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Graphical Abstract

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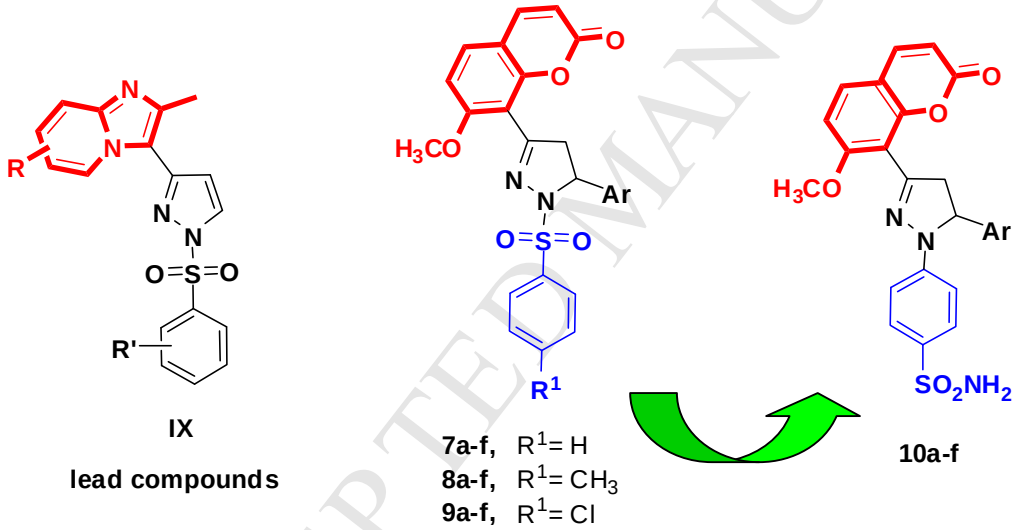
Two groups of coumarin-pyrazoline hybrids bearing either (un)-substituted phenylsulfonyl or terminal sulfamoyl moiety were synthesized as potential antitumor agents utilizing the patent compounds **IX** as lead.



Highlights

- Two groups of coumarin-pyrazoline hybrids were synthesized.
- Selected compounds were tested towards 60 cell lines according to US NCI protocol.
- All compounds were screened against HCT-116.
- The most active compounds were screened for PI3K (P110α/p85α) inhibition.

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Abstract

Two groups of coumarin-pyrazoline hybrids were synthesized. The target compounds were obtained by cyclization of the coumarin chalcones with various substituted hydrazines to produce the corresponding pyrazolines through 1,4-addition on α,β-unsaturated carbonyl system. Selected compounds were investigated for their anticancer activity towards 60 cancer cell lines according to US NCI protocol where breast cancer MCF7 and colon cancer HCT-116 were the most susceptible to the influence of compounds **7d, 8c** and **9c**. Encouraged by this, all final compounds were screened against colorectal cell line HCT-116. The tested compounds exhibited high potency with IC_{50} ranging from 0.01 µM to 2.8 µM. Moreover, compound **9c** which possessed the highest cytotoxicity proved to have weak enzyme inhibitory activity against PI3K (P110α/p85α).

Keywords: Coumarins; pyrazoline; phenylsulfonyl derivatives; antitumor activity.

1. Introduction

Cancer is a notably complex, widespread and lethal disease accounting for 7.6 million deaths (around 13% of all deaths) in 2008, that are projected to continue rising, with an estimated 13.1 million deaths in 2030 [1]. Cancer can affect almost every tissue lineage in the human body and poses great challenges to medical science. Most cancers are characterized by uncontrolled cell proliferation, lack of cell differentiation, and loss of contact inhibition, which confers upon the tumor cell a capability to invade local tissues and metastasize [2,3]. Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells and involve deregulation of essential enzymes and other proteins controlling cell division and proliferation [4]. Several lines of evidence support the view that chemotherapy has become one of the most significant treatment modalities in cancer management. However, the nonselectivity and acute toxicity of many antitumor agents beside the development of cellular drug resistance have been the major deterrent in their usage for treating human cancer, prompting the search for new antitumor agents with improved tumor selectivity, efficiency, and safety [5-7].

Natural and synthetic coumarin derivatives have attracted intense interest in recent years because of their diverse biological and pharmacological properties. Among these properties, their antitumor effects were extensively examined [8-14]. Coumarin and its metabolite,7-hydroxycoumarin **I,** were reported to inhibit the proliferation of a number of human malignant cell lines in vitro [15,16] and in xenograft models [17,18]. Moreover, coumarin was found to produce objective tumor regression in some patients with metastatic renal cell carcinoma, metastatic prostatic carcinoma and malignant melanoma in clinical trials [19]. Osthole **II**, 7-methoxy-8-isopentenyl coumarin, also showed potent activity against lung cancer A549 cells and breast cancer cells by arresting cell cycle in G2 phase followed by inducing apoptosis through modulating PI3K/Akt pathway [20]. Novel coumarin 3-(*N*-aryl) sulfonamides such as **III**, displayed considerable growth inhibition followed by cell death in different cancer cell lines with GI_{50} values less than 100 μM, and proved to be activators of JNK1 alpha protein kinase [21]. The stilbene-coumarin hybrid **IV** was identified as novel anticancer endowed with excellent antiproliferative and proapotosis activities against squamous cell carcinoma A43 and melanoma JR8 [22].

Cytotoxic studies on 7-methoxy-4-methylcoumarin derivatives highlighted their potential as selective antiproliferative and multidrug resistance reversal modulators [23,24] (Fig. 1).

On the other hand, the importance of pyrazoline ring as scaffold for new antineoplastic agents was widely investigated [25,26]. For example, 3,5-diaryl-pyrazoline regioisomers **V**, and their N^1-acetylated derivatives showed potent and selective activity in the NCI 60 human cancer cell lines panel [27, 28]. Another acylated derivative **VI** was chosen among its series as a selective inhibitor of B- Raf Kinase [29, 30]. Some novel N^1-phenylsulfonyl pyrazoline derivatives **VII** exhibited high activity against human gastric cancer cell SGC-7901, liver cancer Hep-G2 and human prostate PC-3 cell lines by virtue of their potential telomerase inhibitory effect [31]. In addition, coumarin-pyrazoline conjugates **VIII** exhibited potentially high activity against human gastric cancer cell SGC-7901 [32] (Fig. 2). Moreover, imidazopyridine derivatives bearing the pyrazole ring, such as the patent compounds **IX** [33] were reported as potent phosphoinositol-3 kinase PI3K inhibitors and antitumor agents [34, 35] (Fig. 3).

Finally, several sulfonamide derivatives were reported to show substantial antitumor activity, both in vitro and/or in vivo [36-38]. Some derivatives are currently being evaluated in clinical trials, and there is much optimism that they might lead to novel alternative drugs, devoid of the side effects of the presently available anticancer agents [39, 40].

In the design of new drugs, the hybridization and bioisosterism approaches might allow obtaining molecules with improved biological activity with respect to the corresponding lead compounds. Thus, adopting these approaches, two series of coumarin-pyrazoline hybrids comprising two types of substitution patterns; one bearing (un)-substituted phenylsulfonyl entities **7-9**, while the other type had a terminal sulfamoyl moiety **10** were synthesized as potential antitumor agents, utilizing the patent compounds **IX** [33] as lead for the design of the present work (Fig. 3).

2. **Results and Discussions**

2.1. Chemistry

The synthesis of the novel coumarin-pyrazoline hybrids **7a-f**, **8a-f**, **9a-f** and **10a-f** was accomplished through reaction of the precursor chalcones, 7-methoxy-8-(arylacryloyl)-2*H*-

chromen-2-ones **6a-f [**41] with the variously (un)-substituted phenylsulfonyl hydrazines viz phenylsulfonyl hydrazine, 4-methylsulfonyl hydrazine, 4-chlorophenylsulfonyl hydrazine or 4-sulfamoylphenyl hydrazine (Scheme 1). These pyrazolines were obtained via 1,4-addition to the α,β-unsaturated carbonyl system, followed by dehydration and rearrangement.

The structure elucidation of the newly synthesized compounds was based on the spectral data (IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry). IR spectra of the prepared pyrazolines **7-10** were characterized by the presence of υ (C=N) stretching at 1604-1558 cm^{-1} due to ring closure and two strong bands in the region of 1342-1290 and 1168-1087cm^{-1} due to (SO_2) stretching. Also, strong broad peaks derived from coumarin (C=O) stretching were observed around 1728-1700 cm^{-1}. In addition, derivatives **10a-f** showed two typical absorption bands at 3392-3367 and 3336-3201 cm^{-1} due to their NH_2 stretching vibrations. ^{1}H NMR spectra recorded for these compounds clearly supported the proposed structures. The pyrazoline protons in compounds **7-10** showed a prominent AMX pattern with three protons H_A, H_M and H_X seen as doublets of doublets. The H_X proton was more deshielded by aryl group (δ 4.80-5.80 *ppm*) and was coupled transoid to the H_M proton and cisoid to the H_A proton. The H_M proton was deshielded to some extent by aryl group (δ 3.40-4.20 *ppm*) and was coupled transoid to the H_X proton and geminally to the H_A proton. The third, H_A proton (δ 2.84-3.45 *ppm*) was coupled cisoid to the H_X proton and geminally to the H_M proton.

There were three coupling constants: geminal (J_{AM} = 16.4-21.9 Hz), trans (J_{MX} = 10.5-16.8 Hz) and cis coupling (J_{AX} = 6.9-8.7 Hz). In case of compounds **7b**, **8b**, **9b** and **10a** simple splitting patterns were observed with pyrazoline protons which appeared as two signals, one doublet equivalent to 2H (δ 3.21-3.92 *ppm*, J = 9.9-11.4 Hz) and a triplet equivalent to 1H (δ 4.30-4.95 *ppm*).

The protons belonging to the aromatic system and phenyl substituents were observed at the expected chemical shifts and integral values. In addition, the derivatives **10a-f** showed a singlet exchangeable signal at δ 4.20-5.10 *ppm* corresponding to NH_2 protons.

2.2. Biological evaluation

2.2.1. Anticancer activity

Seven compounds **7a, 7b, 7d, 7f, 8c, 9c** and **10a** were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) to be screened for their anticancer activity *in vitro*. The anticancer assays were performed in accordance with the protocol of the Drug Evaluation Branch, NCI, Bethesda [42-44].

