An efficient nano CuO-catalyzed synthesis and biological evaluation of quinazolinone Schiff base derivatives and bis-2,3-dihydroquinazolin-4(1*H*)-ones as potent antibacterial agents against *Streptococcus lactis*

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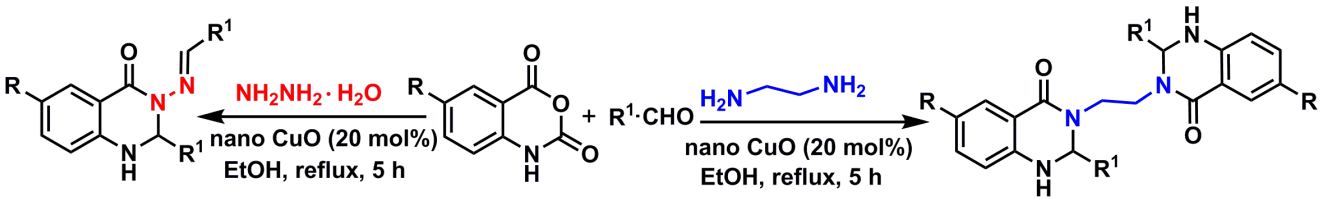

15 examples
Yield up to 87%

4 examples
Yield up to 78%

- Environmentally friendly
- High efficiency
- Short reaction time
- Good inhibitory activity to *Streptococcus lactis*

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An efficient nano CuO-catalyzed synthesis and biological evaluation of quinazolinone Schiff base derivatives and bis-2,3-dihydroquinazolin-4(1*H*)-ones as potent antibacterial agents against *Streptococcus lactis*

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ABSTRACT

An environmentally benign nano CuO catalyzed strategy has been developed for one-pot synthesis of quinazolinone Schiff base derivatives and bis-2,3-dihydroquinazolin-4(1*H*)-ones by use of hydrazine hydrate and ethidene diamine as nitrogen source. The antibacterial activity was investigated using minimum inhibitory concentration (MIC) method against four bacterial strains. Tests have shown that these derivatives have potential activity against *Streptococcus lactis*. The structure-activity relationship of the antibacterial activity evaluation was also explored.

Nitrogen-containing heterocycles are integral part of many drug molecules, physiologically active natural products and synthetic compounds. Quinazolinone represents a class of annulated six-membered nitrogen heterocycles and a core structural component in a variety of natural products and synthesized compounds: febrifugine,[1] 7-hydroxyechinozolinone,[2] 1,2-dihydroxyrutaecarpine,[3] N-methyl dihydroquinazolinone,[4] spiro-oxindole dihydroquinazolinone,[5] *etc*. These compounds show extensively biological and pharmacological activity, for instance, antitumor,[6] anticytotoxicities,[7] antipyretic,[8] analgesic,[9] diuretic,[10] antihistamine,[11] anti-depressant,[12] and vasodilating activities.[13] Due to the importance of such activities of these compounds, the synthesis of quinazolinone and its derivatives have attracted considerable interests. The methodology for synthesis of quinazolinone could be divided into four parts according to different nitrogen sources: (a) the cascade reaction of *o*-aminobenzamides with aldehydes,[12-14] (b) the reductive cyclization of 2-nitrobenzamides formamide to quinazolinones,[15] (c) condensation of phenylhydrazine or benzoylhydrazine with isatoic anhydride,[16] (d) the condensation reaction of primary amine or ammonium salts with aldehydes.[17] Conventionally, quinazolinone Schiff base derivatives were synthesized by condensation of 2-aminobenzhydrazide with aromatic aldehyde.[18] Bis-2,3-dihydroquinazolin-4(1*H*)-ones were synthesized by condensation of isatoic anhydride and diamines in the presence of $KAlSO_4 \cdot 12H_2O$.[19] However, the drawbacks of these protocols were the multiply steps and low yield.

Based on our ongoing interest toward the design and synthesis of novel heterocycles as antibacterial agents,[20] we synthesized the quinazolinone Schiff base derivatives and bis-2,3-dihydroquinazolin-4(1*H*)-ones by using hydrazine hydrate or ethidene diamine as nitrogen source under mild conditions with promoted nano CuO. All the synthesized compounds were screened for four bacteria cultures antibacterial activity by use of minimum inhibitory concentration (MIC).

