European Journal of Medicinal Chemistry 45 (2010) 4058–4064

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

3-Formylchromones: Potential antiinflammatory agents

Khalid M. Khan [a,*], Nida Ambreen [a], Uzma Rasool Mughal [a], Saima Jalil [a], Shahnaz Perveen [b], M. Iqbal Choudhary [a]

[a] H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan
[b] PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan

ARTICLE INFO

Article history:
Received 22 February 2010
Received in revised form
4 May 2010
Accepted 29 May 2010
Available online 8 June 2010

Keywords:
3-Formylchromones
Antiinflammatory activity
Respiratory burst
Neutrophils
COX
LOX inhibition

ABSTRACT

The synthesis and characterization of 3-formylchromone (**1**) and its derivatives **2**–**24** and evaluation of their potential antiinflammatory activities is reported here. These compounds were characterized by ^{1}H NMR, EI MS, IR, and UV spectroscopic techniques and elemental analysis. The synthesized compounds were evaluated by using various *in vitro* and *in vivo* assay models for antiinflammatory activity and their effects were compared with known standard drug such as aspirin and indomethacin. Among all tested compounds, **1**, **2**, **5**, **6**, **9**, **14**, **16**–**19**, **21**–**23**, showed promising antiinflammatory activities.

The results and SAR has been discussed in this report.

1. Introduction

Inflammation is a defensive response of body, which induces physiological adaptations to minimize tissue damage and to remove the pathogenic infections. Such mechanisms involve a complex series of cellular and modular events including dilation of arterioles, venules and capillaries with increased vascular permeability and exudation of fluids containing plasma proteins as well as migration of leukocytes into the inflammatory area. A chronic inflammation is however an important contributory factor in morbidity and mortality. Such inflammatory disorders include rheumatoid arthritis, osteoarthritis, inflammatory bowl diseases, retinitis, multiple sclerosis, psoriasis and atherosclerosis [1].

Inflammation is characterized by the immediate infiltration of key defense cells. Initially neutrophils respond to inflammation, followed by monocytes and lymphocytes [2]. Neutrophils play a key role in inflammatory response by neutralizing the adverse effects of external inflammatory stimuli *e.g.,* chemical, antigens, infections etc. They stored specialized proteins in their granules, which are secreted on their activation. Neutrophils also generate toxic oxygen metabolites including reactive oxygen species (ROS) by various pathways [3,4]. ROS are formed subsequent to the assembly and activation of membrane-based, phagocyte-specific enzyme, NADPH oxidase [5].

Several methods are available to measure the superoxide production by NADPH oxidase of phagocyte cells [5]. WST-1 used to measure the superoxide production by neutrophils which were activated by opsonized Zymosan A, which induces phagocytic activation of neutrophils. This technique is more sensitive and reliable over other available techniques and can be adopted in microplate format. WST-1 is reduced by NADPH oxidase *via* superoxides. It is a very useful assay for measuring the neutrophils functions and antiinflammatory activity of the target compounds [6,7].

In addition to NADPH oxidase, other cellular enzymes are also involved in the inflammatory processes. For example, cyclo-xygenases (COX) catalyze the conversion of arachidonic acid into prostaglandins and thromboxane [8–15]. Lipoxygenase (LOX) is another enzyme which has been reported to induce inflammation *via* the catalysis of arachidonic acid to produce LTs (Leukotriens). These enzymes are, therefore, important targets for the discovery of mechanism-based drugs for the treatment of a variety of inflammatory disorders such as bronchial asthma, inflammation, cancer, and autoimmune diseases. More recently, dual inhibitors of COX and LOX are found to be significantly important for the treatment of inflammatory disorders [9].

Traditionally, the standard treatment for most inflammatory disorders, such as rheumatoid arthritis, is the use of NSAIDs *e.g.,*

* Corresponding author. Tel.: +92 21 34824910; fax: +92 21 34819018.
E-mail addresses: hassaan2@super.net.pk, khalid.khan@iccs.edu (K.M. Khan).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.05.065

aspirin. However, the main side effect of aspirin is gastric ulceration. Therefore, there is a need to discover new and novel inhibitors against these enzymes. Most of the heterocyclic bioactive compounds are used as a source of pharmacophores. For example, a number of chromones and their derivatives have been reported to possess wide spectrum of useful properties of biological as well as pharmacological importance [16]. Chromone derivatives exhibit various biological activities including antimycobacterial, antifungal, anticonvalescent, and antimicrobial mushroom tyrosinase inhibition activities [17–21]. These pharmacological activities have been the major interest behind the syntheses of the chromones and their derivatives. In addition, the importance of lead molecules for the discovery of new synthetic drugs, several chemical structures are used as a starting point for chemical modifications in order to improve potency, selectivity or pharmacokinetic parameters. In drug discovery and drug designing program of our research group, we are working on various classes of compounds in search of potential lead molecules [22–24]. We earlier reported the thymidine phosphorylase inhibitory activities of Schiff bases of 3-formylchromones **2**–**5**, **7**, **8**, **10**, **11**, **14**–**16** and **22** which discloses the excellent thymidine phosphorylase inhibitory activities of compounds **2** and **10** [25]. We also reported the antibacterial and antifungal activities of compounds **1**–**4**, **6**, **8**, **11**, **12**, **15**–**19**, **21**–**23** [26]. To the best of our knowledge only the compound **1** previously reported as an antiinflammatory agent in some other modified assays [27], therefore 3-formylchromone (**1**) is an important and well-studied compound serves as the starting materials for more potent antiinflammatory compounds. Therefore, in view of our aforementioned reports we also screened compounds **2**–**24** for their antiinflammatory potential. This study provides a solid base for further research on these types of compounds.

