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ENVIRONMENTALLY BENIGN SYNTHESIS, COMPUTATIONAL INVESTIGATION, AND MECHANISTIC STUDIES OF NOVEL COUMARIN-CARBONODITHIOATE FRAMEWORKS AS ANTICANCER DRUGS: AN APPROACH TO MICROWAVE SYNTHESIS

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ABSTRACT

In the present study, an easy, rapid protocol for the synthesis of coumarin-carbonodithioate frameworks (1a-1j) under microwave (MW) irradiation and conventional method is described. Further, the efforts underwent towards the development of MW assisted protocol, through which the yields of the compounds were improved drastically in shorter reaction time. The new compounds are characterized by IR, ^{1}H, ^{13}C-NMR, Mass and Elemental analyses. Computational experiments were carried out in order to study the interactions between coumarins and Bcl-2 protein showed promising activity. Amongst, the compounds 1a, 1c, 1d, 1f, 1h, and 1j have been selected for NCI-60 One-Dose screening for their *in-vitro* anticancer activities and displayed excellent growth inhibition. The Structure-Activity Relationship studies explicated the obtained results. The title compounds analyzed for their physicochemical properties set by Lipinski rule and found that none of the compounds violated the rule and fall well in the range of Rule of five [RO5].

KEYWORDS: MW irradiation, computational studies, anticancer activity, SAR studies.

INTRODUCTION

Today, the simpler drug development works with small, chemically invented active molecules. Since these small molecules can be processed easily into ingestible capsules or tablets and if the tablet dissolves in the digestive tract. The dissolved active contents are absorbed as small molecules via the body-fluid system and can reach to almost any desired destination in the body and their chemical composition often help them to easily enter cell membranes,[1,2] Therefore, the majority of pharmaceuticals and biologically active developed drugs are all small in size. Based on these facts, in the year 1997 medicinal chemist Christopher Lipinski and his colleagues[3] analyzed the physicochemical properties of over 2,000 drugs and candidate drugs in clinical trials, and concluded that a compound is more likely to be membrane permeable and easily absorbed by the body if it matches the Rule of five (RO5) criteria. Today the RO5 are widely used by medicinal chemists to predict not only the absorption of compounds but also overall drug-likeness.[4] Hence, keeping these criteria in mind, we designed the structure of the compounds and analyzed for their physicochemical properties set by RO5 and found that none of the compounds violate the RO5 and they fall well within the range as mentioned above (Table I).

The environmentally benign chemical reactions by MW energy as an increasingly very popular theme in the scientific community for organic reactions and synthesis of organic bio-molecules has been continuously modified and explored from decade together.[5] Many organic reactions are carried out in MW for chemical transformations.[6] This technology has played a significant role at laboratory scale with extraordinary features, such as enhanced reaction rates and good yields with high purity.[7] In these conscious days of deteriorating environment, eco-friendly reactions are successful to obtain most important bio-organic molecules such as coumarin hybrids.[8]

On the contrary, there is a dramatic increase in pathogen resistance to pharmaceutical, agrochemical and also antimicrobial agents. Now, new templates are in

necessary to address this situation. Natural products which extensively utilized as medicines, coumarins are amongst them and possess interesting and noteworthy biological activities. Along with the natural sources of coumarins, there are abundant synthetic routes that have been developed while searching for compounds with enhanced properties.[9] Coumarin is considered as a privileged framework because of its abundance in naturally occurring compounds with diverse pharmacological profiles such as lipid-lowering agents, Anticancer agents, HIV integrase inhibitors, radical scavengers as well as anti-invasive compounds because of the inhibition of matrix metalloproteases.[10] The coumarins are members of a huge family of oxygen-containing fused heterocycles, which are widely distributed in nature.[11] Worldwide researchers have designed and synthesized coumarin analogs for the treatment of multifariousness diseases.[12] Moreover, the unique structure of coumarin has a special ability which allows its derivatives to readily interact with a diversity of enzymes and receptors in organisms through weak bond interactions and thereby exhibit wide potential as medicinal drugs. Hence, coumarin-based compounds are attracted as a special interest and their outstanding contributions to the prevention of numerous diseases have become an extremely attractive highlight.[13]

Cancer is one of the frightening chronic disease, and numerous drugs are accessible for its treatment but, it still remains a potential life-threatening lethal and widespread malady,[14,15] because defects in apoptosis regulators always result in tumorigenesis, progressive uncontrollable growth and the number of deaths are increasing.[16] Nearly all types of cancer are caused by abnormalcy in the hereditary material and involves deregulation of necessary enzymes and some proteins responsible for cell division and proliferation of the transformed cells.[17] Over recent years, the design of cancer chemotherapy has become advanced. But, yet there is no treatment effective to disseminated cancer completely.[18] The clear indication from literature is that cancer patients more than 90% die due to chronic tumor metastases the spread and invasion of other organs. Furthermore, tumors that initially respond to chemotherapy can still become refractory to the continuing treatment by developing a multi-drug resistant phenotype (MDR) affecting a broad spectrum of structurally and mechanistically diverse antitumor agents.[19,20] At present cancer therapy involving a single biological drug or pathway has been successfully utilized.[21] Still, the problem of drug resistance and a general belief that agents modulating more than one target could have superior efficacy compared to single target drugs[22,23] has led to the search for molecules modulating multiple targets. The well-known vinca alkaloids and taxanes are common conventional chemotherapeutic drugs, are being used in the development of MDR. Thus, the search for agents capable of circumventing MDR actions is an important area in the research aiming to conflict drug-resistant cancers.[24] As a result, there is increased interest in the development of drugs that concomitantly interacts more than one biological target for cancer treatment. However, due to the heterogeneity of cancer, the search is still on to develop drugs for specific types of cancers.

