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Synthesis, Spectral Characterization and Antibacterial Activity of Quinolone Incorporating 1,2,4-Triazine Rings

Mohammad Shoeb*, Rahimullah Shaikh

Department of Chemistry, Govt. Vidarbha Institute of Science and Humanities, Amravati, (MS), India 444604.
E-mail: shoebchem@gmail.com

Abstract: Simple and effective method of integrating 1,2,4-triazine ring with quinolone ring has been developed in two series (**3a-3d**) and **(3e-h)** starting from coumarins (**1a-4d**). The chemical structures of both the series of quinolone-1,2,4-triazine derivatives have been elucidated by spectral studies (IR, ^{1}H NMR and Mass spectra). The compounds were screened for their antibacterial activity against *Escherichia coli* ATCC 25922, *Shigella flexneri* ATCC 9199, *Staphylococcus aureus* ATCC 12600, *Bacillus cereus* ATCC 10876 by Luria Bertani (LB) media technique.

1. Introduction

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1,2,4-Triazine ring is widely described as scaffold of many biological active compounds, natural or synthetic, with a great variety of pharmacological [1] and medicinal effects, especially active as antitumor agents, anti-AIDS agents [2], CRF receptor antagonists[3], antimicrobial and anti-inflammatory agents[4]. The NCNN sequence of 1,2,4-triazine rings was considered fundamental for various pharmacological activities. Triazine derivatives have also been used as chain lengthening agents in polyurethane polymerization, azodyes, paints,

plastic, rubber and also used as fungicides and insecticides [5]. Quinolones have been reported as potential biologically active agents [6]. Fluoroquinolones are an important kind of synthetic antibacterial agent. They exerted the powerful biological activities by primarily inhibiting the activity of type II topoisomerases or DNA gyrase to stabilize the cleavage complex at specific sites on DNA and preventing the duplication of DNA. However, quinolone drugs were largely developed for treatment of infections caused by Gram-negative bacteria, but only had limited activity against some Gram-positive bacteria. Numerous efforts have

been made in order to obtain more effective derivatives with the goal of further extending the antibacterial spectrum and overcoming the drug-resistance. Amongst, structural modification of the structure of quinolone ring is the most important and meaningful strategy. In this present work bisheterocycles containing (A) 1,2,4-triazine and (B) quinolone ring systems has been synthesized both have been reported as potential biologically active agents. By combining the 1,2,4-triazine and quinolone rings the bacterial activity of drugs will be enhanced. Prompted by these observations, the N-N bisheterocycles, viz. 4-(substituted-4-methyl-2-oxoquinolin-1(2H)-yl)-6-methyl-1,2,4-triazine-3,5(2H,4H)-dione **(3a–h)** have been synthesized and compared to ofloxacin and fluconazole.

Experimental

2.1 Measurements

Melting Points were taken in a Buchi-545 apparatus and are uncorrected. Infra-red Spectrum was recorded on a SHIMADZU FT-IR Tracer100 spectrophotometer using direct KBr Pellets. Proton Nuclear Magnetic Resonance spectrum was recorded on a Varian Mercury YH-300 at 600 MHz spectrophotometer. Agilent 6320 (Quadrupole Mass Analyzer) spectrophotometer. Compounds **1a–d** were prepared according to the literature method.[15]

2.2 Synthesis

2.2.1. Synthesis of 4-(7-hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)-6-methyl-1,2,4-triazine-3,5(2H,4H)-dione **3a**:

A mixture of **1a** (10 mmol) and semicarbohydrazide **2a** (10 mmol) in ethanol (10 ml) was refluxed for about 5 h. Pyruvic acid (10 mmol) and glacial acetic acid (20 ml) fused with sodium acetate (2 g) were added and further refluxed for 5 h. The reaction mixture

was cooled and poured into ice. The solid obtained was filtered off and recrystallized from ethanol gave colourless needles.

Yield: 74.67%, melting point: 173–175 °C,
IR (KBr) cm^{-1}: 1560, 1586, 1655, 1672, 3195.1H NMR (CDCl$_3$): δ 1·98 (3H, s, CH$_3$), 5·14 (1H, s, OH), 6·50–7·15 (3H, m, ArH), 9·0 (1H, bs, NH), MS m/z: 300.0 (M+).