2. Results and discussion

2.1. Chemistry

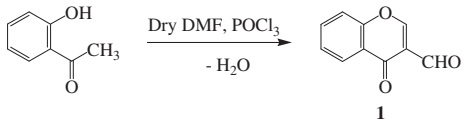
3-Formylchromone (**1**) was prepared by the Vilsmeier–Haack synthesis (Scheme 1) [28]. Derivatives of 3-formylchromone (**1**) were prepared by a condensation reaction of 3-formylchromones with a variety of aromatic, aliphatic amine and hyrazides in ethanol (Scheme 2).

The preparation of derivatives was carried out by stirring the mixture of 3-formylchromone (**1**, 1 mmol) with substituted aromatic, aliphatic amines and hydrazides (1 mmol) in ethanol for 2–4 h, at 50–100 °C. The progress of reaction was monitored by TLC. The resulting products were recrystallized from ethanol and on drying to afford the desired compounds.

The structures of the synthesized compounds were determined by using spectroscopic techniques such as ^{1}H NMR, EI MS, IR and UV. Elemental analysis results were also found to be satisfactory and found within the limit.

2.2. Biological evaluation

Several biological assays were used for the study antiinflammatory potential of 3-formylchromone (**1**) and its derivatives **2**–**24** of which include *in vitro* respiratory burst inhibitory activity, superoxide scavenging activity using xanthine–xanthine oxidase system, cyclooxygenase (COX-1, COX-2) and lipoxygenase inhibitory activities and *in vivo* carrageenan-induced rat paw edema study.



1

Scheme 1. Synthesis of 3-formylchromone (**1**).

Initially, all of the compounds **1**–**24** were screened for their respiratory burst inhibitory activity. The derivatives **2**, **16**, **21**, and **23** exhibited a significant activity in this assay (data not shown). Other derivatives showed a moderate to weak activity. All the derivatives **1**–**24** were also tested for the superoxides scavenging activity and most of them were found to be active in xanthine/xanthine oxidase assay system.

For the evaluation of the enzymes related to the antiinflammatory activity, we selected COX-1 and -2, and LOX. The selection of the compounds for further studies was based on the preliminary studies; the compounds showed very weak studies in primary studies were not further screened. Compounds (**4**, **7**, **13**, **16**, **17**, and **23**) were screened to measure their inhibitory potential against these enzymes. Based on *in vitro* antiinflammatory assay, compound **23** was selected for the study *in vivo* inflammatory carrageenan-induced rat paw edema model. This derivative was found to be most potent candidate in the most of the *in vitro* studies. The results are summarized below:

2.2.1. In vitro respiratory burst inhibitory activity

In *vitro* respiratory burst inhibitory activity of synthetic Schiff bases was evaluated by using the modified method of Berridge et al. [5]. Results, presented in Table 1, show that compound **23** is the most active compound with an IC_{50} value 40.210 ± 1.76 μM. Compound **16** was found to be the second most active compound with an IC_{50} 55.036 ± 6.17 μM. Compound **21** demonstrated an activity with an IC_{50} value 85.540 ± 0.072 μM.

2.2.2. Xanthine/xanthine oxidase superoxides scavenging activity

This is a mechanism-based model for the comparison of the activity of the compounds in the cell-based assay and the cell-free system. The results of this assay are shown in Table 2. Compounds **2**, **3**, **16**, **19**, and **21** showed IC_{50} values in potent range (<5 μM) since below 5 μM always considered as potent range. Other compounds **1**, **5**, **7**, **18**, **16**, **22**, and **23** also appear to be significantly active (less than 30 μM). The remaining derivatives **4**, **6**, **14** and **17** were found to be only weak active in this assay.

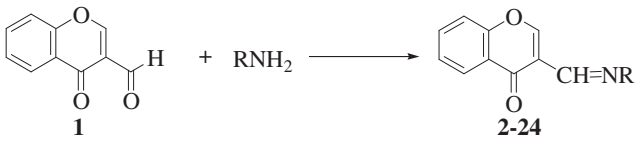
2.2.3. Enzyme inhibition activity

Some of the selected compounds **4**, **7**, **16**, **17**, and **23** were screened for the inhibitory potential against the cyclooxygenases (COX-1 and -2). The results, shown in Table 3, indicate that compounds **16** and **17** were moderate to weakly active against both the enzymes. However, compounds **4** and **23** were very weakly active against only COX-1 and COX-2 (approximate 50% at ~1000 μM), respectively.

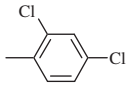
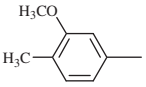
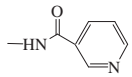
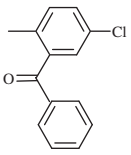
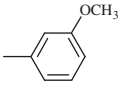
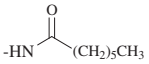
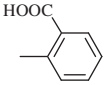
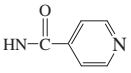
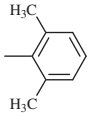
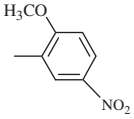
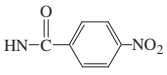
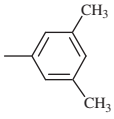
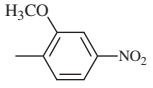
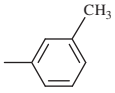
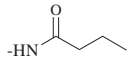
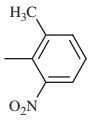
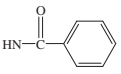
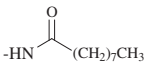
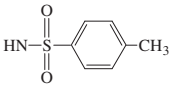
The compounds **4**, **16**, **17**, and **23** were also screened for the lipoxygenase (LOX) enzyme to measure their inhibitory potential. Results shown in Table 4 indicate that none of the compounds appear to inhibit LOX activity, rather all compounds enhanced LOX enzyme activity.

