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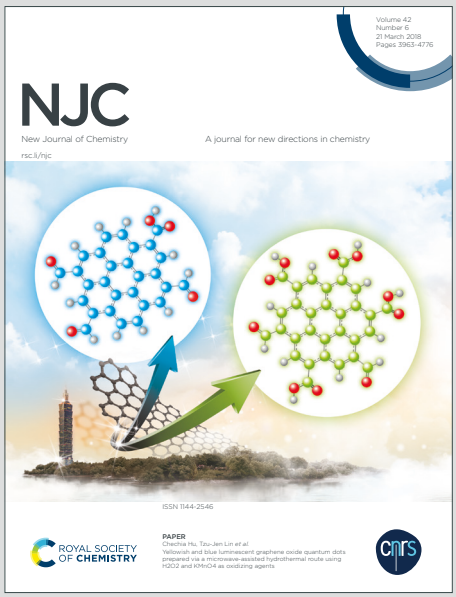

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ARTICLE

Selenopheno[2,3-*f*]coumarins: a novel scaffold with antimetastatic activity against melanoma and breast cancer

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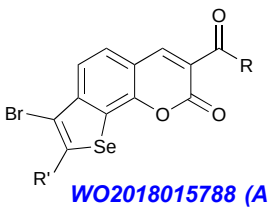
Pavel Arsenyan,[*a] Jelena Vasiljeva,[a] Ilona Domracheva,[a] Iveta Kanepe-Lapsa,[a] Anita Gulbe[a]

General aim of the current research is to find novel non-toxic small molecules with antimetastatic activity. Herein we present series of novel selenopheno[2,3-*f*]coumarins with low cytotoxic profile against cancer cells with different nature (carcinomas, sarcomas, melanoma, neuroblastoma, and hepatoma). Based on SAR, few compounds selectively induce MMP inhibition and suppress angiogenesis *in vivo* up to 57% (10μM). Certain derivatives are excellent agents for *in vivo* prevention of melanoma and breast cancer metastasis: up to 97% with no visual side effects. The present study provides scientific evidence that selenophenocoumarin scaffold is a promising competitor for the development of drug candidates with metastasis-suppressing properties.

Introduction

Cancer is the second leading cause of death globally, and is responsible for an estimated 9.6 million deaths in 2018 (World Health Organization data). Globally, about 1 in 6 deaths is due to cancer. Tumour growth is a complex multistage process. Occurrence and progressive tumour growth is dependent on both the properties of cancer cells, and on the state of immunological reactivity. This determines the diversity of approaches of cancer therapy using one or several basic methods: surgery, radiotherapy, chemotherapy and immunotherapy. Their goal is to minimize the mass of the tumour. Complications caused by metastases account for 90% of deaths from cancer. [1] There are several ways of metastases of malignant tumours: lymphogenous, haematogenous and mixed (through lymphatic, haematogenous or through seeding). Lymphogenous pathway occurs through the lymphatic system, where metastases that penetrate the lymphatic system can then enter the blood stream. Malignant tumours of internal organs (oesophagus, stomach, colon, larynx, cervix) often metastasize to the lymph nodes in this way. Haematogenous path causes the tumour cells to penetrate the blood vessels, and through the blood flow are transferred to other organs (e.g. lungs, liver, bone). Most deaths associated with this pathway are due to increased risk of the spread of tumour cells from the bloodstream after surgical intervention. Malignant tumours of the lymphatic and hematopoietic tissues – sarcoma, hypernephroma, horionepitelioma, metastasize this way. However, most cancers (breast, thyroid, lungs and bronchi, ovaries) can equally metastasize by lymphogenous and haematogenous mechanisms. [2-5] Recently, because of high medical need, there is a clear trend towards search for new antimetastatic drugs. These drugs must block the development of the metastases via elimination of distant cancer cells, but also by normalization of tissue microenvironment. At present, after the successful surgical removal of the primary tumour, the likelihood of recurrence is very high, since the tumour can spread and metastasize to surrounding tissues and organs. Metastasis begins with local invasion of tumour cells from the primary tumour into the surrounding tissue and cells. Entrance into blood or lymphatic vessels occurs as tumours further propagate throughout the body. [6, 7] The 2*H*-chromene system is a structural feature of many natural compounds and pharmacological agents possessing, among other properties, anti-cancer, [8] anti-HIV [9] and antibacterial activity [10]. Coumarin derivatives as medicines with substantial activity *in vitro* and *in vivo* have been discovered (*Psoralen*, *Angelicin*, *Xanthotoxin*, *Bergapten*, *Nodakenetin*, etc). *Imperatorin* shows the ability to inhibit tumour growth. [11, 12] *Osthole* inhibits the migration and invasion of breast cancer cells and effectively blocks matrix metalloproteinases promoter and enzyme activity. [13, 14]

Recently we claimed 2*H*-selenopheno[3,2-*h*]chromenes as antimetastatic agents in treatment of carcinomas and melanoma. These derivatives exhibit low acute toxicity and almost completely (98%) prevent metastases formation of mammary carcinoma 4T1 *in vivo* and melanoma B16-F10 metastasis in lung by 82% without any visual side effects. [15]



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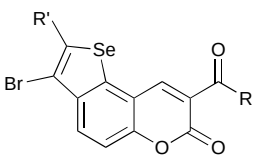

This work

[a] Latvian Institute of Organic Synthesis, Aizkraukles 21, LV-1006, Riga, Latvia
pavel@osi.lv

Electronic Supplementary Information (ESI) available: ^{1}H and ^{13}C NMR copies of compounds are presented in the Supplementary Information]. See DOI: 10.1039/x0xx00000x

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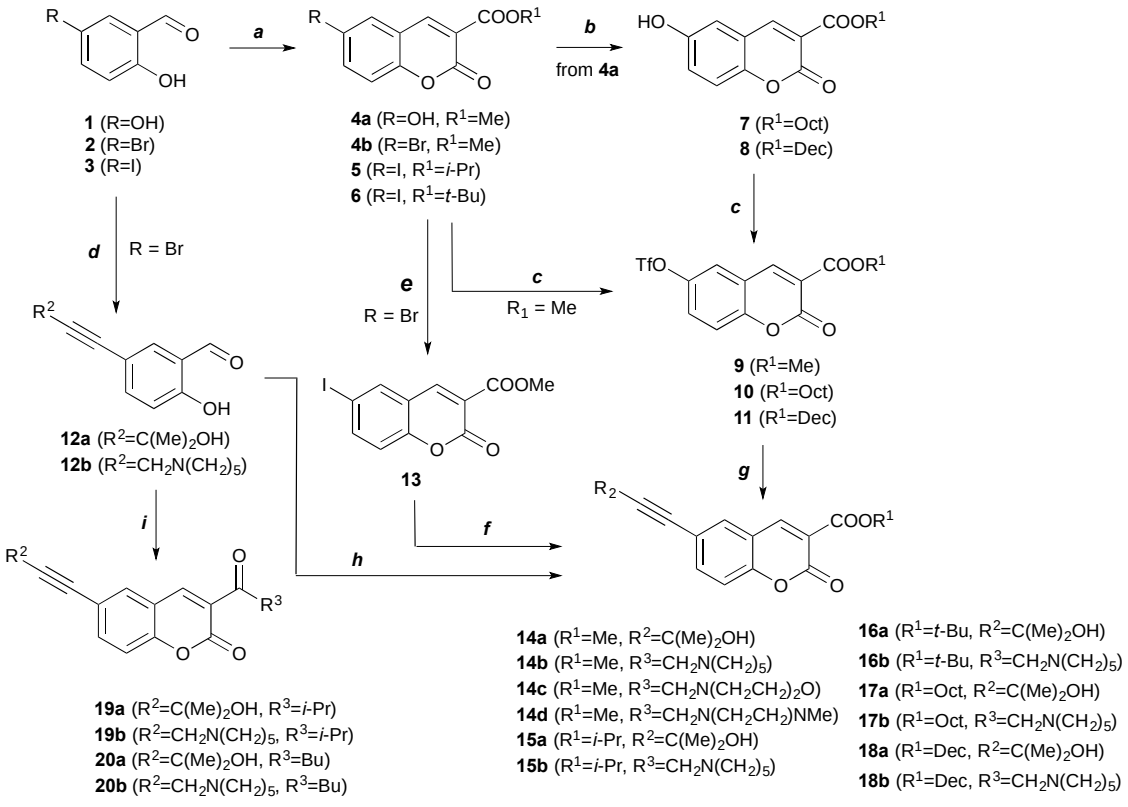
Unfortunately, the number of cancer diagnosis in past few years has increased almost twofold. In clinical practice, the treatment of cancer involves a wide range of chemotherapy drugs. Besides, most of them exhibit various side effects, high toxicity and moderate selectivity. Therefore, a new generation of selective anticancer agents with low toxicity is one of the main tasks for medicinal chemistry and pharmaceutical industries. In continuation of our research, [16-19] herein we would like to report our studies in the field of searching for antimetastatic drug candidate in series of selenopheno[2,3-*f*]coumarins.

Results and discussion

Synthesis

6-Alkynylcoumarins **14a-d**, **15a-b**, **16a-b**, **17a-b**, and **18a-b** were synthesized in few steps using 5-hydroxy, 5-bromo or 5-iodo salicylic aldehydes **1-3** as starting materials (Scheme 1). Methyl 6-hydroxy and 6-bromocoumarin-3-carboxylates **4a** and **4b** were obtained by the reaction of **1** and **2**, correspondingly, with methyl malonate in the presence of few drops of piperidine. [20] Then methyl esters were converted to octyl and decyl analogues **7** and **8** by refluxing the initial compounds in the corresponding alcohol under acidic conditions for 4-5 days. As a result, derivatives **7** and **8** were isolated in 92% and 81%, respectively. Next, hydroxy groups of **4b**, **7** and **8** were converted to triflates **9-11** in purpose to run Sonogashira cross-coupling reactions. The introduction of C≡C triple bond was performed at 60 °C using bis(triphenylphosphino)palladium(II) chloride and copper(I) iodide as catalysts (Scheme 1, *g*). Thus, alkynyl coumarins **14a-d** were isolated in 35-63% yields. Identical products **14b-d** were obtained in higher yields (39-86%) from methyl 6-iodocoumarin-3-carboxylate (**13**, Scheme 1, *f*). In this case, the reaction was performed at lower temperature, however, previously reported **14a** [21] was obtained in 57% yield from the corresponding bromide **5** (Scheme 1, *g*). Isopropyl **17a-b** and *tert*-butyl **18a-b** esters of ethynyl coumarins were synthesized according to *f* conditions in reverse sequence, namely, alkynyl derivatives **12a-b** were obtained from 5-bromo-2-hydroxybenzaldehyde **2** (41-89%) followed by condensation reaction with disopropyl and di(*tert*)butyl malonates (Scheme 1, *h*, 48-56%). 3-Acyl-6-ethynyl coumarins were easily prepared by the treatment of **12a,b** with isobutyrylacetic acid methyl ester or methyl 3-oxoheptanoate (Scheme 1, *i*) at r.t.



