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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, anti-inflammatory, and structure antioxidant activity relationship of novel 4-quinazoline

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Abstract The practice of medicinal chemistry is devoted to the discovery and development of new agents for treating disease. A new derivative of methyl 2-((*E*)-3-(3,4-dihydroxyphenyl)acrylamido)benzoate **2** was synthesized by reacting the amino group of methyl anthranilate **1** with caffeic acid in the presence of PCl_3. Cyclcondensation of **2** with hydrazine hydrate afforded the corresponding 2,3-dihydro-2-(3,4-dihydroxyphenyl) pyrazolo[5,1-*b*]quinazolin-9(1*H*)-one **3**. The median lethal doses (LD_{50s}) of compounds **2** and **3** in mice were 1,135 and 495 mg/kg b.w., respectively. The anti-inflammatory, reducing power, chelating activity on Fe^{2+}, free radical-scavenging, and total antioxidant activities were more pronounced in compound **2** compared to compound **3**. On the other hand, antipyretic activity was more pronounced in compound **3** compared to compound **2**. Antioxidant activity of compounds **2** and **3** increased with increased concentrations. Total antioxidant activity of compounds **2, 3** and both standards decreased in the order of α-tocopherol > compound **2** > trolox > BHA > BHT > compound **3**. Administration of compounds **2** and **3** orally to the rats at dose of 50, 100, and 150 mg/kg b.w., for 10 days showed non-significant changes in serum level of GOT, GPT, ALP, γ-GT, and LDH as compared with the control group. In addition, oral administration of the compound **2** at a concentration of 100 and 150 mg/kg b.w. and compound **3** at a concentration of 150 mg/kg b.w. daily to normal rats for 10 days showed a significant increase in liver GSH, GPx, GR, and GST activities and significant decrease in TBARS level. But, administration of diclofenac sodium (30 mg/kg b.w.) orally to the rats daily for 10 days to rats showed significant increase in serum SGOT, SGPT, ALP, γ-GT, and LDH and significant decrease in liver GSH, GPx, GR, and GST activities. These findings suggest that compounds **2** and **3** exhibited good antioxidant and anti-inflammatory activity and also showed effects on liver enzymes.

Keywords Quinazolines · Caffeic acid · Antioxidant · Anti-inflammatory · Oxidative stress biomarkers

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain, and fever. In medicinal chemistry, quinazolines have been very well known for their therapeutic applications. Quinazolin-ones with 2,3-substitution are reported to possess significant analgesic, anti-inflammatory (Abdel-Rahman *et al.,* 2003; Chambhare *et al.,* 2003), and anticonvulsant activities (Santagati *et al.,* 1995). Recently, we have documented some lead 2-phenyl-3-substituted quinazolines (Hussein, 2012). Caffeic acid, 3,4-dihydroxycinnamic acid exert beneficial effects on human health through prevention of degenerative pathologies such as cardiovascular diseases and cancer (Bendini *et al.,* 2007; Jiang *et al.,* 2005). Caffeic acid is well known to show antioxidant activity (Toda, 2002).

As an extension of our studies on the synthesis of some new biologically active heterocyclic compounds (Hussein, 2011, 2012), now we wish to report the synthesis of combining form of these two structural features into one molecule might produce compounds with promising antioxidant, anti-inflammatory and antipyretic effects.

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Experimental

Chemistry

Melting points were determined on Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu MR 470 infrared spectrophotometer using the KBr pellets. ^{1}H- and ^{13}C NMR spectra were recorded in DMSO-d_6 and CD_3OD, respectively, on a Varian EM 360 (^{1}H NMR at 240 MHz) and (^{13}C NMR at 75 MHz). The chemical shifts are reported in part per million (δ ppm) downfield from internal tetramethylsilane (TMS). Mass spectra were run using HP Model MS-5988. Microanalytical data (C, H, N) were determined at the Microanalytical Center, Cairo University, Egypt.

Caffeic acid, α-tocopherol, butylated hydroxytoluene(BHT), butylated hydroxyanisole (BHA), and trolox were from Sigma, USA.

*1-Synthesis of methyl 2-((E)-3-(3,4-dihydroxyphenyl)acrylamido)benzoate (**2**)*

To a solution of caffeic acid (0.01 mol) and methyl anthranilate **1** (2.31 g, 0.01 mol) in xylene (50 ml), phosphorus trichloride (3 ml) was added. The reaction mixture was heated under reflux for 3–4 h. The crude product was recrystallized from ethanol to give (**2**). Physicochemical and analytical data are listed in Table 1. IR (KBr, cm^{-1}) **2**: 3332 cm^{-1} (OH), 3133 cm^{-1} (NH), 2962 cm^{-1} (CH-arom.), 2854 cm^{-1} (CH-aliph.), 1715, 1685 (2C=O), 1520 cm^{-1} (C=N). MS (*m/z*) **2**: 313 (M^+, 10.57 %), 276 (10.25 %), 224 (14.29 %), 183 (21.42 %), 137 (25.05 %), 97 (100 %), 55 (46.42 %).^{1}H NMR (DMSO-d_6) 2: 2.4 [s, 3H, $COCH_3$], 6.1–6.2 and 7.3–7.4 [d, 2H, CH=CH, two trans-olefinic protons], 6.7–8.0 [m, 7H, Ar–H], 10.2 [s, 1H, NH]. ^{13}C NMR(75 MHz, CD_3OD) **2**:140.322(C-1), 121.392(C-1′), 123.303(C-2),115.529(C-2′), 130.500(C-3), 145.452(C-3′), 125.521(C-4), 145.702(C-4′), 129.859(C-5), 115.929(C-5′), 114.663(C-6), 119.102(C-6′), 165.021(C-7), 144.63(C-7′), 55.520(C-8), 109.21(C-8′), 199.47(C-9′).

Table 1 Physico-chemical properties and molecular formulae of the synthesized compounds

Compd. no.	mp (°C)	Yield (%)	Mol. formula (Mol. Wt.)	Elemental analyses		
				Calcd./found (%)		
				C	H	N
2	178–180	77	$C_{18}H_{17}NO_5$	65.17	4.79	4.47
			(313)	66.20	4.25	4.15
3	245–247	85	$C_{16}H_{12}N_3O_3$	65.30	4.08	14.28
			(294)	65.75	4.15	14.10

*2-Synthesis of 2,3-dihydro-2-(3′,4′-dihydroxyphenyl) pyrazolo[5,1-b]quinazolin-9(1H)-one (**3**)*

