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Pharmacological effects of a synthetic quinoline, a hybrid of tomoxiprole and naproxen, against acute pain and inflammation in mice: a behavioral and docking study

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ABSTRACT

Objective(s): In the present study, we investigated the potential anti-nociceptive activity and acute anti-inflammatory effect of a synthetic quinoline compound (2-(4-Methoxyphenyl)benzo[h]quinoline-4-carboxylic acid, QC), possessing structural elements of both naproxen and tomoxiprole drugs.

Materials and Methods: The anti-nociceptive activity of QC was evaluated using chemical- and thermal-induced nociception models and its acute anti-inflammatory effect was evaluated by xylene-induced ear edema test in mice.

Results: QC displayed a dose dependent effect in both acute anti-nociceptive tests (writhing and hot plate). This compound at dose of 6.562 mg/kg showed a high anti-nociceptive effect near equal to diclofenac 5 mg/kg. It also showed high anti-inflammatory effects (less than 6.562 mg/kg) comparable to those of reference drugs diclofenac (5 mg/kg) and celecoxib (100 mg/kg). Docking study showed that this quinoline derivative could inhibit COX-2 enzyme strongly.

Conclusion: QC showed high anti-nociceptive and anti-inflammatory effects comparable to reference drugs and can exert its anti-nociceptive and anti-inflammatory activities through COX-2 inhibition.

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Introduction

Quinolines have attracted certain consideration due to their various ranges of pharmacological activities including the capability to target numerous causes of inflammation (1). These include cyclooxygenase-2 (COX-2) (2-4), phosphodiesterase 4 (PDE4), tumor necrosis factor (TNF)-α converting enzyme (TACE), and also transient receptor potential vanilloid 1 (TRPV1) antagonists (1). Nowadays many anti-inflammatory drugs are available, which can be divided by their chemical structure into small molecules and biological agents, including steroidal and non-steroidal drugs. In spite of the increasing encouragement of biological drugs particularly in the cancer and autoimmune market, there is still a strong requirement for potent and nontoxic small molecules. Actually, small molecules are less expensive in comparison to biological drugs and can usually be used orally (5).

It is believed that inducible COX may have both pro- and -inflammatory properties through the release of different types of prostaglandins (6) Classical NSAIDs (non-steroidal anti-inflammatory drugs) as non-selective COX inhibitors have been broadly used in the treatment of numerous chronic illnesses for a long time possessing anti- inflammatory, analgesic and anti-pyretic properties, this class of agents is mostly used to treat chronic inflammation illnesses (7). Selective COX-2 inhibitors are drugs that their therapeutic properties are as potent as classical NSAIDs but expected to exert fewer side effects (8). Naproxen is a clinically used drug belonged to NSAIDs family and tomoxiprole is a selective inhibitor of COX-2 (9). In this study we synthesized and evaluated anti-nociceptive and anti-inflammatory effects of a quinolone derivative (QC) possessing structural elements of both these two drugs (Figure 1).

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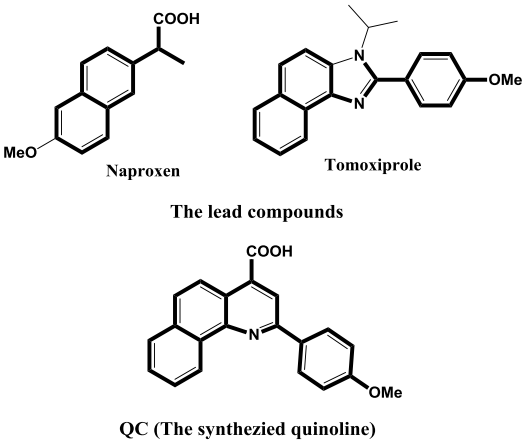

Figure 1. Chemical structures of known anti-nociceptive and anti-inflammatory drugs and the synthesized quinoline

Materials and Methods

Synthesis of compound

A solution of pyruvic acid (1.26 g, 14.3 mmol) and *p*-methoxy benzaldehyde (1.14 ml, 9.45 mmol) in ethanol (10 ml) was refluxed for 15 min then α-naphtylamine (1.35 g, 9.45 mmol) was added to the solution and refluxed for 10 hr. Then, the mixture was cooled and the obtained precipitate was filtered and washed with hexane and ethanol, and recrystallized in methanol to obtained 716 mg of pure QC (10).

