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Original research paper

Novel ethyl 1,5-disubstituted-1*H*-pyrazole-3-carboxylates as a new class of antimicrobial agents

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A series of pyrazole derivatives **9–22** were designed and synthesized. All the newly synthesized compounds were assayed for their antimicrobial activity against the Gram-positive bacteria *Staphyllococcus aureus* and *Bacillius subtilis* and the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, in addition to the fungi organisms, *Candida albicans*, *C. parapsilosis and C. tropicalis*. Ethyl 5-(2,5-dimethylthiophen-3-yl)-1-phenyl-1*H*-pyrazole-3-carboxylate (**21**) ($MIC_{E.coli}$ = 0.038 μmol mL^{-1}, $MIC_{P.\ aerug.}$ = 0.067 μmol mL^{-1}) is nearly as active as ampicillin (*MIC* = 0.033 and 0.067 μmol mL^{-1}), respectively. Ethyl 5-(4-bromo-2-chlorophenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (**16**) (*MIC* = 0.015 μmol mL^{-1}) is more active than fluconazole (0.020 μmol mL^{-1}) as a reference drug against *C. parapsilosis*.

Keywords: pyrazole-3-carboxylates, antimicrobial activity

Nitrogen heterocycles are of special interest because they constitute an important class of natural and non-natural products, many of which exhibit useful biological activities. Pyrazoles and their derivatives exhibit antimicrobial (1–3), anticancer (4), antiinflammatory (5) radioprotective (6), anticonvulsant (7) and antimalarial (8) activities. In the light of these facts, and as a continuation of our efforts towards synthesizing biologically active heterocyclic compounds (9–18), we aimed to prepare new derivatives of pyrazoles. The compounds were designed with the aim of exploring their antimicrobial activity and to study their structure-activity relationship.

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EXPERIMENTAL

Melting points were uncorrected and determined on an electrothermal melting point apparatus (Stuart Scientific, UK). Precoated silica gel plates (Kieselgel 0.25 mm, 60G F254, Merck, Germany) were used for thin layer chromatography. Developing solvent system was composed of chloroform/methanol (8:2) and the spots were detected under UV. IR spectra (KBr discs) were recorded on a FT-IR spectrophotometer (Perkin Elmer, USA). NMR spectra were scanned on a NMR spectrophotometer (Bruker, Switzerland) operating at 500 MHz for ^{1}H- and 125.76 MHz for ^{13}C NMR. Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard, using DMSO-d_6 as a solvent. Elemental analyses were done on a model 2400 CHNSO analyzer (Perkin Elmer, USA). All reagents were purchased from Sigma (USA). Compounds **1** and **9** were prepared according to the reported methods (19, 20).

*Synthesis of ethyl 4-(substituted phenyl)-2-hydroxy-4-oxobut-2-enoates (**1**–**8**)*

Ethanol (100 mL) was converted to sodium ethoxide by portionwise addition of sodium (0.46 g, 0.02 mol) before a solution of diethyl oxalate (2.92 g, 0.02 mol) and acetophenone derivative, namely, 4-chloroacetophenone, 3,4-dimethoxyacetophenone, 2,5-dimethoxyacetophenone, 2,5-dichloroacetophenone, 2-chloro-4-bromoacetophenone, 1-(naphthalene-1-yl)ethanone, 1-(thiophen-2-yl)ethanone and/or 1-(2,5-dimethylthiophene-3-yl)ethanone (0.01 mol) in ethanol (50 mL) was added dropwise at 50 °C. The reaction mixture was heated under reflux for 2-3 h. After cooling the solvent was removed and the residue was taken up in water (200 mL) and acidified with concentrated HCl (1 mL). The aqueous mixture was extracted with diethyl ether (3x150 mL). The combined extracts were washed with brine (100 mL), dried ($MgSO_4$), and concentrated. The obtained solid was recrystallized from methanol to give compounds **1–8**, respectively.

*Synthesis of ethyl 1,5-disubstituted-1*H*-pyrazole-3-carboxylates (**9**–**22**)*

A mixture of compound **1–8** (0.01 mol) with arylhydrazine derivative (0.012 mol) in ethanol (50 mL) was refluxed for 8 h. The reaction mixture was cooled and poured into ice water. The obtained solid was recrystallized from dioxane to give compounds **9–22,** respectively.

Physico-chemical, analytical and spectral data are displayed in Tables I and II. Synthetic pathway is presented in Scheme 1.

Staphyloccocus aureus (NCTC-7447) and *Bacillius subtilis* (NCTC 1040) and Gram-negative bacteria *E. coli* (NCTC 10416), and *Pseudomonas aeruginosa* (ATCC 10145), in addition to the fungi organisms, *Candida albicans* (ATCC-10231), *Candida parapsilosis* (ATCC-22019) and *Candida tropicalis* (ATCC-66029) were tested. All microorganisms were purchased from American Type Culture Collection (Manassas, USA).

