Novel Coumarin Isoxazoline Derivatives: Synthesis and Study of Antibaterial Activities

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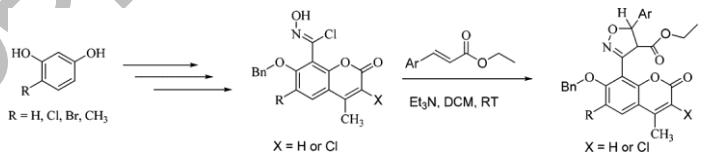
Abstract

A highly efficient and milder protocol for the syntheses of ethyl-3-[7-benzyloxy-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates and ethyl-3-[7-benzyloxy-3-chloro-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates in good yields via [3+2] cycloaddition of **in situ** generated nitrile oxides from 7-Benzyloxy-4-methyl-coumarin hydroxymoylchlorides and 7-Benzyloxy-3-chloro-4-methyl-coumarin hydroxymoylchlorides respectively with ethyl-3-aryl prop-2-enoate has been developed. The new compounds are screened against their antibacterial activity.

GRAPHICAL ABSTRACT



KEYWORDS: [3+2] cycloaddition, 1,3-dipolar cycloaddition, hydroximoyl chloride, nitrile oxide, coumarin isoxazoline, antibacterial activity.

INTRODUCTION

Coumarins (2H-1-benzopyran-2-ones) are most important plant derived metabolites which exhibits an interesting biological and pharmacological activities [1]. Coumarin moiety is used as an important synthetic intermediate and plays a vital role in the synthesis of numerous heterocyclic compounds. Most importantly it is well documented that, large number of pharmaceutical drug products like Novobuocin (Antibiotic), Ensaculin (Anti Dementia)) and Warfarin (Antithrombotic) are marketed widely in USA, Canada and rest of the world which contains 7-hydroxy-4-methyl-2-coumarin as an important structural element [2]. Coumarins containing heterocyclic moieties have significant medicinal value due to their potential pharmacological activities such as antibacterial activities, antifungal and anti-tuberculosis [3].

According to the prior art literature, compounds containing 2-isoxazoline rings showed enormous biological activities such as antinociceptive, anticonvulsant, antipsychotic, anti-stress and analgesic effects [4]. Moreover, isoxazoline derivatives have played an important role, as valuable intermediates in most of the synthetic heterocyclic pharmacological drug products. The traditional way for the synthesis of isoxazolines involve the base catalyzed cyclocondensation of hydroxyl amine hydrochlorides and chalcones. Hemalata Kour et.al teaches the production of isoxazoline coumarin derivatives, which comprises reaction of various substituted chalcone derivatives with hydroxyl amines (free base) in the presence of refluxing methanolic NaOH solution [5]. Desai and his co-workers reported the synthesis of isoxazoline coumarin derivatives by cyclocondensation of hydroxyl amine hydrochlorides and substituted chalcones in

presence of refluxing methanolic NaOH solution for 7.5hours [6]. Recently Imen Zghab and his co-workers speculated the coumarin isoxazoline derivatives by cycloaddition of nitrile oxides with suitable substituted ethyl cinnamates in the presence of triethylamine and refluxing toluene [7]. All these synthesis methods are toilsome; it requires relatively harsh reaction conditions to make coumarin isoxazoline derivatives.

1,3-dipolar cycloaddition was the most effective method for the synthesis of five membered heterocycles which are difficult to be prepared by other means [8]. The most widely used method for the synthesis of isoxazolines is the 1,3-dipolar cycloaddition ([3+2] cycloaddition) of nitrile oxides to activated dipolarophiles [9]. Aryl nitrile oxides are highly reactive and are predominantly generated in situ by either, dehydration of primary nitroalkane derivatives, Mukiyama reaction [10] and halogenation of aldoxime followed by an in situ dehydrohalogenation using a base [11]. The main advantage of the 1,3-dipolar cycloaddition of aryl nitrile oxides to activated double bond is high regioselective with good yields. On the basis of this prior art evidence, we set out to prepare a new series of biological active coumarin appended isoxazoline pharmacophores **11a-d** and **12a-d** via [3+2] cycloaddition.

