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Synthesis and evaluation of Naphthyl bearing 1,2,3-Triazole analogs as Antiplasmodial agents, Cytotoxicity and Docking Studies

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Abstract

Novel series of naphthyl bearing 1,2,3-triazoles (**4a-t**) were synthesized and evaluated for their in vitro antiplasmodial activity against pyrimethamine (Pyr)-sensitive and resistant strains of Plasmodium falciparum. The synthesized compounds were assessed for their cytotoxicity employing human embryonic kidney cell line (HEK-293), and none of them was found to be toxic. Among them **4j**, **4k**, **4l**, **4m**, **4n**, **4t** exhibited significant antiplasmodial activity in both strains, of which compounds **4m**, **4n** and **4t** (~3.0 fold) displayed superior activity to Pyr against resistant strain. Pyr and selected compounds (**4n**, **4p** and **4t**) that repressed parasite development also inhibited PfDHFR activity of the soluble parasite extract, suggesting that anti-parasitic activity of these compounds is a result of inhibition of the parasite DHFR. In silico studies suggest that activity of these compounds might be enhanced due to π-π stacking.

Keywords

Naphthyl 1,2,3-triazoles; Click chemistry; Antiplasmodial activity; Cytotoxicity; Induced fit docking(IFD)

1. Introduction

Malaria remains as one of the world's most widespread and dreadful infectious diseases. Recent estimates indicate approximately 4,38,000 deaths worldwide in 2015, that predominantly trait children under 5 years of age, pregnant women and patients with HIV [1]. Drug resistance is a major cause of morbidity and mortality [2], complicates and impedes current efforts in the treatment of malaria and its control. Antifolates are an important class of antimalarial drugs that target folate metabolism is essential for survival of malarial parasite. Several compounds with Quinoline scaffold are found to be effective against the parasite's Dihydrofolate reductase (DHFR), a key enzyme in the folate biosynthetic pathway [3].

Point mutations in DHFR have been implicated in drug resistance against antifolates [4]. Dihydrofolate reductase-Thymidylate synthase (DHFR-TS) is a bifunctional enzyme of P. falciparum remains as one of the popular targets for malaria chemotherapy [5-10]. This enzyme catalyzes the Nicotinamide Adenine Dinucleotide Phosphate (NADPH) dependent reduction of Dihydrofolate (DHF) to Tetrahydrofolate (THF) that is essential for DNA synthesis. Inhibition of DHFR enzyme interrupts DNA synthesis, which ultimately leads to the death of the parasite.

Triazoles scaffold have garnered much attention in recent times due to their wide range of application in medicinal chemistry. The Huisgen1,3-dipolar cycloaddition reactions of azides and alkynes is an exceptionally atom-economical, forthright route to yield 1,2,3-triazoles [11]. The copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC) has emerged as an outstanding procedure for access to 1,4-disubstituted 1,2,3-triazoles, in a regioselective manner and remains as an attractive scaffold to start with [12-18]. Recently, several naphthyl based compounds [19] and 1,2,3-triazoles [20-23] have been reported for their antimalarial activity. Among the several chemical classes that boast the potential to display the antimalarial activity,

prototype structures of few naphthyl and triazoles possessing significant in vitro antimalarial activity are presented in Fig. 1.

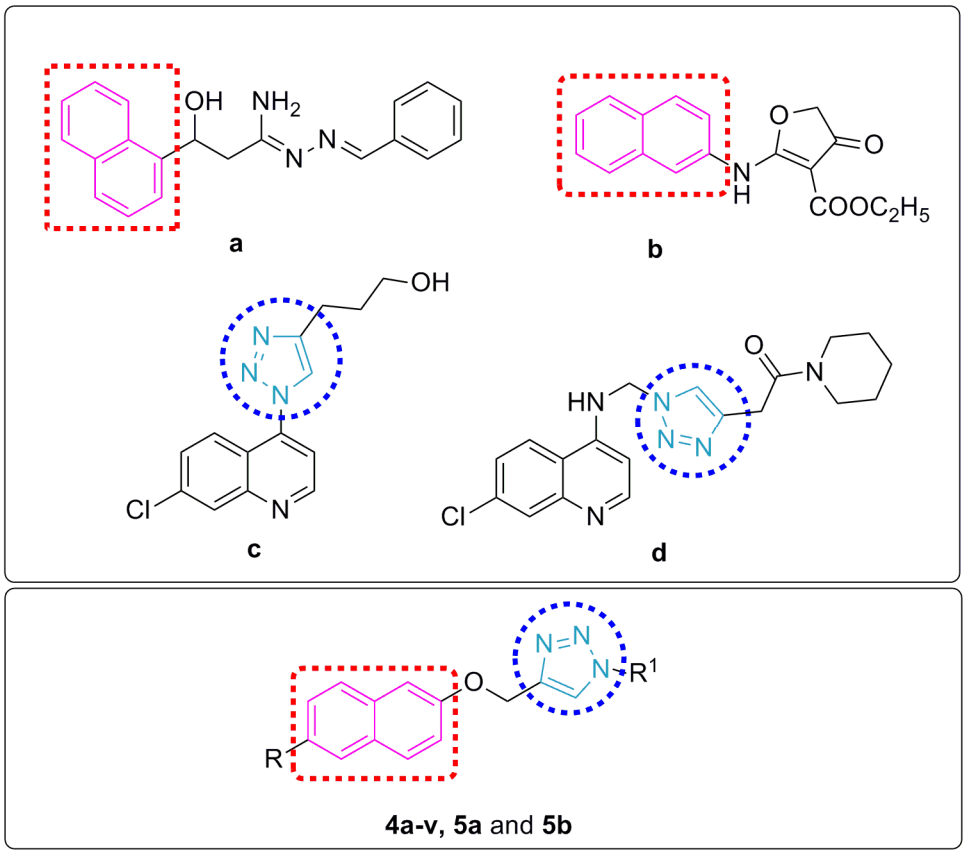


Fig. 1. Bioactive antimalarial agents.

a, b - naphthyl based antimalarials; c,d- triazole based antimalarials

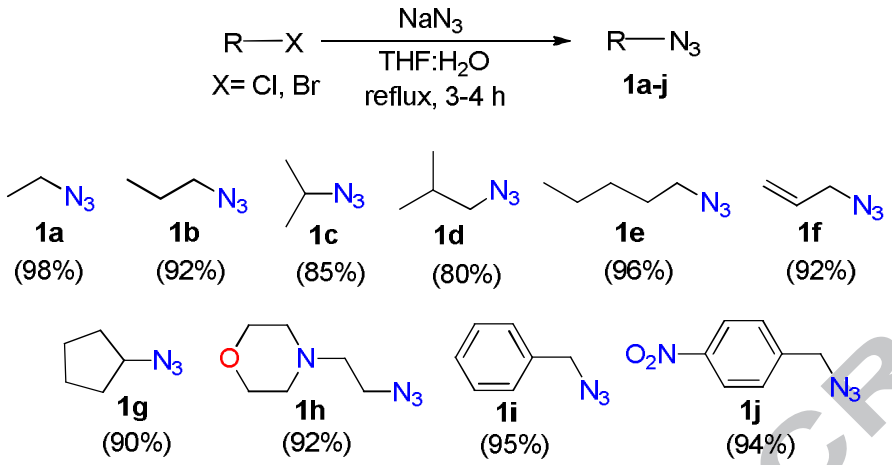
Antimalarial activity of antifolates is suggested to be enhanced by flexibility, aromaticity and hydrophobic nature [24]. In order to explore the significance of aromatic and hydrophobic substituents on 1,2,3-triazole moiety, we have bioisoterically replaced the quinoline moiety with naphthyl and derivatives possessing this substituent's were synthesized. We investigated the effect of our novel naphthyl 1,2,3-triazoles on the growth of cultured P. falciparum parasite, as well as their ability to inhibit parasite DHFR. In silico studies on PfDHFR support us in understanding the basis of drug resistance and are instrumental in developing novel and effective

inhibitors against resistant DHFR [25-28]. The mode of interactions with the actives is emphasized by means of docking studies in both wild type and mutant enzymes.

2. Results and Discussion

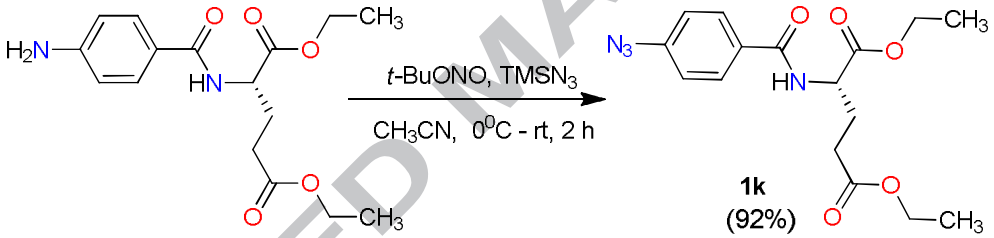
2.1. Chemistry

Naphthyl bearing 1,2,3-triazoles were synthesized using a convenient approach that entails linking of naphthyl alkynyl ether (**3a/b**) with suitable azides (**1a-j**, **1k**) by Click approach, as outlined in Scheme 3. In connection to this, several azides were synthesized by treating alkyl/aryl halides with NaN_3 in presence of THF:H_2O medium (Scheme 1) [29]. A novel (*S)*-diethyl-2-(4-azidobenzamido)pentanedioate (**1k**) was synthesized from its amino derivative using less hazardous, stable and non-explosive reagents such as *t*-butyl nitrite (*t*-BuONO) and azido trimethyl silane ($TMSN_3$) (Scheme 2). The prepared azides are treated with naphthyl alkynyl ether (**3a/b**, that is prepared from naphthols (**2a/b**) by treating with propargyl bromide, K_2CO_3 in DMF), $CuSO_4.5H_2O$, sodium ascorbate in 2:1 mixture of *t*-BuOH and H_2O to capitulate the final products **4a-v**. The reactions were carried out under mild conditions and afforded the final desired products in high yield. The acid compounds **5a** and **5b** were synthesized from its ester derivatives on hydrolysis. The detailed synthesis and spectral data are described in the experimental section.



