Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2014, 3 (2): 541-550

(International Peer Reviewed Journal)

Synthesis And Comparative Bioevalvation Of Aliphatic And Aromatic Triazolyl Derivatives Of Ursolic Acid As Anticancer Agents.

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Accepted on 15th February 2014

ABSTRACT

Two series of ursolic acid triazolyl (alphatic and aromatic) congeners have been designed and synthesized to screen them as anticancer agents. First series of triazolyl derivatives (Aliphatic) was obtained by employing regioselective approach of Huisgen 1,3 –dipolar cycloaddition reaction of ursolic acid – alkyne with aliphatic azides formed from different alcohols whereas the second series of aromatic triazolyl derivatives was obtained by using similar approach but using aromatic azides formed from different anilines through diazotisation process followed by displacement with sodium azide. The structures were confirmed by using various spectral techniques(^{1}H NMR ,^{13}C NMR ,IR and MS analysis) the compounds were evalvated for the anticancer activity against a panel of four human cancer cell lines A-549(Lung), MCF-7(Breast), HCT-116(Colon), THP-1(Leukemia) and normal human epithelial cell line (FR-2) using sulforhodamine- B assay. The pharmacological studies have shown that all triazolyl-derrivatives (aliphatic and aromatic) exhibited more anticancer activities than parent ursolic acid. The aromatic triazolyl derivatives were more active than the alphatic congeners.

Keywords: Anticancer agents, Sulforhodamine, ursolic acid.

INTRODUCTION

Triterpenoids is an important class of natural products widely distributed in medicinal plants. Betulinic acid [1], oleonolic acid [2], ursolic acid [3] are the triterpenes widely exploited on account of their biological activities [1-4]. The derivatives of these triterpenes have been reported to exihibit cytotoxicity to tumor cell lines[5-7], anti HIV [8-13] and HIV protease inhibition [14]. Betulinic acid has been subject to diverse modifications which have resulted in emergence of potential anticancer agents [15-19]. Ursolic acid has also been reported to exihibit anti bacterial, anti inflammatory and cytoxic activities [20-24]. Report by liu et al[25] suggest that incorporation of an acyl piperazine motif at C-28 position improve its cell proliferation inhibition against the gastric cancer and breast cancer cells .We in our institution have also reported the ursolic acid-1-phenyl-1H[1,2,3] triazol-4-yl methyl ester congeners with enhanced cytotoxicity against(human) cancer cell lines and as promising anticancer agents [26].In continuation to our previous work, we devised a scheme to develop a library of urlosic acid –triazolyl derivatives modified at C-28 carboxylic acid using click chemistry. The aliphatic azides involved in the Huisgen [3+2]

cyclo addition with terminal alkyne were formed from alcohols through a one pot reaction using triphenyl phosphine , iodine , imidazole and sodium azide [27]. All the triazolyl derivatives thus formed were screened for anticancer activity against a panel of four human cancer cell lines along with the normal epithelial cell line. Promisingly the derrivatives exhibited good cytotoxic activity. The compounds (6e and 6i) were found to be most potent .However the earlier reported triazolyl congeners with aromatic azides were found to be more cytotoxic against human cancer cell lines than the aliphatic ones.

MATERIALS AND METHODS

General: Melting points were determined on digital buchi B-545 melting point apparatus using glass capillaries. 1R were recorded as cm^{-1} absorption using KBr pellets method of perkin - Elmer FT-1R spectrometer. ^{1}H NMR and ^{13}C NMR were recorded on 400 and 100 MH_Z Bruker daltonics spectrometers. The chemical shifts (δ) are reported in retative to tetramethyl silane (TMS) as internal standard and coupling constant were measured in Hertz (Hz). Mass spectra were recorded on Bruker Daltonics electron spray ionization apparatus. Coloumn chromatography was carried out on sillica gel (Qualigens, 60-120) and precoated sillica gel thin layer plates. Thin layer chromatographic plates (TLC) were viewed with ultra violet at 254nm for fluorescence quenching spots and at 366nm for fluorescent spots. Cericsulphate, anisaldehyde and iodine were used as visualizing agents. Compound(3) used in this study was isolated from dried apple peels in bulk and its characterization was assured by spectral techniques.

Synthesis

Synthesis of compound 4: To a solution of 3(500mg, 1.1 mmol) in acetone 12 ml, 3 drops of Jone's reagent was added and reaction mixture was stirred at room temperature. Reaction was monitored by TLC till its completion in 3h. Crude mixture after quenching was extracted with ethyl acetate (3×20ml). Organic layer was dried over Sodium sulphate and purified through Coloumn chromatography to give pure compound (450mg: 90%yeild). White solid with m.pt 276 -278^{0}C; ^{1}H NMR (400 MH_2, $CDCL_3$) ; 5.29(1H,t), 3.20(1H,d,j=12H_z) 2.28(1H, d j=8Hz), 1.97(2H,m), 1.81(4H,m), 1.52(12H,m), 1.30(4H,m), 1.04(3H,s), 0.91(3H,s), 0.85(3H,s), 0.82(3H,s), 0.80(3H,s), 0.75(3H,s), 0.68(3H,s), ^{13}CNMR (100MH_2,$CDCL_3$): 216.6, 181.5, 151.9, 123.2, 53.6, 52.2, 51.2, 50.6, 49.3, 47.8, 46.8, 41.7, 40.1, 39.8, 39.7, 39.4, 39.3, 39.1, 39.1, 38.9, 38.5, 38.4, 36.6, 28.3, 23.3, 21.2, 17.5, 17.0, 16.1, 15.3 ; 1R(KBr) 2930, 1718, 1657, 1453, 1378, 715cm^{-1} : ESI-MS : 477.46 Calc. for C_{30} H_{48} O_3 ($M+Na^+$) .

