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Synthesis, molecular docking and anti-inflammatory screening of novel quinoline incorporated pyrazole derivatives using the Pfitzinger reaction II

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ABSTRACT

In continuation of our study of novel quinolines with anti-inflammatory activity using the Pfitzinger reaction, several new quinoline derivatives were synthesized and tested for their anti-inflammatory and ulcerogenic effect. A docking study on the COX-2 binding pocket was carried out for the target compounds to rationalize the possible selectivity of them against COX-2 enzyme. The most active compounds (**5a, 8a and 11a**) were found to be superior to celecoxib. Compound **11a** demonstrated the highest anti-inflammatory activity as well as the best binding profiles into the COX-2 binding site. Moreover, compounds **9c, 9e**, **10a** and **11a** were devoid of ulcerogenic activity.

1. Introduction

In our ongoing medicinal chemistry research program [1] we have reported the synthesis and anti-inflammatory activity of a number of fluorinated quinoline derivatives. Since, some fluorinated quinolines incorporated benzimidazole derivatives, such as compound **I** exhibited more potent anti-inflammatory activity than celecoxib, it promoted us soon to modify the established structures on the basis of isosteric replacement [2]. The bioisostere concept is an oversimplification of the role of scaffolds for activity, unless it plays a pivotal role for function or interaction such as for β-lactam in penicillins [2]. Moreover, Lima and Barreiro [3] revealed that bioisosterism is a useful strategy for the lead optimization process and molecular modification for rational drug design. On the basis of these results, we surmised that replacement of fluorine in the 6-position by methoxy group and also replacement of benzimidazole ring system by pyrazole ring system could give compounds **II** with improved anti-inflammatory activity by illustrating the old and well-known isosterism pyrazole/imidazole having similar geometry and electron features [4] (Fig. 1).

Compounds containing N-Aryl-pyrazolone-4-imino and 2,4-imidazolinedione framework displayed a multitude of biological activities, including antitumor, antibacterial, antihypertensive, antiplatelet and anti-inflammatory activities [5–8]. Pyrazolines have also been reported to possess antiparasitic [9], antitubercular [10], insecticidal agents [11], antipyretic [12], anti-inflammatory [13], antidiabetic [14], tranquillizing [15], muscle relaxant [16], psychoanaleptic [17], anticonvulsant [18], antihypertensive [19], antidepressant [20] and anticancer [21] activities. They have also been found to be nitric oxide synthase (NOS) inhibitors and had shown Cannabinoid CB1 receptor antagonist activity [22].

The present work is an extension of our ongoing efforts toward the development and identification of new molecules; by the bioisostere concept [1,23,24], we have designed some trisubstituted quinoline derivatives linked to pyrazoles **(5–11)**. Also, in an attempt to rationalize the possible selective activity of the target compounds against the COX-2 enzyme, a molecular modeling study was conducted to check the ability of these new scaffolds to bind to the active site of COX-2 isozyme.

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2. Results and discussion

2.1. Chemistry

The reaction sequence used to synthesize the target compounds is utilized in Schemes 1–4. The versatile Pfitzinger reaction was employed to synthesize pertinent 6-methoxy-2-(substitutedphenyl)quinoline-4-carboxylic acids **1a–d** in a very good yield by reacting 5-methoxyisatin with appropriate acetophenones in aqueous ethanol at reflux temperature [25], **Scheme 1.** The molecular structure of compounds **1a–d** was confirmed on the basis of their elemental analyses and spectral data. The infrared spectrum of compounds **1** showed a characteristic band at 3419–3300 cm^{-1} which is due to —OH stretching of carboxylic acids in addition to the presence of a stretching band at 1695–1683 cm^{-1} attributed to C=O functional group. The ^{1}H NMR spectrum showed a singlet at δ 13.78 ppm corresponding to the acidic-COOH proton of quinoline. The corresponding esters **2a–d** were obtained by reaction of **1a–d** with ethanol in the presence of concentrated sulfuric acid. These esters were then hydrazinolyed with hydrazine hydrate in ethanol to give the corresponding carbohydrazide precursors **3a–d** in good yields. The structure of hydrazides **3a–d** was confirmed by elemental analysis and spectral data. Infrared spectrum gave a stretching band at 1712–1709 cm^{-1} due to the presence of C=O of ester. ^{1}HNMR spectra gave a triplet at 1.45–1.41 ppm and a quartet at 4.48–4.46 ppm indicating the 5H of $COOCH_2CH_3$. Infrared spectrum of compounds **3a–d** revealed the presence of absorption band at 3372–3368 due to NH-stretching and 1652–1637 cm^{-1} due to a carbonyl group. ^{1}H NMR spectrum of **3a–d** showed a singlet at δ 4.78–4.4 indicating 2H of amino group.

The hydrazide moiety in compound **3a** was exploited to synthesize some pyrazole derivatives through its reaction with some electrophiles. The cyclocondensation of hydrazide derivative **3a** with chalcones **4a, b** [26,27] in ethanolic sodium hydroxide afforded pyrazole derivatives **5a, b,** Scheme 2. Infrared spectrum of **5a, b** revealed the presence of absorption band at 1674–1651 due to a carbonyl group. ^{1}H NMR spectrum of **5a** showed a doublet at δ 3.58–3.66 indicating 2H of pyrazole.

^{1}HNMR spectrum of **5a** in DMSO-d_6 revealed the presence of a singlet signal at 3.58–3.66 corresponding to methylene moiety of pyrazole, a triplet at 4.93–5.04 corresponding to C_5-proton of pyrazole, a singlet at 3.97 ppm corresponding to methoxy group, a singlet at 5.2 ppm corresponding to CH_2 benzylic-H in addition to the presence of aromatic protons at 6.57–7.79 ppm.

Similarly, cyclocondensation of the hydrazides **3a–d** with the appropriate β-dicarbonyl compounds (namely; acetylacetone, benzoylacetone, ethyl acetoacetate & diethylmalonates) gave the corresponding pyrazolyl derivatives **6a–d, 7a–d, 8a–d,** and **9a–h**, respectively, Scheme 3. The structures of compounds **6–9** were confirmed by elemental analyses and spectral data.

Finally, cyclocondensation of the hydrazides **3a–d** with ethoxy ethylidene-malononitriles gave the corresponding pyrazolyl derivatives **10a–d**. On the other hand, the reaction of compounds **3a–d** with ethyl 2-cyano-3-ethoxyacrylate afforded the pyrazolyl derivatives **11a–h**, and the other possible structure **12** was excluded on the basis of elemental analysis and spectral data, Scheme 4.

2.2. Molecular docking results

Considering the promising biological results and for the reason of tackling the interaction mode of the synthesized compounds with the COX-2 enzyme. The protein structure used for docking was prepared using COX2-celecoxib complex (PDB ID: 6COX) as a template by protein modification function of Molecular operating environment (MOE) [28].

The molecular docking studies suggested that all the tested compounds show different bindings modes with the active site amino acids through hydrogen bonding and hydrophobic interactions. Among all the tested compounds, compound **9a**, **10a**, **10b** and **11a** form a common binding mode as indicated from their molecular docking score (−10.24, −10.25, −10.27, −10.26, respectively). The binding modes of compound **11a** and COX-2 were depicted in Fig. 2. All the interactions of compound **11a** with COX-2 were exhibited in Fig. 3. In the binding mode, compound **11a** is potently bound to the binding site of COX-2 *via* hydrophobic interactions and binding is stabilized by hydrogen bonds. The oxygen atom of the methoxy group on compound **11a** formed a hydrogen bond with the Ser530 while, the interaction between Arg120 and p-chlorophenyl group is a cation-pi interaction. Also, the amino group formed a hydrogen bond with Val523 and there is a hydrogen bond interaction between ligand quinoline ring and Arg120. In addition, there are two other hydrogen bond interactions between carbonyl group and Tyr355, and oxygen atom of the methoxy group and Arg120. From this modeling results, it was suggested that compound **11a** have a promising activity on this target (COX-2) enzyme.

2.3. Biological studies

2.3.1. In vitro cyclcooxygenese (COX) inhibition assay

The compounds synthesized in this work were evaluated for their ability to inhibit COX-1 and COX-2 using an ovine COX-1/COX-2 assay kit (Catalog No. 560101, Cayman chemical Inc. Ann Arbor. MI (USA) according to the protocol recommended by the supplier. IC_{50} (μM) are determined as the means of two determination acquired and the deviation from the mean is <100% of the mean value. The selectivity index value (SI values) was defined as IC_{50}% (COX-1)/IC_{50} (COX-2). In the assay system, the IC_{50} values of celecoxib on COX-1 and COX-2 were determined to be >50 and 0.2 μM, respectively indicating that celecoxib is a selective COX-2

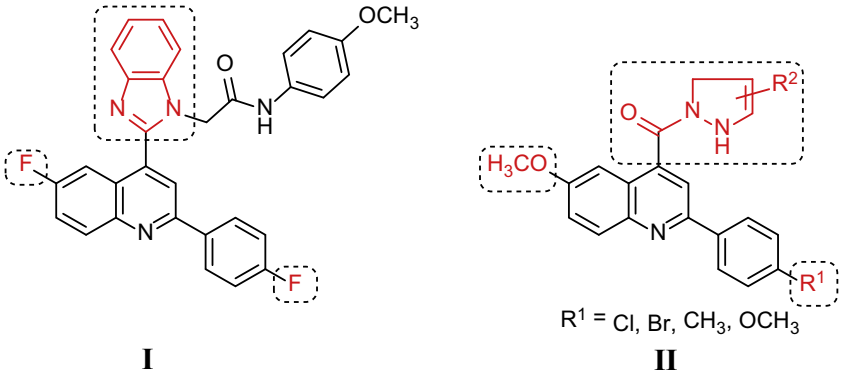
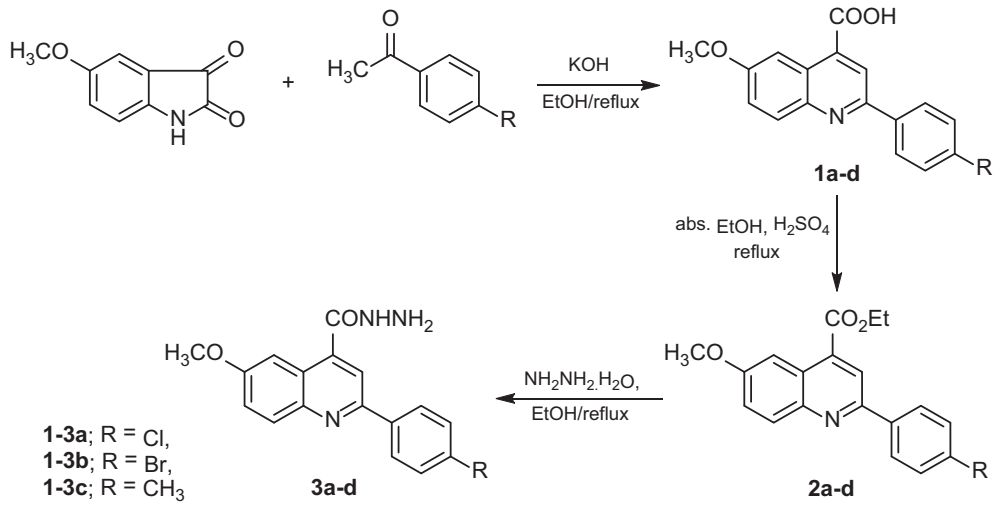
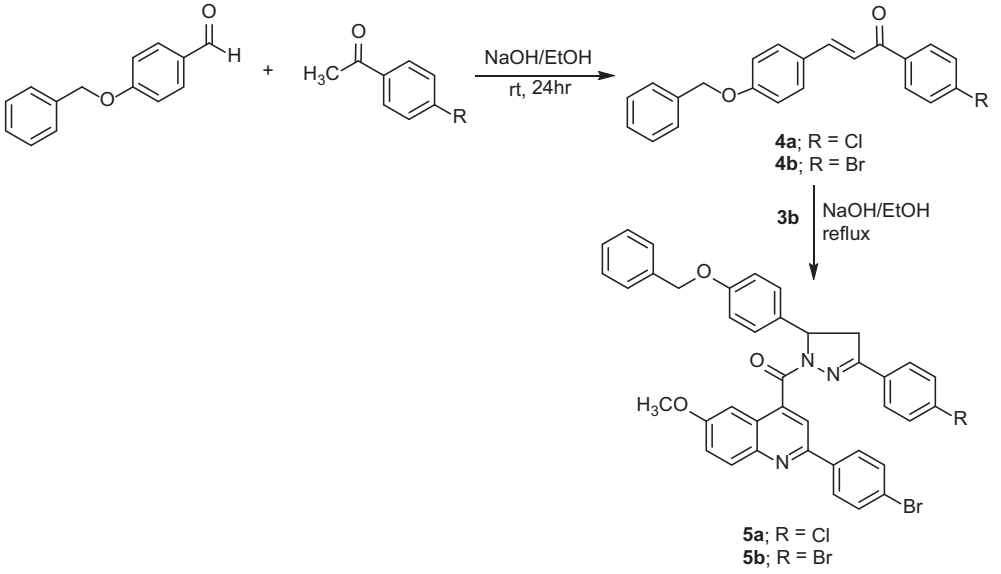


