HETEROCYCLES, Vol. 91, No. 11, 2015, pp. 2126 - 2142. © 2015 The Japan Institute of Heterocyclic Chemistry
Received, 7th September, 2015, Accepted, 19th October, 2015, Published online, 28th October, 2015
DOI: 10.3987/COM-15-13319

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SOME NOVEL THIAZOLE, 1,3,4-THIADIAZOLE AND PYRIDO[2,3-*d*][1,2,4]-TRIAZOLO[4,3-*a*]PYRIMIDINE DERIVATIVES INCORPORATING PYRAZOLE MOIETY

Abdou O. Abdelhamid,* Ahmad S. Shawali, Sobhi M. Gomha, and Waleed A. M. A. El-Enany

Department of Chemistry, Faculty of Science, University of Cairo, Giza 12613, Egypt, e-mail: Abdelhamid45@gmail.com

Abstract – New series of novel functionalized thiazoles, 1,3,4-thiadiazoles and pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidines containing pyrazole moiety were synthesized using 4-acetylpyrazole as a precursor. The structures of the compounds prepared were confirmed by both spectral and elemental analyses and by alternative synthetic routes. The mechanisms of the studied reactions were also discussed. Sixteen compounds were evaluated for their *in vitro* antimicrobial activity. The results proclaimed that some of the tested compounds exhibited moderate to significant antibacterial and antifungal activities. Compounds **11e**, **11a**, and **11d** exhibited high antibacterial activity against *Bacillus subtilis* compared with reference drug (Ampicillin) while compounds **11a**, **6g**, **18e**, **18a**, **11d**, **6a**, **11c**, **11b** and **6d** exhibited higher antifungal activity against *Syncephalastrum racemosum* than reference drug (Amphotericin B).

INTRODUCTION

A survey of literature revealed that many derivatives of 1,3,4-thiadiazole and pyrazole ring systems were reported to have wide range of biological activities.[1,2] Thiazoles can be found in drug development for the treatment of allergies,[3] hypertension,[4] inflammation,[5] schizophrenia,[6] bacterial,[7] HIV infections,[8] hypnotics,[9] and more recently for the treatment of pain,[10] as fibrinogen receptor antagonists with antithrombotic activity[11] and as new inhibitors of bacterial DNA gyrase B.[12] The 1,2,4-triazolopyrimidines have also attracted growing interest due to their important pharmacological activities, such as antitumor potency, antimalarial, antimicrobial, anti-inflammatory, antifungal and macrophage activation.[13-18]

In the light of these findings, it was thought interesting to explore the synthesis of new 1,3-thiazole and 1,3,4-thiadiazole derivatives having a pyrazole moiety. In continuation of our work,[19-24] we wish to report the results of our study of the reactions of hydrazonoyl halides **3** with each of ethylidene-thiosemicarbazone **2**, methyl ethylidenedithiocarbazate **8**, and 2-thioxopyrido[2,3-*d*]pyrimidin-4-one **14** (Chart 1).

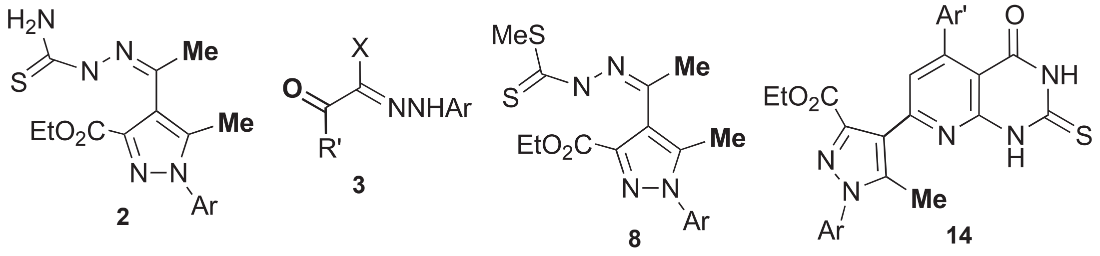
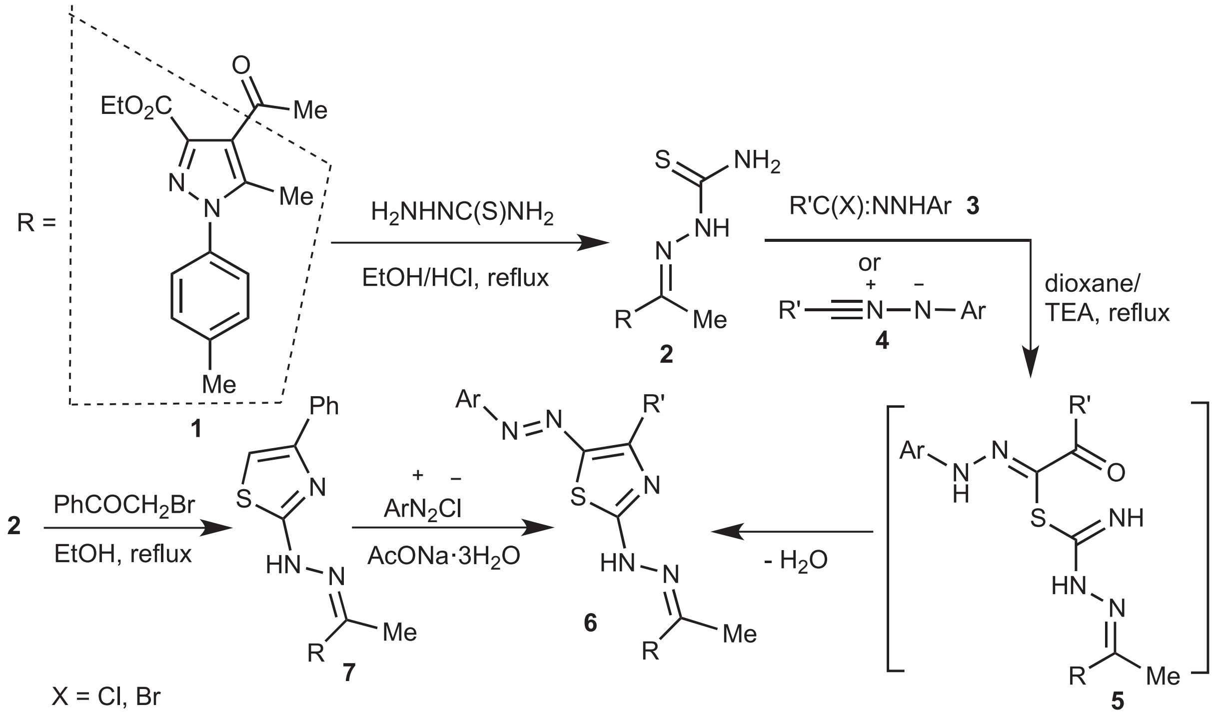


Chart 1

RESULTS AND DISCUSSION

Reaction of compound **2**[25] with each the appropriate hydrazonoyl halides **3a-i** in dioxane containing catalytic amount of triethylamine gave the corresponding 5-methyl-4-(1-(2-(4-substituted 5-(phenyldiazenyl)thiazol-2-yl)hydrazono)-ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate **6a-i** (Scheme 1).



