Synthesis, structure-activity relationship of iodinated-4- aryloxymethyl-coumarins as potential anti-cancer and anti-mycobacterial agents. Eur J Med Chem

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · JANUARY 2014

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2013.12.061 · Source: PubMed

European Journal of Medicinal Chemistry 74 (2014) 225–233

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

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

Mahantesha Basanagouda [a,*], Vishwanath B. Jambagi [a], Nivedita N. Barigidad [a], Sandeep S. Laxmeshwar [b], Venkatesh Devaru [c], Narayanachar [d]

[a] P.G. Department of Chemistry, P.C. Jabin Science College, Hubli 580031, Karnataka, India
[b] P.G. Department of Studies and Research in Polymer Science, Sir, M.V. PG Centre, Mandya 571402, University of Mysore, Karnataka, India
[c] Department of Physics, L.V.D. College, Raichur 584103, Karnataka, India
[d] Department of Chemistry, L.V.D. College, Raichur 584103, Karnataka, India

ARTICLE INFO

Article history:
Received 5 August 2013
Received in revised form
25 December 2013
Accepted 31 December 2013
Available online 11 January 2014

Keywords:
Coumarin
Heterocycles
Iodine
Anticancer
Anti-mycobacterial
SAR

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 are 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.

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 tumour. The early stages of cancer development carry the maximum potential for therapeutic intervention. Therefore, detecting premalignant or premetastatic malignant tumours 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 tumours 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

* Corresponding author. Tel.: +91 9448760184.
E-mail address: mahanteshachem@gmail.com (M. Basanagouda).

0223-5234/$ – see front matter © 2014 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.12.061

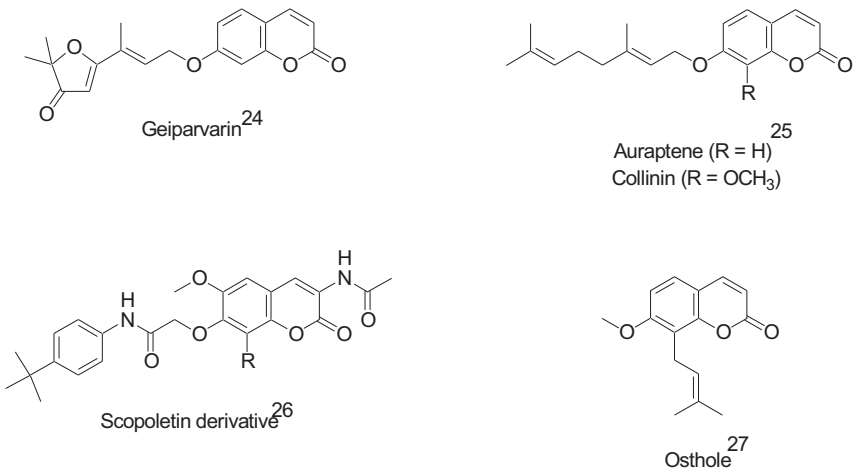


Fig. 1. Structures of some potent anticancer (coumarin ether linkage) compounds.

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, antituberculosis 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 [5,24–26] and anti-tuberculosis [27–30] agents having coumarin moiety are presented in Figs. 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 [31,32]. The biological importance of iodine in the compound have been well reported in the literature such as binding ability [33], cannabinoid receptor antagonists [34], as anti-cancer agent [35]. Amiodarone is an antiarrhythmic drug used for the treatment of tachyarrhythmias [36]. The amiodarone analogue (KB130015) was reported as a antiarrhythmic drug with an improved toxicity profile compared with amiodarone [37]. Three new iodinated tryptophan derivatives, plakohypaphorines have been isolated from the Caribbean sponge *Plakortis simplex* and evaluated for their antihistamine activity [38].

