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1,2,3-Triazole Derivatives as Antitubercular Agents: Synthesis, Biological Evaluation and Molecular Docking Study

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Abstract

Searching for new active molecules against *Mycobacterium tuberculosis* (MTB) H37Ra, a small focused library of 1,2,3-triazoles has been efficiently prepared *via* click chemistry approach. The newly synthesized compounds were tested against drug-sensitive MTB. Several derivatives were found to be promising inhibitors of MTB characterized by lower MIC values (5.8-29.9 μg/mL). Among all the synthesized 31 compounds, **15e** was the most active compound against MTB. Based on the results from the anti-tubercular activity, SAR for the synthesized series has been developed. The active compounds from the anti-tubercular study were further tested for anti-proliferative activity against THP-1, A549 and Panc-1 cell lines using MTT assay and showed no significant cytotoxic activity against these three cell lines except THP-1 at the maximum concentration evaluated. Further, the synthesized compounds were found to have potential antioxidant with IC_{50} range = 10.1-37.3 μg/mL. The molecular docking study of synthesized compounds was performed against DprE1 enzyme of MTB to understand the binding interactions. Moreover, synthesized compounds were also analysed for ADME properties and all the experimental results promote us to consider this series as starting point for the development of novel and more potent anti-tubercular agents in future.

1. Introduction

Tuberculosis (TB), an infection of *Mycobacterium tuberculosis* (MTB), still remains the leading cause of worldwide death among infectious diseases. The World Health Organization (WHO) reported that, more than one-third of the world's population is infected with TB and that resulted in an estimated 1.5 million deaths worldwide in 2013,[1] out of which 0.36 million people were infected with both human immunodeficiency virus (HIV) and TB. The long duration of therapy generally results in noncompliance of the treatment and results in multidrug resistance tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB), which are highly lethal, extremely expensive and complicated to treat, posing new challenges for the prevention, treatment and control of TB.[2] Despite the availability of effective anti-TB drugs, such as isoniazid and rifampicin, there is serious problem emergence as MTB developed resistance against the first line as well as second line drugs.[3] Therefore, there is an urgent need to develop new inhibitors that reduce the complexity and duration of the current therapeutic treatment as well as effectively treat MDR and XDR tuberculosis.

Oxidative stress is the major cause of tissue inflammation in tuberculosis. During illness, due to the poor dietary intake of micronutrients, free radicals burst from activated macrophages and anti-tuberculosis drugs. If these free radicals were not neutralized by the antioxidants, that leads toward the pulmonary inflammation.[4] These antioxidant exerts their effects by scavenging or preventing the generation of reactive oxygen species (ROS)[5] which can protect the formation of free radicals and retard the progress pulmonary inflammation.

1,2,3-Triazole have received much attention, as their intriguing physical and biological properties, as well as their excellent stability, rendering them promising drug core structures. The 1,3-dipolar cycloaddition reaction of a 1,3-dipole to a dipolarophile (i.e. an acetylene or alkyne) for the synthesis of five member heterocycles are well-known transformations in synthetic organic chemistry.[6] Recently, Sharpless[7] and Meldal[8] groups have reported the dramatic rate enhancement (up to 10^7 times) and improved regio-selectivity of the Huisgen 1,3- dipolar cycloaddition reaction of an organic azide and terminal acetylene to afford, regio-specifically, the 1,4-disubstituted-1,2,3-triazole in the presence of Cu (I) catalyst. The Cu (I)-catalyzed azide alkyne cycloaddition (CuAAC) reaction has successfully fulfilled the requirement of "click chemistry" as prescribed by Sharpless and within the past few years has become a premier component of synthetic organic chemistry.[9]

Over the past few years, 1,2,3-triazoles are an important class of compounds because of their wide coverage of biological applications including anti-tubercular,[10] anti-bacterial, anti-allergic, anti-HIV,[11] anti-fungal[12] activity and α-glycosidase inhibitor[13] activity. Moreover, 1,2,3-triazole derivatives have been found to show promising anti-tubercular activity profile.[14] Owing to these significant features, a number of protocols for the synthesis of 1,2,3-triazole compounds have been developed.[15] Among them, the most elegant and useful approach is the Huisgen's 1,3-dipolar cycloaddition of azides and alkynes.[16] In recent years, a library of derivatives with conjugated 1,2,3-triazoles were synthesized and proved to possess different bioactivity. Baltas and coworkers reported,[17] 1,4-disubstituted 1,2,3-triazole derivatives (**A** & **B**) (Figure 1) exhibiting good inhibitory activities against MTB H37Rv. Similarly, clubbed [1,2,3] triazoles with fluorine containing benzimidazole series (**C**) of H37Rv strain inhibitors have been reported.[18] The 1,2,3-triazole based compounds (**D**) are active against different pathogenic and opportunistic *Mycobacteria* including *M. avium* and MTB.[19] Recently, 1,2,3-triazole based isoniazid derivative[10] (**E**) possess antitubercular activity against MTB H37Rv (Figure 1).

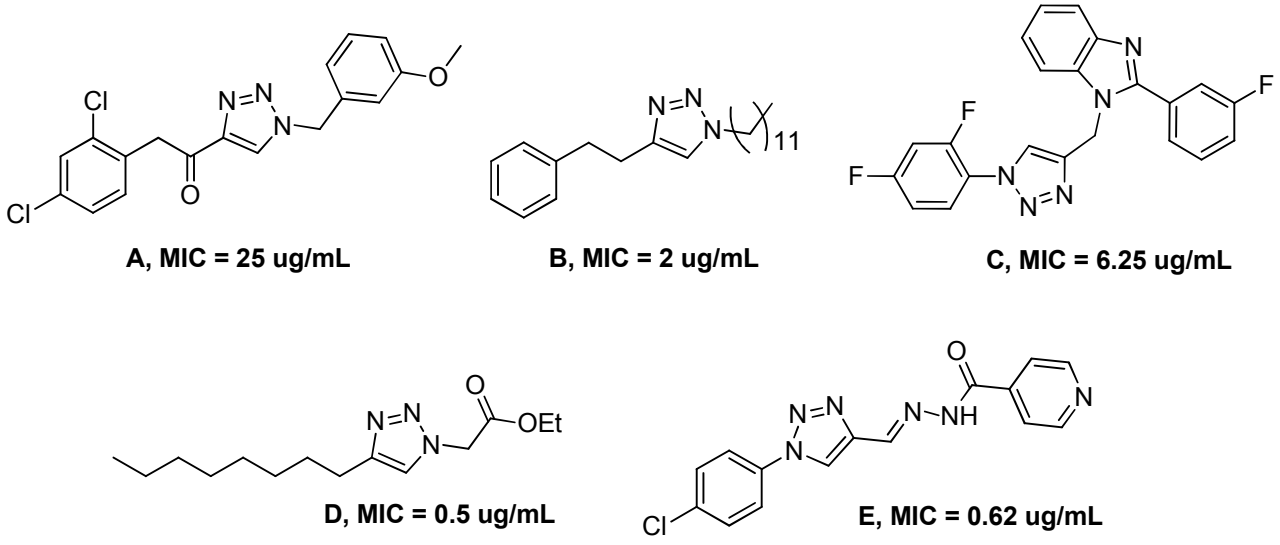


Figure 1. 1,2,3-Triazole containing compounds possesses antitubercular activity

Development of new active molecules against MTB, a small focused library of 1,2,3-triazole incorporated molecules have been efficiently prepared by click chemistry. We were encouraged to design a 1,2,3-triazole based new molecules from commercially available starting

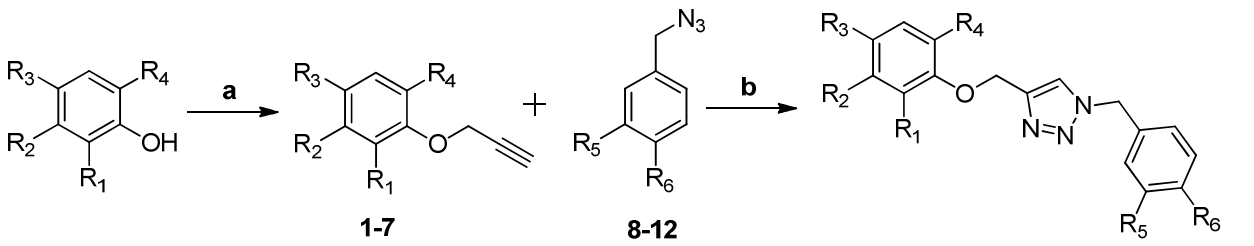
materials in minimum steps with high overall yield. In continuation of our earlier work[20] on synthesis and biological properties of various heterocyclic moieties, herein, we would like to report, the synthesis of 1,4-disubstituted-1,2,3-triazole derivatives and their antitubercular, antioxidant and cytotoxic activities. In addition to this, we have also performed molecular docking study and *In silico* ADME prediction for the synthesized compounds.

2. Results and Discussion

2.1.Chemistry

The 1,4-disubstituted-1,2,3-triazole derivatives **13-19** have been obtained by the fusion of substituted (prop-2-yn-1-yloxy)benzenes **1-7** and benzyl azides **8-12** *via* click chemistry (Scheme 1**)**. The commercially available phenols have been alkylated with propargyl bromide in the presence of K_2CO_3 as a base in *N,N*-dimethylformamide (DMF) afforded the corresponding (prop-2-yn-1-yloxy)benzene derivatives **1-7** in good to excellent yield (Supporting information). The synthesis of benzyl azides **8-12** has been achieved from the corresponding benzaldehydes according to literature procedure.[21] The Huisgen's CuAAC reaction has been performed on (prop-2-yn-1-yloxy)benzene **1** with 4-nitrobenzylazide **8** in the presence of $Cu(OAc)_2$ in t-BuOH-H_2O (3:1) at room temperature for 20 h afforded 1,4-disubstituted-1,2,3-triazole **13a** in 89% yield (Scheme 1).

