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Synthesis and pharmacological evaluation of N-substituted 2-(2-oxo-2*H*-chromen-4-yloxy)propanamide as cyclooxygenase inhibitors

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ABSTRACT

A series of novel N-substituted 2-(2-oxo-2*H*-chromen-4-yloxy)propanamide derivatives were synthesized via converting the readily available 4-hydroxy coumarin to the corresponding ethyl 2-(2-oxo-2*H*-chromen-4-yloxy)propanoate followed by hydrolysis and then reacting with different substituted amines. The molecular structures of two representative compounds, that is, **3** and **5l** were confirmed by single crystal X-ray diffraction study. All the compounds synthesized were evaluated for their cyclooxygenase (COX) inhibiting properties in vitro. The compound **5i** showed balanced selectivity towards COX-2 over COX-1 inhibition and good docking scores when docked into the COX-2 protein.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most extensively used therapeutics worldwide for the treatment of inflammation, pain, fever, and for the prevention of thrombosis.

The mechanisms of their pharmacological effects are based on inhibition of the catalytic domain of cyclooxygenase enzymes by sterically hindering the entrance of arachidonic acid as their physiological binder. This results in a reduced production of prostaglandins and thromboxanes, which contribute as important autocrine and paracrine mediators in many physiologic and pathophysiologic responses.[1–4]

Cyclooxygenases (COXs) are membrane-bound heme proteins which exist in two distinct isoforms, a constitutive form (COX-1) and an inducible form (COX-2). Both COX-1 and COX-2 share the same substrates, produce the same products and catalyze the same reaction using identical catalytic mechanisms. The X-ray crystal structure suggests that both the proteins are very similar in their tertiary conformation.[5,6] Their binding pocket and catalytic site are nearly identical. The COX-1 enzyme is responsible for maintaining homeostasis (gastric and renal integrity) and normal production of eicosanoids. The COX-2 is mainly found in brain and kidney while being virtually absent in most other tissues. However, COX-2 expression is significantly upregulated under various acute and chronic inflammatory conditions. It is also well documented that COX-2 is over expressed in numerous human cancers such as colorectal, gastric, and breast cancer.[7]

Recognition of the importance of COX-2 in inflammation and carcinogenesis has prompted discovery of various COX-2 selective inhibitors over the last two decades. A large number of compounds have been synthesized and investigated as selective inhibitors of COX-2 and many of them belong to the diaryl heterocyclic class of compounds, for example, celecoxib, rofecoxib, valdecoxib, etoricoxib, SC57666 etc. (Fig. 1). [8a] While celecoxib is still in the market other inhibitors are either withdrawn or not launched due to their possible adverse side effects. For example refocoxib was withdrawn based on the fact that its uses may be associated with an increased risk of cardiovascular side effect.[8b] Similarly, lumiracoxib (prexige) was hold back due to concerns that it may cause liver failure.[8c] However, the lack of common reason for withdrawal of these inhibitors suggest that the possible risk associated with their uses may be drug-specific rather than class-specific. Incidentally, all the inhibitors withdrawn are known to be highly selective towards COX-2 compared to celecoxib. It is therefore unclear that which degree of COX-2 selectivity should be considered as safe. It appeared that developing moderately selective inhibitors rather than those possessing high selectivity might be a more balanced approach. Nevertheless, the identification of new inhibitors based on a novel scaffold possessing structural features other than that of the known inhibitors could be beneficial and desirable for the potential treatment of inflammatory diseases.

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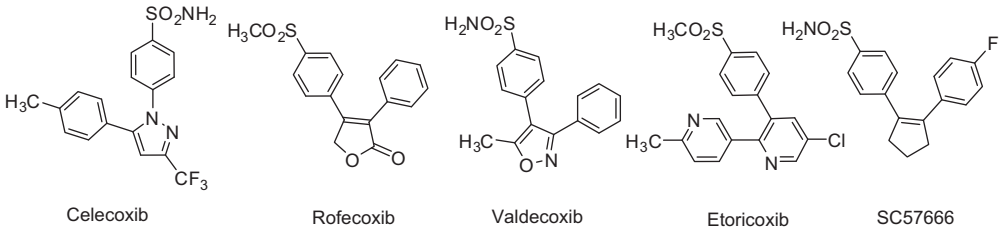


Figure 1. Examples of known COX-2 inhibitors.

