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SYNTHESIS, ANTIBACTERIAL AND ANTI BIO-FILM ACTIVITY OF SOME NEW 1,2,3 TRIAZOLES ON BENZOXAZOLE NUCLEUS

Mood Shilpa, GugulothHanmanthu*

**Department of Chemistry, Kakatiya University, Warangal, Telangana, India-506009.*
Email:hanmanthu.guguloth@yahoo.com

ABSTRACT: A series of benzoxazole fused 1,2,3 triazoles have been synthesised by click chemistry method, The structures of the newly synthesised compounds were assessed by Infra red, NMR and Mass spectroscopic methods. The title compounds were evaluated for their antibacterial activity using zone of inhibition, MIC method and antibiofilm activity, some of the molecules (5e, 5g,5i and 5j) have shown excellent activity and remaining compounds were also exhibit moderate activity against the test organisms employed.

KEY WORDS: Synthesis, 1,2,3, triazoles, Antibacterial , Antibiofilm activity.

INTRODUCTION:

Synthesis of novel heterocyclic molecules being recognized as an urgent materials and become a great challenge for chemists aiming to address the present world demands[i-ii] Benzoxazole derivatives are an important class of heterocyclic moieties, numerous reports have been published on this nucleus and its derivatives exhibit various biological activities[iii] such as antitubercular[iv], antifungal and anti-inflammatory activity[v], anthelmintic[vi], anticonvulsant[vii] and lipoxygenase inhibitors[viii]. Recently, it was found that the 1,2,3 triazoles occupied a unique position in the field of pharmaceutical chemistry.[ix-x] 1,2,3 triazole have been identified as anti-inflammatory[xi], anticonvulsant[xii], anti protozoal[xii],kinage inhibitors[xiv]. The derivatives of 1,2,3 triazoles are also proved promising activity in medicinal chemistry [xv-xvi]. In recent research reveals that the combining 1,2,3 –triazoles and other heterocyclic compounds were showed enhanced biological activity [xvii-xviii] . The current research reveals that the 1,2,3 triazoles and its derivatives has been recognised as most important compounds. Hence, in this direction, efforts have been undertaken to establish the combination of benzoxazole and1,2,3–triazole using click chemistry, the study is the synthesis of 5-(5-methyl-1-((Substituted phenyl-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazol-3-yl)-2-phenylbenzo[*d*] oxazole, which were evaluated for their antibacterial, and antibiofilm activity.

EXPERIMENTAL SECTION:

MATERIALS AND METHODS:

All the solvents and starting materials were purchased from commercial sources and used without further purification. Column chromatography was performed on silica gel 60–120 mesh. Melting points were determined using a Cintex apparatus. Elemental analysis was measured by means of PerkinElmer 2400 CHN elemental analyzer. Infrared (IR) spectra were obtained on a PerkinElmer BX serried FTIR 5000 spectrometer using KBr pellet. A400-MHz nuclear magnetic resonance (NMR) spectrometer was used to acquire 1H-NMR spectra. Mass spectra were recorded by using Electrospray ionisation–Mass Spectrometry (ESI–MS).

ANTIBACTERIAL ACTIVITY:

