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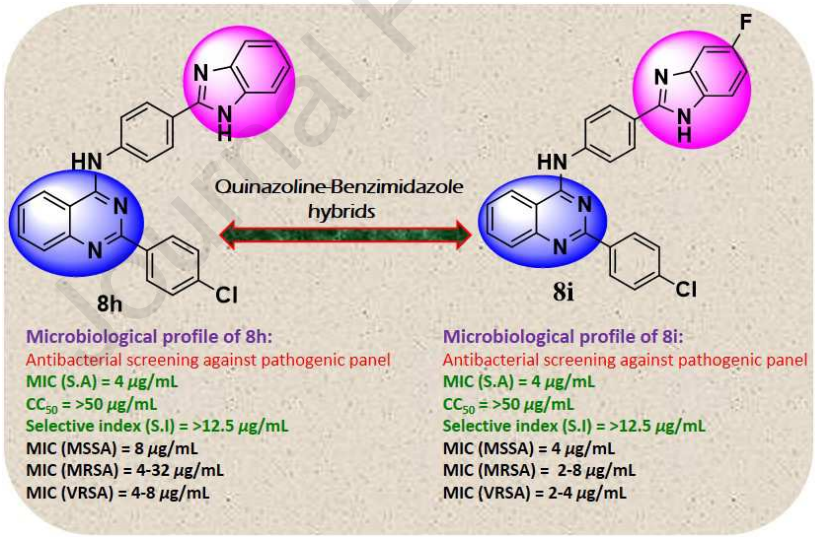
Graphical Abstract:



Synthesis and evaluation of new Quinazoline-benzimidazole hybrids as potent anti-microbial agents against multidrug resistant *Staphylococcus aureus* and *Mycobacterium tuberculosis*

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Abstract: Owing to the rapid rise in antibiotic resistance, infectious diseases have become serious threat to public health. There is an urgent need to develop new antimicrobial agents with diverse chemical structures and novel mechanisms of action to overcome the resistance. In recent years, Quinazoline-benzimidazole hybrids have emerged as a new class of antimicrobial agents active against *S. aureus* and *M. tuberculosis*. In the current study, we designed and synthesized fifteen new Quinazoline-benzimidazole hybrids and evaluated them for their antimicrobial activity against *S. aureus* ATCC 29213 and *M. tuberculosis* H37Rv. These studies led to the identification of nine potent antibacterial agents **8a**, **8b**, **8c**, **8d**, **8f**, **8g**, **8h**, **8i** and **10c** with MICs in the range of 4-64 μg/mL. Further, these selected compounds were found to possess potent antibacterial potential against a panel of drug-resistant clinical isolates which include methicillin and vancomycin-resistant *S. aureus*. The selected compounds were found to be less toxic to Vero cells (CC_{50} = 40-≥200 μg/mL) and demonstrated a favourable selectivity index. Based on the encouraging results obtained these new benzimidazol-2-yl quinazoline derivatives have emerged as promising antimicrobial agents for the treatment of MDR- *S. aureus* and Mycobacterial infections.

Keywords: Quinazoline, Benzimidazole, anti-bacterial agents, MRSA, Tuberculosis, cytotoxicity, resistance.

1. Introduction

Alarming rise of difficult to treat multi drug resistant bacterial infections has emerged as one of the formidable challenges of 21st century [1-5]. The World Health Organization (WHO) has categorised 12 of the pathogens as high-risk priority II pathogens which require immediate attention [6,7]. In particular, *S. aureus* is known to cause a wide range of diseases, from skin infections to serious illnesses like pneumonia, meningitis, bacteraemia and sepsis [8-10]. Among the various resistant strains, methicillin resistant (MRSA) and vancomycin resistant (VRSA) strains are known to be sources of spread of community infections. Multi Drug Resistant Tuberculosis (MDR-TB) caused due to *Mycobacterium tuberculosis* (Mtb) has emerged as another major life-threatening disease. A number of studies indicated that the anti-microbial resistance (AMR) has attained frightening levels globally [11-13]. Around 750,000 deaths are reported annually due to AMR and the toll is likely to rise to 10 million by 2050 [14]. Hence, search for newer treatments for multi drug resistant microbial infections have been the focus of the scientific community in recent years.

Quinazoline is a remarkable scaffold of medicinal importance possessing promising pharmacological activities like antitubercular [16], antibacterial [17], anticonvulsant [18], anti-HIV, antifungal [19], anti-inflammatory, analgesic [20] and anticancer [21] activities. Bouley et al. [22] reported 4(3*H*)-quinazolinones as orally bioavailable antibacterial agents **I**. Our group also reported 3-phenylquinazolin-4(3*H*)-ones and related derivatives **II** [23] and 2-phenylquinazoline derivatives **III** as potent anti-MDR-*S. aureus* agents and as anti-mycobacterial agents [24]. Benzimidazole scaffold has attracted wide attention as an interesting heterocyclic in medicinal chemistry owing to its presence as a core scaffold in a number of drugs such as albendazole, mebendazole, candesartan, astemizole etc. [25]. Several compounds possessing benzimidazole as a core are reported to possess diverse therapeutic properties such as antibacterial, antiulcer, antiviral, antihypertensive, anticancer, antifungal, anti-inflammatory and anticoagulant properties [26]. Dokla et al. [27] reported 1,2-disubstituted benzimidazole scaffold **IV** to possess potent antibacterial activity against *E. coli*. Mustafa et al. [28] reported the benzimidazole-acrylonitrile hybrids **V** as anti-mycobacterial agents. *p*-aminobenzoic acid (PABA) serves as a bioactive linker because of its occurrence in many human pathogens. *p*-Aminosalicylic acid **VI** [29], a derivative of PABA is used to treat active drug resistant tuberculosis together with other anti-tubercular medications.