In our previous work, the nano CuO effectively catalyzed the condensation reactions isatoic anhydride, aromatic aldehydes with aromatic amine at reflux temperature in EtOH.[21] Hence, we commenced our studies by investigating the condensation reaction of isatoic anhydride (**1**), hydrazine hydrate (**2**) and aromatic aldehydes (**3**), which was catalyzed by nano CuO in EtOH at 78°C for 5 hours, and the ratio of reactants was 1: 0.5: 1. Consequently, the desired product **4a** can be obtained in 43% yield, whereas the product **5** was synthesized in poor results due to the steric hindrance. (Table 1, entry 1). When the ratio of reactants changed to 1: 1: 2, the yield of product **4a** was rising up to 88% (Table 1, entry 2). Encouraged by this observation, we investigated the effect of various nano particles on the model reaction at reflux temperature in EtOH. The nano particles such as nano Fe_3O_4, nano TiO_2, and nano CeO_2 (Table 1, entries 3-5) resulted condensation reactions product **4a** in lower yields compared to the yield in the presence of nano CuO as catalyst. Subsequently, other protonic solvents such as H_2O also delivered the product in 48% yield (Table 1, entry 6). Poor to moderate

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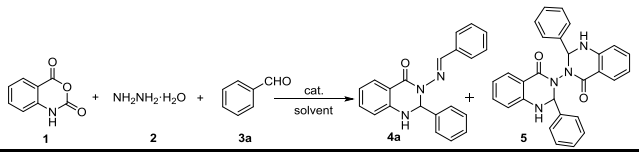
yields were obtained when the reaction was performed in other aprotic organic solvents such as dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), dioxane, acetonitrile and *N*-methyl-2-pyrrolidone (NMP) (Table 1, entries 7-11). The loading of the catalyst to the reaction was also screened (Table 1, entries 12-14). The best result was obtained when 20 mol% nano CuO was used (Table 1, entry 2). The desired product was obtained in 57% yield, when the quantity of nano CuO was decreased to 10 mol% (Table 1, entry 12). Increasing the proportion of nano CuO to 30 mol% did not enhance the yield further (Table 1, entry 13). Trace amount of product was detected when the reaction was conducted in the absence of nano CuO (Table 1, entry 14). The desired product was obtained in 23% yield when CuO powder was used as the catalyst for this protocol (Table 1, entry 15), which implied nano CuO was crucial for this transformation.

Table 1 Optimization of reaction conditions for the formation of **4a**



1 2 3a 4a 5

Entry	Solvent[a]	Catalyst (mol%)	Temperature (°C)	Ratio of reactants	Yield[d] of **4a**	Yield of **5**
1	EtOH	nano CuO (20)	78	1:0.5:1 [b]	43%	-[e]
2	EtOH	nano CuO (20)	78	1:1:2 [c]	88%	-
3	EtOH	nano Fe_3O_4 (20)	78	1:1:2	62%	-
4	EtOH	nano TiO_2 (20)	78	1:1:2	71%	-
5	EtOH	nano CeO_2 (20)	78	1:1:2	68%	-
6	H_2O	nano CuO (20)	100	1:1:2	48%	-
7	DMSO	nano CuO (20)	100	1:1:2	36%	-
8	DMF	nano CuO (20)	100	1:1:2	42%	-
9	Dioxane	nano CuO (20)	100	1:1:2	33%	-
10	CH_3CN	nano CuO (20)	80	1:1:2	14%	-
11	NMP	nano CuO (20)	100	1:1:2	24%	-
12	EtOH	nano CuO (10)	78	1:1:2	57%	-
13	EtOH	nano CuO (30)	78	1:1:2	86%	-
14	EtOH	-	78	1:1:2	-	-
15	EtOH	CuO powder (20)	78	1:1:2	23%	-

[a]solvent 5 mL.
[b]Conditions: isatoic anhydride **1** (1.0 mmol), hydrazine hydrate **2** (0.5 mmol), benzaldehyde **3a** (1.0 mmol), 78 °C.
[c]Conditions: isatoic anhydride **1** (1.0 mmol), hydrazine hydrate **2** (1 mmol), benzaldehyde **3a** (2.0 mmol), 78 °C.
[d] Isolated yield.
[e.] Not detected.

