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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, anti-inflammatory, analgesic screening, and molecular docking of some novel 2-pyridyl (3*H*)-quinazolin-4-one derivatives

Ahmad F. Eweas · Ahmed O. H. El-Nezhawy · Ayman R. Baiuomy · Mohamed M. Awad

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Abstract A novel series of 6-bromo-2-(4-pyridyl)-quinazolin-4(3*H*)-ones were synthesized by reacting 5-bromo anthranilic acid with isonictinoly chloride in the presence of acetic anhydride, which were further reacted with *p*-amino acetophenone to obtain 3-(4-acetylphenyl)-6-bromo-2-(pyridin-4-yl)quinazolin-4(3*H*)-one (**3**). Compound **3** underwent further reactions with different aldehydes to afford chalcone derivatives **4**–**10**, which in turn underwent various cyclization reactions to afford cyclized products **15**–**18**. 2-aminothiazole derivatives **12** obtained by reaction of **3** with bromine then with thiourea. Compound **14** obtained by treatment of **11** with KSCN followed by cyclization. Some of the synthesized compounds **4**, **5**, **12**, **14**, **15** and **18** were screened for both analgesic and anti-inflammatory activities. All tested compounds showed good analgesic and anti-inflammatory activity in comparison to the reference standard indomethacin. Compounds **4** and **5** showed the highest anti-inflammatory activity, while compounds **14** and **15** showed the highest analgesic activity among all the tested compounds.

Keywords (3*H*)-Quinazolin-4-ones · Molecular docking · Anti-inflammatory

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are considered among the most commonly used drugs for the treatment of both acute and chronic inflammation, pain, and fever. Some of the major side effects NSAIDs are gastrointestinal lesions, bleeding, and nephrotoxicity which are commonly associated with long-term medications of NSAIDs. Therefore the development of new analgesic and anti-inflammatory active drugs with less ulcerogenic side effects is still a challenging target for the drug industry (Van Ryn *et al.,* 2000), (Carter, 2000). In recent years 3*H*-quinazolin-4-one and their derivatives have drawn great attention in the field of synthetic medicinal chemistry as they were reported to possess significant activity as antihypertensive (Ram *et al.,* 1990), anti-fibrotic, choleretic, antiphlogistic (Bekhit *et al.,* 2001), antimitotic anticancer (Chandrika *et al.,* 2008) antifungal, (Lopez *et al.,* 2000) (Farghaly and Moharram, 1999) and anticonvulsant agents (Usifoh and Scriba, 2000).

Quinazolinones in general were reported to possess diverse pharmacological activities such as a CNS depressant (Tani *et al.,* 1979), hypnotic, anti-inflammatory (Plescia *et al.*, 1984), antitumor (Singh, 1978), muscle relaxants (Ochiai and Ishida, 1981), and antineoplastic activity (Raffa *et al.*, 1999). Quinazolines and condensed quinazoline derivatives such as hoquizil, prazosin, and buqineran, possess PDE inhibitory (Rotella *et al.*, 2000) antimicrobial (Bekhit *et al.*, 2001), anti-inflammatory (Maggio *et al.*, 2001), anticonvulsant (Lasztoczi *et al.*, 2002), (Lopez-Farre *et al.*, 2002), and antihypertensive activity. Afloqualone and diproqualone which are derivatives of quinazoline-4-one are being used as successful anti-inflammatory agents in the management of lower back pain as well as a centrally acting muscle relaxants (Ochiai and Ishida, 1982) (Fig. 1).

Based on these findings it is rationalized to design and synthesize new substituted 3*H*-quinazolin-4-ones and screen their anti-inflammatory and analgesic activities.

Results and discussion

Chemistry

Synthesis of the starting compound 6-bromo-2-(pyridin-4-yl)-4*H*-benzo[d][1,3]oxazin-4-one **2** was achieved by the reaction of 5-bromoanthranilic acid with isonictionyl chloride to afford 5-bromo-2-(isonicotinamido)benzoic acid **1** which was then refluxed with excess acetic anhydride to afford **2** in good yield (Table 1). Compound **2** underwent fusion reaction with *P*-aminoacetophenone to afford 3-(4-acetylphenyl)-6-bromo-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **3.** The same compound **3** was prepared via alternative method by reacting **2** with *P*-aminoacetophenone in dry pyridine which was considered as a chemical prove of structure of **3**. The structure of **3** was further proved via instrumental analysis including elemental analysis and NMR.

α,β-unsaturated ketones (chalcones), represent active intermediates for several heterocyclic ring systems of

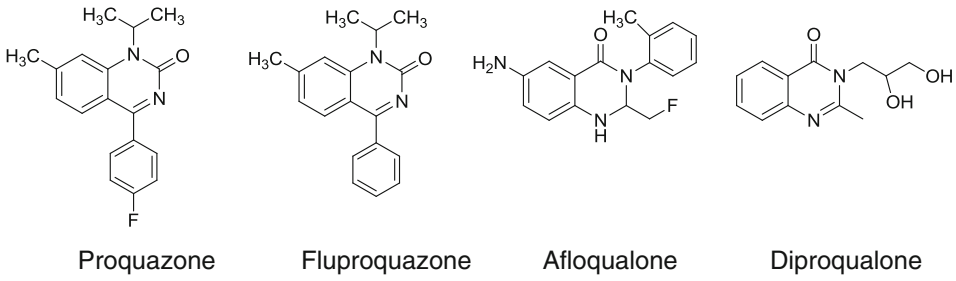


Fig. 1 Quinazoline drugs

Table 1 The docking energy scores of compounds **4**, **5**, **12**, **14**, **15**, and **18** with the amino acid residues forming hydrogen bonds in comparison with reference ligand indomethacin

Cpd. no.	Docking score (Kcal/mol)	No. of hydrogen bonds	Amino acid residues forming hydrogen bonds in Å
Ligand (indomethacin)	−67.53	2	R374 hh21–m M o1 S143 o–m M h13
4	−86.37	1	R374 hh21–m M o1
5	−88.55	3	Arg 374 he–m Res 1 o2 Arg 374 hh22–m Res 1 o2 Asn 537 hd21–m Res 1 o3
12	−79.62	3	R374 hh22–m M o1 R374 he–m M o1 R374 hh22–m M o1
14	−80.06	3	R374 hh22–m M n4 N375 hn–m M o1 R374 hh21–m M o1
15	−92.88	2	R374 hh21–m M o1 R374 hh22–m M o1
18	104.56	4	G225 hn–m M o2 R374 hh21–m M o1 N375 hn–m M o3 N375 hn–m M o1

biological importance, such as pyrazolines, pyridones, and pyrimidone or pyrimidine-thiones (Ram *et al.,* 1990). So, Clause-Schmidt condensation of ketene **3** with the various aromatic aldehydes namely *p*-chloro, *p*-nitro, *p*-fluoro, *p-N, N*-dimethyl amino, *p*-methoxy benzaldehydes, 2-thiophene aldehyde, and 2-furane aldehyde were carried out to produce the corresponding chlacone derivatives **4**–**10** (Scheme 1).