X = Cl, Br

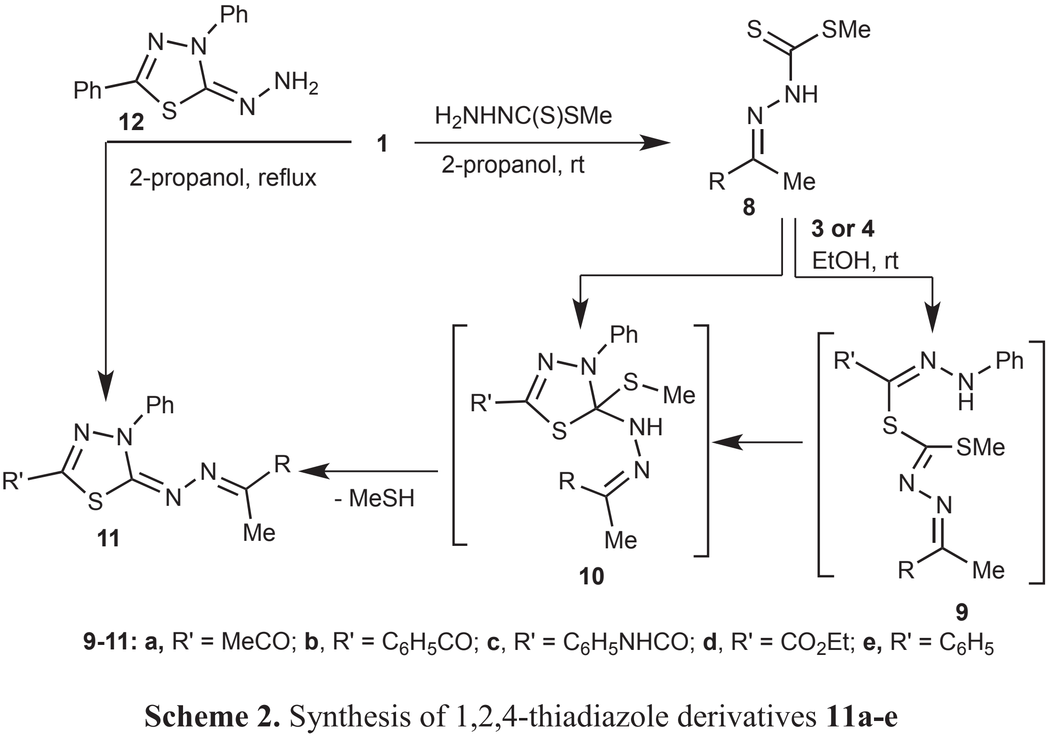
3-6: **a**, R' = MeCO, Ar = C_6H_5; **b**, R' = MeCO, Ar = 4-MeC_6H_4; **c**, R' = MeCO, Ar = 4-ClC_6H_4;
d, R' = MeCO, Ar = 4-BrC_6H_4; **e**, R' = MeCO, Ar = 4-$MeOC_6H_4$; **f**, R' = MeCO, Ar = 3-MeC_6H_4;
g, R' = 2-C_4H_3SCO, Ar = C_6H_5; **h**, R' = C_6H_5CO, Ar = C_6H_5; **i**, R' = 2-$C_{10}H_7CO$, Ar = C_6H_5

Scheme 1. Synthesis of thiazole derivatives **6a-i**

Structures **6a-i** were elucidated by elemental analyses, spectral data and alternate synthesis. Thus, reaction of **2** with ω-bromoacetophenone in ethanol afforded ethyl 5-methyl-4-(1-(2-(4-phenylthiazol-2-yl)hydrazono)ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (**7**). Treatment of the latter product **7** with benzenediazonium chloride in ethanolic sodium acetate solution afforded a product that proved identical in all aspects (mp, mixed mp and spectra) with **6h** (Scheme 1).

To account for the formation **6**, it is suggested as shown in Scheme 1, that the reaction starts with 1,3-addition of the thiol tautomer of **2** to the nitrilimine **4**, generated in situ *via* dehydrohalogenation of hydrazonoyl halide **3**, to give the thiohydrazonate **5** which undergoes in situ dehydration to give the thiazole **6** as end product.

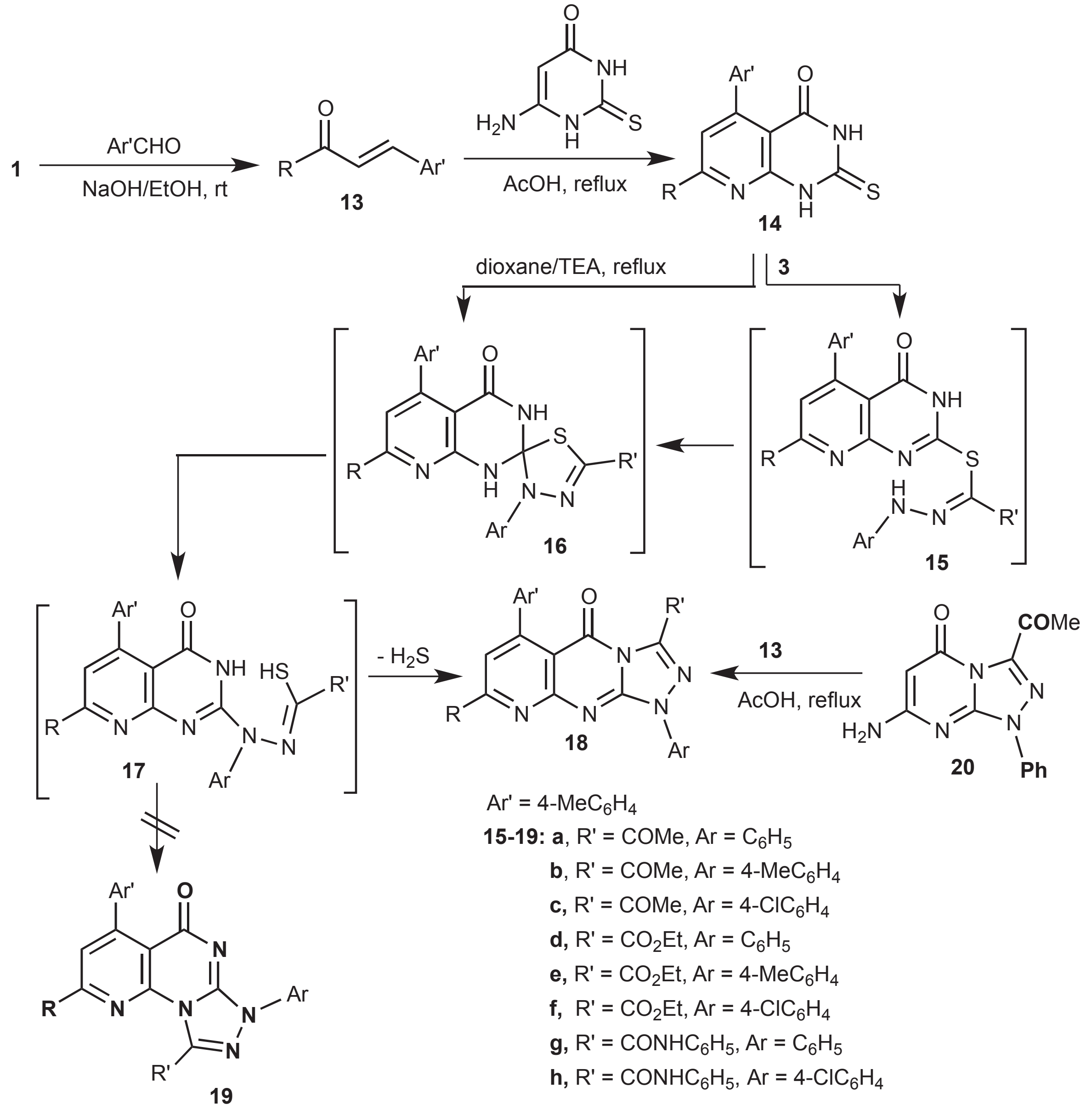
Next, reaction of ethyl 4-acetyl-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (**1**) with methyl hydrazinecarbodithioate in 2-propanol gave ethyl 5-methyl-4-(1-(2-((methylthio)carbonothioyl)-hydrazono)ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate **8** (Scheme 2) Structure **8** was confirmed by elemental analysis, spectral data and chemical transformation (see Experimental). Thus, treatment of **8** with each of the appropriate hydrazonoyl halides **3a-e** afforded ethyl 4-(1-((5-substituted-3-phenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)hydrazono)ethyl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate **11a-e**. Structures **11a-e** were confirmed by elemental analysis, spectral data, and an alternative synthesis. Thus, reaction of 2-hydrazono-3,5-diphenyl-2,3-dihydro-1,3,4-thiadiazole[26] **12** with **1** in ethanol afforded product that proved identical with **11e** in all aspects (mp, mixed mp and spectra) (Scheme 2).