In the light of the above facts and in continuation of our interest in designing oxygen heterocycle based biologically active molecules [39–42], we planned to synthesize a new series of iodinated-4-aryloxymethylcoumarins 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 °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 whereas 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 6th and 7th positions exhibited potent activity (MIC 12.5 μg/mL). Whereas

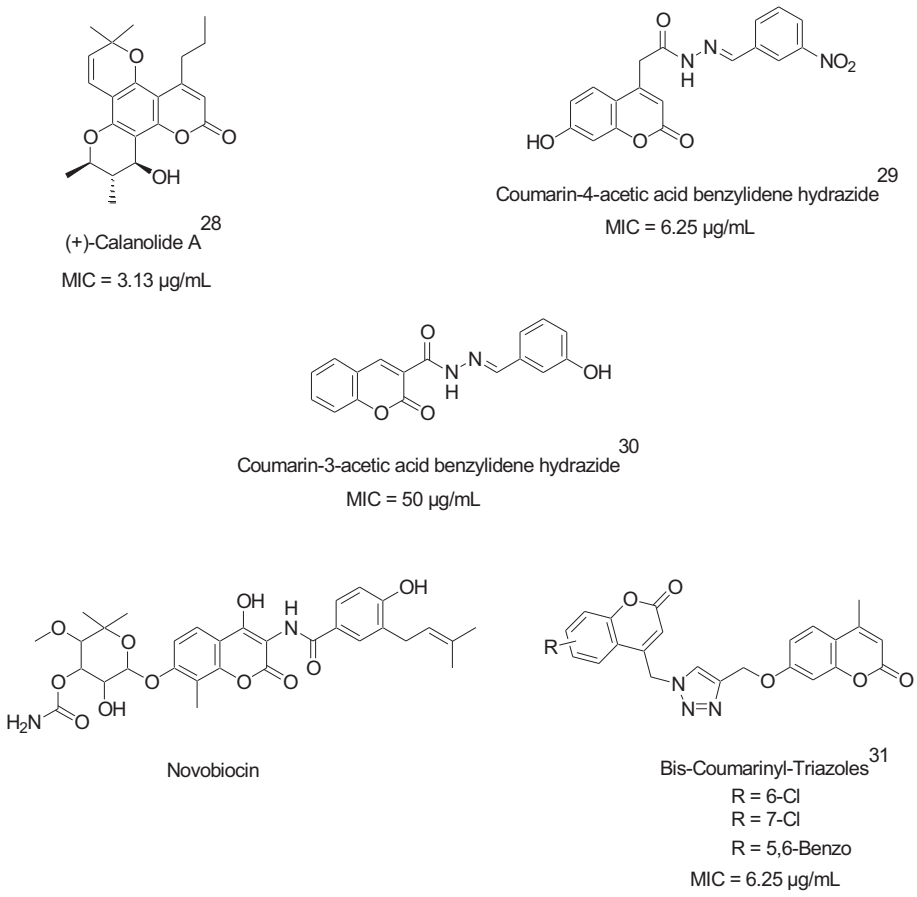


Fig. 2. Some potent anti-mycobacterial agents bearing coumarin and coumarin ether linkage.

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 25–50 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.