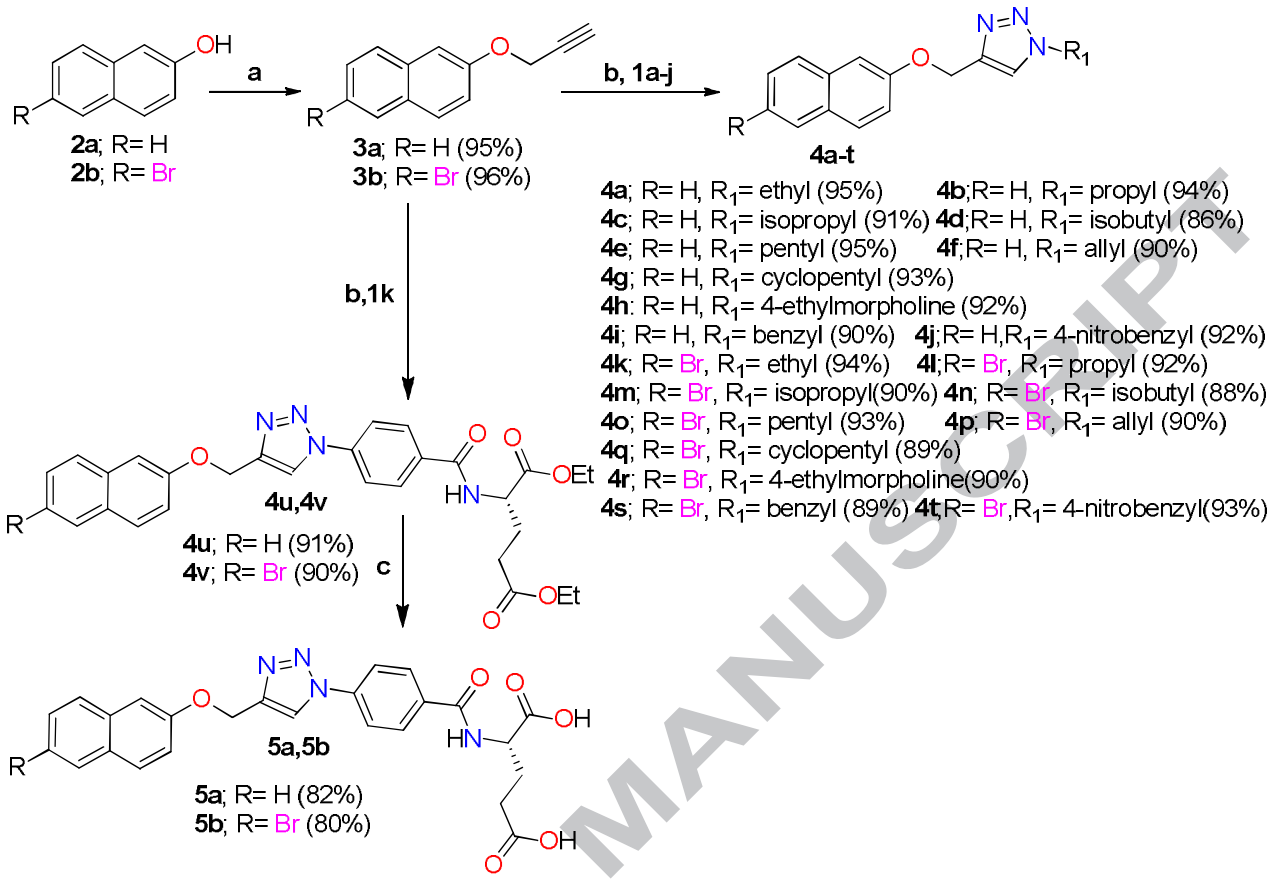
Scheme 1. Synthesis of **1a-j**.

Reagents and conditions: Aryl/alkyl halide (1 equiv), NaN_3 (1.5 equiv), in THF: H_2O (1:4), refluxed at 80-85 ^oC for 3-4 h. Reported yields are for the isolated Alkyl/Aryl azides.



Scheme 2. Synthesis of novel *(S)*-diethyl-2-(4-azidobenzamido)pentanedioate (**1k**).

Reagents and conditions: *(S)*-diethyl-2-(4-aminobenzamido)pentanedioate (1.0 equiv), *t*-BuONO (1.5 equiv), and $TMSN_3$ (1.2 equiv) in acetonitrile at 0 °C to room temperature.



Scheme 3. Synthesis of novel naphthyl 1,2,3-triazole derivatives **4a-v**, **5a** and **5b**. Reagents and conditions; a) K_2CO_3 (1.1 equiv), propargyl bromide (1.1 equiv), in DMF refluxed for 3 h; b) azide **1a-1j**,**1k** (0.75 mmol), alkynes **3a**, **3b** (1.05 equiv), $CuSO_4 \cdot 5H_2O$ (5 mol %), and Na ascorbate (15 mol %), in *t*-BuOH:H_2O (2:1) (10 mL) at room temp. for 12 h; c) THF:H_2O (15 mL), 1N NaOH(7.5 mL) at room temperature for 4 h. Reported yields are for the isolated products.

2.2. X-ray crystallographic studies

All the synthesized compounds are characterized by spectroscopic (^{1}HNMR, ^{13}CNMR, LC-MS and HRMS) data and were found to be in good compliance with their depicted structures. Additionally, the structure of compounds **4c**, **4k**, **4r**, **4s** and **4t** were further confirmed by single crystal X-ray diffraction method. Fig. 2 shows a perspective view of these compounds together with their atomic labeling. Crystallographic data and structure refinement for these compounds is provided in supporting information. Crystallographic data has been deposited for

compounds **4c**, **4k**, **4r**, **4s** and **4t** with the Cambridge Crystallographic Data Centre [CCDC 1037001/2/3/4/5 respectively].

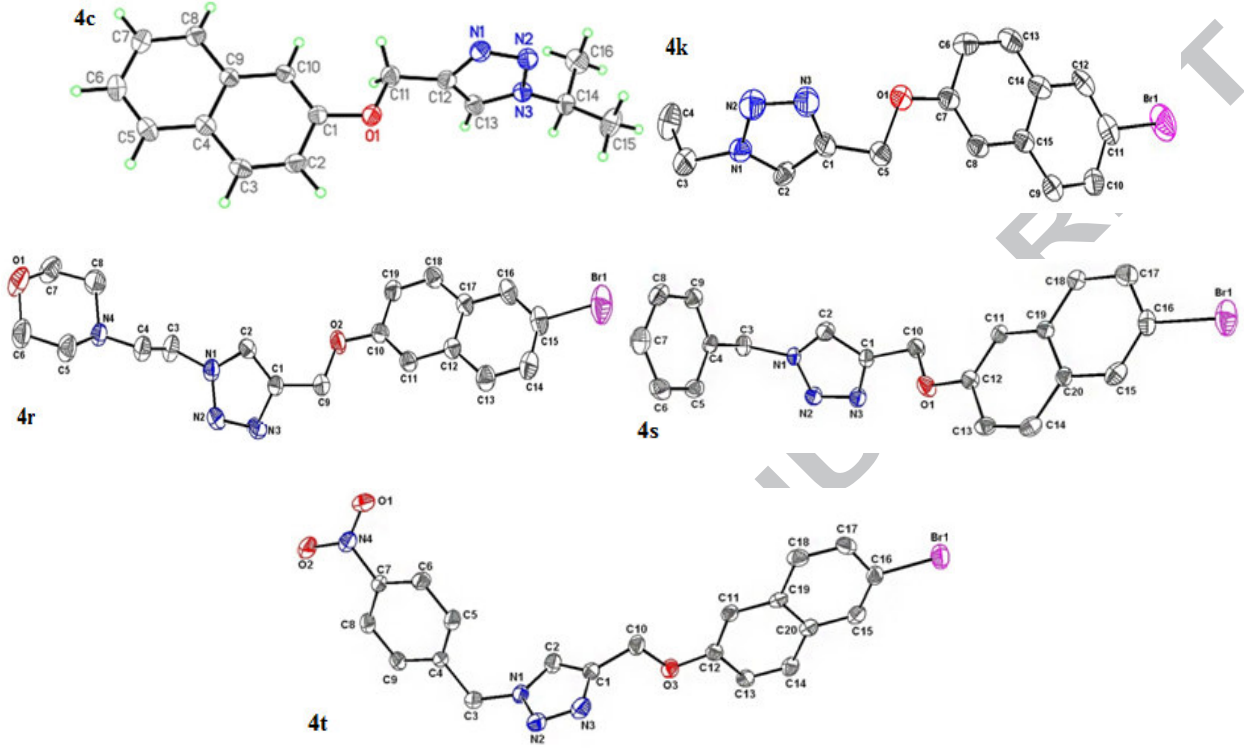


Fig. 2. ORTEP drawings (30% probability level) for **4c**, **4k**, **4r**, **4s** and **4t**.

2.3. In vitro antiplasmodial activity and SAR

The newly synthesized compounds were evaluated for their antiplasmodial activity against Pyr-sensitive (3D7) and resistant (Dd2) P. falciparum strains. Selected 3D7 high active compounds (**4j**, **4k**, **4l**, **4m**, **4n** and **4t**) were assessed for antiplasmodial activity on Dd2 strain. Notably, all these compounds inhibited the growth of Dd2 with similar concentrations to that of wild type strain. The IC_{50} values of these compounds are summarized in Table 1, and their distribution was configured graphically as shown in Fig. 3. The compounds **4m** (IC_{50} of 24.0 µM), **4n** (IC_{50} of 31.03 µM) and **4t** (IC_{50} of 13.6 µM) against Pyr resistant Dd2 strain showed improved antiplasmodial activity than the control drug Pyr (IC_{50} of 33.95 µM).

Table 1: In vitro antiplasmodial activity[a] and Cytotoxicity[b] of the synthesized compounds.

Compound ID	P. falciparum 3D7	P. falciparum Dd2	IC_{50}+SEM (µM)
	IC_{50} in µM	IC_{50} in µM	HEK-293
4a	97.5	–	NC
4b	54.5	–	NC
4c	43.45	–	NC
4d	61.5	–	NC
4e	46.45	–	NC
4f	48.6	–	NC
4g	38.95	–	NC
4h	80	–	NC
4i	NA	–	–
4j	39.65	41.65	NC
4k	23.55	34.16	NC
4l	22.05	36.93	NC
4m*	21.0	24.0	NC
4n*	19.6	31.03	NC
4o	26.25	–	NC
4p	21.25	–	NC
4q	27.3	–	NC
4r	NA	–	–

4s	NA	–	–
4t*	14.7	13.6	NC
4u	NA	–	–
4v	NA	–	–
5a	NA	–	–
5b	41.45	–	NC
Pyr	0.047	33.95	–
Doxorubicin	–	–	3.13 ± 0.01

[a] Pyr was used as a control drug. The IC_{50} values are average with a standard deviation from two independent experiments, each done in duplicates. NA: Not Active (Did not inhibit, even at the highest concentration used), – : not determined. *More potent than Pyr against resistant strain.

[b] Cell line was treated with different concentrations 1µM to 100µM of compounds for 48 h. Cell viability was measured employing MTT assay. IC_{50} values are indicated as the mean +/- SD of three independent experiments. NC: Non Cytotoxic.

