Med Chem Res (2013) 22:4338–4346
DOI 10.1007/s00044-012-0437-8

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

One pot synthesis of some novel coumarins containing 5-(substituted-2-hydroxybenzoyl) pyridine as a new class of antimicrobial and antituberculosis agents

Varun G. Bhila · Chirag V. Patel · Niraj H. Patel · Dinkar I. Brahmbhatt

Received: 29 May 2012 / Accepted: 18 December 2012 / Published online: 8 January 2013

Abstract A novel class of substituted pyridyl coumarin derivatives has been synthesized starting from 3-acetyl coumarin and chromone-3-carbaldehyde via one pot reaction and fully characterized by spectral and elemental analysis. All these derivatives **3a–l** were screened in vitro for antimicrobial activity against a representative panel of pathogenic strains. Compounds demonstrated good to excellent antibacterial activity, while some compounds exhibited equipotent antifungal activity as compared to that of first line standard drug. As a part of investigation of new antitubercular agents, in vitro screening of synthesized compounds against *Mycobacterium tuberculosis* $H_{37}Rv$ has been done. Among the designed molecules, three compounds showed relatively better activity. From the entire study, it has been revealed that compounds appear to be better antimicrobials but relatively poor antituberculars.

Keywords Coumarin · Chromone-3-carbaldehyde · Kröhnke's reaction · Antituberculosis · Antimicrobial

Introduction

Mankind is surrounded by many infectious diseases, and tuberculosis is one of them. TB is a lung infection caused mainly by *Mycobacterium tuberculosis* and is considered as one of the most threatening diseases for public health. This concern is justified by a current report from the World Health Organization, according to which there are 9.4 million new cases of TB and 1.65 million deaths due to TB every year. One-third of the human population is believed to be infected by this pathogen (WHO, 2008). Among HIV-infected people with weakened immune system, TB is a leading killer epidemic. Every year about 2 million people living with HIV die due to TB (Jain *et al*., 2003). A recent estimation by WHO has revealed that within next 20 years, approximately 30 million people will be infected with the bacillus (OMS, 2003). The forging facts reveal that there is a crucial need for the development of new drugs which are synthetically feasible, lack side effects, and have shorter duration of treatment.

The coumarins are of great importance to chemists as well as biologists as it is one of the key building blocks for many naturally occurring compounds (Murray *et al*., 1982; Fylaktakidou *et al*., 2004; O'Kennedy and Thornes, 1997). Among the various heterocyclic moieties of pharmacological interest, coumarin is endowed with various activities such as antituberculosis (Manvar *et al*., 2008), anti-inflammatory (Kontogiorgis and Hadjipavlou-Litina, 2005), anti cancer (Kamal *et al*., 2009), anti HIV (Kaye *et al*., 2004), anti tumor (Suzuki *et al*., 2006), and anti coagulant (Garazd *et al*., 2005). The incorporation of heterocyclic ring in the coumarin nucleus can bring about an extensive modification in the biological activities of the parent compound. Among the heterocyclic substituted coumarins, pyridyl coumarins have a special importance due to their diverse physiological actions. A number of coumarin derivatives having pyridine substituted mainly at 3- and 4-position of the coumarin possess CNS depressant (Moffett, 1964), anti fungal (Garazd *et al*., 2005), antibacterial (Sreenivasulu *et al*., 1974), and moth proofing activity (Moffett, 1965).

V. G. Bhila · C. V. Patel · D. I. Brahmbhatt (✉)
Department of Chemistry, Sardar Patel University, 388120 Vallabh Vidyanagar, Gujarat, India
e-mail: drdib317@gmail.com

N. H. Patel
Oxygen Bioresearch Ltd, Pharmez, Ahmedabad, Gujarat, India

Chromone-3-carbaldehyde has been extensively used in the synthesis of various heterocyclic systems. The synthesis and reactivity of this versatile compound has been reviewed (Ghosh, 1997, 2004). Most of the synthetic utilities of this compound are derived from the reactivity of its electron-deficient centers at C-2, C-4, and formyl group (Sabitha, 1996). Chromone-3-carbaldehyde can give access to compounds where the chromone ring is retained, or to 2-hydroxybenzoyl derivatives resulting from the opening of the pyran-4-one ring.

Considering the importance of pyridyl-substituted coumarins, a variety of substituted coumarins were synthesized from our laboratory (Brahmbhatt *et al*., 2004, 2005, 2007; Patel *et al*., 2010a, b). In continuation of that work, we synthesized 2-hydroxybenzoyl-substituted pyridines attached to coumarins for the first time, which we found here to show good antimicrobial activity.

A frightening rise in pathogenic resistance to existing drugs is a serious problem with antimicrobial therapy and necessitates continuing research into new class of antimicrobials (Woodford, 2003). No new drugs have been developed specifically against mycobacteria since the 1960s, and within the last few years, only some promising drug candidates emerged (Primm and Franzblau, 2007; Médecins Sans Frontières, 2009). So this is our sincere effort in this direction to check activity of the synthesized series of compounds against *M. tuberculosis* $H_{37}Rv$.

Chemistry

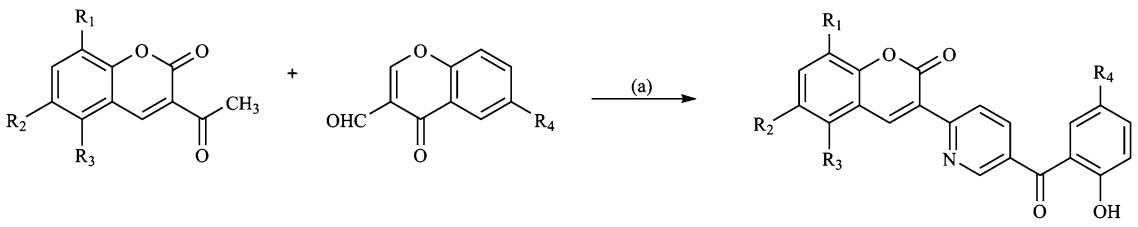
The starting material 3-acetyl coumarins were prepared from various salicylaldehydes by Knoevenagel condensation while chromone-3-carbaldehydes were prepared from various *o*-hydroxy acetophenones by Vilsmeier Haack reaction. The synthetic route employed to produce the library of target compounds 3-(5-(2-hydroxybenzoyl)pyridin-2-yl)coumarins **3a–l** is portrayed in Scheme 1.

