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Synthesis, *in vitro* antiproliferative activity, and *in silico* studies of fused tricyclic coumarin sulfonate derivatives

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Abstract: A series of fused tricyclic coumarin sulfonate derivatives was synthesized. Their in vitro antiproliferative activities against a panel of 57 human cancer cell lines of nine different cancer types were tested at the NCI. Compounds 1e, 1f, 1h, 1i, and 1o showed the highest mean %inhibition values over the 57 cell line panel at 10 μM, and they were further tested in 5-dose testing mode to determine their IC50 values. Compounds 1e, 1f, and 1o were more selective against leukemia and colon cancer subpanels, while compounds 1h and 1i showed broad-spectrum anticancer activities. Compound 1h exerted lethal effect over NCI-H522 NSCLC, SK-MEL-5 melanoma, and A498 renal cancer cell lines with %inhibition values of 114.10%, 103.23%, and 100.52% at 10 μM, respectively. Moreover, the IC50 value of compound 1o against HT29 colon cancer cell line was 532 nM. Compounds 1e, 1f, 1h, 1i, and 1o were tested for inhibitory effect over cyclooxygenase-2 (COX-2) enzyme as a possible mechanism of action. Furthermore, in silico studies were conducted to check the compliance of those five compounds with Lipinski's rule of five, and hence estimate their oral bioavailability.

Dear Prof. Galons,

We have revised and submitted our manuscript entitled "Synthesis, *in vitro* antiproliferative activity, and *in silico* studies of fused tricyclic coumarin sulfonate derivatives" to the *European Journal of Medicinal Chemistry*. I look forward to consideration of this revised manuscript for publication in EJMECH. Thank you so much in advance.

Yours sincerely,

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Thank you so much for your valuable comments which helped us greatly improve our manuscript.

In the manuscript, the corrected sentences have been converted into blue color.

Reviewer #1: This work dealt with the anticancer activities of the synthetic compounds with a cycloketone combining with the coumarin skeleton. The comparison on the anticancer activities leads to the discussion on the structure-activity relationship, in which the influences of the subsituents at R on the biological activities were explored in detail. But some short-comings should be resolved before this work can be accepted by the Journal. As the reference compound, the biological activity of coumarin itself should be determined. The function of the cycloketone on the biological activity should be clarified and whether the cycloketone connected with counarin with a single bond (C-C not the combined style as in the present work) should be emphasized.

The results of coumarin (CAS No.: 91-64-5) against NCI-60 cancer cell line panel was obtained from the NCI datawarehouse index through this link http://dtp.nci.nih.gov/dtpstandard/servlet/MeanGraph?searchtype=NSC&searchlist=8774&outputformat=HTML&outputmedium=page&chemnameboolean=AND&debugswitch=false&assaytype=&testshortname=NCI+Cancer+Screen+Current+Data&dataarraylength=55&endpt=GI50&SVGonly=SVG&button=Mean+Graph&highconc=-4.0 Although many references have reported anticancer activity of coumarin, it didn't show any potential potency against the NCI-60 cancer cell line panel. Upon comparison of its results with those of our potent compounds **1e**, **1f**, **1h**, **1i**, and **1o**, it was found that fused cycloalkane and arylsulfonate moieties are essential for activity of this series of compounds. The results of Coumarin were inserted in the manuscript (Tables 3 and 4), and relevant modifications of the Discussion and Conclusion sections were done as suggested by the reviewer.

Fusion through a single bond instead of a double bond will be investigated in the near future as suggested by the reviewer. But currently, it's almost impossible to do it before the due date for submission of the revised manuscript. We need to find a suitable synthetic pathway, order reagents, synthesize, purify, and analyze the compounds, submit to the NCI, and get results. This process needs long time. Thank you so much in advance for understanding our current situation.

Reviewer #2: The paper of El-Gamal M. I. et al is a sound one and has been improved. I suggest its acceptance after minor revision. General remark: Have these compounds been tested on non-cancerous cell-lines?

The most potent compounds **1e**, **1f**, **1h**, **1i**, and **1o** were tested for cytotoxicity over RAW 264.7 macrophages, and their IC_{50} values were more than 100 μM. These results indicated high selectivity of those compounds towards cancer cell lines than normal cells. This is another merit of those target compounds. Thank you so much for your valuable suggestion.

P4 „%inhibition" use: percentage of inhibition

It was corrected in all parts of the manuscript as suggested by the reviewer.

P9: not now, but in the future for synthesis of 3a and 3b use toluene instead of benzene.

Thank you so much for your valuable recommendation. Yes, toluene is less toxic than benzene.

P20 I would suggest omitting the column graph. Title: " Mean % inhibition" Inhibition of what? Reader does not know 1a, 1b…etc. meaning in the abscissa. The efficiency - if necessary - can be included in the text based on the general formula.

That title was omitted from Figure 2 as suggested by the reviewer.

All parts of the manuscript should be numbered in particular sub-titles as the following:
6-Oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl 4-(tert-butyl) benzenesulfonate (1q)

All parts have been numbered as suggested by the reviewer.

We hope that our revised manuscript will be satisfactory for the editor and reviewers, and it will be accepted for publication in *European Journal of Medicinal Chemistry*.

Synthesis, *in vitro* antiproliferative activity, and *in silico* studies of fused tricyclic coumarin sulfonate derivatives

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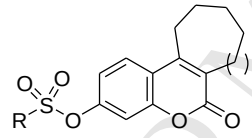
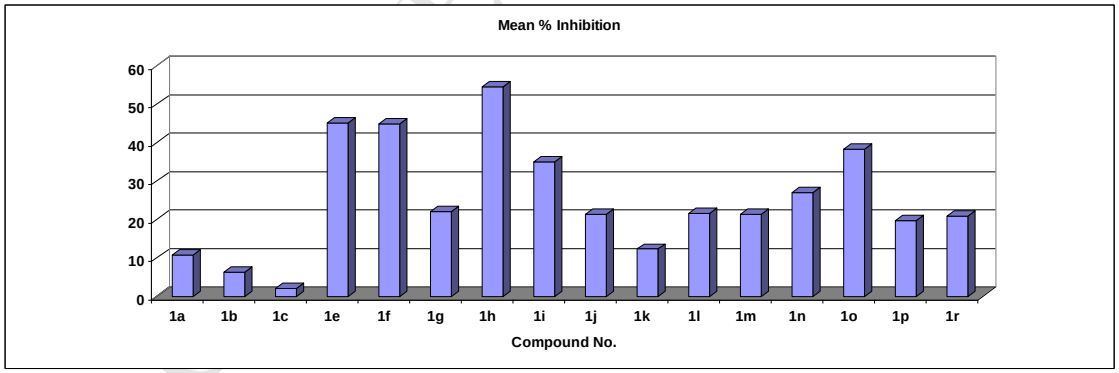
A series of new fused tricyclic coumarin sulfonate was synthesized. Their *in vitro* antiproliferative activities against NCI-60 cancer cell lines, COX-2 inhibition, and *in silico* studies are reported.



n = 1, 2
R = Me, Et, *n*-Pr, *c*-Pr, Ph, *p*-tolyl, *p*-$(CF_3)C_6H_4$, *p*-(*tert*-butyl)C_6H_4, *p*-$(F)C_6H_4$



Synthesis, *in vitro* antiproliferative activity, and *in silico* studies of fused tricyclic coumarin sulfonate derivatives

Mohammed I. El-Gamal, and Chang-Hyun Oh*

► Synthesis and *in vitro* antiproliferative activities of new coumarin sulfonates are reported.

► Compounds **1e**, **1f**, and **1o** were more selective against leukemia and colon cancer subpanels.

► Compounds **1h** and **1i** showed broad-spectrum anticancer activities.

► IC_{50} value of compound **1o** against HT29 colon cancer cell line was 532 nM.

► The antiproliferative effect is almost due to COX-2 inhibition.

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Synthesis, *in vitro* antiproliferative activity, and *in silico* studies of fused tricyclic coumarin sulfonate derivatives

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Abstract- A series of fused tricyclic coumarin sulfonate derivatives was synthesized. Their *in vitro* antiproliferative activities against a panel of 57 human cancer cell lines of nine different cancer types were tested at the NCI. Compounds **1e**, **1f**, **1h**, **1i**, and **1o** showed the highest mean percentage of inhibition values over the 57 cell line panel at 10 μM, and they were further tested in 5-dose testing mode to determine their IC_{50} values. Compounds **1e**, **1f**, and **1o** were more selective against leukemia and colon cancer subpanels, while compounds **1h** and **1i** showed broad-spectrum anticancer activities. Compounds **1e**, **1f**, **1h**, **1i**, and **1o** demonstrated high selectivity towards cancer cell lines than RAW 264.7 macrophages. Compound **1h** exerted lethal effect over NCI-H522 NSCLC, SK-MEL-5 melanoma, and A498 renal cancer cell lines with percentage of inhibition values of 114.10%, 103.23%, and 100.52% at 10 μM, respectively. Moreover, the IC_{50} value of compound **1o** against HT29 colon cancer cell line was 532 nM. Compounds **1e**, **1f**, **1h**, **1i**, and **1o** were tested for inhibitory effect over cyclooxygenase-2 (COX-2) enzyme as a possible mechanism of action. Furthermore, *in silico* studies were conducted to check the compliance of those five compounds with Lipinski's rule of five, and hence estimate their oral bioavailability.

Key words: Anticancer; Antiproliferative; COX-2 inhibition; Fused tricyclic coumarin; *In silico* studies; Sulfonate.

