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Original Article

SYNTHESIS AND CHARACTERIZATION OF NEW IMINE AND PTHALIC ACID DERIVATIVES OF URSOLIC ACID

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ABSTRACT

Objective: The current work envisages synthesis of novel ursolic acid derivatives and characterization by spectral methods that can be possible candidates for anti-inflammatory and anticancer activity.

Methods: A series of imine and phthalic acid derivatives of ursolic acid (3β-hydroxyurs-12-en-28-oic acid), have been synthesized. 3-hydroxyimino-urs-12-en-28-olic acid was treated with alkyl halide in the presence of sodium hydride in ethanol to yield 3-alkyloxyimino-urs-12-en-28-oic acid and further converted to its ester derivatives. Ursolic acid was reacted with phthalic anhydride in pyridine to get mono and di-substituted ester derivatives.

Results: Novel substituted imino and pthalic derivatives were synthesized. The compounds synthesized were characterized by MS, IR, ^{1}H and [13]C-NMR spectroscopy.

Conclusion: The derivatives prepared may facilitate designing of similar newer analogues which may be useful for generating possible candidates from ursolic acid for anti-imflammatory and anti-cancer potential. Ursolic acid oximes and its anhydrides exhibit valuable biological properties and are important starting materials for further transformations.

Keywords: Ursolic acid, Alkyl halide, Nucleophilic substitution, Anti-cancer.

INTRODUCTION

Natural triterpenoids isolated from various medicinal plants now seem to have a prominent role in the chemo-prevention and therapy of a variety of ailments and some have already entered clinical trials. One such important and highly investigated pentacyclic triterpenoid, ursolic acid has attracted great attention of late for its potential as a chemopreventive and chemotherapeutic agent in various types of cancer [1]. Ursolic acid sometimes referred as urson, malol, or 3-β-hydroxy-urs-12-ene-28-oic-acid, is a pentacyclic triterpenoid present in *Nerium indicum* leaf, a common indigenous plant of India. Ursolic acid is present in 1.7- 2.0% concentration in leaf. A simple method to isolate this phytoconstituent has been devised by authors in previous communication [2]. Derivatives of ursolic acid can also be used in cosmetics because triterpenoids transport biologically active compounds deep into tissue and make them more potent [3].

Ursolic acid is known to possess a host of biological activities besides also used in cosmetic industry. Ursolic acid exhibits diverse pharmacological activities, amongst which the anticancer and anti-inflammatory activity has been most exhaustively studied. It inhibits NF-κB activation in both human intestinal epithelial cells and macrophages, and attenuates experimental murine colitis suggesting a potential therapeutic agent for inflammatory bowel disease [4]. Ursolic acid is reported to cause DNA fragmentation, activate caspases and down regulates expression of Bcl-2 in hepatocellular cancer cells [5]. It also induced differentiation of U937 cells by activating the PI3K/Akt pathway, and could be a potential candidate as a differentiation-inducing agent for the therapy of leukemia [6].

Considering the immense anti-cancer potential of ursolic acid an extensive study has been done for synthesis of derivatives of this molecule. A regioselective approach using Huisgen 1,3-dipolar cycloaddition reaction of ursolic acid-alkyne derivative with various aromatic azides was employed to target an array of triazolyl derivatives against MCF-7, HCT-116, FR-2and THP-1 human epithelial cell line [7]. Chemical modification of this scaffold by an isopropyl ester moiety at C-17-COOH and a succinyl moiety at C-3-OH showed potent inhibitory effect on growth of NTUB1 cells suggesting that the presentation of G1 phase arrest and apoptosis mediated through increased amount of ROS in cells [8]. An α,β unsaturated ketone in conjugation with a heterocyclic ring at the ring 3-OH has improved antiproliferative activities against AsPC-1 pancreatic cancer cells by arresting cell cycle in G1 phase and inducing apoptosis with upregulation of p53, p21waf1 and NOXA protein levels [9]. Structural activity relationship (SAR) reveals the C-3, C-28 and C-11 positions of ursolic acid important with respect to the cytotoxic potential. Introduction of an amino group increases the cytotoxicity greatly while 3β-amino increased the potency by several manifold than the parent ursolic acid [10]. The derivatives with a substituted acetyl group at C3 hydroxyl group show better activities than those with an unsubstituted hydroxyl group against Hela cell line [11].

3-hydroxyiminours-12-en-28-oic acid **(A)** and 3-[[2-(hydroxyl carbonyl) benzoyl] oxy] urs-12-en-28-oic acid **(D)** has been reported as key intermediates for the synthesis of different variety of ursolic acid derivatives. In view of the reported SAR considerations the currenty work envisages partial synthesis of imine and phthalic anhydride derivatives of ursolic acid. The derivatives prepared may act as new potential agents for the anti-cancer and anti-inflammatory activity.

