.

*3-(Rhodanine-3-propionyloxyimino)-olean-12-en-28-oic acid methyl ester (**IVe**)* Yield 82%, mp 152–154°C. IR (ν, cm^{-1}): 1720 (C=O, $COOCH_3$), 1700 (C=O, thiazole), 1635 (C=O, COON), 1490 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.69 (s, 3H, CH_3), 0.88 (s, 6H, 2 × CH_3), 0.97 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 2.77 (t, J = 7.4, 2H, N$\underline{CH_2}CH_2$), 3.55 (s, 3H, $COOCH_3$), 4.14 (t, J = 7.4 Hz, 2H, N$\underline{CH_2}CH_2$), 4.24 (s, 2H, CH_2–thiazol) 5.21 (br.s, 1H, C_{12}–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 201.5 (C=S), 177.8 (C-28), 175.5 (C=O, thiazole), 174.7 (C=O, COON), 169.2 (C-3), 144.1 (C-13). 122.4 (C-12), 52.1 ($COO\underline{C}H_3$), 46.7 (C-17). DEPT: 8 × CH_3, 13 × CH_2, 4 × CH, 37 × C at. Anal. calcd. for $C_{37}H_{54}N_2O_5S_2$: C, 66.23; H, 8.11; N, 4.17; Found: C, 66.40; H, 8.23; N, 4.21%.

*3-(Rhodanine-3-hexanoyloxyimino)-olean-12-en-28-oic acid methyl ester (**IVf**)* Yield 87%, mp 134–138°C. IR (ν, cm^{-1}): 1720 (C=O, $COOCH_3$), 1695 (C=O, thiazole), 1640 (C=O, COON), 1500 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.69 (s, 3H, CH_3), 0.88 (s, 6H, 2 × CH_3), 0.98 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 1.42–1.62 (m), 1.98 (m), 2.24 (t, J = 7.2 Hz), 2.41 (t, J = 7.2 Hz), 3.85 (t, J = 7.3 Hz) (10H, $(CH_2)_5$), 2.73–2.85 (m, 1H, C_{18}–H), 3.55 (s, 3H, $COOCH_3$), 4.25 (s, 2H, CH_2-thiazole), 5.21 (br.s, 1H, C_{12}–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 203.1 (C=S), 177.8 (C-28), 175.1 (C=O, thiazole), 171.3 (C=O, COON), 170.1 (C-3), 144.1 (C-13), 122.5 (C-12), 52.1 ($COO\underline{C}H_3$), 46.7 (C-17). DEPT: 8 × CH_3, 16 × CH_2, 4 × CH, 40 × C at. Anal. calcd. for $C_{40}H_{60}N_2O_5S_2$: C, 67.38; H, 8.48; N, 3.93; Found: C, 67.17; H, 8.34; N, 4.19%.

*3-(5-Ethylrhodanine-3-acetyloxyimino)-olean-12-en-28-oic acid methyl ester (**IVg**)* Yield 81%, mp 183–185°C. IR (ν, cm^{-1}): 1720 (C=O, $COOCH_3$), 1700 (C=O, thiazole), 1620 (C=O, COON), 1495 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.70 (s, 3H, CH_3), 0.89 (s, 6H, 2 × CH_3), 0.98 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 2.01–2.10 (m, 2H, C$\underline{H_2}$CH-thiazole), 2.77–2.87 (m, 1H, C_{18}–H), 3.55 (s, 3H, $COOCH_3$), 4.79–4.93 (m, 3H, C$\underline{H_2}$COO, EtC$\underline{H}$-thiazole), 5.18–5.24 (m, 1H, C_{12}–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 202.6 (C=S), 177.8 (C-28), 176.7 (C=O, thiazole), 176.6 (C=O, COON), 165.5 (C-3), 144.2 (C-13), 122.3 (C-12), 52.1 (C-31), 46.7 (C-17). DEPT: 9 × CH_3, 12 × CH_2, 5 × CH, 38 × C at. Anal. calcd. for $C_{38}H_{56}N_2O_5S_2$: C, 66.63; H, 8.24; N, 4.09; Found: C, 66.78; H, 8.32; N, 4.25%.

