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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antimicrobial, antioxidant, anti-inflammatory, and analgesic activities of some new 3-(2′-thienyl)pyrazole-based heterocycles

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Abstract 1-Phenyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carbaldehyde was used as a synthon for the synthesis of new thiazole and pyrazoline heterocycles having 2-thienylpyrazole moiety. The antimicrobial, anti-inflammatory, and analgesic activities of the synthesized compounds were evaluated using agar diffusion method, carrageenan-induced paw edema, and writhing assays, respectively. It was found that the majority of the tested compounds exhibited both analgesic and anti-inflammatory activities.

Keywords 2-Thienylpyrazoles · Thiazoles · Heterocycles · Antimicrobial · Antioxidant · Analgesic · Anti-inflammatory activities

Introduction

Pyrazole derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activities including antibacterial (Sarma *et al*., 2010), antifungal (Zhang *et al*., 2010), herbicidal (Yuhan *et al*., 2010), and insecticidal (Finkelstein and Strock, 1997). They are also found in some commercial pesticides, including fenpyroximate, pyrazolate, and pyrazoxyfen (Li-Rong *et al*., 2004). In addition, pyrazole derivatives are important pharmaceuticals, for instance, they have been found to possess analgesic and anti-inflammatory activities because of their cyclooxygenase and 5-lipoxygenase inhibiting properties (Ochi *et al*., 1999; Chauhan *et al*., 2011). Also, they are also useful as biodegradable agrochemicals (Crosscurt *et al*., 1979) and as intermediates in the dye industry (Lubs, 1970). Furthermore, 5-thienyl pyrazole derivatives have CB1 receptor antagonistic activity (Srivastava *et al*., 2009) and antimicrobial activity by inhibition of cell wall, nucleic acid, and protein in bacteria (Kulikova and Cherkesova, 1974). In view of such applications and in connection with our previous study on synthesis of biologically active heterocycles (Abdel-Aziz *et al*., 2010; Abdel-Wahab *et al*., 2008, 2009a,b), we synthesized various thienylpyrazoles that have antimicrobial, antioxidant, anti-inflammatory, and analgesic activities.

