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Efficient synthesis and in vitro antitubercular activity of 1,2,3-triazoles as inhibitors of *Mycobacterium tuberculosis*

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ABSTRACT

Efficient and rapid synthesis of 1,2,3-triazole derivatives has been achieved via Huisgen's 1,3-dipolar cycloaddition between alkyl/arylazides and diethyl/dimethyl acetylenedicarboxylate in excellent yields under solvent-free conditions. The environmentally friendly solvent-free protocol overcomes the limitations associated with the prevailing time-consuming solution phase protocols and affords the triazoles just in 1–3 min. In vitro antitubercular activity of these triazoles was screened against *Mycobacterium tuberculosis* $H_{37}Rv$ strain. Four of the compounds showed MIC in the range of 1.56–3.13 μg/mL proving their potential activity.

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* and remains as a leading cause of mortality worldwide. Currently, among the infected individuals approximately eight million develop active TB, and almost two million die from this disease. Of the new TB cases reported, 95% occur in developing countries every year. World Health Organization (WHO) estimates that about one-third of the world's population harbors latent infection of TB and thus declared it as a global emergency.[1–3] The mortality and spread of this disease has been further aggravated by its synergy with Human Immunodeficiency Virus (HIV).[4,5] By destroying the two most important cells to the containment of tubercle bacilli (macrophages and CD4-receptor-bearing lymphocytes), HIV vigorously promotes the progression of recent or remotely acquired TB infection to active disease.[6] The deadly synergy between TB and HIV has led to a quadrupling of TB cases in several African and Asian countries. In fact it is estimated that eleven million adults are now co-infected with HIV and tuberculosis worldwide.[3] Thus both the current HIV pandemic and multidrug-resistant *M. tuberculosis* have emerged as major obstacles for treatment and public health control of tuberculosis.[1,2]

Chemotherapy of TB started in the 1940s and since then some agents have been discovered, including *p*-aminosalicylic acid (PAS), isoniazid (INH), pyrazinamide (PZA), cycloserine, ethionamide, rifampicin, and ethambutol.[7] However, the emergence of multiple drug-resistant (MDR) TB revealed the urgent need for new classes of antitubercular drugs.[8]

Internationally efforts are now being made to develop new antitubercular agents.[3] Several studies have indicated the potential of analogues of isoniazid[9,10] and heterocyclic compounds, such as BM212,[11,12] as tuberculostatic agents. In pursuit of this goal, our research efforts herein have been directed toward the discovery of new chemical entities that are effective as antituberculosis agents.

Organic compounds having triazole nucleus inducing antibacterial,[13–15] antifungal,[16] antiviral,[17] antimicrobial, anti inflammatory and analgesic[18] activity have been explored. Especially, 1,2,3-triazole derivatives are found to inhibit tumor proliferation, invasion, metastasis[19] and anti-HIV activity.[20–26] With regard to the antitubercular activity, 1,2,3-triazole derivatives have been found to show promising activity profile.[27] In the present study we report an elegant synthesis of hitherto unknown 1,2,3-triazole derivatives and their antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$.

Owing to these significant features, a number of protocols for the synthesis of 1,2,3-triazole compounds have been developed.[28] Among them, the most elegant and useful approach is the Huisgen's 1,3-dipolar cycloaddition of azides and alkynes.[29]

From perusal of literature, it is understood that solvent-free synthesis of triazoles via azide–alkyne cycloaddition is relatively new. In view of the report of Abu-Orabi et al.[30] on the long time-consuming triazole synthesis, Kenneth Savin et al.[31] have accomplished a facile, solvent-free cycloaddition of DMADC (dimethyl

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acetylenedicarboxylate) with benzyl azide in a minute using microwave irradiation. We, in the present study understand that there is no need for microwave irradiation in this regard, that is we have achieved an instantaneous synthesis of triazole derivatives in excellent yields by conventional heating over a hot-plate (80–120 °C). This substantiates that the increase in the rate of heating is crucial rather than the source of heating. Thus, in this paper we describe a facile methodology to synthesize the 1,2,3-triazole derivatives and their screening for in vitro antitubercular activity against *M. tuberculosis* $H_{37}Rv$ strain.

At the outset, a mixture of benzylazide (**1a**) and DEADC (diethyl aceylenedicarboxylate) in THF at refluxing temperature afford the triazole (**2a**) in 80% yield in 7 h (Scheme 1). For further optimization, the reaction is performed in different solvents at refluxing conditions (Table 1, entries 1–4). Solvent screening indicates that toluene (entry 6) is the right choice giving 88% of **2a** in 3 h.

