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Synthesis of new *N*-phenylpyrazole derivatives with potent antimicrobial activity

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Abstract—The versatile synthons 4-(2-bromoacetyl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**3**) and 4-[(*E*)-3-(dimethylamino)acryloyl]-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**2**) were used as precursors for the synthesis of a series of phenylpyrazoles with different aromatic ring systems at position 4. The antimicrobiological evaluation of the newly synthesized compounds was carried out in vitro assays for antifungal and antibacterial activities. Amongst the tested compounds, 4-acetyl-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**1**), 4-[(*E*)-3-(dimethylamino)acryloyl]-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**2**), 4-(2-bromoacetyl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**3**) and 4-(2-aminothiazol-4-yl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**17**) showed interesting antimicrobial properties. In particular, all tested compounds produced inhibitory effects against pathogenic yeast (*Candida albicans*) similar or superior to those of reference drug. In addition, compound **3** showed excellent activity against pathogenic mould (*Aspergillus*). From structure–activity relationship (SAR) point of view, the attachment of bromoacetyl moiety to pyrazole ring can be considered as a breakthrough in developing a new therapeutic antifungal agent related to phenylpyrazole system.

1. Introduction

Fungi are causing cutaneous, sub-cutaneous or systemic infections, such as oral thrush, *Tenia pedis*, *Tenia corporis* or *Tenia capidis*. The azoles (imidazoles and triazoles) represent a class of versatile antifungal agents which are used to treat generalized systemic fungal infections. The mechanism of their antifungal action includes the inhibition of cytochrome P450 51 (CYP51), which is essential for ergosterol biosynthesis at the step of lanosterol-14-demethylation.[1]

The high affinity of the azole antifungals for CYP51 isozymes appears to be determined primarily by their bulky, polycyclic structure, giving favourable interaction with the hydrophobic residues in the largely non-polar active site of the enzyme.[2]

In recent years, the widespread use of imidazoles and triazoles antifungal agents has resulted in the development of resistance to these drugs by pathogenic microorganisms, causing an increase in morbidity and mortality. Therefore, new trend of antifungal related to phenylpyrazole is developed in order to get an effective antifungal agent without known resistance.[3–17]

Although many reported phenylpyrazoles showed significant activity against pathogenic yeast (*Candida*), unfortunately, no significant effect was obtained against pathogenic moulds such as *Aspergillus*.[3–17]

As part of our ongoing research programme aiming at the synthesis of variety of heterocyclic systems for biological and pharmacological evaluation,[18–27] we report here the synthesis of several phenylpyrazoles having different aromatic bulky structures at position 4 in order to increase the selectivity of this promising new group towards true pathogenic fungi; for example, *Aspergillus*

Keywords: Phenylcarbamoylpyrazole; 1,2,4-Triazolo[4,3-*a*]pyrimidine; Pyrazolo[1,5-*a*]pyrimidine; Imidazo[2,1-*b*]benzothiazole; Enaminones; Antibacterial; Antifungal; Anticandidal; Structure–activity relationship (SAR); 2-Aminothiazole.
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sp. The present study includes also antibacterial evaluation of the newly synthesized compounds.

2. Results and discussion

2.1. Chemistry

Treatment of 4-acetyl-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**1**) with bromine in glacial acetic acid afforded the 4-(2-bromoacetyl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**3**) in a good yield (Scheme 1).

The IR spectrum of compound **3** showed two strong absorption bands at 1651 and 1705 cm^{-1} assignable to amide and ketonic carbonyl groups, respectively. Other important band revealed at 3244 cm^{-1} characterized for amide NH. The ^1H NMR spectrum showed a singlet at δ 2.44 corresponding to pyrazole-attached methyl group and a singlet at δ 4.82 due to active methylene of bromoacetyl moiety. Other important signal appeared at δ 8.8 (D_2O-exchangeable) due to NH protons. Furthermore, the ^{13}C NMR spectrum of compound **3** displayed three important signals at δ 37.17, 161.22 and 189.76 corresponding to the active methylene of bromoacetyl, amide and ketonic carbonyl carbons, respectively. The mass spectrum of compound **3** revealed molecular ion peaks at 397 and 399 reflecting the isotopes of bromine.

The reactivity of 4-[(*E*)-3-(dimethylamino)acryloyl]-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**2**)[18] towards some nitrogen nucleophiles was investigated. Thus, the treatment of compound **2** with hydrazine hydrate, in refluxing ethanol, afforded white crystals of 5-methyl-1-phenyl-3-phenylcarbamoyl-4-(3-pyrazolyl)-*1H*-pyrazole (**4**) (Scheme 2).

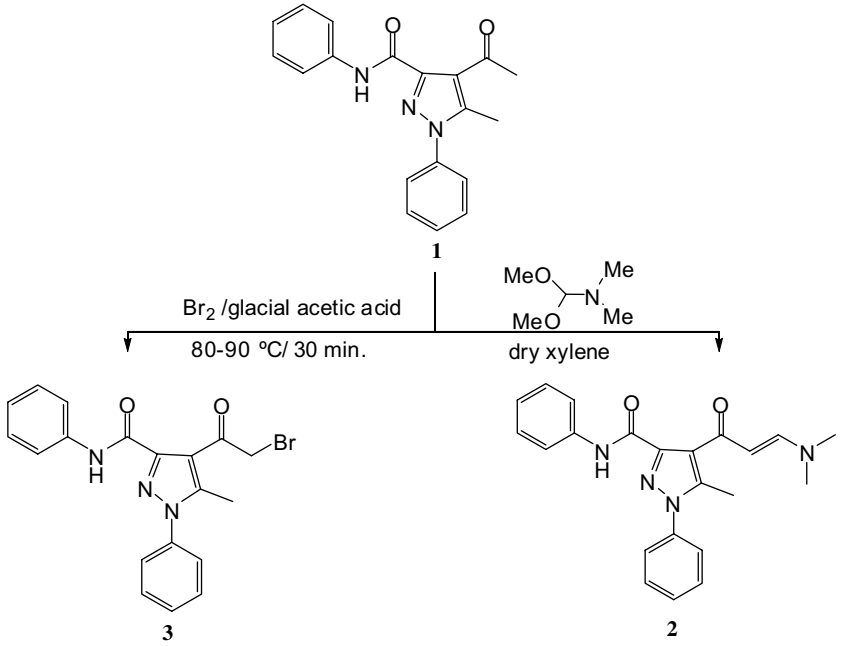
The spectral data of the product recommended the cyclic structure **4** as its ^1H NMR spectrum displayed two broad singlets (D_2O-exchangeable) at δ 10.6 and at δ 13.0 indicating two highly deshielded NH protons. The two protons of the formed pyrazole ring displayed on ^1H NMR spectrum as two doublets at δ 7.985 and δ 7.769 with the same coupling constant (J = 7.5 Hz). The two NH groups displayed on IR spectrum of **4** as two broad bands at 3240 and 3417 cm^{-1}.

