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Synthesis, anti-microbial activity and molecular docking studies on triazolylcoumarin derivatives

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Abstract. A series of triazolylcoumarins was synthesized by the cycloaddition of acetylenic derivatives to azide in the presence of Cu(I) catalyst at room temperature. All the synthesized compounds were evaluated for their anti-microbial activity against Gram-positive (*B. subtilis* and *S. aureus*), Gram-negative bacteria (*K. pneumonia* and *P. vulgaris*) and human pathogenic fungi (*C. tropicalis* and *C. krusei*), with tetracycline and fluconazole as standards for anti-microbial and anti-fungal activity. Triazolylcoumarins exhibit anti-microbial activity against all the tested pathogens, which is further supported by molecular docking studies.

Keywords. Coumarin; 1,2,3-triazole; anti-microbial; molecular docking and ADMET.

1. Introduction

During the last decade, due to the increase in the number of immuno-compromised hosts, the incidence of systemic microbial infection has been increasing drastically. Further, most of the microorganisms develop resistance over a period of time against available drugs. Hence, the available anti-microbial medicines are either less effective or ineffective. There is a need to search for alternative antimicrobial agents. Coumarin, known as 1,2-benzopyrone, occurs naturally in plants, notably in high concentration in the tonka bean, vanilla grass, sweet woodruff and mullein. Coumarin and its derivatives attract great attention due to their wide range of biological activities such as anti-cancer,[1] anti-microbial,[2] anti-HIV,[3] antioxidant,[4] anti-viral,[5] anti-inflammatory,[6] anti-coagulant[7] and as inhibitors of lipoxygenase[8] and cyclooxygenase.[9]

Click chemistry[10–12] has emerged as a reliable approach for the stereo selective synthesis of 1,2,3-triazole with desired properties. Cycloaddition of azide to alkyne in the presence of copper sulphate and sodium ascorbate to give 1,2,3-triazole has drawn considerable attention due to its wide range of biological activities[13,14] as well as in material applications.[15] Click reaction is unique due to high yield and there is no need for a protection or deprotection protocol. Further, 1,2,3-triazole ring system is highly stable under hydrolytic as well as under reductive and oxidative conditions. Recent synthesis have focused on the design of triazole based coumarin compounds using CuAAC reaction.[16,17]

In fact, compounds with the combination of coumarin with triazole systems will be interesting and such molecules may have better bioactivity than coumarin itself. Hence, the aim of the present investigation is to synthesize and characterize triazole-based coumarin derivatives **1–8** (figure 1) and screen them for their *in vitro* anti-microbial activity. Molecular docking and Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of the synthesized coumarin derivatives have also been studied.

2. Experimental

2.1 *General*

All melting points were determined using a Toshniwal melting point apparatus by the open capillary tube

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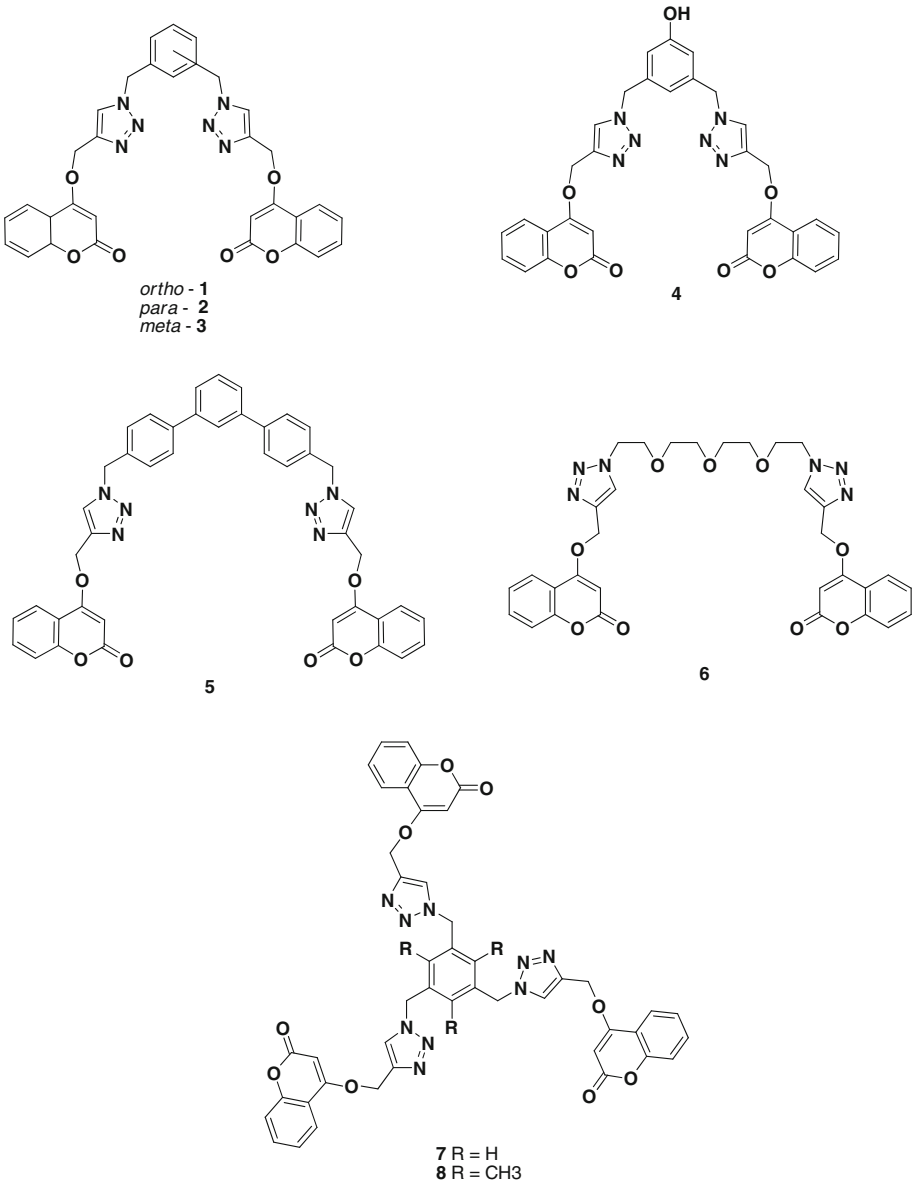


