Synthesis and Anti-inflammatory Activity of Some Benzofuran and Benzopyran-4-one Derivatives

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New series of furosalicylic acids 3a—c, furosalicylanilides 6a—n, furobenzoxazines 8a—f, 1-benzofuran-3-arylprop-2-en-1-ones 12a,b, 6-(aryl-3-oxoprop-1-enyl)-4*H*-chromen-4-ones 16a—c and 6-[6-aryl-2-thioxo-2,5-dihydropyrimidin-4-yl]-4*H*-chromen-4-ones 17a—c were synthesized. Anti-inflammatory activity evaluation was performed using carrageenan-induced paw edema model in rats and prostaglandin E_2 (PGE_2) synthesis inhibition activity. Some of the tested compounds revealed comparable activity with less ulcerogenic effect than Diclofenac at a dose 100 mg/kg. All the synthesized compounds were docked on the active site of cyclooxygenase-2 (COX-2) enzyme and most of them showed good interactions with the amino acids of the active site comparable to the interactions exhibited by Diclofenac.

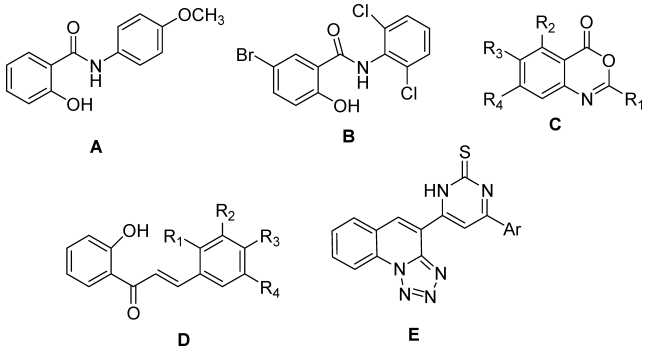
Key words anti-inflammatory activity; prostaglandin E_2; benzofuran; benzopyran-4-one

In spite of the high effectiveness of the steroidal anti-inflammatory drugs, their serious side effects limit their use in common inflammation.[1–3] Although non steroidal anti-inflammatory drugs possess great safety over steroidal drugs, they still have some side effects as development of peptic and duodenal ulcers, kidney and liver malfunctions.[4–6] The rise of selective cyclooxygenase-2 (COX-2) inhibitors reduced the risk of some of these side effects.[7,8] Therefore, seeking new drugs with anti-inflammatory activity and a fewer side effects are still a goal for medicinal chemists.

Benzofuran nucleus is an isostere for indole, the potent basic pharmacodynamic nucleus which has anti-inflammatory activity.[9,10] Many benzofuran derivatives were reported to show potent anti-inflammatory activity and were among COX-2 inhibitors.[11,12] On the other hand, salicylic acid and salicylates exhibit a pronounced anti-inflammatory activity, for example the salicylanilides **A** and **B** are selective inhibitors of interlukin-12 (IL-12) p40 production which is a subunit component of IL-12 and IL-6—23. IL-12 is involved in type 1 helper T cell (TH1) mediated inflammation in both normal immune defence as well as inflammatory diseases such as rheumatoid activities, asthma, psoriases and Crohn's disease.[13] In addition, the antinociceptive and anti-inflammatory activities of 4*H*-2,3-dihydro-1,3-benzoxazines **C** as cyclic acetal like derivatives of salicylamide were accomplished in comparison with aspirin and were of much less ulcerogenic effect.[14] Thus, the present investigation deals with the synthesis of certain substituted furanylsalicylic acids, furanylsalicylanilides and their cyclised furanylbenzoxazines to be evaluated as anti-inflammatory agents.

Several studies indicated that chalcones potently inhibit nitric oxide production by the inducible nitric oxide synthetase (iNOs). Nitric oxide (NO) plays an important role in immune reactions.[15] Also, 2-hydroxy chalcone analogues **D** showed great activity as inhibitors for COX-2 catalyzed prostaglandin production as well as lipooxygenase.[16] On the other hand, thioxopyrimidines **E** were evaluated for their anti-inflammatory activity and the results were comparable with Diclofenac with minimum ulcerogenic activity.[16] With respect to anti-inflammatory activity several studies indicated that benzopyran derivatives (chromones and flavones) are potentially useful anti-inflammatory agents due to their ability to inhibit the protein kinase dependant signal transduction pathway. Furthermore, some natural benzopyran derivatives showed inhibitory activity of prostaglandin E_2 (PGE_2) production.[17]

Thus, the present investigation deals also with the synthesis of certain substituted aminobenzofuranyl hydroxychalcones, benzopyan chalcones and thioxopyrimidine-benzopyrans to be tested for their anti-inflammatory activity. Compounds which showed activity as anti-inflammatory agents in carrageenan-induced paw edema model, their ability to inhibit PGE_2 synthesis were assayed.



Results and Discussion

Chemistry The synthetic pathways adopted for the preparation of the desired new compounds are illustrated in Charts 1—4. Furosalicylic acids **2a** and **3c** were obtained by oxidative cleavage of the naturally occurring furochromones **1** and **5**, respectively.[18,19] Brominating of **2a** in chloroform at room temperature led to the formation of 3,7-dibromo benzofuran **3a**. Alternatively, mono bromo derivative **3b** was obtained by brominating of the furochromone **1** in glacial acetic acid followed by oxidative cleavage of 9-bromofurochromone **4** with hydrogen peroxide in alkaline medium (Chart 1). Furosalicylanilides **6a—n** were prepared from their corresponding furosalicylic acids **3a—c** by reaction with the appropriate aromatic amines in chlorobenzene using phosphorus trichloride

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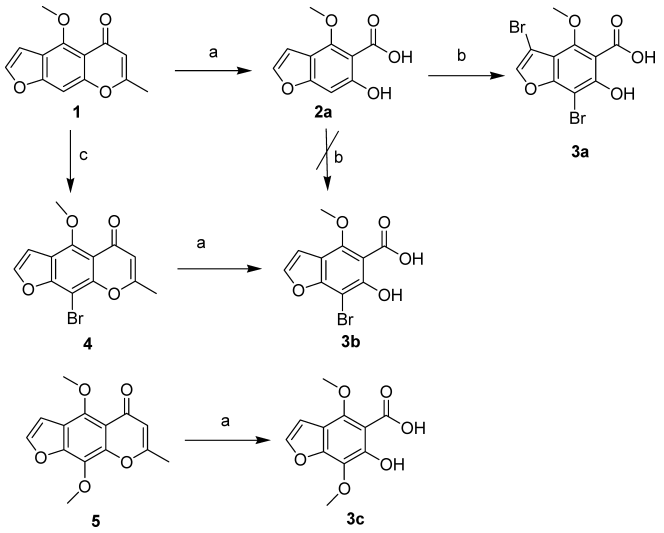
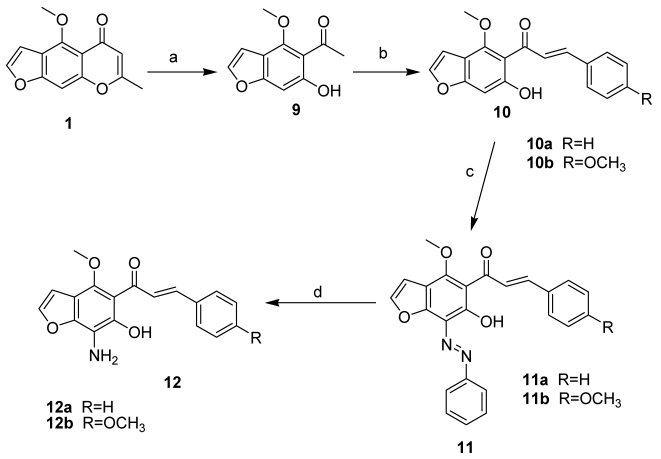
as a condensing agent[20–22] (Chart 2). Furobenzoxazines **8a**—**f** were obtained from the corresponding anilides by reaction with ethyl chloroformate and subsequent cyclization of the resultant esters **7a**—**f** with sodium ethoxide in ethanol (Chart 2). 1-(7-Amino-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-arylprop-2-en-1-ones **12a**, **b** have been synthesized by alkaline cleavage of the furochromone **1** to 5-acetyl-6-hydroxy-4-methoxy benzofuran **9**,[23] which reacted with aromatic aldehydes in prescence of alkali (Claisn–Schmidt condensation) to give 1-(benzofuran-5-yl)-3-arylprop-2-en-1-ones **10a**, **b**. Subsequent introduction of amino group was carried out through coupling with benzenediazonium chloride followed by reductive cleavage of the azo derivatives **11a** and **11b** with sodium dithionite to give **12a**, **b** (Chart 3). A similar sequence was used to prepare (*E*)-8-amino-6-(aryl-3-oxoprop-1-enyl)-4*H*-chromen-4-ones **16a**—**c** from the furchromone **1** which is subjected to oxidative cleavage by potassium dichromate and sulphuric acid to produce 4-oxo-4*H*-chromen-6-carbaldehyde **13**, fol-



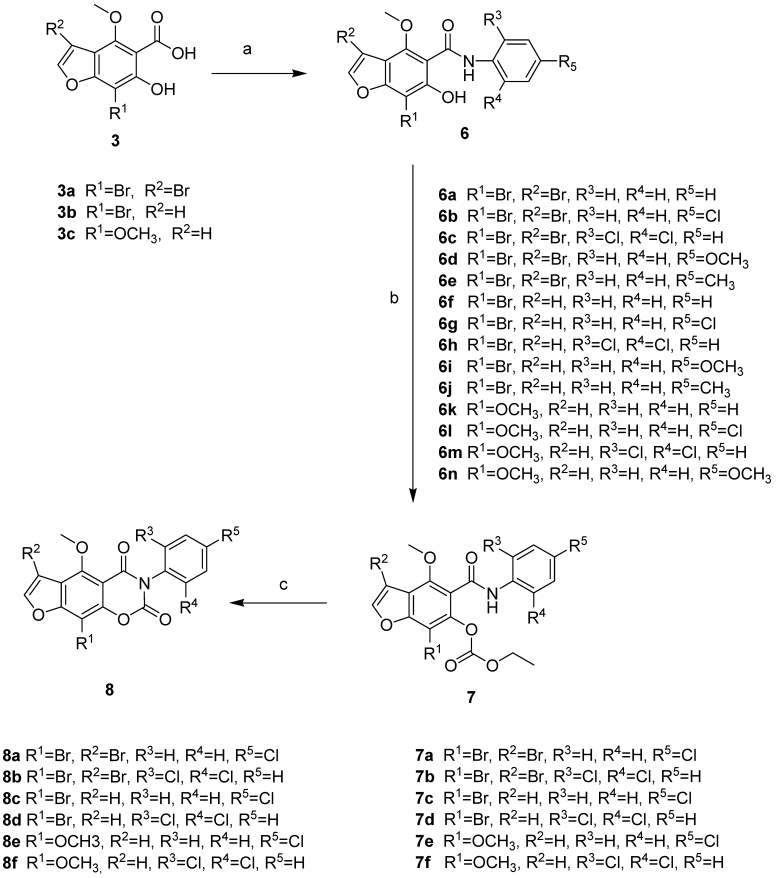
Reagents and solvents: a) 30% H_2O_2, 5% NaOH; b) Br_2, $CHCl_3$; c) Br_2, glacial acetic acid.