9-11: a, R' = MeCO; **b**, R' = C_6H_5CO; **c**, R' = C_6H_5NHCO; **d**, R' = CO_2Et; **e,** R' = C_6H_5

Scheme 2. Synthesis of 1,2,4-thiadiazole derivatives **11a-e**

In the light of these results, the mechanism outlined in Scheme 2 seems to be the most plausible pathway for the formation of **11** from the reaction of the **3** with **8**. This mechanism indicates that the reaction involves initial formation of thiohydrazonate **9**, which undergoes intramolecular cyclization as soon as it is formed to yield the intramediate **10** or via 1,3-dipolar cycloaddition of nitrilimine **6**, generated *in situ* by base-catalyzed dehydrohalogenation of hydrazonoyl halide, to the C=S double bond of **8**. The formation of **9** and **10** are similar to the reactions of hydrazonoyl chloride with 1-phenyl-1,4-dihydrotetrazole-5-thione[27] and 5-phenyl-1,3,4-thiadiazole-2(3*H*)-thione.[28] Compound **10** was converted to **11** by elimination of methanethiol. Reaction of **1** with *p*-tolualdehyde in ethanol in the presence of sodium hydroxide afforded the corresponding enone derivative **13** (Scheme 3).



Ar' = 4-MeC$_6$H$_4$

15-19: a, R' = COMe, Ar = C$_6$H$_5$
b, R' = COMe, Ar = 4-MeC$_6$H$_4$
c, R' = COMe, Ar = 4-ClC$_6$H$_4$
d, R' = CO$_2$Et, Ar = C$_6$H$_5$
e, R' = CO$_2$Et, Ar = 4-MeC$_6$H$_4$
f, R' = CO$_2$Et, Ar = 4-ClC$_6$H$_4$
g, R' = CONHC$_6$H$_5$, Ar = C$_6$H$_5$
h, R' = CONHC$_6$H$_5$, Ar = 4-ClC$_6$H$_4$

Scheme 3. Synthesis of pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5(1*H*)-one derivatives **18a-h**

Heating a mixture of the latter with 6-amino-2-thioxo-2,3,4-trihydro-1*H*-pyrimidin-4-one in glacial acetic acid yielded ethyl 5-methyl-4-(4-oxo-2-thioxo-5-(*p*-tolyl)-1,2,3,4-tetrahydropyrido-[2,3-*d*]pyrimidin-7-yl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate **14** (Scheme 3). The structures of both compounds **13** and **14** were established by their elemental analyses, spectral data (see Experimental) and chemical transformation. Thus, reactions of thione **14** with various hydrazonoyl chlorides **3a-h** were examined. In our hands, reaction of **14** with equimolar amount of the appropriate hydrazonoyl chlorides **3a-h** in refluxing dioxane in the presence of triethylamine gave in each case one isolable product. Both mass spectra and elemental analyses of the isolated products revealed the absence of sulfur in all products. However, the analytical and spectroscopic data of the isolated products were in accordance with either structure **18** or its isomer **19**. Structure **18** was assigned to the isolated products on the basis of alternate synthesis of **18** by reaction of 3-acetyl-7-amino-1-phenyl-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1*H*)-one **20** with the enone derivative **13** (Scheme 3). On this basis, structure **19** was discarded. This structural assignment is also consistent with literature reports which indicate that reaction of hydrazonoyl halides with 2-thioxo-pyrimidin-4-one yielded regioselectively the corresponding 1,2,4-triazolo[4,3-*a*]pyrimidin-5-one derivatives.[29]

ANTIMICROBIAL ACTIVITY

Sixteen compounds of the newly synthesized target compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* and *Bacillis subtilis* as examples of Gram-positive bacteria and *Pseudomonas aeruginosa* and *Escherichia coli* as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal activity against a representative panel of fungal strains i.e. *Aspergillus fumigatus*, *Geotrichum candidum*, *Candida albicans* and *Syncephalastrum racemosum* fungal strains. Ampicillin and Gentamicin are used as reference drugs for *in vitro* antibacterial activity while Amphotericin B is used as reference drug for *in vitro* antifungal activity.

The results of testing for antimicrobial effects are summarized in Tables 1 and 2. These results indicate:

- Compound **11e** exhibited higher antibacterial activity against *Staphylococcus aureus* bacteria than reference drug (Ampicillin) while compounds **11a**, **11d** and **6e** showed excellent activity.
- Compounds **11e**, **11a**, and **11d** exhibited high antibacterial activity against *Bacillus subtilis bacteria* compared with reference drug (Ampicillin).
- Compounds **11e**, **11a**, and **11d** exhibited higher antibacterial activity against *Escherichia coli bacteria* than reference drug (Gentamicin).
- Nine of the tested compounds (**11a**, **6g**, **18e**, **18a**, **11d**, **6a**, **11c**, **11b** and **6d**) exhibited higher antifungal activity against *Syncephalastrum racemosum fungus* than reference drug (Amphotericin B).

- All the tested compounds have no inhibition effect against *Pseudomonas aeruginosa*.
- Compounds **18e**, **18a** and **11a** showed high inhibitory activity against *Aspergillus fumigates fungus compared* with reference drug (Amphotericin B).
- All the tested compounds have no inhibition effect against *Candida albicans*.
- Compounds **18e** and **18a** exhibited high inhibitory effect against *Geotrichum candidum* yeast compared with reference drug (Amphotericin B).
- Rest of the compounds showed good to moderate activity against the tested bacteria and fungi compared with the standard drugs.

Table 1. Mean zone inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic bactrial using (10 mg/mL) concentration of sample.

Tested compounds	**Gram-positive bacteria**		**Gram-negative bacteria**	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
6a	20.8±0.67	19.1±0.44	18.1±0.46	NA*
6b	20.8±0.25	19.1±0.34	18.3±0.58	NA
6c	21.3±0.25	19.6±0.44	19.6±0.33	NA
6d	21.3±0.63	20.9±0.25	16.3±0.63	NA
6e	22.4±0.63	21.3±0.63	19.6±0.46	NA
6f	22.1±1.2	20.3±0.63	18.3±0.37	NA
11a	25.4±0.67	23.2±1.2	20.4±0.46	NA
11b	21.7±0.53	20.5±0.43	16.3±0.33	NA
11c	22.3±0.63	21.2±0.44	17.6±0.25	NA
11d	24.2±0.53	22.6±1.2	19.8±1.5	NA
11e	26.3±0.63	24.5±0.44	22.4±0.25	NA
18a	17.3±1.2	15.6±0.15	15.3±0.53	NA
18b	16.4±0.58	15.2±0.44	14.6±0.58	NA
18c	19.3±0.53	18.1±0.58	15.9±0.25	NA
18d	16.8±0.42	14.3±0.58	13.3±0.53	NA
18e	19.8±0.58	18.6±0.44	16.4±0.19	NA
Ampicillin[a)]	32.4±0.72	23.8±1.2	--	---
Gentamicin[a)]	**--**	**--**	19.9±0.58	17.3±0.63

* **NA**: No antimicrobial activity detected under these experimentation conditions.
[a)] The concentration of the standard drug used is 10 mg/mL.

Table 2. Mean zone inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic fungi and yeast using (10 mg/mL) concentration of tested sample.