The compounds were first evaluated at one dose primary anticancer assay towards a panel of approximately 60 cancer lines (concentration 10^{-5} M). The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers. A 48 h drug exposure protocol was used and sulforhodamine B (SRB) protein assay was applied to estimate the cell viability and growth [45]. Results for each test agent were reported as the percentage growth of the treated cells compared to the untreated control cells and also, presented as mean graph of the growth percent. The preliminary screening results showed that compounds **7a, 7b, 7f** and **10a** had no significant antitumor action. Nevertheless, compounds **7d, 8c** and **9c** possessed considerable activity and were passed on to further evaluation at five-dose assay [0.01 µM-100 µM]. The calculated response parameters for the three compounds -GI_{50}, TGI and LC_{50}- against each cell line were presented in Table 1. GI_{50} corresponds to the molar concentration of the compound causing 50% decrease in net cell growth and is viewed as a growth-inhibitory level of effect; TGI (cytostatic activity) is the molar concentration of the compound leading to total growth inhibition; LC_{50} is the cytotoxicity parameter and reflects the molar concentration needed to cause 50% net cell death. Values were calculated for each of these parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value was expressed as more or less than the maximum or minimum concentration tested. Furthermore, sub-panel and full panel mean graph midpoints (MG-MID) were calculated for each parameter, giving an average activity parameter over individual sub-panels and full panel cell lines toward each compound (Tables 2 and 3). In general, compounds **7d, 8c** and **9c** exhibited superior anticancer activities against the entire panel of tumor cell lines with the chlorophenylsulfonyl analogue **9c** being the most potent. These agents showed effective growth inhibition full

panel GI_{50} (MG-MID) values of 2.18, 1.89 and 1.63 µM, respectively and cytostatic activity full panel TGI (MG-MID) values of 7.32, 7.36 and 5.82 µM, respectively. Regarding sensitivity, the NCI panel cell lines displayed comparatively homogenous response with only few cell lines showing greater sensitivity than the average toward the evaluated compounds. Among the tested cancer cell lines, the colon cancer, melanoma and breast cancer panels were the most susceptible to the influence of **7d, 8c** and **9c** [GI_{50} (MG-MID) 2.09, 1.69 and 1.45 µM for colon cancer, GI_{50} (MG-MID) 2.04, 1.74 and 1.51 µM for melanoma and GI_{50} (MG-MID) 2.03, 1.52 and 1.67 µM for breast cancer]**.**

As for the sensitivity against some individual cell lines, compound **7d** presented an appreciable inhibitory activity toward all tumor subpanels, GI_{50} (MG-MID) range of 2.03-2.65 µM. Also, low degree of differential sensitivity between the most and least sensitive cell lines was observed, GI_{50} range 1.15-3.86 µM. Breast cancer MDA-MB-468, melanoma UACC-62, renal cancer UO-31 as well as colon cancer HCT-116 with GI_{50} range of 1.15-1.62 µM were among the most sensitive cell lines.

Compared to **7d**, the analogue **8c** elicited a better or comparable inhibitory efficacy through out the whole cell line spectrum. It was found especially effective against breast cancer MCF-7 and MDA-MB-468 with GI_{50} 0.99 and 0.50 µM, respectively. Moreover, **8c** revealed a distinctive effectiveness on colon cancer HCT-116, CNS cancer U251, renal cancer RXF 393 and breast cancer BT-549 at both the inhibitory and cytostatic levels. On the other hand, compound **9c** showed the highest growth inhibitory potential against most of the tested cell lines, with an outstanding activity on six different cell lines with GI_{50} lying in the submicromolar range. It exhibited a distinguished sensitivity profile toward leukemia SR, colon cancer HCT-116, CNS cancer U251 and melanoma LOX IMVI, in addition to remarkable high activity against MCF-7 and MDA-MB-468 breast cancer, GI_{50} 0.49 and 0.34 µM, respectively.

The cytotoxicity values associated with the three compounds, measured as LD_{50} were less than 100 µM for most of the tested cell lines except for leukemia.

Concerning selectivity, the index obtained by dividing the full panel MG-MID (µM) of the compounds by their individual sub-panel MG-MID (µM) is considered as a measure of compound selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios greater than six indicate high selectivity towards the corresponding cell line, while compounds not

meeting either of these criteria are rated as nonselective [46]. In this context, compounds **7d, 8c** and **9c** proved to be nonselective with broad spectrum antitumor activity against all tumor sub-panels tested at both GI_{50} and TGI levels, with selectivity ratios ranges of 0.80–1.25 and 0.54–1.70, respectively (Tables 2 and 3).

Colorectal cancer (CRC) is considered one of the most common cancers worldwide and is commonly associated with genes mutation that control growth factor signaling, therefore, new therapeutic agents are being developed to target such tumor [47]. According to the results of the cytotoxicity screening against 60 human tumor cell lines performed at the NCI (Bestheda), it was observed that the tested compounds were potent inhibitors of tumor cell growth and had promising activity against HCT-116 colon cell. Since only seven compounds were selected by the NCI for this screening assay, it was of interest to extend the cytotoxicity study by testing the cell growth inhibitory activity of all target compounds, **7a-f**, **8a-f**, **9a-f** and **10a-f** against human colon cancer cell line (HCT-116). This permitted a good structure activity relationship study. Results were expressed as IC_{50} in µM as shown in Table 4 and activity was compared with that of doxorubicin as reference drug. The data showed that sixteen compounds were found more active than doxorubicin. The most active compounds was **9c** having an IC_{50} of 0.01 µM, (IC_{50} of doxorubicin= 0.63 µM). Other highly potent compounds were **7d**, **8c**, **10e** and **10f** having IC_{50} values of 0.02, 0.02, 0.03 and 0.04 µM, respectively. Correlation of the activity results of the first set of compounds, the 1-(un)substituted phenylsulfonyl-5-arylpyrazolines **7a-f**, **8a-f** and **9a-f** showed that within each individual series there was no absolute relation between the activity and the nature of the aryl substituent (Ar) viz. 5-methyfuranyl, thienyl, phenyl, 4-tolyl, 4-trifluoromethylphenyl and 4-methylthiophenyl. As for the 1-(un)substituted phenylsulfonyl group, it was obvious that compounds having the chloro substituent **9a-c,e,f** were generally the most active especially that all compounds in this series were more active than the reference drug. Also, compared to the other series (**7a-f** and **8a-f**), some derivatives were more potent than their respective analogues as exhibited by compounds **9b**, **9c** and **9f** (IC_{50}= 0.11, 0.01 and 0.11 µM, respectively). The unsubstituted phenylsulfonyl pyrazolines **7a-f** and the 4-methylphenylsulfonyl derivatives **8a-f** interchanged positions regarding their order of activity. From the second set of compounds **10a-f** emerged two promising compounds, **10e** and **10f**, having IC_{50} values of 0.03 and 0.04 µM, respectively. Within this

series, it was apparent that the activity was favored by the phenyl groups having electron withdrawing substituents such as trifluoromethyl and methylthio as in compounds **10e** and **10f**, respectively.

Comparing the activity of the two sets of compounds, namely the 1-(un)-substituted phenylsulfonyl-5-arylpyrazolines (**7a-f**, **8a-f** and **9a-f**) and the sulfamoylphenyl congeners **10a-f**; it was important to explore the effect of flipping the benzene sulfonyl moiety in the first set into a phenyl group with free terminal sulfamoyl function in the second one. Analyzing the data revealed that compounds having 5-methylfuran substitution at position 5 of the pyrazoline ring **7a**, **8a** and **9a** were more active than their corresponding analogue **10a**. The same held true for the compounds with a phenyl substitution (**7c**, **8c**, and **9c** c.f. **10c**). On the other hand, the activity of trifluoromethylphenyl and methylthiophenyl derivatives, **7e,f**, **8e,f** and **9e,f**, was enhanced in the case of their respective analogues **10e,f**.

2.2.2. PI3K (p110α/p85α) protein kinase activity

Genomic studies indicated that phosphoinositide 3-kinase (PI3K) signaling is one of the most frequently deregulated pathways in several human cancers, including CRC. PI3K signaling has an important role in cancer cell proliferation, survival, motility, and metabolism [47,48]. To investigate the possible mechanism of action responsible for the observed cytotoxic effects and due to structure similarity of the newly synthesized compounds with the PI3K inhibitors **IX**, sixteen compounds were selected to be evaluated for their ability to inhibit the activity of PI3K *in vitro*. The compounds were initially screened against PI3K (p110α/p85α) protein kinase at a single dose of 50 µM by employing the standardized ADP-GIo^{TM} assay methodology at Kinexus Labs [49]. The profiling data in Table 5 showed potent to weak inhibition of PI3K (p110α/p85α) activity by the 4-chlorophenylsulfonylpyrazoline derivatives **9c, 9d** and **9f** only. Compound **9c** bearing unsubstituted phenyl group at position 5 of pyrazoline ring exhibited the highest inhibition percentage (100%). Meanwhile the 4-tolyl **9d** and 4-trifluromethylphenyl **9f** analogs inhibited the target enzyme by 20% and 19%, respectively, which suggested poor bulk tolerance regarding substitution on this phenyl. Based on these preliminary results, the IC_{50} value of the most active enzyme inhibitor **9c** was determined in comparison with the known isoform-nonspecific PI3K inhibitor wortmannin [50] as reference. Moreover, the

previously reported data for the p110α inhibitor **IX** was listed in table 5 for comparison [51]. Compound **9c** showed mild inhibitory activity (IC_{50} = 50.78 μM) compared to potent enzyme inhibition demonstrated by wortmannin (IC_{50} =1.61 μM) and the lead compound **IX** (IC_{50} =0.67 μM).

The inconsistency between the enzyme inhibitory activity and cellular efficiency against HCT-116 suggested that the strutcural modifications made in the target compounds might have switched the binding affinity to other biomolecular target involved in cellular signaling pathways. Hence, the definite mechanism needs to be further investigated.

3. Conclusion

Two groups of coumarin-pyrazoline hybrids featuring an (un)substituted phenylsulfonyl moieties, **7a-f**, **8a-f** and **9a-f**, or a terminal sulfamoyl function **10a-f** were prepared. Among other compounds selected and evaluated according to the protocol of the Drug Evaluation Branch, NCI, Bethesda, compounds **7d, 8c** and **9c** exhibited excellent anticancer activities against the entire panel of tumor cell lines with the chlorophenylsulfonyl analogue **9c** being the most potent, in particular against breast cancer MCF7 and colon cancer HCT-116. Subsequently, all target derivatives were screened against human colon cancer cell line (HCT-116) where sixteen compounds were found more potent than doxorubicin especially compound **9c** having an IC_{50} of 0.01 μM (IC_{50} of doxorubicin= 0.63 μM). SAR study showed that grafting an electron-withdrawing chlorine atom to the 4^{th} position of the phenylsulfonyl moiety was profitable to the antitumor activity of the first group of compounds, **7a-f**, **8a-f** and **9a-f**. Meanwhile, there was no absolute relation between the activity and the nature of the aryl substituent at the 5^{th} position of pyrazoline ring in the same group of compounds. Conversely, the cytotoxicity of the second group of compounds **10a-f** was enhanced by the presence of a strong electron-withdrawing group on the 5-phenyl substituent. Moreover, the relation between activity and the location of the sulfonyl group, either internal as in the first group of compounds or free terminal sulfamoyl as in the second group, was not consistent throughout all derivatives.

Finally, the investigation was extended by screening sixteen selected compounds for their ability to inhibit PI3K (P110α/p85α) as potential molecular target. Among them, the most

potent antitumor agent **9c** emerged as a promising enzyme inhibitor (IC_{50}=50.78 μM). Despite of such mild enzyme inhibitory effect, the significance of **9c** is that it might represent a new lead for future optimization of more potent PI3K inhibitors.

4. **Experimental**

4.1. Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Reactions' time and purity of the products were monitored by TLC on FLUKA silica gel TLC aluminum cards (0.2 mm thickness) with fluorescent indicator 254 nm using chloroform: methanol (9:1) as eluent. Melting points were performed on Stuart SMP3 version 5 digital melting point apparatus and were uncorrected. Elemental microanalyses were performed at the microanalytical center, Faculty of Science, Cairo University and the Regional Center for Mycology and Biotechnology, Al-Azhar University. ^{1}HNMR spectra were determined using Varian Mercury VX-300 MHz or 200 MHz NMR Spectrometer (Oxford, England). ^{13}CNMR spectra were run at 75.46 MHz in deuterated chloroform (C*D*Cl_3) or dimethylsulphoxide (DMSO-d_6). Chemical Shifts are given in δ as parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard. Mass spectra were recorded using Hewlett Packard Varian (Varian, Polo, USA), Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX (Shimadzu, Kyoto, Japan) and Finnegan MAT, SSQ 7000 mass spectrophotometer at 70eV. IR spectra were recorded on Bruker FT-IR spectrophotometer as potassium bromide discs.

