1,4-Dihydroquinazolin-3(2H)-yl benzamide derivatives as anti-inflammatory and analgesic agents with an improved gastric profile: Design, synthesis, COX-1/2 inhibitory activity and molecular docking study

Asmaa Sakr\textsuperscript{a,1}, Hend Kothayer\textsuperscript{a,*,1}, Samy M. Ibrahim\textsuperscript{a}, Mohamed M. Baraka\textsuperscript{a}, Samar Rezq\textsuperscript{b}

\textsuperscript{a} Department of Medicinal Chemistry, Faculty of Pharmacy, Zagazig University, Egypt

\textsuperscript{b} Department of Pharmacology, Faculty of Pharmacy, Zagazig University, Egypt

ARTICLE INFO

Keywords:
COX-1/2 inhibition
Dihydroquinazolinyl benzamide
Inflammation
Molecular modeling

ABSTRACT

The design and synthesis of a new series of 1,4-dihydroquinazolin-3(2H)-yl benzamide derivatives (4a–o) as anti-inflammatory and analgesic agents and COX-1/2 inhibitors are reported. The target compounds (4a–o) were synthesized using a two-step scheme, and their chemical structures were confirmed with 1H NMR, 13C NMR, and mass spectra and elemental analysis. Compounds 4b, 4d, 4h, 4l, 4n and 4o showed the best in vitro COX-2 inhibitory activity ($IC_{50}$ 0.04–0.07 $\mu$M), which was nearly the same as that of the reference drug celecoxib ($IC_{50}$ 0.049 $\mu$M), but had a lower selectivity index, as dictated in our target design. In the in vivo anti-inflammatory inhibition assay, compounds 4b, 4c, 4e, 4f, 4m and 4o showed better oedema inhibition percentages, ranging from 38.1% to 54.1%, than did diclofenac sodium (37.8%). An in vivo analgesic assay revealed that compounds 4b and 4n had a potential analgesic effect 4- to 21-fold more potent than that of indomethacin and diclofenac sodium. All the tested compounds showed an improved ulcerogenic index when compared to indomethacin. In the synthesized series, compound 4b showed the best biological activity in all the experiments. The docking study results agreed with the in vitro COX inhibition assay results. Moreover, the predicted in silico studies of all the compounds support their potential as drug candidates.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used for decades worldwide for symptomatic treatment of acute and chronic pain, and these drugs are known for their effects on fever, inflammation and rheumatic disorders [1–3]. The inflammation process is mainly controlled by the cyclooxygenase (COX) enzyme, which is the key enzyme in prostaglandin biosynthesis and is responsible for inflammation and pain [4]. The discovery of two COX isomers, COX-1 and COX-2, provided another facet for the exploration of this enzyme [5,6]. COX-1 and COX-2 are the main targets of most traditional anti-inflammatory drugs [7,8]. Studies of the different effects of the two isomers revealed that COX-1 has a protective mechanism in the stomach and kidney, and blocking this pathway elicits the main side effects of NSAIDs, varying from bleeding to peptic ulcers [9]. Accordingly, the new approach was to make selective COX-2 inhibitors lacking these side effects. Rofecoxib and valdecoxib (Fig. 1) are the most famous and highly selective coxibs, or COX-2 inhibitors; unfortunately, they were withdrawn from the market due to their cardiovascular side effects [10,11]. Celecoxib (Fig. 2) is still on the market, and current evidence supports it as a therapeutic option due to its lower toxicity than other coxibs [12]. Recent studies have also focused on the promising applications of selective COX-2 inhibitors in the treatment of cancer and neurodegenerative disorders, such as Alzheimer’s disease and Parkinson’s disease [13].
Fig. 1. The chemical structures of the withdrawn highly selective COX-2 inhibitors.

Fig. 2. The molecular design through rationale prospective to obtain the novel target compounds (4a–o).

- **Ester Linker**
- **Amide Linker**

**Molecular Design**

**novel compounds (4a–o)**

- **I**
  - Refecoxib
  - SI = 0.48

- **II**
  - Valdecoxib
  - SI = 6.52

- **Etoricoxib**

- **Celecoxib**

- **V: Rutaecarpine**
  - SI = 62

- **VI**
  - Proquazone (R = H)
  - Fluproquazone (R = F)
  - SI = 2.51

- **Heterocyclic ring**
- **Aryl rings**
- **Removed groups**
- **Linker; Ester or Amide**

Fig. 2. The molecular design through rationale prospective to obtain the novel target compounds (4a–o).
The highly selective inhibition of COX-2 leads to potential cardiac side effects, while non-selective (i.e., COX-1/COX-2) inhibition with greater affinity for COX-1 is responsible for gastric and kidney problems [9], albeit with reduced or even absent cardiovascular risk.

Large numbers of compounds have been designed, synthesized and evaluated for their selectivity towards COX-2. The common structural features are the presence of two neighbouring aryl rings attached to a central heterocyclic moiety (V-shape) and a sulfonamide or sulfone substituent at the para position of one of the rings, which provides the high selectivity [14–16]. Recent trials introduced a linker, either an ester as in compound V (Fig. 2) [18], between one of the aryl rings and the central heterocycle. This linker was supposed to improve hydrogen bond formation with the active site of the enzyme, and inclusion of the linker generated potent anti-inflammatory and selective anti-COX-2 compounds, as reported for compounds I and II [17,18].

To avoid the side effects of both highly selective COX-2 inhibitors and non-selective COX inhibitors, we designed compounds with moderately selective COX-2 inhibitory activity [7,9,15,19]. The main goal was achieved by designing compounds that retained some structural features of highly selective COX-2 inhibitors but lacked others. The design included i) the incorporation of a large central anti-inflammatory ring (quinazolinone), ii) the maintenance of the vicinal shape characteristic of selective COX-2 inhibitors, iii) the removal of the carboxylic acid group common to non-selective COX inhibitors, and iv) the removal of the sulfonyl group, which is responsible for the high COX-2 selectivity. The quinazolin-4(3H)-one scaffold was chosen as a central heterocyclic moiety for this study because of its remarkable anti-inflammatory and analgesic activities [20]. Proquazone (structure III, Fig. 2) and fluoroquazone (structure IV, Fig. 2) both contain a quinazolinone moiety and are well-known NSAIDs on the market, especially used to treat gout and rheumatoid arthritis [21]. Rutacearpine (structure V, Fig. 2) is a natural quinazolinone derivative that possesses anti-inflammatory and analgesic activity and high selectivity towards COX-2 inhibition [22–24]. To maintain the common V-shaped structural integrity, two neighbouring phenyl rings were attached at positions 2 and 3 [17–19]. The latter was bounded through an amide linker that may potentiate target interactions and make the compounds slightly bulkier and thus more favourable for entry to the COX-2 active site, which is 20% larger than the COX-1 active site [19,25,26]. On the other hand, we avoided the incorporation of the carboxylic acid group, which is characteristic of many traditional NSAIDs, such as indomethacin and reported compound VI (Fig. 2), and is responsible for the gastric mucosal side effects [27,28]; we also removed the sulfonamide and methyl sulfonyl groups present in celecoxib and etoricoxib, respectively.