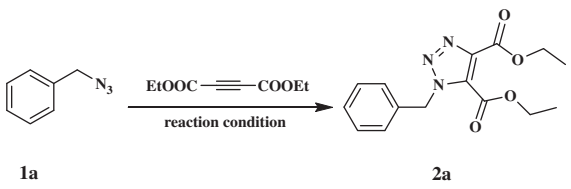
In our next attempt, the reaction is conducted in absence of solvent but with excess of DEADC at 120 °C (entry 5). This results in a very remarkable observation, that is 96% of **2a** is obtained in just one minute, the pure form of which was obtained after column chromatographic technique. A modification of the method wherein **1a** and DEADC in 1:1 mol equiv (entry 6) at 120 °C for just a minute gives **2a** (96%) as a pure product. Other triazole derivatives (**2b–j** and **3a–j**) are synthesized by this optimized condition (Scheme 2) with a variety of azides and DEADC/DMADC (Table 2). Except **2a**,[32] **3a**,[33] **3b**,[34] **3i**[33] and **3j**,[35] all the other fifteen triazoles are new compounds.

The synthesized triazoles (**2a–j** and **3a–j**) are completely characterized by IR and NMR (1D and 2D) spectral techniques.

The ^1H NMR spectrum of a representative triazole, **2a** shows two triplets at 1.26 and 1.38 ppm with $J = 7.2$ Hz for the methyl protons and two quartets at 4.32 and 4.41 ppm with $J = 7.2$ Hz for the methylene protons of the $-OCH_2CH_3$ groups. The N-methylene protons show singlet at 5.79 ppm and the five aromatic protons appeared as multiplet around 7.23–7.34 ppm. In ^{13}C NMR, the signal of two ester carbonyl groups appeared at 160.0 and 158.4 ppm, two OCH_2 carbons at 61.7 and 62.7 ppm, two CH_3 carbons at 13.6 and 14.0 ppm, one N-methylene carbon at 53.6 ppm, three quaternary carbons at 140.3, 133.9 and 129.8 ppm, and three aromatic carbons at 128.7, 128.6 and 127.8 ppm. The IR spectrum showed the band due to the carbonyl group at 1732 cm^{-1}.

All the synthesized compounds were screened for their in vitro antimycobacterial activity against MTB in Middlebrook 7H11 agar medium supplemented with OADC by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in duplicate.[36] The MIC is defined as the minimum concentration of compounds required to completely inhibit the bacterial growth. The MIC values of the synthesized compounds determined in duplicate at 7.4 pH, along with that of standard drugs are listed in Table 3.

Out of fourteen screened compounds, **2a**, **2b**, **2c**, **2d**, **2e**, **3b**, **3c**, **3d** and **3e** display antitubercular activity with MIC ranging from 1.56 to 12.5 μg/mL against the strain *Mycobacterium tuberculosis* $H_{37}Rv$ (Table 3). The remaining compounds, **2i**, **2j**, **3i** and **3j** having alkyl substitution with MIC values >25 μg/mL do not exhibit antitubercular activity. Among the above mentioned active compounds, the more potent antitubercular compounds **2b**, **2d**, **2e** and **3d** having aromatic ring substitution show MIC values 3.13, 1.56, 3.13 and 3.13 μg/mL, respectively. The compounds **2c** and **3e** having substitution on the aromatic ring with MIC values 6.25 μg/mL exhibit reasonable antitubercular activity.

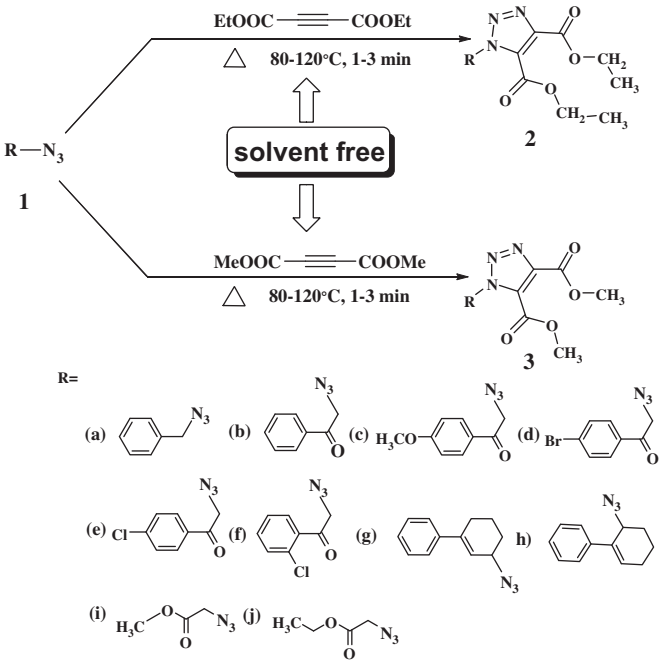


Scheme 1. Synthesis of 1,2,3-triazole (**2a**).

Table 1
Optimization of 1,2,3-triazole (**2a**) synthesis

Entry	Reaction condition		
	Solvent	Time	Yield (%)
1	THF	7 h	80
2	Acetonitrile	5 h	80
3	Benzene	4 h	83
4	Toluene	3 h	88
5	DEADC	1 min[a]	96
6	—	1 min[b]	96

[a] Reaction carried out in DEADC in excess in absence of solvent.
[b] Reaction carried out with 1:1 equiv of **1a** and DEADC without solvent.