The ^{13}C NMR spectrum showed one carbonyl carbon signal at δ 160.93, pyrazole-attached methyl group appeared at δ 12.03, other aromatic carbons arranged between δ 105.59 and 138.85. Mass spectrum of compound **4** showed prominent molecular ion peak at *m*/*z* 343 as the base peak.

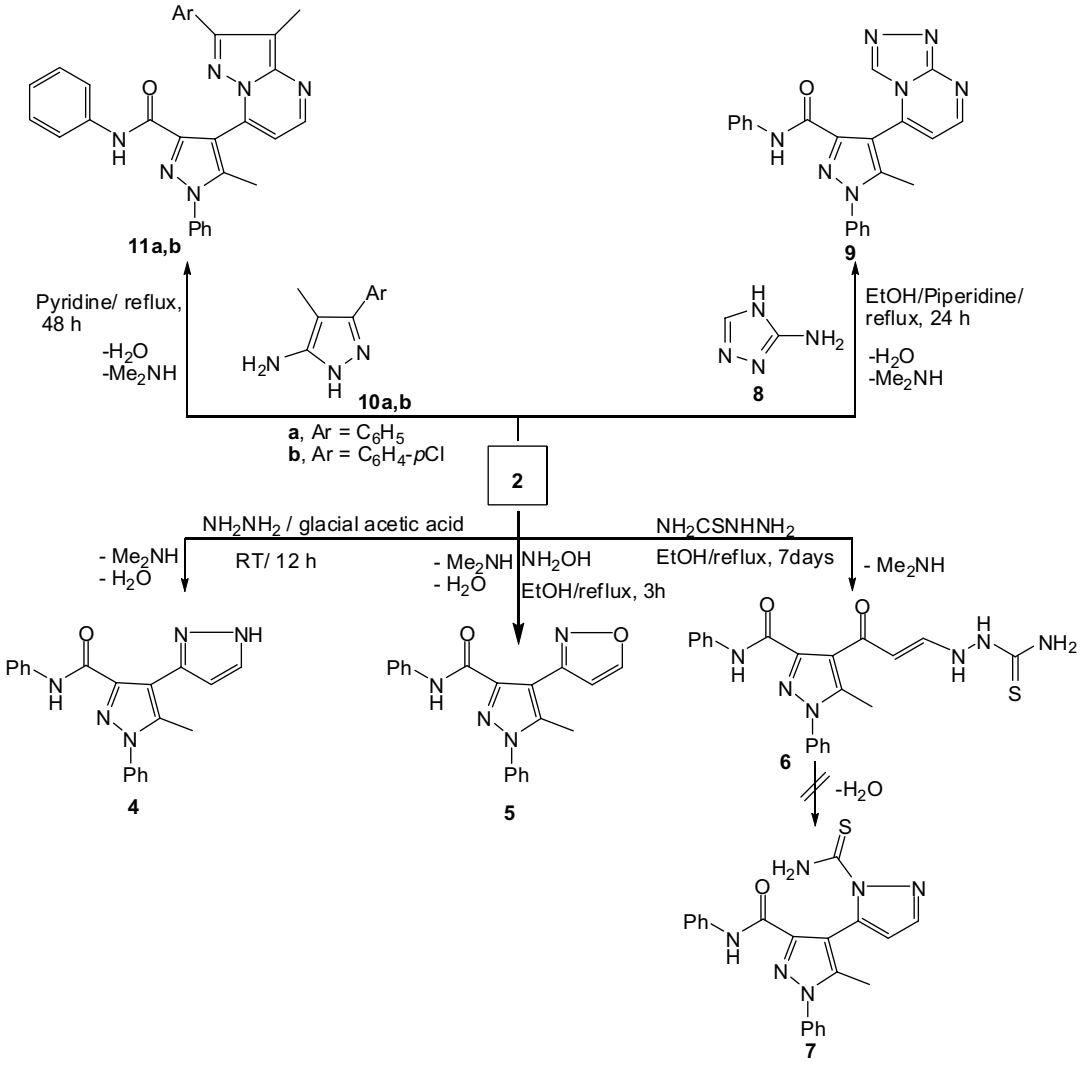
When enaminone **2** was treated with hydroxylamine in refluxing ethanol, it afforded a single product identified as 4-(isoxazol-3-yl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**5**) (Scheme 2).

The ^1H NMR spectrum of compound **5** exhibited two doublets at δ 6.88 and 8.65 with (J = 2.1 Hz) due to isoxazole protons. The mass spectrum of **5** is characterized by the presence of molecular ion peak at *m*/*z* 344, and a fragment ion peak at *m*/*z* 68 corresponding to isoxazolyl radical cation.

On the other hand, when enaminone **2** was treated with thiosemicarbazide in refluxing ethanol, it afforded the open structure 4-[(*E*)-3-(hydrazinothiocarbamido)acryloyl]-5-methyl-1-phenyl-4-phenylcarbamoyl-*1H*-pyrazole (**6**). All attempts to cyclize the 4-side chain of **6** to give 5-methyl-1-phenyl-4-phenylcarbamoyl-4-(1-thiocarbamoyl-*1H*-pyrazol-5-yl)-*1H*-pyrazole (**7**) using different solvents and catalysis were unsuccessful (Scheme 2).



Scheme 1.



Scheme 2.

