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# Design, synthesis, and biological evaluation of ketoprofen analogs as potent cyclooxygenase-2 inhibitors

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ABSTRACT

A new series of ketoprofen analogs were synthesized to evaluate their biological activities as selective cyclooxygenase-2 (COX-2) inhibitors. In vitro COX-1 and COX-2 inhibition studies showed that all compounds were potent and selective inhibitors of the COX-2 isozyme with $IC_{50}$ values in the highly potent 0.057–0.085 μM range, and COX-2 selectivity indexes in the 115 to >1298.7 range. Compounds possessing azido pharmacophore group (**8a** and **8b**) exhibited highly COX-2 inhibitory selectivity and potency even more than reference drug celecoxib. Molecular modeling studies indicated that the azido substituent can be inserted deeply into the secondary pocket of COX-2 active site for interactions with $Arg^{513}$.

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## 1. Introduction

Non-stroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of inflammation, fever, and pain. However, because NSAIDs inhibit both isoforms of cyclooxygenase (COX) (constitutive COX-1, responsible for cytoprotective effects; inducible COX-2, responsible for inflammatory effects), they are associated with well-known side effects such as gastrointestinal side effects and renal function suppression.[1,2] It is known that selective COX-2 inhibitors can provide anti-inflammatory agents devoid of the undesirable effects associated with classical non-selective NSAIDs.[3] In addition to the role of COX-2 in inflammatory disorders such as rheumatoid arthritis and osteoarthritis, it is also implicated in cancer and angiogenesis. In this regard, several epidemiologic studies have been reported that inhibitors of COX-2 enzyme reduce the risk of colorectal, breast, and lung cancer, and COX-2 is expressed in these cancers.[4,5] As a consequence, increasing interest has been devoted to the synthesis of selective inhibitors of COX-2 by means of modification of well-known non-selective agents such as indomethacine,[6–8] zomepirac,[9] flurbiprofen,[10] meclofenamic acid,[11] or ketoprofen.[12–14] On the other hand, we recently reported several investigations describing the design, synthesis, and a molecular modeling study for a group of 2-phenyl-4-carboxyl quinolines (Fig. 1, **A**) possessing a methyl sulfonyl COX-2 pharmacophore at the *para* position C-2 phenyl ring.[15] Our results showed that quinoline ring is a very suitable scaffold for COX-2 inhibitory activity. Therefore, it is interesting to synthesize and evaluate the hybrid structure of ketoprofen as a well-known NSAIDs and our quinoline-4-carboxylic acid scaffold. As part of our research program, aimed at discovering new selective COX-2 inhibitors, we focused our attention on the synthesis, COX inhibitory and some molecular modeling studies of 2-(4-(substituted) phenyl)quinoline-4-carboxylic acid derivatives possessing benzoyl moiety at C-6 or C-8 of quinoline ring. The rational for the design of these compounds was based on ketoprofen structure as a part of 2-aryl-quinoline-4-carboxylic acid scaffold (Fig. 1) in our previously reported COX-2 inhibitors.

## 2. Chemistry

The target 6- or 8-benzoyl-2-(4-(methylthio)phenyl)quinoline-4-carboxylic acid derivatives and 6- or 8-benzoyl-2-(4-(acetamido)phenyl)quinoline-4-carboxylic acid derivatives were obtained employing a Doebner reaction.[16] As illustrated in Scheme 1, appropriate benzaldehyde (**1**), pyrrovic acid (**2**), and appropriate amine (**3**) were heated in acetic acid to provide 4-carboxyl quinolines (**4** and **5**, 15–31%) and then oxidation of methylthio substituent was carried out using oxone by previously reported method[17] to give **6a** and **6b** (67–70%). Hydrolysis of acetamido derivatives **5a** and **5b** under acidic condition gave the corresponding amines **7a** and **7b** (47%). Diazotization of **7a** and **7b** with sodium nitrite followed by treatment with sodium azide afforded the azide derivatives **8a** and **8b** (20–35%).[18]

## 3. Results and discussion

A new group of 2-(4-(substituted)phenyl)quinoline-4-carboxylic acid possessing a benzoyl moiety at C-6 or C-8 position of

