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Synthesis and Biological Evaluation of Novel Pyrazoline Derivatives as Anti-inflammatory and Antioxidant Agents

Nadia A. Khalil[1], Eman M. Ahmed[1], Hala B. El-Nassan[1], Osama K. Ahmed[2], and Ahmed M. Al-Abd[3]

[1]Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt, [2]Biochemistry Department, Faculty of Agriculture, Cairo University*, Cairo, Egypt, and [3]Department of Pharmacology, Medical Division, National Research Center, Dokki, Cairo, Egypt*

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A series of novel 5-aryl-3-cyclopropyl-4,5-dihydropyrazole derivatives **2a**-**p** were synthesized via cyclization of chalcones **1a**-**h** with thiosemicarbazide or semicarbazide HCl and evaluated as anti-inflammatory/antioxidant agents. The structures were confirmed by elemental analyses and spectral data. The free radical scavenging activity toward superoxide was determined. Their effect on hepatocytes viability and nitric oxide (NO) production in LPS-stimulated macrophages was also determined. The results showed that compounds **2e** and **2n** demonstrated the highest free-radical scavenging and anti-inflammatory activities, thus can be useful in the prevention of oxidative stress and inflammation-related disorders.

Key words: Pyrazoline, Anti-inflammatory, Antioxidant, Free-radical scavenging

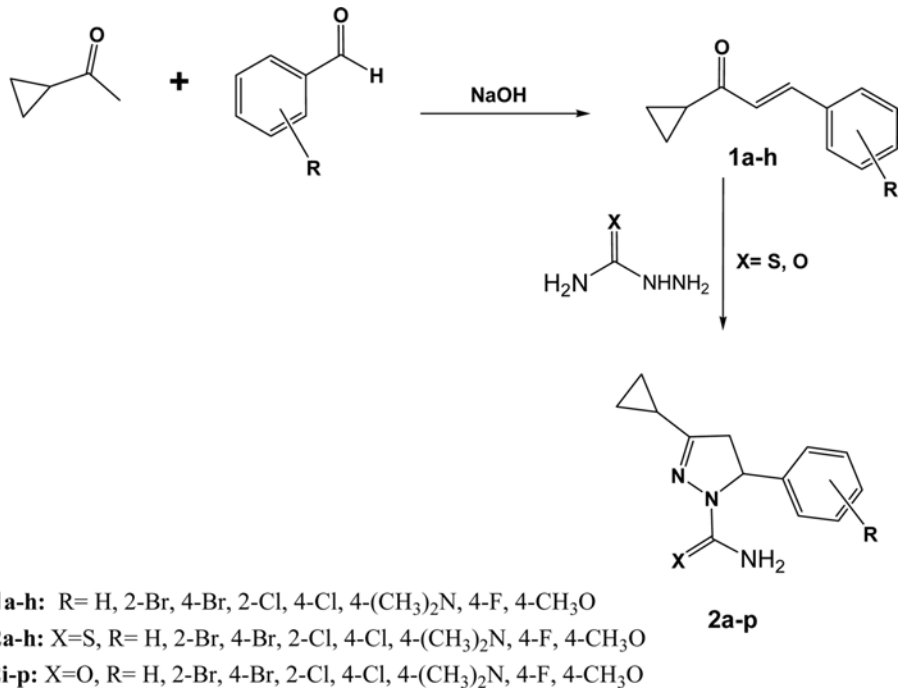
INTRODUCTION

Oxidation and inflammation are two major factors involved in the progression of a wide variety of pathological conditions including cardiovascular diseases (Leopold and Loscalzo, 2009), inflammatory conditions (Halliwell, 1994), atherosclerosis (Cook and Samman, 1996), neurodegeneration, aging (Halliwell, 2001), and cancer (Halliwell et al., 2000). Free radicals, especially reactive oxygen species (ROS) are generated either exogenously from oxidative injuries related to pollution, radiation and food constituents (Foote, 1976) or endogenously in the human body from the metabolic reactions, in addition to oxidative stress (Nohl et al., 2005). Overproduction of these species, such as hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\bullet-}$), nitric oxide ($NO^{\bullet}$), nitrosonium (NO^{+}), and nitroxyl anion (NO^{-}), as well as peroxylnitrite, may contribute to the immunopathological phenomena related to oxidative stress (Awah et al., 2010). Furthermore, it has been established that ROS play an important role in inflammatory conditions by interacting with pro-inflammatory cytokines. The over-produced pro-inflammatory cytokines may lead to inflammation, enhance systemic inflammatory stress and also promote the deterioration of cardiac and/ or renal dysfunctions (Drimal et al., 2008). In this respect, disease progression can be retarded or suppressed by administering protective compounds such as free radical scavengers (Papas, 1999). This had attracted a great deal of research interest in therapeutic antioxidant-based drug formulations. Thus, the development of synthetic compounds capable of scavenging free radicals has been of great importance.

