Potent anti-inflammatory and analgesic activities of new derivatives of chalcone, pyridine, pyrazole, and isoxazole incorporated into 5,6,7,8-tetrahydronaphthalene

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Objective
Synthesis of new series of 5,6,7,8-tetrahydronaphthalene derivatives conjugated with chalcone, pyridine, pyrazole and isoxazole functionalities hoping to circumvent the unwanted ulcerogenic and other side effects of the already used nonsteroidal anti-inflammatory drugs.

Background
Most currently used nonsteroidal anti-inflammatory drugs (NSAIDs) suffer from limitation in their therapeutic uses, since they cause gastrointestinal and renal side effects related to inhibition of cyclooxygenase1 (Cox1) in tissues where prostaglandins exert physiological effects.

Methodology
Reaction of 2-acetyl tetralin (**1**) with some aromatic aldehydes in the presence of malononitrile yielded 2-amino-3-cyanopyridine derivatives **2a–c**. Condensation of compound **1** with aromatic aldehydes afforded the chalcone derivatives **3a–c**. Then, compound **3a** reacted with hydrazine hydrate or phenyl hydrazine and yielded pyrazoline derivatives **4** or **5**, respectively. Also, the reaction of compound **3c** with hydroxylamine hydrochloride afforded the isoxazole derivative **6**. Anti-inflammatory properties of the synthesized compounds were evaluated in vivo utilizing formalin induced paw edema method in rats, analgesic activities were tested via both hot plate and writhing methods.

Results
Derivatives **2c** and **3c** revealed promising results when the anti-inflammatory, analgesic, and ulcerogenic activities of the synthesized compounds were evaluated. All of the compounds induced significant central and peripheral analgesia. The derivatives **2a**, **2c**, **3a**, **3b**, **3c**, **5**, and **6** showed higher activity than the standard ibuprofen.

Keywords:
analgesic activity, anti-inflammatory, isoxazole, pyrazoline, pyridine, tetralin

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Introduction

Importance of the pyridine ring in the chemistry of biological systems has been acknowledged because of its presence in many natural products of therapeutic importance that are involved in the oxidation–reduction process. The potent biological activity of various vitamins and drugs [1–4] is primarily ascribed to the presence of the pyridine ring in their molecular makeup. In contrast, cyanopyridine derivatives have promising antimicrobial [5,6], anticancer [7,8], anti-inflammatory, analgesic, antipyretic [9], and colon tumor cell growth inhibitory [10] activities. Recently, some new heterocyclic compounds containing pyridine moiety were reported as anticancer and anti-inflammatory agents [11,12].

α,β-Unsaturated ketones are useful key intermediates [13,14] bearing the well-known chalcone pharmacophore. Chalcones can be isolated from several plants and are precursors of flavones and anthocyan compounds. Some of them exhibit antioxidant and anticancer properties. In fact, the pharmacological properties of chalcones are due to the presence of both α,β-unsaturation [15] and an aromatic ring. The pyrazole unit is the core structure in a number of natural products [16]. Many pyrazole derivatives are known to exhibit a wide range of biological properties such as antihyperglycemic, analgesic, anti-inflammatory, antipyretic, antibacterial, hypoglycemic, sedative-hypnotic [17,18], and anticoagulant [19] activities. In particular, pyrazoles are important in medicinal chemistry [20] and were reported to have non-nucleoside HIV-1 reverse transcriptase inhibitor [21] and antimicrobial activities [22]. It was also reported that 5-substituted pyrazoles are presently undergoing pharmacological study as antagonists of cannabinoid receptors 1. It was proved that they are useful for the treatment of obesity [23]. Moreover, many pyrazole derivatives have been reported

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as adenosine receptor antagonists having high affinity and selectivity [24]. Furthermore, isoxazoles possess analgesic and anti-inflammatory activities [25].

In view of the above-mentioned facts and in continuation of our search for various biologically active molecules [26–29], we report here the synthesis of some new 3-cyano pyridine, chalcone, pyrazole, and isoxazole derivatives in addition to evaluation of their preliminary anti-inflammatory and analgesic activities.

Subjects and methods

Chemistry

All melting points were uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were obtained using a Vario El-Mentar apparatus (Shimadzu) at the National Research Centre, Cairo, Egypt. Infrared (IR) spectra were recorded using a Biorad FTS 155 FT-IR spectrophotometer (ICB-IR Service Centre, Pozzuoli, Naples, Italy) and recorded as potassium bromide pellets on a Perkin-Elmer 1650 Spectrophotometer at the National Research Centre (Cairo, Egypt). ^{1}H NMR experiments were conducted in DMSO at the ICB-NMR Service Centre (Pozzuoli, Naples, Italy), and shifts were referenced to TMS on a Bruker Avance-400 operating at 400 MHz. ^{1}H NMR spectra were determined in DMSO-d_6 at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR) and determined on a JEOL-Ex-300 NMR spectrometer. Chemical shifts were expressed as parts per million (ppm) (δ values) against TMS as an internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were obtained using an ion-trap MS instrument in electron impact mode at 70 eV (ICB-IR Service Centre) and determined on a Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV (Cairo University, Cairo, Egypt). Compound **1** was prepared according to a method reported previously [30].

General procedure for the preparation of compounds (2a–c)

A mixture of compound **1** (1.74 g, 0.01 mol), the appropriate aldehyde (2-chloro-5-nitrobenzaldehyde, 2-naphthaldehyde, or *p*-isopropyl benzaldehyde) (0.01 mol), malononitrile (0.66 g, 0.01 mol), and ammonium acetate (6.16 g, 0.08 mol) in *n*-butanol (30 ml) was refluxed for 6 h. The reaction mixture was concentrated, allowed to cool, and the separated product was filtered off, washed several times with diethyl ether, and recrystallized from the proper solvent to yield compounds **2a–c**, respectively.

