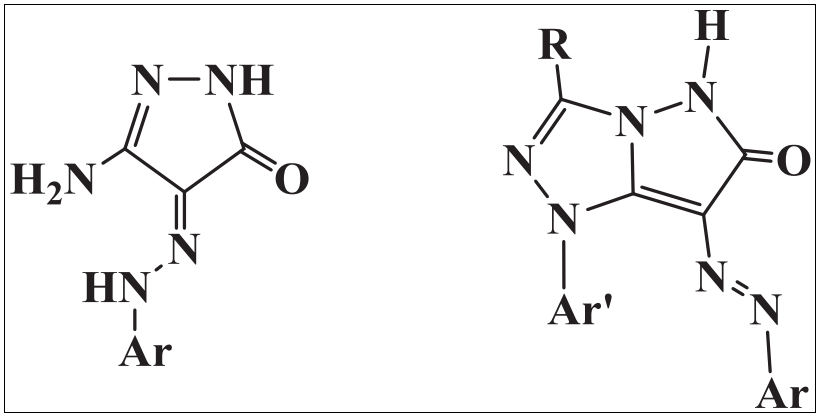
Pyrazolo[5,1-*c*][1,2,4]triazoles: Antimicrobial, Antitumor Activities, and Computational Docking Studies

Thoraya A. Farghaly,[a,b]* Magda A. Abdallah,[a] Huda K. Mahmoud,[a] Nashwa El-Metwaly,[b,c] and Mahmoud Elaasser[d]

[a]Department of Chemistry, Faculty of Science, University of Cairo, Giza 12613, Egypt
[b]Department of Chemistry, Faculty of Applied Science, Umm Al-Qura University, Makkah Almukkarramah, Saudi Arabia
[c]Chemistry Department, Faculty of Science, Mansoura University, Mansoura, Egypt
[d]Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt
*E-mail: thoraya-f@hotmail.com

Received May 20, 2016
DOI 10.1002/jhet.2892
Published online 00 Month 2017 in Wiley Online Library (wileyonlinelibrary.com).



A new series of pyrazolotriazoles **7a–l**, **11**, and **15a–c** derived from the reaction of 3-amino-4-(arylhydrazono)-4,5-dihydropyrazol-5-one **3a**,**b** with various types of hydrazonoyl chlorides **4**, **10**, **12**, and **13** was being synthesized in existence of triethylamine. The spectral data were assured the postulated structures for all compounds. All 7-arylazopyrazolo[5,1-*c*][1,2,4]triazole derivatives **7a–l**, **11**, and **15a–c** have been evaluated for their antimicrobial and antitumor activities, and the results show that some derivatives have good to mild utility as antitumor and antibacterial operators. Moreover, the computational studies using AutoDock tools 4.2 are confirming the results in biological activity.

J. Heterocyclic Chem., **00,** 00 (2017).

INTRODUCTION

In between heterocyclic systems, the 3-aminopyrazole symbolizes an important template. Such system attracts a significant regard because of its long history of implementation in the curative and agrochemical industries [1–4]. Also, 3-aminopyrazole is an important heterocyclic system useful synthon for the synthesis of many bridgehead heterocyclic ring systems [5]. On the other hand, 3-aminopyrazolones are key intermediates in obtaining pyrazolo[5,1-*c*][1,2,4]triazoles, [6] which have a wide range of biological activity [7] such as antimicrobial and antitumor activities [7,8]. Also, they are used as interposes in obtaining color photosensitive reagents as well as toners, inks, and other photographic reagents such as Magenta coupler in an emulsion coat photosensitive [9–12].

Aspect of our interest for synthesizing new bioactive heterocyclic compounds [13–21], we report here the utility of 3-aminopyrazoles as precursors for synthesis of a series of bioactive arylazopyrazolo[5,1-*c*][1,2,4]triazoles pointed to study their inhibition behavior towards bacteria and tumors. The method of preparing the latter compounds depends mainly on reaction of hydrazonoyl halides with 3-amino-4-(arylhydrazono)-4,5-dihydropyrazol-5-one in the presence of triethylamine as considerable stimulation compound. A standard tool used for the verification of biological studies is the AutoDock tool implemented for cell proteins and has a considered interest in medicinal examination. Also, it is extensively used to predict protein [22] interaction countenance and to screen large libraries for molecules that will modulate the qualification of a biological prospector.

RESULTS AND DISCUSSION

The required pyrazole derivatives **3** have been synthesized as previously reported [23] by the reaction of cyanoacetic acid hydrazide **1** with arenediazonium salts **2** in ethyl alcohol in the presence of sodium acetate at 0–5°C. The presence of more than one reactive nucleophilic site in the starting compounds **3** prompted us to investigate their reactivity towards hydrazonoyl

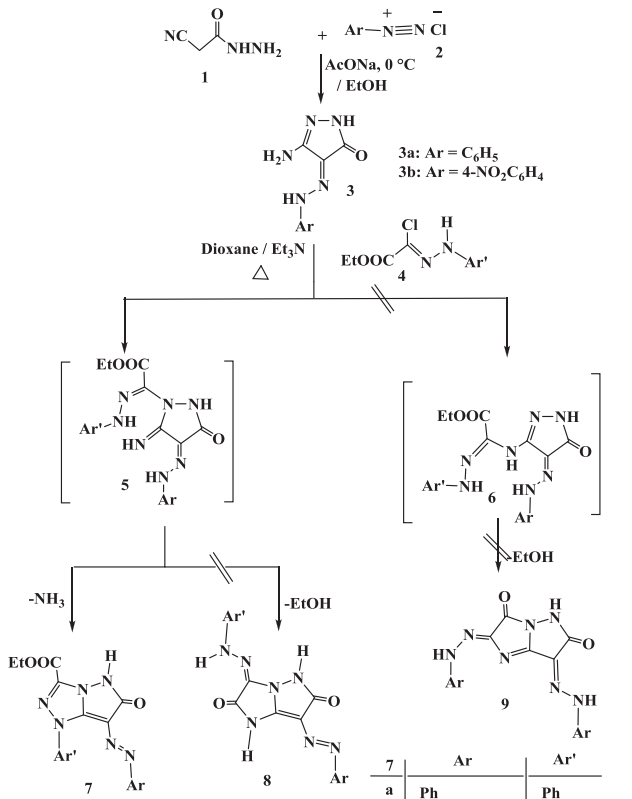
chlorides. Thus, reaction of **3** with ethyl (*N*-arylhydrazono) chloroacetate **4** in refluxing dioxan in the presence of triethylamine yielded one isolable product as evidenced by thin-layer chromatography analysis of the crude product. These compounds were identified to have structure **7** and not **8** or **9** (Scheme 1) on the basis of elemental and spectral analyses data. For example, the mass spectra of the products revealed in each case a molecular ion peak, which is consistent with the proposed structure **7** corresponding to elimination of ammonia molecule. Also, the IR spectra revealed in each case the presence of two absorption bands in the region ύ 1620–1634 and 1736–1739 cm^{-1} assigned for the amide and ester carbonyl groups, in addition to another band at ύ 3427–3447 cm^{-1} assigned for the amide NH group. Their ^{1}H-NMR spectra revealed in each case the characteristic peaks of the ester group as one triplet near δ 1.39 ppm and the quartet near δ 4.50 ppm, in addition to one singlet near 8.50 ppm attributed to the –NH proton of the pyrazole ring. To account for the formation of product **7**, namely, 3-ethoxycarbonyl-1-aryl-7-arylazo-5,6-dihydro-pyrazolo[5,1-*c*][1,2,4]triazol-6-one, it was suggested that intermediate **5** was initially formed by nucleophilic attack of the pyrazole nitrogen atom on the carbon atom (–C*=NNH–) of the hydrazonoyl chloride **4**, followed by intramolecular cyclization with elimination of ammonia to give the final products. It is worthy to mention that Elgemeie *et al*. [24] proved that reaction of another derivatives of aminopyrazoles reacted with *N*-phenylbenzohydrazonoyl chloride in refluxing ethanol in the presence of triethylamine gave pyrazolotriazoles with elimination of ammonia.