Recently, the Δ^2-pyrazoline derivatives have raised a great interest because of their multiple pharmacological applications such as antioxidant (Dora Carrión et al., 2004; Ohyama et al., 2006), anti-inflammatory (Rathish et al., 2009; Fioravant et al., 2010), antimicrobial (Abdel-Wahab et al., 2009), antidepressant (Kaplanciki et al., 2010), anticancer (Shaharyar et al., 2010), and anti-Alzheimer agents (Gökhan-Kelekci et al., 2007). However, little data have been published on the mechanism of action of pyrazoline derivatives as antioxidant or anti-inflammatory agents (Dora Carrión et al., 2004).

In the present work, a novel series of 5-aryl-3-cyclo-

Correspondence to: Eman M. Ahmed, Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt
Tel: 201005270276
E-mail: dr_eman2001@hotmail.com



Scheme 1. General synthetic pathways of derivatives **2a**-**p**

propyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamides **2a-h** and 5-aryl-3-cyclopropyl-4,5-dihydro-1*H*-pyrazole-1-carboxamides **2i-p** were synthesized and evaluated as anti-inflammatory/ antioxidant agents.

MATERIALS AND METHODS

Chemistry

Melting points were determined on Griffin apparatus and the values given are uncorrected. IR spectra were determined on Shimadzu IR 435 spectrophotometer (KBr, cm^{-1}). ^{1}H-NMR spectra were carried out using a Varian Gemini 200 MHz Spectrophotometer and Varian Mercury-300 (300 MHz) Spectrophotometer using TMS as internal standard. Chemical shift values are recorded in ppm on δ scale, Microanalytical Center, Cairo University, Egypt. Mass spectra were recorded on a GCMP-QP1000 EX Mass spectrometer, Microanalytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Microanalytical Center, Cairo University, Egypt. Progress of the reactions was monitored using TLC sheets precoated with UV fluorescent silica gel Merck 60F 254 using acetone/ benzene (1: 9) and were visualized using UV lamp.

All chemicals were obtained from Aldrich, Fluka, or Merck chemicals.

1-Cyclopropyl-3-phenyl-2-propen-1-ones (**1a-h**) were prepared according to reported procedure (Osman et al., 2003).

General procedure for the synthesis of 5-aryl-3-cyclopropyl-4,5-dihydro-1H-pyrazole-1-carbothioamides (2a-h) and 5-aryl-3-cyclopropyl-4,5-dihydro-1H-pyrazole-1-carboxamides (2i-p)

To a solution of an appropriate chalcone **1a-h** (0.01 mol) and thiosemicarbazide or semicarbazide HCl (0.012 mol) in ethanol (25 mL), was added a solution of sodium hydroxide (1 g, 0.025 mol) in water (5 mL), then the mixture was heated under reflux for 8 h. The products were poured into crushed ice and the separated solid was filtered, dried and crystallized from ethanol.

3-Cyclopropyl-5-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2a)

Yield 85%; mp 142-143ºC; IR (cm^{-1}): 3387, 3259 (NH_2), 3143 (C-H aromatic), 2900, 2850 (C-H aliphatic), 1593 (C=N), 1350 (C=S); ^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm) 0.82-0.90 (m, 4H, $2CH_2$), 1.72-1.88 (m, 1H, CH), 2.39 (dd, 1H, H_a), 3.48 (dd, 1H, H_b, J_{ab} = 18 Hz, J_{bx} = 10.8 Hz), 5.75 (dd, 1H, H_x), 7.04-7.34 (m, 5H, Ar-H), 7.39 (br s, 1H, NH, D_2O exchangeable), 7.73 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{13}H_{15}N_3S$: C, 63.64; H, 6.16; N, 17.13; Found: C, 63.85; H, 6.44; N, 17.05.

5-(2-Bromophenyl)-3-cyclopropyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2b)

Yield 82%; mp 153-155ºC; IR(cm^{-1}): 3437, 3248 (NH_2),

3147 (C-H aromatic), 2950, 2855 (C-H aliphatic), 1593 (C=N), 1361 (C=S); ^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm) 0.84-0.87 (m, 4H, $2CH_2$), 1.73-1.85 (m, 1H, CH), 2.32 (dd, 1H, H_a, J_{ab} = 17.9 Hz, J_{ax} = 3.3 Hz), 3.46 (dd, 1H, H_b, J_{ab} = 17.9 Hz, J_{bx} = 11.4 Hz), 5.88 (dd, 1H, H_x, J_{ax} = 3.3 Hz, J_{bx} = 11.4 Hz), 6.82-8.49 (m, 4H, Ar-H), 7.86 (br s, 1H, NH, D_2O exchangeable), 8.10 (br s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{13}H_{14}BrN_3S$: C, 48.16; H, 4.35; N, 12.96; Found: C, 48.50; H, 4.25; N, 13.05.