*3-[5-(4-Methoxyphenylmethylidene)-rhodanine-3-acetyloxyimino)-olean-12-en-28-oic acid methyl ester (**IVi**)* Yield 95%, mp 102–109°C. IR (ν, cm^{-1}): 1725 (C=O, $COOCH_3$), 1695 (C=O, thiazole), 1620 (C=O, COON), 1500 (C=S), 1270 (Ar-OCH_3). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.72 (s, 3H, CH_3), 0.90 (s, 6H, 2 × CH_3), 0.98 (s, 3H, CH_3), 1.11 (s, 6H, 2 × CH_3), 1.20 (s, 3H, CH_3), 2.77–2.87 (m, 1H, C_{18}–H), 3.57 (s, 3H, $COOCH_3$), 3.87 (s, 3H, CH_3O), 5.05 (s, 2H, NCH_2COO), 5.21 (s, 1H, C_{12}–H), 7.14 (d, J = 8.3 Hz, 2H, Ar), 7.64 (d, J = 8.3 Hz, 2H, Ar), 7.81 (s, 1H, Ar–C$\underline{H}$). ^{13}C NMR (100 MHz,

DMSO-d_6) δ: 193.6 (C=S), 177.8 (C-28), 176.9 (C=O, thiazole), 167.0 (C=O), 165,6 (C-3), 144.1 (C-13), 162.5, 133.9, 126.0, 115.9 (Ar), 122.3 (C-12), 135.2 (Ar–CH=), 118.9 (Ar–CH=C), 52.1 ($COO\underline{C}H_3$), 46.7 (C-17). DEPT: 9 × CH_3, 11 × CH_2, 9 × CH, 44 × C at. Anal. calcd. for $C_{44}H_{58}N_2O_6S_2$: C, 68.18; H, 7.54; N, 3.61; Found: C, 68.02; H, 7.72; N, 3.84%.

*3-[5-(4-(N,N-Dimethyl)aminophenylmethylidene)-rhodanine-3-acetyloxyimino)-olean-12-en-28-oic acid methyl ester (**IVj**)* Yield 89%, mp 128–130°C. IR (ν, cm^{-1}): 1715 (C=O, $COOCH_3$), 1700 (C=O, thiazole), 1630 (C=O, COON), 1505 (C=S), 1280 (Ar). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.68 (s, 3H, CH_3), 0.90 (br.s, 9H, 3 × CH_3), 1.07 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 2.70–2.81 (m, 1H, C_{18}–H), 2.85–2.92 (m, 2H, CH_2, $N\underline{CH_2}CH_2$), 3.07 (s, 6H, $N(CH_3)_2$), 3.56 (s, 3H, $COOCH_3$), 4.31–4.37 (m, 2H, CH_2 $NCH_2\underline{CH_2}$), 5,13 (br.s, 1H, C_{12}–H), 6.85 (d, J = 8.7 Hz, 2H, Ar), 7.47 (d, J = 8.7 Hz, 2H, Ar), 7.68 (s, 1H, ArCH=). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 192.9 (C=S), 177.6 (C-28), 175.1 (C=O, thiazole), 168.8 (C=O, COON), 167,2 (C-3), 144.1 (C-13), 152.5, 133.7, 120.4, 112.7 (Ar), 122.2 (C-12), 135.3 (Ar–CH=), 114.7 (Ar–CH=C), 51.9 ($COO\underline{C}H_3$), 46.7 (C-17). DEPT: 10 × CH_3, 11 × CH_2, 9 × CH, 46 × C at. Anal. calcd. for $C_{46}H_{63}N_3O_5S_2$: C, 68.88; H, 7.92; N, 5.24; Found: C, 69.00; H, 8.00; N, 5.35%.

*3-(2,4-Thiazolidinedione-5-acetyloxyimino)-olean-12-en-28-oic acid (**IVk**)* Yield 88%, mp 161–163°C. IR (ν, cm^{-1}): 3440 (OH, COOH and NH), 1700 (C=O, thiazole), 1685 (C=O, thiazole), 1670 (C=O, COOH), 1620 (C=O, COON). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.76 (s, 3H, CH_3), 0.88 (s, 6H, 2 × CH_3), 0.98 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 2.74–2.86 (m, 1H, C_{18}–H), 2.34–2.44 (m, 1H, $\underline{CH_2}$CH-thiazole), 3.24–3.31 (m, 1H, $\underline{CH_2}$CH-thiazole), 4.74–4.81 (m, 1H, $CH_2\underline{CH}$-thiazole), 5.15–5.21 (m, 1H, C_{12}–H), 12.10 (br.s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 179.0 (C-28), 176.0 (C=O, NH$\underline{C}$OCH-thiazole), 172.7 (C=O, COON), 169.1 (C=O, S$\underline{C}$ONH-thiazole), 163.8 (C-3), 144.4 (C-13), 121.8 (C-12), 47.0 (C-17). DEPT: 7 × CH_3, 11 × CH_2, 5 × CH, 35 × C at. Anal. calcd. for $C_{35}H_{50}N_2O_6S$: C, 67.06; H, 8.04; N, 4.47; Found: C, 66.85; H, 8.23; N, 4.60%.

