IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 11, Issue 12 Ver. I (December. 2018), PP 31-39
www.iosrjournals.org

Synthesis and characterization of coumarin-isoxazole conjugate as potent antibacterial and anti-inflammatory agents

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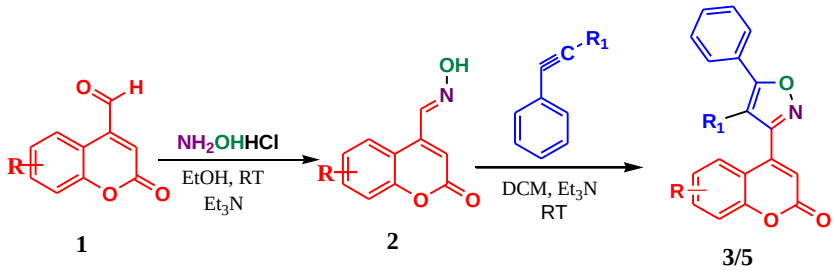
Graphical abstract:



Abstract: *A series of novel coumarin isoxazole (**3a-3f**) and (**5a-5f**) have been synthesized in good yield starting from 4-formylcoumarins. These newly synthesized coumarin-isoxazole conjugates are mimic structure of valdicoxib which is an anti-inflammatory agent. All the synthesized compounds were screened for their antibacterial and anti-inflammatory activity. The antibacterial activity results exhibited high selectivity against gram positive bacterial strains than gram negative bacterial strains. Interestingly, phenylsulphonamide substituted molecules **5a-5f** are more promising against bacterial strains. Further, the anti-inflammatory study showed inhibition of matrix metalloprotiens (MMPs). Particularly, compounds are quite promising with significant inhibition properties against MMP-2 and MMP-9. All the compounds have been characterized by IR, ^{1}HNMR, ^{13}C NMR and mass spectral data.*

Keywords: *Anti-inflammatory, coumarin-oxime, coumarin-isoxazole, phenylsulphonamide.*

Date of Submission: 11-12-2018 Date of acceptance: 27-12-2018

I. Introduction

A wide range of organic compounds containing the coumarin moiety originating natural or unnatural are found to exhibit various important applications, such as: antibacterial[1], antifungal[2],anti-inflammatory[3,4],antioxidant[5], antibiotic[6,7], anticoagulant[8], analgesic[9], antimutagenic[3], anticancer36,[10-15], anti-HIV[16–18], tumor necrosis factor-a inhibitory[19], serine protease inhibitory[20], and steroid 5α-reductase inhibitory[1] activities and also used as fluorescence sensors[21], brightening agents[22].

The isoxazole skeleton is a versatile five-membered heterocyclic motifs present in several natural products and drugs (Figure1). Among all the heterocycles, substituted isoxazoles have attracted great concern owing to their broad range of applications in medicinal chemistry[23]. Isoxazoles are highly selective and more potent agonists at human cloned dopamine D4 receptors[24]. These molecules also displayed GABAA antagonist[25], ulcerogenic, analgesic, anti-inflammatory[26], COX-2 inhibitory[27], antimicrobial, antifungal[28], antinociceptive[29], and anticancer[30] activity. Our previous studies also indicate that coumarin combined with pyranopyrzole[31], pyrans[32] and pyrimidines[33] moieties have exhibited excellent antibacterial and anti-inflammatory activities. Hence, a practical method for the synthesis of such compounds is of great interest in synthetic organic chemistry. Therefore, we designed and synthesized coumarin isoxazoles and screened for their antibacterial and anti-inflammatory activity. **Figure 1**, the structurally similar molecules (**A**-**C**) which have exhibited significant antibacterial and anti-inflammatory activities, while our designed and synthesized coumarin-isoxazoles (**5**) also exhibited promising antibacterial and anti-inflammatory activities.

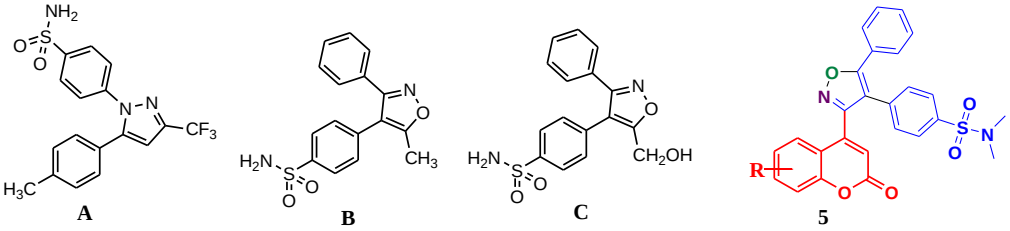
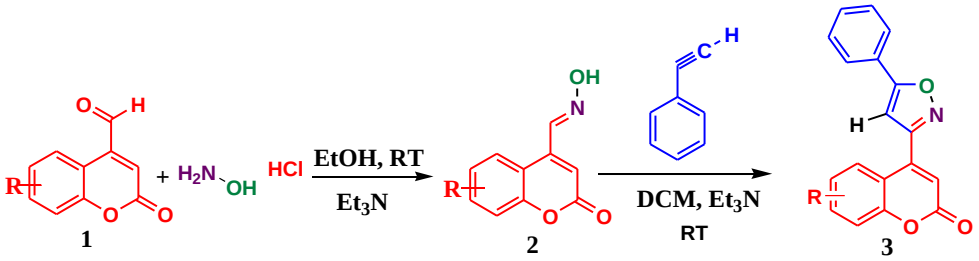


