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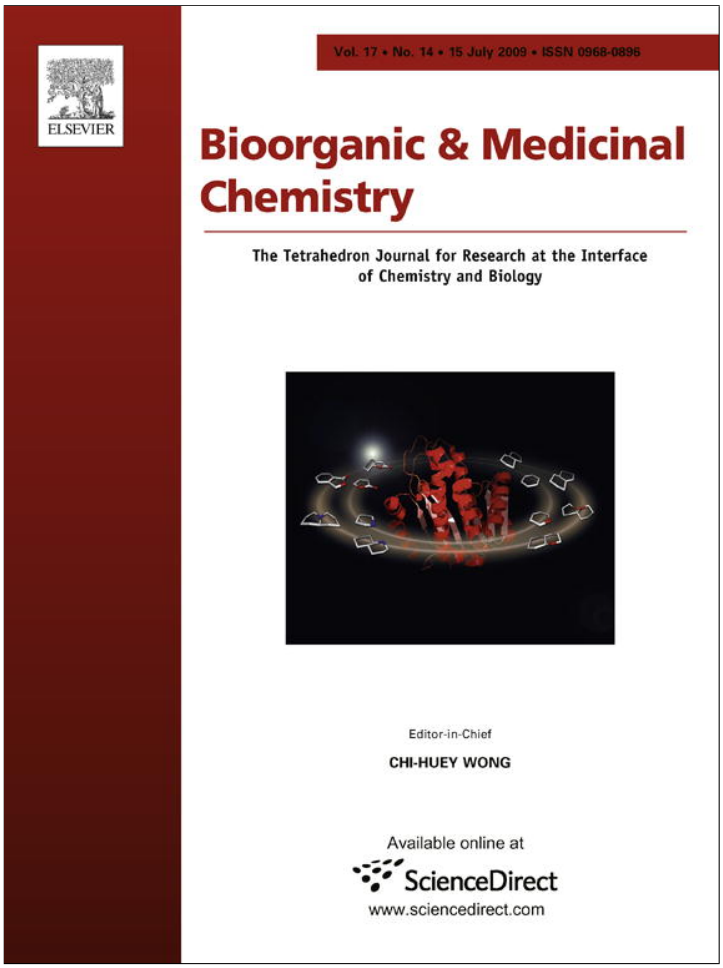

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Synthesis, biological evaluation and docking studies of novel benzopyranone congeners for their expected activity as anti-inflammatory, analgesic and antipyretic agents

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ABSTRACT

8-Acetyl-7-hydroxy-4-phenyl-2*H*-benzopyran-2-one as starting material a number of 8-substituted derivatives (i.e., hydrazones **2a**,**b**, imine **2c**, chalcones **3**, pyrazoles **4**, 3-cyano-2-oxo-dihydropyridines **5**, and/or 3-cyano-2-imino-dihydropyridines **6**) were synthesized and assayed for their anti-inflammatory, analgesic and antipyretic activities. Compounds **3c**, **4b** and **4i** showed significant anti-inflammatory, analgesic and antipyretic activities. In addition, **1**, **3b**, **4d**, **4e**, **5b**, **6a**, **6c**, **6d**, **6e** showed anti-inflammatory activity, **2b**, **4h**, **5e** exhibit analgesic activity, and **2b**, **4h**, **5e** showed antipyretic effect. In addition, molecular modeling and docking of the tested compounds into cyclooxygenase II complexed with its bound inhibitor indomethacin (4COX) using MOLSOFT ICM 3.4-8C program was performed in order to predict the affinity and orientation of the synthesized compounds at the active site. Also, it was found that the active compounds **1**, **4i**, **6a–e** interact with both Serine 530, and Tyrosine 385 amino acids which are the main amino acids involved in the mechanism of cyclooxygenase II inhibition.

The synthesis of the pyrazole-containing new compounds **4** proved a successful hit; also, the 2-imino derivatives of 3-cyano-dihydropyridines were more successful than the 2-oxo derivatives.

According to these results, we can conclude that compounds **1**, **3c**, **4b**, **4i**, and **6c** appear to be the most interesting and seem potentially attractive as anti-inflammatory, analgesic, and antipyretic agents.

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1. Introduction

Several natural products with a coumarinic moiety have been reported to have multiple biological activities, antitumor,[1–3] chronic HBV/HCV,[4] antibacterial and antifungal[5–8] and other activities related to the benzopyranone-containing compounds.[9–17] Hydroxy coumarins might affect the formation and scavenging of reactive oxygen species (ROS) and influence processes involving free radical-mediated injury, possessing antioxidant property,[18] anti-inflammatory[7,9–21] and analgesic[7,21] activities. Moreover coumarin and its 7-hydroxy-derivative known as umbelliferones are present in many plant species in nature,[4] they inhibit prostaglandin biosynthesis, which involves fatty acid hydroperoxy intermediates and protect plants from cellular damage, infestations, trauma and infections.

Natural products like esculetin, fraxetin, daphnetin and other related coumarin derivatives are recognized as inhibitors not only of the lipooxygenase and cyclooxygenase enzymic systems, but also of the neutrophil-dependent superoxide anion generation.

Due to the importance of coumarin derivatives considerable efforts have been made by several investigators, to prepare new compounds bearing single substituent or more complicated systems, including heterocyclic rings mainly at 3-, 4- and/or 7-positions. Also, literature survey reveals an excellent anti-inflammatory activity with some compounds containing heterocyclic ring as pyrazole.[22–28]

Encouraged by these findings we thought of preparing new derivatives 2–6 of 7-hydroxy-4-phenyl-2*H*-benzopyran-2-ones substituted at C-8 with different hydrazones **2a**,b and imine 2c, chalcones 3, pyrazoles 4, 3-cyano-2-oxo-dihydropyridines 5, and/or 3-cyano-2-imino-dihydropyridines 6 in order to screen them for anti-inflammatory, analgesic and antipyretic activities.

Non-steroidal anti-inflammatory drugs (NSAIDs) exert their effects by inhibition of prostaglandin production. The pharmacological target of NSAIDs is cyclooxygenase (COX, also known as prostaglandin synthase), which catalyses the first committed step in arachidonic acid metabolism. Two isoforms of the membrane protein COX are known: COX-1, which is constitutively expressed in most tissues, is responsible for the physiological production of prostaglandins; and COX-2, which is induced by cytokines, mitogens and endotoxins in inflammatory cells, is responsible for the

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elevated production of prostaglandins during inflammation.[29] It was reported that crystal structures of COX enzymes with carboxylic acid-containing NSAIDs shows that the inhibitors are positioned in a similar fashion with their carboxylates coordinates to Arg-120 and their aromatic functionality projecting into the cyclooxygenase active site towards Tyr-385,[30,31] and diarylheterocycles inhibitors of COX-2 bind in the cyclooxygenase active site above Arg-120 and insert their sulfonamide or sulfone groups into a side pocket bordered by Val-523.[1–3]

Recently, X-ray analysis of a cocrystal of arachidonic acid with COX-2 revealed that its carboxylate coordinated to Serine 530 and Tyrosine 385,[32] NSAIDs exert their action through their acetylation of Serine 530, and also hydrogen bonding by Tyrosine 385 is proposed to stabilize the negative charge of the tetrahedral intermediate that developed during Serine 530 acetylation,[33] it was demonstrated that carboxylate group of NSAI binds to COX-2 through hydrogen bonds to Tyrosine 385 and Serine 530,[34] Tyrosine 385 and Serine 530 have a structural and functional evidence for the importance of them in the chelation of the ligands.[34]

Computer docking technique plays an important role in the drug design as well as in the mechanistic study by placing a molecule into the binding site of the target macromolecule in a non-covalent fashion.[35]

Molsoft[36] as flexible docking program enables us to predict favorable protein–ligand complex structures with reasonable accuracy and speed. The docking technique will undoubtedly continue to play an important role in drug discovery.[37]

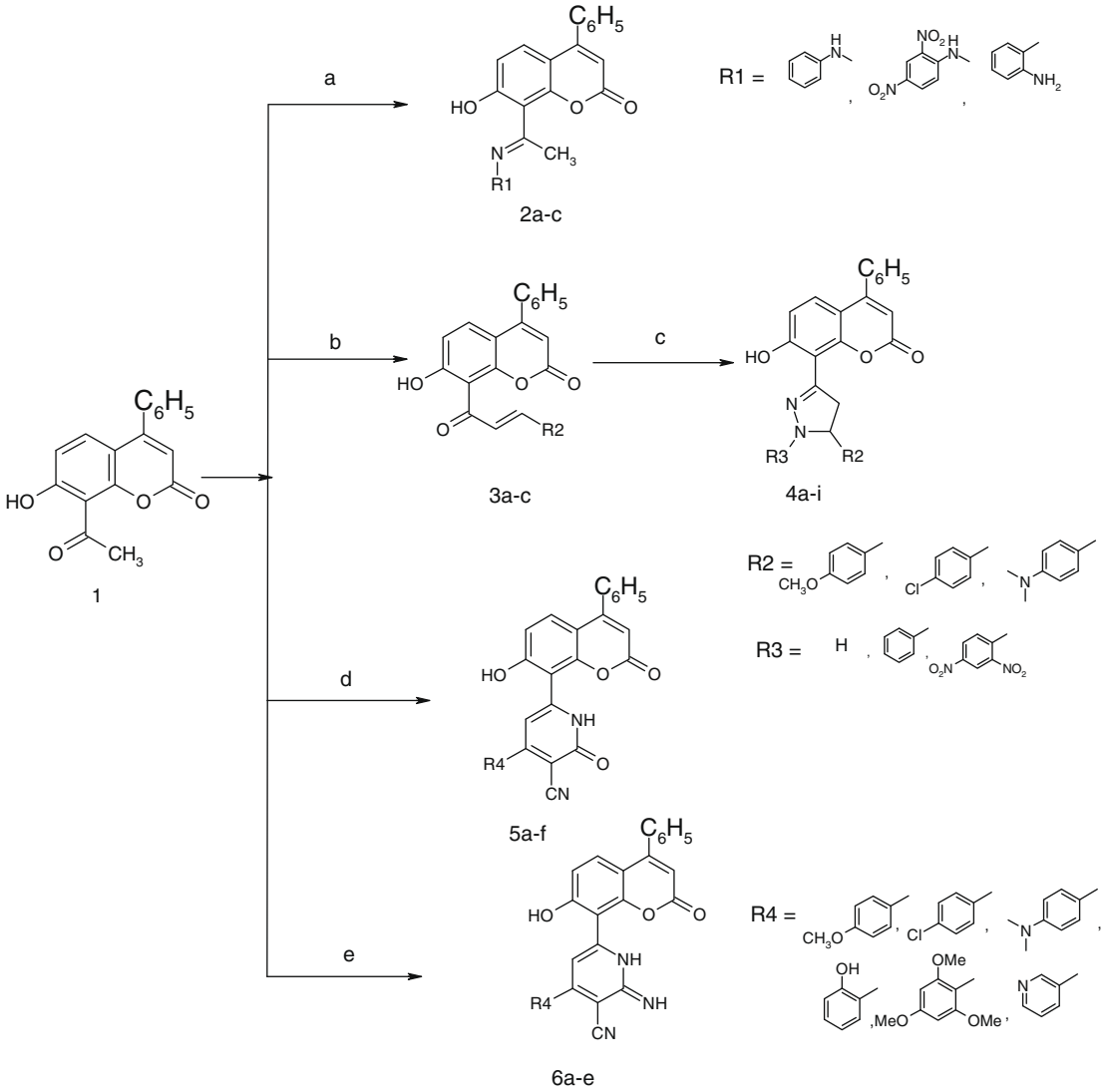
So, we docked the prepared and tested compounds into cycloxygenase II (4cox) active site in order to predict their binding modes, their binding affinities and orientation of these compounds at the active site of the cycloxygenase II enzyme.

