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Synthesis of new imidazopyridine based 1,2,3-triazoles: Evaluation of antibacterial, antibiofilm and time kill studies

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The present communication elaborates the synthesis of 1,2,3-triazoles using click chemistry and their antibacterial studies. Structures of the synthesized compounds have been assessed by infra-red, NMR and mass spectroscopic methods. The title compounds have been screened for their antibacterial, antibiofilm and time kill studies. The molecules **5f**, **5b**, **5e**, **5c** and **3b** have shown excellent activity and remaining compounds have also been found to exhibit moderate activity against the test organisms employed.

Keywords: Synthesis, 1,2,3-Triazole, Antibacterial, Antibiofilm, Time kill studies

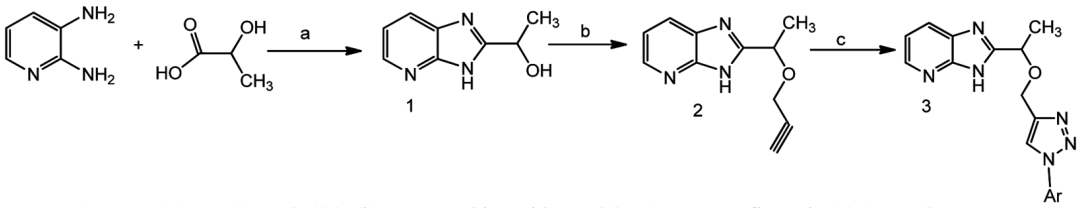
Synthesis and development of novel heterocyclic compounds is a great challenge for medicinal chemists to meet the real-world demands[1,2]. There has been an ever-increasing quest for the synthesis of new heterocycles due to their pharmacological and industrial needs[3,4].

Nitrogen, Oxygen containing heterocycles such as imidazoles, oxazoles, pyridine and triazole derivatives have emerged as important molecules and have been shown to display useful biological as well as engineering applications[5,6].

Imidazopyridine derivatives are an important class of heterocyclic moieties. Numerous reports have been published on this nucleus and its derivatives and it exhibits various biological activities such as antimicrobial[7], c-Met kinase inhibition[8], aromatase inhibition[10], anticancer[11], psychiatric and anti-neurodegenerative activities[9].

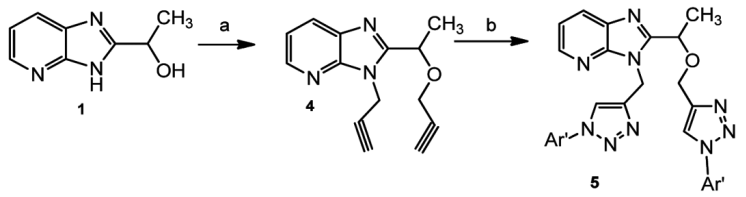
In recent years, there is a growing concern about the synthesis and development of new 1,2,3-triazoles which are associated with diverse pharmacological activities[12-14] identified as anti-inflammatory[15], anticonvulsant[16], antiprotozoal[17], kinase inhibition[18]. The derivatives of 1,2,3-triazoles were also proved to have promising activity in medicinal chemistry[19-21]. Recent research reveals that the combination of 1,2,3–triazoles with other heterocyclic compounds like imidazoles, pyridines, coumerins and benzofurans have enhanced biological activity[22-25] (Fig. 1).

In this line of research, it has been planned to develop new 1,2,3-triazoles on imidazopyridine moiety using click chemistry method. Click chemistry is a modular approach that uses the most practical and reliable chemical transformations. The focus is not only on the yield of the compound but also the eco-friendliness. In this concern Polyethylene glycol (PEG-400) has been used as a solvent in the step-1 (Scheme 1) and step-4 (Scheme 2) because PEG-400 has been found to be a green solvent and environmentally benign protocol has proved in many applications[26-28]. Hence, in this direction, efforts have been undertaken to establish the combination of benzimidazole and 1,2,3–triazole using click chemistry. The present study is the synthesis of 2-(1-((1-substituted phenyl-1*H*-1,2,3-triazol-4-yl)methoxy) ethyl)-3*H*-imidazo[4,5-b]pyridine **3a–f** and 2-(1-((1-substituted phenyl-1*H*-1,2,3-triazol-4-yl)methoxy) ethyl)-1-((1-substitutedphenyl-1*H*-1,2,3-triazol-4-yl)methyl)-3*H*-imidazo[4,5-b] pyridine **5a-f** and were evaluated for their antibacterial, antibiofilm and time kill studies.