*4.1.1. 7-Acetoxy-2H-chromen-2-one (**2**).*

Compound **2** was prepared according to the literature procedure [52] (m.p.147^{o}C, as reported).

*4.1.2. 8-Acetyl-7-hydroxy-2H-chromen-2-one (**4**).*

Compound **4** was prepared according to the literature procedure [52] (m.p.167^{o}C, as reported).

*4.1.3. 8-Acetyl-7-methoxy-2H-chromen-2-one (**5**).*

Compound **5** was prepared according to the literature procedure [53- 55] (m.p.123^{o}C, as reported).

4.1.4. *General procedure for synthesis of 7-Methoxy-8-[arylacryloyl]-2H-chromen-2-ones* **6a-f.**

Compounds of this series (**6a-f**) were prepared according to the literature procedure [41].

4.1.5. General procedure for the preparation of 8-(5-substituted-1-(phenylsulfonyl)-4,5-dihydro-1H-pyrazol-3-yl)-7-methoxy-2H-chromen-2-ones **7a-f**:

Phenylsulphonyl hydrazine (1.72 g, 10 mmol) was added to a solution of the appropriate propenone derivative **6a-f** (10 mmol) in absolute ethanol (50 ml) and refluxed for 12 h. The reaction mixture was filtered while hot and then left overnight. The solid product was filtered and crystallized from ethanol.

*4.1.5.1. 7-Methoxy-8-[5-(5-methylfuran-2-yl)-1-(phenylsulfonyl)-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one (**7a**):*

Yield: 35%, m.p. 194-196^{o}C. IR υ_{max}/cm^{-1}: 3032 (CH aromatic), 2943, 2843 (CH aliphatic), 1720 (C=O), 1620, 1597 (C=C, C=N), 1300, 1141 (SO_2). ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.18 (s, 3H, CH_3), 2.85 (dd, 1H, C4-H_A pyrazoline, J_{AX} = 8.4 Hz and J_{AM} = 18.0 Hz), 3.10 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 11.0 Hz and J_{MA} = 18.0 Hz), 3.88 (s, 3H, OCH_3), 5.80 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 11.0 Hz and J_{XA} = 8.4 Hz), 6.29 (d, 1H, C3-H chromen, *J* = 9.6 Hz), 6.32 (d, 1H, C4-H furan, *J* = 1.2 Hz), 6.51-7.20 (m, 7H, C3-H furan, C6-H chromen and ArH), 7.78 (d, 1H, C5-H chromen, *J* = 8.7 Hz), 8.05 (d,1H, C4-H chromen, *J* = 9.6 Hz). Anal. Calcd for $C_{24}H_{20}N_2O_6S$ (464.29): C, 62.06; H, 4.34; N, 6.03. Found: C, 61.70; H, 4.22; N, 6.33.

*4.1.5.2. 7-Methoxy-8-[1-(phenylsulfonyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one (**7b**):*

Yield: 55%, m.p. 168-170^{o}C. IR υ_{max}/cm^{-1}: 3066 (CH aromatic), 2966, 2939 (CH aliphatic), 1724 (C=O), 1605, 1597 (C=C, C=N), 1342, 1168 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 3.29 (d, 2H, *J* = 10 Hz, CH_2 pyrazoline), 3.80 (s, 3H, OCH_3), 5.34 (t, 1H, C5-H pyrazoline, *J* = 11.0 Hz), 6.31 (d, 1H, C3-H chromen, *J* = 9.6 Hz), 6.94 (t, 1H, C4-H thiophene, *J* = 8.1 Hz), 7.14 (d, 1H, C6-H chromen, *J* = 9.6 Hz), 7.46 (d, 1H, C3-H thiophene, *J* = 8.6 Hz), 7.53 (d,

1H, C5-H thiophene, *J* = 8.7 Hz), 7.58 (d, 1H, C5-H chromen, *J* = 9.6 Hz), 7.69-7.84 (m, 5H, ArH), 7.99 (d, 1H, C4-H chromen, *J* = 9.6 Hz). ^{13}C NMR (DMSO d_6) δ *ppm*: 40.1 (C-4 pyrazoline), 40.4 (C-5 pyrazoline), 56.3 (OCH_3), 106.9 (C-6 chromen), 108.6 (C-3 chromen), 112.8 (C-4a chromen), 113.3 (C-8 chromen), 126.4 -132.5 (C-5 chromen, aromatic Cs and thiophene Cs), 139.6 (C-SO_2), 143.9 (C-4 chromen), 144.9 (C-8a chromen), 152.1 (C=N pyrazoline), 159.1 (C-7 chromen), 159.6 (C=O). MS, m/z 466.15: [M^+]. Anal. Calcd for $C_{23}H_{18}N_2O_5S_2$ (466.53): C, 59.21; H, 3.89; N, 6.00. Found: C, 58.95; H, 4.02; N, 5.84.

*4.1.5.3. 7-Methoxy-8-[5-phenyl-1-(phenylsulfonyl)-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one (**7c**):*

Yield: 65%, m.p. 196-198^{o}C. IR υ_{max}/cm^{-1}: 3066 (CH aromatic), 2947, 2908 (CH aliphatic), 1732 (C=O), 1604, 1592 (C=C, C=N), 1303, 1145 (SO_2). ^{1}HNMR ($CDCl_3$) δ *ppm*: 3.25 (dd, 1H, C4-H_A pyrazoline, J_{AX} =6.9 Hz and J_{AM} = 21.9 Hz), 3.90 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 15.0 Hz and J_{MA} = 21.9 Hz), 3.83 (s, 3H, OCH_3), 4.80 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 15.0 Hz and J_{XA} = 6.9 Hz), 6.21 (d, 1H, C3-H chromen, *J* = 9.3 Hz), 6.84 (d, 1H, C6-H chromen, *J* = 8.7 Hz), 7.18-7.37 (m, 10H, ArH), 7.40 (d, 1H, C5-H chromen, *J* = 8.7 Hz), 7.59 (d, 1H, C4-H chromen, *J* = 8.7 Hz). Anal. Calcd for $C_{25}H_{20}N_2O_5S$ (460.50): C, 65.20; H, 4.38; N, 6.08. Found C, 65.42; H, 4.75; N, 6.01.

*4.1.5.4. 7-Methoxy-8-[1-(phenylsulfonyl)-(4-methylphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one (**7d**):*

Yield: 65%, m.p. 237-239 ^{o}C. IR υ_{max}/cm^{-1}: 3062 (CH aromatic), 2974, 2843 (CH aliphatic), 1728 (C=O), 1647, 1604 (C=C, C=N), 1300, 1145 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 2.28 (s, 3H, CH_3), 3.15 (dd, 1H, C4-H_A pyrazoline, J_{AX} =8.0 Hz and J_{AM} = 20.4 Hz), 3.83 (s, 3H, OCH_3), 3.96 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 16.0 Hz and J_{MA} = 20.4 Hz), 4.80 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 16.0 Hz and J_{XA} = 8.0 Hz), 6.25 (d, 1H, C3-H chromen, *J* = 9.6 Hz), 6.81 (d, 1H, C6-H chromen, *J* = 8.7 Hz), 6.92 (d, 1H, C5-H chromen, *J* = 8.7 Hz), 7.10-7.57 (m, 9H, ArH), 7.67 (d, 1H, C4-H chromen, *J* = 8.6 Hz). MS, m/z 474.10: [M^+]. Anal. Calcd for $C_{26}H_{22}N_2O_5S$ (474.53): C, 65.81; H, 4.67; N, 5.90. Found C, 66.00; H, 5.10; N,5.50.

*4.1.5.5. 7-Methoxy-8-[1-(phenylsulfonyl)-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one (**7e**):*

Yield: 65%, m.p. 222-224^{o}C. IR υ_{max}/cm^{-1}: 3066 (CH aromatic), 2962, 2850 (CH aliphatic), 1732 (C=O), 1645, 1604 (C=C, C=N), 1327, 1119 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 3.20 (dd, 1H, C4-H_A pyrazoline, J_{AX} =8.4 Hz and J_{AM} = 20.4 Hz), 3.83 (s, 3H, OCH_3), 3.94 (dd, 1H,C4-H_M pyrazoline, J_{MX} = 16.0 Hz and J_{MA} = 20.4 Hz), 4.96 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 16.0 Hz and J_{XA} = 8.4 Hz), 6.23 (d, 1H, C3-H chromen, *J* = 9.6 Hz), 6.82 (d, 1H, C6-H chromen, *J* = 8.7 Hz), 7.11 (d, 1H, C5-H chromen, *J* = 8.7 Hz), 7.26-7.59 (m, 9H, ArH), 7.69 (d, 1H, C4-H chromen, *J* = 8.6 Hz). ^{13}C NMR ($CDCl_3$): δ 42.4 (C-4 pyrazoline) , 45.6 (C-5 pyrazoline), 56.5 (OCH_3), 107.7 (C-6 chromen), 112.9 (C-3 chromen), 114.2 (C-4a chromen and C-8 chromen), 125.8-130.5 (aromatic Cs and CF_3), 134.1 (C-5 chromen), 136.6 (C-4 of $C_6H_4CF_3$ and C-4 of $C_6H_5SO_2$), 142.7 (C-SO_2), 142.9 (C-4 chromen), 143.8 (C-1 of $C_6H_4CF_3$), 150.0 (C-8a chromen), 152.1 (C=N pyrazoline), 159.0 (C-7 chromen), 159.7 (C=O). Anal. Calcd for $C_{26}H_{19}F_3N_2O_5S$ (528.50): C, 59.09; H, 3.62; N, 5.30. Found: C, 59.10; H, 3.70; N, 5.40.

*4.1.5.6. 7-Methoxy-8-[5-(4-(methylthio)phenyl)-1-(phenylsulfonyl)-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one (**7f**):*

Yield: 50%, m.p. 189-191 ^{o}C. IR υ_{max}/cm^{-1}: 3020 (CH aromatic), 2950, 2850 (CH aliphatic), 1732 (C=O), 1651, 1604 (C=C, C=N), 1304, 1078 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 2.51 (s, 3H, CH_3), 3.06 (dd, 1H, C4-H_A pyrazoline, J_{AX} =8.0 Hz and J_{AM} = 20.4 Hz), 3.40 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 15.0 Hz and J_{MA} = 20.4 Hz), 3.86 (s, 3H, OCH_3), 4.80 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 15.0 Hz and J_{XA} = 8.0 Hz), 6.26 (d, 1H, C3-H chromen, *J* = 9.6 Hz), 6.84 (d, 1H, C6-H chromen, *J* = 8.7 Hz), 6.93-7.46 (m, 9H, ArH), 7.50 (d, 1H, C5-H chromen, *J* = 8.7 Hz), 7.64 (d, 1H, C4-H chromen, *J* = 9.6 Hz). Anal. Calcd for $C_{26}H_{22}N_2O_5S_2$ (506.59): C, 61.64; H, 4.38; N, 5.53. Found: C, 61.59; H, 4.99; N, 5.56.

