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One pot synthesis of 4-((1-aryl-*1H*-1,2,3-triazol-4-yl) methyl) morpholine-3-carbonitrile derivatives and their biological evaluation

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ABSTRACT

An Cu(I) catalyzed one-pot procedure for the synthesis of 4-((1-aryl-1H-1,2,3-triazol-4-yl) methyl) morpholine-3-carbonitrile derivatives by a three-component reaction of morpholine-3-carbonitrile, propargyl bromide and aryl azides in [BMIM][PF_6]-H_2O system was performed. All the synthesized compounds ***(1a-f)*** *were screened for antimicrobial activity. Among all the synthesized compounds* ***1a****,* ***1c****,* ***1d,*** *and* ***1e*** *showed significant anti-microbial activity against tested organisms.*

Keywords: Click chemistry, morpholine-3-carbonitrile, [BMIM] [PF_6], 1,2,3-Triazoles, anti- microbial activity.

INTRODUCTION

A combination of two or more heterocyclics has been a greater fashion for organic chemists for the synthesis as well as thinking of their biological profile. Many of the drugs are derivatives of heterocyclics. In recent years, 1,2,3-triazoles and related heterocyclic compounds have been identified as bioactive molecules (**Fig. 1&2)**. Morpholine and substituted Morpholine derivatives have emerged as an important class of organic compounds, displaying a vast spectrum of properties and are widely used as pharmaceuticals. Many morpholine derivatives have found medicinal applications, for example, it is a building block in the preparation of the antibiotic linezolid [6] and anticancer agent gefitinib [7] (**Fig. 3**). Morpholine derivatives were also reported to possess central nervous system [8] and antibacterial [9] activities.

Yong-Min Liang [10] have reported a copper (I)-catalyzed three-component reaction forms 1-substituted-1*H*-1,2,3-triazol-4-ylmethyl)-dialkyl amines. Herein, we report the synthesis of copper (I)-catalyzed novel 4-[(1-aryl-1*H*-1,2,3-triazol-4-yl) methyl] morpholine-3-carbonitrile derivatives (**1a-f**) using morpholine-3-carbonitrile, propargyl bromide and various aryl azides in different solvents. The better yields of products (**1a-f**) were achieved in the presence of an ionic liquid [BMIM] [PF_6] -H_2O (**Table 1**).