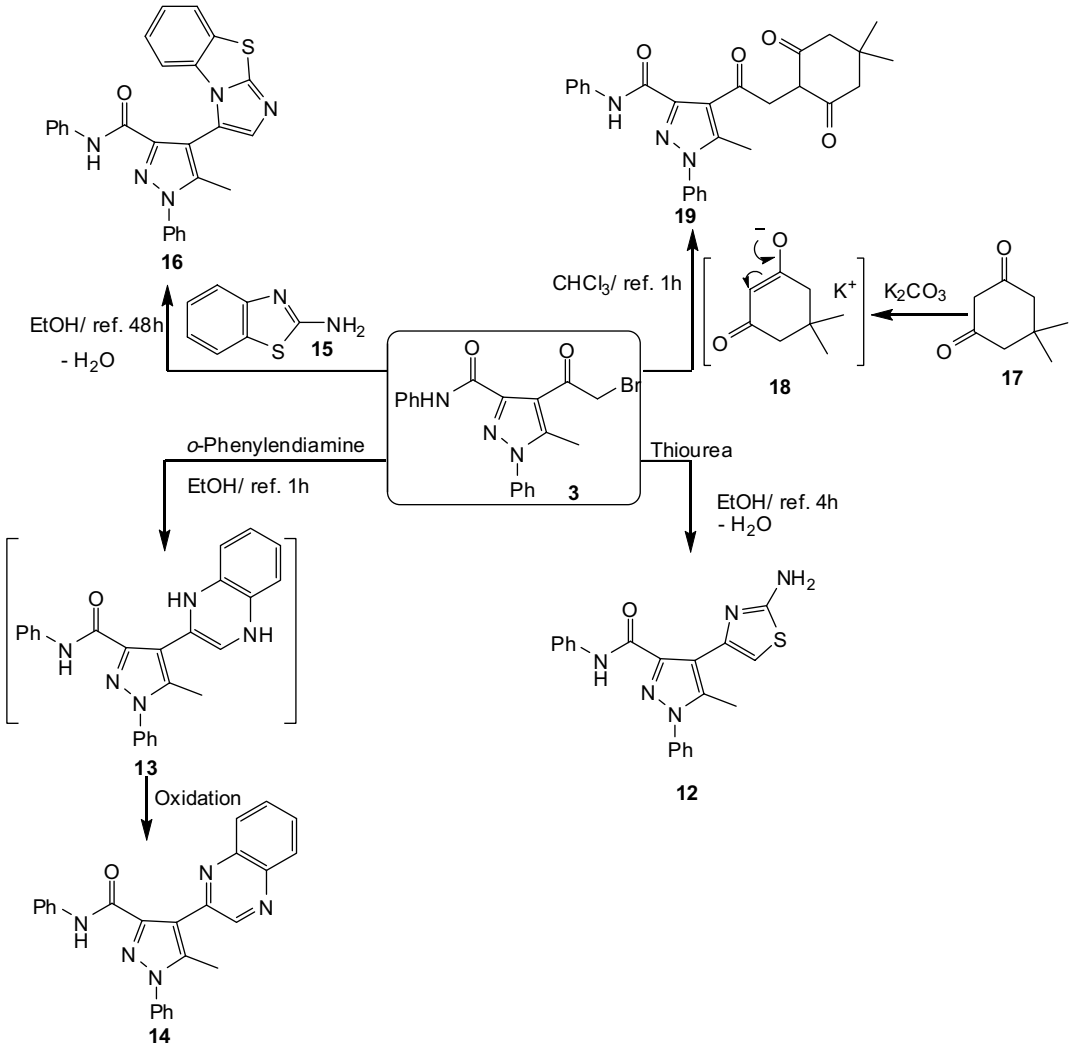
The ^{1}H NMR spectrum of compound **6** revealed four (D_2O-exchangeable) singlets at δ 5.85, 8.21, 8.77 and 10.38 due to amino and three amide protons, respectively.

The reactivity of enaminone **2** towards some heterocyclic amines was also investigated. Thus, the treatment of compound **2** with 3-amino-1,2,4-triazole (**8**) in the presence of a catalytic amount of piperidine afforded 5-methyl-1-phenyl-3-phenylcarbamoyl-4-([1,2,4]triazolo [4,3-*a*]pyrimidin-7-yl)-*1H*-pyrazole (**9**). A possible mechanism for the formation of compound **9** may involve an initial *Michael*-type addition of the amino group of 3-amino-1,2,4-triazole to the activated double bond in enaminone **2** followed by the elimination of dimethylamine and water molecules (Scheme 2).

The absorption band of carbonyl group at position 4 disappeared in the IR spectrum of compound **9** in comparison with the IR spectrum of **2**. The ^{1}H NMR spectrum of **9** showed two doublets, each of one proton, in the region of δ 7.49–8.96 with (J = 4.5 Hz), which can be attributed to the two adjacent protons of pyrimidine ring. The ^{1}H NMR spectrum revealed also a singlet, of one proton, at δ 8.57 corresponding to triazole H-3 proton. Its ^{13}C NMR spectrum revealed eighteen carbon types; the most important signals include triazole-3,5-carbons, which appeared at δ 154.68 and 154.95. Finally, the spectrum showed fourteen aromatic carbons arranged between δ 110.80 and 146.12. The mass spectrum of **9** showed a molecular ion peak at *m*/*z* 395, which loses a phenylamine radical to give the base peak at *m*/*z* 303.

When enaminone **2** was treated with 5-amino-*1H*-pyrazole derivatives **10a**,**b** in refluxing pyridine, it afforded the corresponding 5-methyl-4-(3-methyl-4-phenylpyrazolo[1,5-*a*]pyrimidin-6-yl)-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**11a**) and its chloro derivative **11b** (Scheme 2).

The formation of products **11** is assumed to take place via the addition of the amino group in aminopyrazoles **10a**,**b** to α,β-unsaturated moiety in enaminone **2** followed by the elimination of water and dimethylamine molecules to give the final products **11a**,**b**. The structures of compounds **11a**,**b** were established on the basis of their elemental and spectral data (see Section 3).



Scheme 3.

Next, when bromoacetylpyrazole **3** was treated with thiourea in refluxing ethanol, it afforded a product identified as 4-(2-aminothiazol-4-yl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**12**) (Scheme 3). The IR spectrum of product **12** showed three characteristic absorption bands, two bi-forked bands at 3355 and 3290 cm^{-1} assignable to amino group and a band at 3190 cm^{-1} due to the amide NH group. Its ^1H NMR spectrum revealed a singlet, of one proton, appeared in aromatic region at 6.77 corresponding to thiazole-5-CH, and D_2O-exchangeable singlets at δ 7.553 and δ 10.86 corresponding to amino and amide protons, respectively. The ^{13}C NMR spectrum of the same compound revealed sixteen carbon types; the most important signals appeared at δ 104.98 corresponding to C5 of thiazole ring, δ 160.6 and 168.03 characterized for amide carbonyl and thiazole-C2. The mass spectrum of **12** showed a molecular ion peak at *m/z* 374.

Treatment of compound **3** with *o*-phenylenediamine in refluxing ethanol afforded a crystalline product identified as 5-methyl-1-phenyl-3-phenylcarbamoyl-4-(quinoxalin-2-yl)-*1H*-pyrazole (**14**) in an excellent yield (Scheme 3).

A plausible mechanism may involve the condensation of one of phenylenediamine amino groups with the carbonyl group of bromoacetyl moiety, while the second amino group replaced bromine atom via nucleophilic substitution. The expected product is the dihydroquinoxalinyl derivative **13**; however, the spectral data of the isolated product established that the dihydroquinoxalinyl derivative **13** was oxidized under the reaction conditions to give 5-methyl-1-phenyl-3-phenylcarbamoyl-4-(quinoxalin-2-yl)-*1H*-pyrazole (**15**).

