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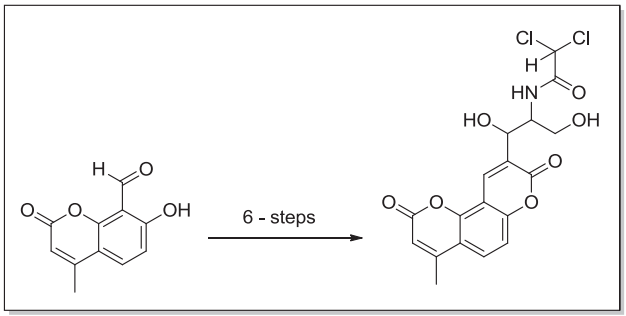


A synthesis, characterization, and antimicrobial study of a novel benzodipyran analog of chloramphenicol was carried out. Structure–antimicrobial activity relationship study indicates that benzodipyran analog of chloramphenicol was most active against Gram-positive bacteria, *Staphylococcus aureus*, and the fungal strains *Rhizoctonia bataticola* and *Penicillium* even at minimum inhibitory concentration 10 μg/mL and showed moderate activity in other bacterial and fungal organisms. All the compounds synthesized during the present investigation were characterized by IR, $^{1}$H NMR, $^{13}$C NMR, ESI-MS, and elemental analysis.

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## INTRODUCTION

Chloramphenicol, the oldest antibacterial agents, which was first isolated from *Streptomyces venezuelae* in 1947 [1]. It is used in the treatment of typhoid, dysentery, salmonella, rickettsia, and ocular bacterial infections. It is only the first antibiotic to be produced in optically active form by chemical synthesis rather than by fermentation. Many new methods of synthesis of this active antibiotic have been reported recently [2–6], and there has been no lack of attempts to relate the structure of chloramphenicol to the structures of biologically important substances to deduce possible antimetabolite–metabolite relationships. Many efforts have been devoted to the synthesis of compounds in which the structural components of chloramphenicol have been varied systematically. The numerous chemical derivatives of this antibiotic have been synthesized and tested for antimicrobial activity so as to clarify the relationship between the structures of this antibiotic and its biological properties. Because no experimental difficulties were encountered in incorporating the active 2-dichloroacetamidopropandiol side chain to various heterocyclic systems, many interesting analogs of chloramphenicol have been synthesized to test their antimicrobial properties [7–10].

Coumarin is the simplest member of group of oxygen heterocycles and a class of lactones, which is an indispensable heterocyclic unit to both the chemists and the biochemists. Coumarins occur naturally in plants and microorganisms, approximately 1000 coumarin derivatives have been isolated from over 800 species of plants and microorganisms [11]. Coumarin itself was first isolated from tonka bean, *Coumarouna odorata* by Vogel in 1820 [12]. Coumarins are structurally diverse, which includes simple substituted coumarins, those that have substituents in the benzene ring, five-membered and six-membered fused coumarins such as furocoumarins and pyranocoumarins and coumarin dimers, which usually consists of two coumarin units linked together such as dicoumarol. A study of antimicrobial properties of such naturally occurring and synthetic coumarins has been reported recently [13]. Several coumarin derivatives have been reviewed recently for their natural occurrence, antimicrobial, anti-inflammatory, anticancer, anti-HIV, and other miscellaneous properties [14]. Many coumarin derivatives have special ability to scavenge reactive oxygen species ROS-free radicals, such as hydroxyl radicals, superoxide radicals, or hypochlorus acid and to influence processes involving free radical injury; moreover, they have been used as inhibitors of lipoxygenase and cyclooxygenase in the arachidonic acid

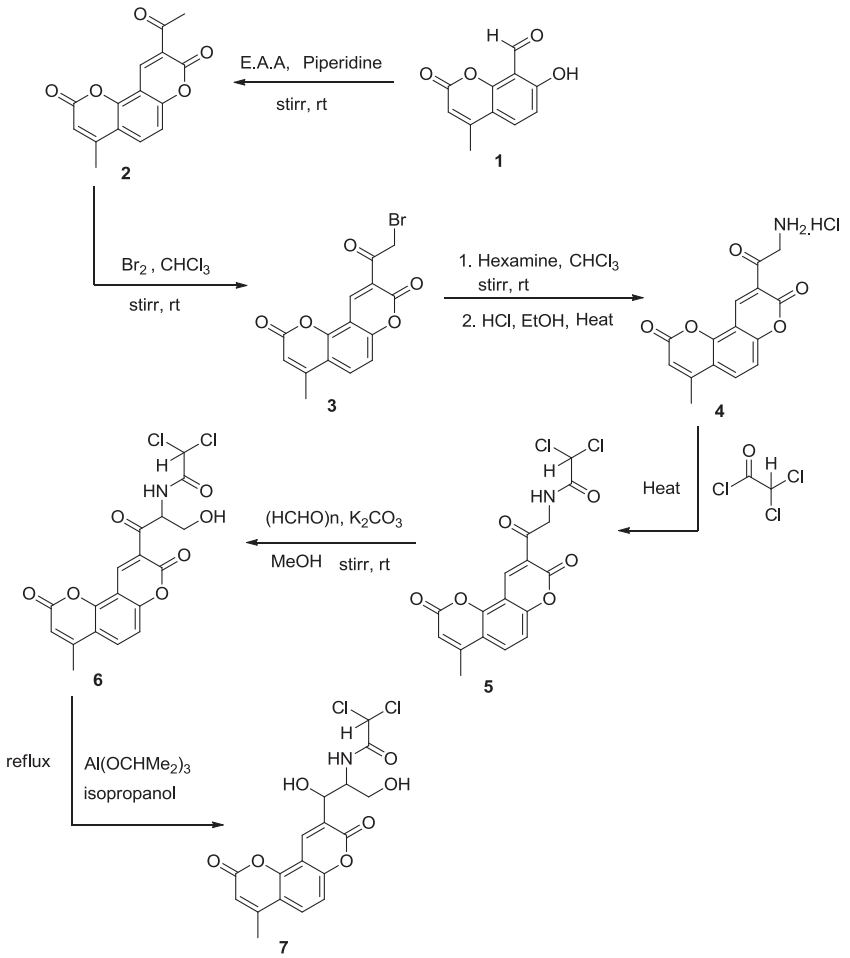
pathway of inflammation suppression [15]. Nonsteroidal anti-inflammatory drugs (NSAIDs) have a broad spectrum of effects in acute pain management and target the cyclooxygenase enzyme. Several coumarin derivatives have been reported for their significant antimicrobial and anti-inflammatory properties and their abilities to inhibit these enzymes in inhibiting inflammation [16–20]. The potential of coumarin derivatives as anti-inflammatory agents has been explored in our laboratory, by incorporating biocompatible pharmacophores such as vanillin, cyanoester, and paracetamol, at the allylic position with respect to C3–C4 double bond of the coumarin moiety [21,22]. The triheterocyclic thiazoles synthesized from 3-bromoacetyl coumarins and 4-aminomethyl quinolinone [23] from our laboratory were found to exhibit promising anti-inflammatory and analgesic activity even after 24 hours. Similarly, we have also reported the introduction of halogens in general and fluorine in particular at position 4 in the aryloxy, and arylamino moieties of coumarin and quinolinone [24–27] were found to enhance the antimicrobial as well as analgesic and anti-inflammatory activities.