Figure 1. Molecular structures of triazolylcoumarins **1–8**.

method and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 300 MHz instrument. The mass spectra (ESI-electron spray ionization) were recorded on a Perkin Elmer Sciex mass spectrophotometer. Elemental analyses were carried out using a Perkin Elmer CHNS 2400 instrument. Column chromatography was performed on silica gel (ACME, 100–200 mesh). Routine monitoring of the reaction was made using thin layer chromatography developed on glass plates coated with silica gel-G (ACME) of 25 mm thickness and visualized with iodine. All the reagents and solvents employed were of the best grade available and used without further purification.

2.2 *General procedure for the synthesis of di and tri azides from di and tri bromides **(18–25)***

To corresponding di or tri bromides (1 mmol) dissolved in dry DMF (20 mL), sodium azide (1.5 mmol) was added and stirring was continued at room temperature for 12 h. The reaction mixture was poured into water (30 mL) and extracted with $CHCl_3$ (3 × 100 mL). The organic layer was washed with water (100 mL) and saturated NaCl (3 × 100 mL), dried ($MgSO_4$). Solvent was evaporated under reduced pressure to afford the crude product, which was purified by column chromatography (SiO_2).

2.2a *4,4″-bis(azidomethyl)-1,1′:3′,1″-terphenyl (**22**)*: Yield 0.25 g (81%); M.p. 96–98°C; ^{1}H NMR: (300 MHz, $CDCl_3$)δ 2.32 (s, 4H), 7.12 (s, 1H), 7.14 (d, 2H), 7.16 (s, 4H), 7.29 (s, 4H), 7.31(s, 1H). ^{13}C NMR: (75 MHz, $CDCl_3$)δ 54.5, 128.4, 128.5, 128.8, 129.2, 129.8, 137.2, 145.5, 148.1. HRMS m/z = 341.40 $(M+1)^+$; Elemental Anal. Calcd. for $C_{20}H_{16}N_6$: C, 70.57; H,4.74; N, 24.69; Found C, 70.27; H, 4.86; N, 24.31. Elemental Analysis: C, 70.57; H, 4.74; N, 24.69

2.3 *General procedure for the Cu(I)-catalyzed Huisgen click reaction (**1–8**)*

Acetylenic derivative (1.0 mmol) was added to azide (0.5 mmol) in a mixture of THF and water (1:1) solution. Sodium ascorbate (10 mol%) was added to the reaction mixture, followed by the addition of $CuSO_4.5H_2O$ (5 mol%). The reaction mixture was stirred overnight at room temperature and after completion of the reaction the solvent was evaporated under reduced pressure and the crude product was dissolved in ethyl acetate (100 mL), washed with water (100 mL), brine solution (50 mL) and dried (Na_2SO_4). Evaporation of the solvent afforded a residue which was purified by column chromatography (silica gel) with $CHCl_3$/MeOH (9:1) as an eluent to give the corresponding triazolylcoumarins.

2.3a *4-((1-(2-((4-(((2-oxo-4a,8a-dihydro-2H-chromen-4-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)benzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one (**1**)*: Yield 0.33 g (70%); M.p. 234–236°C; ^{1}H NMR (300 MHz, DMSO-d_6)δ 5.43 (s, 4H), 5.91 (s, 4H), 6.15 (s, 2H), 7.19–7.22 (m, 2H), 7.30–7.41 (m, 6H), 7.64 (t, 2H, J = 7.8 Hz), 7.75 (d, 2H, J = 7.8 Hz), 8.44 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6)δ 50.0, 62.7, 91.3, 115.0, 116.4, 122.9, 124.2, 125.6, 128.9, 129.3, 132.8, 134.1, 141.3, 152.7, 161.5, 164.3; MALDI-TOF-MS m/z = 611.39 $(M+Na)^+$, 627.39 $(M+K)^+$; Elemental Anal. Calcd. for $C_{32}H_{24}N_6O_6$: C, 65.30; H,4.11; N, 14.28; Found C, 65.26; H, 4.12; N, 14.34.