*2-Amino-4-(2-chloro-5-nitrophenyl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)pyridine-3-carbonitrile (**2a**)*

Yield (74%); m.p. 250–252°C (EtOH/DMF); IR spectrum (KBr, ν, cm^{-1}): 3354, 3227 (NH_2), 2930 (CH, alicyclic), 2217 (CN), 1632 (C = N), 1526, 1345 (NO_2); ^{1}H NMR (DMSO-d_6, δppm): 1.73 (m, 4H, 2CH_2 of tetrahydronaphthalene), 2.74 (m, 4H, 2CH_2 of tetrahydronaphthalene), 6.94 (s, 2H, NH_2, exchangeable with D_2O), 7.15 (d, J = 8.4 Hz, 1H, Ar–H), 7.25 (s, 1H, Ar–H), 7.80 (d, J = 7.6 Hz, 1H, Ar–H), 7.93–7.97 (m, 2H, Ar–H), 8.34–8.37 (m, 2H, Ar–H); ^{13}C NMR (DMSO-d_6): δ (ppm): 22.52, 22.62, 28.70, 28.80 (4CH_2), 127.70 (CN), 109.19, 115.93, 120.75, 124.38, 125.46, 129.18, 131.17, 134.28, 136.90, 137.41, 138.53, 139.40, 146.25, 150.50, 159.17 (aromatic-C). MS *m/z* (%): 404 (M^+, 100), 406 (35.35); Anal. calcd (%) for $C_{22}H_{17}ClN_4O_2$ (404.85): required C, 65.27; H, 4.23; N, 13.84; found C, 65.05; H, 4.43; N, 13.65.

*2-Amino-4-(naphthalen-2-yl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)pyridine-3-carbonitrile (**2b**)*

Yield (78%); m.p. 200–202°C ($CHCl_3$); IR spectrum (KBr, ν, cm^{-1}): 3361, 3212 (NH_2), 2932 (CH, alicyclic), 2202 (CN); ^{1}H NMR (DMSO-d_6, δppm): 1.75 (m, 4H, 2CH_2 of tetrahydronaphthalene), 2.77 (m, 4H, 2CH_2 of tetrahydronaphthalene), 6.92 (s, 2H, NH_2, exchangeable with D_2O), 7.12–8.08 (m, 11H, Ar–H); ^{13}C NMR (DMSO-d_6): δ (ppm): 22.5, 29.5 (4CH_2-tetralin), 113.7 (CN), 109.2, 114.7, 122.5, 125.6, 126.2, 127.7, 128.3, 128.4, 129.1, 129.4, 133.1, 134.4, 135.3, 135.7, 136.2, 154.4, 156.2, 161.9 (aromatic-C). MS *m/z* (%): 375 (M^+, 42), 368 (80), 128 (100); Anal. calcd (%) for $C_{26}H_{21}N_3$ (375.17): required C, 83.17; H, 5.64; N, 11.19; found C, 83.39; H, 5.44; N, 11.02.

*2-Amino-4-(4-isopropylphenyl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)pyridine-3-carbonitrile (**2c**)*

Yield (80%); m.p. 210–212°C (EtOH); IR spectrum (KBr, ν, cm^{-1}): 3324, 3194 (NH_2), 2935 (CH, alicyclic), 2207 (CN); ^{1}H NMR (DMSO-d_6, δppm): 1.23 (d, J = 6.3 Hz, 6H, 2CH_3), 1.74 (m, 4H, 2CH_2 of tetrahydronaphthalene), 2.76 (m, 4H, 2CH_2 of tetrahydronaphthalene), 2.98 (m, 1H, CH), 6.74 (s, 2H, NH_2, exchangeable with D_2O); 6.92–8.28 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6): δ (ppm): 22.5, 29.5 (4CH_2-tetralin), 113.7 (CN), 109.2, 111.4, 114.7, 119.4, 121.4, 121.9, 122.5, 128.4, 128.4, 128.6, 129.1, 129.4, 135.3, 135.5, 136.2, 136.6, 154.4, 156.2, 161.9 (aromatic-C). MS *m/z* (%): 367 (M^+, 100); Anal. calcd (%) for $C_{25}H_{25}N_3$ (367.49): required C, 81.71; H, 6.86; N, 11.43; found C, 81.64; H, 6.63; N, 11.29.

General procedure for the preparation of compounds (3a, b)

To a mixture of compound **1** (4.9 g, 0.028 mol) and 2-chloro-5-nitrobenzaldehyde or 2-naphthaldehyde (0.028 mol) in ethanol (30 ml) was added NaOH solution (15 ml, 30%) dropwise within 15 min. The reaction mixture was stirred for 3 h and left overnight at room temperature. The formed solid was collected and recrystallized from ethanol to yield compounds **3a, b**, respectively.

*(E)3-(2-Chloro-5-nitrophenyl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)prop-2-en-1-one (**3a**)*

Yield (69%); m.p. 118–120°C; IR spectrum (KBr, ν, cm^{-1}): 2934 (CH, alicyclic), 1666 (C = O), 1524, 1347 (NO_2); ^{1}H NMR (DMSO-d_6, δppm): 1.78 (m, 4H, 2CH_2 of tetrahydronaphthalene), 2.80 (m, 4H, 2CH_2 of tetrahydronaphthalene), 7.23 (d, J = 7.8 Hz, 1H, Ar–H), 7.84 (d, J = 8.7 Hz, 1H, COCH =), 7.90–7.97 (m, 3H, Ar–H), 8.15–8.25 (m, 2H, Ar–H, = CH), 8.9 (d, J = 2.7 Hz, 1H, Ar–H); ^{13}C NMR (DMSO-d_6): δ

(ppm): 22.5, 29.5 ($4CH_2$-tetralin), 121.5, 121.6, 122.5, 124.1, 128.2, 128.4, 129.1, 129.4, 132.1, 135.3, 136.2, 139.1, 145.4, 148.3 (aromatic-C), 189.0 (CO). MS *m/z* (%): 341 (M^+, 40), 343 (13), 159 (50), 136 (100); Anal. calcd (%) for $C_{19}H_{16}ClNO_3$ (341.79): required C, 66.77; H, 4.72; N, 4.10; found C, 66.96; H, 4.54; N, 4.31.

*(E)-3-(Naphthalen-2-yl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)prop-2-en-1-one (**3b**)*

Yield (76%); m.p. 154–156°C; IR spectrum (KBr, *v*, cm^{-1}): 2942 (CH, alicyclic), 1651 (C = O), 1606 (C = C); ^1H NMR (DMSO-d_6, δppm): 1.78 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 2.81 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 7.25 (d, *J* = 7.8 Hz, 1H, Ar–H), 7.56–8.32 (m, 11H, 9Ar–H, COCH = , = CH); ^{13}C NMR (DMSO-d_6): δ (ppm): 22.5, 29.5 ($4CH_2$-tetralin), 121.2, 122.5, 125.6, 126.2, 127.7, 128.4, 128.6, 129.1, 129.4, 133.1, 134.4, 135.3, 135.7, 136.2, 145.4 (aromatic-C), 189.0 (CO). MS *m/z* (%): 312 (M^+, 100); Anal. calcd (%) for $C_{23}H_{20}O$ (312.4): required C, 88.43; H, 6.45; found C, 88.25; H, 6.24.