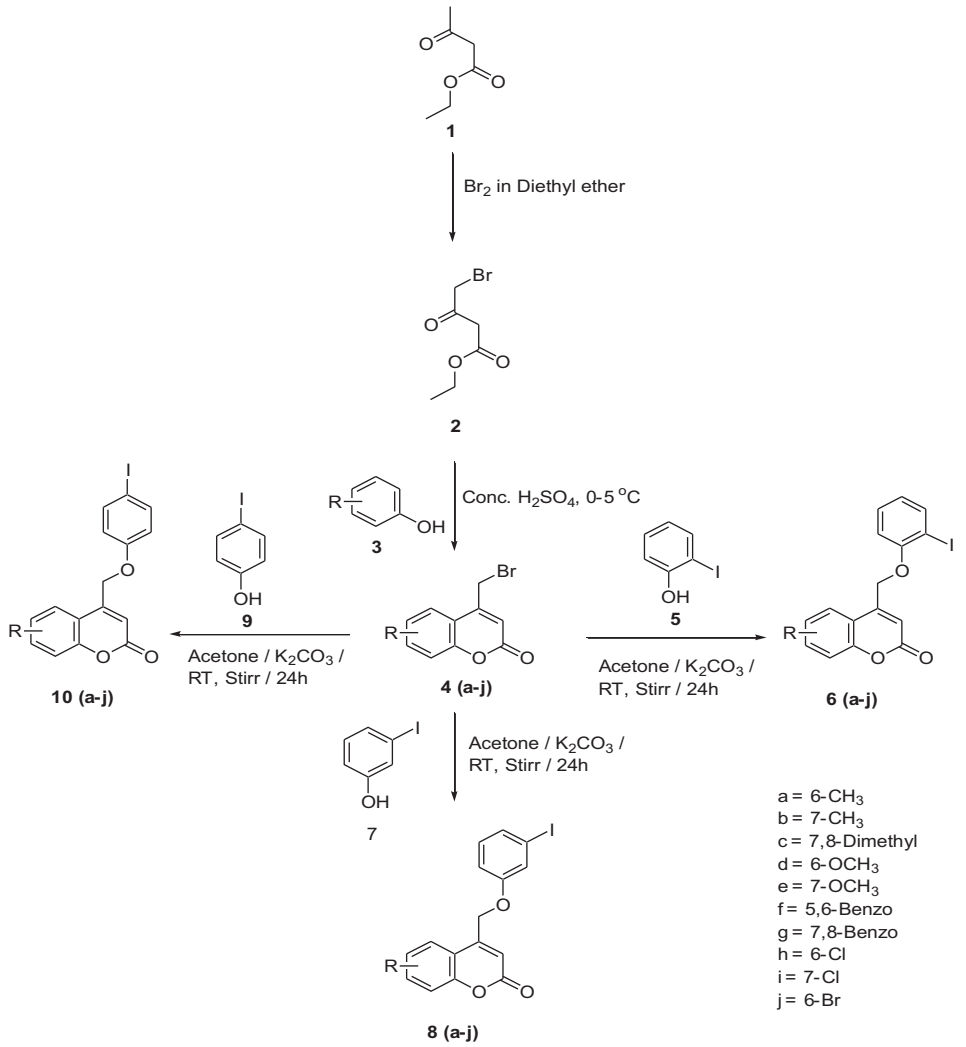
The change in the position of iodine from third to fourth in the phenoxy moiety, i.e., the series of compounds **10**(**a**–**j**), the activity increased in all the compounds, from MIC 6.25 to 3.125 μg/mL for compounds bearing chlorine and bromine (**10h**, **10i**, **10j**). The increase in activity 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*



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

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 6.25–12.5 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 (**10b**), 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 6th and 7th 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 6th and 7th position and bromine at 6th 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 4th position on phenoxy moiety play an important role in enhancing the activity compared to its 3rd and 2nd position.

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

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 ^1H 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 ^1H 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 ^1H 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).

5. 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 6th and 7th position on coumarin and bromine 6th 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 6th and 7th position on coumarin and iodine at 4th 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.

6. Experimental protocols

6.1. 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. ^1H 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

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

were of analytical grade, and were used without further purification unless otherwise stated.

*6.1.1. Synthesis of substituted-4-bromomethylcoumarins **4(a–j)***

The required substituted-4-bromomethyl-coumarins [39,43] have been synthesized by the Pechmann cyclization of substituted phenols with 4-bromoethylacetoacetate [44].

*6.1.2. 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** (1.08 g, 10 mmol) and anhydrous potassium carbonate (1.38 g, 10 mmol) was stirred for 30 min in dry acetone (30 mL). To this, 6-methyl-4-bromomethylcoumarin **4a** (2.53 g, 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.

*6.1.2.1. 4-(2-Iodo-phenoxymethyl)-6-methyl-chromen-2-one (**6a**).* Colourless (Ethanol), m.p. 241 °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.

