Acta Poloniae Pharmaceutica – Drug Research, Vol. 68 No. 5 pp. 665–675, 2011 ISSN 0001-6837
Polish Pharmaceutical Society

SYNTHESIS, BIOLOGICAL EVALUATION AND MOLECULAR DOCKING OF QUINAZOLINE-4(1*H*)-ONE DERIVATIVES AS ANTI-INFLAMMATORY AND ANALGESIC AGENTS

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Abstract: Two series of 2-phenyl-4(3H) quinazolinone derivatives have been synthesized. Most of the tested quinazolinone derivatives showed considerable potent anti-inflammatory and analgesic activity of superior GIT safety profile in experimental rats in comparing to indomethacin as reference drug. Compounds VIa, VIb were the most potent anti-inflammatory in experimental rats in comparing to indomethacin as reference drug. Docking study into COX-2 has been made for derivatives of anti-inflammatory activity.

Keywords: quinazolin-4-ones, anti-inflammatory, analgesic, ulcerogenic effect, molecular docking

Quinazolinone nucleus has been gaining prominence due to the fact that its derivatives have been found to possess wide spectrum of activities like antiviral (1), antibacterial (2, 3), antifungal (4), antimalarial (5), anticancer (6–8), antihypertensive (9), anti-inflammatory, analgesic and COX-II inhibitors (10–12). Substitution pattern by different aryl or heteroaryl moieties at 2/3 position (13) of quinazolinone nucleus markedly influences anti-inflammatory activities. Moreover, 2-oxo (imino) pyridines (14, 15), pyrazoles (16–18) and pyrimidines (19, 20) are other important pharmacodynamic heterocyclic nuclei which, when incorporated into different heterocyclic templates, have been reported to possess potent anti-inflammatory activity.

The enhanced overall lipophilic characteristics of the target compounds could favor their selectivity towards COX-2 enzyme over COX-1 leading to increase of GIT safety margin (21). Based on the above observations and in continuation of our anti-inflammatory and analgesic drug research program (11, 22–24), it was of interest to synthesize a novel series of quinazolinone derivatives with structure modifications involving incorporation of the above mentioned heterocyclic moieties at position 3 and phenyl moiety at position 2 of quinazolinone moiety as a trial to obtain safer and potent anti-inflammatory and analgesic agents.

The anti-inflammatory activity of twelve of the newly synthesized compounds**: 2a, 2b, 3a, 3b, 4a, 4b, 5a, 5b, 6a, 6b, 7** and **8** were evaluated by applying carrageenan-induced paw edema bioassay in rats (25) using indomethacin as a reference standard. The results were expressed as the mean ± SE. Differences between vehicle control and treatment groups were tested using one way ANOVA followed by the least significant difference (LSD) test. Methods of statistical analysis were applied according to Armitage et al. (26). The ulcerogenic effect of the above mentioned twelve derivatives was evaluated (27).

The aim of this work was also to study the crystal structure of COX-2 and to rationalize the obtained biological data and explain the possible interactions that might take place between the tested derivatives and COX-2 enzyme compared to indomethacin in order to estimate the anti-inflammatory effect.

The crystal structure was downloaded (pdb code: 4COX) and our compounds were docked into the active site using plants software (28). Compounds (**2a**,**b**, **3a**,**b**, **4a**,**b**, **5a**,**b**, **6a**,**b**, **7** and **8**) were ranked after docking according to their docking scores and were visualized inside the pocket to view their fitting and closure to the main residues.

The affinity of any small molecule can be considered as a unique tool in the field of drug design.

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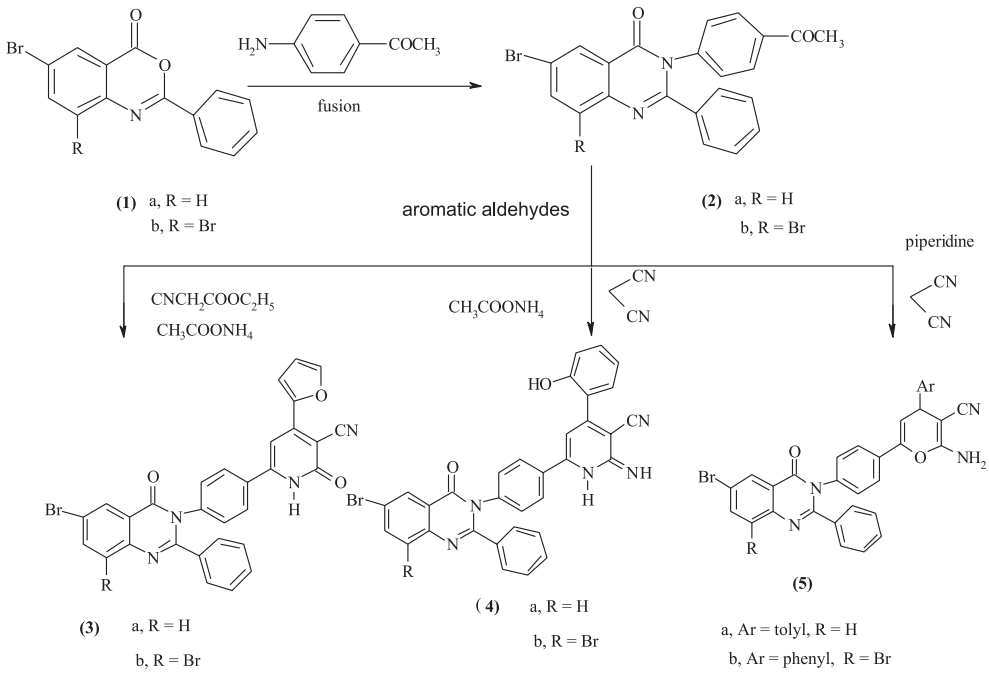
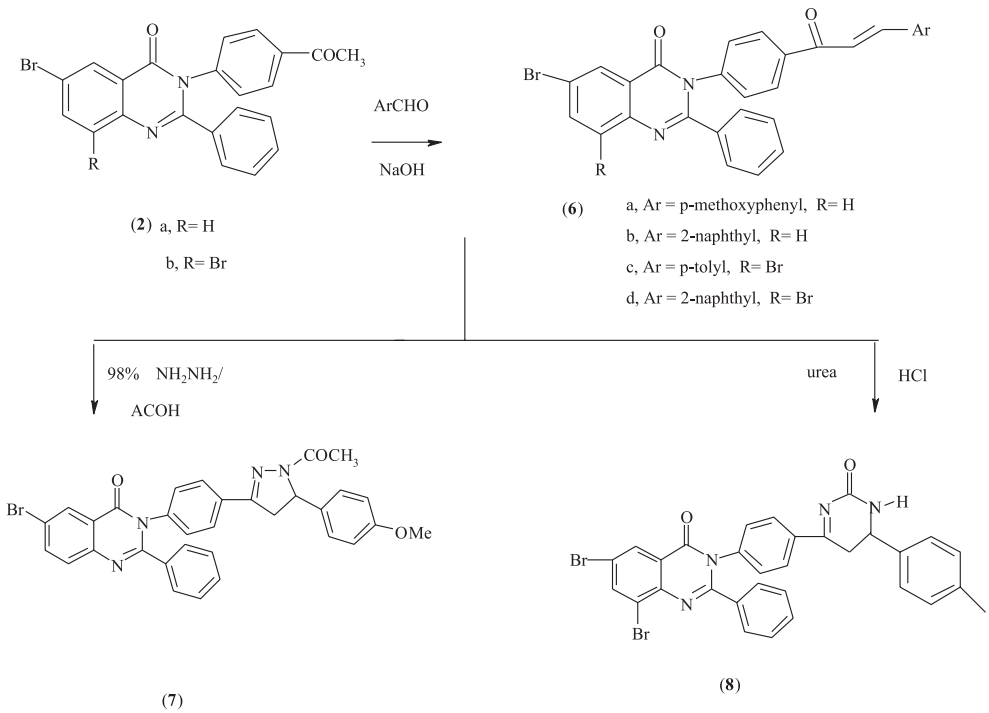


