Synthesis, Characterization and Anti-Inflammatory Study of New Heterocyclic Coumarin Derivatives

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Abstract

The inflammation is one of the most central processes in animal cells defense versus certain injuries or infections of microbes. The most essential metabolic precursor for many inflammatory pathways is PGH2 (prostaglandin), which catalytic synthesis from the AA (arachidonic acid) by COX enzymes. COX can be divided in to three isomer COX-1 has important role in many physiological function like hemostasis, platelet aggregation, and protection of gastric mucosa, COX-2 when stimulate cause formation of PGE2 excessively, with other prostaglandin then decrease the pain threshold and nerve ending sensitization, which induce pain, increase permeability of vascular and then enhance the inflammatory associated diseases pathway, and COX-3 has special characteristic, its higher sensitivity to acetaminophen and present in brain. The NSAIDs are therapeutic agents used for the treatment of inflammation, pain, and fever, they work by decreasing the production of prostaglandins due to inhibiting the function of the cyclooxygenase (COX) enzyme, we have two types nonselective and selective COX-2 inhibitors. In order to design new agents with no or low side effect, the COX-2 selectivity should be increased, this achieved by design molecule structurally similar to approved selective COX-2 inhibitors. The synthetic compound in this study contain three pharmacophores, a nucleus of coumarin and substituted oxazole moiety, separated by a hydrazonoethyl spacer, which have structural similarity properties to selective COX-2 inhibitors.

Keywords: *Heterocyclic coumarin derivatives, anti-inflammatory, selective COX-2 inhibitor*

Introduction

Inflammation is one of the most central processes desired in defense of animal cells versus certain injuries or infections of microbes. Though, inflammation orderly progresses to acute and chronic. Chronic inflammation is caused by a set of diseases like cardiovascular diseases, cancer, and neurodegenerative disorders [(1)]. The driving force for accumulation of fluid may rise only from the actual tissue metabolic activity, the final being a balance between anabolic and catabolic processes in physiological conditions [(2)]. Main proceedings occur through the inflammatory process contain alteration of vascular permeability, recruitment of leukocyte and accumulation, and liberation inflammatory mediator release [(3)]. Inflammation is a popular pathogenesis of numerous chronic diseases, such as cardiovascular diseases, arthritis, diabetes, bowel disease and cancer [(4)]. The most essential metabolic precursor for many inflammatory pathways is the AA (arachidonic acid). many internal and external factor can activate the PLA2 (phospholipase A2). This activation split bounded AA from the membrane phospholipids and makes it ready for the major three inflammatory pathways, which are lipoxygenase, COX pathway, and cytochrome P-450 monooxygenase [(5)]. COX can be divided into, COX-1 has important role in many physiological function like hemostasis, platelet aggregation, and protection of gastric mucosa [(5)]. The stimulation of COX-2 cause formation of PGE2 (major metabolic product) excessively, with other prostaglandin then decrease the pain threshold and nerve ending sensitization, which induce pain, increase permeability of vascular and then enhance the inflammatory associated diseases pathway [(6)]. COX-

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3 has special characteristic, its higher sensitivity to acetaminophen, and it has been reported in brain and heart [7]. NSAIDs are remarkable therapeutic agents used for the treatment of inflammation, pain, and fever. They work by decreasing the production of prostaglandins due to inhibiting the function of the cyclooxygenase (COX) enzyme [8]. The traditional types are correlated with side effects like ulceration of gastrointestinal (GI) and renal toxicity because of their inhibition of COX-1 pathway (nonselective) [9]. coumarins consisting of a benzene ring connect to a pyrone ring, figure (1), so they are classified as a type of the benzopyrone family [10,11].

