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NOVEL SYNTHESIS OF PYRAZOLE-CONTAINING THIOPHENE, 2-ALKYLOXY-PYRIDINE AND THIENO[2,3-*d*]PYRIMIDINE SCAFFOLDS AS ANALGESIC AGENTS

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ABSTRACT. A group of trisubstituted pyrazoles containing thiophen, 2-alkyloxypyridine and thieno[2,3-*d*]pyrimidine heterocycles were synthesized in a study for possible analgesic agents. The desired products were obtained by reaction of 2-((1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)methylene)malononitrile with sulfur in presence of TEA, followed by treatment with different reagents. Newer products were examined for their analgesic properties, among them, analog **7** showed significant analgesic effects in comparison with reference medicines activity.

KEY WORDS: Trisubstituted pyrazoles, Thiophene, Alkyloxypyridine, Fused pyrimidine, Analgesic activities

INTRODUCTION

Pyrazole compounds have an interesting therapeutic effect. Due to the large amount of drugs including this heterocyclic compound, the pharmaceutical properties of the ring were the topic of medicinal studies. Celecoxib and its derivatives are analgesic medicines with a pyrazole nucleus (Figure 1). Diverse bioactive molecules are developed by pyrazole derivatives such as antibacterial, anti-inflammatory, antifungal, antiviral, antimicrobial,and anti-hyperglycemic properties [1-10]. Moreover, pyrazole-based heterocycles as thiophenes, 2-alkyloxypyridines and thieno[2,3-*d*]pyrimidines have been given more attention due to their useful therapeutic fields including, muscle relaxing, antitumor, anti-depressant, antimicrobial, antidiabetic, anti-tubercular, antioxidant, HIV reverse transcriptase inhibitors and also possess significant vasodilation activities [11-17]. They also demonstrated strong anti-inflammatory action with low GIT toxicity and analgesic impacts [18-24]. In the same way, and in the continuing work on synthesis of biologically active heterocycles based pyrazoles [25-29], we focused in this study on designing of new molecules carrying pyrazole substituents as hybrids with various heterocycles aiming to get potent candidates with analgesic properties.

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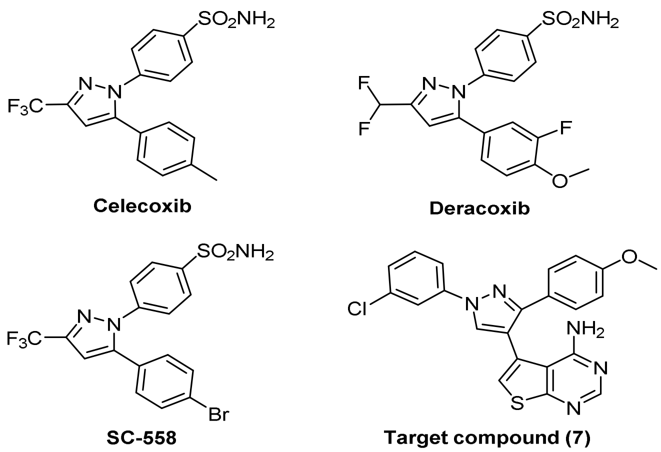


Figure 1. Structure of marketed pyrazole drugs and synthesized compound (**7**).

EXPERIMENTAL

Electrothermal device 9100 has identified melting points. Elementary microanalysis using Vario Elemental was acceptable and performed. The spectrophotometer Shimadzu 435 IR was used to operate infrared spectrum (KBr pellets technique). Varian Gemini 500 MHz NMR Spectrophotometer was used for recording ^{1}H, ^{13}C NMR spectra DMSO-d_6 with TMS as an internal reference. Hewlett Packard 5988 Spectrometer registered the mass spectrum (70 eV).

Animals

The study included albino mice (25-30 g) and wistar rats (150-200 g). Mice and rats used for this experiment were bought from the animal breeding laboratory, NRC, Egypt. Whole animals have been preserved with free access to animal feed under the lights of 12 hours. Prior to testing, the animals were adapted for a week to the lab environment. In line with the Ethics Committee, the animal protocol was performed of (NRC), Egypt.

Central analgesic activity (hot plate test)

By using hot-plate appliance, the central analgesic effects of the examined compounds were achieved. Twelve groups were collected with 6 animals each. The first group was used as (normal control) and the second group was used as (reference) received the vehicle at a dose of (5 mL/kg) and tramadol (40 mg/kg) orally, respectively. Dose levels of (20 mg/kg) were taken orally to the remaining groups from 3rd to 12th. Within an hour of treatment, mice were placed on a hot plate at 53 ± 0.5 °C separately. On mice lick the fore or hind paw or spring away from the location, the reaction time of the thermal stimulus was determined. After oral administration of tested compounds, the response time was recorded (0, 30, 60 and 90 min). The time off for heat stimulation reaction was 60 s to prevent damaging the tissue of the mouse fingers.