The formation of compound **13a** confirmed by ^{1}H NMR, ^{13}C NMR and HRMS spectral analysis. In the ^{1}H NMR spectrum of the compound **13a**, the two methylene groups attached to nitrogen and oxygen showed singlet at δ 5.20 and 5.67 ppm respectively. In addition to this, the signal observed at δ 7.64 ppm indicates the proton present on the triazole ring. In the ^{13}C NMR spectrum for compound **13a**, the signals at δ 53.20 and 62.01 ppm indicates the presence of methylene carbon attached to the nitrogen of triazole ring and oxygen to phenyl ring respectively. For compound **13a**, the calculated mass for $[M+Na]^+$ is 333.0958 and in HRMS, the $[M+Na]^+$ peak observed at 333.0967. Furthermore, to expand the series, 1,4-disubstituted-1,2,3-triazole derivatives **13b-e**, **14a-e**, **15a-e**, **16a-e**, **17a-d**, **18a-e** and **19a-b** with various substituents (Supporting information) have been prepared by the cycloaddition reaction of benzyl azides **8-12** and alkynes **1-7** (Scheme 1 and Table 1) under similar reaction condition in good to excellent yields.



Reagents and conditions: (a) Propargyl bromide, K_2CO_3, DMF; (b) $Cu(OAc)_2$ (20 mol%), *t*-BuOH:H_2O (3:1), rt.

Scheme 1. Synthesis of 1-benzyl-4-(phenoxymethyl)-1*H*-1,2,3-triazole **13-19**.

2.2. Biological Evaluation

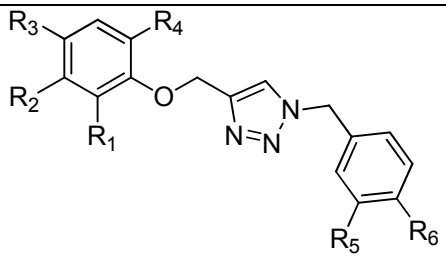
2.2.1. Anti-tubercular activity

All the newly 31 synthesized compounds were screened for *in vitro* anti-tubercular activity, determined by measuring inhibition of growth against avirulent strain of MTB (MTB H37Ra; ATCC 25177) in liquid medium. In a preliminary screening (Supporting information, Table S1 and Table S2), the anti-mycobacterial activity of these compounds was assessed at concentrations of 30, 10 and 3 μg/mL using an established XTT Reduction Menadione assay (XRMA) anti-tubercular screening protocol[22] using first-line antitubercular drugs rifampicin and isoniazid as reference standards and data obtained is presented in Table 1. The triazole derivatives **13a**, **13c**, **13d**, **13e**, **14a**, **14c**, **14d**, **15a**, **15b**, **15c**, **15d**, **15e**, **17a**, **17c**, **18c** and **18e** (MIC range = 5.8-29.9 μg/mL) were found to be particularly active against MTB. The compounds **13b**, **13e**, **14b**, **14e**, **16b**, **17b**, **17d**, **18a**, **18b**, **18d**, **19a** and **19b** (MIC = >30 μg/mL) were found to show less activity against MTB strains. The compounds **16a**, **16c**, **16d** and **16e** (MIC = >100 μg/mL) were found to be less active against MTB strains.

The structure-activity relationship reveals that compounds have shown significant anti-tubercular activity (Table 1). The activity table showed that 1,2,3-triazole ring is important for anti-tubercular activity. The activity varies depending upon the various substituents present on

phenyl rings. The compounds from **13a-13e** series (MIC range = 27.7- >30 μg/mL) do not show any significant activity when compared with standards. Replacement of hydrogen with methyl group at R_3 position (**14a-14e** series, MIC range = 24.2- >30 μg/mL) do not show any significant change in anti-tubercular activity when compare with compounds of **13a-13e** series. The substitution of *chloro*- group at R_3 position (**15a-15e** series) showed increase in anti-tubercular activity (MIC range = 5.8-29.2 μg/mL). The substitution of halogens like *chloro*- and *bromo*- group at R_6 position (compounds **15c**, MIC = 8.2 μg/mL and **15e**, MIC = 5.8 μg/mL) showed sharp increase in anti-tubercular activity. The substitution of *bromo*- at R_6 position (compound **15e**, MIC = 5.8 μg/mL) was more favorable and found to be most active compound among all the 31 synthesized compounds. Replacement of *chloro*- group from R_5 (compound **15d**, MIC = 25.8 μg/mL) to R_6 (compound **15c**, MIC = 8.2 μg/mL) showed 3 fold increase in activity. All the compounds of **16a-16e** series (replacement of hydrogen by *nitro*- group at R_2 position) were found to be least active (MIC range = >30- >100 μg/mL) among all the synthesized series. Substitution of methyl group at R_2 and *chloro*- group at R_3 positions (**17a-17d** series) have shown some significant activity (MIC range = 26.9- >30 μg/mL). Substitution of *chloro*- group at R_1, R_3 and R_4 positions (**18a-18e** series) have not shown significant change in activity (MIC range = 27.8- > 30 μg/mL) except compound **18c** (MIC = 7.7 μg/mL) bearing *chloro*- group at R_6 position. Replacement of *chloro*- group by *iodo*- group at R_1 and R_4 position and *nitro*- group at R_3 position (compounds **19a-19b**) showed no significant alteration in activity (MIC = >30 μg/mL). None of the synthesized compounds showed activity comparable with that of standard rifampicin (MIC = 0.04 μg/mL).

Table 1 *In vitro* antitubercular activity against MTB H37Ra and DPPH Radical scavenging activities of compounds **13-19**



13-19

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	Anti TB MIC (μg/mL)	Anti TB IC_{50} (μg/mL)	DPPH IC_{50} (μg/mL)
13a	H	H	H	H	H	NO_2	28.0	1.7	10.1
13b	H	H	H	H	NO_2	H	>30	2.4	25.3
13c	H	H	H	H	H	Cl	29.9	3.5	26.1
13d	H	H	H	H	Cl	H	27.7	2.4	27.3
13e	H	H	H	H	H	Br	28.9	3.2	18.3
14a	H	H	Me	H	H	NO_2	29.9	1.1	10.9
14b	H	H	Me	H	NO_2	H	>30	2.4	12.1
14c	H	H	Me	H	H	Cl	24.2	2.5	17.3
14d	H	H	Me	H	Cl	H	28.9	3.0	16.3
14e	H	H	Me	H	H	Br	>30	7.8	16.3
15a	H	H	Cl	H	H	NO_2	27.5	3.6	24.2
15b	H	H	Cl	H	NO_2	H	29.2	2.6	21.3
15c	H	H	Cl	H	H	Cl	8.2	0.7	26.1
15d	H	H	Cl	H	Cl	H	25.8	2.9	23.3
15e	H	H	Cl	H	H	Br	5.8	0.5	11.0
16a	H	NO_2	H	H	H	NO_2	>100	>100	17.1

16b	H	NO_2	H	H	NO_2	H	>30	8.3	19.1
16c	H	NO_2	H	H	H	Cl	>100	53.4	37.3
16d	H	NO_2	H	H	Cl	H	>100	26.6	31.2
16e	H	NO_2	H	H	H	Br	>100	43.2	29.4
17a	H	Me	Cl	H	H	NO_2	28.2	0.8	13.4
17b	H	Me	Cl	H	NO_2	H	>30	2.2	15.6
17c	H	Me	Cl	H	H	Cl	26.9	0.2	11.4
17d	H	Me	Cl	H	H	Br	>30	2.4	16.5
18a	Cl	H	Cl	Cl	H	NO_2	>30	2.2	28.9
18b	Cl	H	Cl	Cl	NO_2	H	>30	2.6	29.2
18c	Cl	H	Cl	Cl	H	Cl	7.7	0.8	27.2
18d	Cl	H	Cl	Cl	Cl	H	>30	2.2	16.4
18e	Cl	H	Cl	Cl	H	Br	27.8	0.9	14.0
19a	I	H	NO_2	I	H	NO_2	>30	2.6	16.8
19b	I	H	NO_2	I	H	Br	>30	25.0	18.5
Rifampicin	-	-	-	-	-	-	0.04	0.002	NA
BHT	-	-	-	-	-	-	NA	NA	16.5
AA	-	-	-	-	-	-	NA	NA	12.7

DPPH: 2,2-diphenyl-1-picrylhydrazyl; BHT: Butylated hydroxy toluene; AA: Ascorbic acid; NA: Not applicable.

2.2.2. Antioxidant activity

In tuberculosis, oxidative stress may result in tissue inflammation due to anti-tubercular drugs.[4] The synthesized compounds have shown promising anti-tubercular activity and have potential to develop as lead compounds. Therefore, it is necessary to evaluate the synthesized compounds for their antioxidant activity. Antioxidant activities of the synthesized compounds **13-19** were measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.[23] DPPH radical scavenging activity is the most commonly used method for screening the antioxidant activities of the various natural as well as synthetic antioxidants. A lower IC_{50} value

indicates the greater antioxidant activity. The IC_{50} (concentration required to scavenge 50% of the radicals) were calculated to evaluate the potential antioxidant activities. Butylated hydroxytoluene (BHT) and ascorbic acid (AA) has been used as a standard drug for the comparison of antioxidant activity and the observed results are summarized in Table 1.