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Results and discussion

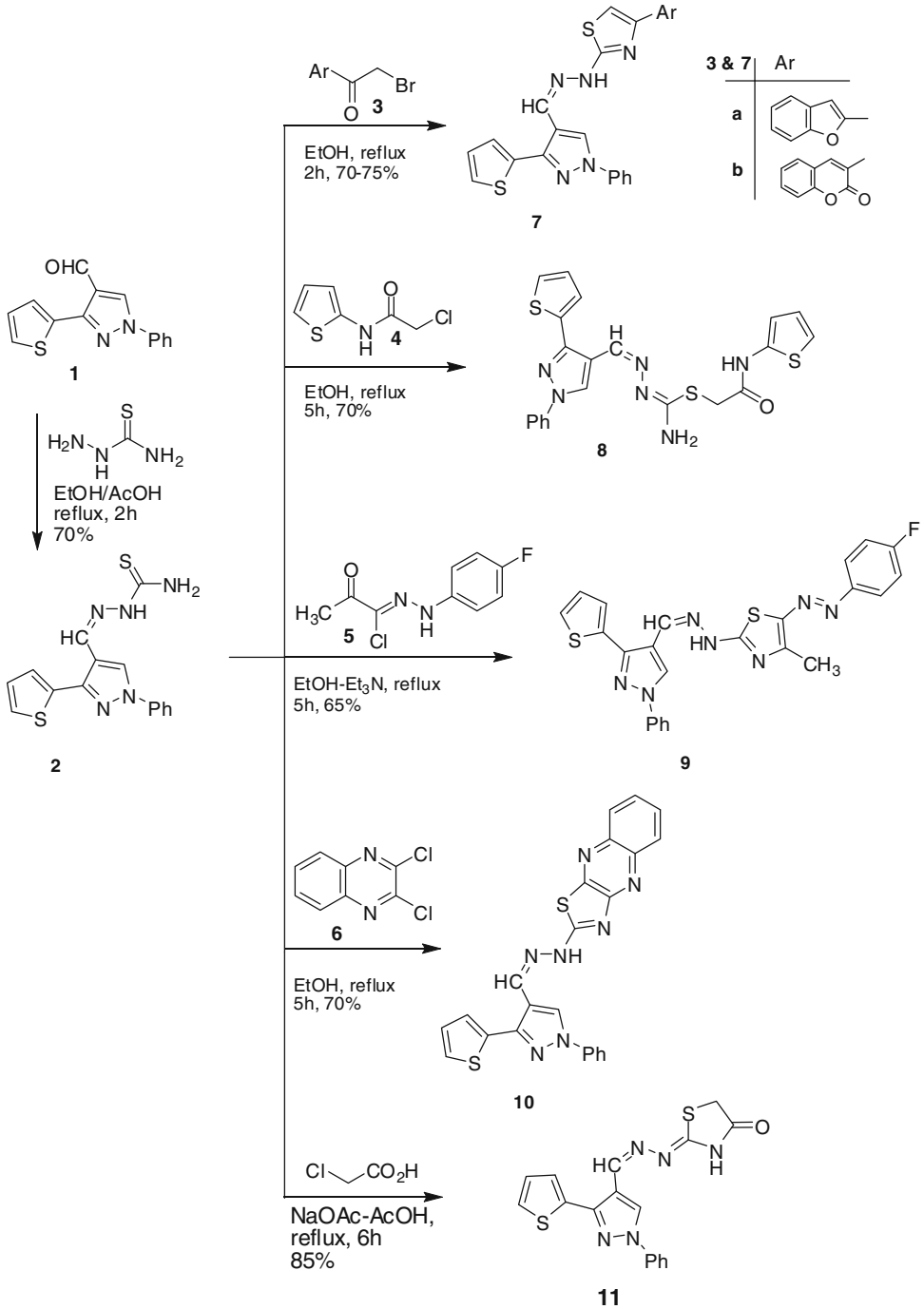
Chemistry

Heating of an equimolar amounts of 1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carbaldehyde **1** (Bratenko *et al*., 2005) with thiosemicarbazide in ethanol in the presence of glacial acetic acid under reflux condition afforded thiosemicarbazone (**2**) in 70% yield. The target compounds **7**–**10** were obtained by reaction of equimolar quantities of thiosemicarbazide **2** with phenacyl bromides **3a**,**b**, 2-chloro-*N*-

(thiophen-2-yl)acetamide **4**, *N*′-(4-fluorophenyl)-2-oxopropanehydrazonoyl chloride **5**, or 2,3-dichloroquinoxaline **6** (Scheme 1).

The structures of thiazoles **7**, **9**, and **10** were established on the basis of their spectral data. For example, the ^{1}H NMR spectra of **7a** showed the thiazole CH proton as a broad singlet that resonated at 5.12 ppm, while the methyl protons of compound **9** resonated as a singlet at 2.67 ppm. Also, the mass spectra of compounds **8** and **10** showed a peak at *m/z* 466 and 453 because of their molecular ions, respectively.

4-Thiazolidinone compound **11** was readily obtained from the reaction of thiazosemicarbazone **2** with chloroacetic acid in glacial acetic acid and in the presence of anhydrous sodium acetate (Scheme 1). The ^{1}H NMR spectrum of **11** is consistent with the assigned structure. For example, the thiazolidinone CH_2 protons resonated as a singlet at 3.91 ppm.



Scheme 1 Reaction of thiosemicarbazone **2** with halogenated compounds

Treatment of **1** with 4-chloroaceteophenone in ethanol and 10% sodium hydroxide at room temperature for 1 h afforded the aldol-type product **12**. Reaction of **12** with hydrazine hydrate produced pyrazoline derivative **13**. Finally, 4-tolylisothiocyanate reacted with **13** to afford thioamide **14** in 45% yield (Scheme 2).

The structures of compounds **12–14** were confirmed by their spectroscopic data (Table 1).

Antimicrobial activity

Table 2 shows the antimicrobial activity of the synthesized compounds against target pathogens using the agar diffusion assay (Perez *et al.,* 1990). Compound **2** showed excellent antibacterial activities for all the tested pathogen including gram-positive, gram-negative bacteria, and yeast with minimal inhibitory concentration (MIC) that ranged between 83.3 and 20.8 μg/ml (Table 2),whereas compound **12** showed antibacterial activities with MIC ranging between 333.3 and 41.6 μg/ml against the tested pathological strains.

Among all the compounds tested, compound **8** showed an excellent (MIC) 20.8 μg/ml against *Staphylococcus aureus* ATCC 29213 and *Enterobacter Cloaca* ATCC3047. Compounds **10**, **11**, and **13** showed moderate antimicrobial activities. However, no compound showed inhibition against fungi *Aspergillus niger* as observed by the absence of inhibition zone (data not shown).