*(E)-3-(1-H-Indol-3-yl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl) prop-2-en-1-one (**3c**)*

A mixture of compound **1** (1.74 g, 0.01 mol) and 3-formyl indole (1.452 g, 0.01 mol) was dissolved in ethylene glycol (10 ml) containing piperidine (0.5 ml). The solution was then heated at 175–180°C for 10 min. After cooling, 5 ml of water and 0.5 ml of acetic acid were added. The crystals that deposited were filtered off and recrystallized from ethanol to yield compound **3c**. Yield (74%); m.p. 208–210°C; IR spectrum (KBr, *v*, cm^{-1}): 3218 (NH), 2934 (CH, alicyclic), 1657 (C = O); ^1H NMR (DMSO-d_6, δppm): 1.76 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 2.78 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 6.94–8.07 (m, 10H, 8Ar–H, = CH, COCH =), 11.69 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6): δ (ppm): 22.5, 29.5 ($4CH_2$-tetralin), 111.4, 119.4, 121.2, 121.4, 121.9, 122.5, 128.4, 128.6, 128.9, 129.1, 129.4, 135.5, 135.6, 136.2, 145.4 (aromatic-C), 189.0 (CO). MS *m/z* (%): 301 (M^+, 100); Anal. calcd (%) for $C_{21}H_{19}NO$ (301.15): required C, 83.69; H, 6.35; N, 4.65; found C, 83.87; H, 6.15; N, 4.56.

*1-(5-(2-Chloro-5-nitrophenyl)-4,5-dihydro-1-(5,6,7,8-tetrahydronaphthalen-7-yl)-1H-pyrazole-1-yl)ethane (**4**)*

A solution of compound **3a** (0.683 g, 0.02 mol) and hydrazine hydrate (1 g, 0.03 mol) in acetic acid (30 ml) was heated under reflux for 5 h. The reaction mixture was cooled and poured onto ice water. The precipitated product was filtered off, washed, and recrystallized from chloroform to yield compound **4**. Yield (69%); m.p. 180–182°C; IR spectrum (KBr, *v*, cm^{-1}): 2936 (CH, alicyclic), 1660 (C = O); ^1H NMR (DMSO-d_6, δppm): 1.72 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 2.35 (s, 3H, $COCH_3$), 2.73 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 3.15 (dd, *J* = 5.1,18.0 Hz, 1H, CH of pyrazoline ring >CHH_a), 3.93 (dd, *J* = 12.0,18.0 Hz, 1H, CH of pyrazoline ring >CH_bH), 5.79 (dd, *J* = 5.4, 12.0 Hz, 1H, CH of pyrazoline ring >CH_cAr), 7.12 (d, *J* = 7.8 Hz, 1H, Ar–H), 7.44 (s, 1H, Ar–H), 7.50 (d, *J* = 8.1 Hz, 1H, Ar–H), 7.80 (d, *J* = 9 Hz, 2H, Ar–H), 8.15 (dd, *J* = 2.7, 8.7Hz, 1H, Ar–H); ^{13}C NMR (DMSO-d_6): δ (ppm): 23.5 (CH_3), 22.5, 29.5 ($4CH_2$-tetralin), 122.5, 127.4, 128.4, 129.1, 129.4, 131.2, 131.6, 133.3, 134.6, 144.3, 145.3, 151.2 (aromatic-C), 168.0 (CO). MS *m/z* (%): 399 ($M^+ + 2$, 33); 397 (M^+, 100); Anal. calcd (%) for $C_{21}H_{20}ClN_3O_3$ (397.12): required C, 63.40; H, 5.07; N, 10.56; found C, 63.21; H, 5.13; N, 10.72.

*5-(2-Chloro-5-nitrophenyl)-4,5-dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-1-phenyl-1H-pyrazole (**5**)*

A solution of compound **3a** (0.673 g, 0.02 mol) and phenyl hydrazine (2.16 g, 0.02 mol) in absolute ethanol (50 ml) and triethylamine (0.5) was refluxed for 6 h. The formed precipitate was filtered off and recrystallized from ethanol to afford compound **5**. Yield (67%); m.p. 268–270°C; IR spectrum (KBr, *v*, cm^{-1}): 2942 (CH, alicyclic), 1596 (C = N); ^1H NMR (DMSO-d_6, δppm): 1.77 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 2.78 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 3.16 (dd, *J* = 6.0, 17.4 Hz, 1H, CH pyrazoline ring >CHH_a), 4.03 (dd, *J* = 12.3, 17.4 Hz, 1H, CH of pyrazoline ring >CH_bH), 5.71 (dd, *J* = 6.0, 12.3 Hz, 1H, CH pyrazoline ring >CH_cAr), 6.76 (t, *J* = 7.5 Hz, 1H, Ar–H), 6.94 (d, *J* = 7.8 Hz, 1H, Ar–H), 7.09–7.28 (m, 3H, Ar–H), 7.42 (s, 1H, Ar–H), 7.49 (d, *J* = 7.8 Hz, 1H, Ar–H), 7.93 (d, *J* = 9.6 Hz, 1H, Ar–H), 8.13–8.29 (m, 4H, Ar–H); ^{13}C NMR (DMSO-d_6): δ (ppm): 22.5, 29.5 ($4CH_2$-tetralin), 116.7, 120.3, 122.5, 125.5, 127.4, 128.4, 129.4, 129.5, 129.7, 131.2, 131.6, 133.3, 134.6, 135.3, 136.2, 143.4, 144.3, 145.3, 151.2 (aromatic-C). MS *m/z* (%): 433 ($M^+ + 2$, 22), 432 ($M^+ + 1$, 19), 431 (M^+, 66), 77 (100); Anal. calcd (%) for $C_{25}H_{22}ClN_3O_2$ (431.14): required C, 69.52; H, 5.13; N, 9.73; found C, 69.42; H, 5.37; N, 9.65.

