Bioorganic Chemistry 83 (2019) 569–579

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis and evaluation of new 4-oxoquinazolin-3(4*H*)-yl)benzoic acid and benzamide derivatives as potent antibacterial agents effective against multidrug resistant *Staphylococcus aureus*

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ARTICLE INFO

Keywords:
Drug resistance
Antibacterial agents
Methicillin-resistant and Vancomycin resistant *Staphylococcus aureus*
Minimum inhibitory concentration
Vero cells

ABSTRACT

Treatment of nosocomial and community acquired *Staphylococcus aureus* infections has become more challenging due to the egression of multi-drug resistance. This has spurred the need for rapid development of new therapeutic agents which can effectively negate the resistance mechanisms. In our current work, several new 4-oxoquinazolin-3(4*H*)-yl)benzoic acid and benzamide derivatives were synthesized and examined for their antimicrobial activity against ESKAP pathogen panel and pathogenic mycobacteria. In the primary screening, compounds **4a**, **4b**, **6′a**, **6′b**, **6′h**, **6′i** and **6′j** were found to demonstrate selective and potent inhibitory activity against *Staphylococcus aureus* (MICs = 0.25–0.5 μg/mL). When tested against Vero cells, all the compounds were found to be non toxic possessing favourable selectivity index (SI > 10), which encouraged us for carrying out further studies. Compound **6′a** (SI > 40) was tested against a number of multiple clinical strains of multi-drug resistant *S. aureus* and was found to exhibit potent activity, irrespective of the resistant status of the strain. Besides, compound **6′a** also exhibited concentration dependent bactericidal activity and synergized with the FDA approved drugs tested. The interesting results obtained suggest the potential utility of the newly synthesized compounds for treatment of multidrug resistant *S. aureus* infections.

1. Introduction

Multidrug resistant *Staphylococcus aureus* (MDR-SA) infections are a significant health crisis globally and have spurred a need for rapid development of new classes of therapeutic agents with a potential to circumvent the drug resistant mechanisms [1]. MDR-SA exhibits resistance to methicillin, β-lactams, glycopeptides, fluoroquinolones, macrolides, oxazolidinones and carbapenems [2-7]. Sensible use and development of new, effective antibiotics is a vital step to palliate the complications associated with MDR-SA infections.

In the quest for new antibacterials, we found quinazolinones to be a privileged structure in modern medicinal chemistry, possessing wide range of biological properties like antibacterial, antifungal, anticonvulsant, anti-inflammatory, anti-HIV, anticancer and analgesic activities [8-18]. Exploration of quinazolinones has gained enormous interest and proven promise with the identification of potent antimicrobial agents. Saravanan et al. [19] and Zayed et al. [20] reported quinazolinones **1** & **2** respectively as potent antibacterial agents. Desai et al. [21] reported the antibacterial properties of 2-styryl-3,4-dihydroquinazolin-6-yl-(1,3,5-triazin-2-yl)-3-methylurea derivatives **3.** Recently, Bouley et al. [22] reported 4(3*H*)-quinazolinones **4** as potent antibacterial agents with good in-vivo activity. Jadhavar *et al.* [23] also reported 2-styryl quinazolinones **5** (Fig. 1) as potent anti-mycobacterial agents. In the present study, we have designed a number of 4-oxoquinazolin-3(4*H*)-yl)benzoic acid and benzamide derivatives (Fig. 2) and evaluated them for their antimicrobial properties against ESKAP pathogen panel and pathogenic mycobacteria.

2. Results and discussion

2.1. Chemistry

The newly designed compounds were synthesized by following the synthetic schemes outlined in Schemes 1 & 2. Substituted anthranilic acids **1**, **1′** were cyclised to the corresponding benzoxazinone intermediates **2**, **2′** by refluxing in acetic anhydride. To a solution of **2**, **2′** in

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https://doi.org/10.1016/j.bioorg.2018.11.007
Received 31 August 2018; Received in revised form 27 October 2018; Accepted 6 November 2018
Available online 08 November 2018
0045-2068/

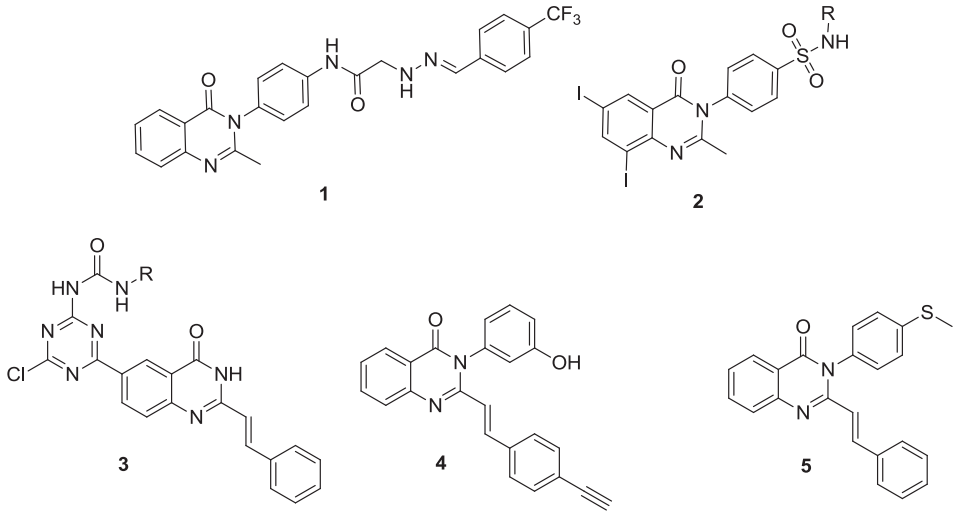


Fig. 1. Structures of some literature reported bioactive quinazolinone compounds having antibacterial and antimycobacterial activity.

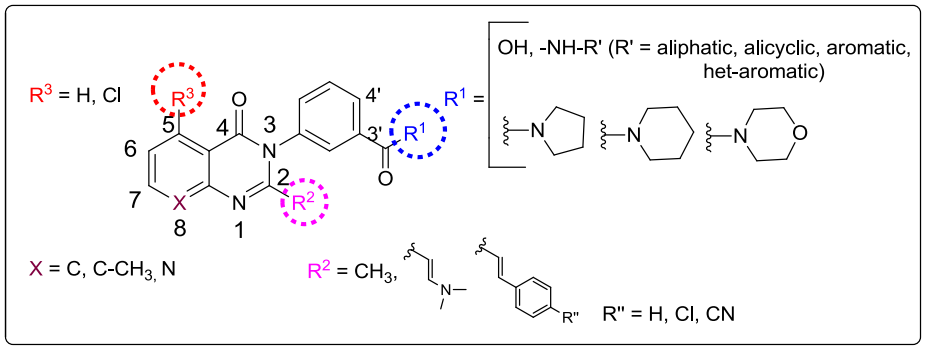
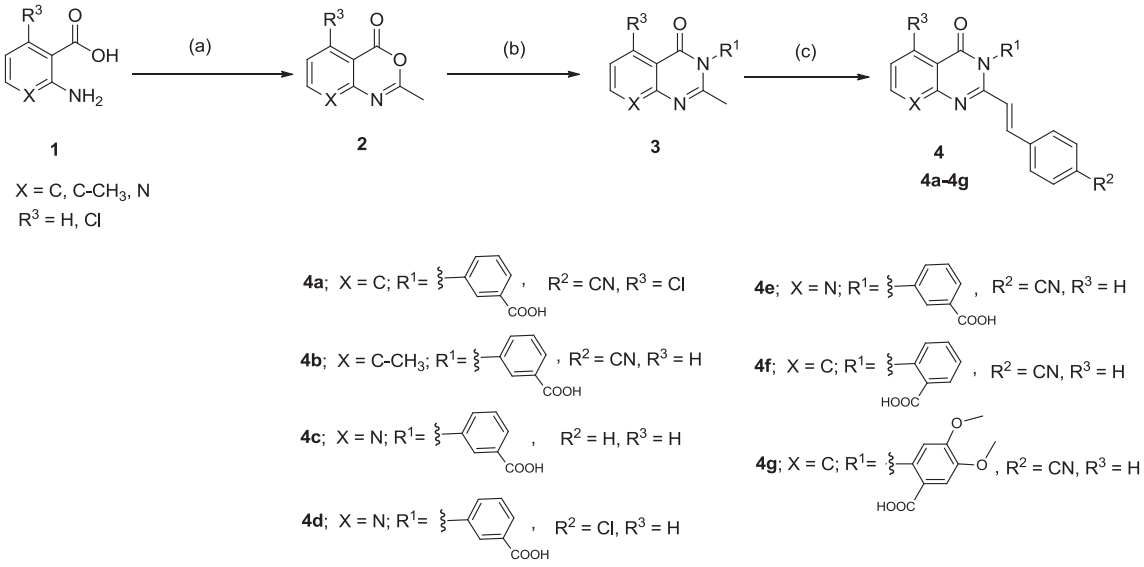
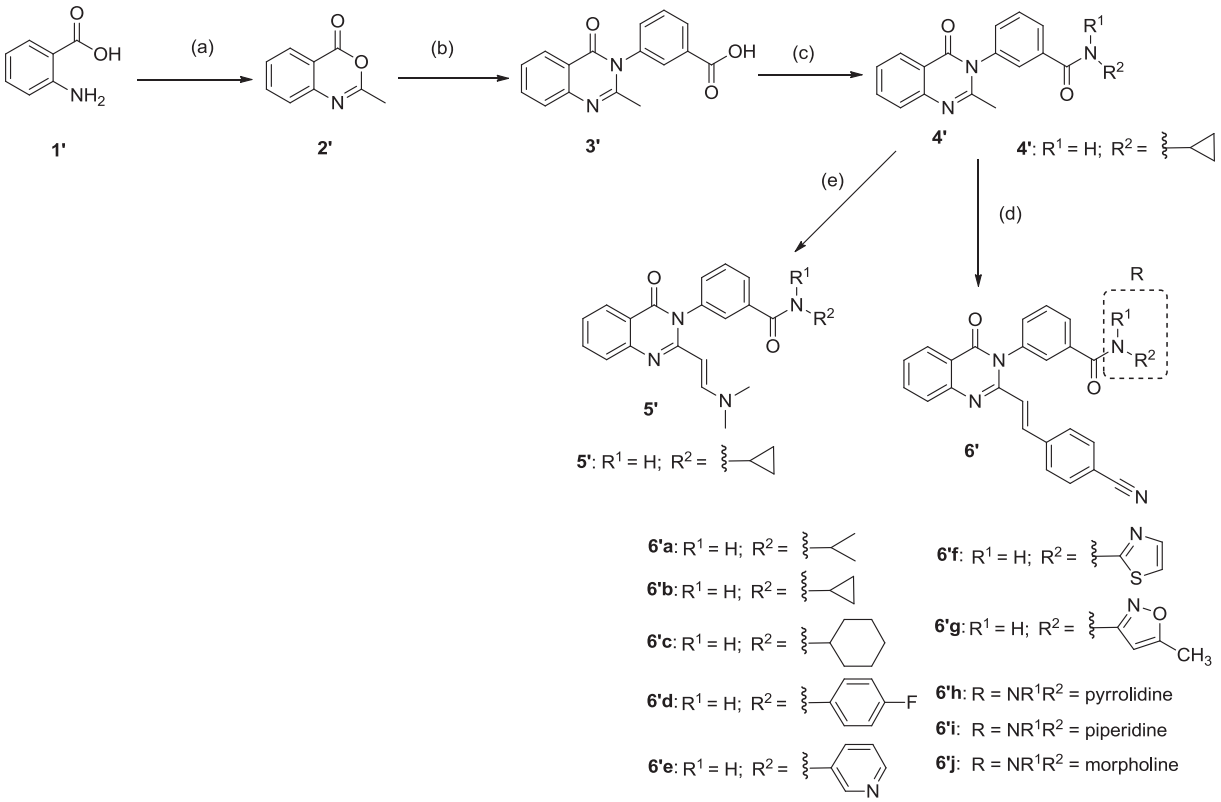