*6.1.2.2. 4-(2-Iodo-phenoxymethyl)-7-methyl-chromen-2-one (**6b**).* Colourless (Ethanol), m.p. 190 °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.

*6.1.2.3. 4-(2-Iodo-phenoxymethyl)-7,8-dimethyl-chromen-2-one (**6c**).* Colourless (Chloroform), m.p. 238 °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.

*6.1.2.4. 4-(2-Iodo-phenoxymethyl)-6-methoxy-chromen-2-one (**6d**).* Colourless (Chloroform), m.p. 214 °C, yield 88%; IR (KBr, ν in cm^{-1}): 1701 (lactone C=O); ^{1}H 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.

*6.1.2.5. 4-(2-Iodo-phenoxymethyl)-7-methoxy-chromen-2-one (**6e**).* Yellow (Ethanol), m.p. 166 °C, yield 86%; IR (KBr, ν in cm^{-1}): 1704 (lactone C=O); ^{1}H 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.

*6.1.2.6. 1-(2-Iodo-phenoxymethyl)-benzo[f]chromen-3-one (**6f**).* Pale yellow (Chloroform), m.p. 226 °C, yield 91%; IR (KBr, ν in cm^{-1}): 1711 (lactone C=O); ^{1}H 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.

*6.1.2.7. 4-(2-Iodo-phenoxymethyl)-benzo[h]chromen-2-one (**6g**).* Yellow (Chloroform), m.p. 218 °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.

*6.1.2.8. 6-Chloro-4-(2-iodo-phenoxymethyl)-chromen-2-one (**6h**).* Colourless (Chloroform), m.p. 244 °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.

*6.1.2.9. 7-Chloro-4-(2-iodo-phenoxymethyl)-chromen-2-one (**6i**).* Colourless (Chloroform), m.p. 225 °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.

*6.1.2.10. 6-Bromo-4-(2-iodo-phenoxymethyl)-chromen-2-one (**6j**).* Colourless (Ethanol), m.p. 208 °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.

*6.1.2.11. 4-(3-Iodo-phenoxymethyl)-6-methyl-chromen-2-one (**8a**).* Colourless (Ethanol), m.p. 164 °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.

*6.1.2.12. 4-(3-Iodo-phenoxymethyl)-7-methyl-chromen-2-one (**8b**).* Colourless (Ethanol), m.p. 170 °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.

*6.1.2.13. 4-(3-Iodo-phenoxymethyl)-7,8-dimethyl-chromen-2-one (**8c**).* Colourless (Ethanol), m.p. 186 °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.

*6.1.2.14. 4-(3-Iodo-phenoxymethyl)-6-methoxy-chromen-2-one (**8d**).* Colourless (Ethanol), m.p. 167 °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.

*6.1.2.15. 4-(3-Iodo-phenoxymethyl)-7-methoxy-chromen-2-one (**8e**).* Colourless (Ethanol), m.p. 158 °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.

*6.1.2.16. 1-(3-Iodo-phenoxymethyl)-benzo[f]chromen-3-one (**8f**).* Grey (Ethanol), m.p. 168 °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.

*6.1.2.17. 4-(3-Iodo-phenoxymethyl)-benzo[h]chromen-2-one (**8g**).* Brown (Acetone), m.p. 206 °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.

*6.1.2.18. 6-Chloro-4-(3-iodo-phenoxymethyl)-chromen-2-one (**8h**).* Grey (Ethanol), m.p. 203 °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.

*6.1.2.19. 7-Chloro-4-(3-iodo-phenoxymethyl)-chromen-2-one (**8i**).* Colourless (Ethanol), m.p. 195 °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.

*6.1.2.20. 6-Bromo-4-(3-iodo-phenoxymethyl)-chromen-2-one (**8j**).* Colourless (Ethanol), m.p. 214 °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.

*6.1.2.21. 4-(4-Iodo-phenoxymethyl)-6-methyl-chromen-2-one (**10a**).* Colourless (Ethyl acetate), m.p. 192 °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.

