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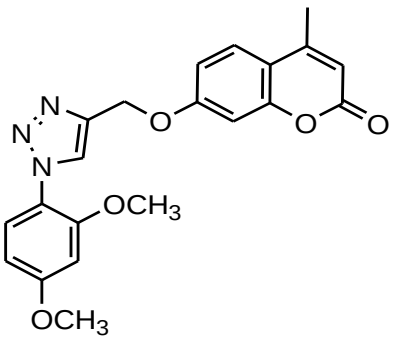
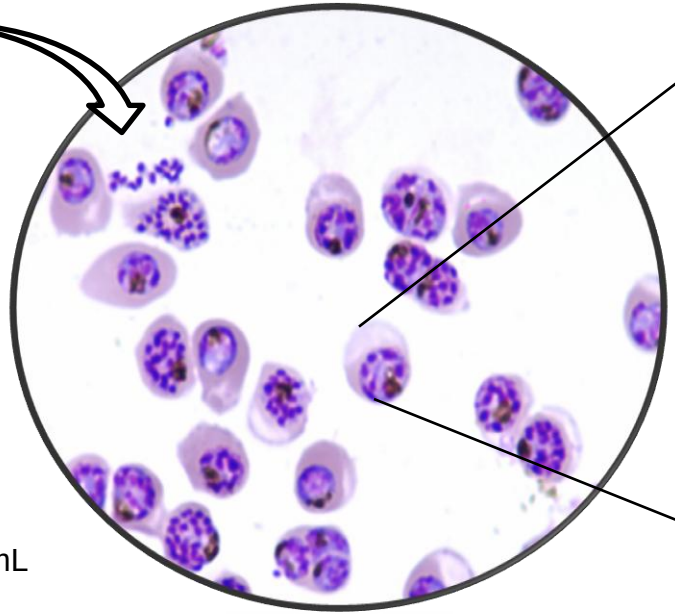
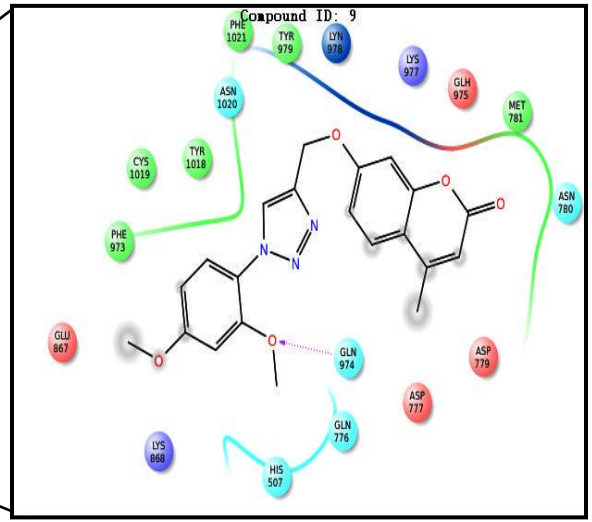


COMPOUND 9

Pf 3D7 IC_{50} = 0.763 + 0.0124 µg/mL

Huh 7 CC_{50} = >100 µg/mL





DNA gyrase binding → Inhibition of supercoiling function

In vitro antiplasmodial efficacy of synthetic coumarin-triazole analogs

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Abstract

Twenty two diverse coumarin-triazole derivatives were synthesized by alkylation of 7-hydroxy-4-methyl-coumarin followed by click chemistry at 7-position. These compounds were evaluated for their *in vitro* antiplasmodial activity against chloroquine sensitive strain of *Plasmodium falciparum* (3D7). Compound 9 (7-[1-(2, 4-dimethoxy-phenyl)-1H-[1, 2, 3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one) was found most active with IC_{50} value 0.763 $\pm$ 0.0124 µg/mL. Further, the structure of compound 20 was characterized by single crystal X-ray diffraction. In view of impressive results, we considered it worthwhile to validate the results of *in vitro* antiplasmodial activity by assessing whether these compounds are capable of hampering the catalytic activity of DNA gyrase, thus preventing its supercoiling function.

Keywords: Coumarin, *Plasmodium falciparum*, antiplasmodial, DNA gyrase.

1. Introduction

Natural products are regarded as rich sources for isolating antiplasmodial compounds such as alkaloids, flavonoids, glycosides, etc. [1-4]. Coumarin, the most abundant naturally occurring secondary metabolite, is found in several plant families, essential oils, microorganisms and a few animal species. Coumarins show certain biochemical properties which exert certain biological effects in the cellular environment. They have also been proposed for use in clinical medicine. Coumarin derivatives possess a remarkably broad spectrum of biological activities including antibacterial [5, 6], antifungal [7-9], anticoagulant [10], anti-inflammatory [11] antitumor [12, 13] and anti-HIV [14]. In addition, these compounds are used as fragrant additives in food and cosmetics [15]. The commercial application of coumarin includes dispersed fluorescent brightening agents and as dyes for tuning lasers [16]. Main representatives of this class are the hydroxy derivatives, viz, 4- and 7-hydroxycoumarins which are biologically active (primarily used as anticoagulants) and act as precursors for the synthesis of other coumarin derivatives.

Pteryxin and Suksdorfin-A, isolated from *Pteryxia terebinthina* and *L. omatium suksdorfii*, respectively, and a coumarin compound isolated from the roots of *Toddalia asiatica*, exhibit antiplasmodial activity [17-19]. Further, Tringali and coworkers reported that 5, 6, 7-trimethoxycoumarin and isofraxidin show moderate activity [20].

The mode of action by which coumarins exert antimalarial activity is, however, not precisely known. However, they have been established to operate at least in part through DNA gyrase inhibition, leading to the accumulation of single-stranded DNA breaks [21]. By this means, self-replication of the organelle where this enzyme is functional in the protozoan parasite, i.e. the apicoplast may also be inhibited, leading to the death of the parasite in the subsequent replication cycle.

Azoles are regarded as one of the most important classes of nitrogen containing heterocyclic compounds that exhibit potent antiplasmodial activity [22]. Among them, the 1, 2, 3-triazole moiety has aroused special interest due to its excellent pharmacokinetic characteristics, favorable safety profile, as well as the latent ability for the formation of hydrogen bonds with other active molecules [23]. A number of triazole drugs including Fluconazole, Voriconazole and Itraconazole have been prevalently used in the anti-infective therapy [24, 25]. Recently, some triazole derivatives have been reported to exhibit good anti-MRSA potency [26], as well as potent antimalarial, anticancer, antidiabetic, and antiinflammatory activities [27]. A series of chalcone and dienone hybrid compounds containing aminoquinoline and nucleoside templates has been screened for *in vitro* antimalarial activity. Amongst the synthesized compounds, three compounds were found to be highly potent against D10, Dd2 and W2 strains of *Plasmodium falciparum* compared with the standard drug chloroquine [28].

Our recent work showed that incorporation of triazole ring in norfloxacin leads to a substantial increase in antimalarial activity [29]. This envisioned us to design and synthesize newer coumarin analogs having altered structural features, with the hypothesis that substituents in the benzopyrone framework would significantly affect the biological activity and may render them more effective against the malaria parasite. In the continuation of our ongoing projects on antimalarial lead identification [30-33] we designed an analog to synthesize different coumarin triazole derivatives involving click chemistry. These derivatives were evaluated for their *in vitro* antiplasmodial activity against *P. falciparum* 3D7 (choroquine-sensitive strain). Further, we attempted to gain insight into their mechanism of action via inhibition of the enzyme DNA gyrase.

2. Results and Discussion

2.1 Synthesis of coumarin-triazole analogs

We synthesized 7-hydroxy-4-methyl-coumarin by the Pechman condensation from resorcinol by reacting with ethylacetoacetate in presence of conc. H_2SO_4 as catalyst. The hydroxyl group of 7-hydroxy-4-methyl-coumarin was alkylated using propargyl bromide and K_2CO_3 in acetone. The compound 7-(prop-2-yn-1-yloxy)-2H-chromen-2-one undergoes cyclo-addition reaction with different azides in presence of $CuSO_4.5H_2O$ and sodium ascorbate as reducing agent yielded a series of 1,4-substituted triazole derivatives 1-22 (figure 1 & table 1). The structures of all synthesized compounds (1-22) were unambiguously established by various spectroscopy techniques viz. FT-IR, ESI-MS, ^{1}H and ^{13}C- NMR. X-Rays analysis was also carried out on compound 20, which validates the feasibility of the reaction.