1. Introduction

Cancer is a major leading cause of death worldwide. According to the American Cancer Society report, 577,190 cancer patients died, and more than 1.6 million new cancer cases were identified in 2012 only in USA [1]. More than 70% of all cancer deaths have occurred in low- and middle-income countries. Deaths from cancer worldwide are estimated to exceed 13 million in 2030 according to the World Health Organization (WHO) report [2]. Despite of the extensive efforts and investment in research, the management of human cancers still constitutes a major challenge for contemporary medicinal chemistry. There has been an urgent need for development of more efficient anticancer agents with minimal side effects.

Natural and synthetic coumarin derivatives have recently attracted much interest because of their diverse biological and pharmacological properties. Among these properties, their anticancer effects were extensively examined [3-13]. Coumarin and its metabolite, 7-hydroxycoumarin (Umbelliferone) (Fig. 1), were reported to inhibit the proliferation of a number of human malignant cell lines *in vitro* [14,15] and in xenograft models [16,17]. 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 [18]. 6-Methoxy-7-hydroxycoumarin (Scopoletin, Fig. 1) was reported to initiate apoptosis in HL-60 cells [19]. It showed dual effects on both concanavalin A-stimulated murine T-cell proliferation and an anti-proliferative activity in a lymphoma cell line [20]. Irusostat (STX-64) has shown potential antiproliferative effect against breast cancer cell lines, and is currently under clinical trials for treatment of breast cancer [4,21]. Other fused tricyclic coumarin compounds have been reported for cytotoxic activity [22].

Cyclooxygenase-2 (COX-2) is an inducible enzyme involved in the conversion of arachidonic acid to prostaglandins and other eicosanoids. COX-2 and its product, prostaglandin E_2 (PGE_2) play a crucial role in tumor microenvironment [23]. Several reports have shown that PGE_2 and COX-2 have a wide range of effects including induction of cellular proliferation, promotion of angiogenesis, promotion of cancer cell

resistance to apoptosis, stimulation of tumor invasion, and suppression of immune responses [24,25]. Molecular pathology studies have revealed that COX-2 is over-expressed in cancer and stroma cells during tumor progression, and anticancer chemo-radiotherapies induce expression of COX-2 in cancer cells [26]. COX-2 has been proven to be over-expressed in several types of human cancer including melanomas [27-30], colon [31], non-small cell lung (NSCLC) [32], intestinal [33], colo-rectal [34], pancreatic [35], cervical [36], breast [37], endometrial [38], laryngeal [39], papillary thyroid [40], and gastric [41] cancers. Much attention has been focused on COX-2 inhibitors as a beneficial avenue for cancer chemotherapy [25,26]. Several experimental and clinical studies have established potent anticancer activity of COX-2 inhibitors such as Celecoxib (Fig. 1). Moreover, we have recently reported dual inhibitors of COX-2 enzyme and ERK pathway as potential antiproliferative agents [42].

In the present study, eighteen fused tricyclic coumarin sulfonate analogs **1a-r** (Fig. 1) were synthesized. The inhibitory effects of compounds **1a-g** and **1j-r** over LPS-induced nitric oxide and prostaglandin E_2 (PGE_2) productions in RAW 264.7 macrophages were recently reported [43]. Herein, their *in vitro* antiproliferative activities were investigated against 57 cancer cell line panel. In addition, the *in vitro* COX-2 inhibitory effect of the most active agents was tested. And *in silico* study was carried out in order to estimate the oral bioavailability of the most active compounds.

[Figure 1]

2. Results and Discussion

2.1. Chemistry

The target compounds were synthesized by the pathway illustrated in Scheme 1. Reaction of cycloheptanone (**2a**) or cyclooctanone (**2b**) with diethyl carbonate in refluxing benzene in the presence of sodium hydride to produce the corresponding ethoxycarbonate derivatives **3a,b** which exist in keto-enol tautomers [44]. Cyclization to the phenolic tricyclic intermediates **4a,b** was achieved by reaction of compounds **3a,b** with resorcinol in the presence of concentrated sulfuric acid and trifluoroacetic acid [45]. Treatment of the phenolic intermediates **4a,b** with the appropriate sulfonyl chloride

derivatives in the presence of triethylamine afforded the target sulfonates **1a-r** [43]. Table 1 illustrates structures of the final compounds and their yield percentages.

[Scheme 1 & Table 1]

2.2. Antiproliferative activities against 57 cell line panel at the NCI

2.2.1. Single-dose testing

Structures of the synthesized target compounds were submitted to National Cancer Institute (NCI), Bethesda, Maryland, USA [46], and the 16 compounds shown in Figure 2 were selected on the basis of degree of structural variation and computer modeling techniques for evaluation of their anticancer activity. The selected compounds were subjected to *in vitro* antiproliferative assay against tumor cells in a full panel of 57 cell lines taken from nine different tissues (blood, lung, colon, CNS, skin, ovary, kidney, prostate, and breast). The compounds were tested at a single-dose concentration of 10 μM, and the percentages of growth inhibition over the 57 tested cell lines were determined. The mean inhibition percentages for each of the tested compounds over the full panel of cell lines are illustrated in Figure 2.

The results showed that aromatic sulfonate derivatives **1e-i**, **1n**, and **1o** exhibited stronger antiproliferative activities than aliphatic and cyclopropyl analogs. Upon comparing the activities of aliphatic sulfonate derivatives **1a-c**, it was found that mean percentage of inhibition decreased with extension of the terminal aliphatic group (Me>Et>*n*-Pr). Aromatic sulfonate derivatives **1e-g** and **1i** possessing cycloheptane ring showed higher mean percentage of inhibition than the corresponding compounds **1n-p** and **1r** with cyclooctanone ring. Compounds **1f** and **1o** with *para*-toluenesulfonate group and compound **1h** containing *para*-(*tert*-butyl)benzenesulfonate were more active than compounds **1g** and **1p** possessing *para*-(trifluoromethyl)benzenesulfonate, and **1i** and **1r** with *para*-fluorobenzenesulfonate moiety. Similarly, compounds **1e** and **1n** possessing terminal benzenesulfonate moiety showed more activities than compounds **1g**, **1i**, **1p**, and **1r**. So it can be concluded that electron-withdrawing groups such *para*-(trifluoromethyl) and *para*-fluoro on the terminal benzenesulfonate moiety is unfavorable for activity.

[Figure 2]

Among all the tested derivatives, compounds **1e**, **1f**, **1h**, and **1o** showed the highest mean inhibitions. The percentages of inhibition of these four compounds over each tested cell line of the NCI-57 panel at 10 μM concentration are depicted in Figure 3. Compounds **1e**, **1f**, and **1o** showed more activities against leukemia and colon cancer cell lines. Compound **1h** possessing fused cycloheptane and *para*-(*tert*-butyl)benzenesulfonate moieties demonstrated broad-spectrum activity with more than 60% inhibition over 21 different cell lines of the nine tested cancer types. It exerted lethal effect with more than 100% inhibition against NCI-H522 non-small cell lung cancer (NSCLC), SK-MEL-5 melanoma, and A498 renal cancer cell lines (114.10%, 103.23%, and 100.52%, respectively). It also inhibited the growth of HOP-92 NSCLC and SF-295 CNS cancer cell lines by 96.06% and 97.29%, respectively, at 10 μM.

[Figure 3]

2.2.2. Five-dose testing

Compounds **1e**, **1f**, **1h**, **1i**, and **1o** with promising results in single-dose testing were further tested in a five-dose testing mode, in order to determine their IC_{50} values over the 57 cancer cell lines. The mean IC_{50} values of those five compounds over the nine cancer types are summarized in Table 2. The results showed that compound **1e** with benzenesulfonate moiety and compounds **1f** and **1o** possessing *para*-toluenesulfonate terminal ring were more selective against leukemia and colon cancer subpanels. The selectivity indices are illustrated in Table 2 as compared with the third most susceptible cancer type. On the other hand, the fused cycloheptane compounds **1h** and **1i** with *para*-(*tert*-butyl)benzenesulfonate and *para*-fluorobenzenesulfonate moieties, respectively, demonstrated broad-spectrum antiproliferative activities.

[Table 2]

The IC_{50} values of compounds **1e**, **1f**, and **1o** against all the tested leukemia and colon cancer cell lines are summarized in Table 3. The results of Coumarin, Scopoletin, and Umbelliferone were obtained from NCI datawarehouse index [47], and are inserted in Table 3. Compounds **1e**, **1f**, and **1o** exerted superior potencies against leukemia and colon cancer cell lines to the three reference compounds, Coumarin, Scopoletin, and

Umbelliferone. Upon comparing the results of those three compounds, it was found that compound **1e** was the most potent against leukemia cell lines but compound **1o** showed the highest potency against colon cancer cell lines. Of special interest, the IC_{50} value of compound **1o** against HT29 colon cancer cell line was in submicromolar scale, 532 nM.

[Table 3]

Furthermore, compounds **1h** and **1i** demonstrated broad-spectrum antiproliferative activities. Their IC_{50} values over the most sensitive cell line of each subpanel are illustrated in Table 4. Both compounds showed higher potencies than Scopoletin and Umbelliferone against the nine cell lines of nine different cancer types. The superior potencies of compounds **1e**, **1f**, **1h**, **1i**, and **1o** to Coumarin indicate that the fused cycloalkane and arylsulfonate moieties are essential for antiproliferative activity of this series of compounds. Compound **1h** with *para*-(*tert*-butyl)benzenesulfonate moiety was generally more potent than compound **1i** possessing *para*-fluorobenzenesulfonate terminal ring. The IC_{50} value of compound **1h** against HOP-92 NSCLC cell line was as low as 1.22 µM. Similarly, compound **1i** showed high potency against HT29 colon and T-47D breast cancer cell lines with IC_{50} values of 1.04 µM and 1.86 µM, respectively. The IC_{50} values of the most active compounds **1e**, **1f**, **1h**, **1i**, and **1o** against RAW 264.7 macrophages were determined [43] in order to investigate their cytotoxicity against normal cells and check their selectivity towards cancer cells over normal cells. All the five compounds did not inhibit 50% of the macrophage growth up to 100 µM. This indicates superior selectivity against cancer cell lines than normal cells.