*3-(4,5-Dihydro-3-(5,6,7,8-tetrahydronaphthalene-7-yl)-isoxazol-5-yl)-1H-indole (**6**)*

A solution of compound **3c** (3.01 g, 0.01 mol) and hydroxylamine hydrochloride (0.7 g, 0.01 mol) in pyridine (40 ml) was refluxed for 8 h. The cooled reaction mixture was acidified with ice-cold dilute hydrochloric acid. The separated solid was filtered off, dried, and recrystallized from ethanol to afford compound **6**. Yield (69%); m.p. 190–192°C (EtOH); IR spectrum (KBr, *v*, cm^{-1}): 3412 (NH), 2949 (CH, alicyclic), 1610 (C = C); ^1H NMR (DMSO-d_6, δppm): 1.74 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 2.74 (m, 4H, $2CH_2$ of tetrahydronaphthalene), 3.53 (dd, *J* = 9.3, 16.8 Hz, 1H, CH of isoxazoline ring >CHH_a), 3.73 (dd, *J* = 10.8, 16.8 Hz, 1H, CH of isoxazoline ring >CH_bH), 5.93 (dd, *J* = 9.3, 10.8 Hz, 1H, CH of isoxazoline ring >CH_cAr), 6.95–7.15 (m, 3H, Ar–H), 7.37–7.47 (m, 5H, Ar–H), 11.2 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6): δ (ppm): 22.44, 22.46, 28.64, 28.94 ($4CH_2$), 39.22, 39.50 ($CHCH_2$), 111.70, 113.83, 118.67, 121.34, 123.34, 123.93, 125.20, 126.85, 126.95, 129.94, 136.79, 137.10, 138.74, 156.22 (aromatic-C). MS *m/z* (%): 316 (M^+, 40), 225 (100); Anal. calcd (%) for $C_{21}H_{20}N_2O$ (316.16): required C, 79.72; H, 6.37; N, 8.85; found C, 79.54; H, 6.44; N, 8.65.

Biological evaluation

Animals

Adult male mice (20–25 g) were used for studying the analgesic activity. Adult male Wister albino rats (150–200 g) were used to study the anti-inflammatory activity. The animals (five per cage) were maintained under standard laboratory conditions (light period of 12 h/day and temperature 27 ± 2°C) with access to food and water *ad libitum*. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations. All experiments were performed in the morning according to the guidelines for the care of laboratory animals [31].

Anti-inflammatory activity

Anti-inflammatory activity screening for the prepared compounds was determined *in vivo* by the standard formalin-induced paw edema method in rats [32]. Wister albino rats of either sex weighing 150–200 g were divided into 11 groups of five animals each. Thickness of the left hind paw of each rat was measured (mm) using a vernier caliper before any drug administration (0 h). The control group received DMSO. Ibuprofen was given orally (50 mg/kg) as reference standard. The tested compounds **2a–c**, **3a–c**, and **4–6** dissolved in DMSO were administered orally (100 mg/kg) to the rest of the groups 1 h before induction of inflammation. Paw edema was induced by subcutaneous injection of 2.5% formalin solution (0.1 ml/rat) into the right hind paw of each rat. Paw thickness of each rat was measured after 30 min and 1, 2, and 3 h following formalin injection. Edema thickness (mm) was calculated by subtracting the zero-hour reading from each time reading. The anti-inflammatory activity was expressed as percentage inhibition of edema thickness in treated animals in comparison with the control group (Table 1):

$$\% \text{ Inhibition of edema} = V_c - V_t/V_c \times 100,$$

where V_c and V_t are the thickness of edema for the control and drug-treated animal groups, respectively.

Analgesic activity

The hot-plate method: Analgesic activity of the tested compounds was determined by the hot-plate method as reported before [33]. A total number of 55 mice were divided into 11 groups of five animals each. The first group was administered DMSO orally (0.2 ml/mice) and kept as negative control. Ibuprofen was given as standard drug (50 mg/kg) to the second group, and the tested compounds **2a–c**, **3a–c**, and **4–6** dissolved in DMSO were administered at a dose of 100 mg/kg body weight to the rest of the groups. Each animal was placed individually on a hot plate and maintained at 55°C. The time taken by the animals to lick the hind paw or jump out of the plate was taken as the reaction time, which was measured at 0, 30, 60, and 120 min. A cut off period of 30 s was considered as maximal latency to avoid paw injury [34]. The pain inhibition percentage (PIP) [35] was calculated according to the following formula:

$$\text{Pain inhibition percentage (PIP)} = (T_t - T_c/T_c) \times 100,$$

where T_c and T_t are the latency for the control and drug-treated animal groups.

The acetic acid-induced writhing test: This test was conducted using the method described by Collier *et al.* [36]. Muscle contractions were induced in 11 groups of mice (five animals per group) by intraperitoneal injection of 0.6% solution of acetic acid (10 ml/kg). Thirty minutes before this administration, the animals in the first group were treated orally with DMSO (0.2 ml/mice) and they served as negative controls. Ibuprofen as the reference standard (50 mg/kg) and the tested compounds (**2a–c**, **3a–c**, and **4–6**) dissolved in DMSO were administered orally (100 mg/kg) to the animals of the rest of the groups. Immediately after administration of acetic acid the animals were placed in glass cages, and the number of 'stretching' per animal was recorded during the course of the next 15 min. Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs. There was significant reduction in the number of writhes in the drug-treated animals as compared with vehicle-treated animals. This was considered a positive analgesic response, and the percentage inhibition of writhing was calculated according to the method described by Collier *et al.* [36].

Ulcerogenic liability: Ulcerogenic liability was determined in albino rats according to the reported standard methods [37].

Table 1 Anti-inflammatory activity of the tested compounds (100 mg/kg, orally) against formalin-induced paw edema

	Increase in paw thickness (mm)				Inhibition (%)			
Treatment	30 min	1 h	2 h	3 h	30 min	1 h	2 h	3 h
Control	0.53 ± 0.03	0.55 ± 0.024	0.57 ± 0.03	0.57 ± 0.028	0	0	0	0
Ibuprofen	0.15 ± 0.004[a]	0.13 ± 0.005[a]	0.09 ± 0.003[a]	0.05 ± 0.002[a]	71.698	76.364	84.211	91.23
2a	0.25 ± 0.014[a,b]	0.32 ± 0.013[a,b]	0.35 ± 0.016[a,b]	0.35 ± 0.014[a,b]	52.83	41.82	38.596	38.596
2b	0.48 ± 0.022[b]	0.45 ± 0.02[a,b]	0.36 ± 0.018[a,b]	0.35 ± 0.019[a,b]	9.434	18.182	36.84	38.596
2c	0.23 ± 0.015[a,b]	0.20 ± 0.013[a,b]	0.18 ± 0.006[a,b]	0.15 ± 0.006[a,b]	56.6	63.64	68.42	73.68
3a	0.25 ± 0.014[a,b]	0.23 ± 0.011[a,b]	0.23 ± 0.013[a,b]	0.22 ± 0.012[a,b]	52.83	58.182	59.65	61.4
3b	0.46 ± 0.022[b]	0.48 ± 0.024[b]	0.49 ± 0.022[b]	0.48 ± 0.023[b]	13.21	12.73	14.04	15.79
3c	0.21 ± 0.013[a,b]	0.20 ± 0.015[a,b]	0.18 ± 0.005[a,b]	0.15 ± 0.002[a,b]	60.38	63.64	68.42	73.68
4	0.43 ± 0.019[a,b]	0.44 ± 0.02[a,b]	0.44 ± 0.02[a,b]	0.45 ± 0.021[a,b]	18.87	20	22.81	21.053
5	0.23 ± 0.012[a,b]	0.28 ± 0.013[a,b]	0.37 ± 0.019[a,b]	0.37 ± 0.016[a,b]	56.6	49.09	35.09	35.09
6	0.35 ± 0.015[a,b]	0.38 ± 0.014[a,b]	0.40 ± 0.019[a,b]	0.42 ± 0.018[a,b]	33.96	30.91	29.82	26.32

