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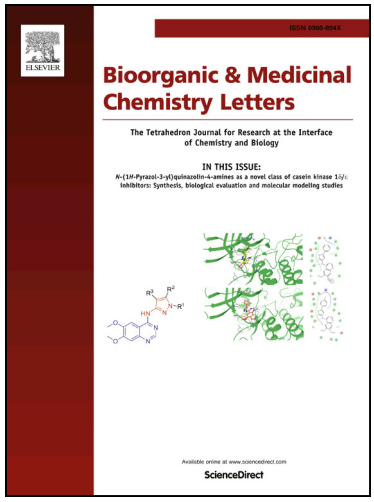

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Synthesis of novel hybrids of pyrazole and coumarin as dual inhibitors of COX-2 and 5-LOX

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Abstract

In our previous study, we designed a series of pyrazole derivatives as novel COX-2 inhibitors. In order to obtain novel dual inhibitors of COX-2 and 5-LOX, herein we designed and synthesized 20 compounds by hybridizing pyrazole with substituted coumarin who was reported to exhibit 5-LOX inhibition to select potent compounds using adequate biological trials sequentially including selective inhibition of COX-2 and 5-LOX, anti-proliferation *in vitro*, cells apoptosis and cell cycle. Among them, the most potent compound **11g** (IC_{50} = 0.23 ± 0.16 μM for COX-2, IC_{50} = 0.87 ± 0.07 μM for 5-LOX, IC_{50} = 4.48 ± 0.57 μM against A549) showed preliminary superiority compared with the positive controls **Celecoxib** (IC_{50} = 0.41 ± 0.28 μM for COX-2, IC_{50} = 7.68 ± 0.55 μM against A549) and **Zileuton** (IC_{50} = 1.35 ± 0.24 μM for 5-LOX). Further investigation confirmed that **11g** could induce human non-small cell lung cancer A549 cells apoptosis and arrest the cell cycle at G2 phase in a dose-dependent manner. Our study might contribute to COX-2, 5-LOX dual inhibitors thus exploit promising novel cancer prevention agents.

Key words: COX-2; 5-LOX; Pyrazole; Coumarin; Cancer

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The incidence rate of malignant tumor which is the leading reason of death worldwide is still increasing [1]. To improve this situation, developing agents aiming at anti-inflammation could be a potential approach for treatment of carcinoma due to the link between inflammation and carcinoma [2]. It is recognized that several inflammatory mediators like chemokines, cytokines and growth factors, activated chronically to promote the development of tumors by regulating the tumor microenvironment. Targeting cancer mediators linked to inflammation in cancer cells, especially regulating vascular programming and microenvironment of solid tumors, might be a good strategy [3].

Prostaglandin E_2 (PGE_2), exerting major effects on the inflammation, is a mediator with profound biological functions on cell proliferation, angiogenesis, apoptosis and metastasis. Therefore, it is necessary to inhibit the biosynthesis of PGE_2 to reduce the adverse effects of long-term use of common nonsteroidal anti-inflammatory drugs (NSAIDs) [4, 5]. As documented, Cyclooxygenases (COXs) pathway, one of the two main metabolic pathways of Rarchidonic acid (AA), contributing to the production of PGE_2, consists of three subtypes: COX-1, COX-2 and COX-3 [6]. COX-1 exists in most normal tissues or cells and COX-3 primarily in the central nervous system, while COX-2, almost undetectable in most normal cells or tissues, in turn, is found up-regulated in various types and stages of tumor. Further study has showed that the peroxidase activity of COX-2 actually promotes the deterioration of tumors [7, 8]. Currently, a number of clinical trials, testing the anti-cancer activity of selective COX-2 inhibitors are under conduction as said in database of clinical trials (www.clinicaltrials.gov) due to the inhibition of COX-1 could cause serious side effects like ulcers [9, 10].

Lipoxygenases (LOXs) pathway is the other main pathway in the AA cascade. To be specific, AA is transformed into hydroxyeicosatetraenioc acids (HETEs) or leukotrienes (LTs), reported to play a major role in the development and progression of human cancers by LOXs [11-14]. 5-LOX, a member of LOXs and pro-inflammatory enzyme, works with COXs jointly to dominate in inflammation and foster tumor development [15, 16]. Emerging evidence suggested that the high expression of 5-LOX

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and its products, particularly leukotriene B_4 (LTB_4) were found in some kinds of human cancer [17]. Similar to COX-2, the overexpression of 5-LOX is significantly associated with cell cycle, blocking apoptosis and stimulating angiogenesis [18]. However, the inhibition of either COX-2 or 5-LOX was reported to promote the other pathway of AA metabolism as compensatory mechanism [19]. Considering the similarities of the two enzymes, the dual inhibition of them to get more efficiency and safer agents for human cancer treatment is promising, albeit the exact mechanism has not been fully illustrated.

Most selective COX-2 inhibitors whose side effects include gastric ulceration and gastrointestinal hemorrhage, comprising with a five-membered core, can be characterized as diarylheterocycles such as **Celecoxib**, **Valdecoxib**, **Parecoxib** and **Rofecoxib** [20, 21]. Meanwhile, as recent study showed, coumarin derivatives, possessing biological activities like antibacterial, antioxidant, anti-inflammatory and anticancer, existing in tonka beans, *Kosteletzkya virginica*, etc, is the redox-active inhibitor of 5-LOX [22, 23]. The previous research has shown the more potent effects and less complexity of the prediction of pharmacodynamic and pharmacokinetic relationships by integrating two drugs together, compared with using the two drugs independently [24]. As reported, however, patients, receiving coumarin alone as a single daily oral dose, caused many side effects, including insomnia, nausea, vomiting, diarrhea, and dizziness. Based on the above, we designed a series of compounds to seek for the most potent and safe agent on treating cancer, beginning with the famous COX-2 selective inhibitors, **Celecoxib**, and integrating with substituted coumarin.

