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Antimalarial and antibacterial activities of ether linked 1,4-disubstituted 1,2,3-triazoles

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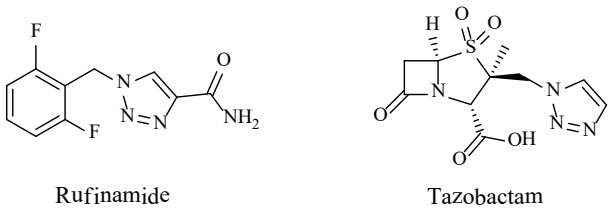
Abstract: A series of twenty seven ether linked 1,4-disubstituted 1,2,3-triazoles was assessed for *in vitro* antimalarial activity against *Plasmodium falciparum*, while antibacterial activity against *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*. Biological evaluation of synthesized triazoles revealed moderate to good antimalarial and antibacterial activity against the tested strains. Molecular docking study has also been investigated for most active compound **19**, that disclosed binding to the active site of E. coli DNA Gyrase.

Keywords: Disubstituted 1,2,3-triazoles, Antimalarial activity, Antibacterial activity, Docking studies

Introduction

Malaria is a curable disease, however, the rapid development of drug resistance has come out as challenge to the frequently used antimalarials such as chloroquine, amodiaquine, mefloquine [1]. Further, the drug resistance of bacteria also hamper the effects of available antibiotics and thereby intensifying the burden of bacterial infections [2]. In this context, the biological activities and other practically useful properties of disubstituted triazole derivatives enabled them to be widely used in various industrial and medical sectors. The triazoles possess antimicrobial [3-5], antiviral [6,7], antioxidant [8-10], anti-inflammatory [11,12], antimalarial [13-15], antidepressant [16], anticonvulsant [17], and cytotoxic [18-21] properties. Some of the drugs containing 1,2,3- triazole scaffolds (Fig 1) are currently in use, such as rufinamide (anticonvulsant agent) and Tazobactum (antibacterial agent). The triazole ring showes the enhanced biological activities are due to its promising properties viz. rigidity, high chemical stability in form of oxidising and reducing agents, dipole moment and *in vivo* hydrogen bonding capability [22].



Rufinamide Tazobactam

Figure1

Encouraged from above considerations, we have screened earlier synthesized twenty seven ether linked 1,4-disubstituted 1,2,3-triazoles [23] for *in vitro* antimalarial activity against *Plasmodium falciparum* and antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*. Molecular docking studies have also been carried out to investigate the interaction modes between the compound 19 and active site of E. coli DNA Gyrase.

Experimental

Biological activity

The antibacterial evaluation was carried out with the help of Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science & Technology, Hisar. The *in vitro* antimalarial was carried out from Microcare laboratory & TRC, Surat, Gujarat.

Antimalarial activity

All the synthesized ether linked 1,4-disubstituted 1,2,3-triazoles **(1**–**27)** were screened for antimalarial activity against *Plasmodium falciparum.* The *in vitro* antimalarial assay was carried out in 96 well microtitre plates according to the micro assay protocol of Rieckmann and co-workers with minor modifications [24]. The cultures of *P. falciparum* strain were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1 % D-glucose, 0.23 % sodium bicarbonate and 10 % heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5 % D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5 % at 3 % haematocrit in a total volume of 200 µL of medium RPMI-1640 was determined by Jaswant Singh Bhattacharji (JSB) staining to assess the percent parasitaemia (rings) and uniformally maintained with 50 % RBCs (O^+). A stock solution of 5 mg/mL of each of the test samples was prepared in dimethylsulfoxide and subsequent dilutions were prepared with culture medium. The diluted samples in 20 µL volume were added to the test wells so as to obtain final concentrations (at five fold dilutions) ranging between 0.4 µg/mL to 100 µg/mL in duplicate well containing parasitized cell preparation. The culture plates were incubated at 38 °C in a candle jar. After 36 to 40 h incubation, thin blood smears from each well were prepared and stained with Jaswant Singh Bhattacharji (JSB) stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Quinine was used as the reference drug. The mean number of rings, trophozoites and schizonts recorded per 100 parasites from duplicate wells after incubation for 38 hours, and percent maturation inhibition with respect to control group. (Table 1)

