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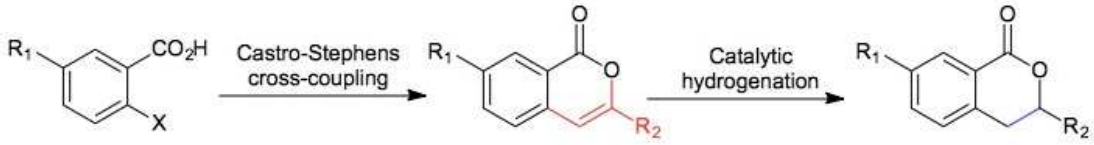

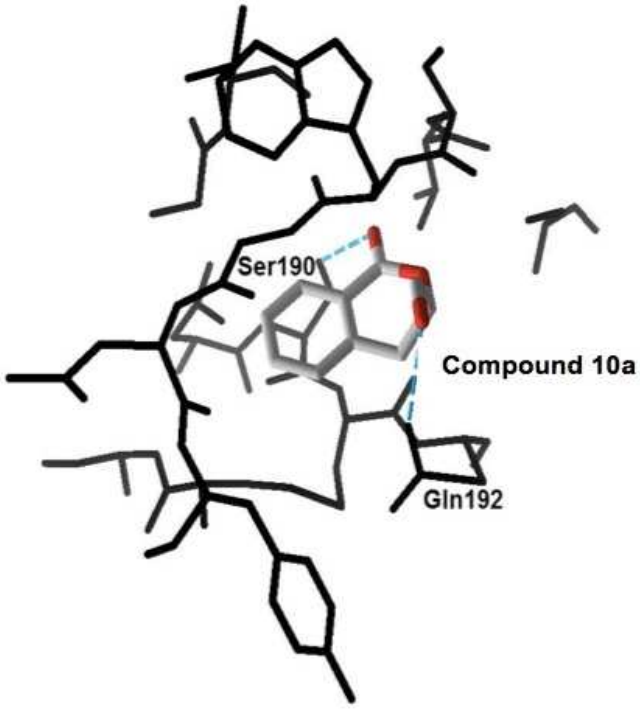

Synthesis, antiproliferative activities, and computational evaluation of novel isocoumarin and 3,4-dihydroisocoumarin derivatives

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Abstract

A series of novel isocoumarin derivatives were synthesized using Castro–Stephens cross-coupling. Moreover, novel 3,4-dihydroisocoumarin derivatives were obtained by catalytic hydrogenation of the corresponding isocoumarin precursors. The antiproliferative activity of all compounds was evaluated *in vitro* in different tumor cells. Furthermore, docking calculations were performed for the kallikrein 5 (KLK5) active site to predict the possible mechanism of action of this series of compounds. Theoretical findings indicate that the 3,4-dihydroisocoumarin derivative **10a** forms hydrogen bonds with Ser190 and Gln192 residues of KLK5. This derivative was the most active compound in the series with potent antiproliferative activity and high selectivity index (SI > **378.79**) against breast cancer cells (MCF-7, GI_{50} = 0.66 µg mL^{-1}). This compound represents a promising matrix for developing new antiproliferative agents.

Keywords: isocoumarin; 3,4-dihydroisocoumarin; Castro–Stephens reaction; antiproliferative

1. Introduction

Cancer is a significant public health problem and a major cause of death in humans [1]. Despite many efforts to fight cancer, successful treatment of certain types of tumors continues to be challenging because of their aggressiveness, the complex mechanisms underlying malignant cell metastasis, chemoresistance, and the lack of selectivity of some drugs [2]. Thus, the development of new, safe, and effective anticancer agents through the synthesis of simple small molecules is necessary.

Isocoumarins, including their 3,4-dihydro derivatives, are isolated from a variety of natural sources and have diverse biological activities such as antifungal, antimicrobial [3], antiallergic [4], immunomodulatory [5], enzyme inhibitory [6-8], antiangiogenic [9], and antioxidant properties [10]. The different biological activities of the natural compounds belonging to this class are thought to be because of the large structural variety found among these compounds [11].

Several natural and synthetic coumarins or 3-substituted- 3,4-dihydroisocoumarins have significant cytotoxic and antitumor activities. For instance, cytogenin **1**, a 3-hydroxymethyl coumarin, first isolated in 1990 from *Streptoverticillium eurocidicum*, was the first natural isocoumarin that showed anticancer activity against experimental tumor cells and human cancer cells [12]. NM-3 **2**, a synthetic analogue of cytogenin in phase I of clinical tests, potentiates the antineoplastic effects of other chemotherapeutic agents and inhibits angiogenesis [13]. 3-Arylisocoumarin **3** has potential anticancer and antimicrobial activity [14]. The compound 185322 **4**, an inhibitor of microtubule assembly, induces mitotic arrest and apoptosis of multiple myeloma cells [15]. The chiral 3-methyl-3,4-ochratoxin A **5**, a mycotoxin isolated from *Aspergillus ochraceus*, shows nephrotoxic, hepatotoxic, carcinogenic, and teratogenic properties [16]; further, the chiral 3-pentyl-3,4-dihydrocoumarin **6** has cytotoxic properties [3,7,8].

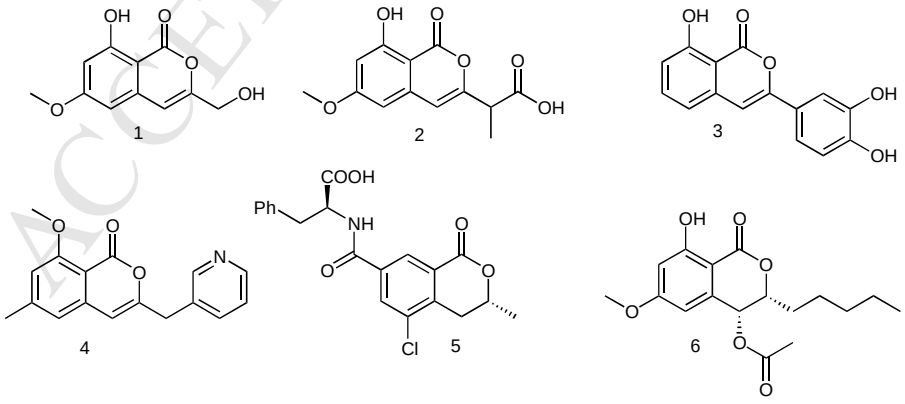


Figure 1. Representative examples of biologically active isocoumarin and 3,4-dihydroisocoumarin.

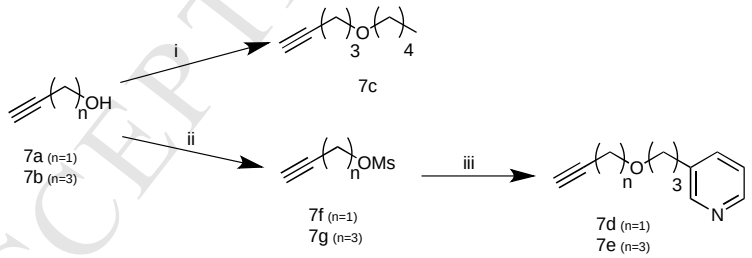
Typically, homophthalic acid derivatives are used as starting materials in the synthesis of isocoumarins and 3-substituted-3,4-dihydrocoumarins [17,18]. The recently developed cross-coupling reactions catalyzed by transition metals have facilitated the development of new methods for the synthesis of isocoumarins and 3,4-dihydroisocoumarins [11,19,20]. A cross-coupling reaction known as the Castro–Stephens reaction [21], catalyzed by Cu (I) has been successfully used to synthesize these compounds. Wang and coworkers modified the methodology of this reaction, making it a one-pot reaction [22].

On the basis of the previously described biological activities of this class of compounds, we designed and synthesized a series of 3-substituted isocoumarin derivatives and a series of 3-substituted 3,4-dihydroisocoumarin derivatives using a modified Castro–Stephens coupling reaction. The antiproliferative activity of the new compounds was evaluated *in vitro* against some tumor cell strains, and we performed theoretical investigations for a potential molecular target.

2. Results and Discussion

2.1. Chemistry

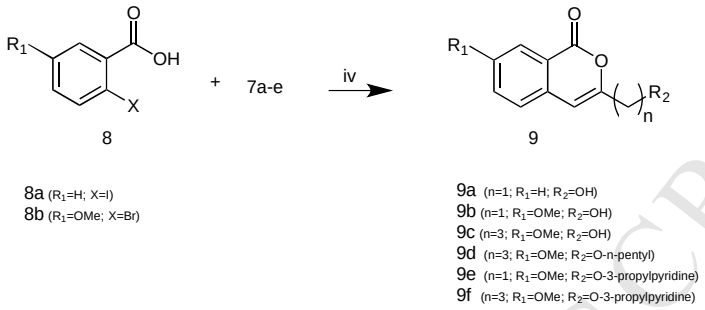
To synthesize novel isocoumarin derivatives, we used a one-pot reaction between a terminal alkyne and a derivative of the 2-halobenzoic acid. The first step was the acquisition of some simple terminal alkynes (Scheme 1). Most of the alkynes were obtained by modification of both the commercially available propargyl alcohol (**7a**) and 4-pentyn-1-ol (**7b**). The choice of different alkynes governed a structural variability in the final products.