Tested compounds	Filamentous fungi		Yeasts	
	Aspergillus fumigatus	*Syncephalastrum racemosum*	*Geotrichum candidum*	*Candida albicans*
6a	18.6±0.25	21.2±0.63	22.3±0.58	NA*
6b	14.3±0.44	17.1±1.2	17.3±0.37	NA
6c	17.8±0.25	19.3±0.58	20.4±0.17	NA
6d	14.6±1.2	16.9±1.5	18.6±0.34	NA
6g	19.3±0.44	22.4±0.58	23.2±0.72	NA
6e	13.1±0.63	16.1±0.25	16.7±0.34	NA
11a	20.6±1.5	22.6±1.2	24.8±0.58	NA
11b	18.3±0.34	20.1±0.25	21.3±0.38	NA
11c	19.4±0.39	20.9±0.58	21.7±0.58	NA
11d	21.3±0.58	21.9±0.25	22.4±0.38	NA
11e	17.3±0.25	19.2±0.44	20.4±0.63	NA
18a	21.6±0.39	22.2±0.16	25.4±0.58	NA
18b	16.3±0.63	18.2±0.27	18.9±0.3	NA
18c	16.6±0.58	19.4±0.25	19.9±0.38	NA
18d	17.3±0.36	17.9±0.44	19.1±0.58	NA
18e	22.3±0.25	22.3±0.25	25.8±0.58	NA
Amphotericin B[a)]	23.7±1.2	19.7±1.5	28.7±0.58	25.4±1.5

* **NA**: No antimicrobial activity detected under these experimentation conditions.
[a)] The concentration of the standard drug used is 10 mg/mL.

CONCLUSION

New series of novel functionalized 1,3-thiazoles, 1,3,4-thiadiazoles and pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidines containing pyrazole moiety were synthesized using 4-acetylpyrazole as a precursor and evaluated for their *in vitro* antibacterial, and antifungal activities. From the screening results, it can be seen that compounds **11e**, **11a**, and **11d** exhibited high antibacterial activity against *Bacillus subtilis* compared with reference drug (Ampicillin) while compounds **11a**, **6g**, **18e**, **18a**, **11d**, **6a**, **11c**, **11b** and **6d** exhibited higher antifungal activity against *Syncephalastrum racemosum* than the reference drug (Amphotericin B).

EXPERIMENTAL

Melting points were measured on an Electrothermal IA 9000 series digital melting point apparatus. IR spectra were recorded using potassium bromide discs on PyeUnicam SP 3300 and Shimadzu FTIR 8101 PC infrared spectrophotometers. NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer operating at 300 MHz (^{1}H-NMR) and run in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. ^{13}C-NMR spectra were recorded on a BRUKER spectrometer at 75 MHz. Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyses were measured by using a German made Elementarvario LIII CHNS analyzer. The biological evaluation of the products was carried out at Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt. Hydrazonoyl chlorides **3** were prepared as previously reported in literature.[30-36]

Synthesis of ethyl 5-methyl-4-(1-(2-(4-methyl-5-(aryldiazenyl)thiazol-2-yl)hydrazono)ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (6a-i). A mixture of thiosemicarbazone **2** (0.359 g, 1 mmol) and the appropriate hydrazonoyl halide **3** (1 mmol) in dioxane (20 mL) containing TEA (0.07 mL) was refluxed for 6 h, allowed to cool and the solid formed was filtered off, washed with EtOH, dried and crystallized from DMF to give the corresponding thiazole derivatives **6a-i**. The products **6a-i** together with their physical constants are listed below.

Ethyl 5-methyl-4-(1-(2-(4-methyl-5-(phenyldiazenyl)thiazol-2-yl)hydrazono)ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (6a): Red solid, (70% yield); mp 194-196 °C; IR (KBr) ν = 3441 (NH), 1715 (C=O), 1600 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.30 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 2.18 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 4.27 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 6.98-7.66 (m, 9H, Ar-H), 10.47 (s, br, 1H, NH); MS, *m/z* (%) 501 (M$^+$, 77), 455 (25), 212 (41), 132 (73), 91 (100). Anal. Calcd for $C_{26}H_{27}N_7O_2S$ (501.60): C, 62.26; H, 5.43; N, 19.55. Found: C, 62.17; H, 5.37; N, 19.42%.

Ethyl 5-methyl-4-(1-(2-(4-methyl-5-(*p*-tolyldiazenyl)thiazol-2-yl)hydrazono)ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (6b): Red solid, (71% yield); mp 171-173 °C; IR (KBr) ν = 3420 (NH), 1716 (C=O), 1598 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.30 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 2.17 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 4.29 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 7.02-7.51 (m, 8H, Ar-H), 10.34 (s, br, 1H, NH); MS, *m/z* (%) 515 (M$^+$, 37), 469 (20), 350 (9), 132 (36), 91 (100). Anal. Calcd for $C_{27}H_{29}N_7O_2S$ (515.63): C, 62.89; H, 5.67; N, 19.01. Found: C, 62.73; H, 5.61; N, 18.92%.

Ethyl 4-(1-(2-(5-((4-chlorophenyl)diazenyl)-4-methylthiazol-2-yl)hydrazono)ethyl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (6c): Red solid, (76% yield); mp 218-220 °C; IR (KBr) ν = 3433 (NH), 1715 (C=O), 1597 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.27 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 2.20 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 4.27 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 7.35-7.45

(m, 8H, Ar-H), 10.55 (s, br, 1H, NH); MS, *m/z* (%) 538 (M+2, 4), 536 (M^+, 12), 489 (20), 254 (59), 212 (32), 132 (53), 91 (100). Anal. Calcd for $C_{26}H_{26}ClN_7O_2S$ (536.05): C, 58.26; H, 4.89; N, 18.29. Found: C, 58.21; H, 4.76; N, 18.05%.

Ethyl 4-(1-(2-(5-((4-bromophenyl)diazenyl)-4-methylthiazol-2-yl)hydrazono)ethyl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (6d): Dark red solid, (76% yield); mp 204-206 °C; IR (KBr) ν = 3440 (NH), 1716 (C=O), 1593 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-*d*$_6$): δ 1.27 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 2.12 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 4.29 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 7.25-7.49 (m, 8H, Ar-H), 10.54 (s, br, 1H, NH); MS, *m/z* (%) 582 (M+2, 8), 580 (M^+, 9), 535 (17), 350 (12), 212 (34), 132 (62), 91 (100). Anal. Calcd for $C_{26}H_{26}BrN_7O_2S$ (580.50): C, 53.79; H, 4.51; N, 16.89. Found: C, 53.72; H, 4.46; N, 16.75%.

Ethyl 4-(1-(2-(5-((4-methoxyphenyl)diazenyl)-4-methylthiazol-2-yl)hydrazono)ethyl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (6e): Orange solid, (72% yield); mp 192-194 °C; IR (KBr) ν = 3441 (NH), 1716 (C=O), 1599 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-*d*$_6$): δ 1.27 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 2.20 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 4.29 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 6.94-7.63 (m, 8H, Ar-H), 10.40 (s, br, 1H, NH); MS, *m/z* (%) 531 (M^+, 36), 485 (28), 350 (11), 212 (29), 132 (60), 91 (100). Anal. Calcd for $C_{27}H_{29}N_7O_3S$ (531.63): C, 61.00; H, 5.50; N, 18.44. Found: C, 60.90; H, 5.54; N, 18.28%.

Ethyl 5-methyl-4-(1-(2-(4-methyl-5-(*m*-tolyldiazenyl)thiazol-2-yl)hydrazono)ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (6f): Orange solid, (78% yield); mp 219-221 °C; IR (KBr) ν = 3435 (NH), 1718 (C=O), 1594 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-*d*$_6$): δ 1.25 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 2.12 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 4.31 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 6.99-7.47 (m, 8H, Ar-H), 10.56 (s, br, 1H, NH); MS, *m/z* (%) 515 (M^+, 12), 489 (15), 254 (72), 127 (69), 91 (100), 65 (69). Anal. Calcd for: $C_{27}H_{29}N_7O_2S$ (515.63): C, 62.89; H, 5.67; N, 19.01. Found: C, 62.82; H, 5.60; N, 18.87%.

Ethyl 5-methyl-4-(1-(2-(5-(phenyldiazenyl)-4-(thiophen-2-yl)thiazol-2-yl)hydrazono)ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (6g): Red solid, (69% yield); mp 231-233 °C; IR (KBr) ν = 3427 (NH), 1714 (C=O), 1598 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-*d*$_6$): δ 1.28 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 2.20 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.29 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 7.21-7.57, 8.05 (m, 12H, Ar-H), 10.22 (s, br, 1H, NH); MS, *m/z* (%) 569 (M^+, 2), 522 (9), 479 (37), 254 (41), 132 (29), 91 (100). Anal. Calcd for $C_{29}H_{27}N_7O_2S_2$ (569.70): C, 61.14; H, 4.78; N, 17.21. Found: C, 61.07; H, 4.64; N, 17.08%.

