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PEG-MEDIATED ONE POT SYNTHESIS OF 1,2,3-BISTRIAZOLE DERIVATIVES BY 1,3-DIPOLAR CYCLOADDITION REACTION AND THEIR BIOLOGICAL EVALUATION

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ABSTRACT

A series of 1,2,3-bistriazole derivatives **4a-i** were synthesized by the treatment of 1, 3 dibromopropane with sodium azide and substituted alkynes by 1,3-dipolar cycloaddition reaction. Solvent PEG-400 is used as a green solvent and potentially reusable. The 1,2,3-bistriazole derivatives have shown good yields and high purity. All the synthesized compounds were characterized by IR, ^{1}H NMR, and Mass spectroscopy and these molecules were evaluated for their *S. aureus, S. epidermidis, S.marscesens, P.aeruginosa and Bacillus substil*is in which 1,2,3-bistriazole derivatives **4a, 4c, 4d,** and **4f** have shown significant zone of inhibition as compared to streptomycin as a standard drug and MIC values were reported. Also in carcinogenicity study of 1,2,3-bistriazoles, except **4e** (only at 1 and 2 mg concentrations) none of the compound shown zone of inhibition in different concentrations like **0.125, 0.250, 0.500, 1.0** and **2.0mg,** which put forward that the study of all other i.e. **4a, 4b, 4c, 4d** and **4f** compounds do not reveal any deleterious effect or toxicity to the *E. coli (AB 1157)* bacterial cell, in this study, Stannous chloride is used as a standard for carcinogenicity study.

KEYWORDS: Click chemistry, Bistriazole, PEG-400, Sodium ascorbate, 1,3 dipolar cycloaddition reaction, Antibacterial activity and Carcinogenicity study.

INTRODUCTION

The various bis-heterocyclic compounds are gaining increased interest in the recent past as the dimeric analogues have proven to be having better and potent biological activity than the corresponding monomer.[1-3] Treatment of emerging infectious diseases and the increasing number of multidrug resistant microbial pathogens are the taunting tasks in the medical

community.[4] The search for an effective compound to potent bacterial pathogencity is the need of an hour. Also pharmacologically very active nitrogen containing molecules can be attributed to the fact that nitrogenous compounds are part and parcel of the bio molecular diversity.[5] The click chemistry approach has been widely used for the synthesis of biologically active molecular frameworks, particularly for the regioselective synthesis of 1,4 disubstituted 1,2,3-triazoles, which involves the copper(I) catalysed cycloaddition reaction between azides and different terminal alkynes (CuAAC), termed as the cream of the crop of click reactions. Thus the development of the copper (I)-catalysed triazole click chemistry has led to many interesting applications in organic synthesis, medicinal chemistry, molecular biology and material science.

Green synthesis of 1,2,3-bistriazoles *via* click chemistry approach has playing attention of the last decades due to rapid, convenient and simple reaction conditions. Therefore it is of interest to use them in organic synthesis. In the present communication we account the synthesis of 1,4-disubstituted 1,2,3-bistriazole derivatives **(4a to 4i)** having general structure and have been characterised by spectral analysis and evaluated for their *in vitro* antibacterial activity by using disc diffusion method.[6]

The objectives of carcinogenicity study are to identify a tumerigenic potential animals and to assess the relevant risk in humans, any cause for concern derived from laboratory investigations (U.S. Department of Health and human services, food and drug administration, center for drug Evaluation and Research) of animal toxicology studies and data in humans may lead to a need for carcinogenicity studies. The practice for requiring carcinogenicity studies in rodents was instituted for pharmaceuticals that were expected to be administered regularly over a substantial part of patients lifetime. Since carcinogenicity studies are time consuming and resource intensive they should only be performed when human exposure warrants the need for information from lifetime studies in animals in order to assess carcinogenic potential.[7]

