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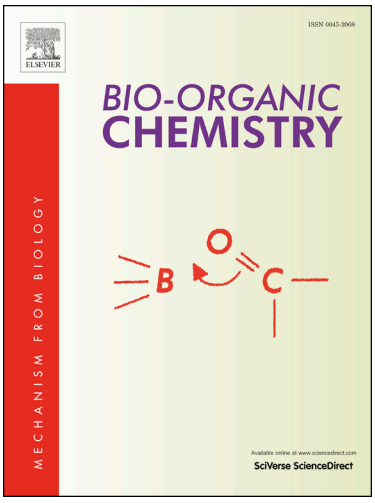

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Synthesis, anti-Inflammatory screening, molecular docking, and COX-1,2/-5-LOX inhibition profile of some novel quinoline derivatives

Ibrahim Chaaban [a], Ola H. Rizk[a, b, *], Tamer M. Ibrahim [c], Shery S. Henen [a], El-Sayeda M. El-Khawass [a], Aida E .Bayad [d], Ibrahim M. El-Ashmawy [d,e], Hisham A. Nematalla [f]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt

[b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Pharos University in Alexandria, 21311, Egypt.

[c] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt

[d] Pharmacology Department, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt

[e] Department of Veterinary Medicine, Faculty of Agricultural and Veterinary Medicine, Qassim University, P.O.Box 1482, Buraydah, Al-Qassim, Saudi Arabia

[f] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Pharos University in Alexandria, 21311, Egypt.

ABSTRACT

New quinoline compounds comprising pyrazole scaffold through different amide linkages were synthesized. The synthesized compounds were evaluated for their anti-inflammatory activity. Eight compounds (**5c, 11b,c, 12c, 14a,b, 20a** and **21a)** were found to exhibit promising anti-inflammatory profiles in acute and sub-acute inflammatory models. They were screened for their ulcerogenic activity and none of them showed significant ulcerogenic activity comparable to the reference drug celecoxib and are well tolerated by experimental animals with high safety margin (ALD_{50}> 0.3 g/kg). Compounds **5c, 11b,c, 12c, 14a,b, 20a** and **21a** showed significant *in vitro* LOX inhibitory activity higher than that of zileuton. In vitro COX-1/COX-2 inhibition study revealed that compounds **12c, 14a,b** and **20a** showed higher selectivity towards COX-2 than COX-1. Among the tested compounds, **12c, 14a** and **14b** showed the highest inhibitory activity against COX-2 with an IC_{50} values of 0.1, 0.11 and 0.11μM respectively. The docking experiments attempted to postulate the binding mode for the

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most active compounds in the binding site of COX-2 enzymes and confirmed the high selectivity binding towards COX-2 enzyme over COX-1.

Key words

Quinoline; pyrazole; anti-inflammatory; COX; 5-LOX; docking

1. Introduction

Inflammation is a complicated response of body tissues that arises in response to various infectious, traumatic or autoimmune diseases, a process that often leads to recovery from infection and healing. However, if not properly faced, inflammation can result in persistent tissue damage by infiltrating leukocytes, lymphocytes or damaged collagen [1]. Targeting cyclooxygenase (COX) enzymes is a well-established strategy to treat inflammation. In this context, inhibiting COX enzyme-associated prostanoid synthesis is established by using non-steroidal anti-inflammatory drugs (NSAIDs) [2-4]. Nevertheless, long-term clinical usage of NSAIDs is usually associated with significant side effects including gastro-duodenal erosions, ulceration and nephrotoxicity [5].

Consequently, selective COX-2 inhibitors with lower gastrointestinal side effects compared with classical NSAIDs were discovered and approved such as celecoxib, valdecoxib and rofecoxib. Nonetheless, many of them has reported cardiovascular side effects, and hence, withdrawn from the market [6,7]. Inhibition of COX-1/COX-2 ensues increased accumulation of leukotrienes through the lipoxygenase (LOX) pathway [8]. LOX catalyzes hydroperoxidation of fatty acids to peroxides [9]. Also, 5-LOX has been connected to several unwanted effects which are part of the progression of inflammation, osteoarthritis and asthma [10,11]. Therefore, dual inhibition of 5-LOX/COX would offer anti-inflammatory effects with lower side effects [12].

Literature survey revealed that substituted quinoline derivatives constitute a promising group in the search for new more effective anti-inflammatory and analgesic drugs **(Figure 1)** [13-17]. On the other hand, pyrazoles were found to occupy a significant position in Medicinal Chemistry because of their capability to exhibit a wide range of bioactivities, particularly as anti-inflammatory [18-20] and analgesic agents [21,22], in addition to their prominent COX-2 inhibitory activity [23,24]. In specific, the selective COX-2 inhibitor celecoxib (**Figure 1**) comprises a substituted diaryl pyrazole as a core structure [25,26].

Encouraged by these findings, it was found interesting to synthesize and evaluate the biological activity of some novel structure hybrids encountering the quinoline backbone bearing a substituted pyrazole moiety aiming to obtain compounds with enhanced anti-inflammatory activity. The targeted substituted quinolines were designed so as to

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comprise the 1,3-diaryl pyrazole, pyrazole-3-carbohydrazide, 3,5-dimethylpyrazole and pyrazolidinedione counterparts through different linkages (Structures A-D, **Figure 1**).The substitution pattern of the synthesized compounds was carefully selected so as to confer different electronic and lipophilic properties to the molecules. All the synthesized compounds were investigated for their *in vivo* anti-inflammatory activity and the most active compounds were further evaluated for their *in vitro* COX/5-LOX inhibition.A molecular docking study was also performed for derivatives of highest anti-inflammatory activity in a trial to investigate the possible mode of action of these compounds.

2. Results and discussion

2.1. Chemistry

The synthetic pathways employed for the synthesis of the intermediate and final compounds are depicted in schemes 1-3.

In scheme 1, Pfitzinger reaction [13] was adopted to synthesize 6-chloro-2-methylquinoline-4-carboxylic acid **1** in a very good yield through the condensation of 5-chloroisatin with acetone in potassium hydroxide solution as described earlier [27]. Structure of compound **1** was confirmed by elemental microanalyses, IR, ^{1}H-NMR in addition to ^{13}C-NMR spectra. IR spectrum revealed absorption bands corresponding to OH and carboxylic C=O at 3413 cm^{-1} and 1707 cm^{-1} respectively, ^{1}H-NMR spectrum was characterized by the presence of CH_3, quinolyl and OH protons at their expected chemical shifts. while ^{13}C-NMR showed signals at 25.13 ppm and 167.51 ppm due to CH_3 and C=O respectively.

Esterification of the acid **1** with ethanol in the presence of concentrated sulfuric acid yielded the key intermediate ethyl 6-chloro-2-methylquinoline-4-carboxylate **2**. ^{1}H-NMR spectrum of compound **2** revealed a triplet at 1.49 ppm and a quartet at 4.52 ppm assigned to $-CH_2CH_3$ protons. Refluxing **2** with 99% hydrazine hydrate in ethanol furnished the corresponding quinoline-4-carbohydrazide derivative **3** [28,29]. ^{1}H-NMR showed the disappearance of $-CH_2CH_3$ protons and the appearance of D_2O exchangeable protons assigned to the NH_2 and NH groups at 4.68 ppm and 9.91 ppm respectively.

On the other hand, 1H-pyrazole-4-carbaldehydes **4a-c** were adequately prepared utilizing a previously reported procedure through Vilsmeier-Haack reaction [30].The IR spectra of the pyrazole 4-carboxaldehydes **4a,c** showed a characteristic peak at 1684-1671 cm^{-1}, which was attributed to the aldehydic C=O group. Their ^{1}H-NMR spectra demonstrated two singlets due to pyrazole-C5-H and the aldehydic proton. Condensing the acid hydrazide **3** with the appropriate aromatic aldehyde **4a-c** in ethanol afforded the target hydrazones **5a-c** [31,32]. The molecular structures of compounds **5a-c** were confirmed on the basis of their elemental analyses and spectral data. ^{1}H-NMR spectra of compounds **5a-c** lacked singlets assigned to NH_2 and aldehydic protons at their previously recorded

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chemical shifts and showed the presence of a singlet assigned to CH=N at 8.43-8.47 ppm and a singlet due to NH at 12.04-12.14 ppm. The MS spectrum of **5c** showed a molecular ion peak ($M^{+\bullet}$) at m/z 530 (15%) which matches its molecular formula $C_{28}H_{21}Cl_2N_5O_2$. Moreover, ^{13}C-NMR for compound **5c** provided an additional proof for the proposed structures of compounds **5a-c**.

Furthermore, cyclocondensation of the hydrazide **3** with 1,3-dicarbonyl compounds namely (acetyl acetone and diethyl malonate) aimed at producing quinoline derivatives bearing pyrazolyl/pyrazolidinyl 1-carbonyl moieties at 4-position. In an attempt to synthesize the 3,5-dimethylpyrazole derivative **7** by heating with acetyl acetone in ethanol containing a catalytic amount of acetic acid, [33-35] the hydrazone **6** was isolated. Whereas, the target compound 6-chloro-4-[(3,5-dimethyl-1H-pyrazol-1-yl)carbonyl]2-methylquinoline **7** was prepared by fusing the hydrazide **3** with excess acetyl acetone at 110 °C. ^{1}H-NMR spectrum of compound **7** was characterized by the disappearance of singlets previously assigned to NH and NH_2 protons and the appearance of two singlets assigned to pyrazole C-3 and C-5 CH_3 protons at 2.73 ppm and 2.12 ppm respectively as well as a singlet corresponding to pyrazole C-4 proton in the aromatic region. Its mass spectrum showed the molecular ion peak ($M^{+\bullet}$) at m/z 299 (37%) corresponding to $C_{16}H_{14}ClN_3O$. An additional confirmation of the proposed structure was achieved through its ^{13}C-NMR spectrum which revealed 2 signals at 13.36 ppm and 13.87 ppm corresponding to pyrazole C-3 and C-5 CH_3. In contrast, fusion of the hydrazide **3** with diethyl malonate at 160˚C [36] afforded the open chain compound **8** while performing the reaction in refluxing ethanol containing sulfuric acid yielded the pyrazolidine-3,5-dione compound **9**. The structure of compound **9** was identified by IR, ^{1}H-NMR and ^{13}C-NMR spectra. ^{1}H-NMR spectrum was characterized by the appearance of a singlet at 3.73 ppm integrated for pyrazolidine C-4 protons. Meanwhile the ^{13}C-NMR spectrum showed 3 signals at 159.36, 165.05 and 165.37 ppm attributed to 3 C=O carbons.

Scheme 2, It is well known that a methyl group positioned α- to an aromatic heterocyclic nitrogen atom, carries acidic hydrogens. Thus this methyl possesses high tendency to form a resonance stabilized carbanion. Consequently, such compounds are able to condense with aldehyde or ketones forming C=C bond. Based on this fact, compound **2** was allowed to condense with 1,3-diaryl pyrazole-4-carbaldehydes **4a-c** through its α-methyl group to afford compounds **11a-c**. This reaction was performed in refluxing acetic anhydride according to reported procedure [37,38]. Similarly, synthesis of compounds **12a-c** was achieved by heating the ester **2** with morpholine under reflux to afford **10** which was subsequently reacted with **4a-c** by heating in acetic anhydride to furnish the desired compounds. The molecular structures of the prepared compounds **11a-c** and **12a-c** were confirmed on the basis of their elemental microanalyses, IR, ^{1}H-NMR spectra for all compounds as well as ^{13}C-NMR and mass spectra for some representative examples.

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^{1}H-NMR spectra of compounds **11a-c** were characterized by triplet and quartet assigned to -CH_2CH_3 protons in addition to aryl, quinolyl, -CH=CH- and pyrazolyl C-5 protons at their expected chemical shifts. The mass spectrum of compound **11a** showed a molecular ion peak ($M^{+\bullet}$) at m/z 548 (41%) corresponding to $C_{29}H_{20}Cl_3N_3O_2$. While the ^{1}H-NMR spectra of compounds **12a-c** revealed multiplets assigned to morpholinyl protons in addition to aryl, quinolyl, -CH=CH- and pyrazolyl C-5 protons at their expected chemical shifts. Investigating the ^{13}C-NMR spectra of compound **11a** and **12c** provided a substantial affirmation for the assigned structure of these compounds. The chalcones **14a,b** were prepared through the reaction of **10** with aryl glyoxal **13a,b** in acetic anhydride[37,38]. The ^{1}H-NMR spectra of chalcones **14a,b** showed the presence of two doublets, each assigned to a vinylic proton with a coupling constant 15.6-15.8 Hz indicating their existence as E isomers [39]. Attempts to cyclize the chalcones **14a,b** into the corresponding *N*-arylpyrazole derivatives **15a-d**using phenyl hydrazine and aryl hydrazines utilizing reaction conditions previously reported for the synthesis of analogous compounds [40] went in vain.

Scheme 3, Knorr pyrazole synthesis [41] was adopted for the synthesis of the pyrazole-3-carboxylate esters **16a-c** which were then reacted with hydrazine hydrate in ethanol to produce the respective hydrazides **17a-c** [42]. IR of compound **17a-c** exhibited strong absorption bands attributed to NH_2, NH, C=O and C=N. Moreover, oxidation of 2-methyl quinoline derivatives **2** and **10** using selenium dioxide in dioxane afforded the corresponding aldehydes **18** and **19** [43]. The latter compounds were then stirred at room temperature with the appropriate 1,5-diaryl-1H-pyrazole-3-carbohydrazide **17a-c** in glacial acetic acid to furnish the target hydrazones **20a-c** and **21a-c** respectively [44]. The structures of the target compounds **20a-c** and **21a-c** were determined by elemental microanalyses and by studying the IR, ^{1}H-NMR spectra for all compounds as well as ^{13}C-NMR and mass spectra for some representative examples. IR spectra of compounds **20a-c** and **21a-c** were characterized by absorption bands assigned to NH, C=O, C=N and C-O-C functional groups. ^{1}H-NMR spectra of compounds **20a-c** and **21a-c** revealed the disappearance of the aldehydic and NH_2 protons and the appearance of singlets assigned to CH=N, D_2O exchangeable NH andpyrazole-C4 protons, which therefore indicates the successful combination of the two heterocyclic moieties. Finally ^{13}C-NMR spectra of **20a** and **21b** provided a further confirmation for their intended structures.

2.2. Biological evaluation

2.2.1. Anti-inflammatory activity

The anti-inflammatory (AI) activity of the synthesized compounds was evaluated by utilizing the formalin-induced paw edema bioassay [45, 46] using celecoxib (20 mg/kg) as a reference standard anti-inflammatory agent. The paw edema was employed as a

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model for acute and sub-acute inflammation. The data obtained were presented in **(Tables 1** and **2)** and expressed as means ± SE. Statistical differences of control and test groups was carried out using the Analysis of Variance (ANOVA) followed by 'Student–Newman–Keuls Multiple Comparison Test. They were performed using computer package of the Statistical Analysis System (SAS, 1987), SAS Incorporation Institute. The difference in results was considered significant when $p < 0.05$.

2.2.1.1. Formalin-induced paw edema bioassay (acute inflammatory model).

In this acute inflammatory model [45,46], each test compound was dosed orally (20 mg/kg body weight) 1 h prior to induction of inflammation by formalin injection. Celecoxib was utilized as a reference drug at a dose of 20 mg/kg, po. The anti-inflammatory activity was then calculated 1–4 h after induction and presented in **(Table 1)** as the mean paw volume (mL) in addition to the percentage anti-inflammatory activity (AI %).