2.2.4. In vivo antiinflammatory study on carrageenan-induced rat paws edema

Derivative **23** was further evaluated for *in vivo* antiinflammatory activity, because of its -potent *in vitro* activities in other assays. Compound **23** showed a promising activity in the *in vivo* model, comparable to the standard drugs, aspirin and indomethacin. This compound showed activity against carrageenan-induced inflammation in a dose and time dependent manners. After the carrageenan injection at the lowest dose (1 mg/kg), the compound **23**



1 2-24

Compds. No.	R	Compds. No.	R	Compds. No.	R
2		10		18	
3		11		19	
4		12		20	
5		13		21	
6		14		22	
7		15		23	
8		16		24	
9	-$(CH_2)_2$-	17			

Scheme 2. Synthesis of chromone schiff bases **2**–**24**.

showed a low inhibitory activity. Highest activity was observed after the second hour of the inflammation (53%). Compound **23** at a dose of 10 mg/kg showed a 7.82% inhibition during the first hour, which reached to 67% after the fourth hour. At the lower dose of 5 mg/kg, activity remains same during the first to fourth hours. However, it was interesting to note that the activity persisted at both the doses. In conclusion, compound **23** was found to possess a significant *in vivo* antiinflammatory activity (Table 5 and 6).

3. Discussion

Synthetic 3-formylchromone (**1**) and its derivatives **2**–**24** were synthesized and assayed for their antiinflammatory activities. Results from respiratory burst inhibitory assay demonstrated that compounds **2**–**24** have a varying degree of activity (IC_{50} = 40.210 ± 1.76–478.39 ± 10.36 μM), which compared with known antiinflammatory compounds, indomethacin and aspirin (IC_{50} values 246.35 ± 12.36 and 518.52 ± 3.68 μM, respectively). Compound **23** found to be the most active compound with a lowest IC_{50} = 40.210 ± 1.76 μM. In addition, compounds **2**, **5**, **16**, and **21** were also exhibited more potent activities than indomethacin and aspirin. Compounds **1**, **5**, **6**, **9**, **14**, and **22** exhibited better activities than aspirin, however, these compounds exhibited moderate activities when compared with indomethacin. Compound **5** exhibited a respiratory burst inhibitory activity. Compound **23**, a butanohydrzide derivative of **1**, was found to be the most potent compound in the series. The difference in inhibitory activity of compounds **16** and **23** may be explained on the basis of substitution on carbonyl moiety. Compound **16** carries a phenyl, whereas compound **23** has a propyl group at the carbonyl residue. The phenyl is an electron withdrawing group, whereas propyl is an electron donating group. The difference in substitution pattern suggests that carbonyl part of hydrazide on chromone moiety with an electron donating group enhances the respiratory burst inhibitory activity. It appears that compound **21** which is a *para*-nitrohydrazide exhibited less activity than compound **16**, perhaps

Table 1
Respiratory burst inhibitory activity of the synthetic chromone hydrazides derivatives at a concentration of 500 μM using activated human neutrophils.

Compound	% Inhibition at 500 μM	IC50 (μM)
1	80.47	399.99 ± 16.92
2	76.37	98.215 ± 32.85
3	71.37	>500
4	+2.41*	>500
5	78.53	130.98 ± 33.98
6	52.59	500
7	92.75	400.85 ± 5.23
8	45.17	>500
9		>500
10	23.72	>1000
11	16.79	>1000
12		>500
13	–	>500
14	84.17	>500
15	80.86	399.357 ± 10.76
16	91.55	55.036 ± 6.17
17	+ 2.41*	–
18	24.48	>1000
21	96.22	85.540 ± 0.072
23	76.55	40.210 ± 1.76
22		288.72 ± 36.51
Aspirin[a]		518.5 ± 3.68
Indomethacine[b]		246.35 ± 12.36

[a] Enhancing activity rather inhibitory.
[b] Standard inhibitor for respiratory burst assay.

due to the same reasons. Similarly compound **2**, a Schiff base with a dicholorophenyl moiety, also showed less activity than compound **16**.

Compound **2**–**24** were screened in cell-free system to measure their superoxide scavenging activity. Most of the compounds found to be potent scavengers of superoxides, produced by the enzymatic system xanthine/xanthine oxidase. In this assay, compounds **2**, **3**, **16**, **19**, and **21** showed IC_{50} values less than 5 μM, which is a potent range. In addition, compounds **1**, **5**, **7**, **18**, **16**, **22**, and **23** were also found to be potent in inhibiting superoxide production at IC_{50} less than 30 μM. The other compounds **4**, **6**, **14** and **17** either demonstrated a weak activity or found to be inactive in this assay. The results of this assay conferred that the possible mechanism of action of the active compounds was their scavenging potential against free radicals rather than inhibition of the enzyme involved in the respiratory burst assay.

Table 2
Superoxide scavenging activity of the synthetic chromone hydrazides derivatives at a concentration of 1000 μM using xanthine–xanthine oxidase system.