Scheme 1.



Scheme 2.

As there is a relationship between the affinity of organic molecules and the free binding energy, this can contribute in prediction and interpretation of the activity of organic compounds toward a specific target protein. Here we used Autodock Vina program (29, 30) for further docking and to obtain the affinities of the tested compounds that have shown anti-inflammatory effect and to compare these data with those obtained from indomethacin docking.

RESULTS AND DISCUSSION

Chemistry

It is well known that the most common method to obtain substituted 3H-quinazolin-4-one derivatives is based on the aminolysis of the corresponding benzoxazin-4-ones (22). Synthesis of the starting compound, namely, 6-bromo and/or 6,8-dibromo-2-phenyl-3-(4-acetylphenyl)-4(3H)-quinazolinone (2**a**) and\or (**2b**), was achieved by fusion of the known, 6,8-dibromo-2-phenyl-4H-3,1-benzoxazin-4-one (**1a**) and\or (**1b**), respectively, with *p*-aminoacetophenone (22). The facile one pot reaction of ketone **2a** and\or **2b** with furfural with ethyl cyanoacetate in the presence of anhydrous ammonium acetate in n-butanol afforded the corresponding pyridine-2(1H)-ones, (**3a**,**b**), respectively, according to our recent reported method (22–24). In the same manner, the one pot reaction of **2a** and\or **2b** with 2-hydroxybenzaldehyde and malononitrile in the presence of anhydrous ammonium acetate in n-butanol afforded the corresponding 2-(1H)-iminopyridine derivatives (**4a,b**). The same one pot reaction (22) of **2a** and\or **2b** with *p*-tolualdehyde and\or benzaldehyde and malononitrile in piperidine, gave the corresponding 2-aminopyrans (**5a**,**b**) (Scheme 1).

On the other hand, α,β-unsaturated ketones (chalcones), represent active intermediates for several heterocyclic ring systems of biological importance (31, 32). So, Claisen-Schmidt condensation of

Table 1. Anti-inflammatory effects.

Group	1 h	2 h	3 h	4 h
Control	74.2 ± 50	80.4 ± 3.6	95.2 ± 2.8	95.8 ± 2.7
2a	58.3 ± 2.4*** (21.4)	60.7 ± 1.4*** (–25.4)	80.4 ±1.7*** (–15.5)	78.2 ± 2.9*** (–18.4)
2**b**	59.4 ± 1.8*** (–19.9)	70.5 ± 1.5** (–12.2)	84.5 ± 2.5** (–11.7)	84.4 ± 2.5** (–11.8)
3a	51.5 ± 0.7*** (–30.6)	67.3 ± 3.4*** (–19.5)	81.9 ± 1.5*** (–14)	80.9 ± 1.1*** (–15.6)
3b	62.2 ± 3.1** (–16.1)	62.6 ± 1.9*** (–22.1)	91.4 ± 1.2 (–3.9)	91.0 ± 2.8 (–5.0)
4a	65.5 ± 1.6* (–11.7)	80.9 ± 1.5 (0.6)	85.9 ± 3.6* (–9.8)	69.3 ± 2.5*** (–27.7)
4b	67.3 ± 4.2 (–9.2)	72.2 ± 2.4* (–10.2)	91.0 ± 2.9 (–4.4)	92.9 ± 4.9 (–3)
5a	60.0 ± 1.1*** (–19.1)	68.3 ± 0.6*** (–15.0)	71.2 ± 2.9*** (–25.2)	85.4 ± 1.9** (–10.9)
5b	64.8 ± 1.1** (–12.6)	72.2 ± 0.9** (–10.1)	87.2 ± 0.9* (–8.4)	87.7 ± 2.1* (–8.4)
6a	58.8 ± 1.3*** (–26.2)	62.6 ± 2.2*** (–22.1)	62.4 ± 1.5*** (–34. 5)	61.7 ± 2.1*** (–35.6)
6b	56.5 ± 2.4*** (–23.8)	57.5 ± 2.8*** (–28.4)	68.2 ± 2.4*** (–28.3)	72.1 ± 1.2*** (–24.7)
7	62.0 ± 0.6*** (–16.4)	66.3 ± 3.6*** (–17.5)	78.9 ± 1.4*** (–17.1)	68.7 ± 1.8*** (–28.3)
8	73.9 ± 3.0 (0.4)	74.6 ± 2.9 (–7.2)	88.2 ± 2.9 (–7.3)	91.1 ± 1.8 (–4.9)
Indo.	45.2 ± 7.1*** (38.8)	40.11 ± 5.4*** (50.1)	46.9 ± 6.0*** (50.7)	48.1 ± 6.2*** (49.7)