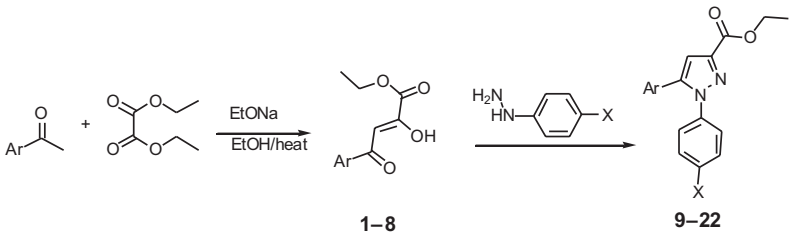
The antimicrobial activity screening of the newly synthesized compounds (**9–22**) was undertaken using the agar cup diffusion (8 mm diameter) assay I (21). The agar media were inoculated with test organisms and a solution of test compound 100 μg mL^{-1} in DMSO. Ampicillin (100 and 50 μg mL^{-1}), and fluconazole (2 and 1 μg mL^{-1}) were used as reference drugs for antibacterial and antifungal activity, respectively. The zones of inhibition were measured after 24 h of incubation.

For determination of minimum inhibitory concentration (*MIC*) (21) by serial plate dilution method, five milligrams of each test compounds were dissolved in 1 mL of dimethylsulfoxide (DMSO) separately to prepare stock solution. From stock solution serial dilutions were prepared. The plates were incubated at 37 °C for 24 h. *MIC* is the lowest concentration (μmol mL^{-1}) of the test compound that resulted in no visible growth on the plates. DMSO was used as a solvent control to ensure that solvent had no effect on bacterial growth. The results of the antimicrobial activities are summarized in Table III.

RESULTS AND DISCUSSION

Chemistry

β-Diketones **1–8** were prepared by Claisen reaction between acetophenone derivatives and diethyl oxalate (Scheme 1). The synthesis of 1,5-diarylpyrazoles **9–22** was done by regioselective cyclization of arylhydrazines with *β*-diketones **1–8** in refluxing ethanol. Tables I and II show the physicochemical and the spectral data. The physicohemical data of compounds **1** and **9** are in accordance with those reported in the literature (19, 20). The structures of compounds **2–8** and **10–22** were confirmed by elemental analyses, IR, ^{1}H NMR and ^{13}C NMR spectral data. IR spectra of compounds **2–8** showed the presence of characteristic bands for OH (3462–3421 cm^{-1}), 2C=O (1751–1708 and 1685–1625 cm^{-1}). ^{1}H NMR spectra of compounds **2–8** revealed the presence of a triplet at 1.2–1.3 ppm assigned to CH_3 ester and a quartet at 4.1–4.4 ppm due to CH_2 ester. Mass spectrum of compound **2** showed



1–8 **9–22**

Compd.	Ar
1	C_6H_4Cl-4
2	$C_6H_3(OCH_3)_2$-3,4
3	$C_6H_3(OCH_3)_2$-2,5
4	$C_6H_3Cl_2$-2,5
5	C_6H_3Cl-2, Br-4
6	1-naphthyl
7	2-thienyl
8	3-thienyl-$(CH_3)_2$-2,5

Compd.	Ar	X
9	C_6H_4Cl-4	H
10	C_6H_4Cl-4	Br
11	$C_6H_3(OCH_3)_2$-3,4	H
12	$C_6H_3(OCH_3)_2$-3,4	Br
13	$C_6H_3(OCH_3)_2$-2,5	H
14	$C_6H_3(OCH_3)_2$-2,5	Br
15	$C_6H_3Cl_2$-2,5	H
16	C_6H_3Cl-2; Br-4	H
17	C_6H_3Cl-2; Br-4	Br
18	1-naphthyl	H
19	2-thienyl	H
20	2-thienyl	Br
21	3-thienyl-$(CH_3)_2$-2,5	H
22	3-thienyl-$(CH_3)_2$-2,5	Br

Scheme 1

a molecular ion peak *m/z* at 280 (M^+, 5.70 %) with a base peak at *m/z* 69 (100 %), and another significant peaks appeared at *m/z* 263 (30 %), 235 (21 %), 190 (12 %). IR spectra of **10**–**22** exhibited the presence of bands at 1702–1739 cm^{-1} for C=O and at 1584–1604 cm^{-1} for C=N. ^1H NMR spectra revealed the presence of a triplet at 1.1–1.6 ppm for CH_3 ester and a quartet at 4.2 –4.5 ppm for CH_2 ester. Mass spectrum of compound **14** showed a molecular ion peak *m/z* at 431 (M^+, 9.25 %), 433 (M^++2, 8.85 %) with a base peak at *m/z* 76 (100 %) in addition to other significant peaks at *m/z* 396 (26 %), 368 (18 %), 288 (31 %), 152 (14 %).