Compound	% Inhibition at 1000 μM	IC50 (μM)
1	70.22	21.36 ± 2.36
2	78.96	1.68 ± 0.58
3	97.25	0.274 ± 0.04
4	32.62	>1000
5	85.69	17.64 ± 1.44
6	65.95	116.12 ± 3.69
7	70.63	18.59 ± 1.85
14	+ 8.47*	Inactive
15	78.32	29.19 ± 12.41
16	99.36	0.439 ± 0.07
17	11.85	>1000
18	–	13.95 ± 5.67
19	93.61	4.63 ± 0.69
21	94.25	1.00 ± 0.20
22	–	20.00 ± 10.43
23	86.25	72.77 ± 12.56
Aspirin[a]		>1000
Indomethacine[b]		>1000

[a] Enhancing activity rather inhibitory.
[b] Standard inhibitor for superoxide scavenging activity.

Table 3
Cyclooxygenase inhibition activity of the synthetic chromone hydrazide derivatives at a concentration of 1000 μM.

Compound	COX-1 Inhibition IC50 (μM)	COX-2 Inhibition IC50 (μM)
7	>1000	+ 25.43*
4	1000	+ 65.72*
17	150.45 ± 4.25	203.69 ± 2.65
16	405.96 ± 4.12	802.32 ± 1.63
23	>1000	1000
Aspirin[a]	750.41 ± 0.54	>1000
Indomethacine[b]	0.05 ± 0.47	0.67 ± 0.98

[a] Enhancing activity rather inhibitory.
[b] Standard inhibitors for cyclooxygenase activity.

Compounds **4**, **7**, **8**, **10**, **13**, **16**, and **23** were also screened for their COX-1 and -2 inhibitory activities. Compounds **4** and **16** showed a weak activity against both the isoforms. None of the compounds (**13**, **16**, **17**, and **23**) were found to be lipoxygenase inhibitory rather they have enhanced the lipoxygenase activity.

The *in vitro* data suggests that compound **23** has the potential to be a potent antiinflammatory compound, therefore, it was further evaluated for its antiinflammatory activity in an *in vivo* system. During the first hour after the carrageenan injection, compound **23** at 10 mg/kg dose inhibited the inflammation only by 7.82% which reached up to 67% after the 4 h of treatment. At the lower dose of 5 mg/kg, activity remains the same during the first to fourth hours (67%). At the lowest dose (1 mg/kg), it showed a reduced inhibitory activity (42%). Highest activity was observed after the second hour of the inflammation (53%). The data indicate that the compound **23** exhibited a very potent activity in the *in vivo* model, comparable to the known antiinflammatory compounds, aspirin and indomethacin.

4. Conclusion

In conclusion, *in vitro* and *in vivo* studies demonstrated that compound **23**, which is butanohydrzide derivative of 3-formylchromone, has potent antiinflammatory activities. This compound has a potential to be further evaluated as a novel drug to reduce inflammation.

4.1. In vitro antiinflammatory assays

4.1.1. Material and methods

Heparinized human blood was obtained from local blood bank. WST-1 was purchased from Dojindo Laboratories (Japan), Ficoll Paque was purchased from Pharmacia Amersham (Uppsala Sweden). Deionized water, which was prepared by using a Millipore system, was used during the entire assay procedure. The colorimetric COX (ovine) inhibitor screening kit (Cat No. 760111) was from Cayman Chemicals, Ann Arbor, USA). Buttermilk xanthine oxidase (EC 1.2.3.2), xanthine, baicalein, and λ-carrageenan, were

Table 4
Lipoxygenase inhibition activity of the synthetic chromone hydrazides derivatives at a concentration of 1000 μM.

Compound	% Inhibition at 1000 μM
17	+137.80*
13	+47.5*
16	+89.65*
23	+80.78*
Baiclein[a]	22.5±0.26

*Enhancing activity rather inhibitory.
[a] Standard inhibitor for lipooxyganase activity.

Table 5
Effect of compound **23** on the carrageenan-induced paw volume in rats with different time intervals against various ranges of the dosage of compound.

Dose (mg/kg)		Paw volume (mL)			
		0–1 h	0–2 h	0–3 h	0–4 h
Control		0.25 ± 0.03	0.42 ± 0.04	0.59 ± 0.05	0.5 ± 0.04
Aspirin	10	0.11 ± 0.09	0.35 ± 0.09	0.47 ± 0.16	0.47 ± 0.17
	5	0.03 ± 0.03	0.20 ± 0.12	0.33 ± 0.07	0.31 ± 0.10
	1	0.40 ± 0.10	0.59 ± 0.03	0.56 ± 0.24	0.59 ± 0.19
(**23**)	10	0.23 ± 0.05	0.27 ± 0.07	0.26 ± 0.07	0.16 ± 0.14
	5	0.14 ± 0.06	0.15 ± 0.11	0.23 ± 0.15	0.16 ± 0.09
	1	0.15 ± 0.06	0.19 ± 0.05	0.36 ± 0.14	0.29 ± 0.13

purchased from Aldrich Chem. Co. Dextran, zymosan A, enzyme lipoxygenase (1.13.11.12 type I-B), arachidonic acid, sodium citrate and all other reagents were purchased from Sigma (St. Louis, MO, USA). Plathysmometer was of (Model 76 014, Ugo, Japan).