MATERIALS AND METHODS

All reagents used were of analytical grade and purchased from S. D. Fine Chemicals, Mumbai. Isolation of ursolic acid was done by extracting leaves of *N. Indicum* with methanol and the extract was basified to separate the triterpinoid, followed by charcoal treatment and subsequent acidification method published previously by the authors. The course of reaction and purity of product was monitored by TLC on Merck 60 F_{254} silica plates using the mobile phase of ethyl acetate: ether (2:8) and observation under UV light (254 nm). IR spectrum was recorded on Perkin Elmer Spectrum10 Mass spectrum of compounds was recorded on Micromass Q-TOF MS mass spectrometer. All ^{1}H NMR and [13]C spectras was recorded on JOEL 300 MHz and 75 MHz instrument respectively, with an internal standard of tetramethylsilane (TMS).

Chemical synthesis

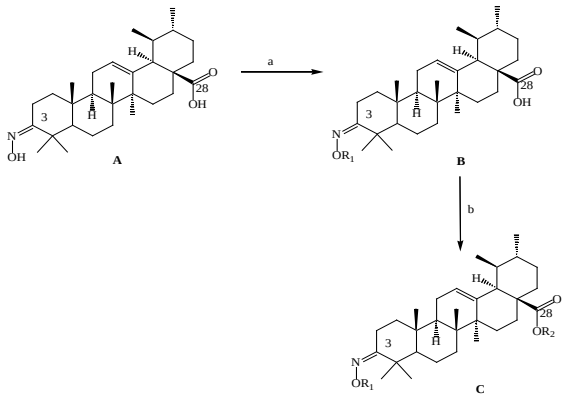
The reaction scheme for substituted derivatives of 3-hydroxyimino-urs-12-en-28-oic acid (Table 1) has been summarized in Scheme-I. The reaction scheme for the substituted derivatives of 3-[[2-(hydroxycarbonyl) benzoyl] oxy] urs-12-en-28-oic acid (Table 2) has been summarized in Scheme-II.

General method for synthesis of 3-alkyloxyimino-urs-12-en-28-oic acid (B) 1-5 compounds (Table 1)

3-hydroxyiminours-12-en-28-oic acid (A) (200 mg, 0.43 mmol) was added in (5 ml) tetrahydrofuran (THF) at 0 - 5°C. The solution was cooled and to it sodium hydride (NaH) (15.48 mg, 0.64 M) was added and stirred for 0.5 hr. Then alkyl substrate was then added to the reaction. The reaction was monitored by TLC to check its completion. The reaction was quenched with water and extracted with ethyl acetate (2 × 20 ml). The product was purified by column chromatography using pet-ether: ethyl acetate (8:2) as eluent and gave white color solid (B) with the good yield.

General method for synthesis 3-alkyloxyiminours-12-en-28-oic acid alkyl ester (C) compounds 6-10 (Table 1)

To a stirred solution of 3-alkyloxyiminours-12-en-28-oic acid (B) (100 mg, 0.20 mmol), potassium carbonate (33.17 mg, 0.24 mmol), 5 ml ethanol was added and refluxed at 65-67°C for 8 hr. Thereafter alkyl substrate was added dropwise, and the reaction was stirred till complete. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was concentrated to remove ethanol and was quenched with water. The product was extracted with ethyl actate (1 × 10 ml) and washed with brine water, dried over sodium sulphate and concentrated. The product was purified by silica gel column chromatography using pet-ether: ethyl acetate (70:30) to give colorless solid product (C).



Scheme I: Synthesis of 3-alkyloxyimino-urs-12-en-28-oic acid (B) and 3-alkyloxyiminours-12-en-28-oic acid alkyl ester (C) [(a) THF, NaH, alkyl substrate 0-5°C; (b) ethanol, K2CO3, alkyl substrate 65-67°C].

Table 1: Substituted imino derivatives of 3-alkyloxyimino-urs-12-en-28-oic acid (B) and 3-alkyloxyiminours-12-en-28-oic acid alkyl ester (C).

