European Journal of Medicinal Chemistry 52 (2012) 263–268

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design and synthesis of new 4-pyrazolin-3-yl-1,2,3-triazoles and 1,2,3-triazol-4-yl-pyrazolin-1-ylthiazoles as potential antimicrobial agents

Bakr F. Abdel-Wahab [a,*], Ehab Abdel-Latif [b], Hanan A. Mohamed [a], Ghada E.A. Awad [c]

[a] Applied Organic Chemistry Department, National Research Centre, Dokki, 12622 Giza, Egypt
[b] Department of Chemistry, Faculty of Science, Mansoura University, ET-35516, Mansoura, Egypt
[c] Chemistry of Natural and Microbial Products, National Research Center, Dokki, 12622 Giza, Egypt

ARTICLE INFO

Article history:
Received 31 January 2012
Received in revised form
13 March 2012
Accepted 13 March 2012
Available online 23 March 2012

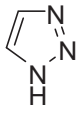
Keywords:
Pyrazolyltriazole
Biheterocycles
Antimicrobial
X-ray crystallography

ABSTRACT

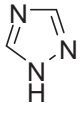
New pyrazolyl-1,2,3-triazoles and 1,2,3-triazol-4-yl-pyrazolylthiazoles were synthesized through multi step reactions using 1-tolylyl-4-acetyl-5-methyl-1,2,3-triazole as a precursor. All the newly synthesized compounds were characterized by spectral and elemental analyses. The structure of **11b** was evidenced by X-ray crystallographic study. The newly synthesized compounds were evaluated for their antimicrobial activities and also their minimum inhibitory concentration (MIC) against most of test organisms was performed. Amongst the tested compounds **5a**, **5c**, **11b** and **11c** displayed excellent antimicrobial activity.

1. Introduction

Triazole refers to either one of a pair of isomeric chemical compounds with molecular formula $C_2H_3N_3$, having a five membered ring of two carbon atoms and three nitrogen atoms. The two isomers are:



1,2,3-triazole



1,2,4-triazole

In recent years, the 1,2,3-triazole ring system has been the subject of considerable research due to its usefulness in synthetic organic chemistry and also because of the pharmacological properties. 1,2,3-Triazoles display biological activities such as anti-HIV activity [1,2], antimicrobial activity against Gram positive bacteria [3], antiviral [4] and antiproliferative [5]. They are used as intermediates at the synthesis of antibiotics [6–8]. The derivatives of 1,2,3-triazole also are applied as insecticides [9], fungicides [10], plant growth regulators [11,12]. 1,2,3-Triazoles have also widely used in industrial applications such as dyes, corrosion inhibition (of copper and copper alloys), photostabilizers, photographic materials, and agrochemicals [13]. Pyrazole derivatives have showed significant biological activities, such as antimicrobial [14], analgesic [15], anti-inflammatory [16] and anticancer [17] activities. Therefore it is important to synthesize new compounds having both 1,2,3-triazole and pyrazoline moieties. In this work, and in continuation of our previous work to discover new biologically active heterocyclic compounds, [18–25] we aimed to the synthesis of novel compounds based on 1,2,3-triazoles-linked pyrazolines.

2. Results and discussion

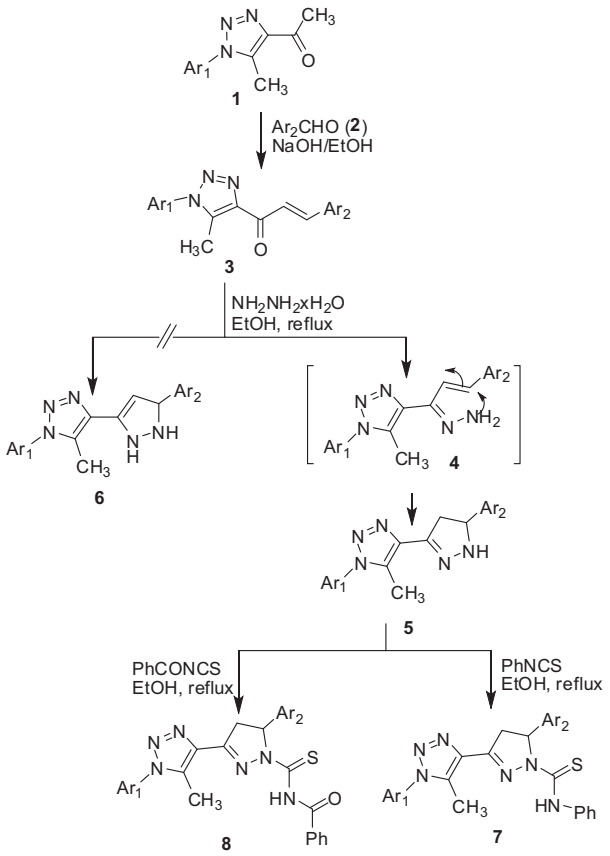
2.1. Chemistry

The title compounds **3**–**14** were prepared from 1-(5-methyl-1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl)ethanone **1**. The starting material **1** was prepared starting from *p*-toluidine and acetylacetone, according to literature [26]. 4-Acetyltriazole **1** was reacted with equivalent of aromatic aldehydes **2** in the presence of 10% alcoholic NaOH in 90% ethanol with stirring at room temperature to give key intermediates **3**. The chalcone **3a** was prepared previously by Dong et al., 2010 (Scheme 1) [27].