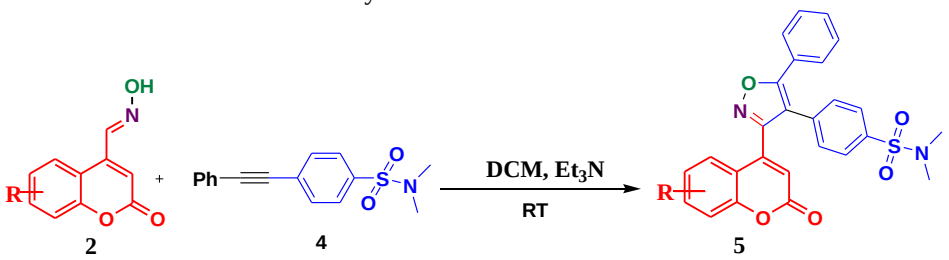
Figure 1. (A-C): Isoxazole derivative having established pharmacological activity. 5: Designed and synthesized molecule.

II. Results And Discussion

The synthetic rout for the target compounds are discussed in **schemes 1** and **2**. Coumarin -4-oxime (**2**) was obtained by the reaction of 4-formylcoumarin (**1**) [34] and hydroxylamine hydrochloride. Further, the reaction of **2** with commercially available phenyl acetylene led to desired coumarin-5-phenyl isoxazole (**3a-3f)** *via* [3+2] cycloaddition approach in the presence of catalytic amount of triethyl amine (TEA) at room temperature in satisfactory yield. Similarly compound (**5a-5f**) was also obtained by similarly procedure in good yield and the plausible mechanism is outlined in **scheme 3**.



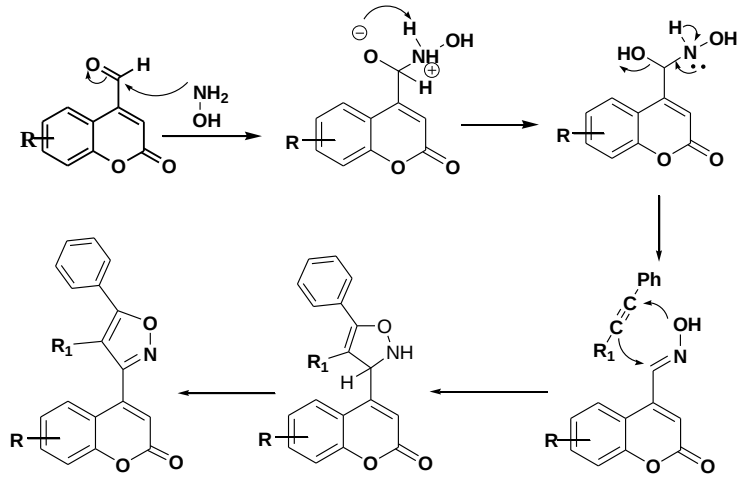
Scheme 1: Synthesis of coumarin isoxazoles **3**