According to the DPPH assay, compounds **13a**, **14a**, **14b**, **14d**, **14e**, **15e**, **17a**, **17b**, **17c**, **17d**, **18d**, **18e** and **19a** exhibited promising radical scavenging activities when compared with synthetic antioxidant BHT (IC_{50} = 16.5 µg/mL), with IC_{50} values of 10.1, 10.9, 12.1, 16.3, 16.3, 11.0, 13.4, 15.6, 11.4, 16.5, 16.4, 14.0 and 16.8 µg/mL, respectively. Similarly, compounds **13a** (IC_{50} = 10.1 µg/mL), **14a** (IC_{50} = 10.9 µg/mL), **14b** (IC_{50} = 12.1 µg/mL), **15e** (IC_{50} = 11.0 µg/mL) and **17c** (IC_{50} = 11.4) show excellent antioxidant activity compared to the standard antioxidant drug ascorbic acid (IC_{50} = 12.7 µg/mL). From synthesized compounds **13a**, **14a**, **15e** and **17c** showed the best DPPH radical scavenging activity, while compound **16c** showed the lowest activity when compared with standards.

From the antioxidant activity data (Table 1), the synthesized compounds had shown moderate to good antioxidant activity. Compound **13a** (IC_{50} = 10.1 µg/mL) from **13a-13e** series bearing R_6 = NO_2 group was found to be most active antioxidant agent among synthesized compounds than standard drugs BHT and ascorbic acid. Replacement of R_6 = NO_2 group to R_5 = NO_2 led to decrease in activity by 2 fold (compound **13b**, IC_{50} = 25.3 µg/mL). Replacement of NO_2 group with *chloro*- group at R_6 (compound **13c**, IC_{50} = 26.1 µg/mL) and R_5 (compound **13d**, IC_{50} = 27.3 µg/mL) position did not show any significant change in activity. The substitution of R_6 = Br (compound **13e**, IC_{50} = 18.3 µg/mL) led to increase in activity when compared with *chloro*- substituted compounds **13c** and **13d**. Among **14a-14e** series, all the five compounds (IC_{50} range = 10.9-17.3 µg/mL) have shown good activity when compared with standards. There is no significant variation observed while varying the substituent like, NO_2, Cl and Br.

From **15a-15e** series, all compounds (IC_{50} range = 21.3-26.1 µg/mL) except **15e** (IC_{50} = 11.0 µg/mL) showed less potent activity when compared with standards. The substitution of R_6 = Br (compound **15e**) led to 2 fold increase in the activity when compared with compounds **15a**, **15b**, **15c** and **15d**. Substitution of R_2 = NO_2 (series **16a-16e**) led to least active (IC_{50} range = 17.1-37.3 µg/mL) series among the synthesized compounds. The compounds (IC_{50} range = 11.4-16.5 µg/mL) with -CH_3 substitution at R_2 position (series **17a-17d**) showed significant better activity than standard BHT (IC_{50} = 16.5 µg/mL). Compound **17c** (IC_{50} = 11.4 µg/mL) with R_2 =

CH_3, R_3 = Cl and R_6 = Cl substitution showed better activity than standards BHT and ascorbic acid. Substitution of halogens like *chloro-* and *iodo-* at R_1 position (compounds **18a-18e** and **19a-19b**) showed moderate activity except compounds **18d** (R_1, R_3, R_4 and R_5= Cl, IC_{50} = 16.4 μg/mL) and **18e** (R_1, R_3 and R_4 = Cl and R_6 = Br, IC_{50} = 14.0 μg/mL) which showed comparable antioxidant activity with BHT.

2.2.3. Cytotoxic activity

The synthesized 1,2,3-triazole derivatives **13a**, **13c**, **13d**, **13e**, **14a**, **14c**, **14d**, **15a**, **15b**, **15c**, **15d**, **15e**, **17a**, **17c**, **18c** and **18e** were further assayed for their cytotoxic activity against the three different human cancer cell lines; THP-1(Human acute monocytic leukemia cell line), A549 (Human lung adenocarcinoma epithelial cell line) and Panc-1 (Human pancreas carcinoma cell line), using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay[24] with 48 h exposure time of the tested compounds and paclitaxel was used as positive control. The observed results were summarized in Table 2. The cytotoxic effect of these compounds was checked on cancer cell lines using the concentration range between 100 and 0.78 μg/mL to determine the Growth inhibition (GI) GI_{50} and GI_{90} (Table 2). The results indicated that, in MTT cytotoxicity studies, most active compounds are leads as antimicrobials owing to no significant cell toxicity against THP-1, A549 and Panc-1 cell lines at the maximum concentration evaluated.

Table 2 Cytotoxicity of selected compounds in 3 human cancer cell lines[a]

Compound	Cytotoxic profile against human cancer cell lines with SD values					
	THP-1		A549		Panc-1	
	GI_{50}	GI_{90}	GI_{50}	GI_{90}	GI_{50}	GI_{90}
13a	62.9± 0.9	>100	>100	>100	>100	>100
13c	36.4± 0.9	>100	>100	>100	>100	>100
13d	14.6± 0.5	54.3± 0.6	>100	>100	>100	>100
13e	16.2± 0.9	68.3± 0.3	>100	>100	>100	>100
14a	14.2± 0.8	66.4± 0.3	>100	>100	>100	>100
14c	74.1± 0.5	>100	>100	>100	>100	>100
14d	36.7± 0.4	93.1± 0.4	>100	>100	>100	>100
15a	31.1± 0.1	88.9± 0.1	>100	>100	>100	>100
15b	34.9± 0.5	97.6± 0.9	>100	>100	>100	>100
15c	17.4± 0.9	89.7± 0.6	>100	>100	>100	>100
15d	20.9± 0.5	>100	>100	>100	>100	>100
15e	52.9± 0.3	>100	>100	>100	>100	>100
17a	27.9± 0.8	84.3± 0.1	>100	>100	>100	>100
17c	31.2± 0.2	88.1± 0.3	>100	>100	>100	>100
18c	25.1± 0.3	>100	>100	>100	>100	>100
18e	58.7± 0.1	>100	>100	>100	>100	>100
[b]Paclitaxel	0.14± 0.5	5.82± 0.1	0.13± 0.9	5.72± 0.2	0.004± 0.7	0.07± 0.6

[a]GI_{50}/GI_{90} in µg/mL, after 48 h. Human cancer cell lines: THP-1(Human acute monocytic leukemia cell line), A549 from lung adenocarcinoma and Panc-1 from pancreas carcinoma. Results shows that Cell viability >80 % at the highest concentration of 100 µg/mL against Panc-1 and A549 cell lines. [b]Standard anticancer drug and positive control. The GI_{50} values were indicated as mean calculated from three independent experiments.

2.2.4. Selectivity Index

According to the study of Hartkoorn and coworkers on drug susceptibility of TB, the anti-mycobacterial activity was considered to be specific when selectivity index >10.[25] Selectivity index was calculated by dividing GI_{50} for cell lines (THP-1, A549, PANC-1) by the MIC for *in vitro* activity against active/dormant MTB.[26] Growth inhibition concentration, 50% (GI_{50}) was taken as the lowest concentration of compound killing 50% of the cells as obtained from the percentage cytotoxicity curve plotted using Origin8. It was found that all the compounds have selectivity index of >3 at dormant state of MTB. However, compound **15c**, **15e** and **18e** showed comparatively higher selectivity index of greater than 10 against A549 and Panc-1 cell lines (Table 3).

Table 3 Selectivity Index on human cell lines against MTB H37Ra

Com-pound	MTB - Dormant MIC (μg/mL)	Selectivity Index (SI)					
		THP1 GI_{50} (μg/mL)	SI	A549 GI_{50} (μg/mL)	SI	Panc1 GI_{50} (μg/mL)	SI
13a	28.0	62.9	2.2	> 100	> 3.6	> 100	> 3.6
13c	29.9	36.4	1.2	> 100	> 3.3	> 100	> 3.3
13d	27.6	14.7	0.5	> 100	> 3.6	> 100	> 3.6
13e	28.8	16.2	0.6	> 100	> 3.5	> 100	> 3.5
14a	29.9	14.2	0.5	> 100	> 3.3	> 100	> 3.3
14c	24.2	74.1	3.1	> 100	> 4.1	> 100	> 4.1
14d	28.9	36.7	1.3	> 100	> 3.5	> 100	> 3.5
15a	27.5	31.1	1.1	> 100	> 3.6	> 100	> 3.6
15b	29.2	34.9	1.2	> 100	> 3.4	> 100	> 3.4
15c	8.2	17.4	2.1	> 100	> 12.2	> 100	> 12.2
15d	25.8	20.9	0.8	> 100	> 3.9	> 100	> 3.9
15e	5.8	52.9	9.2	> 100	> 17.3	> 100	> 17.3

17a	28.2	27.9	1.0	> 100	> 3.6	> 100	> 3.6
17c	26.9	31.2	1.2	> 100	> 3.7	> 100	> 3.7
18c	7.7	25.0	3.2	> 100	> 12.9	> 100	> 12.9
18e	27.8	58.7	2.1	> 100	> 3.6	> 100	> 3.6
RP	0.043	100.0	> 2325.6	100	> 2325.6	> 100	> 2325.6

RP; Rifampicin, Dormant state: A reversible state of bacterial metabolic shutdown. To establish a selectivity index (SI) by (SI = GI_{50}/MIC). Where GI_{50} *in vitro* cytotoxicity on human cancer cell lines and minimum inhibitory concentration (MIC-TB) – *in vitro* activity against dormant MTB $H_{37}Ra$. If the SI is ≥10, the compound is then investigated further.

For the simultaneous detection of both active as well as dormant stage inhibitors against tubercular bacilli, we used XRMA assay protocol developed earlier[22]. The assay basically follows very similar principle of hypoxia model of dormancy.[27] Under these conditions; the incubation was terminated on 8th day and 12th day respectively to identify active and dormant stage inhibitors.