Each group represents the mean ± SE of six animals. Significance *vs*. control group at corresponding hour: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student's *t*-test). In parentheses % of change *vs*. control group at corresponding hour. Indo. = Indomethacin

Table 2. Analgesic effects.

Group	Pre-drug value	1 h		2 h	
	$X^- \pm SE$	$X^- \pm SE$	% of change	$X^- \pm SE$	% of change
Control	6.4 ± 0.16	6.8 ± 0.2	-	7.3 ± 0.5	-
2a	6.0 ± 0.3	8.0 ± 0.6**	33.3	9.0 ± 0.6***	50.0
2b	6.5 ± 0.7	8.46 ± 0.6**	30.3	9.9 ± 0.9***	52.6
3a	7.5 ± 0.4	8.6 ± 0.2	14.7	8.5 ± 0.3	13.3
3b	7.1 ± 0.6	10.6 ± 1.1***	49.4	12.8 ± 0.9***	80.2
4a	5.9 ± 0.0.2	9.4 ± 0.6***	59.3	7.6 ± 0.3*	28.8
4b	6.1 ± 0.3	9.9 ± 0.4***	62.7	11.8 ± 1.2***	94.9
5a	7.9 ± 0.2	9.0 ± 0.5	13.9	9.0 ± 0.4	13.9
5b	7.9 ± 0.4	12.5 ± 0.4***	58.9	14.3 ± 0.3***	81.3
6a	8.0 ± 06	9.0 ± 0.6	12.5	9.2 ± 0.3	15
6b	7.9 ± 0.0.7	12.3 ± 0.8***	55.9	14.1 ± 1.1***	78.6
7	6.0 ± 0.3	7.6 ± 0.2*	26.7	9.0 ± 0.7***	50
8	6.4 ± 0.5	6.2 ± 0.4	-1.9	9.8 ± 0.7***	53.2
Indo.	9.0 ± 0.3	13.6 ± 1.0***	51.1	16.1 ± 0.9***	78.9

Data are presented as the mean ± SE. % of change = from basal (pre-drug) value for each group.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student's *t*-test). Indo. = indomethacin.

Table 3. Effect of newly synthesized compounds on gastric mucosal injury induced by ethanol on rats.

Treatment group	Number of lesions/rat X ± SE	% of change	Severity of lesions/rat X ± SE	% of change
Control	11.0 ± 0.9	-	25.8 ± 3.3	-
2a	2.3 ± 1.4***	–78.9	2.1 ± 0.8***	–91.6
2b	8.7 ± 1.1	–20.9	19.7 ± 0.3***	–23.6
3a	0.86 ± 0.6***	–92.1	1.5 ± 0.8***	–93.8
3b	10.0 ± 0.5	–9.1	26.3 ± 2.2	–1.9
4a	2.3 ± 0.2***	–78.6	3.3 ± 0.9 ***	–86.5
4b	6.0 ± 0.7 ***	–45.5	9.5 ± 1.5 ***	–63.2
5a	1.3 ± 0.7 ***	–87.9	2.0 ± 0.8 ***	–92.1
5b	3.2 ± 0.4 ***	–70.9	4.5 ± 0.5 ***	–82.6
6a	1.6 ± 04 ***	–85.4	2.2 ± 0.5 ***	–91.2
6b	2.3 ± 0.5***	–79.1	8.2 ± 1.7 ***	–68.2
7	2.4 ± 1.8 ***	–78.0	5.0 ± 1.8 ***	–80.3
8	1.5 ± 0.3***	–86.4	5.3 ± 1.2***	–79.5
Indomethacin	7.7 ± 1.7	–29.26	13.0 ± 3.2***	–49.48

Statistical comparison of the difference between the control group and treated groups is indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student's *t*-test).

ketone **2a** and\or **2b** with *p*-anisaldehyde, *p*-tolualdehyde and/or naphthalene-2-carboxaldehyde in the presence of NaOH, afforded the corresponding 1,3-propen-1-one derivatives (chalcones) (**6a-d**), respectively. Compound **6a** was allowed to condense with hydrazine hydrate in the presence of acetic acid to give the corresponding N-acetylpyrazolines (**7**) (Scheme 2).

Table 4. Calculated affinities for all the tested quinazolinone derivatives as anti-inflammatory agents.

Compound	Affinity Kcal/mol	Distance (in Å) from main residue		Functional group
2a	–11.4	2.05	Trp 387	-C=O
2**b**	–11.9	2.69	Tyr 355	-C=O
3a	–11.7	3.02	Ser 353	-C=O
3b	–11.0	2.44	Arg 120	-C=O
4a	–12.6	2.67	Ser 353	-OH
4b	–10.9	1.67	Ser 353	-OH
5a	–12.5	2.23	Tyr 385	-CN
5b	–11.9	……	…….	……
6a	–11.1	2.66	Tyr 355	-C=O
6b	–12.1	2.18	Tyr 385	-CO
7	–13.1	2.71	PHE 518	-C=O
8	–11.7	…..	……	……

All compounds were saved as pdbqt format and docking with Autodock Vina was performed according to the specified condition in which the grid box was adjusted to have center x = 24.15, center y = 22.8, and center z = 12.9. By these centers the pockets with the main residues were involved inside the box.

