Chapter - 3b

Design and Synthesis of Piperazine Derivatives of Coumarin as Anticancer Agents

3b.1 Introduction

In the current landscape of anticancer research, several novel heterocyclic derivatives have emerged and among them, coumarin derivatives such as 7-amino coumarin and 3-aminocoumarin derivatives exhibit unique pharmacological characteristics and have also emerged as promising anticancer agents that make them important in the search of effective cancer treatments [1-2]. Literature studies have highlighted the potential of coumarin derivatives as promising candidates in the fight against cancer. These compounds exhibit cytotoxicity against various cancer cell lines and have shown promise in inhibiting tumor growth through mechanisms such as apoptosis induction, cell cycle arrest, and interference with angiogenesis [3-4]. Researchers are actively exploring the therapeutic potential of coumarin-based anticancer agents, aiming to develop innovative treatments that could provide new hope for cancer patients in the near future [5-7]. 7-amino coumarin derivatives have shown considerable potential due to their ability to target specific pathways involved in cancer progression. These derivatives have demonstrated selective cytotoxicity against cancer cells, making them attractive candidates for targeted therapies. Similarly, 3-aminocoumarin derivatives have garnered interest for their anticancer properties. They possess the ability to induce apoptosis, inhibit tumor growth, and disrupt angiogenesis, thereby impeding cancer progression [8-10]. These derivatives have been investigated extensively for their potential in combating various types of cancer. From our group, coumarin based compounds **1** and **2** have been reported as good anticancer agent against A549 and MCF-7 cell line and also studied their DPP-IV inhibition activity [11]. The pharmacological properties and applications of coumarins can be varied by varying type and position of substitution on coumarin rings like 6-amino coumarin and its derivatives have emerged as crucial compounds with diverse applications in various fields. Mahmoud et al., synthesized the amide derivatives of 6-amino coumarin **3** and **4** for their neurobehavioral investigation and acetylcholinesterase inhibitory activity [12]. According to literature studies 6-aminocoumarin has been less explored for its anticancer activity therefore in continuation of our work on synthesis of coumarin derivatives as an anticancer agent [13-14]. we have designed compounds containing 6-aminocoumarin attached to different substituted phenyl sulphonyl piperazines and phenyl piperazines and studied them for *in vitro* anticancer activity.

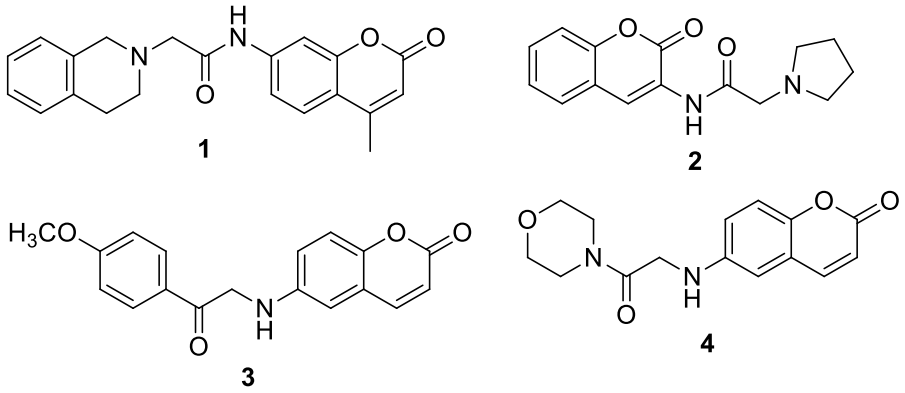
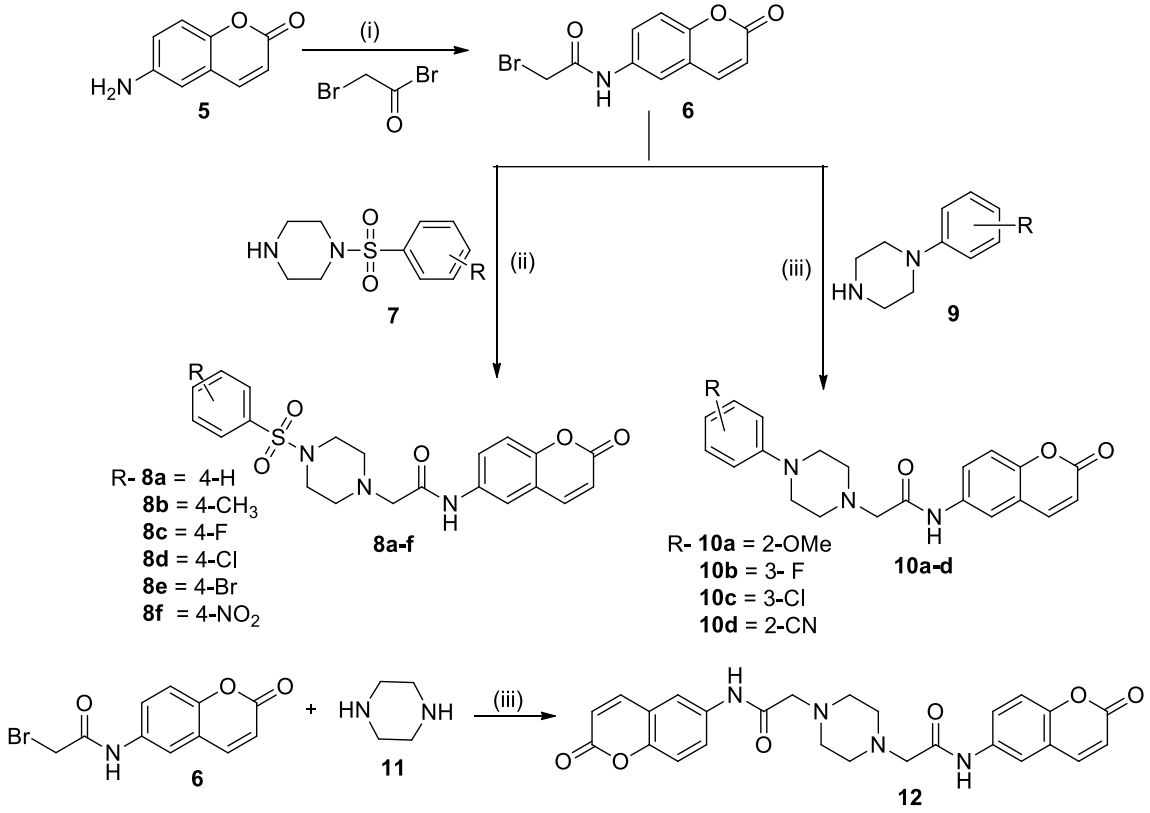


Figure- 3b.1 Aminocoumarin based acetamide derivatives

3b.2 Results and Discussion

3b.2.1 Chemistry

In search of some novel compounds with potent anticancer activity, first synthesis of 6-aminocoumarin **5** was carried out by using same procedure as discuss in **chapter 3a-1.2.** After synthesis of 6-aminocoumarin it reacts with bromoacetyl bromide to give 6-bromoacetamide coumarin **6** (**Scheme-1).** ^{1}H NMR of compound **6 (Fig. 3b.2.1)** showed singlet for two protons of methylene at δ 4.07 and aromatic protons were observed from δ 6.50-8.10 along with one NH proton at δ10.65. In ^{13}C NMR of compound **6 (Fig-3b.2.3)** methylene carbon is appeared at δ 29.26ppm and all aromatic carbons appeared from δ 117-151.08. Amide carbonyl carbon was observed at δ160.44 and lactone carbonyl carbon of coumarin appeared at 160.44ppm. Further reaction of 6-bromoacetamide coumarin **6** with different phenyl sulphonyl piperazines and phenyl piperazines using base in DMF gave desired 6-substituted aminocoumarin based acetamide derivatives **8a-f and 10a-d** and **12** (**Scheme 1**). All synthesized compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, ESI-MS, IR.



Reagents and Conditions- (i) TEA, DCM,RT, 2hrs. (ii)/(iii) TEA, DMF, RT, 4-6hrs.

Scheme- 3b.1 Piperazine derivatives of 6-amino coumarin **8a-f** and **10a-d** and **11**

IR spectra of compounds **8a-f (Fig.-3b.3.1-Fig.3b.8.4)** exhibited N-H stretching frequency of amide range from 3215 -3288 cm^{-1}, -CH stretching observed in the range of 2830-3092. Lactone carbonyl group of coumarin stretching frequency ranges from 1701 to 1737 cm^{-1} and amide stretching frequency was observed in the range of 1615 to 1678 cm^{-1}. ^{1}H-NMR spectrum of compound **8a-f** showed piperazine protons in the range of δ 2.73 to 3.07 as a broad singlet. Methylene protons adjacent to amide carbonyl observed in the range of δ 3.21 to δ 3.28 and all aromatic protons were observed in range of δ 6.41 to 7.99 ppm. One -NH proton was appeared in the range of δ 8.84-9.87 ppm. In ^{13}C-NMR of compound **8a-f** showed peaks of aliphatic carbons in the region of δ 45.36-61.58 ppm of piperazine carbon and methylene carbon and all aromatic carbons appeared from δ 116.57 to 150.00 ppm. Amide carbonyl carbon observed in the range of δ 159.84 to 160.61 ppm. while carbonyl carbon of coumarin lactone ring at δ 167.67-168.26 ppm.

For compound **10a-d,** and **12** IR spectrum **(Fig.-3b.9.1 to 3b.13.4)** exhibited one strong band for -NH proton in the range of 3288-to 3539 cm^{-1}. carbonyl group of coumarin lactone was observed in the rage of 1719 to 1730cm^{-1} and amide protons observed in the range of δ 1618 to δ 1684 cm^{-1}. In ^{1}H-NMR of compounds **10a-d** and **12** aliphatic protons were observed from

δ 2.82- δ3.90 of piperzine and methylene protons. All aromatic protons for compounds **10a-d** and **12** observed in the range of δ 6.47 to δ 7.73, along with one –NH protons at δ 9.26-9.26 ppm. ^{13}C-NMR of **10a-d** and **12** aliphatic carbons were observed in the range of δ 48.99-61.95 ppm for piperazine and methylene carbon. All aromatic carbons observed in the range of δ 106.29-151.96. Amide carbonyl carbon observed in the range of δ 159.86-160.68, while carbonyl carbon of coumarin lactone ring was appeared at δ 168.26-168.68 ppm.

Figure- 3b.2.1 IR of 2-bromo-N-(2-oxo-2H-chromen-6-yl)acetamide **(6)**

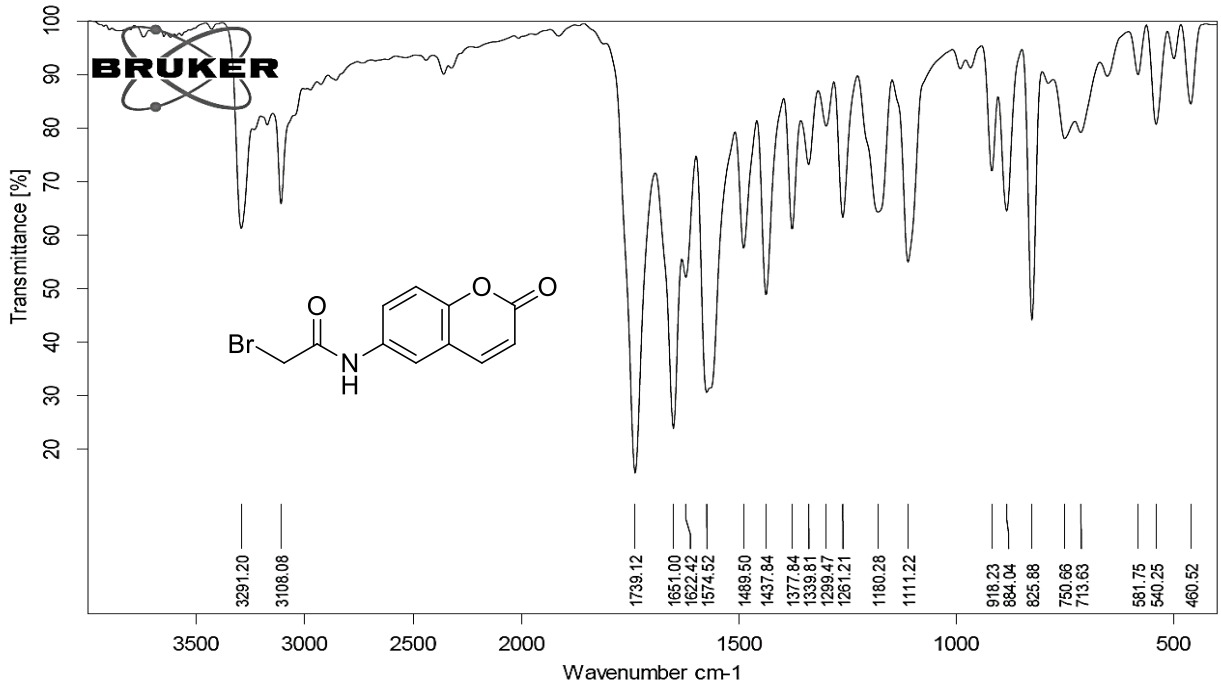


Figure- 3b.2.2 ^{1}H-NMR of 2-bromo-N-(2-oxo-2H-chromen-6-yl)acetamide **(6)**

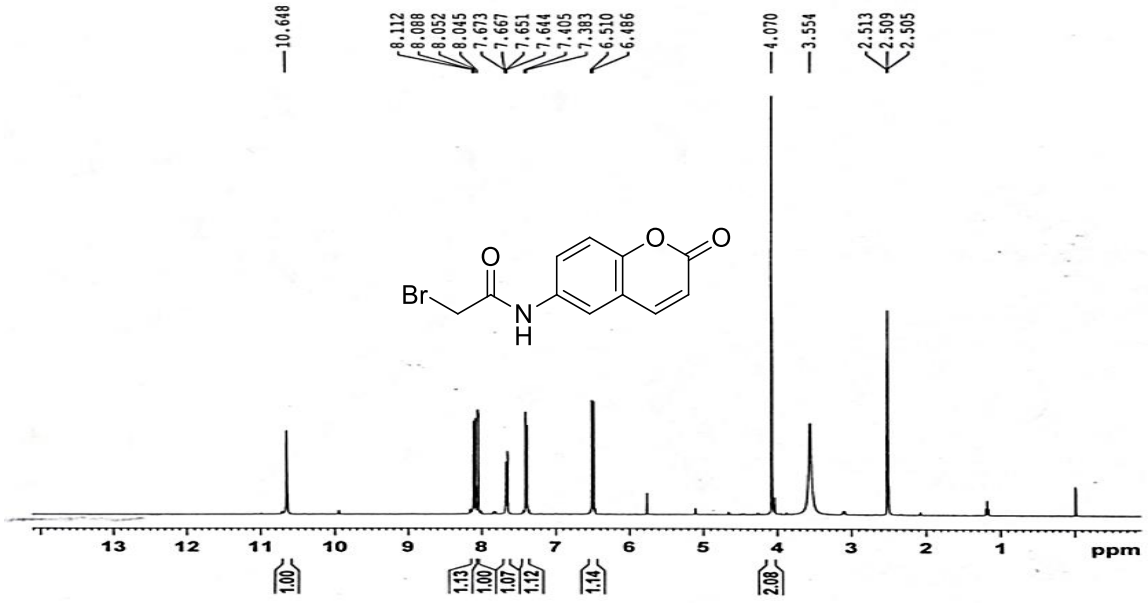


Figure- 3b.2.3 ^{13}C-NMR of 2-bromo-N-(2-oxo-2H-chromen-6-yl)acetamide **(6)**

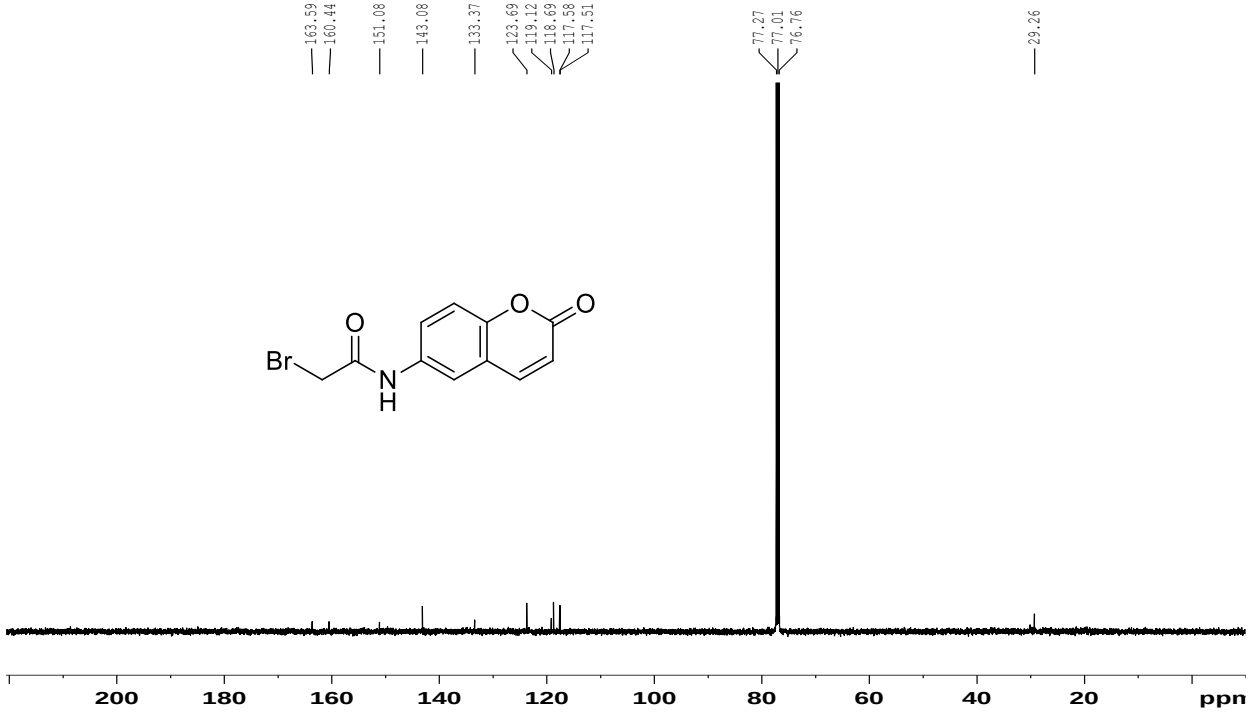


Figure- 3b.2.4 Mass of 2-bromo-N-(2-oxo-2H-chromen-6-yl)acetamide **(6)** M+ peak at 280.1

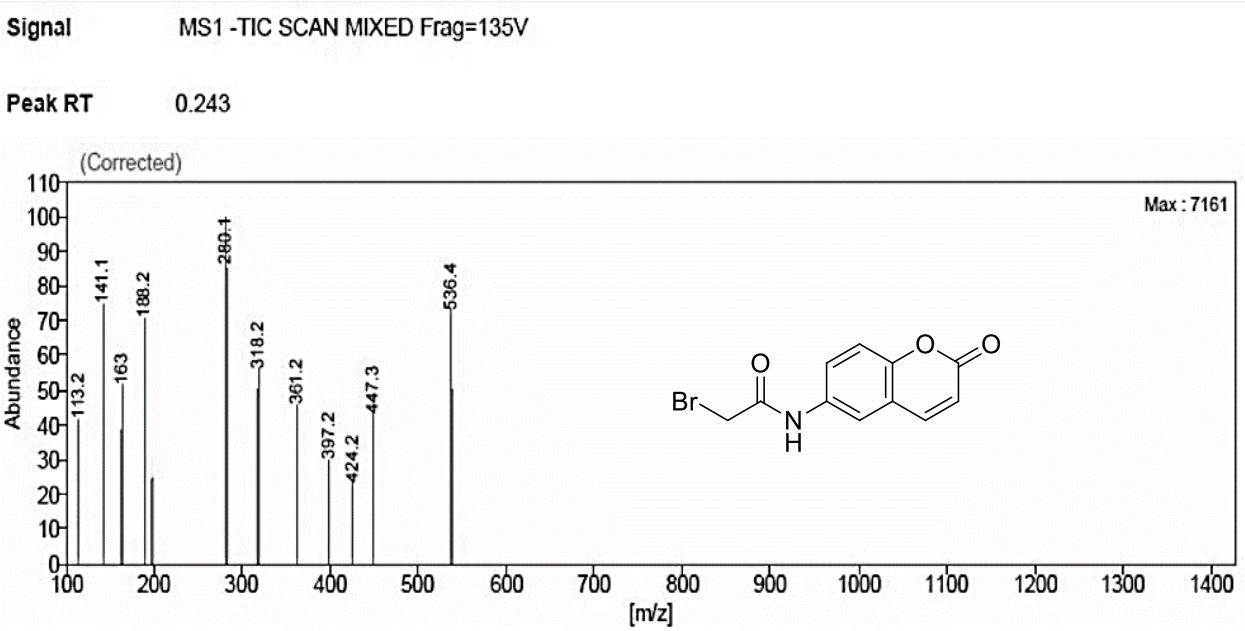


Figure- 3b.3.1 IR of N-(2-oxo-2H-chromen-6-yl)-2-(4-(phenylsulfonyl)piperazin-1-yl)acetamide **(8a)**