Also, under the same reaction condition, compound **3a** reacted with methyl *N*-(4-chlorophenylhydrazono) chloroacetate **10** to give product **11** (Scheme 2). The structure of **11** was also established on the basis of both elemental and spectral analyses data (IR, ^{1}H-NMR, and mass). For example, the IR spectrum of compound **11** showed two absorption bands at 1745 and 1621 cm^{-1} attributed to the two carbonyl groups, in addition to another band at ύ 3430 cm^{-1} assigned for the pyrazole –NH group. ^{1}H-NMR spectrum, also, exhibited one singlet signal at δ 4.01 attributed to the methyl protons of $COOCH_3$ group in addition to the other expected signals for the –NH and the aromatic protons (see the Experimental section). Mass spectrum of product **11** revealed a molecular ion peak at the correct molecular weight of the expected product.

In a similar manner, reaction of compounds **3a**,**b** with hydrazonoyl halides **12** and **13** under the same reaction conditions afforded the respective products **15** (Scheme 3) via the intermediate **14**. The structure of the latter compounds was also confirmed on the basis of both elemental and spectral analyses data (see the Experimental section).

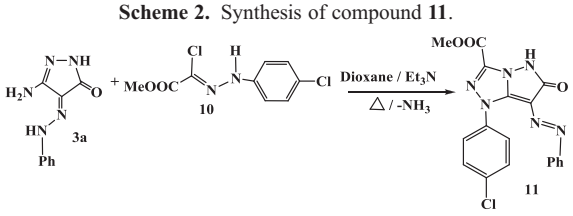
Results of biological activity. ***Antimicrobial screening.*** *In vitro* antimicrobial screening of compounds **7a–l** and **15a–c** was carried out using four fungal strains *Aspergillus fumigatus*, *Syncephalastrum racemosum*, *Geotrichum candidum*, and *Candida albicans*, two Gram-positive bacteria, *Streptococcus pneumonia* and *Bacillus subtilis*, and two Gram-negative bacteria, *Pseudomonas aeruginosae* and *Escherichia coli*. The amphotericin B was used as antifungal agent whereas ampicillin and gentamicin were used as antibacterial

Scheme 1. Synthesis of compounds **7a–l**.

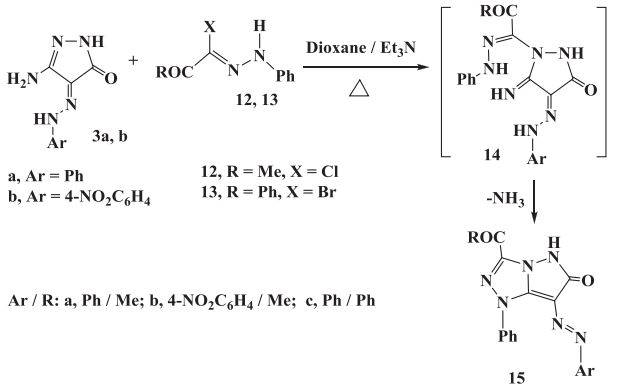


7	Ar	Ar'
a	Ph	Ph
b	Ph	4-ClC_6H_4
c	Ph	4-MeC_6H_4
d	Ph	4-$NO_2C_6H_4$
e	Ph	3-ClC_6H_4
f	Ph	3-$NO_2C_6H_4$
g	4-$NO_2C_6H_4$	Ph
h	4-$NO_2C_6H_4$	4-ClC_6H_4
i	4-$NO_2C_6H_4$	4-MeC_6H_4
j	4-$NO_2C_6H_4$	4-$NO_2C_6H_4$
k	4-$NO_2C_6H_4$	3-ClC_6H_4
l	4-$NO_2C_6H_4$	3-$NO_2C_6H_4$

Scheme 2. Synthesis of compound **11**.



Scheme 3. Synthesis of compounds **15a–c**.



agents for Gram-positive and Gram-negative bacteria, respectively. The results of the investigation showed that among all the tested compounds, **7b**, **7c**, **7e**, and **7g** and **15a–c** have medium to weak activity against only Gram-positive bacteria, namely, *S. pneumonia* and *B. subtilis*, whereas compound **7c** has medium potency against Gram-negative bacteria *E. coli* (Table 1). The results showed also that all the tested compounds **7** and **15** have no activity against all fungi used in this investigation (Table 1). The order of decreasing reactivity towards the tested Gram-positive bacteria *S. pneumonia* and *B. subtilis* is as follows: **7c** < **15b** < **7b** = **15a** < **7e** < **7g** < **15c.**

Table 2

The IC_{50} of the tested compounds **7a–l** and **15a–c** against HEPG-2 liver cancer and HCT-116 colon cancer.