Reaction conditions: (a) PEG-400; (b) Propargyl bromide, K_2CO_3, Acetone, reflux 5 h; (c) ArN_3, CuI, THF, reflux, 6.5h. Ar:a= Phenyl, b=4-nitrophenyl, c=Napthyl, d=2-methylphenyl, e = 3-chlorophenyl, f = 3-methoxyphenyl

Scheme 1 — Synthesis of compounds 3a-f



Reaction conditions: (a) Propargyl bromide, K_2CO_3, Acetone, reflux 6.5 h; (b) ArN_3, CuI, THF, reflux, 8h, Ar': a= Phenyl, b = 3,5 di chloro phenyl, c= 2-nitro phenyl, d = 3,5 dimethyl phenyl, e = 4-bromo phenyl, f = 2-triflouro methyl phenyl

Scheme 2 — Synthesis of compounds 5a-f

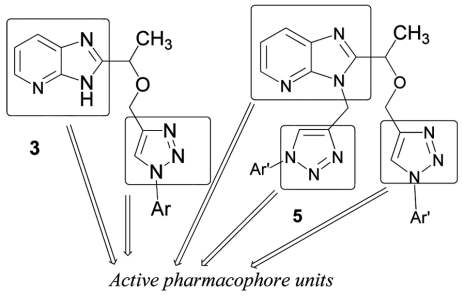


Fig. 1 — Combination of 1,2,3–triazoles with heterocyclic compounds

Experimental Details

All the solvents and materials were purchased from commercial sources. Column chromatography was performed on silica gel 60–120 mesh. The melting points were measured by Cintex apparatus. Elemental analysis was done by CHN analyser (Perkin-Elmer 240). Infrared spectra of compounds were determined by KBr pellet method using Perkin-Elmer BX series (FT IR 500). ^{1}H NMR spectra of the compounds were determined by NMR spectrophotometer (400 MHz), ^{13}C NMR spectra of compounds were determined by using NMR spectrophotometer (125 MHz). Mass spectra were recorded by using electrospray ionisation–Mass Spectrometry (ESI–MS).

General procedure for the preparation of 2-(1-(prop-2-yn-1-yloxy)ethyl)-3*H*-imidazo 4,5-b]pyridine, 2

A mixture of 1-(3*H*-imidazo [4,5-b] pyridin-2-yl) ethanol **1** (0.056mol) and K_2CO_3 (0.10mol) in acetone (30 mL) was treated with propargyl bromide (0.055mol) and refluxed for 6.5 h and completion of the reaction was monitored by TLC method. The reaction mixture was poured into crushed ice. The resulting solid was filtered, washed with excess water and product was extracted with ethylacetae (2×15 mL). The combined organic layers were washed with brine water and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure. Yield 78%.

General procedure for the preparation of 1-(prop-2-yn-1-yl)-2-(1-(prop-2-yn-1-yloxy) ethyl)-1*H*-benzo[*d*]imidazole, 2

A mixture of 1-(3*H*-imidazo[4,5-b]pyridin-2-yl) ethanol (1) (0.05 mol) and K_2CO_3 (0.15 mol) in acetone (30 mL) was treated with propargyl bromide (0.05 mol) and the reaction mixture was stirred at RT for about 8 h. Progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was poured carefully into ice-cold water (50 mL). The resulting solid was filtered, washed and dried under vacuum for about 2 h. Yield 63%.