2. Results and discussion

2.1. Chemistry

In this study we synthesized five new series of 7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one substituted at C-8 with different hydrazones **2a,b** and imine **2c**, chalcones **3**, pyrazoles **4**, 3-cyano-2-oxo-dihydropyridines **5**, and/or 3-cyano-2-imino-dihydropyridines **6** to be tested as anti-inflammatory, analgesic, and antipyretic activities.

The synthesis of our target compounds **2–6** is outlined in Scheme 1. The key intermediate 8-acetyl-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one **1** was previously prepared.[38] Condensation of **1** with hydrazines[39,40] or phenylene diamine gave the



Scheme 1. Reagents: (a) hydrazines/absolute ethanol; (b) aldehydes/alcoholic potassium hydroside; (c) hydrazines; (d) ethyl cyanoacetate/aldehydes/ammonium acetate; (e) malononitrile/aldehydes/ammonium acetate.

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corresponding hydrazones **2a,b** and imine **2c**, respectively. Meanwhile, reaction of **1** with three different aldehydes[39,41–48] yielded the corresponding chalcones **3a–c**, which were further reacted with hydrazine hydrate and/or substituted hydrazines to afford **4a–i** incorporating a mono or disubstituted pyrazole ring system.[23,28,39,41,43–47] Furthermore, the 8-acetyl intermediate **1** was reacted with ethyl cyanoacetate, ammonium acetate, and the appropriate aldehydes in a multicomponent reaction to give **5a–f** in good yield.[42,48] Also, the one pot synthesis was utilized to synthesize compounds **6a–e**, using malononitrile, ammonium acetate, and the appropriate aldehydes.[42,44,45,48–53]

Table 1
Anti-inflammatory effect of the new synthesized compounds in doses equal to 1/10 of their LD_{50} and Celecoxib (20 mg kg^{-1}) in rats ($n = 5$)

Groups	Paw size (mm) (mean ± S.E.M)	Reduction %
Control	29.54 ± 1.15	–
Celecoxib	17.82 ± 1.24***	39.67
1	23.55 ± 1.28**	20.27
2a	25.89 ± 1.24	12.35
2b	29.34 ± 1.25	0.67
2c	26.97 ± 1.34	8.70
3a	25.89 ± 1.55	12.35
3b	23.55 ± 1.26**	20.27
3c	24.40 ± 1.53*	17.40
4a	26.97 ± 1.64	8.70
4b	23.97 ± 1.31**	18.85
4c	26.11 ± 1.24	11.61
4d	24.40 ± 1.48*	17.40
4e	23.97 ± 1.20**	18.85
4f	26.21 ± 1.25	11.27
4g	26.97 ± 1.36	8.70
4h	27.83 ± 1.16	5.78
4i	24.50 ± 1.35**	17.06
5a	27.83 ± 1.26	5.78
5b	24.71 ± 1.24*	16.35
5c	27.40 ± 1.26	7.24
5d	27.83 ± 1.35	5.78
5e	28.26 ± 1.19	4.33
5f	27.42 ± 1.28	7.17
6a	24.83 ± 1.27*	15.94
6b	28.15 ± 1.29	4.70
6c	23.97 ± 1.28**	18.85
6d	24.72 ± 1.27*	16.31
6e	24.75 ± 1.23*	16.21

Significant at: $^{*}P \leqslant 0.05$, $^{**}P \leqslant 0.01$, $^{***}P \leqslant 0.001$.

2.2. Pharmacology

2.2.1. Statistical analysis

All rats treated with different compounds up to 150 mg kg^{-1} were alive during the 24 h of observation. These animals did not show visible signs of acute toxicity. LD_{50} of compounds **3c**, **4f**, **5c**, **5d** and **6b** was 390 mg kg^{-1}. LD_{50} of compounds **2b**, **2c**, **4b**, **4c**, **5f** and **6e** was 370 mg kg^{-1}. LD_{50} of compounds **3b**, **4a**, **4e**, **4h**, **4i**, **5a** and **6a** was 330 mg kg^{-1}. LD_{50} of compounds **4d**, **4g**, **5b**, **6c** and **6d** was 310 mg kg^{-1}. Finally, LD_{50} of compounds **1**, **2a**, **3a** and **5e** was 290 mg kg^{-1}. The tested compounds are considered non-toxic.

2.2.2. Anti-inflammatory activity

All the final new compounds were tested for their in vivo anti-inflammatory efficacy using carrageenan-induced paw edema method.[54]

The tested compounds and the standard drug celecoxib produced significant reduction of paw size as compared to the control group (Table 1).

The key intermediate **1** showed significant anti-inflammatory activity. On the other hand, hydrazone **2a** showed only slight anti-inflammatory activity. As for chalcones **3a–c**, compound **3b** showed very good activity (20.27% reduction) more than **3c**

Table 2
Analgesic effect (represented by number of writhes) of the new synthesized compounds in doses equal to 1/10 their LD_{50} and diclofenac sodium (5 mg kg^{-1}) in mice using acetic acid-induced writhing method ($n = 5$)

Groups	Number of writhes				
	0 h	1 h	2 h	3 h	4 h
Control	42.3 ± 2.5	42.8 ± 2.6	43.7 ± 3.2	45.3 ± 3.1	45.8 ± 3.3
Diclofenac sodium	41.2 ± 2.8	10.7 ± 2.2***	10.2 ± 2.0***	13.0 ± 1.9***	18.2 ± 2.0***
1	42.6 ± 2.9	42.6 ± 2.5	41.6 ± 2.6	40.5 ± 2.0	41.4 ± 2.4
2a	41.5 ± 2.6	41.0 ± 3.1	40.4 ± 3.0	40.3 ± 3.0	40.7 ± 2.7
2b	42.5 ± 2.4	35.4 ± 2.6	34.7 ± 3.2	36.8 ± 2.6	40.6 ± 3.3
2c	42.6 ± 3.2	38.4 ± 2.8	37.3 ± 2.7	38.5 ± 2.7	41.9 ± 3.5
3a	42.5 ± 2.8	38.5 ± 2.4	38.5 ± 2.9	38.5 ± 2.9	41.0 ± 2.5
3b	42.4 ± 3.7	42.4 ± 2.7	42.0 ± 3.3	41.7 ± 3.0	42.0 ± 2.7
3c	42.5 ± 2.6	25.6 ± 3.3***	26.8 ± 3.3***	32.8 ± 2.6**	38.5 ± 3.0
4a	42.0 ± 2.9	37.8 ± 3.2	37.5 ± 3.0	39.8 ± 2.3	41.7 ± 2.8
4b	42.2 ± 3.6	24.8 ± 2.8***	25.7 ± 2.9***	34.5 ± 2.7*	39.4 ± 3.4
4c	41.8 ± 2.8	40.3 ± 3.4	39.8 ± 2.6	40.3 ± 2.5	40.5 ± 2.8
4d	42.2 ± 3.2	41.9 ± 3.1	41.5 ± 2.5	41.5 ± 3.0	41.7 ± 3.0
4e	41.7 ± 3.0	41.4 ± 2.9	40.6 ± 2.8	40.3 ± 3.7	40.4 ± 3.2
4f	42.6 ± 2.8	42.2 ± 2.7	42.0 ± 3.4	42.2 ± 2.5	42.0 ± 2.9
4g	42.5 ± 2.5	41.9 ± 3.1	41.8 ± 3.2	41.5 ± 2.8	41.6 ± 3.2
4h	42.5 ± 3.2	25.6 ± 2.8***	25.4 ± 2.7***	33.8 ± 3.1*	39.6 ± 3.0
4i	42.5 ± 3.4	26.8 ± 2.4***	27.5 ± 3.2**	34.4 ± 3.4**	39.4 ± 3.5
5a	42.2 ± 2.7	41.8 ± 3.0	41.0 ± 2.8	38.2 ± 2.7	41.7 ± 2.9
5b	42.1 ± 3.2	42.1 ± 3.1	41.6 ± 2.9	41.8 ± 2.6	41.4 ± 2.8
5c	42.4 ± 3.3	38.3 ± 2.9	39.2 ± 2.6	39.2 ± 3.0	41.4 ± 2.7
5d	42.4 ± 3.0	42.2 ± 2.6	42.1 ± 3.2	42.1 ± 3.1	41.8 ± 2.9
5e	42.3 ± 2.6	24.6 ± 2.7***	29.4 ± 2.7**	34.2 ± 2.8*	39.6 ± 3.0
5f	42.0 ± 2.8	38.3 ± 2.7	38.3 ± 2.7	40.5 ± 3.0	41.6 ± 2.7
6a	42.5 ± 3.1	42.3 ± 3.1	42.2 ± 2.5	42.0 ± 3.1	41.7 ± 3.0
6b	42.6 ± 2.7	41.2 ± 2.5	41.5 ± 2.9	40.3 ± 2.7	41.1 ± 2.7
6c	42.5 ± 2.8	38.5 ± 2.8	37.4 ± 3.5	37.6 ± 2.7	40.9 ± 2.8
6d	42.6 ± 2.9	38.5 ± 3.1	38.6 ± 3.2	38.5 ± 2.9	41.5 ± 2.7
6e	42.7 ± 2.4	39.5 ± 2.9	39.7 ± 3.0	40.5 ± 3.3	41.5 ± 2.7

Significant at: $^{*}P \leqslant 0.05$, $^{**}P \leqslant 0.01$, $^{***}P \leqslant 0.001$.