Previous studies highlighted the potential essential role of the para-substituted sulfonyl group in boosting selectivity for COX-2 by perfectly fitting in the smaller pocket and interacting with the crucial amino acid Arg513, which is found in the active site of only COX-2 [19,29].

The newly synthesized compounds 4a–o were evaluated for their COX-1/COX-2 selectivity using in vitro and in vivo assays of anti-inflammatory and analgesic activity and for their ulcer index (UI) profile. Molecular modelling and in silico studies were used to predict their binding modes with COX-1/COX2, physicochemical properties and pharmacokinetic profiles.

2. Results and discussion

2.1. Chemistry

The pathways of the synthetic compounds are illustrated in Scheme 1.

N-Methyl-isatoic anhydride is a versatile starting material in the preparation of N'-benzoyl-2-(methylamino)benzohydrazide, an important intermediate for the synthesis of several fused heterocyclic compounds with robust biological activity. Our target intermediate N'-(4-(substituted)-benzyl)-(methylamino)benzohydrazides (3a–c) [30] were prepared by condensation of N-methyl-isatoic anhydride with 4-(substituted)-benzohydrazide and were identified by their reported melting points [30]. The final target compounds, 1,4-dihydroquinazolin-3(2H)-yl benzamide (4a–o), were prepared by cyclization of intermediates 3a–c with different aldehydes.

2.2. Biological activity

2.2.1. In vitro COX inhibition assay

All synthesized compounds were tested by in vitro COX-1/COX-2 inhibition assays using an ovine COX-1/human recombinant COX-2 assay kit as previously reported [31–33]. The half-maximal inhibitory concentration (IC_{50}, μM) values were determined, and the selectivity index (SI) values were calculated as IC_{50} (COX-1)/IC_{50} (COX-2). (Table 1).

Testing of the newly synthesized compounds revealed that they all showed more potent COX-2 inhibitory activity than did the two reference drugs diclofenac sodium and indomethacin; compounds 4b, 4d, 4h, 4l, 4n and 4o showed the greatest COX-2 inhibitory activity, with IC_{50} values ranging from 0.04 to 0.07 μM, which is nearly the value as
the reference drug celecoxib (IC50 0.049μM). All the synthesized compounds, except for 4g, showed greater COX-1 inhibitory activity than the reference drug celecoxib; compounds 4b and 4i showed the most COX-1 inhibitory activity (IC50 4.21μM and 3.89μM, respectively), which was greater than that of diclofenac sodium (IC50 4.23μM). All the synthesized compounds were 7–17 times more selective than diclofenac sodium (reference drug) towards COX-2.

The selectivity of the new compounds was significantly lower than that of celecoxib, which could be envisioned as an advantage in avoiding the cardiovascular side effects of highly selective COX-2 inhibitors [19,34].

2.2.2. In vitro analgesic assay: Acetic acid-induced writhing test

The analgesic activity of the newly synthesized compounds was evaluated by the acetic acid-induced writhing test using indomethacin, diclofenac sodium and celecoxib as positive controls according to the reported method [35]. The significant decrease in the number of acetic acid-induced writhes indicated the activity of each compound. Two compounds, 4b and 4n, had a potential analgesic effect, with 3.4 and 7.9 writhes, respectively; these compounds were more potent than celecoxib (12writhes) and diclofenac sodium (17.4 writhes, respectively), and 8-to-36-fold more effective than celecoxib (28.8 writhes). Additionally, compounds 4m (12 writhes) and 4j (15.2 writhes) showed better analgesic activity than diclofenac sodium or celecoxib. The seven compounds, 4c, 4d, 4g, 4h, 4i, 4l, and 4o, demonstrated analgesic activity with the number of writhes ranging from 20.2 to 28.8, which was slightly higher than or equal to the number after treatment with celecoxib (28.8). (Fig. 3.)

2.2.3. In vivo anti-inflammatory inhibitory assay: Carrageenan-induced rat paw oedema assay

The anti-inflammatory activity of the new compounds was measured by the carrageenan-induced rat paw oedema assay as previously reported [35], and diclofenac sodium and celecoxib were used as positive reference drugs. The test compounds were administered to the animals orally as a suspension in vehicle (1% tween 80 in 10ml H2O per kg), and the mean hind paw oedema thickness of rats pretreated with the test compounds was measured at 0, 1, 2, 3, 4, 5, and 24h after the induction of inflammation. The percentage inhibition of oedema thickness was calculated and compared to that with diclofenac sodium and celecoxib. All tested compounds showed moderate anti-inflammatory activity, with average oedema inhibition percentages from 26.7% to 54.1%, compared to diclofenac sodium and celecoxib, which had average oedema inhibition percentages of 37.8% and 53.7%, respectively (Table 2).

Compounds 4d showed the best oedema inhibition percentage of 54.1%, which was better than that of both reference drugs. Compounds 4b, 4c, 4e, 4f, 4m and 4o showed better oedema inhibition percentages, ranging from 38.1% to 48%, than did diclofenac sodium (37.8%); however, these values were lower than that of celecoxib (53.7%).

2.2.4. Gastric ulcerogenic activity

Ulceration and damage of the gastric mucosa are considered the major serious side effects of NSAIDs [36,37]. Stomach lacerations were detected and examined according to a previously reported method after the oral administration of 100 mmol/kg test compound, 50 mmol/kg celecoxib (first reference drug), or 20 mmol/kg indomethacin (second reference drug) to determine if the test compounds induce ulcers [38,39]. The results revealed that all the test compounds had a magniﬁcant safety proﬁle when compared to indomethacin because the UI range (zero - 10.42) was safer than that for the reference indomethacin (UI = 19.3), as shown in Table 3.