Same procedure was followed for the compounds 3b to 3h.

Table 1. Characterization data of title compounds.

| Compound	Molecular Formula	Calculated (found)		
		C	H	N
3a	C$_{14}$H$_{12}$N$_4$O$_4$	56.00(55.86)	4.03(4.00)	18.66(18.61)
3b	C$_{15}$H$_{14}$N$_4$O$_3$	60.40(60.32)	4.73(4.60)	18.78(18.76)
3c	C$_{15}$H$_{14}$N$_4$O$_3$	60.40(60.28)	4.73(4.70)	18.78(18.76)
3d	C$_{14}$H$_{12}$N$_4$O$_5$	53.17(53.10)	3.82(3.83)	17.71(17.69)
3e	C$_{14}$H$_{12}$N$_4$O$_3$S	53.16(53.14)	3.82(3.79)	17.71(17.70)
3f	C$_{15}$H$_{14}$N$_4$O$_2$S	57.31(57.34)	4.49(4.45)	17.82(17.79)
3g	C$_{15}$H$_{14}$N$_4$O$_2$S	57.31(57.29)	4.49(4.47)	17.82(17.84)
3h	C$_{14}$H$_{12}$N$_4$O$_4$S	50.60(50.57)	3.64(3.61)	16.86(16.87)

2.2.2.4-(4,7-dimethyl-2-oxoquinolin-1(2H)-yl)-6-methyl-1,2,4-triazine-3,5(2H,4H)-dione **3b**:Yield: 77.31%, melting point: 154-155 °C,
IR (KBr) cm^{-1}: 1569, 1585, 1663, 1670, 3216.
^{1}H NMR (CDCl$_3$) : δ 2.12 (3H, s, CH$_3$), 2·20 (3H, s, CH$_3$), 6·40–7·72 (3H, m, ArH), 10·20 (1H, bs, NH), MS m/z: 298.9 (M+).

2.2.3. 4-(4,6-dimethyl-2-oxoquinolin-1(2H)-yl)-6-methyl-1,2,4-triazine-3,5(2H,4H)-dione **3c**:

Yield: 84.02%, melting point: 205–206 °C.
I.R. (KBr) cm^{-1}: 1562, 1576, 1645, 1668, 3210,
^{1}H NMR (CDCl$_3$): δ 2.10 (3H, s, CH$_3$), 2·18

(3H, *s*, CH$_3$), 6·55–7·52 (3H, *m*, ArH), 10·31 (1H, *bs*, NH), MS m/z: 298.5 (M+).
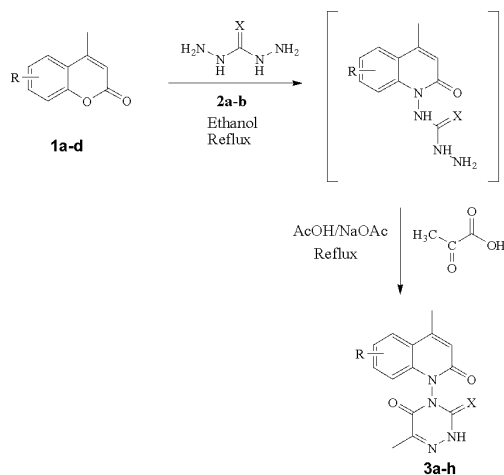
a; R = 7-OH, X = O, **b**; R = 7-CH$_3$, X = O, **c**; R = 6-CH$_3$, X = O, **d**; R = 5,7-di-OH, X = O,
e; R = 7-OH, X = S, **f**; R = 7-CH$_3$, X = S, **g**; R = 6-CH$_3$, X = S, **h**; R = 5,7-di-OH, X = S

Scheme 1

2.2.4. 4-(5,7-dihydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)-6-methyl-1,2,4-triazine-3,5(2H,4H)-dione **3d**:

Yield: 80.37%, melting point: 166–168 °C,
I.R. (KBr) cm—1: 1573, 1592, 1632, 1669, 3263, ^{1}H NMR (DMSO-d$_6$) δ 2.25 (3H, *s*, CH$_3$), 5·2 (1H, *s*, OH), 6·7–7·3 (2H, *s*, ArH), 11·36 (1H, *bs*, NH), MS m/z: 316 (M+).