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(17.40% reduction), while compound **3a** had slight activity (12.35% reduction).

Table 2b
Analgesic effect (represented by % protection against writhing) of the new synthesized compounds in doses equal to 1/10 of their LD_{50} and diclofenac sodium (5 mg kg^{-1}) in mice using acetic acid-induced writhing method ($n = 5$)

Groups	% Protection against writhing				
	0 h	1 h	2 h	3 h	4 h
Control	—	—	—	—	—
Diclofenac sodium	—	74.02	75.24	68.44	55.82
1	—	0.00	2.34	4.92	2.81
2a	—	3.75	2.58	2.81	1.17
2b	—	16.70	18.35	13.41	4.47
2c	—	9.85	12.44	9.62	1.64
3a	—	9.41	9.41	9.41	3.52
3b	—	0.0	0.94	1.65	0.94
3c	—	37.41	36.94	22.82	9.41
4a	—	10.00	10.71	5.23	0.71
4b	—	41.23	39.09	18.24	6.63
4c	—	3.58	4.78	3.58	3.58
4d	—	0.71	1.65	1.65	1.18
4e	—	0.71	2.63	3.35	3.11
4f	—	0.93	1.40	0.93	1.40
4g	—	1.41	1.64	2.35	2.11
4h	—	39.76	40.23	20.47	6.82
4i	—	36.94	35.29	19.05	7.29
5a	—	0.94	2.84	9.47	1.18
5b	—	0.00	1.18	0.71	1.66
5c	—	9.66	7.54	7.54	2.35
5d	—	0.47	0.70	0.70	1.41
5e	—	41.84	30.49	19.14	6.38
5f	—	8.80	8.80	3.57	0.95
6a	—	4.70	0.70	1.17	1.88
6b	—	3.28	2.58	5.39	3.52
6c	—	9.41	12.00	11.52	3.76
6d	—	9.62	9.38	9.62	2.58
6e	—	7.49	7.02	5.15	5.15

On the other hand, screening of substituted pyrazoles **4a–i** revealed that six compounds possess anti-inflammatory activity. Compounds **4b, 4e, 4d, 4i** gave significant activity (18.85%, 18.85%, 17.40%, 17.06% reduction), respectively, while compounds **4c,** and **4f** showed slight activity (11.6%, 11.27% reduction).

Among 2-oxo-dihydropyridine-3-carbonitrile **5a–f**, only compound **5b** showed moderate activity (16.35% reduction). Finally, 2-imino-dihydropyridine-3-carbonitrile **6a–e**, compounds **6c, 6d, 6e, 6a** had significant activity (18.85%, 16.31%, 16.21%, 15.94% reduction), respectively

2.2.3. Analgesic activity

All the newly synthesized compounds were subjected to testing their analgesic activity using the acetic acid-induced writhing method.[55]

Significant protection against writhing was observed in animals treated with compounds **5e, 4b**, **4h**, **3c**, **4i** where number of writhes after 1 h were 24.6, 24.8, 25.6, 25.6, 26.8, respectively compared to 42.3 in the control group (Table 2a).

Also, the tabulated results revealed that compounds **5e**, **4b**, **4h**, **3c**, **4i** and exhibited the highest percentage protection against writhing 41.84, 41.23, 39.76, 37.41 and 36.94, respectively (Table 2b).

2.2.4. Antipyretic activity

The antipyretic activity was evaluated for the newly prepared compounds by using the yeast-induced hyperpyrexia method.[56]

The tabulated results showed that compounds **2b**, **3c**, **4b**, **4h, 4i**, **5e**, and **6c**, significantly decrease the temperature of pyretic rats at 1, 2 and 3 h after compound administration (Table 3). The maximum mean rectal temperatures produced by brewer's yeast in the presence of compounds **2b** and **4h** were (38.05, 38.01 °C), respectively. In addition, compounds **4i**, **4b**, **6c**, **5e**, **3c** again showed a decrease in the rectal temperature after 2 h were

Table 3
Antipyretic effect of the new synthesized compounds in doses equal to 1/10 of their LD_{50} and paracetamol (100 mg kg^{-1}) in hyperthermic rats ($n = 5$)

Groups	Rectal temperature (°C) after compounds administration			
	1 h	2 h	3 h	4 h
Control	39.24 ± 0.22	39.11 ± 0.26	39.20 ± 0.24	39.17 ± 0.20
Paracetamol	37.38 ± 0.19***	37.20 ± 0.17***	37.27 ± 0.18***	37.16 ± 0.19***
1	38.91 ± 0.25	38.91 ± 0.26	39.00 ± 0.28	39.06 ± 0.23
2a	39.11 ± 0.22	38.81 ± 0.22	38.91 ± 0.15	38.91 ± 0.22
2b	38.42 ± 0.22*	38.05 ± 0.27**	38.34 ± 0.19**	38.93 ± 0.19
2c	38.61 ± 0.23	38.41 ± 0.23	38.81 ± 0.15	38.90 ± 0.23
3a	38.91 ± 0.25	38.91 ± 0.22	38.95 ± 0.28	39.11 ± 0.12
3b	39.05 ± 0.22	38.91 ± 0.22	38.84 ± 0.23	38.91 ± 0.23
3c	38.31 ± 0.28*	38.21 ± 0.25*	38.51 ± 0.16*	38.86 ± 0.19
4a	38.81 ± 0.23	38.91 ± 0.23	38.91 ± 0.26	39.15 ± 0.28
4b	38.51 ± 0.23*	38.14 ± 0.27*	38.44 ± 0.23*	38.87 ± 0.21
4c	38.81 ± 0.22	38.91 ± 0.25	38.91 ± 0.23	38.91 ± 0.26
4d	38.90 ± 0.28	38.84 ± 0.29	38.90 ± 0.25	38.91 ± 0.29
4e	38.91 ± 0.22	38.81 ± 0.23	38.91 ± 0.22	39.05 ± 0.25
4f	38.81 ± 0.22	38.91 ± 0.23	38.91 ± 0.22	38.94 ± 0.23
4g	38.91 ± 0.23	38.90 ± 0.25	38.91 ± 0.26	38.91 ± 0.28
4h	38.40 ± 0.28*	38.01 ± 0.29**	38.43 ± 0.24*	38.88 ± 0.21
4i	38.54 ± 0.22*	38.10 ± 0.28*	38.42 ± 0.25*	38.91 ± 0.22
5a	38.81 ± 0.25	38.71 ± 0.26	38.64 ± 0.22	38.91 ± 0.22
5b	38.91 ± 0.33	38.84 ± 0.19	38.81 ± 0.23	38.91 ± 0.23
5c	38.91 ± 0.28	38.91 ± 0.22	38.90 ± 0.22	39.11 ± 0.25
5d	38.91 ± 0.23	38.91 ± 0.22	38.81 ± 0.23	38.91 ± 0.22
5e	38.34 ± 0.29*	38.20 ± 0.28*	38.47 ± 0.20*	38.91 ± 0.22
5f	38.71 ± 0.32	38.81 ± 0.23	38.81 ± 0.22	38.91 ± 0.23
6a	38.90 ± 0.24	38.81 ± 0.22	38.81 ± 0.25	38.91 ± 0.28
6b	38.91 ± 0.22	39.00 ± 0.25	38.84 ± 0.26	38.91 ± 0.22
6c	38.38 ± 0.23*	38.17 ± 0.21*	38.39 ± 0.29*	38.89 ± 0.21
6d	39.01 ± 0.23	38.91 ± 0.23	38.91 ± 0.22	38.94 ± 0.26
6e	39.11 ± 0.31	38.94 ± 0.19	38.91 ± 0.23	38.91 ± 0.29

Significant at: $^{*}P \leqslant 0.05$, $^{**}P \leqslant 0.01$, $^{***}P \leqslant 0.001$.

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(38.10, 38.14, 38.17, 38.20, 38.21 °C), respectively compared to 39.24 °C in the control group.

2.3. Molecular modeling and docking studies

To pre-asses the anti-inflammatory behavior of our benzopyranone derivatives **1–6** on a structural basis,, automated docking studies were carried out using MOLSOFT ICM 3.4-8C program,[36] the scoring functions and hydrogen bonds formed with the surrounding amino acids are used to predict their binding modes, their binding affinities and orientation of these compounds at the active site of the cyclooxygenase II enzyme. The protein–ligand complex was constructed based on the X-ray structure (PDB entry 4cox) cyclooxygenase II with its bound inhibitor indomethacin.[22] The scoring functions of the compounds were calculated from minimized ligand protein complexes.

In order to compare the binding affinity of the newly synthesized benzopyranone analogues, we docked compounds **1–6** into the empty binding site of cyclooxygenase II (4COX), with its bound inhibitor indomethacin, Figures 1–6 show the docking solutions with the highest predicted binding affinity for cyclooxygenase II. Figure 1 shows orientation of indomethacin, Figure 1b, while Figures 2–7 show orientations of compounds **1, 3c, 4b, 4i**, **6c, 6e**, respectively.

