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Synthesis of modified pyridine and bipyridine substituted coumarins as potent antimicrobial agents

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Abstract: In the search for new antimicrobial agents, a series of new modified pyridine and bipyridine substituted coumarins **5a**–**y** was designed and synthesized by adopting a molecular hybridization strategy. All the synthesized compounds were evaluated for their *in vitro* antimicrobial activity using the broth dilution method against selected bacterial (Gram-positive and Gram-negative) and fungal strains. Compounds **5a**, **5f**, **5g**, **5n**, **5r**, **5t**, **5w**, **5x** and **5y** demonstrated promising antibacterial activity, while the other derivatives showed comparable activity to those of the standard drugs used as references.

Keywords: coumarins; bipyridines; Krohnke reaction; antimicrobial activity; broth dilution method.

INTRODUCTION

In recent years, the mounting threat of bacterial resistance has heightened the urgency to discover and develop effective agents with novel mechanisms of action and enhanced activity profiles. Considerable research efforts have been directed to the discovery of high potency local antimicrobial agents with reduced or without systemic adverse effects. The development of potent and effective antimicrobial agents is of utmost importance to overcome the emerging multi-drug resistance strains of bacteria and fungi.

A large number of heterocyclic substituted and heterocyclic fused coumarin derivatives has drawn immense attention among medicinal chemists as they exhibit several significant biological activities, such as anti-alzheimer,[1] antimalarial,[2] anticancer,[3] antioxidant,[4] antitumor,[5] anti-inflammatory,[6] antipyretic,[7] analgesic,[7] antimicrobial,[8] *etc*. Among the heterocyclic-substituted coumarins, pyridine-substituted coumarins constitute an elite class of compounds as they exhibit important biological activities, such as CNS depressant,[9] antifungal,[10] antibacterial,[11] antitubercular,[12] *etc*. During a literature survey, some modified

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pyridine nuclei emerged, such as cyclopenta[*b*]pyridine, 5,6,7,8-tetrahydroquinoline and indeno[1,2-*b*]pyridine, which are endowed with antiproliferative,[13] anti-inflammatory,[14,15] antimicrobial,[16,17] anticancer,[18] antidiabetic,[19] antiplasmodial,[20] anti-alzheimer,[21] cytotoxic,[22] calcium antagonistic,[23] and bovine liver glutathione-*S*-transferase inhibitory activities.[24] Certain bipyridinyl moieties are reported to possess important biological properties, such as antimicrobial,[25] antioxidant,[25] cardiotonic,[26] DNA interacting[27] and cytotoxic[27]. In a literature search, it was observed that hitherto no significant efforts have been made to synthesize coumarin derivatives incorporating such modified pyridine nuclei (cyclopenta[*b*]pyridine, 5,6,7,8-tetrahydroquinoline and indeno[1,2-*b*]pyridine) and to study their biological properties. Encouraged by potential biological activities of modified pyridines and in continuation of previous investigation on biopotent coumarin derivatives,[28] the present efforts were focused on the design and synthesis of biologically potent heterocyclic system *via* the combination of the therapeutically active moieties coumarin and modified pyridine nuclei together in a single scaffold.

RESULTS AND DISCUSSION

Chemistry

The strategy adopted for the synthesis of the key precursors **3a**–**e** and the target compounds is depicted in Scheme 1. The coumarin chalcones **3a**–**e** were synthesized by reacting 8-acetyl-7-hydroxy-4-methylcoumarin **1** with various benzaldehydes **2a**–**e** in ethanol containing piperidine in a catalytic amount.