Also, cyclocondensation of the chalcone **6c** with urea in the presence of HCl according to a reported method (22–24), afforded the corresponding tetrahydropyrimidone derivative (**8**) (Scheme 2).

Biological evaluation

Anti-inflammatory effect:

As shown in Table 1, administration of many of tested compounds 60 min prior to carrageenan injection at a dose of 10 mg/kg b.w. caused significant inhibition of paw edema response. Compounds **2a**, **2b**, **3a**, **3b**, **4a**, **5a**, **5b**, **6a**, **6b** and **7** caused significant decrease in paw edema after 1, 2, 3 and 4 h after drug administration. Compound **4b** showed the effect only after 2 h, while compound **3b** gave its response after 1 h of administration and continued to 2 h only. On the other hand, compound **8** was inactive towards carrageenan-induced edema in comparison to the standard reference – indomethacin,

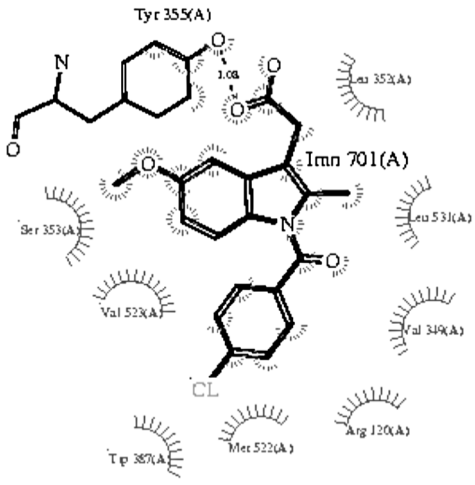


Figure 1. Interaction of Tyr 355 –OH group with the –CO group of indomethacin carboxylic group with a distance of 1.03 Å. The image was taken using Ligplot

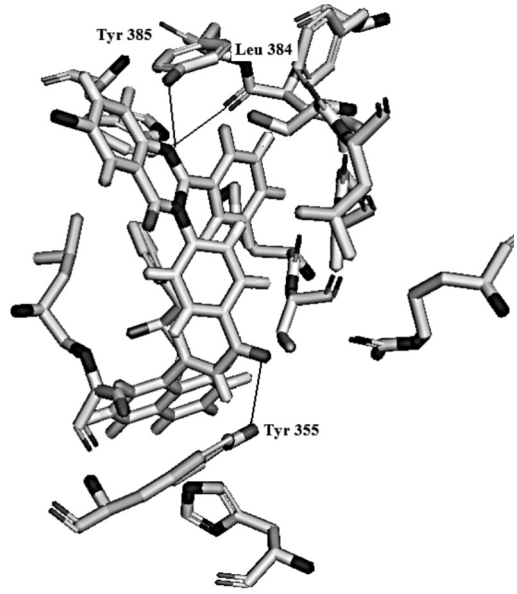


Figure 2. Compound **6a** react by its nitrogen forming hydrogen bond with both Leu 384 –CO group and Tyr 385. Its –CO group interact with the hydroxyl group of Tyr 355

which markedly and significantly inhibited the paw edema after 1, 2, 3 and 4 h of carrageenan injection. Thus, compounds **2a**, **2b**, **3a**, **3b**, **4a**, **4b**, **5a**, **5b**, **6a**, **6b** and **7** have good anti-inflammatory activity and compound **6a** was the most potent derivative.

As shown in Table 2, compounds **4b** and **5b** showed significant analgesic activity higher than that obtained by indomethacin 1 h and 2 h post administration.

Compounds **4a** and **6b** show significant analgesic activity higher than that obtained by indomethacin 1 h post administration only and gave significant analgesic activity 2 h post administration but lower than that obtained by indomethacin. Compound **3b** exhibited analgesic effect higher than that obtained by indomethacin 1 h post administration and showed analgesic effect slightly lower than that of indomethacin after 2 h of administration. Compounds **8** exhibited significant analgesic activity only after 2 h of administration. Compounds **3a**, **5a** and **6a** have non significant analgesic activity in comparison to the base line of the same group 1 and 2 h post administration.

Thus, it can be concluded that compounds **2a, 2b**, **3b**, **4a**, **4b**, **5b**, **6b**, **7** and **8** have significant analgesic activity and compound **4b** is the most potent one.

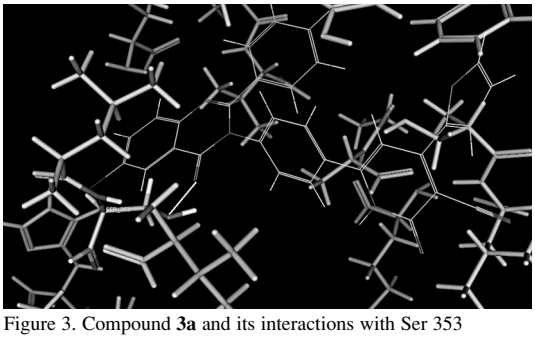


Figure 3. Compound **3a** and its interactions with Ser 353

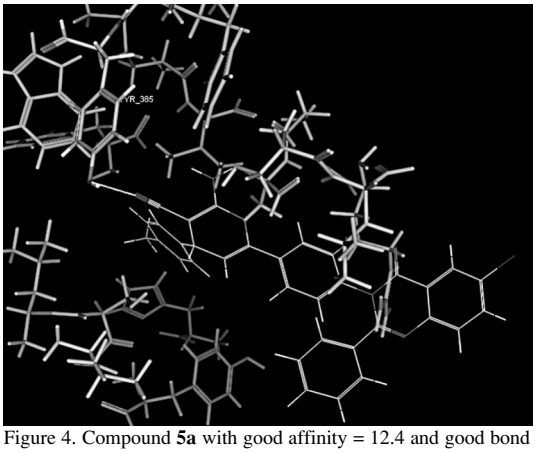


Figure 4. Compound **5a** with good affinity = 12.4 and good bond formation with Tyr 385

Gastric ulcerogenic studies

From Table 3, it has been found that all compounds have very little ulcerogenic effect with better safety margin in comparison to indomethacin except compounds **2b** and **3b** (ulcer lesions in many of the experimental rats). Therefore, there is a potential medicinal value of these compounds as anti-inflammatory and analgesic agents, because they have better safety margin than indomethacin on gastric mucosa.