Compound **3** underwent a substitution reaction with bromine water to afford the α-bromoketone **11** which in turn reacted with thiourea in acetone under reflux conditions to afford the 2-aminothiazole derivatives **12** (Scheme 1). Structure of **12** was elucidated via its correct elemental analysis in addition to NMR spectrum which shows the appearance of peak at δ 6.23 (br, 2H NH_2) and δ 6.99 (s, 1H; methylene H of the thiazole ring).

Compound **11** reacted with potassium thiocynate in ethanol in water bath to afford the methylene thioccynate derivative **13** which was used directly to react with glacial acetic acid under reflux to afford the 2-hydroxythiazole derivative **14.** Structure of **14** was elucidated via its correct elemental analysis in addition to NMR spectrum which shows the appearance of peak at δ 5.53 (s, 1H; methylene H of the thiazole ring) and δ 7.28 (br, 1H OH).

Chalcone derivatives **4**, **5**, and **6** underwent various cyclization reactions with hydroxyl amine hydrochloride, urea, methyl hydrazine, and hydrazine hydrate hydrochloride to afford the corresponding cyclized products **15**, **16**, **17**, and **18**. The structures of the aforementioned compounds were elucidated via their correct elemental analysis in addition to their NMR spectra (Scheme 2).

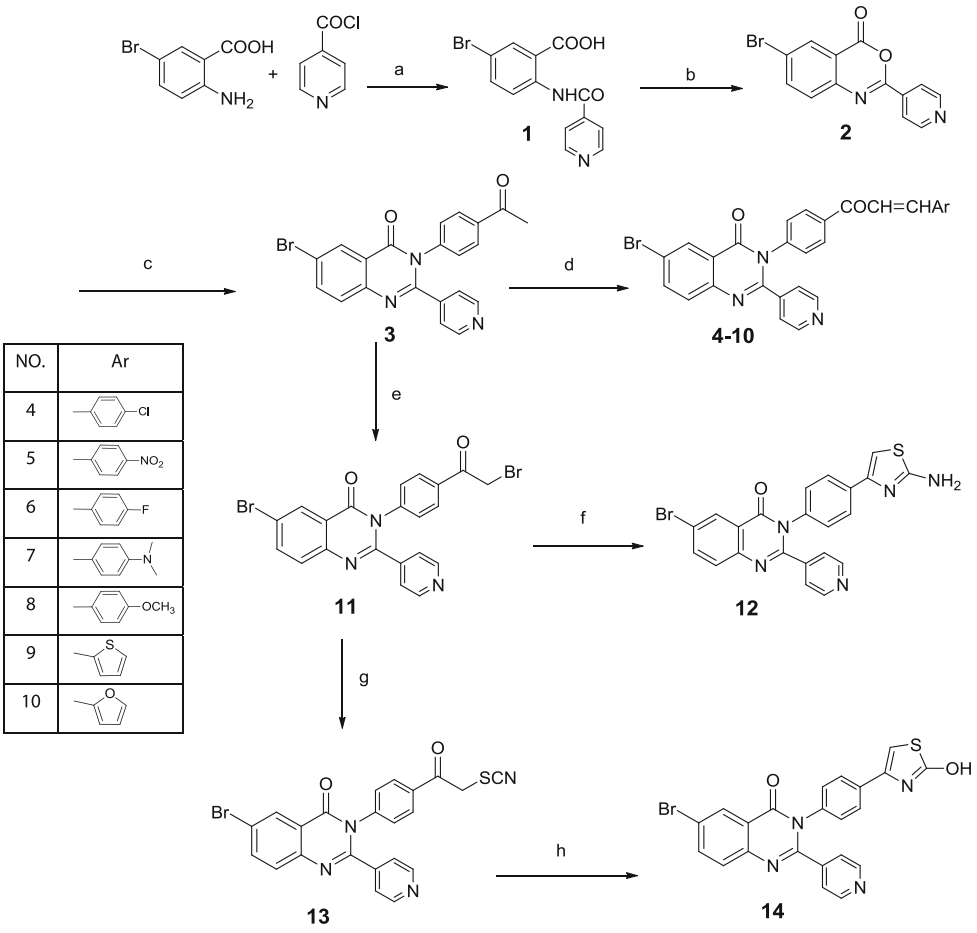
Pharmacology

Anti-inflammatory activity

The activity of the newly synthesized compounds **4**, **5**, **12**, **14**, **15**, and **18** compared to indomethacin as a reference was measured before and 1, 2, 3, and 4 h after carrageenan injection (Fig. 2a, b).

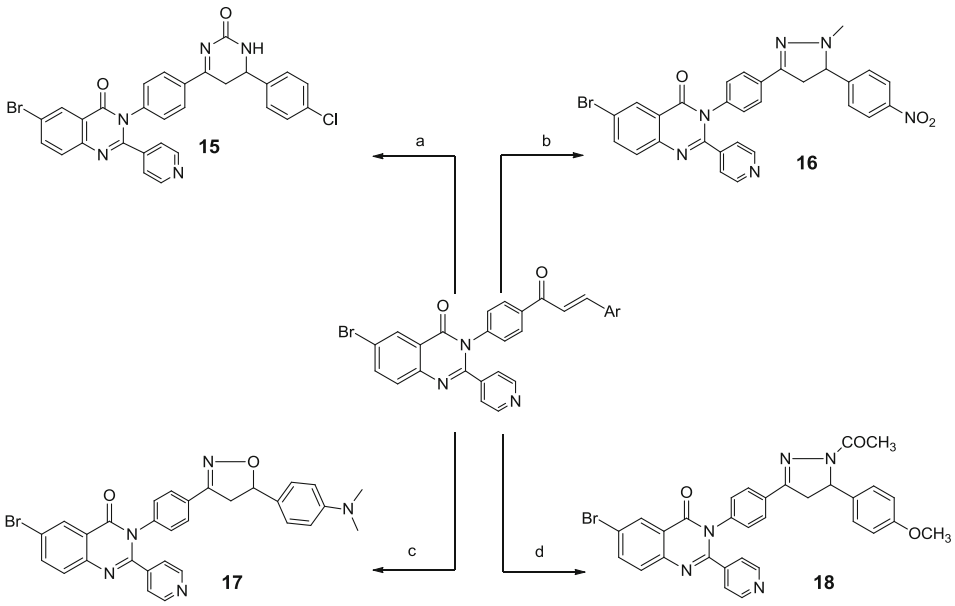
After i.p. administration, compounds **4**, **5**, **12**, **14**, **15**, and **18** (50 mg/kg for each tested compound) significantly inhibited the paw edema response compared with the carrageenan control group at all time points and showed significant anti-inflammatory activities (Fig. 2a, b).