As a part of our research for coumarin isoxazoline derivatives, we proposed to investigate the behavior of coumarin derived aryl nitrile oxides **8** and **9**, which are generated in situ from hydroximoylchlorides (aldehyde chlorooximes) **6** and **7** under basic medium, toward different substituted cinnamates (dipolarophiles) **10**. This led to new series of ethyl-3-[7-benzyloxy-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole

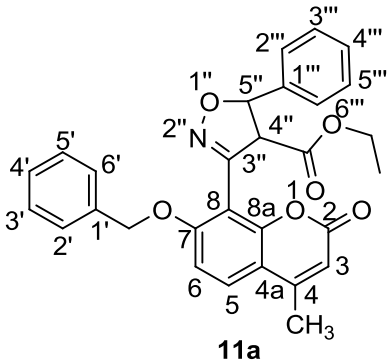
carboxylates **11a-d** and ethyl-3-[7-benzyloxy-3-chloro-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates **12a-d** in good yields. The present study describes the synthesis, characterization and antibacterial activities of novel coumarin isoxazoline derivatives.

RESULTS AND DISCUSSION

The required key intermediates for this study 7-benzyloxy-4-methyl-coumarin aldehyde chlorooximes **6a-d** and 7-Benzyloxy-3-chloro-4-methyl-coumarin aldehyde chlorooximes **7a-d** were prepared as shown in Scheme-1. The first step of the synthesis involves the preparation of 7-hydroxy-4-methyl coumarins **2a-d** via Pechmann condensation of commercially available Resorcinols **1a-d** and ethyl acetoacetate in the presence of H_2SO_4 [12]. Further treatment of 7-hydroxy coumarin derivatives **2a-d** with hexamine in acetic acid undergo Duff formylation [13] to give 8-formyl-7-hydroxy-4-methyl-coumarins **3a-d** which were protected as benzyloxy derivatives **4a-d** with benzyl bromide in the presence of K_2CO_3 in DMF [14]. Compounds **4a-d** was treated with hydroxylamine hydrochloride in the presence of methanol and sodium acetate gets converted in to oximes **5a-d** [15]. Finally, the key intermediates, coumarin derived hydroximoyl chlorides **6a-d** and **7a-d** were synthesized by the chlorination of oximes **5a-d** with equimolar quantities of N-chlorosuccinimide in DMF at 20-25°C [16]. The obtained syrupy mass on purification by column chromatography gave 7-benzyloxy-4-methyl-coumarin aldehyde chloroximes **6a-d** and 3-chlorinated products **7a-d** as off white solids. The absence of oxime proton at δ 11.23 in ^{1}H NMR indicates the formation of hydroxymoylchloride derivatives.

Based on our experimental results and in conjunction with known biological properties for heterocycles as an integral part of the coumarin skeleton we chosen useful and stable coumarin derived hydroximoyl chlorides to synthesize novel coumarin appended isoxazoline derivatives as shown in Scheme-2. Treatment of coumarin derived hydroximoyl chlorides **6a-d** and **7a-d** with triethylamine in dichloromethane at room temperature undergo dehydrohalogenation and results in situ generation of coumarin aryl nitrile oxides **8a-d** and **9a-d** [17] reacted instantaneously with different ethyl cinnamates **10** (dipolarophiles) *via* [3+2] cycloaddition, yielded the corresponding coumarin isoxazoline derivatives **11a-d** and **12a-d.** All these newly synthesized compounds were purified by column chromatography and characterized by Mass, ^{1}H NMR and ^{13}C NMR.

To demonstrate the structure elucidation of the first series of coumarin isoxazoline derivatives **11a-d** we selected compound **11a** which was obtained by the reaction of equimolar quantities of 7-benzyloxy-4-methyl-coumarin hydroximoyl chloride **6a** and ethyl-3-phenylprop-2-enoate **10** in the presence of triethylamine in dichloromethane at room temperature for 24 h. Its positive quasi molecular ion peak was observed at m/z 483.9 (M+H), compatible with the molecular formulae $C_{29}H_{25}NO_{6.}$ In ^{1}H NMR of **11a** the newly formed isoxazoline ring H-4″ appeared at δ 4.68 (d, *J* = 8.8 Hz) and H-5″ at δ 6.07 (d, *J* = 8.8 Hz). In ^{13}C NMR of **11a** isoxazoline ring carbons appeared at δ 60.5 (C-4″), 88.2 (C-5″) and 158.7 (C-3″).