2-(4-Methoxyphenyl)benzo[h]quinoline-4 carboxylic Acid (QC)

Yield: 23%; yellow crystalline powder; mp: 279-280 ^{o}C; IR (KBr): ν (cm^{-1}) 3198-2726 (OH), 1712 (C=O), ^{1}HNMR (DMSO-D6): δ (ppm) 3.83 (s, 3H, OCH_3), 7.11 (d, 2H, 4-methoxy phenyl H_3 & H_5, *J*=8.85 Hz), 7.75-7.78 (m, 2H, benzoquinoline H_8 &H_9), 7.96 (d, 1H, benzoquinoline H_7, *J*=9.2 HZ), 8.01 (d, 1H, benzoquinoline H_6, *J*=9.05), 8.37 (d, 2H, 4-methoxyphenyl H_2 & H_6 , *J*=8.85 Hz), 8.45-8.47 (m, 2H, benzoquinoline H_3 & H_{10}), 9.33 (d, 1H, benzoquinoline H_5, *J*=9.31 Hz), 13.98 (s, 1H, COOH); LCMS (ESI): 330.4 $(M+1)^{+}$100.

Experimental animals

Male mice (BALB/c) weighing 18–20 g were obtained from the animal room of the School of Pharmacy, Mashhad University of Medical Sciences (Mashhad, Iran) and kept in groups of 8 in standard laboratory conditions in the identical center. They were kept at constant room temperature (22±2 °C) under a 12/12 hr light/dark cycle at least ten days before the start of tests. Tap water and commercial food pellets were freely accessible. Male mice were moved to the laboratory at least one hour prior to tests. The assays were done during the light portion between 08:00–12:00 AM to prevent circadian effects. The protocols of all animal experiments have been approved by the University Animal Care Committee.

Preparation of solutions

Appropriate solutions were prepared by dissolving desired amounts of QC in dimethyl sulfoxide (DMSO) then diluted with normal saline to the final concentration of 5% DMSO.

Morphine 10 mg/kg and diclofenac sodium 5 mg/kg and celecoxib 100 mg/kg (Daru Pakhsh, Tehran, Iran) were used as positive controls for anti-nociceptive and anti-inflammatory tests, respectively. Normal saline was used as negative control (10 ml/kg). All the compounds were administered by intraperitoneal (IP) injection.

Acute anti-nociceptive activity using hot-plate test

The hot-plate test was performed according to methods described by Hosseinzadeh *et al* (11). The hot-plate temperature was fixed on 55 °C and cut off time was 20 sec. Jumping, withdrawal of paws and/or licking of paws were counted as positive response to stimuli. 6 male mice were used for each group of tests. Mice responses were determined at the time of injection (time 0) and after 30, 60 and 90 min of administration (normal saline, QC 0.937, 2.812 and 6.562 mg/kg and morphine 10 mg/kg).

Writhing test

30 min after the administration of the single dose of QC 0.937, 2.812 and 6.562 mg/kg, normal saline, diclofenac sodium 5 mg/kg, celecoxib 100 mg/kg and morphine 10 mg/kg to 8 groups of 6 mice, they were given an intraperitoneal injection of 0.5 % (v/v) acetic acid solution (volume of injection 0.1 ml/10 g). The number of writhing produced in these mice was counted 5 min after injection of acid for 30 min (12) and used to express the percentage of analgesia using the following ratio:

Percentage of analgesia = (control group mean)-(test group mean)/control group mean ∗100

Anti-inflammatory studies

Acute anti-inflammatory activity using xylene-induced ear edema test

The acute anti-inflammatory activity of QC was evaluated by xylene-induced ear edema in male mice (12). 30 min after the IP injection (QC 0.937, 2.812 and 6.562 mg/kg, diclofenac 5 mg/kg and normal saline) a drop of xylene (Merck Chemicals, Germany) was applied on the frontal and dorsal sides of the left ear of mice. After 2 hr, a circle of 4.5 mm of both ears were punched out and weight difference between right and left ear was recorded. 8 mice were used for each test compound.

