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**RESEARCH ARTICLE**

# Synthesis, Characterization and Testing of Biological Activity of Some Novel Chalcones Derivatives of Coumarin

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**Abstract:** In the present communication, we herein report the synthesis of some novel derivatives of coumarin *i.e*. 3-((2*E*)-(3-(2-hydroxy-5-((aryl)diazenyl)phenyl)acryloyl)-2*H*-chromen-2-one (**2a-2g**). Various aromatic amines and salicylaldehyde in the presence of sodium nitrite and conc. HCl yielded 2-hydroxy-5-((aryl)diazenyl)benzaldehyde (**1a-1g**). A series of 3-((2*E*)-(3-(2-hydroxy-5-((aryl)diazenyl) phenyl)acryloyl)-2*H*-chromen-2-one were synthesized by the reaction of 2-hydroxy-5-((aryl)diazenyl) benzaldehyde and coumarin in presence of ethanol and piperidine. Different analytical techniques such as elemental analysis, IR spectra, $^1$H NMR spectra and mass spectra were used to elucidate the structures of all newly synthesized compounds (**1a-1g** and **2a-2g**). The anti-bacterial and anti-fungal activity of newly synthesized chalcone derivatives (**2a-2g**) were evaluated against different bacterial and fungal stains. Some of these compounds showed promising activity against different stains.

**Keywords:** Chalcone derivatives, Coumarins, Anti-bacterial activity

## Introduction

Coumarin nucleus is widely distributed in nature in plant kingdom and forms an important class of oxygen heterocycle[1]. Over the years, coumarins have been established as well-known naturally occurring oxygen-heterocyclic compounds isolated from various plants[2]. They are the family of lactones containing benzopyrone skeletal framework that have enjoyed isolation from plant as well as total synthesis in the laboratory. The plant extracts containing coumarin-related heterocycles are employed as herbal remedies in traditional systems of medicine. The synthesis of coumarin (2-oxo-2*H*-chromene) derivatives has attracted considerable attention of organic and medicinal chemists due to its wide usage in food additives, fragrances, pharmaceuticals and agrochemicals. In view of this, coumarins

have attracted intense interest in recent years because of their diverse pharmacological properties. Hence, many researchers have reported different biological activities of coumarins derivatives such as anticoagulant, antitubercular, antileucemic, antimicrobial, anti-inflammatory, anti-HIV, analgesic, anticancer, antitumoral, anticonvulsant, antiplatelet, antifungal, antiviral, antibacterial, antimalarial activities and other pharmacological activities[3-11].

Chalcones are $\alpha,\beta$ -unsaturated ketones which constitute an important group of natural products that serve as precursors for the synthesis of various heterocyclic compounds like pyrimidines, imidazoles, pyrazoles, 2-pyrazoline and flavonoids[12,13]. Cyclization of chalcones, leading to thiazines, pyrimidines, pyrazoline has been a developing field within the realm of heterocyclic chemistry for the past several years because of their ready accessibility and the broad spectrum of biological activity of the products as antibacterial, antifungal, antiprotozoal, anti-inflammatory substances[14]. With this background it has been thought worth to synthesize some novel heterocyclic compounds comprising of chalcone having azo-linkage and coumarin in a single moiety. Also, to evaluate these new compounds for their potency as an anti-bacterial and anti-fungal agent.

## Experimental

All the chemicals and solvents were obtained from E-Merck, India (AR grade) and were used without further purification. Melting points were taken in an open capillary tube. IR spectra were recorded on a Shimadzu Dr-8031 instrument. Elemental analyses were carried out using a Perkin-Elmer, CHN elemental analyzer model 2400. $^1$H NMR spectra of the synthesized compounds were recorded on a Bruker-Avance (300 MHz) and Varian-Gemini (200 MHz) spectrophotometer using $CDCl_3$ solvent and TMS as an internal standard. EI-MS spectra were determined on a LCQ ion trap mass spectrometer (Thermo Fisher, San Jose, CA, USA), equipped with an EI source.