5-(4-Bromophenyl)-3-cyclopropyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2c)
Yield 87%; mp 200-201ºC; IR(cm^{-1}): 3379, 3259 (NH_2), 3143 (C-H aromatic), 2955, 2835 (C-H aliphatic), 1593 (C=N), 1363 (C=S); ^{1}H-NMR (300 MHz, DMSO-d_6): δ (ppm) 0.80-0.90 (m, 4H, $2CH_2$), 1.77-1.83 (m, 1H, CH), 2.45 (dd, 1H, H_a, J_{ab} = 18.75 Hz, J_{ax} = 3.3 Hz), 3.45 (dd, 1H, H_b, J_{ab} = 18.75 Hz, J_{bx} = 11.4 Hz), 5.68 (dd, 1H, H_x, J_{ax} = 3.3 Hz, J_{bx} = 11.4 Hz), 7.01 (d, 2H, Ar-H, J = 8.1 Hz), 7.38 (br s, 1H, NH, D_2O exchangeable), 7.48 (d, 2H, Ar-H, J = 8.1 Hz), 7.75 (br s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{13}H_{14}BrN_3S$: C, 48.16; H, 4.35; N, 12.96; Found: C, 48.35; H, 4.58; N, 12.88.

5-(2-Chlorophenyl)-3-cyclopropyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2d)
Yield 72%; mp 148-149ºC; IR (cm^{-1}): 3417, 3250 (NH_2), 3145 (C-H aromatic), 2916, 2850 (C-H aliphatic), 1589 (C=N), 1365 (C=S); ^{1}H-NMR (300 MHz, DMSO-d_6): δ (ppm) 0.78-0.89 (m, 4H, $2CH_2$), 1.76-1.81 (m, 1H, CH), 2.35 (dd, 1H, H_a, J_{ab} = 18 Hz, J_{ax} = 3.3 Hz), 3.46 (dd, 1H, H_b, J_{ab} = 18 Hz, J_{bx} = 11.4 Hz), 5.92 (dd, 1H, H_x, J_{ax} = 3.3 Hz, J_{bx} = 11.4 Hz), 6.86-7.48 (m, 4H, Ar-H), 7.82 (br s, 1H, NH, D_2O exchangeable), 8.10 (br s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{13}H_{14}ClN_3S$: C, 55.81; H, 5.04; N, 15.02; Found: C, 55.65; H, 5.28; N, 14.85.

5-(4-Chlorophenyl)-3-cyclopropyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2e)
Yield 88%; mp 189-190ºC; IR (cm^{-1}): 3381, 3257 (NH_2), 3145 (C-H aromatic), 2900, 2850 (C-H aliphatic), 1593 (C=N), 1363 (C=S); ^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm) 0.82-0.90 (m, 4H, $2CH_2$), 1.72-1.88 (m, 1H, CH), 2.40 (dd, 1H, H_a), 3.47 (dd, 1H, H_b, J_{ab} = 18.5 Hz, J_{bx} = 11.0 Hz), 5.69 (dd, 1H, H_x), 7.05 (d, 2H, Ar-H, J = 8.2 Hz), 7.34 (d, 2H, Ar-H, J = 8.2 Hz), 7.43 (br s, 1H, NH, D_2O exchangeable), 7.78 (br s, 1H, NH, D_2O exchangeable); MS (EI) m/z (% rel. Int.): 281 (M+2, 21.68), 279 (M^+, 66.58), 60 ($CSNH_2$, 100). Anal. Calcd for $C_{13}H_{14}ClN_3S$: C, 55.81; H, 5.04; N, 15.02; Found: C, 55.45; H, 5.25; N, 15.34.

3-Cyclopropyl-5-(4-*N*,*N*-dimethylaminophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2f)
Yield 93%, mp 159-160ºC; IR (cm^{-1}): 3410, 3251 (NH_2), 3147 (C-H aromatic), 2900, 2812 (C-H aliphatic), 1600 (C=N), 1361 (C=S); ^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm) 0.85-0.92 (m, 4H, $2CH_2$), 2.48-2.53 (m, 1H, CH), 2.95 (s, 6H, $2CH_3$), 2.97 (dd, 1H, H_a), 3.47 (dd, 1H, H_b), 6.67 (m, 1H, H_x), 7.47-7.59 (m, 4H, Ar-H), 7.75 (br s, 1H, NH, D_2O exchangeable), 7.98 (br s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{15}H_{20}N_4S$: C, 62.47; H, 6.99; N, 19.43; Found: C, 62.32; H, 7.25; N, 19.75.