In view of the various biological properties associated with coumarins in general and chloramphenicol in particular, it was thought to be of interest to incorporate the active 2-dichloroacetamidopropandiol side chain of chloramphenicol in to the C-9 position of benzodipyran, and the benzodipyran analog of chloramphenicol is synthesized (Scheme 1). A detail structure–antimicrobial activity relationship study was developed to deduce possible antimetabolite–metabolite relationships between the derivatives of coumarin (**2–6**), benzodipyran analog of chloramphenicol (**7**), and chloramphenicol (drug) in this paper.

## RESULTS AND DISCUSSION

**Synthesis.** The sequence of the reactions employed for the synthesis of the required analog is outlined in Scheme 1. In the first step, the new coumarin derivative **2** was prepared by the condensation of ethylacetoacetate with 8-formyl-7-hydroxy-4-methyl-coumarin [28,29] **1** in the presence of catalytic amount of piperidine. The corresponding bromoacetyl coumarin **3** was synthesized by

**Scheme 1.** Synthesis of benzodipyran analog of chloramphenicol.



the treatment of **2**, with liquid bromine in dry chloroform. In the next step of the reaction, the bromoester **3** was treated with hexamethylenetetramine in dry chloroform, and the corresponding hexamine adduct so formed was subjected to the acid hydrolysis in dry alcohol to obtain the corresponding amine hydrochloride salt **4**. In the third step of the reaction, compound **5** was prepared by the reaction of **4** with dichloroacetyl chloride at 80°C. During the fourth step of the reaction, the incorporation of hydroxymethyl group in compound **5** was carried out by the base catalyzed reaction of **5** with paraformaldehyde, and the corresponding 2-dichloroacetamidopropanone derivative **6** was synthesized. In the last step of the reaction, the reduction of ketone function was carried out by the treatment of **6** with aluminum isopropoxide in dry isopropanol, and the synthesis of benzodipyran analog of chloramphenicol **7** was achieved. Derivatives **6** and final product **7** were isolated as racemic mixtures. All the newly synthesized compounds have been developed by TLC using UV and iodine vapors and are fully characterized by elemental analysis, IR, $^{1}$H-NMR, $^{13}$C-NMR, and EI-MS. All the compounds gave satisfactory analytical and spectroscopic data in full accord with their assigned structures.

**Antibacterial activity.** The antibacterial activity of the coumarin **2** (Table 1) showed 12, 24, and 08% zone of inhibition of *Escherichia coli* (*E.c*), *Staphylococcus* aureus (*S.a*), and *Bacillus cirrosis* (*B.c*) at 50 μg/mL concentration, respectively; however, no antibacterial activity of **2** was observed at reduced concentration. Upon the introduction of bromine to the side chain, coumarin **3** was found to be more active and showed 21% inhibition of *E.c* and 13 and 12% inhibitions of *B.c* and *Neisseria flavescens* (*N.f*), respectively, at 50 μg/mL. Perhaps the same compound was moderately active for *S.a* and showed 31 and 13% inhibitions at 50 and 25 μg/mL, respectively; however, the same was inactive at 10 μg/mL.