2.3. Computational studies

2.3.1. Molecular docking study

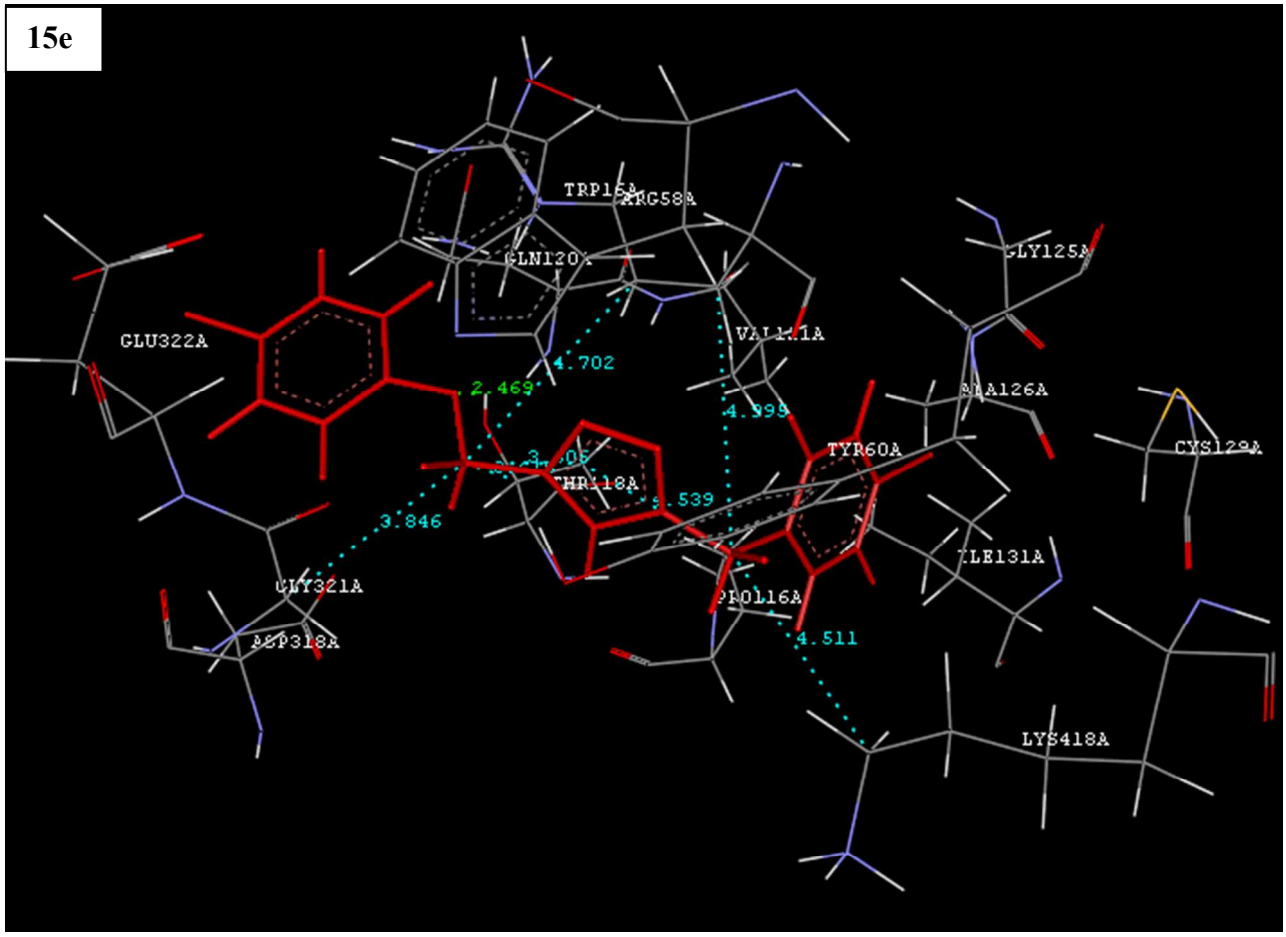
The 1,2,3-triazole derivatives have been reported to inhibit DprE1 (decaprenylphosphoryl-β-D-ribose-2'-epimerase) enzyme of MTB.[28] DprE1 is involved in the biosynthesis of decaprenylphosphoryl-D-arabinose (DPA), as an essential component of the mycobacterial cell wall.[29] In order to investigate the binding interactions, the synthesized compounds **15e** (most active), **14e**, **19b** (moderate active) and **16a** (least active) were docked against DprE1 enzyme (PDB ID:4FDO)[30] using VLife MDS 4.3 package.[31] The binding interactions of docked compounds with DprE1 enzyme is shown in Figure 2. The synthesized compounds have showed binding energy i.e. **15e** (most active, -77.97 kcal/mol), **14e**, **19b** (moderate active -73.79 and -66.58 kcal/mol, respectively) and **16a** (least active, -60.42 kcal/mol). The docking study results for compound **15e** revealed that, it binds to the active site of DprE1 enzyme by forming various hydrophobic bindings with amino acid residues like, ARG58, THR118, GLY321, GLU322 and LYS418 and Van der Waal's interactions with amino acid resides like, TRP16, ARG58, TYR60, PRO116, THR118, GLN120, VAL121, GLY125, ALA126, CYS129, ILE131, ASP318,

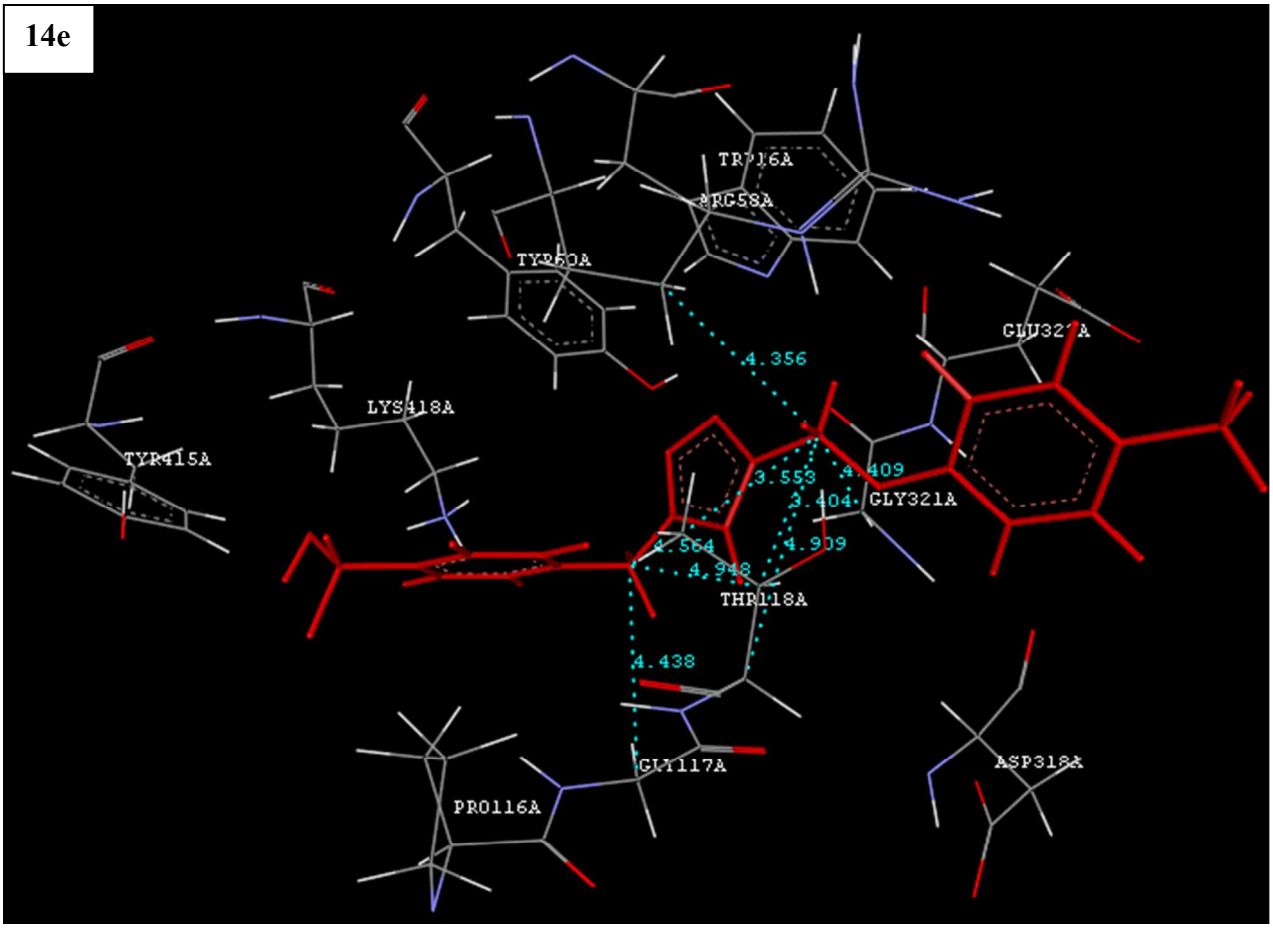
GLY321 and GLY322. The triazole ring was held in active site by forming various interactions with amino acid residues TRP16, ARG58, TYR60 and THR118. The most of the hydrophobic interactions were formed by the presence of active methylene group (-CH_2-). The oxygen atom (-O-) in compound **15e** had shown hydrogen bonding with amino acid THR118 (2.46 Å). The *bromo*- group of compound **15e** had formed the Van der Waal's interctions with amino acid residues GLY125, ALA126 and ILE131. The *chloro*- group had shown the Van der Waal's interctions with amino acid GLU322. Thus, halogen substituents like *chloro*- and *bromo*- groups at R_3 and R_6 positions are important for antitubercular activity.