With the optimized reaction conditions in hand, a variaty of aromatic aldehydes and cyclohexanone were tested. Aromatic aldehydes and heteroaromatic aldehyde were formed desired products in excellent yields. Most of the aldehydes underwent smooth transformation to the corresponding products in satisfied yields (Table 2, entries 1-10). When cyclohexanone was used as substrate, the desired 3'-(cyclohexylideneamino)-1'*H*-spiro[cyclohexane-1,2'-quinazolin]-4'(3'*H*)-one was obtained (Table 2, entry 8). When the reaction was performed in 5-chlorine isatin anhydride and different substituted phenyl aldehyde (Table 2, entries 11-15), the results showed the yield of the unsubstituted isatoic anhydride was higher than the yield of 5-chlorine isatinan hydride, and the substrates at the C-2 position with electron-donating and electron-withdrawing had no significant influences. It was delightful that the compounds such as **4b**, **4g**, **4i**, **4j**, **4k**, **4l**, **4m**, **4n** and **4o** synthesized for the first time. All the compounds synthesized were characterized by spectral analysis. The structure of **4g** was confirmed by X-ray single crystal diffraction analysis (Figure 1).

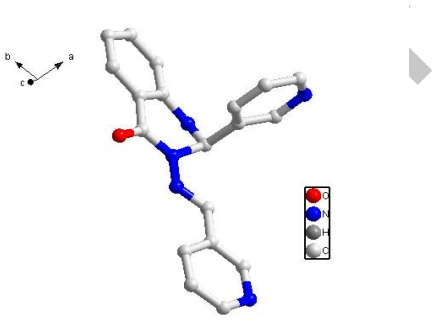
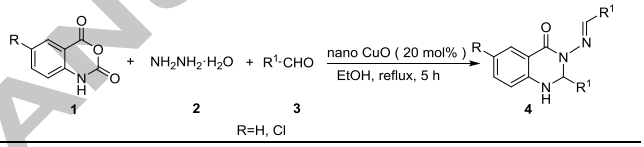


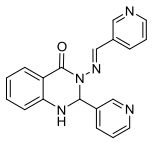
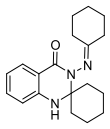
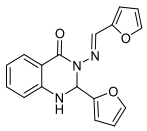
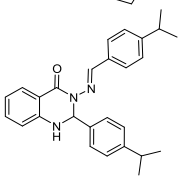
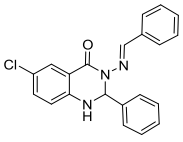
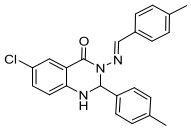
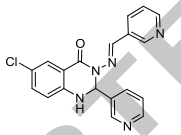
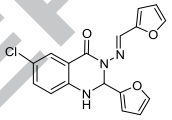
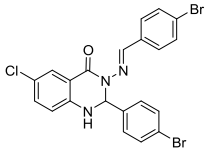
Figure 1. X-ray single crystal diffraction analysis of **4g**

Table 2. The scope of the reaction for synthesis of various dihydroquinazolinone Schiff base[a]



1 2 3 4

Entry	R	R^1	Compounds	Yield[b]
1	H	C_6H_5-	**4a**	83%
2	H	4-CHO-C_6H_4-	**4b**	75%
3	H	2-Br-C_6H_4-	**4c**	81%
4	H	4-CH_3-C_6H_4-	**4d**	78%
5	H	4-CH_3O-C_6H_4-	**4e**	87%
6	H	4-Cl-C_6H_4-	**4f**	83%