Chart 1



Reagents and solvents: a) 10% KOH; b) aromatic aldehydes, 30% NaOH, ethanol; c) aniline, $NaNO_2$, HCl; d) sodium dithionite, ethanol.

Chart 3



Reagents and solvents: a) aromatic amines, phosphorus trichloride, chlorobenzene; b) ethylchloroformate, pyridine; c) sodium ethoxide, absolute ethanol.

Chart 2

lowed by reaction with substituted acetophenones (Claisen–Schmidt reaction) to yield 6-(3-oxo-3-aryllprop-1-en-1-yl)-7-hydroxy-5-methoxy-2-methyl-4*H*-chromen-4-ones **14a—c**. The amino group was introduced in position 8 as mentioned above through the diazo compounds **15a—c**, followed by reductive cleavage to give **16a—c** (Chart 4). 6-(6-Aryl-2-thioxo-2,5-dihydropyrimidin-4-yl)-4*H*-chromen-4-ones **17a—c** were obtained by reaction of 6-(3-aryl-3-oxoprop-1-en-1-yl)-4*H*-chromen-4-ones **14a—c** with thiourea and sulphuric acid (Chart 4).

Pharmacological Screening Pharmacological evaluation was carried in Department of Pharmacology, Faculty of Pharmacy, Cairo University, Egypt.

The experimental tests on animals have been performed in accordance with the Institutional Ethical Committee Approval, Faculty of Pharmacy, Cairo University.

Anti-inflammatory Activity The newly synthesized compounds **3a—c**, **6a—n**, **8a—f**, **12a**, **12b**, **14a**, **15a**, **16a—c**, and **17a—c** were evaluated for their anti-inflammatory activity using carrageenan induced rat paw edema model. The injection of carrageenan to the hind paw of rats causes edema and an increase in paw volume associated with hyperalgesia as reported by Winter *et al.*[24] The development of the paw edema has been described as a biphasic event, where the initial inflammatory phase has been attributed to the release of histamine, serotonin and a kinin-like substance and the second accelerating phase of swelling attributed to the release of a prostaglandin-like substance.[25] The percentage inhibition of edema was calculated and the data were expressed as the mean±S.E.M. Diclofenac was used as a reference (Table 1).

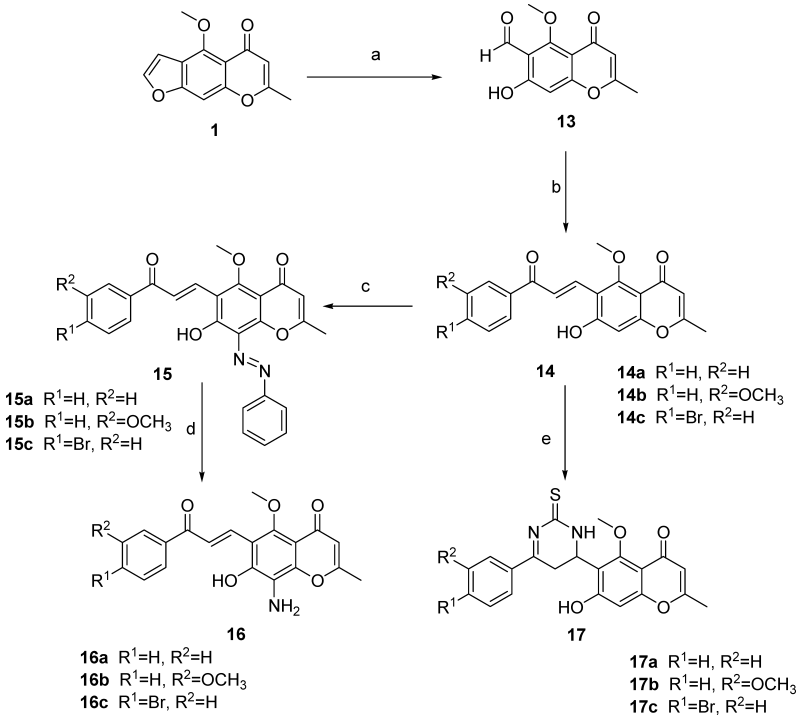
PGE_2 Synthesis Inhibition Activity PGE_2 concentrations were estimated using rat specific immunoassay kit in serum samples prepared by centrifugation of blood of the injected animals.[26] The most active anti-inflammatory compounds **3a—c**, **6d**, **6i**, **6k**, **6n**, **12a**, **12b**, and **16a—c** were assayed using Diclofenac as a reference drug. The percentage inhibition of PGE_2 was calculated and the data were expressed as the mean±S.E.M. All the tested compounds showed inhibitory effect on PGE_2 synthesis. Compounds **3a—c** showed activity comparable to Diclofenac (Table 2).

Compounds **3a—c** and **12a**, **b** which showed the highest percentage inhibition in carrageenan induced rat paw edema model, showed the highest PGE_2 synthesis inhibition. It has been noticed that the PGE_2 inhibitory properties of the tested compounds coincide greatly with our described anti-inflammatory properties.

Ulcerogenic Effect The active compounds as anti-inflammatory agents **3a—c**, **6d**, **6i**, **6k**, **6n**, **12a**, **12b**, and **16a—c** were evaluated for their ulcerogenic potential in rats. Diclofenac was used as reference standard[27] and the results were calculated as ulcer index. Compounds **3a—c** showed more ulcerative effect than Diclofenac. On the other hand, furosalicylanilides **6d**, **6i**, **6k** and **6n** were of much less ulcerogenic effect. The best results were for 1-(7-amino-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-arylprop-2-en-1-ones **12a**, **b** and 6-(aryl-3-oxoprop-1-enyl)-4*H*-chromen-4-ones **16a**, **b** which showed lesser ulcerogenic insults (Table 2).

Molecular Docking Previous literatures showed that series of anti-inflammatory drugs inhibit PGE_2 synthesis by interacting with Tyr385 and Ser530 in the active site of COX-2 enzyme and compounds that could interact with such amino acids can exhibit anti-inflammatory activity.[28–30] Interactions with Arg120 may play also a role in the inhibitory activity of COX-2.[30,31]

Consequently, all the synthesized compounds were docked on the active site of cyclooxygenase-2 enzyme (COX-2) based



Reagents and solvents: a) potassium dichromate, 10% sulphuric acid; b) acetophenones, 5% NaOH, ethanol; c) aniline, $NaNO_2$, HCl; d) sodium dithionite, ethanol; e) thiourea, sulphuric acid.

Chart 4

Table 1. Anti-inflammatory Activity of the Tested Compounds Using Acute Carrageenan Induced Paw Oedema in Rats at a Dose of 100 mg/kg of Body Weight

Compound No.	1 h	2 h	3 h	4 h
Diclofenac	77±2.32	79±2.15	80±2.63	83±2.60
3a	69±3.21	73±1.77	76±3.21	76±4.11
3b	61±1.34	65±2.34	66±2.42	66±2.56
3c	70±2.13	71±2.21	73±3.11	73±2.86
6a	46±2.34	49±2.77	50±3.41	54±2.44
6b	41±1.67	44±3.67	48±1.26	50±2.59
6c	39±2.22	42±2.66	45±2.19	49±3.33
6d	47±1.58	50±3.22	56±2.09	60±2.01
6e	37±2.11	39±4.33	42±3.03	44±5.13
6f	40±3.76	41±2.55	44±1.96	49±3.26
6g	32±3.21	36±1.53	38±4.21	40±4.99
6h	30±2.57	35±2.99	37±3.69	39±5.35
6i	41±2.45	44±3.13	45±2.76	50±2.76
6j	29±2.65	32±2.64	34±2.55	39±5.88
6k	50±1.89	55±2.56	57±2.41	58±2.69
6l	46±2.54	49±4.79	50±2.22	53±4.78
6m	44±1.80	46±2.49	49±3.10	51±3.87
6n	56±1.99	58±2.61	58±1.89	62±2.69
8a	24±2.77	27±4.98	27±2.76	27±5.12
8b	22±5.23	22±3.54	23±3.24	24±4.99
8c	20±4.99	23±2.63	27±3.21	27±4.32
8d	18±3.75	21±1.97	24±3.78	24±4.10
8e	32±6.21	33±3.77	35±4.67	36±2.14
8f	31±3.89	33±2.55	33±1.76	33±3.65
12a	59±2.89	60±4.10	60±1.99	60±1.78
12b	60±3.11	62±2.22	63±2.10	63±2.54
14a	50±1.47	51±2.97	52±2.65	52±1.99
15a	21±2.13	21±2.01	22±2.16	22±1.06
16a	52±1.99	56±6.21	56±2.45	56±2.18
16b	55±2.04	57±5.32	59±3.89	59±4.11
16c	50±3.56	51±4.13	51±2.11	52±2.10
17a	21±2.55	25±1.56	25±2.67	27±4.73
17b	26±4.06	26±3.22	26±2.89	28±2.01
17c	30±5.98	34±4.21	34±4.25	35±4.37

All results are statistically significant at $p<0.05$.