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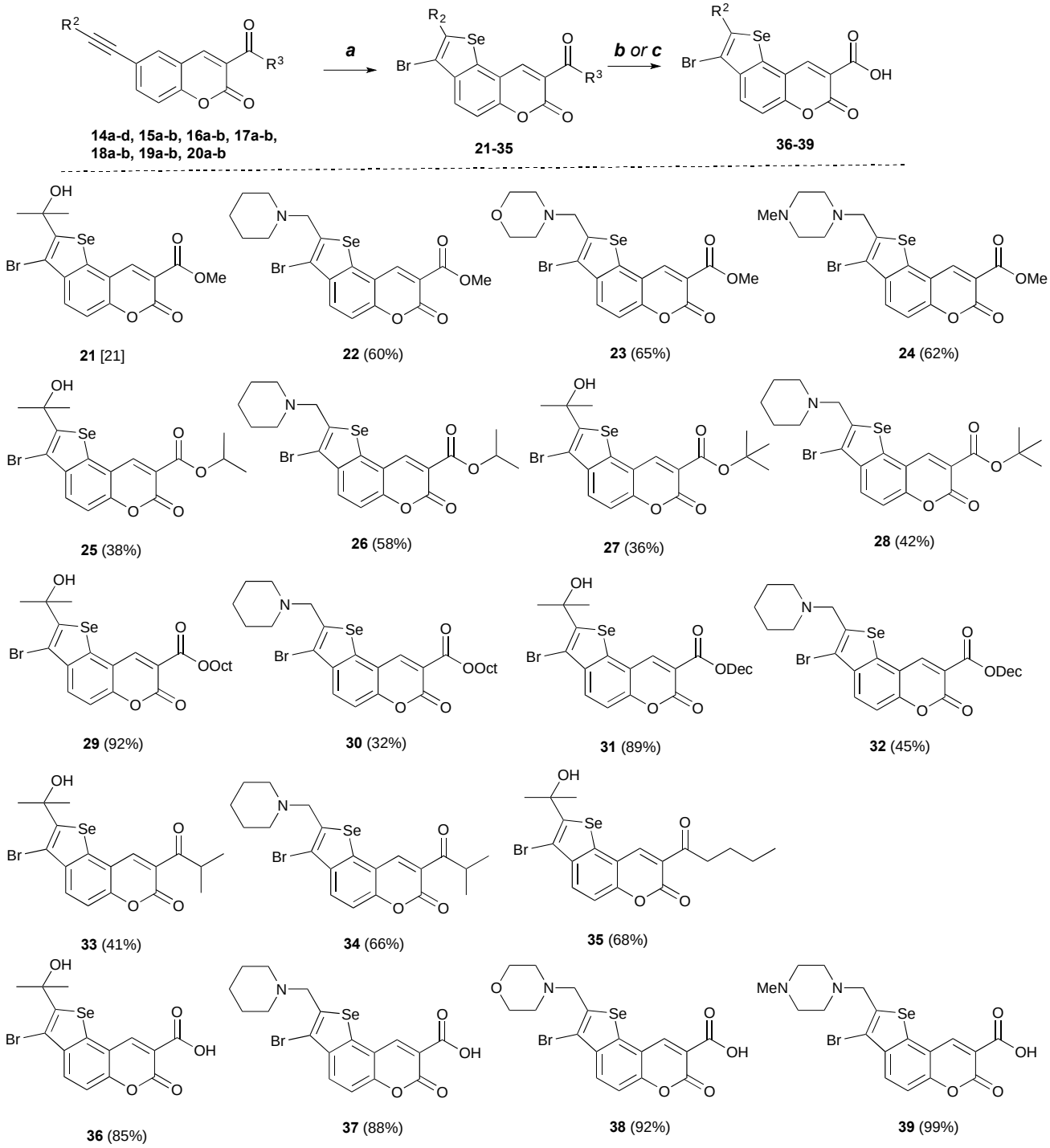
Scheme 1. The synthesis of ethynylcoumarins. Reagents and reaction conditions: **a**: $R_1OOCCH_2COOR_1$ (2 equiv.), MeOH, piperidine (2-4 drops), 60 °C, 24 h.; **b**: $SiMe_3Cl$ (4 mL), octanol or decanol (20 mL), reflux, 4-5 days.; **c**: Tf_2O (3 equiv.), DCM, Et_3N, 48 h.; **d**: terminal acetylene (1.5 equiv.), $PdCl_2(PPh_3)_2$ (7mol%), CuI (7 mol%), THF/ET_3N = 1/1, 60 °C, overnight.; **e**: KI (5 equiv.), CuI (1.2 equiv.), DMEDA (1.0 equiv.), 1,4-dioxane, 120 °C, 48 h.; **f**: **5-6, 13** (1 equiv.), terminal alkyne (1.5 equiv.), $PdCl_2(PPh_3)_2$ (10 mol%), CuI (10 mol%), DMF, Et_3N, 40 °C, overnight. **g**: **9-11** (1 equiv.), terminal alkyne (1.5 equiv.), CuI (15 mol%), $PdCl_2$ (10 mol%), and PPh_3 (20 mol%) in DMF, Et_3N, 60 °C for 18 h. **h**: **12a,b** (1 equiv.), diisopropyl malonate (1.5 equiv.) or di(*tert*)butylmalonate (1.5 equiv.), MeOH, 2 drops of piperidine, r.t., 24h. **i**: **12a,b** (1 equiv.) and isobutyrylacetic acid methyl ester (1.4 equiv.) or methyl 3-oxoheptanoate (1.4 equiv.) MeOH, 2 drops of piperidine, r.t., 24h.



Scheme 2. The synthesis of selenophenocoumarins. Reagents and reaction conditions: **a**: SeO_2 (2 equiv.), HBr, 1,4-dioxane, 0 °C to r.t., 24-48 h. **b**: NaOH (6 equiv.), water, r.t.; **c**: HepG2 (human hepatocellular carcinoma) cells lysate, 37 °C.

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Next, we used previously developed method for the synthesis of selenophene moiety, which is based on treatment of ethynyl-substituted arenes and hetarenes [16] with *in situ* prepared selenium(IV) bromide. A solution of ethynyl coumarins **14–20** in 1,4-dioxane was treated with $SeBr_4$, formed *in situ* from SeO_2 and concentrated hydrobromic acid (48%) at r.t. Selenophenocoumarins **21-35** were prepared in excellent or satisfactory yields (32–95%). In the reaction of 6-(3-hydroxy-3-methylbut-1-ynyl)-3-pentanoyl-2*H*-chromen-2-one (**19a**) with $SeBr_4$, besides cyclization reaction, bromination of pentanoyl substituent proceeded at α-CH_2 position with the formation of bromo-substituted compound, which can be easily debrominated through refluxing with equimolar amount of *N*-acetyl guanidine and sodium acetate yielding **35**. Methyl selenopheno[2,3-*f*]coumarin carboxylates **21-24** were hydrolyzed to their corresponding acids **36-39** by stirring **21-24** in methanol with an excess of aqueous 2N sodium hydroxide solution at r.t. overnight. It should be noted that this step is slow; besides, temperature elevation led to destruction of coumarin cycle. However, hydrolysis of methyl ester by incubation with HepG2 (human hepatocellular carcinoma) cells lysate at 37 ^{o}C was complete in 4h.

Biological evaluation

Cytotoxicity

In vitro cytotoxicity caused by novel selenium analogues was tested on 10 monolayer tumour cell lines: MDA-MB-435s (human melanoma), MCF-7 (human breast adenocarcinoma, estrogen-positive), MES-SA (human uterus sarcoma), HT-1080 (human connective tissue fibrosarcoma), A549 (human lung carcinoma), SH-SY5Y (human neuroblastoma), CCL-8 (mouse sarcoma), 3T3 (Mouse Swiss Albino embryo fibroblasts), MH-22A (mouse hepatoma), HepG2 (human hepatocellular carcinoma), B16-F10 (mouse melanoma). Using the NIH cell line, the borderline concentration which is relevant to the highest tolerated dose, was determined for each compound. The basal cytotoxicity was used to predict starting doses for *in vivo* acute oral LD_{50} values in rodents. [22, 23] The results of these experiments are summarized in Table 1. In the search for a non-toxic antimetastatic drug candidate only derivatives with slight cytotoxic effect can be chosen for the further studies. In general, all novel selenopheno[*f*]coumarins **21**-**39**, with few exceptions, *in vitro* showed medium to low ability to suppress cancer cells' growth. Derivatization of coumarin carboxylic group (**31**) with decyl chain led to a remarkable increase of antiproliferative activity on metastatic mouse melanoma B16-F10 cell line (IC_{50}=8 μM). In addition, human neuroblastoma SH-SY5Y and B16-F10 were sensitive to 3-bromo-2-(morpholinomethyl)-7-oxo-7*H*-selenopheno[2,3-*f*]chromene-8-carboxylic acid (**38**) (IC_{50}=3÷8 μM).

MMP, *in vitro* and *in vivo* angiogenesis inhibition

It is well known that the metastatic process is a multi-step phenomenon by which cells in the primary tumour invade surrounding tissues, penetrate blood and lymphatic circulation and propagate into the organ parenchyma, proliferating to form metastatic colonies at secondary sites. Cancer invasion takes place when the cancer cells respond and migrate towards gradients of stimuli, for example, growth factors require proteolysis of basement membrane (BM), and extracellular matrix (ECM) proteins. It creates a path for migration. Matrix metalloproteinases (MMPs) are a group of enzymes responsible for the proteolysis of BM and ECM proteins. The expression level of MMPs appears to correlate with the invasiveness of cancer cells. [24] MMPs, also designated matrixins, hydrolyse components of the extracellular matrix. These proteinases play important role in different biological processes, such as embryogenesis, normal tissue remodelling, wound healing, angiogenesis, as well as in diseases such as atheroma, arthritis, cancer, and tissue ulceration. [25] Despite their promising abilities [e.g. marimastat (BB-2516)], most rationally designed MMP inhibitors show a broad-spectrum MMP inhibition. Besides, cipemastat (Ro 32-3555), MMP-1 selective inhibitor, have performed poorly in clinical trials. Development of selective and non-toxic MMP inhibitors still lies in uncovered areas of anti-cancer medicine.

Novel selenopheno[*f*]coumarins **21**-**39** passed cytotoxicity test showing medium toxicity. Our next goal was to study their MMP inhibition ability. Inhibition of matrix metalloproteinase enzymes by **21**-**39** was detected on MMP Inhibitor Fluorimetric Profiling kit (Biomol, USA) using NNGH (*N*-isobutyl-*N*-4-methoxyphenylsulfonyl)glycyl-hydroxamic acid) as a standard (Table 2). It was found that selenopheno[*f*]coumarins **21**-**23, 36** and **37** inhibit up to 75% of MMP2 activity. It occurred that MMP8 is sensitive to methyl ester of piperidylmethyl derivative **22**, while hydrolysis of ester group led to a remarkable increase of MMP13 inhibition (61%). Derivatives **21**-**23**, **36** and **37** were chosen for angiogenesis inhibition studies *in vitro* and *in vivo* according to MMPs inhibition data. *In vitro* experiments were performed on Matrigel (BD Biosciences) human umbilical vein endothelial cell line (HUVECs) tube formation model (Table 2). Chosen compounds showed good activity in inhibiting angiogenesis process *in vitro* (49-100% in 20 μM concentration), except for morpholylmethyl derivative **23**. Then *in vivo* studies of angiogenesis were conducted in mice treated with **21**, **22**, **36** and **37**. The angiogenesis *in vivo* model was based on the use of Matrigel implants in Balb/c AnNCrl mice (20 g, 4 weeks of age) and VEGF-induced vessel formation inside the implants. Derivative **21** suppresses angiogenesis by 57%, however its carboxylic acid **36** showed loss in activity (-10%).

Table 1. *In vitro* cytotoxicity caused by selenopheno[*f*]coumarins was tested on monolayer tumour cell lines: MDA-MB-435s (human melanoma), MCF-7 (human breast adenocarcinoma, estrogen-positive), MES-SA (human uterus sarcoma), HT-1080 (human connective tissue fibrosarcoma), A549 (human lung

carcinoma), SH-SY5Y (human neuroblastoma), CCL-8 (mouse sarcoma), 3T3 (Mouse Swiss Albino embryo fibroblasts), MH-22A (mouse hepatoma), HepG2 (human hepatocellular carcinoma), B16-F10 (mouse melanoma).