[Table 4]

2.3. In vitro cyclooxygenase (COX) inhibitory activity

In order to determine the possible mechanism of antiproliferative activity at molecular level, the most active compounds **1e**, **1f**, **1h**, **1i**, and **1o** were tested for COX-2 inhibitory effect. The IC_{50} values were determined, and they are depicted in Table 5. The COX-2 inhibitory effect of compound **1g** [43] encouraged us to conduct this experiment.

The results showed good potency of the five compounds over COX-2 enzyme. The highest potencies were expressed by compounds **1e**, **1f**, and **1o** which are selective for

leukemia and colon cancer subpanels. The most potent compound was **1o** which showed submicromolar IC_{50} value over HT29 colon cancer cell line. Its IC_{50} value over COX-2 enzyme was comparable to that of Celecoxib. It has been reported that COX-2 is up-regulated in HT29 colon cancer cells [48]. So the superior potency of compound **1o** against HT29 colon cancer cell line can be attributed to its strong and selective COX-2 inhibitory effect. Upon comparing the potencies of compounds **1e**, **1f**, **1h**, and **1i**, it was found that the order of potency in correlation with the terminal substituents decreases in the following order ($PhSO_2$ > *para*-tosyl > *para*-fluorobenzenesulfonyl > *para*-(*tert*-butyl)benzenesulfonyl).

In order to examine selectivity of the target compounds towards COX-2 over COX-1, the IC_{50} values over COX-1 were also detected and selectivity indices were determined (Table 5). For instance, the selectivity indices of compounds **1e** and **1o** were more than 50 and 60.61, respectively. So it can be estimated that the target compounds may exert antiproliferative effect due to COX-2 inhibition with diminished or no side effects caused by COX-1 inhibition.

[Table 5]

2.4. Lipinski's rule of five

The bioavailability was assessed using ADME (absorption, distribution, metabolism, and excretion) prediction methods for the most active compounds **1e**, **1f**, **1h**, **1i**, and **1o** with the highest mean percentage of inhibition values, which were selected for 5-dose testing for determination of their IC_{50} values. In particular, we calculated the compliance of those five compounds to the Lipinski's rule of five [49]. This approach has been widely used as a filter for substances that would likely be further developed in drug design programs. In addition, we calculated the total polar surface area (TPSA) since it is another key property that has been linked to drug bioavailability. Thus, passively-absorbed molecules with a TPSA > 140 are thought to have low oral bioavailability [50]. Molecules violating more than one of these rules may have problems with bioavailability. Predictions of ADME properties for the studied compounds are given in Table 6. Compound **1h** violated only one parameter, but the other compounds **1e**, **1f**, **1i**, and **1o** comply with Lipinski's rule of five. Theoretically, those compounds should present good

passive oral absorption and differences in their bioactivity can not be attributed to this property.

[Table 6]

3. Conclusion

In this study, a series of fused tricyclic coumarin analogues possessing sulfonate moiety was synthesized and tested for *in vitro* antiproliferative activities over 57 cancer cell line panel of nine different cancer types. Among them, compounds **1e**, **1f**, **1h**, **1i**, and **1o** showed the most promising results at a single-dose concentration of 10 μM. Of special interest, compound **1h** with *para*-(*tert*-butyl)phenyl and fused cycloheptane moieties demonstrated lethal effect against NCI-H522 NSCLC, SK-MEL-5 melanoma, and A498 renal cancer cell lines with percentage of inhibition values of 114.10%, 103.23%, and 100.52%, respectively, at 10 μM. Compounds **1e**, **1f**, **1h**, **1i**, and **1o** were further tested in 5-dose testing mode in order to determine their IC_{50} values, and the results were compared with those of reference coumarin anticancer agents, Scopoletin and Umbelliferone. The five tested compounds showed higher potencies than Scopoletin and Umbelliferone. Moreover, the superior potency of those five compounds, compared with Coumarin, indicate that fused cycloalkane and arylsulfonate moieties play important roles in antiproliferative activity of this series of compounds. Compounds **1e** possessing benzenesulfonate moiety, and **1f** and **1o** with *para*-toluenesulfonate moiety exhibited the merit of selectivity towards leukemia and colon cancer subpanel more than the other seven tested cancer types. Those compounds can be utilized as leads for future development of potential selective anticancer agents for treatment of leukemia and colon cancer. On the other hand, compounds **1h** and **1i** showed broad-spectrum antiproliferative activities. Among all the five compounds, the highest potency was expressed by compound **1o** against HT29 colon cancer cell line with IC_{50} value of 532 nM. The five compounds **1e**, **1f**, **1h**, **1i**, and **1o** showed higher selectivity towards cancer cell lines than RAW 264.7 macrophages. In order to study the possible mechanism of action of the target compounds at molecular level, compounds **1e**, **1f**, **1h**, **1i**, and **1o** were tested for COX-2 inhibitory activity. They showed high potency and selectivity towards COX-2 than COX-1. So the target compounds might exhibit potential antiproliferative activity

due to COX-2 inhibition. *In silico* ADME prediction indicated that compounds **1e**, **1f**, **1i**, and **1o** comply with Lipinski's rule of five, and they can be passively-absorbed orally. The structure-activity relationship (SAR) studies showed that aromatic sulfonates were more favorable for antiproliferative activities of this series of compounds than aliphatic or alicyclic sulfonates.

4. Experimental

4.1. General

The target compounds were purified by column chromatography using silica gel (0.040-0.063 mm, 230-400 mesh) and technical grade solvents. The melting points were obtained on a Walden Precision Apparatus Electrothermal 9300 apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectroscopy was performed using a Bruker ARX-300, 300 MHz (Bruker Bioscience, Billerica, MA, USA) and a Bruker ARX-400, 400 MHz (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Purities of the target compounds (>95%) were determined by HPLC analysis. LC-MS analysis was conducted using the following system: Waters 2998 photodiode array detector, Waters 3100 mass detector, Waters SFO system fluidics organizer, Waters 2545 binary gradient module, Waters reagent manager, Waters 2767 sample manager, Sunfire^{TM} C18 column (4.6 x 50 mm, 5 μm particle size); Solvent gradient = 95% A at 0 min, 1% A at 5 min; solvent A: 0.035% trifluoroacetic acid (TFA) in water; solvent B: 0.035% TFA in CH_3OH; flow rate = 3.0 mL/min; the area under curve (AUC) was calculated using Waters MassLynx 4.1 software. Unless otherwise noted, all solvents and reagents were commercially available and used without further purification.

*4.2. Synthesis of ethyl 2-oxocycloheptanecarboxylate (**3a**) and ethyl 2-oxocyclooctanecarboxylate (**3b**)* [44]

A 250 mL two-neck, round-bottomed flask equipped with a magnetic stirrer was fitted with a 50 mL pressure-equalizing constant-rate dropping funnel and a condenser. To the flask, sodium hydride (4.5 g, 112 mmol, 60% dispersion in mineral oil) was added. The mineral oil was removed by washing the dispersion four times with 20 mL portions

of dry benzene under nitrogen atmosphere. The benzene was removed with a pipette after the sodium hydride was allowed to settle. After removal of most of the mineral oil, 60 mL of dry benzene was added to the sodium hydride, followed by diethyl carbonate (6.5 g, 55 mmol), this mixture was heated to reflux, and a solution of cycloheptanone (**3a**) or cyclooctanone (**3b**) (30 mmol) in 10 mL of dry benzene was added dropwise over a period of 3-4 h. After the addition was completed, this mixture was allowed to reflux until the evolution of hydrogen gas ceases (15-20 min). The reaction mixture was allowed to cool to room temperature, and 10 mL of glacial acetic acid was added dropwise, a heavy pasty solid separated. Then ice-cold water (about 100 mL) was added dropwise, and the stirring was continued until all the solid material has dissolved. The benzene layer was separated, and the aqueous layer was extracted with benzene (3 x 50 mL). The combined benzene extracts were washed three times with cold water (3 x 50 mL). The organic layer was dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. The title compounds were purified by flash column chromatography. The products were existing in equilibrating mixtures of the keto and enol tautomers.

*4.2.1. Compound **3a**:* it was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 15:1 v/v); yield: 90%; yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.76 (brs, 1H), 4.21-4.15 (m, 2H), 3.55-3.52 (m, 2H), 2.63-2.58 (m, 2H), 2.48-2.36 (m, 1H), 2.08 (t, 1H, *J* = 2.5 Hz), 1.94-1.85 (m, 4H), 1.62-1.61 (m, 2H), 1.49-1.44 (m, 2H), 1.32-1.23 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.8, 179.4, 173.0, 170.4, 101.5, 77.5, 77.2, 76.9, 60.9, 58.8, 43.0, 35.3, 31.9, 29.6, 27.9, 27.5, 27.3, 24.6, 24.3, 14.0.