A comparative study of the anti-inflammatory activity of the test compounds relative to the reference drug celecoxib at different time intervals revealed the following:

After one hour, twelve compounds showed distinctive pharmacokinetic profiles as shown from their rapid onset of action which was higher than celecoxib at a dose of 20 mg/kg, po. A remarkable AI% was recorded for compounds **12c, 14a,b, 20a** (78%); **5c, 11b** (75%); **7, 11c, 12b, 21c** (71%) and **21a** (67%) when compared to celecoxib (60%). In addition, compound **21b** showed good AI% (60%) which was equivalent to the reference drug. After two hours interval, the data indicated that eight compounds namely; **5c, 14a** (75%); **11b** (72%); **11c, 12c** (70%); **20a** (67%) and **21a,b** (64%) showed significant anti-inflammatory activity superior to celecoxib (59%). Whereas, compound **12b** (59%) displayed equipotent activity to the standard drug. The other tested compounds were less potent than the reference drug. Regarding the AI activity after four hours' time interval as a criterion for comparison, it was found that four compounds; **11c** (86%), **11b** (74%), **12c** (70%) and **14a** (66%) showed remarkable effect in inhibiting the paw edema when compared to celecoxib (66%). Whereas, compounds **5c, 14b** (64%), **20a** (62%) and **21a** (60%) showed moderate activity. The most active compounds **5c, 11b,c, 12c, 14a,b**, **20a** and **21a** were further tested at 5, 10, 20, 40 and 50 mg/kg body weight in order to determine their ED_{50} values after two hours. Compound **11c** was found to be more potent (ED_{50} = 10.70 mg/kg, **Table 1**) than celecoxib (ED_{50} = 15.11 mg/kg), whereas the rest of the compounds were found to be nearly equipotent to the reference drug (ED_{50} = 13.15-17.12 mg/kg).

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2.2.1.2. Formalin-induced paw edema bioassay (sub-acute inflammatory model).

For this sub-acute inflammatory model [45,46], inflammation was induced by formalin injection in the first and third days, and test compounds were administered once orally (at 20 mg/kg daily) for 7 days. Again, celecoxib was used as a reference AI agent. In this assay, the anti-inflammatory activity was calculated at 1st and 8th days after induction and presented in **(Table 2)** as the mean paw volume and the percentage anti-inflammatory activity (AI %).

The results obtained revealed that at the first day, six compounds namely; **5c, 14a, 20a, 21c, 21a** and **11b** displayed significant anti-inflammatory activity (74, 62, 62, 62, 60 and 57% respectively) which was higher than celecoxib (51%). Meanwhile, compounds **7** and **14b** were nearly equivalent to the reference celecoxib with AI 48%. The data obtained at day eight revealed that compound **14b** (62%); **5c, 11c** (58%) and **20a** (56%) were found to be nearly as effective as the reference celecoxib (60%). The other compounds exhibited moderate to weak activity.

Further interpretation of the anti-inflammatory activity of the test compounds in pre-mentioned screens **(Tables 1, 2)** revealed that **5c, 11b,c, 14a,b**, **20a** and **21a** showed remarkable activity in both the acute and subacute inflammatory models. On the other hand, compounds **12b, 12c** and **21b** displayed pronounced activity in the acute inflammatory model nevertheless they proved to be less active in the subacute inflammatory model. This fact would suggest that such type of compounds might be effective in managing acute inflammation, while they would be less effective in controlling chronic inflammatory conditions.

2.2.1.3. Ulcerogenic activity

Eight compounds namely; **5c, 11b,c, 12c, 14a,b**, **20a** and **21a** that exhibited promising anti-inflammatory profiles in the pre-mentioned animal models were further evaluated for their ulcerogenic potential in rats [47]. Gross observation of the isolated rat stomach showed a normal stomach texture for compounds **12c** and **14b** with no observable hyperemia indicating a superior GI safety profile (no ulceration) in the population of the test animals at an oral dose of 300 mg/kg, when administered twice daily in fasted rats. Whereas, compounds **5c, 11b, c, 14a, 20a** and **21a** showed weak ulceration effect (10-20%) compared to the reference drug celecoxib which was found to cause no ulceration under the same experimental conditions. It is worth to mention that, indomethacin; the reference standard AI drug was found to cause 100% ulceration.

2.2.1.4. Acute toxicity

The same selected compounds **5c, 11b,c, 12c, 14a,b, 20a** and **21a** were further evaluated for their approximate acute lethal dose ALD_{50} in male rats using a literature method [48].

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The results indicated that all of the tested compounds proved to be non-toxic and are well tolerated by the experimental animals. The compounds showed a high safety margin when screened at graded doses (0.1–0.4 g/kg, po), where their ALD_{50} values were found to be > 0.4 g/kg.

2.2.2. In vitro COX-1/COX-2 inhibition assay

The same selected compounds **5c, 11b,c, 12c, 14a,b, 20a** and **21a** were further evaluated for their ability to inhibit COX-1 and COX-2 isoenzymes using the in vitro colorimetric COX (ovine) inhibitor assay method [49], which utilizes the peroxidase component of cyclooxygenase. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD), which is produced during the reduction of PGG2 to PGH2, at 590 nm. The efficacies of the tested compounds were determined as the concentration causing 50% enzyme inhibition (IC_{50}) and recorded in **Table 3**.

The data revealed that the compounds **12c, 14a,b and 20a** showed higher selectivity towards COX-2 than COX-1. Among the tested compounds, **12c, 14a** and **14b** showed the highest inhibitory activity against COX-2 with an IC_{50} values of 0.1, 0.11 and 0.11μM respectively whereas **11b** was the least selective towards COX-2 inhibition with an IC_{50} value of 0.63 μM. These results indicate that the most active compounds possess selective inhibitory activity towards COX-2 enzyme.

2.2.3. In vitro LOX inhibition assay

The same selected compounds **5c, 11b,c, 12c, 14a,b, 20a** and **21a** were evaluated for their in vitro ability to inhibit 5-lipoxygenase enzyme, using Abnova 5-lipoxygenase inhibitor screening assay kit (catalog no. KA1329, Cayman Chemicals), according to the manufacturer's instructions. The results were recorded in **Table 3** as the compound's concentration causing 50% enzyme inhibition (IC50) and they are the means of three determinations. All compounds showed comparable range of 5-LOX inhibitory activity with IC_{50} values range 1.76 - 2.24 μM. Such activities are comparable to the reference diclofenac sodium. However, comparing their activity to zileuton as a reference drug appears to show zileuton possessing the least inhibitory activity (4.51 μM), nevertheless, with the same range of low micromolar range of activity. In conclusion, these results highlight promising 5-LOX inhibitory activity of the tested compounds.

2.2.4. Molecular docking

The aim of the modeling pat in our study is to rationalize the in vitro biological results observed for the most active compounds towards COX-2 inhibition.

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2.2.4.1. COX-2 docking

Selecting a definite X-ray structure for COX-2 as a docking model does not emerge to be significant according to literature [38, 50]. We selected a celecoxib co-crystallized model (PDB code: 3NL1) since it is used as a reference compounds for our *in vitro* biological evaluation. Also, benchmarking of the widely used docking tools (e.g., GOLD [ChemPLP], Glide [SP] and AutoDock Vina) employing DEKOIS 2.0 benchmark sets showed a good screening performance with all three docking tools [51]. This guided us to employ GOLD (ChemPLP) as scoring function.

Based on the docking fitness distribution of the docking compounds and reference standards, celecoxib appeared to be among the high scored binders while indomethacin and diclofenac showed the lowest docking fitness compared to other compounds (**Table 4**). Compounds **20a** and **21a** demonstrate the highest fitness among the most active compounds. This observation is attributable to the fact that **20a, 21a** and **12c** show the highest topological and size features (e.g. molecular weight, molecular volume and molecular surface area) compared to other biologically active compounds. Such fact would usually reward the docking score based on superior molecular size. [52-54] However, compound **12c** do not benefit from such topological features since its postulated binding mode is dissimilar (data not shown).

Pose prediction employing GOLD (ChemPLP) has been validated by successful pose-retrieval experiment of the co-crystallized ligand (celecoxib) in the binding site of COX-2 (RMSD < 1 Å), as shown in **Figure 2**.

We focus on the most active compounds (**14a**, **14b** and **20a**) for elucidating the binding modes of the docking outcome.

Rationalizing the selectivity of the most active compounds towards COX-2 over COX-1 by docking experiments, we find that these compounds cannot afford a specific binding mode in the binding site of COX-1 (data not shown). This observation is attributable to the fact that the topological features and the general size of the binding site of COX-1 is much smaller than COX-2.[55-57] Besides, the size features (e.g., molecular weight) of **14a**, **14b, 12c** and **20a** (**Table 4**) are generally above the average range of the diverse and representative COX-1 and COX-2 ligands reported in DEKOIS 2.0 benchmark sets (the average molecular weight of COX-1 and COX-2 in DEKOIS 2.0 benchmark set are 333.8 (±62.97) and 373.6 (±50.65), respectively).

The docking poses of the **14a**, **14b** and **20a** compounds showed to access more residues in the binding site compared to the co-crystallized ligand due to scaffold differences and substitution pattern (**Figure 2B**). Since **14a** and **14b** are closely related congeneric

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analogues while **20a** represents a different pyrazole hybrid, we show in **Figure 2B** only docking poses of **14a** and **20a**.

Binding interactions are inspected to be mainly favorable hydrophobic contacts. For instance in **14a,** the quinolinyl core is packed between Val509 and Ser339 residues, **Figure 3**. The chloro group of the quinolinyl moiety is packed between the backbone of Phe504, Ile503 and Ala502. Also, the benzoyl group shows hydrophobic contacts with the side chains of Met99, Leu345, Ile331, Leu517 and Leu103. However, the morpholinyl group established a H-bonding interaction with the side chain of Ser516.

Compound **14b** resembles the binding mode of its congener **14a** elucidated by their optimum overlay of their docking poses, **Figure 3**. Only exception is that the *p*-cholobenzoyl group of **14b** showed a flip to avoid a possible clash with Met99 and Ile331. As a result, the *p*-cholobenzoyl group is directed towards the Tyr101. Overall, this binding mode produced a comparable docking fitness to **14a**.

Compound **20a** interacts with the essential residues of the binding site of COX-2 similarly as celecoxib (**Figure 2B**), however, with extended binding cleft towards the solvent exposed area via the quinolinyl moiety. The nitrogen atom of quinolinyl moiety shows H-bonding interaction with His75, as seen in **Figure 4**. Likewise, the NH group of the hydrazonocarbonyl group demonstrates H-bonding interaction with Ser339. The N-cholorophenyl of the pyrazolyl moiety is packed between Val102, Met99 and Leu517 while the 5-chlorophenyl group is packed between Trp373, Tyr371, Phe367 and Leu370. Unlike celecoxib, the pyrazolyl moiety of **20a** is packed between Val509 and Ser339.

Based on our analysis of the docking fitness and postulated binding modes of different conformers and geometrical isomers we conclude that *E* isomers appeared to be distinctively favored for the docked compounds. This highlights the importance of geometrical isomers for high affinity binding to COX-2.

2.3. Conclusion

The aim of the present study was to synthesize and investigate the anti-inflammatory activity of some novel quinoline derivatives bearing pyrazole moiety through different linkage with the hope of discovering new lead structures devoid of the GI side effects associated with conventional NSAIDs. The obtained results clearly revealed that compounds **5c, 11b,c, 12c, 14a,b**, **20a** and **21a** showed anti-inflammatory activity similar to or even higher than that of celecoxib. In vitro COX-1/COX-2 inhibition study revealed that among the synthesized compounds, compounds **12c, 14a** and **14b** showed the highest inhibitory activity against COX-2 with an IC_{50} values of 0.1, 0.11 and 0.11μM respectively. Additionally, the active compounds showed significant *in vitro* LOX inhibitory activity higher than that of zileuton and good GI safety profile and are well tolerated by experimental animals with high safety margin (ALD_{50}> 0.3 g/kg). The

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docking experiments attempted to postulate the binding mode for the most active compounds in the binding site of COX-2 enzymes and confirmed the high selectivity binding towards COX-2 enzyme over COX-1. Consequently, such type of compounds would represent a fruitful matrix that deserves further investigation and derivatization.

3. Experimental

3.1. Chemistry

All reagents and solvents were purchased from commercial suppliers and were dried and purified when necessary by standard techniques. Melting points were determined in open glass capillaries using Stuart capillary melting point apparatus (Stuart Scientific Stone, Staffordshire, UK) and are uncorrected. Infrared (IR) spectra were recorded on Perkin-Elmer 1430 infrared spectrophotometer (Perkin Elmer, Beaconsfield, UK) and measured by Ú cm¯1 scale using KBr cell. ^{1}H NMR spectra and ^{13}C-NMR were scanned on Mercury 300 MHz spectrophotometer or on Jeol-NMR 500 MHz spectrophotometer in $CDCl_3$ or DMSO-d_6 and are reported as δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard.

3.1.1. 6-Chloro-2-methylquinoline-4-carboxylic acid (1)

Acetone (24 ml, 330 mmol) was gradually added with stirring and cooling to a solution of 5-chloroisatin (4.00 g, 22 mmol) in 33% (w/v) aqueous potassium hydroxide (19 mL). The reaction mixture was heated under reflux with stirring for 16 hours. It was then concentrated to half its original volume, left to attain room temperature, treated with ice (100g) and filtered. The filtrate was acidified with glacial acetic acid whereupon a precipitate was formed. The product was filtered, washed with water, dried and crystallized from ethanol. Yield: 90%; Mp: 265-7 ˚C. IR (KBr, cm^{-1}): broad band at 3413 (OH); 1707 (C=O); 1603 (C=N). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.67 (s, 3H, CH_3); 7.74 (d, 1H, J= 8.6 Hz, quinolyl-C_7-H); 7.88 (s, 1H, quinolyl-C_3-H); 7.97 (d, 1H, J= 8.6 Hz, quinolyl-C_8-H); 8.70 (s, 1H, quinolyl-C_5-H); 13.97 (s, 1H, OH, D_2O exchangeable). ^{13}C-NMR (75.44 MHz, DMSO-d_6, δ ppm): 25.13 (quinoline-C2-CH_3); 124.10, 124.66, 124.71, 130.52, 131.43, 132.07, 135.14, 147.13, 159.94 (quinoline C-5, C-4a, C-6, C-3, C-8, C-7, C-4, C-8a and C-2 respectively); 167.51 (C=O). Anal. Calcd for $C_{11}H_8ClNO_2$ (221.64): C, 59.61; H, 3.64; N, 6.32. Found: C, 59.79; H, 3.70; N, 6.41.

3.1.2. Ethyl 6-chloro-2-methylquinoline-4-carboxylate (2)

A solution of **1** (8.00 g, 36 mmol) in absolute ethanol (60 mL) containing concentrated sulfuric acid (7 mL) was heated under reflux for 15 hours. The reaction mixture was concentrated to a smaller volume, allowed to attain room temperature and then carefully poured with stirring onto excess 10% sodium hydrogen carbonate solution. The ester obtained was filtered, washed with water, dried and crystallized from ethanol. Yield: 85%; Mp: 94-5˚C. Yield 7.62 g (85%), m.p. 94-5˚C. IR (KBr, cm^{-1}):1715 (C=O); 1589

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(C=N); 1249, 1168 (C-O-C).^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.49 (t, 3H, J= 6.9 Hz, -CH_2<u>CH_3</u>); 2.80 (s, 3H, CH_3); 4.52 (q, 2H, J= 6.9 Hz, -<u>CH_2</u>CH_3); 7.68 (d, 1H, J= 9 Hz, quinolyl-C_7-H); 7.85 (s, 1H, quinolyl-C_3-H); 8.03 (d, 1H, J= 9 Hz, quinolyl-C_8-H); 8.79 (s, 1H, quinolyl-C_5-H). Anal. Calcd for $C_{13}H_{12}ClNO_2$ (249.69): C, 62.53; H, 4.84; N, 5.61. Found: C, 62.70; H, 4.90; N, 5.68.