2.2.5. 7-hydroxy-4-methyl-1-(6-methyl-5-oxo-3-thioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)quinolin-2(1H)-one **3e**:

Yield: 81.33% melting point:140-141 °C,
I.R. (KBr) cm—1: 1311, 1565, 1610, 1619, 3250, ^{1}H NMR (DMSO-d$_6$) δ 1.95 (3H, *s*, CH$_3$), 2·3 (3H, *s*, CH$_3$), 6·42–7·5 (3H, *m*, ArH), 10·15 (1H, *bs*, NH), MS m/z: 316.08 (M+).

2.2.6. 4,7-dimethyl-1-(6-methyl-5-oxo-3-thioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)quinolin-2(1H)-one **3f**:

Yield: 76.05%,melting point:195-196 °C,

I.R. (KBr) cm—1: 1323, 1560, 1611, 1670, 3255, ^{1}H NMR (CDCl$_3$) δ 2.00 (3H, *s*, CH$_3$), 2·20 (3H, *s*, CH$_3$), 6·30–7·6 (3H, *m*, ArH), 10·39 (1H, *bs*, NH), MS m/z: 314.0 (M+).

2.2.7. 4,6-dimethyl-1-(6-methyl-5-oxo-3-thioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)quinolin-2(1H)-one **3g**:

Yield: 85.49%,melting point:153-154 °C,
I.R. (KBr) cm^{-1}: 1321, 1564,1620, 3262, ^{1}H NMR (CDCl$_3$) δ 2.01 (3H, *s*, CH$_3$), 2·20 (3H, *s*, CH$_3$), 6·4–7·4 (3H, *m*, ArH), 10·45 (1H, *bs*, NH), MS m/z: 314.0 (M+).

2.2.8. 5,7-dihydroxy-4-methyl-1-(6-methyl-5-oxo-3-thioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)quinolin-2(1H)-one **3h**:

Yield: 82.02%,melting point: 218–219 °C,
I.R. (KBr) cm-1: 1311, 1560, 1610, 1678, 3240, ^{1}H NMR (DMSO-d$_6$): δ 2.12 (3H, *s*, CH$_3$), 6·9–7·8 (2H, *s*, ArH), 11·50 (1H, *bs*, NH), MS m/z: 332.06 (M+).

2.3 Antibacterial screening

The determination of minimum inhibitory concentration (MIC) of the synthesized derivatives (**3a**–**3h**) using ofloxacin and fluconazole as standard drugs against the bacterial species *Escherichia coli* ATCC 25922, *Shigella flexneri* ATCC9199, *Staphylococcus aureus* ATCC 12600, *Bacillus cereus* ATCC10876. All four bacterial strains used were common food borne pathogens, including two Gram –ve bacteria such as *Escherichia coli* ATCC 25922 & *Shigella flexneri* ATCC9199, and two Gram +ve bacteria such as *Staphylococcus aureus* ATCC 12600 & *Bacillus cereus* ATCC 10876.

2.3.1. Preparation of the test compound

The compounds were dissolved at a concentration of 1.0 mg/ml in Di-Methyl Forma-

amide (DMF). The synthesized compounds are soluble only in DMF.

2.3.2. Minimum inhibitory concentration (MIC)
A loop full of the given test strain was inoculated into 25 ml of Luria Bertani broth (LB Broth) and incubated for 24 h in an incubator at 37°C in order to activate the bacterial strain. A petri dish of 100 mm diameter was filled with 28-30 ml of Luria Bertani (LB) media. Inoculation was performed by the pour plate technique. 0.2 ml of the activated strain was inoculated into the media when it had reached a temperature of 40-45°C. The complete procedure of the ditch preparation was done in a laminar airflow to maintain strict sterile and aseptic condition. The media was allowed to solidify. After solidification of the media, a well was made in the media with the help of a cup-borer (0.5 cm) and then 0.03 ml of the synthetic compound (dissolved in DMF) was inoculated into the well. Controls were performed (for each bacterial strain), where 0.03 ml of the pure solvent was inoculated into each well. The plates were incubated for 24 h at 37°C. The inhibition zone formed by the compounds against the particular test bacterial strain determined the antibacterial activities of the synthetic compounds. The observed inhibition zone is presented in Table 2.