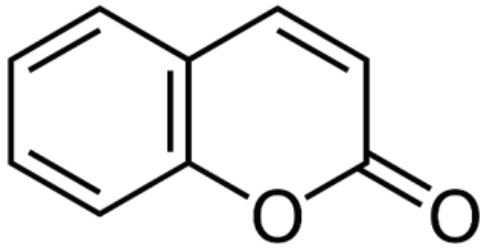


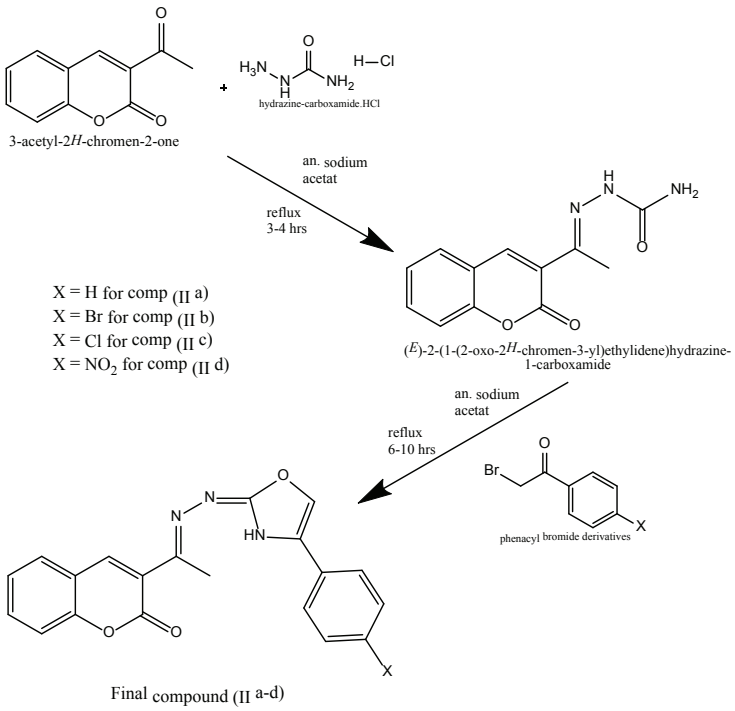
Figure (1) Chemical structure of Coumarin

Both natural and synthetic origin compounds of coumarins have an important. Several compounds which containing the coumarin nucleus show useful and varied biological and pharmaceutical activities, Some of these compounds useful in antitumor, photochemotherapy, anti-HIV therapy, antibacterial, antifungal, anticoagulants, antioxidant agents, as CNS-stimulants, and as dyes. The synthetic, semi-synthetic and natural coumarins are beneficial substances in drug designs and studies [12.13]. This work is focus on Synthesis and anti-inflammatory evaluation of coumarins derivatives containing three pharmacophores a nucleus of coumarin and substituted oxazole moiety, separated by a hydrazonoethyl spacer.

Materials and methods

3-acetyl coumarin bought from SRCT company (Shanghai). Solvent and other reagent that used through reaction were bought from the chemicals store of college of the pharmacy. The monitoring of the reactions was done by thin layer chromatography (TLC), the mobile phase solvent systems used are A: touloene:ethyl (2:1) and B: chloroform:methanol (9.5:0.5). Electronic melting point apparatus (Stuart SMP30) was used to determine all melting points in this study. FTIR spectrophotometer (Schimadzu, Japan), were done by thin film technique. ^{1}HNMR spectra were obtained on BRUKER model Ultra shield 500 MHz spectrophotometer, using Dimethyl sulfoxide (DMSO) as a solvent.

The pathway of synthesis for final compounds was illustrated below in scheme (1)

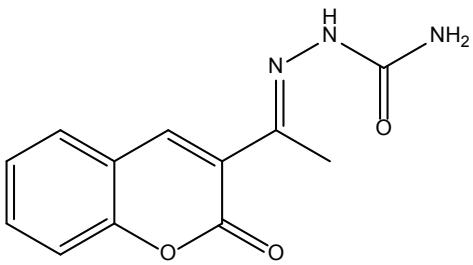


Scheme (1) the overall pathway of synthesis for final compounds

Synthesis of (E)-2-(1-(2-oxo-2H-chromen-3-yl) ethylidene) hydrazine-1-carboxamide compound (I)

A mixture of 3-acetyl coumarin (1.88 g, 0.01mol), hydrazine-carboxamide.HCl (1.11 g, 0.01mol), and anhydrous sodium acetate (0.82 g, 0.01 mol) in (25 mL) absolute ethanol was refluxed for 3–4 hrs. The obtained solid was filtered, dried, and recrystallized from ethanol to give compounds (I) [14, 15, 16].