Fig. 1. Bioisoster concept.



Scheme 1.



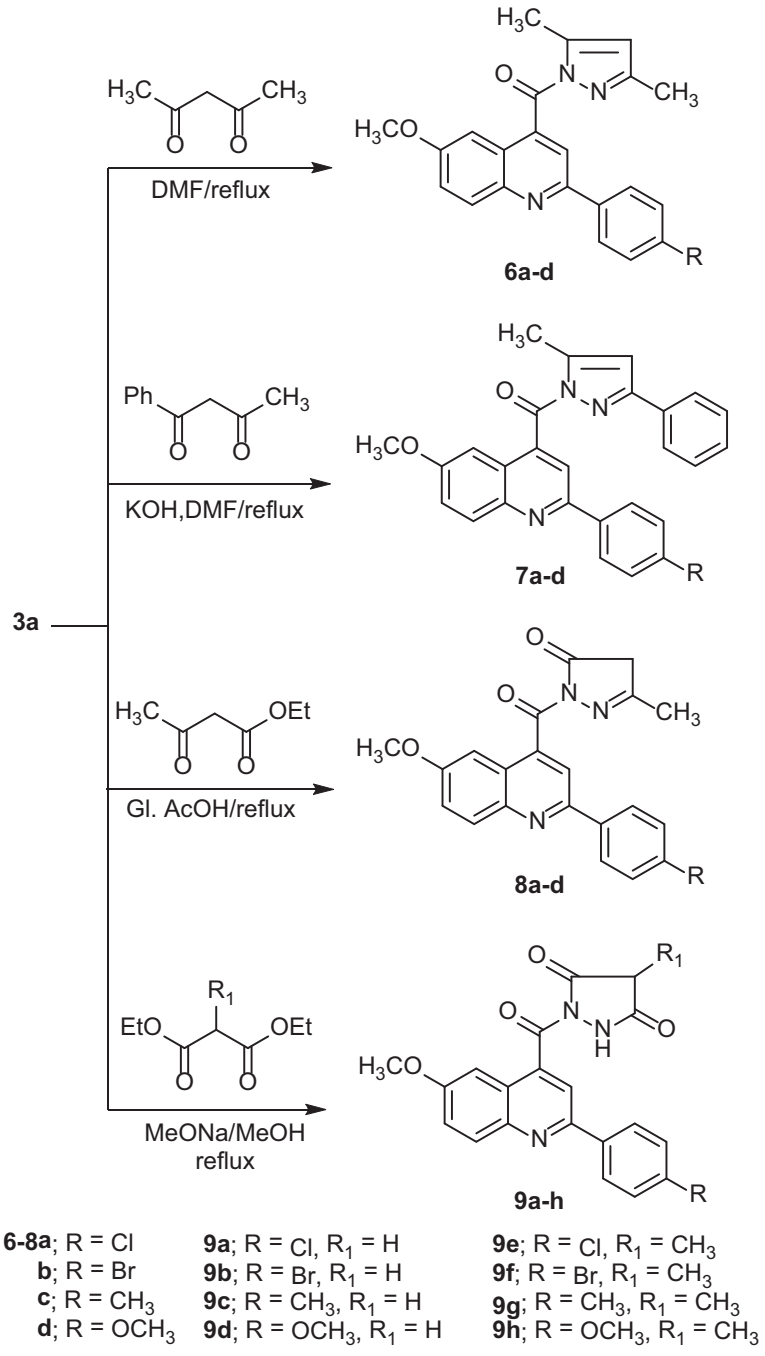
Scheme 2.

inhibitor. None of the tested compounds showed inhibitory activity on COX-1 down to 50 μM. Compounds **9e**, **11c** and **11e** show almost 100-times lower potency (IC_{50}: 40–49 μm) compared with compound **9a**, **10a**, **10b** and **11a** (IC_{50}: 0.26–0.43 μm). Some of the tested compounds (**5a**, **5b**, **9a**, **10a** and **11a**) were found to be potent and selective similar to celecoxib against COX-2. IC_{50} values in μM (Table 1) acquired by the determination of the in vitro ability of the tested compounds to inhibit COX-2 showed that compound **11a** with chlorine substituents of quinolone scaffold on the phenyl ring was more potent and selective COX-2 inhibitor (IC_{50} = 0.26 μM, SI ⩾ 192.33) compared with the reference drug celecoxib (IC_{50} = 0.28 μM, SI ⩾ 178.57). this is in accordance to our previous reported data [1] that the lead compound **I** which show specially for COX-2 receptor that produce the potent anti-inflammatory activity, compound **11a** is a structural analog of the lead compound I with similar electronic and steric characteristics.

Compounds **6a, 6c, 7a, 7e, 8a, 8c** and **8g** have less potency and selectivity compared with compound **11a** due to the presence of bulky methyl group in the position 3 of pyrazole moiety. In addition, the presence of methyl group at C-4 of phenyl ring at 2-position of quinolone scaffold decrease also the potency and selectivity as in compound **9g** and **11c**. this is an evidence that electron withdrawing substituents at C-4 of phenyl group is very important for selective inhibition of COX-2 [30].

2.3.2. In vivo anti-inflammatory studies

Compounds **5a, 5b, 6a, 6c, 7a, 7e, 8a, 8c, 8g, 9a, 9e, 9g, 10a, 10e, 11a, 11c, 11e** and **11g** were evaluated for their anti-inflammatory activity using carragenin induced edema bioassay method in rats [29]. Celecoxib was used as a reference standard representing selective COX-2 inhibitor non-steroidal anti-inflammatory agent. Compounds **5a, 7a, 8a, 8c, 8g, 10a** and **10e**, exhibited good anti-inflammatory activity comparable to or slightly better than celecoxib (Table 2). This is consistent with the reported data [30] that COX-2 activity is sensitive to the lipophilic nature of C-7 and C-8 quinoline substituents. Also, this adds further evidence that these

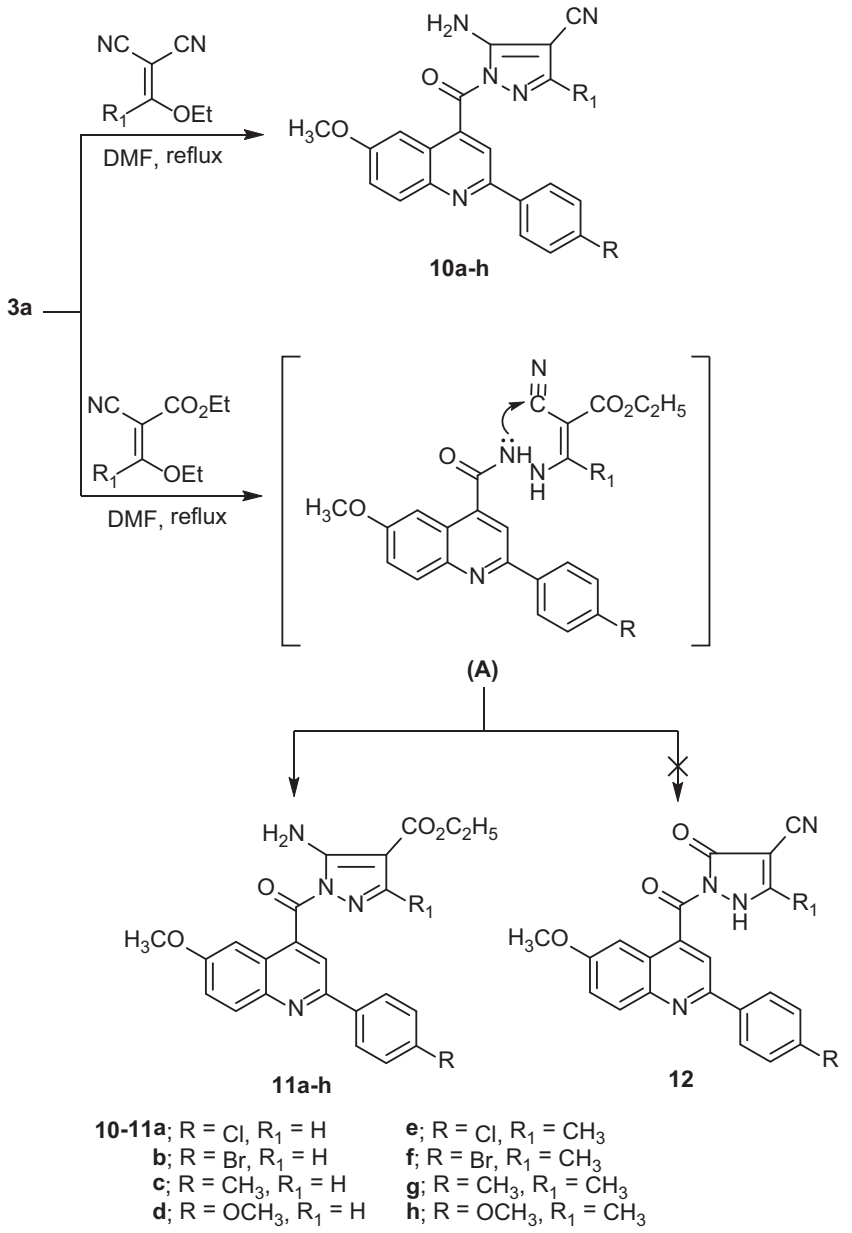


Scheme 3.

compounds may be functioning as COX-2 inhibitors. Moreover, replacement of fluorine in 6-position in quinoline scaffold by methoxy substituent has nearly the same lipophilic nature, thereby enhancing the rates of absorption and transportation in vivo. As all tested compounds exhibited a promising anti-inflammatory activity, this indicates that replacement of fluorine at the *p*-position by another electron withdrawing group like chlorine (compound **11a**) gave a good evidence that designation of COX-2 inhibition is sensitive to the *p*-position of C-2 of phenyl ring [30]. Moreover, the substituents on the pyrazole ring have a great effect on activity; compound **5a** which has 4-bromophenyl at position 3 has more anti-inflammatory activity than compound **5b** which has 4-chlorophenyl substitution at the same side. This may be due to the higher lipophilicty of bromo drugs than that of chlorodrug derivatives [31].