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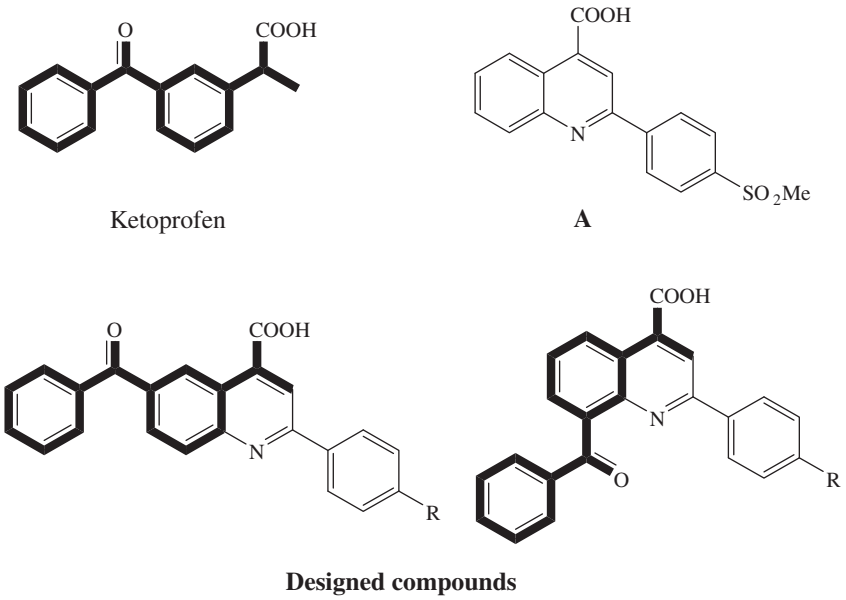
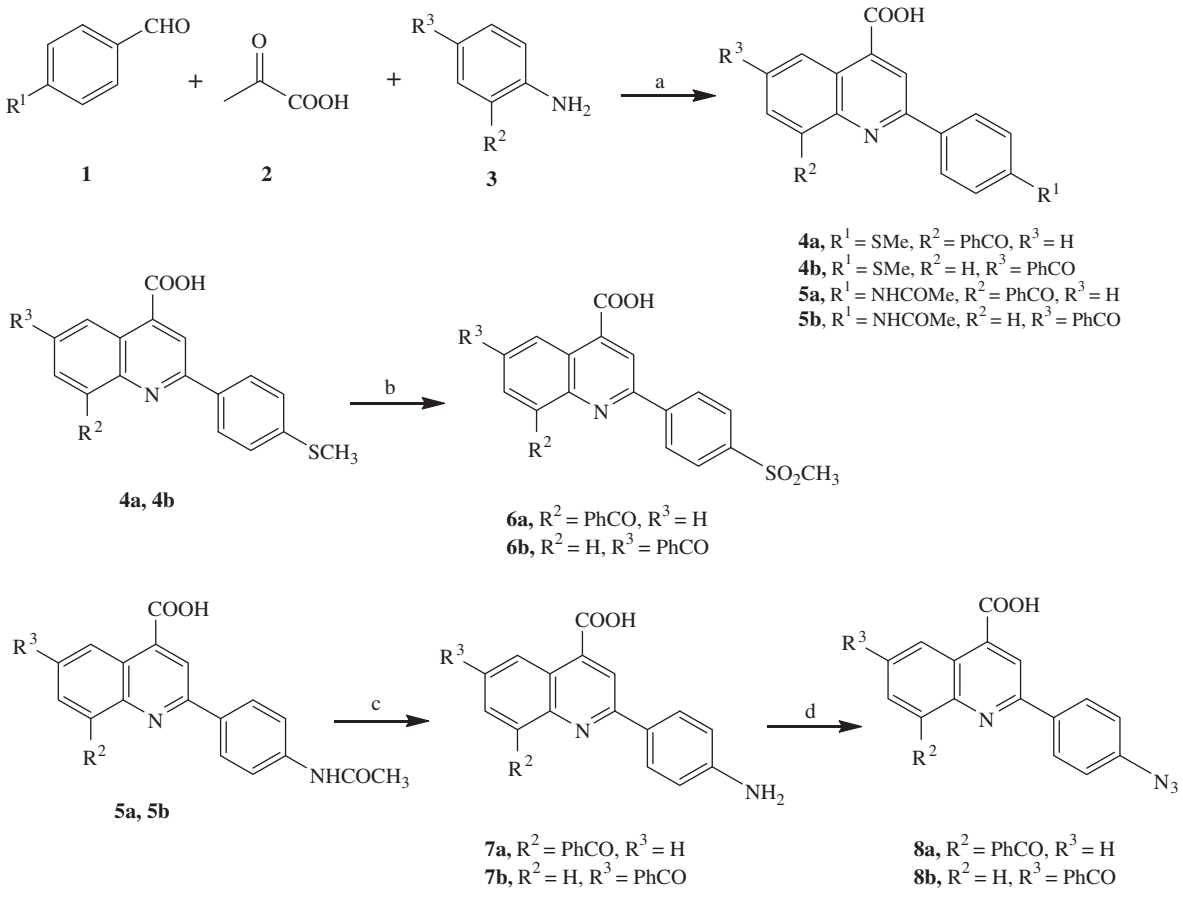


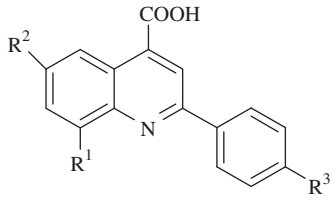

**Figure 1.** Chemical structures of ketoprofen and 4-carboxyl quinoline (**A**) lead compounds and our designed scaffolds.