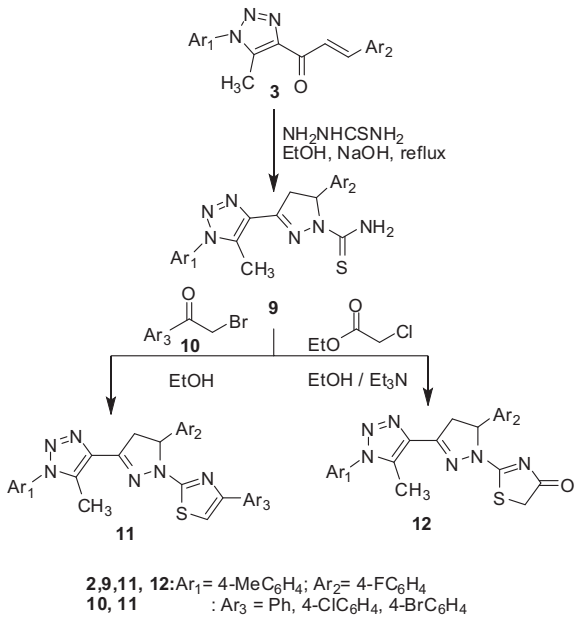
According to Scheme 1, the desired pyrazolinyltriazoles **5** have been prepared by cyclocondensation of hydrazine hydrate with chalcones **3**, which includes formation of hydrazone intermediates **4** and then addition of NH_2 of hydrazone to the double bond.

* Corresponding author.
E-mail address: bakrfatehy@yahoo.com (B.F. Abdel-Wahab).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2012.03.023



Scheme 1. Synthetic routes to new pyrazolyl-1,2,3-triazoles (**5**–**8**).



Scheme 2. Synthetic accesses to 1,2,3-triazol-4-yl-pyrazolylthiazoles (**11**–**12**).

Treatment of pyrazolines **5a**–**c** with phenyl isothiocyanate in anhydrous ethanol led to the formation of 3-(1,2,3-triazol-4-yl)-5-methyl-*N*-phenyl-pyrazole-1-carbothioamides **7a**–**c** in 52–55% yields. Treatment of **5d** with benzoyl isothiocyante in refluxing ethanol results in the *N*-(3-(5-methyl-1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl)-5-(4-(piperidin-1-yl)phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbo nothioyl)benzamide **8** in 69% yield (Scheme 1).

The reaction between the fluorinated chalcone **3b** and the equivalent amount of thiosemicarbazide was performed in ethanol in the presence of 2.5 equivalent of sodium hydroxide to yield 3-(5-methyl-1,2,3-triazol-4-yl)-pyrazole-1-carbothioamide derivative **9**. The resulting product was cyclized to the corresponding 2-(3-(1,2,3-triazol-4-yl)-5-methyl-1*H*-pyrazol-1-yl)thiazole derivatives **11a**–**c** by reaction with phenacyl bromides **10** in anhydrous ethanol. Reaction of **9** with ethyl bromoacetate in refluxing ethanol in the presence of triethylamine gave 2-(3-(1,2,3-triazol-4-yl)-5-methyl-pyrazol-1-yl)thiazol-4-one **12** in high yield (Scheme 2).

Determination of the reaction product structures was performed by means of IR, NMR, MS spectroscopy, and X-ray diffraction. For example ^{1}H NMR spectra of **5** contained two doublet–doublet and one triplet signals due to the presence of CH_2 adjacent asymmetric carbon and presence of a one singlet single at δ 10.19 ppm due to NH proton. While the ^{1}H NMR of compounds **7** and **8** indicate the disappearance of NH signal due to blocking of NH with carbothioamide. The IR spectrum of **5c** exhibits absorption bands at ν_{max}2224 cm^{-1} due to the carbonitrile substituent. The mass spectra of **5b**, **7b** and **8** showed the molecular ion peaks at *m*/*z* 335, 470 and 564 respectively in agreement with the calculated masses.

X-ray crystallography of this tricyclic system **11b** (crystallized from DMF) confirmed the anticipated structure (Fig. 1).

2.2. Antimicrobial activity

All the synthesized compounds were screened for their antibacterial and antifungal activities at 100 μg/ml concentration against four *Gram positive bacteria* (*Staphylococcus aureus* ATCC 29213; *Bacillus subtilis ATCC6633*; *Bacillus megaterium ATCC* 9885; *Sarcina lutea*), three *Gram negative bacteria* (*Klebseilla pneumoniae* ATCC13883; *Pseudomonas aeroginosa* ATCC27953; *Escherichia coli ATCC 25922*) and two yeast (*Saccharomyces cerevisiae* and *Candida albicans* NRRL Y-477). Ciprofloxacin and Ketoconazole were respectively used as standard antibacterial and antifungal reference, respectively. Most of the newly synthesized compounds showed excellent antimicrobial activities with respect to the control drugs. The results of antimicrobial activities were shown in Table 1. Data in Table 1 revealed that most of compounds have superior significant antifungal potency to antibacterial potency. Compounds **5a**, **5c**, **11a** and **11c** exhibited the highest potency against all tested organisms with respect to reference drugs. Compound **5a** inhibited the growth of *S. aureus* ATCC 29213; *B. subtilis ATCC6633* and *S. lutea* with inhibition zones 34, 32 and 30 mm respectively. While compound **5c** showed excellent activity against *K. pneumoniae* ATCC13883; *E. coli ATCC 25922* and *S. cerevisiae* with inhibition zone 30 mm. Also, compound **11c** showed highest activity against *S. aureus* ATCC 29213, *S. cerevisiae* and *C. albicans* NRRL Y-477 with inhibition zone 30 mm.