In this study, we modified the linker between pyrazole ring and substituted coumarin to increase the flexibility and polarity of the synthesized compounds to develop better fitting into the binding pockets of target proteins. Moreover, we used a series of substituted acetophenone considering factors as the same substitution on *ortho*, *meta*, and *para*-position, different substitutes on the same position and the number of substitutes on the same phenyl ring, and a trio of substituted coumarin (3-carboxy coumarin, 4-hydroxycoumarin and 7-hydroxycoumarin) for the integration with a series of pyrazole sulfonamides to select the most effective and safe drug for

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screening *in vitro* as dual inhibitors of COX-2 and 5-LOX (**Fig.1**).

The synthesis route of the intermediate products **3a-3j** has been described in our previous research [5] (**Scheme 1**). 4-(5-(4-fluorophenyl)-3-(hydroxymethyl)-1*H*-pyrazol-1-yl) benzenesulfonamide (**3b**) was reduced by lithium aluminium hydride to give the intermediate product (**4b**) [25], which was linked with coumarin-3-carboxylic acid through esterification reaction to yield target compound (5-(4-fluorophenyl)-1-(4-sulfamoylphenyl)-1*H*-pyrazol-3-yl)methyl 2-oxo-2*H*-chromene-3-carboxylate (**5b**)(**Scheme 2**). Meanwhile, **3a** was hydrolyzed by KOH into pyrazolesulfonamide carboxylic acids **6a**, which was directly integrated with 7-hydroxycoumarin directly after activated to get target compound **7a** (**Scheme 3**).

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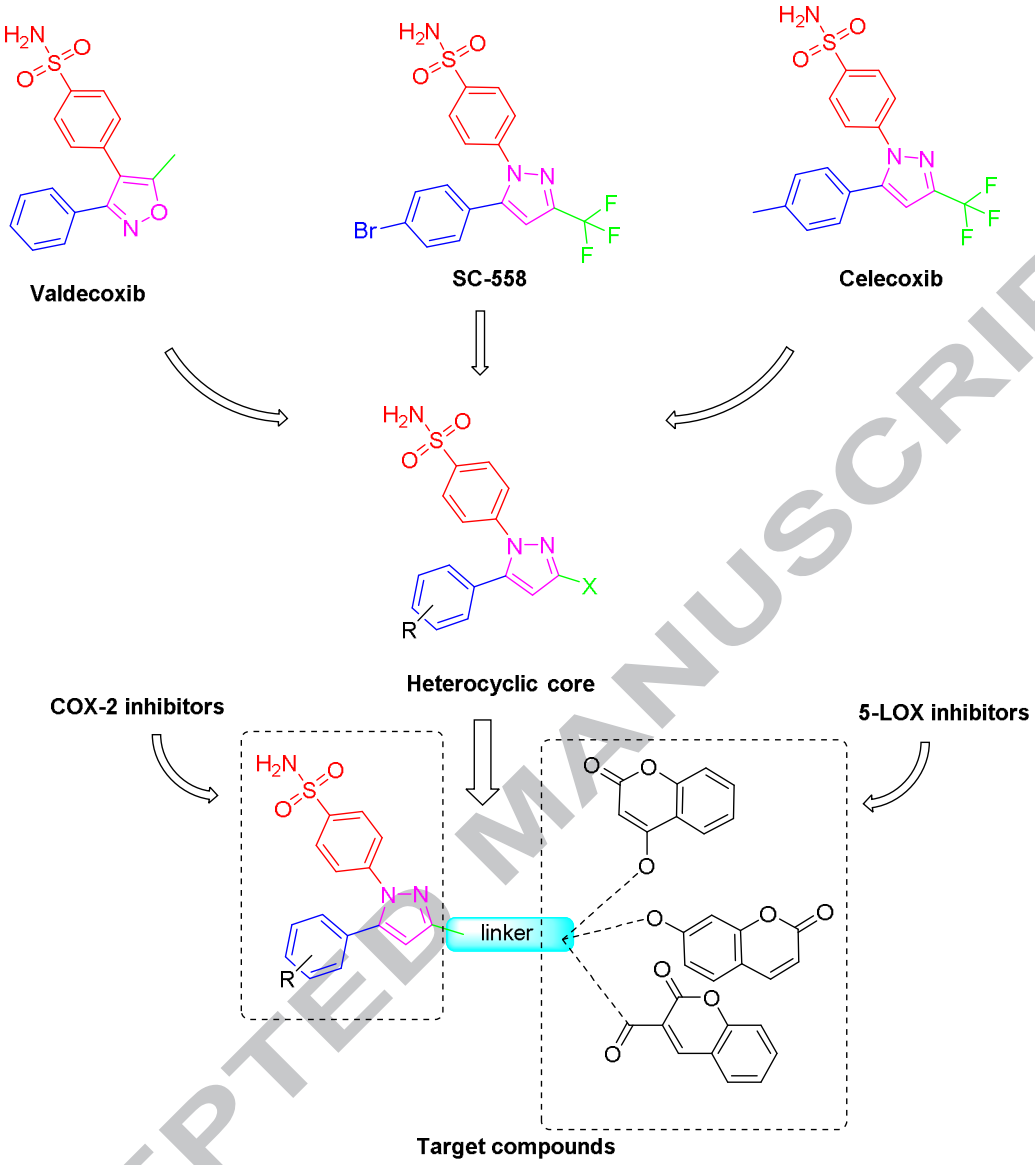
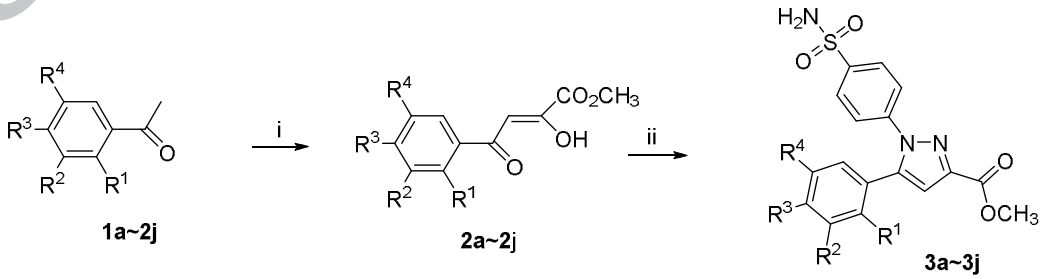