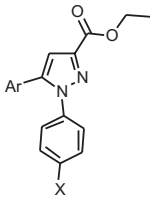


Table I. Physical and analytical data for newly synthesized compounds

Compd.	Ar	X	Formula (M_r)	M. p. (°C)	Yield (%)	Analysis (calcd./found) (%)		
						C	H	N
2	$C_6H_3(OCH_3)_2$-3,4	H	$C_{14}H_{16}O_6$ (280.27)	156–157	76	59.99/59.58	5.75/5.49	–
3	$C_6H_3(OCH_3)_2$-2,5	H	$C_{14}H_{16}O_6$ (280.27)	217–218	69	59.99/59.71	5.75/5.98	–
4	$C_6H_3Cl_2$-2,5	H	$C_{12}H_{10}Cl_2O_{42}$ (289.11)	78–79	88	49.85/49.63	3.49/3.16	–
5	C_6H_3Cl-2, Br-4	H	$C_{12}H_{10}BrClO_4$ (333.56)	90–91	65	43.21/43.46	3.02/3.25	–
6	1-naphthyl	H	$C_{10}H_{14}O_4$ (270.28)	120–121	69	71.10/71.32	5.22/5.46	–
7	2-thienyl	H	$C_{10}H_{10}O_4S$ (226.25)	86–87	78	53.09/53.36	4.46/4.15	–
8	3-thienyl-$(CH_3)_2$-2,5	H	$C_{12}H_{14}O_4S$ (254.30)	208–209	66	56.68/56.36	5.55/4.78	–
10	C_6H_4Cl-4	Br	$C_{18}H_{14}BrClN_2O_2$ (403.99)	117–118	89	53.29/53.54	3.48/3.72	6.91/6.63
11	$C_6H_3(OCH_3)_2$-3,4	H	$C_{20}H_{20}N_2O_4$ (352.38)	177–178	88	68.17/67.82	5.72/5.99	7.95/8.35
12	$C_6H_3(OCH_3)_2$-3,4	Br	$C_{20}H_{19}BrN_2O_4$ (431.28)	> 300	75	55.70/55.94	4.44/4.31	6.50/6.21
13	$C_6H_3(OCH_3)_2$-2,5	H	$C_{20}H_{20}N_2O_4$ (352.38)	156–157	75	68.17/68.51	5.72/5.49	7.95/7.66
14	$C_6H_3(OCH_3)_2$-2,5	Br	$C_{20}H_{19}BrN_2O_4$ (431.28)	136–137	68	55.70/55.45	4.44/4.21	6.50/6.77

15	$C_6H_3Cl_2$-2,5	H	$C_{18}H_{14}Cl_2N_2O_2$ (361.22)	153–154	73	59.85/59.53	3.91/3.65	7.76/7.48
16	C_6H_3Cl-2, Br-4	H	$C_{18}H_{14}BrClN_2O_2$ (405.67)	> 300	70	53.29/53.56	3.48/3.18	6.91/6.68
17	C_6H_3Cl-2, Br-4	Br	$C_{18}H_{13}Br_2ClN_2O_2$ (481.90)	> 300	65	44.62/44.26	2.70/2.51	5.78/5.47
18	1-naphthyl	H	$C_{22}H_{18}N_2O_2$ (341.39)	> 300	82	77.17/77.50	5.30/5.12	8.18/8.48
19	2-thienyl	H	$C_{16}H_{14}N_2O_2S$ (298.36)	176–178	78	64.41/64.12	4.73/4.49	9.39/9.65
20	2-thienyl	Br	$C_{16}H_{13}BrN_2O_2S$ (377.26)	110–111	86	50.94/50.69	3.47/3.72	7.43/7.19
21	3-thienyl-$(CH_3)_2$-2,5	H	$C_{18}H_{18}N_2O_2S$ (326.41)	168–169	77	66.23/66.51	5.56/5.24	8.58/8.91
22	3-thienyl-$(CH_3)_2$-2,5	Br	$C_{18}H_{17}BrN_2O_2S$ (405.31)	191–192	83	53.34/53.65	4.23/4.00	6.91/6.66

