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Synthesis and Characterization of Chlorophenyl-thiazolocoumarinyl Hydrazides as Promising Antimicrobial and Anti-Inflammatory Agents

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Abstract: *Recently the reports of resistance to antibacterial drugs have drastically increased. In the search of novel antimicrobial agents, green facile synthesis of substituted phenyl-thiazolocoumarinyl hydrazides was designed and achieved by one-pot reaction of substituted 4-formylcoumarin, thiosemicarbazide, and various phenacyl bromides. The substituted phenyl-thiazolocoumarinyl hydrazides were evaluated preliminarily for their antimicrobial and anti-inflammatory activities. The title compounds have shown excellent in vitro antibacterial activity against the S. aureus and E. coli bacterial strains with the value of low MIC ranges from 0.4 to 6.25µg/mL. In vitro antifungal activity of compounds against fungal strains A. niger and A. flavus was found to be excellent as compared with the standard fluconazole with the MIC in the range of 0.4 to 3.12 µg/mL. Substituted phenyl-thiazolocoumarinyl hydrazides have shown potent anti-inflammatory activity against both MMP-2 and MMP-9 by gelatinase zymography compared with the standard drug as tetracycline. Docking study was performed for compounds with S. aureus DNA gyrase*.

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I. Introduction

Treatment for microbial infections still remains an important and thought annoying problem for researchers worldwide. However, numerous novel antimicrobial agents and their medical ethics are inadequate to treat an emergent range of life-threatening universal infections due to high risk of toxicity, irrelevance in their antimicrobial activity etc.[1] Active compounds with novel and synergic pharmacodynamic mechanisms are in crucial demand.[2] The most common pathogens, Gram-positive (*S. aureus, S. epidermidis, S. pneumonia,* and *E. faecium)* and Gram-negative (*E. coli, P. aeruginosa, M. pneumonia,* and *Acinetobacter*) bacteria in clinical methods are proficient of causing severe and even toxic infections.[3,4] *S. aureus*, is a bacterium which consists of DNA gyrase and topoisomerase IV are an important target of antibacterial agents to cause bacterial cell death.[5] Type II topoisomerase enzyme of DNA gyrase which involves in the crucial step of the different topological system of DNA during DNA replication.[6]

Inflammation, a complicated phenomenon concerning inter-relative humoral and cellular reactions through many anti-inflammatory mediators remains one of the most important causes of morbidity and mortality around the world. Matrix metalloproteinases (MMPs) are a cluster of proteolytic enzymes that can degrade the extracellular matrix and so they have been suggested to be significant in the improvement of lung disease connected with tissue remodelling. [7] MMPs regulate numerous functions correlated to inflammation including the activity of inflammatory cytokines and chemokines. [8] MMPs play a significant function in the pathogenesis of inflammatory diseases, such as rheumatoid arthritis, liver fibrosis, osteoarthritis, Chronic Venous and cancer progression.[9-12] Gelatinases present in a physiologic system play an important function in inflammation. The number is still inadequate to control this global threat. That is why anti-inflammatory and also antimicrobial agent investigations are very important.

As a part of an endeavour to design improved anti-inflammatory drugs, the biological evaluation of numerous synthesized novel coumarin has been undertaken.[13,14] The substituted Coumarins have been reported to show tremendous pharmacological activities such as anticancer,[15] anticoagulant,[16] anti-inflammatory,[17] antimicrobial,[18] antioxidant,[19] antiviral[20] and antiproliferative activities.[21] They are of unique interest due to their prominent antimicrobial and anti-inflammatory activities.[22-24] Thiazole hybrids are known to have a broad array of biological activities.[25-33] Many researchers have described the enhancement of biological activity by

incorporation of thiazolyl nucleus in various pharmacophore molecules.[34-37] Some examples of thiazole (**A, B Figure 1**)[38,39] and thiazolylcoumarin (**C, D Figure 1**)[40,41] with antibacterial and anti-inflammatory activities have been shown in **Figure 1**.

Inspired by multifarious bioactivity of the thiazole derivatives and in continuation of our ongoing studies on coumarin molecules,[42,43] we were prompted to synthesize substituted phenyl-thiazolocoumarinyl hydrazides and perform antimicrobial as well as anti-inflammatory studies which could furnish better therapeutic results.

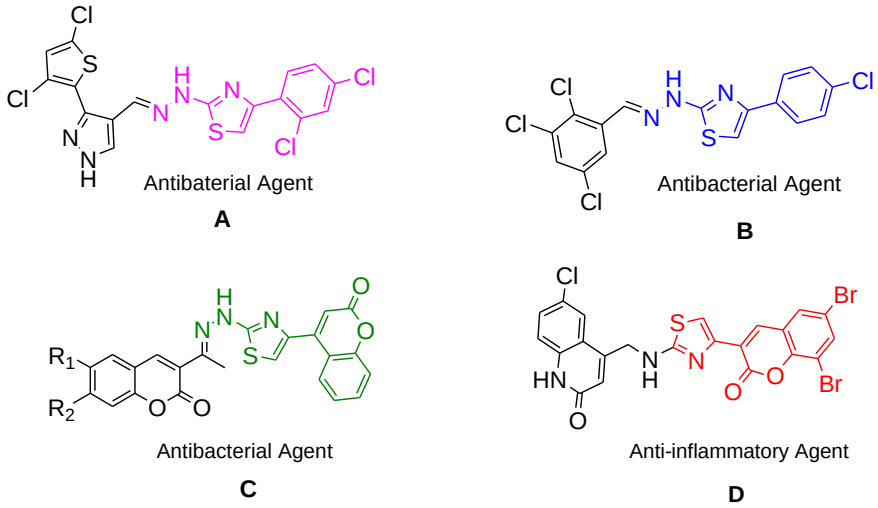


Figure 1. Structurally correlated antibacterial and anti-inflammatory thiazole and coumarin derivatives

II. Material And Methods

The chemicals were obtained commercially and used without additional purification. The melting points were taken by the open capillary technique and are uncorrected. Infrared (IR) spectra were obtained on a VERTEX 70 FT-IR (Bruker Optics) ^{1}H NMR and ^{13}C NMR spectra were measured by a Bruker Advance 400/500 and 100 MHz spectrometer, using DMSO-d_6 and $CDCl_3$ as solvents. Mass spectra on a Shimadzu GCMSQP2010S and CHN elemental analysis of synthesized compounds were measured by LECO TRUSPEC CHN analyzer.

Synthesis

General procedure for the synthesis of substituted phenyl-thiazolocoumarinyl hydrazides 4a-e, 4f-j and 4k -o.

A mixture of substituted 4-formylcoumarin (0.01mol) and thiosemicarbazide (0.01mol) in ethanol (20 ml) were continuously stirred at room temperature for 2h. To this mixture, appropriately substituted phenacyl bromide (0.01mol) was added and the contents were subsequently stirred at room temperature for further 5h. After completion of the reaction mixture was added into ice-cold water the solid separated was decanted, washed with cold water and dried. The product was crystallized from ethanol to give the substituted phenyl-thiazolocoumarinyl hydrazides in pure form.

6-Methyl-4-{(Z)-[2-(4-phenyl-1,3-thiazol-2-yl)hydrazinylidene]methyl}-2H benzopyran-2-one (4a)

Yellow solid; Yield 90%; mp: 217–219 ^{o}C; IR (KBr): 3426, 1724, cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.85 (s, 1H, NH of hydrazine), 8.48 (s, 1H, -CH=N- azomethine), 8.17 (s, 1H, C5H of coumarin), 7.86 (d, *J* = 8 Hz, 1H, C7H of coumarin), 7.40-7.49 (m, 5H, ArH) 7.33 (d, *J* = 7.6 Hz, 1H, C8H of coumarin) 7.19 (s, 1H, C5H of thiazole), 6.64 (s, 1H, C3H of coumarin), 2.44 (s, 3H, C6CH_3 of coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm:169.42, 160.96, 152.70, 152.19, 151.26, 144.04, 143.15, 137.72, 134.14, 133.86, 132.75, 129.25, 128.81, 126.38, 126.31, 116.90, 116.12. 115.35, 104.88, 26.01; MS (m/z): 361 Anal. Calcd. For $C_{20}H_{15}N_3O_2S$ (%): Calcd. C, 66.46; H, 4.18; N, 11.63 Found: C, 66.43; H, 4.15; N, 11.60.

7-Methyl-4-{(Z)-[2-(4-phenyl-1,3-thiazol-2-yl)hydrazinylidene]methyl}-2H-1-benzopyran-2-one (4b)

Yellow solid; Yield 88%; mp: 222–224 ^{o}C; IR (KBr): 3435, 1704, cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.84 (s, 1H, NH of hydrazine), 8.40 (s, 1H, -CH=N- azomethine), 8.41 (d, *J* = 8 Hz, 1H, C5H of coumarin), 7.86 (d, *J* = 7.6 Hz, 1H, C6H of coumarin), 7.26-7.43 (m, 5H, ArH) 7.26 (s, 1H, C8H of coumarin), 7.24 (s, 1H, C5H of thiazole), 6.59 (s, 1H, C3H of coumarin), 2.42 (s, 3H, C6CH_3 of coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm:167.34, 159.97, 153.68, 150.90, 144.43, 142.80, 136.68, 134.26, 128.59, 127.69, 125.88,

125.52, 125.12, 116.89, 113.85, 104, 20.91; MS (m/z): 361 Anal. Calcd. For $C_{20}H_{15}N_3O_2S$ (%): Calcd. C, 66.46; H, 4.18; N, 11.63 Found: C, 66.43; H, 4.15; N, 11.60.

6-Methoxy-4-{(Z)-[2-(4-phenyl-1,3-thiazol-2-yl)hydrazinylidene]methyl}-2H-1-benzopyran-2-one (4c)

Yellow solid; Yield 88%; m.p.: 211–213 ^oC; IR (KBr): 3414, 1716, cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.84 (s, 1H, NH of hydrazine), 8.82 (d, *J* = 2.8 Hz, 1H, C5H of coumarin), 8.17 (s, 1H, -CH=N- azomethine), 7.86 (d, *J* = 8.4 Hz, 1H, C8H of coumarin), 7.26-7.43 (m, 5H, ArH) 7.28 (dd, *J* = 2.8, 9.2 Hz, 1H, C7H of coumarin), 7.31 (s, 1H, C5H of thiazole), 6.67 (s, 1H, C3H of coumarin), 3.94 (s, 3H, C6OCH_3 of coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm:167.10, 159.96, 155.51, 148.01, 143.97, 138.19, 134.20, 133.79, 128.79, 128.61, 127.74, 125.54, 119.54, 117.85, 104.92, 55.93; (m/z): 377 Anal. Calcd. For $C_{20}H_{15}N_3O_3S$ (%): Calcd. C, 63.65; H, 4.01; N, 11.13 Found: C, 63.62; H, 4.01; N, 11.11.

6-Chloro-4-{(Z)-[2-(4-phenyl-1,3-thiazol-2-yl)hydrazinylidene]methyl}-2H-1 benzopyran-2-one (4d)

Yellow solid; Yield 87%; mp: 215–217 ^oC; IR (KBr): 3405, 1724, cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.92 (s, 1H, NH of hydrazine), 8.85 (s, 1H, C5H of coumarin), 8.13 (s, 1H, -CH=N- azomethine), 7.86 (d, *J* = 8.4 Hz, 1H, C7H of coumarin), 7.30-7.50 (m, 5H, ArH), 7.36 (d, *J* = 7.6 Hz 1H C8H of coumarin), 6.70 (s, 1H, C5H of thiazole), 6.67 (s, 1H, C3H of coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm: 167.28, 159.40, 152.22, 150.81, 143.06, 137.50, 134.18, 131.44, 128.59, 128.36, 127.73, 126.44, 125.54, 118.69, 117.34, 105.04; MS (m/z): 381 (M), 383 (M+2). Anal. Calcd. For $C_{19}H_{12}N_3O_2S$ (%): Calcd. C, 59.76; H, 3.17; N, 11.00 Found: C, 59.74; H, 3.15; N, 10.8.

5,7-Dimethyl-4-{(Z)-[2-(4-phenyl-1,3-thiazol-2-yl)hydrazinylidene]methyl}-2H-1-benzopyran-2-one (4e)

Yellow solid; Yield 85%; mp: 233–235 ^oC; IR (KBr): 3393, 1718, cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.67 (s, 1H, NH of hydrazine), 8.54 (s, 1H, -CH=N- azomethine), 7.85 (s, 1H, C8H of coumarin), 7.20-7.42 (m, 5H, ArH), 7.10 (s, 1H, C6H of coumarin) 7.03 (s, 1H, C5H of thiazole), 6.49 (s, 1H, C3H of coumarin), 2.63 (s, 3H, CH3C5 coumarin), 2.35 (s, 3H, CH3C7 coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm: 167.35, 166.21, 159.39, 154.76, 150.47, 147.94, 142.29, 139.47, 138.23, 135.85, 134.32, 133.30, 129.21, 127.78, 125.49, 116.77, 104.21, 23.94, 24.69; MS (m/z): 375 Anal. Calcd. For $C_{21}H_{17}N_3O_2S$ (%): Calcd. C, 67.18; H, 4.56; N, 11.19 Found: C, 67.16; H, 4.54; N, 11.17.

4-[(Z)-{2-[4-(2-Chlorophenyl)-1,3-thiazol-2-yl]hydrazinylidene}methyl]-6-methyl-2H-1-benzopyran-2-one (4f)

Yellow solid; Yield 86%; mp: 221–223 ^oC; IR (KBr): 3440, 1728, cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.88 (s, 1H, NH of hydrazine), 8.23 (d, *J* = 2.8 Hz,1H, C5H of coumarin), 8.19 (s, 1H, -CH=N- azomethine), 7.88 (d, *J* = 8.4 Hz, 1H, C8H of coumarin), 7.40-7.47 (m, 4H, C5H aromatic protons), 7.10 (dd, *J* = 2.8, 8.8 1H, C7H of coumarin) 6.69 (s, 1H, C5H of thiazole), 6.69 (s, 1H, C3H of coumarin), 2.34 (s, 3H, CH_3 C6 coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm: 168.03, 160.48, 152.33, 150.25, 147.17, 144.70, 134.02, 133.33, 129.14, 127.76, 126.96, 117.89, 115.31, 106.43, 21.18; MS (m/z): 395 (M), 397 (M+2). Anal. Calcd. For $C_{20}H_{14}ClN_3O_2S$ (%): Calcd. C, 60.68; H, 3.56; N, 10.61 Found: C, 60.66; H, 3.52; N, 10.59.

4-[(Z)-{2-[4-(2-Chlorophenyl)-1,3-thiazol-2-yl]hydrazinylidene}methyl]-7-methyl-2H-1-benzopyran-2-one (4g)

Yellow solid; Yield 83%; m.p.: 216–218 ^oC; IR (KBr): 3429, 1726, cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.82 (s, 1H, NH of hydrazine), 8.12 (d, *J* = 8.8 1H, C5H of coumarin), 7.92 (s, 1H, -CH=N- azomethine), 7.81 (d, *J* = 8.8 Hz, 1H, C6H of coumarin) 7.74 (s, 1H, C8H of coumarin), 7.43-7.52 (m, 4H, ArH), 6.92 (s, 1H, C5H of thiazole), 6.84 (s, 1H, C3H of coumarin), 2.42 (s, 3H, CH_3 C7 coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm:170.18, 163.04, 156.23, 154.10, 150.21, 140.12, 138.13, 137.36, 133.35, 132.10, 132.20, 129.52, 128.30, 118.01, 105.50, 25.53; (m/z): 395 (M), 397 (M+2). Anal. Calcd. For $C_{20}H_{14}ClN_3O_2S$ (%): Calcd. C, 60.68; H, 3.56; N, 10.61 Found: C, 60.66; H, 3.54; N, 10.59.

4-[(Z)-{2-[4-(2-Chlorophenyl)-1,3-thiazol-2-yl]hydrazinylidene}methyl]-6-methoxy-2H-1-benzopyran-2-one (4h)

Yellow solid; Yield 81%; mp: 219–2221 ^oC; IR (KBr): 3413, 1706, cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 13.03 (s, 1H, NH of hydrazine), 8.61 (s, 1H, C5H of coumarin), 8.36 (s, 1H, -CH=N- azomethine), 8.05 (d, *J* = 7.6 Hz, 1H, C7H of coumarin), 8.05 (d, *J* = 7.6 Hz, 1H, C8H of coumarin), 7.45-7.62 (m, 4H, ArH), 7.43 (s, 1H, C5H of thiazole), 6.78 (s, 1H, C3H of coumarin), 3.50 (s, 3H, C6OCH_3 of coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm:167.58,160.73, 156.42, 156.01, 148.53, 144.49, 129.14, 129.10, 128.27, 126.02, 120.06, 116.40, 105.31, 56.44; MS (m/z): 411 (M), 413 (M+2) Anal. Calcd. For $C_{20}H_{14}ClN_3O_3S$ (%): Calcd. C, 58.32; H, 3.43; N, 10.20 Found: C, 58.30; H, 3.41; N, 10.18.

6-Chloro-4-[(Z)-{2-[4-(2-chlorophenyl)-1,3-thiazol-2-yl]hydrazinylidene}methyl]-2H-1-benzopyran-2-one (4i)
Yellow solid; Yield 88%; mp: 207–209 ^{o}C; IR (KBr): 3425, 1726, cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.75 (s, 1H, NH of hydrazine), 8.21 (s, 1H, C5H of coumarin), 8.12 (s, 1H, -CH=N- azomethine), 7.74 (d, *J* = 9.2 Hz, 1H, C7H of coumarin), 7.65 (d, *J* = 8.2 Hz, 1H C8H of coumarin), 7.20-7.56 (m, 4H ArH), 7.12 (s, 1H, C5H of thiazole), 6.91 (s, 1H, C3H of coumarin), ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm:168.23, 162.21, 157.7, 154.80, 152.21, 150.10, 149.12, 135.40, 133.29, 132.10, 129.50, 129.29, 128.90, 128.15, 127.58, 127.10, 116.20, 103.25; MS (m/z): 414 (M), 416 (M+2), 418 (M+4). Anal. Calcd. For $C_{19}H_{11}Cl_2N_3O_2S$ (%): Calcd. C, 54.82; H, 2.66; N, 10.09 Found: C, 54.80; H, 2.64; N, 10.07.

4-[(Z)-{2-[4-(2-Chlorophenyl)-1,3-thiazol-2-yl]hydrazinylidene}methyl]- 5,7-dimethyl -methyl-2H-1-benzopyran-2-one (4j)
Yellow solid; Yield 86%; mp: 210–212 ^{o}C; IR (KBr): 3382, 1719, cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.72 (s, 1H, NH of hydrazine), 8.12 (s, 1H, -CH=N- azomethine), 7.92 (s, 1H, C8H of coumarin), 7.62 (s, 1H, C6H of coumarin), 7.23-7.45 (m, 4H, ArH), 6.90 (s, 1H, C5H of thiazole), 6.80 (s, 1H, CH of coumarin), 2.41 (s, 3H, CH_3 C5 coumarin), 2.36 (s, 3H, CH_3 C7 coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm:169.20, 161.81, 156.45, 154.23, 152.21, 150.41, 138.12, 137.45, 135.45, 134.92, 133.42, 133.32, 129.46, 128.26, 127.82, 116.60, 106.26, 23.12, 02.12; MS (m/z); 409 (M), 411 (M+2). Anal. Calcd. For $C_{21}H_{16}ClN_3O_2S$ (%): Calcd. C, 61.53; H, 3.93; N, 10.25 Found: C, 61.51; H, 3.91; N, 10.23.