Comp. no.	IC_{50} µg/mL (HEPG-2)	IC_{50} µg/mL (HCT-116)
7a	29	39.7
7b	>100	>100
7c	22.2	23.3
7d	72.5	94.6
7e	45.5	88.6
7f	38.8	40.1
7g	48.1	83.1
7h	22.7	35.2
7i	31.3	59.5
7j	>100	>100
7k	11.3	12.5
7l	23.6	38.9
15a	77.4	>100
15b	46.3	96.5
15c	11.9	18.9
Doxorubicin	0.42	0.46
Imatinib	18.9	9.7
5-Fluorouracil	4.6	4.3

IC_{50}, 50% inhibitory concentration.

Antitumor assay. The *in vitro* antitumor activity of the newly synthesized compounds **7a–l** and **15a–c** was evaluated at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt. Fifteen compounds have tested for their *in vitro* antitumor activity against HEPG-2 (liver cancer cell line) and

Table 1

Antimicrobial activity of the newly synthesized compounds **7a–l** and **15a–c**.

	Microorganism/IZD (mm/µg sample)							
	Fungi				G^+ Bacteria		G^- Bacteria	
Comp. no.	AF	SR	GC	CA	SP	BS	PA	EC
7a	NA	NA	NA	NA	NA	NA	NA	NA
7b	NA	NA	NA	NA	12.9 ± 0.19	15.1 ± 0.44	NA	NA
7c	NA	NA	NA	NA	14.9 ± 0.22	16.2 ± 0.36	NA	12.3 ± 0.27
7d	NA	NA	NA	NA	NA	NA	NA	NA
7e	NA	NA	NA	NA	12.3 ± 0.36	14.1 ± 0.17	NA	NA
7f	NA	NA	NA	NA	NA	NA	NA	NA
7g	NA	NA	NA	NA	11.3 ± 0.19	12.2 ± 0.44	NA	NA
7h	NA	NA	NA	NA	NA	NA	NA	NA
7i	NA	NA	NA	NA	NA	NA	NA	NA
7j	NA	NA	NA	NA	NA	NA	NA	NA
7k	NA	NA	NA	NA	NA	NA	NA	NA
7l	NA	NA	NA	NA	NA	NA	NA	NA
15a	NA	NA	NA	NA	12.9 ± 0.12	15.1 ± 0.34	NA	NA
15b	NA	NA	NA	NA	13.2 ± 0.19	15.7 ± 0.44	NA	NA
15c	NA	NA	NA	NA	NA	10.8 ± 0.34	NA	NA
Amph	23.7 ± 0.1	19.7 ± 0.2	28.7 ± 0.2	25.4 ± 0.1	—	—	—	—
Amp	—	—	—	—	23.8 ± 0.2	32.4 ± 0.3	—	—
Gent	—	—	—	—	—	—	17.3 ± 0.1	19.9 ± 0.3

IZD, inhibition zone diameter; AF, *Aspergillus fumigatus*; SR, *Syncephalastrum racemosu*; GC, *Geotricum candidum*; CA, *Candida albicans*; SP, *Streptococcus pneumonia*; BS, *Bacillus subtilis*; PA, *Pseudomonas aeruginosa*; EC, *Escherichia coli*; Amph, amphotericin B; Amp, ampicillin; Gent, gentamicin.

HCT-116 (colon cell line). The *in vitro* growth inhibitory activity of the synthesized compounds was investigated in comparison with 5-fluorouracil, doxorubicin, and imatinib as standard drugs. Some of the tested compounds showed moderate to weak effects against both cancer cell lines as shown in Table 2. For example, the antitumor screening data against HEPG-2 liver cancer cell revealed that the order of decreasing antitumor activity is as follows: **7k** < **15c** < **7c** < **7h** < **7l** < **7a** < **7i** < **7f** < **15b** < **7e** < **7g** whereas compounds **7b**, **7i**, **7d**, and **15a** have very weak effect (Table 2). On the other hand, the activity of the tested compounds **7a–l** and **15a–c** against HCT-116 colon cancer cell line indicated also the weak effect of these compounds against HCT-116 colon cancer cell line. The order of decreasing antitumor activity is as follows: **7k** < **15c** < **7c** < **7h** < **7l** < **7a** < **7f** < **7i** whereas the rest of the tested compounds **7b**, **7j**, **15a**, **15b**, **7g**, **7d**, and **7e** has very weak effect against HCT-116 cancer cell line.

Molecular docking. Docking is a method that foresees the distinguish orientation of the organic compound (inhibitor) to the protein receptor when bound to form a stable complex. Awareness of the distinguished orientation in turn may be used to foresee the hardness of combination or binding ability between them. Also, docking is considerably used to foresee the binding orientation in between to predict the likeness and activity of the compound that performs an important role in the drugs design. The compounds implemented (**7b**, **7c**, **7e**,

Table 3

Molecular docking energy values for the interaction with protein receptors.

Comp. no.	Receptors	Est. free energy of binding (kcal/mol)	Est. inhibition constant (K_i) (uM)	vdW+ bond+ desolve energy (kcal/mol)	Electrostatic energy (kcal/mol)	Total intercooled energy (kcal/mol)	Frequency (%)	Interact surface
7b	4a34	−4.50	503.47	−6.17	+0.03	−6.14	50	626.435
	3zzq	−4.43	563.21	−6.34	+0.04	−6.31	10	680.049
7c	4a34	−5.05	197.83	−5.64	+0.02	−5.62	20	598.184
	3zzq	−4.63	407.02	−5.36	−0.02	−5.38	30	646.794
7e	4a34	−5.46	99.96	−7.11	−0.30	−7.41	10	636.659
	3zzq	−4.31	689.23	−5.80	−0.06	−5.87	20	639.903
7g	4a34	−4.99	218.13	−6.57	−0.48	−7.05	50	667.103
	3zzq	−3.67	2.03	−5.41	−0.34	−5.75	10	589.128
15a	4a34	−5.25	141.25	−5.75	+0.01	−5.74	20	598.743
	3zzq	−4.86	273.74	−5.13	−0.25	−5.38	10	562.359
7k	2h80	−3.17	4.73	−5.13	−0.41	−5.53	10	725.426
15c	2h80	−5.88	48.58	−7.09	+0.1	−6.98	10	689.84