Compounds 4a, 4b, 4g, and 4n had a UI of 2.3–2.7, similar to the value of the reference drug celecoxib (2.4). Interestingly, compound 4k showed no ulceration (UI = zero) (Table 3, Fig. 4).

2.3. Structure activity relationship (SAR)

To expand the SAR study, we added different substituents to both of the neighbouring phenyl rings present in our compounds (4a–o) R and \( R' \) (as shown in scheme 1). We utilized chloride, fluoride, methoxy or nitro groups as substituents.

In general, substitution of both phenyl rings was favourable as compounds 4a, 4f and 4k with unsubstituted phenyl ring showed lower activities in all the tests compared to their substituted analogues. Compound 4b carrying two chloride atoms on both of the phenyl rings showed the best activities in either the in vitro COX inhibitory activity or the in vivo anti-inflammatory activity.

Substitution with either chloride or methoxy also showed good in vitro and in vivo activities (compounds 4d and 4l). Compound 4h carrying two nitro groups showed good in vitro COX inhibitory activity but with lower in vivo anti-inflammatory activity, this is may be due the high polarity of the two nitro groups that decreases the lipophilicity of the compound and hence decreases its bioavailability.

It is also notable that for a good in vivo anti-inflammatory activity, at least one of the substituents should be a halogen. This is may be due to the lipophilicity of the halogen that improves the bioavailability.

2.4. Molecular modelling and in silico study

2.4.1. Docking study

Docking studies of compounds 4a–o were performed using Molecular Operating Environment (MOE) 2018 software. The 2D/3D tools, scoring function and ligand interactions with the amino acid pool for the selected proteins COX-1 (PDB code: 1EQG) and COX-2 (PDB code: 1CX2) provided a good estimate of the behaviour of each compound in the active sites. The best score was chosen for each compound.

The validation process was performed by re-docking SC-558 into the COX-2 active site and ibuprofen into the COX-1 active site, and their original conformations were reproduced (RMSD: 1.34Å and 1.16Å Score = –9.39 and –7.56 respectively).

Ser353, Tyr355, His90, Arg513 and Arg120 are the key residues in the binding mode of SC-558.

Comparison of the binding sites of COX-1 and COX-2 reveals an additional side pocket in the COX-2 binding site, making it larger. This side pocket is surrounded by His90, Gln192, Tyr355 and Arg513 (the last residue is replaced by His513 in COX-1). Binding with Arg513 is
responsible for the high selectivity of COX-2 inhibitors (usually through the sulfone of sulfonamide groups) \[18,40\]. In contrast, non-selective COX inhibition mainly results from salt bridge formation between Arg120 and the carboxylate ion present in most non-selective inhibitors \[41\].

The docking scores for the series 4a–o on COX-2 ranged from \(-4.3668\) to \(-5.9426\). All the compounds 4a–o showed interactions with Ser353 amino acid except compounds 4g and 4j. (Supp. Data Table 1).

Beside Ser353 compounds 4c, 4e, 4h, 4m and 4o made hydrogen bonds with His90 (Fig. 5), while compounds 4f formed hydrogen bonds with Tyr355 and Gln192.

Compounds 4g and 4j showed only one hydrogen bond interaction with Tyr355 or Gln192 respectively.

Analysis of the docking results and scores for COX-1 with the series (4a–o) revealed that the scores ranged from \(-3.6527\) to \(-1.9031\), whereas the score of SC-558 was \(-1.3609\), indicating a slight increase in selectivity towards COX-1 compared with that of SC-558 (Supp. Data Table 1). Compounds 4a, 4b, 4d, 4g, 4j and 4n had no interactions with the surrounding amino acids. The central quinazolinone moiety made the compounds too bulky to enter the site. Only compounds 4c and 4i interacted with Arg120, Tyr355 and Val116; however, the central ring expansion with the vicinal shape may be responsible for the decreased selectivity and the scores being nearer those of SC-558 than ibuprofen (1EQG).

The results of the comparison of binding modes for each compound with the COX-1/2 active sites met the target rationale of this study: there was no binding to Arg513 in the COX-2 active site (responsible for high selectivity) or Arg120 (responsible for non-selectivity). The limited or absent hydrogen bond formation with COX-1 active site residues, Fig. 3.

### Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean edema thickness (mm) ± SEM</th>
<th>Average edema inhibition%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>1 h</td>
</tr>
<tr>
<td>Control</td>
<td>0.00 ± 0.00</td>
<td>4.18 ± 0.64</td>
</tr>
<tr>
<td>4a</td>
<td>0.00 ± 0.00</td>
<td>2.18 ± 0.12</td>
</tr>
<tr>
<td>4b</td>
<td>0.00 ± 0.00</td>
<td>1.67 ± 0.19</td>
</tr>
<tr>
<td>4c</td>
<td>0.00 ± 0.00</td>
<td>1.09 ± 0.09</td>
</tr>
<tr>
<td>4d</td>
<td>0.00 ± 0.00</td>
<td>1.12 ± 0.09</td>
</tr>
<tr>
<td>4e</td>
<td>0.00 ± 0.00</td>
<td>1.09 ± 0.08</td>
</tr>
<tr>
<td>4f</td>
<td>0.00 ± 0.00</td>
<td>1.19 ± 0.32</td>
</tr>
<tr>
<td>4g</td>
<td>0.00 ± 0.00</td>
<td>2.36 ± 0.18</td>
</tr>
<tr>
<td>4h</td>
<td>0.00 ± 0.00</td>
<td>2.14 ± 0.16</td>
</tr>
<tr>
<td>4i</td>
<td>0.00 ± 0.00</td>
<td>2.35 ± 0.32</td>
</tr>
<tr>
<td>4j</td>
<td>0.00 ± 0.00</td>
<td>1.56 ± 0.14</td>
</tr>
<tr>
<td>4k</td>
<td>0.00 ± 0.00</td>
<td>1.34 ± 0.25</td>
</tr>
<tr>
<td>4l</td>
<td>0.00 ± 0.00</td>
<td>2.09 ± 0.25</td>
</tr>
<tr>
<td>4m</td>
<td>0.00 ± 0.00</td>
<td>2.58 ± 0.43</td>
</tr>
<tr>
<td>4n</td>
<td>0.00 ± 0.00</td>
<td>3.00 ± 0.23</td>
</tr>
<tr>
<td>4o</td>
<td>0.00 ± 0.00</td>
<td>1.05 ± 0.23</td>
</tr>
<tr>
<td>Diclofenac sod</td>
<td>0.00 ± 0.00</td>
<td>1.49 ± 0.16</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>0.00 ± 0.00</td>
<td>1.15 ± 0.13</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.00 ± 0.00</td>
<td>1.99 ± 0.14</td>
</tr>
</tbody>
</table>

All synthesized compounds were tested in comparison of reference drugs diclofenac sodium, celecoxib and indomethacin in dose of 100 mg/kg, 20 mg/kg, 50 mg/kg and 20 mg/kg, respectively. Drugs were given orally 1h before carrageenan injection. Paw edema thickness was measured at 0, 1, 2, 3, 4, 5 and 24 h from the induction of inflammation. The percentage inhibition of edema thickness was calculated from the AUC data of the paw-thickness time course curves. Data are mean ± SEM (n = 5 each).