*General procedure for the synthesis of 2-hydroxy-5-((aryl)diazenyl)benzaldehyde (**1a-1g**)*

Aromatic amines (0.01 mol) was added in conc. HCl (5 mL) boiled for 10 minutes. This solution was then cooled to 0-5 $^0$C in ice water bath. Aqueous sodium nitrite (0.01 mol, 10 mL) solution in cold condition was then added to this solution dropwise with vigorous strirring. The temperature of the reaction mixture was kept to 0-5 $^0$C for 1 h, to give diazonium chloride solution. Then the resulting diazonium solution was poured drop wise with vigorous stirring to alkaline suspension of salicyladehyde in water (10 mL, 0.01 mol) at 0-5 $^0$C. The pH of the reaction mixture was maintained at 8 to 9 by simultaneous addition of 10% aqueous sodium hydroxide (NaOH) solution. The precipitate was collected as a crystalline solid compound after keeping the reaction mixture overnight in the reaction flask. The precipitates were filtered by using Whatmann filter paper (no. 40). The compounds were recrystallized in EtOH.

*General procedure for the synthesis of 3-(3-(2-hydroxy-5-((aryl)diazenyl)phenyl) propanoyl) -2H-chromen-2-one (**2a-2g**)*

A mixture of 2-hydroxy-5-((aryl)diazenyl)benzaldehyde (**1a-1g**) and 3-acetyl-coumarin (0.01 mols, 1:1 ratio) in ethanol was stirred in presence of few drops of piperidine under reflux condition for 10-12 h. Mixture was cooled and poured into ice cold water and stirred vigorously. The compounds were precipitated as a crystalline product. These crude compounds were recrystallized with methanol.

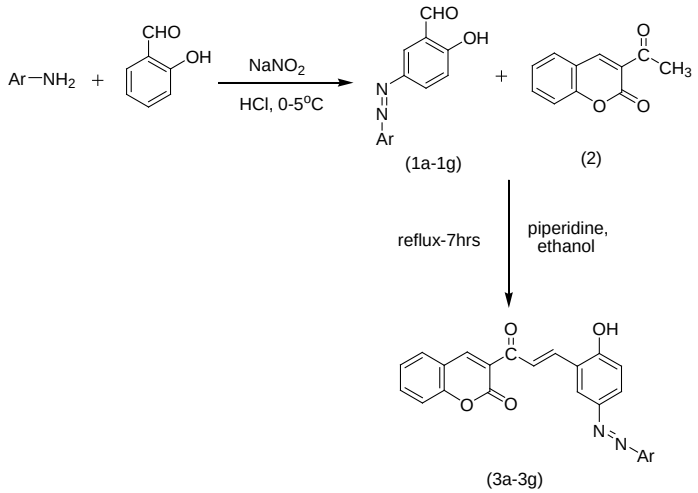
*Evaluation of antimicrobial activity (agar diffusion method)*

Antimicrobial activity of all synthesized compounds was determined by agar diffusion method. All human pathogenic bacteria *viz*. *Bacillus subtilis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* were obtained from the Department of Biotechnology, S. F. S. College, Nagpur. Stock solutions of compounds were diluted in dimethyl sulfoxide (DMSO) to give a final concentration for determining the Minimum inhibitory concentration (MIC) value. MIC was defined as the lowest concentration of compound is required for a complete inhibition of the bacterial growth after incubation time. For antibacterial activity Muller Hinton agar was used. The wells of 6 mm diameter were filled with 0.1 mL of each compound is diluted separately for each test of bacterial strain. The antibiotic Ampicillin used as reference antibacterial agent, for comparison. Inoculated plates were then incubated at 37 $^0$C for antibacterial activity for 24 h. After incubation the antimicrobial activity was measured in terms of the zone of inhibition in mm.

## Results and Discussion

*General*

The synthetic route for the preparation of some novel derivatives of coumarin *i.e*. 3-((2*E*)-(3-(2-hydroxy-5-((aryl)diazenyl)phenyl)acryloyl)-2*H*-chromen-2-one (**2a**-**2g**) have been depicted in Scheme 1. The key intermediate 2-hydroxy-5-((aryl)diazenyl)benzaldehyde (**1a-1g**) was synthesized by the reaction of various aromatic amines and salicylaldehyde in appropriate quantity of conc. HCl and sodium nitrite mixture. The spectral data of newly synthesized compounds have been collected here in the following part.




