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Synthesis and antimicrobial activity of triazolyl analogs of diosgenin

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ABSTRACT

Novel and diverse triazoles of steroidal sapogenin-diosgenin were prepared using click chemistry approach. All the derivatives were subjected to antimicrobial screening to check their antibacterial and antifungal potential. All the tested analogs were inactive towards the tested bacterial strains, while as, some analogs displayed better activity towards the tested fungal strains. Of the synthesized analogs, Dgn-3 and Dgn-6 exhibited antifungal activity against *Candida albicans* with MICs of 12 and 16 µg/ml respectively, while as, Dgn-8 and Dgn-9 showed potency towards both the tested fungal strains: *Candida albicans* and *Aspergillus fumigatus*. The other derivatives showed almost comparable activity with that of the parent molecule. The structure-activity relationship (SAR) revealed that the analogs with electron withdrawing substituents (-NO_2 and -CN) at meta position in R moieties along with analogs bearing bromo substituted R moieties seem to have beneficial impact on the antifungal activity.

Keywords: Diosgenin, click chemistry, antimicrobial activity, triazoles, *Candida albicans*, *Aspergillus fumigates*.

1. INTRODUCTION

Saponins have been found in more than 100 families of plants and a few marine organisms. The name saponin was deduced from the Latin word sapo (soap) reflecting their ability to form stable soap-like foams in aqueous solutions. Saponins are glycosides that consist of a steroidal or triterpenoidaglycone (sapogenin) and one or more sugar chains. The constituents and the connectivity of the saccharides are very diverse and important for the diverse properties of saponins themselves [1,2]. In general, hexoses are the major saccharide component, such as glucose, galactose and rhamnose. Only a few pentoses, such as arabinose and xylose, are found to be the constituents of the saccharide part of saponins, and they are often located on the side chains [3].

Fig 1: Structure of Diosgenin (1)

Diosgenin (25R-spirost-5-en-3β-ol) a steroidal sapogenin belongs to this category, basically hydrosylate of dioscin usually found in the tubers of *Dioscorea*species and widely exists as glucoside in natural plants as triterpene steroidal sapogenin[4].Diosgenin is of a great pharmaceutical importance, found to be a starting material for the generation of corticosteroids, sex harmones, oral contraceptives and other steroidal drugs[5].Diosgenin is among the ten most important sources of steroids and is most oftenly prescribed medicine of plant origin[6]. Click chemistry of natural products has acquired great importance in recent years. Some of the molecules studied include alkaloids[7,8], coumarins[9], saponins[10], steroids[11] and triterpenes such as betulinic acid[12,13]. Triazoles and their derivatives are of great importance in medicinal chemistry and can be used for the synthesis of numerous heterocyclic compounds with different biological activities such as antiviral, antibacterial, antifungal, anti-

tuberculosis, anticonvulsant, antidepressant, anti-inflammatory and anticancer[14,15]. Moreover, synthetic modification of different triazole-thiols was demonstrated as a potent treasure to enhance antimicrobial activities and extend their biological spectrum [16, 17]. Recently, a great number of triazole-thioether and triazole-thione compounds have been reported to demonstrate efficient antibacterial and antifungal activities [18, 19]. Thus, the design and synthesis of novel triazole derivatives is the prospective direction for the development of novel chemotherapeutic agents with better curative effect, lower toxicity as well as higher selectivity.

Based on the above cited findings and inspiration from the potential biological activity of triazoles and our current research on bioprospecting of natural products [15,20], we directed this work towards the synthesis of a diverse series of novel triazolyl derivatives of biological interest using diosgenin as a key template to investigate if these new type of diosgenylsaponins will possess any novel properties. All the newly synthesized compounds were subjected to antimicrobial screening against bacterial and fungal strains to check their antimicrobial potential. This work provides the initial report on structure activity relationship of triazolyl analogs of diosgenin as antimicrobial agents.

2. EXPERIMENTAL

2.1 General methods

All the solvents and reagents for the preparation of extracts, chemical synthesis and biological assays were obtained from Sigma Aldrich.Reactions were carried out in Branson sonicator-3510. All the chemical reactions were monitored by using F_{254} silica gel TLC plates (E. Merck) using ceric ammonium sulphate (charring agent) and UV chamber (366 and 254 nm) for the detection of spots.All the compounds were purified by column chromatography on silica gel (60–120 mesh). ^{1}H NMR and ^{13}C NMR spectra (chemical shifts expressed in δ and coupling constants in Hertz) were recorded on Bruker DPX 400 instrument using $CDCl_3$ as the solvent with TMS as internal standard. High resolution mass spectra (HRMS) were recorded on Agilent Technologies 6540 instrument and melting points of compounds were recorded on Buchi melting point apparatus B-542.

2.1.1 Plant material

The rhizomes of *D. deltoidea* (duly authenticated by the taxonomist) were collected from Sonamarg (J&K), India and the dried voucher specimen (No. DD-13) was deposited in the herbarium of the Institute.

2.1.2 Preparation of plant extract

The air-dried chopped and finely powdered plant material was extracted with DCM:Methanol (1:1) at room temperature for 24 h (three times). The extract was concentrated under reduced pressure at 40 °C using a rotary evaporator (Buchi, Switzerland).