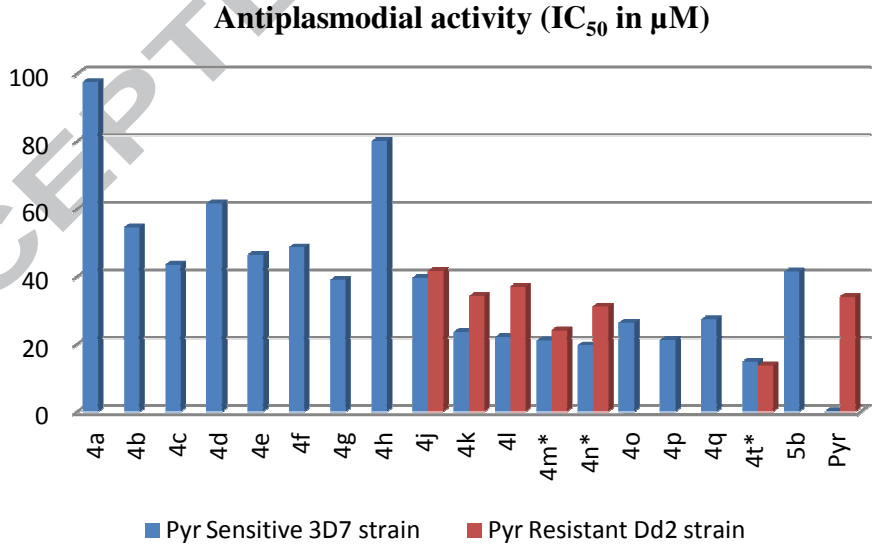


Fig. 3. Graphical representation of antiplasmodial activity.

Twenty four naphthyl bearing 1,2,3-triazole derivatives were synthesized using Click reaction between naphthylalkynylethers and substituted azides. Majority of the synthesized compounds exhibited fair antiplasmodial activity. Dock pose analysis of the potent molecules highlighted the presence of lipophilic groups which aid in anchoring the compounds into hydrophobic pocket. Naphthyl and triazole rings exhibited π-π stacking interactions with Phe58, and aryl substituent on the *N*-1 position of the triazole ring interacted with Phe116 of the enzyme. Rotatable bonds adjacent to the naphthyl and aryl groups allowed the molecules to maneuver effectively into the active site of both wild type (PDB ID: 1J3I) and mutant (PDB ID: 1J3K) PfDHFR.

Structure activity relationship (SAR) studies of reported inhibitors like Trimethoprim and Pyr have revealed that the resistance to Pyr arises due to point mutation in PfDHFR, resulting in the substitution of serine to asparagine (S108N). This mutation is believed to induce steric clashes between the bulky side chain of N108 and Cl atom of the 5-*p*-Cl aryl group of Pyr, reducing the binding affinity between the enzyme and inhibitor. The higher inhibitory potential of newly synthesized naphthyl-1,2,3-triazole derivatives may be attributed to the absence of such steric clashes and the hydrophobic naphthyl framework anticipate in contribution of strong π-π stacking interactions with DHFR.

SAR at the cellular level for these naphthyl 1,2,3-triazole derivatives demonstrated that the compounds with aromatic and aliphatic hydrophobic groups attached to the either side of triazole ring showed remarkable antiplasmodial activity. All the compounds in the series **4a–j** have a simple (naphthalen-6-yloxy) methyl group at one end (C-4) of the triazole ring and hydrophobic groups on the other end, i.e., the *N*-1 position. These compounds thus exhibited moderate activity against the wild strain. While replacement of hydrogen with electronegative

Bromine atom in compounds **4k–t** and **4v**, **5b** with (2-bromonaphthalen-6-yloxy) methyl group at the C-4 position of the triazole ring has shown enhanced activity. Among these, **4k**, **4l**, **4m**, **4n**, **4p** and **4t** compounds showed high antiplasmodial activity on wild and resistant strains. The compound **4t** with a nitro benzyl substituent at the *N*-1 position of triazole moiety exhibited higher antiplasmodial activity when compared to all other compounds. The activity has been observed to increase from compounds **4k** (ethyl), **4l** (propyl), **4m** (isopropyl) to **4n** (isobutyl), and **4p** (allyl) has displayed further increase in activity. This is certainly due to branched alkyl substituent and olefinic character respectively. Compounds **4o** and **4q** with higher hydrocarbon chains showed decreased activity towards wild type strain, despite **4r** and **4s** having 4-ethylmorpholine and benzyl groups attached respectively to the triazole moiety has contributed in enhancement of hydrophobic nature but did not exhibit any activity on wild strain. Hence, moderate hydrobhobicity on the triazole ring (branched alkyl) appears to be optimal for more potent antiplasmodial activity.

While compounds **4u** and **5a** with glutamate side chain on the *N*-1 position and simple (naphthalen-6-yloxy) methyl on the C-4 position has not shown any activity, where as **5b** has shown activity due the presence of electronegative Br group on the naphthyl ring. This emphasizes the importance of an electronegative substituent on the naphthyl ring.

2.4. DHFR inhibition Assay

Three compounds that inhibited parasite development (**4n**, **4p** and **4t**) were also evaluated for inhibition of DHFR activity in the soluble parasite extract. Pyr, a standard parasite DHFR inhibitor, was used as a positive control [30]. Pyr decreased NADPH depletion to nearly 75% compared to control, indicating that 25% of the total NADPH depletion was by DHFR (Fig. 4). **4n** and **4p** decreased NADPH depletion to 59% and 87% of the control, indicating 41% and 13%

inhibition, respectively. However, **4t** did not affect NADPH depletion, most likely due to its rapid precipitation in the assay buffer. Although Pyr is a nanomolar inhibitor of malaria parasites, it inhibited NADPH depletion by 25%, suggesting that DHFR is responsible for 25% of the total NADPH depletion activity. Indeed, several other enzymes, such as thioredoxin reductase and glutathione reductase [31-32], can also utilize NADPH. Alternatively, as Pyr also precipitated during reaction, its effective concentration might have been lower than that used in the assay. Similar was the case with **4t**, which was most potent on parasites among the three compounds. Hence, **4n** and **4p** partially inhibited NADPH depletion activity of the parasite extract, which was similar to that of Pyr, suggesting that these compounds target parasite DHFR.

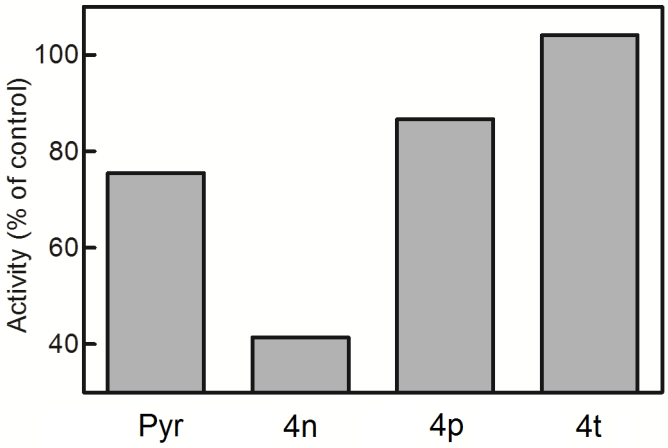


Fig. 4. Inhibition of DHFR activity.

Fig. 4. Inhibition of DHFR activity: 500 µg of soluble parasite extract was incubated with DHFR assay buffer containing 0.05% DMSO (control) or the indicated inhibitors (50 µM Pyr, 25 µM **4n**, 25 µM **4p**, and 25 µM **4t**) for 30 min at room temperature. 60 µM NADPH and 50 µM DHA were added to the reaction, decrease in NADPH absorbance was monitored for 30 min, and expressed as the rate of change in absorbance/min. Data shown are percent activity of control from a representative experiment.

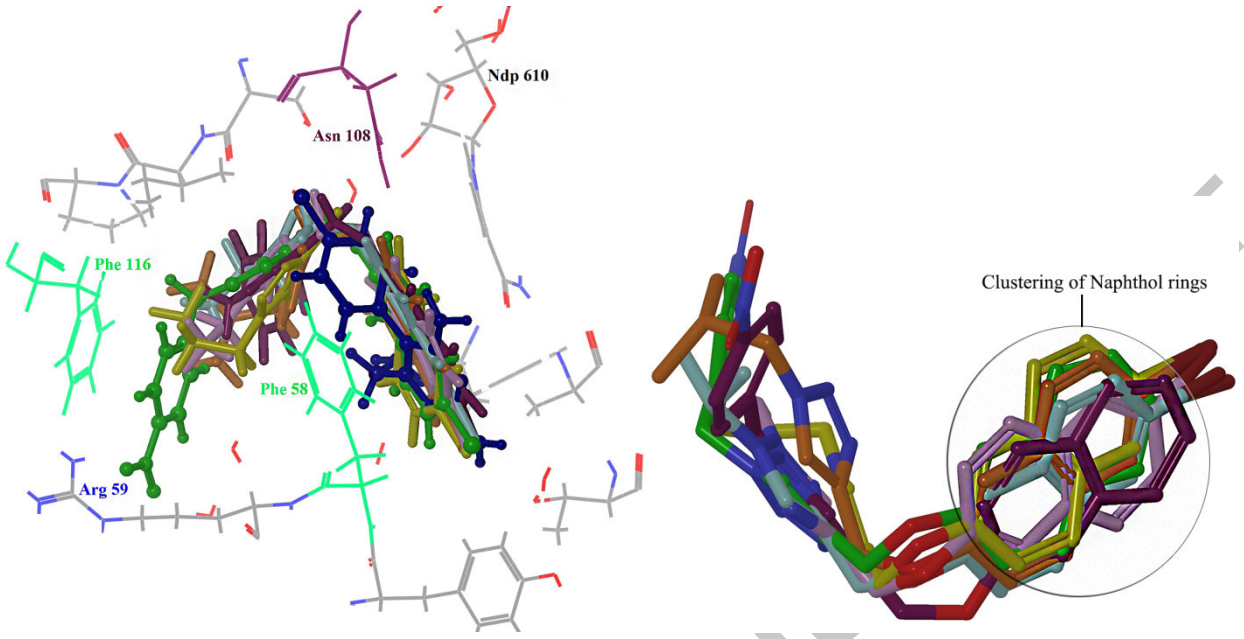
2.5. Cytotoxicity

Cell line used in this study was purchased from the American Type cell Culture. Human embryonic kidney cell line (HEK-293) was maintained in Eagle's minimum essential medium (MEM) containing 10% FBS. Synthesized test compounds were screened on HEK-293 cell line from 1µM - 100µM concentration for their *in vitro* antiproliferative activity. The MTT cell proliferation assay was used to estimate cell viability or growth. None of the compounds inhibited HEK-293 cell growth at maximum concentration checked (100µM) and could be considered non-toxic, shown in Table 1.

2.6. Docking studies on PfDHFR

Pyrimethamine is known to produce effective antiplasmodial activity by binding to PfDHFR [33], We selected this compound to compare the performance of the compounds synthesized in this work by using molecular docking methods. The crystal structures of wild-type (PDB ID:1J3I) and mutant type (PDB ID:1J3K) PfDHFR-TS were considered for molecular docking analysis.