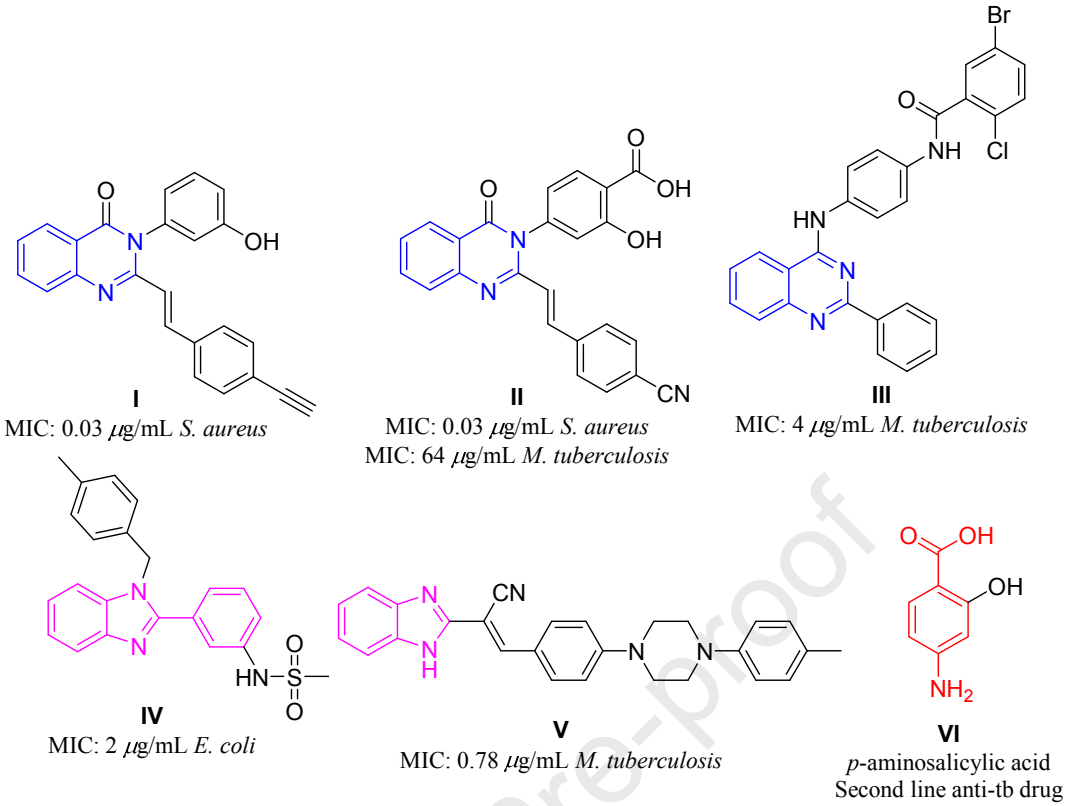


Figure 1. Reported anti-bacterial agents.

Based on the key role being played by quinazoline, benzimidazole and *p*-aminobenzoic acid moieties in a number of anti-microbial agents, we designed and synthesized a number of new quinazoline-benzimidazole hybrids with *p*-aminobenzoic acid as linker.

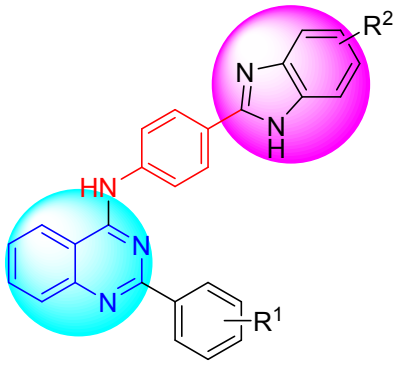
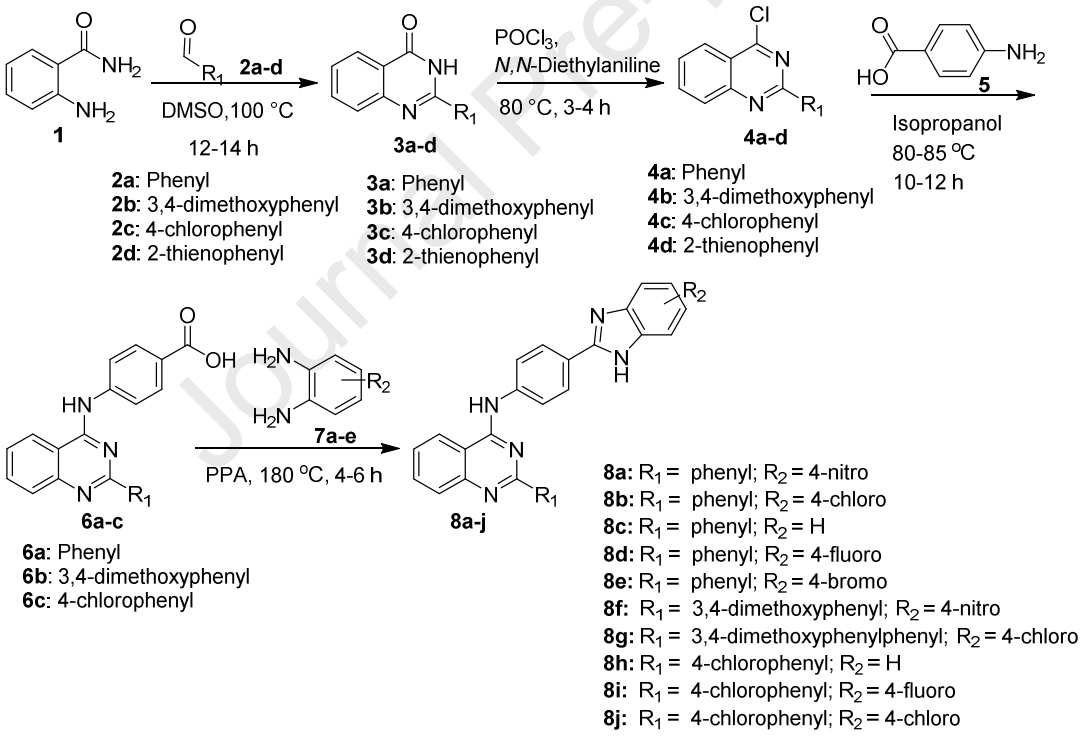


Figure 2. Designed quinazolines-benzimidazole hybrids.

2. Results and Discussion:

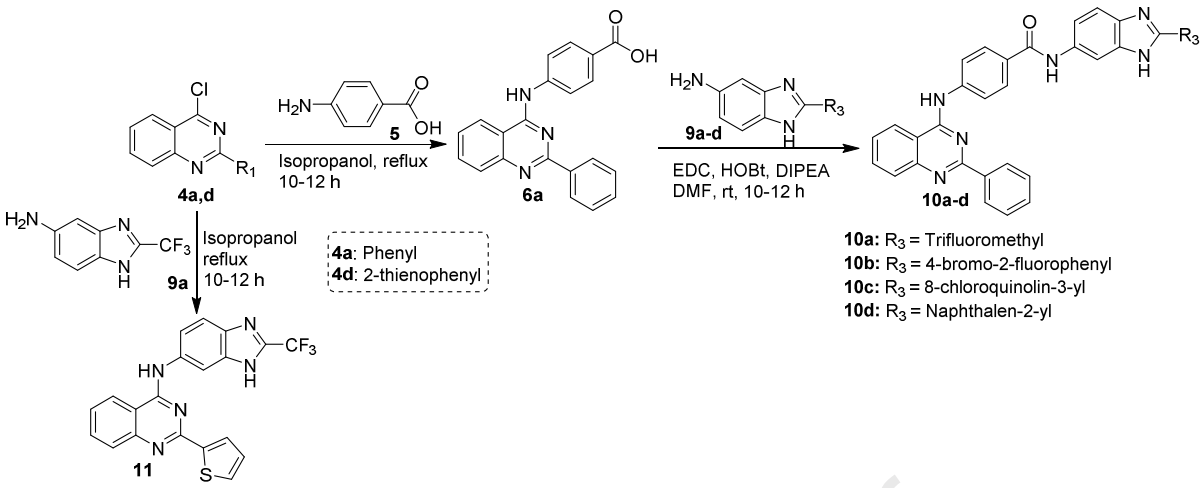
2.1 Chemistry:

A series of new benzimidazolo-2-arylquinazoline derivatives were synthesized as described in **Scheme 1**. 2-aminobenzamide **1** was condensed with different substituted aryl aldehydes **2a-d** for 12-24 h in presence of dimethylsulphoxide to afford 2-arylquinazolinones **3a-d** [30]. These derivatives were treated with phosphorus (V) oxychloride and *N, N*-diethyl aniline to provide 2-aryl 4-chloroquinazoline derivatives **4a-d** [30]. The chlorinated derivatives **4a-d** undergo nucleophillic substitution with *p*-aminobenzoic acid **5** to obtain 4-[(2-phenylquinazolin-4-yl)amino]benzoic acid derivatives **6a-c**. Further, the 4-[(2-phenylquinazolin-4-yl)amino]benzoic acid derivatives **6a-c** were condensed with substituted o-phenylenediamines **7a-e** in presence of polyphosphoric acid at 180 ^{o}C to afford the corresponding benzimidazole derivatives **8a-j** in moderate to excellent yields.



