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Original article

New quinazolinone–pyrimidine hybrids: Synthesis, anti-inflammatory, and ulcerogenicity studies

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ABSTRACT

Two groups of hybrid compounds: the quinazolinone–dihydropyrimidines and quinazolinone–pyrimidines, were synthesized. The starting derivative **3** was reacted with chloroacetyl chloride to give intermediate **5** which was condensed with the 2-mercaptopyrimidines **4a–c** affording compounds **6a–c**. These latter compounds underwent hydrolysis and *N*-alkylation reactions to give the dihydropyrimidine derivatives **7a–c** and **8a–f**, respectively. The chloro derivatives **9a–c** subsequently reacted with various anilines furnishing compounds **10a–i**. The anti-inflammatory activity of the synthesized compounds were evaluated using the carrageenan-induced rat paw oedema model and ulcer indices for the most active compounds were calculated. Five compounds were found more active and less ulcerogenic than diclofenac particularly compound **10g** ($IC_{50} = 116.73$ μmol/kg; ulcer index = 11.38). Compound **10g** was also 2-fold more selective inhibitor of COX-2 than COX-1.

1. Introduction

Inflammation is a multifactorial process. It reflects the response of the organism to various stimuli and is related to many disorders such as arthritis, asthma and psoriasis which require prolonged or repeated treatment [1]. It is well known that non steroidal anti-inflammatory drugs (NSAIDs) are associated with several side effects such as gastrointestinal mucosal damage, bleeding, intolerance and renal toxicity [2,3]. The pharmacological effects of NSAIDs are due to inhibition of a membrane enzyme called cyclo-oxygenase (COX) which is involved in the prostaglandin biosynthesis [4]. There are two isoforms, COX-1 and COX-2. The constitutive COX-1 plays a physiological role in the kidneys and the stomach, whereas, the mainly inducible COX-2 is involved in the production of prostaglandins mediating pain and supporting the inflammatory process [5–7]. Gastric injury associated with classical NSAIDs is attributed to the nonselective inhibition of the two isoenzymes [8,9]. In order to prevent or decrease this side effect, a current strategy consists of designing selective COX-2 inhibitors with an improved gastric safety profile which may allow the use of these new agents for long-term prophylactic use in certain chronic diseases [4,10,11]. This has led intense efforts in search for potent and selective COX-2 inhibitors which could provide anti-inflammatory drugs with fewer risks.

The quinazoline nucleus is considered an important chemical synthon of various physiological significance and pharmaceutical utility. Quinazolines have drawn a great attention due to their wide range of therapeutic activities including antiviral [12], antibacterial [13,14], antifungal [15,16], antimalarial [17], anticancer [18–20], antihypertensive [21], diuretic [22,23], inhibition of derived growth factor receptor phosphorylation [24], anticonvulsant [25], antagonism of ghrelin receptor [26], anti-inflammatory, analgesic and COX-2 inhibitory activities [27–29]. The well known anti-inflammatory drug, proquazone **I** [30], and the recently developed derivatives **II** [31] and **III** [32], are good examples of quinazolinone derivatives with potent anti-inflammatory activity (Fig. 1).

In addition, literature survey revealed that many effective anti-inflammatory agents were derived from the pyrimidine, dihydropyrimidine and tetrahydropyrimidine nuclei as shown in compounds **IV** [33], **V** [34] and **VI** [35], respectively (Fig. 2).

In the view of the facts mentioned above, and in an attempt to design and develop new potential anti-inflammatory agents, a hybrid pharmacophoric approach was adopted in which the quinazolinone and substituted pyrimidine/dihydropyrimidine moieties were hybridized in one structure hoping to synergize the

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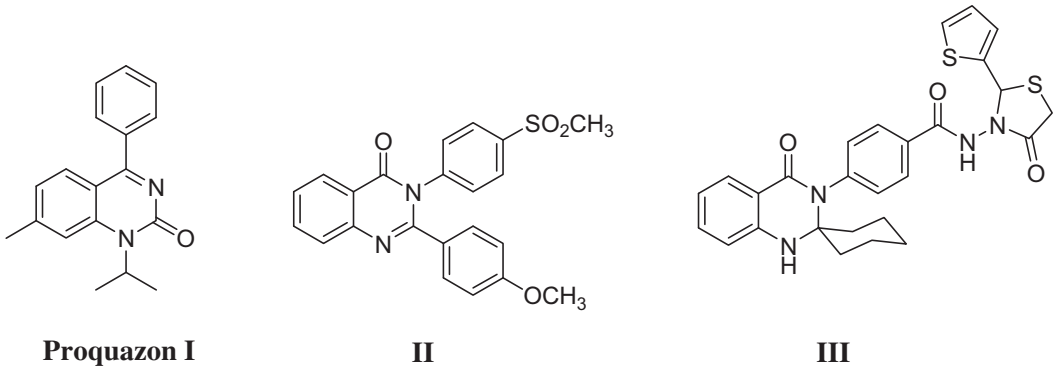


Fig. 1. Examples of some quinazolinone-derived anti-inflammatory agents.

anti-inflammatory potential of both groups. The validity of this design was assessed through preliminary in vivo anti-inflammatory screening and ulcerogenicity study of the target compounds.

2. Results and discussion

2.1. Chemistry

Two groups of target compounds were prepared; the first included the dihydropyrimidinyl-quinazolinone derivatives **6a**–**c**, **7a**–**c** and **8a**–**f** and the second consisted of the pyrimidinyl-quinazolinone derivatives **9a**–**c** and **10a**–**i**. The starting compound 3-amino-2-(2,4-dichlorophenoxy)methyl-3,4-dihydroquinazolin-4-one **3** was prepared from methyl anthranilate **1** which was reacted with 2,4-dichlorophenoxyacetyl chloride to give the intermediate **2** followed by reflux with hydrazine hydrate in n-butanol (Scheme 1) [36].

The first group of compounds was prepared as shown in Scheme 3. Reaction of **3** with chloroacetyl chloride gave the chloroacetyl derivative **5** [37]. The dihydropyrimidinyl compounds **6a**–**c** were obtained through *S*-alkylation of the appropriate 2-mercapto-6-oxo-4-(un)substitutedphenyl-1,6-dihydropyrimidine-5-carbonit riles **4a**–**c** with the chloro intermediate **5** in dry acetone in the presence of potassium carbonate. Compounds **4a**–**c** were prepared as depicted in Scheme 2 following the reported procedure [38]. Primary evidence for the formation of the target compounds **6a**–**c** was drawn from IR spectra that revealed a CN stretching band at about 2200 cm^{-1}. ^1H NMR spectrum **6c** revealed the CH_2S protons as singlet at 4.37 ppm, the CH_2O protons as singlet at 5.09 ppm, in addition to the other aromatic and exchangeable protons appearing as multiplet at 7.29–7.93 ppm. The proton at C-5 of quinazoline ring appeared separately as doublet at 8.15 ppm. It was also noted that the two protons of CH_2O group in compounds **6a** and **6b** were lying in two magnetically unequivalent environments since they appeared as one upfield proton as doublet at 5.01 and 4.78 ppm and one downfield proton as doublet at 5.31 and 4.92 ppm, respectively. The carboxylic acid derivatives **7a**–**c** were obtained upon hydrolysis of their carbonitrile precursors **6a**–**c** using 80% sulphuric acid. IR spectra of compounds **7a**–**c** revealed the disappearance of CN band and alternatively broad carboxylic OH band appeared in the range of 3400–3141 cm^{-1}. *N*-alkylation of **6a**–**c** with the appropriate alkyl halide in absolute ethanol in the presence of potassium carbonate afforded the derivatives **8a**–**f**. The target compounds were essentially characterized by their ^1H NMR spectra that showed the signals assigned to the *N*–CH_3 protons of compounds **8a**, **8c** and **8e** as singlets at 3.24, 3.60 and 3.34, respectively. Likewise, ^1H NMR spectra of compounds **8b**, **8d** and **8f** revealed the benzylic CH_2 protons as singlets at 5.68, 5.08 and 5.28, respectively together with the extra aromatic protons of the benzyl group.