A mixture of methyl 2-((*E*)-3-(3,4-dihydroxyphenyl)acrylamido)benzoate **2** (0.01 mol) and hydrazine hydrate (95 %) (0.05 mol) were dissolved in *n*-butanol (30 ml) and refluxed for 3–5 h. The solvent was concentrated and the residue was recrystallized from ethanol to give **3**. Physicochemical and analytical data are listed in Table 1. IR (KBr, cm^{-1}) **3**: 3500 cm^{-1} (OH), 3146 cm^{-1} (NH), 3041 and 2927 cm^{-1} (CH-arom), 2862 cm^{-1} (CH-aliph.), 1683 (C=O), 1591 cm^{-1} (C=N). MS (*m/z*) **3**: 294 (M^+, 25.11 %), 154 (50.18 %), 103 (46.52 %), 65 (100 %), 55 (46.42 %).^{1}H NMR (DMSO-d_6) **3**: 4.6 [s, 2H, CH_2], 6.6 [s, 1H, CH], 7.3–8.0 [m, 7H, Ar–H], 11.6 [s, 1H, NH]. ^{13}C NMR(75 MHz, CD_3OD) **3**:121.98(C-1′), 146.722 (C-2),115.06(C-2′), 135.72(C-3), 145.10(C-3′), 78.04(C-3″), 147.602(C-4′), 156.225(C-4″), 131.215(C-5), 115.6(C-5′), 127.221(C-6), 119.98(C-6′), 118.214(C-7), 125.568(C-8), 161.515(C-8″), 175.78(C-9).

Biological testing

Animals

Male albino mice weighing around 18–20 g and male Wistar rats weight around 180–200 g were purchased from Faculty of Veterinary Medicine, Cairo University. They were acclimatized to animal house conditions. Animals were provided with standard diet and water adlibrium. Animals were kept under constant environmental condition and observed daily throughout the experimental work.

Anti-inflammatory activity

The anti-inflammatory activity was carried out following the method of Winter *et al.* (1962). Rats (180–200 g) were divided into five different groups each of eight animals. At the beginning, the thickness of the left paw was measured. They were treated orally with the tested compounds, at 50, 100, and 150 mg/kg body weight or diclofenac sodium (30 mg/kg b.w.) as a reference standard. After 30 min of administration, the inflammation was induced by S.C. injection of 0.1 ml of 1 % carrageenan in normal saline. The right hind paw was injected with an equal volume of saline.

The difference in thickness between the two paws gave the swelling induced by formalin. The anti-inflammatory efficacy was estimated by comparing the swelling of the

treated with the control. The difference in thickness was recorded after 30, 60, 90, 120, and 180 min.

Antipyretic activity

Hyperthermia was induced in rats (150–160 g) by s.c. injection of 15 % Brewer's yeast solution at a dose of 20 ml/kg by the method of Loux *et al.* (1972). The body temperature was monitored rectally using a digital thermometer inserted into the rectum for 2 cm distance. The initial temperature was taken just before yeast injection. After elapsing 15 h, the 5 groups of animals, each comprising 8 rats, received oral administration of either acetaminophen (20 mg/100 g) as a reference standard or the tested compounds **2** and **3** of (50, 100 and 150 mg/kg b.w.). The body temperature was recorded at 1, 2, and 3 h after administration.

*Determination of LD_{50} of compounds **2** and **3***

Preliminary experiments were carried out on six main groups (10 mice/each dose/each group). Compounds **2** and **3** were injected in different doses to find out the range of doses which cause 0 and 100 % mortality of animals. A range of doses was determined for each compound.

In group of ten animals each, compound **2** was given i.p. in doses of 650, 800, 1150, 1350, 1500, and 1800 mg/kg b.w. Also, LD_{50} was determined by i.p. injection of compound **3** in different doses 150, 300, 450, 600, 750, and 900 mg/kg b.w. The LD_{50} was evaluated by Spearman and Karber method (Finney 1964) on groups of mice, each of ten animals. The test compounds were administrated i.p. at different doses. The number of animals which died within 24 h was recorded.

The LD_{50} was then calculated by the application of the following formula:

$$LD_{50} = D_m - \frac{\sum (Z\ d)}{n},$$

where D_m is the dose which killed all the mice in the group, Z is half the sum of the dead mice from two successive groups, d is the difference between two successive doses, and n is the number of animals in each group.

Biochemical studies of anti-inflammatory compounds **2** and **3** on liver enzymes in rats

Experimental design

This experiment was carried out to examine the effect of anti-inflammatory compounds, **2** and **3** on liver enzymes at doses of (10, 20 and 30 mg/kg b.w.). A solution of 50, 100, and 150 mg/kg b.w. for each anti-inflammatory compounds, **2** and **3** in dimethyl sulfoxide (DMSO) was prepared for intragastric intubation of rats. Groups of animals each consisting of six rats in each were treated daily for 10 days as follows:

Group A Control (8 rats, was given 1 ml of saline p.o.)
Group B Normal (8 rats, was given 1 ml of DMSO, 1 % p.o.)
Group C 'Test' (consisted of 3 sub groups, 8 rats in each subgroup) received compound **2** (50, 100, and 150 mg/kg orally suspended in DMSO orally in a single daily dose (Lavergne *et al.,* 2005).
Group D 'Test' (consisted of 3 sub groups, 8 rats in each subgroup) received compound **3** (50, 100, and 150 mg/kg orally suspended in DMSO orally in a single daily dose (Lavergne *et al.,* 2005).
Group E Was treated with diclofenac (30 mg/kg b.w.) dissolved in DMSO orally in a single daily dose (Lavergne *et al.,* 2005)

After 10 days of treatment, animals were killed by cervical dislocation, blood samples were withdrawn from the retro-orbital vein of each animal. The separated blood was used for the estimation of SGOT, SGPT, γ-GT, ALP, LDH, and TBARS. Their livers were removed, perfused immediately with ice-cold saline and homogenized in chilled phosphate buffer (0.1 M, pH 7.4) using a Potter–Elvehjem homogenizer. The 10 % of homogenate was used to estimate the activities of the antioxidant enzymes, GSH, GPx, GR, and GST.

Biochemical assays

Serum levels of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvate transaminase (GPT) were carried out by Reitman and Frankel (1975); alkaline phosphatase (ALP) was carried out by King and Armstrong (1988); Gamma glutamyl transferase γ-GT was carried out by Fiala *et al.* (1972); lactate dehydrogenase was carried out by Buhl and Jackson (1978); and TBARS in serum was carried out by Uchiyama and Mihara (1978). Liver Reduced glutathione, glutathione peroxidase (GPx), glutathione reductase, and glutathione-*S*-transferase activities were carried out by Moron *et al.* (1979), Marklund and Marklund (1974), Staal *et al.* (1969), and Habig *et al.* (1974), respectively. Liver total protein was determined according to the method of Lowry *et al.* (1951).

