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Synthesis and evaluation of new Quinazolin-4(3*H*)-one derivatives as potent antibacterial agents against multidrug resistant *Staphylococcus aureus* and *Mycobacterium tuberculosis*

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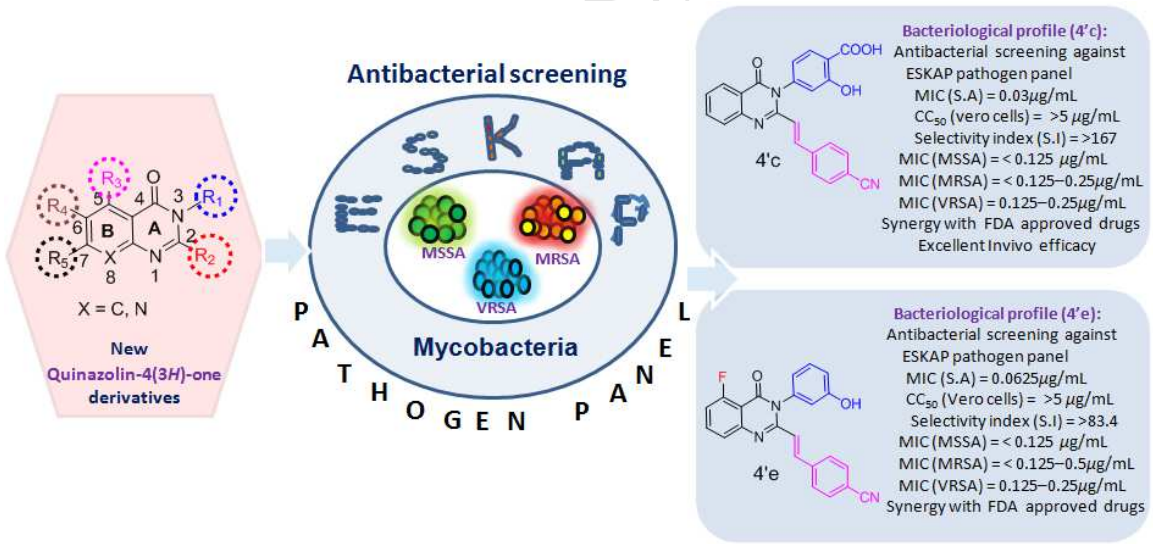
Graphical abstract:



Synthesis and evaluation of new Quinazolin-4(3*H*)-one derivatives as potent antibacterial agents against multidrug resistant *Staphylococcus aureus* and *Mycobacterium tuberculosis*

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Abstract

Staphylococcus aureus and *Mycobacterium tuberculosis* are major causative agents responsible for serious nosocomial and community-acquired infections impacting healthcare systems globally. Over several decades, these pathogens have developed resistance to multiple antibiotics significantly affecting morbidity and mortality. Thus, these recalcitrant pathogens are amongst the most formidable microbial pathogens for which international healthcare agencies have mandated active identification and development of new antibacterial agents for chemotherapeutic intervention. In our present work, a series of new quinazolin-4(3*H*)-one derivatives were designed, synthesized and evaluated for their antibacterial activity against ESKAP pathogens and pathogenic mycobacteria. The experiments revealed that **4'c**, **4'e**, **4'f** and **4'h** displayed selective and potent inhibitory activity against *Staphylococcus aureus* with MIC values ranging from 0.03-0.25 μg/mL. Furthermore, compounds **4'c** and **4'e** were found to be benign to Vero cells (CC_{50} = >5 μg/mL) and displayed promising selectivity index (SI) >167 and >83.4 respectively. Additionally, **4'c** and **4'e** demonstrated equipotent MIC against multiple drug-resistant strains of *S. aureus* including VRSA, concentration dependent bactericidal activity against *S. aureus* and synergized with FDA approved drugs. Moreover, compound **4'c** exhibited more potent activity in reducing the biofilm and exhibited a PAE of ~2 h at 10X MIC which is comparable to levofloxacin and vancomycin. *In vivo* efficacy of **4'c** in murine neutropenic thigh infection model revealed that **4'c** caused a similar reduction in cfu as vancomycin. Gratifyingly, compounds **4d**, **4e**, **9a**, **9b**, **14a**, **4'e** and **4'f** also exhibited anti-mycobacterial activity with MIC values in the range of 2-16 μg/mL. In addition, the compounds were found to be less toxic to

Vero cells (CC_{50} = 12.5->100 μg/mL), thus displaying a favourable selectivity index. The interesting results obtained here suggest the potential utilization of these new quinazolin-4(3*H*)-one derivatives as promising antibacterial agents for treating MDR-Staphylococcal and mycobacterial infections.

Keywords: Quinazolinones, Minimum Inhibitory Concentration, Multidrug-resistant *Staphylococcus aureus* (MDR-*S. aureus*), Vero cells.

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1. Introduction

Staphylococcus aureus and *Mycobacterium tuberculosis* are ubiquitous dreadful pathogens responsible for major human suffering worldwide [1]. Although causing inherently treatable infections, due to rapid acquiring of multidrug-resistance (MDR), their treatment has impacted healthcare systems significantly [2]. In view of the serious crisis, MRSA was recently categorised as high risk Priority II pathogen by World Health Organization (WHO), one among the 12 listed pathogens which imperils human health along with mycobacteria [3]. The incessant rise in drug-resistance exhibited by these pathogens as well as number of nosocomial and community infections caused by these pathogens in recent years has compelled the need to identify and develop new antibacterials for treating infection under hospital and community settings [4-11].

In our search of various chemical classes for developing new antibacterials, we found quinazolinone to be a promising heterocycle which could be considered as a privileged structure in the arena of drug design and development, due to its wide spectrum of biological properties including anti-cancer, anti-bacterial, anti-HIV, anti-fungal, anti-inflammatory, analgesic and anti-convulsant activities and also has broad scope for structural diversity [12-19].

Potential anti-bacterial properties of various quinazolin-4(3*H*)-one derivatives have been cited in the literature. Xioping *et al.* [20] and Zayed *et al.* [21] reported the anti-bacterial properties of 2-methylquinazolinone compounds **I** and **II** respectively (**Figure 1**). Saravanan *et al.* [22] reported the antimicrobial nature of quinazolinone compound **III.** Nandy *et al.* [23] and Jadhavar *et al.* [24] also reported the potent anti-mycobacterial activity of Mannich bases of 2-methyl-3*H*-quinazolin-4-ones **IV** and 2-styrylquinazolinones **V** respectively (**Figure 1**). Desai *et al.*[25] reported the antibacterial properties of series of new 2-styryl-3,4-dihydroquinazolin-6-yl)-1,3,5-triazin-2-yl)-3-methylurea derivative **VI** (**Figure 1**). Bouley *et al.* [26] reported 4(3*H*)-quinazolinones as potential orally bioavailable antibacterial agent **VII**. Recently, our group has reported 3-phenylquinazolin-4(3*H*)-ones **VIII**, 4-oxoquinazolin-3(4*H*)-yl)benzoic acid and benzamide derivatives as potent anti-MDR-*S. aureus* agents [27].

*<Insert **Figure 1** here>*

Many 1,2,3-triazole containing molecules have been explored as interesting therapeutic agents against several biological targets while expressing wide range of biological properties [28] including antimicrobial activity. In addition to the promising pharmacological properties,

triazole heterocycle is also known to have better hydrogen bond forming ability and metabolic stability. Antibiotics cefatrizine (*β*-lactam antibiotic) **IX** and tazobactam [29] (β-lactamase inhibitor) **X** contain 1,2,3-triazole as a key structural feature [30]. We have recently reported 1,2,3-triazole linked 4(3*H*)-quinazolinones **XI** as potent antibacterial agent [31]. (**Figure 2)**.

*<Insert **Figure 2** here>*

1.1 Rationale

Quinazolinone core is considered to be a promising heterocyclic structure in the area of drug design and development, due to its broad spectrum of biological properties including antibacterial activity. In addition, the 1,2,3-triazole heterocycle is found to exhibit good biological properties along with better hydrogen bond forming ability and metabolic stability. We hypothesized that hybridizing the structural features of quinazolinone and 1,2,3-triazole moieties could potentially have advantageous effect on the antibacterial activity. In the present study, we designed and synthesized a number of new quinazolin-4(3*H*)-one derivatives and tested them for their antibacterial and antimycobacterial properties (**Figure 3)**.

*<Insert **Figure 3** here>*

2.0 Results and Discussion

2.1 Chemistry

A series of newly designed quinazolin-4(3*H*)-one derivatives were synthesized as described in **Schemes 1, 2** & **3**. Substituted anthranilic acids were converted into the substituted benzoxazinone intermediate by treating with acetic anhydride under reflux conditions for 2 h. Further, substituted benzoxazinone intermediate was dissolved in glacial acetic acid on gentle heating followed by addition of substituted aniline, and the contents were refluxed for 5 h to give an 2-methylquinazolin-4(3*H*)-one derivatives (**Schemes 1, 2** & **3).**

6 or 7-bromo substituted 2-methyl quinazolin-4(3*H*)-one **3** reacted with substituted boronic acids under Suzuki conditions to afford 6 or 7- phenyl-2-methylquinazolin-4(3*H*)-ones **4**. Besides, 6-hydroxy-2-methyl-3-phenylquinazolin-4(3*H*)-one **7** or 7-amino-2-methyl-3-phenylquinazolin-4(3*H*)-one **13** intermediates was subjected to *O or N*-propargylation by using propargyl bromide under inert conditions to afford corresponding 6-*O*- or 7-*N*-propargylated quinazolin-4(3*H*)-one derivatives **8** or **14.** Treatment of the derivatives **8** and **14** with various substituted azides under click conditions afforded the corresponding 1,2,3-triazole derivatives **9** and **15** respectively.

Intermediates **4** were reacted with benzaldehyde or 4-cyano benzaldehyde in glacial acetic acid under reflux conditions for overnight to afford the new 2-styrylquinazolin-4(3*H*)-one derivatives as final compounds in moderate to excellent yields (**Scheme 1, 2 & 3**).

To the intermediate 2-methyl-7-nitro-4*H*-benzo[d][1,3]oxazin-4-one **11** dissolved in glacial acetic acid, aniline was added and the reaction mixture was refluxed for 5 h to give 2-methyl-7-nitro-3-phenylquinazolin-4(3*H*)-one as intermediate **12**. To obtain the amine **13**, the nitro group of the intermediate **12** was reduced by using tin(II) chloride dihydrate under reflux for 6 h in ethanol. The amine **13** was allowed to undergo azidation reaction by using sodium nitrite and sodium azide in 2N HCl to afford azide intermediate **16**. Finally, preparation of the designed compounds **17** and **18** were accomplished by reacting the azide intermediate **16** with various substituted phenylacetylenes or phenoxymethylacetylene by using Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. Finally, treatment of **17** with 4-cyanobenzaldehyde in glacial acetic acid afforded the 2-styrylquinazolin-4(3*H*)-one compounds **4'u**-**4'v** as final products in moderate to excellent yields (**Schemes 2 & 3)**. Structures of all the newly synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS (ESI) spectroscopic techniques. Representative compounds were also analysed for purity by using HPLC (Agilent Infinity-2 1260 series).

*<Insert **Scheme 1** here>*

*<Insert **Scheme 2** here>*

*<Insert **Scheme 3** here>*

*<Insert **Table 1** here>*

2.2. *In vitro* antibacterial activity

2.2.1. Antibiotic Susceptibility Testing against ESKAP pathogen panel

A series of new quinazolin-4(3*H*)-one derivatives were evaluated for their antibacterial activity against ESKAP pathogen panel by using broth microdilution assay. Minimum Inhibitory Concentration (MIC) was determined as per Clinical Laboratory Standards Institute (CLSI) guidelines [32]. The concentration of new quinazolin-4(3*H*)-one derivatives used is in the range of 64-0.03 μg/mL and Levofloxacin, isoniazid and refampicin were used as reference standards. The results are listed in **Table 2**.

*<Insert **Table 2** here>*

A perusal of the antimicrobial screening results suggested that the compounds **4a**-**4f** (**Scheme 1**) with substituted phenyl group at C-6 or C-7 with methyl group at C-2 position exhibited poor inhibitory activity against the ESKAP pathogen panel (MIC = 32–>64 μg/mL). However, the compounds **4d** and **4e** exhibited modest inhibitory activity against *M. tuberculosis* $H_{37}R_v$. Compounds **9a**-**9g** (**Scheme 1**) with 1,2,3-triazole linked to 3-*N*-phenyl or 3-*N*-benzyl 2-methyl quinazolin-4(3*H*)-one via ether linker at C-6 position did not exhibit activity against the ESKAP pathogen panel. However, the compounds **9a** and **9b** exhibited modest inhibitory activity against *M. tuberculosis* $H_{37}R_v$ (MIC = 16 μg/mL). Replacement of phenyl with benzyl moiety (**9f-9g**) at N-3 on ring A was found to be detrimental on antimycobacterial activity. Interestingly, compound **14a** with prop-2-yn-1-ylamino group at C-7 position of 2-methyl-3-phenyl quinazolin-4(3*H*)-one exhibited good inhibitory activity against *M. tuberculosis* $H_{37}R_v$ (MIC = 4μg/mL). However, **14a** did not display activity against ESKAP pathogen panel. Replacement of prop-2-yn-1-yl group (**14a**) with substituted 1-phenyl-1*H*-1,2,3-triazol-4-yl-methyl at C-7 as in **15a-15c** did not exhibit any activity against both *Staphylococcus aureus* and mycobacteria.

In our continued efforts, we introduced 4-phenyl-1*H*-1,2,3-triazole (compounds **17a**–**17d**) or 4-(phenoxymethyl)-1*H*-1,2,3-triazole (compounds **18a**–**18c**) at C-7 position of quinazolin-4(3*H*)-one on ring B. These compounds did not exhibit inhibitory activity against *S. aureus* and mycobacteria (MIC = >64 μg/mL). Moreover, even after replacement of methyl group at C-2 with styryl moiety (compounds **4'u** & **4'v**), they did not exhibit antibacterial activity against *S. aureus* and mycobacteria (MIC = >64 μg/mL). These broad SAR observations are summarised in Figure 4.

*<Insert **Table 3** here>*

Significantly, (**scheme 3)** compound **4'a** with methyl acetate group, **4'b** with *tert*-butyl phenyl at C-3 position with C-2 styryl moiety (with *p*-C_6H_4-CN group) on ring A was found to be inactive against ESKAP pathogen panel. However, compound **4'c,** with 3-hydroxy-4-carboxy phenyl group at N-3 with styryl moiety (with *p*-C_6H_4-CN group) displayed selective and highly potent inhibitory activity against *S. aureus* (MIC = 0.03 μg/mL). Interestingly, compound **4'd** having 2-carboxy-4-hydroxy phenyl group at N-3 with 4-cyanostyryl moiety at C-2 did not exhibit activity (MIC = >64 μg/mL). Compound **4'e** having hydroxy at *meta* position on the *N*-phenyl with fluoro group at C-5 exhibited selective and potent inhibitory activity against *S. aureus* (MIC = 0.0625 μg/mL). This prompted us to investigate a number of fluoro quinazolinones with 4-cyanostyryl moiety at C-2 position by varying substituents on 3-*N*-phenyl at *meta* position.