2.3. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the synthesized compounds against highly inhibited organisms is reported in Table 2.

Compounds **5a** revealed high MIC (132 μg/ml) against *B. megaterium ATCC* 9885, *E. coli ATCC 25922* respectively. On the other hand, compounds **5a** exhibited low MIC (8.25 μg/ml) against *S.*

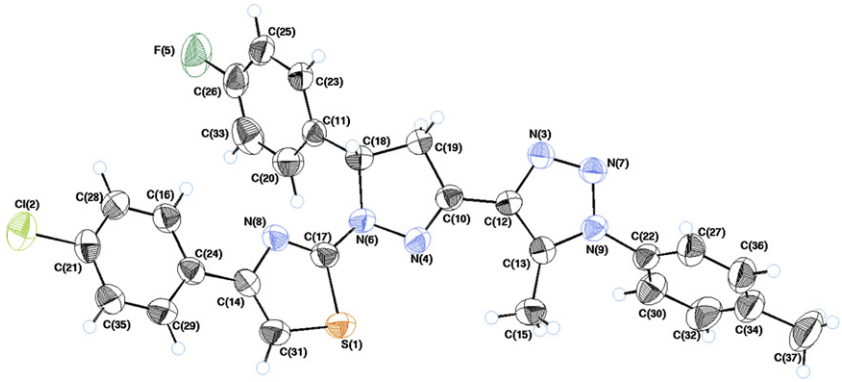


Fig. 1. X-ray structure of compound **11b**.

aureus ATCC 29213; *B. subtilis ATCC6633*; *P. aeroginosa* ATCC27953; *S. cerevisiae* and *C. albicans* NRRL Y-477 respectively. Compound **5c** showed MIC 8.25 μg/ml against *B. subtilis ATCC6633*; *B. megaterium ATCC* 9885; *S. lutea*, *K. pneumoniae* ATCC13883; *P. aeroginosa* ATCC27953; *E. coli ATCC 25922*; *S. cerevisiae* and *C. albicans* NRRL Y-477. Additionally, compounds **11b** and **11c** exhibited MIC 8.25 μg/ml against *S. aureus* ATCC 29213 and also showed MIC 16.5 μg/ml against *K. pneumoniae* ATCC13883 (Table 2).

3. Conclusion

We have synthesized new pyrazolyl-triazole and thiazolyl pyrazolyl triazoles with potential antimicrobial activity, from available 4-acetyl-5-methyl-1,2,3-triazole by routine and facile methods. The new compounds were tested for their antimicrobial activities and most of them show significant activities.

4. Experimental

4.1. Chemistry

4.1.1. General

All melting points were taken on Electrothermal IA 9000 series digital melting point apparatus. Elemental analytical data were carried from the microanalytical unit, Cairo University, Giza, Egypt. The IR spectra were recorded in potassium bromide disks on a JASCO FT/IR-6100. ^{1}H NMR and ^{13}C NMR spectra were run on JOEL-ECA 500 MHz in deuterated dimethylsulphoxide (DMSO-d_6). Chemical shifts values (δ) are given in parts per million (ppm). The mass spectra were performed using mass Varian MAT CH-5 spectrometer at 70 eV. 1-(5-Methyl-1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl) ethanone **1** [26] and 4-(piperidin-1-yl)benzaldehyde **2d** [28] were prepared according to literature.

4.1.2. General procedure for the preparation of *3b–d*

A mixture of the appropriate aromatic aldehydes **2b–d** (12 mmol) and compound **1** (10 mmol, 2.15 g) dissolved in ethanol (50 ml) was added slowly to an aqueous solution of sodium hydroxide (12.8 mmol) in water (10 ml). The reaction mixture was stirred at 20–25 °C for 4 h. The mixture was filtrated and the solid was washed with cold water. The product was crystallized from ethanol to give **3b–d**.

*4.1.2.1. (E)-3-(4-Fluorophenyl)-1-(5-methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)prop-2-en-1-one (**3b**).* Yield 70%; m.p. 148–50 °C; IR (KBr) ν_{max}/cm^{-1} 1652 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.38 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.91 (d, 1H, CH, J = 18.8 Hz), 7.42 (d, 1H, CH, J = 19.1 Hz); 7.29–7.48 (m, 8H, Ar–H) MS *m*/*z* (%): 321 (M^+, 11), 132 (100).

Table 1
Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of chemical compounds against the pathological strains based on well diffusion assay.