Fig.1 Pyrazole coumarin derivatives as dual inhibitors of COX-2 and 5-LOX

Scheme 1 Synthesis of compounds **3a-3j**



Reagents and conditions: (i) dimethyl oxalate, methanol, reflux 6 h; (ii)

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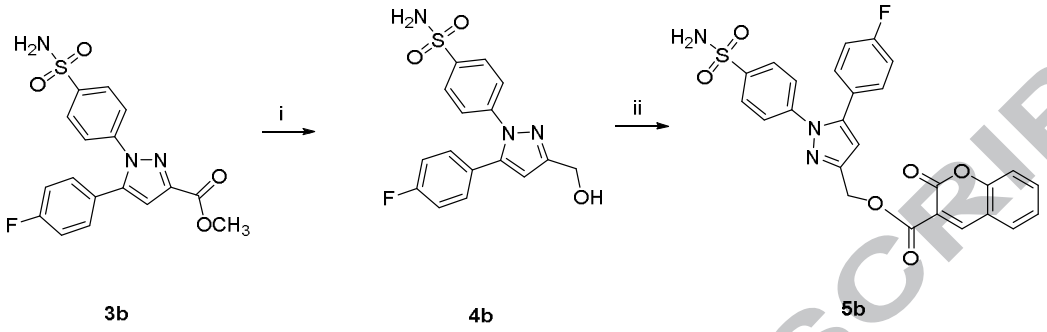
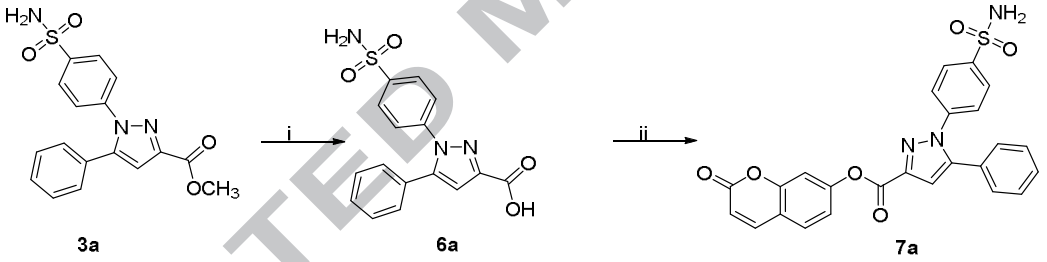
4-hydrazinylbenzenesulfonamide, methanol, reflux, 6 h;

Scheme 2 Synthesis of compounds **5b**



Reagents and conditions: (i) LAH, tetrahydrofuran, r.t., 2 h; (ii) EDC·HCl, HOBt, DMAP, coumarin-3-carboxylic acid, dichloromethane, r.t., overnight.

Scheme 3 Synthesis of compounds **7a**



Reagents and conditions: (i) KOH, methanol, reflux, 2 h; (ii) EDC·HCl, HOBt, DMAP, 7-hydroxycoumarin, dichloromethane, r.t., overnight;

Scheme 4 Synthesis of compounds **9a**, **b**, **c**, **e**, **g**, **h**, **i**, **j, 11a~11i**

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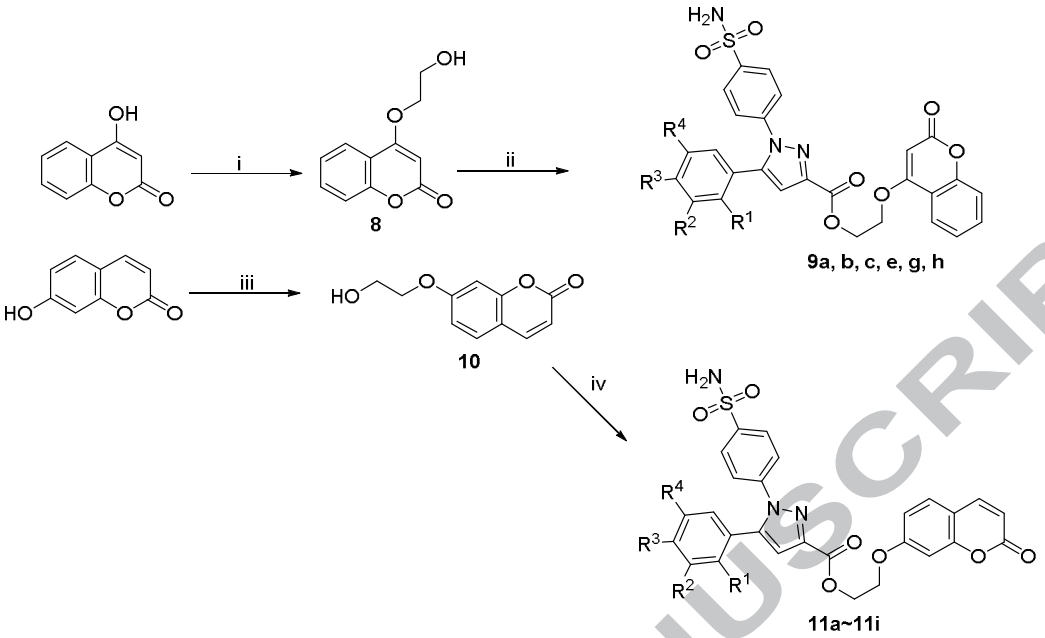
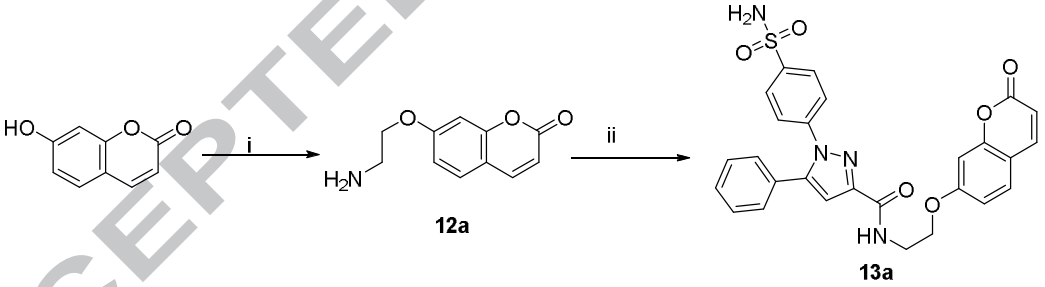