2.3b *4-((1-(4-((4-(((2-oxo-4a,8a-dihydro-2H-chromen-4-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)benzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one (**2**)*: Yield 0.31 g (66%); M.p. 248–250°C; ^{1}H NMR (300 MHz, DMSO-d_6)δ 5.41 (s, 4H), 5.65 (s, 4H), 6.15 (s, 2H), 7.30–7.42 (m, 8H), 7.66 (t, 2H, J = 7.5 Hz), 7.73 (d, 2H, J = 7.8 Hz), 8.44 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6)δ 52.5, 62.7, 91.3, 115.0, 116.4, 122.8, 124.2, 125.3, 128.5, 132.8, 135.9, 141.3, 152.7, 161.5, 164.3; HRMS m/z = 591 $(M+1)^+$; Elemental Anal. Calcd. for $C_{32}H_{24}N_6O_6$: C, 65.30; H,4.11; N, 14.28; Found C, 65.28; H, 4.12; N, 14.31.

2.3c *4-((1-(3-((4-(((2-oxo-4a,8a-dihydro-2H-chromen-4-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)benzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one (**3**)*: Yield 0.34 g (72%); M.p. 213–215°C; ^{1}H NMR (300 MHz, DMSO-d_6)δ 5.40 (s, 4H), 5.67 (s, 4H), 6.12 (s, 2H), 7.31–7.45 (m, 8H), 7.64 (t, 2H, J = 7.8 Hz), 7.72 (d, J = 7.8 Hz, 2H), 8.46 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6)δ 52.6, 62.7, 91.3, 114.9, 116.4, 122.8, 124.2, 125.4, 127.5, 127.9, 129.3, 132.8, 136.5, 141.2, 152.7, 161.5, 164.3; MALDI-TOF-MS m/z = 611.49 $(M+Na)^+$, 627.49 $(M+K)^+$; Elemental Anal. Calcd. for $C_{32}H_{24}N_6O_6$: C, 65.30; H,4.11; N, 14.28; Found C, 65.35; H, 4.16; N, 14.29.

2.3d *4,4′-(((1,1′-((5-hydroxy-1,3-phenylene)bis(methylene))bis(1H-1,2,3-triazole-4,1-diyl))bis(methylene))bis(oxy))bis(2H-chromen-2-one) (**4**)*: Yield 0.42 g (71%); M.p. 237–239°C; ^{1}H NMR (300 MHz, DMSO-d_6)δ 5.40 (s, 4H), 5.57 (s, 4H), 6.14 (s, 2H), 6.66 (s, 2H), 6.76 (s, 1H), 7.31–7.41 (m, 4H), 7.64 (t, 2H, J = 7.2 Hz), 7.73 (d, 2H, J = 7.5 Hz), 8.44 (s, 2H), 9.77 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6)δ 52.6, 62.7, 91.2, 114.5, 114.9, 116.4, 117.8, 122.8, 124.2, 125.4, 132.8, 137.7, 141.2, 152.7, 157.9, 161.6, 164.3; MALDI-TOF-MS m/z = 627.72 $(M+Na)^+$, 643.71 $(M+K)^+$; Elemental Anal. Calcd. for $C_{32}H_{24}N_6O_7$: C, 63.57; H,4.00; N, 13.90; Found C, 63.58; H, 4.00; N, 13.88.

2.3e *4,4′-(((1,1′-(1,1′:3′,1″-terphenyl-4,4″-diylbis(methylene))bis(1H-1,2,3-triazole-4,1-diyl))bis(methylene))bis(oxy))bis(2H-chromen-2-one) (**5**)*: Yield 0.50 g (76%); M.p. 222–224°C; ^{1}H NMR (300 MHz, DMSO-d_6)δ 5.45 (s, 4H), 5.73 (s, 4H), 6.18 (s, 2H), 7.20–7.27 (m, 8H), 7.38–7.42 (m, 8H), 7.65 (d, 2H, J = 7.5 Hz), 7.76 (d, 2H, J = 7.8 Hz), 8.54 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6)δ 52.6, 62.8, 91.3, 115.0, 116.4, 122.9, 124.2, 125.5, 127.5, 128.5, 129.0, 129.6, 135.1, 136,7, 141.3, 142.2, 146.9, 152.7, 161.5, 164.3; HRMS m/z = 741.59 $(M+1)^+$; Elemental Anal. Calcd. for $C_{44}H_{32}N_6O_6$: C, 71.34; H,4.35; N, 11.35; Found C, 71.35; H, 4.39; N, 11.31.

2.3f *4,4′-(((1,1′-(((oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(1H-1,2,3-triazole-4,1-diyl))bis(methylene))bis(oxy))bis(2H-chromen-2-one) (**6**)*: Yield 0.30 g (75%); M.p. 138–140°C; ^{1}H NMR (300 MHz, DMSO-d_6)δ 3.39–3.45 (m, 4H), 3.48–3.50 (m, 4H), 3.83 (t, 4H, J = 4.8 Hz), 4.57 (t, 4H, J = 4.8 Hz),

5.42 (s, 4H), 6.17 (s, 2H), 7.31 (t, 2H, J = 7.5 Hz), 7.39 (d, 2H, J = 8.1 Hz), 7.64 (t, 2H, J = 7.5 Hz), 7.71 (d, 2H, J = 7.8 Hz), 8.35 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6)δ 49.5, 62.7, 68.6, 69.5 (2C), 91.3, 115.0, 116.4, 122.7, 124.1, 125.6, 132.7, 140.8, 152.7, 161.5, 164.3; MALDI-TOF-MS m/z = 667.52 $(M+Na)^+$, 683.52 $(M+K)^+$; Elemental Anal. Calcd. for $C_{32}H_{32}N_6O_9$: C, 59.62; H,5.00; N, 13.04; Found C, 59.60; H, 5.01; N, 13.00.