Chemical compound	Gram positive bacteria				Gram negative bacteria			Yeast	
	Staphylococcus aureus ATCC 29213	*B. subtilis ATCC6633*	*B. megaterium ATCC* 9885	*Sarcina lutea*	*Klebseilla pneumoniae* ATCC13883	*Pseudomonas aeroginos* ATCC27953	*E. coli ATCC 25922*	*Saccharomyces cerevisiae*	*Candida albicans* NRRL Y-477
3a	22	26	N.A.	26	16	28	32	22	24
3b	20	26	24	16	16	16	N.A.	23	22
3c	23	28	N.A.	24	24	26	16	30	24
3d	16	18	20	28	N.A.	16	28	20	18
5a	34	32	16	30	N.A.	28	16	28	28
5b	20	N.A.	16	18	16	30	16	18	16
5c	N.A.	26	24	28	30	28	30	30	28
5d	18	16	N.A.	18	N.A.	N.A.	N.A.	18	18
7a	18	26	24	20	18	24	N.A.	26	24
7b	16	27	20	20	22	N.A.	N.A.	24	22
7c	20	N.A.	N.A.	24	N.A.	22	N.A.	24	22
8	16	24	30	23	N.A.	26	32	30	30
9	N.A.	N.A.	18	16	N.A.	26	22	30	26
11a	26	24	24	22	26	28	16	22	20
11b	28	30	N.A.	22	28	24	29	28	25
11c	30	26	22	22	24	N.A.	22	30	30
12	N.A.	N.A.	30	16	N.A.	20	28	18	16
Ciprofloxacin	20	22	24	20	25	24	23	N.A.	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	23	22

The experiment was carried out in triplicate and the average zone of inhibition was calculated; (N.A. = no activity).

Table 2
Minimum inhibitory concentration (μg/ml) against the pathological strains based on two fold serial dilution technique.

Chemical compound	Gram positive bacteria				Gram negative bacteria			Yeast	
	Staphylococcus aureus ATCC 29213	*B. subtilis ATCC6633*	*B. megaterium ATCC* 9885	*Sarcina lutea*	*Klebseilla pneumoniae* ATCC13883	*Pseudomonas aeroginosa* ATCC27953	*E. coli ATCC 25922*	*Saccharomyces cerevisiae*	*Candida albicans* NRRL Y-477
3a	16.5	33	–	16.5	132	8.25	8.25	66	16.5
3b	16.5	16.5	16.5	132	132	132	–	16.5	16.5
3c	16.5	8.25	–	16.5	16.5	8.25	132	8.25	16.5
3d	132	33	33	33	–	132	8.25	66	16.5
5a	8.25	8.25	132	16.5	–	8.25	132	8.25	8.25
5b	33	–	132	66	132	8.25	132	66	132
5c	–	33	33	8.25	8.25	8.25	8.25	8.25	8.25
5d	132	132	–	33	–	–	–	66	132
7a	8.25	–	8.25	66	16.5	132	8.25	16.5	16.5
7b	132	16.5	16.5	16.5	16.5	–	–	16.5	16.5
7c	33	–	–	33	–	66	–	8.25	33
8	132	33	8.25	66	–	33	8.25	8.25	8.25
9	–	–	132	132	–	16.5	16.5	8.25	16.5
11a	16.5	33	33	66	33	8.25	132	33	66
11b	8.25	8.25	–	66	16.5	16.5	33	16.5	16.5
11c	8.25	33	33	66	16.5	–	16.5	8.25	8.25
12	–	–	8.25	132	–	33	8.25	66	132
Ciprofloxacin	8.25	8.25	8.25	16.5	16.5	16.5	16.5	–	–
Ketoconazole	–	–	–	–	–	–	–	8.25	8.25

*4.1.2.2. (E)-4-(3-(5-Methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-3-oxoprop-1-enyl)benzonitrile (**3c**).* Yield 72%; m.p. 222–3 °C; IR (KBr) ν_{max}/cm^{-1} 1650 (C═O), 2220 (CN); ^{1}H NMR (DMSO-d$_6$) δ 2.38 (s, 3H, CH$_3$), 2.48 (s, 3H, CH$_3$), 6.88 (d, 1H, CH, J = 18.7 Hz), 7.45 (d, 1H, CH, J = 18.9 Hz); 7.31–7.51 (m, 8H, Ar–H) MS m/z (%): 328 (M$^+$, 8), 132 (100).

*4.1.2.3. (E)-1-(5-Methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-3-(4-(piperidin-1-yl)phenyl)prop-2-en-1-one (**3d**).* Yield 68%; m.p. 153–4 °C; IR (KBr) ν_{max}/cm^{-1} 1661 (C═O); ^{1}H NMR (DMSO-d$_6$) δ 1.48–1.56 (m, 6H, piperidine), 3.37–3.42 (m, 4H, piperidine), 2.35 (s, 3H, CH$_3$), 2.46 (s, 3H, CH$_3$), 6.85 (d, 1H, CH, J = 17.8 Hz), 7.46 (d, 1H, CH, J = 18.0 Hz), 7.16–7.49 (m, 8H, Ar–H); MS m/z (%): 386 (M$^+$, 16), 132 (100).

*4.1.3. General procedure for the preparation of **5a–d***

To a solution of appropriate chalcones **3a–d** (2 mmol) in ethanol (30 ml), hydrazine hydrate 80% (5 mmol) was added. The reaction mixture was refluxed for 6 h. Left to cool to room temperature, and the formed solid product was filtered and washed with ethanol.