Scheme 2: Synthesis of coumarin isoxazolo-phenyl sulphonamide **5**

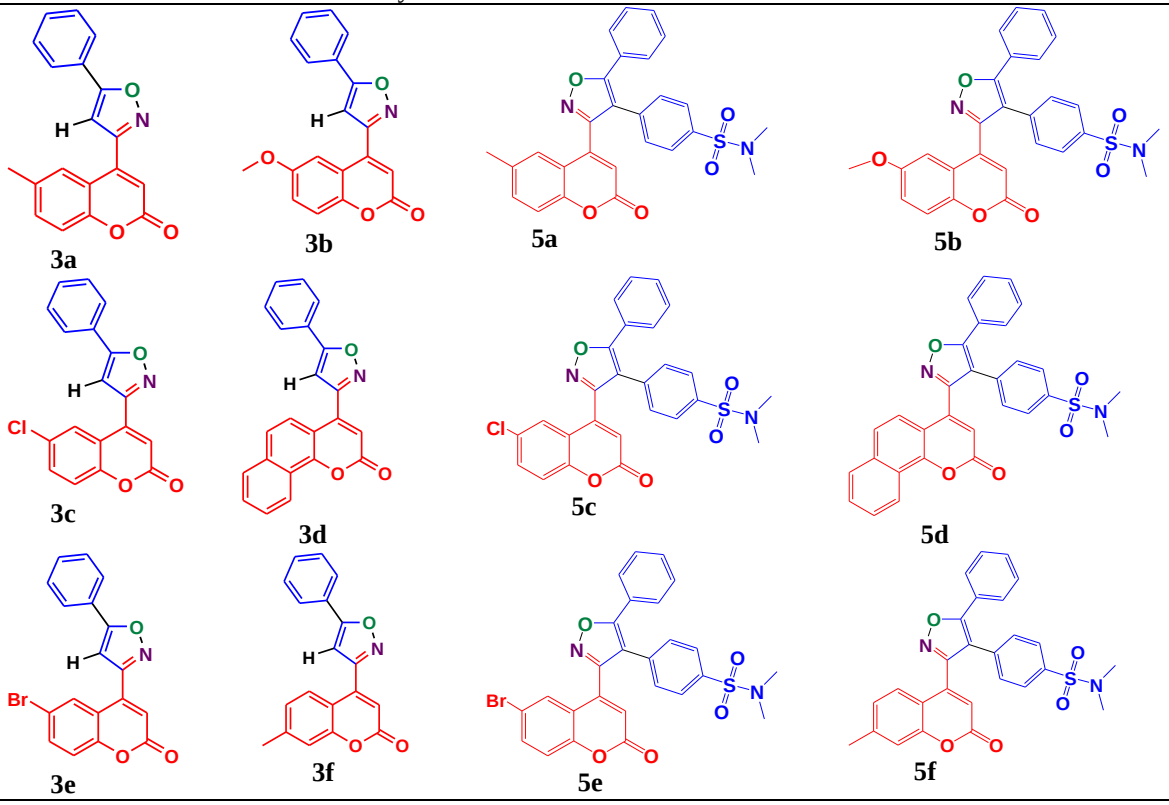
All the newly synthesized compounds were confirmed by spectral analysis and structures are listed in **Table 1**. In the case of compound **3a**, IR spectrum exhibited two intense stretching bands at 1724 and 1640 cm^{-1} due to lactone carbonyl and C=N of coumarin and isoxazolic moiety respectively.^{1}H NMR spectrum of compound **3a** shows down filed singlet at δ8.10ppm due C4-H of isoxazole. Whereas, coumarin C7-H and C8-H resonated as doublet of doublet at δ7.94ppm (J =4Hz) and δ7.96ppm (J =3.6Hz) respectively. Singlet resonated at δ7.71ppm is due to C5-H of coumarin. Two multiplets were observed at δ7.62-7.51ppm and δ7.42-7.38ppm due to phenyl ring protons respectively. The C3-H and C6-CH_3 of coumarin resonated as two singlets at δ6.95 and ppm δ2.39ppm respectively. Molecular mass of the compound **3a** m/z 303confirms the formation of product.

IR of the compound **5a** showed stretching bands at 1722 and 1653 cm^{-1} due to lactone carbonyl and C=N of coumarin and isoxazolic moiety respectively. Further, the formation of compound **5a** was confirmed by ^{1}H NMR spectrum which shows two singlets at δ8.00ppm and δ6.81ppm due to C5-H and C3-H of coumarin, while C6-CH_3 resonated as singlet at δ2.43 ppm. Two singlets resonated at 3.73ppm and 3.85ppm due to N-methyl groups.



Scheme 3. Proposed mechanism for the formation of coumarin-isoxazoles

Table 1: Synthesis of coumarin-isoxazoles **3a-3f** and **5a-5f.**



Pharmacological screening

In vitro antibacterial studies

Preliminary antibacterial screening was performed for all the synthesized compounds (**3a-3f** and **5a-5f**) against two Gram-positive (*S. aureus and E. faecalis*) and two Gram-negative (*E. coli and P. aeruginosa*) bacterial strains by Broth microdilution method using ciprofloxacin as comparator [32]. Preliminary antibacterial results showed that compounds are highly active against tested organisms. The minimum inhibitory concentrations (MIC) of all the compounds were determined and obtained results are presented in **Table 2.**

Results of compounds **3a-3f** indicated that most of the compounds are highly promising against S. *aureus and E. faecalis* bacterial stains with MIC values ranges from 2.10 to 4.25µg/mL and standard ciprofloxacin MIC value 8.00 µg/mL. Among these series compound **3d** is less active against both gram positive bacterial strains. Similarly, compounds **3a-3f** showed good to moderate activity against *E. coli*, while, these compounds are not found active against *P. aeruginosa.* In general, most of the compounds form the series **3a-3f** exhibited better activity against gram positive than gram negative bacterial strains.