2.1.3 Isolation of Diosgenin (1)

Diosgenin (**1**) was isolated in bulk from the rhizomes of *D. deltoidea*. Diosgenin used in present study was of 98% purity (HPLC analysis) achieved through repeated column chromatography over silica gel 60–120 mesh and the natural product was characterized by spectral data analysis in light of literature [21].

2.1.4 Synthesis of 3-(prop-2-ynyloxy) diosgenin (2) (Scheme 1a)

To a solution of diosgenin (2 g, 12.3 mmol) in dry acetonitrile (10 ml), NaH (1.87 g, 13.1 mmol) was added portion wise and stirred for 15 min at 0 °C. Solution of propargyl bromide (1.1 ml, 14.8 mmol) in dry acetonitrile was then added and the suspension was stirred for 2 h at room temperature. Progress of reaction was monitored using TLC in hexane:ethyl acetate (8:2) at regular intervals. After the completion of reaction, the product was extracted with ethyl acetate (30 mL x 3) which on subsequent purification over silica gel column resulted in the isolation of pure product (2) in 95 % yield. Colourless solid; mp. 117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.79 (s, 3H), 0.80 (d, J = 6.20 Hz; 3H), 0.81 (d, J = 7.10 Hz; 3H), 1.02 (s, 3H), 1.18 (m, 2H) 1.26-2.10 (m, 22H), 2.46 (s, 1H), 3.36 (t, J = 10.6 Hz; 1H), 3.48 (m, 2H), 4.25 (s, 2H), 4.48 (q, J = 7.0 Hz; 1H), 5.33 (br. d, J = 5.30 Hz; 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.55, 16.28, 17.18, 19.46, 20.89, 28.77, 30.32, 31.35, 31.38, 31.43, 31.61, 31.88, 36.64, 37.20, 39.78, 41.60, 41.61, 42.26, 50.04, 56.51, 58.90, 62.84, 66.84, 71.716, 75.82, 78.89, 80.819, 109.29, 121.42, 140.79; ESI-MS *m/z*: Calcd. for $C_{30}H_{44}O_3$ 453.33 $[M+H]^+$, found 453.35.

2.1.5 Development of co-solvent for synthesis of triazolyl analogs

The reaction was performed in various organic co-solvents under sonication, to improve the reaction outcome [22,23,24] and the synthesis of Dgn-1 was successfully accomplished in 2:1 t-BuOH:H_2O solvent system with excellent yield (90%) and the results are summarized in Table 1.

Table1: Development of co-solvent system for the reaction of Dgn-1

Entry	Substrate	product	solvent	co-solvent	yield (%)	time (h)
1	2	Dgn-1	H_2O	t-BuOH	90	0.5
2	2	Dgn-1	H_2O	DMF	35	3
3	2	Dgn-1	H_2O	THF	42	6
4	2	Dgn-1	H_2O	DCM	65	4
5	2	Dgn-1	H_2O	DMSO	76	2

2.1.6 General procedure for synthesis of azides

To a solution of particular aromatic amine in 1,4-Dioxane (at the concentration of 50 mg/mL) at -15.0 °C, 5 equivalents of 2M Sulphuric acid was added in small instalments while stirring. After 5 minutes 2 equivalents of 3M sodium nitrite was added drop wise and after 30 minutes 3 equivalents of 3M sodium azide was added drop wise carefully. Reaction was brought to room temperature and extracted with diethyl ether for at least three times. Organic layers were washed with saturated sodium bicarbonate solution two times, dried over anhydrous sodium sulphate and concentrated to a minimum volume under reduced pressure on Rotary evaporator without making use of heating from water bath [25].

2.1.7 General procedure for synthesis triazolyl derivatives DGN-1 to DGN-17

To a solution of 2(3 mmol, 1 eq) in *t*-BuOH:H_2O (2:1, 5mL), sodium ascorbate (4.0 mg, 0.024 mmol) and $CuSO_4.5H_2O$ (4 mg, 0.015 mmol) were added at room temperature. To this mixture, freshly

prepared organic azide (3 mmol) was added and the reaction mixture was sonicated till its completion. The crude mixture was extracted with ethyl acetate (3×20 mL) and the combined organic layer was dried over sodium sulphate and purified through column chromatography to give pure DGN-1 to DGN-17in 85-92 % yield.

2.1.7.1 ***1-phenyl-(1H-1,2,3-triazol-4yl)-methoxydiosgenin (DGN-1)***

Cream coloured solid; Yield: 89%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.71 (m, 2H), 7.49 (t, J = 8.0 Hz, 2H), 7.41 (t, J = 8.0 Hz, 1H), 5.35 (Br s, 1H), 4.75 (s, 2H), 4.38 (m, 1H), 3.36 (m, 4H), 2.46 (Br d, 1H), 2.42 (m, 1H), 1.98 (m, 4H), 1.23 – 1.95 (17 H), 1.01 (s, 3H), 0.96 (s, 3H), 0.77 (Br d, 3H), 0.75 (Br d, 3H): ^{13}C NMR (101 MHz, CDCl3) δ: 143.30, 140.88, 129.96 (3C), 128.92, 121.84, 120.81 (3C), 109.50, 81.14, 79.21, 67.07, 62.35, 61.86, 61.17, 56.74, 50.32, 41.84, 40.50, 40.00, 39.26, 37.37, 37.23, 32.32, 32.08, 32.03, 30.53, 29.04, 28.55, 21.09, 19.62, 17.35, 16.50, 14.74; ESI-MS *m/z*: Calcd. for $C_{36}H_{49}N_3O_3$ 572.38 $[M+H]^+$, found 572.37.