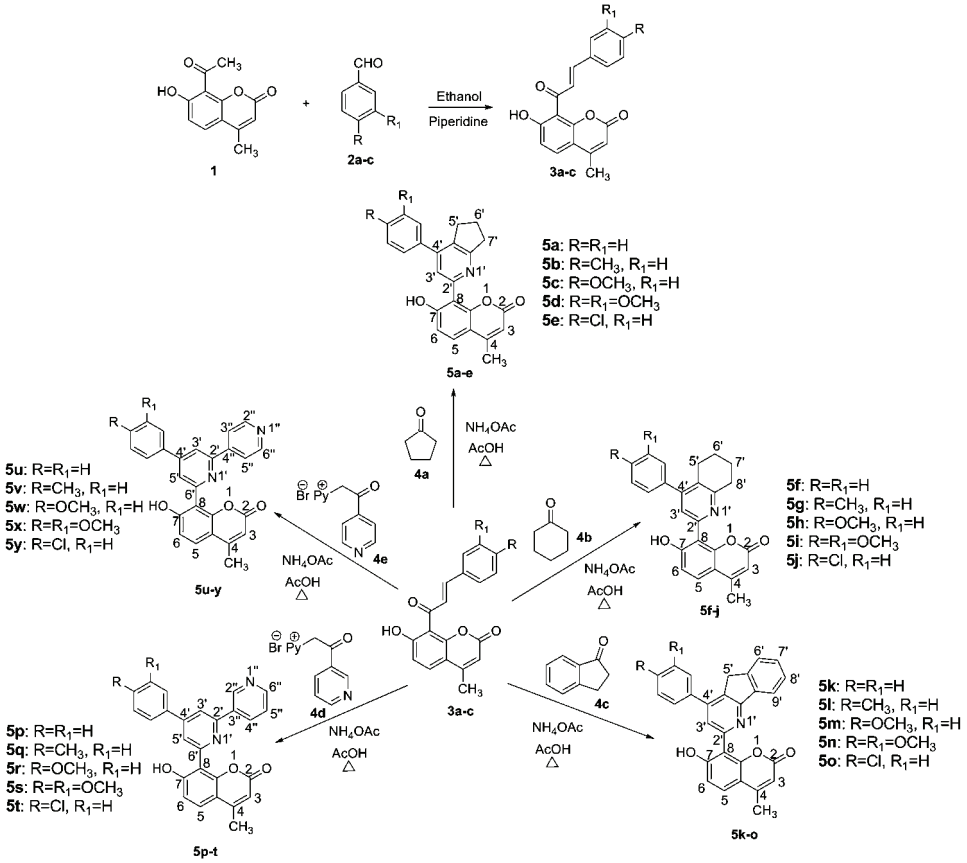
The synthesis of title compounds **5a**–**y** was realized by reacting coumarin chalcones **3a**–**e** with cyclopentanone **4a**, cyclohexanone **4b**, indanone **4c** and (pyridinecarbonylmethyl)pyridinium iodide salts **4d** and **4e** under Krohnke reaction condition.[29] The reaction proceeded *via* a Michael addition by the nucleophilic addition of active methylene group in **4a**–**e** to the α,β-unsaturated carbonyl functionality in **3a**–**e** to afford the 1,5-dicarbonyl intermediates. The corresponding intermediate undergoes cyclization in presence of ammonia and subsequent loss of water afforded target compounds **5a**–**y**.

The analytic and spectroscopic data of all the synthesized compounds are given in the Supplementary material to this paper.

Evaluation of antimicrobial activity

The antimicrobial activity of the synthesized compounds **5a**–**y** was determined by the broth dilution method as described by NCCLS.[30] The antibacterial activity was screened against two Gram-positive (*Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 441) and two Gram-negative (*Escherichia coli* MTCC 443 and *Salmonella enterica* subsp. *enterica* serovar typhi MTCC 98) bacteria using ampicillin, ciprofloxacin and chloramphenicol as standard antibacterial drugs. Antifungal activity was screened against two fungal species

(*Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 282) where griseofulvin and nystatin were used as the standard antifungal drugs. The antimicrobial activity data are presented in Table I.



Scheme 1. Strategy adopted for the synthesis of the precursors **3a**–**e** and the target compounds **5a**–**y**.