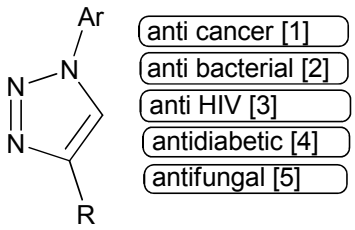


Fig. 1. Potential applications based on the 1,2,3-triazoles

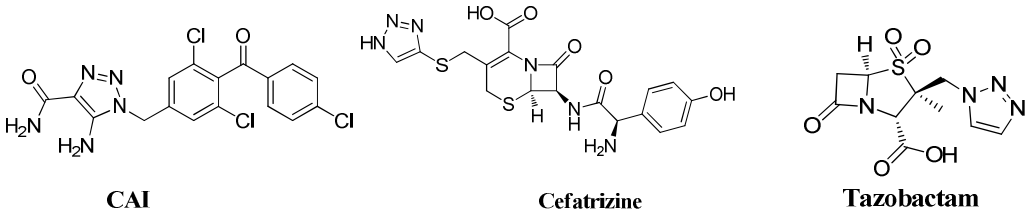


Fig. 2: Some of 1,2,3-triazole containing drugs in the market

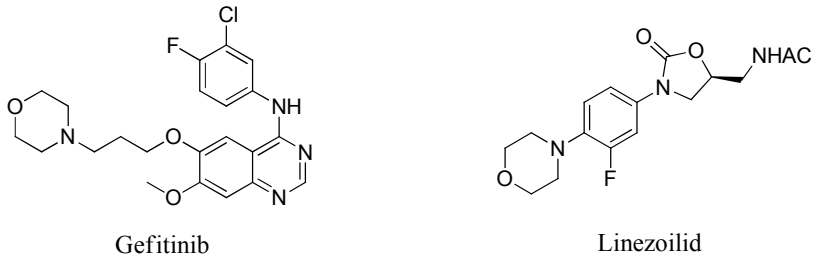


Fig. 3: Some of morpholine ring containing drugs in the market

MATERIALS AND METHODS

All chemicals were purchased from Sigma Aldrich Chemicals / S.D. Fine Chemicals Limited and were used without further purification. The reactions were monitored by thin layer chromatography on silica gel G plates (Merck silica-60 F258).Visualization of the developed chromatogram was performed by UV light (254 nm). Melting points were determined using a Cintex apparatus and were uncorrected. Elemental analysis was measured by means of Perkin Elmer 2400 CHN elemental analyzer. ^{1}H NMR was obtained on Bruker DRX-500 Avance spectrometer operating at 500MHz. Samples were prepared in $CDCl_3$ solvent with TMS as an internal reference. Coupling constant (J) values are presented in Hertz (Hz) and spin multiples are given as s (singlet), d (doublet), t (triplet), and m (multiplet). Fourier-transform infrared (FT-IR) spectra were obtained as KBr discs on Shimadzu spectrometer. Mass spectra were recorded by using ESI–MS. The synthetic route for 1,2,3-triazole derivatives is depicted in **Scheme 1**.

General procedure for the synthesis of 4-[(1-aryl-1*H*-1,2,3-triazol-4-yl) methyl]morpholine-3-carbonitrile derivatives (1a-f).

4-{[1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl]methyl}morpholine-3-carbonitrile(1a):

A mixture of [BMIM][PF6] (1ml), H_2O (3ml), morpholine-3-carbonitrile (1.2 mmol), K_2CO_3 (2 mmol) and propargyl bromide (1.2 mmol) was stirred vigorously for 60 min at room temperature . Then, 1.2 mmol of azide and 10 mol% of CuI were added into the mixture and the complete consumption of the starting materials was monitored by TLC. The reaction was stopped by addition of H_2O and the product was extracted with ethyl acetate. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the crude product. Crude residue washed with hexane to afford ***2a*** **(**76 %) *as* a white solid. The residue ionic liquid was washed with water and reused for the cycloaddition reaction. mp 98-100^{o}C, FTIR(KBr): 1645(C=N), 1554(C=C), 2228 (CN), 3134 (triazole-H) cm^{-1}; ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.828 (s, 1H,Triazole), 7.604 (d, *J* = 9.2 Hz,

2H), 7.026 (d, *J* = 9.2 Hz, 2H), 5.267 (s, 2H, N-CH_2), 4.531 (m, 1 H, NCH), 4.302 (m, 1 H, NCHCH*H*), 3.891 (1 H, m, NCH2CH*H*), 3.866 (s, 3H, OCH_3), 3.760 (m, 1H, NCHCH*H*), 3.628 (m, 3 H, NC*H2*CH*H*); ESI-MS (m/z): 300 [M+H]; Anal. Calcd for $C_{15}H_{17}N_5O_2$: C, 60.19; H, 5.72; N, 23.40; Found: C, 60.01; H, 5.52; N, 23.04.

4-{[1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl]methyl}morpholine-3-carbonitrile(1b):
Light yellow solid**,** mp 89-91^oC, IR (KBr):1640 (C=N),1555(C=C), 2220 (CN), 3131(triazole-H) cm^{-1}; ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.988 (s, 1H, Triazole), 7.690 (d, *J* = 8.5 Hz, 2H, ArH), 7.332 (d, *J*= 8.5 Hz, 2H, ArH), 5.345 (s, 2H, N-CH_2), 4.531 (m, 1H, NCH), 4.302 (m, 1H, NCHCH*H*), 3.891 (m, 1H, NCH2CH*H*), 3.737 (m, 1H, NCHCH*H*), 3.622 (m, 3H, NC*H2*CH*H*); ESI-MS (m/z) : 315 [M+H]: Anal. Calcd for $C_{14}H_{14}N_6O_3$; C, 53.50; H, 4.49; N, 26.74; Found: C, 52.80; H, 4.12; N, 25.94.

