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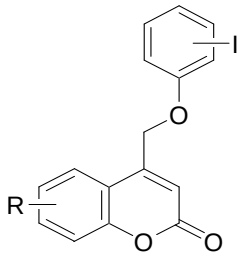
Graphical Abstract:



Iodo-4-aryloxymethylcoumarins

The thirty title compounds were synthesized and screened for their anticancer and anti-mycobacterial activity. Among these **10h** and **10i** having chlorine most effective.

Synthesis, structure–activity relationship of iodinated-4-aryloxymethyl-coumarins as potential anti-cancer and anti-mycobacterial agents

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Abstract:

A series of new iodinated-4-aryloxymethylcoumarins **6**, **8** and **10** have been obtained from the reaction of various 4-bromomethylcoumarins **4** with 2-iodophenol **5**, 3-iodophenol **7** and 4-iodophenol **9** respectively. All the title compounds were screened for anticancer activity against two cancer cell lines (MDA-MB human adenocarcinoma mammary gland and A-549 human lung carcinoma) and two mycobacterial strains (*Mycobacterium tuberculosis* H_{37} RV and *Mycobacterium Phlei*). The SAR results indicate that nine compounds are potent, among these **10h** and **10i** having chlorine most effective. This is the first report assigning *in vitro* anti-mycobacterial, anticancer and structure-activity relationship for this new class of iodinated-4-aryloxymethyl-coumarins.

Keywords: Coumarin; heterocycles; iodine; anticancer; anti-mycobacterial; SAR.

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1. Introduction

The cancer and tuberculosis are called the ‘big killer and intractable diseases’. Cancer, one of the most life-threatening diseases, has more than 200 distinct types associated with it, affecting over 60 human organs. More than 90% of all cancer-related deaths occur from metastasis of the primary cancer tumor. The early stages of cancer development carry the maximum potential for therapeutic intervention. Therefore, detecting premalignant or premetastatic malignant tumors when they are still confined within organ(s) is critical to enable effective treatment and improving survival rate. Among all cancers, lung cancer continues to be the most prevalent and life threatening globally. The disease has a severe impact on the quality of life, due to reduced oxygenation levels and a higher incidence of metastasis due to high blood flow in the lungs. It affects more than a million people worldwide and accounts for about 25% of all cancer deaths [1]. Great advances have been made mapping out the cellular pathways altered in tumors and the pathways that respond to cancer therapeutics. The obvious importance of the components of DNA damage response pathways as potential cancer therapeutic targets has stimulated researchers and pharmaceutical companies to develop numerous chemical inhibitors for many of the proteins involved in these pathways [2].

Tuberculosis (TB) remains the leading cause of mortality due to a bacterial pathogen, *Mycobacterium tuberculosis*. Approximately one-third of the world’s population has been infected with the causative organism *M. tuberculosis* (MTB), eight million become sick with TB every year, and globally it accounts for almost three million deaths annually. One-fifth of all deaths of adults in developing countries are due to TB,

which is a reemerging problem particularly in many industrialized countries. It is estimated that between 2005 and 2020, one billion people will be newly infected, over 125 million people will get sick and 30 million will die of tuberculosis if control is not further strengthened. In addition, the evolution of its new virulent forms like multidrug resistant (MDR-TB) and extremely drug resistant (XDR-TB) has become a major threat to human kind. Among HIV infected people, the resurgence of TB is alarming due to the development of pathogenic synergy. The worsening situation has prompted the World Health Organization (WHO) to declare tuberculosis a global public health crisis [3].

Among the oxygen heterocycles, coumarin derivatives are important motifs, widely found in many natural products, many of them displaying diverse biological activities such as antimicrobial, anti-inflammatory, analgesic, antioxidant, antimalarial, anticancer, antituberclosis and anti-HIV properties, which have been reviewed [4-12]. This type of special coumarin structure enables its derivatives readily interact with a diversity of enzymes and receptors in organisms through weak bond interactions, thereby exhibit wide potentiality as medicinal drugs [12]. The coumarin derivatives in recent studies have been reported to possess the potent anticancer effect through different mechanisms. The tricyclic coumarin sulfamate (STX64), (IC_{50} = 8 nM) a nonsteroid-based irreversible aromatase-steroid sulfatase (STS) inhibitor provides remarkable activity for the cure of prostate cancer, and most encouragingly, its clinical trials have been accomplished in 2011 [13-15]. For instance, 3,8-dibromo-7-hydroxy-4-methyl coumarin (DBC) (IC_{50} = 100 nM) is treated as a CK2 inhibitor to suppress neoplastic growth [16,17]. Novobiocin, a known DNA gyrase inhibitor, binds to a nucleotide-

binding site located on the Hsp90 C-terminus and induces degradation of Hsp90-dependent client proteins at ~700 μM in breast cancer cells [18-21]. 7-O-Alkoxy-4-methylumbelliferone derivatives with longer chains, especially nonyl and decyl have good inhibitory activity against *mycobacterium tuberculosis* [22,23]. Some biologically active anticancer [24-27] and anti-tuberculosis [28-31] agents having coumarin moiety are presented in figure 1 and 2, respectively.

Halogenation is an important approach in lead optimization for drug development and about half of the molecules used in high-throughput screening are halogenated. The biomedical implications of the findings are discussed with respect to vast potential applications in biomolecular design and drug discovery [32,33]. The biological importance of iodine in the compound have been well reported in the literature such as binding ability [34], cannabinoid receptor antagonists [35], as anti-cancer agent [36]. Amiodarone is an antiarrhythmic drug used for the treatment of tachyarrhythmias [37]. The amiodarone analogue (KB130015) was reported as a antiarrhythmic drug with an improved toxicity profile compared with amiodarone [38]. Three new iodinated tryptophan derivatives, plakohypaphorines have been isolated from the Caribbean sponge *Plakortis simplex* and evaluated for their antihistamine activity [39].

In the light of the above facts and in continuation of our interest in designing oxygen heterocycle based biologically active molecules [40-43], we planned to synthesize a new series of iodinated-4-aryloxymethyl coumarins in order to study their structure activity relationship and hoping that the new compounds might show significant anticancer and anti-tuberculosis activity.

2. Chemistry

The required substituted-4-bromomethylcoumarins **4** were prepared by the Pechmann cyclisation of substituted phenols **3** with 4-bromoethylacetoacetate **2** using sulphuric acid as the condensing agent. The 4-bromoethylacetoacetate **2** in turn was obtained by the bromination of ethylacetoacetate **1** in dry ether at 0-5 ^{o}C.

The nucleophilic displacement of 4-bromomethylcoumarins with three phenols viz., 2-iodophenol **5**, 3-iodophenol **7** and 4-iodophenol **9,** resulted the 4-aryloxymethylcoumarins **6**, **8** and **10,** respectively under standard acetone-potassium carbonate conditions at room temperature (Scheme 1). Formation of ethers was indicated by the difference in ^{1}H-NMR chemical shifts. The methylene protons in 4-bromomethylcoumarins **4** resonated around 4.6 δ ppm where as the corresponding 4-aryloxymethylcoumarins **6**, **8** and **10** exhibited this peak around 5.4 ppm. Further these were confirmed by their IR, ^{13}C NMR and mass spectral data.

3. Pharmacology

3.1 Anticancer screening

All the title compounds **6(a-j)**, **8(a-j)** and **10(a-j)** were screened for their *in vitro* anticancer activity against two cancer cell lines MDA-MB human adenocarcinoma mammary gland and A-549 human lung carcinoma by using MTT assay for the determination of MIC values and the results are presented in Table 1. All the thirty compounds were screened in the present study, MIC ranging from 1.56 to 100 µg/mL.

3.1.1 MDA-MB human adenocarcinoma mammary gland

In the series **6(a-j),** the compounds bearing iodine on the second position of the phenoxy moiety with the halogens (**6h, 6i, 6j**) and mono-methyl group (**6a, 6b**) on coumarin at 6^{th} and 7^{th} positions exhibited potent activity (MIC 12.5 µg/mL). Whereas decrease in activity were observed on varying the substituent by methoxy (**6d**, **6e**), benzo (**6f, 6g**) and dimethyl (**6c**) with MIC 25 µg/mL.

The change in the position of iodine from second to third position, i.e., the series of compounds **8(a-j)**, the activity increased from MIC 12.5 to 6.25 µg/mL for compounds bearing chlorine and bromine (**8h, 8i, 8j**). The decrease in activity were observed for mono methyl (**8a**, **8b)** compounds from 12.5 to 25 µg/mL, methoxy (**8d, 8e**) and benzo (**8f, 8g)** compounds from MIC 25 to 50 µg/mL. Whereas the activity of compound **8c** was unaffected.

Further, the change in the position of iodine from third to fourth position, i.e., the series of compounds **10(a-j)**, the activity increased from MIC 6.25 to 1.56 µg/mL for compounds bearing chlorine (**10h, 10i**) and to 3.125 µg/mL bromine (**10j**) on coumarin. The increase in activity were observed for compounds **10a, 10b, 10c** from 25 to 12.5 µg/mL and for compounds **10d, 10e, 10f, 10g** from MIC 50 to 25 µg/mL.

In the library of thirty compounds, **6(a-j)**, **8(a-j)** and **10(a-j)**, the chloro (**10h, 10i)** compounds and bromo (**10j)** compound displayed potent activity with MIC 1.56 and 3.125 µg/mL respectively. The chloro (**8h**, **8i)** and bromo (**8j**) compounds showed moderate activity with MIC 6.25 µg/mL.

3.1.2 A-549 human lung carcinoma

In the series of compounds **6(a-j),** bearing iodine on the second position of the phenoxy moiety with the halogens (**6h, 6i, 6j**) on coumarin at 6th and 7th positions exhibited potent activity with MIC 12.5 µg/mL. The moderate activity was exhibited by the methoxy (**6d**, **6e)** and benzo (**6f**, **6g)** compounds with MIC 25 µg/mL. The mono-methyl (**6a, 6b**) and dimethyl (**6c)** compounds exhibited very weak activity with MIC 50 µg/mL.

The change in the position of iodine from second to third in the phenoxy moiety, i.e., the series of compounds **8(a-j)**, the activity increased from MIC 12.5 to 6.25 µg/mL for compounds bearing chlorine and bromine (**8h, 8i, 8j**). The increase in activity was also observed for compounds **8(d-g)** and **8(a-c)** from MIC 25 to 12.5 µg/mL and MIC 50 to 25 µg/mL, respectively.

The change in the position of iodine from third to fourth in the phenoxy moiety, i.e., the series of compounds **10(a-j)**, the activity increased in all the compounds, from MIC 6.25 to 3.125 µg/mL for compounds bearing chlorine and bromine (**10h, 10i, 10j**). The increase in activity was also observed for compounds **10(d-g)** and **10(a-c)** from MIC 12.5 to 6.25 µg/mL and MIC 25 to 12.5 µg/mL, respectively.

In general the halogenated (**10h**, **10i, 10j**) compounds were exhibited potent activity with MIC 3.125 µg/mL. The halogenated (**8h**, **8i**, **8j**), methoxy (**10d**, **10e**) and benzo (**10f, 10g**) compounds were moderate activity with MIC 6.25 µg/mL.

In concise, the compounds bearing the chlorine at 6th and 7th position and bromine at 6th position on coumarin have the potential impact in improving the anti-cancer activity

compared to methyl, methoxy, benzo-substitutions. The iodine atom at 4th position on phenoxy moiety play an important role in enhancing the activity compared to its 3rd and 2nd position.

3.2 Anti-mycobacterial screening

The *in vitro* results of anticancer activity encouraged us to evaluate their anti-mycobacterial effect against *Mycobacterium tuberculosis* H_{37} RV and *Mycobacterium Phlei* by Microplate Alamar Blue Assay (MABA) for the determination of MIC values of all the synthesized compounds along with standard drugs streptomycin and pyrizanamide for the comparison are presented in Table 2. All the thirty compounds were screened in the present study, MIC ranging from 1.56 to 100 µg/mL.

3.2.1 *Mycobacterium tuberculosis* H_{37} RV

In the series **6(a-j),** the compounds bearing iodine on the second position of the phenoxy moiety with the halogens (**6h, 6i, 6j**) on coumarin at 6th and 7th positions exhibited potent activity with MIC 6.25 µg/mL. The mono-methyl (**6a, 6b**) and methoxy (**6d, 6e)** compounds showed the moderate activity with MIC 12.5 µg/mL. The MIC 25 µg/mL exhibited by dimethyl compound **6c.** The very weak activity were observed by benzo (**6f, 6g**) compounds with MIC 50 µg/mL.

The change in the position of iodine from second to third in the phenoxy moiety, i.e., the series of compounds **8(a-j)**, the activity decreased from MIC 6.25 to 12.5 µg/mL for compounds bearing chlorine and bromine (**8h, 8i, 8j**). The decrease in activity was

also showed by mono methyl (**8a, 8b)** and methoxy (**8d, 8e)** compounds from 12.5 to 25 µg/mL. The increase in activity was observed for compounds **8f, 8g** from MIC 50 to 25 µg/mL. The compound **8c** was unaffected.

The change in the position of iodine from third to fourth in the phenoxy moiety, i.e., the series of compounds **10(a-j)**, the activity increased for chloro (**10h, 10i)** and bromo **(10j)** compounds from MIC 12.5 to 1.56 and 6.25 µg/mL respectively. The increase in activity were also observed for compounds **10a, 10c** with MIC 25 to 12.5 µg/mL. Whereas the methyl (**10 b**), methoxy (**10d**, **10e**) and benzo (**10f**, **10g**) compounds were unaffected in their MIC with 25 µg/mL.

The chloro (**10h, 10i)** compounds showed excellent activity with MIC 1.56 µg/mL and were more potent than standard drugs streptomycin (MIC of 6.25 µg/mL), pyrizanamide (MIC of 3.125 µg/mL). The chloro (**6h**, **6i**) and bromo (**6j, 10j)** compounds showed moderate activity with MIC 6.25 µg/mL against *Mycobacterium tuberculosis.*

3.2.2 *Mycobacterium Phlei*

In the series of compounds **6(a-j)**, bearing iodine on the second position of the phenoxy moiety with the chlorine (**6h, 6i**) on coumarin at 6^{th} and 7^{th} positions exhibited potent activity with MIC 25 µg/mL. The moderate activity was exhibited by the dimethyl (**6c**), methoxy (**6d, 6e**), benzo (**6f**, **6g**), and bromo (**6j**) compounds 50 µg/mL. Whereas the mono methyl (**6a, 6b**) compounds showed least active with MIC greater than 100 µg/mL.

The change in the position of iodine from second to third position, i.e., the series of compounds **8(a-j)**, the chloro (**8h, 8i**) and bromo (**8j**) compounds exhibited potent activity in the series with increase in activity from 25 to 6.25 and 50 to 6.25 µg/mL respectively. The dimethyl (**8c**) and benzo (**8f**, **8g**) compounds exhibited moderate activity in this series with increase in activity MIC from 50 to 25 µg/mL. Similarly the mono methyl (**8a, 8b**) compounds were moderate active in series with increase in activity with MIC from greater than 100 to 12.5 µg/mL. The change in the position of iodine was unaffected on activity of methoxy (**8d, 8e**) compounds MIC 50 µg/mL.