In the presence of ammonium acetate in acetic acid, chromone-3-carbaldehyde gets converted into enamine derivative which upon reaction with 3-acetyl coumarin results in a chalcone type intermediate which in situ gets cyclized to give the final product **3a–l** in good to excellent yields (Table 1). Reaction proceeds in a single step, and the mechanism follows Kröhnke's reaction pathway to afford the target molecule (Krohnke and Zecher, 1961; Krohnke, 1976) (Scheme 2).

Pharmacology

The MICs of synthesized compounds were carried out by broth micro dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS, 2002). All the newly synthesized target compounds were evaluated for their in vitro antibacterial activity against *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 441) as Gram positive bacterial strains while against *Escherichia coli* (MTCC 443) and *Salmonella typhi* (MTCC 98) as Gram negative bacterial strains. They were also evaluated for their in vitro antifungal activity against *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 282) as fungal strains. For comparison, the standard drug used for antibacterial potency of the compounds was ampicillin, a broad spectrum antibiotic, while the drugs used for antifungal potency of the compounds were griseofulvin and nystatin. Minimum inhibitory concentration (MIC) was measured as described in "In vitro evaluation of antimicrobial activity". The screening results (Table 2) indicated that all the tested compounds exhibited different inhibitory effects against different test organisms. All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and tested against above mentioned known drugs. Mueller–Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test. The size of the inoculum for the test strain was adjusted to 10^8 colony forming unit (CFU) per milliliter by comparing the turbidity. DMSO was used as a diluent to get the desired concentration of compounds to test upon standard bacterial strains.

In vitro antituberculosis activity of all the synthesized compounds against *M. tuberculosis* $H_{37}Rv$ strain was determined using Lowenstein–Jensen medium (conventional method) as described by (Rattan, 2000). The observed

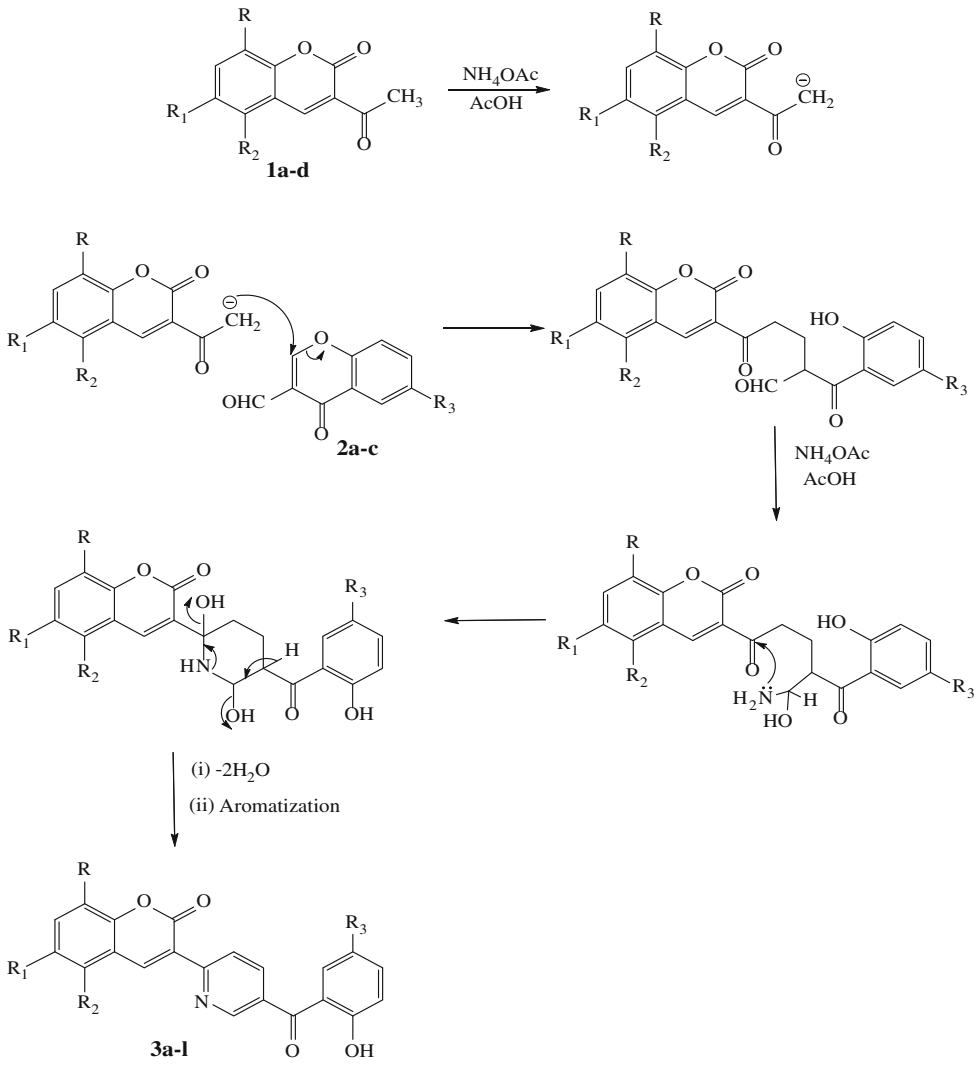


1a-d **2a-c** **3a-l**

Scheme 1 Synthetic pathway for target compounds **3a–l**. Reagents and conditions (*a*) NH_4OAc, AcOH, reflux 8 h