Interestingly, the compound **4'f** with propargyl group and **4'h** with acetylene moiety at *meta* position on the *N*-phenyl displayed potent antibacterial activity with MIC values 0.5 and 0.25 μg/mL respectively. Intriguingly, compound **4'e** and **4'f** exhibited inhibitory activity against *M. tuberculosis* $H_{37}R_v$ (MIC 8 and 2 μg/mL respectively). However, compound **4'g** and **4'i** with allyloxy and nitrile group at *meta* position of the *N*-Phenyl moiety was found to be inactive (MICs = 32 - >64 μg/mL) against both *S. aureus* and mycobacteria. Compound **4'j** with naphthalen-1-yl substitution, compounds **4'k** and **4'l** with 4-fluoro or 3,4-dichloro benzyl substitution, compound **4'm** with 4-(trifluoromethyl)phenyl amino substitution, compound **4'n** with (indol-3-yl)ethyl substitution, compound **4'o** with pyridin-3-yl substitution at N-3 position were found to be inactive (MIC = ≥64 μg/mL). Replacement of X=C with X=N in compounds **4'z-4'aj** did not display any activity except compound **4'ad** with thiol (-SH) group at *para* position of the *N*-Phenyl moiety which exhibited MIC = 2 μg/mL against *S. aureus*. However, all the aza variants of quinazolin-4(3*H*)-one did not display activity against mycobacteria *(*MIC = >64 μg/mL). Compounds **4'p** & **4'q** with methyl acetate group at N-3 with prop-2-yn-1-yloxy or phenyl-1*H*-1,2,3-triazol-4-yl)methoxy group at C-6 also did not exhibit antibacterial activity. Compounds **4'r** (phenyl at C-6), **4's** (prop-2-yn-1-ylamino group at C-7), **4't** (phenyl at C-7), **4'u** (4-phenyl-1*H*-1,2,3-triazole group at C-7), **4'v** ((4-(4-methoxyphenyl)-1*H*-1,2,3-triazole group at C-7)) with styryl moiety at C-2 position on ring A of quinazolin-4(3*H*)-one did not exhibit activity against both *S. aureus* and mycobacteria. Similarly, compounds **4'w, 4'x** & **4'y** with benzyl group at N-3 and prop-2-yn-1-yloxy group at C-6 were found to be inactive. The broad Structure Activity Relationships derived are summarised in **Figure 4 & 5.**

*<Insert **Figure 4** here>*

*<Insert **Figure 5** here>*

2.2.1.1. Brief comparison of results

All the newly synthesized compounds exhibited promising antibacterial activity similar to the compounds reported by various research groups. However, in contrast to 2-methylquinazolin-4(3*H*)-one compounds reported by Xioping *et al.* [20], Zayed *et al.* [21], Saravanan *et al.* [22], Nandy *et al.* [23] and Desai *et al.*[25], compounds **4d** and **4e** with methyl at C-2 and substituted phenyl group at C-7 position exhibited inhibitory activity against *M. tuberculosis* $H_{37}R_v$. In addition, compounds **9a-9b** with 1,2,3-triazole linked to 3-*N*-phenyl or 3-*N*-benzyl 2-methyl quinazolin-4(3*H*)-one via ether linker at C-6 position exhibited modest inhibitory activity against *M. tuberculosis* $H_{37}R_v$ (MIC = 16 μg/mL). Interestingly, compound **14a** with prop-2-yn-1-

ylamino group at C-7 position of 2-methyl-3-phenyl quinazolin-4(3*H*)-one showed potent activity against *M. tuberculosis* $H_{37}R_v$ (MIC = 4 μg/mL). However, they did not show activity against the ESKAP pathogen panel. Unlike 2-styrylquinazolin-4(3*H*)-ones reported by Jadhavar *et al.* [24] and Bouley *et al.* [26], compounds **4'e** and **4'f** with C-2-styryl moiety (*p*-C_6H_4-CN group) exhibited good inhibitory activity against *M. tuberculosis* $H_{37}R_v$ with MIC values 8 and 2 respectively. In comparison with our recent reports on quinazolinones [27a][27b], replacement of 3-(prop-2-yn-1-yloxy) phenyl (MIC = 0.25 μg/mL), 3-allyloxy phenyl (MIC = 0.125 μg/mL), 3-ethynylphenyl (MIC = 0.5 μg/mL), 3-cyanophenyl (MIC = 0.5 μg/mL) group with 3-hydroxy-4-carboxy phenyl group (**4'c**) at N-3 position with styryl moiety (with *p*-C_6H_4-CN group) displayed selective and highly potent inhibitory activity against *S. aureus* (MIC = 0.03 μg/mL). Furthermore, compound **4'c** exhibited 8 and 16 fold potent activity than the secondary amides *N*-isopropylbenzamide (MIC = 0.25 μg/mL) and *N*-cyclopropylbenzamide (MIC = 0. 5 μg/mL) quinazolinone derivatives reported by Gatadi *et al.* [27a]. Recently, Gatadi *et al.* [27b] also reported the compound having hydroxy at *meta* position on the *N*-phenyl with chloro group at C-5, which did not exhibit activity against *S. aureus* (MIC = ≥64 μg/mL). Interestingly, the compound having chloro group, when replaced with fluoro group at C-5 position (**4'e)** exhibited selective and potent activity against *S. aureus* (MIC = 0.0625 μg/mL). Additionally, the 5-fluoro compounds **4'f** with prop-2-yn-1-yloxy group and **4'h** with acetylene moiety at *meta* position on the *N*-phenyl displayed potent antibacterial activity against *S. aureus* with MIC values of 0.5 and 0.25 μg/mL respectively. Intriguingly, compound **4'e** and **4'f** also exhibited inhibitory activity against *M. tuberculosis* $H_{37}R_v$ (MICs 8 and 2 μg/mL respectively). Replacement of X=C with X=N in compound **4'ad** with 4-mercaptophenyl group at N-3 position (MIC = 2 μg/mL) exhibited potent inhibitory activity against *S. aureus* than the compounds reported by Gatadi *et al.* [27a]. Compounds **4'c** and **4'e** exhibited 8-16 fold more potent activity than the compounds with 3-chlorophenyl, 3-toluoyl and 3-fluorophenyl at N-3 position against *Staphylococcus aureus* (MIC = 0.5 μg/mL). Moreover, compounds **4'c** and **4'e** also exhibited 8-16 fold more potent activity than compounds with benzyl (Bz, 3-Br-Bz, 4-OCH_3-Bz) groups on the 1,2,3-triazole moiety (MIC = 0.5 μg/mL) against *Staphylococcus aureus* as reported in the recent literature [31].

2.3. Cytotoxicity against Vero cells

To examine the effect of the active compounds **4'c**, **4'e**, **4'f** and **4'h** on mammalian cells, a cytotoxicity assay was performed against Vero cells (African green monkey kidney cell line,

ATCC CCL-81) using the MTT assay. The 50% cytotoxic concentration (CC_{50}) is defined as the lowest concentration of compound which leads to a 50% reduction in cell viability. Doxorubicin was used as a reference standard and each experiment was performed in triplicate. A perusal of cytotoxicity data suggests that **4'c**, **4'e**, **4'f** and **4'h** are non toxic to Vero cells (CC_{50} = >5–>10 μg/mL) and displayed a promising Selectivity Index (SI) of (>10–>167). In a similar vein, active compounds **4d**, **4e**, **9a**, **9b**, **14a**, **4'e** and **4'f** against mycobacteria were found to be non toxic to Vero cells (CC_{50} = 12.5->100 μg/mL) and exhibited favourable selectivity index. The results are tabulated in **Table 4 & Table 5**.

*<Insert **Table 4** here>*

*<Insert **Table 5** here>*

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

To determine their range of activity against multiple strains of MDR-*S. aureus*, **4'c** and **4'e** were examined against various well defined and characterized clinical isolates of MRSA and VRSA as per standard CLSI guidelines [32]. The results are summarised in **Table 6**. Levofloxacin, meropenem and vancomycin were used as reference standards. From the close examination of the results, it can be inferred that **4'c** and **4'e** displayed potent antibacterial activity with MIC ranging from <0.125–0.5 μg/mL against various clinical strains of MRSA and VRSA.

*<Insert **Table 6** here>*

2.5. Time kill kinetics of 4'c and 4'e

The bactericidal activity was determined 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 **4'c**, **4'e** and vancomycin in MHB in triplicate and incubated at 37 °C. 100 μL samples were collected after time intervals of 0h, 1h, 6h and 24h and serially diluted in Phosphate Buffered Saline (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 stipulated time point in the presence and absence of compound (**Figure 6**).

*<Insert **Figure 6** here>*

2.6. Determination of synergy with FDA approved drugs

The checkerboard method was used to determine the interaction between **4'c**, **4'e** and various FDA approved antibiotics including meropenem, linezolid, ceftriaxone and vancomycin which are typically used for the treatment of staphylococcal infections (**Table 7 & 8**). As can be seen, **4'c** and **4'e** synergized with drugs tested, thus displaying great potential to be a part of multi-drug regimen.

*<Insert **Table 7** here>*

*<Insert **Table 8** here>*

2.7. Determination of Post-Antibiotic effect of 4'c

The post-antibiotic effect of **4'c** was determined with vancomycin and levofloxacin as controls. As can be seen from **Table 9**, **4'c** exhibits a PAE of ~2 h at 10X MIC which is comparable to levofloxacin and vancomycin.

*<Insert **Table 9** here>*

2.8. 4'c eradicates *S. aureus* biofilm

It has been repeatedly demonstrated that bacteria under different stresses make biofilm in order to protect themselves, which often leads to prolonging therapeutic intervention and increasing drug-resistance. Incidentally, most of the approved drugs have very limited activity against pathogens in biofilms owing to an altered physiological state, thus it is imperative to determine the antibiofilm activity of molecules under development. In this context, **4'c** at 10X MIC exhibited potent anti-biofilm activity as it reduced biofilm by 14.1% as compared to untreated. On the other hand, similar reduction in biofilm was achieved at 10X MIC of levofloxacin (30.6% reduction) while vancomycin only reduced by 7.7%. Thus, **4'c** is more potent in reducing the biofilm as compared to vancomycin (**Figure 7**).

*<Insert **Figure 7** here>*

3. *In vivo* animal efficacy of 4'c

The *in vivo* efficacy of **4'c** was tested in murine neutropenic thigh infection model of *S. aureus* infection. This model has been utilized extensively for evaluating the *in vivo* efficacy of several molecules against *S. aureus.* Briefly, the thigh of neutropenic mice was infected with *S. aureus*, followed by two IP injections of **4'c** at 25 and 50 mg/Kg while vancomycin was injected at 25 mg/Kg. Saline and vancomycin treated mice groups were used as a negative and positive control

respectively. As can be seen in **Figure 8**, mice infected with *S. aureus* were treated with vancomycin (25 mg/Kg) and **4'c** (25 and 50 mg/Kg) and the treatment caused significantly equal reduction at 25 mg/Kg ($P < 0.05$). When dosed at 50 mg/Kg, **4'c** reduced significantly more cfu ($P < 0.005$) than vancomycin, thus displaying a concentration dependent reduction in CFU. Taken together, **4'c** is as potently capable of causing reduction in cfu as is vancomycin.

*<Insert **Figure 8** here>*

4. Conclusion

In conclusion, a series of new quinazolin-4(3*H*)-one derivatives have been synthesised and evaluated against ESKAP pathogen panel and pathogenic mycobacteria. Compounds **4'c**, **4'e**, **4'f** and **4'h** displayed selective and potent antibacterial activity against *Staphylococcus aureus* and were found to be non toxic to Vero cells (CC_{50} = >5–>10 μg/mL) with a good selectivity index (SI = >10–>167). **4'c** and **4'e** displayed potent inhibitory activity when screened against clinical MRSA and VRSA isolates. Gratifyingly, compounds **4d**, **4e**, **9a**, **9b**, **14a**, **4'e** and **4'f** also exhibited antimycobacterial activity with MIC values in the range of 2-16 μg/mL. Besides, the compounds were found to be less toxic to Vero cells (CC_{50} = 12.5->100 μg/mL) and exhibited favourable selectivity index. Compounds **4'c** and **4'e** also exhibited concentration dependent bactericidal activity and synergized with the FDA approved drugs tested. Moreover, compound **4'c** exhibited more potent activity in reducing the biofilm and exhibited a PAE of ~2 h at 10X MIC which is comparable to levofloxacin and vancomycin. *In vivo* efficacy of **4'c** in the murine neutropenic thigh infection model, revealed that the compound **4'c** was equipotently capable of causing reduction in cfu as compared to vancomycin. All together, these new compounds display antibacterial potential to be further modified and developed as antimycobacterial and anti-multidrug resistant *S. aureus* leads.

5. Materials and methods

All the chemicals, reagents and starting materials were procured from commercial providers and were used as such. The monitoring of reactions were performed by TLC-MERCK pre-coated silica gel 60-F_{254} (0.5 mm) aluminium plates under UV light. ^{1}H and ^{13}C NMR spectra were obtained 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 DMSO-d_6 was used as an internal reference or TFA-d (δ 164.2 and δ 116.6 for ^{13}C NMR). 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. HPLC purity were determined with Agilent Infinity-2 1260 series instrument (UV–Vis diode-array detector) and were performed at 258-268 nm. Column chromatography was performed using silica gel 60–120 or 100–200 mesh. Melting points were taken using Stuart® SMP30 apparatus.

Intermediates **2**, **3, 6, 7** and **8** (**Scheme 1**) were prepared according to the procedures described in literature [26,27].

*5.1. General reaction procedure for the synthesis of substituted 6 or 7-phenyl-2-methyl-3-phenylquinazolin-4(3H)-one derivatives **4a-4f**.*

To a stirred solution of 6 or 7-bromo-2-methyl-3-phenylquinazolin-4(3*H*)-ones (**3**, 2 mmol) (**scheme 1**), in a schlenk tube, Cs_2CO_3 (2 mmol), substituted phenylacetylenes or phenoxymethyl acetylenes (2 mmol**),** Pd-$(PPh_3)_4$ (5 mol%), followed by LiCl (2 mol%) were added. A septa was placed on the schlenk tube which then was purged with N_2 for 5 min. The solvents 1:1 (5 mL of toluene and 5 mL of ethanol) were added, and the test tube was put in an oil bath previously set at 100 °C and allowed to stir for 12-14 h. The reaction was monitored by TLC. After completion of reaction, it was extracted with ethylacetate and washed with NH_4Cl solution, water and brine and dried by using sodium sulfate (Na_2SO_4). The combined organic extracts were added directly to a plug of silica gel to remove the catalyst, thus yielding the crude product. Further purification was accomplished via column chromatography, yielding a pure white solids ***4a-4f***. All the newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS (ESI). ^{1}H NMR signals was also determined for some representative compounds. The synthesis of the final compounds ***4a-4f*** was followed by general procedure given above.

*5.1.1. 2-methyl-3,6-diphenylquinazolin-4(3H)-one (**4a**):* White solid, Yield 71 %; mp: 133–135 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (d, J = 2.1 Hz, 1He), 8.02 (dd, J = 8.5, 2.2 Hz, 1Hi), 7.79–7.75 (m, 1Hj), 7.71–7.67 (m, 2Hf), 7.61–7.55 (m, 2Hg), 7.55–7.50 (m, 1Hh), 7.49–7.45 (m, 2Hb), 7.41–7.35 (m, 1Hd), 7.31–7.27 (m, 2Hc), 2.27 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.3, 154.3, 146.5, 139.6, 137.8, 133.6, 130.0, 129.4, 129.0, 128.0, 127.9, 127.2,

127.2, 124.9, 121.0, 24.4 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{21}H_{17}N_2O$ 313.1341; found 313.1344.

*5.1.2. 3-(3-chlorophenyl)-6-(4-fluorophenyl)-2-methylquinazolin-4(3H)-one (**4b**):* White solid, Yield 80 %; mp: 165–167 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.27 (d, J = 2.0 Hz, 1Hf), 8.14 (dd, J = 8.4, 2.1 Hz, 1Hb), 7.84–7.78 (m, 2Hij), 7.76–7.72 (m, 1Hd), 7.71 (s, 1He), 7.62 (d, J = 4.4 Hz, 2Hg), 7.53–7.47 (m, 1Hc), 7.35–7.31 (m, 2Hh), 2.17 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.6 (d, J = 244.8 Hz), 161.7, 154.6, 147.0, 139.7, 137.6, 135.8 (d, J = 2,7 Hz), 134.1, 133.6, 131.6, 129.7, 129.3 (d, J = 8.3 Hz), 129.2, 128.0, 128.0, 124.0, 121.2, 116.5 (d, J = 21.5 Hz, 2H), 24.5 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{21}H_{15}FClN_2O$ 365.0857; found 365.0860.

*5.1.3. 6-(4-methoxyphenyl)-2-methyl-3-phenylquinazolin-4(3H)-one (**4c**):* white solid, Yield 68 %; mp: 111–113 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (d, J = 2.1Hz, 1He), 8.00 (dd, J = 8.5, 2.2 Hz, 1Hi), 7.84–7.78 (m, 1Hj), 7.65–7.61 (m, 2Hf), 7.60–7.56 (m, 2Hc), 7.56–7.52 (m, 1Hd), 7.32–7.27 (m, 2Hb), 7.03–6.99 (m, 2Hg), 3.87 (s, 3Hh), 2.32 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.2, 159.6, 154.2, 139.4, 137.6, 134.7, 133.3, 132.0, 130.0, 129.4, 128.3, 128.0, 126.9, 124.2, 120.9, 114.5, 55.4, 24.1 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{22}H_{19}N_2O_2$ 343.1446; found 343.1450.

*5.1.4. 3-(3-chlorophenyl)-2-methyl-7-phenylquinazolin-4(3H)-one (**4d**):* White solid, Yield 85 %; mp: 145–147 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33–8.30 (m, 1H), 7.95–7.92 (m, 1H), 7.76–7.70 (m, 3H), 7.54–7.48 (m, 4H), 7.47–7.42 (m, 1H), 7.34–7.32 (s, 1H), 7.23–7.18 (m, 1H), 2.27(s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.8, 154.3, 147.8, 147.4, 145.8, 139.5, 138.7, 135.7, 131.0, 129.8, 129.1, 128.6, 127.6, 127.4, 126.5, 126.0, 124.7, 119.2, 24.3 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{21}H_{16}ClN_2O$ 347.0951; found 347.0954.

*5.1.5. 3-(3-chlorophenyl)-7-(4-fluorophenyl)-2-methylquinazolin-4(3H)-one (**4e**):* White solid, Yield 76 %; mp: 140–142 °C ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.14 (d, J = 8.3 Hz, 1H), 7.95–7.87 (m, 3H), 7.90 (dd, J = 8.6, 5.5 Hz, 1H), 7.72–7.69 (m, 1H), 7.64–7.59 (m, 2H), 7.52–7.46 (m, 1H), 7.40–7.32 (m, 2H), 2.17 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.04 (d, J = 246.2 Hz), 161.5, 154.9, 148.3, 145.5, 139.6, 135.6 (d, J = 3.1 Hz), 134.1, 131.6, 129.8 (d, J = 8.4 Hz), 129.6, 129.2, 128.0, 127.5, 125.4, 124.6, 119.7, 116.5 (d, J = 21.5 Hz), 24.4 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{21}H_{15}FClN_2O$ 365.0857; found 365.0859.

*5.1.6. 3-(3-chlorophenyl)-7-(4-methoxyphenyl)-2-methylquinazolin-4(3H)-one (**4f**):* White solid, Yield 78 %; mp: 155–157 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (d, J = 8.3 Hz, 1H), 7.91 (s, 1H), 7.73–7.69 (m, 1H), 7.69–7.65 (m, 2H), 7.54–7.50 (m, 2H), 7.33 (s, 1H), 7.23–7.18 (m, 1H), 7.06–7.01 (m, 2H), 3.88 (s, 3H), 2.31 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.5, 160.3, 154.7, 147.5, 138.4, 135.7, 131.6, 131.1, 129.9, 128.6, 127.6, 127.1, 126.5, 125.8, 123.3, 123.3, 118.5, 114.6, 55.4, 24.0 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{22}H_{18}ClN_2O_2$ 377.1057; found 377.1058.