11a

To demonstrate the structure elucidation of the second series of coumarin isoxazoline derivatives **12a-d** we selected the compound **12a** which was obtained by the reaction of equimolar quantities of 7-benzyloxy-3-chloro-4-methyl-coumarin hydroximoyl chloride **7a** and ethyl-3-phenylprop-2-enoate **10.** The compound **12a** showed its quasi molecular ion peaks at m/z 518.1 (M+H) and m/z 520.3 (M+H+2), with 3:1 isotopic peak intensities ratio in mass spectrum confirmed the molecular formulae $C_{29}H_{24}ClNO_6$ with one chlorine atom. The newly formed isoxazoline ring proton H-4″ appeared at δ 4.64 (d, *J* = 8.6 Hz) and H-5″ at δ 6.09 (d, *J* = 8.6 Hz). In ^{13}C NMR of **12a** isoxazoline ring carbons appeared at δ 62.3 (C-4″), 88.7 (C-5″) and 159.7 (C-3″).

Antibacterial Activity

It is well known that coumarin isoxazoline derivatives possess antibacterial activity [18]. Furthermore, halogen substituents were introduced into the basic structure anticipating an improved biological activity because their incorporation was proved to be influencing the biological activity in various heterocycles [19] as well as coumarins [20]. Electron withdrawing groups like halogens will increase bactericidal potential as they alter the nature of the compound in such a way as to promote binding to the target(s) [21]. According to Rajendra Prasad et al in designing the compounds bearing electron

withdrawing substituents (with high degree of binding linearity) results in high molecular weights to exhibit an improved antibacterial activity [22]. Similarly, the significant inhibition shown by substituted isoxazolines. Similarly, the significant inhibition shown by substituted isoxazolines was attributed to substituents like hydroxymethyl / hydroxy / methoxy / ethyl ester groups [23]. Tejaskumar Shah and Vikas Desai also reported that the presence of methoxy-, chloro-, and fluoro groups enhanced the antibacterial activity in isoxazoline derivatives [24].

All these newly synthesized compounds **11a-d** and **12a-d** were evaluated for in vitro antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus* –MTCC 96) and Gram-negative bacteria (*Escherichia coli*– MTCC 40) by agar disc diffusion method [25]. The antibacterial screening data showed that most of the compounds **11a-d** and **12a-d** showed moderate to excellent activities against the used microorganisms (Table-2) compared to the reference drug (Chloramphenicol). These results suggested that the introduction of halogen substituent increased the hydrophobicity of the synthesized compounds and lead to the increase of the antibacterial activity [26]. In the present study, it is observed that good activity was shown by the prepared derivatives against the studied Gram-positive bacteria and very poor activity against Gram-negative bacteria. The cell outer layers of Gram-positive and Gram-negative bacteria may explain the decent antibacterial activities of these prepared derivatives [27]. The permeability of drug constituents in Gram-positive bacteria is due to an ineffective and penetrable outer barrier made of peptidoglycan layer. On the other hand, outer cell wall of Gram-negative bacteria consists multiple impermeable peptidoglycan and phospholipidic layers [28]. Among the compounds screened, **12c** showed high activity. The observed antibacterial

activity profile suggested that the presence of halogen functional group --Bromine had enhanced the activity.

CONCLUSION

In conclusion, we have successfully achieved two important aspects in this work. One is a highly practical method for the synthesis of novel coumarin appended isoxazoline derivatives **11a-d** and **12a-d** in a good yield via [3+2] cycloaddition (1,3-diploar cycloaddition) under mild reaction conditions. Second one is the antibacterial activities of the synthesized compounds. Interestingly all these new compounds are active and showing moderate to good antibacterial activity against Gram-positive bacteria and Gram-negative. The observed antibacterial activity profile suggested that the presence of Bromine had enhanced the activity.

EXPERIMENTAL

Melting points were determined in open glass capillaries on a Fisher–Johns melting point apparatus and are uncorrected. NMR (^{1}H 400 MHz; ^{13}C 125 MHz) were recorded at room temperature in $CDCl_3$ as solvent and TMS as an internal standard (δ = 0 ppm), and the values were reported in the following order: chemical shift (δ in ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, qq = quartet of quartet), coupling constants (J in Hz), and integration. All the reactions were monitored by thin layer chromatography on precoated silica gel 60 F254 (mesh); spots were visualized under UV light at 254 nm.