Table 1 Physical parameters of 3-(5-(2-hydroxybenzoyl)pyridin-2-yl)coumarin derivatives **3a–l**

Compd.	R_1	R_2	R_3	R_4	Formula (mw)	mp (°C)	Yield %
3a	H	H	H	H	$C_{21}H_{13}NO_4$ (343.33)	220	74
3b	H	H	H	CH_3	$C_{22}H_{15}NO_4$ (357.36)	184	69
3c	H	H	H	Cl	$C_{21}H_{12}ClNO_4$ (377.78)	210	75
3d	OCH_3	H	H	H	$C_{22}H_{15}NO_5$ (373.36)	188	83
3e	OCH_3	H	H	CH_3	$C_{23}H_{17}NO_5$ (387.38)	204	77
3f	OCH_3	H	H	Cl	$C_{22}H_{14}ClNO_5$ (407.80)	230	85
3g	H	Br	H	H	$C_{21}H_{12}BrNO_4$ (422.23)	225	72
3h	H	Br	H	CH_3	$C_{22}H_{14}BrNO_4$ (436.25)	190	70
3i	H	Br	H	Cl	$C_{21}H_{11}BrClNO_4$ (456.67)	196	78
3j	H	Benzo		H	$C_{25}H_{15}NO_4$ (393.39)	250	80
3k	H	Benzo		CH_3	$C_{26}H_{17}NO_4$ (407.42)	240	84
3l	H	Benzo		Cl	$C_{25}H_{14}ClNO_4$ (427.84)	260	82



Scheme 2 Plausible mechanistic pathway for the synthesis of compounds **3a–l**

Table 2 In vitro antimicrobial activity of various synthesized coumarin derivatives **3a–l**. (MICs, μg/mL)

Compd.	Gram positive bacteria		Gram negative bacteria		Fungi	
	S.A. MTCC 96	B.S. MTCC 441	E.C. MTCC 443	S.T. MTCC 98	C.A. MTCC 227	A.N. MTCC 282
3a	200	250	100	125	500	1000
3b	100	200	250	200	250	1000
3c	250	200	250	250	500	>1000
3d	500	200	200	250	1000	>1000
3e	250	250	100	100	1000	500
3f	200	200	125	200	>1000	>1000
3g	200	100	50	125	200	>1000
3h	500	500	100	100	500	500
3i	62.5	100	62.5	100	100	>1000
3j	100	100	200	250	250	500
3k	125	100	200	250	500	1000
3l	100	125	250	250	1000	100
Ampi	250	250	100	100	nt[a]	nt[a]
Nyst	nt[a]	nt[a]	nt[a]	nt[a]	100	100
Grise	nt[a]	nt[a]	nt[a]	nt[a]	500	100

S.A. *Staphylococcus aureus*, B.S. *Bacillus subtilis*, E.C. *Escherichia coli*, S.T. *Salmonella typhi*, C.A. *Candida albicans*, A.N. *Aspergillus niger*, *MTCC* Microbial Type Culture Collection, *Ampi* ampicillin, *Nyst* nystatin, *Grise* griseofulvin

[a] *nt* not tested

results of their MICs are presented in Table 3 in μg/mL compared with isoniazid, the standard antitubercular drug.

Results and discussion

Analytical results

A series of 3-(5-(2-hydroxybenzoyl)pyridin-2-yl)coumarins **3a–l** have been synthesized via Kröhnkes pyridine synthesis as exemplified in Scheme 1. The structures of all the newly synthesized compounds were confirmed by elemental analysis and FT-IR, ^{1}H NMR, ^{13}C NMR, and DEPT-90 spectral analysis. A mass spectrum was recorded for one representative compound **3a**.

The IR spectra of compounds **3a–l** exhibited carbonyl stretching band of ketone between 1,612 and 1,628 cm^{-1} and a characteristic δ-lactone carbonyl stretching band between 1,713 and 1,728 cm^{-1}. C=N and C=C aromatic stretching bands were observed in the range of 1,454–1,489 cm^{-1} and 1,566–1,597 cm^{-1}, respectively. The aromatic C–H stretching band was observed between 3,044 and 3,063 cm^{-1}. A characteristic O–H stretching band was observed around 3,420 cm^{-1}. In ^{1}H NMR spectra of compounds **3a–l**, the aromatic protons resonate as multiplets between 6.94 and 8.18 δ (except pyridine ring protons). C_4'–H of pyridine ring resonates at around 8.10 δ as doublet (J = 2, 8.4 Hz), while C_3'–H resonates at around 8.70 δ as a doublet (J = 8.4 Hz). The C_6'–H of pyridine ring appears as poorly resolved doublet at around 9.00 δ in compounds **3g–l** while it merges with C_4–H proton signal in compounds **3a–f**. The C_4–H proton of coumarin ring appears in the downfield region due to peri effect of nitrogen atom of pyridine ring. The phenolic O–H proton appears as a sharp singlet between 11.69 and 11.91 δ and gets exchanged by D_2O. The ^{13}C NMR spectra are in good agreement with the structures assigned. The signals observed at around 20.48 and 56.36 δ can be assigned to CH_3 and OCH_3, respectively. The signals

Table 3 In vitro antituberculosis activity of title compounds against *M. tuberculosis* H_{37}Rv (MICs, μg/mL) compared with isoniazid (0.2 μg/mL)

Compd.	MIC (μg/mL)
3a	1,000
3b	100
3c	250
3d	500
3e	62.5
3f	500
3g	250
3h	100
3i	1,000
3j	250
3k	1,000
3l	500

appearing between 116 and 160 δ are for aromatic carbons. The Carbonyl carbon of δ-lactone ring of coumarin appears around 163.40 δ, while ketonic carbon appears in the most downfield region at around 198.66 δ. It is important to note that in case of compounds **3a**, **3d**, **3e**, and **3f**, one carbon signal in ^{13}C NMR spectra is less than expected. This may be due to identical chemical shifts of two carbon atoms which may appear at the same position. The presence of tertiary carbon atoms was ascertained by DEPT-90 spectra. The selected mass spectrum of compound **3a** showed M^+ peak at *m/z* 343 along with other fragment peaks. The appearance of molecular ion peak at 343 mass units supports the structure of compound **3a**.

