Synthesis and antimicrobial evaluation of some novel thiomorpholine derived 1, 4- disubstituted 1,2,3-triazoles

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Abstract

A convenient synthesis of novel1,4-disubstituted 1,2,3-triazoles (4a-j & 5a-j) is reported via copper(I)-catalyzed one pot [3+2] cycloaddition of various alkyl halides, sodium azide with (prop-2-yn-1-yl)thiomorpholine and 4-(prop-2-yn-1-yl)thiomorpholine 1,1-dioxide. All the synthesized compounds were screened for their antimicrobial activity. Compounds **4a**, **4b**, **4c**, **4g**, **5a** *and* ***5j*** against *S.epidermidis,* **4a, 5a** and ***5d*** against *P.aeroginosa,* ***4a***, ***4b*** *and* ***4g*** *against K.pneumoniae,* **4b**, **5a** and **5d** against *S.aureus* and **5b, 5e** and **5j** against *B.Subtilis* have shown excellent antibacterial activity compared to the standard drugs *Penicillin* and *Streptomycin*. Compounds **4c, 4e**, **4f**, **4j**, **5c**, **5d**, **5g** and **5j** showed moderate antifungal activity compared to standard Ampothericin-*B.*

Keywords: Click chemistry ; one pot synthesis; CuAAC reaction; alkyl azides.

INTRODUCTION

Nowadays, bacterial infection remains a serious threat to human lives due to their increasing resistance towards current antibiotics. Thus, there is big scope for the invention of new antimicrobial agents.

1,4-disubstituted 1,2,3-triazoles are known to possess many biological activities such as anti-HIV,[1,2] anti microbial,[3] anti-cancer, [4-6] anti-inflammatory, [7] Fluorescent activity,[8] inhibitors of kinase-3β[9,10] and other enzyme inhibitors.[11-13] 1, 2, 3-triazole moiety containing drug molecules such as tazobactam,[14] cefatrizine [15] and carboxyamidotriazole [16] are available (**Fig.1**).

There are various methods available for the one pot synthesis of 1.4-disubtituted 1, 2, 3-triazoles.[17-19] However, the copper(I)-catalyzed one pot three component cycloaddition of alkyl

halides with sodium azide and terminal alkynes is one of the best methods for multicomponent regioselective synthesis of 1,4-disubstituted 1,2,3-triazoles.[20] This method was independently pioneered by Fokin et. al [21] in 2004 through dramatic modification of the Huisgen 1,3-dipolar cycloaddition reaction.[22]

Previously, we have reported on the synthesis and antibacterial activity of 1, 2, 3-triazole derivatives of morpholine-3-carboxylic acid ester.[23] Morpholine moiety has played significant role in the medicinal chemistry.[24] Some of Morpholine moieties containing drugs are shown in **Fig.2.** Thiomorpholine derivatives are known to exhibit biological activities including antimycobacterial,[25] antibacterial,[26,27] anticancer,[28] anti-inflammatory and antioxidant agents [29] and dipeptidyl peptidase IV (DPP-IV) inhibitors.[30] The above literature findings and in continuation of our research on synthesis of 1, 4-disubtituted 1, 2, 3-triazole derivatives, we herein report the synthesis of thiomorpholine derived 1,4-disubstituted 1, 2, 3-triazoles and evaluation of their antimicrobial activity.