**Table 1**

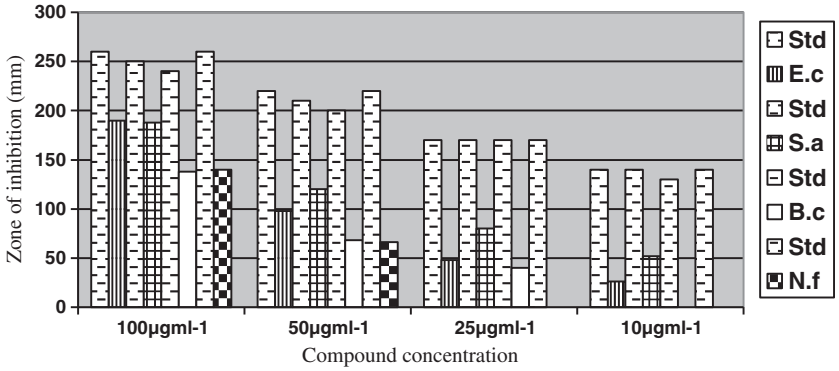
Antibacterial activities of coumarins **2–7**.

| | *Escherichia coli* (Gram-negative) | | | | *Staphylococcus aureus* (Gram-positive) | | | |
|---|---|---|---|---|---|---|---|---|
| Comp | mm (%) | mm (%) | mm (%) | mm (%) | mm (%) | mm (%) | mm (%) | mm (%) |
| **2** | 144 | 045 | Nil | Nil | 126 | 066 | Nil | Nil |
| | (51) | (12) | — | — | (46) | (24) | — | — |
| **3** | 158 | 062 | Nil | Nil | 128 | 080 | 040 | Nil |
| | (57) | (21) | — | — | (47) | (31) | (13) | — |
| **4** | 177 | 086 | 035 | Nil | 180 | 122 | 068 | 034 |
| | (65) | (33) | (10) | — | (69) | (53) | (32) | (11) |
| **5** | 155 | 070 | 026 | Nil | 165 | 110 | 060 | 028 |
| | (56) | (25) | (04) | — | (63) | (47) | (26) | (06) |
| **6** | 178 | 090 | 038 | Nil | 170 | 116 | 064 | 038 |
| | (65) | (35) | (12) | — | (65) | (50) | (29) | (15) |
| **7** | 190 | 098 | 048 | 026 | 188 | 120 | 080 | 052 |
| | (70) | (39) | (18) | (05) | (73) | (52) | (40) | (26) |
| Std[a] | 260 | 220 | 170 | 140 | 250 | 210 | 170 | 140 |
| Ctrl[b] | 020 | 020 | 020 | 020 | 020 | 020 | 020 | 020 |
| Cons[c] | 100 | 50 | 25 | 10 | 100 | 50 | 25 | 10 |
| | *Bacillus cirrhosis* (Gram-positive) | | | | *Neisseria flavescens* (Gram-negative) | | | |
| **2** | 098 | 035 | Nil | Nil | 090 | Nil | Nil | Nil |
| | (35) | (08) | — | — | (29) | — | — | — |
| **3** | 110 | 044 | Nil | Nil | 116 | 044 | Nil | Nil |
| | (40) | (13) | — | — | (40) | (12) | — | — |
| **4** | 122 | 064 | 035 | Nil | 130 | 060 | Nil | Nil |
| | (46) | (24) | (10) | — | (45) | (20) | — | — |
| **5** | 130 | 052 | Nil | Nil | 126 | 055 | Nil | Nil |
| | (50) | (17) | — | — | (44) | (17) | — | — |
| **6** | 130 | 057 | 035 | Nil | 133 | 060 | Nil | Nil |
| | (50) | (20) | (10) | — | (47) | (20) | — | — |
| **7** | 138 | 068 | 040 | Nil | 140 | 066 | Nil | Nil |
| | (53) | (26) | (13) | — | (50) | (23) | — | — |
| Std[a] | 240 | 200 | 170 | 130 | 260 | 220 | 170 | 140 |
| Ctrl[b] | 020 | 020 | 020 | 020 | 020 | 020 | 020 | 020 |
| Cons[c] | 100 | 50 | 25 | 10 | 100 | 50 | 25 | 10 |

[a]Standard: chloramphenicol was used as standard, 100% inhibition at each concentration.
[b]Control: DMF was used as a solvent control.
[c]Concentrations: the compound and the standard were tested in four concentrations, viz. 100, 50, 25, and 10 μg/mL.

The corresponding salt **4** was found to be little more active as compared with **3** and showed 10% zone of inhibition of *E.c* and *B.c* at 25 μg/mL concentration; however, there was no activity found for *N.f* at that concentration. Compound **4** was even active at minimum inhibitory concentration (MIC) 10 μg/mL and showed 11% inhibition at 10 μg/mL for *S.a.*; however, it was inactive for *E.c* and *B.c* at that concentration. Upon the introduction of dichloroacetamido group, coumarin **5** showed poor activity as compared with **4**. Around 4% of inhibition of *E.c* at 25 μg/mL was observed; however, there was no activity for *B.c* and *N.f* at that concentration. Perhaps in compound **5**, 25, and 6% inhibitions were observed at MIC 20 and 10 μg/mL, respectively, for *S.a*. In the introduction of hydroxyl methyl group in **5**, compound **6** was found to be little more active and showed good profile of 12 and 10% inhibitions at 25 μg/mL for *E.c* and *B.c*, respectively. The same was even better active for *S.a* and showed 29% inhibition at 25 μg/mL concentration and 15% inhibition at MIC 10 μg/mL. However, the same compound was not active for other bacteria at that concentration. Upon the reduction of ketone function, benzodipyran analog of chloramphenicol **7** showed potent activity of 40 and 26% inhibitions of *E.c* at 25 and 10 μg/mL, respectively. Compound **7** was also active for *E.c* and showed around 18% inhibition at 25 μg/mL and 5% inhibition at MIC 10 μg/mL. However, the same was inactive for *B.c* and *N.f* at MIC 10 μg/mL; perhaps, it was active at 25 μg/mL for *B.c* and showed 13% inhibition and 23% activity at 50 μg/mL for *N.f.* The graphical representation of antibacterial activities of benzodipyran analog of chloramphenicol **7** and chloramphenicol (Std) at different concentrations is given in Figure 1.