2.3.3. Analgesic activity

All compounds tested for their anti-inflammatory activities and celecoxib reference drug were screened for their analgesic activity using the hot plate method of Jacob and Bsovski [32]. The results presented in Table 3 showed that all tested compounds except celecoxib elicited a non-significant change when compared with control group except compound **11a** which show a significant increase in the reaction time compared with control group.



Scheme 4.

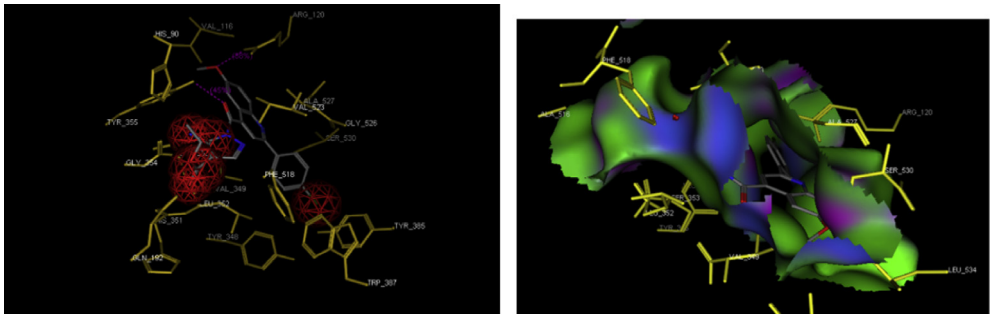


Fig. 2. Docking of compound **11a** into COX-2 active site.

2.3.4. Ulcerogenic activity

The target compounds were evaluated for their ulcerogenic potential in rats using indomethacin as a reference drug. The incidence of ulcer score was calculated according to 1–5 scoring system of Wilhelmi and Menassa-Gdynia [33]. The ulcer index was calculated according to the method of Pauls et al. [34]. All drug

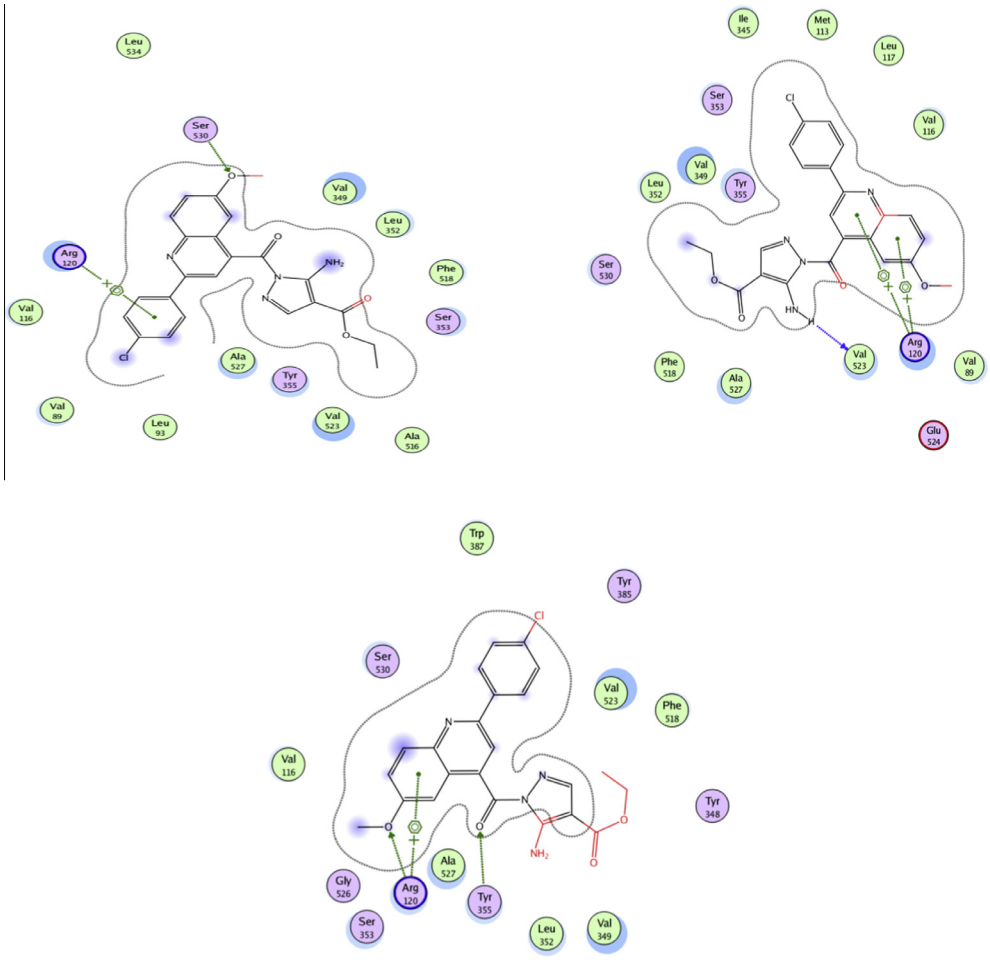


Fig. 3. Interactions of compound **11a** with COX-2.

Table 1
In vitro COX-1/COX-2 enzyme inhibition assay of the designed compounds.

Compd. no.	IC_{50}[a] (μM)		SI[c]
	COX-1[b]	COX-2	
5a	>50	0.50	>100.01
5b	>50	0.48	>104.00
6a	>50	48.0	>1.04
6c	>50	40.2	>1.24
7a	>50	46.0	>1.09
7e	>50	1.2	>47.66
8a	>50	3.3	>15.15
8c	>50	2.1	>28.80
8g	>50	3.2	15.63
9a	>50	0.29	>172.3
9b	>50	3.2	>15.63
9e	>50	49.0	>1.02
9g	>50	4.0	>1.10
10a	>50	0.41	>121.90
10b	>50	0.43	>116.00
11a	>50	0.26	>192.33
11c	>50	40.0	>1.24
11e	>50	46.0	>1.09
11f	>50	3.1	>15.00
Celecoxib	>50	0.28	>178.57

[a] IC_{50} value is the compound concentration required to produce 50% inhibition of COX-1 or COX-2 for means of two determinations and deviation from the mean is <10% of the mean value.
[b] No inhibition of COX-1 up to 50 μM.
[c] Selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

Table 2
Anti-inflammatory evaluation of tested compounds and celecoxib at a dose of 1.8 mg/100 gm body weight of rats on inflamed rat paw (n = 5 rats).

Compd. no.	Thickness of paw skin in mm after				
	Zero time	1st hr.	2nd hr.	3rd hr.	4th hr.
Control	$0.28^{a} \pm 0.01$	$0.76^{a} \pm 0.02$	$0.96^{ab} \pm 0.02$	$1.24^{a} \pm 0.04$	$1.48^{a} \pm 0.03$
Celecoxib	$0.29^{a} \pm 0.01$	$0.48^{g} \pm 0.01$	$0.56^{d} \pm 0.02$	$0.65^{c} \pm 0.02$	$0.70^{g} \pm 0.02$
5a	$0.29^{a} \pm 0.01$	$0.57^{ef} \pm 0.02$	$0.62^{d} \pm 0.02$	$0.67^{f} \pm 0.02$	$0.90^{f} \pm 0.02$
6a	$0.29^{a} \pm 0.01$	$0.77^{a} \pm 0.02$	$0.99^{ab} \pm 0.01$	$1.16^{ab} \pm 0.02$	$1.38^{ab} \pm 0.02$
6c	$0.30^{a} \pm 0.01$	$0.74^{ab} \pm 0.02$	$0.56^{d} \pm 0.02$	$1.12^{b} \pm 0.02$	$1.28^{bc} \pm 0.02$
9a	$0.28^{a} \pm 0.01$	$0.54^{fg} \pm 0.02$	$0.57^{d} \pm 0.03$	$0.72^{ef} \pm 0.02$	$0.80^{fg} \pm 0.02$
9c	$0.29^{a} \pm 0.01$	$0.78^{a} \pm 0.02$	$0.99^{ab} \pm 0.03$	$1.20^{ab} \pm 0.04$	$1.44^{a} \pm 0.02$
9e	$0.30^{a} \pm 0.01$	$0.62^{de} \pm 0.01$	$0.74^{c} \pm 0.02$	$0.81^{de} \pm 0.04$	$1.12^{e} \pm 0.02$
9g	$0.29^{a} \pm 0.01$	$0.77^{a} \pm 0.02$	$1.00^{ab} \pm 0.02$	$1.24^{a} \pm 0.02$	$1.46^{a} \pm 0.02$
10a	$0.29^{a} \pm 0.01$	$0.69^{bc} \pm 0.01$	$0.76^{c} \pm 0.01$	$0.82^{cc} \pm 0.02$	$1.14^{de} \pm 0.02$
10e	$0.29^{a} \pm 0.01$	$0.79^{a} \pm 0.01$	$0.62^{d} \pm 0.03$	$1.22^{ab} \pm 0.02$	$1.46^{g} \pm 0.02$
11a	$0.30^{a} \pm 0.01$	$0.50^{g} \pm 0.02$	$0.92^{b} \pm 0.02$	$0.68^{c} \pm 0.05$	$0.75^{g} \pm 0.03$
11c	$0.29^{a} \pm 0.01$	$0.63^{cde} \pm 0.02$	$0.80^{c} \pm 0.01$	$1.14^{ab} \pm 0.02$	$1.24^{cd} \pm 0.02$
11e	$0.29^{a} \pm 0.01$	$0.76^{a} \pm 0.01$	$0.96^{ab} \pm 0.02$	$1.24^{a} \pm 0.02$	$1.36^{ab} \pm 0.03$
11g	$0.29^{a} \pm 0.01$	$0.65^{cd} \pm 0.02$	$0.80^{c} \pm 0.01$	$0.91^{c} \pm 0.02$	$1.07^{e} \pm 0.05$

Means are expressed as Mean ± S.E.
Means within the same column carrying different letters are significantly different at $p \leqslant 0.05$.

doses were calculated according to Paget and Barnes [35]. As illustrated in table in Table 4, the most active compounds **9c, 9e**, **10a** and **11a** were devoid of any ulcerogenic activity. These data can be added to our previous published data [1] in which,

Table 3
Analgesic activity evaluation of tested compounds and celecoxib at a dose of 1.8 mg/100 gm body weight of mice (n = 5 mice).