The maximal effects of compounds **4**, **15** and **18**, and indomethacin were observed at 1 h time period after



NO.	Ar
4	Cl
5	NO_2
6	F
7	N
8	OCH_3
9	S
10	O

Scheme 1 Reagents and conditions**:** *a* TEA, CH_2Cl_2; *b* $(CH_3CO)O$, reflux 6 h; *c p*-aminoaceophenone, 150 °C or *p*-aminoaceophenone, pyridine, reflux 8 h; *d* appropriate aromatic/heterocyclic aldehyde, 10 % ethanolic sodium hydroxide reflux 1 h; *e* CH_3COOH, Br_2; *f* thiourea, NaOH; *g* KSCN, C_2H_5OH; *h* glacial acetic acid, sulfuric acid



Scheme 2 Reagents and conditions: *a* Urea, C_2H_5OH, KOH, reflux; *b* $CH_3NH_2NH_2$, CH_3COOH, reflux; *c* $NH_2OH.HCl$, NaOH, C_2H_5OH, reflux; *d* NH_2NH_2, CH_3COOH, reflux

carrageenan by 73.8, 62.3, 64.5, and 62.9 % inhibition, respectively. While the maximal effect of compound **5**, **12**, and **14** was observed at 2 h time period after carrageenan by 70.5, 62.8, and 67.3 % inhibition, respectively.

Compound **5** showed the highest anti-inflammatory activity 4 h after carrageenan injection with ED_{50} of 22.3 mg/kg.

Analgesic activity

The anti-nociceptive activity of the newly synthesized compounds **4**, **5**, **12**, **14**, **15**, and **18** compared to indomethacin as a reference was also investigated. It was assessed by the chemical model using acetic acid-induced writhing in mice.

After s.c. administration, compounds **4**, **5**, **12**, **14**, **15**, and **18** (50 mg/kg for each tested compound) significantly inhibited the acetic acid-induced writhes compared with the control untreated group, and showed significant anti-nociceptive activities (Fig. 3).

The percentages of maximal protection were noted with the tested compounds **14** and **15** by 75.67 and 75.40 %, respectively. **14** showed the highest anti-nociceptive activity with PD_{50} of 15 mg/kg[3].

Molecular docking study

To understand both the anti-inflammatory and analgesic data on a structural basis, molecular docking studies were carried out using Mol soft ICM 3.5-0a software. ICM docking is probably the most accurate predictive tool of binding geometry today (Kroemer, 2003), (Cavasotto and Abagyan, 2004). The aim of the flexible docking calculations is prediction of correct binding geometry for each binder. The scoring functions and hydrogen bonds formed with the surrounding amino acids of the receptor COX-1 are used to predict tested compounds binding modes. Indomethacin was used as reference drug for the anti-inflammatory activity of the new (3*H*)-quinazolin-4-one derivatives. It is well known that indomethacin is a nonselective COX inhibitor with preferential binding selectivity for COX-1 over COX-2 receptor (Perrone *et al.*, 2010). We evaluated the highest anti-inflammatory and analgesic active new compounds **4**, **5**, **12**, **14**, **15**, and **18** through molecular modeling and docking techniques against COX-I crystal structure which was downloaded from PDB website PDB id (2OYE) (Fig. 4) shows binding mode of the original ligand (indomethacin) into its binding site while (Figs. 5, 6) show binding modes of compounds **12** and **18**, respectively. As shown in (Table 1). The following results can be drawn.

Indomethacin "the reference drug" docking results to COX-1 reveals docking score of $\Delta G = -67.53$ kcal/mol and 2 H-bonds between O-1 of the ligand and H21 of Arg 374 and H13 of the ligand and O of the carbonyl group of Ser 143 (Fig. 4). The new quinazolinone compounds were docked against COX-1, all docked compounds showed higher binding affinity toward COX-1 compared to indomethacin (Table 1), where compound no. **18** showed the highest binding score of $\Delta G = -104.56$ kcal/mol (Fig. 6) while compound **12** showed the lowest binding score of $\Delta G = -79.62$ kcal/mol (Fig. 5). All docked compounds showed H-bonding with COX-1 Arg 374 residue similar to the reference drug. From that we can assume that COX-1 Arg 374 is essential in fitting the ligand to COX-1 binding site.

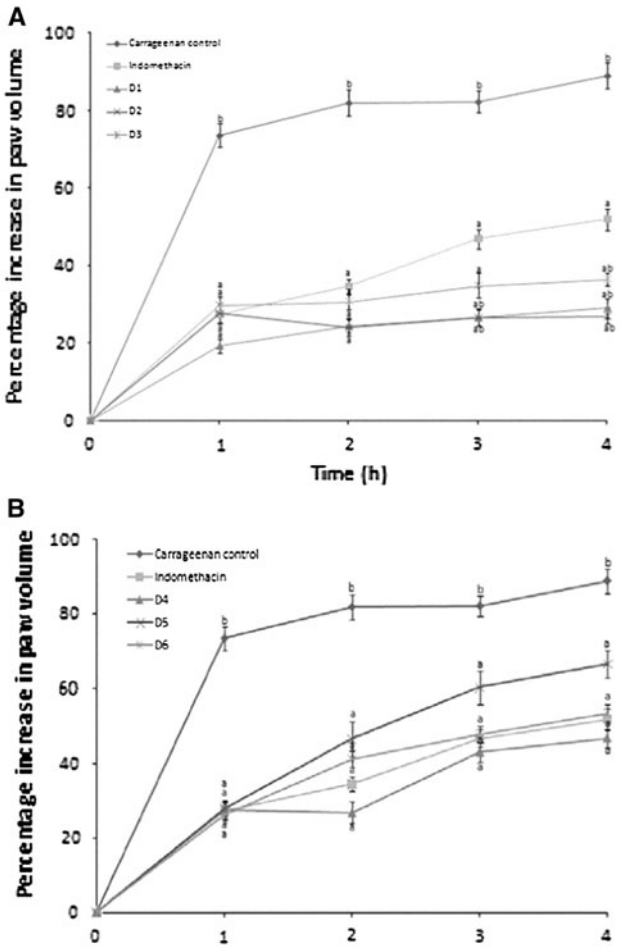


Fig. 2 Anti-inflammatory effect of compounds **4**, **5**, **12** (**a**), **14**, **15**, **18** (**b**) (50 mg/kg, i.p.) and indomethacin (18 mg/kg, i.p.) on the rat paw edema, induced by sub-plantar injection of 100 µl of 1 % sterile carrageenan. Results are expressed as a percentage increase in paw volume from control (pre-drug) values, each point represents mean ± SE of six rats per group. $^{a}p < 0.05$, statistically significant from the carrageenan control group. $^{b}p < 0.05$, statistically significant from the indomethacin-treated group (Tukey test)