Reagents and conditions: (i) KCO_3, KI, 2-bromoethyl alcohol, DMF, 65 ˚C overnight; (ii) EDC·HCl, HOBt, **6a**, **b**, **c**, **e**, **g**, **h**, **i**, **j**, DMAP, dichloromethane, r.t., overnight; (iii) KCO_3, KI, 2-bromoethyl alcohol, DMF, 65 ˚C overnight; (iv) KCO_3, KI, **6a ~ 6i**, DMF, 65 ˚C, overnight;

Scheme 5 Synthesis of compounds **13a**



Reagents and conditions: (i) KCO_3, KI, 2-bromoethylamine, DMF, 65 ˚C, overnight; (ii) EDC·HCl, HOBt, DMAP, **6a**, dichloromethane, r.t., overnight.

The synthesis of pyrazolesulfonamide esters was outlined in **Scheme 4**. Like **Scheme 3**, the compounds **3b-3j** was hydrolyzed into the corresponding pyrazolesulfonamide carboxylic acids **6b-6j** in the presence of KOH. The construction of coumarin esters **8, 10** were achieved by etherification reaction of

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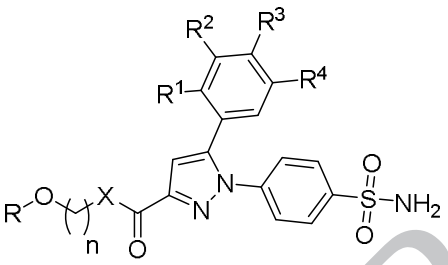
either 4-hydroxycoumarin or 7-hydroxycoumarin with 2-bromoethyl alcohol in the presence of KOH. Next, following the acid activation in the presence of EDC·HCl, HOBt and DMAP, **6a**, **b**, **c**, **e**, **g**, **h** reacted with **8** through esterification condensation reaction gave the corresponding target compounds **9 a**, **b**, **c**, **e**, **g**, **h** while **6a-6h** were transformed into **11a-11h** by condensing with **10** respectively.

And the synthesis route of coumarin amide derivative is shown in **Scheme 5**. Compound **12a** could be directly prepared by treating 7-hydroxycoumarin with 2-bromoethylamine in the presence of KOH in Dimethyl Formamide, by the successive reaction with pyrazolesulfonamide carboxylic acids (**6a**) in the presence of EDC·HCl, HOBt and DMAP in dichloromethane, gave compound **13a**.

Table 1 Structure of target compound



7a, 9a~9h, 11a~11i, 13a

Compds.	R	n	X	R^1	R^2	R^3	R^4
7a	7-coumarin	0	O	H	H	H	H
9a	4-coumarin	2	O	H	H	H	H
9b	4-coumarin	2	O	H	H	F	H
9c	4-coumarin	2	O	H	H	CH_3	H
9e	4-coumarin	2	O	H	Cl	H	H
9g	4-coumarin	2	O	H	OCH_3	OCH_3	OCH_3
9h	4-coumarin	2	O	H	OCH_3	H	H
9i	4-coumarin	2	O	H	F	H	H
9j	4-coumarin	2	O	H	H	NO_2	H
11a	7-coumarin	2	O	H	H	H	H

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11b	7-coumarin	2	O	H	H	F	H
11c	7-coumarin	2	O	H	H	CH_3	H
11d	7-coumarin	2	O	H	H	Cl	H
11e	7-coumarin	2	O	H	Cl	H	H
11f	7-coumarin	2	O	Cl	H	H	H
11g	7-coumarin	2	O	H	OCH_3	OCH_3	OCH_3
11h	7-coumarin	2	O	H	OCH_3	H	H
11i	7-coumarin	2	O	H	F	H	H
13a	7-coumarin	2	N	H	H	H	H

The ability of the tested compounds to inhibit COX-2 and 5-LOX was evaluated *in vitro*, compared with standard compounds **Celecoxib** (COX-2 inhibitor) and **Zileuton** (5-LOX inhibitor). The inhibitory ability of all tested compounds exhibits in **Table 2**. All of the tested compounds showed inhibition of COX-2 and 5-LOX while the majority exhibits little COX-1 inhibitory activities in excess of 40 μM. Among them, the most potent agent, compound **11g**, substituted with a trio of methoxy groups on the phenyl ring, exhibited potential COX-2 inhibitory activity (IC_{50} = 0.23 ± 0.16 μM) *in vitro*, compared with the standard COX-2 selective inhibitor, **Celecoxib** (IC_{50} = 0.41 ± 0.28 μM). Noting that electron-withdrawing groups substituted on *para*-position exhibited better COX-2 inhibition activities than electron-donating groups when comparing compound **9b** (0.44 ± 0.09 μM), **9c** (0.49 ± 0.08 μM) and **9j** (0.33 ± 0.43 μM). According to the different inhibitory COX-2 activity of compound **11d** (0.91 ± 0.29 μM), **11e** (0.49 ± 0.12 μM) and **11f** (0.77 ± 0.76 μM), the substitution on *para*-position were more efficient than other positions of the same phenyl ring. As the phenyl ring substituted by more groups might form more hydrogen bonds with amino acid residues of target protein, the COX-2 inhibition could be more active, comparing compound **11g** (0.23 ± 0.16 μM) and **11h** (0.82 ± 0.26 μM). In this study, we found that the linkage could enhance the inhibitory activity, but the amide linkage showed no extra efficacy than the ester linkage obviously while comparing **13a** and **11a**. Meanwhile, the substitution of 7-hydroxycoumarin, compared with

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4-hydroxycoumarin, exhibited more potent 5-LOX inhibitory activity. Compounds **11g, 9e** and **9j** (IC_{50} = 0.87-1.07 μM) exhibited potent 5-LOX inhibitory activity by the standard of **Zileuton** (IC_{50} = 1.35 ± 0.24 μM).