**Antifungal activity.** The antifungal activity of the compound **2** (Table 2) showed 10 and 27% zones of inhibition of *Aspirgillus niger* (*A.n*) and *Rhizoctonia bataticola* (*R.b*) at 50 μg/mL concentration; the same compound showed 5% inhibition of *Penicillium* (*P*) at that concentration. However, no antifungal activity of **2** was observed at reduced concentration. Upon the introduction of bromine to the side chain, coumarin **3** was found to be little more active and showed 21% inhibition of *A.n* and 15 and 12% inhibitions of *P* and *Candida albicans* (*C.a*), respectively, at 50 μg/mL. Perhaps, the same compound was moderately active for *R.b* and showed 36 and 14% inhibitions at 50 and 25 μg/mL, respectively; however, it was inactive at 10 μg/mL. The corresponding salt **4** was found to be little more active as compared with **3** and showed 31 and 23% inhibitions of *A.n* and *P*, respectively, at 50 μg/mL. The same compound **4** was even active at 25 μg/mL, and hence, 6 and 10% zones of inhibition of *A.n* and *P*, respectively, was observed; however, it was inactive for *C.a* at 25 μg/mL and for *A.n* and *P* at reduced MIC of 10 μg/mL. However, coumarin **4** showed good activity 50, 28, and 8% inhibitions at MIC 50, 25, and 10 μg/mL, respectively, for *R.b*. Upon the introduction of dichloroacetamido group, coumarin derivative **5** showed little poor activity as compared with **4** and showed 30% of inhibition for *A.n* at 50 μg/mL and no activity at reduced concentration. Perhaps, compound **5** was active at 25 μg/mL for *R.b* and showed 24% inhibition. However, the same was not active for *P* and *C.a* even at 25 μg/mL. Towards the introduction of hydroxyl methyl group, compound **6** was found to be more active and showed very good activity of 50, 30, and 12% of inhibition at MIC 50, 25, and 10 μg/mL, respectively, for *R.b*; however, the same compound was not active for other fungi at MIC of 10 μg/mL. Perhaps, around 32 and 6% activities of **6** were observed at 50 and 25 μg/mL, respectively, for *A.n*, and moreover, around 26 and 16% activities of coumarin **6** were observed at 50 and 25 μg/mL, respectively, for *P*. Upon the reduction of ketone function, benzodipyran analog of chloramphenicol **7** showed potent activity of 53, 38, and 24% of inhibition at MIC 50, 25, and 10 μg/mL, respectively, for *R.b* and 31, 22, and 7% of inhibition at MIC 50, 25, and 10 μg/mL, respectively, for *P*. However, coumarin **7** was active for fungi *A.n* and *C.a*




Escherichia coli (E.c), Staphylococcus aureus (S.a), Bacillus cirrosis (B.c), Neisseria flavescns (N.f) and Chloramphenicol (Std).

**Figure 1.** Antibacterial activities of **7** and standard at different concentrations.

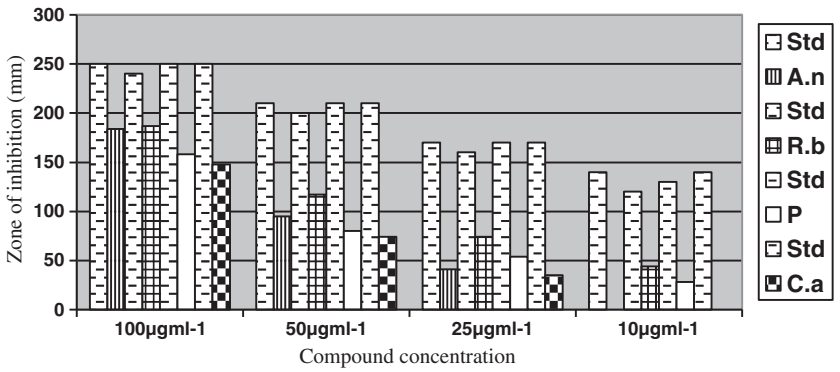
**Table 2**

Antifungal activities of coumarins **2–7**.

| | *Aspirgillus niger* | | | | *Rhizoctonia bataticola* | | | |
|---|---|---|---|---|---|---|---|---|
| Comp | mm (%) | mm (%) | mm (%) | mm (%) | mm (%) | mm (%) | mm (%) | mm (%) |
| **2** | 138 | 040 | Nil | Nil | 120 | 070 | Nil | Nil |
| | (51) | (10) | — | — | (45) | (27) | — | — |
| **3** | 150 | 060 | Nil | Nil | 133 | 086 | 040 | Nil |
| | (56) | (21) | — | — | (51) | (36) | (14) | — |
| **4** | 170 | 080 | 030 | Nil | 166 | 110 | 060 | 028 |
| | (65) | (31) | (06) | — | (66) | (50) | (28) | (08) |
| **5** | 166 | 078 | Nil | Nil | 158 | 102 | 054 | Nil |
| | (63) | (30) | — | — | (67) | (45) | (24) | — |
| **6** | 172 | 082 | 030 | Nil | 164 | 111 | 062 | 032 |
| | (66) | (32) | (06) | — | (65) | (50) | (30) | (12) |
| **7** | 184 | 095 | 041 | Nil | 187 | 117 | 074 | 044 |
| | (71) | (39) | (14) | — | (75) | (53) | (38) | (24) |
| Std[a] | 250 | 210 | 170 | 140 | 240 | 200 | 160 | 120 |
| Ctrl[b] | 020 | 020 | 020 | 020 | 020 | 020 | 020 | 020 |
| Cons[c] | 100 | 50 | 25 | 10 | 100 | 50 | 25 | 10 |
| | *Penicillium* | | | | *Candida albicans* | | | |
| **2** | 080 | 030 | Nil | Nil | 040 | Nil | Nil | Nil |
| | (26) | (05) | — | — | (08) | — | — | — |
| **3** | 120 | 050 | Nil | Nil | 122 | 044 | Nil | Nil |
| | (43) | (15) | — | — | (44) | (12) | — | — |
| **4** | 130 | 064 | 035 | Nil | 130 | 072 | Nil | Nil |
| | (47) | (23) | (10) | — | (47) | (27) | — | — |
| **5** | 133 | 055 | Nil | Nil | 132 | 050 | Nil | Nil |
| | (48) | (18) | — | — | (48) | (16) | — | — |
| **6** | 145 | 070 | 044 | Nil | 140 | 066 | Nil | Nil |
| | (54) | (26) | (16) | — | (52) | (24) | — | — |
| **7** | 158 | 080 | 054 | 028 | 148 | 074 | 035 | Nil |
| | (60) | (31) | (22) | (07) | (55) | (28) | (10) | — |
| Std[a] | 250 | 210 | 170 | 130 | 250 | 210 | 170 | 140 |
| Ctrl[b] | 020 | 020 | 020 | 020 | 020 | 020 | 020 | 020 |
| Cons[c] | 100 | 50 | 25 | 10 | 100 | 50 | 25 | 10 |