Further, the change in the position of iodine from third to fourth position, i.e., the series of compounds **10(a-j)**, the activity increased from MIC 6.25 to 3.125 µg/mL for compounds bearing chlorine (**10h, 10i**). The activity remains same for bromo (**10i)** compound with 6.25 µg/mL. The methyl **(10a**, **10b)** compounds exhibited moderate activity with MIC 12.5 µg/mL which were unaffected. Whereas dimethyl **(10c)** and methoxy **(10d, 10e)** compounds showed moderate activities and increased the activity from 25 to 12.5 and 50 to 12.5 µg/mL respectively. The lease activity was exhibited by benzo (**10f, 10g**) compounds with MIC 25 µg/mL which were unaffected.

The chloro (**10h**,**10i)** compounds showed excellent activity with MIC 3.125 µg/mL and were more potent than standard drugs streptomycin (MIC of 6.25 µg/mL), and equal potent to pyrizanamide (MIC of 3.125 µg/mL). The chloro (**8h**, **8i)** and bromo **(8j, 10j)** compounds with MIC 6.25 µg/mL showed moderate activity against *Mycobacterium Phlei.*

To summarize, it is interesting to note that, the compounds having the chlorine at 6^{th} and 7^{th} position and bromine at 6^{th} position on coumarin have the exhibited potent activity compared to methyl, methoxy, benzo-substitutions. The similar trend was observed as in anti-cancer screening, the iodine atom at 4^{th} position on phenoxy moiety play an important role in enhancing the activity compared to its 3^{rd} and 2^{nd} position.

4. Results and Discussion

The IR spectrum of 4-(2-iodo-phenoxymethyl)-6-methyl-chromen-2-one (**6a**) (R = 6-CH_3) showed lactone carbonyl at 1710 cm^{-1}. The ^{1}H-NMR spectrum exhibited three singlets at 2.41, 5.50 and 6.82 δ ppm due to C6-CH_3, C4-CH_2 and C3-H, respectively. The aromatic protons were resonated as multiplet in the range of 6.84-7.85 δ ppm. This was further confirmed by ^{13}C NMR spectrum which agrees with the number of carbons and by its mass spectrum that shows the molecular ion peak *m/z* 393 (M+1), agrees with the molecular weight of the compound (Supplementary material, spectrum 1-4).

The IR spectrum of 4-(3-iodo-phenoxymethyl)-6-methyl-chromen-2-one (**8a**) (R = 6-CH_3) exhibited lactone carbonyl at 1715 cm^{-1}. The ^{1}H-NMR spectrum showed singlets at 2.40, 5.40 and 6.57 δ ppm due to C6-CH_3, C4-CH_2 and C3-H respectively. The remaining protons were resonated as a multiplet in the range of 7.11-7.77 δ ppm. Formation of the product was confirmed by its ^{13}C NMR and mass spectral data (Supplementary material, spectrum 9-12).

The IR spectrum of 4-(4-iodo-phenoxymethyl)-6-methyl-chromen-2-one (**10a**) (R = 6-CH_3) exhibited lactone carbonyl at 1704 cm^{-1}. The ^{1}H-NMR spectrum showed

singlets at 2.40, 5.40 and 6.53 δ ppm due to C6-CH_3, C4-CH_2 and C3-H respectively. The remaining aromatic protons resonated as a multiplet in the range of 7.01-7.68 δ ppm. The LCMS of compound showed a peak at *m/z* 393 (M+1) confirming its molecular weight (Supplementary material, spectrum 17-20).

Conclusions

The series of synthesized title compounds were characterized by spectral data and evaluated for their anticancer and anti-mycobacterial activity. By results, it is interesting to note that in general the compounds having the chlorine at 6^{th} and 7^{th} position on coumarin and bromine 6^{th} position on coumarin have the exhibited potent activity compared to other substitutions. The careful observation on SAR, the compounds **10h** and **10i** having the chlorine at 6^{th} and 7^{th} position on coumarin and iodine at 4^{th} position on phenoxy moiety exhibited potent anticancer and anti-mycobacterial activity. These two compounds are even more anti-mycobacterial than standard drugs under investigation. The higher activities of these compounds may lead to new anticancer and anti-mycobacterial drugs in future.

5. Experimental protocols

5.1. Chemistry

The melting points were determined by open capillary method and are uncorrected. The IR spectra (KBr disc) were recorded on a Nicolet-5700 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 400 and 300

MHz spectrometer using $CDCl_3$, DMSO-d_6 as solvents and TMS as an internal standard. The chemical shifts are expressed in δ ppm. The mass spectra were recorded using Agilent-single Quartz LC-MS. The elemental analysis was carried out using Heraus CHN rapid analyzer. The purity of the compound was checked by T.L.C. All the chemicals purchased were of analytical grade, and were used without further purification unless otherwise stated.

5.1.1. Synthesis of substituted-4-bromomethyl coumarins 4(a-j)

The required substituted-4-bromomethyl-coumarins [40, 44] have been synthesized by the Pechmann cyclization of substituted phenols with 4-bromoethylacetoacetate [45].

5.1.2 General procedure for the synthesis of iodinated-4-aryloxymethyl-coumarins 6 (a-j), 8 (a-j) and 10 (a-j):

A mixture of 2-iodophenol **5** (1.08g, 10 mmol) and anhydrous potassium carbonate (1.38g, 10 mmol) was stirred for 30 minutes in dry acetone (30 mL). To this, 6-methyl-4-bromomethylcoumarin **4a** (2.53g, 10 mmol) was added and the stirring was continued for 24 h. Then, the resulting reaction mixture was poured to crushed ice. The separated solid was filtered, washed with 1:1 HCl (30 mL) and with water. Then product **6a** was recrystallised from suitable solvent.

The similar procedure was followed for the synthesis of 4-aryloxymethylcoumarins **8** (**a-j**) and **10** (**a-j**) by the reaction of the substituted-4-bromomethylcoumarins **4** (**a-j**) with 3-iodophenol **7** and 4-iodophenol **9** respectively**.**

5.1.2.1 4-(2-Iodo-phenoxymethyl)-6-methyl-chromen-2-one (6a)

Colourless (Ethanol), m.p. 241 ^{o}C, yield 95 %; IR (KBr, ν in cm^{-1}): 1710 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.41 (*s*, 3H, C6-CH_3), 5.50 (*s*, 2H, C4-CH_2), 6.82 (*s*, 1H, C3-H), 6.84-7.85 (*m*, 7H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): 20.97, 66.77, 112.40, 113.80, 113.85, 116.87, 117.15, 123.25, 128.94, 132.96, 133.18, 134.08, 134.23, 149.30, 151.83, 152.05, 160.73. LCMS *m/z:* 393 [M+1]; Anal.calcd. for $C_{17}H_{13}IO_3$; C, 52.06; H, 3.34. Found: C, 52.16; H, 3.42.

5.1.2.2 4-(2-Iodo-phenoxymethyl)-7-methyl-chromen-2-one (6b)

Colourless (Ethanol), m.p. 190 ^{o}C, yield 90 %; IR (KBr, ν in cm^{-1}): 1706 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.42 (*s*, 3H, C7-CH_3), 5.56 (*s*, 2H, C4-CH_2), 6.61 (*s*, 1H, C3-H), 6.88-8.06 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 21.21, 66.79, 110.92, 113.45, 117.05, 117.15, 121.38, 126.41, 129.93, 131.51, 133.11, 133.88, 144.27, 146.09, 152.29, 160.79. LCMS *m/z:* 393 [M+1]; Anal.calcd. for $C_{17}H_{13}IO_3$; C, 52.06; H, 3.34. Found: C, 52.22; H, 3.48.

5.1.2.3 4-(2-Iodo-phenoxymethyl)-7,8-dimethyl-chromen-2-one (6c)

Colourless (Chloroform), m.p. 238 ^{o}C, yield 83 %; IR (KBr, ν in cm^{-1}): 1712 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.40 (*s*, 6H, C7 & C8-CH_3), 5.52 (*s*, 2H, C4-

CH_2), 6.95 (*s*, 1H, C3-H), 7.16-7.94 (*m*, 6H, Ar-H); LCMS *m/z:* 407 [M+1]; Anal.calcd. for $C_{18}H_{15}IO_3$; C, 53.22; H, 3.72. Found: C, 53.36; H, 3.86.

5.1.2.4 4-(2-Iodo-phenoxymethyl)-6-methoxy-chromen-2-one (6d)

Colourless (Chloroform), m.p. 214 ^oC, yield 88 %; IR (KBr, ν in cm^{-1}): 1701 (lactone C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 3.84 (*s*, 3H, C6-OCH_3), 5.58 (*s*, 2H, C4-CH_2), 6.98 (*s*, 1H, C3-H), 7.16-8.01 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 56.74, 66.89, 109.57, 112.83, 113.48, 117.64, 118.87, 119.98, 121.81, 129.14, 132.14, 144.18, 145.26, 148.94, 155.63, 160.16. LCMS *m/z:* 409 [M+1]; Anal.calcd. for $C_{17}H_{13}IO_4$; C, 50.02; H, 3.21. Found: C, 50.24; H, 3.29.

5.1.2.5 4-(2-Iodo-phenoxymethyl)-7-methoxy-chromen-2-one (6e)

Yellow (Ethanol), m.p. 166 ^oC, yield 86 %; IR (KBr, ν in cm^{-1}): 1704 (lactone C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 3.89 (*s*, 3H, C6-OCH_3), 5.61 (*s*, 2H, C4-CH_2), 7.06 (*s*, 1H, C3-H), 7.19-8.24 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 56.92, 66.93, 109.74, 112.92, 113.59, 117.77, 118.94, 120.08, 121.96, 129.46, 132.62, 144.56, 145.69, 148.98, 155.82, 160.28. LCMS *m/z:* 409 [M+1]; Anal.calcd. for $C_{17}H_{13}IO_4$; C, 50.02; H, 3.21. Found: C, 50.32; H, 3.33.

5.1.2.6 1-(2-Iodo-phenoxymethyl)-benzo[f]chromen-3-one (6f)

Pale yellow (Chloroform), m.p. 226 ^oC, yield 91 %; IR (KBr, ν in cm^{-1}): 1711 (lactone C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 5.91 (*s*, 2H, C4-CH_2), 7.36 (*s*, 1H, C3-H), 7.14-8.56 (*m*, 10H, Ar-H); LCMS *m/z:* 429 [M+1]; Anal.calcd. for $C_{20}H_{13}IO_3$; C, 56.10; H, 3.06. Found: C, 50.22; H, 3.11.

5.1.2.7 4-(2-Iodo-phenoxymethyl)-benzo[h]chromen-2-one (6g)

Yellow (Chloroform), m.p. 218 ^{o}C, yield 83 %; IR (KBr, ν in cm^{-1}): 1708 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): 5.66 (*s*, 2H, C4-CH_2), 7.21 (*s*, 1H, C3-H), 7.18-8.45 (*m*, 10H, Ar-H); LCMS *m/z:* 429 [M+1]; Anal.calcd. for $C_{20}H_{13}IO_3$; C, 56.10; H, 3.06. Found: C, 50.36; H, 3.19.

5.1.2.8 6-Chloro-4-(2-iodo-phenoxymethyl)-chromen-2-one (6h)

Colourless (Chloroform), m.p. 244 ^{o}C, yield 61 %; IR (KBr, ν in cm^{-1}): 1712 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.55 (*s*, 2H, C4-CH_2), 7.01 (*s*, 1H, C3-H), 7.11-7.96 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 66.59, 112.32, 118.08, 118.63, 121.01, 126.06, 128.10, 128.17, 129.36, 131.21, 131.49, 143.52, 152.17, 159.64. LCMS *m/z:* 413 [M+1]; Anal.calcd. for $C_{16}H_{10}ClIO_3$; C, 46.57; H, 2.44. Found: C, 46.70; H, 2.49.

5.1.2.9 7-Chloro-4-(2-iodo-phenoxymethyl)-chromen-2-one (6i)

Colourless (Chloroform), m.p. 225 ^{o}C, yield 59 %; IR (KBr, ν in cm^{-1}): 1708 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.57 (*s*, 2H, C4-CH_2), 7.04 (*s*, 1H, C3-H), 7.12-7.99 (*m*, 7H, Ar-H); LCMS *m/z:* 413 [M+1]; Anal.calcd. for $C_{16}H_{10}ClIO_3$; C, 46.57; H, 2.44. Found: C, 46.62; H, 2.56.

5.1.2.10 6-Bromo-4-(2-iodo-phenoxymethyl)-chromen-2-one (6j)

Colourless (Ethanol), m.p. 208 ^{o}C, yield 56 %; IR (KBr, ν in cm^{-1}): 1728 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.56 (*s*, 2H, C4-CH_2), 7.03 (*s*, 1H, C3-H), 7.14-8.01 (*m*, 7H, Ar-H); LCMS *m/z:* 459 [M+2]; Anal.calcd. for $C_{16}H_{10}BrIO_3$; C, 42.05; H, 2.21. Found: C, 42.21; H, 2.29.

5.1.2.11 4-(3-Iodo-phenoxymethyl)-6-methyl-chromen-2-one (8a)

Colourless (Ethanol), m.p. 164 ^{o}C, yield 82 %; IR (KBr, υ in cm^{-1}): 1715 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.40 (*s*, 3H, C6-CH_3), 5.40 (*s*, 2H, C4-CH_2), 6.57 (*s*, 1H, C3-H), 7.11-7.77 (*m*, 7H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz): 21.67, 66.62, 112.27, 112.68, 113.61, 114.67, 117.57, 123.05, 125.54, 128.93, 133.06, 134.18, 143.27, 149.46, 151.98, 153.76, 160.83. LCMS *m/z:* 393 [M+1]; Anal.calc. for $C_{17}H_{13}IO_3$; C, 52.06; H, 3.34. Found: C, 52.24; H, 3.49

5.1.2.12 4-(3-Iodo-phenoxymethyl)-7-methyl-chromen-2-one (8b)

Colourless (Ethanol), m.p. 170 ^{o}C, yield 92 %; IR (KBr, υ in cm^{-1}): 1711 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.43 (*s*, 3H, C7-CH_3), 5.39 (*s*, 2H, C4-CH_2), 6.46 (*s*, 1H, C3-H), 7.05-7.71 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 21.12, 66.08, 110.84, 113.53, 117.24, 117.08, 121.21, 126.36, 129.82, 131.40, 133.02, 133.81, 144.22, 146.01, 152.17, 160.14. LCMS *m/z:* 393 [M+1]; Anal.calc. for $C_{17}H_{13}IO_3$; C, 52.06; H, 3.34. Found: C, 52.28; H, 3.40

5.1.2.13 4-(3-Iodo-phenoxymethyl)-7,8-dimethyl-chromen-2-one (8c)

Colourless (Ethanol), m.p. 186 ^{o}C, yield 83 %; IR (KBr, υ in cm^{-1}): 1714 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.41 (*s*, 6H, C7 & C8-CH_3), 5.40 (*s*, 2H, C4-CH_2), 6.47 (*s*, 1H, C3-H), 7.04-7.92 (*m*, 6H, Ar-H); LCMS *m/z:* 407 [M+1]; Anal.calc. for $C_{17}H_{13}IO_3$; C, 53.22; H, 3.72. Found: C, 53.38; H, 3.80.

5.1.2.14 4-(3-Iodo-phenoxymethyl)-6-methoxy-chromen-2-one (8d)

Colourless (Ethanol), m.p. 167 ^{o}C, yield 83 %; IR (KBr, υ in cm^{-1}): 1707 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.87 (*s*, 3H, C6-OCH_3), 5.38 (*s*, 2H, C4-CH_2), 6.62 (*s*,

1H, C3-H), 6.88-7.39 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 56.09, 66.42, 109.46, 112.38, 113.42, 117.57, 118.37, 119.85, 121.38, 129.89, 132.60, 144.25, 145.78, 148.55, 155.89, 160.83. LCMS *m/z:* 409 [M+1]; Anal.calc. for $C_{17}H_{13}IO_4$; C, 50.06; H, 3.21. Found: C, 50.21; H, 3.25.