* P < 0.05 versus control values.
2.4.2. In silico prediction of pharmacokinetic and physiochemical properties

Molinspiration software [42] was used to predict the oral bioavailability of our compounds through Lipinski’s rule of five. Molinspiration software also gives a clear result not only about violations of the rule of five but also about the topological polar surface area (TPSA) (Å²). The bioavailability is acceptable for a drug with a TPSA value below 140 Å², but drugs with a value below 70–80 Å² will penetrate the BBB and act in the CNS. The TPSA was also used in the calculation of oral bioavailability (%ABS) by the following previously reported equation: (%ABS) = 109−0.345 TPSA [43,44]. The two parameters, TPSA and number of rotatable bonds (NROTB), together affect oral bioavailability in the tested animal; compounds are found to have high oral bioavailability if the NROTB and TPSA values are less than or equal to 10 and 140 Å², respectively. All data for our series are provided in Supp. Data Table 2.

The synthesized compounds 4a–4g and 4i–4o did not violate Lipinski’s rule and had TPSA values of less than 140 Å² (range, 52.65–107.70), which indicated high oral bioavailability. The only exception was compound 4h, which slightly violated the parameters with HBA = 11 and TPSA = 144.29 Å².

MolSoft software [45] was used to test the druglikeness modelscore and drug water solubility. The more positive the number is, the more it behaves as a drug. All the synthesized compounds gave scores from 1.87 to 1.06, indicating that they will act as drugs (Supp.Data Table 3).

All the tested compounds showed good water solubility values > 0.0001 (80% of drugs on the market have a solubility value greater than 0.0001). They also had positive drug likeliness scores, and this supports their potential as drug candidates.

The PreADMET calculator [46] was used in the prediction of five more pharmacokinetic parameters. The first two models, Caco-2 and MDCK, provide parameters of cell permeability, human intestinal absorption (HIA), brain-blood barrier (BBB) partition coefficient, plasma protein binding (PPB) and inhibition of cytochrome P450 2D6 (CYP2D6) (Supp.Data Table 4). All compounds in series 4a–o showed moderate cell permeability in the Caco-2 cell model (17.76–34.44 nm/s) and low cell permeability in the MDCK cell model (0.04–0.26 nm/s).

All the synthesized compounds had high HIA values (96.35–97.74%), which assured high oral bioavailability as observed with the ABS%. Moreover, the compounds had a low to moderate ability to penetrate the BBB (0.01–0.96). Furthermore, all compounds in the series showed strong PPB, with values ranging from 90.30 to 98.77%. Finally, all compounds in the series showed strong PPB, with values ranging from 90.30 to 98.77%. Finally, all compounds in the series showed strong PPB, with values ranging from 90.30 to 98.77%. Finally, all compounds in the series showed strong PPB, with values ranging from 90.30 to 98.77%.

The inhibitory effect of compounds 4a–o on CYP2D6 was the same as that of the 3 reference drugs (celexicib, diclofenac sodium and indomethacin), which do not inhibit CYP2D6; thus, the test compounds are expected to not be included in any drug-drug interactions involving inhibitors and/or inducers of this enzyme. Taken together, the results demonstrated that the newly synthesized compounds had acceptable druglikeness and physicochemical properties and fulfilled Lipinski’s rule of five, except for one drug, 4h, which had one violation. According to the pharmacokinetics predictions, these compounds are future drug candidates.

3. Conclusion

A library of 15 compounds (4a–o) was designed to be moderately selective COX-2 inhibitors and synthesized. Their anti-inflammatory, analgesic, ulcerogenic and in vitro COX inhibition activities were

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Number of rats with ulcer</th>
<th>Lesion incidence (%)</th>
<th>Average Ulcer number</th>
<th>Ulcer Index (UI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>Nil</td>
</tr>
<tr>
<td>4a</td>
<td>1</td>
<td>20</td>
<td>0.2</td>
<td>2.4</td>
</tr>
<tr>
<td>4b</td>
<td>1</td>
<td>20</td>
<td>0.6</td>
<td>2.7</td>
</tr>
<tr>
<td>4c</td>
<td>2</td>
<td>40</td>
<td>1.6</td>
<td>10.42</td>
</tr>
<tr>
<td>4d</td>
<td>3</td>
<td>60</td>
<td>1.6</td>
<td>8.1</td>
</tr>
<tr>
<td>4e</td>
<td>2</td>
<td>40</td>
<td>0.6</td>
<td>5.15</td>
</tr>
<tr>
<td>4f</td>
<td>2</td>
<td>40</td>
<td>0.6</td>
<td>5.6</td>
</tr>
<tr>
<td>4g</td>
<td>2</td>
<td>20</td>
<td>0.2</td>
<td>2.3</td>
</tr>
<tr>
<td>4h</td>
<td>3</td>
<td>60</td>
<td>1.6</td>
<td>8.15</td>
</tr>
<tr>
<td>4i</td>
<td>1</td>
<td>20</td>
<td>0.5</td>
<td>4.7</td>
</tr>
<tr>
<td>4j</td>
<td>2</td>
<td>40</td>
<td>1.4</td>
<td>10.32</td>
</tr>
<tr>
<td>4k</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>Nil</td>
</tr>
<tr>
<td>4l</td>
<td>3</td>
<td>60</td>
<td>1.2</td>
<td>7.9</td>
</tr>
<tr>
<td>4m</td>
<td>1</td>
<td>20</td>
<td>0.3</td>
<td>7.1</td>
</tr>
<tr>
<td>4n</td>
<td>1</td>
<td>20</td>
<td>0.2</td>
<td>2.3</td>
</tr>
<tr>
<td>4o</td>
<td>3</td>
<td>60</td>
<td>0.6</td>
<td>9.7</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>5</td>
<td>100</td>
<td>12.2</td>
<td>23.6</td>
</tr>
<tr>
<td>Colecxib</td>
<td>1</td>
<td>20</td>
<td>1</td>
<td>2.4</td>
</tr>
</tbody>
</table>

where ulcer index is the sum of average severity (US), average number (UN) and % lesion incidence (UP). For orally administrated dose of tested compound 100 mg/kg, celecoxib 50 mg/kg and indomethacin with 20 mg/kg. (N = 5). The ulcer index (UI) was calculated according to the equation (UI = UN + US + UPx10^{-1}).