Table 2. PGE_2 Inhibitory Activity of the Most Promising Prepared Anti-inflammatory Active Agents, Ulcerogenic Activity and LD_{50} of the Tested Compounds

Compound No.	% PGE_2 inhibition	Ulcer index	LD_{50} mg/kg
Diclofenac	68±3.56	40±2.21	370±1.78
3a	60±2.44	49±1.24	127±2.33
3b	54±3.21	47±3.21	118±3.21
3c	61±1.34	49±1.34	170±3.87
6d	29±2.06	40±2.06	109±2.55
6i	21±1.89	39±3.01	115±2.65
6k	39±1.24	41±1.92	133±3.02
6n	37±2.45	41±2.35	124±1.11
12a	49±3.97	33±1.31	214±2.34
12b	49±2.67	30±2.21	221±3.22
16a	40±2.10	32±1.10	225±1.98
16b	47±4.10	30±2.15	216±2.67
16c	46±5.21	31±0.42	219±3.11

All the results are statistically significant at $p<0.05$.

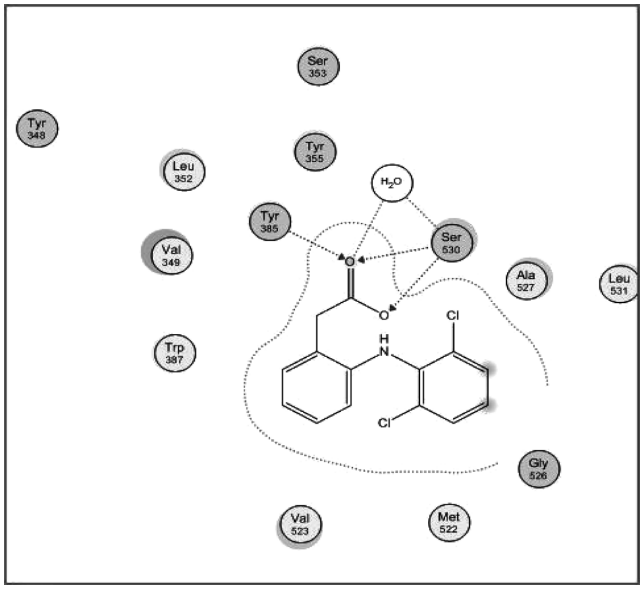


Fig. 1. Interactions of Diclofenac on the Active Site of Cox-2 (2D)

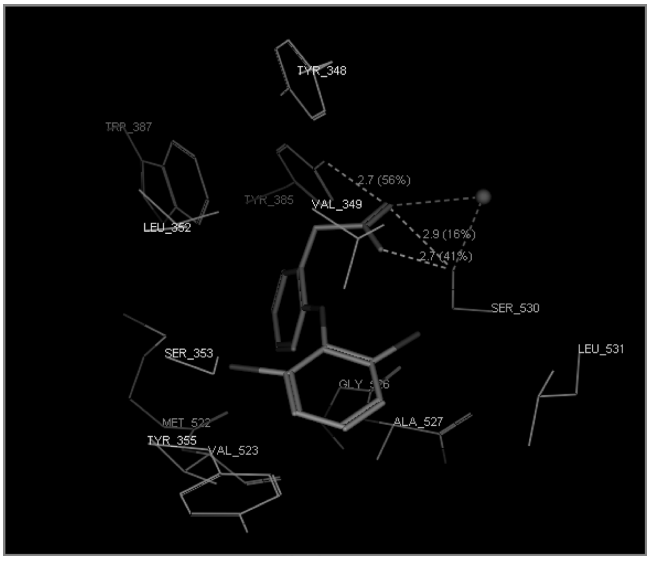


Fig. 2. Interactions of Diclofenac on the Active Site of Cox-2 (3D)

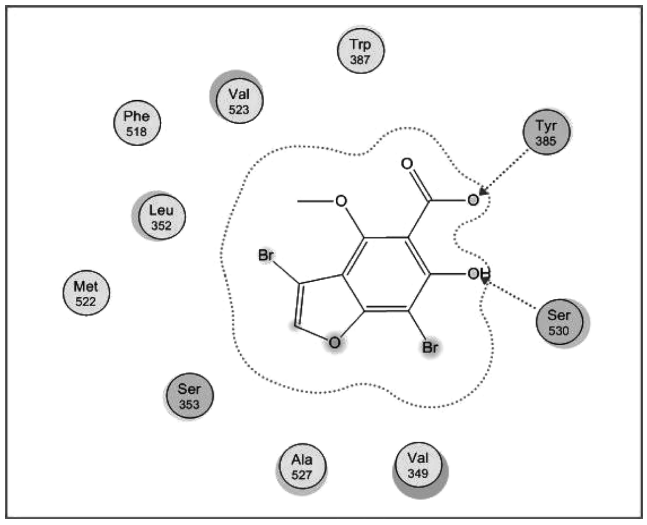


Fig. 3. Compound (**3a**) Interactions 2D

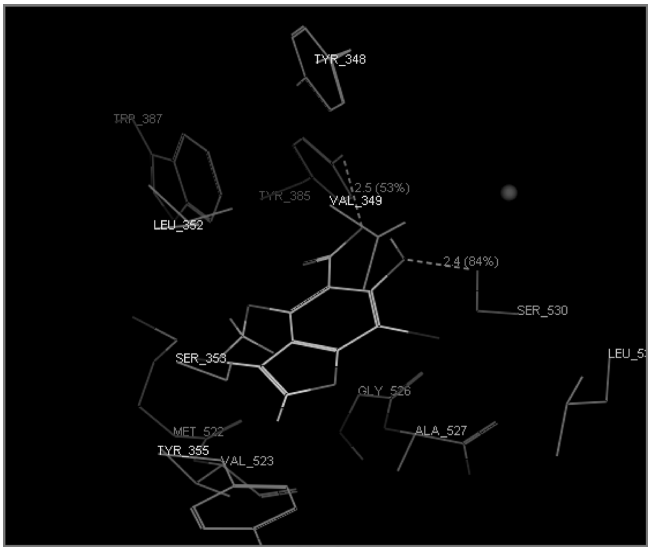


Fig. 4. Compound (**3a**) Interactions 3D

on the protein data bank file code 1PXX. The file contains the COX-2 enzyme with Diclofenac as a co-crystallized ligand using MOE software using force field energy MMFX9 with $S = -7.5072$ kcal/mol and rmsd = 0.3682. Diclofenac interacted with Ser530 with 2 hydrogen bonds 2.7 Å and 2.9 Å and with Tyr385 with one hydrogen bond 2.7 Å as illustrated in Figs. 1 and 2.

Most of the tested compounds showed moderate to good fitting in the active site pocket with interactions of the amino acids, Tyr385 and/or Ser530 except compound **8d** which showed no interactions with any of the amino acids of the pocket. Three compounds **6k**, **6n** and **12b** interacted with the same amino acids as Diclofenac with 3 hydrogen bonds. Three compounds **6b**, **6c** and **12c** interacted with the same amino acids by 2 hydrogen bonds. Only compound **6d** interacted with Ser530 and Arg120. Compounds **12a** and **13a** interacted with the amino acid Tyr355 while compound **13b** interact with Tyr355 and Tyr385. Only compound **12b** interacted with Arg120. The rest of tested compounds interacted with Ser530 only, Table 3.

Correlation of the Anti-inflammatory Activity with the Molecular Docking Study The most active compounds were furosalicylic acids **3a**—**c** (inhibition % 76, 66, 73 respectively). Molecular docking showed that the most active one **3a** interacted with both amino acids Tyr385 and Ser530 with 2 hydrogen bonds—Figs. 3 and 4—while **3c** interacted with one amino acid Ser530 with 2 hydrogen bonds, and the least active derivative **3b** interacted with Ser530 with only one hydrogen bond.

Conversion of the furosalicylic acids into their corresponding anilides **6a**—**n** generally decreased the anti-inflammatory activity. The most active anilides were **6n**, **6d**, and **6k**. From docking study it was noticed that the 2 active anilides **6n** and **6k** interacted with both Tyr385 and Ser530 with 3 hydrogen bonds while **6d** interacted with Ser530, Arg120 with 2 hydrogen bonds. The rest of anilides which showed lower activity

Table 3. Amino Acid Interactions and Hydrogen Bond Lengths

Compound No.	Amino acid	Hydrogen bond length (Å)
3a	Tyr 385, Ser 530	2.50, 2.40
3b	Ser 530	2.30
3c	Ser 530	2.60, 2.90
6a	Ser 530	2.39
6b	Ser 530	3.06
6c	Ser 530	2.93
6d	Ser 530, Arg 120	2.95, 2.69
6e	Ser 530	2.53
6f	Ser 530	2.53
6g	Ser 530	2.93
6h	Ser 530	3.10
6i	Ser 530	3.10
6j	Ser 530	2.44
6k	Tyr 385, Ser 530	2.89, 2.46, 3.01
6l	Ser 530	2.96
6m	Ser 530	2.54
6n	Tyr 385, Ser 530	3.17, 2.66, 2.77
8a	Ser 530	2.51
8b	Ser 530	2.47
8c	Ser 530	2.35
8d	—	—
8e	Ser 530	2.67
8f	Ser 530	2.73
12a	Tyr 355, Ser 530	2.97, 2.79
12b	Tyr 385, Ser 530	2.87, 2.47, 3.12
16a	Tyr 355	2.79
16b	Arg 120	2.79
16c	Tyr 385, Ser 530	3.00, 2.40
17a	Tyr 355	2.99
17b	Tyr 355, Tyr 385	2.59, 2.99
17c	Ser 530	2.67

interacted with Ser530 with one hydrogen bond, Table 3.

Cyclization of the anilides to their corresponding furobenzoxazines **8a**—**f** decreased the anti-inflammatory activity. All these furobenzoxazines interacted with the amino acid Ser530 with only one hydrogen bond. Concerning 1-benzofuran-3-arylprop-2-en-1-ones **12a**,**b** and 6-(aryl-3-oxoprop-1-enyl)-4*H*-chromen-4-ones **16a**—**c**, the benzofuran derivatives were more potent than the benzopyran derivatives. Compound **12b** which showed the highest activity interacted with the 2 amino acids Tyr385 and Ser530. The benzopyran chalcone **16b** which interacted with Arg120 showed better activity than its congeners **16a** which interacted with Tyr355 by one hydrogen bond and **16c** which interacted with Tyr385 and Ser530 by 2 hydrogen bonds.