Table 2 Inhibition activities of target compounds against COX-1/COX-2 and 5-LOX

Compounds	**IC_{50}[a], μM**			COX-1/COX-2
	COX-1	COX-2	5-LOX	ratio
5b	43.28 ± 2.89	3.36 ± 0.31	6.32 ± 0.19	~4
7a	43.61 ± 2.61	2.50 ± 0.95	2.05 ± 0.67	~3
9a	59.23 ± 3.81	0.54 ± 0.30	2.11 ± 0.45	~119
9b	30.41 ± 3.23	0.44 ± 0.08	2.24 ± 0.39	~62
9c	>60	0.49 ± 0.09	2.81 ± 0.57	>300
9e	41.46 ± 5.47	1.43 ± 0.27	1.46 ± 0.61	~12
9g	47.55 ± 4.78	0.29 ± 0.12	1.02 ± 0.52	~163
9h	57.26 ± 1.92	0.43 ± 0.49	2.32 ± 0.40	~173
9i	40.45 ± 2.80	0.30 ± 0.02	1.38 ± 0.84	~134
9j	59.06 ± 1.22	0.33 ± 0.10	1.07 ± 0.41	~137
11a	51.39 ± 1.76	0.78 ± 0.48	2.27 ± 0.11	~65
11b	41.98 ± 2.47	0.53 ± 0.64	1.63 ± 0.81	~79
11c	>60	0.40 ± 0.16	1.40 ± 0.67	>300
11d	>60	0.91 ± 0.29	1.17 ± 0.92	>300
11e	56.52 ± 3.51	0.49 ± 0.12	1.40 ± 0.59	~28
11f	45.35 ± 1.42	0.77 ± 0.76	1.32 ± 1.01	~58
11g	53.08 ± 3.47	0.23 ± 0.16	0.87 ± 0.07	~230
11h	52.67 ± 2.72	0.82 ± 0.26	1.61 ± 1.73	~64
11i	36.14 ± 2.51	0.86 ± 0.64	1.56 ± 0.99	~42
13a	26.25 ± 3.74	0.49 ± 1.08	1.11 ± 0.61	~53
Celecoxib	36.44 ± 2.59	0.41 ± 0.28	-	~88
Zileuton	-	-	1.35 ± 0.24	-

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In general, the target compounds, forming more hydro-keys with target proteins resulted by the substitution of the phenyl ring, could exhibit more inhibitory activities against COX-2 and 5-LOX.

In order to investigate the binding mode and interaction with the binding sites of target enzymes, docking study of compound **11g** that exhibited most potent inhibitory activity of COX-2 was performed. A model of compound **11g**, docked with COX-2 (PDB code: 1CX2), was displayed in (**Fig.2 a**). Compound **11g** showed a highest docking score of 64.38 and was well inserted into the active pocket of the COX-2 by two hydrogen bonds with THR 212 (angle O···H–N = 171.01°, distance = 2.27 Å, angle O···H–N = 120.10°, d = 2.31 Å), one with HIS 386 (angle O···H–N = 135.85°, d = 2.14 Å), two with TYR 385 (angle O···H–C = 124.04°, d = 2.08 Å, angle O··· H–N = 90.97°, d = 2.50 Å), and one with TRP 387 (angle O···H–C = 124.04°, d = 2.02 Å). Meanwhile, sorts of other weaker interactions, like Van Der Waals and carbon-hydrogen bonds, enhanced the binding affinity of compound with COX-2 as well. As illustrated in (**Fig.2 b**), the 3D models suggests the bulge of compound **11g** fits neatly into COX-2's dent.

As documented [26], in 2011, the solution of the crystal structure of human 5-LOX enzyme did great favor in identifying efficient agents in pharmacological screening. Likewise, we also carried out docking study to know the most potent compound **11g**, binding with 5-LOX (PDB code: 3V99), interacted with amino acid residues surrounding the binding domain (**Fig.2 c**). Compound **11g** formed four H-bond with 5-LOX enzyme. Oxygen atoms in phenyl ring and sulfanilamide, H-bond acceptors, formed H-bond with HIS 372 (angle O···H–N = 108.34°, d = 2.38 Å) and GLN 363 (angle N···H–N = 102.08°, d = 3.04 Å), respectively. The carbonyl in the linker and hydrogen atom in the pyrazol ring formed two H-bonds with ASN 180 (angle O··· H–N = 127.50°, d = 2.11 Å, angle O···H–N = 100.70°, d = 2.94 Å). Moreover, weaker interactions such as Carbon Hydrogen Bond, Pi-Cation, Pi-Pi Stacked, Pi-Sigma, Pi-Pi T-shaped contribute to the lowest interaction energy of -66.62 kcal/mol (**Fig.2 d**).