3-Cyclopropyl-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2g)
Yield 75%; mp 195-196ºC; IR (cm^{-1}): 3383, 3255 (NH_2), 3143 (C-H aromatic), 2900, 2812 (C-H aliphatic), 1593 (C=N), 1361 (C=S); ^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm) 0.85-0.93 (m, 4H, $2CH_2$), 1.78-1.95 (m, 1H, CH), 2.45 (dd, 1H, H_a, J_{ab} = 18 Hz, J_{ax} = 3 Hz), 3.44 (dd, 1H, H_b, J_{ab} = 18 Hz, J_{bx} = 11.2 Hz), 5.73 (dd, 1H, H_x, J_{ax} = 3 Hz, J_{bx} = 11.2 Hz), 7.12-7.16 (m, 4H, Ar-H), 7.44 (s, 1H, NH, D_2O exchangeable), 7.79 (s, 1H, NH, D_2O exchangeable); MS (EI) m/z (% rel. Int.): 263 (M^+, 1.66), 60 ($CSNH_2$, 100); Anal. Calcd for $C_{13}H_{14}FN_3S$: C, 59.29; H, 5.36; N, 15.96; Found: C, 59.45; H, 5.12; N, 15.78.

3-Cyclopropyl-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (2h)
Yield 89%; mp 164-165ºC; IR (cm^{-1}): 3379, 3251 (NH_2), 3143 (C-H aromatic), 2951, 2831 (C-H aliphatic), 1593 (C=N), 1361 (C=S); ^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm) 1.22-1.38 (m, 4H, $2CH_2$), 2.14-2.16 (m, 1H, CH), 2.89 (dd, 1H, H_a, J_{ab} = 17.9 Hz, J_{ax} = 3 Hz), 3.67 (dd, 1H, H_b, J_{ab} = 17.9 Hz, J_{bx} = 11 Hz), 4.17 (s, 3H, OCH_3), 6.21 (dd, 1H, H_x, J_{ax} = 3 Hz, J_{bx} = 11 Hz), 7.26 (d, 2H, Ar-H, J = 8.4 Hz), 7.45 (d, 2H, Ar-H, J = 8.4 Hz), 7.40 (s, 1H, NH, D_2O exchangeable), 7.79 (s, 1H, NH, D_2O exchangeable); MS (EI) m/z (% rel. Int.): 275 (M^+, 60.82), 56 (100); Anal. Calcd for $C_{14}H_{17}N_3OS$: C, 61.06; H, 6.22; N, 15.26; Found: C, 61.35; H, 6.12; N, 15.55.

3-Cyclopropyl-5-phenyl-4,5-dihydro-1*H*-pyrazole-1-carboxamide (2i)
Yield 84%; mp 184-185ºC; IR (cm^{-1}): 3460, 3282 (NH_2), 3190 (C-H aromatic), 2935, 2862 (C-H aliphatic), 1651 (C=O), 1597 (C=N); ^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm) 0.75-0.83 (m, 4H, $2CH_2$), 1.70-1.83 (m, 1H, CH), 2.43 (dd, 1H, H_a, J_{ab} = 17.9 Hz, J_{ax} = 5.3 Hz), 3.20-3.29 (m, 1H, H_b), 5.18 (dd, 1H, H_x, J_{ax} = 5.3 Hz, J_{bx} = 11.8 Hz), 6.50 (s, 2H, NH_2, D_2O exchangeable), 7.10-7.85 (m, 5H, Ar-H); Anal. Calcd for $C_{13}H_{15}N_3O$: C, 68.10; H, 6.59; N, 18.33; Found: C, 68.35; H, 6.22; N, 18.60.

5-(2-Bromophenyl)-3-cyclopropyl-4,5-dihydro-1*H*-pyrazole-1-carboxamide (2j)
Yield 78%, mp 214-215ºC; IR (KBr): 3471, 3298 (NH_2), 3159 (C-H aromatic), 2931, 2850 (C-H aliphatic), 1654 (C=O), 1600 (C=N); ^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm) 0.72-0.85 (m, 4H, $2CH_2$), 1.72-1.80 (m, 1H, CH), 2.42 (dd, 1H, H_a), 3.15 (dd, 1H, H_b), 5.45 (dd, 1H, H_x), 6.59 (s, 2H, NH_2, D_2O exchangeable), 7.23-8.20 (m, 4H, Ar-H); Anal. Calcd for $C_{13}H_{14}BrN_3O$: C, 50.67; H, 4.58; N, 13.64; Found: C, 50.35; H, 4.70; N, 13.77.

5-(4-Bromophenyl)-3-cyclopropyl-4,5-dihydro-1*H*-pyrazole-1-carboxamide (2k)
Yield 80%, mp 159-160ºC; IR (KBr): 3464, 3437 (NH_2), 3155 (C-H aromatic), 2950, 2855 (C-H aliphatic), 1666 (C=O), 1585 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ (ppm) 0.74-0.82 (m, 4H, $2CH_2$), 1.70-1.82 (m, 1H, CH), 2.43 (dd, 1H, H_a), 3.27 (dd, 1H, H_b), 5.19 (dd, 1H, H_x), 6.49 (s, 2H, NH_2, D_2O exchangeable), 7.06-7.80 (m, 4H, Ar-H); MS (EI) m/z (% rel. Int.): 309 (M+2, 46.34), 307 (M^+, 48.36), 265 (81.30), 263 (78.60); Anal. Calcd for $C_{13}H_{14}BrN_3O$: C, 50.67; H, 4.58; N, 13.64; Found: C, 50.33; H, 4.84; N, 13.42.