Cyclization of 6-(aryl-3-oxoprop-1-enyl)-4*H*-chromen-4-ones **16a**—**c** to 6-[6-aryl-2-thioxo-2,5-dihydropyrimidin-4-yl]-4*H*-chromen-4-ones **17a**—**c** was not of much benefit as the anti-inflammatory activity decreased, **17a** and **17c** interacted with Tyr355 and Ser530 respectively with one hydrogen bond while **17b** is the only derivative which interacted with Tyr355 and Tyr385 by 2 hydrogen bonds.

Conclusion

All the newly synthesized compounds showed anti-inflammatory activity, using carrageenan induced rat paw edema model. Furosalicylic acids **3a**—**c** and 1-(7-amino-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-arylprop-2-en-1-ones **12a**,**b** showed the highest percentage inhibition in carrageenan induced rat paw edema model, and the highest PGE_2 synthesis inhibition.

Conversion of the furosalicylic acids into anilides **6a**—**n** generally decreased the anti-inflammatory activity. Also, cyclization of the anilides **6a**—**n** to their corresponding furobenzoxazines **8a**—**f** and 6-(aryl-3-oxoprop-1-enyl)-4*H*-chromen-4-ones **16a**—**c** to 6-[6-aryl-2-thioxo-2,5-dihydropyrimidin-4-yl]-4*H*-chromen-4-ones **17a**—**c** decreased the anti-inflammatory activity. Concerning 1-benzofuran-3-arylprop-2-en-1-ones **12a**,**b** and 6-(aryl-3-oxoprop-1-enyl)-4*H*-chromen-4-ones **16a**—**c**, the benzofuran derivatives were more potent than the benzopyran derivatives.

Compounds **3a**—**c** showed more ulcerative effect than Diclofenac. On the other hand, furosalicylanilides **6d**, **6i**, **6k** and **6n** were of much less ulcerogenic effect. The best results were for **12a**,**b** and **16a**—**b** which showed lesser ulcerogenic insults.

Experimental

Chemistry Melting points were determined on Electro thermal Stuart $5MP_3$ digital melting point apparatus and were uncorrected. Elemental microanalyses were performed at the micro analytical centre, Faculty of Science, Cairo University. IR spectra were recorded on a Bruker Fourier transform (FT)-IR spectrophotometer as KBr discs. ^{1}H-NMR spectra were recorded in DMSO-d_6 on a Varian Mercury spectrometer (200, 300 MHz). Mass spectra were performed on HP MODEL: MS_5988 mass spectrometer and on Shimadzu QP-2010 Plus (EI, 70 eV). Silica gel used for column chromatography was obtained from Fluka, 70—230 mesh thin layer chromatography was carried out on silica gel TLC plates with fluorescence indicator (F_{254}).

3,7-Dibromo-6-hydroxy-4-methoxy-1-benzofuran-5-carboxylic Acid (3a) To a solution of **2a** (2.08 g, 10 mmol) in chloroform (50 mL), a mixture of bromine (0.54 mL, 1.58 g, 10 mmol) in chloroform (25 mL) was added with stirring. The mixture was left for 24 h and the solvent was evaporated under reduced pressure. The residue was washed by ethanol and crystallized from benzene, yield 60—65%.

Yield 60%, mp 215—217°C. ^{1}H-NMR (DMSO-d_6) δ: 4.00 (3H, s, OCH_3), 7.97 (1H, s, furan H), 10.90 (1H, s, OH exch. D_2O), 16.00 (1H, s, COOH exch. D_2O). IR (KBr) cm^{-1}: 3150 (OH phenolic), 2850 broad (OH carboxylic), 1702 (C=O). MS *m/z*: 364 (M^+), 366 (M^++2), 368 (M^++4). *Anal.* Calcd. for $C_{10}H_6Br_2O_5$ (365.96): C, 32.82; H, 1.65. Found: C, 32.62; H, 1.79.

7-Bromo-6-hydroxy-4-methoxy-1-benzofuran-5-carboxylic acid (3b) To a suspension of **4** (3.08 g, 10 mmol) in 5% NaOH (50 mL), 30% H_2O_2 (6 mL) was added with stirring till dissolved. The solution was stirred at room temperature for 30 min. and neutralized with acetic acid. The reaction mixture was left over night in the fridge, filtered, dried and crystallized from ethanol.

Yield 65%, mp 204—206°C. ^{1}H-NMR (DMSO-d_6) δ: 4.02 (3H, s, OCH_3), 7.27 (1H, d, furan H, J=2 Hz), 7.94 (1H, d, furan H, J=2 Hz), 10.85 (1H, s, OH exch. D_2O), 16.00 (1H, s, COOH exch. D_2O). IR (KBr) cm^{-1}: 3140 (OH phenolic), 2955 broad (OH carboxylic), 1751 (C=O). MS *m/z*: 286 (M^+), 288

(M^++2). *Anal.* Calcd for $C_{10}H_7BrO_5$ (287.06): C, 41.84; H, 2.46. Found: C, 41.91; H, 2.37.

General Procedure for the Preparation of 6a—n To a solution of the appropriate furosalicylic acids **3a—c** (10 mmol) and the appropriate aniline derivative (10 mmol) in chlorobenzene (50 mL), phosphorus trichloride (1.37 g, 1.27 mL, 10 mmol) was added. The solution was refluxed for 3 h, filtered while hot and the filtrate was evaporated till dryness. The solid obtained was washed with ethanol and crystallized from benzene, yield 50—70%.

3,7-Dibromo-6-hydroxy-4-methoxy-*N*-phenyl-1-benzofuran-5-carboxamide (**6a**): Yield 60%, mp 212—214°C. ^{1}H-NMR (DMSO-d_6) δ: 4.16 (3H, s, OCH_3), 7.09—7.70 (5H, m, ArH), 7.98 (1H, s, furan H), 10.33 (1H, s, NH exch. D_2O), 11.6 (1H, s, OH exch. D_2O). IR (KBr) cm^{-1}: 3323 (NH), 3153 (OH), 1678 (C=O), 1635 (NH bending). MS *m/z*: 439 (M^+), 441 (M^++2). *Anal.* Calcd for $C_{16}H_{11}Br_2NO_4$ (441.07): C, 43.57; H, 2.51; N, 3.18. Found: C, 43.50; H, 2.79; N, 3.23.

3,7-Dibromo-6-hydroxy-4-methoxy-*N*-(4-chlorophenyl)-1-benzofuran-5-carboxamide (**6b**): Yield 70%, mp 240—243°C. ^{1}H-NMR (DMSO-d_6) δ: 4.03 (3H, s, OCH_3), 7.03 (2H, d, ArH, J=9.2 Hz), 7.74 (2H, d, ArH, J=9.2 Hz), 7.97 (1H, s, furan H), 10.44 (1H, s, NH exch. D_2O), 11.0 (1H, s, OH exch. D_2O). IR (KBr) cm^{-1}: 3331 (NH), 3142 (OH), 1680 (C=O), 1641 (NH bending). MS *m/z*: 473 (M^+), 475 (M^++2), 477 (M^++4). *Anal.* Calcd. for $C_{16}H_{10}Br_2ClNO_4$ (475.52): C, 40.41; H, 2.12; N, 2.95. Found: C, 40.29; H, 2.29; N, 2.52.

3,7-Dibromo-6-hydroxy-4-methoxy-*N*-(2,6-dichlorophenyl)-1-benzofuran-5-carboxamide (**6c**): Yield 70%, mp 273—275°C. ^{1}H-NMR (DMSO-d_6) δ: 4.02 (3H, s, OCH_3), 7.41 (1H, m, ArH), 7.61 (1H, d, ArH, J=9.2 Hz), 7.80 (1H, s, furan H), 8.01 (1H, d, ArH, J=9.2 Hz), 10.40 (1H, s, NH exch. D_2O), 13.80 (1H, s, OH exch. D_2O). IR (KBr) cm^{-1}: 3313 (NH), 3145 (OH), 1682 (C=O), 1645 (NH bending). MS *m/z*: 507 (M^+), 509 (M^++2), 511(M^++4). *Anal.* Calcd for $C_{16}H_9Br_2Cl_2NO_4$ (509.96): C, 37.68; H, 1.78; N, 2.75. Found: C, 37.80; H, 2.04; N, 2.31.

3,7-Dibromo-6-hydroxy-4-methoxy-*N*-(4-methoxyphenyl)-1-benzofuran-5-carboxamide (**6d**): Yield 58%, mp 216—218°C. ^{1}H-NMR (DMSO-d_6) δ: 3.95 (3H, s, OCH_3), 4.18 (3H, s, OCH_3), 6.89 (2H, d, ArH, J=9.2 Hz), 7.51 (2H, d, ArH, J=9.2 Hz), 7.88 (1H, s, furan H), 10.22 (1H, s, NH exch. D_2O), 11.22 (1H, s, OH exch. D_2O). IR (KBr) cm^{-1}: 3340 (NH), 3133 (OH), 1643 (C=O), 1600 (NH bending). MS *m/z*: 469 (M^+), 471 (M^++2), 473 (M^++4). *Anal.* Calcd for $C_{17}H_{13}Br_2NO_5$ (471.10): C, 43.34; H, 2.78; N, 2.97. Found: C, 43.80; H, 2.99; N, 2.86.

3,7-Dibromo-6-hydroxy-4-methoxy-*N*-(4-methylphenyl)-1-benzofuran-5-carboxamide (**6e**): Yield 55%, mp 216—218°C. ^{1}H-NMR (DMSO-d_6) δ: 2.29 (3H, s, CH_3), 4.18 (3H, s, OCH_3), 7.14 (2H, d, ArH, J=8.2 Hz), 7.56 (2H, d, ArH, J=8.2 Hz), 7.98 (1H, s, furan H), 10.26 (1H, s, NH exch. D_2O), 11.95 (1H, s, OH exch. D_2O). IR (KBr) cm^{-1}: 3350 (NH), 3150 (OH), 1673 (C=O), 1605 (NH bending). *Anal.* Calcd for $C_{17}H_{13}Br_2NO_4$ (455.10): C, 44.87; H, 2.88; N, 3.08. Found: C, 45.05; H, 2.61; N, 2.95.