Similarly, the antibacterial activity results of compounds **5a-5f** are depicted in table 1, it reveals that remarkable enhancement of activity against both tested gram positive and gram negative bacterial stains. The MIC values of some of the compounds are as low as 1.10µg/mL against gram positive bacterial strains and 4.00 µg/mL against gram negative bacterial strains as compared with standard ciprofloxacin MIC value 8 µg/mL and 12 µg/mL respectively. The improvement of antibacterial activity of compounds **5a-5f** was noticed in comparison with the series **3a-3f**, it may be the introduction of phenyl *p*-sulphonamide group on isoxazole nucleus. In this series we also identified that mono substitution on coumarin nucleus are more promising and potent drug like molecules. The results also compared with standard drug molecule are presents in **Figure 2.**

Table 2. *in vitro* antibacterial data of compounds **3a-3f** and **5a-5f**

Entry	**R**	**Minimum inhibitory concentration In µg/ml (MIC)**			
		Gram +bacteria		Gram –bacteria	
		S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*
Control	DMSO	-	-	-	-
3a	6-CH_3	4.25	4.25	10.12	>20
3b	6-OCH_3	2.12	2.10	6.20	>20
3c	6-Cl	2.12	2.20	8.10	>20
3d	7,8-Benzo	9.20	9.82	12.10	>30
3e	6-Br	2.20	2.18	8.00	>20
3f	7-CH_3	4.20	2.82	6.20	>20
5a	6-CH_3	1.12	1.25	2.00	8.00
5b	6-OCH_3	1.12	1.10	2.00	8.20
5c	6-Cl	1.12	1.20	2.10	8.10
5d	7,8-Benzo	8.10	6.22	6.10	12.00
5e	6-Br	1.20	1.18	2.00	10.00
5f	7-CH_3	1.12	1.82	2.10	8.20
Ciprofloxacin		8.00	8.00	12.00	12.00

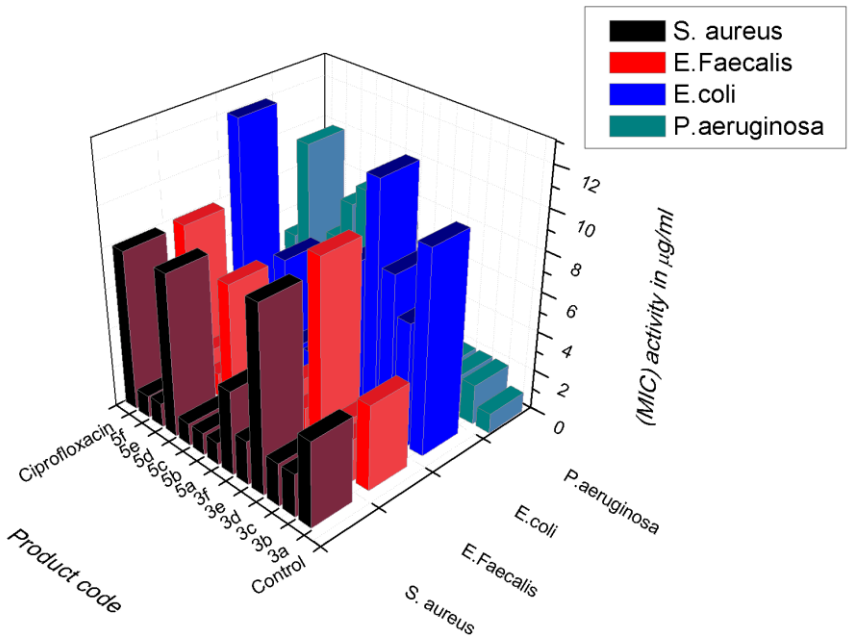


Figure 2. Graphical representation of antibacterial activity.

4.2. ***Anti-inflammatory study***

Anti-inflammatory activity of all the synthesized compounds **3a-3f** and **5a-5f** was screened using gelatin gemography method[33] and tetracycline as a standard drug molecule. Two key members of matrix metelloproteins (MMPs) such as gelatin A (MMP-2) and gelatin B (MMP-9) have more effective gelatin degrading properties. These two MMPs remain inactive when they are with pro-domain, they need to be activate by denaturation and it would be detected on gelatin zymograms as two bands (one pro-band and one active form

of band) after staining with coosmassie blue. The in-vitro anti-inflammatory screening results obtained are % band of MMP-2 and MMP-9 which are listed in **Table 3**. Each sample is detected from the gelatin zymogram by gel electrophoresis apparatus. The % inhibition of MMP-2 ns MMP-9 for each compound is calculated by subtracting from 100 with the % band of MMP-2 and MMP-9 respectively. From **Table 3** results reveal that all the compounds of both series 3a-3h and **5a-5f** are highly active against both MMP-2 and MMP-9. Interestingly, compounds **5a-5h** are more promising than **3a-3f**. The enhanced activity of series **5a-5h** is may be due to the introduction of phenyl p-sulphonamide group on isoxazole nucleus. The ant-inflammatory activity results are also presented in **Figure 3** with standard tetracycline.