Synthesis of compound 5: To a solution of 4(450mg, 0.99 mmol) in THF caesium carbonate (1.6 m mol) and propargyl bromide (4 mmol) were added and reaction mixture was stirred at room temperature for 5h. Reaction was monitored by TLC and crude product was subjected to Coloumn chromatography to give pure 5 (410mg, 91.1% yield), white solid; m.pt: 279-280 ^{0}C : ^{1}H NMR (400MH_2, $CDCL_3$). 5.30(1H,m), 4.66(1H, d, j=12.0Hz), 4.58(1H, d, j=12.5Hz), 2.54(1H, m), 2.40(2H, m),2.26(1H, d, j=8.0Hz), 1.95(4H,m), 1.75(3H,m), 1.60(3H, m), 1.45(3H,m), 1.37(3H, m), 1.30(3H,m), 1.15(3H, s), 1.05(3H,s), 1.00(6H, s), 0.94(3H, s),0.92(3H, s), 0.87(3H, s);^{13} C NMR(100 MHz, $CDCl_3$), 217.9, 176.6, 137.9, 125.6, 78.1, 74.4, 55.3, 52.9, 51.6, 48.2, 47.4, 46.8, 42.2, 39.6, 39.1, 39.0, 38.8, 36.7, 36.4, 34.2, 32.6, 30.6, 28.0, 26.6, 24.2, 23.5, 23.4, 21.5, 21.1, 19.6, 17.2, 17.0, 15.3; IR(KBr): 2939, 2565, 2455, 2417, 1717, 1654, 1464, 1378, 1246, 1042, 779, 718 cm^{-1}; ESI-MS: 493.36, calc. for $C_{33}H_{48}O_3$ $[M+H]^+$.

Synthesis Of Compound 6a-6i: To a solution of compound 5,(20 mg, 0.04 mmol)in 2 ml of t-BuOH: H_2O(2:1) Sodium ascorbate(1.2mg,0.006 mmol) and copper sulphate(1.2mg, 0.0045 mmol), were added at room temperature. To this aliphatic azide(0.06 mmol) was added and the reaction mixture was sonicated at 35^{o}C till completion.It was monitored by TLC.The crude mixture was extracted with ethylacetate (3 aliquots of 20ml each),dried over sodium sulphate and purified by coloumn chromatography to give pure 6a-6i in 75-90% yield.

Compound 6a: white solid m.pt 292-294^{0}C ^{1}H NMR(400 MHz, $CDCl_3$); 7.27 (1H,s), 5.25 (2H, s), 5.18 (1H,t J=12.0 Hz), 4.32 (2H, t, j=16.0 Hz) 2.56 (1H, d, j=12.0Hz), 2.52 (1H,t, j=8.0Hz), 1.95(4H, m),

1.75(3H,m), 1.60(3H, m), 1.45(3H,m), 1.37(3H, m), 1.30(3H,m), 1.15(3H, s), 1.05(3H,s), 1.03(3H,s), 1.02(10H, m), 1.00(6H, s), 0.94(3H, s),0.92(3H, s), 0.87(3H, s);^{13} C NMR (100Hz, $CDCl_3$)211.4, 173.51, 140.57, 133.61, 124.26, 118.84, 55.30, 52.90, 51.60, 48.20, 47.42, 46.81, 42.12, 39.60, 39.10, 39.00, 38.81, 36.72, 36.42, 34.20, 32.61, 30.60, 28.00, 27.00, 26.80, 26.70, 26.60,26.00, 25.00, 24.71, 24.45, 24.20, 23.51, 23.40, 21.50, 21.10, 19.60, 17.20, 17.00, IR(KBr): 2925, 2856, 1713, 1690, 1620, 1530, 1440, 1390, 1320, 1240, 970; ESI-MS:624.40. calc. for $C_{39}H_{59}O_3N_3[M+H]^+$

Compound 6b: white solid m.pt 293-295^{o}C ^{1}H NMR(400MHz, $CDCl_3$ 7.27 (1H,s), 5.25 (2H, s), 5.18 (1H,t J=12.0 Hz), 4.32 (2H, t, j=16.0 Hz) 2.56 (1H, d, j=12.0Hz), 2.52 (1H,t, j=8.0Hz), 1.95(4H, m), 1.75(3H,m), 1.60(3H, m), 1.45(3H,m), 1.37(3H, m), 1.30(3H,m), 1.15(3H, s), 1.05(3H,s), 1.03(3H,s), 1.02(10H, m), 1.00(6H, s), 0.94(3H, s),0.92(3H, s), 0.87(3H, s);^{13} C NMR (100Hz, $CDCl_3$)211.4, 173.51, 140.57, 133.61, 124.26, 118.84, 55.30, 52.91, 51.60, 48.22, 47.40, 46.80, 42.21, 39.60, 39.10, 39.00, 38.80, 36.71, 36.41, 34.20, 32.60, 30.60, 28.00, 27.00, 26.81, 26.70, 26.60, 26.00, 25.00, 24.70, 24.41, 24.21, 23.52, 23.40, 21.51, 21.10, 19.60, 17.21, 17.00, 16.90, 16.80, IR(KBr): 2925, 2856, 1713, 1690, 1620, 1530, 1440, 1390, 1320, 1240, 970;), ESI-MS: 652.50 calc. for $C_{41}H_{63}O_3N_3[M+H]^+$.

Compound 6c: white solid m.pt 296-297^{o}C ^{1}H NMR(400MHz, $CDCl_3$ 7.27 (1H,s), 5.25 (2H, s), 5.18 (1H,t J=12.0 Hz), 4.32 (2H, t, j=16.0 Hz) 2.56 (1H, d, j=12.0Hz), 2.52 (1H,t, j=8.0Hz), 1.95(4H, m), 1.75(3H,m), 1.60(3H, m), 1.45(3H,m), 1.37(3H, m), 1.30(3H,m), 1.15(3H, s), 1.05(3H,s), 1.03(3H,s), 1.02(12H, m), 1.00(8H, s), 0.94(3H, s),0.92(3H, s), 0.87(3H, s);^{13} C NMR (100Hz, $CDCl_3$)211.4, 173.51, 140.57, 133.61, 124.26, 118.84, 55.46, 54.32, 52.00, 51.72, 50.64, 50.54, 48.20, 47.15, 46.99, 40.70, 39.76, 36.37, 36.4, 34.35, 32.81, 31.64, 30.26, 29.06, 28.95, 27.55, 26.96, 24.71, 24.33,22.93, 21.: 632.70, Calcd.for $C_{43}H_{67}O_3[M+H]^+$. 93, 20.21, 19.48 ,18.11, 17.20, 17.00, 16.90, 16.85, 16.83, 16.80, 16.70,16.39, 16.20. IR(KBr): 2925, 2856, 1713, 1690, 1620, 1530, 1440, 1390, 1320, 1240, 970;), ESI-MS: 680.70, Calc. for $C_{43}H_{67}O_3N_3[M+H]^+$.