(a) (b)

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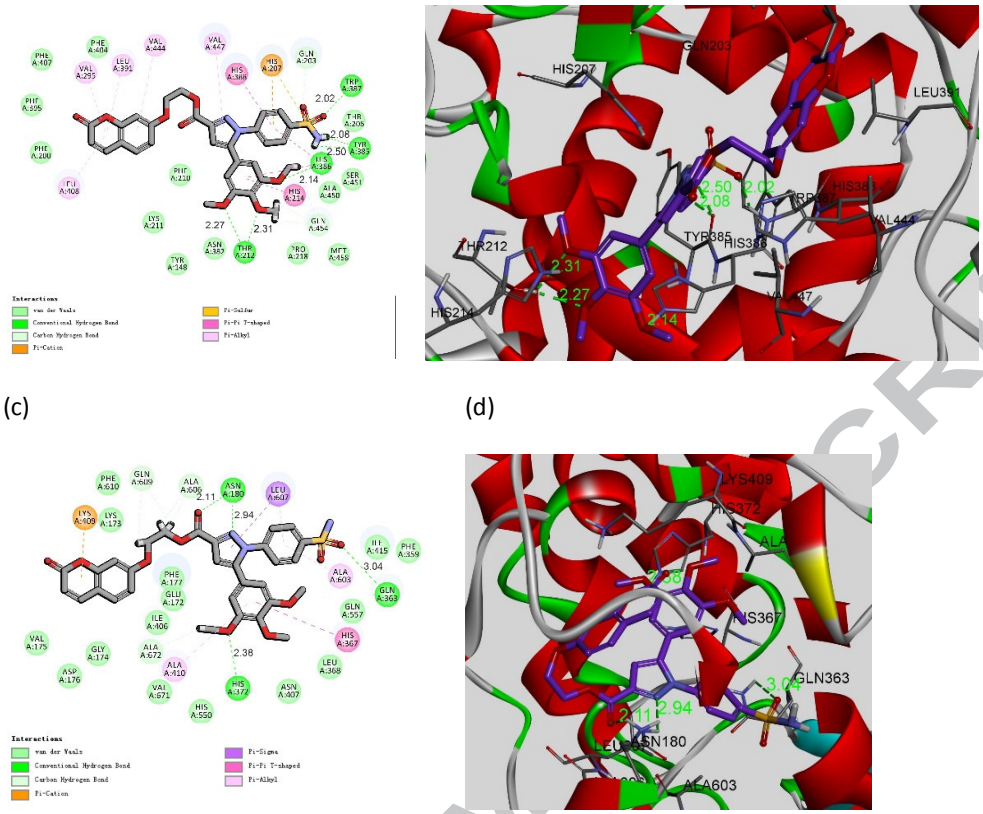

Fig.2 Binding mode of compound **11g** with the COX-2 (PDB code: 1CX2) and 5-LOX (PDB code: 3V99). (a) 2D diagram of the interaction between compound **11g** and COX-2; (b) 3D models of compound **11g** binding with the active site ; (c) 2D diagram of the interaction between compound **11g** and 5-LOX (PDB code: 3V99); (d) 3D models of compound **11g** in the 5-LOX pocket. Green lines represent hydrogen bonds.

In summary, the trimethoxy acylhydrazone, benzenesulfonamide, pyrazole ring, coumarin moieties and the carbonyl linkage of compound **11g** contributed to its inhibitory activities of COX-2 and 5-LOX. As the docking studies shown, it might be a promising way to develop potent agents that the hybrids of coumarin andpyrazole sulphonamide act as COX-2/5-LOX dual inhibitors.

All synthesized compounds were evaluated for anti-proliferative activities by MTT assay against four different cancer cell lines (A549: human lungs cell line, HeLa: human cervix cell line, SMMC-7721: human liver cell line, HT-29: human colorectal cell line) and one non-cancer cell line, 293T: human kidney epithelial cell) which

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indicates a considerable safety profile, compared with intermediate products (**6a**), 7-hydroxycoumarin (7-C) and **Celecoxib**. As shown in **Table 3**, most target compounds, superior to the parent compounds (7-C and 6a), exhibit anti-proliferative activities and low cytotoxic effects through the safety trial on 293T. Moreover, it has been demonstrated that the introduction of substituted coumarin was very necessary to enhance the anti-proliferative activity and safety profile, and it was the same as linkage while comparing others with compound 7a. The reason that compound **5b** showed the lowest anti-cancer effects may be the short linkage which decreased the polarity of the compound, affecting the solubleness in water. Among them, the compound **11g**, comparing with the positive control **Celecoxib** (IC_{50} = 7.68 μM), exhibited the most potent activity against A549 with IC_{50} of 4.48 μM for the reason that a trio of methoxy groups would increase the hydrogen bonding of the compound, affecting the affinity of drugs and protein.

Table 3 Proliferation inhibitory activities of compounds and **Celecoxib** against cancer cells

Compound	IC_{50},[a,b] μM ± SD				
	HT29	Hela	A549	SMMC-7721	293T
5b	9.64 ± 1.20	5.13 ± 0.50	28.50 ± 0.48	16.82±0.34	115.54±0.67
7a	16.76 ± 1.06	16.21 ± 0.88	14.67 ± 0.34	24.11 ± 0.92	134.08 ± 0.56
9a	17.27 ± 1.25	6.83 ± 0.53	9.72 ± 0.71	22.21 ± 0.31	145.8 ± 1.18
9b	11.30 ± 0.38	7.20 ± 0.43	7.94 ± 0.77	20.27 ± 0.76	114.4 ± 0.64
9c	13.66 ± 0.32	6.26 ± 1.80	15.83 ± 0.70	16.54 ± 0.66	225.44 ± 0.86
9e	7.43 ± 0.73	5.65 ± 0.71	25.78 ± 1.07	11.48 ± 1.23	93.06 ± 0.69
9g	17.60 ± 0.17	2.31 ± 0.94	15.65 ± 0.61	12.30 ± 0.85	126.44 ± 0.93
9h	12.99 ± 1.02	5.61 ± 1.55	8.45 ± 1.05	5.90 ± 0.88	122.9 ± 0.61
9i	17.83 ± 1.06	7.29 ± 1.28	22.54 ± 0.17	16.29 ± 0.90	168.57 ± 1.36
9j	9.61 ± 1.11	4.31 ± 0.37	5.52 ± 1.22	12.21 ± 0.52	199.8 ± 0.89
11a	7.29 ± 0.28	8.73 ± 0.69	21.95 ± 0.92	9.60 ± 0.13	207.06 ± 0.97
11b	11.73 ± 0.46	6.40 ± 1.17	15.21 ± 0.81	15.76 ± 0.61	188.76 ± 1.46
11c	12.60 ± 0.91	9.69 ± 0.68	18.83 ± 0.63	9.82 ± 0.84	227.52 ± 0.62