Peripheral analgesic activity (Writhing test)

Acetic acid writhing test was achieved on mice and aspirin was used as a reference control. 72 mice, each with six animals, have been divided into 12 sets. Vehicle (5 mL/kg) and aspirin (150 mg/kg) were used orally for treatment of mice of 1st group (control) and 2nd group (reference). Dose of (20 mg/kg) of the 3rd to 12th groups of mouse was administered orally to the test compound. Writhes were produced after a 30 min dose of intraperitoneal injection of acetic acid (0.7% aqueous acetic acid) at a dosage of (10 mL/kg). Mice were then placed in transparent boxes and the mean number of Writhes was calculated for each group in comparison to control group during 20 min according to the equation:

Protection (%) = [(Control mean - Treated mean)/Control mean] x 100

*2-Amino-4-(1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)thiophene-3-carbonitrile (**2**)*

In ethyl alcohol (50 mL), an equimolar mix (0.03 mol) of both ylidenemalononitrile **1** and sulfur were added and the mix was cooled to 10 ^oC, followed by dropwise addition of TEA (0.03 mol). The reaction mixture was heated for 2 h at 80 ^oC, and cooled afterwards. The solid residues generated were ethanol crystallized. Yield: 67%, m.p. 209-211 ^oC. IR (KBr, cm^{-1}) *ν*: 3386 (NH_2), 2210 (CN). ^1H NMR (DMSO-d_6): δ 3.81 (s, 3H, OCH_3), 4.52 (s, 2H, NH_2), 6.54 (s, 1H, CH-thiophene), 7.02-8.56 (m, 8H, ArH), 9.07 (s, 1H, CH). ^{13}C NMR (DMSO-d_6): δ 56.14, 84.51, 103.58, 113.99, 116.16, 116.28, 117.55, 125.18, 126.35, 128.63, 129.10, 130.67, 131.12, 135.01, 138.94, 140.86, 146.22, 150.38, 161.01, 163.45. MS, m/z (%): 406 (M^+, 14). Anal. calcd for $C_{21}H_{15}ClN_4OS$ (406.89): C, 61.99; H, 3.72; N, 13.77; found: C, 61.84; H, 3.59; N, 13.61.

*5-Amino-3-(1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-1-(quinolin-2-yl)-1H-pyrazole-4-carbonitrile (**3**)*

In (30 mL) of dry ethanol, an equimolar (0.01 mol) of starting **1** and 2-hydrazinylquinoline were refluxed for 6 h. After cooling, the precipitate formed was purified from MeOH. Yield: 63%, m.p. 210-212 ^oC. IR (KBr, cm^{-1}) *ν*: 3374 (NH_2), 2214 (CN). ^1H NMR (DMSO-d_6): δ 3.81 (s, 3H, OCH_3), 7.05-7.99 (m, 14H, H-arom), 8.54 (s, 2H, NH_2 exch.), 8.96 (s, 1H, CH). ^{13}C NMR (DMSO-d_6): δ 56.72, 92.38, 101.35, 104.56, 113.67, 115.42, 115.64, 117.75, 123.58, 124.89, 125.94, 126.18, 126.87, 128.16, 128.37, 128.92, 129.79, 130.47, 131.08, 135.01, 136.29, 140.95, 143.82, 145.90, 153.72, 157.93, 161.76. MS, m/z (%): 517 (M^+, 9). Anal. calcd for $C_{29}H_{20}ClN_7O$ (517.97): C, 67.25; H, 3.89; N, 18.93; found: C, 67.08; H, 3.72; N, 18.76.

*General method for synthesis of 2-alkyloxypyridine-3-carbonitrile derivatives **4a-h***

In an appropriate ethyl or methyl alcohol (15 mL) containing (0.003 mol) of potassium hydroxide, a mix of starting **1** (0.003 mol) and aryl ketones (0.003 mol) were stirred at ambient temperature for almost 67 h (monitored by TLC). The produced residue was purified with butanol.

*4-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-2-methoxy-6-(pyridine-3-yl)-pyridine-3-carbonitrile (**4a**)*. Yield: 46%, m.p. 234-236 ^oC. IR (KBr, cm^{-1}) ν: 2227 (CN). ^1H NMR (DMSO-d_6): δ 3.79, 3.81 (2s, 6H, 2OCH_3), 7.01-8.45 (m, 13H, ArH + H-5 pyridine), 8.95 (s, 1H, CH). ^{13}C NMR (DMSO-d_6): δ 55.68, 56.12, 95.01, 102.84, 112.73, 114.58, 115.39, 115.64, 119.22, 120.17, 123.62, 125.34, 126.41, 129.03, 131.10, 134.70, 135.08, 139.24, 140.99, 146.13, 149.26, 152.04, 154.92, 157.46, 161.01, 164.10. MS, m/z (%): 493 (M^+, 6). Anal. calcd for $C_{28}H_{20}ClN_5O_2$ (493.94): C, 68.08; H, 4.08; N, 7.18; found: C, 67.91; H, 3.89; N, 7.04.