Scheme 1. Synthesis of *N*-(4-(1*H*-benzoimidazol-2-yl)aryl)-2-arylquinazolin-4-amine derivatives.

4-[(2-phenylquinazolin-4-yl) amino] benzoic acid derivatives **6a-c** were also coupled with different substituted 2-aryl-benzoimidazol-6-amines **9a-d** to afford the corresponding benzimidazoloamide derivatives **10a-d** in excellent yields (**Scheme-2**).



Scheme 2. Synthesis of 4-((2-phenylquinazolin-4-yl)amino)-*N*-(2-(aryl)-1*H*-benzoimidazol-6-yl)benzamide derivatives.

2.2 *In vitro* antibacterial activity

2.2.1 Antibiotic susceptibility testing against *E. coli*, *S. aureus*, *K. pneuminiae*, *A. baumannii*, and *P. aeruginosa* pathogen panel.

The newly synthesized benzimidazol-2-yl quinazoline derivatives were subjected to antibiotic susceptibility testing against *E. coli*, *S. aureus*, *K. pneuminiae*, *A. baumannii*, and *P. aeruginosa* pathogen panel. The minimum inhibitory concentrations (MICs) against gram positive and gram-negative bacterial strains viz., *E. coli*, *S. aureus*, *K. pneuminiae*, *A. baumannii*, and *P. aeruginosa* pathogen panel were determined by using broth microdilution assay. In addition, the synthesized compounds were also evaluated against *M. tuberculosis* (ATCC 27294), *M. abscessus* (ATCC 19977), *M. fortuitum* (ATCC 6841) and *M. chelonae* (ATCC 35752). The results are tabulated in **Tables 1** & **2**. As per our previous studies [24], 2-phenylquinazolines are reported to possess potent anti-bacterial and anti-mycobacterial activities. Based on this observation and also the promising antimicrobial activities of benzimidazole, we explored a number of 2-aryl-quinazolines with benzimidazole hybrids containing a number of electron withdrawing and electron donating substituents. We studied the effect of 3,4-dimethoxyphenyl and 4-chlorophenyl as aryl substituents at C-2 of quinazolines. Compounds with 4-nitro, 4-bromo, 4-chloro and 4-fluoro substituents on benzimidazole moiety were studied. Based on this, a total of 15 new quinazoline-benzimidazole hybrids were synthesized and evaluated for their anti-bacterial and anti-mycobacterial activities.

The antimicrobial screening results suggested that the synthesized compounds exhibited selective inhibitory activity against *S. aureus*. Compounds **8d**, **8h** and **8i** exhibited equipotent inhibitory activity with MIC of 4 μg/mL. Compounds **8b** and **8c** have shown anti-bacterial inhibition with MIC of 8 μg/mL. Compounds **8g, 10a**, **10b** and **11** have exhibited moderate inhibition with MIC of 16-64 μg/mL. The remaining compounds are found to be inactive against *E. coli, K. pneuminiae, A. baumannii, and P. aeruginosa* pathogens. With phenyl group at C-2 position, the substitutions on benzimidazole were altered such as 4-nitro **8a,** 4-chloro **8b,** unsubstituted **8c,** 4-fluoro **8d** and 4-bromo **8e**. While, the compound **8a** did not show any inhibitory activity against *S. aureus*, the compounds **8b, 8c** and **8d** exhibited good inhibitory activity against *S. aureus* with MIC 8, 8 and 4 μg/mL respectively. Further, with 3,4-dimethoxyphenyl at C-2 position compounds **8f** with 4-nitro and **8g** with 4-chloro substitutions on benzimidazole were synthesized. While the compound **8g** inhibited *S. aureus* with MIC value 16 μg/mL, the compound **8f** did not show any inhibitory activity. With 4-chlorophenyl at C-2 position, compounds **8h**, **8i** and **8j** were synthesized. While, the compounds **8h** and **8i** were found to possess potent inhibitory activity against *S. aureus* with MIC: 4 μg/mL, the compound **8j** was found to be inactive. Next, we focused our attention on the modification of the carboxyl moiety at para position of the C-4 phenyl by modifying to various amides by coupling with substituted benzimidazoloamines as shown in **Scheme 2**. Thus, the compounds **10a**, **10b**, **10c** and **10d** were synthesized. The synthesized compounds were found to exhibit moderate inhibition against *S. aureus* with MIC values of 32->64 μg/mL. Compound **11** was synthesized by direct substitution of 2-trifluoromethyl benzimidazolo-5-amine at C-4. The compound **11** was found to be inactive.

The synthesized compounds were tested for their anti-mycobacterial potential with four different mycobacterial strains. The inhibitory screening results are tabulated in **Table 2**. Compounds **8a-e** showed moderate inhibitory activity against Mtb (MIC = 8–>64 μg/mL). The compound **8a** exhibited inhibitory activity against *M. tuberculosis* with MIC value of 8 μg/mL. The compound **8b** showed moderate activity against *M. tuberculosis, M. fortuitum and M. chelonae*. The compounds **8c** and **8d** did not show any inhibitory activity against *M. tuberculosis.* Interestingly, compound **8f** exhibited good inhibitory activity against *M. tuberculosis* (H37Rv) with MIC value of 8 μg/mL. The compound **8f** also showed good inhibition against *M. fortuitum* (ATCC 6841) with the MIC value of 4 μg/mL and *M. chelonae* (ATCC 35752) with the MIC value of 8 μg/mL. Compound **8g** exhibited moderate activity against *M. tuberculosis* with MIC value of 32 μg/mL. Compound **10c** exhibited good

inhibition activity with MIC 16 μg/mL against *M. tuberculosis*. The compounds **10a** and **10d** were found to be moderately active and compound **11** showed moderate inhibition with the MIC 64 μg/mL.

Table 1. MIC values (μg/mL) of the tested compounds against gram positive and gram negative pathogen panel.

S. No	Sample code	*E. coli* ATCC 25922	*S. aureus* ATCC 29213	*K. pneumoniae* BAA 1705	*A. baumannii* BAA 1605	*P. aeruginosa* ATCC 27853
1	**8a**	>64	>64	>64	>64	>64
2	**8b**	>64	**8**	>64	>64	>64
3	**8c**	>64	**8**	>64	>64	>64
4	**8d**	>64	**4**	>64	>64	>64
5	**8e**	>64	>64	>64	>64	>64
6	**8f**	>64	>64	>64	>64	>64
7	**8g**	>64	**16**	>64	>64	>64
8	**8h**	>64	**4**	>64	>64	>64
9	**8i**	>64	**4**	>64	>64	>64
10	**8j**	>64	>64	>64	>64	>64
11	**10a**	>64	**32**	>64	>64	>64
12	**10b**	>64	**64**	>64	>64	>64
13	**10c**	>64	>64	>64	>64	>64
14	**10d**	>64	>64	>64	>64	>64
15	**11**	>64	**64**	>64	>64	>64
16	**Levofloxacin**	**0.125**	**0.015**	**64**	**8**	**0.5**

Table 2. MIC values (μg/mL) of the tested compounds against Mtb and other mycobacterium species.