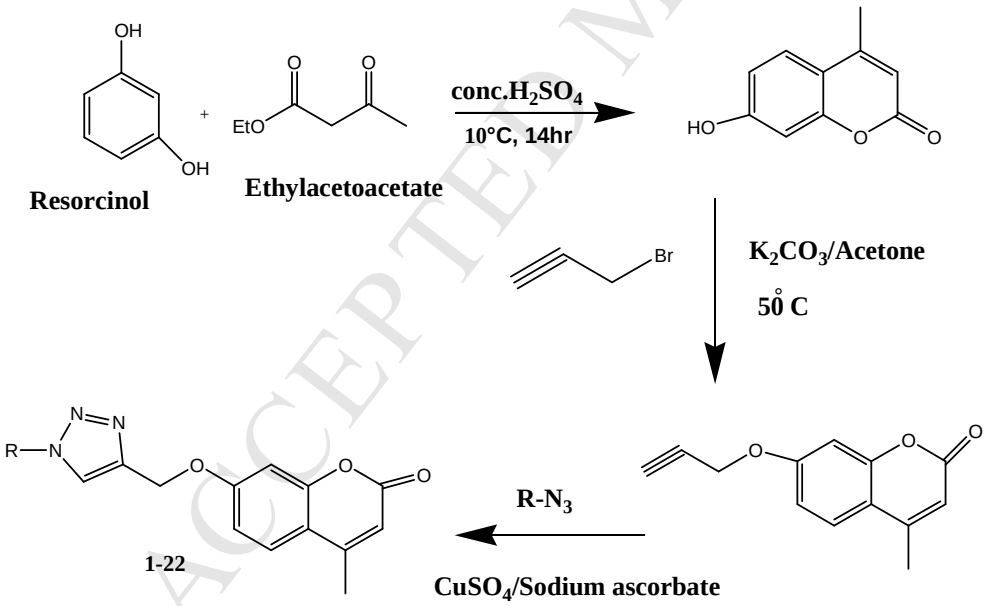
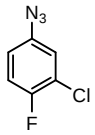
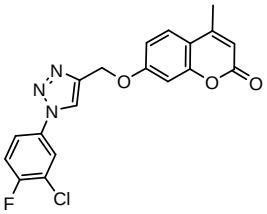
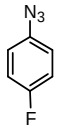
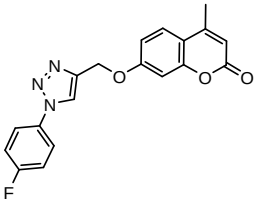
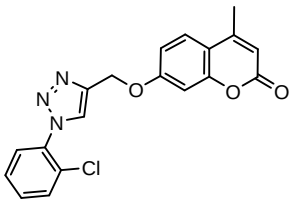
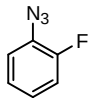
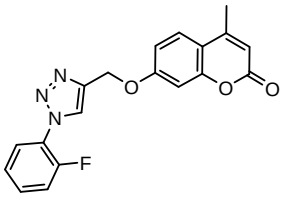
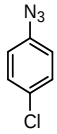
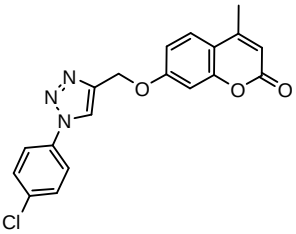
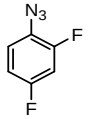
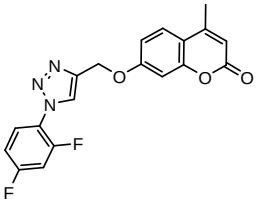
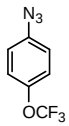
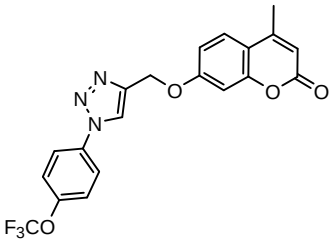
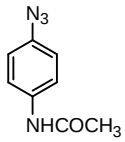
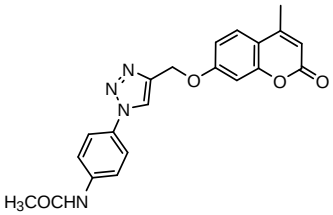
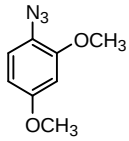
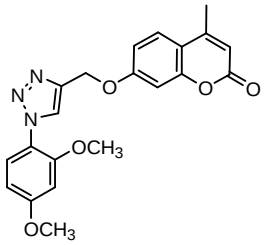
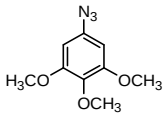
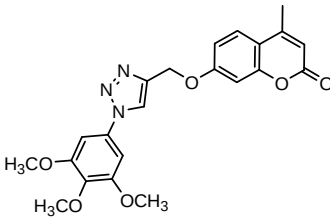
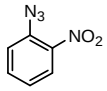
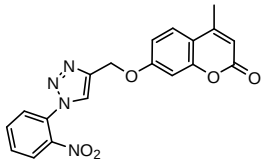
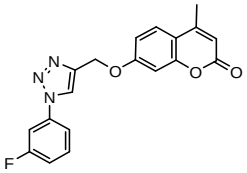
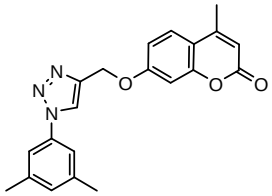
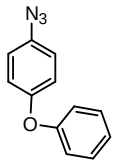
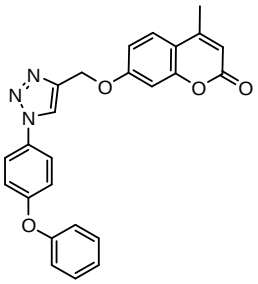
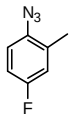
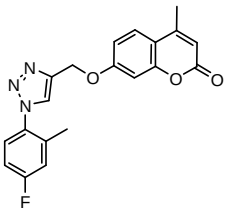
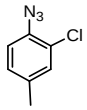
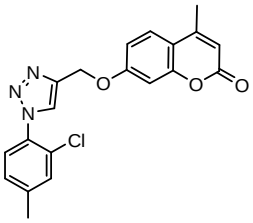
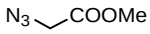
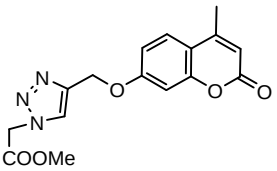
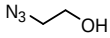
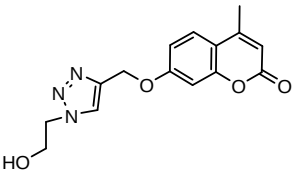
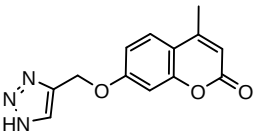
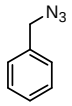
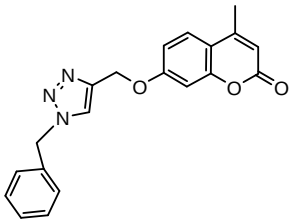


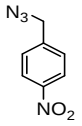
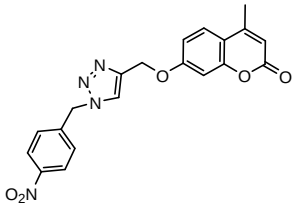
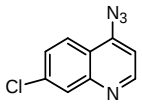
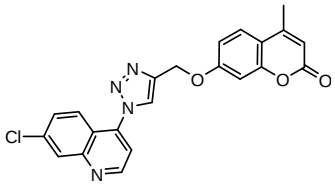
Figure 1. Scheme showing reagents and conditions used in synthesis of coumarin compounds 1-22

Table 1. Inhibitory activity of coumarin-triazole compounds against *P. falciparum* 3D7, and various azides used for their synthesis

Compound	Azide	Product	Mean IC_{50} ± SE[a] (µg/mL)	Mean CC_{50} ±SE[a] (µg/mL)
1			7.43 ± 0.3179	>100
2			6.43 ± 0.1453	>100
3			8.94 ± 0.6472	>100
4			2.48 ± 1.7401	>100
5			8.43 ± 0.3283	>100
6			15.40 ± 0.8718	>100

7			5.00 ± 0.9849	>100
8			4.16 ± 0.6173	>100
9			0.763 ± 0.0124	>100
10			11.06 ± 0.333	>100
11			6.86 ± 0.2403	>100
12			9.46 ± 2.5983	>100
13			0.893 ± 0.0455	>100

14			4.56 ± 0.9261	>100
15			12.80 ± 0.1527	>100
16			1.496 ± 1.0315	>100
17			3.30 ± 0.4041	>100
18			3.36 ± 0.2185	>100
19	N_3H		1.817 ± 0.033	>100
20			6.4 ± 0.1732	>100

21			2.23 ± 1.4438	>100
22			10.60 ± 0.1732	>100

[a] Standard error (n=3).

2.2 The X-ray crystal structure

Single crystals suitable for X-ray diffraction were grown by slow evaporation in ethanol at room temperature. The data were collected on an Oxford Diffraction Excalibur CCD diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71073 Å) at 298(2) K. A total of 3144 reflections were measured, out of which 2607 were found unique. The structure was solved by direct method using SHELXL-97 and refined by full matrix least-squares method on F2 (SHELXL-97). All calculations were carried out using the WinGX package of the crystallographic program and PLATON. For the molecular graphics, program ORTEP-3 and Mercury were used. The final residual indices are: R 0.0907, for the observed and R 0.2855 for all the reflections using 236 parameters. Packing diagram is given in figure 2, whereas the ortep diagram has been included in the supplementary information.

Crystallographic data collection, crystal data and the refinement details are summarized in table 2. The bond distance, bond angles, atomic coordinates and anisotropic displacement parameters are given in the supporting information table.

X-Ray diffraction study was carried out on compound 20 and data were analyzed. The unit cell parameters obtained for the single crystal are; a = 5.5405(0.0006) Å, α = 90.14°; b =

10.5423(0.0016) Å β = 90.01°, c = 14.7610(0.0020) Å, γ = 96.94°and volume =845.09(0.20) $Å^3$, which clearly indicates that compound 20 is Triclinic crystal with the space group of *P-1*. Further, there is intermolecular hydrogen bonding and antiperiplanar structure. The detailed structural data have been deposited with Cambridge Crystallographic Data Centre No. 846613.

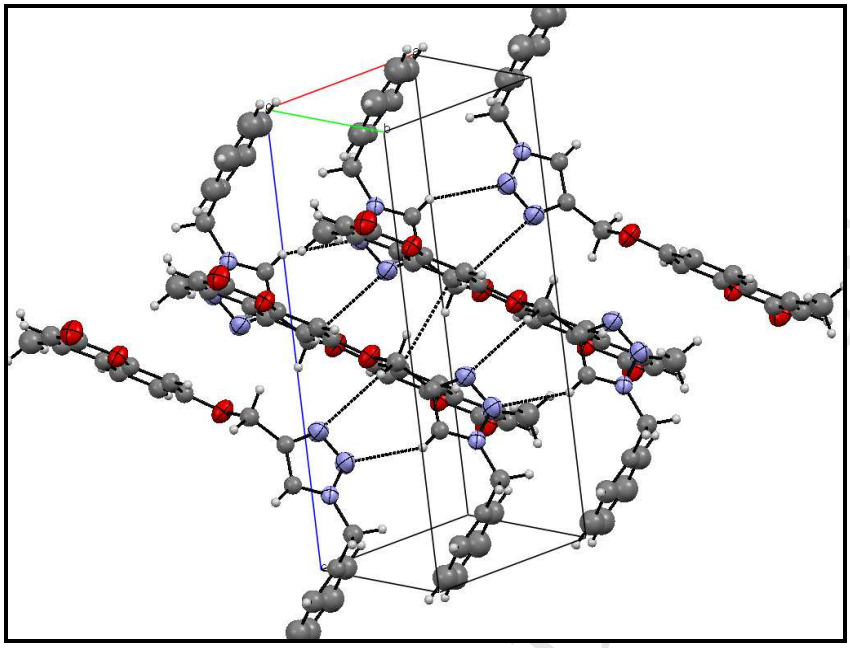


Figure 2. Packing diagram of compound 20

Table 2. Crystal data and structure refinement for compound (20)