4-[(Z)-{2-[4-(2,4-Dichlorophenyl)-1,3-thiazol-2-yl]hydrazinylidene}methyl]-6-methyl-2H-1-benzopyran-2-one (4k)
Yellow solid; Yield 82%; mp: 215–217 ^{o}C; IR (KBr): 3326, 1725, cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.72 (s, 1H NH of hydrazine), 8.21 (d, *J* = 2.4 Hz 1H, C5H of coumarin), 8.12(s, 1H, -CH=N- azomethine), 7.75 (dd, *J* = 2.3, 8.4 Hz, 1H, C7H of coumarin), 7.62 (s, 1H, ArC3H), 7.58 (d, *J* = 8.8 Hz, 1H, ArC5H), 7.48 (d, *J* = 8.7 Hz, 1H, C6H of coumarin), 7.42 (d, *J* = 8.6 Hz, 1H, ArC6H), 6.87 (s, 1H, C5H of thiazole), 6.68 (s, 1H, C3H of coumarin), 2.51 (s, 3H, C6CH_3 of coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm:169.23, 158.47, 154.21, 152.21, 149.20, 148.01, 138.82, 138.21, 137.18, 137.21, 135.23, 132.18, 129.12, 129.14, 128.40, 125.38, 115.62, 25.60; MS (m/z): 430 (M), 432 (M+2), 434 (M+4). Anal. Calcd. For $C_{20}H_{13}Cl_2N_3O_2S$ (%): Calcd. C, 55.82; H, 3.05; N, 9.77 Found: C, 55.80; H, 3.03; N, 9.75.

4-[(Z)-{2-[4-(2,4-Dichlorophenyl)-1,3-thiazol-2-yl]hydrazinylidene}methyl]-7-methyl-2H-1-benzopyran-2-one (4l)
Yellow solid; Yield 86%; mp: 214–216 ^{o}C; IR (KBr): 3425, 1709, cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.78 (s, 1H, NH of hydrazine), 8.30 (d, *J* = 8.4 Hz, 1H, C5H of coumarin), 8.21 (s, 1H, -CH=N- azomethine), 7.92 (d, *J* = 8.6 Hz, 1H, C6H of coumarin), 7.82 (s, 1H, ArC3H), 7.72 (d, *J* = 8.7 Hz, 1H, ArC5H), 7.68 (d, *J* = 8.6 Hz, 1H, ArC6H), 7.32 (s, 1H, C8H of coumarin) 6.94 (s, 1H, C5H of thiazole), 6.72 (s, 1H, C3H of coumarin), 2.38 (s, 3H, C6CH_3 of coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm: 170.40, 157.68, 156.49, 153.21, 148.72, 148.32, 139.20, 138.93, 137.58, 137.55, 136.32, 132.62, 130.10, 129.50, 128.78, 125.59, 115.82, 24.69; MS (m/z): 430 (M), 432 (M+2), 434 (M+4). Anal. Calcd. For $C_{20}H_{13}Cl_2N_3O_2S$ (%): Calcd. C, 55.82; H, 3.05; N, 9.77 Found: C, 55.80; H, 3.03; N, 9.75.

4-[(Z)-{2-[4-(2,4-Dichlorophenyl)-1,3-thiazol-2-yl]hydrazinylidene}methyl]-6-methoxy-2H-1-benzopyran-2-one (4m)
Yellow solid; Yield 84%; m.p: 220–222 ^{o}C; IR (KBr): 3414, 1716, cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.83 (s, 1H, NH of hydrazine), 8.23 (d, *J* = 2.8 1H, C5H of coumarin), 8.19 (s, 1H, -CH=N- azomethine), 7.88 (d, *J* = 8.4 Hz, 1H, C8H of coumarin), 7.29 (dd, *J* = 2.8,8.8 Hz, 1H, C7H of coumarin), 7.40 (s, 1H, ArC3H), 7.43 (d, *J* = 8.8 Hz, 2H, ArC5H and C6), 6.69 (s, 1H, C5H of thiazole), 6.69 (s, 1H, C3H of coumarin), 3.93 (s, 3H C6OCH_3 of coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm:166.12, 159.23, 152.95, 143.70, 142.07, 135.95, 133.52, 127.86, 126.96, 125.15, 124.79, 116.16, 113.11, 112.24, 104.25, 58.51; MS (m/z): 446 (M), 448 (M+2), 450 (M+4). Anal. Calcd. For $C_{20}H_{13}Cl_2N_3O_3S$ (%): Calcd. C, 53.82; H, 2.94; N, 9.42 Found: C, 53.80; H, 3.92; N, 9.40.

6-Chloro-4-[(Z)-{2-[4-(2,4-dichlorophenyl)-1,3-thiazol-2-yl]hydrazinylidene}methyl]-2H-1-benzopyran-2-one (4n)
Yellow solid; Yield 81%; mp: 216–218 ^{o}C; IR (KBr): 3399, 1724, cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 13.06 (s, 1H, NH of hydrazine), 7.92 (d, *J* = 2.4 Hz 1H, C5H of coumarin), 7.82 (s, 1H, -CH=N- azomethine), 7.72 (dd, *J* = 2.5, 8.7 Hz, 1H, C7H of coumarin), 7.62 (s, 1H, ArC3H), 7.56 (d, *J* = 8.6 Hz, 1H, ArC5H), 7.49 (d, *J* = 8.8 Hz, 1H, ArC6H), 7.32 (d, *J* = 8.8 Hz, 1H, C8H of coumarin) 7.12 (d, *J* = 2.7 Hz, 1H, C5H of thiazole), 6.82 (s, 1H, C3H of coumarin), ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm: 168.18, 159.93, 158.49,

152.75, 143.59, 138.03, 137.75, 134.70, 134.70, 131.98, 129.12, 128.89, 128.27, 126.17, 126.07, 119.21, 117.12, 105.61; MS (*m/z*): 450 (M), 452 (M+2), 454 (M+4), 456 (m+6). Anal. Calcd. For $C_{19}H_{10}Cl_3N_3O_3S$ (%): Calcd. C, 50.63; H, 2.24; N, 9.32 Found: C, 50.61; H, 3.2.22; N, 9.30.

4-[(Z)-{2-[4-(2,4-Dichlorophenyl)-1,3-thiazol-2-yl]hydrazinylidene}methyl]-5,7-dimethyl-2H-1-benzopyran-2-one(4o)

Yellow solid; Yield 87%; mp: 204-206 ^oC; IR (KBr): 336, 1712, cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.62 (s, 1H, NH of hydrazine), 8.59 (s, 1H, -CH=N- azomethine), 8.23 (s, 1H, C6H of coumarin) 7.66 (s, 1H, C8H of coumarin), 7.52 (s, 1H, ArC3H), 7.48 (dd, *J* = 2.9, 8.3 Hz, 1H, ArC5H), 7.34 (d, *J* = 9.3 Hz, 1H, ArC6H)7.33 (s, 1H, C5H of thiazole), 6.53 (s, 1H, C3H of coumarin), 2.60 (s, 3H, CH_3C5 coumarine), 2.31 (s, 3H, CH_3C7 coumarin); ^{13}C NMR (100 M Hz, DMSO-d_6) δ ppm:167.86, 155.36, 148.50, 147.73, 138.73, 136.37, 134.83, 129.13, 129.10, 128.81, 128.10, 125.99, 115.85, 114.85, 116.90, 105.11, 24.47, 21.21; MS (*m/z*): 444 (M), 446 (M+2), 448 (M+4). Anal. Calcd. For $C_{21}H_{15}Cl_2N_3O_3S$ (%): Calcd. C, 56.76; H, 3.40; N, 9.46 Found: C, 56.74; H, 3.38; N, 9.44.

Biological evaluation

Antimicrobial screening

The antibacterial and antifungal activities of all the synthesized compounds were performed by broth dilution methods.[44] The MIC determination of the tested compounds was carried out in comparison with ciprofloxacin and fluconazole as standard drugs. For MIC assay fourteen dilutions of each drug were prepared with BHI (brain heart infusion). Initially, 20 µL of a drug was added to the 380 µL of BHI broth. Then from this 200 µL was transferred to the first tube containing 200 µL of BHI broth and it was considered as 10^1 dilution. From 10^1 diluted tube 200 µL was transferred to the second tube, to make 10^2 dilution. The sequential dilution was repeated up to 10^{14} dilution for each drug. From the retained stock cultures of required organisms, 5 µL was taken and added to 2 µL of BHI broth. In each serially diluted tube, 200 µL of above culture suspension was added. The tubes were incubated for 24 h at 37^o C in the incubator and observed for turbidity.

Anti-inflammatory screening

Detection of MMP-2 and MMP-9 by gelatin zymography through gel electrophoresis

Apparatus. Electrophoresis equipment (vertical gel electrophoresis power pack and trough by TechnoSource) was cleaned with warm water and glass plates with methanol. Plates were arranged with the large plate at the bottom, then two spacers (Little petroleum jelly was spread on both sides of the Spacers) and a small plate on top. Plates were assembled into the clamp and were gently tightened with screws. Agarose gel was heated and poured between the two glass plates to seal the bottom surface and was left for 5–10 min. An appropriate resolving gel mixture was mixed and added between the glass plates to avoid bubbles. Plates were filled about 80% way up leaving space for the stacking gel and comb. Small quantity of water was poured to attain a completely flat interface between resolving gel and stacking gel. It was left to set for about 45 min. 5% stacking gel was prepared with bisacrylamide, stacking gel buffer, 10% Sodium Dodecyl Sulfate, Ammonium persulfate 1.5%, Tetramethylethylenediamine 0.2% (TEMED), and Water. When resolving gel solidifies, excess water was poured off. Distilled water was added to wash the plates. Stacking gel 5% prepared was poured and the comb was inserted to avoid bubbles. Then, it was allowed to set for about 30 min. After setting the stacking gel, the comb was removed gently, the wells were washed with distilled water and the gel was assembled onto the electrode/gasket section of the gel apparatus. The top and bottom of the tank was filled with reservoir buffer, i.e., upper tank with 100 ml and lower tank with 150.

Method

Inflamed tissue samples were collected from clinically diagnosed patients of chronic periodontitis and chopped it completely. The patients had no background of dental treatment or antibiotic or anti-inflammatory therapy for at least 3 months prior to the study. 5 mL of tris buffer was centrifuged along with the inflamed tissue samples at 3000 rpm for 15 min and stored at 20^{o}C for additional use. It was named as MMP extract. 10 mg of each target compounds **(4a-e**, **4f-j** and **4k -o**) was dissolved in 1 ml of DMSO separately. Hence each µL contains 10 µg of the compound. For the initial evaluation, three trials were carried out with non-identical concentrations namely 100 µg, 250 µg, and 500 µg/ml and found 500 µg/ml with good inhibition. So 50 µL of each sample which contained 500 µg/ml was prepared for all samples and used. 50 µL of MMP extract and 50 µL of the compounds **(4a-e**, **4f-j** and **4k-o**) were mixed separately and then incubated for 15–30 min. 29 non reducing buffer in equal volume was added, mixed and 20 µL was loaded into each well using gel loading tips and 10 µL molecular weight marker was added in last well. 50 µL of the tissue sample with 0.9% normal saline is used as the control.

Electrodes were coupled and the tank was closed with cover and cables were stippled into the power supply. Initially, the gel electrophoresis apparatus was run at about 50 V for 15 min and then at 100 V until the bromophenol blue reached the bottom of the plates. After electrophoresis, the equipment was disassembled and

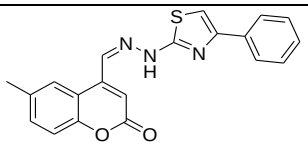
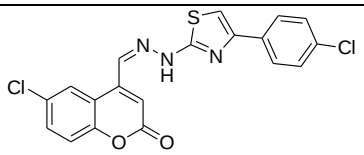
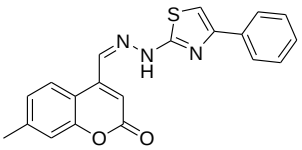
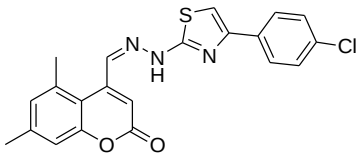
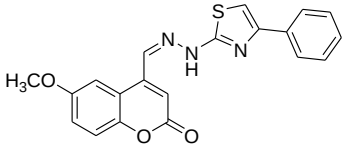
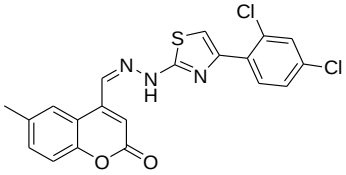
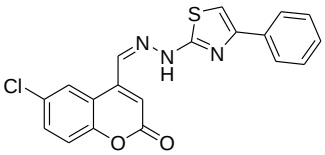
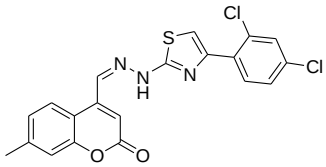
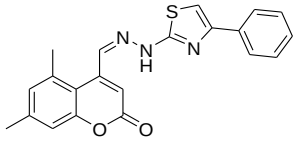
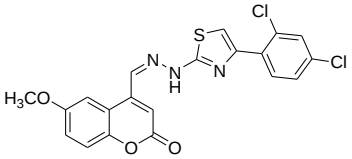
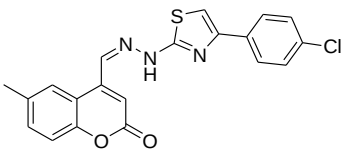
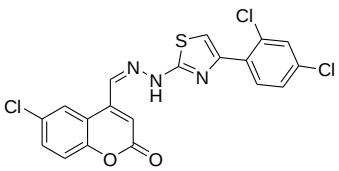
the gel was gently removed and the equipment was washed with zymogram renaturing buffer, i.e., 2.5% Triton x-100 for one hour to remove SDS from the gel and let the proteins to denature. The zymogram renaturing buffer was removed and the gel was incubated in zymogram incubation buffer at 37^{o}C overnight. The gel was staines with Coomassie blue R-250 for one hour. Gels should be smeared with an appropriate Coomassie R-250 smearing solution for about 2 h. The background stains blue with Coomassie stain where the gelatin is degraded.

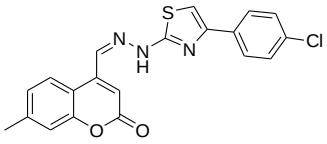
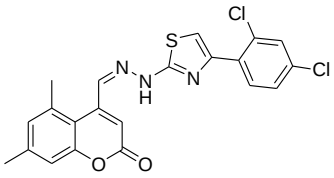
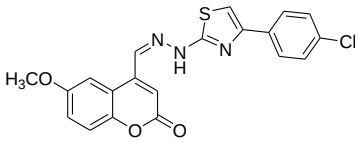
Docking studies

The X-ray Crystal Structure of *S. aureus* 24 kDa Domain in Complex with ciprofloxacin (PDB code: 1KZN; resolution 2.30 Å; http://www. rcsb.org) was taken from protein data bank in PDB method the initial point. Unlabelled atom types from the PDB file were corrected, consequently, proline F angles were fixed at 70 ^{o}C side chain amides were checked to exploit potential hydrogen bonding, side chains were checked for close van der Waals contacts, and important hydrogens were added. The model was checked for conformational attempt by using the component ProTable from Sybyl. The protein was exposed to energy minimization subsequent the gradient achievement of the Powell system for 3000 iterations using Kollman united forcefield the dielectric constant set at 4.0 for synthesized compounds, including ciprofloxacin were carried out using Tripos forcefield and Gasteiger charge with nonbonding cut-off set at 9.0 and the dielectric constant set at 4.0. The compounds were docked to DNA gyrase (PDB code: 1KZN) using Surflex-Dock method in Sybyl software by incremental creation advance of structure the construction in the active site so as to support the binding similarity. At last, the docked ligands were ranked based on a diversity of scoring function that has been compiling into the single C-score.[45]

III. Result

Table 1: Structures of synthesized phenyl-thiazolocoumarinyl hydrazides **4a-e**, **4f-j** and **4k-o**.

Entry	Product Code	Structure	Entry	Product Code	Structure
1	4a		9	4i	
2	4b		10	4j	
3	4c		11	4k	
4	4d		12	4l	
5	4e		13	4m	
6	4f		14	4n	

7	4g		15	4o	
8	4h				

Formation of compounds **4a-e**, **4f-j** and **4k-o** were confirmed by spectral analysis. Compound **4a** shows IR stretching band showed at 3426 cm^{-1} due to NH of the hydrazide and intense stretching band at 1724 cm^{-1} due to lactone stretching of coumarin moiety. In GCMS spectrum [M+H] peak of the compound **4a** was observed at m/z 361. ^{1}H NMR of compound **4a**, NH of hydrazide appeared as a singlet at δ 12.85 ppm and -CH=N- azomethine as a singlet at δ 8.48 ppm. Singlet at δ 8.17 ppm is due to C5H of coumarin, whereas C7H of coumarin resonated at δ 7.86 ppm as a doublet (d, J = 8 Hz). The D_2O exchangeable singlet at δ 12.85 ppm confirmed the NH proton of hydrazide. The aromatic protons appeared as multiplet around δ 7.40-7.49 ppm and C8H of coumarin appears as a doublet at δ 7.33 (d, J = 7.6 Hz) ppm. The C5H of thiazole proton resonated as a singlet at δ 7.19 ppm and C3H of coumarin appears as a singlet at δ 6.64 ppm. Whereas, C6CH_3 resonated as a singlet at δ 2.44 ppm.

Biological activity

Antibacterial screening

The *in vitro* antibacterial activity of title compounds **4a-e**, **4f-j** and **4k-o** were screened against two Gram-positive (*S. aureus* and *Bacillus sps*) and two Gram-negative (*E. coli* and *Pseudomonas*) bacterial strains by using Broth dilution method.[44] The results were compared with known antibiotic Ciprofloxacin were used as the standard drugs. All the fifteen compounds were screened in the present study, MIC ranging from 0.4 to 100 mg/mL. The results revealed that the compounds are more potent against Gram-positive *S. aureus* and Gram-negative *E.Coli* bacterial strain with low MIC values ranging from 0.4 to 6.25 µg/mL. The results of antibacterial activity are presented in **Table 2**. In the series (**4a-e**, **4f-j** and **4k-o**), the compounds bearing chlorosubstitution on phenyl ring exhibited potent activity (MIC µg/mL). In case of *Bacillus sps* and *Pseudomonas* bacterial strains the synthesized compounds **4a-e**, **4f-j** and **4k-o** have shown less activity. The antibacterial MIC values of compounds **4a-e** are less active against four bacterial strains compared to the standard. The antibacterial MIC activity results of compounds **4f-j** are surprisingly high where chloro substitution on phenyl ring of thiazolophenyl skeleton enhanced the activity two to three-fold as compared to standard Gram-positive *S. aureus* and Gram-negative *E.Coli* bacterial strains. Compounds **4f-j** are less active against Gram-positive *Bacillus sps* and Gram-negative *Pseudomonas* bacterial strains. Dichloro substituted compounds **4k-o** have shown low MIC values similar to monochloro derivatives against Gram-positive *S. aureus* and Gram-negative *E.Coli* bacterial strains. The antibacterial results of all the compounds are represented in bar diagram (**Figure 2**).