*3-(2,4-Thiazolidinedione-5-acetyloxyimino)-olean-12-en-28-oic acid methyl ester (**IVl**)* Yield 88%, mp 172–174°C. IR (ν, cm^{-1}): 3420 (NH), 1720 (C=O, $COOCH_3$), 1695 (C=O, thiazole), 1680 (C=O, thiazole), 1620 (C=O, COON). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.70 (s, 3H, CH_3), 0.88 (s, 6H, 2 × CH_3), 0.98 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 2.75–2.87 (m, 1H, C_{18}–H), 3.24–3.30 (m, 1H, $C\underline{H_2}$CH-thiazole), 3.54–3.62 (m, 1H, $C\underline{H_2}$CH-thiazole), 4.77 (dd, J = 4.3, 7.2 Hz, 1H, $CH_2\underline{CH}$-thiazole), 3.57 (s, 3H, $COOCH_3$), 5.17–5.24 (m, 1H, C_{12}–H), 12.1 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 177.6 (C-28), 176.0 (C=O, NH$\underline{C}$OCH-thiazole), 175.8 (C=O, COON), 172.3 (C=O, S$\underline{C}$ONH-thiazole), 169.0 (C-3), 144.0 (C-13), 122.1 (C-12), 51.9 ($COO\underline{C}H_3$), 46.8 (C-17). DEPT: 7 × CH_3, 10 × CH_2, 5 × CH, 36 × C at. Anal. calcd. for $C_{36}H_{52}N_2O_6S$: C, 67.47; H, 8.18; N, 4.37; Found: C, 67.38; H, 8.09; N, 4.22%.

*3-[(2,4-Thiazolidinedione-5-ylidene)-carboxylimino]olean-12-en-28-oic acid methyl ester (**IVm**)* Yield 96%; mp 134–138°C. IR (ν, cm^{-1}): 3430 (NH), 1720 (C=O, CO-OCH_3), 1700 (C=O, thiazole), 1680 (C=O, thiazole), 1625 (C=O, COON). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.70 (s, 3H, CH_3), 0.88 (s, 6H, 2 × CH_3), 0.99 (s, 3H, CH_3), 1.10 (s, 6H, 2 × CH_3), 1.20 (s, 3H, CH_3), 2.72–2.95 (m, 1H, C_{18}–H), 3.55 (s, 3H, $COOCH_3$), 5.21 (br.s, 1H, C_{12}–H), 6.97 (s, 1H,=CHCOO). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 177.6 (C-28), 177.0 (C=O, NH$\underline{C}$OCH-thiazole), 169.9 (C=O, COON), 166.8 (C=O, S$\underline{C}$ONH-thiazole), 164.15 (C-3), 144.4 (C=$\underline{C}$H-thiazole), 144.0 (C-13), 122.2 (C-12), 115.7 (=$\underline{C}$H-COON), 51.9 ($COO\underline{C}H_3$), 46.9 (C-17). DEPT: 8 × CH_3, 10 × CH_2, 5 × CH, 36 × C at. Anal. calcd. for $C_{36}H_{50}N_2O_6S$: C, 68.47; H, 7.89; N, 4.38; Found: C, 67.54; H, 8.05; N, 4.48%.

*3-(2,4-Thiazolidinedione-5-acetyloxyimino)-olean-12-en-28-oic acid morpholide (**IVn**)* Yield 86%, mp 195–197°C. IR (ν, cm^{-1}): 3400 (NH), 1705 (C=O, thiazole), 1695 (C=O, thiazole), 1630 (C=O, amide), 1620 (C=O, COON), 990 (C–O, morph.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.70 (s, 3H, CH_3), 0.88 (s, 6H, 2 × CH_3), 0.97 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 2.80–2.90 (m, 1H, C_{18}–H), 2.93–3.03 (m, 1H, $C\underline{H_2}$CH-thiazole), 3.25–3.31 (m, 1H, CH_2CH-thiazole), 4.77 (dd, J = 4.7, 7.2 Hz, 1H, $CH_2\underline{CH}$-thiazole), 3.46–3.60 (m, 8H, morph.), 5.09–5.14 (m, 1H, C_{12}–H), 12.10 (br.s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 176.2 (C-28), 176.0 (C=O, NH$\underline{C}$OCH-thiazole), 174.7 (C=O, COON), 173.0 (C=O, S$\underline{C}$ONH-thiazole), 169.3 (C-3), 145.5 (C-13), 121.2 (C-12), 67.0, 47.3 (C-morph.), 47.0 (C-17). DEPT: 7 × CH_3, 15 × CH_2, 5 × CH, 39 × C at. Calcd. for $C_{39}H_{57}N_3O_6S$: C, 67.31; H, 8.26; N, 6.04; Found: C, 67.54; H, 8.36; N, 6.21%.