7-Bromo-6-hydroxy-4-methoxy-*N*-phenyl-1-benzofuran-5-carboxamide (**6f**): Yield 58%, mp 228—230°C. ^{1}H-NMR (DMSO-d_6) δ: 4.00 (3H, s, OCH_3), 6.80 (1H, d, furan H, J=2.0 Hz), 7.00—7.70 (5H, m, ArH), 7.98 (1H, s, furan H, J=2.0 Hz), 10.13 (1H, s, NH exch. D_2O), 11.5 (1H, s, OH exch. D_2O). IR (KBr) cm^{-1}: 3206 (NH), 3159 (OH), 1680 (C=O), 1617 (NH bending). MS *m/z*: 361 (M^+), 363 (M^++2). *Anal.* Calcd for $C_{16}H_{12}BrNO_4$ (362.17): C, 53.06; H, 3.34; N, 2.87. Found: C, 53.00; H, 3.38; N, 2.87.

7-Bromo-6-hydroxy-4-methoxy-*N*-(4-chlorophenyl)-1-benzofuran-5-carboxamide (**6g**): Yield 63%, mp 249—251°C. ^{1}H-NMR (DMSO-d_6) δ: 4.13 (3H, s, OCH_3), 6.74 (1H, d, furan H, J=2.0 Hz), 7.11 (2H, d, ArH, J=8 Hz), 7.65 (2H, d, ArH, J=8 Hz), 7.88 (1H, d, furan H, J=2 Hz), 10.44 (1H, s, NH exch. D_2O), 11.20 (1H, s, OH exch. D_2O). IR (KBr) cm^{-1}: 3332 (NH), 3150 (OH), 1641 (C=O), 1600 (NH bending). MS *m/z*: 395 (M^+), 397 (M^++2). *Anal.* Calcd for $C_{16}H_{11}BrClNO_4$ (396.62): C, 48.45; H, 2.80; N, 3.53. Found: C, 48.67; H, 3.01; N, 3.47.

7-Bromo-6-hydroxy-4-methoxy-*N*-(2,6-dichlorophenyl)-1-benzofuran-5-carboxamide (**6h**): Yield 69%, mp 276—278°C. IR (KBr) cm^{-1}: 3328 (NH), 3133 (OH), 1643 (C=O), 1605 (NH bending). MS *m/z*: 431 (M^+). *Anal.* Calcd for $C_{16}H_{10}BrCl_2NO_4$ (431.06): C, 44.58; H, 2.34; N, 3.25. Found: C, 44.50; H, 2.64; N, 3.45.

7-Bromo-6-hydroxy-4-methoxy-*N*-4-methoxyphenyl-1-benzofuran-5-carboxamide (**6i**): Yield 50%, mp 219—221°C. ^{1}H-NMR (DMSO-d_6) δ: 3.57 (3H, s, OCH_3), 4.18 (3H, s, OCH_3), 6.37 (1H, d, furan H, J=2.2 Hz), 6.92 (2H, d, ArH, J=9.2 Hz), 7.58 (2H, d, ArH, J=9.2 Hz), 8.14 (1H, d, furan H, J=2.2 Hz), 10.24 (1H, s, NH exch. D_2O), 13.75 (s, 1H, OH exch. D_2O). IR (KBr) cm^{-1}: 3315 (NH), 3196 (OH), 1682 (C=O), 1616 (NH bending). *Anal.* Calcd for $C_{17}H_{14}BrNO_5$ (392.20): C, 52.06; H, 3.60; N, 3.57. Found: C, 51.54; H, 3.09; N, 3.14.

7-Bromo-6-hydroxy-4-methoxy-*N*-4-methoxyphenyl-1-benzofuran-5-carboxamide (**6j**): Yield 52%, mp 202—204°C.^{1}H-NMR (DMSO-d_6) δ: 3.35 (3H, s, CH_3), 4.17 (3H, s, OCH_3), 6.27 (1H, d, furan H, J=2.0 Hz), 6.65—7.88 (4H, m, ArH), 8.12 (1H, d, furan H, J=2.0 Hz), 10.27 (1H, s, NH exch. D_2O), 13.72 (1H, s, OH exch. D_2O). *Anal.* Calcd for $C_{17}H_{14}BrNO_4$ (376.20): C, 54,27; H, 3.75; N, 3.72. Found: C, 53.79; H, 3.58; N, 3.25.

4,7-Dimethoxy-6-hydroxy-*N*-phenyl-1-benzofuran-5-carboxamide (**6k**): Yield 58%, mp 210—212°C. ^{1}H-NMR (DMSO-d_6) δ: 3.89 (3H, s, OCH_3), 4.18 (3H, s, OCH_3), 7.24 (1H, d, furan H, J=2.1 Hz), 7.41 (2H, m, ArH), 7.61 (3H, m, ArH), 7.93 (1H, d, furan H, J=2.1 Hz), 10.31 (1H, s, NH exch. D_2O), 12.50 (1H, s, OH exch. D_2O). IR (KBr) cm^{-1}: 3366 (NH), 3118 (OH), 1646 (C=O), 1598 (NH bending). *Anal.* Calcd for $C_{17}H_{15}NO_5$ (313.30): C, 65.17; H, 4.83; N, 4.47. Found: C, 65.39; H, 4.69; N, 4.45.

4,7-Dimethoxy-6-hydroxy-*N*-(4-chlorophenyl)-1-benzofuran-5-carboxamide (**6l**): Yield 63%, mp 226—228°C. ^{1}H-NMR (DMSO-d_6) δ: 3.90 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 7.09 (1H, d, furan H, J=1.8 Hz), 7.40 (2H, d, ArH, J=8.4 Hz), 7.73 (2H, d, ArH, J=8.4 Hz), 7.89 (1H, d, furan H, J=1.8 Hz), 9.87 (1H, s, NH exch. D_2O), 10.36 (1H, s, OH exch. D_2O). IR (KBr) cm^{-1}: 3366 (NH), 3118 (OH), 1646 (C=O), 1598 (NH bending). MS *m/z*: 347 (M^+), 349 (M^++2). *Anal.* Calcd for $C_{17}H_{14}ClNO_5$ (347.75): C, 58.72; H, 4.06; N, 4.03. Found: C, 58.97; H, 4.19; N, 3.82.

4,7-Dimethoxy-6-hydroxy-*N*-(2,6-dichlorophenyl)-1-benzofuran-5-carboxamide (**6m**): Yield 69%, mp 258—260°C. ^{1}H-NMR (DMSO-d_6) δ: 3.89 (3H, s, OCH_3), 4.18 (3H, s, OCH_3), 7.14 (1H, d, furan H, J=2.1 Hz), 7.41 (1H, t, ArH, J=8.7 Hz), 7.61 (2H, d, ArH, J=8.7 Hz), 7.93 (1H, d, furan H, J=2.1 Hz), 10.31 (1H, s, NH exch. D_2O), 12.51 (1H, s, OH exch. D_2O). IR

(KBr) cm^{-1}: 3329 (NH), 3150 (OH), 1641 (C=O), 1598 (NH bending). *Anal.* Calcd for $C_{17}H_{13}Cl_2NO_5$ (382.19): C, 53.42; H, 3.43; N, 3.66. Found: C, 53.40; H, 3.50; N, 3.16.

4,7-Dimethoxy-6-hydroxy-*N*-(4-methoxyphenyl)-1-benzofuran-5-carboxamide (**6n**): Yield 50%, mp 223—225°C. ^{1}H-NMR (DMSO-d_6) δ: 3.85 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.18 (3H, s, OCH_3), 6.85 (1H, d, furan H, J=2.1 Hz), 6.98 (2H, d, ArH, J=9.2 Hz), 7.51 (2H, d, ArH, J=9.2 Hz), 7.88 (1H, d, furan H, J=2.1 Hz), 10.50 (1H, s, NH exch. D_2O), 11.51 (1H, s, OH exch. D_2O). IR (KBr) cm^{-1}: 3396 (NH), 3129 (OH), 1654 (C=O), 1605 (NH bending). MS *m/z*: 343 (M^+), 345 (M^++2). *Anal.* Calcd for $C_{18}H_{17}NO_6$ (343.33): C, 62.79; H, 4.66; N, 4.08. Found: C, 62.60; H, 4.75; N, 4.43.

General Procedure for the Preparation of 7a—f To a solution of the appropriate anilide **6b**, **6c**, **6g**, **6h**, **6l** or **6m** (10 mmol) in dry pyridine (20 mL), ethyl chloroformate (1.08 g, 0.94 mL, 10 mmol) was added at 0°C and stirred for 30 min. The solution was refluxed for 1 h, set aside to cool, poured on ice/water and left for 24 h in ice chest. The solid separated was filtered, dried and crystallized from ethanol, yield 60—70%.

3,7-Dibromo-4-methoxy-5-{[(4-chlorophenyl)amino]-carbonyl}-1-benzofuran-6-yl Ethyl Carbonate (**7a**): Yield 62%, mp 293—295°C. ^{1}H-NMR (DMSO-d_6) δ: 1.27 (3H, t, CH_2CH_3), 4.16 (3H, s, OCH_3), 4.29 (2H, q, CH_2CH_3), 7.23 (2H, d, ArH), 7.51 (1H, t, ArH), 8.07 (1H, s, furan H), 9.8 (1H, s, NH exch. D_2O). I MS *m/z*: 545 (M^+), 547 (M^++2), 549 (M^++4). *Anal.* Calcd for $C_{19}H_{14}Br_2ClNO_6$ (547.58): C, 41.67; H, 2.58; N, 2.65. Found: C, 41.51; H, 2.19; N, 2.78.

3,7-Dibromo-4-methoxy-5-{[(2,6-chlorophenyl)amino]-carbonyl}-1-benzofuran-6-yl Ethyl Carbonate (**7b**): Yield 60%, mp 302—304°C. ^{1}H-NMR (DMSO-d_6) δ: 1.27 (3H, t, CH_2CH_3), 4.16 (3H, s, OCH_3), 4.29 (2H, q, CH_2CH_3), 7.23—7.51 (3H, m, ArH), 8.07 (1H, s, furan H), 9.8 (1H, s, NH exch. D_2O). IR (KBr) cm^{-1}: 3250 (NH), 1702 (C=O ester), 1605 (C=O amide). MS *m/z*: 579 (M^+), 581 (M^++2), 583 (M^++4). *Anal.* Calcd for $C_{19}H_{13}Br_2Cl_2NO_6$ (582.02): C, 39.21; H, 2.25; N, 2.41. Found: C, 38.99; H, 2.82; N, 2.45.

