INTRODUCTION

Over the past decade, the incidence of bacterial infections has increased worldwide, particularly in Indian subcontinent, portions of South America, and tropical fraction of Africa [1,2]. In spite of the availability of a large number of antibiotics and chemotherapeutics, the treatment of bacterial diseases still remains a challenging problem due to a number of factors such as the emergence of drug-resistant strains, severe adverse effects, narrow antibacterial spectrum, and association of microbial infections with other diseases mainly in immunocompromised patients [3-5]. Despite the major cause of morbidity and mortality, these infections are given low priority, and most importantly, the pipeline for new antibiotics has paradoxically experienced a long-term decline [6].

Fungal infections also pose a continuous and serious threat to human health and life. It is estimated that about 1.2 billion people worldwide suffer from fungal diseases [7]. The opportunistic fungal pathogens belonging to Candida spp., Cryptococcus spp., and Aspergillus spp. account for the majority of documented invasive fungal infections in humans. These infections are responsible for high morbidity and mortality in immune-compromised patients such as those undergoing organ transplant or anticancer chemotherapy and patients with AIDS [8]. The existing mainstay antifungal drugs such as fluoroquinolones, amphoterin-B (Amp), and azoles are associated with a number of severe side effects and development of resistance [9]. Thus, there is a pressing need for the development of alternative antimicrobial drugs involving new molecules with a broader spectrum and less side effects.

Coumarins are a group of oxygen-containing heterocyclic compounds with a benzoannelated α-pyrene ring. They are found in a large number of bioactive natural and non-natural molecules. Due to structure variability, low toxicity, and low cost, coumarins play an important role in modern drug discovery and synthetic chemistry. Coumarin derivatives display a wide range of biological activities such as antimalarial, antimicrobial, anticancer, anti-inflammatory, antioxidant, antiviral, antitubercular, and antifilarial [10,11]. The pharmacological, biochemical, and therapeutical potential of coumarin compounds mainly depend on the substituent they bear on their basic chemical skeleton [12]. Another, unique feature of coumarin, which makes it a privileged structural framework, is its ability to interact readily with a diversity of enzymes and receptors in organisms through weak bond interactions. Therefore, substitution on the lactone and aromatic rings of a coumarin motif provides opportunities for modulation of biological activities and enhancement in pharmacokinetic properties. In recent years, coumarin derivatives have gained tremendous attention due to their applications in drug design and discovery [13,14].

Keeping in view of the above and in continuation of our work on the design and synthesis of coumarin compounds possessing antimicrobial and antifilarial properties [15,16], we report herein the synthesis and antimicrobial activity of a series of 7-benzamidocoumarins. The synthesized compounds were also evaluated for their chitinase inhibitory activity. To carry out this study, first, we screened six simple coumarin scaffolds for their antimicrobial properties. Out of these, the 4-methyl- and 4-chloromethyl-7-amino coumarins which showed the best activity were chosen as starting material for further optimization study. We hope this study may be useful in further design of coumarin-based molecules as antimicrobial drugs or prodrugs.

MATERIALS AND METHODS

Unless otherwise stated, all materials were obtained from commercial suppliers (SRL and Spectrochem Pvt., Ltd.) and were used without

ANTIMICROBIAL AND CHITINASE INHIBITORY ACTIVITIES OF 7-BENZAMIDOCOUMARINS

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ABSTRACT

Objective: The aim of this study was to examine in vitro antimicrobial activity of a series of 7-benzamidocoumarin derivatives against three Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus, and Bacillus pumilus), three Gram-negative bacteria (Escherichia coli, Klebsiella pneumonia, and Salmonella typhi), and three fungi (Candida albicans, Candida tropicalis, and Aspergillus niger) by 2-fold serial dilution method.

Materials and Methods: The compounds were synthesized by amide coupling of 7-amino-4-methyl coumarin 5 and 7-amino-4-chloromethyl coumarin 6 with different aromatic acids 7 (a-h) and heteroaromatic acids 8 (i-j) in the presence of phosphorous trichloride as an acid catalyst in acetonitrile. The synthesized compounds were evaluated for antimicrobial and chitinase inhibitory activity.