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Nr.	Cytotoxicity IC_{50}, μM											Estimated LD_{50}	
	MDA-MB-431s	MCF-7	MES-SA	HT-1080	A549	SHSY5Y	CCL-8	3T3	MH-22A	HepG2	B16-F10	μM/kg	mg/kg
21	176±6	219±6	ne	108±6	153±6	81±2	72±2	153±6	86±2	164±2	108±20	3200	1421
22	110±5	52±2	46±2	91±3	83±2	50±2	43±6	79±2	72±4	136±6	20±1	1900	918
23	ne	ne	ne	ne	>206	>206	ne	ne	ne	ne	63±10	>4200	>2000
24	48±2	40±2	36±2	44±5	66±3	38±2	36±2	70±2	52±4	74±2	60±1	1700	847
25	98±2	ne	59±6	110±11	54±2	62±1	108±12	120±9	163±14	51±3	79±8	1800	839
27	105±16	97±16	58±10	64±4	132±8	45±4	51±16	84±8	107±16	111±4	43±10	1000	476
28	ne	137±27	ne	ne	ne	>190	190±8	>190	ne	ne	>195	5000	2467
29	76±11	133±20	65±2	46±2	70±4	59±4	79±13	142±13	68±6	100±6	48±7	1000	587
31	19±2	107±25	30±2	26±2	37±4	81±7	42±11	137±5	60±2	32±4	8±1	1000	671
32	>154	ne	ne	>154	154±6	154±8	46±12	39±5	154±5	>154	>154	3000	2061
33	126±5	ne	120±11	13±2	34±2	76±4	76±1	113±8	46±6	10±1	51±10	5800	2644
36	ne	228±3	70±4	193±2	232±12	232±5	86±2	139±5	70±3	>232	84±16	2500	1075
37	85±4	63±5	ne	109±9	198±3	12±1	117±2	198±6	89±2	ne	138±22	>4000	>2000
38	ne	ne	32±6	>197	167±5	8±1	ne	130±2	>197	118±2	3±0	2700	1371
39	49±5	38±2	35±6	42±2	63±2	36±1	35±2	67±3	50±2	71±5	>192	1600	833

ne- no effect

Table 2. Matrix metalloproteinases, *in vitro* and *in vivo* angiogenesis inhibition caused by **21 – 39**

Nr.	Inhibition of MMP subtypes, %										Angiogenesis inhibition, %	
	MMP 1	MMP 2	MMP 3	MMP 7	MMP 8	MMP 9	MMP 10	MMP 12	MMP 13	MMP 14	*in vitro,***	*in vivo,****
NNGH*	100	100	100	7.2	100	100	100	100	100	100		
21	23±3	43±3	14±3	14±2	17±1	19±6	15±2	20±1	13±3	25±3	92±11	57±19
22	21±2	56±3	16±2	14±3	30±4	22±2	24±3	19±2	14±2	27±3	49±11	-51±39
23	16±2	45±3	9±1	11±3	13±2	18±3	15±2	19±2	7±2	20±3	ne	
24	17±1	11±2	21±3	15±2	20±2	18±3	15±2	16±3	11±3	19±2		
25	13±1	11±5	12±1	0±7	0±2	3±2	9±4	5±2	3±1	12±7		
27	16±3	0±1	15±4	8±1	16±8	8±3	13±8	10±4	12±8	14±3		
28	22±5	2±4	11±4	9±4	18±1	8±5	17±4	8±2	14±2	11±5		
29	21±2	3±3	21±4	11±2	23±2	12±2	20±2	17±4	14±6	15±2		
31	22±1	7±2	16±2	15±5	24±6	12±3	20±1	16±1	15±3	22±3		
32	20±1	10±2	26±3	16±2	25±4	13±3	22±1	16±2	19±4	14±6		
33	12±5	3±2	12±2	6±7	4±1	9±2	13±3	10±2	10±1	12±4		
36	21±3	44±3	21±3	17±3	18±2	17±1	19±3	19±5	19±1	28±2	100±1	-10±7
37	8±1	75±4	22±2	14±3	18±2	15±4	21±1	17±2	61±5	33±2	73±11	-37±5
38	14±1	7±1	11±2	11±3	16±3	13±2	13±1	12±3	4±2	22±1		
39	9±1	17±1	14±1	8±1	15±4	9±2	7±2	13±4	4±1	15±5		

* *N*-isobutyl-N-(4-methoxyphenylsulfonyl)-glycylhydroxamic acid (1.3 μM);
** *In vitro* inhibition of HUVEC tube formation (compound dose is 20 μM);
*** *In vivo* inhibition of vessel formation into Matrigel implant (compound dose is 10 μM).
ne- no effect.

In vivo studies

There are various *in vivo* experimental models for studying growth and metastasis of different tumours after transplantation. The injection site and the tumour tropism in the selected cell line were mainly determined by the primary and secondary metastases and their growth. Pulmonary metastases model is widely used to assess the treatment of various *in vivo* tumour models. B16F10 Mice melanoma and 4T1 mammary carcinoma serve as models for highly metastatic melanoma and stage IV breast cancer. B16F10 (ATCC catalogue, CCL-6475) is a highly metastatic murine melanoma cell line from a C57BL/6J mouse. The cells are adherent to an epithelial morphology. 4T1 is a transplantable tumour cell line that is highly tumorigenic and invasive. Unlike most tumour models, it can spontaneously metastasize from the primary tumour in the mammary gland to multiple distant sites including lymph nodes, blood, liver, lungs, brain, and bones (American Type Culture Collection (ATCC) catalogue no CRL-2539, 2004).

Due to many of obvious reasons, those animal studies could not be conducted for all selenopheno[*f*]coumarins; derivatives **21**, **22**, **23**, **36**, **37** were picked as representative compounds, as they had already demonstrated MMP inhibiting activity (see above). One of the most aggressive forms of skin cancer – melanoma B16-F10 model was selected for intravenous administration in the tail vein. [26] C57BL/6JOlaHsd 6-weeks old female mice in cohorts of 6-8 animals were injected with 100,000 B16-F10 melanoma cells via the tail vein and then treated with **21**, **22**, **23**, **36**, **37** in 5 and 20 mg/kg doses *s.c*. according to the following scheme: Day 1, 7, 8, 9, 10, 11, 14, 15, 16th. Total dose reached 120 mg/kg. On day 18 experiments were terminated (Table 3).

Indeed, after the treatment with **21** (dose 5 mg/kg) in cohorts of 7 mice, 4 animals had no signs of metastasis nodules in lungs with overall metastasis inhibition by 85%. Therapeutic dose increase to 20 mg/kg led to impressive 96% of metastasis prevention (p=0.002). *In vitro* hydrolysis of ester group in derivative **21**, which was detected during incubation of **21** in HepG2 cells lysate at 37 ^{o}C for 4h, resulted in slight decrease of the antimetastatic activity till 85% (compound **36**) Again, no signs of metastatic nodules were observed in two mice lungs out of six in cohort. Experiments with **22** and **23** in 5 mg/kg dose resulted with modest effect of inhibition (44% and 17%, correspondingly). However, hydrolysis of the ester group in derivative **22** to carboxylic acid (**37**) considerably improved prevention of metastasis development. Compound **37** suppresses metastasis by 60% in 5 mg/kg dose. Notably, 4 mice out of 8 had no metastasis nodules at all. Increase of dose to 20 mg/kg led to almost complete prevention of metastasis formation (97%, p=0.002).

Next, compound **21** was studied in BALB/c female mice cohorts, consisting of five animals each, with innoculated 4T1 tumours (Figure 1B). Compound **21** in 20 mg/kg dose (administration: *s.c.* according to the following scheme: day 1, 4, 7, 9, 11 and 14th; total dose 120 mg/kg dose) exhibited ability to suppress 4T1 metastasis up to 97 % after 18 days. Described effect was observed for each mouse that participated in the experiment. Those results were particularly encouraging, bearing in mind that this compound did not induce any major side effects either: all animals treated with this compound stayed healthy and active, weight loss was virtually non-existent. Transplantation of breast cancer tumour cells to mice developed splenomegaly, which is associated with the induction of tumour leukemoid reaction and massive granulocytic infiltrates of the red pulp. [27–29] The results of spleen weight changes in the animals with tumour are shown in Figure 2C. The tumours in the control animals develop a 167% tumour spleen weight increase. Notably, spleen volume is significantly smaller and only 73% weight increase is observed in treatment with **21**.

Additionally, we tested two most active compounds **21** and **37** for their ability to induce haemolysis of rat erythrocytes (Figure 1D). Widely used detergent Triton x100 was used as positive control. According to received results, both compounds are not harmful to red blood cells in concentrations less than 50 μM. Moreover, only 10% of cells were haemolysed at extra high concentration (>300 μM) in the case of selenophenocoumarin **21**. To determine the possible mechanism of action of **21** and **37**, which causes the death of tumour cells, we tested the effect of **21** and **37** on cell apoptosis. Determination of apoptotic and necrotic cells was performed using AO/EB (Figure 1, E-H). An increase in number of apoptotic cells was observed in case of **21** at a dose of 100 μM after only 24h. The ratio of apoptotic cells reached 21%, while the number of necrotic cells did not increase. In the case of compound **37**, the appearance of apoptotic cells plateaued at 27% after 6h.

Conclusions

To sum up, methods for the preparation of selenopheno[2,3-*f*]coumarins that are variously modified in position 3 of coumarin ring and selenophene moiety were elaborated and series of 14 new derivatives was obtained. Based on structure-activity relationship data it can be concluded that these compounds exhibit medium to low *in vitro* cytotoxicity. Notably, few compounds suppress angiogenesis *in vivo* up to 57% (10μM). Exploring the intracellular processes in tumour cells allows us to conclude that their low toxicities and potent antiproliferating activities depend on ability to induce apoptosis. The most impressive data received from *in vivo* experiments confirming scientific evidence that selenophenocoumarin scaffold is promising trend for the development of drug candidates with metastasis-suppressing properties. It was found that certain derivatives are excellent for *in vivo* prevention of melanoma and breast cancer metastasis development up to 97% with no visual side effects. Our next task will relate to determination of mechanism of action.

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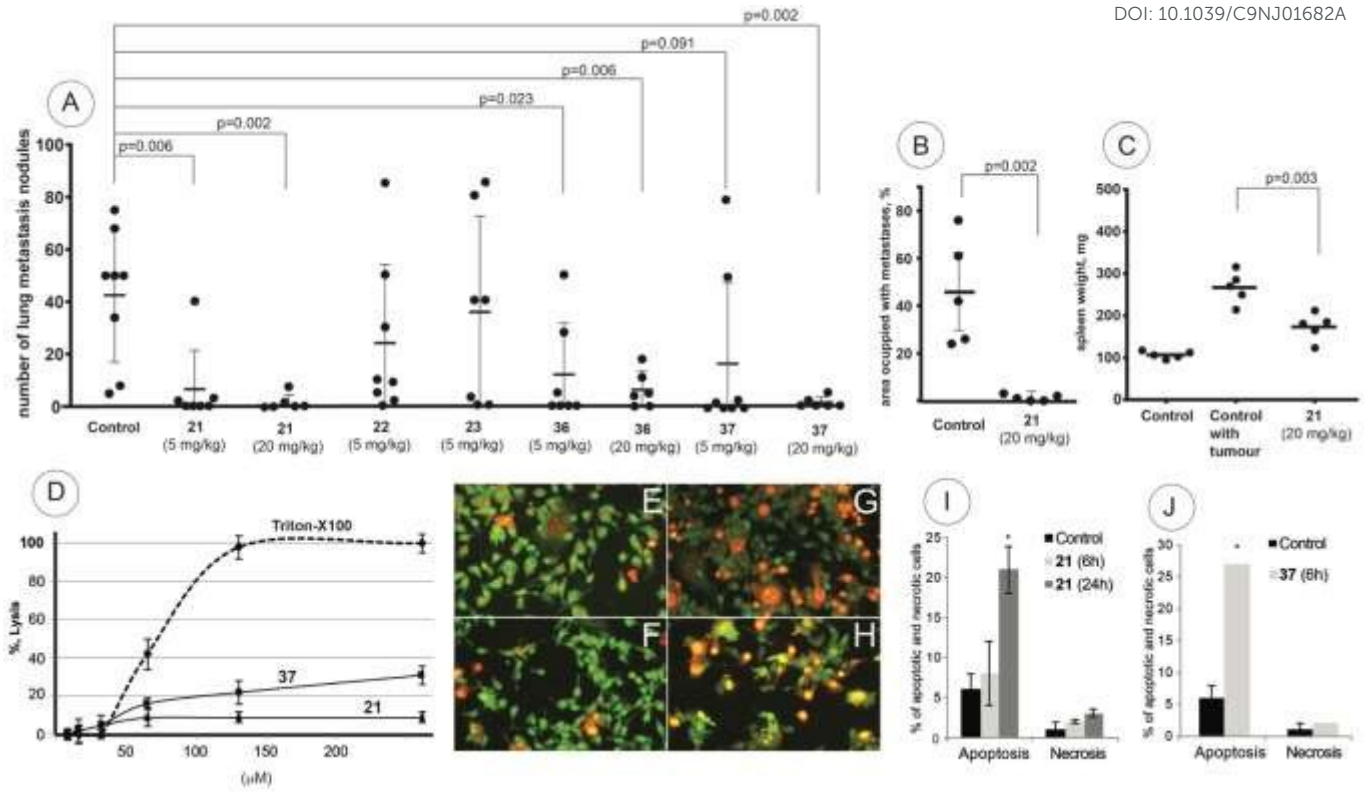

Figure 1. A: Melanoma B16F10 antimetastatic activity caused by **21** – **23**, **36** and **37** (5 and 20 mg/kg doses *s.c.* according to the following scheme: Day 1, 7, 8, 9, 10, 11 and 14th; total dose 120 mg/kg, the number of macroscopic lung metastases was determined 18 days later); B and C: inhibition of 4T1 metastasis in lung as well as spleen weight in BALB/c mice (number of lung metastasis nodules at day 18 is shown for each mouse); D: haemolysis of rat erythrocytes caused by **21** and **37**; E–F: the effects of **21** and **37** on morphology of B16-F1. Cells were stained by acridine orange/ethidium bromide and observed under fluorescence microscope: (E) Control; (F) in the presence of **21** after 6h incubation; (G) in the presence of **21** after 24h incubation; (H) in the presence of **37** after 6h incubation (×200); I and J: Number of apoptotic and necrotic cells after incubation with **21** and **37**.