Identification code	**Shelxl**
Empirical formula	$C_{20}\ H_{17}\ N_3\ O_3$
Formula weight	347.373
Temperature	293 K
Wavelength	0.71073 Å
Crystal system	Triclinic

Space group	P -1	
Unit cell dimensions	a = 5.5405(0.0006) Å	α = 90.14°.
	b = 10.5423(0.0016) Å	β = 90.01°.
	c = 14.7610(0.0020) Å	γ = 96.94°.
Volume	845.09(0.20) $Å^3$	
Z	2	
Density (calculated)	1.3651 Mg/m3	
Absorption coefficient	0.094 mm-1	
F(000)	364	
Index ranges	-6<=h<=6, -10<=k<=12, -17<=l<=16	
Reflections collected	3144	
Independent reflections	2607	
Completeness to theta = 25.50°	99.80%	
Absorption correction	Multiscan	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	236	
Final R indices [I>2sigma(I)]	0.0970(2607)	
R indices (all data)	0.2855(3144)	

2.3 *In vitro* efficacies of coumarin-triazole analogs against *P. falciparum*

It is well documented that coumarin analogs interact with bacterial DNA gyrase, which induces bacterial death [34]. Since the malaria parasite also harbours gyrase enzyme of the bacterial origin, antiplasmodial

activity of coumarin analogs can be attributed to DNA gyrase inhibition, which is suggested to be the new target for antimalarials in future. Moreover, triazole moiety is a very important pharmacophore unit which is widely seen in many clinical drugs and critical for wide range of activity, ie. antifungal: clotrimazole, fluconazole, miconazole; antibacterial: metronidazole, azithromycin. However, their inhibitory role against the malaria parasites has not yet been explored thoroughly except few known drugs. Keeping all these facts in mind, we designed an analog and synthesized simple coumarin-triazole derivatives exploiting click chemistry. Since coumarin and triazole moieties are present in the same molecule, their antiplasmodial activity would be a result of synergistic interaction. Various substituted coumarin-triazole derivatives were tested *in vitro* against chloroquine sensitive 3D7 strain of *P. falciparum* using artemisinin as a standard drug. Analysis reveals that some of the compounds show very promising activity. Out of twenty two compounds, eighteen compounds show IC_{50} less than 10 µg/mL. Compound 6, 10, 15 and 22 show IC_{50} more than 10 µg/mL. In compound 22 we inserted 7- chloro quinoline moiety in expecting better result because quinolones have proven to be successful antimicrobial agents, but we got IC_{50} 10.60 $\pm$ 0.1732 µg/mL. This may be due to steric hindrance, which might have prevented its binding with DNA gyrase enzyme. Same steric factor is evident in compound 10 containing 3, 4, 5-trimethoxy phenyl ring. Compound 6 containing 2, 4-difluoro phenyl ring shows least antimalarial activity with IC_{50} 15.40 $\pm$ 0.8718 µg/mL and compound 15 having 4-fluoro-2-methyl phenyl ring also shows weak activity with IC_{50} 12.80 $\pm$ 0.1527 µg/mL. Here, the stereochemical approach may not have been appropriate with DNA gyrase enzyme. Compound 1, 2, 3, 5, 11 and 12 exhibit moderate activity with IC_{50} ranging 5-10 µg/mL. Here activity data of compound 1 (IC_{50} 7.43 $\pm$ 0.3179 µg/mL) compound 2 (IC_{50} 6.43 $\pm$ 0.1453 µg/mL) cleared fluoro substituent in ring gave better result than chloro substituent as compound 3 (IC_{50} 8.94 $\pm$ 0.6472 µg/mL) and compound 5 (IC_{50} 8.43 $\pm$ 0.3283 µg/mL). Further, in compound 12 (IC_{50} 9.46 $\pm$ 2.5983 µg/mL)

fluoro substituent present at meta position did not give better result. Compound 11 (IC_{50} 6.86 $\pm$ 0.2403 µg/mL) having nitro group at ortho position exhibited moderate activity.

Further, compounds 7, 8 and 14 containing trifluoromethoxy, acetamide and phenoxy subtituent showed better results with IC_{50} ranging 4-5 µg/mL.

Now, if we compare benzyl derivative like compound 20 (IC_{50} 6.4$\pm$ 0.1732 µg/mL) and compound 21 (IC_{50} 2.23 $\pm$ 1.14438 µg/mL), compound 21 (containing 4-Nitro benzyl derivative) shows significant result may be due to electron withdrawing nature of nitro group. Similarly, increase in activity was observed when alkyl chain having polar group such as methoxy ethanoate (compound 17; IC_{50} 3.30 $\pm$ 0.4041µg/mL) and 2-hydroxyethyl (compound18; IC_{50} 3.36 $\pm$ 0.2185 µg/mL) were introduced on triazole ring.

Interestingly, compound 21 in which position-1 of triazole ring was left unsubstituted exhibited promising antimalarial activity with IC_{50} 1.817 $\pm$ .033 µg/mL, suggesting that the hydrogen of triazole ring might be forming H- bond with the target site, thus interfering with the normal function of parasite.

In this series, three most active compounds are compound 9 (IC_{50} 0.763 $\pm$ .0124 µg/mL), compound 13 (IC_{50} 0.893 $\pm$ 0.0455 µg/mL) and compound 16 (IC_{50} 1.496 $\pm$ 1.0315 µg/mL). Compound 16 contains 2-choloro-4-methyl phenyl ring and compound 13 contains 5 –dimethyl phenyl ring. The result suggests that presence of electron releasing group methyl enhances the antimalarial activity. Compound 9 having 2, 4-dimethoxy phenyl ring was the most potent compound; presence of two electron releasing methoxy group at ortho and para position of phenyl ring enhanced the antimalarial activity quite significantly.

Thus, the results presented in table 1 show that most of the new compounds of coumarin-triazole series exhibited very promising activity ranges, depending on the substitution on position-1 of triazole ring, like alkyl chain having polar group and phenyl ring having different functional group. The presence of electron releasing group and electron withdrawing group, their position (ortho, meta and para) in phenyl ring and their steric hindrance, all the factors might affect the activity, but varying behavior of compounds with

similar nature of functional groups is still elusive. This study clearly demonstrates that there is need of systematic SAR study to establish meaningful correlation among various functional groups. Work in this direction is under progress.

2.4 Effect of coumarin-triazole analogs on the activity of *E. coli* DNA gyrase

The molecular details of the gyrase-coumarin complex are extensively studied [35]. The coumarins inhibit the gyrase action by competitive binding to the ATP-binding site of DNA gyrase B (GyrB) protein. In contrast, the quinolones act in a different manner, by trapping the cleavage complex and interacting primarily with GyrA and to some extent with GyrB and DNA [36]. Hence, DNA gyrase has been hypothesized to be a new target for development of newer antimalarial drugs in future. Substantiating this hypothesis is the fact that the malaria parasite harbours the bacterial type DNA gyrase enzyme. It requires the apicoplast, a chloroplast-like organelle for survival. Genome of the apicoplast (plDNA) is a 35 kb circular DNA that is served by gyrase, a prokaryotic type II topoisomerase [37].

In the present study, we investigated the influence of coumarin-triazole analogs on the supercoiling activity of DNA gyrase exerted on relaxed plasmid DNA. The results revealed inhibition of the supercoiling activity, which may be attributed to prevention of ATP hydrolysis required for the enzymatic cycle and inhibition of the gyrase DNA binding. Figure 3 shows the relative positions of the relaxed topoisomers (A), linear (B) and supercoiled (C) pHOT1 plasmid DNA, gyrase treated relaxed DNA (D), and gyrase treated relaxed DNA in the presence of IC_{50} concentrations of test compounds 9, 13 and 16, respectively (E-G) and the positive control, ciprofloxacin (H).

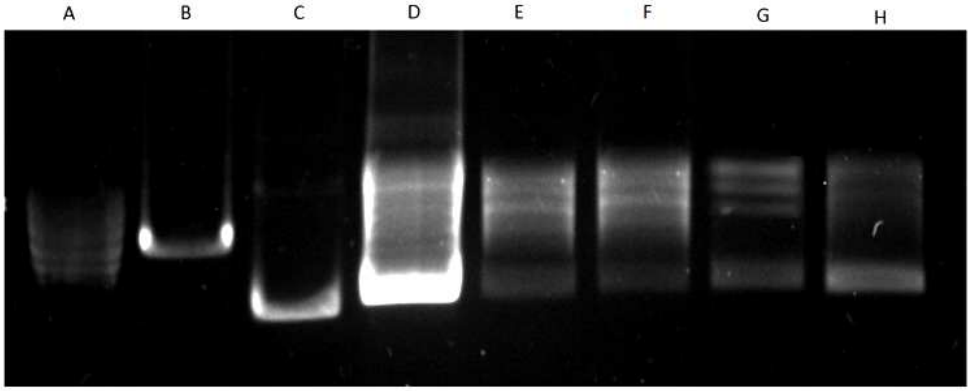


Figure 3. Effect of coumarin-triazole analogs 9, 13 and 16 on *E. coli* DNA gyrase, using relaxed pHOT1 plasmid DNA as a substrate. The lower band shows supercoiled DNA. Samples were analysed by gel electrophoresis.

3. Conclusion

We have synthesized a series of novel coumarin-triazole derivatives (1-22) by exploiting Click Chemistry on 7-(prop-2yn-1-yloxy)-2H-chromen-2-one. Most of the compounds demonstrated promising *in vitro* antiplasmodial activities. However, three compounds with the lowest inhibitory concentration values were 9, 13, 16, with IC_{50} values of 0.763 $\pm$ 0.0124, 0.893 $\pm$ 0.0455 and 1.496 $\pm$ 1.0315, respectively. Convincing results were obtained on account of analyzing their inhibitory effect on the supercoiling activity of the enzyme gyrase, employing *E. coli* DNA gyrase and relaxed plasmid DNA. Thus, we can conclude that these compounds can be used as potential leads to synthesize new antimalarial drugs that target the DNA gyrase enzyme. Furthermore, it would be interesting to study their behavior in Artemisinin-based Combination Therapy (ACT), as combination partners to the artemisinins.