Table 2. Results of *in vitro* antibacterial activity MIC of the compounds. **4a-e**, **4f-j** and **4k-o**

Product Code	R	R^1	R^2	Minimum inhibitory concentrations (MIC) (µg/ml)			
				Gram positive		Gram negative	
				S.aureus	***Bacillus sps***	***E.coli***	***Pseudomonas***
4a	6-CH_3	H	H	6.25	50	6.25	50
4b	7-CH_3	H	H	3.12	50	3.12	100
4c	6-OCH_3	H	H	3.12	25	3.12	50
4d	6-Cl	H	H	6.25	12.6	6.1	50
4e	5,7-di CH_3	H	H	6.25	25	6.25	50
4f	**6-CH_3**	Cl	H	**0.4**	6.25	**0.8**	50
4g	**7-CH_3**	Cl	H	**0.4**	12.6	**1.6**	100
4h	**6-OCH_3**	Cl	H	**0.8**	25	**1.6**	25

4i	**6-Cl**	Cl	H	**0.4**	12.6	**0.8**	50
4j	**5,7-di CH_3**	Cl	H	**1.6**	50	**0.8**	25
4k	**6-CH_3**	Cl	Cl	**0.4**	25	**0.8**	25
4l	**7-CH_3**	Cl	Cl	**0.4**	25	**0.4**	50
4m	**6-OCH_3**	Cl	Cl	**0.4**	12.6	**0.4**	100
4n	**6-Cl**	Cl	Cl	**0.8**	25	**0.4**	100
4o	**5,7-diCH_3**	Cl	Cl	**1.6**	25	**1.6**	50
Ciprofloxacin				2	2	2	<4

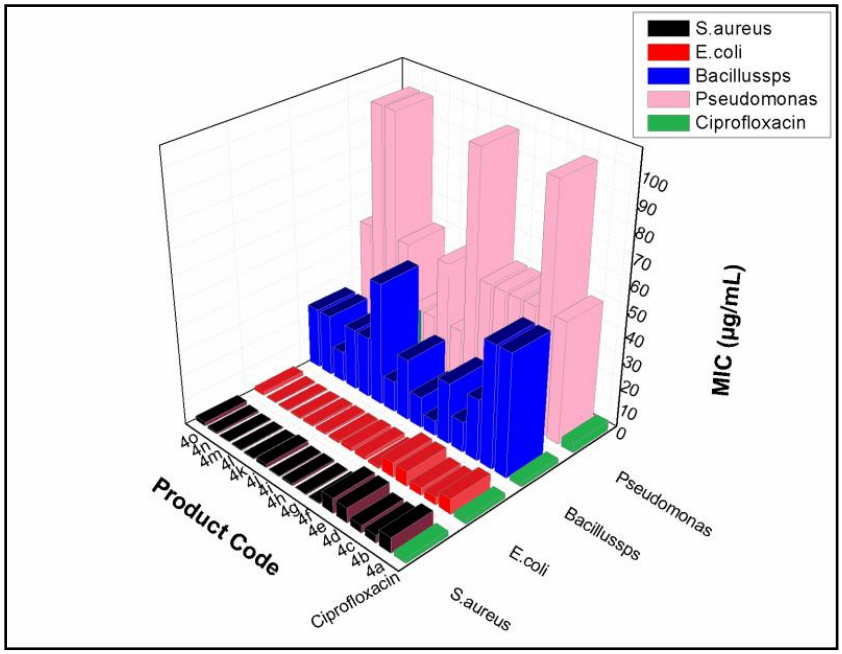


Figure 2. Graphical presentation of the MIC (µg/mL) of the compounds against four bacterial stains

3.3.2 Antifungal screening

The substituted phenyl-thiazolocoumarinyl hydrazides **4a-e**, **4f-j** and **4k-o** were screened for their antifungal activity against *A. flavus* and *A. fumigates* with fluconazole as a standard using Broth dilution method.[44] The preliminary antifungal results showed that all the compounds **4a-e**, **4f-j** and **4k-o** are potential inhibitors, subsequently MIC of all the synthesized hybrids were determined with low MIC values ranges from 0.4 to 3.12 µg/mL. The results of *in vitro* antifungal activities are presented in **Table 3**. The antifungal screening of compounds **4a-e** reveals that almost all the substituted phenyl-thiazolocoumarinyl hydrazides are highly active against *A. fumigatus* at a concentration of 0.4-0.8 µg/mL. The compounds **4a**, **4b** and **4e** are less active compared to compound **4c** and **4d** but more active than standard fluconazole *against A. flavus.*

Whereas compounds **4f-j** are more active against both fungal strains around 0.4-0.8 µg/mL. It has been observed that compounds **4f, 4g, 4h, 4i** and **4j** containing chloro substitution at C2 position of aromatic ring shown excellent activity against two fungal stains. The antifungal activity of dichloro substituted thiazole phenyl coumarins **4k-o** revealed that all compounds are shown excellent activity at lower concentration (0.4-0.8 µg/mL). The results are represented in bar diagram (**Figure 3**).

The overall structure activity relationship (SAR) draws the inference that the mono and dichloro substituted compounds **4f-j** and **4k-o** are more potent than the *S. aureus* and *E.Coli*. Whereas, all the compounds **4f-j** and **4k-o** are found promising against used fungal strains.

Table 3. The MIC results of compounds **4a-e**, **4f-j** and **4k-o**

Product Code	**R**	**R^1**	**R^2**	**% minimum Inhibitory Concentrations (MIC) µg/mL**	
				A. flavus	***A. fumigatus***

4a	6-CH_3	H	H	3.12	0.4
4b	7-CH_3	H	H	3.12	0.8
4c	6-OCH_3	H	H	0.8	0.4
4d	6-Cl	H	H	0.8	0.4
4e	5,7-di CH_3	H	H	3.12	0.4
4f	6-CH_3	Cl	H	0.8	0.4
4g	7-CH_3	Cl	H	0.8	0.4
4h	6-OCH_3	Cl	H	0.4	0.4
4i	6-Cl	Cl	H	0.4	0.4
4j	5,7-di CH_3	Cl	H	0.8	0.4
4k	6-CH_3	Cl	Cl	0.8	0.4
4l	7-CH_3	Cl	Cl	0.4	0.4
4m	6-OCH_3	Cl	Cl	0.4	0.4
4n	6-Cl	Cl	Cl	0.4	0.4
4o	5,7-diCH_3	Cl	Cl	0.4	0.4
Fluconazole				8	8

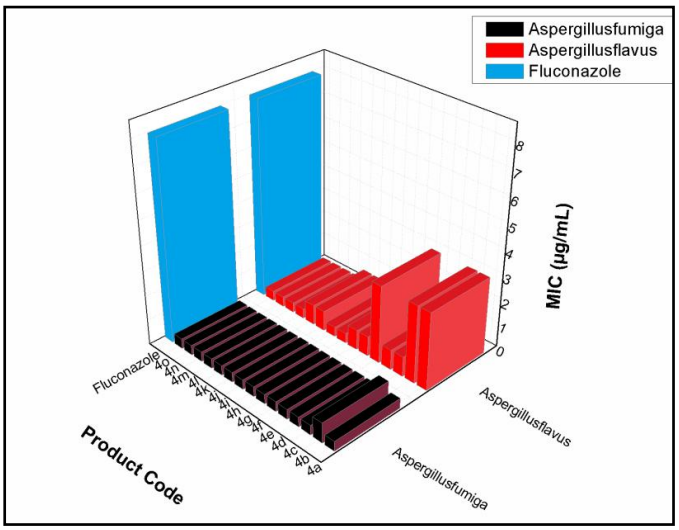


Figure 3. Graphical representation of MIC (µg/mL) of all the compounds against *A. flavus* and *A. fumigates.*

3.3.3 In vitro anti-inflammatory screening

The substituted phenyl-thiazolo-coumarinylhydrazides were evaluated for their anti-inflammatory studies against MMP-2 and MMP-9 by gelatin zymography method using tetracycline as the reference drug. The activity results are compiled in **Table 4** and the percentage inhibitions of compounds **4a-e**, **4f-j** and **4k-o** have shown comparably good activity. MMP-2 and MMP-9 are quite significant in the regulation of inflammation because of their potent gelatine degradation properties. In addition, gelatin presents in biological systems play an important function in inflammation. The screening results have shown that compounds **4a-e**, **4f-j** and **4k-o** are more potent against MMP-2. The mono and dichloro substituted series **4f-j** and **4k-o** exhibited outstanding activity against MMP-9, Whereas compounds **4a-e** are less active against MMP-9 in comparison with the standard. The results are shown in bar diagram (**Figure 4**). These, results revealed that inhibition of MMP-2 is higher than MMP-9. Interestingly, chloro substituted compounds shown excellent anti-inflammatory activity.

Table 4. The *in vitro* anti-inflammatory results of synthesized compounds **4a-e**, **4f-j** and **4 k-o**

Product Code	R	R^1	R^2	% Inhibition of MMP	
				MMP-9	MMP-2
4a	6-CH_3	H	H	50	72
4b	7-CH_3	H	H	62	60
4c	6-OCH_3	H	H	40	85
4d	6-Cl	H	H	53	76

4e	5,7-di CH_3	H	H	30	81
4f	6-CH_3	Cl	H	70	88
4g	7-CH_3	Cl	H	78	96
4h	6-OCH_3	Cl	H	88	87
4i	6-Cl	Cl	H	65	92
4j	5,7-di CH_3	Cl	H	70	93
4k	6-CH_3	Cl	Cl	80	85
4l	7-CH_3	Cl	Cl	69	89
4m	6-OCH_3	Cl	Cl	85	95
4n	6-Cl	Cl	Cl	78	92
4o	5,7-diCH_3	Cl	Cl	80	95
Tetracycline				100	100
DMSO Control					

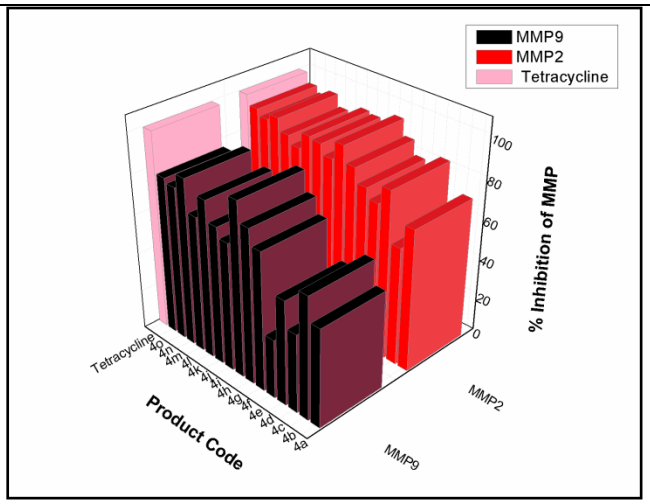


Figure 4. Graphical presentation of % inhibitions of all the compounds **4a-e**, **4f-j** and **4k -o** against MMP- 9 and MMP-2

3.3.4 Molecular Modeling studies

To understand the mechanism of antimicrobial activity of the compounds, molecular modeling and docking studies were carried out on X-ray crystal structure of *S. aureus* 24 kDa area in complex with ciprofloxacin (PDB code: 1KZN; resolution 2.30 Å) by Surflex-Dock programme of Sybyl-X software. All the fifteen title compounds were docked into the dynamic site of the enzyme as given in **Figure 5**. The predicted binding energies of the compounds are tabulated in **Table 5**. The docking results exposed that all the compounds have shown very good docking score against *S. aureus.* As depicted in the **Figure 6,** compound **4j** makes three hydrogen bonding interactions at the active site of the enzyme (PDB ID: 1KZN), among those two interactions, are oxygen atoms of chromene ring of carbonyl group ARG136 (-O------H-ARG136, 2.07 Å; C=O------H-ARG136, 2.00 Å) and one more hydrogen bonding interaction raised from the nitrogen atom of CH=N group with hydrogen of ARG76 (N----- H-ARG76, 2.66 Å) respectively.

As depicted in **Figure 7**, compound **4m**, makes four hydrogen bonding interactions at the active site of the enzyme (PDB ID: 1KZN), among those, two interactions are of oxygen atom of chromene ring with hydrogens of ARG136 (O------H-ARG136, 2.54 Å; 2.45 Å), and oxygen atom of carbonyl group present on chromene ring makes a hydrogen bonding interaction with hydrogen of ARG136 (C=O------H-ARG136, 2.30 Å). The methoxy group on chromene ring has one hydrogen bonding with hydrogen of THR165 (H_3CO-----H-THR165, 2.31 Å). **Figure 8** (**A-B**) presents the hydrophobic and hydrophilic amino acids bounded to the studied compound **4j** and **4m.**

All the compounds have shown c score in the series 7.17-4.98. These scores specify that molecules mainly bind to the protein in assessment to the reference ciprofloxacin (**Table 5**). The hydrogen bonds formed with ASP73, ARG136 and THR165 amino acid residues may be dependable for the antibacterial activity. Considering these results, we can conclude that compound **4m** appears to be the most appealing compound among the newly synthesized and seem potentially attractive as chemotherapeutic drug against gram positive and gram-negative bacteria.

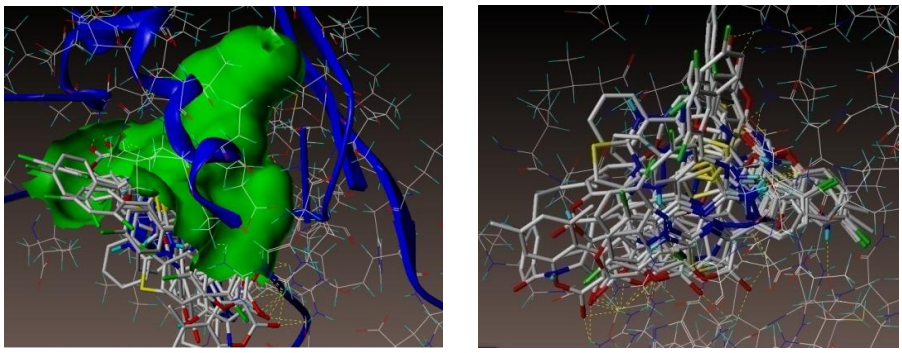


Figure 5. Docked view of the compounds PDB ID: 1KZN

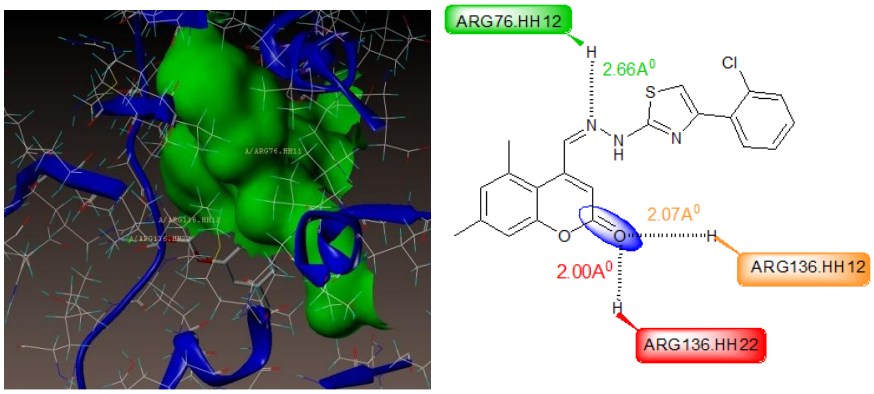


Figure 6. Docked study of the compound **4j**, which shows the C-score of 6.73 and illustration of **4j** PDB: 1KZN subunit.

Table 5. Surflex Docking score (kcal/mol) of compounds 4a-e, 4f-j and 4k-o.

Compounds	C Scorea	Crash Scoreb	Polar Scorec	D Scored	PMF Scoree	G Scoref	Chem Scoreg
4a	4.98	-1.27	1.23	-132.124	-15.674	-194.692	-27.995
4b	5.63	-1.09	0.74	-128.331	-31.189	-199.283	-21.665
4c	5.77	-1.02	1.21	-129.288	-9.231	-183.979	-22.315
4d	5.61	-1.39	1.15	-149.864	-34.189	-213.443	-24.635
4e	6.43	-0.96	1.07	-119.384	-6.775	-207.982	-24.872
4f	6.58	-0.93	2.46	-115.311	-38.917	-211.415	-25.428
4g	5.57	-2.23	0.93	-154.583	-41.029	-246.129	-26.232
4h	5.16	-1.26	0.66	-122.752	-37.753	-200.094	-22.397
4i	5.42	-0.81	2.08	-121.328	-25.735	-187.823	-22.441
4j	6.73	-1.70	0.94	-150.589	-16.339	-252.030	-28.449
4k	5.41	-0.95	1.15	-129.091	-9.671	-187.556	-24.838
4l	5.17	-2.09	0.28	-148.219	-28.059	-237.493	-24.564
4m	7.17	-1.40	2.28	-125.317	-38.553	-218.557	-26.961
4n	6.39	-1.17	1.78	-149.235	-25.512	-238.715	-27.296
4o	5.29	-0.91	0.98	-131.804	-11.086	-181.086	-25.223

[a] CScore integrates a number of accepted scoring functions for rank the similarity of ligands bound to the active site of a receptor and reports the productivity of total score.
[b] Crash-score representing the improper diffusion into the binding site. Crash scores close to 0 are favourable. Negative numbers designate saturation
[c] Polar signifying the contribution of the polar interactions to the total score.
[d] D-score for charge and van der Waals interactions between the protein and the ligand.
[e] PMF-score signifying the Helmholtz free energies of interactions for protein-ligand atom pairs
[f] G-score representing hydrogen bonding, complex, and internal energies.

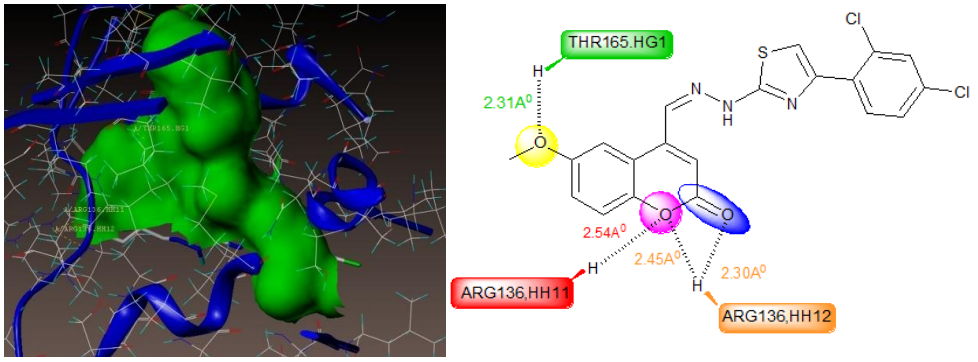
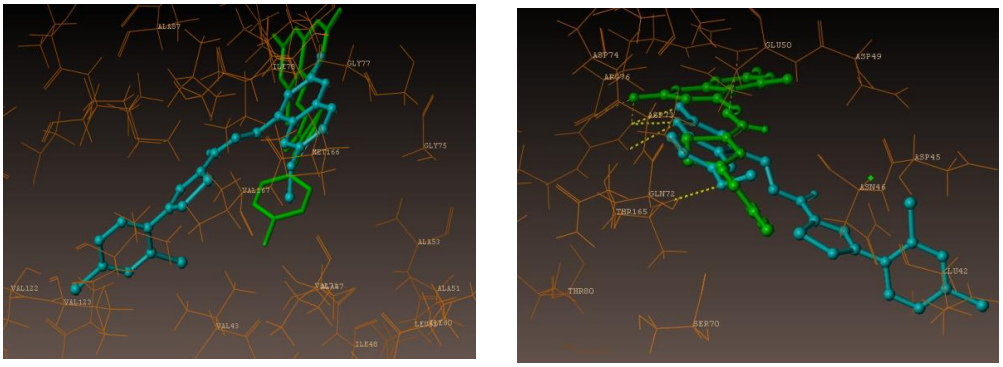


Figure 7. Docked study of the compound **4m**, which shows the C-score of 7.17 and illustration of 4m PDB: 1KZN subunits



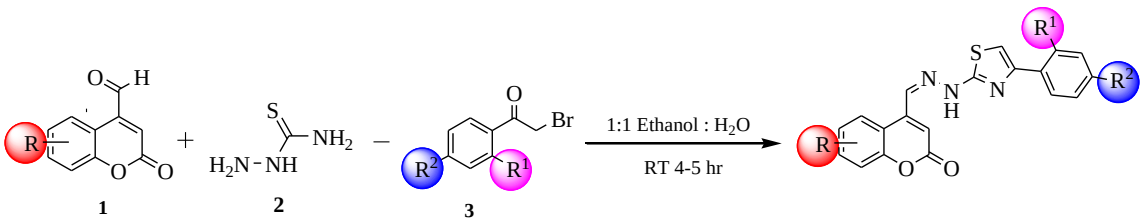
A B

Figure. 8 A) Hydrophobic amino acids bounded to **4j** and **4m**. B) Hydrophilic amino acids bounded to **4j** and **4m**

IV. Discussion

Chemistry

The synthetic approaches for the formation of substituted phenyl-thiazolocoumarinyl hydrazides are illustrated in **Scheme 1**. The synthetic sequence begins with the synthesis of key structure substituted 4-formylcoumarin (**1**) was carried out by the Substituted 4-bromomethylcoumarin was treated with DMSO in aqueous base. [46] The synthesis of substituted phenyl-thiazolocoumarinyl hydrazides **4a-e**, **4f-j** and **4k-o** were achieved by one-pot approach. The reaction proceeds via the cyclization of thiosemicarbazone with substituted phenacyl bromides to give the desired title compound and the plausible mechanism is given in **Scheme-2**. The product were further confirmed by spectroscopic (FT-IR, NMR and Mass) and elemental analysis. The structures of compounds are represented in **Table 1.**

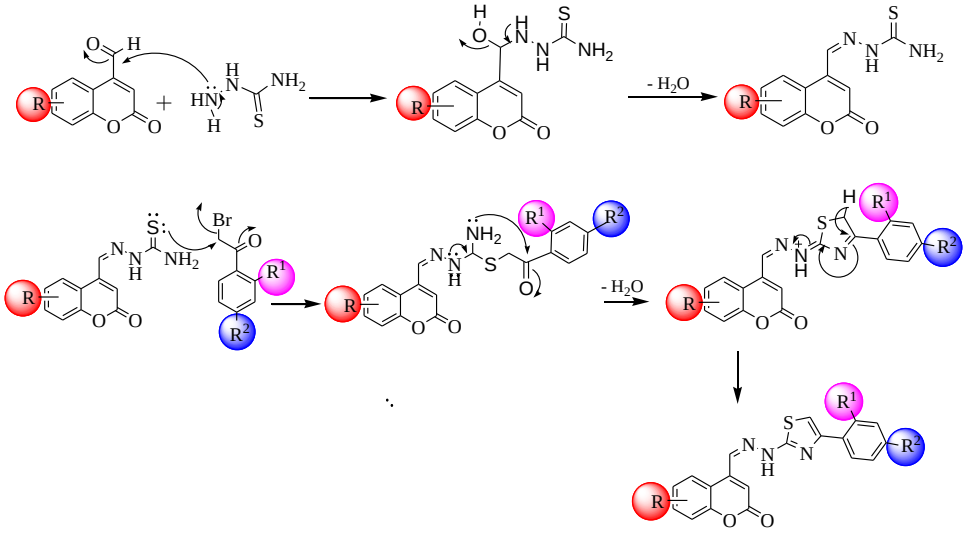


1 2 3

R = 6-CH_3, 7-CH_3, 6-OCH_3, 6-Cl, 5,7-diCH_3
R^1 = Cl , R^2 = Cl

R^1 = H, R^2 = H 4 a-e
R^1 = H, R^2 = Cl 4f-j
R^1 = Cl, R^2 = Cl 4k-o

Scheme 1. Synthesis of substituted phenyl-thiazolocoumarinyl hydrazides.