Ethyl 5-methyl-4-(1-(2-(4-phenyl-5-(phenyldiazenyl)thiazol-2-yl)hydrazono)ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (6h): Dark red solid, (75% yield); mp 256-257 °C; IR (KBr) ν = 3439 (NH), 1715 (C=O), 1596 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-*d*$_6$): δ 1.29 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 2.22 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.29 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 7.31-8.29 (m, 14H, Ar-H), 10.22 (s, br, 1H, NH); MS, *m/z* (%) 563 (M^+, 9), 516 (14), 413 (13), 224 (19), 91 (100). Anal. Calcd for $C_{31}H_{29}N_7O_2S$ (563.67): C, 66.05; H, 5.19; N, 17.39. Found: C, 66.01; H, 5.13; N, 17.25%.

Ethyl 5-methyl-4-(1-(2-(4-(naphthalen-2-yl)-5-(phenyldiazenyl)thiazol-2-yl)hydrazono)ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (6i): Dark red solid, (63% yield); mp 224-226 °C; IR (KBr) ν = 3370 (NH), 1713 (C=O), 1613 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.30 (3H, t, *J* = 7.2, $\underline{CH_3}CH_2$), 2.22 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.28 (2H, q, *J* = 7.2, $CH_3\underline{CH_2}$), 7.30-8.14 (m, 16H, Ar-H), 10.32 (s, br, 1H, NH); MS, *m/z* (%) 613 (M^+, 1), 523 (20), 224 (15), 127 (56), 91 (100). Anal. Calcd for $C_{35}H_{31}N_7O_2S$ (613.73): C, 68.49; H, 5.09; N, 15.98. Found: C, 68.31; H, 5.02; N, 15.74%.

Synthesis of ethyl 5-methyl-4-(1-(2-(4-phenylthiazol-2-yl)hydrazono)ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (7). A mixture of thiosemicarbazone **2** (3.59 g, 10 mmol) and phenacyl bromide (2.00 g, 10 mmol) in EtOH (50 mL) was refluxed for 2 h then cooled to room temperature. The solid product was filtered off, washed with EtOH and recrystalized from EtOH to afford pure thiazole **7** as white solid, (76% yield); mp 168-170 °C; IR (KBr) ν = 3448 (NH), 1678 (C=O), 1576 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.28 (3H, t, *J* = 7.2, $\underline{CH_3}CH_2$), 2.42 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 4.28 (2H, q, *J* = 7.2, $CH_3\underline{CH_2}$), 7.36-7.53, 7.96-8.01 (m, 10H, Ar-H and thiazole H-5), 10.33 (s, br, 1H, NH); MS, *m/z* (%) 459 (M^+, 2), 413 (100), 224 (17), 132 (31), 91 (51). Anal. Calcd for $C_{25}H_{25}N_5O_2S$ (459.56): C, 65.34; H, 5.48; N, 15.24. Found: C, 65.28; H, 5.41; N, 15.16%.

Alternate synthesis of 6h

To a solution of thiazole **7** (0.459 g, 1 mmol) in EtOH (20 mL) was added sodium acetate trihydrate (0.138 g, 1 mmol), and the mixture was cooled to 0-5 °C in an ice bath. To the resulting cold solution was added portionwise a cold solution of arenediazonium chloride [prepared by diazotizing aniline derivative (1 mmol) dissolved in hydrochloric acid (6 M, 1 mL) with a solution of sodium nitrite (0.07 g, 1 mmol) in water (2 mL)]. After complete addition of the diazonium salt, the reaction mixture was stirred for a further 30 min in an ice bath. The solid that separated was filtered off, washed with water and finally crystallized from DMF to give the corresponding product, **6h** which was identical in all aspects (mp, mixed mp and IR spectra) with those obtained from reaction of **2** with hydrazonoyl bromide **3h**.

Synthesis of ethyl 5-methyl-4-(1-(2-((methylthio)carbonothioyl)hydrazono)ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (8). To a solution of ethyl 4-acetyl-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate **1** (2.86 g, 10 mmol) in 2-PrOH (20 mL), methyl hydrazinecarbodithioate (1.22 g, 10 mmol) was added. The mixture was stirred at room temperature for 2 h. The solid product was filtered off, crystallized from EtOH to afford **8** as yellow solid in 81% yield; mp 171-173 °C; IR (KBr): ν = 3293 (NH), 1744 (C=O), 1604 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.32 (3H, t, *J* = 7.2, $\underline{CH_3}CH_2$), 2.40 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.77 (3H, s, SCH_3), 4.34 (2H, q, *J* = 7.2, $CH_3\underline{CH_2}$), 7.36-7.43 (m, 5H, Ar-H and NH); MS *m/z* (%): 390 (M^+, 4), 263 (50), 182 (43), 107 (75), 91

(100). Anal. Calcd for $C_{18}H_{22}N_4O_2S_2$ (390.52): C, 55.36; H, 5.68; N, 14.35. Found C, 55.31; H, 5.53; N, 14.20%.

General procedure for synthesis of ethyl 4-(1-((5-substituted-3-phenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)hydrazono)ethyl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (11a-e). To a mixture of alkyl carbodithioate **8** (0.390 g, 1 mmol) and the appropriate hydrazonoyl halides **3** (1 mmol) in EtOH (20 mL), triethylamine (0.5 mL) was added, the mixture was stirred at room temperature for 2 h. The resulting solid was collected and crystallized from DMF to give the corresponding 1,3,4-thiadiazolines **11a-e**. The products **11a-e** together with their physical constants are listed below.

Ethyl 4-(1-((5-acetyl-3-phenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)hydrazono)ethyl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (11a): Yellow solid, (70% yield); mp 242-244 °C; IR (KBr): v = 1744, 1655 (2C=O), 1604 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 1.30 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 2.34 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.50 (3H, s, CH_3), 4.34 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 7.29-7.44 (m, 9H, Ar-H); MS, *m/z* (%) 502 (M^+, 21), 436 (14), 241 (61), 132 (61), 91 (100), 77 (93). Anal. Calcd for $C_{26}H_{26}N_6O_3S$ (502.59): C, 62.13; H, 5.21; N, 16.72. Found: C, 62.07; H, 5.10; N, 16.63%.

Ethyl 4-(1-((5-benzoyl-3-phenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)hydrazono)ethyl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (11b): Yellow solid, (71% yield); mp 224-226 °C; IR (KBr): v = 1744, 1652 (2C=O), 1600 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 1.30 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 2.34 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.46 (3H, s, CH_3), 4.32 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 7.36-7.44 (m, 14H, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 11.6, 13.8, 20.6, 30.6 (CH_3), 61.1 (CH_2), 121.7, 122.5, 124.3, 125.4, 128.7, 129.7, 130.3, 132.7, 134.5, 135.3, 137.3, 138.9, 139.3, 139.8, 140.2, 142.5, 149.0, 153.5 (Ar-C), 162.4, 194.61 (CO). MS, *m/z* (%) 564 (M^+, 37), 303 (38), 113 (40), 87 (100), 59 (83). Anal. Calcd for $C_{31}H_{28}N_6O_3S$ (564.66): C, 65.94; H, 5.00; N, 14.88. Found: C, 65.74; H, 4.87; N, 14.69%.