DPPH free radical scavenging activity

DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action (Niki, 1987). Therefore, the DPPH radical scavenging activity was determined by the decrease in absorbance at 517 nm, due to the reduction by the antioxidant (AH) or the reaction with a radical species, as shown in the Eq. 1 (Gordon, 2001).

$$DPPH^{\bullet} + R^{\bullet} \rightarrow DPPH - R \quad (1)$$

According to the results of DPPH free radical scavenging activity showed in Table 3, compounds **7a**,

Table 1 Characteristic data of the synthesized compounds

Compd. no.	Mol. formula (M. Wt.)	Calcd. found			Mp. °C	Yield %
		C%	H%	N%		
2	$C_{15}H_{13}N_5S_2$	55.02	4.00	21.39	226–227	70
	(327.43)	55.13	3.92	21.43		
7a	$C_{25}H_{17}N_5OS_2$	64.22	3.66	14.98	178–179	70
	(467.57)	64.13	3.72	15.03		
7b	$C_{26}H_{17}N_5O_2S_2$	63.01	3.46	14.13	242–243	75
	(495.58)	62.89	3.51	13.99		
8	$C_{21}H_{18}N_6OS_3$	54.06	3.89	18.01	275–276	70
	(466.60)	53.98	3.98	18.20		
9	$C_{24}H_{18}FN_7S_2$	59.12	3.72	20.11	140–142	65
	(487.58)	59.33	3.79	20.26		
10	$C_{23}H_{15}N_7S_2$	60.91	3.33	21.62	244–245	70
	(453.54)	61.10	3.46	21.73		
11	$C_{17}H_{13}N_5OS_2$	55.57	3.57	19.06	273–274	85
	(367.45)	55.49	3.63	18.97		
12	$C_{22}H_{15}ClN_2OS$	67.60	3.87	7.17	130–131	85
	(390.89)	67.51	3.91	7.26		
13	$C_{22}H_{17}ClN_4S$	65.26	4.23	13.84	184–185	70
	(404.92)	65.39	4.49	14.01		
14	$C_{30}H_{24}ClN_5S_2$	65.02	4.37	12.64	245–246	45
	(554.13)	65.18	3.46	12.50		

Scheme 2 Route to bipyrazole derivative **14**

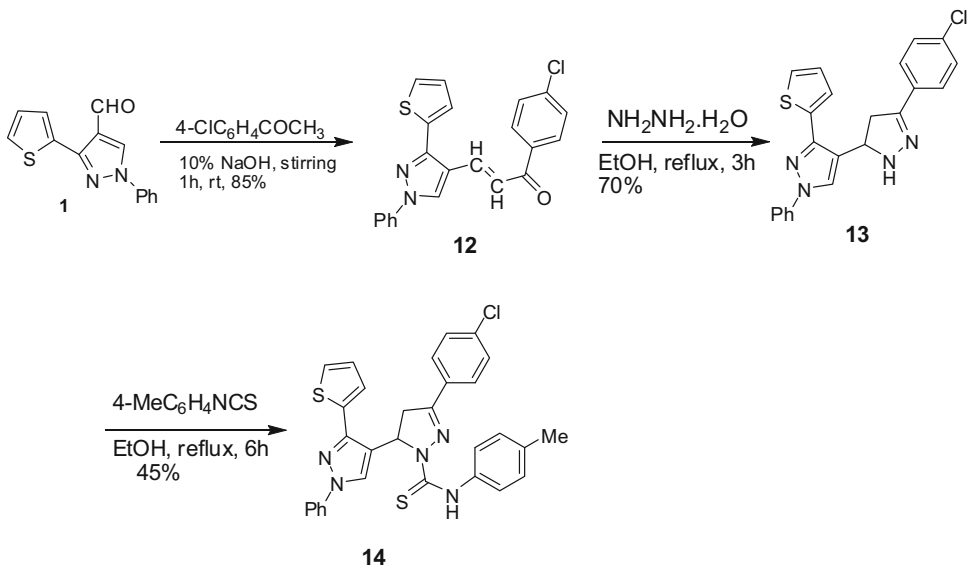


Table 2 Antimicrobial activity & Minimum inhibitory concentration (μg/ml)[a]