The IR spectrum of product **15** showed one absorption band at 3274 cm^{-1} assignable to one NH group. The ^1H NMR spectrum showed a singlet, of one proton, at δ 9.12 due to quinoxaline-3-C*H*. The presence of 19 aromatic carbon types on ^{13}C NMR spectrum of the isolated product between δ 118.29 and 148.29, in addition to one aliphatic carbon at δ 11.34 and one carbonyl carbon at δ 160.42, confirmed structure **15**.

Cyclocondensation reaction of bromoacetylpyrazole **3** with some heterocyclic amine was also examined. Thus, when compound **3** was allowed to react with 2-aminobenzothiazole (**15**) in ethanol, at reflux temperature, it

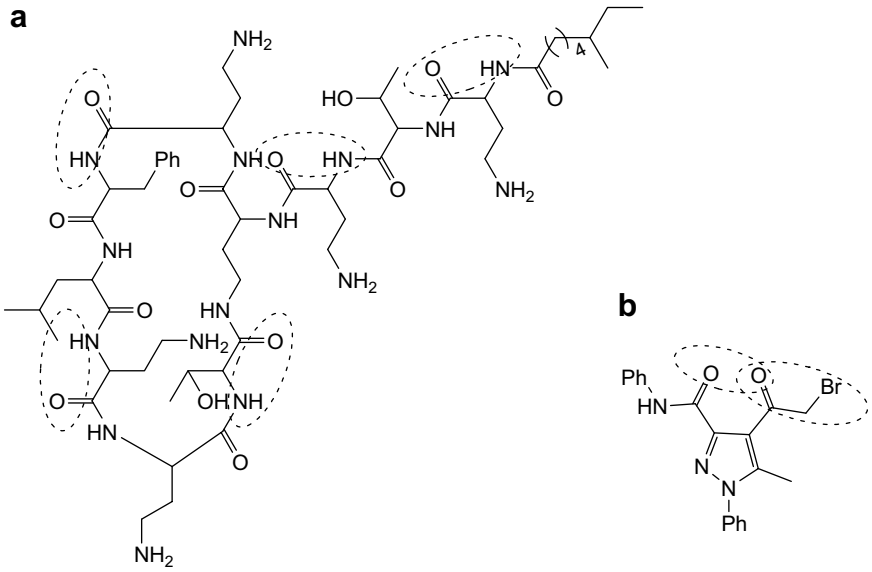


Figure 1. Chelation parts of polymyxin B_1 and compound **3.**

afforded 4-(imidazo[2,1-*b*]benzothiazole-3-yl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**16**) (Scheme 3).

The preliminary antimicrobial test showed unexpected result that the phenylpyrazole derivative that carries the least non-polar side chain, that is, bromoacetylpyrazole **3**, has good activity against both fungi and bacteria, which is assumed that it exerts its action via chelation of some vital cellular divalent cations as observed in other anti-infective agents such as polymyxin B_1[28] (Fig. 1a). In other words, the bromoacetyl moiety acts as a chelating part (Fig. 1b). Therefore, we decided to synthesize a phenylpyrazole derivative with a strong chelating moiety at position 4 to prove this assumption. Thus bromoacetylpyrazole **3** was allowed to react with 5,5-dimethylcyclohexane-1,3-dione (dimedone) (**17**) in refluxing chloroform in the presence of potassium carbonate, which reaction afforded a product identified as 4-[2-(4,4-dimethyl-2,6-dioxocyclohexyl)-1-oxoethyl]-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**19**).

The suggested reaction mechanism involves the conversion of dimedone (**17**) into its enolate potassium salt **18** in the presence of potassium carbonate. The latter intermediate is considered as a strong carbon nucleophile that can replace bromine atom from bromoacetylpyrazole **3** to produce the final product **19** as shown in Scheme 3. The ^{1}H NMR spectrum of the reaction product displayed five signals at δ 0.95, 2.13, 2.36, 2.48 and 3.64 corresponding to methyl and methylene groups of dimedone, methylene protons of acetyl moiety, pyrazole-attached methyl group and dimedone-CH, respectively. The mass spectrum of compound **19** is characterized by the presence of molecular ion peak at *m*/*z* 457.

The antimicrobial tests showed that the activity of compound **19** is lower than compound **3**; therefore, the previous assumption can be excluded. The second assumption is that this compound exerts its action on bacteria and fungi via two different mechanisms; to confirm this suggestion, further study is in progress based on molecular modeling.

2.2. Biological testing

2.2.1. Antimicrobial screening. The results of the antimicrobial evaluation of the tested compounds are presented in Table 1. They showed that most of the tested compounds have a significant antibacterial effect against *Staphylococcus aureus* (*S. A.*), and *Pseudomonas aeruginosa* (*P. A.*). Acetylpyrazole **1** showed the highest potency against Gram-positive bacteria *S. A.*, while pyrazolopyrimidines **11a**,**b** exhibited the lowest activity. Antipseudomonal activity was observed with compounds **1**, **3** and **12** (Table 1 and Fig. 2). These compounds also possess a potent antifungal activity against *Candida albicans* (*C. A.*), mostly higher than that of reference drug (terbinafine). However, they have no effect against *Aspergillus fumigatus* (*A. F.*) except compound **3**. The latter compound was found to be more effective than terbinafine against *A. F.* (Table 1 and Fig. 3). Moreover, compound **3** showed an antifungal activity against *C. A.*, 1.6 higher than that of the reference drug.

Based on the antimicrobial evaluation, compound **3** was selected for further assessment. The minimum inhibitory concentration (MIC) of compound **3** against *A. F.* was 25 μg/ml, which is less than that of miconazole and higher than amphoteracin B as reference drugs as depicted in Table 2.