Where: Ar = (a) 4-$NO_2$-$C_6H_4$, (b) 3-$NO_2$-$C_6H_4$, (c) 2-$NO_2$-$C_6H_4$, (d) *o*-$CH_3$-$C_6H_4$, (e) *p*-$CH_3$-$C_6H_4$, (f) *m*-$CH_3$-$C_6H_4$, (g) *p*-Cl-$C_6H_4$

**Scheme 1.** Synthetic route to obtain 3-((2*E*)-(3-(2-hydroxy-5-((aryl)diazenyl)phenyl) acryloyl)-2*H*-chromen-2-one

*Characterization data of 2-hydroxy-5-((4-nitrophenyl)diazenyl)benzaldehyde (**1a**)*

Yield: 75; m.p.: 112 $^o$C, IR (KBr): 1530 (C=C), 1570 (N=N);1710 (aldehydic C=O); 2920 (Ar-CH), 2990 cm$^{-1}$ (Ar-OH); $^1$H NMR: δ = 7.40 (d, 1H, Ar-CH); 7.90 (d, 2H, (Ar-CH); 8.10

(d, 2H, Ar-CH); 8.40 (d, 2H, Ar-CH); 10.40 (s, 1H, Ar-CHO); 11.90 (s, 1H, Ar-OH); Anal. Calcd. For $C_{13}H_9N_3O_4$: C, 57.57; H, 3.34; N, 15.49; Found: C, 57.40; H, 3.20; N, 15.20; Mass spectra, *m/z* = 271 (100%).

*Characterization data of 2-hydroxy-5-((3-nitrophenyl)diazenyl)benzaldehyde (**1b**)*

Yield: 68; m.p.: 105 $^oC$, IR (KBr): 1550 (C=C), 1580 (N=N);1730 (aldehydic C=O); 2930 (Ar-CH), 3110 $cm^{-1}$ (Ar-OH); $^1H$ NMR: δ = 7.10 (d, 1H, Ar-CH); 7.60 (d, 2H, (Ar-CH); 8.30 (d, 2H, Ar-CH); 8.60 (d, 2H, Ar-CH); 10.60 (s, 1H, Ar-CHO); 11.70 (s, 1H, Ar-OH); Anal. Calcd. For $C_{13}H_9N_3O_4$ : C, 57.57; H, 3.34; N, 15.49; Found: C, 57.10; H, 3.40; N, 15.30; Mass spectra, *m/z* = 271.10 (100%).

*Characterization data of 2-hydroxy-5-((2-nitrophenyl)diazenyl)benzaldehyde (**1c**)*

Yield: 70; m.p.: 110 $^oC$, IR (KBr): 1510 (C=C), 1570 (N=N);1740 (aldehydic C=O); 2960 (Ar-CH), 3140 $cm^{-1}$ (Ar-OH); $^1H$ NMR: δ = 7.20 (d, 1H, Ar-CH); 7.70 (d, 2H, (Ar-CH); 8.50 (d, 2H, Ar-CH); 8.80 (d, 2H, Ar-CH); 10.80 (s, 1H, Ar-CHO); 11.90 (s, 1H, Ar-OH); Anal. Calcd. For $C_{13}H_9N_3O_4$ : C, 57.57; H, 3.34; N, 15.49; Found: C, 57.20; H, 3.30; N, 15.10; Mass spectra, *m/z* = 271.00 (100%).

*Characterization data of 2-hydroxy-5-((o-tolyldiazenyl)benzaldehyde (**1d**)*

Yield: 73; m.p.: 172 $^oC$, IR (KBr): 1540 (C=C), 1560 (N=N);1730 (aldehydic C=O); 2990 (Ar-CH), 3180 $cm^{-1}$ (Ar-OH); $^1H$ NMR: δ = 2.40 (s, 3H, Ar-$CH_3$); 7.10-7.40 (m, 4H, Ar-CH); 7.90 (d, 1H, (Ar-CH); 8.10 (d, 2H, Ar-CH); 10.40 (s, 1H, Ar-CHO); 11.70 (s, 1H, Ar-OH); Anal. Calcd. For $C_{14}H_{12}N_2O_2$ : C, 69.99; H, 5.03; N, 11.66; Found: C, 69.50; H, 5.00; N, 11.40; Mass spectra, *m/z* = 240.09 (100%).