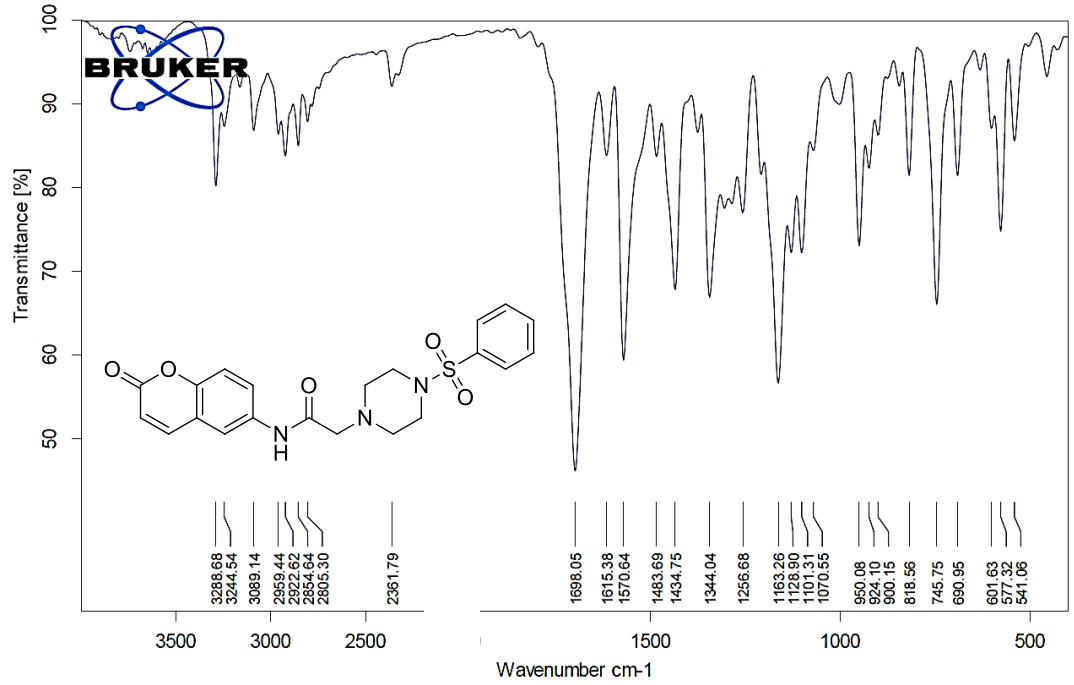


Figure-

Figure- 3b.3.2 ^{1}H-NMR of N-(2-oxo-2H-chromen-6-yl)-2-(4-(phenylsulfonyl)piperazin-1-yl)acetamide **(8a)**

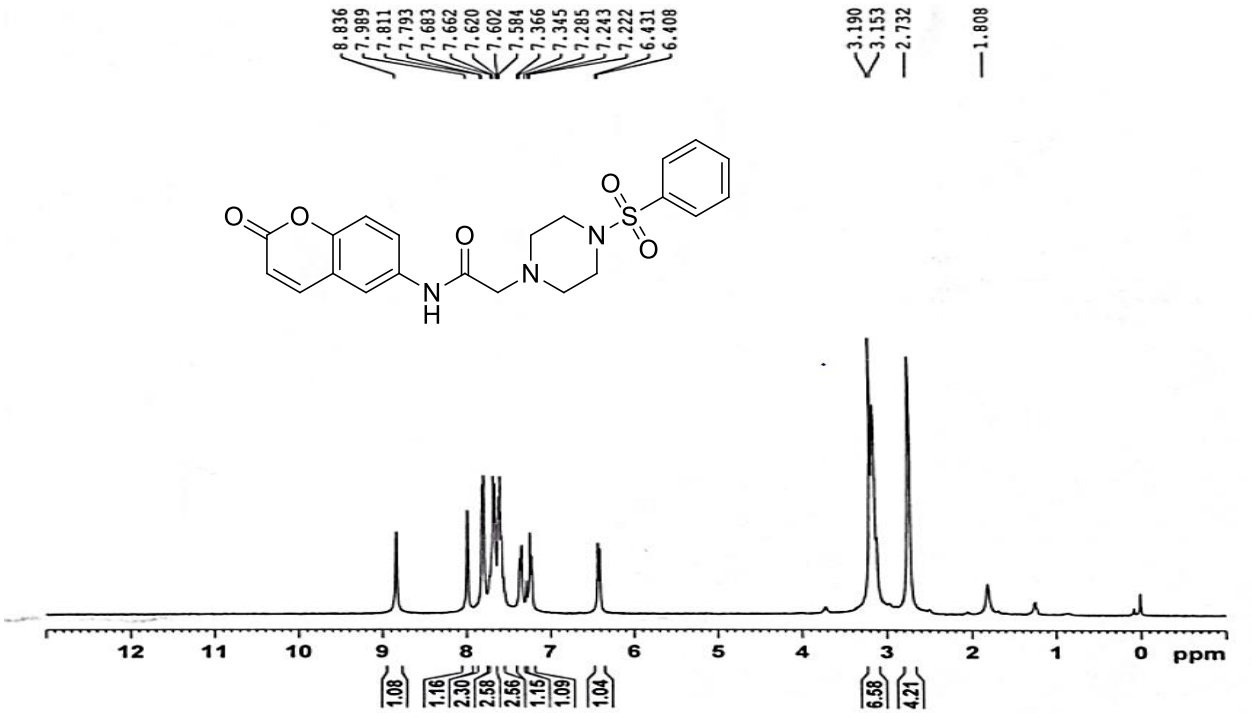


Figure- 3b.3.3 ^{13}C-NMR of N-(2-oxo-2H-chromen-6-yl)-2-(4-(phenylsulfonyl)piperazin-1-yl)acetamide **(8a)**

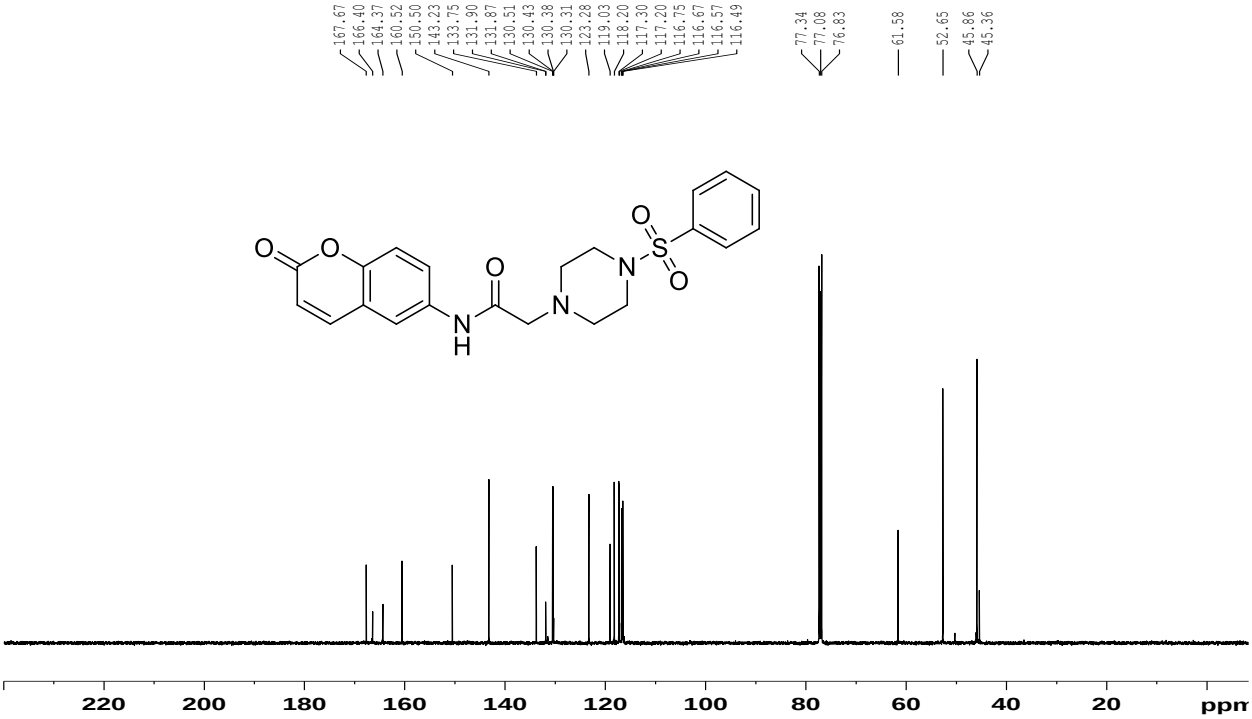


Figure- 3b.3.4 Mass of N-(2-oxo-2H-chromen-6-yl)-2-(4-(phenylsulfonyl)piperazin-1-yl)acetamide **(8a)** M+H peak at 428.3

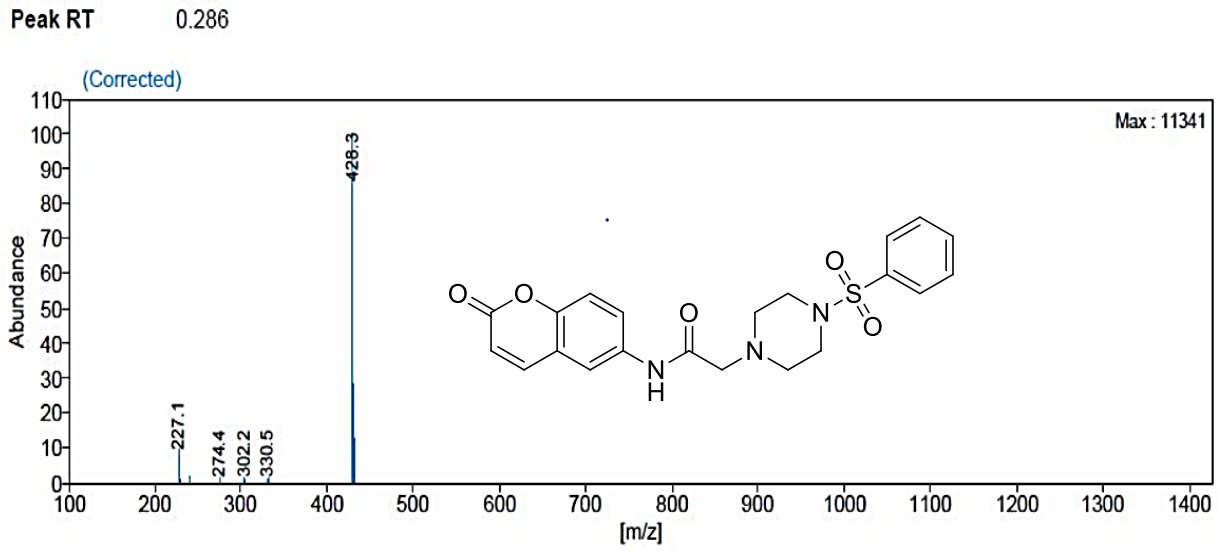


Figure- 3b.4.1 IR of N-(2-oxo-2H-chromen-6-yl)-2-(4-tosylpiperazin-1-yl)acetamide **(8b)**

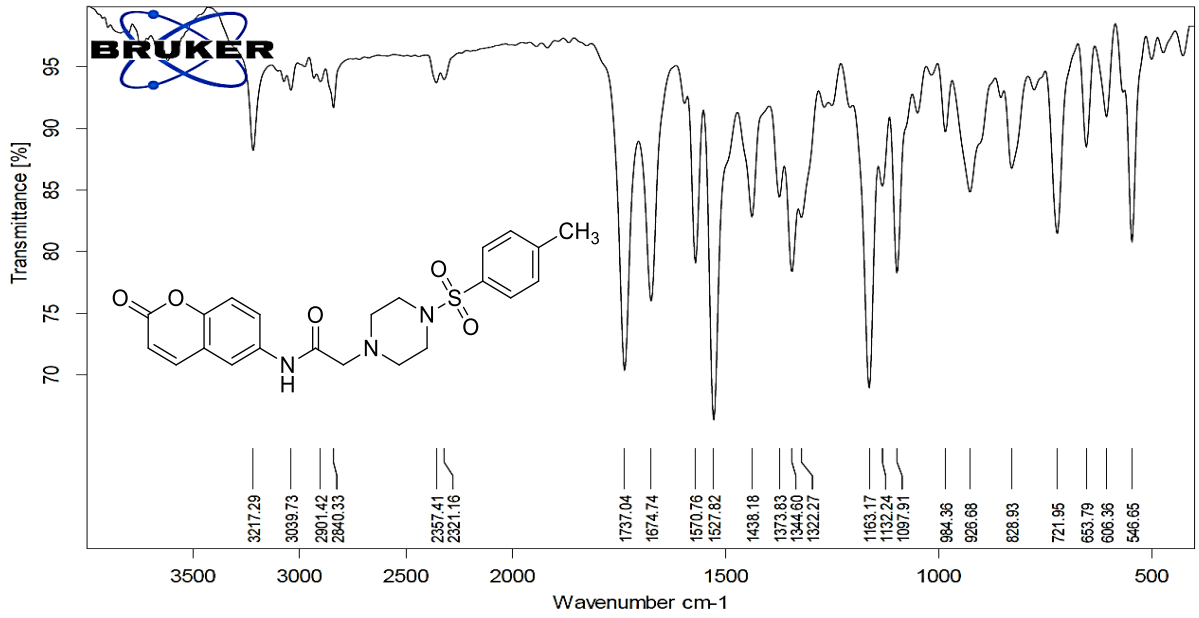


Figure- 3b.4.2 ^{1}H-NMR of N-(2-oxo-2H-chromen-6-yl)-2-(4-tosylpiperazin-1-yl)acetamide **(8b)**

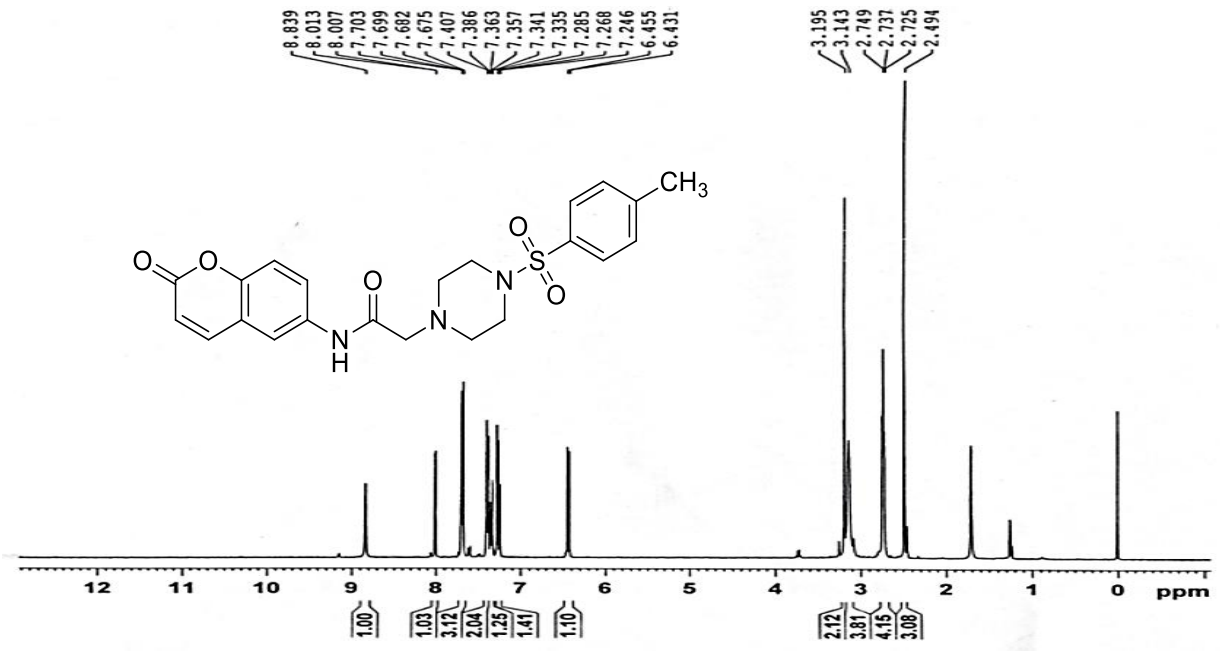


Figure- 3b.4.3 ^{13}C-NMR of N-(2-oxo-2H-chromen-6-yl)-2-(4-tosylpiperazin-1-yl)acetamide **(8b)**

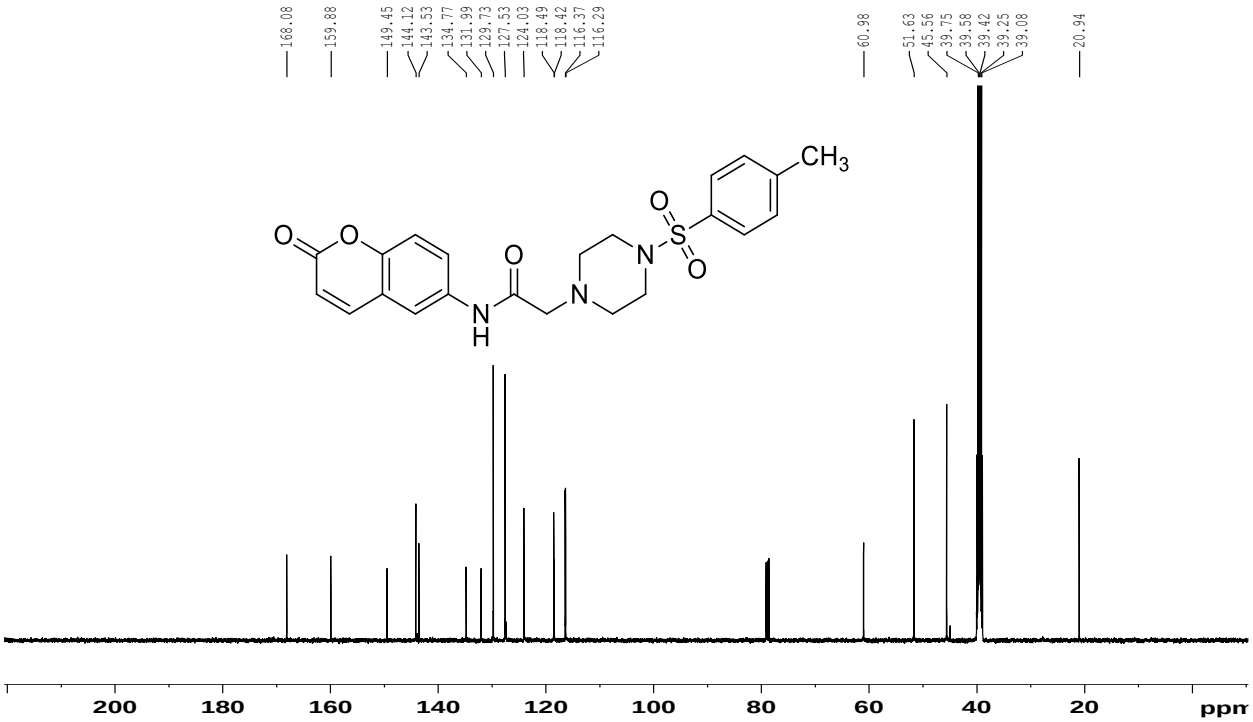


Figure- 3b.4.4 Mass of N-(2-oxo-2H-chromen-6-yl)-2-(4-tosylpiperazin-1-yl)acetamide **(8b)** M+H peak at 442.4