**Scheme 1.** Reagents and conditions: (a) acetic acid, reflux, 20 h; (b) oxone/THF, 3–5 h; (c) HCl, reflux, 5 h; (d) $NaNO_2$/HCl and then $NaN_3$, 0–5 °C, 2 h.

quinoline ring was designed based on ketoprofen structure. SAR data ($IC_{50}$ values) acquired by determination of the in vitro ability of the title compounds to inhibit the COX-1 and COX-2 isozymes showed that all compounds were potent and selective inhibitors of the COX-2 isozyme with $IC_{50}$ values in the highly potent 0.057–0.085 μM range, and COX-2 selectivity indexes in the 115 to >1298.7 range (Table 1). Our results showed that the COX inhibition was sensitive to the position of benzoyl group on the quinoline ring. Accordingly, compounds possessing benzoyl on the C-6 (**5b, 6b**, and **8b**) were more selective COX-2 inhibitors compared with those

**Table 1**
In vitro COX-1 and COX-2 enzyme inhibition assay data




| Compound | $R^1$ | $R^2$ | $R^3$ | $IC_{50}$[a] (μM) | | Selectivity index (SI)[b] |
|---|---|---|---|---|---|---|
| | | | | COX-1 | COX-2 | |
| **5a** | PhCO | H | $NHCOCH_3$ | 9.2 | 0.080 | 115.0 |
| **5b** | H | PhCO | $NHCOCH_3$ | 21.8 | 0.085 | 256.5 |
| **6a** | PhCO | H | $SO_2Me$ | 15.9 | 0.069 | 231.1 |
| **6b** | H | PhCO | $SO_2Me$ | 24.4 | 0.077 | 316.8 |
| **8a** | PhCO | H | $N_3$ | 65.55 | 0.057 | 1150 |
| **8b** | H | PhCO | $N_3$ | >100 | 0.077 | >1298.7 |
| **A** | H | H | $SO_2Me$ | 13.4 | 0.091 | 147.2[c] |
| Celecoxib | | | | 24.3 | 0.06 | 405 |

[a] Values are means of two determinations acquired using an ovine COX-1/COX-2 assay kit and the deviation from the mean is <10% of the mean value.
[b] In vitro COX-2 selectivity index (COX-1 $IC_{50}$/COX-2 $IC_{50}$).
[c] Ref. 15.

having benzoyl on the C-8 (**5a, 6a**, and **8a**) of quinoline ring. This effect may be explained by steric parameter for interaction with COX-1 active binding site. However, the isomers having C-8 benzoyl moiety showed more potency for COX-2 inhibitory activity. SAR data also indicated the COX inhibition was very sensitive to the type of substituent at the *para* position of C-2 phenyl ring so that it can affect both potency and selectivity on COX-2 inhibitory activity. The relative COX-2 potency and COX-2 selectivity profiles for ketoprofen derivatives with respect to the substituents at the *para* position of C-2 phenyl ring was $N_3 \gg SO_2Me > NHCOMe$. Interestingly, compounds possessing $N_3$ substituent (**8a** and **8b**) exhibited high potency and selectivity on COX-2 inhibition which may be due to the insertion of $N_3$ deeply into the secondary pocket of COX-2 binding site where it undergoes electrostatic interaction with $Arg^{513}$. Accordingly, the binding interactions of the most selective COX-2 inhibitor (**8b**) within the COX-2 binding site were investigated. The most stable enzyme–ligand complex of 2-(4-(azido)phenyl)-6-benzoyl-quinoline-4-carboxylic acid possessing a $N_3$ COX-2 pharmacophore group within the COX-2 binding site (Fig. 2) shows that the *para*-$N_3$-phenyl moiety is oriented towards the COX-2 secondary pocket ($Arg^{513}$, $Phe^{518}$, and $Val^{523}$). All the nitrogen atoms of $N_3$ can form remarkable binding interactions with N*H* groups of $Arg^{513}$ (distances <4 Å). The azido substituent has the potential to undergo electrostatic binding interactions with amino acid residues, particularly $Arg^{513}$, lining the secondary pocket of COX-2.[18] In addition, our docking studies showed that one of the *O*-atoms of carboxylic acid group can form hydrogen binding interaction with amino group of $Arg^{120}$ (distance = 2.0 Å) whereas the other *O*-atom is about 2.8 Å away from OH of $Tyr^{355}$. Also, the distance between the *O*-atom of C=*O* group attached to C-6 quinoline ring and O*H* of $Ser^{530}$ is 3.4 Å which can provide a hydrogen binding interaction. On the other hand, the nitrogen atom of quinoline ring is close to N*H* group of $Ala^{527}$ (distance = 4.7 Å) and the phenyl ring of benzoyl moiety is close to hydrophobic side chain of $Leu^{117}$. These data together with experimental results can explain the high potency and selectivity of compound **8b**. Similarly, molecular modeling study of compound **8a** shows that it binds in the primary binding site such that the *para*-$N_3$ substituent is inserted into the secondary pocket present in COX-2 and forms hydrogen bonding interaction with N*H* groups of $Arg^{513}$ (distances <4 Å). Also, the carboxylic acid substituent of compound **8a** is close to N*H* groups of $Arg^{120}$ and can bind to this amino acid in the same manner as compound **8b**. However, the orientation of benzoyl moiety of **8a** is different from that of **8b** into the active site of COX-2, which may explain the selectivity and potency differences of these isomers. The binding interactions