Chemical compound	*Staphylococcus aureus* ATCC 29213	*B. subtilis* ATCC6633	*Salmonella typhi*	*Enterobacter cloaca* ATCC3047	*Klebseilla peneumoniae* ATCC13883	*Pseudomonas. aeroginosa* ATCC27953	*E. coli* ATCC 25922	*Enterococcus faecalis* ATCC29212	*Mycobacterium phlei*	*Saccharomyces cervesia*	*Candida Albicans* NRRL Y-477
2	21(83.3)	29(20.8)	28(20.8)	26(20.8)	28(20.8)	15(-)	N.A.(-)	13(-)	16(-)	30(20.8)	16(-)
7a	21(166.6)	23(166.6)	N.A.(-)	15(-)	15(-)	14(-)	N.A.(-)	15(-)	N.A.(-)	N.A.(-)	N.A.(-)
7b	11(-)	21(166.6)	N.A.(-)	14(-)	14(-)	N.A.(-)	N.A.(-)	22(83.3)	N.A.(-)	N.A.(-)	N.A.(-)
8	28(20.8)	23(83.3)	N.A.(-)	28(20.8)	18(166.6)	15(-)	N.A.(-)	21(83.3)	N.A.(-)	N.A.(-)	15(-)
9	21(41.8)	22(166.6)	N.A.(-)	26(41.6)	18(166.6)	19(333.3)	N.A.(-)	16(-)	N.A.(-)	31(20.8)	17(166.6)
10	21(333.3)	26(41.6)	N.A.(-)	18(333.3)	N.A.(-)	28(41.6)	N.A.(-)	21(166.6)	15(-)	28(41.6)	N.A.(-)
11	29(20.8)	19(166.6)	N.A.(-)	13(-)	N.A.(-)	N.A.(-)	N.A.(-)	15(-)	N.A.(-)	29(41.6)	N.A.(-)
12	21(166.6)	21(166.6)	24(83.3)	24(41.6)	18(166.6)	19(333.3)	N.A.(-)	21(83.3)	15(-)	29(20.8)	18(166.6)
13	20(333.3)	17(333.3)	N.A.(-)	21(83.3)	N.A.(-)	26(166.6)	N.A.(-)	21(41.6)	N.A.(-)	31(20.8)	17(166.6)
14	15(-)	21(166.6)	N.A.(-)	16(-)	21(333.3)	N.A.(-)	N.A.(-)	21(166.6)	13(-)	N.A.(-)	15(-)
Ciprofloxacin	20(10.4)	22(10.4)	21(41.6)	25(20.8)	25(20.8)	24(20.8)	23(20.8)	23(20.8)	22(20.8)	N.A.(-)	N.A.(-)
Ketoconazole	N.A.(-)	N.A.(-)	N.A.(-)	N.A.(-)	N.A.(-)	N.A.(-)	N.A.(-)	N.A.(-)	N.A.(-)	23(5.2)	22(10.4)

[a] Antimicrobial activity expressed as inhibition diameter zones in millimeters; () Minimum inhibitory concentration (μg/ml);the experiment was carried out in triplicate and the average zone of inhibition was calculated

Table 3 CI_{50} for DPPH inhibition of chemical compounds

Chemical compounds	DPPH IC_{50} (μg/ml)
2	500
7a	25
7b	25
8	1000
9	25
10	500
11	500
12	1000
13	750
14	500

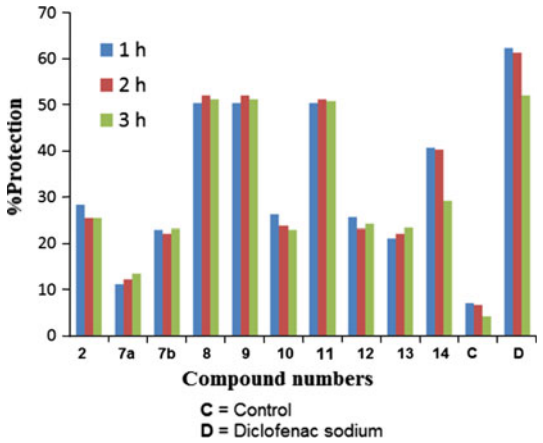


Fig. 1 Percent anti-inflammatory activity of the tested compounds (carrageenan-induced paw edema test in rats)

7b, and **9** were the strongest radical scavenger among the tested compounds with CI_{50} 25 μg/ml, followed by **2**, **10**, **11**, and **14** with CI_{50} 500 μg/ml. However, the other compounds were moderate radical scavengers with CI_{50} that ranged between 750 and 1000 μg/ml.

The anti-inflammatory activity

The anti-inflammatory activity of the synthesized pyrazoles was evaluated using carrageenan-induced paw edema test in rats. The anti-inflammatory activity data (Fig. 1) indicated that the most compounds protected rats from carrageenan-induced inflammation. Compounds **8**, **9**, and **11** were equipotent as diclofenac sodium in terms of anti-inflammatory activity. However, other compounds showed moderate activity. Compound **7a** was proved to be the least active among tested compounds.