3.1.3. 6-Chloro-2-methylquinoline-4-carbohydrazide (3)

A solution of ethyl 6-chloro-2-methylcinchoninate **2** (2.50 g, 10 mmol) in absolute ethanol (20 mL) was added with stirring to 99% hydrazine hydrate (2 ml, 40 mmol). The reaction mixture was heated under reflux for 2 hours whereupon white crystals started to separate out. After attaining room temperature, the crystalline product was filtered, washed thoroughly with water, dried and recrystallized from ethanol. Yield: 98 %; Mp: 245-6 ˚C. IR (KBr, cm^{-1}): 3334, 3257, 3180 (NH_2, NH); 1642 (C=O); 1588 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.68 (s, 3H, CH_3); 4.68 (s, 2H, NH_2, D_2O exchangeable); 7.49 (s, 1H, quinolyl-C_3-H); 7.77 (d, 1H, J= 9 Hz, quinolyl -C_7-H); 8.00 (d, 1H, J= 9 Hz, quinolyl-C_8-H); 8.19 (s, 1H, quinolyl-C_5-H); 9.91 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (75.44 MHz, DMSO-d_6, δ ppm): 25.09 (quinoline C-2 CH_3); 121.54, 124.03, 124.57, 130.62, 131.23, 131.30, 140.34, 146.54, 159.67 (quinoline C-5, C-4a, C-6, C-3, C-8, C-7, C-4, C-8a and C-2 respectively); 165.93 (C=O). Anal. Calcd for $C_{11}H_{10}ClN_3O$ (235.67): C, 56.06; H, 4.28; N, 17.83. Found: C, 56.13; H, 4.26; N, 18.02.

3.1.4. 1,3-Diaryl-1H-pyrazole-4-carbaldehydes (4a-c)

To a solution of phenylhydrazine hydrochloride or 4-chlorophenylhydrazine hydrochloride (70 mmol) in ethanol containing anhydrous sodium acetate (70 mmol), was added an equimolar amount of p-substituted acetophenone (70 mmol). The reaction mixture was heated under reflux for 2 hours, filtered while hot to remove the sodium chloride formed and set aside to attain room temperature. The reaction mixture was then cooled in an ice bath whereupon a precipitate started to separate. The obtained hydrazones were filtered, washed with ether and allowed to dry. Under anhydrous conditions, phosphorus oxychloride (13.80 g, 8.40 mL, 90 mmol) was dropwise added over a period of 30 minutes to a cold well stirred dry dimethylformamide (38.00 g, 40 ml, 520 mmol). Cooling and stirring were continued for further 45 minutes. The appropriate previously prepared hydrazone (40 mmol) was portionwise added to the well stirred cold mixture. After attaining room temperature, the reaction mixture was heated for 3 hours at 75 °C, allowed to attain room temperature and then poured onto crushed ice (55 g) and water (100 mL). The reaction mixture was further boiled whereupon a white precipitate separated out. After attaining room temperature, the crude product was filtered, washed with water, dried and crystallized from ethanol.

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3.1.4.1. 1,3-bis(4-chlorophenyl)-1H-pyrazole-4-carbaldehyde (4a). Yield: 96%; Mp: 169-170 °C. IR (KBr, cm^{-1}): 2947, 2842 (CH aldehyde), 1684 (C=O); 1607 (C=N). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 7.5 (dd, 4H, J1= 8.4, J2 = 4.2 Hz, pyrazolyl-C3,4-chlorophenyl-$C_{3,5}$-H and pyrazolyl-*N*1-4-chlorophenyl-$C_{3,5}$-H); 7.75 (d, 2H, J= 8.7 Hz, pyrazolyl-C3-4-chlorophenyl-$C_{2,6}$-H); 7.83 (d, 2H, J= 8.4 Hz, pyrazolyl-*N*1-4-chlorophenyl-$C_{2,6}$-H); 8.51 (s, 1H, pyrazolyl-C_5-H); 10.05 (s, 1H, CHO). Anal. Calcd for $C_{l6}H_{10}Cl_2N_2O$ (317.17): C, 60.59; H, 3.18; N, 8.83. Found: C, 60.71; H, 3.21; N, 9.04.

3.1.4.2. 3-(4-methoxyphenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (4b). Yield: 96%; Mp: 169-170 °C [58].

3.1.4.3. 1-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazole-4-carbaldehyde (4c). Yield: 88%; Mp: 136-7 °C. IR (KBr, cm^{-1}):2921, 2815 (CH aldehyde), 1671 (C=O); 1605 (C=N); 1219, 1176 (C-O-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.88 (s, 3H, OCH_3); 7.04 (d, 2H, J= 8.1 Hz, 4-methoxyphenyl-$C_{3,5}$-H); 7.48 (d, 2H, J= 8.1 Hz, 4-methoxyphenyl-$C_{2,6}$-H); 7.73-7.80 (m, 4H, 4-chlorophenyl-H); 8.49 (s, 1H, pyrazolyl-C_5-H); 10.05 (s, 1H, CHO). Anal. Calcd for $C_{17}H_{13}ClN_2O_2$ (312.75): C, 65.29; H, 4.19; N, 8.96. Found: C, 65.37; H, 4.24; N, 9.08.

3.1.5. N'-[(1,3-Diaryl-1H-pyrazol-4-yl)methylene]6-chloro-2-methylquinoline-4-carbohydrazides (5a-c)

A solution of the appropriate 1,3-diaryl-1*H*-pyrazole-4-carbaldehyde **4a-c**(2 mmol) in absolute ethanol (30 ml) was gradually added to a solution containing an equimolar amount of the acid hydrazide **3** (0.47 g) in absolute ethanol (30 ml). The reaction mixture was heated under reflux for 2 hours and then set aside for an overnight whereupon white crystals separated out. The products obtained were filtered, washed with water, dried and recrystallized from ethanol.

3.1.5.1. N'-[(1,3-bis(4-chlorophenyl)-1H-pyrazol-4-yl)methylene]6-chloro-2-methylquinoline-4-carbohydrazide (5a). Yield: 71%; Mp: 298-300 °C. IR (KBr, cm^{-1}): 3182 (NH); 1652 (C=O); 1595 (C=N).^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.70 (s, 3H, CH_3); 7.39-7.85 (m, 9H, the two chlorophenyl-H and quinolyl-C_3-H); 8.00-8.07 (m, 2H, quinolyl-$C_{7,8}$-H); 8.17 (s, 1H, quinolyl-C_5-H); 8.43 (s, 1H, CH=N); 9.12 (s, 1H, pyrazolyl-C_5-H); 12.11 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{27}H_{18}Cl_3N_5O$ (534.82): C, 60.63; H, 3.39; N, 13.09. Found: C, 60.72; H, 3.43; N, 13.32.

3.1.5.2. 6-Chloro-N'-[(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)methylene]-2-methylquinoline-4-carbohydrazide (5b). Yield: 94%; Mp: 275-6 °C. IR (KBr, cm^{-1}): 3190 (NH); 1652 (C=O); 1599 (C=N); 1250-1175 (C-O-C).^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.73 (s, 3H, CH_3); 3.82 (s, 3H, OCH_3); 7.07 (d, 2H, J= 9 Hz, 4-methoxyphenyl-$C_{3,5}$-H); 7.29-7.86 (m, 8H, 4-methoxyphenyl-$C_{2,6}$-H, phenyl-H and

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quinolyl-C_3-H); 8.03 (d, 2H, J= 8.7 Hz, quinolyl-$C_{7,8}$-H); 8.20 (s, 1H, quinolyl-C_5-H); 8.47(s, 1H, CH=N); 9.04 (s, 1H, pyrazolyl-C_5-H). 12.04 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{28}H_{22}ClN_5O_2$ (495.96): C, 67.81; H, 4.47; N, 14.12. Found: C, 67.87; H, 4.49; N, 14.19.

3.1.5.3. 6-Chloro-N'-[(1-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)methylene]-2-methylquinoline-4-carbohydrazide (5c).(Yield: 94%; Mp: 270-2 °C. IR (KBr, cm^{-1}): 3187 (NH); 1653 (C=O); 1596 (C=N); 1249-1175 (C-O-C).^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.72 (s, 3H, CH_3); 3.82 (S, 3H, OCH_3); 7.08 (d, 2H, J= 8.7 Hz, 4-methoxyphenyl-$C_{3,5}$-H) ; 7.60 (d, 2H, J= 8.4 Hz, 4-chlorophenyl-$C_{3,5}$-H); 7.67 (d, 2H, J= 8.4 Hz, 4-chlorophenyl-$C_{2,6}$-H); 7.71 (s, 1H, quinolyl-C_3-H); 7.78-8.11 (m, 4H, 4-methoxyphenyl-$C_{2,6}$-H and quinolyl-$C_{7,8}$-H); 8.20 (s, 1H, quinolyl-C_5-H); 8.46 (s, 1H, CH=N); 9.09 (s, 1H, pyrazolyl-C_5-H); 12.05 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (75.44 MHz, DMSO-d_6, δ ppm): 24.71 (CH_3); 55.22 (OCH_3); 114.14 (4-methoxyphenyl C-3/5); 120.17 (4-chlorophenyl C-2/6); 120.42 (pyrazole C-4); 121.29 (4-methoxyphenyl C-1); 123.37, 123.80 and 124.02 (quinoline C-5, C-3 and C-7 respectively); 127.51 (4-chlorophenyl C-3/5); 129.44 (4-methoxyphenyl C-2/6); 129.70 (pyrazole C-5); 130.30, 130.87 (quinoline C-8 and C-6 respectively); 131.02 (4-chlorophenyl C-4); 131.10 (quinoline C-4a); 137.83 (4-chlorophenyl C-1); 139.13 (quinoline C-4); 142.34 (CH=N); 146.12 (quinoline C-8a); 152.12 (quinoline C-2); 159.23 (pyrazole C-3); 159.69 (4-methoxyphenyl C-4); 161.81 (C=O). MS (m/z, %): 532 ($M^{+\bullet}$ +2) (5); 531($M^{+\bullet}$ +1) (13); 530 ($M^{+\bullet}$)(15) 176 (100) Anal. Calcd for $C_{28}H_{21}Cl_2N_5O_2$ (530.40): C, 63.40; H, 3.99; N, 13.20. Found: C, 63.48; H, 3.97; N, 13.31).

3.1.6. 6-Chloro-2-methyl-N'-(1-methyl-3-oxobutylidene)quinoline-4-carbohydrazide (6).

Acetylacetone (0.15 mL, 1.5 mmol) was added to a solution containing an equimolar amount of acid hydrazide **3** (0.35g) in ethanol (30 mL). The reaction mixture was heated under reflux for 4 hours and then set aside to attain room temperature whereupon a precipitate was formed. The precipitate obtained was filtered, washed with 50% cold ethanol, dried and crystallized from ethanol. Yield: 89 %; Mp: 160-1˚C. IR (KBr, cm^{-1}): 3232 (NH); 1633 (C=O); 1598 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.78 (s, 3H, $\underline{CH_3}$-C=N-NH); 1.99 (s, 3H, $\underline{CH_3}$-C=O); 2.67 (s, 3H, quinolyl-C_2-CH_3); 2.90 (d, 1H, J= 18.9 Hz, CH_2); 3.05 (d, 1H, J= 18.9 Hz, CH_2); 6.97 (s, 1H, NH, D_2O exchangeable); 7.38 (s, 1H, quinolyl-C_3-H); 7.71 (s, 1H, quinolyl-C_5-H) ; 7.76 (d, 1H, J= 9 Hz, quinolyl-C_7-H); 7.99 (d, 1H, J= 9.3 Hz, quinolyl-C_8-H). ^{13}C-NMR (75.44 MHz, DMSO-d_6, δ ppm): 15.64($\underline{CH_3}$-C=N-NH); 24.70 (quinoline C-2 $\underline{CH_3}$); 25.82 ($\underline{CH_3}$-C=O); 52.21 (CH_2); 91.02 (quinoline C-5), 119.54, 122.91, 123.38, 130.06, 130.57, 130.74 and 142.92 (C-4a, C-3, C-7, C-8, C-6, C-4 and C-8a respectively); 145.69 (CH_3-$\underline{C}$=N); 156.86 (quinoline C-2); 159.19 (NH-$\underline{C=O}$); 164.07 (CH_3-$\underline{C=O}$). Anal. Calcd for $C_{16}H_{16}ClN_3O_2$ (317.77): C,60.47; H, 5.08; N, 13.22. Found: C, 60.56; H, 5.13; N, 13.34.

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3.1.7. 6-Chloro-4-[(3,5-dimethyl-1H-pyrazol-1-yl)carbonyl]2-methylquinoline (7)

A mixture of acid hydrazide **3** (0.47 g, 2 mmol) and excess acetylacetone (3 mL, 30 mmol) was heated at 110 °C for 6 hours. The reaction mixture was set aside to attain room temperature whereupon a precipitate was formed. The product obtained was triturated with diethyl ether to break the lumps formed, filtered, washed with diethyl ether, dried and crystallized from ethanol. Yield: 68 %; Mp: 199-200˚C. IR (KBr, cm^{-1}): 1710 (C=O); 1598 (C=N).^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 2.12 (s, 3H, pyrazolyl-C_5-CH_3); 2.73 (s, 3H, pyrazolyl-C_3-CH_3); 2.78 (s, 3H, quinolyl-C_2-CH_3); 6.11 (s, 1H, pyrazolyl-C_4-H); 7.42 (s, 1H, quinolyl-C_3-H); 7.63 (d, 1H, J= 8.4 Hz, quinolyl-C_7-H); 7.71 (s, 1H, quinolyl-C_5-H); 8.02 (d, 1H, J= 8.4 Hz, quinolyl-C_8-H). ^{13}C-NMR (75.44 MHz, DMSO-d_6, δ ppm): 13.36 and 13.87 (pyrazole C-3 and C-5 CH_3); 24.76 (quinoline C-2 CH_3); 112.28 (pyrazole C-4); 121.37, 122.86, 123.44, 130.43, 130.86, 131.18 and 140.23 (quinoline C-5, C-4a, C-3, C-7, C-8, C-6 and C-4 respectively); 144.70 (pyrazole C-5); 145.67 (quinoline C-8a); 153.34 (pyrazole C-3); 158.95 (quinoline C-2); 166.48 (C=O). MS (m/z, %):301 ($M^{+\bullet}$ +2) (13); 300 ($M^{+\bullet}$ + 1) (8); 299 ($M^{+\bullet}$) (37).Anal. Calcd for $C_{16}H_{14}ClN_3O$ (299.75): C, 64.11; H, 4.71; N, 14.02. Found: C, 64.23; H, 4.68; N, 14.13.

3.1.8.. Ethyl 3-[2-(6-chloro-2-methylquinolin-4-ylcarbonyl)hydrazino]-3-oxopropanoate (8)

A mixture of acid hydrazide **3** (0.35 g, 1.5 mmol) and excess diethyl malonate (2 mL, 13.2 mmol) was heated at 160˚C for half an hour. The reaction mixture was set aside to attain room temperature whereupon a precipitate was formed. The product obtained was triturated with diethyl ether, filtered, washed with diethyl ether, air dried and crystallized from ethanol. Yield: 90 %; Mp: 200-2˚C. IR (KBr, cm^{-1}): 3184 (NH); broad band centered at 1744 (C=O); 1268, 1164 (C-O-C). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm):1.22 (t, 3H, J= 7.2 Hz, -$CH_2\underline{CH_3}$); 2.70 (s, 3H, CH_3); 3.42 (s, 2H, CH_2); 4.14 (q, 2H, J= 7.2 Hz, -$\underline{CH_2}CH_3$); 7.57 (s, 1H, quinolyl-C_3-H); 7.80 (d, 1H, J= 9 Hz, quinolyl-C_7-H); 8.02 (d, 1H, J= 9 Hz, quinolyl-C_8-H); 8.31 (s, 1H, quinolyl-C_5-H); 10.39, 10.73 (2s, 2H, 2NH, D_2O exchangeable). Anal. Calcd for $C_{16}H_{16}ClN_3O_4$ (349.77): C, 54.94; H, 4.61; N, 12.01. Found: C, 55.13; H, 4.67; N, 12.14.