2.1.7.2 ***1-[2-nitrophenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-2)***

Yellow solid; Yield: 92%; ^{1}H NMR (400 MHz, $CDCl_3$) δ:8.09 (d, J = 8.0 Hz, 1H), 7.98 (s, 1H), 7.81 (t, J = 8.0 Hz,1H), 7.70 (m, 2H), 5.37 (Br s, 1H), 4.79 (s, 2H), 4.40 (m, 1H), 3.35 (m, 4H), 2.45 (Br d, 1H), 2.39 (m, 1H), 2.01 (m, 4H), 1.20 – 1.96 (17 H), 0.99 (s, 3H), 0.95 (s, 3H), 0.78 (Br d, 3H), 0.76 (Br d, 3H); ^{13}C NMR (101 MHz, CDCl3) δ: ^{13}C NMR (100 MHz, CDCl3) δ: 146.47, 146.49, 144.41, 139.88, 133.80, 130.63, 130.37, 127.62, 121.08, 125.45, 124.21,80.49, 79.20, 66.81,62.31, 62.10, 61.72, 56.69, 49.96, 41.44, 40.22, 39.82, 39.35, 37.30, 37.22, 32.41, 32.12, 32.05, 31.13, 29.21, 28.50, 20.83, 19.55, 17.31, 16.62, 14.72; ESI-MS *m/z*: Calcd. for $C_{36}H_{48}N_4O_5$ 617.36 $[M+H]^+$, found 617.37.

2.1.7.3 ***1-[3-nitrophenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-3)***

Yellow solid; Yield: 88%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.58 (s, 1H), 8.28 (d, J = 8.0 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.08 (s, 1H), 7.72 (m, 1H), 5.36 (Br s, 1H), 4.77 (s, 2H), 4.39 (Br s, 1H), 3.35 (m, 3H), 2.42 (m, 1H), 2.26 (m, 1H), 1.97 (m, 3H), 1.85 (m, 2H), 1.05 - 1.74 (17H), 1.01 (s, 3H), 0.95 (Br s, 3H)) 0.77 (Br s, 6H);^{13}C NMR (101 MHz, $CDCl_3$) δ: 149.19, 140.76, 138.03, 131.20, 131.20, 126.14, 123.37, 121.96, 120.60, 115.44, 109.51, 81.03, 79.47, 67.07, 62.34, 61.73, 56.73, 50.31, 41.84, 40.50, 39.99, 39.23, 37.34, 37.23, 32.31, 32.07, 31.66, 30.52, 29.57, 29.03, 22.90, 21.09, 19.61, 17.34, 16.50, 14.73;ESI-MS *m/z*: Calcd. for $C_{36}H_{48}N_4O_5$ 617.36 $[M+H]^+$, found 617.37.

2.1.7.4 ***1-[4-nitrophenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-4)***

Yellow solid; Yield: 86%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.42 (d, J = 9.06 Hz, 2H), 8.25 (s, 1H), 7.98 (d, J = 9.06 Hz, 2H), 5.34 (Br s, 1H), 4.75 (s, 2H), 4.37 (Br s, 1H), 3.36 (m, 3H), 2.45 (m, 1H), 2.29 (m, 1H), 1.95 (m, 3H), 1.82 (m, 2H), 1.01 - 1.75 (17H), 1.01 (s, 3H), 0.93 (Br s, 3H) 0.78 (Br s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 147.61, 147.12, 141.24, 140.44, 125.58, 125.58, 120.56, 120.30, 120.30, 119.26, 109.77, 81.05, 79.42, 67.07, 62.34, 61.73, 56.75, 50.35, 41.83, 40.55, 39.89, 39.25, 37.39, 37.41, 32.33, 32.05, 31.53, 30.55, 29.51, 29.13, 22.89, 21.19, 19.62, 17.41, 16.45, 14.83;ESI-MS *m/z*: Calcd. for $C_{36}H_{48}N_4O_5$ 617.36 $[M+H]^+$, found 617.37.

2.1.7.5 ***1-[2-cyanophenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-5)***

Yellow solid; Yield: 88%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.35 (s, 1H), 7.86 (m, 3H), 7.63 (m, 1H),5.33 (Br s, 1H), 4.78 (s, 2H), 4.39 (Br s, 1H), 3.40 (m, 3H), 2.48 (m, 1H), 2.30 (m, 1H), 1.99 (m, 3H), 1.80 (m, 2H), 1.02 - 1.78 (17H), 1.03 (s, 3H), 0.91 (Br s, 3H) 0.79 (s, 3H), 0.77 (Br s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ:146.70, 140.02, 138.69, 134.39, 134.39, 129.48, 125.39,123.17, 120.21, 116.20, 115.72, 109.88, 81.21, 80.13, 67.12, 62.45, 62.01, 56.77, 50.53, 42.03, 40.75, 39.99, 39.41, 37.48, 37.31, 32.35, 31.95, 31.32, 30.88, 29.72, 29.20, 23.12, 21.02, 19.48, 17.15, 16.47, 14.62; ESI-MS *m/z*: Calcd. for $C_{37}H_{48}N_4O_3$ 597.37 $[M+H]^+$, found 597.36.