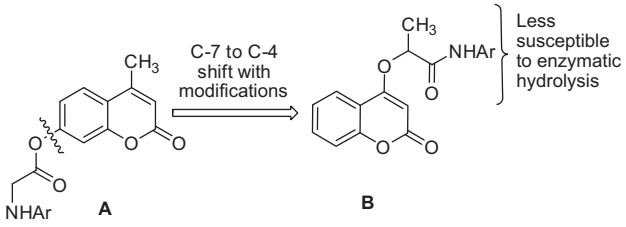
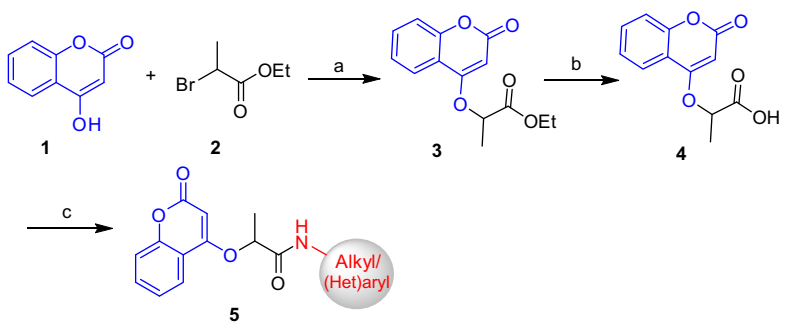


Figure 2. The design of 4-substituted coumarin derivative (B) from the known 7-substituted analogue (A).

Our long standing interest in the identification of new COX-2 inhibitors[9] prompted us to explore the use of coumarin framework for the design of non archetype inhibitors. We were particularly encouraged by the recent report on docking studies of 7-substituted coumarin derivatives (**A**, Fig. 2) with COX-2 enzyme and their promising anti-inflammatory activities.[10] These compounds containing (hetero)aryl amine connected through a linker at C-7 of the coumarin ring were shown to interact mainly with Arg 44 amino acid, which was thought to be involved when COX-2 was inhibited. We anticipated that a similar group at C-4 instead of at C-7 of the coumarin ring (**B**, Fig. 2) would maintain the interaction with COX-2. Moreover, an amide moiety (i.e., –CONHAr of **B**) possessing a α-methyl group was thought to provide possibility of better pharmacokinetic stability rather than an ester (i.e., $-OCOCH_2-$ of **A**) containing a α-amino moiety (Fig. 2). Herein we report our preliminary results on the synthesis and COX-2 inhibiting properties of 4-oxyalkyl substituted coumarin derivatives which to the best of our knowledge were not explored as inhibitors of COX earlier.

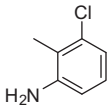
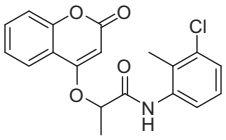
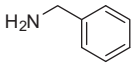
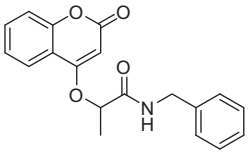
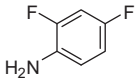
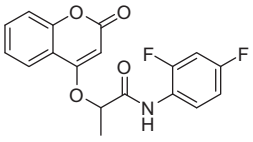
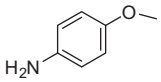
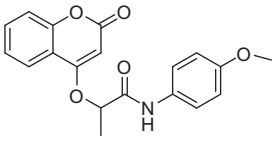
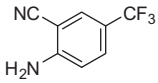
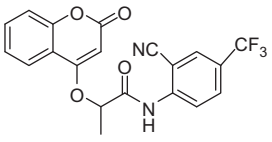
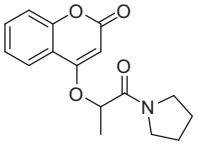
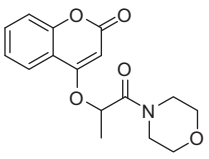
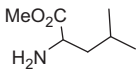
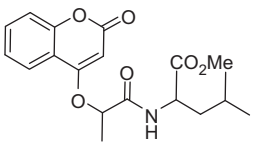
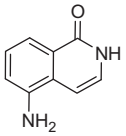
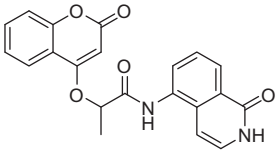
The synthesis of our target compounds **B** is outlined in Scheme 1. Thus 4-hydroxy coumarin on reaction with ethyl 2-bromopropanoate yielded ethyl 2-(2-oxo-2*H*-chromen-4-yloxy) propanoate (**3**), which on hydrolysis using NaOH solution afforded the key starting material 2-(2-oxo-2*H*-chromen-4-yloxy)propanoic acid (**4**). Further amidation of the compound **4** with different amines yielded N-substituted-2-(2-oxo-2*H*-chromen-4-yloxy)propanamide **5** (Table 1). This reaction was carried out in DMF using the coupling reagent HATU [2-(7-aza-1*H*- benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate]. A variety of alkyl/(hetro)aryl amines were employed to prepare a range of target amides that are shown in Table 1. All the compounds synthesized were mixture of enantiomers whereas the compound **5h** was a mixture of diastereomers. All these compounds were characterized by ^1H and ^{13}C NMR as well as MS spectra. Additionally, the molecular structure of the intermediate **3** and a representative compound **5l** were established unambiguously by single crystal X-ray diffraction study (Figs. 3 and 4).[11,12]