Results: Compounds 9, 11, 12, 21, and 23 showed good antibacterial activity with minimum inhibitory concentration (MIC) ranging from 6.25 to 50 µg/mL. Among them, compound 21 was the most active molecule with MIC 6.25 µg/mL against S. aureus and 6.25-25 µg/mL against B. pumilus and S. typhi. Compounds 11 and 21 were the most potent antifungal candidates with MIC 6.25-25 µg/mL against C. albicans and C. tropicalis. All the compounds were also evaluated for their chitinase inhibitory property and among them; compound 9 emerged as strong inhibitor of the enzyme.

Conclusion: Some of the compounds showed very good antimicrobial activities. Among these, compound 21 showing potent antimicrobial activities against five of the nine microbial strains examined in this study, was the most active compound of the series. Some of the compounds also showed chitinase inhibitory properties. This study also provides a starting point for investigating the structure-activity relationships (SARs) of synthetic 7-benzamidocoumarins.
further purification. Melting points were determined on a Buchi 510 apparatus and are uncorrected. Silica gel (60–120 mesh) from commercial supplier Spectrochem Pvt. Ltd. was used for column chromatography. Thin-layer chromatography was used to monitor the reactions, and the spots were visualized by spraying KMnO₄ reagent. IR spectra were recorded on JASCO FTIR 5300 in KBr from 400 to 4000 cm⁻¹. NMR spectra were recorded on JEOL AL 300 FT-NMR spectrometer using tetramethylsilane as an internal standard. The chemical shift values are on δ scale and the coupling constant (J) is in hertz (Hz).

Synthetic procedures

4-methyl-7-hydroxy-6-nitrocumarin 4

4-Methyl-7-hydroxycoumarin (250 mg) was dissolved in 2 mL of conc. sulfuric acid at room temperature and cooled to 0°C. To this solution, a chilled nitrating mixture (3 mL of concentrated nitric acid added to 9 mL of concentrated sulfuric acid) was added. The solution was stirred at 0°C for 3 h and then poured over crushed ice. The precipitated yellow product was filtered off, washed with ice cold water and recrystallized from ethanol to give compound 4 as yellow green crystals [17].

7-Amino-4-methyl-2H-chromen-2-one 5

First, ethyl [3-hydroxyphenyl] carbamate was prepared by the following method: To stirred suspension of 3-aminophenol (5 g, 45.5 mmol) in 20 mL of anhydrous diethyl ether, ethyl chloroformate (6 g, 54.6 mmol) was added. The reaction mixture was stirred for 2 h at room temperature. After the completion of the reaction, the amine hydrochloride was removed by filtration. The filtrate was then evaporated to give white solid, which was recrystallized from petroleum ether to afford carbamate derivative as white crystalline solid. To the stirred solution of the carbamate (2.0 g, 11.0 mmol) in 25 mL of 70% H₂SO₄ in ethanol (18 mL H₂SO₄, 7 mL EtOH), ethyl acetacetate (218 g, 13.25 mmol) was added and the reaction mixture was stirred at room temperature for 4 h. The mixture was then poured in 50 mL of ice cold water, giving ethyl [4-(methyl-2-oxo-2H-chromen-7-yl)] carbamate white precipitate that was crystallized from absolute EtOH. Ethyl (4-chloromethyl-2-oxo-2H-chromen-7-yl) carbamate was also prepared by the same procedure using ethyl chloroacetate.

In the next sequence of reaction, 7-carbethoxyaminocoumarin 1.5 g, 5.33 mmol) was dissolved in a solution of 10 mL of concentrated H₂SO₄ and 10 mL of glacial acetic acid. The mixture was heated at reflux for 2 h. After cooling to room temperature, the mixture was poured in 50 mL of ice cold water. At 0°C, 50% NaOH was added until the solution resulted slightly alkaline (pH = 9–10). The obtained precipitate was filtered and washed with ice cold water (10 mL) to afford the desired 7-amino-4-methyl-2H-chromen-2-one 5 as light yellow solid [18,19].

7-amino-4-chloromethyl-2H-chromen-2-one 6

The reaction of 7-carbethoxyaminocoumarin (2.5 g, 13.8 mmol) in the presence of 2 mL of concentrated H₂SO₄ and 2 mL of glacial acetic acid under reflux condition for a period of 2 h as described above gave 7-amino-4-chloromethyl-2H-chromen-2-one 6 as light brown solid.