All the newly synthesized compounds **5a**–**y** exerted significant inhibitory activity against all the employed strains. The antimicrobial assessment data of compounds **5a**–**y** revealed that compounds **5n** and **5w** (*MIC* = 50 μg mL^{-1}); compounds **5a** and **5x** (*MIC* = 62.5 μg mL^{-1}) exhibited excellent activity against *S. aureus* compared to ampicillin (*MIC* = 250 μg mL^{-1}) and comparable activity to chloramphenicol and ciprofloxacin (*MIC* = 50 μg mL^{-1}). In addition, compounds **5d**, **5f**, **5g**, **5i** and **5r** (*MIC* = 100 μg/mL) also showed significant activity against *S. aureus* compared to ampicillin (*MIC* = 250 μg mL^{-1}). Against *B. subtilis*, compound **5f** (*MIC* = 50 μg mL^{-1}) demonstrated remarkable activity compared to ampicillin (*MIC* = 250 μg mL^{-1}) and was equipotent to chloramphenicol

and ciprofloxacin (*MIC* = 50 μg mL^{-1}). Compounds **5a**, **5j** and **5n** (*MIC* = 100 μg mL^{-1}) also showed superior activity to ampicillin (*MIC* = 250 μg mL^{-1}) against *B. subtilis*. Compound **5r** (*MIC* = 12.5 μg mL^{-1}) depicted excellent activity against *E. coli* compared to all the drugs used as standards. Compounds **5t**, **5y** (*MIC* = 50 μg mL^{-1}) and **5g** (*MIC* = 62.5 μg mL^{-1}) showed excellent activity compared to ampicillin (*MIC* = 100 μg mL^{-1}) and comparable activity to chloramphenicol and ciprofloxacin (*MIC* = 50 μg mL^{-1}) against *E. coli*. Against *S. typhi*, compound **5r** (*MIC* = 50 μg mL^{-1}) and **5g** (*MIC* = 62.5 μg mL^{-1}) showed significant activity compared to ampicillin (*MIC* = 100 μg mL^{-1}) and comparable activity to chloramphenicol (*MIC* = 50 μg mL^{-1}).

TABLE I. Antimicrobial activity (*MIC* / μg mL^{-1}) of compounds **5a–y**; AMP: ampicillin, CHL: chloramphenicol, CIP: ciprofloxacin, GRIS: griseofulvin, NYT: nystatin. –: not tested

Compd.	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	S. aureus MTCC 96	*B. subtilis* MTCC 441	*E. coli* MTCC 443	*S. enterica* MTCC 98	*C. albicans* MTCC 227	*A. niger* MTCC 282
5a	62.5	100	100	200	1000	1000
5b	500	500	500	500	500	1000
5c	500	250	250	250	1000	500
5d	100	250	100	200	250	>1000
5e	250	500	500	500	500	1000
5f	100	50	200	200	>1000	>1000
5g	100	250	62.5	62.5	1000	1000
5h	250	250	250	500	500	1000
5i	100	200	250	100	>1000	>1000
5j	250	100	500	500	500	500
5k	250	250	100	200	>1000	500
5l	250	500	100	250	500	500
5m	200	500	100	100	1000	>1000
5n	50	100	250	250	250	250
5o	250	200	250	500	>1000	250
5p	500	250	100	200	250	1000
5q	250	250	250	250	1000	>1000
5r	100	200	12.5	50	500	1000
5s	500	200	100	100	>1000	200
5t	250	200	50	200	1000	>1000
5u	250	500	250	200	1000	500
5v	500	250	100	250	500	1000
5w	50	500	200	500	1000	>1000
5x	62.5	250	200	100	500	1000
5y	200	250	50	100	1000	1000
AMP	250	250	100	100	–	–
CHL	50	50	50	50	–	–
CIP	50	50	25	25	–	–
GRIS	–	–	–	–	100	100
NYT	–	–	–	–	500	100