Scheme 1. Reagents and conditions: (i) **7b** (1.0 equiv), NaH (3.0 equiv), 1-iodopentane (3.0 equiv), THF, 0 ^{o}C-r.t., 16h (**7c**: 87% yield); (ii) **7a** or **7b** (1.0 equiv), Et_3N (1.5 equiv), MsCl (1.7 equiv), CH_2Cl_2, -40 °C, 1h (**7f**: 97% yield, **7g**: quant.) ; (iii) **7f** or **7g** (1.0 equiv), NaH (3.0 equiv), 3-pyridinepropanol (1.5 equiv), THF, 0 ^{o}C-r.t., 16h (**7d**: 79% yield, **7e**: 83% yield).

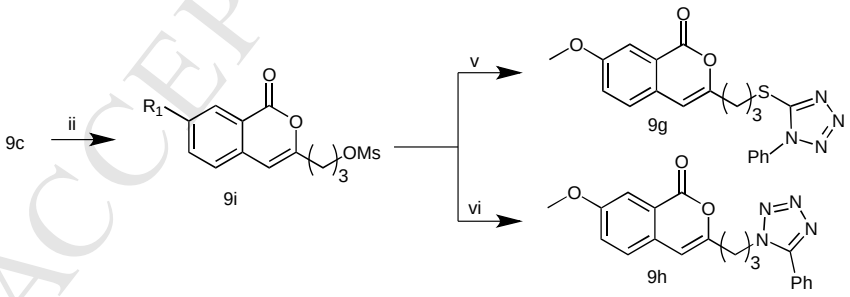
After obtaining the desired alkynes, we synthesized the isocoumarin derivatives in a one-pot reaction catalyzed by Cu (I) and *trans*-4-hydroxy-L-proline as a ligand. The presence of an amino acid ligand is thought to inhibit the formation of phthalides, which are common byproducts in these kinds of reactions. We did not detect these

byproducts during our experiments. All the planned isocoumarins were obtained successfully (Scheme 2). The benzoic acid derivatives 2-iodobenzoic acid (**8a**) and 2-bromo-5-methoxybenzoic acid (**8b**) are commercially available.



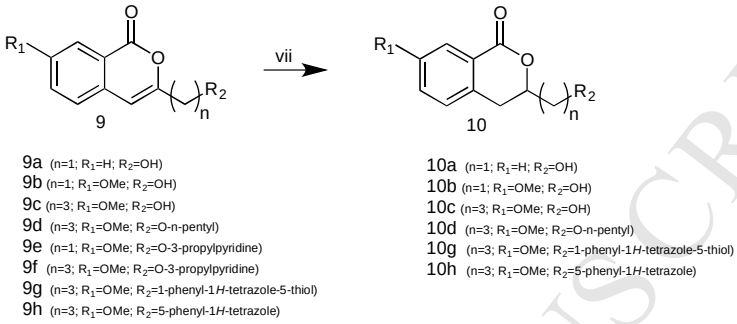
Scheme 2 - Reagents and conditions: (iv): **8a** or **8b** (1.0 equiv), **7** (1.0 equiv), CuI (20 mol%), *trans*-4-hydroxy-L-proline (20 mol%), K_2CO_3 (2.0 equiv), DMSO, 70 ^oC, 16h (isolated yields: **9a**: 54%; **9b**: 51%; **9c**: 73%; **9d**: 69%; **9e**: 65%; **9f**: 71%).

The observed yields of the compounds **9a** (54%), **9b** (51%), **9c** (73%), and **9d** (69%) suggest that the size of the alkyne chain influences the performance of the reaction. Compounds **9e** and **9f** were obtained in moderate to good yields (65% and 71%, respectively). Compound **9c** was subjected to a subsequent mesylation reaction, which resulted in an intermediate **9i**. Subsequently, **9i** reacted with both the commercial 1-phenyl-1*H*-tetrazole-5-thiol and 5-phenyl-1*H*-tetrazole, thus resulting in compounds **9g** and **9h** (Scheme 3). We did not detect any isomer for compound **9h**.



Scheme 3. Reagents and conditions: (ii) **9c** (1.0 equiv), Et_3N (1.5 equiv), MsCl (1.7 equiv), CH_2Cl_2, -40 °C, 1h (**9i**: 93% yield); (v) **9i** (1.0 equiv), 1-phenyl-1*H*-tetrazole-5-thiol (1.5 equiv), K_2CO_3 (3.0 equiv), acetone, reflux, 24h (**9g**: 90% yield); (vi) **9i** (1.0 equiv), 5-phenyl-1*H*-tetrazole (1.5 equiv), K_2CO_3 (3.0 equiv), acetone, reflux, 24h (**9h**: 83% yield).

The obtained isocoumarins were then submitted to Pd/C-catalyzed hydrogenation under pressure, generating their respective 3,4-dihydroisocoumarins as racemic mixtures (**10**) in good to high yields (Scheme 4). The compounds bearing the substituent *O*-3-propylpyridine, **9e** and **9f**, did not react under the catalytic hydrogenation conditions used. The results of the catalytic hydrogenation are shown in **Table 1**.



Scheme 4. Reagents and conditions: (vii) **9** (1.0 equiv), Pd/C 10% (10%w/w), H_2 (250-500 psi), methanol or THF, 8-24h. For yields: see Table 1.

Table 1 – Reduction of the isocoumarins (**9**) to their respective 3,4-dihydroisocoumarins (**10).**

Starting material	Pressure of H_2 (psi)	Solvent	Reaction time (h)	Product	Isolated yield (%)
9a	250	MeOH	8	**10a**	98
9b	250	MeOH	8	**10b**	Quant.
9c	250	MeOH	8	**10c**	94
9d	250	MeOH	8	**10d**	89
9e	500	THF	24	**-**	-
9f	500	THF	24	**-**	-
9g	300	MeOH	16	**10g**	84
9h	300	MeOH	16	**10h**	81

2.2. Antiproliferative studies

We examined the antiproliferative activities of compounds from series (**9a-h**, **10a-d**, and **10g-h**) according to the methodology described by Developmental Therapeutics Program NCI/NIH (http://http://dtp.nci.nih.gov/; Monks *et al*. 1990); we examined the antiproliferative activities against various tumor cells (U251 [glioma], MCF-7 [breast cancer], NCI-ADR/RES [ovarian phenotype of multidrug resistance], 786-0 [kidney], NCI H460 [lung, non-small cells], PC-3 [prostate], and HT29 [colon]) and the non-tumor human immortalized keratinocytes (HaCaT). Doxorubicin was used as a standard drug.

Here, the antiproliferative activity is expressed as the concentration producing 50% of cell growth inhibition or cytostatic effect (GI_{50} μg mL^{-1}) for each cell type (Table 2). Almost all the evaluated compounds showed some inhibition of cell growth. Some compounds showed dose-dependent inhibition of cell proliferation.

Results of the average activity (mean $logGI_{50}$) indicated that compounds belonging to series **9** were more promising as cytostatic agents than those belonging to series **10**, because unlike one compound belonging to series **10** (**10d**), four compounds belonging to series **9** (**9c-f**) showed the lowest values of mean $logGI_{50}$ (Table 2). These results indicate that the unsaturation between C-3 and C-4 in the isocoumarin structure contributes to the cytostatic effect.

Among isocoumarins of series **9**, isocoumarin **9d** was the most active compound (mean $logGI_{50}$ = 1.3), which showed selective inhibition of breast (MCF-7), multidrug resistant ovarian (NCI-ADR/RES), and prostate (PC-3) cancer cell lines (Table 2). Isocoumarins **9c** and **9e** showed a mean antiproliferative effect (mean $logGI_{50}$ = 1.4) similar to that of **9d** without exhibiting selective inhibition of any lineage (Table 2). These results suggested that introduction of a methoxy group on C-8 (compounds **9c-e**) contributes to the cytostatic effect especially when an alkyl ether is introduced on the side chain at C-3 (compound **9d**) (Table 2).

Results from the 3,4-dihydroisocoumarins series (**10a-d**, **10g-h**) indicated that the presence of a methoxy group on C-8 and an alkyl ether on the side chain at C-3 (compound **10d**) resulted in an active compound with a mean inhibition (mean $logGI_{50}$ = 1.5) similar to that observed for **9d** without exhibiting selective inhibition of any lineage (Table 2) unlike that observed for **10d**.

Considering the effect on each tumor cell line, the most active of all the evaluated compounds was the 3,4-dihydroisocoumarin **10a**. Interestingly, this molecule was active only against the breast cancer cell line (MCF-7) with a very low GI_{50} (0.66 μg mL^{-1}) and a very high selectivity index (SI) >378. Its respective isocoumarin, **9a**, showed much lower activity against this cell line (Table 2).