Ethyl 5-methyl-4-(1-((3-phenyl-5-(phenylcarbamoyl)-1,3,4-thiadiazol-2(3*H*)-ylidene)hydrazono)-ethyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (11c): Yellow solid, (73% yield); mp 278-280 °C; IR (KBr): v = 3386 (NH), 1744, 1656 (2C=O), 1602 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 1.30 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 2.34 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.46 (3H, s, CH_3), 4.34 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 7.33-7.44 (m, 14H, Ar-H), 11.23 (1H, s, NH); MS, *m/z* (%) 579 (M^+, 10), 499 (39), 241 (34), 119 (85), 77 (100). Anal. Calcd for $C_{31}H_{29}N_7O_3S$ (579.67): C, 64.23; H, 5.04; N, 16.91. Found: C, 64.19; H, 5.02; N, 16.73%.

Ethyl 5-((1-(3-(ethoxycarbonyl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)ethylidene)hydrazono)-4-phenyl-4,5-dihydro-1,3,4-thiadiazole-2-carboxylate (11d): Yellow solid, (71% yield); mp 236-238 °C; IR (KBr): v = 3386 (NH), 1744, 1655 (2C=O), 1602 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 1.19 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 1.30 (3H, t, J = 7.2, $\underline{CH_3}CH_2$), 2.34 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.63 (3H, s, CH_3), 4.12 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 4.32 (2H, q, J = 7.2, $CH_3\underline{CH_2}$), 7.36-7.44 (m, 9H, Ar-H); MS, *m/z* (%) 532 (M^+, 19), 468 (32), 258 (21), 135 (75), 87 (100). Anal. Calcd for $C_{27}H_{28}N_6O_4S$ (532.61): C, 60.89; H, 5.30; N, 15.78. Found: C, 60.71; H, 5.22; N, 15.71.

Ethyl 4-(1-((3,5-diphenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)hydrazono)ethyl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (11e): Yellow solid, (72% yield); mp 249-251 °C; IR (KBr): ν = 1744, 1655 (2C=O), 1602 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): ν = 1.30 (3H, t, *J* = 7.2, $\underline{CH_3}CH_2$), 2.34 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 2.50 (3H, s, CH_3), 4.32 (2H, q, *J* = 7.2, $CH_3\underline{CH_2}$), 7.36-7.44 (m, 14H, Ar-H); MS, *m/z* (%) 536 (M^+, 6), 347 (20), 101 (41), 59 (100). Anal. calcd for $C_{30}H_{28}N_6O_2S$ (536.65): C, 67.14; H, 5.26; N, 15.66; found: C, 67.10; H, 5.21; N, 15.48%.

Alternate synthesis of 11e

To a solution of 4-acetyl-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate **1** (0.286 g, 1 mmol) in 2-PrOH (10 mL), 2-hydrazono-3,5-diphenyl-2,3-dihydro-1,3,4-thiadiazole **12**[34] (0.268 g, 1 mmol) was added. The mixture was refluxed for 2 h then cooled to room temperature. The solid precipitated was filtered off, washed with water, dried and crystallized from DMF to give pure **11e** which was identical in all aspects (mp, mixed mp and spectra) with those obtained from reaction of **1** with **3e**.

Synthesis of ethyl 5-methyl-1-(*p*-tolyl)-4-(3-(*p*-tolyl)acryloyl)-1*H*-pyrazole-3-carboxylate (13). A mixture of ethyl 5-methyl-1-(*p*-tolyl)-4-(3-(*p*-tolyl)acryloyl)-1*H*-pyrazole-3-carboxylate (3.88 g, 10 mmol), 4-methylbenzaldehyde (1.2 g, 10 mmol), and 10% aqueous sodium hydroxide (10 mL) in EtOH (30 mL) was stirred at room temperature for about 6 h. The resulting solid was washed, dried, and crystallized from EtOH to give pure product **13** as white crystals, (74% yield); mp 184-186 °C; IR (KBr) ν= 1712, 1671 (2C=O), 1598 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.20 (t, *J* = 7.2, 3H, $\underline{CH_3}CH_2$), 2.29 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 4.28 (q, *J* = 7.2, 2H, $CH_3\underline{CH_2}$), 7.79 (d, 1H, *J* = 16 Hz, CO-CH=), 7.09-7.57 (m, 8H, Ar-H), 8.08 (d, *J* = 16 Hz, 1H, =CH-Ar); MS, *m/z* (%) 388 (M^+, 12), 335 (13), 254 (100), 132 (24), 91 (59), 65 (36); Anal. Calcd for $C_{24}H_{24}N_2O_3$ (388.46): C, 74.21; H, 6.23; N, 7.21. Found: C, 74.14; H, 6.17; N, 7.09%.

Synthesis of ethyl 5-methyl-4-(4-oxo-2-thioxo-5-(*p*-tolyl)-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidin-7-yl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (14). A mixture of ethyl 5-methyl-1-(*p*-tolyl)-4-(3-(*p*-tolyl)acryloyl)-1*H*-pyrazole-3-carboxylate **13** (3.88 g, 10 mmol) and 6-amino-2-thioxo-2,3,4-trihydro-1*H*-pyrimidin-4-one (1.43 g, 10 mmol) in glacial AcOH (30 mL) was heated under reflux for 6 h. After cooling, the reaction mixture was poured into ice/HCl mixture and the formed solid was collected and crystallized from DMF to give thione **14** as yellow crystals, (78 yield); mp 270-272 °C; IR (KBr) ν = 3419, 3227 (2NH), 1705, 1663 (2C=O), 1601 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.24 (t, *J* = 7.2, 3H, $\underline{CH_3}CH_2$), 2.27 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.27 (q, *J* = 7.2, 2H, $CH_3\underline{CH_2}$), 7.02-7.44 (m, 8H, Ar-H), 8.06 (s, 1H, pyridine-H), 11.43 (br., 1H, NH), 11.83 (br., 1H, NH); MS, *m/z* (%) 511 (M^+, 5), 488 (63), 364 (7), 219 (9), 149 (6), 80 (100); Anal. Calcd for $C_{28}H_{25}N_5O_3S$ (511.59): C, 65.74; H, 4.93; N, 13.69. Found: C, 65.69; H, 4.88; N, 13.48%.

Synthesis of ethyl 4-(3-substituted-5-oxo-1-aryl-6-(*p*-tolyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo-[4,3-*a*]pyrimidin-8-yl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate 18a-h. To a solution of **14** (0.412 g, 1 mmol) and the appropriate hydrazonoyl chlorides **3** (1 mmol) in dioxane (20 mL) was added triethylamine (0.14 mL, 1 mmol). The reaction mixture was refluxed till all of the starting materials had disappeared (20-24 h, monitored by TLC). The solvent was evaporated and the residue was triturated with MeOH. The solid formed was collected and crystallized from the appropriate solvent to give products **18a-h**. The products **18a-h** together with their physical constants are listed below.

Ethyl 4-(3-acetyl-5-oxo-1-phenyl-6-(*p*-tolyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-8-yl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (18a): Yellow solid, (76% yield), mp 268-270 °C; IR (KBr) ν = 1705, 1678, 1620 (3C=O), 1599 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.09 (t, J = 7.2, 3H, $\underline{CH_3}CH_2$), 2.25 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 4.21 (q, J = 7.2, 2H, $CH_3\underline{CH_2}$), 7.01-7.67 (m, 13H, Ar-H), 8.23 (s, 1H, pyridine-H); MS, *m/z* (%) 641 (M$^+$, 2), 468 (18), 318 (8), 223 (2), 199 (5), 132 (3), 80 (100). Anal. Calcd for $C_{37}H_{31}N_7O_4$ (637.69): C, 69.69; H, 4.90; N, 15.38. Found: C, 69.61; H, 4.82; N, 15.27%.

Ethyl 4-(3-acetyl-5-oxo-1,6-di-*p*-tolyl-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-8-yl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (18b): Yellow solid, (77% yield), mp 258-260 °C; IR (KBr) ν = 1708, 1676, 1641 (3C=O), 1597 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.10 (t, J = 7.2, 3H, $\underline{CH_3}CH_2$), 2.21 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 4.28 (q, J = 7.2, 2H, $CH_3\underline{CH_2}$), 7.03-7.51 (m, 12H, Ar-H), 8.08 (s, 1H, pyridine-H); MS, *m/z* (%) 651 (M$^+$, 1), 579 (7), 488 (2), 199 (2), 80 (3), 64 (100). Anal. Calcd for $C_{38}H_{33}N_7O_4$ (651.71): C, 70.03; H, 5.10; N, 15.04. Found: C, 70.01; H, 5.03; N, 14.84%.