Compound 6d: white solid m.pt 298-299^{o}C ^{1}H NMR(400MHz, $CDCl_3$), 7.27 (1H,s), 5.25 (2H, s), 5 .18 (1H,t J=12.0 Hz), 4.32 (2H, t, j=16.0 Hz) 2.56 (1H, d, j=12.0Hz), 2.52 (1H,t, j=8.0Hz), 1.95(4H, m), 1.75(3H,m), 1.60(3H, m), 1.45(3H,m), 1.37(3H, m), 1.30(3H,m), 1.15(3H, s), 1.05(3H,s), 1.03(3H,s), 1.02(12H, m), 1.00(8H, s), 0.94(3H, s),0.92(3H, s), 0.87(3H, s);^{13} C NMR (100Hz, $CDCl_3$)211.4, 173.31, 140.57, 133.61, 124.26, 118.84, 55.46, 54.32, 52.00, 51.72, 50.64, 50.54, 48.20, 47.15, 46.99, 40.70, 39.76, 36.37, 36.4, 34.35, 32.81, 31.64, 30.26, 29.06, 28.95, 27.55, 26.96, 24.71, 24.33, 22.93, 21.93, 20.21, 19.48 ,18.11, 17.20, 17.00, 16.90, 16.85, 16.83, 16.80, 16.70 ,16.39, 16.20. 16.15, 16.10, IR(KBr): 2925, 2856, 1713, 1690, 1620, 1530, 1440, 1390, 1320, 1240, 970, ESI-MS; 708.70, Calc. for $C_{45}H_{71}O_3N_3[M+H]^+$.

Compound 6e: white solid m.pt 292-293^{o}C ^{1}H NMR(400 MHz, $CDCl_3$); 7.37(5H,s), 7.27 (1H,s), 5.56(2H,s), 5.25 (2H, s), 5.16 (1H,t J=12.0 Hz), 4.32 (2H, t, j=16.0 Hz) 2.56 (1H, d, j=12.0Hz), 2.52 (1H,t, j=8.0Hz), 1.95(4H, m), 1.75(3H,m), 1.60(3H, m), 1.45(3H,m), 1.37(3H, m), 1.30(3H,m), 1.15(3H, s), 1.05(3H,s), 1.03(3H,s), 1.02(10H, m), 1.00(6H, s), 0.94(3H, s),0.92(3H, s), 0.87(3H, s);^{13} C NMR (100Hz, $CDCl_3$)211.4, 172.89, 145.30. 136.22, 133.61, 128.87, 128.57, 127.54, 121.01, 118.84, 55.46, 54.32, 52.00, 50.64, 50.17, 47.15, 46.99, 40.07, 39.76, 39.03, 37.95, 37.79, 37.53, 36.37, 34.35, 32.81, 30.26, 24.71, 24.45, 24.20, 23.51, 23.40, 21.50, 21.10, 19.60, 19.48, 18.11, 17.20, 17.00, 16.39: IR(KBr): 2925, 2856, 1713, 1690, 1620, 1530, 1440, 1390, 1320, 1240, 970, ESI-MS; 631.40, Calc. for $C_{40}H_{54}O_3N_3[M+H]^+$.

Compound 6f: white solid m.pt 280-282^{o}C ^{1}H NMR(400 MHz, $CDCl_3$); 7.27 (1H,s), 5.25 (2H, s), 5.18 (1H,t J=12.0 Hz), 4.32 (2H, t, j=16.0 Hz) 2.56 (1H, d, j=12.0Hz), 2.52 (1H,t, j=8.0Hz), 1.95(4H, m), 1.75(3H,m), 1.60(3H, m), 1.45(3H,m), 1.37(3H, m), 1.30(3H,m), 1.15(3H, s), 1.05(3H,s), 1.03(3H,s), 1.02(6H, m), 1.00(4H, s), 0.94(3H, s),0.92(3H, s), 0.87(3H, s);^{13} C NMR (100Hz, $CDCl_3$)211.4, 173.51, 140.57, 133.61, 124.26, 118.84, 55.30, 52.90, 51.60, 48.20, 47.42, 46.81, 42.12, 39.60, 39.10, 39.00,

38.81, 36.72, 36.42, 34.20, 32.61, 30.60, 28.00, 27.00, 26.80, 26.70, 26.60 ,26.00, 25.00, 24.71, 24.45, 24.20, 23.51, 23.40, 21.50, 17.10, IR(KBr): 2925, 2856, 1713, 1690, 1620, 1530, 1440, 1390, 1320, 1240, 970, ESI-MS; 582.40, Calc. for $C_{34}H_{53}0_3N_3[M+H]^+$.

Compound 6g: white solid m.pt 283-284^oC ^1H NMR(400 MHz, $CDCl_3$); 7.27 (1H,s), 5.25 (2H, s), 5.18 (1H,t J=12.0 Hz), 4.32 (2H, t, j=16.0 Hz) 2.56 (1H, d, j=12.0Hz), 2.52 (1H,t, j=8.0Hz), 1.95(4H, m), 1.75(3H,m), 1.60(3H, m), 1.45(3H,m), 1.37(3H, m), 1.30(3H,m), 1.15(3H, s), 1.05(3H,s), 1.03(3H,s), 1.02(6H, m), 1.00(6H, s), 0.94(3H, s),0.92(3H, s), 0.87(3H, s);^{13} C NMR (100Hz, $CDCl_3$)211.4, 173.51, 140.57, 133.61, 124.26, 118.84, 55.30, 52.90, 51.60, 48.20, 47.42, 46.81, 42.12, 39.60, 39.10, 39.00, 38.81, 36.72, 36.42, 34.20, 32.61, 30.60, 28.00, 27.00, 26.80, 26.70, 26.60 ,26.00, 25.00, 24.71, 24.45, 24.20, 23.51, 23.40, 21.50, 17.10, 16.10, IR(KBr): 2925, 2856, 1713, 1690, 1620, 1530, 1440, 1390, 1320, 1240, 970, ESI-MS; 596.50, Calc. for $C_{37}H_{55}O_3N_3[M+H]^+$.