The second group of compounds, the pyrimidinyl-quinazoline derivatives **9a**–**c** and **10a**–**i**, was prepared through chlorination of **6a**–**c** with phosphorous oxychloride to give the chloro intermediates **9a**–**c** which were reacted with the appropriate anilines in absolute ethanol affording the target anilino derivatives **10a**–**i** (Scheme 3). Compounds **10a**–**i** were characterized by their ^1H NMR spectra that declared an increase in the integration of aromatic protons attributed to the anilino aromatic protons together with the exchangeable anilino NHs.

2.2. Anti-inflammatory screening

Evaluation of the anti-inflammatory activity of the synthesized compounds was performed using the carrageenan-induced rat paw oedema model using diclofenac sodium as the reference drug [39–42]. Mean changes in paw oedema thickness of animals

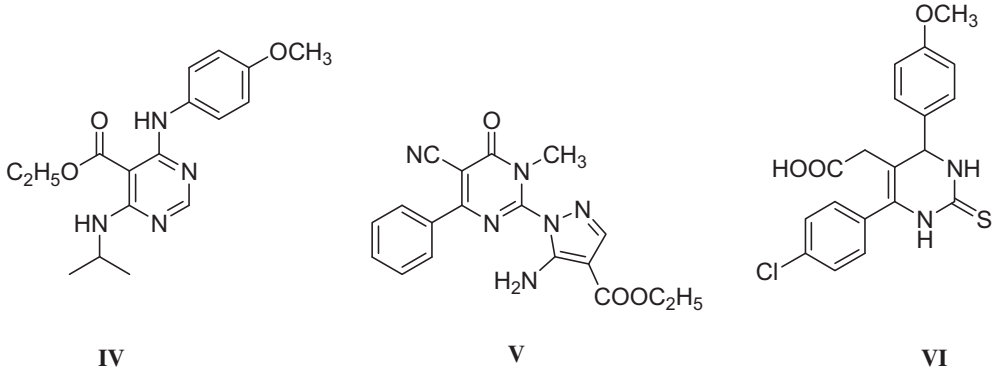
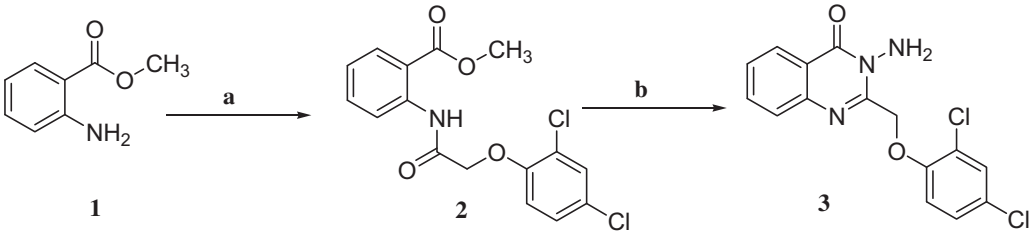


Fig. 2. Examples of some pyrimidine-, dihydropyrimidine- and tetrahydropyrimidine-based anti-inflammatory agents.



Scheme 1. Synthesis of compound **3**. **Reagents and conditions**. **a**: 2,4-dichlorophenoxyacetyl chloride, dry ether, TEA, rt. 24 h; **b**: hydrazine hydrate (85%), n-butanol, reflux 10 h.

pretreated with the tested compounds after 0.5, 1, 2 and 3 h from induction of inflammation was measured, together with the inhibition percent of oedema by the tested compounds (Table 1).

The preliminary anti-inflammatory activity of the first group of compounds comprising the dihydropyrimidinyl-quinazolinone derivatives showed that unsubstituted phenyl group at position 4 of the dihydropyrimidine ring, compound **6a**, was befitting to high activity. This held true in the corresponding carboxyl derivatives **7a**–**c** in which compound **7a** exhibited higher activity than **7b** and **7c**. Regarding the introduction of a substituent on N^1 of the dihydropyrimidine ring, activity of **6a** decreased when either a methyl group, compound **8a**, or a benzyl group, compound **8b**, were introduced. The opposite held true for compound **6b** whose activity increased by the presence of an *N*-substitution as in compounds **8c** and **8d**. However, with compound **6c** there was no consistent relation since the activity increased in the *N*-methyl derivative **8e** and decreased in the *N*-benzyl derivative **8f**.

In the pyrimidinyl-quinazolinone derivatives, the presence of the 4-chloro group on the pyrimidine ring of compounds **9b** and **9c**, unlike **9a**, was associated with higher activity compared to their precursors **6b** and **6c**. As per the 4-(un)substitutedanilino-pyrimidine derivatives **10a**–**i**, the presence of an unsubstituted anilino group, compound **10a**, positively affected the activity compared to its precursor **9a**; while the reverse was true with the substituted anilino moieties. The activity of **9b** was greatly enhanced with the *p*-hydroxyanilino group as shown by derivative **10f**, which exhibited an activity similar to diclofenac. Regarding compound **9c**, introduction of an (un)substituted anilino group at position 4 of the pyrimidine ring contributed to the increase in activity in all derivatives **10g**–**i**. It is noteworthy that compound **10g** was equipotent to diclofenac.

In conclusion, five, out of all synthesized compounds, showed potency above 90% of that of diclofenac: **6a**, **8e**, **10f**, **10g** and **10i**. In particular, the 4-anilinopyrimidine derivatives **10f** and **10g** were equipotent to diclofenac. The highest overall activity was observed in the 4-anilinopyrimidinyl-quinazolinone series **10a**–**i**, especially in all compounds possessing 4-hydroxyphenyl group (electron donating, hydrophilic), compounds **10g**–**i**, or having a 4-chloro anilino group (electron withdrawing, lipophilic) compromised by the 6-(4-hydroxyphenyl) ring (electron donating, hydrophilic), compound **10f**, or unsubstituted on both the 4-anilino and 6-phenyl rings, compound **10a**. The IC_{50} (μmol/kg) of these five compounds were calculated and presented in Table 2. In terms of IC_{50}, all compounds except **6a** were more active than diclofenac sodium. Also, it could be observed that the 4-anilino derivatives (**10f**, **10g** and **10i**) were more active than both **8e** and **6a**. The most active compound was **10f** having an IC_{50} of 114.03 μmol/kg.

2.3. Ulcerogenicity study

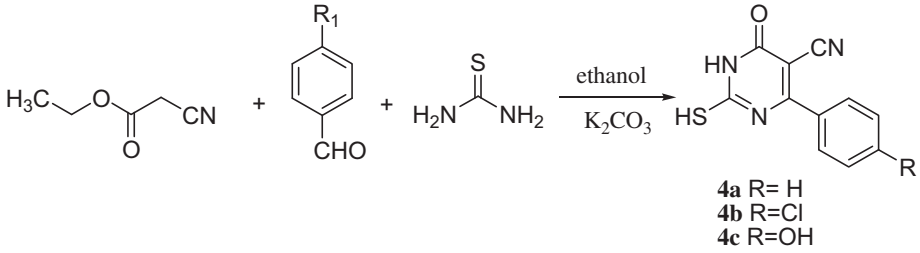
Compounds **6a**, **8b**, **8d**, **8e**, **10a**, **10f**–**i** with best overall profile in animal efficacy model were evaluated for gastric ulcerogenic potential in rats (Table 3) [43,44]. Ulcerogenic effect was compared to a classical NSAID, diclofenac sodium and to a non-ulcerogenic drug, celecoxib. Results revealed that all of the tested compounds were less ulcerogenic than diclofenac sodium and that compounds **8b**, **8d**, **8e**, **10g** and **10h** were less ulcerogenic than celecoxib.