*6.1.2.22. 4-(4-Iodo-phenoxymethyl)-7-methyl-chromen-2-one (**10b**).* Green (Ethyl acetate), m.p. 148 °C, yield 89%; IR (KBr, υ in cm^{-1}): 1708 (lactone C=O); ^{1}H 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.

*6.1.2.23. 4-(4-Iodo-phenoxymethyl)-7,8-dimethyl-chromen-2-one (**10c**).* Colourless (Ethyl acetate), m.p. 232 °C, yield 83%; IR (KBr, υ in cm^{-1}): 1708 (lactone C=O); ^{1}H 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.

*6.1.2.24. 4-(4-Iodo-phenoxymethyl)-6-methoxy-chromen-2-one (**10d**).* Pale yellow (Ethyl acetate), m.p. 196 °C, yield 94%; 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), 5.25 (*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.

*6.1.2.25. 4-(4-Iodo-phenoxymethyl)-7-methoxy-chromen-2-one (**10e**).* Pale yellow (Ethyl acetate), m.p. 188 °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.

*6.1.2.26. 1-(4-Iodo-phenoxymethyl)-benzo[f]chromen-3-one (**10f**).* Green (Ethyl acetate), m.p. 188 °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.

*6.1.2.27. 4-(4-Iodo-phenoxymethyl)-benzo[h]chromen-2-one (**10g**).* Yellow (Ethyl acetate), m.p. 240 °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.

*6.1.2.28. 6-Chloro-4-(4-iodo-phenoxymethyl)-chromen-2-one (**10h**).* Yellow (Ethyl acetate), m.p. 196 °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.

*6.1.2.29. 7-Chloro-4-(4-iodo-phenoxymethyl)-chromen-2-one (**10i**).* Pale yellow (Ethyl acetate), m.p. 210 °C, yield 71%; IR (KBr, υ in cm^{-1}): 1713 (lactone C=O); ^{1}H 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.

*6.1.2.30. 6-Bromo-4-(4-iodo-phenoxymethyl)-chromen-2-one (**10j**).* Colourless (Ethyl acetate), m.p. 179 °C, yield 66%; IR (KBr, υ in cm^{-1}): 1713 (lactone C=O); ^{1}H 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.

6.2. Anti-cancer activity

In all the experiments, different cell lines were seeded at a final density of 2×10^4 cells/well, in 96 well microtiter plates. Cytotoxicity was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, according to the method of Mosmann (1983) [45]. Briefly, the cells (2×10^4) 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 °C 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.

6.3. 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) [46]. 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 °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.

Acknowledgements

The author Dr. Mahantesha B., thank the Principal, P.C. Jabin Science College, Hubli and K.L.E. Society, Belgaum. Dr. Venkatesh Devaru thank the Vision Group of Science and Technology (VGST), Department of IT, BT, Science & Technology, Government of Karnataka for the financial support vide sanction No: VGST/P-15/K-FIST Level-1/2010-11/744 dated 21-03-2011.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.12.061.