Experimental

Chemistry

The purity of the newly synthesized compounds was evidenced by TLC. All solvents which were used for crystallization were of analytical grade. All melting points were determined on a Gallenkamp apparatus and uncorrected. Elemental analysis were carried out at the Micro analytical Laboratory of the National Research Centre, Cairo, Egypt (satisfactory microanalyses were obtained C; ±0.40, H; ±0.02; and N; ±0.30, The 1H NMR were recorded (DMSO-d6), on JOEL-JNM-EX 270 FTNMR system (NRC) and chemical shifts (δ) are expressed in ppm using TMS as an internal standard. Splitting patterns are indicated as s, singlet; d, doublet; m, multiple, b, broad signal.

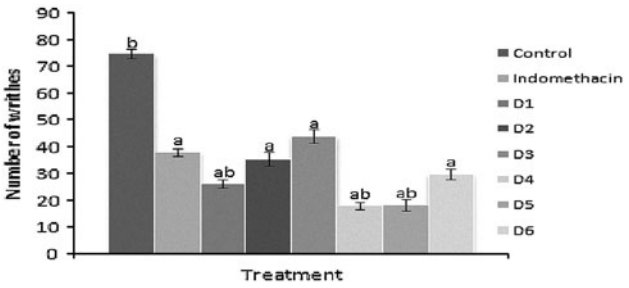


Fig. 3 Effect of administration of compounds **4**, **5**, **12**, **14**, **15**, and **18** (50 mg/kg, s.c.) and indomethacin (18 mg/kg, s.c.) on writhing induced in mice by intraperitoneal injection of acetic acid. Each *bar* represents mean ± SE of six mice per group. $^{a}p < 0.05$, statistically significant from the control group. $^{b}p < 0.05$, statistically significant from the indomethacin-treated group (Tukey test)

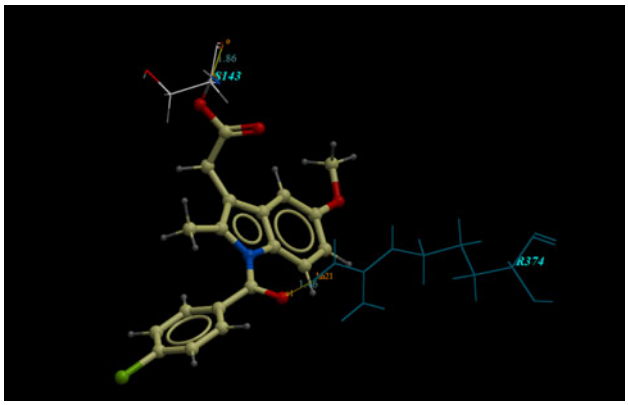


Fig. 4 Binding mode of the reference ligand indomethacin into its binding site of COX-1

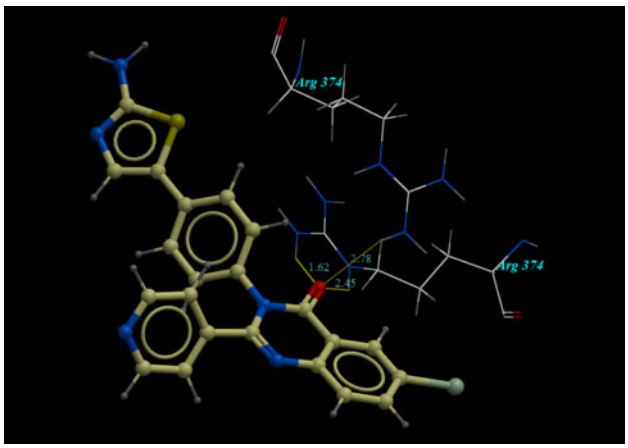


Fig. 5 Binding mode of the Compound **12** into its binding site of COX-1

5-Bromo-2-(isonicotinamido)benzoic acid **1**

Isonicotinyl chloride (0.2 mol) was added drop wise to a stirred solution of 5-bromoanthranilic acid (0.2 mol) with

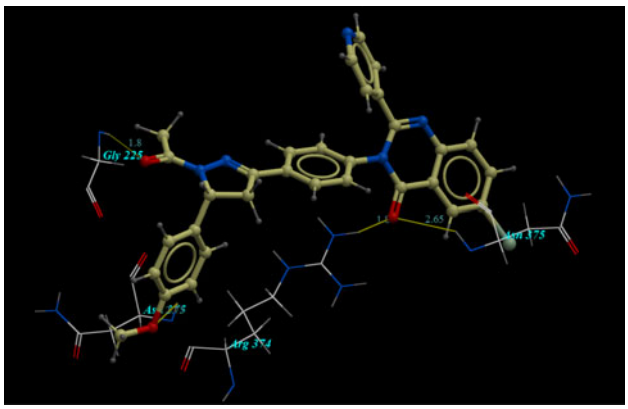


Fig. 6 Binding mode of the Compound **18** into its binding site of COX-1

(0.2 mol) triethyl amine in 200 ml dichloromethane. The reaction mixture was stirred at room temperature for 10–12 h. The solid formed was filtered off, washed with water several times and crystallized from DMF to give **1** as pale yellow crystals yield 72 %; m.p. 297 °C. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm] = 7.80–8.89 (m, 7H), 9.15 (s, br., NH); 11.01(s, br., OH) Anal. Calcd. For $C_{13}H_9BrN_2O_3$. C, 48.62; H, 2.82; N, 8.72. Found: C, 48.65; H, 2.87; N, 8.73.

6-Bromo-2-(pyridin-4-yl)-4*H*-benzo[d][1,3]oxazin-4-one **2**

A mixture of 5-bromo-2-(isonicotinamido)benzoic acid **1** (3.2 g, 0.01 mol) and acetic anhydride (10 ml) was heated under reflux for 5 h. The excess acetic anhydride was distilled off under reduced pressure the residue was triturated with petroleum ether 40–60 and the solid formed was filtered off, dried, and crystallized from ethanol to afford **2** as colorless crystals yield 75 %; m.p. 169–171 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm] = 7.6 (d, 1H); 8–8.7 (m, 6H); Anal. Calcd. For $C_{13}H_7BrN_2O_2$, 51.51; H, 2.33; N, 9.24. Found: C, C, 51.53; H, 2.39; N, 9.27.