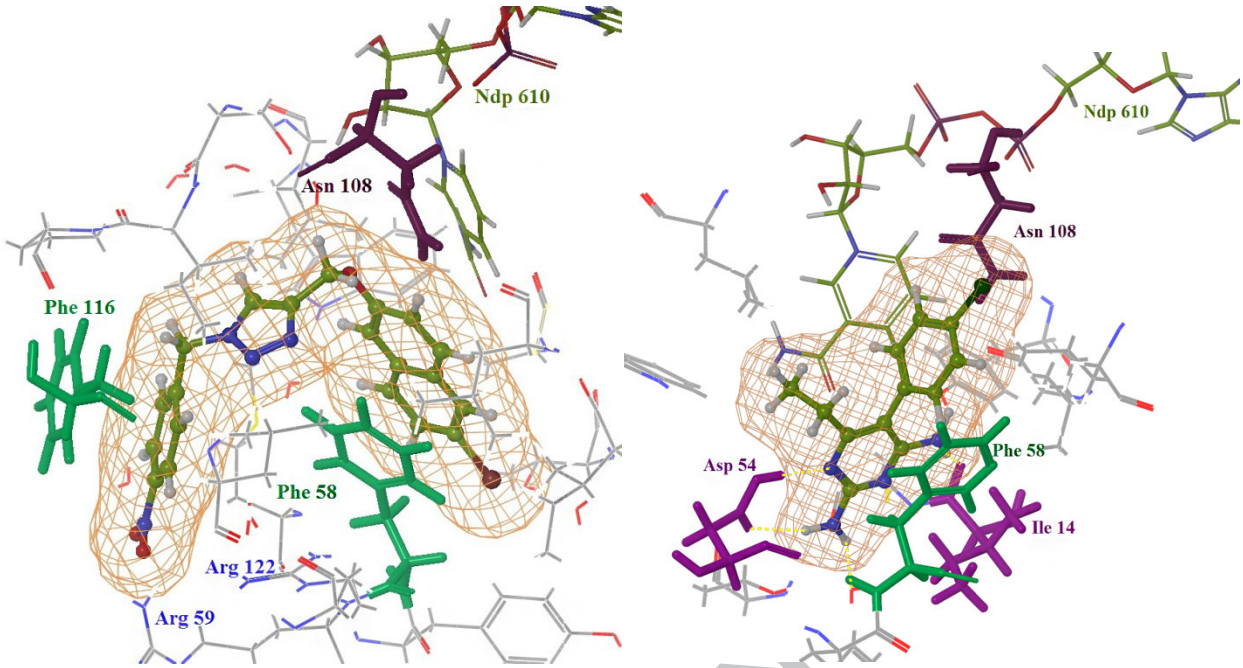
Docking simulations (Induced Fit Docking, a computationally intensive docking protocol) were performed on active compounds (**4j**, **4k**, **4l**, **4m**, **4n**, **4p**, **4t**) against DHFR to validate the SAR of triazole derivatives. All these compounds were found to occupy the central region in the active site similar to Pyr and an overlay of the dock poses clustered at the same position i.e., naphthyl ring is shown in Fig. 5.



(a) **(b)**

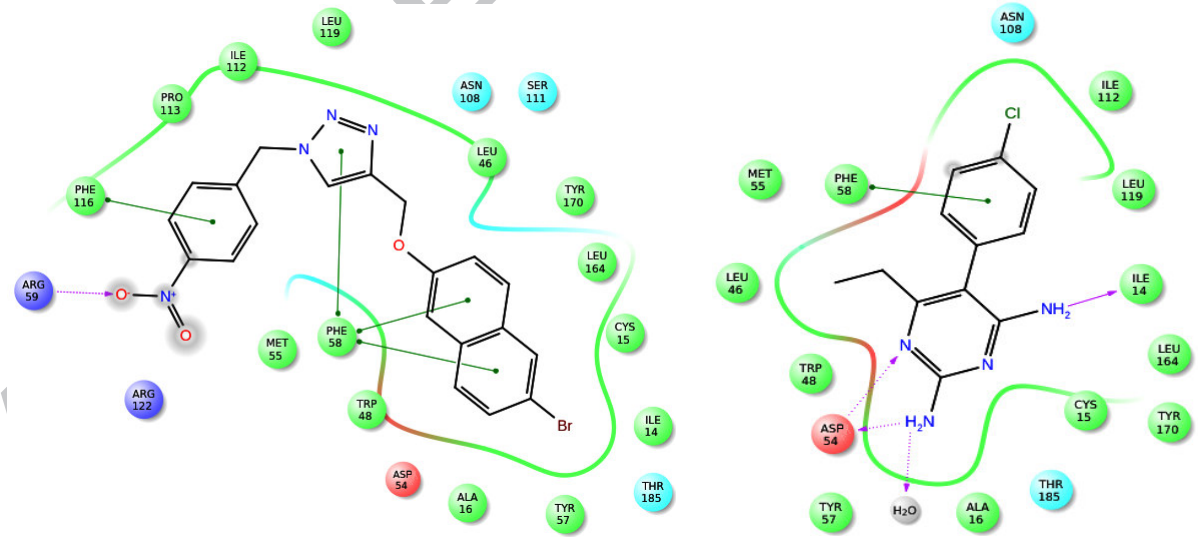
Fig. 5. (a) All the docked conformations occupy the same position in the center of the active site with respect to Pyr (blue in color). (b) Overlay of dock poses of **4t** (green), **4n** (orange), **4m** (turquoise), **4l** (yellow), **4k** (plum), and **4j** (maroon).

The dock poses of **4t** and Pyr in the crystal structure of PfDHFR (PDB ID: 1J3K) are represented in Fig. 6a and 6b. Docking analysis revealed that amino acids Asn108, Phe58, Phe116, Arg59 and Arg122, in the binding pocket of mutant enzyme (1J3K) play a vital role in stabilizing the conformation of **4t**, by π-π stacking interactions (Fig. 6a). Triazole and naphthyl rings of **4m** showed two π–π stacking interactions with Phe58. Only one π–π interaction with Phe58 was observed in **4j**, **4k**, **4l**, **4n** and **4p**. From the ligand interaction diagram (Fig. 7a and 7b), it can be clearly seen that Br group on naphthyl ring is oriented diametrically opposite to Asn108 unlike the Pyr, reducing the steric repulsions between bulky side chain of Asn108 and the Br atom on bromo naphthyl ring of triazole derivatives. Additionally, the bromo naphthyl ring is projecting into the hydrophobic pocket and exhibits strong π-π interactions with the active site amino acid Phe58.



(a) **(b)**

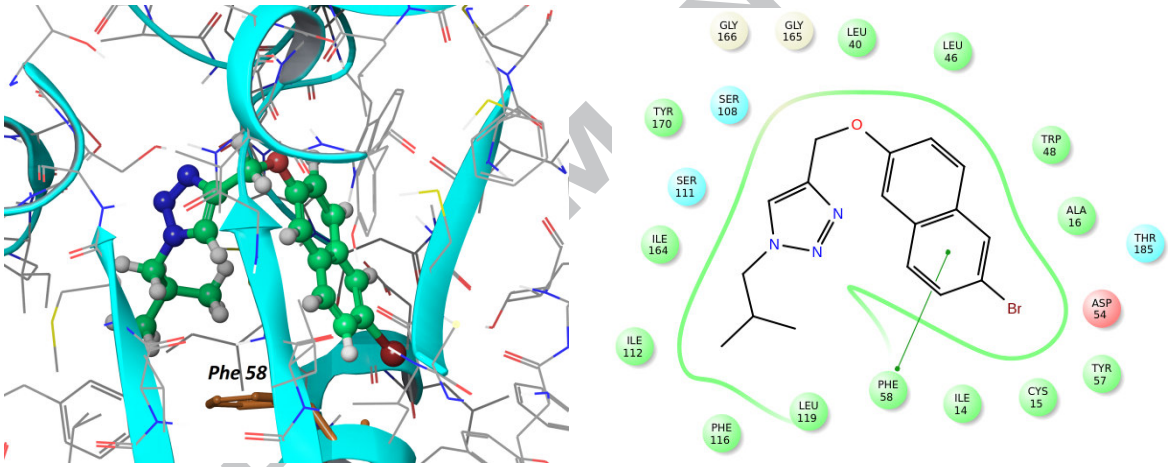
Fig. 6. (a) Docked conformation of **4t** in the active site of 1J3K, showing π-π stacking with Phe116 and Phe58, -NO_2 group of **4t** interacting with Arg59 and Arg122. The Br atom on **4t** is away from Asn108. (b) Docked conformation of Pyr in the active site showing π-π stacking with Phe58, four hydrogen bonds with Ile14, Asp54, and H_2O.The Cl atom of Pyr close to Asn108.



(a) **(b)**

Fig. 7. (a) Ligand interaction diagram of compound **4t**, (b) Ligand interaction diagram of Pyr.

The high inhibitory potential of **4t** may be explained on the basis of additional π-π interaction between benzene ring of *p*-nitro benzyl group and amino acid Phe116. Dock pose analysis of **4t** also shows polar interactions of $–NO_2$ group with Arg59 and Arg122 of PfDHFR. The computational parameters like IFD score and Emodel (-9.40 & -83.69) of a potent molecule **4t** against mutant PfDHFR (1J3K), surpass that of Pyr (-9.05 & -69.14). While in wild type PfDHFR (1J3I), IFD score and Emodel (-8.65 & -79.27) of **4t** are less compared to Pyr (-10.05 & -84.96) and this is in good agreement with the antiplasmodial activity. Dock pose of **4n** in the active site of wild type PfDHFR (1J3I) showed π-π stacking interaction with Phe58 and IFD score and Emodel (-9.12 & -80.24), shown in Fig 8.



(a) **(b)**

Fig. 8: (a) Docked conformation of compound **4n**. (b) Ligand interaction diagram of **4n**.

3. Conclusion

In summary, a new series of naphthyl bearing 1,2,3-triazoles were synthesized by [3+2] cycloaddition of azides with a terminal alkyne in excellent yields. Preparation of triazoles (**4u** and **4v**) bearing *p*-aminoglutamate ester substituent adds another dimension to multifaceted Click chemistry. Most of the synthesized 1,2,3-triazoles exhibited good antiplasmodial activity against wild type strain, among them few compounds are assessed against the resistant strain of P.

falciparum and exhibited similar potency. Significantly, the compound **4t** was found to be more potent against the resistant strain than the standard antifolate Pyr. The compounds **4n** and **4p** inhibited NADPH depletion activity, suggesting that these compounds target parasite DHFR as the standard antifolate pyrimethamine. The SAR studies of these 1,2,3-triazole derivatives throw light on structural features responsible for antiplasmodial activity. This work will promote further studies in synthesis of naphthyl 1,2,3-triazole derivatives for developing new drugs to combat the widespread drug resistance.