From the structure–activity relationship (SAR), we can conclude that the bromoacetyl moiety is essential for the activity against true fungi (moulds). Amongst the dozens of synthesized phenylpyrazoles found in recent

Table 1. Antimicrobial activity of new compounds and reference drugs

Sample	Inhibition zone diameter (mm/mg sample)							
	(G +ve)		(G –ve)		Mould		Yeast	
	S. A.	Pot.[a]	*P. A.*	Pot.[a]	*A. F.*	Pot.[a]	*C. A.*	Pot.[a]
1	21 ± 1.3	1	17 ± 2	0.85	0	0	15 ± 1.2	1.5
2	14 ± 0.7	0.66	14 ± 0.9	0.7	0	0	15 ± 1.2	1.5
3	18 ± 3	0.85	20 ± 1.3	1	12 ± 0.5	1.2	16 ± 1.2	1.6
4	13 ± 1.1	0.61	13 ± 1	0.65	0	0	12 ± 1	1.2
5	13 ± 1.2	0.61	13 ± 2	0.65	0	0	13 ± 1.2	1.3
6	10 ± 0.5	0.47	8 ± 1	0.4	0	0	12 ± 1	1.2
9	13 ± 1.2	0.61	13 ± 1	0.65	0	0	12 ± 1	1.2
11a	8 ± 0.5	0.38	0	0	0	0	10 ± 1	1
11b	10 ± 1	0.47	0	0	0	0	10 ± 1	1
12	13 ± 1.0	0.61	18 ± 1.3	0.9	0	0	13 ± 1	1.3
14	11 ± 1.0	0.52	10 ± 1.0	0.5	0	0	10 ± 1	1
16	12 ± 1.0	0.57	10 ± 1.1	0.5	0	0	12 ± 1	1.2
19	11 ± 0.8	0.52	0	0	0	0	10 ± 1	1
Control	0	0	0	0	0	0	0	0
Chloramphenicol	21 ± 2.2	1	20 ± 1.4	1	Nt[b]	—	Nt[b]	—
Terbinafine	Nt[b]	—	Nt[b]	—	10 ± 0.5	1	10 ± 0.5	1

[a] Potency.
[b] Not tested.

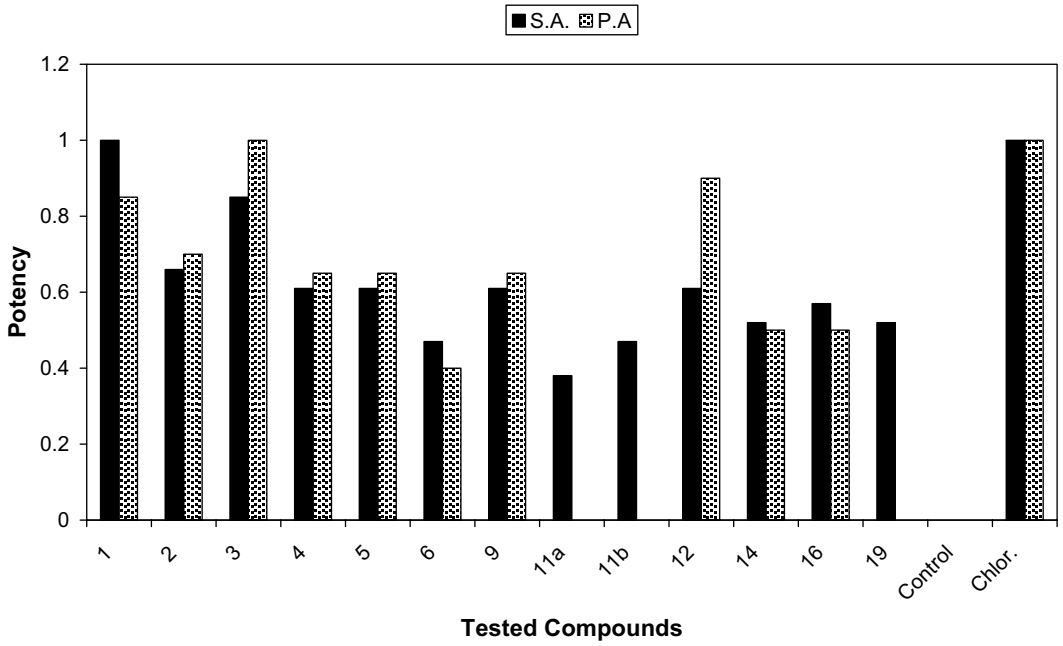


Figure 2. Antibacterial potency of the tested compounds in comparison with reference drug.

few years,[3–17] compound **3** showed one of the highest observed activities against *Aspergillus*; so that, it can be considered as a lead compound in this field. Further studies are in progress on the same compound to increase its efficacy and understand its QSAR. Also, aminothiazolyl moiety is important to increase anti-pseudomonal activity as observed in compound **12**.

The overall results of the present study can be considered very promising in the perspective of new drugs discovery, with respect to the medical importance of the tested microorganisms. *P. A.* has emerged as one of the most problematic Gram-negative pathogens, with the alarmingly high antibiotics resistance rates.[29,30] Even with the most effective antibiotics against this pathogen, namely carbapenems (imipenem and meropenem), the resistance rates were detected as 15–20.4% amongst 152 *P. A.* strains.[29,30] This pathogen was found to be sensitive to compounds **1**, **3** and **12** (Table 1 and Fig. 2). *Candida albicans* and other *Candida* species causing candidiasis are increasingly important diseases that are distributed worldwide due to the fact that they are frequent opportunistic pathogens in AIDS patients.[31] This fungus was found to be sensitive to most of the synthesized compounds especially bromoacetylpyrazole **3** (Table 1 and Fig. 3). *Aspergillus*

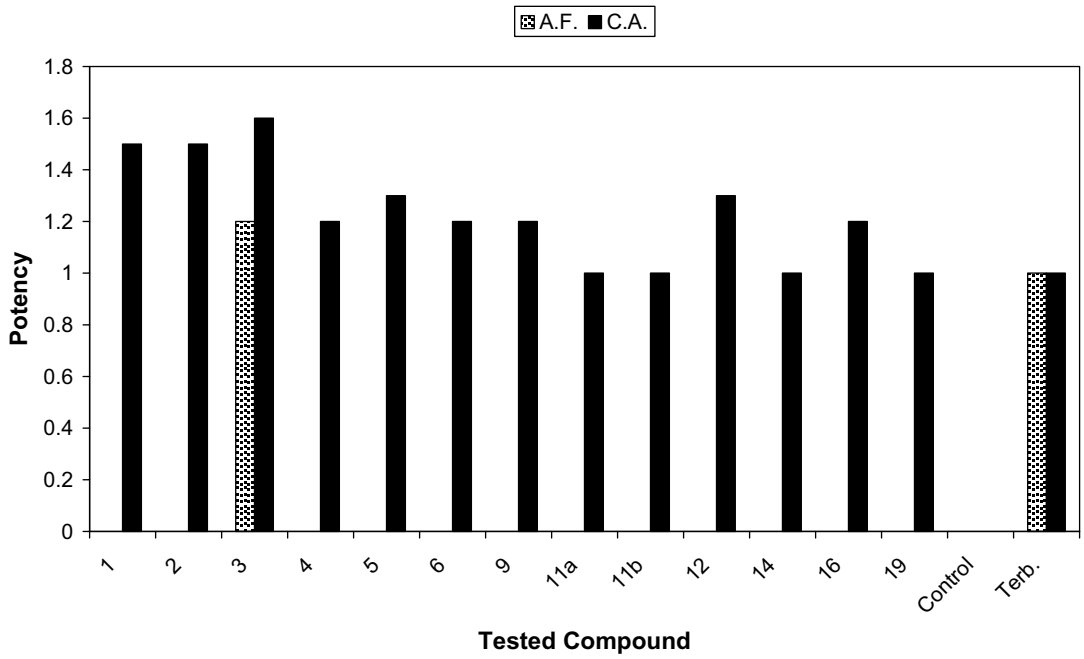


Figure 3. Antimycotic effect of the tested compounds in comparison with reference drug.