2.3g *4,4′,4″-(((1,1′,1″-(benzene-1,3,5-triyltris(methylene))tris(1H-1,2,3-triazole-4,1-diyl))tris(methylene))tris(oxy))tris(2H-chromen-2-one) (**7**)*: Yield 0.51 g (74%); M.p. 232–234°C; ^{1}H NMR (300 MHz, DMSO-d_6)δ 5.37 (s, 6H), 5.67 (s, 6H), 6.08 (s, 3H), 7.29–7.38 (m, 9H), 7.62 (t, 3H, J = 7.8 Hz), 7.72 (d, 2H, J = 7.8 Hz), 8.46 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6)δ 52.4, 62.7, 91.2, 114.9, 116.4, 122.9, 124.1, 125.4, 127.4, 132.7, 137.2, 141.2, 152.7, 161.5, 164.2; HRMS m/z = 845.3 $(M+1)^+$; Elemental Anal. Calcd. for $C_{45}H_{33}N_9O_9$: C, 64.05; H,3.94; N, 14.94; Found C, 64.07; H, 3.89; N, 14.98.

2.3h *4,4′,4″-(((1,1′,1″-((2,4,6-trimethylbenzene-1,3,5-triyl)tris(methylene))tris(1H-1,2,3-triazole-4,1-diyl))tris(methylene))tris(oxy))tris(2H-chromen-2-one) (**8**)*: Yield 0.26 g (70%); M.p. 102–104°C; ^{1}H NMR (300 MHz, $CDCl_3$)δ 2.39 (s, 9H), 5.28 (s, 6H), 5.66 (s, 6H), 5.82 (s, 3H); 7.25–7.28 (m, 6H), 7.48–7.55 (m, 6H), 7.73 (d, 3H, J = 7.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$)δ 20.1, 48.8, 62.5, 91.1, 115.4, 116.7, 122.9, 123.9, 129.2, 131.8, 139.5, 141.3, 153.3, 164.9; HRMS m/z = 886.7 $(M+1)^+$; Elemental Anal. Calcd. for $C_{48}H_{39}N_9O_9$: C, 65.08; H,4.44; N, 14.23; Found C, 65.10; H, 4.41; N, 14.25.

2.4 *Anti-bacterial activity*

The *in vitro* antibacterial activity of the triazole-based coumarin derivatives **1–8** was determined by the well diffusion method.[18] The Muller Hinton Agar (MHA) medium was used for the preparation of the plates. The medium was poured onto sterile petri dishes of 90 mm diameter. The agar was allowed to set at ambient temperature. Fresh human pathogenic bacterial cultures inoculated into the Muller Hinton Broth (MHB) of two Gram-positive bacteria, *Bacillus subtilis* (MTCC-441) and *Staphylococcus aureus* (MTCC-98) and two Gram-negative bacteria, *Klebsiella pneumoniae* (MTCC-109) and *Proteus vulgaris* (MTCC-742) were spread on the surface of the MHA plate with swabs. They were allowed to incubate; after incubation, using a sterile cork (9 mm diam) borer, wells were cut from the MHA in the petri dishes. The compounds were weighed (5 mg/mL) and dissolved in dimethyl sulfoxide (DMSO). Different volumes of 25 μL, 50 μL and 75 μL test solution were poured into the wells using a sterile micropipette. The inoculated plates were initially incubated for 15 min at room temperature and then they were incubated at 37°C for 24 h. Turbidity was adjusted with a sterile broth so as to correspond to 0.5 McFarland standards. Inhibition zones were recorded as the diameter of the growth free zones, including the diameter of the well in mm at the end of the incubation period. The percentage of inhibition was calculated by the formula: % of inhibition = {I (Diameter of the inhibition zone)/90 (Diameter of the petri-plate in mm)} × 100, where I = Zone of inhibition.

2.5 *Anti-fungal activity*

Anti-fungal activity of compounds **1–8** was determined,[19] Sabouraud Dextrose Agar (SDA) medium was used for the preparation of plates. The anti-fungal activities of compounds **1–8** were tested against two human pathogenic fungi *Candida tropicalis* and *Candida krusei*. The medium was poured into sterile petriplates of 90 mm diameter. The agar was allowed to set at ambient temperature. Fresh fungal culture inoculated into the Sabouraud Dextrose Broth (SDB) was spread on the surface of the SDA plate with the swab. After incubation, the SDA plates were allowed pre-incubation for 10 min, after that using the cork borer (9 mm diameter) well was cut on the agar plate. The compounds were weighed (5 mg/mL) and dissolved in dimethyl sulfoxide (DMSO). The solution was poured (25 μL, 50 μL and 75 μL) using a sterile micropipette. The inoculated plates were initially incubated for 15 min at room temperature and then they were incubated at 37°C for 24 h. Then, the plates were examined for the growth inhibition zone. Inhibition zones were measured as the diameter of the growth free zones in mm including the diameter of the well at the end of the incubation period.