S.No	Sample code	Mtb H37Rv ATCC 27294	*M. abscessus* ATCC 19977	*M. fortuitum* ATCC 6841	*M. chelonae* ATCC 35752
1	**8a**	**8**	>64	>64	>64
2	**8b**	**32**	>64	**32**	**32**
3	**8c**	>64	>64	>64	>64
4	**8d**	>64	>64	>64	**32**
5	**8e**	NT	NT	NT	NT
6	**8f**	**8**	>64	**4**	**8**
7	**8g**	**32**	>64	>64	>64
8	**8h**	NT	NT	NT	NT
9	**8i**	NT	NT	NT	NT
10	**8j**	NT	NT	NT	NT
11	**10a**	**64**	>64	>64	>64
12	**10b**	>64	>64	>64	>64
13	**10c**	**16**	>64	>64	>64
14	**10d**	**64**	>64	>64	>64
15	**11**	**64**	>64	>64	>64
16	**Isoniazid**	**0.03**	NT	NT	NT
17	**Rifampicin**	**0.06**	NT	NT	NT
18	**Streptomycin**	**1**	NT	NT	NT
19	**Ethambutol**	**1**	NT	NT	NT
20	**Levofloxacin**	NT	1	0.03	0.03

*NT=not tested

The Structure-Activity Relationships derived are explained in **Figure 3**.

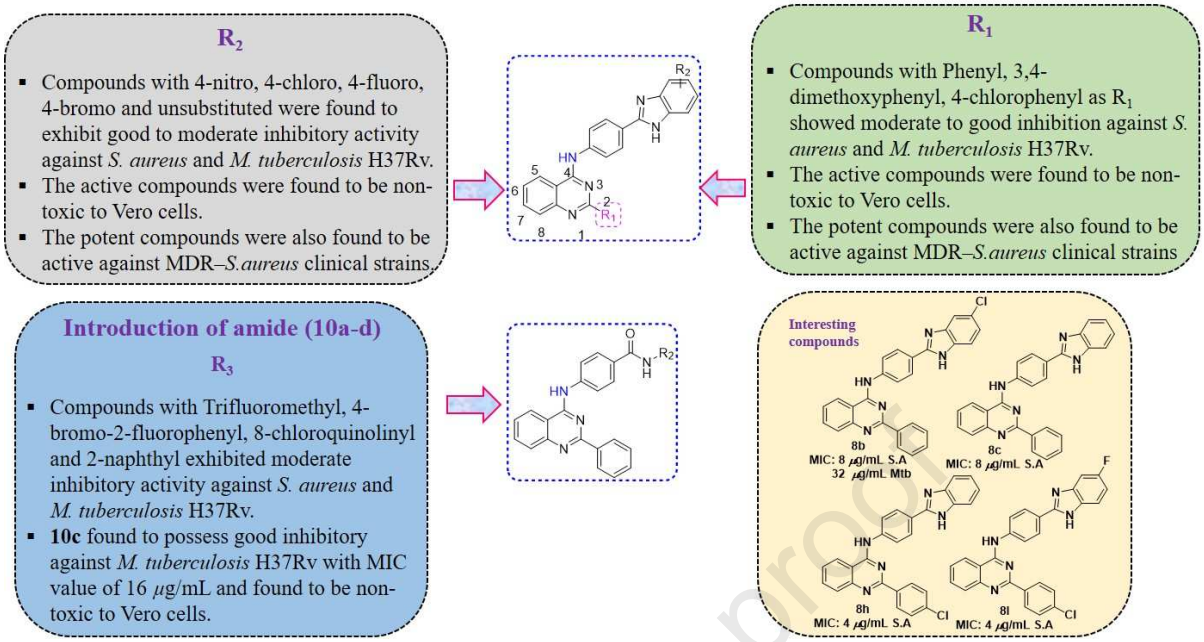


Figure 3. Structure-Activity Relationship of new quinazoline-benzimidazole hybrids.

2.2.2 Cytotoxicity against Vero cells:

The selected compounds were further subjected to cytotoxicity assay against Vero cells (African green monkey kidney cell line, ATCC CCL-81) using MTT assay. The CC_{50} (50% cytotoxic concentration) was defined as the reduction of cell viability to 50% by compound. Doxorubicin and Levofloxacin were used as a reference standard and each experiment was performed in triplicate. The cytotoxicity data demonstrates that **8a**, **8b, 8c, 8d, 8f, 8g, 8h, 8i** and **10c** were nontoxic to Vero cells (CC_{50} = 40->100 μg/mL) and displayed favourable selectivity index. The results are tabulated in **Tables 3.**

Table 3. Cytotoxicity profile against Vero cells and Selectivity Index (SI).

S. No	Sample code	*S. aureus* ATCC 29213	CC_{50} (μg/mL)	Selective index (CC_{50}/MIC)
1.	**8a**	**8**	100	12.5
2.	**8b**	**8**	100	12.5
3.	**8c**	**8**	80	10
4.	**8d**	**4**	40	10
5.	**8f**	**8**	100	12.5
6.	**8g**	**16**	>100	>6.25
7.	**8h**	**4**	50	12.5
8.	**8i**	**4**	50	12.5
9.	**10c**	**16**	>100	>6.25

2.2.3 Determination of MIC against MDR-*S. aureus* including VRSA

To further determine their potential against multiple strains of MDR *S. aureus*, **8b, 8c, 8h** and **8i** were tested against various well defined and characterized clinical isolates of MSSA, MRSA and VRSA as per standard CLSI guidelines. The results are summarised in **Table 4**. The compounds **8b**, **8c**, **8h** and **8i** were found to be active against various resistant strains. From the close examination of the results, it can be inferred that **8b, 8c, 8h** and **8i** displayed potent antibacterial activity with MIC 4–16 μg/mL against a number of clinical strains of MSSA, MRSA and VRSA. The obtained results suggest that compounds **8b, 8c, 8h** and **8i** are less resistant to all the MRSA and VRSA isolates with MIC 4–16 μg/mL in comparison with Methicillin**.** Levofloxacin and Meropenam. The compounds **8b, 8c, 8h** and **8i** were also found to be active against VRSA clinical isolates with MIC 4–16 μg/mL.

Table 4. MIC of a compound **8b**, **8c**, **8h** and **8i** against MRSA and VRSA strains.