Fig. 2. Newly designed 4-oxoquinazolin-3(4*H*)-yl)benzoic acid and benzamide derivatives.



Scheme 1. Synthesis of 4-oxoquinazolin-3(4*H*)-yl)benzoic acid derivatives 4a-4 g: Reagents and conditions: (a) Ac_2O, reflux, 2 h (b) R^1-NH_2, AcOH, reflux, 5 h (c) 4-R^2-PhCHO, AcOH, reflux, 18–20 h, 83–68%.

glacial acetic acid, 3-amino benzoic acid was added and the reaction mixture was refluxed for 5 h to afford 3-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)benzoic acid as intermediates **3, 3′.** To obtain the amide derivatives **4′**, the intermediate **3′** was reacted with various amines by using N,N-dimethyl amino pyridine (DMAP), N-(3-Dimethylamino-propyl)-N′ethylcarbodiimide hydrochloride (EDC) in dichloromethane and allowed to react at room temperature for 12–14 h. Further intermediate **3** or **4′** were reacted with benzaldehyde or 4-chloro or 4-cyano benzaldehyde in glacial acetic acid or N,N-Dimethylformamide dimethylacetal (DMF-DMA) in N,N-dimethyl formamide (DMF) under reflux conditions overnight (18–20 h) to afford the final compounds in moderate to excellent yields. The structures of the newly synthesized



Scheme 2. Synthesis of 4-oxoquinazolin-3(4*H*)-yl)benzamide derivatives **5′**, **6′a-6′j**: Reagents and conditions: (a) Ac_2O, reflux, 2 h (b) 3-amino benzoic acid, AcOH, reflux, 5 h (c) R^1-NH_2 or R^1R^2NH, EDC.HCl, DMAP, DCM, rt., 12–14 h (d) 4-cyanobenzaldehyde, AcOH, reflux, 18 h (e) DMF-DMA, DMF, reflux, 18–20 h, 50–70%.

compounds were confirmed by ^{1}H NMR, ^{13}C NMR (given in supplementary data B**)** and HRMS (ESI) spectroscopic techniques.

2.2. In vitro antibacterial activity

2.2.1. Antibiotic susceptibility testing against ESKAP panel of bacteria

All the newly synthesised 4-oxoquinazolin-3(4*H*)-yl)benzoic acid and benzamide derivatives were evaluated for their antibacterial activity against ESKAP panel of pathogens. Antibiotic susceptibility testing was performed by determining minimum inhibitory concentration (MIC) according to the CLSI guidelines [24]. The newly synthesized derivatives were tested in the range of 64–0.03 μg/mL with Levofloxacin as a control and the results are given in Table 1. In order to find their spectrum of activity, these compounds were also tested against pathogenic *M. tuberculosis* H37Rv strain, wherein all the compounds were found to be inactive (Supplementary data A).

Compound **4a** with chloro at C-5 position and carboxy group at *meta* position on the N-phenyl with styryl moiety (with p-C_6H_4-CN group) at C-2 exhibited selective activity against *S. aureus* (MIC = 8 μg/mL). Compound **4b** having C-CH_3 at C-8 position carboxy group at *meta* position on the N-phenyl and with styryl moiety (p-C_6H_4-CN group) at C-2 displayed potent activity against *S. aureus* with MIC of 2 μg/mL (Scheme 1). This observation prompted us to investigate a number of quinazolinones with 4-cyano styryl moiety at C-2 position by varying R^1, R^2, R^3 & X. The corresponding aza derivatives **4c-4e** (pyrido pyramidinones) with carboxy group at *meta* position on the N-phenyl and styryl moiety (with p-C_6H_4-R^2 group) at C-2 were found to be devoid of activity. Besides, compound **4f** with 2-carboxy group and compound **4 g** with 2-carboxy-4, 5-dimethoxy group on the N-Phenyl moiety were found to be inactive against ESKAP pathogen panel. Based on the potent activity shown by the 4-oxoquinazolin-3(4*H*)-yl) benzoic acid derivatives, a number of corresponding amide derivatives were prepared and evaluated for their antibacterial activity and some of the highlights are discussed below.

Table 1
MIC values (μg/mL) of the tested compounds against ESKAP panel of bacteria.

Compound	*S. aureus* ATCC 29213	*E. coli* ATCC 25922	*K. pneumoniae* BAA-1705	*A. baumannii* BAA-1605	*P. aeruginosa* ATCC 27853
4a	**8**	> 64	> 64	> 64	> 64
4b	**2**	> 64	> 64	> 64	> 64
4c	> 64	> 64	> 64	> 64	> 64
4d	> 64	> 64	> 64	> 64	> 64
4e	> 64	> 64	> 64	> 64	> 64
4f	> 64	> 64	> 64	> 64	> 64
4g	> 64	> 64	> 64	> 64	> 64
3′	> 64	> 64	> 64	> 64	> 64
4′	> 64	> 64	> 64	> 64	> 64
5′	> 64	> 64	> 64	> 64	> 64
6′a	**0.25**	> 64	> 64	> 64	> 64
6′b	**0.5**	> 64	> 64	> 64	> 64
6′c	**32**	> 64	> 64	> 64	> 64
6′d	> 64	> 64	> 64	> 64	> 64
6′e	**64**	> 64	> 64	> 64	> 64
6′f	> 64	> 64	> 64	> 64	> 64
6′g	**64**	> 64	> 64	> 64	> 64
6′h	**4**	> 64	> 64	> 64	> 64
6′i	**4**	> 64	> 64	> 64	> 64
6′j	**8**	> 64	> 64	> 64	> 64
Levofloxacin	0.125	0.015	64	8	0.5

Compounds **4′** and **5′** with methyl and (*E*)-N,N-dimethylprop-1-en-1-amine group at C-2 respectively and cyclopropyl amide at *meta* position on the N-phenyl moiety were found to be inactive. However, when the C-2 methyl group is replaced with styryl moiety (with p-C_6H_4-CN group) as in **6′b**, the compound exhibited selective and potent inhibitory activity against *S. aureus* (MIC = 0.5 μg/mL), suggesting the usefulness of styryl moiety (with p-C_6H_4-CN group). Replacement of cyclopropyl amide with cyclohexyl (**6′c**), 4-fluoro phenyl (**6′d**), 3-pyridyl (**6′e**), thiazolyl (**6′f**), 5-methylisoxazolyl (**6′g**) amides led to loss of