References

[1] S.K. Arya, S. Bhansali, Lung cancer and its early detection using biomarker-based biosensors, Chem. Rev. 111 (2011) 6783–6809.
[2] M. Ljungman, Targeting the DNA damage response in cancer, Chem. Rev. 109 (2009) 2929–2950.
[3] M. Jeyachandran, P. Ramesh, D. Sriram, P. Senthilkumar, P. Yogeeswari, Synthesis and *in vitro* antitubercular activity of 4-aryl/alkylsulfonylmethylcoumarins as inhibitors of mycobacterium tuberculosis, Bioorg. Med. Chem. Lett. 22 (2012) 4807–4809.
[4] A. Lacy, Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer, Curr. Pharm. Des. 10 (2004) 3797–3811.
[5] F. Borges, F. Roleira, N. Milhazes, L. Santana, E. Uriarte, Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity, Curr. Med. Chem. 12 (2005) 887–916.
[6] I. Kostova, Synthetic and natural coumarins as cytotoxic agents, Anti-Cancer Agents Med. Chem. 5 (2005) 29–46.
[7] M.V. Kulkarni, G.M. Kulkarni, C.H. Lin, C.M. Sun, Recent advances in coumarins and 1-azacoumarins as versatile biodynamic agents, Curr. Med. Chem. 13 (2006) 2795–2818.
[8] M.A. Musa, J.S. Cooperwood, M.O.F. Khan, A review of coumarin derivatives in pharmacotherapy of breast cancer, Curr. Med. Chem. 15 (2008) 2664–2679.
[9] L. Wu, X. Wang, W. Xu, F. Farzaneh, R. Xu, The structure and pharmacological functions of coumarins and their derivatives, Curr. Med. Chem. 16 (2009) 4236–4260.
[10] M.E. Riveiro, N.D. Kimpe, A. Moglioni, R. Vazquez, F. Monczor, C. Shayo, C. Davio, Coumarins: old compounds with novel promising therapeutic perspectives, Curr. Med. Chem. 17 (2010) 1325–1338.
[11] C. Kontogiorgis, A. Detsi, D.H. Litina, Coumarin-based drugs: a patent review (2008-present), Expert Opin. Ther. Pat. 22 (2012) 437–454.
[12] X.M. Peng, G.L.V. Damu, C.H. Zhou, Current developments of coumarin compounds in medicinal chemistry, Curr. Pharm. Des. 19 (2013) 3884–3930.
[13] L.W.L. Woo, A. Purohit, B. Malini, M.J. Reed, B.V.L. Potter, Potent active site-directed inhibition of steroid sulphatase by tricyclic coumarin-based sulphamates, Chem. Biol. 7 (2000) 773–791.
[14] B. Malini, A. Purohit, D. Ganeshapillai, L.W.L. Woo, B.V.L. Potter, M.J. Reed, Inhibition of steroid sulphatase activity by tricyclic coumarin sulphamates, J. Steroid Biochem. Mol. Biol. 75 (2000) 253–258.

[15] A. Purohit, L.W.L. Woo, B.V.L. Potter, M.J. Reed, In vivo inhibition of estrone sulfatase activity and growth of nitrosomethylureainduced mammary tumors by 667 COUMATE, Cancer Res. 60 (2000) 3394–3396.

[16] F. Meggio, M.A. Pagano, S. Moro, G. Zagotto, M. Ruzzene, S. Sarno, G. Cozza, J. Bain, M. Elliott, A.D. Deana, A.M. Brunati, L.A. Pinna, Inhibition of protein kinase CK2 by condensed polyphenolic derivatives. An in vitro and in vivo study, Biochemistry 43 (2004) 12931–12936.

[17] A. Chilin, R. Battistutta, A. Bortolato, G. Cozza, S. Zanatta, G. Poletto, M. Mazzorana, G. Zagotto, E. Uriarte, A. Guiotto, L.A. Pinna, F. Meggio, S. Moro, Coumarin as attractive casein kinase 2 (CK2) inhibitor scaffold: an integrate approach to elucidate the putative binding motif and explain structure-activity relationships, J. Med. Chem. 51 (2008) 752–759.

[18] G.L. Bras, C. Radanyi, J.F. Peyrat, J.D. Brion, M. Alami, V. Marsaud, B. Stella, J.M. Renoir, New novobiocin analogues as antiproliferative agents in breast cancer cells and potential inhibitors of heat shock protein 90, J. Med. Chem. 50 (2007) 6189–6200.

[19] A.C. Donnelly, J.R. Mays, J.A. Burlison, J.T. Nelson, G. Vielhauer, J. Holzbeierlein, B.S.J. Blagg, The design, synthesis, and evaluation of coumarin ring derivatives of the novobiocin scaffold that exhibit antiproliferative activity, J. Org. Chem. 73 (2008) 8901–8920.