3.1.9. 1-(6-Chloro-2-methylquinolin-4-ylcarbonyl) pyrazolidine-3,5-dione (9)

Diethyl malonate (0.5 mL, 3.28 mmol) was added to a solution containing an equimolar amount of acid hydrazide **3** (0.77 g, 3.28 mmol) in absolute ethanol (40 mL) containing one drop of concentrated sulphuric acid. The reaction mixture was heated under reflux for 3hours whereupon a white precipitate started to form. The reaction mixture was set aside to attain room temperature for complete precipitation and the precipitate formed was filtered, washed with cold ethanol, dried and crystallized from ethanol. Yield: 70 %; Mp: 168-170˚C. IR (KBr, cm^{-1}): 3251 (NH); 1702, 1641 (C=O). ^{1}H-NMR (300 MHz, DMSO-

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d_6, δ ppm):2.69 (s, 3H, quinolyl-C_2-CH_3); 3.73 (s, 2H, pyrazolidinyl-C_4-H); 7.65 (s, 1H, quinolyl-C_3-H); 7.85 (d, 1H, J= 8.7 Hz, quinolyl-C_7-H); 8.05 (d, 1H, J= 8.7 Hz, quinolyl-C_8-H); 8.18 (s, 1H, quinolyl-C_5-H); 11.05 (s, 1H, NH, D_2O exchangeable).^{13}C-NMR (75.44 MHz, DMSO-d_6, δ ppm): 24.41 (quinoline C-2 CH_3); 40.32 (pyrazolidine C-4, under DMSO); 121.47, 121.83, 122.96, 123.61, 130.38, 130.89, 131.68, 137.28 and 145.33 (quinoline C-); 159.36 ,165.05 , 165.37 (3 C=O). Anal. Calcd for $C_{14}H_{10}ClN_3O_3$ (303.70): C, 55.37; H, 3.32; N, 13.84. Found: C, 55.48; H, 3.35; N, 13.92.

3.1.10. 6-Chloro-2-methyl-4-(morpholin-4-yl) carbonylquinoline (10)

A mixture of ethyl 6-chloro-2-methylquinoline-4-carboxylate **2** (3.25 g, 13 mmol) and excess morpholine (6.10 g, 6.00 mL, 70 mmol) was heated under reflux for 24 hours, the reaction mixture was left to attain room temperature and then poured onto cold water (50 mL). The product separated was filtered, washed several times with water, dried and crystallized from ethanol. Yield: 70 %; Mp: 144-5˚C. IR (KBr, cm^{-1}): 1637(C=O); 1594 (C=N); 1108, 1066 (C-O-C).^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.78 (s, 3H, CH_3); 3.17-3.26 (m, 2H, morpholin-$C_{3a,5a}$-H); 3.54-3.62 (m, 2H, morpholin-$C_{3e,5e}$-H); 3.79-4.06 (m, 4H, morpholin-$C_{2,6}$-H); 7.25 (s, 1H, quinolyl-C_3-H); 7.69 (d, 1H, J= 9 Hz, quinolyl-C_7-H); 7.77 (s, 1H, quinolyl-C_5-H); 8.06 (d, 1H, J= 9 Hz, quinolyl-C_8-H). Anal. Calcd for $C_{15}H_{15}ClN_2O_2$ (290.74): C, 61.97; H, 5.20; N, 9.64. Found: C, 62.08; H, 5.25; N, 9.79.

3.1.11. Ethyl 2-[2-(1,3-diaryl-1H-pyrazol-4-yl)ethenyl]-6-chloroquinoline-4-carboxylates (11a-c)

To a solution of (1.00 g, 4 mmol) of ethyl 6-chloro-2-methylcinchoninate **2** in acetic anhydride (20 mL), was added an equimolar amount of the appropriate 1,3-diaryl-1*H*-pyrazole-4-carbaldehyde **4a-c**.The reaction mixture was heated under reflux for 20 hours and was then set aside to attain room temperature. The precipitates formed were filtered, washed with ethanol, dried and crystallized from glacial acetic acid.

3.1.11.1. Ethyl 2-[2-(1,3-bis(4-chlorophenyl)-1H-pyrazol-4-yl)ethenyl]-6-chloroquinoline-4-carboxylate (11a). Yield: 71%; Mp: 180-2 °C. IR (KBr, cm^{-1}): 1722 (C=O); 1590 (C=N); 1239-1172 (C-O-C). ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 1.47 (t, 3H, J= 6.9 Hz, $CH_2\underline{CH_3}$); 4.49 (q, 2H, J= 6.9 Hz, $\underline{CH_2}CH_3$); 7.15 (d, 1H, J= 16.00 Hz, -$\underline{CH}$=CH-); 7.41-7.47 (m, 4H, pyrazolyl-*N*1-4-chlorophenyl-$C_{3,5}$-H and pyrazolyl-C3-4-chlorophenyl-$C_{3,5}$-H); 7.62-7.67 (m, 6H, quinolyl-C_3-H, -CH=$\underline{CH}$-, pyrazolyl-*N*1-4-chlorophenyl-$C_{2,6}$-H and pyrazolyl-C3-4-chlorophenyl-$C_{2,6}$-H); 7.94-8.03 (m, 2H, quinolyl-C-$_{7,8}$-H); 8.21 (s, 1H, quinolyl-C_5-H); 8.71 (s, 1H, pyrazolyl-C_5-H). ^{13}C-NMR (75.44 MHz, $CDCl_3$, δ ppm):14.54 (-$CH_2\underline{CH_3}$); 62.41 (-$\underline{CH_2}CH_3$); 119.95 (quinoline C-3); 120.50 (pyrazole C-4); 121.82 (pyrazole-*N*1-4-chlorophenyl C-2/6); 124.85, 124.96 and 125.44 (quinoline C-5, C-4a and C-6 respectively); 125.69 (pyrazole-C3-4-chlorophenyl C-2/6); 128.41 (pyrazole-*N*1-4-chlorophenyl C-3/5 and pyrazole-C3-4-

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chlorophenyl C-3/5); 129.26 (pyrazole C-5); 129.94 (quinoline C-8 and pyrazole-C3-4-chlorophenyl C-1); 130.17 (pyrazole-*N*1-4-chlorophenyl C-4); 131.27 (quinoline C-7); 132.84 (-CH=CH-); 133.96 (quinoline C-4); 134.93 (pyrazole-C3-4-chlorophenyl C-4); 138.30 (pyrazole-*N*1-4-chlorophenyl C-1); 147.72 (quinoline C-8a); 151.96 (pyrazole C-3); 155.56 (quinoline C-2); 165.98 (C=O). MS (m/z, %): 550($M^{+\bullet}$+2) (31); 549($M^{+\bullet}$+1) (39); 548($M^{+\bullet}$) (41). Anal. Calcd for $C_{29}H_{20}Cl_3N_3O_2$ (548.85): C, 63.46; H, 3.67; N, 7.66. Found: C, 63.62; H, 3.74; N, 7.82.

3.1.11.2. Ethyl 2-[2-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)ethenyl]-6-chloroquinoline-4-carboxylate (11b). Yield: 64%; Mp: 210-2 °C. IR (KBr, cm^{-1}): 1725 (C=O); 1595 (C=N); 1242-1172 (C-O-C, ester); 1092-1027 (C-O-C, OCH_3). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.41 (t, 3H, J= 6.9 Hz, $CH_2\underline{CH_3}$); 3.85 (s, 3H, OCH_3); 4.47 (q, 2H, J= 6.9 Hz, $\underline{CH_2}CH_3$); 7.14 (d, 2H, J= 6.9 Hz, 4-methoxyphenyl-$C_{3,5}$-H); 7.37 (t, 1H, J= 7.8 Hz, phenyl-C_4-H); 7.46 (d, 1H, J= 16.20 Hz, -$\underline{CH}$=CH-); 7.57 (t, 2H, J= 7.8 Hz, phenyl-$C_{3\&5}$-H); 7.67 (d, 2H, J= 9 Hz, 4-methoxyphenyl-$C_{2,6}$-H); 7.74 (s, 1H, quinolyl-C_3-H); 7.75-8.15 (m, 5H, phenyl-$C_{2,6}$-H, quinolyl-$C_{7,8}$-H and -CH=$\underline{CH}$-); 8.61 (s, 1H, quinolyl-C_5-H); 9.17 (s, 1H, pyrazolyl-C_5-H). Anal. Calcd for $C_{30}H_{24}ClN_3O_3$ (509.98): C, 70.65; H, 4.74; N, 8.24. Found: C, 70.73; H, 4.75; N, 8.31.

3.1.11.3. Ethyl 2-[2-(1-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)ethenyl]-6-chloroquinoline-4-carboxylate (11c). Yield: 60%; Mp: 208-9 °C. IR (KBr, cm^{-1}): 1726 (C=O); 1594 (C=N); 1242-1175 (C-O-C, ester); 1088-1027 (C-O-C, OCH_3). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.51 (t, 3H, J= 7.2 Hz, $CH_2\underline{CH_3}$); 3.91 (s, 3H, OCH_3); 4.55 (q, 2H, J= 7.2 Hz, $\underline{CH_2}CH_3$); 7.08 (d, 2H, J= 8.7 Hz, 4-methoxyphenyl-$C_{3,5}$-H); 7.32 (d, 1H, J= 16.50 Hz, -$\underline{CH}$=CH-); 7.48 (d, 2H, J= 8.7 Hz, 4-chlorophenyl-$C_{3,5}$-H); 7.69-7.79 (m, 6H, 4-methoxyphenyl-$C_{2,6}$-H, 4-chlorophenyl-$C_{2,6}$-H, -CH=$\underline{CH}$- and quinolyl-C_3-H); 8.10-8.14 (m, 2H, quinolyl-$C_{7,8}$-H); 8.32 (s, 1H, quinolyl-C_5-H); 8.76 (s, 1H, pyrazolyl-C_5-H). Anal. Calcd for $C_{30}H_{23}Cl_2N_3O_3$ (544.43): C, 66.18; H, 4.26; N, 7.72. Found: C, 66.24; H, 4.28; N, 7.88.

3.1.12. 6-Chloro-2-[2-(1,3-diaryl-1H-pyrazol-4-yl)ethenyl]-4-(morpholin-4-ylcarbonyl)quinolines (12a-c)

The appropriate 1,3-diaryl-1*H*-pyrazole-4-carbaldehyde **4a-c** (3.5 mmol) was added to a solution containing an equimolar amount of 6-chloro-2-methyl-4-morpholinocarbonylquinoline **10** (1.02 g) in acetic anhydride (20 mL). The reaction mixture was heated under reflux for 20 hours and was then set aside to attain room temperature. The precipitates obtained were filtered, washed with ethanol, dried and crystallized from glacial acetic acid.

3.1.12.1. 6-Chloro-2-[2-(1,3-bis(4-chlorophenyl)-1H-pyrazol-4-yl)ethenyl]-4-(morpholin-4-ylcarbonyl) quinoline (12a). Yield: 50%; Mp: 235-7 °C. IR (KBr, cm^{-1}):

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1643 (C=O); 1535 (C=N); 1249-1113 (C-O-C). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.13-3.25 (m, 2H, morpholin-$C_{3a,5a}$-H); 3.39-3.64 (m, 2H, morpholin-$C_{3e,5e}$-H); 3.70-3.88 (m, 4H, morpholin-$C_{2,6}$-H); 7.40 (d, 1H, J= 16.20 Hz, -CH=CH-); 7.59-7.63 (m, 4H, pyrazolyl-*N*1-4-chlorophenyl-$C_{3,5}$-H and pyrazolyl-C3-4-chlorophenyl-$C_{3,5}$-H); 7.71-7.81 (m, 5H, pyrazolyl-*N*1-4-chlorophenyl-$C_{2,6}$-H, pyrazolyl-C3-4-chlorophenyl-$C_{2,6}$-H and -CH=CH); 7.88 (s, 1H, quinolyl-C_3-H); 8.00 (d, 2H, J= 8.7 Hz, quinolyl-$C_{7,8}$-H); 8.04 (s, 1H, quinolyl-C_5-H); 9.22 (s, 1H, pyrazolyl-C_5-H). Anal. Calcd for $C_{31}H_{23}Cl_3N_4O_2$ (589.90): C, 63.12; H, 3.93; N, 9.50. Found: C, 63.18; H, 3.97; N, 9.58.

3.1.12.2.6-Chloro-2-[2-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)ethenyl]-4-(morpholin-4-ylcarbonyl)quinoline (12b). Yield: 60%; Mp: 251-2 °C. IR (KBr, cm^{-1}): 1639 (C=O); 1533 (C=N); 1247-1115 (C-O-C); 1176-1056 (C-O-C, OCH_3). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.14-3.25 (m, 2H, morpholin-$C_{3a,5a}$-H); 3.40-3.60 (m, 2H, morpholin-$C_{3e,5e}$-H); 3.72-3.80 (m, 4H, morpholin-$C_{2,6}$-H); 3.85 (s, 3H, OCH_3); 7.12 (d, 2H, J= 8.85 Hz, 4-methoxyphenyl-$C_{3,5}$-H); 7.32-7.43 (m, 2H, -CH=CH- and phenyl-C_4-H); 7.55 (t, 2H, J= 8.4 Hz, phenyl-$C_{3,5}$-H); 7.68 (d, 2H, J= 8.85 Hz, 4-methoxyphenyl-$C_{2,6}$-H); 7.73-7.81 (m, 3H, -CH=CH- and phenyl-$C_{2,6}$-H); 7.82 (s, 1H, quinolyl-C_3-H); 7.93-8.03 (m, 3H, quinolyl-$C_{5,7,8}$-H); 9.14 (s, 1H, pyrazolyl-C_5-H). Anal. Calcd for $C_{32}H_{27}ClN_4O_3$ (551.03): C, 69.75; H, 4.94; N, 10.17. Found: C, 69.83; H, 4.96; N, 10.32.

3.1.12.3. 6-Chloro-2-[2-(1-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)ethenyl]-4-(morpholin-4-ylcarbonyl)quinoline (12c). Yield: 65%; Mp: 240-1 °C. IR (KBr, cm^{-1}): 1639 (C=O); 1533 (C=N); 1249-1114 (C-O-C); 1177-1087 (C-O-C, OCH_3). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.15-3.26 (m, 2H, morpholin-$C_{3a,5a}$-H); 3.56-3.58 (m, 2H, morpholin-$C_{3e,5e}$-H); 3.73-3.82 (m, 4H, morpholin-$C_{2,6}$-H); 3.85 (s, 3H, OCH_3); 7.11 (d, 2H, J= 9 Hz, 4-methoxyphenyl-$C_{3,5}$-H); 7.38 (d, 1H, J= 16.20 Hz, -CH=CH-); 7.61 (d, 2H, J= 9 Hz, 4-chlorophenyl-$C_{3,5}$-H); 7.67 (d, 2H, J= 9 Hz, 4-methoxyphenyl-$C_{2,6}$-H); 7.72-7.80 (m, 3H, -CH=CH- and 4-chlorophenyl-$C_{2,6}$-H); 7.83 (s, 1H, quinolyl-C_3-H); 7.97-8.02 (m, 3H, quinolyl-$C_{5,7,8}$-H); 9.17 (s, 1H, pyrazolyl-C_5-H). ^{13}C-NMR (75.44 MHz, DMSO-d_6, δ ppm): 47.01 (morpholin C-3/5); 55.20 (OCH_3); 66.14 (morpholin C-2/6); 114.28 (quinoline C-3); 117.36 (4-methoxyphenyl C-3/5); 119.00 (pyrazole C-4); 119.95 (4-chlorophenyl C-2/6); 123.30 (quinoline C-4a); 123.51 (4-methoxyphenyl C-1); 124.63, 125.46 (quinoline C-5 and C-6); 127.27 (4-methoxyphenyl C-2/6); 128.20 (4-chlorophenyl C-3/5); 129.39 (pyrazole C-5); 129.49 (quinoline C-8); 129.57 (4-chlorophenyl C-4); 130.66 (quinoline C-7); 130.83 (CH=CH); 131.29 (CH=CH); 137.97 (4-chlorophenyl C-1); 141.41, 146.30 (quinoline C-4 and C-8a); 151.56 (pyrazole C-3); 155.99 (quinoline C-2); 159.54 (4-methoxyphenyl C-4); 165.25 (C=O). Anal. Calcd for $C_{32}H_{26}Cl_2N_4O_3$ (585.48): C, 65.65; H, 4.48; N, 9.57. Found: C, 65.72; H, 4.52; N, 9.65.