General procedure for the synthesis of 7-benzamidocoumarin derivatives 9–23

Amino coumarins 5 and 6 (1 mmol) and appropriate aromatic acids 7 (a–h) or hetero-aromatic acids 8 (i–j) (1.2 mmol) were dissolved in acetonitrile (5 mL), PCI₃ (1 mmol) and the reaction mixture was refluxed for 3–6 h. After completion of the reaction, the reaction mixture was quenched with a few drops of water. The solvent was evaporated; the residue was dissolved in ethyl acetate (10 mL) and washed twice with saturated NaHCO₃ solution. The organic layer was dried over Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using 2% CH₃OH in CH₂Cl₂ solvent system as mobile phase [20].

Determination of MIC

Synthesized compounds were evaluated on the basis of their antimicrobial activities examined in terms of MIC against six multiple drug-resistant bacterial test strains, namely B. subtilis MTCC 121, S. aureus MTCC 96, B. pneumoniae MTCC 3384, S. typhi MTCC 537, and E. coli MTCC1304 and three fungi C. albicans MTCC 3017, C. tropicalis MTCC 184, and A. niger MTCC 1344. MIC is the lowest concentration of the compound that will be able to inhibit the microbial growth under experimental conditions. Erythromycin (Ery) and Amp were used as standards for antibacterial and antifungal activity, respectively.

MIC of the synthesized compounds was determined by the micro broth dilution method using 96 well plates according to the method described by Vipra et al. [21] with some modification. The bacterial test organisms were grown in sterile nutrient broth (NB) at 30°C. From this stock solution certain inoculums were used to inoculate fresh medium, and the final inoculum size for test strains was adjusted to 10⁶ cfu/mL (colony-forming unit per milliliter). Fresh NB was taken as a negative control in the first well, whereas NB with test organisms were considered as a positive control in the last well. Stock solutions of 1 mg/mL of compounds were prepared. From this, 100 μg/mL concentrations were taken as starting solution in the second well and diluted serially up to the second last well. Microtiter plates were incubated at 30°C for overnight under the static condition and read using ELISA plate reader. Well containing the lowest concentration of the compounds at which no turbidity was observed considered as MIC.

Chitinase assay for coumarin derivatives

Chitinase inhibitory activity of the coumarin compounds was determined by Dinitrosalicylic acid (DNS) as mentioned by Miller using colloidal chitin as a substrate [22]. Colloidal chitin was prepared from the chitin powder. Five grams of chitin powder (C₃H₇O₆N) were gradually mixed with 6 mL concentrated HCl (32%) and incubated at room temperature with vigorous stirring for about 2 h on an orbital shaker. Ice-cold water was added to precipitate the chitin and this solution was further incubated overnight. After incubation, it was centrifuged at 10,000 rpm for 20 min at 4°C. The pellet was washed repeatedly with distilled water until the pH of the chitin suspension reached the pH of distilled water. Finally, the substrate was autoclaved and stored at 4°C.

Reaction mixture containing 100 μl of coumarin compound (stock solution 1 mg/mL), 1 ml colloidal chitin and 1 ml chitinase extracted from actinomycetes strain were taken in test tubes and incubated for 30 min at 37°C in shaking water bath. The reaction was stopped by adding 2 ml DNS reagent and kept in a boiling water bath for 5 min to develop the color. After cooling the test tubes for 10 min; color was observed against control. The concept lies in DNS reaction is the change in color from yellowish orange to brownish orange.

RESULTS AND DISCUSSION

Chemistry

Compounds 1–3 were purchased from the commercial supplier and used without further purification. Compound 4 was synthesized by nitration of 4-methyl-7-hydroxycoumarin under cold condition by earlier reported method [17]. Compounds 5 and 6 were prepared by following method. In the first step, protected amino phenol was nitration of 4-methyl-7-hydroxycoumarin under cold condition by earlier reported method [17]. Compounds 5 and 6 were prepared by following method. In the first step, protected amino phenol was