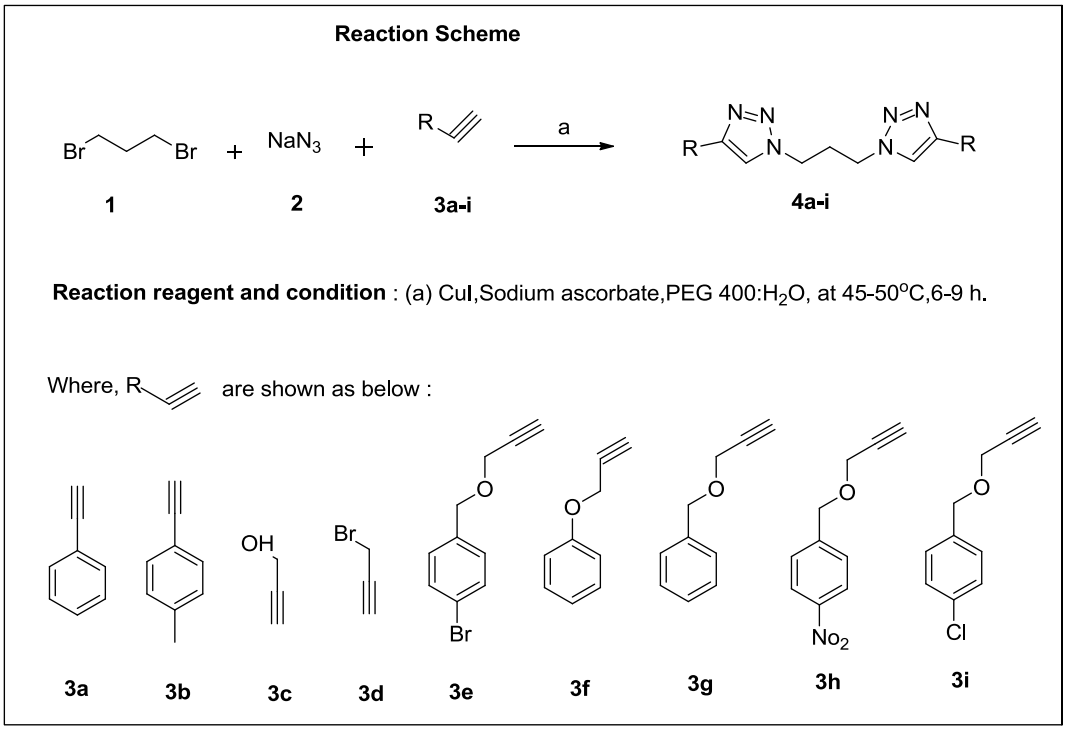
Importance of poly(ethylene glycol) (PEG) solvent as a reaction medium

S. Chandrasekhar *et al*.,[8] have been reported poly(ethylene- glycol) (PEG-400) as an efficient reaction medium for pd-catalyzed c-c bond formation, namely, the Heck reaction.[9,10] They have noticed that this transformation is more rapid and high yielding and the catalyst is easily recycled with high efficiency. Jin-Heng Li *et al*.,[11] have reported that

the PEG is recyclable and environmental concerns, a more facile, nonvolatile solvent. There are also significant of PEG as economical, environmental, benign and industrial perspectives.

MATERIALS AND METHODS

Keeping these observations in mind we report herein the synthesis and *in vitro* Antibacterial activity as well as carcinogenicity study of certain 1,2,3 Bis-triazoles. The melting points were determined in open glass capillaries and are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8400-S spectrophotometer by KBr pellet technique. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on AMX-400 NMR spectrophotometer at 400 MHz using DMSO-d^{6} as the solvent and tetra methyl silane (TMS) as internal standard. The chemical shifts are expressed in δ ppm. The splitting patterns were designated as follows; s: singlet; d: doublet; q: quartet; m: multiplet. LCMS were recorded by using Shimadzu LCMS- 2010A instrument by ESI. Molecular ion (M+) Synthesis of the target compounds was accomplished according to the steps depicted in **Scheme.1**.



Scheme 1: Synthesis of 1,2,3-bistriazoles via 1,3-dipolar cycloaddition reactions (4a-i).