The percentage of inhibition was calculated by the formula: % of inhibition = {I (Diameter of the inhibition zone)/90 (Diameter of the petri-plate in mm)} × 100, where I = Zone of inhibition.

2.6 *Docking studies*

The protein in complex with simocyclinone (PDB ID: 2Y3P) was used as the template for molecular docking studies. GLIDE 9.5 and IFD script from Schrödinger, LLC (New York) was employed as our primary docking engine.[20] A hierarchical search protocol was utilized by

the docking algorithm of the GLIDE program. The scoring function, called the GLIDE score, for computing the binding affinity based Chem-Score function[20] is used. OPLS is the molecular mechanism whose potential energy function was used throughout the calculations. The extra precision mode of GLIDE, which has higher penalties for unfavourable and unphysical interactions, was used for docking. Computations were carried out on a Linux system with CentOS-5 computer platform. The pictures were generated using LIGPLOT.[21]

3. Results and Discussion

The reaction of di and tri bromides **10–17** with sodium azide at room temperature afforded di and tri azides[22] **18–25** in good yields respectively (scheme 1).

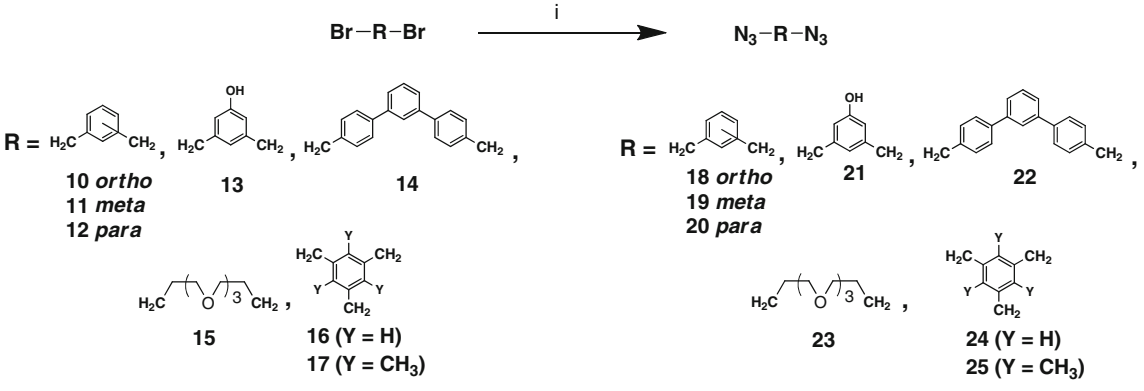
The reaction of propargyloxy coumarin **9** with 0.5 equivalents of azides **18–23** under the click reaction conditions of $CuSO_4.5H_2O$ (5 mol%) and sodium ascorbate (10 mol%), in a mixture of water-THF (1:1) at room temperature gave the triazolylcoumarin **1–6** in good yield (scheme 2).

The ^{1}H NMR spectrum of compound **1** displayed two singlets at δ 5.43 and δ 5.91 for *N*-methylene and *O*-methylene protons, respectively in addition to aromatic proton signals. The ^{13}C NMR spectrum of compound **1** displayed singlets at δ 50.0 and δ 62.7 for *N*-methylene and *O*-methylene carbons, respectively, along with aromatic carbon signals. The appearance of a molecular ion peak at m/z 611.39 confirmed the structure of the triazolylcoumarin **1**. Similarly, the structure of the compounds **2–6** was also confirmed from the spectral and analytical data.

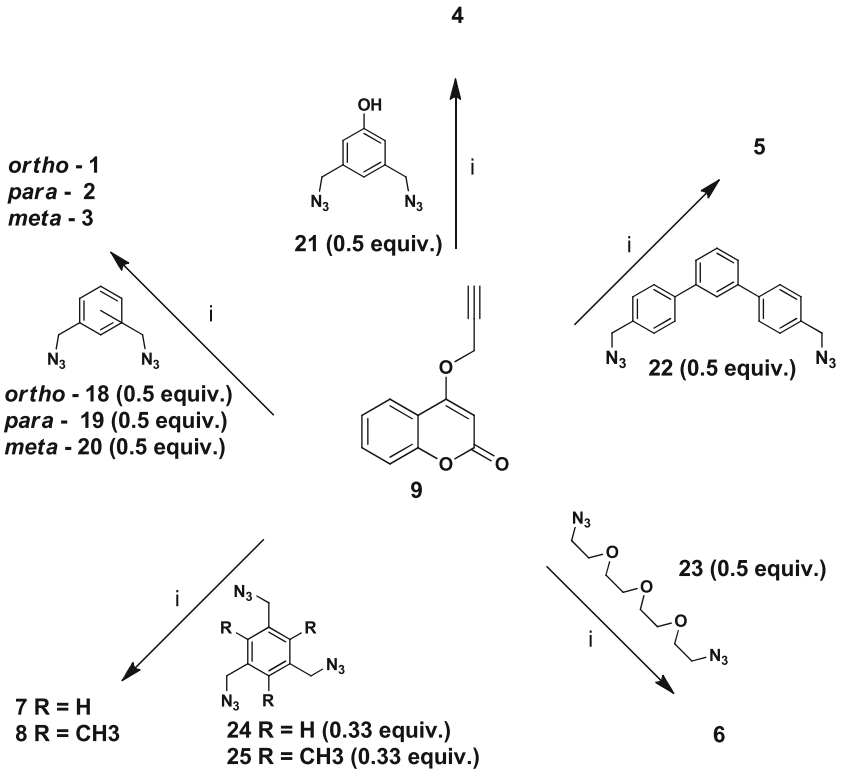
Further, the reaction of propargyloxy coumarin **9** with 0.33 equivalents of azides **24** and **25** under Cu (I) catalyzed click reaction conditions, offered the compound **7** and **8** in 74% and 70% yields, respectively (scheme 2). Compound **7** in ^{1}H NMR spectrum displayed two singlets at δ 5.37 and δ 5.67 for *N*-methylene and *O*-methylene protons, respectively in addition to aromatic proton signals. The ^{13}C NMR spectrum showed the carbon signals at δ 52.4 and δ 62.7 for *N*-methylene and *O*-methylene carbons, respectively along with the signals for aromatic carbons. Similarly, the structure of the compound **8** was also confirmed from spectral and analytical data.