*3-[(2,4-thiazolidinedione-5-ylidene)-carboxylimino]olean-12-en-28-oic acid morpholide (**IVo**)* Yield 85%, mp 164–166°C. IR (ν, cm^{-1}): 3449 (NH), 1710 (C=O, thiazole), 1695 (C=O, thiazole), 1630, 1620 (2 × C=O, COON and amide), 990 (C–O, morph.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.70 (s, 3H, CH_3), 0.87 (s, 6H, 2 × CH_3), 0.98 (s, 3H, CH_3), 1.10 (s, 6H, 2 × CH_3), 1.19 (s, 3H, CH_3), 2.43 (m, 1H, C_2–H), 2.89–3.00 (m, 1H, C_{18}–H),

3.46–3.60 (m, 8H, morph.), 5.08–5.14 (m, 1H, C_{12}–H), 7.03 (s, 1H, =CHCOO). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 177.1 (C-28), 176.8 (C=O, NHCOCH-thiazole), 175.2 (C=O, COON), 174.1 (C=O, SCONH-thiazole), 169.6 (C-3), 145.7 (CH=C-thiazole), 144.9 (C-13), 121.2 (C-12), 116.0 (=CH–COON), 67.0, 46.6 (C-morph.), 47.1 (C-17). DEPT: 7 × CH_3, 14 × CH_2, 5 × CH, 39 × C at. Anal. calcd. for $C_{39}H_{55}N_3O_6S$: C, 67.31; H, 8.26; N, 6.04; Found: C, 67.54; H, 8.36; N, 6.21%.

*12α-Bromo-3-(2,4-thiazolidinedione-5-acetyloxyimino)-olean-28β → 13β olide (**IVp**)* Yield 83%, mp 152–154°C. IR (ν, cm^{-1}): 1760 (C=O, lacton) 1710 (C=O, thiazole), 1690 (C=O, thiazole), 1635 (C=O, COON). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.87 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 2.72–2.82 (m, 1H, C_{18}–H), 3.26–3.30, 3.57–3.59, 4.54–4.58 (3 × m, 3H, CH_2CH-thiazole), 4.76–4.82 (m, 1H, C_{12}–H), 12.10 (br.s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 178.3 (C-28), 176.1 (C=O, NHCOCH-thiazole), 172.9 (C=O, COON), 172.2 (C=O, SCONH-thiazole), 169.2 (C-3), 91.5 (C-13), 57.2 (C-12), 45.5 (C-17). DEPT: 7 × CH_3, 11 × CH_2, 5 × CH, 35 × C at. Anal. calcd. for $C_{35}H_{49}BrN_2O_6S$: C, 59.57; H, 7.00; N, 3.97; Found: C, 59.74; H, 7.18; N, 4.10%.

*12α-Bromo-3-[(2,4-thiazolidinedione-5-ylidene)-carboxylimino]olean-28β → 3β olide (**IVq**)* Yield 87%, mp 183–185°C. IR (ν, cm^{-1}): 1755 (C=O, lacton) 1700 (C=O, thiazole), 1690 (C=O, thiazole), 1635 (C=O, COON). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.88 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 2.37–2.45 (m, 1H, C_{18}–H), 4.54–4.60 (br.s, 1H, C_{12}–H), 7.02 (s, 1H, =CH-thiazole) 12.33 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 178.3 (C-28), 174.8 (C=O, NHCOCH-thiazole), 173.6 (C=O, COON), 171.5 (C=O, SCONH-thiazole), 170.2 (C-3), 155.7 (CH=C-thiazole), 119.6 (=CH–COON), 91.6 (C-13), 57.4 (C-12), 45.4 (C-17). DEPT: 7 × CH_3, 10 × CH_2, 5 × CH, 35 × C at. Anal. calcd. for $C_{35}H_{47}BrN_2O_6S$: C, 59.74; H, 6.73; N, 3.98; Found: C, 58.00; H, 6.98; N, 4.12%.

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