[a]Standard: chloramphenicol was used as standard, 100% inhibition at each concentration.
[b]Control: DMF was used as a solvent control.
[c]Concentrations: the compound and the standard were tested in four concentrations, viz. 100, 50µ, 25, and 10 µg/mL.

only upto MIC of 25 µg/mL and showed, respectively, 14 and 10% activities, and at reduced concentration, it was found to be inactive. All the compounds inhibit the bacteria and fungi via the same mechanism as control and standard. The graphical representation of antifungal activities of benzodipyran analog of chloramphenicol **7** and chloramphenicol (Std) at different concentrations is given in Figure 2.




Aspirgillus niger (A.n), Rhizoctonia bataticola (R.b), Penicillium (p), Candida albicans (C.a) and Chloramphenicol (Std).

**Figure 2.** Antifungal activities of **7** and standard at different concentrations.

## CONCLUSION

The present study has shown that for both antibacterial and antifungal activities of series of coumarin derivatives (**2–7**), involving four strains of bacteria and fungi, in four concentrations (100, 50, 25, and 10 μg/mL, respectively), it is observed that the benzodipyran analog of chloramphenicol **7** was most active against Gram-positive bacteria, *S.a*, and the fungal strains *R.b* and *P* even at 10 μg/mL and showed moderate activity for other bacterial and fungal organisms when compared with their precursors (**2–6**). In this paper, we have established the detail structure–antimicrobial activity relationship of **2–7** with standard chloramphenicol (drug sample). In observation, we found that introduction of active 2-dichloroacetamidopropandiol side chain to benzodiparan enhances both antibacterial and antifungal activities of compound. Our findings will have high impact on chemists and biochemists for further investigation in this field in search of chloramphenicol-containing antimicrobial agents.

## EXPERIMENTAL

**General.** The melting points of the products were determined by open capillaries on a Buchi apparatus (Thomas Scientific, Swedesboro, NJ) and are uncorrected. The IR spectra were recorded on a Nicolet Impact-410 FTIR (Thomas Scientific, Swedesboro, NJ) spectrophotometer, using KBr pellets. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AC-300 F, 300 MHz spectrometer in $CDCl_3$, and DMSO-$d_6$ by using TMS as an internal standard with $^1$H resonant frequency of 300 MHz and $^{13}$C resonant frequency of 75 MHz. $D_2O$ exchange was applied to confirm the assignment of the signals of NH protons. TFA was also used in $CDCl_3$ for insoluble compounds. The mass spectra were recorded on an Autospec ESI-MS (Thomas Scientific, Swedesboro, NJ). The elemental analysis was carried out by using Heraus CHN rapid analyzer (Bamko Surplus Process EQUIPMENT LLC,Texas City, TX). All the compounds gave C, H, and N analysis within ±0.4% of the theoretical values. The homogeneity of the compounds was described by TLC on aluminum silica gel 60 $F_{254}$ (Merck) detected by UV light (254 nm) and iodine vapors. The reagents were all analytical reagent-grade or chemically pure. All solvents were dried, deoxygenated, and redistilled before use.

***9-Acetyl-4-methyl-pyrano (2,3-f) chromene-2,8-dione (2).*** A mixture of 7-hydroxy-4-methyl-2-oxo-2*H*-chromene-8-carbaldehyde **1** (1.5 mmol) and ethylacetoacetate (1.5 mmol) in absolute alcohol (15 mL) and catalytic amount of piperidine was stirred for 4 h at room temperature. The reaction was monitored by TLC; after completion of the reaction, the separated solid was filtered, washed with excess of cold alcohol, dried, and crystallized from ethanol and dioxan mixture. Nature: pale green crystalline solid (ethanol + dioxan); yield: 78%; mp 140–142°C; IR (KBr): 1747, 1723, 1572 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$ + TFA, δppm): 2.55 (s, 3H, C4-$CH_3$), 2.74 (s, 3H, acetyl-$CH_3$), 6.45 (s, 1H, C3-H), 7.34–7.37 (d, 1H, C6-H, $J$ = 9 Hz), 7.93–7.96 (d, 1H, C5-H, $J$ = 9 Hz), 9.06 (s, 1H, C10-H); $^{13}$C NMR (75 MHz, $CDCl_3$ + TFA, δppm): 196.30, 162.40, 160.10, 159.30, 153.60, 151.20, 143.80, 132.60, 125.66, 116.20, 114.28, 113.45, 109.17, 30.10, 19.08; ESI-MS: *m/z* 271 (M + 1) (100), *Anal*. Calcd for $C_{15}H_{10}O_5$ (%): C, 66.67, H, 3.73; found (%): C, 66.40, H, 3.42.