Further information and details are not provided in the given text.
substituted salicylic acids 7-a-c in CH$_2$CN in the presence of PCl$_3$ under reflux condition while compounds 12-14 and 15 were prepared by the reaction of 7-amino-4-chloromethyl coumarin 6 with substituted salicylic acids 7-a-c and unsubstituted salicylic acid 7d, respectively. Further, the synthesis of amidocoumarins 16-19 was carried out by reaction between compound 6 and benzoic acid derivatives 7 (e-h) bearing electron releasing and electron withdrawing groups as described above (Scheme 1). In the next sequence of reactions, few heteroaromatic acids such as 2-picolinic acid 8i and 2-pyrazinoic acid 8j were selected to synthesize 7-amidocoumarins 20-23 (Scheme 1). The spectroscopic data of all the compounds reported herein were found to be in good agreement with previously published data [20]. As a representative example, the structure elucidation of compound 19, obtained by the reaction between 7-amino-4-chloromethyl coumarin 6 and 2-methyl benzoic acid 7 h is described here. The compound exhibited absorption bands at 3331 and 1714 cm$^{-1}$ in the IR spectrum for N-H stretching and 5-lactone, respectively. In $^1$H NMR spectrum, -NH proton appeared at δ 10.3 as broad singlet while signal corresponding to H-6' proton of benzamide ring was observed as multiplet at δ 6.79. Doublet appeared at δ 7.81 with coupling constant of 2.4 Hz was due to the H-5 and H-6 protons of benzopyrone ring, respectively. Signal due to H-4' was found as multiplet at δ 7.50 with a coupling constant of 8.7 Hz were due to the H-5 and H-6 protons of benzopyrone ring, respectively. Signal due to H-4' was found as multiplet at δ 7.29 while multiplet at δ 7.38 was ascribed to the H-3' proton appeared at δ 7.17 as broad singlet while signal corresponding to H-3 of lactone ring, methylene protons (−C=CH$_2$) and methyl group at C-2' carbon, respectively.

4-Methyl-7-hydroxy-6-nitrocoumarin (4)
Yield: 78%; $^1$H NMR (DMSO-d$_6$, 300 MHz): δ 2.38 (s, 3H, CH$_3$), 6.18 (s, 1H), 6.37 (s, 1H), 6.58 (m, 1H), 7.36 (d, $J = 8.8$ Hz, 1H).

7-amino-4-chloromethyl-2H-chromen-2-one (5)
Yield 70%; $^1$H NMR (DMSO-d$_6$, 300 MHz): δ 2.40 (s, 3H, CH$_3$), 5.92 (s, 1H), 6.10 (brs, 2H), 6.37 (s, 1H), 6.58 (m, 1H), 7.36 (d, $J = 8.8$ Hz, 1H).

2-hydroxy-3-(4-methyl-2-oxo-2H-chromen-7-yl)-3,5-dinitrobenzamide (9)
Light yellow solid; yield: 75%; $^1$H NMR (CDCl$_3$, 300 MHz): δ 2.36 (s, 3H, CH$_3$), 6.46 (s, 1H), 7.25 (d, $J = 1.5$ Hz, 1H), 7.88 (d, $J = 8.5$ Hz, 1H), 7.93 (m, 2H), 8.57 (s, 1H), 9.65 (s, 1H).

- 2-hydroxy-3,5-diido-N-(4-methyl-2-oxo-2H-chromen-7-yl) benzamide (10)
Off white solid; yield: 79%; $^1$H NMR (DMSO-d$_6$, 500 MHz) & $^13$C NMR (DMSO-d$_6$, 500 MHz): δ 2.38 (s, 3H, CH$_3$), 6.18 (s, 1H), 7.36 (d, $J = 9.0$ Hz, 1H), 7.67 (d, $J = 8.5$ Hz, 1H), 7.73 (d, $J = 2.0$ Hz, 1H), 7.92 (s, 1H), 7.95 (s, 1H).

3,5-dibromo-2-hydroxy-N-(4-methyl-2-oxo-2H-chromen-7-yl) benzamide (11)
Off white solid; yield: 80%; $^1$H NMR (DMSO-d$_6$, 500 MHz) & $^13$C NMR (DMSO-d$_6$, 500 MHz): δ 2.38 (s, 3H, CH$_3$), 6.18 (s, 1H), 7.34 (d, $J = 8.5$ Hz, 1H), 7.66 (d, $J = 8.5$ Hz, 1H), 7.81 (m, 2H), 7.95 (s, 1H), 15.21 (s, 1H).

N-(4-chloromethyl)-2-oxo-2H-chromen-7-yl-2-hydroxy-3,5-dinitro benzamide (12)
Yellow solid; yield: 78%; $^1$H NMR (DMSO-d$_6$, 500 MHz) & $^13$C NMR (DMSO-d$_6$, 500 MHz): δ 4.95 (s, 2H), 6.72 (s, 1H), 7.47 (m, 2H), 7.79 (s, 1H), 8.02 (d, $J = 2.0$ Hz, 1H), 8.61 (s, 1H), 10.05 (s, 1H, NH).