The synthesized compounds **1–8** were evaluated for *in vitro* anti-bacterial and anti-fungal activity against two Gram-positive bacteria, *B. subtilis* and S. *aureus,* two Gram-negative bacteria, *K. pneumoniae* and *P. vulgaris* and two fungal pathogens, *C. tropicalis* and *C. krusei* using the well diffusion method. Standard anti-bacterial and anti-fungal drugs, *viz.*, tetracycline and fluconazole were also screened for comparison. The inhibition percentages of the various microbial strains are shown in table 1. All the triazole based coumarin derivatives showed various levels of inhibitory effects against human pathogenic bacteria and fungi. The anti-microbial activities of these compounds were dose dependent and found to be significant at 75 μL addition.

The synthesized triazolylcoumarins; compound **1** to **8** were screened for anti-bacterial and anti-fungal activity against human pathogens. Compound **1** showed anti-bacterial activity against Gram-positive bacterium; *S. aureus*, Gram-negative bacteria, *K. pneumonia*, *P. vulgaris*, anti-fungal activity against *C. tropicalis* and *C. krusei*. Compound **1** didn't show any activity against Gram-positive bacterium; *B. subtilis*. Compounds **2** and **3** showed activity against *K. pneumonia*, *P. vulgaris*, *C. tropicalis* and *C. krusei* but didn't show any activity against *B. subtilis* and *S. aureus*. Compound **5** showed activity against *B. subtilis*, *S. aureus*, *P. vulgaris*, *C. tropicalis* and *C. krusei*, but didn't show activity against



Scheme 1. i)NaN_3, DMF, rt., **12** h, **18** (84%), **19** (82%), **20** (81%), **21** (80%), **22** (81%), **23** (89%), **24** (87%) and **25** (89%).



Scheme 2. i) $CuSO_4.5H_2O$ (5 mol%), sodium ascorbate (10 mol%), H_2O-THF (1:1, v/v), rt., 10 h, **1** (70%), **2** (66%), **3** (72%), **4** (71%), **5** (76%), **6** (75%), **7** (74%) and **8** (70%).

Table 1. Anti-microbial activity of triazolylcoumarins **1–8** (values given are mean values with triplicate (n=3).

	Well diffusion method (Zone of inhibition (dia. in mm)																	
	Standard[a]		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
Microbial strains	I[b]	I%[c]	I	I%	I	I%	I	I%	I	I%	I	I%	I	I%	I	I%	I	I%
B. subtilis	25	26.66	NI	NI	NI	NI	NI	NI	12	13.33	12	13.33	11	12.22	15	16.66	14	15.55
S. aureus	22	23.33	12	13.33	NI	NI	NI	NI	12	13.33	12	13.33	12	13.33	NI	NI	NI	NI
K. pneumoniae	NI[d]	NI	14	15.55	15	16.66	13	14.44	19	20	NI	NI	16	17.77	17	18.88	17	18.88
P. mirabilis	20	21.11	14	15.55	12	13.33	NI	NI	13	14.44	12	13.33	12	13.33	12	13.33	13	14.44
C. krusei	NI	NI	15	16.66	15	16.66	14	15.55	15	16.66	14	15.55	15	16.66	12	13.33	13	14.44
C. tropical	NI	NI	15	16.66	15	16.66	14	15.55	19	20	12	13.33	15	16.66	16	17.77	14	15.55

[a]Standard: antibacterial drug-Tetracycline; anti-fungal drug-Fluconacole are used
[b]Zone of inhibition
[c]Percentage of zone of inhibition
[d]No Inhibition

K. pneumonia. Compounds **7** and **8** showed activity against *B. subtilis*, *K. pneumonia*, *P. vulgaris*, *C. tropicalis* and *C. krusei*, but didn't show activity against *S. aureus*. Tetracycline and fluconazole were used as internal standard for anti-bacterial activity and fungal activity, respectively.