Compound **14a** (**Scheme 1**) were prepared according to the procedures described in literature [26,27].

5.2. General reaction procedure for the synthesis of 6-((1H-1,2,3-triazol-4-yl)methoxy)-2-methylquinazolin-4(3H)-one derivatives **9a-9g** *or 7-(((1H-1,2,3-triazol-4-yl)methyl)amino)-2-methyl-3-phenylquinazolin-4(3H)-one derivatives* ***15a-15c***.

Compound (**8** or **14**, 2 mmol) (**scheme 1 & 2**) and phenyl or benzyl azides (2 mmol) were suspended in 10 mL of a 1:1 *tert*-BuOH/H_2O mixture. To the heterogeneous mixture, sodium ascorbate (10 mol%), copper (II) sulfate pentahydrate (2 mol%) were added and the reaction mixture was allowed to stir for 12-14 h to give crude white or pale yellow precipitates, which were filtered, washed with water, followed by hexane and finally purified by using column chromatography to obtain pure solid compounds **9a-9g** in 61-77% yields and **15a-15c** in 51-56% yields. All the newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS (ESI). The synthesis of the final compounds **9a-9g** and **15a-15c** was followed by general procedure given above.

*5.2.1. 6-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-methyl-3-phenylquinazolin-4(3H)-one (**9a**):* White solid, Yield 72 %; mp: 177–179 °C ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (s, 1Hg), 7.81–7.78 (m, 1Hk), 7.76 (d, J = 2.7 Hz, 1He), 7.67–7.62 (m, 2Hhj), 7.59–7.53 (m, 2Hb), 7.53–7.49 (m, 1Hm), 7.49–7.40 (m, 3Hci), 7.31–7.26 (m, 2Hld), 5.36 (s, 2Hf), 2.23 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.5, 156.6, 152.7, 144.3, 142.6, 138.5, 138.0, 134.7, 132.1, 130.1, 129.4, 129.1, 128.9, 128.9, 124.8, 123.6, 121.7, 120.4, 119.2, 108.4, 62.1, 24.3 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{19}ClN_5O_2$ 444.1227; found 444.1229.

*5.2.2. 6-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-methyl-3-phenylquinazolin-4(3H)-one (**9b**):* White solid, Yield 74 %; mp: 171–173 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.01 (s, 1Hg), 7.99–7.94 (m, 2Hh), 7.71–7.66 (m, 3Hie), 7.66–7.63 (m, 1Hk), 7.61–7.56 (m,

2Hb), 7.56–7.50 (m, 2Hc), 7.46–7.42 (m, 2Hjd), 5.37 (s, 2Hf), 2.10 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.5, 156.7, 152.7, 144.3, 142.6, 138.5, 135.8, 133.6, 130.4, 130.1, 129.4, 128.9, 128.9, 124.8, 123.5, 122.3, 121.7, 108.4, 62.1, 24.3 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{19}ClN_5O_2$ 444.1227; found 444.1231.

*5.2.3. 2-methyl-3-phenyl-6-((1-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)quinazolin-4(3H)-one (**9c**):* White solid, Yield 66 %; mp: 178–180 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (s, 1Hg), 7.81 (d, *J* = 2.8 Hz, 1He), 7.75–7.70 (m, 1Hk), 7.63–7.57 (m, 2Hb), 7.56–7.52 (m, 1Hj), 7.51–7.47 (dd, *J* = 8.9, 2.9 Hz, 1Hd), 7.31–7.27 (m, 2Hc), 6.97 (s, 2Hh), 5.39 (s, 2Hf), 3.95 (s, 6Hi), 3.91 (s, 3Hi'), 2.29 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.7, 157.0, 153.9, 152.9, 143.9, 141.3, 138.5, 137.4, 132.8, 130.1, 129.5, 128.1, 127.9, 125.0, 121.6, 121.4, 108.1, 98.7, 62.3, 61.1, 56.5, 23.8 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{27}H_{26}N_5O_5$ 500.1934; found 500.1941.

*5.2.4. 2-methyl-7-(4-(((2-methyl-4-oxo-3-phenyl-3,4-dihydroquinazolin-6-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)-3-phenylquinazolin-4(3H)-one (**9d**):* White solid, Yield 61 %; mp: 255–257 °C ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.25 (s, 1H), 8.28 (d, *J* = 8.6 Hz, 1H), 8.23 (d, *J* = 2.1 Hz, 1H), 8.13 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.71 (d, *J* = 2.9 Hz, 1H), 7.66 (d, *J* = 8.9 Hz, 1H), 7.62–7.52 (m, 7H), 7.52–7.47 (m, 2H), 7.47–7.43 (m, 2H), 5.41 (s, 2H), 2.16 (s, 3H), 2.11 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.5, 161.1, 156.7, 156.7, 152.7, 149.0, 144.6, 142.6, 141.2, 138.5, 138.1, 130.1, 130.0, 129.6, 129.4, 129.2, 129.0, 128.9, 128.9, 124.8, 123.7, 121.7, 120.6, 118.2, 116.9, 108.5, 62.1, 24.6, 24.3 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{33}H_{26}N_7O_3$ 568.2097; found 568.2102.

*5.2.5. 6-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-methyl-3-phenylquinazolin-4(3H)-one (**9e**):* White solid, Yield 71 %; mp: 155–157 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.32 (s, 1H), 8.13–8.09 (m, 1H), 7.88–7.82 (m, 1H), 7.69–7.65 (m, 1H), 7.61–7.56 (m, 2H), 7.55–7.50 (m, 1H), 7.50–7.46 (m, 1H), 7.31–7.26 (m, 2H), 7.20–7.15 (m, 2H), 7.05–7.01 (m, 1H), 5.61 (s, 2H), 5.23–5.14 (m, 2H), 2.14 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.7, 159.2, 154.9, 147.8, 143.2, 139.4, 135.8, 135.1, 132.2, 130.8, 130.7, 127.1, 126.9, 126.8, 125.4, 121.9, 121.3, 121.0, 116.0, 115.4, 61.7, 52.6, 24.3 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{25}H_{21}BrN_5O_2$ 502.0878; found 502.0880.

*5.2.6. 3-benzyl-6-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-methylquinazolin-4(3H)-one (**9f**):* White solid, Yield 70 %; mp: 240–242 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.06 (s, 1Hh), 8.08 (s, 1Hf), 7.97–7.93 (m, 1Hl), 7.75 (d, *J* = 2.8 Hz, 1Hi), 7.66–7.59 (m, 2Hkn),

7.59–7.56 (m, 1Hj), 7.55–7.50 (m, 1Hm), 7.40–7.32 (m, 2Hc), 7.31–7.25 (m, 1Hd), 7.20 (d, *J* = 7.6 Hz, 2Hb), 5.39 (s, 4Hge), 2.47 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.7, 156.7, 153.4, 144.3, 142.5, 138.1, 137.0, 134.7, 132.1, 129.3, 129.1, 129.0, 127.8, 126.8, 124.9, 123.6, 121.1, 120.5, 119.3, 108.4, 62.1, 46.9, 23.2 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{25}H_{21}ClN_5O_2$ 458.1384; found 458.1384.

*5.2.7. 3-benzyl-6-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-methylquinazolin-4(3H)-one (**9g**):* White solid, Yield 74 %; mp: 235–237 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.01 (s, 1Hh), 8.02–7.93 (m, 2Hi), 7.75 (d, *J* = 2.8 Hz, 1Hf), 7.71–7.66 (m, 2Hj), 7.63–7.58 (m, 1Hl), 7.52 (dd, *J* = 8.9, 2.9 Hz, 1Hd), 7.39–7.32 (m, 2Hc), 7.31–7.25 (m, 1Hk), 7.20 (d, *J* = 7.4 Hz, 2Hd), 5.39 (s, 4Heg), 2.47 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.7, 156.7, 153.4, 144.3, 142.5, 137.0, 135.8, 133.6, 130.3, 129.3, 128.9, 127.8, 126.8, 124.9, 123.5, 122.3, 121.1, 108.3, 62.1, 46.9, 23.2 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{25}H_{21}ClN_5O_2$ 458.1384; found 458.1387.

*5.2.8. 2-methyl-3-phenyl-7-(prop-2-yn-1-ylamino)quinazolin-4(3H)-one (**14a**):* White solid, Yield 77 %; mp: 155–157 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 7.80 (d, *J* = 8.7 Hz, 1He), 7.57–7.51 (m, 2Hb), 7.50–7.46 (m, 1Hd), 7.40–7.35 (m, 2Hc), 7.00 (t, *J* = 5.9 Hz, 1Hi), 6.81 (dd, *J* = 8.7, 2.3 Hz, 1Hf), 6.69–6.67 (m, 1Hj), 4.01 (dd, *J* = 5.9, 2.3 Hz, 2Hg), 3.15 (t, *J* = 2.3 Hz, 1Hh), 2.06 (s, 3Ha). ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.3, 154.6, 153.4, 149.9, 138.7, 129.9, 129.1, 127.7, 114.9, 110.4, 105.5, 81.6, 73.9, 49.1, 32.2, 24.5 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{18}H_{16}N_3O$ 290.1293; found 290.1295.

*5.2.9. 7-(((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-2-methyl-3-phenylquinazolin-4(3H) (**15a**):* White solid, Yield 53 %; mp: 122-124 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.83 (s, 1Hi), 8.27–8.22 (m, 1He), 8.01–7.95 (m, 1Hf), 7.78 (d, *J* = 8.7 Hz, 1Hj), 7.68 (t, *J* = 9.0 Hz, 1Hk), 7.57–7.51 (m, 2Hb), 7.51–7.46 (m, 1Hd), 7.38–7.33 (m, 2Hc), 7.26 (t, *J* = 5.7 Hz, 1Hg), 6.87 (dd, *J* = 8.8, 2.1 Hz, 1Hm), 6.67 (d, *J* = 2.0 Hz, 1Hl), 4.53 (d, *J* = 5.6 Hz, 2Hh), 2.04 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.9, 157.9, 155.9, 154.1, 153.4, 149.6, 146.3, 138.3, 133.7, 129.5, 128.7, 127.3, 122.2, 121.7, 120.9, 120.8 (d, *J* = 7.8 Hz), 118.2 (d, *J* = 22.7 Hz), 114.5, 109.7, 104.4, 38.1, 24.0 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{19}ClFN_6O$ 461.1293; found 461.1294.

*5.2.10. 2-methyl-7-(((1-(3-methyl-4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-3-phenylquinazolin-4(3H)-one (**15b**):* White solid, Yield 56 %; mp: 176-178 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.61 (s, 1Hi), 8.40 (d, *J* = 2.3 Hz, 1Hk), 8.25 (dd, *J* = 8.7, 2.5 Hz, 1Hj), 7.79–

7.74 (m, 2Hb), 7.56–7.52 (m, 2Hc), 7.50–7.46 (m, 1Hd), 7.39–7.33 (m, 2Hem), 7.22 (t, *J* = 5.6 Hz, 1Hg), 6.90–6.86 (m, 1Hf), 6.73 (d, *J* = 2.1 Hz, 1Hn), 4.55 (d, *J* = 5.6 Hz, 2Hh), 2.35 (s, 3Hl), 2.05 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.3, 154.5, 153.9, 150.0, 148.0, 145.8, 141.4, 138.7, 135.5, 129.9, 129.1, 127.7, 127.6, 126.9, 125.3, 122.7, 114.8, 110.1, 105.0, 38.4, 24.4, 18.4 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{25}H_{22}N_7O_3$ 468.1784; found 468.1784.

*5.2.11. 2-methyl-3-phenyl-7-(((1-(3,4,5-trimethylphenyl)-1H-1,2,3-triazol-4yl)methyl)amino)quinazolin-4(3H)-one (**15c**):* White solid, Yield 51 %; mp: 126-128 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (s, 1H), 7.78 (d, *J* = 8.7 Hz, 1H), 7.55–7.52 (m, 2H), 7.51–7.49 (m, 1H), 7.39–7.36 (m, 2H), 7.18 (t, *J* = 5.5 Hz, 1H), 7.09 (s, 2H), 6.90–6.86 (m, 1H), 6.74–6.71 (m, 1H), 4.53 (d, *J* = 5.5 Hz, 2H), 2.33 (s, 3H), 2.05 (s, 3H), 1.88 (s, 6H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.3, 154.4, 154.0, 150.0, 145.0, 139.9, 138.7, 134.9, 133.9, 129.9, 129.3, 129.1, 127.7, 125.6, 114.7, 110.0, 106.9, 105.0, 38.6, 24.4, 21.1, 17.3 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{27}H_{27}N_6O$ 451.2246; found 451.2250.

*5.3. General reaction procedure for the synthesis of 2-methyl-3-phenyl-7-(1H-1,2,3-triazol-1-yl)quinazolin-4(3H)-one derivatives **17a-17d**, **18a-18c**.*

7-azido-2-methyl-3-phenylquinazolin-4(3*H*)-one (**16**, 2 mmol) (**scheme 2**) and substituted phenylacetylenes or phenoxymethyl acetylenes (2 mmol**)** were mixed in a 10 mL volume of 1:1 *tert*-BuOH/H_2O mixture. To the heterogeneous mixture, sodium ascorbate (10 mol%), followed by copper(II)sulfate pentahydrate (2 mol%) were added and the reaction mixture was allowed to stir for 12-14 h to give crude white precipitates, which were filtered, washed with water, followed by hexane and finally purified by using column chromatography to obtain as pure white solid compounds ***17a-17d***, ***18a-18c*** in 58-82% yields. All the newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS (ESI). The synthesis of the final compounds ***17a-17d***, ***18a-18c*** was followed by general procedure given above.

*5.3.1. 2-methyl-3-phenyl-7-(4-phenyl-1H-1,2,3-triazol-1-yl)quinazolin-4(3H)-one (**17a**):* White solid, Yield 78 %; mp: 210–212 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.58 (s, 1Hg), 8.31 (d, *J* = 8.6 Hz, 1He), 8.28–8.25 (m, 1Hf), 8.19–8.14 (m, 1Hj), 8.01–7.96 (m, 2Hh), 7.63–7.57 (m, 2Hb), 7.57–7.47 (m, 5Hcik), 7.44–7.39 (m, 1Hd), 2.17 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.2, 156.7, 149.0, 148.2, 141.3, 138.1, 130.4, 130.1, 129.6, 129.5, 129.2, 128.9, 128.9, 125.9, 120.5, 120.3, 118.0, 116.7, 24.7 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{23}H_{18}N_5O$ 380.1511; found 380.1516.

*5.3.2. 7-(4-(4-bromophenyl)-1H-1,2,3-triazol-1-yl)-2-methyl-3-phenylquinazolin-4(3H)-one (**17b**):* White solid, Yield 82 %; mp: 204–206 °C; ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.65 (s, 1Hg), 8.33 (d, J = 8.6 Hz, 1He), 8.26 (d, J = 2.1 Hz, 1Hf), 8.16 (dd, J = 8.6, 2.2 Hz, 1Hj), 7.95–7.91 (m, 2Hh), 7.77–7.73 (m, 2Hi), 7.63–7.58 (m, 2Hb), 7.58–7.52 (m, 1Hd), 7.52–7.48 (m, 2Hc), 2.18 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.1, 156.8, 149.0, 147.1, 141.2, 138.1, 132.5, 130.1, 129.7, 129.6, 129.3, 128.9, 127.8, 122.0, 120.7, 120.6, 118.0, 116.7, 24.8 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{23}H_{17}BrN_5O$ 458.0616; found 458.0619.

*5.3.3. 2-methyl-3-phenyl-7-(4-(p-tolyl)-1H-1,2,3-triazol-1-yl)quinazolin-4(3H)-one (**17c**):* White solid, Yield 77 %; mp: 200–202 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (d, J = 8.6 Hz, 1H), 8.33 (s, 1H), 8.09 (s, 1H), 8.06–8.03 (m, 1H), 7.87–7.83 (m, 2H), 7.65–7.59 (m, 2H), 7.59–7.54 (m, 1H), 7.35–7.30 (m, 4H), 2.44 (s, 3H), 2.31 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.4, 156.1, 149.0, 148.7, 141.5, 138.7, 137.4, 130.2, 129.7, 129.6, 129.4, 127.9, 127.0, 125.9, 122.4, 120.4, 118.3, 117.0, 24.5, 21.4 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{20}N_5O$ 394.1668; found 394.1670.

*5.3.4. 7-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)-2-methyl-3-phenylquinazolin-4(3H)-one (**17d**):* White solid, Yield 69 %; mp: 198–200 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (d, J = 8.5 Hz, 1H), 8.28 (s, 1H), 8.08 (s, 1H), 8.06–8.02 (m, 1H), 7.9 1–7.86 (m, 2H), 7.65–7.59 (m, 2H), 7.59–7.53 (m, 1H), 7.34–7.30 (m, 2H), 7.07–7.02 (m, 2H), 3.89 (s, 3H), 2.30 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.4, 160.0, 156.1, 148.7, 141.5, 137.4, 132.2, 130.2, 129.6, 129.4, 127.9, 127.3, 122.5, 120.4, 118.3, 116.9, 116.5, 114.4, 55.4, 24.6 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{20}N_5O_2$ 410.1617; found 410.1619.