Scheme 2. plausible mechanism of substituted phenyl-thiazolo coumarinyl hydrazides.

V. Conclusion

In summary, we have synthesized and characterized novel substituted phenyl-thiazolo coumarinyl hydrazides with the simple experimental method at short reaction time, without a catalyst under normal reaction conditions, in high yield. The antibacterial activities of compounds **4a- o** are less active against *Baciluss sps* and *Pseudomonas*, whereas active against *S. aureus* and *E. coli*. Surprisingly among these series, chloro substituted derivatives **4f-o** are selectively most promising against *S. aureus* and *E.coli* with MIC values ranges 0.4 to 1.6 µg/mL. Among all the scaffolds, compounds 4j and 4m are fourteen to sixteen times more potent than standard drug ciprofloxacin against *S. aureus*. The activity of compound **4j** and **4m** is supported by docking study. *In vitro* antifungal activity showed that the compounds **4a-e**, **4f-j** and **4k-o** showed tremendous activity against *A. flavus* and *A. fumigates* fungal strains with the MIC values from 0.4 to 3.12 µg/mL. The anti-inflammatory activity against MMP-2 and MMP-9 has exhibited outstanding result. These results also suggested a new and potential route in the discovery of drug against antimicrobial gram-positive and gram-negative bacteria.

Acknowledgments

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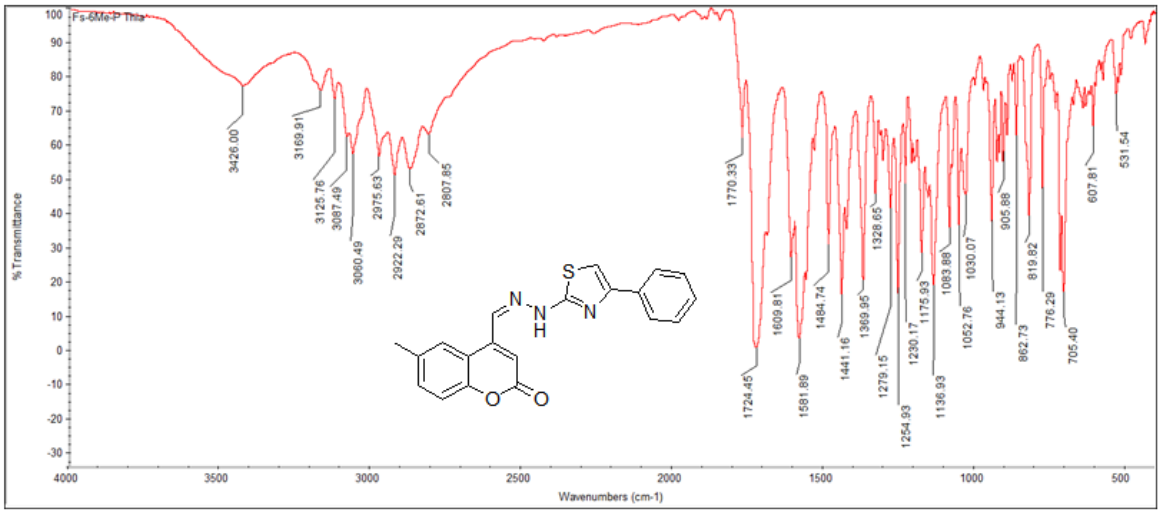
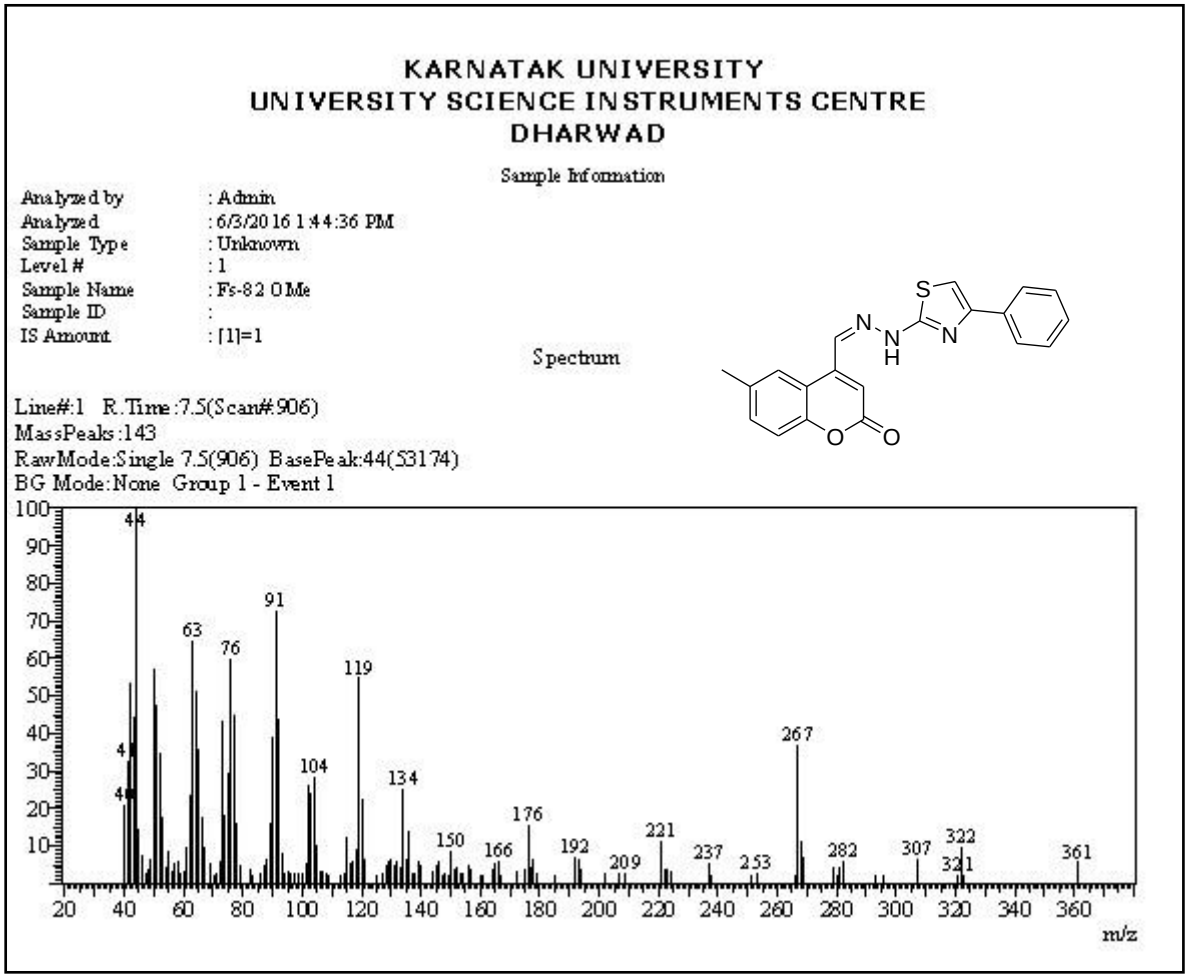
Supplimentary Information

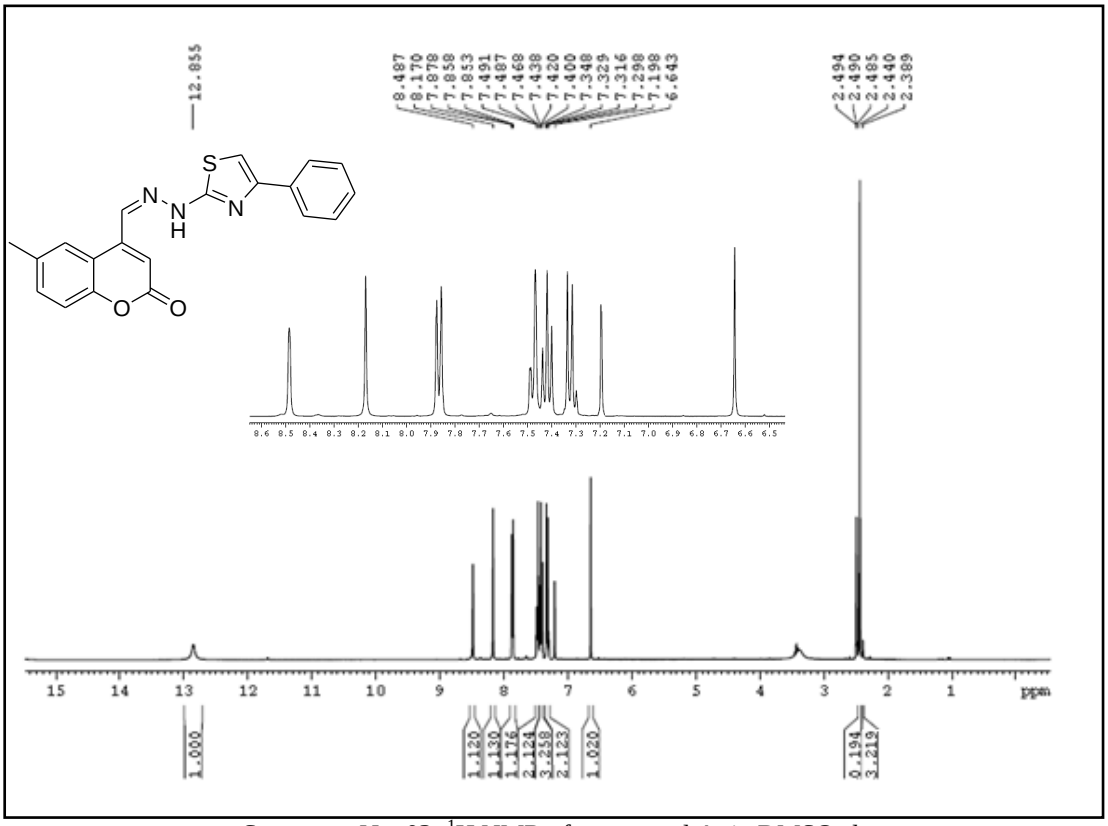
Spectral Data of Chlorophenyl-thiazolocoumarinyl hydrazides derivatives



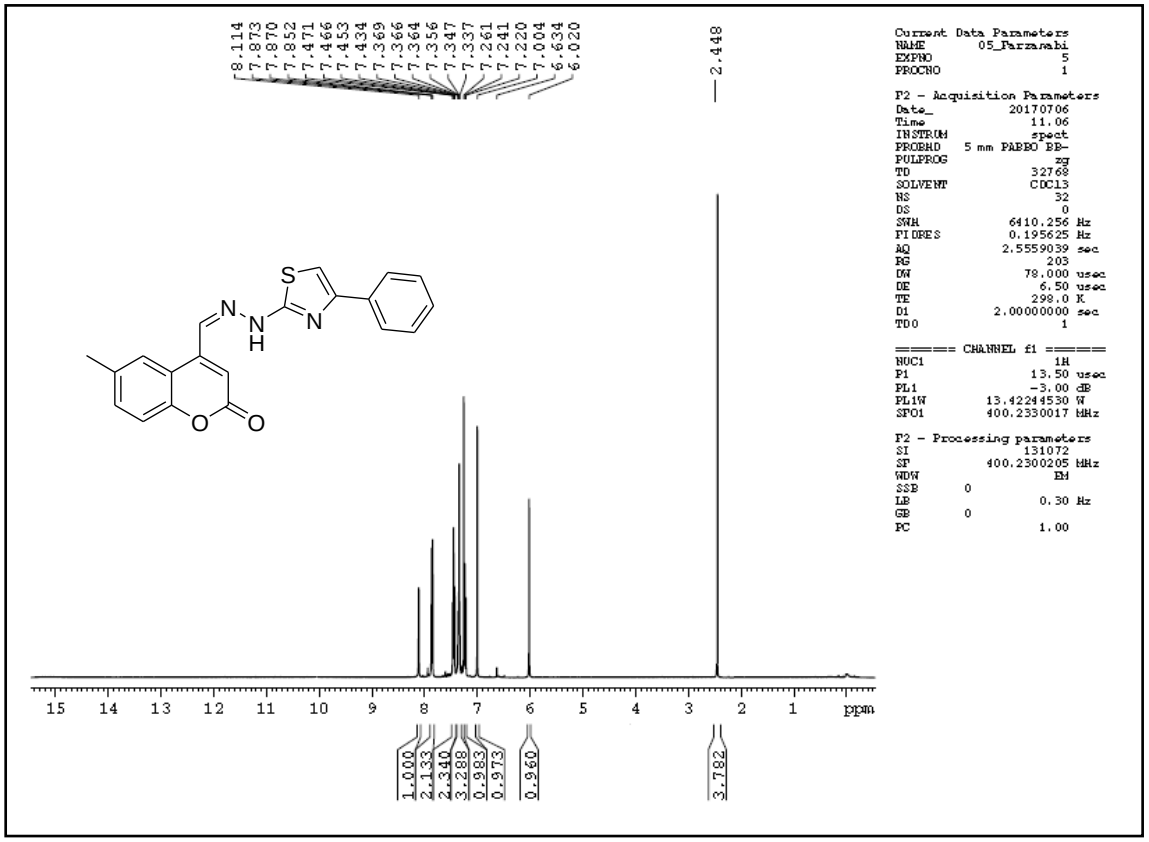
Spectrum No. 01: IR of compound **4a** in KBr



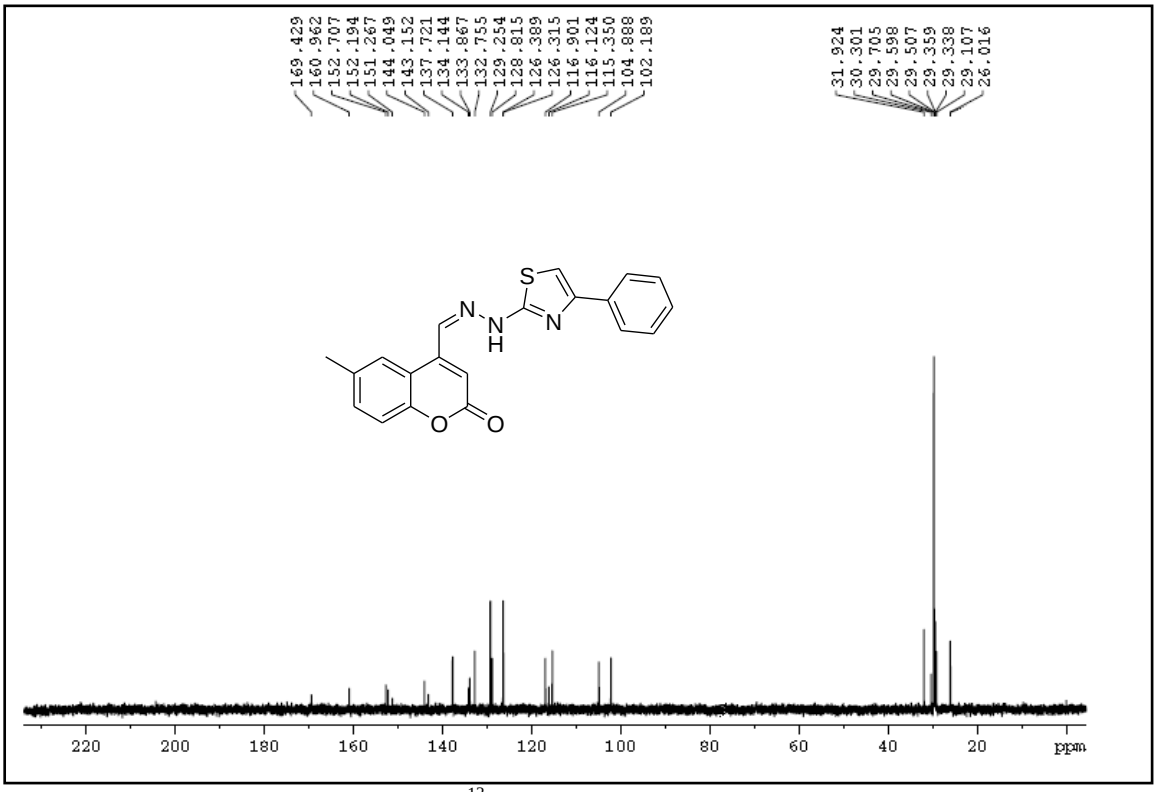
Spectrum No. 2: GM-MS of Compound **4a**



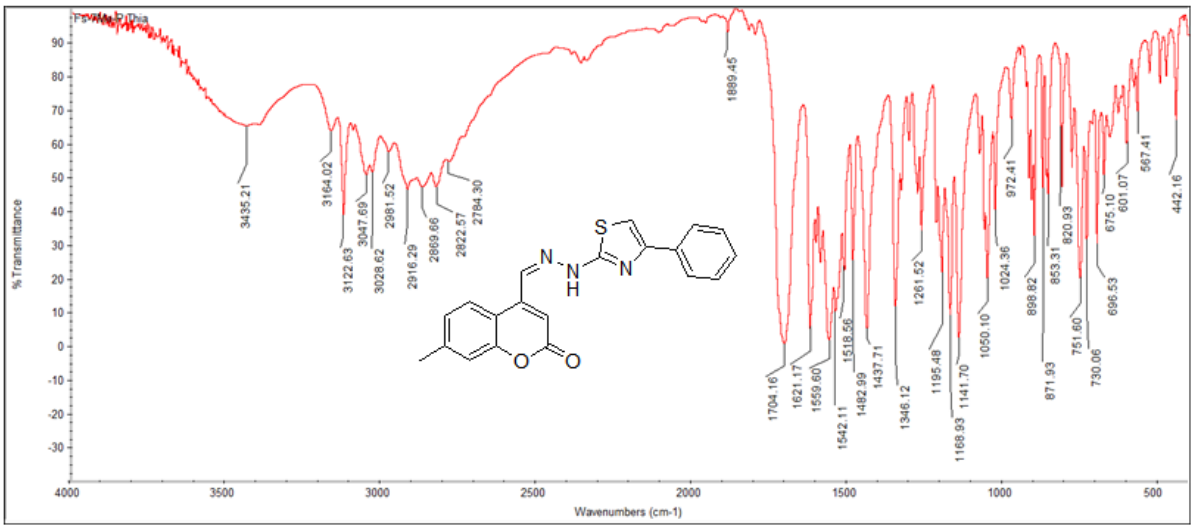
Spectrum No. 03: ^{1}H NMR of compound **4a** in DMSO-d_6