5.1.2.15 4-(3-Iodo-phenoxymethyl)-7-methoxy-chromen-2-one (8e)

Colourless (Ethanol), m.p. 158 ^{o}C, yield 69 %; IR (KBr, υ in cm^{-1}): 1709 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.88 (*s*, 3H, C7-OCH_3), 5.39 (*s*, 2H, C4-CH_2), 6.60 (*s*, 1H, C3-H), 6.97-7.67 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 56.97, 66.82, 109.62, 112.81, 113.52, 117.70, 118.83, 120.03, 121.83, 129.39, 132.51, 144.48, 145.60, 148.84, 155.80, 160.16. LCMS *m/z:* 409 [M+1]; Anal.calc. for $C_{17}H_{13}IO_4$; C, 50.06; H, 3.21. Found: C, 50.29; H, 3.31.

5.1.2.16 1-(3-Iodo-phenoxymethyl)-benzo[f]chromen-3-one (8f)

Grey (Ethanol), m.p. 168 ^{o}C, yield 91 %; IR (KBr, υ in cm^{-1}): 1717 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.46 (*s*, 2H, C4-CH_2), 6.63 (*s*, 1H, C3-H), 7.19-8.69 (*m*, 10H, Ar-H); LCMS *m/z:* 429 [M+1]; Anal.calc. for $C_{20}H_{13}IO_3$; C, 56.10; H, 3.06. Found: C, 56.29; H, 3.22

5.1.2.17 4-(3-Iodo-phenoxymethyl)-benzo[h]chromen-2-one (8g)

Brown(Acetone), m.p. 206 ^{o}C, yield 90 %; IR (KBr, υ in cm^{-1}): 1713 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.47 (*s*, 2H, C4-CH_2), 6.64 (*s*, 1H, C3-H), 7.15-8.65 (*m*, 10H, Ar-H); LCMS *m/z:* 429 [M+1]; Anal.calc. for $C_{20}H_{13}IO_3$; C, 56.10; H, 3.06. Found: C, 56.32; H, 3.26.

5.1.2.18 6-Chloro-4-(3-iodo-phenoxymethyl)-chromen-2-one (8h)

Grey (Ethanol), m.p. 203 ^{o}C, yield 65 %; IR (KBr, υ in cm^{-1}): 1712 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.38 (*s*, 2H, C4-CH_2), 6.51 (*s*, 1H, C3-H), 7.10-7.76 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 66.02, 112.26, 118.04, 118.57, 121.16, 126.18, 128.24, 128.11, 129.18, 131.16, 131.40, 143.48, 152.08, 159.57. LCMS *m/z:* 413 [M+1]; Anal.calc. for $C_{16}H_{10}ClIO_3$; C, 46.57; H, 2.44. Found: C, 46.36; H, 2.36

5.1.2. 19 7-Chloro-4-(3-iodo-phenoxymethyl)-chromen-2-one (8i)

Colourless (Ethanol), m.p. 195 ^{o}C, yield 62 %; IR (KBr, υ in cm^{-1}): 1710 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.38 (*s*, 2H, C4-CH_2), 6.53 (*s*, 1H, C3-H), 7.06-7.68 (*m*, 7H, Ar-H); LCMS *m/z:* 413 [M+1]; Anal.calc. for $C_{16}H_{10}ClIO_3$; C, 46.57; H, 2.44. Found: C, 46.29; H, 2.31.

5.1.2.20 6-Bromo-4-(3-iodo-phenoxymethyl)-chromen-2-one (8j)

Colourless (Ethanol), m.p. 214 ^{o}C, yield 58 %; IR (KBr, υ in cm^{-1}): 1708 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.24 (*s*, 2H, C4-CH_2), 6.49 (*s*, 1H, C3-H), 7.13-7.89 (*m*, 7H, Ar-H); LCMS *m/z:* 459 [M+2]; Anal.calc. for $C_{16}H_{10}BrIO_3$; C, 42.05; H, 2.21. Found: C, 42.31; H, 2.34.

5.1.2.21 4-(4-Iodo-phenoxymethyl)-6-methyl-chromen-2-one (10a)

Colourless (Ethyl acetate), m.p. 192 ^{o}C, yield 94 %; IR (KBr, υ in cm^{-1}): 1704 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.40 (*s*, 3H, C6-CH_3), 5.40 (*s*, 2H, C4-CH_2), 6.53 (*s*, 1H, C3-H), 7.01-7.68 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 20.69, 66.90, 112.93, 116.53, 116.63, 120.86, 125.88, 129.41, 130.99, 132.59, 133.36, 143.75,

145.57, 151.77, 160.26.LCMS *m/z:* 393 [M+1]; Anal.calc. for $C_{17}H_{13}IO_3$; C, 52.06; H, 3.34. Found: C, 52.24; H, 3.38.

5.1.2.22 4-(4-Iodo-phenoxymethyl)-7-methyl-chromen-2-one (10b)

Green (Ethyl acetate), m.p. 148 ^oC, yield 89 %; IR (KBr, υ in cm^{-1}): 1708 (lactone C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 2.40 (*s*, 3H, C7-CH_3), 5.48 (*s*, 2H, C4-CH_2), 6.72 (*s*, 1H, C3-H), 7.07-7.69 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 21.10, 66.12, 110.83, 113.21, 117.01, 117.12, 121.23, 126.36, 129.62, 131.36, 133.02, 133.69, 144.16, 146.01, 152.18, 160.68. LCMS *m/z:* 393 [M+1]; Anal.calc. for $C_{17}H_{13}IO_3$; C, 52.06; H, 3.34. Found: C, 52.49; H, 3.44.

5.1.2.23 4-(4-Iodo-phenoxymethyl)-7,8-dimethyl-chromen-2-one (10c)

Colourless (Ethyl acetate), m.p. 232 ^oC, yield 83 %; IR (KBr, υ in cm^{-1}): 1708 (lactone C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 2.43 (*s*, 3H, C7 & C8-CH_3), 5.41 (*s*, 2H, C4-CH_2), 6.61 (*s*, 1H, C3-H), 6.80-7.85 (*m*, 7H, Ar-H); LCMS *m/z:* 407 [M+2]; Anal.calc. for $C_{18}H_{15}IO_3$; C, 53.22; H, 3.72. Found: C, 53.06; H, 3.60.

5.1.2.24 4-(4-Iodo-phenoxymethyl)-6-methoxy-chromen-2-one (10d)

Pale yellow (Ethyl acetate), m.p. 196 ^oC, yield 94 %; IR (KBr, υ in cm^{-1}): 1707 (lactone C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 3.87 (*s*, 3H, C6-OCH_3), 5.25 (*s*, 2H, C4-CH_2), 6.68 (*s*, 1H, C3-H), 6.91-7.86 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 56.02, 66.30, 109.18, 112.29, 113.40, 117.51, 118.33, 119.76, 121.24, 129.68, 132.46, 144.21, 145.62, 148.61, 155.77, 160.12. LCMS *m/z:* 409 [M+1]; Anal.calc. for $C_{17}H_{13}IO_3$; C, 50.02; H, 3.21. Found: C, 50.14; H, 3.32.

5.1.2.25 4-(4-Iodo-phenoxymethyl)-7-methoxy-chromen-2-one (10e)

Pale yellow (Ethyl acetate), m.p. 188 ^{o}C, yield 88 %; IR (KBr, υ in cm^{-1}): 1711 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.89 (*s*, 3H, C7-OCH_3), 5.29 (*s*, 2H, C4-CH_2), 6.70 (*s*, 1H, C3-H), 6.73-7.91 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 57.08, 66.10, 109.18, 112.24, 113.46, 117.58, 118.80, 120.01, 121.78, 129.21, 132.58, 144.41, 145.73, 148.69, 155.76, 160.10. LCMS *m/z:* 409 [M+1]; Anal.calc. for $C_{17}H_{13}IO_3$; C, 50.02; H, 3.21. Found: C, 50.26; H, 3.36.

5.1.2.26 1-(4-Iodo-phenoxymethyl)-benzo[f]chromen-3-one (10f)

Green (Ethyl acetate), m.p. 188 ^{o}C, yield 86 %; IR (KBr, υ in cm^{-1}): 1706 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.88 (*s*, 2H, C4-CH_2), 7.34 (*s*, 1H, C3-H), 7.11-8.12 (*m*, 10H, Ar-H); LCMS *m/z:* 429 [M+1]; Anal.calc. for $C_{20}H_{13}IO_3$; C, 56.10; H, 3.06. Found: C, 56.01; H, 3.02.

5.1.2.27 4-(4-Iodo-phenoxymethyl)-benzo[h]chromen-2-one (10g)

Yellow (Ethyl acetate), m.p. 240 ^{o}C, yield 84 %; IR (KBr, υ in cm^{-1}): 1701 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.65 (*s*, 2H, C4-CH_2), 7.28 (*s*, 1H, C3-H), 6.93-8.05 (*m*, 10H, Ar-H); LCMS *m/z:* 429 [M+1]; Anal.calc. for $C_{20}H_{13}IO_3$; C, 56.10; H, 3.06. Found: C, 56.03; H, 3.04.

5.1.2.28 6-Chloro-4-(4-iodo-phenoxymethyl)-chromen-2-one (10h)

Yellow (Ethyl acetate), m.p. 196 ^{o}C, yield 76 %; IR (KBr, υ in cm^{-1}): 1716 (lactone C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.17 (*s*, 2H, C4-CH_2), 6.74 (*s*, 1H, C3-H), 6.90-7.71 (*m*, 7H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 66.70, 112.94, 118.02, 118.79,

121.06, 126.13, 128.14, 128.26, 129.44, 131.34, 131.72, 143.63, 152.30, 159.73. LCMS *m/z:* 413 [M+1]; Anal.calc. for $C_{16}H_{10}ClIO_3$; C, 46.57; H, 2.44. Found: C, 46.36; H, 2.38.

5.1.2.29 7-Chloro-4-(4-iodo-phenoxymethyl)-chromen-2-one (10i)

Pale yellow (Ethyl acetate), m.p. 210 ^oC, yield 71 %; IR (KBr, υ in cm^{-1}): 1713 (lactone C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 5.21 (*s*, 2H, C4-CH_2), 6.81 (*s*, 1H, C3-H), 6.97-7.85 (*m*, 7H, Ar-H); LCMS *m/z:* 413 [M+1]; Anal.calc. for $C_{16}H_{10}ClIO_3$; C, 46.57; H, 2.44. Found: C, 46.41; H, 2.40.

5.1.2.30 6-Bromo-4-(4-iodo-phenoxymethyl)-chromen-2-one (10j)

Colourless (Ethyl acetate), m.p. 179 ^oC, yield 66 %; IR (KBr, υ in cm^{-1}): 1713 (lactone C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 5.42 (*s*, 2H, C4-CH_2), 6.72 (*s*, 1H, C3-H), 6.90-7.74 (*m*, 7H, Ar-H); LCMS *m/z:* 459 [M+2]; Anal.calc. for $C_{16}H_{10}BrIO_3$; C, 42.05; H, 2.21 Found: C, 42.16; H, 2.34.

5.2 Anti-Cancer activity

In all the experiments, different cell lines were seeded at a final density of 2 x 10^4 cells/well, in 96 well microtiter plates. Cytotoxicty was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, according to the method of Mosmann (1983) [46]. Briefly, the cells (2 x 10^4) were seeded in each well containing 0.1 mL of medium in 96 well plates. After overnight incubation, the cells were treated with different test concentrations of test compounds (5-200 mg/mL) at identical conditions.. The cell viability was assessed after 24 h, by adding 10 mL of MTT (5 mg/mL) per well. The plates were incubated at 37 ^oC for additional 3 h. The medium was

discarded and the formazan blue, which formed in the cells, was dissolved with 100 mL of DMSO. The rate of colour production was measured at 570 nm in a spectrophotometer (Spectra MAX Plus; Molecular Devices; supported by SOFTmax PRO-5.4). The percent inhibition of cell viability was determined with reference to the control values (without test compound). The data were subjected to linear regression analysis and the regression lines were plotted for the best straight-line fit. The IC_{50} (inhibition of cell viability) concentrations were calculated using the respective regression equation.

5.3 Anti-mycobacterial activity

The anti-mycobacterial activity of compounds was assessed against *M. tuberculosis* H_{37}RV and *M. phlei* using Microplate Alamar Blue Assay (MABA) [47]. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µL of sterile deionized water was added to all outer perimeter wells of sterile 96-well plate to minimize evaporation of medium in the test wells during incubation. The 96-well plate received 100 µL of the Middlebrook 7H9 broth, and serial dilution of compounds was made directly on plate. The final drug concentrations tested were 100–0.2 µg/mL. Plates were covered and sealed with parafilm and incubated at 37 ^{o}C for 5 days. After this time, 25 µL of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 were added to the plate and incubated for 24 h. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as lowest drug concentration that prevented the colour change from blue to pink.