[a]Significantly different from the control value at $P < 0.05$.
[b]Significantly different from the ibuprofen value at $P < 0.05$; results are means of five experiments ± SE.

Rats were divided into 11 groups of five animals each. The animals were fasted 18 h before drug administration. Animals in the first group were treated orally with 2 ml of DMSO aqueous suspension (1% w/v) and considered as the control group; ibuprofen was administered (50 mg/kg body weight) as a reference standard to the second group. The tested compounds **2a–c**, **3a–c**, and **4–6** were administered in the form of DMSO aqueous suspensions (100 mg/kg body weight) to the rest of the groups. Treatment was continued once daily for three successive days in all groups. An hour after the last dose, the animals were killed by cervical dislocation and the stomach was removed, opened along the greater curvature, and rinsed with saline. The gastric mucosa was examined with a magnifying lens (× 10) for the presence of lesions in the form of hemorrhages or linear breaks and erosions. The ulcer index was calculated (Table 3) and the degree of ulcerogenic effect was expressed in terms of:

(1) percentage incidence of ulcer divided by 10;
(2) average number of ulcers per stomach; and
(3) average severity of ulcers.

The ulcer index is the value that resulted from the sum of the above three values.

Statistical analysis

Results of anti-inflammatory and analgesic activities were represented as mean ± SE. The significant difference between the groups was tested using one-way analysis of variance, followed by Dunnett's test at P less than 0.05.

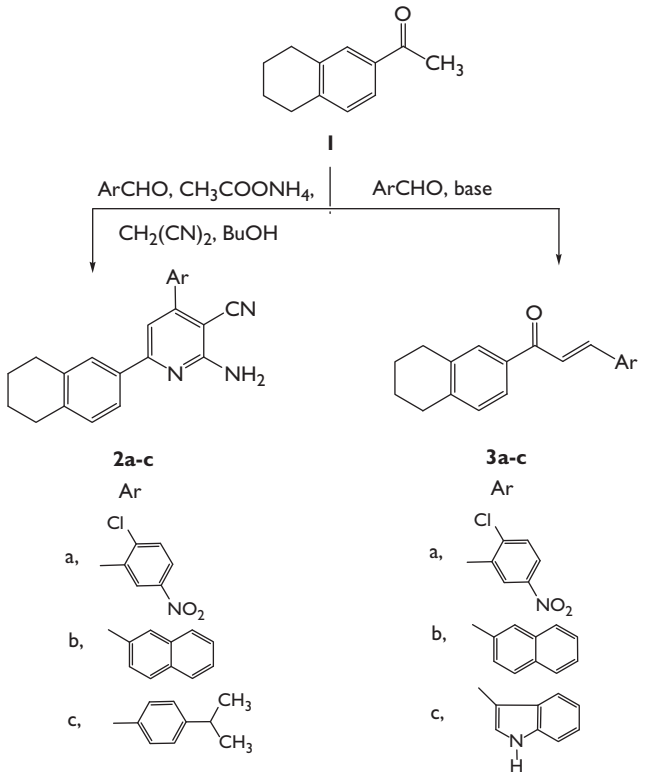
Results and discussion

Chemistry

The general synthesis of the 3-cyano-2-aminopyridine derivatives **2a–c** is illustrated in Scheme 1. We used the in-solution one-pot synthesis. In this respect, 2-acetyl tetralin (**1**) was reacted with the appropriate aldehyde (2-chloro-5-nitrobenzaldehyde, 2-naphthaldehyde, or 4-isopropyl benzaldehyde) in the presence of malononitrile and excess ammonium acetate in *n*-butanol. The respective pyridine derivatives **2a–c** were obtained. ^{1}H NMR spectra of these compounds showed singlet signals at δ 6.94, 6.92, and 6.74 ppm, respectively, which corresponded to the NH_2 group in addition to the signals due to the aromatic protons. Mass spectra of the synthesized compounds showed molecular ion peaks [M^+] corresponding to the molecular weights of the target compounds. The chlorine containing derivative **2a** showed molecular ion peaks for [M^+] and [$M^+ + 2$] at a ratio of 3 : 1 because of the isotopic nature of the chlorine atom. Infrared spectra of all compounds showed bands at 3194–3361 cm^{-1} region due to NH stretching vibrations of the amino group, in addition to a band around 2200 cm^{-1} (CN, stretching).

2-Acetyl-5,6,7,8-tetrahydronaphthalene (**1**) also condensed with aromatic aldehydes (2-chlorobenzaldehyde, or 2-naphthaldehyde) in ethanolic sodium hydroxide under Claisen–Schmidt conditions to yield 1-[2-(5,6,7,8-tetrahydronaphthyl)]-3-aryl propenones **3a, b**

Scheme 1.



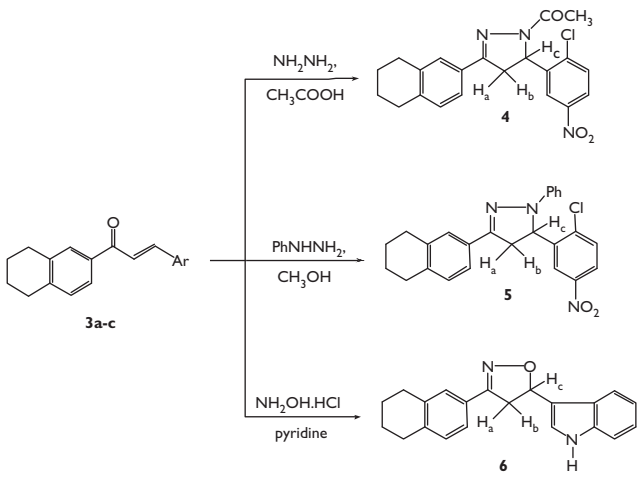
Synthesis of pyridine-3-carbonitrile **2a–c** and chalcones **3a–c**.