Yield = 89%, R*f* = 0.42(B), IR: (3483 &3147 cm^{-1}) N-H stretching of 1º & 2º amine, (3074 cm^{-1}) C-H stretching of Ar ring, (2974 cm^{-1}) C-H stretching of alkene, (1747 cm^{-1}) C=O stretching of lactone ring, (1705 cm^{-1}) C=O stretching of amid, (1585 cm^{-1}) C=N stretching, (1608, 1492 &1435 cm^{-1}) C=C stretching of Ar ring (1238 & 1265 cm^{-1}) C-O-C stretching of cyclic ether, (1118 & 740 cm^{-1}) in and out of plane of Ar ring. **^{1}HNMR:** 2.13 (3H, s, $-CH_3$), 6.53 (2H, s, $-NH_2$), 7.38 (2H, m, Ar C-H), 7.61 (1H, m, Ar C-H), 7.76 (1H, m, Ar C-H), 8.32 (1H, m, Ar C-H), 9.53 (1H, s, $-NH-NH_2$).



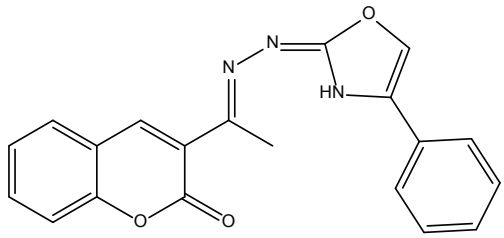
Synthesis of final compounds (II a- II d)

A mixture of compounds I (2.45 g, 0.01 mol), one of following phenacyl bromide or its derivatives: phenacyl bromide (1.99 g, 0.01 mol) for compound (II a), p- bromo phenacyl bromide (2.79 g, 0.01 mol) for compound (II b), p-chloro phenayl bromid (2.33 g, 0.01 mol) for compound (II c), p-nitro phenacyl bromid (2.44 g, 0.01 mol) for compound (II d), and anhydrous sodium acetate (0.82 g, 0.01 mol) in (30 mL) absolute ethanol, was refluxed for 6–10 hrs. The mixture was filtered after cooling, the filtrate was evaporated, the remaining powder washed with water, and dried. The final compounds were isolated and purified by column chromatography by using (silica gel) as stationary phase and (n-hexane with ethyl acetate 2:1) as mobile phase.

Then crystallized from ethanol to give compounds (III a-d) [14, 17].

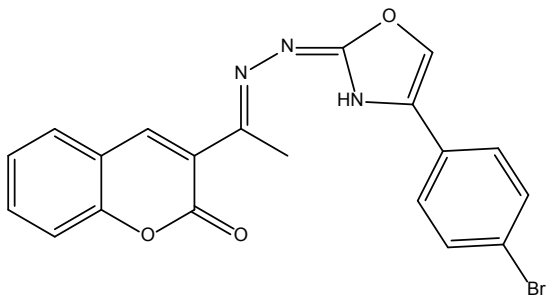
(II a) 3-((E)-1-(((E)-4-phenyloxazol-2(3H)-ylidene)hydrazineylidene)ethyl)-2H-chromen-2-one