[20] H.P. Zhao, A.C. Donnelly, B.R. Kusuma, G.E.L. Brandt, D. Brown, R.A. Rajewski, G. Vielhauer, J. Holzbeierlein, M.S. Cohen, B.S.J. Blagg, Engineering an antibiotic to fight cancer: optimization of the novobiocin scaffold to produce anti-proliferative agents, J. Med. Chem. 54 (2011) 3839–3853.

[21] H.P. Zhao, B. Yan, L.B. Peterson, B.S.J. Blagg, 3-Arylcoumarin derivatives manifest anti-proliferative activity through Hsp90 inhibition, ACS Med. Chem. Lett. 3 (2012) 327–331.

[22] S. Ananthan, E.R. Faaleolea, R.C. Goldman, J.V. Hobrath, C.D. Kwong, B.E. Laughon, J.A. Maddry, A. Mehta, L. Rasmussen, R.C. Reynolds, J.A. Secrist III, N. Shindo, D.N. Showe, M.I. Sosa, W.J. Suling, E.L. White, High-throughput screening for inhibitors of mycobacterium tuberculosis H37Rv, Tuberculosis 89 (2009) 334–353.

[23] R.C. Reynolds, S. Ananthan, E. Faaleolea, J.V. Hobrath, C.D. Kwong, C. Maddox, L. Rasmussen, M.I. Sosa, E. Thammasuvimol, E.L. White, W. Zhang, J.A. Secrist III, High throughput screening of a library based on kinase inhibitor scaffolds against *Mycobacterium tuberculosis* H37Rv, Tuberculosis 92 (2012) 72–83.

[24] H. Kohno, R. Suzuki, M. Curini, F. Epifano, F. Maltese, S.P. Gonzales, T. Tanaka, Dietary administration with prenyloxycoumarins, auraptene and collinin, inhibits colitis-related colon carcinogenesis in mice, Int. J. Cancer 118 (2006) 2936–2942.

[25] W. Liu, J. Hua, J. Zhou, H. Zhang, H. Zhu, Y. Cheng, R. Gust, Synthesis and in vitro antitumor activity of novel scopoletin derivatives, Bioorg. Med. Chem. Lett. 22 (2012) 5008–5012.

[26] K.V. Sashidhara, S.R. Avula, K. Sharma, G.R. Palnati, S.R. Bathula, Discovery of coumarin-monastrol hybrid as potential antibreast tumor-specific agent, Eur. J. Med. Chem. 60 (2013) 120–127.

[27] Z.Q. Xu, W.W. Barrow, W.J. Suling, L. Westbrook, E. Barrow, Y.M. Lin, M.T. Flavin, Anti-HIV natural product (+)-calanolide A is active against both drug-susceptible and drug-resistant strains of *Mycobacterium tuberculosis*, Bioorg. Med. Chem. 12 (2004) 1199–1207.

[28] A. Manvar, A. Malde, J. Verma, V. Virsodia, A. Mishra, K. Upadhyay, H. Acharya, E. Coutinho, A. Shah, Synthesis, anti-tubercular activity and 3D-QSAR study of coumarin-4-acetic acid benzylidene hydrazides, Eur. J. Med. Chem. 43 (2008) 2395–2403.

[29] S.H. Cardoso, M.B. Barreto, M.C.S. Lourenco, M.G.M.O. Henriques, A.L.P. Candea, C.R. Kaiser, M.V.N. Souza, Antitubercular activity of new coumarins, Chem. Biol. Drug Des. 77 (2011) 489–493.

[30] R.J. Naik, M.V. Kulkarni, K.S.R. Pai, P.G. Nayak, Click chemistry approach for bis-chromenyl triazole hybrids and their antituberculosis activity, Chem. Biol. Drug Des. 80 (2012) 516–523.

[31] Y. Lu, Y. Wang, W. Zhu, Nonbonding interactions of organic halogens in biological systems: implications for drug discovery and biomolecular design, Phys. Chem. Chem. Phys. 12 (2010) 4543–4551.