4. Experimental Section

4.1. General information

All chemicals and reagents used in the study were of analytical grade. Reactions progress was monitored by Merck pre coated plates (60 F254); using UV light (254 nm). Column chromatography was performed on silica gel (200-300 mesh) using solvent mixtures specified in the corresponding experiments. Spots were visualized by UV light (254 nm). Melting points (mp) are reported in degree centigrade, using open capillaries and are uncorrected. Single-crystal structures of X-ray data were collected on a Bruker SMART APEX CCD X-ray diffractometer; using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker (Bio-spin) Ultra shield Arance-III Nano Bay 400MHz NMR spectrometer (400.13 MHz for ^{1}H; 100.61 MHz for ^{13}C) in $CDCl_3$, and DMSO-d_6 as solvents, at room temperature with TMS as internal standard. Chemical shift values were reported in δ (ppm). LC-MS were recorded on SHIMADZU 2010A mass spectrometer, HRMS were recorded on ‘‘High Resolution QSTAR XL hybrid MS/MS system, Applied bio systems’’ under Electron Spray Ionization conditions preparing sample solutions in methanol. The purity (≥95%) of all compounds evaluated in this work was determined by ^{1}H NMR.

*4.2. General procedure for the synthesis of azides (**1a-j**) [29]*

To a stirred solution of the corresponding aryl/alkyl halide (1.0 equiv) in a 50 mL water/THF mixture (1:4), NaN_3 (1.5 equiv) was added. The resulting suspension was refluxed at 80-85 ^oC for 3-4 h. The aqueous layer was extracted with DCM (3 x 10 mL) and the combined organic solvent was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, leaving the azide, which was sufficiently pure and used for further step without purification.

*4.3. Typical procedure for the synthesis of (S)-diethyl 2-(4-azidobenzamido)pentanedioate (**1k**)*

(S)-diethyl 2-(4-aminobenzamido)pentanedioate (1 g, 2.87 mmol) was dissolved in acetonitrile (20 mL) in a 50 mL round bottomed flask and cooled to 0°C in an ice bath. To this stirred mixture *t*-BuONO (444 mg, 512 µL, 4.31 mmol) was added followed by $TMSN_3$ (397 mg, 457 µL, 3.44 mmol) in a dropwise manner. The mixture was warmed to room temperature and stirred for 2 h. The resulting mixture was concentrated under vacuum and the crude product was purified by column chromatography on silica gel, eluted with absolute hexane to afford **1k** as light yellow solid (Yield:92%); mp: 42-44 ºC ; ^1H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, J = 8.8 Hz, 2H), 7.09 (d, J = 8.8 Hz, 2H), 4.79-4.75 (m, 1H), 4.27-4.23 (m, 2H), 4.14-4.10 (m, 2H), 2.54-2.41 (m, 2H), 2.32-2.29 (m, 1H), 2.20-2.12 (m, 1H), 1.32 (t, J = 7.2 Hz, 3H), 1.24 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 171.9, 166.1, 143.7, 130.1, 128.9, 119.0, 61.8, 60.9, 52.5, 30.5, 27.1, 14.2, 14.1; ESI-MS: m/z 349 $(M+H)^+$. HRMS (ESI): m/z calcd for $C_{16}H_{21}O_5N_4$ $[M+H]^+$ 349.15065, found 349.15157. $[\alpha]^{25}_D$ +25.680 deg cm^3 g^{-1} dm^{-1} (c 0.0016 g cm^{-3} in Chloroform).

*4.4. General procedure for the synthesis of alkynes **3a** and **3b** [34]*

To a suspension of K_2CO_3 (10.5 g, 76.3 mmol for **2a** ; 6.8 g, 49.3 mmol for **2b**) in DMF (15 mL) was added a solution of appropriate 2-naphthol (10 g, 69.4 mmol of **2a** ; 10 g, 44.8 mmol of **2 b**) in DMF (15 mL). After the reaction was heated to 60°C for 30 min, a solution of 3-bromoprop-1-yne (9 g, 6.8 mL for **2a** ; 5.9 g, 4.4 mL for **2b**) in DMF (10 mL) was added in a dropwise manner and the mixture was refluxed for 3 h. (monitored by TLC; Hexane:EtOAc-9:1). The mixture was neutralized with dil. aqueous HCl, then extracted with EtOAc(3x30 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum, purified by column chromatography (silica gel, hexane:EtOAc-9:1 as developing solvent) to obtain the desired product.

*4.4.1. 2-(Porp-2-yn-1-yloxy)naphthalene (**3a**)*

Light orange solid (Yield:95%); mp: 48-50 ºC ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79-7.75 (m, 3H), 7.45 (td, J = 5.6, 1.2 Hz, 1H), 7.36 (td, J = 5.6, 1.2 Hz, 1H), 7.24 (d, J = 2.4 Hz, 1H), 7.19 (dd, J = 6.4, 2.4 Hz, 1H), 4.81 (d, J = 2.4 Hz, 2H), 2.55 (t, J = 2.4 Hz, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.5, 134.3, 129.6, 129.3, 127.7, 126.9, 126.5, 124.0, 118.7, 107.5, 78.5, 75.7, 55.9.

*4.4.2. 2-Bromo-6-(porp-2-yn-1-yloxy)naphthalene (**3b**)*

Color less solid (Yield:96%); mp: 70-72 ºC ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, J = 2.0 Hz, 1H), 7.64 (m, 2H), 7.51 (dd, J = 6.4, 2.0 Hz, 1H), 7.22-7.19 (m, 2H), 4.80 (d, J = 2.4 Hz, 2H), 2.56 (t, J = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.7, 132.8, 130.4, 129.8, 129.7, 128.7, 128.6, 119.8, 117.5, 107.5, 78.2, 75.9, 55. 9.

*4.5. General procedure for the synthesis of compounds (**4a-v**)*

In a round bottomed flask, Aryl/ Alkyl azide **1a-j**,**1k** (0.75 mmol) and naphthalen-2-ylprop-2-yn-1-ylether (**3a**) or 6-bromo naphthalen-2-ylprop-2-yn-1-ylether (**3b**) (1.05 equiv) were added to a mixture of copper(II) sulfate pentahydrate (9.4 mg, 0.05 equiv),and sodium

ascorbate (23 mg, 0.15 equiv) dissolved in 2:1 of *t*-BuOH and H_2O (10 mL) at room temperature. The reaction mixture was stirred for 12 h at room temperature and was poured into ice cold water (20 mL). The aqueous layer was extracted with CH_2Cl_2 (10 mL) thrice. The combined organic layer was concentrated in vacuo. The residue was purified by short column chromatography on silica gel (70–230 mesh) eluted with hexane/ EtOAc (4/1) to afford the desired products **4a-v**.

*4.5.1. 1-Ethyl-4-((naphthalen-6-yloxy)methyl)-1H-1,2,3-triazole (**4a**)*

Light yellow solid (Yield:95%); mp: 97-99 ºC ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.74 (m, 3H), 7.65 (s, 1H), 7.45 (td, J = 5.6, 1.2 Hz, 1H), 7.35 (td, J = 5.6, 1.2 Hz, 1H), 7.28 (d, J = 2.4 Hz, 1H), 7.19 (dd, J = 6.4, 2.4 Hz, 1H), 5.342 (s, 2H), 4.43 (q, J = 7.2 Hz, 2H), 1.57 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 144.1, 134.4, 129. 6, 129.2, 127.6, 126.9, 126.5, 123.9, 122.1, 118.8, 107.2, 62.1, 45.4, 15.5; LC-MS (ESI), m/z 254.15 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{15}H_{16}ON_3$ $[M+H]^+$ 254.12879, found 254.12841.

*4.5.2. 4-((Naphthalen-6-yloxy)methyl)-1-propyl-1H-1,2,3-triazole (**4b**)*

Light yellow solid (Yield:94%); mp: 86-88 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.74 (m, 3H), 7.62 (s, 1H), 7.44 (td, J = 5.6, 1.2 Hz, 1H), 7.34 (td, J = 5.6, 1.2 Hz, 1H), 7.27 (d, J = 2.4Hz, 1H), 7.19 (dd, J = 6.4, 2.4 Hz, 1H), 5.342 (s, 2H), 4.33 (t, J = 7.2 Hz, 2H), 1.99-1.90 (sextet, J = 7.2 Hz, 2H), 0.96 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 144.0, 134.4, 129.6, 129.2, 127.6, 126.9, 126.5, 123.9, 122.6, 118.8, 107.2, 62.2, 52.0, 23.7, 11.1; LC-MS (ESI), m/z 268.15 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{16}H_{18}ON_3$ $[M+H]^+$ 268.14444, found 268.14399.

*4.5.3. 1-Isopropyl-4-((naphthalen-6-yloxy)methyl)-1H-1,2,3-triazole (**4c**)*

Light red crystals (Yield:91%); mp: 110-112 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.75 (m, 3H), 7.67 (s, 1H), 7.45 (td, J = 5.6, 1.2 Hz, 1H), 7.35 (td, J = 5.6, 1.2 Hz, 1H), 7.29 (d, J = 2.4

Hz, 1H), 7.20 (dd, J = 6.4, 2.4 Hz, 1H), 5.33 (s, 2H), 4.85 (septet, J = 6.4 Hz, 1H), 1.60 (d, J = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 143.8, 134.5, 129.5, 129.2, 127.6, 126.9, 126.5, 123.9, 120.2, 118.8, 107.2, 62.3, 51.1, 23.0; LC-MS (ESI), m/z 266 $(M-H)^+$, HRMS (ESI): m/z calcd for $C_{16}H_{18}ON_3$ $[M+H]^+$ 268.14444, found 268.14396.

*4.5.4. 1-Isobutyl-4-((naphthalen-6-yloxy)methyl)-1H-1,2,3-triazole (**4d**)*

Colorless solid (Yield:86%); mp: 82-84 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.73 (m, 3H), 7.59 (s, 1H), 7.44 (td, J = 5.6, 1.2 Hz, 1H), 7.34 (td, J = 5.6, 1.2 Hz, 1H), 7.27 (d, J = 2.4 Hz, 1H), 7.19 (dd, J = 6.4, 2.4 Hz, 1H), 5.35 (s, 2H), 4.17 (d, J = 7.2 Hz, 2H), 2.28-2.17 (septet, J = 6.8 Hz, 1H), 0.95 (d, J = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 143.9, 134.4, 129.5, 129.2, 127.6, 126.9, 126.5, 123.9, 122.9, 118.8, 107.3, 62.2, 57.7, 29.7, 19.9, 10.4; LC-MS (ESI), m/z 282.40 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{17}H_{20}ON_3$ $[M+H]^+$ 282.16009, found 282.15952.