*4.1.3.1. 5-Methyl-4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-1-p-tolyl-1H-1,2,3-triazole (**5a**).* Yield 55%; m.p. 139–40 °C; IR (KBr) ν_{max}/cm^{-1} 3334 (NH); ^{1}H NMR (DMSO-d$_6$) δ 2.33 (s, 3H, CH$_3$), 2.46 (s, 3H, CH$_3$), 2.89, 2.93 (dd, 1H, CH, J = 10.7 Hz, J = 9.95 Hz), 3.54, 3.56 (dd, 1H, CH, J = 10.7 Hz, J = 10.7 Hz), 4.80 (t, 1H, CH, J = 10.7 Hz, J = 9.95 Hz), 7.13–7.43 (m, 9H, Ar–H), 10.19 (s, 1H, NH, D$_2$O-exchangeable); MS m/z (%): 317 (M$^+$, 9), 65 (100).

*4.1.3.2. 4-(5-(4-Fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-5-methyl-1-p-tolyl-1H-1,2,3-triazole (**5b**).* Yield 68%; m.p. 135–6 °C; IR (KBr) ν_{max}/cm^{-1} 3336 (NH); ^{1}H NMR (DMSO-d$_6$) δ 2.33 (s, 3H, CH$_3$), 2.46 (s, 3H, CH$_3$), 2.91, 2.94 (dd, 1H, CH, J = 10.7 Hz, J = 9.95 Hz), 3.53, 3.55 (dd, 1H, CH, J = 10.7 Hz, J = 10.7 Hz), 4.82 (t, 1H, CH, J = 10.7 Hz, J = 9.95 Hz), 7.14–7.43 (m, 8H, Ar–H), 10.11 (s, 1H, NH, D$_2$O-exchangeable); MS m/z (%): 335 (M$^+$, 8), 65 (100).

*4.1.3.3. 4-(3-(5-Methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazol-5-yl)benzonitrile (**5c**).* Yield 65%; m.p. 168–70 °C; IR (KBr) ν_{max}/cm^{-1} 2224 (CN), 3336 (NH); ^{1}H NMR (DMSO-d$_6$) δ 2.35 (s, 3H, CH$_3$), 2.45 (s, 3H, CH$_3$), 2.93, 2.95 (dd, 1H, CH, J = 10.7 Hz, J = 9.95 Hz), 3.62, 3.65 (dd, 1H, CH, J = 10.7 Hz, J = 10.7 Hz), 4.89 (t, 1H, CH, J = 10.7 Hz, J = 9.95 Hz), 7.38–7.57 (m, 8H, Ar–H), 10.19 (s, 1H, NH, D$_2$O-exchangeable); MS m/z (%): 342 (M$^+$, 7), 90 (100).

*4.1.3.4. 1-(4-(3-(5-Methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazol-5-yl)phenyl)piperidine (**5d**).* Yield 66%; m.p. 159–61 °C; IR (KBr) ν_{max}/cm^{-1} 3336 (NH); ^{1}H NMR (DMSO-d$_6$) δ 1.48–1.56 (m, 6H, piperidine), 2.35 (s, 3H, CH$_3$), 2.45 (s, 3H, CH$_3$), 3.08–3.37 (m, 4H, piperidine), 3.38, 3.41 (dd, 1H, CH, J = 10.7 Hz, J = 9.95 Hz), 4.03, 4.05 (dd, 1H, CH, J = 10.7 Hz, J = 10.7 Hz), 5.93 (t, 1H, CH, J = 10.7 Hz, J = 9.95 Hz), 7.38–7.57 (m, 8H, Ar–H), 10.19 (s, 1H, NH, D$_2$O-exchangeable); MS m/z (%): 400 (M$^+$, 11), 56 (100).

*4.1.4. General procedure for the preparation of **7a–c** and **8***

A mixture of **5a–d** (2 mmol) and phenyl isothiocyanate (2 mmol, 0.27 g) {or benzoylisothiocyanate (2 mmol, 0.33 g) in case of **8**} in dry ethanol (30 ml) was refluxed for 5 h. Cool to the room temperature and the formed solid product was collected by filtration to give products **7a–c** or **8**.

*4.1.4.1. 3-(5-Methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-N,5-diphenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**7a**).* Yield 55%; m.p. 197–9 °C; ^{1}H NMR (DMSO-d$_6$) δ 2.39 (s, 3H, CH$_3$), 2.47 (s, 3H, CH$_3$), 3.02, 3.24 (dd, 1H, CH, J = 15.3 Hz, J = 6.9 Hz), 4.08, 4.10 (dd, 1H, CH, J = 15.3 Hz, J = 6.9 Hz), 6.03 (t, 1H, CH, J = 3.05 Hz, J = 8.04 Hz), 7.15–7.48 (m, 14H, Ar–H), 9.85 (s, 1H, NH, D$_2$O-exchangeable); MS m/z (%): 452 (M$^+$, 19), 77 (100).

*4.1.4.2. 5-(4-Fluorophenyl)-3-(5-methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-N-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**7b**).* Yield 52%; m.p. 155–7 °C; ^{1}H NMR (DMSO-d$_6$) δ 2.39 (s, 3H, CH$_3$), 2.47 (s, 3H, CH$_3$), 3.02, 3.24 (dd, 1H, CH, J = 15.3 Hz, J = 6.9 Hz), 4.08, 4.10 (dd, 1H, CH, J = 15.3 Hz, J = 6.9 Hz), 6.03 (t, 1H, CH, J = 3.05 Hz, J = 8.04 Hz), 7.15–7.48 (m, 13H, Ar–H), 9.85 (s, 1H, NH, D$_2$O-exchangeable); MS m/z (%): 470 (M$^+$, 20), 77 (100).