[32] Y. Lu, T. Shi, Y. Wang, H. Yang, X. Yan, X. Luo, H. Jiang, W. Zhu, Halogen bonding-a novel interaction for rational drug design? J. Med. Chem. 52 (2009) 2854–2862.

[33] S. Arnold, F. Goglia, B. Kadenbach, 3,5-Diiodothyronine binds to subunit Va of cytochrome-*c* oxidase and abolishes the allosteric inhibition of respiration by ATP, Eur. J. Biochem. 252 (1998) 325–330.

[34] R. Lan, Q. Liu, P. Fan, S. Lin, S.R. Fernando, D. McCallion, R. Pertwee, A. Makriyannis, Structure-activity relationships of pyrazole derivatives as cannabinoid receptor antagonists, J. Med. Chem. 42 (1999) 769–776.

[35] R. Wilcken, X. Liu, M.O. Zimmermann, T.J. Rutherford, A.R. Fersht, A.C. Joerger, F.M. Boeckler, Halogen-enriched fragment libraries as leads for drug rescue of mutant p53, J. Am. Chem. Soc. 134 (2012) 6810–6818.

[36] V.Y. Su, Y.W. Hu, K.T. Chou, S.M. Ou, Y.C. Lee, E.Y. Lin, T.J. Chen, C.H. Tzeng, C.J. Liu, Amiodarone and the risk of cancer, Cancer (2003) 1699–1705.

[37] B. Carlsson, B.N. Singh, M. Temciuc, S. Nilsson, Y.L. Li, C. Mellin, J. Malm, Synthesis and preliminary characterization of a novel antiarrhythmic compound (KB130015) with an improved toxicity profile compared with amiodarone, J. Med. Chem. 45 (2002) 623–630.

[38] F. Borrelli, C. Campagnuolo, R. Capasso, E. Fattorusso, O.T. Scafati, Iodinated indole alkaloids from *plakortis simplex* - new plakohypaphorines and an evaluation of their antihistamine activity, Eur. J. Org. Chem. (2004) 3227–3232.

[39] M. Basanagouda, M.V. Kulkarni, D. Sharma, V.K. Gupta, Pranesha, P. Sandhyarani, V.P. Rasal, Synthesis of some new 4-aryloxymethylcoumarins and examination of their antibacterial and antifungal activities, J. Chem. Sci. 121 (2009) 485–495.

[40] M. Basanagouda, K. Shivashankar, M.V. Kulkarni, V.P. Rasal, H. Patel, S.S. Mutha, A.A. Mohite, Synthesis and antimicrobial studies on novel sulfonamides containing 4-azidomethylcoumarin, Eur. J. Med. Chem. 45 (2010) 1151–1157.

[41] M. Basanagouda, V.B. Jadhav, M.V. Kulkarni, R.N. Rao, Computer aided prediction of biological activity spectra: study of correlation between predicted and observed activities for coumarin-4-acetic acids, Indian J. Pharm. Sci. 73 (2011) 88–92.

[42] S.N. Makandar, M. Basanagouda, M.V. Kulkarni, Pranesha, V.P. Rasal, Synthesis and anti-microbial studies of some 4-aryloxymethylcoumamrins obtained by reaction of 4-bromomethyl coumarins with aromatic bidentate nucleophiles, Med. Chem. Res. 21 (2012) 2603–2614.

[43] M. Basanagouda, M.V. Kulkarni, Novel one-pot synthesis for 2,5-diaryl and 5-aryl-pyridazin-3(*2H*)-ones, Synth. Commun. 41 (2011) 2569–2582.

[44] A. Burger, G.E. Ullyot, Analgesic studies. β-ethyl and β-isopropylamine derivatives of pyridine and thiazole, J. Org. Chem. 12 (1947) 342–355.

[45] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.

[46] M.C.S. Lourenco, M.V.N. deSouza, A.C. Pinheiro, M.L. Ferreira, R.B. Goncalves, T.C.M. Nogneira, M.A. Peralta, Evaluation of anti-tubercular activity of nicotinic and isoniazid analogues, Arkivoc 15 (2007) 181–191.