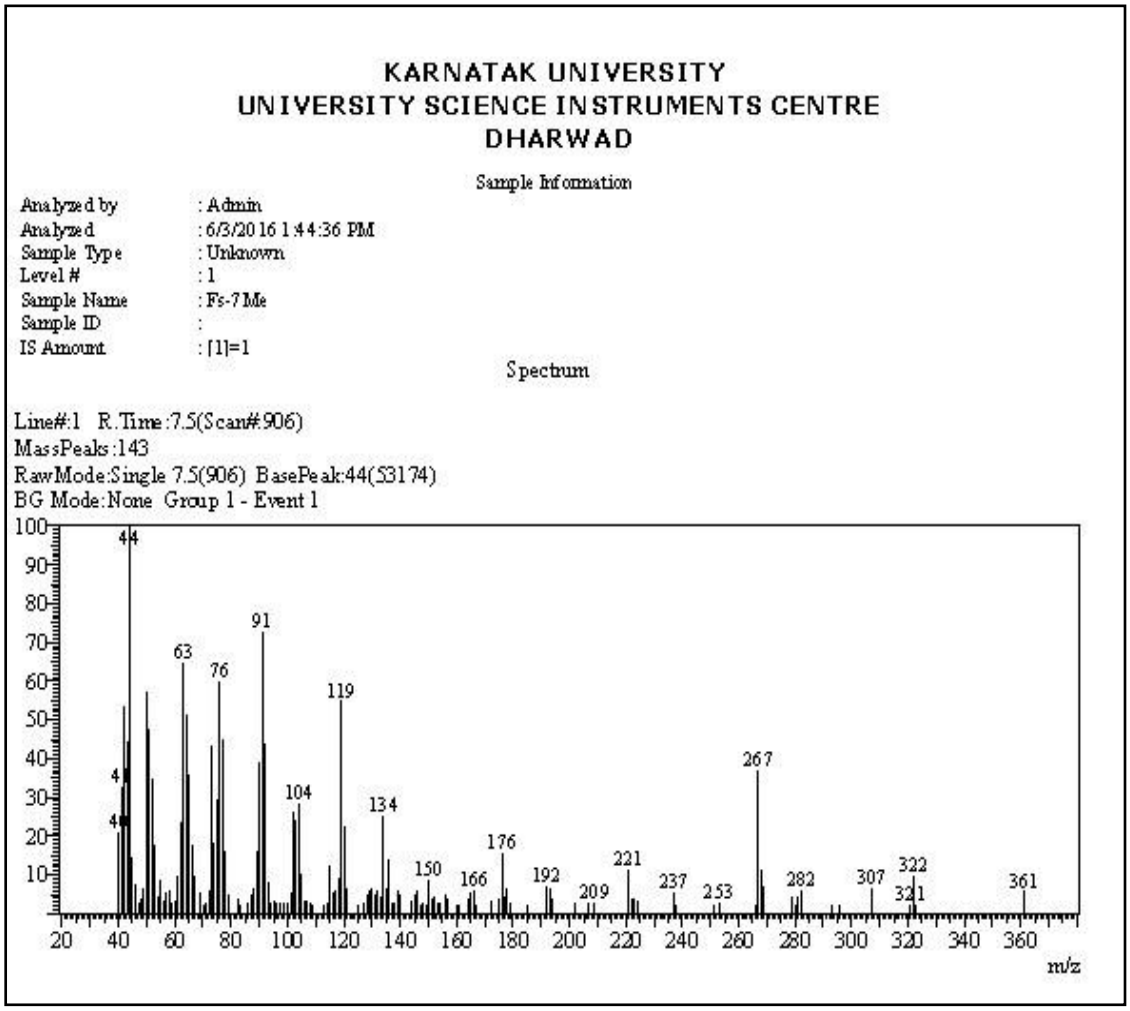
Spectrum No. 04: D_2O-Exchange of compound **4a** in $CDCl_3$



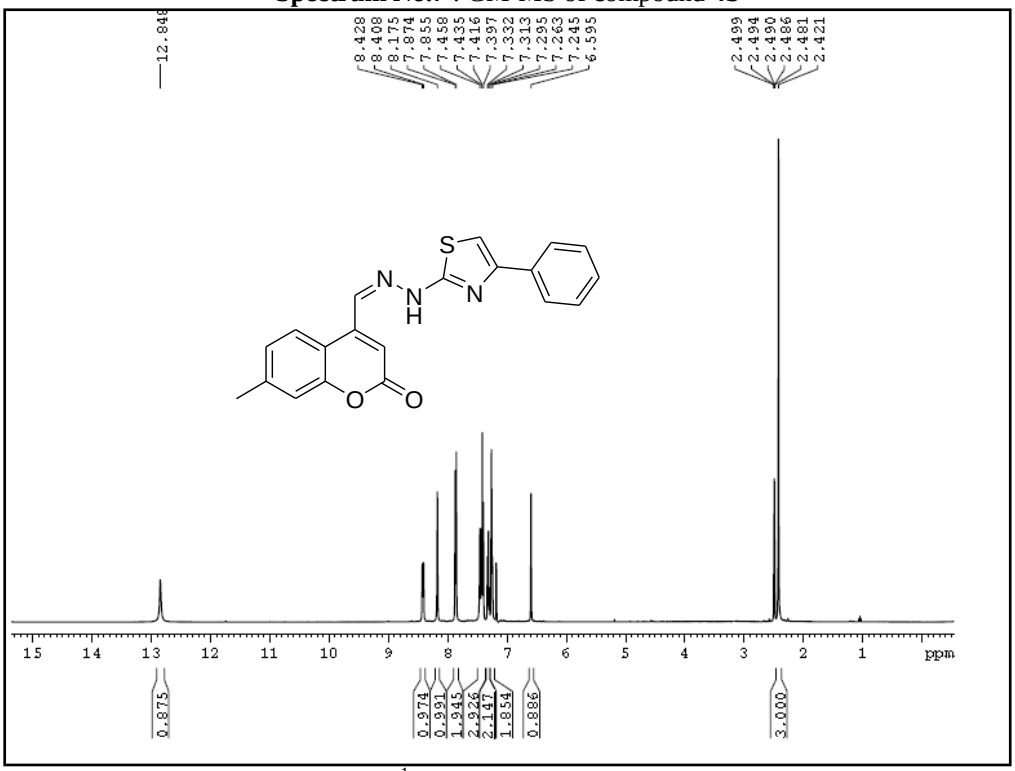
Spectrum No. 05: ^{13}C NMR of compound **4a** in DMSO-d_6



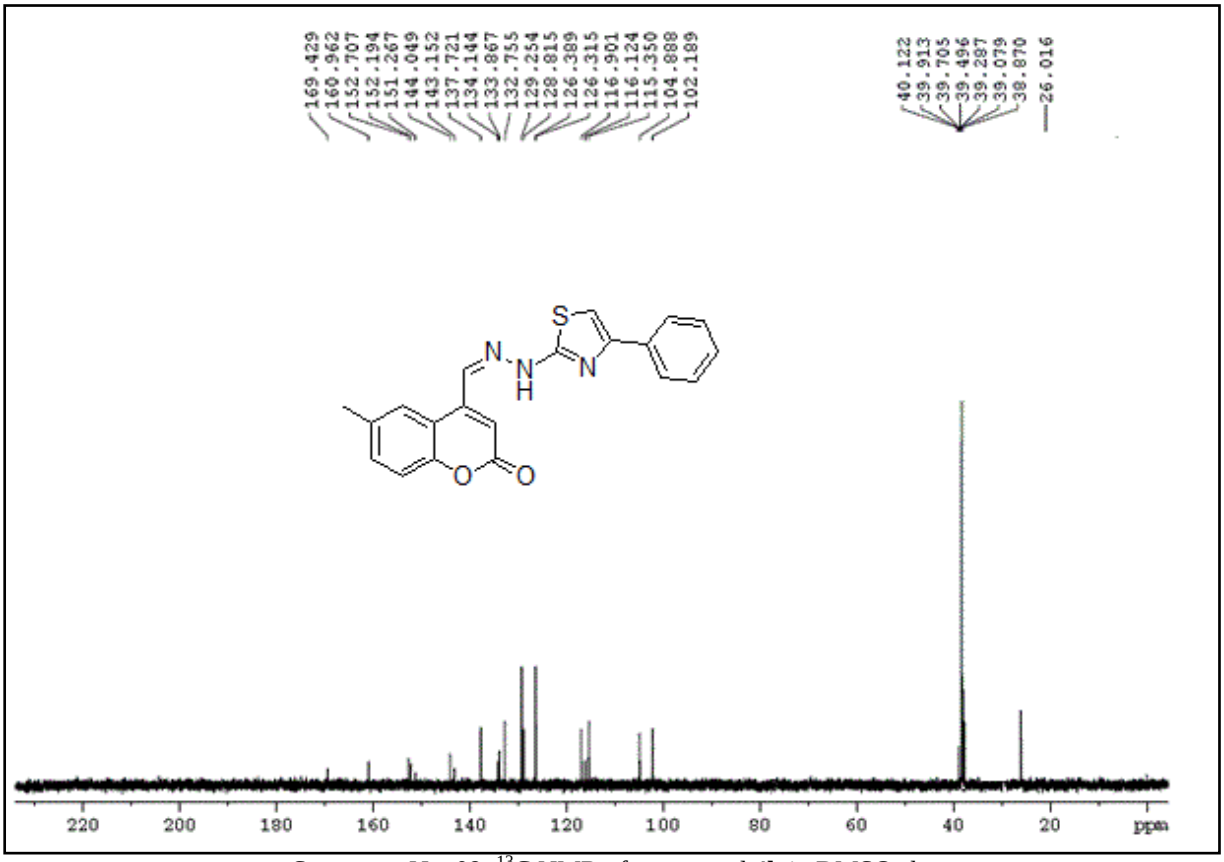
Spectrum No. 06: IR of compound **4b** in KBr



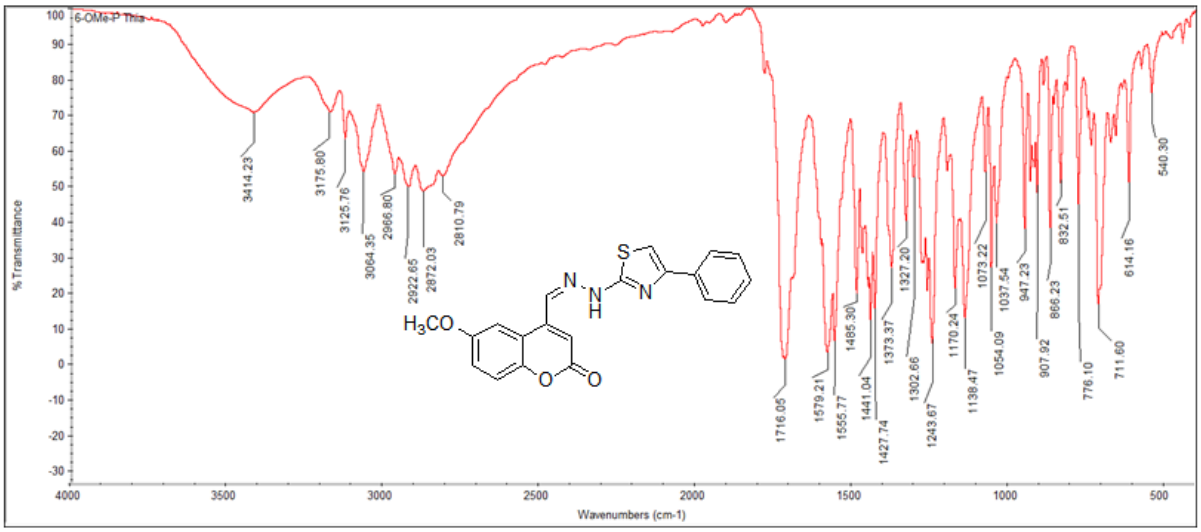
Spectrum No.7 : GM-MS of compound **4b**



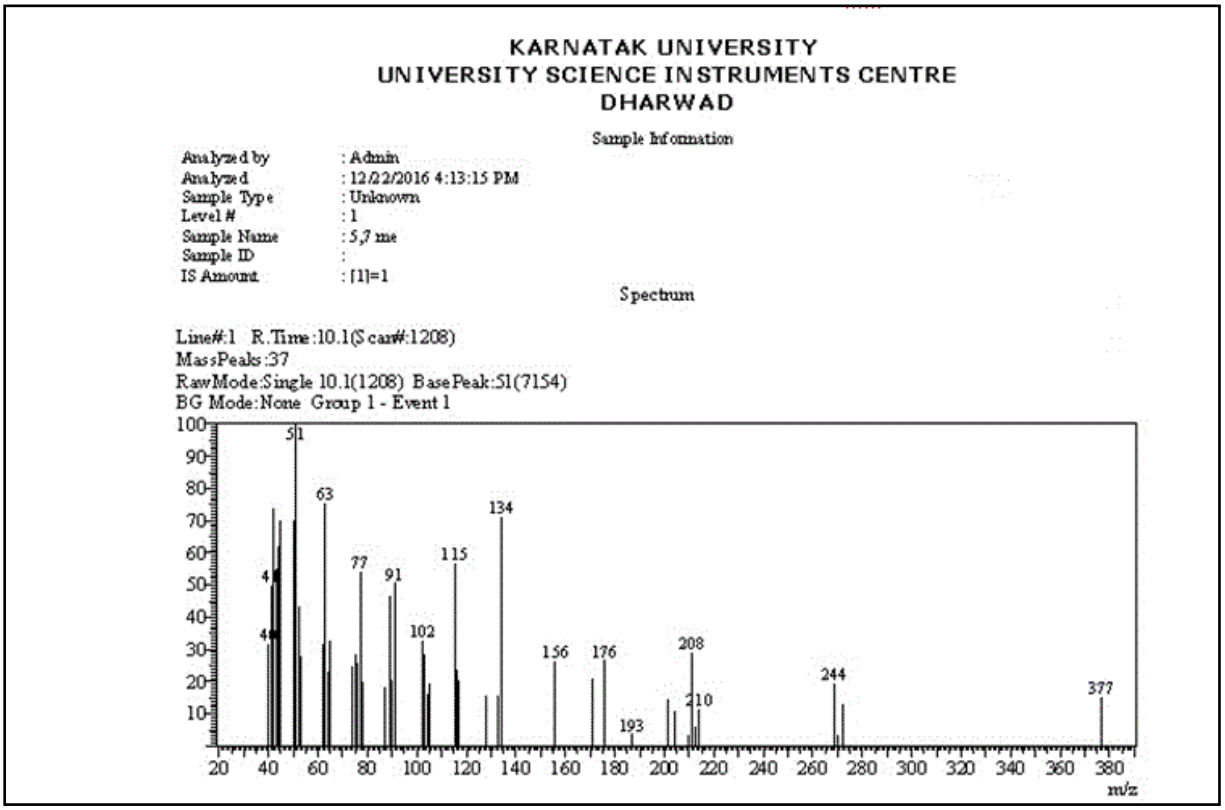
Spectrum No. 08: ^{1}H NMR of compound **4b** in DMSO-d_6



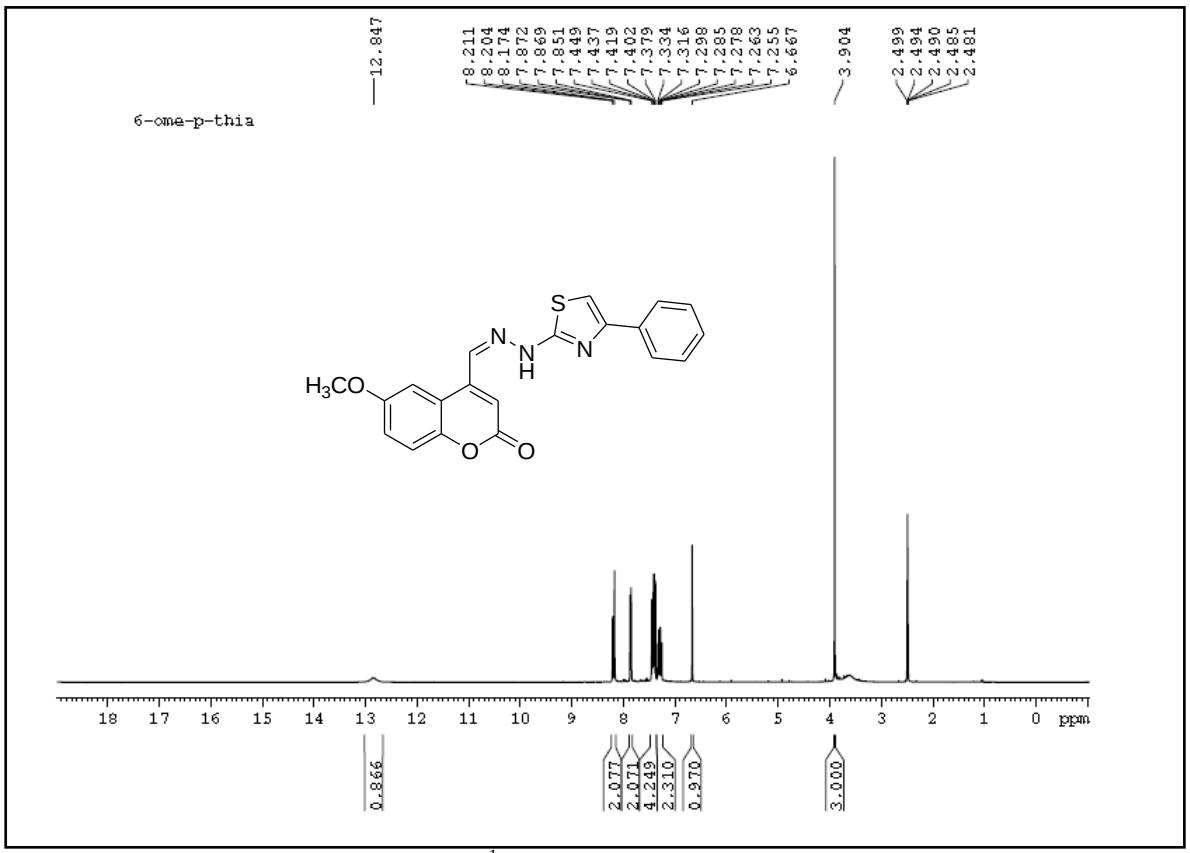
Spectrum No. 09: ^{13}C NMR of compound **4b** in DMSO-d_6



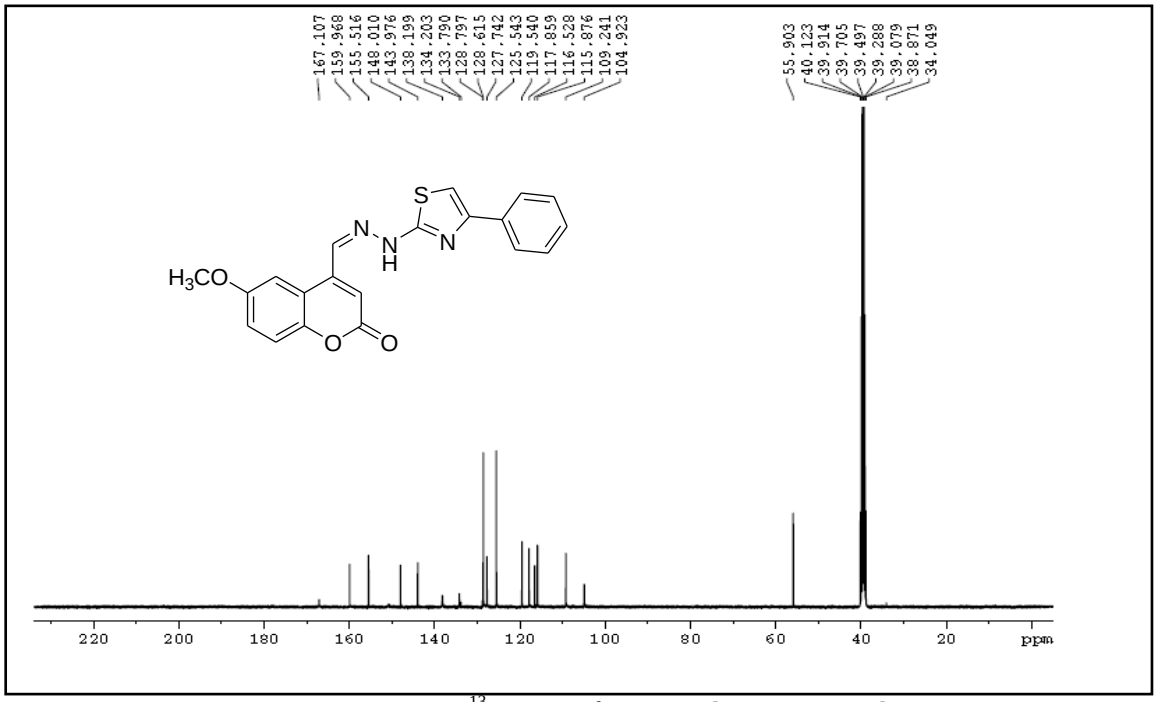
Spectrum No. 10: IR of compound **4c** in KBr