4. Experimental procedure

4.1 General methods

Various chemicals and solvents used in this study were purchased from E. Merk (India) and Sigma-Aldrich Chemicals. Melting points werejhu8iky determined by using open capillary method and are uncorrected. ^{1}H NMR spectral data were recorded on a BruckerAvance 300MHz and Jeol JNM 400MHz spectrometers respectively using TMS as an internal standard. The chemical shift values are recorded on δ scale and coupling constants (J) are in hertz. The following abbreviations were used in reporting spectra: s =singlet, d = doublet, t = triplet, q = quartet, m = multiple. IR spectra were obtained on a Perkin Elmer Fourier-transform infrared (FT-IR) spectrophotometer (Spectrum 2000) in potassium bromide disk. ESI-MS spectra were obtained on Waters Micromass LCT spectrometer. Elemental analysis was done on Elementar GmbH VarioElanalyzer.

4.2 Preparation of 4-methyl-7-hydroxy coumarin

Concentrated H_2SO_4 (20mL) was taken into a 250mL round flask and cooled in an ice bath at 0°C. A solution of resorcinol (2.0g, 18.16 mmol) in ethyacetoacetate (2.4mL, 19mmol) was added dropwise to the flask with constant stirring while the temperature was carefully kept below at 10°C. Then the mixture was continued to stir overnight at room temperature. After this period, the reaction mixture was poured into crushed ice with vigorous stirring. The solid product was filtered and washed with cold water. Crude product was recrystallized from absolute ethanol to obtain pure 4-methyl-7-hydroxycoumarin with an approximately 60% yield [38].

4.3 Synthesis of 7-(Prop-2-yn-1-yloxy)-2H-chromen-2-one

To a stirred solution of 4-methyl-7-hydroxy-coumarins (1.76g, 10mmol) in dry acetone, anhydrous potassium carbonate (1.38g, 10mmol) and propargyl bromide (1.19g, 10mmol) were added. The resultant mixture was stirred at 50ºC for 18h, then the mixture was cooled and the solvent was removed under reduced pressure. The residue was redissolved into 15mL of water and extracted with ethyl acetate. The combined organic phases was washed with water, dried over anhydrous sodium sulphate and evaporated in vacuum. The crude product was purified by crystallization from ethyl acetate/hexane mixture to yield light yellow solid product [38].

Yield: 90%. mp: 130-132ºC. ^{1}H NMR ($CDCl_3$, 400MHz) δ ppm: 2.40(s, 3H, 4-CH_3), 2.57(s, 1H, CH of alkyne), 4.76(s, 2H, -O-CH_2- methylene proton), 6.16(s, 1H, pyran ring), 6.93(m, 2H, H_5 and H_6), 7.5 (s, 1H, H_8). ^{13}C NMR ($CDCl_3$, 100MHz) δ ppm: 18.56(CH_3), 56.08(-CH_2-O), 76.41(C_3' alkyne), 77.31 ($C_{2'}$ alkyne), 102.04(C_3), 112.27(C_8), 114.14(C_6), 125.55(C_5), 152.35(C_4 pyran ring), 154.90(C_7-O), 160.24 (C=O).

4.4 General procedure for synthesis of alkylazide

Alkyl halide (5.8 mmol) in DMSO (8 mL) was taken in a round bottom flask and sodium azide was (760mg, 11.69mmol) added and stirred overnight. The reaction mixture was quenched in ice-water and extracted with diethyl ether, washed successively with 5% bicarbonate solution and dried over anhydrous sodium sulphate. The solvent was removed by rotary evaporated to give alkyl azide, yield 70% [39a].

4.5 General procedure for synthesis of arylazide

Aniline (7.25 mmol) was dissolved in 4.57mL (1:1 ratio HCl:water) and stirred at 0-5˚C. Sodium azide (468g) was dissolved in 5.67mL of water and added drop wise and then reaction was allowed to reach at room temperature and stirred further for 30 minutes. The resultant precipitate was extracted with $CHCl_3$ and washed successively with water. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed in vacuum to get arylazide in good yield [39a].

4.6 Synthesis of 4-azido-7-chloro-quinoline

To 4,7-dichloroquinoline (1.0gm, 5mmol) solution in anhydrous DMF (4mL) was added sodium azide (0.650gm, 10mmol) and resulting mixture was stirred at 70˚C for 6h. After completion of reaction as checked by TLC, the reaction mixture was allowed to cool to room temperature and diluted with DCM (100mL), washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. The resulting product residue was crystallized from DCM / hexane mixture which yielded pure colourless needle like crystals in 80% yield [39b].

4.7 General procedure for synthesis of compounds (1-22)

To a solution of compound 7-(Prop-2-yn-1-yloxy)-2H-chromen-2-one (214 mg, 1.0 mmol) in tert. BuOH/H_2O (1:1), $CuSO_4.5H_2O$ (37.45mg, 0.15mmol) and sodium ascorbate (59.4mg, 0.30mmol) were added. The mixture was stirred at room temperature for 15 minute and then azide (1.0 mmol) was added. The resulting mixture was stirred at room temp. until the starting material was consumed. After 24h, on completion of the reaction as seen by TLC, the reaction mixture was extracted with ethylacetate. The combined organic phase washed with water, dried over anhydrous sodium sulphate and evaporated in vacuum. The crude product was purified by column chromatography [39a].

4.7.1. 7-[1-(3-Chloro-4-fluoro-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(1)**

Yield: 40%. Yellow solid. mp: 182-184°C. MS: m/z = 385.34 (M^+), 387 (M+2), 386 (M+1), 210 (M-175) . ^{1}H NMR (DMSO-d_6, 300MHz) δppm: 2.36(s, 3H, -CH_3), 5.38(s, 2H, -O-CH_2-) 6.25(s, 1H, H_3 coumarin pyran ring), 7.07(d, 1H, H_5, J = 8.1Hz), 7.19(s, 1H, H_8 coumarin ring), 7.67(d, 1H, H_6, J = 4.2Hz), 7.87(m, 2H, H_6'& H_5' benzene ring), 9.04(s, 1H, H_6, benzene ring). 9.05(s, 1H, triazole ring). ^{13}C NMR ((DMSO-d_6, 75MHz) δ ppm: 18.64(CH_3), 62.00(-CH_2-O), 80.38, 102.12(C_3), 111.80(C_8), 113.08(C_6), 121.60($C_{5'}$ benzene ring), 123.04($C_{6'}$), 124.03($C_{2'}$), 127.06(C_5 coumarin ring & C_1' benzene ring) 129.76(CH, ethylene C of triazole), 139.10($C_{3'}$-Cl), 143.84(C, quaternary carbon of triazole ring), 149.93($C_{4'}$), 153.13(C-O Fused in pyran ring), 155.13(C_4 pyran ring), 160.60(C_7-O), 161.36(C=O). IR (KBr, cm^{-1}) ν_{max}: 2929.50(C-H stretching), 1718.44(α ,β-unsaturated ketonic C=O stretching), 1617.95, 1389.05, 1270.58(C-O-C stretching), 1153.71(C-N stretching in 1,2,3-triazole ring), 1072.62(C-O-C stretching), 845.78. Anal. Calcd for $C_{19}H_{13}ClFN_3O_3$**:** C, 59.15; H, 3.40; N, 10.89. Found: C, 59.12; H, 5.19; N, 12.49%.

4.7.2. 7-[1-(4-Fluoro-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(2)**

Yield: 40%. Yellow solid, mp: 172-174°C. MS: m/z = 351.23 (M^+), 352.23 (M+1), 353.22(M+2), 176.11(M-175). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.39(s, 3H, -CH_3), 5.36(s, 2H, -O-CH_2-), 6.22(s, 1H, H_3 coumarin pyran ring), 7.04(d, 1H, H_5, 7.15(s, 1H, H_8 coumarin ring), J = 7.8Hz), 7.49(m, 2H, H_6' & H_2' benzene ring), 7.67(d, 1H, H_6 coumarin ring, J = 8.4Hz), 7.9(br, s, 2H, $H_{3'}$ & $H_{5'}$ benzene ring), 9.02(s, 1H, triazole ring). ^{13}C NMR ((DMSO-d_6, 75MHz) δ ppm: 18.12(CH_3), 61.85(-CH_2-O), 101.57(C_3), 111.36(C_8), 112.53(C_6), 113.45($C_{3'}$ & $C_{5'}$), 116.94(C

quaternary fused C of coumarin ring), 122.53 ($C_{1'}$), 123.62 ($C_{5'}$), 126.52 (CH, ethylene C of triazole), 132.98($C_{2'}$ & $C_{6'}$), 143.32(C, quaternary carbon of triazole ring), 153.37(C-O Fused in pyran ring), 154.61(C_4 pyran ring), 160.11($C_{4'}$-F benzene ring), 160.85 (C_7-O), 163.40(C=O). IR (KBr, cm^{-1}) v_{max}: 2925.95(C-H stretching), 1717.95 (α, β-unsaturated ketonic C=O stretching), 1609.70, 1516.59, 1388.08(C-O-C stretching), 1158.17 (C-N stretching in 1,2,3-triazole ring), 1071.92(C-O-C stretching), 845.5. Anal. Calcd for $C_{19}H_{14}FN_3O_3$: C, 64.95; H, 4.02; N, 11.96. Found: C, 64.32 ; H, 4.22; N, 11.82%.

4.7.3. 7-[1-(2-Chloro--phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(3)**

Yield: 36%. Yellow solid. mp: 118-120°C. MS: m/z = 367.89 (M^+), 369.88 (M+2), 368.86 (M+1), 193(M-175) . ^{1}H NMR ((DMSO-d_6, 300MHz) δppm: 2.41(s, 3H, -CH_3), 5.38 (s, 2H, -O-CH_2-), 6.25(s, 1H, H_3 coumarin pyran ring), 7.09(d, 1H, H_5, J = 8.0Hz), 7.2 (s, 1H, H_8 coumarin ring), 7.6-7.8(m, 5H, 4H benzene ring, H_6 coumarin ring),8.7(s, 1H, triazole ring). ^{13}C NMR ((DMSO-d_6, 75MHz) δ ppm: 18.62(CH_3), 61.88(-CH_2-O), 102.10(C_3), 111.83(C_8), 113.13(C_6), 113.92 (C quaternary fused C of coumarin ring), 127.03(C_5), 127.70 ($C_{5'}$), 129.00 ($C_{1'}$ & $C_{3'}$), 131.03 ($C_{4'}$ & $C_{6'}$), 132.27 (CH, ethylene C of triazole), 134.83($C_{2'}$-Cl), 142.59(C, quaternary carbon of triazole ring), 153.92(C-O Fused in pyran ring), 155.14(C_4 pyran ring), 160.61(C_7-O), 161.45(C=O). IR (KBr, cm^{-1}) v_{max} : 2922.33(C-H stretching), 1718.21(α, β-unsaturated ketonic C=O stretching), 1617.78, 1387.97, 1282.08(C-O-C stretching), 1143.18 (C-N stretching in 1,2,3-triazole ring), 1070.38(C-O-C stretching), 837.28, 761.91. Anal. Calcd for $C_{19}H_{14}ClN_3O_3$: C, 62.05; H, 3.84; N, 11.43. Found: C, 62.01; H, 3.89; N, 11.22%.