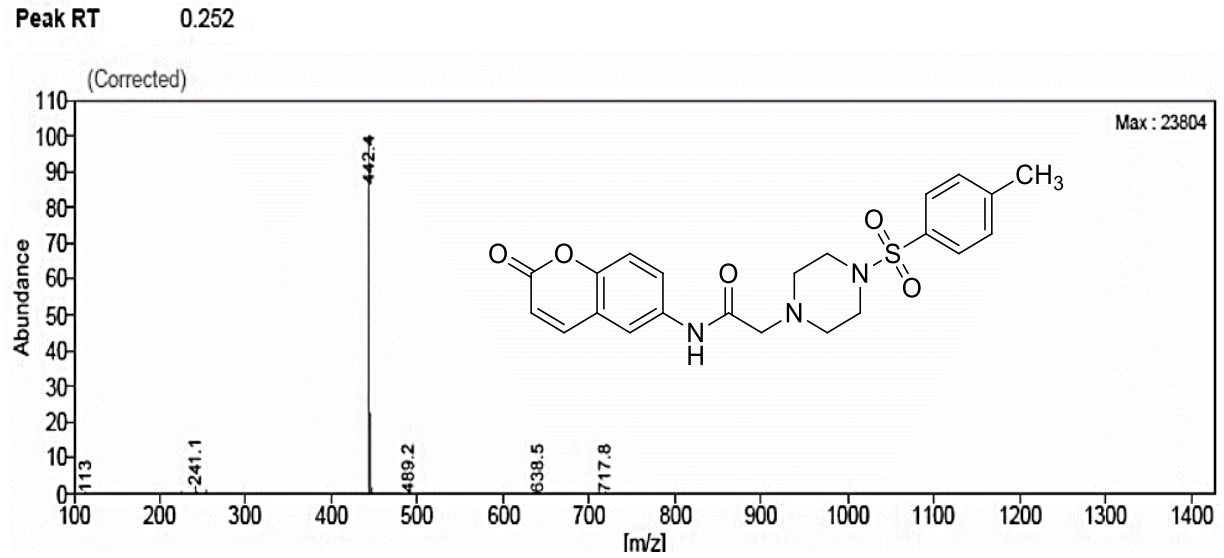


Figure- 3b.5.1 IR of 2-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8c)**

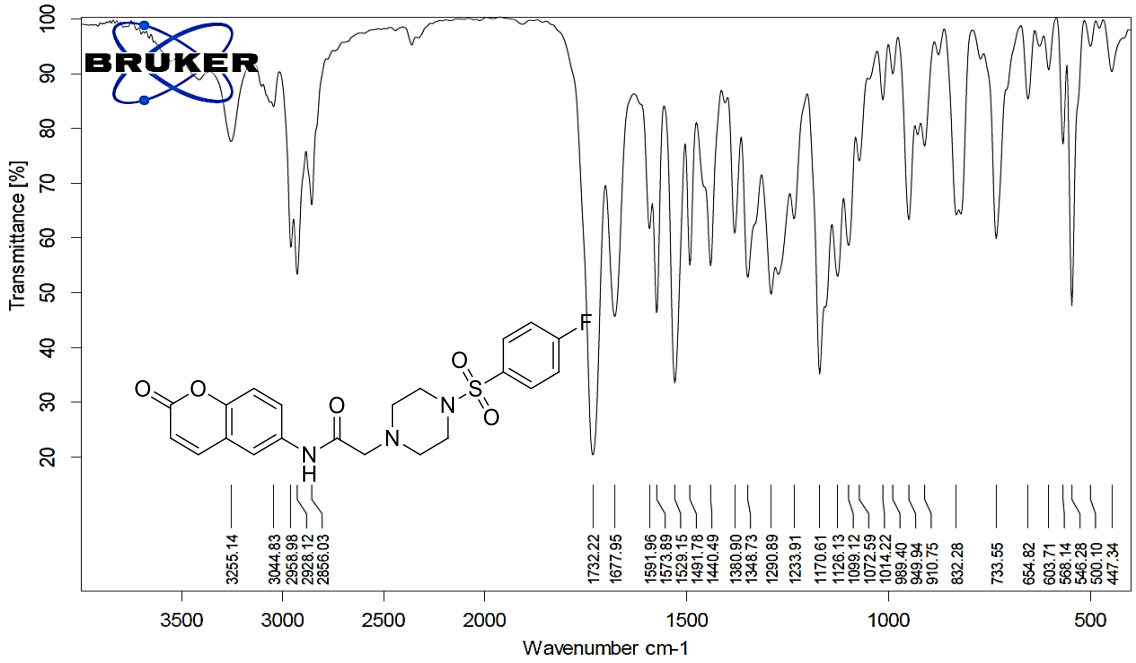


Figure- 3b.5.2 ^{1}H-NMR of 2-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8c)**

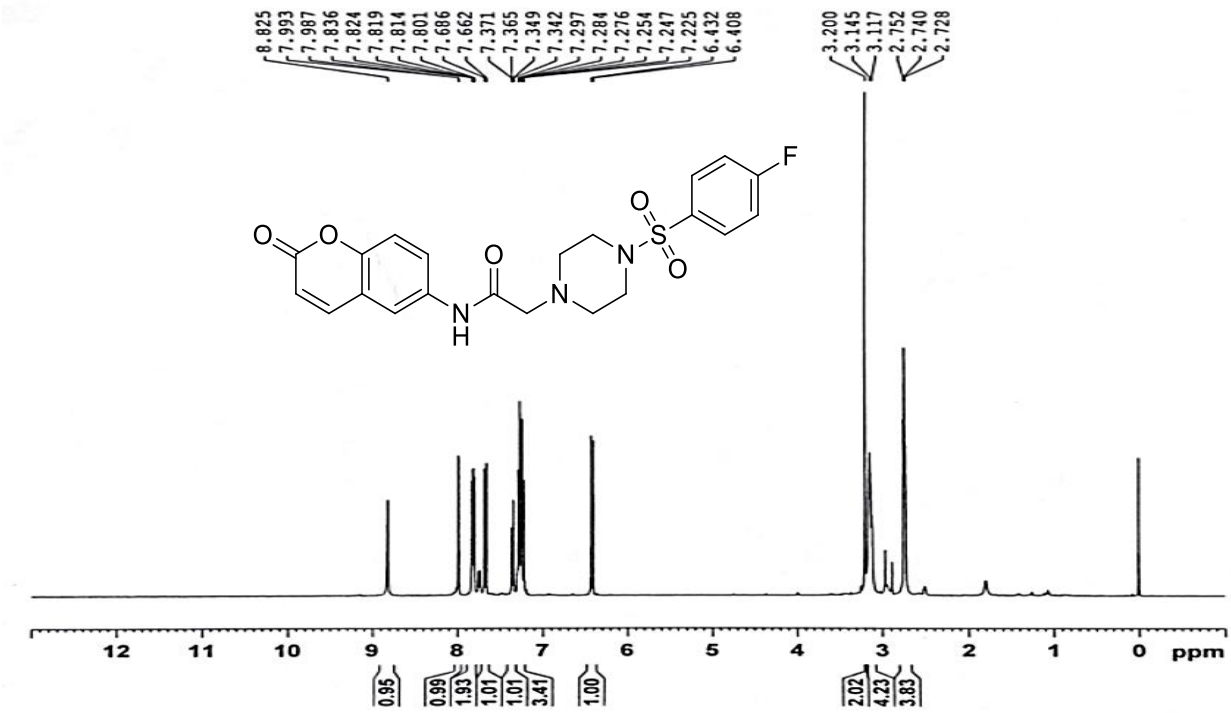


Figure- 3b.5.3 ^{13}C-NMR of 2-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8c)**

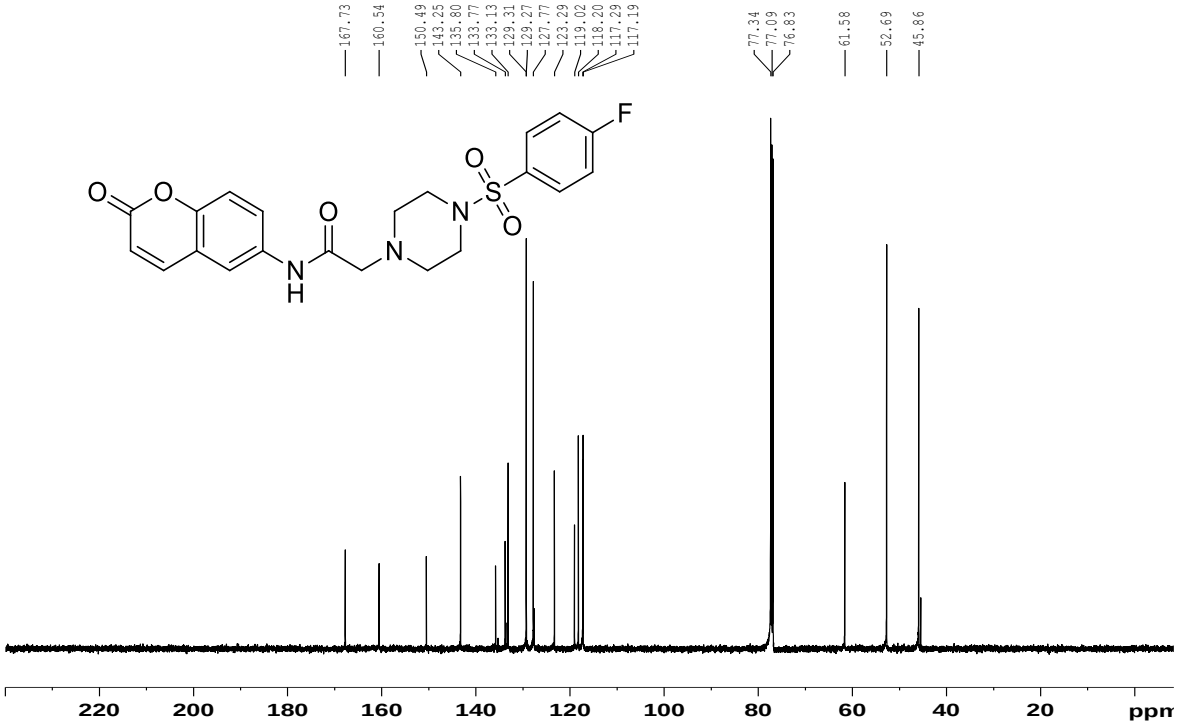


Figure- 3b.5.4 Mass of 2-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide (8c) M+H peak at 446.4

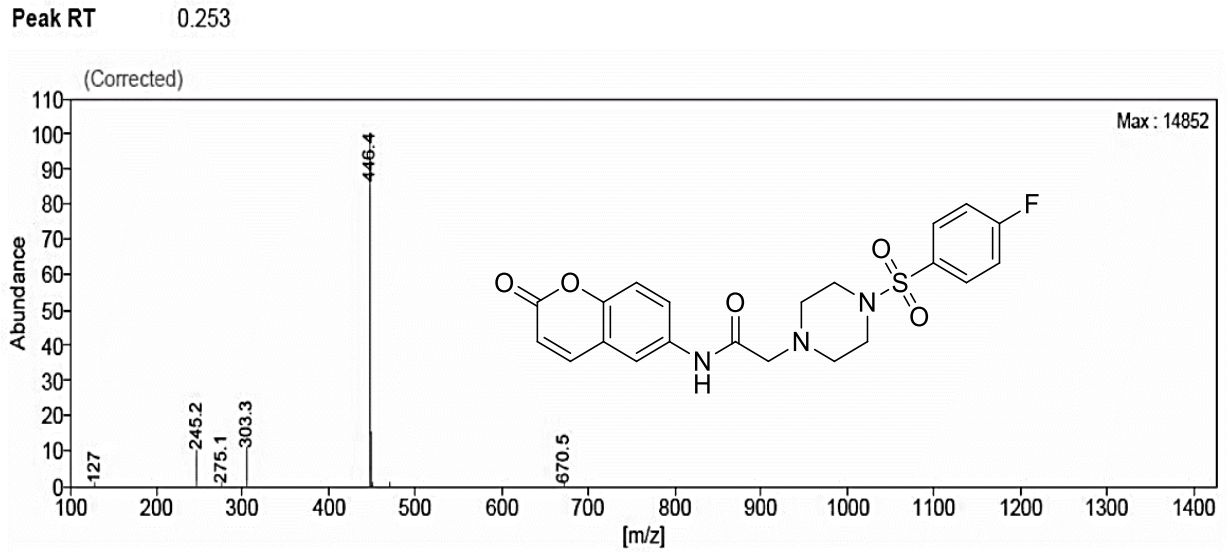


Figure- 3b.6.1 IR of 2-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8d)**

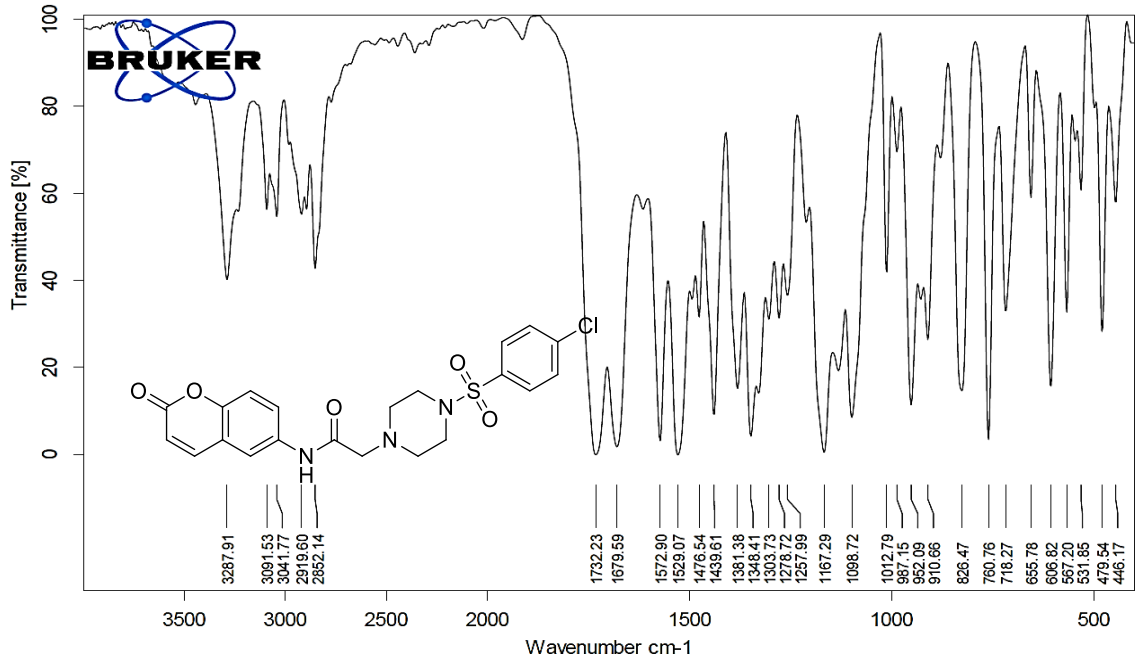


Figure- 3b.6.2 ^{1}H-NMR of 2-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8d)**

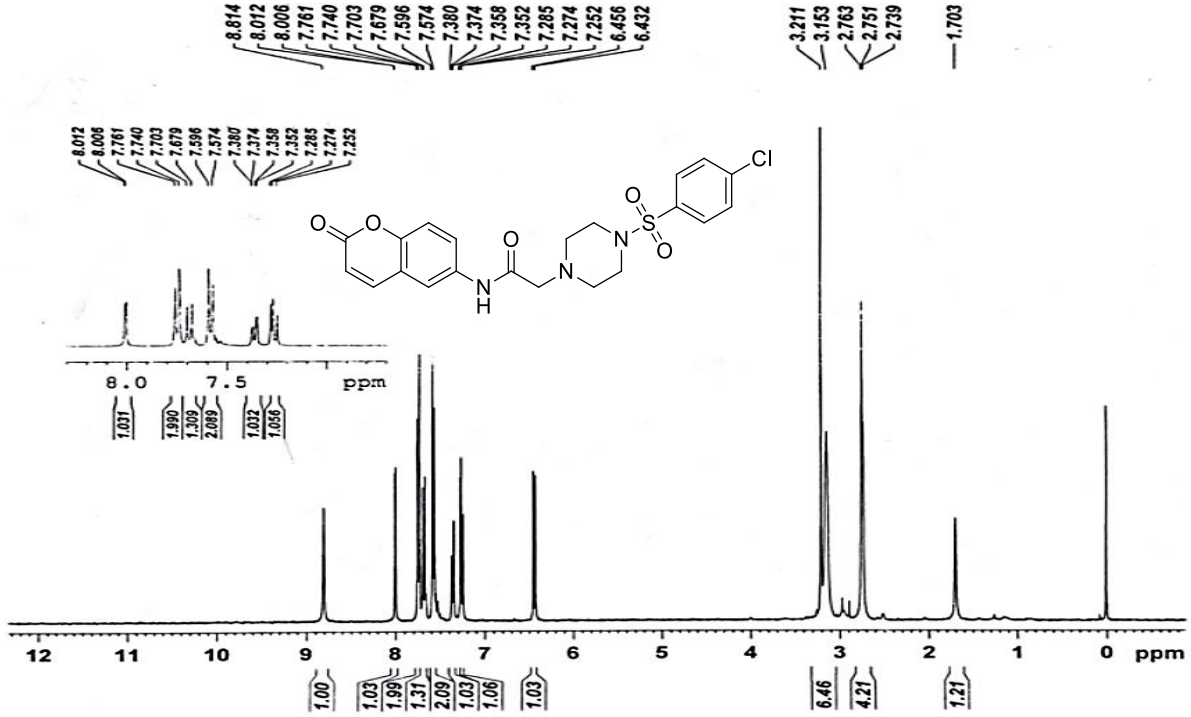


Figure- 3b.6.3 ^{13}C-NMR of 2-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8d)**

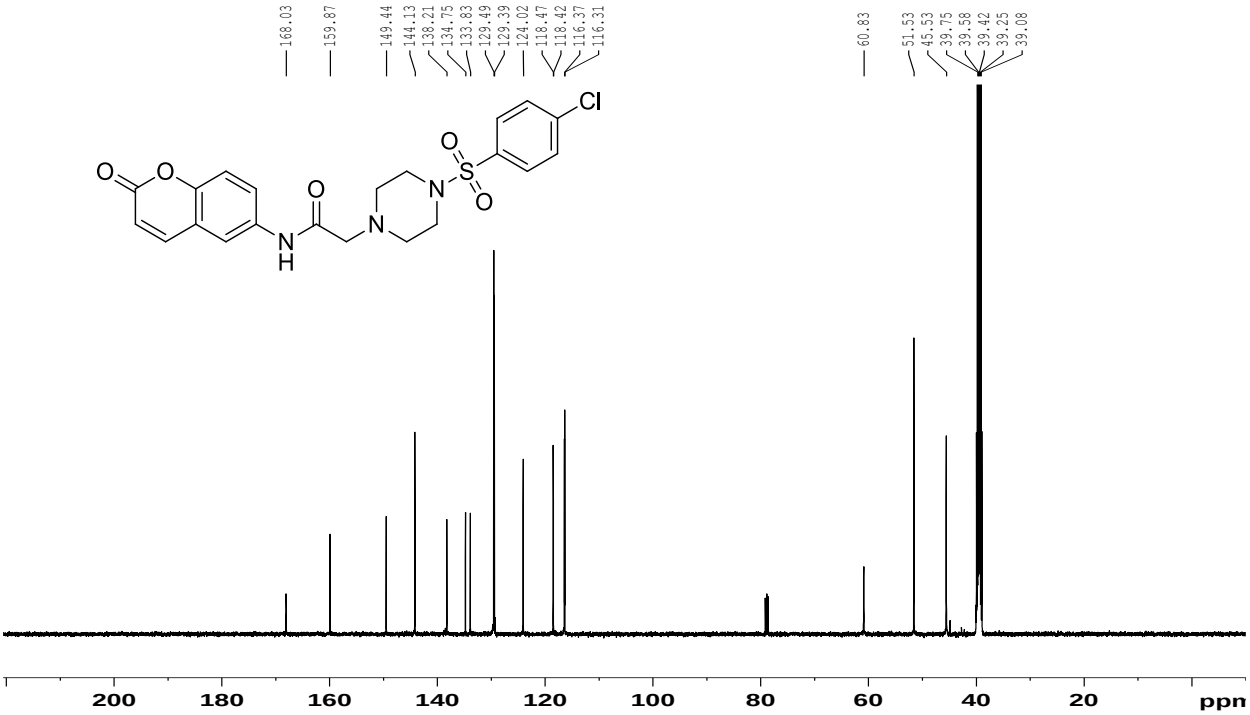


Figure- 3b.6.4 Mass of 2-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8d)** M+H peak at 462.3