*4.2.2. Compound **3b**:* it was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 10:1 v/v); yield: 85%; yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.60 (brs, 1H), 4.23-4.12 (m, 3H), 2.41-2.33 (m, 4H), 2.15-2.08 (m, 1H), 1.93-1.86 (m, 1H), 1.75-1.65 (m, 3H), 1.57-1.45 (m, 8H), 1.30 (t, 3H, *J* = 7.1 Hz), 1.24 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 212.2, 176.0, 172.9, 170.1, 99.2, 61.1, 60.1, 57.0, 41.7, 32.3, 29.9, 28.9, 28.7, 27.0, 26.5, 26.0, 25.5, 25.2, 24.5, 23.8, 14.3, 14.0.

*4.3. Synthesis of the hydroxyl intermediates **4a,b*** [45]

Resorcinol or 4-methoxyresorcinol or 4-chlororesorcinol or hydroquinone (11.0 mmol) was dissolved in hot ethyl 2-oxocycloheptanecarboxylate (**3a**) or ethyl 2-oxocyclooctanecarboxylate (**3b**) (11.0 mmol). To this stirred mixture at ice-water temperature was added dropwise a mixture of trifluoroacetic acid (1.7 mL, 22.0 mmol) and conc. sulfuric acid (2.2 mL, 22.0 mmol) at such a rate that the reaction temperature was kept below 10ºC (about 30 min). The reaction mixture was then allowed to warm to room temperature and then stirred for an additional 3 h before being quenched cautiously with ice-water. The product was then extracted with ethyl acetate (3 x 20 mL), and the combined organic layer extracts were washed with brine (3 x 25 mL) and dried over anhydrous sodium sulfate. The organic solvent was evaporated under reduced pressure, and the product was used in the next step without further purification.

*4.4. Synthesis of the target cycloalkane-fused coumarin sulfonates **1a-r***

A solution of the appropriate intermediate hydroxyl compound (0.1 mmol) and triethylamine (0.0165 mL, 0.2 mmol) in dry dichloromethane (5 mL) was cooled in an ice bath. A solution of the appropriate sulfonyl chloride derivative (0.11 mmol) in dry dichloromethane (2 mL) was added dropwise at the same temperature. The reaction mixture was allowed to warm to room temperature, and the stirred for an additional 1 h. The reaction mixture was washed with brine (3 x 5 mL) and dried over anhydrous sodium sulfate. The organic solvent was evaporated under reduced pressure, and the product was purified by flash column chromatography.

*4.4.1. 6-Oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-3-yl methanesulfonate (**1a**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 5:1 v/v); mp: 172-3 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72 (d, 1H, *J* = 9.4 Hz), 7.27-7.24 (m, 2H), 3.22 (s, 3H), 2.97-2.91 (m, 4H), 1.92 (d, 2H, *J* = 5.4 Hz), 1.70-1.62 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.4, 153.1, 152.8, 150.0, 129.2, 125.5, 119.1, 118.0, 110.6, 37.9, 31.9, 28.2, 26.9, 25.5, 24.9.

*4.4.2. 6-Oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-3-yl ethanesulfonate (**1b**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 4:1 v/v); mp: 169-72 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (t, 1H, *J* = 4.6 Hz), 7.24-7.23 (m, 2H), 3.35 (q, 2H, *J* = 7.4 Hz), 2.96-2.90 (m, 4H), 1.91 (d, 2H, *J* = 5.5 Hz), 1.70-1.62 (m, 4H), 1.58 (t, 3H, *J* = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.4, 153.1, 152.8, 150.0, 129.0, 125.4, 118.9, 117.9, 110.7, 45.6, 1.9, 28.2, 26.9, 25.5, 24.9, 8.2.

*4.4.3. 6-Oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-3-yl propane-1-sulfonate (**1c**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 5:1 v/v); mp: 133-5 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.72-7.68 (m, 1H), 7.22-7.21 (m, 2H), 3.32-3.25 (m, 2H), 2.95-2.89 (m, 4H), 2.06-1.98 (m, 2H), 1.91 (d, 2H, *J* = 6.0 Hz), 1.69-1.61 (m, 4H), 1.17-1.12 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.4, 153.1, 150.1, 129.0, 125.4, 118.9, 118.1, 110.6, 52.7, 31.9, 28.3, 26.9, 25.5, 24.9, 17.4, 12.9.

*4.4.4. 6-Oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-3-yl cyclopropanesulfonate (**1d**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 5:1 v/v); mp: 128-30 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (d, 1H, *J* = 8.4 Hz), 7.27-7.25 (m, 2H), 3.33 (q, 1H, *J* = 7.1 Hz), 2.97-2.92 (m, 4H), 2.69-2.63 (m, 1H), 1.93 (brs, 2H), 1.71-1.63 (m, 4H), 1.25-1.18 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.5, 153.0, 152.8, 150.6, 129.0, 125.3, 118.9, 118.3, 110.8, 41.5, 31.9, 28.2, 26.9, 25.5, 24.9, 14.0, 6.4.

*4.4.5. 6-Oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-3-yl benzenesulfonate (**1e**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 7:1 v/v then switching to hexanes:ethyl acetate 5:1 v/v); mp: 125-8 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.86 (d, 2H, *J* = 7.8 Hz), 7.71 (t, 2H, *J* = 7.4 Hz), 7.63 (d, 1H, *J* = 8.9 Hz), 7.56 (t, 3H, *J* = 7.6 Hz), 2.93-2.87 (m, 4H), 1.90 (d, 2H, *J* = 5.5 Hz), 1.68-1.58 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.4, 152.9, 152.8, 150.5, 135.0, 134.7, 129.4, 129.0, 128.4, 125.2, 118.9, 118.4, 110.7, 31.8, 28.2, 26.8, 25.4, 24.8; LC-MS: 371.1 (M^{+} + 1).

*4.4.6. 6-Oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-3-yl 4-methylbenzenesulfonate (**1f**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 10:1 v/v then switching to hexanes:ethyl acetate 7:1 v/v); mp: 149-52 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (d, 2H, *J* = 7.2 Hz), 7.64-7.62 (m, 1H), 7.35-7.32 (m, 2H), 7.06 (d, 1H, *J* = 8.8 Hz), 6.82 (s, 1H), 2.94-2.88 (m, 4H), 2.47 (s, 3H), 1.91-1.89 (m, 2H), 1.69-1.60 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.5, 152.9, 152.8, 150.6, 146.0, 132.0, 130.0, 128.9, 128.5, 125.2, 118.8, 118.6, 110.7, 31.9, 28.2, 26.8, 25.5, 24.8, 21.8.

*4.4.7. 6-Oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-3-yl 4-(trifluoromethyl) benzenesulfonate (**1g**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 10:1 v/v); mp: 137-40 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.03 (d, 2H, *J* = 9.0 Hz), 7.85 (d, 2H, *J* = 9.0 Hz), 7.66 (d, 1H, *J* = 9.0 Hz), 7.04-6.95 (m, 2H), 2.95-2.88 (m, 4H), 1.93-1.88 (m, 2H), 1.72-1.59 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.3, 152.9, 152.7, 150.1, 138.7, 136.4, 136.1, 129.3, 129.0, 126.6, 125.4, 119.2, 118.1, 110.7, 31.9, 28.2, 26.9, 25.4, 24.8; LC-MS: 439.1 (M^+ + 1).

*4.4.8. 6-Oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-3-yl 4-(tert-butyl) benzenesulfonate (**1h**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 20:1 v/v then switching to hexanes:ethyl acetate 15:1 v/v); mp: 94-7 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (d, 2H, *J* = 8.5 Hz), 7.64 (d, 1H, *J* = 8.9 Hz), 7.56 (d, 2H, *J* = 8.5 Hz), 7.08 (dd, 1H, *J* = 2.1 Hz, 8.8 Hz), 6.90 (d, 1H, *J* = 2.2 Hz), 2.91 (dt, 4H, *J* = 10.6 Hz, 9.8 Hz), 1.90 (q, 2H, *J* = 6.0 Hz), 1.69-1.59 (m, 4H), 1.36 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.5, 158.8, 152.9, 152.8, 150.7, 132.2, 128.9, 128.3, 126.4, 125.1, 118.8, 118.5, 110.7, 35.4, 31.9, 31.0, 28.2, 26.8, 25.5, 24.9; LC-MS: 427.2 (M^+ + 1).

*4.4.9. 6-Oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromen-3-yl 4-fluorobenzenesulfonate (**1i**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 30:1 v/v then switching to hexanes:ethyl acetate 25:1 v/v); mp: 147-9 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.91-7.88 (m, 2H), 7.64 (d, 1H, *J* = 8.8 Hz), 7.26-7.22 (m, 2H), 7.03 (dd, 1H, *J* = 2.3 Hz, 8.8 Hz), 6.89 (d, 1H, *J* = 2.2 Hz), 2.94-2.88 (m, 4H), 1.90 (q, 2H, *J* = 6.0 Hz), 1.69-1.60 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.4, 152.8, 152.7, 150.4, 131.4, 131.3, 129.1, 125.3, 119.0, 118.3, 117.0, 116.8, 110.7, 31.8, 28.2, 26.8, 25.4, 24.8.

4.4.10. 6-Oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl methanesulfonate ***(1j)***

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 7:1 v/v then switching to hexanes:ethyl acetate 4:1 v/v); mp: 146-8 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66 (d, 1H, *J* = 8.6 Hz), 7.27-7.22 (m, 2H), 3.22 (s, 3H), 2.99 (t, 2H, *J* = 6.5 Hz), 2.82 (t, 2H, *J* = 6.1 Hz), 1.85-1.71 (m, 4H), 1.56-1.42 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.8, 153.3, 149.8, 149.5, 127.3, 125.7, 118.6, 118.1, 110.7, 37.9, 29.7, 29.1, 26.9, 26.6, 26.4, 25.9.