*Characterization data of 2-hydroxy-5-((p-tolyldiazenyl)benzaldehyde (**1e**)*

Yield: 70; m.p.: 170 $^oC$, IR (KBr): 1510 (C=C), 1530 (N=N);1760 (aldehydic C=O); 2970 (Ar-CH), 3230 $cm^{-1}$ (Ar-OH); $^1H$ NMR: δ = 2.20 (s, 3H, Ar-$CH_3$); 7.20-7.50 (m, 4H, Ar-CH); 7.70 (d, 1H, (Ar-CH); 8.40 (d, 2H, Ar-CH); 10.60 (s, 1H, Ar-CHO); 11.80 (s, 1H, Ar-OH); Anal. Calcd. For $C_{14}H_{12}N_2O_2$ : C, 69.99; H, 5.03; N, 11.66; Found: C, 69.70; H, 5.10; N, 11.50; Mass spectra, *m/z* = 240.00 (100%).

*Characterization data of 2-hydroxy-5-((m-tolyldiazenyl)benzaldehyde (**1f**)*

Yield: 65; m.p.: 140 $^oC$, IR (KBr): 1520 (C=C), 1550 (N=N);1730 (aldehydic C=O); 2980 (Ar-CH), 3210 $cm^{-1}$ (Ar-OH); $^1H$ NMR: δ = 2.30 (s, 3H, Ar-$CH_3$); 7.30-7.50 (m, 4H, Ar-CH); 7.60 (d, 1H, (Ar-CH); 8.30 (d, 2H, Ar-CH); 10.70 (s, 1H, Ar-CHO); 11.60 (s, 1H, Ar-OH); Anal. Calcd. For $C_{14}H_{12}N_2O_2$ : C, 69.99; H, 5.03; N, 11.66; Found: C, 69.40; H, 5.00; N, 11.30; Mass spectra, *m/z* = 240.00 (100%).

*Characterization data of 5-((4-chlorophenyl)diazenyl)-2-hydroxybenzaldehyde (**1g**)*

Yield: 70; m.p.: 130 $^oC$, IR (KBr): 1510 (C=C), 1560 (N=N);1760 (aldehydic C=O); 2960 (Ar-CH), 3260 $cm^{-1}$ (Ar-OH); $^1H$ NMR: δ = 7.40 (d, 1H, Ar-CH); 7.70 (d, 2H, Ar-CH); 7.90-8.10 (m, 4H, (Ar-CH); 10.40 (s, 1H, Ar-CHO); 11.90 (s, 1H, Ar-OH); Anal. Calcd. For $C_{13}H_9N_2O_2Cl$ : C, 59.90; H, 3.48; N, 10.75; Found: C, 59.50; H, 3.20; N, 10.60; Mass spectra, *m/z* = 260.00 (100%).

*Characterization data of 3-((2E)-(3-(2-hydroxy-5-((4-nitrophenyl)diazenyl)- phenyl) acryloyl)-2H-chromen-2-one (**2a**)*

Yield: 69; m.p.: 145 $^oC$, IR (KBr): 1490 (C=C), 1530 (N=N); 1680 $cm^{-1}$ (ketonic C=O); 2960 (Ar-CH), 3210 $cm^{-1}$ (Ar-OH); $^1H$ NMR: δ = 6.80 (d, 1H, Ar-CH); 7.10 (d, 1H, Ar- CH);

7.40-7.60 (m, 5H, (Ar-CH); 7.80 (t, 3H, Ar-CH); 8.10 (d, 1H, Ar-CH); 8.40 (d, 2H, Ar-CH); 8.60 (s, 1H, Ar-CH); 11.90 (s, 1H, Ar-OH); Anal. Calcd. For $C_{24}H_{15}N_3O_6$ : C, 65.31; H, 3.43; N, 9.52; Found: C, 65.20; H, 3.30; N, 9.40; Mass spectra, *m/z* = 441.00 (100%).

*Characterization data of 3-((2E)-(3-(2-hydroxy-5-((3-nitrophenyl)diazenyl)- phenyl) acryloyl)-2H-chromen-2-one (**2b**)*

Yield: 74; m.p.: 151 $^oC$, IR (KBr): 1470 (C=C), 1540 (N=N); 1690 $cm^{-1}$ (ketonic C=O); 2940 (Ar-CH), 3270 $cm^{-1}$ (Ar-OH); $^1H$ NMR: δ = 6.50 (d, 1H, Ar-CH); 7.20 (d, 1H, Ar-CH); 7.50-7.60 (m, 5H, (Ar-CH); 7.90 (t, 3H, Ar-CH); 8.30 (d, 1H, Ar-CH); 8.50 (d, 2H, Ar-CH); 8.70 (s, 1H, Ar-CH); 11.60 (s, 1H, Ar-OH); Anal. Calcd. For $C_{24}H_{15}N_3O_6$ : C, 65.31; H, 3.43; N, 9.52; Found: C, 65.10; H, 3.20; N, 9.30; Mass spectra, *m/z* = 441.00 (100%).