As shown from Tables 1–4 and Figures 1–7 the following results can be drawn: indomethacin (the original ligand) reveals ICM score of −88.93 and form four hydrogen bonds between carboxylic acid moiety and Arg-120, and another two hydrogen bonds between carbonyl oxygen with Ser-530, and another bond between OH of carboxylic acid with Tyr-355 (Table 4, Fig. 1b). Compound **1** exhibits relatively weak binding affinity with ICM score of −66.01 but form two hydrogen bonds between 2-oxo of benzopyranone with Tyr-385, and Ser-530, and another bond between O-1 of benzopyran-2-one with Ser-530, and between OH-7 with Met-522 (Table 4, Fig. 2). Hydrazones **2a–b**, and imine **2c** possess ICM scores of ranges from −69.70 to −85.87, and the most biologically active anti-inflammatory is compound **2b** with lowest ICM score −85.87 and form two hydrogen bonds between OH-7 with 2-NH of Arg-120, and O of 2′-nitro phenyl group and Gly-526 (Table 4). Chalcones **3a–c** have ICM scores of ranges from −78.09 to −86.62, and the most biologically active one is compound **3c** with

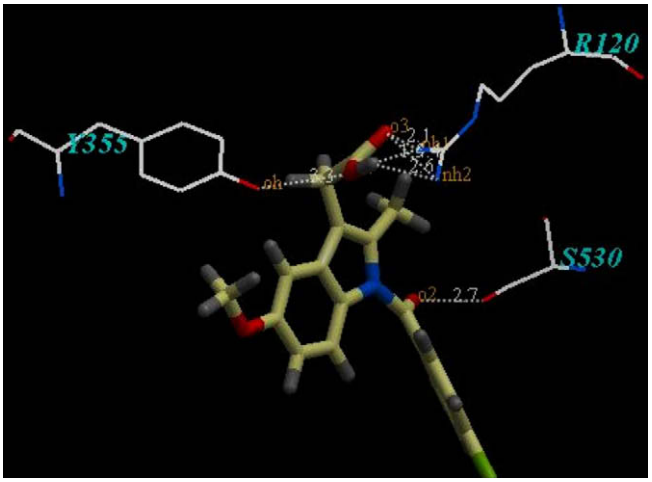

Figure 1. Binding mode of the original ligand Indomethacin (Imn) into its binding site of cycloxygenase II, it has ICM score −88.93, and form 6 hydrogen bonds shown as white dotted lines (Table 4), showing two hydrogen bonds between O (*C*OOH), O of (*COOH*) with NH-1 of Arg-120 distance 1.60 A and 2.63 A, respectively, and another two hydrogen bonds between O (*C*OOH), O of (*COOH*) with NH-2 of Arg-120 distance 2.13 A and 2.43 A, another two hydrogen bonds between O of oxo-2 and Ser-530 of distance 1.94 A, and between O of (*COOH*) and Tyr-355 distance 2.43 A.

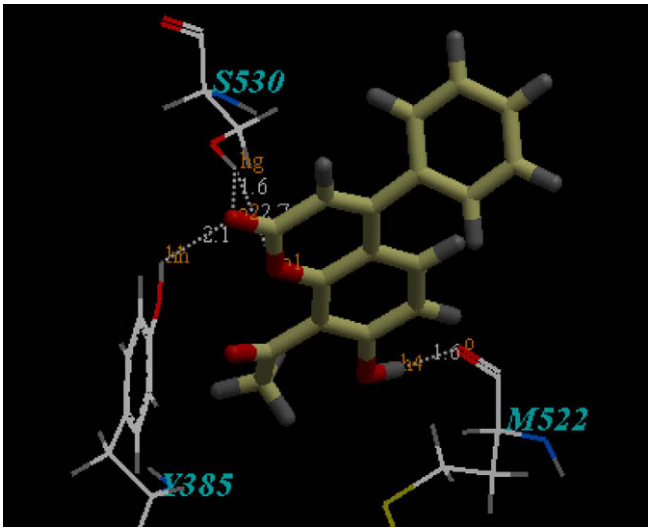

Figure 2. Binding mode of compound **1** into the binding site of cycloxygenase II, it has ICM score −66.01, and form 4 hydrogen bonds shown as white dotted lines (Table 4), showing two hydrogen bonds between O of 2-oxo of benzopyranone moiety withTyr-385, and Ser-530 of distances 2.15 A and 1.62 A, respectively, and another bond between O-1 and Ser-530 of distance 2.71 A and between H of OH-7 and O of Met-522 of distance 1.66 A.

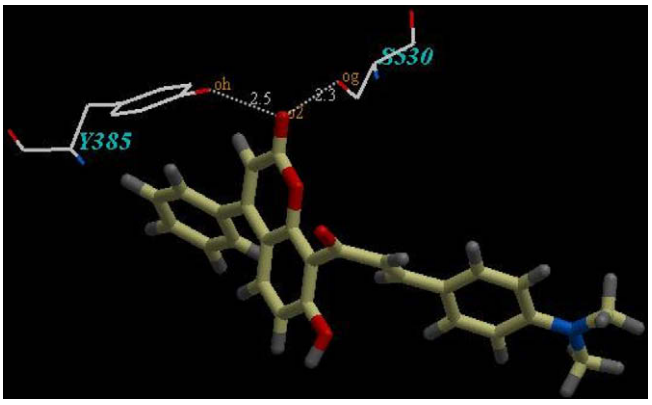

Figure 3. Binding mode of compound **3c** into the binding site of cycloxygenase II, it has ICM score −80.29, and form 2 hydrogen bonds shown as white dotted lines (Table 4), showing one hydrogen bond between O of 2-oxo of benzopyranone moiety with S-530 and Tyr-385 of distances of 1.43 A, 2.04 A, respectively.

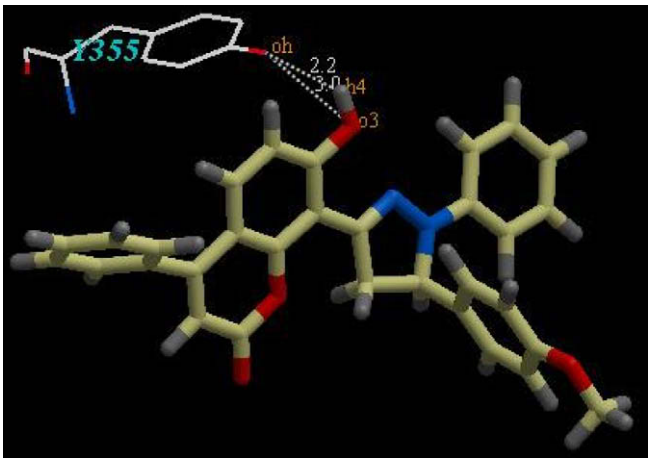

Figure 4. Binding mode of compound **4b** into the binding site of cycloxygenase II, it has ICM score −97.27 A, and form 2 hydrogen bonds shown as white dotted lines (Table 4), showing two hydrogen bonds between O of OH-7 and H of OH-7 with Tyr-355 of distances of 2.23 A, and 2.76 A, respectively.

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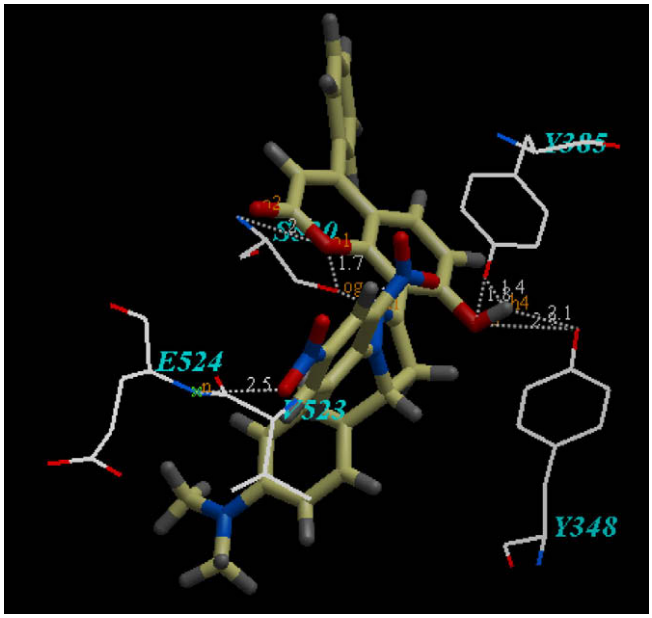

Figure 5. Binding mode of compound **4i** into the binding site of cycloxygenase II, it has ICM score −105.59 A, and form 10 hydrogen bonds shown as white dotted lines (Table 4), showing one hydrogen bonds between O of 2-oxo of benzopyranone moiety with S-530 of distance 1.03 A, and two bonds of O-1 with S-530 of distances 2.77 A, and 2.29 A, and another one between N of pyrazole ring with Ser-530 of distance 2.49 A, and two hydrogen bonds between O of OH-7 with Tyr-385, and Tyr-348 of distances 1.77 A, and 2.16 A, respectively, and another two hydrogen bonds between H of OH-7 with Tyr-385, and Tyr-348 of distances1.39 A, and 2.12 A, respectively, and between O of NO_2 with Val-523, and E-524 of distances 1.58 A, and 2.47 A, respectively.

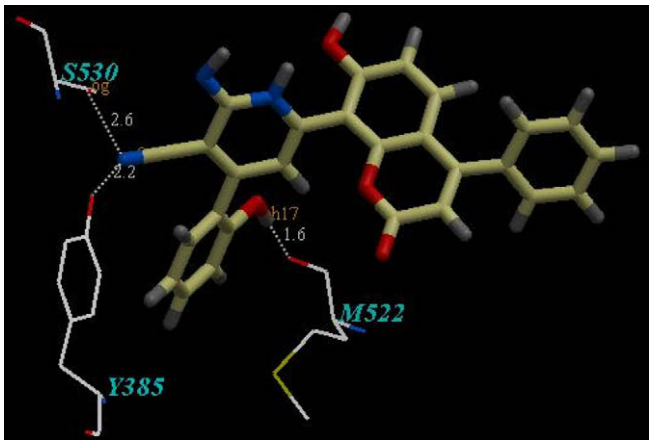

Figure 6. Binding mode of compound **6c** into the binding site of cycloxygenase II, it has ICM score −84.50 A, and form 3 hydrogen bonds shown as white dotted lines (Table 4), showing two hydrogen bonds between N of 3-cyano moiety with Tyr-385, and S-530 of distance 1.68 A, and 2.00 A, respectively, and another bond of H of OH of 4-(2′-hydroxy) phenyl group with Met-522 of distances 1.55 A.

ICM score −82.21 which form two hydrogen bonds between 2-oxo moiety with Ser-530, and Tyr-385 (Table 4, Fig. 3). Substituted pyrazoles **4a–i** possess ICM scores of ranges from −84.58 to −105.59, where compounds **4i, 4h, 4b** with ICM score −105.59, −100.02 and −97.27, respectively, where they have significant biological activities, compound **4i** is the most active one in the series, it form ten hydrogen bonds, two bonds with O-1 of benzopyranone moiety with Ser-530, another one between 2-oxo and Ser-530, between N of pyrazole ring and Ser-530, four hydrogen bonds between OH-7 and Tyr-348, and Tyr-385, and two bonds between O of nitro group and Val-523, and E-524 (Table 4, Fig. 5). 2-Oxo-dihydropyridine-3-carbonitile **5a–f** have ICM scores of ranges from −78.95 to −88.96, but the most biologically active one is compound **5b** with ICM score −78.98. Finally, 2-imino-dihydropyridine-3-carbonitile **6a–e** have ICM scores of ranges from −78.08 to −89.67, the biologically activity ranges from compounds **6c**, **6d**, **6e**, **6a** where ICM scores are −84.50, −86.96, −79.70, and −89.67, where **6c** is the most active one, it forms three hydrogen bonds, two of them between 3-cyano moiety with Ser-530, and Tyr-385, and another bond between 2′-OH with Met-522 (Table 4, Fig. 6).

