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Article

Molecular Hybrids of Pyrazolo[3,4-*b*]Pyridine and Triazole: Design, Synthesis and *in vitro* Antibacterial Studies

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Abstract: Antimicrobial resistance is on the rise, and there aren't enough new treatments to combat it. This might send the modern world back to the pre-antibiotic age. The molecular hybrids of pyrazolo[3,4-b]pyridine and triazole have been designed, synthesized, and analyzed for their drug-like molecule nature and *in vitro* analyses for their inhibition potentials against *S. Aureus* and *K. Pneumoniae*. The compounds CY-R2-25 and CY-R2-28 have been identified as the high potential molecules in this series based on *in vitro* experiments. CY-R2-25 has zone of inhibition values of 15 0.82 and 14 0.7, whilst CY-R2-28 has the zone of inhibition values of 18 0.95 and 16 0.82 against *S. Aureus* and *K. Pneumoniae*, respectively. MIC and MIB values for CY-R2-25 and CY-R2-28 against *S. Aureus* and *K. Pneumoniae* are 0.25 and 0.5, respectively.

Keywords: pyrazolo[3,4-*b*]pyridine; triazole; *in vitro*; anti-bacterial; *S. Aureus*; *K. Pneumoniae*

Highlights:

- The molecular hybrids of pyrazolo[3,4-*b*]pyridine and triazole with a wide range of structural and functional group changes have been designed.
- Physico-chemical properties have been evaluated to assess the DLM nature.
- The potent molecules, CY-R2-25 and CY-R2-28 have been identified.
- CY-R2-25 has exhibited a zone of inhibition with values of 15 ± 0.82 and 14 ± 0.7, whereas CY-R2-28 has shown 18 ± 0.95 and 16 ± 0.82 against the *S. Aureus* and *K. Pneumoniae* respectively.
- CY-R2-25 and CY-R2-28 have shown equivalent MIC and MIB values of 0.25 and 0.5 against the *S. Aureus* and *K. Pneumoniae* respectively.

1. Introduction

The emergence of antimicrobial resistance (AMR) bacterial strains, combined with a limited arsenal of viable treatments, threatens to return the modern world to a pre-antibiotic era when simple infections were fatal. A lack of clean water, sanitation, and hygiene (WASH) for both humans and animals, as well as inadequate health-care facilities, a lack of awareness and knowledge, and a lack of legislative enforcement have all contributed to AMR becoming one of the top ten global health threats. *Klebsiella pneumonia (K. pneumoniae)* is a bacterium that can cause potentially fatal infections in newborns and patients in intensive care units, as well as pneumonia and bloodstream and gastrointestinal infections, which are frequently brought on by *K. pneumoniae*. More than half of the patients

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treated for *K. pneumoniae* infections in various countries are unable to benefit from carbapenem medications due to the spread of carbapenem antibiotic-resistant strains of *K. pneumoniae*. Another most prevalent organism in hospitals and the general public, *Staphylococcus aureus* (*S. aureus*), has been linked to a variety of clinically significant illnesses, from superficial skin infections to deeply seated invasive infections.[1, 2] *S. aureus* may avoid the effects of antibacterial medications using several methods *Viz.* are alteration of the target protein, improved efflux, lower antibiotic penetration, enzymatic drug degradation, and plasmids.[3, 4] Because bacteria with resistance genes, such as methicillin-resistant *S. aureus* (MRSA), can spread between humans, animals, and the environment, drug-resistant illnesses pose a serious threat to public health yet have few effective therapies.[5,6] Vancomycin is still one of the first-line therapies for MRSA infections, but newly discovered vancomycin-completely resistant MRSA isolates [7,8] have made the urgent need for the identification of new antibacterial drug classes apparent. Finding medicines with high therapeutic action against both *K. pneumoniae* and *S. aureus* germs is therefore urgently needed.[1]

The synthesis of small compounds with a variety of therapeutic potential has been widely researched using 1*H*-pyrazolo[3,4-*b*]pyridine, one of the most fascinating medicinal chemical scaffolds. Examples include, among others, anti-cancer drugs, anti-diabetic drugs, cardiovascular drugs, enzyme inhibitors, anti-inflammatory drugs, and drugs for the neurological system.[2] Similar to this, the 1,2,3-triazole ring is a five-membered heterocyclic motif that can be a bioisostere of amide, ester, carboxylic acid, and other heterocycles. This moiety is easily able to interact with a variety of proteins, enzymes, and receptors in organisms via non-covalent interactions, and numerous structural modifications have resulted in a broad spectrum of activity. With shown anticancer, antibacterial, antitubercular, antiviral, antidiabetic, antimalarial, anti-leishmanial, and neuroprotective activities, it is one of the most prevalent frameworks found in bioactive compounds.

2. Results and Discussion

2.1. Design and evaluation of physico-chemical properties

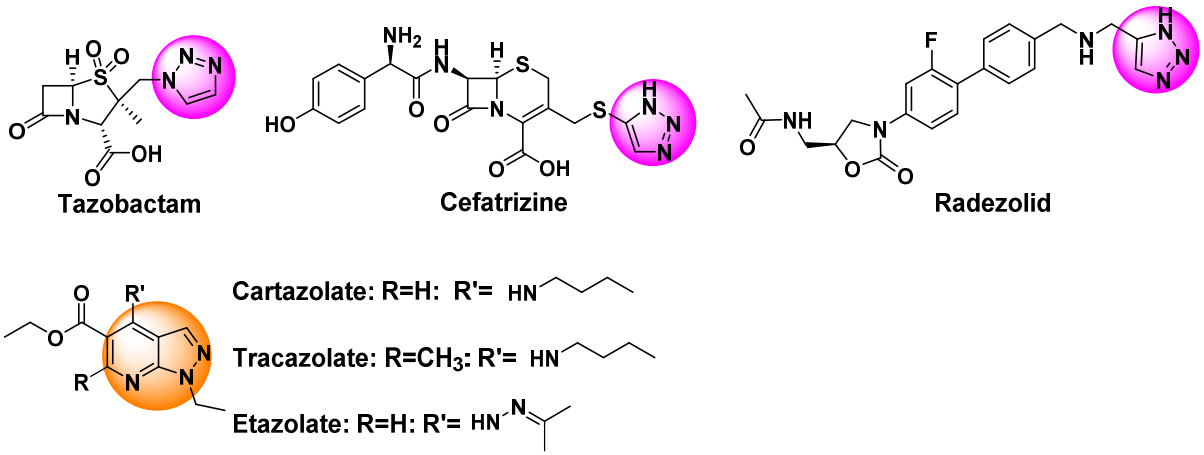

Figure 1. Structures of important pyrazolo[3,4-*b*]pyridine and triazole antibacterial agents.

According to the literature, triazole and pyrazolo[3,4-*b*]pyridine scaffolds have been the most extensively studied for their medicinal characteristics among nitrogen-containing heterocycles. To combat drug resistance, there has recently been a surge in interest in the meticulous use of 1,2,3-triazole in the creation of new antibacterial agents. Some 1,2,3-triazole-containing hybrids, including cefatrizine, tazobactam, and radezolid, have already been used in clinics or are currently undergoing clinical trials for the treatment of infections brought on by a variety of bacteria, including MRSA.[3] To create new anti-MRSA candidates, the rational creation of hybrids incorporating 1,2,3-triazoles is an appealing method. Therefore, a thoughtful design of molecular hybrids of 1,2,3-triazole derivatives

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and pyrazolo[3,4-*b*]pyridine may create prospects for the creation of new anti-MRSA drugs. In this study, we continued our attempts to develop anti-infective drugs by designing molecular hybrids of pyrazolo[3,4-*b*]pyridine and triazole with structural and functional alterations, synthesizing them, and evaluating how effective they were against the bacteria *K. pneumoniae* and *S. aureus*.

