SYNTHESIS AND CHARACTERIZATION OF NEW IMINE AND PHTHALIC 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-oic 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 phthalic derivatives were synthesized. The compounds synthesized were characterized by MS, IR, 1H and 13C-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-inflammatory 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 3β-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-kB 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 P38/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 (B) has been reported as key intermediates for the synthesis of different variety of ursolic acid derivatives. In view of the reported SAR considerations the current 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 F254 silica plates using the mobile phase of ethyl acetate: ether (2:9) and observation under UV light (254 nm). IR spectrum was recorded on Perkin Elmer Spectrum 10 Mass spectrometry of compounds was recorded on Micromass Q-TOF MS mass spectrometer. All 1H NMR and [13C] spectra 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-alkyloxyimino-urs-12-en-28-oic acid alkyl ester (C) compounds 6-10 (Table 1)**

To a stirred solution of 3-alkyloxyimino-urs-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 acetate (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 product (C).

![Chemical structure](image)

**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).**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Alkyl substrate</th>
<th>R1</th>
<th>R2</th>
<th>Alkyl substrate (mg/mmol)</th>
<th>Derivatives B [1-5]</th>
<th>Reaction time</th>
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<td>1</td>
<td></td>
<td>CH3</td>
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<td>109.86/ 0.77</td>
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<td>2</td>
<td></td>
<td></td>
<td>H</td>
<td>78.03/ 0.64</td>
<td>3-allyloxyimino-urs-12-en-28-oic acid 2</td>
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</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>H</td>
<td>132/ 0.77</td>
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<tr>
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<td></td>
<td>H</td>
<td>143.62/ 0.86</td>
<td>3-[2-ethoxy-2-oxoethoxy]imino]-urs-12-en-28-oic acid 4</td>
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<tr>
<td>5</td>
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<td></td>
<td>H</td>
<td>171.17/ 0.86</td>
<td>3-[2-oxo-2-phenylethoxy]imino]-urs-12-en-28-oic acid 5</td>
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<tr>
<td>6</td>
<td></td>
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<td>7</td>
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<td>15</td>
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<td>8</td>
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<td></td>
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<tr>
<td>10</td>
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<td></td>
<td>67.00/ 0.34</td>
<td>3-[2-oxo-2-phenylethoxy]imino]-urs-12-en-28-oic acid (2-oxo-2-phenylethyl) ester</td>
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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)

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<td>15</td>
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<td></td>
<td>H</td>
<td>67.00/ 0.34</td>
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<td>28</td>
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<tr>
<td>16</td>
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<td>CH₃</td>
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<tr>
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<td>49.22/ 0.28</td>
<td>3-[[2-[benzoyl]oxy]benzoyl]oxy]urs-12-en-28-oic acid benzyl ester</td>
<td></td>
<td>27</td>
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<td>20</td>
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<td>77.44/ 0.38</td>
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<td>30</td>
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</tbody>
</table>

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 of ethanol and refluxed for 8 hrs. After completion of reaction, the reaction mixture 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) compounds 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 of ethanol and refluxed for 8-10 hrs to make potassium salt and water was removed by Dean Stark apparatus. Alkyl substrate was added.
3-methoxymyricins-12-en-18-oic acid (1)

Yield: 63%, m. p. 120-122°C. IR (KBr): 3423, 1680 cm⁻¹; 'H NMR (300 MHz, CHCl₃): δ 3.8 (3H, OCH₃), 5.2 (1H, alkene proton). [13]C NMR (75 MHz, CHCl₃): δ 58 (O-CH₃), 125 and 135 (alkene group carbons). MS: m/z 483.50 [M⁺], calcd for C₃₇H₄₃O₇ (483.73).

3-allyloxymyricins-12-en-18-oic acid (2)

Yield: 64%, m. p. 111-113°C. IR (KBr, cm⁻¹): 3448 (O-H of acid group), 1685 (C=O of acidic group). 'H NMR (300 MHz, CHCl₃): δ 3.8 (d, 2H, OCH₂), 5.1, 5.7, 5.8 (3H, alkene protons, H-R). [13]C NMR (75 MHz, CHCl₃): δ 65 (O-CH₃ allylic carbon), 118-130 (alkene group carbon), 170 (acid group carbon). MS: m/z 510.60 [M⁺], calcd for C₃₇H₄₅O₇ (509.76).

3-benzyloxymyricins-12-en-18-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). 'H NMR (300 MHz, CHCl₃): δ 5.0 (3H, OCH₃), 6.7-7.6 (8H, aromatic protons). [13]C NMR (75 MHz, CHCl₃): δ 60 (O-CH₃ benzyl carbon), 125-150 (aromatic carbons), 185 (acid group carbon). MS: m/z 559.40 [M⁺], calcd for C₄₀H₄₇O₉ (559.82).

3-[[2-ethoxy-2-oxoethoxy]iminours-12-en-28-oic acid allyl ester (7)

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 (C=O of ester group). IR (KBr cm⁻¹): 3448 (O-H of acid group), 1700 (C=O of acid group), 1450-1600 (C=C of aromatic ring). 'H NMR (300 MHz, CHCl₃): δ 4.7 (t, 3H, CH₃-CH₂-O), 4.5 (s, 2H, -OCH₂-C=O, H-R1), 4.8 (q, 2H, -OCH₂-CH₂); [13]C NMR (75 MHz, CHCl₃): δ 60 (O-CH₃, 60 (O-CH₃), 170-178 (acid and ester group carbon); MS: m/z 555.60 [M⁺], calcd for C₃₇H₄₅O₇ (555.79).