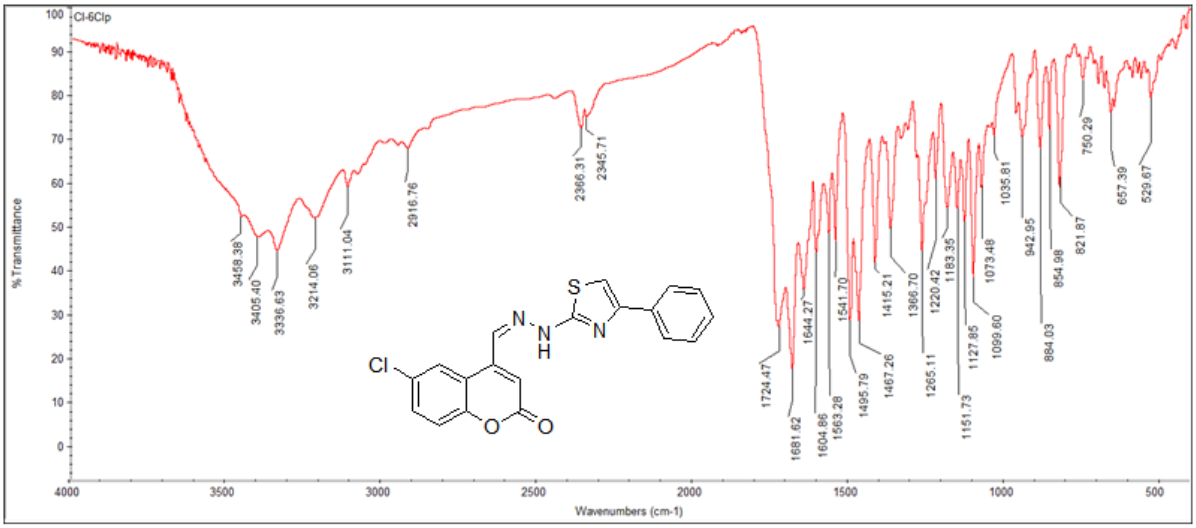
Spectrum No.11: GM-MS of compound **4c**



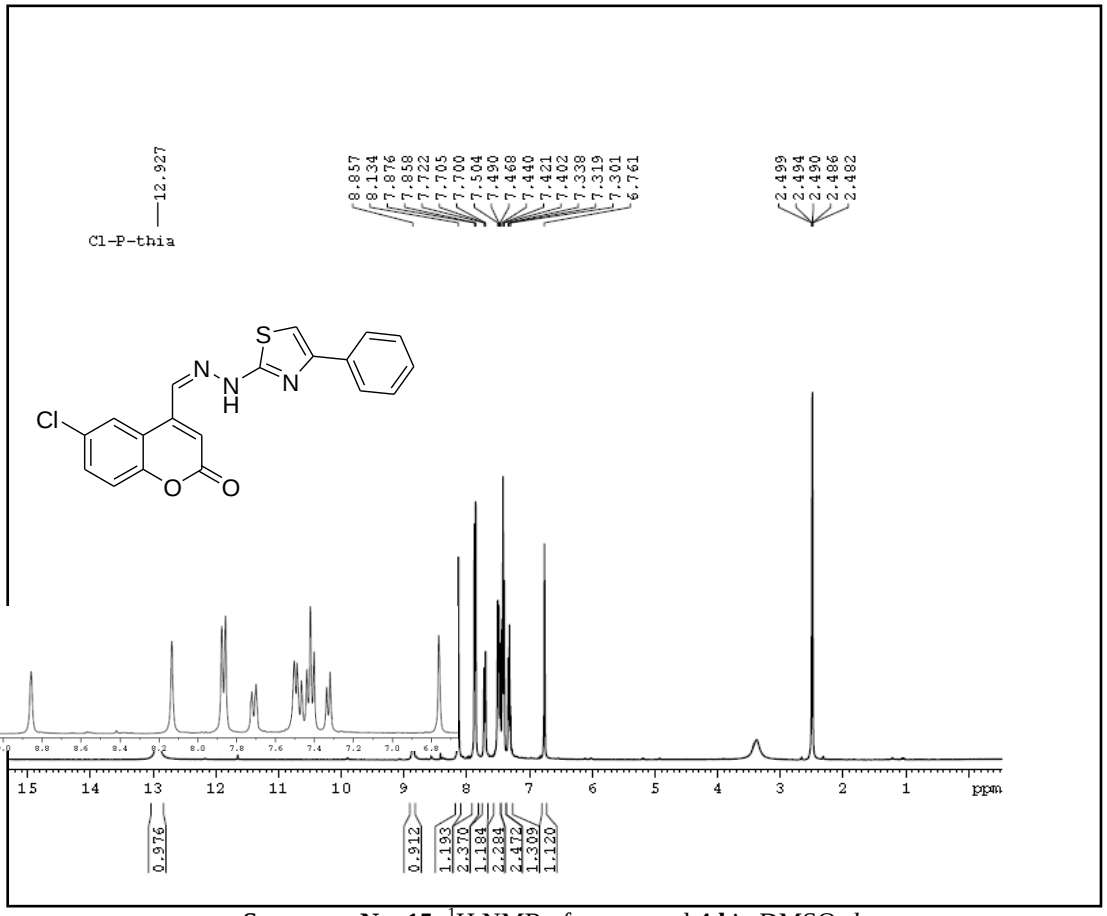
Spectrum No. 12: ^{1}H NMR of compound **4c** in DMSO-d_6



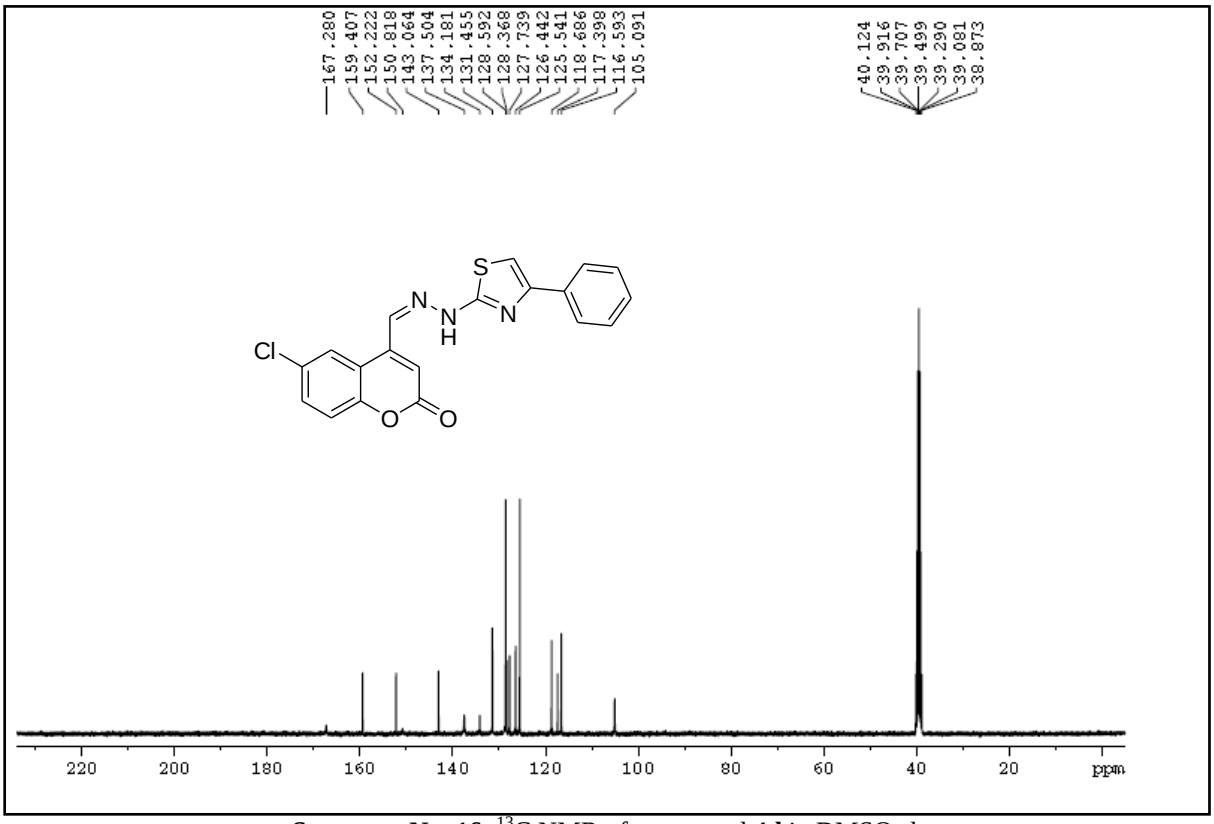
Spectrum No. 13: ^{13}C NMR of compound **4c** in DMSO-d_6



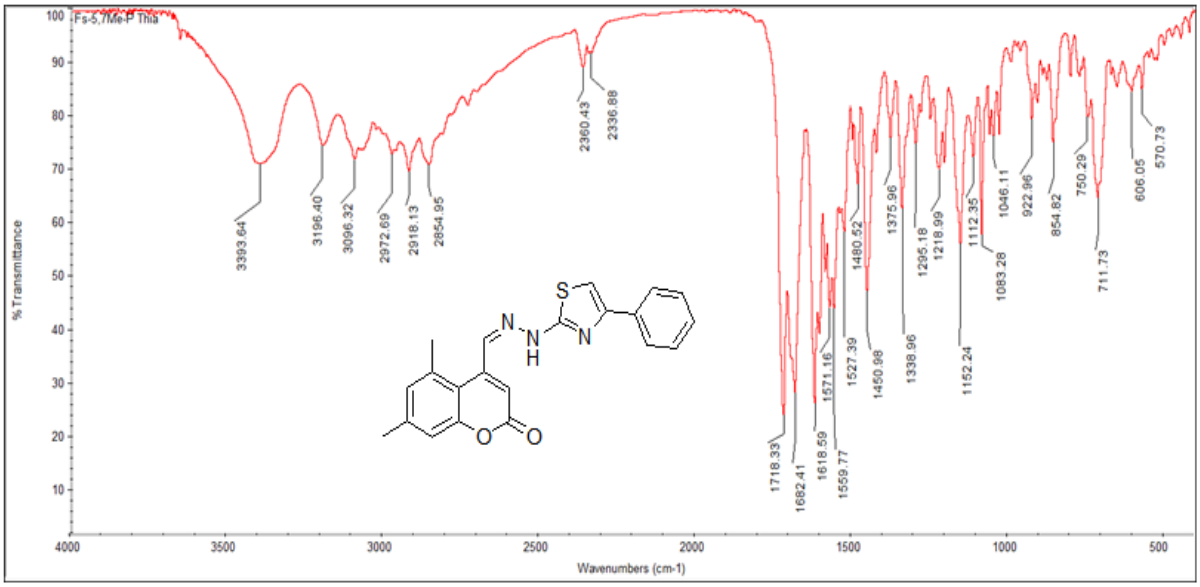
Spectrum No. 14: IR of compound **4d** in KBr



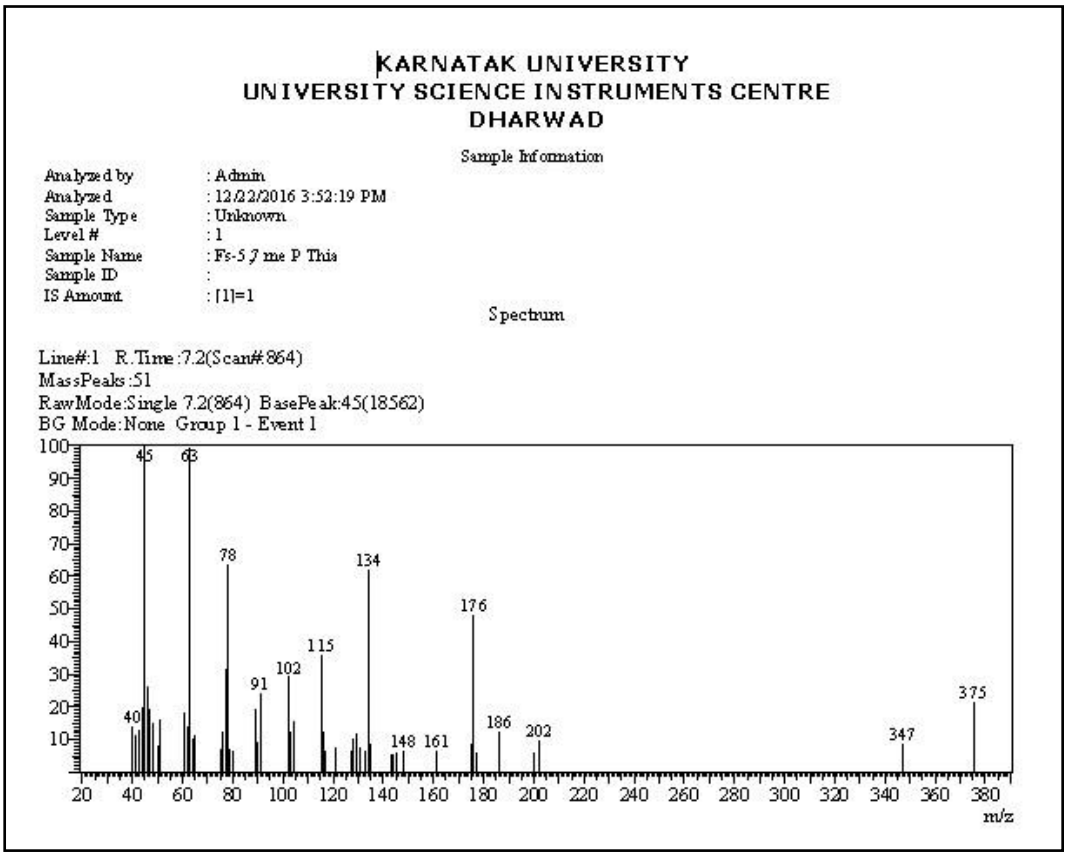
Spectrum No. 15: ^{1}H NMR of compound **4d** in DMSO-d_6



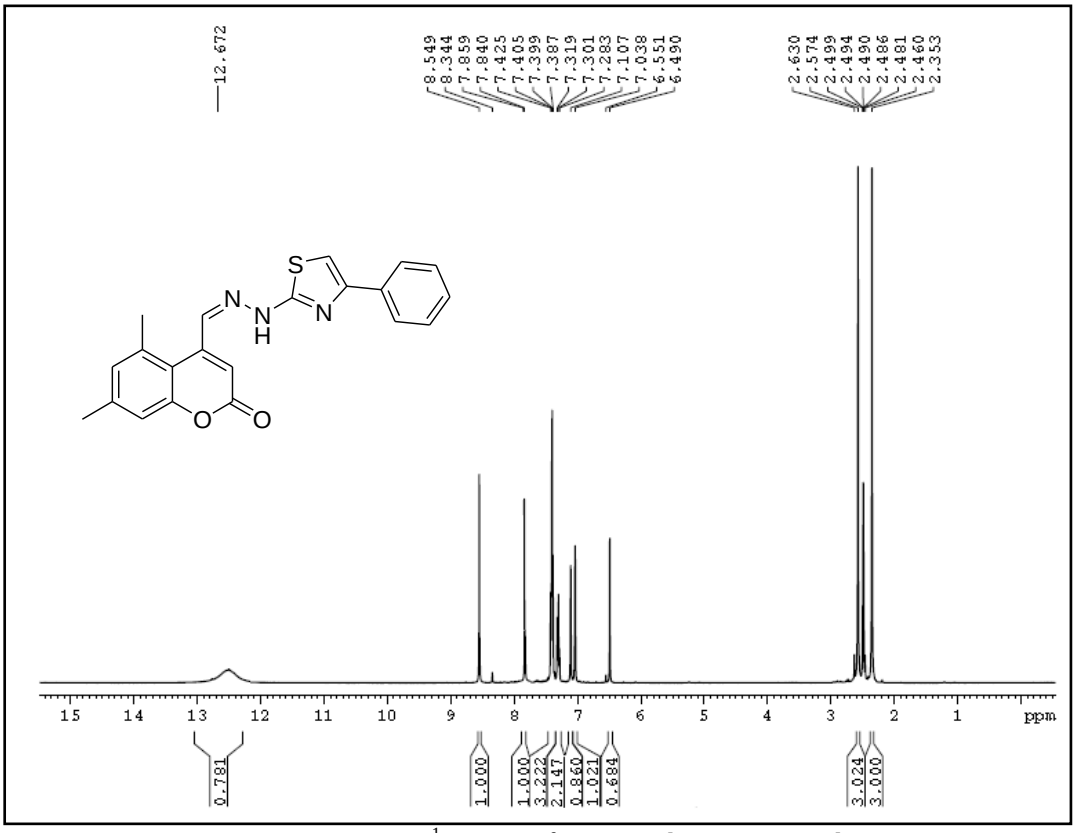
Spectrum No. 16: ^{13}C NMR of compound **4d** in DMSO-d_6



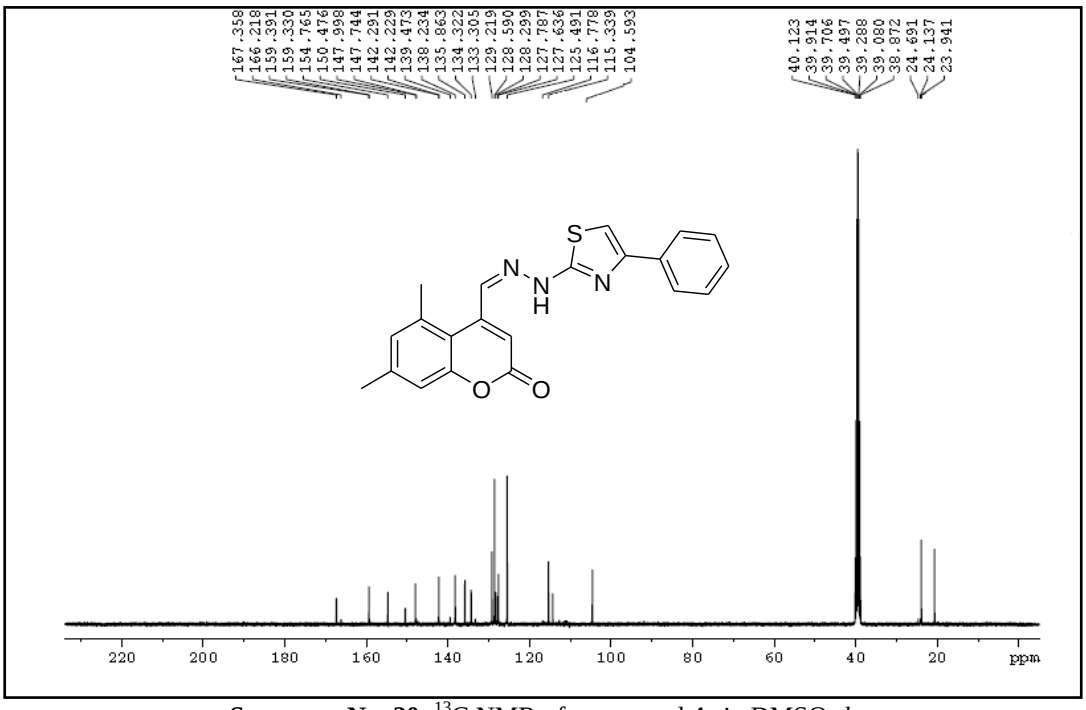
Spectrum No. 17: IR of compound **4e** in KBr