4.1.2. Animals

Wistar rats (180–210 g) of either sex were obtained from the animal house facility of the International Center for Chemical and Biological Sciences, University of Karachi. The animals were kept under standardized conditions *i.e.* temperature 22 ± 2 °C, humidity 60% ± 4%, 12/12 h light and dark cycles. Animals were kept on free excess of water and normal diet.

4.1.3. Assay for respiratory burst inhibitory activity

Respiratory burst inhibitory activity of synthetic Schiff's bases were determine by using the modified method of Berridge et al. Briefly, 15–20 mL heparinized fresh human volunteer blood was used to isolate neutrophils, as described elsewhere (Siddiqui et. al.). The cells were re-suspended with MHS at a concentration of 1×10^6 cells/mL. Freshly prepared human serum (preferably AB blood group) was mixed with zymosan A (20 mg/mL in 1:1PBS). Tube was shaken vigorously with vortex mixer and kept in a shaking water bath at 37 °C for 20 min. The tube was then centrifuged at 3000 rpm for 10 min at room temperature, pallets were re-suspended and kept at −20 °C until used. All the test compounds were prepared as stock at a concentration of 500 μM in 5% DMSO with MHS. Aspirin and indomethacin were used as standard antiinflammatory agents. Cells (1×10^6 cells/mL) were incubated with various concentrations of test samples and 250 μM of WST-1 in a total volume 200 μL in 96-well plates. Pre-read absorbance was taken at 450 nm on microplate reader (SpectraMax 340, Molecular Devices). After 30 min of incubation at 37 °C, cells were induced to generate reactive oxygen species by adding opsonized zymosan (10 μL/well); and kinetic absorbance was taken for 30 min. At the end of the reaction, endpoint reading was taken. IC_{50} was calculated by using Ez-fit 5.0 (Perrella Scientific Inc., Amherst, USA). All the reactions were carried out in five replicates to reduce the variability of the values.

Table 6
Percentage inhibition of different concentrations of compound **23** at different time intervals on carrageenan-induced rat paw edema model.

Dose (mg/kg)		% Edema inhibition			
		1 h	2 h	3 h	4 h
Control	–	–	–	–	–
Aspirin	10	64.23	52.06	51.37	1.43
	5	89.82	72.45	66.21	32.60
	1	25.26	19.17	42.38	28.80
(**23**)	10	7.82	36.31	55.37	67.17
	5	41.50	64.48	61.87	67.33
	1	39.80	53.97	38.84	42.17

4.1.4. Cell viability assay

Cytotoxicity of the active compounds were measured by using freshly isolated human neutrophils, as described by Berridge et al. and Ishiyama et al. Freshly isolated human neutrophils (1×10^7 cells/mL) were incubated in a total reaction volume of 200 μL containing test compound with the various concentrations (200–12.5 μM). The absorbance was pre-read at 450 nm. After 30 min of incubation with a moderate shaking in a water bath at 37 °C, WST-1 (250 μM) was added and the plate was re-incubated with shaking for 3 h. Absorbance was measured at 450 nm with a microplate reader (SpectraMax 340, Molecular Devices). Percentage cell viability was calculated by using the following formula:

$$\%\text{Cell Viability} = \{(\text{OD test compound} \times 100/\text{OD control}) - 100\}$$

4.1.5. Cyclooxygenses inhibition assay

The assay was performed as per manufacturer's instruction (Cayman Chemicals, Ann Arbor, USA). The activity was assayed colorimetrically by monitoring the appearance of oxidized *N, N, N′, N′*-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm. Inhibition of COX activity by a variety of selective inhibitors was calculated based on their IC_{50} values. Five replicates of each experiment were performed.

4.1.6. Lipoxygense inhibition assay

Lipoxygenase inhibitory activity was measured by slightly modifying the spectrophotometric method developed by Tappel et al. [29]. In a total volume of 200 μL, test compound, and lipoxygenase solutions were mixed with sodium phosphate (100 mM) buffer (pH 8.0) and incubated for 10 min at 25 °C. The reaction was then initiated by the addition of 10 μL linoleic acid solution and the change of absorbance at 234 nm was measured for next 10 min due to formation of (9*Z*, 11*E*)-(13*S*)-13- hydroperoxyoctadeca-9, 11-dienoate. Baicalein (Aldrich Chem. Co.) was used as a positive control in the assay as described by Ellis and Luscombe [30]. The IC_{50} values were then calculated by using the Ez-Fit 5.0.

4.1.7. Platelet aggregation assay

Venous blood was mixed with sodium citrate (3.8%) solution in 9:1 ratio, in order to prevent coagulation. The whole blood was centrifuged at 1000 rpm for 15 min at 37 °C to obtain platelet rich plasma (PRP). The upper part of the plasma which was rich in platelets was taken. The remaining plasma was again centrifuged for 10 min at 37 °C to obtain platelet poor plasma (used as blank). This plasma hardly contained any platelet.

Aggregation was measured by using a Dual Channel Lumi-aggregometer with 230 μL aliquots of PRP in small glass cuvette. Various concentrations (200–10 μM) of test compounds and aspirin, (10 μL), were dissolved in DMSO (10% with normal saline) and incubated at for 1 min before challenged with platelet aggregation agonist (Arachidonic acid). Aggregation was recorded for 4 additional minutes, so the total exposure time of the platelets to inhibitors was 5 min. Percent inhibitions were expressed in the dose-response curves between the % aggregation and the time in minutes.

4.1.8. In vitro superoxide scavenging assay by using xanthine/xanthine oxidase system

In a total 200 μL reaction volumes, various concentrations of the test samples were tested with xanthine oxidase (0.011 units/well).