Table 3. The number of individual metastatic nodules in whole lung assessed at endpoint. N=6-8 mice per group. Points represent individual animals and bars represent the mean number of metastatic nodules (B16F10) and percent of total lung area occupied with metastases (4T1).

	N1	N2	N3	N4	N5	N6	N7	N8	Average ± SD	Inhibition %	TTEST
B16F10											
Control	5	50	34	8	50	68	50	75	43±26		
21, 5 mg/kg	3	0	0	2	40	0	0		6.4±15	85	0.006
21, 20 mg/kg	0	0	0	8	2	0			1.7±3.2	96	0.002
22, 5 mg/kg	10	50	30	85	2	0	5	9	24±30	44	0.202
23, 5 mg/kg	85	40	3	0	80	40	0		35±37	17	0.668
36, 5 mg/kg	0	0	0	5	28	50	0		12±20	72	0.023
36, 20 mg/kg	11	5	18	0	4	0			6.3±7.0	85	0.006
37, 5 mg/kg	3	80	2	0	0	0	50	0	17±30	60	0.091
37, 20 mg/kg	0	2	5	0	0	0			1.2±2.0	97	0.002
4T1											
Control	42	24	76	26	61				46±22		
21, 20 mg/kg	1	3	0	2	0				1±1	97	0.002

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Experimental

General remarks

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Thin layer chromatography (TLC) was performed using MERCK Silica gel 60 F254 plates and visualized by UV (254 nm) fluorescence. ZEOCHEM silica gel (ZEOprep 60/35-70 microns – SI23501) was used for column chromatography. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 Mercury spectrometer at 400.0 and 100.58 MHz correspondingly at 298 K in $CDCl_3$ or DMSO-d_6. The melting points were determined on a "Digital melting point analyser" (Fisher), and the results are given without correction.

Synthesis of initial compounds

General procedure for the preparation of methyl 6-hydroxy- or 6-bromo-coumarin-3-carboxylate **(4a-b)** [20]: to 2,5-dihydroxybenzaldehyde (4 g, 29 mmol) or 5-bromo-2-hydroxybenzaldehyde (4 g, 19.9 mmol) and dimethyl malonate (5.78 g, 43 mmol) solution in dry methanol (20 mL) 3 drops of piperidine was added. The reaction mixture was heated at 60 ^{o}C for 24h. The mixture was then cooled to -15 ^{o}C and precipitates were filtered off, washed with cold methanol (50 mL) and dried to give pure methyl 6-hydroxycoumarin-3-carboxylate **4a** in 84% yield or 6-bromocoumarin-3-carboxylate **4b** in 81% yield. ^{1}H NMR (**4a**) (400 MHz, DMSO-d_6) δ (ppm): 3.82 (3H, s, CH_3), 7.15-7.29 (3H, m, Ar), 8.69 (1H, s, 5-CH), 9.89 (1H, s, OH).

General procedure for the preparation of isopropyl ***5*** *or tert-butyl 6-iodo-coumarin-3-carboxylates* **(6)**: to a solution of 2-hydroxy-5-iodo-benzaldehyde (2 g, 8 mmol) and di-isopropyl malonate (3.01 g, 16 mmol) or di-tert-butyl malonate (3.45 g, 16 mmol) in dry methanol (12 mL) 3 drops of piperidine was added. The reaction mixture was stirred at r.t for 24h. The mixture was then cooled to -15 ^{o}C and precipitates were filtered off, washed with cold methanol (50 mL) and dried to give pure isopropyl 6-iodo-coumarin-3-carboxylate (**5**) or tert-butyl 6-iodo-coumarin-3-carboxylate (**6**) in 82-86% yield.

Isopropyl 6-iodo-coumarin-3-carboxylate (**5**). Yield: 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.38 (6H, d, *J* = 6.2 Hz, CH_3), 5.23-5.30 (1H, m, CH), 7.28 (1H, d, *J* = 8.6 Hz, 8-CH), 7.63-7.66 (2H, m, 5,7-CH), 8.37 (1H, s, 4-CH).

Tert-butyl 6-iodo-coumarin-3-carboxylate (**6**). Yield: 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.53 (9H, s, CH_3), 7.25 (1H, d, *J* = 8.8 Hz, 8-CH), 7.98 (1H, dd, *J* = 8.8, 2.0 Hz, 7-CH), 8.29 (1H, d, *J* = 2.0 Hz, 5-CH), 8.59 (1H, s, 4-CH).

General procedure for the re-esterification of methyl 6-hydroxycoumarin-3-carboxylate (**4**) to the corresponding octyl and decyl esters **7-8**: Methyl 6-hydroxycoumarin-3-carboxylate **4a** (5 g, 22.72 mmol) was suspended in octanol or decanol (20 mL), then chlorotrimethylsilane (4 mL) was added and vial was closed. The reaction mixture was heated at 120 ^{o}C for 4-5 days. Then the mixture was cooled and petroleum ether (100 mL) was added. Precipitates were filtered off, washed with petroleum ether and dried to give pure esters **7** and **8**.

Octyl 6-hydroxycoumarin-3-carboxylate **(7)** Yield: 92%. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.86 (3H, t, *J* = 6.8 Hz, CH_3), 1.20-1.45 (10H, m, CH_2), 1.76 (2H, qui, *J* = 14.0, 6.8 Hz, CH_2), 4.33 (2H, t, *J* = 6.8 Hz, OCH_2), 7.12 (1H, d, *J* = 2.4 Hz, 8-CH), 7.21-7.27 (2H, m, 5,7-CH), 8.46 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.0, 22.6, 25.9, 28.5, 29.1, 29.2, 31.7, 66.3, 113.7, 117.7, 118.0, 118.3, 123.3, 148.7, 149.2, 153.2, 157.7, 163.3.

Decyl 6-hydroxycoumarin-3-carboxylate **(8)** Yield: 81%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.82 (3H, t, *J* = 6.6 Hz, CH_3), 1.21-1.41 (14H, m, CH_2), 1.66 (2H, qui, *J* = 14.0, 6.6 Hz, CH_2), 4.22 (2H, t, *J* = 6.6 Hz, OCH_2), 7.13-7.29 (3H, m, 5,7,8-CH), 8.63 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ (ppm): 13.8, 22.0, 25.2, 27.9, 28.5, 28.6, 28.81, 28.83, 31.2, 65.0, 113.7, 117.7, 118.2, 118.3, 122.5, 147.8, 148.3, 153.9, 156.2, 162.8.

General procedure for the preparation of alkyl 6-(((trifluoromethyl)sulfonyl)oxy)coumarin-3-carboxylates **9-11**: solution of alkyl 6-hydroxycoumarin-3-carboxylate (**4**, **7-8**) (45 mmol) in dry dichloromethane (100 mL) and Et_3N (10 mL) was cooled to 0 ^{o}C, and then trifluoromethanesulfonic anhydride (15.11 mL, 90 mmol) was added dropwise. After 24h of stirring at rt mixture was cooled to 0 ^{o}C and additional portion of trifluoromethanesulfonic anhydride (7.5 mL, 45 mmol) was added dropwise and reaction was continued for 24h. Then mixture was cooled to 0 ^{o}C and 1N HCl (100 mL) and dichloromethane (100 mL) were added. Organic phase was separated and washed with brine (100 mL), dried over sodium sulphate. After removal of the solvent under reduced pressure, the crude product **9-11** was purified by flash chromatography on silica gel using the mixture of petroleum ether/dichloromethane as an eluent.

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Methyl 6-(((trifluoromethyl)sulfonyl)oxy)coumarin-3-carboxylate **(9)**. Yield: 56%. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 3.91 (3H, s, CH_3), 7.42 (1H, d, *J* = 9.0 Hz, 8-CH), 7.52 (1H, dd, *J* = 2.8 Hz, 9.0 Hz, 7-CH), 7.58 (1H, d, *J* = 2.8 Hz, 5-CH), 8.53 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 52.9, 118.6, 118.8, 119.7, 120.1, 121.6, 126.9, 145.1, 147.2, 153.9, 155.4, 162.7.

Octyl 6-(((trifluoromethyl)sulfonyl)oxy)coumarin-3-carboxylate **(10)** Yield: 68% Foam. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.86 (3H, t, *J* = 6.8 Hz, CH_3), 1.22-1.45 (10H, m, CH_2), 1.76 (2H, qui, *J* = 14.0, 6.8 Hz, CH_2), 4.34 (2H, t, *J* = 6.8 Hz, OCH_2), 7.42-7.45 (1H, m, 8-CH), 7.51-7.57 (2H, m, 5,7-CH), 8.46 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.0, 22.6, 25.8, 28.5, 29.0, 29.1, 31.7, 66.4, 117.0, 118.7, 118.8, 120.3, 121.6, 126.8, 145.1, 146.5, 153.9, 155.4, 162.3.

Decyl 6-(((trifluoromethyl)sulfonyl)oxy)coumarin-3-carboxylate **(11)** Yield: 71% ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.87 (3H, t, *J* = 6.8 Hz, CH_3), 1.23-1.46 (14H, m, CH_2), 1.77 (2H, qui, *J* = 13.9, 6.8 Hz, CH_2), 4.35 (2H, t, *J* = 6.6 Hz, OCH_2), 7.43-7.46 (1H, m, 8-CH), 7.51-7.56 (2H, m, 5,7-CH), 8.46 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.1, 22.6, 25.8, 28.5, 29.2, 29.3, 29.4, 29.5, 31.9, 66.5, 118.7, 118.9, 120.4, 121.5, 126.8, 145.2, 146.5, 153.9, 155.4, 162.3.

General procedure for the preparation of 2-hydroxy-5-(alkynyl)-benzaldehydes (**12a-b**): A solution of 5-bromo-2-hydroxybenzaldehyde **4b** (1 g, 5 mmol) and terminal alkyne (7.5 mmol) in dry THF (8 mL) was added to a solution of bis(triphenylphosphine)palladium(II) chloride (0.245 g, 0.35 mmol) and copper(I) iodide (0.67 g, 0.35 mmol) in mixture of THF/Et_3N (7 mL/15 mL). The reaction mixture obtained was heated to 60 °C overnight. The mixture was then poured into ethyl acetate (150 mL); the organic layer was washed with water and dried over sodium sulphate. After removal of the solvent under reduced pressure, the crude product was purified by flash chromatography on silica gel.

2-Hydroxy-(hydroxy-3-methylbutyn-1-yl)-benzaldehyde **(12a)** Yield: 89%. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.62 (6H, s, CH_3), 1.97 (1H, s, OH), 6.94 (1H, d, *J* = 8.6 Hz, 3-CH), 7.56 (1H, dd, *J* = 8.6, 2.1 Hz, 4-CH), 7.65 (1H, d, *J* = 2.1 Hz, 6-CH), 9.86 (1H, s, CHO), 11.08 (1H, s, OH).

2-Hydroxy-(3-piperid-1-yl-prop-1-ynyl)-benzaldehyde **(12b)** Yield: 41%. ^{1}H NMR (400 Hz, $CDCl_3$/TMS) δ (ppm): 1.44-1.48 (2H, m, CH_2), 1.61-1.67 (4H, m, CH_2), 2.52-2.60 (4H, m, CH_2), 3.45 (2H, s, CH_2), 6.92 (1H, d, *J* = 8.6 Hz, 3-CH), 7.55 (1H, dd, *J* = 8.6, 2.1 Hz, 4-CH), 7.64 (1H, d, *J* = 2.1 Hz, 6-CH), 9.84 (1H, s, CHO). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 23.9, 25.9, 48.4, 53.5, 83.1, 84.6, 115.2, 117.9, 120.3, 136.9, 139.9, 161.2, 196.0.