Table 2 - GI_{50}: Growth Inhibition 50 - concentration required to inhibit 50% of cellular growth (μg mL^{-1})

	U251	**MCF-7**	**NCI-ADR/RES**	**786-0**	**NCI-H460**	**PC-3**	**HT29**	**Mean $logGI_{50}$ #**	**HaCat**
Doxo.	<0.025	<0.025	0.14	0.025	<0.025	0.085	0.13	-1.2	<0.025
9a	*	89.3	*	*	*	117.8	168.4	>2.3	222.0
9b	167.3	32.6	160.4	37.6	93.2	49.2	80.3	1.9	134.4
9c	28.9	25.8	28.1	26.9	29.1	25.9	29.1	1.4	29.0
9d	19.4	6.0	7.4	25.5	32.0	8.0	28.3	1.3	5.5
9e	28.5	26.5	28.0	28.6	29.6	25.0	25.8	1.4	26.2
9f	26.3	23.8	19.3	28.7	25.2	26.3	25.0	1.4	27.0
9g	43.3	45.7	*	51.1	*	144.5	*	>2.2	*
9h	67.4	75.2	*	224.2	*	179.8	*	>2.3	*
10a	*	0.66	*	*	*	*	*	>2.3	*

10b	*	250	*	*	*	175.4	*	>2.4	*
10c	135.0	80.8	93.3	101.6	135.6	70.7	132.0	2.0	130.3
10d	32.2	32.3	42.3	31.9	33.7	29.5	43.5	1.5	32.3
10g	*	18.6	132.1	*	*	45.1	13.7	>2.1	*
10h	226.3	28.2	142.1	*	210.8	23.9	31.2	>2.1	*

Tumor human cell lines: U251 = glioma; MCF-7 = breast; NCI-ADR/RES = multidrug resistance ovary; 786-0 = kidney; NCI-H460 = lung, non-small cells; PC-3 = prostate; HT29 = colon. Non-tumor human cell line: HaCat = human immortalized keratinocytes; * = >250 μg mL^{-1}; # = arithmetical mean value of GI_{50} considering only tumor cell lines.

Finally, almost all SI values calculated for both series **9** and **10** were below or equal to 1.00 (Table 3). Doxorubicin, a chemotherapy agent currently used in cancer treatment, also exhibits a low SI value; therefore, the SI results for the compounds of series **9** and **10** suggest that *in vivo* toxicological studies should be performed to confirm the safety of these compounds.

Table 3 - Selective index (SI) for each synthesized compound

Compounds	Cell lines						
	U251	**MCF-7**	**NCI-ADR/RES**	**786-0**	**NCI-H460**	**PC-3**	**HT29**
9a	-	2.49	-	-	-	1.88	1.32
9b	0.80	4.12	0.84	3.57	1.44	2.73	1.67
9c	1.00	1.12	1.03	1.08	1.00	1.12	1.00
9d	0.28	0.92	0.74	0.22	0.17	0.69	0.19
9e	0.92	0.99	0.94	0.92	0.89	1.05	1.02
9f	1.03	1.13	1.40	0.94	1.07	1.03	1.08
9g	>5.77	>5.47	-	>4.89	-	>1.73	-
9h	>3.71	>3.32	-	>1.12	-	>1.39	-
10a	-	**>378.79**	-	-	-	-	-
10b	-	>1.00	-	-	-	>1.43	-
10c	0.97	1.12	1.40	1.28	0.96	1.84	0.99
10d	1.00	1.00	0.76	1.01	0.96	1.09	0.74
10g	-	>13.44	>1.89	-	-	>5.54	>18.25

10h	>2.29	>8.87	>1.76	-	>1.19	>10.46	>8.01

Tumor human cell lines: U251 = glioma; MCF-7 = breast; NCI-ADR/RES = multidrug resistance ovary; 786-0 = kidney; NCI-H460 = lung, non-small cells; PC-3 = prostate; HT29 = colon.

2.3 Docking Studies

To determine the potential mode of action of all compounds synthesized in this study, we docked the novel isocoumarins derivatives obtained into the kallikrein 5 (KLK5) active site to simulate a binding mode of this kind of ligand into that target protein. Human KLK5 is a member of the human tissue kallikrein family, which comprises 15 kallikrein-like serine peptidases (KLKs) [23]. Recent studies report that isocoumarins are powerful KLK5 inhibitors and a strong correlation has been observed between the biological activity of isocoumarin, KLK5 inhibition and the antiproliferative activity against tumor cell lines [24]. Although kallikreins are attractive targets for the development of novel therapeutics for many pathological conditions, including neoplastic diseases, an in-depth understanding of the mechanism of action of kallikreins is required, which requires an interplay of experimental and theoretical techniques. Molecular docking techniques are suitable tools to adjust ligands at target sites to estimate interaction energy [25]. Currently, those molecular-mechanics methods are well-established techniques applied on numerous occasions [26]. Although the function of the KLKs in tissues is not clear, growing evidence indicates that overexpression of KLK5 occurs in endocrine-related malignancies, including ovarian, breast, and testicular cancer [27]. Hence, a strong correlation between the biological activity of isocoumarins and the KLK5 inhibition has been observed [27]. Thus, new inhibitors for KLK5 should be developed as novel anticancer drugs. We calculated the interaction energies between each isocoumarin and KLK5 residue within a radii of 6 Å around the ligand and the results were then compared with the predicted GI_{50}. Partial least square (PLS) regression was used to model the relationship between the independent energy term values and GI_{50} values. The determination coefficient of 0.81 guarantees that GI_{50} values are well reproduced by the docking scores and, therefore, this receptor-based approach can be used to understand the interactions governing their biological activities. The experimental and predicted activity and the residual values for the compound set, including the MolDock score and hydrogen bond energies are shown in Table 4. We analyzed the hydrogen bonds formed between the 14 compounds and KLK5, and we observed that: (i) all compounds interact through the carbonyl group of the isocoumarin ring hydrogen bonds and Ser190; (ii) all compounds, except **9g**, **10a**, and **10g**, interact through the oxygen atom at the R2 position with Ser 195; (iii) compounds **9a** and **9b** interact with Cys191; (iv) compounds **9g**, **9h**, **10a**, **10c**, **10d**, and **10g** interact with Gln192; and (v) compound **9h** interacts with Ala221. The superimposition of the best conformation of isocoumarin inside the KLK5 active site and the amino acid residues that interact through hydrogen bonds is shown in Figure 2. Compound **9d** exhibited the lowest E_{score} (more stable complexes), which indicated that

further studies are required on compounds with alkyl functional group at the R2 position. However, **9d** is a very toxic compound with a very low SI (Table 3). Compound **10a**, which was the most active against breast cancer cells (0.66 µg mL^{-1}), was the most selective (SI > **378.79)** compound of the studied series. Despite recent developments in chemotherapy, breast cancer continues to be one the most serious cancers in humans with a great impact on society and is especially shocking and challenging for young women. Our findings indicate that **10a** forms hydrogen bonds with Ser190 and Gln192. The differences in the morphology, histological type, tumor grade, and lymph node status among the different cancer cell lines can lead to differences in the pharmacokinetics of the studied compounds [28].

Table 4 - Experimental (GI_{50Exp}) and predicted (GI_{50Pred}) activity and residual (GI_{50Exp}-GI_{50Pred}) values for the compound set. MolDock score and hydrogen bond energies (kcal mol^{-1}) from docking studies.

Compounds	GI_{50Exp}	GI_{50Pred}	Residue	Energy score	Hydrogen bond energy
9a	3.93	3.86	0.07	-81.2	1.18
9b	4.31	4.16	0.15	-90.55	-2.02
9c	4.59	4.55	0.04	-120.25	-2.60
9d	5.10	5.12	-0.03	-143.79	-6.11
9e	4.60	4.75	-0.15	-118.33	-2.18
9f	4.58	4.49	0.09	-130.82	-2.96
9g	3.84	3.76	0.08	-118.39	-0.50
9h	3.75	3.98	-0.24	-115.94	-8.40
10a	3.60	3.77	-0.16	-133.162	-4.00
10b	3.76	3.90	-0.14	-91.53	0.58
10c	4.15	3.99	0.16	-109.28	-0.95
10d	4.53	4.14	0.39	-122.98	-3.78
10g	4.35	4.52	-0.18	-138.81	-14.49
10h	4.62	4.71	-0.09	-137.20	-0.15

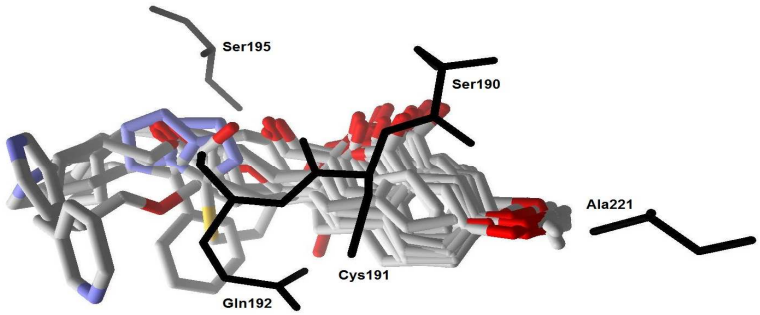


Figure 2 - Structure of isocoumarin analogs docked into the KLK5 active site. The residues shown are involved in hydrogen bonding with the compounds.