7-Bromo-4-methoxy-5-{[(4-chlorophenyl)amino]carbonyl}-1-benzofuran-6-yl Ethyl Carbonate (**7c**): Yield 65%, mp 289—291°C. IR (KBr) cm^{-1}: 3404 (NH), 1762 (C=O ester), 1699 (C=O amide). MS *m/z*: 467 (M^+), 469 (M^++2). *Anal.* Calcd for $C_{19}H_{15}BrClNO_6$ (468.68): C, 48.69; H, 3.23; N, 2.99. Found: C, 48.59; H, 3.01; N, 2.89.

7-Bromo-4-methoxy-5-{[(2,6-dichlorophenyl)amino]-carbonyl}-1-benzofuran-6-yl Ethyl Carbonate (**7d**): Yield 60%, mp 295—297°C. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (3H, t, CH_2CH_3), 4.00 (3H, s, OCH_3), 4.20 (2H, q, CH_2CH_3), 6.80 (1H, d, furan H, J=2.1 Hz), 7.23—7.95 (4H, m, ArH, furan H), 10.1 (1H, s, NH exch. D_2O). I *Anal.* Calcd for $C_{19}H_{14}BrCl_2NO_6$ (503.13): C, 45.36; H, 2.80; N, 2.78. Found: C, 45.21; H, 2.99; N, 2.78.

4,7-Dimethoxy-5-{[(4-chlorophenyl)amino]carbonyl}-1-benzofuran-6-yl Ethyl Carbonate (**7e**): Yield 67%, mp 238—240°C. ^{1}H-NMR (DMSO-d_6) δ: 1.23 (3H, t, CH_2CH_3), 3.90 (3H, s, OCH_3), 4.09 (3H, s, OCH_3), 4.22 (2H, q, CH_2CH_3), 7.07 (1H, d, furan H, J=1.8 Hz), 7.34 (2H, d, ArH, J=8.5 Hz), 7.68 (2H, d, ArH, J=8.5 Hz), 8.15 (1H, d, furan H, J=1.8 Hz), 9.86 (1H, s, NH exch. D_2O). IR (KBr) cm^{-1}: 3210 (NH), 1702 (C=O ester), 1607 (C=O amide). MS *m/z*: 419 (M^+). *Anal.* Calcd for $C_{20}H_{18}ClNO_7$ (419.81): C, 57.22; H, 4.32; N, 3.34. Found: C, 57.35; H, 3.94; N, 3.35.

4,7-Dimethoxy-5-{[(2,6-dichlorophenyl)amino]carbonyl}-1-benzofuran-6-yl Ethyl Carbonate (**7f**): Yield 70%, mp 254—256°C. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (3H, t, CH_2CH_3), 3.90 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 4.24 (2H, q, CH_2CH_3), 7.14 (1H, d, furan H, J=2.0 Hz), 7.33—7.90 (3H, m, ArH), 8.16 (1H, d, furan H, J=2.0 Hz), 9.86 (1H, s, NH exch. D_2O). MS *m/z*: 453 (M^+). *Anal.* Calcd for $C_{20}H_{17}Cl_2NO_7$ (454.62): C, 52.83; H, 3.77; N, 3.08. Found: C, 53.10; H, 3.21; N, 3.35.

General Procedure for the Preparation of 8a—f To a solution of the appropriate ethyl ester **7a—f** (10 mmol) in absolute ethanol (25 mL), sodium metal (0.25 g) was added; the mixture was stirred for 30 min till no effervescence observed then refluxed for 24 h. The solution was left aside to cool down poured on ice/water and neutralized by dilute acetic acid. The separated solid was filtered off, dried and crystallized from ethanol, yield 55—65%.

6,9-Dibromo-5-methoxy-3-(4-chlorophenyl)-2*H*-furo[3,2-*g*][1,3]benzoxazine-2,4(3*H*)-dione (**8a**): Yield 60%, mp 269—271°C. ^{1}H-NMR (DMSO-d_6) δ: 4.00 (3H, s, OCH_3), 7.40 (2H, d, ArH, J=8.7 Hz), 7.73 (2H, d, ArH, J=8.7 Hz), 7.89 (1H, s, furan H). IR (KBr) cm^{-1}: 1770 (C=O ester), 1699 (C=O amide). MS *m/z*: 501 (M^++2). *Anal.* Calcd for $C_{17}H_8Br_2ClNO_5$ (501.51): C, 40.71; H, 1.61; N, 2.79. Found: C, 40.52; H, 1.89; N, 2.65.

6,9-Dibromo-5-methoxy-3-(2,6-dichlorophenyl)-2*H*-furo[3,2*g*][1,3]benzoxazine-2,4(3*H*)-dione (**8b**): Yield 65%, mp 284—286°C. ^{1}H-NMR (DMSO-d_6) δ: 4.24 (3H, s, OCH_3), 7.37 (2H, d, ArH), 7.59 (1H, t, ArH), 7.95 (1H, s, furan H). IR (KBr) cm^{-1}: 1773 (C=O ester), 1692 (C=O amide). MS *m/z*: 535 (M^++2). *Anal.* Calcd for $C_{17}H_7Br_2Cl_2NO_5$ (535.96): C, 38.10; H, 1.32; N, 2.61. Found: C, 38.33; H, 1.52; N, 1.96.

9-Bromo-5-methoxy-3-(4-chlorophenyl)-2*H*-furo[3,2-*g*][1,3]-benzoxazine-2,4(3*H*)-dione (**8c**): Yield 63%, mp 263—265°C. ^{1}H-NMR (DMSO-d_6) δ: 3.81 (3H, s, OCH_3), 6.77 (1H, d, furan H, J=2.1 Hz), 7.27 (2H, d, ArH, J=8.7 Hz), 7.49 (1H, d, furan H, J=2.1 Hz), 7.71 (2H, d, ArH, J=8.7 Hz). IR (KBr) cm^{-1}: 1720 (C=O ester), 1672 (C=O amide). *Anal.* Calcd for $C_{17}H_9BrClNO_5$ (422.61): C, 48.31; H, 2.15; N, 3.31. Found: C, 47.33; H, 2.65; N, 3.00.

9-Bromo-5-methoxy-3-(2,6-dichlorophenyl)-2*H*-furo[3,2-*g*][1,3]benzoxazine-2,4(3*H*)-dione (**8d**): Yield 60%, mp 267—269°C. ^{1}H-NMR (DMSO-d_6) δ: 4.31 (3H, s, OCH_3), 7.40 (1H, d, furan H, J=2.4 Hz), 7.44—7.63 (3H, m, ArH), 8.02 (1H, d, furan H, J=2.4 Hz). IR (KBr) cm^{-1}: 1768 (C=O ester), 1692 (C=O amide). *Anal.* Calcd for $C_{17}H_8BrCl_2NO_5$ (457.06): C, 44.76; H, 1.67; N, 3.06. Found: C, 44.79; H, 2.00; N, 2.73.

5,9-Dimethoxy-3-(4-chlorophenyl)-2*H*-furo[3,2-*g*][1,3]benzoxazine-2,4(3*H*)-dione (**8e**): Yield 63%, mp 222—224°C. ^{1}H-NMR (DMSO-d_6) δ: 3.89 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 7.13 (1H, d, furan H, J=2.1 Hz), 7.39 (2H, d, ArH, J=8.4 Hz), 7.76 (2H, d, ArH, J=8.4 Hz), 7.88 (1H, d, furan H, J=2.1 Hz). IR (KBr) cm^{-1}: 1770 (C=O ester), 1693 (C=O amide). MS *m/z*: 373 (M^+). *Anal.* Calcd for $C_{18}H_{12}ClNO_6$ (373.74): C, 57.85; H, 3.24; N, 3.75. Found: C, 57.35; H, 3.84; N, 3.25.

5,9-Dimethoxy-3-(2,6-dichlorophenyl)-2*H*-furo[3,2-*g*][1,3]-benzoxazine-2,4(3*H*)-dione (**8f**): Yield 55%, mp 229—231°C. ^{1}H-NMR (DMSO-d_6) δ: 3.88 (3H, s, OCH_3), 4.17 (3H, s, OCH_3), 7.24 (1H, d, furan H, J=2.4 Hz), 7.41—7.61 (3H, m, ArH), 7.93 (1H, d, furan H, J=2.4 Hz). IR (KBr) cm^{-1}: 1768 (C=O ester), 1700 (C=O amide). MS *m/z*: 407 (M^+). *Anal.* Calcd for $C_{18}H_{11}Cl_2NO_6$ (408.19): C, 52.96; H, 2.72; N, 3.34.

Found: C, 53.16; H, 3.02; N, 3.11.

General Procedure for the Preparation of 11a,b A solution of aniline (0.93 mL, 10 mmol) in conc. HCl (8 mL) and water (8 mL) was stirred in ice bath and $NaNO_2$ (2 g) in water (5 mL) was added in portions to the stirred solution during 30 min till red fumes appear. The previous solution was added in portions to a solution of the appropriate chalchone **10a**, **b** (10 mmol) in ethanolic 10% NaOH (20 mL). The mixture was kept in ice chest for 1 h and neutralized with dilute acetic acid. The deeply colour solid was filtered off, washed with water, dried and crystallized from ethanol, yield 55—60%.

(2*E*)-1-{6-Hydroxy-4-methoxy-7-[(*E*)-phenyldiazenyl]-1-benzofuran-5-yl}-3-phenylprop-2-en-1-one (**11a**): Yield 60%, mp 165—167°C. IR (KBr) cm^{-1}: 3155 (OH), 1630 (C=O), 1575 (N=N). MS *m/z*: 399 (M^++1). *Anal.* Calcd for $C_{24}H_{18}N_2O_4$ (398.41): C, 72.35; H, 4.55; N, 7.03. Found: C, 72.53; H, 4.24; N, 7.42.

(2*E*)-1-{6-Hydroxy-4-methoxy-7-[(*E*)-phenyldiazenyl]-1-benzofuran-5-yl}-3-(4-methoxyphenyl)prop-2-en-1-one (**11b**): Yield 55%, mp 172—174°C. ^{1}H-NMR (DMSO-d_6) δ: 3.89 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.66 (1H, d, furan H, J=2 Hz), 6.79—7.40 (9H, m, ArH), 7.60 (1H, d, furan H, J=2 Hz), 7.80 (1H, d, C<u>H</u>=C<u>H</u>, J=15 Hz), 8.20 (1H, d, CH=C<u>H</u>, J=15 Hz), 10.00 (1H, s, OH exch. D_2O). MS *m/z*: 429 (M^+ +1). *Anal.* Calcd for $C_{25}H_{20}N_2O_5$ (428.44): C, 70.08; H, 4.81; N, 6.54. Found: C, 69.78; H, 4.48; N, 6.73.