Typical Experimental Procedure For The Synthesis Of Hydroximoyl Chlorides (6a-D) And (7a-D):

To a stirred solution of 7-benzyloxy-4-methyl-coumarin aldehyde oxime **5a** (8.0g, 25.8 mmol) in DMF (200.0 mL) was added N-chlorosuccinimide (4.1g, 31.0 mmol) at 0°C, raised to RT over 2 hours and quenched the reaction with ice cold water (600.0 mL), extracted with ethyl acetate (3×200.0 mL). The combined organic layer was washed with saturated NaCl solution (200.0 mL), dried over Na_2SO_4, concentrated and purified by column chromatography to give **6a,** 5.6 g (63%). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 2.41 (3H, s, 4-C$\underline{H}_3$); 5.37 (s, 2H, OC$\underline{H}_2C_6H_5$); 6.13 (s, 1H, H-3); 7.24-7.35 (5H, m, $OCH_2C_6\underline{H}_5$); 7.41 (1H, d, *J* = 8.8 Hz, H-6); 7.8 (1H, d, *J* = 8.8 Hz, H-6); 12.4 (1H, s, N-O$\underline{H}$) and **7a,** 2.6 g (27%); ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 2.45 (3H, s, 4-C$\underline{H}_3$); 5.3 (s, 2H, OC$\underline{H}_2C_6H_5$); 7.34-7.46 (5H, m, $OCH_2C_6\underline{H}_5$); 7.8 (1H, d, *J* = 8.8 Hz, H-6); 7.9 (1H, d, *J* = 8.8 Hz, H-6); 12.4 (1H, s, N-O$\underline{H}$).

Following the same procedure as depicted in a **6a** and **7a**, the other hydroximoyl chlorides **6b-d** and **7b-d** were prepared from the corresponding aldehyde oximes**.** Hydroximoyl chlorides are highly unstable and are readily prepared whenever required.

Typical Experimental Procedure For The Synthesis Of Coumarin Isoxazoline Compounds (11a-D):

To a stirred solution of hydroximoyl chloride **6a** (0.5g, 1.45 mmol) in Dichloromethane (12.5 mL) and Et_3N (0.19g, 1.89 mmol) was added the corresponding ethyl-3-phenyl-prop-2-enoate **10** (0.29g, 1.66 mmol) dissolved in Dichloromethane (12.5

mL) and the reaction mixture was stirred for 24 h at room temperature. Water (30.0 mL) was added and extracted in to Dichloromethane (2x30.0 mL). The combined organic layer was washed with saturated NaCl solution (60.0 mL), dried over Na_2SO_4, and concentrated to give the crude isoxazoline which was purified by column chromatography using silica gel in ethyl acetate: pet ether (6:4), obtained **11a,** 0.46 g, 65.7 %, yield.

Following the same procedure as illustrated in **11a,** the other coumarin isoxazoline derivatives **11b-d** were prepared from the corresponding hydroximoyl chlorides**.** The physical, spectral and analytical data for these compounds are mentioned as follows.

Ethyl-3-[7-(Benzyloxy)-4-Methyl-2-Oxo-2H-8-Chromenyl]-5-Phenyl-4,5-Dihydro-4-Isoxazole Carboxylate (11a)

Off white solid; Yield 0.46 g (66%); MR: 130-132°C; ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 0.95 (3H, t, *J* = 7.2,$OCH_2C\underline{H}_3$); 2.42 (3H, s, 4-$C\underline{H}_3$); 3.95 (2H, q, *J* = 7.2,$OC\underline{H}_2CH_3$); 4.68 (1H, d, *J* = 8.8 Hz, H-4″); 5.21 (2H, s, -$OC\underline{H}_2C_6H_5$); 6.07 (1H, d, *J* = 8.8 Hz, H-5″); 6.29 (s, 1H, H-3); 6.91 (1H, d, *J* = 9.2 Hz, H-6); 7.23-7.38 (5H, m, $OCH_2C_6\underline{H}_5$); 7.51-7.62 (5H, m, Ar); 7.72 (1H, d, *J* = 9.2 Hz, H-5); ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 13.7 ($OCH_2\underline{C}H_3$); 18.7 ($\underline{C}H_3$); 60.5 (C-4″); 63.1 ($O\underline{C}H_2CH_3$); 70.1 ($O\underline{C}H_2C_6H_5$); 88.2 (C-5″); 103.9 (C-6); 104.2 (C-3); 112.6 (C-4a); 113.9 (C-8); 127.1 (C-2′,6′,4′,2‴,6‴,4‴ Ar); 129.5 (C-3′,5′,3‴,5‴ Ar); 142.1 (C-5); 150.3 (C-1‴, 1′); 153.5 (C-8a); 158.7 (C-3″, 4); 166.9 (C-2,7); 167.8 (COOEt); DIPMS: m/z= 483.9