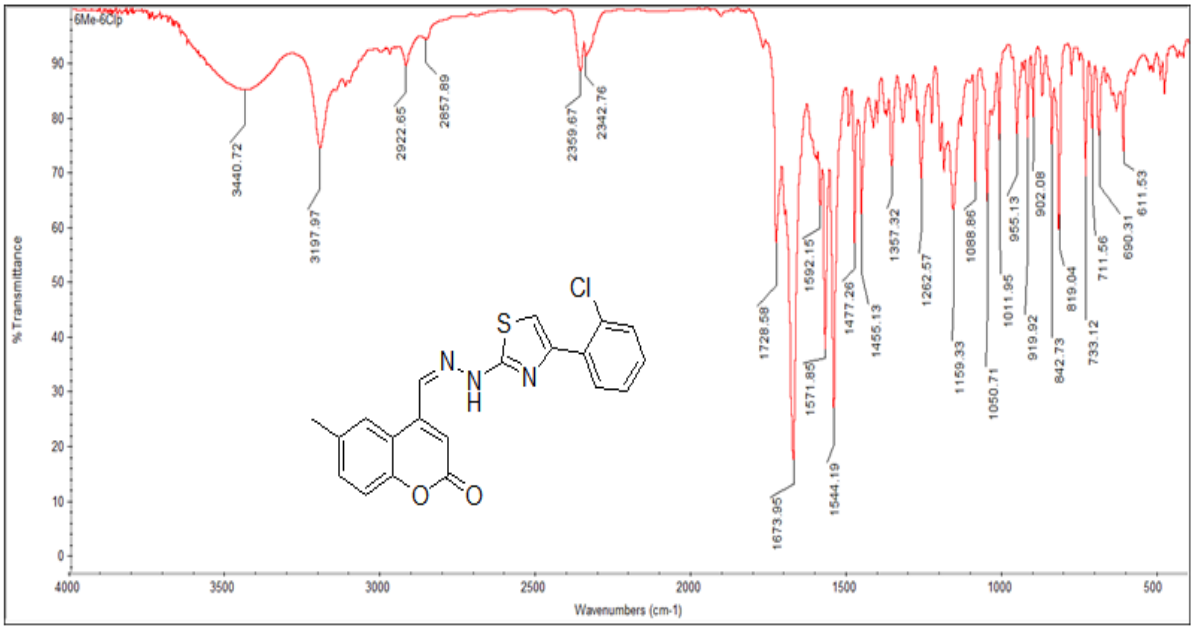
Spectrum No. 18: GM-MS of compound **4e**



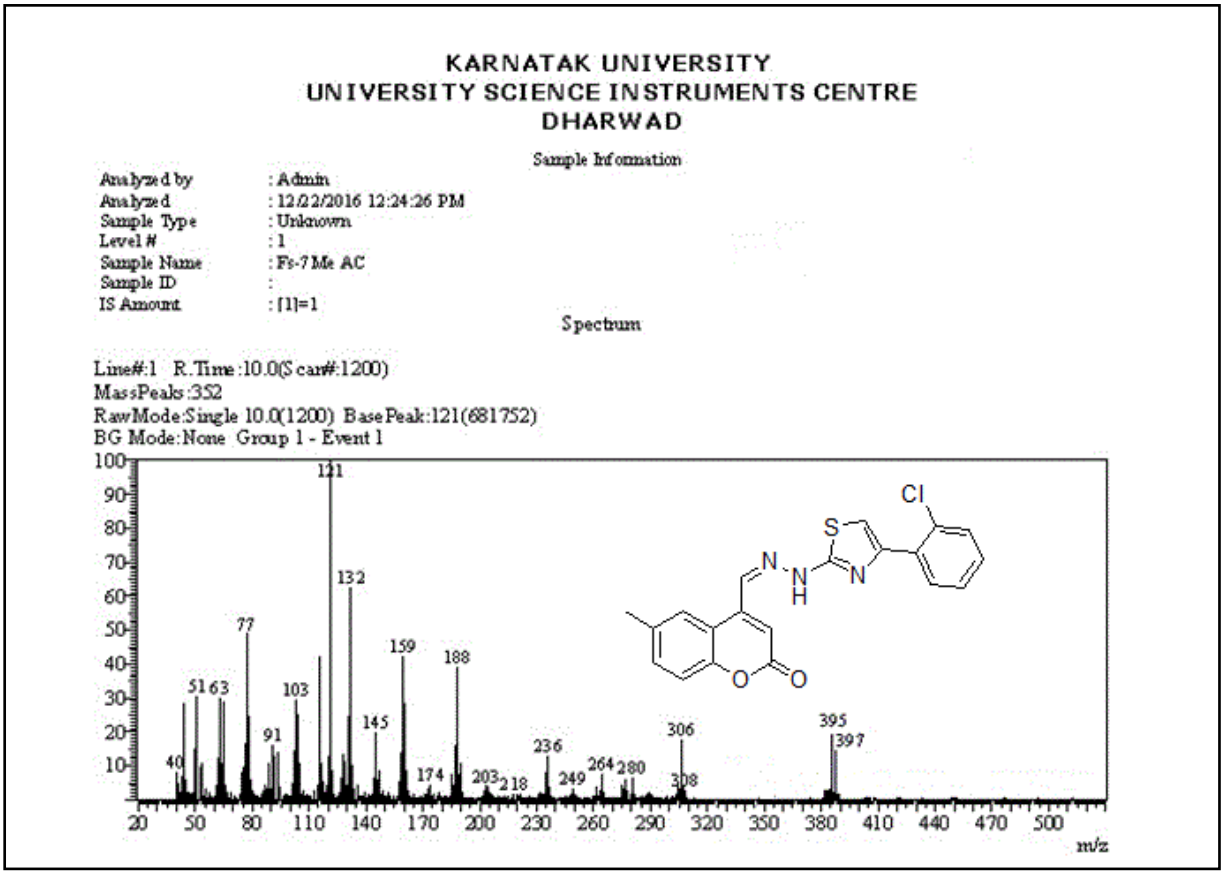
Spectrum No. 19: ^{1}H NMR of compound **4e** in DMSO-d_6



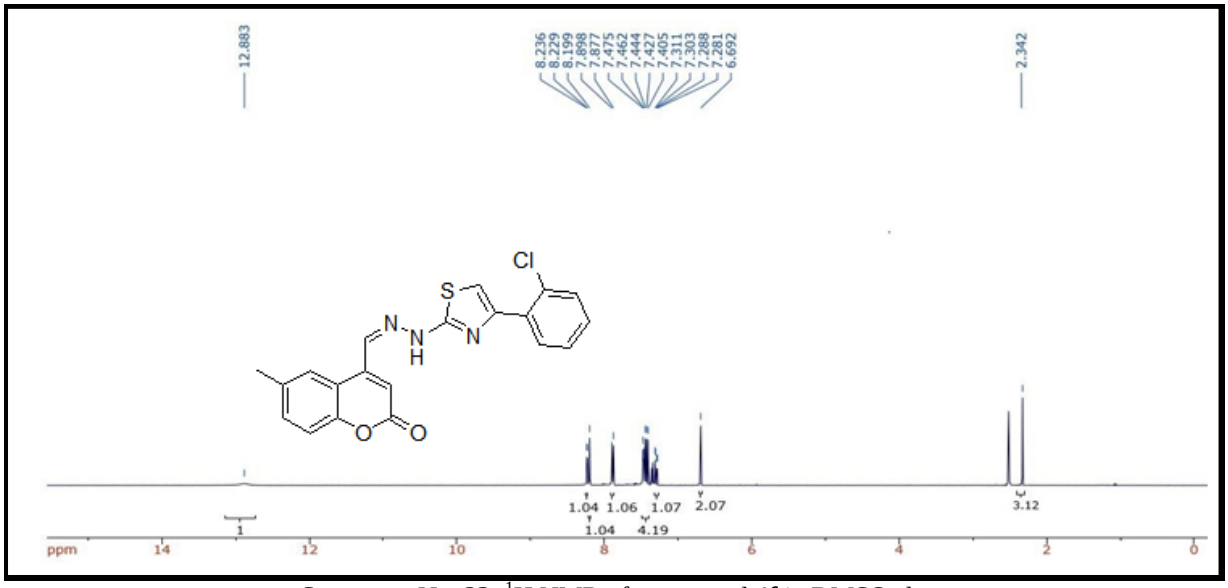
Spectrum No. 20: ^{13}C NMR of compound **4e** in DMSO-d_6



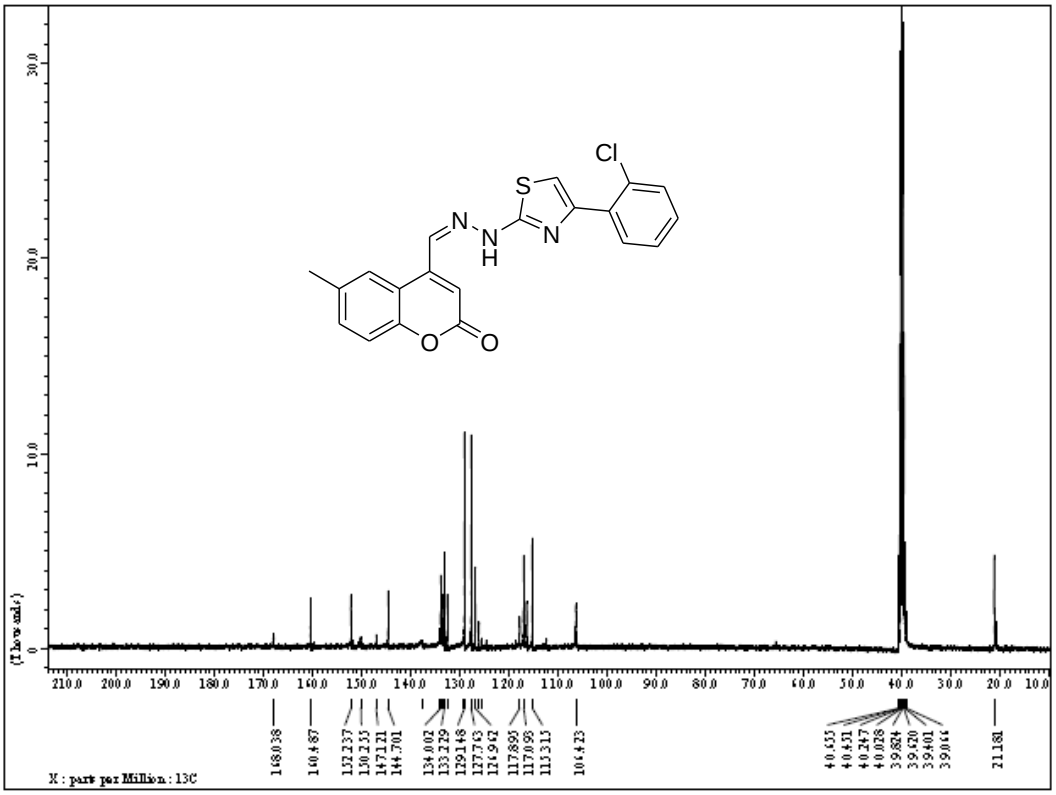
Spectrum No. 21: IR of compound 4**f** in KBr



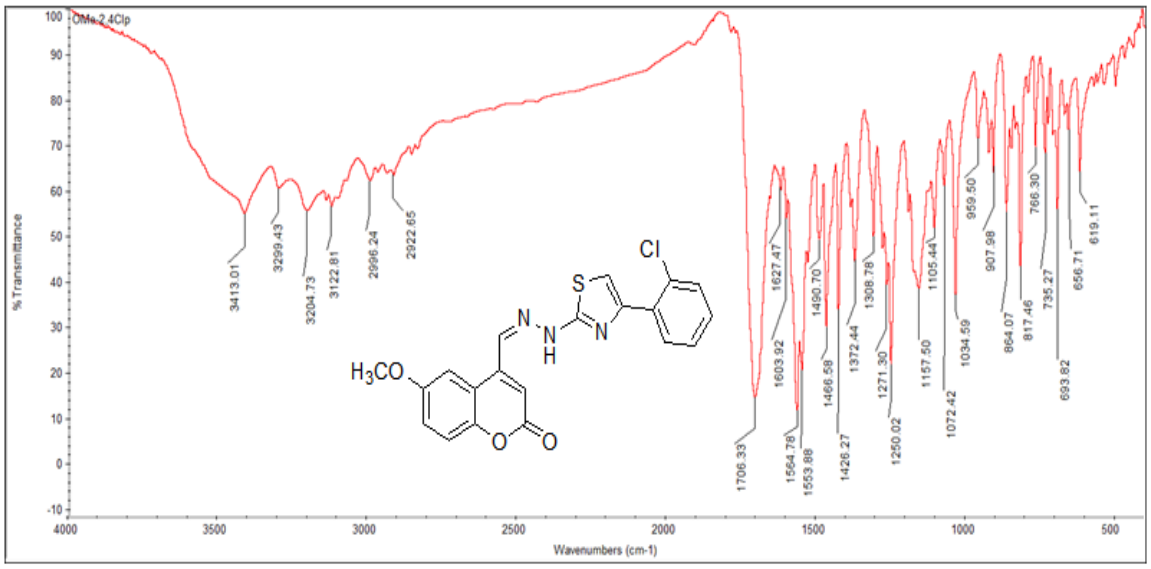
Spectrum No. 22: GM-MS of compound **4f**



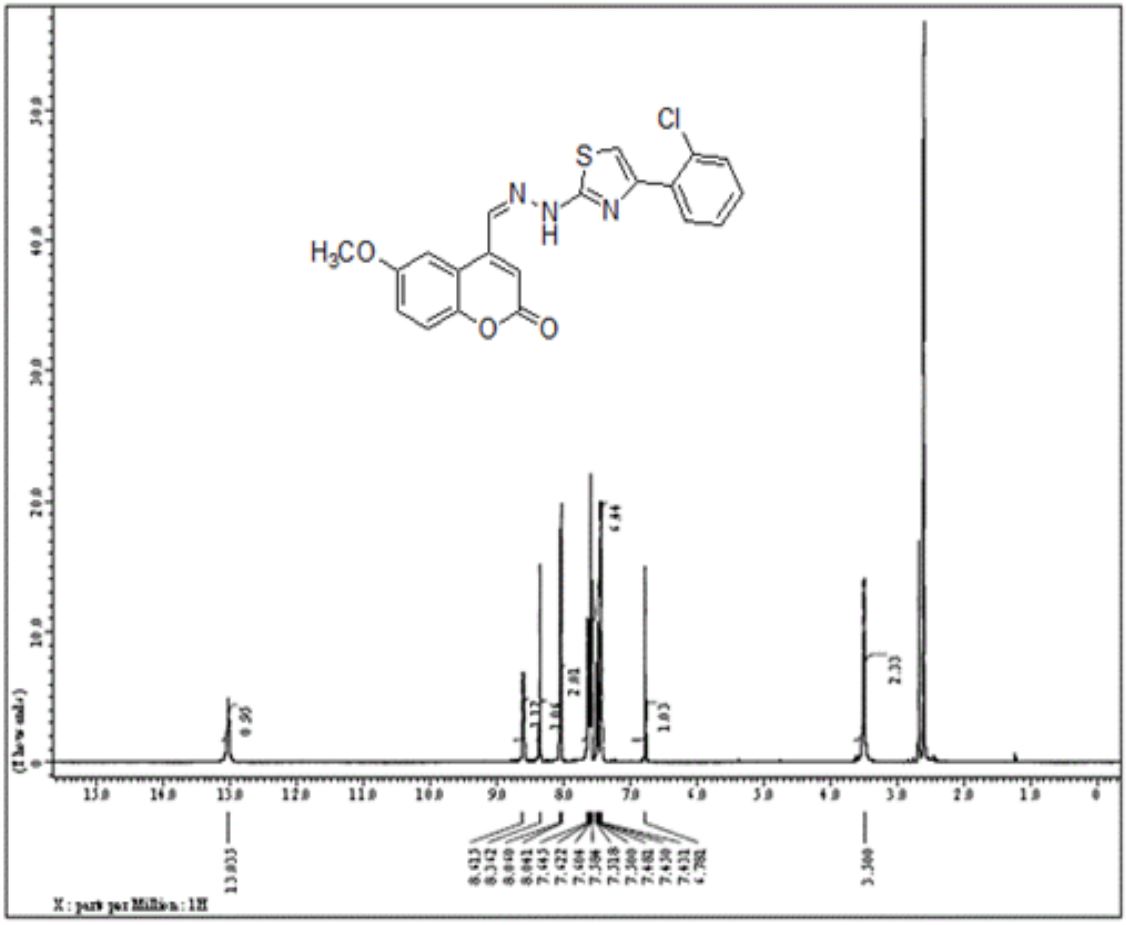
Spectrum No. 23: ^{1}H NMR of compound **4f** in DMSO-d_6



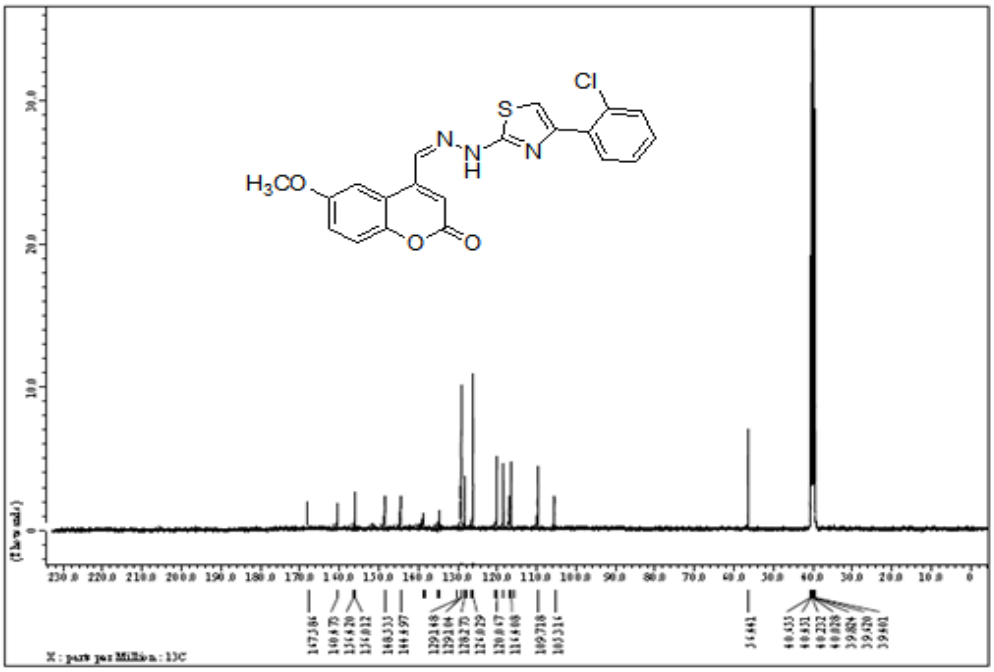
Spectrum No. 24: ^{13}C NMR of compound **4f** in DMSO-d_6



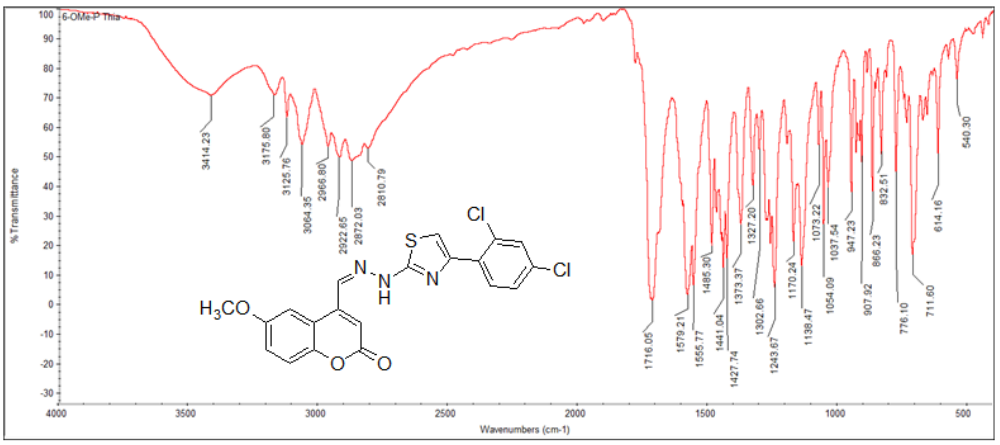
Spectrum No. 25: IR of compound **4h** in KBr