4.7.4. 7-[1-(2-Fluoro-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(4)**

Yield: 35%. Yellow solid. mp: 128-130°C. MS: m/z = 351.13 (M^+), 352.23(M+1), 353.22(M+2), 176.11 (M-175). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.38(s, 3H, -CH_3), 5.30(s, 2H, -O-CH_2-)

6.23(s, 1H, H_3 coumarin pyran ring), 7.10-8.19(br, 7H, 4H benzene ring & 3H coumarin ring H_5, H_6, H_8), 8.84(s, 1H, triazole ring). ^{13}C NMR ((DMSO-d_6,75MHz) δ ppm: 23.36(CH_3), 66.54(-CH_2-O), 106.81 (C_3), 116.57 (C_8), 117.82 (C_6), 118.66 (C, quaternary fused C of coumarin ring), 122.24($C_{1'}$), 122.50 ($C_{3'}$), 129.78(C_5), 129.92($C_{5'}$), 130.85($C_{4'}$), 131.26($C_{6'}$), 131.77 (CH, ethylene C of triazole), 147.87 (C, quaternary carbon of triazole ring), 157.40 (C-O Fused in pyran ring), 158.64 (C_4 pyran ring), 160.72(C_7-O), 165.30(C_2-F), 166.16(C=O). IR (KBr, cm^{-1}) ν_{max}: 2924.28 (C-H stretching), 1718.40 (α, β-unsaturated ketonic C=O stretching), 1617.57, 1389.35, 1282.36 (C-O-C stretching), 1140.09 (C-N stretching in 1,2,3-triazole ring), 1044.25 (C-O-C stretching), 834.21, 765.51. Anal. Calcd for $C_{19}H_{14}FN_3O_3$: C, 64.95; H, 4.02; N, 11.96. Found: C, 64.31; H, 4.27; N, 11.83%.

4.7.5. 7-[1-(4-Chloro-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(5)**

Yield: 40%. Yellow solid. mp: 200-202°C. MS: m/z = 367.13 (M^+),369.07(M+2), 368.07(M+1) 193(M-175), 176 . ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.40(s, 3H, -CH_3), 5.36(s, 2H, -O-CH_2-) 6.23(s, 1H, H_3 coumarin pyran ring), 7.07(d, 1H, H_5, J = 6.3Hz), 7.18(s, 1H, H_8, coumarin ring), 7.69(m, 3H, H_6 coumarin ring, $H_{2'}$ and $H_{6'}$ benzene ring), 7.95(d, 2H, $H_{3'}$ and $H_{5'}$ benzene ring), 9.02(s, 1H, triazole ring). ^{13}C NMR ((DMSO-d_6, 75MHz) δ ppm: 18.60(CH_3), 62.00(-CH_2-O), 102.12(C_3), 111.94(C_8), 113.06(C_6), 113.95(C quaternary fused C of coumarin ring), 122.36($C_{3'}$ &$C_{5'}$), 123.73($C_{2'}$ & $C_{6'}$), 127.03(C_5), 130.36(CH, ethylene C of triazole), 133.57($C_{1'}$), 135.77($C_{4'}$-Cl), 143.82 (C, quaternary carbon of triazole ring), 153.86(C-O Fused in pyran ring), 155.11(C_4 pyran ring), 160.57(C_7-O), 161.37(C=O). IR (KBr, cm^{-1}) ν_{max}: 2924.41(C-H stretching), 1718.39 (α, β-unsaturated ketonic C=O stretching), 1612.28, 1501.14, 1388.50, 1265.51(C-O-C stretching), 1155.15(C-N stretching in 1,2,3-triazole ring), 1070.28(C-O-C stretching), 839.48, 504.28. Anal. Calcd for $C_{19}H_{14}ClN_3O_3$: C, 62.05; H, 3.84; N, 11.43. Found: C, 62.31; H, 3.90; N, 11.20%.

4.7.6. 7-[1-(2,4-Difluoro-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(6)**

Yield: 35%. Yellow solid. mp: 170-172°C. MS: m/z = 369.17 (M^+), 370.16(M+1), 371.18(M+2), 177.1(7-hydroxy coumarin cation). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.36(s, 3H, -CH_3), 5.39(s, 2H, -O-CH_2-) 6.24(s, 1H, H_3 coumarin pyran rings), 7.09-7.20(br, 2H, H_5 and H_6), 7.37(s, 1H, $H_{3'}$, benzene ring), 7.71(s, br, 2H, $H_{5'}$ and $H_{6'}$, benzene ring), 7.93(s, 1H, H_8), 8.80(s, 1H, triazole). IR (KBr, cm^{-1}) ν_{max}: 3069.46, 2925.08(C-H stretching), 1710.86(α, β-unsaturated ketonic C=O stretching), 1612.87, 1517.46, 1388.02, 1279.07(C-O-C stretching), 1198.58, 1142.00(C-N stretching in 1,2,3-triazole ring), 1067.42(C-O-C stretching), 1013.82, 836.90, 609.44. Anal. Calcd for $C_{19}H_{13}F_2N_3O_3$: C, 61.79; H, 3.55; N, 11.38. Found: C, 61.61; H, 3.89, N, 11.22%.

4.7.7. 7-[1-(4-Trifluoromethoxy-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(7)**

Yield: 35%. Yellow solid. mp: 154-156°C. MS: m/z = 417.17 (M^+), 332(M-OCF_3), 177 (7-hydroxy coumarin cation). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.40(s, 3H, -CH_3), 5.38(s, 2H, -O-CH_2-) 6.24(s, 1H, H_3 coumarin pyran rings), 7.08(d, 2H, H_5 and H_6, J = 8.7Hz), 7.62-772(m, 3H, $H_{2'}$ and $H_{6'}$ benzene ring and H_8), 8.06(d, 2H, $H_{3'}$ and $H_{5'}$ benzene ring , J = 8.7Hz), 9.05(s, 1H, triazole). ^{13}C NMR ((DMSO-d_6, 75MHz) δ ppm: 18.62(CH_3), 61.96(-CH_2-O), 102.07(C_3), 111.87(C_8), 113.08(C_6), 113.94(C quaternary fused C of coumarin ring), 122.68(CF_3), 123.16($C_{3'}$ &$C_{5'}$), 123.97($C_{1'}$), 127.05(C_5), 135.83($C_{2'}$ &$C_{6'}$), 143.83(C, quaternary carbon of triazole ring), 148.41($C_{4'}$-O), 153.92(C-O Fused in pyran ring), 155.10(C_4 pyran ring), 160.62(C_7-O), 161.32(C=O). IR (KBr, cm^{-1}) $\nu_{max:}$ 2925.22(C-H stretching), 1743.20 (α, β-unsaturated ketonic C=O stretching), 1612.50, 1517.53, 1389.32, 1261.94 (C-O-C stretching), 1156.10 (C-N stretching in

1,2,3-triazole ring), 1027.39(C-O-C stretching), 839.99, 502.60. Anal. Calcd for $C_{20}H_{14}F_3N_3O_4$: C, 57.56; H, 3.38; N, 10.07. Found: C, 57.53; H, 3.86; N, 10.22%.

4.7.8. N-{4-[4-(4-Methyl-2-oxo-2H-chromen-7-yloxylmethyl)-[1,2,3] triazol-1-yl]-phenyl}-acetamide **(8)**

Yield: 38%. Yellow solid. mp: 150-152°C. MS: m/z = 390.16(M^+), 391.16(M+1), 392(M+2). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.04(s, 3H, -CO-CH_3), 2.4(s, 3H, -CH_3), 4.94(s, 2H, -O-CH_2-), 6.26(s, 1H, H_3 coumarin pyran ring), 7.03(d, 2H, H_5 and H_6, J = 12.0Hz), 7.46(d, 2H, $H_{2'}$ and $H_{6'}$, J = 8.7Hz), 7.56(d, 2H, $H_{3'}$ and $H_{5'}$, J = 8.7Hz), 9.01(s, 1H, triazole), 10.095(s, 1H, NH). ^{13}C NMR ((DMSO-d_6,75MHz) δ ppm: 18.20(CH_3,coumarin ring methyl C), 24.08(CH_3 amide methyl C), 56.11(-CH_2-O), 78.98, 78.59, 101.87(C_3), 111.58(C_8), 112.63(C_6), 113.73($C_{1'}$&C quaternary fused C of coumarin ring), 114.55($C_{3'}$ &$C_{5'}$), 120.89($C_{2'}$ &$C_{6'}$), 126.63(C_5), 131.54 (CH, ethylene C of triazole), 138.71(C4′-N & quaternary carbon of triazole ring), 153.47(C-O Fused in pyran ring), 154.53(C_4 pyran ring), 160.15(C=O), 168.57(C=O of amide). IR (KBr, cm^{-1}) ν_{max}: 3305.17(N-H Stretching in amide), 2926.13(C-H stretching), 1718.81(α, β-unsaturated ketonic C=O stretching), 1390.01, 1364.04, 1282.09, 1262.03(C-O-C stretching), 1155.58(C-N stretching in 1,2,3-triazole ring), 1070.35(C-O-C stretching), 1017.94, 822.01, 504.20. Anal. Calcd for $C_{21}H_{18}N_4O_4$: C, 64.61; H, 4.65; N, 14.35. Found: C, 64.59; H, 4.97; N, 14.32%.

