Copper mediated one-pot synthesis of quinazolinones and exploration of piperazine linked quinazoline derivatives as anti-mycobacterial agents†

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A facile method was developed for the synthesis of quinazolinone derivatives in a one-pot condensation reaction via in situ amine generation using ammonia as the amine source and with the formation of four new C–N bonds in good to excellent yields. With the optimised method, we synthesized a library of piperazine linked quinazoline derivatives and the synthesized compounds were evaluated for their inhibitory activity against Mycobacterium tuberculosis. The compounds 8b, 8e, 8f, 8m, 8n and 8v showed potent anti-mycobacterial activity with MIC values of 2–16 μg mL⁻¹. All the synthesized compounds follow Lipinski’s rules for drug likeness.

Introduction

Nitrogen-containing heterocycles are present in a wide range of bioactive natural products and synthetic drug candidates.¹ Among them, quinazolines and their derivatives represent medicinally important structural cores present in a number of drug candidates.² They possess a wide range of biological activities including anticancer,³ antiviral,⁴ antitubercular⁵ and antibacterial⁶ properties.

Recently, ammonia has attracted wide attention as a cost-effective and efficient nitrogen source.⁷ A number of homogeneous transition-metal catalysed reactions for the synthesis of organic amines using gaseous or liquid ammonia are reported.⁸⁹ Owing to its safety and ease of handling, aqueous ammonia is even more attractive as a substrate.

In view of the medicinal and pharmacological importance of quinazolinones, several methods on the synthesis of this class of compounds have been reported. Zhan and co-workers¹⁰ in 2013 reported an interesting approach by condensation of substituted anthranilamide with different aldehydes in presence of copper oxide for the synthesis of substituted quinazolinone derivatives. In 2014, Hung and co-workers¹¹ reported synthesis of quinazolinone by using 2-bromobenzoic acid and substituted amidines as starting materials. Abe et al.¹² reported the synthesis of quinazolinones by using 2-amino benzoic acid with substituted nitriles (Scheme 1).

Tuberculosis is a transmissible disease caused by Mycobacterium tuberculosis (Mtb) complex and recognized to have high mortality rate globally¹³. According to WHO reports, 10 million cases were reported in the year 2017, where India is the leading country with the highest burden of TB.¹⁴ Emergence of drug-resistant tuberculosis (DR-TB) has been an increasing threat over the years. Although historically tuberculosis was treated with a single drug, the current multidrug-resistant tuberculosis (MDR-TB) requires a combination of at least four or more drugs to cure the disease.¹⁵–¹⁶ New drugs and drug combinations against MDR-TB are required to combat resistance. New quinazolinone derivatives are being developed as potential candidates due to their biological properties, such as anticancer, antiviral, antitubercular and antibacterial activities.¹⁷–¹⁹ Copper mediated one-pot synthesis of quinazolinones is an easy, rapid and efficient method for the synthesis of new quinazolinone derivatives.
resistant TB or accompanying chronic diseases like HIV and diabetes certainly limits the current treatment options and hence drives the researchers to fulfill the growing demand for new agents (Fig. 1) that are effective against drug resistant TB.\textsuperscript{15} Bedaquiline (A, Fig. 1)\textsuperscript{16} led the drug discovery efforts towards the exploration of different heterocycles as anti-mycobacterial agents. Gatifloxacin (B, Fig. 1) and delamanid (C, Fig. 1) are the 2\textsuperscript{nd} line anti-TB drugs.\textsuperscript{17}a,b Wang et al. reported 4- (aminopyrazolyl)-substituted quinazolines (D, Fig. 1) as inhibitors of protein kinases (PknA & PknB) of Mycobacterium tuberculosis;\textsuperscript{17}c Tran et al. developed 4-aminoquinazolines (E, Fig. 1), as inhibitors of uridyl transferase activity of M. tuberculosis GlmU\textsuperscript{17}d and Naik et al. reported the quinolone based derivatives as potent anti-mycobacterial agents (F, Fig. 1).\textsuperscript{17}e

In the present method, we have developed a copper mediated oxidative coupling of different aldehydes and 2-bromobenzoic acid, using aq. ammonia as a less expensive nitrogen source. We explored the double amination of aryl halides to the corresponding amines and also acids to amides at the same substrate. With the established method, we could successfully synthesize 4-substituted piperazine/piperidine linked C2-aryl/ heteroaryl quinazolines. The synthesized compounds were evaluated for their \textit{in vitro} inhibitory activity against \textit{Mycobacterium tuberculosis} H37Rv. clog \textit{P} values were determined using SwissADME.