*4-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-2-ethoxy-6-(pyridine-3-yl)-pyridine-3-carbonitrile (**4b**)*. Yield: 39%, m.p. 182-184 ^oC. IR (KBr, cm^{-1}) ν: 2221 (CN). ^1H NMR (DMSO-d_6): δ 1.52 (t, 3H, *J* = 7.2 Hz, CH_3), 3.80 (s, 3H, OCH_3), 4.60 (q, 2H, *J* = 7.0 Hz, CH_2), 7.01-8.52 (m, 13H, ArH + H-5pyridine), 8.95 (s, 1H, CH). ^{13}C NMR (DMSO-d_6): δ 14.49, 56.05, 63.12, 94.78, 102.65, 112.80, 114.83, 115.40, 115.69, 118.02, 120.38, 123.55, 125.32, 126.19, 128.67, 131.06, 134.48, 135.01, 138.60, 141.15, 145.85, 147.91, 149.98, 155.16, 157.66, 161.01, 164.25. MS, m/z (%): 507 (M^+, 4). Anal. calcd for $C_{29}H_{22}ClN_5O_2$ (507.97): C, 68.57; H, 4.37; N, 13.79; found: C, 68.39; H, 4.20; N, 13.63.

*4-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-2-methoxy-6-(thiophen-2-yl)-pyri -dine-3-carbonitrile (**4c**)*. Yield: 28%; m.p. 243-245 °C. IR (KBr, cm^{-1}) ν: 2223 (CN). ^{1}H NMR (DMSO-d_6): δ 3.78, 3.81 (2s, 6H, 2OCH$_3$), 7.01-8.45 (m, 12H, ArH + H-5 pyridine), 8.95 (s, 1H, CH). ^{13}C NMR (DMSO-d_6): δ 55.64, 56.08, 93.89, 104.50, 112.81, 114.63, 115.56, 116.04, 118.21, 125.28, 125.79, 126.16, 127.11, 127.88, 128.46, 130.95, 133.82, 135.01, 139.68, 142.31, 145.76, 153.14, 154.27, 161.01, 163.96.MS, m/z (%): 498 (M^+, 2). Anal. calcd for $C_{27}H_{19}ClN_4O_2S$ (498.98): C, 64.99; H, 4.08; N, 11.23; found: C, 64.80; H, 3.68; N, 11.07.

*4-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-2-ethoxy-6-(thiophen-2-yl)-pyridine-3-carbonitrile (**4d**)*. Yield: 32%, m.p.147-149 °C, IR (KBr, cm^{-1}) ν: 2219 (CN), ^{1}H NMR (DMSO-d_6): δ 1.49 (t, 3H, J = 6.8 Hz, CH$_3$), 3.81 (s, 3H, OCH$_3$), 4.62 (q, 2H, J = 6.8 Hz, CH$_2$), 7.01-8.55 (m, 12H, ArH + H-5 pyridine), 8.96 (s, 1H, CH). ^{13}C NMR (DMSO-d_6): δ 14.52, 55.60, 63.24, 94.17, 104.45, 112.78, 114.71, 115.47, 115.95, 118.34, 124.90, 125.84, 126.10, 127.12, 127.74, 128.54, 131.08, 134.29, 135.01, 140.18, 142.49, 147.02, 152.60, 155.13, 161.01, 164.20.MS, m/z (%): 513 (M^+, 4). Anal. calcd for $C_{28}H_{21}ClN_4O_2S$ (513.01): C, 65.55; H, 4.13; N, 10.92; found: C, 65.37; H, 3.98; N, 10.76.

*4-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-6-(4-hydroxyphenyl)-2-methoxy-pyridine-3-carbonitrile (**4e**)*. Yield: 31%, m.p. 257-259 °C; IR (KBr, cm^{-1}) ν: 3412 (OH), 2220 (CN). ^{1}H NMR (DMSO-d_6): δ 3.77, 3.80 (2s, 6H, 2OCH$_3$), 7.01-8.65 (m, 13H, ArH + H-5 pyridine), 8.98 (s, 1H, CH), 10.36 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 55.71, 56.10, 94.16,104.45, 112.80, 114.65, 115.38, 115.85, 116.26, 118.34, 125.29, 128.44, 128.79, 129.04, 130.67, 134.15, 135.01, 140.96, 146.03, 157.11, 157.24, 161.01, 164.22. MS, m/z (%): 509 (M^+, 6). Anal. calcd for $C_{29}H_{21}ClN_4O_3$ (508.96): C, 68.44; H, 4.16; N, 11.01; found: C, 68.28; H, 3.98; N, 10.87.