General procedure for the preparation of 2-(1-(1-substituted phenyl 1*H*-1,2,3,triazole-4-yl)methoxy)ethyl)-3*H*-imidazo[4,5-b]pyridine, 3a–f

Equimolar solution of terminal alkyne **2** (0.05 mmol) and aryl azide (0.05 mmol) in solution of PEG-400 (15 mL) added to (5mmol %) of Cu(I), then the reaction mixture was stirred for about 4-5 h. After

completion of the reaction, the resulting mixture was diluted with water (15 mL) and the crude product was extracted with ethyl acetate (2×5 mL). The combined organic layers were washed with brine and dried with anhydrous sodium sulphate. After filtration, the solvent was evaporated under vacuum and the product was obtained, purified by column chromatography.

General procedure for the preparation of 2-(1-((1-substituted phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)ethyl)-3-((1-substituted-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-3*H*-imidazo[4,5b] pyridine, 5a–f

To a stirred solution of compound **4** (1.0 mmol) and aryl azide (2.0 mmol) in PEG-400 (15 mL) was added Cu (I) halide (10 mol%). The reaction mixture was stirred at RT for about 8–10 h. After completion of the reaction, the reaction mixture was diluted with water (15 mL) and the product was extracted with ethyl acetate (2 × 15 mL). The combined organic layers were washed with brine water and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under vacuum and the crude compounds were purified by column chromatography using silica gel (100–200 mesh) and hexane/ethyl acetate gradient system as an eluent to afford the title compound.

Methodology for antibacterial, antibiofilm and time kill studies

Bacterial strains

Escherichia coli (ATCC 8739), Vancomycin-Resistant *Enterococcus* (ATCC 2365), Methicillin-resistant *Staphylococcus aureus* (MRSA,NCTC 1616) and *Klebselia pneumoniae* (ATCC 13883) were obtained from Kakatiya Medical College, Warangal. 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 nutrient agar medium for bioassays examination. The cultures were grown and the turbidity was adjusted with sterile broth to obtain half of Mc Farland standared (1×10^8-5×10^8 CFU/mL). This was used as starting inoculums for the assay.

Growing a biofilm

The ability of the selected compounds to prevent biofilm development or destruction of preformed biofilm was investigated by the standard method[29]. A 100 µL aliquot of standardized concentration of cultures with OD_{560} = 0.05 (5×10^3 CFU/mL) was added to individual flat-bottomed 96-well micro titre plates containing LB medium. The micro titre plate was incubated to develop a multilayer biofilm for about 24 h (irreversible attachment phase) and 48 h (mature biofilm) at 37°C. Compounds in different concentrations (1000-0 µg/mL) were added into 96-well micro titre plates and the plates were incubated further at 37°C for 24 h. Wells with media is served as negative control. The biofilm and biomass were assayed with crystal violet (CV) staining[30].

Crystal violet staining assay

Staining the Biofilm

The cells were dumped by shaking and turning the plate briefly. The micro titre plates were washed gently and repeatedly for 3-4 times with sterile distilled water, air dried and then oven dried at 60°C for 35-45 min. This step removes unattached cells and media components that can be stained in the following step and mainly minimises the background staining of well. 125 µL of 0.1% solution of crystal violet was added to each well and incubated at RT for 10-15 min. The plates were rinsed 4-5 times with sterilised distilled water to get rid the plates of all excess cells and dye. At this stage, biofilm was observed as purple rings at the sidewall of the well. The plates were dried overnight and followed by quantitative assessment.

Quantitative assessment of biofilm

A quantitative assessment of biofilm formation was done by adding 125 µL of 30% acetic acid to each well. The micro titre plate was incubated at RT for 10-15 min. A 125 µL aliquot of the solubilised solution was transferred to a fresh and sterile micro titre plate and the absorbance was measured at 590 nm using a micro plate reader[31]. The mean absorbance of the samples was determined and the percentage inhibition of biofilm was determined using the equation below: Percentage (%) of inhibition = [(OD Negative control – OD Experimental)/OD of Negative control]× 100.