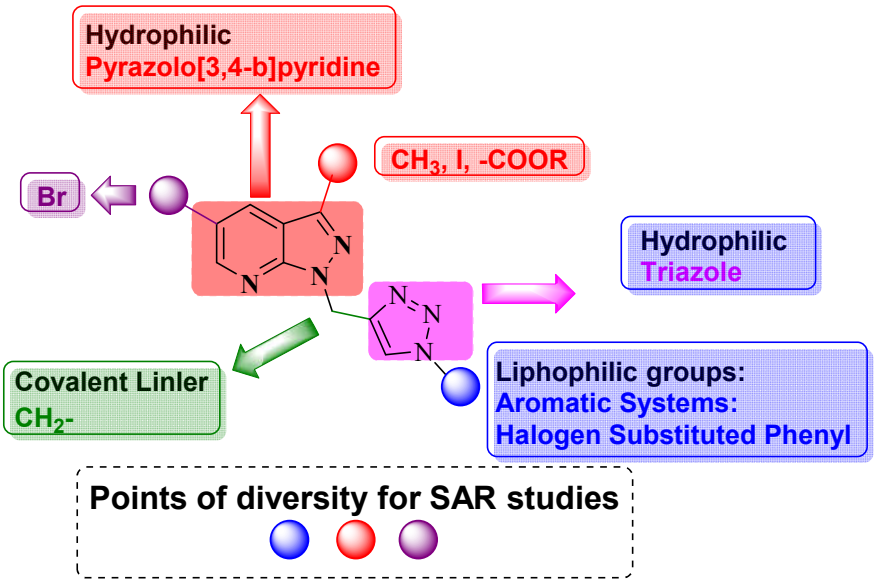

Figure 2. Design of the molecular hybrids of pyrazolo[3,4-b]pyridine and triazole.

Enhanced paracellular and transcellular absorption characteristics of compounds with lower molecular weight and lipophilicity are anticipated[4], as well as a transporter influence on clearance, leading to improved renal excretion[5] and mild toxicity.[6] A molecule becomes a drug-like molecule (DLM) when it meets the requirements outlined by the "rule of 5" (Ro5). When a molecule meets the Ro5 requirements, it has a molecular mass of less than 500, a log*P* of less than 5, and less than 5 and 10 donors and acceptors of hydrogen bonds, respectively.[7] According to the Veber rule, oral bioavailability is greater for molecules with rotatable bonds, polar surface area, and H-bond donors and acceptors that are, respectively, equal to or less than 10, 140, and 12.[8] In addition, Leeson and colleagues found that approved molecules between 1983 and 2002 contain 16% to 23% more molecular mass, rings, rotatable bonds, and hydrogen bonding groups.[9] Additionally, these traits aid in the creation of molecules with enhanced absorption, distribution, metabolism, excretion, and toxicity (ADMET).[10] Due to this, physicochemical characteristics and how precisely they are regulated now play a crucial role in deciding whether a molecule may be used as a therapeutic candidate and how well it performs throughout key stages of drug development. In light of the aforementioned elements, pyrazolo[3,4-*b*]pyridine and triazole molecular hybrids with various structural and functional alterations have been created with and without obeying.

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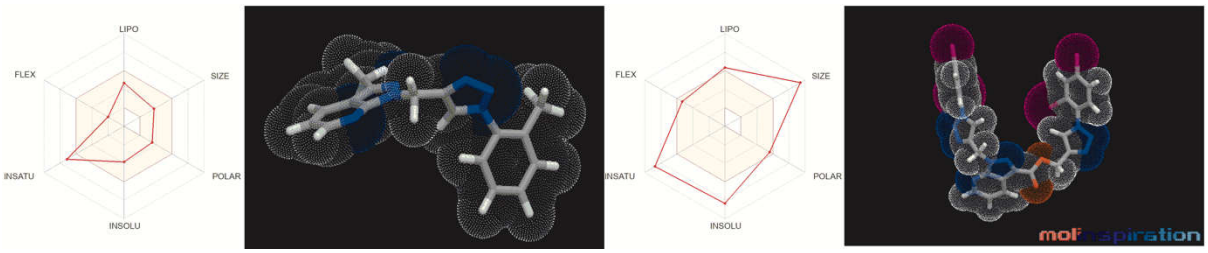

(a) **(b)**

Figure 3. Web and MLP representation of physico-chemical properties of (**a**) 14 and (**b**) 32.

With CY-R2-14 and CY-R2-32, respectively, the suggested compounds have molecular weights that range from 304.35 to 798.16. Additionally, it is discovered that the molecular weights of CY-R2-29, CY-R2-27, CY-R2-33, and CY-R2-31 are, respectively, 574.42, 562.53, 546.37, and 513.46, which do not obey the Ro5. With CY-R2-17 being the lowest and CY-R2-32 being the highest, the iLogP values varied from 2.7 to 4.71. There are no hydrogen bond donors and a range of 4 to 10 hydrogen bond acceptors (HA) (HD). Except for molecules CY-R2-29 to CY-R2-33, all compounds have three rotatable bonds. Like this, all of the compounds have PTSA values of 61.42, whereas molecules CY-R2-29 to CY-R2-33 have values of 118.43. A variety of molecules with a variety of physicochemical characteristics have been produced consequently, and they have been evaluated to see whether they agree with the theories put forward by Lipinski, Veber, and Leeson and the results have been summarized in table 1. Additionally, it has been expected that certain pharmacokinetic behaviors will occur, including GIA and BBB (brain-blood barrier). By calculating the lipophilicity (WLOGP versus TPSA), the Brain Or IntestinaL EstimateD Permeation method (BOILEDEgg) has been visually shown.[11] It is projected that all molecules that fall inside the white ellipse and none that fall inside the yellow ellipse would have superior GIA but poorer BBB properties. Since none of the molecules is in the grey region, they cannot pass through the BBB or the GIA. All substances whose efflux activity in the Central Nervous System (CNS) was predicted by the P-glycoprotein (PGP).

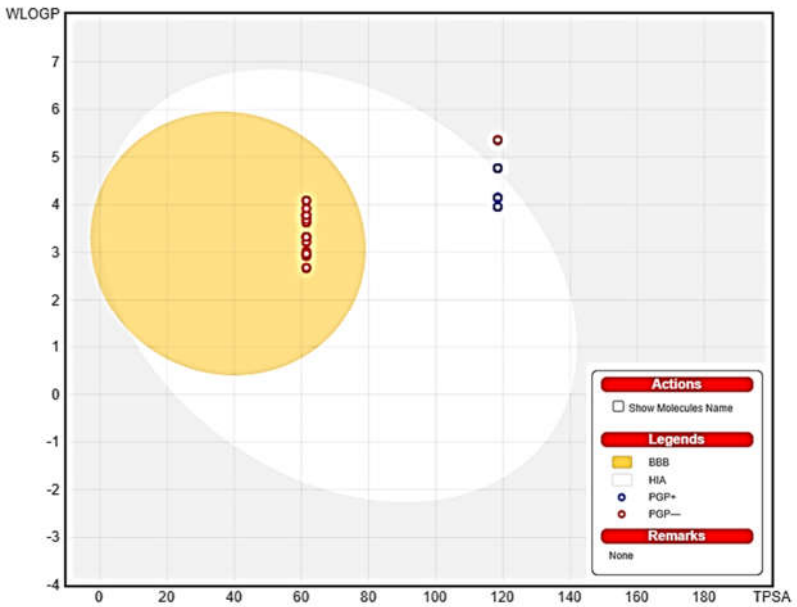

Figure 4. Boiled egg model for molecular hybrids of pyrazolo[3,4-*b*]pyridine and triazole.