*4-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-2-ethoxy-6-(4-hydroxyphenyl)-pyridine-3-carbonitrile (**4f**)*. Yield: 37%, m.p. 192-194 °C. IR (KBr, cm^{-1}) ν: 3398 (OH), 2226 (CN). ^{1}H NMR (DMSO-d_6): δ 1.40 (t, 3H, J = 7.6 Hz, CH$_3$), 3.81 (s, 3H, OCH$_3$), 4.51 (q, 2H, J = 7.6 Hz, CH$_2$), 7.01-8.70 (m, 13H, ArH + H-5 pyridine), 8.95 (s, 1H, CH), 10.45(s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 14.82, 56.04, 63.20, 94.06, 104.56, 112.82, 114.54, 115.27, 115.93, 116.10, 118.31, 125.17, 128.35, 128.90, 129.01, 130.74, 133.87, 135.01, 141.08, 145.97, 157.05, 157.34, 161.01, 164.10; MS, m/z (%): 523 (M^+, 7). Anal. calcd for $C_{30}H_{23}ClN_4O_3$ (522.98): C, 68.90; H, 4.43; N, 10.71; found: C, 68.71; H, 4.25; N, 10.62.

*4-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-2-methoxy-6-(4-nitrophenyl)-pyridine-3-carbonitrile (**4g**)*. Yield: 39%, m.p. 262-264 °C. IR (KBr, cm^{-1}) ν: 2221 (CN). ^{1}H NMR (DMSO-d_6): δ 3.78, 3.81 (2s, 6H, 2OCH$_3$), 7.05-8.30 (m, 13H, ArH + H-5 pyridine), 8.95 (s, 1H, CH). ^{13}C NMR (DMSO-d_6): δ 55.69, 56.15, 93.89, 104.34, 112.83, 114.60, 115.42, 115.90, 118.24, 121.47, 125.37, 126.18, 128.52, 128.68, 130.71, 133.75, 135.01, 141.04, 143.12, 145.85, 147.10, 153.02, 157.23, 161.01, 164.30. MS, m/z (%): 538 (M^+, 5). Anal. calcd for $C_{29}H_{20}ClN_5O_4$ (537.95): C, 64.75; H, 3.75; N, 13.02; found: C, 64.58; H, 3.60; N, 12.89.

*4-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-2-ethoxy-6-(4-nitrophenyl)-pyridine-3-carbonitrile (**4h**)*. Yield: 33%, m.p.178-180 °C; IR (KBr, cm^{-1}) ν: 2228 (CN). ^{1}H NMR

(DMSO-d_6): δ 1.42 (t, 3H, J = 7.4 Hz, CH_3), 3.80 (s, 3H, OCH_3), 4.56 (q, 2H, J = 7.4 Hz, CH_2), 7.05-8.32 (m, 13H, ArH + H-5 pyridine), 8.95 (s, 1H, CH). ^{13}C NMR (DMSO-d_6): δ 14.76, 56.09, 63.31, 94.15 104.45, 112.80, 114.57, 115.48, 115.78, 118.20, 121.64, 125.44, 126.10, 128.49, 128.79, 130.62, 134.02, 135.01, 165.01. MS, m/z (%): 552 (M^+, 8). Anal. calcd for $C_{30}H_{22}ClN_5O_4$ (551.98): C, 65.28; H, 4.02; N, 12.69; found: C, 65.09; H, 3.90; N, 12.52.

*N-(4-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-3-cyanothiophen-2-yl)-acetamide (**5**)*

In (7 mL) of acetic anhydride, starting **2** has been heatedfor 4 h under reflux. The solid product separated after being poured into cold water was gathered and purified from dioxane. Yield 51%, m.p. 161-163 °C. IR, ν: 3446 (NH), 2218 (CN), 1658 (CO). ^1H NMR (DMSO-d_6): δ 2.38 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 7.05-7.56 (m, 10H, ArH + H-5 thiophene + NH exch.), 8.99 (s, 1H, CH). ^{13}C NMR (DMSO-d_6): δ 22.54, 55.70, 92.46, 104.26, 114.61, 115.20, 115.59, 118.09, 125.31, 126.52, 128.42, 130.69, 132.90, 135.04, 137.67, 140.78, 145.92, 148.16, 150.73, 150.84, 161.01, 168.54. MS, m/z (%): 449 (M^+, 8). Anal. calcd for $C_{23}H_{17}ClN_4O_2S$ (448.92): C, 61.54; H, 3.82; N, 12.48; found: C, 61.35; H, 3.67; N, 12.33.