Time-kill kinetics assay

Time-kill kinetics of **5f** was evaluated by according to standard procedure[32]. Aliquots of compound 5f at 1 × MIC, 2 × MIC, 4 × MIC were prepared. An inoculum size of 1.0×10^6 CFU/mL was transferred to tubes containing nutrient broth and incubated 37°C for 24 h. A control test was performed for the organisms without the compound or reference antibiotics. Aliquots of 1.0 mL of the medium were taken at time intervals of 0, 1, 2, 3, 4, 5, 6, 12, and

24 h and inoculated aseptically into freshly prepared 20 mL nutrient agar plates and incubated at 37°C for 24 h. The CFU of the organisms was determined and the experiments were performed in triplicate. A graph was plotted between log CFU/mL *versus* time.

Results and Discussion

The present study reports a mild and efficient method for the synthesis of 1,2,3-triazoles using Cu(I) as catalyst and 1,3-dipolar cyclo addition of terminal alkynes with aryl azides. Copper (I) catalysed 1,2,3-triazole formation from azide and terminal alkynes is a powerful tool due to its high degree of dependability, complete specificity and bio-compatibility of the reactants.

Cuprous iodide acts as a useful precursor of organocopper compounds due to its unique chemo selectivity and reactivity and it occupies a special position in organic synthesis.

Synthesis of triazoles **3a–f** and bis-triazoles **5a–f** has been developed and accomplished by a sequence shown in Scheme 1 and Scheme 2. The intermediate 2-(1-(prop-2-ynyloxy)ethyl)-3*H*-imidazo[4,5-b] pyridine **2** was prepared by treating 1-(3*H*-imidazo[4,5b]pyridin-2-yl)ethanol **1** with selective propargylation with propargyl bromide in presence of K_2CO_3 in acetone. The final step is the synthesis of dipolar cycloaddition of terminal alkyne **3** with different substituted aryl azides using a catalytic amount of Cu (I) halide and 15 mL of PEG-400 at room temperature to afford 1,2,3-triazoles (3a–3f) in an excellent yield.

In the Scheme 2, the compound **4** was synthesized by 1:2 mole ratio of 3-(prop-2-ynyl)-2-(1-(prop-2-ynyloxy)ethyl)-3*H*-imidazo [4,5-b]pyridine with propargyl bromide. Later, this compound **4** was cyclized with substituted aryl azide using catalytic amount of Cu (I) halide in 15 mL of PEG-400 at 60°C to afford the corresponding bis 1,2,3-triazoles **5a–f** in an excellent yield. This method is easy and requires simple experimental setup with mild reaction conditions and it uses green solvent PEG-400 obtaining the compound in appreciable yield.

The chemical structures of the synthesized compounds **3a-f** were confirmed by IR, NMR and mass spectroscopy and elemental (C, H and N) analysis. From the IR spectrum, the sharp band at 3144 cm^{-1} indicates the presence of =C–H stretching, a sharp band at 1592 cm^{-1} shows the C=C stretching. From the ^{1}H NMR spectrum, the appearance of signal at δ 8.10 indicates a triazole proton and a multiple signal at δ 6.89-7.10 and 7.21-7.35 indicate the presence of aromatic protons. The singlet at δ 4.16 shows –O-CH_2- protons. Mass spectra of the title compounds were determined by molecular ion peak at *m/z* of the corresponding molecular weights.

Structures of the compounds (5a-f) were also established with their spectral (IR, ^{1}H NMR and mass) and elemental (C, H and N) analysis. IR spectrum of the compounds showed sharp absorption bands at 3167 (CH, triazole), 3078 (CH, Ar), 1610 (C=C) cm^{-1}. In the ^{1}H NMR spectrum, multiplets appear in the aromatic region at δ 7.12-7.32,7.38-7.53 and 7.56-7.68, two singlets at δ 8.17 and 8.21 show the traizole protons. Mass spectra of the title compounds were determined by molecular ion peak at *m/z* of corresponding molecular weights.