General method for the preparation of 1,2,3-bistriazoles (4a-i)

To a stirred solution of 1, 3-dibromopropane (1.5 mmol) in PEG 400: H_2O (4:1) 15 ML, NaN_3 (3.2 mmol), CuI (0.6 mmol), sodium ascorbate (2.2 mmol) and phenyl acetylene (3.1mmol) were added. The reaction mixture was stirred at 45-50^{O}C temperature for 6-9

hours. The progress of reaction monitored by TLC. Then, aqueous NH_4OH and CH_2Cl_2 were added in the reaction mixture and the organic layer was separated and washed with water, brine solution and dried by $MgSO_4$. The organic solvent was evaporated under reduced pressure to get crude product. The isolated crude product was recrystallized from ethanol to obtain pure compound (4a). Other derivatives were prepared by similar method.

Spectral data of representative compound

Synthesis of 1, 3-bis (4-phenyl-*1H*-1, 2, 3-triazol-1-yl) propane (4a)

Yield: 85%, **MF/FWt**: $C_{19}H_{18}N_6$ /330.16, **MP:** 130-132^oC, **IR** (cm^{-1}): 2981, 2889, 1595, 1335, 1107, 707:^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.83 (d, 2H *J*=3Hz, Ar-H), 7.33 (t, 1H, Ar-H), 7.44 (t, 2H, *J*=9Hz, Ar-H), **8.61 (s, 1H, triazoles)**, 4.45 (t, 2H, N-CH_2-CH_2-), 3.41 (quintet, 2H,-CH_2-CH_2-CH_2-): **MS:** m/e 330

Synthesis of 1, 3-bis (4-p-tolyl-*1H*-1, 2, 3-triazol-1yl) propane (4b)

Yield: 52 %,**MF/FWt**: $C_{21}H_{22}N_6$ / 358.19, **MP:** 135-137^oC,**IR** (cm^{-1}) : 2950, 1628, 1588, 1430, 1265, 1233, 797. **^{1}H NMR** (300 MHz, $CDCl_3$, δ ppm): 7.44 (d, 2H, Ar-H), 7.83 (d, 2H, *J*=3Hz, Ar-H), **8.61 (s, 1H, triazole)**, 4.41 (t, 2H, N-CH_2-CH_2-), 2.48 (quintet, 2H, -CH_2-CH_2-CH_2-), 2.56 (s, 3H, Ar-CH_3).

Synthesis of (1, 1'- (propane-1, 3-diyl) bis (*1H*-1, 2, 3-triazol-4,1diyl) dimethanol (4c)

Yield: 75 %:**MF/FWt:** $C_9H_{14}N_6O_2$ / 238.12: **MP:** 140-142^oC.**IR (cm^{-1})**: 3648, 2922, 1617, 1588, 1430, 1265, 1233, 797:**^{1}H NMR** (300 MHz, $CDCl_3$, δ ppm): **8.14 (s, 1H, triazole),** 3.35 (s, 1H,-OH), 5.34 (s, 2H-CH_2-), 4.39 (t, 2H, N-CH_2-CH_2-), 2.47 (quintet, 2H, -CH_2-CH_2-CH_2-).

Synthesis of 1, 3 –bis (4-bromomethyl)-*1H*-1, 2, 3-triazol-1yl) propane (**4d**)

Yield: 69%; **MF/FWt:** $C_9H_{12}Br_2N_6$ / 361.95.; **MP:** 142-144^oC; **IR (cm^{-1}):** 2988, 1655, 1578, 1430, 1235, 1273, 707; **^{1}H NMR** (300 MHz, $CDCl_3$, δ ppm): **8.18 (s, 1H, triazole)**, 5.68 (s, 2H, -CH_2-), 4.47 (t, 2H, N-CH_2-CH_2-), 3.42 (quintet, 2H, -CH_2-CH_2-CH_2-).