Table 3

Acute Ulcerogenicity study of the new compounds with two reference drugs indomethacin and celecoxib.

Fig. 4. Showed the presence or absence of ulcer comparing with two reference drugs indomethacin and celecoxib.
evaluated. Compound 4b showed the best results in most aspects because it had an oedema inhibition percentage (48%) better than that of the reference drug diclofenac sodium (37.8%) and an analgesic effect (3.4 writhes) better than that of indomethacin (12.6 writhes) and diclofenac sodium (17.4 writhes). Moreover, its UI of 2.7 was 7-fold safer than that of indomethacin (19.3) and nearly the same as that of celecoxib (2.4). These results were in complete accordance with the in vitro COX-1/COX-2 enzyme assay results: COX-1 IC50 = 4.21, COX-2 IC50 = 0.05, and SI = 84.20. Moreover, a docking study revealed that these compounds recognized the essential amino acids in the COX-2 active site, except for Arg120 and Arg513, and their inability to enter COX-1 with zero reactions with amino acids; moreover, the physico-chemical and pharmacodynamic studies predicted these compounds to have promising medicinal value.

4. Materials and methods

4.1. Chemistry

All reagents were obtained commercially with the high percent purity, especially for synthesis, unless otherwise mentioned. Melting points (°C) were determined using open capillary tubes on a Gallenkamp melting point apparatus (London, UK) using dimethyl sulfoxide (DMSO)-d6. All NMR spectra were generated on a Varian Mercury-300 (300 MHz) (Palo Alto, CA, USA) using dimethyl sulfoxide (DMSO)-d6 as the solvent. Chemical shifts are reported in δ ppm units with respect to TMS as an internal standard (chemical shift in δ, ppm). Mass spectra were detected using a GC/MSShimadzu Qp-2010 plus (Shimadzu Corporation, Tokyo, Japan). Elemental analyses were determined using the Vario EL III (Elementar) CHNS analyzer (Hanau, Germany). All reactions were observed continuously by thin layer chromatography (TLC) (RF) on silica gel 60 GF245 (E-Merck, Germany) and were detected by a UV-lamp at a wavelength (λ) of 254 nm.

Compounds 1, 2a–c and 3a–c were synthesized according to previously reported methods [47 48 30].

4.1.1. General method for the synthesis of 1,4-dihydroquinazolin-3(2H)-yl benzamide (4a–o)

A mixture of N-(4-(substituted)-benzoyl)-2-(methylamino) benzohydrazide (3a–c, 0.003 mol) and the appropriate aldehyde (0.003 mol) in glacial acetic acid was heated under reflux for 8 h. After cooling, the formed precipitate was filtered, washed with cold petroleum ether, dried and then crystallized from dioxiane/H2O drops to generate the desired products 4a–o at 60–90% yield.

4.1.2. 4-Chloro-N-(1-methyl-4-oxo-2-phenyl-1,4-dihydroquinazolin-3(2H)-yl)benzamide (4a)

White powder, 90% yield. M.p., 243–248°C. 1H NMR (DMSO-d6) δ 2.81 (3H, s, NCH3), 5.93 (1H, s, NCH), 6.76 (1H, d, J = 8.28 Hz, ArH), 6.88 (1H, t, J = 7.48 Hz, ArH), 7.32–7.36 (5H, m, ArH), 7.47 (1H, t, J = 8.48 Hz, ArH), 7.57 (2H, d, J = 8.56 Hz, ArH), 7.79–7.84 (3H, m, ArH), 10.91 (1H, s, CONH, exch). 13C NMR (DMSO-d6) δ 35.62 (NCH3), 80.28 (NCH), 112.96 (ArCH), 115.40 (ArC), 118.40 (ArCH), 127.35 (ArC), 128.50 (ArCH), 128.98 (ArCH), 129.05 (ArCH), 129.52 (ArCH), 130.09 (ArCH), 131.31 (ArCH), 135.07 (ArCH), 137.41 (ArC), 137.55 (ArC), 147.56 (ArCH), 160.00 (ArC), 164.98 (ArC). MS, m/z: 392 (M+) + 394 (M+ + 2). Analysis calcd. for C18H18ClN3O2: C, 67.43; H, 4.63; N, 10.72. Found: C, 67.54; H, 4.61; N, 10.75.

4.1.3. 4-Chloro-N-(2-(4-chlorophenyl)-1-methyl-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)benzamide (4b)

Pale yellow powder, 71% yield. M.p., 257–261°C. 1H NMR (DMSO-d6) δ 2.81 (3H, s, NCH3), 5.97 (1H, s, NCH), 6.76 (1H, d, J = 8.28 Hz, ArH), 6.88 (1H, t, J = 7.40 Hz, ArH), 7.35 (2H, d, J = 8.52 Hz, ArH), 7.42 (2H, d, J = 8.48 Hz, ArH), 7.47 (1H, t, J = 8.52 Hz, ArH), 7.58 (2H, d, J = 8.56 Hz, ArH), 7.79–7.86 (3H, m, ArH), 10.90 (1H, s, CONH, exch). 13C NMR (DMSO-d6) δ 35.55 (NCH3), 79.53 (NCH), 112.96 (ArCH), 115.40 (ArC), 118.40 (ArCH), 127.35 (ArC), 128.50 (ArCH), 128.98 (ArCH), 129.05 (ArCH), 129.52 (ArCH), 130.09 (ArCH), 131.31 (ArCH), 135.07 (ArCH), 137.41 (ArC), 137.55 (ArC), 147.56 (ArCH), 160.00 (ArC), 164.98 (ArC). MS, m/z: 392 (M+) + 394 (M+ + 2). Analysis calcd. for C22H17Cl2N3O2: C, 61.99; H, 4.03; N, 10.72. Found: C, 61.81; H, 4.03; N, 10.00.