Table 2. MIC (μg/ml) of compound 3 and fluconazole against *A. F.*

	MIC[a] (μg/ml) *A. F.*
Compound **3**	25 ± 0.2
Miconazole	27.5 ± 0.1
Amphoteracin B	11 ± 0.1

[a] Minimum inhibitory concentration.

causes chronic necrotizing pulmonary aspergillosis (CNPA),[32] which is a subacute infection seen in patients with an underlying lung disease. About 50% of reported cases have shown no response to amphotericin B.[31] Such a pathogen was found to be highly sensitive to compound **3** (Table 1 and Fig. 3).

3. Experimental

3.1. Chemistry

3.1.1. General. All melting points were measured on a Gallenkamp melting point apparatus. The infrared spectra were recorded in potassium bromide discs on a Pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}H (300 MHz) and ^{13}C NMR (75.46 MHz) were run in deuterated chloroform ($CDCl_3$) or dimethylsulphoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 eV. Elemental analyses were carried out at the Microanalytical Center of Cairo University, Giza, Egypt. Acetylphenylpyrazole **1**,[18] and enaminone **2**[18] were prepared following the procedures reported in the literature.

3.1.2. 4-(2-Bromoacetyl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (3). A solution of 4-acetyl-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**1**) (31.9 g, 100 mmol) in glacial acetic acid (100 ml) was heated to 80–90 °C with vigorous stirring. To this hot solution, bromine (16 g, 100 mmol) in glacial acetic acid (20 ml) was added dropwise over a period of 30 min with stirring and maintaining the temperature at 80–90 °C. After complete addition of bromine, the reaction mixture was stirred vigorously at room temperature for further 1 h till the evolution of hydrogen bromide gas was ceased, then poured onto crushed ice with stirring. The solid that formed was collected, washed with water and dried. Crystallization ethanol from 70% afforded compound **3** in 78% yields, mp 140–141 °C. $C_{19}H_{16}BrN_3O_2$ (398.25), Analysis % Calcd (Found): C: 57.30 (57.39), H: 4.05 (4.02), N: 10.55 (10.52). IR (KBr) υ_{max}/cm^{-1}: 3244 (NH), 1705 (C=O), 1651 (C=O), 1600 (C=N). ^{1}H NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 4.82 (s, 2H, CH_2), 7.14–7.66 (m, 10H, ArH's), 8.8 (D_2O-exchangeable) (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 12.59 (CH_3, aliphatic), 37.17 (CH_2), 118.32, 120.82, 121.23, 124.66, 126.42, 129.79, 130.12, 138.4, 139.02, 145.57, 146.7 (11 CH, ArC's), 161.22 (C=O, amide), 189.76 (C=O, ketonic). MS (*m*/*z*, aband. %): 399 (M^{+2}, 4.1), 397 (M^+, 3.6), 318 (100), 307(13.0), 305 (13.9), 304 (12.7), 279 (0.7), 277 (0.9), 276 (0.7), 227 (22.0), 199 (1.1), 157 (3.4), 123 (0.4), 121(0.4), 118 (27.7), 95 (5.7), 93 (5.7), 81 (0.5), 79 (0.7), 77 (40.4).

3.1.3. 5-Methyl-1-phenyl-3-phenylcarbamoyl-4-(3-pyrazolyl)-*1H*-pyrazole (4). Hydrazine hydrate (2 ml) was added to a stirred solution of enaminone **2** (0.748 g, 2 mmol) dissolved in acetic acid (10 ml). Stirring was continued for 12 h at room temperature and the solid product obtained was filtered off, washed with cold water, dried and finally recrystallized from ethanol/

DMF to afford 5-methyl-1-phenyl-3-phenylcarbamoyl-4-(3-pyrazolyl)-*1H*-pyrazole (**4**) in 85% yield, mp 197–198 °C. $C_{20}H_{17}N_5O$ (343.4), Analysis % Calcd (Found): C: 69.96 (70.04), H: 4.99 (5.00), N: 20.40 (20.42). IR (KBr) υ_{max}/cm^{-1}: 3417 (NH), 3240 (NH), 1654 (C=O), 1596 (C=N). ^1H NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 6.56–7.78 (m, 12H, ArH's), 7.595 (d, 1H, pyrazole-4-C*H*, J = 7.5 Hz), 7.769 (d, 1H, pyrazole-3-C*H*, J = 7.5 Hz), 10.6 (D_2O-exchangeable) (s, 1H, NH), 13.0 (D_2O-exchangeable) (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 12.03 (CH_3, aliphatic), 105.59, 119.84, 123.69, 125.41, 128.7, 129.39, 138.85 (14 ArC's), 160.93 (C=O, amide). MS (*m*/*z*, aband. %): 343 (M^+, 100), 251 (97.8), 93 (21), 118 (14.5%), 77 (54.3).

3.1.4. 4-(Isoxazol-3-yl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (5). To a solution of **2** (0.748 g, 2 mmol), in ethanol (10 ml), were added hydroxylamine hydrochloride (0.14 g, 2 mmol) and ammonium acetate (0.3 g). The reaction mixture was heated under reflux for 3 h, then poured onto ice cold water. The resulting solid was filtered off, washed with cold water, dried and recrystallized from 70% ethanol to afford 4-(3-isoxazolyl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**5**) in 88% yield, mp 183–185 °C. $C_{20}H_{16}N_4O_2$ (344.4). Analysis % Calcd (Found): C: 69.76 (69.81), H: 4.68 (4.74), N: 16.27 (16.23). IR (KBr) υ_{max}/cm^{-1}: 3359 (NH), 1670 (C=O), 1600 (C=N). ^1H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 7.1–7.78 (m, 10H, ArH's), 6.879 (d, 1H, isoxazole-4-C*H*, J = 2.1 Hz), 8.625 (d, 1H, isoxazole-5-C*H*, J = 2.1 Hz), 10.34 (D_2O-exchangeable) (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 12.02 (CH_3, aliphatic), 120.31, 123.89, 125.61, 125.72, 128.67, 129.01, 129.17, 129.46, 138.49, 138.59, 140.9, 144.12, 150.98 (13 ArC's), 154.97 (isoxazole-5-*C*H), 160.31 (C=O, amide). MS (*m*/*z*, aband. %): 344 (M^+, 29.2), 252 (76), 118 (58.4), 93 (19.8), 77 (100), 68 (15.9).