Compounds **4** and **6** showed anti-bacterial activity against *B. subtilis*, *S. aureus*, *K. pneumonia*, *P. vulgaris* and anti-fungal activity against *C. tropicalis* and *C. krusei*. Compared to all the compounds **1** to **8**, compound **4** and **6** exhibited anti-bacterial and anti-fungal activity against all the tested human pathogens. All the coumarin triazole derivatives **1–8** showed excellent anti-fungal activity against *C. tropicalis* and *C. krusei* at a volume of 75 μL which is comparable to that of standard viz., fluconazole. Within 6 h, fluconazole showed activity against fungal pathogens, after 12 h fluconazole's activity was reduced against fungal pathogens. Triazolylcoumarins **4** and **6** exhibit good activity among the synthesized compounds due to the combined effect of coumarin and triazole, with phenolic and tetraethyleneglycol units, respectively.

The structures of the compounds **1–8** were employed for docking studies with the target DNA gyrase B (PDB ID: 2Y3P).[23] The ligands maintain an average four hydrogen bond with the DNA gyrase. 10–15 Poses were obtained for all the compounds and based on the top score selected for each compound. Among all the compounds, synthesized triazolylcoumarins **4**, **6** and **7** show high binding affinity towards the DNA gyrase B and the results are compared with simocyclinone (D8), which was also docked with the DNA gyrase using a similar protocol and the results are shown in table 2. Figure 2 shows hydrogen bonding and hydrophobic interactions of the triazolylcoumarins **1–8** with the DNA gyrase B, respectively and it has be seen that for each compound, the binding sites and their hydrogen bonding interactions vary.

The variation in the bioactivity is mainly due to the difference in the binding site. The activity study shows that triazolylcoumarins **1–8** shows comparable results with simocyclinone (D8) in the case of *E. coli*. It may be due to the fact that the binding sites of triazolylcoumarins are similar to that of the simocyclinone (D8) binding sites. The triazolylcoumarins **4, 6** and **7** show interaction with the key residues of the receptors Arg 91, Lys 42, Val 268, His 45, Arg 32, Gln 94 and Gln 114 with Glide energy of −101.548 kcal mol^{-1}, −101.002

Table 2. Binding free energy docking simulation results of triazolylcoumarins **1–8**.

Compounds	Glide Score	Glide Energy (kcal/mol)	Hydrogen Bond	
			D-H. . .A	Distance(Å)
1	−10.291	−81.071	Lys N-H. . .N	3.19
			Lys N-H. . .O	3.17
			Arg N-H. . .N	3.07
			Gln N-H. . .O	3.01
2	−8.963	−77.302	Lys N-H. . .O	3.29
			Ser O-H. . .O	2.89
3	−10.115	−83.188	Ser O-H. . .O	3.26
			Ser N-H. . .O	3.17
			Tyr N-H. . .O	3.24
			Asn N-H. . .O	3.30
			Gln N-H. . .O	3.06
4	−10.238	−101.548	Arg N-H. . .O	2.82
			Arg N-H. . .N	3.13
			Arg N-H. . .O	2.98
			Lys N-H. . .O	2.87
			Val O-H. . .O	2.98
5	−9.705	−87.672	Lys N-H. . .N	3.31
			Lys N-H. . .O	3.30
			His N-H. . .N	3.01
			Gly N-H. . .O	3.12
6	−11.057	−101.002	Lys N-H. . .N	3.05
			His N-H. . .N	3.28
			Arg N-H. . .O	3.07
			Gln N-H. . .O	307
7	−10.238	−100.056	Lys N-H. . .O	3.06
			Gln N-H. . .N	3.21
			Arg N-H. . .O	305
8	−9.112	−80.123	Lys N-H. . .N	3.13
			Ala N-H. . .O	2.90
			Arg N-H. . .N	3.07
Simicyclinone	−6.565	−64.715	Asp N-H. . .O	3.30
			Arg N-H. . .O	2.70
			Gln N-H. . .O	2.20
			Ala N-H. . .O	2.70
			Ser N-H. . .O	3.50
			Lys N-H. . .O	3.23