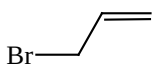
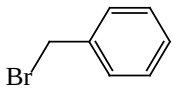
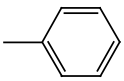
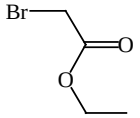
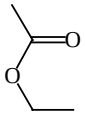
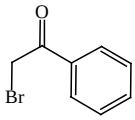
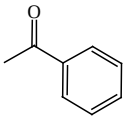
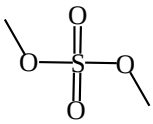
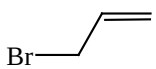
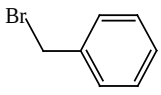
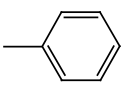
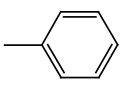
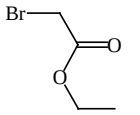
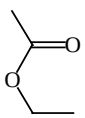
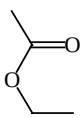
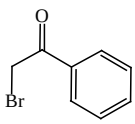
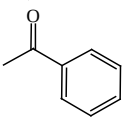
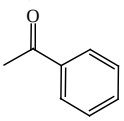
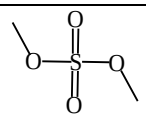
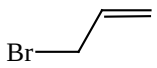
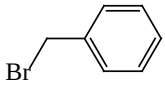
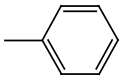
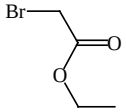
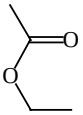
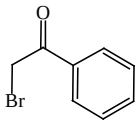
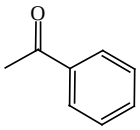
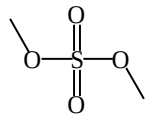
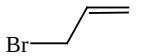
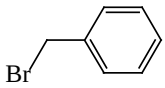
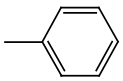
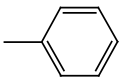
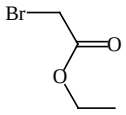
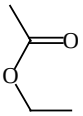
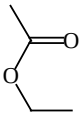
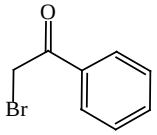
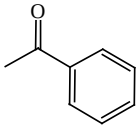
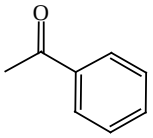
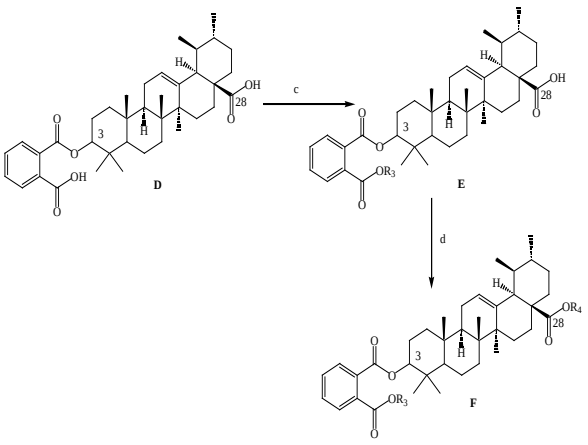
S. No.	Alkyl substrate R_1X/R_2X	R_1	R_2	Alkyl substrate (mg/mmol)	Derivatives B [1-5] C [6-10]	Reaction time
1		CH_3	H	109.86/ 0.77	3-methoxyiminours-12-en-28-oic acid	20
2			H	78.03/ 0.64	3-allyloxyiminours-12-en-28-oic acid 2	15
3			H	132/ 0.77	3-benzyloxyiminours-12-en-28-oic acid	17
4			H	143.62/ 0.86	3-[(2-ethoxy-2-oxoethoxy)imino]-urs-12-en-28-oic acid 4	10
5			H	171.17/ 0.86	3-[(2-oxo-2-phenylethoxy)imino]-urs-12-en-28-oic acid 5	28
6		CH_3	CH_3	26.4/ 0.21	3-Methoxyiminours-12-en-28-oic acid methyl ester	18
7				36.29/ 0.30	3-allyloxyiminours-12-en-28-oic acid allyl ester	15
8				55.41/ 0.32	3-benzyloxyiminours-12-en-28-oic acid benzyl ester	28
9				45.07/ 0.26	3-((2-ethoxy-2-oxoethoxy)imino)-urs-12-en-28-oic acid (2-ethoxy-2-oxoethyl) ester	20
10				67.00/ 0.34	3-((2-oxo-2-phenylethoxy)imino)-urs-12-en-28-oic acid (2-oxo-2-phenylethyl) ester	32

Table 2: Pthalic acid derivatives of 3-[[2-(alkyloxycarbonyl)benzoyl]oxy]urs-12-en-28-oic acid (E) and 3-[[2-(alkyloxycarbonyl)benzoyl]oxy]urs-12-en-28-oic acid alkyl ester (F)

S. No.	Alkyl substrate R_1X/R_2X	R_1	R_2	Alkyl substrate mg/mmol	Derivatives E [11-15] F [16-20]	Reaction time
11		CH_3	H	26.49/ 0.21	3-[[2-(methoxycarbonyl)benzoyl]oxy]urs-12-en-28-oic acid	20
12			H	36.29/ 0.30	3-[[2-[(allyloxy)carbonyl]benzoyl]oxy]urs-12-en-28-oic acid	17
13			H	55.41/ 0.32	3-[[2-[(benzyloxy)carbonyl]benzoyl]oxy]urs-12-en-28-oic acid	25
14			H	45.07/ 0.26	3-[[2-[(2-ethoxy-2-oxoethoxy)carbonyl]benzoyl]oxy]urs-12-en-28-oic acid	15
15			H	67.00/ 0.34	3-[[2-[(2-oxo-2-phenylethoxy)carbonyl]benzoyl]oxy]urs-12-en-28-oic acid	28
16		CH_3	CH_3	14.26/ 0.70	3-[[2-(methoxycarbonyl) benzoyl]oxy]urs-12-en-28-oic acid methyl ester	22
17				27.91/ 0.23	3-((2-((allyloxy)carbonyl) benzoyl)oxy)urs-12-en-28-oic acid allyl ester	25
18				49.22/ 0.28	3-[[2[(benzyloxy)carbonyl] benzoyl]oxy]urs-12-en-28-oic acid benzyl ester	27
19				60.03/ 0.36	3-[[2-[(2-ethoxy-2-oxoethoxy)carbonyl]benzoyl]oxy]urs-12-en-28-oic acid [2-ethoxy-2-oxoethyl] ester	20
20				77.44/ 0.38	3-[(2-oxo-2-phenylethoxy) carbonyl]urs-12-en-28-oic acid-[2-oxo-2-phenylethyl] ester	30