4-{[1-(4-butylphenyl)-1H-1,2,3-triazol-4-yl]methyl}morpholine-3-carbonitrile(1c):
White solid**,** mp 85-87^oC, IR(KBr): 1642(C=N), 1545(C=C), 2222 (CN), 3141(triazole-H) cm^{-1}; ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.988 (s, 1H, Triazole), 7.658 (d, *J* =9.2Hz, 2H, ArH), 7.33 (d, *J*= 9.2Hz, 2H, ArH), 5.213 (s, 2H, N-CH_2), 4.531 (m,1H, NCH), 4.302 (m, 1H, NCHCH*H*), 3.891 (m, 1H, NCH2CH*H*), 3.737 (m, 1H, NCHCH*H*), 3.622 (m, 3H, NC*H2*CH*H*), 2.701(t, *J*= 6 Hz , 2H), 1.325-1.654 (m, 4H), 0.961 (t, *J*=8 Hz, 3H); ESI-MS (m/z): 326 [M+H] Anal. Calcd for $C_{18}H_{23}N_5O$; C, 66.44; H, 7.12; N, 21.52; Found: C, 66.11; H, 6.91; N, 21.02;

4-((1-(o-tolyl)-1H-1,2,3-triazol-4-yl)methyl)morpholine-3-carbonitrile (1d):
White solid**,** mp 94-96^oC, IR(KBr): 1655(C=N), 1550(C=C), 2220 (CN), 3136(triazole-H) cm^{-1}; ^{1}HNMR(400MHz, CDCl3) δ 7.854 (s, 1H, Triazole), 7.421 (m, 4H, Ar), 5.257 (s, 2H, N-CH_2), 4.525 (m, 1H, NCH), 4.312 (m, 1H, NCHCH*H*), 3.875 (m, 1H, NCH2CH*H*), 3.741 (m, 1H, NCHCH*H*), 3.631 (m, 3H, NC*H2*CH*H*), 2.259 (s, 3H, Ar-*CH3*); ESI-MS (m/z): 284 [M+H]. Anal. Calcd for $C_{15}H_{17}N_5O$; C, 63.59; H, 6.05; N, 24.72; found: C, 63.05; H,5.90; N, 24.02.

4-{[1-(3,5-dimethylphenyl)-1H-1,2,3-triazol-4-yl]methyl}morpholine-3-carbonitrile(1e):
Yellow solid**,** mp 88-90 ^oC; IR(KBr): 1640 (C=N), 1545(C=C), 2230 (CN), 3148 (triazole-H), cm^{-1}; ^{1}H-NMR (400MHz,$CDCl_3$) δ 7.832 (s, 1H, Triazole), 7.541 (s, 2H, Ar), 7.512 (s, 1H, Ar), 5.267 (s, 2H, N-CH_2),4.523 (m, 1H, NCH), 4.312 (m, 1H, NCHCH*H*), 3.898 (m,1H, NCH2CH*H*), 3.722 (m, 1H, NCHCH*H*), 3.615 (m, 3H, NC*H2*CH*H*), 2.781 (s, 6H, Ar-CH_3) ; ESI-MS (m/z): 298 [M+H]. Anal. Calcd for $C_{16}H_{19}N_5O$: C, 64.63; H, 6.44; N, 23.55 ; Found: C, 63.83; H, 6.14; N, 23.01.

4-{[1-(naphthalen-1-yl)-1H-1,2,3-triazol-4-yl]methyl}morpholine-3-carbonitrile(1f):
Red solid, mp110-112^oC; IR (KBr): 1660 (C=N), 1550 (C=C), 2220 (CN), 3140 (triazole-H) cm^{-1}; ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.874 (s, 1H, triazole-*H*), 7.782 (m ,4H), 7.608 (m, 3H), 5.067 (s, 2H, N-CH_2), 4.524 (m, 1H, NCH), 4.201 (m, 1H, NCHCH*H*), 3.888 (m, 1H, NCH2CH*H*), 3.729 (m, 1H, NCHCH*H*), 3.618 (m, 3H, NC*H2*CH*H*); ESI-MS (m/z): 320 [M+H]. Anal. Calcd for $C_{18}H_{17}N_5O$: C, 67.70; H, 5.37; N, 21.93; Found: C, 66.89; H, 5.01; N, 21.30.