4.4.11. 6-Oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl ethanesulfonate ***(1k)***

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 7:1 v/v then switching to hexanes:ethyl acetate 4:1 v/v); mp: 157-60 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (d, 1H, *J* = 9.4 Hz), 7.26-7.22 (m, 2H), 3.35 (q, 2H, *J* = 7.4 Hz), 2.98 (t, 2H, *J* = 4.6 Hz), 2.81 (t, 2H, *J* = 3.9 Hz), 1.81-1.73 (m, 4H), 1.59-1.44 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.8, 153.3, 149.8, 149.5, 127.2, 125.6, 118.4, 118.1, 110.6, 45.6, 29.7, 29.1, 26.9, 26.6, 26.4, 25.9, 8.3.

4.4.12. 6-Oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl propane-1-sulfonate ***(1l)***

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 7:1 v/v); mp: 114-7 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (d, 1H, *J* = 5.9 Hz), 7.25-7.22 (m, 2H), 3.31-3.24 (m, 2H), 2.99 (t, 2H, *J* = 6.0 Hz), 2.82 (t, 2H, *J* = 5.8 Hz), 2.09-1.98 (m, 2H), 1.82-1.73 (m, 4H), 1.53-1.44 (m, 4H), 1.15 (t, 3H, *J* = 7.4 Hz); ^{13}C NMR ($CDCl_3$,

100 MHz) δ 160.8, 153.3, 149.9, 149.6, 127.2, 125.6, 120.9, 118.1, 110.6, 52.7, 29.7, 29.1, 26.9, 26.6, 26.4, 25.9, 17.4, 12.8.

*4.4.13. 6-Oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl cyclopropanesulfonate (**1m**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 7:1 v/v then switching to hexanes:ethyl acetate 5:1 v/v); mp: 133-6 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (d, 1H, *J* = 8.5 Hz), 7.27-7.24 (m, 2H), 3.00 (t, 2H, *J* = 6.6 Hz), 2.82 (t, 2H, *J* = 6.1 Hz), 2.71-2.65 (m, 2H), 1.83-1.74 (m, 6H), 1.54-1.46 (m, 6H), 1.34-1.19 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.9, 153.2, 150.4, 149.6, 127.2, 125.6, 118.4, 110.9, 29.7, 29.1, 28.2, 26.9, 26.6, 26.4, 25.9, 6.4.

*4.4.14. 6-Oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl benzenesulfonate (**1n**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 10:1 v/v then switching to hexanes:ethyl acetate 6:1 v/v); mp: 108-11 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.88 (d, 2H, *J* = 8.0 Hz), 7.72 (t, 1H, *J* = 7.0 Hz), 7.57 (t, 3H, *J* = 7.6 Hz), 7.04 (dd, 1H, *J* = 2.1 Hz, 8.8 Hz), 6.85 (d, 1H, *J* = 2.1 Hz), 2.96 (t, 2H, *J* = 6.8 Hz), 2.79 (t, 2H, *J* = 6.1 Hz), 1.81-1.72 (m, 4H), 1.52-1.44 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.8, 153.0, 150.3, 149.6, 135.1, 134.7, 129.4, 128.4, 127.2, 125.5, 118.5, 118.4, 110.8, 29.6, 29.1, 26.9, 26.6, 26.4, 25.9.

*4.4.15. 6-Oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl 4-methylbenzenesulfonate (**1o**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 12:1 v/v then switching to hexanes:ethyl acetate 6:1 v/v); mp: 107-10 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (d, 2H, *J* = 8.2 Hz), 7.58 (d, 1H, *J* = 8.8 Hz), 7.36-7.29 (m, 2H), 7.05 (dd, 1H, *J* = 1.9 Hz, 8.8 Hz), 6.82 (d, 1H, *J* = 1.9 Hz), 2.95 (t, 2H, *J* = 5.7 Hz), 2.78 (t, 2H, *J* = 5.0 Hz), 2.47 (s, 3H), 1.80-1.71 (m, 4H), 1.50-1.42 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.9, 152.9, 150.4, 149.7, 146.0, 132.0, 130.1, 128.4, 127.1, 125.4, 118.7, 118.3, 110.8, 29.6, 29.1, 26.9, 26.6, 26.4, 25.8, 21.8; LC-MS: 399.2 (M^+ + 1).

4.4.16. 6-Oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl 4-(trifluoromethyl)

*benzenesulfonate (**1p**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 12:1 v/v then switching to hexanes:ethyl acetate 8:1 v/v); mp: 154-7 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04 (d, 2H, *J* = 8.2 Hz), 7.85 (d, 2H, *J* = 8.3 Hz), 7.60 (d, 1H, *J* = 8.8 Hz), 7.03 (dd, 1H, *J* = 2.3 Hz, 8.8 Hz), 6.94 (d, 1H, *J* = 2.2 Hz), 2.97 (t, 2H, *J* = 6.4 Hz), 2.81 (t, 2H, *J* = 6.0 Hz), 1.81-1.73 (m, 4H), 1.53-1.44 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.7, 153.1, 150.0, 149.5, 138.8, 136.4, 129.0, 127.5, 126.6, 125.6, 118.7, 118.2, 110.7, 29.6, 29.1, 26.9, 26.6, 26.4, 25.9.

*4.4.17. 6-Oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl 4-(tert-butyl) benzenesulfonate (**1q**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 20:1 v/v then switching to hexanes:ethyl acetate 15:1 v/v); mp: 166-9 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81 (d, 2H, *J* = 8.5 Hz), 7.59-7.56 (m, 3H), 7.07 (dd, 1H, *J* = 2.2 Hz, 8.8 Hz), 6.91 (d, 1H, *J* = 2.2 Hz), 2.96 (t, 2H, *J* = 6.4 Hz), 2.79 (t, 2H, *J* = 6.0 Hz), 1.83-1.69 (m, 4H), 1.52-1.43 (m, 4H), 1.36 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.9, 158.8, 153.0, 150.5, 149.7, 132.2, 128.3, 127.1, 126.5, 125.4, 118.6, 118.3, 110.7, 35.4, 31.0, 29.7, 29.1, 26.9, 26.6, 26.4, 25.9.

*4.4.18. 6-Oxo-7,8,9,10,11,12-hexahydro-6H-cycloocta[c]chromen-3-yl 4-fluorobenzenesulfonate (**1r**)*

It was purified by flash column chromatography (silica gel, hexanes:ethyl acetate 30:1 v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.92-7.89 (m, 2H), 7.58 (d, 1H, *J* = 8.8 Hz), 7.24-7.22 (m, 2H), 7.05 (dd, 1H, *J* = 2.2 Hz, 8.7 Hz), 6.88 (d, 1H, *J* = 2.2 Hz), 2.97 (t, 2H, *J* = 6.5 Hz), 2.81 (t, 2H, *J* = 6.1 Hz), 1.81-1.73 (m, 4H), 1.53-1.44 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.8, 153.0, 150.2, 149.5, 131.4, 131.3, 127.3, 125.5, 118.5, 118.4, 117.0, 116.8, 110.8, 29.6, 29.1, 26.9, 26.6, 26.4, 25.9.

4.5. Cancer cell line screening at the NCI

Screening against the cancer cell lines was carried out at the National Cancer Institute (NCI), Bethesda, Maryland, USA [46] applying the standard protocol of the NCI [51,52].

4.6. MTT assay for RAW 264.7 macrophage cell viability

RAW 264.7 macrophages were plated at a density of 105 cells/well in 96-well plates. To determine the appropriate concentration not toxic to cells, cytotoxicity studies were performed 24 h after treating cells with various concentrations of tested compounds. Cell viabilities were determined using colorimetric MTT assays, as described previously [53].

4.7. In vitro cyclooxygenase (COX) inhibition assay

The ability of the test compounds to inhibit bovine COX-1 and COX-2 was determined using an enzyme immunoassay (EIA) (kit catalog number 560101, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Cyclooxygenase catalyzes the first step in the biosynthesis of arachidonic acid to PGH_2. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, was measured by enzyme immunoassay (ACETM competitive EIA). Stock solutions of test compounds were dissolved in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (960 µL, 0.1 M Tris–HCl pH 8.0 containing 5 mM EDTA and 2 mM phenol) with either COX-1or COX-2 (10 µL) enzyme in the presence of heme (10 µL) were added 10 µL of various concentrations of test drug solutions (0.01, 0.1, 1, 10, and 50 µM in a final volume of 1 mL). These solutions were incubated for a period of 5 min at 37 ºC after which 10 µL of arachidonic acid (100 µM) solution were added and the COX reaction was stopped by the addition of 50 µL of 1 M HCl after 2 min. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride was measured by enzyme immunoassay. This assay is based on the competition between PGs and a PG-acetylcholinesterase conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells since the concentration of PG tracer is held constant while the concentration of PGs varies. This antibody–PG complex bound to a mouse anti-rabbit monoclonal antibody that was previously attached to the well. The plate was washed to remove any unbound

reagents and then Ellman's reagent, which contains the substrate to acetylcholine esterase, was added to the well. The product of this enzymatic reaction produced a distinct yellow color that absorbs at 405 nm. The intensity of this color, determined spectrophotometrically, was proportional to the amount of PG tracer bound to the well, which was inversely proportional to the amount of PGs present in the well during the incubation: Absorbance α [Bound PG Tracer] α1/PGs. Percent inhibition was calculated by the comparison of compound treated to various control incubations. The concentration of the test compound causing 50% inhibition (IC_{50}, μM) was calculated from the concentration–inhibition response curve (duplicate determinations).

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References

[1] http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-031941.pdf Retrieved on Apr. 29th, 2014.

[2] http://www.who.int/mediacentre/factsheets/fs297/en/ Retrieved on Apr. 29th, 2014.