***9-(2-Bromoacetyl)-4-methyl-pyrano (2,3-f) chromene-2,8-dione (3).*** Liquid bromine (6 mmol) in dry chloroform (10 mL) was added dropwise to the mixture of 9-acetyl-4-methyl-pyrano (2,3-*f*) chromene-2,8-dione **2** (6 mmol) in dry (alcohol free) chloroform (8 mL). The reaction mixture was stirred for 8 h at room temperature. The reaction was monitored by TLC; after completion of the reaction, the separated solid was filtered, washed with excess of cold alcohol, dried, and crystallized from acetic acid. Nature: pale yellow crystalline solid (acetic acid); yield: 70%; mp 190–192°C; IR (KBr): 1738, 1681, 1569 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$, δppm): 2.48 (s, 3H, C4-$CH_3$), 4.96 (s, 2H, Br-$CH_2$), 6.55 (s, 1H, C3-H), 7.44–7.47 (d, 1H, C6-H, $J$ = 9 Hz), 8.13–8.16 (d, 1H, C5-H, $J$ = 9 Hz), 8.82 (s, 1H, C10-H); $^{13}$C NMR (100 MHz, DMSO-$d_6$, δppm): 196.33, 158.48, 157.37, 156.04, 153.40, 150.63, 139.40, 131.07, 122.70, 115.70, 113.40, 112.27, 107.22, 68.06, 18.29; ESI-MS: *m/z* 349 (100), 351 (M + 2) (100); *Anal*. Calcd for $C_{15}H_9BrO_5$ (%): C, 51.60, H, 2.60; found (%): C, 51.40, H, 2.32.

***9-(2-Amino-acetyl)-4-methyl-pyrano (2,3-f) chromene-2,8-dione-hydrocloride (4).*** Powdered anhydrous hexamine (5 mmol) was dissolved in dry chloroform (25 mL), and the solution was stirred for 30 min at room temperature. To this stirred solution, the solution of 9-(2-bromoacetyl)-4-methyl-pyrano (2,3-*f*) chromene-2,8-dione **3** (5 mmol) in dry chloroform (30 mL) was added. The stirring was continued for 3 h at room temperature. The reaction was monitored by TLC; after completion of the reaction, the separated bright yellow colored hexamine adduct was filtered, washed with excess of chloroform, and dried. To the dry hexamine adduct, conc HCl (20 mL) and dry alcohol (30 mL) was added, the reaction mixture was stirred for 6 h at 85°C, and the resulting clear solution was left overnight. The separated solid was filtered and washed with chilled solution of ethanol and ether (8:2) to obtain 95% of the corresponding pure colorless amine hydrochloride salt. Nature: colorless crystalline solid; yield: 78%; mp 340–343°C; IR (KBr): 3320, 1740, 1670, 1569 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$, δppm): 2.50 (s, 3H, C4-$CH_3$), 4.10 (s, 2H, $NH_2$-$C\underline{H}_2$), 6.60 (s, 1H, C3-H), 7.32–7.35 (d, 1H, C6-H, $J$ = 8.5 Hz), 7.48 (s, 2 H, $NH_2$), 7.95–7.98 (d, 1H, C5-H, $J$ = 8.5 Hz), 8.80 (s, 1H, C10-H); $^{13}$C NMR (100 MHz, DMSO-$d_6$, δppm): 195.20, 159.18, 158.27, 156.04, 153.40, 150.40, 140.40, 131.07, 122.70, 115.70, 113.40, 112.27, 109.22, 65.06, 17.30; ESI-MS: *m/z* 321, *Anal*. Calcd for $C_{15}H_{12}O_5NCl$ (%): C, 56.07, H, 3.87; found (%): C, 56.02, H, 3.82.

***2, 2-Dichloro-N-{2-[4-methyl-2, 8-dioxo-2H,8H-pyrano (2,3-f)-chromene-9-yl]-2-oxoethyl}-acetamide (5).*** A mixture of 9-(2-amino-acetyl)-4-methyl-pyrano (2,3-*f*) chromene-2,8-dione-hydrocloride **4** (3 mmol) and dichloroacetyl chloride (4 mmol) was stirred for 2 h at 80–85°C. The reaction was monitored by TLC; after completion of the reaction, the reaction mixture was allowed to attain the room temperature. The separated solid was filtered, washed with excess of cold water, dried, and crystallized from ethanol and dioxan mixture. Nature: colorless crystalline solid (ethanol + dioxan); yield: 62%; mp 231–233°C; IR (KBr): 3432, 1728, 1698, 1662, 1560 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$, δppm): 2.42 (s, 3H, C4-$CH_3$), 4.47 (s, 2H, $COCH_2$), 6.57 (s, 1H, C3-H), 6.81 (s, 1H, $COC\underline{H}Cl_2$), 7.31–7.34 (d, 1H, C6-H, $J$ = 9 Hz), 7.51–7.54 (d, 1H, C5-H, $J$ = 9 Hz), 7.69 (s, 1H, NH), 8.33 (s, 1H, C10-H); $^{13}$C NMR (75 MHz, $CDCl_3$, δppm): 193.40, 165.20, 162.44, 159.88, 149.77, 143.44, 141.40, 133.40, 130.17, 128.65, 126.38, 122.70, 119.42, 108.12, 73.60, 51.22, 18.20; ESI-MS: *m/z* 396 (M + 1) (50),

398 (M + 2) (15) 400 (M + 4) (12); *Anal*. Calcd for $C_{17}H_{11}Cl_2NO_6$ (%): C, 51.54, H, 2.80, N, 3.54; found (%): C, 51.20, H, 2.62, N, 3.38.