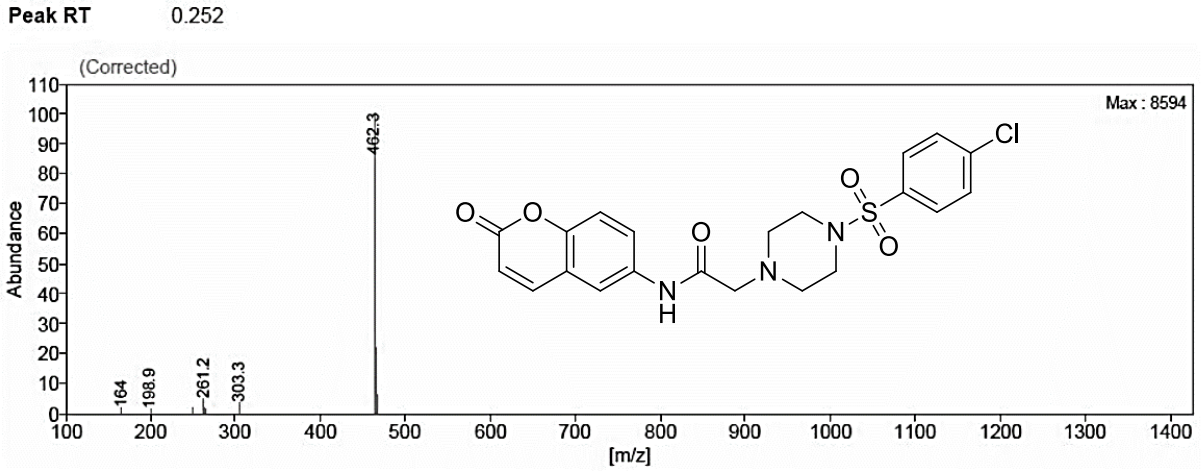


Figure- 3b.7.1 IR of 2-(4-((4-bromophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8e)**

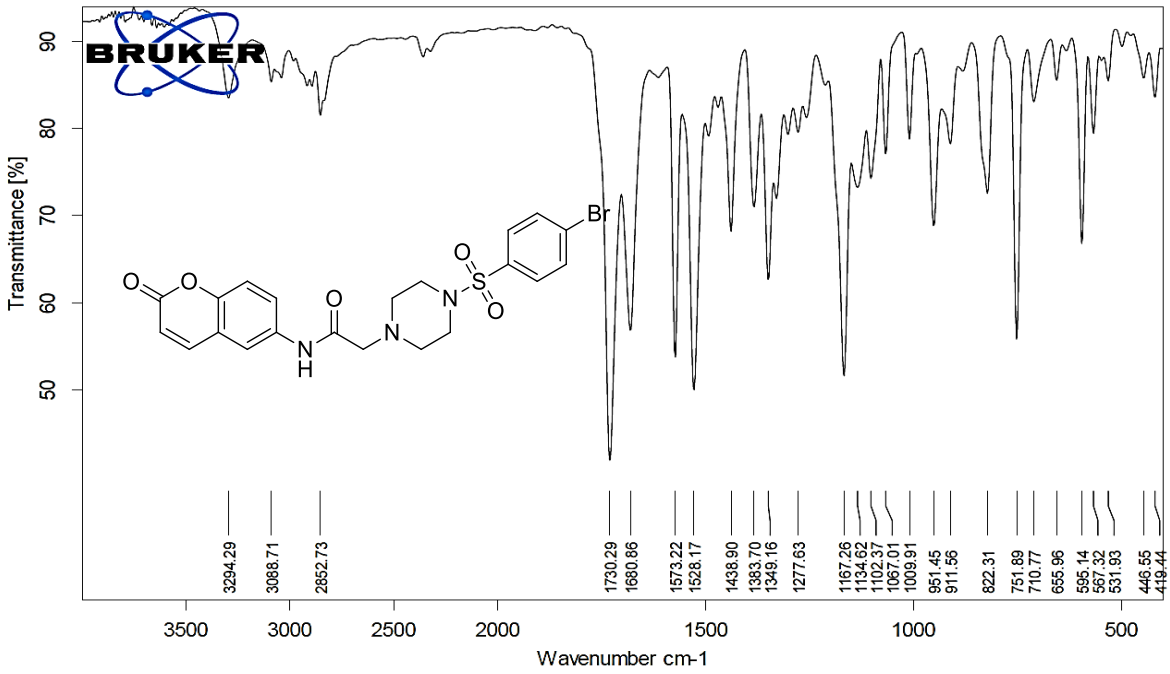


Figure- 3b.7.2 ^{1}H-NMR of 2-(4-((4-bromophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8e)**

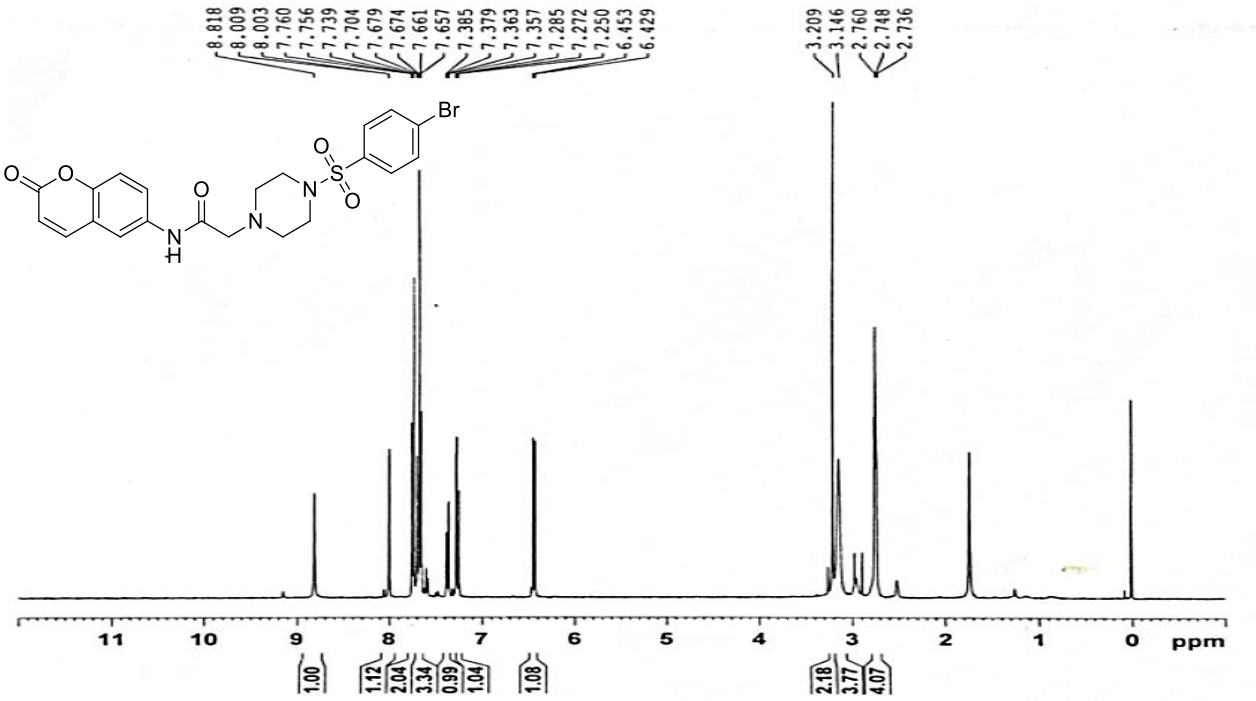


Figure- 3b.7.3 ^{13}C-NMR of 2-(4-((4-bromophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8e)**

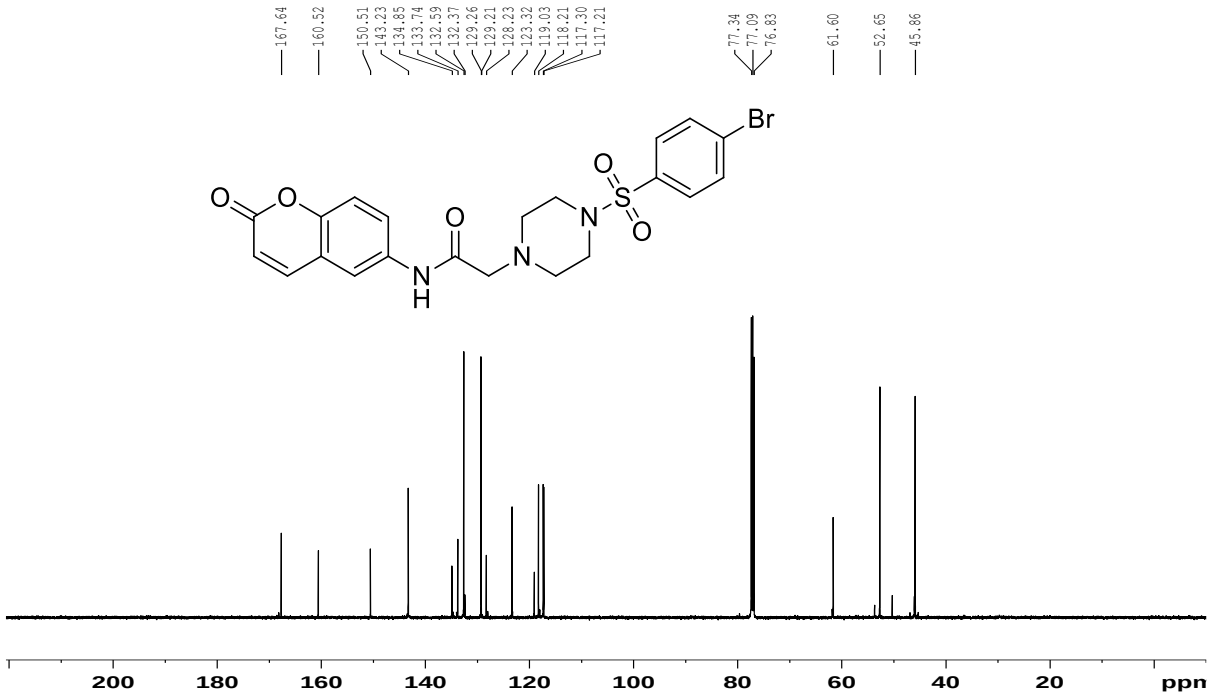


Figure- 3b.8.1 IR of 2-(4-((4-nitrophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8f)**

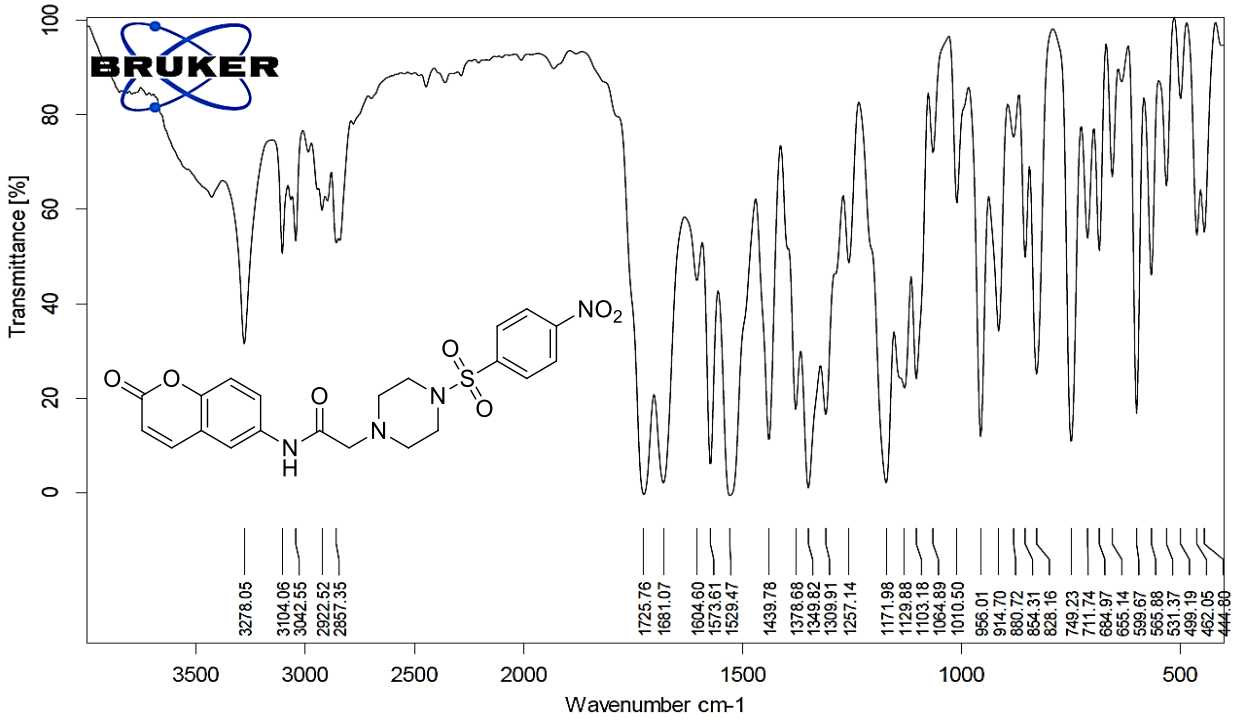


Figure- 3b.8.2 ^{1}H-NMR of 2-(4-((4-nitrophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8f)**

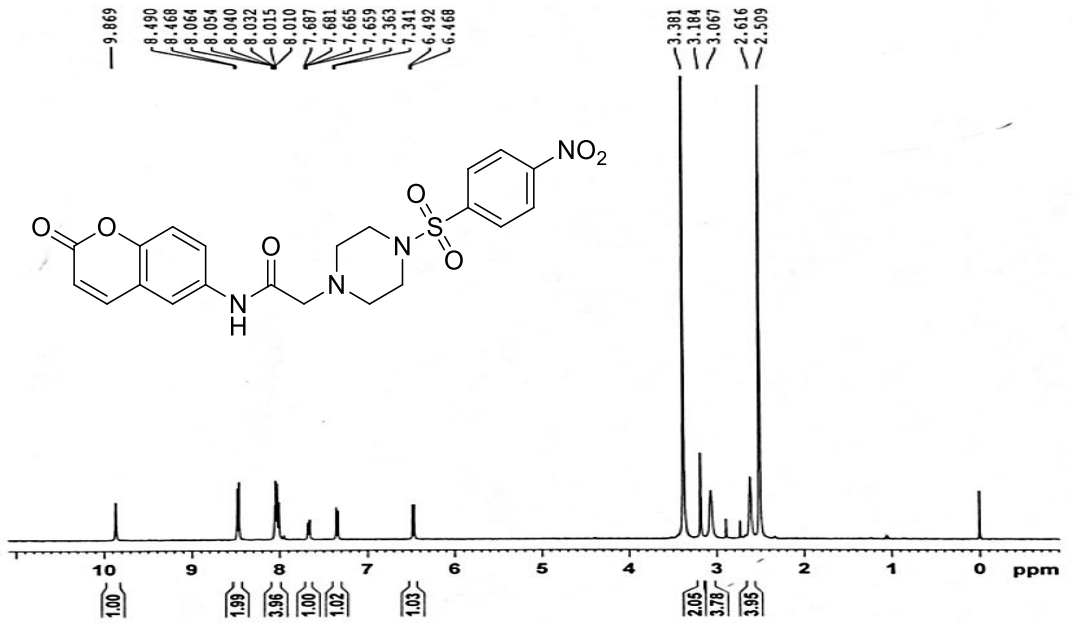


Figure- 3b.8.3 ^{13}C-NMR of 2-(4-((4-nitrophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8f)**

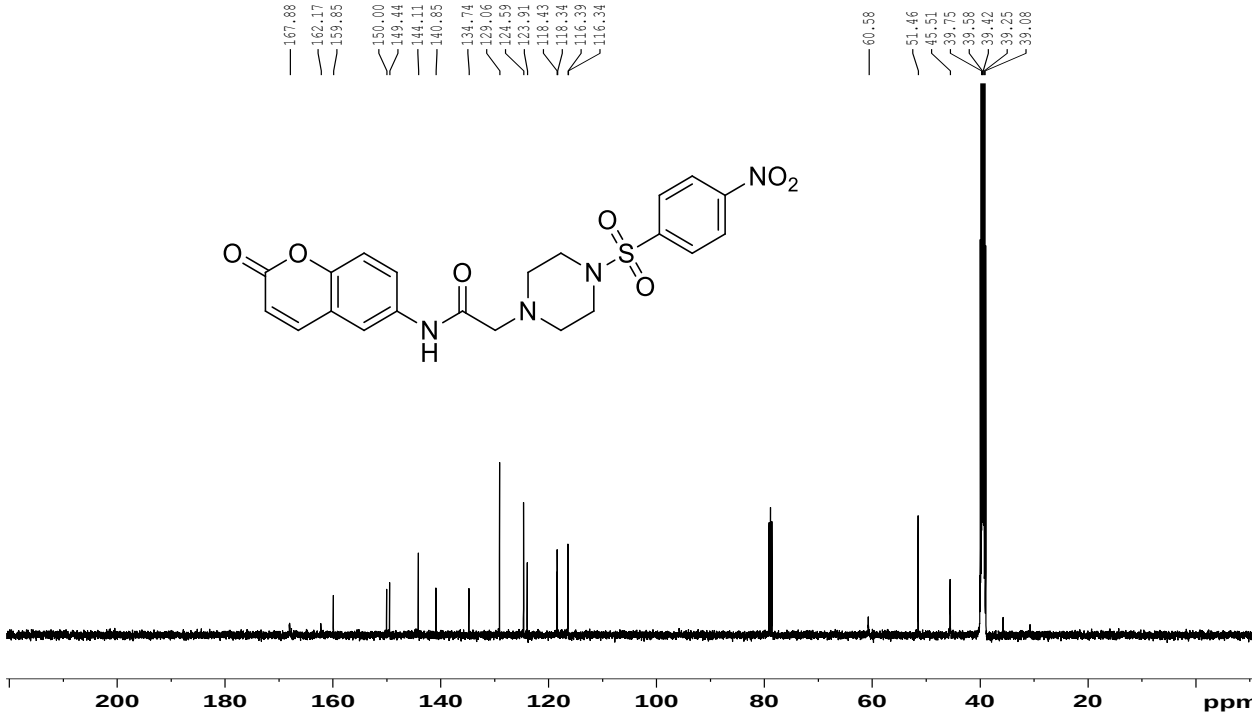


Figure- 3b.8.4 Mass of 2-(4-((4-nitrophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(8f)** M+H peak at 473.3

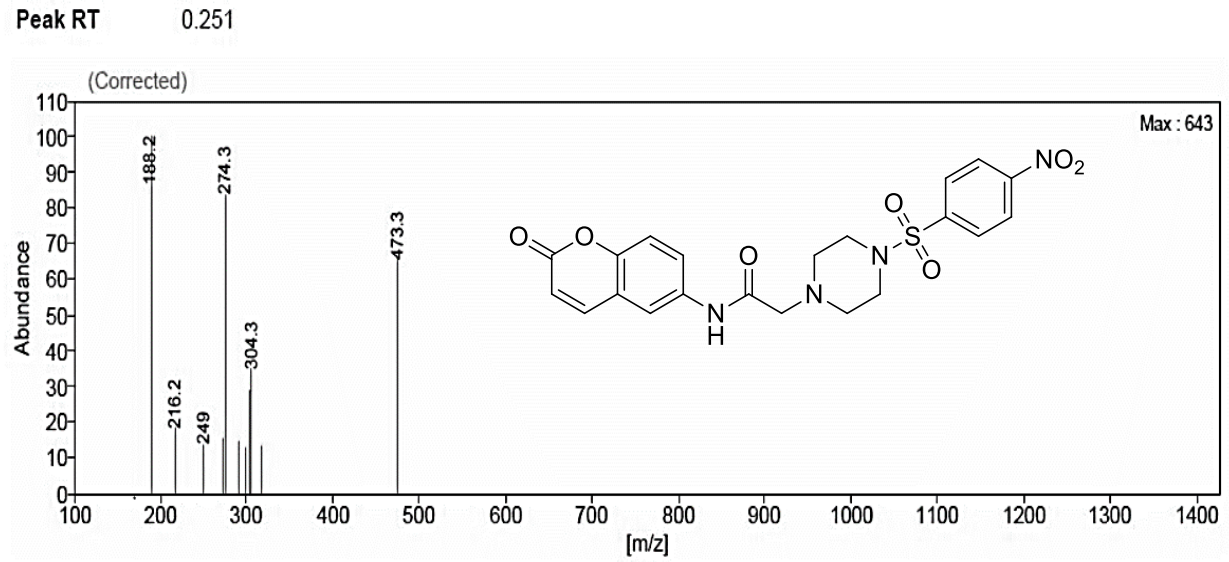


Figure- 3b.9.1 IR of 2-(4-(2-methoxyphenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(10a)**