(Scheme 1). The structures of **3a** and **b** were confirmed by their IR, ^{1}H NMR, ^{13}C NMR, and mass spectra. IR spectra showed absorption bands at 1666–1651 cm^{-1} (C = O) and 1612–1606 cm^{-1} (C = C). Mass spectra showed ion peaks [M^+] corresponding to their molecular weights. ^{1}H NMR spectra of **3a** and **b** showed signals at δ 7.23–8.32 ppm corresponding to the aromatic protons, in addition to the ethylene protons.

In contrast, condensation of compound **1** with 3-formyl indole takes place in ethylene glycol in the presence of piperidine on heating to 180°C for 20 min, according to the reported method [38], to yield **3c**.IR spectrum showed absorption bands at 3218 cm^{-1} (NH) and 1657 cm^{-1} (C = O). Its mass spectrum showed molecular ion peak [M^+] corresponding to the molecular weight, which is also the base peak; ^{1}H NMR showed multiplet signals at δ 6.94–8.07 ppm corresponding to aromatic protons in addition to the ethylene protons and singlet signal at δ 11.6 ppm for NH.

Reaction of **3a** with hydrazine hydrate in boiling acetic acid led to the formation of 1-acetyl-5-(2-chloro-5-nitrophenyl)-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-4,5-dihydro-1*H*-pyrazole (**4**) (Scheme 2). Structure of **4** was assigned on the basis of its spectral data and elemental analysis. For example, IR spectrum revealed the carbonyl absorption band at 1660 cm^{-1}, whereas its ^{1}H NMR spectrum revealed singlet signal at δ 2.35 ppm for $COCH_3$, δ 3.15, 3.93 ppm (d, d for unsymmetrical 2H of pyrazoline ring), and 5.79 (d, d for 1H of pyrazoline ring),

Scheme 2.



Synthesis of the pyrazole derivatives **4**, **5**, and the isoxazole derivative **6**.

in addition to the aromatic protons in the region δ 7.12–8.15 ppm. Its mass spectrum showed the molecular ion peak [M^+] as the base peak at *m/z* (397).

Meanwhile, reaction of the same compound **3a** with phenyl hydrazine in absolute ethanol in the presence of a few drops of triethylamine yielded 5-(2-chloro-5-nitrophenyl)-1-phenyl-3-(5,6,7,8-tetrahydronaphthlen-2-yl)-4,5-dihydro-1*H*-pyrazole (**5**). Its structure was confirmed on the basis of the disappearance of the carbonyl group in the IR spectrum. Its ^{1}H NMR spectrum revealed signals at δ 3.16, 4.03 ppm (d, d for unsymmetrical 2H of pyrazoline ring), and 5.71 (d, d for 1H of pyrazoline ring), in addition to the aromatic protons in the region δ 6.76–8.29 ppm. In addition, the mass spectrum showed molecular ion peak [M^+] at *m/z* (431).

Moreover, the reaction of chalcone **3c** with hydroxylamine hydrochloride in boiling pyridine yielded 3-[2-(5,6,7,8-tetrahydronaphthyl)-5-indolyl]-4,5-dihydroisoxazoline (**6**). Its IR spectrum showed absorption bands characteristic for the C = N and NH, whereas its ^{1}H NMR spectrum showed the absence of the ethylenic protons present in chalcone **3c**.

Biological evaluation

Anti-inflammatory activity

The newly synthesized pyridine compounds **2a–c** (100 mg/kg, orally) exhibited significant anti-inflammatory activity in formalin-induced rat paw edema. 2-Amino-4-(4-isopropylphenyl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl) pyridine-3-carbonitrile (**2c**) exhibited the highest activity among this series (Table 1). It showed 73.68% edema inhibition 3 h after formalin injection compared with 91.23% for the standard ibuprofen after the same period of time. Cyanopyridine derivatives were previously found to influence the inflammatory mediators nitric oxide (NO), tumor necrosis factor-α (TNF-α), prostaglandin E-2 (PGE-2), cycloxygenase-2 (COX-2), and 5-lipoxygenase (5-LOX) [28,39].

Chalcone derivatives **3a** and **3c** showed enhanced anti-inflammatory activity, with maximum edema inhibition percentage of 61.40 and 73.68, respectively, 3 h after formalin injection, whereas **3b** showed complete loss of activity. This result was in agreement with the previous report that showed the presence of a reactive α,β-unsaturated ketone group in the propenone side chain as being responsible for the anti-inflammatory and analgesic activities [15]. In addition, it was also reported that the value of the correlation coefficient of in-vitro COX-2 inhibition versus the in-vivo anti-inflammatory activity for these compounds is 0.61, which indicates that COX-2 inhibition may not be the sole mechanism by which these compounds act as anti-inflammatory agents and that other mechanisms such as inhibition of the lipoxygenase and hemeoxygenase-1 might be included [15]. Other chalcone derivatives were found to have related dual COX-1/2- and 5/15-LOX-inhibiting effects [40].

The reaction of **3a** with hydrazine hydrate to yield 1-(5-(2-chloro-5-nitrophenyl)-4,5-dihydro-1-(5,6,7,8-tetrahydronaphthalen-7-yl)-1*H*-pyrazole-1-yl)ethane (**4**) greatly diminished its anti-inflammatory activity from 61.40% for the parent compound **3a** to 22.81% for the obtained pyrazoline derivative **4**. However. the reaction of **3a** with phenyl hydrazine hydrate to obtain 5-(2-chloro-5-nitrophenyl)-4,5-dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-1-phenyl-1*H*-pyrazole (**5**) did not alter its recorded potency but strongly affected its kinetic manner by enhancement of metabolism and/or excretion. The pyrazoline derivative **5** exerted its maximum activity with 56.60% inhibition of edema 30 min after formalin injection, which decreased stepwise to 35.09% after 3 h. According to the activity relationship, it could be suggested that the attachment of a phenyl group to the pyrazoline moiety could improve the anti-inflammatory and analgesic activities of the derivatives **4** and **5**. Similar findings were recorded in the previous studies of different pyrazoline analogs bearing phenyl groups [41,42]. Despite its moderate activity at 30 min, compound **5** might have a poor kinetic pattern, resulting in an abrupt decrease in its activity.