It has elicited many researchers interests and current activities are focusing on the design and development of various coumarin containing anticancer drugs.[25] Such coumarin hybrids are investigated as potential candidates for cancer therapy, through different modes of action; by hindering the telomerase enzyme,[26] induce cell apoptosis,[27] reduced metastases from intestinal carcinomas,[28] suppress neoplastic growth by inhibiting CK2,[29] inhibits tubulin polymerization and arrest cells in mitotic phase by inhibiting microtubule formation,[30] suppress cancer cell proliferation by arresting cell cycle in G2/M phase.[31] So far some coumarin derivatives for e.g., Warfarin (anticoagulant, I), Armillarisin A (antibiotic, II), Hymecromone (choleretic and antispasmodic, III), Carbochromen (coronary disease, IV), Phenprocoumon (anticoagulant, V) and Novobiocin (anti-biotic, VI) have made their way to clinics (Fig. 1).[32]

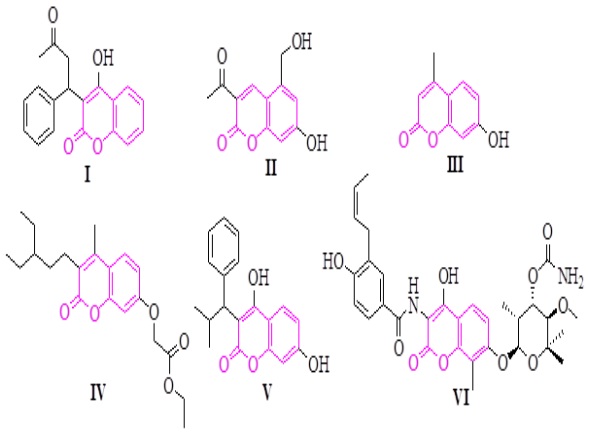
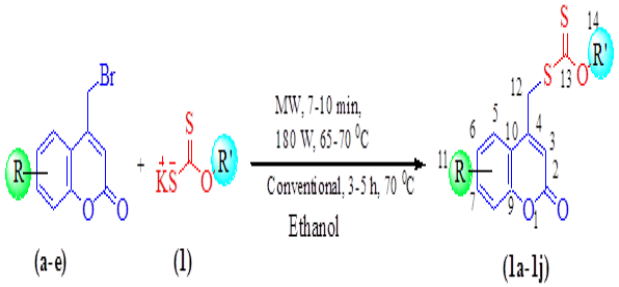


Fig. 1 Coumarin containing commercially available anticancer drugs.



R = a) 6-Me, b) 7-Me, c) 6-OMe, d) 6-Cl, e) 7-OH

R' = Ethyl, Methyl

Scheme - I: Syntheses of coumarin-carbonodithioate derivatives

Hence, considering the most valuable aspects, we extended our studies on the reactions for title compounds under conventional and MW assisted synthesis in one hand and evaluated for their immune-modulatory potential. Here, we describe a novel multi-purpose tool for selecting to perform nucleophilic condensation reactions of coumarins leading to substituted coumarin-carbonodithioates through thioether link based on reaction conditions, with a short reaction time, acutely non-toxic, easy to handle and easy work up without using any purification techniques. We also found the variations in substituent at coumarin skeleton are responsible for varying inhibitory properties of this library of compounds along with other structural features of the molecule. (Scheme-I).

CHEMISTRY

The substituted 4-bromomethyl coumarins (a-e) were synthesized by the Pechman cyclisation of phenols with Ethyl 4-bromoacetoacetate.[12] Condensation of 4-bromomethyl coumarins (a-e) (0.01mol) with potassium O-ethyl/methyl carbonodithioate[33] (1) (0.01mol) using absolute ethanol as solvent afforded *O-ethyl/methyl S-(2-oxo-2H-chromen-4-yl) methyl carbonodithioate derivatives* (1a-1j) under microwave irradiation and conventional method as outlined in Scheme-1. It was observed that microwave approach proved to be an extremely fast method, providing good to excellent yields (75-90%) as compared to the conventional method (55-75%). The results are summarized analytical data in Table I. The most marked improvement was the speed with which the reaction proceeded. The reaction was completed within 7-9 minutes, being 30-40 times faster than the conventional method. All the newly synthesized compounds were characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR, and Mass and elemental analysis. The spectral data of newly synthesized compounds (1a-1j) are given in the experimental section, while the ^{1}H and ^{13}C NMR spectra are in good agreement with the proposed structure of the compounds.

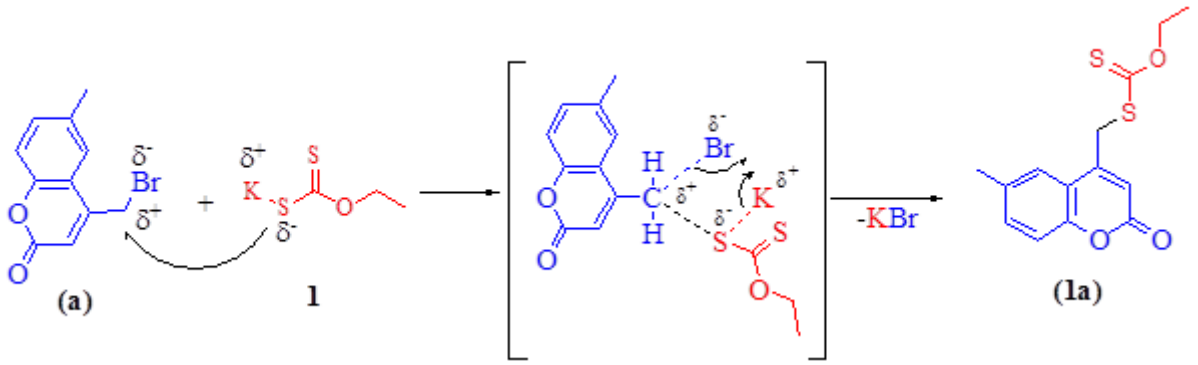
Table I. Analytical data of synthesized compounds (1a-1j).

Products	R	Yield (%) [a]C [b]M		Time C (h) M (min)		Melting Pt. (°C)	LogP*
1a	6-Me	75	94	3.5	7	110-112	3.63
1b	7-Me	73	86	4.00	7	120-122	3.63
1c	6-OMe	71	81	4.66	8.30	122-124	3.24
1d	6-Cl	74	92	4.5	9	104-106	3.86
1e	7-OH	72	83	6.0	10	176-178	2.70
1f	6-Me	70	91	3.75	7.30	118-120	3.25
1g	7-Me	76	85	4.25	8	114-116	3.25
1h	6-OMe	69	90	5.5	9	120-122	2.86
1i	6-Cl	71	89	4. 0	9	126-128	3.48
1j	7-OH	68	85	5.5	10	144-146	2.32

[a] = conventional method, [b] = MW method * http://www.molinspiration.com/cgi-bin/properties

Mechanism

The reaction between 6-methyl-4-bromomethyl coumarin and carbonodithioate in ethanol solvent has a 1:1 stoichiometry. On the experimental results, a plausible reaction mechanism has been proposed for which all the observed steps in each constituent may be well accommodated.



Initially, the 6-methyl-4-bromomethyl coumarin (a), where $-CH_2$ and Bromine groups are occupied with partially δ^+ and δ^- charges respectively. In another moiety, carbonodithioate (1) where sulfur and potassium are occupied with partially δ^- and δ^+ charges respectively. When both the reactants come in closer to each other, the sulfur as a nucleophile initially attacks to the methyl carbon, which is bearing leaving group

bromine. In the intermediate step, the dissociation of –Br from methyl group and potassium attached to sulfur groups takes place simultaneously, while the bond formation between sulfur and methyl group with elimination of KBr, forms the stable compound (1a).