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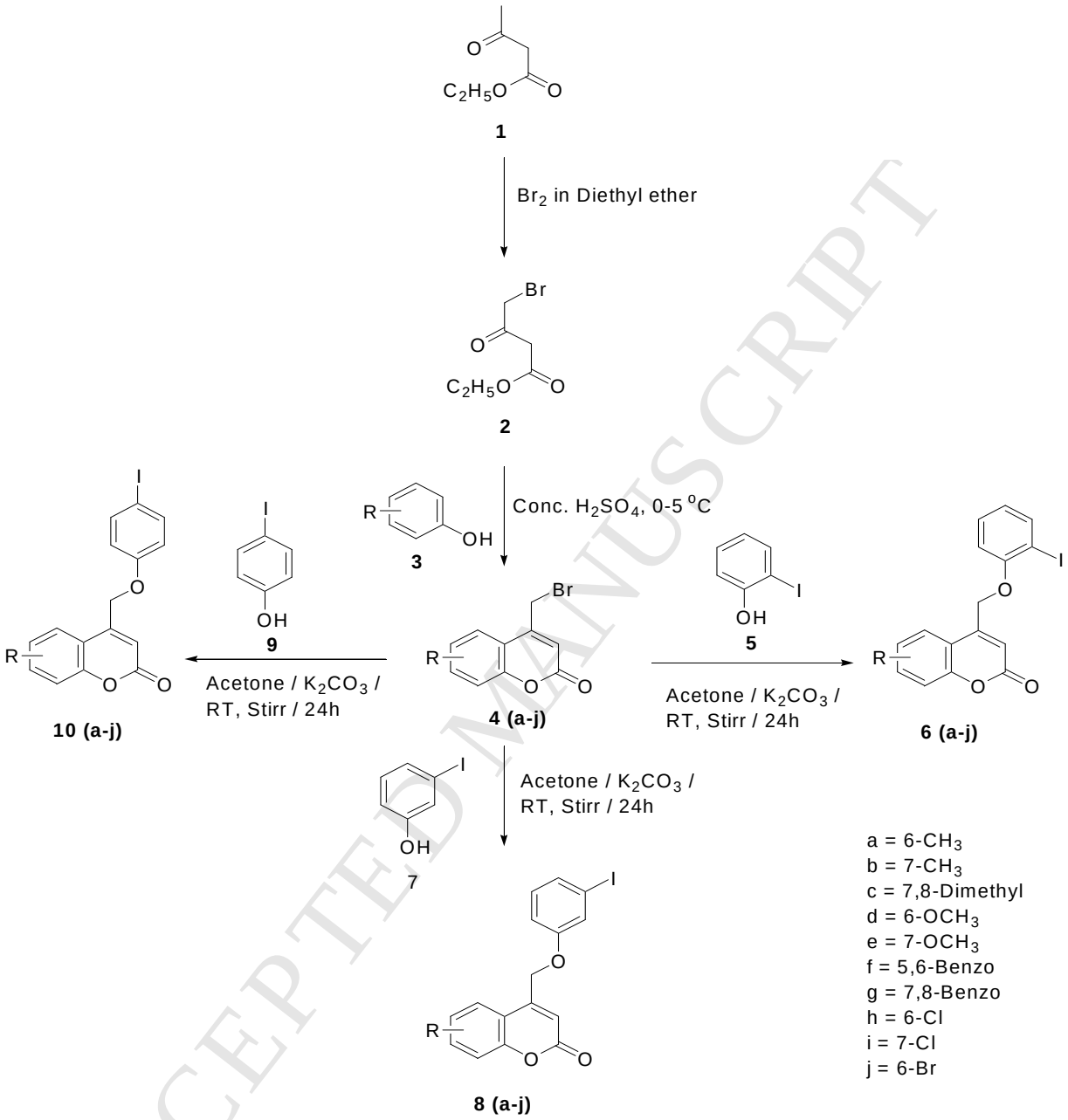
Table 1. Results of anti-cancer activity of compounds **6(a-j)**, **7(a-j)** and **10(a-j)** MICs (µg/mL)

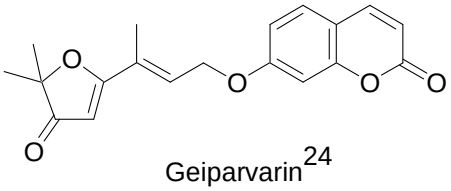
Compound	R	R2	Cancer cell lines	
			MDA-MB human adenocarcinoma mammary gland	***A-549 human lung carcinoma***
6a	6-CH_3	2-I	12.5	50
6b	7-CH_3	2-I	12.5	50
6c	7,8-diCH_3	2-I	25	50
6d	6-OCH_3	2-I	25	25
6e	7-OCH_3	2-I	25	25
6f	5,6-Benzo	2-I	25	25
6g	7,8-Benzo	2-I	25	25
6h	6-Cl	2-I	12.5	12.5
6i	7-Cl	2-I	12.5	12.5
6j	6-Br	2-I	12.5	12.5
8a	6-CH_3	3-I	25	25
8b	7-CH_3	3-I	25	25
8c	7,8-diCH_3	3-I	25	25
8d	6-OCH_3	3-I	50	12.5
8e	7-OCH_3	3-I	50	12.5
8f	5,6-Benzo	3-I	50	12.5
8g	7,8-Benzo	3-I	50	12.5
8h	6-Cl	3-I	6.25	6.25
8i	7-Cl	3-I	6.25	6.25
8j	6-Br	3-I	6.25	6.25
10a	6-CH_3	4-I	12.5	12.5
10b	7-CH_3	4-I	12.5	12.5
10c	7,8-diCH_3	4-I	12.5	12.5
10d	6-OCH_3	4-I	25	6.25
10e	7-OCH_3	4-I	25	6.25
10f	5,6-Benzo	4-I	25	6.25
10g	7,8-Benzo	4-I	25	6.25
10h	6-Cl	4-I	1.56	3.125
10i	7-Cl	4-I	1.56	3.125
10j	6-Br	4-I	3.125	3.125

Table 2. Results of anti-mycobacterial activity of compounds **6(a-j)**, **7(a-j)** and **10(a-j)** MICs (µg/mL)

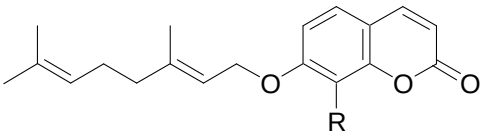
Compound	R	R2	*Mycobacterium tuberculosis* ($H_{37}RV$)	*Mycobacterium Phlei*
6a	6-CH_3	2-I	12.5	>100
6b	7-CH_3	2-I	12.5	>100
6c	7,8-diCH_3	2-I	25	50
6d	6-OCH_3	2-I	12.5	50
6e	7-OCH_3	2-I	12.5	50
6f	5,6-Benzo	2-I	50	50
6g	7,8-Benzo	2-I	50	50
6h	6-Cl	2-I	6.25	25
6i	7-Cl	2-I	6.25	25
6j	6-Br	2-I	6.25	50
8a	6-CH_3	3-I	25	12.5
8b	7-CH_3	3-I	25	12.5
8c	7,8-diCH_3	3-I	25	25
8d	6-OCH_3	3-I	25	50
8e	7-OCH_3	3-I	25	50
8f	5,6-Benzo	3-I	25	25
8g	7,8-Benzo	3-I	25	25
8h	6-Cl	3-I	12.5	6.25
8i	7-Cl	3-I	12.5	6.25
8j	6-Br	3-I	12.5	6.25
10a	6-CH_3	4-I	12.5	12.5
10b	7-CH_3	4-I	25	12.5
10c	7,8-diCH_3	4-I	12.5	12.5
10d	6-OCH_3	4-I	25	12.5
10e	7-OCH_3	4-I	25	12.5
10f	5,6-Benzo	4-I	25	25
10g	7,8-Benzo	4-I	25	25
10h	6-Cl	4-I	1.56	3.125
10i	7-Cl	4-I	1.56	3.125
10j	6-Br	4-I	6.25	6.25
Standard	Streptomycin	-	6.25	6.25
	Pyrizanamide	-	3.125	3.125



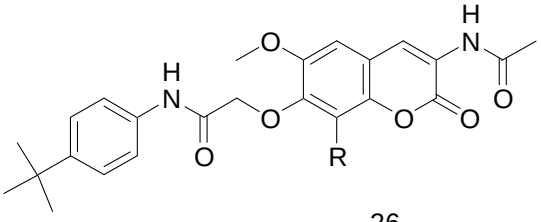
Scheme 1. Synthesis of Iodinated-4-aryloxymethylcoumarins [**6**], **[8]** and **[10].**



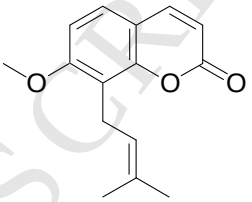
Geiparvarin[24]



Auraptene (R = H)[25]
Collinin (R = OCH_3)

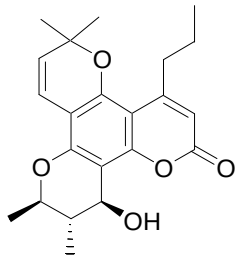


Scopoletin derivative[26]



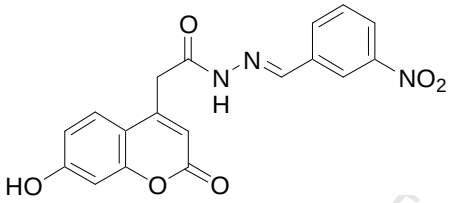
Osthole[27]

Figure 1. Structures of some potent anticancer (coumarin ether linkage) compounds.



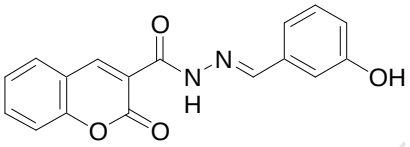
(+)-Calanolide A [28]

MIC = 3.13 µg/mL



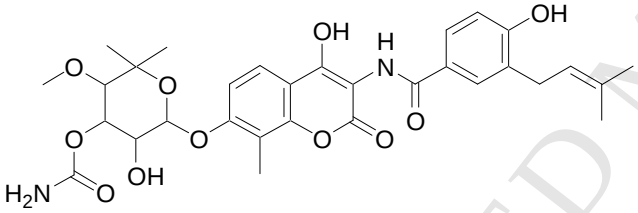
Coumarin-4-acetic acid benzylidene hydrazide [29]

MIC = 6.25 µg/mL

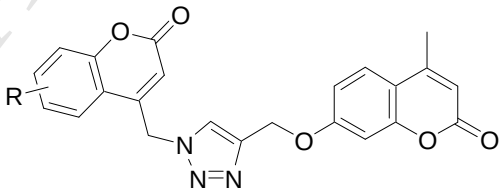


Coumarin-3-acetic acid benzylidene hydrazide [30]

MIC = 50 µg/mL



Novobiocin



Bis-Coumarinyl-Triazoles [31]

R = 6-Cl

R = 7-Cl

R = 5,6-Benzo

MIC = 6.25 µg/mL

Figure 2. Some potent anti-mycobacterial agents bearing coumarin and coumarin ether linkage.

Highlights

- Thirty new iodinated-4-aryloxymethyl coumarin derivatives were synthesized.
- Evaluated for their anticancer and anti-mycobacterial activities for all the synthesized compounds
- The compounds **10h** and **10i** bearing chlorine showed significant activity.

Supplementary material

Spectrum No. 1. IR (KBr) of compound **6a**.

Spectrum No. 2. ^{1}H NMR (DMSO-d_6) of compound **6a**.

Spectrum No. 3. ^{13}C NMR ($CDCl_3$) of compound **6a**.

Spectrum No. 4. LCMS of compound **6a**.

Spectrum No. 5. IR (KBr) of compound **6b**.

Spectrum No. 6. ^{1}H NMR (DMSO-d_6) of compound **6b**.

Spectrum No. 7. ^{13}C NMR (DMSO-d_6) of compound **6b**.

Spectrum No. 8. LCMS of compound **6b**.

Spectrum No. 9. IR (KBr) of compound **8a**.

Spectrum No. 10. ^{1}H NMR (DMSO-d_6) of compound **8a**.

Spectrum No. 11. ^{13}C-NMR ($CDCl_3$) of compound **8a**.

Spectrum No. 12. LCMS of compound **8a**.

Spectrum No. 13. IR (KBr) of compound **8d**.

Spectrum No. 14. ^{1}H NMR (DMSO-d_6) of compound **8d**.

Spectrum No. 15. ^{13}C NMR (DMSO-d_6) of compound **8d**.

Spectrum No. 16. LCMS of compound **8d**.

Spectrum No. 17. IR (KBr) of compound **10a**.

Spectrum No. 18. ^{1}H NMR (DMSO-d_6) of compound **10a**.

Spectrum No. 19. ^{13}C NMR (DMSO-d_6) of compound **10a**.

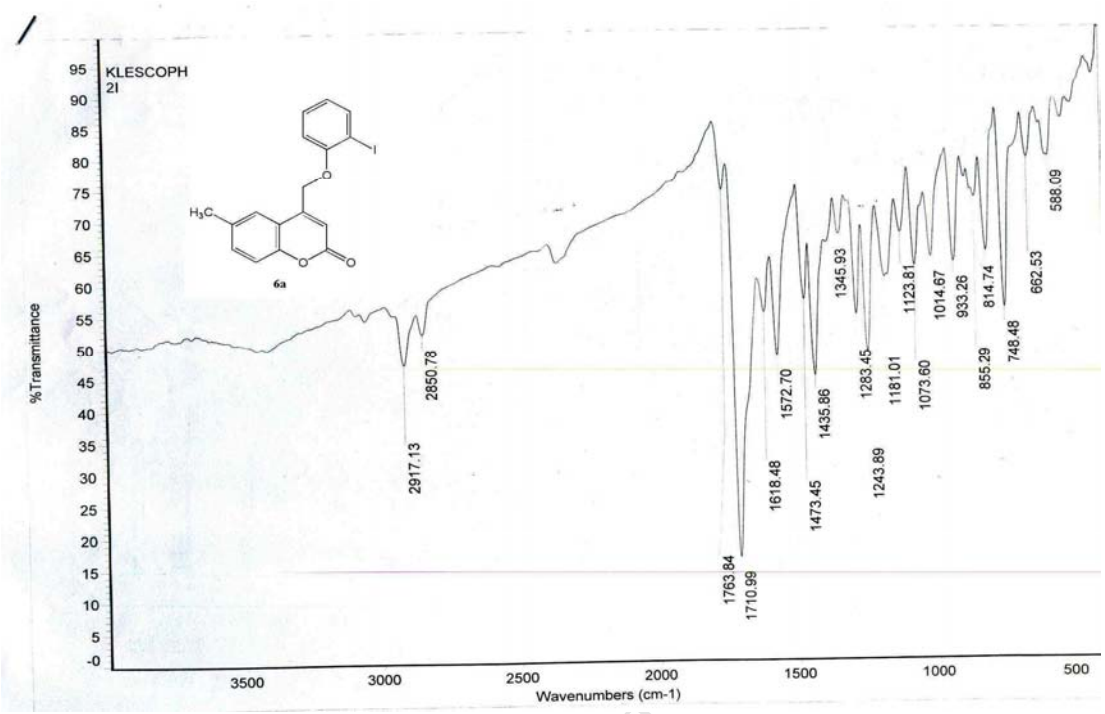
Spectrum No. 20. LCMS of compound **10a**.

Spectrum No. 21. IR (KBr) of compound **10h**.

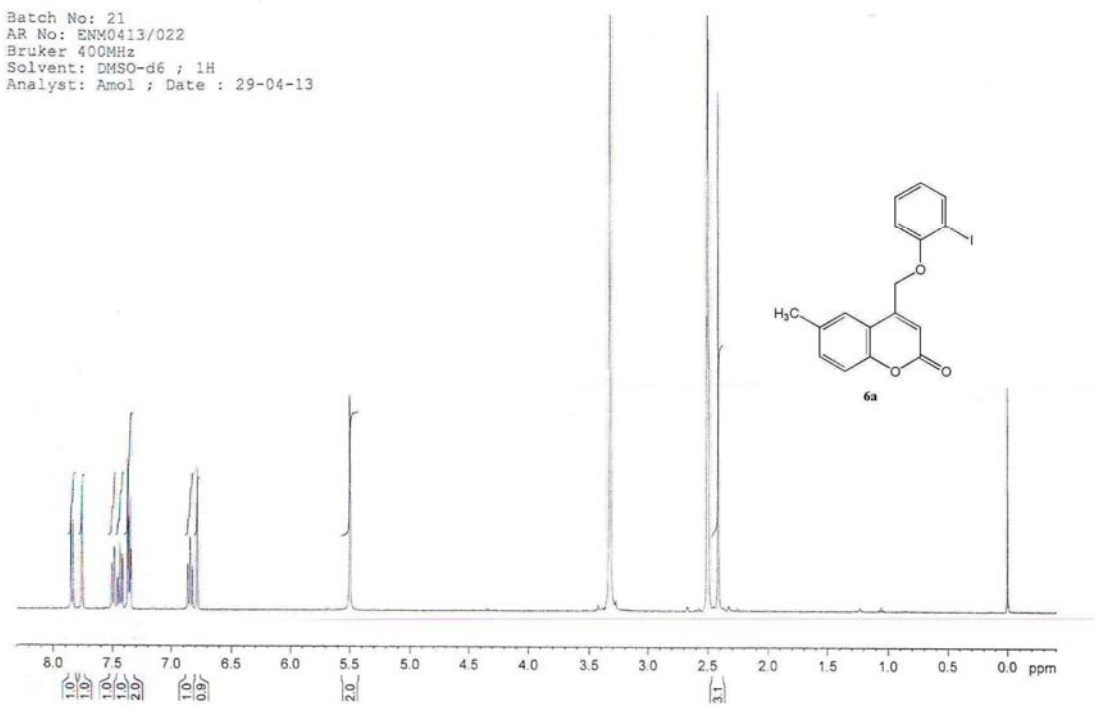
Spectrum No. 22. ^{1}H NMR (DMSO-d_6) of compound **10h**.