### Results and discussion

Direct employment of ammonia as a reagent in transition-metal catalysis is generally a challenging task. In the current optimised method, copper mediated oxidative coupling is developed between aldehydes and 2-bromobenzoic acid, using aq. ammonia as a nitrogen source. Various conditions for the conversion of 1 to 3 are studied and the results are discussed in Table 1. We initiated our studies by using copper oxide and NMP as solvent in the absence of oxygen at 80°C for 24 h (Table 1, entry 1). The reaction did not proceed. However, we observed the formation of the product in small quantities when the

### Table 1  Optimization of reaction conditions\textsuperscript{a}

| Entry | Catalyst | Solvent | \( \text{O}_2 \) | Temperature (°C) | Time (h) | Yield (%) |
|-------|----------|---------|----------------|----------------|----------|-----------|
| 1     | \( \text{Cu}_2\text{O} \) | NMP     | No            | 80             | 24       | 0         |
| 2     | \( \text{Cu}_2\text{O} \) | NMP     | Yes           | 80             | 24       | 12        |
| 3     | \( \text{Cu}_2\text{O} \) | NMP     | Yes           | 80             | 12       | 25        |
| 4     | \( \text{Cu}_2\text{O} \) | NMP     | Yes           | 100            | 10       | 40        |
| 5     | \( \text{CuCl} \) | NMP     | Yes           | 100            | 10       | >20       |
| 6     | \( \text{CuCl} \) | DMSO    | Yes           | 100            | 10       | 24        |
| 7     | \( \text{CuBr} \) | NMP     | Yes           | 100            | 10       | Trace     |
| 8     | \( \text{CuBr} \) | DMSO    | Yes           | 100            | 10       | >20       |
| 9     | \( \text{Cu}_2\text{O} \) | DMSO    | Yes           | 100            | 10       | 55        |
| 10    | \( \text{Cul} \) | DMSO    | Yes           | 100            | 10       | 70        |
| 11    | \( \text{Cul} \) | ACN     | Yes           | 100            | 10       | Trace     |
| 12    | \( \text{Cul} \) | Toluene | Yes           | 100            | 10       | Trace     |
| 13    | \( \text{Cul} \) | DMF     | Yes           | 100            | 10       | 40        |
| 14    | \( \text{Cul} \) | NMP     | Yes           | 100            | 10       | 50        |
| 15    | \( \text{Cu(OAc)}_2 \) | DMSO    | Yes           | 100            | 10       | 38        |
| 16    | \( \text{Cu(OAc)}_2 \) | DMF     | Yes           | 100            | 10       | 26        |
| 17    | \( \text{Cu(OTf)}_2 \) | DMSO    | Yes           | 100            | 10       | 33        |

\textsuperscript{a} Reaction conditions: 1 (1 mmol), aq. ammonia (2 mmol), aldehyde (1 mmol), catalyst (5.0 mol%), solvent (5 mL) the reaction was performed at 100°C for 10 h under oxygen atmosphere.

### Scheme 1  Methodologies for the synthesis of quinazolinones.

### Scheme 2  Reaction scope.
reaction was conducted in presence of oxygen (Table 1, entry 2). With changes in time and temperature we observed the product formation to be improved (Table 1, entry 3–4). Moderate yields were observed with the change of catalyst to CuCl or CuBr with DMSO or NMP as solvents (Table 1, entry 5–8). With copper oxide and CuI as catalysts in presence of oxygen, NMP and DMSO as the solvent, the reaction proceeded smoothly. DMSO as a solvent was found to be more favourable (Table 1, entry 9–10). The reaction was studied with different solvents viz., ACN, toluene, DMF and the reaction proceeded with altered yields (Table 1, entry 11–14). When the catalyst was changed to copper diacetate and coppertriflate the reaction was sluggish and resulted in low yields (Table 1, entry 15–17). The reaction was found to proceed with optimal yields with CuI as catalyst and DMSO as the solvent (entry 10).

After optimizing the reaction conditions, we focussed on expanding the substrate scope of this transformation and the results are summarized in (Scheme 2). It is observed that electron-donating substituents such as methoxy, amino, and methyl on 2-phenyl were well tolerated under the optimal reaction conditions, with 70–78% yields (3b, 3c, 3g and 3h). Similarly, halogen substituents like 2,4-dichloro, 2,6-dichloro...
and 3-nitro are also tolerated, yielding the desired products (3j, 3i and 3l) in good to high yields (75–79%). Heterocycles like pyridyl, furan, thiophene and isoxazole at C-2 position (3d, 3e, 3f and 3m) are also well tolerated with good to moderate yields (78–82%). With 4-bromo and 4-cyano (3o and 3n) substituents, the reactions proceeded smoothly (Scheme 2).

We performed the gram scale synthesis with 1.5 gm of 2-bromo benzoic acid and benzaldehyde as the starting materials which resulted in 1.2 g of the final product (Scheme 3). As the reaction with TEMPO did not show the product formation, the free radical mediated mechanism is observed and conventional mechanism in one-pot protocol is proposed (Scheme 3). Based on the control experiments, a plausible reaction mechanism is proposed and depicted in Scheme 4. Our mechanistic investigation was supported by ESI-QTOF-MS technique and collected the mass data at different time intervals with <5 ppm error. Initially, under copper catalysis substrate 1 gets converted to intermediate I. Next addition of aq. ammonia gives the intermediate II, the observed mass was [M+H] at m/z of 198.8620 after 30 min. The replacement of halo atom from the 2nd position with amine will give the intermediate III, the obtained mass result was [M+] at m/z of 198.8620. After that another equivalent of aq. ammonia will be addition to the intermediate III to give the intermediate IV, the mass was shown with [M + H] at m/z of 223.0896. The product formation was observed after 6 h but not completely, further preceding the reaction for 10–12 h to get the complete conversion (Fig. 2).