5-(2-Chlorophenyl)-3-cyclopropyl-4,5-dihydro-1*H*-pyrazole-1-carboxamide (2l)
Yield 68%; mp 219-220ºC; IR (KBr): 3468, 3278 (NH_2), 3159 (C-H aromatic), 2931, 2854 (C-H aliphatic), 1658 (C=O), 1600 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ (ppm) 0.72-0.81 (m, 4H, $2CH_2$), 1.70-1.75 (m, 1H, CH), 2.36 (dd, 1H, H_a), 3.43 (dd, 1H, H_b), 5.40 (dd, 1H, H_x), 6.54 (s, 2H, NH_2, D_2O exchangeable), 7.73-8.23 (m, 4H, Ar-H); MS (EI) m/z (% rel. Int.): 265 (M+2, 1.29), 263 (M^+, 2.94), 228 (6.70), 219 (6.74); Anal. Calcd for $C_{13}H_{14}ClN_3O$: C, 59.21; H, 5.35; N, 15.93; Found: C, 59.52; H, 5.10; N, 16.15.

5-(4-Chlorophenyl)-3-cyclopropyl-4,5-dihydro-1*H*-pyrazole-1-carboxamide (2m)
Yield 85%; mp 171-172ºC; IR (KBr): 3302, 3276 (NH_2), 3105 (C-H aromatic), 2970, 2850 (C-H aliphatic), 1639 (C=O), 1597 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 0.74-0.94 (m, 4H, $2CH_2$), 1.71-1.80 (m, 1H, CH), 2.51 (dd, 1H, H_a), 3.27 (dd, 1H, H_b), 5.27 (s, 2H, NH_2, D_2O exchangeable), 5.30 (dd, 1H, H_x), 7.12 (d, 2H, Ar-H, J = 8.4 Hz), 7.31 (d, 2H, Ar-H, J = 8.4 Hz); Anal. Calcd for $C_{13}H_{14}ClN_3O$: C, 59.21; H, 5.35; N, 15.93; Found: C, 59.50; H, 5.15; N, 15.85.

3-Cyclopropyl-5-(4-N,N-dimethylaminophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (2n)
Yield 90%, mp 209-210ºC; IR (KBr): 3464, 3290 (NH_2), 3167 (C-H aromatic), 2927, 2854 (C-H aliphatic), 1643 (C=O), 1604(C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ (ppm) 0.85-0.90 (m, 4H, $2CH_2$), 1.77-1.84 (m, 1H, CH), 2.67 (dd, 1H, H_a), 2.93 (s, 6H, $2CH_3$); 3.15 (dd, 1H, H_b), 5.42 (dd, 1H, H_x), 6.28 (s, 2H, NH_2, D_2O exchangeable), 6.67 (dd, 2H, Ar-H), 7.47 (dd, 2H, Ar-H); MS (EI) m/z (% rel. Int.): 272 (M^+, 35.02). Anal. Calcd for $C_{15}H_{20}N_4O$: C, 66.15; H, 7.40; N, 20.57; Found: C, 66.35; H, 7.58; N, 20.22.

3-Cyclopropyl-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (2o)
Yield 72%; mp 214-215ºC; IR (KBr): 3460, 3282 (NH_2), 3163 (C-H aromatic), 2989, 2850 (C-H aliphatic), 1647 (C=O), 1604 (C=N); ^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm) 0.78-0.90 (m, 4H, $2CH_2$), 1.72-1.76 (m, 1H, CH), 2.44 (dd, 1H, H_a), 3.46 (dd, 1H, H_b), 5.20 (dd, 1H, H_x), 6.54 (s, 2H, NH_2, D_2O exchangeable), 7.23 (dd, 2H, Ar-H, J = 8.6 Hz), 7.80 (dd, 2H, Ar-H, J = 8.6 Hz); MS (EI) m/z (% rel. Int.): 247 (M^+, 15.4); Anal. Calcd for $C_{13}H_{14}FN_3O$: C, 63.15; H, 5.71; N, 16.99; Found: C, 63.10; H, 5.55; N, 17.23.