[3] I. Kostova, Curr. Med. Chem. 5 (2005) 29-46.

[4] S.J. Stanway, A. Purohit, L. W. L. Woo, S. Sufi, D. Vigushin, R. Ward, R.H. Wilson, F. Z. Stanczyk, N. Dobbs, E. Kulinskaya, M. Elliott, B.V.L. Potter, M. J. Reed, R.C. Coombes, Clin. Cancer Res. 12 (2006) 1585-1592.

[5] W. Li, Y.N. Sun, X.T. Yan, S.Y. Yang, E.-J. Kim, H.K. Kang, Y.H. Kim, J. Agric. Food Chem. 61 (2013) 10730-10740.

[6] R. M. Mohareb, S. M. Sherif, A. Abou Elkair, Curr. Org. Chem. 17 (2013) 1910-1918.

[7] T. Nasr, S. Bondock, M. Youns, Eur. J. Med. Chem. 76 (2014) 539-548.

[8] K. Paul, S. Bindal, V. Luxami, Bioorg. Med. Chem. Lett. 23 (2013) 3667-3672.

[9] K.B. Puttaraju, K. Shivashankar, C.M. Mahendra, V.P. Rasal, P.N.V. Vivek, K. Rai, M.B. Chanu, Eur. J. Med. Chem. 69 (2013) 316-322.

[10] O. Dömötör, T. Tuccinardi, D. Karcz, M. Walsh, B. S. Creaven, É. A. Enyedy, Bioorg. Chem. 52 (2014) 16-23.

[11] M. Basanagouda, V.B. Jambagi, N.N. Barigidad, S.S. Laxmeshwar, V. Devaru, Narayanachar, Eur. J. Med. Chem. 74 (2014) 225-233.

[12] B.R.V. Avin, P. Thirusangu, V.L. Ranganatha, A. Firdouse, B.T. Prabhakar, S.A. Khanum, Eur. J. Med. Chem. 75 (2014) 211-221.

[13] K.M. Amin, A.A.M. Eissa, S.M. Abou-Seri, F.M. Awadallah, G.S. Hassan, Eur. J. Med. Chem. 60 (2013) 187-198.

[14] C.M. Elinos-Báez, F. León, E. Santos, Cell. Biol. Int. 29 (2005) 703-708.

[15] M.E. Marshall, J.L. Mohler, K. Edmonds, B. Williams, K. Butler, M. Ryles, L. Weiss, D. Urban, A. Beuschen, M. Markiewicz, J. Cancer Res. Clin. Oncol. 120 (1994) 39-42.

[16] G.J. Finn, B.S. Creaven, D.A. Egan, Eur. J. Pharm. Sci. 26 (2005) 16-25.

[17] L.D. Raev, E. Voinova, I.C. Ivanov, D. Popov, Pharmazie 45 (1990) 696-702.

[18] J.L. Mohler, L.G. Gomella, E.D. Crawford, L.M. Glode, C.D. Zippe, W.R. Fair, M.E. Marshall, Prostate 20 (1992) 123-131.

[19] E.K. Kim, K.B. Kwon, B.C. Shin, E.A. Seo, Y.R. Lee, J.S. Kim, J.W. Park, B.H. Park, D.G. Ryu, Life Sci. 77 (2005) 824-836.

[20] M.G. Manuele, G. Ferraro, A.M.L. Barreiro, P. Lopez, G. Cremaschi, C. Anesini, Life Sci. 79 (2006) 2043-2048.

[21] http://clinicaltrials.gov/ct2/results?term=stx-64&Search=Search Retrieved on April 29th, 2014.

[22] M. Khoobi, A. Foroumadi, S. Emami, M. Safavi, G. Dehghan, B.H. Alizadeh, A. Ramazani, S.K. Ardestani, A. Shafiee, Chem. Biol. Drug Des. 78 (2011) 580-586.

[23] S. Funakoshi, H. Takaishi, T. Hibi, Surg. Trauma Immunol. Responses 20 (2011) 166-168.

[24] D. Wang, R.N. Dubois, Gut 55 (2006) 115-122.

[25] A. Krishna, A.K.C. Divvela, S.R. Challa, I.K. Tagaram, 56 (2010) 502-516.

[26] Z. Khan, N. Khan, R.P. Tiwari, N.K. Sah, G.B.K.S. Prasad, P.S. Bisen, Curr. Drug Targets 12 (2011) 1082-1093.

[27] C. Denkert, M. Kobel, S. Berger, A. Siegert, A. Leclere, U. Trefzer, S. Hauptmann, Cancer Res. 61 (2001) 303-308.

[28] F. Bianchini, D. Massi, C. Marconi, A. Franchi, G. Baroni, M. Santucci, A. Mannini, G. Mugnai, L. Calorini, Prostaglandins Other Lipid Mediat. 83 (2007) 320-328.

[29] B.W. Chwirot, L. Kuzbicki, Melanoma Res. 17 (2007) 139-145.

[30] C.C. Johansson, S. Egyhazi, G. Masucci, H. Harlin, D. Mougiakakos, I. Poschke, B. Nilsson, L. Garberg, R. Tuominen, D. Linden, M.F. Stolt, J. Hansson, R. Kiessling, Cancer Immunol. Immunother. 58 (2009) 1085-1094.

[31] M. Tsujii, S. Kawano, R.N. Dubois, Proc. Natl. Acad. Sci. USA 94 (1997) 3336-3340.

[32] M. Huang, M. Stolina, S. Sharma, J.T. Mao, L. Zhu, P.W. Miller, J. Wollman, H. Herschman, S.M. Dubinett, Cancer Res. 58 (1998) 1208-1216.

[33] C.S. Williams, R.L. Shattuk-Brandts, R.N. Dubois, Expert Opin. Investig. Drugs 8 (1999) 1-12.

[34] K.M. Sheehan, K. Sheahan, D.P. O'Donoghue, F. MacSweeny., R.M. Conroy, D.J. Fitzgerald, F.E. Murray, JAMA 282 (1999) 1254-1257.

[35] M.A. Molina, M. Sitja-Arnau, M.G. Lemoine, M.L. Frazier, F.A. Sinicrope, Cancer Res. 59 (1999) 4356-4362.

[36] H.S. Ryu, K.H. Chang, H.W. Yang, M.S. Kim, H.C. Kown, K.S. Oh, Gynecol. Oncol. 76 (2000) 320-325.

[37] L.R. Howe, K. Subbaramaiah, A.M. Brown, A.J. Dannenberg, Endocr. Relat. Cancer 8 (2001) 97-114.

[38] R. Fujikawi, K. Iida, H. Kanasaki, T. Ozaki, K. Hata, K. Miyazaki, (2002) Cyclooxygenase-2 expression in endometrial cancer: correlation with microvessel count and expression of vascular endothelial growth factor and thymidine phosphorylase. Hum. Pathol. 33 (2002) 213-219.

[39] P. Nix, M. Lind, J. Greenman, N. Stafford, L. Cawkwell, Ann. Oncol. 15 (2004) 797-801.

[40] P. Siironen, A. Ristimaki, S. Nordling, J. Louhimo, R. Haapiainen, C. Haglund, Histopathology 44 (2004) 490-497.

[41] C. Wu, H.F. Tsai, W.C. Lin, A.H. Chou, H.T. Chen, J.C. Yang, P.I. Hsu, P.N. Hsu, World J. Gastroenterol. 11 (2005) 3197-3203.

[42] M.I. El-Gamal, H.S. Choi, K.H. Yoo, D. Baek, C.-H. Oh, Chem. Biol. Drug. Des. 82 (2013) 336-347.

[43] H.-L. Jang, M.I. El-Gamal, H.-E. Choi, H.-Y. Choi, K.-T. Lee, C.-H. Oh, Bioorg. Med. Chem. Lett. 24 (2014) 571-575.

[44] C.J. Li, D.L. Chen, Y.Q. Lu, J.X. Haberman, J.T. Mague, Tetrahedron 54 (1998) 2347-2364.

[45] L.W.L. Woo, A. Purohit, B. Malini, M.J. Reed, B.V.L. Potter, Chemistry & Biology 7 (2000) 773-791.

[46] NCI website: www.dtp.nci.nih.gov.

[47] DTP Data Search: ‹http://dtp.nci.nih.gov/dtpstandard/dwindex/index.jsp›.

[48] S. Battu, J.L. Beneytout, M. Pairet, M. Rigaud, FEBS Lett. 437 (1998) 49-55.

[49] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Adv. Drug Deliv. Rev. 46 (2001) 3-26.

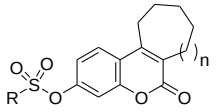
[50] D.E. Clark, S.D. Pickett, Drug Discov. Today 5 (2000) 49-58.

[51] DTP Data Search: http://dtp.nci.nih.gov/dtpstandard/dwindex/index.jsp. Retrieved on Apr. 29th, 2014.

[52] DTP Human Tumor Cell Line Screen Process: http://www.dtp.nci.nih.gov/branches/btb/ivclsp.html. Retrieved on Apr. 29th, 2014.

[53] J.-Y. Kim, S.J. Park, K.-J. Yun, Y.-W. Cho, H.-J. Park, K.-T. Lee, Eur. J. Pharmacol. 584 (2008) 175-184.

Table 1. Structures of the target compounds and their yield percentages.