Ethyl 4-(3-acetyl-1-(4-chlorophenyl)-5-oxo-6-(*p*-tolyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-8-yl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (18c): Yellow solid, (73% yield), mp 237-239 °C; IR (KBr) ν = 1714, 1655, 1641 (3C=O), 1591 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.11 (t, J = 7.2, 3H, $\underline{CH_3}CH_2$), 2.28 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 4.23 (q, J = 7.2, 2H, $CH_3\underline{CH_2}$), 7.05-7.75 (m, 12H, Ar-H), 8.28 (s, 1H, pyridine-H); MS, *m/z* (%) 672 (M$^+$, 5), 599 (25), 219 (23), 91 (26), 64 (100). Anal. Calcd for $C_{37}H_{30}ClN_7O_4$ (672.13): C, 66.12; H, 4.50; N, 14.59. Found: C, 66.13; H, 4.39; N, 14.48%.

Ethyl 8-(3-(ethoxycarbonyl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-5-oxo-1-phenyl-6-(*p*-tolyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-3-carboxylate (18d): Yellow solid, (71% yield), mp 220-222 °C; IR (KBr) ν = 1719, 1660, 1648 (3C=O), 1590 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.11 (t, J = 7.2, 3H, $\underline{CH_3}CH_2$), 1.33 (t, J = 7.2, 3H, $\underline{CH_3}CH_2$), 2.28 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.63 (s, 3H,

CH_3), 4.16 (q, *J* = 7.2, 2H, $CH_3\underline{CH_2}$), 4.43 (q, *J* = 7.2, 2H, $CH_3\underline{CH_2}$), 7.04-7.67 (m, 13H, Ar-H), 8.18 (s, 1H, pyridine-H); MS, *m/z* (%) 667 (M^+, 3), 633 (8), 444 (23), 321 (21), 80 (100), 64 (36). Anal. Calcd for $C_{38}H_{33}N_7O_5$ (667.71): C, 68.35; H, 4.98; N, 14.68. Found: C, 68.17; H, 4.91; N, 14.42%.

Ethyl 8-(3-(ethoxycarbonyl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-5-oxo-1,6-di-*p*-tolyl-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-3-carboxylate (18e): Yellow solid, (74% yield), mp 217-219 °C; IR (KBr) ν = 1748, 1688, 1640 (3C=O), 1593 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.14 (t, *J* = 7.2, 3H, $\underline{CH_3}CH_2$), 1.33 (t, *J* = 7.2, 3H, $\underline{CH_3}CH_2$), 2.23 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 4.14 (q, *J* = 7.2, 2H, $CH_3\underline{CH_2}$), 4.43 (q, *J* = 7.2, 2H, $CH_3\underline{CH_2}$), 7.04-7.50 (m, 12H, Ar-H), 8.04 (s, 1H, pyridine-H); MS, *m/z* (%) 681 (M^+, 7), 657 (28), 277 (45), 149 (34), 80 (100), 64 (48). Anal. Calcd for $C_{39}H_{35}N_7O_5$ (681.74): C, 68.71; H, 5.17; N, 14.38. Found: C, 68.58; H, 5.11; N, 14.27%.

Ethyl 1-(4-chlorophenyl)-8-(3-(ethoxycarbonyl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-5-oxo-6-(*p*-tolyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-3-carboxylate (18f): Yellow solid, (77% yield), mp 226-228 °C; IR (KBr) ν = 1718, 1683, 1620 (3C=O), 1591 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.13 (t, *J* = 7.2, 3H, $\underline{CH_3}CH_2$), 1.32 (t, *J* = 7.2, 3H, $\underline{CH_3}CH_2$), 2.23 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 4.14 (q, *J* = 7.2, 2H, $CH_3\underline{CH_2}$), 4.43 (q, *J* = 7.2, 2H, $CH_3\underline{CH_2}$), 7.24-7.74 (m, 12H, Ar-H), 8.25 (s, 1H, pyridine-H); MS, *m/z* (%) 704 (M+2, 1), 702 (M^+, 3), 629 (100), 556 (32), 132 (40), 91 (64), 80 (22). Anal. Calcd for $C_{38}H_{32}ClN_7O_5$ (702.16): C, 65.00; H, 4.59; N, 13.96. Found: C, 64.93; H, 4.51; N, 13.83%.

Ethyl 5-methyl-4-(5-oxo-1-phenyl-3-(phenylcarbamoyl)-6-(*p*-tolyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]-triazolo[4,3-*a*]pyrimidin-8-yl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (18g): Yellow solid, (71% yield), mp 272-274 °C; IR (KBr) ν = 3383 (NH), 1725, 1693, 1628 (3C=O), 1598 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.10 (t, *J* = 7.2, 3H, $\underline{CH_3}CH_2$), 2.27 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 4.20 (q, *J* = 7.2, 2H, $CH_3\underline{CH_2}$), 7.04-7.73 (m, 18H, Ar-H), 8.26 (s, 1H, pyridine-H), 11.06 (s, br, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 9.4, 13.6, 20.6, 20.8 (CH_3), 61.4 (CH_2), 105.8, 119.6, 120.5, 124.8, 125.2, 127.3, 127.5, 127.7, 128.2, 128.4, 128.7, 129.1, 129.3, 129.7, 135.3, 136.3, 137.5, 138.3, 140.7, 146.3, 152.6, 154.7 (Ar-C), 159.5, 160.0, 171.1 (CO). MS, *m/z* (%) 714 (M^+, 1), 629 (9), 279 (7), 149 (80), 57 (100). Anal. Calcd for $C_{42}H_{34}N_8O_4$ (714.77): C, 70.58; H, 4.79; N, 15.68. Found: C, 70.58; H, 4.70; N, 15.53%.

Ethyl 4-(1-(4-chlorophenyl)-5-oxo-3-(phenylcarbamoyl)-6-(*p*-tolyl)-1,5-dihydropyrido[2,3-*d*][1,2,4]-triazolo[4,3-*a*]pyrimidin-8-yl)-5-methyl-1-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (18h): Yellow solid, (79% yield), mp 283-285 °C; IR (KBr) ν = 3396 (NH), 1717, 1689, 1628 (3C=O), 1597 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.08 (t, *J* = 7.2, 3H, $\underline{CH_3}CH_2$), 2.28 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 4.28 (q, *J* = 7.2, 2H, $CH_3\underline{CH_2}$), 7.04-7.74 (m, 17H, Ar-H), 8.19 (s, 1H, pyridine-H), 11.13 (s, br, 1H, NH); MS, *m/z* (%) 751 (M+2, 1), 749 (M^+, 4), 562 (67), 240 (22), 180 (44), 80 (10), 64 (100). Anal. Calcd for $C_{42}H_{33}ClN_8O_4$ (749.22): C, 67.33; H, 4.44; N, 14.96 Found: C, 67.31; H, 4.37; N, 14.87%.

Alternate synthesis of 18a

A mixture of ethyl 5-methyl-1-(*p*-tolyl)-4-(3-(*p*-tolyl)acryloyl)-1*H*-pyrazole-3-carboxylate **13** (0.388 g, 1 mmol) and 3-acetyl-7-amino-1-phenyl-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1*H*)-one **20** (0.269 g, 1 mmol) in glacial AcOH (10 mL) was refluxed for 6 h. After cooling, the reaction mixture was poured onto ice/HCl mixture and the precipitated solid was collected and crystallized from DMF. The isolated product proved identical in all aspects (mp, mixed mp and spectra) with the corresponding **18a**.