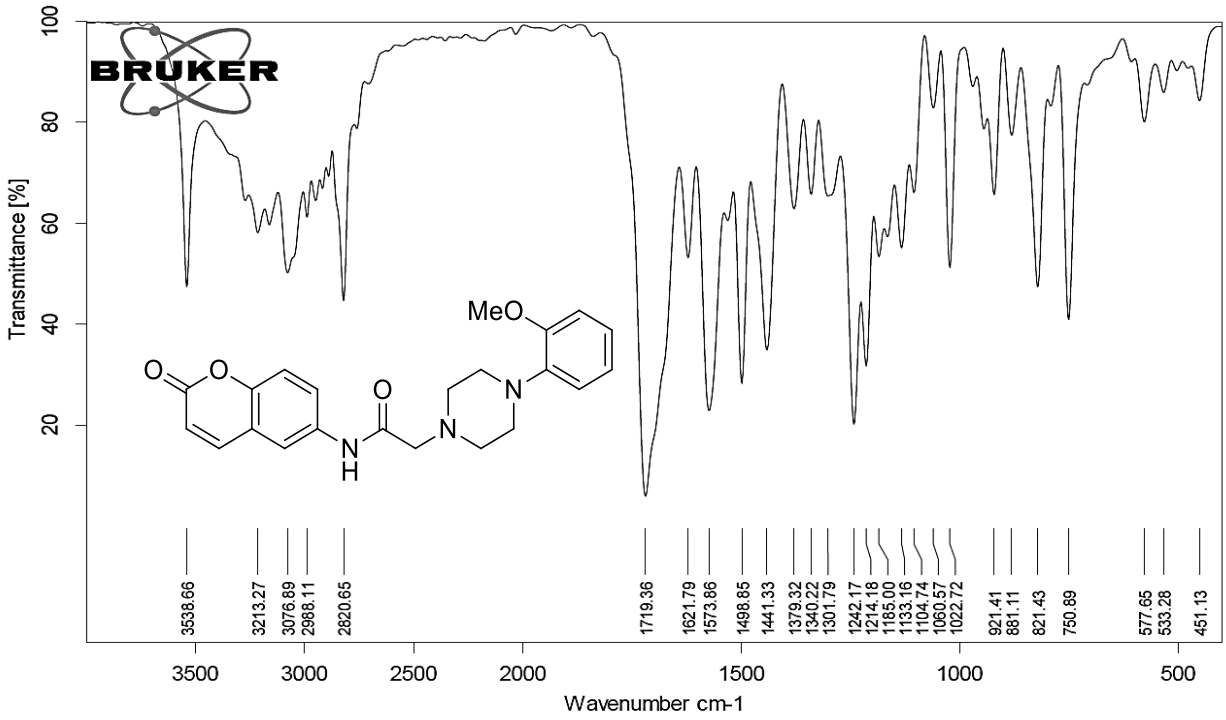


Figure- 3b.9.2 ^{1}H-NMR of 2-(4-(2-methoxyphenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(10a)**

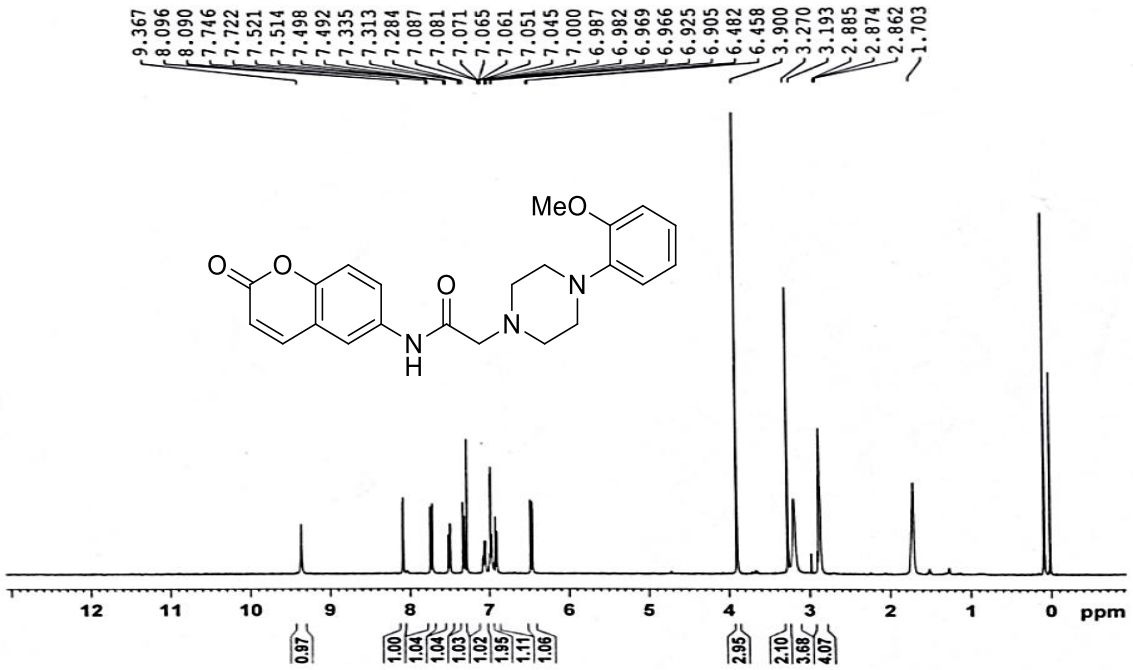


Figure- 3b.9.3 ^{13}C-NMR of 2-(4-(2-methoxyphenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide**(10a)**

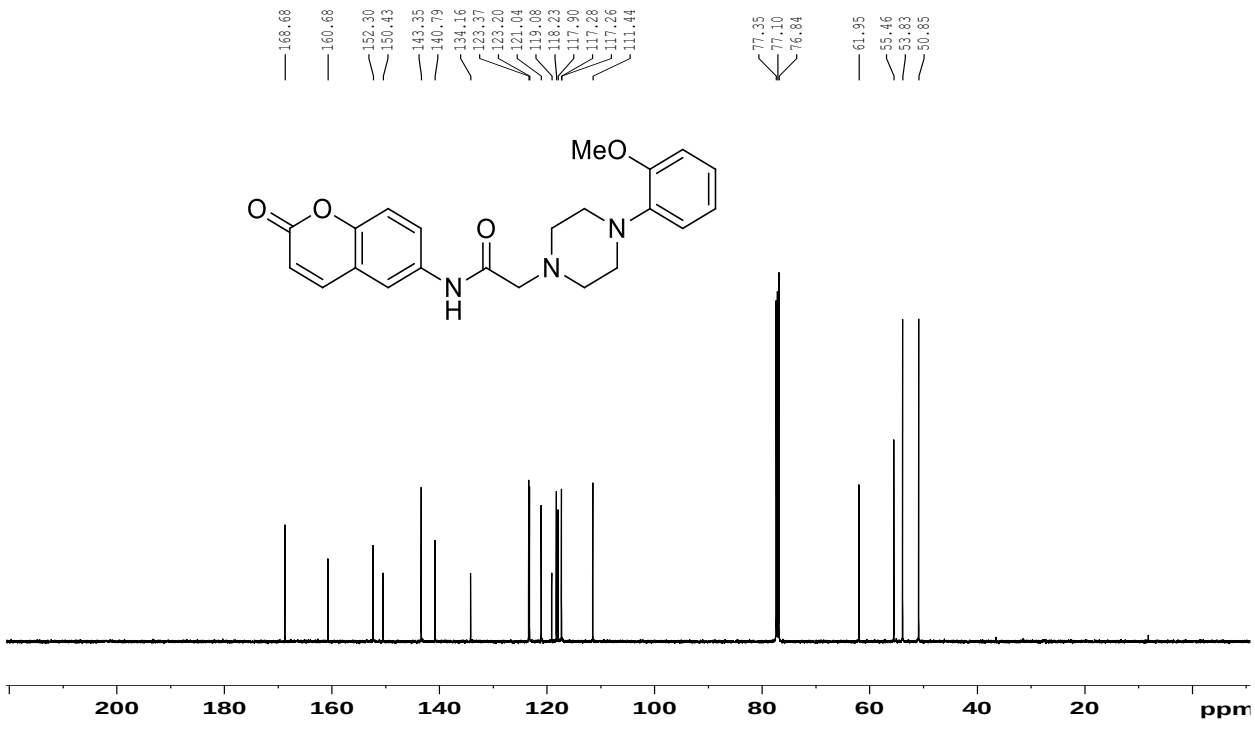


Figure- 3b.9.4 Mass of 2-(4-(2-methoxyphenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(10a)** M+H peak at 394.4

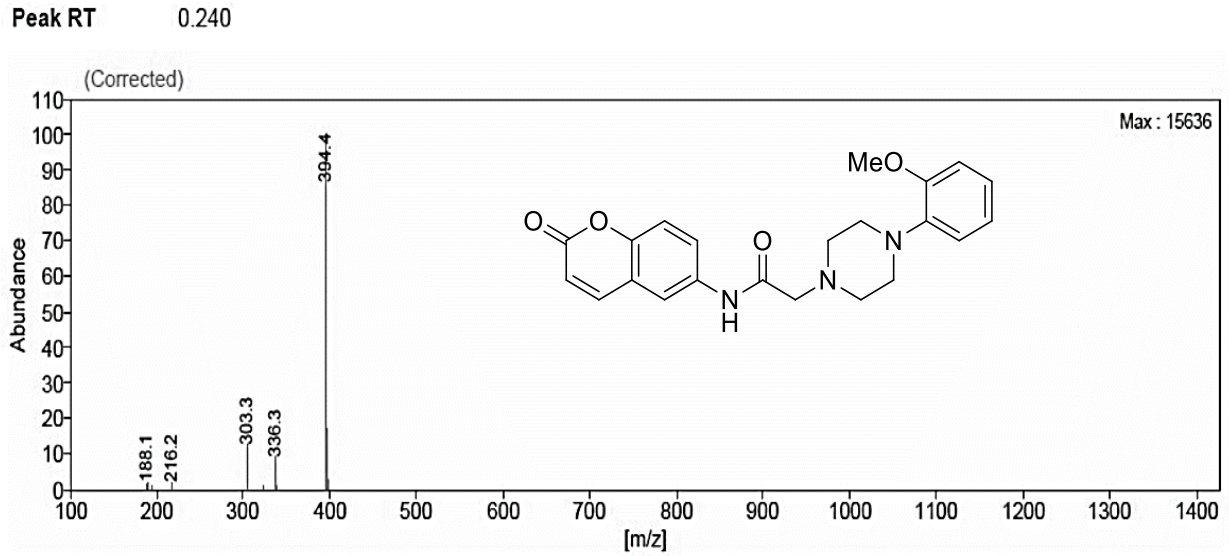


Figure- 3b.10.1 IR spectra of 2-(4-(4-fluorophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide**(10b)**

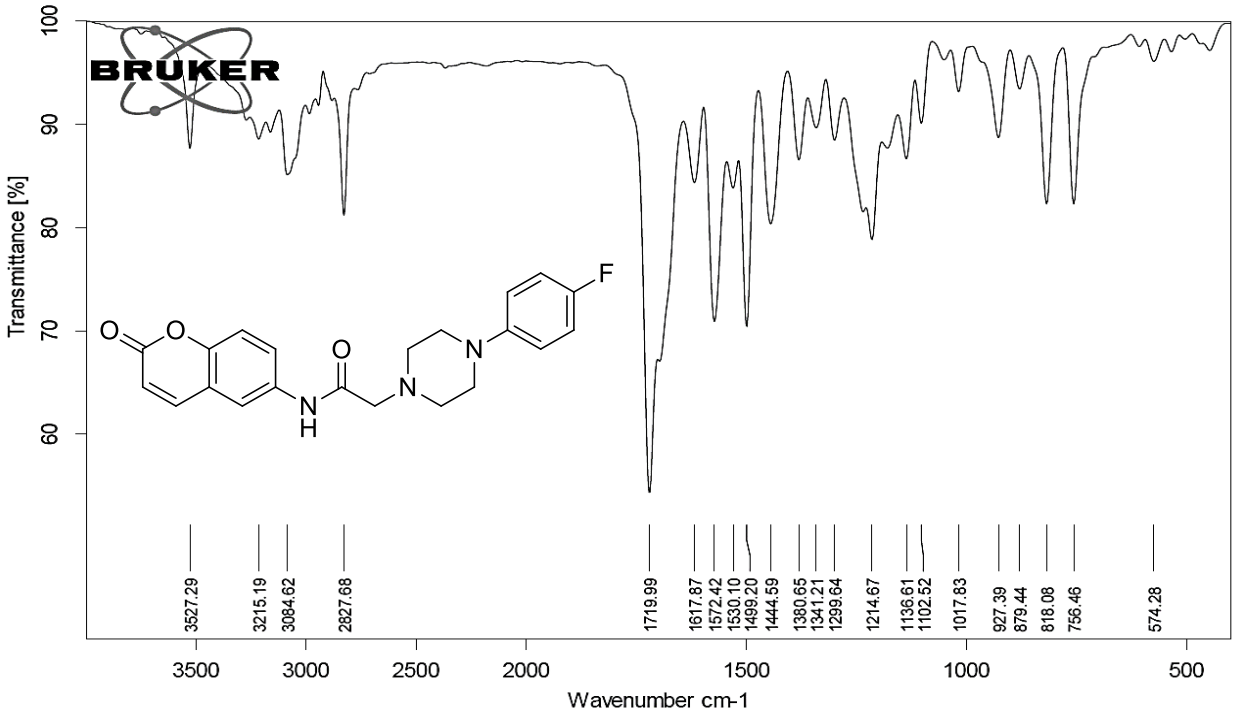


Figure- 3b.10.2 ^{1}H-NMR of 2-(4-(4-fluorophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(10b)**

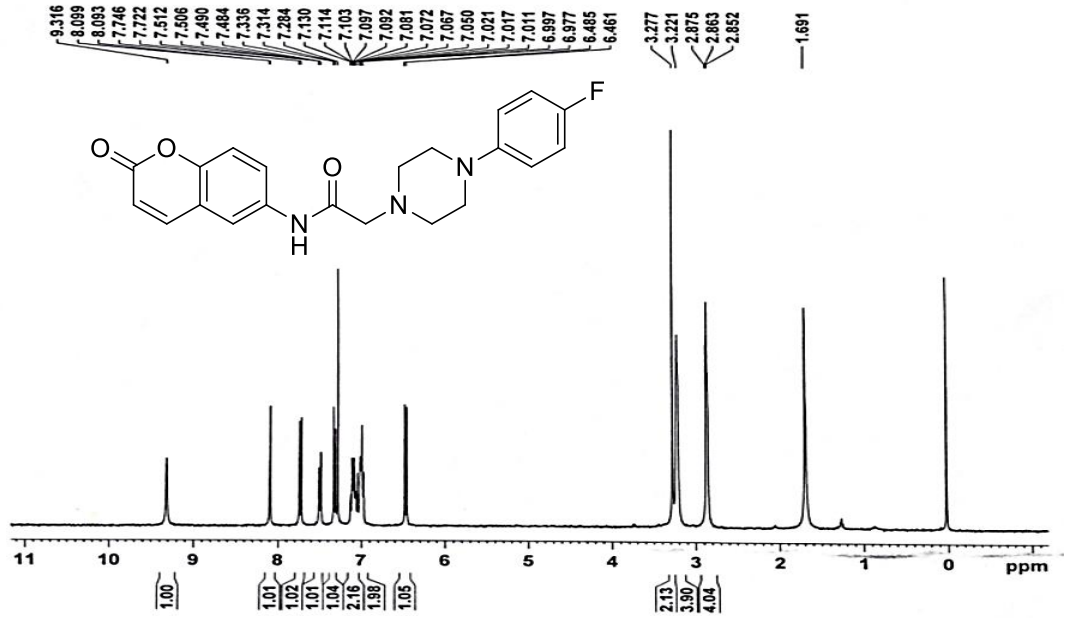


Figure-3b.10.3 ^{13}C-NMR of 2-(4-(4-fluorophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(10b)**

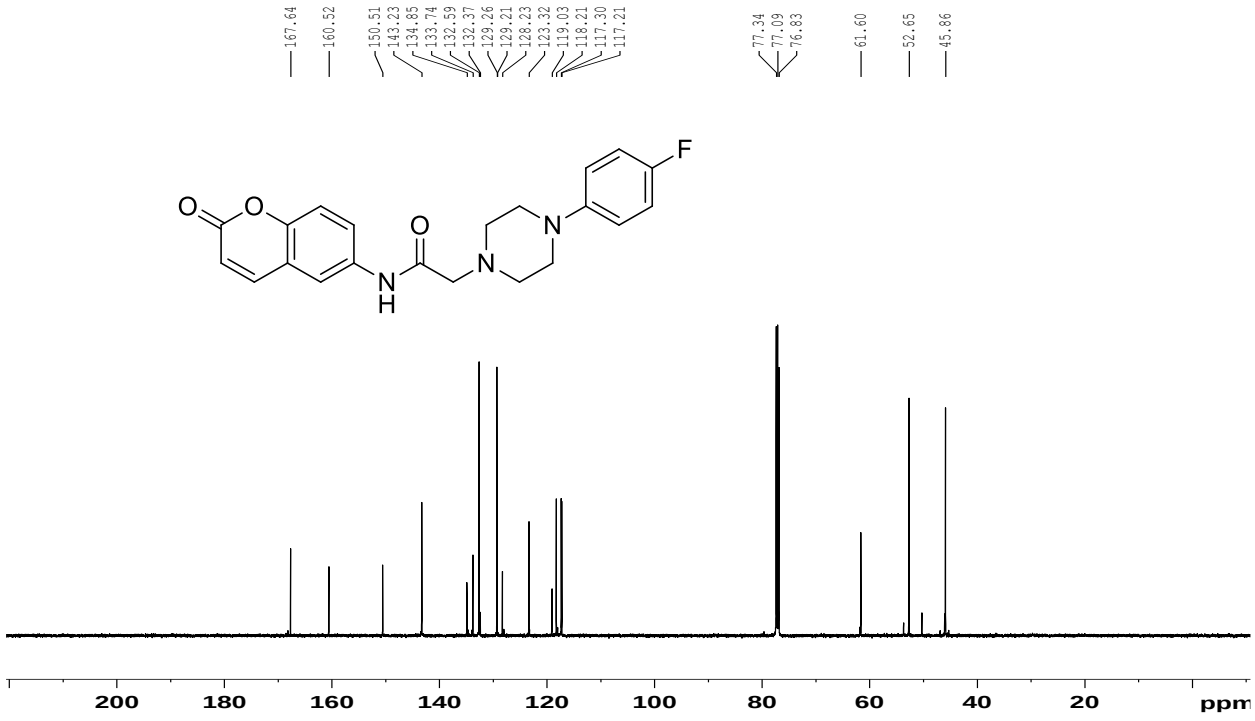


Figure- 3b.10.4 Mass of 2-(4-(4-fluorophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(10b)** M+H peak at 382.3

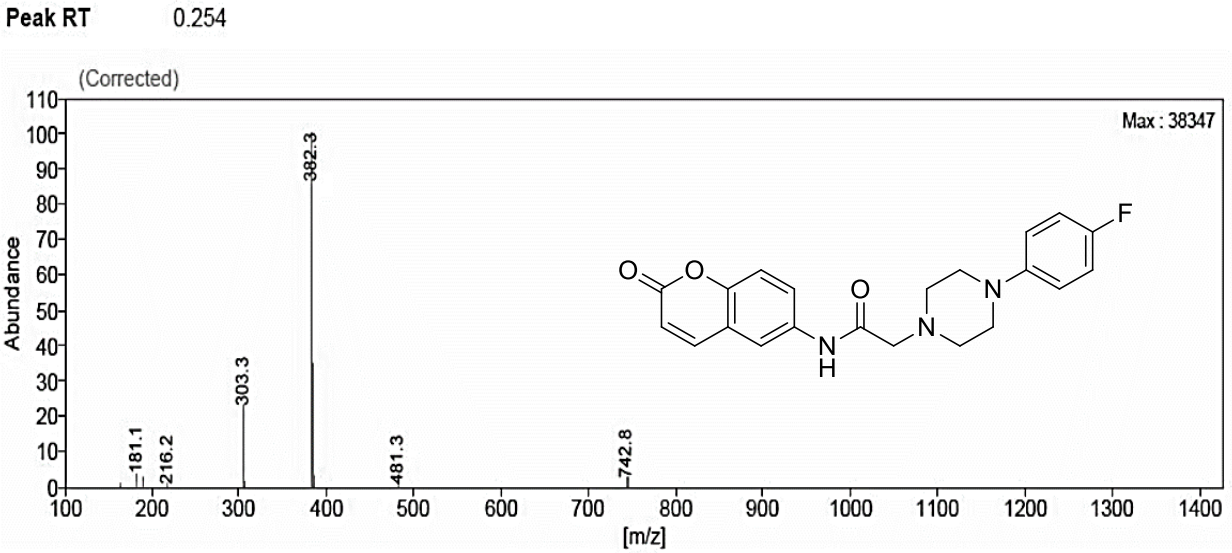


Figure- 3b.11.1 IR of 2-(4-(3-chlorophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl) acetamide **(10c)**