*4.5.5. 4-((Naphthalen-6-yloxy)methyl)-1-pentyl-1H-1,2,3-triazole (**4e**)*

Colorless solid (Yield:95%); mp: 76-78 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.74 (m, 3H), 7.62 (s, 1H), 7.45 (td, J = 5.6, 1.2 Hz, 1H), 7.35 (td, J = 5.6, 1.2 Hz, 1H), 7.27 (d, J = 2.4 Hz, 1H), 7.19 (dd, J = 6.4, 2.4 Hz, 1H), 5.34 (s, 2H), 4.35 (t, J = 7.2 Hz, 2H), 1.95-1.88 (quintet, 2H), 1.40-1.27 (m, 4H), 0.89 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 143.9, 134.5, 129.5, 129.2, 127.6, 126.9, 126.5, 123.9, 122.6, 118.8, 107.3, 62.1, 50.4, 29.9, 28.6, 22.1, 13.8; LC-MS (ESI), m/z 296.20 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{18}H_{22}ON_3$ $[M+H]^+$ 296.17574, found 296.17503.

*4.5.6. 1-Allyl-4-((naphthalen-6-yloxy)methyl)-1H-1,2,3-triazole (**4f**)*

Light yellow solid (Yield:90%); mp: 79-80 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77-7.74 (m, 3H), 7.64 (s, 1H), 7.44 (td, J = 6.4, 1.2 Hz, 1H), 7.35 (td, J = 6.4, 1.2 Hz, 1H), 7.27 (d, J = 2.4 Hz,

1H), 7.18 (dd, J = 6.4, 2.4 Hz, 1H), 6.07-5.97 (m, 1H), 5.37-5.29 (tq, J=6.8/1.2/0.8Hz, 2H), 5.34 (s, 2H), 4.98 (dt, J = 3.6, 1.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 144.4, 134.4, 131.1, 129.6, 129.2, 127.6, 126.9, 126.5, 123.9, 122.6, 120.4, 118.8, 107.3, 62.1, 52.8; LC-MS (ESI), m/z 266.20 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{16}H_{16}ON_3$ $[M+H]^+$ 266.12879, found 266.12831.

*4.5.7. 1-Cyclopentyl-4-((naphthalen-6-yloxy)methyl)-1H-1,2,3-triazole (**4g**)*

Colorless solid (Yield:93%); mp: 108-110 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.75(m, 3H), 7.65 (s, 1H), 7.45 (td, J = 6.4, 1.2 Hz, 1H), 7.35 (td, J = 6.4, 1.2 Hz, 1H), 7.29 (d, J = 2.4 Hz, 1H), 7.20 (dd, J = 6.4, 2.4 Hz, 1H), 5.33 (s, 2H), 4.98-4.91 (quintet, 1H), 2.32-2.23 (m, 2H), 2.11-2.02 (m, 2H), 1.95-1.86 (m, 2H), 1.82-1.73 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 143.8, 134.5, 129.5, 129.2, 127.6, 126.9, 126.5, 123.9, 121.2, 118.8, 107.2, 62.2, 61.9, 33.4, 24.1; LC-MS (ESI), m/z 294.25 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{18}H_{19}ON_3Na$ $[M+Na]^+$ 316.14203, found 316.14122.

*4.5.8. 4-(2-(4-((Naphthalen-6-yloxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)morpholine (**4h**)*

Light yellow solid (Yield:92%); mp: 94-96 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.77-7.73 (m, 3H), 7.45 (td, J = 6.4, 1.2 Hz, 1H), 7.35 (td, J = 6.4, 1.2 Hz, 1H), 7.27 (brs, 1H), 7.19 (dd, J = 6.4, 2.4 Hz, 1H), 5.36 (s, 2H), 4.47 (t, J = 6.0 Hz, 2H), 3.62 (t, J = 4.8 Hz, 4H), 2.81 (t, J = 6.0 Hz, 2H), 2. 46 (t, J =4.8 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 144.0, 134.4, 129.6, 129.2, 127.6, 126.9, 126.5, 123.9, 123.3, 118.8, 107.3, 66.7, 62.1, 57.8, 53.4, 47.5; LC-MS (ESI), m/z 339.25 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{19}H_{23}O_2N_4$ $[M+H]^+$ 339.18155, found 339.18114.

*4.5.9. 1-Benzyl-4-((naphthalen-6-yloxy)methyl)-1H-1,2,3-triazole (**4i**)*

Colorless solid (Yield:90%); mp: 140-144 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77-7.73 (m, 3H), 7.56 (s, 1H), 7.45 (t, J = 6.4 Hz, 1H), 7.40-7.33 (m, 4H), 7.29-7.27 (m, 2H), 7.25 (d, J = 2.4 Hz, 1H), 7.16 (dd, J = 6.4, 2.4 Hz, 1H), 5.54 (s, 2H), 5.31 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 144.6, 134.4, 134.4, 129.5, 129.2, 128.8, 128.1, 127.6, 126.9, 126.5, 123.9, 122.6, 118.8, 107.3, 62.2, 54.3; LC-MS (ESI), m/z 313.95 $(M-H)^+$, HRMS (ESI): m/z calcd for $C_{20}H_{18}ON_3$ $[M+H]^+$ 316.14444, found 316.14393.

*4.5.10. 1-(4-Nitrobenzyl)-4-((naphthalen-6-yloxy)methyl)-1H-1,2,3-triazole (**4j**)*

Yellowish solid (Yield:92%); mp: 102-104 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 8.8 Hz, 2H), 7.78-7.72 (m, 3H), 7.63 (s, 1H), 7.45 (td, J = 6.4, 1.2 Hz, 1H), 7.41-7.34 (m, 3H), 7.24 (d, J = 2.4 Hz, 1H), 7.16 (dd, J = 6.4, 2.4 Hz, 1H), 5.64 (s, 2H), 5.35 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 148.1, 145.2, 141.5, 134.3, 129.6, 129.2, 128.6, 127.7, 126.9, 126.6, 124.3, 124.0, 122.9, 118.7, 107.4, 62.1, 53.2; LC-MS (ESI), m/z 360.95 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{20}H_{17}O_3N_4$ $[M+H]^+$ 361.12952, found 361.12906.

*4.5.11. 4-((2-Bromonaphthalen-6-yloxy)methyl)-1-ethyl-1H-1,2,3-triazole (**4k**)*

Colorless needles (Yield:94%); mp: 118-120 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 2.0 Hz, 1H), 7.67-7.61 (m, 3H), 7.51 (dd, J = 6.8, 2.0 Hz, 1H), 7.25 (d, J = 2.4 Hz, 1H), 7.20 (dd, J = 6.4, 2.4 Hz, 1H), 5.32 (s, 2H), 4.43 (q, J = 7.2 Hz, 2H), 1.58 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 143.9, 132.9, 130.2, 129.7, 129.6, 128.6, 128.6, 122.0, 119.9, 117.4, 107.2, 62.2, 45.4, 15.5; LC-MS (ESI), m/z 330.80 $(M-H)^+$, HRMS (ESI): m/z calcd for $C_{15}H_{15}ON_3Br$ $[M+H]^+$ 332.03930, found 332.04021.

*4.5.12. 4-((2-Bromonaphthalen-6-yloxy)methyl)-1-propyl-1H-1,2,3-triazole (**4l**)*

Colorless solid (Yield:92%); mp: 100-102 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 2.0 Hz, 1H), 7.67-7.61 (m, 3H), 7.50 (dd, J = 6.8, 2.0 Hz, 1H), 7.24 (d, J = 2.4 Hz, 1H), 7.20 (dd, J =

6.4, 2.4 Hz, 1H), 5.32 (s, 2H), 4.33 (t, J = 7.2 Hz, 2H), 2.0-1.91 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 143.8, 132.9, 130.2, 129.7, 129.6, 128.6, 128.6, 122.5, 119.9, 117.4, 107.2, 62.2, 52.1, 23.7, 11.1; LC-MS (ESI), m/z 347.25 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{16}H_{17}ON_3Br$ $[M+H]^+$ 346.05495, found 346.05586.

*4.5.13. 4-((2-Bromonaphthalen-6-yloxy)methyl)-1-isopropyl-1H-1,2,3-triazole (**4m**)*

Colorless solid (Yield:90%); mp: 122-124 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 2.0Hz, 1H), 7.67-7.62 (m, 3H), 7.51 (dd, J = 6.4, 2.0 Hz, 1H), 7.30 (brs, 1H), 7.21(dd, J = 6.4, 2.4 Hz, 1H), 5.31 (s, 2H), 4.90-4.80 (m, 1H), 1.60 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 143.6, 132.9, 130.2, 129.7, 129.6, 128.6, 128.6, 120.2, 119.9, 117.4, 107.2, 62.3, 53.2, 23.0; LC-MS (ESI), m/z 347.30 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{16}H_{17}ON_3Br$ $[M+H]^+$ 346.05495, found 346.05596.

*4.5.14. 4-((2-Bromonaphthalen-6-yloxy)methyl)-1-isobutyl-1H-1,2,3-triazole (**4n**)*

Colorless solid (Yield:88%); mp: 88-90 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 2.0 Hz, 1H), 7.67-7.60 (m, 3H), 7.51 (dd, J = 6.4, 2.0 Hz, 1H), 7.24 (d, J = 2.4 Hz, 1H), 7.20 (dd, J = 6.4, 2.4 Hz, 1H), 5.33 (s, 2H), 4.18 (d, J = 7.2 Hz, 2H), 2.30-2.16 (m, 1H), 0.95 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 143.7, 132.9, 130.2, 129.7, 129.6, 128.6, 128.6, 123.0, 119.9, 117.4, 107.3, 62.236, 57.7, 29.7, 19.9; ESI-MS: m/z 360$(M)^+$, 362 $(M+2)^+$, HRMS (ESI): m/z calcd for $C_{17}H_{19}ON_3Br$ $[M+H]^+$ 360.07060, found 360.07169.

*4.5.15. 4-((2-Bromonaphthalen-6-yloxy)methyl)-1-pentyl-1H-1,2,3-triazole (**4o**)*

Colorless solid (Yield:93%); mp: 96-98 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94(d, J = 2.0 Hz, 1H), 7.69-7.63 (m, 3H), 7.52 (dd, J = 6.4, 2.0 Hz, 1H), 7.27 (d, J = 2.4 Hz, 1H), 7.22 (dd, J = 6.4, 2.4 Hz, 1H), 5.34 (s, 2H), 4.37 (t, J = 7.2 Hz, 2H), 1.95-1.92 (quintet, 2H), 1.37-1.27 (m, 4H), 0.91 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 143.85, 132.95, 130.25, 129.75,

129.65, 128.65, 128.7, 122.5, 119.9, 117.4, 107.3, 62.23, 50.5, 29.9, 28.6, 22.1, 13.8; LC-MS (ESI), m/z 375.30 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{18}H_{21}ON_3Br$ $[M+H]^+$ 374.08625, found 374.08750.