Anti-bacterial activity

All the synthesized compounds exhibited antibacterial activity against multidrug resistant pathogens. The activity was found to be concentration dependent. However, certain compounds tested have shown poor activity or did not exhibit the activity. Based on the results, the compounds **5f**, **5b** and **5d** showed significant ($p<0.01$) minimum inhibitory concentration (MIC) 2.95, 3.62, 4.92 μg/mL, respectively, against *E. coli*. In addition, these compounds were also found to be having significant activity ($p<0.01$) against Methicillin resistant *Staphylococcus aureus* (MRSA), Vancomycin resistant *Enterococcus* (VRE) and *K. pneumoniae* with MIC 3.11, 5.22, 6.23 and 4.63, 6.74, 7.14 and 3.25, 5.58, 6.25 μg/mL, respectively. Similar to these, compound **3e** has also exhibited good activity ($p<0.05$) against *K. pneumoniae* with MIC 6.44 μg/mL. The results were compared with known reference drug ampicillin and it was noted that compound **5f** is highly competitive with standard in the inhibition of multidrug resistant bacteria (Fig. 2, Table 1, Table 2 and Table 3). The compounds **5f**, **5b** and **5d** were further screened for their ability to inhibit formation of biofilm and time kill studies by the selected pathogens in view of their significant antibacterial activity.

Antibiofilm activity

Antibiofilm activity of the compounds **5f**, **5b**, **5d** at different concentrations (0-1000 μg/mL) were screened against multidrug resistant bacterial strains. As per Table 4, the biofilm inhibition concentration (BIC) was found to be significant with compound **5f**. Compound **5f** exhibited immense inhibition