4.7.9. 7-[1-(2,4-Dimethoxy-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(9)**

Yield: 30%. Yellow solid. mp:170-172°C. MS: m/z = 393.12(M^+), 394.13(M+1), 395.11 (M+2), 218 (M-175). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.41(s, 3H, 4-CH_3 coumarin ring), 3.78 (s, 6H, 2′ and 4′-OCH_3), 5.36(s, 2H, -OCH_2-), 6.24(s, 1H, H_3 pyran ring), 7.13(m, 2H, aromatic), 7.43(m, 3H, aromatic), 7.80(s, 1H, $H_{3'}$), 8.68(s, 1H, triazole ring). ^{13}C NMR ((DMSO-d_6,75MHz) δ

ppm: 18.60(CH_3), 56.28(2′-OCH_3), 57.01(4′-OCH_3), 61.91(-CH_2-O), 102.11(C_3), 111.58(C_8), 111.80(C_6), 113.13(C quaternary fused C of coumarin ring), 113.88(C3′), 114.74($C_{1'}$), 116.36($C_{5'}$), 126.27($C_{6'}$), 127.00(C_5), 127.44(CH, ethylene C of triazole), 142.28(C, quaternary carbon of triazole ring), 145.96($C_{2'}$-O), 153.61($C_{4'}$-O), 153.88(C-O Fused in pyran ring), 155.15(C_4 pyran ring), 160.60(C_7-O), 161.48(C=O). IR (KBr, cm^{-1}) ν_{max}: 2926.01(C-H stretching), 1728.48(α, β-unsaturated ketonic C=O stretching), 1615.75, 1511.97, 1511.97, 1389.79, 1292.63, 1281.18(C-O-C stretching), 1146.77(C-N stretching in 1,2,3-triazole ring), 1071.82, 1042.84(C-O-C stretching), 862.58, 810.45, 705.16. Anal. Calcd for $C_{21}H_{19}N_3O_5$; C, 64.12; H, 4.87; N, 10.60. Found: C, 64.01; H, 4.89; N, 10.22%.

4.7.10. 7-[1-(3,4,5-Trimethoxy-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(10)**

Yield: 28%. Yellow solid, mp: 142-144°C. MS: m/z = 423.16 (M^+), 248(M-175), 177(hydroxy coumarin). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.39(s, 3H, -CH_3), 3.71(s, 3H, 4-OCH_3), 3.87(s, 6H, 3-OCH_3 and 4-OCH_3), 5.3(s, 2H, -CH_2-O-), 6.23(s, 1H, H_3 coumarin pyran ring), 7.07(d, 1H, H_5, J = 8.4Hz), 7.08-7.17(m, 3H, H_8 coumarin ring, $H_{2'}$ and $H_{6'}$ benzene ring), 7.09(d, 1H, H_6 J = 8.7Hz), 9.03(s, 1H, triazole ring), ^{13}C NMR ((DMSO-d_6,75MHz) δ ppm: 18.18(CH_3), 56.31(3′-OCH_3 &5′-OCH_3), 60.23(4′-OCH_3), 61.72(-CH_2-O), 98.13, 101.65, 102.20(C_3), 110.27(C_8), 111.41(C_6), 112.64($C_{2'}$), 112.90($C_{4'}$), 113.51(C quaternary fused C of coumarin ring), 123.47($C_{1'}$), 126.59(C_5), 132.43(CH, ethylene C of triazole), 137.39($C_{4'}$), 143.09(C, quaternary carbon of triazole ring), 153.45($C_{3'}$&$C_{5'}$), 153.56(C-O Fused in pyran ring), 154.68(C_4 pyran ring), 160.18(C_7-O), 160.97(C=O). IR (KBr, cm^{-1}) ν_{max}: 2930.68(C-H stretching), 1717.53(α, β-unsaturated ketonic C=O stretching), 1610.74, 1510.30, 1389.06, 1277.49, 1265.72(C-O-C stretching), 1126.41(C-N

stretching in 1,2,3-triazole ring), 1073.56(C-O-C stretching), 861.26, 820.30. Anal. Calcd for $C_{22}H_{21}N_3O_6$: C, 62.41; H, 5.00; N, 9.92. Found: C, 62.21; H, 5.64; N, 10.13%.

4.7.11. 7-[1-(2-Nitro-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(11)**

Yield: 35%. Yellow solid. mp: 146-148°C. MS: m/z = 378.10 (M^+), 173(M-175). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.41(s, 3H, -CH_3), 5.39(s, 2H, -O-CH_2-), 6.29(s, 1H, H_3 coumarin pyran ring), 8.91(s, 1H, triazole ring), 7.20(s, 1H, H_8 coumarin ring), 7.09(d, 1H, H_5, J = 6.0Hz), 7.72(d, 1H, H_6, J = 6.9Hz) , 7.86-7.95(m, 3H, H_4', H_5' & H_6' benzene ring), 8.24(d, 1H, H_3' benzene ring, J = 6.6Hz). ^{13}C NMR ((DMSO-d_6, 75MHz) δ ppm: 18.63(CH_3), 61.83(-CH_2-O), 102.05(C_3), 111.84(C_8), 113.10(C_6), 115.90(C quaternary fused C of coumarin ring), 119.62($C_{1'}$), 126.08($C_{3'}$), 127.04(C_5), 128.16($C_{4'}$), 129.47($C_{6'}$), 131.81(CH, ethylene C of triazole), 134.97($C_{5'}$), 136.21, 143.26(C, quaternary carbon of triazole ring), 144.50($C_{2'}$-NO_2), 153.92(C-O Fused in pyran ring) , 155.13(C_4 pyran ring), 160.62, 161.40(C=O). IR (KBr, cm^{-1}) ν_{max}: 2920.02(C-H stretching), 1735.98(α ,β-unsaturated ketonic C=O stretching), 1617.96, 1524.91, 1345.94(-NO_2 sym stretching), 1278.65(C-O-C stretching), 1154.55(C-N stretching in 1,2,3-triazole ring), 1017.41(C-O-C stretching), 839.57, 740.83. Anal. Calcd for $C_{19}H_{14}N_4O_5$: C, 60.32; H, 3.73; N, 14.81. Found: C, 60.17; H, 3.89; N,14.65%.

4.7.12. 7-[1-(3-Fluoro-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(12)**

Yield: 40%. Yellow solid. mp: 178-180°C. MS: m/z = 351.10 (M^+) 352.23(M+1), 353.22(M+2), 176.11 (M-175). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.41(s, 3H, -CH_3), 5.39(s, 2H, -CH_2-O-), 6.24(s, 1H, H_3), 7.08(d, 1H, J = 9.0Hz), 7.18(s, 1H, H_8), 7.39(d, 1H, H_6, J = 6.9Hz), 7.66-7.72(m, 2H, $H_{4'}$ and $H_{5'}$), 7.81-7.88(m, 2H, $H_{2'}$ and $H_{6'}$). ^{13}C NMR ((DMSO-d_6,75MHz) δ ppm: 18.51(CH_3), 62.00(-CH_2-O), 102.09(C_3), 107.95, 108.30, 111.83(C_8), 113.03(C_6), 113.94($C_{4'}$&$C_{2'}$), 115.87(C

quaternary fused C of coumarin ring), 116.51($C_{5'}$), 123.76($C_{6'}$), 126.90(C_5), 132.27(CH, ethylene C of triazole), 138.20($C_{1'}$), 143.84(C, quaternary carbon of triazole ring), 153.82(C-O Fused in pyran ring), 155.09(C_4 pyran ring), 160.58(C_7-O), 161.30($C_{3'}$-F), 164.48(C=O). IR (KBr, cm^{-1}) v_{max}: 3161.20(C-H stretching), 1700.21(α, β-unsaturated ketonic C=O stretching), 1612.81, 1384.34, 1296.92(C-O-C stretching), 1146.58(C-N stretching in 1,2,3-triazole ring), 1072.17(C-O-C stretching), 1037.88, 868.38, 853.48, 801.18, 529.35. Anal. Calcd for $C_{19}H_{14}FN_3O_3$: C, 64.95; H, 4.02; N, 11.96. Found: C, 64.30 ; H, 4.29; N, 11.72%.

4.7.13. 7-[1-(3,5-Dimethyl-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(13)**

Yield: 30%. Yellow solid. mp :202-204°C. MS: m/z = 361.14 (M^+). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.41(s, 9H, 3-CH_3), 5.36(s, 2H, -CH_2-O,), 6.24(s, 1H, pyran ring), 7.10(m, 3H, aromatic proton), 7.53(s, 2H, $H_{2'}$ and $H_{5'}$), 7.72(d, 1H, aromatic proton, J = 8.1Hz), 8.93(s, 1H, triazole ring). IR (KBr, cm^{-1}) v_{max}: 2919.65 (C-H stretching), 1717.61(α, β-unsaturated ketonic C=O stretching), 1612.81, 1390.28, 1370.28, 1265.88(C-O-C stretching), 1199.40, 1138.71(C-N stretching in 1,2,3-triazole ring), 1070.91(C-O-C stretching), 1017.93, 841.30, 808.35, 681.12, 439.12. Anal. Calcd for $C_{21}H_{19}N_3O_3$: C, 69.79; H, 5.30; N, 11.63. Found: C, 69.65; H, 5.98, N, 11.61%.

4.7.14. 4-Methyl-7-[1-(4-phenoxy-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-chromen-2-one **(14)**

Yield: 30%. Yellow solid. mp: 144-146°C. MS: m/z = 425.67(M^+), 332 (M- Ph^+), 93 (Ph-O),77 (Ph^+). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.41(s, 3H, 4-CH_3 aromatic ring) 5.37(s, 2H, -CH_2-O-), 6.24(s, 1H, H_3 coumarin pyarn ring), 7.10(m, 3H, aromatic proton), 7.21 (m, 4H aromatic proton), 7.45(t, 2H, aromatic ring, J = 15.6Hz, J′=7.8Hz, J”=7.8Hz), 7.2 (d, 2H, aromatic ring, J = 8.7Hz), 7.90 (d, 2H, J = 8.7Hz), 8.96 (s, 1H, triazole ring). ^{13}C NMR ((DMSO-d_6,75MHz) δ ppm:

18.08(CH_3), 61.56(-CH_2-O), 101.61 (C_3), 111.32 (C_8), 112.55(C_6), 113.42 (C quaternary fused C of coumarin ring), 119.15(2C aromatic), 119.18(2C aromatic), 122.21(2C aromatic), 123.20(2C aromatic), 124.12(1C aromatic), 126.49(C_5), 130.20($C_{1'}$), 131.87(CH, ethylene C of triazole), 143.09 (C, quaternary carbon of triazole ring), 153.32 (C-O Fused in pyran ring), 154.61 (C_4 pyran ring), 155.89 ($C_{4'}$-O), 157.00 (C-O phenoxy), 160.05(C_7-O), 160.89(C=O). IR (KBr, cm^{-1}) v_{max}: 2935.57 (C-H stretching), 1733.94 (α, β-unsaturated ketonic C=O stretching), 1612.08, 1513.81, 1388.29, 1241.30(C-O-C stretching), 1155.12 (C-N stretching in 1,2,3-triazole ring), 1071.14(C-O-C stretching), 1024.15, 833.68, 699.06. Anal. Calcd for $C_{25}H_{19}N_3O_4$: C, 70.58; H, 4.50; N, 9.88. Found: C, 70.12; H, 4.84; N, 9.78%.