4.1.4. 4-Chloro-N-(1-methyl-2-(4-nitrophenyl)-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)benzamide (4c)

White powder, 90% yield. M.p., 243–248°C. 1H NMR (DMSO-d6) δ 2.88 (3H, s, NCH3), 6.17 (1H, s, NCH), 6.78 (1H, d, J = 8.32 Hz, ArH), 6.91 (1H, t, J = 7.44 Hz, ArH), 7.49 (1H, t, J = 8.40 Hz, ArH), 7.62 (4H, q, J = 8.70 Hz, ArH), 7.82–7.87 (3H, m, ArH), 8.22 (2H, d, J = 8.72 Hz, ArH), 10.98 (1H, s, CONH, exch). 13C NMR (DMSO-d6) δ 35.76 (NCH3), 79.22 (NCH), 113.29 (ArCH), 115.36 (ArC), 118.87 (ArC), 124.19 (ArCH), 128.65 (ArCH), 128.73 (ArCH), 129.11 (ArCH), 130.08 (ArC), 131.12 (ArCH), 135.25 (ArC), 137.57 (ArC), 144.75 (ArC), 147.06 (ArC), 148.38 (ArC), 160.71 (ArC), 165.05 (ArC). MS, m/z: 437.25 (M+) + 439.30 (M+ + 2). Analysis calcd. for

Fig. 5. 2D and 3D interaction image of compound 4a in the active site of COX-2.
Yellow powders, 70% yield. M.p., 267–271 °C. 1H NMR (DMSO-d6) δ 2.89 (3H, s, NCH3), 6.21 (1H, s, NCHN), 6.80 (1H, d, J = 8.28 Hz, ArH), 6.92 (1H, t, J = 7.48 Hz, ArH), 7.50 (1H, t, J = 8.52 Hz, ArH), 7.64 (2H, d, J = 8.72 Hz, ArH), 7.83 (1H, d, J = 9.08 Hz, ArH), 8.05 (2H, d, J = 8.8 Hz, ArH), 8.23 (2H, d, J = 8.72 Hz, ArH), 8.35 (2H, d, J = 8.84 Hz, ArH), 11.23 (1H, s, CONH, exch.). 13C NMR (DMSO-d6) δ 35.79 (NCH3), 79.13 (NCHN), 113.36 (ArCH), 115.24 (ArC), 118.92 (ArCH), 124.20 (ArCH), 124.24 (ArCH), 128.67 (ArCH), 128.73 (ArCH), 129.69 (ArC), 135.35 (ArC), 137.99 (ArC), 144.66 (ArCH), 147.09 (ArC), 148.42 (ArC), 150.03 (ArC), 160.65 (ArC), 164.59 (ArC). MS, m/z: 447.10 (M+). Analysis calc'd. for C22H16N2O6C: 59.06; H, 3.83; N, 15.65. Found: C, 59.30; H, 3.91; N, 15.62.

4.1.10. N-(2-(4-Fluorophenyl)-1-methyl-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)-4-nitrobenzamide (4i)

Yellow crystals, 60% yield. M.p., 245–250 °C. 1H NMR (DMSO-d6) δ 2.79 (3H, s, NCH3), 3.73 (3H, s, OCH3), 5.92 (1H, s, NCHN), 6.76 (1H, d, J = 8.32 Hz, ArH), 6.86–6.91 (3H, m, ArH), 7.27 (2H, d, J = 8.64 Hz, ArH), 7.47 (1H, t, J = 7.74 Hz, ArH), 7.82 (1H, d, J = 7.64 Hz, ArH), 8.03 (2H, d, J = 8.76 Hz, ArH), 8.34 (2H, d, J = 8.76 Hz, ArH), 11.12 (1H, s, CONH, exch.). 13C NMR (DMSO-d6) δ 35.46 (NCH3), 55.57 (OCH3), 79.91 (NCHN), 112.94 (ArC), 114.31 (ArC), 115.25 (ArC), 118.32 (ArC), 121.42 (ArCH), 128.51 (ArCH), 128.74 (ArCH), 129.57 (ArC), 129.69 (ArC), 135.08 (ArCH), 147.63 (ArC), 149.95 (ArC), 160.27 (ArC), 161.00 (ArC), 164.43 (ArC). MS, m/z: 432 (M+). Analysis calc'd. for C24H15FNO6C: 63.88; H, 4.66; N, 12.96. Found: C, 64.06; H, 4.91; N, 13.14.

4.1.11. N-(2-(4-Fluorophenyl)-1-methyl-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)-4-nitrobenzamide (4j)

Yellow powder, 75% yield. M.p., 230–236 °C. 1H NMR (DMSO-d6) δ 2.81 (3H, s, NCH3), 6.00 (1H, s, NCHN), 6.78 (1H, d, J = 8.32 Hz, ArH), 6.89 (1H, t, J = 7.44 Hz, ArH), 7.19 (2H, t, J = 8.88 Hz, ArH), 7.38–7.41 (2H, m, ArH), 7.49 (1H, t, J = 8.48 Hz, ArH), 7.81 (1H, d, J = 7.64 Hz, ArH), 8.03 (2H, d, J = 8.76 Hz, ArH), 8.34 (2H, d, J = 8.76 Hz, ArH), 11.17 (1H, s, CONH, exch.). 13C NMR (DMSO-d6) δ 35.54 (NCH3), 79.48 (NCHN), 113.09 (ArCH), 115.16 (ArC), 115.98 (d, J = 22 Hz, ArCH), 118.56 (ArCH), 124.16 (ArCH), 128.56 (ArCH), 129.59 (d, J = 8 Hz, ArCH), 133.90 (d, J = 8 Hz, ArCH), 135.23 (ArC), 138.15 (ArCH), 147.42 (ArC), 149.98 (ArC), 160.85 (ArC), 164.17 (d, J = 245 Hz, ArC), 164.49 (ArC). MS, m/z: 420 (M+). Analysis calc'd. for C22H16FNO6C: 62.85; H, 4.08; N, 13.33. Found: C, 62.70; H, 4.09; N, 13.32.