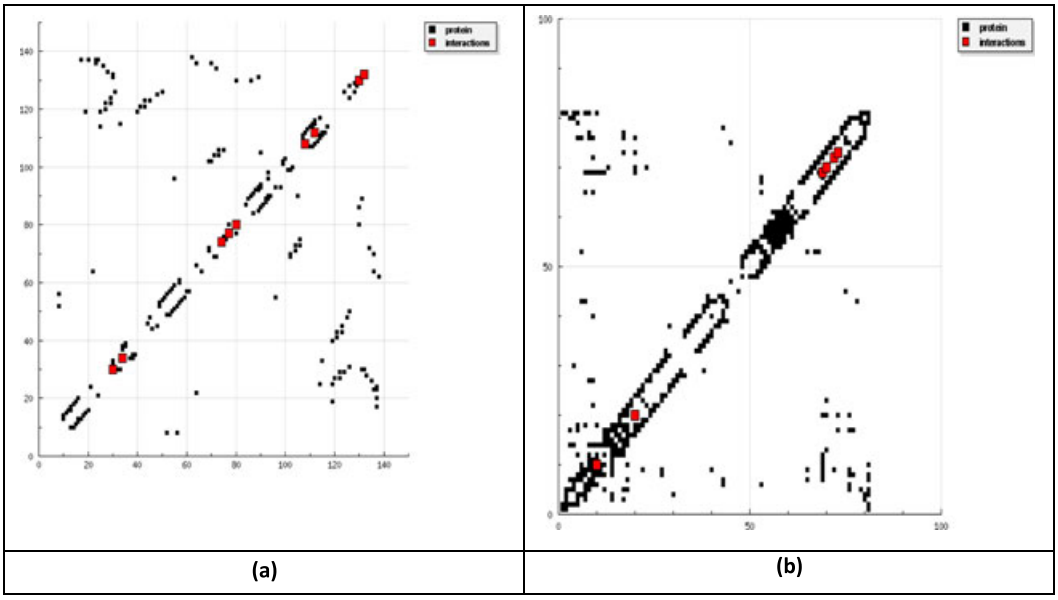


Figure 1. HB plot of (a) **7b**–4a34 and (b) **7k**–2h80 interactions. [Color figure can be viewed at wileyonlinelibrary.com]

7g, and **15a**) towards bacteria receptors were chosen referring to their activity results. The chosen host–guest protein receptors are 4a34 as the isomerase type of crystal structure of the fucose mutarotase in complex with L-fucose from *S. pneumonia*, while 3zzq as transcription-type engineered 12-subunit *B. subtilis* tryptophan RNA-binding attenuation protein and 2h80 as lipid-binding protein-type NMR structures of self-assembled monolayer domain deleted in liver cancer 2. The biological data display their mild toxicity against *S. pneumonia* (4a34) and *B. subtilis* (3zzq) bacteria, while compounds **7k** and **15c** represent an excellent activity against the liver cancer (2h80). The energies executed were tabulated in Table 3, corresponding to the obtained results, demonstrate respectable low-binding energy values beside wide interact surfaces reflecting the interaction facility. HB plot curves (Fig. 1) display that all the computed compounds were interacted with 4a34 and 3zzq receptors through one to two hydrogen bonds, which considered a case of mild interaction, while the HB plot of 2h80 docking protein is displaying multi-hydrogen bonds with the **7k** and **15c** compounds. Inspections of results are in compatible with experimental data. The data propose the interaction of receptors with the investigated compounds by variable degrees, a strong to moderate states. This interaction could deactivate or apoptosis the microorganisms. Binding energies are most exceedingly utilized as a measuring vector for the compound binding affinity towards protein. Thus, the binding affinity towards protein receptors will be elevated through the reduction in binding energies because of mutation [25]. The features of the used compounds were represented in presence of several active sites available for hydrogen bonding interaction.

CONCLUSIONS

In this report, a series of 7-arylazopyrazolo[5,1-*c*][1,2,4] triazoles was synthesized in one step via reaction of 3-amino-4-arylhydrazono-4,5-dihydropyrazol-5-one with a variety of hydrazonoyl halides using triethylamine as basic catalyst. The mechanism of formation of the products was discussed, and their structures were established on the basis of elemental and spectral data. The antimicrobial evaluation of the newly synthesized compounds indicated the medium activity against Gram-positive bacteria, whereas all the tested compounds have no activity against fungi species. On the other hand, the antitumor screening of the products against HEPG-2 and HCT-116 indicated that some derivatives have good activity against HEPG-2 and HCT-116 cancer cell lines. Moreover, the computational studies using AutoDock tools 4.2 are confirming the results in biological activity.

EXPERIMENTAL

Melting points were determined on a Gallenkamp apparatus. IR spectra were recorded in potassium bromide using Perkin Elmer FTIR 1650 and Pye-Unicam SP300 IR spectrophotometers. NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer operating at 300 MHz (^{1}H-NMR) or 75 MHz (^{13}C-NMR) and run in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a GCMS-QP 1000 EX Shimadzu and GCMS 5988-A HP spectrometers. Elemental analyses were carried out using German-made Elementar vario LIII CHNS analyzer at the Microanalytical Laboratory of Cairo University, Giza, Egypt. Antitumor and antimicrobial activities were carried out at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt**.** Compounds **3, 4, 10, 12**, and **13** were prepared by the method previously reported [23,26–29].

Hydrazonoyl chlorides. ***3-Amino-4-(phenylhydrazono)-4,5-dihydropyrazol-5-one (3a)***. Dark red solid, yield (0.49 g, 97%), mp 246–248°C (ethanol/dioxane) IR ύ:, 3411, 3333, 3191 (2NH, NH_2), 1669 (CO), 1625, 1584, 1487, 1439, 1327, 1249, 1161, 1105 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 5.8 (s, 2H, NH_2), 7.1–7.53 (m, 6H, NH, Ar–H), 10.53 (s, 1H, NH). MS m/z (%) 204 (M^++1, 12), 203 (M^+, 100), 126 (67), 93 (38), 77 (60), 65 (61). *Anal*. Calcd for $C_9H_9N_5O$ (203.08): C, 53.20; H, 4.46; N, 34.47. Found: C, 53.49; H, 4.34; N, 34.75%.

3-Amino-4-((4-nitrophenyl)hydrazono)-4,5-dihydropyrazol-5-one (3b). Brown solid, yield (0.59 g, 96%), mp >300°C (ethanol/dioxane) IR ύ:, 3450, 3380, 3234 (2NH, NH_2), 1691 (CO), 1640, 1605, 1537, 1505, 1347, 1308, 1247, 1196, 1171, 1108, 1002 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 6.02 (s, 2H, NH_2), 7.73 (d, J = 9 Hz, 2H, Ar–H), 7.95 (s, 1H, NH), 8.22 (d, J = 9 Hz, 2H, Ar–H), 10.66 (s, 1H, NH). MS m/z (%) 249 (M^++1, 13), 248 (M^+, 100), 138 (12), 126 (94), 122 (16), 108 (10), 92 (10), 68 (22), 64 (24). *Anal*. Calcd for $C_9H_8N_6O_3$ (248.07): C, 43.55; H, 3.25; N, 33.86. Found: C, 43.49; H, 3.34; N, 33.75%.