*5-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-2-methylthieno[2,3-d]pyrimidin-4(3H)-one (**6**)*

In a mixture of HCl/AcOH (3:9 mL), starting **2** and/or derivative **5** (0.01 mol) was refluxed for 2 h. The reaction mixture could cool down, the precipitation formed after it was pour in cold water was dried, and dioxan crystallized. Yield 60%, m.p. 192-194 °C, IR, ν: 3205 (NH), 1672 (CO). ^1H NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 7.01-7.52 (m, 9H, ArH + H-5 thiophene), 8.12 (s, 1H, NH ex.), 8.95 (s, 1H, CH). ^{13}C NMR (DMSO-d_6): δ 21.04, 55.76, 98.35, 114.27, 115.46, 117.98, 125.09, 126.10, 126.89, 128.31, 129.93, 131.19, 135.01, 139.77, 140.28, 140.87, 143.99, 153.68, 159.48, 160.14, 161.03. MS, m/z (%): 448 (M^+, 11). Anal. calcd for $C_{23}H_{17}ClN_4O_2S$ (448.92): C, 61.54; H, 3.82; N, 12.48; found: C, 61.41; H, 3.90; N, 12.39.

*General method for preparation of derivatives **7**, **8***

A solution of starting **2** in (15 mL) of formamide or formic acid was reflux for 3-4 h. After cooling, the solid produced was washed and AcOH-crystallized.

5-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)thieno[2,3-d]pyrimidin-4-amine (7). Yield 60%, m.p. >300 °C. IR, ν: 3470, 3236 (NH_2). ^1H NMR (DMSO-d_6): δ 3.79 (s, 3H, OCH_3), 7.05-7.45 (m, 9H, ArH + H-5 thiophene), 8.12 (s, 1H, H-2pyrimidine), 8.34 (s, 2H, NH_2), 8.95 (s, 1H, CH). ^{13}C NMR (DMSO-d_6): δ 55.63, 102.85, 114.59, 115.68, 118.22, 121.46, 123.96, 125.18, 126.48, 128.37, 130.74, 133.15, 134.99, 140.69, 141.90, 143.89, 146.13, 156.21, 158.02, 161.03. MS, m/z (%): 433 (M^+, 11). Anal. calcd for $C_{22}H_{16}ClN_5OS$ (433.91): C, 60.90; H, 3.72; N, 16.14; found: C, 60.72; H, 3.58; N, 15.97.

*5-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (**8**)*. Yield 57%, m.p. >300 °C. IR, ν: 3195 (NH), 1667 (CO). ^1H NMR (DMSO-d_6): δ 3.81 (s, 3H, OCH_3), 7.05-7.43 (m, 9H, ArH + H-5 thiophene), 8.14 (s, 1H, H-2 pyrimidine), 8.95 (s, 1H, CH), 12.36 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 55.79, 102.60, 114.67, 115.53, 118.10, 125.38, 125.95, 127.14, 128.46, 130.62, 132.90, 135.10, 139.88, 140.98, 141.08, 145.34, 147.01, 159.87, 158.02, 161.01, 161.23.MS, m/z (%): 435 (M^+, 11). Anal. calcd for $C_{22}H_{15}ClN_4O_2S$ (434.9): C, 60.76; H, 3.48; N, 12.88; found: C, 60.57; H, 3.29; N, 12.70.

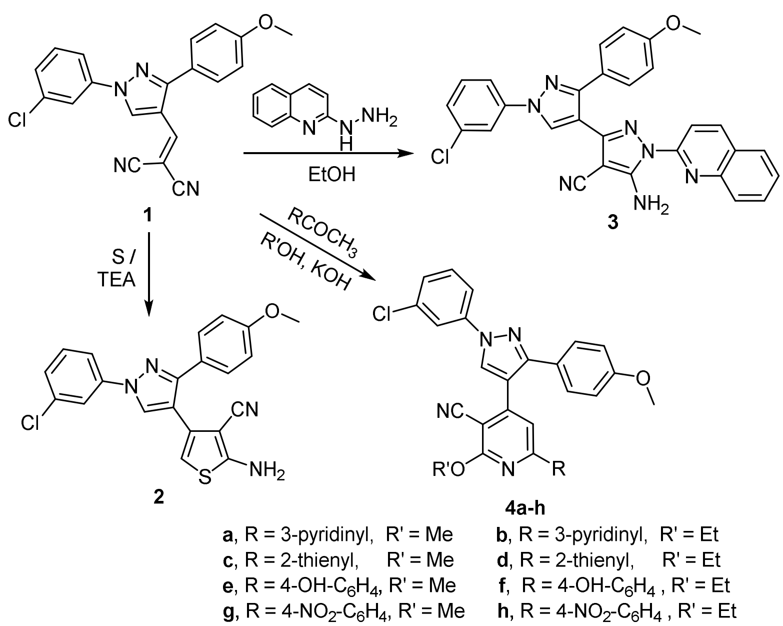
*1-(4-(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-3-cyanothiophen-2-yl)-3-phenylthiourea (**9**)*