Scheme II: Synthesis of 3-[[2-(alkyloxycarbonyl)benzoyl] oxy]urs-12-en-28-oic acid (E) and 3-[[2-(alkyloxycarbonyl) benzoyl]oxy]urs-12-en-28-oic acid alkyl ester (F) [(c) ethanol, K_2CO_3, alkyl substrate 55-60^{0}C; (d) ethanol, K_2CO_3, alkyl substrate 65 -67^{0}C].

General method for synthesis of 3-[[2-(alkyloxycarbonyl) benzoyl] oxy]-urs-12-en-28-oic acid (E) compounds 11-15 (Table 2)

To a solution of 3-[[2-(hydroxycarbonyl) benzoyl] oxy] urs-12-en-28-oic acid (D) (200 mg, 0.33 mmol) and potassium carbonate (31.99 mg, 0.23 mmol) was added into 10 ml ethanol and refluxed for 8 hrs. Alkyl substrate was added and mixture was stirred at 55-60°C till completion of reaction. After completion, the reaction was quenched with ice at room temperature and extracted with ethyl acetate (2× 20 ml). The combined organic layer was washed with brine, dried over sodium sulphate and the solvent evaporated to dryness. The crude product was purified by column chromatography using pet-ether: ethyl acetate (80:20) as mobile phase to give off white product (E).

General method for synthesis 3-[[2-(alkyloxycarbonyl) benzoyl]oxy]urs-12-en-28-oic acid alkyl ester (F) compunds 16-20 (Table 2)

3-[[2-(alkyloxycarbonyl)benzoyl]oxy]urs-12-en-28-oic acid (E) and potassium carbonate (15.99 mg, 0.11 mmol) was added into 5 mL ethanol and refluxed for 8-10 hrs to make potassium salt and water was removed by Dean Stark apparatus. Alkyl substrate was added

and mixture was stirred at 65-67°C till reaction was complete. After completion (monitored by TLC), the reaction was quenched with ice at room temperature, extracted with ethyl acetate (2× 20 ml), the combined organic layers washed with brine, dried over sodium sulphate and the solvent evaporated to dryness. The crude product was purified by column chromatography using pet-ether: ethyl acetate (80:20) to give off white product (F).

3-methoxyiminours-12-en-28-oic acid (1)

Yield: 63%, m. p. 120-122°C. IR (KBr): 3423, 1680 cm^{-1}; ^1H NMR (300 MHz, $CHCl_3$): δ 3.8 (s, 3H, OCH_3, H-R_1), 5.2 (s, 1H, alkene proton); [13]C NMR (75 MHz $CHCl_3$): δ 58 (-OCH_3), 125 and 135 (alkene group carbons), 165 (acid group carbon). MS: *m/z* 483.50 $[M]^+$, calcd for $C_{31}H_{49}NO_3$ (483.73).

3-allyloxyiminours-12-en-28-oic acid (**2**)

Yield: 64%, m. p. 111-113°C. IR (KBr, cm^{-1}): 3420 (-O-H of acidic group), 1685 (-C=O of acidic group); ^1H NMR (300 MHz, $CHCl_3$): δ 3.8 (d, 2H, -OCH_2 allylic protons, H-R_1), 5.1, 5.7, 5.8 (m, 3H, alkene protons, H-R_1); [13]C NMR (75 MHz, $CHCl_3$): δ 65 (-OCH_2 allylic carbon), 118-130 (alkene group carbon), 170 (acid group carbon); MS: *m/z* 510.60 $[M]^+$, calcd for $C_{33}H_{51}NO_3$ (509.76).

3-benzyloxyiminours-12-en-28-oic acid (**3)**

Yield: 50%. m. p. 125-127°C. IR (KBr cm^{-1}): 3448 (-O-H of acid group), 1700 (-C=O of acid group), 1458-1603 (C=C of aromatic ring). ^1H NMR (300 MHz, $CHCl_3$): δ 3.0 (s, 2H, -CH_2 benzylic proton), δ 7.6-7.8 (m, 5H, corresponds to aromatic protons); [13]C NMR (75 MHz, $CHCl_3$): δ 60 (-OCH_2 benzyl carbon), 125-150 (aromatic carbons), 185 (acid group carbon); MS: *m/z* 559.40 $[M]^+$, calcd for $C_{37}H_{53}NO_3$ (559.82).