2.1.7.6 ***1-[3-cyanophenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-6)***

Pale yellow solid; Yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04 (s, 1H), 8.01 (m, 2H), 7.64 (m, 2H), 5.35 (t, J = 2.40 Hz, 1H), 4.75 (s, 2H), 4.38 (m, 1H), 3.36 (m, 3H), 2.40 (m, 1H), 2.25 (m, 1H), 1.15 - 2.02 (m, 22H), 1.01 (s, 3H), 0.94 (s, 3H), 0.77 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 147.84, 140.76, 137.86, 132.19, 131.10, 124.62, 123.76, 121.94, 120.42, 117.58, 114.43, 109.49, 81.02, 79.42, 67.06, 62.34, 62.16, 61.74, 56.72, 50.31, 41.83, 40.49, 39.99, 39.22, 37.33, 37.22, 32.30, 32.06, 31.65, 30.51, 29.02, 28.53, 21.08, 19.60, 17.34, 16.49, 14.73; ESI-MS *m/z*: Calcd. for $C_{37}H_{48}N_4O_3$ 597.37 $[M+H]^+$, found 597.36.

2.1.7.7 ***1-[4-cyanophenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-7)***

Pale yellow solid; Yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ:8.20 (s, 1H), 7.93 (d, J = 8.8 Hz, 2H), 7.86 (d, J = 8.8 Hz, 2H), 5.37 (m, 1H), 4.70 (s, 2H), 4.33 (m, 1H), 3.34 (m, 3H), 2.41 (m, 1H), 2.23 (m, 1H), 1.10 - 2.00 (m, 22H), 1.03 (s, 3H), 0.95 (s, 3H), 0.78 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ:147.49, 140.21, 139.93, 133.96, 133.96, 120.44, 120.44, 120.40, 120.18, 117.71, 112.35, 109.76,80.92, 79.31, 67.12, 62.28, 61.96, 61.62, 56.55, 50.36, 42.12, 40.68, 40.08, 39.28, 37.35, 37.22, 32.31, 32.00, 31.55, 30.23, 29.12, 28.88, 21.42, 19.62, 17.43, 16.52, 14.62; ESI-MS *m/z*: Calcd. for $C_{37}H_{48}N_4O_3$ 597.37 $[M+H]^+$, found 597.36.

2.1.7.8 ***1-[2-Bromophenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-8)***

Creamy white solid; Yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06 (s, 1H), 7.75 (m, 1H), 7.55 (m, 1H), 7.49 (m, 1H), 7.38 (m, 1H), 5.35 (m, 1H), 4.70 (s, 2H), 4.32 (m, 1H), 3.31 (m, 3H), 2.38 (m, 1H), 2.25 (m, 1H), 1.08 - 2.02 (m, 22H), 1.01 (s, 3H), 0.98 (s, 3H), 0.79 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 145.29, 141.08 136.70, 133.94, 131.07, 128.48, 128.20, 124.73, 120.22, 118.48, 109.78, 81.44, 79.78, 67.22, 62.17, 61.68, 61.60, 56.62, 50.51, 42.18, 40.54, 40.08, 39.32, 37.29, 37.16, 32.30, 32.06, 31.61, 30.22, 29.18, 28.72, 21.45, 19.62, 17.38, 16.50, 14.71; ESI-MS *m/z*: Calcd. for $C_{36}H_{48}BrN_3O_3$ 650.29 $[M+H]^+$, found 650.29.

2.1.7.9 ***1-[4-Bromophenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-9)***

Yellowish solid; Yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09 (s, 1H), 7.66 (d, J = 9.1 Hz, 2H), 7.63 (d, J = 9.1 Hz, 2H), 5.32 (Br s, 1H), 4.77 (s, 2H), 4.40 (Br s, 1H), 3.41 (m, 3H), 2.47 (m, 1H), 2.31

(m, 1H), 1.99 (m, 3H), 1.81 (m, 2H), 1.02 - 1.78 (17H), 1.04 (s, 3H), 0.91 (Br s, 3H) 0.79 (s, 3H), 0.76 (Br s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 146.15, 140.44, 135.12, 131.91, 131.91, 121.28, 120.80, 120.80, 120.02, 119.59, 108.72, 81.15, 79.22, 67.09, 62.39, 61.86, 61.18, 56.75, 50.30, 41.85, 40.52, 40.01, 39.26, 37.38, 37.22, 32.31, 32.07, 32.02, 30.53, 29.12, 28.62, 21.12, 19.55, 17.56, 16.48, 14.90; ESI-MS *m/z*: Calcd. for $C_{36}H_{48}BrN_3O_3$ 650.29 $[M+H]^+$, found 650.29.