Strains		Antibiotics resistant to	Details about the strain	MIC (µg/mL)								
				8b	8c	8h	8i	Levofloxacin	Meropenem	Vancomycin	Daptomycin	Methicillin
MSSA	*S. aureus* ATCC 29213	None		8	8	8	4	0.125	0.0625	1	1	1
MRSA	NR 100	Methicillin, Ceftriaxone, Meropenem, Gentamycin and Linezolid	Resistant to tetracycline, contains mec subtype I cassette, Large variety of virulence factors	8	32	8	4	0.25	32	2	1	>64
	NR 119	Methicillin, Ceftriaxone, Meropenem, Gentamycin and Linezolid	Contains mec subtype IV cassette, G2576T mutation in domain V in one or more 23 S rRNA genes	8	8	32	4	16	64	2	1	>64
	NR 10129	Methicillin, Ceftriaxone, Meropenem	Also called as TCH60	8	16	8	4	0.25	4	1	0.5	>64
	NR 10198	Methicillin, Ceftriaxone, Meropenem	USA100 Community acquired-MRSA, Contains mec type II cassette, Negative for PVL virulence factor	4	4	8	8	8	4	1	0.5	>64
	NR 10192	Methicillin, Ceftriaxone, Meropenem	Community acquired-MRSA, Contains mec type II cassette, Negative for PVL virulence factor	8	32	4	2	16	64	1	1	>64
	NR 10186	Methicillin, Ceftriaxone, Meropenem	USA 300 Community acquired-MRSA, Contains mec type IV cassette, Positive for PVL virulence factor	8	8	16	4	8	8	1	0.5	>64
	NR 10193	Methicillin, Ceftriaxone, Meropenem	Community acquired-MRSA, Contains mec type II cassette, Negative for PVL virulence factor	16	16	8	8	32	64	2	1	>64
	NR 10194	Methicillin, Ceftriaxone	Community acquired-MRSA, Contains mec type V cassette, Positive for PVL virulence factor	8	8	8	4	0.125	1	1	0.5	32
	NR 10191	Methicillin, Ceftriaxone, Meropenem	USA 600 Community acquired-MRSA Contains mec type II cassette, Negative for PVL virulence factor	16	16	8	8	32	64	2	1	>64
VRSA	VRS1	Methicillin, Ceftriaxone, Meropenem, Gentamycin, Vancomycin, Teicoplanin	USA100 and contains mec (subtype II) and *van A.*, Negative for *van B, van C1, van C2, van D, van E,* PVL and arginine catabolic mobile element (ACME)	4	8	4	4	32	64	>64	1	>64
	VRS4	Methicillin, Ceftriaxone, Meropenem, Vancomycin and Teicoplanin	USA100 and contains mec (subtype II) and *van A.* Negative for *van B, van C1, van C2, van D, van E,* PVL and arginine catabolic mobile element (ACME)	8	8	8	2	>64	64	>64	0.5	>64
	VRS12	Methicillin, Ceftriaxone, Meropenem, Vancomycin and Teicoplanin	NA*	8	16	8	4	32	16	>64	0.5	>64

2.3 ADMET properties

ADMET properties of the synthesized compounds were calculated using Qikprop program (Qikprop, version 6.5, Schrödinger, LLC, New York, NY, 2014). As can be seen below, the partition coefficient (QPlogPo/w), hydrogen bond donors (donor HB), hydrogen bond acceptors (acceptor HB), molecular weight (mol. Wt.) and percent human oral absorption exhibited satisfactory results. The compounds also followed Lipinski rule of five. ADMET properties for Isoniazid and Rifampicin were also calculated and compared with the results of the synthesized compounds. The predicted results are shown in **Table 5**.

Table 5. ADMET properties of **8a-j, 10a-d** and **11.**

Descriptors	**Recommended values**	**8a**	**8b**	**8c**	**8d**	**8e**	**8f**	**8g**	**8h**	**8i**	**8j**	**10a**	**10b**	**10c**	**10d**	**11**	**Levofloxacin**	**Isoniazid**	**Rifampicin**
Molecular weight	130.0–725.0	458.47		413.48	431.47	492.37	518.53	507.97	447.92	465.91	465.37	524.50	629.49	618.09	582.66	411.40	361.37	137.141	820.978
Dipole moment	1.0–12.5	10.49	9.71	9.30	5.25	8.15	8.13	5.16	9.33	7.32	7.33	7.79	9.61	8.30	5.25	4.15	5.33	3.32	4.33
Total SASA	300–1000	969.54	899.24	789.81	819.49	773.04	909.35	721.15	687.16	829.909	1005.33	855.54	979.24	1021.81	1011.49	653.04	587.16	329.909	1018.33
No. of rotatable bonds	0–15	2	3	4	3	4	4	4	1	2	2	2	3	4	3	4	1	2	25
Donor HB	0.0–6.0	2	2	2	2	2	2	2	2	2	2	3	3	3	3	2	0	3	6
Acceptor HB	2.0–20.0	5	4	4	4	4	6.5	5.5	6.5	4	4	6.5	6.5	7.5	6.5	4	7.25	4.5	20.35
QP Polarizability	13.0–70.0	44.20	44.42	46.05	46.08	48.52	55.81	52.87	36.38	56.80	68.01	54.20	41.42	40.05	56.01	38.52	36.38	13.80	68.019
QP logP o/w	2.0–6.5	8.08	7.66	9.81	7.54	5.08	7.82	5.04	8.39	7.67	5.89	7.85	5.16	7.66	7.87	7.72	-0.39	-0.653	2.893
QP log BB	−3.0 and 1.2	-1.01	-1.41	-0.513	-0.112	-0.940	-0.96	-0.137	-0.42	-0.87	-2.430	-1.39	-0.61	-1.01	-1.11	-1.07	-0.42	-0.87	-2.430
Human Oral Absorption	1–3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1
Percent Human Oral Absorption	> 80% is high	91.49	100	100	100	100	79.59	100	100	100	100	100	100	100	100	100	48.823	66.30	33.90
Rule of Five violations	< 25% is low	1	1	1	1	1	1	1	1	1	1	2	2	2	2	1	0	0	3

3. Conclusion:

Rapid rise in multidrug resistance is one of the alarming threats to public health worldwide. In order to alleviate the situation, there is an urgent need to develop new antimicrobial drugs with novel mechanisms of action. In this work, we designed and synthesized a library of 15 new Quinazoline-Benzimidazole hybrids and evaluated them against a panel of gram positive and gram negative bacterial strains consisting of against *E. coli, S. aureus, K. pneuminiae, A. baumannii,* and *P. aeruginosa, M. tuberculosis* H37Rv and other mycobacterial strains. Among the studied compounds, **8a**, **8b**, **8c**, **8d**, **8f**, **8g**, **8h**, **8i** and **10c** exhibited good to moderate inhibitory activity against *S. aureus* and different *mycobacterium species*. The compounds were found to be non-toxic to Vero cells (CC_{50} 40->100 μg/mL) with favourable selectivity index (SI <10->25). Further, the compounds **8b, 8c**, **8h** and **8i** were found to display potent inhibitory activity against clinical MRSA and VRSA isolates. In a separate study, the compounds **8a**, **8f** and **10c** were found to exhibit potent anti-mycobacterial activity with MIC values in the range of 8-16 μg/mL. The compounds were also found to be less toxic to Vero cells (CC_{50} ≥100 μg/mL) and exhibited promising selectivity index. Based on the above studies, the newly synthesized quinazoline-benzimidazole hybrids were found to possess promising anti-bacterial and anti-mycobacterial activities and possess the potential for further development.