Synthesis of 1, 3-bis (4-(((4-bromobenzyl)oxy)methyl) *1H*-1, 2, 3-triazol-1-yl) propane (4e)

Yield: 76%; **MF/FWt:** $C_{23}H_{24}Br_2N_6O_2$ / 576.28; **MP:** 150-152^oC; **IR (cm^{-1})** : 2938, 1622, 1588, 1430,1265, 1233, 797; **^{1}H NMR** (300 MHz, $CDCl_3$, δ ppm): 8.22 (d, 2H, *J*=6Hz, Ar-

H), 7.26 (d, 1H, *J* =9Hz, Ar-H), **8.31 (s, 1H, triazole)**, 5.32 (s, 2H, -CH_2-O-), 4.62 (s, 2H, -O-CH_2-).4.45 (t, 2H, N-CH_2-CH_2-), 2.39 (quintet, 2H, -CH_2-CH_2-CH_2-).

Synthesis of 1, 3-bis (4-(phenoxymethyl) *1H*-1, 2, 3-triazol-1-yl) propane (4f)

Yield: 78 %; **MF/FWt:** $C_{21}H_{22}N_6O_2$ /390.44; **MP:** 150-152^oC; **IR (cm^{-1})** : 2981, 1652, 1588, 1405, 1227, 858; **^{1}H NMR** (300 MHz, $CDCl_3$, δ ppm): 7.32 (t, 1H, *J*=6Hz, Ar-H), 7.44 (t, 2H, *J*=9Hz, Ar-H), 7.84 (d, 2H, *J*=6Hz, Ar-H), **8.16 (s, 1H, triazole),** 5.16(s, 2H, -CH_2-O-), 4.35 (t, 2H, N-CH_2-CH_2-), 2.42 (quintet, 2H, -CH_2-CH_2-CH_2-).

Synthesis of 1, 3-bis (4-((benzyloxy)methyl)-*1H*-1, 2, 3-triazol-1yl) propane (4g)

Yield: 65%; **MF/FWt:** $C_{23}H_{26}N_6O_2$ / 418.49; **MP:** 155-157^oC; **IR (cm^{-1})** : 2938, 1622, 1588, 1430,1265, 1233, 797; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.22 (d, 2H, *J*=6Hz, Ar-H), 8.31(d, 2H, *J*=3Hz, Ar-H), 7.26 (t, 1H, *J*=9Hz, Ar-H), **8.31 (s, 1H, triazole)**, 5.32 (s, 2H, -CH2-O-), 4.62 (s, 2H, -O-CH_2-) 4.43 (t, 2H, N-CH_2-CH_2-), 2.40 (quintet, 2H, -CH_2-CH_2-CH_2-); **^{13}C NMR** (400 MHz, $CDCl_3$, δ ppm): 127.8, 128.4, 127.9, 137.78, 145.55, 72.70, 63.63, **128.46**, 46.71, 30.58.

RESULTS AND DISCUSSION

Chemistry

Melting points of all 1,2,3-bistriazole derivatives were determined in open glass capillaries and uncorrected. These 1,2,3-bistriazole derivatives were synthesized by reacting equimolar amount of 1,3 dibromopropane, sodium azide and different substituted alkynes. These 1,2,3-bistriazole **(4a-i)** were characterized by ^{1}H NMR spectra were recorded in $CDCl_3$ and DMSO-d6 on Broker Avance-400 MHZ spectrometer operating procedure using TMS as an internal standard (Chemical shift are given in ð ppm). The mass spectra (MS) were recorded on EIMS 40-600 mass spectrometer and DI-NFDD.

IR spectra of the 1,2,3-bistriazoles in KBr pellets exhibit v 2981, 2889, 1595, 1107, 707 (in cm^{-1}). In IR spectrum azido and alkyne peak are disappeared to confirmed 1, 2, 3-triazole formation of compounds **(4a-4i).** These assignments are in agreement with those by research groups.[12] The ^{1}H-NMR spectra of the 1, 2, 3-bistriazole were recorded in $CDCl_3$ and DMSO-d6 using TMS as the standard. The chemical shift value of triazole ring proton significantly observed at ð 8.61-8.14 ppm. These findings are in agreements with those observed by different workers.[13]

GC-MS of the 1, 2, 3-bistriazole compounds shown the expected molecular ion peaks which confirm the molecular formula of each compound and found to be agreement with the literature.[14]

Biological Evaluation

***In vitro* antibacterial Activity**

The 1, 2, 3-bistriazole derivatives **(4a to 4f)** were dissolved in dimethyl sulfoxide (DMSO) with required concentrations for bioassay. Antibacterial activity was evaluated by screening of the compounds by standard method i.e. agar disc diffusion method[15] against *S. aureus, S. epidermidis, S.marscesens, P.aeruginosa and B. substilis* (Shown in **Table No.1**).