N-(4-chloromethyl)-2-oxo-2H-chromen-7-yl-2-hydroxy-3,5-diido benzamide (13)
Off white solid; yield: 80%; $^1$H NMR (DMSO-d$_6$, 500 MHz) & $^13$C NMR (DMSO-d$_6$, 500 MHz): δ 4.96 (s, 2H), 6.57 (s, 1H), 7.71 (d, $J = 2.0$ Hz, 1H), 7.80 (m, 2H), 8.20 (m, 2H).

3,5-dibromo-N-(4-chloromethyl)-2-oxo-2H-chromen-7-yl)-2-hydroxy-3,5-dinitro benzamide (14)
Off white solid; yield: 79%; $^1$H NMR (DMSO-d$_6$, 500 MHz) & $^13$C NMR (DMSO-d$_6$, 500 MHz): δ 4.94 (s, 2H), 6.53 (s, 1H), 7.54 (dd, $J = 8.5$ and 2.0 Hz, 1H), 7.68 (dd, $J = 8.0$ Hz, 1H), 7.72 (d, $J = 8.5$ Hz, 1H), 8.34 (d, $J = 2.0$ Hz, 1H), 8.46 (d, $J = 2.0$ Hz, 1H), 10.85 (s, 1H, NH).

N-(4-chloromethyl)-2-oxo-2H-chromen-7-yl) benzamide (15)
Colorless solid; yield: 82%; $^1$H NMR (CDCl$_3$, 300 MHz) & $^13$C NMR (CDCl$_3$, 300 MHz): δ 4.60 (s, 2H), 6.56 (s, 1H), 6.94 (m, 2H), 7.39 (m, 1H), 7.67 (m, 2H), 7.89 (m, 2H), 10.84 (s, 1H).

N-(4-chloromethyl)-2-oxo-2H-chromen-7-yl) benzamide (16)
Colorless solid; yield: 81%; $^1$H NMR (CDCl$_3$, 300 MHz) & $^13$C NMR (CDCl$_3$, 300 MHz): δ 4.60 (s, 2H), 6.56 (s, 1H), 6.94 (m, 2H), 7.39 (m, 1H), 7.67 (m, 2H), 7.89 (m, 2H), 10.84 (s, 1H).

Scheme 1: Synthesis of 7-amidocoumarins
2-chloro-N-(4-chloromethyl)-2-oxo-2H-chromen-7-yl) benzamide (17)
Colorless solid; yield: 81%; mp: 146°C–147°C; IR (cm\(^{-1}\), KBr) \(\nu_{\text{max}}\) 3421, 1732, 1591, 1261; \(\delta\) NMR (CDCl\(_3\), 300 MHz) \(\delta\): 6.42 (s, 2H), 6.60 (s, 1H), 7.19 (m, 2H), 7.41 (m, 4H), 7.69 (d, \(J = 8.4\) Hz, 1H).

N-(4-chloromethyl)-2-oxo-2H-chromen-7-yl)-2-methoxy benzamide (18)
Colorless solid; yield: 80%; mp: 160°C–162°C; IR (cm\(^{-1}\), KBr) \(\nu_{\text{max}}\) 3317, 1726, 1587, 1296; \(\delta\) NMR (CDCl\(_3\), 300 MHz) \(\delta\): 3.18 (s, 3H), 4.27 (s, 2H), 5.86 (s, 1H), 6.37 (m, 1H), 6.50 (m, 1H), 6.82 (m, 2H), 7.12 (d, \(J = 8.5\) Hz, 1H), 7.24 (m 1H), 9.87 (s, 1H).

N-(4-chloromethyl)-2-oxo-2H-chromen-7-yl)-2-methylbenzamide (19)
Colorless solid; yield: 84%; mp: 149°C–151°C; IR (cm\(^{-1}\), KBr) \(\nu_{\text{max}}\) 3331, 1713, 1582, 1250; \(\delta\) NMR (CDCl\(_3\), 300 MHz) \(\delta\): 2.38 (s, 3H), 4.69 (s, 2H), 6.45 (s, 1H), 7.29 (m, 2H), 7.38 (m, 1H), 7.50 (d, \(J = 8.7\) Hz, 1H), 7.61 (d, \(J = 8.7\) Hz, 1H), 7.81 (d, \(J = 2.4\) Hz, 1H), 7.94 (m, 1H), 10.3 (s, 1H).