4.1.12. 4-Methoxy-N-(1-methyl-4-oxo-2-phenyl-1,4-dihydroquinazolin-3(2H)-yl)-4-nitrobenzamide (4k)

White powder, 77% yield. M.p., 279–284 °C. 1H NMR (DMSO-d6) δ 2.80 (3H, s, NCH3), 3.82 (3H, s, OCH3), 5.91 (1H, s, NCHN), 6.75 (1H, d, J = 8.28 Hz, ArH), 6.87 (1H, t, J = 7.44 Hz, ArH), 7.01 (2H, d, J = 8.8 Hz, ArH), 7.34 (5H, s, ArH), 7.46 (1H, t, J = 8.48 Hz, ArH), 7.79–7.83 (3H, m, ArH), 10.66 (1H, s, CONH, exch.). 13C NMR (DMSO-d6) δ 35.61 (NCH3), 55.88 (OCH3), 80.38 (NCHN), 112.89 (ArCH), 114.11 (ArCH), 115.57 (ArC), 118.33 (ArCH), 124.67 (ArC), 127.36 (ArCH), 128.46 (ArCH), 128.93 (ArCH), 129.45 (ArCH), 130.13 (ArCH), 134.96 (ArCH), 137.66 (ArCH), 147.52 (ArC), 161.05 (ArC), 162.65 (ArC), 165.36 (ArC). MS, m/z: 387 (M+). Analysis calc'd. for C23H18F2N2O6C: 71.30; H, 5.46; N, 10.85. Found: C, 71.00; H, 5.35; N, 10.53.

4.1.13. N-(2-(4-Chlorophenyl)-1-methyl-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)-4-nitrobenzamide (4l)

Yellow powder, 85% yield. M.p., 255–259 °C. 1H NMR (DMSO-d6) δ 2.81 (3H, s, NCH3), 3.82 (3H, s, OCH3), 5.95 (1H, s, NCHN), 6.75 (1H,
d, J = 8.28 Hz, ArH), 6.88 (1H, t, J = 7.40 Hz, ArH), 7.02 (2H, d, J = 8.88 Hz, ArH), 7.34 (2H, d, J = 8.52 Hz, ArH), 7.42 (2H, d, J = 8.52 Hz, ArH), 7.47 (1H, t, J = 8.16 Hz, ArH), 7.79–7.84 (3H, m, ArH), 10.66 (1H, s, CONH, exch). 13C NMR (DMSO-d6) δ 35.53 (NCH3), 55.89 (OCH3), 79.62 (NCHN), 112.98 (ArCH), 114.15 (ArCH), 115.47 (ArC), 118.49 (ArCH), 124.60 (ArCH), 128.51 (ArCH), 128.95 (ArCH), 129.26 (ArCH), 130.12 (ArC), 130.08 (ArCH), 130.03 (ArC), 130.71 (ArC), 147.28 (ArC), 160.88 (ArCH), 128.51 (ArCH), 128.95 (ArCH), 129.26 (ArCH), 130.12 (ArC), 130.08 (ArCH), 130.03 (ArC), 136.71 (ArC).


4.1.14. 4-Methoxy-N-(1-methyl-2-(4-nitrophenyl)-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)benzamide (4m)

White powder, 71% yield. M.p., 193–197°C. 1H NMR (DMSO-d6) δ 2.77 (3H, s, NCH3), 3.73 (3H, s, OCH3), 3.82 (3H, s, OCH3), 5.85 (1H, s, NCHN), 6.73 (1H, d, J = 8.32 Hz, ArH), 6.85–6.89 (3H, m, ArH), 7.01 (2H, d, J = 8.84 Hz, ArH), 7.24 (2H, d, J = 8.68 Hz, ArH), 7.46 (1H, t, J = 8.44 Hz, ArH), 7.78–7.83 (3H, m, ArH), 10.62 (1H, s, CONH, exch). 13C NMR (DMSO-d6) δ 35.72 (NCH3), 55.89 (OCH3), 79.32 (NCHN), 113.24 (ArCH), 114.18 (ArCH), 115.52 (ArC), 118.81 (ArCH), 124.15 (ArCH), 124.47 (ArCH), 128.61 (ArCH), 128.75 (ArCH), 130.13 (ArC), 130.15 (ArC), 144.87 (ArC), 147.94 (ArC), 148.35 (ArC), 160.77 (ArC), 162.76 (ArC), 165.42 (ArC). MS, m/z: 432 (M+). Analysis calcd: for C32H22ClN4O3: C, 63.88; H, 4.66; N, 12.96. Found: C, 64.00; H, 4.66; N, 12.98.

4.1.15. 4-Methoxy-N-[2-(4-fluorophenyl)-1-methyl-4-oxo-1,4-dihydroquinazolin-3(2H)-yl]benzamide (4n)

White powder, 65% yield. M.p., 265–269°C. 1H NMR (DMSO-d6) δ 2.79 (3H, s, NCH3), 3.73 (3H, s, OCH3), 3.82 (3H, s, OCH3), 5.85 (1H, s, NCHN), 6.73 (1H, d, J = 8.32 Hz, ArH), 6.85–6.89 (3H, m, ArH), 7.01 (2H, d, J = 8.84 Hz, ArH), 7.24 (2H, d, J = 8.68 Hz, ArH), 7.46 (1H, t, J = 8.44 Hz, ArH), 7.78–7.83 (3H, m, ArH), 10.62 (1H, s, CONH, exch). 13C NMR (DMSO-d6) δ 35.72 (NCH3), 55.89 (OCH3), 79.32 (NCHN), 113.24 (ArCH), 114.18 (ArCH), 115.52 (ArC), 118.81 (ArCH), 124.15 (ArCH), 124.47 (ArCH), 128.61 (ArCH), 128.75 (ArCH), 130.13 (ArC), 130.15 (ArC), 144.87 (ArC), 147.94 (ArC), 148.35 (ArC), 160.77 (ArC), 162.76 (ArC), 165.42 (ArC). MS, m/z: 432 (M+). Analysis calcd: for C32H22ClN4O3: C, 63.88; H, 4.66; N, 12.96. Found: C, 64.00; H, 4.66; N, 12.98.

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

The inhibition of both cyclooxygenase enzymes (COX-1/COX-2) was evaluated using the colorimetric ovine COX-1/human recombinant COX-2 assay (catalogue No. 560131; Cayman Chemicals Inc., Ann Arbor, MI, USA) according to the manufacturer’s instructions and previously reported studies [31–33]. All the compounds were tested in comparison with the reference drugs at the same time. The experiment was repeated three times (n = 3), and the data are presented as the average of three values ± standard error of the mean (SEM). The half-maximal inhibitory concentration (IC50) was measured, and the SI values were calculated as IC50 (COX-1)/IC50 (COX-2).