In absolute ethanol (30 mL) containing (0.5 mL) of TEA, an equimolar mix (0.01 mol) of compound **2** and phenyl isothiocyanate were reflux for 5 h. The solid produced was methanol crystallized. Yield 70%, m.p. 238-240 °C. IR, ν: 3428, 3190 (2NH), 2210 (CN). ^{1}H NMR (DMSO-*d*$_6$): δ 3.81 (s, 3H, OCH_3), 4.45 (s, 1H, NH), 6.90-7.49 (m, 14H, ArH + H-5 thiophene), 8.95 (s, 1H, CH), 11.26 (s, 1H, NH). ^{13}C NMR (DMSO-*d*$_6$): δ 55.74, 102.86, 110.46, 114.53, 115.26, 115.65, 118.29, 123.98, 125.16, 125.94, 126.48, 128.54, 129.03, 129.14, 130.77, 133.62, 134.99, 136.89, 139.25, 140.85, 143.67, 146.08, 161.01, 182.35. MS, m/z (%): 542 (M^+, 17). Anal. calcd for $C_{28}H_{20}ClN_5OS_2$ (542.07): C, 62.04; H, 3.72; N, 12.92; found: C, 61.91; H, 3.56; N, 12.79.

RESULTS AND DISCUSSION

Chemistry

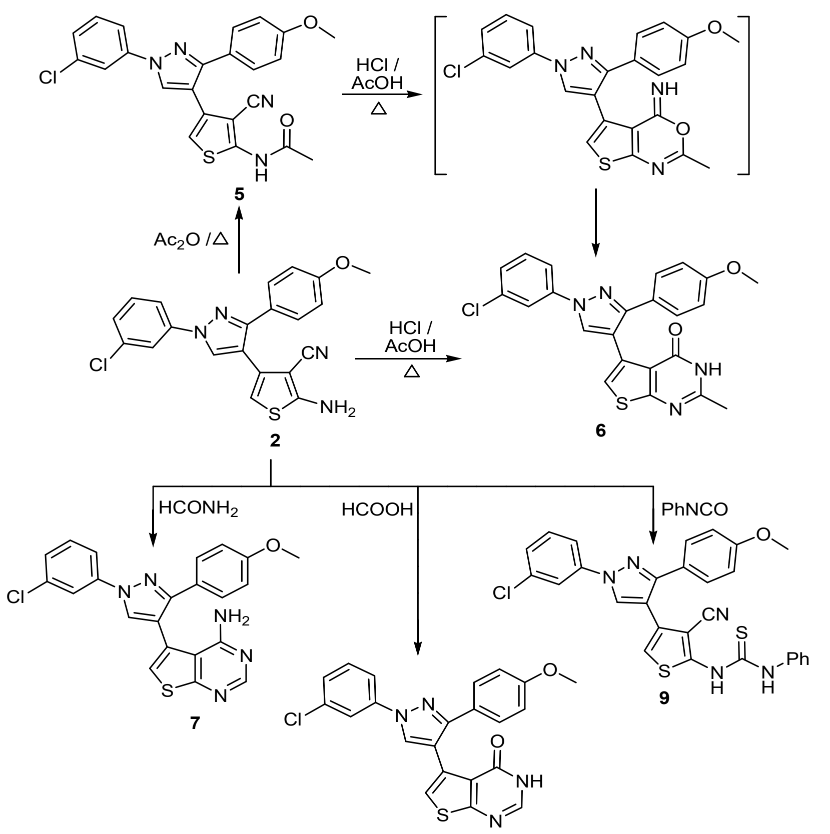
The synthetic routes to pyrazoles **2-9** have been identified in (Schemes 1 and 2). The starting ylidenemalononitrile **1** treated in basic conditions with sulfur to provide 2-aminothiophene-3-carbonitrile derivative **2**. Compound **2** showed two characteristic bands at 3386 and 2210 cm^{-1} corresponding to amino and nitrile functions, whereas the ^{1}H NMR spectrum exhibited two singlets peaks at δ 4.52 and 6.54 ppm for the amino protons and H-5 of thiophene ring. Also, peaks at δ 84.51, 103.58, 150.38 and 163.45 ppm proved C-5, C-3, C-2 and C-4 of the new thiophene ring in the ^{13}C NMR spectrum. On the other hand, the starting **1** reacted with 2-hydrazinylquinoline to afford 5-amino-4-cyano-3-aryl-pyrazole derivative **3**. The later compound showed two different bands at 3374 and 2214 cm^{-1} belonging to amino and cyano functions, meanwhile ^{1}H NMR spectrum showed two singlets at δ 3.81 and 8.54 ppm referred to



Scheme 1. Synthesis of compounds **2**, **3** and **4a-h**.

methoxy and amino protons. Furthermore, the precursor **1** condensed with various aryl ketones according to Michael addition to provide the desired 2-alkyloxy pyridine-3-carbonitriles **4a-h** (Scheme 1). The later products exhibited strong bands in the range of 2219-2228 cm^{-1} referred to nitrile function in the IR spectrum. Also, the appearance of peaks corresponding to alkoxide in ^{1}H NMR spectrum confirming the cyclization form of pyridine moiety. Moreover, ^{13}C NMR and MS confirmed the carbons at their expected regions and molecular formula of the title products.