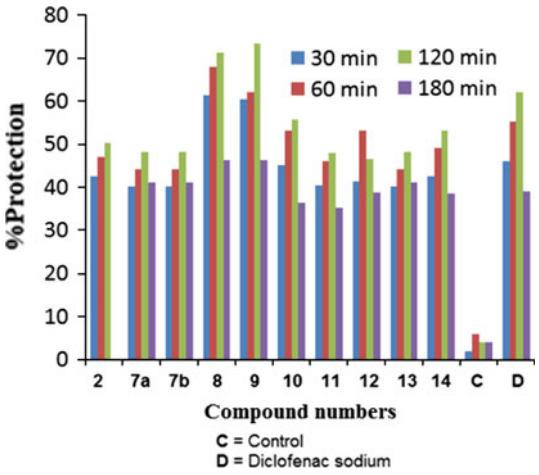


Fig. 2 Percent analgesic activity (peripheral, writhing test)

Analgesic activity

The analgesic activity was determined by the hot plate test (central analgesic activity) and acetic acid induced writhing assay. The obtained data (Fig. 2) revealed that all tested compounds exhibited good analgesic activity. Most of the tested compounds have comparable activity to diclofenac sodium. Compounds **8** and **9** exhibit activities higher than that of the reference drug in peripheral analgesic activity testing. The remaining compounds have strong to moderate activity.

Structure–activity Relationship (SAR) correlations

- Pyrazole nucleus is essential for all of the biological activities
- The antimicrobial and antioxidant activities of the synthesized thien-2-ylpyrazole derivatives are due to the presence of thiosemicarbazide fragment (compound **2**), chalcone (**12**), and thiazole substitute (**8**).
- The anti-inflammatory and analgesic activities are due to the presence of thiosemicarbazone moiety (compound **8**); fluorine substituent (compounds **9**), 4-thiazolidinone nucleus (compounds **11**), and coumarin residue (compounds **7b**).

Conclusion

The results of this investigation revealed that the observed increase in analgesic, anti-inflammatory, and antimicrobial activities are attributed to the presence of 3-thien-2-ylpyrazole nucleus, thiosemicarbazone, and 4-F in phenyl of synthesized compounds containing pyrazole. The data reported in this article may be a helpful guide for the medicinal chemists who are working in this area.

Experimental

Chemistry

All melting points were taken on Electrothermal IA 9000 series digital melting point apparatus. Elemental analytic data were carried from the microanalytic unit, National Research Centre, Dokki, Giza, Egypt. The IR spectra were recorded in potassium bromide disks on a Shimadzu CVT-04 spectrophotometer. The ^{1}H NMR spectra were recorded at 270 MHz on a Varian EM-360 spectrometer using TMS as an internal standard. Chemical shifts values (δ) are given in parts per million (ppm). The electron impact mass spectra were performed using mass Varian MAT CH-5 spectrometer at 70 eV. 1-Phenyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carbaldehyde **1** (Bratenko *et al.*, 2005), 1-(benzofuran-2-yl)-2-bromoethanone **3a** (Shriner and Anderson, 1939), 3-(2-bromoacetyl)-2*H*-chromen-2-one **3b** (Koelsch, 1950), 2-chloro-*N*-(thiazol-2-yl)acetamide **4** (Abdel-Rahman *et al.*, 1981), *N*′-(4-fluorophenyl)-2-oxopropanehydrazonoyl chloride **5** (Biere *et al.*, 1982), and 2,3-dichloroquinoxaline **6** (Krishnan *et al.*, 1999) were prepared according to the reported procedures.

*2-((1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)methylene)hydrazinecarbothioamide (**2**)*

Compound **1** (2.54 g, 10 mmol) was dissolved in absolute ethanol (20 ml) and thiosemicarbazide (0.91 g, 10 mmol) was added. Three drops of glacial acetic acid was also added. The reaction mixture was refluxed for 5 h. The solid obtained was isolated by filtration, washed with ethanol, dried, and recrystallized from (EtOH-DMF; 2:1 by v/v) to give **2**.

IR (KBr) ν_{max}/cm^{-1} 3330–3172 (NH_2, NH); ^{1}H NMR (DMSO-d_6) δ 7.48–7.69 (m, 8H, Ar–H), 7.80 (s, 1H, pyrazole-H), 8.32 (s, 1H, C<u>H</u> = N), 9.16 (s, 2H, NH_2, D_2O-exchangeable), 11.51 (s, H, NH, D_2O-exchangeable); MS *m/z* (%): 327 (M^+, 33), 63 (100%).