Molecular modeling

Mode of interaction between QC ligand and COX-2 was examined by docking. 2D structure of QC was

prepared using Chem Draw Ultera 8.0 software and 3D structures were prepared in Hyperchem 7 software using molecular mechanic force filed pre-optimization followed by AM1 semi empirical calculation. The X-ray crystal structure of COX-2 (PDB ID: 1cx2) was downloaded from the Protein Data Bank (www.rcsb.org). Further modification such as water molecules removal and polar hydrogen addition was completed by MOE software. QC was docked into the binding site of COX-2 using MOE software. All atoms inside a 5 Å around the co-crystallized ligand in crystal coordinates of COX-2 was selected as active site. The docking simulations were completed employing triangle matcher placement algorithm in combination with London dG scoring function and force field as refinement method. The top-score docking poses were selected for final ligand–target interaction analysis using LigX module in MOE Software (7).

Statistical analysis

Results are presented as mean±SEM. Statistical analyses were done with one-way ANOVA followed by Tukey–Kramer test to compare the differences between means. Differences were considered statistically significant when $P<0.05$.

Results

Acute anti-nociceptive activity using hot plate test

QC demonstrated a moderate dose related anti-nociceptive activity which was in general the highest at 60 min and lasted up to 90 min. Morphine as a positive control, showed significant anti-nociceptive activities in comparison to negative control which was the highest at 60 min and lasted up to 90 min ($P < 0.001$) (Figure 2).

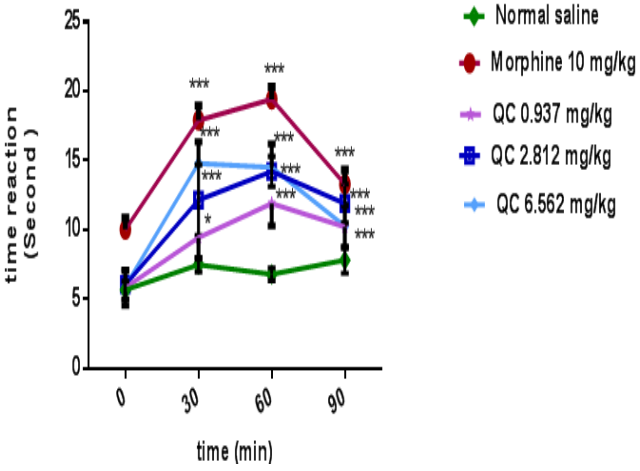

Figure 2. Anti-nociceptive effects of QC 0.937, 2.812 and 6.562 mg/kg and morphine 10 mg/kg using hot-plate test in mice (n = 6 mice in each group). Different groups were compared with negative control. Each point represents mean±standard error *$P<0.05$, ***$P < 0.001$. QC=quinoline compound

Acute anti-nociceptive activity using writhing test

The numbers of writhings of all treated groups were decreased considerably in comparison to negative control ($P<0.001$) and QC showed a dose dependent effect. QC at dose of 6.562 mg/kg showed high anti-nociceptive effect near equal to those of diclofenac 5 mg/kg and celecoxib 100 mg/kg (Figure 3).

Anti-inflammatory studies

Acute anti-inflammatory activity using xylene-induced ear edema test

QC caused considerable reductions in the edema in all doses in comparison to the negative control ($P<0.001$) (Figure 3). It also showed high anti-inflammatory effect comparable to those of reference drugs diclofenac and celecoxib (Figure 4).