A series of 2-arylquinazoline derivatives were synthesized with the optimized method as described in Scheme 2. The obtained quinazoliones (3a-f) were further chlorinated using POCl₃ and N,N-dimethyl aniline to provide the corresponding 2-aryl chloroquinazoline intermediates 4a-f. The chlorinated intermediates 4a-f were treated with piperazine 5 to yield 2-aryl-4-(piperazin-1-yl)quinazoline 6a-f. Coupling of 6a-f with a number of carboxylic acids 7a-i using HATU as coupling reagent afforded the corresponding amide derivatives 8a–z in moderate to excellent yields. Structures of all the newly synthesized compounds were confirmed by ¹H NMR, ¹³C NMR and HRMS (ESI) spectroscopic techniques (Scheme 5).

The synthesized derivatives were evaluated for their antimicrobial activity against ESKAP panel (results included in the ESf) and Mycobacterium tuberculosis H37Rv strain. While, the compounds were found to be inactive against ESKAP.

### Table 2

| Sample code | S. aureus ATCC 29213 | E. coli ATCC 25922 | K. pneumoniae BAA 1705 | A. baumannii BAA 1605 | P. aeruginosa ATCC 27853 | Mtb H37Rv ATCC 27294 | clog P |
|-------------|----------------------|---------------------|-------------------------|------------------------|--------------------------|-----------------------|--------|
| 8a          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 3.84   |
| 8b          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 3.65   |
| 8c          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 3.9    |
| 8d          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 3.77   |
| 8e          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 4.77   |
| 8f          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 4.49   |
| 8g          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 4.95   |
| 8h          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 4.54   |
| 8i          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 4.45   |
| 8j          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 4.92   |
| 8k          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 4.7    |
| 8l          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 3.56   |
| 8m          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 3.56   |
| 8n          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 4.76   |
| 8o          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 3.13   |
| 8p          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 4.05   |
| 8q          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 3.14   |
| 8r          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 3.02   |
| 8s          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 3.76   |
| 8t          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 2.25   |
| 8u          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 4.22   |
| 8v          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 3.77   |
| 8w          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 4.93   |
| 8x          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 3.07   |
| 8y          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 2.99   |
| 8z          | >64                  | >64                 | >64                     | >64                    | >64                      | >64                   | 2.48   |

Levofloxacin 0.125 0.015 64 8 0.5 Not tested

Isoniazid — — — — — 0.03

Rifampicin — — — — — 0.06
pathogenic panel, some of the compounds were found to exhibit promising inhibitory activity against M. tuberculosis H37Rv strain (Table 2). A perusal of the results indicated that among the amide derivatives, compounds 8f exhibited potent anti-mycobacterial activity with MIC of 2 µg mL⁻¹ and 8n showed an MIC of 4 µg mL⁻¹. Further, the results indicated that four compounds 8b, 8e, 8m and 8v showed MIC of 16 µg mL⁻¹ whereas other molecules were found to be moderately active or inactive. Presence of methoxy group on 2-phenyl moiety as in 8k and 8m resulted in moderate activity with MIC of 16–64 µg mL⁻¹. Replacement of C2-phenyl with 4-pyridyl was found to be a favourable lead. Substitution of R₂ position with electron withdrawing groups like 2-chloro-5-bromo phenyl 8t showed inhibitory activity with MIC of 32 µg mL⁻¹. Compounds having 2-phenyl with electron donating groups like 3,5-dimethoxy and 3,4,5-trimethoxy 8s and 8q were found to be inactive but 4-N,N-dimethylaniline was found to be good lead with MIC of 2 µg mL⁻¹. Compounds with halogen containing groups like 5-bromo-2-chlorophenyl 8y exhibited moderate activity with MIC of 32 µg mL⁻¹ whereas 4-iodo 8r was inactive and unfortunately rest of the molecules were devoid of activity (Table 2 and Fig. 3).

Conclusions

In conclusion, we have developed an operationally simple, one-pot and cost-efficient method for the preparation of quinazolines with diverse substituents. This method uses mild catalytic system which enables effective construction of four C-N bonds in one pot operation through in situ amine generation, confirmed through the ESI-MS technique. By using the optimised method, we generated a library of new piperazine linked 2-aryl/hetero-aryl-quinazoline derivatives which were evaluated for their anti-microbial activity against ESUKAP pathogen panel and also against M. tuberculosis. Among the tested compounds, 8f exhibited selective and potent anti-mycobacterial activity with MIC value 2 µg mL⁻¹. Compounds 8b, 8e, 8m and 8v exhibited moderate anti-mycobacterial activity with MIC value 16 µg mL⁻¹. All the synthesized compounds obey the Lipinski rule of clog P values.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

S. M is thankful to Department of Pharmaceuticals, Ministry of Chemicals & Fertilizers, Govt. of India, for the award of NIPER fellowship. This study was supported by the DST grant from Department of Science and Technology, Govt. of India to S. N. and S. C. (EMR/2017/000220).

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