Biological results

In general, the compounds showed an improved antibacterial activity when compared to their antifungal activity. The deduced patterns of antimicrobial activity of the synthesized target compounds are in the following order: Antibacterial activity > antifungal activity.

A close investigation of the in vitro antibacterial and antifungal activity profiles of the 3-(5-(2-hydroxyaroyl)pyridin-2-yl)coumarins gives a clear picture of the structure activity relations (SAR) among the compounds under study.

Upon reviewing antimicrobial data, it has been observed that compound **3i** (MIC = 62.5 μg/mL) showed excellent activity against gram positive bacteria *S. aureus* as compared with ampicillin (MIC = 250 μg/mL). Compounds **3g** (MIC = 50 μg/mL) and **3i** (MIC = 62.5 μg/mL) showed excellent activity against gram negative bacteria *E. coli* as compared with ampicillin (MIC = 100 μg/mL).

Compounds **3b**, **3j**, **3l** (MIC = 100 μg/mL), **3k** (MIC = 125 μg/mL), and **3a**, **3f**, **3g** (MIC = 200 μg/mL) were more potent when compared with ampicillin (MIC = 250 μg/mL) against *S. aureus* while **3c**, **3e** were equipotent compared with ampicillin (MIC = 250 μg/mL) against *S. aureus*. Compounds **3g**, **3i**, **3j**, **3k** (MIC = 100 μg/mL), **3l** (MIC = 125 μg/mL), and **3b**, **3c**, **3d**, **3f** (MIC = 200 μg/mL) were found to be more active while compounds **3a**, **3e** (MIC = 250 μg/mL) were found to be equally active as compared with ampicillin (MIC = 250 μg/mL) against *B. subtilis*.

Compounds **3a**, **3e**, **3h** (MIC = 100 μg/mL) were found to be equipotent against gram negative bacteria *E. coli* as compared with ampicillin (MIC = 100 μg/mL) while on the other hand, compounds **3e**, **3h**, **3i** (MIC = 100 μg/mL) showed equipotent activity against gram negative bacteria *S. typhi* as compared with ampicillin (MIC = 100 μg/mL).

Compounds **3i**, **3l** (MIC = 100 μg/mL) were the only candidates which showed equal activity against anti fungal strains *C. albicans* and *A. niger*, respectively as compared with nystatin (MIC = 100 μg/mL). Compounds **3g** (MIC = 200 μg/mL) and **3b**, **3j** (MIC = 250 μg/mL) showed higher activity as compared with griseofulvin (MIC = 500 μg/mL) against *C. albicans*.

The observation indicates that the compounds **3j**, **3k**, and **3l** having the presence of fused benzene ring at 5,6 position of the chromene ring show good activity against Gram positive bacterial strain but poor activity against Gram negative bacterial strain. The compounds **3g** and **3h** containing bromine at 6th position of chromene ring are more potent toward Gram negative bacterial strain as compared with Gram positive bacterial strain, while compound **3i** having bromine at 6th position and chlorine in hydroxybenzoyl ring shows excellent activity against all the four bacterial strains. It is interesting to note that the values of MIC are considerably increased with the presence of chlorine atom in the hydroxybenzoyl ring in compounds **3c**, **3f**, **3i**, and **3l**. The compounds having methoxyl group at 8th position in chromene ring as well as compounds having no substitution in chromene ring show good to moderate activity.

The encouraging results from the antimicrobial studies prompted us to go for the screening of the title compounds for their in vitro antituberculosis activity against *M. tuberculosis* $H_{37}Rv$. The results of the screening showed relatively good anti-TB activity for compounds **3b** and **3h** having $R_4 = CH_3$, as compared to the most potent activity for compound **3e** having $R_4 = CH_3$ and $R_1 = OCH_3$. The rest of the compounds showed poor anti-TB activity.

Conclusion

The present research study reports the successful synthesis, antibacterial and antituberculosis studies of a new series of the 3-(5-(2-hydroxyaroyl)pyridin-2-yl)coumarin derivatives carrying biologically active heterocyclic entities. We are reporting for the first time that coumarin containing 5-(substituted-2-hydroxybenzoyl)pyridines might be a suitable pharmacophore for developing novel antimicrobial and antitubercular agents. Their screening results revealed that all the compounds showed moderate to very good antimicrobial activities against various pathogenic strains and moderate to poor antituberculosis activity against *M. tuberculosis* $H_{37}Rv$ strain. Compounds **3g** and **3i** exhibited excellent antimicrobial activity and are said to be the most proficient members of the series.

Experimental

Chemistry

All reactions were performed with commercially available reagents, and they were used without further purification.