*General procedure for the preparation of methyl 6-iodocoumarin-3-carboxylate (**13**):* Argon gas was bubbled for 15 min through a dry 1,4-dioxane solution (15 mL) of copper(I) iodide (4.04 g, 21.20 mmol), grounded potassium iodide (14.67 g, 88.35 mmol). Then subsequently DMEDA (1.9 mL, 17.67 mmol) and after 5 min a solution of methyl 6-bromocoumarin-3-carboxylate (**4b**) (5.00 g, 17.67 mmol) in 1,4-dioxane (15 mL) were added. The reaction mixture was heated at 120 °C for 48 h. The mixture was then cooled to rt, poured into ethyl acetate (200 ml); the organic layer was washed with saturated ammonium chloride solution (2x100 mL), brine (100 mL), water (100 mL) and dried over sodium sulphate. After removal of the volatiles under reduced pressure, the crude product was purified by washing with diethyl ether. Yield: 3.4 g (58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.83 (3H, s, CH_3), 7.24 (1H, d, *J* = 8.6 Hz, 8-CH), 7.87 (1H, dd, *J* = 8.6, 2.0 Hz, 7-CH), 8.28 (1H, d, *J* = 2.0 Hz, 5-CH), 8.69 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ (ppm): 52.5, 88.3, 116.2, 118.3, 119.5, 119.9, 136.6, 137.9, 147.5, 155.4, 162.8.

*General procedures for the preparation of methyl (**14a-d**), isopropyl (**15a-b**), tert-butyl (**16a-b**) octyl (**17a-b**), and decyl (**18a-b**) 6-(alkynyl)-coumarin-3-carboxylates.*

f: A solution of 6-iodocoumarin **5-6**, or **13** (2.0 mmol) and terminal alkyne (3.0 mmol) in dry DMF (10 mL) was added to a solution of bis(triphenylphosphine)palladium(II) chloride (0.07 g, 0.1 mmol) and copper(I) iodide (0.019 g, 0.1 mmol) in dry DMF (2 mL) and Et_3N (2 mL). Then reaction mixture was heated at 40 °C overnight. After consumption of initial **5-6**, or **13** the mixture was poured into ethyl acetate (100 mL). The organic layer was washed with water and dried over sodium sulphate. After removal of the solvent under reduced pressure the crude product was purified by flash chromatography on silica gel using mixture of DCM/MeOH as eluent for **14b-d**, **15b**, **16b**; and DCM/EtOAc for **16a**.

g: Argon gas was bubbled through a mixture of copper(I) iodide (32 mg, 0.17 mmol), palladium(II) chloride (20 mg, 0.11 mmol) and triphenylphosphine (58 mg, 0.22 mmol) in DMF/Et_3N (8mL/4mL) for 10 min at 40 °C. Then solution of corresponding 6-(((trifluoromethyl)sulfonyl)oxy)coumarin-3-carboxylate (**9-11**) (1.11 mmol) followed by terminal alkyne in dry DMF (12 mL) were added. The reaction mixture was heated at 60 °C for 18 h. The mixture was then cooled to rt, poured into ethyl acetate (200 ml); the organic layer was washed with saturated ammonium chloride solution (1x100 mL), brine (3x100 mL), water (100 mL) and dried over sodium sulphate. After removal of the volatiles under reduced pressure, the crude product was purified by flash chromatography on silica gel using mixture of DCM/MeOH for **14b-d**, **15b**, **16b**, DCM/EtOAc for **16a**, petroleum ether/EtOAc for **17a, 18a**, petroleum ether/DCM/EtOAc **17b, 18b** – petrol ether/DCM/EtOAc (10/1/2) for **17b** and **18b**.

h: To a solution of 2-hydroxy-(alkynyl)-benzaldehyde **12a,b** (2.94 mmol) and diisopropyl malonate (0.83 g, 4.41 mmol) or di(*tert*)butylmalonate in dry methanol (10 mL) 2 drops of piperidine was added. The reaction mixture was stirred at rt

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for 24h, the mixture was then cooled to -15 °C and precipitates were filtered off, washed with cold methanol (25 mL) and dried to give pure products **15a,b** and **16a,b** in 48-56% yields.

Methyl ester of 6-(3-hydroxy-3-methylbutyn-1-yl)coumarin-3-carboxylic acid (**14a**) [Ref. 21]. Yield: 38% (**f**), 57% (**g**).

Methyl ester of 6-(3-piperid-1-yl-prop-1-ynyl)coumarin-3-carboxylic acid **(14b)** Yield: 74% (**f**), 63% (**g**), m.p. = 150 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.44-1.47 (2H, m, $CH_2CH_2CH_2$), 1.61-1.67 (4H, m, NCH_2CH_2), 2.52-2.57 (4H, m, NCH_2), 3.46 (2H, s, CH_2), 3.94 (3H, s, CH_3), 7.27 (1H, d, *J* = 9.2 Hz, 8-CH), 7.64-7.67 (2H, m, 5-CH, 7-CH), 8.47 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 23.8, 25.8, 48.4, 52.9, 53.6, 82.6, 86.8, 116.9, 117.7, 118.5, 120.4, 132.3, 137.4, 148.3, 154.4, 156.2, 163.4.

Methyl ester of 6-(3-morphol-4-yl-prop-1-ynyl)coumarin-3-carboxylic acid **(14c)** Yield: 86% (**f**), 54% (**g**), m.p. = 185 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 2.64 (4H, t, *J* = 4.8 Hz, CH_2NCH_2), 3.51 (2H, s, CH_2), 3.77 (4H, t, *J* = 4.8 Hz, CH_2OCH_2), 3.95 (3H, s, CH_3), 7.29 (1H, d, *J* = 9.2 Hz, 8-CH), 7.65-7.68 (2H, m, 5,7-CH), 8.48 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 47.9, 52.5, 53.0, 66.8, 83.2, 85.7, 116.9, 117.7, 118.7, 120.1, 132.3, 137.4, 148.2, 154.5, 156.1, 163.4.

Methyl ester of 6-(3-(4-methylpiperaz-1-yl)-prop-1-ynyl)coumarin-3-carboxylic acid **(14d)** Yield: 39% (**f**), 35% (**g**), m.p. = 140 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 2.29 (3H, s, NCH_3), 2.45-2.55 (4H, m, CH_2NCH_3), 2.61-2.69 (4H, m, NCH_2CH_2), 3.51 (2H, s, CH_2), 3.92 (3H, s, CH_3), 7.25 (1H, d, *J* = 9.2 Hz, 8-CH), 7.61-7.64 (2H, m, 5-CH, 7-CH), 8.44 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 45.8, 47.5, 51.9, 52.9, 54.8, 83.0, 85.9, 116.9, 117.7, 118.5, 120.1, 132.4, 137.3, 148.2, 154.4, 156.1, 163.4.

Isopropyl ester of 6-(3-hydroxy-3-methylbutyn-1-yl)coumarin-3-carboxylic acid **(15a)** Yield: 50% (**f**), 56% (**h**). ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.39 (6H, d, *J* = 6.2 Hz, CH_3), 1.63 (6H, s, CH_3), 2.02 (1H, s, OH), 5.22-5.31 (1H, m, CH), 7.28 (1H, d, *J* = 8.6 Hz, 8-CH), 7.63 (1H, dd, *J* = 8.6, 1.9 Hz, 7-CH), 7.65 (1H, d, *J* = 1.9 Hz, 5-CH), 8.38 (1H, s, 4-CH).

Isopropyl ester of 6-(3-piperid-1-yl-prop-1-ynyl)coumarin-3-carboxylic acid **(15b)** Yield: 44% (**f**), 48% (**h**). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.31 (6H, d, *J* = 6.2 Hz, CH_3), 1.35-1.46 (1H, m, piperidine), 1.70-1.86 (5H, m, piperidine), 2.95-3.06 (2H, m, piperidine), 3.48-3.55 (2H, m, piperidine), 4.32 (2H, s, CH_2), 5.08-5.15 (1H, m, CH), 7.49 (1H, d, *J* = 8.7 Hz, 8-CH), 7.86 (1H, dd, *J* = 8.7, 2.0 Hz, 7-CH), 8.14 (1H, d, *J* = 2.0 Hz, 5-CH), 8.69 (1H, s, 4-CH).

Tert-butyl ester of 6-(3-hydroxy-3-methylbutyn-1-yl)coumarin-3-carboxylic acid **(16a)** Yield: 42% (**f**), 49% (**h**). ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.59 (9H, s, CH_3), 1.62 (6H, s, CH_3), 7.23-7.26 (1H, m, 8-CH), 7.59-7.62 (m, 2H, 5,7-CH), 8.29 (s, 1H, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 28.0, 31.3, 65.5, 79.9, 83.1, 94.9, 116.8, 117.8, 119.6, 120.4, 132.1, 136.7, 146.6, 154.3, 156.3, 161.7.

Tert-butyl ester of 6-(3-piperid-1-yl-prop-1-ynyl)coumarin-3-carboxylic acid **(16b)** Yield: 48% (**f**), 55% (**h**). ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.42-1.49 (2H, m, piperidine), 1.59 (9H, s, $3CH_3$), 1.61-1.67 (4H, m, piperidine), 2.53-2.58 (4H, m, piperidine), 3.47 (2H, s, CH_2), 7.24-7.27 (1H, m, 8-CH), 7.62-7.64 (2H, m, 5,7-CH), 8.31 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 23.8, 25.9, 28.0, 48.4, 53.6, 82.7, 83.0, 86.5, 116.8, 117.8, 120.1, 120.3, 132.1, 136.9, 146.6, 154.2, 156.3, 161.7.

Octyl ester of 6-(3-hydroxy-3-methylbutyn-1-yl)coumarin-3-carboxylic acid **(17a)** Yield: 47% (**g**). m.p. = 145-146 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.88 (3H, t, *J* = 6.8 Hz, CH_3), 1.25-1.46 (10H, m, $5CH_2$), 1.63 (6H, s, $2CH_3$), 1.77 (2H, qui, *J* = 13.8 Hz, CH_2), 2.04 (1H, br s, OH), 4.34 (2H, t, *J* = 6.8 Hz, CH_2), 7.28 (1H, d, *J* = 8.4 Hz, 8-CH), 7.63-7.65 (2H, m, 5,7-CH), 8.42 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.1, 22.6, 25.9, 28.5, 29.1, 29.2, 31.4, 31.8, 65.6, 66.3, 79.9, 95.0, 116.9, 117.8, 119.1, 119.8, 132.3, 137.1, 147.6, 154.5, 156.1, 162.9.

Octyl ester of 6-(3-piperid-1-yl-prop-1-ynyl)coumarin-3-carboxylic acid **(17b)** Yield: 32% (**g**). Foam. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.87 (3H, t, *J* = 6.8 Hz, CH_3), 1.24-1.49 (12H, m, $5CH_2$, 2H-piperidine), 1.62-1.67 (4H, m, piperidine), 1.76 (2H, qui, *J* = 13.8 Hz, CH_2), 2.54-2.58 (4H, m, piperidine), 3.47 (2H, s, CH_2), 4.33 (2H, t, *J* = 6.8 Hz, CH_2), 7.26-7.29 (1H, m, 8-CH), 7.64-7.67 (2H, m, 5,7-CH), 8.42 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, CDCl3/TMS) δ (ppm): 14.1, 22.6, 23.8, 25.9, 28.5, 29.1, 29.2, 31.7, 48.4, 53.6, 66.2, 82.7, 86.6, 116.9, 117.7, 119.0, 120.3, 132.3, 137.2, 147.7, 154.4, 156.2, 162.9.

Decyl ester of 6-(3-hydroxy-3-methylbutyn-1-yl)coumarin-3-carboxylic acid **(18a)** Yield: 45% (**g**). m.p. = 87-88 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.87 (3H, t, *J* = 6.8 Hz, CH_3), 1.25-1.44 (14H, m, $7CH_2$), 1.63 (6H, s, $2CH_3$), 1.76 (2H, qui, *J* = 13.7 Hz, CH_2), 2.08 (1H, br s, OH), 4.34 (2H, t, *J* = 6.8 Hz, CH_2), 7.28 (1H, d, *J* = 8.4 Hz, 8-CH), 7.62-7.66 (2H, m, 5,7-CH), 8.42 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.1, 22.6, 25.8, 28.5, 29.2, 29.3, 29.4, 29.5, 31.4, 31.8, 65.5, 66.3, 79.9, 95.0, 116.9, 117.8, 119.1, 119.7, 132.3, 137.1, 147.6, 154.5, 156.1, 162.9.

Decyl ester of 6-(3-piperid-1-yl-prop-1-ynyl)coumarin-3-carboxylic acid **(18b)** Yield: 40% (**g**). m.p. = 83-84 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.87 (3H, t, *J* = 6.8 Hz, CH_3), 1.26-1.48 (16H, m, $7CH_2$, 2H-piperidine), 1.62-1.68 (4H, m, piperidine), 1.76 (2H, qui, *J* = 13.7 Hz, CH_2), 2.53-2.59 (4H, m, piperidine), 3.47 (2H, s, CH_2), 4.33 (2H, t, *J* = 6.8 Hz, CH_2), 7.27-7.29 (1H, m, 8-CH), 7.64-7.67 (2H, m, 5,7-CH), 8.42 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.1, 22.6, 23.9, 25.8, 25.9, 28.5, 29.2, 29.3, 29.4, 29.5, 31.8, 48.4, 53.6, 66.2, 82.7, 86.7, 116.9, 117.8, 119.0, 120.3, 132.3, 137.2, 147.6, 154.4, 156.2, 162.9.