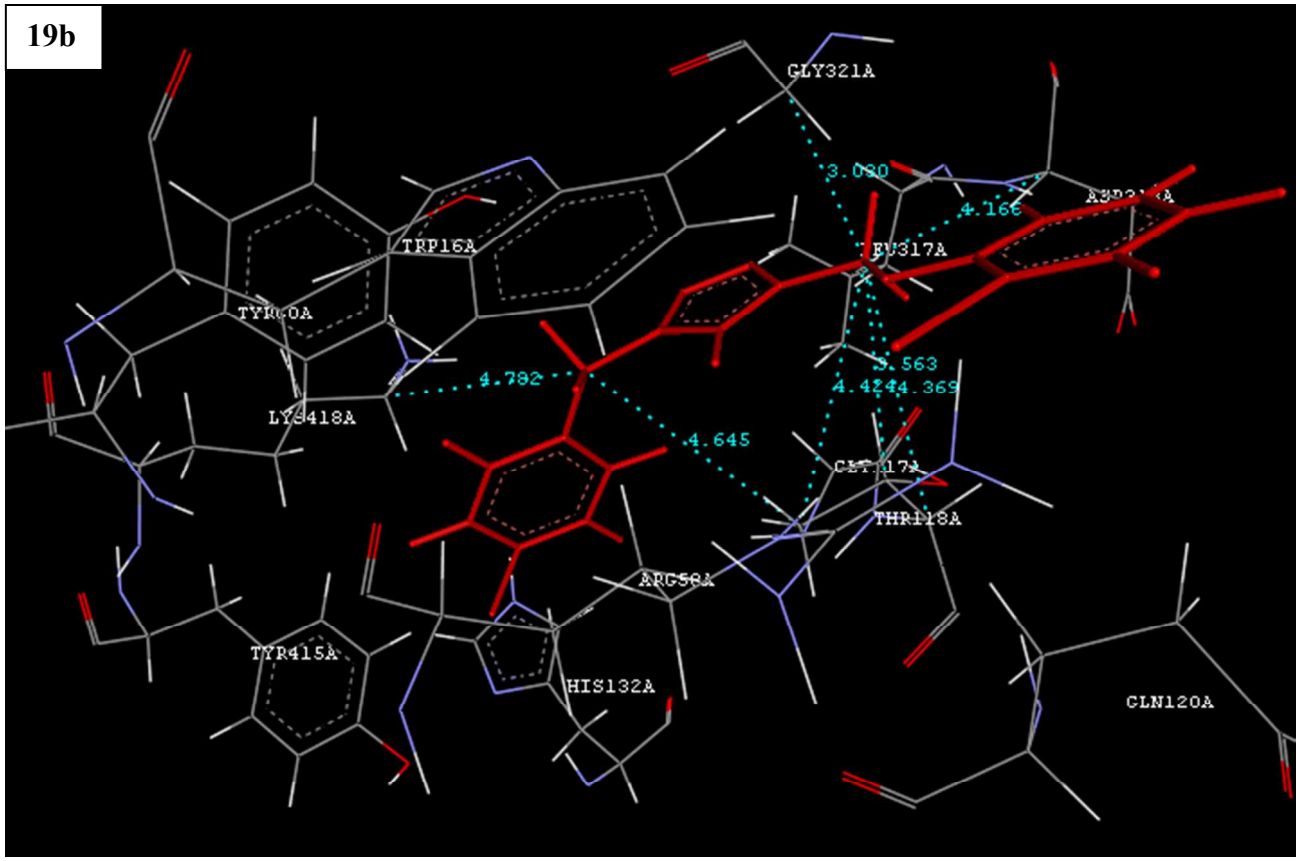
The moderate active compounds **14e** and **19b** were docked to understand the binding interactions against DprE1 enzyme. The compound **14e** were held in active site by forming various hydrophobic interaction with amino acid residues like, ARG58, GLY117, THR118 and GLY321 and Van der Waal's interactions with amino acids TRP16, ARG58, TYR60, PRO116, GLY117, THR118, ILE131, ASP318, GLY321, GLU322 and TYR415. The triazole ring held in active site by forming various interactions with amino acids residues like, TRP16, TYR60, THR118 and GLY321. The amino acid TYR60 had formed two hydrogen bonding with 2^{nd} (2.31 Å) and 3^{rd} (2.45Å) *nitrogen*- atom of triazole ring. The *bromo*- group at R_6 position had shown the Van der Waal's interactions with amino acids PRO116, ILE131 and TYR415. The replacement of *chloro*- group (electron withdrawing group) with –CH_3 group (electron donating group) at R_3 position may led to decrease in binding with active site of enzyme compared to the most active compound **15e**. The compound **19b** had shown the hydrophobic and the Van der Waal's interactions with amino acid residues like, TRP16, ARG58, TYR60, GLY117, THR118, GLN120, ASP318, GLY321, GLU322, TYR415, ALA417 and LYS418. The triazole ring is held in active site by forming various interactions with amino acids TRP16, ARG58, TYR60 and THR118. The *bromo*- and *iodo*- group had formed the Van der Waal's interaction with amino acid GLN120 and GLY117, respectively. The *nitro*- group (-NO_2) did not show any interaction with the active sites of enzyme and this may be the reason for less activty of compound **19b**.

We have also docked the least active compound **16a** against DprE1 enzyme. The compound had shown least binding energy that is -60.42 kcal/mol. The compound had shown good hydrophobic and the Van der Waal's interactions but it did not formed any hydrogen bonding with the enzyme. The various amino acid residues like ARG58, THR118, VAL121, CYS129, ILE131 and ALA417 had formed the hydrophobic interactions with compound **16a**. The amino acids like,

GLY55, GLY57, ARG58, SER59, TYR60, ASN63, ALA64, THR118, VAL121, GLY125, CYS129, ILE131, GLY321, ALA417 and LYS418 showed the Van der Waal's interactions with compound **16a**. The traizole ring held in active site by forming only weak Van der Waal's interactions with amino acids ARG58, THR118, VAL121 and ALA417. The triazole ring and *nitro-* groups ($-NO_2$) had not shown any hydrophobic interaction. Due to poor binding interactions with DprE1 enzyme, compound **16a** may have shown least anti-mycobacterial activity among the synthesized compound. When we compare the docking pose of compounds **15e** (most active) and **16a** (least active), it was found that in compound **16a**, the phenyl ring with R_5= H and R_6= NO_2 substituent was out of the active cavity of enzyme. In compound **15e**, the phenyl ring with R_5= H and R_6= Br was held deep into the active cavity of enzyme and this may be the reason, that the compound **15e** is more active than **16a**. On the basis of activity data and docking results, the compound **15e** has potential to inhibit DprE1 enzyme can be processed further to develop as a lead compound.


15e
TRP15A
ARG58A
GLN120A
GLY125A
GLU322A
VAL111A
4.702
2.469
4.999
TYR60A
CYS129A
3.846
THR118A
5.539
ILE131A
GLY321A
PRO116A
ASP318A
4.511
LYS418A


14e
TRP16A
ARG58A
TYR60A
GLU321A
4.356
LYS418A
TYR415A
3.553
4.409
3.404
GLY321A
4.564
4.909
4.948
THR118A
4.438
GLY117A
PRO116A
ASP318A


19b
GLY321A
3.030
4.166
TRP16A
4.782
4.645
4.369
THR118A
ARG58A
TYR415A
HIS132A
CLN120A

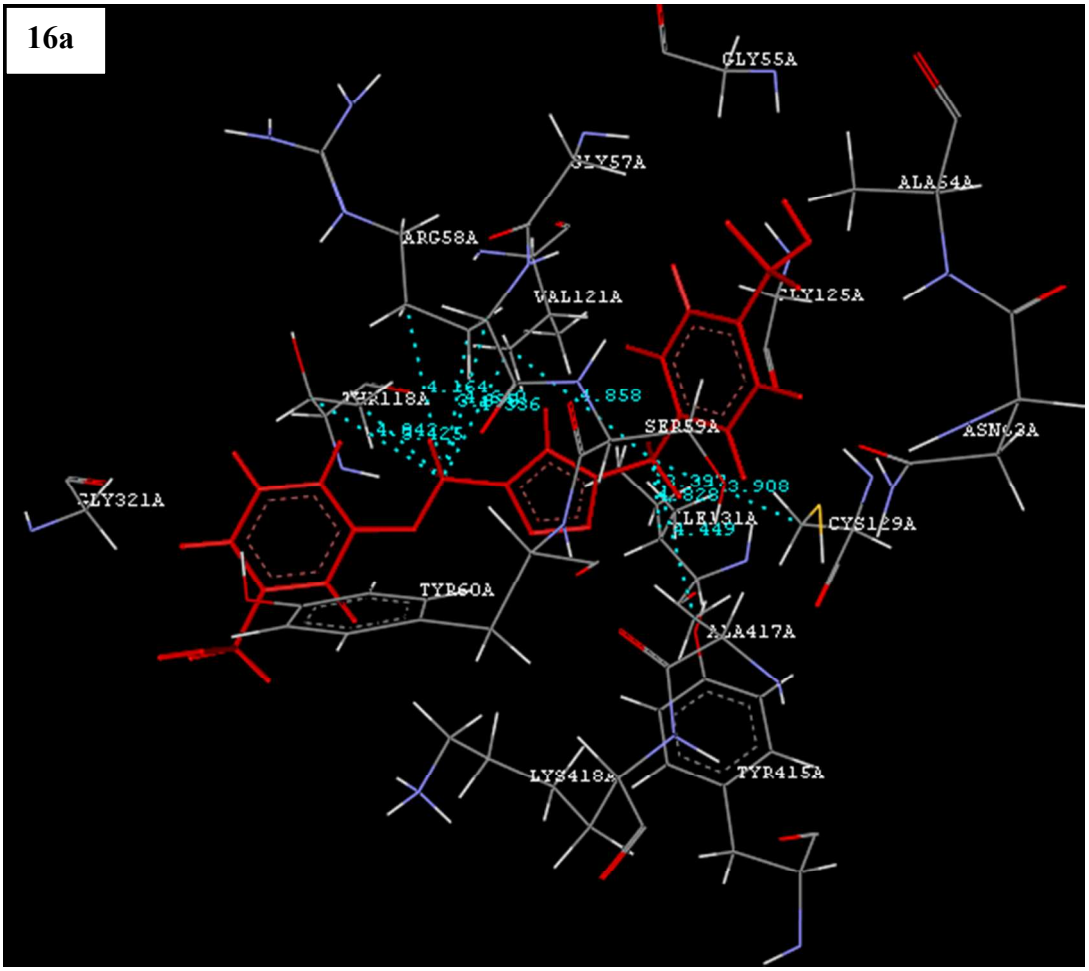


Figure 2. Docking image of compounds **15e** (most active), **14e**, **19b** (moderate active) and **16a** (most inactive). Ligands are shown in red color. Hydrophobic bonds are shown in sky blue color.