3-(4-Acetylphenyl)-6-bromo-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **3**

Method 1

A mixture of *p*-aminoaceophenone (0.01 mol) and 6-bromo-2-(pyridin-4-yl)-4*H*-benzo[d][1,3]oxazin-4-one 2 (0.01 mol) was fused at 150–160 °C in an oil path for 1 h. The mixture was then allowed to cool to ambient temperature, washed with diluted HCl then several times with water. The solid formed was filtered off dried and crystallized from ethanol/H_2O to give **3** in 72 % yield mp: 218–220 °C.

Method 2

A mixture of equimolar amounts 6-bromo-2-(pyridin-4-yl)-4*H*-benzo[d][1,3]oxazin-4-one **2** and *P*-aminoaceophenone (0.01 mol) in dry pyridine (15 ml) was heated under reflux in a sand bath for 8 h. The mixture was then cooled to ambient temperature, acidified with diluted HCl. The solid formed was filtered off, dried and crystallized from ethanol/H_2O to give **3** in 75 % yield; ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm] = 2.50 (s, 3H); 7.28–8.66 (m, 11H); Anal. Calcd. For $C_{21}H_{14}BrN_3O_2$: C, 60.02; H, 3.36; N, 10.00. Found: C, 60.13; H, 3.32; N, 10.12.

General procedure for 6-bromo-2-(pyridin-4-yl)-3-(4-(3-(Aryl/Heteryl)acryloyl) phenyl) quinazolin-4(3*H*)-ones **4–10**

A mixture of 3-(4-acetylphenyl)-6-bromo-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **3** (2.1 g, 0.005 mol) and (0.005 mol) of the appropriate aromatic/heterocyclic aldehyde in 10 % ethanolic sodium hydroxide (20 ml) was stirred at room temperature for 10 h then refluxed for 1 h. The reaction mixture was then poured over crushed ice (100 g), neutralized with diluted HCl. The resulted solid was filtered off, washed with cold water, dried, and crystallized from the suitable solvent (Table 1) to give compounds **4–10**.

(E)-6-Bromo-3-(4-(3-(4-chlorophenyl)acryloyl)phenyl)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **4**

Crystallized from EtOH/H_2O; colorless solid; yield: 76% m.p. 174–76 °C; ^{1}H NMR (DMSO-d6) δ (ppm): 7.36–8.22 (m, 15H); 8.66 (d, 2H, 2,2′-pyridyl protons); C^{13} NMR (DMSO-d6) δ (ppm): 55.3, 114.4, 120.9, 122.2, 122.9, 124.3, 124.9, 127.8, 130.4, 131.6,132.3, 136.0, 136.7, 138.5, 143.5, 145.1, 149.7, 156.2, 159.8, 160.6, 189.7; Anal. Calcd. For $C_{28}H_{17}BrClN_3O_2$; C, 61.96; H, 3.16; N, 7.74. Found: C, 61.93; H, 3.19; N, 7.70.

(E)-6-Bromo-3-(4-(3-(4-nitrophenyl)acryloyl)phenyl)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **5**

Crystalized from EtOH/H_2O; yellowish solid; yield 59 % m.p. 150–151 °C; ^{1}H NMR (DMSO-d6) δ (ppm): 7.36–8.22(m, 15H); 8.66 (d, 2H, 2,2′-pyridyl protons); Anal. Calcd. For $C_{28}H_{17}BrN_4O_4$; C, 60.77; H, 3.10; N, 10.12. Found: 60.75; H, 3.08; N, 10.09.

(E)-6-Bromo-3-(4-(3-(4-fluorophenyl)acryloyl)phenyl)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **6**

Crystalized from EtOH; colorless solid, yield 62 % m.p. 155 °C; ^{1}H NMR (DMSO-d6) δ (ppm): 7.19–8.22(m, 15H); 8.67 (d, 2H, 2,2′-pyridyl protons); Anal. Calcd. For $C_{28}H_{17}BrFN_3O_2$; C, 63.89; H, 3.26; N, 7.98. Found: C, 63.29; H, 3.23; N, 7.93.

(E)-6-Bromo-3-(4-(3-(4-(dimethylamino)phenyl)acryloyl)phenyl)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **7**

Crystalized from MeOH; colorless solid, yield 73 % m.p. 137–139 °C; ^{1}H NMR (DMSO-d6) δ (ppm): 3.04 (s, 6H *N,N*-dimethyl protons); 6.78 (d, 2H); 7.36–8.21(m, 13H); 8.67(d, 2H, 2,2′-pyridyl protons); C13 NMR (DMSO-d6) δ (ppm): 41.5, 110.8, 121.3, 122.5, 123.6, 124.3, 124.9, 125.4, 125.9, 129.7, 131.7, 132.8, 133.1, 133.9, 136.0, 136.7, 143.5, 145.1, 150.1, 151.3, 156.2, 160.5, 189.9; Anal. Calcd. For $C_{30}H_{23}BrN_4O_2$; C, 65.34; H, 4.20; N, 10.16. Found: C, 65.30; H, 4.23; N, 10.13.

(E)-6-bromo-3-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **8**

Crystalized from AcOH/H_2O; pale yellow solid; yield 68 %; m.p. 216–218 °C; ^{1}H NMR (DMSO-d6) δ (ppm): 3.84 (s, 3H *o*-methyl protons); 6.96–8.22 (m, 15H); 8.62(d, 2H, 2,2′-pyridyl protons); Anal. Calcd. For $C_{29}H_{20}BrN_3O_3$; C, 64.69; H, 3.74; N, 7.80. Found: C, 64.64; H, 3.72; N, 7.48.

(E)-6-Bromo-2-(pyridin-4-yl)-3-(4-(3-(thiophen-2-yl)acryloyl)phenyl)quinazolin-4(3*H*)-one **9**

Crystalized from AcOH/H_2O; white solid; yield 53 %; m.p. 212–214 °C; ^{1}H NMR (DMSO-d6) δ (ppm): 7.53–8.25 (m, 14H); 8.68 (d, 2H, 2,2′-pyridyl protons); Anal. Calcd. For $C_{26}H_{16}BrN_3O_2S$; C, 60.71; H, 3.14; N, 8.17. Found: C, 60.86; H, 3.17; N, 8.18.