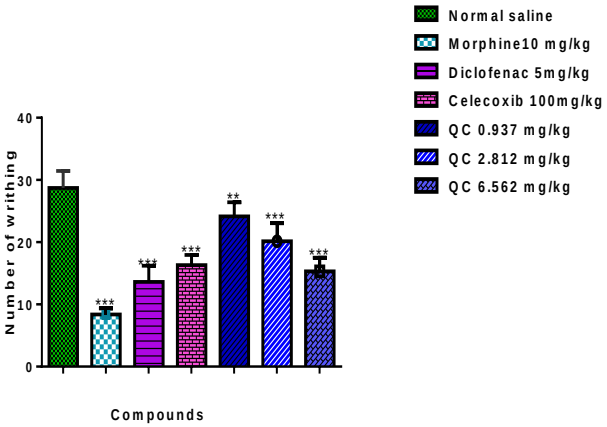

Figure 3. Anti-nociceptive effects of QC 0.937, 2.812 and 6.562 mg/kg, morphine 10 mg/kg, diclofenac 5mg/kg and celecoxib 100 mg/kg using writhing test in mice (n=6 mice in each group). Different groups were compared with negative control. Each point represents mean±standard error. **$P<0.01$, ***$P<0.001$. QC= quinoline compound

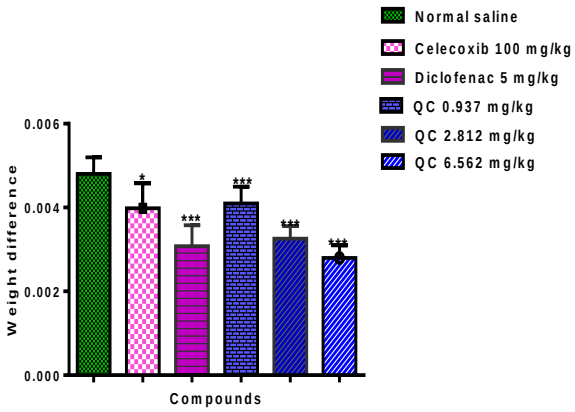

Figure 4. Anti-inflammatory effects of QC 0.937, 2.812 and 6.562 mg/kg, diclofenac 5 mg/kg and celecoxib 100mg/kg using xylene-induced ear edema test in mice (n = 6 mice in each group). Different groups were compared with negative control. Each point represents mean±standard error. **$P<0.01$, ***$P<0.001$. QC= quinoline compound

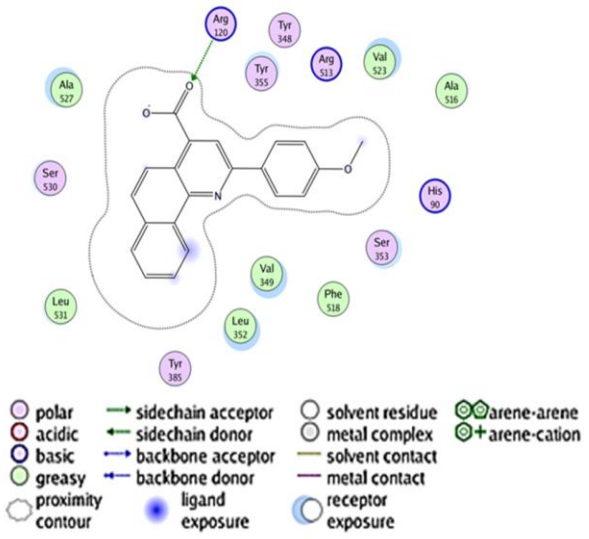

Figure 5. The 2D representation of the interaction between QC in the crystal structure of COX-2 (PDB ID: 1cx2) using LigX in MOE

Molecular modeling

As QC arises from combining structural elements of naproxen; a non-selective COX inhibitor and tomoxiprole; a selective COX-2 inhibitor, the binding interactions of QC within the COX-2 binding site were investigated. The most stable enzyme–ligand complex of QC within the COX-2 binding site (Figures 5, 6) showed that the methoxyphenyl moiety of QC occupied the additional pocket of the cyclooxygenase-2 active site (Arg513, Phe518, and Val523) that the phenylsulfonamide or phenylsulfone of other COX-2 inhibitors is supposed to occupy. In addition, our docking studies showed that one of the O-atoms of carboxylic acid group could make hydrogen binding interaction with amino group of Arg120. The other parts of QC could make hydrophobic interaction with different amino acid residue such as Phe 518, Val 349, Leu 531, Ser 530, Ala 527.