3.1.5. 5-Methyl-1-phenyl-4-phenylcarbamoyl-4-[(*E*)-3-(thiocarbamoylhydrazino)acryloyl]-*1H*-pyrazole (6). To a solution of enaminone **2** (0.748 g, 2 mmol), in ethanol (10 ml), was added thiosemicarbazide (0.182 g, 10 mmol). The reaction mixture was heated under reflux for 7 days, and then poured onto ice cold water. The resulting solid product was filtered off, washed with cold water, dried and crystallized from ethanol 70% to afford compound **6** in 60% yield, mp 191–192 °C. $C_{21}H_{20}N_6O_3$ (404.42). Analysis % Calcd (Found): C: 59.98 (60.12), H: 4.79 (4.73), N: 19.99 (19.89). IR (KBr) υ_{max}/cm^{-1}: 3151, 3232, 3409 (3NH, NH_2 overlapped), 1681 (conjugated C=O), 1655 (amide C=O), 1596 (C=N). ^1H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3), 5.60 (d, 1H, J value = 12.6 Hz), 5.85 (D_2O-exchangeable) (br s, 2H, NH_2), 7.1–7.77 (m, 10H, ArH's), 7.8 (d, 1H, J value = 12.5 Hz), 8.21 (D_2O-exchangeable) (br s, 1H, NH), 8.77 (D_2O-exchangeable) (br s, 1H, NH), 10.38 (D_2O-exchangeable) (br s, 1H, NH).

3.1.6. 5-Methyl-1-phenyl-3-phenylcarbamoyl-4-([1,2,4]triazolo[4,3-*a*]pyrimidin-5-yl)-*1H*-pyrazole (9). A mixture of enaminone **2** (0.748 g, 2 mmol) and the 3-amino-*1H*-1,2,4-triazole (**8**) (0.184 g, 2.2 mmol), in ethanol (10 ml), was refluxed for 24 h in the presence of catalytic amount of piperidine, then allowed to cool. The solid that formed was filtered off, washed with cold water and dried. Crystallization from DMF afforded compound **9** in 65% yield, mp 278–279 °C. $C_{22}H_{17}N_7O$ (396.4), Analysis % Calcd (Found): C: 66.82 (66.89), H: 4.33 (4.34), N: 24.80 (24.84). IR (KBr) υ_{max}/cm^{-1}: 3417 (NH), 1670 (C=O), 1596 (C=N). ^1H NMR (DMSO-d_6): δ 2.37 (s, 3H, CH_3), 7.05–7.77 (m, 11H, ArH's), 7.49 (d, 1H, pyrimidine-5-CH, J = 4.5 Hz), 8.961 (d, 1H, pyrimidine-6-CH, J = 4.5 Hz), 8.57 (s, 1H, (1,2,4-triazole-5-*C*H)), 10.36 (D_2O-exchangeable) (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 11.22 (CH_3, aliphatic), 110.80, 111.48, 120.30, 123.78, 125.57, 128.59, 129.23, 129.58, 138.26, 138.49, 141.61, 142.22, 146.01, 146.12 (14 ArC's), 154.68, 154.95 (triazole-3,5-Carbons), 159.57 (C=O, amide). MS (*m*/*z*, aband. %): 396 (M^+, 4.7), 303 (100), 184 (0.5), 118 (3.3), 93 (3), 77 (17.7), 65 (5.5).

3.1.7. 5-Methyl-4-(3-methyl-4-(aryl)pyrazolo[1,5-*a*]pyrimidin-6-yl)-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (11a,b): General procedure. A mixture of the enaminone **2** (0.374 g, 1 mmol) and the appropriate aminopyrazole derivatives **10a**,**b** (1 mmol), in dry pyridine (20 ml), was refluxed for 48 h. The formed solid product was filtered off, washed with ethanol and crystallized from ethanol/DMF to afford the pyrazolo[1,5-*a*]pyrimidine derivatives **11a**,**b** in 65–70% yield.

3.1.7.1. 5-Methyl-4-(2-methyl-3-phenylpyrazolo[1,5-*a*]pyrimidin-7-yl)-1-phen-yl-3-phenylcarbamoyl-*1H*-pyrazole (11a). Yield (65%), mp. 222–223 °C (ethanol/DMF). $C_{30}H_{24}N_6O$ (484.2). Analysis % Calcd (Found): C: 74.36 (74.41), H: 4.99 (4.98), N: 17.34 (17.33). IR (KBr) υ_{max}/cm^{-1}: 3259 (NH), 1666 (C=O), 1596 (C=N). ^1H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 6.92–7.78 (m, 17H, ArH's), 10.56 (D_2O-exchangeable) (br s, 1H, NH). MS (*m*/*z*, aband. %): 484 (M^+, 2.9), 392 (100), 364 (66.2), 118 (3.7), 77 (32.5).

3.1.7.2. 5-Methyl-4-(2-methyl-3-(4-chlorophenyl)pyrazolo[1,5-*a*]pyrimidin-7-yl)-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (11b). Yield (70%), mp. 230–231 °C (ethanol/DMF). $C_{30}H_{23}ClN_6O$ (518.16). Analysis % Calcd (Found): C: 69.43 (69.39), H: 4.47 (4.37), N: 16.19 (16.15). IR (KBr) υ_{max}/cm^{-1}: 3132 (NH), 1666 (C=O), 1600 (C=N). ^1H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 7.09–7.78 (m, 16H, ArH's), 10.56 (D_2O-exchangeable) (br s, 1H, NH). MS (*m*/*z*, aband. %): 520 (M^{+2}, 2.6), 518 (M^+, 6.8), 426 (100), 398 (55.0), 118 (13.4), 77 (40.1).