RESULT AND DISCUSSION

Coumarin derived products were confirmed by spectroscopic analysis, as in case of compound *O-ethyl S-(6-methyl-2-oxo-2H-chromen-4-yl)methyl carbonodithioate* (1a).

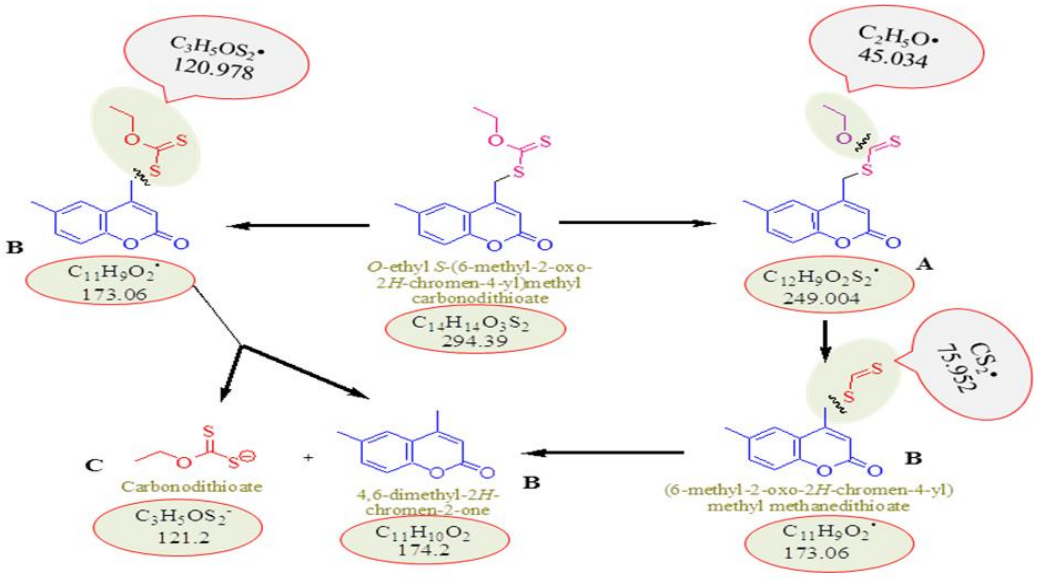
The IR spectrum exhibited band at 1712cm^{-1} assignable to lactone carbonyl stretching, whereas the –C=S (thiocarbonyl) stretching appeared at 1044 cm^{-1}, S-OEt (thioester) appeared stretching band at 942cm^{-1}. Formation of product (1a) was further established by ^{1}H NMR spectrum, wherein one triplet corresponding to –C7-H and –C8-H coumarin resonates in the downfield region at δ 7.33-7.37 ppm (J = 6.2Hz). Adjacent to it, one singlet corresponding to –C5-H appeared at δ 7.22 ppm of coumarin, one more singlet corresponding to –C3-H of coumarin appeared at δ 6.54 ppm. One quartet was resonated at δ 4.67 ppm corresponding to methylene protons of –C14 (J = 6.8Hz), one more singlet resonated at δ 4.46 ppm corresponding to methylene protons of –C12, one sharp singlet and triplet was observed at δ 2.41 & 1.43 ppm which is assigned to methyl protons of –C6 and –C15 respectively. ^{13}C NMR provides additional support for the structure of the compound 1a. The carbon of (-C=S) and the lactone carbonyl (-C=O) group resonates at δ 211.86 and 160.66 ppm respectively. The –C9 carbon adjacent to coumarin oxygen resonates at δ 152.01 ppm whereas peak at δ 149.26ppm corresponds to the –C4 carbon. The -C6 carbon were resonated at δ 134.23 ppm and adjacent to this, peak at δ 133.23 ppm is assigned to –C7 carbon, whereas –C5 carbon resonates at δ 124.04 ppm. The two methylene carbons, one attached to oxygen, –C14 carbon and –C12 are resonated at δ 71.1 and 36.13 ppm respectively. The two methyl carbons, –C11 and –C15 carbons are resonated at δ 21.16 and 13.89 ppm respectively. The remaining aromatic carbons have shown signals in between δ 115.97 to 118.05 ppm which is in agreement with the expected values. The m/z ratios of molecular ions of the whole compounds are in good agreement with the expected molecular weight and are an indication of the stability of ion. That is the reason why most fragmentations are derived from the molecular ion obtained by this impact. The molecular ion peak at 294 [M^{+}] in the GC-MS proved an additional support to the architecture of compound 1a. The bond between C_{12} and S heteroatom, $C_{13,}$ and O heteroatom are the bearers of the most negative charge. The significant fragmentations take place on these carbons or are induced by heteroatoms (oxygen, or sulfur). These carbons are the most electron-donating carbons. Some fragmentation have been obtained giving the fragment A, which m/z ratio was M-45 (-OEt) and other fragments B and C with m/z=174 (-CS_2) and 120 (-CS_2OEt) respectively. The mechanistic mass fragmentation of compound (1a) is given in Scheme II. Rest all the compounds gave satisfactory spectroscopic data, which are in accordance with their assigned structures and have been tabulated in the experimental section.

COMPUTATIONAL STUDY

The computational study approach was used to study the interaction between a small molecule and a protein, which allow us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes. For the molecular docking studies, the crystal l structure of Bcl-2 in complex with a BAX BH3 peptide (PDB ID: 2XA0)[34] was used, obtained from the Protein Data Bank. The file was prepared for docking by adding polar hydrogen atom with Gasteiger-Huckel charges and water molecules were removed. The 3D structure of the ligands was generated by the SKETCH module implemented in the SYBYL program and its energy-minimized conformation was obtained with the help of the Tripos force field using Gasteiger-Huckel[35] charges and molecular docking was performed with Surflex-Dock program that is interfaced with SYBYL-X 2.0,[36] and other miscellaneous parameters were assigned with the default values given by the software.

Inhibition of microtubule polymerization, as a result, the formation of the mitotic spindle of dividing cells, cannot occur, which leads to activation of c-Jun NH_2-terminal Kinase (JNK), phosphorylation of Bcl-2, followed by the G2/M arrest and finally apoptosis.[37] In order to understand binding and inhibition of the synthesized hybrid molecules with the active site of the enzyme Bcl-2 and thereby sensitize cancer cells to apoptosis, in *silico* docking studies were performed. The Bcl-2 protein is monomeric in it stoichiometry and is present as a dimer with two chains A and B. Each monomeric unit has a ligand peptide BAX bound to the protein. The chain B of the structure of Bcl-2 PDB ID 2XA0 was selected for docking.