Analgesic activity

The analgesic activity of the synthesized compounds was evaluated by hot-plate and acetic acid writhing methods as central [34] and peripheral [36] antinociceptive methods, respectively. From the obtained results (Tables 2 and 3) it could be concluded that all of the tested compounds showed significant activity ($P < 0.05$). Results obtained by the pyridines **2a–c** revealed that (2-amino-4-(2-chloro-5-nitrophenyl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl) pyridine-3-carbonitrile) **2a** showed the highest analgesic activity using both hot-plate and acetic acid writhing methods. The peak of its analgesic activity against thermal stimuli was demonstrated 1 h after oral dosing with PIP 311.84%, which is superior to the value of 221.45% exerted by the standard ibuprofen 3 h after dosing. Therefore, despite this promising activity of compound **2a**, the activity was not sustained on the same potency for a long time as it decreased to 98.38 and 60.25% (2 and 3 h after dosing), which is expected to be because of its rapid metabolism and/or excretion of the

Table 2 Analgesic activity of the tested compounds following oral administration (100 mg/kg, orally) in mice using hot-plate method

	Latency time (s)				Pain inhibition (%)			
Compound	30 min	1 h	2 h	3 h	30 min	1 h	2 h	3 h
Control	3.15 ± 0.15[b]	3.21 ± 0.18[b]	3.09 ± 0.18[b]	3.17 ± 0.16[b]	–	–	–	–
Ibuprofen	5.63 ± 0.18 [a]	7.29 ± 0.23[a]	8.34 ± 0.25[a]	10.19 ± 0.24[a]	78.73	127.103	169.903	221.45
2a	10.42 ± 0.35[a,b]	13.22 ± 0.45[a,b]	6.13 ± 0.26[a,b]	5.08 ± 0.21[a,b]	230.794	311.84	98.382	60.25
2b	5.40 ± 0.22[a]	5.18 ± 0.24[a,b]	5.33 ± 0.27[a,b]	4.15 ± 0.302[a,b]	71.429	72.492	61.371	30.915
2c	8.42 ± 0.37[a,b]	8.15 ± 0.32[a]	4.38 ± 0.26[a,b]	3.44 ± 0.15[b]	167.302	153.89	41.748	8.517
3a	12.41 ± 0.503[a,b]	5.47 ± 0.31[a,b]	5.62 ± 0.27[a,b]	5.13 ± 0.24[a,b]	293.968	81.877	70.405	61.83
3b	13.25 ± 0.42[a,b]	15.43 ± 0.46[a,b]	15.35 ± 0.55[a,b]	15.28 ± 0.49[a,b]	320.635	380.685	396.764	382.02
3c	7.20 ± 0.25[a,b]	8.53 ± 0.301[a,b]	10.24 ± 0.33[a,b]	11.08 ± 0.37[a,b]	128.57	165.732	231.392	249.527
4	5.37 ± 0.22[a]	5.39 ± 0.24[a,b]	5.25 ± 0.22[a,b]	5.12 ± 0.23[a,b]	70.48	74.434	63.55	61.514
5	7.24 ± 0.29[a,b]	7.63 ± 0.32[a]	13.08 ± 0.48[a,b]	10.28 ± 0.37[a,b]	129.84	137.695	323.301	224.29
6	5.33 ± 0.17[a]	7.14 ± 0.24[a]	17.25 ± 0.62[a,b]	15.82 ± 0.48[a,b]	69.206	122.43	458.25	399.05

[a]Significantly different from the control value at $P < 0.05$.
[b]Significantly different from the ibuprofen value at $P < 0.05$; results are means of five experiments ± SE.

Table 3 Analgesic activity of the tested compounds (100 mg/kg, orally) on acetic acid writhing abdominal contractions

Treatment	Number of contractions/15 min	Inhibition (%)
Control	59.28 ± 3.49	0
Ibuprofen	5.18 ± 0.46[a]	91.26
2a	3.74 ± 0.28[a,b]	93.691
2b	19.27 ± 2.06[a,b]	67.49
2c	15.38 ± 1.26[a,b]	74.06
3a	6.49 ± 0.42[a]	89.052
3b	5.83 ± 0.51[a]	90.17
3c	13.72 ± 1.05[a,b]	76.86
4	21.08 ± 1.63[a,b]	64.44
5	12.53 ± 0.816[a,b]	78.863
6	10.27 ± 0.85[a,b]	82.68

[a]Significantly different from the control value at $P < 0.05$.
[b]Significantly different from the ibuprofen value at $P < 0.05$; results are means of five experiments ± SE.

compound. Other previous studies have indicated that tetralin-2-aminopyridine carbonitrile derivatives exert significant pain perception in the hot-plate test rather than in writhing response [43].

The synthetic derivative 2-amino-4-(4-isopropylphenyl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)pyridine-3-carbonitrile **(2c)** showed moderate analgesic activity using the hot-plate method with PIP 167.302 and 153.89% after 30 min and 1 h, respectively. These activities abruptly decreased afterward, in the same manner as that of compound **2a**, indicating a similar kinetic profile for these two pyridine derivatives. It also exerted considerable activity in reducing numbers of abdominal contractions (74.06%) in the writhing test. The lowest analgesic activity of these tested pyridine series following either thermal or chemical stimulus was recorded for 2-amino-4-(naphthalen-2-yl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)pyridine-3-carbonitrile **2b**, with PIP 72.492 and 67.49%, respectively, revealing that introduction of a naphthalene moiety in the amino pyridine nucleus greatly diminishes its analgesic activity.

Chalcone derivatives **3a–c** showed analgesic activity, with the highest activity for **3b**, and their PIP ranged from 320.64 to 382.02% 30 min–3 h after administration (orally). This was followed by **3c** with the highest PIP at 323.301% 2 h after dosing. These recorded activities were significantly ($P < 0.05$) higher than the 221.45% obtained by the standard ibuprofen 3 h after administration. Meanwhile, compound **3a** exhibited maximum activity of 293.968% 30 min after treatment, followed by abrupt decrease to 61.83% within 3 h. In the acetic acid writhing test, all of the prepared chalcones significantly inhibited the number of abdominal contractions, with the highest activity for compound **3b** at 90.17%, followed by **3a** at 89.05%, and finally for **3c** at 76.86%. Thus, it is clear that the analgesic activity of these chalcones is mediated by both central and peripheral mechanisms. The exact mechanism of the recorded analgesic activity is not a point of this study. However, the previous findings demonstrated that the antinociceptive mechanism of the chalcone series is varied according to their chemical structures. In this regard, it was found that different chalcone analogs were found to be potent cyclooxygenase inhibitors [44] and others exhibited anti-inflammatory activity through 1,2 lipoxygenase inhibition [45]. Furthermore, chalcone derivatives containing he flurophenyl group act through selective inhibition of COX-2. However, replacing the flurophenyl group by the isopropylphenyl group in the same compound resulted in an optimal combination of in-vitro COX-1/2 and 5/15-LOX inhibitory effects [40]. Some phenylsulfonyl urenyl chalcone derivatives exert their antinociceptive responses through dual inhibition of COX-2 and 5-LOX activities [46].