***2, 2-Dichloro-N-{1-hydroxymethyl-2-[4-methyl-2,8-dioxo-2H, 8H-pyrano (2,3f)-chromene-9-yl]-2-oxoethyl}-acetamide (6).*** A mixture of 2, 2-dichloro-*N*-{2-[4-methyl-2, 8-dioxo-2*H*,8*H*-pyrano (2,3-*f*)-chromene-9-yl]-2-oxoethyl}-acetamide **5** (3 mmol), paraformaldehyde (3 mmol), and powered anhydrous $K_2CO_3$ (4 mmol) in dry methanol (15 mL) was stirred for 3 h at room temperature. The reaction was monitored by TLC; after completion of the reaction, the separated brown-colored solid was filtered, washed with 20 mL dilute HCl (10%) and then with excess of cold water, dried, and chromatographed with hexane and ethyl acetate mixture (80:20) and crystallized from ethanol and dioxan mixture. Nature: brown solid (ethanol + dioxan); yield: 58%; mp 198–200°C; IR (KBr): 3418, 3271, 1710, 1706, 1651, 1568 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δppm): 2.42 (s, 3H, C4-$CH_3$), 3.72 (s, 1H, OH, $D_2O$ exchangeable), 4.25–4.71 (m, 3H, COC$\underline{H}$C$\underline{H}_2$), 6.15 (s, 1H, C3-H), 6.83 (s, 1H, COC$\underline{H}$Cl$_2$), 6.90–6.93 (d, 1H, C6-H, $J$ = 9 Hz), 7.49–7.52 (d, 1H, C5-H, $J$ = 9 Hz), 7.61 (s, 1H, NH, $D_2O$ exchangeable), 8.21 (s, 1H, C10-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δppm): 192.20, 167.33, 163.24, 160.18, 157.42, 148.57, 145.66, 134.80, 131.72, 126.45, 124.18, 121.20, 117.32, 107.88, 72.40, 63.78, 55.51, 19.08; ESI-MS: *m/z* 426 (M + 1) (50), 428 (M + 2) (16), 430 (M + 4) (13); *Anal*. Calcd for $C_{18}H_{13}Cl_2NO_7$ (%): C, 50.73, H, 3.07, N, 3.29; found (%): C, 50.50, H, 2.92, N, 3.10.

***2, 2-Dichloro-N-{2-hydroxy-1-hydroxymethyl-2-[4-methyl-2,8-dioxo-2H, 8H-pyrano (2,3-f)-chromene-9-yl]-2-oxoethyl}-acetamide (7).*** A mixture of 2, 2-dichloro-*N*-{1-hydroxy-methyl-2-[4-methyl-2, 8-dioxo-2*H*, 8*H*-pyrano (2, 3-*f*)-chro-mene-9-yl]-2-oxoethyl}-acetamide **6** (2 mmol) and aluminum isopropoxide (3 mmol) in isopropanol (10 mL) was refluxed on a water bath for 6 h. After cooling the reaction mixture, a 5 mL NaCl solution (10%) was added and the refluxed for another 30 min; the aluminum hydroxide so precipitated was removed by filtration. The filtrate was washed with excess of ether (30 mL) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the reddish brown paste so obtained was chromatographed with hexane and ethyl acetate mixture (80:20) and then crystallized with benzene and ethyl acetate mixture. Nature: brown solid (benzene + ethyl acetate); yield: 20%; mp 178–180°C; IR (KBr): 3444, 1734, 1683, 1547 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δppm): 2.44 (s, 3H, C4-$CH_3$), 3.72 (s, 2H, OH, $D_2O$ exchangeable), 4.39–5.14 (m, 4H, C$\underline{H}$OHC$\underline{H}$C$\underline{H}_2$), 6.23 (s, 1H, C3-H), 6.98 (s, 1H, COC$\underline{H}$Cl$_2$), 7.07–7.10 (d, 1H, C6-H, $J$ = 9 Hz), 7.58–7.61 (d, 1H, C5-H, $J$ = 9 Hz), 7.78 (s, 1H, NH, $D_2O$ exchangeable), 8.12 (s, 1H, C10-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δppm):165.43, 161.14, 160.10, 158.02, 147.17, 144.66, 134.80, 131.42, 126.41, 123.98, 121.20, 117.30, 109.18, 71.51, 59.20, 49.45, 18.24; ESI-MS: *m/z* 428 (M + 1) (50%), *m/z* 430 (M + 2) (17%), *m/z* 432 (M + 4) (15%).