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i: *General procedure for the preparation of alkynyl ketones **19a,b** and **20a,b**.* To 2-hydroxy-(alkynyl)-benzaldehyde **12a,b** (2.94 mmol) and isobutyrylacetic acid methyl ester (0.59 g, 4.11 mmol) or methyl 3-oxoheptanoate (0.65 g, 4.11 mmol) solution in dry methanol (10 mL) 2 drops of piperidine was added. The reaction mixture was stirred at rt for 24h. The mixture was then cooled to -15 ^{o}C and precipitates were filtered off, washed with cold methanol (25 mL) and dried to give pure products **19a,b** and **20a,b** in 46-61% yield.

6-(3-Hydroxy-3-methylbut-1-ynyl)-3-isobutyryl-2H-chromen-2-one **(19a)** Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.17 (6H, d, *J* = 6.8 Hz, CH_3), 1.62 (6H, s, CH_3), 1.97 (1H, s, OH), 3.75-3.87 (1H, m, CH), 7.29 (1H, d, *J* = 8.6 Hz, 8-CH), 7.63 (1H, dd, *J* =8.6, 1.9 Hz, 7-CH), 7.66 (1H, d, *J* = 1.9 Hz, 5-CH), 8.34 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 18.2, 31.3, 38.6, 65.5, 79.8, 95.1, 116.7, 118.2, 119.9, 125.3, 132.7, 136.9, 146.7, 154.4, 158.3, 202.1.

3-Isobutyryl-6-(3-(piperidin-1-yl)prop-1-ynyl)-2H-chromen-2one **(19b)** Yield: 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.09 (6H, d, *J* = 6.8 Hz, CH_3), 1.32-1.41 (2H, m, CH_2), 1.50-1.56 (2H, m, CH_2), 2.46-2.52 (4H, m, CH_2), 3.48 (2H, s, CH_2), 3.57-3.68 (1H, m, CH), 7.45 (1H, d, *J* = 8.6 Hz, 8-CH), 7.74 (1H, dd, *J* = 8.6, 1.9 Hz, 7-CH), 8.03 (1H, d, *J* = 1.9 Hz, 5-CH), 8.58 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ (ppm): 17.9, 23.5, 25.4, 37.7, 47.4, 52.6, 82.5, 86.9, 116.7, 118.4, 119.0, 125.2, 132.9, 136.5, 146.8, 153.8, 157.7, 201.8.

6-(3-Hydroxy-3-methylbut-1-ynyl)-3-pentanoyl-2H-chromen-2-one **(20a)** Yield: 61%. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.94 (3H, t, *J* = 7.4 Hz, CH_3), 1.35-1.44 (2H, m, CH_2), 1.63 (6H, s, CH_3), 1.63-1.71 (2H, m, CH_2), 2.01 (1H, s, OH), 3.12 (2H, t, *J* = 7.4 Hz, CH_2), 7.30 (1H, d, *J* = 8.6 Hz, 8-CH), 7.64 (1H, dd, *J* = 8.6, 1.9 Hz, 7-CH), 7.69 (1H, d, *J* = 1.9 Hz, 5-CH), 8.39 (1H, s, 4-CH).

3-Pentanoyl-6-(3-(piperidin-1-yl)prop-1-ynyl)-2H-chromen-2-one **(20b)** Yield: 34%. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.93 (3H, t, *J* = 7.2 Hz, CH_2), 1.33-1.43 (2H, m, CH_2-piperidine), 1.42-1.50 (2H, m, CH_2), 1.61-1.69 (6H, m, CH_2, CH_2-piperidine), 2.53-2.58 (4H, m, CH_2- piperidine), 3.11 (2H, t, *J* = 7.2 Hz, CH_2), 3.47 (2H, s, CH_2), 7.28 (1H, d, *J* = 8.6 Hz, 8-CH), 7.65 (1H, dd, *J* = 8.6, 1.9 Hz, 7-CH), 7.69 (1H, d, *J* = 1.9 Hz, 5-CH), 8.39 (1H, s, 4-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 13.9, 22.3, 23.8, 25.8, 25.9, 42.2, 48.3, 53.6, 82.7, 86.6, 116.7, 118.2, 120.4, 125.3, 132.9, 137.1, 146.5, 154.4, 158.6, 197.9.

Synthesis of selenopheno[2,3-f]coumarins **(21-35)**

*General procedure for the preparation of selenopheno[2,3-f]coumarins (**21-35**):* 6-Alkynylcoumarin **14-20** (1.0 mmol) in dioxane was added to a solution of *in situ* prepared $SeBr_4$ from selenium dioxide (0.22 g, 2.0 mmol) in HBr (2 mL) at 0 ^{o}C. The resulting mixture was stirred at rt for 24–48h. After the consumption of substrate (LC-MS monitoring), the reaction mixture was basified with aqueous Na_2CO_3 and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, concentrated, and the residue was purified by flash chromatography on silica gel.

Methyl 3-bromo-2-(2-hydroxypropan-2-yl)-7-oxo-7H-selenopheno[2,3-f]chromene-8-carboxylate **(21)** **[**Ref. 21**]** HRMS (ESI) calcd for $C_{16}H_{13}BrNO_4Se$ [M+H] 444.9184, found 444.9194. HPLC: 96.2% (RT=8.34 min, Apollo C18-8 (4.6x150mm), mobile phase 5→95% Acetonitrile+0.1%H_3PO_4, 1 mL/min, 40 ^{o}C).

Methyl 3-bromo-7-oxo-2-(piperidin-1-ylmethyl)-7H-selenopheno[2,3-f]chromene-8-carboxylate **(22)** Yield: 60%, m.p. = 160 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.47-1.50 (2H, m, piperidine), 1.59-1.64 (4H, m, piperidine), 2.56-2.61 (4H, m, piperidine), 3.76 (2H, s, CH_2), 3.97 (3H, s, CH_3), 7.33 (1H, d, *J* = 9.0 Hz, 5-CH), 7.91 (1H, d, *J* = 9.0 Hz, 4-CH), 8.58 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 23.9, 26.0, 52.9, 55.1, 59.2, 104.7, 114.7, 114.9, 117.4, 129.7, 138.1, 139.9, 146.4, 147.4, 153.4, 156.5, 163.5. LC-MS *m/z* 484 [M+1]. HRMS (ESI) calcd for $C_{19}H_{18}BrNO_4Se$ [M+H] 483.9657, found 483.9661.

Methyl 3-bromo-2-(morpholinomethyl)-7-oxo-7H-selenopheno[2,3-f]chromene-8-carboxylate **(23)** Yield: 65%, m.p. = 197-199 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 2.68 (4H, t, *J* = 4.6 Hz CH_2N), 3.76 (4H, t, *J* = 4.6 Hz OCH_2), 3.86 (2H, s, CH_2), 2.56-2.61 (4H, m, piperidine), 3.99 (3H, s, CH_3), 7.39 (1H, d, *J* = 8.9 Hz, 5-CH), 7.98 (1H, d, *J* = 8.9 Hz, 4-CH), 8.63 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 53.0, 54.0, 58.9, 67.0, 106.0, 115.0, 117.7, 130.0, 138.0, 139.9, 145.0, 146.4, 153.7, 156.5, 163.5. LC-MS *m/z* 486 [M]. HRMS (ESI) calcd for $C_{18}H_{16}BrNO_5Se$ [M+H] 485.9450, found 485.9460.

Methyl 3-bromo-2-((4-methylpiperazin-1-yl)methyl)-7oxo-7H-selenopheno[2,3-f]chromene-8-carboxylate **(24)** Yield: 62%, m.p. = 174-176 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 2.33 (3H, s, CH_3), 2.41-2.77 (8H, m, piperazine), 3.85 (2H, s, CH_2), 3.99 (3H, s, CH_3), 7.39 (1H, d, *J* = 8.9 Hz, 5-CH), 7.98 (1H, d, *J* = 8.9 Hz, 4-CH), 8.64 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 45.9, 53.0, 53.6, 55.1, 58.4, 105.5, 114.9, 115.0, 117.6, 130.0, 138.1, 139.9, 146.1, 146.5, 153.6, 156.5, 163.6. LC-MS *m/z* 499 [M+1]. HRMS (ESI) calcd for $C_{19}H_{19}BrN_2O_4Se$ [M+H] 498.9766, found 498.9779.

Isopropyl 3-bromo-2-(2-hydroxypropan-2-yl)-7-oxo-7H-selenopheno[2,3-f]chromene-8-carboxylate **(25)** Yield: 38%, m.p. = 194-196 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.42 (6H, d, *J* = 6.2 Hz, CH_3CHCH_3), 1.85 (6H, s, CH_3), 2.78 (1H, br s, OH), 5.26-5.32 (1H, m, CH), 7.37 (1H, d, *J* = 8.9 Hz, 5-CH), 7.97 (1H, d, *J* = 8.9 Hz, 4-CH), 8.52 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 21.8, 29.1, 70.1, 74.3, 101.4, 114.6, 115.0, 118.6, 130.3, 138.3, 139.1, 145.3, 153.6, 154.7, 156.5, 162.5. LC-MS *m/z* 472 [M]. HRMS (ESI) calcd for $C_{18}H_{17}BrO_5Se$ [M+H] 472.9497, found 472.9517. HPLC: 99.4% (RT=10.93 min,

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Apollo C18-8 (4.6x150mm), mobile phase 5→95% Acetonitrile+0.1%H_3PO_4, 1 mL/min, 40 ^oC).

Isopropyl 3-bromo-7-oxo-2-piperidin-1-ylmethyl)-7H-selenopheno[2,3-f]chromene-8-carboxylate **(26)** Yield: 58%, m.p. = 174-176 ^oC. ^1H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.42 (6H, d, *J* = 6.2 Hz, CH_3CHCH_3), 1.47-1.53 (2H, m, piperidine), 1.60-1.66 (4H, m, piperidine), 2.58-2.61 (4H, m, piperidine), 3.78 (2H, s, CH_2), 5.26-5.32 (1H, m, CH), 7.37 (1H, d, *J* = 8.8 Hz, 5-CH), 7.95 (1H, d, *J* = 8.8 Hz, 4-CH), 8.52 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 21.8, 24.0, 26.1, 55.1, 59.3, 69.9, 104.9, 114.8, 115.0, 118.5, 129.5, 138.1, 139.8, 145.4, 147.0, 153.4, 156.5, 162.4. LC-MS *m/z* 512 [M+1]. HRMS (ESI) calcd for $C_{21}H_{22}BrNO_4Se$ [M+H] 511.9970, found 511.9982. HPLC: 97.9% (RT=3.28 min, Apollo C18-8 (4.6x150mm), mobile phase 5→95% Acetonitrile+0.1%H_3PO_4, 1 mL/min, 40 ^oC).

Tert-butyl 3-bromo-2-(2-hydroxypropan-2-yl)-7-oxo-7H-selenopheno[2,3-f]chromene-8-carboxylate **(27)** Yield: 36%, foam. ^1H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.62 (9H, s, CH_3), 1.85 (6H, s, CH_3), 2.78 (1H, br s, OH), 7.32 (1H, d, *J* = 8.8 Hz, 5-CH), 7.92 (1H, d, *J* = 8.8 Hz, 4-CH), 8.44 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 28.1, 29.0, 74.2, 83.2, 101.3, 114.6, 114.9, 119.5, 130.0, 138.1, 139.0, 144.7, 153.4, 154.8, 156.7, 162. LC-MS *m/z* 487 [M+1]. HRMS (ESI) calcd for $C_{19}H_{19}BrO_5Se$ [M+H] 486.9654, found 486.9492.

Tert-butyl 3-bromo-7-oxo-2-piperidin-1-ylmethyl)-7*H*-selenopheno[2,3-*f*]chromene-8-carboxylate **(28)** Yield: 42%. ^1H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.46-1.51 (2H, m, piperidine), 1.59-1.65 (4H, m, piperidine), 1.63 (9H, s, CH_3), 2.56-2.60 (4H, m, piperidine), 3.77 (2H, s, CH_2), 7.35 (1H, d, *J* = 8.8 Hz, 5-CH), 7.92 (1H, d, *J* = 8.8 Hz, 4-CH), 8.45 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 24.0, 26.0, 28.1, 55.1, 59.2, 83.1, 114.7, 115.0, 119.5, 129.3, 137.9, 139.6, 144.8, 146.8, 156.6, 162.0. LC-MS *m/z* 526 [M+1]. HRMS (ESI) calcd for $C_{22}H_{24}BrNO_4Se$ [M+H] 526.0127, found 526.0143. HPLC: 98.3% (RT=9.70 min, Apollo C18-8 (4.6x150mm), mobile phase 5→95% Acetonitrile+0.1%H_3PO_4, 1 mL/min, 40 ^oC).