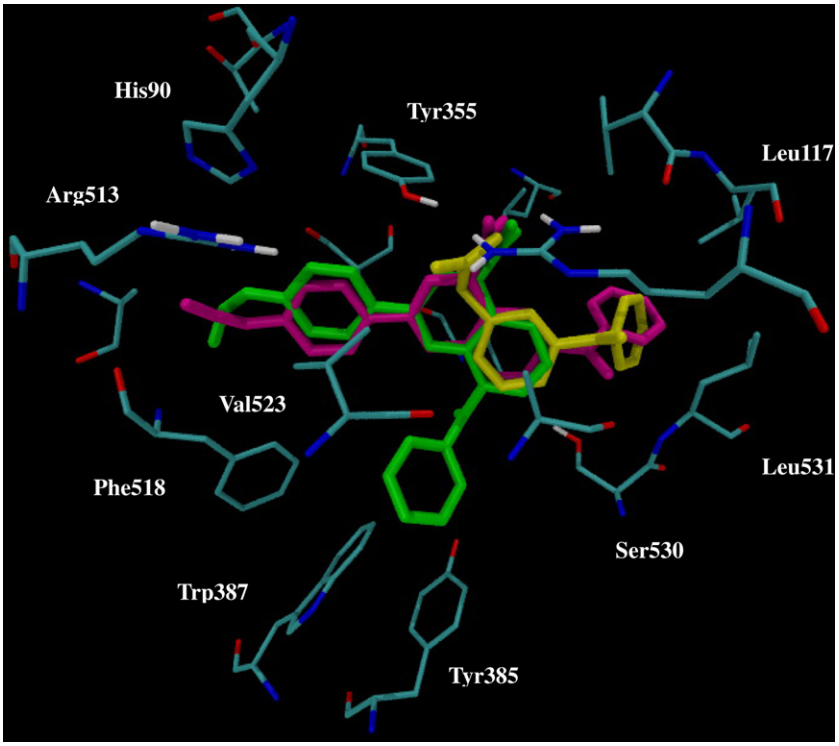



**Figure 2.** Docking 2-(4-(azido)phenyl)-8-phenylcarbonylquinoline-4-carboxylic acid (**8a**, in green), 2-(4-(azido)phenyl)-6-phenylcarbonylquinoline-4-carboxylic acid (**8b**, in pink) and ketoprofen (in yellow) in the active site of murine COX-2.

of ketoprofen molecule within the COX-2 binding site were also investigated and interestingly the binding interactions of ketoprofen are similar to compound **8b** into the COX-2 binding site (Fig. 2). These results showed that when the benzoyl group is at C-6 position of quinoline ring, the molecule is more like ketoprofen, hence showing better selectivity than the isomer possessing C-8 benzoyl group.

## 4. Conclusions

This study indicates that (i) 2-aryl-4-carboxyl quinoline is a suitable scaffold (template) to design COX-1/COX-2 inhibitors, (ii) COX-1/COX-2 inhibition is very sensitive to the substituent of the *para* position of C-2 phenyl ring, (iii) COX-1/COX-2 inhibition is affected by the position of benzoyl moiety on the quinoline ring, and (iv) 2-(4-(azido)phenyl)-6 or 8-benzoyl-quinoline-4-carboxylic acid (**8a** or **8b**) exhibited highly COX-2 inhibitory potency and selectivity even more than celecoxib.

## 5. Experimental section

All chemicals and solvents used in this study were purchased from Merck AG and Aldrich Chemical. Melting points were determined with a Thomas-Hoover capillary apparatus. Infrared spectra were acquired using a Perkin Elmer Model 1420 spectrometer. A Bruker FT-500 MHz instrument (Brucker Biosciences, USA) was used to acquire $^1$H NMR spectra with TMS as internal standard. Chloroform-D and DMSO-$d_6$ were used as solvents. Coupling constant ($J$) values are estimated in hertz (Hz) and spin multiples are given as s (singlet), d (double), t (triplet), q (quartet), m (multiplet), and br (broad). The mass spectral measurements were performed on an 6410Agilent LCMS triple quadrupole mass spectrometer (LCMS) with an electrospray ionization (ESI) interface. Microanalyses, determined for C and H, were within ±0.4% of theoretical values.

### 5.1. General procedure for preparation of 6- or 8-benzoyl-2-(4-(substituted)phenyl)quinoline-4-carboxylic acid (4 and 5)

To a 50 ml round-bottom flask was added appropriate aldehyde (**1**, 4.73 mmol) and pyrrovic acid (**2**, 0.82 g, 9.35 mmol), immediately a white precipitate produced, then 20 ml acetic acid was added and the mixture was heated at 100 °C to solve the precipitate then refluxed for 30 min. After that 2- or 4-aminobenzophenone (**3**, 1.86 g, 9.45 mmol) was added and refluxed for 3–6 h. After cooling, the produced precipitate was filtered and washed with acetic acid, ethanol, ether, and hexane and crystallized in ethanol (yields: 15–31%).