2.4. COX-2/COX-1 selectivity assay

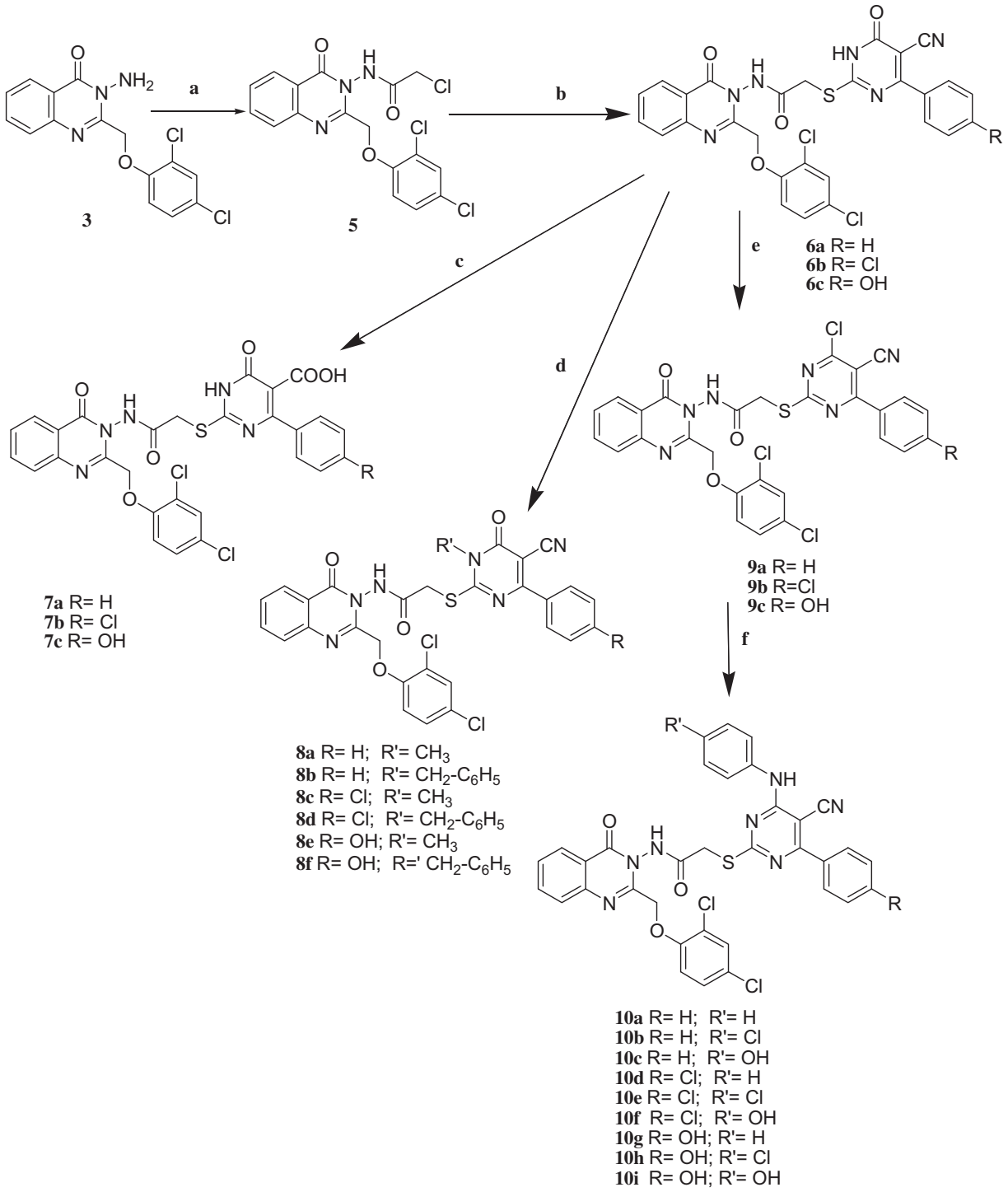
Compounds **6a**, **8e**, **10f**, **10g**, **10i** were evaluated for their selectivity to inhibition of COX-2 and COX-1 isoenzymes using COX (ovine) inhibitor screening assay kit according to the manufacturer's instructions. The ratio of IC_{50} of COX-2 to IC_{50} of COX-1 (COX-2/COX-1) would suggest the selectivity of the compound and hence its gastric liability. The COX-2/COX-1 ratio of the above compounds showed that compounds **8e, 10f** and **10g** are selective COX-2 inhibitor with a ratio of 0.62, 0.40 and 0.44, respectively (Table 4).

3. Conclusion

Two sets of quinazolinone hybrids were prepared: the dihydropyrimidinyl-quinazolinone and the pyrimidinyl-quinazolinone hybrids which were tested for their anti-inflammatory activity and ulcerogenic potential. Preliminary activity revealed that the pyrimidinyl-quinazolinones possessing an anilino group in position 4 of the pyrimidine ring were the most active especially compounds **10f**–**i**. The dihydropyrimidinyl derivatives were generally less active except for compounds **6a** and **8e**. These compounds possessed IC_{50} values and ulcer indices lower than



Scheme 2. Synthesis of compounds **4a**–**c**.



Scheme 3. Synthesis of compounds **6a**–**c**, **7a**–**c**, **8a**–**f**, **9a**–**c** and **10a**–**i**. **Reagents and conditions**. **a**: chloroacetyl chloride, dry benzene, TEA, reflux, 4 h; **b**: compounds **4a**–**c**, dry acetone, anhydrous K_2CO_3, reflux, 6 h; **c**: sulphuric (80%), stir, 24 h; **d**: alkyl halide, absolute ethanol, anhydrous K_2CO_3, reflux, 8 h; **e**: phosphorous oxychloride, stir, rt, 1/2 h, reflux, 6 h; **f**: appropriate aniline, absolute ethanol, stir, rt, 24 h then reflux, 5 h.

diclofenac and compounds **8e** and **10g** were even less ulcerogenic than celecoxib at the same dose level. The most active and safest compound is **10g** (IC_{50} = 116.73 μmol/kg; ulcer index = 11.38). Also, COX-2/COX-1 selectivity assay suggested that compounds **8e**, **10f** and **10g** were 2-fold selective inhibitors to COX-2 than COX-1. According to these results, it could be claimed that our aim was achieved through the development of compounds more active and of less ulcerogenic side effects than standard drugs.

4. Experimental

4.1. Chemistry

Melting points were uncorrected and were carried out by open capillary tube method using IA 9100 MK-Digital Melting Point Apparatus. Elemental microanalyses were carried out on Heraew and Vario El III (elementar), CHNS analyzer (Germany) at the

Table 1
Oedema thickness and inhibition percent in oedema thickness of control, diclofenac sodium and tested compounds.