Antibacterial activity

In vitro antibacterial activity of all the compounds **(1-27)** was carried out against bacteria – *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 3160) and *Escherichia coli* (MTCC 443) by standard serial dilution method [5] using a stock solution of 200 µg/mL concentrations. Nutrient broth was employed as culture media and Norfloxacin was used as a standard drug. A

stock solution of testing compound and control drug was serially diluted to get concentration of 100, 50, 25, 12.5, 6.25 µg/mL. All these dilutions were inoculated with their respective bacteria in saline solution and incubated at 37 °C for 24 h. After incubation, the results were recorded visually in terms of minimum inhibitory concentration (MIC). **(Table 2)**

Docking studies

The docking studies were carried out as per the protocols of our previously reported procedure [25]. The docking simulations were performed using Autodock vina [26] module in VEGAZZ [27]. The results were visualized using discovery studio and PyMOL.

Result and discussion

The 1,4-disubstituted 1,2,3-triazoles with ether functionality **(Figure 2)** were synthesized by click reaction of various aromatic azides and 1-substituted-4-(prop-2-yn-1-yloxy)benzene in dimethylformamide using catalytic amount of copper sulphate pentahydrate and sodium ascorbate.

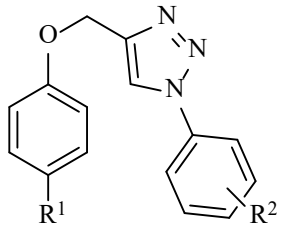


Figure 2

Compound	R^1	R^2
1	H	2-OH
2	H	3-OH
3	H	4-OH
4	H	2-$OCH_2C_6H_5$
5	H	3-$OCH_2C_6H_5$
6	H	4-$OCH_2C_6H_5$
7	H	2-$OCH_2CH_2C_6H_5$
8	H	3-$OCH_2CH_2C_6H_5$
9	H	4-$OCH_2CH_2C_6H_5$
10	CH_3	2-OH
11	CH_3	3-OH
12	CH_3	4-OH
13	CH_3	2-$OCH_2C_6H_5$
14	CH_3	3-$OCH_2C_6H_5$
15	CH_3	4-$OCH_2C_6H_5$
16	CH_3	2-$OCH_2CH_2C_6H_5$
17	CH_3	3-$OCH_2CH_2C_6H_5$
18	CH_3	4-$OCH_2CH_2C_6H_5$
19	NO_2	2-OH
20	NO_2	3-OH
21	NO_2	4-OH
22	NO_2	2-$OCH_2C_6H_5$
23	NO_2	3-$OCH_2C_6H_5$
24	NO_2	4-$OCH_2C_6H_5$
25	NO_2	2-$OCH_2CH_2C_6H_5$
26	NO_2	3-$OCH_2CH_2C_6H_5$
27	NO_2	4-$OCH_2CH_2C_6H_5$

Ether linked 1,4-disubstituted 1,2,3-triazoles

Antimalarial activity

All the synthesized triazoles **(1–27)** were assessed *in vitro* for antimalarial activity against *P. falciparum* by using micro assay procedure of Rieckmann and coworker with minor modification [24]. Experiment was carried out in duplicate and IC_{50} values are calculated in µmol/mL). Quinine was used as standard drug. Results are summarized in **Table 1**

It can be depicted from antimalarial screening data (**Table 1)**, synthesized triazoles exhibited moderate antimalarial activity against *P. falciparum.* Among the synthesized derivatives, compound **6** (IC_{50} = 0.1987 µmol/mLx10^{-2}), **7** (IC_{50} = 0.1885 µmol/mLx10^{-2}), **19**(IC_{50} = 0.1783

μmol/mLx10^{-2}), **24** (IC_{50} = 0.1989 μmol/mLx10^{-2}) and **25** (IC_{50} = 0.1873 μmol/mLx10^{-2}) possess average antimalarial activity.