#### 5.1.1. 8-Benzoyl-2-(4-(methylthio)phenyl)quinoline-4-carboxylic acid (4a)

Yield: 25%; pale brown crystalline powder; mp: 261–263 °C; IR (KBr): $\nu$ (cm$^{-1}$) 3295–2335 (OH), 1682, 1669 (C=O), $^1$H NMR (DMSO-$d_6$): $\delta$ 2.50 (s, 3H, Me), 7.18 (d, 2H, 4-methylthiophenyl $H_3$ & $H_5$, $J$ = 8.4 Hz), 7.45 (t, 2H, phenyl $H_3$ & $H_5$, $J$ = 7.7 Hz), 7.59 (t, 1H, phenyl $H_4$, $J$ = 7.4 Hz), 7.65–7.67 (m, 4H, 4-methylthiophenyl $H_2$ & $H_6$ & phenyl $H_2$ & $H_6$), 7.77 (t, 1H, quinoline $H_6$, $J$ = 7.8 Hz), 7.91 (d, 1H, quinoline $H_7$, $J$ = 6.8 Hz), 8.40 (s, 1H, quinoline $H_3$), 8.75 (d, 1H, quinoline $H_5$, $J$ = 8.5 Hz), 14.09 (s, 1H, COOH), LCMS (ESI): 400.6 (M+1)$^+$ 100.

#### 5.1.2. 6-Benzoyl-2-(4-(methylthio)phenyl)quinoline-4-carboxylic acid (4b)

Yield: 16%; orange crystalline powder; mp: 221–223 °C; IR (KBr): $\nu$ (cm$^{-1}$) 3490–2500 (OH), 1696, 1652 (C=O), $^1$H NMR (DMSO-$d_6$): $\delta$ 2.53 (s, 3H, Me), 7.41 (d, 2H, 4-methylthiophenyl $H_3$ & $H_5$, $J$ = 8.4 Hz), 7.57 (t, 2H, phenyl $H_3$ & $H_5$, $J$ = 7.6 Hz), 7.69 (t, 1H, phenyl $H_4$, $J$ = 7.4 Hz), 7.80 (d, 2H, phenyl $H_2$ & $H_6$, $J$ = 8.1 Hz), 8.10 (d, 1H, quinoline $H_8$, $J$ = 9.4 Hz), 8.22 (d, 1H, quinoline $H_7$, $J$ = 8.4 Hz), 8.26 (d, 2H, 4-methylthiophenyl $H_2$ & $H_6$, $J$ = 8.4 Hz), 8.51 (s, 1H, quinoline $H_3$), 9.06 (s, 1H, quinoline $H_5$), 14.2 (s, 1H, COOH), LCMS (ESI): 400.6 (M+1)$^+$ 100.

#### 5.1.3. 2-(4-(Acetamido)phenyl)-8-benzoyl-quinoline-4-carboxylic acid (5a)

Yield: 31%; yellow crystalline powder; mp: 310–311 °C; IR (KBr): $\nu$ (cm$^{-1}$) 3347 (NH) 2973–2263 (OH), 1708, 1657, 1646 (C=O), $^1$H NMR (DMSO-$d_6$): $\delta$ 2.01 (s, 3H, Me), 7.46 (t, 2H, phenyl $H_3$ & $H_5$, $J$ = 7.70 Hz), 7.53 (d, 2H, 4-acetamidophenyl $H_3$ & $H_5$, $J$ = 8.7 Hz), 7.58–7.69 (m, 5H, phenyl $H_2$, $H_4$ & $H_6$ & 4-acetamidophenyl $H_2$ & $H_6$), 7.77 (t, 1H, quinoline $H_6$, $J$ = 7.8 Hz), 7.91 (d, 1H, quinoline $H_7$, $J$ = 7.0 Hz), 8.38 (s, 1H, quinoline $H_3$), 8.75 (d, 1H, quinoline $H_5$, $J$ = 9.4 Hz), 10.04 (s, 1H, NH), 14.01 (s, 1H, COOH), $^{13}$C NMR (DMSO-$d_6$): $\delta$ 24.9, 119.5, 119.7, 123.8, 127.9, 128.4, 128.5, 129.3, 130.0, 130.2, 132.5, 133.7, 138.5, 139.5, 139.7, 142.1, 146.8, 155.7, 168.2, 169.4, 198.5, LCMS (ESI): 411.6 (M+1)$^+$ 100. Anal. Calcd for $C_{25}H_{18}N_2O_4$: C, 73.16; H, 4.42; N, 6.83. Found: C, 73.38; H, 4.20; N, 6.81.