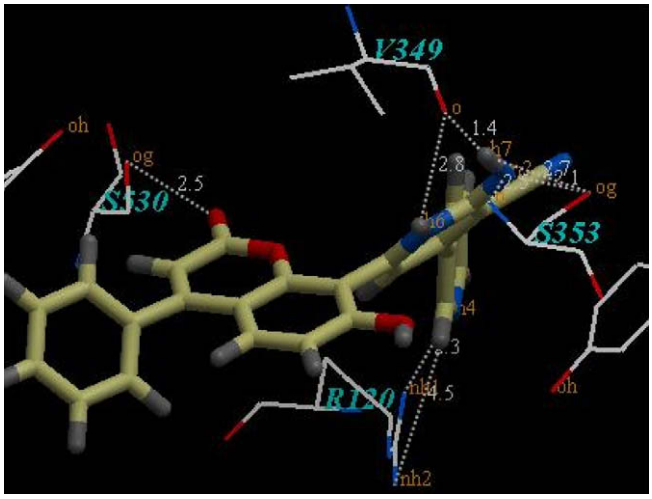

Figure 7. Binding mode of compound **6e** into the binding site of cycloxygenase II, it has ICM score −79.70 A, and form 10 hydrogen bonds shown as white dotted lines (Table 4), showing one hydrogen bond between O of 2-oxo of benzopyranone moiety with S-530 of distance 2.14 A, and another three bonds of NH of imino group , and two of 3-cyano group with S-353 of distances 2.70 A, 1.95 A, and 1.90 A, respectively, and two bonds of NH of dihydropyridine ring , and NH of imino group with Val-349 of distances 2.80 A, and 1.41 A, respectively, and two bonds of N of 4-(3-pyridinyl) with Arg-120 of distances 1.61 A, and 2.45 A, and finally, bond with H of OH-7with Leu-352 of distance 2.66 A.

3. Conclusions

In our study we focused on substitution in position 8 of 7-hydroxy-2*H*-1-benzopyran-2-one moiety. Reaction of 8-acetyl-7-hydroxy derivatives **1** with hydrazines, or phenylene diamine to give hydrazone **2a,b** and imine **2c**, respectively, only **2a** retains anti-inflammatory activity, while **2b** reveals antipyretic activity. Reaction of **1** with different aldehydes in alcoholic potassium hydroxide to give chalcones **3a–c**, all the chalcones retain anti-inflammatory activity in the order of **3b** > **3c** > **3a**, and **3c** reveals also analgesic and antipyretic effects. Cyclization of chalcones **3a–c** through reaction with different hydrazine derivatives to give substituted pyrazoles **4a–i** retains the anti-inflammatory effect of **4b, 4d, 4e, 4f, 4i** , also, compounds **4b, 4i** possess analgesic, and antipyretic activities. Reaction of **1** with ethyl cyanoacetate, and ammonium acetate with different aldehydes to afford the substituted 2-oxo-dihydropyridine-3-carbonitrile derivatives **5a–f**, abolish the anti-inflammatory activity except compound **5b** which retain the activity, and compound **5e** which possesses analgesic and antipyretic effects. Finally the reaction of **1** with malononitrile, and ammonium acetate with different aldehydes to afford the substituted 2-imino-dihydropyridine-3-carbonitrile derivatives **6a–e**, retain the anti-inflammatory activity of all members of the series except compound **6b** Shows slight activity, and compound **6c** possesses antipyretic effect.

The active compounds **1, 4i, 6a–e** interact with Ser-530, and Tyr-385 which are the amino acids involved in the mechanism of cyclooxygenase II inhibition.[34]

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Table 4
ICM Scores of indomethacin, the compounds, and hydrogen bonds formed with amino acid residues and their lengths

Compounds	ICM scores	No. of hydrogen bonds	Involved group of amino acid	Atom of ligand involved	Length of hydrogen bond (A)
Indomethacin	−88.93	6	Y355···HH	OH of COOH	2.43
			S530···HG	CO of benzoyl	1.94
			R120···NH	CO of COOH	1.60
			R120···NH	OH of COOH	2.63
			R120···NH	CO of COOH	1.33
			R120···NH	OH of COOH	2.43
1	−66.01	4	Y385···HH	2-Oxo	2.15
			S530···HG	2-Oxo	1.62
			S530···HG	–O–	2.71
			M522···O	H of 7-OH	1.66
2a	−79.70	1	V349···O	H of 7-OH	2.15
2b	−85.87	3	R120···(NH)	O of 7-OH	2.53
			R120···H12	O of 7-OH	1.37
			G526···NH	O of 7-OH	2.60
2c	−69.95	3	S530···HG	2-Oxo	2.59
			M522···O	H of NH_2	1.97
			M522···O	H of NH_2	1.45
3a	−86.62	3	Y355···HH11	O of 7-OH	2.76
			R120···NH	O of 7-OH	1.64
			R120···NH	O of 7-OH	2.76
3b	−78.09	1	V349···O	H of 7-OH	2.05
3c	−80.29	2	S530···HG	2-Oxo	1.43
			Y38···HH	2-Oxo	2.04
4a	−86.92	1	S530···HG	2-Oxo	2.21
4b	−97.27	2	Y355···HH	O of 7-OH	2.76
			Y355···OB	H of 7-OH	2.23
4c	−100.17	5	R120···HH 11	MO6	2.75
			Y355···HH	O of 7-OH	2.47
			Y355···OH	H of 7-OH	1.91
			L531···NH	O of nitro group	2.43
			L531···NH	O of nitro group	1.31
4d	−84.58	1	S530···HG	2-Oxo	2.19
4e	−95.01	0	—	—	—
4f	−95.59	4	S530···HG	MN1	1.84
			S530···HG	MO4	2.11
			S530···HG	MO5	2.62
			L531···NH	MO5	2.66
4g	−89.67	1	Y355···HH	O of 7-OH	2.69
4h	−100.02	2	Y355···HH	O of 7-OH	2.61
			Y355···OH	H of 7-OH	2.06
4i	−105.59	10	V523···NH	O of nitro group	1.58
			Y348···HH	O of 7-OH	2.16
			Y348···OH	H of 7-OH	2.12
			Y385···HH	O of 7-OH	1.77
			Y385···OH	H of 7-OH	1.39
			E524···NH	O of nitro group	2.47
			S530···NH	2-Oxo	1.03
			S530···HG	N of pyrazole	2.49
			S530···NH	–O–	2.77
			S530···HG	–O–	2.29
5a	−88.95	3	S530···HG	N of 3-cyano group	2.49
			Y385···OH	H of 7-OH	1.39
			Y348···OH	H of 7-OH	2.12
5b	−78.98	3	Y355···HH	H of 7-OH	2.17
			Y355···OH	O of 7-OH	1.66
			S530···HG	N of 3-cyano group	1.50
5c	−85.23	4	Y355···HH	N of 3-cyano group	1.73
			S530···HG	N of 3-cyano group	1.97
			M522···O	H of 4 (2'-hydroxy phenyl)	1.56
			V523···O	H of 4 (2′-hydroxy phenyl)	2.80
5d	−86.58	3	Y355···HH	O of 7-OH	1.60
			S530···HG	N of 3-cyano	2.27
			Y355···OH	H of 7-OH	1.28
5[e]	−88.96	5	Y355···HH	O of 7-OH	1.77
			Y355···OH	H of 7-OH	1.36
			S530···HG	N of 3-cyano	2.08
			S530···HG	O of 2′-methoxy	2.80
			A527···NH	O of 6′-methoxy	2.65
5f	−79.41	3	Y385···HH	N of 3-cyano	1.44
			W387···HH	N of pyridine	2.36
			S530···HG	N of 3-cyano	2.11
6a	−89.67	3	Y355···HH	O of 7-OH	1.70
			Y355···HG	N of 3-cyano	2.14
			S530···OH	H of 7-OH	1.35

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Table 4 (*continued*)

Compounds	ICM scores	No. of hydrogen bonds	Involved group of amino acid	Atom of ligand involved	Length of hydrogen bond (A)
6b	−78.08	3	Y385···HH	N of 3-cyano	1.88
			S530···HG	NH of imino group	1.70
			S530···HG	N of 3-cyano	2.57
6c	−84.50	3	Y385···HH	N of 3-cyano	1.68
			S530···HG	N of 3-cyano	2.00
			M522···O	H of 4 (2′-hydroxy phenyl)	1.55
6d	−86.96	3	S530···HG	N of 3-cyano	2.30
			Y355···HH	O of 7-OH	1.60
			Y355···OH	H of 7-OH	1.29
6e	−79.70	10	R120···NH	N of 4-(3-pyridyl)	1.61
			R120···NH	N of 4-(3-pyridyl)	2.45
			S353···NH	N of 3-cyano	1.95
			S353···HG	N of 3-cyano	1.90
			S353···OG	NH of imino group	2.70
			S530···HG	2-Oxo	2.14
			V349···O	NH of dihydropyridine	2.80
			V349···O	NH of imino group	1.41
			S530···O	NH of imino group	2.49
			L352···O	H of 7-OH	2.66

The synthesis of the pyrazole **4** containing new compounds proved a successful hit, since most of them showed anti-inflammatory, analgesic or antipyretic activities, also, concerning the compounds containing the 3-cyano-dihydropyridine ring; the 2-imino derivatives were more successful than the 2-oxo one.

4. Experimental

4.1. General

Melting points were determined on Gallenkamp and Kofler melting point apparatus and are uncorrected. Elemental analyses (C, H, N) were performed by Micro Analytical Center, Faculty of Science, Cairo University, the values were found to be within ±0.4% of the theoretical ones unless otherwise indicated. Infrared spectra were recorded on Shimadzu IR 435 Spectrophotometer or on a Genesis II FTIR TM, Mattson, 5225, Verona Road, Madison wi. 53711 USA, using KBr discs. ^{1}H NMR spectra were scanned on Varian 360 MHz and 90 MHz spectrometers (chemical shifts are given in part per million (ppm) downfield from TMS). Mass spectra were made on a Finnigan Mat 212-spectrometer (EI. 120 eV, R 1000). Analytical thin-layer chromatography (TLC) was performed on precoated silica gel plates 60-F-254 (Merck; 0.25 mm), developing with chloroform.