*Synthesis of thiazoles **7–10***

Equimolar amounts of **2** (0.33 g, 1 mmol) and appropriate phenacyl bromides **3**; 2-chloro-*N*-(thiophen-2-yl)acetamide **4**, *N*′-(4-fluorophenyl)-2-oxopropanehydrazonoyl chloride **5**, or 2,3-dichloroquinoxaline **6** (1 mmol) in absolute ethanol (30 cm^3) {in the presence of few drops of triethylamine as a catalyst in the case of **5**} was heated under reflux for 3 h, then left to cool. The solid formed was

isolated by filtration, washed with ethanol, dried, and recrystallized from EtOH-DMF.

*4-(Benzofuran-2-yl)-2-{[(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)methylene]hydrazono}-2,5-dihydrothiazole (**7a**)*

IR (KBr) ν_{max}/cm^{-1} 3185 (NH); ^1H NMR (DMSO-d_6) δ 5.12 (s, 1H, thiazole-CH), 7.02 (s, 1H, benzofuryl-H), 7.21–7.81 (m, 12H, Ar–H), 7.91 (s, 1H, pyrazole-H), 8.27 (s, 1H, C<u>H</u> = N), 11.33 (s, H, NH, D_2O-exchangeable); MS *m/z* (%): 467 (M^+, 2.5), 76 (100%).

*3-(2-{[(1-Phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)methylene]hydrazono}-2,5-dihydrothiazol-4-yl)-2H-chromen-2-one (**7b**)*

IR (KBr) ν_{max}/cm^{-1} 1679 (C=O), 3181 (NH); ^1H NMR (DMSO-d_6) δ 6.32 (s, 1H, thiazole-CH), 7.19–7.76 (m, 12H, Ar–H), 7.90 (s, 1H, pyrazole-H), 8.10 (s, 1H, coumarinyl-H), 8.62 (s, 1H, C<u>H</u> = N), 11.99 (s, H, NH, D_2O-exchangeable); MS *m/z* (%): 495 (M^+, 4.5), 76 (100%).

*2-Oxo-2-(thiophen-2-ylamino)ethyl N′-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)methylenecarbamohydrazonothioate (**8**)*

IR (KBr) ν_{max}/cm^{-1} 1668 (C=O), 3181–3390 (NH_2,NH); ^1H NMR (DMSO-d_6) δ 3.91 (s, 2H, CH_2), 7.15 (d, 1H, thiazole-CH), 7.16 (d, 1H, thiazole-H), 7.36–7.61 (m, 8H, Ar–H), 7.88 (s, 1H, pyrazole-H), 8.52 (s, 1H, C<u>H</u> = N), 8.95 (s, 2H, NH_2, D_2O-exchangeable), 11.76 (s, H, NH, D_2O-exchangeable); MS *m/z* (%): 466 (M^+, 0.5), 76 (100%).

*5-[2-(4-Fluorophenyl)hydrazono]-4-methyl-2-{[(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)methylene]hydrazono}-2,5-dihydrothiazole (**9**)*

IR (KBr) ν_{max}/cm^{-1} 3199 (NNH); ^1H NMR (DMSO-d_6) δ 2.67 (s, 3H, CH_3), 7.38–7.92 (m, 12H, Ar–H), 8.08 (s, 1H, pyrazole-H), 9.01 (s, 1H, C<u>H</u> = N), 10.56 (s, 1H, NH, D_2O-exchangeable); MS *m/z* (%): 487 (M^+, 8), 62 (100%).

*2-{2-[(1-Phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)methylene]hydrazinyl}thiazolo[4,5-b]quinoxaline (**10**)*

IR (KBr) ν_{max}/cm^{-1} 3218 (NNH); ^1H NMR (DMSO-d_6) δ 7.23–7.90 (m, 12H, Ar–H), 8.79 (s, 1H, pyrazole-H), 9.11 (s, 1H, C<u>H</u> = N), 11.15 (s, 1H, NH, D_2O-exchangeable); MS *m/z* (%): 453 (M^+, 2), 76 (100%).

*2-{[(1-Phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)methylene]hydrazono}thiazolidin-4-one (**11**)*

A mixture of **2** (0.33 g, 1 mmol) and chloroacetic acid (0.1 g, 1 mmol) in glacial acetic acid (30 ml) containing anhydrous sodium acetate (0.33 g, 4 mmol) was heated under reflux for 6 h. The reaction mixture was cooled, and the solid formed was filtered and recrystallized from ethanol to give **11**. IR (KBr) ν_{max}/cm^{-1} 3193 (NNH), 1689 (C=O); ^1H NMR (DMSO-d_6) δ 3.91 (s, 2H, thiazolidinone-CH_2), 7.14–7.90 (m, 8H, Ar–H), 8.51 (s, 1H, pyrazole-H), 8.95 (s, 1H, C<u>H</u> = N), 11.82 (s, 1H, NH, D_2O-exchangeable); MS *m/z* (%): 367 (M^+, 30), 62 (100%).