Compound	Oedema thickness (mm) ± SEM (oedema inhibition %)			
N°	0.5 h	1 h	2 h	3 h
Control	0.520 ± 0.023	0.550 ± 0.022	0.570 ± 0.024	0.570 ± 0.024
Diclofenac sodium	0.120 ± 0.008^{c} (76.92)	0.090 ± 0.004^{c} (83.64)	0.070 ± 0.005^{c} (87.72)	0.050 ± 0.003^{c} (91.23)
6a	0.100 ± 0.006^{c} (80.77)	0.100 ± 0.007^{c} (81.82)	0.080 ± 0.004^{c***} (85.96)	0.080 ± 0.004^{c***} (85.96)
6b	0.200 ± 0.009^{c***} (61.54)	0.200 ± 0.009^{c***} (63.64)	0.200 ± 0.008^{c***} (64.91)	0.200 ± 0.008^{c**} (64.91)*
6c	0.200 ± 0.013^{c***} (61.54)	0.200 ± 0.013^{a***} (63.64)	0.180 ± 0.012^{a} (68.42)	0.180 ± 0.012** (68.42)*
7a	0.200 ± 0.014^{c***} (61.54)	0.170 ± 0.012^{c***} (69.09)	0.180 ± 0.013^{c***} (68.42)	0.200 ± 0.015^{c***} (64.91)
7b	0.250 ± 0.012^{c***} (51.92)	0.230 ± 0.014^{a***} (58.18)	0.220 ± 0.01^{a} (61.40)	0.220 ± 0.01*** (61.40)
7c	0.350 ± 0.018^{c***} (32.69)	0.350 ± 0.018^{a***} (36.36)	0.350 ± 0.017^{a} (38.59)	0.350 ± 0.017*** (38.59)
8a	0.250 ± 0.020^{c***} (51.92)	0.250 ± 0.020^{c***} (54.55)	0.230 ± 0.020^{c***} (59.65	0.230 ± 0.018^{c***} (59.65)
8b	0.125 ± 0.008^{c} (75.96)	0.125 ± 0.008^{c**} (77.27)	0.120 ± 0.009^{a***} (78.95	0.120 ± 0.009*** (78.95)
8c	0.100 ± 0.007^{c} (80.77)	0.130 ± 0.009^{a**} (76.36)	0.150 ± 0.009^{a***} (73.68)	0.150 ± 0.01*** (73.68)
8d	0.200 ± 0.01^{c***} (61.54)	0.150 ± 0.006^{c***} (72.73)	0.130 ± 0.008^{a***} (77.19)	0.130 ± 0.008^{a} (77.19)
8e	0.120 ± 0.01^{c} (76.92)	0.110 ± 0.010 (80.00)	0.080 ± 0.006^{c} (85.96)	0.080 ± 0.006** (85.96)
8f	0.250 ± 0.012^{c***} (51.92)	0.250 ± 0.012^{a***} (54.55)	0.250 ± 0.012^{a***} (56.14)	0.220 ± 0.013*** (61.40)
9a	0.240 ± 0.016^{c***} (53.85)	0.220 ± 0.014^{c***} (60.00)	0.190 ± 0.012^{c***} (66.67)	0.180 ± 0.012^{c***} (68.41)
9b	0.200 ± 0.013^{c***} (61.54)	0.200 ± 0.013^{a***} (63.64)	0.180 ± 0.008^{a} (68.42)	0.180 ± 0.007*** (68.42)
9c	0.170 ± 0.011^{c**} (67.31)	0.150 ± 0.08^{c***} (72.73)	0.150 ± 0.07^{c***} (73.68	0.150 ± 0.07^{c***} (73.68)
10a	0.150 ± 0.009^{c*} (71.15)	0.125 ± 0.007^{a**} (77.27)	0.125 ± 0.007^{a***} (78.07)	0.125 ± 0.007*** (78.07)
10b	0.250 ± 0.013^{c***} (51.92)	0.250 ± 0.013^{a***} (54.55)	0.240 ± 0.015^{a} (57.89)	0.230 ± 0.016*** (59.65)
10c	0.200 ± 0.012^{c***} (61.54)	0.220 ± 0.013^{a***} (72.73)	0.230 ± 0.016^{a} (59.65)	0.230 ± 0.015*** (59.65)
10d	0.330 ± 0.023^{c***} (36.54)	0.310 ± 0.024^{c***} (43.64)	0.280 ± 0.022^{c***} (50.88)	0.250 ± 0.02^{c***} (56.14)
10e	0.280 ± 0.023^{c***} (46.15)	0.250 ± 0.022^{c***} (54.55)	0.220 ± 0.021^{c***} (61.40)	0.200 ± 0.016^{c***} (64.91)
10f	0.150 ± 0.008^{c} (71.15)	0.050 ± 0.003^{a***} (90.91)	0.050 ± 0.003^{a} (91.23)	0.050 ± 0.003^{c} (91.23)
10g	0.050 ± 0.003^{c***} (90.38)	0.050 ± 0.003^{c***} (90.91)	0.050 ± 0.003^{a**} (91.23)	0.050 ± 0.003^{c} (91.23)
10h	0.180 ± 0.02^{c*} (65.38)	0.150 ± 0.016^{c**} (72.73)	0.130 ± 0.012^{c**} (77.19)	0.120 ± 0.01^{c***} (78.95)
10i	0.100 ± 0.007^{c} (80.77)	0.075 ± 0.005^{c*} (86.36)	0.075 ± 0.004^{c} (86.84)	0.075 ± 0.004^{a***} (86.84)

Values with superscript letter a significantly different with control value at $P \leq 0.05$, c are significantly different with control value at $P \leq 0.001$, values with stars are significantly different with diclofenac-sodium values * at $P \leq 0.05$, ** at $P \leq 0.01$ and *** at $P \leq 0.001$. Results are mean ± S.E. of five animals.

Microanalytical Center, Faculty of Science, Cairo University. Infrared spectra were made on Bruker Vector 22 (Japan), infrared spectrophotometers and were expressed in wave number (cm^{-1}) using potassium bromide disc. ^{1}H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 300 MHz and ^{13}C NMR were recorded at 75 MHz in the specified solvent. Chemical shifts were reported on the d scale and were related to that of the solvent and *J* values are given in Hz. Mass spectra were recorded on Shimadzu Qp-2010 plus, at 70 eV (EI). IUPAC chemical nomenclature were assigned using CS Chemdraw ultra version 5.0. Thin layer chromatography was performed using MachereyeNagel Alugram Sil G/UV254 silica gel plates and petroleum ether: ethyl acetate (9:1) as the eluting system.

Table 2
IC_{50} values of the anti-inflammatory activity for compounds **6a**, **8e**, **10f**, **10g**, **10i** and diclofenac sodium.

Compound N°	Dose(mg/kg)	Oedema inhibition % after 3 h	IC_{50} mg/kg (IC_{50} μmol/kg)
Diclofenac-sodium	20	42.25	45.01 (141.03)
	50	87.26	
	100	91.23	
6a	50	68.42	86.74 (143.26)
	100	85.96	
	200	91.23	
8e	50	70.42	82.89 (130.44)
	100	85.96	
	200	97.75	
10f	50	61.40	83.36 (114.03)
	100	91.23	
	200	96.47	
10g	50	77.19	81.31 (116.73)
	100	91.23	
	200	96.49	
10i	50	56.14	88.27 (123.88)
	100	86.84	
	200	91.23	

*4.1.1. General procedure for the preparation of compounds (**6a**–**c**)*

A mixture of the chloro derivative **5** (4.1 g, 10 mmol), the appropriate 2-mercapto-dihydropyrimidine derivative **4a**–**c** (10 mmol) and anhydrous potassium carbonate (1.37 g, 10 mmol) was refluxed in dry acetone (20 ml) for 6 h. The reaction mixture was filtered while hot and the residue was washed with hot acetone. The organic solution was concentrated and the separated crude product was filtered off and recrystallized from chloroform–ethanol.

*4.1.1.1. 2-(5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-ylsulfanyl)-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**6a**).* Yellow powder; m.p. 170–172 °C, yield, 60%. IR ν_{max}/

Table 3
Ulcer index of tested compounds of the most active compounds, diclofenac sodium and celecoxib.

Compound N°	No. of animals with ulcer	% Incidence /10	Average no of ulcer	Average severity	Ulcer index
Control	0/5	0	0	0	0
Diclofenac-sodium	5/5	10	5.60	1.42	17.02
Celecoxib	4/5	8	3.20	1.31	12.51
6a	4/5	8	5.20	1.20	14.40
8b	4/5	8	2.20	0.70	10.90
8d	4/5	8	2.80	0.50	11.30
8e	4/5	8	2.60	0.62	11.22
10a	4/5	8	4.20	1.06	13.26
10f	5/5	10	3.60	0.83	14.43
10g	4/5	8	3.00	0.38	11.38
10h	4/5	8	3.40	0.68	12.08
10i	5/5	10	3.40	0.82	14.22

Table 4
COX-2/COX-1 ratio of compounds **6a**, **8e**, **10f**, **10g**, **10i** and celecoxib.