2.1.7.10 ***1-[4-Fluorophenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-10)***

White solid; Yield: 89%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (s, 1H), 7.83 (Br d, J = 9.1 Hz, 2H), 7.69 (Br d, J = 9.1 Hz, 2H), 5.36 (Br s, 1H), 4.78 (s, 2H), 4.44 (Br s, 1H), 3.45 (m, 3H), 2.49 (m, 1H), 2.33 (m, 1H), 2.08 (m, 3H), 1.85 (m, 2H), 1.01 - 1.80 (17H), 1.05 (s, 3H), 0.92 (Br s, 3H) 0.80 (s, 3H), 0.77 (Br s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 161.82, 147.18, 141.20, 135.12, 132.22, 132.22, 121.25, 121.25, 120.82, 119.68, 109.29, 81.17, 80.16, 67.12, 61.91, 61.59, 61.20, 56.72, 50.32, 42.24, 40.61, 40.22, 39.26, 37.34, 37.28, 32.36, 32.18, 32.17, 30.50, 29.15, 28.52, 21.22, 19.60, 17.59, 16.62, 14.92; ESI-MS *m/z*: Calcd. for $C_{36}H_{48}FN_3O_3$ 590.37 $[M+H]^+$, found 590.37.

2.1.7.11 ***1-Napthalen-1-yl-(1H-1,2,3-triazol-4yl)-methoxydiosgenin (DGN-11)***

White solid; Yield: 87%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.32 (m, 1H), 7.93 (m, 1H), 7.70 (m, 2H), 7.55 (m, 3H), 7.33 (m, 1H), 5.35 (Br s, 1H), 4.75 (s, 2H), 4.46 (Br s, 1H), 3.48 (m, 3H), 2.51 (m, 1H), 2.35 (m, 1H), 2.08 (m, 3H), 1.86 (m, 2H), 1.01 - 1.82 (17H), 1.03 (s, 3H), 0.94 (s, 3H) 0.82 (s, 3H), 0.79 (Br s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 145.5, 140.32, 139.06, 137.48, 132.33, 129.19, 129.19, 127.83, 126.93, 126.32, 126.32, 125.28, 120.20, 119.16, 109.77,80.92, 79.44, 67.31, 62.52, 61.83, 61.54, 56.53, 50.12, 42.14, 40.68, 40.12, 39.22, 37.27, 37.15, 32.30, 32.06, 31.52, 30.38, 29.16, 28.80, 21.46, 19.74, 17.42, 16.78, 14.72; ESI-MS *m/z*: Calcd. for $C_{40}H_{51}N_3O_3$ 622.39 $[M+H]^+$, found 622.40.

2.1.7.12*1-[2-Methoxyphenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-12)*

Yellowish solid; Yield: 92%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.2 (s, 1H), 7.77 (d, J =8 Hz, 1H), 7.42 (m, 1H), 7.10 (m, 2H), 5.35 (Br s, 1H), 4.78 (s, 2H), 4.41 (m, 1H), 3.88 (s, 3H), 3.36 (m, 4H), 2.48 (Br d, 1H), 2.38 (m, 1H), 2.02 (m, 4H), 1.21 – 1.96 (17 H), 0.99 (s, 3H), 0.96 (s, 3H), 0.79 (Br d, 3H), 0.77 (Br d, 3H); ^{13}C NMR (100 MHz, CDCl3) δ: 151.12, 140.11 130.04, 126.32, 125.67, 125.50, 121.34, 121.22, 120.10, 112.19, 109.74, 81.22, 80.14, 67.12, 62.48, 62.02, 56.79, 55.92, 50.53, 42.05, 40.76, 39.96, 39.43, 37.47, 37.30, 32.42, 31.88, 31.31, 30.63, 29.70, 29.20, 23.13, 21.06, 19.49, 17.20, 16.48, 14.70; ESI-MS *m/z*: Calcd. for $C_{37}H_{51}N_3O_4$ 602.39 $[M+H]^+$, found 602.40.

2.1.7.13 ***1-[3-Methoxyphenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-13)***

Yellowish solid; Yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11 (s, 1H), 7.38 (m, 2H), 7.26 (m, 1H), 6.98 (m, 1H), 5.33 (Br s, 1H), 4.80 (s, 2H), 4.44 (m, 1H), 3.85 (s, 3H), 3.34 (m, 4H), 2.49 (Br d, 1H), 2.42 (m, 1H), 1.99 (m, 4H), 1.20 – 1.98 (17 H), 0.98 (s, 3H), 0.95 (s, 3H), 0.78 (Br d, 3H), 0.76 (Br d, 3H); ^{13}C NMR (101 MHz, CDCl3) δ: ^{13}C NMR (100 MHz, CDCl3) δ: 145.96, 139.88, 137.91, 130.49, 120.91, 120.45, 119.76, 114.49, 112.29, 109.65, 106.21, 80.89, 80.02, 67.42, 63.12, 62.03, 56.60, 56.12, 50.88, 42.13, 41.17, 39.82, 39.30, 36.98, 36.87, 32.33, 32.02, 31.44, 30.65, 29.88, 29.22, 23.43, 21.14, 19.65, 17.22, 16.62, 14.71; ESI-MS *m/z*: Calcd. for $C_{37}H_{51}N_3O_4$ 602.39 $[M+H]^+$, found 602.40.