*1-(4-Chlorophenyl)-3-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)prop-2-en-1-one (**12**)*

A suspension of **1** (2.5 g, 10 mmol) and 4-chloroacetopheneone (1.54 g, 10 mmol) in ethanol (20 ml) containing sodium hydroxide (10%; 5 ml) was stirred for 1 h at room temperature and then left overnight to stand. The solid obtained was filtered, dried, and recrystallized from EtOH-DMF (5:1 by volume) to give **12**. IR (KBr) ν_{max}/cm^{-1} 1732 (C=O); ^1H NMR (DMSO-d_6) δ 6.54 (d, 1H, CH, J = 8.5 Hz), 6.62 (d, 1H, CH, J = 8.5 Hz), 7.15–7.64 (m, 12H, Ar–H), 8.31 (s, H, pyrazole-H); MS *m/z* (%): 392 (M^+, 52), 63 (100).

*4-(3-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-3-(thiophen-2-yl)-1H-pyrazole (**13**)*

A mixture of **12** (0.39 g, 1 mmol), hydrazine hydrate (0.5 g, 10 mmol) in absolute ethanol (20 ml) was heated under reflux for 3 h. The reaction mixture was left to cool and the solid formed was filtered, washed with ethanol, dried and recrystallized from ethanol to give **13**.^1H NMR (DMSO-d_6) δ 3.38 (dd, 1H, CH, J = 16.8; 7.02 Hz), 4.05 (dd, 1H, CH, J = 16.7; 6.98 Hz), 6.21 (t, 1H, CH), 7.15–7.98 (m, 12H, Ar–H), 8.15 (s, H, pyrazole-H), 10.01 (s, H, NH, D_2O-exchangeable); MS *m/z* (%): 404 (M^+, 18), 63 (100).

*3-(4-Chlorophenyl)-5-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-N-p-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**14**)*

A mixture of **13** (0.4 g, 1 mmol), *p*-tolyl isothiocyanate (1 mmol) in absolute ethanol (20 ml) was heated under reflux for 6 h. The solid was filtered, washed with ethanol, dried, and recrystallized from EtOH-DMF (1:2 by volume) to give **14**. ^1H NMR (DMSO-d_6) δ 2.46 (s, 3H, CH_3), 3.36 (dd, 1H, CH, J = 16.8; 7.02 Hz), 4.02 (dd, 1H, CH, J = 16.7; 6.98 Hz), 6.23 (t, 1H, CH), 7.16–7.97 (m, 16H,

Ar–H), 8.16 (s, H, pyrazole-H), 10.11 (s, H, NH, D_2O-exchangeable); MS *m/z* (%): 554 (M^+, 3), 63 (100).

Antimicrobial activity

The synthetic compounds were tested for their antimicrobial activities against the following microorganisms: *Staphylococcus aureus* ATCC 29213; *B. subtilis* ATCC6633; *Salmonella typhi*; *Enterobacter Cloaca* ATCC3047; *Klebseilla peneumoniae* ATCC13883; *Pseudomonas. Aeroginosa* ATCC27953; *E. coli* ATCC 25922; *Enterococcus faecalis* ATCC29212; *Mycobacterium phlei*; *Saccharomyces Cervesia*, and *Candida Albicans* NRRL Y-477. The agar well diffusion method (Perez *et al.,* 1990) was applied using 100 µl of suspension containing 1×10^8 CFU/ml of pathological tested bacteria, 1×10^6 CFU/ml of yeast, and 1×10^4 spore/ml of fungi spread on nutrient agar (NA), Sabourand dextrose agar (SDA), and potato dextrose agar (PDA) medium, respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 µl of tested compound solution prepared by dissolving 50 mg of the chemical compound in one ml of dimethyl sulfoxide (DMSO) as solvent. The inculcated plates were then incubated for 24 h at 37°C for bacteria and 48 h at 28°C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. Ciprofloxacin (50 µg/ml) and Ketoconazole (50 µg/ml) were used as standards for antibacterial and antifungal activity, respectively. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. The observed zone of inhibition is presented in Table 2. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Minimal Inhibitory Concentration (MIC)