Compound	COX-2/COX-1
6a	20.60
8e	0.62
10f	0.40
10g	0.44
10i	7.30
Celecoxib	0.02

cm^{-1}: 3308 (NHs); 3198 (CH aromatic); 2923 (CH aliphatic); 2216 (C≡N); 1681 (COs); 1608, 1477 (C=N, C=C); 769 (C–Cl). ^{1}H NMR (DMSO-d_6): δ 3.69 (s, 2H, CH_2S); 5.01(d, 1H, upfield proton of CH_2O, $J = 16.2$ Hz); 5.31(d, 1H, downfield proton of CH_2O, $J = 16.2$ Hz); 7.05–7.76 (m, 13H, 11 aromatic H and 2 NHs exchangeable); 8.06 (d, 1H, C5-H of quinazoline, $J = 7.8$ Hz). MS, *m/z* (%): 606 (M + 1, 3.03); 161 (100). Anal. Calcd. for $C_{28}H_{18}Cl_2N_6O_4S$ (605.45): C, 55.55; H, 3.00; N, 13.88. Found: C, 55.50; H, 3.39; N, 14.01.

*4.1.1.2. 2-[4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**6b**).* Yellow powder; m.p. 190–192 °C, yield, 70%. IR ν_{max}/cm^{-1}: 3414 (NHs); 3076 (CH aromatic); 2927 (CH aliphatic); 2215 (C≡N); 1689 (COs); 1611, 1551 (C=N, C=C); 772 (C–Cl). ^{1}H NMR (DMSO-d_6): δ 4.25 (s, 2H, CH_2S); 4.78(d, 1H upfield proton of CH_2O, $J = 15.9$ Hz); 4.92(d, 1H, downfield proton of CH_2O, $J = 15.6$ Hz); 6.84–7.95 (m, 10H, aromatic H); 8.13 (d, 1H, C5-H of quinazoline, $J = 7.95$ Hz); 11.40 (s, 2H, 2NHs exchangeable). ^{13}C NMR (DMSO-d_6): δ 32.5 (CH_2S); 65.8 (CH_2O); 115.0–130.2 (aromatic C); 135.2 (C-1 chlorophenyl); 145.8 (C-1 dichlorophenyl); 152.2 (C-2 pyrimidine); 158.4 (C=O quinazoline); 164.0 (C-2 quinazoline); 165.6 (C=O pyrimidine); 168.1 (C=O carboxamide). Anal. Calcd. for $C_{28}H_{17}Cl_3N_6O_4S$ (639.90): C, 52.56; H, 2.68; N, 13.13. Found: C, 52.60; H, 2.71; N, 13.16.

*4.1.1.3. 2-[5-Cyano-4-(4-hydroxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**6c**).* Off-White crystals; m.p. 200–202 °C, yield, 55%. IR ν_{max}/cm^{-1}: 3404 (br., OH, NHs); 3108 (CH aromatic); 2918 (CH aliphatic); 2197 (C≡N); 1607 (COs); 1555 (C=N, C=C); 771 (C–Cl). ^{1}H NMR (DMSO-d_6): δ 4.37 (s, 2H, CH_2S); 5.09 (s, 2H, CH_2O); 7.29–7.93 (m, 13H, 10 aromatic H, 2 NHs and OH exchangeable); 8.15 (d, 1H, C5-H of quinazoline, $J = 7.8$ Hz). Anal. Calcd. for $C_{28}H_{18}Cl_2N_6O_5S$ (621.45): C, 54.12; H, 2.92; N, 13.52. Found: C, 53.82; H, 3.05; N, 13.20.

*4.1.2. General procedure for the preparation of compounds (**7a**–**c**)*

A mixture of the cyano derivatives **6a**–**c** (6.02 mmol) in 80% sulphuric (5 ml) was stirred at room temperature for 24 h. The reaction mixture was diluted with cold water, neutralized with diluted ammonia solution and filtered. The separated solid was dried and crystallized from ethanol.

*4.1.2.1. 2-{[2-(2,4-Dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-ylcarbamoyl]-methylsulfanyl}-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carboxylic acid (**7a**).* Orange powder; m.p. 250–252 °C, yield, 55%. IR ν_{max}/cm^{-1}: 3374 (br., OH, NHs); 3041 (CH aromatic); 2923 (CH aliphatic); 1662 (COs); 770 (C–Cl).^{1}H NMR (DMSO-d_6): δ 5.10 (s, 2H, CH_2S); 5.80 (s, 2H, CH_2O); 7.30–7.76 (m, 11H, aromatic H); 8.15 (d, 1H, C5-H of quinazoline, $J = 7.8$ Hz); 10.05 (s, 3H, 2 NHs and COOH exchangeable). MS, *m/z* (%): 625 (M + 1, 3.53); 627 ((M + 3), 1.71); 77 (100). Anal. Calcd. for $C_{28}H_{19}Cl_2N_5O_6S$ (624.45): C, 53.86; H, 3.07; N, 11.22. Found: C, 54.04; H, 3.37; N, 10.88.

*4.1.2.2. 4-(4-Chlorophenyl)-2-{[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-ylcarbamoyl]-methylsulfanyl}-6-oxo-1,6-dihydropyrimidine-5-carboxylic acid (**7b**).* Buff powder; m.p. 265–267 °C, yield, 60%. IR ν_{max}/cm^{-1}: 3400 (br., OH, NHs); 3095 (CH aromatic); 2922 (CH aliphatic); 1607 (COs); 772 (C–Cl). ^{1}H NMR (DMSO-d_6): δ 4.28 (s, 2H, CH_2S); 4.72 (s, 2H, CH_2O); 6.74–7.80 (m, 10H, aromatic H); 8.13 (d, 1H, C5-H of quinazoline, $J = 7.9$ Hz); 11.40 (s, 2H, 2 NHs exchangeable with D_2O); 11.45 (s, 1H, COOH, exchangeable). Anal. Calcd. for $C_{28}H_{18}Cl_3N_5O_6S$ (658.90): C, 51.04; H, 2.75; N, 10.63. Found: C, 51.26; H, 2.90; N, 10.01.

*4.1.2.3. 2-{[2-(2,4-Dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-ylcarbamoyl]-methylsulfanyl}-4-(4-hydroxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carboxylic acid (**7c**).* Buff powder; m.p. 245–247 °C, yield, 62%. IR ν_{max}/cm^{-1}: 3141 (br., OHs, NHs); 3085 (CH aromatic); 2929 (CH aliphatic); 1613 (COs); 769 (C–Cl).^{1}H NMR (DMSO-d_6): δ 4.14 (s, 2H, CH_2S); 5.14 (s, 2H, CH_2O); 6.82–7.82 (m, 14H, 10 aromatic H, 2 NHs and 2 OHs exchangeable); 8.26 (d, 1H, C5-H of quinazoline, $J = 7.8$ Hz). Anal. Calcd. for $C_{28}H_{19}Cl_2N_5O_7S$ (640.45): C, 52.51; H, 2.99; N, 10.94. Found: C, 52.59; H, 3.29; N, 11.28.

*4.1.3. General procedure for the preparation of compounds (**8a**–**f**)*

Methyl iodide/benzyl chloride (10 mmol) was added to a mixture of the appropriate compound **6a**–**c** (10 mmol) and anhydrous potassium carbonate (2.1 g, 15 mmol) in absolute ethanol (20 ml). The reaction mixture was refluxed for 8 h. The solvent was evaporated under reduced pressure and the residue was treated with water, filtered and the crude product was crystallized from chloroform–ethanol.

*4.1.3.1. 2-(5-Cyano-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-ylsulfanyl)-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**8a**).* Brown powder; m.p. 260–262 °C, yield, 70%. IR ν_{max}/cm^{-1}: 3368 (NH); 3194 (CH aromatic); 2918 (CH aliphatic); 2207 (C≡N); 1633 (COs); 768 (C–Cl). ^{1}H NMR (DMSO-d_6): δ 3.24 (s, 3H, CH_3); 4.63 (s, 2H, CH_2S); 5.37 (s, 2H, CH_2O); 7.10–7.94 (m, 11H, aromatic H); 8.30 (d, 1H, C5-H of quinazoline, $J = 8.1$ Hz); 11.2 (s, 1H, NH exchangeable). Anal. Calcd. for $C_{29}H_{20}Cl_2N_6O_4S$ (619.48): C, 56.23; H, 3.25; N, 13.57. Found: C, 56.32; H, 3.70; N, 14.01.