Table 3: The *in vitro* anti-inflammatory results of compounds **3a-3f** and **5a-5f**with % bands and % inhibition of MMP-2 and MMP-9

Product Code	R	% Bands of MMPs		% Inhibition of MMPs	
		MMP-9	MMP-2	MMP-9	MMP-2
3a	6-CH_3	10	05	**90**	**95**
3b	6-OCH_3	10	05	**90**	**95**
3c	6-Cl	05	10	**95**	**90**
3d	7,8-Benzo	70	40	30	**60**
3e	6-Br	10	10	**90**	**90**
3f	7-CH_3	20	08	**80**	**92**
5a	6-CH_3	05	02	**95**	**98**
5b	6-OCH_3	02	02	**98**	**98**
5c	6-Cl	05	05	**95**	**95**
5d	7,8-Benzo	30	40	70	**60**
5e	6-Br	20	10	**80**	**90**
5f	7-CH_3	10	05	**90**	**95**
Tetracycline		**00**	**00**	**100**	**100**
DMSO Control		**-**	**-**	**-**	**-**

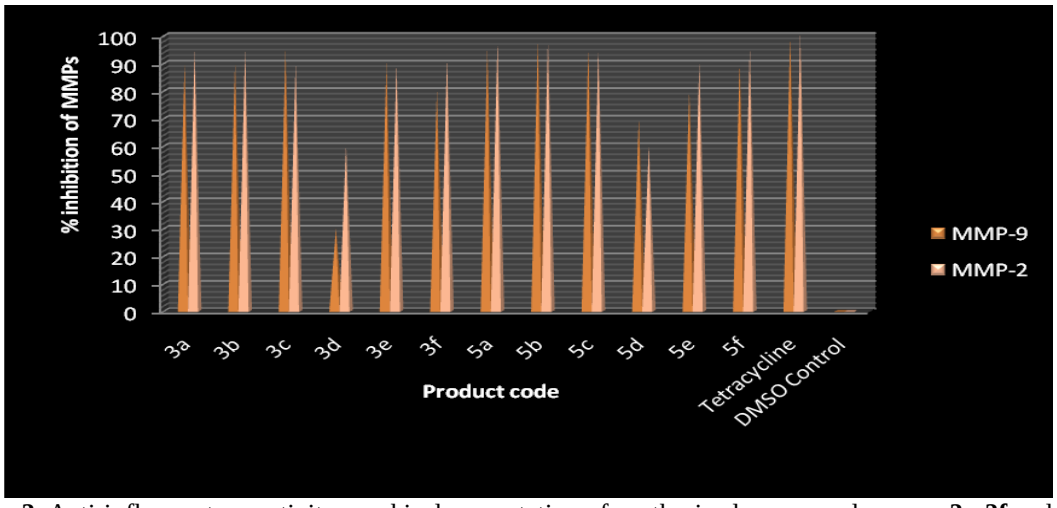


Figure 3. Anti-inflammatory activity graphical presentation of synthesized compounds **3a-3f** and **5a-5f**

III. Conclusion

As part of our interest and continuing efforts to identify inhibitors with greater potency and high specificity for antibacterial and anti-inflammatory agents, we have synthesized a series of mono (**3a-3f**) and diarylisoxazole (**5a-5f**) coumarin molecules. The synthesized molecules showed exceptional higher potency toward gram positive compared to gram negative bacterial strains. The molecules are highly promising, more efficient and potential anti-inflammatory agents.

EXPERIMENTAL PROTOCOL

All the chemicals were purchased by commercial source and were used without further purification unless otherwise stated. The reaction monitored by thin-layer chromatography (TLC) was performed with Merck Kieselgel 60 F254 plates and visualized using UV light at 254 nm and $KMnO_4$ staining or iodine vapour. The melting points were determined by open capillary method and are uncorrected. ^{1}H NMR and ^{13}C NMR

spectra were measured on a Bruker 400 MHz and Jeol solution for innovation 500 MHz spectrometer using DMSO-d_6 as solvent and TMS as an internal standard. Chemical shifts are reported in δ ppm relative to internal tetramethylsilane standard (TMS). The coupling constant 'J' is reported in Hertz (Hz). The IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer in KBr disc method and mass spectra were recorded using Shimadzu GCMSQP2010S.