Spectrum No. 23. ^{13}C NMR (DMSO-d_6) of compound **10h**.

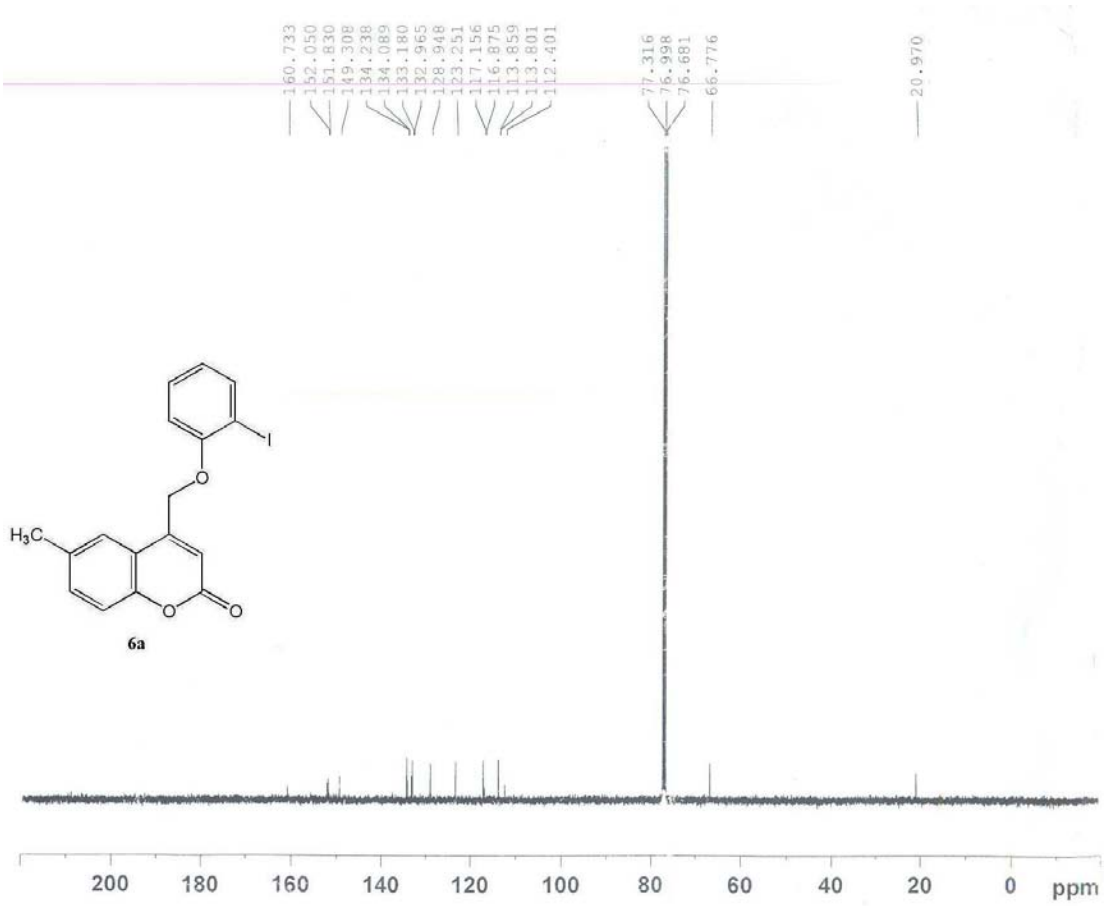
Spectrum No. 24. LCMS of compound **10h**.



Spectrum No. 1. IR (KBr) of compound **6a**.



Spectrum No. 2. ^{1}H NMR (DMSO-d_6) of compound **6a**.

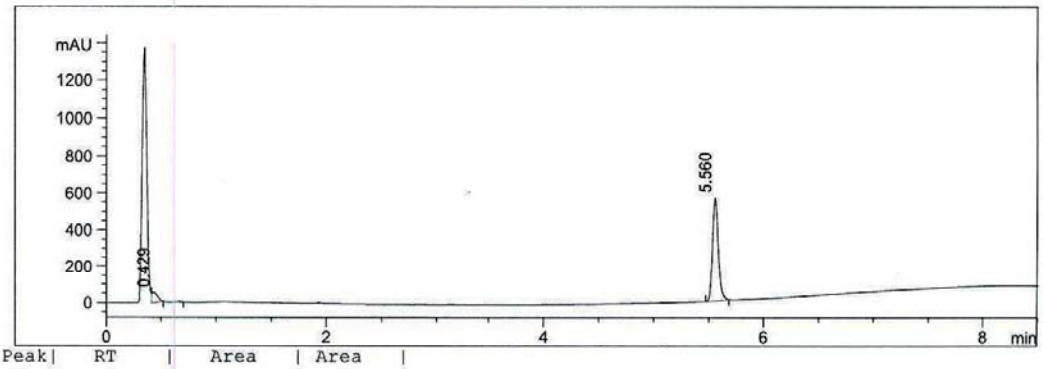


Spectrum No. 3. ^{13}C NMR ($CDCl_3$) of compound **6a**.

LC-MS REPORT

Method info : A: 0.1% TFA IN H2O , B:0.1% TFA IN ACN ; Flow Rate:2.0 ml/min
COLUMN:XBridge C8 (50x4.6mm, 3.5µm), +ve mode

TIME	%B
0	05
8.0	100
8.1	100
8.5	05
10	05



Peak No	RT min	Area	Area %
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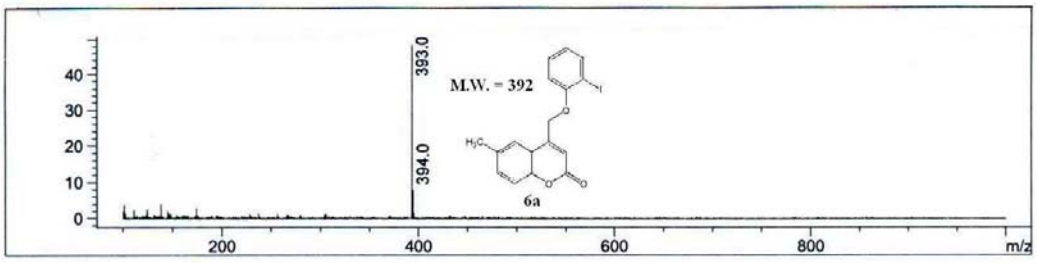
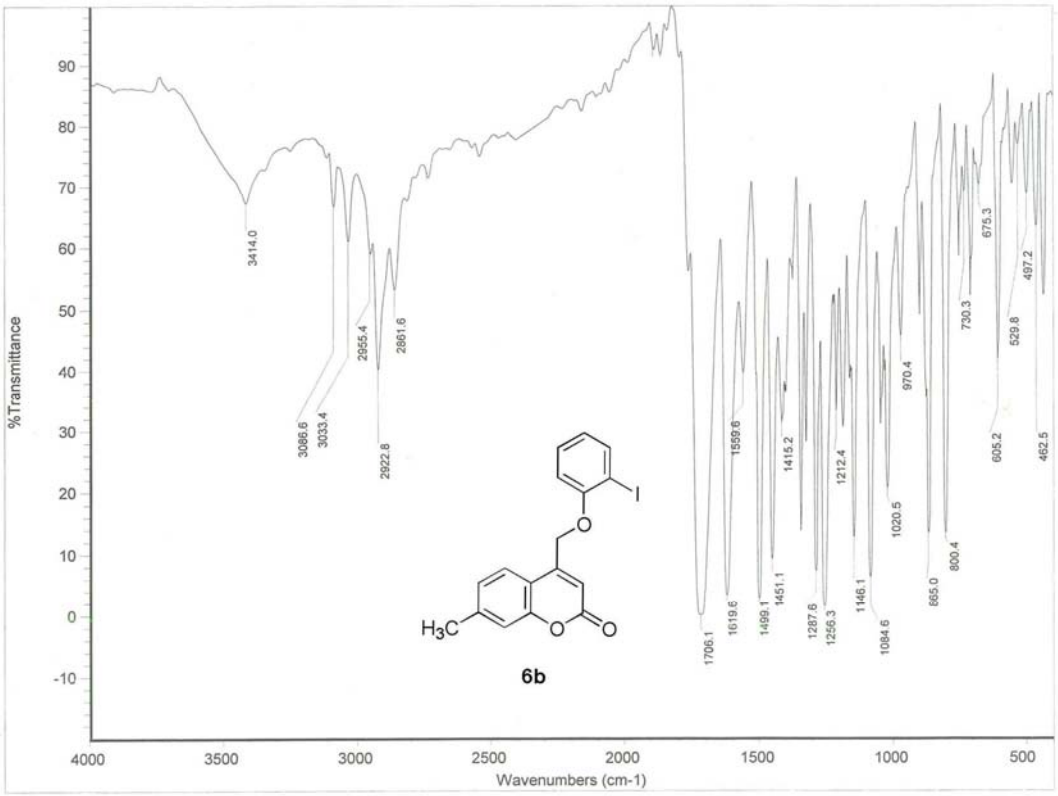
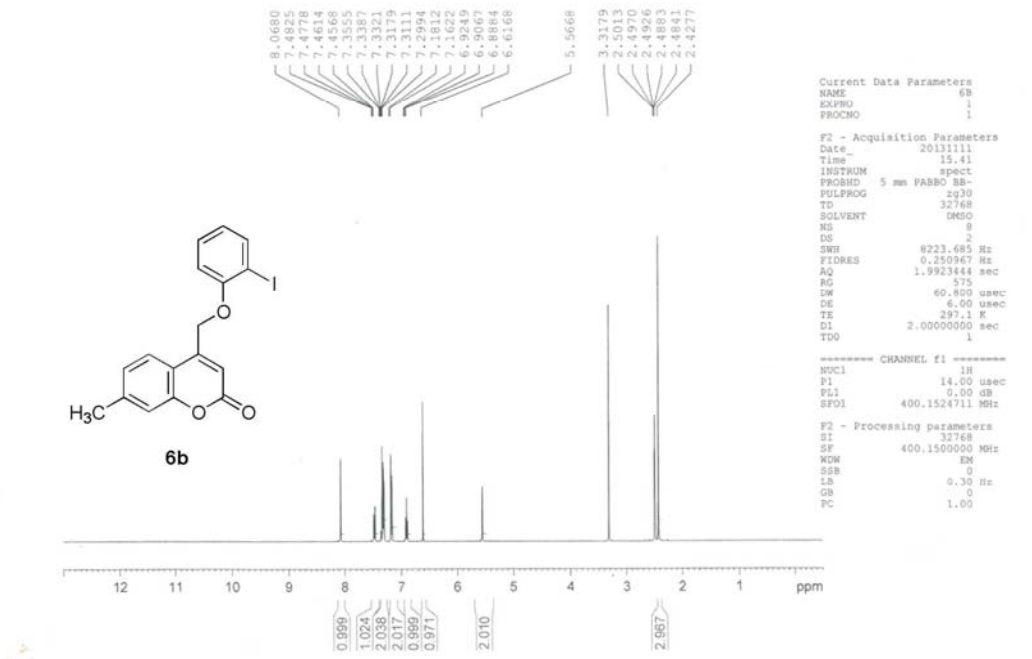
2	5.560	2.086e+003	99.476



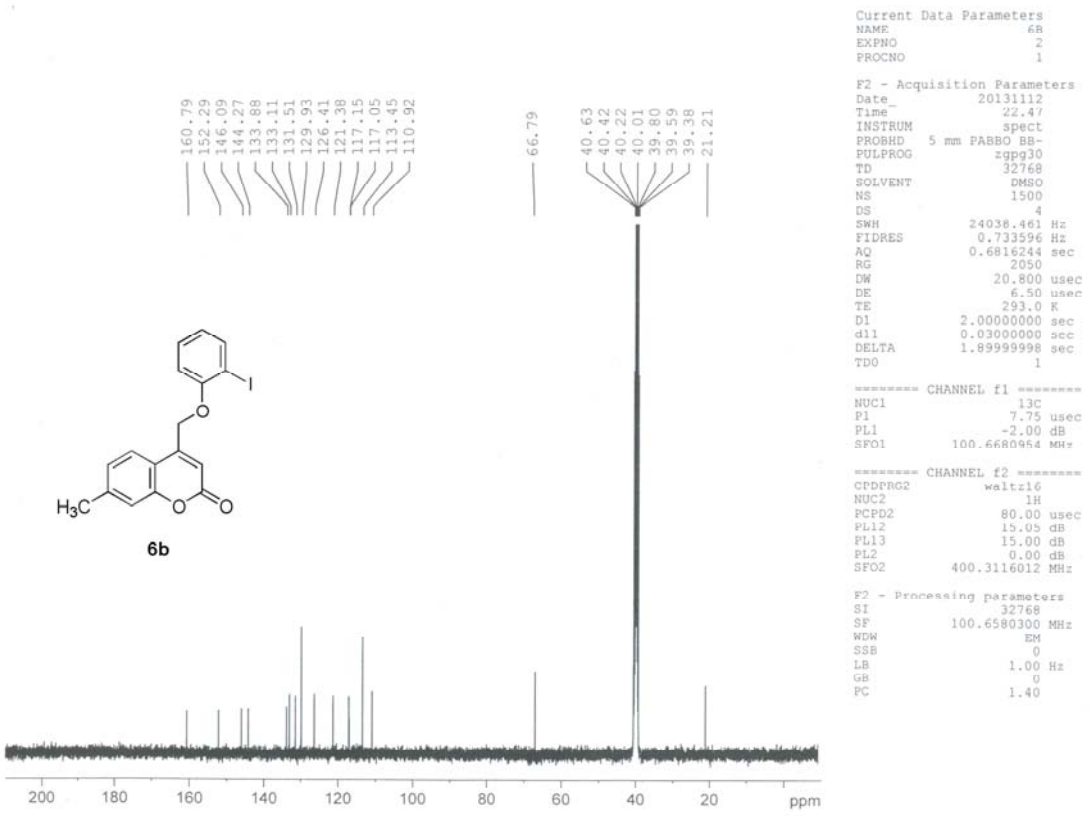
Spectrum No. 4. LCMS of compound **6a**.



Spectrum No. 5. IR (KBr) of compound **6b**.



Spectrum No. 6. ^{1}H NMR (DMSO-d_6) of compound **6b**.

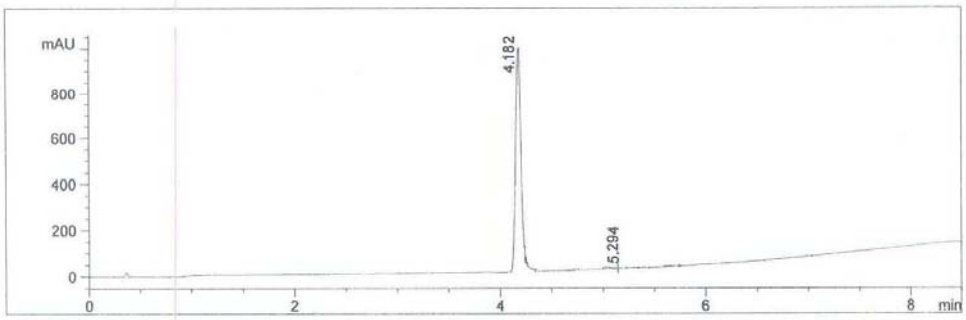


Spectrum No. 7. ^{13}C NMR (DMSO-d_6) of compound **6b**.