#### 5.1.4. 2-(4-(Acetamido)phenyl)-6-benzoyl-quinoline-4-carboxylic acid (5b)

Yield: 15%; yellow crystalline powder; mp: 286–288 °C; IR (KBr): $\nu$ (cm$^{-1}$) 3348 (NH), 3094–1995 (OH), 1721, 1663, 1633 (C=O), 1311, 1150 ($SO_2$), $^1$H NMR (DMSO-$d_6$): $\delta$ 2.04 (s, 3H, Me), 7.57 (t, 2H, phenyl $H_3$ & $H_5$, $J$ = 7.7 Hz), 7.68 (t, 1H, phenyl $H_4$, $J$ = 7.4 Hz), 7.77 (d, 2H, 4-acetamidophenyl $H_3$ & $H_5$, $J$ = 8.7 Hz), 7.80 (d, 2H, phenyl $H_2$ & $H_6$, $J$ = 7.2 Hz), 8.10 (d, 1H, quinoline $H_8$, $J$ = 10.5 Hz), 8.20 (d, 1H, quinoline $H_7$, $J$ = 8.7 Hz), 8.27 (d, 2H, 4-acetamidophenyl $H_2$ & $H_6$, $J$ = 8.7 Hz), 8.49 (s, 1H, quinoline $H_3$), 9.06 (s, 1H, quinoline $H_5$), 10.18 (s, 1H, NH), 14.2 (s, 1H, COOH), $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.0, 119.9, 120.7, 123.2, 129.0, 129.5, 129.9, 130.4, 130.6, 130.9, 132.6, 133.7, 135.7, 137.8, 139.2, 142.5, 150.8, 158.4, 168, 169.5, 196.1, LCMS (ESI): 411.6 (M+1)$^+$ 100. Anal. Calcd for $C_{25}H_{18}N_2O_4$: C, 73.16; H, 4.42; N, 6.83. Found: C, 73.34; H, 4.24; N, 6.89.

### 5.2. General procedure for preparation of 6- or 8-benzoyl-2-(4-(methylsulfonyl)phenyl)quinoline-4-carboxylic acid (6)

One gram of 2-(4-(methylthio)phenyl)-6- or 8-benzoyl-quinoline-4-carboxylic acid was dissolved in 10 ml THF and 5 g oxone in THF/water was added. The mixture was stirred at room temperature for 3–5 h, after evaporation of THF, the residue was extracted with ethyl acetate and dried with sodium sulfate and then evaporated, the product was recrystallized in ethanol (yields: 67–70%).

#### 5.2.1. 8-Benzoyl-2-(4-(methylsulfonyl)phenyl)quinoline-4-carboxylic acid (6a)

Yield: 67%; cream crystalline powder; mp: 228–230 °C; IR (KBr): $\nu$ (cm$^{-1}$) 3419–2678 (OH), 1739, 1695 (C=O), 1300, 1140 ($SO_2$), $^1$H NMR (DMSO-$d_6$): $\delta$ 3.24 (s, 3H, Me), 7.52 (t, 2H, phenyl $H_3$ & $H_5$, $J$ = 7.7 Hz), 7.67 (t, 1H, phenyl $H_4$), 7.71 (d, 2H, phenyl $H_2$ & $H_6$, $J$ = 8.3 Hz), 7.89–7.93 (m, 3H, quinoline $H_6$ & 4-methylsulfonylphenyl $H_2$ & $H_6$), 7.97 (d, 2H, 4-methylsulfonylphenyl $H_3$ & $H_5$, $J$ = 8.4 Hz), 8.03 (d, 1H, quinoline $H_7$, $J$ = 7.1 Hz), 8.57 (s, 1H, quinoline $H_3$), 8.86 (d, 1H, quinoline $H_5$, $J$ = 9.4 Hz), 14.2 (s, 1H, COOH), $^{13}$C NMR (DMSO-$d_6$): $\delta$ 44.2, 120.5, 124.4, 128.2, 128.6, 128.7, 129.0, 129.5, 130.0, 130.5, 133.9, 139.2, 139.4, 140.0, 142.7, 146.6, 154.5, 168.1, 198.1, LCMS (ESI): 432.5 (M+1)$^+$ 100. Anal. Calcd for $C_{24}H_{17}NO_5S$: C, 66.81; H, 3.97; N, 3.25. Found: C, 66.99; H, 3.80; N, 3.22.