2.3.2. *In silico* ADME prediction:

The success of a drug is determined not only by good efficacy but also by an acceptable ADME (absorption, distribution, metabolism and excretion) profile. In the present study, we have calculated molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (miLog *P*), number of hydrogen bond acceptors (n-ON), number of hydrogen bonds donors (n-OHNH), topological polar surface area (TPSA), number of rotatable bonds (n-ROTB) and Lipinski's rule of five[32] using Molinspiration online property calculation toolkit.[33] Absorption (% ABS) was calculated by: % ABS = 109-(0.345×TPSA)[34] Drug-likeness model score (a collective property of physic-chemical properties, pharmacokinetics and

pharmacodynamics of a compound is represented by a numerical value) was computed by MolSoft[35] software.

Table 4 Pharmacokinetic parameters important for good oral bioavailability.

Entry	% ABS	TPSA (A^2)	n-RO TB	MV	MW	miLog *P*	n-ON	n-OH NH	Lipinski's violations	Drug-likeness model score
Rule	-	-	-	-	< 500	≤ 5	< 10	< 5	≤ 1	-
13a	79.40	85.77	6	269.83	310.31	3.29	7	0	0	-0.72
14a	79.40	85.77	6	286.39	324.3	3.74	7	0	0	-0.62
15c	95.21	39.95	5	273.57	334.20	4.69	4	0	0	-0.20
15e	95.21	39.95	5	277.92	378.65	4.82	4	0	0	-0.46
17a	79.40	85.77	6	299.93	358.78	4.35	7	0	0	-0.01
17c	95.21	39.95	5	290.13	348.23	4.89	4	0	0	0.27
18c	95.21	39.95	5	300.64	403.09	5.90	4	0	1	0.21
18e	95.21	39.95	5	304.99	447.54	6.03	4	0	1	-0.06

A computational study of active compounds (compounds showing IC_{50} values ≤ 2.00 μg/mL) **13a**, **14a**, **15c**, **15e**, **17a**, **17c**, **18c** and **18e** was performed for prediction of ADME properties and the value obtained is presented in Table 4. It is observed that, the compounds exhibited a good % ABS (% absorption) ranging from 79.40 to 95.21%. Furthermore, only compounds **18c** and **18e** violated Lipinski's rule of five (miLog *P* ≤ 5). The compounds **13a**, **14a**, **15c**, **15e**, **17a** and **17c** did not violated Lipinski's rule of five. A molecule likely to be developed as an orally active drug candidate should show no more than one violation of the following four criteria: miLog *P* (octanol-water partition coefficient) ≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10 and number of hydrogen bond donors ≤ 5.[36] The larger the value of the drug likeness model score, the higher is also probability that the particular molecule will be active. All the tested compounds followed the criteria for orally active drug and therefore, these compounds may have a good potential for eventual development as oral agents.

3. Experimental

All the solvents and reagents were purchased from commercial suppliers, Spectrochem Pvt. Ltd., Rankem India Ltd. and Sigma Aldrich which was used without further purification. The progress of each reaction was monitored by ascending thin layer chromatography (TLC) using TLC aluminum sheets, silica gel F_{254} precoated, Merck, Germany and locating the spots using UV light as the visualizing agent or Iodine vapors. Melting points were taken in open capillary method and are uncorrected. ^{1}H NMR spectra were recorded ($CDCl_3$/DMSO-d_6) on Bruker Avance 200 NMR Spectrometer. ^{13}C NMR and DEPT 135 spectra were recorded ($CDCl_3$/DMSO-d_6) on Bruker Avance 200 NMR Spectrometer and JEOL ECX 400 NMR Spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. The splitting pattern abbreviations are designed as singlet (s); doublet (d); double doublet (dd); triplet (t); quartet (q) and multiplet (m).The mass spectra were recorded on Q-TOF micromass (YA-105) spectrometer in the ESI (Electrospray Ionization) modes.

3.1. General procedure for the synthesis of 1-(prop-2-ynyloxy)benzene or substituted 1-(prop-2-ynyloxy)benzene (1-7):

To the stirred solution of phenol or substituted phenol (20 mmol) in *N,N*-dimethylformamide (DMF) (20 mL), K_2CO_3 (24 mmol) was added. The reaction mixture was stirred at room temperature for 30 minutes, which results into the corresponding oxyanion. To this mixture, propargyl bromide (20 mmol) was added and stirred for 2 h. The progress of the reaction was monitored by TLC using ethyl acetate:hexane as a solvent system. The reaction was quenched by crushed ice. In case of solid product, it was filtered and the obtained crude solid product was crystallized using ethanol. The crystallized products were taken for next step. When the products are liquid, it has been extracted in ethyl acetate (20 mL × 3). The combined organic layers were dried over $MgSO_4$. The solvent was removed under a reduced pressure and used for further reaction without purification.

3.2. General experimental procedure for the Synthesis of substituted 1-benzyl-4-(phenoxymethyl)-1H-1,2,3-triazole (13-19):

To the stirred solution of (prop-2-yn-1-yloxy)benzenes **1-7** (0.5 mmol), substituted benzyl azide **8-12** (0.5 mmol) and copper diacetate ($Cu(OAc)_2$) (20 mole %) in *t*-BuOH-H_2O (3:1, 8 mL) were added and the resulting mixture was stirred at room temperature for 19-27 h. The progress of the reaction was monitored by TLC using ethyl acetate:hexane as a solvent system. The reaction mixture was quenched with crushed ice and extracted with ethyl acetate (2×15 mL). The organic extracts were washed with brine solution (2×15 mL) and dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure to afford the corresponding crude compounds. The obtained crude compounds were recrystallized using ethanol.

3.2.1. 1-(4-Nitrobenzyl)-4-(phenoxymethyl)-1*H*-1,2,3-triazole (13a).

The compound **13a** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **8** and alkyne **1** in 20 h with 89% yield, mp: 96-98 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.23 (s, N-CH_2), 5.65 (s, O-CH_2), 6.93-7.02 (m, 3H, Ar *H*), 7.29-7.44 (m, 4H, Ar *H*), 7.61 (s, 1H, triazole *H*), and 8.20-8.27 (m, 2H, Ar *H*). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 53.2, 62, 114.8, 121.4, 124.4, 128.5, 129.6 and 141.5. HRMS calculated $[M+Na]^+$ for $C_{16}H_{14}N_4O_3Na$: 333.0958, found: 333.0967.

3.2.2. 1-(3-Nitrobenzyl)-4-(phenoxymethyl)-1*H*-1,2,3-triazole (13b).

The compound **13b** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **9** and alkyne **1** in 20 h with 90% yield, mp: 98 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.23 (s, N-CH_2), 5.65 (s, O-CH_2), 6.94-7.01 (m, 3H), 7.24-7.35 (m, 2H), 7.57-7.63 (m, 3H), and 8.17-8.26 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 53.2, 62, 114.8, 121.4, 122.9, 123.8, 129.6, 130, 133.9. HRMS calculated $[M+H]^+$ for $C_{16}H_{15}N_4O_3$: 311.1160 found: 311.1155, $[M+Na]^+$ for $C_{16}H_{14}N_4O_3Na$: 333.0980, found: 333.0980.

3.2.3. 1-(4-Chlorobenzyl)-4-(phenoxymethyl)-1*H*-1,2,3-triazole (13c).

The compound **13c** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **10** and alkyne **1** in 21 h with 88% yield, mp: 90-92 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.19 (s, N-CH_2), 5.49 (s, O-CH_2), 6.94-7.00 (m, 3H), 7.12-7.17 (d, 2H), 7.29-7.33 (m, 2H) and 7.47-7.53

(m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 53.5, 62.1, 114.8, 121.3, 122.6, 129.4, 129.6, 133, 134.9, 145 and 158.2. HRMS calculated $[M+H]^+$ for $C_{16}H_{15}N_3OCl$: 300.0898, found: 300.0905, $[M+Na]^+$ for $C_{16}H_{14}N_3OClNa$: 322.0718, found: 322.0725.

3.2.4. 1-(4-Bromobenzyl)-4-(phenoxymethyl)-1*H*-1,2,3-triazole (13e).

The compound **13e** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **12** and alkyne **1** in 21 h with 89% yield, mp: 103-104 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.19 (s, N-CH_2), 5.49 (s, O-CH_2), 6.94-7.00 (m, 3H), 7.12-7.17 (m, 2H), 7.28-7.33 (m, 2H), and 7.47-7.53 (m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 53.6, 62.1, 114.8, 121.4, 122.6, 123.1, 129.7, 129.8, 132.4, 133.6, 145.1 and 158.2. HRMS calculated $[M+Na]^+$ for $C_{16}H_{14}N_3OBrNa$: 366.0212, found: 366.0212.

3.2.5. 1-(4-Nitrobenzyl)-4-((*p*-tolyloxy)methyl)-1*H*-1,2,3-triazole (14a).

The compound **14a** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **8** and alkyne **2** in 19 h with 90% yield, mp: 90-91 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 17 (s, 3H), 5.22 (s, N-CH_2), 5.65 (s, O-CH_2), 6.94-7.01 (m, 3H), 7.29-7.42 (m, 3H), 7.63 (s, 1H, triazole *H*), and 8.19-8.24 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 31.1, 53.3, 62, 114.8, 121.5, 123, 124.4, 128.7, 129.7, 141.7, 145.4, 148.1, and 158.1.