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3.1.13. Phenyl glyoxal or 4-chlorophenyl glyoxal (13a,b).

Compound13*a* (R = H), yield: 91%; Mp: 75-6 ˚C [59].Compound13*b* (R = Cl), yield: 82%; Mp:115-6 ˚C [60]

3.1.14. 3-[6-Chloro-4-(morpholin-4-ylcarbonyl)quinolin-2-yl]-1-arylprop-2-en-1-ones (14a,b)

Phenyl glyoxal or 4-chlorophenyl glyoxal **13a**,**b** (3.75 mmol) was added to a solution of 6-chloro-2-methyl-4-morpholinocarbonylquinoline **10** (1.00 g, 3.5 mmol) in acetic anhydride (20 mL).The reaction mixture was heated under reflux for 5 hours, set aside to attain room temperature and then poured onto ice. The precipitates formed were filtered, washed with water and crystallized from 70% aqueous ethanol**.**

3.1.14.1. 3-[6-Chloro-4-(morpholin-4-ylcarbonyl) quinolin-2-yl]-1-phenylprop-2-en-1-one (14a). Yield: 91%; Mp: 143-5 °C. IR (KBr, cm^{-1}): 1709 (C=C-C=O); 1634 (N-C=O); 1546 (C=N); 1111-1100 (C-O-C). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.10-3.35 (m, 2H, morpholin-$C_{3a,5a}$-H); 3.40-3.65 (m, 2H, morpholin-$C_{3e,5e}$-H); 3.70-3.95 (m, 4H, morpholin-$C_{2,6}$-H); 7.46-7.91 (m, 7H, CH=CH, quinolyl-C_3-H and phenyl-H); 8.15-8.23 (m, 2H, quinolyl-$C_{7,8}$-H); 8.31 (s, 1H, quinolyl-C_5-H); 8.37 (d, 1H, J= 15.6 Hz, -CH=CH-). Anal. Calcd for $C_{23}H_{19}Cl_1N_2O_3$ (406.86): C, 67.90; H, 4.71; N, 6.89. Found: C, 67.98; H, 4.68; N, 6.96.

3.1.14.2. 3-[6-Chloro-4-(morpholin-4-ylcarbonyl)quinolin-2-yl]-1-(4-chlorophenyl)prop-2-en-1-one (14b). Yield: 91%; Mp: 259-260 °C. IR (KBr, cm^{-1}): 1714 (C=C-C=O); 1641 (N-C=O); 1592 (C=N); 1248-1239 (C-O-C).^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.17-3.26 (m, 2H, morpholin-$C_{3a,5a}$-H); 3.40-3.64 (m, 2H, morpholin-$C_{3e,5e}$-H); 3.72-3.90 (m, 4H, morpholin-$C_{2,6}$-H); 7.68 (d, 2H, J= 8.4 Hz, 4-chlorophenyl-$C_{3,5}$-H); 7.80 (d, 1H, J= 15.75, -CH=CH-); 7.85-7.90 (m, 2H, quinolyl-$C_{3,7}$-H); 8.16 (d, 1H, J= 9 Hz, quinolyl-C_8-H); 8.20 (d, 2H, J= 8.4 Hz, 4-chlorophenyl-$C_{2,6}$-H); 8.31 (s, 1H, quinolyl-C_5-H); 8.35 (d, 1H, J= 15.75 Hz, -CH=CH-). Anal. Calcd for $C_{23}H_{18}Cl_2N_2O_3$ (441.31): C, 62.60; H, 4.11; N, 6.35. Found: C, 62.67; H, 4.13; N, 6.49.

3.1.15. Ethyl 1,5-diaryl-1H-pyrazole-3-carboxylates (16a-c).

Compound16*a* (R = R^1 = 4-Cl-C_6H_4), yield: 57%; Mp: 102-2 ˚C [61]. Compound16*b* (R = C_6H_5, R^1 = 4-OCH_3-C_6H_4), yield: 87%; Mp: 67-9˚C [62]. Compound16*c* (R = 4-Cl-C_6H_4, R^1 = 4-OCH_3-C_6H_4), yield: 64%; Mp: 116-7˚C [62].

3.1.16. 1,5-Diaryl-1H-pyrazole-3-carbohydrazides (17a-c)

A solution of the appropriate ethyl 1,5-diaryl-1*H*-pyrazole-3-carboxylate **16a-c** (10 mmol) in ethanol (30 mL) was gradually added with stirring to 99% hydrazine hydrate (5 mL, 100 mmol). The reaction mixture was heated under reflux for 5 hours, concentrated

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under diminished pressure to (15 mL) and allowed to attain room temperature. The products separated were filtered, washed with cold 50% aqueous ethanol, dried and crystallized from ethanol.

3.1.16.1. 1,5-Bis(4-chlorophenyl)-1H-pyrazole-3-carbohydrazide (17a). Yield: 91%; Mp: 110-2 °C. IR (KBr, cm^{-1}): 3345, 3274, 3131 (NH_2, NH); 1676 (C=O); 1618 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 4.50 (s, 2H, NH_2, D_2O exchangeable); 7.04 (s, 1H, pyrazolyl-C_4-H); 7.29 (d, 2H, J= 8.4 Hz, pyrazolyl-*N*1-4-chlorophenyl-$C_{3,5}$-H); 7.36 (d, 2H, J= 8.7 Hz, pyrazolyl-C5-4-chlorophenyl-$C_{3,5}$-H); 7.46 (d, 2H, J= 8.7 Hz, pyrazolyl-C5-4-chlorophenyl-$C_{2,6}$-H); 7.53 (d, 2H, J= 8.4 Hz, pyrazolyl-*N*1-4-chlorophenyl-$C_{2,6}$-H); 9.59 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{16}H_{12}Cl_2N_4O$ (347.20): C, 55.35; H, 3.48; N, 16.14. Found: C, 55.43; H, 3.50; N, 16.22.

*3.1.16.2. 5-(4-Methoxyphenyl)-1-phenyl- 1H-pyrazole-3-carbohydrazide (17b).*Yield: 66%; Mp: 162-3 °C. IR (KBr, cm^{-1}): 3325, 3271, 3100 (NH_2, NH); 1649 (C=O); 1592 (C=N); 1245, 1171 (C-O-C). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.75 (s, 3H, OCH_3); 4.49 (s, 2H, NH_2, D_2O exchangeable); 6.92-6.95 (m, 3H, pyrazolyl-C_4-H and 4-methoxyphenyl-$C_{3,5}$-H); 7.18 (d, 2H, J= 8.7 Hz, 4-chlorophenyl-$C_{3,5}$-H); 7.35 (d, 2H, J= 8.7 Hz, 4-methoxyphenyl-$C_{2,6}$-H); 7.50 (d, 2H, J= 9 Hz, 4-chlorophenyl-$C_{2,6}$-H); 9.55 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{17}H_{16}N_4O_2$ (308.33): C, 66.22; H, 5.23; N, 18.17. Found: C, 66.35; H, 5.21; N, 18.34.

3.1.16.3. 1-(4-chlorophenyl)-5-(4-methoxyphenyl)-1H-pyrazole-3-carbohydrazide (17c). Yield: 82%; Mp: 140-2 °C. IR (KBr, cm^{-1}): 3325, 3271, 3100 (NH_2, NH); 1649 (C=O); 1592 (C=N); 1245, 1171 (C-O-C). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.75 (s, 3H, OCH_3); 4.49 (s, 2H, NH_2, D_2O exchangeable); 6.92-6.95 (m, 3H, pyrazolyl-C_4-H and 4-methoxyphenyl-$C_{3,5}$-H); 7.18 (d, 2H, J= 8.7 Hz, 4-chlorophenyl-$C_{3,5}$-H); 7.35 (d, 2H, J= 8.7 Hz, 4-methoxyphenyl-$C_{2,6}$-H); 7.50 (d, 2H, J= 9 Hz, 4-chlorophenyl-$C_{2,6}$-H); 9.55 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{17}H_{15}ClN_4O_2$ (342.78): C, 59.57; H, 4.41; N, 16.34. Found: C, 59.65; H, 4.44; N, 16.51.

3.1.17. Ethyl 6-chloro-2-formylquinoline-4-carboxylate (18)

A mixture of equimolar amounts of ethyl 6-chloro-2-methylcinchoninate **2** (1.12 g, 4.5 mmol) and selenium dioxide (0.50 g) in dioxane (40 mL) was heated under reflux with stirring for 4 hours. The reaction mixture was then carefully filtered while hot to get rid of the precipitated selenium metal. The filtrate was concentrated to a smaller volume and left to attain room temperature. The product separated was filtered, washed with cold ethanol, dried and crystallized from ethanol. Yield: 56%; Mp: 160-2 °C. IR (KBr, cm^{-1}):2988, 2863 (CH aldehyde); broad band centered at 1707 for two (C=O); 1599 (C=N); 1242, 1164 (C-O-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.50 (t, 3H, J= 7.2 Hz, -$CH_2\underline{CH_3}$); 4.55 (q, 2H, J= 7.2 Hz, -$\underline{CH_2}CH_3$); 7.82 (d, 1H, J= 9 Hz, quinolyl-C_7-H); 8.25

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(d, 1H, J= 9 Hz, quinolyl-C_8-H); 8.55 (s, 1H, quinolyl-C_3-H); 8.97 (s, 1H, quinolyl-C_5-H); 10.24 (s, 1H, CHO). Anal. Calcd for $C_{13}H_{10}ClNO_3$ (263.68): C, 59.22; H, 3.82; N, 5.31. Found: C, 59.35; H, 3.85; N, 5.39.

3.1.18. 6-Chloro-4-(morpholin-4-ylcarbonyl)quinoline-2-carbaldehyde (19)

A mixture of equimolar amounts of 6-chloro-2-methyl-4-morpholinocarbonylquinoline **10 (**2.04 g, 7 mmol) and selenium dioxide (0.78 g)in dioxane (40 mL) was heated under reflux with stirring for 4 hours. The reaction mixture was carefully filtered while hot to get rid of the precipitated selenium metal. The filtrate was concentrated to a smaller volume, left to attain room temperature and then allowed to cool in an ice bath. The product separated was filtered, washed with ethanol, dried and crystallized from dioxane. Yield: 67%; Mp: 224-5 °C. IR (KBr, cm^{-1}): 2913, 2851 (CH aldehyde); 1708 (HC=O); 1635 (C=O); 1554 (C=N); 1271-1115 (C-O-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.15-3.33 (m, 2H, morpholin-$C_{3a,5a}$-H); 3.52-3.73 (m, 2H, morpholin-$C_{3e,5e}$-H); 3.81-4.07 (m, 4H, morpholin-$C_{2,6}$-H); 7.83 (d, 1H, J= 9 Hz, quinolyl-C_7-H); 7.92 (s, 1H, quinolyl-C_3-H); 7.94 (s, 1H, quinolyl-C_5-H); 8.26 (d, 1H, J= 9 Hz, quinolyl-C_8-H); 10.21 (s, 1H, CHO). Anal. Calcd for $C_{15}H_{13}ClN_2O_3$ (304.73): C, 59.12; H, 4.30; N, 9.19. Found: C, 59.19; H, 4.37; N, 9.34.

3.1.19. Ethyl 6-chloro-2-{[(1,5-diaryl-1H-pyrazol-3-ylcarbonyl)hydrazono] methyl} quinoline-4-carboxylates (20a-c)

The appropriate 1,5-diaryl-1*H*-pyrazole-3-carbohydrazide **17a-c** (1 mmol) was gradually added to a solution containing an equimolar amount of the aldehyde **18** (0.26 g) in glacial acetic acid (20 mL). The reaction mixture was stirred at 25˚C until a precipitate started to form and was then set aside for an overnight to complete precipitation. The precipitates formed were filtered, washed with ethanol, dried and crystallized from glacial acetic acid.

3.1.19.1. Ethyl 6-chloro-2-{[(1,5-bis(4-chlorophenyl)-1H-pyrazol-3-ylcarbonyl)hydrazono]methyl}quinoline-4-carboxylate (20a). Yield: 82%; Mp: 240-2 °C. IR (KBr, cm^{-1}): 3186 (NH); 1718 (C=O); 1665 (NH-C=O); 1592 (C=N); 1236-1087 (C-O-C). ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 1.50 (t, 3H, J= 6.9 Hz, -CH_2CH_3); 4.53 (q, 2H, J= 6.9 Hz, -CH_2CH_3); 7.14-7.52 (m, 9H, CH=N, two chlorophenyl-H); 7.76 (s, 1H, quinolyl-C_3-H); 7.96 (d, 1H, J= 8.4 Hz, quinolyl-C_7-H); 8.10 (s, 1H, quinolyl-C_5-H); 8.85-8.88 (m, 2H, quinolyl-C_8-H and pyrazolyl-C_4-H); 10.58 (s, 1H, D_2O exchangeable, NH). ^{13}C-NMR (75.44 MHz, DMSO-d_6, δ ppm): 13.94 (-CH_2CH_3); 62.20 (-CH_2CH_3); 107.98 (pyrazole C-4); 119.44 (quinoline C-3); 123.96 (pyrazole-*N*1-4-chlorophenyl C-2/6); 126.10, 126.25 (quinoline C-5 and C-6 respectively); 128.55 (pyrazole-C3-4-chlorophenyl C-2/6); 128.62 (pyrazole-*N*1-4-chlorophenyl C-3/5); 128.86 (pyrazole-C3-4-chlorophenyl C-3/5); 129.19 (pyrazole-C3-4-chlorophenyl C-1); 129.47, 130.59 (quinoline C-4a and C-8 respectively); 130.89 (pyrazole-*N*1-4-chlorophenyl C-4);

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133.40, 134.25 (quinoline C-7 and C-4 respectively); 135.21 (pyrazole-C3-4-chlorophenyl C-4); 137.20 (pyrazole-*N*1-4-chlorophenyl C-1); 139.32 (pyrazole C-3); 144.58 (pyrazole C-5); 145.16 (CH=N); 145.76, 151.83 (quinoline C-8a and C-2 respectively); 158.16 (HN-C=O); 164.42 (C=O-OC_2H_5). Anal. Calcd for $C_{29}H_{20}Cl_3N_5O_3$ (592.86): C, 58.75; H, 3.40; N, 11.81. Found: C, 58.88; H, 3.47; N, 11.88.