(E)-6-Bromo-3-(4-(3-(furan-2-yl)acryloyl)phenyl)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **10**

Crystalized from EtOH/H_2O; colorless solid; yield 65 %; m.p. 120–122 °C; ^{1}H NMR (DMSO-d6) δ (ppm): 6.85–8.27 (m, 14H); 8.25 (d, 2H, 2,2′-pyridyl protons); Anal. Calcd. For $C_{26}H_{16}BrN_3O_3$; C, 62.67; H, 3.24; N, 8.43. Found: C, 62.62; H, 3.21; N, 8.39.

6-Bromo-3-(4-(2-bromoacetyl)phenyl)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **11**

To a solution of **3** (4.3 g, 0.01 mol) in acetic acid 25 ml was added bromine in water 8 ml. The reaction mixture was allowed to stir for 5 h at 40 °C. The resultant solid was filtered off, washed with cold ethanol several times, air dried, and crystallized from Ethanol/water to give compound **11** as a colorless solid, yield 75 % m.p. 167–169 °C; ^{1}H NMR (DMSO-d6) δ (ppm): 4.58 (s, 2H); 7.28–8.23 (m, 9H); 8.66 (d, 2H, 2,2′-pyridyl protons); Anal. Calcd. For $C_{21}H_{13}Br_2N_3O_2$; C, 50.53; H, 2.63; N, 8.42. Found: C, 50.56; H, 2.67; N, 8.38.

3-(4-(2-Aminothiazol-4-yl)phenyl)-6-bromo-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **12**

A mixture of **11** (0.005 mol) and thiourea (0.5 g) in acetone 30 ml was refluxed for 10 h. The excess solvent was removed under reduced pressure. The residue was poured into crushed ice (100 g). Sodium hydroxide solution (10 %) was added and the mixture was heated to boiling for few minutes. The mixture was then neutralized with dilute hydrochloric acid. After cooling to ambient temperature the mixture was rendered just alkaline with ammonia solution. The formed precipitate was filtered off, dried, and crystallized from ethanol to give compound **12** as a colorless solid, Yield 79 % m.p. 228–230 °C; ^{1}H NMR (DMSO-d6) δ (ppm): 6.23 (br, 2H NH_2); δ 6.99 (s, 1H; methylene H of the thiazole ring); 7.12–8.66 (m, 11H); C^{13} NMR (DMSO-d6) δ (ppm): 100.3, 121.1, 122.5, 123.4, 124.6, 127.7, 129.2, 130.4, 132.3, 136.4, 143.5, 149.7, 156.2, 160.6, 189.7; Anal. Calcd. For $C_{22}H_{14}BrN_5OS$; C, 55.47; H, 2.96; N, 14.70. Found: C, 55.42; H, 3.02; N, 14.73.

6-Bromo-2-(pyridin-4-yl)-3-(4-(2-thiocyanatoacetyl)phenyl)quinazolin-4(3*H*)-one **13**

A solution of (0.001 mol, 0.5 g) of **12** in 10 ml absolute ethanol was added to a solution of (0.007 mol, 0.87 g) of potassium thiocyante in 5 ml absolute ethanol. The reaction mixture was heated over steam bath for 4 h. the mixture was then allowed to cool to ambient temperature. The formed solid was filtered off, air dried and recrystallized from isopropanol to afford compound 13 in 83 % yield; m.p.: 205–206 °C; ^{1}H NMR (DMSO-d6) δ (ppm): 5.02 (s, 2H); 7.28–8.66 (m, 11H); Anal Calcd. For $C_{22}H_{13}BrN_4O_2S$; C, 55.36; H, 2.75; N, 11.74. Found: C, 55.41; H, 2.72; N, 11.81.

6-Bromo-3-(4-(2-hydroxythiazol-4-yl)phenyl)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **14**

A mixture of (0.001 mol, 0.477 g) of compound 13 in 20 ml of glacial acetic acid and 0.15 ml of conc. Sulfuric acid was heated under reflux for 3 h. during stirring. The reaction mixture was then allowed to cool to ambient temperature, the solid formed solid was filtered off and recrystallized from $EtOH/H_2O$ (1:1) to afford colorless crystals of compound 14 in 68 % yield; m.p.: 261–263°C; ^1H NMR (DMSO-d6) δ (ppm): δ 5.53 (s, 1H; methylene H of the thiazole ring); δ 7.18 (br, 1H OH); δ 7.32–8.66 (m, 11H); C^{13} NMR (DMSO-d6) δ (ppm): 108.9, 121.7, 122.8, 123.4, 124.6, 127.5, 128.6, 129.7, 132.7, 136.4, 143.5, 149.5, 152.3, 155.0, 156.4, 160.4; Anal Calcd. For $C_{22}H_{13}BrN_4O_2S$; C, 55.36; H, 2.75; N, 11.74. Found: C, 55.42; H, 2.68; N, 11.79.

6-Bromo-3-(4-(6-(4-chlorophenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **15**

Alcoholic solution of urea (0.01 mol) in 15 ml absolute ethanol was added to a solution of **4** (0.01 mol) and potassium hydroxide (0.01 mol) in 15 ml absolute ethanol. The reaction mixture was heated under reflux for 15 h during stirring. The mixture was then allowed to cool down to ambient temperature and the excess solvent was evaporated under reduced pressure. The residue was dissolved in 50 ml distilled water and the formed solid was filtered off, dried and crystallized from ethyl alcohol/water mixture 1:1 to produce 13 as yellow crystals; 67 % yield m.p.: 212–213 °C. ^1H NMR (300 MHz, DMSO-d6) δ [ppm] = 2.8–2.9 (dd, dd 2H pyrimidinone ring, J = 12.8, 7.0 Hz); 7.0-8.8 (m, 17H, aromatic protons, overlapped with CH proton of pyrimidinone ring); 10.95 (1H, s, NH); Anal. Calcd. For $C_{29}H_{19}BrClN_5O_2$ C, 59.56; H, 3.27; N, 11.97. Found: C, 59.49; H, 3.22; N, 11.96.