*5.3.5. 2-methyl-7-(4-(phenoxymethyl)-1H-1,2,3-triazol-1-yl)-3-phenylquinazolin-4(3H)-one (**18a**):* White solid, Yield 58 %; mp: 203–205 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (d, J = 8.5 Hz, 1He), 8.25 (s, 1Hg), 8.08 (s, 1Hl), 8.04–8.00 (m, 1Hf), 7.65–7.59 (m, 2Hj), 7.59–7.55 (m, 1Hd), 7.38–7.33 (m, 2Hb), 7.33–7.30 (m, 2Hc), 7.09–7.05 (m, 2Hi), 7.05–7.01(m, 1Hk), 5.36 (s, 2Hh), 2.33 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.2, 158.1, 156.7, 148.1, 141.4, 137.1, 130.2, 129.7, 129.5, 127.9, 121.5, 121.1, 120.5, 118.6, 116.8, 114.8, 113.3, 111.3, 61.8, 24.3 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{20}N_5O_2$ 410.1617; found 410.1621.

*5.3.6. 3-(3-chlorophenyl)-7-(4-((3,5-dimethylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-2-methylquinazolin-4(3H)-one (**18b**):* White solid, Yield 65 %; mp: 255–257 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (d, J = 8.6 Hz, 1Hf), 8.22 (s, 1Hh), 8.13–8.09 (m, 1Hb), 8.07–8.02 (m, 1Hg), 7.61–7.54 (m, 2Hed), 7.38–7.36 (m, 1Hm), 7.27–7.23 (m, 1Hc), 6.70–6.66 (m, 3Hjl), 5.33

(s, 2Hi), 2.40 (s, 3Ha), 2.33 (s, 6Hk) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.8, 158.1, 156.2, 147.6, 146.1, 141.6, 139.5, 138.0, 135.9, 131.2, 130.2, 129.5, 128.4, 126.4, 123.3, 120.7, 120.1, 118.8, 116.5, 112.5, 61.8, 24.2, 21.5 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{26}H_{23}ClN_5O_2$ 472.1540; found 472.1544.

*5.3.7. 3-(3-chlorophenyl)-2-methyl-7-(4-((4-(tert-pentyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)quinazolin-4(3H)-one (**18c**):* White solid, Yield 65 %; mp: 255–257 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.24 (s, 1H), 8.30 (d, J = 8.6 Hz, 1H), 8.25 (d, J = 1.8 Hz, 1H), 8.15 (dd, J = 8.6, 1.9 Hz, 1H), 7.75–7.71 (m, 1H), 7.66–7.60 (m, 2H), 7.56–7.50 (m, 1H), 7.31–7.24 (m, 2H), 7.05–6.99 (m, 2H), 5.25 (s, 2H), 2.19 (s, 3H), 1.59 (q, J = 7.4 Hz, 2H), 1.23 (s, 6H), 0.62 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.1, 156.3, 156.1, 148.9, 145.1, 141.8, 141.3, 139.4, 134.2, 131.7, 129.8, 129.2, 129.1, 128.0, 127.3, 123.5, 120.5, 118.2, 116.9, 114.7, 61.4, 37.4, 36.7, 28.9, 24.6, 9.5 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{29}H_{29}ClN_5O_2$ 514.2010; found 514.1975.

*5.4. General reaction procedure for the synthesis of (E)-2-styrylquinazolin-4(3H)-one derivatives and (E)-2-styrylpyrido[2,3-d]pyrimidin-4(3H)-one **4' (4'a-4'z)*** and ***(4'aa-4'aj).***

Compound (**3'**, 1 mmol) (**scheme 3**) was dissolved in glacial acetic acid on gentle heating, to which benzaldehyde or 4-cyanobenzaldehyde (1 mmol) was added and the homogeneous mixture was allowed to reflux overnight (16-18 h). The reaction was monitored by using TLC. After completion of reaction, the reaction mixture was allowed to cool to room temperature and then 10 mL water was added to give crude precipitates, which were filtered under vacuum, washed with water, methanol and finally washed with hexane to afford a pure yellow to pale yellow crystal products ***4' (4'a-4'z)*** and ***(4'aa-4'aj)*** in 48–78% yields. All the newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS (ESI). Some representative potent compounds were also analysed for purity, which showed >95 %, by using Agilent Infinity-2 1260 series instrument (UV–Vis diode-array detector) at 258–268 nm. The synthesis of the final compounds ***4' (4'a-4'z)*** and ***(4'aa-4'aj)*** was followed by general procedure given above.

*5.4.1. (E)-methyl 2-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)acetate (**4'a**):* Yellow solid, Yield 52 %; mp: 144–146 °C. ^{1}H NMR (500 MHz, DMSO–d_6+ 2-3 drops of $CDCl_3$) δ 8.16 (dd, J = 8.0, 1.1 Hz, 1Hg), 7.99 (d, J = 15.3 Hz, 1Hc), 7.97–7.94 (m, 2Hdf), 7.85–7.80 (m, 1He), 7.81–7.77 (m, 2Hj), 7.75–7.72 (m, 1Hh), 7.53–7.48 (m, 2Hi), 5.20 (s, 2Hb), 3.76 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, DMSO–d_6 + $CDCl_3$) δ 168.8, 161.6, 151.8, 147.4, 139.9, 139.4, 134.9,

132.7, 129.1, 127.6, 127.1, 126.7, 122.9, 120.2, 118.8, 112.3, 52.7, 45.1 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{20}H_{16}N_3O_3$ 346.1191; found 346.1194.

*5.4.2. (E)-4-(2-(3-(4-(tert-butyl)phenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'b**):* Yellow solid, Yield 60 %; mp: 211-213 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.15 (d, J = 7.9 Hz, 1H), 7.92–7.85 (m, 2H), 7.84–7.76 (m, 3H), 7.64–7.60 (m, 2H), 7.57 (t, J = 7.5 Hz, 1H), 7.54–7.50 (m, 2H), 7.41–7.35 (m, 2H), 6.46 (d, J = 15.6 Hz, 1H), 1.38 (s, 9H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.7, 152.2, 151.6, 147.7, 139.9, 137.0, 135.3, 134.6, 133.4, 128.9, 128.5, 127.8, 127.5, 127.0, 126.9, 124.6, 124.2, 121.2, 119.0, 112.0, 35.1, 31.6 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{27}H_{24}N_3O$ 406.1919; found 406.1922.

*5.4.3. (E)-4-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)-2-hydroxybenzoic acid (**4'c**):* Yellow solid, Yield 48 %; mp: 138-140 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.93 (s, 1Hb), 8.18–8.13 (m, 1Hf), 7.95–7.87 (m, 2Hkd), 7.86–7.81 (m, 2Hm), 7.79 (d, J = 8.1 Hz, 1Hh), 7.61–7.53 (m, 2Hl), 7.44–7.36 (m, 1Hg), 7.01–6.96 (m, 1Hi), 6.89–6.83 (d, J = 8.4 Hz, 2Hea), 6.55 (d, J = 15.6 Hz, 1Hj) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.5, 158.8, 151.3, 147.7, 139.9, 138.2, 137.1, 135.3, 133.4, 130.9, 129.1, 128.6, 127.8, 127.4, 127.0, 124.0, 121.3, 119.8, 119.1, 116.9, 116.4, 112.0 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{16}N_3O_4$ 410.1141; found 410.1139; HPLC purity 96.3%.

*5.4.4. (E)-2-(2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)-5-hydroxybenzoic acid (**4'd**):* Yellow solid, Yield 52 %; mp: 258–260 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 12.93 (s, 1H), 10.28 (s, 1H), 8.13–8.10 (m, 1H), 7.91–7.86 (m, 2H), 7.84–7.77 (m, 3H), 7.64–7.58 (m, 2H), 7.56–7.50 (m, 2H), 7.35–7.30 (m, 1H), 7.18–7.13 (m, 1H), 6.54 (d, J = 15.6 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 166.0, 161.9, 158.4, 152.1, 147.8, 139.9, 137.2, 135.2, 133.3, 132.2, 128.7, 128.5, 128.0, 127.7, 127.2, 126.9, 124.0, 121.2, 120.6, 119.1, 118.4, 112.0 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{16}N_3O_4$ 410.1141; found 410.1145.

*5.4.5. (E)-4-(2-(5-fluoro-3-(3-hydroxyphenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'e**):* Yellow solid, Yield 49 %; mp: 188-190 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.93 (s, 1Hb), 7.91 (d, J = 15.6 Hz, 1Hj), 7.88–7.79 (m, 3Hgl), 7.61–7.55 (m, 3Hfk), 7.45–7.35 (m, 1Hh), 7.33–7.37 (m, 1Hd), 7.00–6.93 (m, 1He), 6.91–6.81 (m, 2Hac), 6.49 (d, J = 15.6 Hz, 1Hi) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 160.6 (d, J = 263.4 Hz), 158.3, 158.0 (d, J = 4.0 Hz), 151.8, 149.3, 139.2, 137.2 (d, J = 17.0 Hz), 135.3 (d, J = 10.7 Hz), 132.9, 130.4, 128.1, 123.4 (d, J = 3.6 Hz), 123.2, 119.3, 118.6, 116.4, 115.9, 113.2 (d, J = 20.5 Hz),

111.7, 110.3, 110.3 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{23}H_{15}FN_3O_2$ 384.1148; found 384.1153; HPLC purity 98.3 %.

*5.4.6. (E)-4-(2-(5-fluoro-4-oxo-3-(3-(prop-2-yn-1-yloxy)phenyl)-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'f**):* Yellow solid, Yield 51 %; mp: 220–222 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 7.91 (d, J = 15.6 Hz, 1H), 7.89–7.79 (m, 3H), 7.62–7.59 (m, 1H), 7.58–7.51 (m, 3H), 7.34–7.28 (m, 1H), 7.23–7.16 (m, 2H), 7.11–7.07 (m, 1H), 6.46 (d, J = 15.6 Hz, 1H), 4.89–4.84 (m, 2H), 3.55 (m, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 160.7 (d, J = 263.4 Hz), 158.1, 151.9, 149.4, 139.3, 137.6, 137.5, 135.5 (d, J = 11.0 Hz), 133.3, 133.0, 130.6, 130.0, 128.3, 123.5 (d, J = 3.7 Hz), 123.2, 122.0, 118.6, 116.0 (d, J = 2.9 Hz), 113.3 (d, J = 20.5 Hz), 111.8, 110.4 (d, J = 5.3 Hz), 78.9, 78.5, 55.9 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{26}H_{17}FN_3O_2$ 422.1305; found 422.1309; HPLC purity 95.9 %.

*5.4.7. (E)-4-(2-(3-(3-(allyloxy)phenyl)-5-fluoro-4-oxo-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'g**):* Yellow solid, Yield 56 %; mp: 200–202 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 7.91 (d, J = 15.6 Hz, 1Hl), 7.89–7.80 (m, 3Hnh), 7.63–7.54 (m, 3Hmj), 7.51 (t, J = 8.1 Hz, 1Hi), 7.34–7.27 (m, 1Hf), 7.19–7.12 (m, 2Hga), 7.06–7.01 (m, 1He), 6.47 (d, J = 15.6 Hz, 1Hk), 6.09–5.96 (m, 1Hc), 5.42 –5.35 (m, 1Hd), 5.26–5.19 (m, 1Hd'), 4.65–4.58 (m, 2Hb) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 162.2, 160.1, 159.5, 158.5 (d, J = 4.2 Hz), 152.3, 149.8, 139.7, 137.9 (d, J = 23.0 Hz), 135.9 (d, J = 10.5 Hz), 133.8, 133.4, 130.9, 128.7, 123.9 (d, J = 3.7 Hz), 123.7, 121.7, 119.0, 118.2, 116.4, 116.0, 113.7 (d, J = 20.7 Hz), 112.2, 110.8, 110.8, 68.9 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{26}H_{19}FN_3O_2$ 424.1461; found 424.1466.

*5.4.8. (E)-4-(2-(3-(3-ethynylphenyl)-5-fluoro-4-oxo-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'h**):* Yellow solid, Yield 69 %; mp: 171-173 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 7.91 (d, J = 15.6 Hz, 1H), 7.89–7.84 (m, 1H), 7.83–7.79 (m, 2H), 7.71–7.67 (m, 2H), 7.64 (t, J = 7.8 Hz, 1H), 7.62–7.57 (m, 3H), 7.57–7.53 (m, 1H), 7.33–7.27 (m, 1H), 6.43 (d, J = 15.6 Hz, 1H), 4.36 (s, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 162.1, 160.0, 158.6, 152.1, 149.7, 139.6, 138.0, 137.2, 136.0, 135.9, 133.3, 133.2, 132.8, 130.6, 130.3, 128.7, 123.9, 123.6, 123.5, 119.0, 113.8, 113.7, 112.2, 110.7, 110.7, 82.9, 82.7 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{25}H_{15}FN_3O$ 392.1199; found 392.1202; HPLC purity 98.8 %.

*5.4.9. (E)-3-(2-(4-cyanostyryl)-5-fluoro-4-oxoquinazolin-3(4H)-yl)benzonitrile (**4'i**):* Yellow solid, Yield 70 %; mp: 180-182 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.12-8.06 (m, 2H), 7.93 (d, J = 15.5 Hz, 1H), 7.90–7.86 (m, 2H), 7.85 (d, J = 7.8 Hz, 1H), 7.82–7.77 (m, 2H), 7.67–7.63 (m, 2H), 7.62 (d, J = 8.2 Hz, 1H), 7.35–7.27 (m, 1H), 6.47 (d, J = 15.5 Hz, 1H) ppm; ^{13}C NMR

(125 MHz, DMSO–d_6) δ 162.1, 160.0, 158.6, 152.0, 149.6, 139.5, 138.4, 137.7, 136.2, 136.1, 134.8, 133.8, 133.6, 133.2, 131.5, 129.0, 123.9, 123.9, 123.4, 119.0, 118.5, 114.0, 113.8, 113.2, 112.2, 110.6, 110.5 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{14}FN_4O$ 393.1151; found 393.1156.

*5.4.10. (E)-4-(2-(3-(naphthalen-1-yl)-4-oxo-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'j**):* Yellow solid, Yield 74 %; mp: 199-201 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (m, 1H), 8.08 (d, J = 8.3 Hz, 1H), 8.01 (d, J = 8.2 Hz, 1H), 7.97 (d, J = 15.5 Hz, 1H), 7.90–7.84 (m, 2H), 7.69–7.63 (m, 1H), 7.60–7.52 (m, 2H), 7.51–7.45 (m, 5H), 7.26–7.18 (d, J = 8.3 Hz, 2H), 6.28 (d, J = 15.5 Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.1, 151.5, 147.7, 139.4, 137.9, 136.0, 134.5, 133.2, 132.4, 130.4, 130.1, 128.8, 128.1, 128.0, 127.6, 127.4, 127.3, 127.2, 127.0, 125.7, 122.9, 121.9, 121.1, 118.4, 112.5 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{27}H_{18}N_3O$ 400.1450; found 400.1453.

*5.4.11. (E)-4-(2-(3-(4-fluorobenzyl)-4-oxo-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'k**):* Yellow solid, Yield 71 %; mp: 238–240 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.22–8.18 (m, 1Hd), 7.95–7.85 (m, 6Hkefgi), 7.77–7.72 (m, 1Hh), 7.60–7.52 (m, 2Hj), 7.38–7.31(m, 2Ha), 7.18–7.11(m, 2Hb), 5.65 (s, 2Hc) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 162.8, 161.9, 160.8, 152.0, 147.5, 140.0, 138.7, 135.2, 133.8, 133.2, 129.3 (d, J = 8.3 Hz), 129.1, 127.7, 127.4, 127.1, 123.9, 120.6, 119.1, 116.0 (d, J = 21.5 Hz), 112.1, 45.1 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{17}FN_3O$ 382.1355; found 382.1359.

*5.4.12. (E)-4-(2-(3-(3,4-dichlorobenzyl)-4-oxo-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'l**):* Yellow solid, Yield 66 %; mp: 231–233 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.21–8.18 (m, 1H), 7.98–7.85 (m, 6H), 7.78–7.74 (m, 1H), 7.65 (d, J = 2.0 Hz, 1H), 7.60–7.50 (m, 3H), 7.22 (dd, J = 8.4, 2.0 Hz, 1H), 5.64 (s, 2H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.9, 151.9, 147.5, 140.0, 139.0, 138.8, 135.3, 133.2, 131.7, 131.4, 130.5, 129.5, 129.2, 127.8, 127.5, 127.4, 127.1, 123.7, 120.6, 119.1, 112.1, 45.0 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{16}Cl_2N_3O$ 432.0670; found 432.0694.

*5.4.13. (E)-4-(2-(4-oxo-3-((4-(trifluoromethyl)phenyl)amino)-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'm**):* Yellow solid, Yield 63 %; mp: 200-202 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.77 (s, 1H), 8.13 (d, J = 7.8 Hz, 1H), 8.08 (d, J = 15.8 Hz, 1H), 7.92 (t, J = 7.4 Hz, 1H), 7.87–7.78 (m, 5H), 7.61–7.48 (m, 4H), 6.90 (d, J = 8.3 Hz, 2H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 160.4, 153.8, 151.0, 147.1, 139.8, 139.1, 135.6, 133.3, 129.0, 128.1, 127.5,

127.1, 126.9, 126.2, 124.0, 121.9, 121.7, 121.3, 121.0, 119.1, 113.3, 112.2 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{16}F_3N_4O$ 433.1276; found 433.1283.

*5.4.14. (E)-4-(2-(3-(2-(1H-indol-3-yl)ethyl)-4-oxo-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'n**):* Yellow solid, Yield 55 %; mp: 229–231 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 10.83 (s, 1H), 8.20 (d, *J* = 7.8 Hz, 1H), 7.83 (t, *J* = 7.4 Hz, 1H), 7.75 (d, *J* = 7.4 Hz, 2H), 7.71–7.59 (m, 3H), 7.54 (t, *J* = 7.4 Hz, 1H), 7.40–7.34 (m, 2H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.12 (t, *J* = 7.4 Hz, 1H), 7.07 (m, 1H), 7.01 (t, *J* = 7.3 Hz, 1H), 6.96–6.89 (m, 1H), 4.49 (t, *J* = 5.8 Hz, 2H), 3.15 (t, *J* = 6.3 Hz, 2H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 160.5, 151.3, 146.4, 139.0, 136.7, 135.7, 133.8, 131.9, 127.7, 126.5, 126.5, 126.0, 125.8, 123.1, 122.7, 120.7, 119.6, 118.1, 118.1, 117.4, 111.0, 110.5, 109.7, 43.4, 23.6 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{27}H_{21}N_4O$ 417.1715; found 417.1719.