**Antimicrobial screening.** All the newly synthesized coumarin derivatives were tested for *in vitro* antibacterial and antifungal activity by cup plate method [30–32]. Four bacterial microorganisms *E.c* (Gram-negative), *S.a* (Gram-positive), *B.c* (Gram-negative), and *N.f* (Gram-negative) and four fungal microorganisms *A.n*, *R.b*, *P*, and *C.a* were used. DMF was used as a solvent control, and chloramphenicol (drug) was used as a standard. The tests were carried out at concentrations of 100, 50, and 25, and 10 μg/mL. After 48 h of incubation at 37°C, the zone of inhibition was measured in mm. The percentage inhibition of test compounds was related to the standard whose zone of inhibition was taken as 100%. It was calculated as percentage of reciprocal of zone of inhibition of test compound to standard with respect to control, in other words by relating the zone of inhibition of the test compound ($ZOI_{\text{test compound}}$) to those of the standard ($ZOI_{\text{standard}}$, taken as 100%) and control ($ZOI_{\text{control}}$) as follows:

$$\% \text{ inhibition} = \frac{ZOI_{\text{test compound}} - ZOI_{\text{control}}}{ZOI_{\text{standard}} - ZOI_{\text{control}}} \times 100\%$$

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## REFERENCES AND NOTES

[1] Ehrlich, J.; Bartz, Q. R.; Smith, R. M.; Josylyn, D. A.; Burkholder, P. R. Science 1947, 106, 417.

[2] Saumen, H.; Karmakar, A.; Maji, T.; Medda, A. K. Tetrahedron 2006, 62, 8959.

[3] Veeresa, G.; Datta, A. Tetrahedron Lett 1998, 39, 8503.

[4] George, S.; Srinivasarao, V.; Sudalai, A. Tetrahedron 2006, 62, 10202.

[5] Boruwa, J.; Jagat, C.; Gogoi, S.; Barua, N. C. Tetrahedron Lett 2005, 46, 1743.

[6] Bhasker, G.; Satish Kumar, V.; Venkatesh Rao, B. Tetrahedron: Asymmetry. 2004, 15, 1279.

[7] Loncaric, C.; Wulff, W. D. Org Lett 2001, 23, 3675.

[8] Rossi, S.; Trave, R. IlFarmaco (Pavia) Ed. Sci. 1990, 15, 396.

[9] Van der Meer, S.; Kofman, H.; Veldastarg, R. A. Rec. Trav. Chim. 1993, 72, 236.

[10] Bevinkatti, H. S.; Badiger, V. V. Arch. Pharm (Weinheim). 1981, 314, 162.

[11] National Toxicology Program. Toxicology and carcinogenesis studies of coumarin. (CAS No. 91-64-5) in F344/N rats and B6C3F1 mice. Technical Report NTP TR 422. Research Triangle Park, NC: US Department of Health and Human Services: 1992, NIH Publication No. 92–3153.

[12] Vogel Gilbert's, A. Ann Phys 1820, 64, 161.

[13] Smyth, T.; Ramachandran, V. N.; Smyth, W. F. International J. Antimicrobial Agents. 2009, 33, 421.

[14] Kulkarni, M. V.; Kulkarni, G. M.; Sun, C. M. Curr Med Chem 2006, 13, 2795.

[15] Fylaktakidou, K. C.; Hadjipavlou-Litina, D. J.; Litinas, K. E.; Nicolaides, D. N. Curr Pharm Des 2004, 10, 3813.

[16] Fylaktakidou, K. C.; Hadjipavlou-Litina, D. J.; Litinas, K. E.; Nicolaides, D. N. J. Heterocycl. Chem. 1996, 33, 967.

[17] Fylaktakidou, K. C.; Hadjipavlou-Litina, D. J.; Litinas, K. E.; Nicolaides, D. N. Eur J Med Chem 1998, 33, 715.

[18] Fylaktakidou, K. C.; Hadjipavlou-Litina, D. J.; Litinas, K. E.; Nicolaides, D. N. J. Heterocycl. Chem. 1998, 35, 619.

[19] Georgia, M.; Antreas, A.; Igglessi-Markopoulou, O.; Hadjipavlou-Litina, D. J. Eur J Med Chem 2009, 44, 3020.

[20] Mulwad, V. V.; Ali Mir, A J. Korean. Chem. Soc. 2008, 52, 6.

[21] Ghate, M. D.; Kulkarni, M. V.; Shobha, R.; Kattimani, S. Y. Eur J Med Chem 2003, 38, 297.

[22] Shastri, L. A.; Ghate, M. D.; Kulkarni, M. V. Ind.J. Chem. 2004, 43B, 2416.

[23] Kalkhambkar, R. G.; Kulkarni, G. M.; Rao, N. R.; Shivakumar, H. Eur J Med Chem 2007, 42, 1272.

[24] Kalkhambkar, R. G.; Kulkarni, G. M.; Kamanavalli, C. M.; Premkumar, N.; Asdaq, S. M. B.; Sun, C. M. Eur J Med Chem 2008, 43, 2178.

[25] Patil, M. B.; Kulkarni, M. V.; Shivashankar, K.; Rasal, V. P.; Patel, H.; Mutha, S. S.; Mohite, A. A. Eur J Med Chem 2010, 45, 1151.

[26] Kulkarni, G. M.; Kulkarni, M. V.; Patil, V. D. J. Ind. Chem. Soc. 1990, 67, 693.

[27] Kulkarni, G. M.; Kulkarni, M. V.; Patil, V. D. Ind.J. Chem. Soc. 1991, 30B, 970.

[28] Fitton, A. O.; Smalley, R. K. *Practical Heterocyclic Chemistry*; Acad Press: London, 1998; p P97.

[29] Duff, J. C. J Chem Soc 1941, 547. DOI: 10.1039/JR9410000547

[30] Hugo, W.B.; Russel, A. B.; Denyer, S. P.; Hodges, N. A.; German, S. P.; *Pharmaceutical Microbiology* 4th Ed.; Blackwell Scientific Publication: London, 1997; p 187.

[31] Casman, E. Am.J. Clin. Path. 1997, 17, 281.

[32] Dughid, J. P.; Marimon, B. P.; Swain, R. H. *Medicinal Microbiology*; ELBS: London, 1993; p P12.