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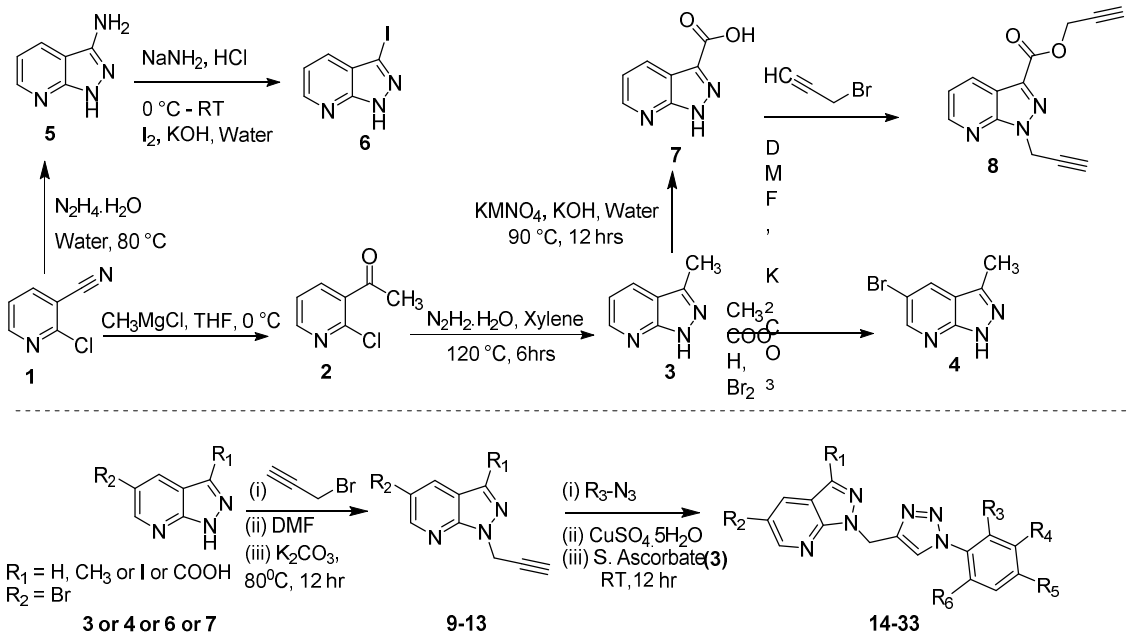
S. No.	Molecule	Physico-Chemical Properties						*In vitro* studies					
								Zone of Inhibition		MIC and MBC			
										S. aureus		*K. pneumoniae*	
		MW	iLog*P*	HA	HD	RB	TPSA	*S. aureus*	*K. pneumoniae*	MIC	MBC	MIC	MBC
1.	CY-R2-14	304.35	2.87	4	0	3	61.42	NI	11 ± 0.15	0.25	0.5	0.25	0.5
2.	CY-R2-15	338.79	3.08	4	0	3	61.42	11 ± 0.12	13 ± 0.28	0.25	0.5	0.25	0.5
3.	CY-R2-16	450.66	3.04	4	0	3	61.42	15 ± 0.59	12 ± 0.22	0.25	0.5	0.25	0.5
4.	CY-R2-17	308.31	2.7	5	0	3	61.42	NI	11 ± 0.19	0.25	0.5	0.25	0.5
5.	CY-R2-18	324.77	2.95	4	0	3	61.42	NI	11 ± 0.09	0.5	1.0	0.5	1.0
6.	CY-R2-19	417.69	3.67	4	0	3	61.42	11 ± 0.17	13 ± 0.41	0.25	0.5	0.25	0.5
7.	CY-R2-20	387.21	3.18	5	0	3	61.42	NI	13 ± 0.39	0.25	0.5	0.25	0.5
8.	CY-R2-21	403.66	3.34	4	0	3	61.42	12 ± 0.19	NI	0.5	1.0	0.25	0.5
9.	CY-R2-23	403.66	3.3	4	0	3	61.42	11 ± 0.09	NI	0.5	1.0	0.5	1.0
10.	CY-R2-24	403.66	3.3	4	0	3	61.42	11 ± 0.16	10 ± 0.12	0.25	0.5	0.25	0.5
11.	**CY-R2-25**	416.22	2.87	4	0	3	61.42	**15 ± 0.82**	**14 ± 0.75**	**0.25**	**0.5**	**0.25**	**0.5**
12.	CY-R2-26	420.18	3.02	5	0	3	61.42	12 ± 0.18	15 ± 0.65	0.25	0.5	0.25	0.5
13.	CY-R2-27	562.53	3.26	4	0	3	61.42	13 ± 0.27	14 ± 0.45	0.25	0.5	0.25	0.5
14.	**CY-R2-28**	436.64	3.06	4	0	3	61.42	**18 ± 0.95**	**16 ± 0.82**	**0.25**	**0.5**	**0.25**	**0.5**
15.	CY-R2-29	574.42	4.63	8	0	8	118.43	10 ± 0.15	11 ± 0.15	0.5	1.0	0.25	0.5
16.	CY-R2-31	513.46	4.08	10	0	8	118.43	12 ± 0.21	14 ± 0.28	0.25	0.5	0.25	0.5
17.	CY-R2-32	798.16	4.71	8	0	8	118.43	10 ± 0.13	NI	0.25	0.5	0.25	0.5
18.	CY-R2-33	546.37	4.51	8	0	8	118.43	12 ± 0.19	NI	0.25	0.5	0.25	0.5
19.	Ciprofloxacin							32 ± 0.40	31 ± 0.20	0.062	0.125	0.031	0.062

S. aureus: *Staphylococcus aureus; K. pneumonia: Klebsiella pneumonia;* NI: No Inhibition. Data are means (n = 3) ± Standard deviation of three replicates; Zone of inhibition (Diameter in mm) at a concentration of 100 µg; MIC and MBC data with a stock concentration of 1.0 mg/ml.

2.2. Chemistry



Scheme 1. Synthesis of molecular hybrids of pyrazolo[3,4-*b*]pyridine and triazole.

The synthesis was initiated by reacting the 2-chloronicotinonitrile, 1 with methyl magnesium chloride at 0 °C for about 3 hours, followed by the acidic workup yielding compound 2 with 60% yield.[12] Which further treated with hydrazine hydrate at 120 °C in the presence of xylene for 6 hours followed by cooling the reaction mass to 0°C obtained compound 3 in 95% yield.[13] Compound 3 was subjected to bromination in the presence of

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a mixture of acetic acid and bromine at 0 – 5 °C. Upon completion of the reaction, the solid obtained is filtered to get compound 4 in 73% yield.[14] Following the method described in the literature with minor modifications, the amine substituted pyrazolo[3,4-*b*]pyridine, 5 has been achieved by treating compound 4 with hydrazine hydrate with 90% yield and which has been subsequently undergone nucleophilic substitution with the Iodine to generate compound 6 with 93% yield.[15] The compound 3 oxidised to generate the corresponding acid 7 with 80% yield by following the method described in the literature with minor modification.