N-(4-methyl-2-oxo-2H-chromen-7-yl) picolinamide (20)
Off white solid; yield: 82%; mp: 184°C–186°C; IR (cm\(^{-1}\), KBr) \(\nu_{\text{max}}\) 3299, 1745, 1623, 1332; \(\delta\) NMR (DMSO-d\(_6\), 500 MHz) \(\delta\): 2.4 (s, 3H), 3.18 (s, 3H), 4.27 (s, 2H), 6.60 (s, 1H), 7.69 (d, \(J = 9.0\) Hz, 1H), 7.92 (d, \(J = 9.0\) Hz, 1H), 8.1 (s, 1H), 8.09 (m, 1H), 8.17 (d, \(J = 7.5\) Hz, 1H), 8.72 (d, \(J = 4.0\) Hz, 1H), 11.05 (s, 1H, NH).

N-(4-methyl-2-oxo-2H-chromen-7-yl) pyrazine-2-carboxamide (21)
Light yellow solid; yield: 83%; mp: 170°C–171°C; IR (cm\(^{-1}\), KBr) \(\nu_{\text{max}}\) 3428, 1710, 1615, 1391; \(\delta\) NMR (DMSO-d\(_6\), 500 MHz) \(\delta\): 2.39 (s, 3H), 4.27 (s, 2H), 6.23 (s, 1H), 7.64 (m, 1H), 7.75 (m, 1H), 7.96 (s, 1H), 8.69 (s, 1H), 8.84 (s, 1H), 9.46 (s, 1H), 10.21 (s, 1H).

N-(4-chloromethyl)-2-oxo-2H-chromen-7-yl) pyrazine-2-carboxamide (22)
Off white solid; yield: 81%; mp: 179°C–180°C; IR (cm\(^{-1}\), KBr) \(\nu_{\text{max}}\) 3304, 1708, 1617, 1332; \(\delta\) NMR (DMSO-d\(_6\), 500 MHz) \(\delta\): 4.95 (s, 2H), 6.54 (s, 1H), 7.68 (m, 1H), 7.79 (d, \(J = 9.0\) Hz, 1H), 7.92 (d, \(J = 9.0\) Hz, 1H), 8.1 (s, 1H), 8.09 (m, 1H), 8.17 (d, \(J = 7.5\) Hz, 1H), 8.72 (d, \(J = 4.0\) Hz, 1H), 11.05 (s, 1H, NH).

N-(4-chloromethyl)-2-oxo-2H-chromen-7-yl) benzamide (23)
Light yellow solid; yield: 83%; mp: 184°C–186°C; IR (cm\(^{-1}\), KBr) \(\nu_{\text{max}}\) 3299, 1745, 1623, 1332; \(\delta\) NMR (CDCl\(_3\), 500 MHz) \(\delta\): 2.39 (s, 3H), 4.27 (s, 2H), 6.23 (s, 1H), 7.64 (m, 1H), 7.75 (m, 1H), 7.96 (s, 1H), 8.69 (s, 1H), 8.84 (s, 1H), 9.46 (s, 1H), 10.21 (s, 1H).

Antimicrobial activity
First, we evaluated six simple coumarins 1–6 against three Gram-positive bacterial strains B. subtilis MTCC 121(Bs), S. aureus MTCC 96 (Sa), and B. pumilus MTCC1607 (Bp) and three Gram-negative strains E. coli MTCC1304 (Ec), K. pneumoniae MTCC 3394 (Kp), and S. typhi MTCC 537 (St) to determine the best skeleton for further modification. Out of these compounds, aminocoumarins 5 and 6 exhibited superior activity against the tested strains as compared to compounds 1–4 (Table 1). Therefore, compounds 5 and 6 were selected for further molecular optimization. Using amino coumarins 5 and 6 as basic skeleton, 7-amidocoumarins 9–23 were synthesized and examined for their MIC by the two-fold serial microdilution method using clinically relevant bacterial strains. The results are shown in Table 2 and Figs. 1 and 2. The MIC was defined as the lowest concentration of the tested compound at which no growth of the strain was observed in a period of time and under specified experimental conditions.