Compounds **5m** and **5y** (*MIC* = 200 μg mL^{-1}) showed better activity, while compounds **5e**, **5h**, **5j**, **5k**, **5l**, **5o**, **5q**, **5t** and **5u** (*MIC* = 250 μg mL^{-1}) were found equipotent compared to ampicillin (*MIC* = 250 μg mL^{-1}) against *S. aureus*. Compounds **5i**, **5o**, **5r**, **5s** and **5t** (*MIC* = 200 μg mL^{-1}) exhibited better activity and compounds **5c**, **5d**, **5g**, **5h**, **5k**, **5p**, **5q**, **5v**, **5x** and **5y** (*MIC* = 250 μg mL^{-1}) were found equipotent to ampicillin (*MIC* = 250 μg mL^{-1}) against *B. subtilis*. Compounds **5a**, **5d**, **5k**, **5l**, **5m**, **5p**, **5s** and **5v** (*MIC* = 100 μg mL^{-1}) against *E. coli* and compounds **5i**, **5m**, **5s**, **5x** and **5y** (*MIC* = 100 μg mL^{-1}) against *S. enterica* subsp. *enterica* serovar typhi were found equipotent to ampicillin (*MIC* = 100 μg mL^{-1}).

A close look at the SAR (structure–activity relationship) of these compounds clearly indicated the influence of peripheral substituents on the aryl ring (*i.e.*, R and R_1) and the nature of the pyridine moiety on antimicrobial potency. It is interesting to note that almost all the compounds **5a**–**y** possessed promising antibacterial activity against Gram-positive bacteria *B. subtilis* and *S. aureus*. In the case of compounds **5a**–**e** bearing cyclopenta[*b*]pyridine moiety, introduction of electron donating group, *i.e.*, compounds **5b** (R = CH_3) and **5c** (R = OCH_3), reduced the antibacterial activity significantly. Upon introduction of a second methoxyl group, *i.e.*, compound **5d** (R = R_1= OCH_3), enhanced the antibacterial activity against *S. aureus*. Replacement of cyclopenta[*b*]pyridine moiety with 5,6,7,8-tetrahydroquinoline moiety boosts the antibacterial potency against all the bacterial strains. The enhancement in the antibacterial activity of the compounds **5f**–**j** may be attributed to the increased lipophilicity due to insertion of an additional –CH_2– group in the modified pyridine moiety than those of compounds **5a**–**e**. In compounds **5f**–**j**, altering the substitution on the appended aryl ring did not affect the activity against *S. aureus* but it reduced the activity against *B. subtilis*. Introduction of methyl group, *i.e.*, compound **5g** (R = CH_3), remarkably intensified the antibacterial potency against Gram-positive bacterial strains, which may be credited to further enhancement in lipophilicity. A marked reduction in the antibacterial potency against the Gram-positive bacteria *B. subtilis* was observed when the pyridine moiety was modified to indeno[1,2-*b*]pyridine, *i.e.*, compounds **5k**–**o**, but the antibacterial potency was appreciably enhanced against *E. coli*.

Surprisingly, upon replacement of the modified pyridine moiety with bipyridine, *i.e.*, compounds **5p**–**y**, the antibacterial effectiveness increased significantly. In fact, compound **5r** (*MIC* = 12.5 μg mL^{-1}) emerged as the most potent derivative of the series. In addition, compounds **5t**, **5w**, **5x** and **5y** exhibited appreciable antibacterial activity. The compounds bearing a bipyridine moiety with a 2-3′ linkage (compounds **5p**–**t**) depicted better antibacterial potency than compounds with a 2-4′ linkage (compounds **5u–y**). Among the compounds **5p–y**, derivatives bearing two methoxyl group or a chlorine group, *i.e.*, compounds **5s**,

5x (R = R_1 = OCH_3), **5t** and **5y** (R = Cl) showed better activity than the other analogs. The compounds **5p**–**y** bearing a bipyridine moiety, exhibited enhanced antibacterial activity compared to compounds **5a**–**o** bearing a modified pyridine moiety.