(M+H); Elemental analysis: Found, %: C 71.92; H 5.18; N-2.81. $C_{29}H_{25}NO_6$. Calculated, %: C 72.04; H 5.21; N 2.90.

Typical Experimental Procedure For The Synthesis Of 3-Chlorinated Coumarin Isoxazoline Compounds (12a-D)

To a stirred solution of hydroximoyl chloride **7a** (0.5 g, 1.32 mmol) in Dichloromethane (10.0 mL), triethylamine (0.17 g, 1.71 mmol) was added the corresponding ethyl-3-phenyl-prop-2-enoate **10** (0.26 g, 1.51 mmol) dissolved in Dichloromethane (10.0 mL) and reaction mixture was stirred for 24 h at room temperature. Added water (50.0 mL) and extracted in to Dichloromethane (2x50.0 mL). The combined organic layer was washed with saturated NaCl solution (60.0 mL), dried over Na_2SO_4, and concentrated to give the crude isoxazoline which was purified by column chromatography using silica gel in ethyl acetate: pet ether (8: 2), obtained **12a,** 0.51 g, 74.5 % yield.

Following the same procedure as illustrated in **12a** coumarin isoxazoline derivatives **12b-d** were prepared from the corresponding hydroximoyl chlorides. The physical, spectral and analytical data for these compounds are mentioned as follows.

Ethyl-3-[7-(Benzyloxy)-3-Chloro-4-Methyl-2-Oxo-2H-8-Chromenyl]-5-Phenyl-4,5-Dihydro-4-Isoxazole Carboxylate (12a)

Off white solid; Yield 0.51 g (75%); MR: 118-120°C; ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 1.03 (3H, t, *J* = 7.3,$OCH_2C\underline{H}_3$); 2.45 (3H, s, 4-$C\underline{H}_3$); 3.97 (2H,

q, *J* = 7.3,OC$\underline{H_2}$CH$_3$); 4.64 (1H, d, *J* = 8.6 Hz, H-4″); 5.24 (2H, s, OC$\underline{H_2}$C$_6$H$_5$); 6.09 (1H, d, *J* = 8.6 Hz, H-5″); 6.38 (1H, d, *J* = 9.1 Hz, H-6); 7.28-7.37 (5H, m, OCH$_2$C$_6\underline{H_5}$); 7.51-7.59 (5H, m, Ar); 7.81 (1H, d, *J* = 9.1 Hz, H-5); ^{13}C NMR spectrum (125 MHz, CDCl$_3$), δ, ppm: 14.1 (OCH$_2\underline{C}$H$_3$); 18.9 ($\underline{C}$H$_3$); 62.3 (C-4″); 64.7 (O$\underline{C}$H$_2$CH$_3$); 72.3 (O$\underline{C}$H$_2$C$_6$H$_5$); 88.7 (C-5″); 105.3 (C-6); 113.7 (C-4a); 115.9 (C-8); 119.3 (C-3); 122.5 (C-2′,6′,4′,2‴,6‴,4‴ Ar); 123.5 (C-3′,5′,3‴,5‴ Ar); 142.6 (C-5); 148.2 (C-1‴, 1′); 151.8 (C-8a); 154.2 (C-3″); 159.7 (C-4); 163.8 (C-7); 166.9 (C-2,); 169.8 ($\underline{C}$OOEt); DIPMS: m/z= 518.1 (M+H), 520.3 (M+H+2); Elemental analysis: Found, %: C 67.02; H 4.67; N 2.69. C$_{29}$H$_{24}$ClNO$_6$. Calculated, %: C 67.25; H 4.67; N 2.70.