Table II. Spectral characterization of the newly synthesized compounds

Compd.	IR (ν_{max}, cm^{-1})	^1H NMR (DMSO-d_6) ^{13}CNMR (DMSO-d_6) (δ, ppm)	Mass (*m*/*z*, %)
2	3421 (OH), 3083 (CH arom.), 2939, 2836 (CH aliph.), 1721, 1625 (2C=O)	1.2 [t, 3H, CH_3, *J* = 7.0 Hz], 3.9 [s, 6H, $2OCH_3$], 4.3 [q, 2H, CH_2, *J* = 6.9 Hz], 6.9 [s, 1H, CH], 7.0-7.5 [m, 3H, Ar-H], 15.3 [s, 1H, OH, D_2O exchangeable] 14.1, 56.1(2), 62.5, 97.9, 109.9, 110.4, 122.8, 128.0, 149.3, 154.1, 162.4, 167.2, 190.7	280 [M^+], 69 (100)
3	3426 (OH), 3098 (CH arom.), 2953, 2840 (CH aliph.), 1719, 1678 (2C=O)	1.3 [t, 3H, CH_3, *J* = 7.2 Hz], 3.8 [s, 6H, $2OCH_3$], 4.3 [q, 2H, CH_2, *J* = 6.9 Hz], 7.1-7.3 [m, 4H, Ar-H + CH], 14.8 [s, 1H, OH, D_2O exchangeable] 13.7, 56.0(2), 62.9, 113.2, 113.6, 114.2, 124.3, 125.2, 153.0, 153.1, 163.3, 189.2, 190.7. Mass (*m*/*z*, %) 280 (M^+), 165 (100)	471 [M^+] (31.6), 65 (100)
4	3421 (OH), 3100 (CH arom.), 2939, 2876 (CH aliph.), 1730, 1638 (2C=O), 779 (C-Cl)	1.3 [t, 3H, CH_3, *J* = 7.1 Hz], 4.3 [q, 2H, CH_2, *J* = 7.2 Hz], 6.8-7.5 [m, 4H, Ar-H + CH], 14.6 [s, 1H, OH, D_2O exchangeable] 14.0, 62.8, 102.7, 130.0, 130.3, 132.1, 132.5, 133.3, 136.9, 161.6, 168.4, 191.1	289 [M^+], 93 (100)
5	3421 (OH), 3100 (CH arom.), 2950, 2860 (CH aliph.), 1708, 1683 (2C=O), 723 (C-Cl)	1.3 [t, 3H, CH_3, *J* = 6.8 Hz], 4.1 [q, 2H, CH_2, *J* = 7.0 Hz], 6.7-8.0 [m, 4H, Ar-H + CH], 15.2 [s, 1H, OH, D_2O exchangeable] 15.0, 58.8, 109.4, 126.4, 127.9, 128.7, 129.1, 130.1, 135.1, 165.3, 187.6, 188.1	333 [M^+], 43 (100)