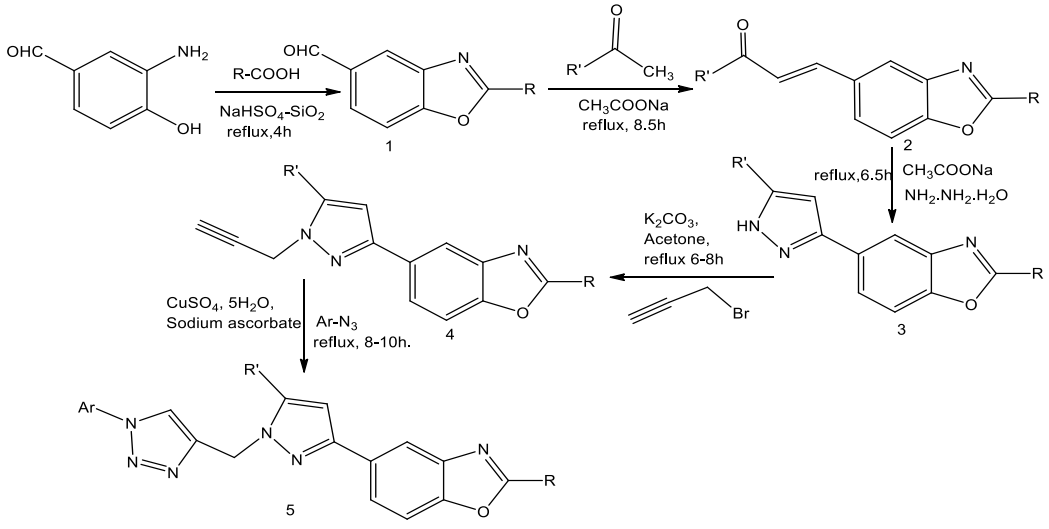
BACTERIAL STRAINS

Escherichia coli (ATCC 8739), *Klebselia pneumoniae* (ATCC13883), *Methicillin-resistantStaphylococcus aureus* (MRSA, NCTC 13616), *Salmonella typhi* (ATCC 4420) were obtained from the Kakatiya Medical College, Warangal Urban. MRSA was cultured and maintained on Mannitol Salt Agar medium augmented with 7.5% sodium chloride. The other bacterial strains were maintained on Luria-Bertani (LB) medium (purchased from Hi-media Laboratories, Mumbai, India). All the bacterial cultures were incubated at 37°C for 24 h. All strains were sub cultured on to nutrient agar medium for bioassays examination. The cultures were grown and the turbidity was adjusted with sterile broth to obtain a half of MC Farland standard (1x108 - 5x108 cfu/ml). This was used as starting inoculum for the assay.

ANTIBIOFILM ACTIVITY:GROWING A BIO-FILM

The ability of the selected compounds to prevent biofilm development or destruction of preformed biofilm was investigated by the standard method[xix]. A 100µl aliquot of standardized concentration of cultures with OD560 =0.05(5×10^3 CFU/ml) was added into individual flat-bottomed 96-well micro titre plates containing LB medium. The micro titre plate was incubated to develop a multilayer biofilm for about 24h (irreversible attachment phase) and 48h (mature biofilm) at 37°C. Following, different concentrations of the compounds (1000-0 µg/mL) were added into the wells of a 96-well micro titre plate and the plates were incubated further at 37°C for 24h. Wells with only media is served as negative control. The biofilm biomass was assayed using the crystal violet (CV) staining assay[xx].

SCHEME:



R= C_6H_5, R'= CH_3,

Table 1: Substituents of compounds (5a-5j):

Compd	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	**5j**
Ar	C_6H_5	3,5-dichloro	$2NO_2$	3,5dimethyl	4-Br	2-CF3	2,4-di Chloro	4-Cl	4-OH	$3OCH_3$
Yield(%)	**63**	**56**	**59**	**64**	**52**	**48**	**53**	**72**	**67**	**71**

Synthesis of 2-phenylbenzo[d]oxazole-5-carbaldehyde (1):

Equimolar mixture of 2-amino phenol (0.03 mmol), benzoic acid (0.03 mmol) and $NaHSO_4$-SiO_2 (25% wt.) in 10 mL of ethanol was heated under reflux at 180ºC for 4 h. The reaction mixture was partially cooled, poured on to crushed ice and neutralized with10% NaOH solution. The precipitated product was collected by vacuum filtration, washed with excess 10% NaOH solution was dried and recrystallized from ethanol.

Synthesis of 4-(2-phenylbenzo[d]oxazol-5-yl) but-3-en-2-one (2):

To a solution of 2-phenylbenzo[*d*]oxazole-5-carbaldehyde(1) (0.01 mol) in ethanol (60 ml), acetone (0.01 mol) and a few drops of glacial acetic acid were added and the mixture refluxed for 8.5 hr. It was then cooled concentrated and poured into crushed ice and filtered. The solid thus obtained was purified by recrystallisation from ethanol.