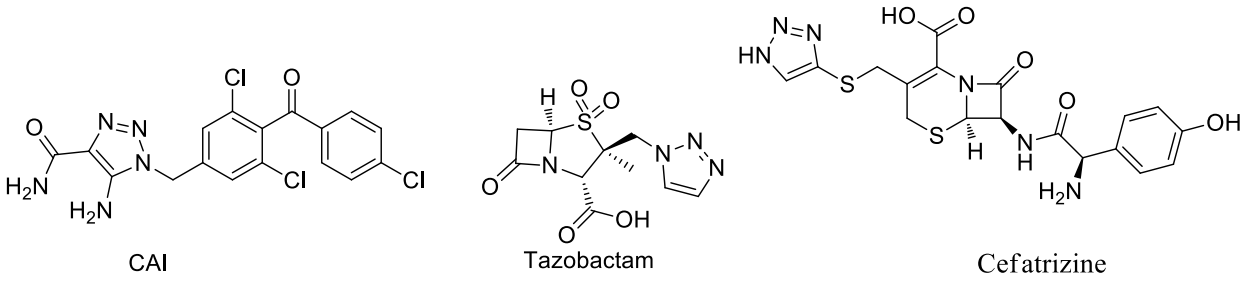


Fig. 1: Some of 1, 2, 3-triazole ring containing drugs in the market

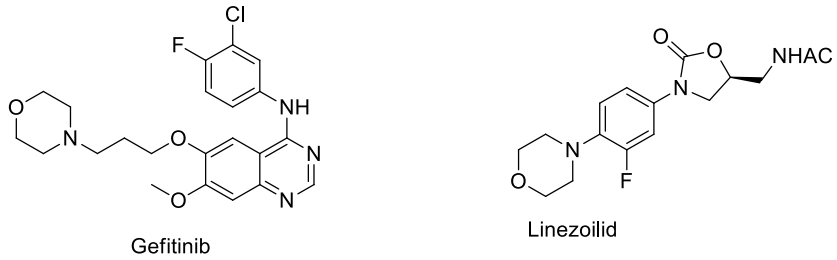
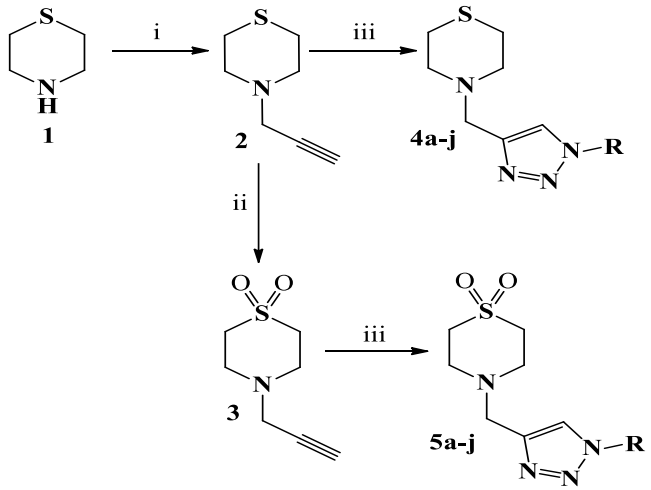


Fig. 2: Some of morpholine ring containing drugs in the market

RESULTS AND DISCUSSION

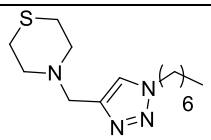
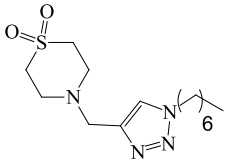
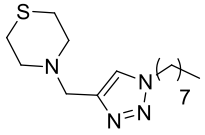
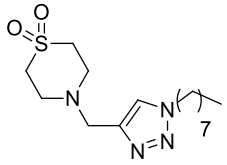
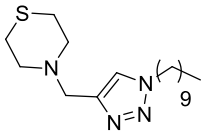
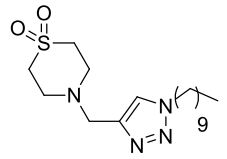
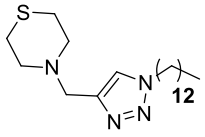
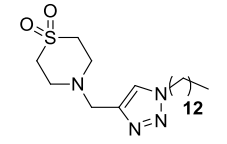
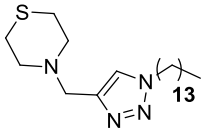
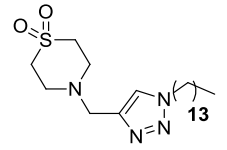
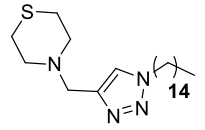
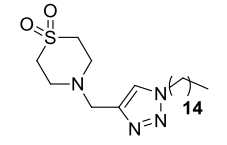
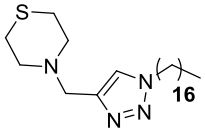
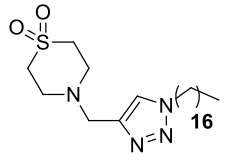
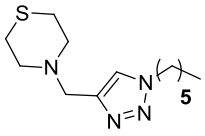
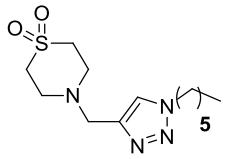
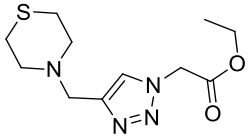
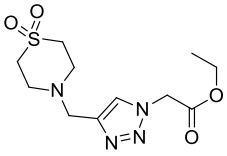
Chemistry