6	3446 (OH), 3085 (CH arom.), 2950, 2825 (CH aliph.), 1743, 1654 (2C=O)	1.3 [t, 3H, CH_3, *J* = 7.1 Hz], 4.2 [q, 2H, CH_2, *J* = 6.9 Hz], 6.8-8.1 [m, 7H, Ar-H], 8.2 [s, 1H, CH], 15.1 [s, 1H, OH, D_2O exchangeable] 13.8, 62.2, 102.6, 123.0, 127.1, 127.6, 128.9, 129.8, 130.1, 132.1, 133.3, 134.1, 135.2, 161.7, 190.2, 198.2	270 [M^+], 54 (100)
7	3462 (OH), 3110 (CH arom.), 2963, 2861 (CH aliph.), 1751, 1685 (2C=O)	1.3 [t, 3H, CH_3, *J* = 6.9 Hz], 4.3 [q, 2H, CH_2, *J* = 7.2 Hz], 6.8-7.7 [m, 4H, Ar-H + CH], 14.8 [s, 1H, OH, D_2O exchangeable] 13.7, 62.1, 97.9, 129.1, 130.3, 133.0, 139.0, 161.4, 168.9, 188.8	226 [M^+], 69 (100)
8	3430 (OH), 3100 (CH arom.), 2930, 2871 (CH aliph.), 1708, 1641 (2C=O)	1.3 [t, 3H, CH_3, *J* = 7.3 Hz], 2.3 [s, 6H, $2CH_3$], 4.4 [q, 2H, CH_2, *J* = 7.1 Hz], 6.6-8.0 [m, 2H, Ar-H + CH], 11.4 [s, 1H, OH, D_2O exchangeable] 10.0, 13.8, 14.3, 61.3, 109.5, 123.0, 135.1, 143.5, 155.2, 160.4, 186.1, 188.3	254 [M^+], 43 (100)
10	3098 (CH arom.), 2965, 2861 (CH aliph.), 1714, (C=O), 1608 (C=N), 765 (C-Cl)	1.3 [t, 3H, CH_3, *J* = 7.6 Hz], 4.1 [q, 2H, CH_2, *J* = 7.5 Hz], 6.9-8.1 [m, 9H, Ar-H + CH pyrazole] 14.0, 63.2, 115.2, 120.8 (2), 126.1, 126.9 (2), 127.7, 128.6, 129.6 (2), 130.6, 133.5, 138.1, 141.0, 144.1, 163.5	403 [M^+], 78 (100)
11	3091 (CH arom.), 2982, 2951 (CH aliph.), 1721, (C=O), 1589 (C=N)	1.1 [t, 3H, CH_3, *J* = 7.6 Hz], 3.6 [s, 6H, $2OCH_3$], 4.2 [q, 2H, CH_2, *J* = 7.4 Hz], 6.9-8.0 [m, 9H, Ar-H + CH pyrazole] 12.5, 56.4, 55.8, 63.2, 110.1, 112.1, 115.2, 116.8, 118.1, 123.1 (2), 126.2, 128.6 (2), 140.4, 140.6, 146.8, 150.6, 151.3, 165.7	352 [M^+], 77 (100)
12	3078 (CH arom.), 2950, 2835 (CH aliph.), 1722, (C=O), 1610 (C=N)	1.4 [t, 3H, CH_3, *J* = 7.3 Hz], 3.5 [s, 6H, 2 OCH_3], 4.4 [q, 2H, CH_2, *J* = 7.3 Hz], 6.9-8.1 [m, 9H, Ar-H + CH pyrazole] 14.2, 56.2, 56.3, 62.4, 111.6, 112.6, 114.6, 118.3, 119.7, 122.1, 126.3 (2), 127.5 (2), 139.7, 139.8, 144.6, 151.5, 153.1, 164.2	431 (M^+), 42 (100)
13	3100 (CH arom.), 2948, 2835 (CH aliph.), 1739, (C=O), 1584 (C=N)	1.3 [t, 3H, CH_3, *J* = 7.1 Hz], 3.8 [s, 6H, $2OCH_3$], 4.4 [q, 2H, CH_2, *J* = 7.2 Hz], 6.6-7.8 [m, 9H, Ar-H + CH pyrazole] 13.8, 55.2, 55.3, 62.9, 110.7, 111.5, 115.8, 116.3, 119.0, 123.7 (2), 127.8, 128.4 (2), 140.0, 140.9, 144.2, 150.1, 152.8, 163.1	352 [M^+], 59 (100)
14	3100 (CH arom.), 2978, 2886 (CH aliph.), 1718, (C=O), 1602 (C=N)	1.1 [t, 3H, CH_3, *J* = 7.5 Hz], 3.7 [s, 6H, 2 OCH_3], 4.5 [q, 2H, CH_2, *J* = 7.4 Hz], 6.7-7.5 [m, 9H, Ar-H + CH pyrazole] 13.8, 55.2, 55.3, 62.9, 110.7, 112.8, 115.8, 116.7, 119.0, 123.7, 127.8 (2), 128.4 (2), 140.0, 140.9, 144.2, 150.1, 152.8, 163.1	431 [M^+], 76 (100)
15	3095 (CH arom.), 2983, 2872 (CH aliph.), 1702, (C=O), 1587 (C=N), 757 (C-Cl)	1.2 [t, 3H, CH_3, *J* = 7.2 Hz], 4.2 [q, 2H, CH_2, *J* = 7.1 Hz], 6.8-8.0 [m, 9H, Ar-H + CH pyrazole] 14.2, 62.9, 111.5, 121.7 (2), 124.3, 128.5, 129.5 (2), 130.2, 130.4, 131.1, 131.8, 132.6, 136.4, 140.4, 143.4, 161.3	361 [M^+], 77 (100)
16	3098 (CH arom.), 2970, 2836 (CH aliph.), 1708, (C=O), 1604 (C=N), 779 (C-Cl)	1.1 [t, 3H, CH_3, *J* = 7.4 Hz], 4.4 [q, 2H, CH_2, *J* = 7.4 Hz], 7.5-8.2 [m, 9H, Ar-H + CH pyrazole] 15.0, 64.8, 115.5, 117.9 (2), 126.8, 128.0, 129.0, 129.1 (2), 133.5, 133.6, 134.3, 134.6, 135.3, 145.7, 146.4, 162.1	405 [M^+], 46 (100)