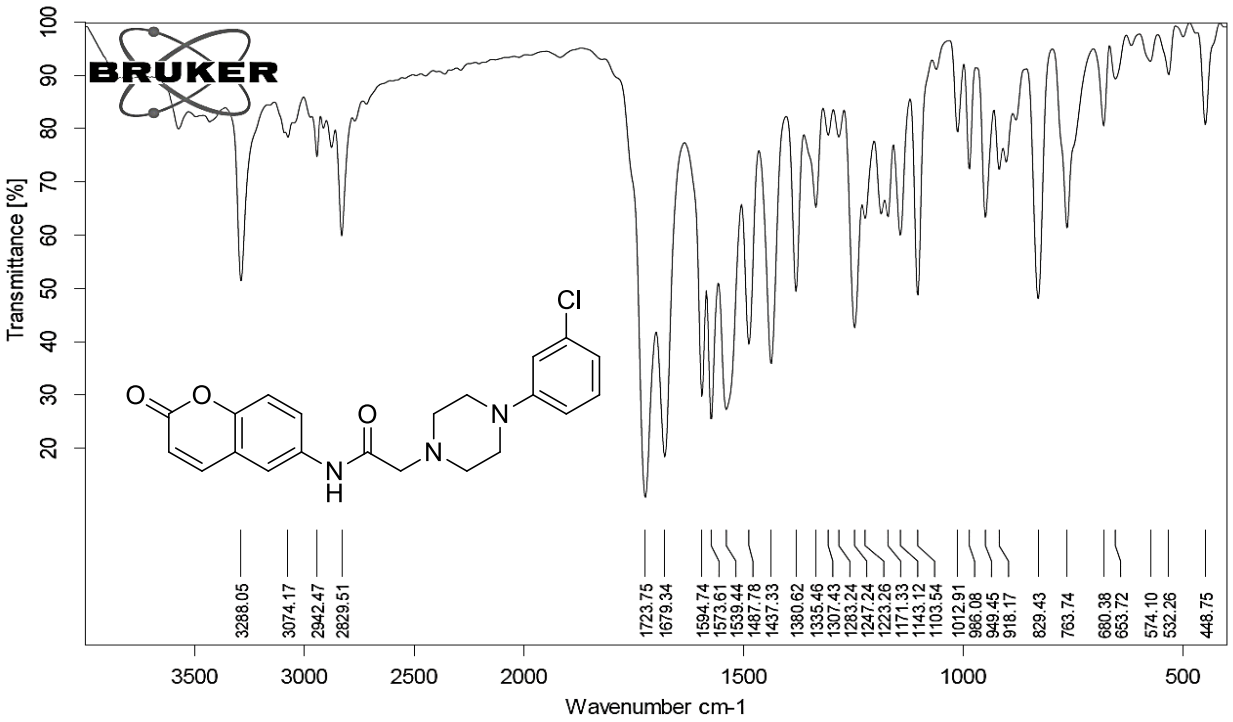


Figure- 3b.11.2 ^{1}H-NMR of 2-(4-(3-chlorophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(10c)**

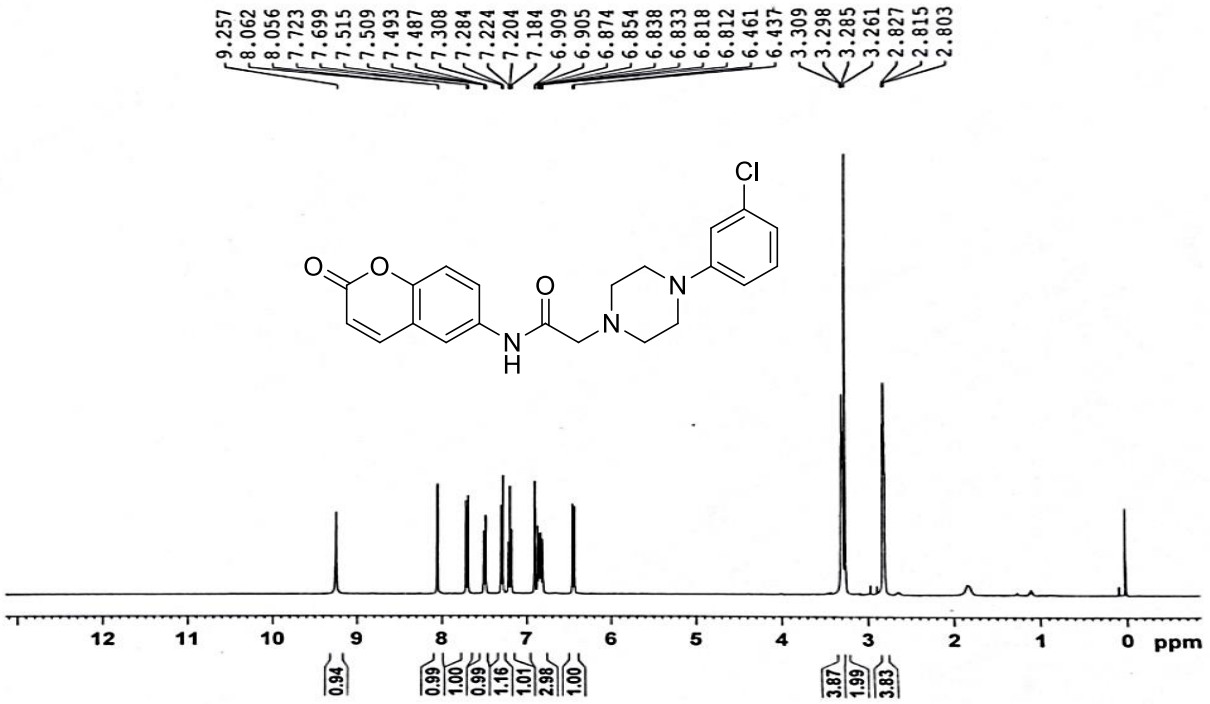


Figure- 3b.11.3 ^{13}C-NMR of 2-(4-(3-chlorophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(10c)**

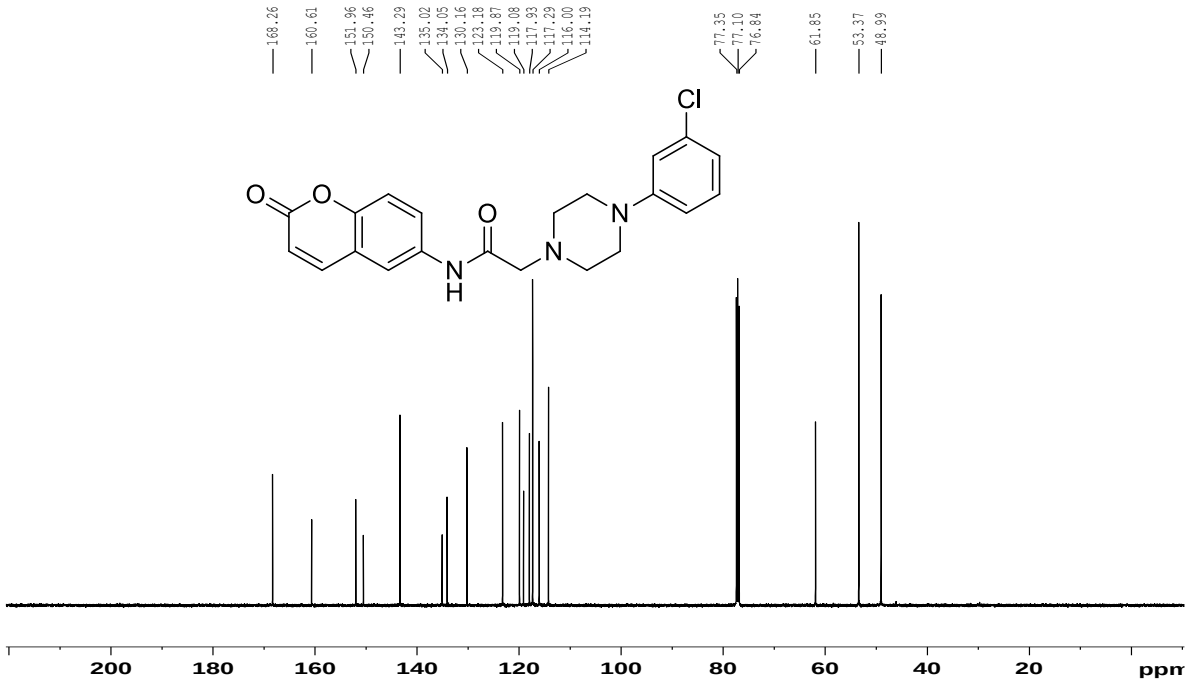


Figure- 3b.11.4 Mass of 2-(4-(3-chlorophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide**(10c)** M+H peak at 308.3

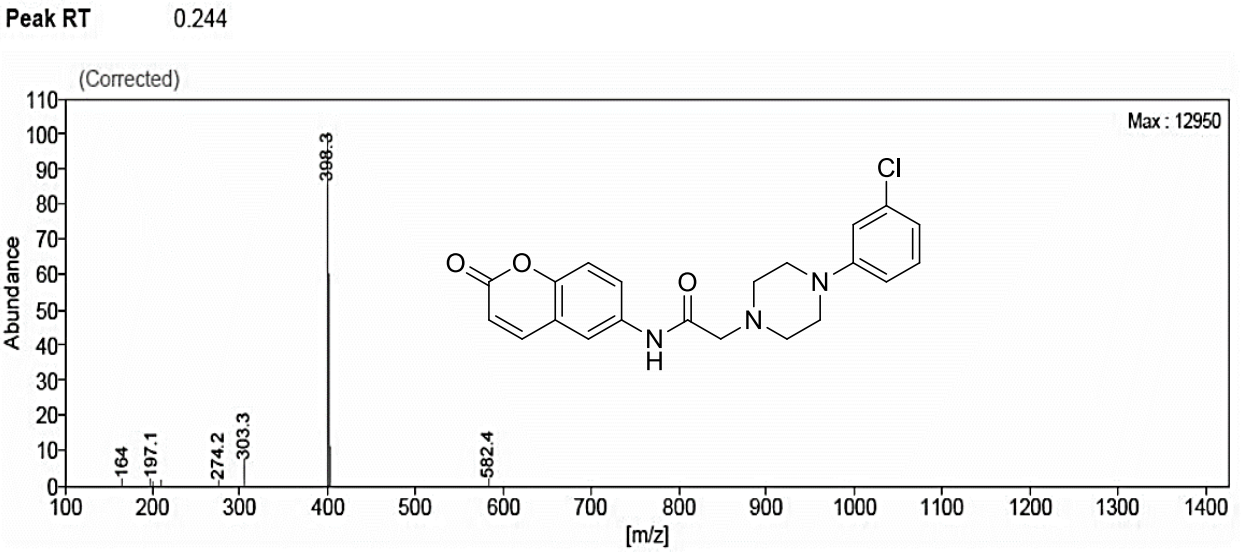


Figure- 3b.12.1 IR of 2-(4-(2-cyanophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(10d)**

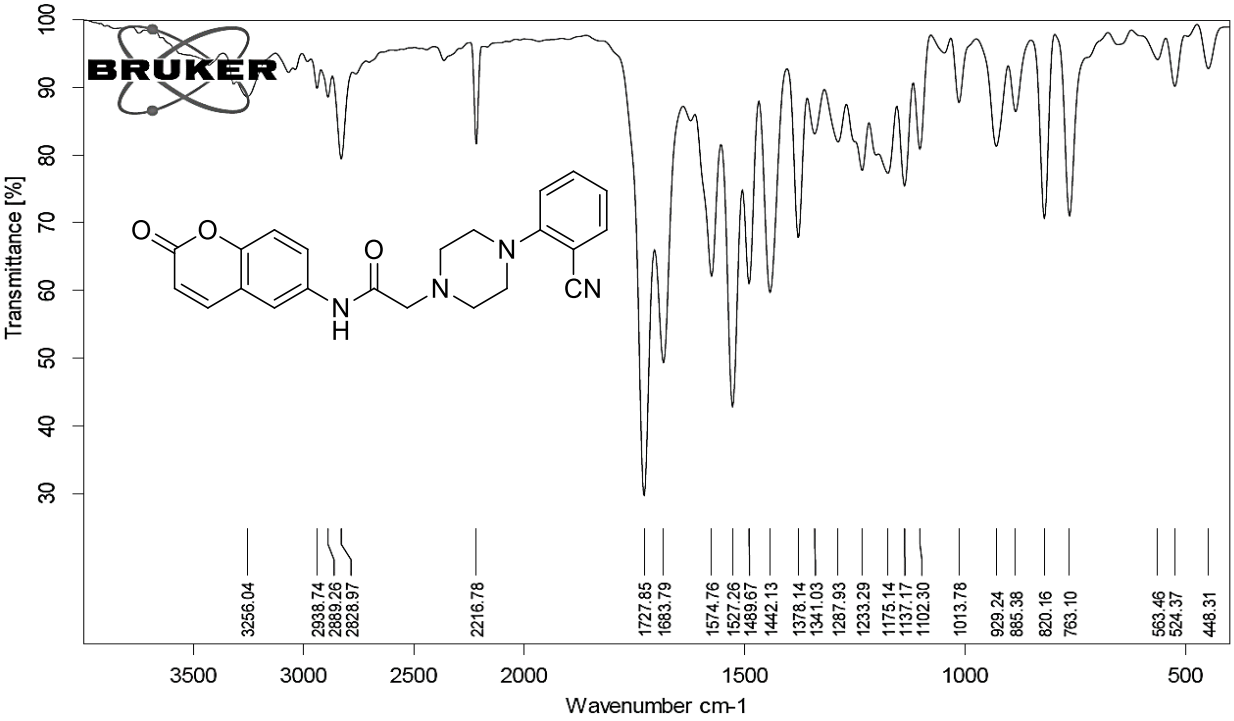


Figure- 3b.12.2 ^{1}H-NMR of 2-(4-(2-cyanophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(10d)**

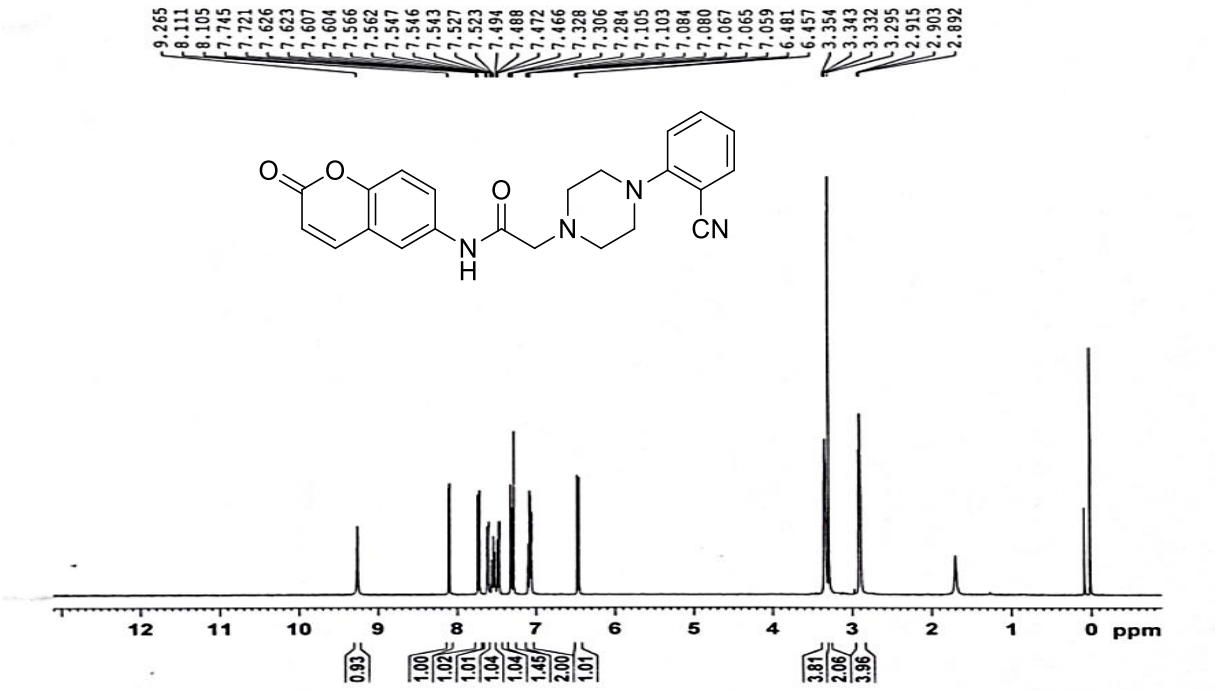


Figure- 3b.12.3 ^{13}C-NMR of 2-(4-(2-cyanophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(10d)**

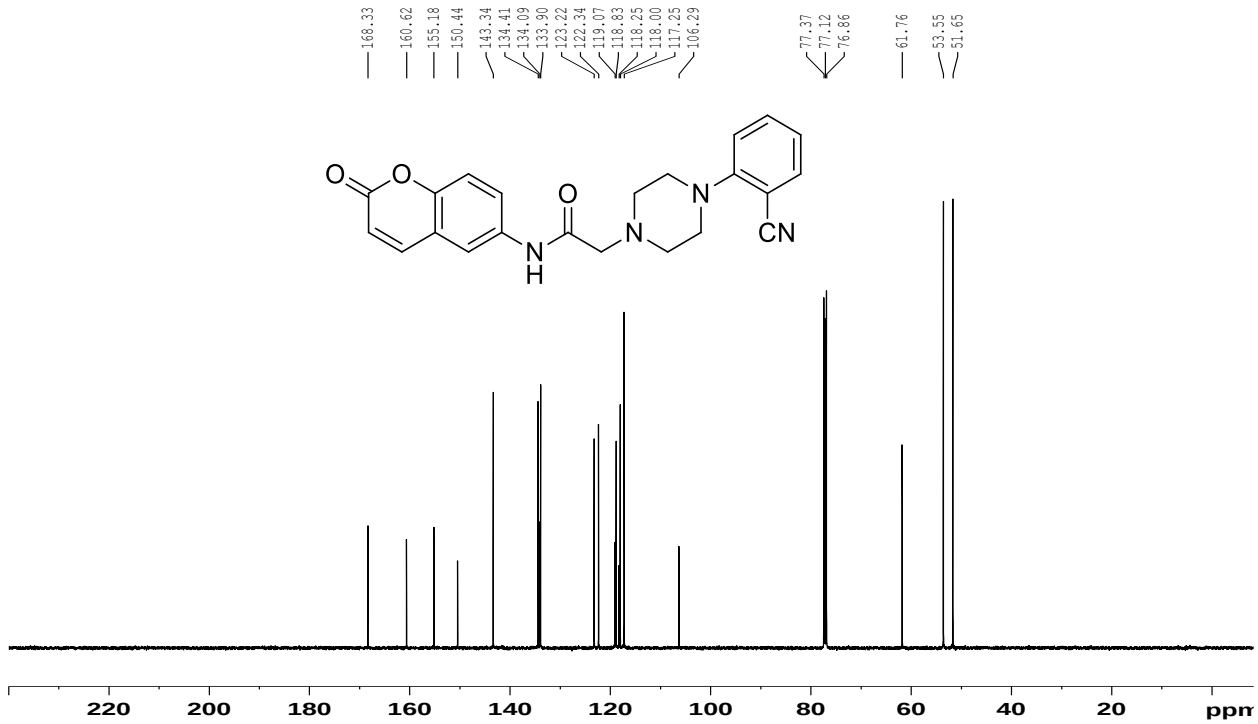


Figure- 3b.12.4 Mass of 2-(4-(2-cyanophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide **(10d)** M+H peak at 389.3