Synthesis of 5-(5-methyl-1H-pyrazol-3-yl)-2-phenylbenzo[d]oxazole (3):

Equimolar mixture of 2 (0.03 mol), hydrazine hydrate (0.03mol) and anhydrous CH_3COONa (0.001mol) in glacial acetic acid (30ml) were heated under reflux for about 6.5 hours, the resulting compound was cooled at room temperature and poured in to crushed ice. The product was filtered, washed with water and recrystallized with ethanol to afford the pure compound.

Synthesis of 5-(5-methyl-1-(prop-2-yn-1-yl)-1H-pyrazol-3-yl)-2-phenylbenzo[d]oxazole (4):

A mixture of compound 3 (0.05mol) and K_2CO_3 (0.15mol) in acetone (30 mL) was treated with propargyl bromide (0.05mol) and the reaction mixture was stirred at room temperature for about 6-8 h. The Progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was poured carefully in ice-cold water (50mL) and extracted with ethyl acetate (2×15mL). The combined organic layer was washed with brine water and dried over anhydrous Na_2SO_4, and then the organic layer was filtered, washed and dried under *vacuum* to give the corresponding compound.

5-(5-methyl-1-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazol-3-yl)-2-phenyl benzo[d]oxazole (5):

To a stirred solution of compound (4) (1.0 mmol) and aryl azide (2.0 mmol) in aq (1:1) tBuOH (15mL) was added $CuSO_4$ (10mol %) and sodium ascorbate. The reaction mixture was stirred at room temperature for about 8–10 h. After completion of the reaction, the reaction mixture was diluted with water (15mL) and the product was extracted with ethyl acetate (2×15mL). The combined organic layer was washed with brine water and dried over anhydrous Na_2SO4. After filtration, the solvent was evaporated under vacuum and the crude compounds were purified by column chromatography using silica gel (60-120 mesh) and hexane/ethyl acetate gradient system as an eluent to afford the title compounds.

The remaining compound (5b-h) was prepared by similar procedure with minor changes as per the reaction conditions.

5-(5-methyl-1-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazol-3-yl)-2-phenylbenzo[d] oxazole (5a):

^{1}HNMR (400MHz, DMSO-d_6,δ ppm): 2.45 (s, 3H,CH_3),5.42(s,2H,CH_2), 6.88 (s,1H, CH-Ar-pyrazole), 7.24-7.36(m,4H,Ar-H),7.44-7.54(m,5H,Ar-H),7.66-7.85(m,4H,Ar-H), 8.08 (s,1H, triazole): IR (KBr) λ_{max} in (cm^{-1}): 3135 (C-H,triazole), 3086(C-H-alkyl), 1588 (C=C), 1545 (C=N): MS: *m*/*z* 432 (M+); Anal. Calcd for $C_{26}H_{20}N_6O$: C, 72.21; H, 4.66; N, 19.43 %. Found: C, 72.05; H, 4.43; N, 19.05%.

5-(1-((1-(3,5-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-1H-pyrazol-3-yl)-2-phenylbenzo[d]oxazole (5b):

^{1}HNMR (400MHz, DMSO-d_6,δ ppm): 2.42(s, 3H,CH_3),5.27(s,2H,CH_2), 6.28(s,1H,CH-Ar-pyrazole),7.48(t, 3H,Ar-H),7.69(s, 3H,Ar-H),7.80-7.84(m, 3H,Ar-H), 8.14(dd,*J*= 7.6 Hz, 2H,Ar-H),8.21 (s,1H, triazole): IR (KBr) λ $_{max}$ in (cm^{-1}): 3135 (C-H,triazole), 3086(C-H-alkyl), 1588 (C=C), 1545 (C=N), 744(C-Cl halo),: MS: *m*/*z*501 (M+); Anal. Calcd for $C_{26}H_{18}Cl_2N_6O$: C, 62.29; H, 3.62; N, 16.76 %. Found: C, 62.05; H, 3.43; N, 16.05%.