To support the interaction and preferred binding mode of coumarin derivatives. The predicted binding energies of all the compounds are listed in Table - II which shows that the compound 1c and 1a exhibit the highest C-score value of 7.98 & 7.11 respectively, which is in agreement with our observed results.



Scheme - II. Fragmentation pattern of the product (1a)

Table II: Surflex Docking score (kcal/mol) of the coumarin-carbonodithioate derivatives (1a-1j).

Compounds	C Score[a]	Crash Score[b]	Polar Score[c]	D Score[d]	PMF Score[e]	G Score[f]	Chem Score[g]
1a*	7.11	-0.97	3.14	-128.04	-33.47	-61.38	-19.44
1b	6.03	-0.86	2.37	-162.62	-46.52	-61.37	-23.29
1c*	7.98	-1.58	3.72	-309.23	-48.60	-130.28	-19.16
1d*	6.94	-1.88	2.92	-275.62	-28.83	-141.88	-27.29
1e	5.69	-0.81	3.02	-225.13	-44.07	-89.48	-21.22
1f	6.86	-1.23	3.13	-167.08	-51.26	-88.24	-16.61
1g	5.85	-0.85	1.91	-165.83	-46.25	-73.03	-22.79
1h	7.02	-1.18	1.29	-278.36	-29.51	-102.74	-17.90
1j	6.38	-0.97	3.31	-152.96	-42.36	-90.96	-14.84
1i	5.43	-0.76	3.24	-98.43	-37.51	-144.36	-17.39

*Asterisk indicates compounds with better CScore.
[a]CScore (Consensus Score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor and reports the output of total score.
[b]Crash-score revealing the inappropriate penetration into the binding site.
[c]Polar indicating the contribution of the polar interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds.
[d]D-score for charge and van der Waals interactions between the protein and the ligand (work of Kuntz).
[e]PMF-score indicating the Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF) (work of Muegge and Martin).
[f]G-score showing hydrogen bonding, complex (ligand-protein) and internal (ligand-ligand) energies.
[g]Chem-score points for H-bonding, lipophilic contact and rotational entropy, along with an intercept term.

The hydrogen bonding interactions of compound 1c are depicted in Fig. 2 and Fig. 3 wherein, Compound 1c shows five binding interaction at the active site of the enzyme, out of which two interaction raised from oxygen atom of terminal -OEt group binds with hydrogen atom of ARG415 and LYS411 (H-ARG415, 2.22 Å and H-LYS411, 1.84 Å), oxygen atom of -OMe group present at 6th position of coumarin makes hydrogen bonding interaction with hydrogen atom of ARG415 (H-ARG415, 1.98Å) and remaining two bonding interaction raised from the oxygen atom of –C=O group of coumarin with hydrogen atom of ASN238 and LYS168 (H-ASN238, 2.35Å and H-LYS168, 2.63Å).

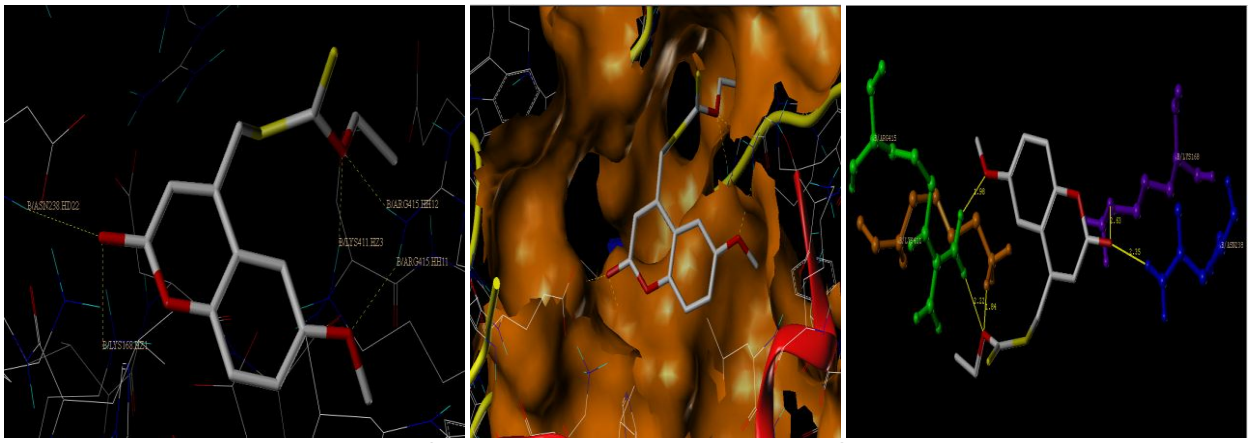


Fig 2. Interaction of compound 1c at the binding site of the enzyme 2XA0.

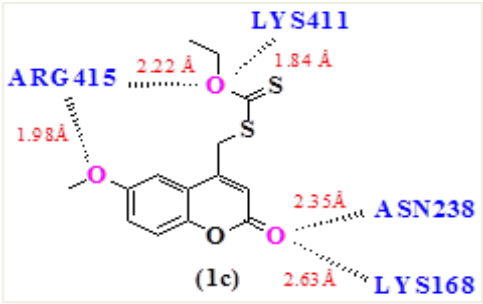


Fig 3. Binding interaction of compound 1c at the binding site of the enzyme 2XA0.

As depicted in Fig. 4 and Fig. 5, the compound 1a shows four binding mode of interaction, out of which three interaction raised from the oxygen atom of –C=O group of coumarin with hydrogen atom of ARG130 and GLY20 and remaining one interaction is raised from the oxygen atom of terminal -OEt group with hydrogen atom of LYS200.