General procedure for the synthesis of coumarin oxime 2:
A mixture of 4-formylcoumarin (1 mmol) and hydroxylamine hydrochloride (1 mmol) was dissolved in aqueous ethanol (1:4). To this add 2-3 drops of Et_3N and stirred for 2-3 hrs at room temperature. The oxime formation was corroborated by thin-layer chromatographic (TLC) analysis. The solid product formed was filtered, repeatedly washed with water and dried to get pure product.

Synthesis of coumarin-5-phenylisoxazole 3:
To a solution of coumarin oxime **2** (1 mmol) and phenylacetylene (1 mmol) in DCM was prepared and to this 2-3 drops of Et_3N was added. The reaction mixture was allowed to stir for 4-5 hrs at room temperature. The product formation was confirmed by thin-layer chromatographic (TLC) analysis. After completion of reaction DCM was removed by rota evaporator. The solid obtained was washed with cold ethanol and purified by column chromatography using ethyl acetate-hexane (2:8).

6-Methyl-4-(5-phenylisoxazol-3-yl)-2H-chromen-2-one (3a): Yield: 82%; pale yellow solid; m.p. 202-204 ^{0}C; IR(KBr) ν: 1724, 1640 cm^{-1}; ^{1}H NMR (400Hz, DMSO-d_6): δ (ppm): 2.39 (3H, s, C6-CH_3 of coumarin), 6.95 (s,1H, C3-H of coumarin), 7.42-7.38 (m,1H), 7.62-7.52(m,4H), 7.71(s,1H, C5-H of coumarin), 7.94 and 7.96 (dd, 2H J = 4 and 3.6 Hz), 8.10(s,1H,C4 of isoxazole); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm): 169.84, 159.73, 159.42, 153.68, 143.55, 141.38, 130.89, 129.32, 126.99, 126.13, 125.78, 125.69, 116.96, 115.99, 113.79, 100.93, 20.96; GC-MS: m/z 303.

6-Methoxyl-4-(5-phenylisoxazol-3-yl)-2H-chromen-2-one (3b): Yield: 80%; light green solid; m.p. 182-184 ^{0}C; IR(KBr) ν: 1718, 1654 cm^{-1}; ^{1}H NMR (400Hz, DMSO-d_6): δ (ppm): 3.89 (3H, s, C6-OCH_3 of coumarin), 6.85 (s,1H, C3-H of coumarin), 7.46-7.40 (m,1H), 7.64-7.53(m, 4H), 7.75(s,1H, C5-H of coumarin), 7.95 and 7.98 (dd, 2H J = 6.2 and 4.2 Hz), 8.12(s,1H,C4 of isoxazole); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm): 170.82, 160.72, 159.80, 154.78, 144.51, 142.41, 131.52, 130.30, 127.62, 127.56, 126.22, 126.10, 117.12, 116.16, 114.17, 101.48, 58.68; GC-MS: m/z 319.

6-Chloro-4-(5-phenylisoxazol-3-yl)-2H-chromen-2-one (3c): Yield: 72%; white solid; m.p. 214-218 ^{0}C; IR(KBr) ν: 1725, 1658 cm^{-1}; ^{1}H NMR (400Hz, DMSO-d_6): δ (ppm): 6.81 (s,1H, C3-H of coumarin), 7.48-7.44 (m,1H), 7.70-7.66(m, 4H), 7.79(s,1H, C5-H of coumarin), 7.96 and 7.99 (dd, 2H J = 5.2 and 4.1 Hz), 8.11(s,1H,C4 of isoxazole); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm): 168.96, 161.12, 158.72, 154.86, 144.66, 142.57, 131.74, 130.56, 128.42, 128.24, 126.16, 126.12, 118.18, 117.21, 115.11, 102.18; GC-MS: m/z 323.

7,8-Benzo-4-(5-phenylisoxazol-3-yl)-2H-benzo[h]chromen-2-one (3d): Yield: 76%; light brown solid; m.p. 168-170 ^{0}C; IR(KBr) ν: 1718, 1656 cm^{-1}; ^{1}H NMR (400Hz, DMSO-d_6): δ (ppm): 6.78 (s,1H, C3-H of coumarin), 7.14-7.19 (m, 4H), 7.41-7.43 (m, 3H), 7.78-7.74(m, 4H), 8.14(s,1H, C4 of isoxazole); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm): 167.92, 162.10, 159.11, 154.52, 144.72, 142.81, 132.18, 131.48, 130.88, 130.42, 127.48, 127.21, 125.71, 125.52, 117.92, 117.79, 115.92, 102.18; GC-MS: m/z 339.