Plate was pre-incubated for 10 min at room temperature. WST-1 (15 μM) was added and pre-read absorbance was recorded at 450 nm. Continues kinetics analysis was initiated by adding xanthine (20 μL) and the kinetic absorbance was read for 30 min. At the end of reaction, endpoint absorbance was recorded. Each experiment was the mean of triplicates.

4.1.9. *In vivo carrageenan-induced rat paw edema antiinflammatory activity measurement*

In vivo antiinflammatory activity was measured by using modified method of Winter et al. [16]. Various doses of the test compounds (0.5, 1, 5, and 10 mg/mL/kg body weight) were injected intra-peritoneally (*i.p.*). After 30 min, edema was induced by injecting 0.05 mL of 0.1% carrageenan (dissolved in 0.9% saline) in the sub-planter region of the right paw of each rat. Control group of animals received only vehicle (10% DMSO with 0.9% saline). Paw volume was measured by using plathysmometer after 30 min, and 1, 2, 3, and 4 h, after the carrageenan injection. 0 h measurement was taken prior to the *i.p.* injection of test compounds. The percentages of the inhibition in the treated animals versus control group were calculated by using following formula:

$$\%\ \text{inhibition} = (V_T - V_C/V_C) \times 100$$

Where, V_T = Volume of paw for test sample and V_C = Volume of paw for control group

5. Experimental

Melting points were determined on a Büchi 434 melting point apparatus and were uncorrected. NMR was performed on a Bruker AM 300, 400 and 500 MHz, respectively. CHN analysis was performed on a Carlo Erba Strumentazion-Mod-1106, Italy. Ultraviolet (UV) spectra were recorded on Perkin–Elmer Lambda-5 UV/VIS spectrometer in MEOH. Infrared (IR) spectra were recorded on JASCO IR-A-302 Spectrometer as KBr (disc). Electron impact mass spectra (EIMS) were recorded on a Finnigan MAT-311A, Germany. Thin layer chromatography (TLC) was performed on pre-coated silica gel glass plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm or by iodine vapors.

5.1. 4-Oxo-4H-chromene-3-carbaldehyde (**1**)

Yield: 82%; mp = 142 °C; R_f: 0.56 (EtOAc: Hex, 3:7) [25].

5.2. 3-{[(2-Benzoyl-4-chlorophenyl) imino] methyl}-4H-chromen-4-one (**3**)

Yield = 76%; mp = 138 °C; R_f = 0.52 (EtOAc:Hex, 3:7) [25].

5.3. 2-{[(4-Oxo-4H-chromen-3-yl) methylidene] amino}benzoic acid (**4**)

Yield = 64%; mp = 96 °C; R_f = 0.42 (EtOAc:Hex, 3:7) [25].

5.4. 3-{[(2,6-Dimethylphenyl)imino]methyl}-4H-chromen-4-one (**5**)

Yield = 53%; mp = 84 °C; R_f = 0.61(EtOAc:Hex, 3:7) [25].

5.5. 3-{[(3, 5-Dimethylphenyl)imino]methyl}-4H-chromen-4-one (**6**)

Yield = 72%; mp = 184 °C; R_f = 0.51 (EtOAc:Hex, 3: 7) [26].

5.6. 3-{[(3-Methylphenyl) imino] methyl}-4H-chromen-4-one (**7**)

Yield = 60%; mp = 116 °C; R_f = 0.46 (EtOAc:Hex, 3:7) [25].

5.7. 3-[Bis(tert-butylamino)methyl]-4H-chromen-4-one (**8**)

Yield = 46%; mp = 138 °C; R_f = 0.45 (EtOAc:Hex, 5:5) [25].

5.8. 3-{[(2-{[(4-Oxo-4H-chromen-3-yl)methylidene]amino}ethyl) imino] methyl}-4H-chromen-4-one (**9**)

Yield = 23%; mp = 188 °C; R_f = 0.35 (EtOAc:Hex, 3:7) [25].

5.9. 3-{[(3-Methoxy-4-methylphenyl) imino] methyl} -4H-chromen-4-one (**10**)

Yield = 72%; mp = 86 °C; R_f = 0.42 (EtOAc:Hex, 3:7) [25].

5.10. 3-{[(3-Methoxyphenyl)imino]methyl}-4H-chromen-4-one (**11**)

Yield = 43%; mp = 178 °C; R_f = 0.35 (EtOAc:Hex, 5:5) [25].

5.11. 3-[(Phenylimino) methyl]-4H-chromen-4-one (**12**)

Yield = 69%; mp = 125 °C; R_f = 0.52 (EtOAc:Hex, 3:70) [25].

5.12. 3-{[(2-Methoxy-5-nitrophenyl) imino] methyl}-4H-chromen-4-one (**13**)

Yield = 78%; mp = 266 °C; R_f = 0.38 (EtOAc:Hex, 3:7) [25].

5.13. 3-{[(2-Methoxy-4-nitrophenyl) imino] methyl}-4H-chromen-4-one (**14**)

Yield = 63%; mp = 268 °C; R_f = 0.46 (EtOAc:Hex, 3:7) [25].

5.14. 3-{[(3-Hydroxy-2-pyridinyl)imino]methyl}-4H-chromen-4-one (**15**)

Yield = 62%; mp = 144 °C; R_f = 0.5 (EtOAc:Hex, 3:7) [25].