4. Experimental section:

4.1 General Methods. All the reagents and solvents were obtained from commercial suppliers and were used without further purification. Analytical thin layer chromatography (TLC) was performed on MERCK pre-coated silica gel 60-F254 (0.5 mm) aluminum plates. Visualization of the spots on TLC plates was achieved by UV light. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 500 MHz by making a solution of samples in the DMSO-d_6 as solvent using tetramethyl silane (TMS) as the internal standard. Chemical shifts for ^{1}H and ^{13}C are reported in parts per million (ppm) downfield from tetra methyl silane. Spin multiplicities are described as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constant (*J*) values are reported in hertz (Hz). HRMS were determined with Agilent QTOF mass spectrometer 6540 series instrument. Wherever required, column chromatography was performed using silica gel (60-120). The reactions are carried under nitrogen positive pressure using freshly distilled solvents wherever anhydrous conditions required. All evaporation of solvents was carried out under reduced pressure using

rotary evaporator below 45 □. Melting points were determined with an electro thermal digital melting point apparatus IA9100 and are uncorrected. The names of all the compounds given in the experimental section were taken from ChemBioDraw Ultra, Version 12.0.

General Experimental Procedure for the Synthesis of 8a-j, 10a-d and 11.

Procedure for 8a-j: To the mixture of **6** (1 mmol), substituted o-phenylenediamines (**7a-f**, 1 mmol) was added to the RBF, then polyphospheric acid (5gm) was added to the reaction mixture and stir the contents at 180 ^{o}C for 4-6 h. The reaction mixture was stirred for 20 minutes at room temperature, followed by the addition of ice cold water. Then neutralise the reaction mixture by using the aqueous solution of NaOH. After neutralization of the reaction, the suspension was extracted with ethyl acetate (3x5.0 mL), washed with 1:1 mixture of brine. The combined organic extracts were dried over anhydrous sodium sulphate. After removal of the solvent under reduced pressure, the crude product was purified by using column chromatography, EtOAc:Hexane (2:8) as eluent on silica gel to afford the pure products.

Procedure for 10a-d: To the mixture of **6** (1 mmol) and HATU (1mmol), DMF (3mL) was added slowly under nitrogen atmosphere. The reaction mixture was then stirred for 20 minutes at 0 ^{o}C, followed by the addition of substituted benzimidazole amines (**9a-d**, 1mmol). The reaction mixture was stirred for 20 minutes at room temperature, followed by the addition of DIPEA. Upon completion of the reaction as monitored by TLC, crushed ice was added to the reaction mixture. The resulting solid was then subjected to vacuum filtration, excess of water was used to wash off the insoluble solids to obtain crude powder which was purified using column chromatography (elution with hexane/EtOAc = 3:7). The pure products were collected as white color solids in good yields.

*4.1.1 N-(4-(5-nitro-1H-benzo[d]imidazol-2-yl)phenyl)-2-phenylquinazolin-4-amine (**8a**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.57 (s, 1H), 10.14 (s, 1H), 8.65 (d, J = 8.3 Hz, 1H), 8.59-8.42 (m, 3H), 8.34 (d, J = 8.7 Hz, 2H), 8.29 (d, J = 8.7 Hz, 2H), 8.15 (d, J = 8.2 Hz, 1H), 7.93 (d, J = 3.2 Hz, 2H), 7.76 (s, 1H), 7.69-7.61 (m, 1H), 7.60–7.53 (m, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 159.4, 158.2, 151.0, 143.0, 142.5, 138.6, 133.9, 130.9, 129.0, 128.9, 128.7, 128.4, 128.2, 127.9, 126.6, 126.3, 124.1, 123.5, 122.2, 118.4, 114.6; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.2 N-(4-(5-chloro-1H-benzo[d]imidazol-2-yl)phenyl)-2-phenylquinazolin-4-amine (**8b**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.07 (d, J = 14.8 Hz, 1H), 10.10 (s, 1H), 8.64 (d, J = 8.3 Hz, 1H), 8.58–8.44 (m, 2H), 8.33–8.19 (m, 4H), 7.99–7.87 (m, 2H), 7.74–7.64 (m, 2H), 7.55 (t, J = 7.2 Hz, 4H), 7.24 (t, J = 9.1 Hz, 1H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 159.4, 158.2, 151.0, 141.7, 138.7, 133.8, 130.8, 129.0, 128.9, 128.7, 128.4, 128.2, 127.4, 126.6, 124.9, 123.5, 122.8, 122.3, 121.8, 118.4, 114.6, 114.0, 112.8; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.13 N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-2-phenylquinazolin-4-amine (**8c**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.83 (s, 1H), 10.53-10.46 (m, 1H), 10.14 (s, 1H), 8.66 (d, J = 5.8 Hz, 1H), 8.53 (s, 2H), 8.40 (s, 1H), 8.25 (s, 2H), 8.18 (s, 2H), 7.93 (s, 2H), 7.79 (d, J = 33.7 Hz, 2H), 7.68 (s, 1H), 7.56 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 159.4, 158.2, 151.2, 151.0, 141.6, 138.7, 133.8, 130.9, 128.9, 128.7, 128.4, 128.2, 127.3, 126.6, 123.5, 122.3, 117.4, 114.6, 114.0, 109.5; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.4 N-(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-2-phenylquinazolin-4-amine (**8d**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.69 (s, 1H), 8.78–8.14 (m, 3H), 8.14–8.02 (m, 2H), 7.98-7.85 (m, 2H), 7.85–7.67 (m, 3H), 7.75–7.17 (m, 6H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 164.6, 158.4, 157.9, 150.6, 143.1, 141.0, 135.4, 135.2, 134.9, 133.9, 132.9, 131.4, 129.1, 128.9, 128.7, 127.1, 123.5, 123.3, 120.2, 119.3, 118.0, 114.6, 112.9; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.5 N-(4-(5-bromo-1H-benzo[d]imidazol-2-yl)phenyl)-2-phenylquinazolin-4-amine (**8e**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.60 (s, 1H), 9.98 (d, J = 8.9 Hz, 1H), 8.76–8.51 (m, 3H), 8.02 (t, J = 9.3 Hz, 2H), 7.98–7.85 (m, 4H), 7.82 (d, J = 8.9 Hz, 2H), 7.78 (d, J = 8.6 Hz, 1H), 7.75 (t, J = 4.7 Hz, 1H), 7.70–7.66 (m, 1H), 7.53 (d, J = 8.6, 1H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 165.5, 159.4, 158.2, 151.0, 143.0, 138.6, 133.9, 130.9, 130.5, 129.6,