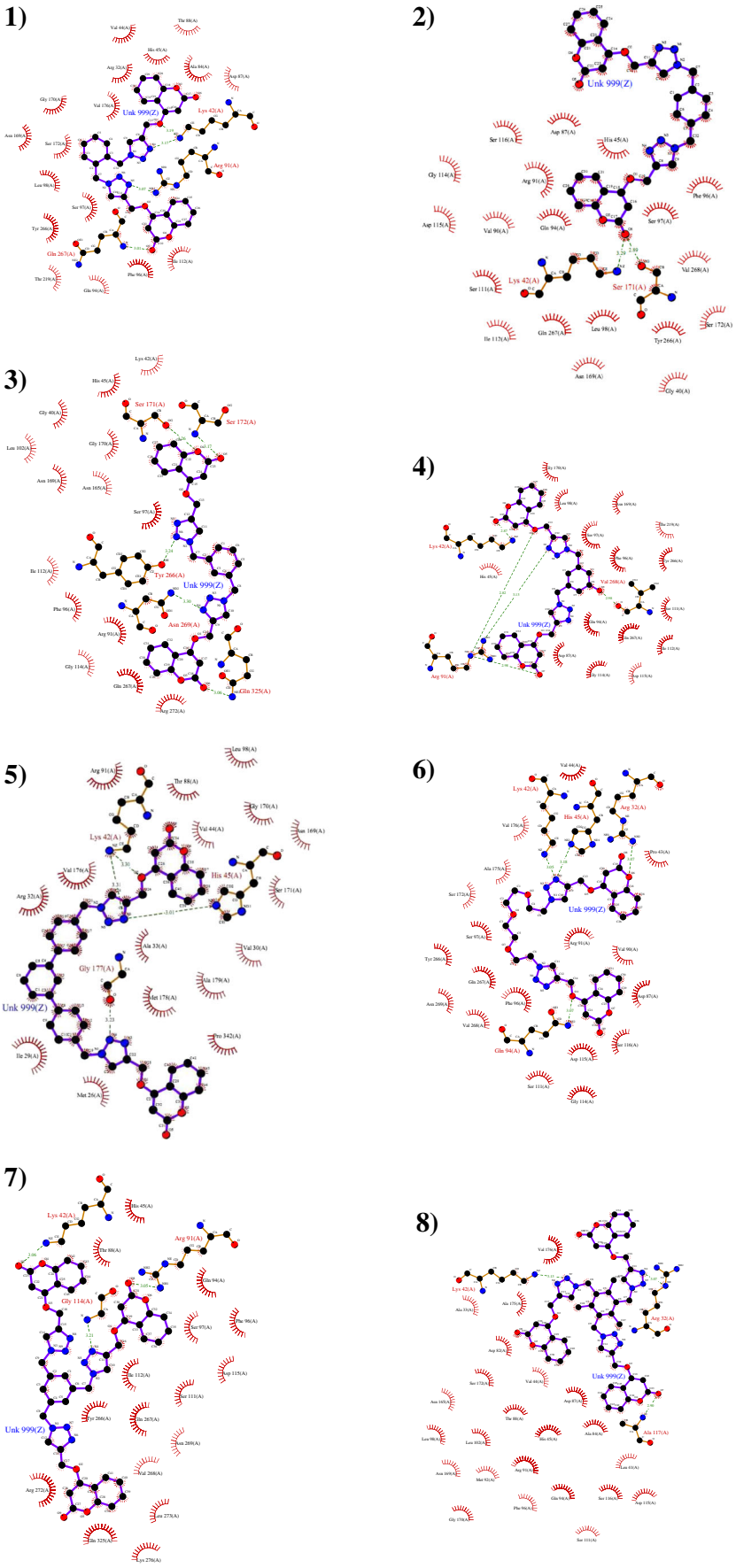


Figure 2. Interaction of triazolylcoumarins **1–8** with DNA gyrase B.

Table 3. Theoretical ADMET properties of triazolylcoumarins **1–8**.

Compounds	CNS[a]	SASA[b]	Hydrogen Bonding		QPlogS[c]
			Donor	Acceptor	
1	−2	962.55	0	12	−6.546
2	−2	970.04	0	11	−7.212
3	−2	1010.42	0	11.5	−7.715
4	−2	1023.68	1	12.25	−8.206
5	−2	1187.89	0	11.5	−10.753
6	−2	958.69	0	17.25	−2.91
7	−2	1010.09	0	17.25	−3.884
8	−2	1010.91	0	17.25	−3.9
Tetracylcine	−	641.47	2	7.5	−4.53
Flucanacole	+/−	514.69	1	6	−3.740

[a]Predicted central nervous system (CNS) activity on a −2 (inactive) to +2 (active) scale.
[b]Total solvent accessible surface area (SASA) in square Angströms using a probe with a 1.4 Å radius.
[c]Predicted aqueous solubility, log S. S. in moles/liter is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid (QplogS).

kcal mol^{-1} and −100.056 kcal mol^{-1}, respectively. The activity study clearly reveals that all the synthesized triazolylcoumarins **1–8** show excellent results comparable with that of simocyclinone (D8). Hence, the docking studies would of great help in the design of the novel triazolylcoumarins drug targets of bacterial proteins. Further, the theoretical prediction of the ADMET properties based on the numbers of hydrogen donors and acceptors have been studied for compounds **1–8** and the results are presented in table 3. The ADMET parameter shows that the reported triazolylcoumarins have good oral absorption and do not affect the central nervous systems (CNS) and hence could be favourable drug candidates after a systematic *in vivo* analysis.

4. Conclusions

We have synthesized a series of triazole based coumarin derivatives by the click chemistry approach. The anti-microbial activity showed that the coumarin derivatives **4** and **6** could be developed as potential anti-bacterial agents against *B. subtilis*, *S. aureus*, *K. pneumonia*, *P. mirabilis* and also anti-fungal agents against *C. krusei* and *C. tropicalis*. The molecular docking studies also revealed that compounds **4** and **6** may be good inhibitors of the DNA gyrase B enzyme and also showed strong hydrogen bonding with key residues of chain A of the enzyme dimer DNA gyrase B with novel binding conformation.

Supplementary Information

NMR and Mass data are available at www.ias.ac.in/chemsci.

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