Octyl 3-bromo-2-(2-hydroxypropan-2-yl)-7-oxo-7H-selenopheno[2,3-f]chromene-8-carboxylate **(29)** Yield: 92%, m.p. = 119-120 ^oC. ^1H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.88 (3H, t, *J* = 6.8 Hz, CH_3), 1.25-1.48 (10H, m, CH_2), 1.76-1.83 (2H, m, CH_2), 1.85 (6H, s, CH_3), 2.72 (1H, br s, OH), 4.37 (2H, t, *J* = 6.8 Hz, CH_2), 7.39 (1H, d, *J* = 9.0 Hz, 5-CH), 7.99 (1H, d, *J* = 9.0 Hz, 4-CH), 8.58 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.1, 22.6, 25.9, 28.5, 29.10, 29.16, 29.19, 31.8, 66.4, 74.3, 101.5, 114.6, 115.0, 118.2, 130.5, 138.4, 139.1, 145.8, 153.7, 154.7, 156.5, 163.2. LC-MS *m/z* 543 [M+1]. HRMS (ESI) calcd for $C_{23}H_{27}BrO_5Se$ [M+H] 543.0280, found 543.0286. HPLC: 96.4% (RT=9.93 min, Apollo C18-8 (4.6x150mm), mobile phase 5→95% Acetonitrile+0.1%H_3PO_4, 1 mL/min, 40 ^oC).

Octyl 3-bromo-7-oxo-2-piperidin-1-ylmethyl)-7H-selenopheno[2,3-f]chromene-8-carboxylate **(30)** Yield: 32%, oil. ^1H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.85-0.90 (1H, m, piperidine), 0.88 (3H, t, *J* = 6.6 Hz, CH_3), 1.25-1.54 (12H, m, CH_2), 1.60-1.65 (4H, m, piperidine), 1.76-1.84 (2H, m, CH_2), 2.58-2.62 (4H, m, piperidine), 3.79 (2H, br s, CH_2), 4.37 (2H, t, *J* = 6.6 Hz, CH_2), 7.38 (1H, d, *J* = 8.8 Hz, 5-CH), 7.97 (1H, d, *J* = 8.8 Hz, 4-CH), 8.59 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.1, 22.6, 24.0, 25.9, 26.1, 28.6, 29.17, 29.20, 31.8, 55.1, 59.3, 66.3, 72.4, 114.8, 115.0, 118.1, 129.7, 145.9, 153.5, 156.5, 163.1. LC-MS *m/z* 582 [M+1]. HRMS (ESI) calcd for $C_{26}H_{32}BrO_4Se$ [M+H] 582.0753, found 582.0773.

Decyl 3-bromo-2-(2-hydroxypropan-2-yl)-7-oxo-7H-selenopheno[2,3-f]chromene-8-carboxylate **(31)** Yield: 89%, m.p. = 105-106 ^oC. ^1H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.87 (3H, t, *J* = 6.8 Hz, CH_3), 1.26-1.48 (14H, m, CH_2), 1.76-1.83 (2H, m, CH_2), 1.86 (6H, s, CH_3), 2.66 (1H, br s, OH), 4.38 (2H, t, *J* = 6.8 Hz, CH_2), 7.40 (1H, d, *J* = 8.8 Hz, 5-CH), 7.99 (1H, d, *J* = 8.8 Hz, 4-CH), 8.59 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.1, 22.6, 25.8, 28.5, 29.0, 29.2, 29.3, 29.48, 29.51, 31.0, 31.8, 66.4, 74.3, 101.4, 114.6, 114.9, 118.1, 130.4, 138.3, 139.1, 145.7, 153.6, 154.9, 156.5, 163.2. LC-MS *m/z* 571 [M+1]. HRMS (ESI) calcd for $C_{25}H_{31}BrO_5Se$ [M+H] 571.0593, found 571.0589. HPLC: 97.7% (RT=12.17 min, Apollo C18-8 (4.6x150mm), mobile phase 5→95% Acetonitrile+0.1%H_3PO_4, 1 mL/min, 40 ^oC).

Decyl 3-bromo-7-oxo-2-piperidin-1-ylmethyl)-7H-selenopheno[2,3-f]chromene-8-carboxylate hydrochloride **(32)** Yield: 45%, m.p. = 149-150 ^oC. ^1H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.85 (3H, t, *J* = 7.0 Hz, CH_3), 1.19-1.52 (16H, m, CH_2), 1.76-1.83 (2H, m, CH_2), 1.90-1.97 (2H, m, piperidine), 2.15-2.25 (2H, m, piperidine), 2.77-2.82 (2H, m, piperidine), 3.57-3.62 (2H, m, piperidine), 4.36 (2H, t, *J* = 7.0 Hz, CH_2), 4.63 (2H, br s, CH_2), 7.42 (1H, d, *J* = 8.8 Hz, 5-CH), 8.03 (1H, d, *J* = 8.8 Hz, 4-CH), 8.57 (1H, s, 9-CH), 12.6 (0.5H, br s, HCl). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 14.0, 22.0, 22.6, 22.9, 25.8, 28.5, 29.19, 29.24, 29.45, 29.49, 31.8, 52.8, 56.2, 66.4, 114.9, 115.9, 116.0, 118.8, 128.9, 131.0, 135.4, 142.9, 144.7, 154.6, 155.9, 162.1. LC-MS *m/z* 610 [M+1]. HRMS (ESI) calcd for $C_{28}H_{36}BrO_4Se$ [M+H] 610.1066, found 610.1075.

3-Bromo-2-(2-hydroxypropan-2-yl)-8-isobutyryl-7H-selenopheno[2,3-f]chromen-7-one **(33)** Yield: 41%, m.p. > 200 ^oC. ^1H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.21 (6H, d, *J* = 6.8 Hz, CH_3), 1.85 (6H, s, CH_3), 2.71 (1H, br s, OH), 3.85-3.93 (1H, m, CH), 7.42 (1H, d, *J* = 8.9 Hz, 5-CH), 7.99 (1H, d, *J* = 8.9 Hz, 4-CH), 8.56 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 18.3, 29.1, 38.6, 74.3, 101.4, 114.9, 115.2, 124.3, 130.3, 138.9, 139.2, 145.0, 153.7, 154.8, 158.7, 202.2. LC-MS *m/z* 457 [M+1]. HRMS (ESI) calcd for $C_{18}H_{17}BrO_4Se$ [M+H] 456.9548, found 456.9561.

3-Bromo-8-isobutyryl-2-(piperidin-1-ylmethyl)-7H-selenopheno[2,3-f]chromen-7-one **(34)** Yield: 66%, m.p. = 160-162 ^oC. ^1H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.20 (6H, d, *J* = 6.8 Hz, CH_3), 1.47-1.51 (2H, m, piperidine), 1.59-1.65 (4H, m, piperidine), 2.58-2.62 (4H, m, piperidine), 3.77 (2H, s, CH_2),

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3.84-3.91 (1H, m, CH), 7.36 (1H, d, *J* = 8.8 Hz, 5-CH), 7.93 (1H, d, *J* = 8.8 Hz, 4-CH), 8.51 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 18.3, 23.9, 26.1, 38.5, 55.1, 59.2, 114.5, 115.5, 124.2, 129.4, 138.1, 140.4, 145.0, 153.4, 158.7, 202.1. LC-MS *m/z* 496 [M+1]. HRMS (ESI) calcd for $C_{21}H_{22}BrNO_3Se$ [M+H] 495.9948, found 496.0017. HPLC: 96.4% (RT=3.93 min, Apollo C18-8 (4.6x150mm), mobile phase 5→95% Acetonitrile+0.1%H_3PO_4, 1 mL/min, 40 °C).

3-Bromo-2-(2-hydroxypropan-2-yl)-8-(2-bromo-pentanoyl)-7*H*-selenopheno[2,3-*f*]chromene-7-one **(35-Br)** Yield: 95%, m.p. = 178-180 °C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.02 (3H, t, *J* = 7.4 Hz, CH_3), 1.44-1.69 (2H, m, CH_2), 1.86 (6H, s, CH_3), 2.00-2.09 (1H, m, CH_2), 2.14-2.23 (1H, m, CH_2), 2.62 (1H, br s, OH), 5.82-5.86 (1H, m, CH), 7.44 (1H, d, *J* = 8.9 Hz, 5-CH), 8.03 (1H, d, *J* = 8.9 Hz, 4-CH), 8.69 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 13.6, 20.7, 29.10, 29.11, 34.7, 51.4, 74.4, 101.4, 115.0, 115.2, 122.5, 130.9, 139.4, 146.3, 153.9, 155.1, 158.5, 191.2. LC-MS *m/z* 550 [M+1].

3-Bromo-2-(2-hydroxypropan-2-yl)-8-pentanoyl-7H-selenopheno[2,3-f]chromene-7-one **(35)**: The bromide **35-Br** (0.2 g, 0.36 mmol), *N*-acetylguanidine (0.036 g, 0.36 mmol) and sodium acetate (0.030 g, 0.36 mmol) were refluxed in anhydrous ethanol for 12 h and cooled to rt. Reaction mixture was then evaporated and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc = 1/1). Yield: 68%. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ (ppm): 0.95 (3H, t, *J* = 7.2 Hz, CH_3), 1.36-1.47 (2H, m, CH_2), 1.65-1.73 (2H, m, CH_2), 1.86 (6H, s, CH_3), 2.93 (1H, br s, OH), 3.16 (2H, t, *J* = 7.2 Hz, CH_2), 7.39 (1H, d, *J* = 8.8 Hz, 5-CH), 7.98 (1H, d, *J* = 8.8 Hz, 4-CH), 8.57 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, $CDCl_3$/TMS) δ (ppm): 13.9, 22.3, 25.9, 29.0, 42.3, 74.3, 101.3, 114.8, 115.1, 124.2, 130.4, 139.0, 139.2, 144.6, 153.7, 155.0, 159.0, 198.1. LC-MS *m/z* 471 [M+1]. HRMS (ESI) calcd for $C_{21}H_{24}BrNO_4Se$ [M] 470.9705, found 470.9716.

Hydrolysis of methyl selenopheno[2,3-f]chromene-8-carboxylates: Methyl selenopheno[2,3-*f*]chromene-8-carboxylate (0.4 mmol) was suspended in methanol (30 mL). Then sodium hydroxide (4 mmol) dissolved in water (10 mL) was added. After the consumption of the initial compound (12-24 h), reaction mixture was acidified with 2N HCl till pH=3 and evaporated. The residue was purified by washing solids with water or flash chromatography on silica gel.

3-bromo-2-(2-hydroxypropan-2-yl)-7-oxo-7H-selemopheno[2,3-f]chromene-8-carboxylic acid **(36)** Yield: 85%, m.p. > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.68 (6H, s, CH_3), 6.50 (1H, br s, OH), 7.51 (1H, d, *J* = 8.8 Hz, 5-CH), 7.93 (1H, d, *J* = 8.8 Hz, 4-CH), 8.69 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ (ppm): 28.4, 72.2, 99.4, 114.6, 114.9, 119.0, 129.2, 137.8, 138.5, 144.6, 152.6, 156.4, 157.8, 163.8. ^{77}Se-NMR (DMSO-d_6, 76.36 MHz) δ (ppm): 570.3. LC-MS *m/z* 431 [M+1]. HRMS (ESI) calcd for $C_{15}H_{11}BrO_5Se$ [M+H] 430.9028, found 430.9026. HPLC: 97.2% (RT=6.71 min, Apollo C18-8 (4.6x150mm), mobile phase 5→95% Acetonitrile+0.1%H_3PO_4, 1 mL/min, 40 °C).