129.0, 128.8, 128.7, 128.4, 126.6, 123.6, 121.4, 121.3, 119.1, 118.5, 114.6; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.6 2-(3,4-dimethoxyphenyl)-N-(4-(5-nitro-1H-benzo[d]imidazol-2-yl)phenyl)quinazolin-4-amine (**8f**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^1H NMR (500 MHz, DMSO-d_6): δ 13.63 (s, 1H), 10.09-10.03 (m, 1H), 8.61 (s, 1H), 8.49-8.41 (d, 1H), 8.32 (s, 3H), 8.14 (d, J = 16.2 Hz, 2H), 8.00 (s, 1H), 7.89-7.80 (m, 2H), 7.76 (s, 1H), 7.65-7.55 (m, 2H), 7.06-7.00 (m, 1H), 4.20–3.88 (m, 3H), 3.94–3.68 (m, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.4, 161.06, 159.2, 158.5, 152.3, 149.6, 146.0, 140.3, 134.8, 133.5, 132.3, 128.6, 128.5, 127.7, 127.5, 126.6, 126.2, 122.8, 121.2, 115.1, 114.1, 106.3, 99.0, 56.5, 56.0; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.7 N-(4-(5-chloro-1H-benzo[d]imidazol-2-yl)phenyl)-2-(3,4-dimethoxyphenyl)quinazolin-4-amine (**8g**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^1H NMR (500 MHz, DMSO-d_6): δ 13.63 (s, 1H), 10.09-10.03 (m, 1H), 8.61 (s, 1H), 8.49-8.41 (d, 1H), 8.32 (s, 3H), 8.14 (d, J = 16.2 Hz, 2H), 8.00 (s, 1H), 7.89-7.80 (m, 2H), 7.76 (s, 1H), 7.65-7.55 (m, 2H), 7.06-7.00 (m, 1H), 4.20–3.88 (m, 3H), 3.94–3.68 (m, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.6, 163.4, 161.6, 159.2, 152.3, 149.6, 146.0, 139.8, 134.8, 132.8, 132.3, 128.7, 128.1, 127.7, 126.6, 126.2, 122.7, 121.2, 118.8, 115.0, 114.1, 113.8, 106.3, 99.0, 56.5, 56.0; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.8 N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-2-(4-chlorophenyl)quinazolin-4-amine (**8h**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^1H NMR (500 MHz, DMSO-d_6): δ 10.57 (s, 1H), 10.03 (s, 1H), 8.60 (d, J = 8.4 Hz, 3H), 8.16 (d, J = 8.1 Hz, 2H), 8.06 (d, J = 8.1 Hz, 2H), 8.00 (d, J = 8.2 Hz, 2H), 7.94–7.88 (m, 5H), 7.70–7.66 (m, 1H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 164.4, 159.5, 158.3, 139.5, 135.8, 135.0, 133.7, 132.9, 130.7, 128.9, 128.8, 128.3, 126.4, 123.4, 123.1, 121.0, 118.8, 114.4, 114.2; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.9 2-(4-chlorophenyl)-N-(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)phenyl)quinazolin-4-amine (**8i**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.56 (s, 1H), 9.92 (s, 1H), 8.97 (s, 1H), 8.59 (d, J = 8.2 Hz, 1H), 8.46 (d, J = 6.2 Hz, 2H), 8.33–8.24 (m, 1H), 7.97 (d, J = 8.7 Hz, 2H), 7.87 (t, J = 6.0 Hz, 4H), 7.63 (d, J = 7.8 Hz, 1H), 7.52 (d, J = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 159.5, 159.1, 158.3, 150.9, 139.8, 138.8, 135.8, 134.5, 134.2, 133.6, 130.7, 129.4, 128.8, 128.7, 128.5, 128.3, 126.3, 123.4, 123.1, 121.0, 114.4; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.10 N-(4-(5-chloro-1H-benzo[d]imidazol-2-yl)phenyl)-2-(4-chlorophenyl)quinazolin-4-amine (**8j**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.36 (s, 1H), 9.90 (s, 1H), 8.59 (d, J = 8.2 Hz, 1H), 8.50–8.43 (m, 2H), 7.95 (d, J = 8.6 Hz, 4H), 7.88 (d, J = 3.7 Hz, 3H), 7.81 (d, J = 8.2 Hz, 2H), 7.62 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 7.1 Hz, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 162.4, 154.1, 149.2, 135.0, 133.6, 132.2, 130.8, 130.7, 128.8, 128.1, 127.9, 127.8, 127.5, 127.2, 126.8, 126.3, 125.7, 125.5, 125.3, 121.7; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.11 4-((2-phenylquinazolin-4-yl)amino)-N-(2-(trifluoromethyl)-1H-benzo[d]imidazol-5-yl)benzamide (**10a**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.38 (s, 1H), 11.33 (s, 1H), 8.47 (d, J = 5.2 Hz, 1H), 8.31 (t, J = 7.5 Hz, 1H), 8.23–8.12 (m, 1H), 8.03–7.86 (m, 2H), 7.76 (d, J = 7.6 Hz, 2H), 7.59 (d, J = 7.7 Hz, 3H), 7.48 (t, J = 7.4 Hz, 1H), 7.36–7.28 (m, 1H), 7.19 (d, J = 7.6 Hz, 2H), 6.91 (t, J = 7.7 Hz, 2H), 6.76 (t, J = 7.4 Hz, 1H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 163.6, 159.5, 158.3, 150.9, 139.1, 138.8, 135.7, 135.0, 134.2, 133.6, 133.4, 133.0, 132.1, 131.9, 130.7, 129.9, 128.9, 128.5, 128.3, 126.3, 123.4, 123.1, 120.5, 120.2, 114.4; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.12 N-(2-(4-bromo-2-fluorophenyl)-1H-benzo[d]imidazol-5-yl)-4-((2-phenylquinazolin-4-yl)amino)benzamide (**10b**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.78 (s, 1H), 10.13 (s, 1H), 8.66 (d, J = 8.2 Hz, 1H), 8.59–8.51 (m, 1H), 8.49 (d, J = 6.8 Hz, 1H), 8.44 (s, 1H), 8.20 (d, J = 8.5 Hz, 2H), 8.07 (d, J = 8.6 Hz, 2H), 7.99 (d, J = 9.4 Hz, 2H), 7.89 (d, J = 6.9 Hz, 2H), 7.70 (d, J = 9.6 Hz, 2H), 7.64–7.34 (m, 6H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 162.7, 159.3, 158.3, 152.8, 150.7, 149.1, 135.0, 134.1, 133.2, 131.8, 131.1, 129.0, 129.0, 128.4, 128.2, 128.1, 127.8, 127.5, 127.0, 126.8, 126.3, 124.1, 123.6, 122.4, 121.4, 114.5; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.13 N-(2-(8-chloroquinolin-3-yl)-1H-benzo[d]imidazol-5-yl)-4-((2-phenylquinazolin-4-yl)amino)benzamide (**10c**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.00 (s, 1H), 12.54 (s, 1H), 10.09 (s, 1H), 8.64 (d, J = 8.2 Hz, 1H), 8.52 (d, J = 6.0 Hz, 2H), 8.41–8.05 (m, 4H), 7.93 (s, 2H), 7.83 (d, J = 7.9 Hz, 1H), 7.68 (s, 2H), 7.56 (d, J = 6.7 Hz, 3H), 7.50–7.26 (m, 2H), 7.09-7.00 (m, 2H), 6.67 (d, J = 8.0 Hz, 1H), 5.62 (s, 1H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 165.9, 163.6, 159.5, 158.3, 150.9, 139.2, 138.9, 138.8, 135.7, 135.5, 135.0, 134.2, 134.2, 133.7, 133.4, 133.0, 132.2, 131.9, 130.8, 130.7, 129.9, 128.9, 128.6, 128.3, 126.4, 123.4, 123.1, 120.5, 120.3, 120.2, 120.1, 114.4, 106.6. HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.14 N-(2-(naphthalen-2-yl)-1H-benzo[d]imidazol-5-yl)-4-((2-phenylquinazolin-4-yl)amino)benzamide (**10d**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.54 (s, 1H), 10.23 (d, J = 9.6 Hz, 1H), 8.65 (d, J = 8.4 Hz, 1H), 8.52–8.50 (m, 1H), 8.30 (d, J = 7.1 Hz, 2H), 8.21–8.16 (m, 4H), 7.95 (t, J = 4.4 Hz, 2H), 7.86–7.84 (m, 1H), 7.80–7.65 (m, 4H), 7.61–7.52 (m, 8H), 7.40–7.35 (m, 1H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 167.1, 159.2, 157.9, 151.4, 151.2, 150.4, 149.0, 147.9, 146.8, 143.0, 142.6, 133.7, 133.1, 132.5, 131.2, 129.3, 128.9, 128.7, 128.5, 128.0, 127.8, 126.1, 124.0, 123.5, 122.2, 122.0, 121.6, 120.3, 118.3, 114.3, 112.2, 111.9, 111.5; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