5-(5-methyl-1-((1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazol-3-yl)-2-phenylbenzo[d]oxazole(5c):

^{1}HNMR (400MHz, DMSO-d_6,δ ppm): 2.44(s, 3H,CH_3),5.22(s,2H,CH_2), 6.36 (s,1H,CH-Ar-pyrazole),7.42-7.52(m, 3H,Ar-H),7.75-7.94(m, 5H,Ar-H), 8.34(dd,*J*= 9.8 Hz, 2H,Ar-H), 8.06 (s, 1H,Ar-H),8.08 (s,1H, triazole), 8.25(s, 1H,Ar-H): IR (KBr) λ max in (cm^{-1}): 3136 (C-H,triazole), 3086(C-H-alkyl), 1588 (C=C), 1545 (C=N): MS: *m*/*z*478 (M+); Anal. Calcd for $C_{26}H_{19}N_7O_3$: C, 65.40; H, 4.01; N, 20.53 %. Found: C, 65.05; H, .83; N, 19.95%.

5-(1-((1-(3,5-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-1H-pyrazol-3-yl)-2-phenylbenzo[d]oxazole (5d):

^{1}HNMR (400MHz, DMSO-d_6,δ ppm):2.35(s, 6H,CH_3), 2.42(s, 3H,CH_3),5.34(s,2H,CH_2), 6.14(s,1H,CH-Ar-pyrazole), 7.35(s, 1H,Ar-H), 7.38(s, 2H,Ar-H),7.42-7.53(m, 3H,Ar-H),7.79-7.90 (m, 2H, Ar-H), 7.96(dd,*J*= 7.1 Hz, 2H,Ar-H),8.02 (s,1H, triazole) 8.09(s,1H,Ar-H): IR (KBr) λ $_{max}$ in (cm^{-1}): 3135 (C-H,triazole), 3086(C-H-alkyl), 1588 (C=C), 1545 (C=N): MS: *m*/*z*461 (M+); Anal. Calcd for $C_{28}H_{24}N_6O$: C, 73.02; H, 5.25; N, 18.25%. Found: C, 72.85; H, 5.03; N, 18.05%.

5-(1-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-1H-pyrazol-3-yl)-2 phenyl benzo[d]oxazole (5e):

^{1}HNMR (400MHz, DMSO-d_6,δ ppm):2.51(s, 3H,CH_3),5.06(s,2H,CH_2) 6.27 (s,1H,CH-Ar-pyrazole), 7.41(dd, *J* = 8.1 Hz, 1H, Ar-H), 7.51-7.60(m, 6H,Ar-H),7.79-7.90(m, 2H,Ar-H), 8.36(dd,*J*= 8.5 Hz, 2H,Ar-H),8.18 (s,1H, triazole), 8.09(s,1H,Ar-H): IR (KBr) λ $_{max}$ in (cm^{-1}): 3135 (C-H,triazole), 3086(C-H-alkyl), 1588 (C=C), 1545 (C=N)744(C-Br halo),: MS: *m*/*z*511 (M+); Anal. Calcd for $C_{26}H_{19}BrN_6O$: C, 61.07; H, 3.74; N, 16.43%. Found: C, 60.95; H, 3.63; N, 16.05%.

5-(1-((1-(2-trifluoro methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-1H-pyrazol-3-yl)-2-phenylbenzo[d]oxazole (5f):

^{1}HNMR (400MHz, DMSO-d_6,δ ppm):2.38(s, 3H,CH_3),5.26(s,2H,CH_2) 6.31(s,1H,CH-Ar-pyrazole), 7.41(dd, *J* = 8.1 Hz, 1H, Ar-H), 7.38-7.58(m, 5H,Ar-H),7.79-7.85(m, 2H,Ar-H), 8.23(dd,*J*= 8.8 Hz, 2H,Ar-H),8.22 (s,1H, triazole) 8.09(s, 1H,Ar-H): IR (KBr) λ $_{max}$ in (cm^{-1}): 3135 (C-H,triazole), 3086(C-H-alkyl), 1588 (C=C), 1545 (C=N)744(C-F halo),: MS: *m*/*z*501 (M+); Anal. Calcd for $C_{27}H_{19}F_3N_6O$: C, 64.80; H, 3.83; N, 16.79%. Found: C, 64.65; H, 3.63; N, 16.65%.