6-Bromo-3-(4-(1-methyl-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **16**

A mixture of (0,82 g, 0.003 mol) of compound **5** and (6.15 ml, 0.006 mol) of methyl hydrazine in 10 ml acetic acid was heated under reflux for 10 h after cooling to ambient temperature the reaction mixture was poured into ice cold water (50 ml). The formed solid was filtered off, extracted with chloroform (75 ml), dried over magnesium sulfate anhydrous. The excess solvent was removed under reduced pressure. The solid residue was collected, crystallized from Methyl alcohol/water mixture 1:1 to produce **16** as yellowish crystals; 62 yield m.p.: 162–163 °C. ^1H NMR (300 MHz, DMSO-d6) δ [ppm] = 2.2 (s, 3H, N–CH_3); 3.1, 3.7 (d, d, 2H CH_2 of the dihydro-1*H*-pyrazol ring); 4.6 (t, 1H, CH of the dihydro-1*H*-pyrazol ring); 7.1–8.5 (m, 15H, aromatic protons). C^{13} NMR (DMSO-d6) δ (ppm): 39.2, 41.1, 60.2, 121.7, 122.6, 123.2, 123.9, 125.7, 129.8, 132.0, 135.4, 136.4, 141.3, 143.5, 146.4, 149.8, 151.6, 157.2, 160.4; Anal. Calcd. For C, 59.91; H, 3.64; N, 14.45. Found C, 59.95; H, 3.60; N, 14.48.

6-Bromo-3-(4-(5-(4-(dimethylamino)phenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **17**

A mixture of **7** (0.003 mol), hydroxylamine hydrochloride (0.35 g, 0.005 mol) and ethanolic sodium hydroxide (0.5 g in 60 ml absolute ethanol) was refluxed for 8 h. The reaction mixture was then cooled to ambient temperature, poured into ice cold water. The formed solid was filtered off, dried, and crystallized from ethanol/water 1:1 to give compound **17**; 58 % yield; m.p.: 167–168 °C. ^1H NMR (300 MHz, DMSO-d6) δ [ppm] = 3.1 (s, 6H); 3.6, 3.8 (d, d, 2H, CH_2 of dihydroisoxazol ring); 5.9 (t, 1H, CH of the dihydroisoxazol ring); 6.8–8.7 (m, 15H, aromatic protons) Anal. Calcd. For $C_{30}H_{24}BrN_5O_2$: C, 63.61; H, 4.27; N, 12.36. Found: C, 63.63; H, 4.29; N, 12.33.

3-(4-(1-Acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl)-6-bromo-2-(pyridin-4-yl)quinazolin-4(3*H*)-one **18**

A mixture of (1.6 g, 0.003 mol) of compound **8** and hydrazine hydrate (0.006 mol) in 10 ml glacial acetic acid was heated under reflux for 8 h. after cooling to ambient temperature the reaction mixture was poured into ice cold water (50 ml). The formed precipitate was filtered off, dried, and crystallized from methanol/water 1:1 to give compound **18** as yellow crystals, yield 59 %, m.p.: 212–214 °C. ^1H NMR (300 MHz, DMSO-d6) δ [ppm] = 2.3 (s, 3H, CO–CH_3); 2.5, 3.0(d, d, 2H, CH_2 of the dihydro-1*H*-pyrazole ring); 3.9 (s, 3H, O–CH_3); 5.5 (dd, 1H, CH of the dihydro-1*H*-pyrazole ring); 7.0–8.5 (m, 15H, aromatic protons); C^{13} NMR (DMSO-d6) δ (ppm): 23.5, 40.1, 55.8, 66.2, 114.1, 121.6, 122.2, 123.0, 124.5, 126.6, 129.6, 132.2, 134.0, 136.6, 143.5, 149.8, 151.6, 156.4, 160.5, 168.3; Anal. Calcd. For $C_{31}H_{24}BrN_5O_3$ C, 62.63; H, 4.07; N, 11.78. Found: C, 62.69; H, 4.10; N, 11.73.

Pharmacological assay

Animals

Adult albino Sprague-Dawely rats (120–150 g) and Swiss mice (20–25 g) of either sex, supplied by the Animal

House Colony of the National Research Centre, Cairo, Egypt were used throughout the experiments.

Animals were kept in raised mesh bottom colony cages to prevent coprophagy; and maintained at 25 ± 2 °C, relative humidity 50–55 %, and under 12:12 h light and dark cycle. They were fed with standard animal feed and water ad libitum. Animals were divided into groups, six animals each in all experiments.

All animal procedures were performed after approval from the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals.

Anti-inflammatory activity

Carrageenan-induced paw edema assay

Paw edema was induced by sub-plantar injection of 100 µl of 1 % sterile carrageenan lambda in saline into the right hind paw of rats (Winter *et al.*, 1962). Contralateral paw received an equal volume of saline. Paw volume was determined immediately before carrageenan injection and at selected times thereafter using a plethysmometer (Ugo Basile, Milan, Italy). The edema component of inflammation was quantified by measuring the paw volume (ml) at zero time (before carrageenan injection) and at 1, 2, 3, and 4 h after carrageenan injection and comparing it with the pre-injection value for each animal. The effect of systemic administration of tested compounds **4**, **5**, **12**, **14**, **15**, and **18** (50 mg/kg, i.p., 0.2 ml, $n = 6$/group) given 30 min before induction of inflammation by sub-plantar carrageenan was studied. The control group of carrageenan-treated rats received an equal volume of saline 30 min before sub-plantar carrageenan injection ($n = 6$ each). A further group administered indomethacin (18 mg/kg, i.p.) served as a positive control. Edema was expressed as a percentage increase in paw volume from control (pre-drug, zero time) values. The % inhibition was calculated for each point in order to compare the potencies of the tested compounds.

Several doses of the tested compounds were used to construct the dose–response curve for each compound by plotting the dose against the % inhibition. The effective dose fifty (ED_{50}) for each one was determined after 4 h from carrageenan injection.

Analgesic activity

Acetic acid-induce writhing in mice

The analgesic investigation was carried out according to the method (Koster *et al.*, 1959). The mice were divided into different groups (six mice each). They were pretreated with the tested compounds **4**, **5**, **12**, **14**, **15**, and **18** (50 mg/kg, s.c.), indomethacin (18 mg/kg, s.c.) and control normal saline (10 ml/kg, s.c.). After 1 h pretreatment interval, an i.p. injection of 0.6 % acetic acid was carried out (1 ml/100 g, i.p.). Each mouse was then placed in an individual clear plastic observational chamber, and 5 min after the administration of acetic acid the total number of writhes (abdominal constrictions) made by each mouse was counted for 30 min. The results of the treatment groups were compared with those of normal saline pre-treated control.

Several doses of the tested compounds were used to construct the dose–response curve for each compound. The protective dose fifty (PD_{50}) for each one was determined.

Statistical analysis

Results expressed as mean ± SEM significance of the difference of the responses to treatment group in comparison to controls was determined by one-way analysis of variance (ANOVA) followed by Tukey test $p < 0.05$ was considered significant.

Molecular modeling studies

All docking studies were performed using 'Internal Coordinate Mechanics (Molsoft ICM 3.4–8C).