LC-MS REPORT

Method info : A: 0.1% TFA IN H2O , B:0.1% TFA IN ACN ; Flow Rate:2.0 ml/min
COLUMN:XBridge C8 (50x4.6mm, 3.5µ), +ve mode

TIME	%B
0	05
8.0	100
8.1	100
8.5	05
10.0	05



Peak No	RT min	Area	Area %
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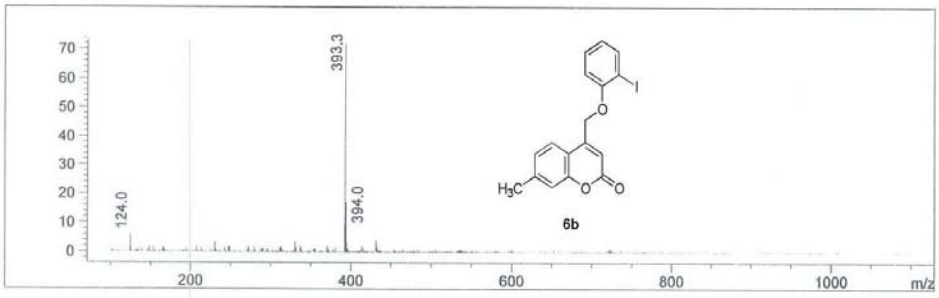
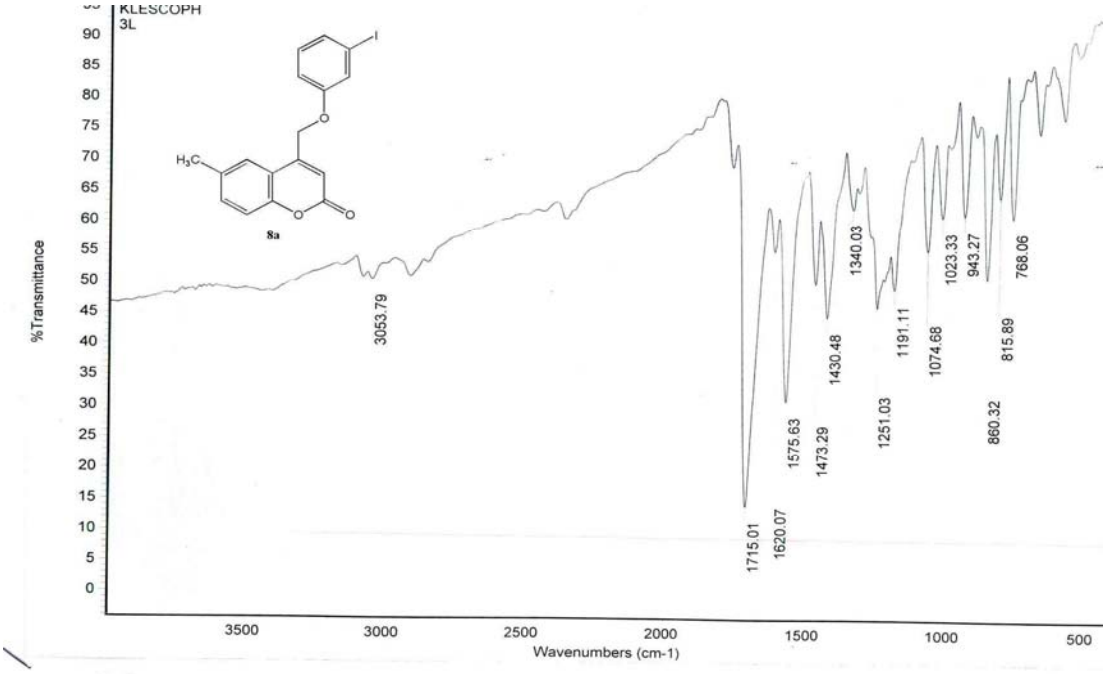
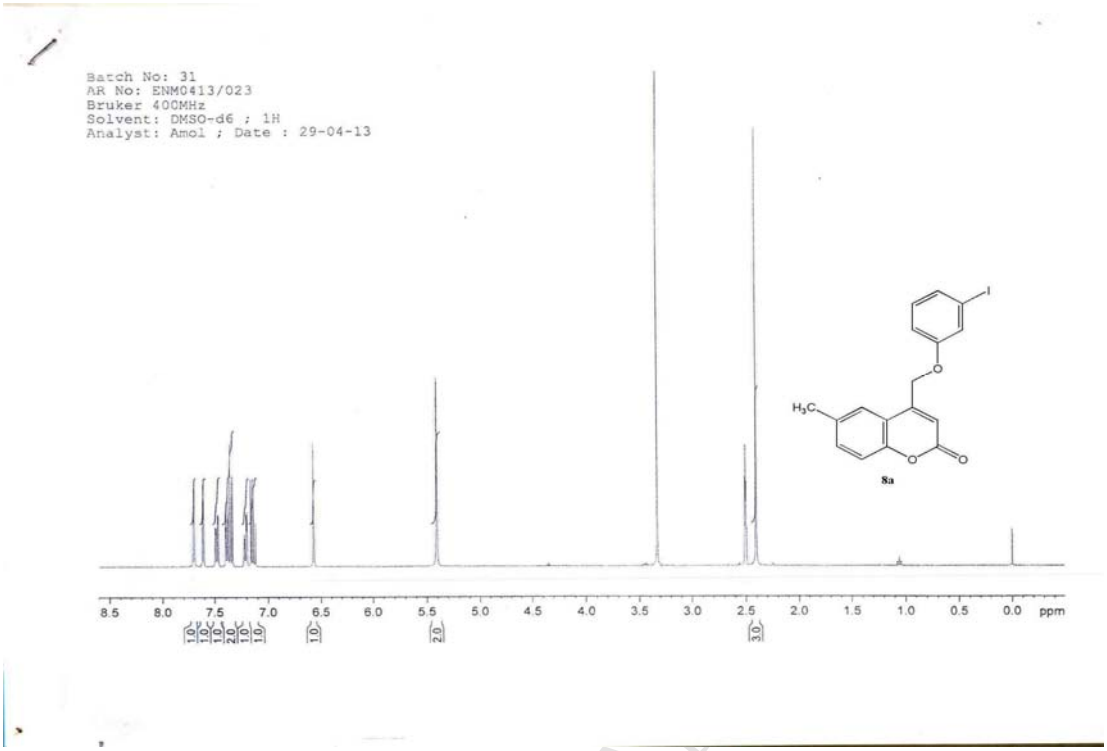


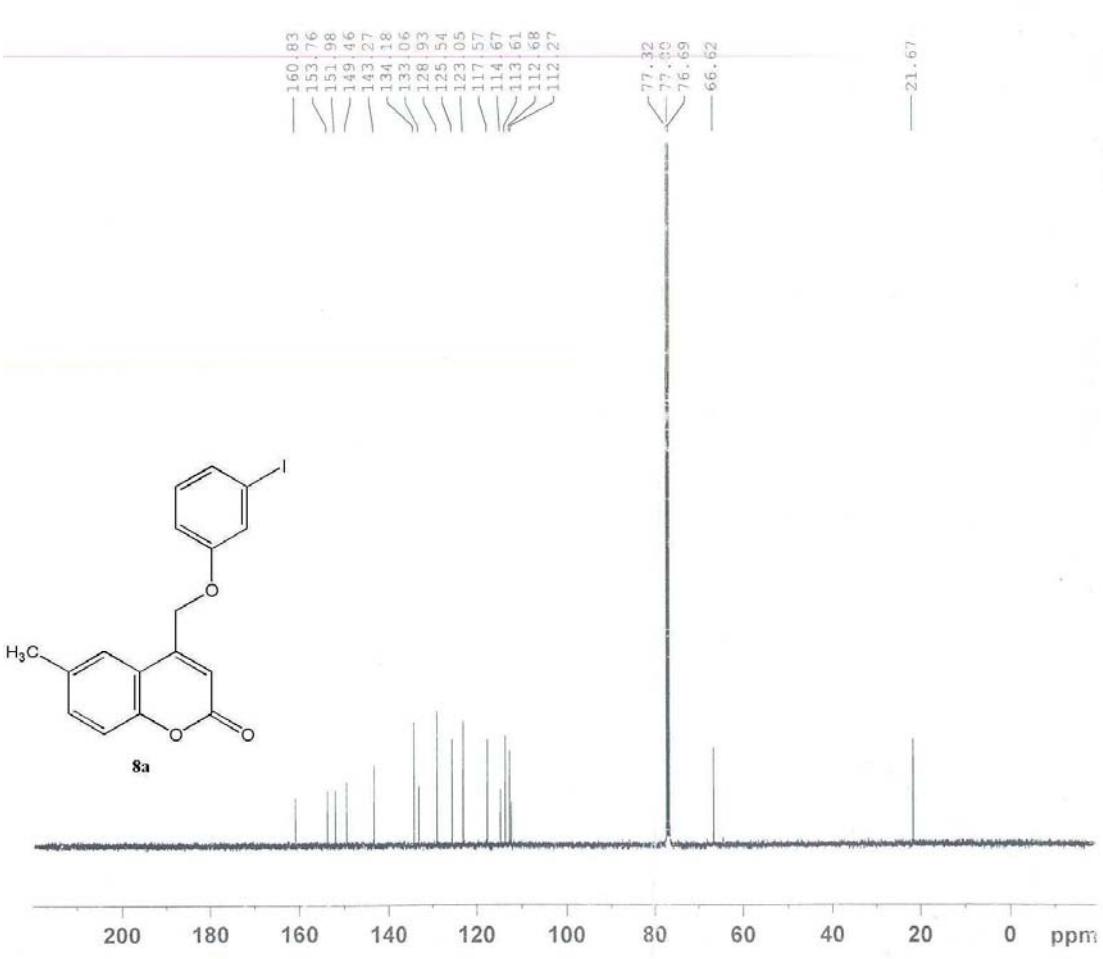
Spectrum No. 8. LCMS of compound **6b**.



Spectrum No. 9. IR (KBr) of compound **8a**.



Spectrum No. 10. ^{1}H NMR (DMSO-d_6) of compound **8a**.

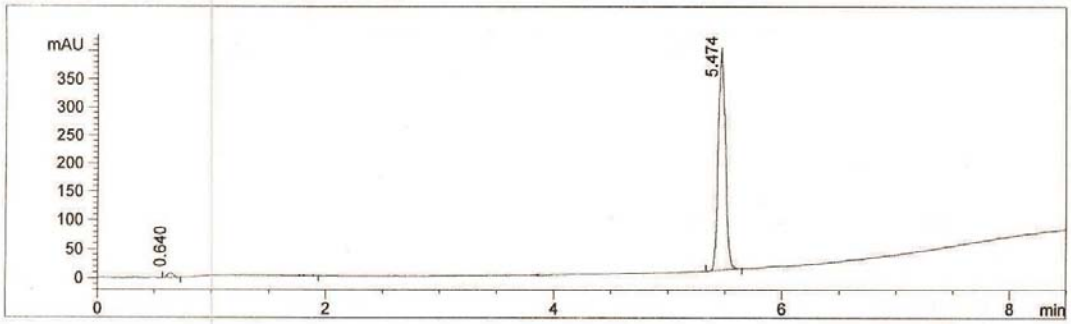


Spectrum No. 11. ^{13}C-NMR ($CDCl_3$) of compound **8a**.

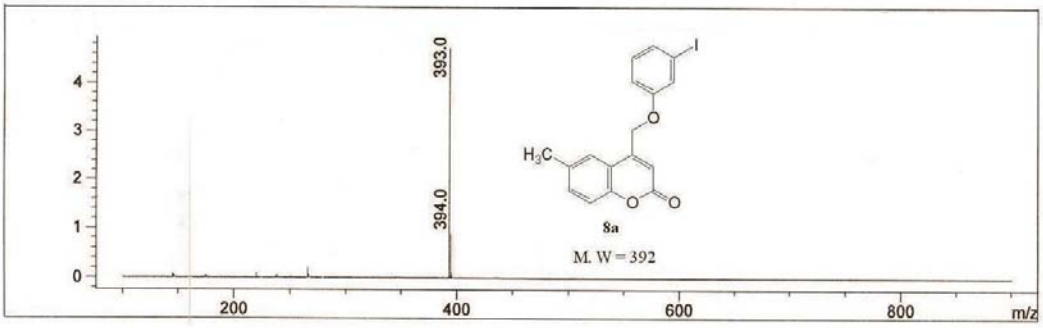
LC-MS REPORT

Method info : A: 0.1% TFA IN H2O , B:0.1% TFA IN ACN ; Flow Rate:2.0 ml/min
COLUMN:XBridge C8 (50x4.6mm, 3.5µ), +ve mode

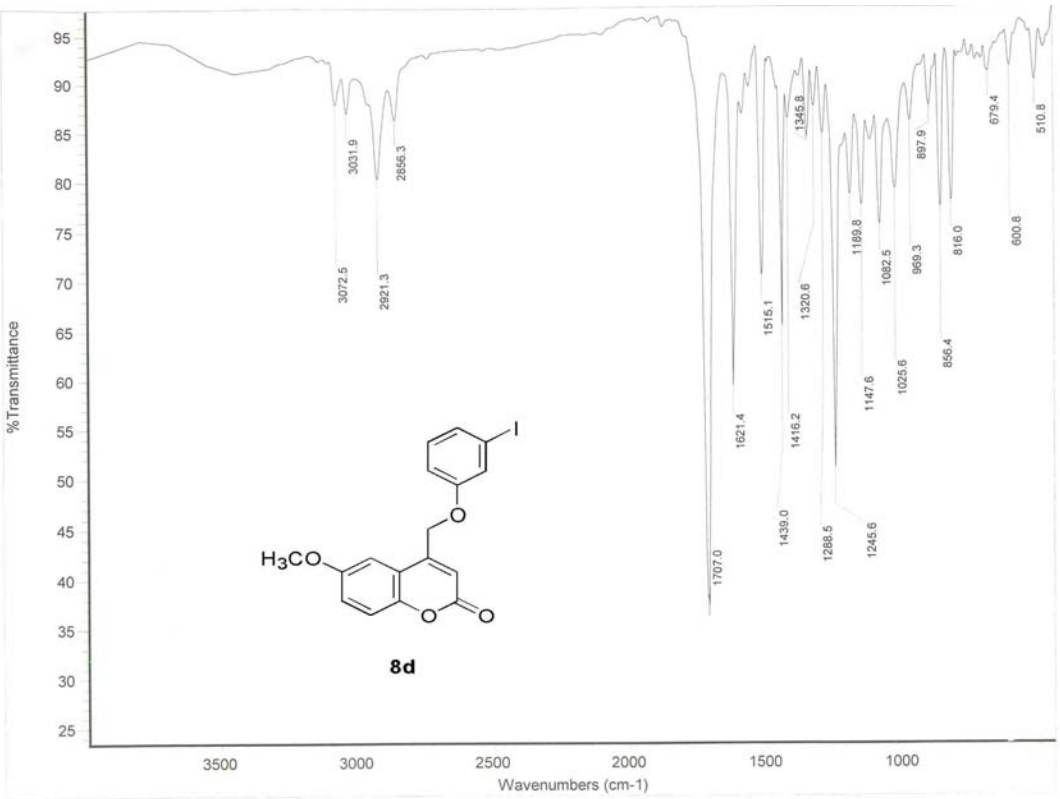
TIME	%B
0	05
8.0	100
8.1	100
8.5	05
10.0	05