*5.4.15. (E)-4-(2-(4-oxo-3-(pyridin-3-yl)-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'o**):* Yellow solid, Yield 67 %; mp: 110–112 °C; ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.79–8.75 (m, 1Hb), 8.72–8.69 (m, 1He), 8.19–8.12 (m, 1Hh), 8.03–7.98 (m, 1Hf), 7.95–7.87 (m, 2Hgj), 7.85–7.78 (m, 3Hla), 7.68 (dd, *J* = 8.0, 4.8 Hz, 1Hd), 7.64 (d, *J* = 8.2 Hz, 2Hk), 7.59 (t, *J* = 7.5 Hz, 1Hc), 6.52 (d, *J* = 15.5 Hz, 1Hi) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.8, 151.3, 150.7, 150.1, 147.7, 139.7, 137.7, 137.5, 135.6, 134.0, 133.3, 128.8, 127.9, 127.7, 127.0, 124.9, 123.9, 121.0, 119.1, 112.1 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{22}H_{15}N_4O$ 351.1246; found 351.1249.

*5.4.16. (E)-methyl 2-(2-(4-cyanostyryl)-4-oxo-6-(prop-2-yn-1-yloxy)quinazolin-3(4H)-yl)acetate (**4'p**):* Yellow solid, Yield 61 %; mp: 210–212 °C; ^{1}H NMR (500 MHz, DMSO–d_6) ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.06–8.01 (m, 2H), 7.96–7.89 (m, 3H), 7.73 (d, *J* = 9.0 Hz, 1H), 7.62 (d, *J* = 2.9 Hz, 1H), 7.60–7.55 (m, 1H), 7.54–7.50 (m, 1H), 5.19 (s, 2H), 4.97 (d, *J* = 2.1 Hz, 2H), 3.72 (s, 3H), 3.64 (t, *J* = 2.2 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 169.2, 161.3, 156.3, 150.2, 142.4, 140.2, 138.6, 133.1, 129.6, 129.3, 125.6, 123.2, 120.9, 111.9, 108.3, 79.3, 79.2, 56.5, 52.9, 45.5, 31.1 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{23}H_{18}N_3O_4$ 400.1297; found 400.1300.

*5.4.17. (E)-methyl-2-(6-((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-(4-cyanostyryl)-4-oxoquinazolin-3(4H)-yl)acetate (**4'q**):* Yellow solid, Yield 51 %; mp: 198–200 °C; ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.03 (s, 1H), 8.27–8.22 (m, 1H), 8.06–8.02 (m, 2H), 8.01–7.97 (m, 1H), 7.96–7.90 (m, 3H), 7.76–7.67 (m, 3H), 7.61–7.55 (m, 2H), 5.41 (s, 2H), 5.20 (s, 2H), 3.73 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 168.8, 160.9, 158.0, 156.8, 156.0,

143.8, 141.9, 139.8, 138.1, 133.6 (d, *J* = 3.4 Hz), 132.7, 129.3, 128.9, 125.0, 123.5, 122.9, 122.6, 121.1 (d, *J* = 8.0 Hz), 120.9, 120.8, 120.6, 118.8, 118.3, 118.2, 111.5, 107.9, 61.7, 52.5, 45.1 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{29}H_{21}ClFN_6O_4$ 571.1297; found 571.1300.

*5.4.18. (E)-6-(4-methoxyphenyl)-3-phenyl-2-styrylquinazolin-4(3H)-one (**4'r**):* Yellow solid, Yield 55 %; mp: 220–222 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.29 (d, *J* = 2.0 Hz, 1H), 8.19–8.15 (m, 1H), 7.90 (d, *J* = 15.5 Hz, 1H), 7.84 (d, *J* = 8.5 Hz, 1H), 7.77–7.71 (m, 2H), 7.67–7.57 (m, 3H), 7.52–7.46 (m, 2H), 7.40–7.30 (m, 5H), 7.12–7.04 (m, 2H), 6.33 (d, *J* = 15.6 Hz, 1H), 3.82 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.8, 159.8, 151.5, 146.7, 139.1, 138.4, 137.5, 135.3, 133.3, 131.6, 130.3, 130.2, 129.7, 129.5, 129.4, 128.4, 128.4, 127.9, 123.3, 121.4, 120.5, 115.1, 55.7 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{29}H_{23}N_2O_2$ 431.1759; found 431.1765.

*5.4.19. (E)-4-(2-(4-oxo-3-phenyl-7-(prop-2-yn-1-ylamino)-3,4-dihydroquinazolin-2yl)vinyl)benzonitrile (**4's**):* Yellow solid, Yield 53 %; mp: 200–202 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 7.91–7.73 (m, 4H), 7.66–7.48 (m, 5H), 7.44–7.38 (d, *J* = 6.6 Hz, 2H), 7.10 (t, 1H), 6.90–6.85 (m, 1H), 6.79 (s, 1H), 6.43 (d, *J* = 15.5 Hz, 1H), 4.09–4.01 (m, 2H), 3.19 (s, 1H). ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.1, 153.6, 151.2, 149.7, 140.0, 137.6, 136.6, 133.3, 130.0, 129.6, 129.5, 128.5, 127.8, 124.3, 119.1, 115.8, 111.9, 110.8, 105.7, 81.7, 74.0, 32.2 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{26}H_{19}N_4O$ 403.1559; found 403.1567.

*5.4.20. (E)-3-(3-chlorophenyl)-7-phenyl-2-styrylquinazolin-4(3H)-one (**4't**):* Yellow solid, Yield 60 %; mp: 219–221°C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 8.20 (d, *J* = 8.3 Hz, 1He), 8.04 (d, *J* = 1.6 Hz, 1Ha), 7.92–7.85 (m, 4Hjnl), 7.74–7.62 (m, 3Hfm), 7.60–7.53 (m, 4Hgh), 7.52–7.46 (m, 2Hic), 7.42–7.36 (m, 2Hdb), 6.40 (d, *J* = 15.5 Hz, 1Hk) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.5, 151.8, 148.3, 146.8, 139.1, 138.7, 138.4, 134.5, 134.2, 132.5, 132.2, 131.8, 131.7, 130.0, 129.9, 129.7, 129.2, 128.5 127.7, 127.7, 125.9, 125.1, 123.6, 121.1, 119.9 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{29}H_{19}ClN_3O$ 460.1216; found 460.1219.

*5.4.21. (E)-4-(2-(4-oxo-3-phenyl-7-(4-phenyl-1H-1,2,3-triazol-1-yl)-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'u**):* Yellow solid, Yield 58 %; mp: 225–227°C ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.64 (s, 1H), 8.39–8.32 (m, 2H), 8.22–8.17 (m, 1H), 7.99 (d, *J* = 7.4 Hz, 2H), 7.93 (d, *J* = 15.6 Hz, 1H), 7.82 (d, *J* = 7.9 Hz, 2H), 7.69–7.58 (m, 3H), 7.58–7.46 (m, 6H), 7.41 (t, *J* = 7.1 Hz, 1H), 6.48 (d, *J* = 15.6 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.0, 152.9, 148.9, 148.2, 141.4, 139.5, 137.9, 137.0, 133.4, 130.4, 130.2, 130.0, 129.8, 129.5, 129.4, 128.9,

128.6, 125.9, 123.7, 120.7, 120.3, 119.0, 118.4, 117.1, 112.2 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{31}H_{21}N_6O$ 493.1777; found 493.1780.

*5.4.22. (E)-4-(2-(7-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'v**):* Yellow solid, Yield 53 %; mp: 229–231°C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.54 (s, 1H), 8.39–8.34 (m, 2H), 8.20 (dd, J = 8.7, 2.1 Hz, 1H), 7.98–7.88 (m, 3H), 7.86–7.81 (m, 2H), 7.68–7.58 (m, 3H), 7.58–7.50 (m, 4H), 7.14–7.07 (m, 2H), 6.50 (d, J = 15.6 Hz, 1H), 3.83 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.0, 159.9, 152.9, 151.2, 148.9, 148.2, 141.5, 139.6, 137.9, 137.0, 133.4, 130.2, 130.0, 129.4, 128.7, 127.3, 123.8, 122.9, 120.7, 119.3, 119.0, 118.4, 117.0, 115.0, 112.2, 55.7 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{32}H_{23}N_6O_2$ 523.1882; found 523.1887.

*5.4.23. (E)-4-(2-(3-benzyl-4-oxo-6-(prop-2-yn-1-yloxy)-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'w**):* Yellow solid, Yield 62 %; mp: 171–173°C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 7.92–7.81 (m, 5H), 7.75–7.66 (m, 2H), 7.56–7.47 (m, 2H), 7.37–7.20 (m, 5H), 5.67 (s, 2H), 4.99 (d, J = 1.9 Hz, 2H), 3.67 (t, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.6, 156.3, 150.2, 142.4, 140.2, 137.8, 137.7, 133.2, 129.6, 129.2, 128.9, 127.8, 127.0, 125.34, 123.9, 121.3, 119.2, 111.9, 108.5, 79.3, 79.3, 56.5, 45.8 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{27}H_{20}N_3O_2$ 418.1555; found 418.1559.

*5.4.24. (E)-4-(2-(3-benzyl-6-((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-oxo-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'x**):* Yellow solid, Yield 64 %; mp: 229–231 °C; ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.04 (s, 1H), 8.28–8.24 (m, 1H), 8.03–7.97 (m, 1H), 7.91–7.84 (m, 4H), 7.84–7.78 (m, 2H), 7.76–7.66 (m, 2H), 7.60–7.50 (m, 2H), 7.35–7.30 (m, 2H), 7.30–7.20 (m, 3H), 5.68 (s, 2H), 5.43 (s, 2H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.6, 157.2, 150.1, 144.2, 142.3, 140.2, 137.7 (d, J = 5.7 Hz), 134.0, 133.2, 129.7, 129.2, 129.0, 127.8, 127.0, 125.2, 125.0, 123.9, 123.0, 121.6, 121.5 (d, J = 8.2 Hz), 121.4, 121.2, 118.6 (d, J = 22.6 Hz), 111.9, 108.5, 62.1, 45.8 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{33}H_{23}ClFN_6O_2$ 589.1555; found 589.1563.

*5.4.25. (E)-4-(2-(3-benzyl-6-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-oxo-3,4-dihydroquinazolin-2-yl)vinyl)benzonitrile (**4'y**):* Yellow solid, Yield 68 %; mp: 200–202 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.08 (s, 1H), 8.08 (t, J = 2.0 Hz, 1H), 7.98–7.93 (m, 1H), 7.88–7.82 (m, 5H), 7.80 (d, J = 3.0 Hz, 1H), 7.73 (d, J = 8.9 Hz, 1H), 7.65 (t, J = 8.1 Hz, 1H), 7.61–7.56 (m, 2H), 7.53 (d, J = 15.4 Hz, 1H), 7.35–7.26 (m, 4H), 7.26–7.21 (m, 1H), 5.68 (s, 2H), 5.42 (s, 2H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.6, 157.2, 150.1, 144.2, 142.3, 140.2,

138.0, 137.7, 137.7, 134.7, 133.2, 132.1, 129.7, 129.2, 129.1, 128.9, 127.8, 127.0, 125.2, 123.9, 123.7, 121.4, 120.5, 119.2, 119.2, 111.9, 108.5, 62.1, 45.8 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{33}H_{24}ClN_6O_2$ 571.1649; found 571.1650.

*5.4.26. (E)-4-(2-(3-(3-hydroxyphenyl)-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)vinyl)benzonitrile (**4'z**):* Yellow solid, Yield 73 %; mp: 229–231 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.99 (s, 1Hb), 9.00 (dd, *J* = 4.6, 2.0 Hz, 1Hh), 8.49 (dd, *J* = 7.8, 2.0 Hz, 1Hf), 8.02 (d, *J* = 15.6 Hz, 1Hj), 7.83 (d, *J* = 8.4 Hz, 2Hl), 7.58 (d, *J* = 8.3 Hz, 2Hk), 7.55 (dd, *J* = 7.8, 4.6 Hz, 1Hj), 7.42 (t, *J* = 8.3 Hz, 1Hd), 7.06–6.94 (m, 1He), 6.95–6.84 (m, 2Hac), 6.55 (d, *J* = 15.6 Hz, 1Hi) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 162.2, 158.8, 157.6, 156.7, 154.2, 139.6, 138.6, 137.8, 136.5, 133.4, 131.0, 128.8, 123.5, 122.8, 119.7, 119.0, 117.1, 116.7, 116.3, 112.3 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{22}H_{15}N_4O_2$ 367.1195; found 367.1198.

*5.4.27. (E)-4-(2-(3-(3-ethynylphenyl)-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)vinyl)benzonitrile (**4'aa**):* Yellow solid, Yield 70 %; mp: 229–231 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.03 (d, *J* = 2.6 Hz, 1H), 8.52 (d, *J* = 6.8 Hz, 1H), 8.04 (d, *J* = 15.5 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.71 (s, 2H), 7.65 (d, *J* = 7.1 Hz, 3H), 7.61–7.53 (m, 2H), 6.52 (d, *J* = 15.5 Hz, 1H), 4.37 (s, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.3, 156.6, 155.8, 153.1, 138.4, 137.8, 136.1, 135.4, 132.3, 132.2, 131.5, 129.6, 129.1, 127.9, 122.5, 122.4, 121.9, 118.0, 115.6, 111.3, 81.8, 81.8 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{15}N_4O$ 375.1246; found 375.1253.

*5.4.28. (E)-3-(2-(4-cyanostyryl)-4-oxopyrido[2,3-d]pyrimidin-3(4H)-yl)benzonitrile (**4'ab**):* Yellow solid, Yield 66 %; mp: 229–231 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.03 (dd, *J* = 4.5, 2.0 Hz, 1Hg), 8.48 (dd, *J* = 7.8, 2.0 Hz, 1He), 8.15 (d, *J* = 1.7 Hz, 1Hf), 8.10 (dd, *J* = 10.9, 3.2 Hz, 1Hb), 8.03 (d, *J* = 15.4 Hz, 1Hi), 7.96–7.92 (m, 1Hd), 7.86 (t, *J* = 7.9 Hz, 1Ha), 7.82 (d, *J* = 8.4 Hz, 2Hj), 7.68 (d, *J* = 8.4 Hz, 2Hk), 7.56 (dd, *J* = 7.8, 4.6 Hz, 1Hc), 6.55 (d, *J* = 15.5 Hz, 1Hh) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 162.4, 157.6, 157.0, 154.0, 139.4, 139.3, 137.7, 136.5, 134.7, 133.9, 133.4, 133.3, 131.6, 129.2, 123.3, 123.0, 119.0, 118.5, 116.5, 113.2, 112.4 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{23}H_{14}N_5O$ 376.1198; found 376.1200.

*5.4.29. (E)-4-(2-(3-(4-cyanophenyl)-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)vinyl)benzonitrile (**4'ac**):* Yellow solid, Yield 63 %; mp: 229–231 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.04 (dd, *J* = 4.5, 1.9 Hz, 1H), 8.53 (dd, *J* = 7.8, 1.7 Hz, 1H), 8.13 (d, *J* = 8.3 Hz, 2H), 8.05 (d, *J* = 15.5 Hz, 1H), 7.83 (d, *J* = 8.2 Hz, 2H), 7.80–7.74 (m, 2H), 7.70 (d, *J* = 8.2 Hz, 2H), 7.58 (dd, *J* = 7.8, 4.6 Hz, 1H), 6.51 (d, *J* = 15.5 Hz, 1H) ppm; ^{13}C NMR (125 MHz,

DMSO–d_6) δ 162.2, 157.6, 156.9, 153.7, 141.2, 139.4, 136.5, 134.4, 133.3, 130.8, 130.7, 129.2 123.2, 123.0, 119.0, 118.8, 116.5, 112.9, 112.4 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{23}H_{14}N_5O$ 376.1198; found 376.1206.

*5.4.30. (E)-4-(2-(3-(4-mercaptophenyl)-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)vinyl)benzonitrile (**4'ad**):* Yellow solid, Yield 65 %; mp: 210–212°C. ^{1}H NMR (500 MHz, DMSO–d_6) δ ^{1}H NMR (500 MHz, DMSO) δ 9.02 (d, *J* = 2.2 Hz, 1H), 8.52 (d, *J* = 7.4 Hz, 1H), 7.96 (d, *J* = 15.5 Hz, 1H), 7.83–7.78 (m, 4H), 7.60–7.54 (m, 6H), 6.49 (d, *J* = 15.5 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 162.4, 157.6, 156.9, 139.4, 138.9, 137.3, 136.5, 136.0, 133.3, 130.5, 128.9, 128.5, 128.3, 123.3, 122.9, 118.9, 116.6, 112.3 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{22}H_{15}N_4OS$ 383.0966; found 383.0970; HPLC purity 98.7 %.