3.1.19.2. Ethyl 6-chloro-2-{[(5-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-3-ylcarbonyl)hydrazono]methyl}quinoline-4-carboxylate (20b). Yield: 74%; Mp: 200-2 °C. IR (KBr, cm^{-1}): 3210 (NH); 1723 (C=O); 1705 (NH-C=O); 1609 (C=N); 1296-1081 (C-O-C); 1198-1032 (C-O-C, OCH_3). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.51 (t, 3H, J= 7.2 Hz, -CH_2CH_3); 3.83 (s, 3H, OCH_3); 4.54 (q, 2H, J= 7.2 Hz, -CH_2CH_3); 6.87 (d, 2H, J= 7.8 Hz, 4-methoxyphenyl-$C_{3,5}$-H); 7.12-7.60 (m, 8H, CH=N, 4-methoxyphenyl-$C_{2,6}$-H and phenyl-H); 7.76 (s, 1H, quinolyl-C_3-H); 8.04 (d, 1H, J= 7.5 Hz, quinolyl-C_7-H); 8.10 (s, 1H, quinolyl-C_5-H); 8.81-8.87 (m, 2H, quinolyl-C_8-H and pyrazolyl-C_4-H); 10.60 (s, 1H, D_2O exchangeable, NH). Anal. Calcd for $C_{30}H_{24}ClN_5O_4$ (554.00): C, 65.04; H, 3.37; N, 12.64. Found: C, 65.16; H, 4.41; N, 12.78.

3.1.19.3. Ethyl 6-chloro-2-{[(1-(4-chlorophenyl)-5-(4-methoxyphenyl)-1H-pyrazol-3-ylcarbonyl)hydrazono]methyl}quinoline-4-carboxylate (20c). Yield: 78%; Mp: 220-2 °C. IR (KBr, cm^{-1}): 3157 (NH); 1723 (C=O); 1664 (NH-C=O); 1612 (C=N); 1202-1083 (C-O-C); 1172-1033 (C-O-C, OCH_3). ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 1.49 (t, 3H, J= 6.9 Hz, -CH_2CH_3); 3.82 (s, 3H, OCH_3); 4.53 (q, 2H, J= 6.9 Hz, -CH_2CH_3); 6.85-7.49 (m, 9H, CH=N, 4-methoxyphenyl-H and4-chlorophenyl-H); 7.72 (s, 1H, quinolyl-C_3-H); 7.94 (d, 1H, J= 8.4 Hz, quinolyl-C_7-H); 8.07 (s, 1H, quinolyl-C_5-H); 8.82-8.86 (m, 2H, quinolyl-C_8-H and pyrazolyl-C_4-H); 10.62 (s, 1H, D_2O exchangeable, NH). Anal. Calcd for $C_{30}H_{23}Cl_2N_5O_4$ (588.44): C, 61.23; H, 3.94; N, 11.90. Found: C, 61.39; H, 4.01; N, 12.04.

3.1.20. N'-[6-Chloro-4-(morpholin-4-ylcarbonyl)quinolin-2-ylmethylene]-1,5-diaryl-1H-pyrazole-3-carbohydrazides (21a-c)

The appropriate 1,5-diaryl-1*H*-pyrazole-3-carbohydrazide **17a-c**(1 mmol) was gradually added to a solution containing an equimolar amount of 6-chloro-4-(morpholin-4-ylcarbonyl)quinoline-2-carbaldehyde **19** (0.30g)in glacial acetic acid (25 mL).The reaction mixture was stirred at 25˚C until a precipitate started to form and was left for an overnight to complete precipitation. The precipitates formed were filtered, washed with ethanol, air dried and crystallized from glacial acetic acid.

*3.1.20.1. N'-[6-Chloro-4-(morpholin-4-ylcarbonyl)quinolin-2-ylmethylene]-1,5-bis(4-chlorophenyl)-1H-pyrazole-3-carbohydrazide (21a).*Yield: 50%; Mp: 190-2 °C. IR (KBr,

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cm^{-1}): 3247 (NH); 1687 (C=O); 1634 (NH-C=O); 1534 (C=N); 1208-1113 (C-O-C).^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 3.12-3.34 (m, 2H, morpholin-$C_{3a,5a}$-H); 3.47-3.52 (m, 2H, morpholin-$C_{3e,5e}$-H); 3.81-4.11 (m, 4H, morpholin-$C_{2,6}$-H); 7.11-7.68 (m, 9H, CH=N and two chlorophenyl-H); 7.78 (s, 1H, quinolyl-C_3-H); 7.90 (d, 1H, J= 9.2 Hz, quinolyl-C_7-H); 8.12 (d, 1H, J= 9.2 Hz, quinolyl-C_8-H); 8.30 (s, 1H, quinolyl-C_5-H); 8.58 (s, 1H, pyrazolyl-C_4-H); 10.86 (s, 1H, D_2O exchangeable, NH). Anal. Calcd for $C_{31}H_{23}Cl_3N_6O_3$ (633.91): C, 58.74; H, 3.66; N, 13. 26. Found: C, 58.88; H, 3.62; N, 13.44.

3.1.20.2. N'-[6-Chloro-4-(morpholin-4-ylcarbonyl)quinolin-2-ylmethylene]-5-(4-methoxyphenyl)-1-phenyl-1H-pyrazole-3-carbohydrazide (21b). Yield: 63%; Mp: 287-9 °C. IR (KBr, cm^{-1}): 3279 (NH); 1702 (C=O); 1644 (NH-C=O); 1603 (C=N); 1244-1103 (C-O-C); 1193-1077 (C-O-C, OCH_3).^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 3.12-3.38 (m, 2H, morpholin-$C_{3a,5a}$-H); 3.47-3.65 (m, 2H, morpholin-$C_{3e,5e}$-H); 3.80 (s, 3H, OCH_3); 3.82-4.04 (m, 4H, morpholin-$C_{2,6}$-H); 6.82-6.85 (m, 2H, 4-methoxyphenyl-$C_{3,5}$-H); 7.13-7.54 (m, 8H, CH=N, phenyl-H and 4-methoxyphenyl-$C_{2,6}$-H); 7.67 (s, 1H, quinolyl-C_3-H); 7.76 (d, 1H, J= 8.4 Hz, quinolyl-C_7-H); 7.84 (s, 1H, quinolyl-C_5-H); 8.00 (d, 1H, J= 8.4 Hz, quinolyl-C_8-H); 8.36 (s, 1H, pyrazolyl-C_4-H); 10.60 (s, 1H, D_2O exchangeable, NH). ^{13}C-NMR (125.75 MHz, $CDCl_3$, δ ppm): 42.40 (morpholin C-3/5); 47.64 (OCH_3); 66.92 (morpholin C-2/6); 109.02 (pyrazole C-4); 116.59 (quinoline C-3); 119.89 (4-methoxyphenyl C-3/5); 123.37 (4-methoxyphenyl C-1); 123.83, 124.10 (phenyl C-2/6); 125.47 (phenyl C-4); 126.15, 128.76 and 128.86 (quinoline C-4a, C-5 and C-6 respectively); 129.24 (4-methoxyphenyl C-2/6); 131.63, 131.96 (phenyl C-3/5); 135.33, 136.74 (quinoline C-8 and C-7 respectively); 139.33 (phenyl C-1); 140.01, 142.59 (pyrazole C-3 and C-5 respectively); 145.00, 145.61 (quinoline C-4 and C-8a respectively); 146.43 (CH=N); 151.74 (quinoline C-2); 158.17 (NH-C=O); 159.86 (4-methoxyphenyl C-4); 165.58 (C=O). Anal. Calcd for $C_{32}H_{27}ClN_6O_4$ (595.05): C, 64.59; H, 4.57; N, 14.12. Found: C, 64.68; H, 4.61; N, 14.21.

*3.1.20.3. N'-[6-Chloro-4-(morpholin-4-ylcarbonyl)quinolin-2-ylmethylene]-1-(4-chlorophenyl)-5-(4-methoxyphenyl)-1H-pyrazole-3-carbohydrazide (21c).*Yield: 64%; Mp: 185-6 °C. IR (KBr, cm^{-1}): 3257 (NH); 1688 (C=O); 1623 (NH-C=O); 1531 (C=N); 1207-1112 (C-O-C); 1175-1085 (C-O-C, OCH_3).^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 3.14-3.40 (m, 2H, morpholin-$C_{3a,5a}$-H); 3.50-3.75 (m, 2H, morpholin-$C_{3e,5e}$-H); 3.80 (s, 3H, OCH_3); 3.81-4.08 (m, 4H, morpholin-$C_{2,6}$-H); 6.82-7.48 (m, 9H, CH=N, 4-methoxyphenyl-H and 4-chlorophenyl-H); 7.77 (d, 1H, J= 9.15, quinolyl-C_7-H); 7.84 (s, 1H, quinolyl-C_3-H); 7.94 (d, 1H, J= 9.15 Hz, quinolyl-C_8-H); 8.40 (s, 1H, quinolyl-C_5-H); 8.81 (s, 1H, pyrazolyl-C_4-H); 11.03 (s, 1H, D_2O exchangeable, NH). Anal. Calcd for $C_{32}H_{26}Cl_2N_6O_4$ (629.49): C, 61.06; H, 4.16; N, 13. 35. Found: C, 61.13; H, 4.19; N, 13.51.

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3.2. Anti-inflammatory (AI) activity

3.2.1. Formalin-induced paw edema bioassay (acute inflammatory model)

Male albino rats weighing 180–200 g were used throughout the assay [45,46]. They were kept in the animal house under standard condition of light and temperature with free access to food and water. The animals were randomly divided into groups each of five rats. One group of five rats was kept as a control and another group received the standard drug celecoxib [at a dose of 20 mg/kg body weight per os (po)]. A solution of formalin (2%, 0.1 mL) was injected into the subplanter region of the left hind paw under light ether anesthesia 1h after oral administration (po) of the test compound (at a dose level of 20 mg/kg body weight). The paw volume (mL) was measured by means of water plethysmometer and re-measured again 1, 2 and 4 h after administration of formalin. The edema was expressed as an increase in the volume of paw, **(Table 1)** and the percentage of edema inhibition for each rat and each group was obtained as follows:

% Inhibition = (Vt–Vo) control–(Vt–Vo) tested compound / (Vt–Vo) control ×100

Where Vo = volume of edema at zero time interval.

Vt = volume of edema at specific time interval.

3.2.2. Formalin-induced paw edema bioassay (sub-acute inflammatory model)

Rats in the first experiment were given the same test compounds at a dose level of 20 mg/kg body weight daily for 7 consecutive days. A solution of formalin (2%, 0.1 mL) was injected into the subplanter region of the left hind paw under light ether anesthesia 1 h after oral administration (po) of the test compound. A second injection of formalin (2%, 0.1 mL) was given on the third day. The changes in the volume of paw were measured plethymographically at the first and eighth days [45,46]. **(Table 2)**

3.2.3. Ulcerogenic activity

Male albino rats (180–200 g) were divided into groups each of five animals and were fasted for 12 h prior to the administration of the test compounds. Water was given ad libitum. Control group received 1% gum acacia orally. Other groups received celecoxib or the test compounds orally in two equal doses at 0 and 12 h for three successive days at a dose of 300 mg/kg per day. Animals were sacrificed by diethyl ether 6 h after the last dose and their stomachs were removed. An opening at the greater curvature was made and the stomach was cleaned by washing with cold saline and inspected with a 3×magnifying lens for any evidence of hyperemia, hemorrhage, definite hemorrhagic erosion or ulcer [47].

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3.2.4. Acute toxicity

Twelve groups of rats (180–200 g) each consists of five animals, were used in this test [48]. The animals were fasted for 24 h prior to administration of the test compounds. The compounds were given orally in graded doses of 0.1–0.4 g/kg body weight, po. The compounds were screened at graded doses for their acute lethal doses (ALD_{50}) and the mortalities were recorded at each dose level after 24 h.

3.2.5. Determination of effective dose 50 (ED_{50})

The selected compounds were further tested at 5, 10, 20, 40, and 50 mg/Kg body weight and the ED_{50} was determined by measuring the inhibition of the edema volume 2 h after formalin injection. **(Table 1)**

3.2.6. Statistical analysis of data

The data obtained are presented as means ± SE of the mean. The concentration-dependent effects of various drugs were evaluated statistically by the randomized block design analysis of Variance (ANOVA) followed by Student–Newman–Keuls Multiple Comparison Test. The difference in results was considered significant when $P < 0.05$.

3.2.7. In vitro COX-1/COX-2 inhibition assay

The inhibitory COX activity of the tested compounds and the reference was assayed using Cayman colorimetric COX (ovine) inhibitor screening assay kit (Catalog No. 760111, Cayman Chemicals, Ann Arbor, MI) according to the manufacturer's instructions [49]. Aliquots (170 mL) of the assay buffer (0.1N Tris- HCl, pH 8.0), heme, and enzyme ovine (COX-1 or COX-2) were placed in a 96-well plate. The test compounds were added to the aliquots. The plate was carefully shaken for a few seconds and then incubated for 5 min at 25 °C. The colorimetric substrate N,N,N',N'-tetramethyl-p-phenylenediamine (20 mL, TMPD) and arachidonic acid (20 mL) were added to the aliquots. The plate was carefully shaken for a few seconds and then incubated for 5 min at 25 °C. The absorbance was measured at 590 nm using a 96-well Tecan Safire plate reader. The mixture of 160 mL of assay buffer and 10 mL of heme served as a background control. The mixture of 150 mL of assay buffer, 10 mL of heme, and 10 mL of ovine COX-1 or COX-2 showed 100% initial activity as a control experiment in the absence of inhibitor. Diclofenac, celecoxib and indomethacin were used as reference standards in the study. The assays were performed in triplicate and the IC_{50} values were calculated from the concentration curves by means of GraphPad software PRISM.

3.2.8. In vitro 5-LOX inhibitory assay

The inhibitory activity of the test compounds against soya bean 5-LOX was evaluated by using Abnova lipoxygenase assay kit (catalog no. KA1329, Cayman Chemicals). IC50

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values of the tested compounds were carried out according to procedures and instructions given with the assay kit and in accordance to previously reported methods [63].

3.3. Docking Studies

Preparation of COX crystal structures:

Coordinates for COX-1 and COX-2 crystal structures was retrieved from the Protein Data Bank (PDB code: 1EQG and 3LN1 respectively) and handled consequently with Molecular Operating Environment program (MOE) [64]. Redundant chains, non-essential ions, water molecules and ligands were discarded. The crystal structures were treated with "QuickPrep" module at default settings. This includes additions of bond orders, formal charges and explicit hydrogen atoms to the complex structure. Subsequently, the most appropriate protonation states and optimization of the H-bond network were performed employing "Amber: 10EHT" force field. Prepared protein structures were saved as PDB files which were further used for GOLD docking. The ligand-protein in-place minimization was also performed.

Preparation of the selected compounds for docking:

The molecules were built and prepared by MOE. 'Molecule wash' function was used to generate meaningful protonation states by deprotonating strong acids and protonating strong bases (if required). Energy minimization of all molecules was performed using the Amber: 10EHT force field at a gradient of 0.01 RMSD (i.e. if the gradient falls below RMSD, the minimization terminates). Conformational search was conducted to sample maximum number of minimized conformations including different stereoisomers and ring conformations (if necessary) using "Stochastic" method, with option "Allow unconstrained double bond rotation" enabled to sample different geometrical isomers of the double bond-containing compounds. All other options were set at default levels. This ended up to total of 4254 conformations of the compounds under investigation. The resulting conformers were saved as SD file for the docking experiments.

Docking experiments:

GOLD (version 5.2) [65-70] was used for docking experiments against COX-1 and COX-2 crystal structures. Residues of the binding site were defined by specifying the coordinates of the co-crystallized ligands and using a cutoff radius of 8 Å, with the 'detect cavity' option enabled. The scoring function used for GOLD docking experiments was ChemPLP. The search efficiency of the genetic algorithm was at 50% to handle the 4254 conformations in a meaningful time period. The best docking solution per conformation were saved consequently. This docking approach was validated by successful pose-retrieval of the co-crystallized ligands of COX-2 crystal structures. All graphical representations in Figures 2-4 were rendered using MOE.