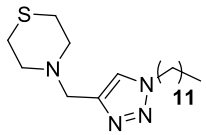
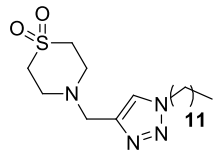
In our present work, we have synthesized novel thiomorpholine derived1, 4-disubtituted 1, 2, 3-triazoles, employing the copper catalyzed azide alkyne cycloaddition (CuAAC) reaction . 1,4-disubstituted 1,2,3-triazole derivatives were synthesized by one-pot reaction, starting from alkyne, alkyl halide and sodium azide in presence of Cu(I) catalyst. 4-(prop-2-yn-1-yl) thiomorpholine (2) was synthesized by reacting thiomorpholine with propargyl bromide in presence of CS_2CO_3 in acetone at room temperature.[31] 4-(prop-2-yn-1-yl) thiomorpholine 1,1-dioxide(**3**) was obtained by oxidation of sulfur group in 4-(prop-2-yn-1-yl)thiomorpholine with 3-chloroperbenzoic acid.[32] Cycloaddition of compound-**2** and **3** with insitu prepared alkyl azides in presence of Cu (1) catalyst yielded 1, 4-disubstituted 1, 2, 3-triazole derivatives (**4a-5j**) in good yields (**Table I**). The structures of the synthesized compounds were confirmed by spectral techniques such as IR, ^{1}HNMR, ^{13}C NMR and ESI-MS.



Scheme 1: i) propargyl bromide / CS_2CO_3, acetone, rt, 6h; ii) mCPBA / DCM, rt, 12h; iii) R-Br / NaN_3, CuI, THF-H_2O (1:1), rt-50 ^oC, 8-12h.

Table I: Synthesized 1, 4- disubstituted 1, 2, 3-triazoles (**4a-5j**) from different alkyl bromides.

Entry	R_1-Br	Product	Entry	Product
4a	$C_7H_{15}Br$		**5a**	
4b	C_8H_{17} Br		**5b**	
4c	$C_{10}H_{21}$ Br		**5c**	
4d	$C_{13}H_{27}$ Br		**5d**	
4e	$C_{14}H_{29}$ Br		**5e**	
4f	$C_{15}H_{31}$ Br		**5f**	
4g	$C_{17}H_{35}$ Br		**5g**	
4h	$C_6H_{13}N_3$		**5h**	
4i	$C_2H_5OCOCH_2Br$		**5i**	

4j	$C_{12}H_{25}N_3$		**5j**	

Antibacterial activity

All the synthesized compounds **(4a-5j)** were screened for their invitro antibacterial activity against various gram-positive microorganisms such as *Bacillus subtilis, Staphylococcus aureus***,** *Staphylococcus epidermidis* and gram-negative microorganisms such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia.* Penicillin and Streptomycin were used as standard drugs for comparison. The screening data (**Table II**) revealed that compounds **4a**, **4b**, **4c, 4g, 5a** and **5j** showed excellent activity against *S.epidermidis* with MIC values 2.34, 1.17, 2.34,1.17, 1.1718, 2.34 and 9.37 (µg/ml) respectively. Compounds **4a**, **4b** and **4g** showed very good antibacterial activity against *K.pneumoniae* with MIC values 9.37, 2.34, and 4.68 (µg/ml). Compounds **4a, 5a** and **5d** against *P.aeroginosa* with MIC values 2.34, 9.37 and 4.68 (µg/ml), **4b**, **5a** and **5b** against *S.aureus* with MIC values 2.34, 2.34 and 4.68 (µg/ml) and **5b, 5e** and **5j** against *B.Subtilis* showed very good antibacterial activity with MIC values 2.34, 9.37 and 2.34 (µg/ml).