5-(1-((1-(2,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-1H-pyrazol-3-yl)-2-phenylbenzo[d]oxazole (5g):

^{1}HNMR (400MHz, DMSO-d_6,δ ppm):2.51(s, 3H,CH_3),5.21(s,2H,CH_2), 6.46(s,1H,CH-Ar-pyrazole), 7.41(dd, *J* = 8.1 Hz, 1H, Ar-H), 7.38-7.68(m, 5H,Ar-H),7.79-7.85(m, 2H,Ar-H), 8.21(dd,*J*= 7.3 Hz, 2H,Ar-H), 7.95 (s,1H, triazole) 8.09(s, 1H,Ar-H): IR (KBr) λ $_{max}$ in (cm^{-1}): 3135 (C-H,triazole), 3086(C-H-alkyl), 1588 (C=C), 1545 (C=N)744(C-Cl halo),: MS: *m*/*z*501 (M+); Anal. Calcd for $C_{26}H_{18}Cl_2N_6O$: C, 62.29; H, 3.62; N, 16.76%. Found: C, 62.05; H, 3.43; N, 16.35%.

5-(1-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-1H-pyrazol-3-yl)-2-phenylbenzo[d]oxazole(5h):

^{1}HNMR (400MHz, DMSO-d_6,δ ppm):2.32(s, 3H,CH_3),5.16(s,2H,CH_2), 6.02 (s,1H,CH-Ar-pyrazole), 7.41(dd, *J* = 8.1 Hz, 1H, Ar-H), 7.49-7.56(m, 6H,Ar-H),7.79-7.86(m, 2H,Ar-H), 8.31(dd,*J*= 8.6 Hz, 2H,Ar-H),8.21(s,1H, triazole), 8.09(s, 1H,Ar-H): IR (KBr) λ_{max} in (cm^{-1}): 3135 (C-H,triazole), 3086(C-H-alkyl), 1588 (C=C), 1545 (C=N)744(C-Cl halo),: MS: *m*/*z*467 (M+); Anal. Calcd for $C_{26}H_{19}ClN_6O$: C, 66.88; H, 4.10N, 18.00%. Found: C, 66.65; H, 4.03; N, 17.85%.

5-(1-((1-(4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-1H-pyrazol-3-yl)-2-phenylbenzo[d]oxazole(5i):

^{1}HNMR (400MHz, DMSO-d_6,δ ppm):2.48(s, 3H,CH_3),5.03(s,2H,CH_2), 5.35(s, broad,1H, phenolic), 6.16(s,1H,CH-Ar-pyrazole), 6.96(dd, *J* = 7.8Hz, 2H, Ar-H), 7.42-7.51(m,5H,Ar-H),7.79-7.86(m, 2H,Ar-H), 8.12(dd,*J*= 7.4 Hz, 2H,Ar-H),8.02(s,1H, triazole) 8.09(s, 1H,Ar-H): IR (KBr) λ_{max} in (cm^{-1}): 3554(O-H hydroxy), 3135 (C-H,triazole), 3086(C-H-alkyl), 1588 (C=C), 1545 (C=N), 1152 (C-O) : MS: *m*/*z*489 (M+); Anal. Calcd for $C_{26}H_{20}N_6O_2$: C, 69.63; H, 4.49; N, 18.74%. Found: C, 69.55; H, 4.33; N, 18.45%.