Compound 6h: white solid m.pt 285-287^oC ^1H NMR(400 MHz, $CDCl_3$); 7.27 (1H,s), 5.25 (2H, s), 5.18 (1H,t J=12.0 Hz), 4.32 (2H, t, j=16.0 Hz) 2.56 (1H, d, j=12.0Hz), 2.52 (1H,t, j=8.0Hz), 1.95(4H, m), 1.75(3H,m), 1.60(3H, m), 1.45(3H,m), 1.37(3H, m), 1.30(3H,m), 1.15(3H, s), 1.05(3H,s), 1.03(3H,s), 1.02(6H, m), 1.00(8H, s), 0.94(3H, s),0.92(3H, s), 0.87(3H, s);^{13} C NMR (100Hz, $CDCl_3$)211.4, 173.51, 140.57, 133.61, 124.26, 118.84, 55.30, 52.90, 51.60, 48.20, 47.42, 46.81, 42.12, 39.60, 39.10, 39.00, 38.81, 36.72, 36.42, 34.20, 32.61, 30.60, 28.00, 27.00, 26.80, 26.70, 26.60 ,26.00, 25.00, 24.71, 24.45, 24.20, 23.51, 23.40,22.50, 21.50, 17.10, 16.10, IR(KBr): 2925, 2856, 1713, 1690, 1620, 1530, 1440, 1390, 1320, 1240, 970, ESI-MS; 610.50, Calc. for $C_{38}H_{57}O_3N_3[M+H]$.

Compound 6i: white solid m.pt 298-299^oC, ^1H NMR(400MHz, $CDCl_3$); 7.37 (5H, s), 7.27(1H, s), 5.36(1H,m), 5.30(2H,d), 5.25(1H,d), 5.16 (1H,t J=12.0 Hz), 4.32 (2H, t, j=16.0 Hz) 2.56 (1H, d, j=12.0Hz), 2.52 (1H,t, j=8.0Hz), 1.95(4H, m), 1.75(3H,m), 1.60(3H, m), 1.45(3H,m), 1.37(3H, m), 1.30(3H,m), 1.15(3H, s), 1.05(3H,s), 1.03(3H,s), 1.02(10H, m), 1.00(6H, s), 0.94(3H, s),0.92(3H, s), 0.87(3H, s); ^{13} C NMR (100Hz, $CDCl_3$)211.4, 172.89, 145.30, 136.22, 133.61, 128.87, 128.57, 127.40, 127.54, 121.01, 118.84, 115.00, 110.60, 55.46, 54.32, 52.00, 50.64, 50.17, 47.15, 46.99, 40.07, 39.76, 39.03, 37.95, 37.79, 37.53, 36.37, 34.35, 32.81, 30.26, 24.71, 24.45, 24.20, 23.51, 23.40, 21.50, 21.10, 19.60, 19.48, 18.11, 17.20, 17.00, 16.39: IR(KBr): 2925, 2856, 1713, 1690, 1620, 1530, 1440, 1390, 1320, 1240, 970, ESI-MS; 682.40, Calc. for $C_{43}H_{56}O_3N_4[M+H]$.

Compound 7a: White Solid , 1H NMR (400 MHz, CDCl3): 8.02 (1H, s), 7.28 (1H, m), 6.82 (1H, m), 6.65 (1H, m), 5.23 (2H, s),5.19 (1H, m), 2.78 (1H, d, J ¼ 12.0 Hz), 2.43 (2H, m), 2.26 (4H, m),1.77 (2H, m), 1.60 (2H, m), 1.54 (2H, m), 1.47 (2H, m), 1.42 (2H, m),1.21 (3H, s), 1.04 (2H, m), 1.01 (3H, s), 0.99 (3H, s), 0.94 (2H, m), 0.90(3H, s), 0.87 (3H, s), 0.81 (2H, m), 0.77 (3H, s), 0.45 (3H, s); 13C NMR(100 MHz, CDCl3): d 217.4, 177.2, 168.4, 166.1, 144.4, 139.6, 137.0,123.4, 118.8, 110.9, 110.1, 103.6, 58.4, 55.4, 54.2, 53.6, 51.0, 49.8, 48.4,46.3, 43.5, 41.2, 39.8, 38.4, 36.8, 36.4, 34.2, 32.6, 30.6, 28.0, 26.2, 24.2, 23.4, 21.8, 21.1, 19.6, 17.2, 17.0, 16.8; IR (KBr): 2922, 2857, 1717,1703, 1628, 1540, 1432, 1341, 986 cm 1; ESI-MS: 670.48 calc. for $C_{39}H_{51}N_3O_3$ [M + Na].

Compound 7b: White solid, mp: 287-288^oC; 1H NMR (400 MHz, $CDCl_3$): 7.98 (1H, s), 7.50 (1H, s), 741 (1H, d, J ¼ 8.0 Hz),7.25 (1H, d, J ¼ 8.0 Hz), 5.26 (2H, s), 5.21 (1H, m), 2.89 (1H, d,J ¼ 12.0 Hz), 2.52 (2H, m), 2.37 (3H, s), 2.34 (3H, s), 2.27 (2H, m),1.99,(2H, m), 1.88 (4H, m), 1.57 (4H, m), 1.43 (4H, m), 1.33 (2H, m), 1.26,(3H, s), 1.10 (3H, s), 1.06 (3H, s), 1.00 (3H, s), 0.95 (3H, s), 0.91 (2H,m), 0.86 (3H, s), 0.48 (3H, s); 13C NMR (100 MHz, CDCl3): d 217.7,177.8, 143.7, 138.3, 138.1, 137.6, 130.6, 125.5, 122.7, 122.3, 121.7, 117.8,57.6, 56.2, 55.2, 54.4, 47.4, 46.8, 41.8, 41.0, 39.2, 39.1, 36.7, 34.1, 33.1, 31.9, 31.6, 30.7, 30.3, 29.7, 29.3, 26.5, 25.6, 23.6, 22.7, 21.4, 19.9, 19.4,16.7, 14.9, 14.1; IR (KBr): 2925, 2856, 1714, 1700, 1620, 1539, 1444,1391, 1325, 1230, 972 cm 1; ESI-MS: 662.34 calc. for $C_{41}H_{57}N_3O_3$ [M +Na]$^+$.