*4.1.6. General procedure for the preparation of 8-[5-substituted-1-(4-methylbenzene sulfonyl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-ones **8a-f**:*

The appropriate propenone derivative **6a-f** (10 mmol) was dissolved in absolute ethanol (50 ml), then 4-methylphenylsulphonyl hydrazine (1.86, 10 mmol) was added and the reaction mixture was heated under reflux for 12 h. The mixture was filtered while hot, the filtrate was concentrated under vacuum and the separated solid was crystallized from methanol.

*4.1.6.1. 8-[1-(4-Methylphenylsulfonyl)-5-(5-methylfuran-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**8a**):*

Yield: 55%, m.p. 184-186 ^{o}C. IR υ_{max}/cm^{-1}: 3062 (CH aromatic), 2943, 2920 (CH aliphatic), 1732 (C=O), 1631, 1604 (C=C, C=N), 1296, 1145 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 2.26 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 3.20 (dd, 1H, C4-H_A pyrazoline, J_{AX} =8.0 Hz and J_{AM} = 20.4 Hz), 3.91 (s, 3H, OCH_3), 4.15 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 16.0 Hz and J_{MA} = 20.4 Hz), 4.95 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 16.0 Hz and J_{XA} = 8.0 Hz), 6.15 (d, 1H, furan H, *J* = 1.2 Hz), 6.20 (d, 1H, C3-H chromen, *J* = 9.4 Hz), 6.50 (d, 1H, furan H, *J* = 1.2 Hz), 6.84 (d, 1H, C6-H chromen, *J* = 8.7 Hz), 7.26-7.57 (m, 4H, Ar H), 7.62 (d, 1H, C5-H chromen, *J* = 8.7 Hz), 7.81 (d, 1H, C4-H chromen, *J* = 9.6 Hz). Anal. Calcd for $C_{25}H_{22}N_2O_6S$ (478.52): C, 62.75; H, 4.63; N, 5.85. Found: C, 62.79; H, 5.51; N, 5.85.

*4.1.6.2. 8-[1-(4-Methylphenylsulfonyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**8b**):*

Yield: 50%, m.p. 158-160 ^{o}C. IR υ_{max}/cm^{-1}: 3093 (CH aromatic), 2970, 2939 (CH aliphatic), 1724 (C=O), 1604, 1558 (C=C, C=N), 1290, 1168 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 2.45 (s, 3H, CH_3), 3.50 (d, 2H, *J* = 10 Hz, CH_2 pyrazoline), 3.86 (s, 3H, OCH_3), 4.95 (t, 1H, C5-H pyrazoline, *J* = 11.2 Hz), 6.20 (d, 1H, C3-H thiophene, *J* = 8.6 Hz), 6.30 (d, 1H, C3-H chromen, *J* = 9.6 Hz), 6.90 (t, 1H, C4-H thiophene, *J* = 8.1 Hz), 7.10 (d, 1H, C5-H thiophene, *J* = 8.7 Hz), 7.30 (d, 1H, C6-H chromen, *J* = 9.6 Hz), 7.69 (m, 3H, C5-H chromen and ArH), 7.81 (d, 1H, C4-H chromen, *J* = 9.6 Hz), 7.91 (d, 2H, ArH, *J* = 8.1 Hz). ^{13}C NMR($CDCl_3$): 22.0 (CH_3), 46.0 (C-4 pyrazoline), 47.5 (C-5 pyrazoline), 56.4 (OCH_3), 107.9 (C-6 chromene), 112.9 (C-3 chromen), 113.7 (C-4a chromen), 117.3 (C-8 chromen), 126.7-132.0 (C-5 chromen and aromatic Cs), 138.7 (C-SO_2), 139.5 (C-4 of $C_6H_4CH_3$), 143.1 (C-4 chromen), 151.8 (C-8a chromen and C=N pyrazoline), 159.3 (C-7 chromen), 159.9 (C=O). MS, m/z 481.50: [M^++1]. Anal. Calcd for $C_{24}H_{20}N_2O_5S_2$ (480.56): C, 59.98; H, 4.19; N, 5.83. Found: C, 59.83; H, 4.08; N, 5.60.

*4.1.6.3. 8-[1-(4-Methylphenylsulfonyl)-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**8c**):*

Yield: 68%, m.p. 186-188^{o}C. IR υ_{max}/cm^{-1}: 3090 (CH aromatic), 2947, 2912 (CH aliphatic), 1739 (C=O), 1602, 1562 (C=C, C=N), 1292, 1111 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 2.40

(s, 3H, CH_3), 3.10 (dd, 1H, C4-H_A pyrazoline, J_{AX} =8.7 Hz and J_{AM} = 20.4 Hz), 3.82 (s, 3H, OCH_3), 3.91 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 15.6 Hz and J_{MA} = 20.4 Hz), 4.80 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 15.6 Hz and J_{XA} = 8.7 Hz), 6.23 (d, 1H, C3-H chromen, J = 9.6 Hz), 6.80 (d, 1H, C6-H chromen, J = 8.4 Hz), 7.17-7.27 (m, 9H, ArH), 7.41 (d, 1H, C5-H chromen, J = 8.4 Hz), 7.58 (d, 1H, C4-H chromen, J = 9.6 Hz). Anal. Calcd for $C_{26}H_{22}N_2O_5S$ (474.53): C, 65.81; H, 4.67; N, 5.90. Found: C, 66.00; H, 4.58; N, 5.96.

*4.1.6.4. 8-[1-(4-Methylphenylsulfonyl)-5-(4-methylphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**8d**):*

Yield: 65%, m.p. 204-207^{o}C. IR υ_{max}/cm^{-1}: 3093 (CH aromatic), 2947, 2912 (CH aliphatic), 1732 (C=O), 1600, 1562 (C=C, C=N), 1292, 1168 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 2.41 (s, 3H, CH_3), 2.91 (s, 3H, CH_3), 3.40 (dd, 1H, C4-H_A pyrazoline, J_{AX} =8.0 Hz and J_{AM} = 20.4 Hz), 3.82 (s, 3H, OCH_3), 4.10 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 16.0 Hz and J_{MA} = 20.4 Hz), 4.95 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 16.0 Hz and J_{XA} = 8.0 Hz), 6.20 (d, 1H, C3-H chromen, J = 9.3 Hz), 6.82 (d, 1H, C6-H chromen, J = 8.7 Hz), 7.27-7.60 (m, 8H, Ar H), 7.76 (d, 1H, C5-H chromen, J = 8.4 Hz), 7.80 (d, 1H, C4-H chromen, J = 9.6 Hz). ^{13}C NMR ($CDCl_3$): 21.4 (CH_3), 37.3 (C-4 pyrazoline), 42.5 (C-5 pyrazoline), 56.1 (OCH_3), 107.6 (C-6 chromen), 113.0 (C-3 chromen), 114.0 (C-4a chromen and C-8 chromen), 124.8-130.6 (C-5 chromen and aromatic Cs), 136.0 (C-4 of $C_6H_4CH_3$), 140.0 (C-SO_2), 142.8 (C-4 of $C_6H_4CH_3SO_2$), 144.0 (C-4 chromen), 151.1 (C-8a chromen and C=N pyrazoline), 158.7 (C-7 chromen), 159.4 (C=O). Anal. Calcd for $C_{27}H_{24}N_2O_5S$ (488.55): C, 66.38; H, 4.95; N, 5.73. Found: C, 66.10; H, 5.43; N, 5.35.

*4.1.6.5. 8-[1-(4-Methylphenylsulfonyl)-5-(4-(trifluromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**8e**):*

Yield: 60%, m.p. 165-168^{o}C. IR υ_{max}/cm^{-1}: 3028 (CH aromatic), 2893, 2839 (CH aliphatic), 1732 (C=O), 1600, 1558 (C=C, C=N), 1296, 1165 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 2.44 (s, 3H, CH_3), 3.45 (dd, 1H, C4-H_A pyrazoline, J_{AX} =8.4 Hz and J_{AM} = 20.2 Hz), 3.89 (s, 3H, OCH_3), 4.15 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 16.8 Hz and J_{MA} = 20.2 Hz), 4.80 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 16.8 Hz and J_{XA} = 8.4 Hz), 6.10 (d, 1H, C3-H chromen, J = 9.3 Hz), 6.40 (d, 1H, C6-H chromen, J = 8.7 Hz), 6.80-7.40 (m, 8H, Ar H), 7.69 (d, 1H, C5-H chromen, J = 8.7 Hz), 7.81 (d, 1H, C4-H chromen, J = 9.6 Hz). Anal. Calcd for $C_{27}H_{21}F_3N_2O_5S$ (542.53): C, 59.77; H, 3.90; N, 5.16. Found: C, 60.04; H, 4.20; N, 4.83.

*4.1.6.6. 8-[1-(4-Methylphenylsulfonyl)-5-(4-(methylthio)phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**8f**):*

Yield: 65%, m.p. 170-172°C. IR υ_{max}/cm^{-1}: 3059 (CH aromatic), 2989, 2920 (CH aliphatic), 1732 (C=O), 1604, 1562 (C=C, C=N), 1265, 1087 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 2.40 (s, 3H, CH_3), 2.49 (s, 3H, S-CH_3), 3.20 (dd, 1H, C4-H_A pyrazoline, J_{AX} =8.1 Hz and J_{AM} = 20.4 Hz), 3.80 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 16.2 Hz and J_{MA} = 20.4 Hz), 3.85 (s, 3H, OCH_3), 4.80 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 16.2 Hz and J_{XA} = 8.1 Hz), 6.21 (d, 1H, C3-H chromen, *J* = 9.3 Hz), 6.82 (d, 1H, C6-H chromen, *J* = 8.4 Hz), 7.07-7.43 (m, 8H, ArH), 7.45 (d, 1H, C5-H chromen, *J* = 8.4 Hz), 7.66 (d, 1H, C4-H chromen, *J* = 9.6 Hz). ^{13}C NMR ($CDCl_3$): 15.0 (SCH_3), 21.6 (CH_3), 41.5 (C-5 pyrazoline), 56.5 (OCH_3), 65.9 (C-4 pyrazoline), 108.6 (C-6 chromen), 112.7 (C-3 chromen), 112.9 (C-4a chromen), 116.6 (C-8 chromen), 125.4-130.7 (C-5 chromen and aromatic Cs) , 139.4 (C-SO_2), 142.9 (C-1 of $C_6H_4SCH_3$), 144.8 (C-4 chromen), 145.8 (C-4 of $C_6H_4CH_3SO_2$), 151.8 (C-8a chromen and C=N pyrazoline), 158.7 (C-7 chromen), 159.4 (C=O). MS m/z 521.25: [M^+]. Anal. Calcd for $C_{27}H_{24}N_2O_5S_2$ (520.62): C, 62.29; H, 4.65; N, 5.38. Found: C, 62.41; H, 3.88; N, 4.96.

*4.1.7. General procedure for the preparation of 8-[5-substituted-1-(4-chlorophenyl sulfonyl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-ones **9a-f**:*

4-Chlorophenylsulphonyl hydrazine (2.06 g, 10 mmol) was added to a solution of the appropriate propenone derivative **6a-f** (10 mmol) in absolute ethanol (50 ml) and heated under reflux for 8 h. The mixture was filtered while hot, the filtrate was concentrated under vacuum and the separated solid was crystallized from absolute ethanol.