yellow powder, yield = 50%, M.P. = (40-43) ºC, R*f* = 0.73 (A), IR: (3066 cm^{-1}) N-H stretching of 2º amine, (3016 cm^{-1}) C-H stretching of Ar ring, (2981 & 2939 cm^{-1}) C-H stretching of alkane, (1735 cm^{-1}) C=O stretching of lactone ring, (1693 cm^{-1}) C=N stretching, (1597 &1423 cm^{-1}) C=C stretching of Ar ring (1222, 1242 & 1280 cm^{-1}) C-O-C stretching of cyclic ether, (1076 & 752 cm^{-1}) in and out of plane of Ar ring. **^{1}HNMR** 2.14 (3H, s, $-CH_3$), 5.43 (1H, s, C-NH-C), 7.54 (2H, m, Ar C-H), 7.57 (2H, m, Ar C-H), 7.68 (2H, m, Ar C-H), 7.94 (1H, m, Ar C-H), 7.9 (3H, m, Ar C-H), 7.97 (1H, m, Ar C-H).



(II b) 3-((E)-1-(((E)-4-(4-bromophenyl)oxazol-2(3H)-ylidene)hydrazineylidene)ethyl)-2H-chromen-2-one

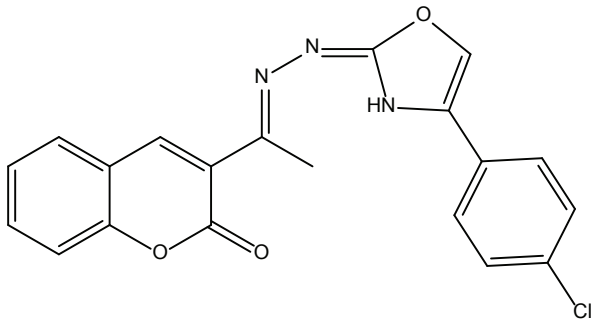
yellow powder, yield = 62%, M.P. = (78-81) ºC, R*f* = 0.76 (A), IR: (3089 cm^{-1}) N-H stretching of 2º amine, (3059 cm^{-1}) C-H stretching of Ar ring, (2989 & 2951 cm^{-1}) C-H stretching of alkane, (1743 cm^{-1}) C=O stretching of lactone ring, (1689 cm^{-1}) C=N stretching, (1585, 1485 & 1431 cm^{-1}) C=C stretching of Ar ring (1219 & 1246 cm^{-1}) C-O-C stretching of cyclic ether, (1068 & 813 cm^{-1}) in and out of plane of Ar ring. **^{1}HNMR** 2.13 (3H, s, $-CH_3$), 5.43 (1H, s, C-NH-C), 7.75 (2H, m, Ar C-H), 7.77 (1H, m, Ar C-H), 7.78 (1H, m, Ar C-H), 7.87 (3H, m, Ar C-H), 7.89 (2H, m, Ar C-H), 7.92 (1H, m, Ar C-H).



(II c) 3-((E)-1-(((E)-4-(4-chlorophenyl)oxazol-2(3H)-ylidene)hydrazineylidene)ethyl)-2H-chromen-2-

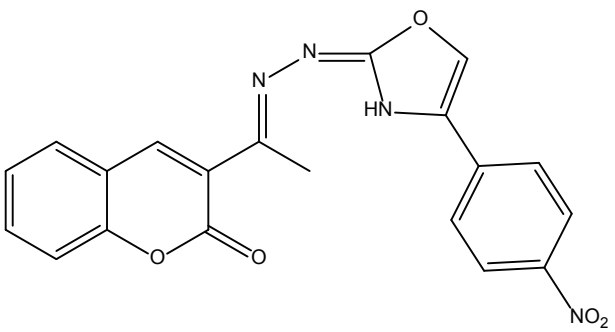
one

Yellowish to orang powder, yield = 65 %, M.P. = (66-69) ºC, R*f* = 0.76 (A), IR: (3078 cm^{-1}) N-H stretching of 2º amine, (3020 cm^{-1}) C-H stretching of Ar ring, (2981 & 2939 cm^{-1}) C-H stretching of alkane, (1739 cm^{-1}) C=O stretching of lactone ring, (1693 cm^{-1}) C=N stretching, (1589, 1489 & 1423 cm^{-1}) C=C stretching of Ar ring (1219 & 1238 cm^{-1}) C-O-C stretching of cyclic ether, (1083 & 821 cm^{-1}) in and out of plane of Ar ring. **^{1}HNMR** 2.14 (3H, s, -CH_3), 5.44 (1H, s, C-NH-C), 7.61 (1H, m, Ar C-H), 7.63 (4H, m, Ar C-H), 7.96 (4H, m, Ar C-H), 7.99 (1H, m, Ar C-H).