*5.4.31. (E)-4-(2-(3-(4-(tert-butyl)phenyl)-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)vinyl)benzonitrile (**4'ae**):* Yellow solid, Yield 78 %; mp: 229–231 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.02 (m, 1Hf), 8.52 (m, 1Hd), 8.00 (d, *J* = 15.6 Hz, 1Hh), 7.84 (d, *J* = 8.4 Hz, 2Hj), 7.70–7.59 (m, 2Hi), 7.57 (m, 3Hae), 7.45–7.34 (m, 2Hb), 6.49 (d, *J* = 15.6 Hz, 1Hg), 1.38 (s, 9Hc) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 162.4, 157.7, 156.8, 154.5, 152.5, 139.7, 138.5, 136.5, 134.2, 133.4, 128.8, 128.7, 127.0, 123.8, 122.9, 119.0, 116.6, 112.3, 35.1, 31.6 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{26}H_{23}N_4O$ 407.1872; found 407.1880.

*5.4.32. (E)-4-(2-(3-(2,3-dimethylphenyl)-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)vinyl)benzonitrile (**4'af**):* Yellow solid, Yield 69 %; mp: 229–231 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.05 (dd, *J* = 4.6, 2.0 Hz, 1Hh), 8.54 (dd, *J* = 7.9, 2.0 Hz, 1Hf), 8.07 (d, *J* = 15.5 Hz, 1Hj), 7.84 (d, *J* = 8.4 Hz, 2Hl), 7.62–7.57 (m, 3Hkg), 7.41 (d, *J* = 7.4 Hz, 1He), 7.32 (t, *J* = 7.7 Hz, 1Hd), 7.25 (d, *J* = 7.5 Hz, 1Hc), 6.45 (d, *J* = 15.5 Hz, 1Hi), 2.37 (s, 3Hb), 1.95 (s, 3Ha) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.9, 157.8, 157.0, 154.4, 139.5, 139.3, 138.9, 136.6, 135.7, 134.8, 133.4, 131.5, 128.9, 127.2, 126.8, 123.0, 122.8, 119.0, 116.5, 112.4, 20.5, 14.1 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{19}N_4O$ 379.1559; found 379.1561.

*5.4.33. (E)-4-(2-(3-(2,5-dimethylphenyl)-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)vinyl)benzonitrile (**4'ag**):* Yellow solid, Yield 71 %; mp: 229–231 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.05 (dd, *J* = 4.6, 2.0 Hz, 1H), 8.54 (dd, *J* = 7.9, 2.0 Hz, 1H), 8.09 (d, *J* = 15.5 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 7.63–7.57 (m, 1H), 8.51–6.95 (m, 3H), 7.40 (d, *J* = 7.9 Hz, 1H), 7.33 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.25 (s, 1H), 6.47 (d, *J* = 15.5 Hz, 1H), 2.35 (s, 3H), 2.01 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 161.8, 157.7, 157.0, 154.2, 139.5, 137.5, 136.6, 135.6,

133.4, 132.8, 131.5, 131.0, 129.5, 128.9, 123.0, 122.6, 119.0, 116.5, 112.4, 20.9, 17.0 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{19}N_4O$ 379.1559; found 379.1563.

*5.4.34. (E)-4-(2-(4-oxo-3-(3,4,5-trimethoxyphenyl)-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)vinyl)benzonitrile (**4'ah**):* Yellow solid, Yield 63 %; mp: 229–231 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.02 (s, 1H), 8.52 (d, J = 3.9 Hz, 1H), 8.06 (d, J = 15.3 Hz, 1H), 7.86 (d, J = 6.3 Hz, 2H), 7.67 (d, J = 6.0 Hz, 2H), 7.57 (s, 1H), 6.92 (s, 2H), 6.64 (d, J = 15.2 Hz, 1H), 3.78 (s, 3H), 3.76 (s, 6H) ppm; ^{13}C NMR (125 MHz, d-TFA) δ 156.8, 153.6, 150.1, 149.7, 149.6, 140.7, 140.5, 135.0, 132.5, 131.3, 124.6, 121.4, 120.7, 118.0, 117.2, 115.8, 114.9, 107.6, 63.0, 57.8 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{25}H_{21}N_4O_4$ 441.1563; found 441.1565.

*5.4.35. (E)-4-(2-(3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)vinyl)benzonitrile (**4'ai**):* Yellow solid, Yield 77 %; mp: 229–231 °C. ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.00 (dd, J = 4.5, 1.9 Hz, 1Hg), 8.50 (dd, J = 7.8, 1.8 Hz, 1He), 8.02 (d, J = 15.5 Hz, 1Hi), 7.85 (d, J = 8.3 Hz, 2Hk), 7.66 (d, J = 8.3 Hz, 2Hj), 7.55 (dd, J = 7.8, 4.6 Hz, 1Hc), 7.08 (dd, J = 12.9, 5.4 Hz, 2Hda), 6.94 (dd, J = 8.5, 2.4 Hz, 1Hf), 6.62 (d, J = 15.6 Hz, 1Hh), 4.34 (d, J = 7.4 Hz, 4Hbb') ppm; ^{13}C NMR (125 MHz, DMSO–d_6) δ 162.5, 157.6, 156.7, 154.7, 144.6, 144.3, 139.7, 138.6, 136.5, 133.4, 129.6, 128.9, 123.6, 122.8, 121.9, 119.1, 118.2, 116.6, 112.3, 64.6, 64.5 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{24}H_{17}N_4O_3$ 409.1300; found 409.1309.

*5.4.36. (E)-4-(2-(3-(4-fluorobenzyl)-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)vinyl)benzonitrile (**4'aj**):* Yellow solid, Yield 61 %; mp: 229–231°C ^{1}H NMR (500 MHz, DMSO–d_6) δ 9.01 (s, 1H), 8.57 (d, J = 2.5 Hz, 1H), 8.12–7.98 (m, 2H), 7.93 (d, J = 13.6 Hz, 3H), 7.59 (d, J = 15.1 Hz, 2H), 7.37 (s, 2H), 7.15 (s, 2H), 5.65 (s, 2H) ppm; ^{13}C NMR (125 MHz, d-TFA) δ 166.0 (d, J = 248.1 Hz), 165.0, 164.8, 163.8, 163.7, 154.0, 150.8 (d, J = 26.4 Hz), 150.3, 141.6, 135.8, 133.4, 131.9, 130.8 (d, J = 8.5 Hz), 125.4, 122.1, 121.1, 119.1 (d, J = 22.5 Hz), 115.8, 50.6 ppm; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{23}H_{16}FN_4O$ 383.1308; found 383.1310.

6. Bacterial strains and media

The ESKAP 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% (v/v) ADC (Bovine Serum Albumin, Dextrose, NaCl), 0.2% (v/v) glycerol and 0.05% (v/v) Tween-80 (ADC-Tween-80).

6.1. Antibiotic susceptibility testing against ESKAP pathogen panel

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 [32,33]. 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 64-0.03 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.

6.2. Antibiotic Susceptibility Testing against pathogenic mycobacteria

Antimycobacterial susceptibility testing was carried out on newly synthesized compounds (given in Supplementary data, **1**) by using broth microdilution assay [34]. 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 [35]. 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 microtitre 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.

6.3. Cell Cytotoxicity Assay

The active newly synthesized compounds were screened for their cell toxicity against Vero cells using MTT assay [36]. ~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 was over, 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.

6.4. Time Kill Study

The bactericidal activity was assessed by the time-kill method [37]. *S. aureus* ATCC 29213 cells were diluted up to ~10^6 cfu/mL and treated with compound for concentrations corresponding to 1X and 10X of MIC of **4'c**, **4'e** and vancomycin in MHB in triplicate and incubated at 37 °C. 100 μL samples were collected after time intervals of 0h, 1h, 6h and 24h 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.

6.5. Synergy Screen

Checkerboard method was used to determine synergy between **4'c**, **4'e** and the antibiotics that included linezolid, meropenem, ceftriaxone and vancomycin [34]. 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 ~10^6 CFU/mL was prepared and inoculated with 100 µL of a bacterial inoculum and plates were incubated at 37 °C for 24 hours under aerobic conditions. 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 [38].

6.6. Determination of activity of 4'c against *S. aureus* biofilm

The determination of **4'c** anti-biofilm activity was performed as described earlier [39]. Briefly, *S. aureus* ATCC 29213 were grown overnight in 1% TSB with shaking (180 RPM) at 37^0C. The overnight culture was diluted in fresh TSB broth (1:100) and 0.2mL of freshly diluted culture was transferred into 96 well polystyrene flat bottom plate, covered with adhesive foil lid for maintaining low oxygen and incubated in static condition for 48 h at 37^0C. After incubation, media was decanted and plate was rinsed gently 3 times with the 1X PBS (pH 7.4) to remove the planktonic bacteria. Plates were refilled with TSB with different drug concentration and incubated for 24 h at 37^0C. After drug treatment, the media was decanted, washed 3 times with 1X PBS (pH 7.4) and biofilm was fixed by incubating the plate at 60^0C for 1 h. After fixing, the biofilm is stained by 0.06% crystal violet for 10 minute, rinsed with PBS and . dried at room temperature. For quantification of biofilm, the bound crystal violet was eluted by 30% acetic acid (0.2 mL). Absorbance was taken on microtiter plate reader at 600nm for biofilm quantification.

6.7. Determination of Post antibiotic effect (PAE) of 4'c. To determine the PAE of **4'c**, overnight culture of *S. aureus* ATCC 29213 was diluted in MHBII ~10^5 cfu/mL and exposed to 1X and 10X MIC of VANCO, LEVO, **4'c** and incubated at 37°C for 1 h. Following the incubation period, culture was centrifuged and washed 2 times with pre-warmed MHBII to remove any traces of antibiotics. Finally, cells were resuspended in drug free MHBII and incubated further at 37°C. Samples were taken after every 1h, serially diluted and plated on TSA for enumeration of CFU. The PAE was calculated as PAE = T – C; where, T is referred to the difference in time required for 1 Log_{10} increase in CFU versus CFU observed immediately after the removal of drug and C in a similarly treated drug free control [40].

6.8. Animals experiments

Animal experiments were performed on six-eight week old Balb/c mice procured from National Laboratory Animal Facility of CSIR-Central Drug Research Institute, Lucknow. The experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee of CSIR-Central Drug Research Institute, Lucknow. Animal experiments were performed in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Govt. of India).

6.9. Murine neutropenic thigh infection model

For *in vivo* antimicrobial activity evaluation of **4'c**, female BALB/c mice weighing approximately 18-20 g were used throughout the study. Mice were rendered neutropenic by a series of cyclophosphamide injections given intraperitoneally (IP) 1 day and 1 h before infection. This was followed by injection of *S. aureus* ATCC 29213 in the right thigh of mice to establish infection. After 3 h post infection, **4'c** and vancomycin at 25 and 50 mg/Kg and 25 mg/Kg of body weight respectively, were injected IP into mice twice at an interval of 3 h between injections. Control animals were administered saline in the same volume and frequency as those receiving treatment. After 24 h, the mice were sacrificed, thigh tissue were collected from the animal and weighed. Collected tissue was homogenized in 5 mL of saline, serially diluted followed by plating on MHA plates for CFU determination. After incubation for 18–24 h at 37°C, the CFU were enumerated. Each experiment was repeated three times in duplicate and the mean data is plotted [41][42].

6.10. Statistical analysis. Statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, La Jolla, CA, USA). Comparison between three or more groups was analyzed using one-way ANOVA, with post-hoc Tukey's multiple comparisons test. P-values of <0.05 were considered to be significant.

Ethics statement

The use of mice for infectious studies (IAEC/2014/139 dated 03.12.2014) was approved by Institutional Animal Ethics Committee at CSIR-CDRI, Lucknow.

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Competing financial interests

The authors declare no competing financial interests.

Abbreviations

FDA**,** Food and Drug Administration; MDR-SA, Multi Drug Resistant *Staphylococcus aureus*; CuAAC, Cu(I)-catalyzed azide-alkyne cycloaddition; WHO, World Health Organisation; SA, *Staphylococcus aureus*; MRSA, Methicillin Resistant *Staphylococcus aureus;* CLSI, Clinical Laboratory Standards Institute; MIC, Minimum Inhibitory Concentration; $CC_{50,}$ 50% Cytotoxic Concentration; ATCC, American Type Culture Collection; SI, Selectivity Index; PBS, Phosphate Buffered Saline; TSA, Tryptic Soy Agar; VRSA, Vancomycin Resistant *Staphylococcus aureus;* MHB, Mueller-Hinton cation supplemented broth; MHA, Mueller-Hinton Agar; FIC, Fractional Inhibitory Concentration; CPCSEA, Committee for the Purpose of Control and Supervision of Experiments on Animals.

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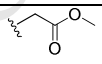
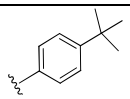
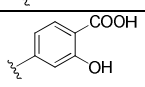
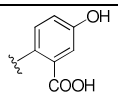
Table, Figures and Schemes

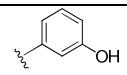
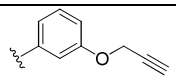
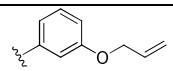
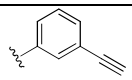
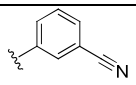
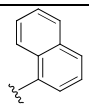
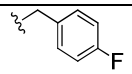
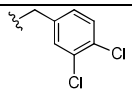
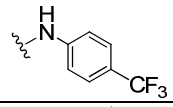
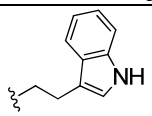
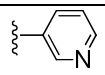
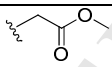
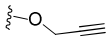
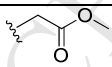
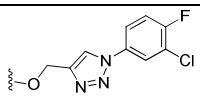
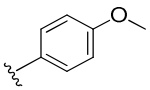
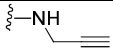
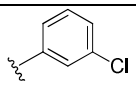
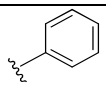
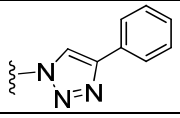
Table 1. Structures of new 2-styryl quinazolin-4(3*H*)-one derivatives **4'a-4'z and 4'aa-4'aj**

Table2. MIC (μg/mL) of 2-methyl-3-phenylquinazolin-4(3*H*)-one derivatives against ESKAP pathogen panel and *M. tuberculosis*

Table 3. MIC (μg/mL) of the (*E*)-2-styrylquinazolin-4(3*H*)-one derivatives against ESKAP pathogen panel and *M. tuberculosis*

Table 1. Structures of new 2-styryl quinazolin-4(3*H*)-one derivatives **4'a-4'z and 4'aa-4'aj**

Compound	X	R_1	R_2	R_3	R_4	R_5	% Yield
4'a	C		CN	H	H	H	52
4'b	C		CN	H	H	H	60
4'c	C		CN	H	H	H	48
4'd	C		CN	H	H	H	52

4'e	C		CN	F	H	H	49
4'f	C		CN	F	H	H	51
4'g	C		CN	F	H	H	56
4'h	C		CN	F	H	H	69
4'i	C		CN	F	H	H	70
4'j	C		CN	H	H	H	74
4'k	C		CN	H	H	H	71
4'l	C		CN	H	H	H	66
4'm	C		CN	H	H	H	63
4'n	C		CN	H	H	H	55
4'o	C		CN	H	H	H	67
4'p	C		CN	H		H	61
4'q	C		CN	H		H	51
4'r	C		H	H		H	55
4's	C		CN	H	H		53
4't	C		CN	H	H		60
4'u	C		CN	H	H		58

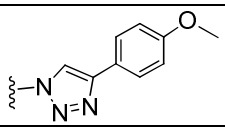
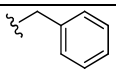
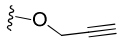
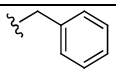
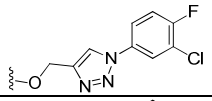
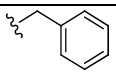
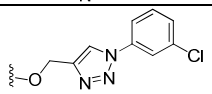
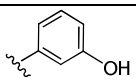
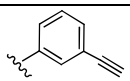
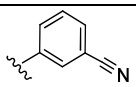
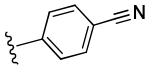
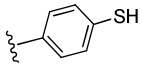
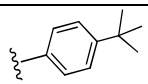
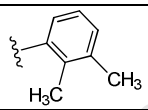
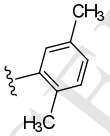
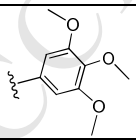
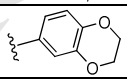
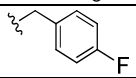
4'v	C		CN	H	H		53
4'w	C		CN	H		H	62
4'x	C		CN	H		H	64
4'y	C		CN	H		H	68
4'z	N		CN	H	H	H	73
4'aa	N		CN	H	H	H	70
4'ab	N		CN	H	H	H	66
4'ac	N		CN	H	H	H	63
4'ad	N		CN	H	H	H	65
4'ae	N		CN	H	H	H	78
4'af	N		CN	H	H	H	69
4'ag	N		CN	H	H	H	71
4'ah	N		CN	H	H	H	63
4'ai	N		CN	H	H	H	77
4'aj	N		CN	H	H	H	61

Table2. MIC (μg/mL) of 2-methyl-3-phenylquinazolin-4(3*H*)-one derivatives against ESKAP pathogen panel and *M. tuberculosis*

Compound	Gram +*ve*	Gram -*ve*				
	***S. aureus* ATCC 29213**	***E. coli* ATCC 25922**	***K. pneumoniae* BAA-1705**	***A. baumannii* BAA 1605**	***P. aeruginosa* ATCC 27853**	***M. tuberculosis* H37Rv**
4a	>64	>64	>64	>64	>64	>64
4b	>64	>64	>64	>64	>64	>64
4c	**32**	>64	>64	>64	>64	>64
4d	>64	>64	>64	>64	>64	**16**
4e	>64	>64	>64	>64	>64	**16**
4f	>64	>64	>64	>64	>64	>64
9a	>64	>64	>64	>64	>64	**16**
9b	>64	>64	>64	>64	>64	**16**
9c	>64	>64	>64	>64	>64	>64
9d	>64	>64	>64	>64	>64	>64
9e	**64**	>64	>64	>64	>64	>64
9f	>64	>64	>64	>64	>64	>64
9g	>64	>64	>64	>64	>64	>64
14a	>64	>64	>64	>64	>64	**4**
15a	>64	>64	>64	>64	>64	>64
15b	>64	>64	>64	>64	>64	>64
15c	>64	>64	>64	>64	>64	>64
17a	>64	>64	>64	>64	>64	>64
17b	**64**	>64	>64	>64	>64	**64**
17c	>64	>64	>64	>64	>64	>64
17d	>64	>64	>64	>64	>64	**64**
18a	>64	>64	>64	>64	>64	>64
18b	>64	>64	>64	>64	>64	>64
18c	>64	>64	>64	>64	>64	>64
Levofloxacin	0.125	0.015	64	8	0.5	NT
Isoniazid	NT	NT	NT	NT	NT	0.03
Rifampicin	NT	NT	NT	NT	NT	0.03