Molecular docking study

First of all, the main interactions of indomethacin with COX-2 were determined and we found that the main residue is Tyr 355 (–OH group) forming interactions with the carboxylate group of indomethacin in a distance equal to 1.03 Å. From Figure 1, we can also determine the other residues, which are present in the pocket and can be involved in the interactions, such as Arg 120, Leu 531, Val 349, and Ser 353.

In this work the 3-phenyl- 3,4-dihydro-4-quinazolinone scaffold has different substitutions in the 3-phenyl *para* position what gave us a variety in the type of interactions in the docking process. It also clarified that there are some other residues in the site of action which could be involved in ligand-receptor reaction and may be potential in determination of the activity of the compounds. It was found that Tyr 355, Phe 318, Tyr 385 and Arg 120 are the most widely distributed residues in the interactions and bond formation.

The calculated affinities may be a good tool for interpretation of the difference of activity together with the measured distances between specific functional groups in the compounds with the previously mentioned residues (Table 4). The best observation here was that all the measured distances are within the range 1.67–3.02 Å, which is considered as a good sign that indicate the absence of clashes between ligands and protein.

Considering compound **6a,** which is the most potent anti-inflammatory agent of the tested compounds comparing to indomethacin as the refrence drug, its activity may be referred to the conformation that could react by its nitrogen atom found in position number one of the quinazoline ring forming hydrogen bond with both Leu 384 –CO group and Tyr 385. The flexibility of this compound allows also its –CO group found in the 2-propen-1-one moiety to interact with the hydroxyl group of

Tyr 355, giving the chance to this compound to be fixed in a centered position in the active site (Figure 2).

CONCLUSIONS

This study includes the synthesis of two series of quinazolinone derivatives attached to various aromatic and/or heterocyclic ring systems such as: oxo(imino)pyridines, pyrazoles and pyrimidines.

Different twelve derivatives were evaluated as anti-inflammatory and analgesic agents in experimental animals. It has been found that most of the tested quinazolinone derivatives showed considerable potent anti-inflammatory and analgesic activity of superior GIT safety profile in experimental rats in comparison to indomethacin as the reference drug. Compounds **6a**, **6b** were the most potent anti-inflammatory agents in experimental rats with superior gastrointestinal safety profile when compared to indomethacin. So, we can conclude that the presence of α,β-unsaturated ketones (chalcones) increases anti-inflammatory activity for both mono and dibromo quinazolinone derivatives. The results also show that mono bromo derivatives **2a**, **3a**, **4a** and **6a** have higher anti-inflammatory activity than dibromo derivatives **2b, 3b, 4b, 6b,** so it may be concluded that the substitution of quinazolinone ring with only one bromine atom results in higher anti-inflammatory activity than that of dibromo substituted derivatives. Compound **8** has no anti-inflammatory effect in rats in comparison to indomethacin, so tetrahydropyrimidone group might decrease anti-inflammatory effect of quinazolinone derivative. Compounds **3b**, **4b**, **5b** and **6b** were the most potent analgesic agents in experimental rats in comparison to indomethacin. Compounds **3a**, **5a** and **6a** have no significant analgesic activity in comparison to indomethacin, so we concluded that substitution with two bromine atoms is essential for analgesic activity in chalcones **6** and pyridine-2(1H)-ones **3**. The potential medicinal value of these compounds as anti-inflammatory and analgesic agents, may lay in the fact that they have better safety margin than indomethacin on gastric mucosa.

Twelve quinazoline compounds that were tested for their anti-inflammatory activity were docked into the same pocket of indomethacin in COX-2 and have shown good docking results and good fitting into the active site. It has been found that a majority of these compounds have good anti-inflammatory effect compared to that of indomethacin. By the use of molecular modeling we speculated on the mechanism of their effects that could be their interactions with the same residues that interact with indomethacin.

EXPERIMENTAL

Chemistry

All melting points are uncorrected, elemental analyses were carried out in the microanalytical units of National Research Centre and Cairo University, Egypt. The IR spectra were recorded on FTIR-Nexus 670-Nicolet, USA and Perkin Elmer-9712 spectrophotometers. The ^{1}H-NMR spectra were determined on a Varian-Gemmi-300 MHz and Jeol-Ex270 MHz NMR spectrometers using TMS as an internal standard. Mass spectra were recorded on Finnigan Mat SSQ 7000 mode EI 70 eV (Thermo Inst. Sys. Inc. USA). Thin layer chromatography was carried out on silica gel 60 F_{254} (Merck) thin layer chromatography plates using a chloroform, petroleum ether, methanol mixture (7:4:1 v/v/v) as the mobile phase.

6-Bromo-2-phenyl-4H-3,1-benzoxazin-4-one (1a)

This compound was prepared according to reported method (33), m. p. 180^{o}C.

6,8-Dibromo-2-phenyl-4H-3,1-benzoxazin-4-one (1b)

This compound was prepared according the reported method (33).

6-Bromo-2-phenyl-3-(4-acetylphenyl)-4(3H)-quinazolinone (2a)

A mixture of compound **1a** (2.9 g, 0.01 mol) and *p*-aminoacetophenone (1.35 g, 0.01 mol) was heated upon fusion at 150^{o}C on sand bath for 2 h. After cooling, the crude mass was crystallized twice from ethanol to give dark brown crystals of **2a**, m. p. 215^{o}C in 82% yield. Analysis: for $C_{22}H_{15}BrN_2O_2$ (m.w. 419.2) calcd.: C, 63.0, H, 3.6, N, 6.7%; found: C, 63.32, H, 3.63, N, 6.55%. IR (KBr, cm^{-1}): 3302 (C-H aromatic), 1762 (C=O of acetyl), 1685 (C=O of quinazolone), 1633 (C=N), 1590 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.45 (3H, s, $COCH_3$), 7.4–7.7 (m, 9H, Ar-H), 7.9–8.4 (m, 3H, quinazoline ring). MS (m/z): M^+ 418, 420 (75%, 73%), 417 (100%).