Compound 7c: White solid, mp: 280-282^{o}C; 1H NMR (500 MHz, CDCl3): d 8.01 (1H, d, J ¼ 10.0 Hz), 7.77 (1H, m), 7.56 (1H,m), 7.50 (1H, m), 7.41 (1H, m), 5.28 (3H, m), 2.51 (1H, m), 2.45 (2H,m), 2.28 (1H, d, J ¼ 15.0 Hz), 2.01 (2H, m), 1.95 (3H, m), 1.74 (2H, m),1.67 (4H, m), 1.47 (2H, m), 1.27 (2H, m), 1.25 (3H, s), 1.20 (3H, s), 1.17(3H, s), 1.08 (3H, s), 0.99 (3H, s), 0.87 (4H, m), 0.84 (3H, s), 0.68 (3H, s); 13C NMR (100 MHz, CDCl3): d 217.8, 177.5, 142.9, 138.2, 136.4,134.0, 131.2, 128.5, 128.1, 126.0, 125.5, 118.3, 57.2, 55.2, 52.9, 48.2,47.4, 46.8, 46.7, 42.2, 41.2, 39.5, 39.3, 39.2, 39.1, 38.8, 36.7, 34.1, 30.6,30.5, 28.3, 26.6, 24.4, 23.5, 22.6, 22.4, 19.6, 17.0, 15.2; IR (KBr): 3015,2951, 1704, 1692, 1522, 1462, 1434, 1372, 1322, 1285, 982 cm 1; ESIMS:713.44 calc. for $C_{39}H_{52}BrN_3O_3$ $[M + Na]^+$.

Compound 7d: Yellow paste: 1H NMR (500 MHz, CDCl3):d 8.16 (1H, d, J ¼ 5.0 Hz), 7.89 (1H, d, J ¼ 5.0 Hz), 7.42 (1H, t,J ¼ 5.0 Hz), 7.10 (2H, t, J ¼ 10.0 Hz), 5.27 (3H, m), 3.90 (3H, s), 2.50(1H, m), 2.38 (1H, m), 2.27 (1H, d, J ¼ 15.0 Hz), 2.00 (2H, m), 1.92(4H, m), 1.70 (6H, m), 1.58 (2H, m), 1.46 (2H, m), 1.32 (6H, s), 1.20(2H, m), 1.12 (3H, s), 1.05 (3H, s), 0.95 (6H, s), 0.89 (2H, m), 0.62 (3H,s); 13C NMR (100 MHz, CDCl3): d 218.0, 177.3, 143.9, 142.4, 138.1,130.1, 126.3, 125.4, 125.3, 122.2, 121.1, 112.2, 57.7, 56.4, 56.0, 53.2,48.2, 47.4, 46.7, 42.1, 39.4, 39.2, 39.1, 38.8, 36.6, 34.1, 32.0, 31.0, 29.7,27.2, 26.6, 25.1, 23.3, 22.4, 21.1, 19.5, 17.0, 16.7, 15.2, 14.1; IR (KBr):3010, 2940, 2852, 1703, 1512, 1465, 1450, 1420, 1385, 972 cm 1; ESIMS:642.78 calc. for $C_{40}H_{55}N_3O_3$: $[M+ H]^+$.

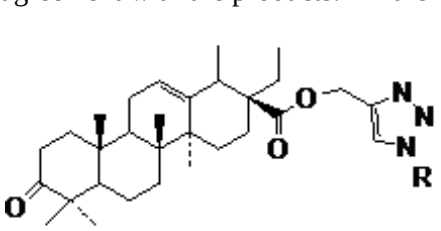
Compound 7e: Yellow solid, mp: 282-285^{o}C: 1H NMR (500 MHz, CDCl3): d 8.10 (1H, m), 7. 91 (1H, d, J ¼ 10.0 Hz), 7.80 (1H,m), 7.73 (1H, m), 7. 63 (1H, m), 5.29 (3H, m), 2.51 (1H, m), 2.39 (1H, m), 2.27 (1H, d, J ¼ 15.0 Hz), 2.08 (2H, m), 1.95 (4H, m), 1.72 (4H, m),1.65 (2H, m), 1.48 (2H, m), 1.33 (2H, m), 1.30 (3H, s), 1.22 (3H, s), 1.12(3H, s), 1.08 (3H, s), 0.99 (3H, s), 0.92 (3H, s), 0.88 (4H, m), 0.68 (3H,s); 13C NMR (100 MHz, CDCl3): d 217.9, 177.4, 144.3, 143.7, 138.1,133.8, 130.9, 130.1, 127.8, 125.7, 125.4, 122.2, 57.5, 57.4, 55.1, 52.8,48.2, 47.4, 46.8, 46.7, 42.2, 41.9, 39.5, 39.3, 39.0, 38.7, 36.6, 34.1, 30.7,29.7, 26.7, 25.5, 23.3, 21.5, 21.1, 19.5, 17.0, 15.2, 15.0; IR (KBr): 3117,2630, 1715, 1519, 1440, 1418, 1378, 981 cm 1; ESI-MS: 657.58 calc.for $C_{39}H_{52}N_4O_5$: [M+ H].

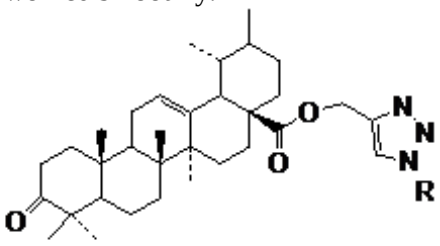
Compound 7f: White paste: 1H NMR (500 MHz, CDCl3): 8.05 (1H, d, J ¼ 10.0 Hz), 7. 60 (2H, m), 7.46 (2H, m), 5.29 (3H, m),2.51 (1H, m), 2.40 (1H, m), 2.27 (1H, d, J ¼ 15.0 Hz), 2.00 (2H, m),1.93 (4H, m), 1.73 (4H, m), 1.65 (2H, m), 1.45 (2H, m), 1.32 (6H, s),1.20 (2H, m), 1.18 (3H, s), 1.10 (3H, s), 0.98 (3H, s), 0.95 (3H, s), 0.8(4H, m), 0.65 (3H, s); ^{13}CNMR (100 MHz, CDCl3): d 217.8, 177.5,143.7, 143.0, 138.2, 134.7, 130.9, 128.4, 127.7, 126.1, 126.0, 125.5, 57. 3,55.2, 52.2, 48.2, 47.4, 46.8, 46.7, 42.2, 41.8, 39.5, 39.3, 39.1, 38.7, 36.7,36.6, 34.1, 32.3, 32.2, 30.9, 29.8, 29.7, 26.7, 23.4, 21.8, 19.5, 17.0, 15.3,IR (KBr): 2994, 2708, 1705, 1512, 1462, 1421, 1405, 1380, 1367,975 cm 1; ESI-MS: 669.12 calcd. for $C_{39}H_{52}ClN_3O_3$ [M + Na].