The next step involves the alkylation of compound 3, 4, 6 and 7 with 3-bromoprop-1-yne using potassium carbonate as base and DMF as a solvent and stirring the reaction at 80 °C for 12 hours to get compounds, 9-13 in 75- 95% yield by using the method described in the literature with minor modification.[16] The final step of the synthesis involves the application of click chemistry on compounds 9-13, which has been carried out by heating with the corresponding azide for one hour. After the reaction is complete, water is added.[16] The precipitated solid is filtered to get crude compounds, CY-R2-14 to 33, which were further purified by column chromatography to obtain the spectrally pure desired compounds of this study. All the synthetic pathways are outlined in scheme 1 and the structures of all the synthesised molecular hybrids of pyrazolo[3,4-b]pyridine and triazole have been represented in Table 2.

2.3. In vitro anti-bacterial studies

All the synthesized compounds, CY-R2-14 to 33 were classified into four categories and subjected to their *in vitro* antibacterial activity against Gram-positive; *S. aureus* and Gram-negative; *K, pneumoniae* bacterial strains by using standard *in vitro* methods *Viz.* are Zone of inhibition, MIC and MBC. The class I molecules (CY-R2-14 to 18) contains a methyl group substitution at the 3rd position of pyrazolo[3,4-*b*]pyridine moiety and with variations of functional group substitutions on phenyl system of phenyl -1*H*-1,2,3-triazole. All the molecules were found to be in an almost equipotent zone of inhibition, MIC and MBC with 11, 0.25, and 0.5 respectively against both *K. pneumonia* and *S. aureus*. The class II molecules (CY-R2-18 to 23) contain a bromo and methyl substitutions at the 5th and 3rd positions of pyrazolo[3,4-*b*]pyridine moiety and with a similar variation of functional group substitutions on the phenyl system of phenyl -1H-1,2,3-triazole as that of group I molecules. Surprisingly similar to group I molecules, all the molecules were found to be almost equipotent with a zone of inhibition, MIC and MBC values of 11, 0.25, and 0.5 respectively against both *K. pneumonia* and *S. aureus*.

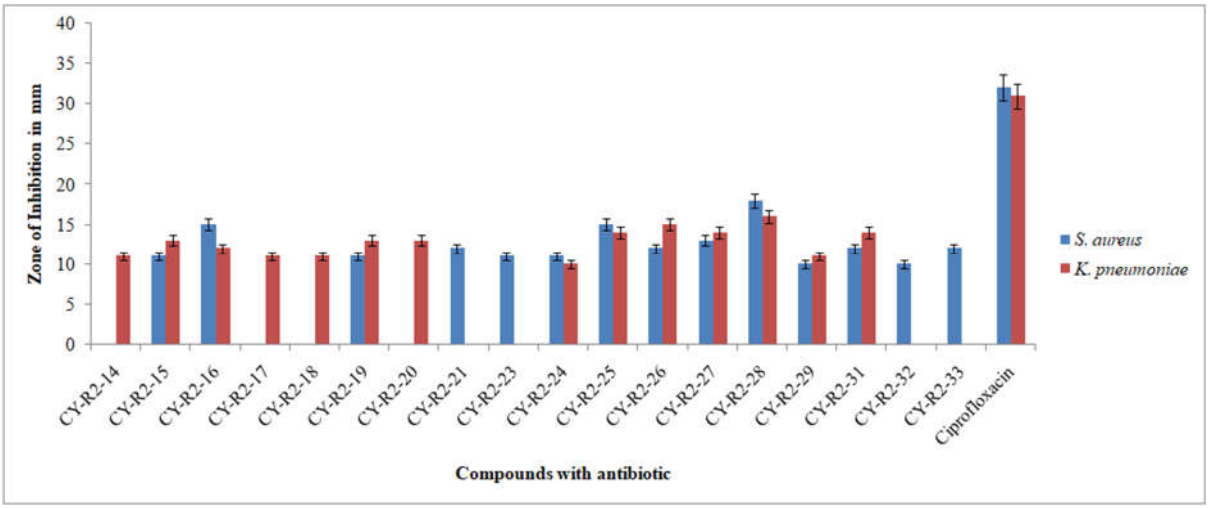

Figure 5. Zone of inhibition of pyrazolo[3,4-*b*]pyridine and triazole molecular hybrids.

The interesting results are found in the group III molecules, where iodo substitutions at 3rd positions of pyrazolo[3,4-*b*]pyridine moiety have been introduced and with similar

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variations of functional group substitutions on phenyl system of phenyl -1H-1,2,3-triazole. The CY-R2-25, which has the methyl group substitution at the 2nd position of the phenyl ring has exhibited the zone of inhibition values of 15 ± 0.82 and 14 ± 0.75 against *S. aureus* and *K. pneumonia* respectively. The MIC and MIB values of the molecule, CY-R2-25 has found to be 0.25 and 0.5 against *S. aureus* and *K. pneumonia* respectively. With these results, molecule CY-R2-25 was found to be the second-best potent molecule after molecule CY-R2-28. The best potent of this study is CY-R2-28, which has the structural modification of chloro substitution at the 3rd position of the phenyl ring. With the zone of inhibition values of 18 ± 0.95 and 16 ± 0.82 against *S. aureus* and *K. pneumonia* respectively the molecule CY-R2-28 stand out to be the best potent molecule in this group. Similarly, The MIC and MIB values of the molecule, CY-R2-28 has found to be 0.25 and 0.5 against *S. aureus* and *K. pneumonia* respectively. The group-IV molecules contain two substituted phenyl-1H-1,2,3-triazole attachments at 1st and 3rd position with alkyl and ester functional group linkers. The group IV molecules, all the molecules found to be almost equipotent with a zone of inhibition, MIC and MBC values of 11, 0.25, and 0.5 respectively against both *K. pneumonia* and *S. aureus*.

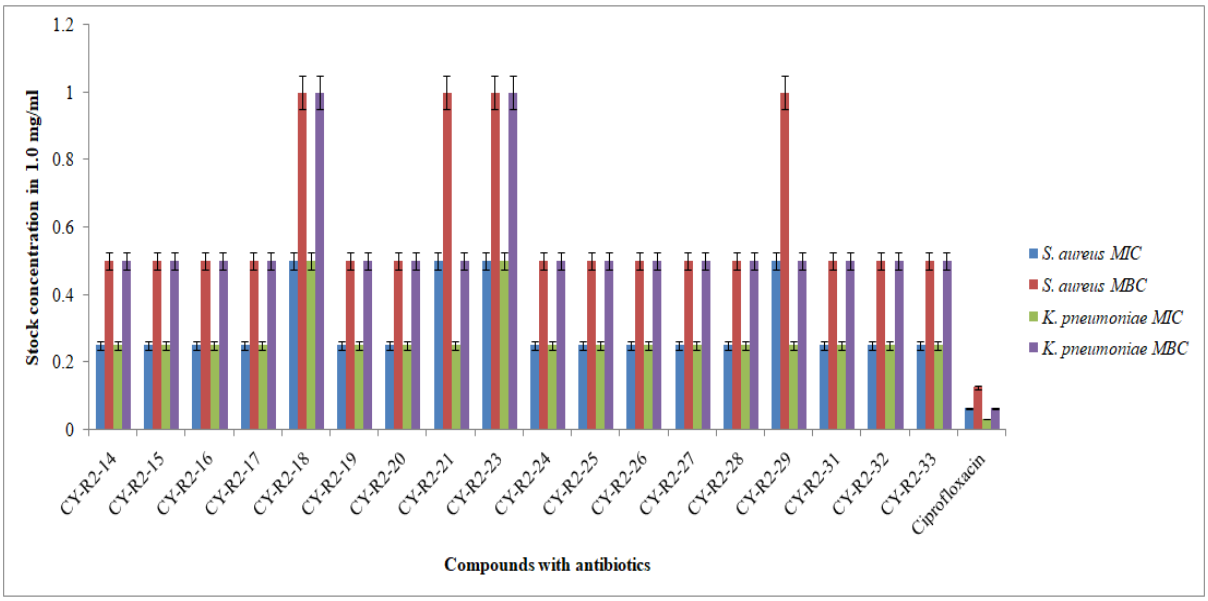

Figure 6. MIC and MBC of pyrazolo[3,4-*b*]pyridine and triazole molecular hybrids.