2.1.7.14*1-[4-Methoxyphenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-14)*

Yellowish solid; Yield: 88%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.02 (s, 1H), 7.63 (d, J= 8.8 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 5.37 (Br s, 1H), 4.82 (s, 2H), 4.48 (m, 1H), 3.83 (s, 3H), 3.36 (m, 4H), 2.42 (m, 1H), 2.38 (m, 1H), 1.89 (m, 4H), 1.17 – 2.00 (17 H), 1.02 (s, 3H), 0.95 (s, 3H), 0.79 (Br d, 3H), 0.77 (Br d, 3H); ^{13}C NMR (100 MHz, CDCl3) δ: 159.73, 146.16, 140.02, 130.58, 122.11, 122.11, 121.03, 120.22, 114.74, 114.74, 109.34, 81.20, 80.11, 67.21, 64.17, 62.23, 56.62, 56.23, 49.93, 42.14, 41.04, 39.91, 39.32, 36.56, 36.44, 32.35, 31.96, 31.41, 30.52, 30.06, 29.47, 23.40, 21.31, 19.60, 17.22, 16.60, 14.72; ESI-MS *m/z*: Calcd. for $C_{37}H_{51}N_3O_4$ 602.39 $[M+H]^+$, found 602.40.

2.1.7.15*1-[3,4,5-Trimethoxyphenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-15)*

Yellowish solid; Yield: 82%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.05 (s, 1H), 6.96 (s, 2H), 5.35 (Br s, 1H), 4.85 (s, 2H), 4.43 (m, 1H), 3.93 (s, 6H) 3.89 (s, 3H), 3.32 (m, 4H), 2.45 (m, 1H), 2.39 (m, 1H), 1.92 (m, 4H), 1.20 – 2.04 (17 H), 1.02 (s, 3H), 0.94 (s, 3H), 0.78 (Br d, 3H), 0.77 (Br d, 3H); ^{13}C NMR (100 MHz, CDCl3) δ: 153.88, 153.88, 146.42, 140.10, 138.12, 133.00, 121.09, 120.28, 109.29, 98.42, 98.42, 81.43, 79.85, 68.12, 64.33, 62.47, 59.93, 56.42, 56.42, 56.21, 50.11, 42.24, 40.82, 39.97, 39.33, 36.59, 36.50, 32.57, 32.06, 31.38, 30.35, 30.08, 29.72, 24.22, 21.89, 19.62, 17.20, 16.63, 14.75; ESI-MS *m/z*: Calcd. for $C_{39}H_{55}N_3O_6$ 661.41 $[M+H]^+$, found 661.42.

2.1.7.16*1-[2,5-Dimethylphenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-16)*

Whitish solid; Yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.82 (s, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.07 (m, 1H), 6.97 (m, 1H), 5.39 (s, 1H), 4.83 (s, 2H), 4.49 (m, 1H), 3.35 (m, 4H), 2.48 (m, 1H), 2.42 (m, 1H), 2.37 (s, 3H), 2.19 (s, 3H), 1.93 (m, 4H), 1.20 – 2.02 (17 H), 1.02 (s, 3H), 0.95 (s, 3H), 0.79 (Br d, 3H), 0.76 (Br d, 3H); ^{13}C NMR (100 MHz, CDCl3) δ: 145.87, 140.18, 138.69, 137.68, 134.51, 134.51, 133.35, 133.05, 123.97, 120.28, 109.29, 81.45, 79.83, 68.10, 64.25, 62.19, 56.20, 49.55, 42.45, 40.39, 39.82, 39.32, 36.66, 36.49, 33.15, 32.19, 31.48, 30.33, 30.16, 29.88, 25.33, 25.30,24.35, 21.92, 19.65, 17.20, 16.65, 14.74; ESI-MS *m/z*: Calcd. for $C_{38}H_{53}N_3O_3$ 599.41 $[M+H]^+$, found 599.41.

2.1.7.17 ***1-[4-Hydroxymethylphenyl-(1H-1,2,3-triazol-4yl)]-methoxydiosgenin (DGN-17)***

White solid; Yield: 88%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.03 (s, 1H), 7.68 (s, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.41 (t, J = 8.0 Hz, 1H), 7.36 (t, J = 8.0 Hz, 1H), 5.36 (m, 1H), 4.73 (s, 2H), 4.60 (s, 2H), 4.39 (m, 1H), 3.36 (m, 3H), 2.41 (m, 1H), 2.23 (m, 1H), 1.12 - 2.02 (m, 22H), 1.03 (s, 3H), 0.95 (s, 3H), 0.79 (Br s, 3H), 0.77 (Br s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 146.46, 143.11, 140.21, 137.31, 129.87, 126.81, 120.88, 119.40, 118.67, 114.1, 109.77, 81.21, 80.13, 67.12, 64.41, 62.24, 61.46, 56.72, 49.73, 42.15, 41.08, 39.97, 39.33, 37.52, 37.31, 32.42, 31.90, 31.66, 30.83, 29.28, 28.31, 23.27, 21.16, 19.50, 17.16, 16.55, 14.72; ESI-MS *m/z*: Calcd. for $C_{37}H_{51}N_3O_4$ 602.39 $[M+H]^+$, found 602.38.