*4. 5.16. 4-((2-Bromonaphthalen-6-yloxy)methyl)-1-allyl-1H-1,2,3-triazole (**4p**)*

Light yellow solid (Yield:90%); mp: 92-94ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 2.0 Hz, 1H), 7.66-7.60 (m, 3H), 7.50 (dd, J = 6.4, 2.0 Hz, 1H), 7.23 (d, J = 2.4 Hz, 1H), 7.19 (dd, J = 6.4, 2.4 Hz, 1H), 6.07-5.97 (m, 1H), 5.38-5.29 (m, 2H), 5.31 (s, 2H), 4.99 (dt, J = 3.6, 1.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 144.1, 132.9, 131.1, 130.2, 129.7, 129.6, 128.6, 128.6, 122.6, 120.5, 119.9, 117.4, 107.2, 62.1, 52.8; LC-MS (ESI), m/z 345.15 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{16}H_{15}ON_3Br$ $[M+H]^+$ 344.03930, found 344.04036.

*4.5.17. 4-((2-Bromonaphthalen-6-yloxy)methyl)-1-cyclopentyl-1H-1,2,3-triazole (**4q**)*

Colorless solid (Yield:89%); mp: 112-114 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 2.0 Hz, 1H), 7.67-7.61 (m, 3H), 7.50 (dd, J = 6.8, 2.0 Hz, 1H), 7.25 (s, 1H), 7.21 (dd, J = 6.4, 2.4Hz, 1H), 5.30 (s, 2H), 4.97-4.90 (m, 1H), 2.32-2.23 (m, 2H), 2.10-2.02 (m, 2H), 1.96-1.86 (m, 2H), 1.81-1.74 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 143.6, 132.9, 130.2, 129.7, 129.6, 128.6, 128.5, 121.2, 119.9, 117.4, 107.2, 62.2, 61.9, 33.4, 24.1; LC-MS (ESI), m/z 373.20 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{18}H_{19}ON_3Br$ $[M+H]^+$ 372.07060, found 372.07173.

*4.5.18. 4-(2-(4-((2-Bromonaphthalen-6-yloxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)morpholine (**4r**)*

Light yellow needles (Yield:90%); mp: 152-154 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 2.0 Hz, 1H), 7.78 (s, 1H), 7.67-7.60 (m, 2H), 7.50 (dd, J = 6.8, 2.0 Hz, 1H), 7.24 (d, J = 2.4 Hz, 1H), 7.21 (dd, J = 6.4, 2.4 Hz, 1H), 5.34 (s, 2H), 4.47 (t, J = 6.0 Hz, 2H), 3.63 (t, J = 4.8 Hz, 4H), 2.82 (t, J = 6.0 Hz, 2H), 2.46 (t, J = 4.8 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 143.8,

132.9, 130.2, 129.8, 129.6, 128.6, 128.5, 123.3, 119.9, 117.4, 107.3, 66.9, 62.1, 57.8, 53.5, 47.5; LC-MS (ESI), m/z 417.45 $(M-H)^+$, HRMS (ESI): m/z calcd for $C_{19}H_{22}O_2N_4Br$ $[M+H]^+$ 417.09207, found 417.09338.

*4.5.19. 4-((2-Bromonaphthalen-6-yloxy)methyl)-1-benzyl-1H-1,2,3-triazole (**4s**)*

White needles (Yield:89%); mp: 134-136 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 2.0 Hz, 1H), 7.65-7.59 (m, 2H), 7.56 (1H, s), 7.50 (dd, J = 6.8, 2.0 Hz, 1H), 7.38-7.36 (m, 3H), 7.29-7.27 (m, 2H), 7.22 (d, J = 2.4 Hz, 1H), 7.17 (dd, J = 6.4, 2.4 Hz, 1H), 5.54 (s, 2H), 5.29 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 144.3, 134.4, 132.9, 130.2, 129.7, 129.6, 129.2, 128.9, 128.6, 128.5, 128.1, 122.6, 119.8, 117.4, 107.2, 62.2, 54.3; LC-MS (ESI), m/z 393.25 $(M-H)^+$, HRMS (ESI): m/z calcd for $C_{20}H_{17}ON_3Br$ $[M+H]^+$ 394.05495, found 394.05625.

*4.5.20. 4-((2-Bromonaphthalen-6-yloxy)methyl)-1-(4-nitrobenzyl)-1H-1,2,3-triazole (**4t**)*

Yellow needles (Yield:93%); mp: 118-120 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 2.0 Hz, 1H), 7.66-7.59 (m, 3H), 7.51 (dd, J = 6.8, 2.0 Hz, 1H), 7.40 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 2.4 Hz, 1H), 7.17 (dd, J = 6.4, 2.4 Hz, 1H), 5.66 (s, 2H), 5.33 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 148.1, 144.9, 141.4, 132.8, 130.2, 129.8, 129.7, 128.7, 128.6, 128.5, 124.4, 122.9, 119.8, 117.5, 107.3, 62.0, 53.2; LC-MS (ESI), m/z 440 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{20}H_{16}O_3N_4Br$ $[M+H]^+$ 439.04003, found 439.04152.

*4.5.21. (S)-Diethyl2-(-4-(4-((naphthalen-6-yloxy)methyl)-1H-1,2,3-triazol-1-yl)benzamido)pentane dioate (**4u**)*

Colorless solid (Yield:91%); mp: 146-148 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 8.01 (d, J = 8.8 Hz, 2H), 7.87 (d, J = 8.8 Hz, 2H), 7.79-7.76 (m, 3H), 7.46 (td, J = 6.4, 1.2 Hz, 1H), 7.36 (td, J = 6.4, 1.2 Hz, 1H), 7.31 (d, J = 2.4 Hz, 1H), 7.30 (brs, 1H), 7.22 (dd, J = 6.4, 2.4 Hz, 1H), 5.44 (s, 2H), 4.81-4.76 (m, 1H), 4.29-4.22 (m, 2H), 4.17-4.09 (m, 2H), 2.59-2.43 (m, 2H),

2.37-2.29 (m, 1H), 2.23-2.14 (m, 1H), 1.32 (t, J = 7.2 Hz, 3H), 1.24 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 171.8, 165.7, 156.0, 145.4, 139.2, 134.4, 133.9, 129.7, 129.2, 128.9, 127.7, 126.9, 126.6, 124.0, 120.7, 120.2, 118.7, 107.2, 62.0, 61.9, 61.0, 52.7, 30.5, 26.9, 14.2, 14.1; LC-MS (ESI), m/z 530.35 $(M-H)^+$, HRMS (ESI): m/z calcd for $C_{29}H_{31}O_6N_4$ $[M+H]^+$ 531.22381, found 531.22505.

*4.5.22. (S)-Diethyl2-(4-(4-(((6-bromonaphthalen-2-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)benzamido)pentane dioate (**4v**)*

Light yellow solid (Yield:90%); mp: 166-168 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 8.01 (d, J = 8.8 Hz, 2H), 7.93 (d, J = 2.0 Hz, 1H), 7.86 (d, J = 8.8 Hz, 2H), 7.69-7.63 (m, 2H), 7.52 (dd, J = 6.8, 2.0 Hz, 1H), 7.32 (d, J = 7.2 Hz, 1H), 7.28 (ds, 1H), 7.24 (dd, J = 6.4, 2.4 Hz, 1H), 5.42 (s, 2H), 4.81-4.76 (m, 1H), 4.29-4.23 (m, 2H), 4.16-4.10 (m, 2H), 2.59-2.43 (m, 2H), 2.37-2.29 (m, 1H), 2.23-2.14 (m, 1H), 1.32 (t, J = 7.2 Hz, 3H), 1.24 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 171.8, 165.6, 156.3, 145.1, 139.1, 133.9, 132.9, 130.3, 129.8, 129.7, 128.9, 128.7, 128.6, 120.8, 120.2, 119.8, 117.5, 107.3, 62.0, 61.9, 61.0, 52.8, 30.6, 26.9, 14.2, 14.1; LC-MS (ESI), m/z 609.25 $(M-H)^+$, HRMS (ESI): m/z of $C_{29}H_{29}O_6N_4Br$ $[M+2]^+$ found 611.13506.

*4.6. General procedure for the synthesis of **5a** and **5b** [35]*

A solution of corresponding ester (**4u/v**) (0.5 g) in H_2O:THF (1:1, 15 mL) was treated with 1N aqueous NaOH solution (7.5 mL) at room temperature for 4 h. THF was evaporated under reduced pressure and 1N HCl solution was added drop wise to the residue. The white crystalline precipitate was collected by filtration, washed with water, and dried over $CaCl_2$ under vacuum to give **5a/b**.

*4.6.1.(S)-2-(4-(4-((Naphthalene-2-yloxy)methyl)-1H-1,2,3-triazol-1-yl)benzamido)pentanedioic acid (**5a**)*

White solid (Yield:82%); mp:172-174ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H), 8.81 (d, J = 7.6 Hz, 1H), 8.10 (q, J = 8.8, 6.0 Hz, 4H), 7.87-7.84 (m, 3H), 7.57 (d, J = 2.4 Hz, 1H), 7.49 (td, J = 6.4, 1.2 Hz, 1H), 7.37 (td, J = 6.4, 1.2 Hz, 1H), 7.24 (dd, J = 6.4, 2.4 Hz, 1H), 5.38 (s, 2H), 4.46-4.40 (m, 1H), 2.38 (t, J = 7.2 Hz, 2H), 2.16-2.08 (m, 1H), 2.02-1.93 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.2, 165.2, 156.3, 144.5, 138.8, 134.6, 129.9, 129.6, 129.1, 128.0, 127.3, 126.9, 124.3, 123.5, 120.1, 119.1, 112.2, 107.7, 104.7, 61.6, 53.0, 32.1, 27.5; LC-MS (ESI), m/z 475.25 $(M+H)^+$, HRMS (ESI): m/z calcd for $C_{25}H_{23}O_6N_4$ $[M+H]^+$ 475.16121, found 475.16324.

*4.6.2. (S)-2-(4-(4-(((6-Bromonaphthalen-2-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)benzamido) pentanedioicacid (**5b**)*

Colorless solid (Yield:80%); mp:180-182ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H), 8.80 (d, J = 7.6 Hz, 1H), 8.14 (d, J = 2.0 Hz, 1H), 8.10 (q, J = 8.8, 6.8 Hz, 4H), 7.88-7.81 (m, 2H), 7.61-7.59 (m, 2H), 7.30 (dd, J = 6.4, 2.4 Hz, 1H), 5.38 (s, 2H), 4.46-4.40 (m, 1H), 2.38 (t, J = 7.2 Hz, 2H), 2.16-2.07 (m, 1H), 2.02-1.92 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.7, 165.4, 156.8, 144.3, 138.8, 134.6, 133.3, 130.3, 129.9, 129.8, 129.6, 129.5, 129.2, 123.6, 120.3, 120.1, 117.0, 107.8, 61.6, 53.0, 31.8, 27.2; LC-MS (ESI), m/z 553 $(M)^+$, HRMS (ESI): m/z of $C_{25}H_{21}O_6N_4Br$ $[M+2]^+$ found 555.07176.