5-(1-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-5-methyl-1H-pyrazol-3-yl)-2-phenylbenzo[d]oxazole(5j):

^{1}HNMR (400MHz, DMSO-d_6,δ ppm):2.36(s, 3H,CH_3), 3.84(s, 3H, OCH_3),5.09(s,2H,CH_2), 6.38(s,1H,CH-Ar-pyrazole), 6.99(dd, *J* = 7.8Hz, 2H, Ar-H), 7.42-7.51(m, 5H,Ar-H),7.79-7.86(m, 2H,Ar-H), 8.05(dd,*J*= 9.2 Hz, 2H,Ar-H),8.16(s,1H, triazole), 8.09(s, 1H,Ar-H): IR (KBr) λ_{max} in (cm^{-1}): 3135 (C-H,triazole), 3086(C-H-alkyl), 1588 (C=C), 1545 (C=N), 1152 (C-O): MS: *m*/*z*463 (M+); Anal. Calcd for $C_{27}H_{22}N_6O_2$: C, 70.02; H, 4.79; N, 18.17 %. Found: C, 71.85; H, 4.63; N, 17.95%.

RESULTS AND DISCUSSION:

The synthesis of target compounds (**5 a–j)** was accomplished by a sequence shown in Scheme **1**.The target compounds (**5**) were synthesized by dipolar cyclo addition of terminal alkyne **(4)** with different substituted aryl azides using a catalytic amount of $CuSO_4.5H_2O$ and sodium ascorbate refluxed for about 6-8hours using (1:1) aq. ^{t}BuOH as a green solvent to afford the title compound, disubstituted 1,2,3-triazoles in moderate to good yields. The compound (**4**) was prepared by treating the compound (**3**) with selective propargylation of 3-bromoprop-1-yne in the presence of K_2CO_3 in acetone. The synthesis of compound**1** is a new and ecofriendly method using $NaHSO_4$-SiO_2 as a catalyst. It is heterogeneous and ecofriendly catalyst. The structures of all the synthesized compounds 5(a-j) were established with the aid of their spectral (IR, NMR and mass) and elemental (C, H and N) analysis. Analytical and spectral data of all the synthesized compounds were in full agreement with the proposed structures and also discussed for a representative compound 5a. From the IR spectrum the sharp bands at 3135,1545 cm^{-1} indicate the presence of C-H triazole group and C=N stretching, a sharp band at 1585 cm^{-1} shows the C=C. From the ^{1}H NMR spectrum, the appearance of signal at δ 8.08 ppm indicates a traizole proton and a multiplate signals at δ 6.89-7.10 and 7.21-7.35ppm indicates the presence of aromatic protons. The singlet at δ 5.42 ppm shows -CH_2- protons and 6.27 ppm shows aromatic –CH proton of pyrazole. Mass spectrum of the title compounds were determined by molecular ion peak at m/z of corresponding molecular weights.

ANTIBACTERIAL ACTIVITY:

Table:2 Antibacterial activity of compound 5a-5j:

Comp.	**Conc.** (µg/mL)	**MRSA**	**S.Typi**	**K. *Pneumoniae***	**E.*coli***
		Zoe of Inhibition (mm)			
5a	50	2.40±0.08	--	4.30±0.11	4.12±0.15
	100	2.74±0.05	--	7.14±0.14	5.26±0.12
5b	50	5.50±0.09	--	5.51±0.06	6.16±0.10
	100	8.64±0.11	--	7.24±0.15	8.60±0.12
5c	50	3.42±0.10	3.75±0.08	7.10±0.06	4.17±0.21
	100	5.35±0.13	4.15±0.15	10.5±0.12	6.35±0.13
5d	50	5.60±0.08	--	3.16±0.15	3.10±0.10
	100	8.23±0.14	--	5.12±0.12	5.18±0.13
5e	50	8.51±0.10	3.37±0.15	11.8±0.21	15.17±0.14
	100	11.5±0.21	5.55±0.17	13.0±0.12	20.32±0.24
5f	50	5.12±0.16	--	7.22±0.21	9.28±0.22
	100	6.55±0.11	--	10.28±0.17	14.27±0.12
5g	50	17.20±0.22	11.6±0.15	15.25±0.20	19.14±0.20
	100	22.12±0.19	15.4±0.12	19.23±0.24	24.15±0.26
5h	50	3.21±0.26	--	3.25±0.26	8.06±0.21
	100	5.23±0.12	--	4.42±0.18	10.25±0.11
5i	50	14.15±0.14	8.57±0.09	14.6±0.33	18.23±0.21
	100	19.15±0.17	12.7±0.17	18.54±0.19	22.25±0.12
5j	50	4.25±0.18	--	5.55±0.28	6.21±0.12
	100	5.18±0.28	--	7.14±0.12	8.41±0.09
Ampicillin	50	19.16±0.1	15.21±0.31	17.05±0.12	21.23±0.23
	100	23.05±0.22	21.3±0.15	21.28±0.12	27.05 ±0.2