3-Cyclopropyl-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (2p)
Yield 86%; mp 194-195ºC; IR (KBr): 3452, 3282 (NH_2), 3163 (C-H aromatic), 2954, 2858 (C-H aliphatic), 1647 (C=O), 1604 (C=N); ^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm) 0.78-0.90 (m, 4H, $2CH_2$), 1.72-1.76 (m, 1H, CH), 2.62 (dd, 1H, H_a), 3.25 (dd, 1H, H_b), 3.78 (s, 3H, OCH_3), 5.35 (dd, 1H, H_x), 6.41 (s, 2H, NH_2, D_2O exchangeable); 6.96 (dd, 2H, Ar-H, J = 8.8 Hz), 7.67 (dd, 2H, Ar-H, J = 8.8 Hz); MS (EI) m/z (% rel. Int.): 259 (M^+, 28.11), 215 (M-44, 100); Anal. Calcd for $C_{14}H_{17}N_3O_2$: C, 64.85; H, 6.61; N, 16.20; Found: C, 64.50; H, 6.75; N, 16.42.

Pharmacological and biochemical tests

Superoxide radical scavenging assay

The assay was based on the capacity of compounds to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system (Beauchamp and Fridovich, 1971). Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 mg riboflavin, and 12 mM EDTA, 0.1 mg NBT and 1 mL sample solution. The reaction was started by illuminating the reaction mixture with different concentrations of the sample (25-100 mg/mL) for 90 sec. Immediately after illumination, the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminum foil. Identical tubes with the reaction mixture were kept in the dark and served as blanks. The percentage inhibition of superoxide anion generation was calculated. The antioxidant activity of the synthesized com-

pounds was measured in terms of superoxide radical scavenging activity at different concentrations (10, 50 and 100 μg/mL) (Table I).

***In vitro* anti-inflammatory activity**

Macrophage cells were cultured in phenol red free Dulbecco's modified Eagle's medium (DMEM) containing 50 units/mL penicillin, 50 mg/mL streptomycin, 44 mM sodium bicarbonate and 10% fetal bovine serum at 37°C in humidied air containing 5% CO_2. Macrophage cells were plated in 1 mL of the aforementioned media in 24 well plates, cultured for 2 days to approximately 1×10^6 cells/well) then treated with 10 μg/mL LPS and the test compounds at different concentrations (10, 50 and 100 μg/mL). In all cases, cells were washed, and the fresh complete media was added before the indicated treatments. Stock solutions of each compound were prepared in DMSO so that the nal concentration of DMSO did not exceed 0.5%. Six hours after LPS-treatment, the stable end products of L-arginine-dependent NO synthesis, nitrate and nitrite were measured in the cell culture medium using Griess reaction, (Green et al., 1982) (Table II).

Hepatocyte viability assay

Liver cell culture preparation for viability test

The effect of the synthesized compounds on hepatocyte viability were assessed in cultured cells (Moldeus et al., 1978). Cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco, Invitrogen Corporation) with glucose content of 4.5 g/L and supplemented with the following: inactivated fetal calf serum 10% (v/v), non-essential amino acids (1%), glutamine (1%), penicillin (100 U/mL) and streptomycin (10 mg/mL). The medium was adjusted at pH 7.4 and maintained in a humidified atmosphere containing CO_2 (5%) at 37°C. At 70-80% confluence, cells were trypsinized, centrifuged (250 × g for 5 min at 4°C), resuspended in fresh medium and plated in microtiter wells (2×10^4 cells/well). After attachment, they were incubated for 24 h at 37°C in serum-free medium containing different concentrations of the test compounds (10, 50 and 100 μg/mL).

Methylthiazolyl tetrazolium (MTT) assay

The metabolic competence in viable cells was relied on the conversion of yellow MTT to the purple formazan derivative by mitochondrial succinate dehydrogenase (Heras et al., 2001). The incubated cells (2×10^4 cells/well) in serum-free medium were treated with different concentrations of test compounds (10, 50 and 100 μg/mL) for 24 h at 37°C, washed with phosphate buffered saline and incubated in serum-free medium to which MTT (0.5 mg/mL, 100 μL) was added. After incubation for 4 h, the medium was removed and 100 μL of acidic isopropanol (0.08 N HCl) was added to dissolve the formazan crystals. The absorbance was determined spectrophotometrically at 570 nm. Viability was defined as the ratio (expressed as a percentage) of absorbance of treated cells to untreated cells that served as control (Table III, Fig. 1).

RESULTS AND DISCUSSION

As shown in Scheme 1, the intermediate chalcones **1a**-**h** have been synthesized by Claisen-Schmidt condensation of cyclopropyl methyl ketone and the appropriate substituted aromatic aldehydes in ethanol containing sodium hydroxide as a catalyst (Osman et al. 2003). Cyclization of **1a**-**h** with thiosemicarbazide or semicarbazide HCl afforded **2a**-**h** and **2i**-**p**, respectively.