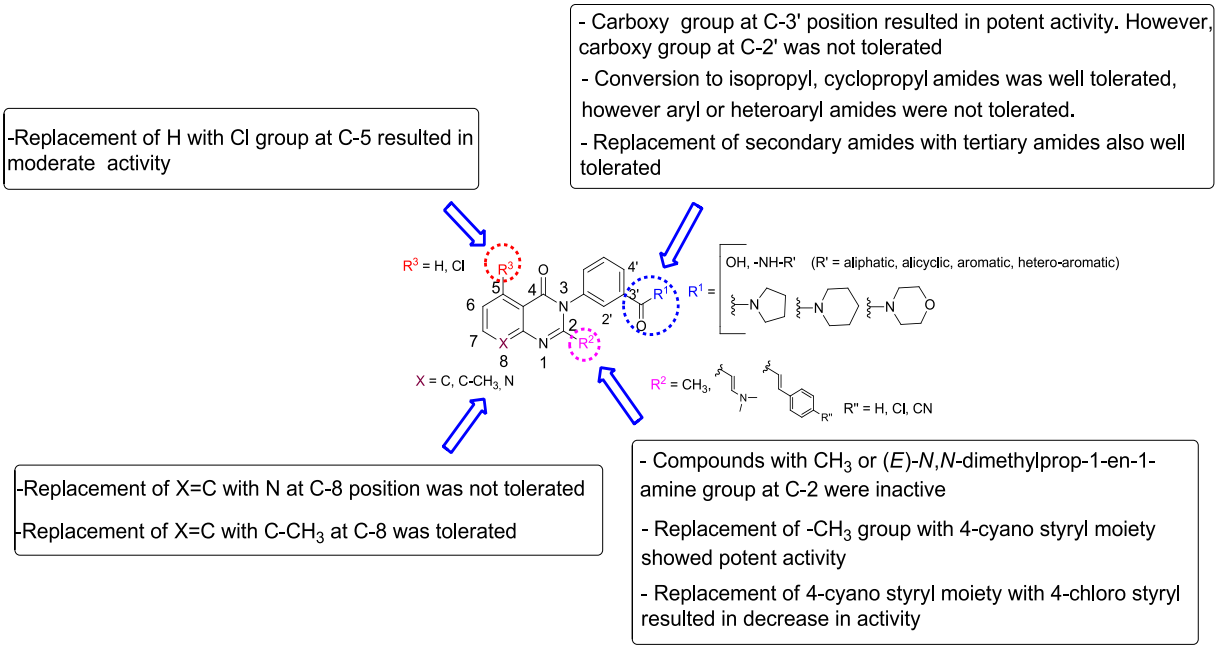


Fig. 3. Structure Activity Relationship (SAR) of 4-oxoquinazolin-3(4*H*)-yl)benzoic acid and benzamide derivatives.

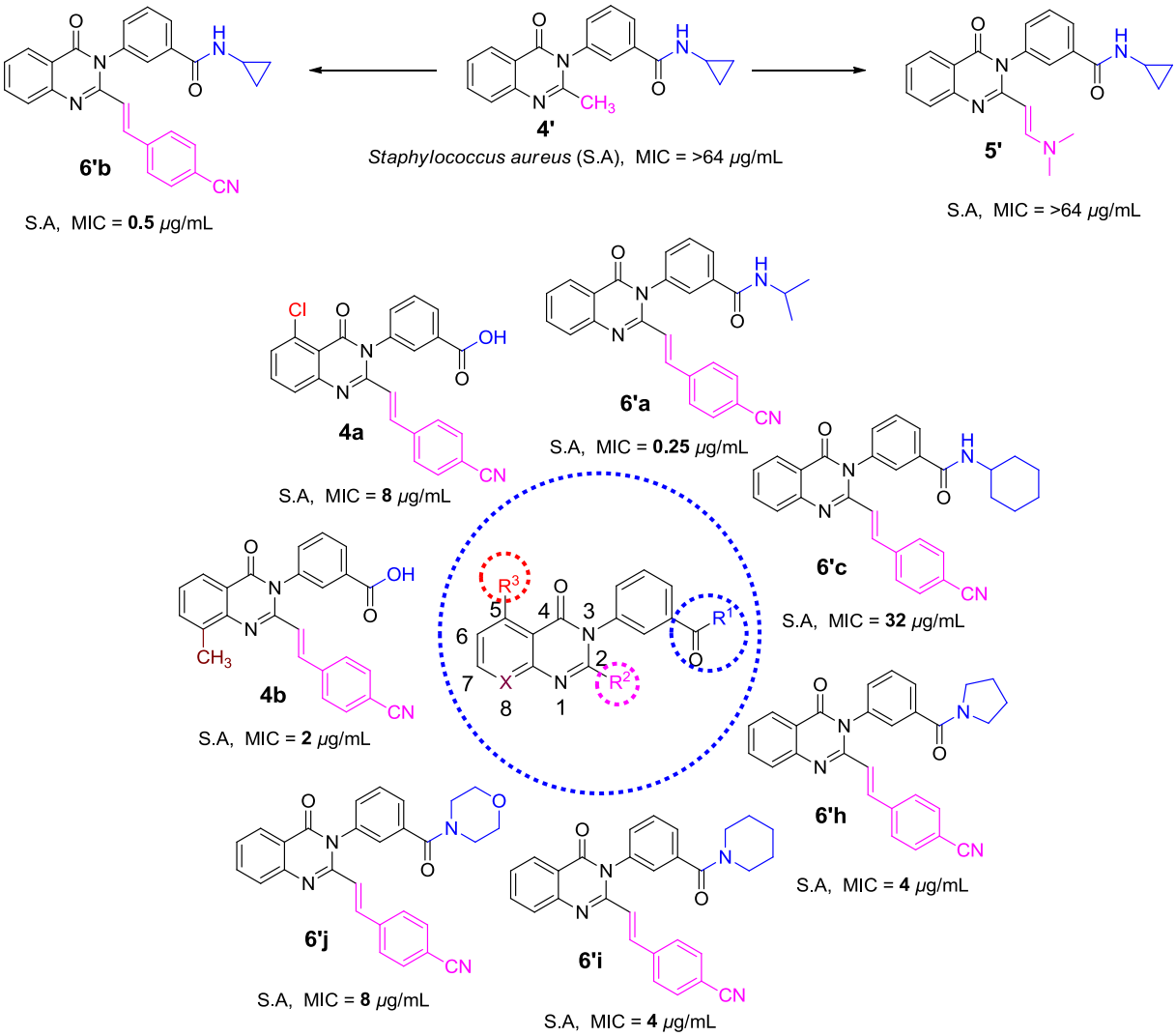


Fig. 4. New 4-oxoquinazolin-3(4*H*)-yl)benzoic acid and benzamide derivatives selectively active against *Staphylococcus aureus*.

Table 2
Cytotoxicity profile against Vero cells and SI.

Compound	*S. aureus* ATCC 29213 MIC (μg/mL)	CC_{50} (μg/mL)	Selectivity Index (CC_{50}/MIC)
4a	8	> 10	> 1.25
4b	2	> 20	> 10
6′a	0.25	> 10	> 40
6′b	0.5	> 5	> 10
6′h	4	> 10	> 2.5
6′i	4	> 10	> 2.5
6′j	8	> 10	> 1.25

activity. Interestingly, compound **6′a** with isopropyl amide was found to be the most potent compound with a MIC of 0.25 μg/mL. Gratifyingly, when secondary amides were replaced with tertiary amides at C-3′ of N-phenyl as in compound **6′h** (pyrrolidinyl), **6′i** (piperidinyl), **6′j** (morpholinyl), the compounds were found to exhibit potent and selective activity against *S. aureus* with MIC values 4–8 μg/mL (Figs. 3 & 4).

2.3. Cytotoxicity assay against Vero cells

The compounds **4a**, **4b**, **6′a**, **6′b**, **6′h**, **6′i** and **6′j** were tested for cytotoxicity against Vero cells using the MTT assay [25]. CC_{50} is defined as the lowest concentration of compound which leads to a 50% reduction in cell viability with doxorubicin as positive control. A perusal of results (Table 2) indicate that **4a**, **4b**, **6′a**, **6′b**, **6′h**, **6′i** and **6′j** are non toxic to Vero cells and exhibited a selectivity index (SI) of (> 1.25- > 40).

Among the tested compounds, **6′a** was found to be promising with potent activity against *S. aureus* and also possessing favourable selectivity index (SI > 40), which encouraged us to carry further studies.

2.4. Determination of MIC against MDR-SA including VRSA

To determine the spectrum of activity of a compound **6′a** against multiple strains of MDR-SA, it was tested against various well defined and characterized clinical strains of MRSA and VRSA. The results are summarised in Table 3. Levofloxacin, Meropenem and Vancomycin were used as reference standards. From the examination of the results, it can be inferred that the compound **6′a** exhibited potent antibacterial activity with MIC = < 0.125–0.5 μg/mL against various clinical strains of MRSA and VRSA. Thus, **6′a** exhibits equipotent activity against MDR-SA irrespective of its drug-resistance status.

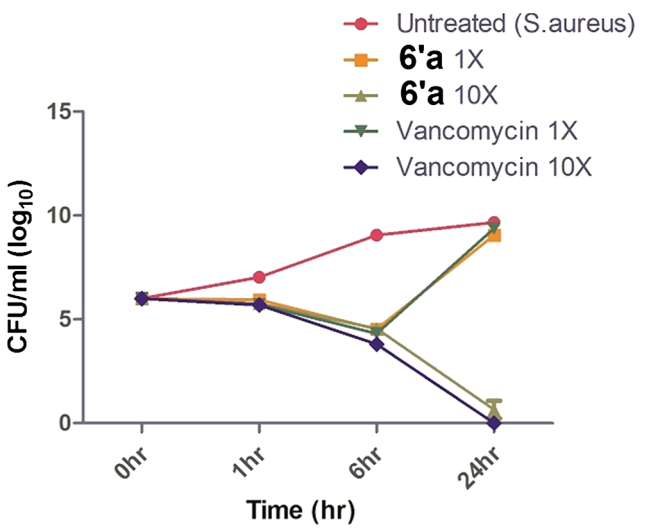


Fig. 5. Bacterial Time kill kinetics of a compound **6′a**.

3. Time kill kinetics of a compound 6′a

The bactericidal activity was assessed by the time-kill method. *S. aureus* ATCC 29213 cells were diluted up to ~10^5 CFU/mL and treated with compound for concentrations corresponding to 1X and 10X of MIC of a compound **6′a** and vancomycin in MHB in triplicate and incubated at 37 °C. 0.1 mL samples were collected after time intervals of 0, 1, 6 and 24 h, serially diluted in PBS and plated on TSA followed by incubation at 37 °C for 18–20 h. Kill curves were constructed by counting the colonies from plates and plotting the CFU/mL of surviving bacteria at each time point in the presence and absence of compound (Fig. 5). Compound **6′a** exhibits concentration dependent bactericidal activity.