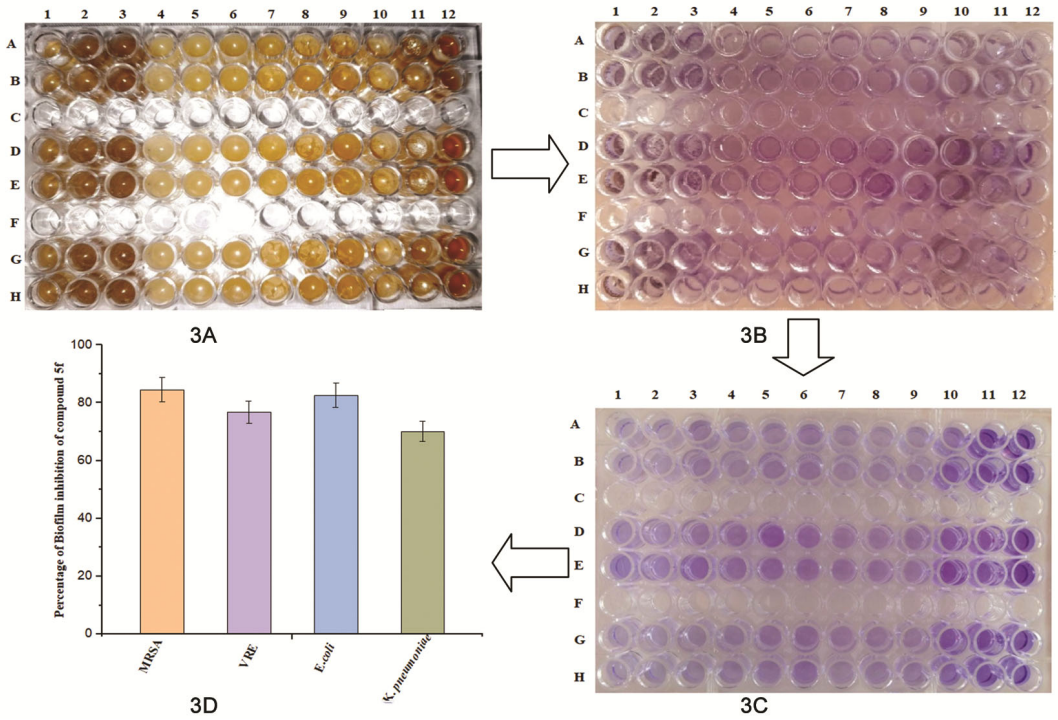


Fig. 2 — Plate 3A- Inoculation of selected multidrug resistant pathogenic bacteria; (1-3 wells are filled with Methicillin Resistant *Staphylococcus aureus* (MRSA), 4-6 wells are filled with Vancomycin resistant *Enterococcus* (VRE), 7-9, wells are filled with *K. pneumoniae*, 10-12 wells are filled with *E. coli*), in a multi well plate at the rate of 5×104^3 cells per well along with different concentration of 5f compound. 3 B- Plate showing the result after treatment of cells with 0.1% Crystal Violet (CV), Plate 3 C- Solubilisation of CV with 30% of acetic acid used for quantification, 2 D- Percentage of Biofilm inhibition by compound 5f against selected pathogenic bacteria

Table 1 — Antibacterial activity of compounds **3a-f**

Compd	Conc. (µg/mL)	Zone of Inhibition (mm)			
		MRSA	VRE	*K. pneumoniae*	*E. coli*
	25	1.05±0.17	–	2.72±0.17	3.25±0.09
	50	2.42±0.09	–	4.35±0.12	4.2±0.18
	100	2.77±0.09	–	7.17±0.12	5.6±0.14
3b	25	3.45±0.05	–	3.15±0.12	4.21±0.09
	50	5.52±0.09	–	5.52±0.09	6.13±0.12
	100	8.62±0.12	–	7.25±0.12	8.62±0.18
3c	25	2.42±0.09	2.21±0.12	4.25±0.12	2.17±0.15
	50	3.47±0.12	3.18±0.12	7.12±0.09	4.17±0.23
	100	5.37±0.12	5.20±0.17	10.0±0.17	6.42 ±0.17
3d	25	3.2±0.11	–	2.12±0.09	2.25±0.12
	50	5.62±0.09	–	3.17±0.17	3.15±0.12
	100	8.2±0.18	–	5.15±0.12	5.22±0.17
3e	25	6.45±0.12	2.15±0.12	7.35±0.12	8.4±0.28
	50	8.55±0.12	3.37±0.15	11.2±0.22	15.2±0.18
	100	11.1±0.23	5.55±0.17	13.2±0.16	20.4±0.29
3f	25	2.47±0.15	–	2.12±0.09	3.12±0.12
	50	3.67±0.12	–	3.15±0.12	4.6±0.14
	100	5.17±0.17	–	3.65±0.12	5.35±0.23
Ampicillin	10	31.5±0.5	28.2±0.9	30.12±1.2	32.02±0.5

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

Table 2 — Antibacterial activity of compound **5a-f**

Compd	Conc. (µg/mL)	Zone of Inhibition (mm)			
		MRSA	VRE	*K. pneumoniae*	*E. coli*
	25	3.17±0.12	–	4.32±0.27	6.52±0.25
	50	5.15±0.12	–	7.25±0.25	9.27±0.22
	100	6.57±0.15	–	10.3±0.17	14.3±0.12
5b	25	10.1±0.15	6.65±0.20	10.4±0.17	11.3±0.23
	50	17.2±0.28	12.6±0.15	15.3±0.20	19.2±0.20
	100	22.6±0.14	16.4±0.12	19.5±0.24	24.3±0.26
5c	25	2.22±0.17	–	2.35±0.20	5.22±0.22
	50	3.22±0.22	–	3.35±0.26	8.3±0.21
	100	5.22±0.17	–	4.4±0.18	10.4±0.11
5d	25	7.6±0.14	5.3±0.11	10.3±0.22	11.3±0.22
	50	14.2±0.17	9.57±0.09	14.4±0.33	18.5±0.21
	100	19.3±0.14	14.7±0.17	18.45±0.19	22.4±0.12
5e	25	2.75±0.12	–	3.42±0.22	3.42±0.17
	50	4.45±0.12	–	5.55±0.28	6.25±0.12
	100	5.3±0.25	–	7.15±0.12	8.42±0.09
5f	25	10.2±0.14	8.37±0.17	11.3±0.21	12.3±0.20
	50	19.2±0.18	15.5±0.31	17.5±0.12	21.3±0.23
	100	23.3±0.27	21.3±0.15	21.6±0.12	27.3±0.2
Ampicillin	10	31.5±0.5	28.2±0.9	30.12±1.2	32.02±0.5