5.15. N′-[(4-Oxo-4H-chromen-3-yl) methylidene] benzohydrazide (**16**)

Yield = 58%; mp = 201 °C; R_f = 0.42 (EtOAc:Hex, 3:7) [26].

5.16. 4-Methyl-N′-[(4-Oxo-4H-chromen-3yl) methylidene] benzenesulfonohydrazide (**17**)

Yield = 56%; mp = 208 °C; R_f = 0.41 (EtOAc:Hex, 3:7) [26].

5.17. N′-[(4-Oxo-4H-chromen-3-yl) methylene] isonicotinohydrazide (**18**)

Yield = 56%; mp = 278 °C; R_f = 0.45 (EtOAc:Hex, 3:7); UV (MeOH); λ_{max} 206.1 (log ε = 4.2) nm; IR (KBr): υ_{max} 3448 (N–H), 3050 (Ar CH), 2924 (CH_3), 2862 (CH_2), 1702 (C=O) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ: 10.45 (s, 1H N–H), 8.80 (d, $J_{5,6}$ = 8.1 Hz, 1H, H-5), 8.62 (s, 1H, CH=N), 8.22 (s,1H, H-2), 7.83 (d, $J_{3',2'} = J_{5',6'}$ = 6.4 Hz, 2H, H-3′,5′), 7.69 (d, $J_{2',3'} = J_{6',5'}$ = 6.4 Hz, 2H, H-2′, 6′), 7.47 (td, $J_{7,5}$ = 1.5, $J_{7,6} = J_{7,8}$ = 8.2 Hz, 1H, H-7), 7.0 (d, $J_{8,7}$ = 8.2 Hz, 1H, H-8), 6.91 (t, $J_{6,5} = J_{6,7}$ = 8.2 Hz, 1H, H-6); EIMS: *m*/*z* (rel. abund. %) 292 (M^+, 12), 188 (80), 172 (3.2), 120 (100); Anal. Calc. for $C_{13}H_{11}N_3O_3$: (293): [Found: C, 65.52; H, 3.77; N, 14.34. Requires: C, 65.53; H, 3.78; N, 14.33].

*5.18. N′-[(4-Oxo-4H-chromen-3-yl) methylene] heptanohydrazide (**19**)*

Yield = 78%; mp = 201 °C; R_f = 0.52 (EtOAc:Hex, 5:5) [26].

*5.19. N′-[(4-Oxo-4H-chromen-3-yl)methylidene] nicotinohydrazide (**20**)*

Yield = 52%; mp = 278 °C; R_f = 0.45 (EtOAc:Hex, 3:7) [26].

*5.20. N-Hydroxy-N-oxo-4-({2-[(4-oxo-4H-chromen-3-yl) methylidene]hydrazino}carbonyl)benzenaminium (**21**)*

Yield = 74%; mp = 208 °C; R_f = 0.43 (EtOAc:Hex, 3:7) [26].

*5.21. N′-[(4-Oxo-4H-chromen-3-yl) methylidene] propanohydrazide (**22**)*

Yield = 46%; mp = 278 °C; R_f = 0.42 (EtOAc:Hex, 3:7) [26].

*5.22. N′-[(4-Oxo-4H-chromen-3-yl) methylidene] butanohydrazide (**23**)*

Yield = 61%; mp = 197 °C; R_f = 0.48 (EtOAc:Hex, 2:8) [26].

*5.23. N′-[(4-Oxo-4H-chromen-3-yl)methylidene] nonanohydrazide (**24**)*

Yield = 46%; mp = 278 °C; R_f = 0.44 (EtOAc:Hex, 3:7) [26].

Acknowledgements

This work was financially supported by the Higher Education Commission (HEC) Pakistan under the National Research Support Program for Universities. The help of the Fatimid Foundation is highly acknowledged for providing fresh healthy blood for this research study.