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Dedication

This work is dedicated to the memory of El-Sayeda M. El-Khawass.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the contents and writing the paper.

Ethical conduct of research

The protocols used in the present study followed the guidelines set in 'The Guide for the Care and Use of Laboratory Animals', and got approval By ACUC, Faculty of Pharmacy, Alexandria University, Project No. 82 at 23/11/2016. Copy of approval is available upon request.

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Table 1: Anti-inflammatory activity (AI) of synthesized compounds in formalin-induced rat paw edema bioassay (acute inflammatory model).

Compound[a]	Volume of edema (ml)[b]				
	0	1h	2h	4h	ED_{50} (mg/Kg)
Control	0.31±0.01	0.59±0.03	0.68±0.03	0.81±0.04	
5*a*	0.33±0.01	0.51±0.02* (35)[c]	0.56±0.02* (37)	0.69±0.03* (28)	
5*b*	0.32±0.01	0.51±0.02* (32)	0.55±0.02* (38)	0.67±0.03* (30)	
5*c*	0.42±0.02	0.49±0.02* (75)	0.51±0.03* (75)	0.60±0.02* (64)	14.25
7	0.39±0.01	0.47±0.01* (71)	0.58±0.03* (48)	0.70±0.04* (38)	
9	0.30±0.01	0.49±0.01* (32)	0.54±0.03* (35)	0.66±0.03* (28)	
11*a*	0.32±0.01	0.50±0.03* (36)	0.55±0.02* (38)	0.67±0.02* (30)	
11*b*	0.39±0.01	0.46±0.03* (75)	0.49±0.02* (72)	0.52±0.02* (74)	13.15
11*c*	0.41±0.03	0.49±0.02* (71)	0.52±0.02* (70)	0.58±0.03* (86)	10.70
12*a*	0.37±0.02	0.57±0.02* (29)	0.62±0.03* (32)	0.74±0.02* (26)	
12b	0.32±0.01	0.40±0.01* (71)	0.47±0.02* (59)	0.55±0.02* (54)	
12*c*	0.36±0.01	0.42±0.02* (78)	0.47±0.01* (70)	0.51±0.02* (70)	13.50
14*a*	0.38±0.01	0.44±0.01* (78)	0.47±0.03* (75)	0.55±0.02* (66)	15.25
14*b*	0.41±0.01	0.47±0.02* (78)	0.60±0.02* (48)	0.69±0.04* (64)	13.90
20*a*	0.39±0.01	0.45±0.02* (78)	0.51±0.01* (67)	0.58±0.02* (62)	16.50
20*b*	0.36±0.02	0.56±0.03* (28)	0.62±0.03* (29)	0.75±0.04* (22)	
20*c*	0.34±0.03	0.50±0.03* (42)	0.52±0.02* (51)	0.66±0.04* (36)	
21*a*	0.38±0.03	0.47±0.02* (67)	0.51±0.03* (64)	0.58±0.02* (60)	17.12
21*b*	0.37±0.01	0.48±0.01* (60)	0.50±0.02* (64)	0.62±0.03* (50)	
21*c*	0.38±0.01	0.46±0.02* (71)	0.56±0.02* (51)	0.63±0.03* (50)	

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Diclofenac Na.	0.32±0.01	0.44±0.02* (57)	0.52±0.02* (45)	0.53±0.02* (58)	16.80
Celecoxib	0.34±0.02	0.45±0.02* (60)	0.49±0.03* (59)	0.51±0.02* (66)	15.11

*Significantly different compared to corresponding control $p \leq 0.05$.

[a] Dose levels, po: test compounds (20 mg/Kg body weight), celecoxib (20 mg/Kg body weight).

[b] Values are expressed as mean ± S.E. (Number of animals N=5 rats).

[c] Between parentheses (percentage anti-inflammatory, AI%).

Table 2: Anti-inflammatory activity (AI) of synthesized compounds in formalin-induced rat paw edema bioassay (sub-acute inflammatory model).

Compound[a]	**Volume of edema (ml)[b]**		
	0	**1st day**	**8th day**
Control	0.31±0.01	0.66±0.02	0.82±0.03
5*a*	0.33±0.01	0.60±0.03* (22)[c]	0.71±0.03* (25)
5*b*	0.32±0.01	0.59±0.03* (23)	0.69±0.03* (27)
5*c*	0.42±0.02	0.51±0.01* (74)	0.63±0.02* (58)
7	0.39±0.01	0.57±0.02* (48)	0.64±0.03* (50)
9	0.35±0.01	0.60±0.03* (29)	0.70±0.03* (31)
11*a*	0.38±0.02	0.62±0.03* (31)	0.72±0.03* (33)
11*b*	0.39±0.01	0.54±0.02* (57)	0.63±0.02* (52)
11*c*	0.41±0.03	0.60±0.03* (45)	0.62±0.02* (58)
12*a*	0.33±0.01	0.56±0.02* (34)	0.65±0.03* (37)
12*b*	0.32±0.01	0.53±0.02* (40)	0.67±0.02* (31)
12*c*	0.36±0.01	0.56±0.03* (42)	0.66±0.02* (41)

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14*a*	0.38±0.01	0.51±0.02* (62)	0.62±0.02* (52)
14*b*	0.41±0.01	0.59±0.02* (48)	0.60±0.03* (62)
20*a*	0.39±0.01	0.52±0.02* (62)	0.61±0.02* (56)
20*b*	0.36±0.02	0.63±0.03* (22)	0.72±0.03* (29)
20*c*	0.34±0.03	0.60±0.03* (22)	0.68±0.02* (33)
21*a*	0.38±0.03	0.52±0.02* (60)	0.61±0.02* (54)
21*b*	0.37±0.01	0.58±0.02* (40)	0.66±0.02* (43)
21*c*	0.38±0.01	0.51±0.02* (62)	0.64±0.03* (49)
Diclofenac Na.	0.32±0.01	0.53±0.02* (40)	0.56±0.03* (52)
Celecoxib	0.34±0.02	0.51±0.02* (51)	0.54±0.02* (60)

*Significantly different compared to corresponding control $p \leq 0.05$.

[a] Dose levels, po: test compounds (20 mg/Kg body weight), celecoxib (20 mg/Kg body weight).

[b] Values are expressed as mean ± S.E. (Number of animals N=5 rats).

[c] Between parentheses (percentage anti-inflammatory, AI%).

Table 3: *In vitro* COX-1, COX-2 and LOX enzymatic inhibition assay of the active compounds.

Compound	**IC_{50}/µM[a)]**			
	COX-1[b)]	**COX-2[b)]**	**COX-1/COX-2 SI [c)]**	**5-LOX[d)]**
5c	8.41	0.34	24.74	2.09
11b	6.98	0.63	11.08	1.76
11c	8.52	0.56	15.21	2.24
12c	11.41	0.10	114.10	1.87
14a	10.14	0.11	92.18	1.91

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14b	12.33	0.11	112.09	1.93
20a	10.22	0.14	73.00	1.78
21a	7.21	0.54	13.35	2.11
Diclofenac Na.	3.9	0.8	4.88	2.11
Celecoxib	14.8	0.05	296.00	ND
Indomethacin	0.039	0.49	0.08	ND
Zileuton	ND	ND	ND	4.51

ND: not determined.

[a)] IC_{50} value is the compound concentration required to produce 50% inhibition of COX-1, COX-2 or LOX for means of three determinations.

[b)] Values are means of three determinations acquired using an ovine COX-1/COX-2 assay kit (catalog no. 760111, Cayman Chemicals) and the deviation from the mean is <10% of the mean value.

[c)] *In vitro* COX-2 selectivity index (IC_{50} of COX-1/COX-2).

[d)] Values are means of three determinations acquired using Abnova 5-lipoxygenase assay kit (catalog no. KA1329, Cayman Chemicals) and the deviation from the mean is <10% of the mean value.

Table 4: Distribution of docking fitness (score) of the active compounds onto COX-2 enzyme as well as some of their topological parameters.

Compound	Fitness (SD[a]) COX-2	Molecular weight	Molecular volume	Molecular surface area
21a	99.17 (0.62)	633.9	385.9	561.7
20a	97.89 (0.54)	592.9	359.5	528.3
14a	76.04 (1.42)	406.9	260.7	378.1
14b	71.91 (2.25)	441.3	276.8	401.2
11c	72.58 (3.81)	544.4	347.1	500.7
5c	69.93 (2.04)	530.4	332.0	484.8

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11b	69.62 (2.35)	510.0	334.4	477.6
12c	69.00 (4.42)	585.5	368.0	534.1
Celecoxib	91.51 (0.43)	381.4	220.5	328.3
Indomethacin	66.87 (0.50)	356.8	226.0	340.5
Diclofenac	59.27 (0.89)	296.2	197.2	266.3
Zileuton	ND	236.3	151.3	227.0

[a]SD is the standard deviation of three consecutive runs. The docking score is expressed as fitness of ChemPLP scoring function of GOLD (v 5.2) [64-69]. [b]ND is not determined. The fitness values are for best-scored poses.

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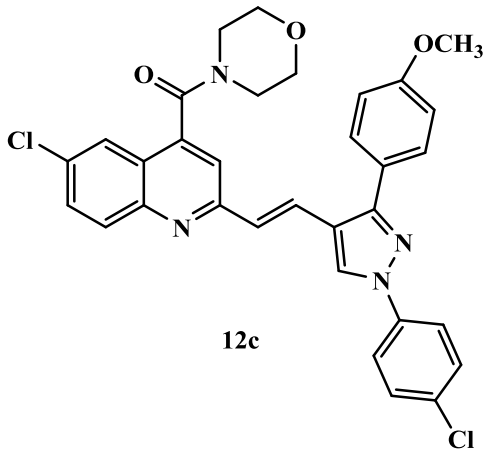
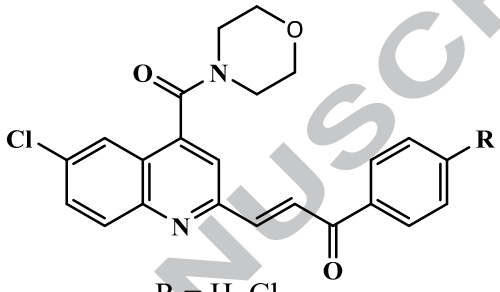
Graphical Abstract

New quinoline compounds comprising pyrazole scaffold through different linkers were synthesized and evaluated for their anti-inflammatory activity. In vitro COX-1/COX-2 inhibition study revealed that compounds **12c, 14a,b** and **20a** showed relatively higher selectivity towards COX-2 than COX-1. All compounds showed significant *in vitro* LOX inhibitory activity.

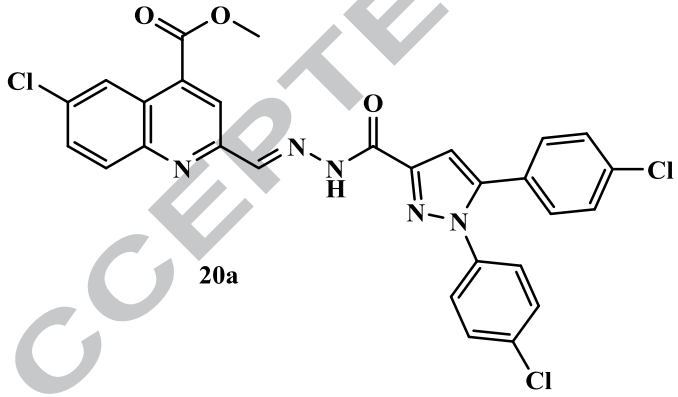


12c



R = H, Cl

14a,b



20a

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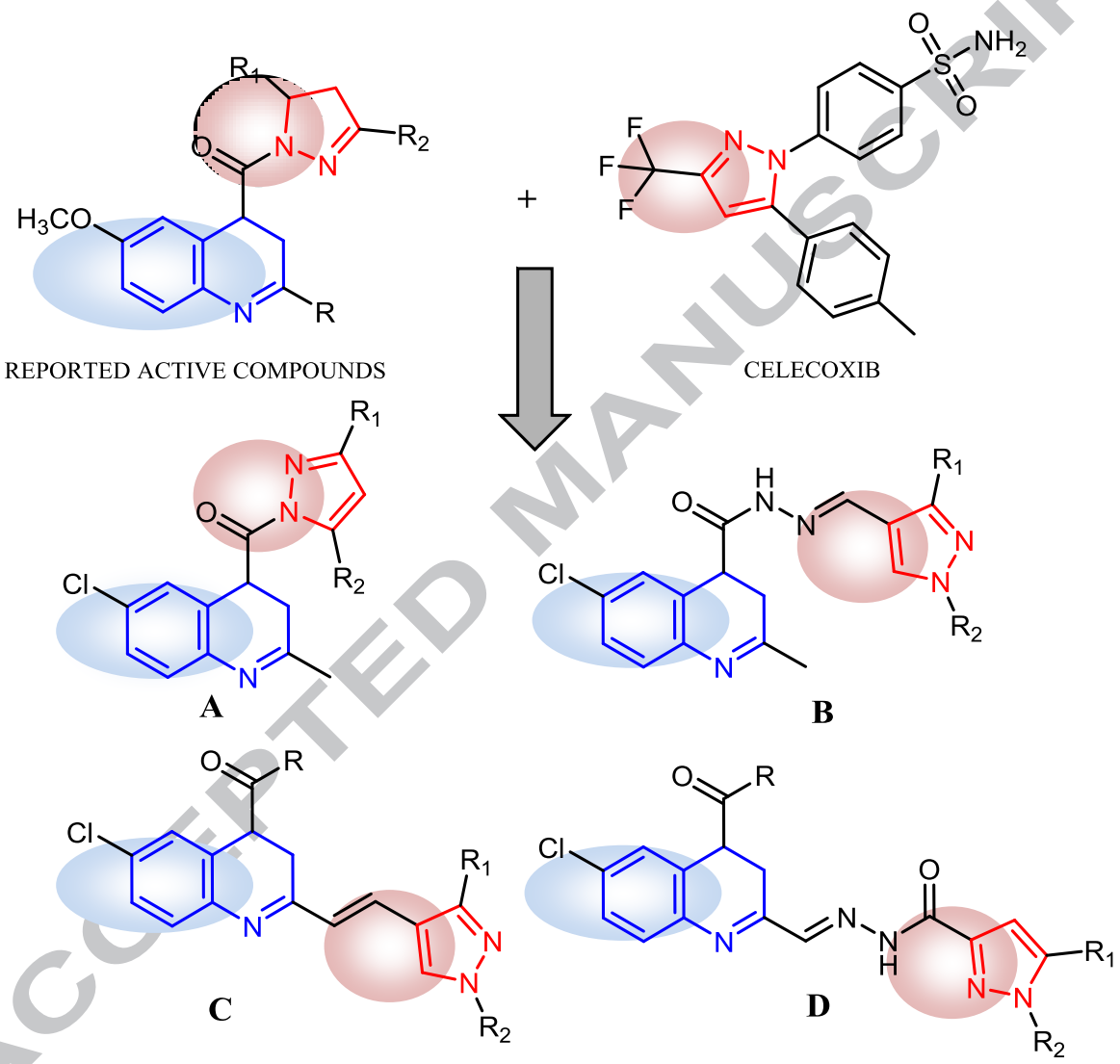

Figure 1: Structures of Celecoxib, previously synthesized anti-inflammatory pyrazolylquinolines and the general structures of the target compounds (A-D)