3. Results and discussion

1. Chemistry

Synthesis of N–N bisheterocycles, which is effected sequentially, requires bifunctional precursors, which are not always readily accessible. Coumarins **1a–d** can be served as important synthetic precursors for the synthesis of title compounds. When the compounds **1a–d** was refluxed with **2a** for about 5 h whereby transient ring opening, insertion of terminal nitrogen gave intermediates which underwent cyclization *in-situ* readily with pyruvic acid in presence of acetic acid fused with

sodium acetate to 4-(substituted-4-methyl-2-oxoquinolin-1(2H)-yl)-6-methyl-1,2,4-triazine-3,5(2H,4H)-dione (**3a–d**). Similarly, the compound **3e–h** was prepared using **2b** (scheme 1). The mechanism of the reaction involves the nucleophilic attack of **2** to carbonyl carbon atom of **1**, which proceeds to the formation of a condensed product that in turn eliminates a molecule of water. The other end of the **2** reacts with pyruvic acid followed by intramolecular cyclocondensation giving N-N bicyclic product (**3a–h**). The IR, ^{1}HNMR and Mass spectra of the title compounds were in complete agreement with the assigned structure. In case of IR spectral analyses abroad the band due to NH group appeared in all the title compounds between 3185 and 3401 cm–1. The amide carbonyl functions appeared as sharp band in the range 1591–1680 cm–1. Another band around 1275–1315 cm–1 appeared in the compounds **3e–h** due to C=S group. ^{1}H NMR spectral analyses of the title compounds exhibited two singlets at δ 1.95-2.60 ppm assigned to the methyl group. All the title compounds have shown abroad singlet in the range δ 9.0-12.0 ppm due to NH. Aromatic protons appeared in the expected range as multiplets.

4. Conclusion

The SAR analyses showed that the activity is significantly dependent on the electron density of the hydroxyl group, which is enhanced by the introduction of electron-donating substituents. Compounds **3d** and **3h** containing hydroxyl group are showing higher degree of inhibition in biological investigation. Fusion of two biologically active nuclei i.e. 1,2,4-triazine and quinolone increases the potentiality of prepared compounds towards pathogens.

3.2. Biological investigation

The inhibition zone formed by the compounds against the particular test bacterial strain

Table 2 Antibacterial activity of the synthesized compounds.

Compound   *In vitro* activity – zone of inhibition in mm (MIC in lg/ml)

	E.coli ATCC 25922	S.flexneri ATCC 9199	S. aureus ATCC 12600	B.cereus ATCC 2853
3a	+	-	+	+
3b	++	+	-	-
3c	++	++	-	+
3d	++	++	+	++
3e	-	+	-	++
3f	++	-	+	-
3g	+	++	-	+
3h	++	++	+	+

++ zone of inhibition (0. 5 -1.5 cm diameter); + zone of inhibition (>1.0 cm diameter);
- no zone of inhibition

determined the antibacterial activities of the synthetic compounds. Test compounds viz; **3d** and **3h** were found to show antibacterial activity against Gram +ve and Gram –ve bacteria, indicating a broad range spectrum of activity. Compounds **3c** and **3g** were found to show antibacterial activity (zone of inhibition more than 1.0 cm) against *Escherichia coli* and *Shigella flexneri* both belonging to Gm –ve genotypes. However growth of *Bacillus cereus* (Gram +ve) was also inhibited by **3e** compound but *Staphylococcus aureus* (Gram +ve) growth was not affected, indicating a narrow spectrum of antibacterial activity. Other compounds viz; **3a**, **3b** and **3f** showed variable antibacterial activity.

Acknowledgements

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