From the antimalarial activity results, the following structure activity relationships can be summarized: The ortho derivatives showed better inhibition in comparison to meta and para derivatives. It also has been observed that generally triazoles with nitro substituent (compound 19, 20, 21, 23, 24, 25, 26, 27) possess better activity than the unsubstituted ones or methyl substituents.

Table 1. *In vitro* antimalarial activity of 1,4-disubstituted 1,2,3-triazoles **(1-27)**

	Mean Inhibitory Concentration (IC_{50}, μmol/ mLx10^{-2})
Compound	*Plasmodium falciparum*
1	0.2769
2	0.2769
3	0.2769
4	0.2574
5	0.2854
6	**0.1987**
7	**0.1885**
8	0.2558
9	0.2962
10	0.3164
11	0.4088
12	0.4088
13	0.2423
14	0.2962
15	0.2262
16	0.2179
17	0.2465
18	0.2465
19	**0.1783**
20	0.2562
21	0.2210
22	0.2735
23	0.2238
24	**0.1989**
25	**0.1873**
26	0.2521
27	0.2642
Quinine	0.0826

The bold numbers represents the activity of synthesized compound comparable to standard drug used.

Antibacterial activity

The synthesized triazoles **(1-27)** were examined for *in vitro* antibacterial activity against *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 3160) and *Escherichia coli* (MTCC 443) by employing serial dilution method [5]. Minimum inhibitory concentrations were expressed in μmol/mL as represented in **Table 2**. Norfloxacin was used as a standard drug.

It has been revealed from data presented in **Table 2** that most of compounds exhibited moderate to good activities. Compound **1** (MIC = 0.0468 μmol/mL), **2** (MIC = 0.0468 μmol/mL), **3** (MIC = 0.0468 μmol/mL), **19** (MIC = 0.0400 μmol/mL), **20** (MIC = 0.0400 μmol/mL), **21** (MIC = 0.0400 μmol/mL) showed good activity against *B. cereus*. Compound **1** (MIC = 0.0468 μmol/mL) and **19** (MIC = 0.0400 μmol/mL) showed remarkable activity against *S. aureus*, while, Compound **19** (MIC = 0.0400 μmol/mL) and **20** (MIC = 0.0400 μmol/mL) showed appreciable activity against *E. coli.*

From the above results, it can be summarized that free OH (Compound 1, 2, 3, 10, 11, 12, 19, 20 and 21) group containing triazoles showed better activity than others. It has also been reflected that compound with nitro substituent (compound 19, 20, 21, 23, 24, 25, 26, 27 have better activity than the unsubstituted one or with methyl substitution. Triazoles having phenyl ethyl moiety (Compounds 7, 8, 9, 16, 17, 18, 25, 26 and 27) showed enhanced antibacterial activity in comparison to benzyl moiety. In most of cases ortho derivatives showed better inhibition in comparison to meta and para derivatives.

Table 2. *In vitro* antibacterial activity of 1,4-disubstituted 1,2,3-triazoles **(1-27)**

	Minimum Inhibitory Concentration (MIC, µmol/mL)		
Compound	*Bacillus cereus*	*Staphylococcus aureus*	*Escherichia coli*
1	**0.0468**	**0.0468**	0.0935
2	**0.0468**	0.0935	0.0935
3	**0.0468**	0.0935	0.0935
4	0.1399	0.1399	0.1399
5	0.1399	0.1399	0.1399
6	0.1399	0.1399	0.1399
7	0.1346	0.1346	0.1346
8	0.1346	0.1346	0.1346
9	0.1346	0.1346	0.1346
10	0.0888	0.0888	0.1777
11	0.0888	0.1777	0.0888
12	0.0888	0.0888	0.0888
13	0.1346	0.2692	0.2692
14	0.1346	0.1346	0.1346
15	0.1346	0.1346	0.1346
16	0.1297	0.1297	0.1297
17	0.1297	0.1297	0.1297
18	0.1297	0.1297	0.1297
19	**0.0400**	**0.0400**	**0.0400**
20	**0.0400**	0.0801	**0.0400**
21	**0.0400**	0.0801	0.0801
22	0.1243	0.1243	0.1243
23	0.1243	0.1243	0.1243
24	0.1243	0.1243	0.1243
25	0.1200	0.1200	0.1200
26	0.1200	0.1200	0.1200
27	0.1200	0.1200	0.1200
Norfloxacin	0.0391	0.0783	0.0391