The key intermediate **2** was reacted with acetic anhydride to afford acyclic 3-cyanothiophen-2-acetamide derivative **5**. Compounds **2** or **5** treated with HCl/AcOH mixture (3:9 mL) to afford the pyrimidinone derivative **6**. ^{1}H NMR spectrum showed peaks for methyl and amino protons at δ 2.41, 8.12 ppm besides peaks appeared in the ^{13}C NMR spectrum attributed to methyl and carbonyl groups at δ 21.04, 160.14 ppm.



Scheme 2. Synthesis of compounds **5-9**.

The desired thieno[2,3-*d*]pyrimidine derivatives **7**, **8** could be achieved through cyclization reaction ofcompound **2** and formamide or formic acid. Compound **7** indicated the disappearance of nitrile band and appearance of new bands for the amino group at 3470, 3236 cm^{-1} in IR spectrum, besides two singlet peaks for H-2 of pyrimidine and amino protons at δ 8.12 and 8.34 ppm respectively in the ^{1}HNMR spectrum. In the same time, compound **8** reveled lack of nitrile function with the appearance of new bands for amino and carbonyl groups.

Furthermore, the key intermediate **2** was reacted with phenyl isothiocyanate to give the corresponding 3-cyanothiophen-2-phenylthiourea derivative **9** (Scheme 2). New bands were shown in the later compound **9** at 3428, 3190 and 2210 cm^{-1} due to (2NH) and (CN) functions in IR spectra, besides two singlets signals appeared at δ 4.45 and 11.26 ppm assigned to D_2O-exchangeable (2NH) protons in the ^{1}H NMR spectrum.

Analgesic activity evaluation

The analgesic profile of compounds **2**–**9**, acquired from hot plate test and acetic acid induced writhing test was performed using the techniques previously mentioned [30,31]. The findings are presented in (Tables 1 and 2).

The compounds tested showed remarkable analgesic effects in the hot plate and writhing assays in mice. Regarding central analgesic activity (hot plate test): The latency of the examined products improved compared to fundamental levels by oral administration. The resulting data revealed **4d**, **4e**, **4f**, **4g**, **4h**, **6**, **7** and **9** derivatives showing significant analgesic activity (70-159 %) increase in pain threshold after 90 min following the administration. Compound **7** which contains the fused pyrimidine moiety showed highest core analgesic characteristics (159.6%) at 90 min, which was statistically equipotent to the control drug (174.6%). The central analgesic properties of the active products next 90 min, sorted in descending way, were 159.6, 115.28, 102.1, 93.8, 85.6, 84.2, 80.1, 76.0 and 70.5 % for derivatives **7**, **4g**, **4e**, **4f**, **4d**, **3**, **4**, **4h** and **9**, respectively, comparable to the reference tramadol (Table 1).

Table 1. Central analgesic activity of synthesized compounds in mice.

Compds	0 min	30 min		60 min		90 min	
	Reaction time (s)	Reaction time (s)	Protection (%)	Reaction time (s)	Protection (%)	Reaction time (s)	Protection (%)
Control	12.7±1.04	12.8±0.91 ǂ	0	14.5±0.45 ǂ	0	14.6±0.58 ǂ	0
2	10.3±0.80	12.6±1.00 ǂ	0.8	15.4±0.81 ǂ	6.2	21.1±1.10 ǂ	44.5
3	11.6±0.65	22.8±0.97 *ǂ	79.5	24.0±1.12 *ǂ	65.5	26.9±2.38 *ǂ	84.2
4a	10.4±0.79	15.2±0.20 ǂ	19.6	18.2±0.94 ǂ	25.5	22.4±0.91 ǂ	53.4
4b	11.0±0.38	12.9±1.19 ǂ	1.6	15.2±1.04 ǂ	4.8	20.8±1.57 *ǂ	42.4
4c	10.6±0.41	13.9±0.42 ǂ	9.4	19.2±0.36 ǂ	32.4	19.5±1.00 ǂ	33.6
4d	11.1±0.90	18.3±0.85 *ǂ	44.0	22.6±1.35 *ǂ	55.9	27.1±2.14 *ǂ	85.6
4e	10.5±0.54	18.5±1.00 *ǂ	45.7	21.1±2.09 *ǂ	45.5	29.5±2.42 *ǂ	102.1
4f	11.2±0.61	19.1±1.12 *ǂ	50.4	24.0±0.87 *ǂ	65.5	28.3±0.98 *ǂ	93.8
4g	10.7±0.86	24.9±0.51 *	96.0	28.5±1.45 *	96.6	31.5±1.40 *	115.8
4h	12.1±0.57	15.8±0.45 ǂ	24.4	22.6±0.67 *ǂ	55.9	25.7±2.05 *ǂ	76.0
5	10.1±0.48	17.6±1.00 ǂ	38.6	25.1±1.00 *ǂ	73.1	23.1±2.67 ǂ	58.2
6	11.5±0.69	15.9±0.56 ǂ	25.2	19.3±1.23 ǂ	33.1	26.3±1.04 *ǂ	80.1
7	10.4±0.82	26.1±1.23 *ǂ	105.5	31.5±0.10 ǂ	117.2	37.9±0.37 ǂ	159.6
8	10.0±0.94	17.0±1.34 *ǂ	33.8	20.8±2.05 ǂ	43.4	19.8±0.87 *ǂ	35.6
9	10.1±1.02	20.3±0.73 *	59.8	25.4±0.90 *ǂ	75.1	24.9±2.51 ǂ	70.5
Tramadol	10.8±0.53	29.6±1.57 *	131.2	33.0±1.00 *	127.5	40.1±2.28 *	174.6