4.7.15. 7-[1-(4-Fluoro-2-methyl-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(15)**

Yield: 30%. Yellow solid. mp: 140-142°C. MS m/z = 365.12 (M^+),190(M-175), 177(hydroxy coumarin cation). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 1.23(s, 3H, 2′-CH_3 of azide ring), 2.08(s, 3H, 4-CH_3 of coumarin ring), 5.37(s, 2H, -CH_2-O-), 6.07(s, 1H, H_3 pyran ring), 6.80(d, 2H, aromatic proton J = 6.6Hz), 7.21(s, 1H), 7.41(s, 1H), 7.80(m, 3H, aromatic), 9.53(s, 1H, triazole ring). IR (KBr, cm^{-1}) v_{max}: 2923.48(C-H stretching), 1715.21(α, β-unsaturated ketonic C=O stretching), 1611.91, 1509.45, 1379.85, 1266.27(C-O-C stretching), 1203.52, 1139.56(C-N stretching in 1,2,3-triazole ring), 1021.98(C-O-C stretching), 827.87, 683.55, 456.47. Anal. Calcd for $C_{20}H_{16}FN_3O_5$: C, 65.75; H, 4.41; N, 11.50. Found: C, 65.18; H, 4.74; N,11.48%.

4.7.16. 7-[1-(2-Chloro-4-methyl-phenyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(16)**

Yield: 40%. Yellow solid. mp :148-150°C. MS: m/z = 381.10 (M^+), 383.11(M+2), 382(M+1), 206.05(M-175). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.41(s, 3H, 4-CH_3 pyran ring), 2.50(s, 3H,-CH_3 aromatic ring attached with triazole) 5.37(s, 2H, -CH_2-O-), 6.24(s, 1H, H_3 coumarin pyran ring), 7.08(d, 1H, H_6 coumarin ring , J = 8.7Hz), 7.2(s, 1H, H_8 coumarin ring), 7.39(d, 1H, $H_{5'}$, J = 7.8Hz), 7.5-7.6(m, 2H, $H_{3'}$ and $H_{6'}$), 7.71(d, 1H, H_5, J = 8.7Hz) , 8.71(s, 1H, triazole ring). ^{13}C NMR ((DMSO-d_6,75MHz) δ ppm: 18.61(CH_3 coumarin ring), 20.91(CH_3 benzene ring), 61.93(-CH_2-O), 102.12(C_3), 111.83(C_8), 113.13(C_6), 113.92(C quaternary fused C of coumarin ring), 127.02(C_5), 127.67($C_{1'}$), 128.50($C_{5'}$), 128.60($C_{6'}$), 129.42($C_{3'}$), 131.10(CH, ethylene C of triazole), 132.38($C_{2'}$), 142.53(C, quaternary carbon of triazole ring), 142.65($C_{4'}$), 153.89(C-O Fused in pyran ring), 155.15(C_4 pyran ring), 160.59(C_7-O), 161.47(C=O). IR (KBr, cm^{-1}) v_{max}: 3144.15, 2923.48(C-H stretching), 1715.21(α, β-unsaturated ketonic C=O stretching), 1611.91, 1509.45, 1379.85, 1266.27(C-O-C stretching), 1203.52, 1139.56(C-N stretching in 1,2,3-triazole ring), 1021.98(C-O-C stretching), 827.87, 456.47. Anal. Calcd for $C_{20}H_{16}$ Cl N_3O_3: C, 62.91; H, 4.22; N, 11.50. Found: C, 62.12; H, 4.52; N,14.38%.

4.7.17. [4-(4-Methyl-2-oxo-2H-chromen-7-yloxymethyl)-[1,2,3] triazol-1-yl]-acetic acid methyl ester **(17)**

Yield: 42%. Yellow solid. mp: 138-140°C. MS: m/z = 329.16 (M^+), 315(M-CH_3), 271 (M-COOMe). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.39(s, 3H, -CH_3), 3.7(s, 3H, -OCH_3), 5.30(s, 2H, -CH_2-N-), 5.44(s, 2H, CH_2-O-), 7.04(d, 1H, H_5, J = 9.0Hz), 7.15(s, 1H, H_8), 7.67(d, 1H, H_6, J = 8.7Hz), 8.30(s, 1H, triazole ring). ^{13}C NMR ((DMSO-d_6,75MHz) δ ppm: 18.59(CH_3), 50.79(-OCH_3), 53.02, 56.52(CH_2-N), 61.97(-CH_2-O), 101.92, 102. 26(C_3), 111.75(C_8), 113.00(C_6), 114.00(C quaternary fused C of coumarin ring), 126.95(C_5 &CH, ethylene C of triazole), 142.60(C, quaternary carbon of triazole ring), 153.82(C-O, Fused in pyran ring), 154.00(C_4, pyran ring),

160.52(C_7-O), 161.44(C=O), 168.16 (C=O, ester). IR (KBr, cm^{-1}) v_{max}: 2960.23(C-H stretching), 1733.95(α, β-unsaturated ketonic C=O stretching), 1717.58(C=O of methyl ester), 1611.95, 1396.82, 1375.73, 1279.70(C-O-C stretching), 1254.58, 1155.44(C-N stretching in 1,2,3-triazole ring), 1138.37, 1070.82(C-O-C stretching), 988.59, 840.22, 800.16, 458.77. Anal. Calcd for $C_{16}H_{15}N_3O_5$: C, 58.36; H, 4.59; N, 12.76. Found: C, 58.12; H, 4.81; N, 12.74%.

4.7.18. 7-[1-(2-Hydroxy-ethyl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(18)**

Yield: 38%. Yellow solid. mp: 140-142°C. MS: m/z = 301.16 (M^+), 282.10(M-H_2O). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.39(s, 3H, -CH_3), 3.7(s, 2H, -CH_2OH), 4.42(s, br, 2H, -CH_2-N), 5.07(s, 1H, -OH), 5.26(s, 2H, -CH_2-O-), 6.21(s, 1H, H_3, coumarin pyran ring), 7.03(d, 1H, H_5, J = 8.4Hz), 7.13(s, 1H, H_8, coumarin ring), 7.67(d, 1H, H_6, J = 8.4Hz), 8.25(s, 1H, triazole ring). ^{13}C NMR ((DMSO-d_6, 75MHz) δ ppm: 18.11(CH_3), 52.27(-CH_2-N-,ethyl), 59.78(-CH_2-OH,ethyl) 61.64(-CH_2-O), 101.50(C_3), 112.24(C_8), 112.57(C_6), 113.31(C quaternary fused C of coumarin ring), 125.44(C_5), 126.46 (CH, ethylene C of triazole), 153.40(C-O Fused in pyran ring), 154.62(C_4 pyran ring), 160.14(C_7-O), 161.04(C=O). IR (KBr, cm^{-1}) v_{max}: 3096.95(C-H stretching), 1716.70(α, β-unsaturated ketonic C=O stretching), 1612.62, 1390.23, 1294.71(C-O-C stretching), 1150.24(C-N stretching in 1,2,3-triazole ring), 1075.75(C-O-C stretching), 1044.00, 849.32, 707.14. Anal. Calcd for $C_{15}H_{15}N_4O_3$: C, 59.79; H, 5.02; N, 13.95. Found: C, 59.75; H, 5.33; N,13.92%.

4.7.19. 4-Methyl-7-(1H-[1,2,3] triazol-4-ylmethoxy)-chromen-2-one **(19)**

Yield: 30%. Dark green solid. mp: 186-188°C. MS: m/z = 257.33 (M^+), 177 (7-hydroxy coumarin cation). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.18(s, 3H, -CH_3), 5.3(s, 2H, -O-CH_2-), 6.23(s, 1H, H_3 coumarin pyran ring), 7.16(d, 1H, H_5, J = 12.3Hz), 8.07(s, 1H, H_8), 8.29(d, 1H, H_6, J = 12.9Hz), 9.18(s, 1H, triazole ring). ^{13}C NMR ((DMSO-d_6, 75MHz) δ ppm: 18.60(CH_3), 61.95(-CH_2-O),

102.09(C_3), 111.84(C_8), 113.07(C_6), 122.34(C quaternary fused C of coumarin ring), 123.75, 127.04(C_5), 130.37(CH, ethylene C of triazole), 135.75, 143.84(C, quaternary carbon of triazole ring), 153.89(C-O Fused in pyran ring), 155.11(C_4 pyran ring), 160.60(C_7-O), 161.35(C=O) . IR (KBr, cm^{-1}) v_{max}: 3422.71, 2925.89(C-H stretching), 1718.63 (α, β-unsaturated ketonic C=O stretching), 1612.22, 1501.95, 1388.70, 1265.54(C-O-C stretching), 1155.63 (C-N stretching in 1,2,3-triazole ring), 1071.67(C-O-C stretching), 1008.03, 839.59. Anal. Calcd for $C_{13}H_{11}N_3O_3$: C, 60.79; H, 4.31; N, 16.33. Found: C,60.65; H, 4.34; N,16.23%.