6,8-Dibromo-2-phenyl-3-(4-acetylphenyl)-4(3H)-quinazolinone (2b)

A mixture of the benzoxazine **1b** (3.8 g, 0.01 mol) and *p*-aminoacetophenone (1.35 g, 0.01 mol) was heated at 150^{o}C on sand bath for 2 h. After cool-

ing, the crude mass was crystallized twice from ethanol to give dark brown crystals of **2b**, m. p. 240°C in 85% yield. Analysis: for $C_{22}H_{14}Br_2N_2O_2$ (m.w. 498.17) calcd.: C, 53.04, H, 2.83, N, 5.62%; found: C, 52.99, H, 3.63, N, 5.55%. IR (KBr, cm^{-1}): 3302 (C-H aromatic), 1736 (C=O of acetyl), 1685 (C=O of quinazolinone), 1633 (C=N), 1590 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.5 (3H, s, $COCH_3$) and 7.5–7.8 (m, 9H, Ar-H) , 8.1, 8.44 (s, s, H, H, at 7 and 5 positions of the quinazolinone ring). MS (m/z): M^+ 495,497,499 (71%, 100%, 69%).

6-{4-[6-Bromo and\or 6,8-Dibromo-2-phenyl-4-oxo-4H-quinazolin-3-yl]-phenyl}-2-oxo- (furan-2-yl)]1,2-dihydropyridine-3-carbonitriles (3a,b)

General method

A mixture of ketone **2a** or **2b** (0.002 mol), ethyl cyanoacetate (0.23 mL, 0.002 mol), anhyd. ammonium acetate (1.24 g, 0.016 mol) and furan-2-carboxaldehyde (0.002 mol) in 10 mL of n-butanol was refluxed for 6 h. The reaction mixture was concentrated to half its volume under reduced pressure. After cooling, the formed precipitate was filtered, air dried, and recrystallized from the proper solvent to give compounds **3a,b**, respectively.

6-{4-[6-Bromo-2-phenyl-4-oxo-4H-quinazolin-3-yl]-phenyl}-2-oxo-4-(furan-2-yl)]-1,2-dihydropyridine-3-carbonitrile (3a)

Crystallized from acetic acid to give brown crystals, m.p. 295–297°C and yield 0.8 g, 71%. Analysis: for $C_{30}H_{17}BrN_4O_3$ (m.w. 561.38) calcd.: C, 64.18, H, 3.05, N, 9.98%; found: C, 64.15, H, 3.11, N, 9.95%. IR (KBr, cm^{-1}): 3360 (NH), 2200(-CN), 1720 (C=O of quinazolone), 1660 (C=O pyridone), 1580 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.0–8.1 (16H, m, aromatic protons including 1 H of pyridine), 9.0 (1 H, s, NH).

6-{4-[6,8-Dibromo-2-phenyl-4-oxo-4H-quinazolin-3-yl]-phenyl}-2-oxo-4-(furan-2-yl)]-1,2-dihydropyridine-3-carbonitrile (3b)

Crystallized from acetic acid to give dark brown crystals, m. p. 275–277°C and yield 0.95 g, 75%. Analysis: for $C_{30}H_{16}Br_2N_4O_3$ (m.w. 637.96) calcd.: C, 56.28, H, 2.52, N, 8.75%; found: C, 56.20, H, 2.49, N, 8.71%. IR (KBr, cm^{1}): 3350 (NH), 2204 (-CN), 1722, 1678(2 C=O groups), 1580 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.25–8.2 (15H, m, aromatic protons including 1 H of pyridine), 9.25 (1 H, s, NH).

6-{4-[6 Bromo and\or 6,8-Dibromo-2-phenyl-4-oxo-4H-quinazolin-3-yl]phenyl}-2-imino- (2-hydroxyphenyl)-1,2-dihydropyridine-3-carbonitriles (4a,b)

General method

A mixture of compound **2a** or **2b** (0.002 mol) and\or malononitrile (0.12 mL, 0.002 mol), anhyd. ammonium acetate (1.24 g, 0.016 mol) and 2-hydroxybenzaldehyde (0.002 mol)**,** was refluxed for 5 h. After cooling, the reaction mixture was filtered and crystallized from the proper solvent to give iminopyridines **4 a**,**b**, respectively.

6-{4-[6-Bromo-2-phenyl-4-oxo-4H-quinazolin-3-yl]-phenyl}-2-imino-4-(2-hydroxyphenyl)-1,2-dihydropyridine-3-carbonitrile (4a)

Crystallized from acetic acid to give reddish brown crystals with m. p. 330–332°C yield 0.75 g, 63%. Analysis for $C_{32}H_{20}BrN_5O_2$, (m.w. 586.44) calcd.: C, 65.54, H, 3.44, N, 11.94%; found: C, 65.50, H, 3.40, N, 11.91%. IR (KBr, cm^{-1}): 3350 (NH), 3345–3240 (OH), 2205 (-CN), 1730 (C=O, quinazolone), 1606 (C=N). MS (m/z): M^+ 569, 571 (5%, 4.8%), 187 (100%).

6-{4-[6,8-Dibromo-2-phenyl-4-oxo-4H-quinazolin-3-yl]-phenyl}-2-imino-4-(2-hydroxyphenyl)-1,2-dihydropyridine-3-carbonitrile (4b)

Crystallized from ethanol to give reddish brown crystals with m.p. 190–192°C, yield 0.93 g, 70%. Analysis: for $C_{32}H_{19}Br_2N_5O_2$, (m.w. 665.33) calcd.: C, 57.77, H, 2.88, N, 10.53%; found: C, 57.73, H, 2.84, N, 10.50%. IR (KBr, cm^{-1}): 3345 (NH), 3340–3240 (OH), 2205 (-CN), 1690 (C=O, quinazolinone), 1600 (C=N). MS (m/z, R.I.): M^+ 647, 649, 651 (5%, 10, 4.8%), 187 (100%).