*4.1.4.3. 3-(5-Methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-N-phenyl-5-(4-(piperidin-1-yl)phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**7c**).* Yield 53%; m.p. 168–70 °C; ^{1}H NMR (DMSO-d$_6$) δ 1.48–1.56

(m, 6H, piperidine), 2.39 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.08–3.37 (m, 4H, piperidine), 3.39, 3.41 (dd, 1H, CH, J = 10.7 Hz, J = 6.85 Hz), 4.08, 4.10 (dd, 1H, CH, J = 10.7 Hz, J = 6.85 Hz), 5.93 (t, 1H, CH, J = 3.05 Hz, J = 8.04 Hz), 7.15–7.48 (m, 13H, Ar–H), 10.19 (s, 1H, NH, D_2O-exchangeable); MS m/z (%): 535 (M^+, 20), 56 (100).

*4.1.4.4. N-(3-(5-Methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-5-(4-(piperidin-1-yl)phenyl)-4,5-dihydro-1H-pyrazole-1-carbonothioyl)benzamide (**8**).* Yield 69%; m.p. 178–80 °C; ^{1}H NMR (DMSO-d_6) δ 1.48–1.56 (m, 6H, piperidine), 2.39 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.08–3.37 (m, 4H, piperidine), 3.39, 3.41 (dd, 1H, CH, J = 10.7 Hz, J = 6.85 Hz), 4.08, 4.10 (dd, 1H, CH, J = 10.7 Hz, J = 6.85 Hz), 5.93 (t, 1H, CH, J = 3.05 Hz, J = 8.04 Hz), 6.85–7.90 (m, 13H, Ar–H), 10.97 (s, 1H, NH, D_2O-exchangeable); MS m/z (%): 564 (M^+ + 1, 50), 70 (100).

*4.1.5. 5-(4-Fluorophenyl)-3-(5-methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**9**)*

To a suspension of chalcone **3b** (10 mmol, 3.21 g) and sodium hydroxide (25 mmol, 1.0 g) in ethanol (50 ml), thiosemicarbazide (12 mmol, 1.1 g) was added. The mixture was refluxed for 6 h, then left to cool; the solid product was filtered off, washed with ethanol and dried.

Yield 46%; m.p. 240–2 °C; IR (KBr) ν_{max}/cm^{-1} 3475, 3345 (NH_2); ^{1}H NMR (DMSO-d_6) δ 2.38 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 3.14, 3.17 (dd, 1H, CH, J = 3.05 Hz, J = 3.05 Hz), 3.99, 4.03 (dd, 1H, CH, J = 11.45 Hz, J = 11.5 Hz), 5.90 (t, 1H, CH, J = 3.05 Hz, J = 7.65 Hz), 7.13–7.43 (m, 9H, Ar–H), 8.09 (s, 2H, NH_2, D_2O-exchangeable); ^{13}C NMR (DMSO-d_6): δ 10.25, 14.46, 19.88, 21.27, 39.71, 39.87, 40.20, 40.36, 41.24, 55.39, 56.41, 75.98, 87.79, 88.55, 93.12, 100.74, 106.17, 109.82, 113.32, 120.18, 125.84, 130.55, 133.58, 135.95, 140.12, 141.13, 152.95, 155.38, 163.63, 168.017, 176.96; MS m/z (%): 394 (M^+, 36), 91 (100).

*4.1.6. General procedure for the preparation of **11a–c** and **12***

To a suspension of compound **9** (1 mmol, 0.39 g) in ethanol (15 ml) the appropriate 1-aryl-2-bromoethanones **10a–c** (1 mmol) {or ethyl chloroacetate (1 mmol, 0.12 g) in case of **12**} was added and heated under reflux for 1.5 h. After cooling, the precipitate was collected by suction filtration.

*4.1.6.1. 2-(5-(4-Fluorophenyl)-3-(5-methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-phenylthiazole (**11a**).* Yield 86%; m.p. 196–8 °C; ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 3.39, 3.41 (dd, 1H, CH, J = 11.45 Hz, J = 12.25 Hz), 4.15, 4.10 (dd, 1H, CH, J = 11.45 Hz, J = 10.33 Hz), 5.64 (t, 1H, CH, J = 5.35 Hz, J = 6.1 Hz), 7.25 (s, 1H, thiazole-H), 7.29–7.51 (m, 13H, Ar–H); ^{13}C NMR (DMSO-d_6): δ 10.25, 14.46, 21.27, 125.84, 130.55, 133.11, 133.58, 140.12, 140.22, 142.23, 145.75, 168.023; MS m/z (%): 494 (M^+, 12), 91 (100).

*4.1.6.2. 4-(4-Chlorophenyl)-2-(5-(4-fluorophenyl)-3-(5-methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazol-1-yl)thiazole (**11b**).* Yield 88%; m.p. 205–7 °C; ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 3.39, 3.41 (dd, 1H, CH, J = 11.45 Hz, J = 12.25 Hz), 4.15, 4.10 (dd, 1H, CH, J = 11.45 Hz, J = 10.33 Hz), 5.64 (t, 1H, CH, J = 5.35 Hz, J = 6.1 Hz), 7.27 (s, 1H, thiazole-H), 7.30–7.56 (m, 12H, Ar–H); MS m/z (%): 530 (M^+ + 1, 38), 529 (M^+, 38), 528 (M^+ − 1, 78), 91 (100).