3-[(2-ethoxy-2-oxoethoxy) imino]-urs-12-en-28-oic acid (4)

Yield: 57%. m. p. 140-142°C. IR (KBr, cm-1): 3441 (-O-H of acid group), 1670-1730 (-C=O of acid and ester group), 1100-1300 (-O-CH2 of ester group); 1H NMR (300MHz, CHCl3): δ 4.2 (t, 3H, CH3-CH2, H-R1), 4.5 (s, 2H, -OCH2-C=O, H-R1), 4.8 (q, 2H, -OCH2-CH3); [13]C NMR (75 MHz, CHCl3): δ 20 (CH3), 60 (O-CH2), 170-178 (acid and ester group carbons); MS: m/z 555.60 [M]+, calcd for C34H53NO5 (555.79).

3-[(2-oxo-2-phenylethoxy) imino]-urs-12-en-28-oic acid (5)

Yield: 60%. m. p. 133-135°C. IR (KBr, cm^{-1}): 3448 (O-H of acid group), 1680-1725 (C=O of acid and ketone group), 1450-1600 (C=C of aromatic ring); ^1H NMR (300MHz, $CHCl_3$): δ 4.7 (s, 2H, -O-CH2-C=O), 7.2-7.8 (m, 5H, aromatic protons); [13]C NMR ($CHCl_3$, 75 MHz): δ 60 (-OCH_2), 125-140 (aromatic carbans), 165, 185 (acid and ester group carbon); MS: *m/z* 587.60 $[M]^+$, calcd for $C_{38}H_{53}NO_4$ (587.83).

3-Methoxyiminours-12-en-28-oic acid methyl ester (6)

Yield: 61%. m. p. 105-106°C. IR (KBr, cm^{-1}): 1720 (-C=O of ester group), 1448 (-C=C of alkene); ^1H NMR (300 MHz, $CHCl_3$): δ 3.8 (s, 3H, -OCH_3, H-R_1), 3.9 (s, 3H, -OCH_3 ester group protons, H-R_2), 5.1 (s, 1H, -CH= alkene proton); [13]C NMR (75 MHz, $CHCl_3$): δ 58-65 (two methoxy group carbons), 125-140 (alkene carbans), 168 (-C=O corresponds to ester carbon); MS: *m/z* 497.20 $[M]^+$, calcd. for $C_{32}H_{51}NO_3$ (497.75).

3-allyloxyiminours-12-en-28-oic acid allyl ester (7)

Yield: 64%. m. p. 112-114°C. IR (KBr, cm^{-1}): 1730 (-C=O of ester group), 1400-1500 (-C=C of allyl group); ^1H NMR (300MHz, $CHCl_3$): δ 2.8 (m, 2H, -OCH_2 allylic protons, H-R_1), 4.7 (m, 2H, -OCH2 allylic methyl group protons attached to ester group, H-R_2), 5.1, 5.7, 5.9, 6.1, 6.3, 6.7 (m, 6H, 2(-CH=CH_2)). [13]C NMR (75 MHz, $CHCl_3$): δ 64 (CH_2), 65 (CH_2), 118, 125, 130, 135 (2(-CH=CH_2)), 165 (C=O); MS: *m/z* 549.60 $[M]^+$, calcd. for $C_{36}H_{55}NO_3$ (549.83).

3-benzyloxyiminours-12-en-28-oic acid benzyl ester (**8**)

Yield: 60%. M. p. 110-115°C. IR (KBr, cm^{-1}): 1735 (C=O), 1500-1600 (aromatic -C=C); ^1H NMR (300 MHz, $CHCl_3$): δ 3.7 (s, 2H, CH_2, H-R_1), 3.9 (s, 2H, CH_2, H-R_2), 7.8 (s 5H, Ar-H, H-R_1), 7.9 (s 5H, Ar-H, H-R_2); [13]C NMR (75 MHz, $CHCl_3$): δ 58 (CH_2), 59 (CH_2), 120, 122, 130, 135, 140, 142, 150, 155 (aromatic carban), 160 (C=O); MS: *m/z* 648.70 $[M]^+$, calcd. for $C_{44}H_{59}NO_3$ (649.93).