As shown in Table 2, among the 4-methyl series, compounds 9 and 11 bearing dimethyl and dibromo substituted salicylic acid residue, respectively, showed MIC 12.5 \(\mu\)g/mL against B. pumilus and K. pneumonia. Compound 11 showed superior activity against B. subtilis (6.25 \(\mu\)g/mL) as compared to compound 9 (25 \(\mu\)g/mL). Compounds 12–15 bearing 4-chloromethyl group showed low order of activity against all the tested strain with MICs in the range of 50 to >200 \(\mu\)g/mL. Further, the role of salicylic acid residue was explored with the synthesis and compounds 20–23 bearing heteroaromatic acid substituent. The activity was improved with the introduction of pyridinyl and piperezinyl ring, but surprisingly, in this case, 4-chloromethyl group bearing compounds exhibited superior activity than the 4-methyl group-containing compounds (21 and 23 vs. 20 and 22). Compound 21 bearing 4-chloromethyl and pyridyl substituent showed superior activity among all the tested compounds and was found to be the most active compound of the series. It showed activity against four bacterial strains with MIC ranging from 6.25 to 25 \(\mu\)g/mL. Its potency against S. typhi was comparable to the standard drug Ery. Compound 23 exhibited activity at a concentration of 6.25 and 12.5 \(\mu\)g/mL against B. pumilus and K. pneumoniae, respectively. Altogether, in these compounds, SARs revealed that the antibacterial activity improved by replacing the benzoic acid substituent with a salicylic acid substituent. Further, compounds containing 4-methyl substituent showed superior activity than the corresponding 4-chloromethyl group bearing compounds (compounds 9-11 vs. compounds 12-14). Interestingly, compounds 21 and 23 containing heteroaromatic ring and chloromethyl group as a substituent at the fourth position of coumarin ring were found to be more active than the corresponding 4-methyl group-containing compounds 20 and 22.

The synthesized compounds were also evaluated against three fungal strains C. albicans, C. tropicalis, and A. niger. The results are summarized in Table 2 and Fig. 3. For C. albicans, MIC values of these compounds vary from 6.25 to 200 \(\mu\)g/mL. Among these, compounds 9, 11, 21, and

Fig. 1: Antibacterial activity of amidocoumarins against Gram-positive bacteria

Fig. 2: Antibacterial activity of amidocoumarins against Gram-negative bacteria
CONCLUSION
In summary, we synthesized a series of 7-benzamidocoumarins and reported their antimicrobial activity for the first time. Some of these compounds were found to inhibit the enzyme chitinase because, after incubation with these compounds, the enzyme became ineffective to breakdown the colloidal chitin and the basic color of DNS did not change. However, the other coumarin compounds of the series exhibited non-inhibitory behavior toward chitinase, i.e., the compounds did not interfere in the normal reaction between chitinase and colloidal chitin and color change was observed. Among the tested compounds, compound 9 was found to be the most effective chitinase inhibitor.

Chitinase Inhibitory activity
Chitin, the linear polymer of N-acetylglucosamine, is an essential structural component of the fungal cell wall. Fungal chitinases play an important role in exogenous chitin decomposition, fungal cell wall degradation, and remodeling. These enzymes have been proposed as an important target for the design and development of novel antifungals [24]. Several chitinase inhibitors have been reported in literature, but none of them has yet successfully reached the clinic for the treatment of fungal infections [25,26]. Therefore, there is an urgent need for the identification of novel chitinase inhibitors. In this study, the coumarin derivatives 9–23 were also tested for their anti-chitinase activity. Chitinase activity of the compounds was determined by DNS, as mentioned by Miller using colloidal chitin as a substrate [22]. Here, the concept lies in DNS color change from yellowish orange to brownish orange. During the reaction, chitinase breaks colloidal chitin into N-acetyl glucosamine (NAG), a reducing sugar liberated from the hydrolysis of chitin polymer. This reducing sugar binds with DNS to impart a brown color to the solution. If the compound is inhibitory in nature toward chitinase the basic color of DNS does not change and NAG impart a brown color to the solution. If the compound is inhibitory in nature toward chitinase the basic color of DNS does not change and NAG is not formed but if the compound is noninhibitory in nature; it does not interfere in the normal reaction between chitinase and colloidal chitin and change in color is reflected. In our study, compounds 9, 16, 18, 21, and 23 were found to inhibit the enzyme chitinase because, after incubation with these compounds, the enzyme became ineffective to breakdown the colloidal chitin and the basic color of DNS did not change. However, the other coumarin compounds of the series exhibited non-inhibitory behavior toward the chitinases, i.e., the compounds did not interfere in the normal reaction of chitinase with colloidal chitin and color change was observed. Among the tested compounds, compound 9 was found to be the most effective chitinase inhibitor.