*4.1.6.3. 4-(4-Bromophenyl)-2-(5-(4-fluorophenyl)-3-(5-methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazol-1-yl)thiazole (**11c**).* Yield 87%; m.p. 224–5 °C; ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 3.39, 3.41 (dd, 1H, CH, J = 11.45 Hz, J = 12.25 Hz), 4.15, 4.10 (dd, 1H, CH, J = 11.45 Hz, J = 10.33 Hz), 5.64 (t, 1H, CH, J = 5.35 Hz, J = 6.1 Hz), 7.27 (s, 1H, thiazole-H), 7.30–7.56 (m, 12H, Ar–H); MS m/z (%): 574 (M^+ + 2, 28), 573 (M^+ + 1, 18), 572 (M^+, 38), 91 (100).

*4.1.6.4. 2-(5-(4-Fluorophenyl)-3-(5-methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4(5H)-one (**12**).* Yield 58%; m.p. 228–30 °C; IR (KBr) ν_{max}/cm^{-1} 1671 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.38 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 3.90 (s, 2H, thiazolidinone-CH_2) 3.17, 3.18 (dd, 1H, CH, J = 11.45 Hz, J = 3.05 Hz), 3.99, 4.03 (dd, 1H, CH, J = 11.45 Hz, J = 3.05 Hz), 5.91 (t, 1H, CH, J = 3.05 Hz, J = 7.65 Hz), 7.13–7.43 (m, 9H, Ar–H), MS m/z (%): 434 (M^+ − 1, 6) 433 (M^+, 16), 91 (100).

4.2. X-ray crystallography

A single crystal of compound **11b** was obtained by slow evaporation at room temperature, from dimethylformamide (DMF). The crystal structure was solved and refined using MaXus (Bruker Nonius, Deflt and MacScience, Japan) [29] Mo K_α radiation (λ = 0.71073 Å) and a graphite monochromator were used for data collection. The chemical formula and ring labeling system is shown in Fig. 1. Crystal data for compound **11b**: $C_{28}H_{22}ClFN_6S$, Mr, 529.041; system, orthorhombic; space group, $P2_12_12_1$; unit cell dimensions, a, 11.4339 (2) Å; b, 14.1131 (4) Å; c, 16.0647 (4) Å; α, 90.00°; β, 90.00°; γ, 90.00°; V, 2592.32 (11) Å^3; Z, 4; D_x, 1.356 Mg m^{-3}; θ range for data collection, 2.910–27.485°; μ (Mo-K_α), 0.27 mm^{-1}; T = 298 K; independent reflections, 3705; measured reflections, 5953; observed reflections, 1934; R_{int}, 0.025; R(all), 0.087; R(gt), 0.035; wR(ref), 0.061; wR(all), 0.078; wR(gt), 0.061; S(ref), 1.464; S(all), 1.802; S(gt), 1.464; Δ/σ_{max}, 0.043, $\Delta\rho_{max}$, 0. 0.48 e Å^3; $\Delta\rho_{min}$ −0.45 e Å^3.

Crystallographic data for the structures **11b** have been deposited with the Cambridge Crystallographic Data Center (CCDC) under the number 858991. Copies of the data can be obtained, free of charge, on application to CCDC 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or at www.ccdc.cam.ac.uk].

4.3. Antimicrobial activity

Chemical compounds were individually tested against a panel of gram positive and gram negative bacterial pathogens, yeast and fungi. Antimicrobial tests were carried out by the agar well diffusion method [30] using 100 μL of suspension containing 1×10^8 CFU/ml of pathological tested bacteria and 1×10^6 CFU/ml of yeast spread on nutrient agar (NA) and Sabourand dextrose agar (SDA) 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 100 mg of the chemical compound in one ml of dimethyl sulfoxide (DMSO). 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 standard 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 1. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

4.4. Minimal inhibitory concentration (MIC) measurement

The bacteriostatic activity of the active compounds (having inhibition zones (IZ) ≥ 16 mm) was then evaluated using the two

fold serial dilution technique [31]. Two fold serial dilutions of the tested compounds solutions were prepared using the proper nutrient broth. The final concentration of the solutions was 132; 66; 33; 16.5; and 8.25 μg/ml. The tubes were then inoculated with the test organisms, grown in their suitable broth at 37 °C for 24 h for bacteria (about 1×10^8 CFU/ml), 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 minimum inhibitory concentration (MIC).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2012.03.023.