Antioxidant activity

Determination of reducing power

The reducing power of compounds **2** and **3** were measured according to the method of Oyaizu (1986). Various concentrations of compounds **2** and **3** (20–140 μg) in 1 ml of

distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 7.6) and 2.5-ml potassium ferricyanide [$K_3Fe(CN)_6$] (1 %, w/v), and then the mixture was incubated at 50 °C for 30 min. Afterward, 2.5 ml of trichloroacetic acid (10 %, w/v) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. Finally, 2.5 ml of upper-layer solution was mixed with 2.5-ml distilled water and 0.5-ml $FeCl_3$ (0.1 %, w/v), and the absorbance was measured at 700 nm. α- tocopherol, BHA and BHT were used as standard antioxidants. Higher absorbance of the reaction mixture indicated greater reducing power.

Determination of chelating activity on Fe^{2+}

The chelating activity of compounds **2** and **3** on ferrous ions (Fe^{2+}) were measured according to the method of Decker and Welch (1990). Aliquots of 1 ml of different concentrations (0.25, 0.50, 1.0, 1.25, and 1.5 mg/ml) of the samples were mixed with 3.7 ml of deionized water. The mixture was incubated with $FeCl_2$ (2 mM, 0.1 ml) for 5, 10, 30, and 60 min. After incubation, the reaction was initiated by addition of ferrozine (5 mM and 0.2 ml) for 10 min at room temperature, and then the absorbance was measured at 562 nm in a spectrophotometer. A lower absorbance indicates a higher chelating power. The chelating activity of compounds **2** and **3** on Fe^{2+} were compared with that of EDTA at a level of 0.037 mg/ml. Chelating activity was calculated using the following formula:

$$\text{Chelating activity } (\%) = [1 - (\text{Absorbance of sample/Absorbance of control})] \times 100]$$

Control test was performed without addition of compounds **2** and **3.**

Determination of free radical-scavenging activity

The free radical-scavenging activity of compounds **2** and **3** were measured with 1,1-diphenyl-2-picrylhydrazil ($DPPH^{\cdot}$) using the slightly modified methods of Brand-Williams *et al.* (1995) and Takashira and Ohtake (1998). Briefly, 6×10^{-5} mol/l $DPPH^{\bullet}$ solution in ethanol was prepared and 3.9 ml of this solution was added to 0.1 ml of the compounds **2** and **3** (2–6 mg/ml) and trolox solution (0.02–0.06 mg/ml). The mixture was shaken vigorously and the decrease in absorbance at 517 nm was measured at 5, 10, 30, and 60 min. Water (0.1 ml) in place of compounds **2** and **3** were used as control. The percent inhibition activity was calculated using the following equation:

$$\text{Inhibition activity } (\%) = [(A_0 - A_1)/A_0 \times 100],$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of compounds **2** and **3** samples.

Determination of total antioxidant activity

The antioxidant activity was determined according to the thiocyanate method of Osawa and Namiki (1981) with slight modifications. For the stock solution, 10 mg of compounds **2** and **3** was dissolved in 10 ml water. Then, the solution of compounds **2** and **3** or standards samples (tocopherol, trolox, BHA and BHT) [100 mg/l] in 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.6) was added to 2.5 ml of linoleic acid emulsion. Fifty-ml linoleic acid emulsion contained Tween-20 (175 µg), linoleic acid (155 µl), and potassium phosphate buffer (0.04 M, pH 7.6). On the other hand, 5.0 ml of control contained 2.5 ml of linoleic acid emulsion and 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.6). Each solution was then incubated at 37 °C in a glass flask in the dark. At 24 h intervals during incubation, 0.1 ml of this incubation solution was added to 4.7 ml of 75 % (v/v) ethanol and 0.1 ml of 30 % (w/v) ammonium thiocyanate. Precisely, 3 min after 0.1 ml of 0.02 M $FeCl_2$ in 3.5 % (w/v) HCl was added to the reaction mixture, the absorbance of the red color was measured at 500 nm in a spectrophotometer. The solutions without added compounds **2** and **3** or standards were used as the control. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\text{Inhibition } \% = [(A_0 - A_1)/A_0 \times 100],$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of compounds **2** and **3** or standards.

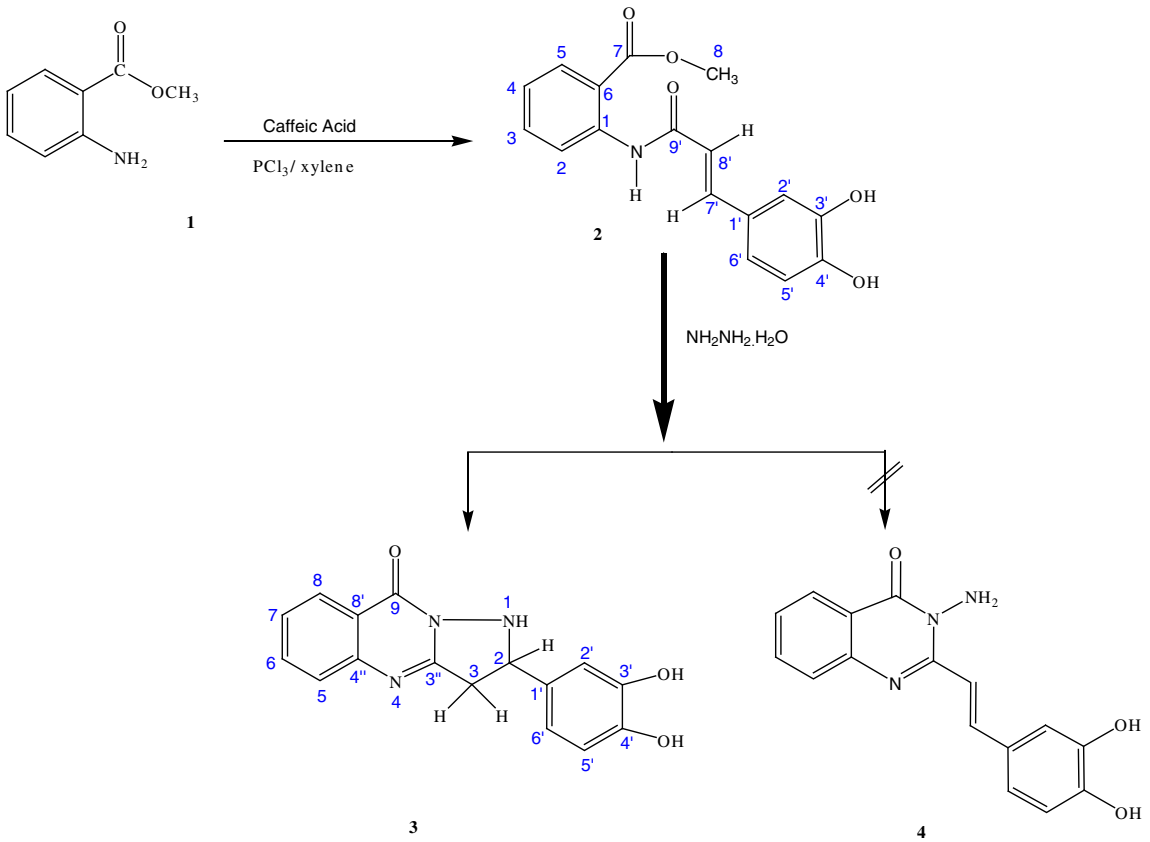
Statistical analysis

All the grouped data were statistically evaluated with SPSS/13 software. Hypothesis testing methods included one way analysis of variance (ANOVA). $P < 0.05$ was considered to indicate statistical significance. All the results were expressed as mean ± SD for eight animals in each group.