*4.1.3.2. 2-(1-Benzyl-5-cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-ylsulfanyl)-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**8b**).* Light-brown powder; m.p. 190–192 °C, yield, 69%. IR ν_{max}/cm^{-1}: 3419 (NH); 3040 (CH aromatic); 2916 (CH aliphatic); 2213 (C≡N); 1669, 1605 (COs); 762 (C–Cl). ^{1}H NMR (DMSO-d_6): δ 4.76 (s, 2H, CH_2S); 5.50 (s, 2H, CH_2O); 5.68 (s, 2H, CH_2-C_6H_5); 7.00–7.83 (m, 17H, 16 aromatic H and NH exchangeable); 8.15 (d, 1H, C5-H of quinazoline, $J = 8.1$ Hz). Anal. Calcd. for $C_{35}H_{24}Cl_2N_6O_4S$ (695.58): C, 60.44; H, 3.48; N, 12.08. Found: C, 60.98; H, 3.91; N, 12.48.

*4.1.3.3. 2-[4-(4-Chlorophenyl)-5-cyano-1-methyl-6-oxo-1,6-dihydro pyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**8c**).* Brown powder; m.p. 200–202 °C, yield, 73%. IR ν_{max}/cm^{-1}: 3435 (NH); 3091 (CH aromatic); 2924 (CH aliphatic); 2219 (C≡N); 1687 (COs); 777 (C–Cl). ^{1}H NMR (DMSO-d_6): δ 3.60 (s, 3H, CH_3); 5.05 (s, 2H, CH_2S); 5.42 (s, 2H, CH_2O); 7.19–7.93 (m, 10H, aromatic H); 8.13 (d, 1H, C5-H of quinazoline, $J = 6.9$ Hz); 11.44 (s, 1H, NH exchangeable). Anal. Calcd. for $C_{29}H_{19}Cl_3N_6O_4S$ (653.92): C, 53.26; H, 2.93; N, 12.85. Found: C, 53.23; H, 3.22; N, 12.55.

*4.1.3.4. 2-[1-Benzyl-4-(4-chlorophenyl)-5-cyano-6-oxo-1,6-dihydro-pyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**8d**).* Orange powder; m.p. 220–222 °C, yield, 70%. IR ν_{max}/cm^{-1}: 3436 (NH); 3105 (CH aromatic); 2924 (CH

aliphatic); 2209 (C≡N); 1686 (COs); 1548, 1467 (C=N, C=C); 774 (C–Cl). ^{1}H NMR (DMSO-d_6): δ 3.91 (d, 1H, upfield proton of CH_2S, $J = 15$ Hz); 4.04 (d, 1H, downfield proton of CH_2S, $J = 15.9$ Hz); 4.94 (d, 1H, upfield proton of CH_2O, $J = 15.6$ Hz); 5.08 (s, 2H, CH_2-C_6H_5); 5.14 (d, 1H, downfield proton of CH_2O, $J = 15.6$ Hz); 6.94–7.86 (m, 15H, aromatic H); 8.12 (d, 1H, C5-H of quinazoline, $J = 7.8$ Hz); 11.38 (s, 1H, NH exchangeable). ^{13}C NMR (DMSO-d_6): δ 32.5 (CH_2S); 41.2 (CH_2 benzyl); 65.9 (CH_2O); 115.2–129.8 (aromatic C); 135.8 (C-1 chlorophenyl); 145.9 (C-1 dichlorophenyl); 152.3 (C-2 pyrimidine); 158.4 (C=O quinazoline); 164.1 (C-2 quinazoline); 165.6 (C=O pyrimidine); 169.0 (C=O carboxamide). Anal. Calcd. for $C_{35}H_{23}Cl_3N_6O_4S$ (730.02): C, 57.58; H, 3.18; N, 11.51. Found: C, 57.25; H, 3.33; N, 11.28.

*4.1.3.5. 2-[5-Cyano-4-(4-hydroxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**8e**).* Brown powder; m.p. 235–237 °C, yield, 65%. IR ν_{max}/cm^{-1}: 3282 (br., NH, OH); 3085 (CH aromatic); 2927 (CH aliphatic); 2230 (C≡N); 1702 (COs); 1589, 1512 (C=N); 1473 (C=C); 770 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 3.34 (s, 3H, CH_3); 4.29 (s, 2H, CH_2S); 5.09 (s, 2H, CH_2O); 6.92–8.23 (m, 12H, 10 aromatic H, NH and OH exchangeable); 8.10 (d, 1H, C5-H of quinazoline, $J = 8.0$ Hz). Anal. Calcd. for $C_{29}H_{20}Cl_2N_6O_5S$ (635.48): C, 54.81; H, 3.17; N, 13.22. Found: C, 55.07; H, 3.03; N, 12.85.

*4.1.3.6. 2-[1-Benzyl-5-cyano-4-(4-hydroxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**8f**).* Brown powder; m.p. 240–242 °C, yield, 60%. IR ν_{max}/cm^{-1}: 3377 (OH); 3280 (NH); 3051 (CH aromatic); 2926 (CH aliphatic); 2214 (C≡N); 1683, 1603 (COs); 1506 (C=N); 1476 (C=C); 770 (C–Cl). ^{1}H NMR (DMSO-d_6): δ 4.24 (s, 2H, CH_2S); 5.07 (s, 2H, CH_2O, $J = 3.9$ Hz); 5.28 (s, 2H, CH_2-C_6H_5); 6.57–7.82 (m, 17H, 15 aromatic H, NH and OH exchangeable); 8.20 (d, 1H, C5-H of quinazoline, $J = 8.2$ Hz). ^{13}C NMR (DMSO-d_6): δ 32.6 (CH_2S); 40.0 (CH_2 benzyl); 65.8 (CH_2O); 115.8–131.3 (aromatic C); 135.1 (C-1 chlorophenyl); 144.8 (C-1 dichlorophenyl); 153.8 (C-2 pyrimidine); 155.7 (C-4 hydroxyphenyl); 158.3 (C=O quinazoline); 163.8 (C-2 quinazoline); 165.5 (C=O pyrimidine); 168.1 (C=O carboxamide). Anal. Calcd. for $C_{35}H_{24}Cl_2N_6O_5S$ (635.48): C, 59.08; H, 3.40; N, 11.81. Found: C, 59.25; H, 3.70; N, 12.03.

*4.1.4. General procedure for the preparation of compounds (**9a**–**c**)*

Compound **6a**–**c** (11.1 mmol) was added portion wise with stirring to ice-cooled phosphorous oxychloride (10 ml). The reaction mixture was stirred at room temperature for 30 min. The mixture was then heated to reflux for 6 h. The cooled reaction mixture was poured on crushed ice and the separated solid was filtered off, washed with water, dried and crystallized from aqueous ethanol.

*4.1.4.1. 2-(4-Chloro-5-cyano-6-phenylpyrimidin-2-ylsulfanyl)-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**9a**).* Yellow crystals; m.p. 230–232 °C, yield, 57%. IR ν_{max}/cm^{-1}: 3240 (NH); 3067 (CH aromatic); 2916 (CH aliphatic); 2230 (C≡N); 1697 (COs); 760 (C–Cl). ^{1}H NMR (*C*DCl$_3$): δ 4.12 (s, 2H, CH_2S); 4.73 (s, 2H, CH_2O); 6.92–8.13 (m, 11H, aromatic H); 8.81 (d, 1H, C5-H of quinazoline, $J = 8.4$ Hz); 11.62 (s, 1H, NH exchangeable). MS, *m*/*z* (%): 624((M)$^+$, 2.27); 625 ((M + 1), 2.07); 162(100). Anal. Calcd. for $C_{28}H_{17}Cl_3N_6O_3S$ (623.90): C, 53.90; H, 2.75; N, 13.47. Found: C, 53.63; H, 2.32; N, 13.95.