The structures of the new compounds **2a**-**p** were confirmed by elemental analyses and spectral data. The IR spectra of the carbothioamide derivatives **2a**-**h** revealed a forked band in the region of 3437-3248 cm^{-1} corresponding to NH_2 group as well as a band at 1365-1350 cm^{-1} corresponding to C=S group. While, the IR spectra of the carboxamide derivatives **2i**-**p** demonstrated NH_2 forked band at 3471-3276 cm^{-1} and a band in the region of 1666-1639 cm^{-1} corresponding to the carbonyl group. All new compounds, **2a**-**p** displayed absorption bands in the region around 1600 cm^{-1} corresponding to C=N stretching because of ring closure. Moreover, the formation of 2-pyrazoline ring was confirmed by the appearance of ABX system in ^{1}H-NMR due to *geminal-vicinal* coupling between the two unequivalent protons of the methylene group H_a and H_b at C-4 and a methine proton H_x at C-5. The proton H_a which appeared as doublet of doublets around δ 2.32-2.97 ppm is the proton *trans* to H_x and *geminal* to H_b. The *cis* proton, H_b, *vicinal* to H_x appeared as doublet of doublets in the range of δ 3.15-3.48 ppm. The upfield shifted proton around δ 5.18-6.67 ppm of methylene residue has been shown to be coupled with the *vicinal* methine proton (H_x), indicating the presence of *trans*-configuration. Besides, the amino protons appeared as two exchangeable signals at δ 7.38-8.10 ppm in compounds **2a**-**h** and as an exchangeable broad singlet signal at δ 6.28-6.59 ppm in compounds **2i**-**p**. The mass spectrum of compound **2g** showed the molecular ion peak at m/z 263 and the base peak at m/z 60 corresponding to $CSNH_2$. However, the mass spectra of compounds **2h**, **2o** and **2p** displayed molecular ion peaks at m/z 275, 247, and 259, respectively. In addition, the mass spectra of compounds **2k** and **2l**

showed characteristic M+2 peaks. Besides, a peak corresponding to M-$CONH_2$ appeared in the mass spectrum of compounds **2k**, **2l** and **2p**. The synthetic routes for the preparation of the new compounds **2a**-**p** are outlined in Scheme 1.

Biochemical assay

Superoxide radical scavenging activity

Superoxide anions are precursors to active free radicals that have potential of reacting with the biological macromolecules and thereby inducing tissue damage (Halliwell and Gutteridge, 1984). Moreover, superoxide has been observed to initiate lipid peroxidation either directly or through transformation into more reactive species such as hydroxyl radical (Wickens, 2001). Rutin is a flavonoid with strong anti-oxidant activity which has been used herein as positive control anti-oxidant for comparison (Mitrović et al., 2011).

The superoxide-scavenging activity (%) of test compounds was calculated as follows:

$$\text{The superoxide-scabenging activity (\%)} = \frac{\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}} \times 100$$

The results revealed a significant concentration-dependent free radical scavenging activity of all test compounds. Generally, most of the carbothioamide derivatives displayed higher activity than the corresponding carboxamide derivatives may be due to presence of 'S' atom which was reported to act as good radical scavenger (Sankaran et al., 2010). The highest activity was observed with the carbothioamide derivative **2e** and the carboxamide derivative **2n** compared to rutin as a reference standard (Table I). The pyrazoline derivatives exhibited their anti-oxidative behavior in the riboflavin-NBT system assay and no significant effect in the NO-based cell assay. Superoxide-derived damage of NMT signal, would be potentially studied in the future.

In vitro anti-inflammatory activity

The anti-inflammatory activity of the synthesized compounds was studied *in vitro* for their inhibitory effects on chemical mediators release (LPS-induced NO production) from macrophages (Table II). Activated macrophages produce large amounts of chemical mediators that indicate inflammation. Nitric oxide (NO), a bioactive free radical, is one of these critical mediators which is produced by inducible NO synthase (iNOS) in inflamed macrophages when stimulated by lipopolysaccharide (LPS). Excessive production of NO is indicated both in chronic and acute inflammation. In fact, NO production induced by LPS through iNOS induction may reflect the degree of inflammation and provide a measure for assessing the effect of the test compounds on the inflammatory process. The data in Table II represented NO content six hours after LPS-treatment. The stable end products of L-arginine-dependent NO synthesis, nitrate and nitrite were measured in the