Preparation of small molecule

A set of (3*H*)-quinazolin-4-one derivatives synthesized to inhibit cyclooxygenase I was compiled by us using ChemDraw 3D structures were constructed using Chem 3D ultra 12.0 software [Molecular Modeling and Analysis; Cambridge Soft Corporation, USA (2010)], and then they were energetically minimized by using MOPAC (semi-empirical quantum mechanics), Job Type with 100 iterations and minimum RMS gradient of 0.01, and saved as MDL MolFile (*.mol).

Generation of ligand and enzyme structures

The crystal structure of target protein cyclooxygenase (2OYE) is a COX-I complexed with indomethacin was retrieved from the Protein Data Bank (http://www.rcsb.org/pdb/welcome.do). All bound waters ligands and cofactors were removed from the protein.

Docking using Molsoft ICM 3.4-8C program

1- Convert our PDB file into an ICM object: This conversion involves addition of hydrogen bonds, assignment of atoms types, and charges from the residue templates.

2- To perform ICM small molecule docking:
a) Setup docking project:
1) Set project name,
2) Setup the receptor,
3) Review and adjust binding site,
4) Make receptor maps,
b) Start docking simulation:
3- Display the result: ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that include five grid potentials describing interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved (Table 1).

The mode of interaction of the Indomethacin within COX-1 was used as a standard docked model. All inhibitors were compared according to the best binding-free energy (minimum) obtained among all the run.

References

Bekhit AA, Habbib NS, El. Bekhit A (2001) Synthesis and antimicrobial evaluation of chalcone and syndrome derivatives of 4(3*H*)-quinazolinone. Boll Chim Farm 140:297–301

Carter JS (2000) Inhibition of cyclooxygenase-2. Expert Opin Ther Pat 10:1011–1020

Cavasotto CN, Abagyan RA (2004) Protein flexibility in ligand docking and virtual screening to protein kinases. J Mol Biol 337:209–225

Chandrika M, Yakaiah T, Rao ARR, Narsaiah B, Chakra eddy N, Sridhar V, Rao JV (2008) Synthesis of novel 4,6-disubstituted quinazoline derivatives, their anti-inflammatory and anti-cancer activity (cytotoxic) against U937 leukemia cell lines. Ear J Med Chem 43:846–852

Farghaly AO, Moharram AM (1999) Synthesis and in vitro antifungal activity of some *N,N*-disubstituted dithiocarbamic acid esters derived from 2-methylquinazolinones. Boll Chim Farm 138:280–284

Koster R, Anderson M, De Beer EJ (1959) Acetic acid for analgesic screening. Fed Proc 18:412–417

Kroemer RT (2003) Molecular modeling probes: docking and scoring. Biochem Soc 31:980–984

Lasztoczi B, Kovacs R, Nyikos L (2002) A glutamate receptor subtype antagonist inhibits seizures in rat hippocampal slices. NeuroReport 13:351–365

Lopez SE, Rosales ME, Canelon EC, Valverode AE, Narvaez RC, Charris JE, Giannini FA, Enriz RD, Carrasco M, Zacchino S (2000) Synthesis and preliminary cytotoxic and antifungal evaluation of some 6-*N,N*-dialkyl 2-aryl-4(3*H*)-quinazolinone derivatives. Heterocycl Commun 7:473–480

Lopez-Farre A, Rodriguez-Feo JA, Garcia-Colis E (2002) Reduction of the soluble cyclic GMP vasorelaxing system in the vascular wall of stroke-prone spontaneously hypertensive rats: effect of the α1-receptor blocker doxazosin. J Hypertens 20:463

Maggio B, Daidone G, Raffa D (2001) Synthesis and pharmacological study of ethyl 1-methyl-5-(substituted 3,4-dihydro-4-oxoquinazolin-3-yl)-1*H*-pyrazole-4-acetates. Eur J Med Chem 36:737–742

Ochiai T, Ishida R (1981) Effects of afloqualone, a new centrally acting muscle relaxant, on DRL response and CER in rats. Jpn J Pharmacol 78:381–392

Ochiai T, Ishida R (1982) Pharmacological studies on 6-amino-2-fluoromethyl-3-(O-tolyl)-4(3*H*)-quinazolinone (afloqualone), a new centrally acting muscle relaxant. (II) Effects on the spinal reflex potential and the rigidity. Jpn J Pharmacol 32(3):427–438

Perrone MG, Scilimati A, Simone L, Vitale P (2010) Selective COX-1 inhibition: a therapeutic target to be reconsidered. Curr Med Chem 17:3769

Plescia S, Daiolone G, Ceraulo L, Bajardr ML, Reina R (1984) Studies in organic mass spectrometry. IV. Electron impact induced fragmentation of 2-substituted 3-(5-isoxazolyl)-4(3H)-quinazolinones of pharmaceutical interest. Farmaco ED Sci 39:120

Raffa D, Daidone G, Schillaci D, Maggio B, Plescia F (1999) Synthesis of new 3-(3-phenyl-isoxazol-5-yl) or 3-[(3-phenyl-isoxazol-5-yl)-amino] substituted 4(3*H*)-quinazolinone derivatives with antineoplastic activity. Pharmazie 54:251–254

Ram VJ, Srimal RC, Kushwaha DS, Mishra L (1990) Chemotherapeutic agents. XIX. Synthesis of [1,2,4]-triazoloquinazolinones and related compounds as antihypertensive agents. J Prakt Chem 323:629–639

Rotella DP, Sun Z, Zhu Y, Krupinski J, Pongrac R, Seliger L, Normandin D, Macor JE (2000) N-3-substituted imidazoquinazolinones: potent and selective PDE5 inhibitors as potential agents for treatment of erectile dysfunction. J Med Chem 43:1257–1263

Singh P (1978) Study in nitrogen mustards. Part II: synthesis of some 2-Alkyl-3-aryl-4(3H)-quinazolinone derivatives as possible anti-tumour agents. J Ind Chem Soc 55:801–805

Tani J, Yamada Y, Oine T, Ochiai T, Ishida R, Inoue I (1979) Studies on biologically active halogenated compounds. 1. Synthesis and central nervous system depressant activity of 2-(fluoromethyl)-3-aryl-4(3*H*)-quinazolinone derivatives. J Med Chem 22:59–99

Usifoh CO, Scriba GKE (2000) Synthesis and anticonvulsant activity of acetylenic quinazolinone derivatives. Arch Pharm (Weinheim) 333:261–266

Van Ryn J, Trummlitz G, Pairet M (2000) COX-2 selectivity and inflammatory processes. Curr Med Chem 7:1145–1161

Winter CA, Risely EA, Nuss GV (1962) Carrageenan induced edema in hind paw of the rats as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 111:544–547