3-[(2-ethoxy-2-oxoethoxy) imino]-urs-12-en-28-oic acid [2-ethoxy-2-oxoethyl] ester (9)

Yield: 57%. m. p. 105-107 °C. IR (KBr, cm^{-1}): 1728 (C=O), 1400-1500 (-C=C); ^1H NMR (300 MHz, $CHCl_3$): δ 2.5 (s, 2H, CH_2, H-R_1), 2.8 (s, 2H, CH_2, H-R_2), 3.3 (q, 2H, CH2, H-R_1), 3.8 (q, 2H, CH2, H-R_2), 3.7 (t, 3H, CH_3, H-R_1), 4.4 (t, 3H, CH_3, H-R_2), 5.1 (s, 1H,CH=C); [13]C NMR ($CHCl_3$, 300 MHz): δ 20, 25 (CH_3), 58, 59, 63, 65 (OCH_2), 170 (C=O); MS: *m/z* 641.3 $[M]^+$, calcd. for $C_{38}H_{59}NO_7$ (641.88).

3-[(2-oxo-2-phenylethoxy) imino]-urs-12-en-28-oic acid [2-oxo-2-phenylethyl] ester (10)

Yield: 61%. m. p.: 116-118°C. IR (KBr): 1730 (C=O), 1500-1600 (Ar-C=C); ^1H NMR (300 MHz, $CHCl_3$): δ 3.5 (s, 2H, CH_2, H-R_1), 3.7 (s 2H, CH_2, H-R_2), 5.1 (s, 1H, CH=C), 7.4 (m, 5H, Ar-H, H-R_1), 7.8 (m, 5H, Ar-H, H-R_2); [13]C NMR (75 MHz, $CHCl_3$): δ 57, 58 (CH_2), 115, 120, 118, 125, 138 (Ar-C), 170, 185 (C=O); MS: *m/z* 705.30 (M^+), calcd. for $C_{46}H_{59}NO_5$ (705.96).

3-[[2-(methoxycarbonyl)benzoyl]oxy]urs-12-en-28-oic acid (11)

Yield: 73%. m. p. 150-152°C. IR (KBr, cm^{-1}): 1735 (C=O), 1500-1600 (Ar-C=C), 1448 (C=C); ^1H NMR (300 MHz, $CHCl_3$): δ 3.1 (s, 3H, CH_3, H-R_3), 3.8 (s, 1H, CH), 7.3-7.8 (m, 4H, Ar-H); [13]C NMR (75 MHz, $CHCl_3$): δ 55, 59 (O-CH_3), 125, 140 (Ar-C), 165 (C=O); MS: *m/z* 618.00 $[M]^+$, calcd. for $C_{39}H_{54}O_6$ (618.84).

3-[[2-[(allyloxy) carbonyl]benzoyl]oxy]urs-12-en-28-oic acid (12)

Yield: 61%. m. p. 152-154°C. IR (KBr, cm^{-1}): 1740 (C=O), 1500-1600 (Ar-C=C), 1400-1500 (C=C); ^1H NMR (300 MHz, $CHCl_3$): δ 5.5, 5.7, 5.9 (m, 3H, OCH=CH_2, H-R_3), 7.3-7.9 (m, 4H, Ar-H); [13]C NMR (75 MHz, $CHCl_3$): δ 62 (OCH_2), 118, 130, 135, 139 (-CH=CH_2), 170 (C=O); MS: *m/z* 644.40 $[M]^+$, calcd. for $C_{41}H_{56}O_6$ (644.88).

3-[[2-[(benzyloxy) carbonyl] benzoyl] oxy] urs-12-en-28-oic acid (13)

Yield: 61%. m. p. 161-163°C. IR (KBr, cm^{-1}): 1730 (C=O), 1500-1600 (Ar-C=C); ^1H NMR (300MHz, $CHCl_3$): δ 4.0 (s, 2H, CH_2, H-R_3), 7.2-7.5 (m, 4H, Ar-H), 7.9 (s, 5H, Ar-H, H-R_3); [13]C NMR (75 MHz, $CHCl_3$): δ 55 (O-CH_3), 59 (O-CH_3), 120, 125, 130, 140, 150 (Ar-C), 185(C=O); MS: *m/z* 693.90 $[M]^+$, calcd. for $C_{45}H_{58}O_6$ (694.94).

3-[[2-[(2-ethoxy-2-oxoethoxy) carbonyl] benzoyl] oxy] urs-12-en-28-oic acid (**14)**

Yield: 65%. m. p. 155-157°C. IR (KBr, cm^{-1}): 1725 (C=O), 1500-1600 (Ar-C=C) cm^{-1}, 1400-1500 (C=C); ^1H NMR (300MHz, $CHCl_3$): δ 4.2 (t, 3H, CH_3, H-R_3), 4.4 (q, 2H, CH_2, H-R_3), 7.5, 79 (m, 4H, Ar-H); [13]C NMR (75 MHz, $CHCl_3$): δ 30 (-CH_3), 55 (O-CH_2), 62 (O-CH_2), 140 (Ar-C), 177 (C=O); MS: *m/z* 690.70 $[M]^+$, calcd. for $C_{42}H_{58}O_8$ (690.91).