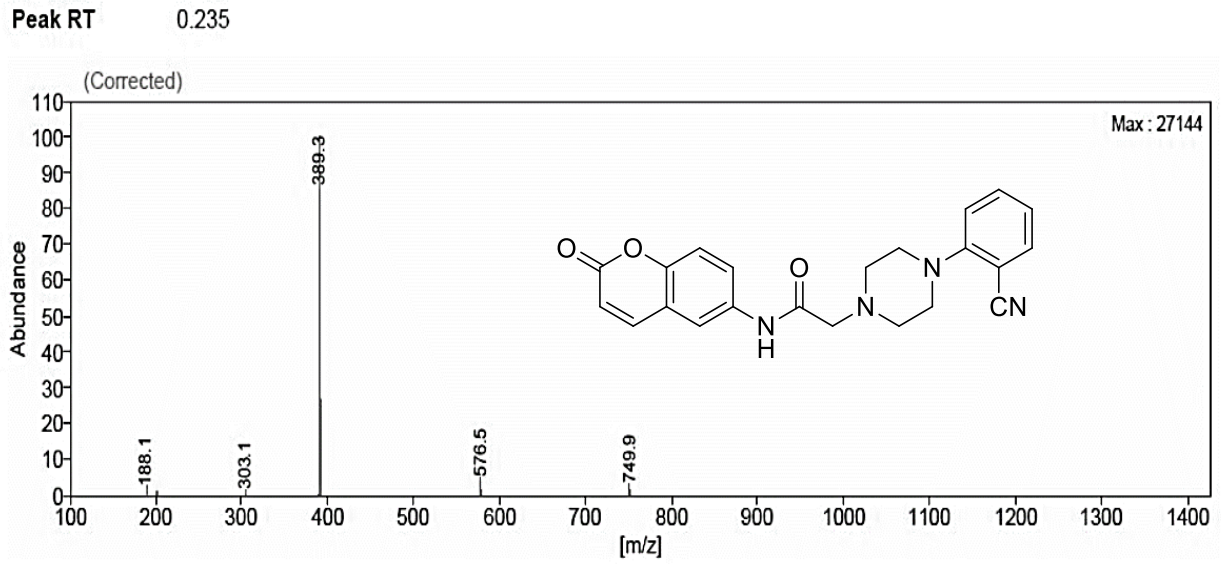


Figure- 3b.13.1 IR of 2,2'-(piperazine-1,4-diyl)bis(N-(2-oxo-2H-chromen-6-yl)acetamide) **(12)**

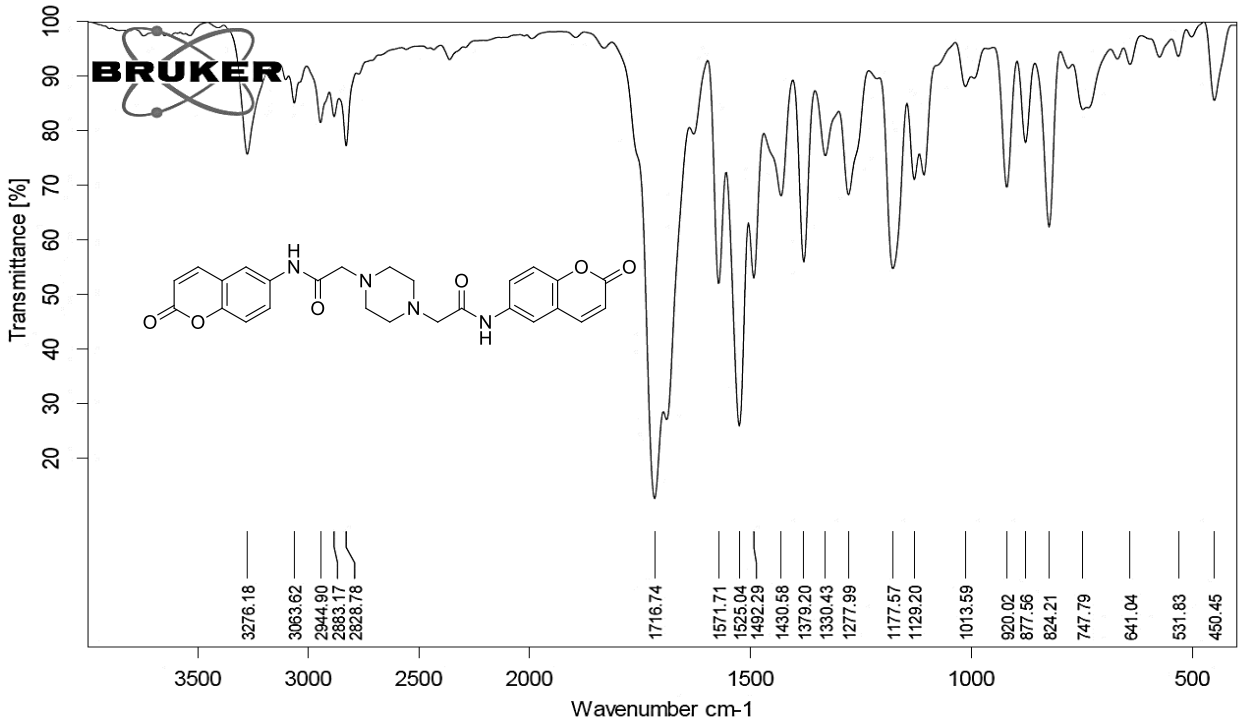


Figure- 3b.13.3 ^{1}H-NMR of 2,2'-(piperazine-1,4-diyl)bis(N-(2-oxo-2H-chromen-6-yl)acetamide) **(12)**

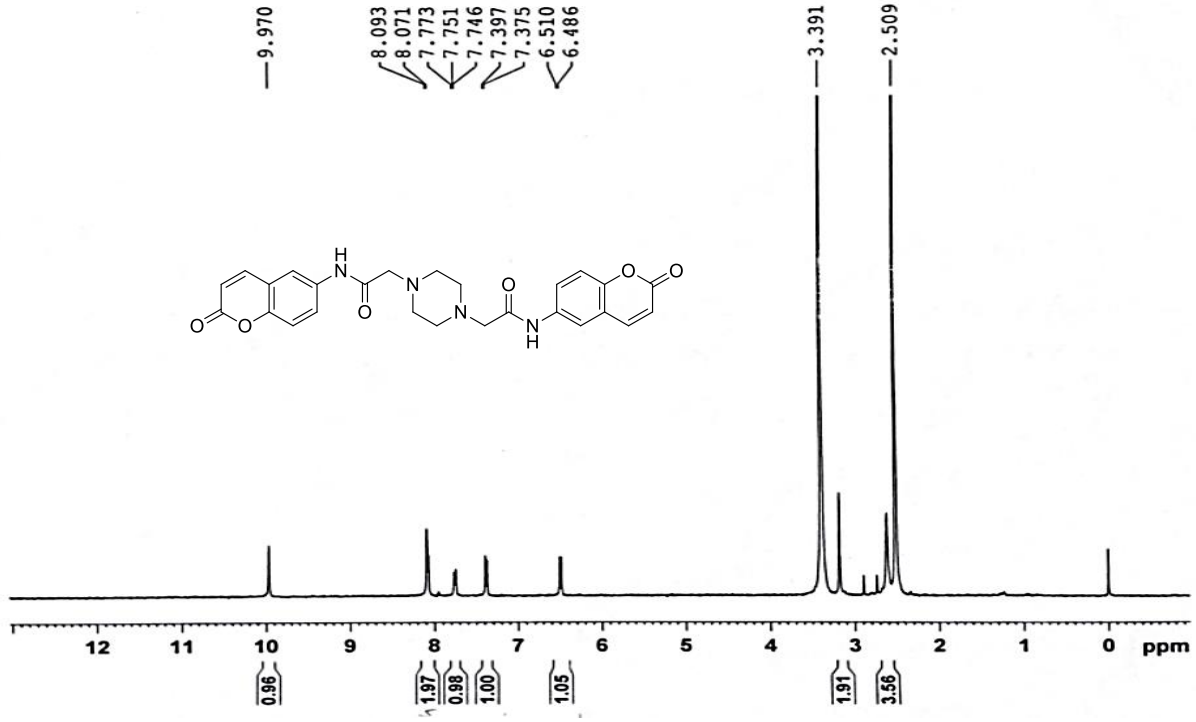
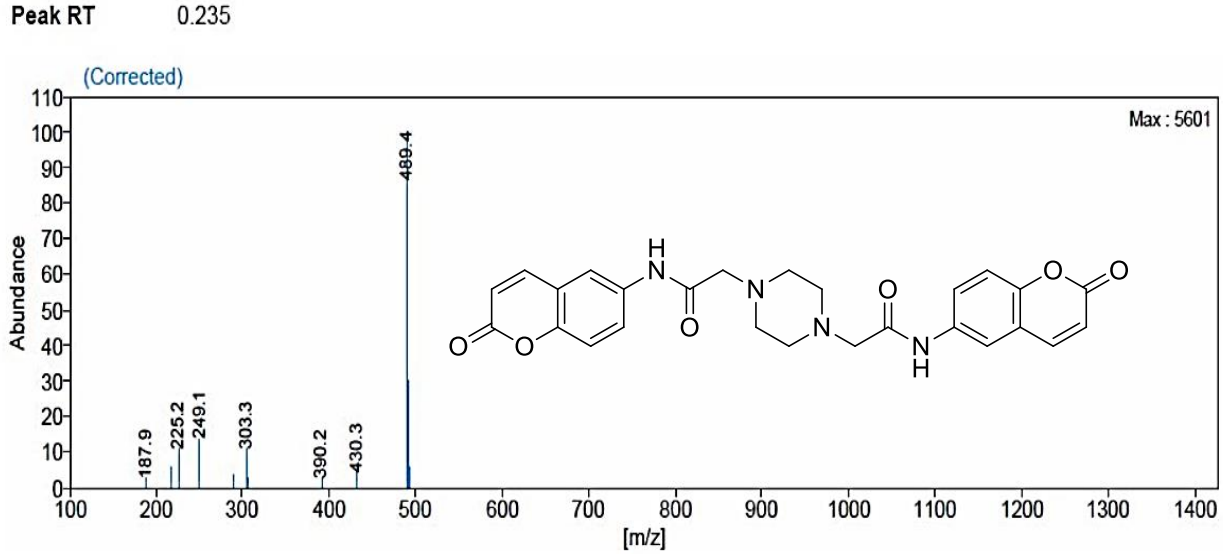


Figure- 3b.13.4 Mass of 2,2'-(piperazine-1,4-diyl)bis(N-(2-oxo-2H-chromen-6-yl)acetamide) **(12)** M+H peak at 489.4



3b.2.1Biological Evaluation

3b.2.2.1 Anticancer activity by MTT assay:

Acetamide derivatives of 6-aminocoumarin **8a-f and 10a-d, and 12** on reaction with substituted penyl piperazine sulphonamide and phenyl piperazine were studied for their anticancer activity using MTT assay against A549 (Lung cancer cell line), MCF-7 (Breast cancer cell line) and compared the results with that of the standard drug Fluorouracil **(Table 3b.1).**

3b.2.2.2 Structure activity relationship (SAR) for anticancer activity

Compound **8a** without any substituent on phenyl ring showed good activity against A549 cell line with IC_{50} value 3.93±0.15µM whereas showed moderate activity against MCF-7 cell line with IC_{50} value 8.14±0.033µM. When electron donating $–CH_3$ group was attached on phenyl ring in compound **8b** drops its anticancer activity against both tested cell lines and showed poor activity against both cell lines whereas $–CH_3$ is replaced by –Fluro compound **8c** showed improvement in the activity against A549 cell line but for MCF-7 cell line it loss of activity and showed poor activity **(Table-3.1)**.

Table 3b.1: Anticancer activity against A549 (Lungs cancer cell line), MCF-7 (Breast cancer cell line) for compounds **8a-f and 10a-d and 12**

Compounds	**R**	**IC_{50} µM[a]**	
		A549 µM[a]	MCF-7 µM[a]
8a	4-H	3.93±0.15	8.14±0.033
8b	4-CH_3	460±5.66	50.41±1.71
8c	4-F	5.62±0.053	338.1±2.52
8d	4-Cl	2.06±0.057	4.93±0.029
8e	4-Br	92.62±1.23	**0.85±0.03**
8f	4-NO_2	3.5±0.045	3.95±0.027
10a	2-OMe	1.7±0.059	39.17±1.6
10b	3-F	7.74±0.038	4.74±0.042
10c	3-Cl	**0.4±0.032**	**0.51±0.031**
10d	2-CN	14.5±0.12	11.15±0.019
12		5.04±0.071	11.43±0.014
Fluorouracil		11.13 ±0.083	45.04 ±1.02

[a]IC_{50} values were determined based on MTT assay using GraphPad Prism software

When Fluoro is replaced by Chloro substituent then it was observed that there is improvement in the activity and compound **8d** showed good activity against both A549 with IC_{50} value 2.06±0.057 µM and in MCF-7 with IC_{50} value 4.93±0.029 µM. In coumpound **8e** where –F is replaced by –Bromo drops its activity against A549 cell line and showed poor activity whereas improvement in the anticancer activity against MCF-7 cell line and showed very excellent activity against breast cancer cell line MCF-7 with IC_{50} value 0.85±0.03 µM However, -Fluoro in **8e** was replace by strong electron withdrawing -NO_2 compound **8f** showed improvements in the anticancer activity against A549 cell line showed moderate activity with IC_{50} value 3.5±0.045 µM and drops activity against MCF-7 with 3.95±0.027 µM IC_{50} value.

In another variation when different phenyl pierazines attached to compound **6** i.e. **10a-d**, compound **10a** with –OMe group attached to phenyl group showed good activity against A549 cell line with IC_{50} value 1.7±0.059 µM and drop the anticancer activity against MCF-7 cell line. When methoxy was replaced by –Fluoro in compound **10b** showed that loss of anticancer activity against A549 cell line whereas showed improvement in the activity against MCF-7 cell line with IC_{50} value 4.74±0.042µM. On replacing Fluro by -Chloro in compound **10c** showed very good improvement in the anticancer activity and showed very excellent activity against both tested cell line i.e. 0.4±0.032 µM A549 cell line and 0.51±0.031µM for MCF-7 cell line. However, -Chloro is replaced by –CN in compound **10d** drop its anticancer activity and showed moderate activity against both tested cell lines with IC_{50} value 14.5±0.12 µM and 11.15±0.019 µM against A549 cell line and MCF-7 cell line respectively. Compound **12** coumarin dimer with piperazine showed improvement in anticancer activity against A549 cell line with IC_{50} value 5.04±0.071 µM and remains moderate for MCF-7 cell line with IC_{50} value 11.43±0.014 µM.

3b.3 Conclusion

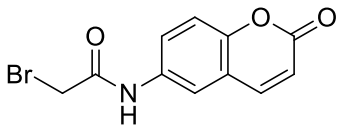
In this work different phenyl pierazine derivatives of 6-amino coumarins were synthesized and their *In-Vitro* anticancer activity were studied against two cancer cell lines A549-Lungs cancer cell line and MCF-7 breast cancer cell line. For phenyl sulphonyl piperazine derivatives of coumarin **8a-f**, compound **8e** showed very good activity against MCF-7 breast cancer cell line and other compounds showed good to moderate activity against A549 cell line and MCF-7 cell line while compound **8b** was found very poor active against both cell line. From compounds **10a-d** phenyl piperazines attached to coumarin, compound **10c** showed very excellent activity against both lungs cancer cell line (A549) and breast cancer cell line (MCF-7) cell line and compound **10a, 10b, 10d and 12** showed good to moderate activity against both lungs cancer A549 and MCF-7 breast cancer cell line.

3b.4 Experimental

3b.4.1 General Chemistry

A commercial supplier was used to obtain reagent-grade chemicals and solvents after purification. TLC was done on silica gel F254 plates from Merck. For column chromatographic purification, Acme's silica gel (60–120 mesh) was used. The melting points of open capillary tubes were calculated using a Rolex melting point instrument. KBr pellet IR spectra were recorded on the Perkin Elmer RX 1 spectrometer. Spectral data for ^{1}H NMR and ^{13}C NMR were gathered on an Advance Bruker 400 spectrometer (400 MHz) using CDCl3 or DMSO-d6 as the solvent and TMS as the internal standard. *J* values are in Hz. A Shimadzu LCMS 2020 equipment was utilised to determine the mass spectra using ESI-MS. The findings of the elemental analysis were recorded using a Thermosinnigan Flash 11–12 series EA. 6-amino coumarin, different phenyl sulphonyl piperazines were prepared according to literature method [15-16].

2-bromo-N-(2-oxo-2H-chromen-6-yl)acetamide (6)



To a solution of 6-aminocoumarin **5** (10.0 mmol) in dichloromethane (DCM) (25 mL) was added triethylamine (TEA) (15.0 mmol) and stirred for 5–10 min. To this bromoacetyl bromide (12.0 mmol) was added dropwise over a period of 10 min. The resulting solution was stirred at room temperature for 2 hours. The reaction mixture filtered, washed with DCM, water and acetone to give compound **6** as a pale white solid. The substituted bromoacetamide **6** thus obtained, used directly for next step without any purification.

White Solid; Yield: 70 %; M.P: 174-176 ºC; IR (KBr) 3291, 3108, 1739, 1651, 1622, 1575, 1490, 1438, 1378, 1340, 1299, 1261, 1180, 1111, 918, 884, 826, 751, 714 cm-^{1}**;** ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.07(s, 2H), 6.50 (d, *J* = 9.6Hz, 1H), 7.39 (d, *J* = 8.8Hz, 1H), 7.66 (dd, *J* = 2.4Hz, 8.8Hz, 1H), 8.05 (d, *J* = 2.8Hz, 1H), 8.10 (d, *J* = 9.6Hz, 1H), 10.65 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 29.26, 117.51, 117.58, 118.69, 119.12, 123.69, 133.37, 143.08, 151.08, 160.44, 163.59.

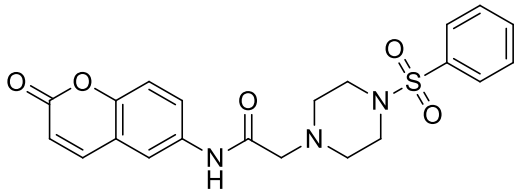
General procedure for the synthesis of compounds 10a-f and 10a-d, 12

To a Solution of 2-bromo-N-(2-oxo-2H-chromen-6-yl)acetamide **6** (**1.1 eq**) and phenyl sulphonyl piperazines **7**/phenyl piperazines **9** /piperazine **11 (1 eq)** in DMF (20 mL) was added triethylamine (1.5 eq). The resulting mixture was stirred at room temperature for 16 hr and then poured into cold water. The aqueous layer thus obtained was extracted using ethyl acetate

and/or dichloromethane (checked by TLC) and solvent evaporated to give crude product. The crude compound was purified by column chromatography.

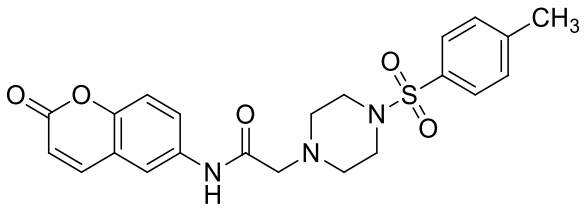
Characteristics data of compounds 8a-f and 10a-d, 12:

N-(2-oxo-2H-chromen-6-yl)-2-(4-(phenylsulfonyl)piperazin-1-yl)acetamide (8a)



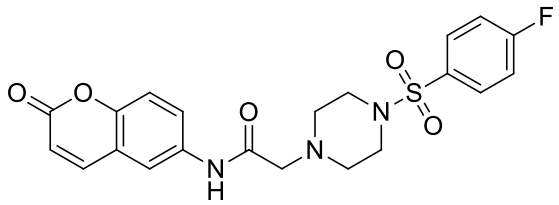
White Solid; Yield: 72 %; M.P: 184-186 ºC; IR (KBr) 3289, 3089, 2959, 2923, 2855, 2805, 1698, 1615, 1571, 1484, 1435, 1344, 1257, 1163, 1129, 1101, 1071, 950, 924, 900, 819, 746, 691cm^{-1}**;** ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.73 (broad s, 4H), 3.15 (broad s, 4H), 3.19 (s, 2H), 6.42 (d, *J* = 9.2Hz, 1H), 7.23 (d, *J* = 8.4Hz, 1H), 7.35 (d, *J* = 8.4Hz, 1H), 7.58-7.68 (m, 5H), 7.80(d, *J* = 7.2, 2H), 7.99 (s, 1H), 8.84 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 45.36, 45.86, 52.65, 61.58, 116.57, 116.75, 117.20, 117.30, 118.20, 119.20, 123.28, 130.31,131.97, 143.23, 150.50, 160.52, 166.40, 167.67 Anal. calc. for $C_{21}H_{21}N_3O_5S$; C,59.00; H,4.95; N,9.83; found: C,59.03; H,5.60; N,6.25; ESI-MS: 428.3 $[M+H]^+$.