3.2.6. 1-(4-Chlorobenzyl)-4-((*p*-tolyloxy)methyl)-1*H*-1,2,3-triazole (14c).

The compound **14c** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **10** and alkyne **2** in 21 h with 92% yield, mp: 84-85 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 2.17 (s, 3H), 5.22 (s, N-CH_2), 5.65 (s, O-CH_2), 6.94-7.01 (m, 3H), 7.29-7.42 (m, 3H), 7.64 (s, 1H, triazole *H*), and 8.19-8.24 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 29.8, 53.6, 62.1, 114.8, 121.4, 122.7, 129.4, 129.5, 129.6, 133.1, 134.9, 145 and 158.2.

3.2.7. 1-(3-Chlorobenzyl)-4-((*p*-tolyloxy)methyl)-1*H*-1,2,3-triazole (14d).

The compound **14d** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **11** and alkyne **2** in 22 h with 91% yield, mp: 70 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 2.17 (s, 3H), 5.20 (s, N-CH_2), 5.67 (s, O-CH_2), 6.95-6.99 (m, 3H), 7.18-7.33 (m, 4H), 7.42-7.44 (d, 1H), and

7.64 (s, 1H, triazole *H*). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 31.1, 51.6, 62.1, 114.9, 121.4, 123.1, 127.8, 129.8, 130, 130.4, 132.4, 133.6, 144.7 and 158.3.

3.2.8. 1-(4-Bromobenzyl)-4-((*p*-tolyloxy)methyl)-1*H*-1,2,3-triazole (14e).

The compound **14e** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **12** and alkyne **2** in 21 h with 93% yield, mp: 106 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 2.17 (s, 3H), 5.18 (s, N-CH_2), 5.46 (s, O-CH_2), 6.93-6.97 (m, 3H), 7.11-7.15 (m, 2H), 7.32 (m, 1H), and 7.47-7.54 (m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 29.8, 53.5, 62.1, 114.8, 121.4, 122.7, 123.1, 129.7, 129.8, 132.4, 133.6, 145 and 158.2.

3.2.9. 1-(4-Nitrobenzyl)-4-((4-chlorophenoxy)methyl)-1*H*-1,2,3-triazole (15a).

The compound **15a** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **8** and alkyne **3** in 22 h with 90% yield, mp: 94 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.25 (s, N-CH_2), 5.53 (s, O-CH_2), 7.21-7.28 (m, 2H), 7.34-7.48 (m, 4H) 7.61 (s, 1H, triazole *H*) and 7.81-7.85 (m, 2H). NMR (100 MHz, $CDCl_3$, δ ppm): 53.6, 62.3, 116.1, 122.6, 123, 126.2, 129.4, 129.7, 132.4, 133.4, 144.5 and 156.7. HRMS calculated $[M+H]^+$ for $C_{16}H_{14}N_4O_3Cl$: 345.0748, found: 345.0739, $[M+Na]^+$ for $C_{16}H_{13}N_4O_3Cl$ Na: 367.0568 found: 367.0559.

3.2.10. 1-(3-Nitrobenzyl)-4-((4-chlorophenoxy)methyl)-1*H*-1,2,3-triazole (15b).

The compound **15b** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **9** and alkyne **3** in 23 h with 89% yield, mp: 96 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 5.21 (s, N-CH_2), 5.67 (s, O-CH_2), 6.91-6.93 (m, 2H), 7.24-7.29 (m, 2H), 7.60-7.65 (m, 3H), 8.19 (s, 1H, triazole *H*) and 8.24-8.27 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 53.2, 62.2, 116.1, 122.8, 122.9, 123.9, 126.3, 129.5, 130.4, 133.9, 136.5, 148.6 and 156.7. HRMS calculated $[M+H]^+$ for $C_{16}H_{14}N_4O_3Cl$: 345.0749, found: 345.075, $[M+Na]^+$ for $C_{16}H_{13}N_4O_3ClNa$: 367.0571 found: 367.0567.

3.2.11. 1-(4-Chlorobenzyl)-4-((4-chlorophenoxy)methyl)-1*H*-1,2,3-triazole (15c).

The compound **15c** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **10** and alkyne **3** in 23 h with 88% yield, mp: 80 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.18 (s, N-CH_2), 5.51 (s, O-CH_2), 6.91-6.93 (m, 2H), 7.16-7.18 (m, 2H), 7.24-7.29 (m, 2H) and 7.52-7.54

(m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): δ 53.6, 62.3, 116.2, 126.3, 129.5, 132.9, 135 and 156.8. HRMS calculated $[M+H]^+$ for $C_{16}H_{14}N_3OCl_2$: 334.0508, found: 334.0504, for $[M+Na]^+$ $C_{16}H_{13}N_3OCl_2Na$: 356.0358 found: 356.0324.

3.2.12. 1-(3-Chlorobenzyl)-4-((4-chlorophenoxy)methyl)-1*H*-1,2,3-triazole (15d).

The compound **15d** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **11** and alkyne **3** in 24 h with 89% yield, mp: 104 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.21 (s, N-CH_2), 5.67 (s, O-CH_2), 6.91-6.93 (m, 2H), 7.24-7.29 (m, 2H), 7.60-7.65 (m, 3H), 8.19 (s, 1H, triazole *H*) and 8.24-8.27 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 51.8, 62.3, 116.2, 126.3, 127.8, 129.5, 129.8, 130.5, 130.6, 132.3, 133.6 and 156.8. HRMS calculated $[M+H]^+$ for $C_{16}H_{14}N_3OCl_2$: 334.0508, found: 334.0504, $[M+Na]^+$ for $C_{16}H_{13}N_3OCl_2Na$: 356.0358 found: 356.0324.

3.2.13. 1-(4-Bromobenzyl)-4-((4-chlorophenoxy)methyl)-1*H*-1,2,3-triazole (15e).

The compound **15e** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **12** and alkyne **3** in 24 h with 90% yield, mp: 98 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 5.18 (s, N-CH_2), 5.51 (s, O-CH_2), 6.91-6.93 (m, 2H), 7.16-7.18 (m, 2H), 7.24-7.29 (m, 2H) and 7.52-7.54 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 53.6, 62.3, 116.1, 122.6, 123, 126.2, 129.4, 129.7, 132.4, 133.4, 144.5 and 156.7. HRMS calculated $[M+H]^+$ for $C_{16}H_{14}N_3OClBr$: 379.9948, found: 379.9948, for $[M+Na]^+$ $C_{16}H_{13}N_3OClBrNa$: 401.9768, found: 401.9767.

3.2.14. 4-((3-Nitrophenoxy)methyl)-1-(4-chlorobenzyl)-1*H*-1,2,3-triazole (16c).

The compound **16c** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **10** and alkyne **4** in 26 h with 89% yield, mp: 123-124 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.25 (s, N-CH_2), 5.53 (s, O-CH_2), 7.21-7.28 (m, 2H), 7.34-7.48 (m, 4H) 7.61 (s, 1H, triazole *H*) and 7.81-7.85 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 53.6, 62.4, 109.6, 116.3, 121.6, 122.9, 129.4, 129.5, 130.2, 132.9, 135, 143.6, 149.2 and 158.7. HRMS calculated $[M+H]^+$ for $C_{16}H_{14}N_4O_3Cl$: 345.0748, found: 345.0739, $[M+Na]^+$ for $C_{16}H_{13}N_4O_3Cl$ Na: 367.0568 found: 367.0559.

3.2.15. 4-((3-Nitrophenoxy)methyl)-1-(3-chlorobenzyl)-1*H*-1,2,3-triazole (16d).

The compound **16d** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **11** and alkyne **4** in 25 h with 88% yield, mp: 86 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.25 (s, N-CH_2), 5.68 (s, O-CH_2), 7.21-7.44 (m, 6H), 7.33 (bs, 1H, triazole *H*) and 7.81-7.83 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 51.8, 62.4, 109.6, 116.3, 121.6, 127.7, 130, 130.1, 130.5, 130.6, 132.2, 133.7, 149.2 and 158.7. HRMS calculated $[M+H]^+$ for $C_{16}H_{14}N_4O_3Cl$: 345.0748, found: 345.0739, $[M+Na]^+$ for $C_{16}H_{13}N_4O_3Cl$ Na: 367.0568 found: 367.0559.

3.2.16. 4-((3-Nitrophenoxy)methyl)-1-(4-bromobenzyl)-1*H*-1,2,3-triazole (16e).

The compound **16e** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **12** and alkyne **4** in 26 h with 87% yield, mp: 131-132 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 5.23 (s, N-CH_2), 5.50 (s, O-CH_2), 7.14-7.16 (m, 2H), 7.29-7.36 (d, 1H), 7.40-7.51 (m, 3H), 7.63 (bs, 1H, triazole *H*) and 7.80-7.83 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 53.8, 62.5, 109.6, 116.3, 121.5, 123.2, 129.8, 130, 132.4, 133.3, 149.2 and 158.7. HRMS calculated $[M+H]^+$ for $C_{16}H_{14}N_4O_3Br$: 389.0250, found: 389.0257, $[M+Na]^+$ for $C_{16}H_{13}N_4O_3Br$ Na: 411.0063 found: 411.0070.

3.2.17. 4-((4-Chloro-3-methylphenoxy)methyl)-1-(3-nitrobenzyl)-1*H*-1,2,3-triazole (17b).