4.2.2. In vivo analgesic activity: acetic acid-induced writhing test

The analgesic activity was measured using an acetic acid-induced abdominal writhing test in mice as described by Nakamura et al. [49]. The test was performed in groups of five mice each. The control group (1) was given vehicle, which consisted of 1% Tween 80 (10 ml/kg, p.o.). The newly synthesized drugs were administered p.o. at 100 mg/kg 1 h before the injection of 0.7% acetic acid (1 ml/100 mg, i.p.). Then, the number of writhes over 30 min was counted. Diclofenac and indomethacin were used as reference drugs (20 mg/kg).

4.2.3. In vivo anti-inflammatory activity: carrageenan-induced rat paw oedema test

The anti-inflammatory activity of the test compounds was investigated using carrageenan-induced rat paw oedema as described by Sobeh et al. [35]. Albino male rats weighing 200–250 g were housed at 23–25°C (room temperature) with good ventilation, an appropriate dark/light cycle and ad libitum access to food/water. The rats were divided into 18 groups (five rats each). Group 1 was the control group and was given vehicle (1% Tween 80, 10 ml/kg). The remaining groups each received one of the synthesized drugs (100 mg/kg) or one of the two reference drugs, diclofenac sodium (20 mg/kg) and celecoxib (50 mg/kg). All drugs were administered orally once a day prior to an injection of carrageenan solution (1% in 0.9% NaCl, 0.1 ml) (Sigma-Aldrich, USA) into the sub-planter tissue of the right hind paw. The increase in thickness (mm) was determined using callipers before and after the carrageenan injection at 0, 1, 2, 3, 4, 5 and 24 h. The % inhibition of paw oedema thickness was calculated as (control−drug/ control) × 100. The cumulative anti-inflammatory effect during the entire observation period was estimated by calculating the area under the curve (AUC). Both the analgesic and anti-inflammatory screening were carried out in accordance with the guidelines of the Faculty of Pharmacy, Zagazig University, Egypt, and the whole study was approved by the local authorities, the Ethical Committee for Animal Handling at Zagazig University (ECAHZU), Faculty of Pharmacy, Zagazig University, Egypt, with a registration number (P15-12-2017).

4.2.4. Acute ulcerogenic activity

All new test compounds (4a–o) were subjected to an investigation of their ulcerogenic activity using indomethacin and celecoxib as reference drugs [38]. The rats from the previous experiment were fasted for 12 h, followed by the administration of additional doses of the test compounds (4a–o) for two additional days. Six hours after the last dose, the animals were sacrificed, and the stomachs were removed and examined for ulceration after being washed with saline solution (0.9%). The ulcer scores were calculated according to the method prescribed by Kulkarni and in a previous study [38] as follows:

- Zero for normal colour stomach
- (0.5) for red colouration
- for a spot ulcer
- (1.5) for haemorrhagic streaks
- for an ulcer > 3 but < 5 mm
- for ulcers > 5 mm.

The UI was calculated according to the following equation: [UI = UN + US + UP × 10−1], where UN is the average number of ulcers, US is the average severity score, and UP is the percentage of animals with an ulcer.
4.3. Molecular modelling and in silico study

4.3.1. Docking study
A molecular modelling study was performed to provide a further explanation of all possible binding interactions of the new series of active compounds with the active sites of both COX-1 and COX-2. We used MOE version 2018 (Chemical Computing Group, Montreal, CA). We chose COX-2 was crystallized in complex with the ligand SC-558 (PDB code 1CX2), which has the best known performance in recognizing COX-2 inhibitors from other decoys [50], and COX-1 (PDB code 1EQG), as in a previous study [51]. Both PDB structures were chosen according to the reported Benchmarking Sets study [52] and downloaded from the online protein data bank (www.rcsb.org). The non-selective COX inhibitor ibuprofen and the selective inhibitor SC-558 were used as reference drugs. Moreover, to validate the method, the selective inhibitor (SC-558) and ibuprofen were re-looked. The target compound data were prepared by adding hydrogen, calculating partial charges and minimizing energy (MMF94). The COX proteins were prepared by deleting the repeated chains, ligand, undesired surfactants and H2O molecules. Finally, hydrogen and calculated partial charges were added. The docking process of the ligand within the active site resulted in scores between ligand positions and enzyme binding sites (Kcal/mol). Many conformations were obtained, and the ligand–enzyme interaction with the best score was chosen.

4.3.2. In silico prediction of pharmacokinetic and physiochemical properties
All the compounds in the series confirmed previously as active were subjected to screening assays using three software packages, Molinspiration Chemoinformatics server [42], MolSoft software [45] and PreADMET calculator [46]. Lipinski’s rule of five for drug likeliness, TPSA and oral bioavailability were determined by Molinspiration, while MolSoft was used to measure druglikeness and water solubility. The PreADMET calculator was used to evaluate some essential pharmacometric parameters of the synthesized drugs inside the body and to compare with them by reference drugs.

4.4. Statistical analysis
Statistical analysis was performed using statistical software (Graph Pad Prism version 5). One-way analysis of variance (ANOVA) or repeated-measures analysis of variance (RM-ANOVA) was used to detect significance among group means, followed by Tukey’s post hoc test for pair-wise comparisons between group means, along with Student’s t test. Differences were considered significant at p < 0.05. All data are presented as the mean ± SEM.

Acknowledgements
The authors appreciate the efforts of Dr. Waleed Ali, Biochemistry lab, Cairo General hospital for his participation in in vitro COX1/2 enzyme assays.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest
No conflict of interest to declare.

Appendix A. Supplementary material
Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.11.030.

References
[2] D. Chiumello, M. Gotti, G. Vergani, Paracetamol in fever in critically ill pa-
[5] B.S. Fletcher, D. Kajubu, D. Perrin, H. Hershman, Structure of the mitogen-in-
ducible TIS10 gene and demonstration that the TIS10-encoded protein is a func-
flammatory drugs: an update of gastrointestinal, cardiovascular and renal compli-

quinazolinone and 3-amino-triazoloquinazolinone derivatives, 2014.
Azaz, Design, synthesis of 2, 3-dimethyl-4 (3H)-quinazolinone derivatives as anti-inflammatory and analgesic agents: COX-1/2 inhibitory activities and mole-
[24] T. Charier, C. Michaux, Dual inhibition of cyclooxygenase-2 (COX-2) and 5-li-
poxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-


https://preadmet.bmdrc.kr.