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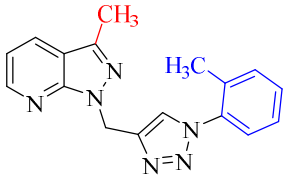
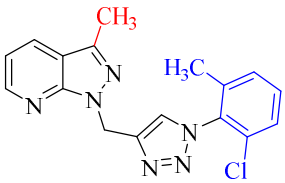
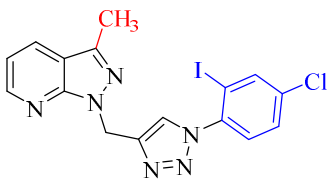
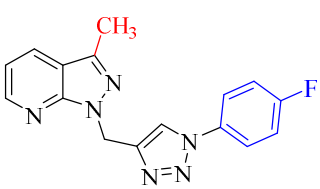
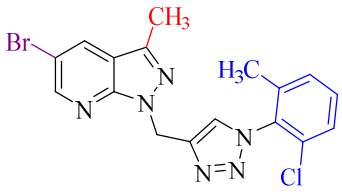
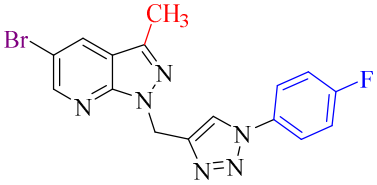
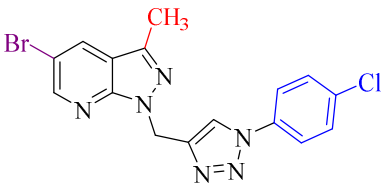
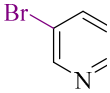
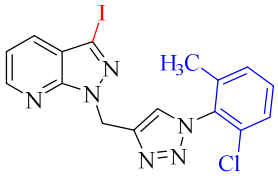
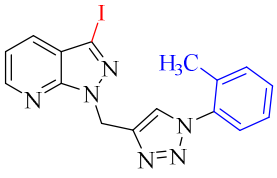
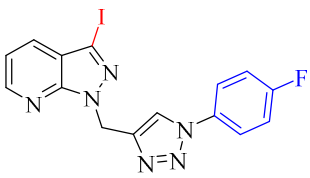
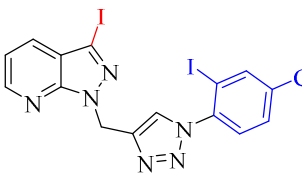
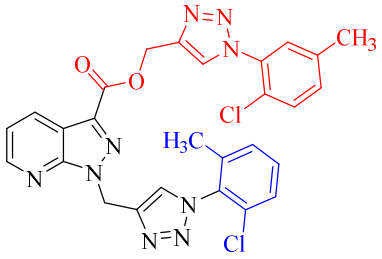
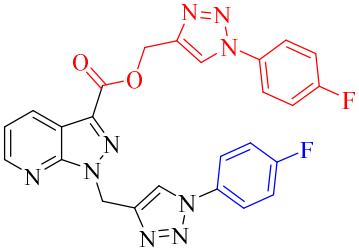
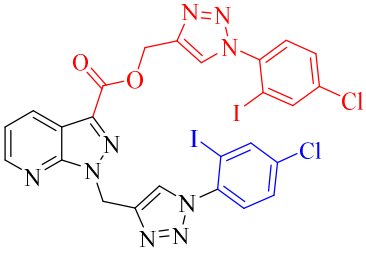
Table 2. The structures of synthesised pyrazolo[3,4-*b*]pyridine and triazole molecular hybrids.

Class-I				
	CY-R2-14	**CY-R2-15**	**CY-R2-16**	**CY-R2-17**
Class-II				
	CY-R2-19	**CY-R2-20**	**CY-R2-21**	
Class-III				
	CY-R2-24	**CY-R2-25**	**CY-R2-26**	**CY-R2-27**
Class-IV				
	CY-R2-29	**CY-R2-31**	**CY-R2-32**	

2.4. Materials and Methods

2.4.1. Chemistry

Oven-dried glassware was used to carry out all the reactions and the progression of reactions was monitored by thin-layer chromatography (TLC). VEEGO VMP-DS melting point apparatus was used to determine melting points. Melting points were uncorrected and determined in open-end capillaries. Nicolet-6700 spectrometer was used to record IR spectra using KBr. ^{1}H, ^{13}C and dept-135 NMR spectra were recorded on Bruker Advance 400 spectrometer using DMSO-d_6 and CCl_4 in a 1:1 ratio as solvent. DEPT spectral data were used to ascertain the number of hydrogen atoms on each carbon atom. The chemical shift value (δ) is expressed in parts per million units and is measured relative to $SiMe_4$ (δ = 0.00) as the internal standard. Coupling constants (J) are measured in Hz. Multiplicities are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or broad (br). UV spectra were recorded using a Shimadzu UV-2450 double-beam spectrometer. HPLC analyses were carried out by using the SCL-10ATVP SHIMADZU instrument.

General procedure for the synthesis of 14-33: To a solution of alkyne derivatives (0.14 mmol) dissolved in THF, $CuSO_4.5H_2O$ (0.014 mmol) added in portion wise followed by a catalytic amount of sodium ascorbate. After stirring for 12 hours at room temperature, the reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAc. The combined organic phases afforded, after usual workup and the crude has been purified by the column chromatography.

3-methyl-1-((1-(o-tolyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazolo[3,4-b]pyridine ***(14)****:* Cream colour solid; Yield %; IR (KBr) cm^{-1}: 3138.99, 3105.1, 2921.76, 2904, 2877.7,

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2842.55, 1591.28, 1562.99, 1502.14, 1383.70, 1359.98, 1329.80, 1255.38, 1176.62, 1102.07,1040.63, 778.85, 768.18, 685.36; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.57 (d, *J* = 2.1 Hz, 1H), 8.13 (d, *J* = 2.1 Hz, 1H), 7.91 (s, 1H), 7.71 (t, *J* = 2.0 Hz, 1H), 7.59 (dt, *J* = 7.7, 1.8 Hz, 1H), 7.47 – 7.35 (m, 2H), 5.83 (s, 2H), 2.55 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 149.68, 149.25, 143.42, 136.35, 133.65, 131.41, 131.34, 129.80, 126.73, 125.92, 123.99, 116.74, 111.82, 77.20, 42.36, 17.87, 12.42; HRMS (ESI) Calcd for $C_{17}H_{16}N_6$ [M + H] 304.14 amu, found 305.15 amu.