Organic solvents were purified by standard methods (Furniss *et al*., 2004) 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. Melting points were determined in open capillaries and are uncorrected. Infrared spectra were recorded on Shimadzu FTIR 8401 spectrophotometer using potassium bromide pellets in the range 4,000–400 cm^{-1}, and frequencies of only characteristic peaks are expressed in cm^{-1}. ^1H and ^{13}C Nuclear Magnetic Resonance spectra were recorded in $CDCl_3$ on a Bruker Avance 400 (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using TMS signal as an internal standard at 400 and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm). The coupling constants (*J*) are given in Hertz (Hz). Mass spectrum of compound **3a** was scanned on a Shimadzu QP 2010 spectrometer (Shimadzu, Tokyo, Japan). The compounds were purified by column chromatography using silica gel (60–120 mesh). Reference drugs ampicillin, griseofulvin, nystatin, and isoniazid were commercial.

Starting precursors 3-acetyl coumarins (Bischler, 1892; Bui-Hoi *et al*., 1957; Murthi and Basak, 1993) **1a–d** and chromone-3-carbaldehydes (Zhao *et al*., 2007) **2a–c** were prepared using the reported procedures.

*General procedure for the synthesis of 3-(5-(2-hydroxybenzoyl)pyridin-2-yl)coumarins **3a–l***

In a 100 mL three-necked flask equipped with a dropping funnel, condenser, guard tube, and magnetic needle, an appropriate chromone-3-carbaldehyde **2a–c** (0.004 mol) was taken in glacial acetic acid (15 mL). To this, ammonium acetate (0.04 mol) was added with stirring at room temperature. Then a solution of appropriate 3-acetyl coumarin **1a–d** (0.004 mol) in glacial acetic acid (15 mL) was added with stirring at room temperature. The reaction mixture was further stirred for 45 min and then refluxed in an oil bath at 140–150 °C for 8 h. It was then allowed to come to room temperature and was poured into ice cold water (100 mL). The solid product obtained was extracted with chloroform (3 × 30 mL). The combined chloroform extract was washed with 10 % sodium bicarbonate solution (3 × 20 mL) and then with water (3 × 20 mL). It was then dried over anhydrous sodium sulfate. The removal of chloroform under vacuum gave a solid product which was purified by column chromatography using silica gel and chloroform–petroleum ether (60°–80°) (6:4) as an eluent. Thus, 3-(5-(2-hydroxyaroyl)pyridin-2-yl)coumarins **3a–l** were obtained as a solid material, which were recrystallized from chloroform–hexane.

*3-(5-(2-Hydroxybenzoyl)pyridin-2-yl)coumarin **(3a)*** IR (KBr, ν_{max}, cm^{-1}): 1454 (C=N aromatic stretching), 1597 (C=C aromatic stretching), 1625 (C=O ketone stretching), 1722 (C=O δ-lactone stretching), 3065 (C–H aromatic stretching), 3421 (OH stretching); ^1H NMR (400 MHz, $CDCl_3$, δ): 6.95–7.75 (8H, m, Ar–H), 8.14 (1H, dd, $J = 8.4$ and 2.4 Hz, C_4'–H), 8.69 (1H, d, $J = 8.4$ Hz, C_3'–H), 9.02 (2H, merged singlet, C_4–H and C_6'–H), 11.88 (1H, s, OH, D_2O-exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 116.53 (CH), 118.80 (CH), 118.98 (C), 119.15 (CH), 119.29 (C), 123.44 (CH), 124.90 (C), 129.34 (CH), 132.96 (C), 133.04 (CH), 137.11 (CH), 137.85 (CH), 144.35 (CH), 149.10 (CH), 151.22 (C), 153.79 (C), 154.24 (C), 159.99 (C), 163.40 (δ-lactone CO), 198.66 (CO); Anal. Calcd. for $C_{21}H_{13}NO_4$: C, 73.46; H, 3.82; N, 4.08 %. Found: C, 73.61; H, 3.74; N, 4.16 %.

*3-(5-(2-Hydroxy-5-methylbenzoyl)pyridin-2-yl)coumarin **(3b)*** IR (KBr, ν_{max}, cm^{-1}): 1489 (C=N aromatic stretching), 1589 (C=C aromatic stretching), 1628 (C=O ketone stretching), 1720 (C=O δ-lactone stretching), 3063 (C–H aromatic stretching), 3420 (OH stretching); ^1H NMR (400 MHz, $CDCl_3$, δ): 2.31 (3H, s, CH_3), 7.03–7.75 (7H, m, Ar–H), 8.12 (1H, dd, $J = 8.4$ and 2 Hz, C_4'–H), 8.67 (1H, d, $J = 8.4$ Hz, C_3'–H), 9.00 (2H, merged singlet, C_4–H and C_6'–H), 11.72 (1H, s, OH, D_2O-exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 20.48 (CH_3), 116.53 (CH), 118.53 (CH), 118.73 (C), 119.34 (C), 123.26 (CH), 124.88 (CH), 128.38 (C), 129.25 (CH), 132.53 (CH), 132.91 (CH), 137.57 (CH), 138.16 (CH), 144.04 (CH), 144.79 (C), 149.29 (CH), 152.69 (C), 153.77 (C), 154.19 (C), 160.06 (C), 161.28 (δ-lactone CO), 198.76 (CO); Anal. Calcd. for $C_{22}H_{15}NO_4$: C, 73.94; H, 4.23; N, 3.92 %. Found: C, 74.06; H, 4.29; N, 3.87 %.