2.2 Biology

2.2.1Chemicals

Acetylcholine (ACh), dimethyl sulfoxide (DMSO), dipotassium hydrogen phosphate ($K_2HPO_4 \cdot 3H_2O$), disodium hydrogen orthophosphate,ethylenediaminetetraacetic acid (EDTA), ethanol, glucose agar, imipenin, linoleic acid, lipoxygenase (1.13.11.12) type I-B, lithium chloride ($LiCl_2$), permethrin, phenol, sabouraud, sodium hydroxide, sodium nitropruside, sodium hypochlorite (NaOCl), sodium dihydrogen orthophosphate and ureases were purchased from Sigma Aldrich, USA. Bacterial and fungal strains were collected from Division of Pharmaceutical Sciences, IIIM Srinagar India. The solvents: n-butanol, ethyl acetate, hexane, chloroform and methanol were obtained from Merck, Darmstadt, Germany. Chemicals used for making physiological salt solutions were: potassium chloride (Sigma Aldrich, USA), calcium chloride, glucose, magnesium chloride, sodium bicarbonate, sodium dihydorgen phosphate (Merck, Darmstadt, Germany) and sodium chloride from S D Fine Chem Limited, Mumbai India. All chemicals used were of the analytical grade available.

2.2.2 Microbial Strains and culture media

The antibacterial activity was tested against a panel of 6 bacterial and 2 fungal strains. The bacterial strains used were Gram positive *Staphylococcus aureus*ATCC-25923 and *Bacillus subtilis* ATCC-25955, Gram negative bacteria such as *Escherichia coli* ATCC-25922, *Pseudomonas aeruginosa*ATCC-27853, *Klebseilla pneumonia* ATCC-25924, *Shigellaflexneri*ATCC-12022obtained from American Type Cultures Collection (Manassas, VA, USA).The medium used was Muller Hinton Agar and Muller Hinton Broth (Becton-Dickinson, Cockeysville, MD, USA; DIFCO laboratories). The cultures were maintained on Tryptone soya agar and stored at -70°C containing 50% glycerol (Himedia, Mumbia, India). The five fungal strains i.e. *Candida albicans* and*Aspergillusfumigatus* obtained from Mycology section, Department of Microbiology, SKIMS, J&K, India and Institute of Basic Medical Sciences (IBMS) were grown on Sabouraud dextrose agar (SDA) plates at 25°C and maintained on SDA slants. Cell suspension of microorganisms in 0.9% NaCl was adjusted at 0.5 McFarland to obtain approximately 10^6 CFU/mL.

2.2.3 Antibacterial activity assay

Antibacteria activity was determined as per the guidelines ofClinical and Laboratory Standards Institute (Formerly the National Committee for Clinical Laboratory Standards) (Clinical and Laboratory Standards Institute, 2006) [26]. Bacterial suspensions were prepared by suspending 18 h grown bacterial culture in sterile normal saline. The turbidity of the bacterial suspension was adjusted to 0.5 McFarland standards (equivalent to 1.5x108 CFU/mL) at wavelength 625nm. The 2-fold serial of the essential oil and individual constituents (stock solution prepared in dimethylsulphoxide) were prepared in Mueller Hinton Broth (MHB; DIFCO laboratories) in 100 µL volume in 96-well U bottom microtitre plates (Tarson, Mumbai, India). The above mentioned bacterial suspension was further diluted in the MHB and 100 µL volume of this diluted inoculum was added to each well of the plate resulting in the final inoculum of $5x10^5$ CFU/mL in the well and the final concentrations of samples ranged from 2000 to 3.90 µg/mL till 10th column. Column 11 and column 12, containing 100 µL and 200 µL of medium without drug, served as growth and medium control respectively. Gentamicin, streptomycin and ciprofloxacin (Sigma-Aldrich) were used as positive control. The plates were incubated at 37 °C for 18 h. The plates were visually read and the minimum concentration of the compound showing no turbidity was recorded as MIC.

2.2.4 Antifungal activity assay

A macrodilution broth method was used to determine the Minimal Inhibitory Concentrations (MIC) according to NCCLS references [26]. The serial doubling dilution of each oil was prepared in dimethyl sulfoxide (DMSO), with concentrations ranging from 0.05 to 130 µg/mL. Final concentration of DMSO never exceeded 2%. Recent cultures of each strain were used to prepare the cell suspension adjusted 1-2 × 10^4 cells/mL. The concentration of cells was confirmed by viable count on Sabouraud agar. The test tubes were incubated aerobically at 35 °C for 48 h/72 h and MICs were determined. In addition, two reference antifungal compounds, amphotericin B (Fluka) and fluconazole (Pfizer) were used to control the sensitivity of tested microorganisms. All tests were performed in RPMI medium. For each strain tested, the grow conditions and the sterility of the medium were checked in two control tubes. The innocuity of the DMSO was also checked at the highest tested concentration. All experiments were performed in triplicate and repeated if the results differed.

3. RESULTS AND DISCUSSION

3.1 Chemistry

The bioactivity profile of diosgenin (**1**) prompted us to exploit its unique pharmacophore as a basic template to synthesise its novel analogs with improved antimicrobial activity keeping its stereochemistry rich framework intact. In this perspective, a click reaction involving copper(I)-catalysed union of terminal alkynes with organic azides has been exploited for the synthesis of novel 1,4-disubstituted 1,2,3-triazolyl derivatives of diosgenin in good yields. The copper(I)-catalysed click chemistry procedure (Huisgen 3+2 cycloaddition) is the best click reaction [27] known to date and exhibits remarkably broad scope and exquisite selectivity and has contrasting applications in chemistry, biology, and materials science [28].