The compound **17b** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **9** and alkyne **5** in 26 h with 88% yield, mp: 106-108 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 2.32 (s, 3H), 5.17 (s, N-CH_2), 5.65 (s, O-CH_2), 6.73-6.84 (m, 2H), 7.20-7.22 (m, 1H), 7.57-7.63 (m, 3H) and 8.16-8.23 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 20.4, 53.3, 62.3, 113.4, 117.4, 123, 123.9, 126.6, 129.8, 130.5, 134, 136, 137.3, 145.1, 148.6 and 156.6. HRMS calculated $[M+H]^+$ for $C_{17}H_{16}N_4O_3Cl$: 359.0905, found: 359.0903, $[M+Na]^+$ for $C_{17}H_{15}N_4O_3ClNa$: 381.0725, found: 381.0720.

3.2.18. 4-((4-Chloro-3-methylphenoxy)methyl)-1-(4-chlorobenzyl)-1*H*-1,2,3-triazole (17c).

The compound **17c** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **10** and alkyne **5** in 25 h with 90% yield, mp: 108-110 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 2.37 (s, 3H), 5.18 (s, N-CH_2), 5.55 (s, O-CH_2), 6.75-6.88 (m, 2H), 7.20-7.30 (m, 3H), 7.37-7.42 (m, 2H) and 7.60 (bs, 1H, triazole *H*). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 20.5, 53.7, 62.3, 113.4,

117.5, 126.6, 129.6, 129.7, 129.8, 133, 135, 137.3 and 156.7. HRMS calculated $[M+H]^+$ for $C_{17}H_{16}N_3OCl_2$: 348.0664, found: 348.0664, $[M+Na]^+$ for $C_{17}H_{15}N_3OCl_2Na$: 370.0484, found: 370.0486.

3.2.19. 4-((2,4,6-Trichlorophenoxy)methyl)-1-(3-nitrobenzyl)-1*H*-1,2,3-triazole (18b).

The compound **18b** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **9** and alkyne **6** in 27 h with 89% yield, mp: 170 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.21 (s, N-CH_2), 5.70 (s, O-CH_2), 7.31-7.34 (m, 2H), 7.56-7.66 (m, 2H), 7.87 (bs, 1H) and 8.19-8.27 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 53.2, 66.3, 122.9, 123.8, 128.8, 130.2, 130.3, 133.9, 136.6, 148.5 and 149.4. HRMS calculated $[M+H]^+$ for $C_{16}H_{12}N_4O_3Cl_3$: 404.9802, found: 404.9802, $[M+Na]^+$ for $C_{16}H_{11}N_4O_3Cl_3Na$: 426.9622, found: 426.9622.

3.2.20. 4-((2,4,6-Trichlorophenoxy)methyl)-1-(4-chlorobenzyl)-1*H*-1,2,3-triazole (18c).

The compound **18c** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **10** and alkyne **6** in 27 h with 87% yield, mp: 106 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 5.20 (s, N-CH_2), 5.53 (s, O-CH_2), 7.20-7.37 (m, 6H) and 7.63 (s, 1H, triazole *H*). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 53.6, 66.6, 123.4, 128.9, 129.5, 130, 130.4, 133.1, 135, 143.9 and 149.6. HRMS calculated $[M+H]^+$ for $C_{16}H_{12}N_3OCl_4$: 403.9628, found: 403.9704, $[M+Na]^+$ for $C_{16}H_{11}N_3OCl_4Na$: 425.9526, found: 425.9526.

3.2.21. 4-((2,6-Diiodo-4-nitrophenoxy)methyl)-1-(4-nitrobenzyl)-1*H*-1,2,3-triazole (19a).

The compound **19a** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **8** and alkyne **7** in 26 h with 86% yield, mp: 176 °C. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ_H 5.21 (s, N-CH_2), 5.70 (s, O-CH_2), 7.31-7.34 (m, 2H), 7.60-7.63 (m, 2H), 7.87 (bs, 1H) and 8.19-8.26 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$, ppm): δ 53.2, 66.2, 122.8, 123.8, 128.8, 130.2, 130.3, 133.9 and 136.6.

3.2.22. 4-((2,6-Diiodo-4-nitrophenoxy)methyl)-1-(4-bromobenzyl)-1*H*-1,2,3-triazole (19b).

The compound **19b** was obtained *via* 1,3-dipolar cycloaddition reaction between azide **12** and alkyne **7** in 24 h with 89% yield, mp: 146-148 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.26 (s, N-CH_2), 5.54 (s, O-CH_2), 7.17-7.21 (m, 3H), 7.51-7.55 (m, 2H) and 8.64 (s, 2H). ^{13}C NMR (50

MHz, $CDCl_3$, δ ppm): 53.2, 66.2, 122.8, 128.8, 130.2, 130.3, 133.9, 136.6, 148.5 and 149.4. HRMS calculated $[M+H]^+$ for $C_{16}H_{12}N_4O_3BrI_2$: 640.8197, found: 640.8197, $[M+Na]^+$ for $C_{16}H_{11}N_4O_3BrI_2Na$: 662.8017, found: 662.8017.

3.3. Experimental protocol for biological activity

3.3.1. Antitubercular testing using XRMA protocol

The compounds **13–19** were evaluated for their *in vitro* effects against dormant phase *M. tuberculosis* H37Ra using XRMA protocol.[22] All experiments were performed in triplicates and IC_{50} and MIC values were calculated from their dose–response curves.

% Inhibition = 100-(A1-Blank)/(A2-Blank)*100.

where,

A1 - Culture absorbance at 470 nm in the presence of the compound after addition of menadione

A2 - Culture absorbance at 470 nm (DMSO solvent control) after addition of menadione

Blank - Culture absorbance at 470 nm of the respective data points before addition of XTT/menadione.

3.3.2. General procedure for evaluation of DPPH radical scavenging activity

The hydrogen atom or electron donation ability of the some compounds were measured from the bleaching of the purple colored methanol solution of 1,1-diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 mL of various concentrations of the test compounds (5, 10, 25, 50 and 100 μg/mL) in methanol was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. The reaction mixture was incubated at 37 °C. The scavenging activity on DPPH was determined by measuring the absorbance at 517 nm after 30 min. All tests were performed in triplicate and the mean values were entered. The percent of inhibition (I %) of free radical production from DPPH was calculated by the following equation

% of scavenging = $[(A_{control} - A_{sample})/(A_{sample} \times 100)]$.

Where, $A_{control}$ is the absorbance of the control (DPPH radical without test sample)

A_{sample} is the absorbance of the test sample (DPPH radical with test sample). The control contains all reagents except the test samples.

3.4. Cytotoxic activity assay

3.4.1. Anti-proliferative activity against THP-1, A549 and PANC-1 cell lines using MTT assay [37]

Effect of the compounds 13–19 were evaluated on cell growth in acute monocytic leukemia cell line THP-1, lung A549 adenocarcinoma, pancreatic PANC-1 adenocarcinoma cell line. In vitro cytotoxicity of all test samples were assessed on a panel of three human cancer cell lines using a standard MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay[24] for measuring cellular proliferation. Each concentration was tested in triplicates in a single experiment. The viability and growth in the presence of test material is calculated by following formula:

% cytotoxicity = (Average of control-Average of compound) / (Average of control-Average of blank) X 100).

Where control is culture medium with cells and DMSO and blank is culture medium without cells.

IC_{50} and MIC value was calculated by plotting the percentage survival versus the concentrations, using OriginPro Software.

3.4.2. Selectivity Index[38]

The selectivity index (SI) was calculated by dividing IC_{50} for human cancer cell lines (THP-1, A549, PANC-1) by the MIC for *in vitro* activity against active/dormant MTB H37Ra; if the SI is ≥10, the compound is then investigated further.

Selectivity index (SI) = (IC50 - *in vitro* cytotoxicity on human cancer cell lines) / (minimum inhibitory concentration (MIC-TB) *in vitro* activity against MTB H37Ra.

3.4.3. Molecular docking study

Molecular docking study was performed using VLife MDS 4.3 package. With this purpose, crystal structure of DprE1 (decaprenylphosphoryl-β-D-ribose-2'-epimerase) enzyme of MTB (PDB ID:4FDO) was obtained from the Protein Data Bank in order to prepare protein for docking study. Docking procedure was followed using the standard protocol implemented in VLife MDS 4.3 package and the compounds were docked against three dimensional structure of DprE1 enzyme.

4. Conclusions

We have synthesized 1,4-disubstituted-1,2,3-triazole derivatives from commercially available starting materials. Out of 31 screened compounds, **13a**, **13c**, **13d**, **13e**, **14a**, **14c**, **14d**, **15a**, **15b**, **15c**, **15d**, **15e**, **17a**, **17c**, **18c** and **18e** displays antitubercular activity with MIC ranging from 5.8 to 29.9 μg/mL against the strain MTB H37Ra. Among the above mentioned active compounds, the more potent antitubercular compound **15e** having *chloro*- group at R_3 and *bromo*- group at R_6 position of phenyl ring shows MIC values 5.8 μg/mL. Furthermore, the compounds **15c** and **18c** with MIC 8.2 and 7.7 μg/mL is endowed with better potency, being more active than remaining compounds. Further, the active antitubercular compounds were evaluated for their cytotoxic effect against three human cancer cell lines THP-1, A549 and Panc-1. The cytotoxic study revealed that the compounds **13a**, **13c**, **13d**, **13e**, **14a**, **14c**, **14d**, **15a**, **15b**, **15c**, **15d**, **15e**, **17a**, **17c**, **18c** and **18e** shown any cytotoxicity against A549 and Panc-1cell lines at the maximum concentration evaluated. Compound **13a**, **14a**, **14b**, **15e** and **17c** shows potential antioxidant activity as compared with standards BHT and ascorbic acid. In addition to this, molecular docking study of these synthesized triazole derivatives have a high affinity towards the active site of DprE1 enzyme which provides a strong platform for new structure-based design efforts. Furthermore, analysis of the ADME parameters for synthesized compounds showed good drug like properties and can be developed as oral drug candidate. Thus, suggesting that compounds from present series **13a**, **14a**, **14b**, **15c**, **15e**, **17a**, **17c**, **18c** and **18e** can be further optimized and developed as a lead molecule.

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