6-Bromo-4-(5-phenylisoxazol-3-yl)-2H-chromen-2-one (3e): Yield: 78%; light yellow solid; m.p. 190-192^{0}C; IR(KBr) ν: 1716, 1660 cm^{-1}; ^{1}H NMR (400Hz, DMSO-d_6): δ (ppm): 6.66 (s,1H, C3-H of coumarin), 7.42-7.46 (m,1H), 7.69-7.66(m, 4H), 7.85(s,1H, C5-H of coumarin), 7.94 and 7.98 (dd, 2H J = 5.2 and 4.1 Hz), 8.19(s, 1H, C4 of isoxazole); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm): 170.11, 162.21, 159.54, 155.36, 144.62, 142.59, 131.78, 130.59, 128.51, 128.21, 126.24, 126.17, 118.56, 117.49, 115.33, 103.10; GC-MS: m/z 367.

7-Methyl-4-(5-phenylisoxazol-3-yl)-2H-chromen-2-one (3f): Yield: 82%; pale yellow solid; m.p. 178-180 ^{0}C; IR(KBr)ν: 1717, 1652 cm^{-1}; ^{1}H NMR (400Hz, DMSO-d_6): δ (ppm): 2.42 (3H, s, C7-CH_3 of coumarin), 6.91 (s,1H, C3-H of coumarin), 7.32 (d, 1H, J = 4.8 Hz, C6-H of coumarin), 7.36 (d, 1H, J = 3.8 Hz, C5-H of coumarin), 7.38-7.41(m,3H), 7.58-7.62(m,2H), 7.80(s,1H, C8-H of coumarin), 8.16(s,1H,C4 of isoxazole); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm): 170.11, 160.27, 159.32, 153.57, 143.88, 141.47, 130.92, 129.51, 127.11, 126.98, 125.92, 125.83, 117.18, 116.21, 114.10, 101.14, 22.11; GC-MS: m/z 303.

Synthesis of coumarin-phenylisoxazolosulphonamide 5:
To a solution of coumarin oxime **2** (1 mmol) and phenyl(phenyl-4-N,N,-dimethylaminosulphonamide)acetylene **4** (1 mmol) in DCM was prepared and to this 2-3 drops of Et_3N was added. Then the reaction mixture was allowed to stir for 6-8 hrs at room temperature. The product formation was confirmed by thin-layer

chromatographic (TLC) analysis. After completion of reaction DCM was removed by rota evaporator. The solid obtained was washed with cold ethanol and purified by column chromatography using ethyl acetate-hexane (3:7).

6-Methyl-4-(5-phenyl(4-(4-N,N-dimethylsulphonamidophenyl)isoxazol-3-yl)-2H-chromen-2-one (5a): Yield: 82% as a light green solid; m.p. 242-244 ^{0}C; IR (K Br)ν: 1728, 1658 cm^{-1}; ^{1}H NMR (400Hz, DMSO-d_6): δ(ppm): 2.43 (3H, s, C6-CH_3 of coumarin), 3.73(s.3H, N-CH_3), 3.77(s, 3H, N-CH_3), 6.70 (s,1H, C3-H of coumarin), 6.81(d, 3H, J = 5.2 Hz), 7.33-7.52 (m, 2H), 7.54-7.58(m, 4H), 7.86-7.89(m, 2H), 7.96(s,1H, C5-H of coumarin); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm): 171.11, 160.27, 159.38, 158.61, 156.15, 152.33, 142.80, 134.49, 133.53, 130.94, 129.32, 129.22, 127.45, 127.07, 126.55, 126.01, 117.13, 117.02, 116.79, 116.65, 34.88, 34.79, 21.01; GC-MS: m/z 486.

6-Methoxy-4-(5-phenyl(4-(4-N,N-dimethylsulphonamidophenyl)isoxazol-3-yl)-2H-chromen-2-one (5b): Yield: 78% as a green solid; m.p. 232-234 ^{0}C; IR (K Br)ν: 1714, 1644 cm^{-1}; ^{1}H NMR (400Hz, DMSO-d_6): δ(ppm): 3.72(s.3H, N-CH_3), 3.76(s, 3H, N-CH_3), 3.89 (3H, s, C6-OCH_3 of coumarin), 6.72 (s,1H, C3-H of coumarin), 6.80(d, 4H, J = 4.8 Hz), 7.34-7.46 (m, 3H), 7.58-7.62(m, 4H), 7.85-7.90(m, 2H), 7.94(s,1H, C5-H of coumarin); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm): 170.18, 160.67, 159.71, 158.78, 156.56, 152.57, 142.88, 134.75, 133.88, 131.10, 129.92, 129.53, 127.73, 127.41, 126.41, 126.11, 117.38, 117.14, 116.83, 116.79, 58.23, 35.52, 35.33; GC-MS: m/z 502.