Compound no.	Reaction time in seconds after					
	10 min	20 min	30 min	60 min	90 min	120 min
Control	$12.00^{cd} \pm 0.70$	$11.20^{c} \pm 0.20$	$12.40^{e} \pm 0.50$	$12.80^{b} \pm 0.37$	$12.40^{de} \pm 0.50$	$13.40^{b} \pm 0.50$
Celecoxib	$33.00^{e} \pm 0.70$	$36.20^{a} \pm 0.58$	$38.60^{a} \pm 0.50$	$39.20^{a} \pm 0.37$	$40.20^{a} \pm 0.37$	$41.80^{a} \pm 0.96$
5a	$14.40^{bc} \pm 0.81$	$13.80^{b} \pm 0.58$	$12.80^{cde} \pm 0.58$	$13.20^{b} \pm 0.58$	$13.40^{bcde} \pm 0.50$	$15.40^{b} \pm 0.43$
6a	$13.80^{bcd} \pm 0.58$	$13.80^{b} \pm 0.51$	$13.00^{cde} \pm 0.50$	$13.80^{b} \pm 0.37$	$13.80^{bcd} \pm 0.37$	$14.00^{b} \pm 0.44$
6c	$13.80^{cd} \pm 0.58$	$13.80^{b} \pm 0.58$	$13.00^{cde} \pm 0.70$	$13.80^{b} \pm 0.58$	$13.40^{bcde} \pm 0.40$	$13.60^{b} \pm 0.50$
9a	$12.60^{cd} \pm 0.50$	$13.80^{b} \pm 0.58$	$14.60^{bc} \pm 0.24$	$14.20^{b} \pm 0.37$	$14.00^{bc} \pm 0.63$	$13.80^{b} \pm 0.58$
9c	$13.20^{bcd} \pm 0.37$	$13.40^{b} \pm 0.50$	$13.40^{bcde} \pm 0.50$	$13.20^{b} \pm 0.37$	$13.20^{bcde} \pm 0.37$	$14.20^{b} \pm 0.37$
9e	$13.40^{bcd} \pm 0.50$	$13.60^{b} \pm 0.50$	$13.40^{bcde} \pm 0.50$	$13.40^{b} \pm 0.50$	$13.40^{bcde} \pm 0.50$	$14.00^{b} \pm 0.70$
9g	$14.40^{bc} \pm 0.60$	$13.20^{b} \pm 0.58$	$13.20^{bcde} \pm 0.58$	$13.20^{b} \pm 0.58$	$14.40^{b} \pm 0.40$	$13.80^{b} \pm 0.58$
10a	$14.00^{b} \pm 0.40$	$13.60^{b} \pm 0.24$	$14.20^{bcd} \pm 0.37$	$14.20^{b} \pm 0.73$	$13.40^{bcde} \pm 0.50$	$13.40^{b} \pm 0.50$
10e	$13.80^{bcd} \pm 0.58$	$14.00^{b} \pm 0.63$	$12.60^{cde} \pm 0.40$	$13.00^{b} \pm 0.31$	$12.00^{e} \pm 0.31$	$13.20^{b} \pm 0.80$
11a	$14.20^{bc} \pm 0.37$	$14.40^{b} \pm 0.40$	$14.80^{b} \pm 0.20$	$13.60^{b} \pm 0.40$	$13.80^{bcd} \pm 0.37$	$14.00^{b} \pm 0.44$
11c	$13.80^{bcd} \pm 0.73$	$13.60^{b} \pm 0.40$	$13.20^{bcde} \pm 0.58$	$13.20^{b} \pm 0.58$	$12.80^{cde} \pm 0.37$	$13.40^{b} \pm 0.50$
11e	$13.80^{bcd} \pm 0.73$	$13.60^{b} \pm 0.40$	$13.20^{bcde} \pm 0.58$	$13.20^{b} \pm 0.58$	$12.80^{cde} \pm 0.37$	$13.40^{b} \pm 0.50$
11g	$13.60^{bcd} \pm 0.40$	$13.80^{b} \pm 0.37$	$13.80^{bcde} \pm 0.31$	$13.80^{b} \pm 0.20$	$13.40^{bcd} \pm 0.24$	$13.60^{b} \pm 0.50$

Means are expressed as Mean ± S.E.
Means within the same column carrying different letters are significantly different at $p \leqslant 0.05$.

Table 4
Ulcerogenic activity of the tested compounds and indomethacin (n = 5 rats).

Compound no.	Ulcer score	Incidence of gastric ulceration	Ulcer index
Ulcerogenic activity			
Control	0.0%	0.0	0.0
Celecoxib	0.0%	0.0	0.0
Indomethacin	$4.40^{a} \pm 0.24$	100%	440
5a	$1.80^{d} \pm 0.20$	75%	135.0
6a	$3.8^{b} \pm 0.20$	75%	285.0
6c	$2.80^{c} \pm 0.20$	100%	280.0
9a	$2.80^{c} \pm 0.20$	75%	210.0
9c	0.0	0.0	0.0
9e	0.0	0.0	0.0
9g	$0.60^{f} \pm 0.20$	25%	15.0
10a	0.0	0.0	0.0
10e	$1.20^{e} \pm 0.20$	50%	60.0
11a	0.0	0.0	0.0
11c	$1.80^{d} \pm 0.20$	75%	135
11e	$1.20^{e} \pm 0.20$	50%	60.0
11g	$0.4^{g} \pm 0.20$	25%	10.0

Means are expressed as Mean ± S.E.

incorporation quinoline skeleton with different azoles may provide lead compounds devoid of any ulcerogenic activity.

3. Conclusion

The importance of the potent quinoline class of compounds has been established in the search for effective anti-inflammatory agents [36]. In the current investigation, a new scaffold of quinoline linked to pyrazoles were synthesized and evaluated as anti-inflammatory agents. Also, fluorine at 6-position of quinoline scaffold has been replaced by methoxy group and also fluorine at *p*-position of 2-phenyl group has been replaced by electron withdrawing and electron donating substituents. The results of this study revealed that, lipophilic substituents at 6-position and electron withdrawing substituents at *p*-position of phenyl group are very important to act as selective COX-2 inhibitor [1]. Compound **11a** was found to have the best anti-inflammatory activity as well as the best binding profiles with COX-2 isozyme. Moreover, some of the synthesized and evaluated compounds were devoid of any ulcerogenic activity. Therefore, these derivatives may represent good lead compounds for the development of potent and selective anti-inflammatory agents.

4. Experimental

4.1. General

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra were recorded on a Pye Unicam SP 1000 infrared spectrophotometer and SHIMADZU infrared spectrophotometer using potassium bromide discs and expressed in wave number (cm^{-1}). ^1H NMR spectra were recorded on a Varian-Mercury 200 MHz or 300 MHz spectrometer in DMSO-d_6 and BRUKER 400 MHz spectrometer in DMSO-d_6. Chemical shifts were expressed in parts per million (ppm) with tetramethylsilane (TMS) as an internal standard. As for the proton magnetic resonance, D_2O was carried out for NH and OH protons. ^{13}C NMR spectra were obtained in DMSO-d_6 on Avance 125 MHz spectrometers; the chemical shifts are expressed in δ units. MS spectra were measured with an HP 5995 instrument and Hewlett Packard 5988 spectrometer Micro Analytical Center, Egypt. Elemental analyses (C, H, and N) were performed on VARIO El ELEMENTER apparatus at the Micro Analytical Centre, Cairo University, Giza, Egypt. All compounds were routinely checked by thin-layer chromatography (TLC) on aluminum-backed silica gel plates. All solvents were dried by standard methods.

4.1.1. General method for the synthesis of compounds (**1a**–**d**)

A mixture of 5-methoxyisatin, (10 mmol), 33% potassium hydroxide (10 ml) in ethanol (20 ml), and 4-substituted acetophenone (10 mmol) was heated under reflux for 9–18 h. The reaction mixture was concentrated under reduced pressure and the residue was diluted with water (50 ml) and extracted with ether (3 × 50 ml). The aqueous layer was neutralized with 1 M hydrochloric acid. The precipitated solid was filtered, washed with water, dried and crystallized from ethanol.

*4.1.1.1. 2-(4-Chlorophenyl)-6-methoxyquinoline-4-carboxylic acid (**1a**).* Yield 70%; m.p. 264–266 °C; IR (KBr, cm^{-1}): 3300–2700 (br, OH), 3064–3003 (CH aromatic), 2968, 2837 (CH aliphatic), 1689 (C═O), 1618 (C═N), 1587 (C═C). ^1H NMR (200 MHz DMSO-d_6, δ ppm): 3.97 (s, 3H, OCH_3) 7.6–8.4 (m, 8H, aromatic H), 13.78 (s, 1H, OH, D_2O exchangeable); Anal. Calcd. For $C_{17}H_{12}ClNO_3$ (313.5): C, 65.08; H, 3.86; N, 4.46. Found: C, 65.19; H, 4.07; N, 4.15.

*4.1.1.2. 2-(4-Bromophenyl)-6-methoxyquinoline-4-carboxylic acid (**1b**).* Yield 71%; m.p. 279–280 °C; IR (KBr, cm^{-1}): 3400 (OH),

3007 (CH aromatic), 2964–3835 (CH, aliphatic), 1689 (C=O), 1616 (C=N), 1589 (C=C). ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 3.97 (s, 3H, OCH_3), 7.55–8.47 (m, 8H, aromatic H), 13.62 (s, 1H, OH, D_2O exchangeable); Anal. Calcd. For $C_{17}H_{12}BrNO_3$ (358): C, 57.00; H, 3.38; N, 3.91, Found: C, 56.90; H, 3.32; N, 3.84.

*4.1.1.3. 6-Methoxy-2-p-tolylquinoline-4-carboxylic acid (**1c**).* Yield 65%; m.p. 243–245 °C; IR (KBr, cm^{-1}): 3400 (OH), 3064, 3007 (CH aromatic), 2993, 2841 (CH aliphatic), 1695 (C=O), 1618 (C=N), 1587 (C=C). ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 2.45 (s, 3H, CH_3), 3.98 (s, 3H, OCH_3), 7.42–8.49 (m, 8H, aromatic H), 13.64 (s, 1H, OH, D_2O exchangeable); Anal. Calcd. For $C_{18}H_{15}NO_3$ (293.32): C, 73.71; H, 5.15; N, 4.78, Found: C, 73.50; H, 5.37; N, 4.47.

*4.1.1.4. 6-Methoxy-2-(4-methoxyphenyl)quinoline-4-carboxylic acid (**1d**).* Yield 60%; m.p. 243.7–244 °C; IR (KBr, cm^{-1}): 3419 (OH), 3032, 3005 (CH aromatic), 2968, 2841 (CH aliphatic), 1683 (C=O), 1616 (C=N), 1581 (C=C). ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 3.91 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 7.15–8.47 (m, 8H, aromatic H), 13.6 (s, 1H, OH, D_2O exchangeable); Anal. Calcd. For $C_{18}H_{15}NO_4$ (309.32): C, 69.89; H, 4.89; N, 4.53, Found: C, 69.77; H, 4.77; N, 4.48.

*4.1.2. General method for the synthesis of compounds (**2a–d**)*

A mixture of the appropriate 4-carboxylic acids (**1a–d**) (10 mmol), absolute ethanol (20 ml) and concentrated sulfuric acid (2 ml) was refluxed for 10 h. Excess ethanol was distilled under reduced pressure; the mixture was allowed to cool, diluted with water then rendered alkaline with sodium bicarbonate solution. The precipitate was filtered, washed with water and crystallized from ethanol.