RESULTS AND DISCUSSION

Chemistry: Encouraged by our previous work, we have undertaken a programme-directed towards structural modification of ursolic acids at C-28 again. It was oxidized by using Jone's reagent at 0^{o}C to get C-3 oxidised derivative (4) in quantitative yield. Propargylation of carboxylic acid group at C-28 was carried out using Cs_2CO_3 base in dry condition and THF as solvent to give the alkyne derivative (5) in excellent yield. Aliphatic azide were formed by highly exothermic paste formation by mixing of different alcohols with triphenyl phosphine, imidazole and iodine followed by displacement with ice cold solution of sodium azide in sodium thiosulphate. Aromatic azides were formed from different anilines by diazolisation with sodium nitrate in acidic conditions followed by displacement with sodium azide in good yield. The dipolar cycloaddition of (5) with different aliphatic azides in presence of $CuSO_4$ $5H_2O$ and sodium ascorbate in t-BUOH H_2O (2:1) resulted in the formation of 1,4-Substituted triazolyl derivates 6a-6i with excellent yields. The dipolar cycloaddition of (5) with aromatic azides yielded second series of 1,4-substituted trizolyl derivates 7a-7f. All the reactions were carried out at 35^{0}C under sonication and completed within 0.5-6h.The products were confirmed by ^{1}H NMR, ^{13}C NMR, 1R and MS analysis. The

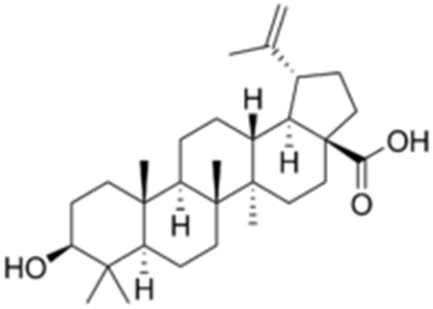
resonance of H-5 of trizole in aromatic region in ^{1}H NMR confirmed cyclisation of azides with terminal alkyne. ^{13}C NMR showed all the expected signals corresponding to ursolic acid trizolyl derivates. ESI-MS was also in agreement with the products. All the reactions worked smoothly.



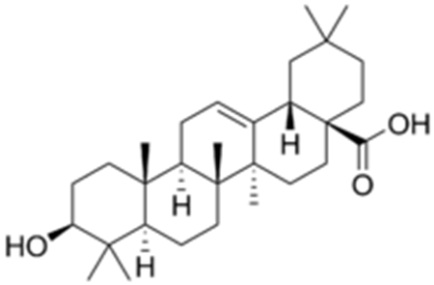


R=

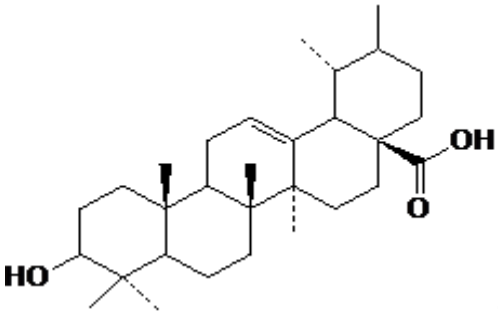
R = 



(1)



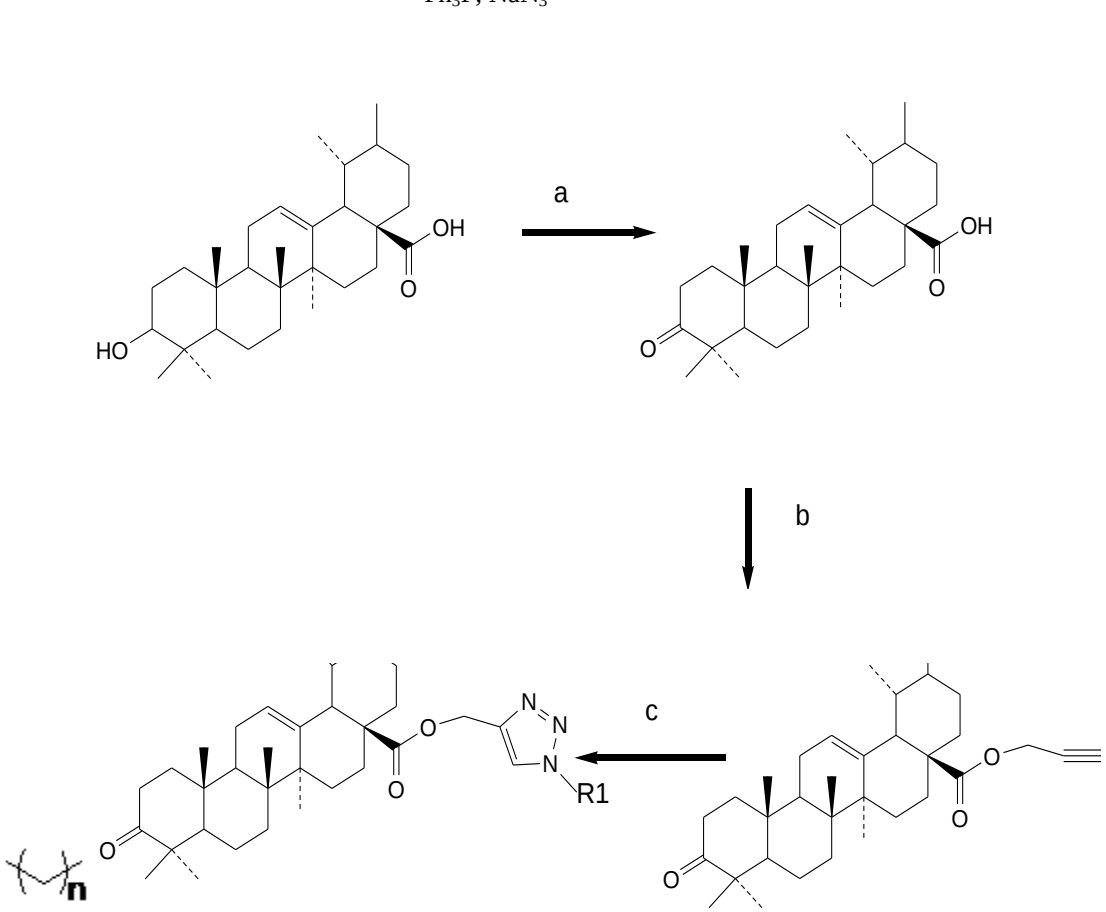
(2)