References

[1] R. Alvarez, S. Velazquez, F. San, S. Aquaro, C. De, C.F. Perno, A. Karlsson, J. Balzarini, M.J. Camarasa, J. Med. Chem. 37 (1994) 4185–4194.
[2] S. Velazquez, R. Alvarez, C. Perez, F. Gago, C. De, J. Balzarini, M.J. Camarasa, Antivir. Chem. Chemother. 9 (1998) 481–489.
[3] M.J. Genin, D.A. Allwine, D.J. Anderson, M.R. Barbachyn, D.E. Emmert, S.A. Garmon, D.R. Graber, K.C. Grega, J.B. Hester, D.K. Hutchinson, J. Morris, R.J. Reischer, C.W. Ford, G.E. Zurenko, J.C. Hamel, R.D. Schaadt, D. Stapert, B.H. Yagi, J. Med. Chem. 43 (2000) 953–970.
[4] A.K. Jordão, V.F. Ferreira, T.M. Souza, G.G. Faria, V. Machado, J.L. Abrantes, M.C. de Souza, A.C. Cunha, Bioorg. Med. Chem. 19 (2011) 1860–1865.
[5] S.G. Agalave, S.R. Maujan, V.S. Pore, Chem. Asian J. 6 (2011) 2696–2718.
[6] C. Peto, G. Batta, Z. Gyorgydeak, F. Sztaricskai, J. Carbohydr. Chem. 15 (1996) 465–483.
[7] M. Kume, T. Kubota, Y. Kimura, H. Nakashimizu, K. Motokawa, M. Nakano, J. Antibiot. 46 (1993) 177–182.
[8] R.C. Mearman, C.E. Newall, A.P. Tonge, J. Antibiot. (Tokyo) 37 (1984) 885–891.
[9] I.K. Boddy, G.G. Briggs, R.P. Harrison, T.H. Jones, M.J. O'Mahony, I.D. Marlow, B.G. Roberts, R.J. Willis, R. Bardsley, J. Reid, Pestic. Sci. 48 (1996) 189–196.
[10] K.H. Buechel, H. Gold, P.E. Frohberger, H. Kaspers, German Patent 2407305, 1975; Chem. Abstr. 83 (1975) 206290.
[11] F. Reisser, British Patent 8101239, 1981; Chem. Abstr. 96 (1981) 69006.
[12] H.R. Krueger, U. Schroeer, D. Baumert, H. Joppien, German Patent 2936951, 1981; Chem. Abstr. 96 (1981) 52509.
[13] W.-Q. Fan, A.R. Katritzky, in: A.R. Katritzky, C.W. Rees, E.F.V. Scriven (Eds.), Comprehensive Heterocyclic Chemistry II, vol. 4, Elsevier Science, Oxford, 1996, pp. 1–126.
[14] A.M. Isloor, B. Kalluraya, P. Shetty, Eur. J. Med. Chem. 44 (2009) 3784–3787.
[15] A.M. Isloor, B. Kalluraya, M. Rao, J. Saudi Chem. Soc. 4 (3) (2000) 265–270.
[16] B. Kalluraya, A.M. Isloor, P.V. Frank, R.L. Jagadesha, S. Shenoy, Indian J. Heterocycl. Chem. 11 (2001) 159–162.
[17] D. Sunil, A.M. Isloor, P. Shetty, Der Pharma Chemica 1 (2) (2009) 19–26.
[18] B.F. Abdel-Wahab, R.E. Khidre, G.E.A. Awad, Eur. J. Med. Chem. 50 (2012) 55–62.
[19] B.F. Abdel-Wahab, H.A. Mohamed, A.A. Farahat, K.M. Dawood, Heterocycles 83 (2011) 2731–2767.
[20] B.F. Abdel-Wahab, H.A. Mohamed, J. Sulfur Chem. 32 (2011) 543–556.
[21] R.E. Khidre, B.F. Abdel-Wahab, F.A. Badria, Lett. Drug Des. Discov. 8 (2011) 640–648.
[22] B.F. Abdel-Wahab, G.E.A. Awad, F.A. Badria, Eur. J. Med. Chem. 46 (2011) 1505–1511.
[23] B.F. Abdel-Wahab, M. Farghaly, F.A. Badria, Pharm. Chem. J. 45 (2011) 30–35.
[24] H.A. Abdel-Aziz, B.F. Abdel-Wahab, F.A. Badria, Arch. Pharm. 343 (2011) 152–159.
[25] B.F. Abdel-Wahab, A.-A.S. El-Ahl, Phosphorus, Sulfur Silicon Relat. Elem. 185 (2010) 249–260.
[26] N.T. Pokhodylo, R.D. Savka, V.S. Matiichuk, N.D. Obushak, Russ. J. Gen. Chem. 79 (2009) 309–314.
[27] H.-S. Dong, H.-C. Wang, Z.-L. Gao, R.-S. Li, F.-H. Cui, J. Heterocycl. Chem. 47 (2010) 389–395.
[28] D.J. Gale, J.F.K. Wilshire, Aust. J. Chem. 23 (1970) 1063–1068.
[29] S. Mackay, C.J. Gilmore, C. Edwards, N. Stewart, K. Shankland, maXus Computer Program for the Solution and Refinement of Crystal Structures (1999) Bruker Nonius, The Netherlands, MacScience, Japan & The University of Glasgow.
[30] A.C. Scott, Laboratory Control of Antimicrobial Therapy, in: , thirteenth ed.., in: J.G. Collee, J.P. Duguid, A.G. Fraser, B.P. Marmion (Eds.), Mackie and McCartney Practical Medical Microbiology, 2 Churchill, Livingstone, 1989, pp. 161–181.
[31] C. Perez, M. Pauli, P. Bazevque, Acta Biol. Med. Exp. 15 (1990) 113–115.