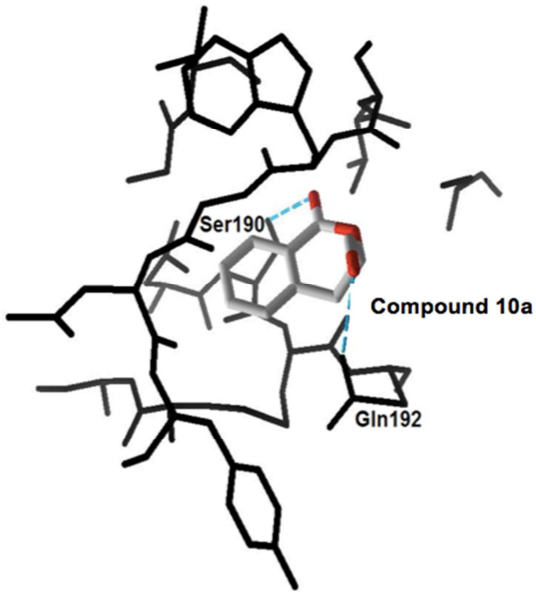


Figure 3 - Structure of compound **10a** docked into the KLK5 active site.

3. Conclusions

We synthesized a range of new isocoumarin and 3,4-dihydroisocoumarin derivatives. Some of these compounds exhibited antiproliferative activity *in vitro*; compound **10a**, in particular, showed an excellent activity against the breast cancer cell line MCF-7 with an excellent SI value.

Results from our docking studies reinforce the observation that the binding orientation of the inhibitors in the active site of KLK5 is governed by the interaction between the carbonyl group of the isocoumarin ring and Ser190 and Gln192. However, it is important to mention that some compounds also interact with Cys191 or Ala221.

Our theoretical and experimental results indicate that compound **10a** (Figure 3) is a promising compound and has selective activity against breast cancer. Furthermore, our findings indicate that structural modifications aiming to maximize interaction with Ser190 and Gln192 can lead to a better biological activity against cancer cells. In fact, the determination coefficient of 0.81 between the interaction energy data and experimental results guarantees that GI_{50} values are well reproduced by the docking scores.

4. Materials and Methods

4.1. Reagents and equipment

Commercially available reagents were purchased from Sigma–Aldrich and used without further purification. The solvents were purified by distillation. The melting points were determined using a Büchi apparatus and were uncorrected. Column chromatography was performed on Silica Gel 60 (70–230 mesh, Merck). The progress of the reactions was monitored by thin-layer chromatography using Merck silica plates (GF_{254}). ^{1}H and ^{13}C NMR spectra were recorded using a Bruker AVANCE DRX 200 MHz spectrometer by using tetramethylsilane as an internal standard. The results are presented as the chemical shift δ in ppm, number of protons, multiplicity, J values in Hertz, proton position, and carbon position. Multiplicities are abbreviated as follows: s, singlet; brs, broad signal; d, doublet; dd, double doublet; t, triplet; m, multiplet; qn, quintet; and td, triple doublet. Infrared spectra were recorded on a Perkin–Elmer Spectrum One SP-IR Spectrometer. High-resolution mass spectra were recorded using an LC-MS Bruker Daltonics Micro electrospray ionization-time-of-flight mass spectrometer (solvent: MeOH/H_2O, 1:1). All the compounds were purified by instrumental flash chromatography using a Biotage Isolera™ Dalton system. Chromatography was performed in SNAP Ultra 10 g columns using gradient elution with hexane and ethyl acetate. The elemental analyses were performed on a Perkin-Elmer CHNS model 2400. A mechanically stirred stainless steel Parr 4560 bomb coupled with a 4282 control module with a PID temperature controller and tachometer was used as the reaction vessel in catalytic hydrogenation reactions.

4.2. Synthesis

4.2.1 General procedure 1

CuI (0.2 equivalent), *trans*-4-hydroxy-L-proline (0.2 equivalent), K_2CO_3 (2.0 equivalents) and the derivative of 2-halogenated benzoic acid (1.0 equivalent) were added to a 50 mL round-bottom flask. The flask was sealed with a rubber septum and purged with nitrogen. Then DMSO enough to obtain a 0.2 mol/L solution of the benzoic acid derivative was added. Lastly the alkyne (1.0 equivalent) was added. The solution was stirred under nitrogen atmosphere at 70 °C for 16 hours. The reaction was monitored by TLC. After 16 hours, the reaction flask was cooled and its contents transferred to a separatory funnel where EtOAc (20 mL) was added. The

mixture was partitioned with water, collecting the organic phase, which was washed with HCl 2 mol/L, saturated $NaHCO_3$ and brine. The organic phase was dried over anhydrous sodium sulfate and removed by performing distillation. The remaining residue was purified by column chromatography on silica gel.

4.2.2 General procedure 2

NaH (3.0 equivalents), prewashed with hexane, was added to a 50 mL round-bottom flask. The flask was sealed with a rubber septum and attached to a bubbler containing mineral oil. The system was cooled in an ice bath. Then, a solution 0.2 mol/L of the alcohol (1.0 mmol in 5 mL of dry THF) was added dropwise. The mixture was stirred until gas release stopped. Lastly the electrophile (3.0 equivalents) was added dropwise. The ice bath was removed after an hour and the reaction was stirred for 16 hours at room temperature. The reaction was monitored by TLC. After 16 hours, the reaction was quenched by slow addition of ice water, followed by extraction with DCM (3 × 25 mL). The organic phase was washed with HCl 2 mol/L, saturated $NaHCO_3$ and brine, dried over anhydrous sodium sulfate and removed by performing distillation. The remaining residue was purified by column chromatography on silica gel to produce pure compound.

4.2.3 General procedure 3

The alcohol (1.0 equivalent) and triethylamine (1.5 equivalent) were added to a 50 mL round-bottom flask containing dry DCM enough to a 0.3 mol/L concentration. The flask was sealed with a rubber septum and purged with nitrogen. The mixture was cooled to -40 °C, and methanesulfonyl chloride (1.7 equivalent) was added to the mixture while being stirred vigorously. After one hour, the mixture was washed with HCl 2 mol/L, saturated $NaHCO_3$ and brine, dried over anhydrous sodium sulfate and removed by performing distillation, obtaining the pure mesylate product.

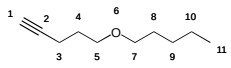
4.2.4 General procedure 4

Tetrazolium derivative (1.5 mmol in 10 mL of acetone) and potassium carbonate (3.0 equivalents) were added to a 50 mL round-bottom flask. The reaction mixture was stirred under nitrogen flow for 10 minutes. The mesylated isocoumarin (1.0 mmol in 10 mL of acetone) was added dropwise. The reaction mixture was stirred under reflux for 24 hours. After 24 hours, the acetone was removed by performing distillation and water was added to the residue, followed by extraction with DCM (3 × 25 mL). The organic phase was washed with HCl 2 mol/L, saturated $NaHCO_3$ and brine, dried over anhydrous sodium sulfate and removed by performing distillation. The remaining residue was purified by column chromatography on silica gel.

4.2.5 General procedure 5

The isocoumarin (50 mg), Pd/C (5 mg) and 25 mL of the solvent were added to the reaction vessel. The reactor was closed and three cycles of vacuum/argon were made. The vessel was pressurized with hydrogen up to the reported pressure. The reaction mixture was stirred for 8 to 24 hours. The reaction was monitored by TLC every 8 hours. At the end, the mixture was filtered in Celite® and the solvent was removed by performing distillation. The remaining residue was purified by column chromatography on silica gel.

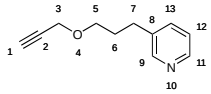
*Synthesis of 5-(pentyloxy)pent-1-yne (**7c**)*



4-pentyn-1-ol (1.0 mmol) and 1-iodopentane (3 mmol) were used according to **general procedure 2** to produce the compound **7c.**

Colorless oil; yield 87%; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (3H, t, J = 6.4 Hz, H-11), 1.27-1.36 (4H, m, H-9 and H-10), 1.57 (2H, qn, J = 6.2 Hz, H-8), 1.77 (2H, qn, J = 6.6 Hz, H-4), 1.94 (1H, t, J = 2.4 Hz, H-1), 2.29 (2H, td, J = 6.6 and 2.4 Hz, H-3), 3.40 (2H, t, J = 6.6 Hz, H-5), 3.49 (2H, t, J = 6.2 Hz, H-7); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.1 (C-11), 15.4 (C-3), 22.7 (C-10), 28.5, 28.8 and 29.6 (C-4, C-8 and C-9), 68.5 (C-1), 69.1 (C-7), 71.1 (C-5), 84.0 (C-2).

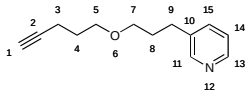
*Synthesis of 3-(3-(prop-2-yn-1-yloxy)propyl)pyridine (**7d**)*



Prop-2-yn-1-ol (2 mmol) was used according to **general procedure 3** to produce the mesylate intermediate **7f** in 97% yield. 3-pyridinepropanol (1.5 mmol) and **7f** (1.0 mmol) were used according to **general procedure 2** to produce compound **7d**.

Pale brown oil; yield: 79%; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.60-1.90 (2H, m, H-6), 2.35 (1H, s, H-1), 2.57 (2H, t, J = 7.5 Hz, H-7), 3.38 (2H, t, J = 6.0 Hz, H-5), 4.00 (2H, s, H-3), 7.00-7.10 (1H, m, H-12), 7.37 (1H, d, J = 6.3 Hz, H-13), 8.32 (2H, br s, H-9 and H-11); ^{13}C NMR (50 MHz, $CDCl_3$): δ 29.3 and 30.7 (C-6 and C-7), 58.0 (C-3), 68.6 (C-5), 74.4 (C-1), 79.7 (C-2), 123.2 (C-12), 135.8 (C-13), 136.9 (C-8), 147.2 (C-11), 149.9 (C-9).