Zone of inhibitions are represented as Mean Standard deviation (SD). n=4

Table 3: Minimum Inhibitory Concentration (MIC/µg/mL) Compounds 5a-5j

Comp.	**MRSA**	**S.Typi**	**K. pneumoniae**	**E.coli**
5a	23.6	--	25.8	21.4
5b	20.8	--	22.6	22.5
5c	25.6	--	16	28.2
5d	14.9	--	23.1	19.1
5e	9.22	14.7	5.44	14.2
5f	24.05	--	26.4	16.6
5g	15.1	13.7	16.5	13.9
5h	25.25	--	30.3	18.4
5i	6.15	8.14	6.22	4.62
5j	3.18	3.63	3.22	3.95
Standard	2.55	3.4	2.85	2.62

All the synthesized compounds exhibited significant antibacterial activity against multidrug resistance pathogens. The activity was found to be concentration dependent. However, certain compounds tested were have shown poor activity or did not exhibit the activity. Based on results, the compounds 5i and 5j showed significant activity ($p<0.01$) in Minimum Inhibitory Concentration (MIC) 3.18, 4.62 µg/mL respectively against MRSA and E.Coli (table 2).In addition, these compounds were also found to having exhibit significant activity ($p<0.01$) the compounds 5i & 5g shown 19.15, 22.12mm against MRSA, the compounds 5g &5i shown 19.23, 18.54mm against K.Pne, compounds 5e & 5i shown 22.25, 20.32 mm against E.coli respectively (table1). Ampicillin was taken as reference drug and the results were compared with it. With comparison of the results, it can be seen that compound 5j is highly competing with the standard in the inhibition of multidrug resistance bacteria (Table 2, and 3). Compounds 5i and 5j were further screened for their capability to inhibit the formation of biofilm in view of significant antibacterial activity of them.

ANTI BIOFILM ACTIVITY:

Table 4 : Bio-film Inhibitory Concentration (BIC/µg/mL) Compounds 5d&5e:

Comp.	**MRSA**	**S.Typi**	**K. pneumoniae**	**E.coli**
5i	8.23± 0.5	7.56±0.11	6.25±0.5	6.62±1.5
5j	2.22±0.56	3.05±0.7	3.25±1.0	2.03±0.02
Std	1.33±0.02	2.66±0.5	3.11±0.5	3.22±0.05

Anti biofilm activity of the compounds 5i, 5j, at different concentrations (0-1000 µg/mL) were screened against multidrug resistance bacterial strains. As per table 3, the biofilm inhibition concentration (BIC) was found significant with compound 5j. Compound 5j exhibited immense inhibition 2.22±0.56 µg/mL ($P<0.001$) against MRSA. In addition, 5j was also found significant against E. *coli* and S.typi wih BIC 2.03±0.02 and 3.05±0.7 µg/mL ($P<0.001$) respectively, whereas, antibiofilm activity of 5j against K. *Pneumonia* also found high with BIC 3.25±1.0 ($P<0.001$). By the results obtained in the study it is clear that the compound 5j was equally compete in the inhibition of biofilm with the reference drug ampicillin against the bacterial strains (Table 4).

CONCLUSION:

In conclusion, a new series of 1,2,3 triazoles were synthesized by click chemistry method. The present study reports the synthesis of triheterocyclic compounds were furnishing excellent yield. It was concluded that all the compounds were characterized by Spectroscopic techniques and evaluation of antibacterial activity of compounds (5a-j) were examined by zone of inhibition, MIC method and antibiofilm (5i&5j) activity of compounds were proven excellent activity.

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