17	3060 (CH arom.), 2955, 2866 (CH aliph.), 1704, (C=O), 1610 (C=N), 850 (C-Cl)	1.2 [t, 3H, CH_3, J = 7.3 Hz], 4.3 [q, 2H, CH_2, J = 7.4 Hz], 6.5-7.9 [m, 8H, Ar-H + CH pyrazole] 13.6, 62.4, 109.3, 120.3, 123.0 (2), 124.5, 128.4, 130.6, 131.0, 134.0, 134.5, 135.1, 136.7, 138.2, 144.1, 144.5, 160.3	481 [M^+], 43 (100)
18	3053 (CH arom.), 2926, 2843 (CH aliph.), 1700, (C=O), 1570 (C=N)	1.2 [t, 3H, CH_3, J = 7.3 Hz], 4.3 [q, 2H, CH_2, J = 7.3 Hz], 6.9-8.0 [m, 13H, Ar-H + CH pyrazole] 13.8, 62.1, 102.6, 123.0 (2), 124.9, 126.6 (2), 127.1, 127.7, 128.7 (2), 128.8, 129.8 (2), 130.1, 132.2, 133.2, 135.4, 148.6, 148.9, 161.8	342 [M^+], 129 (100)
19	3100 (CH arom.), 2977, 2862 (CH aliph.), 1728, (C=O), 1599 (C=N)	1.2 [t, 3H, CH_3, J = 7.5 Hz], 4.3 [q, 2H, CH_2, J = 7.3 Hz], 6.4-7.9 [m, 9H, Ar-H + CH pyrazole] 13.7, 62.1, 111.6, 119.6, 121.4 (2), 128.2, 128.8, 129.1, 129.7, 131.9 (2), 138.5, 140.0, 145.3, 163.4	298 [M^+], 77 (100)
20	3099 (CH arom.), 2981, 2886 (CH aliph.), 1709, (C=O), 1590 (C=N)	1.2 [t, 3H, CH_3, J = 7.2 Hz], 4.3 [q, 2H, CH_2, J = 7.2 Hz], 7.0-7.7 [m, 8H, Ar-H + CH pyrazole] 14.1, 60.6, 109.0, 122.7, 127.7 (2), 128.6, 128.7, 128.8, 131.5, 132.3 (2), 137.9, 138.7, 143.4, 161.7	377 [M^+], 76 (100)
21	3085 (CH arom.), 2968, 2840 (CH aliph.), 1710, (C=O), 1560 (C=N)	1.1 [t, 3H, CH_3, J = 7.4 Hz], 2.5 [s, 6H, $2CH_3$], 4.4 [q, 2H, CH_2, J = 7.5 Hz], 6.8-7.9 [m, 7H, Ar-H + CH pyrazole] 11.6, 14.5, 14.8, 62.6, 109.4, 120.8 (2), 123.0, 126.5, 127.1, 128.0 (2), 134.5, 135.2, 136.8, 139.3, 144.4, 160.4	326 [M^+], 91 (100)
22	3098 (CH arom.), 2949, 2871 (CH aliph.), 1708, (C=O), 1599 (C=N)	1.6 [t, 3H, CH_3, J = 7.1 Hz], 2.6 [s, 6H, $2CH_3$], 4.5 [q, 2H, CH_2, J = 7.1 Hz], 6.5-7.9 [m, 6H, Ar-H + CH pyrazole] 11.1, 13.9, 15.8, 57.4, 115.5, 121.3, 123.1 (2), 126.5, 127.1, 128.8 (2), 130.3, 135.2, 136.9, 139.9, 143.5, 160.5	405 [M^+], 155 (100)

Antimicrobial screening

Table III lists the results of the antimicrobial screening of the new compounds against some Gram-positive and some Gram-negative bacteria and fungi. The antibacterial screening revealed that compound **21** ($MIC_{E.\ coli}$ = 0.038 μmol mL^{-1}, $MIC_{P.\ aeruginosa}$ = 0.076 μmol mL^{-1}) is nearly as active as ampicillin ($MIC_{E.\ coli}$ = 0.033, $MIC_{P.\ aeruginosa}$ 0.067 μmol mL^{-1}). The antifungal screening showed that compound **16** (MIC = 0.015 μmol mL^{-1}) exhibited more potency activity than fluconazole (0.020 μmol mL^{-1}) against *C. parapsilosis*. Also, compound **16** showed lower activity than fluconazole (0.005 μmol mL^{-1}) against *C. albicans* and *C. tropicalis* ($MIC_{C.\ albicans}$ = 0.015 μmol mL^{-1}, $MIC_{C.\ tropicalis}$ = 0.030 μmol mL^{-1}). From these results it was found that the presence of 2,5-dimethylthienyl moiety at 5-position and unsubsituted phenyl ring at 1-position in compound **21** enhanced the antibacterial activity against *E. coli*, while the presence of (un)substituted aromatic hydrocarbon moiety or unsubstituted thienyl at 5-position and (un)substituted phenyl ring at 1-position in compounds **9–20, 22** revealed a moderate or weak activity. Dihalogenated phenyl at 5-position and unsubstituted phenyl at 1-position in compound **16** revealed significant activity compared with fluconazole as reference drug against *C. parapsilosis*. The remaining compounds were found either weak or inactive.