Results and discussion

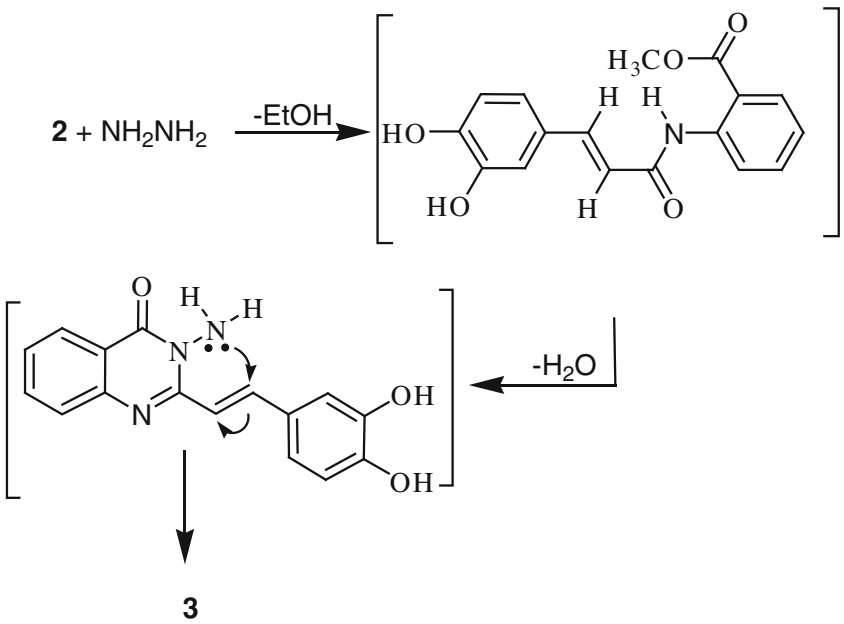
Chemistry

The synthesis of the target compounds methyl 2-((*E*)-3-(3,4-dihydroxyphenyl)acrylamido)benzoate **2** and



Scheme 1 Rationalized formation of compounds **2** and **3**

Scheme 2 Mechanism of compound **3** formation



2,3-dihydro-2-(3,4-dihydroxyphenyl)pyrazolo[5,1-*b*]quinazolin-9(1H)-one **3** were achieved by the route depicted in Scheme 1.

The structures of compounds **2** and **3** were proved on the basis of elemental analyses, IR, mass and ^{1}H NMR spectral data. The IR spectrum of compound **2** showed bands at

Table 2 Anti-inflammatory activity of the different doses of compounds **2** and **3**

Experimental group dose (orally)	Time (in min) and paw diameter (in mm)				
	30	60	90	120	180
Control Group A (untreated)	20.27 ± 5.78	24.61 ± 6.28	27.11 ± 4.22	33.70 ± 4.75	29.16 ± 2.56
Control Group B (distilled water-treated) 3 ml/kg	22.67 ± 5.23	25.66 ± 4.46	30.62 ± 3.75	34.11 ± 4.36	28.52 ± 4.22
Group C compound **2** (mg/kg)					
50	15.26 ± 1.84* (24.72 %)	14.38 ± 1.96* (41.56 %)	12.71 ± 3.85** (53.12 %)	12.21 ± 2.61** (63.77 %)	10.11 ± 3.15** (65.33 %)
100	14.84 ± 2.51* (26.78 %)	13.44 ± 1.82* (45.39 %)	13.75 ± 1.08** (49.28 %)	12.04 ± 2.08** (64.27 %)	8.53 ± 2.28** (70.74 %)
150	12.58 ± 1.08* (37.94 %)	11.63 ± 1.68* (52.74 %)	10.14 ± 2.01** (62.59 %)	8.07 ± 1.27** (76.05 %)	6.14 ± 1.16** (78.94 %)
Group D compound **3** (mg/kg)					
50	20.53 ± 2.45 (1.28 %)	17.7 ± 3.43* (28.08 %)	16.4 ± 0.67* (39.51 %)	15.6 ± 2.84** (53.71 %)	15.46 ± 2.32** (46.98 %)
100	19.34 ± 2.37 (4.58 %)	15.26 ± 1.38* (37.99 %)	14.66 ± 2.60* (45.92 %)	15.26 ± 2.26** (54.72 %)	15.08 ± 2.08** (48.28 %)
150	18.55 ± 1.24 (8.48 %)	16.20 ± 1.33* (34.17 %)	15.12 ± 1.45* (44.22 %)	14.10 ± 2.27** (58.16 %)	13.25 ± 0.2** (54.56 %)
Group E (diclofenac) (mg/kg)					
30	11.89 ± 0.03* (41.34 %)	4.43 ± 0.05*** (81.99 %)	3.72 ± 0.05*** (86.28 %)	2.44 ± 0.04*** (92.76 %)	0.00 ± 0.00*** (100 %)

Diclofenac sodium is used as a reference

Significant at * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$

Table 3 Antipyretic activity of the different doses of compounds **2** and **3**

Experimental group dose (orally)	Initial body temp.	Recorded temperature after (h)		
		1	2	3
Control Group A (untreated)	37.00 ± 2.30	37.00 ± 3.55	37.00 ± 1.70	36.80 ± 1.90
Control Group B (distilled water-treated) 3 ml/kg	38.30 ± 2.5	38.50 ± 1.25	38.70 ± 3.40	38.80 ± 3.50
Group C compound **2** (mg/kg)				
50	38.10 ± 2.80	37.70 ± 3.50	37.00 ± 2.30	36.80 ± 3.10
100	37.70 ± 2.50	37.20 ± 1.90	36.70 ± 3.55	36.80 ± 2.60
150	38.50 ± 3.4	36.90 ± 3.60	36.50 ± 3.10*	36.50 ± 1.95*
Group D compound **3** (mg/kg)				
50	37.60 ± 2.90	37.60 ± 3.25	36.50 ± 2.70	36.10 ± 2.40
100	38.35 ± 2.50	37.80 ± 2.10	36.00 ± 3.00*	36.80 ± 1.35
150	38.45 ± 2.25	37.50 ± 2.30	37.00 ± 2.60	36.60 ± 1.90*
Group E (acetaminophen) (mg/kg)				
20	37.75 ± 1.90	37.00 ± 2.30	36.80 ± 2.30*	36.40 ± 2.45*