Table II: Invitro antibacterial activity data of compounds **(4a–5j)** as MIC (µg/ml)

Compound	Gram-positive bacteria			Gram-negative bacteria		
	B.Subtilis	*S.aureus*	*S.epidermidis*	*E.coli*	*P.aeroginosa*	*K.pneumoniae*
4a	>150	75	2.3435	>150	2.3435	9.375
4b	>150	2.3435	1.1718	>150	>150	2.3435
4c	>150	>150	2.3435	>150	>150	>150
4d	>150	>150	>150	>150	>150	>150
4e	>150	>150	>150	>150	>150	>150
4f	>150	>150	>150	>150	>150	>150
4g	>150	>150	1.1718	>150	>150	4.6875
4h	>150	>150	>150	>150	>150	>150
4i	>150	>150	>150	>150	>150	>150
4j	>150	>150	>150	>150	>150	>150
5a	>150	2.3435	2.3435	>150	9.375	>150

5b	2.3435	4.6875	>150	>150	>150	>150
5c	>150	>150	>150	>150	>150	>150
5d	>150	>150	>150	>150	4.6875	>150
5e	9.375	75	>150	>150	>150	>150
5f	>150	>150	>150	>150	>150	>150
5g	>150	>150	>150	>150	>150	>150
5h	>150	>150	>150	>150	>150	>150
5i	>150	>150	>150	>150	>150	>150
5j	2.3435	>150	9.375	>150	>150	>150
Penicillin	1.562	1.562	3.125	12.5	12.5	6.25
Streptomycin	6.25	6.25	3.125	6.25	1.562	3.125

Antifungal activity

All the synthesized compounds **(4a-5j)** were screened for in vitro antifungal activity against the fungal strains such as *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger* and *Aspergillus flavus*. Amphotericin-B was used as a standard. Invitro antifungal activity results (**Table III**) revealed that compounds **4c**, **4e**, **4f, 4j**, **5c**, **5d**, **5g** and **5j** exhibited moderate antifungal activity against *C.albicans*, *A. niger* and *A. flavus.* Rest of the compounds inactive against all fungal strains.

Table III: Invitro antifungal activity data of compounds **(4a-5j)** as zone of inhibition (mm)

Compound	*C. albicans*		*S. cerevisiae*		*A. niger*		*A. flavus*	
	100µg	150µg	100µg	150µg	100µg	150µg	100µg	150µg
4a	0	0	0	0	0	0	0	0
4b	0	0	0	0	0	0	0	0
4c	10	13	0	0	10	14	9	12
4d	0	0	0	0	0	0	0	0
4e	9	12	0	0	15	16	10	13
4f	8	11	0	0	12	14	0	0
4g	0	0	0	0	0	0	0	0
4h	0	0	0	0	0	0	0	0
4i	0	0	0	0	0	0	0	0
4j	10	13	0	0	10	14	0	0
5a	0	0	0	0	0	0	0	0

5b	0	0	0	0	0	0	0	0
5c	8	10	0	0	10	12	0	0
5d	10	12	0	0	10	13	0	0
5e	0	0	0	0	0	0	0	0
5f	0	0	0	0	0	0	0	0
5g	11	13	0	0	0	0	8	11
5h	0	0	0	0	0	0	0	0
5i	0	0	0	0	0	0	0	0
5j	10	14	0	0	12	15	10	13
Ampothericin-B	23.5		22		25		25	

Zone of inhibition values (mm) for analogs (**4a-5j**) were measured at 100 and 150 µg/ml.

MATERIALS AND METHODS

All the reagents and solvents were purchased from Aldrich/Merck and used without further purifications. Thin-layer chromatography (TLC) was performed using Merck silica gel 60 F254 precoated plates (0.25 mm) and Silica gel (100-200 mesh) was used for column chromatography. The progress of the reactions as well as purity of the compounds was monitored by thin layer chromatography with using ethylacetate /hexane (7/3) as eluent. Phosphomolybdic Acid (PMA) stain was used for detection. Melting points were determined using a Cintex apparatus and were uncorrected. 300MHz and 500Mz spectrometers were used for ^{1}HNMR and 125 MHz NMR spectrometer was used for ^{13}C NMR (**4a-4j**) spectra respectively. Coupling constant (J) values are presented in Hertz, spin multiples are given as **s** (singlet), **d** (doublet), **t** (triplet), and **m** (multiplet). Mass spectra were recorded by using ESI–MS method.