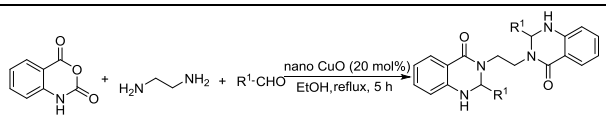
7	H	3-pyridyl		**4g**	78%
8	H	cyclohexanone		**4h**	82%
9	H	2-furanyl		**4i**	76%
10	H	4-^{i}Pr-C_6H_4-		**4j**	51%
11	Cl	C_6H_5-		**4k**	77%
12	Cl	4-CH_3-C_6H_4-		**4l**	70%
13	Cl	3-pyridyl		**4m**	72%
14	Cl	2-furanyl		**4n**	77%
15	Cl	4-Br-C_6H_4-		**4o**	74%

[a]Conditions: isatoic anhydride **1** (1.0 mmol), hydrazine hydrate **2** (1.0 mmol), **3** (2.0 mmol), nano CuO (20 mol%), reflux in EtOH at 78 °C for 5 hours.

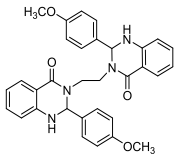
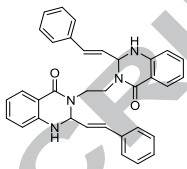
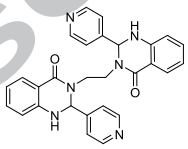
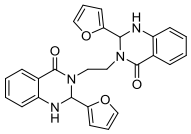
[b]Isolated yields.

To well understand the influence of the nitrogen source on reaction yield better, the study was extended to ethidene diamine. Substrates bearing 4-CH_3O-C_6H_4-, cinnamyl, 4-pyridyl, 2-furnayl moieties gave the desired bis-2,3-dihydroquinazolin-4(1*H*)-ones in moderate to good yields (Table 3, entries 1-4). Notably, some of these products were difficult to prepare using regular synthetic methods from easily available substrates. It was worth mentioning that compounds **7b**, **7c**, **7d** were synthesized for the first time.

Table 3 The scope of the reaction for synthesis various bis-quinzolinones

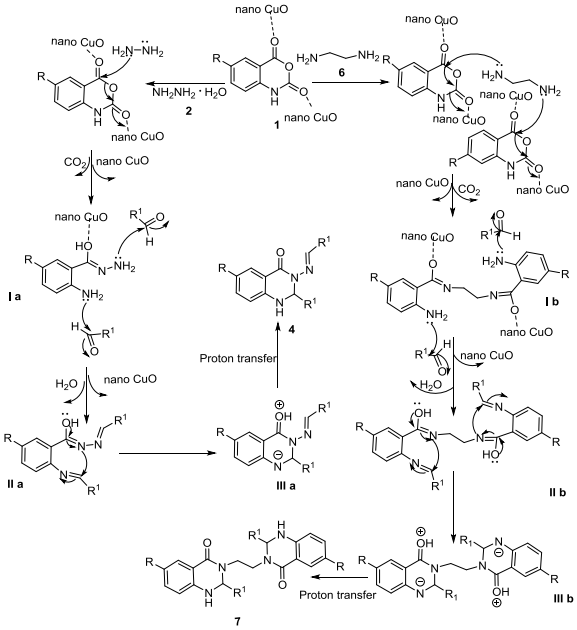


1 6 3 7

Entry	R^1	Compounds	Yield[b]
1	4-CH_3O-C_6H_4-	 **7a**	75%
2	cinnamyl	 **7b**	78%
3	4-pyridyl	 **7c**	62%
4	2-furanyl	 **7d**	70%

[a] Conditions: isatoic anhydride **1** (1.0 mmol), ethidene diamine **6** (0.5 mmol), **3** (1.0 mmol), nano CuO (20 mol%), reflux in EtOH at 78 °C for 5 hours.

[b] Isolated yields



Scheme 1. A plausible mechanism to the reaction

On the basis of our experimental results and by referring to the literature,[20-22] a plausible mechanism of the three components condensation reaction was proposed in Scheme 1. Initially, the carbonyl of the isatoic anhydride might be activated by nano CuO particles, which underwent the nucleophilic attack of the hydrazine hydrate and the decarboxylation to generate the 2-aminobenzoyl hydrazide intermediate **Ia**. **Ia** was simultaneously attacked by two aromatic aldehydes formed the intermediate **IIa**

with eliminating an equivalent amount of H_2O. The intramolecular nucleophilic addition of **IIa** generated the adduct **IIIa**. Finally, the products **4** formed from **IIIa** by proton transfer. As similar as the generation of the compound **4**, the nucleophilic nitrogen atom of ethidene diamine attacked on the carbonyl of the isatoic anhydride to form the bisanthranilamide intermediate **Ib**. The intermediate **Ib** was subject to a nucleophilic attack by two aromatic aldehydes simultaneously to form the intermediate **IIb**, which would undergo intramolecular cyclization to afford intermolecular **IIIb**, then a proton transfer transformed **IIIb** into the final product **7**.