Acetaminophen is used as a reference

Significant at * $P < 0.05$; ** $P < 0.01$

3332 cm^{-1} (OH), 3133 cm^{-1} (NH), 2962 cm^{-1} (CH-arom.), 2854 cm^{-1} (CH-aliph.), 1715, 1685 (2C=O), 1520 cm^{-1} (C=N). Mass spectrum of compound **2** exhibited molecular ion peak 313 (M^+, 10.57 %) with base peak at 97 (100 %) and other significant peaks at 276 (10.25 %), 224 (14.29 %), 183 (21.42 %), 137 (25.05 %) and 55 (46.42 %). ^{1}H NMR spectrum of compound **2** in (DMSO-d_6) exhibited signals at 2.4 [s, 3H, $COCH_3$], 6.2 [d, 2H, CH=CH, two trans-olefinic protons], 6.7–8.0 [m, 7H, Ar–H], 10.2 [s, 1H, NH]. Also, ^{13}C NMR spectrum of compound **2** in (CD_3OD) exhibited signals at 144.63(C-7′), 109.21(C-8′) and 199.47(C-9′) indicated that the presence of double bond between C-7′ and C-8′ as well as the presence of (C=O) group at (C-9′).

The formation of compound **3** is assumed to proceed via formation of intermediate (acid hydrazide) followed by

Table 4 Determination of LD_{50} of compound **2** given orally in adult mice

Group number	Dose (mg/kg)	No. of animals/group	No. of dead animals	(*Z*)	(*d*)	(*Z.d*)
1	650	10	0	1.5	150	225
2	800	10	3	4.0	350	1,400
3	1,150	10	5	6.0	200	1,200
4	1,350	10	7	7.5	150	1,125
5	1,500	10	8	9.0	300	2,700
6	1,800	10	10	0	00	00

$$LD_{50} = D_m - \frac{\sum(Zd)}{n}$$
$$LD_{50} = 1800 - \left(\frac{6650}{10}\right) = 1135\ \text{mg/kg b. w.}$$

Table 5 Determination of LD50 of compound **3** given orally in adult mice

Group number	Dose (mg/kg)	No. of animals/group	No. of dead animals	(*Z*)	(*d*)	(*Z.d*)
1	150	10	0	1.0	150	150
2	300	10	2	3.0	150	450
3	450	10	4	5.5	150	825
4	600	10	7	8.0	150	1,200
5	750	10	9	9.5	150	1,425
6	900	10	10	0	00	00

$$LD_{50} = D_m - \frac{\sum(Z \cdot d)}{n}$$
$$LD_{50} = 900 - \left(\frac{4050}{10}\right) = 495\ \text{mg/kg b. w.}$$

nucleophilic addition with elimination of mole of water, to give the intermediate which was cyclized via nucleophilic addition to give compound **3** Scheme 2.

The IR spectrum of compound **3** showed bands at 3500 cm^{-1} (OH), 3146 cm^{-1} (NH), 3041 and 2927 cm^{-1} (CH-arom.), 2862 cm^{-1} (CH-aliph.), 1683 (C=O), 1591 cm^{-1} (C=N). Mass spectrum of compound **3** exhibited molecular ion peak 294 (M^+, 25.11 %) at with base peak at 65 (100 %) and other significant peaks at 154 (50.18 %), 103 (46.52 %),, 55 (46.42 %). ^{1}H NMR spectrum of compound **3** in (DMSO-d_6) exhibited signals at 4.6 [s, 2H, CH_2], 6.6 [s, 1H, CH], 7.3–8.0 [m, 7H, Ar–H], 11.6 [s, 1H, NH]. ^{13}C NMR spectrum of compound **3** in (CD_3OD) exhibited signals at 121.98(C-1′), 146.722(C-2), 115.06(C-2′), 135.72(C-3), 145.10(C-3′) and 78.04(C-3″), and disappear of 144.63(C-7′), 109.21(C-8′) and 199.47(C-9′) signals due to formation of pyrazol ring.

Biological activity

It is well known from the literature that the tested quinazolines exhibit a wide range of biological activities. So, it was of interest to design new compounds containing both these biologically active phenolic moieties and to study their anti-inflammatory activities.

Anti-inflammatory activity

Table 2 listed that compounds **2** and **3** have promising anti-inflammatory activity compared with the reference

Table 6 Levels of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvate transaminase (GPT), alkaline phosphatase (ALP), Gamma glutamyl transferase (γ-GT), lactate dehydrogenase (LDH), and lipid peroxides (TBARS) in serum of normal and experimental groups of rats

Group	SGOT (U/l)	SGPT (U/l)	ALP (U/l)	γ-GT (U/l)	LDH (U/l)	TBARs (nmol/ml)
Group A normal (distilled water-treated) 1 ml/kg	145.50 ± 13.86	38.77 ± 4.25	39.50 ± 3.86	0.57 ± 0.04	249.89 ± 13.26	0.82 ± 0.073
Group B control (1 ml DMSO, 1 %)	146.76 ± 9.11	39.18 ± 5.81	42.26 ± 6.24	0.56 ± 0.026	245.37 ± 10.27	0.81 ± 0.050
Group C compound **2** (mg/kg)						
50	135.62 ± 10.94	42.47 ± 6.38	38.30 ± 5.84	0.53 ± 0.032	240.38 ± 15.07	0.77 ± 0.084
100	133.79 ± 15.26	43.21 ± 4.57	35.64 ± 4.99	0.51 ± 0.046	239.19 ± 13.58	0.75 ± 0.062
150	129.32 ± 14.65	40.84 ± 3.92	31.17 ± 6.24	0.49 ± 0.052	232.48 ± 17.28	0.70 ± 0.054
Group D compound **3** (mg/kg)						
50	139.53 ± 12.36	41.97 ± 5.08	40.77 ± 3.15	0.55 ± 0.0081	246.29 ± 9.16	0.82 ± 0.09250
100	136.34 ± 13.55	36.15 ± 4.36	35.64 ± 5.62	0.51 ± 0.063	243.46 ± 7.08	0.83 ± 0.07450
150	134.55 ± 10.06	33.26 ± 3.84	37.58 ± 4.78	0.50 ± 0.057	238.21 ± 11.65	0.80 ± 0.0650
Group E (diclofenac) (mg/kg)						
30	201.27 ± 9.74*	84.43 ± 4.35**	75.25 ± 6.46**	1.37 ± 0.064*	320.69 ± 15.47*	1.41 ± 0.130*

Compounds **2**, **3** and diclofenac sodium were given orally as a single daily dose for 10 days. Control group was compared to normal group. Experimental groups were compared to control group. Values are given as mean ± SD for groups of eight animals each

* Significantly different from control group at $P < 0.05$

** Significantly different from control group at $P < 0.01$

Table 7 Level of reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-*S*- transferase (GST) activities in liver of normal and experimental groups of rats