1-((1-(2-chloro-6-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine ***(15)***: Brown colour solid; Yield 80%; mp 65 - 68 °C; IR (KBr) cm^{-1}: 3138.99, 3105.1, 2921.76, 2904, 2877.7, 2842.55, 1591.28, 1562.99, 1502.14, 1383.70, 1359.98, 1329.80, 1255.38, 1176.62, 1102.07,1040.63, 778.85, 768.18, 685.36; 1H NMR (400 MHz, Chloroform-d) δ 8.55 (d, J = 2.1 Hz, 1H), 8.12 (d, J = 2.2 Hz, 1H), 7.67 (s, 1H), 7.39 – 7.30 (m, 2H), 7.28 – 7.21 (m, 2H), 5.87 (s, 2H), 2.54 (s, 3H), 2.02 (s, 3H); 13C NMR (101 MHz, cdcl3) δ 149.64, 149.27, 143.43, 140.84, 138.12, 131.74, 131.32, 130.91, 129.32, 127.70, 124.70, 116.75, 111.81, 42.40, 17.83, 12.42; HRMS (ESI) Calcd for $C_{17}H_{15}ClN_6$ [M + H] 338.80 amu, found 340.10 amu.

1-((1-(4-chloro-2-iodophenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine ***(16)****:* White colour solid; Yield 82%; mp 140-142 °C; IR (KBr) cm^{-1}: 3142.36, 3086.46, 2987.7, 2950, 1598.97, 1577.48 1488.29, 1379.42, 1358.95, 1343.93, 1266.73, 1242.93, 1142.93, 1040.61, 870-850, 772.90, 696.71, 600, 500; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.56 (dd, *J* = 4.6, 1.5 Hz, 1H), 8.04 – 7.93 (m, 2H), 7.79 (s, 1H), 7.44 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.26 (s, 3H), 7.11 (dd, *J* = 8.0, 4.6 Hz, 1H), 5.90 (s, 2H), 2.58 (s, 3H); ^{13}C NMR (101 MHz, cdcl$_3$) δ 150.87, 148.88, 144.09, 141.53, 139.48, 138.64, 136.52, 129.43, 129.29, 128.28, 124.40, 116.19, 115.31, 94.06, 42.07, 12.46; HRMS (ESI) Calcd. for $C_{17}H_{15}ClN_6$ [M + H] 450.66 *amu*, found 451.0 *amu*.

1-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine ***(17)****:* Off-white colour solid; Yield 81%; mp 134-135 °C; IR (KBr) cm^{-1}: 3132.47, 3005.1, 2921.76, 2894, 2877.7, 2852.55, 1737.99, 1600.67, 1575.13, 1503.70, 1385.84, 1362.75, 1340.90, 1270.43, 1175.01, 1140.30, 1044.84, 770.49, 701.53; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.56 (dd, *J* = 4.6, 1.5 Hz, 1H), 8.01 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.85 (s, 1H), 7.64 (dd, *J* = 9.0, 4.6 Hz, 2H), 7.20 – 7.10 (m, 3H), 5.88 (s, 2H), 2.58 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 148.92, 144.91, 141.57, 129.46, 122.54, 122.45, 120.78, 116.66, 116.43, 116.21, 115.28, 42.02, 12.45; HRMS (ESI) Calcd for $C_{16}H_{13}FN_6$ [M + H] 308.31 amu, found 309.1 amu.

1-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine ***(18)****:* White colour solid; Yield 83%; mp 91-92 °C; IR (KBr) cm^{-1}; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.56 (d, *J* = 4.6 Hz, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.89 (s, 1H), 7.71 (s, 1H), 7.58 (d, *J* = 7.7 Hz, 1H), 7.41 (dd, *J* = 13.9, 6.1 Hz, 2H), 7.12 (dd, *J* = 8.0, 4.6 Hz, 1H), 5.88 (s, 2H), 2.59 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 148.91, 145.04, 141.60, 137.71, 135.37, 130.66, 129.47, 128.67, 120.64, 120.52, 118.42, 116.22, 115.27, 41.96, 12.43; HRMS (ESI) Calcd for $C_{16}H_{13}ClN_6$ [M + H] 323.76 amu, found 325.1 amu.

5-bromo-1-((1-(2-chloro-6-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine ***(19):*** White colour solid; Yield 85%; mp 140-142 °C; IR (KBr) cm^{-1}:3138.99, 3105.1, 2921.76, 2904, 2877.7, 2842.55, 1591.28, 1562.99, 1502.14, 1383.70, 1359.98, 1329.80, 1255.38, 1176.62, 1102.07,1040.63, 778.85, 768.18, 685.36; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.55 (d, *J* = 2.1 Hz, 1H), 8.12 (d, *J* = 2.2 Hz, 1H), 7.67 (s, 1H), 7.39 – 7.30 (m, 2H), 7.28 – 7.21 (m, 2H), 5.87 (s, 2H), 2.54 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (101 MHz, cdcl$_3$) δ 149.64, 149.27, 143.43, 140.84, 138.12, 131.74, 131.32, 130.91, 129.32, 127.70, 124.70, 116.75, 111.81, 42.40, 17.83, 12.42; ^{13}C NMR (101 MHz, cdcl$_3$) δ 149.64, 149.27, 143.43, 140.84, 138.12, 131.74, 131.32, 130.91, 129.32, 127.70, 124.70, 116.75, 111.81, 42.40, 17.83, 12.42; HRMS (ESI) Calcd for $C_{17}H_{14}BrClN_6$ [M + H] 418.70 amu, found 419.0 amu.

5-bromo-1-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine ***(20)****:* Light Yellow colour solid; Yield 84%; mp 140-141 °C; IR (KBr) cm^{-1}: 3476.57, 3430.47, 3253.82, 3131.82, 3082.62, 2923.88, 2853.04, 1667.12, 1592.96, 1516.16, 1455.84, 1370.85, 1328.98, 1255.61, 1176.23, 1101.01,1060.19, 775.80, 681.58; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.57 (d, *J* = 2.1 Hz, 1H), 8.12 (d, *J* = 2.1 Hz, 1H), 7.87 (s, 1H), 7.67 – 7.63 (m,

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2H), 7.21 – 7.15 (m, 2H), 5.83 (s, 2H), 2.54 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 149.72 , 149.20, 144.41, 140.97, 133.18, 131.36, 122.56, 122.48, 120.85, 116.72, 116.49, 111.86, 42.20, 12.41; HRMS (ESI) Calcd for $C_{16}H_{12}BrFN_6$ [M + H] 387.21 amu, found 389.0 amu.

5-bromo-1-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine **(21)**: White colour solid; Yield 86%; mp 147-148 °C; IR (KBr) cm^{-1}: 3150.13, 3139.14, 3083.70, 3066.83, 3052.62, 2922.65, 2873.04, 1591.75, 1566.11, 1487.11, 1462.27, 1384.87, 1328.31, 1236.95, 1184.11, 1096.73,1076.14, 770.25, 671.36; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.57 (d, *J* = 2.1 Hz, 1H), 8.13 (d, *J* = 2.1 Hz, 1H), 7.89 (s, 1H), 7.65 – 7.61 (m, 2H), 7.48 – 7.44 (m, 2H), 5.83 (s, 2H), 2.54 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 149.75, 144.54, 141.01, 135.37, 134.53, 131.38, 129.83, 121.67, 120.57, 116.73, 111.89, 42.19, 12.42; HRMS (ESI) Calcd for $C_{16}H_{12}BrClN_6$ [M + H] 403.66 amu, found 405.0 amu.