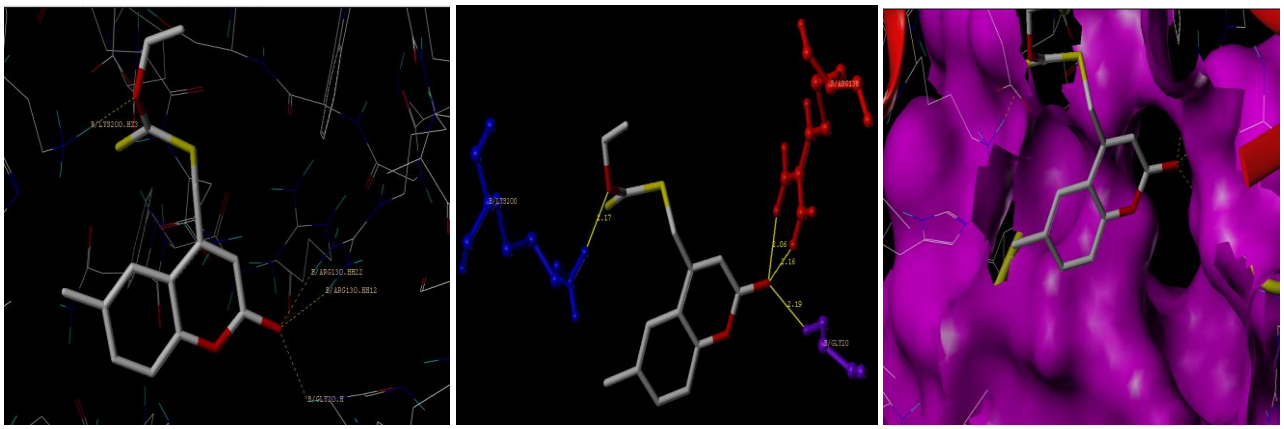


Fig 4. Interaction of compound 1a at the binding site of the enzyme 2XA0.

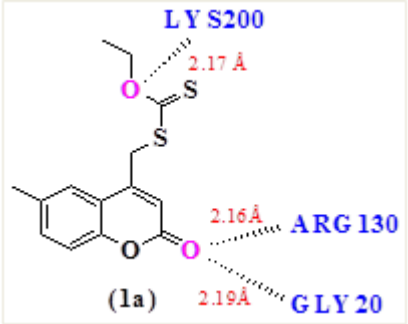


Fig 5. Binding interaction of compound 1a at the binding site of the enzyme 2XA0.

In practically, the anti-cancer assays against Bcl-2 strain demonstrated excellent results with compounds 1c and 1a with methoxy and methyl groups respectively. They have been found to be excellent compared with the standard. Incorporation of the carbonodithioate group has enhanced the binding ability of the new molecular entities at the active site of the enzyme as revealed by *in silico* studies. The enhanced activities of the compounds 1a, 1c, 1d, 1f, 1h and 1i could be attributed to the presence of methyl, methoxy and chlorine groups varied at coumarin ring. However, based on this promising observation, it is immature to arrive at the conclusion on structure-activity aspect of these molecules and further evaluation is needed to use them for clinical use.

BIOLOGICAL SCREENING

The newly synthesized compounds were evaluated for their anticancer activity against the nine different human cancers and screened for full 60 human cancer cell lines at NCI, USA.[38] Among (1a-1j), compounds 1a, 1c, 1d, 1f, 1h and 1i were selected to *in vitro* anticancer screening in a single high dose (10^{-5} M) concentration and exhibited well to moderate activity.

In vitro anticancer activity at a single high dose concentration (10^{-5} M)

The newly synthesized compounds were selected to *in vitro* anticancer screening in a single high dose against full 60 human cancer cell lines at NCI under DTP drug discovery program. The output from the single dose screen is reported as a graph of mean growth percent of the treated cancer cells. This provides detection of both growth inhibition values (between 0 and 100) and cytotoxicity values (less than 0). Compounds 1a (NSC: 793634/1), 1c (NSC: 793636/1), 1d (NSC: 793635/1), 1f (NSC: 794044/1), 1h (NSC: 794046/1) and 1i (NSC: 794045/1), have been screened for single high dose (10^{-5} M) concentration on all the 60 human cancer cell lines organized into nine sub-panels derived from nine different human cancer types: leukemia, lung, colon, CNS, melanoma, renal, ovarian, prostate and breast cancer cell lines. The overall results as shown in Table III.

The results discussed below are the percentage growth inhibition (GI%) of the treated cells at 10^{-5} M concentration. Compound (1c) showed excellent growth inhibition. The percentage growth inhibition of compound (1c) over the full panel of tumor cell lines is given in Fig. 6.

Table III. Anticancer activity of 1a, 1c, 1d, 1f, 1h and 1i compounds with one dose Mean Growth Inhibition Percent.

	Growth inhibition (GI %)					
Panel/ Cell line	6-Me-OEt	6-OMe-OEt	6-Cl-OEt	6-Me-OMe	6-OMe-OMe	6-Cl-OMe
Leukemia						
CCRF-CEM	27.77	47.35	22.12	-6.28	23.46	-1.80
HL-60(TB)	46.85	52.55	34.86	22.47	35.35	25.85
K-562	52.55	62.48	44.81	12.10	18.22	38.31
MOLT-4	15.91	38.98	-1.52	-0.05	55.60	4.75
RPMI-8226	48.07	72.06	43.48	14.92	16.29	19.51
SR	73.25	83.16	55.95	17.88	34.28	23.54
Non-Small Cell Lung Cancer						
A549/ATCC	27.16	20.80	25.57	-3.32	-1.79	-0.82
EKVX	-5.09	-3.10	8.57	-4.58	11.91	15.52
HOP-62	-10.03	24.32	-5.19	20.37	20.00	13.36
HOP-92	17.76	47.08	13.57	28.51	31.15	11.73
NCI-H226	53.77	-5.37	32.17	-16.68	-12.25	-2.60
NCI-H23	28.70	26.97	50.74	5.86	38.25	8.75
NCI-H322M	40.41	-8.59	13.15	7.33	-6.47	2.86
NCI-H460	-2.29	59.62	30.19	-11.43	57.38	-1.28
NCI-H522	34.11	28.62	26.32	9.32	11.69	19.87
Colon Cancer						
COLO 205	-4.50	-3.61	-3.78	-25.43	-11.33	-7.72
HCC-2998	-3.69	37.84	-3.84	15.75	-0.69	5.47
HCT-116	21.90	26.05	67.83	-5.01	24.37	13.51
HCT-15	36.90	33.55	34.24	2.76	25.10	17.47
HT29	59.44	77.38	28.90	11.04	61.04	-6.18
KM12	36.76	43.99	35.45	1.74	33.18	2.23
SW620	42.72	50.39	27.72	-7.74	-2.09	-9.38
CNS Cancer						