Carcinogenicity study

In carcinogenicity study of 1,2,3-bistriazoles, except **4e** (only at 1mg and 2mg concentrations) none of the compounds showed MIC in the minimum concentrations, suggests that these compounds do not exhibit any deleterious effect or toxicity to bacteria cell in this study.

Stannous chloride is a toxic chemical which induces free radicals, showed MIC at 0.25mg, shown in **Table2.** A carcinogen is an agent that can cause cancer and carcinogens can be chemicals, viruses, hormone, ionizing radiation or solid materials. Carcinogens produce cancer by changing the information that cells receive from their DNA, causing immature cells to accumulate in the body rather than differentiate into normal functional cells. Carcinogens may be *genotoxic* carcinogen. Other carcinogens may change how DNA expresses its information without changing its structure directly or may create a situation in a cell or tissue that makes it more susceptible to DNA damage from other sources. These are known as nongenotoxic carcinogens or promoters.

Table No. 1: Antibacterial activity of 1,2,3-bistriazoles (4a-f).

Sr.No.	Comp. Code	*S.aureus*	*S.epidermidis*	*S.marscesens*	*P.aeruginosa*	*B. substilis*
1	4a	14(10)	10(10)	**30(10)**	-	12(10)
2	4b	10(10)	-	18(10)	13(10)	10(10)
3	4c	12(10)	13(10)	**29(10)**	-	**29(10)**
4	4d	-	**29(10)**	12(15)	-	-
5	4e	-	-	12 (15)	12(15)	-
6	4f	**18(25)**	-	22(25)	-	12(25)
streptomycin		17(10)	25(10)	25(10)	15(10)	20(10)

a. Bold values indicate better results.

b. (-) not found results

c. Values indicate zone of inhibition in mm.

d. Bracket values indicate concentration of solution (µg/ml).

Table No. 2: Carcinogenicity for 1, 2, 3-bistriazoles (4a-f).

E. coli AB 1157						
Zone of Inhibition (mm)						
Compounds code	**0.125mg**	**0.25mg**	**0.5mg**	**1.0mg**	**2.0mg**	**MIC in mg**
4a	0	0	0	0	0	1.0
4b	0	0	0	0	0	>2.0
4c	0	0	0	0	0	>2.0
4d	0	0	0	0	0	>2.0
4e	0	0	0	**2**	**5**	>2.0
4f	0	0	0	0	0	>2.0
Stannous chloride	0	2	6	9	11	0.25

CONCLUSION

Applying the principles of 'Green Chemistry for these reactions, **PEG-400** used as solvent is not only economical but also environmental friendly and in adherence to the various principles of green chemistry. PEG-400 is a benign reaction medium than other organic solvents as well PEG is potentially reusable and nontoxic or non hazardous reaction medium.

The importance of such work lies in the possibility that the new compounds might be more efficacious drugs against bacterial activity, which could be helpful in the designing of non carcinogenic and more potent antibacterial agents for therapeutic use.

We have concluded that 1, 2, 3-bistriazoles derivatives **4a** against *S.marscesens*, **4c** *S.marscesens and Bacillius substilis*, **4d** against *S. epidermidis* and **4f** against *S.aureus* were found potent antibacterial shown in **Table 1.** In carcinogenicity study of 1, 2, 3-bistriazole derivatives except **4e** (only at 1mg and 2mg concentrations) none of the compounds do not exhibit any deleterious effect or toxicity to the E.*coli* bacteria as shown in **Table 2**.

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