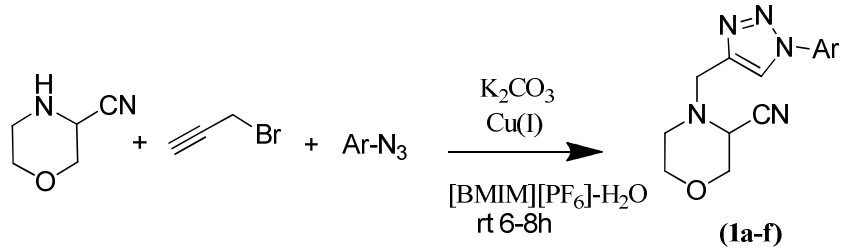
Antimicrobial activity
The newly synthesized compounds **(1a-f)** were evaluated for in-vitro antibacterial and antifungal activity against various Gram-positive, Gram-negative bacteria, and fungal strains using broth dilution method [11] and agar cup bioassay method respectively. The results are shown in **Table 2** and **3**. Penicillin, Streptomycin and Ampothericin-B were used as standard drugs for comparison. The minimum inhibitory concentrations (MIC) of synthesized compounds **(1a-f)** were tested against three representative Gram-positive organisms such as *Bacillussubtilis***),** *Staphylococcus aureus* , *Staphylococcus epidermidis* and Gram-negative organisms such as *Escherichia coli*, *Pseudomonas aeruginosa* , and *Klebsiella pneumoniae* by broth dilution method recommended by National Committee for Clinical Laboratory (NCCL) standards (1). Standard antibacterial agents like Penicillin and Streptomycin were also screened under identical conditions for comparison. The minimum inhibitory concentration (MIC) values are presented in **Table 2**. All the synthesized compounds **(1a-f)** were tested for In-vitro antifungal activity against the fungal strains such as *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus Niger* and *Aspergillus flavus*, by Agar Well Diffusion Method (2). Agar well bioassay was employed for testing antifungal activity [12]. 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 a pressure of 15 lb/INC^2 for 20 min. The medium was poured into sterile Petri dishes under aseptic conditions in a laminar airflow 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 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°C for 48 h. Inhibition zones were measured and the diameter was calculated in millimeter (**Table 3**). Three to four replicates were maintained for each treatment. Amphotericin was used as a standard.

RESULTS AND DISCUSSION

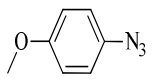
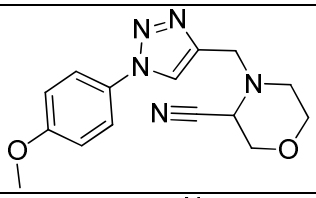
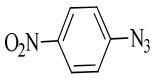
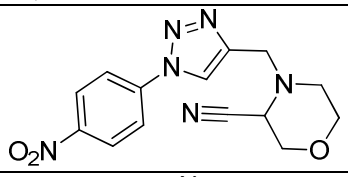
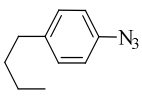
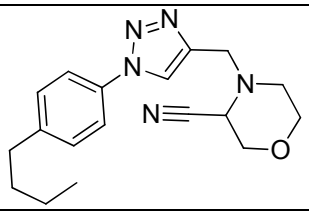
Chemistry

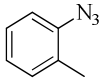
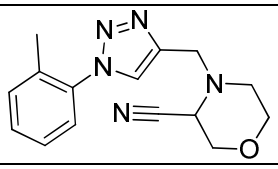
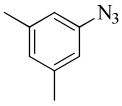
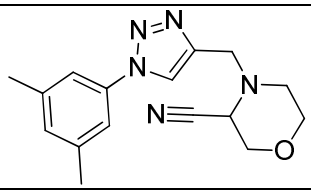
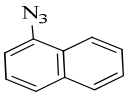
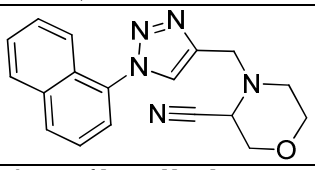
This three-component reaction proceeds via in-situ formation of N-propagation by the reaction of morpholine-carbonitrile, K_2CO_3 and propargyl bromide. The alkyne then undergoes 1, 3-dipolar cycloaddition with aryl azide to afford 1, 4-disubstituted 1,2,3-triazoles. Herein, we describe a process in which CuI (I) efficiently catalyzes azide–alkyne cycloaddition in the presence of [BMIM] [PF_6] and H_2O mixture (**Scheme 1**). Initially, the effect of solvent on the formation of the 1,2,3-triazoles (yield) was investigated (**Table 1**). Among all the solvents used, [BMIM] [PF_6] -H_2O requires shorter reaction time and afforded corresponding triazoles in good to excellent yields.The [BMIM] [PF_6] effects can be explained to solvophobic interactions that generate an internal pressure, which promotes the association of the reactants in a solvent cavity during the activation process and showed an acceleration of the multi-component reactions (MCRs) in comparison to conventional solvents [13].