5-bromo-1-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine ***(23):*** White colour solid; Yield 82%; mp 130-131 °C; IR (KBr) cm^{-1}: 3146.63, 3139.14, 3083.70, 3066.83, 3052.62, 2922.65, 2873.04, 1590.57, 1560.71, 1499.08, 1460.47, 1377.57, 1332.59, 1253.01, 1227.11, 1114.14,1076.14, 767.86, 715.26; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.56 (d, *J* = 4.6 Hz, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.89 (s, 1H), 7.71 (s, 1H), 7.58 (d, *J* = 7.7 Hz, 1H), 7.41 (dd, *J* = 13.9, 6.1 Hz, 2H), 7.12 (dd, *J* = 8.0, 4.6 Hz, 1H), 5.88 (s, 2H), 2.59 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 148.91, 145.04, 141.60, 137.71, 135.37, 130.66, 129.47, 128.67, 120.64, 120.52, 118.42, 116.22, 115.27, 41.96, 12.43; HRMS (ESI) Calcd for $C_{16}H_{12}ClBrN_6$ [M + H] 402.0 amu, found 403.67 amu.

1-((1-(2-chloro-6-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-iodo-1H-pyrazolo[3,4-b]pyridine ***(24)****:* White colour solid; Yield 85%; mp 159-160 °C; IR (KBr) cm^{-1}: 3146.63, 3139.14, 3083.70, 3066.83, 3052.62, 2922.65, 2873.04, 1595.99, 1571.67, 1487.59, 1454.69, 1389.47, 1317.59, 1271., 1235.88, 1159.72,1081.56, 1037.47, 767.86, 715.26; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.61 (dd, *J* = 4.5, 1.5 Hz, 1H), 7.82 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.72 (s, 1H), 7.39 – 7.31 (m, 2H), 7.26 – 7.19 (m, 2H), 5.99 (d, *J* = 0.7 Hz, 2H), 2.03 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 150.40, 150.24, 143.16, 138.12, 134.08, 131.73, 130.93, 130.56, 129.33, 127.70, 124.97, 117.82, 90.58, 42.91, 17.83.

3-iodo-1-((1-(o-tolyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazolo[3,4-b]pyridine ***(25):*** White colour solid; Yield 82%; mp 132-133 °C; IR (KBr) cm^{-1}: 3150.11, 3101.14, 3045.57, 2965.88, 2921.16, 2822.65, 2349.41, 1596.90, 1568.54, 1498.31, 1457.62, 1391.17, 1315.18, 1285.46, 1227.52, 1167.31, 1127.22, 792.23, 712.88; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.78 – 8.49 (m, 1H), 8.10 – 7.65 (m, 2H), 7.56 – 7.08 (m, 5H), 5.97 (s, 2H), 2.18 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 150.26, 143.16, 136.31, 133.64, 131.40, 130.58, 129.81, 126.73, 125.93, 124.24, 120.56, 117.82, 42.87, 17.86; HRMS (ESI) Calcd for $C_{16}H_{13}IN_6$ [M + H] 416.22 amu, found amu 417.1.

1-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-iodo-1H-pyrazolo[3,4-b]pyridine ***(26):*** Off-white colour solid; Yield 85%; mp 145-146 °C; IR (KBr) cm^{-1}: 3732.25, 3147.36, 3088.43, 2922.43, 2851.50, 188.25, 1738.55, 1597.50, 1566.32, 1514.32, 1446.09, 1412.16, 1316.58, 1295.03, 1233.57, 1152.91, 1126.68, 829.53, 762.77, 712.88; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.62 (dd, *J* = 4.6, 1.6 Hz, 1H), 7.93 (s, 1H), 7.82 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.69 – 7.63 (m, 2H), 7.24 – 7.16 (m, 3H), 5.96 – 5.93 (m, 2H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 150.33, 144.18, 130.63, 122.63, 122.55, 121.14, 120.59, 117.90, 116.75, 116.52, 90.74, 42.74; HRMS (ESI) Calcd for $C_{15}H_{10}FIN_6$ [M + H] 420.18 amu, found 421.1 amu.

1-((1-(4-chloro-2-iodophenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-iodo-1H-pyrazolo[3,4-b]pyridine ***(27)****:* White colour solid; Yield 81%; mp 163-164 °C; IR (KBr) cm^{-1}: 3142.07, 3085.18, 3058.23, 2920.25, 2851.14, 1882.34, 1732.58, 1595.44, 1563.48, 1484.28, 1443.77, 1384.84, 1321.94, 1302.73, 1244.54, 1172.57,1121.19, 814.28, 795.89, 737.79; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.62 (dd, *J* = 4.5, 1.6 Hz, 1H), 7.97 (d, *J* = 2.2 Hz, 1H), 7.88 (s, 1H), 7.82 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.46 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.21 (dd, *J* = 8.1, 4.5 Hz, 1H), 5.97 (s, 2H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 150.37, 150.28, 143.33, 139.53, 138.55, 136.65, 130.61, 129.36, 128.30, 124.79, 120.59, 117.86, 94.08, 42.74; HRMS (ESI) Calcd for $C_{15}H_9ClI_2N_6$ [M + H] 562.53 amu, found 564.9 amu.

1-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-iodo-1H-pyrazolo[3,4-b]pyridine ***(28):*** White colour solid; Yield 86%; mp 140-142 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ

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8.63 (d, *J* = 5.4 Hz, 1H), 7.96 (s, 1H), 7.86 – 7.79 (m, 1H), 7.73 (s, 1H), 7.59 (d, *J* = 7.3 Hz, 1H), 7.42 (dd, *J* = 13.6, 5.9 Hz, 2H), 7.22 (dd, *J* = 8.1, 4.6 Hz, 1H), 5.95 (s, 2H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 150.35, 144.33, 137.70, 135.49, 130.75, 130.64, 128.85, 120.86, 120.79, 120.60, 118.53, 117.91, 90.79, 42.71.

(1-(2-chloro-5-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl 1-((1-(2-chloro-6-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazolo[3,4-b]pyridine-3-carboxylate **(29):** White colour solid; Yield 84%; mp 112-113 °C; IR (KBr) cm^{-1}: 3138.21, 2955.45, 2923.47, 2853.24, 1736.87, 1713.12, 1597.98, 1573.06, 1487.06, 1460.86, 1371.33, 1266.60, 1225.39, 1150.05, 1120.13, 866.91, 771.83, 721.84; ^{1}H NMR (400 MHz, Chloroform-d) δ 8.66 (d, J = 5.1 Hz, 1H), 8.52 (d, J = 8.5 Hz, 1H), 7.92 (s, 1H), 7.77 (s, 1H), 7.45 – 7.31 (m, 5H), 7.26 (q, J = 7.6, 6.8 Hz, 4H), 6.09 (s, 2H), 5.73 (s, 2H), 2.08 (s, 2H), 2.01 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-d) δ 161.64, 149.83, 142.46, 142.42, 138.06, 138.01, 133.96, 131.65, 131.44, 131.05, 130.94, 129.41, 129.32, 127.75, 127.66, 126.35, 125.18, 119.52, 115.53, 58.18, 43.45, 29.63, 17.83, 17.78; HRMS (ESI) Calcd for $C_{27}H_{21}Cl_2N_9O_2$ [M + H] 574.42 amu, found 576.1 amu.