General Procedure for the Preparation of 12a,b To a solution of the appropriate azo derivative **11a**, **b** (10 mmol) in 70% ethanol (30 mL) a solution of sodium dithionite (8.4 g, 40 mmol) in water (25 mL) was added in portions while refluxing for 1 h. The deep intense color disappeared and the solution was kept aside to cool, filtered and the filtrate was concentrated to half its volume. The filtrate was poured into 3 times its volume of cold water. The separated solid was filtered off, dried and crystallized from ethanol, yield 35—40%.

(2*E*)-1-(7-Amino-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-phenylprop-2-en-1-one (**12a**): Yield 40%, mp 176—178°C. ^{1}H-NMR (DMSO-d_6) δ: 3.90 (2H, broad, NH_2 exch. D_2O), 4.00 (3H, s, 3H, OCH_3), 6.70—8.20 (m, 9H, ArH, furan H, C<u>H</u>=C<u>H</u>), 13.20 (s, 1H, OH exch. D_2O). IR (KBr) cm^{-1}: 3357—3200 (broad, NH_2, OH), 1600 (C=O) chalcone. MS *m/z*: 311 (M^++2). *Anal.* Calcd for $C_{18}H_{15}NO_4$ (309.32): C, 69.89; H, 4.89; N, 4.53. Found: C, 69.65; H, 4.81; N, 4.62.

(2*E*)-1-(7-Amino-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (**12b**): Yield 35%, mp 189—191°C. ^{1}H-NMR (DMSO-d_6) δ: 3.79 (2H, broad, NH_2 exch. D_2O), 3.91 (3H, s, OCH_3), 4.15 (3H, s, OCH_3), 6.80—8.20 (8H, m, ArH, furan H, C<u>H</u>=C<u>H</u>), 14.00 (1H, s, OH exch. D_2O). IR (KBr) cm^{-1}: 3156, 3129 (NH_2), 3074 (OH), 1691 (C=O) chalcone. MS *m/z*: 339 (M^+). *Anal.* Calcd for $C_{19}H_{17}NO_5$ (339.34): C, 67.25; H, 5.05; N, 4.13. Found: C, 67.45; H, 5.09; N, 4.27.

7-Hydroxy-5-methoxy-2-methyl-4-oxo-4*H*-chromene-6-carbaldehyde (13) To a solution of visnagin **1** (2.3 g, 10 mmol) in 10% w/v hot sulphuric acid (60 mL), 10% w/v potassium dichromate solution (40 mL) was gradually added at a temprature (70—80°C). The mixture was left at room temprature for 30 min. The white ppt was filtered washed with water, dried and crystallized from hot water. Yield 65—70%, mp 189°C (as reported).[18]

General Procedure for the Preparation of 14a—c To a solution of **13** (2.34 g, 10 mmol) in ethanol (20 mL). 5% NaOH (10 mL) and the appropriate acetophenone (10 mmol) were added and stirred for 24 h at room temperature. The red solution was then neutralized by drops of dilute acetic acid and the separated solid was filtered off, washed by ethanol, dried and crystallized from *N*,*N*-dimethylformamide (DMF)/water, yield 78—85%.

6-[(1*E*)-3-Oxo-3-phenylprop-1-en-1-yl]-7-hydroxy-5-methoxy-2-methyl-4*H*-chromen-4-one (**14a**): Yield 78%, mp 260—262°C. ^{1}H-NMR (DMSO-d_6) δ: 2.09 (3H, s, CH_3), 3.79 (3H, s, OCH_3), 6.00 (1H, s, γ-pyrone H), 6.75 (1H, s, ArH), 7.41—7.68 (5H, m, ArH), 7.99 (1H, d, C<u>H</u>=CH, J=16 Hz), 8.14 (1H, d, CH=C<u>H</u>, J=16 Hz), 11.70 (1H, s, OH, exch. D_2O). IR (KBr) cm^{-1}: 3100 (OH), 1655 (C=O) chalcone, 1631 (C=O) chromone. MS *m/z*: 337 (M^++1). *Anal.* Calcd for $C_{20}H_{16}O_5$ (336.34): C, 71.42; H, 4.79. Found: C, 71.64; H, 4.64.

6-[(1*E*)-3-(3-Methoxyphenyl)-3-oxoprop-1-en-1-yl]-7-hydroxy-5-methoxy-2-methyl-4*H*-chromen-4-one (**14b**): Yield 80%, mp 272—274°C. ^{1}H-NMR (DMSO-d_6) δ: 2.24 (3H, s, CH_3), 3.75 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 5.99 (1H, s, γ-pyrone H), 6.72 (1H, s, ArH), 7.22—7.52 (4H, m, ArH), 7.96 (1H, d, <u>CH</u>=CH, J=16 Hz), 8.11 (1H, d, CH=<u>CH</u>, J=16 Hz), 12.00 (1H, s, OH, exch. D_2O). IR (KBr) cm^{-1}: 3076 (OH), 1654 (C=O) chalcone, 1645 (C=O) chromone. MS *m/z*: 366 (M^+). *Anal.* Calcd for $C_{21}H_{18}O_6$ (366.36): C, 68.85; H, 4.95. Found: C, 69.02; H, 4.81.

6-[(1*E*)-3-(4-Bromophenyl)-3-oxoprop-1-en-1-yl]-7-hydroxy-5-methoxy-2-methyl-4*H*-chromen-4-one (**14c**): Yield 85%, mp 279—281°C. ^{1}H-NMR (DMSO-d_6) δ: 2.26 (3H, s, CH_3), 3.91 (3H, s, OCH_3), 5.97 (1H, s, γ-pyrone H), 6.68 (1H, s, ArH), 7.76—7.89 (4H, m, ArH), 8.04 (1H, d, C<u>H</u>=CH, J=16 Hz), 8.21 (1H, d, CH=C<u>H</u>, J=16 Hz), 12.00 (1H, s, OH, exch. D_2O). IR (KBr) cm^{-1}: 3100 (OH), 1653 (C=O) chalcone, 1600 (C=O) chromone. MS *m/z*: 415 (M^+). *Anal.* Calcd for $C_{20}H_{15}BrO_5$ (415.23): C, 57.85; H, 3.64. Found: C, 58.09; H, 3.67.

General Procedure for the Preparation of 15a—c A solution of aniline (0.93 mL, 10 mmol) in conc. HCl (8 mL) and water (8 mL) was stirred in ice bath and $NaNO_2$ (2 g) in water (5 mL) was added to the stirred solution during 30 min till red fumes appear. The previous solution was added to a solution of the appropriate chalchone **14a—c** (10 mmol) in ethanolic 10% NaOH (20 mL). The mixture was kept in ice chest for 1 h and neutralized with acetic acid. The deeply colure solid was filtered off, washed with water, dried and crystallized from ethanol, yield 50—55%.

6-[(1*E*)-3-Phenyl-3-oxoprop-1-en-1-yl]-7-hydroxy-5-methoxy-2-methyl-8-((*E*)-phenyldiazenyl)-4*H*-chromen-4-one (**15a**): Yield 55%, mp 180—182°C. ^{1}H-NMR (DMSO-d_6) δ: 2.24 (3H, s, CH_3), 3.81 (3H, s, OCH_3), 6.04 (1H, s, γ-pyrone H), 6.81—6.94 (4H, m, ArH), 7.35—7.67 (6H, m, ArH),7.95 (d, 1H, C<u>H</u>=CH, J=15 Hz), 8.16 (d, 1H, CH=CH, J=15 Hz), 11.91 (1H, s, OH, exch. D_2O). IR (KBr) cm^{-1}: 3178 (OH), 1705 (C=O) chalcone, 1640 (C=O) chromone. MS *m/z*: 440 (M^+). *Anal.* Calcd for $C_{26}H_{20}N_2O_5$ (440.45): C, 70.90; H, 4.58; N, 6.36. Found: C, 71.20; H, 4.89; N, 6.78.

6-[(1*E*)-3-(3-Methoxyphenyl)-3-oxoprop-1-en-1-yl]-7-hydroxy-5-methoxy-2-methyl-8-((*E*)-phenyldiazenyl)-4*H*-chromen-4-one (**15b**): Yield 50%, mp 188—190°C. ^{1}H-NMR (DMSO-d_6) δ: 2.21 (3H, s, CH_3), 3.81 (3H, s, OCH_3), 4.19 (3H, s, OCH_3), 5.97 (1H, s, γ-pyrone H), 6.42 (1H, s, ArH), 7.22—7.52 (8H, m, ArH), 7.96 (1H, d, CH=CH, J=16 Hz),

8.10 (1H, d, CH=CH, J=16 Hz), 12.00 (1H, s, OH, exch. D_2O). IR (KBr) cm^{-1}: 3100 (OH), 1682 (C=O) chalcone, 1664 (C=O) chromone. *Anal.* Calcd for $C_{27}H_{22}N_2O_6$ (470.74): C, 68.93; H, 4.71; N, 5.95. Found: C, 69.43; H, 4.73; N, 6.33.

6-[(1*E*)-3-(4-Bromophenyl-3-oxoprop-1-en-1-yl]-7-hydroxy-5-methoxy-2-methyl-8-((*E*)-phenyldiazenyl)-4*H*-chromen-4-one (**15c**): Yield 50%, mp 279—281°C. ^{1}H-NMR (DMSO-d_6) δ: 2.24 (3H, s, CH_3), 4.12 (3H, s, OCH_3), 5.90 (1H, s, γ-pyrone H), 7.05—7.50 (5H, m, ArH), 7.75 (2H, d, ArH, J=8 Hz), 7.86 (d, 2H, ArH, J=8 Hz), 8.13 (d, 1H, CH=CH, J=15 Hz), 8.77 (d, 1H, CH=C<u>H</u>, J=15 Hz), 14.20 (1H, s, OH, exch. D_2O). *Anal.* Calcd for $C_{26}H_{19}BrN_2O_5$ (519.34): C, 60.13; H, 3.69; N, 5.39. Found: C, 60.43; H, 3.69; N, 5.59.