Scheme 1: Synthetic route of 4-((1-aryl-1H-1,2,3-triazol-4-yl)methyl)morpholine-3-carbonitrile derivatives (1a-f)

Table 1: Synthesized novel 4-((1-aryl-1H-1,2,3-triazol-4-yl)methyl)morpholine-3-carbonitrile derivatives (1a-f)

Entry	azide	Product	Time	Product/ M.F	Yield(%)
1			**6**	**1a/** $C_{15}H_{17}N_5O_2$	22[a], 25[b], 50[c], 76
2			**8**	**1b/** $C_{14}H_{14}N_6O_3$	70
3			**8**	**1c/** $C_{18}H_{23}N_5O$	72

4			**7**	**1d/** $C_{15}H_{17}N_5O$	67
5			**6**	**1e/** $C_{16}H_{19}N_5O$	68
6			**8**	**1f/** $C_{18}H_{17}N_5O$	62

Reaction conditions: In absence of [BMIM][PF_6] , a = CH_3CN, b = Acetone and c = THF

Biological Activity

Antimicrobial Activity.

Antibacterial activity results (**Table 2**) revealed that, among the synthesized compounds **1a, 1c, 1d** and **1e** showed potent activity against gram-positive bacterial strains *S. Aureus*, *S. Epidermidis*, and gram-negative bacterial strains *P. Aeroginosa*, *K. Pneumonia* on comparing with the standard drugs Streptomycin and Penicillin. Compound **1b** and **1f** showed moderate activity against gram-positive bacterial strains *S. aureus and* gram-negative bacterial strains *P. Aeroginosa.* The antifungal screening data of **(1a-f)** revealed that all the tested compounds showed moderate to good antifungal activities against the tested fungal strains. The antifungal screening results revealed that 7**a, 7c, and 7e** showed appreciable activity against *S. cerevisiae* and *A. flavus* organism **(Table 3).** Compound **7d** showed good activity against *S. Cerevisiae* orgasm.

Table 2: Antibacterial activity data of synthesized compounds (1a-f)

	MIC (µg/ml)					
Compound Code	***B.Subtilis***	***S.aureus***	***S.epidermidis***	***E.coli***	***P.aeroginosa***	***K.pneumoniae***
1a	>150	9.375	2.3435	37.5	2.3435	9.375
1b	>150	37.5	75	>150	75	75
1c	>150	18.75	9.375	75	37.5	18.75
1d	>150	9.375	2.3435	>150	9.375	18.75
1e	>150	9.375	18.75	75	9.375	9.375
1f	>150	75	37.5	>150	75	37.5
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

Table 3: Antifungal activity data of synthesized compounds (1a-f)

	Zone of inhibition (mm)							
S.No	***Candida albicans***		***Sacharromyces cerevisiae***		***Aspergillus niger***		***Aspergillus flavus***	
	100µg	150µg	100µg	150µg	100µg	150µg	100µg	150µg
7a	0	0	15	19	0	0	13	19
7b	0	0	0	0	0	0	0	7
7c	0	0	12	17	0	0	14	18
7d	0	0	10	15	0	0	0	0
7e	0	0	11	16	0	0	14	18
7f	0	0	0	0	0	0	0	0
Ampothericin-B	23.5	23.5	22	22	25	25	25	25

CONCLUSION

In conclusion, the present work demonstrated an environmentally benign and a convenient method for the synthesis of biologically active novel 1, 2, 3-triazole derivatives. All the compounds were screened for antimicrobial activity.

Among all the synthesized compounds, **1a**, **1c**, **1d**, and **1e** showed significant antibacterial activity against both gram positive and negative bacterial strains. Compounds **1a**, **1c**, and **1e** showed better anti-fungal activity than all other synthesized compounds. These results suggest that the synthesized compounds can be good candidates for future investigations.

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