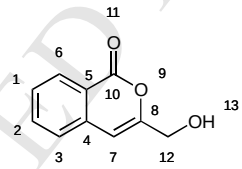
*Synthesis of 3-(3-(pent-4-yn-1-yloxy)propyl)pyridine (**7e**)*



Pent-4-yn-1-ol (2 mmol) was used according to **general procedure 3** to produce the mesylate intermediate **7g** in 97% yield. 3-pyridinepropanol (1.5 mmol) and **7g** (1.0 mmol) were used according to **general procedure 2** to produce compound **7e**.

Pale brown oil; yield: 83%; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.72 (2H, qn, J = 7.2 Hz, H-8), 1.81 (2H, dt, J = 7.0 and 6.2 Hz, H-4), 1.90 (1H, t, J = 2.7 Hz, H-1), 2.23 (2H, td, J = 7.0 and 2.7 Hz, H-3), 2.63 (2H, t, J = 7.2 Hz, H-9), 3.35 (2H, t, J = 6.2 Hz, H-5 or H-7), 3.43 (2H, t, J = 6.2 Hz, H-5 or H-7), 7.13 (1H, dd, J = 7.8 and 4.7 Hz, H-14), 7.44 (1H, dt, J = 7.8 and 1.8 Hz, H-15) 8.36 (1H, dd, J = 4.7 and 2.0 Hz, H-13), 8.39 (1H, d, J = 2.0 Hz, H-11); ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.3 (C-3), 28.7 and 29.6 (C-4 and C-8), 31.1 (C-9), 68.6 (C-1), 69.1 and 69.6 (C-5 and C-7), 84.0 (C-2), 123.4 (C-14), 136.1 (C-15), 137.3 (C-10), 147.3 (C-13), 150.0 (C-11).

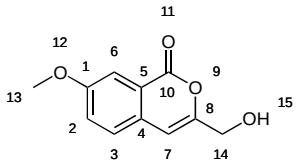
*Synthesis of 3-(hydroxymethyl)-1H-isochromen-1-one (**9a**)*



2-Iodobenzoic acid (1 mmol) and prop-2-yn-1-ol (1 mmol) were used according to **general procedure 1** to produce compound **9a**.

Yellow solid; 54% yield; m.p. (hexane) 92.2-93.6 ^{o}C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.75 (1H, br s, OH), 4.45 (2H, s, H-12), 6.52 (1H, s, H-7), 7.32 (1H, d, J = 8.0 Hz, H-3), 7.40 (1H, t, J = 8.0 Hz, H-1), 7.62 (1H, t, J = 8.0 Hz, H-2), 8.13 (1H, d, J = 8.0 Hz, H-6); ^{13}C NMR (50 MHz, $CDCl_3$): δ 61.3 (C-12), 103.2 (C-7), 120.4 (C-5), 125.8 (C-1), 128.4 (C-6), 129.6 (C-3), 135.1 (C-2), 137.0 (C-4), 156.0 (C-8), 162.9 (C-10); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{10}H_8O_3$, 177.0552; found, 177.0558; elemental analysis calcd for $C_{10}H_8O_3$: C 68.177, H 4.577; found: C 68.701, H 4.817.

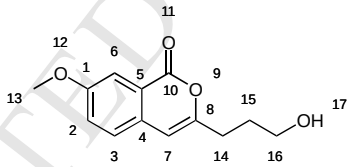
*Synthesis of 3-(hydroxymethyl)-7-methoxy-1H-isochromen-1-one (**9b**)*



2-Bromo-5-methoxybenzoic acid (1 mmol) and prop-2-yn-1-ol (1 mmol) were used according to **general procedure 1** to produce compound **9b**.

Yellow solid; 51% yield; m.p. (hexane) 128.4-129.0 ^{o}C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.66 (1H, br s, OH), 3.77 (3H, s, H-13), 4.36 (2H, s, H-14), 6.39 (1H, s, H-7), 7.10-7.30 (2H, m, H-2 and H-3), 7.52 (1H, s, H-6); ^{13}C NMR (50 MHz, $CDCl_3$): δ 55.9 (C-13), 61.6 (C-14), 103.3 (C-7), 110.3 (C-6), 121.8 (C-5), 124.8 (C-2), 127.5 (C-3), 130.7 (C-4), 153.6 (C-8), 159.8 (C-1), 162.9 (C-11); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{11}H_{10}O_4$, 207.0657; found, 207.0237; elemental analysis calcd for $C_{11}H_{10}O_4$: C 64.074, H 4.888; found: C 63.799, H 4.821.

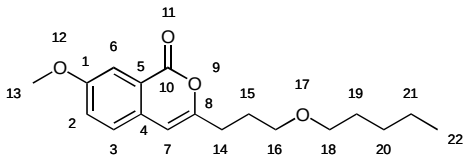
*Synthesis of 3-(3-hydroxypropyl)-7-methoxy-1H-isochromen-1-one (**9c**)*



2-Bromo-5-methoxybenzoic acid (1 mmol) and pent-4-yn-1-ol (1 mmol) were used according to **general procedure 1** to produce compound **9c**.

Yellow oil; 73% yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.70-2.10 (2H, m, H-15), 2.58 (2H, t, J = 8.0 Hz, H-14), 3.66 (2H, t, J = 6.0 Hz, H-16), 3.81 (3H, s, H-13), 7.10-7.30 (2H, m, H-2 and H-3), 7.55 (1H, s, H-6); ^{13}C NMR (50 MHz, $CDCl_3$): δ 29.8 and 30.0 (C-14 and C-15), 55.8 (C-13), 61.5 (C-16), 103.1 (C-7), 109.9 (C-6), 121.1 (C-5), 124.6 (C-2), 126.8 (C-3), 131.3 (C-4), 155.4 (C-8), 159.2 (C-1), 163.4 (C-10); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{13}H_{14}O_4$, 235.0970; found, 235.0888; elemental analysis calcd for $C_{13}H_{14}O_4$: C 66.656, H 6.024; found: C 66.484, H 5.917.

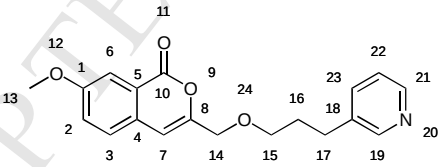
*Synthesis of 7-methoxy-3-(3-(pentyloxy)propyl)-1H-isochromen-1-one (**9d**)*



2-Bromo-5-methoxybenzoic acid (1 mmol) and **7c** (1 mmol) were used according to **general procedure 1** to produce compound **9d**.

Colorless oil; 69% yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.92 (3H, t, J = 6.0 Hz, H-22), 1.25-1.40 (4H, m, H-20 and H-21), 1.40-1.70 (2H, m, H-19), 1.80-2.15 (2H, m, H-15), 2.64 (2H, t, J = 8.0 Hz, H-14), 3.46 (4H, t, J = 6.0 Hz, H-16 and H-18), 3.90 (3H, s, H-13), 6.26 (1H, s, H-7), 7.10-7.30 (2H, m, H-2 and H-3), 7.66 (1H, s, H-6); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.1 (C-22), 22.6 (C-21), 27.2 and 28.4 (C-19 and C-20), 29.5 and 30.2 (C-14 and C-15), 55.7 (C-13), 69.4 and 71.1 (C-16 and C-18), 102.9 (C-7), 109.9 (C-6), 121.2 (C-5), 124.5 (C-2), 126.7 (C-3), 131.3 (C-4), 155.6 (C-8), 159.1 (C-1), 163.2 (C-10); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{18}H_{24}O_4$, 305.1747; found, 305.1744; elemental analysis calcd for $C_{18}H_{24}O_4$: C 71.027, H 7.947; found: C 70.899, H 7.971.

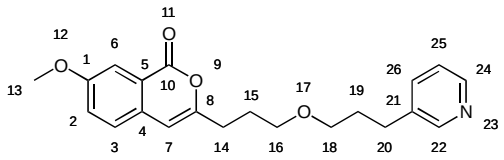
*Synthesis of 7-methoxy-3-((3-(pyridin-3-yl)propoxy)methyl)-1H-isochromen-1-one (**9e**)*



2-Bromo-5-methoxybenzoic acid (1 mmol) and **7d** (1 mmol) were used according to **general procedure 1** to produce compound **9e**.

Pale brown oil; 65% yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.70-1.90 (2H, m, H-16), 2.61 (2H, t, J = 6.0 Hz, H-17), 3.43 (2H, t, J = 7.0 Hz, H-15), 3.75 (3H, s, H-13), 4.15 (2H, s, H-14), 6.33 (1H, s, H-7), 7.00-7.30 (3H, m, H-2, H-3 and H-22), 7.38 (1H, d, J = 8.0 Hz, H-23), 7.54 (1H, d, J = 2.0 Hz, H-6); ^{13}C NMR (50 MHz, $CDCl_3$): δ 29.5 (C-16), 31.0 (C-17), 55.9 (C-13), 69.2 and 70.1 (C-14 and C-15), 104.2 (C-7), 110.3 (C-6), 122.0 (C-5), 123.5 (C-22), 124.6 (C-2), 127.4 (C-3), 130.5 (C-4), 136.1 (C-23), 137.1 (C-18), 147.5 (C-21), 150.0 (C-19), 151.6 (C-8), 159.8 (C-1), 162.6 (C-10); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{19}H_{19}NO_4$, 326.1392; found, 326.1716; elemental analysis calcd for $C_{19}H_{19}NO_4$: C 70.139, H 5.886, N 4.305; found: C 69.932, H 6.214; N 4.160.