4. Determination of synergy with FDA approved drugs

The checkerboard method was used to determine synergy between compound **6′a** and various antibiotics linezolid, meropenem, ceftriaxone and vancomycin for the treatment of staphylococcal infections. As can be seen in Table 4, compound **6′a** synergized with the FDA approved drugs tested, thus exhibiting great potential to be a part of multi-drug regimen.

5. Conclusion

In conclusion, a series of new 4-oxoquinazolin-3(4*H*)-yl)benzoic

Table 3
MIC of a compound **6′a** against MRSA and VRSA strains.

Strains		Antibiotics resistant to	MIC (μg/mL)				
			6′a	Levofloxacin	Meropenem	Vancomycin	Methicillin
MSSA	*S. aureus* ATCC 29213	None	< 0.125	< 0.5	< 0.5	1	1
MRSA	NR 119	Methicillin, Ceftriaxone, Meropenem, Gentamycin and Linezolid	0.125	16	> 64	1	32 - > 64
	NR 10129	Methicillin, Ceftriaxone, Meropenem	< 0.125	< 0.5	16	1	32 - > 64
	NR 100	Methicillin, Ceftriaxone, Meropenem	0.25–0.5	< 0.5	> 64	1	32 - > 64
	NR 10198	Methicillin, Ceftriaxone, Meropenem	< 0.125	32	32	1	32 - > 64
	NR 10192	Methicillin, Ceftriaxone, Meropenem	< 0.125	4–8	4–8	1	32 - > 64
	NR 10186	Methicillin, Ceftriaxone, Meropenem	< 0.125	4–8	16–32	1	32 - > 64
	NR 10193	Methicillin, Ceftriaxone, Meropenem	< 0.125	32	32	1	32 - > 64
	NR 10194	Methicillin, Ceftriaxone	< 0.125	< 0.5	< 0.5	1	32 - > 64
	NR 10191	Methicillin, Ceftriaxone, Meropenem	< 0.125	16–32	> 64	1	32 - > 64
VRSA	VRS1	Methicillin, Ceftriaxone, Meropenem, Gentamycin, Vancomycin, Teicoplanin	0.25–0.5	32	> 64	> 64	> 64
	VRS4	Methicillin, Ceftriaxone, Meropenem, Vancomycin and Teicoplanin	0.125	> 64	> 64	> 64	32 - > 64
	VRS12	Methicillin, Ceftriaxone, Meropenem, Vancomycin and Teicoplanin	0.125–0.25	32 - > 64	> 64	> 64	32 - > 64

Table 4
Synergy screen data of a compound **6′a.**

Drug	*S. aureus* ATCC 29213 MIC (μg/mL)	MIC of 6′a in the presence of drug (μg/mL) A	MIC of drug in the presence of 6′a (μg/mL) B	FIC A	FIC B	ΣFIC = FIC A + FIC B	Inference
6′a	0.25						
Ceftazidime	8	0.007813	0.03125	0.03125	0.00390625	0.035156	Synergistic
Daptomycin	1	0.007813	0.00195	0.03125	0.00195	0.0332	Synergistic
Gentamycin	0.25	0.007813	0.00195	0.03125	0.0078	0.03905	Synergistic
Linezolid	2	0.007813	0.0075	0.03125	0.00375	0.035	Synergistic
Levofloxacin	0.25	0.007813	0.0009	0.03125	0.0036	0.03485	Synergistic
Meropenem	0.5	0.007813	0.0009	0.03125	0.0018	0.03305	Synergistic
Minocycline	0.125	0.007813	0.0009	0.03125	0.0072	0.03845	Synergistic
Rifampicin	0.015	0.007813	0.00003	0.03125	0.002	0.03325	Synergistic
Vancomycin	1	0.007813	0.0039	0.03125	0.0039	0.03515	Synergistic

acid and benzamide derivatives were synthesised and evaluated against ESKAP pathogen panel. Compounds **4a**, **4b**, **6′a**, **6′b**, **6′h**, **6′i** and **6′j** displayed selective and potent antibacterial activity against *Staphylococcus aureus*. They were found to be non toxic to Vero cells (CC_{50} = > 5- > 20 μg/mL) with good selectivity index. In further studies, compound **6′a** displayed potent inhibitory activity when screened against clinical MRSA and VRSA isolates. Besides, compound **6′a** exhibited concentration dependent bactericidal activity and synergized with the FDA approved drugs tested. With the promising results obtained, the synthesized new 4-oxoquinazolin-3(4*H*)-yl)benzoic acid and benzamide derivatives present potential for further development as anti-staphylococcal leads.

6. Materials & experimental methods

All the chemicals, reagents and starting materials were procured from commercial providers. The thorough monitoring of reactions were performed by thin layer chromatography (TLC-MERCK pre-coated silica gel 60-F254 aluminium plates) under UV light. Melting points were checked using Stuart® SMP30 apparatus and are uncorrected. ^{1}H and ^{13}C NMR were taken on Bruker Avance 500 MHz spectrometer using tetramethylsilane (TMS) as the internal standard and chemical shifts are reported in ppm. Chemical shifts are referenced to TMS (δ 0.00 for ^{1}H NMR and ^{13}C NMR), DMSO-d_6 (δ 2.50 for ^{1}H NMR and 39.7 for ^{13}C NMR) or $CDCl_3$ (δ 7.26 for ^{1}H NMR and 77.00 or 77.16 for ^{13}C NMR) or combination of $CDCl_3$ and DMSO-d_6, in which $CDCl_3$ was used as an internal reference. Spin multiplicities are reported as s (singlet), brs (broad singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). Coupling constant (*J*) values are reported in hertz (Hz). HRMS were determined with Agilent QTOF mass spectrometer 6540 series instrument and were performed in the ESI techniques at 70 eV. Column chromatography was performed using silica gel 60–120 or 100–200 mesh.

Intermediates **2**, **3** (Scheme 1) were prepared according to the procedures described in literature [22].

6.1. General reaction procedure for the synthesis of (E)-3-(4-oxo-2-styrylquinazolin-3(4H)-yl)benzoic acid and its corresponding aza derivatives *4a–4 g*.

Substituted 3-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)benzoic acid (**3,** 2 mmol) was dissolved in 15 mL of glacial acetic acid, to which 4-substituted benzaldehyde (2 mmol) was added. The reaction was refluxed for 18–20 h, and monitered by using TLC. After completion of reaction, the reaction mixture was allowed to cool at room temperature and then 5 mL volume of water was added to give crude precipitate. The resulting crude precipitate was filtered and washed with water followed by cold methanol and hexane to obtain a (*E*)-3-phenyl-2-styrylquinazolin-4(3*H*)-ones and its corresponding aza derivatives ***4a–4 g*** as pure yellow to pale yellow solids in 68–83% yields. All the newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS (ESI).

6.1.1. (E)-3-(5-chloro-2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl) benzoic acid (*4a*):

Yellow solid, Yield 74%; mp: 191–193 °C, ^{1}H NMR (500 MHz, DMSO–d_6) δ 13.25 (s, 1H), 8.17–8.12 (m, 1H), 8.09–8.06 (m, 1H), 7.90 (d, *J* = 15.5 Hz, 1H), 7.82–7.73 (m, 5H), 7.71 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.57–7.52 (m, 3H), 6.40 (d, *J* = 15.6 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 167.00, 159.77, 151.85, 150.16, 139.51, 137.95, 137.41, 135.03, 133.93, 133.40, 133.27, 132.88, 130.63, 130.55, 130.53, 129.74, 128.76, 127.39, 123.48, 119.01, 118.17, 112.18 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{15}ClN_3O_3$ 428.0802; found 428.0808.

6.1.2. (E)-3-(2-(4-cyanostyryl)-8-methyl-4-oxoquinazolin-3(4H)-yl) benzoic acid (*4b*)

Yellow solid, Yield 81%; mp: 193–195 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 13.13 (s, 1H), 8.17–8.13 (m, 1H), 8.05–8.03 (m, 1H), 7.99–7.93 (m, 2H), 7.81–7.77 (m, 2H), 7.77–7.71 (m, 3H), 7.60–7.56 (m, 2H), 7.44 (t, *J* = 7.6 Hz, 1H), 6.49 (d, *J* = 15.5 Hz, 1H), 2.70 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 166.99, 161.96, 149.92, 145.99, 139.78, 137.52, 137.48, 136.11, 135.61, 133.86, 133.25, 132.85, 130.56, 130.52, 130.41, 128.70, 127.02, 124.59, 124.06, 121.11, 119.04, 111.97, 17.49 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{25}H_{18}N_3O_3$ 408.1348; found 408.1352.

6.1.3. (E)-3-(4-oxo-2-styrylpyrido[2,3-d]pyrimidin-3(4H)-yl)benzoic acid (*4c*)

Yellow solid, Yield 83%; mp: 229–231 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 13.29 (s, 1H), 9.02 (d, *J* = 2.6 Hz, 1H), 8.51 (d, *J* = 6.9 Hz, 1H), 8.16 (d, *J* = 6.7 Hz, 1H), 8.09 (s, 1H), 8.02 (d, *J* = 15.4 Hz, 1H), 7.82–7.73 (m, 2H), 7.55 (dd, *J* = 7.6, 4.6 Hz, 1H), 7.39 (d, *J* = 17.3 Hz, 5H), 6.35 (d, *J* = 15.4 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 166.97, 162.56, 157.82, 156.76, 154.62, 141.08, 137.42, 136.44, 135.03, 133.84, 132.91, 130.70, 130.64, 130.59, 130.39, 129.55, 128.31, 122.56, 119.99, 116.45 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{22}H_{16}N_3O_3$ 370.1191; found 370.1209.