*4.1.7.1. 8-[1-(4-Chlorophenylsulfonyl)-5-(5-methylfuran-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**9a**)*:

Yield: 50%, m.p. 180-182°C. IR υ_{max}/cm^{-1}: 3032 (CH aromatic), 2943, 2843 (CH aliphatic), 1720 (C=O), 1620, 1597 (C=C, C=N), 1300, 1141 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 2.34 (s, 3H, CH_3), 3.10 (dd, 1H, C4-H_A pyrazoline, J_{AX} =8.0 Hz and J_{AM} = 20.4 Hz), 3.70 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 16.0 Hz and J_{MA} = 20.4 Hz), 3.88 (s, 3H, OCH_3), 5.90 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 16.0 Hz and J_{XA} = 8.0 Hz), 6.10 (d, 1H, furan H, *J* = 1.2 Hz), 6.30 (d, 1H, C3-H chromen, *J* = 9.4 Hz), 6.50 (d, 1H, furan H, *J* = 1.2 Hz), 6.80 (d, 1H, C6-H chromen, *J* = 8.4 Hz), 7.30 (d, 1H, C5-H chromen, *J* = 8.4 Hz), 7.50 (d, 2H, Ar H, *J* = 8.1

Hz), 7.66 (d, 2H, ArH, *J* = 9.0 Hz), 7.80 (d, 1H, C4-H chromen, *J* = 9.6 Hz). Anal. Calcd for $C_{24}H_{19}ClN_2O_6S$ (498.93): C, 57.77; H, 3.84; N, 5.61. Found: C, 58.04 H, 4.26; N, 5.24.

*4.1.7.2. 8-[1-(4-Chlorophenylsulfonyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**9b**):*

Yield: 50%, m.p. 178-180°C. IR υ_{max}/cm^{-1}: 3032 (CH aromatic), 2943, 2843 (CH aliphatic), 1724 (C=O), 1600, 1581 (C=C, C=N), 1320, 1168 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 3.21 (d, 2H, *J* = 11.4 Hz, CH_2 pyrazoline), 3.87 (s, 3H, OCH_3), 4.30 (t, 1H, C5-H pyrazoline, *J* = 11.2 Hz), 6.22 (d, 1H, C3-H chromen, *J* = 9.0 Hz), 6.66 (d, 1H, C3-H thiophene, *J* = 8.7 Hz), 6.76 (d, 1H, C6-H chromen, *J* = 9.6 Hz), 6.90 (t, 1H, C4-H thiophene, *J* = 8.6 Hz), 7.10 (d, 1H, C5-H thiophene, *J* = 8.7 Hz), 7.30 (d, 1H, C5-H chromen, *J* = 9.6 Hz), 7.69 (d, 2H, ArH, *J* = 8.1 Hz), 7.81 (d, 1H, C4-H chromen, *J* = 9.0 Hz), 7.99 (d, 2H, ArH, *J* = 8.1 Hz). Anal. Calcd for $C_{23}H_{17}ClN_2O_5S_2$ (500.17): C, 55.14; H, 3.42; N, 5.59. Found: C, 55.50; H, 3.73; N, 5.89.

*4.1.7.3. 8-[1-(4-Chlorophenylsulfonyl)-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**9c**):*

Yield: 68%, m.p. 202-204°C. IR υ_{max}/cm^{-1}: 3032 (CH aromatic), 2943, 2843 (CH aliphatic), 1735 (C=O), 1604, 1560 (C=C, C=N), 1310, 1114 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 3.10 (dd, 1H, C4-H_A pyrazoline, J_{AX} = 6.9 Hz and J_{AM} = 20.4 Hz), 3.82 (s, 3H, OCH_3), 3.93 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 15.6 Hz and J_{MA} = 20.4 Hz), 4.80 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 15.6 Hz and J_{XA} = 6.9 Hz), 6.23 (d, 1H, C3-H chromen, *J* = 9.6 Hz), 6.80 (d, 1H, C6-H chromen, *J* = 8.4 Hz), 7.17-7.27 (m, 9H, ArH), 7.41 (d, 1H, C5-H chromen, *J* = 8.4 Hz), 7.58 (d, 1H, C4-H chromen, *J* = 9.6 Hz). MS, m/z 494.95: [M^+]. Anal. Calcd for $C_{25}H_{19}ClN_2O_5S$ (494.94): C, 60.67; H, 3.87; N, 5.66. Found: C, 60.69; H, 3.63; N, 5.60.

*4.1.7.4. 8-[1-(4-Chlorophenylsulfonyl)-5-(4-methylphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**9d**):*

Yield: 65%, m.p. 177-179°C. IR υ_{max}/cm^{-1}: 3032 (CH aromatic), 2943, 2843 (CH aliphatic), 1732 (C=O), 1604, 1560 (C=C, C=N), 1292, 1168 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 2.30 (s, 3H, CH_3), 3.21 (dd, 1H, C4-H_A pyrazoline, J_{AX} = 8.4 Hz and J_{AM} = 20.4 Hz), 3.82 (s, 3H, OCH_3), 3.90 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 16.8 Hz and J_{MA} = 20.4 Hz), 4.95 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 16.8 Hz and J_{XA} = 8.4 Hz), 6.25 (d, 1H, C3-H chromen, *J* = 9.6 Hz), 6.85 (d, 1H, C6-H chromen, *J* = 8.7 Hz), 6.80-7.50 (m, 8H, Ar H), 7.60 (d, 1H, C5-H

chromen, J = 8.7 Hz), 7.76 (d, 1H, C4-H chromen, J = 9.6 Hz). ^{13}C NMR($CDCl_3$): 21.4 (CH_3), 37.3 (C-4 pyrazoline), 42.5 (C-5 pyrazoline), 56.1 (OCH_3), 107.6 (C-6 chromen), 113.0 (C-3 chromen), 114.0 (C-4a, C-8 chromen), 124.8-130.6 (C-5 chromen and aromatic Cs), 138.0 (C-Cl and C-SO_2), 142.6 (C-1 of $C_6H_4CH_3$ and C-4 chromen, 151.1 (C-8a chromen and C=N pyrazoline), 158.7 (C-7 chromen), 159.4 (C=O). Anal. Calcd for $C_{26}H_{21}ClN_2O_5S$ (508.97): C, 61.35; H, 4.16; N, 5.50. Found: C, 61.62; H, 5.89; N, 5.74.

*4.1.7.5. 8-[1-(4-Chlorophenylsulfonyl)-5-(4-(trifluromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**9e**):*

Yield: 60%, m.p. 232-234^{o}C. IR υ_{max}/cm^{-1}: 3032 (CH aromatic), 2943, 2843 (CH aliphatic), 1732 (C=O), 1604, 1562 (C=C, C=N), 1327, 1118 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 3.20 (dd, 1H, C4-H_A pyrazoline, J_{AX} = 8.4 Hz and J_{AM} = 20.4 Hz), 3.83 (s, 3H, OCH_3), 3.91 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 16.8 Hz and J_{MA} = 20.4 Hz), 4.80 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 16.8 Hz and J_{XA} = 8.4 Hz), 6.10 (d, 1H, C3-H chromen, J = 9.3 Hz), 6.40 (d, 1H, C6-H chromen, J = 8.7 Hz), 6.80-7.40 (m, 8H, ArH), 7.60 (d, 1H, C5-H chromen, J = 8.7 Hz), 7.81 (d, 1H, C4-H chromen, J = 9.6 Hz). Anal. Calcd for $C_{26}H_{18}ClF_3N_2O_5S$ (562.94): C, 55.47; H, 3.22; N, 4.98. Found: C, 55.81; H, 3.18; N, 4.88.

*4.1.7.6. 8-[1-(4-Chlorophenylsulfonyl)-5-(4-(methylthio)phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**9f**):*

Yield: 65%, m.p. 206-208^{o}C. IR υ_{max}/cm^{-1}: 3032 (CH aromatic), 2943, 2843 (CH aliphatic), 1730 (C=O), 1600, 1586 (C=C, C=N), 1303, 1145 (SO_2). ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.40 (s, 3H, S-CH_3), 2.84 (dd, 1H, C4-H_A pyrazoline, J_{AX} = 8.1 and J_{MA} = 20.4 Hz), 3.20 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 16.2 Hz and J_{MA} = 20.4 Hz), 3.85 (s, 3H, OCH_3), 4.10 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 16.2 Hz and J_{XA} = 8.1 Hz), 6.33 (d, 1H, C3-H chromen, J = 9.3 Hz), 7.06 (d, 1H, C6-H chromen, J = 8.4 Hz), 7.11-7.33 (m, 8H, ArH), 7.79 (d, 1H, C5-H chromen, J = 8.4 Hz), 8.06 (d, 1H, C4-H chromen, J = 9.6 Hz). ^{13}C NMR (DMSO- d_6): 14.0 (SCH_3), 40.3 (C-4 pyrazoline), 41.5 (C-5 pyrazoline), 56.5 (OCH_3), 108.6 (C-6 chromen), 112.7 (C-3 chromen), 112.9 (C-4a chromen), 116.6 (C-8 chromen), 125.4-130.3 (C-5 chromen and aromatic Cs), 137.0 (C-4 of $C_6H_4SCH_3$), 142.6 (C-Cl and C-SO_2), 144.2 (C-1 of $C_6H_4SCH_3$), 145.8 (C-4 chromen), 151.1 (C-8a chromen and C=N), 158.8 (C-7 chromen), 159.4 (C=O). MS, m/z 540.60: [M^+]. Anal. Calcd for $C_{26}H_{21}ClN_2O_5S_2$ (541.03): C, 57.72; H, 3.91; N, 5.18. Found: C, 57.99; H, 4.12; N, 4.80.

*4.1.8. General procedure for the preparation of 8-[5-substituted-1-(4-sulfamoyl phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-ones **10a-f**:*

4-Sulphamoylphenyl hydrazine (1.87 g, 10 mmol) was added to a solution of the appropriate propenone derivative **6a-f** (10 mmol) in absolute ethanol (50 ml), refluxed for 16 h. The reaction mixture was concentrated under vacuum and the residue was crystallized from ethanol.

*4.1.8.1. 8-[4,5-Dihydro-5-(5-methylfuran-2-yl)-1-(4-sulfamoylphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**10a**):*

Yield: 70%, m.p.244-246^{o}C. IR υ_{max}/cm^{-1}: 3367, 3253 (NH_2), 3062 (CH aromatic), 2960, 2903 (CH aliphatic), 1728 (C=O), 1602, 1560 (C=C, C=N), 1301, 1163 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 2.35 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 3.92 (d, 2H, *J* = 9.9 Hz, CH_2 pyrazoline), 4.80 (t, 1H, C5-H pyrazoline, *J* = 11.3 Hz), 5.10 (s, 2H, NH_2, exch.), 6.09 (d, 1H, furan H, *J* = 1.2 Hz), 6.24 (d, 1H, C3-H chromen, *J* = 9.4 Hz), 6.56 (d, 1H, furan H, *J* = 1.2 Hz), 6.80-7.08 (m, 3H, C6-H chromen, ArH), 7.45 (d, 1H, C5-H chromen, *J* = 8.4 Hz), 7.66 (d, 1H, C4-H chromen, *J* = 9.4 Hz), 8.01 (d, 2H, ArH, *J* = 7.8 Hz). Anal. Calcd for $C_{24}H_{21}N_3O_6S$ (479.51): C, 60.12; H, 4.41; N, 8.76. Found: C, 60.32; H, 4.29; N, 9.03.