General Procedure for the Preparation of 16a—c To a solution of the appropriate azo derivative **15a—c** (10 mmol) in 70% ethanol (50 mL) a solution of sodium dithionite (8.4 g, 40 mmol) in water (25 mL) was added in portions while refluxing for 1 h. The deep intense color of azo dye disappeared and the solution was kept aside to cool, filtered and the filtrate was concentrated to half its volume. The filtrate was poured into 3 times its volume of cold water. The separated solid was filtered off, dried and crystallized from ethanol, yield 45—55%.

(*E*)-8-Amino-6-(phenyl-3-oxoprop-1-enyl)-7-hydroxy-5-methoxy-2-methyl-4*H*-chromen-4-one (**16a**): Yield 50%, mp 172—174°C. ^{1}H-NMR (DMSO-d_6) δ: 2.25 (3H, s, CH_3), 3.56—3.66 (2H, broad, NH_2 exch. D_2O), 3.81 (3H, s, OCH_3), 6.00 (1H, s, γ-pyrone H), 6.66—7.85 (7H, m, ArH and C<u>H</u>=C<u>H</u>), 11.80 (1H, s, OH, exch. D_2O). IR (KBr) cm^{-1}: 3168, 3144 (NH_2), 3081 (OH), 1695 (C=O) chalcone, 1619 (C=O) chromone. MS *m*/*z*: 351 (M^+). *Anal.* Calcd for $C_{20}H_{17}NO_5$ (351.35): C, 68.37; H, 4.88; N, 3.99. Found: C, 68.43; H, 4.68; N, 4.08.

(*E*)-8-Amino-6-(3-methoxyphenyl-3-oxoprop-1-enyl)-7-hydroxy-5-methoxy-2-methyl-4*H*-chromen-4-one (**16b**): Yield 55%, mp 179—181°C. ^{1}H-NMR (DMSO-d_6) δ: 2.24 (3H, s, CH_3), 3.59—3.65 (2H, broad, NH_2 exch. D_2O), 3.76 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 6.00 (1H, s, γ-pyrone H), 6.68—7.91 (6H, m, ArH and C<u>H</u>=C<u>H</u>), 11.70 (1H, s, OH, exch. D_2O). IR (KBr) cm^{-1}: 3156, 3129 (NH_2), 3074 (OH), 1691 (C=O) chalcone, 1676 (C=O) chromone. *Anal.* Calcd for $C_{21}H_{19}NO_6$ (381.83): C, 66.13; H, 5.02; N, 3.67. Found: C, 66.45; H, 4.88; N, 3.26.

(*E*)-8-Amino-6-(4-bromophenyl-3-oxoprop-1-enyl)-7-hydroxy-5-methoxy-2-methyl-4*H*-chromen-4-one (**16c**): Yield 45%, mp 196—198°C. ^{1}H-NMR (DMSO-d_6) δ: 2.29 (3H, s, CH_3), 3.68 (2H, s, NH_2 exch. D_2O), 3.86 (3H, s, OCH_3), 5.92 (1H, s, γ-pyrone H), 6.60—8.07 (6H, m, ArH and C<u>H</u>=C<u>H</u>), 11.70 (1H, s, OH, exch. D_2O). IR (KBr) cm^{-1}: 3156, 3126 (NH_2), 3074 (OH), 1690 (C=O) chalcone, 1625 (C=O) chromone. MS *m*/*z*: 429 (M^+). *Anal.* Calcd for $C_{20}H_{16}BrNO_5$ (430.25): C, 55.83; H, 3.75; N, 3.26. Found: C, 55.35; H, 3.81; N, 3.11.

General Procedure for the Preparation of 17a—c To a solution of the appropriate chalcone **16a—c** (10 mmol) in dry DMF (30 mL), thiourea (0.76 g, 10 mmol) and H_2SO_4 (0.5 mL) were added. The solution was refluxed for 6 h, cooled and poured into ice-water. The separated solid was filtered off, washed with ethanol, dried and crystallized from DMF/water, yield 60—65%.

6-[6-Phenyl-2-thioxo-2,5-dihydropyrimidin-4-yl]-7-hydroxy-5-methoxy-2-methyl-4*H*-chromen-4-one (**17a**): Yield 65%, mp >350°C. ^{1}H-NMR (DMSO-d_6) δ: 2.27 (3H, s, CH_3), 2.88 (2H, d, pyrimid.C^5-H), 3.73 (1H, m, pyrimid.C^6-H), 3.92 (3H, s, OCH_3), 6.03 (1H, s, γ-pyrone H), 7.39—7.92 (7H, m, ArH and NH). IR (KBr) cm^{-1}: 3421 (NH), 3061 (OH), 1654 (C=O) pyrone, 1112 (C=S). MS *m*/*z*: 392 (M^+−2). *Anal.* Calcd for $C_{21}H_{18}N_2O_4S$ (394.44): C, 63.94; H, 3.06; N, 7.10. Found: C, 64.32; H, 3.72; N, 7.45.

6-[6-(3-Methoxyphenyl)-2-thioxo-2,5-dihydropyrimidin-4-yl]-7-hydroxy-5-methoxy-2-methyl-4*H*-chromen-4-one (**17b**): Yield 62%, mp >350°C. IR (KBr) cm^{-1}: 3430 (NH), 3080 (OH), 1650 (C=O) pyrone, 1115 (C=S). MS *m*/*z*: 422 (M^+−2). *Anal.* Calcd for $C_{22}H_{20}N_2O_5S$ (424.47): C, 62.25; H, 4.75; N, 6.60. Found: C, 61.62; H, 4.31; N, 6.23.

6-[6-(4-Bromophenyl)-2-thioxo-2,5-dihydropyrimidin-4-yl]-7-hydroxy-5-methoxy-2-methyl-4*H*-chromen-4-one (**17c**): Yield 60%, mp >350°C. ^{1}H-NMR (DMSO-d_6) δ: 2.27 (3H, s, CH_3), 2.89 (2H, d, pyrimid.C^5-H), 3.77 (1H, m, pyrimid.C^6-H), 3.98 (3H, s, OCH_3), 6.03 (1H, s, γ-pyrone H), 6.49-8.00 (6H, m, ArH, NH). IR (KBr) cm^{-1}: 3446 (NH), 3099 (OH), 1654 (C=O) pyrone and 1114 (C=S). MS *m*/*z*: 475 (M^++2). *Anal.* Calcd for $C_{21}H_{17}BrN_2O_4S$ (473.34): C, 53.29; H, 3.62; N, 5.92. Found: C, 53.34; H, 3.85; N, 6.02.

Pharmacological Studies. Animals Male Wister rats, each weighing 120—180 g, were purchased from the animal breeding unit of the National Ophthalmology Institute, Egypt. They were housed under appropriate conditions of controlled humidity, temperature and light. The animals were allowed free access to water and were fed a standard pellet rat diet. The animals were kept at an ambient temperature of 22±2°C and a humidity of 65—70%. The study was conducted according to the guidelines for animal experiments set by Faculty of Pharmacy, Cairo University in accordance with the international guidelines.

Anti-inflammatory Activity A suspension (1%) of carrageenan was prepared by sprinkling 1 g carrageenan powder in small amounts over the surface of 100 mL saline (NaCl 0.9% w/v) and the particles allowed to soak between additions. The suspension was then left at 37°C for 2—3 h before use. Right paw is marked with ink at the level of lateral malleolus; basal paw volume is measured plethysmographically by volume displacement method using Plethysmometer (UGO Basile 7140) by immersing the paw till the level of lateral malleolus. 0.05 mL of this suspension was injected into the subplantar surface of the right hind paw of the rat. The paw volume is measured again at 1, 2, 3 and 4 h after challenge. The increase in paw volume is calculated as percentage compared with the basal volume. The animals were randomized and divided into groups consists of 6 animals per group. The tested compounds and the reference drug (Diclofenac) were given orally 30 min prior to the intraplatar injection of carrageenan. The difference of average values between treated animals and control group is calculated for each time interval and evaluated statistically. The percent Inhibition is calculated using the formula as follows.

$$\% \text{ edema inhibition} = [1 - (V_t/V_c)] \times 100$$

V_t and V_c are edema volume in the drug treated and control groups respectively.

PGE_2 Inhibition Activity PGE_2 concentrations were measured in serum samples prepared by centrifugation of blood of the injected animals using rat specific immunoassay kit.[26] The assay was performed following the instructions in

the leaflet of the kit. The duplicate readings for each standard, control and sample were averaged and subtracted from the average zero standard optical density. A standard curve was constructed by plotting the mean optical density on a logarithmic *y*-axis against the concentrations (pg/mL) on a logarithmic *x*-axis. The best fitting line was then determined using regression analysis. These standard curves were used to determine the concentration of PGE_2 (pg/mL) in the samples.

Ulcerogenic Effect Male albino rats (100–120 g) were fasted for 12 h prior to the administration of the compounds. The animals were divided into 14 groups, each of 6 animals. The control group received 1% gum acacia orally. Other groups received Diclofenac or the tested compounds orally in 2 equal doses at 0 and 12 h for 3 successive days at a dose of 100 mg/kg per day. Animals were sacrificed by diethyl ether 6 h after the last dose and the stomach was removed. An opening at the greater curvature was made and the stomach was cleaned by washing with cold saline and inspected with a 3 Å magnifying lens for any evidence of hyperaemia, haemorrhage, definite hemorrhagic erosion, or ulcer. An arbitrary scale was used to calculate the ulcer index which indicates the severity of the stomach lesions. Ulcers were classified into levels: level I, in which the ulcer area is less than 1 mm^2; level II, in which the ulcer area is in the range from 1 to 3 mm^2; and level III, in which the ulcer area equals 3 mm^2.[27] The ulcer index was calculated as 1 × (number of ulcers of level I) + 2 × (number of ulcers of level II) + 3 × (number of ulcers of level III).

Statistical Analysis of Data Data obtained from animal experiments were expressed as mean ± standard error (± S.E.M.). Statistical differences between the treatments and the control were tested by one-way analysis of variance (ANOVA) followed by *post hoc* test using SPSS 11.0 software. A value of $p < 0.05$ was considered to be significant.

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