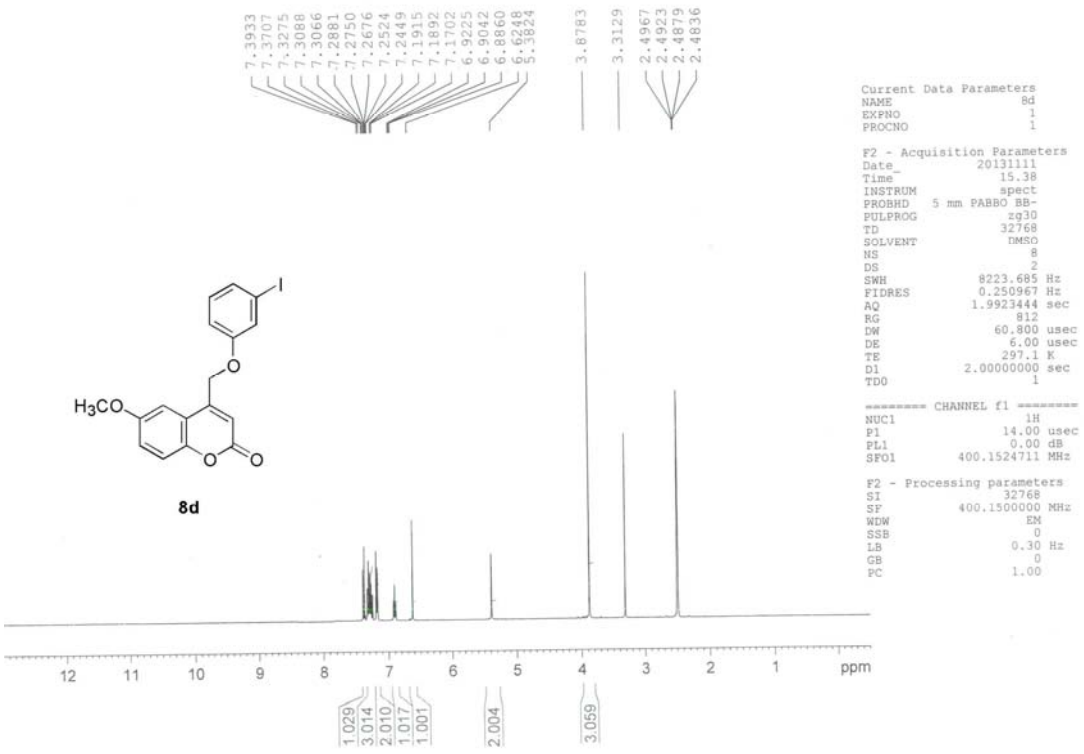
Peak No	RT min	Area	Area %
1	0.640	2.781e+001	1.569
2	5.474	1.691e+003	98.432



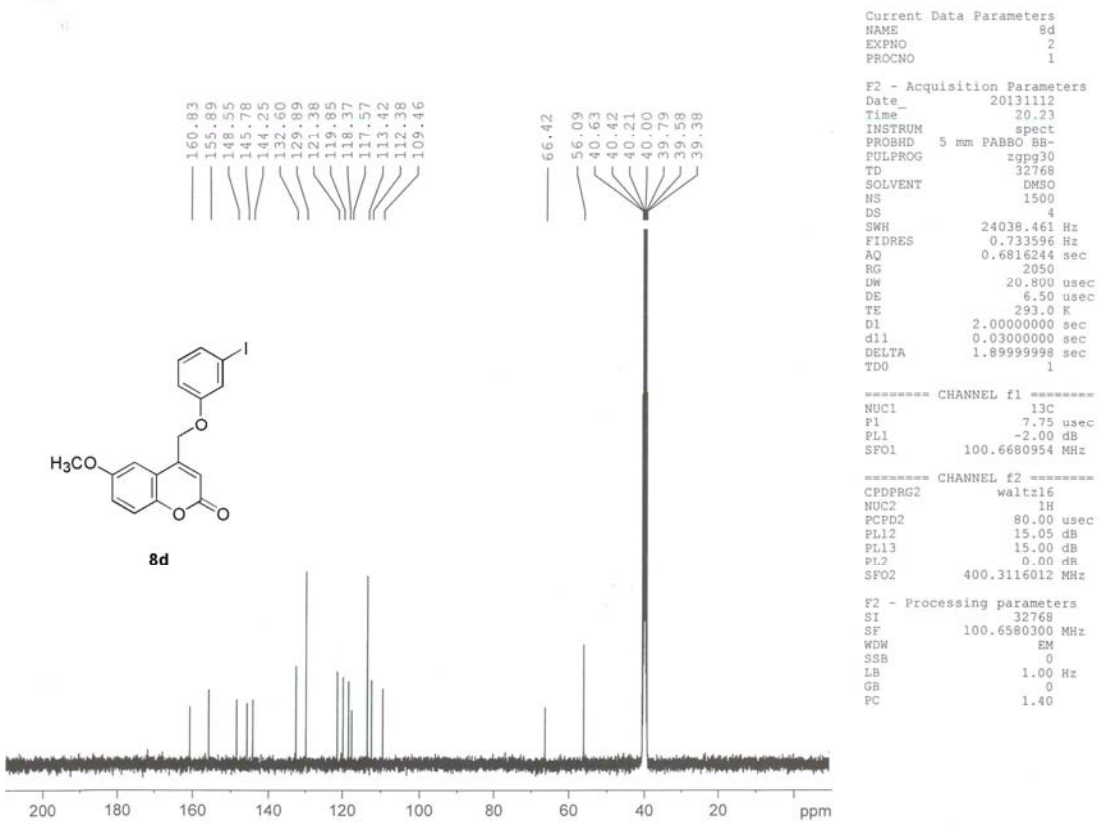
Spectrum No. 12. LCMS of compound **8a**.



Spectrum No. 13. IR (KBr) of compound **8d**.



Spectrum No. 14. ^{1}H NMR (DMSO-d_6) of compound **8d**.

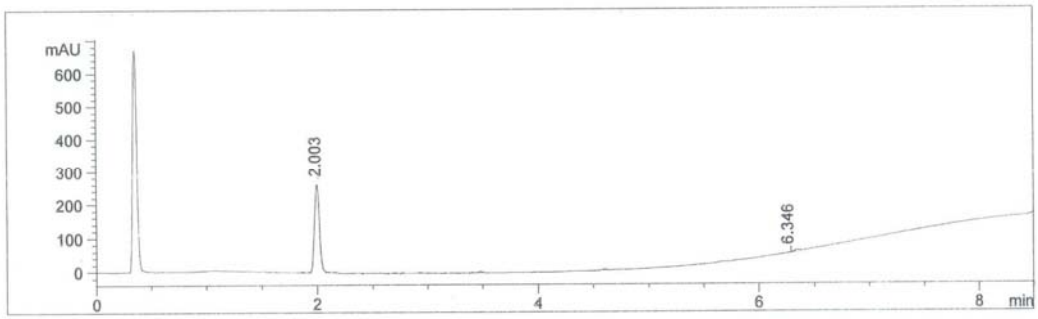


Spectrum No. 15. ^{13}C NMR (DMSO-d_6) of compound **8d**.

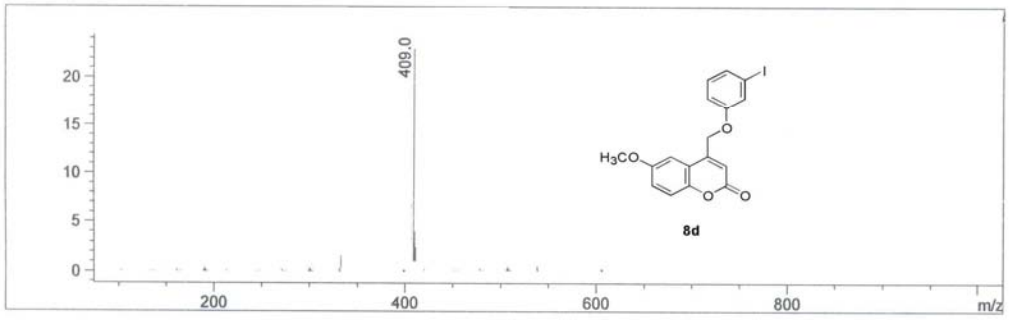
LC-MS REPORT

Method info : A: 0.1% TFA IN H2O , B:0.1% TFA IN ACN ; Flow Rate:2.0 ml/min
COLUMN:XBridge C8 (50x4.6mm, 3.5µm), +ve mode

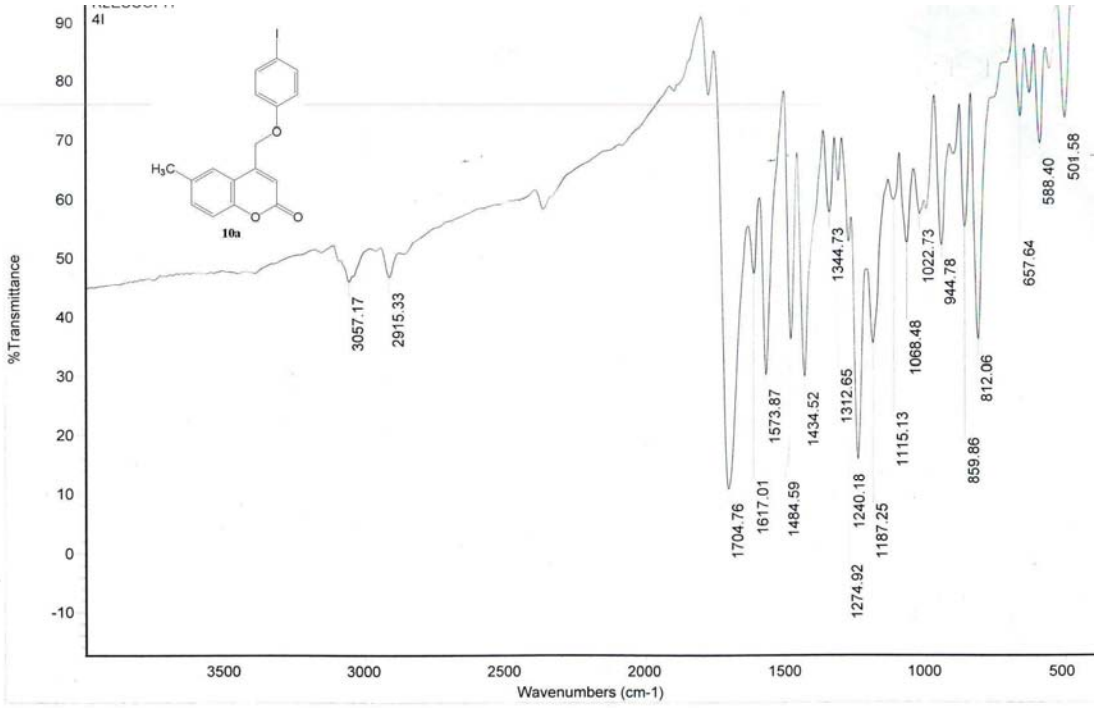
TIME	%B
0	05
8.0	100
8.1	100
8.5	05
10	05



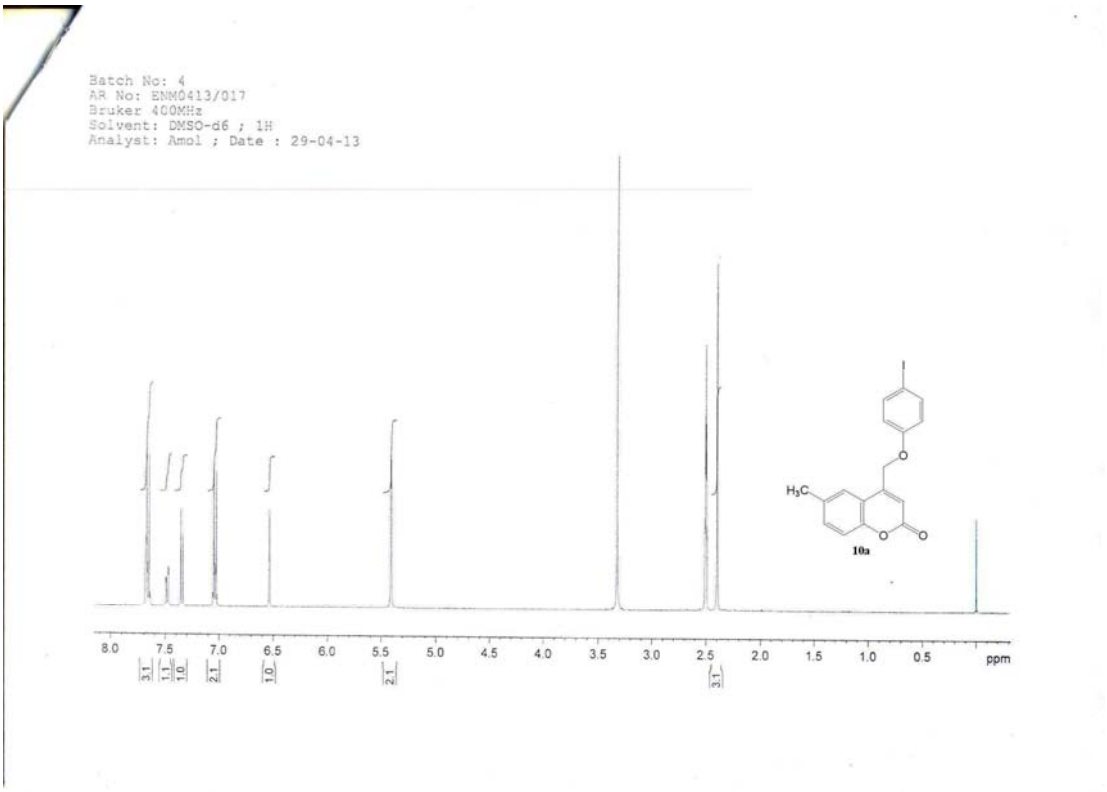
Peak No	RT min	Area	Area %
1	2.003	1.951e+001	99.718
2	6.346	2.082e+001	0.282



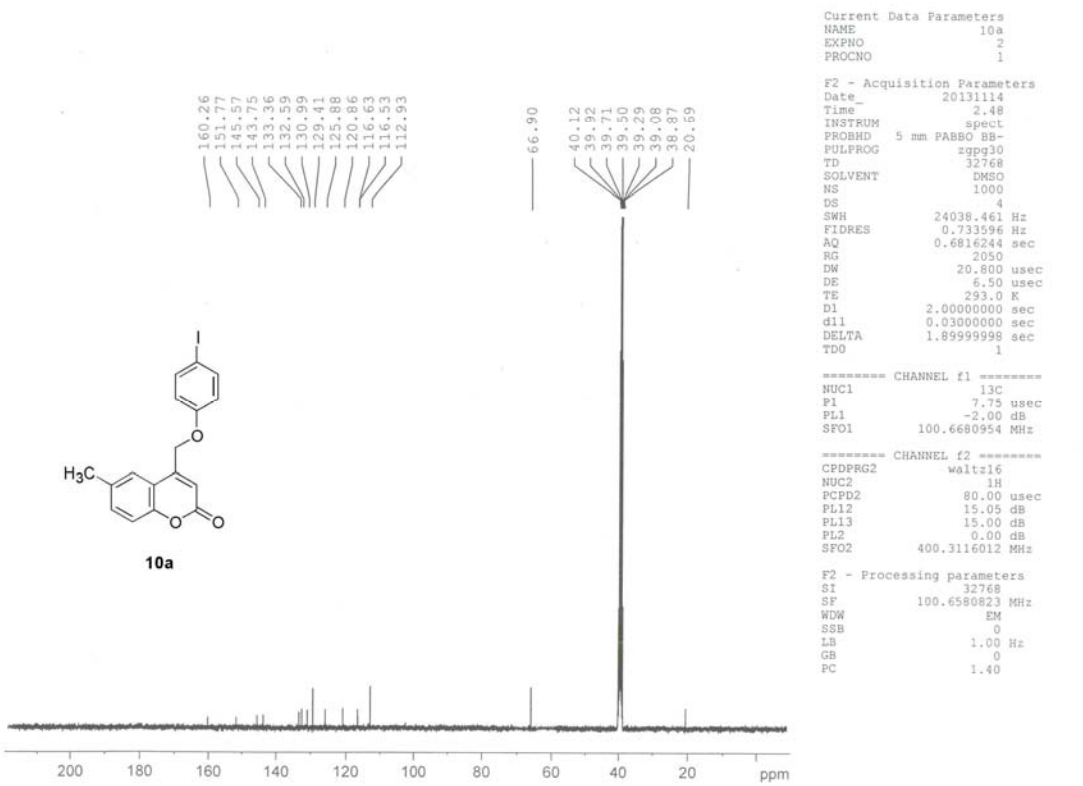
Spectrum No. 16. LCMS of compound **8d**.