Table I. Superoxide radical scavenging activity expressed as % inhibition

Compd No.	Superoxide scavenging activity %		
	0.04 μM	0.2 μM	0.4 μM
Rutin (Standard)	70.40 ± 2.49	90.32 ± 0.68	97.82 ± 0.37
2a	59.20 ± 1.36	80.03 ± 0.77	87.43 ± 0.77
2b	30.06 ± 0.49	50.27 ± 0.54	60.23 ± 0.74
2c	59.04 ± 0.85	81.34 ± 0.45	87.54 ± 0.69
2d	39.92 ± 0.65	57.76 ± 0.34	65.57 ± 0.64
2e	68.26 ± 0.69	90.24 ± 0.74	94.03 ± 0.45
2f	59.26 ± 1.06	81.56 ± 0.35	88.19 ± 0.70
2g	59.90 ± 1.10	83.46 ± 0.60	91.69 ± 0.62
2i	26.55 ± 0.49	48.46 ± 0.49	56.52 ± 0.58
2j	33.11 ± 0.91	57.11 ± 0.16	64.59 ± 0.49
2k	37.40 ± 0.67	59.60 ± 0.41	67.69 ± 0.55
2l	59.75 ± 1.00	81.29 ± 0.49	89.63 ± 0.60
2m	46.08 ± 0.91	67.29 ± 0.65	75.37 ± 0.59
2n	68.23 ± 0.74	87.48 ± 0.62	95.52 ± 0.65
2p	39.56 ± 0.53	58.16 ± 0.49	68.39 ± 0.62

Data are presented as mean ± S.D., n = 3.

Table II. Effect of test compounds on NO content after six hour treatment with LPS

Compd No.	NO content (μM/10^6 cells)		
	0.04 μM	0.2 μM	0.4 μM
Control	5.62 ± 0.08	5.58 ± 0.13	5.58 ± 0.06
LPS (10 μg/mL)	77.49 ± 0.16	73.49 ± 0.65	72.99 ± 0.40
2a	52.18 ± 0.78	45.13 ± 0.12	40.80 ± 0.57
2b	59.44 ± 0.48	52.07 ± 1.05	47.29 ± 0.31
2c	48.76 ± 0.47	40.40 ± 0.43	34.94 ± 0.20
2d	60.41 ± 0.78	56.93 ± 0.41	53.72 ± 0.23
2e	43.28 ± 0.40	39.11 ± 0.55	30.71 ± 0.40
2f	51.28 ± 0.49	44.77 ± 0.11	44.59 ± 0.26
2g	45.57 ± 0.41	37.03 ± 0.11	29.98 ± 0.06
2i	66.64 ± 0.47	55.37 ± 0.38	51.54 ± 0.36
2j	64.38 ± 0.84	54.45 ± 0.44	50.53 ± 0.43
2k	58.50 ± 0.33	47.43 ± 0.43	48.51 ± 0.33
2l	52.53 ± 0.60	46.69 ± 0.50	45.00 ± 0.82
2m	55.47 ± 1.07	49.68 ± 0.35	44.54 ± 0.38
2n	40.40 ± 0.94	36.15 ± 0.37	29.65 ± 0.29
2p	62.42 ± 0.93	54.66 ± 0.37	50.39 ± 0.31

Data are presented as mean ± S.D., n = 3.

cell culture medium of different compounds. All test compounds exhibited reduction of NO level. The lowest NO content was observed with the carbothioamide derivatives **2e**, **2g** and the carboxamide derivative **2n** compared to other compounds.

Hepatocyte viability assay

The effect of the synthesized compounds on hepatocyte viability was assessed in cultured cells using methylthiazolyl tetrazolium (MTT) assay (Heras et al., 2001). Compound **2d** displayed the lowest toxicity on cell viability in all tested doses. However, compounds **2e** and **2n** that have shown the most potent superoxide scavenging activity and the lowest NO content, were found to exhibit moderate toxicity on hepatocyte viability. On the other hand, the highest toxicity on cell viability, was observed with compounds **2i** at a concentration of 10 μg/mL, **2l** at a concentration of 50 μg/mL and **2a** at a concentration of 100 μg/mL (Table III).

In summary, *in vitro* assays revealed that the test compounds exhibited a significant concentration-dependent free radical scavenging activity as well as significant reduction of nitric oxide level. The carbothioamide derivatives showed higher radical scavenging activity than the corresponding carboxamide derivatives especially compounds **2e** and **2n**, which contain chloro or N,N-dimethyl substituents in the para position of the phenyl ring. Liver and kidney toxicity tests of the most active compounds, **2e** and **2n**, showed that these compounds have no significant toxicity on liver and kidney tissues. The results reflected the safe usage of these compounds at the recommended doses against the control rats. Further data will be published on the activity and mode of action of compounds **2e** and **2n** as antioxidant and anti-inflammatory agents.

Table III. Effect of the test compounds on liver cell viability

Compd No.	Liver cell viability (%)		
	0.04 μM	0.2 μM	0.4 μM
Control	100	100	100
2a	90.22	88.17	76.62
2b	93.55	90.55	82.18
2c	92.55	88.94	80.54
2d	95.34	92.51	87.54
2e	91.35	88.07	78.51
2f	93.87	89.65	82.50
2g	94.86	91.73	83.54
2i	90.08	87.92	78.51
2j	94.21	92.56	79.95
2k	91.23	87.08	80.95
2l	90.97	86.85	80.77
2m	94.21	92.62	84.64
2n	91.50	86.92	81.66
2p	94.87	91.00	84.34

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