Screening Of Antibacterial Activity

All these newly synthesized compounds **11a-d** and **12a-d** were evaluated for in vitro antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus* –MTCC 96) and Gram-negative bacteria (*Escherichia coli*– MTCC 40) by agar disc diffusion method. All the derivatives at the concentration of 50 µg/ml, 100 µg/ml and 200 µg/ml were tested against Gram-positive bacteria (*Staphylococcus aureus* –MTCC 96) and Gram-negative bacteria (*Escherichia coli* – MTCC 40). The molten nutrient agar was inoculated with 100 µl of the inoculum (1 x 108 cfu/ml) and poured into the Petri plate, the disc (5mm) (Hi-Media), was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated at about 37ºC and microbial progress was determined by measuring the diameter of zone of inhibition after 24 hours. Pure solvents were used as control and inhibitory zones were nearly negligible compared to the inhibition zones of the samples.

To clarify any solvent effect on the biological screening, separate studies were carried out using Dimethyl sulfoxide (DMSO) as a control and it showed no activity against any bacterial strains. Chloramphenicol was used as standard drug for the purpose of comparison of antibacterial activities of the derivatives. The antibacterial activities were carried out in triplicate and average values were compiled.

SUPPLEMENTAL MATERIAL

Elemental analysis, Mass, ^{1}H NMR, ^{13}C NMR Spectra and characterization data for all newly synthesized coumarin isoxazoline derivatives can be accessed on the publisher's website.

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Table-1. Yields of coumarin isoxazoline derivatives

Entry	***R***	***R_1***	***Product***	***Yield* (%)***
1	H	H	**11a**	66
2	Cl	CF_3	**11b**	74
3	Br	CF_3	**11c**	77
4	CH_3	3,5-difluoro	**11d**	69
5	H	H	**12a**	75
6	Cl	CF_3	**12b**	74
7	Br	CF_3	**12c**	69
8	CH_3	3,5-difluoro	**12d**	72

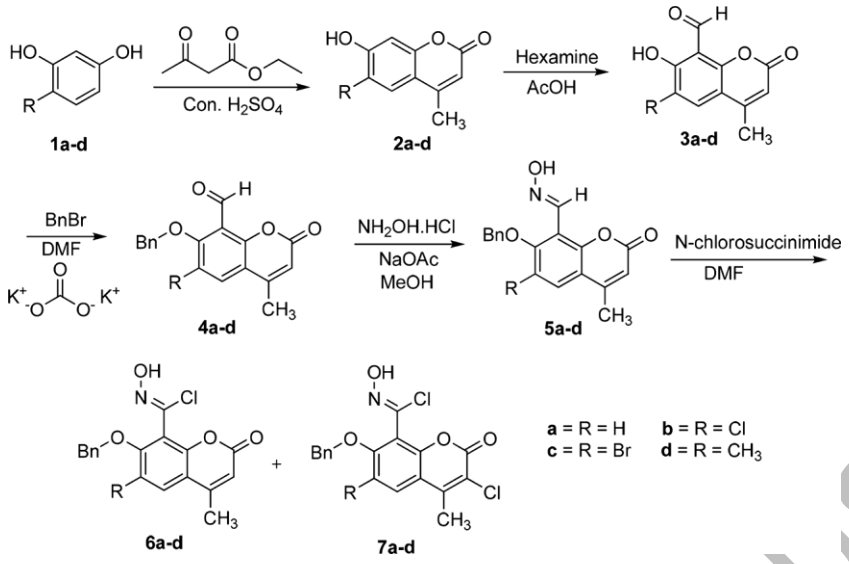
*****yields are after column purification

Table-2. Antibacterial activity data of the synthesized coumarin isoxazoline derivatives

Comp.	***Zone of inhibition (mm)****					
	Escherichia coli ***(MTCC 40)*** *(Gram-negative)*			*Staphylococcus aureus* ***(MTCC 96)***		
	(Conc. µg/ml)			*(Gram-positive)* (Conc. µg/ml)		
	200	***100***	***50***	***200***	***100***	***50***
11a	15	11	5	17	12	5
11b	18	12	8	18	13	10
11c	27	20	19	29	21	19
11d	15	12	11	21	19	4
12a	22	11	6	22	18	7
12b	11	13	14	24	18	26
12c	28	24	20	31	29	24
12d	22	22	16	24	22	20
Chloramphenicol	31	30	21	33	30	23

** indicates average of triplicate*

Scheme-1. Synthesis of coumarin derived hydroximoyl chlorides 6a-d and 7a-d.



Scheme-2. Synthesis of coumarin isoxazoline derivatives