Synthesis of compounds 7, **11**, and **15**. To a mixture of compound **3** (2.5 mmol) and the appropriate hydrazonoyl halides **4, 10, 12**, or **13** (2.5 mmol of each) in dioxane (15 mL) was added triethylamine (0.35 mL), and the mixture was heated to reflux for 3 h, then cooled. The solid produced was collected by filtration and crystallized from the appropriate solvent to give the corresponding products **7**, **11**, or **15**, respectively. The products **7**, **11**, and **15** together with their physical constants are listed next.

3-Ethoxycarbonyl-1-phenyl-7-phenylazo-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (7a). Red solid, yield (0.88 g, 94%), mp 230–232°C (ethanol/dioxane) IR ύ:,

3442 (NH), 2927, 1737 (CO), 1633(CO), 1544, 1495, 1416, 1244, 1127 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 1.39 (t, J = 7 Hz, 3H, CH_3), 4.51 (q, J = 7 Hz, 2H, CH_2), 7.29–8.09 (m, 11H, NH, Ar–H). MS m/z (%) 377 (M^++1, 4), 376 (M^+, 17), 284 (26), 256 (15), 197 (5), 129 (18), 91 (13), 77 (100), 65 (14). *Anal*. Calcd for $C_{19}H_{16}N_6O_3$ (376.38): C, 60.63; H, 4.28; N, 22.33. Found: C, 60.55; H, 4.34; N, 22.05%.

1-(4-Chlorophenyl)-3-ethoxycarbonyl-7-phenylazo-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (7b). Orange solid, yield (0.97 g, 95%), mp 210–212°C (ethanol/dioxane) IR ύ:, 3431 (NH), 2979, 2928, 1736 (CO), 1634(CO), 1587, 1541, 1488, 1444, 1403, 1303, 1245, 1134, 1012 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 1.39 (t, J = 7 Hz, 3H, CH_3), 4.47 (q, J = 7 Hz, 2H, CH_2), 7.31–7.58 (m, 6H, NH, Ar–H), 7.78 (d, J = 8 Hz, 2H, Ar–H), 8.07 (d, J = 8 Hz, 2H, Ar–H). ^{13}C-NMR (DMSO-d_6) 13.91 (CH_3), 62.65 (CH_2), 113.15, 118.37, 121.83, 127.59, 129.40, 129.51, 133.02, 133.46, 134.94, 138.94, 146.02, 154.57 (CO), 166.44 (CO). MS m/z (%) 413 (M^++2, 2), 412 (M^++1, 7), 411 (M^+, 4), 411 (M^+-1, 17), 318 (20), 290 (14), 231 (12), 163 (13), 125 (17), 113 (16), 111 (38), 91 (23), 79 (16), 77 (100), 69 (36). *Anal*. Calcd for $C_{19}H_{15}ClN_6O_3$ (410.82): C, 55.55; H, 3.68; N, 20.46. Found: C, 55.29; H, 3.88; N, 20.24%.

3-Ethoxycarbonyl-1-(4-methylphenyl)-7-phenylazo-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (7c). Red solid, yield (0.92 g, 95%), mp 192–194°C (ethanol/dioxane) IR ύ:, 3445 (NH), 2978, 2925, 1738 (CO), 1631 (CO), 1593, 1550, 1505, 1446, 1403, 1236, 1127, 1018 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 1.39 (t, J = 7 Hz, 3H, CH_3), 2.45 (s, 3H, CH_3), 4.49 (q, J = 7 Hz, 2H, CH_2), 7.31–8.0 (m, 10H, NH, Ar–H). MS m/z (%) 391 (M^++1, 14), 390 (M^+, 53), 298 (86), 270 (43), 143 (27), 105 (20), 91 (99), 77 (100), 65 (71). *Anal*. Calcd for $C_{20}H_{18}N_6O_3$ (390.40): C, 61.53; H, 4.65; N, 21.53. Found: C, 61.32; H, 4.83; N, 21.71%.

3-Ethoxycarbonyl-1-(4-nitrophenyl)-7-phenylazo-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (7d). Orange solid, yield (0.94 g, 90%), mp 228–230°C (ethanol/dioxane) IR ύ:, 3437 (NH), 2986, 2932, 1736 (CO), 1619 (CO), 1590, 1543, 1517, 1446, 1341, 1246, 1138, 1011 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 1.41 (t, J = 7 Hz, 3H, CH_3), 4.51 (q, J = 7 Hz, 2H, CH_2), 7.39–7.7 (m, 6H, NH, Ar–H), 8.35 (d, J = 9 Hz, 2H, Ar–H), 8.56 (d, J = 9 Hz, 2H, Ar–H). MS m/z (%) 422 (M^++1, 6), 421 (M^+, 25), 329 (32), 301 (24), 242 (12), 143 (10), 122 (8), 92 (18), 77 (100), 64 (63). *Anal*. Calcd for $C_{19}H_{15}N_7O_5$ (421.38): C, 54.16; H, 3.59; N, 23.27. Found: C, 54.22; H, 3.82; N, 23.57%.

3-Ethoxycarbonyl-1-(3-chlorophenyl)-7-phenylazo-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (7e). Orange solid, yield (0.94 g, 92%), mp 206–208°C (ethanol/dioxane) IR ύ:, 3430 (NH), 2980, 2933, 1738 (CO), 1631(CO), 1590, 1545, 1482, 1434, 1414, 1328, 1244, 1169, 1131, 1099 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 1.4 (t, J = 7 Hz, 3H, CH_3), 4.5 (q, J = 7 Hz, 2H, CH_2), 7.32–8.01 (m, 9H, Ar–H), 8.32 (s, 1H, NH). MS m/z (%) 413 (M^++2, 2), 412 (M^++1, 11), 411 (M^+, 7), 411 (M^+-1, 25), 318 (38), 290 (24), 231 (11), 163 (18), 125 (10), 111 (48), 91 (21), 77 (100), 65 (37). *Anal*. Calcd for $C_{19}H_{15}ClN_6O_3$ (410.82): C, 55.55; H, 3.68; N, 20.46. Found: C, 55.39; H, 3.86; N, 20.28%.