#### 5.2.2. 6-Benzoyl-2-(4-(methylsulfonyl)phenyl)quinoline-4-carboxylic acid (6b)

Yield: 70%; pale yellow crystalline powder; mp: 246–248 °C; IR (KBr): $\nu$ ($cm^{-1}$) 3542–2650 (OH), 1696, 1661 (C=O), 1311, 1150 ($SO_2$), $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.21 (s, 3H, Me), 7.57 (t, 2H, phenyl $H_3$ & $H_5$, $J$ = 7.6 Hz), 7.69 (t, 1H, phenyl $H_4$, $J$ = 7.4 Hz), 7.81 (d, 2H, phenyl $H_2$ & $H_6$, $J$ = 7.4 Hz), 8.09 (d, 2H, 4-methylsulfonylphenyl $H_2$ & $H_6$, $J$ = 8.4 Hz), 8.13 (d, 1H, quinoline $H_8$, $J$ = 10.1 Hz), 8.27 (d, 1H, quinoline $H_7$, $J$ = 8.7 Hz), 8.55 (d, 2H, 4-methylsulfonylphenyl $H_3$ & $H_5$, $J$ = 8.4 Hz), 8.6 (s, 1H, quinoline $H_3$), 9.08 (s, 1H, quinoline $H_5$), 14.2 (s, 1H, COOH), $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 44.3, 121.5, 123.9, 128.5, 129.2, 129.5, 129.8, 130.7, 131.3, 133.8, 136.6, 137.6, 139.7, 142.8, 142.9, 150.5, 157.1, 167.7, 196.0, LCMS (ESI): 432.5 $(M+1)^+$ 100. Anal. Calcd for $C_{24}H_{17}NO_5S$: C, 66.81; H, 3.97; N, 3.25. Found: C, 70.03; H, 3.79; N, 3.23.

### 5.3. General procedure for preparation of 2-(4-(amino)phenyl)-6- or 8-benzoyl-quinoline-4-carboxylic acid (7)

To a 50 ml round-bottom flask was added 2-(4-(acetamido)phenyl)-6- or 8-benzoyl-quinoline-4-carboxylic acid (1 mmol), then 1 ml HCl (1 N) and 3 ml HCl (12 N) were added and refluxed for 5 h then the solution was neutralized with NaOH (10%), the pH was adjusted to 5–6, the dark red precipitate was formed. The precipitate was filtered and washed with water and used without further purification (yields: 47%).

#### 5.3.1. 2-(4-(Amino)phenyl)-8-benzoyl-quinoline-4-carboxylic acid (7a)

Yield: 47%; red crystalline powder; mp: 348–350 °C (decomposed); IR (KBr): $\nu$ ($cm^{-1}$) 3467, 3352 ($NH_2$), 3232 (OH), 1657, 1645 (C=O), $^1H$ NMR (DMSO-$d_6$): $\delta$ 6.42 (s, 2H, $NH_2$), 7.39–7.43 (m, 5H, 4-aminophenyl $H_3$ & $H_5$ & phenyl $H_3$–$H_5$), 7.62–7.78 (m, 5H, 4-aminophenyl $H_2$ & $H_6$ & phenyl $H_2$ & $H_6$ & quinoline $H_6$), 7.88 (s, 1H, quinoline $H_3$), 8.13 (s, 1H, quinoline $H_7$), 8.78 (s, 1H, quinoline $H_5$), LCMS (ESI): 369.7 $(M+1)^+$ 100.

#### 5.3.2. 2-(4-(Amino)phenyl)-6-benzoyl-quinoline-4-carboxylic acid (7b)

Yield: 47%; red crystalline powder; mp: 267–270 °C; IR (KBr): $\nu$ ($cm^{-1}$) 3420, 3351 ($NH_2$), 3239 (OH), 1662, 1644 (C=O), $^1H$ NMR (DMSO-$d_6$): $\delta$ 6.67 (s, 2H, $NH_2$), 7.56–7.78 (m, 5H, 4-aminophenyl $H_3$ & $H_5$ & phenyl $H_3$–$H_5$), 7.9–8.2 (m, 6H, 4-aminophenyl $H_2$ & $H_6$ & phenyl $H_2$ & $H_6$ & quinoline $H_7$ & $H_8$), 8.35 (s, 1H, quinoline $H_3$), 9.01 (s, 1H, quinoline $H_5$), LCMS (ESI): 369.7 $(M+1)^+$ 100.

### 5.4. General procedure for preparation of 2-(4-(azido)phenyl)-6- or 8-benzoyl-quinoline-4-carboxylic acid (8)

2-(4-(Amino)phenyl)-6- or 8-benzoyl-quinoline-4-carboxylic acid (0.5 mmol) was suspended in 10 ml water in a 50 ml flask. The flask was placed in ice bath so that the temperature of the mixture reached to 0 and 3 ml of HCl (12 N) was added. Then 0.5 g (7.2 mmol) $NaNO_2$ was dissolved in water and added to the mixture drop wisely. Then 5 ml THF was added, the temperature was kept between 0 and 5, the solution was stirred for 1.5 h. After that $NaN_3$ (0.5 g, 7.6 mmol) was dissolved in water and slowly added to the solution and the mixture was stirred for 2 h, THF was evaporated and the precipitate was filtered and washed with water, the solid was added to a hot solution of HCl (3 N), after cooling, the solid was filtered and washed with water and $CHCl_3$ (yields: 20–35%).