*4.1.8.2. 8-[4,5-Dihydro-1-(4-sulfamoylphenyl)- 5-(thiophen-2-yl)-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**10b**):*

Yield: 66%, m.p.214-216 ^{o}C. IR υ_{max}/cm^{-1}: 3390, 3253 (NH_2), 3086 (CH aromatic), 2970, 2918 (CH aliphatic), 1730 (C=O), 1602, 1582 (C=C, C=N), 1300, 1087 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 3.40 (dd, 1H, C4-H_A pyrazoline, J_{AX} = 8.1 Hz and J_{AM} = 16.5 Hz), 3.90 (s, 3H, OCH_3), 4.30 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 10.5 Hz and J_{MA} = 16.5 Hz), 4.60 (s, 2H, NH_2, exch.), 4.80 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 10.5 Hz and J_{XA} = 8.1 Hz), 6.27 (d, 2H, C3-H thiophene, C3-H chromen, *J* = 9.6 Hz), 6.80 (t, 1H, C4-H thiophene, *J* = 8.3 Hz), 6.95 (d, 1H, C6-H chromen, *J* = 8.4 Hz), 7.17-7.40 (m, 3H, C5-H thiophene, ArH), 7.73 (d, 1H, C5-H chromen, *J* = 8.4 Hz), 7.81 (d, 1H, C4-H chromen, *J* = 9.6 Hz), 7.95 (d, 2H, ArH, *J* = 7.8 Hz). MS, m/z 483.55: [M^++2]. Anal. Calcd for $C_{23}H_{19}N_3O_5S_2$ (481.54): C, 57.37; H, 3.98; N, 8.73. Found: C, 57.53; H, 4.12; N, 9.18.

*4.1.8.3. 8-[4,5-Dihydro-1-(4-sulfamoylphenyl)- 5-phenyl-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**10c**):*

Yield: 70%, m.p.240-242 ^{o}C. IR υ_{max}/cm^{-1}: 3392, 3261 (NH_2), 3032 (CH aromatic), 2932, 2889 (CH aliphatic), 1739 (C=O), 1593, 1558 (C=C, C=N), 1328, 1153 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 3.20 (dd, 1H, C4-H_A pyrazoline, J_{AX} = 8.4 Hz and J_{AM} = 16.8 Hz), 3.93 (s, 3H, OCH_3), 4.20 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 10.5 Hz and J_{MA} = 16.8 Hz), 4.56 (s, 2H, NH_2, exch.), 5.60 (dd, 1H,C5-H_X pyrazoline, J_{XM} = 10.5 Hz and J_{XA} = 8.4 Hz), 6.28 (d, 1H, C3-H chromen, *J* = 9.6 Hz), 6.91 (d, 1H, C6-H chromen, *J* = 8.7 Hz), 7.05 (d, 1H, C5-H chromen, *J* = 8.6 Hz), 7.21-7.46 (m, 7H, ArH), 7.64 (d, 1H, C4-H chromen, *J* = 9.6 Hz), 7.80 (d, 2H, ArH, *J* = 7.8 Hz). Anal. Calcd for $C_{25}H_{21}N_3O_5S$ (475.52): C, 63.15; H, 4.45; N, 8.84. Found: C, 63.10; H, 3.90; N, 8.63.

*4.1.8.4. 8-[4,5-Dihydro-5-(4-methylphenyl)-1-(4-sulfamoylphenyl)-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**10d**):*

Yield: 68%, m.p.262-264 ^{o}C. IR υ_{max}/cm^{-1}: 3383, 3253 (NH_2), 3084 (CH aromatic), 2945, 2920 (CH aliphatic), 1732 (C=O), 1602, 1562 (C=C, C=N), 1328, 1089 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 2.33 (s, 3H, CH_3), 3.20 (dd, 1H, C4-H_A pyrazoline, J_{AX} = 8.4 Hz and J_{AM} = 16.5 Hz), 3.89 (s, 3H, OCH_3), 4.15 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 10.5 Hz and J_{MA} = 16.5 Hz), 4.63 (s, 2H, NH_2, exch.), 5.30 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 10.5 Hz and J_{XA} = 8.4 Hz), 6.20 (d, 1H, C3-H chromen, *J* = 9.6 Hz), 6.40 (d, 1H, C6-H chromen, *J* = 8.7 Hz), 6.80-7.40 (m, 5H, C5-H chromen and ArH), 7.50 (d, 2H, ArH, *J* = 7.8 Hz), 7.64 (d, 1H, C4-H chromen, *J* = 9.6 Hz), 7.80 (d, 2H, ArH, *J* = 7.8 Hz). Anal. Calcd for $C_{26}H_{23}N_3O_5S$ (489.54): C, 63.79; H, 4.74; N, 8.58. Found: C, 63.96; H, 4.95; N, 8.44.

*4.1.8.5. 8-[4,5-Dihydro-1-(4-sulfamoylphenyl)- 5-(4-(trifluromethyl)phenyl)-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**10e**):*

Yield: 70%, m.p.180-182^{o}C. IR υ_{max}/cm^{-1}: 3255, 3201 (NH_2), 3068 (CH aromatic), 2943, 2850 (CH aliphatic), 1732 (C=O), 1604, 1562 (C=C, C=N), 1325, 1168 (SO_2). ^{1}H NMR ($CDCl_3$) δ *ppm*: 3.40 (dd, 1H, C4-H_A pyrazoline, J_{AX} = 8.2 Hz and J_{AM} = 16.4 Hz), 3.87 (s, 3H, OCH_3), 4.10 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 10.5 Hz and J_{MA} = 16.4 Hz), 4.63 (s, 2H, NH_2, exch.), 5.30 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 10.5 Hz and J_{XA} = 8.2 Hz), 6.30 (d, 1H, C3-H chromen, *J* = 9.6 Hz), 6.40 (d, 1H, C6-H chromen, *J* = 8.7 Hz), 7.10-7.73 (m, 6H, Ar H), 7.76 (d, 1H, C5-H chromen, *J* = 8.7 Hz), 7.86 (d, 1H, C4-H chromen, *J* = 9.6 Hz), 8.09 (d, 2H, ArH, *J* = 7.8 Hz). ^{13}C NMR ($CDCl_3$): 40.3 (C-4 pyrazoline), 51.0 (C-5 pyrazoline), 56.5 (OCH_3), 108.7 (C-6 chromen), 112.7 (C-3 chromen), 112.9 (C-4a and C-8

chromen), 116.3 (C-2 and C-6 of $C_6H_4SONH_2$), 120.6-130.7 (C-5 chromen, aromatic Cs and CF_3), 135.8 (C-4 of $C_6H_4SONH_2$), 138.0 (C-4 of $C_6H_4CF_3$), 143.7 (C-4 chromen), 144.1 (C-1 of $C_6H_4CF_3$), 148.4 (C-8a chromen and C-1 of $C_6H_4SONH_2$), 151.1 (C-8a chromen and C=N pyrazoline), 158.8 (C-7 chromen), 159.3 (C=O). MS, m/z 543.20: [M^+]. Anal. Calcd for $C_{26}H_{20}FN_3O_5S$ (543.51): C, 57.46; H, 3.71; N, 7.73. Found: C, 57.91; H, 4.15; N, 8.03.

*4.1.8.6. 8-[4,5-Dihydro-5-(4-(methylthio)phenyl)-1-(4-sulfamoylphenyl)-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (**10f**):*

Yield: 65%, m.p.239-242 ^oC. IR υ_{max}/cm^{-1}: 3392, 3210 (NH_2), 3032 (CH aromatic), 2920, 2850 (CH aliphatic), 1724 (C=O), 1602, 1587 (C=C, C=N), 1303, 1145 (SO_2). ^1H NMR ($CDCl_3$) δ *ppm*: 2.20 (s, 3H, S-CH_3), 2.80 (dd, 1H, C4-H_A pyrazoline, J_{AX} = 8.1 Hz and J_{AM} = 16.2 Hz), 3.20 (dd, 1H, C4-H_M pyrazoline, J_{MX} = 10.5 Hz and J_{MA} = 16.2 Hz), 3.85 (s, 3H, OCH_3), 4.20 (s, 2H, NH_2, exch.), 5.60 (dd, 1H, C5-H_X pyrazoline, J_{XM} = 10.5 Hz and J_{XA} = 8.1 Hz), 6.31 (d, 1H, C3-H chromen, *J* = 9.3 Hz), 6.95 (d, 1H, C6-H chromen, *J* = 8.4 Hz), 7.18-7.33 (m, 6H, ArH), 7.64 (d, 2H, ArH, *J* = 7.8 Hz), 7.80 (d, 1H, C5-H chromen, *J* = 8.4 Hz), 8.04 (d, 1H, C4-H chromen, *J* = 9.3 Hz). ^{13}C NMR: 15.8 (SCH_3), 44.8 (C-4 pyrazolin), 56.5 (OCH_3), 63.4 (C-5 pyrazolin), 108.6 (C-6 chromen), 111.0 (C-3 chromen), 112.7 (C-4a and C-8 chromen), 112.9 (C-2 and C-6 of $C_6H_4SONH_2$), 123.4-130.3 (C-5 chromen and aromatic Cs), 136.0 (C-4 of $C_6H_4SCH_3$), 140.0 (C-1 of $C_6H_4SCH_3$), 143.5 (C-4 chromen), 145.6 (C-1 of $C_6H_4SONH_2$), 151.0 (C-8a chromen and C=N), 158.8 (C-7 chromen), 159.3 (C=O). Anal. Calcd for $C_{26}H_{23}N_3O_5S_2$ (521.61): C, 59.87; H, 4.44; N, 8.06. Found: C, 60.33; H, 4.71; N, 8.10.

4.2. Biological evaluation

4.2.1. Anticancer activity [45]

The cytotoxicity assays were performed at National Cancer Institute (NCI), Bethesda, USA (against 60 cell lines) and at Department of Pharmacology, Faculty of Pharmacy, Ain Shams University (against HCT-116 cell line). The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well

microtiter plates in 100 µl at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed *in situ* with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations. Triplicate wells were prepared for each individual dose. Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5 % CO_2, 95 % air, and 100 % relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each well, and plates were incubated for 10 minutes at room temperature. After staining, unbound dye was removed by washing five times with 1 % acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

Three dose response parameters were calculated for each experimental compound: Growth inhibition of 50 % (GI_{50}) was calculated when [(Ti-Tz)/(C-Tz)] x 100 = 50. The compound concentration resulting in total growth inhibition (TGI) was calculated when Ti = Tz. The LC_{50} indicating a net loss of cells following treatment was calculated when [(Ti-Tz)/Tz] x 100 = -50.
The results reported are means of at least three separate experiments in case of cytotoxic activity evaluated at Ain Shams University. Statistical differences were analyzed according to one way ANOVA test wherein the differences were considered to be significant at $p < 0.05$.

4.2.2. PI3K (p110 α /p85 α) protein kinase assay [49]

The PI3K (p110α/p85 α) assay was performed using the ADP-Glo™ assay kit from Promega which measured the generation of ADP by the PI3K (p110 α /p85 α). Generation of ADP by the PI3K reaction led to increase in luminescence signal in the presence of ADP-Glo™ assay kit.
The PI3K assay was performed in duplicate in a reaction mixture of final volume of 25 µl containing: 5 µl of diluted active PI3K kinase, 5 µl of stock solution of peptide substrate, 5 µl of kinase assay buffer, 5 µl of compound (250 µM) or 10% DMSO and 5 µl of 250 µM ATP stock solution. The assay was started by incubating the reaction mixture in a 96-well plate at 30 ºC for 30 minutes. The reaction was then terminated by the addition of 25 µl of ADP-Glo™ reagent (Promega). The plate was shaken and then incubated for 40 minute at ambient temperature. 50 µl of kinase detection reagent was added; the plate was shaken and incubated for further 30 minute at ambient temperature. The reaction plate was then read using the ADP-Glo™ Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031)). Blank and control were set up that included all the assay components except the addition of the substrate in blank (replaced with equal volume of kinase assay buffer) and the addition of compound in control. The corrected activity for PI3K (p110α/p85α) was

determined by removing the blank control value. For IC_{50} Determination for compound **9c** and wortmannin, a graph of log inhibitor concentrations versus normalized responses with variable slope was generated using the Prism software.