3-[[allyloxy]urs-12-en-28-oic acid (11)

Yield: 73% m. p. 150-152°C. IR (KBr cm⁻¹): 3448 (O-H of acid group), 1448 (C=O); 'H NMR (300 MHz, CHCl₃): δ 3.1 (3H, CH₃), 3.8 (3H, OCH₃), 7.3-7.8 (4H, 4H, Ar-H); [13]C NMR (75 MHz, CHCl₃): δ 55, 59 (O-CH₃), 125, 140 (Ar-C), 165 (C=O); MS: m/z 618.00 [M⁺], calcd for C₃₇H₄₅O₇ (618.84).

3-[[allyloxy]urs-12-en-28-oic acid (12)

Yield: 61% m. p. 152-154°C. IR (KBr cm⁻¹): 3440 (O-H of acid group), 1700 (C=O of acid group). IR (KBr cm⁻¹): 3448 (O-H of acid group), 1700 (C=O of acid group), 1450-1600 (C=C of aromatic ring). 'H NMR (300 MHz, CHCl₃): δ 4.0 (s, 2H, CH₂-O), 7.2-7.5 (4H, Ar-H), 7.9 (s, 5H, Ar-H), 8.0 (3H, OCH₃, 171.5 [C=O]); MS: m/z 644.40 [M⁺], calcd for C₃₇H₄₅O₇ (644.88).

3-[[benzoyloxy]urs-12-en-28-oic acid (13)

Yield: 61% m. p. 161-163°C. IR (KBr cm⁻¹): 3438 (O-H of acid group), 1700 (C=O of acid group). IR (KBr cm⁻¹): 3448 (O-H of acid group), 1700 (C=O of acid group), 1450-1600 (C=C of aromatic ring). 'H NMR (300 MHz, CHCl₃): δ 4.0 (s, 2H, CH₂-O), 7.1-7.5 (4H, Ar-H), 7.7 (s, 5H, Ar-H), 8.0 (3H, OCH₃). [13]C NMR (75 MHz, CHCl₃): δ 62 (O-CH₃), 118, 130, 135, 139 (CH=CH₂); MS: m/z 693.90 [M⁺], calcd for C₃₇H₄₅O₇ (694.94).

3-[[benzoyloxy]urs-12-en-28-oic acid (14)

Yield: 65% m. p. 151-157°C. IR (KBr cm⁻¹): 3438 (O-H of acid group), 1700 (C=O of acid group). IR (KBr cm⁻¹): 3448 (O-H of acid group), 1700 (C=O of acid group), 1450-1600 (C=C of aromatic ring). 'H NMR (300 MHz, CHCl₃): δ 4.0 (s, 2H, CH₃, H-R), 7.2-7.9 (m, 5H, Ar-H), 7.8 (s, 5H, Ar-H), 8.0 (3H, OCH₃). [13]C NMR (75 MHz, CHCl₃): δ 80 (O-C₃H₃), 59 (O-CH₂), 120, 125, 130, 140, 150 (Ar-C), 185 (C=O); MS: m/z 722.30 [M⁺], calcd for C₃₇H₄₇O₉ (722.30).

3-[[benzoyloxy]urs-12-en-28-oic acid (15)

Yield: 54% m. p. 142-144°C. IR (KBr cm⁻¹): 3432 (O-H of acid group), 1700 (C=O of acid group). IR (KBr cm⁻¹): 3448 (O-H of acid group), 1700 (C=O of acid group), 1450-1600 (C=C of aromatic ring). 'H NMR (300 MHz, CHCl₃): δ 4.1 (s, 2H, CH₃, H-R), 7.2-7.9 (m, 4H, Ar-H), 8.0 (3H, OCH₃). [13]C NMR (75 MHz, CHCl₃): δ 55 (CH₃), 121, 136, 145 (Ar-C), 165, 185 (C=O); MS: m/z 722.30 [M⁺], calcd for C₃₇H₄₅O₇ (722.30).

3-[[benzoyloxy]urs-12-en-28-oic acid (16)

Yield: 72% m. p. 130-132°C. IR (KBr cm⁻¹): 1725 (C=O), 1500-1600 (Ar-C=O), 1400-1500 (C=C); 'H NMR (300 MHz, CHCl₃): δ 4.2 (s, 3H, CH₃-H), 4.4 (t, 3H, CH₃-H), 7.2-7.5 (4H, Ar-H), 8.0 (3H, OCH₃). [13]C NMR (75 MHz, CHCl₃): δ 55 (OCH₃), 122, 125, 120, 135, 136 (Ar-C), 165, 170, 180 (C=O); MS: m/z 632.70 [M⁺], calcd for C₆₀H₅₅O₂6 (632.87).

3-[[allyloxy]carboxyloxy]urs-12-en-28-oic acid (17)

Yield: 64% m. p. 132-134°C. IR (KBr cm⁻¹): 1730 (C=O), 1500-1600 (Ar-C=O), 1400-1500 (C=C); 'H NMR (300 MHz, CHCl₃): δ 5.1 (s, 1H, 563

CONCLUSION

In conclusion, two derivatives comprising of phthalid 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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REFERENCES