Group	GSH	GPx	GR	GST
Group A normal (distilled water-treated) 1 ml/kg	1.84 ± 0.092	47.47 ± 3.86	4.19 ± 0.42	0.86 ± 0.08
Group B control (1 ml DMSO, 1 %)	1.76 ± 0.085	46.83 ± 5.24	4.07 ± 0.35	0.87 ± 0.06
Group C compound **2** (mg/kg)				
50	1.94 ± 0.069	49.11 ± 4.9	4.85 ± 0.28	1.30 ± 0.13
100	2.39 ± 0.17*	53.74 ± 5.17*	5.59 ± 0.19*	1.54 ± 0.19*
150	2.95 ± 0.15*	57.89 ± 4.85*	6.36 ± 0.26*	1.97 ± 0.14*
Group D compound **3** (mg/kg)				
50	1.80 ± 0.18	49.25 ± 4.6	4.33 ± 0.19	0.97 ± 0.09
100	1.85 ± 0.094	51.38 ± 5.22	4.25 ± 0.21	1.15 ± 0.16
150	2.39 ± 0.19*	53.45 ± 4.74*	5.14 ± 0.34*	1.34 ± 0.14*
Group E (diclofenac) (mg/kg)				
30	0.79 ± 0.15*	32.09 ± 4.55*	3.28 ± 0.12*	0.63 ± 0.16*

Compounds **2**, **3** and diclofenac sodium were given orally as a single daily dose for 10 days. Control group was compared to normal group. Experimental groups were compared to control group. Values are given as mean ± SD for groups of eight animals each. GSH is expressed as mg/mg protein, GPx as mmoles of GSH oxidized/min per mg protein, GR as nmoles of NADPH oxidized/min per mg protein, and GST as mmoles of CDNB conjugated/min per mg protein

* Significantly different from control group at $P < 0.05$

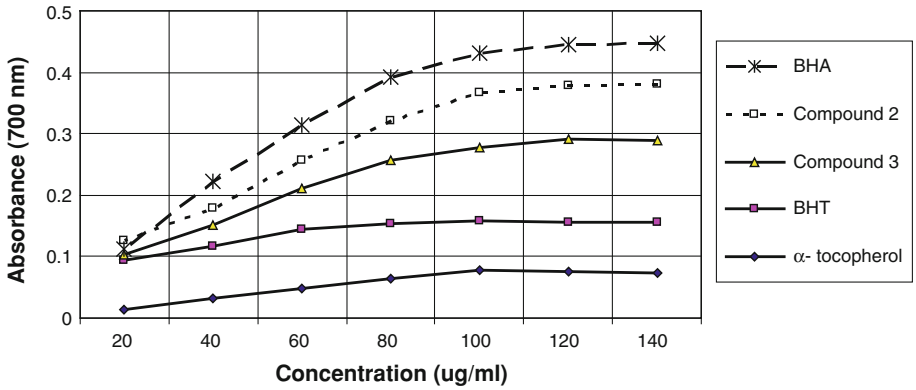


Fig. 1 Reducing power of compounds **2** and **3**, BHA, BHT and α-tocopherol

anti-inflammatory drug, diclofenac sodium. Also, compound **2** more pronounced anti-inflammatory activity than compound **3**.

Antipyretic activity

Table 3 showed that compounds **2** and **3** have good antipyretic activities compared with the reference antipyretic acetaminophen. Also, compound **3** more pronounced anti-inflammatory activity than compound **2**.

*Determination of LD_{50} of compounds **2** and **3** in adult mice*

The results are given in Table 4 shows that i.p. injection of compound **2** in doses of 650, 800, 1150, 1350, 1500, and 1800 mg/kg b.w. resulted in mortalities of 0, 3, 5, 7, 8, and 10 respectively. The dose of compound **2** that killed half of the mice (LD_{50}) was 1130 mg/kg b.w. The results are given in Table 5 shows that i.p. injection of compound **3** in doses of 150, 300, 450, 600, 750, and 900 mg/kg b.w. resulted in mortalities of 0, 2, 4, 7, 9, and 10, respectively. The dose of compound **3** that killed half of the mice (LD_{50}) was 495 mg/kg b.w.

Toxic symptoms Compounds **2** and **3** injected mice exhibited an increase in heart rate, rapid respiration with in 1–2 h. There is a general depression in activity with tremors in hind limbs. The mucous of the eye become brownish in color and the skin and toes bluish. The temperature of the animals extremities dropped with the toes and tail being cool.

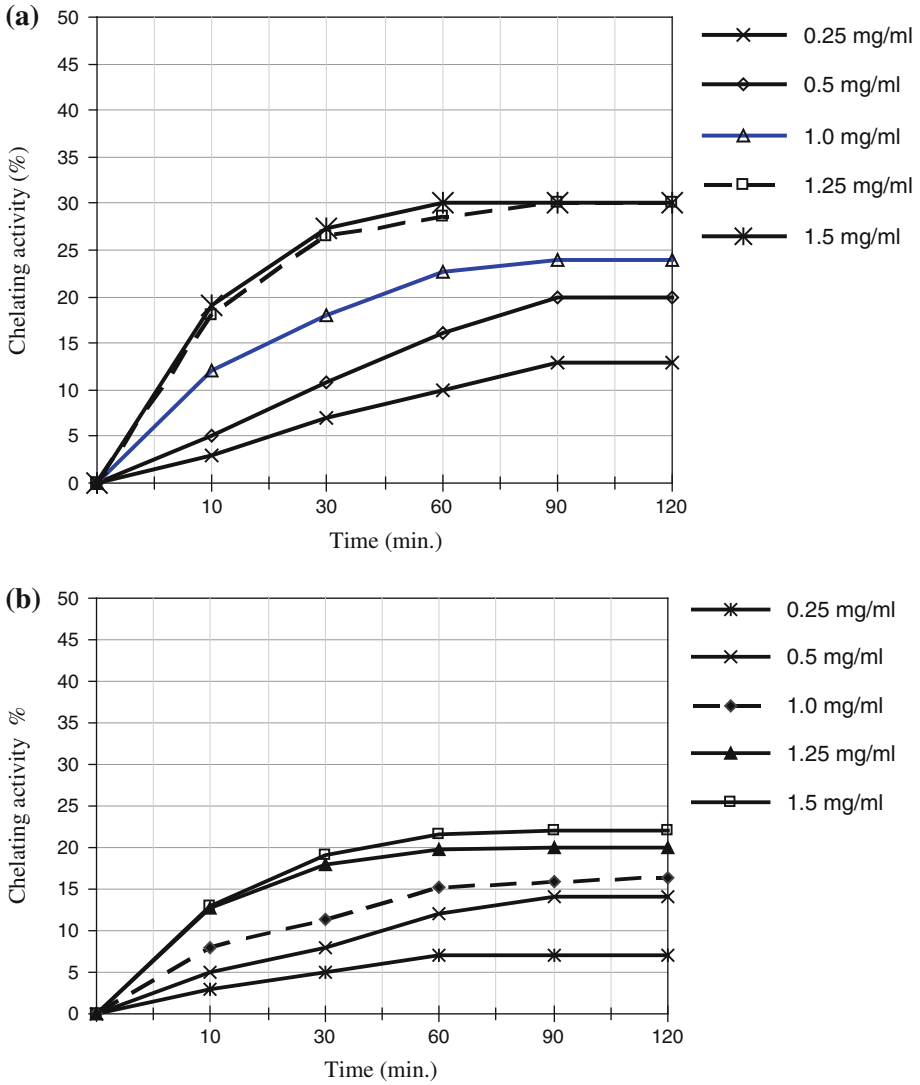


Fig. 2 **a** Chelating effects of different concentrations of compound **2** on Fe^{2+} ions at different incubation times with $FeCl_2$. **b** Chelating effects of different concentrations of compound **3** on Fe^{2+} ions at different incubation times with $FeCl_2$