SF-268	25.11	-6.37	12.69	-0.39	17.54	13.22
SF-295	37.06	55.28	14.76	21.90	23.83	17.23
SF-539	51.68	63.50	21.09	13.57	57.04	45.39
SNB-19	09.06	-8.24	14.41	-8.64	27.03	10.88
SNB-75	23.25	34.29	21.12	11.41	17.92	26.56
U251	15.10	-4.25	11.39	-8.58	-0.79	21.29
Melanoma						
LOX IMVI	21.26	27.85	23.56	6.78	27.90	39.16
MALME-3M	11.20	-12.72	-12.65	-0.53	39.36	-0.15
M14	10.72	37.89	27.66	0.22	52.13	10.78
MDA-MB-435	20.14	-3.04	51.08	-4.78	43.98	42.04
SK-MEL-2	33.72	40.34	29.39	1.47	14.60	20.23
SK-MEL-28	-7.72	-8.23	-9.25	-2.31	-0.45	-2.18
SK-MEL-5	37.59	54.96	38.09	37.22	-8.02	5.46
UACC-257	15.34	67.31	26.02	-14.68	24.05	-1.12
UACC-62	9.40	-6.39	6.48	19.03	15.53	16.69
Ovarian Cancer						
IGROV-1	24.80	63.89	18.96	-9.02	20.76	-1.99
OVCAR-3	30.27	-5.64	-1.26	27.46	54.50	32.65
OVCAR-4	-6.28	48.30	-9.94	-3.92	21.54	-7.98
OVCAR-5	-18.56	79.30	42.87	25.78	-0.01	21.28
OVCAR-8	22.76	34.47	-2.29	-5.18	21.19	21.34
NCI/ADR-RES	30.26	-5.65	32.49	18.59	-3.00	13.24
SK-OV-3	-10.31	47.29	2.97	-3.74	27.93	-5.36
Renal Cancer						
786-0	30.75	34.64	50.12	-3.98	-2.70	-5.31
A498	23.79	46.25	-10.45	10.76	29.42	27.34
RXF 393	14.32	-19.57	-24.90	-25.35	-11.82	-24.46
SN12C	42.69	53.99	21.29	-5.19	-5.84	-2.24
TK-10	2.94	-3.75	-0.13	20.91	32.64	-27.84
UO-31	29.40	54.08	28.85	17.07	21.77	20.96
Prostate Cancer						
PC-3	15.62	64.01	10.60	-1.88	45.66	3.28
DU-145	8.03	-4.33	11.51	-6.06	14.08	21.17
Breast Cancer						
MCF7	71.40	80.45	31.34	18.19	17.66	18.01
MDA-MB-231/ATCC	46.90	52.15	59.49	-11.49	13.58	37.70
HS578T	50.79	44.04	-14.65	48.08	52.04	-5.95
BT-549	60.45	65.23	57.82	-12.33	25.96	-2.79
T-47D	68.38	78.15	58.40	61.81	63.50	56.33
MDA-MB-468	45.01	52.01	35.67	-6.51	32.69	-0.37

Compound with $-CH_3$ at C6 position of coumarin 1a (6-Me coumarin) showed good growth inhibition and the same substitution in case of (1f) showed comparative moderate activity. In case of compound (1d) and (1i) 6-chlorine substitution of coumarin, showed less growth inhibition compared to methoxy and methyl substitutions. The obtained results revealed that amongst the selected compounds methoxy substituted compounds were shown excellent activity due to electron donating groups substitution at 6-position of coumarin while others were good active at single high dose (10^{-5} M) concentration.

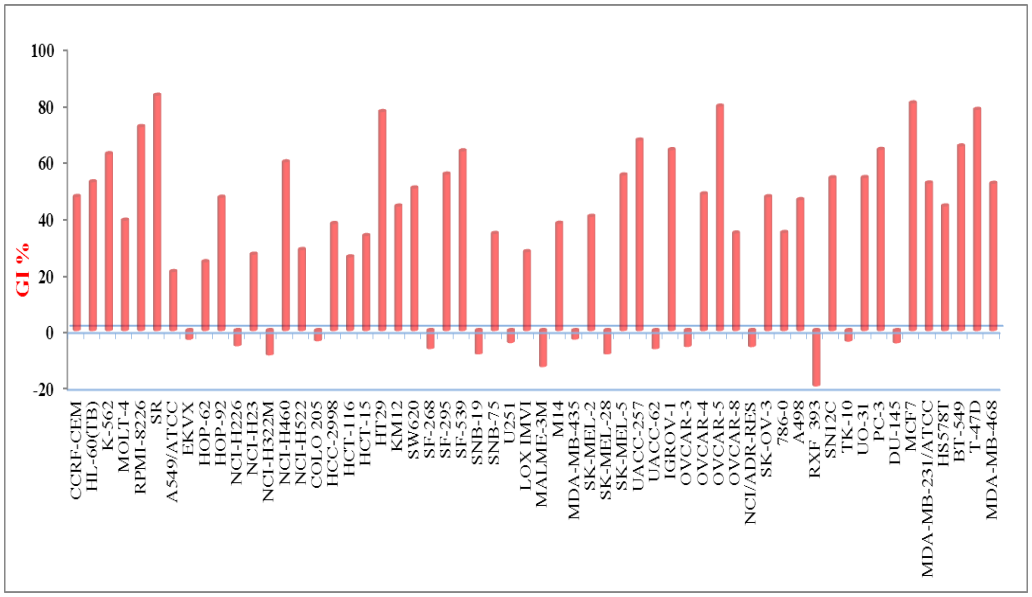


Fig 6. The percentages growth inhibition of compound (1c) over the full panel of tumor cell lines.

Structure-Activity Relationship (SAR) Studies

Although the number of compounds examined here is limited, a few key features regarding structural requirements for this *O-ethyl S-(6-methyl-2-oxo-2H-chromen-4-yl)methyl carbonodithioate* (1a-1j) to exert their anticancer activity can be determined. The design of coumarin-carbonodithioate analogs began with substitutions at the 6-position of the coumarin pharmacophore with small groups of varying lipophilicity. Specifically, methyl, methoxy and chloro-substituents were explored at the 6-position, affording analog 1a, 1c and 1d respectively. Similarly, in another set of five compounds, same substituents were explored to afford 1f, 1h and 1i, respectively. The substitution at 7-position with methyl and hydroxyl, 1b, 1e, 1g and 1j were inactive.

Our initial strategy was to identify the key sub unity required for activity such as; Coumarin (Active pharmacophore, which allows its derivatives readily interact with a diversity of enzymes & receptors) & Carbonodithioate group (Influence both the pharmacodynamic and pharmacokinetic properties of drugs). Further essential substituent's like, [R = -CH_3 (electron donating), -OCH_3 (electron releasing), -*Cl* (halogen)] groups were at the 6^{th} position of the coumarin ring to get the optimum results.