(II d) 3-((E)-1-(((E)-4-(4-nitrophenyl)oxazol-2(3H)-ylidene)hydrazineylidene)ethyl)-2H-chromen-2-one

Orang powder, yield = 37%, M.P. = (98-101) ºC, R*f* = 0.68 (A), IR: (3120 cm^{-1}) N-H stretching of 2º amine, (3082 cm^{-1}) C-H stretching of Ar ring, (2981 & 2947 cm^{-1}) C-H stretching of alkane, (1743 cm^{-1}) C=O stretching of lactone ring, (1701 cm^{-1}) C=N stretching, (1600 & 1419 cm^{-1}) C=C stretching of Ar ring, (1519 & 1346 cm^{-1}) N-O stretching (asymmetrical and symmetrical), (1215 & 1246 cm^{-1}) C-O-C stretching of cyclic ether, (1080 & 856 cm^{-1}) in and out of plane of Ar ring. **^{1}HNMR** 2.15 (3H, s, -CH_3), 5.52 (1H, s, C-NH-C), 8.18 (2H, m, Ar C-H), 8.19 (3H, m, Ar C-H), 8.35 (3H, m, Ar C-H), 8.37 (2H, m, Ar C-H).



Anti-inflammatory Study

The inflammatory model that used to evaluate final compounds (II a-d) for the *in vivo* acute anti-inflammatory effects exploited egg-white induced rat paw edema, for comparison with the anti-inflammatory activity of celecoxib. The decrease of paw thickness is the basis of screening of the newly synthesized compounds for their anti-inflammatory activity.

Methods of Anti-inflammatory study

Both sex albino rats (200 ± 10) were provided by Iraqi Center for Cancer & Medical Genetic Research / Al-Mustansiriyah University, and housed under standardized conditions in animal house. Animals were divided into six groups (six rats per group) as follow: **Group 1**: injected with the vehicle (in DMSO) with a dose of 2 ml/kg and served as a control group. **Group 2**: injected with celebrax with a dose of 5mg/kg [18], dissolved in DMSO, and served as a reference group. **Group 3-6**: each group of six rats injected with the tested compounds (III a-d) respectively, in doses (4.5 mg/kg for comp II a, 5.5 mg/kg for comp II b, 5 mg/kg for comp II c, and 5.1 mg/kg for comp II d), dissolved in DMSO. The reference substance celecoxib was administered both with the tested compounds by the intra-peritoneal route (i.p.). Egg albumin was used to induce rat paw edema as an acute inflammatory model for studying the activity of the final compounds. 0.05mL of undiluted ovalbumin was subcutaneously injected into the left hand paw of the rats; preceded by a half hour of intraperitoneal injection of the drugs or their vehicle [19]. Digital vernier used for measuring paw thickness at 7 periods (0, 30, 60, 120, 180, 240, and 300 minutes) and these measurements were taken after the intra-peritoneal administration of the tested compounds or DMSO (control), considered as time zero [20].

In this study there was no significant difference among the tested compounds compared to celecoxib and control at baseline, after 30 and 60 minutes. However, after 120 to 300 minutes, compounds (II b, II c, and II d) showed more activities to reduce paw thickness compared to celecoxib, in which (III d) showed best reduction in paw thickness compared to the other compounds. Compounds (II a) showed less activities to reduce paw thickness when compared to the standard celecoxib after. Among them, compounds (II b, II c, and II d) was found to exhibit the best reduction in paw

thickness relative to the other compounds as illustrated in Figure (2) and Table (1).