*4.1.4.2. 2-(4-Chloro-6-(4-chlorophenyl)-5-cyanopyrimidin-2-ylsulfanyl)-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**9b**).* Yellow crystals; m.p. 240–242 °C, yield, 60%. IR ν_{max}/cm^{-1}: 3309 (NH); 3077 (CH aromatic); 2921 (CH aliphatic); 2200 (C≡N); 1678 (COs); 771 (C–Cl). ^{1}H NMR (*C*DCl$_3$): δ 4.11 (s, 2H, CH_2S); 4.85 (d, 1H, upfield proton of CH_2O, $J = 12$ Hz); 4.95 (d, 1H, downfield proton of CH_2O, $J = 11.4$ Hz); 6.90–8.08 (m, 10H, aromatic H); 8.23 (d, 1H, C5-H of quinazoline, $J = 8.1$ Hz); 9.09 (s, 1H, NH exchangeable). Anal. Calcd. for $C_{28}H_{16}Cl_4N_6O_3S$ (658.34): C, 51.08; H, 2.45; N, 12.77. Found: C, 51.50; H, 2.11; N, 12.31.

*4.1.4.3. 2-(4-Chloro-5-cyano-6-(4-hydroxyphenyl)pyrimidin-2-ylsulfanyl)-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**9c**).* Buff powder; m.p. 225–227 °C, yield, 55%. IR ν_{max}/cm^{-1}: 3369 (br., OH, NH); 3084 (CH aromatic); 2922 (CH aliphatic); 2208 (C≡N); 1633 (COs); 769 (C–Cl). ^{1}H NMR (*C*DCl$_3$): δ 4.72 (d, 1H, upfield proton of CH_2S, $J = 16.2$ Hz); 4.92 (d, 1H, downfield proton of CH_2S, $J = 16.5$ Hz); 5.34 (s, 2H, CH_2O); 7.16–7.87 (m, 10H, aromatic H); 8.23 (d, 1H, C5-H of quinazoline, $J = 7.8$ Hz); 9.09 (s, 1H, NH exchangeable with D_2O); 11.64 (s, 1H, OH exchangeable). Anal. Calcd. for $C_{28}H_{17}Cl_3N_6O_4S$ (639.90): C, 52.56; H, 2.68; N, 13.13. Found: C, 52.67; H, 2.88; N, 12.85.

*4.1.5. General procedure for the preparation of compounds (**10a**–**i**)*

To a stirred solution of the appropriate aniline (10 mmol) and triethylamine (0.5 ml) in absolute ethanol (10 ml), a solution of compound **9a**–**c** (10 mmol) in absolute ethanol (10 ml) was added portion wise. The reaction mixture was stirred for 24 h at room temperature, and then refluxed for 5 h. The solvent was removed by distillation under vacuum, the residue was triturated with cold water and the solid was filtered off and crystallized from methanol.

*4.1.5.1. 2-(5-Cyano-6-phenyl-4-phenylaminopyrimidin-2-ylsulfanyl)-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**10a**).* Brown powder; m.p. 225–227 °C, yield, 60%. IR ν_{max}/cm^{-1}: 3393 (NHs); 3058 (CH aromatic); 2925 (CH aliphatic); 2210 (C≡N); 1697 (COs); 763 (C–Cl). ^{1}H NMR (*C*DCl$_3$): δ 3.03 (s, 2H, CH_2S), 4.68 (s, 2H, CH_2O), 6.91–7.98 (m, 18H, 16 aromatic H and 2 NHs exchangeable); 8.79 (d, 1H, C5-H of quinazoline, $J = 7.9$ Hz). ^{13}C NMR (DMSO-d_6): δ 32.1 (CH_2S); 65.9 (CH_2O); 115.3–130.7 (aromatic C); 142.2 (C-1 anilino); 149.5 (C-1 dichlorophenyl); 160.3 (C=O quinazoline); 163.7 (C-2 quinazoline); 170.3 (C=O carboxamide); 172.5 (C-4 pyrimidine); 174.1 (C-2 pyrimidine). MS, *m*/*z* (%): 681 (M$^+$, 0.57); 63 (100). Anal. Calcd. for $C_{34}H_{23}Cl_2N_7O_3S$ (680.56): C, 60.00; H, 3.41; N, 14.41. Found: C, 60.29; H, 3.89; N, 14.11.

*4.1.5.2. 2-[4-(4-Chlorophenylamino)-5-cyano-6-phenylpyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**10b**).* Buff powder; m.p. 230–232 °C, yield, 65%. IR ν_{max}/cm^{-1}: 3398 (NHs); 3063 (CH aromatic); 2924 (CH aliphatic); 2213 (C≡N); 1697 (COs); 764 (C–Cl). ^{1}H NMR (*C*DCl$_3$): δ 3.72 (s, 2H, CH_2S), 4.98 (s, 2H, CH_2O), 6.86–7.80 (m, 15H, aromatic H); 8.01 (d, 1H, C5-H of quinazoline, $J = 7.8$ Hz); 11.90 (s, 2H, NHs exchangeable). Anal. Calcd. for $C_{34}H_{22}Cl_3N_7O_3S$ (715.01): C, 57.11; H, 3.10; N, 13.71. Found: C, 56.89; H, 3.45; N, 14.04.

*4.1.5.3. 2-[5-Cyano-4-(4-hydroxyphenylamino)-6-phenylpyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**10c**).* Buff crystals; m.p. 235–237 °C, yield, 55%. IR ν_{max}/cm^{-1}: 3385 (br., OH, NHs); 3065 (CH aromatic); 2929 (CH aliphatic); 2211 (C≡N); 1688 (COs); 764 (C–Cl). ^{1}H NMR (DMSO-d_6): δ 4.20 (s, 2H, CH_2S); 5.58 (s, 2H, CH_2O); 6.42–7.85 (m, 15H, aromatic H); 8.16 (d, 1H, C5-H of quinazoline, $J = 8.1$ Hz); 9.62 (s, 3H, 2 NHs and OH exchangeable). Anal. Calcd. for $C_{34}H_{23}Cl_2N_7O_4S$ (696.56): C, 58.63; H, 3.33; N, 14.08. Found: C, 58.24; H, 3.65; N, 14.28.

*4.1.5.4. 2-[6-(4-Chlorophenyl)-5-cyano-4-phenylaminopyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**10d**).* Brown powder; m.p. 235–237 °C, yield, 65%. IR ν_{max}/cm^{-1}: 3394, 3298 (NHs); 3054 (CH aromatic); 2924 (CH aliphatic); 2212 (C≡N); 1695 (COs); 756 (C–Cl). ^{1}H NMR (DMSO-

d_6): δ 4.19 (s, 2H, CH_2S); 4.68 (d, 1H upfield proton of CH_2O, $J = 16.5$ Hz); 4.81 (d, 1H, downfield proton of CH_2O, $J = 16.4$ Hz); 6.81–8.13 (m, 15H, aromatic H); 8.57 (d, 1H, C5-H of quinazoline, $J = 7.95$ Hz); 11.20 (s, 1H, NH, exchangeable with D_2O); 11.30 (s, 1H, NH, exchangeable). Anal. Calcd. for $C_{34}H_{22}Cl_3N_7O_3S$ (715.01): C, 57.11; H, 3.10; N, 13.71. Found: C, 57.09; H, 3.22; N, 13.44.

*4.1.5.5. 2-[6-(4-Chlorophenyl)-4-(4-chlorophenylamino)-5-cyanopyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**10e**).* Orange crystals; m.p. 240–242 °C, yield, 57%. IR ν_{max}/cm^{-1}: 3410, 3307 (NHs); 3075 (CH aromatic); 2924 (CH aliphatic); 2213 (C≡N); 1689, 1606 (COs); 772 (C–Cl). ^{1}H NMR ($CDCl_3$): δ 3.95 (d, 1H upfield proton of CH_2S, $J = 11.1$ Hz); 4.03 (d, 1H, downfield proton of CH_2S, $J = 12.4$ Hz); 4.72 (d, 1H upfield proton of CH_2O, $J = 12.5$ Hz); 4.80 (d, 1H, downfield proton of CH_2O, $J = 13.4$ Hz); 6.76–8.02 (m, 14H, aromatic H); 8.26 (d, 1H, C5-H of quinazoline, $J = 8.2$ Hz); 9.24 (s, 2H, NHs exchangeable). Anal. Calcd. for $C_{34}H_{21}Cl_4N_7O_3S$ (749.45): C, 54.49; H, 2.82; N, 13.08. Found: C, 54.40; H, 2.52; N, 12.89.