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Figure Captions

Table 1: In vitro testing expressed as growth inhibition of cancer cell lines for compounds **7d, 8c** and **9c**.

Table 2: Median growth inhibitory concentrations [a] (GI_{50}, µM) of in vitro subpanel tumor cell lines.

Table 3: Median total growth inhibitory concentrations [a] (TGI, µM) of in vitro subpanel tumor cell lines.

Table 4: In vitro cytotoxic activity of the final compounds against human colon cancer HCT-116 cell line.

Table 5: % Inhibition of PI3K (p110α/p85α) protein kinase at 50 µM (IC_{50} values) for the selected compounds

Figure 1. Chemical structures of coumarin derivatives mentioned in this study.

Figure 2: Chemical structures of some pyrazoline derivatives mentioned in this study.

Figure 3: Design of the new coumarin-pyrazoline hybrids based on the lead compounds **IX**.

Scheme 1. Reagents and conditions: (i) acetic anhydride / reflux 5h; (ii) $AlCl_3$ / heat 145 ^oC 1h; (iii) CH_3I / dry actone / reflux 24h; (iv) Ar-CHO, 10% NaOH / ethanol / r.t. 24 h; (v) 4-(un) substituted phenylsulfonyl hydrazines / absolute ethanol / reflux 8-12h; (vi) 4-sulfamoylphenyl hydrazine /absolute ethanol / reflux 16h.

Table 1: In vitro testing expressed as growth inhibition of cancer cell lines for compounds **7d, 8c** and **9c**.

Subpanel /tumor cell lines	Compound								
	7d			8c			9c		
	GI_{50}(μM)	TGI(μM)	LC_{50}(μM)	GI_{50}(μM)	TGI(μM)	LC_{50}(μM)	GI_{50}(μM)	TGI(μM)	LC_{50}(μM)
Leukemia									
CCRF-CEM	1.95	7.06	> 100	1.93	9.17	> 100	1.31	6.11	> 100
HL60(TB)	2.43	7.18	> 100	2.59	9.99	> 100	2.24	7.44	> 100
K-562	2.45	10.40	> 100	1.97	10.00	> 100	1.20	5.19	> 100
MOLT-4	2.02	11.20	> 100	2.27	15.40	> 100	1.42	7.54	> 100
RPMI-8226	2.42	6.94	> 100	2.27	7.76	> 100	1.62	6.34	> 100
SR	1.64	5.52	> 100	1.50	7.78	> 100	0.77	3.96	> 100
Non-Small Cell Lung Cancer									
A549/ATCC	2.43	7.00	71.10	2.59	10.30	> 100	2.27	9.75	45.30
EKVX	2.14	13.10	51.50	2.09	11.20	67.40	1.50	6.56	37.10
HOP-62	1.96	6.01	23.00	1.46	4.11	13.40	1.33	3.18	7.64
HOP-92	1.83	6.70	54.60	1.40	5.82	31.00	1.65	6.04	27.70
NCI-H226	2.14	5.45	29.50	2.01	5.00	85.40	1.68	4.35	23.20
NCI-H23	1.74	5.54	24.10	1.51	4.75	27.10	1.22	3.42	9.59
NCI-H322M	2.68	9.48	32.20	2.68	11.00	33.40	2.39	7.96	28.30
NCI-H460	1.55	3.42	7.56	1.53	4.00	12.20	1.42	4.65	32.90
NCI-H522	1.80	4.09	9.30	1.39	3.61	9.38	1.24	3.50	9.87
Colon Cancer									
COLO 205	1.43	2.80	5.51	1.48	3.12	6.56	1.51	3.07	6.22
HCC-2998	1.97	3.73	7.08	1.74	3.18	5.81	1.74	3.14	5.68
HCT-116	1.62	3.42	7.24	1.27	2.06	3.32	0.94	2.19	4.89
HCT-15	2.65	10.10	42.70	2.11	10.40	39.20	1.42	4.30	17.30
HT29	3.37	10.20	96.20	2.37	6.00	58.20	1.78	4.65	21.30
KM12	1.66	3.32	6.63	1.35	2.91	6.23	1.26	3.02	7.23
SW-620	1.94	4.50	11.50	1.54	3.39	7.47	1.48	3.59	8.71
CNS Cancer									
SF-268	1.95	6.93	30.60	2.26	9.45	38.60	1.65	6.08	27.50
SF-295	3.43	11.50	45.90	2.85	11.10	42.20	2.93	10.90	36.00
SF-539	2.89	8.47	> 100	2.20	5.44	28.70	2.14	5.07	17.10
SNB-19	2.77	11.90	37.10	1.96	6.58	26.60	1.59	4.67	18.00
SNB-75	2.11	7.79	31.40	2.23	11.90	34.80	1.62	5.08	19.50
U251	1.45	3.33	7.69	1.17	2.66	6.06	0.90	2.33	5.58
Melanoma									
LOX IMVI	1.43	3.10	6.72	1.21	2.99	7.39	0.87	2.37	5.91
MALME-3M	3.29	11.40	47.70	1.94	5.29	28.70	1.68	4.22	13.00
M14	1.95	3.99	8.18	2.08	5.59	28.30	1.69	4.42	14.80
MDA-MB-435	2.26	7.36	32.10	2.01	7.81	65.80	1.78	11.40	53.10
SK-MEL-2	2.05	5.18	30.60	1.94	5.02	40.00	1.85	4.32	10.50
SK-MEL-28	1.84	4.25	98.10	1.83	3.84	8.04	1.61	3.53	7.72
SK-MEL-5	1.68	4.14	10.60	1.49	2.83	5.38	1.43	2.75	5.30

UACC-257	2.48	12.30	36.00	1.76	8.85	34.50	1.47	5.43	24.30
UACC-62	1.36	3.28	7.91	1.37	2.98	6.51	1.21	3.02	7.52
Ovarian Cancer									
IGROV1	2.92	11.70	> 100	2.80	10.80	55.20	2.23	9.79	54.30
OVCAR-3	1.68	3.53	7.41	1.41	3.34	7.95	1.17	2.89	7.13
OVCAR-4	3.08	11.30	45.90	2.53	9.72	31.60	2.29	9.31	30.70
OVCAR-8	1.91	5.18	28.00	2.08	8.88	41.40	1.92	1.17	43.90
NCI/ADR-RES	2.10	5.48	22.30	1.84	7.30	32.60	1.47	7.28	43.80
SK-OV-3	1.67	4.47	14.50	1.78	6.01	23.50	1.48	4.83	19.00
Renal Cancer									
786-0	2.78	7.37	38.80	2.19	4.69	10.30	1.77	3.68	7.65
A498	2.58	6.43	22.60	1.86	4.70	14.50	1.72	4.45	13.60
CAKI-1	1.84	5.80	76.00	2.29	9.35	38.20	2.38	12.90	41.00
RXF 393	1.74	3.90	8.72	1.21	2.63	5.69	1.09	2.54	5.93
SN12C	2.58	11.50	44.40	1.76	6.78	37.30	1.62	6.72	35.20
TK-10	3.86	9.23	42.20	3.38	8.89	40.00	3.05	7.84	28.80
UO-31	1.40	3.03	6.57	1.32	2.65	5.33	1.25	2.53	5.13
Prostate Cancer									
PC-3	2.98	16.50	> 100	2.85	12.20	81.30	2.40	8.79	44.80
DU-145	2.31	7.27	26.40	1.85	8.50	29.70	1.11	3.08	8.51
Breast Cancer									
MCF7	1.92	11.80	35.70	0.99	11.50	34.10	0.49	2.49	12.70
MDA-MB-231/ATCC	2.91	14.20	65.20	1.87	7.13	55.20	1.93	9.49	40.70
HS 578T	2.90	21.60	> 100	3.05	47.70	> 100	4.89	42.90	> 100
BT-549	1.62	4.23	12.70	1.25	2.93	6.85	1.26	2.91	6.68
T-47D	1.68	5.01	21.70	1.45	5.68	50.60	1.08	4.57	44.90
MDA-MB-468	1.15	3.00	7.82	0.50	2.31	8.38	0.34	1.87	8.82

Table 2: Median growth inhibitory concentrations [a] (GI_{50}, μM) of in vitro subpanel tumor cell lines.

Subpanel tumor cell line[b]	Compound					
	7d		8c		9c	
	MG-MID	Selectivity index	MG-MID	Selectivity index	MG-MID	Selectivity index
I	2.15	1.01	2.09	0.90	1.43	1.14
II	2.03	1.07	1.85	1.02	1.63	1.00
III	2.09	1.04	1.69	1.12	1.45	1.12
IV	2.43	0.90	2.11	0.90	1.81	0.90
V	2.04	1.07	1.74	1.09	1.51	1.08
VI	2.23	0.98	2.07	0.91	1.76	0.93
VII	2.40	0.91	2.00	0.95	1.84	0.89
VIII	2.65	0.82	2.35	0.80	1.76	0.93
IX	2.03	1.07	1.52	1.25	1.67	0.98
Full panel MG-MID[c]	2.18		1.89		1.63	

[a] Median value calculated according to the data obtained from NCI's in vitro disease-oriented human tumor cell screen.

[b] I, Leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer.

[c] GI_{50} (μM) full panel mean-graph mid point(MG-MID)= the average sensitivity of all cell lines toward the test agents.

Table 3: Median total growth inhibitory concentrations [a] (TGI, μM) of in vitro subpanel tumor cell lines.

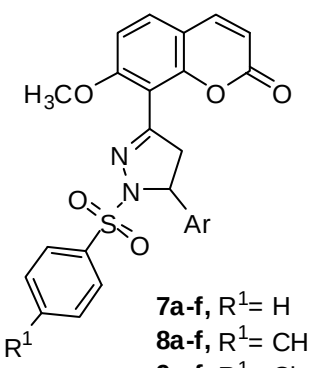
Subpanel tumor cell line[b]	Compound					
	7d		8c		9c	
	MG-MID	Selectivity index	MG-MID	Selectivity index	MG-MID	Selectivity index
I	8.05	0.91	10.02	0.73	6.10	0.95
II	6.75	1.09	6.64	1.11	5.49	1.06
III	5.44	1.35	4.44	1.66	3.42	1.70
IV	8.32	0.88	7.86	0.94	5.69	1.02
V	6.11	1.20	5.02	1.47	4.61	1.26
VI	6.94	1.06	7.68	0.96	5.88	0.99
VII	6.75	1.08	5.67	1.30	5.81	1.00
VIII	11.89	0.62	10.35	0.71	5.94	0.98
IX	9.97	0.73	12.88	0.57	10.71	0.54
Full panel MG-MID[c]	7.32		7.36		5.82	

[a] Median value calculated according to the data obtained from NCI's in vitro disease-oriented human tumor cell screen.

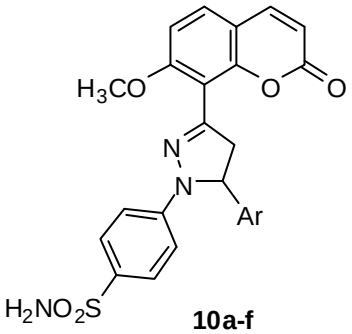
[b] I, Leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer.