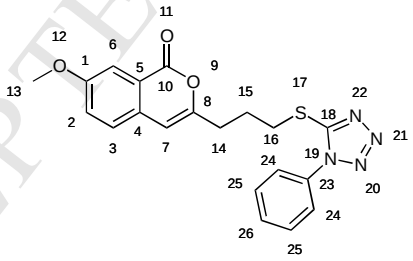
*Synthesis of 7-methoxy-3-(3-(3-(pyridin-3-yl)propoxy)propyl)-1H-isochromen-1-one (**9f**)*



2-Bromo-5-methoxybenzoic acid (1 mmol) and **7e** (1 mmol) were used according to **general procedure 1** to produce compound **9f**.

Pale brown oil; 71% yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.60-1.95 (4H, m, H-15 and H-19), 2.50-2.70 (4H, m, H-14 and H-20), 3.20-3.45 (4H, m, H-16 and H-18), 3.76 (3H, s, H-13), 6.14 (1H, s, H-7), 6.70-7.10 (3H, m, H-2, H-25 and H-26), 7.19 (1H, d, J = 7.8 Hz, H-3), 7.34 (1H, d, J = 2.4 Hz, H-6), 8.00-8.20 (2H, m, H-22 and H-24); ^{13}C NMR (50 MHz, $CDCl_3$): δ 26.8 and 29.2 (C-15 and C-19), 29.0 and 30.6 (C-14 and C-20), 55.3 (C-13), 69.2 (C-16 and C-18), 102.5 (C-7), 109.5 (C-6), 120.8 (C-5), 123.0 (C-25), 124.2 (C-2), 126.3 (C-3), 130.9 (C-4), 135.6 (C-26), 136.9 (C-21), 146.9 (C-24), 149.7 (C-22), 155.0 (C-8), 158.7 (C-1), 162.8 (C-10); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{21}H_{23}NO_4$, 354.1705; found, 354.1948; elemental analysis calcd for $C_{21}H_{23}NO_4$: C 71.369, H 6.560, N 3.963; found: C 71.805, H 6.638; N 4.044.

Synthesis of 7-methoxy-3-(3-((1-phenyl-1*H*-tetrazol-5-yl)thio)propyl)-1*H*-isochromen-1-one ***(9g)***

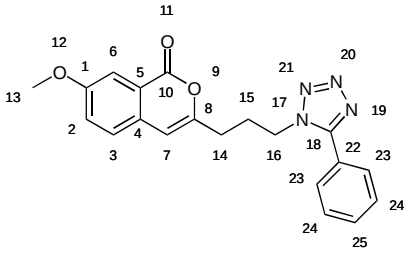


9c (3 mmol) was submitted to **general procedure 3** to produce compound **9i** in 93% yield. **9i** (1 mmol) and 1-phenyl-1*H*-tetrazole-5-thiol (1.5 equiv) were used according to **general procedure 4** to produce compound **9g**.

Colorless solid; 90% yield; m.p. (hexane/DCM 9:1) 109.1-110.7 ^{o}C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.00-2.30 (2H, m, H-15), 2.61 (2H, t, J = 7.0 Hz, H-14), 3.37 (2H, t, J = 7.0 Hz, H-16), 3.78 (3H, s, H-13), 6.23 (1H, s, H-7), 7.10-7.30 (2H, m, H-2, H-3), 7.40-7.60 (4H, m, H-6, H-24, H-25 and H26); ^{13}C NMR (50 MHz, $CDCl_3$): δ 26.4 (C-15), 32.0 (C-14), 32.2 (C-16), 55.7 (C-13), 103.5 (C-7), 109.9 (C-6), 121.1 (C-5), 123.7 (C-24), 124.5 (C-2), 126.8 (C-3), 129.8 and 130.2 (C-25 and C-26), 130.9 (C-4), 133.5 (C-23), 153.8 (C-18), 154.0 (C-8),

159.2 (C-1), 162.9 (C-10); HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{20}H_{18}N_4O_3S$, 417.0997; found, 417.1012; elemental analysis calcd for $C_{20}H_{18}N_4O_3S$: C 60.899, H 4.600, N 14.204; found: C 60.637, H 4.490; N 13.953.

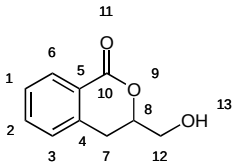
*Synthesis of 7-methoxy-3-(3-(5-phenyl-1H-tetrazol-1-yl)propyl)-1H-isochromen-1-one (**9h**)*



9i (1 mmol) and 5-phenyl-1*H*-tetrazole (1.5 equiv) were used according to **general procedure 4** to produce compound **9h**.

Colorless solid; 83% yield; m.p. (hexane/DCM 9:1)105.1-106.4 ^oC; ^1H NMR (200 MHz, $CDCl_3$): δ 2.30-2.70 (4H, m, H-14 and H-15), 3.86 (3H, s, H-13), 4.69 (2H, t, J = 6.0 Hz, H-16), 6.19 (1H, s, H-7), 7.20-7.30 (2H, m, H-2, H-3), 7.30-7.50 (2H, m, H-24 and H-25), 7.90-8.10 (1H, m, H-23); ^{13}C NMR (50 MHz, $CDCl_3$): δ 26.5 (C-15), 30.4 (C-14), 52.1 (C-16), 55.8 (C-13), 104.0 (C-7), 110.0 (C-6), 121.3 (C-5), 124.6 (C-25), 126.8 (C-3 and 24), 127.3 (C-22), 128.9 (C-23), 130.4 (C-25), 130.8 (C-4), 153.1 (C-8), 159.4 (C-1), 162.9 (C-18), 165.3 (C-10); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{20}H_{18}N_4O_3$, 363.1457; found, 363.1543; elemental analysis calcd for $C_{20}H_{18}N_4O_3$: C 66.288, H 5.007, N 15.461; found: C 66.007, H 4.983; N 15.239.

*Synthesis of 3-(hydroxymethyl)isochroman-1-one (**10a**)*

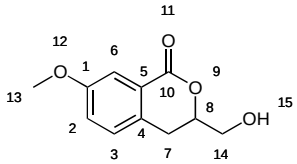


9a (0.5 mmol) was used according to **general procedure 5** to produce compound **10a**.

Colorless oil; 98% yield; ^1H NMR (200 MHz, $CDCl_3$): δ 3.00-3.25 (3H, m, H-12 and OH), 3.70-3.90 (2H, m, H-7), 4.40-4.70 (1H, m, H-8), 6.90-7.20 (1H, m, H-3), 7.10-7.40 (1H, m, H-1), 7.44 (1H, t, J = 8.0 Hz, H-2), 7.96 (1H, d, J = 8.0 Hz, H-6); ^{13}C NMR (50 MHz, $CDCl_3$): δ 29.3 (C-7), 64.2 (C-12), 79.3 (C-8), 124.7 (C-5), 127.8 (C-1 and C-3), 130.4 (C-6), 134.1 (C-2), 139.1 (C-4), 165.6 (C-10); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for

$C_{10}H_{10}O_3$, 179.0708; found, 179.0417; elemental analysis calcd for $C_{10}H_{10}O_3$: C 67.406, H 5.657; found: C 67.876, H 5.671.

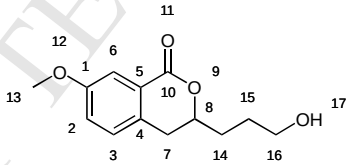
*Synthesis of 3-(hydroxymethyl)-7-methoxyisochroman-1-one (**10b**)*



9b (0.5 mmol) was used according to **general procedure 5** to produce compound **10b**.

Colorless solid; quantitative; m.p. (hexane)99.8-100.1 ^{o}C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.60-3.20 (1H, m, H-7), 3.34 (1H, s, OH), 3.70-3.90 (2H, m, H-14), 3.77 (3H, s, H-13), 4.40-4.60 (1H, m, H-8), 6.90-7.20 (2H, m, H-2 and H-3), 7.48 (1H, s, H-6); ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.4 (C-7), 55.7 (C-13), 64.1 (C-14), 79.6 (C-8), 113.0 (C-6), 121.9 (C-5), 125.5 (C-2), 128.9 (C-3), 131.2 (C-4), 159.0 (C-1), 165.6 (C-10); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{11}H_{12}O_4$, 209.0814; found, 209.1013; elemental analysis calcd for $C_{11}H_{12}O_4$: C 63.454, H 5.809; found: C 63.050, H 5.931.

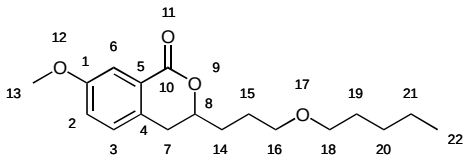
*Synthesis of 3-(3-hydroxypropyl)-7-methoxyisochroman-1-one (**10c**)*



9c (0.5 mmol) was used according to **general procedure 5** to produce compound **10c**.