Zone of inhibitions are represented as Mean+standarddeviation (SD) n=4

Table 3 — Minimum inhibitory concentration (µg/mL) of compounds **3a-f** and **5a-f**

Compd	MRSA	VRE	*K. pneumoniae*	*E. coli*
3a	24.6	–	27.8	22.4
3b	21.8	–	24.6	23.5
3c	24.6	–	19.0	29.2
3d	22.9	–	31.1	20.1
3e	9.20	15.7	6.44	15.2
3f	23.1	–	28.8	7.7
5a	24.1	–	25.4	17.6
5b	5.22	6.74	5.58	4.92
5c	25.8	–	30.3	19.4
5d	6.23	7.14	6.25	3.62
5e	25.3	–	24.6	23.8
5f	3.11	4.63	3.25	2.95
Std	2.99	2.40	2.81	2.22

Std- Ampicillin

Table 4 — Biofilm inhibitory concentration (µg/mL) of compounds **3a-f** and **5a-f**

Compd	MRSA	VRE	*K. pneumoniae*	*E. coli*
5b	6.58±1.5	5.21±1.2	5.22±1.5	3.70±1.5
5d	8.23± 0.5	7.56±0.11	6.25±0.5	6.62±1.5
5f	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

2.22+0.56 µg/mL ($p<0.01$) against MRSA. In addition, **5f** was also found to be significant against *E. coli* and VRE with BIC 2.03+0.02 and 3.05+1.0 µg/mL($p<0.01$) respectively, whereas anti biofilm activity of **5f** against *K. pneumoniae* was also found to be high with BIC 3.25+1.0 µg/mL($p<0.01$). With the results obtained in the study, it is clear that compound **5f** was equally competent in the inhibition of biofilm with the reference drug ampicillin against the bacterial strains (Table 4). On the other hand, compounds **5b** and **5d** were found to be slightly lesser efficient in the inhibition of biofilm compared to compound **5f** (Table 4).

Time kill studies

The bactericidal activity of the compounds **5f**, **5b** and **5d** were evaluated. The compounds exhibited