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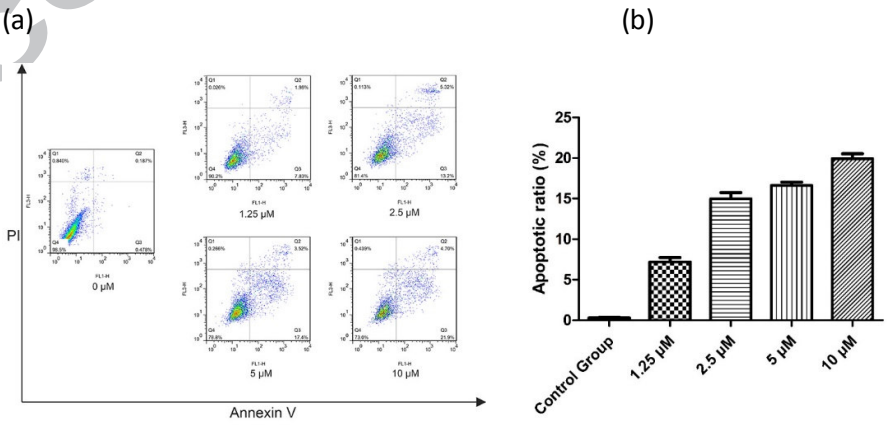
11d	8.85 ± 0.16	7.08 ± 0.54	9.28 ± 0.51	9.96 ± 1.83	>300
11e	11.37 ± 1.27	3.53 ± 1.24	7.31 ± 0.34	13.31 ± 0.83	205.95 ± 1.55
11f	11.76 ± 1.71	10.41 ± 0.72	8.42 ± 0.71	15.66 ± 0.59	189.63 ± 0.27
11g	8.46 ± 0.85	5.51 ± 1.28	4.48 ± 0.57	9.62 ± 0.35	121.86 ± 0.39
11h	11.20 ± 0.71	6.28 ± 1.33	26.96 ± 0.16	6.87 ± 1.59	220.17 ± 1.94
11i	6.82 ± 1.21	14.69 ± 0.33	16.84 ± 0.89	8.92 ± 0.85	195.66 ± 0.89
13a	16.04 ± 1.62	15.22 ± 0.48	37.66 ± 1.33	9.15 ± 0.47	179.4 ± 0.96
6a	21.98 ± 1.03	51.26 ± 1.24	14.31 ± 0.25	25.51 ± 1.13	205.95 ± 0.59
7-C	22.67 ± 1.36	42.35 ± 0.59	21.11 ± 0.51	19.90 ± 0.92	189.63 ± 0.82
Celecoxib	8.47 ± 0.31	11.06 ± 0.93	7.68 ± 0.55	5.96 ± 0.76	111.86 ± 1.51

[a] IC 50 : Concentration inhibits 50% of cell growth

[b] Data are shown as the mean ± SD of three independent experiments (n = 3) run in triplicate

Flow cytometry analysis was applied into verifying whether the potent anti-proliferative and COX-2/5-LOX inhibitory activity of compound **11g** related to the apoptosis of cancer cell like A549 cell line. The results of **Fig.3** indicated that compound **11g** could induce apoptosis of A549 cells in a varying concentrations (0 μM, 1.25 μM, 2.5 μM, 5 μM and 10 μM) for 24 h and the percentage of apoptotic cells increased significantly in a dose-dependent manner. Compound **11g**, obviously, could induce apoptosis in A549 cells in a dose-dependent manner.

Fig.3 Different concentrations of compound **11g** induces apoptosis in A549 cells for 24 h. (a) Flow cytometry analysis of apoptotic A549 cells; (b) Apoptotic ratio.



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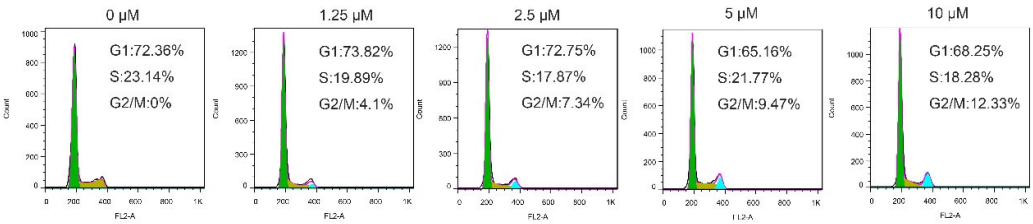
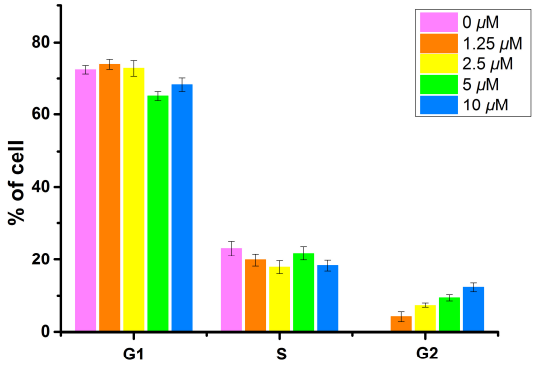
We conducted further study to evaluate the cytostatic effects of compound **11g** on A549 cells. After treated with different concentrations (0 μM, 1.25 μM, 2.5 μM, 5 μM and 10 μM) of compound **11g** for 24 h, G2-block arrest of A549 was observed in a dose-dependent manner (**Fig.4**). As illustrated in previous studies, EGFR, co-overexpressing and co-localizing with COX-2 in cancer cells, decreasing along with the inhibition of COX-2, induced cell cycle arrest in G2 [27]. In summary, increasing concentration of compound **11g** could enhance the accumulation of cells in G2/M through the mechanism need to be further understood.