3-bromo-2-(piperidin-1-ylmethyl)-7-oxo-7H-selemopheno[2,3-f]chromene-8-carboxylic acid **(37)** Yield: 88%, m.p. > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.48-1.63 (2H, m, piperidine), 1.76-1.79 (4H, m, piperidine), 2.99-3.20 (4H, m, piperidine), 4.56 (2H, br s, CH_2), 7.59 (1H, d, *J* = 8.8 Hz, 5-CH), 8.06 (1H, d, *J* = 8.8 Hz, 4-CH), 8.74 (1H, s, 9-CH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ (ppm): 21.5, 22.8, 51.9, 114.8, 115.6, 119.3, 130.1, 135.5, 144.4, 153.5, 156.4, 163.8. LC-MS *m/z* 470 [M+1]. HRMS (ESI) calcd for $C_{18}H_{16}BrNO_4Se$ [M+H] 469.9501, found 469.9512.

3-bromo-2-(morpholinomethyl)-7-oxo-7H-selemopheno[2,3-f]chromene-8-carboxylic acid **(38)** Yield: 92%, m.p. > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.40-3.62 (4H, m, morpholine), 3.72-4.04 (4H, m, morpholine), 4.75 (2H, br s, CH_2), 7.59 (1H, d, *J* = 8.8 Hz, 5-CH), 8.07 (1H, d, *J* = 8.8 Hz, 4-CH), 8.76 (1H, s, 9-CH). LC-MS *m/z* 472 [M+1]. HRMS (ESI) calcd for $C_{17}H_{14}BrNO_5Se$ [M+H] 471.9293, found 471.9304.

3-bromo-2-((4-methylpiperazin-1-yl)methyl)-7-oxo-7H-selemopheno[2,3-f]chromene-8-carboxylic acid **(39)** Yield: 99%, m.p. > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.75 (3H, s, CH_3), 2.86-2.97 (2H, m, piperazine), 3.07-3.26 (4H, m, piperazine), 3.42-3.45 (2H, m, piperazine), 4.17 (2H, br s, CH_2), 7.58 (1H, d, *J* = 8.8 Hz, 5-CH), 8.03 (1H, d, *J* = 8.8 Hz, 4-CH), 8.69 (1H, s, 9-CH). LC-MS *m/z* 485 [M+1]. HRMS (ESI) calcd for $C_{18}H_{17}BrN_2O_4Se$ [M+H] 484.9610, found 484.9660. HPLC: 96.9% (RT=7.26 min, Apollo C18-8 (4.6x150mm), mobile phase 5→95% Acetonitrile+0.1%H_3PO_4, 1 mL/min, 40 °C).

In Vitro cytotoxicity assay: Monolayer tumour cell line: MDA-MB-435s (human melanoma), MCF-7 (human breast adenocarcinoma, oestrogen-positive), MES-SA (human uterus sarcoma), HT-1080 (human connective tissue fibrosarcoma), A549 (human lung carcinoma), SH-SY5Y (human neuroblastoma), CCL-8 (mouse sarcoma), 3T3 (Mouse Swiss Albino embryo fibroblasts), MH-22A (mouse hepatoma), HepG2 (human hepatocellular carcinoma), B16-F10 (mouse melanoma). All cell lines from ATCC. The cells were seeded in 96-well plates in DMEM and cultivated for 72 h while exposed to different concentrations of the compounds. Cell viability was measured via MTT assay [28]. Briefly, after incubation with the compounds, the culture medium was removed, and fresh medium containing 0.2-mg/ml MTT was added. After incubation (3 h, 37°C, 5% CO_2), the MTT-containing medium was removed, and 200 µL of dimethyl sulfoxide (DMSO) was immediately added to each sample. The samples were assessed at 540 nm on a Tecan Infinite1000 microplate reader. The half-maximal inhibitory concentration (IC_{50}) of each compound was calculated using Graph Pad Prism® 3.0.

Basal cytotoxicity test [22]: The Neutral Red Uptake (NRU) assay was performed according to the standard protocol of Stokes modified by the NICEATM-ECVAM validation study. The NRU cytotoxicity assay procedure is based on the ability of viable cells to incorporate and bind Neutral Red, a supravital

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dye. Balb/c 3T3 (Mouse Swiss Albino embryo fibroblast) cells (9000 cells/well) were placed into 96-well plates for 24 h in Dulbecco's modified Eagle's (DMEM) medium containing 5% fetal bovine serum. The cells were then exposed to the test compounds over a range of eight concentrations (1000, 316, 100, 31, 10, 3, 1 μg/ml) for 24 h. Untreated cells were used as a control. After 24 h, the medium was removed from all plates. Then, 250 μl of Neutral Red solution were added (0.05 mg/ml). Plates were incubated for 3 h and then cells were washed three times with PBS. The dye within viable cells was released by extraction with a mixture of acetic acid, ethanol and water (1:50:49). Absorbance of Neutral Red was measured using a spectrophotometer multiplate reader (TECAN, Infinite M1000) at 540 nm. The optical density (OD) was calculated using the following formula: $OD_{treated\ cells}$ x 100/$OD_{control\ cells}$. The IC_{50} values were calculated using the computer program Graph Pad Prism® 3.0.

Estimation of LD_{50} from IC_{50} values [23]: Data from the *in vitro* NRU tests were used to estimate the starting dose for acute oral systemic toxicity tests in rodents. The *in vivo* starting dose is an estimated LD_{50} value calculated by inserting the *in vitro* IC_{50} value into a regression formula: log LD_{50} (mM/kg) = 0.439 log IC_{50} (mM) + 0.621. The value is recalculated to mg/kg and compounds are evaluated in accordance with four toxicity categories: category 1: $LD_{50} \leq 5$ mg/kg (highly toxic); category 2: $5 < LD_{50} \leq 50$ mg/kg (moderately toxic); category 3: $50 < LD_{50} \leq 300$ mg/kg (slightly toxic); category 4: $300 < LD_{50} \leq 2000$ mg/kg (practically non-toxic). Using this alternative *in vitro* method allows comparisons of possible toxicity of new compounds and to select compounds for further study, thus vastly reducing the number of animal experiments.

MMP assay: Inhibitors of matrix metalloproteinase enzymes were detected with the use of MMP Inhibitor Fluorimetric Profiling kit (Biomol, USA) accordingly to manufacturer's instructions. MMP activity assays were performed in 96-well plates using the recombinant human MMP-1–10, 12, 13 and 14 catalytic domains and OmniMMP™ fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2. The test compounds (20μM) were dissolved in DMSO. The compound NNGH (N-isobutyl-*N*-(4-methoxyphenylsulfonyl)-glycylhydroxamic acid) was used as a prototypic control inhibitor. The rate of substrate hydrolysis was determined by fluorescence intensity measuring for 10 minutes at 37 °C temperature using a fluorescence plate reader (Tecan infinite M1000, Austria) with excitation at 328 nm and emission at 420 nm.

In vitro angiogenesis assay: HUVEC cells were grown in Vascular Cell Basal medium with 2% fetal bovine serum, bovine brain extract, hydrocortisone, human endothelial growth factor, and gentamicin/amphotericin B (ATCC) in incubator with 5% CO_2 and at 37°C. Matrigel was dispensed into 96-well plates (50 μL/well). Plates were incubated at 37°C for 1 hour to allow polymerization of matrigel. HUVEC cultures were added into each matrigel-coated well at a final concentration of 10,000 cells/well. After 8 h incubation at 37°C cells were labelled with calcein-AM (Molecular Probes) and photographed at ×4 magnification using an inverted fluorescence microscope (Nicon) calculating the length of capillary tubes.

In vitro haemolytic assay: Haemolytic effect of the compounds on rat erythrocytes was evaluated by using washed erythrocytes. [30] Blood samples from the rats were collected from Charles foster strain, (each weighing 130-180 g) in citrated tubes. The cells were then washed three times with phosphate-buffered saline (150 mM NaCl, 1.9 mM NaH_2PO_4, and 8.1 mM Na_2HPO_4, pH 7.4) and 2% erythrocyte suspension was prepared. The haemolytic activities of the compounds were tested in 96-well plates. PBS solution was used as negative control, 1% solution of Triton X-100 in PBS was a positive control. Compounds were tested in various concentrations (from 5 to 300 μg/mL). After an incubation for 1h at 37°C, the plate was centrifuged at 1500 rpm for five minutes and 50 μl of supernatant was transferred to a fresh 96-well plate. The supernatant was used to measure the absorbance of the liberated haemoglobin at 405 nm. The average value was calculated from triplicate assay. The OD of cells exposed to 1% Triton X-100 represented 100% lysis; the OD of cells incubated in PBS represented 0%.

Hydrolysis of selenophenocoumarin methyl esters with HepG2 cells lysate: Human hepatocellular carcinoma HepG2 cells were seeded into six-well plates at a density of $2x10^6$ cells/ml and grown in DMEM with 10% FBS. After cells reached 80 to 90% of confluence, the medium was replaced with serum-free culture medium with **21** or **37** (100 μM) and exposed for 6 h. Then the medium was separated and cells were sonicated. The existence of compounds and its metabolites in the cells lysate and conditioned medium was analysed using UPLC-MS.

In vivo experiments: Six-week-old female C57BL/6JOlaHsd and BALB/cOlaHsd mice were purchased from the Envigo (Lab Animal, Nederland). The mice were housed five to a cage, and under standard conditions (21–23°C, 12 h light: dark cycle), fed ad libitum (R3 diet, Lactamin AB, Kimstad, Sweden) and observed daily. The experimental procedures involving experimental animals were carried out in accordance with the guidelines of the European Community, local laws and policies, and were approved by the Latvian Animal Protection Ethical Committee, the Food and Veterinary Service, Riga, Latvia.

In vivo angiogenesis assay: The matrigel plug angiogenesis assay is a simple *in vivo* technique to detect the newly formed blood vessels in the transplanted gel plugs in mice (5 animals, BALB/c). The antiangiogenic potential of compounds was assessed using a modified Matrigel plug assay as described in Ref. 40. The female Balb/c mice were anaesthetized, placed on a heated pad (37°C) and 0.5 ml of ice-cold Matrigel supplemented with 10 μg/ml of basic fibroblast growth factor (bFGF) and 100 ng/ml of vascular endothelial growth factor (VEGF) without or with compounds in dose 10 μM were injected subcutaneously into the flanks of each mouse. Control

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mice received Matrigel without bFGF and VEGF. After 7 days, mice were euthanized and the matrigel implant was recovered and frozen gel were cut with cryostat. Sections cut and stained with haematoxylin and eosin was studied by light microscopy. Results were expressed as percentage±SD of the vessel area to the total Matrigel area.

Melanoma model of lung metastasis: *In vivo* studies were performed utilizing the B16-F10 mouse melanoma (ATCC® CRL-6475™). The cells were grown in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal calf serum (Sigma). C57BL/6 mice were injected with 10,000 B16-F10 melanoma cells into the mice via the tail vein and treated 24 hr later with subcutaneous injections (s.c.) of compounds administered according to the following scheme: 1, 7, 8, 9, 10, 11 and 14th days in dose 20 mg/kg and 5 mg/kg. Control mice were injected with tumour cells only. 18 days later, all mice were anesthetized with ketamine/xylazine and euthanized, the mice were sacrificed and the black melanoma nodules on the lungs were calculated. In each experiment, mice were weighed twice weekly.

4T1 model of lung metastasis: *In vivo* studies were performed utilizing the 4T1 mouse mammary carcinoma (ATCC® CRL-2539™). The cells were grown in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal calf serum (Sigma). Balb/c mice were injected with 10,000 4T1 breast cancer cells into the mice via the tail vein and treated 24 h later with subcutaneous injections (*s.c.*) of compounds administered according to the following scheme: 1, 4, 7, 9, 11 and 14th days in dose 20 mg/kg. 18 days later all mice were anesthetized with ketamine/xylazine and euthanized, mice were sacrificed, lung was removed. [38] Pulmonary metastases were enumerated by intra-tracheal injection of India ink (15% India Ink, 85% water, 3 drops $NH_3(H_2O)$/100 ml). India ink injected lungs were washed in Feket's solution (300 ml 70% EtOH, 30 ml 37% formaldehyde, 5 ml glacial acetic acid) and then placed fresh Feket's solution overnight. Tumour nodules do not absorb India ink, which results in the normal lung tissue staining black and the tumour nodules remaining white. White tumour nodules against a black lung background were measured.

Conflicts of interest

There are no conflicts to declare.

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The experimental procedures involving experimental animals were carried out in accordance with the guidelines of the European Community, local laws and policies, and were approved by the Latvian Animal Protection Ethical Committee, the Food and Veterinary Service, Riga, Latvia.

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Selenopheno[2,3-*f*]coumarins: a novel scaffold with antimetastatic activity against melanoma and breast cancer

Pavel Arsenyan*, Jelena Vasiljeva, Ilona Domracheva, Iveta Kanepe-Lapsa, Anita Gulbe