*4.1.2.1. Ethyl 2-(4-chlorophenyl)-6-methoxyquinoline-4-carboxylate (**2a**).* Yield 65%; m.p. 128–129 °C; IR (KBr, cm^{-1}): 3032–3007 (CH aromatic), 2981, 2902 (CH aliphatic), 1712 (C=O), 1624 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.44 (t, 3H, CH_3), 3.92 (s, 3H, OCH_3), 4.5 (q, 2H, CH_2), 7.51–8.45 (m, 8H, aromatic H). Anal. Calcd. For $C_{19}H_{16}ClNO_3$ (341.5): C, 66.77; H, 4.72; N, 4.10; Found: C, 66.46; H, 4.65; N, 3.84.

*4.1.2.2. Ethyl 2-(4-Bromophenyl)-6-methoxyquinoline-4-carboxylate (**2b**).* Yield 75%; m.p. 134–136 °C; IR (KBr, cm^{-1}): 3010 (CH aromatic), 2981, 2900 (CH aliphatic), 1712 (C=O), 1622 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.44 (t, 3H, CH_3), 3.93 (s, 3H, OCH_3), 4.5 (q, 2H, CH_2), 7.5–8.46 (m, 8H, aromatic H). Anal. Calcd. For $C_{19}H_{16}BrNO_3$ (386.24): C, 59.08; H, 4.18; N, 3.63; Found: C, 58.81; H, 4.36; H, 3.97.

*4.1.2.3. Ethyl 6-methoxy-2-p-tolylquinoline-4-carboxylate (**2c**).* Yield 60%; m.p. 115–117 °C; IR (KBr, cm^{-1}): 3007 (CH aromatic), 2984, 2842 (CH aliphatic), 1712 (C=O), 1614 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.45 (t, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 4.5 (q, 2H, CH_2), 7.52–8.48 (m, 8H, aromatic H). Anal. Calcd. For $C_{20}H_{19}NO_3$ (321.37): C, 74.75; H, 5.96; N, 4.36; Found: C, 74.92; H, 6.18; N, 4.18.

*4.1.2.4. Ethyl 6-methoxy-2-(4-methoxyphenyl)quinoline-4-carboxylate (**2d**).* Yield 59%; m.p. 96–98 °C; IR (KBr, cm^{-1}): 3112 (CH aromatic), 2948, 2829 (CH aliphatic), 1708 (C=O), 1609 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.45 (t, 3H, CH_3), 3.90 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 4.48 (q, 2H, CH_2), 7.52–8.48 (m, 8H, aromatic H). Anal. Calcd. For $C_{20}H_{19}NO_4$ (337.37): C, 71.20; H, 5.68; N, 4.15; Found: C, 70.93; H, 5.70; N, 3.84.

*4.1.3. General method for the synthesis of compounds (**3a–d**)*

To a solution of **2a–d** (10 mmol) in ethanol (20 ml), hydrazine hydrate (97%, 3 ml) was added and refluxed for 3–7 h. After cooling, the precipitate was filtered, washed with water dried and crystallized from ethanol.

*4.1.3.1. 2-(4-Chlorophenyl)-6-methoxyquinoline-4-carbohydrazide (**3a**).* Yield 75%; m.p. 233–234 °C. IR (KBr, cm^{-1}): 3269 (NHs), 3050 (CH aromatic), 2950, 2800 (CH aliphatic), 1637 (C=O), 1618 (C=N). ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 3.94 (s, 3H, OCH_3), 4.78 (s, 2H, NH_2, D_2O exchangeable), 7.57–8.37 (m, 8H, aromatic H), 10.08 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. For $C_{17}H_{14}ClN_3O_2$ (327.5): C, 62.30; H, 4.31; N, 12.82; Found: C, 62.49; H, 4.73; N, 12.69.

*4.1.3.2. 2-(4-Bromophenyl)-6-methoxyquinoline-4-carbohydrazide (**3b**).* Yield 82%; m.p. 242–244 °C; IR (KBr, cm^{-1}): 3313, 3271 (NH, NH_2), 3050 (CH aromatic), 2956, 2860 (CH aliphatic), 1640 (C=O). ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 3.97 (s, 3H, OCH_3), 4.4 (br. s, 2H, NH_2, D_2O exchangeable), 7.65–8.28 (m, 8H, aromatic H), 11.85 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. For $C_{17}H_{14}BrN_3O_2$ (372.22): C, 54.86; H, 3.79; N, 11.29; Found: C, 54.72; H, 3.53; N, 11.12.

*4.1.3.3. 6-Methoxy-2-p-tolylquinoline-4-carbohydrazide (**3c**).* Yield 78%; m.p. 206–208 °C; IR (KBr, cm^{-1}): 3372, 3287 (NH_2), 3175 (NH), 3000 (CH aromatic), 2975, 2838 (CH aliphatic), 1652 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.5 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 4.70 (s, 2H, NH_2, D_2O exchangeable), 7.09–8.23 (m, 8H, aromatic H), 10.01 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. For $C_{18}H_{17}N_3O_2$ (307.35): C, 70.34; H, 5.58; N, 13.67; Found: C, 70.19; H, 5.38; N, 13.52.

*4.1.3.4. 6-Methoxy-2-(4-methoxyphenyl)quinoline-4-carbohydrazide (**3d**).* Yield 70%; m.p. 159–161 °C; IR (KBr, cm^{-1}): 3368, 3286 (NH_2), 3177 (NH), 3010 (CH aromatic), 2978, 2838 (CH aliphatic) 1650 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.84 (s, 3H, OCH_3), 3.87 (S, 3H, OCH_3), 4.71 (S, 2H, NH_2, D_2O exchangeable), 7.08–8.23 (m, 8H, aromatic H), 10.0 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. For $C_{18}H_{17}N_3O_3$ (323.35): C, 66.86; H, 5.30; N, 13.00; Found: C, 66.54; H, 5.12; N, 12.71.

*4.1.4. General procedure for the synthesis of compounds (**5a**,**b**)*

A mixture of the acid hydrazide **(3b)** (10 mmol) and the corresponding chalcones **(4a–b)** (10 mmol) was refluxed in ethanol (30 ml) containing NaOH (10 mmol) for 6 h. The mixture was cooled and the precipitated product was filtered, washed and crystallized from aqueous ethanol.

*4.1.4.1. (5-(4-(Benzyloxy)phenyl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) (2-(4-bromophenyl)-6-methoxyquinolin-4-yl)methanone (**5a**).* Yield 60%; m.p. 189–191 °C; IR (KBr, cm^{-1}): 3062, 3032 (CH aromatic), 2916, 2850 (CH aliphatic), 1651 (C=O), 1600 (C=N), 1589 (C=C). ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 3.33–3.44 (d, 2H, C_4—H of pyrazole), 3.92 (s, 3H, OCH_3), 4.27 (t, 1H, C_5—H of pyrazole), 5.20 (s, 2H, benzylic-H), 6.88–7.78 (m, 21H, aromatic H). Anal. Calcd. For $C_{39}H_{29}BrClN_3O_3$ (703.02): C, 66.63; H, 4.16; N, 5.98; Found: C, 66.41; H, 4.40; N, 5.79.

*4.1.4.2. (5-(4-(Benzyloxy)phenyl)-3-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl) (2-(4-bromophenyl)-6-methoxnyquinolin-4-yl)methanone (**5b**).* Yield 65%; m.p. 180–182 °C; IR (KBr, cm^{-1}): 3062, 3032 (CH aromatic), 2920, 2862 (CH aliphatic), 1674 (C=O), 1604 (C=N), 1581 (C=C). ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 3.58–3.66 (d, 2H, C_4—H of pyrazole), 3.97 (s, 3H, OCH_3), 4.93–5.04 (t, 1H, C_5—H of pyrazole), 5.2 (s, 2H, benzylic-H),

6.57–7.79 (m, 21H, aromatic H); Anal. Calcd. For $C_{39}H_{29}Br_2N_3O_3$ (745.06): C, 62.67; H, 3.91; N, 5.62; Found: C, 62.60; H, 4.12; N, 5.31.

*4.1.5. General procedure for the synthesis of compounds (**6a–d**)*

A solution of acid hydrazide **(3a–d)** (10 mmol) and acetylacetone (10 mmol) in dry DMF (5 ml) was heated for 6 h. The mixture was cooled and poured on ice water (20 ml). The separated solid was filtered, washed with water and crystallized from ethanol.

*4.1.5.1. (2-(4-Chlorophenyl)-6-methoxyquinolin-4-yl)(3,5-dimethyl-1H-pyrazol-1-yl)-methanone (**6a**).* Yield 83%; m.p. 280–282 °C; IR (KBr, cm^{-1}): 3062 (CH aromatic), 2924, 2843 (CH aliphatic), 1700 (C=O), 1620 (C=N), 1588 (C=C). ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.50 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 6.3 (s, 1H, pyrazole H), 7.00–8.29 (m, 8H, aromatic H). Anal. Calcd. For $C_{22}H_{18}ClN_3O_2$ (391.11): C, 67.43; H, 4.63; N, 10.72; Found: C, 67.09; H, 4.74; N, 10.46.

*4.1.5.2. (2-(4-Bromophenyl)-6-methoxyquinolin-4-yl)(3,5-dimethyl-1H-pyrazol-1-yl)-methanone (**6b**).* Yield 82%; m.p. 295–297 °C; IR (KBr, cm^{-1}): 3019 (CH aromatic), 2929, 2842 (CH aliphatic), 1692 (C=O), 1617 (C=N), 1588 (C=C). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.80 (s, 3H, CH_3), 3.16 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 6.96 (s, 1H, Pyrazole H), 7.50–8.45 (m, 8H, aromatic H). Anal. Calcd. For $C_{22}H_{18}BrN_3O_2$ (435.06): C, 60.56; H, 4.16; N, 9.63; Found: C, 60.50; H, 4.14; N, 9.85.

*4.1.5.3. (3,5-dimethyl-1H-pyrazol-1-yl)(6-methoxy-2-p-tolylquinolin-4-yl)methanone (**6c**).* Yield 70%; m.p. 242–244 °C; IR (KBr, cm^{-1}): 3062 (CH aromatic), 2930, 2839 (CH aliphatic), 1688 (C=O), 1626 (C=N), 1544 (C=C). ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.50 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 2.81 (s, 3H, CH_3) 3.92 (s, 3H, OCH_3), 6.97 (s, 1H, pyrazole H), 7.48–8.46 (m, 8H, aromatic H). Anal. Calcd. For $C_{23}H_{21}N_3O_2$ (371.43): C, 74.37; H, 5.70; N, 11.31; Found: C, 74.66; H, 5.98; N, 11.20.

*4.1.5.4. (3,5-dimethyl-1H-pyrazol-1-yl)(6-methoxy-2-(4-methoxyphenyl)quinolin-4-yl)methanone (**6d**).* Yield 73%; m.p. 260–262 °C; IR (KBr, cm^{-1}): 3090 (CH aromatic), 2968, 2925, 2831 (CH aliphatic), 1627 (C=O), 1547 (C=C). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.50 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.93 (s, 1H, pyrazole H), 7.07–8.22 (m, 8H, aromatic H). Anal. Calcd. For $C_{23}H_{21}N_3O_3$ (387.43): C, 71.30; H, 5.46; N, 10.85; Found: C, 71.36; H, 5.81; N, 10.71.