(3)

Scheme-1

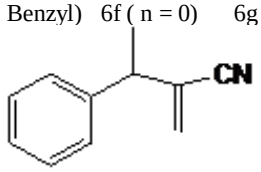
$$R_1\,OH \xrightarrow[I_2,\,I_m]{} R_1N_3$$



Experimental Conditions; a= CrO_3 aquous + H_2SO_4 2hr, b = $CHCCH_2Br$, Cs_2CO_3, THF, 3h
C= $CuSO_4$/sod-Ascorbate, R_1N_3, t-BuOH+H_2O(2:1), 1-3h

R_1 =

6a (n = 4) 6b (n = 6) 6c (n= 8) 6d (n= 10)
6e (R_1 = Benzyl) 6f (n = 0) 6g (n = 2) 6h (n= 3)

6i = 

Biology : The cytotoxic activity of compounds was studied using cultured A-549(lung), MCF-7(breast), HCT-116(colon) and THP-1(leukemia) cancer cell lines along with normal cell lines(FR-2) by using sulforhodamine –B assay. 5-fluoro uracil and mitomycin-C along with compound 3 were taken as reference standards in this study table 1. The cell lines were exposed to 50 and 10 µmol concentrations of compounds for 48 h and percentage of dead cells was evaluated. . From aromatic series congener with ortho substitution in the benzene ring were found to be most active were as from aliphatic series, The derrivates from benzylic alcohol (6e, 6i) were found to be potent.

We concluded that -

- In series 1 of compounds, compound 6e is most active whereas in series 2, the compound 7f is most active.

- All the active compounds exhibited less cytotoxic activity towards the normal epithelial cell line (FR-2) compared to their anti cancer – potential against cancer cell lines as in evident from $1C_{50}$ values. All the derivatives were found to be more cytotoxic against used cancer cell lines than the parent compound (3).

APPLICATIONS

The compounds synthesized in the paper have been screened for anticancer activity against four cancer cell lines and have shown a promising potential as anticancer agents as compared to the parent compound with reference to the positive controls used.

CONCLUSIONS

Two series of the ursolic acid - trizole -4-yl methyl derrivates have been synthesized by employing Cu(1) catalyzed Huisgen 1,3-dipolar cycloaddition reaction of terminal alkyne derivative of (3) with aliphatic and aromatic azides. All the compounds synthesized were screened for anti cancer activity against a panel of 4 human cancer cell lines including A-549(lung),MCF-7(breast),HCT-116(colon) and THP-1(leukemia) and a normal epithelial cell line (FR-2) using sulforhodamine – B assay. From the data it was evident that most of the compounds exhibited better anti cancer activity against all cancer lines compared to positive controls 3, 5-fluorouracil and mitomycin C used in this study. Derivatives from aromatic azides were more active than those from aliphatic azides.

Table 1 : Percentage inhibition data of compounds 4, 6a-6i and 7a-7f against four cancer cell lines and a normal cell line.

Tissue		Lung	Breast	Colon	Leukemia	Normal cell line
Cell line		A-549	MCF-7	HCT-116	THP-1	FR-2
Code	Conc(µmol)					
4	50	61±o.3	57±o.4	53±o.3	84±0.4	66±0.2
	10	28±0.2	36±0.2	55±0.2	31±0.3	31±0.2
6a	50	66±0.4	74±0.3	76±0.4	79±0.4	56±0.3
	10	42±0.2	53±0.2	42±0.3	55±0.3	34±0.4
6b	50	68±0.2	67±0.3	68±0.4	78±0.3	54±0.2
	10	40±0.4	51±0.2	44±0.3	58±0.2	32±0.4
6c	50	45±0.4	59±0.3	59±0.4	80±0.3	53±0.4
	10	28±0.2	45±0.2	56±0.2	56±0.2	30±0.4
6d	50	62±0.4	67±0.2	68±0.3	75±0.2	59±0.4
	10	42±0.3	52±0.2	45±0.4	56±0.3	38±0.2
6e	50	86±0.4	85±0.3	86±0.3	87±0.4	40±0.4
	10	70±0.3	74±0.2	76±0.2	57±0.2	20±0.4
6f	50	47±0.4	52±0.3	56±0.2	62±0.4	55±0.3
	10	30±0.2	35±0.2	38±0.2	40±0.3	35±0.2
6g	50	55±0.4	53±0.3	55±0.2	58±0.2	50±0.4
	10	36±0.2	38±0.2	40±0.3	42±0.3	34±0.2
6h	50	60±0.4	64±0.4	68±0.2	65±0.4	42±0.4
	10	44±0.2	51±0.4	47±0.3	50±0.2	25±0.2
6i	50	84±0.4	88±0.3	83±0.4	87±0.3	38±0.3

	10	75±0.2	76±0.2	74±0.2	56±0.2	18±0.3
7a	50	64±0.2	73±0.2	91±0.1	86±0.1	78±0.3
	10	57±0.3	17±0.3	69±0.3	82±0.4	2±0.2
7b	50	93±0.3	99±0.1	99±0.2	94±0.2	85±0.4
	10	74±0.1	69±0.3	91±0.1	82±0.4	68±0.5
7c	50	99±0.3	99±0.1	99±0.3	99±0.3	99±0.3
	10	85±0.2	80±0.2	41±0.2	92±0.5	2±0.2
7d	50	94±0.4	91±0.1	94±0.3	93±0.5	28±0.1
	10	79±0.5	87±0.4	77±0.5	82±0.2	5±0.5
7e	50	39±0.2	42±0.4	65±0.2	54±0.4	12±0.2
	10	3±0.3	4±0.2	45±0.4	24±0.4	9±0.1
7f	50	91±0.5	95±0.6	93±0.4	96±0.1	35±0.5
	10	77±0.3	81±0.3	54±0.5	80±0.3	9±0.4
5-FU	20	86	ND	76	96	ND
Mito-C	01	70	82	ND	ND	ND

All experiments were carried out in triplicate. ND = Not determined. 5-FU = 5Fluorouracil. Mito-C Mitomycin

ACKNOWLEDGEMENTS

We are thankful to Director IIIM, CSIR Lab, Jammu and Kashmir, INDIA for providing us with the facilities to carry out the synthetic modifications at medicinal chemistry Division at Branch office Srinagar.