*Characterization data of 3-((2E)-(3-(2-hydroxy-5-((2-nitrophenyl)diazenyl)phenyl-acryloyl)-2H-chromen-2-one (**2c**)*

Yield: 72; m.p.: 141 $^oC$, IR (KBr): 1480 (C=C), 1580 (N=N); 1710 $cm^{-1}$ (ketonic C=O); 2960 (Ar-CH), 3290 $cm^{-1}$ (Ar-OH); $^1H$ NMR: δ = 6.30 (d, 1H, Ar-CH); 7.30 (d, 1H, Ar-CH); 7.50-7.70 (m, 5H, (Ar-CH); 7.90 (t, 3H, Ar-CH); 8.20 (d, 1H, Ar-CH); 8.60 (d, 2H, Ar-CH); 8.90 (s, 1H, Ar-CH); 11.30 (s, 1H, Ar-OH); Anal. Calcd. For $C_{24}H_{15}N_3O_6$ : C, 65.31; H, 3.43; N, 9.52; Found: C, 65.20; H, 3.10; N, 9.40; Mass spectra, *m/z* = 441.00 (100%).

*Characterization data of 3-((2E)-(3-(2-hydroxy-5-((o-tolyldiazenyl)phenyl) acryloyl-2H-chromen-2-one (**2d**)*

Yield: 68; m.p.: 170 $^oC$, IR (KBr): 1460 (C=C), 1590 (N=N); 1730 $cm^{-1}$ (ketonic C=O); 2980 (Ar-CH), 3260 $cm^{-1}$ (Ar-OH); $^1H$ NMR: δ = 2.40 (s, 3H, Ar-$CH_3$); 6.70 (d, 1H, Ar-CH); 7.10 (d, 1H, Ar-CH); 7.40-7.70 (m, 7H, (Ar-CH); 7.90 (d, 2H, Ar-CH); 8.10 (d, 1H, Ar-CH); 8.60 (s, 1H, Ar-CH); 11.90 (s, 1H, Ar-OH); Anal. Calcd. For $C_{25}H_{18}N_2O_4$ : C, 73.16; H, 4.42; N, 6.83; Found: C, 73.10; H, 4.20; N, 6.50; Mass spectra, *m/z* = 410.10 (100%).

*Characterization data of 3-((2E)-(3-(2-hydroxy-5-((p-tolyldiazenyl)phenyl)acryloyl)-2H-chromen-2-one (**2e**)*

Yield: 74; m.p.: 160 $^oC$, IR (KBr): 1480 (C=C), 1560 (N=N); 1750 $cm^{-1}$ (ketonic C=O); 2970 (Ar-CH), 3280 $cm^{-1}$ (Ar-OH); $^1H$ NMR: δ = 2.20 (s, 3H, Ar-$CH_3$); 6.50 (d, 1H, Ar-CH); 7.20 (d, 1H, Ar-CH); 7.40-7.60 (m, 7H, (Ar-CH); 7.80 (d, 2H, Ar-CH); 8.30 (d, 1H, Ar-CH); 8.70 (s, 1H, Ar-CH); 11.50 (s, 1H, Ar-OH); Anal. Calcd. For $C_{25}H_{18}N_2O_4$ : C, 73.16; H, 4.42; N, 6.83; Found: C, 72.90; H, 4.30; N, 6.70; Mass spectra, *m/z* = 410.00 (100%).