3-Ethoxycarbonyl-1-(3-nitrophenyl)-7-phenylazo-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (7f). Light brown solid, yield (0.99 g, 95%), mp 212–214°C (ethanol/dioxane) IR ύ:, 3435 (NH), 3047, 1736 (CO), 1623 (CO), 1536, 1482, 1444, 1348, 1256, 1141, 1011 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 1.4 (t, J = 7 Hz, 3H, CH_3), 4.5 (q, J = 7 Hz, 2H, CH_2), 7.32–8.44 (m, 9H, Ar–H), 8.81 (s, 1H, NH). MS m/z (%) 422 (M^++1, 7), 421 (M^+, 24), 329 (32), 301 (22), 242 (10), 174 (15), 162 (10), 106 (26), 91 (40), 77 (100), 64 (58). *Anal*. Calcd for $C_{19}H_{15}N_7O_5$ (421.38): C, 54.16; H, 3.59; N, 23.27. Found: C, 54.20; H, 3.79; N, 23.47%.

3-Ethoxycarbonyl-1-phenyl-7-(4-nitrophenylazo)-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (7g). Brown solid, yield (0.93 g, 89%), mp 264–266°C (ethanol/dioxane) IR ύ:, 3434 (NH), 2928, 1739 (CO), 1633(CO), 1551, 1498, 1427, 1336, 1229, 1125, 1017 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 1.39 (t, J = 7 Hz, 3H, CH_3), 4.51 (q, J = 7 Hz, 2H, CH_2), 7.66–7.75 (m, 6H, NH, Ar–H), 8.02 (d, J = 8 Hz, 2H, Ar–H), 8.32 (d, J = 8 Hz, 2H, Ar–H). MS m/z (%) 422 (M^++1, 6), 421 (M^+, 24), 284 (50), 256 (30), 214 (3), 197 (12), 144 (6), 129 (31), 91 (17), 77 (100). 64 (18). *Anal*. Calcd for $C_{19}H_{15}N_7O_5$ (421.38): C, 54.16; H, 3.59; N, 23.27. Found: C, 54.39; H, 3.38; N, 23.54%.

1-(4-Chlorophenyl)-3-ethoxycarbonyl-7-(4-nitrophenylazo)-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (7h). Brown solid, yield (1 g, 89%), mp 220–222°C (ethanol/dioxane) IR ύ:, 3427 (NH), 2982, 1736 (CO), 1620 (CO), 1598, 1550, 1508, 1406, 1336, 1235, 1146, 1105, 1013 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 1.40 (t, J = 7 Hz, 3H, CH_3), 4.51 (q, J = 7 Hz, 2H, CH_2), 7.7–8.47 (m, 9H, NH, Ar–H). MS m/z (%) 457 (M^++2, 21), 456 (M^++1, 10), 455 (M^+, 31), 358 (7), 318 (70), 290 (37), 248 (15), 231 (29), 178 (14), 163 (33), 138 (12), 122 (39), 111 (100), 99 (12), 90 (47), 80 (66), 75 (96), 64 (95). *Anal*. Calcd for $C_{19}H_{14}ClN_7O_5$ (455.82): C, 50.07; H, 3.10; N, 21.51. Found: C, 50.32; H, 3.35; N, 21.36%.

3-Ethoxycarbonyl-1-(4-methylphenyl)-7-(4-nitrophenylazo)-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (7i). Red solid, yield (0.94 g, 87%), mp 260–262°C (ethanol/dioxane) IR ύ:, 3438 (NH), 2926, 1738 (CO), 1631 (CO), 1555, 1510, 1337, 1233, 1135 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 1.40 (t, J = 7 Hz, 3H, CH_3), 2.47 (s, 3H, CH_3), 4.50 (q, J = 7 Hz, 2H, CH_2), 7.54 (d, J = 8 Hz, 2H, Ar–H), 7.61 (d, J = 8 Hz, 2H, Ar–H), 7.93 (d, J = 8 Hz, 2H, Ar–H), 8.33 (d, J = 8 Hz, 2H, Ar–H), 8.41 (s, 1H, NH). MS m/z (%) 436 (M^++1, 10), 435 (M^+, 31), 298 (70), 270 (33), 228 (17), 211 (31), 105 (29), 91 (100), 80 (17), 76 (27),

65 (70). *Anal*. Calcd for $C_{20}H_{17}N_7O_5$ (435.40): C, 55.17; H, 3.94; N, 22.52. Found: C, 55.32; H, 3.65; N, 22.36%.

3-Ethoxycarbonyl-1-(4-nitrophenyl)-7-(4-nitrophenylazo)-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (7j). Dark orange solid, yield (0.94 g, 87%), mp 170–172°C (ethanol/dioxane) IR ύ:, 3432 (NH), 2926, 1735 (CO), 1625 (CO), 1595, 1516, 1427, 1339, 1242, 1164, 1108, 1014 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 1.40 (t, J = 7 Hz, 3H, CH_3), 4.52 (q, J = 7 Hz, 2H, CH_2), 7.04–8.50 (m, 9H, NH, Ar–H). MS m/z (%) 467 (M^++1, 4), 466 (M^+, 6), 394 (13), 369 (11), 242 (22), 189 (13), 163 (9), 149 (10), 143 (14), 136 (16), 122 (45), 116 (12), 106 (22), 90 (67), 76 (100), 64 (80). *Anal*. Calcd for $C_{19}H_{14}N_8O_7$ (466.37): C, 48.93; H, 3.03; N, 24.03. Found: C, 48.65; H, 3.25; N, 24.26%.

1-(3-Chlorophenyl)-3-ethoxycarbonyl-7-(4-nitrophenylazo)-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (7k). Brown solid, yield (1.0 g, 88%), mp 250–252°C (ethanol/dioxane) IR ύ:, 3424 (NH), 2981, 1738 (CO), 1630 (CO), 1593, 1550, 1513, 1337, 1236, 1144, 1111, 1014 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 1.40 (t, J = 7 Hz, 3H, CH_3), 4.51 (q, J = 7 Hz, 2H, CH_2), 7.71–8.47 (m, 9H, NH, Ar–H). MS m/z (%) 457 (M^++2, 14), 456 (M^++1, 6), 455 (M^+, 22), 318 (40), 290 (24), 231 (27), 163 (25), 138 (11), 125 (22), 122 (27), 111 (83), 90 (32), 80 (91), 75 (78), 64 (100). *Anal*. Calcd for $C_{19}H_{14}ClN_7O_5$ (455.82): C, 50.07; H, 3.10; N, 21.51. Found: C, 50.31; H, 3.30; N, 21.39%.