4.7. Crystallographic studies

Single-crystal structures of X-ray data were collected for **4c**, **4k**, **4r**, **4s**, and **4t** on a Bruker SMART APEX CCD X-ray diffractometer, using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). Data were reduced using SAINTPLUS [36] and a multi-scan

absorption correction using SADABS [37] was performed. The structure was solved and refined against F^2 using SHELX-97 [38]. All ring hydrogen atoms were assigned on the basis of geometrical considerations and were allowed to ride upon the respective carbon atoms. All hydrogen atoms were assigned fixed U_{iso} values, equal to $1.2U_{eq}$. of the parent atom. The molecular structures of **4c**, **4k**, **4r**, **4s**, and **4t** are illustrated in Fig. 2 with 30% probability displacement ellipsoid.

4.8. Antiplasmodial activity, DHFR inhibition assay and Cytotoxicity

4.8.1. Antiplasmodial activity

P. falciparum 3D7 and Dd2 strains were obtained from the Malaria Research and Reference Reagent Resource Centre (MR4), cell culture reagents and SYBR Green 1 were from Invitrogen, Pyr and DMSO were from Sigma, human serum was from Millipore. Both the strains were grown in RPMI 1640 medium (containing 2 g/litre sodium bicarbonate, 2 g/litre glucose, 25 µg/ml gentamicin, 300 mg/litre glutamine, 10 % human serum, and human erythrocytes at 2% haematocrit [39]) at 37°C in the presence of a gas mixture (5% O_2, 5% CO_2, 90% N_2). Parasites were synchronized by treatment with 5% D-sorbitol at ring stage [40]. Stocks of all the compounds and Pyr were prepared in DMSO at 20-50 mM concentrations, and the stocks were serially diluted 2-fold in 50 µl culture medium across rows of a 96 well tissue culture plate. DMSO (0.05%) or chloroquine (500 nM) was added to the control wells. 50 µl parasite suspension (1% ring-infected erythrocytes at 4% haematocrit) was added to each well. The plate was incubated in a modular incubator chamber (Billups-Rothenberg, Inc.) with the gas mixture at 37°C for 50 hours. At the end of incubation, 100 µl lysis buffer (20 mM Tris-Cl, 5 mM EDTA, 0.008% saponin, 0.08% Triton X-100, pH 7.5) with SYBR Green 1 (at the manufacturer's recommended dilution) was added to each well, the plate was incubated at 37°C for 30 min, and

fluorescence was measured (Ex: 485 nm, Em: 530 nm, gain setting: 50) using an Infinite M200 multimode microplate reader (TECAN) as described previously [41]. The fluorescence of chloroquine-treated culture was subtracted from the fluorescence values of cultures treated with compounds/Pyr and DMSO, which adjusted for the background fluorescence. Fluorescence values of cultures treated with compounds or Pyr were normalized as percentage of the fluorescence of DMSO-treated cultures, plotted against concentrations, and analyzed using nonlinear regression analysis to determine IC_{50} concentrations as described earlier [42].

4.8.2. DHFR inhibition assay

P. falciparum was cultured as described above. Trophozoite stage parasites were isolated from a 100 ml culture with 15-16% parasitaemia by saponin lysis, and soluble parasite extract was prepared as has been described earlier [41]. Briefly, the culture was centrifuged at 894g for 5 min, supernatant was aspirated, and the pellet was suspended in 20 ml cold lysis buffer (0.05% saponin in PBS) and incubated in ice for 5 min. The suspension was centrifuged at 12,096g for 5 min at 4°C. The supernatant was discarded, the pellet was suspended in 20 ml lysis buffer, incubated in ice for 5 min, and centrifuged at 12,096g for 5 min at 4°C. The supernatant was discarded, and the pellet was washed three times with cold PBS to remove residual hemoglobin. The final pellet, which contained parasites, was suspended in 10x volume of the DHFR assay buffer (DHFR Assay Kit from Sigma-Aldrich, Product Code CS0340). The parasite suspension was subjected to three cycles of freeze-thaw using liquid N2. The lysate was sonicated (pulses of 9 sec on/9 sec off) for 3 min at 4˚C, centrifuged at 13000g for 15 min at 4˚C. The supernatant, which contained cytosolic proteins was transferred to a fresh micro-centrifuge tube, and centrifuged again at 13000g for 30 min at 4˚C. The supernatant was called soluble parasite extract and its protein concentration was estimated by Bradford Reagent (Bio-Rad Protein Assay,

Catalog #500-0006) using BSA as standard. As DHFR is a cytosolic protein, the soluble parasite extract was assessed for DHFR activity using the DHFR Assay Kit (DHFR Assay Kit from Sigma-Aldrich, Product Code CS0340) according to the manufacturer's instructions. Briefly, reactions were set up in 1 ml assay buffer with 500 µg soluble parasite extract and DMSO (Control, 0.05% final) or inhibitors (50 µM Pyr, 25 µM **4n**, 25 µM **4p** and 25 µM **4t**). The reactions were incubated for 30 min at room temperature, and centrifuged at 13000g for 1 min to remove any precipitate. The reaction supernatant was supplemented with 60 µM NADPH and 50 µM DHA, and DHFR activity was monitored at room temperature for 30 min at 15 sec intervals as decrease of NADPH absorbance at 340 nm using a UV/Vis spectrophotometer (PerkinElmer). DHFR activity was determined by linear regression analysis as the rate of change in absorbance per min using the Graphpad Prism software and expressed as the percent activity of control reaction.

4.8.3. Cytotoxicity

Cells were maintained in 60mm dishes, trypsinised when the cells were confluent and seeded at a density of 10,000 cells/well in 96 well plates. The seeded 96 well plates were incubated for 24 hrs at37 °C incubator with 5% CO_2 and 100% relative humidity. After overnight incubation, cells were treated with compounds **4a-h**, **4j-q**, **4t** and **5b** at different concentrations ranging from 1µM to 100 µM in triplicates along with control (DMSO) and Doxorubicin as standard inhibitor in the same plate. After 48hr of incubation, the assay was terminated by the addition of 10µl of 5%MTT and incubated for 1hr at 37 °C. The medium was discarded and the plates were air dried. To these plates 100µl of DMSO was added per well. Absorbance was measured at 562 nm in a multimode microplate reader (TecanGENios).The sensitivity of the cell lines to the test compound was expressed in terms of IC_{50}, a value defined

as the concentration of compound that produced 50% reduction as compared to the control absorbance. IC_{50} values are indicated as means ± Standard Deviation of three independent experiments.

4.9. Experimental protocol of docking study

Molecular docking of compounds **4j**, **4k**, **4l**, **4m**, **4n**, **4p**, **4t** and Pyr into the three dimensional X-ray structures of PfDHFR (PDB ID: 1J3I and1J3K) was carried out using the Schrodinger suite version 5.6 as implement through the graphical user interface, IFD protocol. For this purpose, the X-ray crystal structure of P. falciparum wild type and quadruple mutant DHFR-TS (1J3I and 1J3K) [43] were selected respectively. The crystal structures contain the third-generation PfDHFR inhibitor WR99210 bound to the active site in the presence of NADPH. To consider the flexibility of both ligand and enzyme in the docking studies, the IFD [44] protocol was adopted. In IFD protocol, ligands were docked into the rigid protein using softened potential docking in the Glide program with the van der Waals radii scaling of 0.9Å for the proteins. The resulted top 20 poses of each ligand were then used to sample the protein plasticity using the Prime program in the Schrödinger suite. Residues having at least one atom within 5Å of any of the 20 ligand poses were subjected to a conformational search and energy minimization process, although residues outside this zone were fixed and hence the protein is considered to be flexible and resulting 20 new receptor conformations were redocked. In this redocking stage, Glide docking parameters were set to the default hard-potential function. The Glide XP (extra precision) was used for all the docking calculations and binding affinity of each complex was reported as the Glide Score (provided in supporting information).

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Synthesis and evaluation of Naphthyl bearing 1,2,3-Triazole analogs as Antiplasmodial agents, Cytotoxicity and Docking Studies

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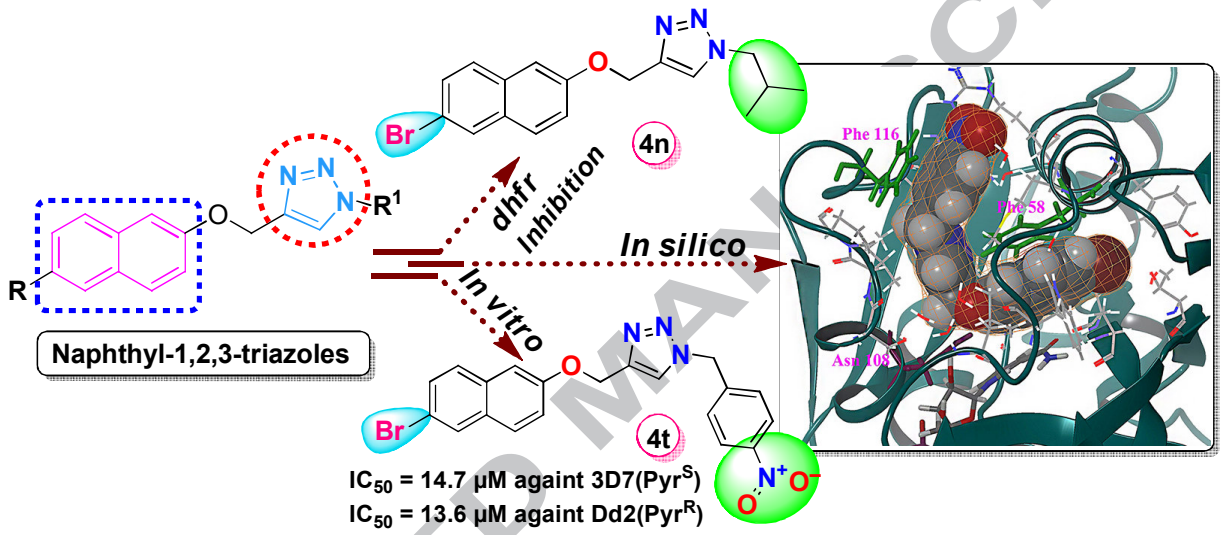

- New series of naphthyl bearing 1,2,3-triazoles were synthesized with good yields.
- Novel azide of *(S)*-diethyl-2-(4-azidobenzamido)pentanedioate was synthesized.
- Structures of **4c**, **4k**, **4r**, **4s** and **4t** have been studied by single crystal XRD.
- **4m**, **4n** and **4t** showed potent antiplasmodial activity compared to Pyrimethamine.
- Induced fit docking studies were performed for active compounds.