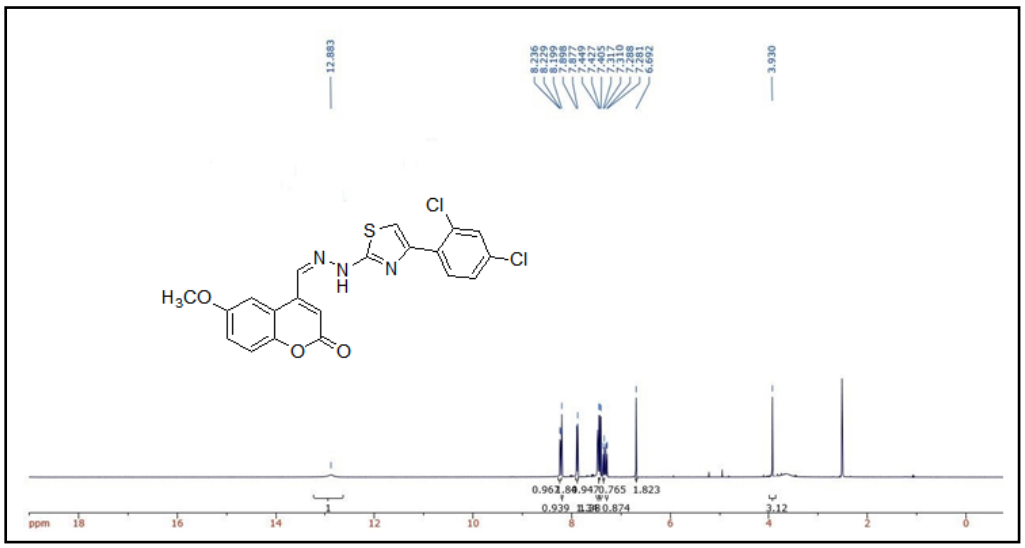
Spectrum No. 26: 1H NMR of compound **4h** in DMSO-d_6



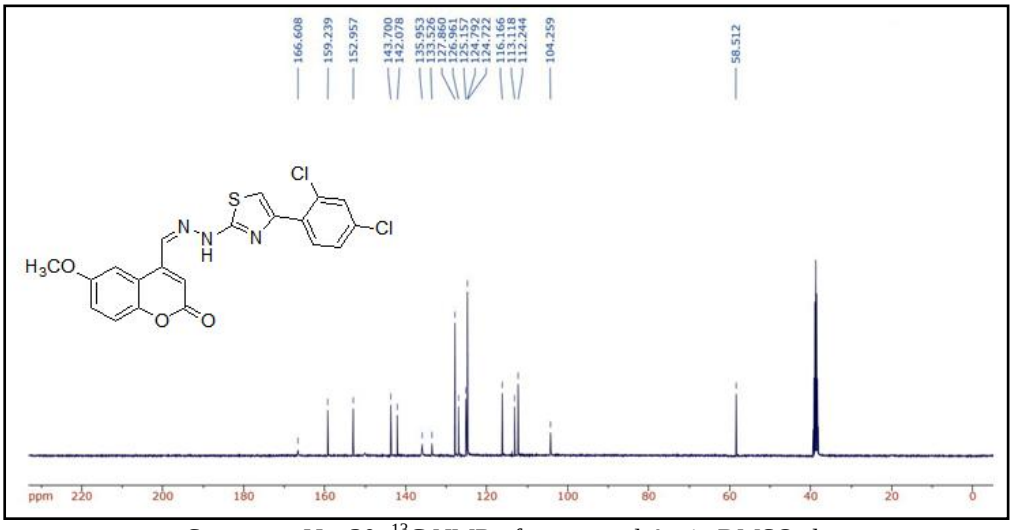
Spectrum No. 27: ^{13}C NMR of compound **4h** in DMSO-d_6



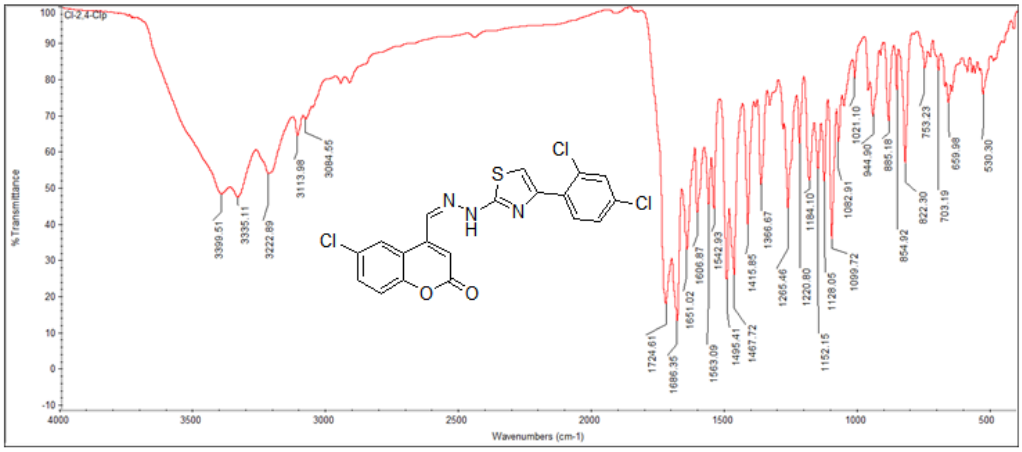
Spectrum No. 28: IR of compound **4m** in KBr



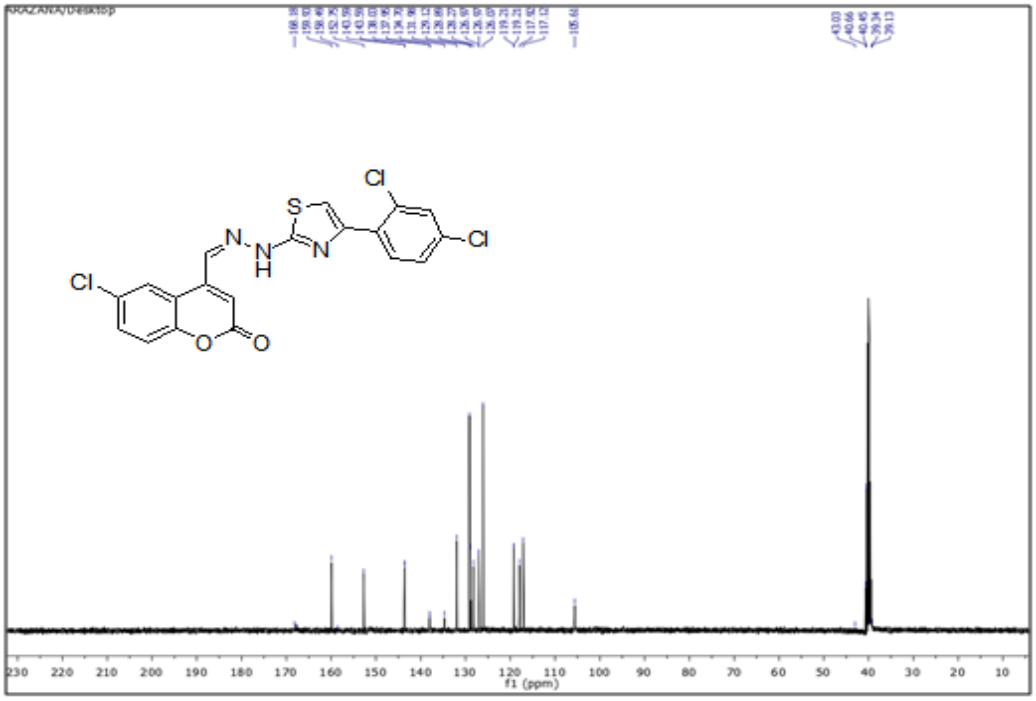
Spectrum No. 29: 1H NMR of compound **4m** in DMSO-d_6



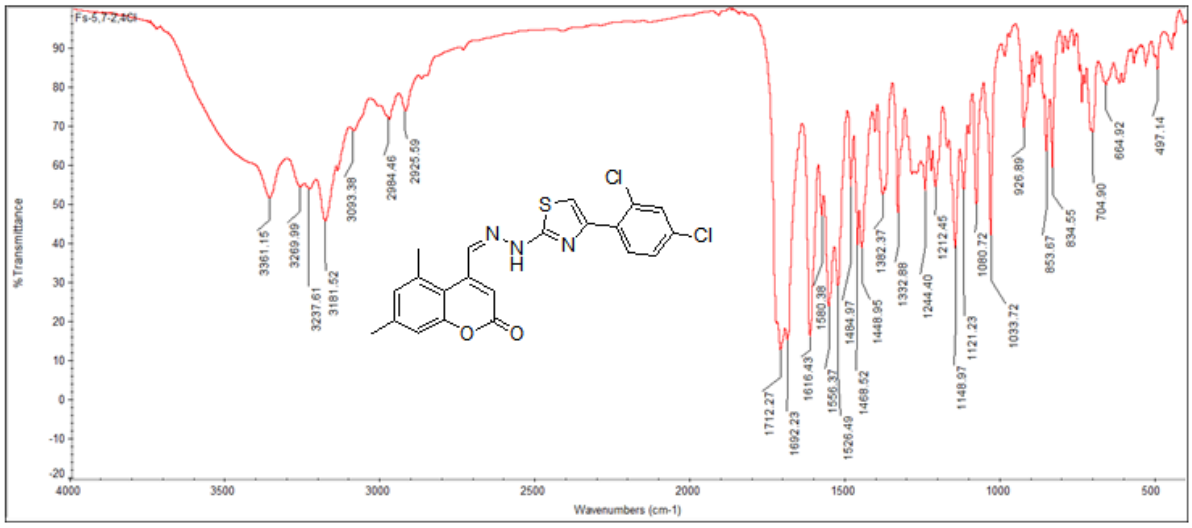
Spectrum No. 30: ^{13}C NMR of compound **4m** in DMSO-d_6



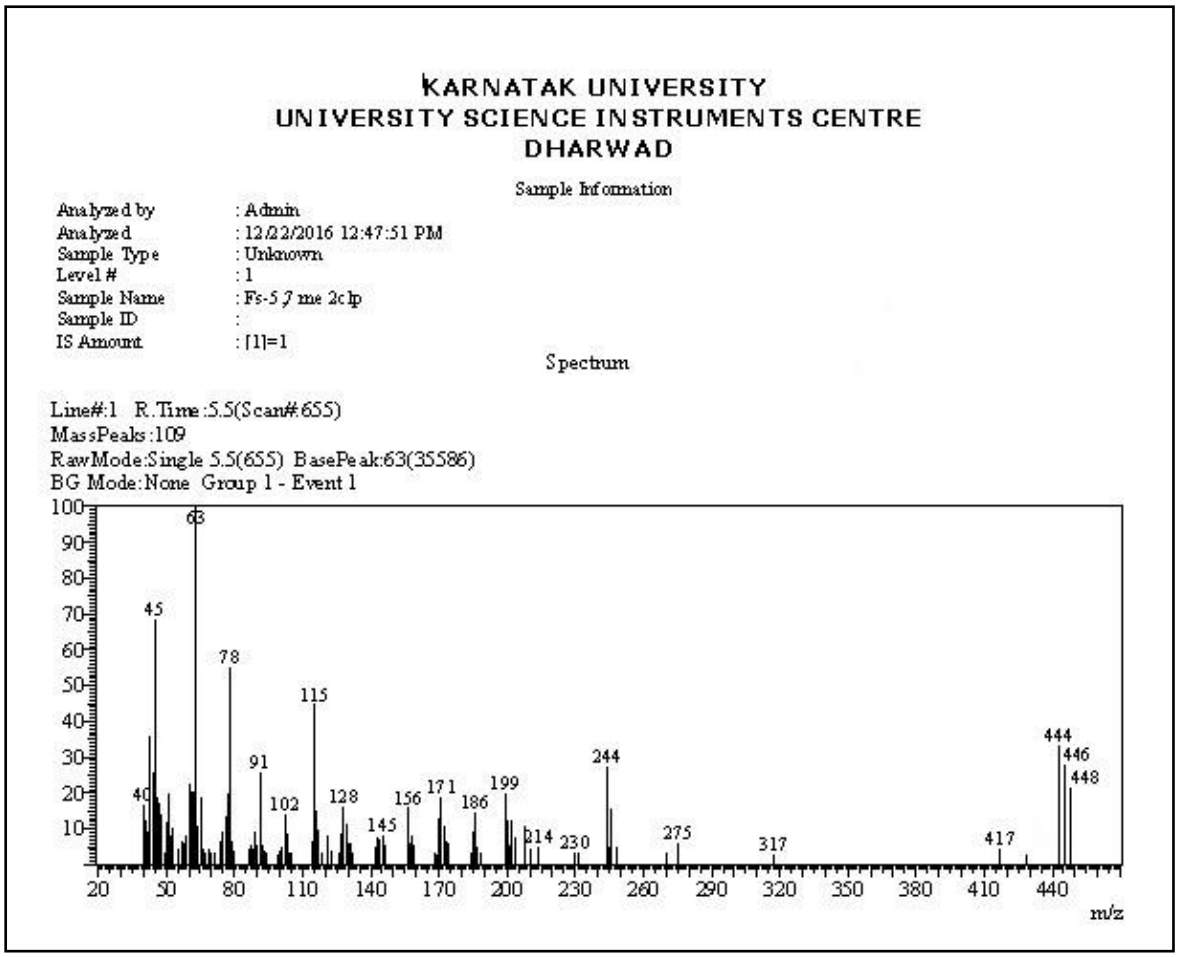
Spectrum No. 31: IR of compound **4n** in KBr



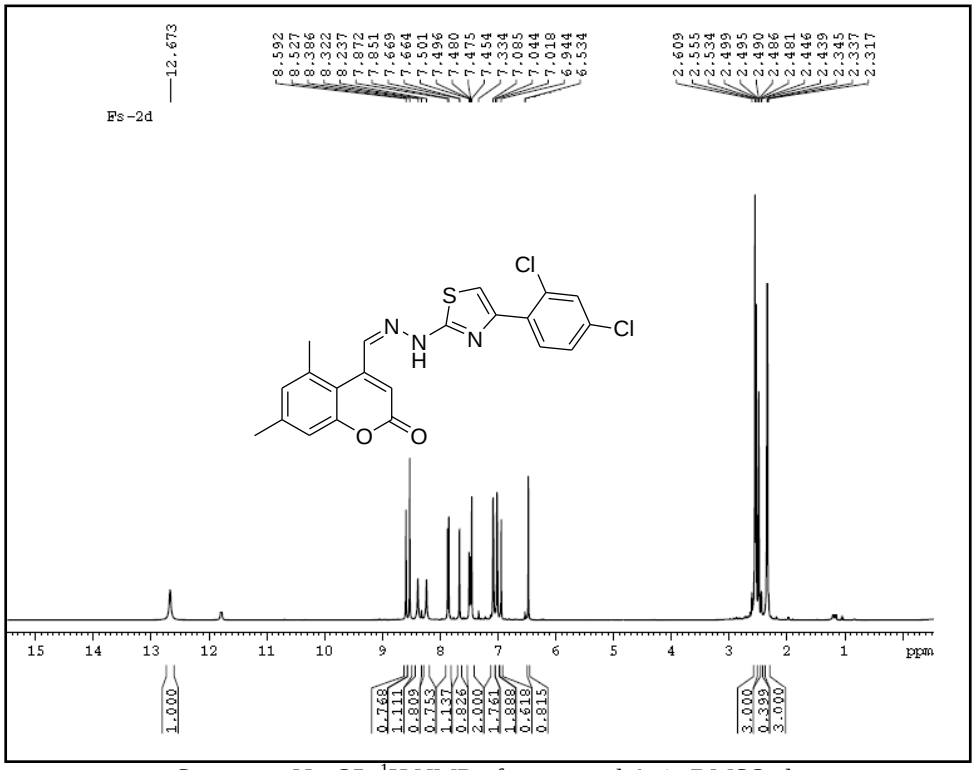
Spectrum No. 32: ^{13}C NMR of compound **4n** in DMSO-d_6



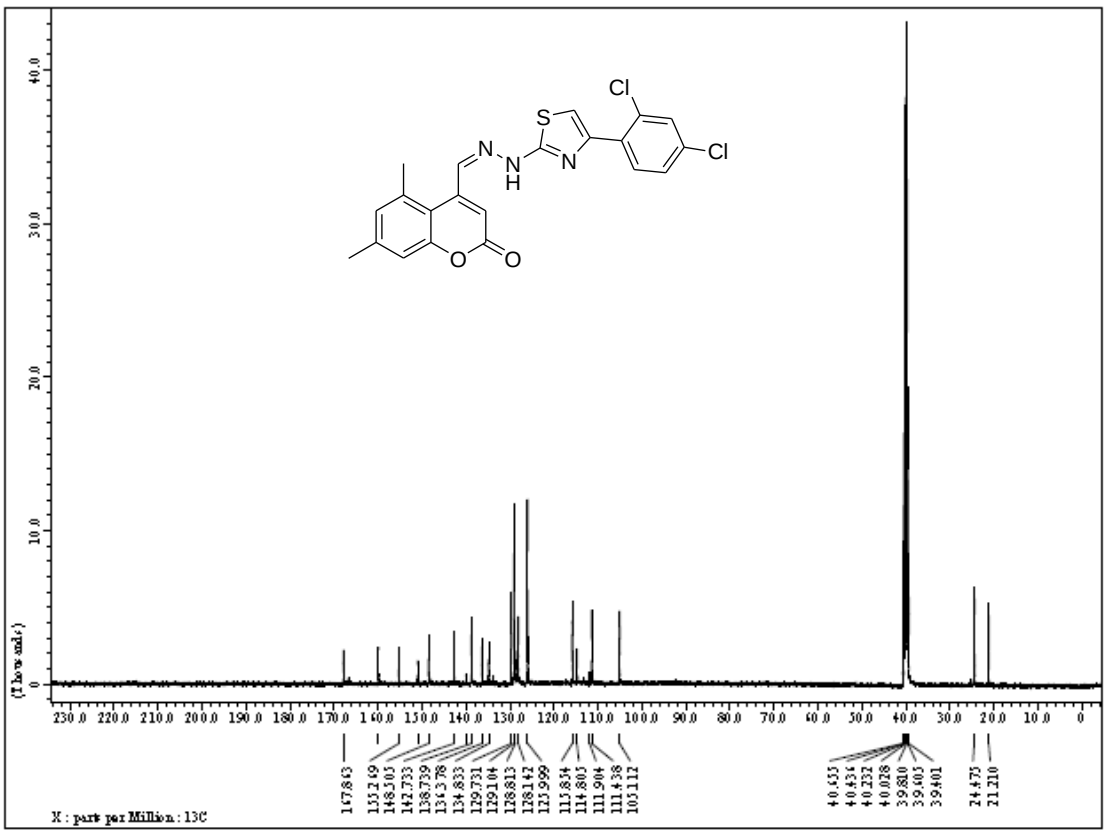
Spectrum No. 33: IR of compound **4o** in KBr



Spectrum No. 34: GM-MS of compound **4o**



Spectrum No. 35: ^{1}H NMR of compound **4o** in DMSO-d_6



Spectrum No. 36: ^{13}C NMR of compound **4o** in DMSO-d_6

(Dr. L. A. Shastri)" Synthesis and Characterization of Chlorophenyl- thiazolocoumarinyl Hydrazides as Promising Antimicrobial and Anti-Inflammatory Agents." IOSR Journal of Applied Chemistry (IOSR-JAC) 11.7 (2018): 09-39.