4.2. Chemistry

4.2.1. 8-Acetyl-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 1

Compound **1** was previously reported.[38]

4.2.2. General procedure for synthesis of 8-(1-(substituted phenylazo) ethyl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 2a,b

To a solution of **1** (0.01 mol) in absolute ethanol (30 ml), phenyl hydrazine, or 2,4-dinitrophenyl hydrazine (0.011 mol) was added. The reaction was left to reflux for 16 h, and then left overnight at room temperature. The precipitated product was filtered, left to dry and crystallized from ethanol/chloroform 9:1.

4.2.2.1. 8-(1-Phenylazo) ethyl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 2a. Yield 85%; mp 194–195 °C. IR: 3450, 3300 (OH, NH), 1710 (C=O), 1600, 1540, 1500 (NH, C=N, C=C), 1515 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 2.63 (s, 3H, CH_3), 6.19 (s, 1H, C3–H), 6.86–7.74 (m, 12H aromatic), 8.10 (s, 1H, NH exchangeable), 9.80 (s, 1H, OH exchangeable). MS (*m*/*z*): 370 (M^+, 100), Anal. Calcd for $C_{23}H_{18}N_2O_3$: C, 74.58; H, 4.89; N, 7.56. Found: C, 74.62; H, 4.98; N, 7.37.

4.2.2.2. 8-(1-(2,4-Dinitrophenylazo) ethyl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 2b. Yield 83%; mp 203–204 °C. IR: 3400–3300 (br) (OH, NH), 2900 (CH_3), 1720 (C=O), 1620, 1590, 1550 (NH, C=N, C=C), 1515, 1340 (NO_2). cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 6.25 (s, 1H, C3–H), 6.86–8.07 (m, 10H aromatic), 8.90 (s, 1H, NH exchangeable), 9.59 (s, 1H, OH exchangeable). MS (*m*/*z*, %): 460 (M^+, 68.2), Anal. Calcd for $C_{23}H_{16}N_4O_7$: C, 60.00; H, 3.50; N, 12.17. Found: C, 60.20; H, 3.60; N, 12.10.

4.2.2.3. 8-(1-(2-Aminophenylimino) ethyl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 2c. *ortho*-Phenylene diamine (0.011 mol) was added to a solution of **1** (0.01 mol) in absolute ethanol (30 ml). The reaction was refluxed for 16 h, and left overnight at room temperature to separate. The precipitated product was collected, dried and recrystallized from ethanol/chloroform.

Yield 73%; mp above 360 °C. IR: 3450, 3350, 3325 (OH, NH_2), 2900 (br) (CH_3), 1722 (C=O), 1610, 1591 (NH_2, C=N,C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 2.51 (s, 3H, CH_3), 6.24 (s, 1H, C3–H), 6.80–7.80 (m, 11H aromatic), 8.08 (s, 2H, NH_2 exchangeable), 9.59 (s, 1H, OH exchangeable). MS (*m*/*z*): 370 (M^+, 100), Anal. Calcd for $C_{23}H_{18}N_2O_3$: C, 74.58; H, 4.89; N, 7.56. Found: C, 74.50; H, 4.79; N, 7.77.

4.2.3. General procedure for synthesis of 8-(3-(4-Aryl)acetyloyl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 3a–c

A 30 ml ethanolic solution of the appropriate aromatic aldehyde (0.01 mol) was added with continuous stirring and cooling to a solution of 8-acyl-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one **1** (2.8 g, 0.01 mol) in 2.5% solution of sodium hydroxide. The reaction mixture was stirred at room temperature for 3 h and left overnight. It was then poured onto crushed ice; the separated precipitate was filtered, left to dry and recrystallized from ethanol/chloroform 9:1.

4.2.3.1. 8-(3-(4-Anisoyl)acetyloyl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 3a. Yield 75%; mp 134–135 °C. IR: 3329, 3276 (OH), 2895, 2836 (CH_3), 1729, 1624 (C=$O_{(s)}$), 1591, 1511 (C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 3.87 (s, 3H, OCH_3), 6.18 (s, 1H, C3–H), 6.81–7.89 (m, 13H aromatic and C*H*=C*H*), 9.87 (s, 1H, OH exchangeable). MS (*m*/*z*): 398 (M^+, 67), Anal. Calcd for $C_{25}H_{18}O_5$: C, 75.37; H, 4.55. Found: C, 75.44; H, 4.34.

4.2.3.2. 8-(3-(4-Chlorophenyl)acetyloyl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 3b. Yield 78%; mp 196–197 °C. IR: 3400 (OH), 1720, 1660 (C=$O_{(s)}$), 1600, 1560 (C=C), 700 (Cl) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 6.35 (s, 1H, C3–H), 7.09–7.61 (m, 13H,

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aromatic and C*H*=C*H*), 12.50 (s, 1H, OH exchangeable). MS (*m*/*z*): 402 (M^+, 47), 404 (M^++2, 27)), Anal. Calcd for $C_{24}H_{14}Cl\ O_4$: C, 71.74; H, 3.51. Found: C, 71.9; H, 4.00.

4.2.3.3. 8-(3-(4-*N*,*N*-Dimethylaminophenyl)acetyloyl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 3c. Yield 75%; mp 211–213 °C. IR: 3319, 3275 (OH), 2863 (br) ($CH3_{(s)}$), 1728, 1650 ($C{=}O_{(s)}$), 1595, 1500 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$) δ: 3.07 (s, 6H, $2CH_3$), 6.24 (s, 1H, C3–H), 6.71–8.19 (m, 13H aromatic and C*H*=C*H*), 14.45 (s, 1H, OH exchangeable). MS (*m*/*z*): 411 (M^+, 41.5), Anal. Calcd for $C_{26}H_{21}NO_4$: C, 75.90; H, 5.14; N, 3.40. Found: C, 75.73; H, 4.56; N, 3.75.

4.2.4. General procedure for synthesis of 8-(5-aryl-1-substituted-4,5-dihydro-1*H*-pyrazol-3-yl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 4a–i

A solution of **3a–c** (0.01 mol) in absolute ethanol (30 ml), was heated, then the suitable hydrazine derivative (0.011 mol) was added. The reaction mixture was heated for 16 h under reflux conditions. It was left overnight at room temperature. The aggregated precipitate was filtered, dried and recrystallized from suitable solvent.

4.2.4.1. 8-(5-*p*-Anisoyl-4,5-dihydro-1*H*-pyrazol-3-yl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 4a. Crystallization solvent ethanol/water, yield 83%; mp 182–184 °C. IR: 3450–3350 (br) (OH, NH), 2900 (CH_3), 1720 (C=O), 1600, 1570, 1520, 1500 (NH, C=N, C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 2.50, 2.67 (m, 2H, CH_2 of pyrazole), 3.48 (m, 1H, CH of pyrazole), 3.68 (s, 3H, OCH_3), 6.35 (s, 1H, C3–H), 6.58–7.80 (m, 10H aromatic), 9.60 (s, 1H, NH exchangeable), 10.90 (s, 1H, OH exchangeable). MS (*m*/*z*): 412 (M^+, 100), Anal. Calcd for $C_{25}H_{20}N_2O_4$: C, 72.80; H, 4.89; N, 6.79. Found: C, 72.58; H, 5.00; N, 6.80.

4.2.4.2. 8-(5-*p*-Anisoyl-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 4b. Crystallization solvent ethanol, yield 96%; mp 120–122 °C. IR: 3450 (OH), 2925, 2850 (CH_3), 1720 (C=O), 1600, 1510 (C=N, C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 2.27, 2.49 (m, 2H, CH_2 of pyrazole), 3.28 (m, 1H, CH of pyrazole), 3.87 (s, 3H, OCH_3), 6.21 (s, 1H, C3–H), 6.83–7.54 (m, 15H, aromatic), 15.29 (s, 1H, OH exchangeable). Anal. Calcd for $C_{31}H_{24}N_2O_4$: C, 76.22; H, 4.95; N, 5.73. Found: C, 75.98; H, 5.14; N, 5.71.

4.2.4.3. 8-(5-*p*-Anisoyl-1-(2,4-dinitrophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 4c. Crystallization solvent dimethyl formamide/ethanol/water 6:2:2, yield 98%; mp 151–152 °C. IR: 3300 (OH), 2900, 2825 (CH_3), 1720 (C=O), 1620, 1590 (C=N, C=C), 1510, 1340 (NO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 2.52, 2.74 (m, 2H, CH_2 of pyrazole), 3.22 (m, 1H, CH of pyrazole), 3.79 (s, 3H, OCH_3), 6.14 (s, 1H, C3–H), 6.98–7.56 (m, 13H aromatic), 15.60 (s, 1H, OH exchangeable).. MS (*m*/*z*): 578 (M^+, 18.3), Anal. Calcd for $C_{31}H_{22}N_4O_8$: C, 64.36; H, 3.83; N, 9.68. Found: C, 64.45; H, 4.12; N, 9.82.

4.2.4.4. 8-(5-*p*-Chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 4d. Crystallization solvent ethanol/water, yield 53%; mp 159–160 °C. IR: 3399–3300 (br) (OH, NH), 1716 (C=O), 1603, 1489, 1480 (NH, C=N,C=C), 699 (Cl) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 2.34, 2.52 (m, 2H, CH_2 of pyrazole), 3.81 (m, 1H, CH of pyrazole), 6.15 (s, 1H, C3–H), 7.46–7.54 (m, 10H aromatic), 9.63 (s, 1HNH exchangeable), 15.60 (s, 1H, OH exchangeable). MS (*m*/*z*): 416 (M^+, 36.8) and 418 (M^++2, 24.9) Anal. Calcd for $C_{24}H_{17}ClN_2O_3$: C, 69.15; H, 4.11; N, 6.72. Found: C, 69.00; H, 3.49; N, 6.65.