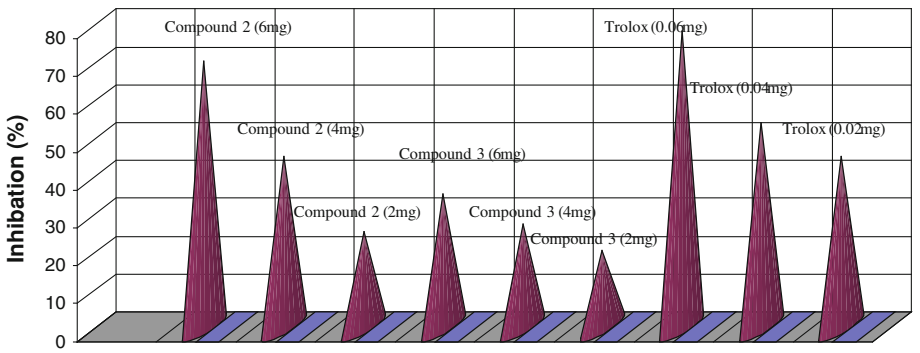


Fig. 3 Scavenging activities of different concentrations of compounds **2** and **3** and trolox against the 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\cdot}$) radical

Biochemical studies of anti-inflammatory compounds **2** and **3**

Administration of compounds **2** and **3** orally to the rats at dose of 50, 100, and 150 mg/kg b.w., for 10 days showed non-significant changes in serum level of GOT, GPT, ALP, γ-GT, LDH, and TBARS as compared with the control group (Table 6). In addition, oral administration of the compound **2** at a concentration of 100 and 150 mg/kg b.w. and compound **3** at a concentration of 150 mg/kg b.w. daily to normal rats for 10 days showed a significant increase in liver GSH, GPx, GR, and GST activities and significant decrease in TBARS level (Table 7). But, administration of diclofenac sodium (30 mg/kg b.w.)

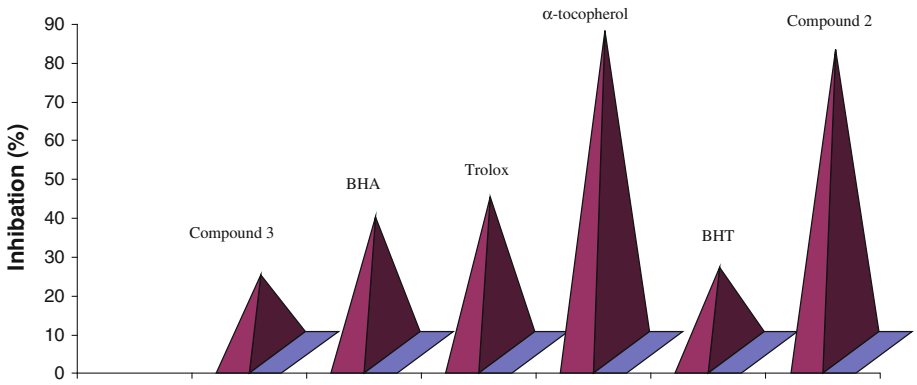


Fig. 4 Total antioxidant activities of compounds **2** and **3**, α-tocopherol, trolox and BHA, BHT (100 mg/l concentration) on peroxidation of linoleic acid emulsion

orally to the rats daily for 10 days to rats showed significant increase in serum SGOT, SGPT, ALP, γ-GT, and LDH and significant decrease in liver GSH, GPx, GR, and GST activities (Tables 6 and 7).

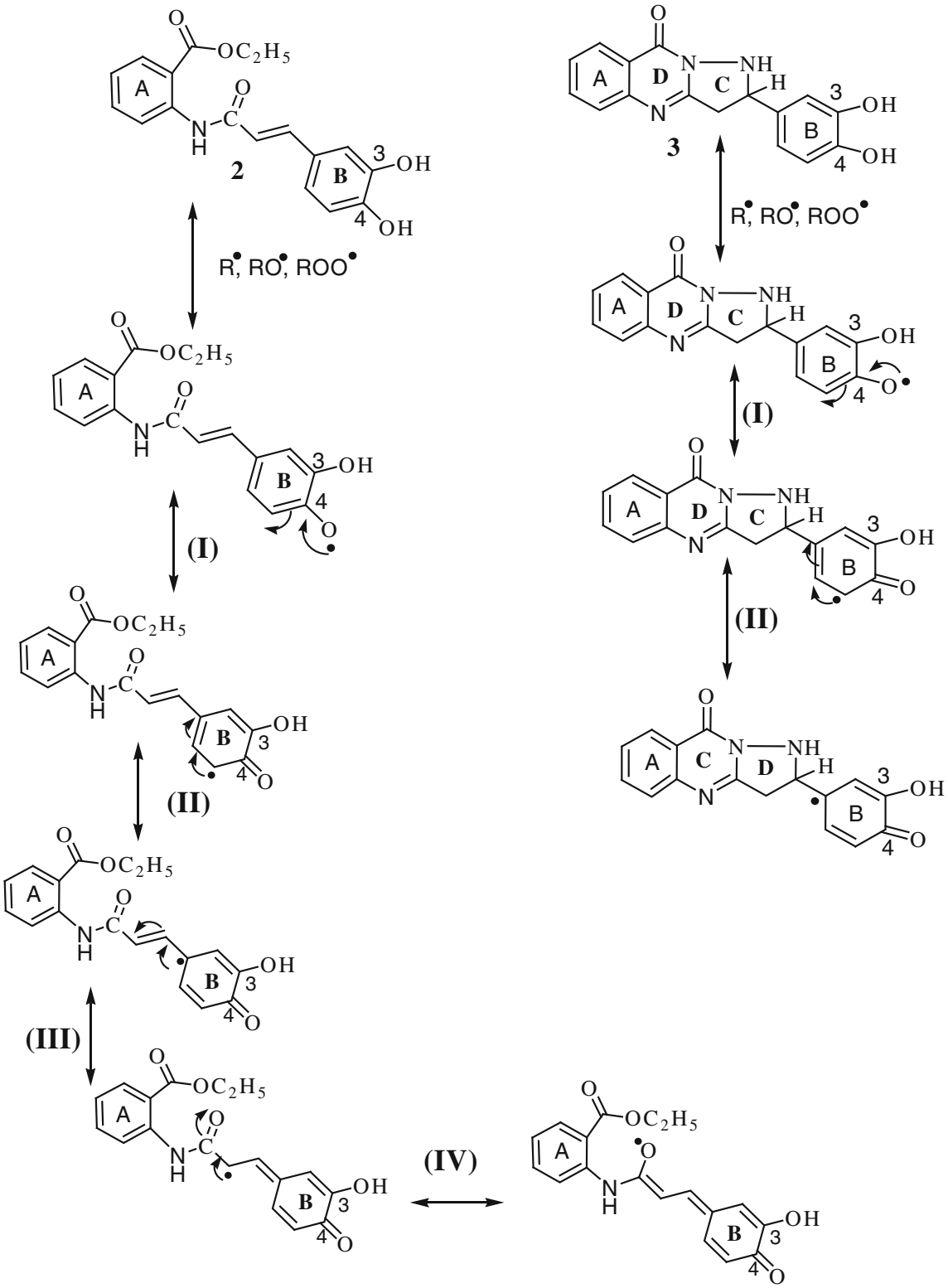
Antioxidant activity of compounds **2** and **3**