The antifungal screening data (Table I) revealed that compounds **5d**, **5n** and **5p** (*MIC* = 250 μg mL^{-1}); compounds **5b**, **5e**, **5h**, **5j**, **5l**, **5r**, **5v** and **5x** (*MIC* = = 500 μg mL^{-1}) showed comparable activity to griseofulvin against *C. albicans*. None of the compounds showed promising antifungal activity against *A. niger*.

EXPERIMENTAL

All reactions were performed with commercially available reagents and they were used without further purification. Organic solvents were purified by standard methods and stored over molecular sieves. All reactions were monitored by thin-layer chromatography (TLC, on aluminum plates coated with silica gel 60 F_{254}, 0.25 mm thickness, Merck) and detection of the components was made by exposure to UV light. The compounds were purified by column chromatography using silica gel (60–120 mesh). Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on a Shimadzu FTIR 8401 spectrophotometer using potassium bromide pellets in the range 4000–400 cm^{-1} and frequencies of only characteristic peaks are expressed in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 400 (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) operating at 400 MHz and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm) using $CDCl_3$ as solvent and calibrated with the standard solvent signal. The coupling constants (*J*) are given in Hertz (Hz). Mass spectra of representative compounds were scanned on a Shimadzu QP 2010 spectrometer (Shimadzu, Tokyo, Japan). The precursors 8-acetyl-7-hydroxy-4-methylcoumarin **1**,[31] 8-(3-arylacryloyl)-7-hydroxy-4-methyl--chromen-2-ones **3a**, **3c** and **3d**,[32] (pyridinecarbonylmethyl)pyridinium iodide salts **4d** and **4e**[33] were prepared using reported procedures.

*General procedure for the synthesis of **5a**–**y***

In a 100-mL round bottom flask equipped with a condenser, guard tube and magnetic needle, an appropriate active methylene compound **4a**–**e** (*i.e.*, cyclopentanone **4a**, cyclohexanone **4b**, 1-indanone **4c**, (pyridinecarbonylmethyl)pyridinium iodide salts **4d** and **4e** (0.003 mol) in glacial acetic acid (15mL) was taken. To this, ammonium acetate (0.03 mol) was added under stirring at room temperature. Then a solution of an appropriate 8-(3-arylacryloyl)-7-hydroxy-4-methyl-chromen-2-ones **3a**–**e** (0.003 mol) in glacial acetic acid (15 mL) was added under stirring and the reaction mixture was further stirred for 1 h at room temperature and then refluxed for 8 h at 140 °C. The mixture was then allowed to cool to room temperature and then poured into ice-cold water (75 mL). The crude solid obtained was extracted with chloroform (3×30 mL). The organic layer was washed with 5 % sodium bicarbonate solution (3×20 mL) and water (2×20 mL), and dried over anhydrous sodium sulfate. The removal of chloroform under reduced pressure gave a crude material that was subjected to column chromatography using silica gel and chloroform–petroleum ether (60–80, 1:4) as the eluent to give the targeted compounds **5a**–**y**.

Biological assay

The *in vitro* antimicrobial activities of all the compounds and standard drugs were assessed against Gram-positive bacteria, *viz. Staphylococcus aureus* (MTCC 96) and *Bacillus*