In this study, diosgenin (**1**) was used as a key template, alkylated at the only hydroxyl (C-3) position using propargyl bromide and NaH (base) in dry acetonitrile to form its propargylated product (**2**) (Scheme-1). Proton singlets (apparent) at δ 2.48 ppm and 4.26 ppm (integrating for one and two protons respectively) represent terminal alkyne proton and two methylenic protons respectively. The other proton signals along with ^{13}C NMR and ESIMS (453.33) data confirmed the structure of **2**. Compound **2** was subjected to 1,3-dipolar cycloaddition reaction typically called Huisgencycloaddition with various freshly prepared organic azides under Sharpless click chemistry conditions [$CuSO_4.5H_2O$ and sodium ascorbate in *t*-BuOH:H_2O (2:1)] to afford regioselectively 1,4-disubstituted 1,2,3-triazole analogs (Dgn-1 to Dgn-17) in excellent yields (Scheme-1). The solvent system *t*-BuOH:H_2O (2:1) was optimized for this reaction after working out several permutations and combinations [Table 1]. A series of Dgn analogs was prepared to look for a possible structure-activity relationship. The structures of all the synthesized triazolyl derivatives were established by analytical and spectral data analysis. Formation of products was confirmed by a downfield H-5 proton singlet (almost around 8.0 ppm) of triazole moiety and other proton resonances in the aromatic region along with the aliphatic signals of

the starting material (diosgenin). Further characterization of all the products was done using ^{13}C NMR-DEPT and ESI-MS data.

Scheme 1: Preparation of triazolyl analogues of diosgenin usingCu-(I)-catalyzed Huisgen 1, 3-dipolar cycloaddition.

3.2 Biology

Natural products (NPs) have offered a vast source of compounds throughout ages, that have found distinct applications in the fields of medicine, pharmacy and biology [29]. A number of important new commercialised drugs have been obtained from natural sources, by structural modification of natural compounds, or by the synthesis of new compounds, designed following a natural compound as template [30]. Moreover, semi-synthesis processes of new compounds, obtained by molecular modification of the functional groups of lead compounds, are able to generate structural analogs with better pharmacological activity and with fewer side effects [31]. As part of our on-going research program on bio-prospection of natural products, all the newly synthesized analogs (Dgn-1 to Dgn-17) prepared through click reaction were screened for antimicrobial activity against bacterial (*S. aureus* and *E. coli*) and fungal pathogens (*C. albicans* and *A. fumigatus*) via agar tube dilution method. Preliminary antimicrobial screening of the analogs was carried out at 3mg/ml concentration and activity was determined. All the analogs were further assayed at different concentrations (0.1 – 3mg/ml) to determine the MIC values (Table-2). Ciprofloxacin and Amphotericin B (potent broad-spectrum antimicrobial agents) were used as positive control in this method. Interesting to note that diosgenin and all the synthesised analogs were impotent towards bacterial pathogens and diosgenin as such was found to be inactive against fungal pathogens as well.

Table 2: MIC (µg/ml) determination of the Active sample

S.No.	Sample Code	MIC (µg/ml)	
		C. albicans	*A. fumigatus*
1	Dgn-3	12	NA
2	Dgn-6	16	NA
3	Dgn-8	32	28
4	Dgn-9	64	64
5	Amphotericin B	**0.5**	**0.5**

NA: Not active

However some of the synthesized analogs were active against fungal pathogens. Among the active analogs, Dgn-3 and Dgn-6 exhibited antifungal activity against *C. albicans* with MIC of 12 and 16 µg/ml respectively, while as, Dgn-8 and Dgn-9 showed potency towards both the tested fungal strains: *C. albicans* and *A. fumigatus*. The other derivatives showed almost comparable activity with that of the parent molecule. The structure-activity relationship (SAR) revealed that the analogs with electron withdrawing substituents (-NO_2 and -CN) at meta position in R moieties along with analogs bearing bromo substituted R moieties seem to have beneficial impact on the antifungal activity. It is noteworthy to mention that the analogs with electron withdrawing substituents (-NO_2 and -CN) at ortho/para position in R moieties were inactive towards both the tested fungal strains. The SAR studies of diosgenin revealed an interesting finding that these steroidal triazolyl analogs will have no effect on bacterial pathogens but have potential to be called antifungal agents.

4. CONCLUSION

In conclusion, the current study demonstrates the antimicrobial activity of a novel library of 3-*O*-tethered triazolyl derivatives of diosgenin, created through regioselective click chemistry approach and characterized by spectral data analysis. All the derivatives displayed negligible antibacterial activity, while as, Dgn-3 and Dgn-6 exhibited selective antifungal activity against *C. Albicans* with MIC 12 and 16 µg/ml respectively. In addition to this, Dgn-8 and Dgn-9 exhibited potent antimicrobial activity against both the tested fungal pathogens. However the efficacy of these analogs does not overpower the efficacy of control. Systematic change of position of the functionalities in R moiety might be of interest to optimize these diosgenin analogs against the targets of therapeutic relevance.

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