4.7.20. 7-(1-Benzyl-1H-[1,2,3] triazol-4-ylmethoxy)-4-methyl-chromen-2-one **(20)**

Yield: 55%. White crystals. mp :118-120°C. MS: m/z = 377.17 (M^+), 256.17(M-91), 91(benzyl cation) . ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.36(s, 3H, -CH_3), 5.20(s, 2H, -Benzylic –CH_2-), 5.50(s, 2H, -O-CH_2-) 6.11(s, 1H, H_3 coumarin pyran ring), 6.87 (s, br, 2H, H_2 and H_6 coumarin ring), 7.23-7.56(m, 7H, 5 benzene ring, 1H triazole ring, H_8 coumarin ring). ^{13}C NMR ((DMSO-d_6, 75MHz) δ ppm: 18.64(CH_3), 54.33(-CH_2-N), 62.29(-CH_2-O), 102.10(C_3), 112.27(C_8), 114.09(C_6), 122.83(C quaternary fused C of coumarin ring), 125.66($C_{3'}$, $C_{4'}$ &$C_{5'}$), 128.53(C_5), 128.90($C_{2'}$ & $C_{6'}$), 129.18($C_{1'}$), 134.37(CH, ethylene C of triazole), 143.57(C, quaternary carbon of triazole ring), 152.33(C-O Fused in pyran ring), 155.25(C_4 pyran ring), 160.86(C=O). IR (KBr, cm^{-1}) v_{max}: 2925.77(C-H stretching), 1708.09(α, β-unsaturated ketonic C=O stretching), 1609.84, 1388.99, 1264.87(C-O-C stretching), 1198.08, 1141.98 (C-N stretching in 1,2,3-triazole ring), 1071.99(C-O-C stretching), 1001.73, 844.33, 726.90, 706.24. Anal. Calcd for $C_{20}H_{17}N_3O_3$: C, 69.15; H, 4.93; N, 12.10. Found: C, 69.12; H, 4.99; N, 12.06%.

4.7.21. 7-[1-(4-Nitro-benzyl)-1H-[1,2,3] triazol-4-yl-methoxy]-4-methyl-chromen-2-one **(21)**

Yield: 36%. Yellow solid. mp: 144-146°C. MS: m/z = 392.64 (M^+), 256.24, 136.11(nitrobenzyl cation). ^{1}H NMR ((DMSO-d_6, 300MHz) δ ppm: 2.39(s, 3H, -CH_3), 5.29(s, 2H, -CH_2-O-), 5.80(s, 2H, -CH_2-N- nitrobenzyl), 6.23(s, 1H, H_3 coumarin pyran ring), 7.03(d, 1H, H_5, J = 8.1Hz), 7.14(s, 1H, H_8 coumarin ring), 7.54(d, 2H, $H_{2'}$ and $H_{6'}$, J = 7.8Hz), 7.68(s, 1H, H_6 , J = 8.4Hz), 8.24(d, 2H, $H_{3'}$ and $H_{5'}$, J = 7.8Hz), 8.43(s, 1H, triazole ring). ^{13}C NMR ((DMSO-d_6, 75MHz) δ ppm : 18.18(CH_3), 59.16(-CH_2-N), 61.61(-CH_2-O), 101.62(C_3), 111.36(C_8), 112.71(C_6), 113.42(C quaternary fused C of coumarin ring), 124.00($C_{3'}$ & $C_{5'}$), 125.52($C_{2'}$ &$C_{6'}$), 126.57(C_5), 129.12(CH, ethylene C of triazole), 142.51(C, quaternary carbon of triazole ring), 143.59($C_{1'}$ nitrobenzyl), 147.29($C_{4'}$-NO_2), 153.48(C-O Fused in pyran ring), 154.67(C_4 pyran ring), 160.20(C_7-O), 160.99(C=O). IR (KBr, cm^{-1}) ν_{max}: 2924.63(C-H stretching), 2107.12, 1718.32(α, β-unsaturated ketonic C=O stretching), 1618.14, 1521.64 (-NO_2 asym stretching), 1388.25, 1346.73(-NO_2 sym stretching), 1292.29 (C-O-C stretching), 1150.52 (C-N stretching in 1,2,3-triazole ring), 1074.12(C-O-C stretching), 852.71, 814.34, 727.81, 504.01. Anal. Calcd for $C_{20}H_{16}N_4O_5$: C, 61.22; H, 4.11; N, 14.28. Found: C, 61.12; H, 4.34; N, 14.23%.

4.7.22. 7-[1-(7-Chloro-quinolin-4-yl)-1H-[1,2,3] triazol-4-ylmethoxy]-4-methyl-chromen-2-one **(22)**

Yield: 40%. Yellow crystals. mp: 150-152°C. MS: m/z = 418.60 (M^+), 257.06 (M-quinoline ring), 162 (quinoline cation), ^{1}H NMR (DMSO-d_6, 300MHz) δ ppm: 2.39(s, 3H, -CH_3), 5.37(s, 2H, -O-CH_2-) 6.21(s, 1H, H_3 coumarin pyran ring), 6.69-8.2(m, 8H, 5H quinoline and 3H coumarin ring), 8.99(s, 1H, triazole ring), ^{13}C NMR ((DMSO-d_6, 75MHz) δ ppm: 18.62(CH_3), 62.09(-CH_2-O), 80.54, 102.11(C_3), 111.59(C_8), 113.06(C_6), 114.18(C quaternary fused C of

coumarin ring), 122.35(quinoline aromatic carbon), 124.65(C_5), 127.81(CH, ethylene C of triazole), 130.37($C_{4'}$-N), 143.50 (C, quaternary carbon of triazole ring), 149.53(C-N, quinoline), 151.78(C-O Fused in pyran ring), 154.02(C_4 pyran ring), 155.40 (CH-N, quinoline), 160.57(C_7-O), 161.61(C=O). IR (KBr, cm^{-1}) v_{max}: 2128.31(C-H stretching), 1726.16(α ,β-unsaturated ketonic C=O stretching), 1610.32, 1388.36, 1304.50(C-O-C stretching), 1201.14, 1155.88(C-N stretching in 1,2,3-triazole ring), 1071.74(C-O-C stretching), 820.52. Anal. Calcd for $C_{22}H_{15}ClN_4O_3$: C, 63.09; H, 3.61; N, 13.38. Found: C, 63.03; H, 3.75; N, 11.31%.

4.8 Estimation of *in vitro* antiplasmodial activity

4.8.1 Parasite culture

Culture of erythrocytic stages of chloroquine sensitive *P. falciparum* strain 3D7 was procured from International Centre of Genetic Engineering and Biotechnology (ICGEB), New Delhi. It was continuously maintained as stocks in 25 cm^2 tissue culture flasks, on human O+ red blood cells under low-oxygen concentration (3%) and high carbon dioxide atmosphere (4%) along with nitrogen (93%), at a temperature of 37 °C, in RPMI 1640 (Invitrogen) with 25 mM HEPES, 25 mM $NaHCO_3$, 2 mM L-glutamine, 50 mg/L gentamicin (Gibco), 5 g/L Albumax II (Life Technologies). The stock cultures were started with 5% hematocrit and parasitemia less than 1%. Parasitemia was kept between 2 and 4% with sub-culturing done beyond 5%. Medium was changed once a day and percentage parasitemia was monitored by Giemsa stained slides.

4.8.2. Stock solution of compounds

Artemisinin (Sigma–Aldrich, USA), and the compounds 1 to 22 were prepared separately in DMSO to get the concentration of stock solution of 1 mg/ml strength. The stock solution was further diluted on the day

of experiment to get the desired concentrations for each drug. The amount of DMSO in diluted concentrations used had no effect on parasite growth.

4.8.3. Compound (drug) concentration response assay

The concentration of an individual compound required to inhibit multiplication of parasites by 50% (IC_{50}) against *P. falciparum* was determined using concentration response assay in 24-well tissue culture plates in triplicates. Parasite cultures were subjected to graded concentration of a compound, prepared in gentamicin-free RPMI culture medium, for 48 h at 37 °C in a CO_2 incubator. Medium was changed in each well after 24 h with or without the compound. The results were expressed as the mean percentage inhibition ± standard error in relation to control; examined by thin smear Giemsa stained slides. IC_{50} values were computed from semi-log plots.

4.8.4. Slide preparation, staining and assessment

Thin blood smear slides were air dried, methanol fixed, and stained in Giemsa solution for 40 min. After staining, the slides were removed from coupling jar, washed in running tap water and air dried. The Giemsa stained slides were examined for counting the number of parasites in random adjacent microscopic fields, equivalent to about 4000 erythrocytes at 1000x magnification. Percent parasitemia was calculated. Reproducibility of counts was checked by two other readers to maintain the quality control.

4.9 MTT assay for cell viability

Various human hepatoma cells (Huh-7) were maintained as monolayer at 37 °C in 5% CO_2 using DMEM medium. Approximately, 20,000 cells/well were seeded in 96-well plate containing 200 mL of medium and incubated for 24 h. The culture medium was replaced by fresh medium containing 1, 10, 20, 30, 50 and 100 µg/mL concentration of each of the compounds and incubated for 72 h. The cell viability was determined

by the MTT assay following the procedure described by Price and McMillan [40]. The light absorbance was measured at 570 nm wave length using a microplate reader.

4.10 Inhibition of DNA gyrase

1 U of enzyme gyrase converts 0.5 μg of relaxed plasmid DNA to the supercoiled form [41]. *Escherichia coli* DNA gyrase and relaxed plasmid DNA substrate (pHOT1, a derivative of pBR322) were purchased as a kit from TopoGEN. The *E. coli* DNA gyrase supercoiling inhibition assay was performed according to the instructions of the manufacturer, as follows: The assay buffer (1X recipe) consisted of 35 mM Tris-HCl, pH 7.5, 24 mM KCl, 4 mM MgCl2, 1 mM ATP, 2 mM DTT, 1.8 mM spermidine, 6.5% glycerol, 1 mM ATP and 100 μg/mL BSA. A typical reaction mixture (20 μl final volume) consisted of sterile distilled water, 5X assay buffer, relaxed DNA substrate, and approximately IC_{50} concentrations of the coumarin analogs. The fluoroquinolone antibiotic ciprofloxacin was used as a positive control. Following the addition of gyrase, the reactions were moved to 37 °C for 1 h. Termination was carried out by addition of 10% SDS. Next, proteinase K was added, and the assay was incubated an additional 30 min at 37 °C, followed by addition of loading dye. Organic extraction was carried out to withdraw the aqueous phase, which was loaded onto 1% agarose gel prepared without ethidium bromide (EtBr). Bands were visualized by staining with EtBr (0.5 μg/ml) for 30 min, followed by destaining with distilled water, and finally UV analysis on a Bio-Rad GelDoc apparatus.

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Conflicts of interest: none

Ethical approval: NA

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Highlights

- A series of coumarin-triazole derivatives were synthesized by alkylation of 7-hydroxy-4-methyl-coumarin followed by click chemistry.
- Most of the compounds demonstrated promising *in vitro* antiplasmodial activities.
- They displayed inhibition of the supercoiling activity of DNA gyrase enzyme.