ANTIMICROBIAL ACTIVITY ASSAY

The preliminary antimicrobial activity was investigated on a dozen of newly synthesized compounds in order to increase the selectivity of these derivatives towards test microorganisms. All microbial strains were provided from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

The antimicrobial profile was tested against two Gram-positive bacterial species (*Bacillus subtilis*, *Staphylococcus aureus*), two Gram negative bacterial species (*Escherichia coli*, *Pseudomonas aeruginosa*), two fungi (*Aspergillus fumigatus* and *Candida albicans*) using a modified well diffusion method.[37] Briefly, 100 µL of the test bacteria/fungi were grown in 10 mL of fresh media Mueller-Hinton and Sabaroud agar (Oxoid, UK) until they reached a count of approximately 10^8 cells/mL for bacteria or 10^5 cells/mL for fungi. One hundred µL of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained and tested for susceptibility by well diffusion method. One hundred µL of each sample (at 5 mg/mL) was added to each well (10 mm diameter holes cut in the agar gel). The plates were incubated for 24-48 h at 37 °C (for bacteria and yeast) and for 48 h at 28 °C (for filamentous fungi). After incubation, the microorganism's growth was observed. The plates were done in triplicate and the resulting inhibition zone diameters were measured in millimeters and used as criterion for the antimicrobial activity. The size of the clear zone is proportional to the inhibitory action of the compound under investigation. Solvent control (DMSO) was included in every experiment as negative control. The standard drugs were also screened for antibacterial and antifungal activities under similar conditions, for comparison.

REFERENCES

1. A. S. Shawali *J. Adv. Res.*, 2014, **5**, 1.
2. A. Jamwal1, A. Javed, and V. Bhardwaj, *J. Pharm. BioSci.*, 2013, **3**, 114.
3. K. D. Hargrave, F. K. Hess, and J. T. Oliver, *J. Med. Chem.*, 1983, **26**, 1158.
4. W. C. Patt, H. W. Hamilton, M. D. Taylor, M. J. Ryan, D. G. Taylor, C. J. C. Connolly, A. M.

Doherty, S. R. Klutchko, I. Sircar, B. A. Steinbaugh, B. L. Batley, C. A. Painchaud, S. T. Rapundalo, B. M. Michniewicz, and S. C. J. Olson, *J. Med. Chem.*, 1992, **35**, 2562.

5. R. N. Sharma, F. P. Xavier, K. K. Vasu, S. C. Chaturvedi, and S. S. Pancholi, *J. Enzym. Inhib. Med. Chem.*, 2009, **24**, 890.
6. J. C. Jaen, L. D. Wise, B. W. Caprathe, H. Tecle, S. Bergmeier, C. C. Humblet, T. G. Heffner, L. T. Meltzner, and T. A. Pugsley, *J. Med. Chem.*, 1990, **33**, 311.
7. K. Tsuji and H. Ishikawa, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1601; F. W. Bell, A. S. Cantrell, M. Hogberg, S. R. Jaskunas, N. G. Johansson, C. L. Jordon, M. D. Kinnick, P. Lind, J. M. Morin, R. Noreen, B. Oberg, J. A. Palkowitz, C. A. Parrish, P. Pranc, C. Sahlberg, R. J. Ternansky, R. T. Vasileff, L. Vrang, S. J. West, H. Zhang, and H. X.-X. Zhou, *J. Med. Chem.*, 1995, **38**, 4929.
8. N. Ergenc, G. Capan, N. S. Gunay, S. Ozkirimli, M. Gungor, S. Ozbey, and E. Kendi, *Arch. Pharm. Pharm. Med. Chem.*, 1999, **332**, 343.
9. J. S. Carter, S. Kramer, J. J. Talley, T. Penning, P. Collins, M. J. Graneto, K. Seibert, C. Koboldt, J. Masferrer, and B. Zweifel, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1171.
10. A. Badorc, M. F. Bordes, P. de Cointet, P. Savi, A. Bernat, A. Lale, M. Petitou, J. P. Maffrand, and J. M. Herbert, *J. Med. Chem.*, 1997, **40**, 3393.
11. J. Rudolph, H. Theis, R. Hanke, R. Endermann, L. Johannsen, and F. U. Geschke, *J. Med. Chem.*, 2001, **44**, 619.
12. M. Fares, S. M. Abou-Seri, H. A. Abdel-Aziz, S. E. S. Abbas, M. M. Youssef, and R. A. Eladwy, *Eur. J. Med. Chem.*, 2014, **83**, 155.
13. A. V. Astakhov and V. M. Chernyshev, *Chem. Heterocycl. Compd.*, 2014, **50**, 319.
14. X. H. Liu, Z. H. Sun, M. Y. Yang, C. X. Tan, J. Q. Weng, Y. G. Zhang, and Y. Ma, *Chem. Biol. Drug Des.*, 2014, **84**, 342.
15. T. A. Farghaly and H. M. E. Hassaneen, *Arch. Pharm. Res.*, 2013, **36**, 564.
16. S. M. Gomha, *Monatsh. Chem.*, 2009, **140**, 213.
17. S. M. Gomha and M. G. Badrey, *Eur. J. Chem.*, 2013, **4**, 180.
18. A. O. Abdelhamid, A. A. Fahmi, and K. N. M. Halim, *Synth. Commun.*, 2013, **43**, 1101.
19. A. Abdelazim, M. S. Ei-Gendy, and A. O. Abdelhamid, *Eur. J. Chem.*, 2012, **3**, 455.
20. S. M. Gomha, A. S. Shawali, and A. O. Abdelhamid, *Turk. J. Chem.*, 2014, **38**, 865.
21. S. A. Ahmad, N. A. Abdelreiheem, and A. O. Abdelhamid, *Eur. Bull. Chem.*, 2014, **5**, 334.
22. S. A. Ahmad, O. M. Ahmad, and A. O. Abdelhamid, *Eur. J. Chem.*, 2014, **5**, 334.
23. S. M. Gomha, K. D. Khalil, A. M. El-Zanate, and S. M. Riyadh, *Heterocycles*, 2013, **87**, 1109.
24. S. M. Gomha and H. M. Abdel-aziz, *Heterocycles*, 2015, **91**, 583.
25. A. Abdelhamid, A. S. Shawali, S. M. Gomha, and W. A. M. A. El-Enany, *World J. Pharm.*

Pharmaceut. Sci., 2015, **4**, 1695.

26. H. A. Emam, H. F. Zohdi, and A. O. Abdelhamid, *J. Chem. Res. (M)*, 1998, 169.
27. R. N. Butler, *Comprehensive Heterocyclic Chemistry*, ed. by A. R. Katritzky, C. W. Rees, and E. F. V. Scriven, Pergamon Press: New York, NY, USA, 1996, Vol. 4, p 621.
28. R. Huisgen, R. Grashey, M. Seidel, H. Knupfer, and R. Schmidt, *Liebigs Ann. Chem.*, 1962, **658**, 169.
29. M. A. N. Mosselhi, *Monatsh. Chem.*, 2002, **133**, 1297.
30. A. M. Asiri, M. E. M. Zayed, and S. W. Ng, *Acta Cryst.*, 2011, **67**, o1962.
31. N. F. Eweiss and A. Osman, *J. Heterocycl. Chem.*, 1980, **17**, 1713.
32. A. S. Shawali and A. Osman, *Bull. Chem. Soc. Jpn.*, 1976, **49**, 321.
33. A. S. Shawali and A. Osman, *Tetrahedron*, 1971, **27**, 2517.
34. A. O. Abdelhamid and F. H. H. El-Shiatey, *Phosphorus, Sulfur Silicon Relat. Elem.*, 1988, **39**, 45.
35. H. M. Hassaneen, A. S. Shawali, N. M. Elwan, and N. M. Abounada, *Sulfur Lett.*, 1992, **13**, 273.
36. A. O. Abdelhamid, H. F. Zohdi, and N. M. Rateb, *J. Chem. Res. (S)*, 1999, **184**, 920.
37. M. A. Pfaller, L. Burmeister, M. A. Ghorab, and M. G. Rinaldi, *J. Clin. Microbiol.*, 1988, **26**, 1437.