Scheme 2. Synthesis of 1,2,3-triazoles (**2a–j** and **3a–j**).

In particular, the compound **2d**, with MIC 1.56 μg/mL is endowed with maximum potency, being 2.08 times more active than the standard drug ethambutol (MIC 3.25 μg/mL). However, none of the compounds screened in the present work is found to be more potent than the standard drug isoniazid (MIC 0.75 μg/mL). The halogen substitution on aromatic ring generally leads to the compounds having better activity in comparison to other compounds with unsubstituted aromatic ring and the compounds with alkyl substitution do not display any significant activity.

In conclusion, we have developed an efficient and a practical protocol for the synthesis of 1,2,3-triazoles instantaneously from the alkyl/aryl azides and alkynes under solvent-free conditions. The note-worthy features of our method are it is very simple and gives excellent yields. In vitro antitubercular activity of the triazoles against *MTB* $H_{37}Rv$ strain has been evaluated. Four of the compounds showed MIC in the range of 1.56–3.13 μg/mL proving their potential activity.

Table 2
Synthesis of 1,2,3-triazoles (**2a–j** and **3a–j**) from alkyl/arylazides and DEADC/DMADC

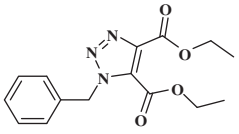
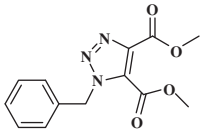
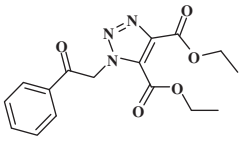
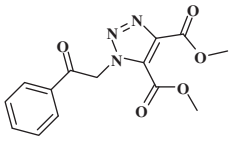
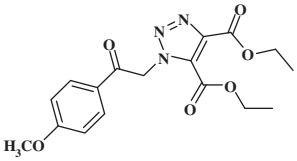
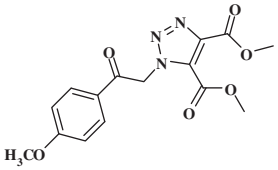
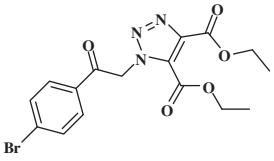
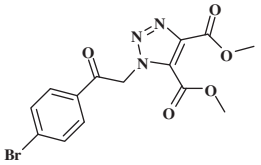
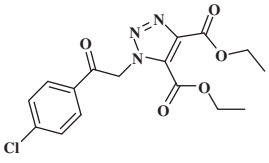
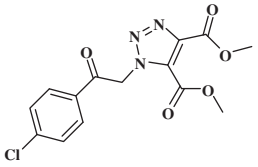
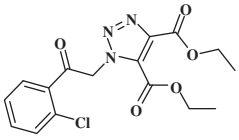
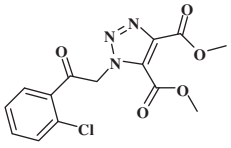
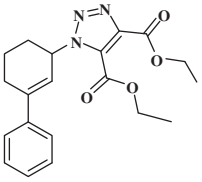
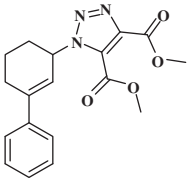
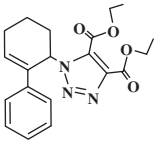
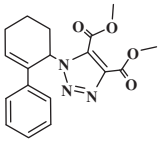
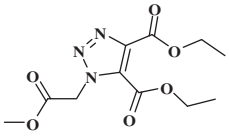
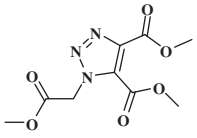
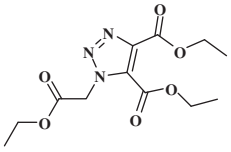
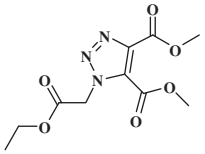
Azides (**1a–j**)	1,2,3-Triazoles (**2** and **3**)				
	2a–j	**3a–j**	Temp. (°C) **2**/**3**	Time (min) **2**/**3**	Yield (%) **2**/**3**
1a			120/120	1/1	96/96
1b			80/80	1/1	98/98
1c			80/80	1/1	98/98
1d			80/80	1/1	99/99
1e			80/80	1/1	99/99
1f			80/80	1/1	97/97
1g			100/100	3/3	99/99
1h			100/100	3/3	97/97
1i			90/90	2/2	96/96
1j			90/90	2/2	96/96

Table 3
Experimentally determined MIC values of 1,2,3-triazoles

Triazoles	MIC (μg/mL)
2a	12.5
2b	3.13
2c	6.25
2d	1.56[a]
2e	3.13
2i	>25
2j	>25
3a	25
3b	12.5
3c	12.5
3d	3.13
3e	6.25
3i	>25
3j	>25
Isoniazid	0.75
Ethambutal	3.25

[a] High potent antitubercular compound.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.048.

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