#### 5.4.1. 2-(4-(Azido)phenyl)-8-benzoyl-quinoline-4-carboxylic acid (8a)

Yield: 20%; orange crystalline powder; mp: 202–204 °C; IR (KBr): $\nu$ ($cm^{-1}$) 3336–2517 (OH), 2136, 2103 ($N_3$), 1693, 1670 (C=O), $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.05 (d, 2H, 4-azidophenyl $H_2$ & $H_6$, $J$ = 8.6 Hz), 7.46 (t, 2H, phenyl $H_3$ & $H_5$, $J$ = 7.7 Hz), 7.60 (t, 1H, phenyl $H_4$, $J$ = 7.4 Hz), 7.65 (d, 2H, phenyl $H_2$ & $H_6$, $J$ = 7.3 Hz), 7.77 (d, 2H, 4-azidophenyl $H_3$ & $H_5$, $J$ = 8.6 Hz), 7.80 (t, 1H, quinoline $H_6$, $J$ = 8.4 Hz), 7.92 (d, 1H, quinoline $H_7$, $J$ = 7.0 Hz), 8.42 (s, 1H, quinoline $H_3$), 8.75 (d, 1H, quinoline $H_5$, $J$ = 8.5 Hz), 14.2 (s, 1H, COOH), $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 119.8, 120.3, 124.0, 128.2, 128.4, 129.4, 129.4, 130, 130.1, 133.9, 134.8, 138.8, 139.3, 139.8, 142.2, 146.7, 155.2, 168.2, 198.3, LCMS (ESI): 417.5 $(M+23)^+$ 100. Anal. Calcd for $C_{23}H_{14}N_4O_3$: C, 70.05; H, 3.58; N, 14.21. Found: C, 69.88; H, 3.70; N, 14.02.

#### 5.4.2. 2-(4-(Azido)phenyl)-6-benzoyl-quinoline-4-carboxylic acid (8b)

Yield: 35%; orange crystalline powder; mp: 207–209 °C; IR (KBr): $\nu$ ($cm^{-1}$) 3602–2415 (OH), 2132, 2105 ($N_3$), 1700, 1659 (C=O), $^1H$ NMR (DMSO-$d_6$): $\delta$ 7.25 (d, 2H, 4-azidophenyl $H_2$ & $H_6$, $J$ = 8.5 Hz), 7.57 (t, 2H, phenyl $H_3$ & $H_5$, $J$ = 7.6 Hz), 7.68 (t, 1H, phenyl $H_4$, $J$ = 7.4 Hz), 7.80 (d, 2H, phenyl $H_2$ & $H_6$, $J$ = 7.4 Hz), 8.10(d, 1H, quinoline $H_8$, $J$ = 10.1 Hz), 8.20 (d, 1H, quinoline $H_7$, $J$ = 8.7 Hz), 8.33 (d, 2H, 4-azidophenyl $H_3$ & $H_5$, $J$ = 8.5 Hz), 8.5 (s, 1H, quinoline $H_3$), 9.05 (s, 1H, quinoline $H_5$), 14.2 (s, 1H, COOH), $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 120.5, 120.8, 123.4, 129.5, 129.9, 130.0, 130.4, 130.6, 130.9, 133.7, 135, 136.0, 137.7, 139.4, 142.5, 150.6, 157.8, 167.9, 196.0, LCMS (ESI): 417.5 $(M+23)^+$ 100. Anal. Calcd for $C_{23}H_{14}N_4O_3$: C, 70.05; H, 3.58; N, 14.21. Found: C, 70.24; H, 3.73; N, 14.43.

## 6. Molecular modeling and biological evaluation

Docking studies were performed using Autodock software Version 3.0. The coordinates of the X-ray crystal structure of the selective COX-2 inhibitor SC-558 bound to the murine COX-2 enzyme was obtained from the RCSB Protein Data Bank (1cx2) and hydrogens were added. The ligand molecules were constructed using the Builder module and were energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The energy minimized ligands were superimposed on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. The purpose of docking is to search for favorable binding configuration between the small flexible ligands and the rigid protein. Protein residues with atoms greater than 7.5 Å from the docking box were removed for efficiency. These docked structures were very similar to the minimized structures obtained initially. The quality of the docked structures was evaluated by measuring the intermolecular energy of the ligand–enzyme assembly.[19,20]

## 7. In vitro cyclooxygenase (COX) inhibition assays

The assay was performed using an enzyme chemiluminescent kit (Cayman Chemical, MI, USA) according to our previously reported method.[21] The Cayman chemical chemiluminescent COX (ovine) inhibitor screening assay utilizes the heme-catalyzed hydroperoxidase activity of ovine cyclooxygenases to generate luminescence in the presence of a cyclic naphthalene hydrazide and the substrate arachidonic acid. Arachidonate-induced luminescence was shown to be an index of real-time catalytic activity and demonstrated the turnover inactivation of the enzyme. Inhibition of COX activity, measured by luminescence, by a variety of selective and non-selective inhibitors showed potencies similar to those observed with other in vitro and whole cell methods.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.094. These data

include MOL files and InChiKeys of the most important compounds described in this article.

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