*4.1.15 2-(thiophen-2-yl)-N-(2-(trifluoromethyl)-1H-benzo[d]imidazol-5-yl)quinazolin-4-amine (**11**)*

Off-white solid; yield 80 %; mp:160–164 °C; FT-IR (cm^{-1}): 3325, 3062, 1650, 1585, 780, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.32 (s, 1H), 8.22–8.11 (m, 1H), 7.89–7.80 (m, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.67–7.39 (m, 2H), 7.40 (t, J = 1.9 Hz, 1H), 7.26 (t, J = 13.6 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H), 6.77 (d, J = 7.9, 1H), 5.36 (s, 2H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 167.5, 159.4, 158.2, 151.0, 144.1, 138.5, 134.0, 130.9, 130.5, 129.0, 128.6, 128.4, 128.2, 127.8, 127.8, 126.7, 125.5, 123.6, 121.4, 114.5; HRMS (ESI): *m/z* calculated for $C_{31}H_{22}N_4O$ 467.1872 found 467.1896 $[M+H]^+$.

4.2 Bacterial strains and media

The gram positive and gram-negative pathogen panel of bacteria consisted of *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (BAA-1705), *Acinetobacter baumannii* (BAA1605), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 29213). NRS199, NRS129, NRS186, NRS191, NRS192, NRS193, NRS194, NRS198 are MRSA strains while VRS1, VRS4, VRS12 are VRSA strains. These strains were obtained from BEI/NARSA/ATCC (Biodefense and Emerging Infections Research Resources Repository/Network on Antimicrobial Resistance in *Staphylococcus aureus*/American Type Culture Collection, USA) and routinely cultivated on Mueller-Hinton Agar (MHA). Prior to the experiment, a single colony was picked from MHA plate, inoculated in Mueller-Hinton cation supplemented broth II (CA-MHB) and incubated overnight at 37 °C with shaking for 18–24 h to get the starter culture.

M. tuberculosis H37Rv ATCC 27294 was cultured in Middlebrook 7H9 (Difco, Becton, NJ, USA) media supplemented with 10% ADC (Bovine Serum Albumin, Dextrose, NaCl), 0.2% glycerol and 0.05% Tween-80 (ADC-Tween-80).

4.2.1 Antibiotic susceptibility testing against pathogen panel consisting *E. coli, S. aureus, K. pneuminiae, A. baumannii,* and *P. aeruginosa.*

Antibiotic susceptibility testing was carried out on the newly synthesized compounds by determining the Minimum Inhibitory Concentration (MIC) with reference to the standard CLSI guidelines [31, 32]. MIC is defined as the minimum concentration of compound at which visible bacterial growth is inhibited. Bacterial cultures were grown in Mueller-Hinton cation supplemented broth (CA-MHB). Optical density (OD_{600}) of the cultures was measured, followed by dilution for ~10^6 cfu/mL. This inoculum was added into a series of test wells in a microtitre plate that contained various concentrations of compound under test ranging from

64-4 μg/mL. Controls i.e., cells alone and media alone (without compound+cells) and levofloxacin used as a reference standard. Plates were incubated at 37 °C for 16-18 h followed by observations of MIC values by the absence or presence of visible growth. For each compound, MIC determinations were performed independently thrice using duplicate samples each time.

4.2.2 Antibiotic susceptibility testing against pathogenic mycobacteria

Antimycobacterial susceptibility testing was carried out on the newly synthesized compounds by using broth micro dilution assay [33]. 1g/100 mL stock solutions of test and control compounds were prepared in DMSO and stored in −20°C. Mycobacterial cultures were inoculated in Middlebrook 7H9 enriched (Difco, Becton, NJ, USA) media supplemented with 10% ADC-Tween-80 (Bovine Serum Albumin, Dextrose, 0.2% glycerol and 0.05% Tween-80) and OD_{600} of the cultures was measured, followed by dilution to achieve ~10^6 cfu/mL [34]. The newly synthesized compounds were tested from 0.0064–0.00005 g/100 mL in two-fold serial diluted fashion with 2.5 µL of each concentration added per well of a 96-well round bottom micro titre plate. Later, 97.5 µL of bacterial suspension was added to each well containing the test compound along with appropriate controls. Presto blue (Thermo Fisher, USA) resazurin-based dye was used for the visualized identification of active compounds. MIC of active compound was determined as lowest concentration of compound that inhibited visible growth after incubation period. For each compound, MIC determinations were replicated thrice using duplicate samples. The MIC plates were incubated at 37 °C for 7 days for Mtb.

4.2.3 Cell Cytotoxicity Assay

The active newly synthesized compounds were screened for their cell toxicity against Vero cells using MTT assay [35]. ~10^3 cells/well were seeded in 96 well plate and incubated at 37 °C with an 5% CO_2 atmosphere. After 24 h, compound was added ranging from 100–5 mg/L and incubated for 72 h at 37 °C with 5% CO_2 atmosphere. After the incubation period, MTT was added at 5 mg/L in each well, incubated at 37 °C for further 4 hours, residual medium was discarded, 0.1 mL of DMSO was added to solubilise the formazan crystals and OD was taken at 540 nm for the calculation of CC_{50}. CC_{50} is defined as the lowest concentration of compound which leads to a 50% reduction in cell viability. Doxorubicin was used as positive control and each experiment was repeated in triplicate.

4.2.4 ADMET properties calculation

ADMET properties of the synthesized compounds were calculated using Qikprop program (Qikprop, version 6.5, Schrödinger, LLC, New York, NY, 2014). As can be seen below, the partition coefficient (QPlogPo/w), hydrogen bond donors (donor HB), hydrogen bond acceptors (acceptor HB), molecular weight (mol. Wt.) and percent human oral absorption exhibited satisfactory results. The compounds also followed Lipinski rule of five. ADMET properties for Isoniazid and Rifampicin were also calculated and compared with the results of the synthesized compounds.

Conflicts of interest

The authors declare no conflicts of interest.

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Synthesis and evaluation of new Quinazoline-benzimidazole hybrids as potent anti-microbial agents against multidrug resistant *Staphylococcus aureus* and *Mycobacterium tuberculosis*

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Highlights:

1. Series of new quinazoline-benzimidazole hybrids were designed, synthesized and evaluated for antibacterial activity against ESKAP pathogens and pathogenic mycobacteria.
2. Compounds **8b, 8c, 8d, 8g, 8h, 8i** and **10a** displayed selective and potent inhibitory activity against *Staphylococcus aureus*.
3. Compounds **8a, 8b, 8f, 8g,** and **10c** exhibited potent inhibitory activity against different strains of *Mycobacterium tuberculosis*.
4. The selected compounds were found to show less cytotoxicity towards Vero cells with favorable selectivity index.
5. The selected compounds were found to possess potent inhibitory activity against multiple drug-resistant strains of *S. aureus* including VRSA.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflicts of interest

The authors declare no conflicts of interest.