The bold numbers represents the activity of synthesized compound comparable to standard drug used

Docking studies

Compound **19** was found to be most active against E. coli and its binding conformation was determined by docking it into the active site of E. coli DNA Gyrase (PDB ID: 1KZN). The most favorable binding conformation of compound **19** interacted by different types of interactions with the active site residues. Oxygen atom of hydroxyl group created hydrogen bond with Arg76 while its hydrogen atom made a hydrogen bond with Gly77. Thus, hydroxyl group acted as both donor as well as acceptor of hydrogen bond. Oxygen atoms of nitro and ether groups acted as hydrogen bond acceptor and exhibited hydrogen bond interactions with Val167 and Asn46 respectively. Further, pi orbitals of triazole ring showed pi-anion interactions with Glu50. All the interacting residues of the active site along with binding conformation of compound 19 are displayed in figure 3. The cartoon diagram of DNA gyrase containing co-crystalized ligand clorobiocin and docked compound 19 is shown in figure 4.

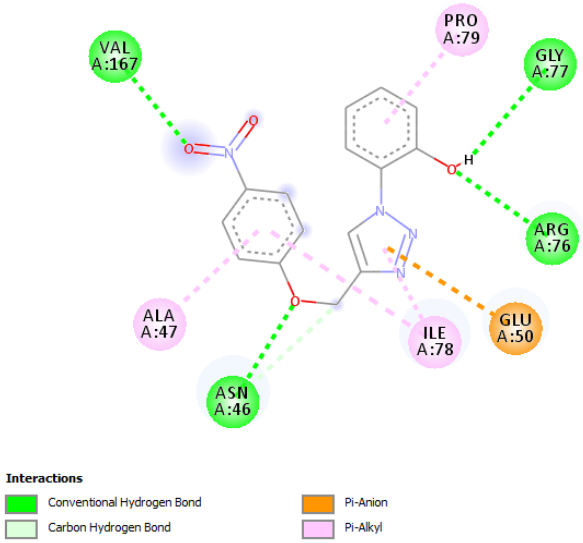


Figure 3. Interactions of compound 19 with active site of e. coli DNA Gyrase.

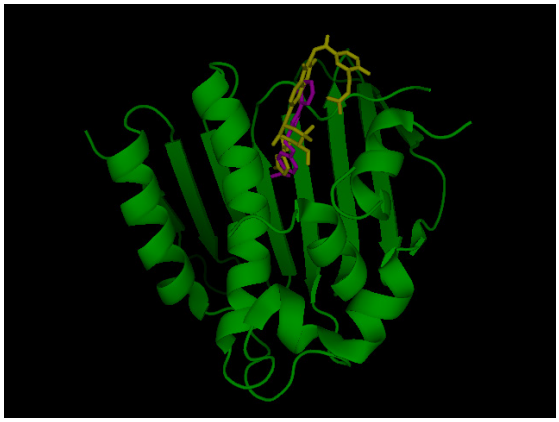


Figure 4. DNA gyrase along with docked compound 19 (magenta) and co-crystalized ligand clorobiocin.

Conclusion

A series of 1,4-disubstituted 1,2,3-triazoles **(1–27)** was assessed for *in vitro* antimalarial activity against *Plasmodium falciparum*, while antibacterial activity against *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*. Biological evaluation of synthesized 1,2,3-triazoles revealed that molecules having free OH showed better activity and in most of cases ortho derivatives showed better inhibition in comparison to meta and para derivatives.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgment

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