6.1.4. (E)-3-(2-(4-chlorostyryl)-4-oxopyrido[2,3-d]pyrimidin-3(4H)-yl) benzoicacid (*4d*)

Yellow solid, Yield 73%; mp: 220–222 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 13.30 (s, 1H), 9.02 (dd, *J* = 4.5, 1.9 Hz, 1H), 8.53–8.49 (m, 1H), 8.18–8.11 (m, 1H), 8.08 (s, 1H), 8.00 (d, *J* = 15.4 Hz, 1H),

7.81–7.72 (m, 2H), 7.55 (dd, J = 7.8, 4.6 Hz, 1H), 7.47–7.43 (m, 4H), 6.37 (d, J = 15.5 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 166.97, 162.54, 157.76, 156.77, 154.48, 139.66, 137.31, 136.45, 135.09, 133.95, 133.83, 132.90, 130.74, 130.59, 130.40, 130.03, 129.58, 122.65, 120.75, 116.49 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{22}H_{15}ClN_3O_3$ 404.0802; found 404.0815.

6.1.5. (E)-3-(2-(4-cyanostyryl)-4-oxopyrido[2,3-d]pyrimidin-3(4H)-yl) benzoic acid (**4e**)

Yellow solid, Yield 68%; mp: 242–244 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.03 (dd, J = 4.5, 1.8 Hz, 1H), 8.52 (dd, J = 7.8, 1.8 Hz, 1H), 8.19–8.11 (m, 1H), 8.09 (s, 1H), 8.04 (d, J = 15.5 Hz, 1H), 7.82 (d, J = 8.2 Hz, 2H), 7.79–7.71 (m, 2H), 7.64 (d, J = 8.3 Hz, 2H), 7.60–7.53 (m, 1H), 6.52 (d, J = 15.5 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 172.49, 166.96, 162.51, 157.69, 156.84, 154.20, 139.51, 138.93, 137.16, 136.51, 133.81, 133.35, 132.89, 130.60, 130.41, 129.00, 123.53, 122.93, 119.05, 116.71, 112.32 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{23}H_{15}N_4O_3$ 395.1144; found 395.1151.

6.1.6. (E)-2-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)benzoic acid (**4f**)

Yellow solid, Yield 78%; mp: 154–156 °C; ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.20–8.11 (m, 2H), 8.95–8.87 (m, 2H), 7.87–7.77 (m, 4H), 7.76–7.71 (m, 1H), 7.62–7.53 (m, 4H), 6.46 (d, J = 15.6 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 165.05, 160.67, 150.41, 146.75, 138.66, 136.37, 135.99, 134.27, 133.19, 132.24, 131.16, 130.10, 129.33, 128.79, 127.60, 126.71, 126.29, 125.86, 122.63, 120.11, 117.99, 110.98 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{24}H_{16}N_3O_3$ 394.1191; found 394.1194.

6.1.7. (E)-2-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)-4,5-dimethoxybenzoic acid (**4 g**)

Yellow solid, Yield 71%; mp: 185–187 °C;. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.14 (d, J = 7.8 Hz, 1H), 7.96–7.85 (m, 2H), 7.84–7.76 (m, 3H), 7.60 (d, J = 8.2 Hz, 2H), 7.55 (t, J = 7.5 Hz, 1H), 7.16–7.10 (m, 2H), 7.00–6.96 (m, 1H), 6.59 (d, J = 15.6 Hz, 1H), 3.86 (s, 3H), 3.76 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.79, 151.86, 149.82, 149.64, 147.68, 139.94, 137.09, 135.21, 133.34, 129.74, 128.65, 127.75, 127.36, 126.97, 124.14, 121.47, 121.30, 119.07, 113.05, 112.35, 111.98, 56.29, 56.18 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{26}H_{20}N_3O_5$ 454.1403; found 454.1406.

Intermediates **2′**, **3′** (Scheme 2) were prepared according to the procedures described in literature [22].

6.2. General reaction procedure for the synthesis of 2-methyl-4-oxoquinazolin-3(4H)-yl)benzamide derivatives **4′** .

To a mixture of the amine (2 mmol) (Scheme 2) and N,*N*-dimethyl amino pyridine (DMAP) (2.6 mmol) in dichloromethane was added the 3-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)benzoic acid (**3′**, 2 mmol), followed by N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) (2.7 mmol.), and was allowed to stir 12–14 h at room temperature. After completion of reaction, the reaction mixture was extracted with ethylacetate and the organic layer was washed with 10% aq citric acid, water, sat. aq $NaHCO_3$, and brine. The organic layer was dried with sodium sulphate (Na_2SO_4) and concentrated *in vacuum* to afford the amides **4′** as pure white products in good yields without further purification.

6.2.1. N-cyclopropyl-3-(2-methyl-4-oxoquinazolin-3(4H)-yl)benzamide (**4′**)

White solid, Yield 61%; mp: 140–142 °C. ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6) δ 8.11 (d, J = 7.6 Hz, 1H), 7.90–7.86 (m, 1H), 7.75–7.69 (m, 1H), 7.67–7.65 (m, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.40 (t, J = 7.4 Hz, 1H), 7.31(d, J = 7.7 Hz, 1H), 6.90 (brs, 1H), 2.89–2.79 (m, 1H), 2.17 (s, 3H), 0.79–0.72 (m, 2H), 0.57–0.49 (m, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 171.96, 166.68, 158.87, 152.23, 142.49, 141.25, 139.45, 135.57, 134.57, 133.29, 133.14, 132.04, 131.67, 131.45, 125.36, 29.18, 28.00, 10.82, 10.78 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{19}H_{18}N_3O_2$ 320.1399; found 320.1402.

6.3. General reaction procedure for the synthesis of (E)-N-cyclopropyl-3-(2-(2-(dimethylamino)vinyl)-4-oxoquinazolin-3(4H)-yl)benzamide **5′**, or (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl-benzamide derivatives **6′a–6′j**

Compound (**4′**, 2 mmol) was reacted with 4-cyano benzaldehyde (2 mmol) in acetic acid or N,N-Dimethylformamide dimethyl acetal (2 mmol) in N,N-dimethyl formamide (DMF) under reflux conditions (18–20 h). The reaction was monitered by using TLC. After completion of reaction, 5 mL volume of water was added to the cooled reaction mixture. The resulting crude precipitate was filtered and washed with water followed by cold methanol and hexane to obtain a quinazolin-3(4*H*)-yl)benzamide derivatives **5′, 6′a–6′j** derivatives as pure yellow to pale yellow solids in 50–80% yields. All the newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS (ESI).

6.3.1. (E)-N-cyclopropyl-3-(2-(2-(dimethylamino)vinyl)-4-oxoquinazolin-3(4H)-yl)benzamide (**5′**)

White solid, Yield 50%; mp: 156–158 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.52 (s, 1H), 8.03–7.91 (m, 2H), 7.87 (d, J = 12.1 Hz, 1H), 7.75 (s, 1H), 7.71–7.61 (m, 2H), 7.52–7.39 (m, 2H), 7.25–7.17 (m, 1H), 3.97 (d, J = 12.0 Hz, 1H), 2.82–2.78 (m, 7H), 0.64 (m, 4H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 166.68, 162.24, 155.43, 150.59, 149.55, 138.48, 136.06, 134.84, 132.10, 130.01, 128.00, 127.94, 126.72, 125.86, 123.41, 118.89, 86.70, 23.61, 6.18, 6.04 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{22}H_{23}N_4O_2$ 375.1821; found 375.1828.

6.3.2. (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)-N-isopropylbenzamide (**6′a**)

Yellow solid, Yield 58%; mp: 188–189 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.34 (d, J = 7.6 Hz, 1H), 8.18–8.14 (m, 1H), 8.09–8.05 (m, 1H), 7.98–7.88 (m, 3H), 7.84–7.77 (m, 3H), 7.70 (t, J = 7.8 Hz, 1H), 7.67–7.63 (m, 1H), 7.61–7.53 (m, 3H), 6.50 (d, J = 15.6 Hz, 1H), 4.13–4.09 (m, 1H), 1.19–1.15 (m, 6H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 164.58, 161.69, 151.26, 147.67, 139.77, 137.51, 137.16, 136.65, 135.44, 133.31, 131.99, 130.11, 128.70, 128.26, 127.86, 127.58, 126.96, 123.89, 121.16, 119.03, 112.10, 41.69, 22.77, 22.72 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{27}H_{23}N_4O_2$ 435.1821; found 435.1827.

6.3.3. (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)-N-cyclopropylbenzamide (**6′b**)

Pale yellow solid, Yield 68%; mp: 167–169 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.59 (s, 1H), 8.15 (d, J = 7.7 Hz, 1H), 8.03 (d, J = 7.6 Hz, 1H), 7.99–7.87 (m, 3H), 7.83–7.77 (m, 3H), 7.72–7.68 (m, 1H), 7.66–7.62 (m, 1H), 7.60–7.56 (m, 3H), 6.48 (d, J = 15.6 Hz, 1H), 2.88–2.84 (m, 1H), 0.72–0.68 (m, 2H), 0.60–0.56 (m, 2H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 166.82, 161.72, 151.25, 147.64, 139.73, 137.49, 137.17, 136.20, 135.49, 133.32, 132.15, 130.23, 128.71, 128.60, 128.22, 127.85, 127.63, 126.96, 123.85, 121.10, 119.06, 112.07, 23.59, 6.23, 6.06 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{27}H_{21}N_4O_2$ 433.1664; found 433.1667.