6-{4-[6-Bromo-2-phenyl-4-oxo-4H-quinazolin-3-yl]-phenyl}-2-amino-4-(*p*-tolyl)-4,4-dihydropyran-3-carbonitrile (5a)

A mixture of compound **2a** (0.99 g, 0.002 mol), malononitrile (0.12 mL, 0.002 mol), and *p*-tolualdehyde with few drops of piperidine was refluxed for 4 h. The reaction mixture was reduced to half its volume under reduced pressure, and cooled. The crude precipitate was filtered, washed with cold water and crystallized from ethanol to give dark brown crystals with m.p. 200–202°C. Analysis for $C_{33}H_{23}BrN_4O_2$ (m.w. 587.47) calcd.: C, 67.47, H, 3.95, N, 9.54%; found: C, 67.42.11, H, 3.93, N, 9.51%. IR (KBr, cm^{-1}): 3362–3342 (NH2), 2205 (C=N), 1730 (C=O quinazolone), 1600 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.34 (3H, s, C-CH_3), 3.9 (1H, d, pyran), 4.9 (1H, d, pyran), 7.25–8.00 (16H, m, aromatic protons), 9.6 (2H, s, NH_2). MS (m/z, R.I.): M^+ 586, 588 (20.1%, 19.2%), 189 (100%).

6-{4-[6,8-Dibromo-2-phenyl-4-oxo-4H-quinazolin-3-yl]-phenyl}-2-amino-4-phenyl-4,4-dihydropyran-3-carbonitrile (5b)

A mixture of compound **2b** (0.99 g, 0.002 mol), malononitrile (0.12 mL, 0.002 mol), and benzaldehyde in the presence of few drops of piperidine was refluxed for 4 h. The reaction mixture was reduced to half its volume under reduced pressure and cooled. The precipitate was filtered, washed with cold water and crystallized from ethanol to give brown crystals with m.p. 150–152°C, yield 0.8 g, 60%. Analysis: for $C_{32}H_{20}Br_2N_4O_2$, (m.w. 652.33) calcd.: C, 58.92, H, 3.09, N, 8.59%; found: C, 58.89, H, 2.99, N, 8.53%. IR (KBr, cm^{-1}): 3430–3347 (NH_2), 2220 (-CN), 1680 (C=O quinazolinone), 1600 (C=N).

6 Bromo and\or 6,8-Dibromo-2-phenyl-3-{4-[(E)-3-substituted aryl-acryloyl]-phenyl}-3H-quinazolin-4-ones (6a-d) (Chalcones)

General method

To a mixture of ketone **2a** and\or **2b** (0.002 mol) and the appropriate aromatic aldehyde (0.002 mol) in ethyl alcohol (10 mL) 5% NaOH in ethyl alcohol (10 mL) was added dropwise within 15 min. The reaction mixture was refluxed for 3 h then cooled and the precipitate was filtered, air dried and then crystallized from the proper solvent to give chalcones **6a-d**, respectively.

6-Bromo-2-phenyl-3-{4-[(E)-3-(4-methoxyphenyl-acryloyl]-phenyl}-3H-quinazolin-4-one (6a)

Crystallized from ethanol to give yellowish brown crystals with m.p. 150–151°C and yield 0.8 g,74%. Analysis: for $C_{30}H_{21}BrN_2O_3$ (m.w. 537.40) calcd.: C, 67.05; H, 3.94; N, 5.51%; found: C, 67.00; H, 3.89, N, 5.40%. IR (KBr, cm^{-1}): 1725 (C=O quinazolone), 1670 (C=O of the α,β-unsaturated ketone), 1610 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.83 (3H, s, OCH_3), 6.60–6.80 (2H, dd, CH=CH), 7.3–8.6 (16H, m, aromatic protons including that of quinazoline ring). MS (m/z): M^+ 536, 538 (0.1%, 0.09%), 301, 303 (50.8%, 55.3%), 224, 226 (5%, 5%), 106 (9%), 105 (100%).

6-Bromo-2-phenyl-3-{4-[(E)-3-(naphthalene-2yl)-acryloyl]-phenyl}-3H-quinazolin-4-one (6b)

Crystallized from methanol to give brown crystals with m.p.190–192°C and yield 0.75 g, 67%. Analysis: for $C_{33}H_{21}BrN_2O_2$ (m.w. 556.08) calcd.: C, 71.10, H, 3.80, N, 5.03%; found: C, 70.99, H, 3.76, N, 5.00%. IR (KBr, cm^{-1}): 1733 (C=O quinazolone), 1665 (C=O of α,β-unsaturated ketone), 1610 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.70–6.90 (2H, dd, CH=CH), 7.3–8.6 (19H, m, aromatic protons including that of quinazoline ring).

6,8-Dibromo-2-phenyl-3-{4-[(E)-3-(p-tolyl-acryloyl]-phenyl}-3H-quinazolin-4-one (6c)

Crystallized from ethanol to give yellowish brown crystals with m.p. 280–282°C and yield 0.9 g, 75%. Analysis: for $C_{30}H_{20}BrN_2O_2$ (m.w. 600.30) calcd.: C, 60.02, H, 3.36, N, 4.67%; found: C, 60.00, H, 3.31, N, 4.63%. IR (KBr, cm^{-1}): 1685 (C=O quinazolinone), 1674 (C=O of the α,β-unsaturated ketone), 1610 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.34 (3H, s, C-CH_3) 6.6–6.80 (2H, dd, CH=CH), 7.4–8.6 (15H, m, aromatic protons including that of quinazoline ring). MS (m/z): M^+–2 596, 598, 600 (3.1%, 5.2%, 3.8%), 429 (100%).