*$p < 0.05$: Statistically significant from control (Dunnett's test). ǂ$p < 0.05$: statistically significant from tramadol (Dunnett's test).

According to acetic acid induced writhing test, peripheral analgesic activity was found in all the compounds examined versus acetic acid induced writhing conduct related to vehicle-treated mice. Aconsiderable decrease in the writhing response was noticed in compounds **4e** (70.29%),

4g (75.25) and **7** (82.43%). In addition, the peripheral analgesic impact of pyrimidine analog **7** (82.43%) showed superior to those of aspirin (78.47%) (Table 2, Figure 2).

Table 2. Peripheral analgesic activity of synthesized compounds in mice.

Compds	No. of writhes /20 min	Protection (%)	Compds	No. of writhes /20 min	Protection (%)
Control	80.8±4.5ǂ	-----	**4g**	20.8±1.3*	74.25
2	28.4±2.1*	64.85	**4h**	28.6±2.9*	64.60
3	34.6±1.4*ǂ	57.18	**5**	38.2±1.7*	52.72
4a	41.2±3.2*ǂ	49.00	**6**	31.5±2.1*ǂ	61.01
4b	39.8±1.8*ǂ	50.74	**7**	14.2±1.0*ǂ	82.43
4c	36.5±1.8*ǂ	54.83	**8**	43.6±2.9*ǂ	46.04
4d	27.1±2.5*	66.46	**9**	27.3±2.5*ǂ	66.21
4e	24.0±1.6*	70.29	**Aspirin**	17.4±1.6*	78.47
4f	25.3±3.3*ǂ	68.69			

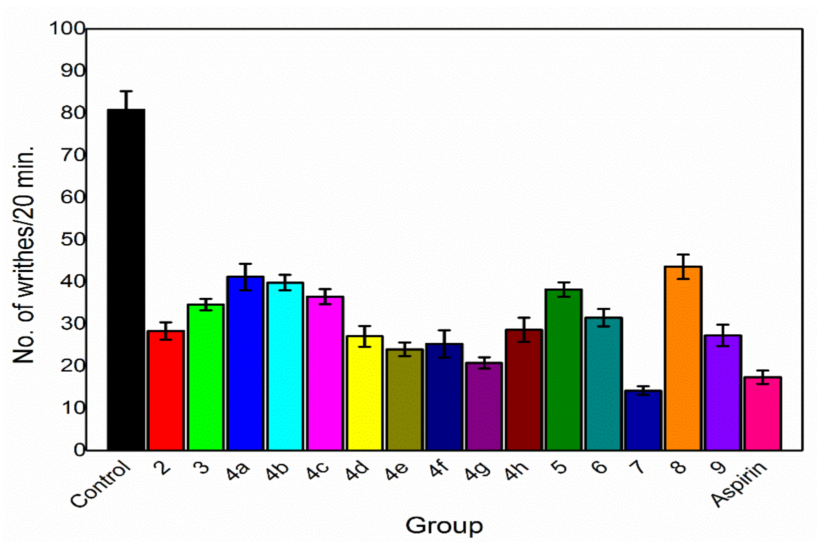


Figure 2. Peripheral analgesic activity of the synthesized products **2-9** in mice.

CONCLUSION

New pyrazole derivatives including thiophene, 2-alkyloxy-pyridine and thieno[2,3-*d*]pyrimidines have been synthesized and analgesical activities and have been performed and discussed. The products acquired inhibited the restriction of acetic acid and the response of hot plate device relative to conventional aspirin control. Our results suggest that it is favorable to analgesic activity to incorporate substituted pyridine and fused thieno[2,3-*d*]pyrimidinemoieties with pyrazole backbone.

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