Compound No.	R	n	Yield%
1a	Me	1	95
1b	Et	1	92
1c	*n*-Pr	1	93
1d	Cyclo-Pr	1	85
1e	Ph	1	93
1f	*p*-Tolyl	1	95
1g	*p*-(CF_3)C_6H_4	1	90
1h	*p*-(*tert*-butyl)C_6H_4	1	95
1i	*p*-(F)C_6H_4	1	82
1j	Me	2	94
1k	Et	2	90
1l	*n*-Pr	2	88
1m	Cyclo-Pr	2	87
1n	Ph	2	92
1o	*p*-Tolyl	2	95
1p	*p*-(CF_3)C_6H_4	2	90
1q	*p*-(*tert*-butyl)C_6H_4	2	94
1r	*p*-(F)C_6H_4	2	90

Table 2. Mean IC_{50} values (μM) of the tested compounds over *in vitro* subpanel cancer cell lines[a]

Cancer Subpanel	No. of cell line in each subpanel	**Compound No.**				
		1e	**1f**	**1h**	**1i**	**1o**
Leukemia	6	3.60 **(8.37)**[c]	4.54 **(8.06)**[c]	67.73	75.93	6.26 **(8.46)**[c]
NSCLC[b]	8	40.56	60.68	15.92	78.45	76.21
Colon	6	8.48 **(3.55)**[c]	20.82 **(1.76)**[c]	46.13	27.36	17.46 **(3.03)**[c]
CNS	6	54.68	79.70	21.17	86.60	>100
Melanoma	9	30.14	76.06	18.78	88.21	76.46
Ovarian	6	65.87	68.98	19.12	86.54	86.44
Renal	8	41.68	89.46	16.88	>100	75.96
Prostate	2	51.31	52.13	20.06	>100	52.99
Breast	6	36.20	36.60	12.48	40.23	67.85

[a] Mean IC_{50} values were calculated by dividing the summation of IC_{50} values of the compound over cell lines of the same cancer type by the number of cell lines in the subpanel.

[b] Non-Small Cell Lung Cancer.

[c] Selectivity index.

Table 3. IC_{50} values of compounds **1e**, **1f**, and **1o** over leukemia and colon cancer cell lines, and RAW 264.7 macrophages

Compound No.		**1e**	**1f**	**1o**	**Coumarin**	**Scopoletin**	**Umbelliferone**
Leukemia	CCRF-CEM	**4.56**	**5.18**	**8.92**	>100	31.62	100
	HL-60(TB)	**2.05**	Not Tested	**7.17**	>100	31.62	100
	K-562	**4.06**	**3.51**	**3.88**	>100	>100	Not Tested
	MOLT-4	**4.34**	**6.16**	**9.57**	>100	31.62	100
	RPMI-8226	**3.58**	**4.55**	**2.33**	>100	50.12	100
	SR	**3.01**	**3.30**	**5.66**	Not Tested	Not Tested	Not Tested
Colon Cancer	COLO 205	**3.51**	**4.05**	**2.92**	>100	>100	100
	HCC-2998	**32.10**	>100	>100	>100	100	79.43
	HCT-116	**3.62**	**6.00**	**8.04**	>100	63.10	100
	HCT-15	**3.92**	**3.66**	**3.16**	>100	25.12	100
	HT29	**2.84**	**4.06**	**0.532**	>100	>100	100
	KM12	Not Tested	**7.16**	**4.78**	>100	39.81	100
	SW-620	**4.89**	Not Tested	**2.76**	>100	63.10	100
RAW 264.7 macrophages		>100	>100	>100	Not Tested	Not Tested	Not Tested

Table 4. IC_{50} values (μM) of compounds **1h**, **1i**, Scopoletin, and Umbelliferone over the most sensitive cell line of each subpanel and RAW 264.7 macrophages

Cell Line	Cancer Type	Compound No.				
		1h	**1i**	**Coumarin**	**Scopoletin**	**Umbelliferone**
RPMI-8226	Leukemia	**3.18**	**3.70**	>100	50.12	100
HOP-92	NSCLC	**1.22**	**4.05**	>100	>100	100
HT29	Colon	**7.95**	**1.04**	>100	>100	100
U251	CNS	**12.50**	**>100**	>100	25.12	100
SK-MEL-5	Melanoma	**5.48**	**5.69**	Not Tested	79.43	100
OVCAR-4	Ovarian	**8.13**	**5.76**	>100	63.10	100
A498	Renal	**6.52**	**>100**	>100	79.43	100
PC-3	Prostate	**2.22**	Not Tested	Not Tested	Not Tested	100
T-47D	Breast	**5.17**	**1.86**	Not Tested	Not Tested	100
RAW 264.7 macrophages		>100	>100	Not Tested	Not Tested	Not Tested

Table 5. Data of the *in vitro* COX-1/COX-2 enzyme inhibition assay of the target compounds.

Compound No.	IC_{50} (μM)[a]		Selectivity Index[c]
	COX-2	COX-1[b]	
1e	0.40±0.05	>20	>50.00
1f	1.85±0.13	>20	>10.81
1h	4.80±0.42	>20	>4.17
1i	2.72±0.17	>20	>7.35
1o	0.33±0.02	>20	>60.61
Celecoxib	0.30±0.02	>20	>66.67

[a] IC_{50} value is the compound concentration required to produce 50% inhibition of COX-1 or COX-2. The IC_{50} values are expressed as means of two determinations ± standard deviation.
[b] No inhibition of COX-1 up to 20 μM.
[c] Selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

Table 6. Calculated Lipinski's rule of five for the most active target compounds

Compd. No.	cLog P[a]	Log*S*[b]	TPSA[c]	MW[d]	*n*ON[e]	*n*OHNH[f]	*n*Violations
1e	3.63	-3.89	78.05	370.42	5	0	0
1f	3.97	-4.24	78.05	384.45	5	0	0
1h	5.21	-5.05	78.05	426.53	5	0	1
1i	3.73	-4.21	78.05	388.41	5	0	0
1o	4.99	-4.51	78.05	398.47	5	0	0

[a] Calculated lipophilicity.

[b] Solubility parameter.

[c] Total polar surface area ($Å^2$).

[d] Molecular weight.

[e] Number of hydrogen bond acceptors.

[f] Number of hydrogen bond donors.

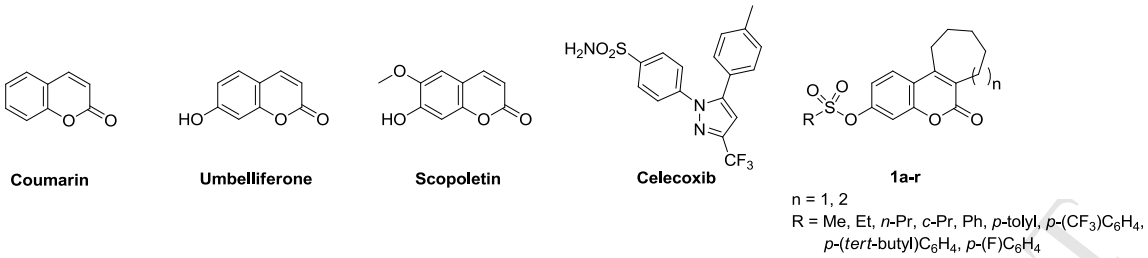


Figure 1. Structures of Coumarin, Umbelliferone, Scopoletin, Celecoxib, and the target compounds **1a-r**.

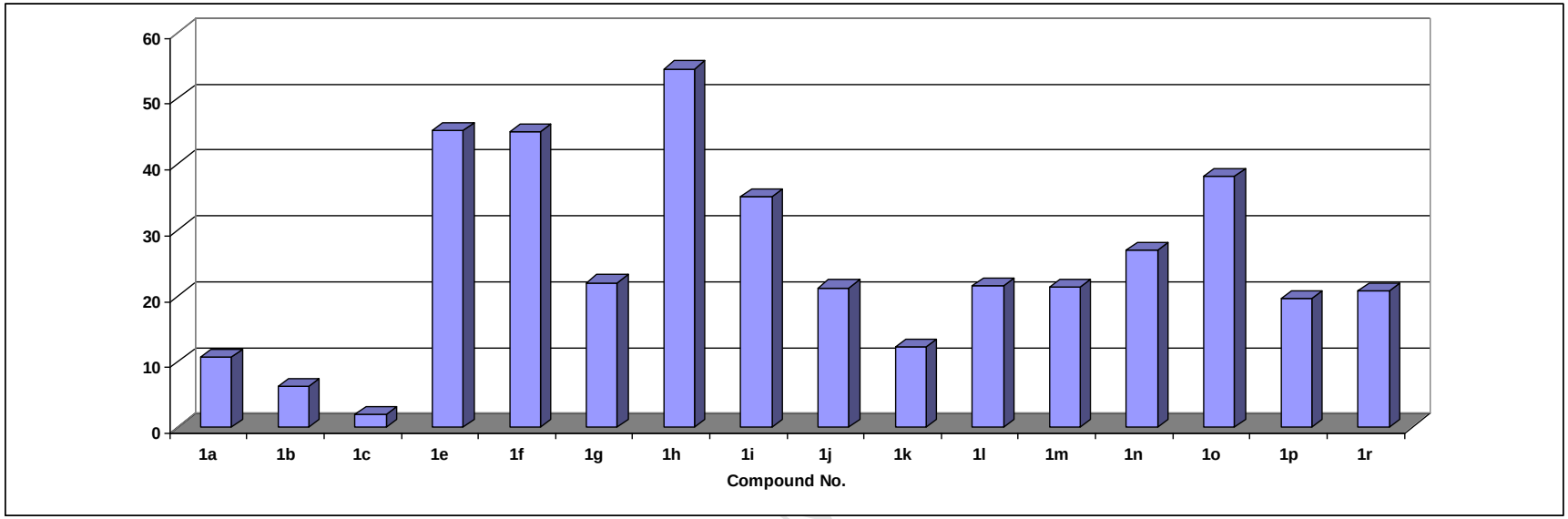


Figure 2. Mean inhibition percentages observed with the final compounds in single-dose (10 μM) 60-cancer cell line screening. Mean % inhibition represents the mean inhibition percentages over the 60 cell lines. The inhibition percentages were calculated by subtracting the growth percentages from 100.