(1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl 1-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazolo[3,4-b]pyridine-3-carboxylate **(31)**: Off-white colour solid; Yield 83%; mp 139-140 °C; IR (KBr) cm^{-1}: 3148.24, 3080.43, 2922.39, 2851.18, 1725, 1710, 1601.80, 1572.52, 1515.13, 1466.92, 1367.26, 1269.27, 1224.70, 1166.86, 1119.28, 831.28, 769.66, 695.47; ^{1}H NMR (400 MHz, Chloroform-d) δ 8.66 (dd, J = 4.5, 1.6 Hz, 1H), 8.52 (dd, J = 8.1, 1.6 Hz, 1H), 8.16 (s, 1H), 7.96 (s, 1H), 7.74 – 7.69 (m, 2H), 7.66 – 7.61 (m, 2H), 7.33 (dd, J = 8.1, 4.5 Hz, 1H), 7.25 – 7.13 (m, 5H), 6.04 (s, 2H), 5.68 (s, 2H); ^{13}C NMR (101 MHz, Chloroform-d) δ 161.71, 150.67, 149.97, 143.45, 143.24, 133.06, 131.48, 122.83, 122.62, 122.59, 122.53, 122.50, 121.32, 119.62, 116.86, 116.75, 116.63, 116.52, 115.47, 58.10, 43.24; HRMS (ESI) Calcd for $C_{25}H_{17}F_2N_9O_2$ [M + H] 513.46 amu, found 514.2 amu.

(1-(4-chloro-2-iodophenyl)-1H-1,2,3-triazol-4-yl)methyl 1-((1-(4-chloro-2-iodophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazolo[3,4-b]pyridine-3-carboxylate **(32):** Off-white colour solid; Yield 80%; mp 147-149 °C; IR (KBr) cm^{-1}: 1730.87, 1707.76, 1573.88, 1487.06, 1462.35, 1371.13, 1266.70, 1227.39, 1153.32, 1118,39, 868.85, 771.49, 694.47; ^{1}H NMR (400 MHz, Chloroform-d) δ 8.66 (dd, J = 4.4, 1.4 Hz, 1H), 8.52 (dd, J = 8.1, 1.6 Hz, 1H), 8.07 (s, 1H), 8.00 (d, J = 2.2 Hz, 1H), 7.96 (d, J = 2.2 Hz, 1H), 7.93 (s, 1H), 7.47 (ddd, J = 17.3, 8.5, 2.2 Hz, 2H), 7.40 – 7.29 (m, 3H), 6.07 (s, 2H), 5.70 (s, 2H); ^{13}C NMR (101 MHz, Chloroform-d) δ 161.66, 149.96, 142.63, 142.52, 139.65, 139.59, 131.49, 129.49, 129.40, 128.30, 126.30, 125.03, 119.62, 94.11, 58.05, 43.27; HRMS (ESI) Calcd for $C_{25}H_{15}Cl_2I_2N_9O_2$ [M + H] 798.16 amu, found 799.9 amu.

(1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl 1-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazolo[3,4-b]pyridine-3-carboxylate **(33):** Off-white colour solid; Yield 84%; mp 171-172 °C; IR (KBr) cm^{-1}: 3090.90, 2853.24, 1720.87, 1710.12, 1593.65, 1573, 1488.92, 1463.45, 1371.33, 1266.16, 1225.79, 1166.22, 1120.71, 872.35, 774.49, 672.68; ^{1}H NMR (400 MHz, Chloroform-d) δ 8.67 (dd, J = 4.5, 1.6 Hz, 1H), 8.52 (dd, J = 8.1, 1.6 Hz, 1H), 8.21 (s, 1H), 8.00 (s, 1H), 7.79 (t, J = 2.0 Hz, 1H), 7.71 (t, J = 1.8 Hz, 1H), 7.64 (ddd, J = 7.8, 2.2, 1.5 Hz, 1H), 7.60 – 7.54 (m, 1H), 7.51 – 7.37 (m, 4H), 7.34 (dd, J = 8.1, 4.5 Hz, 1H), 6.04 (s, 2H), 5.69 (s, 2H); ^{13}C NMR (101 MHz, Chloroform-d) δ 161.70, 150.70, 150.01, 143.63, 143.41, 137.60, 135.62, 135.50, 131.48, 130.84, 130.76, 129.01, 128.90, 122.59, 121.06, 120.83, 120.76, 119.66, 118.49, 115.48, 58.08, 43.24; HRMS (ESI) Calcd for $C_{25}H_{17}Cl_2N_9O_2$ [M + H] 545.37 amu, found 548.1 amu.

2.4.2. In vitro assay for evaluation of antibacterial activity

2.4.2.1. Qualitative test (agar well diffusion method)

All the Gram-negative and Gram-positive bacterial strains used for the present study were obtained from the Department of Microbiology, Osmania General Hospital, Hyderabad. All strains were tested for purity by standard microbiological methods. The bacterial stock cultures were maintained on Mueller-Hinton agar slants and stored at 4°C. An agar-well diffusion method (B. Venkanna et al., 2013 and Venkanna Banothu et al., 2017) was employed to an evaluation of antibacterial activities of test compounds. DMSO was

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used as a negative control. The bacterial strains were reactivated from stock cultures by transferring them into Mueller-Hinton broth and incubating at 37 ˚C for 18 h. A final inoculum containing 106 colonies forming units (1 x 106 CFU/ml) was added aseptically to the MHA medium and poured into sterile Petri dishes. Different test compounds at a concentration of 100 µg per well were added to wells (8 mm in diameter) punched on an agar surface. Plates were incubated overnight at 37 ºC and the diameter of the inhibition zone (DIZ) around each well was measured in mm. Experiments were performed in triplicates.

2.4.2.2 Quantitative estimation (Minimum inhibitory concentration)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the micro-broth dilution method done in 96 well plates according to standard protocol (Banothu V et al., 2017). A 2-fold serial dilution of the compounds, with the appropriate antibiotic, was prepared. Initially, 100 µl of MH broth was added to each well plate. Then 100 µl of compound or antibiotic was taken from the stock solution and dissolved in the first well plate. Serial dilution was done to obtain different concentrations. The stock concentrations of 1.0 mg/ml. 24 hr culture turbidity was adjusted to match 0.5 McFarland standards which correspond to 1×108 CFU/ml. The standardized suspension (100 µl) of bacteria was added to all the wells except the antibiotic control well and the 96 well plates were incubated at 37 °C for 24 h. After 24 h of incubation 40 µl of MTT (3-(4,5-dimethlthiazol-2-yl)-2,5-diphenyltrazolium bromide) reagent (0.1 mg/ml in 1x PBS) was added to all the wells. MIC was taken as the lowest concentration which did not show any growth which was visually noted from the blue colour developed by MTT. Subcultures were made from clear wells and the lowest concentration that yielded no growth after subculturing was taken as the MBC.

3. Conclusion

In summary, diversely functionalized pyrazolo[3,4-b]pyridine and triazole molecular hybrids have been designed and synthesised using multistep synthetic strategies. The analyses of anti-bacterial properties of the synthesised molecules evaluated against gram-positive and negative pathogens. The compound CY-R2-25 and CY-R2-28 showed potential antibacterial activity with the zone of inhibition values of 15 ± 0.82 and 18 ± 0.95 respectively against *S. aureus.* Similarly, CY-R2-25 and CY-R2-28 showed 14 ± 0.75 and 16 ± 0.82 zone of inhibition values respectively against *K. pneumonia.* The MIC and MIB values of the molecule, CY-R2-25 has found to be 0.25 and 0.5 against *S. aureus* and *K. pneumonia* respectively. Similarly, The MIC and MIB values of the molecule, CY-R2-28 has found to be 0.25 and 0.5 against *S. aureus* and *K. pneumonia* respectively.

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Sample Availability: Samples of the compounds are not available from the authors.

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