Results of new pyrazoline derivatives 1-(5-(2-chloro-5-nitrophenyl)-4,5-dihydro-1-(5,6,7,8-tetrahydronaphthalen-7-yl)-1*H*-pyrazole-1-yl)ethane **(4)** and 5-(2-chloro-5-nitrophenyl)-4,5-dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-1-phenyl-1*H*-pyrazole **(5)** obtained by the hot-plate test revealed that compound **5** demonstrated better analgesic activity ($P < 0.05$) compared with standard ibuprofen along the different time intervals, with maximum effect (323.30%) 2 h after dosing. However, mild activity was recorded for compound **4**, with PIP 74.434–61.514%. Similar findings obtained using the acetic acid writhing test as higher inhibition of writhing response (78.863%) were recorded for compound **5** as compared with compound **4** (64.44%). Depending on the structure–activity relationship, it could be predicted that substitution of a phenyl group at position 1 of the pyrazole nucleus of compound **5** increases its antinociceptive activity. Similar suggestions were reported previously by Tabarelli *et al.* [47], and the authors suggested

that some pyrazole derivatives involved antinociceptive activity through opioid mechanisms. Hence, our synthesized pyrazole derivatives showed analgesic activity using both the hot-plate and acetic acid writhing tests; hence, it was safe to decide that their pain perception inhibitory effects were through both central and peripheral mechanisms. A similar investigation was recorded for other benzimidazole–pyrazole series [48]. Other earlier preliminary findings evaluated some of the previously synthesized 4,5-dihydro-1*H*-pyrazole derivatives as promising antinociceptive agents using both the acetic acid writhing model and the hot-plate test [49,50].

The synthesized isoxazole derivative 3-(4,5-dihydro-3-(5,6,7,8-tetrahydronaphthalen-7-yl)-isoxazol-5-yl)-1*H*-indole **(6)** exhibited a promising analgesic activity that was higher than that of the parent compound **3c** using both hot-plate and writhing tests. Thus, it can be deduced that the reaction of the chalcone derivative (E)-3-(1-H-indol-3-yl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)prop-2-en-1-one (**3c**) with hydroxylamine hydrochloride improves its analgesic activity. The latency time was significantly ($P<0.05$) prolonged against thermal stimulus 2 and 3 h after treatment (with 393.53 and 335.96% PIP, respectively), compared with ibuprofen (169.903 and 221.45%, respectively). However, the analgesic activity demonstrated 82.68% reduction in writhing response compared with ibuprofen (91.26%). Analgesic activities recorded for other isoxazole derivatives were found to be varied according to their mechanisms [51] depending on their chemical structures and the substituted groups even in the same compound. In this regard, 4,5-phenyl-4-isoxazolines exhibited potent analgesic activity, and most of these compounds were nonselective COX-2 inhibitors. However, those with methylsulfonyl or flourine substituents at the *para* position of the phenyl group were potent and selective COX-2 inhibitors [25].

In the same manner, 3,4-diarylisoxazole analogs of valdecoxib [4-(5-methyl-3-phenylisoxazol-4-yl)-benzensulfonamide] were found to be selective (COX-2) inhibitors. However, the removal of the sulfonamide group resulted in selective COX-1 inhibitors [52]. Further studies are needed to determine the exact mechanism of the newly synthesized isoxazole compound **6**.

Ulcerogenic liability

The ulcerogenic liability for the tested compounds in each series was determined in albino rats according to previously reported methods [37]. The obtained data revealed that all of the tested compounds possessed less ulcerogenic potentialities (ulcer indexes of 10.14 ± 0.45–13.5 ± 0.47) compared with that of the standard drug ibuprofen (ulcer index of 20.96 ± 0.88) (Table 4). The obtained results of the tested pyridine derivatives were consistent with the results obtained by Fathalla *et al.* [43], which indicated that similar tetrahydronaphthalene compounds exhibited reduced gastric ulcerogenic activities compared with indomethacin. The lowest ulcerogenic activity among all of the tested compounds was recorded for compound **2c**, which belongs to the pyridine series. Reduced ulcerogenic activity recorded for the tested pyrazole derivatives **4** and **5** might be attributed to their phenolic moiety, which is responsible for the reduced ulcerogenic activity of other related compounds.

Table 4 Ulcerogenic liability of the synthesized compounds

Treatment	Number of animals with ulcers	Incidence divided by 10 (%)	Average number of ulcers	Average severity	Ulcer index
Control	0/5	0	0	0	0
Ibuprofen	5/5	10	9.13	1.83	21.96 ± 0.88
2a	3/5	6	3.6	1.45	10.65 ± 0.58[b]
2b	4/5	6	4.3	1.27	11.57 ± 0.50[b]
2c	4/5	6	3	1.14	10.14 ± 0.45[b]
3a	4/5	6	4.6	1.5	12.1 ± 0.67[b]
3b	4/5	8	2.5	1	11.5 ± 0.54[b]
3c	4/5	8	2	1.2	11.2 ± 0.40[b]
4	3/5	6	3.7	1.13	10.83 ± 0.32[b]
5	4/5	8	3.4	1.27	12.67 ± 0.45[b]
6	4/5	8	4.2	1.3	13.5 ± 0.47[b]

[b]Significantly different from the ibuprofen value at $P<0.05$; results are means of five experiments ± SE.

Conclusion

New 3-cyano pyridine and chalcone derivatives were synthesized. Chalcone derivatives of **3** were converted to pyrazole and isoxazole derivatives **4–6**. Maximum anti-inflammatory activities were recorded for **2c** and **3c**. However, promising analgesic activity was established by the hot-plate method for most of the tested compounds, with higher activity for **2a**, **2c**, **3b**, **3c**, **5**, and **6** compared with standard ibuprofen. Interestingly, compounds **3b–c**, **5**, and **6** possessed more pronounced activities compared with standard ibuprofen. In addition, these derivatives showed pronounced analgesia by the writhing test, with the highest activity for **2a**, **3a**, and **3b**. All of the tested compounds showed reduced ulcerogenic potentialities.

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Conflicts of interest

There are no conflicts of interest.

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