Colorless oil; 94% yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.60-1.90 (4H, m, H-14 and H-15), 2.38 (1H, s, H-17), 2.80-2.90 (1H, m, H-7), 3.66 (2H, t, J = 6.0 Hz, H-16), 3.78 (3H, s, H-13), 4.40-4.60 (1H, m, H-8), 6.90-7.20 (1H, m, H-2 and H-3), 7.50 (1H, d, J = 2.0 Hz, H-6); ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.1 (C-15), 31.5 (C-14), 32.5 (C-7), 55.7 (C-13), 62.3 (C-16), 79.1 (C-8), 113.0 (C-6), 121.7 (C-5), 125.8 (C-2), 128.7 (C-3), 131.6 (C-4), 159.0 (C-1), 166.0 (C-10); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{13}H_{16}O_4$, 237.1127; found, 237.0953; elemental analysis calcd for $C_{13}H_{16}O_4$: C 66.087, H 6.826; found: C 66.118, H 6.879.

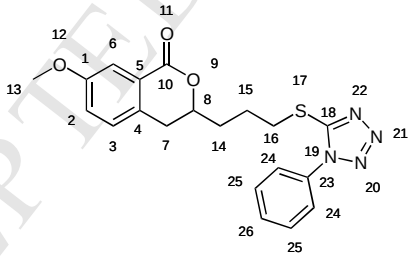
*Synthesis of 7-methoxy-3-(3-(pentyloxy)propyl)isochroman-1-one (**10d**)*



9d (0.5 mmol) was used according to **general procedure 5** to produce compound **10d**.

Colorless oil; 89% yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.80 (3H, t, J = 6.6 Hz, H-22), 1.10-1.35 (4H, m, H-19 and H-20), 1.30-1.60 (2H, m, H-19), 1.70-2.00 (2H, m, H-15), 2.52 (2H, t, J = 7.2 Hz, H-14), 2.70-2.95 (2H, m, H-7), 3.20-3.50 (4H, m, H-6 and H-18), 3.78 (3H, s, H-13), 4.40-4.60 (1H, m, H-8), 6.90-7.20 (2H, m, H-2 and H-3), 7.49 (1H, d, J = 2.4 Hz, H-6); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.0 (C-22), 22.5 (C-21), 27.1, 28.3, 29.4 and 30.1 (C-14, C-15, C-19 and C-20), 32.5 (C-7), 55.6 (C-13), 69.3 and 70.9 (C-16 and C-18), 79.0 (C-8), 112.9 (C-6), 121.6 (C-3), 125.7 (C-5), 128.6 (C-2), 131.5 (C-4), 158.9 (C-1), 165.9 (C-10); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{18}H_{26}O_4$, 307,1909; found, 307,2127; found, 237.0953; elemental analysis calcd for $C_{18}H_{26}O_4$: C 70.560, H 8.553; found: C 70.168, H 8.651.

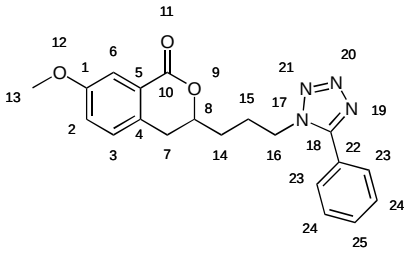
*Synthesis of 7-methoxy-3-(3-((1-phenyl-1H-tetrazol-5-yl)thio)propyl)isochroman-1-one (**10g**)*



9g (0.5 mmol) was used according to **general procedure 5** to produce compound **10g**.

Colorless solid; 84% yield; m.p. (hexane/DCM 9:1) 98.3-100.7 ^{o}C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.70-2.20 (4H, m, H-15 and H-16), 2.60-2.90 (2H, m, H-14), 3.37 (2H, dd, J = 8.0 and 6.0 Hz, H-7), 3.73 (3H, s, H-13), 4.30-4.60 (1H, m, H-8), 6.90-7.20 (2H, m, H-2 and H-3), 7.40-7.60 (4H, m, H-6, H-24, H-25 and H-26); ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.9 (C-15), 32.3 (C-14), 32.8 (C-7), 33.7 (C-16), 55.6 (C-13), 78.3 (C-8), 112.9 (C-6), 121.6 (C-5), 123.8 (C-24), 125.7 (C-2), 128.6 (C-3), 129.8 (C-25), 130.2 (C-26), 131.2 (C-4), 133.6 (C-23), 154.7 (C-18), 158.9 (C-1), 165.4 (C-10); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{20}H_{20}N_4O_3S$, 397.1334; found, 397.0883; elemental analysis calcd for $C_{20}H_{20}N_4O_3S$: C 60.589, H 5.085, N 14.132; found: C 60.503, H 5.185, N 14.132.

*Synthesis of 7-methoxy-3-(3-(5-phenyl-1H-tetrazol-1-yl)propyl)isochroman-1-one (**10h**)*



9h (0.5 mmol) was used according to **general procedure 5** to produce compound **10h**.

Colorless solid; 81% yield; m.p. (hexane/DCM 9:1) 92.5-94.2 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.55-2.20 (2H, m, H-15), 2.10-2.60 (2H, m, H-14), 2.60-3.00 (2H, m, H-7), 3.77 (3H, s, H-13), 4.30-4.65 (1H, m, H-8), 4.70 (2H, t, J = 6.0 Hz, H-16), 6.90-7.20 (2H, m, H-2 and H-3), 7.30-7.60 (3H, m, H-6, H-24 and H-25), 7.90-8.20 (1H, m, H-23); ^{13}C NMR (50 MHz, $CDCl_3$): δ 25.1 (C-15), 31.8 (C-14), 32.5 (C-7), 52.8 (C-16), 55.7 (C-13), 78.0 (C-8), 113.0 (C-6), 121.8 (C-2), 125.7 (C-5), 126.9 (C-23), 127.4 (C-22), 128.6 (C-3), 129.0 (C-24), 130.4 (C-25), 131.1 (C-4), 159.1 (C-1), 165.4 (C-10); HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{20}H_{20}N_4O_3$, 365.1608; found, 365.2039; elemental analysis calcd for $C_{20}H_{20}N_4O_3$: C 65.921, H 5.532, N 15.375; found: C 65.558, H 5.752, N 15.065.

4.3. Antiproliferative evaluation

4.3.1. Cell lines

Human tumor cell lines U251 (glioma, CNS), MCF-7 (human breast adenocarcinoma), NCI-ADR/RES (ovarian expressing phenotype multiple drug resistance), 786-0 (kidney), NCI-H460 (lung, non-small cells), PC-3 (prostate), HT-29 (human colon adenocarcinoma) were kindly provided by the National Cancer Institute (NCI-USA). The immortalized human keratinocyte (HaCaT) cell line was kindly was provided by prof. Dr. Ricardo Della Coletta (University of Campinas, UNICAMP).

4.3.2. Antiproliferative activity assay

The *in vitro* antiproliferative activity assay was performed as described by Monks et al. (1991). All cell lines were maintained in RPMI 1640 (Gibco, USA) supplemented with 5% (v/v) fetal bovine serum (FBS, Gibco) and 1% (v/v) penicillin:streptomycin (Nutricell, 1000 U/mL:1000 g/mL) in a humidified atmosphere with 5% CO2, at 37°C. For the experiments, cell lines were used between passages 5 to 12. Cells in 96 well plates (100 µL cells/well, 3 to 5 x 10^4 cells mL^{-1}) were exposed to sample concentrations in DMSO/RPMI (0.25, 2.5, 25 and

250 μg/ml) at 37 °C, in a humidified atmosphere with 5% CO2, for 48 h. Doxorubicin (DOX; 0.025 – 25 μg/mL) was used as positive control. The final DMSO concentration (≤ 0.25%) did not affect cell viability. Before (T_0) and after sample addition [compound-free (*C*) and tested (*T*) cells], cells were fixed with 50% trichloroacetic acid, and cell proliferation was determined by the spectrophotometric quantification (540 nm) of cellular protein content using sulforhodamine B assay. Cell proliferation was determined according to the equation 100 x [(T-T_0)/C-T_0], for $T_0 < T \leq C$, and 100 x [(T-T_0)/T_0], for $T \leq T_0$ and a concentration-response curve for each cell line was plotted using software ORIGIN 7.5 (OriginLab Corporation).

4.3.3. Data analysis

Using the concentration-response curve for each cell line, GI50 (concentration that inhibits cell growth by 50%) was determined through non-linear regression analysis using the software ORIGIN 8.0 (OriginLab Corporation) (Shoemaker, 2006). The average activity (mean of log GI_{50}) (Shoemaker, 2006) and the selectivity index (SI) (relation GI_{50} HaCat/GI_{50} tumor cell line) were calculated for each compound using MS Excel software.