*6.3.4. (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)-N-cyclohexylbenzamide (**6′c**)*

Yellow solid, Yield 62%; mp: 190–192 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.33 (d, J = 7.9 Hz, 1H), 8.16 (dd, J = 7.9, 1.2 Hz, 1H), 8.08–8.03 (m, 1H), 7.96–7.90 (m, 3H), 7.84–7.80 (m, 3H), 7.70 (t, J = 7.8 Hz, 1H), 7.66–7.62 (m, 1H), 7.61–7.57 (m, 3H), 6.51 (d, J = 15.6 Hz, 1H), 3.85–3.69 (m, 1H), 1.89–1.77 (m, 2H), 1.77–1.67 (m, 2H), 1.64–1.55 (m, 1H), 1.38–1.20 (m, 4H), 1.19–1.04 (m, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 164.58, 161.71, 151.24, 147.65, 139.73, 137.49, 137.14, 136.63, 135.45, 133.31, 132.00, 130.12, 128.77, 128.70, 128.31, 127.85, 127.59, 126.96, 123.86, 121.13, 119.05, 112.07, 49.02, 32.87, 32.82, 25.72, 25.38 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{30}H_{27}N_4O_2$ 475.2134; found 475.2140.

*6.3.5. (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)-N-(4-fluorophenyl)benzamide (**6′d**)*

Yellow solid, Yield 70%; mp: 245–247 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 10.43 (s, 1H), 8.20–8.16 (m, 2H), 8.10–8.06 (m, 1H), 7.98–7.89 (m, 2H), 7.85–7.76 (m, 6H), 7.75–7.71 (m, 1H), 7.63–7.56 (m, 3H), 7.25–7.17 (m, 2H), 6.55 (d, J = 15.6 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 164.69, 161.74, 158.89 (d, $J^1_{C\text{-}F}$ = 240.5 Hz), 151.26, 147.67, 139.75, 137.54, 137.36, 136.54, 135.70 (d, $J^4_{C\text{-}F}$ = 2.6 Hz), 135.48, 133.31, 132.71, 130.37, 129.09, 128.78, 128.74, 127.87, 127.62, 126.98, 123.90, 122.83 (d, $J^3_{C\text{-}F}$ = 7.9 Hz), 121.16, 119.06, 115.72 (d, $J^2_{C\text{-}F}$ = 22.2 Hz), 112.09 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{30}H_{20}FN_4O_2$ 487.1570; found 487.1576.

*6.3.6. (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)-N-(pyridin-3-yl)benzamide (**6′e**)*

Yellow solid, Yield 69%; mp: 280–282 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 10.59 (s, 1H), 8.92 (d, J = 2.4 Hz, 1H), 8.33 (dd, J = 4.7, 1.4 Hz, 1H), 8.23–8.15 (m, 3H), 8.10 (t, J = 1.7 Hz, 1H), 7.98–7.90 (m, 2H), 7.86–7.78 (m, 4H), 7.77–7.73 (m, 1H), 7.66–7.56 (m, 3H), 7.41 (dd, J = 8.3, 4.7 Hz, 1H), 6.55 (d, J = 15.6 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 165.22, 161.76, 151.26, 147.67, 145.28, 142.55, 139.75, 137.58, 137.41, 136.12, 136.04, 135.51, 133.30, 132.98, 130.46, 129.20, 128.88, 128.75, 128.02, 127.88, 127.65, 126.98, 124.07, 123.90, 121.14, 119.05, 112.09 ppm; HRMS (ESI): m/z calcd for $[M+H]+$ $C_{29}H_{20}N_5O_2$ 470.1617; found 470.1624.

*6.3.7. (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)-N-(thiazol-2-yl)benzamide (**6′f**)*

Yellow solid, Yield 64%; mp: 220–222 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 12.77 (s, 1H), 8.30–8.26 (m, 1H), 8.23–2.20 (m, 1H), 8.17 (dd, J = 7.9, 0.9 Hz, 1H), 7.97–7.89 (m, 2H), 7.86–7.73 (m, 5H), 7.65–7.54 (m, 4H), 7.30 (s, 1H), 6.58 (d, J = 15.6 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 164.48, 161.72, 151.29, 147.72, 139.82, 137.49, 137.45, 135.46, 133.52, 133.30, 133.42, 130.54, 129.51, 129.35, 128.78, 127.89, 127.60, 126.99, 124.07, 121.19, 119.82, 119.04, 114.54, 112.71, 112.08 ppm; HRMS (ESI): m/z calcd for $[M+H]+$ $C_{27}H_{18}N_5O_2S$ 476.1181; found 476.1182.

*6.3.8. (E)-3-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)-N-(5-methylisoxazol-3-yl)benzamide (**6′g**)*

Yellow solid, Yield 66%; mp: 240–242 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 11.45 (s, 1H), 8.21–8.18 (m, 1H), 8.17 (dd, J = 7.9, 1.3 Hz, 1H), 8.14–8.11 (m, 1H), 7.96–7.89 (m, 2H), 7.84–7.79 (m, 3H), 7.77–7.71 (m, 2H), 7.63–7.56 (m, 3H), 6.77 (s, 1H), 6.56 (d, J = 15.6 Hz, 1H), 2.42 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 170.03, 164.80, 161.71, 158.98, 151.28, 147.71, 139.81, 137.48, 137.37, 135.44, 135.16, 133.29, 130.44, 129.42, 129.25, 128.76, 127.88, 127.58, 126.98, 126.77, 124.05, 121.18, 119.04, 112.09, 97.41, 12.60 ppm; HRMS (ESI): m/z calcd for $[M+H]+$ $C_{28}H_{20}N_5O_3$ 474.1566; found 474.1568.

*6.3.9. (E)-4-(2-(4-oxo-3-(3-(pyrrolidine-1-carbonyl)phenyl)-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**6′h**)*

Yellow solid, Yield 51%; mp: 258–260 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 15.5 Hz, 1H), 7.89–7.81 (m, 2H), 7.75 (d, J = 7.7 Hz, 1H), 7.65 (t, J = 7.8 Hz, 1H), 7.62–7.58 (m, 2H), 7.55–7.51 (m, 2H), 7.45–7.41 (m, 2H), 7.38–7.34 (m, 1H), 6.49 (d, J = 15.5 Hz, 1H), 3.71–3.59 (m, 2H), 3.53–3.43 (m, 2H), 2.01–1.92 (m, 2H), 1.92–1.80 (m, 2H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 193.42, 167.56, 161.61, 151.47, 147.70, 139.79, 138.89, 137.20, 136.89, 135.41, 133.31, 130.78, 129.83, 128.62, 128.07, 127.83, 127.55, 127.01, 124.26, 121.24, 119.06, 112.02, 49.38, 46.36, 26.17, 24.34 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{28}H_{23}N_4O_2$ 447.1821; found 447.1826.

*6.3.10. (E)-4-(2-(4-oxo-3-(3-(piperidine-1-carbonyl)phenyl)-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**6′i**)*

Yellow solid, Yield 65%; mp: 280–282 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32–8.28 (m, 1H), 7.99 (d, J = 15.5 Hz, 1H), 7.85–7.81 (m, 2H), 7.67–7.58 (m, 4H), 7.55–7.50 (m, 1H), 7.45–7.41 (m, 2H), 7.40–37 (m,1H), 7.37–7.33 (m, 1H), 6.50 (d, J = 15.5 Hz, 1H), 3.86–3.73 (m, 1H), 368–3.55 (m, 1H), 3.50–3.32 (m, 2H), 1.58–1.54 (m, 6H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 168.14, 161.62, 151.38, 147.69, 139.79, 138.23, 137.16, 137.11, 135.40, 133.32, 130.50, 130.39, 128.53, 128.06, 127.82, 127.61, 127.55, 127.00, 124.12, 121.23, 119.04, 112.04, 48.43, 42.77, 26.11, 25.63, 24.36 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{29}H_{25}N_4O_2$ 461.1977; found 461.1982.

*6.3.11. (E)-4-(2-(3-(3-(morpholine-4-carbonyl)phenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**6′j**)*

Yellow solid, Yield 62%; mp: 298–300 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.18–8.14 (m, 1H), 7.93–7.89 (m, 1H), 7.87 (d, J = 15.6 Hz, 1H), 7.84–7.78 (m, 3H), 7.71 (t, J = 7.8 Hz, 1H), 7.64–7.58 (m, 4H), 7.58–7.52 (m, 2H), 6.52 (d, J = 15.6 Hz, 1H), 3.67–3.51 (m, 4H), 3.43–3.22 (m, 4H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 168.36, 161.59, 151.41, 147.68, 139.74, 137.23, 137.20, 135.41, 133.33, 130.78, 130.53, 128.72, 128.61, 128.53, 128.04, 127.83, 127.55, 127.01, 124.19, 121.21, 119.04, 112.06, 66.32, 60.24, 21.23, 14.55 ppm; HRMS (ESI): m/z calcd for $[M+H]^+$ $C_{28}H_{23}N_4O_3$ 463.1770; found 463.1767.

7. Bacterial strains and media

The ESKAP panel of bacteria consisted of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Klebsiella pneumoniae* (BAA-1705), *Acinetobacter baumannii* (BAA-1605) and *Pseudomonas aeruginosa* (ATCC 27853). NRS100, NRS199, NRS129, NRS186, NRS191, NRS192, NRS193, NRS194, NRS198 were the MRSA strains while VRS1, VRS4, VRS12 were the 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).