NT = Not Tested

Table 3. MIC (μg/mL) of the (*E*)-2-styrylquinazolin-4(3*H*)-one derivatives against ESKAP pathogen panel and *M. tuberculosis*

Compound	Gram +ve	Gram -ve				
	***S. aureus* ATCC 29213**	***E. coli* ATCC 25922**	***K. pneumoniae* BAA-**	***A. baumannii* BAA 1605**	***P. aeruginosa* ATCC 27853**	***M. tuberculosis* H37Rv**

			1705			
4'a	>64	>64	>64	>64	>64	>64
4'b	>64	>64	>64	>64	>64	>64
4'c	**0.03**	>64	>64	>64	>64	**64**
4'd	>64	>64	>64	>64	>64	>64
4'e	**0.0625**	>64	>64	>64	>64	**8**
4'f	**0.5**	>64	>64	>64	>64	**2**
4'g	**64**	>64	>64	>64	>64	>64
4'h	**0.25**	>64	>64	>64	>64	>64
4'i	**32**	>64	>64	>64	>64	>64
4'j	>64	>64	>64	>64	>64	>64
4'k	>64	>64	>64	>64	>64	>64
4'l	>64	>64	>64	>64	>64	>64
4'm	>64	>64	>64	>64	>64	>64
4'n	>64	>64	>64	>64	>64	>64
4'o	**64**	>64	>64	>64	>64	>64
4'p	>64	>64	>64	>64	>64	>64
4'q	>64	>64	>64	>64	>64	>64
4'r	>64	>64	>64	>64	>64	**64**
4's	>64	>64	>64	>64	>64	>64
4't	>64	>64	>64	>64	>64	>64
4'u	>64	>64	>64	>64	>64	>64
4'v	>64	>64	>64	>64	>64	>64
4'w	>64	>64	>64	>64	>64	>64
4'x	>64	>64	>64	>64	>64	>64
4'y	>64	>64	>64	>64	>64	>64
4'z	>64	>64	>64	>64	>64	>64
4'aa	>64	>64	>64	>64	>64	>64
4'ab	>64	>64	>64	>64	>64	>64
4'ac	**64**	>64	>64	>64	>64	>64
4'ad	**2**	>64	>64	>64	>64	>64
4'ae	>64	>64	>64	>64	>64	>64
4'af	>64	>64	>64	>64	>64	>64
4'ag	>64	>64	>64	>64	>64	>64
4'ah	**64**	>64	>64	>64	>64	**64**
4'ai	>64	>64	>64	>64	>64	>64
4'aj	>64	>64	>64	>64	>64	>64
Levofloxacin	0.125	0.015	64	8	0.5	NT
Isoniazid	NT	NT	NT	NT	NT	0.03
Rifampicin	NT	NT	NT	NT	NT	0.03

NT = Not Tested

Table 4. Cytotoxicity profile against Vero cells and Selectivity index (SI) of selected compounds

Compound	***S. aureus* ATCC 29213 MIC (μg/mL)**	**CC_{50} (μg/mL)**	**SI (CC_{50}/MIC)**
4'c	0.03	>5	>167
4'e	0.0625	>5	>83.4
4'f	0.5	>5	>10
4'h	0.25	>10	>40
4'ad	2	>10	>5

Table 5. Cytotoxicity profile of antimycobacterial agents against Vero cells and SI

Compound	**Mtb H37Rv MIC (μg/mL)**	**CC_{50} (μg/mL)**	**SI (CC_{50}/MIC)**
4d	16	12.5	0.78
4e	16	>15	>0.93
9a	16	>100	>6.25
9b	16	>33.3	>2.08
14a	4	>100	>25
4'e	8	>100	>12.5
4'f	2	>10	>5

Table 6. MIC (μg/mL) of **4'c** and **4'e** against MRSA and VRSA strains

Strains		Antibiotics resistant to	**Details about strains**	MIC (μg/mL)				
				4'c	**4'e**	Levofloxacin	Meropenem	Vancomycin
MSSA	*S. aureus* ATCC 29213	None		<0.125	<0.125	<0.5	<0.5	1

MRSA	NR 100	Methicillin, Ceftriaxone, Meropenem, Gentamycin and Linezolid	Resistant to tetracycline Contains mec subtype I cassette Large variety of virulence factors	0.125-0.25	0.5	<0.5	>64	1
	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	0.125-0.25	<0.125	16	>64	1
	NR 10129	Methicillin, Ceftriaxone, Meropenem	Also called as TCH60	<0.125	<0.125	0.5	16	1
	NR 10198	Methicillin, Ceftriaxone, Meropenem	USA100 Community acquired-MRSA Contains mec type II cassette Negative for PVL virulence factor	<0.125	<0.125	32	32	1
	NR 10192	Methicillin, Ceftriaxone, Meropenem	Community acquired-MRSA Contains mec type II cassette Negative for PVL virulence factor	<0.125	<0.125	4-8	4-8	1
	NR 10186	Methicillin, Ceftriaxone, Meropenem	USA 300 Community acquired-MRSA Contains mec type IV cassette Positive for PVL virulence factor	<0.125	<0.125	4-8	16-32	1
	NR 10193	Methicillin, Ceftriaxone, Meropenem	Community acquired-MRSA Contains mec type II cassette Negative for PVL virulence factor	<0.125	<0.125	32	32	1
	NR 1019	Methicillin,	Community acquired-MRSA	<0.125	0.125-0.25			

	4	Ceftriaxone	Contains mec type V cassette Positive for PVL virulence factor			0.5	0.5	1
	NR 10191	Methicillin, Ceftriaxone, Meropenem	USA 600 Community acquired-MRSA Contains mec type II cassette Negative for PVL virulence factor	<0.125	<0.125	16-32	>64	1
VRSA	VRS 1	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)	0.125-0.25	0.25	32	>64	>64
	VRS 4	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)	0.125	0.125	>64	>64	>64
	VRS 12	Methicillin, Ceftriaxone, Meropenem, Vancomycin and Teicoplanin	NA*	0.125	0.125	32 - >64	>64	>64

NA*: Not available

Table 7. Determination of synergy of **4'c** with approved antibiotics

Drug	***S.***	**MIC of**	**MIC of**	**FIC A**	**FIC B**	**∑FIC=**	**Inference**

	aureus **ATCC 29213 MIC (*μ*g/mL)**	**4'c in the presence of drug (*μ*g/mL) A**	**drug in the presence of 4'c (*μ*g/mL) B**			**FIC A+ FIC B**	
4'c	0.03						
Ceftazidime	16	0.0019	0.0312	0.032	0.0019	0.034	Synergistic
Daptomycin	0.5	0.0019	0.0019	0.032	0.003	0.036	Synergistic
Gentamycin	0.25	0.0019	0.0019	0.032	0.007	0.040	Synergistic
Linezolid	2	0.0019	0.0075	0.032	0.003	0.036	Synergistic
Levofloxacin	0.25	0.0019	0.0009	0.032	0.003	0.036	Synergistic
Meropenem	0.25	0.0019	0.0009	0.032	0.003	0.036	Synergistic
Minocycline	0.125	0.0019	0.0009	0.032	0.007	0.039	Synergistic
Rifampicin	0.015	0.0019	0.00003	0.032	0.002	0.034	Synergistic
Vancomycin	1	0.0019	0.0039	0.032	0.003	0.036	Synergistic

Table 8. Determination of synergy of **4'e** with approved antibiotics

Drug	***S. aureus*** **ATCC 29213 MIC (*μ*g/mL)**	**MIC of 4'e in the presence of drug (*μ*g/mL) A**	**MIC of drug in the presence of 4'e (*μ*g/mL) B**	**FIC A**	**FIC B**	**∑FIC= FIC A+ FIC B**	**Inference**
4'e	0.0625						
Ceftazidime	8	0.0078	0.0312	0.0625	0.0039	0.0664	Synergistic
Daptomycin	1	0.0078	0.0019	0.0625	0.0019	0.0644	Synergistic
Gentamycin	0.25	0.0078	0.0019	0.0625	0.0078	0.0703	Synergistic
Linezolid	2	0.0078	0.0075	0.0625	0.0037	0.0662	Synergistic
Levofloxacin	0.25	0.0078	0.0009	0.0625	0.0036	0.0661	Synergistic
Meropenem	0.5	0.0078	0.0009	0.0625	0.0018	0.0643	Synergistic

Minocycline	0.125	0.0078	0.0009	0.0625	0.0072	0.0697	Synergistic
Rifampicin	0.015	0.0078	0.00003	0.0625	0.002	0.0645	Synergistic
Vancomycin	1	0.0078	0.0039	0.0625	0.0039	0.0664	Synergistic

Table 9. *in vitro* Post Antibiotic effect (PAE) of **4'c**

Treatments	**Time for 1 log_{10} (h)**	**PAE (h)**
S. aureus ATCC 29213 (Untreated)	2	0
4'c 1X MIC	~2	0
4'c 10X MIC	~4	~2
Levofloxacin 1X MIC	~3	~1
Levofloxacin 10X MIC	~3	~1
Vancomycin 1X MIC	~3	~1
Vancomycin 10X MIC	~4	~2

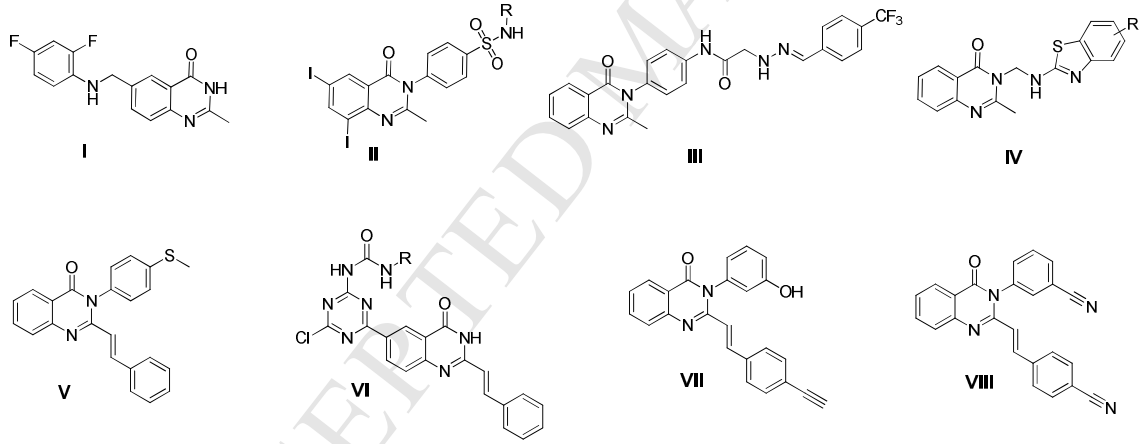


Figure 1. Structure of some literature reported quinazolin-4(3*H*)-one as antibacterial and anti-mycobacterial agents.

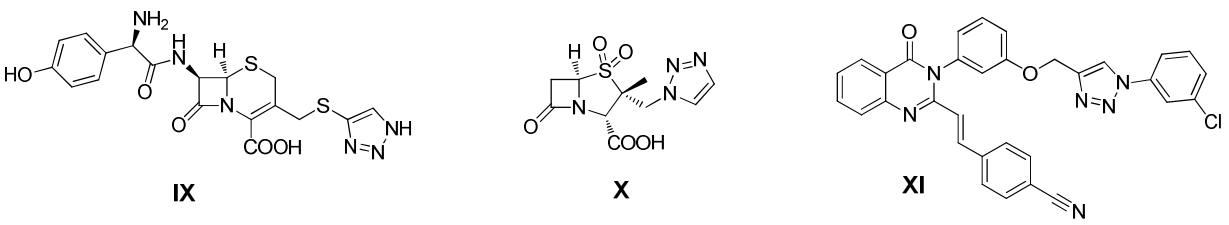


Figure 2. Structures of 1,2,3-triazole containing antibacterial agents

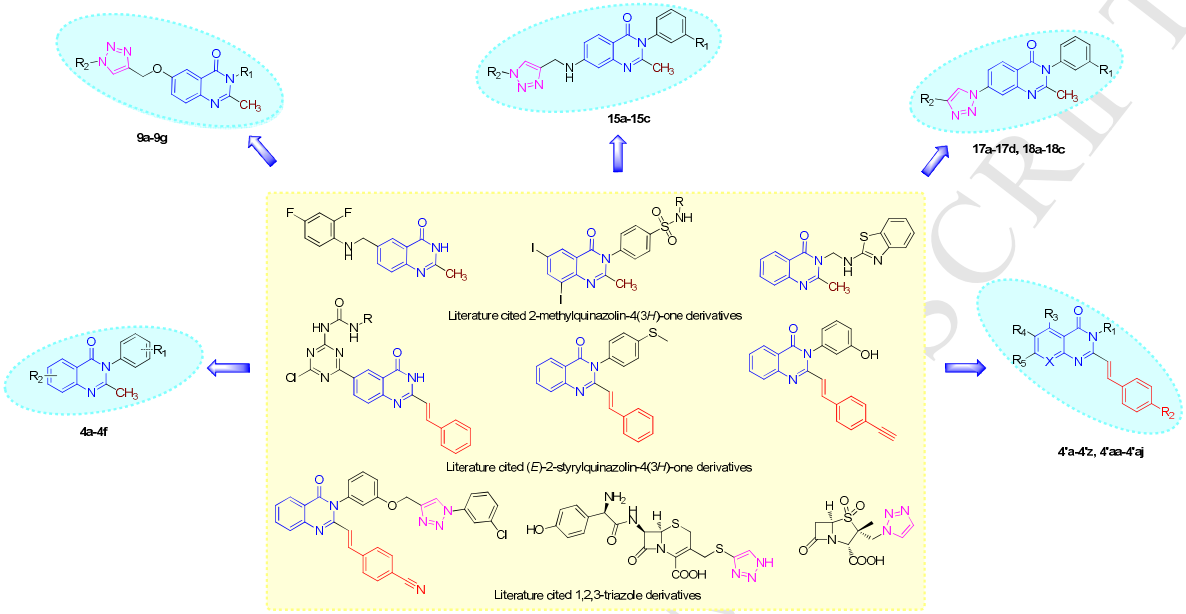


Figure 3. Rationale of design of new quinazolin-4(3*H*)-one derivatives as antibacterial agents

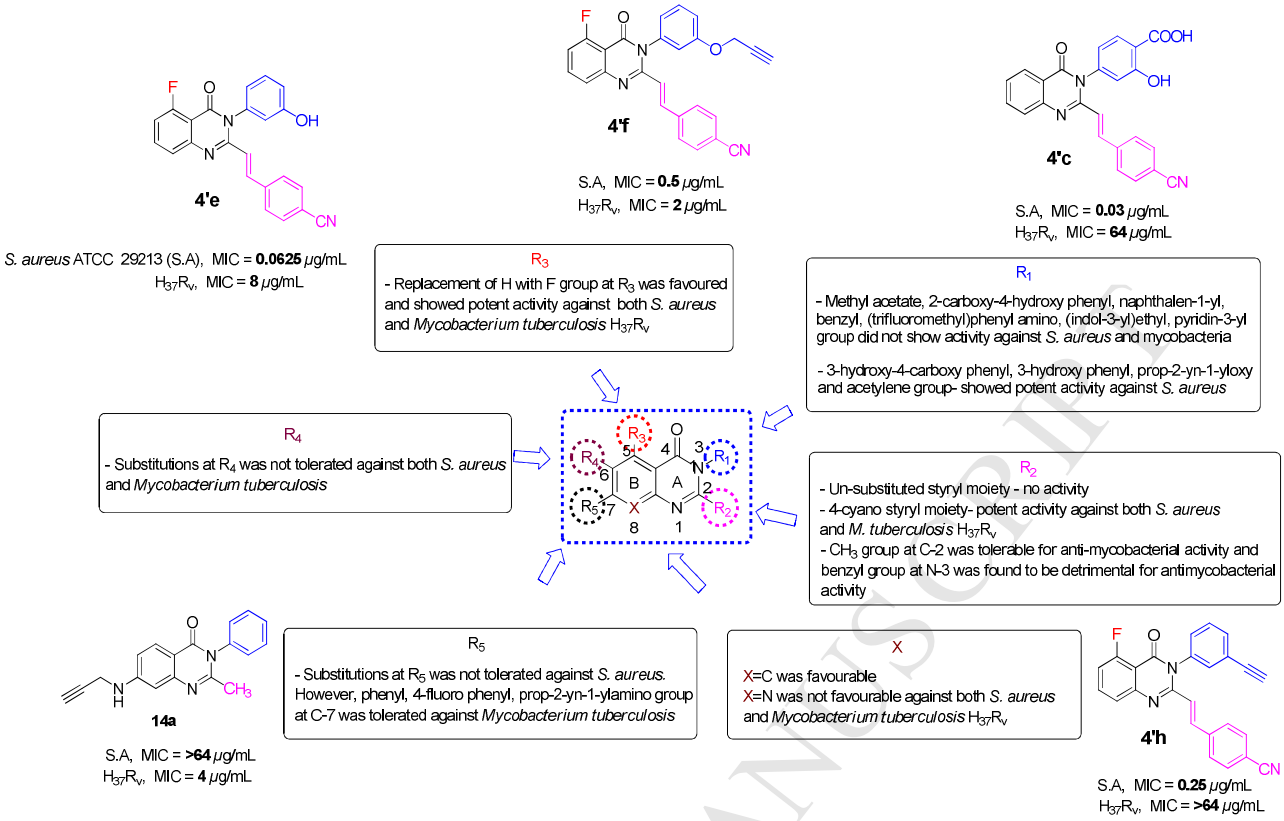


Figure 4. Broad Structure Activity Relationship (SAR) of quinazolin-4(3*H*)-one derivatives