References

[1] J.M. Alvaro-Gracia, Licofelone-clinical update on a novel LOX/COX inhibitor for the treatment of osteoarthritis. Rheumatology 43 (2004) 121.
[2] S. Cuzzocrea, Inflammation and PARP. Shock Pharm. Res. 52 (2005) 72.
[3] A. Roussin, V.L. Cabec, M. Lonchampt, J. De Nadaý, E. Canet, I.M. Parini, Neutrophil-associated inflammatory responses in rats are inhibited by phenylarsine oxide. Eur. J. Pharmacol. 322 (1997) 91.
[4] Q. Liu, K. Suzuki, S. Kudo, M. Yamada, K. Kowatari, T. Umeda, S. Nakaji, K. Sugawara, Effect of decaglycerol monooleate on of human neutrophils: an *in vitro* study. Food Chem. Toxicol. 38 (2000) 423.
[5] A.S. Tan, M.V. Berridge, Superoxide produced by activated neutrophils efficiently reduces the tetrazolium salt, WST-1 to produce a soluble formazan: a simple colorimetric assay for measuring respiratory burst activation and for screening antiinflammatory agents. J. Immunol. Meth. 238 (2000) 59.
[6] G. Costantino, S. Cuzzocrea, E. Mazzon, A.P. Caputi, Protective effects of melatonin in zymosan-activated plasma-induced paw inflammation. Eur. J. Pharmacol. 363 (1998) 57.
[7] A.M. Francoeur, A. Assalian, A novel cell proliferation and cytotoxicity assay based on WST-1. Microcat: Biochemica. 3 (1996) 19.
[8] R.A. Siddiqui, D. English, Y. Cui, M.I. Martin, J. Wentlands, L. Akard, Phorbol ester-induced priming of superoxide generation by phosphatidic acid-stimulated neutrophils and granule-free neutrophil cytoplasts. J. Leukoc. Biol. 58 (1995) 189.
[9] C. Charlier, C. Michaux, Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. Eur. J. Med. Chem. 38 (2003) 645.
[10] C.D.W. Brooks, A.O. Stewart, A. Basha, P. Bhatia, J.D. Ratajczyk, J.G. Martin, R. A. Craig, T. Kolasa, J.B. Bouska, C. Lanni, R.R. Harris, P.E. Malo, G.W. Carter, R. L. Bell, Preparation of (*R*)-(+)-*N*-[3-[5-[(4-fluor-ophenyl) methyl]-2-thienyl]-1-methyl-2-propynyl]-N-hydroxyurea (ABT-761), a second-generation 5-lipoxygenase inhibitor. J. Med. Chem. 38 (1995) 4768.
[11] M.V. Berridge, A.S. Tan, K.D. Mccoy, R. Wang, The biochemical and cellular basis of cell proliferation assay that use tetrazolium salts. Biochemica 4 (1996) 14.
[12] M. Ishiyama, H. Tominaga, M. Shiga, K. Sasamoto, Y. Ohkura, K. Ueno, A combined assay of cell viability and *in vivo* toxicity with a highly water-soluble tetrazolium salt, neutral red and crystal violet. Biol. Pharm. Bull. 19 (1996) 1518.
[13] D. Steinhilber, A target for antiinflammatory drug revisited. Curr. Med. Chem. 6 (1999) 71.
[14] D. Nie, K.V. Honn, Cyclooxygenase, lipoxygenase and tumor angiogenesis. Cell. Mol. Life Sci. 59 (2002) 799.
[15] G.P. Ellis, D.K. Luscombe, Progress in Medicinal Chemistry, vol. 29, Elsevier Science, 1992.
[16] G.P. Ellis, in: A. Weissberger, E.C. Taylor (Eds.), Chromenes, Chromanones, and Chromones, John Wiley and Sons, New York, 1977, pp. 1–10.
[17] R. Kumar, M. Yousuf, Chromones and bischromones: an account of photoinduced reactions. ARKIVOC 9 (2006) 239.
[18] G. Athanasellis, G. Melagraki, A. Afantitis, K. Makridima, O. Igglessi-Markopoulou, A simple synthesis of functionalized 2-amino-3-cyano-4-chromones by application of the *N*-hydroxybenzotriazole methodology. ARKIVOC 10 (2006) 28.
[19] G.R. Singh, N.K. Singh, M.P. Girdhar, S. Ishar, A versatile route to 2-alkyl-/aryl-amino-3-formyl and hetero annelated chromones through a facile nucleophilic substitution at C-2 in 2-(*N*-methylanilino)-3-formylchromones. Tetrahedron 58 (2002) 2471.
[20] L.Z. Piao, H.R. Park, Y.K. Park, S.K. Lee, J.H. Park, M.K. Park, Mushroom tyrosinase inhibition activity of some chromones. Chem. Pharm. Bull. 50 (2002) 309.
[21] H. Harnisch, Chromone-3-carboxaldehyde. Liebigs Ann. Chem. 765 (1972) 8.
[22] K.M. Khan, N. Fatima, M. Rasheed, S. Jalil, N. Ambreen, S. Perveen, M. I. Choudhary, 1,3,4-Oxadiazole-2(3*H*)-thione and its analogues: a new class of non-competitive nucleotide pyrophosphatases/phosphodiesterases 1 inhibitors. Bioorg. Med. Chem. 17 (2009) 7816.
[23] K.M. Khan, U.R. Mughal, N. Ambreen, A. Khan, S. Perveen, M.I. Choudhary, Schiff bases of istain: antiglycation activity. Lett. Drug Des. Discov. 6 (2009) 358.
[24] K.M. Khan, U.R. Mughal, N. Ambreen, Samreen, S. Perveen, M.I. Choudhary, Synthesis and lishmenicidal activity of 2,3,4-substituted-5-imidazolon. J. Enzym. Inhib. Med. Chem. (2009).
[25] K.M. Khan, N. Ambreen, S. Hussain, S. Perveen, M.I. Choudhary, Schiff bases of 3-formylchromone as thymidine phosphorylase inhibitors. Biorg. Med. Chem. 17 (2009) 2983.
[26] K.M. Khan, A. Ahmad, N. Ambreen, A. Afroz, S. Perveen, S.A. Khan, M.I. Choudhary, Schiff bases of 3-formylchromones as phytotoxic, antibacterial, and antifungal agents. Lett. Drug Des. Discov. 6 (2009) 363.
[27] L. Margita, L. Dusan, F. Mikulas, M. Maria, H.M. Elshaaer, Synthesis and reactions of new 4-oxo-4*H*-benzopyran-3-carboxaldehydes containing hydroxy groups or 2-oxopyran cycles. Molecules 3 (1998) 149.
[28]. C.A. Winter, E.A. Risley, G.W. Nuss, Carrageenan-induced edema in the hind paw of the rat as an assay for antiinflammatory drugs. Proc. Soc. Exp. Biol. Med. 111 (1962) 544.
[29]. A.L. Tappel, Methods of Enzymology, vol. 5, Academic Press, New York, 1962.
[30]. G.P. Ellis, D.K. Luscombe, Progress in Medicinal Chemistry. Elsevier Science Pub Co, 1992.