*Table III. Minimal inhibitory concentration (*MIC, *μmol mL^{-1}) of the newly synthesized pyrazole derivatives*

Compd.	*Bacillus subtilis* (NCTC 1040)	*Staphylococcus aureus* (NCTC-7447)	*E. coli* (NCTC 10416)	*Pseudomonas aeruginosa* (ATCC 10145)	*Candida albicans* (ATCC-10231)	*Candida tropicalis* (ATCC-66029)	*Candida parapsilosis* (ATCC-22019)
9	0.306	0.153	0.153	0.306	0.153	0.153	0.306
10	0.124	0.248	0.124	0.248	0.124	0.062	0.124
11	0.142	0.142	0.142	0.142	0.071	0.142	0.142
12	0.232	0.116	0.232	0.232	0.058	0.116	0.116
13	0.142	0.142	0.142	0.142	0.284	NA*	NA
14	0.116	0.232	0.116	0.116	0.232	NA*	NA
15	0.138	0.138	0.138	0.138	0.069	0.069	0.034
16	0.246	0.123	0.123	0.246	0.015	0.030	0.015
17	0.103	0.103	0.103	0.103	NA	NA	NA
18	0.293	NA	0.146	NA	0.293	0.293	0.293
19	NA	0.167	NA	0.325	0.167	0.167	0.167
20	NA	NA	0.265	NA	0.132	0.132	0.132
21	0.076	0.076	0.038	0.076	NA	0.153	NA
22	0.123	0.246	0.123	0.123	0.061	0.061	NA
Ampicillin	0.033	0.008	0.033	0.067	NA	0.067	0.134
Fluconazole	–	–	–	–	0.005	0.005	0.020

NA – Compounds having *MIC* > 0.5 μmol mL^{-1}.

CONCLUSIONS

This study showed that the newly synthesized ethyl 5-(2,5-dimethylthiophen-3-yl)-1-phenyl-1*H*-pyrazole-3-carboxylate (**21**) was found to be of comparable activity as ampicillin against *E. coli* and *P. aeruginosa*. The same is true for ethyl 5-(4-bromo-2-chlorophenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (**16**) *vs.* fluconazole against *Candida parapsilosis*. This is due to the presence of dihalogenated phenyl at 5-position and unsubstituted phenyl at 1-position of the pyrazole ring.