subtilis (MTCC 441), Gram-negative bacteria, *viz*., *Salmonella enterica* subsp. *enterica* serovar typhi (MTCC 98) and *Escherichia coli* (MTCC 443) and two fungi, *viz*., *Aspergillus niger* (MTCC 282) and *Candida albicans* (MTCC 227) by the broth microdilution *MIC* (Minimum inhibitory concentration) method according to NCCLS (National Committee for Clinical Laboratory Standards). The employed strains were procured from (MTCC – micro type culture collection) Institute of Microbial Technology, Chandigarh. Mueller–Hinton Broth was used as the nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud dextrose broth was used for fungal nutrition. Ampicillin, chloramphenicol and ciprofloxacin were used as standard antibacterial drugs, while griseofulvin and nystatin were used as standard antifungal drugs. The bacterial strains were primarily inoculated into Mueller–Hinton agar and, after overnight growth, a number of colonies were directly suspended in saline solution until the turbidity matched the turbidity of the McFarland standard (approximately 10^8 CFU mL^{-1}). *i.e*., the inoculum size for test strain was adjusted to 10^8 CFU mL^{-1} (colony forming unit) per milliliter well by comparing the turbidity (turbidimetric method). Similarly, the fungi were inoculated on Sabouraud dextrose broth; the procedures of inoculum standardization were also similar. DMSO was used as diluent to obtain the desired concentration of the synthesized compounds and the standard drugs for the test upon the standard microbial strains, *i.e*., the compounds were dissolved in DMSO and the solutions were diluted with a culture medium. Each compound and standard drug was diluted to obtain 2000 μg mL^{-1} stock solutions. By further progressive dilutions with the test medium, the required concentrations were obtained for primary and secondary screening. In primary screening, 1000, 500, and 250 μg mL^{-1} concentrations of the synthesized compounds were taken. The active compounds found in this primary screening were further diluted to obtain 200, 100, 62.5, 50, 25, 12.5 and 6.25 μg mL^{-1} concentrations for secondary screening to test in a second set of dilution against all microorganisms. Briefly, the control tube containing no antibiotic was immediately sub-cultured (before inoculation) by spreading a loopful evenly over a quarter of a plate of medium suitable for the growth of the test organism. The tubes were then incubated at 37 °C for 24 h for the bacteria and 48 h for the fungi. Growth or a lack of growth in the tubes containing the antimicrobial agent was determined by comparison with the growth control, indicated by turbidity. The lowest concentration that completely inhibited visible growth of the organism was recorded as the minimum inhibitory concentration (*MIC* / μg mL^{-1}), *i.e*., the amount of growth from the control tube before incubation (which represents the original inoculum) was compared. A set of tubes containing only seeded broth and the solvent controls were maintained under identical conditions to ensure that the solvent had no influence on strain growth. The protocols are summarized in Table I as the minimum inhibitory concentration (*MIC* / μg mL^{-1}).

CONCLUSIONS

In conclusion, a series of modified pyridine and bipyridine substituted coumarins, which emerged as a new and important class of antimicrobial agents, was successfully designed and synthesized. The results revealed the positive contribution of methoxyl substituents at the *meta* and *para* positions of the phenyl ring to the observed antimicrobial activity. The results also indicated that coumarins bearing the bipyridine moiety showed marked enhancement in their antibacterial activity than those bearing a modified pyridine moiety. It is believed that the

observed results may be useful in guiding future global efforts to discover new compounds with improved antimicrobial activity.

SUPPLEMENTARY MATERIAL

General procedure for the synthesis of **3a**–**e** and physical, analytical and spectral data of the synthesized compounds **5a**–**y** are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА ДЕРИВАТА КУМАРИНА СУПСТИТУИСАНИХ МОДИФИКОВАНИМ ПИРИДИНИМА И БИПИРИДИНИМА КАО ПОТЕНЦИЈАЛНИХ АНТИМИКРОБНИХ АГЕНАСА

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Током истраживања нових антимикробних агенаса синтетисана је серија деривата кумарина **5a**–**y** супституисаних модификованим пиридинима и бипиридинима. Свим синтетисаним дериватима испитана је антимикробна активност применом методе разблаживања у бујону (*broth dilution method*) према одабраним сојевима бактерија (грам-позитивне и грам-негативне) и гљива. Једињења **5a**, **5f**, **5g**, **5n**, **5r**, **5t**, **5w**, **5x** и **5y** показују значајне антибактеријске активности, док преостали деривати имају активности блиске стандардним лековима.

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