4.4. Docking studies

In order to obtain a molecular interaction profile of isocoumarin derivatives as antitumor agents, the crystal structure of kallikrein 5 (KLK5) was obtained [29] from the Protein Data Bank (PDB code: 2PSX) and used for docking and alignment of the structures synthetized (Table 4). The calculation of the docking energies of the ligands inside the hK5 active site was performed using the software Molegro Virtual Docker® (MVD®) [30]. This program is able to predict the most likely conformation of how a ligand will bind to a macromolecule [31, 32]. In summary, MolDock scoring function is used to automatically superimpose a flexible molecule onto a template molecule. The docking search algorithm used in MVD is based on an evolutionary algorithm, where interactive optimization techniques inspired by Darwinian evolution theory and a new hybrid search algorithm called guided differential evolution are employed [33, 34]. The guided differential evolution algorithm combines the differential evolution optimization technique with a cavity prediction algorithm during the search process, which allows for a fast and accurate identification of potential binding modes (poses). The active site exploited in docking studies was defined, with a subset region of 8.0 Å from the center of the ligand. The interaction modes of the ligand with the enzyme active site were determined as the highest energy scored protein-ligand complex used during docking [35].

Appendix A. Supplementary data

Supplementary data (S1: images of the antiproliferative activity assay, S2: ^{1}H and ^{13}C NMR spectra, and S3: high resolution mass spectra related to this article can be found at http://...

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The authors have declared no conflict of interest.

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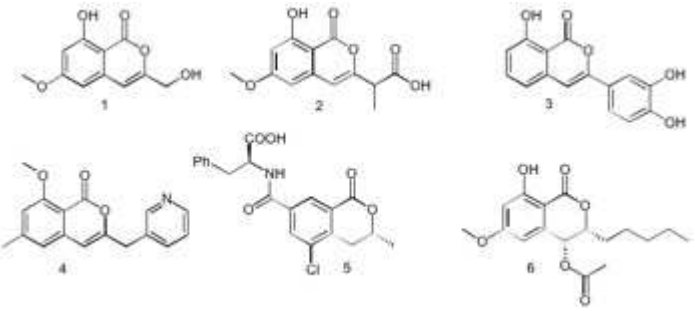

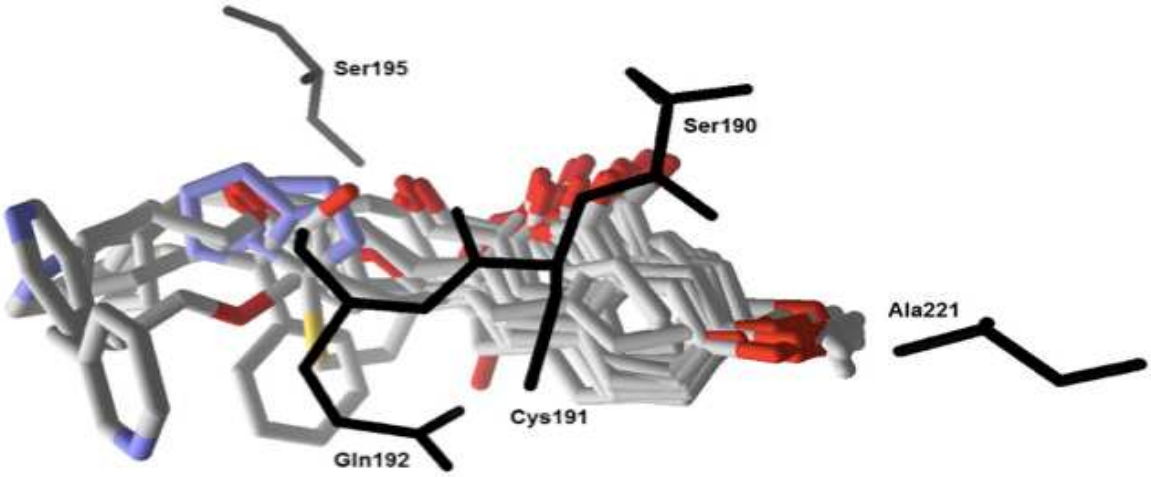

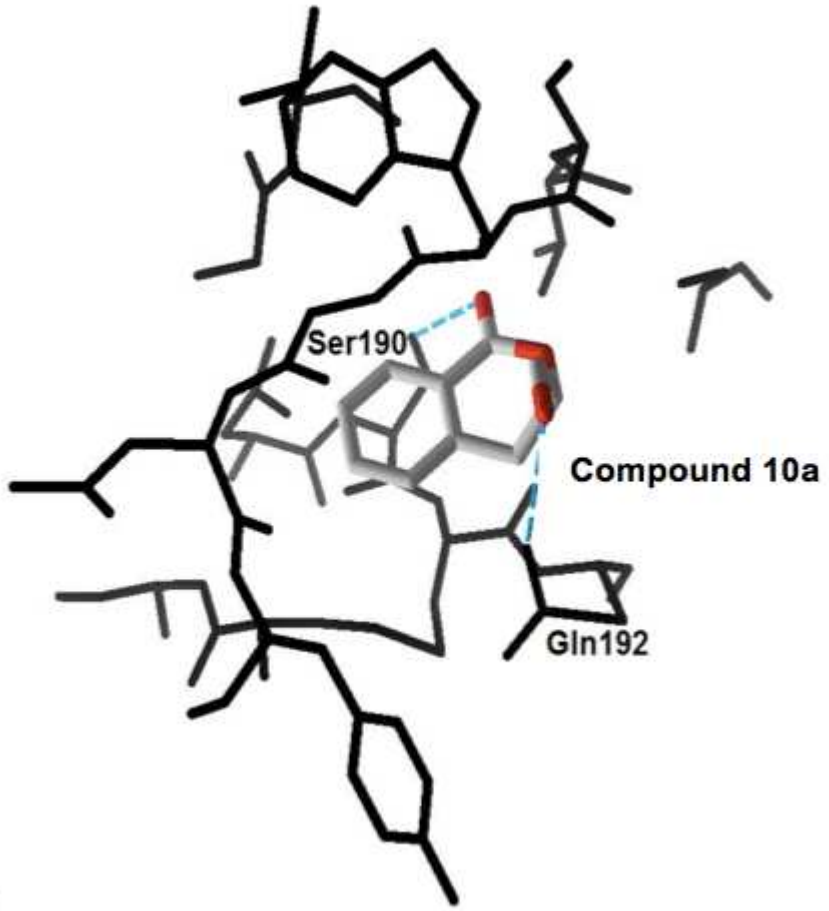

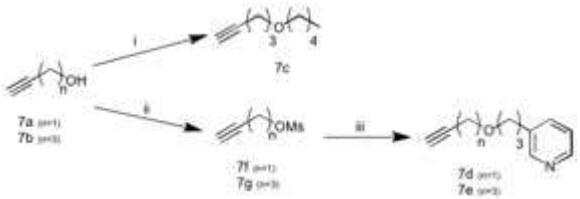

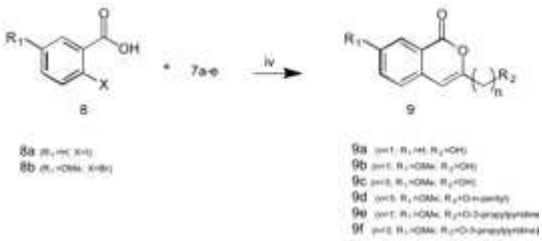

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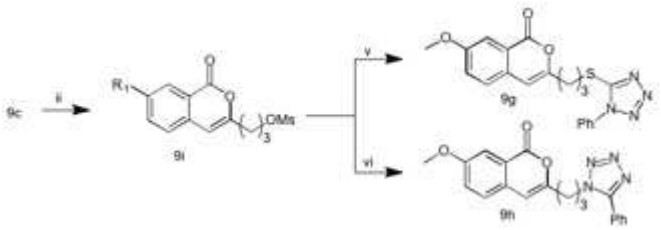

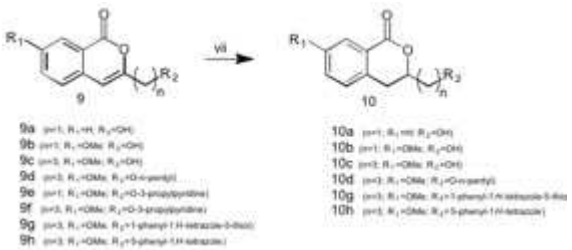

9a (n=1, R_1=H, R_2=OH)
9b (n=1, R_1=OMe, R_2=OH)
9c (n=3, R_1=OMe, R_2=OH)
9d (n=3, R_1=OMe, R_2=O-n-pentyl)
9e (n=1, R_1=OMe, R_2=O-3-propylpyridine)
9f (n=3, R_1=OMe, R_2=O-3-propylpyridine)
9g (n=3, R_1=OMe, R_2=1-phenyl-1*H*-tetrazole-5-thiol)
9h (n=3, R_1=OMe, R_2=5-phenyl-1*H*-tetrazole)

10a (n=1, R_1=H, R_2=OH)
10b (n=1, R_1=OMe, R_2=OH)
10c (n=3, R_1=OMe, R_2=OH)
10d (n=3, R_1=OMe, R_2=O-n-pentyl)
10g (n=3, R_1=OMe, R_2=1-phenyl-1*H*-tetrazole-5-thio
10h (n=3, R_1=OMe, R_2=5-phenyl-1*H*-tetrazole)

HIGHLIGHTS

- Novel isocoumarins and 3,4-dihydroisocoumarins were synthesized.
- *In vitro* antiproliferative activity was evaluated.
- Docking calculations were performed to predict the possible mechanism of action.
- Compound **10a** exhibits potent antiproliferative activity against breast cancer cells.
- This compound also shows an excellent selectivity index for that cell type.