3-Ethoxycarbonyl-1-(3-nitrophenyl)-7-(4-nitrophenylazo)-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (7l). Dark orange solid, yield (1.0 g, 86%), mp 238–240°C (dioxane) IR ύ:, 3427 (NH), 2983, 1740 (CO), 1628 (CO), 1599, 1533, 1342, 1234, 1144, 1110, 1012 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 1.40 (t, J = 7 Hz, 3H, CH_3), 4.52 (q, J = 7 Hz, 2H, CH_2), 7.69–8.50 (m, 8H, Ar–H), 8.74 (s, 1H, NH). MS m/z (%) 467 (M^++1, 0.3), 466 (M^+, 0.8), 98 (1), 90 (2), 80 (100), 64 (59). *Anal*. Calcd for $C_{19}H_{14}N_8O_7$ (466.37): C, 48.93; H, 3.03; N, 24.03. Found: C, 48.75; H, 3.22; N, 24.30%.

1-(4-Chlorophenyl)-3-methoxycarbonyl-7-phenylazo-5,6-dihydro-pyrazolo[5,1-c][1,2,4]triazol-6-one (11). Yellow solid, yield (0.93 g, 94%), mp 190–192°C (ethanol/dioxane) IR ύ:, 3430 (NH), 2959, 1745 (CO), 1621 (CO), 1570, 1496, 1455, 1418, 1332, 1292, 1243, 1204, 1151, 1131, 1098, 1014 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 4.01 (s, 3H, OCH_3), 7.27–8.17 (m, 10H, NH, Ar–H). MS m/z (%) 398 (M^++2, 24), 396 (M^+, 43), 304 (13), 263 (20), 231 (47), 125 (20), 111(34), 105 (22), 77 (100), 64 (65). *Anal*. Calcd for $C_{18}H_{13}ClN_6O_3$ (396.80): C, 54.49; H, 3.30; N, 21.18. Found: C, 54.29; H, 3.58; N, 21.24%.

3-Acetyl-1-phenyl-7-(2-phenylazo)-1H-pyrazolo[5,1-c][1,2,4]triazol-6(5H)-one (15a). Brown solid, yield (0.8 g, 93%), mp 220–222°C (dioxane) IR ύ:, 3432 (NH), 2918, 1704 (CO), 1630(CO), 1548, 1491, 1437, 1240, 1152, 1116 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 2.75 (s, 3H, CH_3), 7.32–8.12 (m, 11H, NH, Ar–H). MS m/z (%) 347 (M^++1, 13), 346 (M^+, 59), 254 (41), 213 (33), 171 (18), 91 (49), 80 (16), 77 (100), 64 (24). *Anal*. Calcd for $C_{18}H_{14}N_6O_2$ (346.34): C, 62.42; H, 4.07; N, 24.27. Found: C, 62.70; H, 4.27; N, 24.54%.

3-Acetyl-7-(2-(4-nitrophenyl)azo)-1-phenyl-1H-pyrazolo[5,1-c][1,2,4]triazol-6(5H)-one (15b). Brown solid, yield (0.89 g, 92%), mp 212–214°C (ethanol/dioxane) IR ύ:, 3423 (NH), 2925, 1709 (CO), 1635(CO), 1597, 1553, 1500, 1336, 1232, 1167, 1110 cm^{-1}. ^{1}H-NMR (DMSO-d_6) 2.75 (s, 3H, CH_3), 7.62–7.77 (m, 6H, NH, Ar–H), 8.04 (d, J = 8 Hz, 2H, Ar–H), 8.30 (d, J = 8 Hz, 2H, Ar–H). MS m/z (%) 392 (M^++1, 13), 391 (M^+, 60), 254 (25), 213 (56), 198 (16), 171 (32), 122 (16), 118 (13), 91 (38), 77 (100), 64 (45). *Anal*. Calcd for $C_{18}H_{13}N_7O_4$ (391.34): C, 55.24; H, 3.35; N, 25.05. Found: C, 55.50; H, 3.27; N, 25.34%.

3-Benzoyl-1-phenyl-7-(2-phenylazo)-1H-pyrazolo[5,1-c][1,2,4]triazol-6(5H)-one (15c). Red solid, yield (0.96 g, 95%), mp 218–220°C (dioxane) IR ύ:, 3431 (NH), 2925, 1662 (CO), 1629 (CO), 1588, 1546, 1486, 1447, 1395, 1329, 1286, 1237, 1153, 1080, 1027, cm^{-1}. ^{1}H-NMR (DMSO-d_6) 7.31–8.31 (m, 16H, NH, Ar–H). MS m/z (%) 409 (M^++1, 4), 408 (M^+, 12), 316 (16), 275 (3), 197 (3), 105 (76), 91 (7), 77 (100), 64 (7). *Anal*. Calcd for $C_{13}H_{16}N_6O_2$ (408.42): C, 67.64; H, 3.95; N, 20.58. Found: C, 67.42; H, 4.20; N, 20.38%.

Antimicrobial activity assay. All microbial strains were provided from the culture collection of the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. The preliminary antimicrobial activity was investigated on a dozen newly synthesized compounds in order to increase the selectivity of these derivatives towards test microorganisms. Briey, 100 μL of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 108 cells/mL for bacteria or 105 cells/mL for fungi [30]. Then 100 μL of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained and tested for susceptibility by the well-diffusion method. The National Committee for Clinical Laboratory Standards recommends Mueller–Hinton and Sabouraud agar as they result in good batch-to-batch reproducibility. The diffusion method for filamentous fungi and yeast was tested by using the approved standard methods (M38-A and M44-P, respectively) developed by the National Committee for Clinical Laboratory Standards-48 for evaluating susceptibilities to antifungal agents. One hundred micro liters 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 resulting inhibition zone diameters were measured in millimeters and used as the criterion for antimicrobial activity. If an

organism is placed on the agar, it will not grow in the area around the well if it is susceptible to the chemical. This area of no growth around the disk is known as a zone of inhibition. The size of the clear zone is proportional to the inhibitory action of the compound under investigation. Solvent controls (DMSO) were included in every experiment as negative controls. DMSO was used for dissolving the tested compounds and showed no inhibition zones, confirming that it has no influence on growth of the tested microorganisms.