Table (1): Effect of Dimethyl sulfoxide (control), celecoxib (standard) and target compounds (II a-II d) on induced paw edema

	Paw Thickness (mm)					
Time (min)	**Control**	**Standard**	**II a**	**II b**	**II c**	**II d**
0	3.70±0.03a	3.51±0.03ab	3.63±0.06ab	3.53±0.16ab	3.46±0.04ab	3.40±0.07b
30	5.56±0.03ab	5.50±0.04ab	5.48±0.08d	5.33±0.11bc	5.31±0.04bc	5.10±0.03c
60	6.6±0.06b	6.11±0.06b	6.55±0.05a	5.96±0.08b	5.83±0.03d	5.86±0.04e
120	6.95±0.06a	5.11±0.07d	6.08±0.06d	4.75±0.08e	4.50±0.04f	4.41±0.07f
180	6.08±0.06a	4.86±0.08c	5.11±0.07d	4.35±0.10e	4.35±0.05e	3.98±0.04f
240	5.76±0.06a	4.55±0.07b	4.66±0.06c	3.80±0.11e	4.10±0.05d	3.76±0.07 e
300	5.48±0.07a	4.21±0.07b	4.40±0.04b	3.55±0.09d	3.80±0.06c	3.70±0.06cd

Data are expressed in mm paw thickness as mean ± SEM.

Means with a different letter in the same row are significantly different (P<0.05)

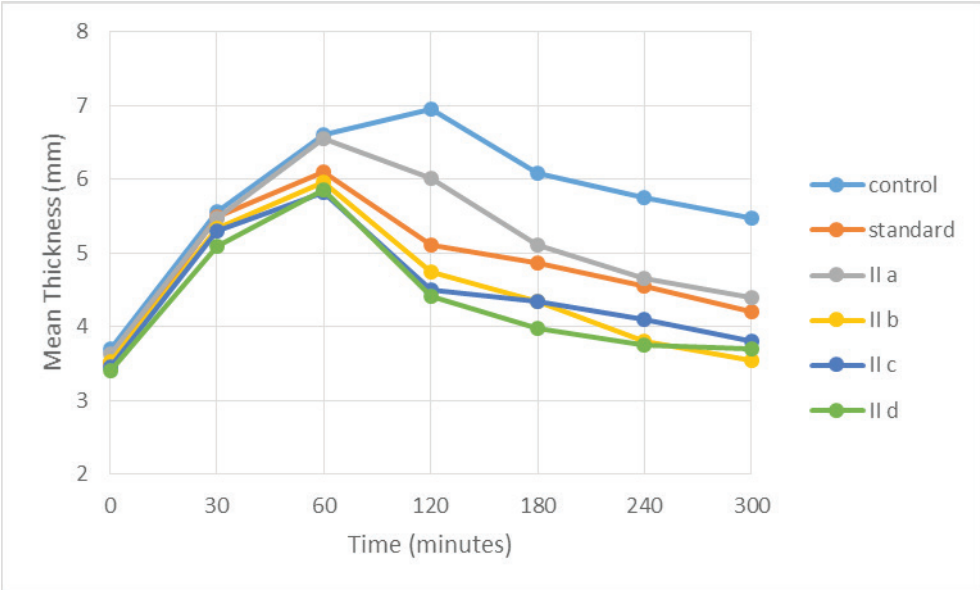


Figure (2): Effect of celecoxib (reference), DMSO (control), and compounds III a-III f on induced paw

edema in rats.