The results revealed the following assumptions about the structural activity relationship (SAR) studies. From the results, it is evident that in a group of compounds having -OCH_3 substituent's (1c and 1h) were influencing the anticancer activity very significantly, particularly at -6^{th} position (1c) was found to be the most active, *in-vitro* exhibiting 78.15% growth inhibition and in (1h), lowers the activity to 63.5% growth inhibition over breast cancer cell lines. The second line of activity was flaunted by the -CH_3 substituent's at -6^{th} position (1a) and (1f) exhibiting the comparatively good activity of 68.38% and 61.81% growth inhibition, respectively. The difference in the structures slightly lowers the activity of growth inhibition in (1i). Whereas the halogen substituent's (1d) and (1i) were found to be moderate activity against breast cancer cell lines of 58.40% and 56.33% growth inhibition respectively. The growth inhibitory results of all the compounds are in good agreement with the computational studies. The molecular docking studies of the compound (1c) showed good interaction towards the BCl_2 protein. The docking scores revealed good binding affinity towards all the compounds with same protein. It also suggests that better the C score value, the interaction will be more. Thus, from docking score table it concluded 1c showed excellent interaction with protein and displayed effectively *in vitro* growth inhibition of cancer cells.

Overall it can be hypothesized that compounds bearing -OCH_3 substituent have shown more significant growth inhibition of anticancer activity than -CH_3 and -*Cl*, substituent's at the coumarin ring, the second line of activity were flaunted by methyl substituent, while the -*Cl* substituent were found to be moderate. From the above results, it is also evident that the substituent's at the 6^{th} position of the coumarin ring showed much better activity when compared with the same substituent at 7^{th} position. The results from the preliminary structure-activity analysis have led to the determination of some key structural requirements for the title hybrids to exert their anticancer activity, which provides insights into further structural modifications.

CONCLUSION

In summary, we have designed, synthesized and characterized a series of new *O-ethyl S-(6-methyl-2-oxo-2H-chromen-4-yl)methyl carbonodithioate* (1a-1j) under MW conditions with a short reaction time, non-toxic, easy to handle and simple work up without any purification techniques. It was observed that MW

approach proved to be an extremely fast method, providing good to excellent yields (75-90%) as compared to the conventional method (55-75%). The *in vitro* anticancer activity assays revealed the significant growth inhibition of the tumor cells against various cell lines by six compounds have shown nearly 50-99%. Structural modifications of these title compounds may lead to the discovery of more effective anticancer agents in future. Molecular docking studies provided binding insights consistent with acceptor and donor of coumarin-carbonoditioates.

EXPERIMENTAL

Instrumentation

All the chemicals purchased were of analytical grade and used without further purification. Melting points were determined with the open capillary method on a Buchi apparatus and are uncorrected. IR spectra were recorded on a Nicolet 5700 FT-IR instrument using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on JEOL 400MHz Spectrometer using $CDCl_3$ as solvents and TMS as internal standard and chemical shifts were reported as δ values (ppm). Mass spectra were recorded using Shimadzu GCMSQP2010S. The elemental analysis was carried out using Hereaus CHN rapid analyzer.

Representative procedure for synthesis of Coumarin-carbonodithioates (1a-1j)

MW method: A mixture of substituted 4-bromomethyl coumarins (0.01mol) and Potassium O-ethyl dithiocarbonate (0.01mol) in 10ml MW vial with 5 ml of dry ethanol was run into MW irradiator at 65-70^{o}C for 7 to 10 min and cooled. The progress of the reaction was confirmed by thin layer chromatography (TLC). The reaction mixture was quenched onto the crushed ice; the solid product obtained was filtered and washed with water.

Conventional method: A mixture of substituted 44-bromomethyl coumarins (0.01mol) and Potassium O-ethyl dithiocarbonate (0.01mol) in 10ml RB flask with 5 ml of dry ethanol was refluxed at around 70^{o}C for 3-6 h and cooled. The progress of reaction was confirmed by thin layer chromatography (TLC). The reaction mixture was quenched onto crushed ice; the solid product obtained was filtered and washed with water recrystalised using ethanol.

O-ethyl S-(6-methyl-2-oxo-2H-chromen-4-yl)methyl carbonodithioate (1a)

Light yellow crystals; Mp 110-112^{o}C; IR (KBr) (ν_{max}/cm^{-1}): 1712 (C=O of coumarin), 1044(C=S of thiocarbonyl) cm^{-1} and 942 (S-OEt of thio-ester) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.33-7.37 (t, 2H, *J* = 6.4Hz), 7.22 (s, 1H), 6.54 (s, 1H), 4.67 (q, 2H), 4.46 (s, 2H), 2.41 (s, 3H), 1.43 (t, 3H); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 211.86, 160.66, 152.01, 149.26, 134.23, 133.23, 124.04, 118.05, 117.29, 115.97, 71.10, 36.13, 21.16, 13.89; ESI-MS: 294 $[M]^+$; Anal. calcd for $C_{14}H_{14}O_3S_2$: C, 57.12; H, 4.79%. found: C, 56.98; H, 4.92%.

O-ethyl S-(7-methyl-2-oxo-2H-chromen-4-yl)methyl carbonodithioate (1b)

Light yellow solid; Mp 120-122^{o}C; IR (KBr) (ν_{max}/cm^{-1}: 1710 (C=O of coumarin), 1045(C=S of thiocarbonyl) cm^{-1} and 857 (S-OEt of thio-ester) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.10-7.49 (m, 3H, *J* = 8.4Hz), 6.48 (s, 1H), 4.66 (q, 2H), 4.44 (s, 2H), 2.43 (s, 3H), 1.42 (t, 3H); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 211.90, 160.73, 153.98, 149.39, 143.56, 125.69, 123.94, 117.69, 115.94, 114.96, 71.07, 36.17, 21.72, 13.88; ESI-MS: 294 $[M]^+$; Anal. calcd for $C_{14}H_{14}O_3S_2$: C, 57.12; H, 4.79%. found: C, 56.03; H, 4.91%.

O-ethyl S-(6-methoxy-2-oxo-2H-chromen-4-yl)methyl carbonodithioate (1c)

Light yellow crystals; Mp 122-124^{o}C; IR (KBr) (ν_{max}/cm^{-1}: 1710 (C=O of coumarin), 1052(C=S of thiocarbonyl) cm^{-1} and 844 (S-OEt of thio-ester) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.02 - 7.29 (m, 3H, *J* = 8.8, 8.0 & 2.8Hz), 6.54 (s, 1H), 4.67 (q, 2H), 4.47 (s, 2H), 3.83 (s, 3H), 1.42 (t, 3H); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 212.00, 160.61, 156.13, 149.07, 148.26, 119.40, 118.72, 118.48, 116. 51, 107.28, 71.23, 56.06, 36.42, 13.88; ESI-MS: 310 $[M]^+$; Anal. calcd for $C_{14}H_{14}O_4S_2$:C, 54.17; H, 4.55%. found: C, 54.28; H, 4.72%.