Figure 1 shows the reducing power of compounds **2** and **3**. The reducing power of compounds **2** and **3** increased with increasing concentration. Based on a comparison of the absorbance at 700 nm, the reducing power of compounds **2** and **3** at a concentration of 20 μg/ml were nearly similar to that of BHA and BHT, respectively. This indicates that compounds **2** and **3** were electron donors and could also react with free radicals, converting them to more stable products and terminate the radical chain reaction. Also, 120 μg/ml compounds **2** and **3** are the best concentration which exhibits the most reducing power. In the reducing power assay, the presence of reductants (antioxidants) resulted in the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}) Fig. 1. The amount of Fe^{2+} complex can therefore be monitored by measuring the formation of Perl's Prussian Blue at 700 nm^2. The reducing power of compounds **2** and **3** and both standards decreased in the order of BHA > compound **2** > compound **3** > BHT > α-tocopherol.

Figure 2a, b shows the chelating effect of compounds **2** and **3**. All samples at 1.50 mg/ml concentration showed the best chelating effect 30 and 22 % on ferrous ions at an incubation time of 60 min, respectively. The chelating activity of samples increased with increasing incubation times with $FeCl_2$. However, the chelating activity of compounds **2** of 1.25 mg/ml (30 %) was lower than EDTA at 0.037 mg/ml (43.67 %) for an incubation time of 90 min. This indicates that the chelation property of the compounds **2** on Fe^{2+} ions may afford protection against oxidative damage. The results in Fig. 2a, b indicated that a significant property of compounds **2** and **3** is its capability for blocking the oxidative activity of systems with transition metal ion (Fe^{2+}/Fe^{3+}) that play an essential role in the formation of reactive oxygen species in Fenton's reactions.

The DPPH· radical-scavenging effects of compounds **2** and **3** are presented in Fig. 3 and showed appreciable free radical-scavenging activities. The free radical-scavenging activity of compounds **2** and **3** were compared to trolox, as a synthetic antioxidant. Compounds **2** and **3** of 6 mg/ml had the highest radical-scavenging activity when compared with 0.06 mg/ml trolox. The effects of 100 mg/l of compounds **2** and **3** on peroxidation of linoleic acid emulsion are shown in Fig. 4. Compound **2** showed higher antioxidant activity when compared to trolox, BHA, and BHT. Total antioxidant activity of compounds **2** and **3** and both standards decreased in the order of α-tocopherol > compound **2** > trolox > BHA > compound **3** > BHT.

The antioxidant activity of compounds **2** (four resonating structures) more pronounced than compound **3** (two resonating structures) due to the presence of conjugated double bond, which makes the electrons more delocalized from C ring to the carbonyl group (Scheme 3). Free radicals are known to be a major factor in biological damages, and DPPH$^{\bullet}$ has been used to evaluate the free radical-scavenging activity of natural antioxidants (Yokozawa *et al.,* 1998). DPPH$^{\bullet}$, which is a molecule containing a stable free radical with a purple color, changes into a stable compound with a yellow color by reacting with an antioxidant which can donate an electron to DPPH$^{\bullet}$. In such case, the purple color typical of the free DPPH$^{\bullet}$ radical decays, a change which can be followed either spectrophotometrically (517 nm). The proton radical-scavenging action is known as an important mechanism of antioxidation. 1, 1-Diphenyl-2-picrylhydrazil (DPPH$^{\bullet}$) is used as a free radical to evaluate the antioxidative activity of some natural sources (Chung *et al.,* 2005). The DPPH$^{\bullet}$ radical-scavenging effects of compounds **2** and **3** are presented in Fig. 3. From these results, it can be stated that compounds **2** have the ability to scavenge free radicals and could serve as a strong free radical inhibitor or scavenger according to trolox. Many attempts at explaining the structure–activity relationships of some phenolic compounds



Scheme 3 Proposal mechanism of compounds **2** and **3** antioxidant activity

have been reported in the literature. It has been reported that the antioxidant activity of phenolic compounds may result from the neutralization of free radicals initiating oxidation processes, or from the termination of radical chain reactions, due to their hydrogen donating ability (Baumann *et al.,* 1979). It is also known that the antioxidant activity of phenolic

compounds is closely associated with their structures, such as substitutions on the aromatic ring and side chain structure. Their accessibility to the radical center of DPPH$^{\bullet}$ could also influence the order of the antioxidant power. Free radical-scavenging activity of phenolic compounds is believed to be influenced by the number and position of phenolic hydrogen in their molecules (Rice-Evans *et al.,* 1996). It is also proposed that the higher antioxidant activity of compound **2** and **3** is related to the presence of hydroxyl groups (Cao *et al.,* 1997). The structural requirement considered essential for effective radical scavenging by compounds **2** and **3** is the presence of *P*-dihydroxyl groups in B ring and conjugated double bond. The presence of double bond between B ring and carbonyl group in compound **2** makes the electrons more delocalized to form quinone structure which possesses electron donating properties and is a radical target (Hussein and Samir 2010) (Scheme 3).

Synthesis and structure antioxidant activity relationship effect of newly anti-inflammatory quinazolines bearing caffeic acid moiety has not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind.

In conclusion, quinazoline derivatives when combining with caffeic acid exhibited promising antioxidant, anti-inflammatory activity, high LD_{50} value, and more safe on liver enzymes. Also, the present study showed that the effects of antioxidative activity of compound **2** more pronounced than **3** depend on their resonating structures. More studies are needed to prove their medicinal and biological importance which may pave the way for possible therapeutic applications.

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