Evaluation of the antitumor activity using a viability assay. All human anticancer cell lines were obtained from the American Type Culture Collection. The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 μg/mL gentamicin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week.

Gangadevi and Muthumary's method [24] was used for evaluation of the potential cytotoxicity of the tested compounds. The number of surviving cells was determined by staining the cells with crystal violet [31,32] followed by cell lysing using 33% glacial acetic acid and reading the absorbance at 590 nm using a microplate reader (SunRise, TECAN, Inc, USA) after mixing well. The 50% inhibitory concentration, the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots.

Molecular docking. Docking Server was used for carrying out the docking calculations using Gasteiger partial charges added to the ligand atoms. Non-polar hydrogen atoms were conjoined, and rotatable bonds were illustrated. The calculations were performed on the ligand–protein pattern. AutoDock tools were implemented after the addition of fundamental hydrogen atoms, Kollman united atom type charges, and solvation parameters [33]. Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were created applying the AutoGrid program [34]. Calculating van der Waals and the electrostatic terms was carried out using AutoDock parameter set and distance-dependent dielectric functions, respectively. Docking simulations were executed using the Solis & Wets local search method and the Lamarckian genetic algorithm [35]. Initial position, orientation, and torsions of the ligand molecules were set indiscriminately. All rotatable torsions were emitted during docking. Each docking experiment was derived from 10 different runs that were set to close after a maximum of 250 000 energy assessments. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of five were applied.

REFERENCES AND NOTES

[1] Anwar, H. F.; Elnagdic, M. H. ARKIVOC 2009, i, 198.

[2] Yachevskii, D. S.; Chizhov, D. L.; Charushin, V. N. Russ J Org Chem 2006, 42, 142.

[3] Kost, A. N.; Grandberg, I. I. In Advances in Heterocyclic ChemistryKatritzky, A. R.; Boulton, A. J. Eds.; Academic Press: New York, 1966; Vol 6, p. 347 ff.

[4] Lee, K. Y.; Kim, J. M.; Kim, J. N. Tetrahedron Lett 2003, 44, 6737.

[5] Aggarwal, R.; Kumar, V.; Kumar, R.; Singh, S. P. Beilstein J Org Chem 2011, 7, 179.

[6] Shawali, A. S.; Edrees, M. M. ARKIVOC 2006, ix, 292.

[7] Katsunori, K.; Takayuki, S.; Hiromi, I.JP 2002167305 (Konica Co., Japan). "Industrial antibacterial and antifungal agent with reduced human toxicity", 2002.

[8] Abdallah, M. A.; Riyadh, S. M.; Abbas, I. M.; Gomha, S. M. J Chinese Chem Soc 2005, 52, 987.

[9] Bailey, J. J Chem Soc Perkin Trans I 1977, 18, 2047.

[10] Bailey, J.; Bowes, E.; Marr, P. A.*Brit. 1247493*, "Photographic colour processes", 1967.

[11] Diehl, D. R.; Mbiya, K.; Wray, C. S. EP 20000628 (Eastman Kodak Co. USA), "Photographic element containing pyrazolazole coupler", 2001.

[12] Tawara, K.; Akira, O. EP 0763569A1 (Konica Co. Japan), "Metal complex methin dye", 1997.

[13] Farghaly, T. A.; Abdallah, M. A.; Masaret, G. S.; Muhammad, Z. A. Eur J Med Chem 2015, 97, 320.

[14] Abdel Hafez, N. A.; Farghaly, T. A.; Al-Omar, M. A.; Abdalla, M. M. Eur J Med Chem 2010, 45, 4838.

[15] Farghaly, T. A.; Mahmoud, H. K. Arch Pharm Chem Life Sci 2013, 346, 392.

[16] Farghaly, T. A.; Gomha, S. M.; Abbas, E. M.; Abdalla, M. M. Arch Pharmazie 2012a, 345, 117.

[17] Farghaly, T. A.; Abdallah, M. A.; Aziz, M. R. A. Molecules 2012b, 17, 14625.

[18] Riyadh, S. M.; Farghaly, T. A.; Abdallah, M. A.; Abdalla, M. M.; Abd El-Aziz, M. R. Eur J Med Chem 2010, 45, 1042.

[19] Farghaly, T. A.; Abbas, E. M. H.; Dawood, K. M.; El-Naggar, T. B. A. Molecules 2014, 19, 740.

[20] Shawali, A. S.; Farghaly, T. A.; Shehata, M. R.; Hussein, S. M. J Heterocyclic Chem 2015, 52, 545.

[21] Farghaly, T. A.; Mahmoud, H. K. J Heterocyclic Chem 2015, 52, 86.

[22] Cheng, T.; Li, Q.; Zhou, Z.; Wang, Y.; Bryant, S. AAPS J 2012a, 14, 133.

[23] Dubenko, R. G.; Gorbenko, E. F. Chim Geterocikl Soedin 1967, 5, 923.

[24] Elgemeie, G. E. H.; Elghandour, A. H.; Elshimy, H. M. J Prakt Chem 1989, 331, 466.

[25] Cheng, T.; Li, Q.; Zhou, Z.; Wang, Y.; Bryant, S. AAPS J 2012b, 14, 133.

[26] Hassaneen, H. M.; Hilal, R. H.; Elwan, N. M.; Harhash, A.; Shawali, A. S. J Heterocyclic Chem 1984, 21, 1013.

[27] Coda, A. C.; Desimoni, G.; Invernizzi, A. G.; Quadrelli, P.; Rightetti, P. P.; Tacconi, G. Tetrahedron 1987, 43, 2843.

[28] Eweiss, N. F.; Osman, A. O. J Heterocyclic Chem 1980, 17, 1713.

[29] Shawali, A. S.; Abdelhamid, A. O. Bull Chem Soc Jpn 1976, 49, 321.

[30] Pfaller, M. A.; Burmeister, L.; Bartlett, M. A.; Ghorab, M. A.; Rinaldi, M. G. J Clin Microbiol 1988, 26, 1437.

[31] Gangadevi, V.; Muthumary, J. Afr J Biotechnol 2007, 6, 1382.

[32] Mosmann, T. J Immunol Methods 1983, 65, 55.

[33] Halgren, T. A. J Comput Chem 1998, 17, 490.

[34] Morris, G. M.; Goodsell, D. S.; et al. J Comput Chem 1998, 19, 1639.

[35] Solis, F. J.; Wets, R. J. B. Math Oper Res 1981, 6, 19.