*3-(5-(5-Chloro-2-hydroxybenzoyl)pyridin-2-yl)coumarin **(3c)*** IR (KBr, ν_{max}, cm^{-1}): 1466 (C=N aromatic stretching), 1589 (C=C aromatic stretching), 1612 (C=O ketone stretching), 1720 (C=O δ-lactone stretching), 3063 (C–H aromatic stretching), 3425 (OH stretching); ^1H NMR (400 MHz, $CDCl_3$, δ): 7.10–7.75 (7H, m, Ar–H), 8.14 (1H, dd, $J = 8.4$ and 2 Hz, C_4'-H), 8.72 (1H, d, $J = 9.2$ Hz, C_3'-H), 9.01 (2H, merged singlet, C_4–H and C_6'–H), 11.79 (1H, s, OH, D_2O-exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 116.55 (CH), 119.32 (C), 119.63 (C), 120.44 (CH), 123.35 (CH), 123.93 (C), 124.06 (C), 124.89 (CH), 129.29 (CH), 131.74 (CH), 132.15 (C), 132.99 (CH), 136.90 (CH), 137.49 (CH), 144.21 (CH), 149.35 (CH), 154.25 (C), 154.40 (C), 160.02 (C), 161.77 (δ-lactone CO), 198.01 (CO); Anal. Calcd. for $C_{21}H_{12}ClNO_4$: C, 66.77; H, 3.20; N, 3.71 %. Found: C, 66.61; H, 3.11; N, 3.78 %.

*8-Methoxy-3-(5-(2-hydroxybenzoyl)pyridin-2-yl)coumarin (**3d**)* IR (KBr, ν_{max}, cm^{-1}): 1478 (C=N aromatic stretching), 1582 (C=C aromatic stretching), 1623 (C=O ketone stretching), 1723 (C=O δ-lactone stretching), 3056 (C–H aromatic stretching), 3419 (OH stretching); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 4.04 (3H, s, OCH_3), 6.94–7.62 (7H, m, Ar–H), 8.14 (1H, dd, $J = 8.4$ and 2 Hz, C_4'–H), 8.71 (1H, d, $J = 8.4$ Hz, C_3'–H), 9.01 (2H, merged singlet, C_4–H and C_6'–H), 11.87 (1H, s, OH, D_2O-exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 56.36 (OCH_3), 114.73 (CH), 118.79 (CH), 118.99 (C), 119.17 (CH), 119.90 (C), 120.67 (CH), 123.57 (CH), 124.77 (CH), 132.95 (CH), 137.12 (CH), 138.00 (CH), 142.37 (C), 143.92 (C), 144.58 (CH), 147.04 (C), 148.92 (CH), 153.63 (C), 159.42 (C), 163.34 (δ-lactone CO), 198.59 (CO); Anal. Calcd. for $C_{22}H_{15}NO_5$: C, 70.77; H, 4.05; N, 3.75 %. Found: C, 70.91; H, 3.98; N, 3.69 %.

*8-Methoxy-3-(5-(2-hydroxy-5-methylbenzoyl)pyridin-2-yl)-coumarin (**3e**)* IR (KBr, ν_{max}, cm^{-1}): 1481 (C=N aromatic stretching), 1582 (C=C aromatic stretching), 1628 (C=O ketone stretching), 1720 (C=O δ-lactone stretching), 3060 (C–H aromatic stretching), 3422 (OH stretching); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.30 (3H, s, CH_3), 4.04 (3H, s, OCH_3), 7.03–7.41 (6H, m, Ar–H), 8.09 (1H, dd, $J = 8.4$ and 2.4 Hz, C_4'–H), 8.68 (1H, d, $J = 8.4$ Hz, C_3'–H), 8.97 (2H, merged singlet, C_4–H and C_6'–H), 11.72 (1H, s, OH, D_2O-exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 20.49 (CH_3), 56.36 (OCH_3), 114.83 (CH), 118.58 (C), 118.62 (C), 119.84 (CH), 120.74 (CH), 123.38 (C), 123.80 (C), 124.83 (CH), 128.50 (CH), 132.43 (CH), 133.20 (C), 138.31 (CH), 143.91 (CH), 144.83 (CH), 147.02 (CH), 148.56 (C), 153.28 (C), 159.36 (C), 161.29 (δ-lactone CO), 198.22 (CO); Anal. Calcd. for $C_{23}H_{17}NO_5$: C, 71.31; H, 4.42; N, 3.62 %. Found: C, 71.43; H, 4.49; N, 3.69 %.

*8-Methoxy-3-(5-(5-chloro-2-hydroxybenzoyl)pyridin-2-yl)-coumarin (**3f**)* IR (KBr, ν_{max}, cm^{-1}): 1472 (C=N aromatic stretching), 1589 (C=C aromatic stretching), 1628 (C=O ketone stretching), 1728 (C=O δ-lactone stretching), 3056 (C–H aromatic stretching), 3421 (OH stretching); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 4.05 (3H, s, OCH_3), 7.09–7.58 (6H, m, Ar–H), 8.15 (1H, dd, $J = 8.4$ and 2 Hz, C_4'–H), 8.76 (1H, d, $J = 8.4$ Hz, C_3'–H), 9.03 (2H, merged singlet, C_4–H and C_6'–H), 11.75 (1H, s, OH, D_2O-exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 56.37 (OCH_3), 114.77 (CH), 119.58 (C), 119.89 (C), 120.46 (CH), 120.70 (CH), 123.65 (CH), 123.78 (C), 123.98 (C), 124.78 (CH), 131.71 (CH), 132.25 (C), 136.96 (CH), 137.86 (CH), 143.95 (C), 144.72 (CH), 147.05 (C), 148.98 (CH), 154.15 (C), 159.41 (C), 161.76 (δ-lactone CO), 197.76 (CO); Anal. Calcd. for $C_{22}H_{14}ClNO_5$: C, 64.79; H, 3.46; N, 3.43 %. Found: C, 64.94; H, 3.40; N, 3.50 %.