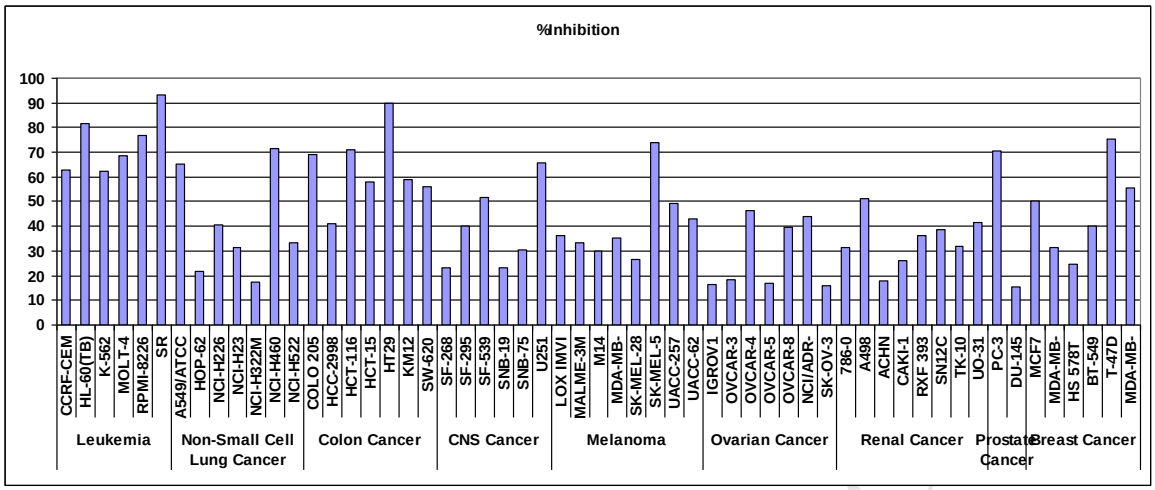


Fig. 3a (Comp. **1e**)

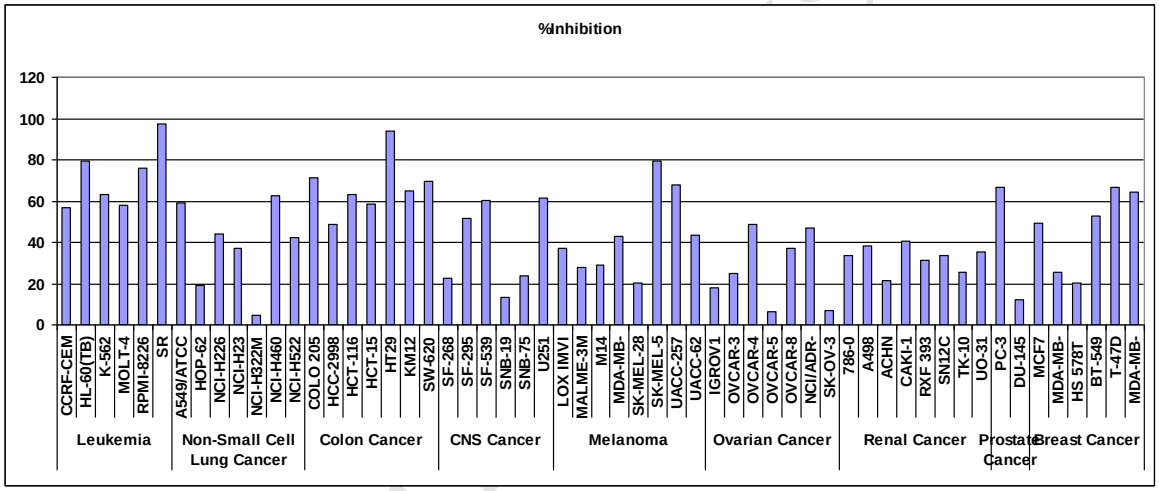


Fig. 3b (Comp. **1f**)

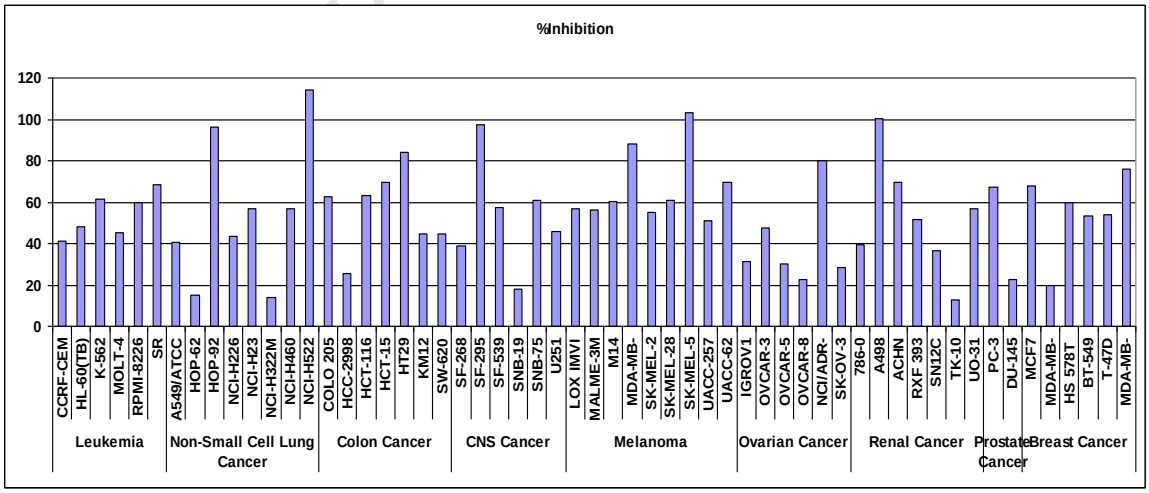


Fig. 3c (Comp. **1h**)

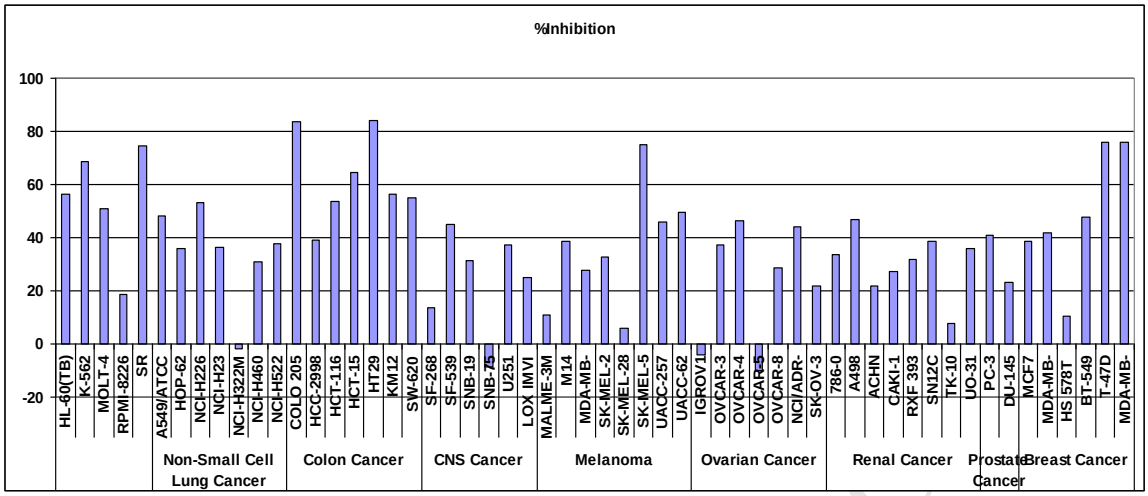
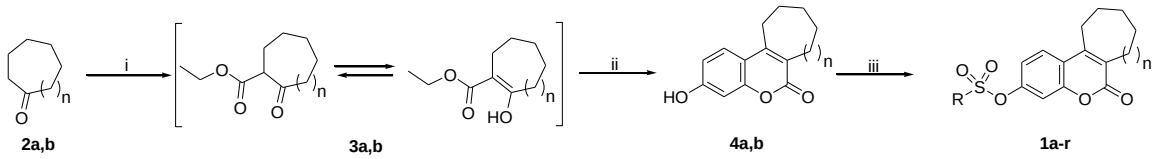


Fig. 3d (Comp. **1o**)

Figure 3. % Inhibition expressed by compounds **1e** (Fig. 3a), **1f** (Fig. 3b), **1h** (Fig. 3c), and **1o** (Fig. 3d) at a single-dose concentration of 10 µM over all cell lines of the NCI cancer cell line panel of nine different cancer types.



n = 1, 2
R = Me, Et, *n*-Pr, *c*-Pr, Ph, *p*-tolyl, *p*-$(CF_3)C_6H_4$, *p*-(*tert*-butyl)C_6H_4, *p*-$(F)C_6H_4$

Scheme 1. Reagents and conditions: i) diethyl carbonate, NaH, benzene, reflux, 90% (**3a**, n = 1), 85% (**3b**, n = 2); ii) resorcinol, CF_3COOH, conc. H_2SO_4, 0 ºC; rt, 3h; iii) appropriate sulfonyl chloride derivative, triethylamine, CH_2Cl_2, 0 ºC; rt, 1h.