All the synthesized compounds were screened for their in vitro antibacterial activity against two Gram-negative bacterial strains: *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) and two Gram-positive bacterial strains: *Streptococcus lactis* (*S. lactis*) and *Staphylococcus aureus* (*S. aureus*) using the minimum inhibitory concentration (MIC) method with penicillin and streptomycin sulfate as the positive controls. The MIC values were presented in Table 4.

Table 4. Antibacterial activity of compounds **4a**-**4o** and **7a**-**7d**

Compounds	MIC (μg/mL)			
	Gram-negative		Gram-positive	
	E.coli	*P. aeruginosa*	*S.lactis*	*S. aureus*
4a	64	128	128	64
4b	128	128	128	128
4c	128	128	32	128
4d	128	128	128	128
4e	>256	64	64	128
4f	128	128	16	128
4g	64	128	16	128
4h	128	128	16	64
4i	128	64	16	128
4j	128	128	32	128
4k	128	128	128	128
4l	128	128	16	128
4m	64	128	16	128
4n	128	64	32	128
4o	>256	128	32	128
7a	128	64	64	128
7b	>256	128	32	128
7c	128	128	32	128
7d	64	64	64	128
penicillin	-	-	4	4
streptomycin sulfate	4	4	-	-

These data in Table 4 suggested that most of the compounds presented more considerable potency against *S.lactis*. than other bacteria. It was noted that compounds **4c**, **4j**, **4n**, **4o** and **7c** exhibited moderate inhibitory activities (MICs: 32μg/mL), and compounds **4f**-**4j**, **4l** and **4m** had significant activity against *S. lactis*. (MICs: 16 μg/mL) which were comparable to the positive control penicillin. The structure-activity relationship of the antibacterial activity evaluation against *S. lactis* was also explored. In the series of quinazolinone derivatives, the moieties at the C-2 position were especially important for the activity. Unsubstituted benzyl was moderately active against *S.lactis*. (compounds **4a**,**4k** and **7b**). Introduction of formyl or methyl (compounds **4b**, **4d** and **4l**) at benzene ring did not improve the activity. Replacement of methyl with methoxyl led to slight increment in activity (compounds **4e** and **7a**). Interestingly, compounds **4c**, **4f** and **4o** with halogenated substituent exhibited strong antibacterial activity against *S.lactis*. with MIC values ranging from 16 to 32 μg/mL. The improvements of antibacterial activity were likely due to the presence of halogen, which was known to enhance lipophilicity and stability of the molecule.[23] Changing the benzene ring to pyridine or furan ring resulted highly active analogues **4g**, **4i**, **4m**, **4n**, **7c** and **7d** unexpectedly. Substituents such as cyclohexyl (**4h**) and 4-isopropylphenyl (**4j**) at C-2 position resulted strong antibacterial activity. From the structure-activity relationship study of these compounds, it was established that combination of quinazolinone with halogenated phenyl, pyridyl and furanyl was favorable for the antibacterial activity.

In summary, we have demonstrated a concise, mild, and facile protocol for the synthesis of quinazolinone Schiff base derivatives and bis-2,3-dihydroquinazolin-4(1*H)*-ones with hydrazine hydrate and ethidene diamine as nitrogen source. It was found that the combination of quinazolinone with halogenated phenyl, pyridyl and furanyl was favorable as the potent antibacterial agents against *S.lactis* from the structure-activity relationship study.

Acknowledgements

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Supplementary Material

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tetlet.XXXX

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Highlights

- Environmentally Friendly
- High efficiency
- Short reaction time
- Good inhibitory activity to *Streptococcus lactis*