*Characterization data of 3-((2E)-(3-(2-hydroxy-5-((m-tolyldiazenyl)phenyl)acryloyl)-2H-chromen-2-one (**2f**)*

Yield: 69; m.p.: 152 $^oC$, IR (KBr): 1450 (C=C), 1550 (N=N); 1760 $cm^{-1}$ (ketonic C=O); 2960 (Ar-CH), 3250 $cm^{-1}$ (Ar-OH); $^1H$ NMR: δ = 2.30 (s, 3H, Ar-$CH_3$); 6.20 (d, 1H, Ar-CH); 6.90 (d, 1H, Ar-CH); 7.20-7.50 (m, 7H, (Ar-CH); 7.70 (d, 2H, Ar-CH); 8.50 (d, 1H, Ar-CH); 8.90 (s, 1H, Ar-CH); 11.70 (s, 1H, Ar-OH); Anal. Calcd. For $C_{25}H_{18}N_2O_4$ : C, 73.16; H, 4.42; N, 6.83; Found: C, 73.00; H, 4.20; N, 6.40; Mass spectra, *m/z* = 410.00 (100%).

*Characterization data of 3-((2E)-3-(5-(2-(4-chlorophenyl)diazenyl)-2-hydroxyphenyl) acryloyl)-2H-chromen-2-one (**2g**)*

Yield: 78; m.p.: 145 $^{o}$C, IR (KBr): 1460 (C=C), 1580 (N=N); 1790 cm$^{-1}$ (ketonic C=O); 2980 (Ar-CH), 3190 cm$^{-1}$ (Ar-OH); $^{1}$H NMR: $\delta$ = 6.80 (d, 1H, Ar-CH); 7.10-7.20 (m, 4H, (Ar-CH); 7.40 (d, 2H, Ar-CH); 7.60 (d, 1H, Ar-CH); 7.80-7.90 (m, 4H, Ar-CH); 8.40 (s, 1H, Ar-CH); 11.80 (s, 1H, Ar-OH); Anal. Calcd. For $C_{24}H_{15}N_2O_4Cl$: C, 66.91; H, 3.51; N, 6.50; Found: C, 66.50; H, 3.30; N, 6.20; Mass spectra, *m/z* = 430.00 (100%).

*Biological activity*

From the investigation of antibacterial screening data depicted in Table 1, it is revealed that most of the tested compounds showed moderate to good bacterial inhibition.

**Table 1.** Antibacterial activity of newly synthesized chalcone derivatives (**2a-2g**) (zone of inhibition in mm)

| Compound | Anti-bacterial activity (MIC 500 μg/mL) | | | | |
|---|---|---|---|---|---|
| | Gram (+ve) bacteria | | | Gram (-ve) Bacteria | |
| | A | B | C | D | E |
| **2a** | ++ | - | - | ++ | ++ |
| **2b** | + | ++ | ++ | + | + |
| **2c** | ++ | - | +++ | + | - |
| **2d** | + | ++ | + | + | ++ |
| **2e** | +++ | + | ++ | ++ | - |
| **2f** | + | + | + | + | +++ |
| **2g** | **+++** | **+++** | **+++** | **+** | **+** |
| Standard Ampicillin | +++ | **+++** | **+++** | **+++** | **+++** |

*Gram +ve Bacteria: (A) Bacillus subtilis; (B) Proteus vulgaris; (C) Staphylococcus aureus. Gram -ve Bacteria: (D) Escherichia coli; (E) Klebsiella pneumonia. Zone of Inhibitions: Inactive = - (inhibition zone < 5 mm); Slightly active = + (inhibition zone 5-12 mm); Moderately active = + + (inhibition zone 13-17 mm); Highly active = + + + (inhibition zone > 17 mm)*

Compound **2a** have shown moderate activity against *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia* bacteria. Compound **2b** were found to be moderately active against *Proteus vulgaris* and *Staphylococcus aureus*. Compound **2c** was highly active against *Staphylococcus aureus* whereas moderately active against *Bacillus subtilis.* Compound **2d** was found to be moderately active against *Proteus vulgaris* and *Klebsiella pneumonia*. Compound **2e** showed promising good activity against *Bacillus subtilis* and moderate activity against *Staphylococcus aureus* and *Escherichia coli.* Compound **2f** have shown good activity against *Klebsiella pneumonia*. Among all the newly synthesized compounds, compound **2g** have shown good activity against gram positive bacteria *i.e*. *Bacillus subtilis*, *Proteus vulgaris* and *Staphylococcus aureus* whereas this compound shown slight activity against both gram negative bacteria *i.e*. *Escherichia coli* and *Klebsiella pneumonia*. Thus, it can be concluded that compound **2g** can be used a promising anti-bacterial agent.

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