*4.1.6. General procedure for the synthesis of compounds (**7a–d**)*

To a solution of compound (**3a–d**) (10 mmol) and potassium hydroxide (20 mmol) in ethanol (20 ml), benzoylacetone (10 mmol) was added. The mixture was refluxed for 15 h. The reaction mixture was cooled, diluted with water and neutralized with hydrochloric acid. The separated solid was filtered, dried and crystallized from ethanol.

*4.1.6.1. (2-(4-Chlorophenyl)-6-methoxyquinolin-4-yl)(5-methyl-3-phenyl-1H-pyrazol-1-yl)-methanone (**7a**).* Yield 88%; m.p. 352–354 °C; IR (KBr, cm^{-1}): 3062 (CH aromatic), 2928, 2839 (CH aliphatic), 1680 (C=O), 1620 (C=N), 1544 (C=C). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.48 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 7.48–8.47 (m, 13H aromatic H + pyrazole H). Anal. Calcd. For $C_{27}H_{20}ClN_3O_2$ (453.92): C, 71.44; H, 4.44; N, 9.26; Found: C, 71.36; H, 4.56; N, 9.36.

*4.1.6.2. (2-(4-Bromophenyl)-6-methoxyquinolin-4-yl)(5-methyl-3-phenyl-1H-pyrazol-1-yl)-methanone (**7b**).* Yield 82%; m.p. > 360 °C; IR (KBr, cm^{-1}): 3089 (CH aromatic), 2925, 2830 (CH aliphatic), 1691 (C=O), 1616 (C=N), 1500 (C=C). ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.50 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 6.23 (s, 1H, pyrazole H), 7.47–8.23 (m, 13H, aromatic H). Anal. Calcd. For $C_{27}H_{20}BrN_3O_2$ (498.37): C, 65.07; H, 4.04; N, 8.43; Found: C, 64.79; H, 3.90; N, 8.12.

*4.1.6.3. (6-Methoxy-2-p-tolylquinolin-4-yl)(5-methyl-3-phenyl-1H-pyrazol-1-yl) methanone (**7c**).* Yield 60%; m.p. 320–322 °C; IR (KBr, cm^{-1}): 3066 (CH aromatic), 2920, 2830 (CH aliphatic), 1685 (C=O), 1620 (C=N), 1540 (C=C). ^1H NMR (DMSO-d_6, 400 MHz): δ 2.03 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 6.87 (s, 1H, pyrazole H), 7.34–8.44 (m, 13H, aromatic H). Anal. Calcd. For $C_{28}H_{23}N_3O_2$ (433.50): C, 77.58; H, 5.35; N, 9.69; Found: C, 77.50; H, 5.29; N, 9.60.

*4.1.6.4. (6-Methoxy-2-(4-methoxyphenyl)quinolin-4-yl)(5-methyl-3-phenyl-1H-pyrazol-1-yl) methanone (**7d**).* Yield 63%; m.p. 332–334 °C; IR (KBr, cm^{-1}): 3063 (CH aromatic), 2935, 2830 (CH aliphatic), 1690 (C=O), 1635 (C=N), 1560 (C=C). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.00 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3) 6.85 (s, 1H, pyrazole H), 7.11–8.28 (m, 13H, aromatic H). Anal. Calcd. For $C_{28}H_{23}N_3O_3$ (449.50): C, 74.82; H, 5.16; N, 9.35; Found: C, 74.78; H, 5.40; N, 9.24.

*4.1.7. General procedure for the synthesis of compounds (**8a–d**)*

Ethyl acetoacetate (3 mmol) was added to a solution of the requisite acid hydrazide **3a–d** (2 mmol) in glacial acetic acid (15 ml). The reaction mixture was refluxed for 18 h. The reaction mixture was then cooled and the precipitated solid was filtered, washed with water and crystallized from aqueous ethanol.

*4.1.7.1. 1-(2-(4-Chlorophenyl)-6-methoxyquinoline-4-carbonyl)-3-methyl-1H-pyrazol -5(4H)-one (**8a**).* Yield 86%; m.p. 277–279 °C; IR (KBr, cm^{-1}): 3174 (CH aromatic), 3005 (CH aromatic), 2974 (CH aliphatic), 1696 (C=O), 1658 (C=O). ^1H NMR (DMSO-d_6, 300 MHz): δ 1.99 (s, 3H, CH_3), 3.44 (s, 2H, CH_2), 3.92 (s, 3H, OCH_3), 7.48–8.31 (m, 8H, aromatic H). Anal. Calcd. For $C_{21}H_{16}ClN_3O_3$ (393.82): C, 64.05; H, 4.09; N, 10.67; Found: C, 64.20; H, 4.43; N, 10.82.

*4.1.7.2. 1-(2-(4-Bromophenyl)-6-methoxyquinoline-4-carbonyl)-3-methyl-1H-pyrazol -5(4H)-one (**8b**).* Yield 82%; m.p. 284–286 °C; IR (KBr, cm^{-1}): 3086 (CH aromatic), 2974, 2846 (CH aliphatic), 1696 (C=O), 1657 (C=O). ^1H NMR (DMSO-d_6, 300 MHz): δ 1.99 (s, 3H, CH_3), 3.40 (s, 2H, CH_2), 3.92 (s, 3H, OCH_3), 7.48–8.24 (m, 8H, aromatic H). Anal. Calcd. For $C_{21}H_{16}BrN_3O_3$ (438.27): C, 57.55; H, 3.68; N, 9.59; Found: C, 57.09; H, 3.93; N, 9.00.

*4.1.7.3. 1-(6-Methoxy-2-p-tolylquinoline-4-carbonyl)-3-methyl-1H-pyrazol-5(4H)-one (**8c**).* Yield 72%; m.p. 264–266 °C; IR (KBr, cm^{-1}): 3005 (CH aromatic), 2927, 2837 (CH aliphatic), 1695 (C=O), 1661 (C=O). ^1H NMR (DMSO-d_6, 400 MHz): δ 1.99 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.38 (s, 2H, CH_2), 3.91 (s, 3H, OCH_3), 7.35–8.22 (m, 8H, aromatic H). Anal. Calcd. For $C_{22}H_{19}N_3O_3$ (373.40): C, 70.76; H, 5.13; N, 11.25; Found: C, 71.11; H, 5.73; N, 11.46.

*4.1.7.4. 1-(6-Methoxy-2-(4-methoxyphenyl)quinoline-4-carbonyl)-3-methyl-1H-pyrazol-5(4H)-one (**8d**).* Yield 70%; m.p. 271–272 °C; IR (KBr, cm^{-1}): 3000 (CH aromatic), 2964, 2833 (CH aliphatic), ^1H NMR (DMSO-d_6, 400 MHz): δ 1.99 (s, 3H, CH_3), 3.40 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 7.49–8.28 (m, 8H, aromatic H). Anal. Calcd. For $C_{22}H_{19}N_3O_4$ (389.40): C, 67.86; H, 4.92; N, 10.79; Found: C, 67.90; H, 5.11; N, 10.79.

*4.1.8. General procedure for the synthesis of compounds (**9a–h**)*

A mixture of the requisite acid hydrazide **3a–d** (2 mmol) and diethyl malonate or diethyl 2-bromomalonate (4 mmol) and sodium methoxide (0.22 g, 4 mmol) was refluxed in methanol (20 ml) for 18 h. Excess solvent was removed under reduced pressure; the remaining residue was dissolved in water (20 ml). The aqueous solution was acidified with 2 N hydrochloric acid to pH 3–4. The precipitated solid was filtered, washed with water, dried and crystallized from ethanol.

*4.1.8.1. 1-(2-(4-Chlorophenyl)-6-methoxyquinoline-4-carbonyl)pyrazolidine-3,5-dione (**9a**).* Yield 86%; m.p. 236–238 °C; IR (KBr, cm^{-1}): 3171 (NH), 3018 (CH aromatic), 2932, 2839 (CH aliphatic), 1693 (3 C=O), 1614 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.92 (s, 3H, OCH_3), 4.03 (s, 2H, CH_2), 7.53–8.46 (m, 8H, aromatic H), 10.43 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.42 (CO—$\underline{C}H_2$—CO), 55.59 ($\underline{C}H_3$—O—), 103.42 ($\underline{C}$=C—OCH_3), 116.96 ($\underline{C}$=C), 122.63–131.87 (aromatic carbons), 134.42 ($\underline{C}$—Cl), 136.87 (C—$\underline{C}$ of phenyl), 144.60 ($\underline{C}$—CO), 152 ($\underline{C}$-phenyl), 158.43 ($\underline{C}$-OCH_3), 165.29 (HN—$\underline{C}$O—CH_2), 165.87 (N—$\underline{C}$O—CH_2), 167.66 (C—$\underline{C}$O—N). Anal. Calcd. For $C_{20}H_{14}ClN_3O_4$ (395.80): C, 60.69; H, 3.57; N, 10.62; Found: C, 60.64; H, 4.04; N, 10.36.

*4.1.8.2. 1-(2-(4-Bromophenyl)-6-methoxyquinoline-4-carbonyl)pyrazolidine-3,5-dione (**9b**).* Yield 73%; m.p. 242–244 °C; IR (KBr, cm^{-1}): 3163 (NH), 3008 (CH aromatic), 2924, 2840 (CH aliphatic), 1656 (3 C=O), 1614 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.92 (s, 3H, OCH_3), 4.02 (s, 2H, CH_2), 7.49–8.25 (m, 8H, aromatic H), 10.49 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. For $C_{20}H_{14}BrN_3O_4$ (440.25): C, 54.56; H, 3.21; N, 9.54; Found: C, 55.00; H, 3.36; N, 9.53.

*4.1.8.3. 1-(6-Methoxy-2-p-tolylquinoline-4-carbonyl)pyrazolidine-3,5-dione (**9c**).* Yield 65%; m.p. 224–226 °C; IR (KBr, cm^{-1}): 3250 (NH), 3050 (CH aromatic), 2930 (CH aliphatic), 1689 (3 C=O), 1615 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.37 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 3.98 (s, 2H, CH_2), 7.49–8.25 (m, 8H, aromatic H), 8.41 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. For $C_{21}H_{17}N_3O_4$ (375.38): C, 67.19; H, 4.56; N, 11.19; Found: C, 67.58; H, 4.70; N, 11.05.

*4.1.8.4. 1-(6-Methoxy-2-(4-methoxyphenyl)quinoline-4-carbonyl)pyrazolidine-3,5-dione (**9d**).* Yield 63%; m.p. 230–232 °C; IR (KBr, cm^{-1}): 3183 (NH), 3025 (CH aromatic), 2999, 2838 (CH aliphatic), 1674 (3 C=O), 1617 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.84 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.94 (s, 2H, CH_2), 7.08–8.27 (m, 8H, aromatic H), 10.43 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. For $C_{21}H_{17}N_3O_5$ (391.38): C, 64.45; H, 4.38; N, 10.74; Found: C, 64.33; H, 4.13; N, 10.72.