Molecular details of Multi Drug Resistant (MDR) strains

MDR-Strains		Molecular details of strains
MRSA	NR	Community acquired-MRSA
	10194	positive for the *Panton-Valentine leucocidin* (PVL) virulence factor Contains staphylococcal chromosome cassette mec type V
	NR	Community acquired-MRSA
	10186	positive for the *Panton-Valentine leucocidin* (PVL) virulence factor Contains staphylococcal chromosome cassette mec type IV Pulse-field gel electrophoresis (PFGE) typed as USA 300
	NR	Community acquired-MRSA
	10193	Negative for the *Panton-Valentine leucocidin* (PVL) virulence factor Contains staphylococcal chromosome cassette mec type II
	NR	Community acquired-MRSA
	10191	Pulse-field gel electrophoresis (PFGE) typed as USA 600 Contains staphylococcal chromosome cassette mec type II Negative for the *Panton-Valentine leucocidin* (PVL) virulence factor
	NR	Community acquired-MRSA
	10192	Pulse-field gel electrophoresis (PFGE) typed not as USA100-1100 Contains staphylococcal chromosome cassette mec type II Negative for the *Panton-Valentine leucocidin* (PVL) virulence factor
	NR	Community acquired-MRSA
	10198	Pulse-field gel electrophoresis (PFGE) typed as USA100 Contains staphylococcal chromosome cassette mec type II Negative for the *Panton-Valentine leucocidin* (PVL) virulence factor
	NR 100	Resistant to tetracycline Positive for mec (subtype I) Large variety of virulence factors
	NR 10129	Also called as TCH60
	NR 119	G2576T mutation in domain V in one or more 23 S rRNA genes Positive for mec (subtype IV)
VRSA	VRS1 VRS4	Positive for 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)
	VRS12	NA*

NA*- Not Available

7.1. Antibiotic susceptibility testing against ESKAP pathogen panel

Antibiotic susceptibility testing was performed on the newly synthesized compounds by calculating the Minimum Inhibitory Concentration (MIC) according to standard CLSI guidelines [24]. 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 (MHB) [26]. Optical density (OD_{600}) of the cultures was measured, followed by dilution for $\sim 10^6$ 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 to 0.03 μg/mL. Controls i.e., cells alone and media alone (without compound + cells) and drug levofloxacin used as a reference standard in the whole experiment. 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 carried independently three times using duplicate samples each time.

7.2. Antibiotic susceptibility testing against pathogenic mycobacteria

Antimycobacterial susceptibility testing was performed on newly synthesized compounds (given in Supplementary data), by using broth microdilution assay [27,28]. 10 mg/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 [29] 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 $\sim 10^6$ CFU/mL. The newly synthesized compounds were tested from 64 to 0.5 mg/L in two-fold serial diluted fashion with 2.5 μL of each concentration added per well of a 96-well round bottom microtiter 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 newly synthesized 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.

7.3. Cell cytotoxicity assay

The active newly synthesized compounds were screened for their cell toxicity against Vero cell using the MTT assay [30]. $\sim 10^3$ 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 to 5 mg/L and incubated for 72 h at 37 °C with an 5% CO_2 atmosphere. After the incubation was over, MTT was added at 5 mg/L in each well, incubated at 37 °C for further 4 h, residual medium was discarded, 0.1 mL of DMSO was added to solubilise the formazan crystals and optical density (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.

7.4. Bacterial Time kill Kinetics

The bactericidal activity was assessed by the time-kill method [31]. *S. aureus* ATCC 29213 cells were diluted up to $\sim 10^5$ CFU/ml and treated with compound for concentrations corresponding to 1X and 10X of MIC of **6′a** and vancomycin in MHB in triplicate and incubated at 37 °C. 100 μL samples were collected after time intervals of 0 h, 1 h, 6 h and 24 h and serially diluted in PBS and plated on TSA followed by incubation at 37 °C for 18–20 h. Kill curves were constructed by counting the colonies from plates and plotting the CFU/mL of surviving bacteria at each time point in the presence and absence of compound.

7.5. Synergy screen

Checkerboard method was used to determine synergy between compound and the antibiotics included in the study that included linezolid, meropenem, ceftriaxone and vancomycin against a panel of MRSA and VRSA strains [27]. According to the recommendations of CLSI, serial two-fold dilutions of each drug to at least double the MIC were freshly prepared prior to testing. The compounds was serially diluted along the ordinate ranged from 0.03 to 4 μg/mL while the antibiotics were serially diluted as shown along the abscissa ranged from 0.03 to 64 μg/mL /ml in 96 well microtiter plate. An inoculum equal to 10^5 or 10^6 CFU/mL was prepared from each MRSA and VRSA isolate in MHB. Each microtiter well was inoculated with 100 μL of a bacterial inoculum of 10^5 or 10^6 CFU/mL, and plates were incubated at 37 °C for 24 h under aerobic conditions. According to the CLSI guidelines for broth microdilution, the MIC was defined as the lowest concentration of antibiotic that completely inhibited the growth of the organism as detected with the naked eye. The ΣFICs (fractional inhibitory concentrations) were calculated as follows: ΣFIC = FIC A + FIC B, where FIC A is the MIC of drug A in the combination/MIC of drug A alone, and FIC B is the MIC of drug B in the combination/MIC of drug B alone. The combination is considered synergistic when the ΣFIC is ≤0.5, indifferent when the ΣFIC is > 0.5 to 4, and antagonistic when the ΣFIC is > 4.

Acknowledgements

G. S. conveys warm regards to DoP, Ministry of Chemicals &

Fertilizers, Govt. of India, for the award of NIPER fellowship. M.S. would like to thank UGC for her fellowship while G.K. thanks DST for her fellowship. This study was supported by the DST grant from Department of Science and Technology, Govt. of India to S.C. and S.N. (EMR/2017/000220). The following reagents were provided by the Network on Antimicrobial Resistance in *S. aureus* (NARSA) for distribution by BEI Resources, , : NR 119, NR 10129, NR 10198, NR 10192, NR 10191, NR 10193, NR 10186, NR 10194, VRS1, VRS4 and VRS12.

Competing financial interests

The authors declare no competing financial interests.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.11.007.

References

[1] (a) J.P. Marcel, M. Alfa, F. Baquero, J. Etienne, H. Goossens, S. Harbarth, W. Hryniewicz, W. Jarvis, M. Kaku, R. Leclercq, S. Levy, D. Mazel, P. Nercelles, T. Perl, D. Pittet, C. Vandenbroucke-Grauls, N. Woodford, V. Jarlier, Clin. Microbiol. Infect. 14 (2008) 895–907;
(b) E. Toner, A. Adalja, G.K. Gronvall, A. Cicero, T.V. Inglesby, Antimicrobial resistance is a global health emergency, Health Secur. 13 (2015) 153–155.

[2] (a) T.L. Smith, M.L. Pearson, K.R. Wilcox, C. Cruz, M.V. Lancaster, B. Robinson-Dunn, F.C. Tenover, M.J. Zervos, J.D. Band, E. White, Emergence of vancomycin resistance in Staphylococcus aureus, N. Engl. J. Med. 340 (1999) 493–501;
(b) S. Gardete, A. Tomasz, Mechanisms of vancomycin resistance in Staphylococcus aureus, J. Clin. Investig. 124 (2014) 2836–2840;
(c) W. Noble, Z. Virani, R.G. Cree, Co-transfer of vancomycin and other resistance genes from Enterococcus faecalis NCTC 12201 to Staphylococcus aureus, FEMS Microbiol. Lett. 93 (1992) 195–198;
(d) K. Hiramatsu, Vancomycin-resistant Staphylococcus aureus: a new model of antibiotic resistance, Lancet Infect. Dis. 1 (2001) 147–155;
(e) H.S. Sader, R.E. Mendes, L.R. Duncan, M.A. Pfaller, R.K. Flamm, Antimicrobial activity of dalbavancin against Staphylococcus aureus with decreased susceptibility to glycopeptides, daptomycin, and/or linezolid from U.S. medical centers, Antimicrob. Agents, Chemother. 62 (2018) e02397–e2417.

[3] H. Lee, S. Ahn, N.Y. Hwang, K. Jeon, O.J. Kwon, H.J. Huh, N.Y. Lee, C.K. Kim, W.J. Koh, Limited effect of later-generation fluoroquinolones in the treatment of ofloxacin-resistant and moxifloxacin-susceptible multidrug-resistant tuberculosis, Antimicrob. Agents Chemother. 62 (2018) e01784–e1817.

[4] S. Tsiodras, H.S. Gold, G. Sakoulas, G.M. Eliopoulos, C. Wennersten, L. Venkataraman, R.C. Moellering Jr, M.J. Ferraro, Linezolid resistance in a clinical isolate of Staphylococcus aureus, Lancet 358 (2001) 207–208.

[5] M.A. Fischbach, C.T. Walsh, Antibiotics for emerging pathogens, Science 325 (2009) 1089–1093.

[6] M. Barber, Editorial, J. Clin. Pathol. 14 (1961) 385–393.

[7] D.J. Payne, M.N. Gwynn, D.J. Holmes, D.L. Pompliano, Drugs for bad bugs: confronting the challenges of antibacterial discovery, Nat. Rev. Drug Discovery. 6 (2007) 29–40.

[8] (a) A. Hameed, M. Al-Rashida, M. Uroos, S. Abid Ali, M. Arshia, K.M Khan Ishtiaq, Quinazoline and quinazolinone as important medicinal scaffolds: a comparative patent review (2011–2016), Expert Opin. Ther. Pat. 28 (2018) 281–297;
(b) D. Hea, M. Wanga, S. Zhaoa, Y. Shua, H. Zenga, C. Xiaob, C. Luc, Y. Liua, Pharmaceutical prospects of naturally occurring quinazolinone and its derivatives, Fitoterapia. 119 (2017) 136–149.

[9] (a) B. Dash, S. Dash, D. Laloo, C. Medhi, Design, synthesis and preliminary pharmacological screening (antimicrobial, analgesic and anti-inflammatory activity) of some novel quinazoline derivatives, J. appl. pharm. sci. 7 (2017) 083–096;
(b) M. Dinari, F. Gharahi, P. Asadi, Synthesis, spectroscopic characterization, antimicrobial evaluation and molecular docking study of novel triazine–quinazolinone based hybrids, J. Mol. Struct. 1156 (2018) 43–50.