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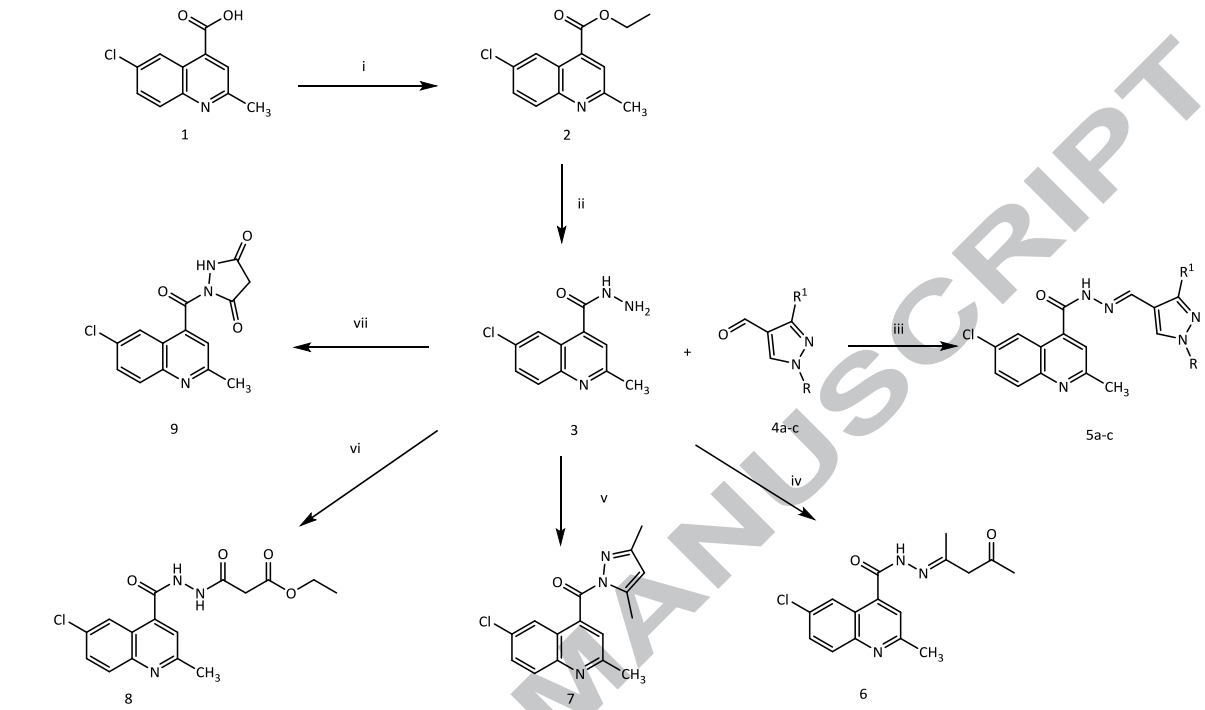

Scheme 1: Synthetic pathways for compounds **5a-c** and **6-9**

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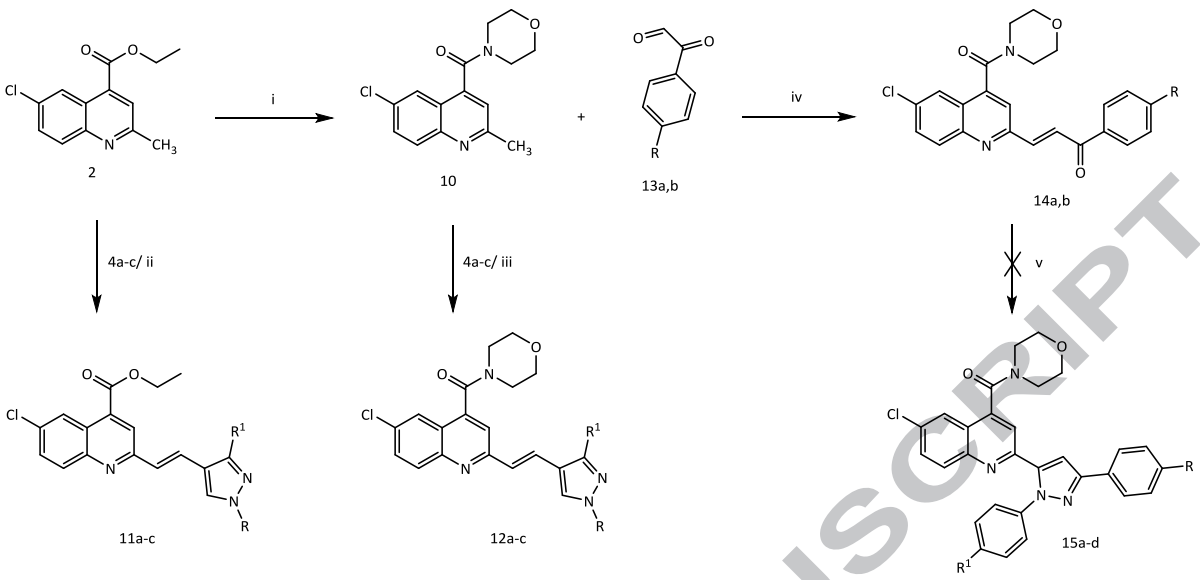

Reagents and conditions: i) excess morpholine/ reflux; ii) Ac_2O / reflux; iii) Ac_2O / reflux; iv) Ac_2O / reflux; v) $R^1C_6H_4NHNH_2$ / Ethanol.

For 11 and 12: a: R = R^1 = 4-Cl-C_6H_4; b: R = C_6H_5, R^1 = 4-OCH_3-C_6H_4 ; c: R = 4-Cl-C_6H_4, R^1 = 4-OCH_3-C_6H_4.
For 13 and 14: a: R = H; b: R = Cl.
For 15: a: R = R^1= H; b: R= H, R^1 = Cl; c: R=Cl, R^1= H; d : R = Cl, R^1 = Cl.

Scheme 2

Scheme 2: Synthetic pathways for compounds **11a-c, 12a-c** and **14a,b**

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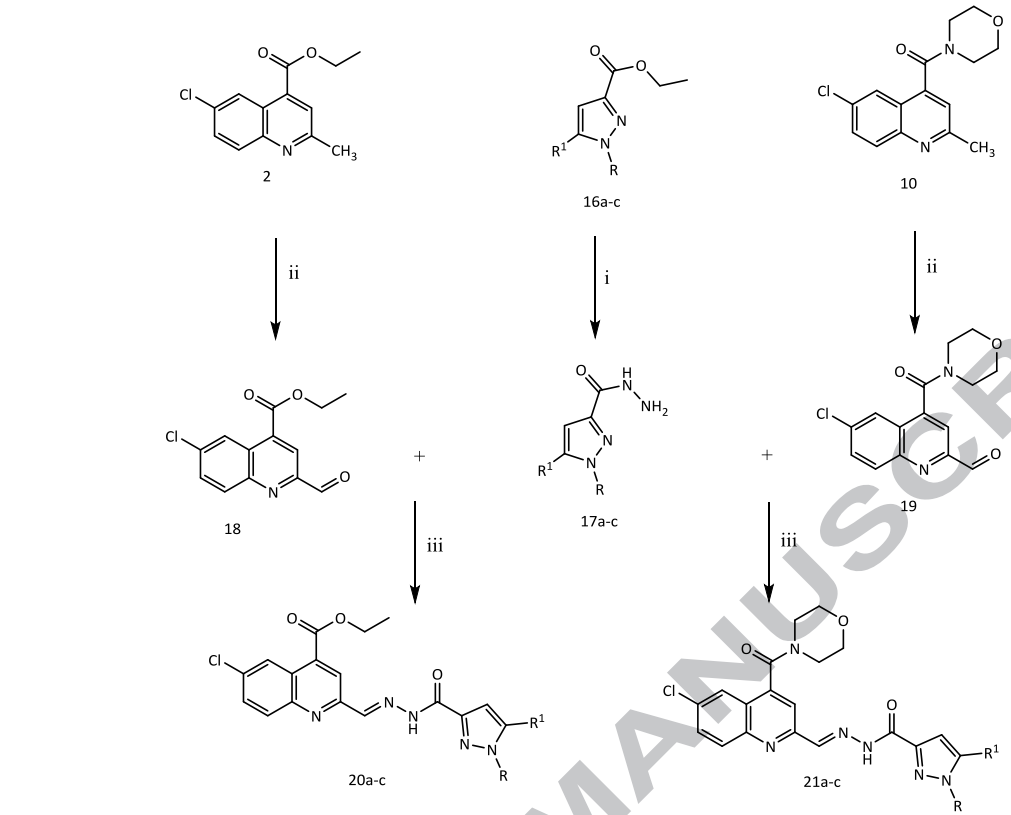

Reagents and conditions: i) N_2H_4 /Ethanol / Reflux; ii) SeO_2 / Dioxane / Reflux; iii) gl. Acetic acid / 25 °C/ stir.

For 17, 20 and 21: a: R = R^1 = 4-Cl-C_6H_4; b: R = C_6H_5, R_1 = 4-OCH_3-C_6H_4; c: R = 4-Cl-C_6H_4, R^1 = 4-OCH_3-C_6H_4.

Scheme 3

Scheme 3: Synthetic pathways for compounds **20a-c** and **21a-c**

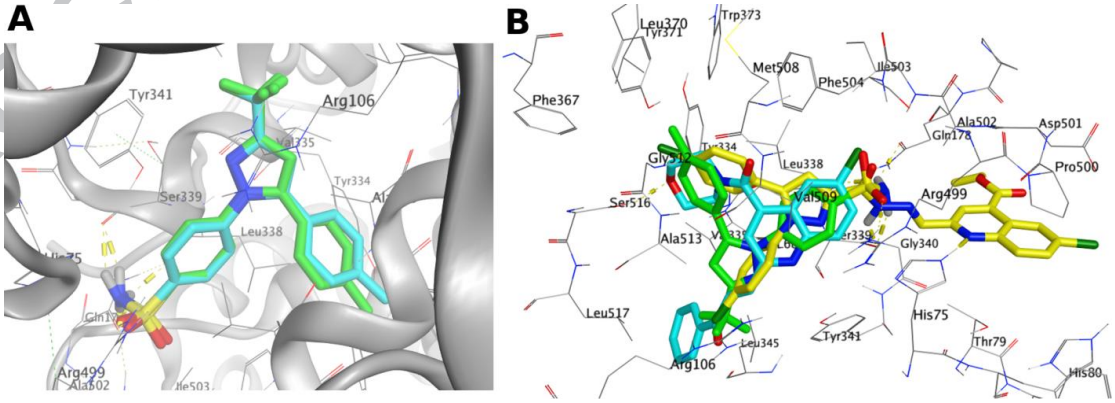

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Figure 2: (A) Overlay of the co-crystal ligand (celecoxib – green sticks) and its docked pose (cyan sticks) in the binding site of COX-2 (PDB: 3LN1). (B) Overlay of the co-crystal ligand (celecoxib – green sticks) and the docking poses of **14a** (cyan sticks) and **20a** (yellow sticks) indicating the general binding mode in the binding site of COX-2. Polar hydrogen atoms were omitted for clarity.

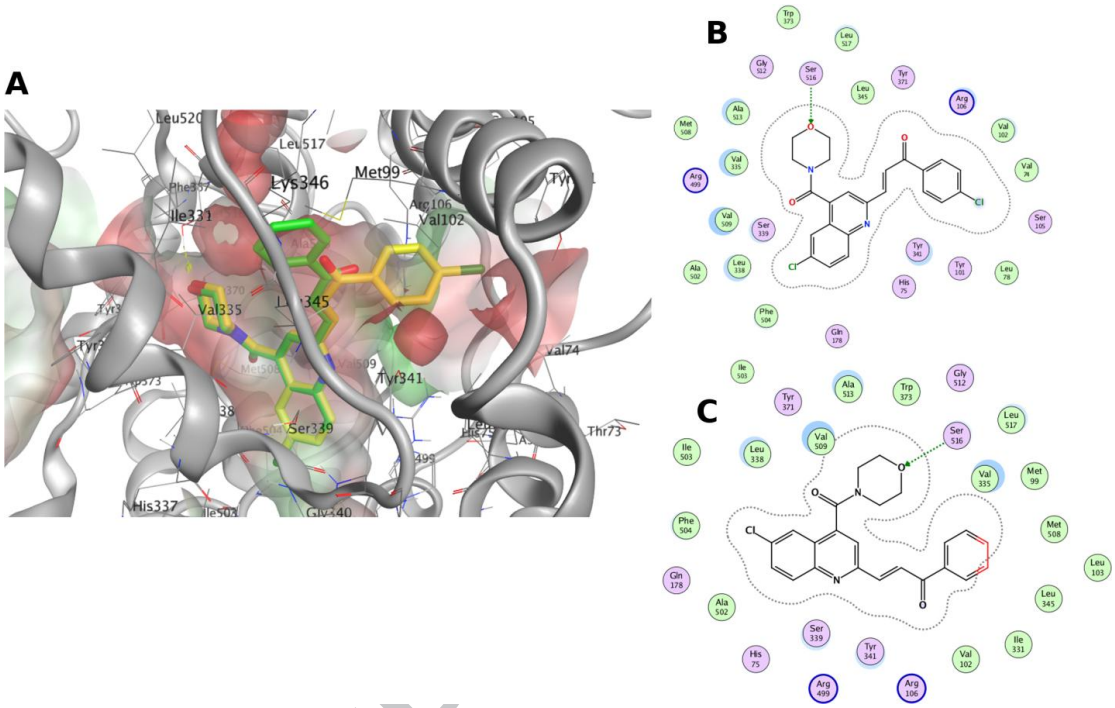

Figure 3: (A) Overlay of the docking poses of **14a** (green sticks) and **14b** (yellow sticks) in the binding site of COX-2. (B) 2D interaction pattern of the docking poses of **14b** and **14a** in the binding site of COX-2 for (B) and (C), respectively. The red and green colors of the surface representation of (A) are indicating hydrophobic and hydrophilic areas, respectively.

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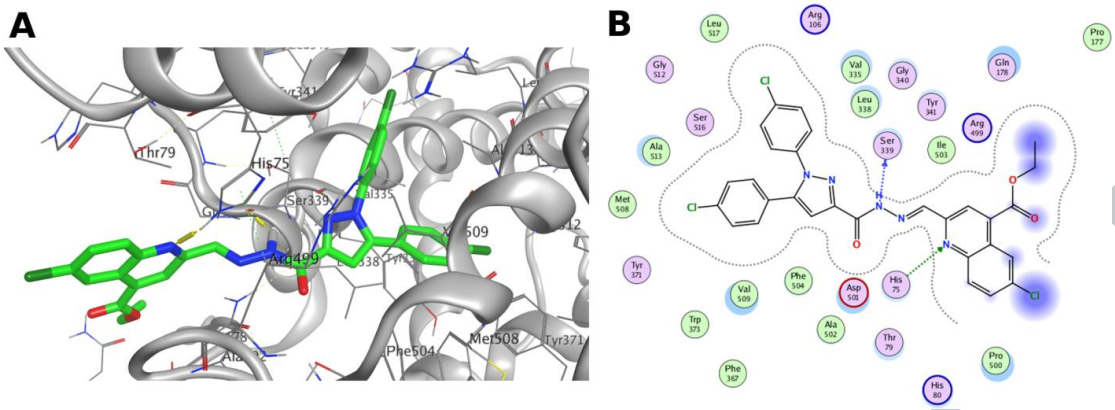

Figure 4: Docking pose of **20a** (green sticks) in the binding site of COX-2, in 3D and 2D representation for (A) and (B), respectively.

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Highlights

- Synthesis of some new quinoline compounds comprising pyrazole scaffold through different amide linkages.
- Eight compounds were found to exhibit promising anti-inflammatory profiles in acute and sub-acute inflammatory models.
- Compounds **12c, 14a,b, 20a** showed relatively higher selectivity towards COX-2 than COX-1.
- All Compounds showed significant *in vitro* LOX inhibitory activity higher than that of zileuton.

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