*4.1.8.5. 4-Bromo-1-(2-(4-chlorophenyl)-6-methoxyquinoline-4-carbonyl) pyrazolidine-3,5-dione (**9e**).* Yield 89%; m.p. 260–262 °C; IR (KBr, cm^{-1}): 3377 (NH), 3025 (CH aromatic), 2932 (CH aliphatic), 1654 (3 C=O), 1621 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.88 (s, 3H, OCH_3), 4.21 (s, 1H, pyrazole—C_4—H), 7.47–8.31 (m, 9H, 8 aromatic H + 1H, NH D_2O exchangeable). Anal. Calcd. For $C_{20}H_{13}BrClN_3O_4$ (474.69): C, 50.60; H, 2.76; N, 8.85; Found: C, 50.68; H, 2.63; N, 8.85.

*4.1.8.6. 4-Bromo-1-(2-(4-bromophenyl)-6-methoxyquinoline-4-carbonyl)-pyrazo-lidine-3,5-dione (**9f**).* Yield 82%; m.p. 282–284 °C; IR (KBr, cm^{-1}): 3385 (NH), 2928 (CH aliphatic), 1652 (3 C=O), 1621 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.91 (s, 3H, OCH_3), 3.96 (s, 1H, pyrazole—C_4—H), 7.47–8.33 (m, 9H, 8 aromatic H + 1H, NH). Anal. Calcd. For $C_{20}H_{13}Br_2N_3O_4$ (519.14): C, 46.27; H, 2.52; N, 8.09; Found: C, 46.77; H, 2.75; N, 7.90.

*4.1.8.7. 4-Bromo-1-(6-methoxy-2-p-tolylquinoline-4-carbonyl)pyrazolidine-3,5-dione (**9g**).* Yield 74%; m.p. 250–252 °C; IR (KBr, cm^{-1}): 3363 (NH), 3022 (CH aromatic), 2925 (CH aliphatic), 1649 (3 C=O), 1619 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.38 (s, 3H, CH_3), 3.92 (s, 1H, OCH_3), 4.02 (s, 1H, pyrazole—C_4—H), 7.34–8.32 (m, 8H, aromatic H), 10.00 (s, 1H, NH) Anal. Calcd. For $C_{21}H_{16}BrN_3O_4$ (454.27): C, 55.52; H, 3.55; N, 9.25; Found: C, 55.96; H, 4.15; N, 9.53.

*4.1.8.8. 4-Bromo-1-(6-methoxy-2-(4-methoxyphenyl)quinoline-4-carbonyl)pyrazo-lidine-3,5-dione (**9h**).* Yield 76%; m.p. 257–259 °C; IR (KBr, cm^{-1}): 3384 (NH), 3000 (CH aromatic), 2927, 2837 (CH aliphatic), 1659 (3 C=O), 1614 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.85 (s, 3H, OCH_3), 3.95 (s, 1H, OCH_3), 4.00 (s, 1H, pyrazole—C_4—H), 7.06–8.39 (m, 8H, aromatic H), 10.19 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. For $C_{21}H_{16}BrN_3O_5$ (470.27): C, 53.63; H, 3.43; N, 8.94; Found: C, 53.42; H, 3.91; N, 8.91.

*4.1.9. General procedure for the synthesis of compounds (**10a–h**)*

To a solution of the requisite **3a–d** (10 mmol) in dry DMF (10 ml), an equimolar amount of 2-(ethoxymethylene)malononitrile or 2-(1-ethoxyethylidene)-malononitrile (10 mmol) was added. The mixture was refluxed for 8 h. The reaction mixture was then cooled and poured on ice water (20 ml). The separated solid was filtered and crystallized from ethanol.

*4.1.9.1. 5-Amino-1-(2-(4-chlorophenyl)-6-methoxyquinoline-4-carbonyl)-1H-pyrazole -4-carbonitrile (**10a**).* Yield 79%; m.p. 300–302 °C; IR (KBr, cm^{-1}): 3418, 3255 (NH_2), 3020 (CH aromatic), 2928, 2833 (CH aliphatic), 2218 (CN), 1665 (C=O), 1620 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.92 (s, 3H, OCH_3), 4.79 (s, 2H, NH_2 D_2O-exchangeable), 7.46–8.29 (m, 9H, 8H aromatic H + 1H, pyrazole H). Anal. Calcd. For $C_{21}H_{14}ClN_5O_2$ (403.82): C, 62.46; H, 3.49; N, 17.34; Found: C, 62.56; H, 3.47; N, 17.24.

*4.1.9.2. 5-Amino-1-(2-(4-bromophenyl)-6-methoxyquinoline-4-carbonyl)-1H-pyrazole -4-carbonitrile (**10b**).* Yield 73%; m.p. 325–327 °C; IR (KBr, cm^{-1}): 3424, 3292 (NH_2), 3006 (CH aromatic), 2958, 2832 (CH aliphatic), 2222 (CN), 1679 (C=O) 1617 (C=N), 1578 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.95(s, 3H, OCH_3), 5.03 (s, 2H, NH_2 D_2O exchangeable), 7.12 (s,1H, pyrazole H), 7.46–8.39 (m, 8H, aromatic H). Anal. Calcd. For $C_{21}H_{14}BrN_5O_2$ (448.27): C, 56.27; H, 3.15; N, 15.62; Found: C, 56.57; H, 3.05; N, 15.28.

*4.1.9.3. 5-Amino-1-(6-methoxy-2-p-tolylquinoline-4-carbonyl)-1H-pyrazole-4-carbonitrile (**10c**).* Yield 68%; m.p. 290–292 °C; IR (KBr, cm^{-1}): 3232 (NH_2), 3018 (CH aromatic), 2930, 2832 (CH aliphatic), 2200 (CN), 1668 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.38 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 4.8 (s, 2H, NH_2 D_2O-exchangeable), 7.34–8.17 (m, 9H, 8H, aromatic H + 1H, pyrazole H). Anal. Calcd. For $C_{22}H_{17}N_5O_2$ (383.40): C, 68.92; H, 4.47; N, 18.27; Found: C, 69.17; H, 4.80; N, 18.90.

*4.1.9.4. 5-Amino-1-(6-methoxy-2-(4-methoxyphenyl)quinoline-4-carbonyl)-1H-pyrazole-4-carbonitrile (**10d**).* Yield 70%; m.p. 286–288 °C; IR (KBr, cm^{-1}): 3170 (NH_2), 3020 (CH aromatic), 2928, 2836 (CH aliphatic), 2216 (CN), 1671 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.88 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 6.00 (s, 2H, NH_2 D_2O-exchangeable), 7.09 (s, 1H, pyrazole H), 7.47–8.26 (m, 8H, aromatic H). Anal. Calcd. For $C_{22}H_{17}N_5O_3$ (399.40): C, 66.16; H, 4.29; N, 17.53; Found: C, 66.12; H, 4.07; N, 17.62.

*4.1.9.5. 5-Amino-1-(2-(4-chlorophenyl)-6-methoxyquinoline-4-carbonyl)-3-methyl-1H-pyrazole-4-carbonitrile (**10e**).* Yield 83%; m.p. 232–234 °C; IR (KBr, cm^{-1}): 3432, 3300 (NH_2), 3099 (CH aromatic), 2933, 2834 (CH aliphatic), 2198 (CN), 1695 (C=O), 1616 (C=N),

1572 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.00 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 7.50–8.43 (m, 8H, aromatic H), 11.12 (s, 2H, NH_2, D_2O-exchangeable). Anal. Calcd. For $C_{22}H_{16}ClN_5O_2$ (417.85): C, 63.24; H, 3.86; N, 16.76; Found: C, 62.92; H, 3.96; N, 16.78.

*4.1.9.6. 5-Amino-1-(2-(4-bromophenyl)-6-methoxyquinoline-4-carbonyl)-3-methyl-1H-pyrazole-4-carbonitrile (**10f**).* Yield 77%; m.p. 246–248 °C; IR (KBr, cm^{-1}): 3430, 3295 (NH_2), 3099 (CH aromatic), 2932, 2834 (CH aliphatic), 2223 (CN), 1694 (C=O), 1617 (C=N), 1572 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.05 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 6.43 (s, 2H, NH_2, D_2O-exchangeable), 7.55–8.50 (m, 8H, aromatic H). Anal. Calcd. For $C_{22}H_{16}BrN_5O_2$ (462.30): C, 57.16; H, 3.49; N, 15.15; Found: C, 57.20; H, 3.60; N, 15.25.

*4.1.9.7. 5-Amino-1-(6-methoxy-2-p-tolylquinoline-4-carbonyl)-3-methyl-1H-pyrazole-4-carbonitrile (**10g**).* Yield 74%; m.p. 221–223 °C; IR (KBr, cm^{-1}): 3414, 3299 (NH_2), 3097 (CH aromatic), 2921, 2840 (CH aliphatic), 2224 (CN), 1696 (C=O), 1617 (C=N), 1571 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.99 (s, 3H, CH_3), 2.10 (s, 3H, CH_3)m 3.94 (s, 3H, OCH_3), 6.85 (s, 2H, NH_2, D_2O-exchangeable), 7.36–8.46 (m, 8H, aromatic H). Anal. Calcd. For $C_{23}H_{19}N_5O_2$ (397.43): C, 69.51; H, 4.82; N, 17.62; Found: C, 69.80; H, 5.05; N, 17.64.

*4.1.9.8. 5-Amino-1-(6-methoxy-2-(4-methoxyphenyl)quinoline-4-carbonyl)-3-methyl-1H-pyrazole-4-carbonitrile (**10h**).* Yield 76%; m.p. 226–228 °C; IR (KBr, cm^{-1}): 3423, 3297 (NH_2), 3020 (CH aromatic), 2931, 2841 (CH, aliphatic), 2216 (CN), 1693 (C=O), 1626 (C=N), 1562 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.00 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 7.45–8.44 (m, 10H, aromatic H + NH_2, D_2O-exchangeable). Anal. Calcd. For $C_{23}H_{19}N_5O_3$ (413.43): C, 66.82; H, 4.63; N, 16.94; Found: C, 66.91; H, 4.82; N, 16.62.

*4.1.10. General procedure for the synthesis of compounds (**11a–h**)*

To a solution of **3a–d** (1 mmol) in dry DMF (4 ml), an equimolar amount of ethyl 2-cyano-3-ethoxyacrylate or ethyl 2-cyano-3-ethoxybut-2-enoate (1 mmol) was added. The mixture was refluxed for 8 h. The reaction mixture was then cooled and poured on ice water (20 ml). The separated solid was filtered and crystallized from ethanol.

*4.1.10.1. Ethyl 5-amino-1-(2-(4-chlorophenyl)-6-methoxyquinoline-4-carbonyl)-1H-pyrazole-4-carboxylate (**11a**).* Yield 88%; m.p. 229–231 °C; IR (KBr, cm^{-1}): 3452, 3345 (NH_2), 3020 (CH aromatic), 2983, 2936 (CH aliphatic), 1700 (2 C=O), 1614 (C=N), 1551 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.27 (t, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.26 (q, 2H, CH_2), 7.13 (s, 1H, pyrazole H), 7.50–8.41 (m, 8H, aromatic H), 9.51 (s, 2H, NH_2, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.40 ($CH_2\underline{C}H_3$), 55.70 ($\underline{C}H_3$—O), 59.38 ($\underline{C}H_2CH_3$), 93.64 ($\underline{C}$=C—OCH_3), 102.78 ($\underline{C}$=C—NH_2), 118.14 ($\underline{C}$=C), 123–139.07 (aromatic carbons), 136.75 ($\underline{C}$—Cl) 139.07 (C—$\underline{C}$ of phenyl), 143.74 (pyrazole carbon), 144.08 ($\underline{C}$—CO), 151.46 ($\underline{C}$—NH_2), 153.77 ($\underline{C}$–phenyl), 158.27 ($\underline{C}$—OCH_3), 162.85 (C=O ester), 169.11 (C—$\underline{C}$O—N). Anal. Calcd. For $C_{23}H_{19}ClN_4O_4$ (450.87): C, 61.27; H, 4.25; N, 12.43; Found: C, 61.16; H, 4.30; N, 12.61.