[c] TGI (μM) full panel mean-graph mid point(MG-MID)= the average sensitivity of all cell lines toward the test agent

Table 4: In vitro cytotoxic activity of the final compounds against human colon cancer HCT-116 cell line.



7a-f, R^1= H
8a-f, R^1= CH_3
9a-f, R^1= Cl



10a-f

Compound	Ar	R^1	IC_{50} µM
Doxorubicin	-	-	0.63
7a	5-CH_3-C_4H_2O	H	0.40
7b	C_4H_3S	H	1.90
7c	C_6H_5	H	0.10
7d	4-CH_3-C_6H_4	H	0.02
7e	4-CF_3-C_6H_4	H	0.10
7f	4-SCH_3-C_6H_4	H	1.40
8a	5-CH_3-C_4H_2O	CH_3	1.30
8b	C_4H_3S	CH_3	1.20
8c	C_6H_5	CH_3	0.02
8d	4-CH_3-C_6H_4	CH_3	ND
8e	4-CF_3-C_6H_4	CH_3	ND
8f	4-SCH_3-C_6H_4	CH_3	0.30
9a	5-CH_3-C_4H_2O	Cl	0.50
9b	C_4H_3S	Cl	0.11
9c	C_6H_5	Cl	0.01
9d	4-CH_3-C_6H_4	Cl	0.24
9e	4-CF_3-C_6H_4	Cl	0.20
9f	4-SCH_3-C_6H_4	Cl	0.11
10a	5-CH_3-C_4H_2O	-	1.40
10b	C_4H_3S	-	0.50
10c	C_6H_5	-	2.80
10d	4-CH_3-C_6H_4	-	0.10
10e	4-CF_3-C_6H_4	-	0.03
10f	4-SCH_3-C_6H_4	-	0.04

ND not determined

Table 5: % Inhibition of PI3K (p110α/p85α) protein kinase at 50 µM (IC_{50} values) for the selected compounds

Compound	**Ar**	**R^1**	**p110α inhibition [%] (IC_{50}, µM)**
Wortmanin	-	-	-- (1.61)
IX [51]	R' = 4F	R=H	-- (0.67)
7b	C_4H_3S	H	NA
7c	C_6H_5	H	NA
7d	4-CH_3-C_6H_4	H	NA
7f	4-SCH_3-C_6H_4	H	NA
8b	C_4H_3S	CH_3	NA
8c	C_6H_5	CH_3	5
8d	4-CH_3-C_6H_4	CH_3	NA
8f	4-SCH_3-C_6H_4	CH_3	10
9b	C_4H_3S	Cl	NA
9c	C_6H_5	Cl	100 (50.78)
9d	4-CH_3-C_6H_4	Cl	20
9f	4-SCH_3-C_6H_4	Cl	19
10b	C_4H_3S	-	NA
10c	C_6H_5	-	7
10d	4-CH_3-C_6H_4	-	NA
10f	4-SCH_3-C_6H_4	-	NA

NA= no activity (% inhibition is less than 5%).

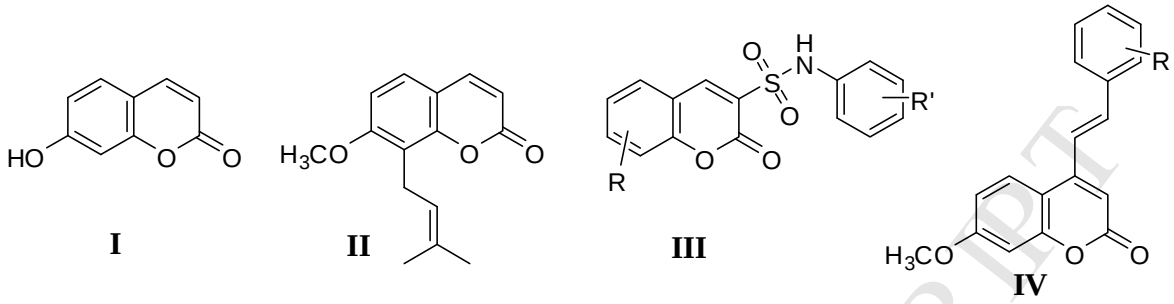


Figure 1. Chemical structures of antitumor coumarin derivatives mentioned in this study.

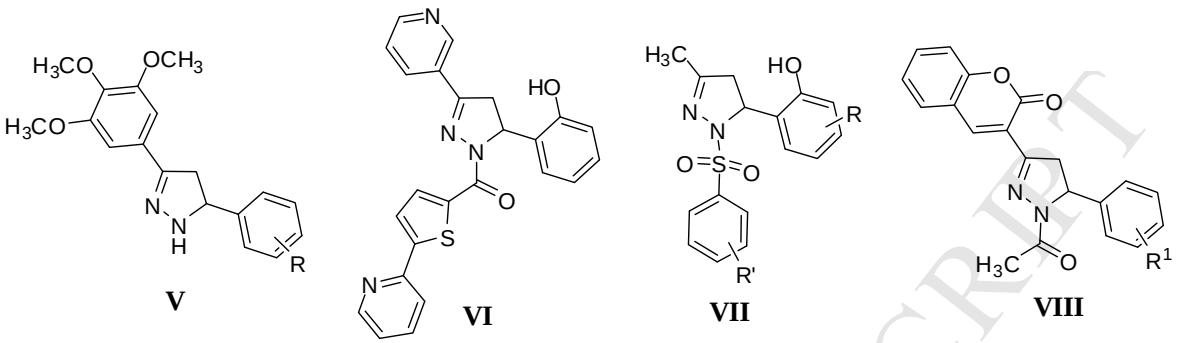


Figure 2: Chemical structures of some antitumor pyrazoline derivatives mentioned in this study.

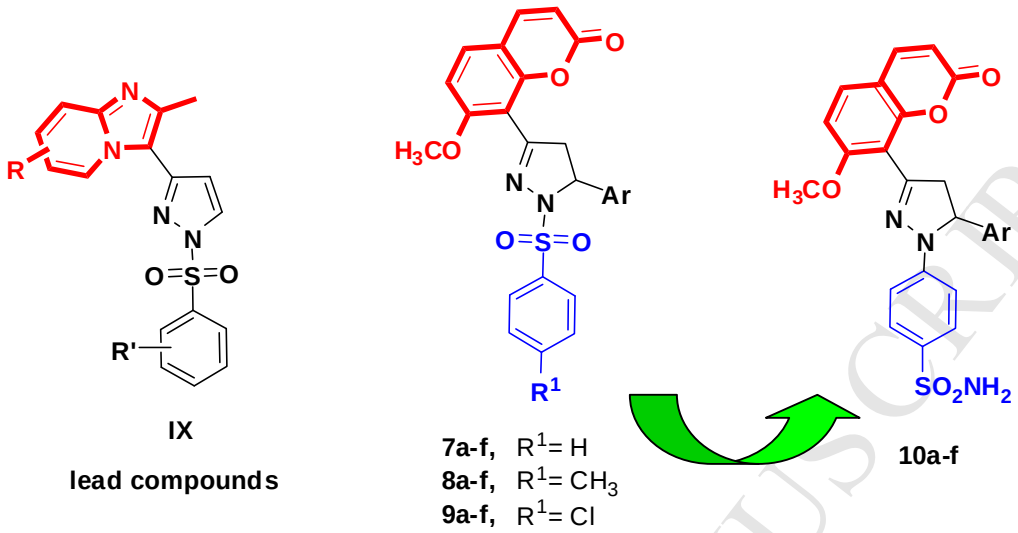
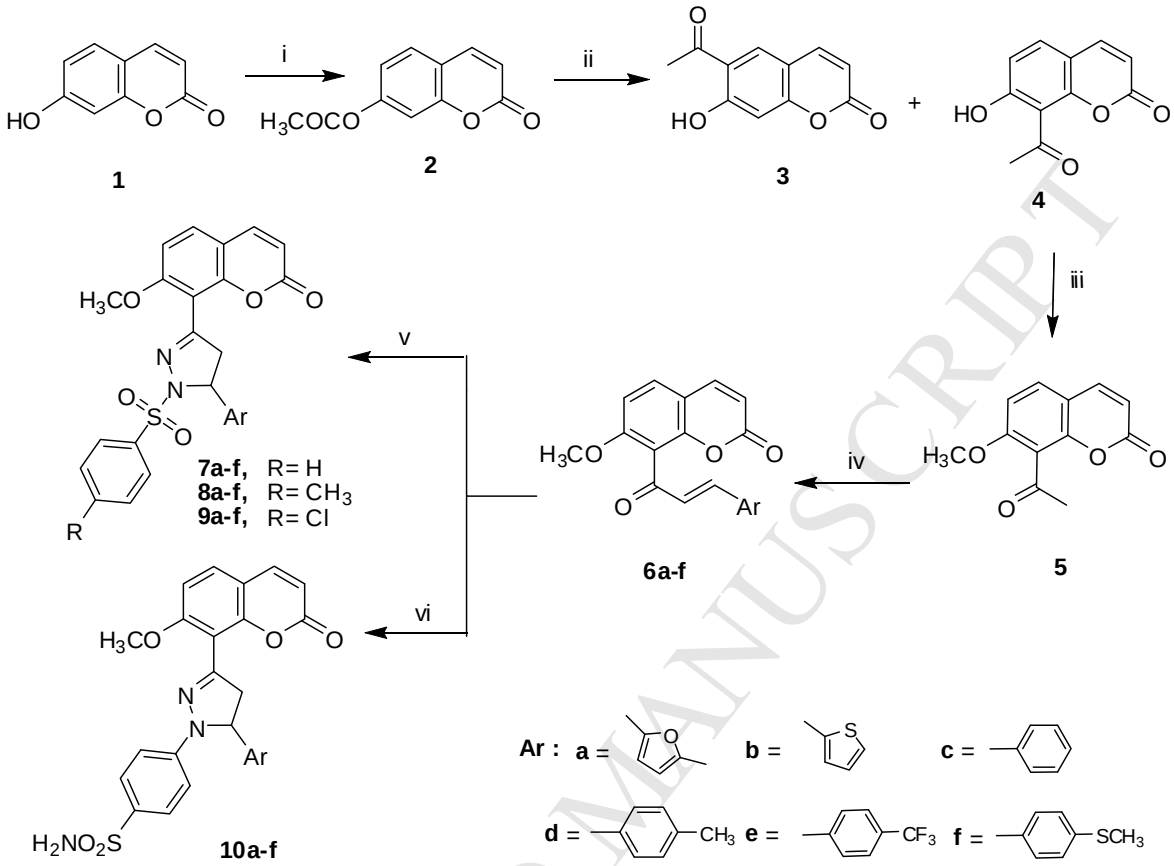


Figure 3: Design of the new coumarin-pyrazoline hybrids based on the lead compounds **IX**.



Scheme 1. Reagents and conditions: (i) acetic anhydride / reflux 5h; (ii) $AlCl_3$ / heat 145 ^oC 1h; (iii) CH_3I / dry actone / reflux 24h; (iv) Ar-CHO, 10% NaOH / ethanol / r.t. 24 h; (v) 4-(un) substituted phenylsulphonyl hydrazines / absolute ethanol / reflux 8-12h; (vi) 4-sulphamoylphenyl hydrazine / absolute ethanol / reflux 16h.