REFERENCES

[1] S.B. Mahato, S.K. Sarkar, G. Poddar, *Phytochemistry*, **1988,** 27, 3037 - 3067.
[2] H. Nishino, A. Nishino, J. Takayasu, A. Iwashima, K. Hirabayashi, S. Iwata,S. Shibata, *Cancer Res.*, **1988,** 48, 5210 - 5215.
[3] K.R. Price, I.K. Johnson, G.R. Fenwick, *Crit. Rev. Food Sci. Nutr.* **1987,** 26, 27 - 135.
[4] J.F. Mayaux, A. Bousseau, R. Pauwels, T. Huet, Y. Henin, N. Dereu, M. Evers,F. Soler, C. Poujade, E.D. Clercq, J.B. Le Pecq, *Proc. Natl. Acad. Sci.* U.S.A. **1994**, 91, 3564-3568.
[5] M. Evers, C. Poujade, F. Soler, Y. Ribeill, C. James, Y. Lelièvre, J.C. Gueguen,D. Reisdorf, I. Morize, R. Pauwels, E.D. Clercq, Y. Hénin, A. Bousseau,J.F. Mayaux, J.B.L. Pecq, N. Dereu,, *J. Med. Chem*. **1996,** 39, 1056-1068.
[6] Y. Kashiwada, T. Nagao, A. Hashimoto, Y. Ikeshiro, H. Okabe, L.M. Cosentino,K.H. Lee, , *J. Nat. Prod*. **2000**, 63, 1619-1622.
[7] Y.M. Zhu, J.K. Shen, H.K. Wang, L.M. Cosentino, K.H. Lee, *Bioorg. Med. Chem. Lett*., **2001,** 11, 3115-3118.
[8] C. Ma, N. Nakamura, H. Miyashiro, M. Hattori, K. Shimotohno, *Chem. Pharm. Bull*. **1999,** 47, 141-145.
[9] J. Liu, *J. Ethnopharmacol*. **1995,** 49, 57-68.

[10] K.H. Lee, Y.M. Lin, T.S. Wu, D.C. Zhang, T. Yamagishi, T. Hayashi, I.H. Hall, J.J. Chang, R.Y. Wu, T.H. Yang, *Planta Med.* **1988,** 54, 308 -311.
[11] C.N. Lin, C.M. Lu, M.K. Cheng, K.H. Gan, S.J. Won, *J. Nat. Prod*. **1990,** 53, 513-516.
[12] E. Pisha, H. Chai, I.S. Lee, T.E. Chagwedera, N.R. Farnsworth, G.A. Cordell,C.W. Beencher, H.H. Fong, A.D. Kinghorn, D.M. Brown, M.C. Wani, M.E. Wall, T.J. Hieken, T.K.D. Gupta, J.M. Pezzuto, *Nat. Med*., **1995,** 1 ,1046-1051.
[13] I.C. Sun, C.H. Chen, Y. Kashiwada, J.H. Wu, H.K. Wang, K.H. Lee, *J. Med. Chem.* **2002**, 45, 4271-4275.
[14] S.L. Holz-Smith, I.C. Sun, L. Jin, T.J. Matthews, K.H. Lee, C.H. Chen,, *Antimicrob. Agents Chemother*. **2001,** 45, 60-66.
[15] E. Pisha, H. Chai, I.S. Lee, T.E. Chagwedera, N.R. Farns-worth, G.A. Cordell,C.W.W. Beecher, H.H.S. Fong, A.D. Kinghorn, D.M. Brown, M.C. Wani,M.E. Wall, T.J. Hieken, T.K.D. Gupta, J.M. Pezzuto, *Nat. Med*. **1995,** 1, 1046-1051.
[16] R. Majeed, P.L. Sangwan, P.K. Chinthakindi, I. Khan, N.A. Dangroo, N. Thota,A.H. Dar, P.R. Sharma, A.K. Saxena, S. Koul, *Eur. J. Med. Chem.* **2013,** 63, 782-792.
[17] S. Fulda, C. Friesen, M. Los, C. Scaffidi, W. Mier, M. Benedict, G. Nuñez,P.H. Krammer, M.E. Peter, K.M. Debatin, *Cancer Res.* **1997,** 57, 4956-4964.
[18] M.N. Laszczyk, *Planta Med.* **2009,** 75, 1549-1560.
[19] D.S.H.L. Kim, J.M. Pezzuto, E. Pisha, *Bioorg. Med. Chem. Lett*. **1998,** 8, 1707-1712.
[20] H.J. Jeong, H.B. Chai, S.Y. Park, D.S.H.L. Kim, *Bioorg. Med.Chem. Lett*. **1999,** 9, 1201-1204.
[21] C.M. Ma, S.Q. Cai, J.R. Cui, R.Q. Wang, P.F. Tu, M. Hattori, M. Daneshtalab, *Eur. J. Med. Chem.* **2005,**40 , 582-589.
[22] J. Li, W.J. Guo, Q.Y. Yang, *World J. Gastroenterol.* **2002,** 8,493-495.
[23] D.K. Kim, J.H. Baek, C.M. Kang, M.A. Yoo, J.W. Sung, D.K. Kim, H.Y. Chung,N.D. Kim, Y.H. Choi, S.H. Lee, K.W. Kim, *Int. J. Cancer* ,**2000,** 87,629-636.
[24] P.O Harmand, R. Duval, C. Delage, A. Simon, *Int. J. Cancer* , **2005,**114 , 1-11.
[25] M. Kanjoormana, G. Kuttan, Integr. Cancer Ther., **2010**, 9, 224-235
[26] Showkat Rashid, Bilal Ahmad Dar, Rabia Majeed, Abid Hamid,Bilal Ahmad Bhat. *Eur.J .Med.chem*, **2013**, 66 238-245.
[27] Y.H. Choi, J.H. Baek, M.A. Yoo, H.Y. Chung, N.D. Kim, K.W. Kim, *Int. J. Oncol.*, **2000**, 17, 565-571.
[28] M. Kanjoormana, G. Kuttan, *Integr. Cancer Ther.*, **2010**, 9, 224-235.