*4.1.10.2. Ethyl 5-amino-1-(2-(4-bromophenyl)-6-methoxyquinoline-4-carbonyl)-1H-pyrazole-4-carboxylate (**11b**).* Yield 89%; m.p. 238–239 °C; IR (KBr, cm^{-1}): 3459, 3348 (NH_2), 3020 (CH aromatic), 2943, 2838 (CH aliphatic), 1692 (2 C=O), 1616 (C=N), 1552 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.28 (t, 3H, CH_3), 3.91 (s, 3H, OCH_3) 4.26 (q, 2H, CH_2), 7.11 (s, 1H, pyrazole H), 7.50–8.39 (m, 10H, aromatic H + NH_2, D_2O exchangeable). Anal. Calcd. For $C_{23}H_{19}BrN_4O_4$ (495.33): C, 55.77; H, 3.87; N, 11.31; Found: C, 55.49; H, 3.73; N, 11.40.

*4.1.10.3. Ethyl 5-amino-1-(6-methoxy-2-p-tolylquinoline-4-carbonyl)-1H-pyrazole-4-carboxylate (**11c**).* Yield 65%; m.p. 223–225 °C; IR (KBr, cm^{-1}): 3493, 3224 (NH_2), 3005 (CH aromatic), 2926, 2837 (CH aliphatic), 1689 (2 C=O), 1617 (C=N), 1544 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.43 (t, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3) 4.49 (q, 2H, CH_2), 7.34–8.42 (m, 9H, aromatic H, 1H, pyrazole H), 9.61 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. For $C_{24}H_{22}N_4O_4$ (430.46): C, 66.97; H, 5.15; N, 13.02; Found: C, 67.03; H, 5.37; N, 13.31.

*4.1.10.4. Ethyl 5-amino-1-(6-methoxy-2-(4-methoxyphenyl)quinoline-4-carbonyl)-1H-pyrazole-4-carboxylate (**11d**).* Yield 62%; m.p. 224–226 °C; IR (KBr, cm^{-1}): 3466, 3347 (NH_2), 3084 (CH aromatic), 2942, 2834 (CH aliphatic), 1712 (2 C=O), 1616 (C=N), 1554 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.26 (t, 3H, CH_3), 3.91 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 4.26 (q, 2H, CH_2), 7.07 (s, 1H, pyrazole H), 7.46–8.31 (m, 8H, aromatic H), 9.31 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. For $C_{24}H_{22}N_4O_5$ (446.46): C, 64.57; H, 4.97; N, 12.55; Found: C, 64.39; H, 4.81; N, 12.50.

*4.1.10.5. Ethyl 5-amino-1-(2-(4-chlorophenyl)-6-methoxyquinoline-4-carbonyl)-3-methyl-1H-pyrazole-4-carboxylate (**11e**).* Yield 86%; m.p. 230–232 °C; IR (KBr, cm^{-1}) 3464, 3345 (NH_2), 3062 (CH aromatic), 2933, 2836 (CH aliphatic), 1703 (2 C=O), 1616 (C=N), 1550 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.28 (t, 3H, CH_3), 2.08 (s, 3H, CH_3) 3.83 (s, 3H, OCH_3), 4.27 (q, 2H, CH_2), 7.09–8.38 (m, 8H, aromatic H), 9.56 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. For $C_{24}H_{21}ClN_4O_4$ (464.90): C, 62.00; H, 4.55; N, 12.05; Found: C, 61.80; H, 4.47; N, 11.85.

*4.1.10.6. Ethyl 5-amino-1-(2-(4-bromophenyl)-6-methoxyquinoline-4-carbonyl)-3-methyl-1H-pyrazole-4-carboxylate (**11f**).* Yield 81%; m.p. 232–234 °C; IR (KBr, cm^{-1}): 3463, 3344 (NH_2), 3063 (aromatic H), 2932, 2838 (CH aliphatic), 1701 (2 C=O), 1615 (C=N), 1550 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.28 (t, 3H, CH_3), 2.09 (s, 3H, CH_3) 3.83 (s, 3H, OCH_3), 4.25 (q, 2H, CH_2), 7.50–8.39 (m, 8H, aromatic H), 9.22 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. For $C_{24}H_{21}BrN_4O_4$ (509.35): C, 56.59; H, 4.16; N, 11.00; Found: C, 56.37; H, 4.00; N, 10.83.

*4.1.10.7. Ethyl 5-amino-1-(6-methoxy-2-p-tolylquinoline-4-carbonyl)-3-methyl-1H-pyrazole-4-carboxylate (**11g**).* Yield 64%; m.p. 228–229 °C; IR (KBr, cm^{-1}): 3466, 3344 (NH_2), 3021 (CH aromatic), 2917, 2840 (CH aliphatic), 1692 (2 C=O), 1614 (C=N), 1550 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.28 (t, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.38 (s, 3H, CH_3) 3.82 (s, 3H, OCH_3), 4.25 (q, 2H, CH_2), 7.34–8.31 (m, 8H, aromatic H), 9.15 (s, 2H, NH_2, D_2O-exchangeable). Anal. Calcd. For $C_{25}H_{24}N_4O_4$ (444.48): C, 67.55; H, 5.44; N, 12.60; Found: C, 67.32; H, 5.33; N, 12.76.

*4.1.10.8. Ethyl 5-amino-1-(6-methoxy-2-(4-methoxyphenyl)quinoline-4-carbonyl)-3-methyl-1H- pyrazole-4-carboxylate (**11h**).* Yield 66%; m.p. 220–221 °C; IR (KBr, cm^{-1}): 3444, 3327 (NH_2), 3066 (CH aromatic), 2926, 2835 (CH aliphatic), 1698 (2 C=O), 1610 (C=N), 1551 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.28 (t, 3H, CH_3), 2.09 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.28 (q, 2H, CH_2), 7.11–8.30 (m, 8H, aromatic H), 9.44 (s, 2H, NH_2, D_2O exchangeable). Anal. Calcd. For $C_{25}H_{24}N_4O_5$ (460.48): C, 65.21; H, 5.25; N, 12.17; Found: C, 65.05; H, 5.39; N, 12.30.

4.2. Molecular docking methodology

All the molecular modeling studies were carried out on Intel Pentium 1.6 GHZ processor, 512 MB memory with Windows XP operating system using Molecular Operating Environment (MOE 2007-09 Chemical Computing Group, Canada) 24 as the computational software.

4.2.1. Target compounds optimization

The compounds were constructed using builder interface of MOE program, and then were subjected to energy minimization with MMFF94X force and the partial charges were automatically calculated. Systematic conformational import of the MOE is used to produce different conformers for each compound. The obtained database was then saved as mdb files be used in the docking calculations.

4.2.2. Preparation of the enzyme structure

The 3D coordinations of the inhibitor celecoxib of murine COX-2 enzyme (PDB code: 6cox) were obtained from the freely accessible Protein data bank and used for molecule docking studies. In this structure, three ligands are bound to the enzyme: HEM (protoporphyrin IX containing FE), NAG (N-acetyl-D-glucosamine) and celecoxib. The non-relevant compounds HEM and NAG were removed for the study.

4.2.3. Docking of the target molecules to the COX-2 active site

Docking of the conformational database of the compounds was done using MOE-dock software through the following methodology: (i) the enzyme active site was loaded and the dock tool was initiated where the program parameters were adjusted as follow: ligand atoms as the docking site, triangle matcher as the placement methodology, London dG as scoring methodology, and Forcefield as refinement (default values used) and (ii) the mdb file of the ligands to be docked was loaded and Dock calculations were run. The obtained ligand-enzyme complex model was then used in the calculation of the energy parameters using the MMFF94x force filed.

4.3. Pharmacology

4.3.1. In vitro cyclcooxygenese (COX) inhibition assay

The ability of the test compounds to inhibit ovine COX-1 and COX-2 was determined using an enzyme immune assay [EIA] (catalog No. 560101, Cayman Chemicals, Ann Arbor. MI) according to the reported procedure [37].

4.3.2. In vivo Anti-inflammatory activity

The rat hind paw oedema method [29] was applied to determine the anti-inflammatory activity of the test compounds using celecoxib as a standard. Mature albino rats of both sexes weighing 200–250 gm were used. The animals were divided into 15 equal groups (each of 5). The first was left as control, while the second group was injected (i.p.) with celecoxib at a dose of 1.8 mg/100 gm. The test compounds were injected (i.p.) to the remaining groups at a dose of 1.8 mg/100 gm. One hour later, oedema in the right hind paw was induced by injection of 0.1 ml of 10% carragenin. The thickness of the paw was measured at 60, 120, 180 and 240 min after carragenin injection to determine the anti-inflammatory activity of the test compounds (Table 1).

4.3.3. Analgesic activity

The hot plate method of Jacob and Bsovski [32] was used to evaluate the analgesic activity. Mature albino mice of both sexes weighing 20–25 gm were classified into 15 groups (each of 5). The first group was left as control and injected (i.p.) with the solvent (DMSO), whereas, the second group was injected (i.p.) with celecoxib at a dose of 1.8 mg/100 gm. Each of the remaining groups was injected (i.p.) with the test compound in the same dose 1.8 mg/100 gm. Ten minutes later, each mouse was placed in a two liter–beaker immersed in a water bath thermostatically at 56 °C. The time elapsed till the mouse licks its paw or jumps was considered as the reaction time and was taken as a measure of the analgesic effect. Readings were taken at 10, 20, 30, 60, 90 and 120 min post treatment (Table 2).

4.3.4. Ulcerogenic activity

The anti-inflammatory tested compounds and celecoxib were tested for their ulcerogenic activity using indomethacin as reference drug. Male albino rats weighing 180–200 gm were fasted for 12 h prior to drug administration. The animals were divided into 16 equal groups (each of 5). The first received 1% gum acacia (suspending vehicle) orally once a day and was left as a control, whereas, the second group received indomethacin at a dose of 1.8 mg/100 gm/day orally. The third group received celecoxib at a dose of 1.8 mg/100 gm/day orally. The remaining groups received the test compounds at a dose of 1.8 mg/100 gm/day orally. The drugs were administered once a day for three successive days. The animals were killed by overdose of ether 6 h after the last dose. The stomach was removed, opened along the great curvature and examined for ulceration. The number and severity of discrete areas of damage in the glandular mucosa were scored (Table 3). The ulcer score was calculated according to the 1 to 5 scoring system of Wilhelmi and Menassa-Gdynia [33] as follow: (1) 1 or 2 min sporadic punctuate lesions, (2) several small lesions, (3) one extensive lesion or multiple moderate-sized lesions, (4) several large lesions, and (5) several large lesions with stomach perforation. Stomach ulceration was expressed in terms of ulcer index (U.I. = mean ulcer score of a group of animals similarly tested % of ulcerated animals of this group) [34].

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