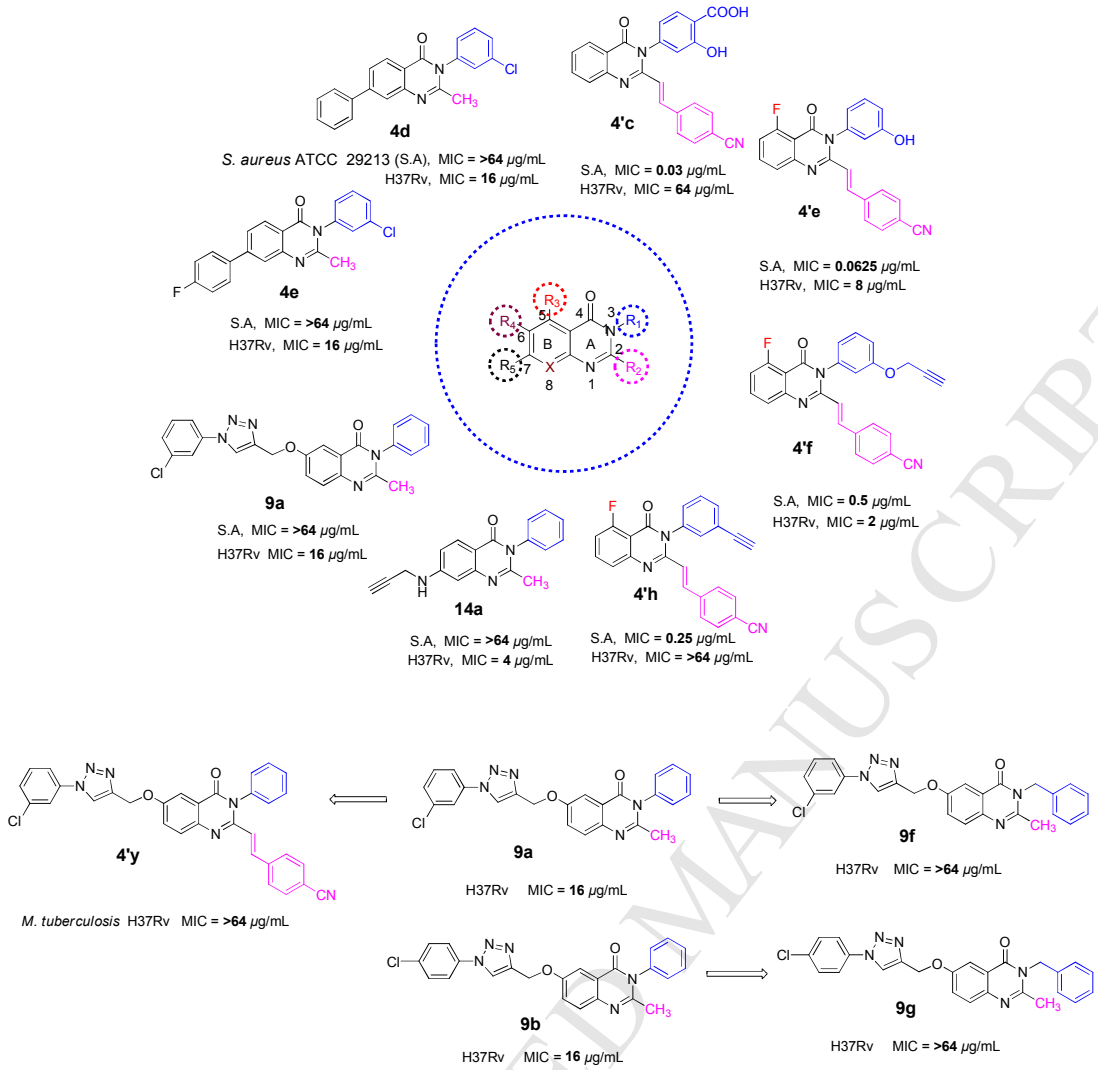


Figure 5. Effect of substitution on antibacterial and antimycobacterial activity

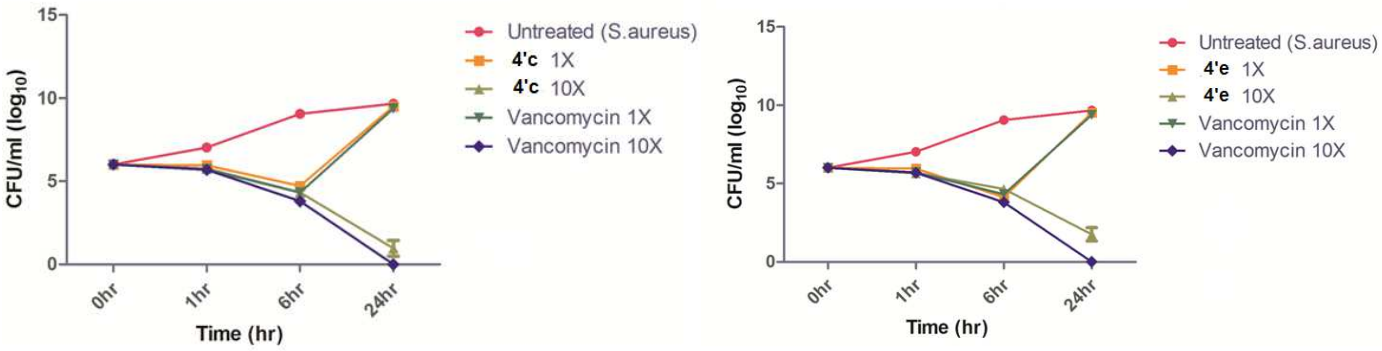


Figure 6. Time kill kinetics of compounds **4'c** and **4'e**

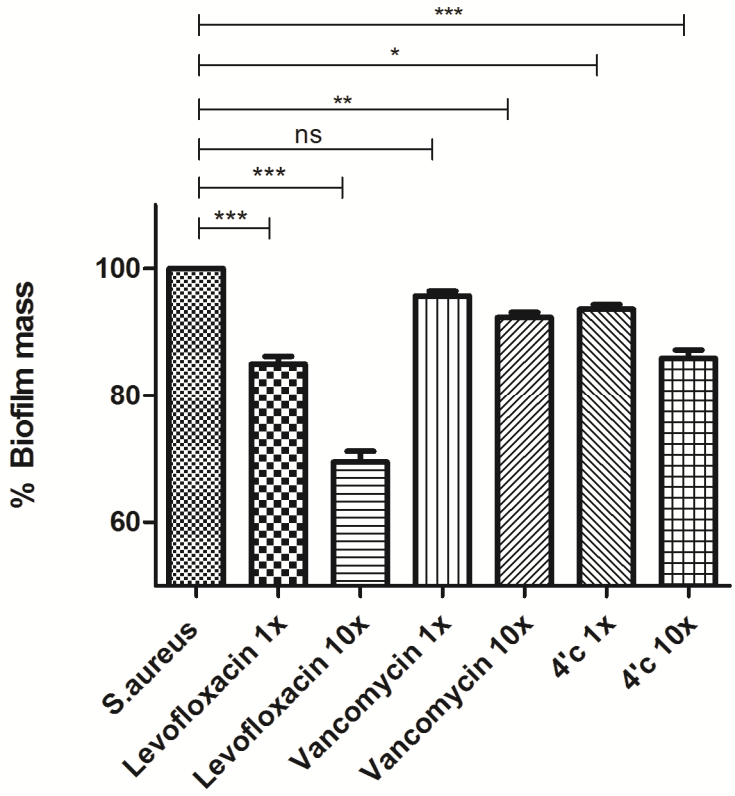


Figure 7. Activity of **4'c** against *S. aureus* pre-formed biofilm

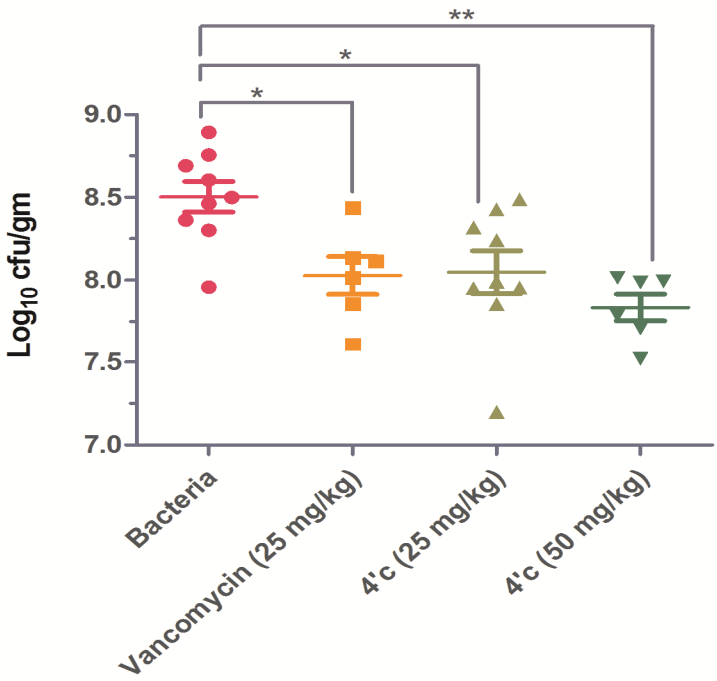
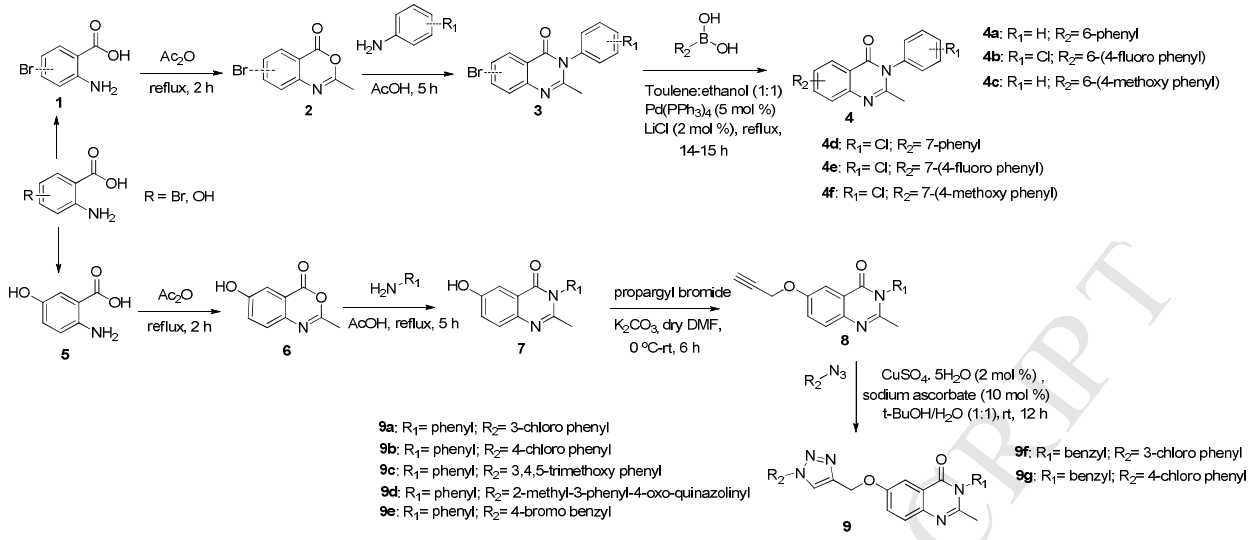
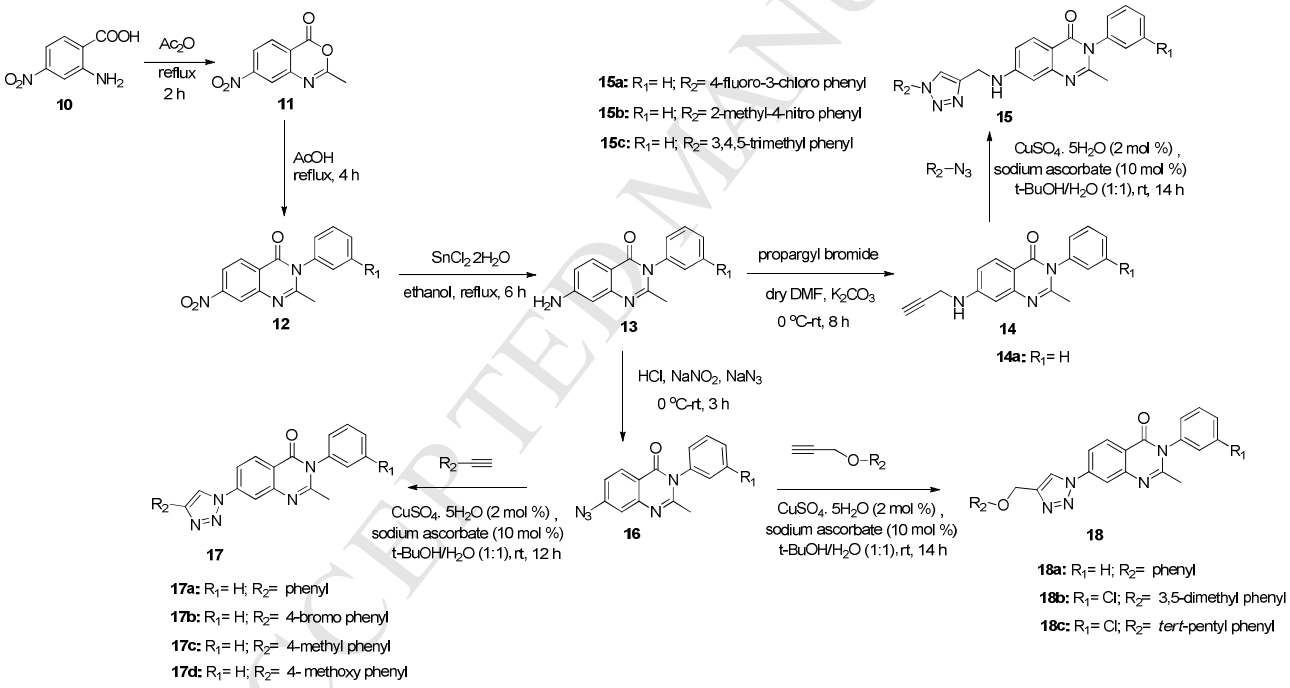


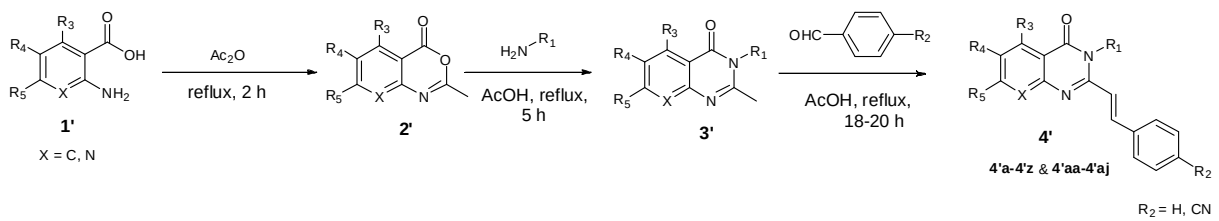
Figure 8. *In vivo* efficacy of **4'c** in murine neutropenic thigh infection model. The reduction in cfu/gm of tissue is plotted. The mice were treated with two IP doses at 3h and 6h of **4'c** and vancomycin post-infection



Scheme 1. Structures of new 2-methyl quinazolin-4(3*H*)-one derivatives **4a-4f** and **9a-9g**



Scheme 2. Structures of new 2-methyl quinazolin-4(3*H*)-one derivatives **15(a-c), 14a, 17(a-d) and 18(a-c)**



Scheme 3. Structures of new quinazolin-4(3*H*)-one derivatives **4'a-4'z and 4'aa-4'aj**

Synthesis and evaluation of new Quinazolin-4(3*H*)-one derivatives as potent antibacterial agents against multidrug resistant *Staphylococcus aureus* and *Mycobacterium tuberculosis*

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Highlights:

1. Series of new quinazolin-4(3*H*)-one derivatives were designed, synthesized and evaluated for antibacterial activity against ESKAP pathogens and pathogenic mycobacteria.
2. **4'c**, **4'e**, **4'f** and **4'h** displayed selective and potent inhibitory activity against *Staphylococcus aureus*
3. **4'c** and **4'e** were found to be benign to Vero cells and displayed promising selectivity index.
4. **4'c** and **4'e** demonstrated equipotent MIC against multiple drug-resistant strains of *S. aureus* including VRSA, concentration dependent bactericidal activity against *S. aureus* and synergized with FDA approved drugs.
5. **4'c** exhibited more potent activity in reducing the biofilm and exhibited a PAE of ~2 h at 10X MIC which is comparable to Levofloxacin and Vancomycin.
6. *In vivo* efficacy of **4'c** in murine neutropenic thigh infection model revealed that **4'c** caused a similar reduction in cfu as Vancomycin.