3-[[2-[(2-oxo-2-phenylethoxy) carbonyl] benzoyl] oxy] urs-12-en-28-oic acid (15)

Yield: 54%. m. p. 142-144°C. IR (KBr, cm^{-1}): 1732 (C=O), 1500-1600 (Ar-C=C), 1400-1500 (C=C). ^1H NMR (300 MHz, $CHCl_3$): δ 4.1 (s, 2H, CH_2, H-R_3), 7.2-7.9 (m, 4H, Ar-H), 8.0 (m, 5H, Ar-H, H-R_3); [13]C NMR (75 MHz, $CHCl_3$): δ 55 (O-CH_2), 121, 138, 145 (Ar-C), 165, 185 (C=O); MS: *m/z* 722.30 $[M]^+$, calcd. for $C_{46}H_{58}O_7$ (722.30).

3-[[2-(methoxycarbonyl)benzoyl]oxy]urs-12-en-28-oic acid methyl ester (16)

Yield: 72%. m. p. 130-132°C. IR (KBr, cm-1): 1725 (C=O), 1500-1600 (Ar-C=C) cm-1, 1400-1500 (C=C); 1H NMR (300MHz, CHCl3): δ 4.2 (s, 3H, CH3, H-R3), 4.4 (s 3H, CH3, H-R4), 7.3-7.5 (m, 4H, Ar-H); [13]C NMR (75 MHz, CHCl3): δ 55 (OCH3), 59 (OCH3), 122, 125, 130, 135 (Ar-C), 165, 170, 180 (C=O); MS: m/z 632.70 [M]+, calcd. for C40H56O6 (632.87).

3-[[2-[(allyloxy) carbonyl] benzoyl] oxy] urs-12-en-28-oic acid allyl ester (17)

Yield: 64%. m. p. 132-134°C. IR (KBr, cm^{-1}): 1730 (C=O), 1500-1600 (Ar-C=C), 1400-1500 (C=C); ^1H NMR (300 MHz, $CHCl_3$): δ 5.1 (s, 1H,

CH=C, H-R_3), 5.3 (s, 1H, CH=C, H-R_4), 5.5-6.1 (m, 3H, CH=CH_2, H-R_3), 7.2-7.9 (m, 4H, Ar-H, H-R_4); [13]C NMR (75 MHz, $CHCl_3$): δ 65 (O-CH_2), 70 (O-CH_2), 110, 125, 130, 140 (Ar-C), 161, 170 (C=O); MS: *m/z* 684.30 $[M]^+$, calcd. for $C_{44}H_{60}O_6$ (684.94).

3-[[2-[(benzyloxy) carbonyl] benzoyl] oxy]urs-12-en-28-oic acid benzyl ester [18]

Yield: 54%. m. p. 121-123°C. IR (KBr, cm^{-1}): 1735 (C=O), 1500-1600 (Ar-C),1400 (C=C); ^{1}H NMR (300 MHz, $CHCl_3$): δ 3.8 (s, 1H, OCH_2, H-R_3), 4.1 (s, 1H, OCH_2, H-R_4), 7.1-7.3 (m, 4H, Ar-H), 7.5 (s 5H Ar-H, H-R_3), 8.1 (s, 5H, Ar-H, H-R_4); [13]C NMR (75 MHz, $CHCl_3$): δ 62 (O-CH_2), 65 (O-CH_2), 125, 130, 135, 140, 145 (Ar-C), 168, 180, 185 (C=O); MS: *m/z* 784.40 $[M]^+$, calcd. for $C_{52}H_{64}O_6$ (785.06).

3-[[2-[(2-ethoxy-2-oxoethoxy) carbonyl]benzoyl]oxy]urs-12-en-28-oic acid [2-ethoxy-2-oxoethyl] ester (19)

Yield: 62%. M. p.121-123°C. IR (KBr, cm^{-1}): 1740 (C=O), 1500-1600 (Ar-C), 1450 (C=C); ^{1}H NMR (300 MHz, $CHCl_3$): δ 3.6 (t, 3H, CH_3, H-R_3), 3.8(s, 2H, OCH_2, H-R_3), 4.1 (q, 2H, OCH_2, H-R_3), 4.2 (t, 3H, CH_3, H-R_4), 4.3 (s, 2H, OCH_2, H-R_4), 4.4 (q, 2H, OCH_2, H-R_4); [13]C NMR (75 MHz, $CHCl_3$): δ 15, 22 (CH_3), 55, 59, 60, 68 (OCH_2) 122, 125, 125, 130, 135 (Ar-C), 175, 180, 185 (C=O); MS: *m/z* 776.60 $[M]^+$, calcd. for $C_{46}H_{64}O_{10}$ (776.99).