6,8-Dibromo-2-phenyl-3-{4-[(E)-3-(naphthalene-2-yl)-acryloyl]-phenyl}-3H-quinazolin-4-one (6d)

Crystallized from ethanol to give dark brown crystals with m.p. 265–267°C and yield 0.8 g, 65%. Analysis: for $C_{33}H_{20}Br_2N_2O_2$ (m.w. 636.33) calcd.: C, 62.29, H, 3.17, N, 4.40%; found: C, 62.23, H, 3.12, N, 4.30%. IR (KBr, cm^{-1}): 1690 (C=O quinazolone), 1670 (C=O of the α,β-unsaturated ketone), 1612 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.50–6.80 (2H, dd, CH=CH), 7.3–8.6 (18H, m, aromatic protons including that of quinazoline ring). MS (m/z): M^+ 638, 636, 634 (6.1%, 10.2%, 5.9%), 429 (100%).

6-Bromo-2-phenyl-3-{4-[5-(*p*-anisyl)-4,5-dihydro-1-acetyl-1H-pyrazol-3-yl]-phenyl}-3H-quinazolin-4-one (7)

A mixture of chalcone **6a** (0.005 mol) and hydrazine hydrate (2.5 mL, 0.005 mol, 98%) in the presence of (10 mL) of glacial acetic acid was refluxed for 6 h. The precipitate was filtered and crystallized from ethanol to give yellow crystals with m.p. 142–144°C, yield 1.6 g, 54%. Analysis: for $C_{32}H_{25}BrN_4O_3$ (m.w. 593.47) calcd.: C, 64.76, H,4.25 , N, 9.44%; found: C, 64.63, H, 4.11, N, 9.32%. IR (KBr, cm^{-1}): 1750 (C=O quinazolone), 1685 (C=O, acetyl), 1635 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.1 (3H, s, COCH3), 3.8 (3H, s, OCH_3), 4.0, 4.4 (2H, dd, dd, CH_2 of pyrazoline), 4.1 (1H, dd, CH of pyrazoline), 7.28–8.2 (16H, m, aromatic protons including those of quinazolinone ring). MS (m/z): M^+ is unstable toward electron impact. It showed [(M^+) – $C_8H_{10}O_2$] at m/z 452, 454 (9.4%, 9.2%) and a base peak at m/z 77 (100%).

3-{4-[6-(*p*-Tolyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl]-phenyl}-6,8-dibromo-2-phenyl-3H-quinazolin-4-one (8)

A mixture of chalcone **6c** (0.005 mol) and urea (0.5 g, 0.005 mol) in ethanol (20 mL) and conc. HCl (5 mL) was refluxed for 7 h. The reaction mixture

was concentrated to 1/2 of its volume, cooled and neutralized with NH_4OH solution. The precipitated solid was filtered, washed with water, air dried and crystallized from ethanol to give yellowish brown crystals with m.p. 112–114°C and yield 2.24 g, 70%. Analysis: for $C_{31}H_{22}Br_2N_4O2$ (m.w. 642.34) calcd.: C, 57.96, H, 3.45, N, 8.72%; found: C, 57.93 H, 3.41 N,8.69%. IR (KBr, cm^{-1}): 3200–3600 (OH enolic of pyrimidine), 1685 (C=O quinazolinone), 1618 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.3 (3H, s, CH_3), 3.5 (2H, d, CH_2 of pyrimidinone) 5.1 (1H, t, CH of pyrimidinone) 7.2–8.5 (16H, m, aromatic protons including that of quinazolone rings). MS (m/z,): M^+ 640, 642, 646 (4%, 10.1%, 5%), 339 (100%).

Biological screening

Materials and methods

Animals – adult rats of both sexes weighing 150–200 g and adult mice weighing 20–25 g were used in the experiments. Animals were housed under standardized conditions for light and temperature and received standard rat chow and tap water *ad libitum*.

Animals were randomly assigned to different experimental groups, each kept in a separate cage. All animal procedures were performed after approval from the Ethics Committee of the National Research Center and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No.85-23, revised 1985).

Anti-inflammatory testing

The carrageenan rat paw edema model of inflammation was used to evaluate the anti-inflammatory properties of the tested compounds. Rats were randomly assigned to treatment groups and sterile carrageenan lambda (Sigma–Aldrich Co., USA) (100 mL of a 1% solution in saline) was injected sub-plantarly into right hind paw of the rat. Carrageenan caused visible redness and pronounced swelling that was well developed by 4 h and persisted for more than 48 h. Right hind paw was measured with a planimeter (34, 35) before and at 1, 2, 3 and 4 h after carrageenan injection. The tested compound was injected *i.p.* (10 mg/kg b.w.). The control animals were injected (*i.p.*) with 1 mL saline. The standard drug was indomethacin (Khahira Pharmaceutical and Chemical Co. Cairo, Egypt, 10 mg/kg b.w.). Different compounds or indomethacin were given 1 h before carrageenan injection.

Analgesia testing

The hot-plate test was performed on mice by using an electronically controlled hot-plate (Ugo Basile, Italy) heated to 52°C (± 0.1°C), for possible centrally mediated analgesic effect of the drugs. Groups of fourteen rats each were given vehicle and/or the different compounds and the last group received indomethacin (10 mg/kg b.w.) 60 min prior to testing. Latency to lick a hind paw or jumping (36) was recorded sequentially before and at 1 and 2 h post treatment.

Ulcerogenic effects

Gastric lesions were induced in rats by absolute ethanol (1 mL of 100%, orally) (37). Animals were fasted for 24 h and then divided into seven groups, one group received ethanol and served as control, and the remaining groups received 10 mg/100 g/b.w. of different compounds 1 h before the ethanol was given. Rats were killed 1 h after ethanol administration by cervical dislocation after being lightly anesthetized with diethyl ether and the stomach was excised, opened along the greater curvature, rinsed with saline, extended on a plastic board and examined for mucosal lesions. The number and severity of mucosal lesions were noted and lesions were scaled as follows: petechial lesions = 1, lesions less than 1 mm = 2, lesion between 1 and 2 mm = 3, lesions between 2 and 4 mm = 4, lesions more than 4 mm = 5. A total lesion score for each animal is calculated as the total number of lesions multiplied by the respective severity scores. The results are expressed as the severity of lesions/rat.

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Received: 24. 07. 2010