EXPERIMENTAL

Synthesis of *4-(prop-2-yn-1-yl) thiomorpholine* (**2**)

The title compound was prepared according to known literature procedure.

4-(prop-2-yn-1-yl) thiomorpholine 1,1-dioxide **(3)**

To a stirred solution of 4-(prop-2-yn-1-yl) thiomorpholine (3g, 0.021mol) in DCM (100 ml), was added mCPBA (10.9g, 0.0638mol) at 0^{o}C. The reaction mixture was stirred at room temperature for 12h. After completion of the reaction, solvent was removed under reduced pressure to afford crude compound. The crude compound was partitioned between ethyl acetate and

aqueous $NaHCO_3$ solution. Then organic layer was separated, washed with brine solution, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford 2.8 g (77 %) of 4-(prop-2-yn-1-yl)thiomorpholine 1,1-dioxide (3).

General Procedure for the synthesis of 1,4-disubstituted 1,2,3-triazoles (**4a-5j**)

To a stirred solution of alkyl bromide (1.67 mmol, 1eq) in aqueous THF solution (H_2O/THF, 1:1) was added sodium azide (2.01mmol, 1.2 eq) and mixure was stirred at room temperature for 2h. Then, alkyne (1.67mmol, 1 eq), 10 mol % Cu (I) were added to the reaction mixture and stirred at ambident temperature for 8–12 h. After completion of the reaction, water was added, extracted with ethyl acetate, dried over Na_2SO_4 and evaporated under reduced pressure to afford crude compounds. The crude compounds were purified by column chromatography using silica (100-200 mesh) and (20 -30%) ethyl acetate in n-hexane as eluent. The evaporation of solvent afforded compounds **4a**- **5j** in good yields.

Antibacterial activity

The minimum inhibitory concentrations (MIC) of synthesized compounds were tested against Gram-positive organisms such as *Bacillus subtilis* **(MTCC 441),** *Staphylococcus aureus* **(MTCC 96),** *Staphylococcus epidermidis* **(MTCC 2639**) and Gram-negative organisms such as *Escherichia coli* **(MTCC 443),** *Pseudomonas aeruginosa* **(MTCC 741),** and *Klebsiella pneumoniae* **(MTCC 618**) using broth dilution method.[33] Penicillin and Streptomycin were also screened under identical conditions for comparison.

Antifungal activity

In vitro antifungal activity of synthesized compounds was carried out against the fungal strains such as *Candida albicans* **(MTCC 227)**, *Saccharomyces cerevisiae* **(MTCC 36)**, *Aspergillus niger* **(MTCC 282)** and *Aspergillus flavus* **(MTCC 92)** by Agar Well Diffusion Method.[34] The ready-made Potato Dextrose Agar (PDA) medium (Hi-media, 39 g) was suspended in distilled water (1000 ml) and heated to boiling until it dissolved completely, the medium and Petri dishes were autoclaved at pressure of 15 lb/inc for 20 min. The medium was poured into sterile Petri dishes under aseptic conditions in a laminar air flow chamber. When the medium in the plates was solidified, 0.5 ml of (week old) culture of test organism was inoculated and

uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving the compound in DMSO and different concentrations (100 &150 µg/ml) were made. After inoculation, the wells were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each well different concentrations of test solutions were added and controls were maintained. The treated samples and the controls were kept at 27^{o}C for 48 h. Inhibition zones were measured and the diameter was calculated in millimeter. Amphotericin B was used as a standard drug for comparison.

Acknowledgments

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CONCLUSION

In summary, we have synthesized some novel thiomorpholine derived 1,4-disubstituted 1, 2, 3-triazols and screened for their antimicrobial activity. Majority of the compounds showed excellent antibacterial activity compared to the standard drugs *Penicillin* and *Streptomycin*. Some of the synthesized compounds showed moderate antifungal activity. These active compounds can be very good candidates for further antimicrobial investigations.

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