6-Chloro-4-(5-phenyl(4-(4-N,N-dimethylsulphonamidophenyl)isoxazol-3-yl)-2H-chromen-2-one (5c): Yield: 70% as a white solid; m.p. 247-249 ^{0}C; IR (K Br)ν: 1721, 1651 cm^{-1}; ^{1}H NMR (400Hz, DMSO-d_6): δ(ppm): 3.73(s.3H, N-CH_3), 3.77(s, 3H, N-CH_3), 6.75 (s,1H, C3-H of coumarin), 7.11(d, 3H, J = 6.1 Hz), 7.47-7.56 (m, 4H), 7.59-7.64(m, 4H), 7.78-7.94(m, 2H), 7.98(s,1H, C5-H of coumarin); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm): 172.01, 161.13, 159.52, 158.63, 156.44, 152.61, 142.73, 134.51, 133.58, 131.25, 129.83, 129.74, 127.71, 127.38, 126.53, 126.21, 118.13, 117.92, 116.92, 116.85, 35.13, 34.93; GC-MS: m/z 506.

7,8-Benzo-4-(5-phenyl(4-(4-N,N-dimethylsulphonamidophenyl)isoxazol-3-yl)-2H-chromen-2-one (5d): Yield: 72% as a light brown solid; m.p. 192-194 ^{0}C; IR (K Br)ν: 1726, 1648 cm^{-1}; ^{1}H NMR (400Hz, DMSO-d_6): δ(ppm): 3.68(s.3H, N-CH_3), 3.71(s, 3H, N-CH_3), 6.72 (s,1H, C3-H of coumarin), 7.26(d, 4H, J = 6.8 Hz), 7.42-7.53 (m, 4H), 7.58-7.66(m, 3H), 7.74-7.81(m, 3H), 8.01(s,1H, C5-H of coumarin); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm): 168.13, 160.83, 158.91, 158.31, 155.88, 153.17, 148.14, 141.63, 138.18, 133.92, 133.67, 132.69, 130.19, 129.81, 128.67, 128.14, 127.18, 127.12, 119.19, 118.11, 117.13, 117.10, 35.19, 34.98; GC-MS: m/z 522.

6-Bromo-4-(5-phenyl(4-(4-N,N-dimethylsulphonamidophenyl)isoxazol-3-yl)-2H-chromen-2-one (5e): Yield: 62% as a light yellow solid; m.p. 220-222 ^{0}C; IR (K Br)ν: 1714, 1644 cm^{-1}; ^{1}H NMR (400Hz, DMSO-d_6): δ(ppm): 3.70(s.3H, N-CH_3), 3.74(s, 3H, N-CH_3), 6.70 (s,1H, C3-H of coumarin), 7.21(d, 3H, J = 3.6 Hz), 7.49-7.57 (m, 3H), 7.61-7.68(m, 3H), 7.75-7.91(m, 2H), 7.94(s,1H, C5-H of coumarin); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm): 169.28, 162.17, 159.86, 158.73, 156.56, 152.73, 142.81, 134.61, 133.64, 132.21, 130.78, 130.28, 128.53, 128.41, 126.79, 126.63, 119.10, 118.99, 117.11, 117.01, 34.81, 34.43; GC-MS: m/z 552.

7-Methyl-4-(5-phenyl(4-(4-N,N-dimethylsulphonamidophenyl)isoxazol-3-yl)-2H-chromen-2-one (5f): Yield: 84% as a light green solid; m.p. 232-234 ^{0}C; IR (K Br)ν: 1718, 1648 cm^{-1}; ^{1}H NMR (400Hz, DMSO-d_6): δ(ppm): 2.45 (3H, s, C7-CH_3 of coumarin), 3.72(s.3H, N-CH_3), 3.76(s, 3H, N-CH_3), 6.72 (s,1H, C3-H of coumarin), 6.78(d, 4H, J = 6.1 Hz), 6.90-7.01 (m, 3H), 7.42-7.51(m, 3H), 7.64-7.67(m, 1H), 7.88(d,1H, C5-H of coumarin); ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm): 170.77, 160.82, 159.67, 158.32, 157.01, 152.11, 143.16, 134.31, 133.83, 131.17, 129.66, 129.34, 127.65, 127.42, 126.67, 126.23, 117.88, 117.46, 116.54, 116.29, 34.27, 34.06, 22.22; GC-MS: m/z 486.

Acknowledgement

One of the authors Alpana acknowledges UGC-UPE scholarship. We acknowledge the DST and UGC New Delhi, for the financial support. Authors also thank to NMR Research Center, Indian Institute of Science (IISc.), Bangalore and University Sophisticated Instrumentation Center (USIC), Dharwad for spectral analysis.

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