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REFERENCES

1. N. D. Gaikwad, S. V. Patil and V. D. Bobade, Synthesis and antimicrobial activity of novel thiazole substituted pyrazole derivatives, *J. Het. Chem*. **50** (2013) 519–527; DOI: 10.1002/jhet.1513.
2. M. S. Essam and M. M. H Nagwa, Synthesis and antimicrobial evaluation of some pyrazole derivatives, *Molecules* **17** (2012) 4962–4971; DOI: 10.3390/molecules17054962.
3. Z. Chuan-Yu, L. Xing-Hai, W. Bao-Lei, W. Su-Hua and L. Zheng-Ming, Synthesis and antifungal activities of new pyrazole derivatives *via* 1,3-dipolar cycloaddition reaction, *Chem. Biol. Drug Des.* **75** (2010) 489–493; DOI: 10.1111/j.1747-0285.2010.00948.x.
4. E. M. Flefel, W. A. Tantawy, W. A. El-Sayed, H. H. Sayed and N. M. Fathy, Synthesis and anticancer activity of new substituted pyrazoles and their derived 1,2,4-triazoles and sugar derivatives, *J. Het. Chem*. **50** (2013) 344–350; DOI: 10.1002/jhet.1122.
5. S. M. El-Moghazy, F. F. Barsoum, H. M. Abdel-Rahman and A. A. Marzouk, Synthesis and anti-inflammatory activity of some pyrazole derivatives**,** *Med. Chem. Res*. **21** (2012) 1722–1733; DOI: 10.1007/s00044-011-9691-4.
6. M. M. Ghorab, F. A. Ragab, S. I. Alqasoumi, A. M. Alafeefy and S. A. Aboulmagd, Synthesis of some new pyrazolo[3,4-*d*]pyrimidine derivatives of expected anticancer and radioprotective activity, *Eur. J. Med. Chem.* **45** (2010**)** 171–178; DOI: 10.1016/j.bmc.2013.11.042.
7. B. Parashar, A. Jain, S. Bharadwaj and V. K. Sharma, Synthesis and pharmacological properties of some novel pyrazolidine and pyrazole derivatives, *Med. Chem. Res.* **21** (2012) 1692–1699; DOI: 10.1007/s00044-011-9687-0.
8. A. B. Adnan, H. Ariaya, A. Henok and E. A. B. Alaa, Synthesis and biological evaluation of some pyrazole derivatives as anti-malarial agents, *Arch. Pharm. Chem. Life Sci.* **345** (2012) 147–154; DOI: 10.1002/ardp.201100078.
9. M. M. Ghorab, H. I. Zienab, A. Mohamad and A. A. Radwan, Synthesis, antimicrobial evaluation and molecular modelling of novel sulfonamides carrying a biologically active quinazoline nucleus**,** *J. Pharm. Res.* **36** (2013) 660–670; DOI: 10.1007/s12272-013-0094-6.
10. M. M. Ghorab, H. I. Zienab, A. A. Radwan and A. Mohamad, Synthesis and pharmacophore modeling of novel quinazolines bearing a biologically active sulfonamide moiety, *Acta Pharm.* **63** (2013) 1–18; DOI: 10.2478/acph-2013-0006.
11. M. Lindler, W. Sippl and A. A. Radwan, Pharmacophore elucidation and molecular docking studies on 5-phenyl-1-(3-pyridyl)-1H-1,2,4-triazole-3-carboxylic acid derivatives as COX-2 inhibitors, *Sci. Pharm*. **78** (2010) 195–214; DOI: 10.3797/scipharm.0912-19.
12. M. M. Ghorab, F. A. Ragab, H. I. Heiba and M. G. El-Gazzar, Synthesis, *in vitro* anticancer screening and radiosensitizing evaluation of some new 4-[3-(substituted)thioureido]-*N*-(quinoxalin-2-yl)-benzenesulfonamide derivatives, *Acta Pharm.* **61** (2011) 415–425; DOI: 10.2478/v10007-011-0040-4.
13. M. M. Ghorab, F. A. Ragab, H. I. Hieba, H. A. Yousef and M. G. El-Gazzar, Synthesis of novel pyrazole and pyrimidine derivatives bearing sulfonamide moiety as antitumor and radiosensitizing agents, *Med. Chem. Res.* **21** (2012) 1376–1383; DOI 10.1007/s00044-013-0721-2.
14. M. S. Al-Dosari, M. M. Ghorab, M. S. Alsaid, Y. M. Nissan and A. B. Ahmed, Synthesis and anticancer activity of some novel trifluoromethylquinolines carrying a biologically active benzenesulfonamide moiety, *Eur. J. Med. Chem.* **69** (2013) 373–383; DOI: 10.1016/j.ejmech.2013.08.048.
15. A. M. Ali, G. E. Saber, N. M. Mahfouz, M. A. El-Gendy, A. A. Radwan and M. A. E. Hamid, Synthesis and three-dimensional qualitative structure selectivity relationship of 3,5-disubstituted-2,4-thiazolidinedion derivatives as COX2 inhibitors, *Arch. Pharm. Res.* **30** (2007) 1186–1204; DOI: 10.1007/BF02980259.
16. M. M. Ghorab and M. S. Alsaid, Synthesis and antitumor activity of some novel hydrazide, 1,2-dihydropyridine, chromene, and benzochromene derivatives, *J. Heterocyclic Chem.* **49** (2012) 272–280; DOI: 10.1002/jhet.829.

17. M. M. Ghorab, F. A. Ragab, H. I. Hieba and W. M. Ghorab, Design and synthesis of some novel quinoline derivatives as anticancer and radiosensitizing agents targeting VEGFR tyrosine kinase, *J. Heterocyclic Chem.* **48** (2011) 1269–1279; DOI: /10.1002/jhet.749.

18. M. M. Ghorab, M. S. Alsaid and E. M. El-Hossary, *In vitro* cytotoxic evaluation of some new heterocyclic sulfonamide derivatives, *J. Heterocyclic Chem*. **48** (2011) 563–571; DOI: 10.1002/jhet.619.

19. M. Alvarado, G. Pilar, M. Macías-González, F. J. Pavón, A. Serrano, N. Jagerovic, J. Elguero, A. Gutiérrez-Rodríguez, S. García-Granda, M. Suardíaz and F. R. de Fonseca, Antiobesity designed multiple ligands: Synthesis of pyrazole fatty acid amides and evaluation as hypophagic agents, *Bioorg. Med. Chem.* **16** (2008) 10098–10105; DOI: 10.1016/j.bmc.2008.10.023.

20. I. L. Finar and R. J. Hurlock, The skraup reaction with aminopyrazoles, *J. Chem. Soc*. **1958**, 3259–3263; DOI: 10.1039/JR9580003259.

21. H. Naeimi, Z. S. Nazifi, S. M. Amininezhad and M. Amouheidari, Synthesis, characterization and *in vitro* antimicrobial activity of some new Schiff bases and their complexes, *J. Antibiot.* **66** (2013**)** 687–689; DOI: 10.1038/ja.2013.73.