[10] (a) N. Jain, J. Jaiswal, A. Pathak, P.K. Singour, Synthesis, molecular docking and evaluation of 3 -{4 - [2 - amino - 4 - (substituted phenyl) - 2H - [1, 3]oxazin / thiazin-6-yl}- 2 - phenyl - 3H -quinazolin-4-one derivatives for their anticonvulsant activity, Cent. Nerv. Syst. Agents. Med Chem. 18 (2018) 63–73;
(b) S.K. Kashawa, V. Kashawa, P. Mishra, N.K. Jain a, J.P. Stables, Synthesis, anticonvulsant and CNS depressant activity of some new bioactive 1-(4-substituted-phenyl)-3-(4-oxo-2-phenyl/ethyl-4H-quinazolin-3-yl)-urea, Eur. J. Med. Chem. 44 (2009) 4335–4343.

[11] A.M. Alaa, L.A. Abou-Zeid, K.E.H. ElTahir, R.R. Ayyad, A.-A. Magda, A.S. El-Azab, Synthesis, anti-inflammatory, analgesic, COX-1/2 inhibitory activities and molecular docking studies of substituted 2-mercapto-4 (3H)-quinazolinones, Eur. J. Med. Chem. 121 (2016) 410–421.

[12] (a) L. Yang, S. Ge, J. Huang, X. Bao, Synthesis of novel (E)-2-(4-(1H–1, 2, 4-triazol-1-yl) styryl)-4-(alkyl/arylmethyleneoxy) quinazoline derivatives as antimicrobial agents, Mol. Divers. 22 (2018) 71–82;
(b) H.A. Abuelizz, R.A. El-Dib, M. Marzouk, R. Al-Salahi, In vitro evaluation of new 2-phenoxy-benzo [g][1,2,4] triazolo [1,5-a] quinazoline derivatives as antimicrobial agents, Microb. Pathog. 117 (2018) 60–67;
(c) H. Ighachane, M.H. Sedra, H. Lazrek, Synthesis and evaluation of antifungal activities of (3H)-quinazolin-4-one derivatives against tree plant fungi, J. Mater. Environ. Sci. 8 (2017) 134–143.

[13] M. Hrast, K. Rožman, M. Jukič, D. Patin, S. Gobec, M. Sova, Synthesis and structure–activity relationship study of novel quinazolinone based inhibitors of Mur A, Bioorganic Med. Chem. Lett. 27 (2017) 3529–3533.

[14] (a) S.Y. Abbas, K.A. El-Bayouki, W.M. Basyouni, E.A. Mostafa, New series of 4 (3H)-quinazolinone derivatives: syntheses and evaluation of antitumor and antiviral activities, Med. Chem. Res. 27 (2018) 571–582;
(b) W. Dohle, F.L. Jourdan, G. Menchon, A.E. Prota, P.A. Foster, P. Mannion, E. Hamel, M.P. Thomas, P.G. Kasprzyk, E. Ferrandis, Quinazolinone based anticancer agents: synthesis, antiproliferative SAR, antitubulin activity, and tubulin co-crystal structure, J. Med. Chem. 61 (2018) 1031–1044.

[15] (a) A.L. Leivers, M. Tallant, J.B. Shotwell, S. Dickerson, M.R. Leivers, O.B. McDonald, J. Gobel, 30 K.L. Creech, S.L. Strum, A. Mathis, S. Rogers, C.B. Moore, J. Botyanszki, Discovery of selective small molecule type III Phosphatidylinositol 4-kinase alpha (PI4KIIIα) inhibitors as anti hepatitis C (HCV) agents, J. Med. Chem. 57 (2014) 2091–2106;
(b) Z.W. Wang, M.X. Wang, X. Yao, Y. Li, J. Tan, L.Z. Wang, W.T. Qiao, Y.Q. Geng, Y.X. Liu, Q.M. Wang, Design, synthesis and antiviral activity of novel quinazolinones, Eur. J. Med. Chem. 53 (2012) 275–282.

[16] C. Carmi, E. Galvani, F. Vacondio, S. Rivara, A. Lodola, S. Russo, S. Aiello, F. Bordi, G. Costantino, A. Cavazzoni, R.R. Alfieri, A. Ardizzoni, P.G. Petronini, M. Mor, Irreversible inhibition of epidermal growth factor receptor activity by 3-amino-propanamides, J. Med. Chem. 55 (2012) 2251–2264.

[17] X. Zhan, Y. Xu, Q. Qi, Y. Wang, H. Shi, Z. Mao, Synthesis, cytotoxic, and antibacterial evaluation of quinazolinone derivatives with substituted amino moiety, Chem. Biodivers. 15 (2018) e1700513.

[18] (a) I. Khan, A. Ibrar, N. Abbas, A. Saeed, Recent advances in the structural library of functionalized quinazoline and quinazolinone scaffolds: Synthetic approaches and multifarious applications, Eur. J. Med. Chem. 76 (2014) 193–244;
(b) I. Khan, A. Ibrar, W. Ahmed, A. Saeed, Synthetic approaches, functionalization and therapeutic potential of quinazoline and quinazolinone skeletons: The advances continue…, Eur. J. Med. Chem. 90 (2015) 124–169;
(c) I. Khan, S. Zaib, S. Batool, N. Abbas, Z. Ashraf, J. Iqbal, A. Saeed, Quinazolines and quinazolinones as ubiquitous structural fragments in medicinal chemistry: an update on the development of synthetic methods and pharmacological diversification, Bioorg. Med. Chem. 24 (2016) 2361–2381.

[19] (a) G. Saravanan, V. Alagarsamy, C.R. Prakash, Synthesis, analgesic, anti-inflammatory, and in vitro antimicrobial activities of some novel quinazolin-4 (3H)-one derivatives, Med. Chem. Res. 22 (2013) 340–350;
(b) V. Alagarsamy, G. Murugananthan, R. Venkateshperumal, Synthesis, Analgesic, Anti-inflammatory and Antibacterial Activities of Some Novel 2-Methyl-3-substituted Quinazolin-4-(3H)-ones, Biol. Pharm. Bull. 26 (2003) 1711–1714.

[20] M.F. Zayed, M.H. Hassan, Synthesis and biological evaluation studies of novel quinazolinone derivatives as antibacterial and anti-inflammatory agents, Saudi Pharm. J. 22 (2014) 157–162.

[21] K.R. Desai, P.H. Desai, Studies on quinazoline and mercaptoquinazoline. Preparation and antibacterial activity of 2(2'-styryl 4'-oxoquinazolino-6'-ylamino)-4-(arylureido)-6-chloro-s-triazine and 2[2'-mercapto-3'(4"-methylphenyl)]-4'-oxoquinazolino-6'-ylamino)-4,6-bis(arylureido)-s-triazine, J. Indian. Chem. Soc. 65 (1988) 804–805.

[22] R. Bouley, D. Ding, Z. Peng, M. Bastian, E. Lastochkin, W. Song, M.A. Suckow, V.A. Schroeder, W.R. Wolter, S. Mobashery, Structure-Activity Relationship for the 4 (3 *H*)-Quinazolinone antibacterials, J. Med. Chem. 59 (2016) 5011–5021.

[23] P.S. Jadhavar, T.M. Dhameliya, M.D. Vaja, D. Kumar, J.P. Sridevi, P. Yogeeswari, D. Sriram, A.K. Chakraborti, Synthesis, biological evaluation and structure–activity relationship of 2-styrylquinazolones as anti-tubercular agents, Bioorg. Med. Chem. Lett. 26 (2016) 2663–2669.

[24] J.H. Jorgensen, J.F. Hindler, L.B. Reller, M.P. Weinstein, New consensus guidelines from the clinical and laboratory standards institute for antimicrobial susceptibility testing of infrequently isolated or fastidious bacteria, Clin. Infect. Dis. 44 (2007) 280–286.

[25] (a) C.F. Carson, B.J. Mee, T.V. Riley, Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy, Antimicrob. Agents. Chemother. 46 (2002) 1914–1920;
(b) T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol Methods 65 (1983) 55–63.

[26] J.G. Barr, E.T. Smyth, G.M. Hogg, In vitro antimicrobial activity of 299 imipenem in combination with vancomycin or teicoplanin against Staphylococcus 300 aureus and Staphylococcus epidermidis, Eur. J. Clin. Microbiol. Infect. Dis. 9 (1990) 804–809.

[27] (a) M. Pandey, A.K. Singh, R. Thakare, S. Talwar, P. Karaulia, A. Dasgupta, S. Chopra, A.K. Pandey, Diphenyleneiodonium chloride (DPIC) displays broad-spectrum bactericidal activity, Sci. Rep. 7 (2017) 11521;
(b) S. Thangamani, H. Mohammad, M.F. Abushahba, T.J. Sobreira, V.E. Hedrick, L.N. Paul, M.N. Seleem, Antibacterial activity and mechanism of action of auranofin against multi-drug resistant bacterial pathogens, Sci. Rep. 6 (2016) 22571.

[28] I. Wiegand, K. Hilpert, R.E. Hancock, Agar and broth dilution methods to determine

the minimal inhibitory concentration (MIC) of antimicrobial substances, Nat. Protoc. 3 (2008) 163–175.
[29] B. Rivas-Santiago, C.E.R. Santiago, J.E. Castañeda-Delgado, J.C. León-Contreras, R.E. Hancock, R. Hernandez-Pando, Activity of LL-37, CRAMP and antimicrobial peptide-derived compounds E2, E6 and CP26 against *Mycobacterium tuberculosis*, Int. J. Antimicrob. Agents. 41 (2013) 143–148.
[30] S. Patel, N. Gheewala, A. Suthar, A. Shah, In-vitro cytotoxicity activity of *Solanum nigrum* extract against Hela cell line and Vero cell line, Int. J. Pharm. Pharm. Sci. 1 (2009) 38–46.
[31] R. Thakare, A.K. Singh, S. Das, N. Vasudevan, G.R. Jachak, D.S. Reddy, A. Dasgupta, S. Chopra, Repurposing Ivacaftor for treatment of Staphylococcus aureus infections, Int. J. Antimicrob. Agents. 50 (2017) 389–392.