3-[(2-oxo-2-phenylethoxy) carbonyl] urs-12-en-28-oic acid-[2-oxo-2-phenylethyl] ester (20)

Yield: 59%. m. p. 135-137°C. IR (KBr, cm^{-1}): 1730 (C=O), 1500-1600 (aromatic carban), 1400-1500 (C=C); ^{1}H NMR (300MHz, $CHCl_3$): δ 4.5 (s, 2H, OCH_2, H-R_3), 4.8 (s, 2H, OCH_2, H-R_4), 7.1- 7.8 (m, 4H, Ar-H), 8.0 (s, 5H, Ar-H, H-R_3), 8.2 (s, 5H, Ar-H, H-R_4); [13]C NMR (75 MHz, $CHCl_3$): δ 45, 50 (OCH_2), 110, 120, 125, 135 (Ar-C), 165, 180,185 (C=O); MS: *m/z* 840.60 $[M]^+$, calcd. for $C_{54}H_{64}O_8$ (841.08).

RESULTS AND DISCUSSION

The aim of the present work was to study the reactivity of **A** and **D** towards the wide variety of substituted imine and substituted phthalic acid derivatives of ursolic acid and ursolic acid esters. Synthesis of compound **A** was done by the reaction of 3-ketoursolic acid, hydroxyl amine hydrochloride in ethanol as previously reported [12]. Synthesis of compound **D** was done by the reaction of 3-ketoursolic acid and phthalic anhydride in pyridine as reported earlier [13]. Compounds **A** and **D** was identified by MS, ^{1}H and [13]C NMR.

A one pot reaction of 3-hydroxyiminours-12-en-28-oic acid **(A)** and alkyl substrate in the presence of NaH in THF offered substituted imino compounds 3-methoxyiminours-12-en-28-oic acid **1**, 3-allyloxyiminours-12-en-28-oic acid **2**, 3-benzyloxyiminours-12-en-28-oic acid **3**, 3-[(2-ethoxy-2-oxoethoxy)imino]-urs-12-en-28-oic acid **4,** 3-[(2-oxo-2-phenylethoxy)imino]-urs-12-en-28-oic acid **5** in good yields (Scheme I), (Fig. 1). To prepare 3-alkyloxyiminours-12-en-28-oic acid methyl ester **(C)**, the compound **1, 2, 3, 4** and **5** was reacted with alkyl substrate in the presence of K_2CO_3 in ethanol. Esterification occured to form 3-Methoxyiminours-12-en-28-oic acid methyl ester 6, 3-allyloxyiminours-12-en-28-oic acid allyl ester **7**, 3-benzyloxyiminours-12-en-28-oic acid benzyl ester **8**, 3[(2-ethoxy-2-oxoethoxy)imino]-urs-12-en-28-oic acid [2-ethoxy-2-oxoethyl] ester **9**, 3-[(2-oxo-2-phenylethoxy)imino]-urs-12-en-28-oic acid [2-oxo-2-phenylethyl] ester **10** (Scheme I), (Fig. 1). For synthesizing 3-[[2-(alkyloxycarbonyl)benzoyl]oxy]urs-12-en-28-oic acid **(E)**, a one pot reaction of 3-[[2-(hydroxycarbonyl)benzoyl]oxy]urs-12-en-28-oic acid **(D)** and an alkyl substrate in the presence of K_2CO_3 in ethanol was made. The substituted phthalic acid ester compounds synthesized were 3-[[2-(methoxycarbonyl)benzoyl]oxy]urs-12-en-28-oic acid **11**, 3-[[2-[(allyloxy)carbonyl]benzoyl]oxy]urs-12-en-28-oic acid **12,** 3-[[2-[(benzyloxy)carbonyl]benzoyl]oxy]urs-12-en-28-oic acid **13**, 3-[[2-[(2-ethoxy-2-oxoethoxy)carbonyl] benzoyl]oxy] urs-12-en-28-oic acid **14,** 3-[[2-[(2-oxo-2-phenylethoxy)carbonyl] benzoyl]oxy]urs-12-en-28-oic acid **15** white solid obtained (Scheme-II), (Fig. 2). Further compounds **11-15** were used as a starting material in synthesis of acid functionality at C-28.

The synthesized compounds were 3-[[2-(methoxycarbonyl) benzoyl] oxy]urs-12-en-28-oic acid methyl ester **16**, 3-[[2-[(allyloxy) carbonyl]benzoyl]oxy]urs-12-en-28-oic acid allyl ester **17**, 3-[[2-[(benzyloxy) carbonyl] benzoyl] oxy]urs-12-en-28-oic acid benzyl ester **18**, 3-[[2-[(2-ethoxy-2-oxoethoxy)carbonyl] benzoyl]oxy]urs-12-en-28-oic acid [2-ethoxy-2-oxoethyl] ester **19**, 3-[(2-oxo-2-phenylethoxy) carbonyl] urs-12-en-28-oic acid-[2-oxo-2-phenylethyl] ester **20**.

CONCLUSION

In conclusion, twenty derivatives comprising of phthalic acid and imines have been prepared by using a cost effective approach and considering the extensive SAR studies done in the prior art. Prepared derivatives can be screened as leads for anticancer potential that may open the possibility for newer therapeutic actions.

CONFLICT OF INTERESTS

Declared None

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