N-(2-oxo-2H-chromen-6-yl)-2-(4-tosylpiperazin-1-yl)acetamide (8b)



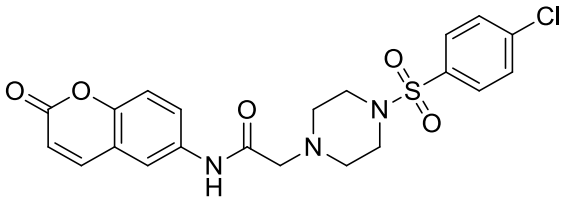
White Solid; Yield: 84 %; M.P: 200-202 ºC; IR (KBr) 3271, 3040, 2901, 2840, 1737, 1675, 1571, 1528, 1438, 1374, 1345, 1322, 1163, 1132, 1098, 984, 927, 829, 722, 654cm^{-1}**;** ^{1}H-NMR (400 MHz, $CDCl_3$): 2.49 (S,3H), 2074 (d, *J* = 4.8Hz, 4H), 3.14 (S, 4H), 3.20(S, 2H), 6.44 (d, *J* = 9.6Hz, 1H), 7.25 (d, *J* = 8.8Hz, 1H), 7.35 (dd, *J* = 2.4Hz & 8.8Hz, 1H), 7.39 (d, *J* = 8.4Hz), 7.68-7.70 (m, 3H), 8.00 (d, *J* = 2.4Hz, 1H), 8.84 (S, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): 20.94, 45.56, 51.63, 60.98, 116.25, 116.37, 118.42, 118.49, 124.03, 127.53, 129.73, 131.99, 134.77, 143.53, 144.12, 149.45, 159.88, 168.08; Anal. calc. for $C_{22}H_{23}N_3O_5S$; C,59.85; H,5.25; N,9.52; found: C,59.87; H,5.28; N,9.55; ESI-MS:442.4 $[M+H]^+$.

2-(4-((4Fluorophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide (8c)



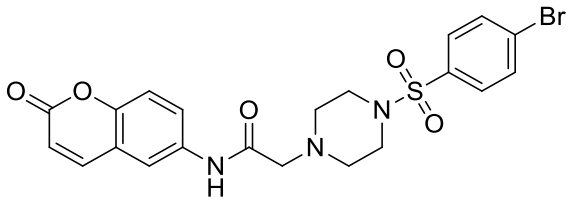
White Solid; Yield: 80 %; M.P: 194-196ºC; IR (KBr) 3255, 3045, 2959, 2928, 2856, 1732, 1678, 1592, 1574, 1529, 1492, 1440, 1381, 1349, 1291, 1234, 1171, 1126, 1099, 1073, 1014, 989, 950, 911, 832, 734, 655cm^{-1}**;** ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.74 (t, *J* = 4.8Hz, 4H), 3.12-3.15 (m,4H), 3.20 (S, 2H), 6.42 (d, *J* = 9.6Hz, 1H), 7.23-7.30 (m, 3H), 7.35 (dd, *J* = 2.4Hz & 8.8Hz, 1H), 7.67 (d, *J* = 9.6Hz, 1H), 7.80-7.84 (m, 2H), 7.99 (d, *J* = 2.4Hz, 1H), 8.83 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 45.86, 52.69, 61.58, 117.19, 117.29, 118.20, 119.02, 123.29, 127.77, 129.27, 129.31, 133.13, 133.77, 135.80, 150.49, 160.54, 167.73; Anal. calc. for $C_{21}H_{20}FN_3O_5S$ C,56.62; H,4.53; N,9.43; found: C,56.60; H,4.52; N,9.40; ESI-MS: 446.4 $[M+H]^+$.

2-(4-((4-Chlorophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide (8d)



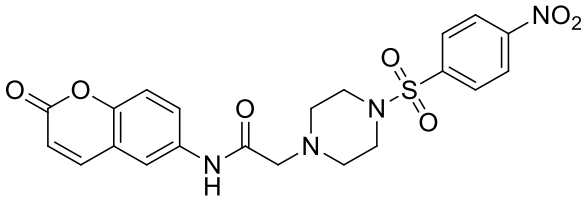
White Solid; Yield: 75 %; M.P: 186-188ºC; IR (KBr) 3288, 3092, 3042, 2920, 2852, 1732, 1680, 1573, 1529, 1477, 1440, 1381, 1348, 1304, 1279, 1258, 1167, 1099, 1013, 987, 952, 911, 826, 761, 718, 656, 607cm^{-1}**;** ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.75 (t, *J* = 4.8Hz, 4H), 3.15 (broad s, 4H), 3.21 (s, 2H), 6.44 (d, *J* = 9.6Hz, 1H), 7.26 (d, *J* = 8.8Hz, 1H), 7.37 (dd, *J* = 2.4Hz, 8.8Hz, 1H), 7.58 (d, *J* = 8.8Hz, 2H), 7.69 (d, *J* = 9.6Hz, 1H), 7.75 (d, *J* = 8.4Hz, 2H), 8.00 (d, *J* = 2.4Hz, 1H), 8.81 (s, 1H) ^{13}C-NMR (100 MHz, DMSO-*d*6): δ ppm 45.53, 51.53, 60.83, 116.31, 116.37, 118.42, 118.47, 124.02, 129.39, 129.49, 133.83, 134.75, 138.21, 144.13, 149.44, 159.87, 168.03; Anal. calc. for $C_{21}H_{20}ClN_3O_5S$ C,54.60; H,4.36; N,9.10; O,17.96; S,6.94; Anal. calc. for $C_{21}H_{20}ClN_3O_5S$ C,54.60; H,4.36; N,9.10; found: C,54.58; H,4.34; N,9.13; O,17.96; S,6.94; ESI-MS: 462.3 $[M+H]^+$.

2-(4-((4-Bromophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide (8e)



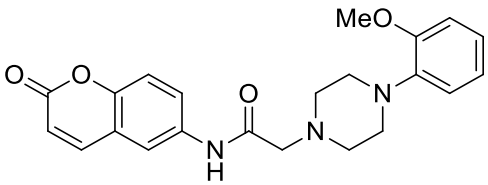
White Solid; Yield: 88 %; M.P: 222-224ºC; IR (KBr) 3294, 3089, 2853, 1730, 1681, 1573, 1528, 1439, 1384, 1349, 1278, 1167, 1135, 1102, 1067, 1010, 951, 912, 822, 752, 711, 656 cm^{-1}**;** ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.75(t J= 4.8Hz, 4H), 3.15 (broad s, 4H), 3.21(s, 2H), 6.44 (d, *J* = 9.6Hz, 1H), 7.26 (d, *J* = 8.8Hz, 1H), 7.37 (dd, *J* = 2.4Hz, 8.8Hz, 1H), 7.66-7.70 (m, 3H), 7.74 (d, *J* = 8.4Hz, 2H), 8.00 (d, *J* = 2.4Hz, 1H), 8.82 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 45.86, 52.65, 61.60, 117.21, 117.30, 118.21, 123.32, 128.23, 129.21, 129.26, 132.59, 133.74, 134.85, 143.23, 150.51, 160.52, 167.64 Anal. calc. for $C_{21}H_{20}BrN_3O_5S$ C,49.81; H,3.98; N,8.30; found: C,49.84; H,3.95; N,8.280; ESI-MS: 506.37 $[M+H]^+$.

2-(4-((4-Nitrophenyl)sulfonyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide (8f)



White Solid; Yield: 88 %; M.P: Above 250-252ºC; IR (KBr) 3278, 3104, 3043, 2923, 2857, 1726, 1681, 1605, 1574, 1529, 1440, 1379, 1350, 1310, 1257, 1172, 1130, 1103, 1065, 1011, 956, 915, 881, 854, 828, 749, 712, 685 cm^{-1}**;** ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.51 (broad s, 4H), 3.07 (broad s, 4H), 3.18 (s, 2H), 6.48 (d, *J* = 9.6Hz, 1H), 7.35 (d, *J* = 8.8Hz, 1H), 7.68 (dd, *J* = 2.4Hz, 8.8Hz, 1H), 8.01-8.06 (m, 4H), 8.48 (d, *J* = 8.8Hz, 2H), 9.87 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ ppm 45.54, 51.43, 60.72, 116.35, 116.39, 118.36, 118.43, 123.93, 124.62, 129.04, 134.74, 140.79, 144.14, 149.40, 150.00, 159.84, 168.03; Anal. calc. for $C_{21}H_{20}N_4O_7S$ C,53.38; H,4.27; N,11.86; found: C,53.40; H,4.25; N,11.89; ESI-MS: 473.3 $[M+H]^+$.

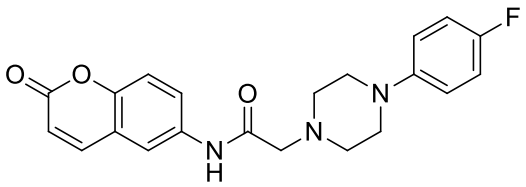
2-(4-(2-Methoxyphenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide (10a)



White Solid; Yield: 88 %; M.P: 234-236 ºC; IR (KBr) 3539, 3213, 3077, 2988, 2821, 1719, 1622, 1574, 1499, 1441, 1379, 1340, 1302, 1242, 1214, 1185, 1133, 1105, 1061, 1023, 921,

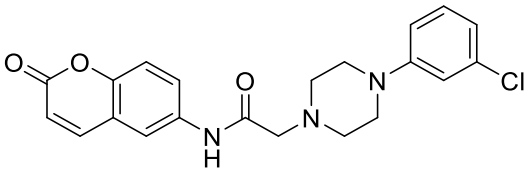
881, 821, 751 cm^{-1}**;** ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.87 (t, *J* = 4.6Hz, 4H), 3.19 (broad s, 4H), 3.27 (s, 2H), 3.90 (s, 3H), 6.47 (d, *J* = 9.6Hz, 1H), 6.91 (d, *J* = 8.0Hz, 1H), 6.97-7.00 (m, 2H), 7.05-7.09 (m, 1H), 7.32 (d, *J* = 8.8Hz, 1H), 7.50 (dd, *J* = 2.4Hz & 8.8Hz, 1H), 7.73 (d, 9.6Hz, 1H), 8.09 (d, *J* = 2.4Hz, 1H), 9.37 (s, 1H); δ NMR (100 MHz, $CDCl_3$): δ ppm 50.85, 53.83, 55.46, 61.95, 111.44, 117.26, 117.28, 117.90, 118.23, 119.08, 121.04, 123.20, 123.37, 134.16, 140.79, 143.35, 150.43, 152.30, 160.68, 168.68; Anal. calc. for $C_{22}H_{23}N_3O_4$ C,67.16; H,5.89; N,10.68; found: C,67.19; H,5.85; N,10.65; ESI-MS: 394.4 $[M+H]^+$.

2-(4-(4-Fluorophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide (10b)



White Solid; Yield: 88 %; M.P: 244-247ºC; IR (KBr) 3527, 3215, 3085, 2828, 1720, 1618, 1572, 1530, 1499, 1445, 1381, 1341, 1300, 1215, 1137, 1103, 1018, 927, 879, 818, 756cm^{-1}**;** ^{1}H-NMR(400 MHz, $CDCl_3$): δ 2.86 (t, *J* = 4.8Hz, 4H), 3.22 (s, 4H), 3.28 (s, 2H), 6.47 (d, *J* = 9.6Hz, 1H), 6.98-7.13 (m, 4H), 7.32 (d, *J* = 8.8Hz, 1H), 7.50 (dd, *J* = 2.4Hz, 8.8Hz, 1H), 7.73 (d, *J* = 9.6Hz, 1H), 8.09 (d, *J* = 2.4Hz, 1H), 9.32 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 50.68, 50.71, 53.66, 61.91, 116.19, 116.36, 117.26, 117.94, 119.05, 119.07, 122.90, 122.97, 123.20, 124.55, 134.11, 139.64, 143.33, 150.44, 160.66, 168.49, Anal. calc. for $C_{21}H_{20}FN_3O_3$; C,66.13; H,5.29; N,11.02; found: C,66.15; H,5.32; N,11.04; ESI-MS: 382.3 $[M+H]^+$.

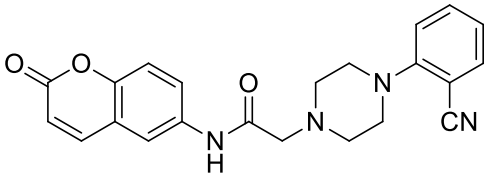
2-(4-(3-Chlorophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide (10c)



White Solid; Yield: 88 %; M.P: 250-252 ºC; IR (KBr) 3288, 3074, 2942, 2830, 1724, 1679, 1595, 1574, 1539, 1488, 1437, 1381, 1335, 1307, 1283, 1247, 1223, 1171, 1143, 1104, 1013, 986, 949, 918, 829, 764, 680 cm^{-1}**;** ^{1}H-NMR (400 MHz, $CDCl_3$):): δ 2.82 (t, *J* = 4.8Hz, 4H), 3.26 (s, 2H), 3.29 (t, *J* = 4.8Hz, 4H), 6.45 (d, *J* = 9.6Hz, 1H), 6.83 (dd, *J* = 2.4Hz, 8.0Hz, 1H), 6.86 (d, *J* = 8.0Hz, 1H), 6.90 (d, *J* = 1.6Hz, 1H), 7.20 (t, *J* = 8.0Hz, 1H), 7.29 (d, *J* = 9.6Hz, 1H), 7.50 (dd, *J* = 8.8Hz, 2.4Hz, 1H), 7.71 (d, *J* = 9.6Hz, 1H), 8.06 (d, *J* = 2.4Hz, 1H), 9.26 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 48.99, 53.37, 61.85, 114.19, 116.00, 117.29, 117.93, 119.08, 119.87, 123.18, 130.16, 134.05, 135.02, 143.29, 150.46, 151.96, 160.61, 168.26; Anal.

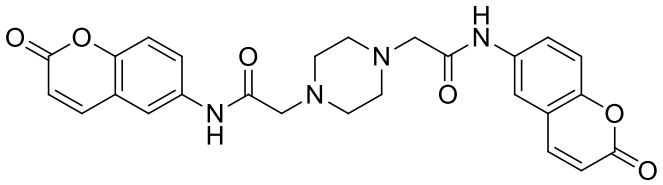
calc. for $C_{21}H_{20}ClN_3O_3$ C,63.40; H,5.07; N,10.56; found C,63.43; H,5.10; N,10.58; ESI-MS: 398.3 $[M+H]^+$.

2-(4-(2-Cyanophenyl)piperazin-1-yl)-N-(2-oxo-2H-chromen-6-yl)acetamide (10d)



White Solid; Yield: 88 %; M.P: 284-286ºC; IR (KBr) 3256, 2939, 2889, 2829, 2217, 1728, 1684,1575, 1527, 1490, 1442, 1378, 1341, 1288, 1233, 1175, 1137, 1102, 1014, 929, 885, 820, 763cm^{-1}**;** ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.90 (t, J = 4.4Hz, 4H), 3.30 (s, 2H), 3.34 (t, *J* = 4.4Hz, 4H), 6.47 (d, *J* = 9.6Hz, 1H), 7.06-7.11(m, 2H), 7.31 (d, *J* = 8.8Hz, 1H), 7.48 (dd, *J* = 2.4Hz & 8.8Hz, 1H), 7.52-7.56 (m, 1H), 7.61 (dd, *J* = 1.2Hz & 8.8Hz, 1H), 7.73 (d, *J* = 9.6Hz, 1H), 8.10 (d, *J* = 2.4Hz, 1H), 9.27 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 51.65, 53.55, 61.76, 106.29, 117.25, 118.00, 118.25, 118.83, 119.07, 122.34, 123.22, 133.90, 134.09, 134.41, 143.34, 150.44, 155.18, 160.62, 168.33 Anal. calc. for $C_{22}H_{20}N_4O_3$; C,68.03; H,5.19; N,14.42; found: C,68.05; H,5.21; N,14.45 ESI-MS: 389.3 $[M+H]^+$.

2,2'-(Piperazine-1,4-diyl)bis(N-(2-oxo-2H-chromen-6-yl)acetamide) (12)



White Solid; Yield: 88 %; M.P: 216-218ºC; IR (KBr) 3276, 3064, 2945, 2883, 2829, 1717, 1572, 1525, 1492, 1431, 1379, 1330, 1278, 1178, 1129, 1014, 920, 878, 824, 748, 641, cm^{-1}**;** ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.50 (broad s, 4H), 3.20 (s, 4H), 3.39 (s, 2H), 6.50 (d, *J* = 9.6Hz, 1H), 7.38 (d, *J* = 8.8Hz, 1H), 7.76 (dd, *J* = 2.0Hz & 8.8Hz, 1H), 8.07-8.09 (m, 2H), 9.97 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 52.48, 61.61, 116.42, 118.09, 118.50, 123.66, 134.90, 144.17, 149.37, 159.86, 168. 34 Anal. calc. for $C_{26}H_{24}N_4O_6$; C,63.93; H,4.95; N,11.47; found: C,63.95; H,4.93; N,11.51; ESI-MS: 489.4 $[M+H]^+$.

3b.5 Biological activity screening

3b.5.1 MTT assay:

The half-minimum inhibitory concentration (IC50) was determined utilizing the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as per the established protocol. Initially, cells were seeded onto a 96-well plate at a density of 1 x 104 cells per well and were allowed to incubate overnight in DMEM medium supplemented with 10% FBS. Subsequently, various concentrations of each chemical (0.5, 1, 10, 25, 50, 75, and 100 M) were added to the cells, followed by a further 48-hour incubation period.

Afterward, the plate underwent an additional 4-hour incubation with the addition of 20 µl of MTT solution (5 mg/mL in PBS). Following the removal of the supernatant solution, 100 µl of acidified isopropanol was employed to dissolve the resulting blue formazan crystals. The absorbance at 570 nm was then measured using a microplate reader (Metertech 960). The cell viability results are expressed as percentages relative to the control group, which is set at 100%. For the determination of IC_{50} values, Graph Pad Prism software was utilized. The formula used to calculate cell viability (%) is as follows:Cell viability (%) = (average absorbance of treated groups / average absorbance of control group) × 100%.

The IC_{50} values were subsequently calculated using Graph Pad Prism, allowing for a comprehensive analysis of the inhibitory concentration of the chemicals. Each experiment was performed in triplicates.

3b.6 References

[1] O.A.E.A. Abd Allah, L.A.M.E. Nassr, *Monatshefte Fur Chemie.*, **2016**, *147*, 735.

[2] E. Krystkowiak, R.A. Bachorz, J. Koput, *Dye. Pigment.*, **2015**, *112*, 335.

[3] M. Ramegowda, *New J. Chem.*, **2013**, *37*, 2648.

[4] E. Krystkowiak, K. Dobek, A. Maciejewski., *Int. J. Mol. Sci.*, **2014**, *15*, 16628.

[5] D. Yang, Y. Yang, Y. Liu, *J. Clust. Sci.*, **2014**, *25*, 467.

[6] S. Guha, S. Lohar, A. Banerjee, A. Sahana, S.K. Mukhopadhyay, J.S. Matalobos, D. Das, *Anal. Methods.*, **2012**, *4*, 3163.

[7] B. Zengin Kurt, F. Sonmez, D. Ozturk, A. Akdemir, A. Angeli, C.T. Supuran, *Eur. J. Med. Chem.*,**2019,** *183*, 111702.

[8] R. Soni, S.S. Soman, Bioorg. Chem., **2018,** *79*, 277.

[9] R. Sharma, S.S. Soman, *Eur. J. Med. Chem.*, **2015,** *90*, 342.

[10] S. Guha, S. Lohar, M. Bolte, D.A. Safin, D. Das, *Spectrosc. Lett.* **2012**, *45*, 225.

[11] R. Soni, S.S. Soman, *Bioorg. Chem.*, **2018,** *79*, 277.

[12] W.R. Mahmoud, Y.M. Nissan, M.M. Elsawah, R.H. Refaey, M.F. Ragab, K.M. Amin, *Eur. J. Med. Chem.*, **2019,** 182, 111651.

[13] J. V. Patil, S. Umar, R. Soni, S.S. Soman, S. Balakrishnan, *Synth. Commun.*, **2023**, *53*, 217–233.

[14] P. Thakare, A. Shinde, S. Dakhane, A. Chavan, V.D. Bobade, P.C. Mhaske, *J. Heterocycl. Chem.*, **2021**, *58*, 1867.

[15] R. L. Atkins, D. E. Bliss, *J. Org. Chem*., **1978**, *43*, 1975.

[16] Y. D. Kulkarni, D. Srivastava, D. A. Bishnoi, P. R. Dua, *J. Indian Chem. Soc*., **1996**, *73*, 173.

[17] A. K. Singh, G. Saxena, S. Dixit, Hamidullah, S. K Singh, S.K. Singh, M. Arshad, R. Konwar, *J. Mol. Str*., **2016**, *1111*, 90.