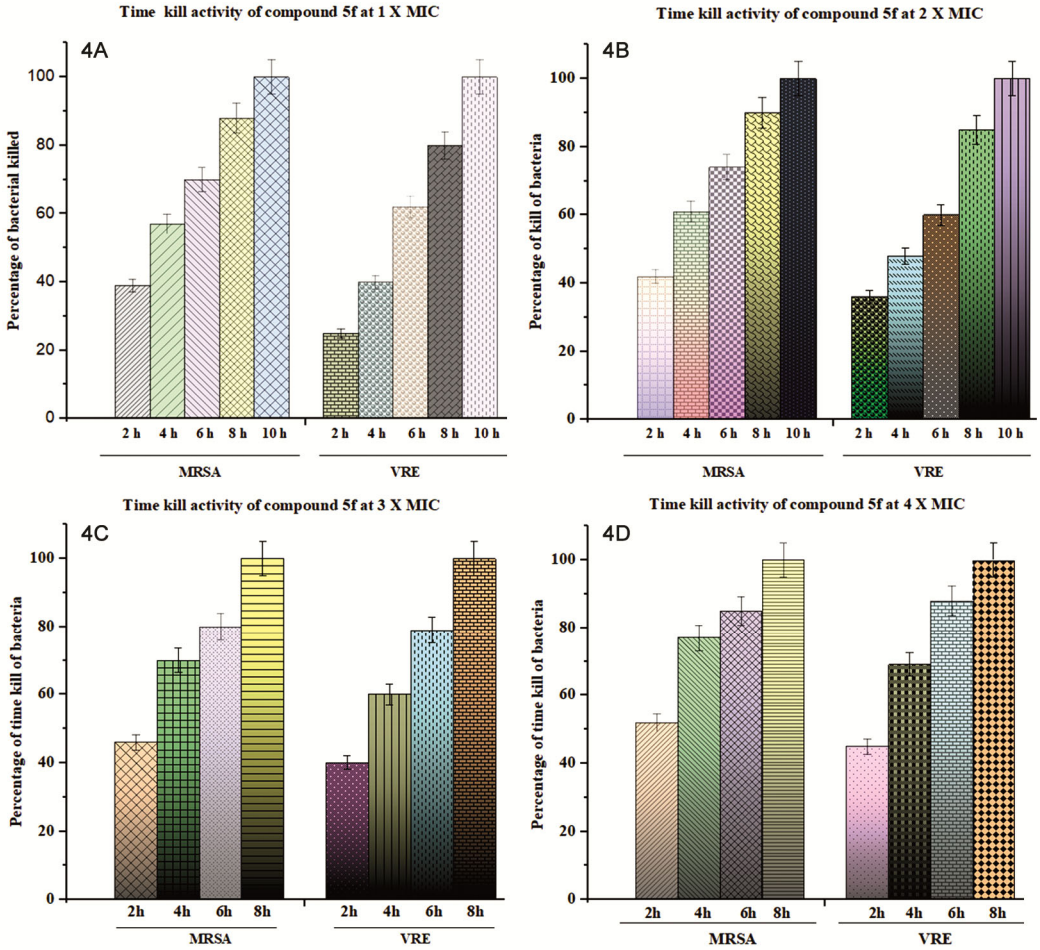


Fig. 3 — 4A-Graph showing bactericidal activity of compound 5f against MRSA and VRE at a concentration of 1×MIC. 4B- Graph showing bactericidal activity of compound 5f against MRSA and VRE at a concentration of 2×MIC. 4C- Graph showing bactericidal activity of compound 5f against MRSA and VRE at a concentration of 3×MIC. 4D- Graph showing bactericidal activity of compound 5f against MRSA and VRE at a concentration of 4×MIC

concentration and time dependent bactericidal effects against selected drug resistance bacterial strains. The time kill studies of the compounds have been conducted at 1 × MIC, 2 × MIC, 3 × MIC, 4 × MIC concentrations. In accordance with our results, compounds **5f** and **5b** initiated bactericidal effects against MRSA and VRE after 2 h of incubation. As shown in Fig. 3, the results noted that **5f** killed (100% $P<0.01$) MRSA and VRE at a concentration of 1 × MIC and 2 × MIC during 10 h of incubation. However, this compound is found more effective in bactericidal activity (100% $P<0.01$) at 3 × MIC and 4 × MIC during 8 h of incubation. In addition to MRSA, this compound is also more active against VRE. The 100% of CFU inhibition was found at 1 × MIC and 2 × MIC at 10 h of incubation ($P<0.01$). However, at 3 × MIC and 4 × MIC this compound killed 100% CFU of VRE at 8 h of incubation (Fig. 3).

By our results, it is clear that compound **5f** shows concentration dependent and as well time dependent activity against MRSA and VRE.

Conclusion

In conclusion, a new series of 1,2,3-triazoles on imidazopyridine nucleus was obtained in excellent yield. It was concluded that all the compounds were characterized by spectroscopic techniques. Evaluation of antibacterial activity of the compounds **5a-j** was done with zone of inhibition and MIC method. Further, the compounds **5f**, **5b** and **5d** were also screened for their anti-biofilm activity and time kill studies which proved that they have excellent activity.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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