Conclusion

The proposed compounds were successfully synthesized, and their chemical structures and purity were determined using IR spectroscopy and ^{1}HNMR. The anti-inflammatory was tested using control (solvent DMSO) and standards (celecoxibe). The compounds (II b, II c, and II d) showed good anti-inflammatory activity, when compared with standard compound.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References

1. Abdulkhaleq LA, Assi MA, Abdullah R, Zamri-Saad M, Taufiq-Yap YH, Hezmee MN. The crucial roles of inflammatory mediators in inflammation: A review. Veterinary world. 2018;11(5):627.
2. Stankov SV. Definition of inflammation, causes of inflammation and possible anti-inflammatory strategies. The open inflammation journal. 2012 Jul;5(1):1-9.
3. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010 Mar 19;140(6):805-20.
4. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018 Jan 23;9(6):7204.
5. Attiq A, Jalil J, Husain K, Ahmad W. Raging the war against inflammation with natural products. Frontiers in Pharmacology. 2018 Sep 7;9:976.
6. Mbonye UR, Yuan C, Harris CE, Sidhu RS, Song I, Arakawa T, Smith WL. Two distinct pathways for cyclooxygenase-2 protein degradation. Journal of Biological Chemistry. 2008 Mar 28;283(13):8611-23.
7. Zarghi A, Arfaei S. Selective COX-2 inhibitors: a review of their structure-activity relationships. Iranian journal of pharmaceutical research: IJPR. 2011;10(4):655.
8. Dr J Sylvester. Nonsteroidal Anti-inflammatory Drugs. Anaesthesia tutorial of week. 2019 june;405:1-5.
9. Deb PK, Mailabaram RP, Al-Jaidi B, Saadh M. Molecular basis of binding interactions of NSAIDs and computer-aided drug design approaches in the pursuit of the development of cyclooxygenase-2 (COX-2) selective inhibitors. Nonsteroidal Anti Inflamm Drugs. 2017: 23;6:101-21.
10. Jain PK, Joshi H. Coumarin: chemical and pharmacological profile. Journal of Applied Pharmaceutical Science. 2012;2(6):236-40.
11. Venugopala KN, Rashmi V, Odhav B. Review on natural coumarin lead compounds for their pharmacological activity. BioMed research international. 2013 Oct;2013.
12. Salem MA, Marzouk MI, El-Kazak AM. Synthesis and characterization of some new coumarins with in vitro antitumor and antioxidant activity and high protective effects against DNA damage. Molecules. 2016 Feb;21(2):249.
13. Rohini K, Srikumar PS. Therapeutic role of coumarins and coumarin-related compounds. J Thermodyn Catal. 2014 : 1;5(2):1-3.
14. Dawood DH, Batran RZ, Farghaly TA, Khedr MA, Abdulla MM. New coumarin derivatives as potent selective COX□2 inhibitors: Synthesis, anti□ Inflammatory, QSAR, and molecular modeling studies. Archiv der Pharmazie. 2015 ;348(12):875-88.
15. Kapoor P, Fahmi N, Singh RV. Microwave assisted synthesis, spectroscopic, electrochemical and DNA cleavage studies of lanthanide (III) complexes with coumarin based imines. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2011: 1;83(1):74-81.
16. Kumar R, Sharma S, Singh RV, Rastogi S. Synthesis, Characterization and Biological Evaluation of Niobium (V) Complexes of Coumarin based IMINES. Rasayan Journal. 2013l;6(3):183-9.
17. Dawood NT. SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 1-(4-ARYL-2-THIAZOLYL)-AND 1-(4-ARYL-2-OXAZOLYL)-3, 5-DIARYL [Delta]^ sup 2^-PYRAZOLINE DERIVATIVES. International Journal of Pharmaceutical Sciences and Research. 2011: 1;2(9):2368.
18. Hu PJ, Yu J, Zeng ZR, Leung WK, Lin HL, Tang BD, Bai AH, Sung JJ. Chemoprevention of gastric cancer by celecoxib in rats. Gut. 2004 Feb

1;53(2):195-200.

19. Okokon JE, Udoh AE, Frank SG, Amazu LU. Anti–inflammatory and analgesic activities of Melanthera scandens. Asian Pacific Journal of Tropical Biomedicine. 2012 1;2(2):144-8.
20. Singh M, Kumar V, Singh I, Gauttam V, Kalia AN. Anti-inflammatory activity of aqueous extract of Mirabilis jalapa Linn. leaves. Pharmacognosy research. 2010 ;2(6):364.