*6-Bromo-3-(5-(2-hydroxybenzoyl)pyridin-2-yl)coumarin (**3g**)* IR (KBr, ν_{max}, cm^{-1}): 1484 (C=N aromatic stretching), 1589 (C=C aromatic stretching), 1622 (C=O ketone stretching), 1723 (C=O δ-lactone stretching), 3044 (C–H aromatic stretching), 3420 (OH stretching); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.95–7.86 (7H, m, Ar–H), 8.13 (1H, dd, $J = 8.4$ and 2 Hz, C_4'–H), 8.66 (1H, d, $J = 8.4$ Hz, C_3'–H), 9.01 (1H, poorly resolved doublet, C_6'–H), 8.91 (1H, s, C_4–H), 11.88 (1H, s, OH, D_2O-exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 117.45 (C), 118.25 (CH), 118.82 (CH), 119.15 (CH), 120.83 (C), 123.46 (CH), 125.10 (C), 131.34 (CH), 132.97 (CH), 133.89 (C), 135.61 (CH), 137.18 (CH), 137.76 (CH), 142.66 (CH), 149.33 (CH), 153.00 (C), 153.28 (C), 159.41 (C), 161.10 (C), 163.34 (δ-lactone CO), 198.74 (CO); Anal. Calcd. for $C_{21}H_{12}BrNO_4$: C, 59.74; H, 2.86; N, 3.32 %. Found: C, 59.87; H, 2.78; N, 3.24 %.

*6-Bromo-3-(5-(2-hydroxy-5-methylbenzoyl)pyridin-2-yl)-coumarin (**3h**)* IR (KBr, ν_{max}, cm^{-1}): 1474 (C=N aromatic stretching), 1582 (C=C aromatic stretching), 1628 (C=O ketone stretching), 1713 (C=O δ-lactone stretching), 3047 (C–H aromatic stretching), 3422 (OH stretching); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.30 (3H, s, CH_3), 7.03–7.84 (6H, m, Ar–H), 8.11 (1H, dd, $J = 8.4$ and 2 Hz, C_4'–H), 8.64 (1H, d, $J = 8.4$ Hz, C_3'–H), 8.89 (1H, poorly resolved doublet, C_6'–H), 8.97 (1H, s, C_4–H), 11.69 (1H, s, OH, D_2O-exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 20.49 (CH_3), 117.42 (C), 118.23 (CH), 118.56 (CH), 118.68 (C), 120.83 (C), 123.37 (CH), 125.18 (C), 128.39 (C), 131.29 (CH), 132.48 (CH), 133.29 (C), 135.53 (CH), 137.58 (CH), 138.22 (CH), 142.47 (CH), 149.35 (CH), 152.94 (C), 153.16 (C), 159.38 (C), 161.30 (δ-lactone CO), 198.65 (CO); Anal. Calcd. for $C_{22}H_{14}BrNO_4$: C, 60.57; H, 3.23; N, 3.21 %. Found: C, 60.73; H, 3.16; N, 3.28 %.

*6-Bromo-3-(5-(5-chloro-2-hydroxybenzoyl)pyridin-2-yl)-coumarin (**3i**)* IR (KBr, ν_{max}, cm^{-1}): 1466 (C=N aromatic stretching), 1582 (C=C aromatic stretching), 1628 (C=O ketone stretching), 1713 (C=O δ-lactone stretching), 3047 (C–H aromatic stretching), 3420 (OH stretching); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.09–7.86 (6H, m, Ar–H), 8.12 (1H, dd, $J = 8.4$ and 2 Hz, C_4'–H), 8.69 (1H, d, $J = 8.4$ Hz, C_3'–H), 8.91 (1H, poorly resolved doublet, C_6'–H), 9.00 (1H, s, C_4–H), 11.77 (1H, s, OH, D_2O-exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 117.44 (C), 118.26 (CH), 119.60 (C), 120.47 (CH), 120.82 (C), 123.47 (CH), 123.93 (C), 125.11 (C), 131.33 (CH), 131.69 (CH), 132.44 (C), 135.64 (CH), 136.94 (CH), 137.53 (CH), 142.66 (CH), 149.42 (CH), 153.01 (C), 153.79 (C), 159.36 (C), 161.82 (δ-lactone CO), 197.92 (CO); Anal. Calcd. for $C_{21}H_{11}BrClNO_4$: C, 55.23; H, 2.43; N, 3.07 %. Found: C, 55.11; H, 2.34; N, 3.13 %.

*3-(5-(2-Hydroxybenzoyl)pyridin-2-yl)-3H-benzo[f]coumarin (**3j**)* IR (KBr, ν_{max}, cm^{-1}): 1481 (C=N aromatic stretching), 1587 (C=C aromatic stretching), 1624 (C=O ketone stretching), 1727 (C=O δ-lactone stretching), 3056 (C–H aromatic stretching), 3419 (OH stretching); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.96–8.12 (9H, m, Ar–H), 8.16 (1H, dd, $J = 8$ and 2 Hz, C_4'–H), 8.57 (1H, d, $J = 8.4$ Hz, C_5–H), 8.80 (1H, d, $J = 8.4$ Hz, C_3'–H), 9.07 (1H, poorly resolved doublet, C_6'–H), 9.88 (1H, s, C_4–H), 11.91 (1H, s, OH, D_2O-exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 113.80 (C), 116.52 (CH), 118.81 (CH), 119.04 (C), 119.17 (CH), 122.14 (CH), 123.33 (CH), 126.47 (CH), 128.81 (CH), 129.11 (CH), 129.71 (C), 130.45 (C), 132.98 (CH), 133.52 (C), 133.87 (C), 134.79 (CH), 137.09 (CH), 138.05 (CH), 140.15 (CH), 149.04 (CH), 153.88 (C), 154.47 (C), 160.02 (C), 163.38 (δ-lactone CO), 198.49 (CO); Anal. Calcd. for $C_{25}H_{15}NO_4$ C, 76.33; H, 3.84; N, 3.56 %. Found: C, 76.47; H, 3.90; N, 3.51 %.