Fig.4 Influence of compound **11g** on the cell cycle distribution of A549 cells in a dose-dependent

(a)



(b)



As a promising strategy for cancer therapy, dual inhibitors of COX-2 and 5-LOX, however, have yet to be fully illustrated. Therefore, for further exploration, we designed a series of novel hybrids of pyrazole and coumarin as dual inhibitors of COX-2 and 5-LOX for the treatment of cancer. After tested in initial experiments, all the synthesized compounds, exhibiting inhibitory activities of COX-2 and 5-LOX

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with satisfying selectivity, have potential to be effective drugs, especially representative compound **11g**. As the docking studies revealed, these compounds has relatively high affinity to both COX-2 and 5-LOX. Most compounds showed potent anti-proliferative activity with high safety by MTT bioassay. For further researches of the anti-proliferative mechanism, compound **11g** could induce apoptosis and G2 phase cell cycle arrest of A549 in a dose-dependent manner. In general, we designed and evaluated a series of hybrids of pyrazole and coumarin so that they could benefit the study to find novel anti-tumor drugs as dual inhibitors of COX-2 and 5-LOX.

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Scheme 1 Reagents and conditions: (i) dimethyl oxalate, methanol, reflux 6 h; (ii) 4-hydrazinylbenzenesulfonamide, methanol, reflux, 6 h;

Scheme 2 Reagents and conditions: (i) LAH, tetrahydrofuran, r.t., 2 h; (ii) EDC·HCl, HOBt, DMAP, coumarin-3-carboxylic acid, dichloromethane, r.t., overnight.

Scheme 3 Reagents and conditions: (i) KOH, methanol, reflux, 2 h; (ii) EDC·HCl, HOBt, DMAP, 7-hydroxycoumarin, dichloromethane, r.t., overnight;

Scheme 4 Reagents and conditions: (i) KCO_3, KI, 2-bromoethyl alcohol, DMF, 65 ˚C overnight; (ii) EDC·HCl, HOBt, **6a, b, c, e, g, h, i, j**, DMAP, dichloromethane, r.t., overnight; (iii) KCO_3, KI, 2-bromoethyl alcohol, DMF, 65 ˚C overnight; (iv) KCO_3, KI, **6a~6i**, DMF, 65 ˚C, overnight;

Scheme 5 Reagents and conditions: (i) KCO_3, KI, 2-bromoethylamine, DMF, 65 ˚C, overnight; (ii) EDC·HCl, HOBt, DMAP, **6a**, dichloromethane, r.t., overnight.

Table 1 Structure of target compounds

Table 2 Inhibition activities of target compounds against COX-1/COX-2 and 5-LOX

Table 3 Proliferation inhibitory activities of compounds and **Celecoxib** against cancer cells

Fig.1 Pyrazole coumarin derivatives as dual inhibitors of COX-2 and 5-LOX

Fig.2 Binding mode of compound **11g** with the COX-2 (PDB code: 1CX2) and 5-LOX (PDB code: 3V99). (a) 2D diagram of the interaction between compound **11g** and COX-2; (b) 3D models of compound **11g** binding with the active site ; (c) 2D diagram of the interaction between compound **11g** and 5-LOX (PDB code: 3V99); (d) 3D models of compound **11g** in the 5-LOX pocket. Green lines represent hydrogen bonds.

Fig.3 Different concentrations of compound **11g** induces apoptosis in A549 cells for 24 h. (a) Flow cytometry analysis of apoptotic A549 cells; (b) Apoptotic ratio.

Fig.4 Influence of compound **11g** on the cell cycle distribution of A549 cells in a dose-dependent

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Synthesis of novel hybrids of pyrazole and coumarin as dual inhibitors of COX-2 and 5-LOX

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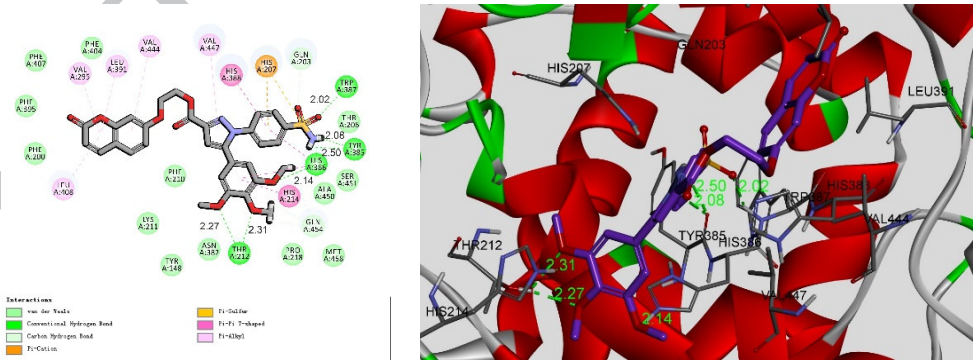
(a) (b)



(c) (d)

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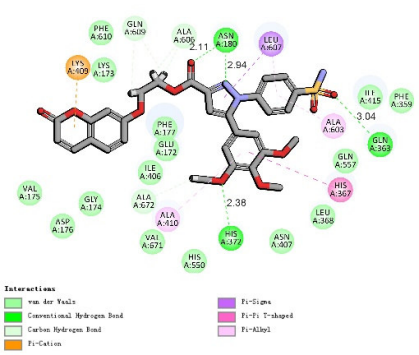

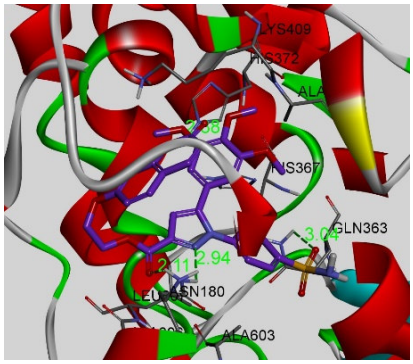

As docking study exhibited, target compound **11g** showed the lowest interaction energy of 64.38 kcal/mol and -66.62 kcal/mol after interacting with COX-2 (PDB code: 1CX2, a, b) and 5-LOX (PDB code: 3V99, c, d), respectively.

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