*4.1.5.6. 2-[6-(4-Chlorophenyl)-5-cyano-4-(4-hydroxyphenylamino) pyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**10f**).* Brown powder; m.p. 242–244 °C, yield, 55%. IR ν_{max}/cm^{-1}: 3391 (OH); 3297 (NHs); 3055 (CH aromatic); 2924 (CH aliphatic); 2211 (C≡N); 1691 (COs); 763 (C–Cl). ^{1}H NMR (DMSO-d_6): δ 3.22 (s, 2H, CH_2S); 4.91 (s, 2H, CH_2O); 6.79–7.93 (m, 15H, aromatic H); 10.5 (s, 1H, NH exchangeable with D_2O); 11.32 (s, 1H, NH exchangeable); 11.50 (s, 1H, OH exchangeable). Anal. Calcd. for $C_{34}H_{22}Cl_3N_7O_4S$ (731.01): C, 55.86; H, 3.03; N, 13.41. Found: C, 56.02; H, 3.27; N, 13.46.

*4.1.5.7. 2-[5-Cyano-6-(4-hydroxyphenyl)-4-phenylaminopyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**10g**).* Light-brown powder; m.p. 245–247 °C, yield, 55%. IR ν_{max}/cm^{-1}: 3370, 3200 (OH, NHs); 3076 (CH aromatic); 2956 (CH aliphatic); 2221 (C≡N); 1693 (COs); 768 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 4.33 (s, 2H, CH_2S); 5.11 (s, 2H, CH_2O); 6.96–7.79 (m, 15H, aromatic H); 8.21 (d, 1H, C5-H of quinazoline, $J = 8.1$ Hz); 8.59 (s, 1H, NH exchangeable); 8.60 (s, 1H, NH exchangeable with D_2O); 9.90 (s, 1H, OH exchangeable). Anal. Calcd. for $C_{34}H_{23}Cl_2N_7O_4S$ (696.56): C, 58.63; H, 3.33; N, 14.08. Found: C, 59.01; H, 3.24; N, 13.99.

*4.1.5.8. 2-[4-(4-chlorophenylamino)-5-cyano-6-(4-hydroxyphenyl) pyrimidin-2-ylsulfanyl]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**10h**).* Brown powder; m.p. 250–252 °C, yield, 53%. IR ν_{max}/cm^{-1}: 3373 (OH, NHs); 3075 (CH aromatic); 2923 (CH aliphatic); 2215 (C≡N); 1694, 1609 (COs); 773 (C–Cl). ^{1}H NMR ($CDCl_3$): δ 4.72 (d, 1H, upfield proton of CH_2S, $J = 12.6$ Hz); 5.08 (d, 1H, downfield proton of CH_2S, $J = 12.3$ Hz); 5.33 (s, 2H, CH_2O); 6.60–7.80 (m, 17H, 14 aromatic H, 2 NHs and OH exchangeable); 8.25(d, 1H, C5-H of quinazoline, $J = 8.2$ Hz). ^{13}C NMR (DMSO-d_6): δ 32.3 (CH_2S); 65.7 (CH_2O); 114.3–132.8 (aromatic C); 140.1 (C-1 chloroanilino); 146.8 (C-1 dichlorophenyl); 156.7 (C-4 hydroxyphenyl); 158.3 (C=O quinazoline); 163.8 (C-2 quinazoline); 170.1 (C=O carboxamide); 172.6 (C-4 pyrimidine); 174.6 (C-2 pyrimidine). Anal. Calcd. for $C_{34}H_{22}Cl_3N_7O_4S$ (731.01): C, 55.86; H, 3.03; N, 13.41. Found: C, 55.55; H, 2.92; N, 13.05.

*4.1.5.9. 2-[5-Cyano-6-(4-hydroxyphenyl)-4-(4-hydroxyphenylamino) pyrimidin-2-ylsulfany]-N-[2-(2,4-dichlorophenoxymethyl)-4-oxo-4H-quinazolin-3-yl]acetamide (**10i**).* Buff powder; m.p. 245–247 °C, yield, 50%. IR ν_{max}/cm^{-1}: 3396 (OHs, NHs); 3095 (CH aromatic); 2941 (CH aliphatic); 2212 (C≡N); 1639 (COs); 789 (C–Cl). ^{1}H NMR (DMSO-d_6): δ 4.94 (s, 2H, CH_2S); 5.22 (s, 2H, CH_2O); 7.15–7.96 (m, 14H, aromatic H); 8.16 (d, 1H, C5-H of quinazoline, $J = 8.3$ Hz); 11.24 (s, 2H, 2 NHs exchangeable); 13.93 (s, 2H, 2 OHs exchangeable). Anal. Calcd. for $C_{34}H_{23}Cl_2N_7O_5S$ (712.56): C, 57.31; H, 3.25; N, 13.76. Found: C, 57.35; H, 3.72; N, 13.80.

4.2. Pharmacological screening

4.2.1. Anti-inflammatory activity

Adult albino rats of both sexes weighing between 120 and 150 g were used. Rats were uniformly hydrated by giving 3 ml water/rat through gastric inoculation to reduce variability to oedema response. Animals were divided into 26 groups each of five animals. The control group was given saline solution containing few drops of Tween 80. Diclofenac sodium (50 mg/kg) was taken as standard drug for comparison and compounds under examination (100 mg/kg) were suspended in distilled water by the aid of few drops of Tween 80 and were given orally 1 h before induction of inflammation. Induction of inflammation was performed by S.C. injection of 50 μl of 1% carrageenan-sodium gel (Sigma–Aldrich, USA), into the sub-plantar region of the right hind paw. The dorso-ventral diameter (thickness) of the right and left hind paw of each rat was measured using a pair of dial thickness gauge callipers accurate to 0.001 cm 0.5, 1, 2 and 3 h after induction of inflammation. The left hind paw diameter served as a control for the degree of inflammation in the right hind paw. The percentage of anti-inflammatory activity (% inhibition of inflammation) was calculated according to the following equation:

$$\%\ \text{inhibition} = (W_c - W_t/W_c) \times 100$$

W_t: is the mean increase in paw thickness in rats treated with the tested compounds.
W_c: is the mean increase in paw thickness in control group.

Data were analyzed by SPSS statistical package version 10. Results are presented in Table 1.

4.2.2. Acute ulcerogenicity study

Adult albino rats of both sexes weighing between 120 and 150 g were used. Animals were divided into groups each of five animals. Rats were fasted 20 h before drug administration. The tested compounds, diclofenac sodium and celecoxib were given orally in a dose of 100, 50 and 50 mg/kg, respectively, suspended in 1% Tween while one group received vehicle (1% Tween). Rats were fasted for 2 h, allowed to feed for 2 h then fasted for another 20 h. Rats were given another two doses in the second and third days. In the fourth day, rats were sacrificed, the stomach removed, opened along with the greater curvature and rinsed with 0.9% saline. The number of mucosal damage (red spots) was counted and their severity (ulcerogenic severity) was graded from 0 to 4 according to the following score assignment: The following figures were calculated:

- % Incidence/10 = [number of rats showing ulcer of any grade divided by total number of rats in the group × 100]/10.
- Average number of ulcers: number of ulcers in the group/total number of rats in the group.
- Average severity: ∑[each ulcer multiplied by its score of severity]/number of ulcers in the group.

Ulcer index = the sum of the 3 figures

Results are tabulated in Table 3.

	Score		Score
Normal (no injury)	0	Slight injury	3
Latent small red spot	1	Severe injury	4
Wide red spot	2		

4.2.3. In-vitro COX study

Cyclo-oxygenase activity was assayed using COX ovine inhibitor screening assay kit (catalogue no 560131, Cayman chemicals, Ann Arbor, MI) by the method of Gierse et al. [45].

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