4.2.4.5. 8-(5-*p*-Chlorophenyl-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 4e. Crystallization solvent ethanol, yield 81%; mp 148–149 °C. IR: 3350 (OH), 1704 (C=O), 1598, 1493 (C=N, C=C), 690 (Cl) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 2.16, 2.60 (m, 2H, CH_2 of pyrazole), 3.80 (m, 1H, CH of pyrazole), 6.17 (s, 1H, C3–H), 6.81–7.70 (m, 15H aromatic), 12.40 (s, 1H, OH exchangeable). MS (*m*/*z*): 492 (M^+, 47.9), 490 (M^+−2, 100), 494 (M^++2, 42.9). Anal. Calcd for $C_{30}H_{21}ClN_2O_3$: C, 73.09; H, 4.29; N, 5.68. Found: C, 73.20; H, 4.50; N, 5.61.

4.2.4.6. 8-(5-*p*-Chlorophenyl-1-(2,4-dinitrophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 4f. Crystallization solvent acetic acid/water, yield 92%; mp 124–126 °C. IR: 3350 (OH), 1720 (C=O), 1620, 1590 (C=N, C=C), 1515, 1340 (NO_2), 700 (Cl) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 2.12, 2.52 (m, 2H, CH_2 of pyrazole), 3.86 (m, 1H, CH of pyrazole), 6.19 (s, 1H, C3–H), 7.34–8.83 (m, 13H aromatic), 12.76 (s, 1H, OH exchangeable). MS (*m*/*z*): 582.2 (M^+, 37,8), 580.2 (M^+−2, 100). Anal. Calcd for $C_{30}H_{19}Cl\ N_4O_7$: C, 61.81; H, 3.29; N, 9.61. Found: C, 61.75; H, 3.53; N, 9.38.

4.2.4.7. 8-(5-*p*-*N*,*N*-Dimethylaminophenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 4g. Crystallization solvent ethanol/water, yield 85%; mp 140–142 °C. IR: 3500–3200 (br) (OH, NH), 2950, 2900 ($CH_{3(s)}$), 1720 (C=O), 1615, 1570, 1530 (NH, C=N, C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 2.24, 2.51 (m, 2H, CH_2 of pyrazole), 2.99 (s, 6H, $2CH_3$), 3.46 (m, 1H, CH of pyrazole), 6.15 (s, 1H, C3–H), 6.69–7.68 (m, 10H aromatic), 8.49 (s, 1H, NH exchangeable), 15.00 (s, 1H, OH exchangeable). MS (*m*/*z*): 425.25 (M^+, 53.4), Anal. Calcd for $C_{26}H_{23}N_3O_3$: C, 73.40; H, 5.45; N, 9.88. Found: C, 73.88; H, 5.04; N, 9.71.

4.2.4.8. 8-(5-*p*-*N*,*N*-Dimethylaminophenyl-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 4h. Crystallization solvent ethanol, yield 98%; mp 145–147 °C. IR: 3300 (OH), 2900, 2825 ($CH_{3(s)}$), 1720 (C=O), 1600, 1540, 1520 (C=N, C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 2.49, 2.60 (m, 2H, CH_2 of pyrazole), 2.85 (s, 6H, $2CH_3$), 3.91 (m, 1H, CH of pyrazole), 6.20 (s, 1H, C3–H), 6.64–7.53 (m, 15H aromatic), 12.10 (s, 1H, OH exchangeable). Anal. Calcd for $C_{32}H_{27}N_3O_3$: C, 76.63; H, 5.43; N, 8.38. Found: C, 76.64; H, 5.50; N, 8.59.

4.2.4.9. 8-(5-*p*-*N*,*N*-Dimethylaminophenyl-1-(2,4-dinitrophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 4i. Crystallization solvent dimethyl formamide/water, yield 98%; mp 197–199 °C. IR: 3300 (OH), 2900, 2850 ($CH_{3(s)}$), 1720 (C=O), 1620, 1590 (C=N, C=C), 1510, 1330 (NO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 2.12, 2.52 (m, 2H, CH_2 of pyrazole), 2.99 (s, 6H, $2CH_3$), 3.66 (m, 1H, CH of pyrazole), 6.18 (s, 1H, C3–H), 6.65–7.57 (m, 13H aromatic), 11.60 (s, 1H, OH exchangeable). Anal. Calcd for $C_{32}H_{25}N_5O_7$: C, 64.97; H, 4.26; N, 11.84. Found: C, 65.14; H, 4.42; N, 10.99.

4.2.5. General procedure for synthesis of 4-(aryl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile 5a–f

To equimolar amounts of 8-acyl-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one **1** (2.8 g, 0.01 mol), ethyl cyanoacetate (1.13 ml, 0.01 mol) and the chosen aldehyde (0.01 mol); ammonium acetate (0.08 mol), was added in absolute ethanol (50 ml). The mixture was stirred and refluxed for 8 h. After cooling, the solid product that separated was gathered and crystallized from suitable solvent.

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4.2.5.1. 4-(Anisoyl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile 5a. Crystallization solvent ethanol, yield 75%; mp 320–321 °C. ^{1}H NMR ($CDCl_3$) δ: 3.84 (s, 3H, OCH_3), 6.05, 6.20 (2s, 2H, C3–H, C5′–H), 6.96–7.99 (m, 11H aromatic), 8.18 (s, 1H, NH exchangeable), 11.60 (s, 1H, OH exchangeable). MS (*m*/*z*): 462 (M^+, 57.8) 461 (M^+-1, 100). Anal. Calcd for $C_{28}H_{18}N_2O_5$: C, 72.72; H, 3.92; N, 6.06. Found: C, 72.41; H, 4.36; N, 5.92.

4.2.5.2. 4-(*p*-Chlorophenyl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile 5b. Crystallization solvent ethanol/chloroform 9:1, yield 78%; mp above 360 °C. IR: 3471, 3345 (OH, NH), 2219 (CN), 1721, 1684 (C=$O_{(s)}$), 1602, 1514, 1458 (NH, C=N, C=C), 702 (Cl) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 6.15, 6.30 (s, 1H, C3–H, C5′–H), 6.83–7.67 (m, 11H aromatic), 9.60 (s, 1H, NH exchangeable), 11.60 (s, 1H, OH exchangeable). MS (*m*/*z*): 467.25 (M^+, 5.0). Anal. Calcd for $C_{27}H_{15}ClN_2O_4$: C, 69.46; H, 3.24; N, 6.00. Found: C, 69.53; H, 3.37; N, 5.80.

4.2.5.3. 4-(*N*,*N*-Dimethylaminophenyl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile 5c. Crystallization solvent acetic acid/water, yield 86%; mp 275–276 °C. IR: 3350–3297 (br) (OH, $NH_{(s)}$), 2909, 2866, 2828 ($CH_{3(s)}$), 2206 (CN), 1704 (C=$O_{(s)}$), 1611, 1567, 1522 (NH, C=N,C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 3.05 (2s, 6H, $2CH_3$), 6.30, 6.60 (2s, 2H, C3–H, C5′–H), 6.76–8.07 (m, 11H aromatic), 9.60 (s, 1H, NH exchangeable), 12.30 (s, 1H, OH exchangeable). Anal. Calcd for $C_{29}H_{21}N_3O_4$: C, 73.25; H, 4.45; N, 8.84. Found: C, 73.01; H, 4.46; N, 8.80.

4.2.5.4. 4-(2,4,6-Trimethoxyphenyl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile 5d. Crystallization solvent ethanol/chloroform, yield 85%; mp 265–266 °C. IR: 3437, 3305, 3189 (OH, $NH_{(s)}$), 2940, 2840 ($CH_{3(s)}$), 2215 (CN), 1714 (C=$O_{(s)}$), 1602, 1459 (NH, C=N, C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 3.70, 3.81, 3.85 (3s, 9H, $3OCH_3$), 6.27, 6.36 (2s, 2H, C3–H, C5′–H), 7.39–8.16 (m, 9H aromatic), 9.60 (s, 1H, NH exchangeable), 15.60 (s, 1H, OH exchangeable). MS (*m*/*z*): 523.4 (M^+, 36.31), Anal. Calcd for $C_{30}H_{22}N_2O_7$: C, 68.96; H, 4.24; N, 5.36. Found: C, 68.93; H, 4.75; N, 5.38.

4.2.5.5. 4-(*o*-Hydroxyphenyl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile 5e. Crystallization solvent acetic acid/water, yield 65%; mp above 360 °C. IR: 3431, 3318, 3277, 3210 ($OH_{(s)}$, $NH_{(s)}$), 2210 (CN), 1705 (C=$O_{(s)}$), 1604, 1486 (NH, C=N, C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 6.14, 6.19 (2s, 2H, C3–H, C5′–H), 6.82–7.54 (m, 11H aromatic), 9.60 (s, 1H, NH exchangeable), 11.60 (s, 2H, 2OH exchangeable). MS (*m*/*z*): 448 (M^+, 100). Anal. Calcd for $C_{27}H_{16}N_2O_5$: C, 72.32; H, 3.60; N, 6.25. Found: C, 72.34; H, 3.66; N, 6.28.

4.2.5.6. 4-(3-Pyridyl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile 5f. Crystallization solvent ethanol/chloroform, yield 55%; mp 228–229 °C. IR: 3333, 3285, 3195 (OH, $NH_{(s)}$), 2210 (CN), 1721 (C=$O_{(s)}$), 1606, 1564 (NH, C=N, C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 6.21, 6.46 (2s, 2H, C3–H, C5′–H), 6.67–8.2 (m, 11H aromatic), 9.70 (s, 1H, NH exchangeable), 11.60 (s, 1H, OH exchangeable). Anal. Calcd for $C_{26}H_{15}N_3O_4$: C, 72.05; H, 3.49; N, 9.69. Found: C, 71.50; H, 3.50; N, 9.61.

4.2.6. General procedure for synthesis of 4-(Aryl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-imino-1,2-dihydropyridine-3-carbonitrile 6a–e

A mixture comprised of 8-acyl-7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one **3** (2.8 g, 0.01 mol), malononitrile (0.66 ml, 0.01 mol), ammonium acetate (0.08 mol), and the appropriate aromatic aldehyde (0.01 mol) in absolute ethanol (50 ml) were stirred and refluxed for 8 h. The reaction mixture was cooled; the solid precipitate that was formed was filtered and recrystallized from the suitable solvent.