All the compounds synthesized were evaluated for their COX inhibiting potential and selectivity by using biochemical COX (COX-1 and COX-2) enzyme based assay. The COX-1 enzyme was isolated from Ram seminal vesicles whereas the recombinant human COX-2 was expressed in insect cell expression system. These enzymes were purified by employing conventional chromatographic techniques. Enzymatic activities of COX-1 and COX-2 were measured according to the method reported earlier,[13] with slight modifications using a chromogenic assay based on the oxidation of *N,N,N,N*,-tetra methyl-*p*-phenylene diamine (TMPD) during the reduction of PGG2 to PGH2.[14,15] The known non-selective inhibitor indomethacin and COX-2 inhibitor celecoxib was used as reference compounds in this assay. The IC_{50} values determined for all the compounds along with their selectivity (COX-2/COX-1 ratio) are listed in Table 2. While most of the compounds showed COX inhibiting properties in vitro only three of them however were found to be COX-2 selective. While it was not clear that which structural features were particularly responsible for COX-2 inhibition the data presented in Table 2 however indicated that the size of the amide side chain perhaps played a key role. The size of this amide moiety seemed to be favorable for compounds **5b**, **5h** and **5i** that showed COX-2 selectivity (entry 2, 8 and 9, Table 2). The compound **5i** was found to have better activity than **5b** and **5h**. It is better than indomethacin though inferior to the diaryl heterocyclic class celecoxib in terms of selectivity. Notably, while celecoxib is still in the market its uses in patients having heart disease (or those who have a risk of developing heart disease) are restricted. The use of paracetamol or certain NSAIDs such as non-selective inhibitor naproxen have been suggested to be safer choices in these patients. The compound **5i**



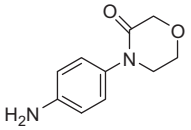
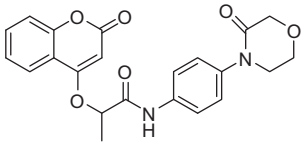
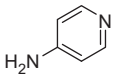
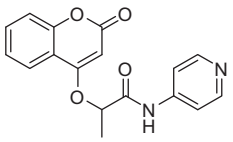
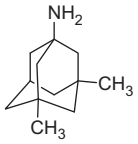
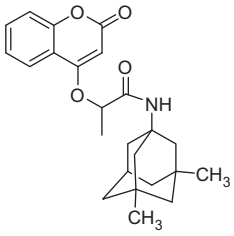
Scheme 1. Reagents and conditions: (a) K_2CO_3, acetone, reflux, 5 h, 98%; (b) NaOH then HCl, EtOH, 2 h, reflux, 99%; (c) amine, HATU, DMF, room temp, 12 h.

Table 1
Preparation of coumarin derivatives (**5**) from 2-(2-oxo-2*H*-chromen-4-yloxy)propanoic acid (**4**) (Scheme 1)[a]

Entry	Amine	Product (**5**)	Yield[b] (%)
1		 **5a**	92
2		 **5b**	90
3		 **5c**	86
4		 **5d**	95
5		 **5e**	87
6		 **5f**	86
7		 **5g**	84
8		 **5h**	81
9		 **5i**	90

(*continued on next page*)

Table 1 (*continued*)

Entry	Amine	Product (**5**)	Yield[b] (%)
10		 **5j**	92
11		 **5k**	85
12		 **5l**	87

[a] All the reactions were carried out using compound **4** (**1** mmol) and an appropriate amine (1.1 mmol) in the presence of HATU (1.1 mmol) in DMF (3.0 mL) for 12 h at room temp.
[b] Isolated yield.