*3-(5-(2-Hydroxy-5-methylbenzoyl)pyridin-2-yl)-3H-benzo[f]coumarin (**3k**)* IR (KBr, ν_{max}, cm^{-1}): 1474 (C=N aromatic stretching), 1566 (C=C aromatic stretching), 1628 (C=O ketone stretching), 1728 (C=O δ-lactone stretching), 3063 (C–H aromatic stretching), 3421 (OH stretching); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.32 (3H, s, CH_3), 7.04–8.10 (8H, m, Ar–H), 8.13 (1H, dd, $J = 8.4$ and 2.4 Hz, C_4'–H), 8.54 (1H, d, $J = 8.4$ Hz, C_5–H), 8.77 (1H, d, $J = 8.4$ Hz, C_3'–H), 9.05 (1H, poorly resolved doublet, C_6'–H), 9.83(1H, s, C_4–H), 11.74 (1H, s, OH, D_2O-exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 20.51 (CH_3), 113.78 (C), 116.52 (CH), 118.54 (CH), 118.75 (C), 122.09 (CH), 122.36 (C), 123.23 (CH), 126.43 (CH), 128.40 (C), 128.74 (CH), 129.11 (CH), 129.64 (C), 130.41 (C), 132.54 (CH), 132.89 (C), 134.63 (CH), 137.82 (CH), 138.16 (CH), 139.91 (CH), 149.17 (CH), 153.87 (C), 154.38 (C), 160.11 (C), 161.29 (δ-lactone CO), 198.67 (CO); Anal. Calcd. for $C_{26}H_{17}NO_4$: C, 76.65; H, 4.21; N, 3.44 %. Found: C, 76.78; H, 4.28; N, 3.50 %.

*3-(5-(5-Chloro-2-hydroxybenzoyl)pyridin-2-yl)-3H-benzo[f]coumarin (**3l**)* IR (KBr, ν_{max}, cm^{-1}): 1466 (C=N aromatic stretching), 1566 (C=C aromatic stretching), 1628 (C=O ketone stretching), 1720 (C=O δ-lactone stretching), 3063 (C–H aromatic stretching), 3418 (OH stretching); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.10–8.13(8H, m, Ar–H), 8.15 (1H, dd, $J = 8.4$ and 2 Hz, C_4'–H), 8.56 (1H, d, $J = 8.4$ Hz, C_5–H), 8.82 (1H, d, $J = 8.4$ Hz, C_3'–H), 9.06 (1H, poorly resolved doublet, C_6'–H), 9.87 (1H, s, C_4–H), 11.82 (1H, s, OH, D_2O-exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 116.57 (CH), 119.68 (C), 120.45 (CH), 122.06 (CH), 122.57 (C), 123.26 (CH), 123.92 (C), 126.42 (CH), 128.75 (CH), 129.13 (CH), 129.67 (C), 130.43 (C), 131.76 (CH), 132.04 (C), 134.62 (CH), 136.87 (CH), 137.61 (CH), 139.98 (CH), 149.40 (CH), 153.21 (C), 154.43 (C), 154.63 (C), 160.15 (C), 161.78 (δ-lactone CO), 198.01 (CO); Anal. Calcd. for $C_{25}H_{14}ClNO_4$: C, 70.18; H, 3.30; N, 3.27 %. Found: C, 70.02; H, 3.22; N, 3.34 %.

Biological assay

In vitro evaluation of antimicrobial activity

The newly prepared compounds were screened for their MICs by broth micro dilution method. DMSO was used as a diluent to get desired concentration of compounds to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the compound concentrations. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). All the tubes showing no visible growth (same as control tube) were subcultured and incubated overnight at 37 °C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show (i) similar number of colonies indicating bacteriostatic (ii) a reduced number of colonies indicating a partial or slow bactericidal activity (iii) no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized compound was diluted obtaining 2,000 μg/mL concentration as a stock solution. In primary screening, 500, 250, and 200 μg/mL concentrations of the synthesized compounds were taken. The compounds which were found active in this primary screening were further tested in a second set of dilution using 100, 62.5, 50, and 25 μg/mL concentrations against all microorganisms. The highest dilution showing at least 99 % inhibition is taken as MIC.

In vitro evaluation of antituberculosis activity

All the compounds were tested for in vitro antituberculosis activity against *M. tuberculosis* $H_{37}Rv$ by Lowensteine–Jensen method, and then media was sterilized by inspissation method. A culture of *M. tuberculosis* $H_{37}Rv$ growing on Lowensteine–Jensen medium was harvested in 0.85 % saline in bijou bottle. Each compound was tested using DMSO as a diluent at a concentration range of 1,000–62.5 μg/mL, and results are presented as the minimal inhibitory concentration (MIC) (Table 3) with respect to the standard drug

isoniazid. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* $H_{37}Rv$ (5×10^4 bacilli per tube). The growth of bacilli was seen after 28 days of incubation. The tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* $H_{37}Rv$. The MIC can be defined as the lowest concentration effecting the reduction in fluorescence of 90 % relative to controls or the concentration at which no development of colonies occurred or <20 colonies occurred, i.e., MIC can be determined either by fluorescence or colony formation method. The colony formation method was utilized to determine the MIC of the compounds **3a–l**. The compound **3e** ($R_1 = OCH_3$ and $R_4 = CH_3$) shows relatively good antituberculosis activity with MIC value 62.5 μg/mL, while the compounds **3b** ($R_1 = H$ and $R_4 = CH_3$) and **3h** ($R_2 = Br$ and $R_4 = CH_3$) show a moderate activity with MIC value 100 μg/mL.

Acknowledgments The authors are thankful to UGC for meritorious research fellowship under RFSMS. The authors are also thankful to the Department of Chemistry, Sardar Patel University for providing research facilities.

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