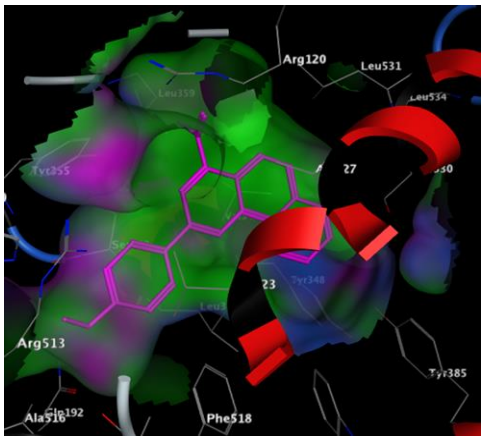

Figure 6. The 3D representation of the interaction between QC in the crystal structure of COX-2 (PDB ID: 1cx2)

Discussion

QC was synthesized using one pot Doebner reaction and the anti-nociceptive effect of QC was assessed in mice by using thermal nociception test model of the hot-plate test and chemical nociception test models of writhing test. These tests were designated such that both peripherally and centrally mediated effects of QC were investigated (13).

It was revealed in this study that IP administration of QC produced significant dose related analgesic effects in both thermal and chemical nociceptive test models used. The hot-plate test is a common test for investigating acute heat pain sensitivity in mice. This test has been found to be appropriate for investigation of centrally but not of peripherally analgesic activity of drugs (14, 15). These, indicate the presence of both centrally and peripherally analgesic activities of the compound. The acetic acid-induced writhing test in mice has been used as a common model for the evaluation of analgesic or anti-inflammatory activities of new agents. This chemical test is very sensitive to distinguish anti-nociceptive properties of NSAIDs, narcotics and other centrally acting drugs (16). Acetic acid causes pain by directly triggering non-selective cation channels placed in the primary sensory pathways (17) or indirectly by stimulating the release of numerous inflammation mediators such as bradykinin, histamine, prostaglandins and others (18, 19). The results reported herein indicated that the administration of the QC significantly decreased the number of abdominal writhing made by acetic acid. In addition, the consequences of xylene made ear edema support the assumption that QC may prevent action or synthesis of inflammation mediators. Increasing in permeability of vascular and release of inflammatory mediators that causes edema is the representative of xylene's toxicity (20). The acute anti-inflammatory activity of QC at 2.812 mg/kg was near equal to those of diclofenac at 5 mg/kg. All QC's doses affected significant reductions in the edema compared to the negative control. The results also showed that diclofenac, which inhibit cyclooxygenase, caused a significant inhibition xylene made ear edema. Compared with diclofenac, QC was about 5-fold more potent in attenuating xylene made ear edema. Therefore, the results of xylene made ear edema test, strongly suggest that the mechanisms of action of QC may be partly mediated by the inhibition of cyclooxygenases activity. Regarding to our quinoline's structure, its anti-inflammatory properties may be due to its COX enzymes inhibition. Our molecular modeling study showed that the quinoline could occupy COX-2 active site and inhibit it strongly.

It is believed that the binding site of COX-2 is larger than COX-1 (about 20%) because of the exchange of a valine at location of 523 in COX-2 for a moderately bulky isoleucine (Ile) residue in COX-1 at the same location of the binding site of enzyme, This

alteration in the COX-2 enzyme permits the access to a further secondary pocket, which is a necessity for COX-2 drug selectivity. Access to this secondary pocket is restricted in those of COX-1 (21). So as our docking shows that the methoxyphenyl moiety of QC occupies the additional pocket of the COX-2 binding site.

Conclusion

QC showed high anti-nociceptive and anti-inflammatory effects comparable to reference drugs. Based on our docking studies, we can conclude that larger structure of our quinoline (QC) rather than naproxen (a non-selective COX inhibitor), is responsible for its possible selective COX-2 inhibitory effect.

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