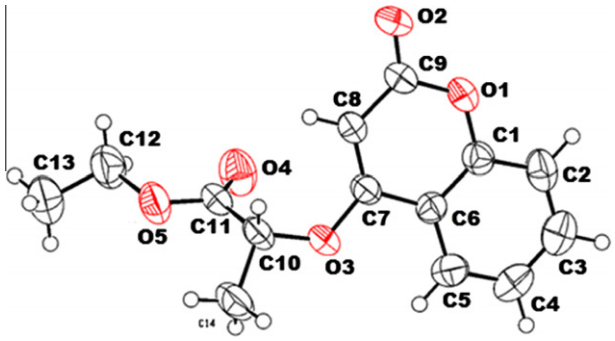


Figure 3. ORTEP representation of the compound **3** (thermal ellipsoids are drawn at 50% probability level).

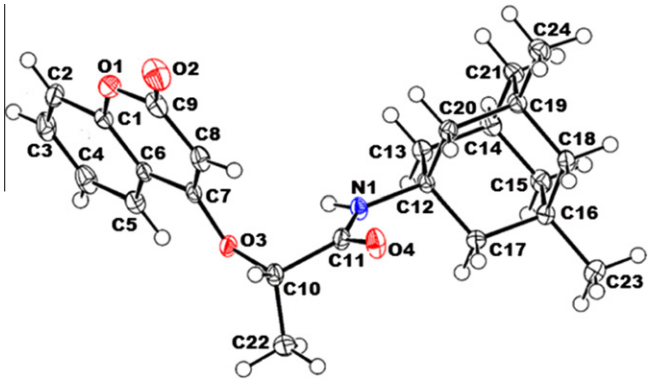


Figure 4. ORTEP representation of the compound **5l** (thermal ellipsoids are drawn at 50% probability level).

therefore was of further interest as the moderate or balanced selectivity displayed by this compound might be beneficial from the view point of adverse side effects shown by rofecoxib or lumiracoxib.

To understand the interaction of compound **5i** with COX-2 enzyme, in silico docking studies were carried out (see ESI). The docking analysis was performed to identify the key amino acid residues involved in making interactions between the compound **5i** and the human COX-2 model. The compound **5i** was docked successfully in the human COX-2 homology model, which showed good docking scores, that is, −9.48 kcal/mol. The docking program AutoDock computes binding energy, RMSD and inhibition constant (K_i) with respect to the docked molecule. The AutoDock computed binding energy; RMSD and K_i values along with H-bond interacting residues are presented in Table 3. The binding 3D conformation of human COX-2 with compound **5i** is shown in Figure 5. It was observed that the interaction of **5i** with human COX-2 occurred in a distinct site, formed by Phe[188], Gln[189], His[193], Tyr[371], Trp[373], Leu[376], Leu[377], Tyr[390], Phe[393] and Ile[394] residues. In a similar docking studies the binding site of celecoxib was found to be formed by Phe[188], Gln[189], His[193], Asp[348], Phe[349], Tyr[371], Trp[373], Ser[516], Gly[519] and Ile[520] residues indicating a different binding pocket for celecoxib which perhaps justify the differences in potencies of celecoxib and **5i** in COX-2 inhibition.

In summary, a series of novel N-substituted 2-(2-oxo-2*H*-chromen-4-yloxy)propanamide derivatives were designed and explored as potential inhibitors of COX. These compounds were synthesized from readily available 4-hydroxy coumarin via a simple and straightforward three step method. The molecular structures of two representative compounds were confirmed by single crystal X-ray diffraction study. All the compounds synthesized were evaluated for their cyclooxygenase (COX) inhibiting properties in vitro. The compound **5i** showed balanced selectivity towards COX-2 over COX-1 inhibition and good docking scores when

Table 2
In vitro COX inhibition by N-substituted 2-(2-oxo-2*H*-chromen-4-yloxy)propanamide derivatives (**5**)

Entry	Compound	COX inhibition IC_{50} (μM)		COX-2/COX-1 Selectivity ratio
		COX-1	COX-2	
1	**5a**	4.61	74.91	16.24
2	**5b**	0	13.19	–
3	**5c**	1.64	16.83	10.26
4	**5d**	2.26	38.71	17.12
5	**5e**	9.60	50.25	5.23
6	**5f**	13.73	0	–
7	**5g**	5.03	69.94	13.90
8	**5h**	0	8.25	–
9	**5i**	2.98	1.02	0.33
10	**5j**	4.60	12.64	2.74
11	**5k**	9.64	52.39	5.43
12	**5l**	3.33	8.43	2.53
13	Indomethacin	0.0067	0.048	7.16
14	Celecoxib	15.0	0.042	0.0028