S-(6-chloro-2-oxo-2H-chromen-4-yl)methyl O-ethyl carbonodithioate (1d)

Light yellow crystals; Mp 104-106^{o}C; IR (KBr) (ν_{max}/cm^{-1}): 1721 (C=O of coumarin), 1045(C=S of thiocarbonyl) cm^{-1} and 881 (S-OEt of thio-ester) cm^{-1}; ^{1}H NMR (400 MHz, CDCl3, δ ppm): 7.42 - 7.59 (m, 3H, *J* = 8.8 & 2.0 Hz), 6.60 (s, 1H), 4.67 (q, 2H, *J* = 7.2, & 6.8Hz), 4.43 (d, 2H), 1.43 (t, 3H); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 211.41, 159.74, 152.28, 148.45, 132.17, 130.01, 123.91, 119.54, 118.95, 116.99, 71.30, 35.96, 13.88; ESI-MS: 314 $[M]^+$; Anal. calcd for $C_{13}H_{11}ClO_3S_2$: C, 49.60; H, 3.52%. found: C, 49.76; H, 3.38%.

O-ethyl S-(7-hydroxy-2-oxo-2H-chromen-4-yl)methyl carbonodithioate (1e)

Light yellow crystals; Mp 176-178^{o}C; IR (KBr) (ν_{max}/cm^{-1}: 3326 (OH), 1701 (C=O of coumarin), 1038(C=S of thiocarbonyl) cm^{-1} and856 (S-OEt of thio-ester) cm^{-1}; ^{1}H NMR (400 MHz, CDCl3, δ ppm): 10.02 (s, 1H), 7.20 - 7.54 (m, 3H), 6.55 (s, 1H),4.63 (q, 2H, *J* = 7.2, & 6.8Hz), 4.39 (d, 2H), 1.38 (t, 3H); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 209.15, 157.45, 149.99, 146.16, 129.89, 127.72, 121.63, 117.26, 116.66, 114.07, 69.02, 33.68, 11.59; ESI-MS: 296 $[M]^+$; Anal. calcd for $C_{13}H_{12}O_4S_2$: C, 52.69; H, 4.08%. found: C, 52.88; H, 4.23%.

O-methyl S-(6-methyl-2-oxo-2H-chromen-4-yl)methyl carbonodithioate (1f)

Light yellow crystals; Mp 118-120^{o}C; IR (KBr) (ν_{max}/cm^{-1}: 1714 (C=O of coumarin), 1044(C=S of thiocarbonyl) cm^{-1} and 891 (S-OEt of thio-ester) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.33-7.37 (m, 2H), 7.22 (s, 1H), 6. 53 (s, 1H), 4.64 (s, 3H), 4.46 (s, 2H), 2.41(s, 3H); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 211.86, 160.65, 152.01,

149.26, 134.23, 133.23, 124.04, 118.05, 117.29, 115.97, 71.09, 36.13, 21.16; ESI-MS: 280 $[M]^+$; Anal. calcd for $C_{13}H_{12}O_3S_2$: C, 55.69; H, 4.31%. found: C, 55.81; H, 4.22%.

O-methyl S-(7-methyl-2-oxo-2H-chromen-4-yl)methyl carbonodithioate (1g)

Light yellow crystals; Mp 114-116°C; IR (KBr) (ν_{max}/cm^{-1}: 1715 (C=O of coumarin), 1047(C=S of thiocarbonyl) cm^{-1} and 891 (S-OEt of thio-ester) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm), 7.23-7.63 (m, 3H), 6. 62 (s, 1H), 4.79 (s, 3H), 4.58 (s, 2H), 2.57 (s, 3H); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm):, 212.00, 160.82, 154.07, 149.49, 143.66, 125.78, 124.04, 117.78, 116.04, 115.06, 71.17, 36.27, 21.82; ESI-MS: 280 $[M]^+$; Anal. calcd for $C_{13}H_{12}O_3S_2$: C, 55.69; H, 4.31%. found: C, 55.61; H, 4.42%.

S-(6-methoxy-2-oxo-2H-chromen-4-yl)methyl O-methyl carbonodithioate (1h)

Light yellow crystals; Mp 120-122°C; IR (KBr) (ν_{max}/cm^{-1}: 1713 (C=O of coumarin), 1047(C=S of thiocarbonyl) cm^{-1} and 846 (S-OEt of thio-ester) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.02 - 7.29 (m, 3H), 6.54 (s, 1H), 4.66 (s, 3H), 4.47 (s, 2H), 3.83 (s, 3H); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 211.80, 160.40, 155.93, 148.87, 148.06, 119.20, 118.52, 118.28, 116.31, 107.09, 71.02, 55.86, 36.22; ESI-MS: 296 $[M]^+$; Anal. calcd for $C_{13}H_{12}O_4S_2$:C, 52.69; H, 4.08%. found: C, 52.48; H, 4.02%.

S-(6-chloro-2-oxo-2H-chromen-4-yl)methyl O-methyl carbonodithioate (1i)

Light yellow crystals; Mp 126-128°C; IR (KBr) (ν_{max}/cm^{-1}: 1722 (C=O of coumarin), 1046(C=S of thiocarbonyl) cm^{-1} and 881 (S-OEt of thio-ester) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm), 7.29 - 7.63 (m, 3H), 6.64 (s, 1H), 4.74 (s, 3H), 4.48 (s, 2H); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 205.95, 154.24, 146.80, 142.65, 126.68, 124.52, 118.42, 114.05, 113.46, 111.5, 65.81, 30.47; ESI-MS: 300 $[M]^+$; Anal. calcd for $C_{12}H_9ClO_3S_2$: C, 47.92; H, 3.02%. found: C, 48.08; H, 3.12%.

S-(7-hydroxy-2-oxo-2H-chromen-4-yl)methyl O-methyl carbonodithioate (1j)

Light yellow crystals; Mp 144-146°C; IR (KBr) (ν_{max}/cm^{-1}): 3325 (OH), 1702 (C=O of coumarin), 1037(C=S of thiocarbonyl) cm^{-1} and 884 (S-OEt of thio-ester) cm^{-1}; ^{1}H NMR (400 MHz , CDCl3, δ ppm): 10.07 (s, 1H), 7.28 - 7.59 (m, 3H), 6.60 (s, 1H), 4.70 (s, 3H), 4.43 (s, 2H); ^{13}C NMR (100MHz, $CDCl_3$, δ ppm): 211.76, 160.06, 152.60, 148.76, 132.49, 130.33, 124.23, 119.86, 119.27, 117.30, 71.62, 36.28; ESI-MS: 282 $[M]^+$; Anal. calcd for $C_{12}H_{10}O_4S_2$: C, 51.05; H, 3.57%. found: C, 51.28; H, 3.72%.

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