Table 1: Antibacterial activity of compounds 1-6 expressed as a minimum inhibitory concentration (μg/mL)

| Bacterial strain | Compound 1 | Compound 2 | Compound 3 | Compound 4 | Compound 5 | Compound 6 | Ery* |
|------------------|------------|------------|------------|------------|------------|------------|------|
| Bs               | 200        | 100        | 100        | 100        | 100        | 50         | >200 |
| Sa               | 200        | 200        | 100        | 50-100     | 50         | 25         | >200 |
| Bp               | 200        | 100        | 100        | 100        | 200        | 200        | >200 |
| Ec               | 200        | 200        | 200        | 200        | >200       | 200        | >200 |
| Kp               | >200       | >200       | >200       | >200       | 200        | 100        | <0.39|
| St               | 200        | 100        | 100        | 100        | 25         | 25         | >200 |

*Erythromycin

Table 2: Antimicrobial activities of 7-amidocoumarins as expressed in minimum inhibitory concentration (μg/mL)

| Compound number | Gram-positive | Gram-negative | Ca* | Ct* | An* |
|-----------------|---------------|---------------|-----|-----|-----|
|                 | Bs* | Sa* | Bp* | Ec* | Kp* | St* |
| 9               | 25  | 100 | 12.5 | 200 | 12.5 | 200 | 12.5 |
| 10              | 50  | 100 | 50   | >200| 25  | 100 | >200 |
| 11              | 6.25| 50  | 12.5 | 50  | 12.5 | 25  | 6.25 |
| 12              | 100 | 100 | 25   | 200 | 200 | 50  | >200 |
| 13              | 100 | 200 | 100  | 100 | >200 | >200 | 200  |
| 14              | 50  | 100 | 50   | >200| 100 | 25  | 100  |
| 15              | 200 | >200| 50   | 100 | 200 | 50  | >200 |
| 16              | 200 | 200 | 200  | >200| >200 | 200 | >200 |
| 17              | 100 | >200| 200  | 100 | 200 | 100 | >200 |
| 18              | 100 | >200| 200  | 100 | 200 | 50  | >200 |
| 19              | 50  | 100 | 100  | 200 | 200 | 100 | >200 |
| 20              | 200 | 50  | 100  | 200 | 100 | 200 | >200 |
| 21              | 12.5| 6.25| 6.25 | 100 | 50  | 6.25| 12.5 |
| 22              | 100 | 100 | 200  | >200| >200 | 100 | 50   |
| 23              | >3.25| 2.75| 1.5  | 9.63| <0.39| 6.78| -    |
| Amp             | -   | -   | -    | -   | -   | -   | 6.25 |

* Bacillus subtilis (Bs, MTCC 121), Staphylococcus aureus (Sa, MTCC 96), Bacillus pumilus (Bp, MTCC 1607), Escherichia coli (Ec, MTCC1304), Klebsiella pneumonia (Kp, MTCC 3384), Salmonella typhi (St, MTCC 537), Candida albicans (Ca, MTCC 3017), Candida tropicalis (Ct, MTCC 184), Aspergillus niger (An, MTCC 1344)

Fig. 3: Antifungal activity of amidocoumarins

23 showed the highest activity (6.25–50 μg/mL). Compound 11 was equipotent to Amp against C. albicans and exhibited two-fold superior activity against C. tropicalis than the standard drug Amp. The MICs of these six compounds against A. niger were found to be in the range of 6.25–50 μg/mL. Compound 9 showed promising activity (6.25 μg/mL) against A. niger.
compounds showed moderate to excellent in vitro activities against different Gram-positive, Gram-negative bacterial strains, and fungi with MIC values between 6.25 and 50 mg/mL. Compound 21 was found to be the most active with MIC 6.25 μg/mL against *B. pumilus*, *S. aureus*, and *S. typhi*. Compound 21 also inhibited the growth of fungi *C. albicans* and *C. tropicalis* at concentration 12.5 and 25 μg/mL, respectively. The other promising antibacterial molecules from this series were compounds 9, 11, and 23. These compounds showed broad-spectrum activity against both bacterial and fungal strains. Compound 9 emerged as best chitinase inhibitor of the series. Our findings suggest that the further derivatization of the amidocoumarin scaffold could give rise to structures with enhanced antimicrobial and anti-chitinase activities.

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**AUTHOR’S CONTRIBUTIONS**

DK and VS conceived and planned the experiments. NT and Priyanka synthesized the compounds. VS carried out biological experiments. DK analyzed the data and wrote the manuscript with input from VS.

**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interest.

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