Table 3
Docking results of compound *N*-(1-oxo-1,2-dihydroisoquinolin-5-yl)-2-(2-oxo-2*H*-chromen-4-yloxy)propanamide onto human COX-2

Compound	Binding energy (kcal/mol)	Estimated inhibition constant (K_i) (nm)	RMSD (Å)
5i	−9.48	13.2	0.56

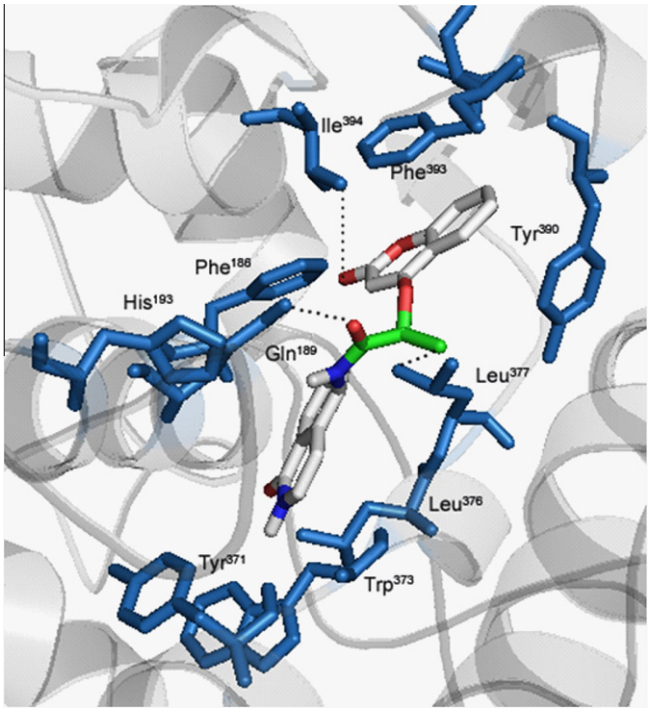


Figure 5. Docking of compound 5i into the human COX-2.

docked into the COX-2 protein. Overall, the coumarin framework presented here could be an attractive template for the identification of novel cyclooxygenase inhibitors and the corresponding synthetic strategy described could be useful for generating diversity based library of small molecules of potential pharmacological interest.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.082.

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11. Crystal data of **3**: Molecular formula = $C_{14}H_{14}O_5$, formula weight = 262.25, Crystal system = Monoclinic, space group = *P*2(1)/*n*, *a* = 11.699 (4) Å, *b* = 8.056 (3)Å, *c* = 14.034 (5) Å, *V* = 1301.6 (8) Å^3, *T* = 296(2) K, *Z* = 4, D_c = 1.339 mg m^{-3}, μ(Mo-Kα) = 0.09 mm^{-1}, 5053 reflections measured, 1659 independent reflections, 1057 observed reflections [$I > 2.0\ \sigma(I)$], R_1_obs = 0.040, Goodness of fit = 0.91. Crystallographic data (excluding structure factors) for **3** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 871213.
12. Crystal data of **5l**: Molecular formula = $C_{24}H_{29}NO_4$, formula weight = 395.48, Crystal system = Monoclinic, space group = *P*$\bar{1}$, *a* = 7.324 (6) Å, *b* = 11.5596 (9) Å, *c* = 13.115 (10) Å, *V* = 1017.67 (14) Å^3, *T* = 296(2) K, *Z* = 2, D_c = 1.291 mg m^{-3}, μ(Mo-Kα) = 0.09 mm^{-1}, 16,879 reflections measured, 4436 independent reflections, 3891 observed reflections [$I > 2.0\ \sigma\ (I)$], R_1_obs = 0.038, Goodness of fit = 0.90. Crystallographic data (excluding structure factors) for **5l** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 871214.
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15. Briefly, the assay mixture containing Tris HCl buffer (100 mM, pH 8.0), hematin (15 mM), EDTA (3 mM), enzyme (100 mg COX-1 or COX-2) and the test compound was pre-incubated at 25 °C for 1 min and then the reaction was initiated by the addition of arachidonic acid and TMPD, in total volume of 1 mL. The enzyme activity was determined by estimating the velocity of TMPD oxidation for the first 25 s of the reaction by following the increase in absorbance at 603 nm. A low rate of nonenzymatic oxidation observed in the absence of COX-1 and COX-2 was subtracted from the experimental value while calculating the percent inhibition.