The bacterio-static activity of the active compounds (having inhibition zones (IZ) $\geq$ 16 mm) was then evaluated using the two fold serial dilution technique (Scott, 1989). Two fold serial dilutions of the tested compound solutions were prepared using the proper nutrient broth. The final concentrations of the solutions were 333.3; 166.6; 83.3 41.6; and 20.8 µg/ml. The tubes were then inoculated with the test organisms, grown in their suitable broth at 37°C for 24 h, which contain 1×10^8 CFU/ml of bacteria; 1×10^6 CFU/ml of yeast; and 1×10^4 spore/ml of fungi), each 5 ml received 0.1 ml of the above inoculum and incubated at 37°C for 24 h. The lowest concentration showing no growth was taken as the MIC.

DPPH free radical and scavenging activity

The hydrogen atom or electron donation ability of the corresponding compounds was measured from the bleaching of purple colored of methanolic solution of DPPH. This spectrophotometric assay uses stable radical diphenylpicrylhydrazyl (DPPH) as a reagent (Cuendet *et al*., 1997; Burits and Bucar, 2000). Different concentrations of the tested compounds were dissolved in methanol to obtain a final concentration (25, 50, 50, 250, 500, 750, and 1000 µg/ml) to determine CI_{50} (concentrations make 50% inhibition of DPPH color). Fifty microliters of various sample concentrations were added to 5 ml of 0.004% methanolic solution of DPPH. After 60 min of incubation at dark, the absorbance was measured against a blank at 517 nm. Inhibition free radical DPPH in percent (*I* %) was calculated using Eq. 2:

$$I\% = \left(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}\right) \times 100 \quad (2)$$

where A_{blank} is the absorbance of the control reaction, and A_{sample} is the absorbance of the test sample.

Anti-inflammatory

Animals

Female Sprague-Dawley rats (150–200 g) were used in the study of anti-inflammatory activity. Both sexes of Swiss mice (25–30 g) were used in analgesic activity, taking into account international principle and local regulations concerning the care and use of laboratory animals. The animals had free access to standard commercial diet and water ad libitum, and were kept in rooms maintained at 22 ± 1°C with 12 h light dark cycle.

Anti-inflammatory activity (carrageenan-induced rat hind paw edema model)

The method adopted resembles essentially that described by (Olfert *et al*., 1993); distilled water was selected as vehicle to suspend the standard drugs and the synthesized compounds. The albino rats weighing between 150 and 180 g was starved for 18 h before the experiment. The animals were weighed, marked for identification, and divided into 15 groups each group containing five animals. Edema was induced in the left hind paw of all rats by subcutaneous injection of 0.1 ml of 1% (W/V) carrageenan in distilled water into their footpads. The first group was kept as control and was given the prescribed volume of the solvent (0.5 ml distilled water). The 2nd–14th groups were orally administered aqueous suspension of the synthesized compounds (dose; 20 mg/kg) 1 h before carrageenan injection. The last group (standard) was administered

diclofenc sodium in a dose of 20 mg/kg, orally as aqueous suspension (Abu-Hashem *et al.*, 2010). The paw volume of each rat was measured immediately by mercury plethysmometer, before carrageenan injection and then hourly for 4-h post administration of aqueous suspension of the synthesized compounds. The edema rate and inhibition rate of each group were calculated as follows, (Edema rate $(E)\% = V_t - V_0/V_0$, Inhibition rate $(I)\% = E_c - E_t/E_c$ where V_t is the volume before carrageenan injection (ml), V_t is the volume at t hours after carrageenan injection (ml), and E_c and E_t are the edema rates of control and treated groups, respectively.

Analgesic activity (acetic acid induced writhing response model)

The compounds were selected for investigating their analgesic activities in acetic acid induced writhing response in Swiss albino mice, following the method of (Collier *et al.*, 1968). One hundred and two mice were divided into 15 groups (5 in each group) starved for 16 h pretreated as follows: the first group which served as control positive was orally administered distilled water in appropriate volumes. The 2nd–14th groups were administered the aqueous suspension of synthesized compounds orally at a specified dose (20 mg/kg). The last group was orally administered diclofenac sodium in a dose of 20 mg/kg. After 30 min, each mouse was administrated 0.7% of an aqueous solution of acetic acid (10 ml/kg) and the mice were then placed in transparent boxes for observation. The number of writhes was counted for 20 min after acetic acid injection. The number of writhes in each treated group was compared to that of a control group. The number of writhing was recorded and the percentage protection was calculated using the following ratio (%) protection = (control mean-treated mean/control mean) × 100

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