Spectrum No. 17. IR (KBr) of compound **10a**.



Spectrum No. 18. ^{1}H NMR (DMSO-d_6) of compound **10a**.

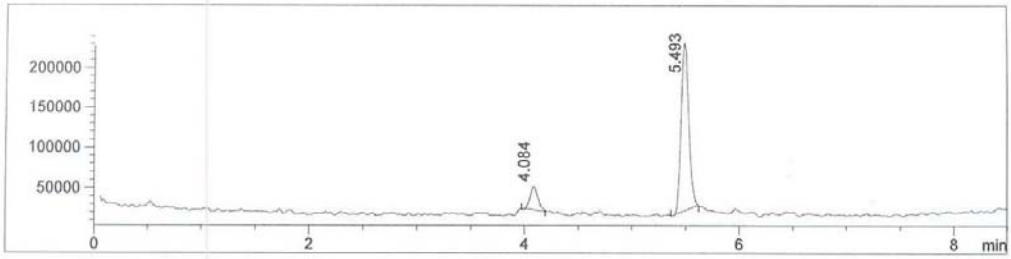


Spectrum No. 19. ^{13}C NMR (DMSO-d_6) of compound **10a**.

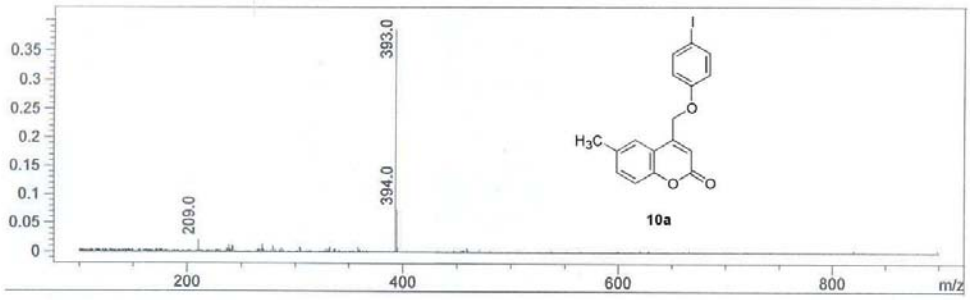
LC-MS REPORT

Method info : A: 0.1% TFA IN H2O , B:0.1% TFA IN ACN ; Flow Rate:2.0 ml/min
COLUMN:XBridge C8 (50x4.6mm, 3.5µ), +ve mode

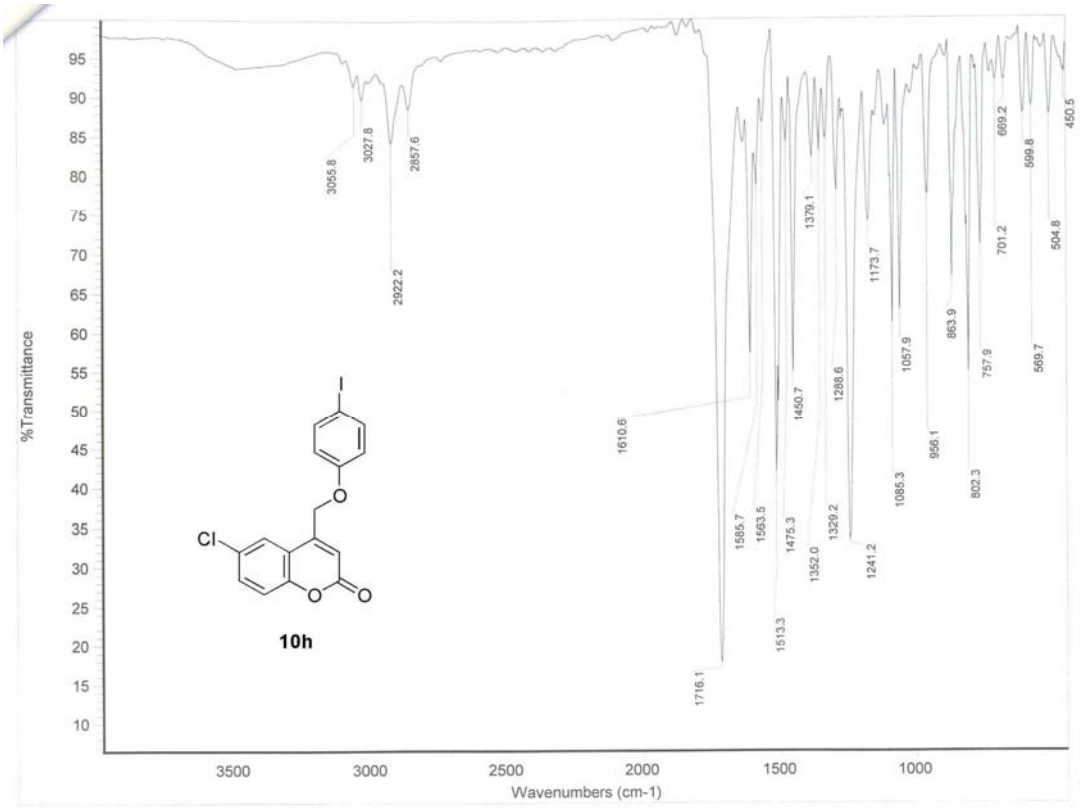
TIME	%B
0	05
8.0	100
8.1	100
8.5	05
10.0	05



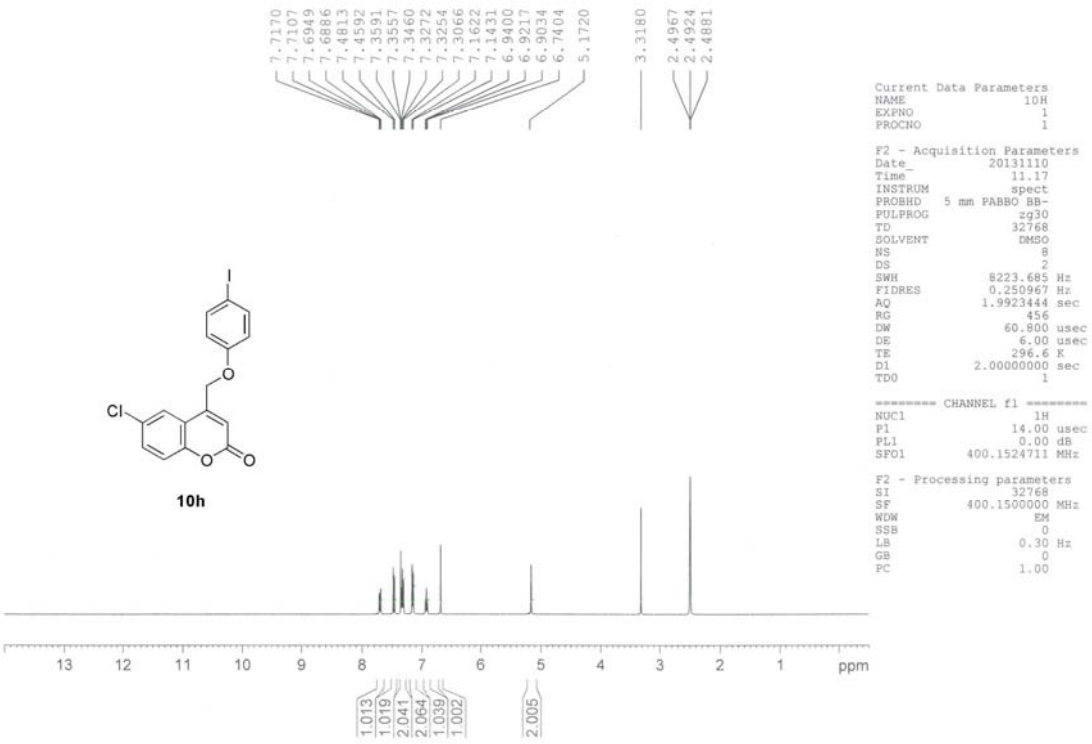
Peak No	RT min	Area	Area %
1	4.084	6.094e+002	0.568
2	5.493	1.691e+003	99.432



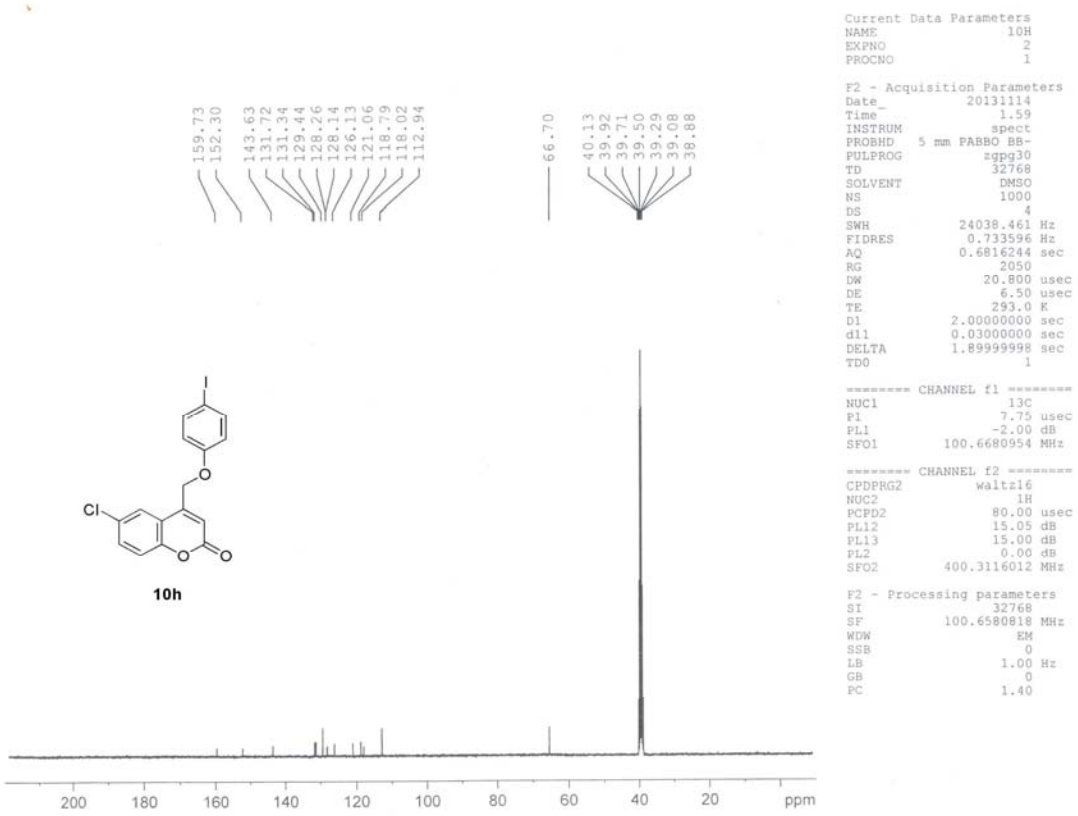
Spectrum No. 20. LCMS of compound **10a**.



Spectrum No. 21. IR (KBr) of compound **10h**.



Spectrum No. 22. ^{1}H NMR (DMSO-d_6) of compound **10h**.

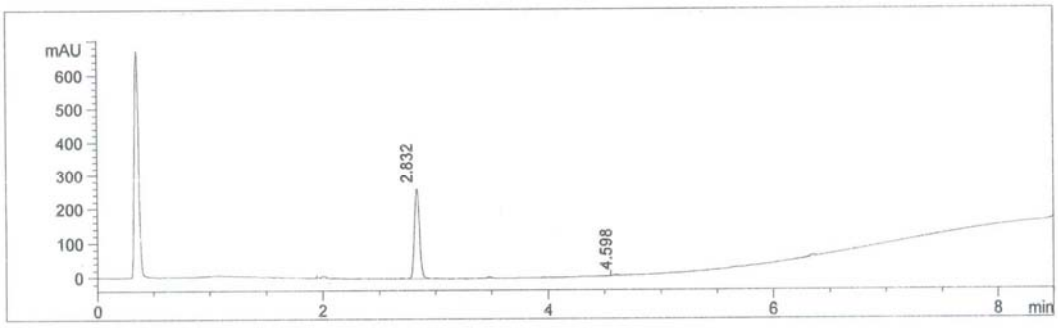


Spectrum No. 23. ^{13}C NMR (DMSO-d_6) of compound **10h**.

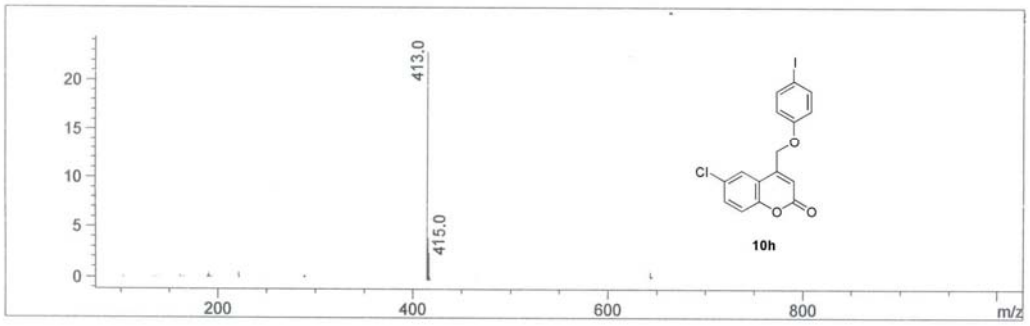
LC-MS REPORT

Method info : A: 0.1% TFA IN H2O , B:0.1% TFA IN ACN ; Flow Rate:2.0 ml/min
COLUMN:XBridge C8 (50x4.6mm, 3.5µm), +ve mode

TIME	%B
0	05
8.0	100
8.1	100
8.5	05
10	05



Peak No	RT min	Area	Area %
1	2.832	8.243e+002	99.360
2	4.598	1.127e+001	0.640



Spectrum No. 24. LCMS of compound **10h**.