4.2.6.1. 4-(Anisoyl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile 6a. Crystallization solvent acetic acid/water, yield 83%; mp 203–204 °C. IR: 3400, 3352, 2220 (OH, $NH_{(s)}$), 2840 ($CH_{3(s)}$), 2210 (CN), 1731 (C=O), 1602, 1514, 1460 (NH, C=N, C=C) cm^{-1}. ^{1}H NMR ($CDCL_3$) δ: 3.85 (s, 3H, OCH_3), 6.19, 6.42 (2s, 2H, C3–H, C5′–H), 7.12–7.64 (m, 11H aromatic), 8.00, 8.60 (2s, 2H, 2NH exchangeable), 12.70 (s, 1H, OH exchangeable). Anal. Calcd for $C_{28}H_{19}N_3O_4$: C, 72.88; H, 4.15 N 9.11. Found: C, 72.49; H, 4.35; N, 8.89.

4.2.6.2. 4-(*p*-Chlorophenyl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-imino-1,2-dihydropyridine-3-carbonitrile 6b. Crystallization solvent ethanol/chloroform, yield 75%; mp 244–246 °C. IR: 3350, 3200 (OH, $NH_{(s)}$), 2200 (CN), 1720 (C=O), 1615, 1560 (NH, C=N, C=C), 700 (Cl) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 6.17, 6.42 (2s, 2H, C3–H, C5′–H), 7.31–7.66 (m, 11H aromatic), 8.10, 8.79 (2s, 2H, 2NH exchangeable), 12.60 (s, 1H, OH exchangeable) MS (*m*/*z*): 465.65 (M^+, 80.27), 467.15 (M^++1, 32.38). Anal. Calcd for $C_{27}H_{16}ClN_3O_3$: C, 69.61; H, 3.46; N, 9.02. Found: C, 69.45; H, 3.59; N, 9.07.

4.2.6.3. 4-(*N*,*N*-Dimethylaminophenyl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-imino-1,2-dihydropyridine-3-carbonitrile 6c. Crystallization solvent ethanol/chloroform, yield 82%; mp 245–246 °C. IR: 3350, 3200 (OH, $NH_{(s)}$), 2925 ($CH_{3(s)}$), 2200 (CN), 1720 (C=O), 1615, 1560, 1520 (NH, C=N, C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 3.09 (s, 6H, $2CH_3$), 6.44, 6.76 (2s, 2H, C3–H, C5′–H), 7.17–7.85 (m, 11H aromatic), 8.00, 8.30 (2s, 2H, 2NH exchangeable), 10.80 (s, 1H, OH exchangeable). MS (*m*/*z*): 474.2 (M^+, 0.67). Anal. Calcd for $C_{29}H_{22}N_4O_3$: C, 73.41; H, 4.67; N, 11.81. Found: C, 73.14; H, 4.89; N, 11.69.

4.2.6.4. 4-*o*-Hydroxyphenyl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-imino-1,2-dihydropyridine-3-carbonitrile 6d. Crystallization solvent acetic acid/water, yield 79%; mp 202–203 °C. IR: 3311, 3204 ($OH_{(s)}$, $NH_{(s)}$), 2204 (CN), 1711 (C=O), 1605, 1485 (NH, C=N, C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ6.17, 6.79 (2s, 2H, C3–H, C5′–H), 7.40–8.00 (m, 11H aromatic), 8.20, 9.60 (2s, 2H, 2NH exchangeable), 11.60, 13.50 (2s, 2H, 2OH exchangeable). MS (*m*/*z*): 446 (M^+-1, 1.47). Anal. Calcd for $C_{27}H_{17}N_3O_4$: C, 72.48; H, 3.83; N, 9.39. Found: C, 72.60; H, 4.29; N, 9.60.

4.2.6.5. 4-(3-Pyridyl)-6-(7-hydroxy-2-oxo-4-phenyl-2*H*-1-benzopyran-8-yl)-2-imino-1,2-dihydropyridine-3-carbonitrile 6e. Crystallization solvent ethanol/chloroform 9:1, yield 75%; mp 260–261 °C. IR: 3350, 3200 (OH, $NH_{(s)}$), 2200 (CN), 1720 (C=O), 1610, 1560, 1520 (NH, C=N, C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 6.18, 6.79 (2s, 2H, C3–H, C5′–H), 6.97–8.05 (m, 11H aromatic), 8.20, 8.70 (2s, 2H, 2NH exchangeable), 11.60 (s, 1H, OH exchangeable) Anal. Calcd for $C_{26}H_{16}N_4O_3$, H_2O: C, 69.33; H, 4.03; N, 12.44. Found: C, 68.96; H, 3.73; N, 12.36.

4.3. Pharmacological screening

All the tested compounds were dissolved in dimethylformamide (DMF) before subcutaneous injection in the tested animals. The control group was similarly injected with DMF. The LD_{50} of the newly synthesized compounds was determined.

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4.3.1. Acute toxicity and lethality (LD_{50}) test

The acute toxicity and lethality (LD_{50}) of the new compounds were estimated in albino mice (25–30 g)[57] In a preliminary test, animals in groups of three, received one of 10, 50, 150, 300, or 600 mg kg^{-1} of the tested compounds by subcutaneous injection. Animals were observed for 24 h for signs of toxicity and number of deaths. From the results of the first test, 150, 300, 450 and 600 mg kg^{-1} doses of the tested compounds were administered to fresh groups of mice. Control animals received the vehicle and were kept under the same conditions without any treatments. Signs of toxicity and number of deaths per dose in 24 h were recorded and the LD_{50} was calculated as the geometric mean of the dose that resulted in 100% mortality and that which caused no lethality at all.

4.3.2. Anti-inflammatory activity

The anti-inflammatory effect of the newly synthesized compounds was evaluated in correspondence to the carrageenan-induced paw edema method.[54] Twenty nine groups of animals each consisting of five rats weighing 180–200 g were selected. The 1st group was treated with the vehicle and left as control while the 2nd one was given celecoxib by subcutaneous injection in a dose of 20 mg kg^{-1} (standard). Other groups were subcutaneously injected with the tested compounds in doses equal to 1/10 their LD_{50}. After 30 min, acute inflammation was induced by subplantar injection of 0.1 ml of 1% suspension of carrageenan in the right hind paw of all rats. Paw size was measured by wrapping a piece of cotton thread round the paw and measuring the circumference with a meter rule. Measurement was carried out immediately before and 3 h following carrageenan injection. Percent inhibition of tested compounds and standard drug was calculated in comparison with vehicle control (100%). Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects.

4.3.3. Analgesic activity

The analgesic validation of tested compounds was evaluated using the acetic acid-induced writhing method[55] Mice (25–30 g) were assigned into 29 groups, each containing five animals. The 1st group was kept as a control (received the vehicle) while the 2nd one was subcutaneously injected with diclofenac sodium in a dose of 5 mg kg^{-1} (standard). The other groups were injected with the tested compounds in doses equal to 1/10 of their LD_{50}. Writhing was induced 30 min later, by intraperitoneal injection of 0.1 ml of 0.6% acetic acid. Numbers of writhes (abdominal contractions) in all animals were counted for 30 min, immediately after acetic acid injection (0 time) and hourly after administration for 4 h. Analgesic activity was expressed as the percentage protection against writhing produced by the new compounds allover the experimental period comparing between control and those pretreated with the new compounds using the ratio:

$$\%\ \text{reduction} = \frac{\text{Writhes mean at (0) time} - \text{Writhes mean at (t) time}}{\text{Writhes mean at (0) time}} \times 100$$

The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors.

4.3.4. Antipyretic activity

The antipyretic activity of the newly synthesized compounds was screened in male rats (180–200 g), in consistent to the yeast-induced hyperpyrexia method.[56] After measuring rectal temperature of each rat by introducing 1.5 cm of digital thermometer in rectum, pyrexia was induced by subcutaneous injection of 1 ml of 15% brewer's yeast suspension in saline solution. After 18 h of yeast injection, rats which showed a rise in temperature of at least 0.6 °C were taken for the study. One hundred and forty five, hyperthermic rats were divided into 29 equal groups. Rats of the 1st group were subcutaneously injected with the vehicle and left as control while the 2nd one was orally given paracetamol in a dose of 100 mg kg^{-1} (standard). The rest of the groups were injected with the tested compounds in doses equal to 1/10 of their LD_{50}. Rectal temperature of each rat was then recorded at 1 h interval after administration for 4 h. The antipyretic efficacy was decided on the basis of the difference in the mean temperature between the control and the tested compounds.

4.3.5. Statistical analysis

All values were expressed as mean ± S.E.M. Statistical significance was determined by comparing the values of the test compounds and the standards with those obtained in the presence of the vehicle using Student's *t*-test.[58,59]

4.4. Drug modeling studies

All docking studies were performed using 'Internal Coordinate Mechanics (MOLSOFT ICM 3.4–8C)'. ICM docking is probably the most accurate predictive tool of binding geometry today.[36,60–62]

4.4.1. Preparation of small molecule

A set of 7-hydroxy-2*H*-1-benzopyran-2-one analogues synthesized to inhibit cyclooxygenase II was compiled by us earlier; ChemDraw 3D structures were constructed using Chem 3D ultra 8.0 software [Molecular Modeling and Analysis; Cambridge Soft Corporation, USA (2004)], and then they were energetically minimized by using MOPAC (semi-empirical quantum mechanics), Jop Type with 100 iterations and minimum RMS gradient of 0.01, and saved as MDL MolFile (*.mol).

4.4.2. Generation of ligand and enzyme structures

The crystal structure of target protein cyclooxygenase (4cox) is a murine COX-II complexed with indomethacin[22] was retrieved from the Protein Data Bank (http://www.rcsb.org/pdb/welcome.do). All bound waters ligands and cofactors were removed from the protein. The amino acids of the binding site where defined using data in pdbsum (http://www.ebi.ac.uk/thoronton-srv/databases/pdbsum/.

4.4.3. Docking using MOLSOFT ICM 3.4-8C program

1- *Convert our PDB file into an ICM object:* This conversion involves addition of hydrogen bonds, assignment of atoms types, and charges from the residue templates.
2- To perform ICM small molecule docking:
   a) Setup docking project:
      1) Set project name:
      2) Setup the receptor:
      3) Review and adjust binding site:
      4) Make receptor maps:
   b) Start docking simulation:
3- Display the result:

ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that include five grid potentials describing interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during

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this process a stack of alternative low energy conformations is saved (Table 4).

The mode of interaction of the Imn (Indomethcin) within (4cox) was used as a standard docked model. All inhibitors were compared according to the best binding free energy (minimum) obtained among all the run.

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