3.1.8. 4-(2-Aminothiazol-4-yl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (12). To a solution of 4-(2-bromoacetyl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (**3**) (3.98 g, 10 mmol) in absolute ethanol (20 ml) was added thiourea (0.76 g, 10 mmol). The mixture was refluxed for 4 h, then afforded to cool and treated with ammonium hydroxide solution till it became alkaline (pH 9). The solid that formed was filtered off, washed with water, dried and finally recrystallized from dioxane to afford aminothiazole derivative **12** in a 75% yield; mp 251–252 °C. $C_{20}H_{17}N_5OS$, (375.45). Analysis

% Calcd (Found): C: 63.98 (63.87), H: 4.56 (4.60), N: 18.65 (18.66). IR (KBr) υ_{max}/cm^{-1}: 3355, 3290 (NH_2), 3190 (NH), 1666 (C=O), 1624, 1600 (2 C=N). ^1H NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 6.77 (s, 1H, thiazole-5-CH), 7.08–7.77 (m, 10H, ArH's), 7.553 (D_2O-exchangeable) (d, 2H, NH_2), 10.86 (D_2O-exchangeable) (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 12.09 (CH_3, aliphatic), 104.98 (thiazole-5-*C*H), 115.50, 119.81, 123.55, 125.32, 128.53, 128.76, 129.35, 138.95, 138.97, 139.01, 141.51, 144.62 (12 ArC's), 168.03 (thiazole-2-*C*H), 160.60 (C=O, amide). MS (*m*/*z*, aband. %): 375 (48), 283 (100), 77 (11.2).

3.1.9. 5-Methyl-1-phenyl-3-phenylcarbamoyl-4-(quinoxalin-2-yl)-*1H*-pyrazole (14). To a solution of bromoacetylpyrazole derivative **3** (0.79 g, 2 mmol), in absolute ethanol (10 ml), *o*-phenylenediamine (0.21 g, 2 mmol) was added. The mixture was refluxed for 1 h, then allowed to cool and treated with ammonium acetate solution. The solid that formed was filtered off, washed with water, dried and finally recrystallized from ethanol/DMF to afford the corresponding quinoxalinylpyrazole derivative **14** in an 87% yield; mp 240–241 °C. $C_{25}H_{19}N_5O$ (405.45). Analysis % Calcd (Found): C: 74.06 (74.08), H: 4.72 (4.70), N: 17.27 (17.30). IR (KBr) υ_{max}/cm^{-1}: 3274 (NH), 1654 (C=O), 1600 (C=N). ^1H NMR (DMSO-d_6): δ 2.48 (s, 3H, CH_3), 7.08–8.13 (m, 14H, ArH's), 9.12 (s, 1H, quinoxaline-3-CH), 10.29 (D_2O-exchangeable) (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 11.34 (CH_3, aliphatic), 118.29, 120.43, 123.84, 125.72, 128.62, 128.81, 128.89, 129.09, 129.48, 129.81, 130.21, 138.50, 138.54, 140.07, 141.32, 141.93, 144.47, 147.75 (18 ArC's), 148.29 (quinoxaline-3-*C*H), 160.42 (C=O, amide). MS (*m*/*z*, aband. %): 405 (54.4), 313 (100), 129 (5), 118 (7.7), 77 (28.5).

3.1.10. 4-(Imidazo[2,1-*b*]benzothiazol-3-yl)-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (26). A mixture of bromoacetylpyrazole derivative**3** (0.79 g, 2 mmol) and 2-aminobenzothiazole (**15**) (0.3 g, 2 mmol) was refluxed in ethanol for 48 h, and then allowed to cool. The solid so formed was filtered off, washed with water and dried. Recrystallization from dioxane afforded the corresponding imidazo[2,1-*b*]benzothiazole derivative **16** in 65% yield; mp 160–161 °C. $C_{26}H_{19}N_5OS$ (449.53). Analysis % Calcd (Found): C: 69.47 (69.50), H, 4.26 (4.28), N: 15.58 (15.59). IR (KBr) υ_{max}/cm^{-1}: 3390 (NH), 1685 (C=O), 1596 (C=N). ^1H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 7.08–8.10 (m, 14H, ArH's), 8.61 (s, 1H, imidazole-4-CH), 10.60 (D_2O-exchangeable) (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 12.91 (CH_3, aliphatic), 112.84, 114.21, 114.89, 118.36, 120.75, 121.57, 124.24, 125.51, 125.77, 126.12, 127.27, 129.30, 129.97, 132.48, 138.89, 139.50, 139.58, 140.08, 144.57, 146.37, (20 ArC's), 161.46 (C=O, amide). MS (*m*/*z*, aband. %): 449 (50.7), 357 (100), 118 (5.1), 77 (20.2).

3.1.11. 4-[2-(4,4-Dimethyl-2,6-dioxocyclohexyl)-1-oxoethyl]-5-methyl-1-phenyl-3-phenylcarbamoyl-*1H*-pyrazole (19). To a solution of bromoacetylpyrazole derivative **3** (0.79 g, 2 mmol), in chloroform (10 ml), were added dimedone (**17**) (0.28 g, 2 mmol) and potassium carbonate (0.345 g, 2.5 mmol). The reaction mixture was refluxed for 1 h, then allowed to cool and the precipitated solid was filtered off, washed with water and dried. Crystallization from 70% ethanol afforded **19** in 58% yield; mp 180–181 °C. $C_{27}H_{27}N_3O_4$, (457.52). Analysis % Calcd (Found): C: 70.88 (71.00), H: 5.95 (5.93), N: 9.18 (9.20). IR (KBr) υ_{max}/cm^{-1}: 3201 (NH), 1722 (C=O), 1670 (C=O), 1596 (C=N). ^1H NMR (DMSO-d_6): δ 0.95 (s, 6H, 2 CH_3), 2.13 (s, 4H, $2CH_2$), 2.36 (d, 2H, CH_2), 2.48 (s, 3H, CH_3), 3.64 (t, 1H, CH), 7.08–7.79 (m, 10H, ArH's), 10.71 (D_2O-exchangeable) (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 11.89, 28.25 (CH_3, aliphatic), 31.68, 37.32, 38.73, 107.08 (aliphatic carbons), 119.90, 121.04, 123.57, 125.63, 128.64, 128.93, 129.43, 128.23, 139.16, 141.16, 147.09 (11 aromatic carbons), 161.46 (C=O, amide), 183.75 (C=O, ketonic), 197.57 (2C=O, ketonic). MS (*m*/*z*, aband. %): 457 (24.4), 439 (21.5), 364 (12.9), 276 (6.1), 185 (26.9), 157 (2.9), 131 (3.9), 120 (5.9), 118 (73.9), 117 (6.0), 92 (9.9), 91 (9.7), 77 (100).

3.2. Biological screening

Antimicrobial activity of eight newly synthesized compounds was tested by measuring the inhibitory effects of such compounds against Gram-positive, Gram-negative bacteria and fungi using agar diffusion technique.

3.2.1. Materials. *Staphylococcus aureus*, *Pseudomonas aeraginosa*, *C. albicans* and *A. fumigatus* were used against the tested compounds and were obtained from the regional center for Mycology and Biotechnology, Faculty of Science, Al-Azhar University. Chloramphenicol, Terbinafine, miconazole and amphoteracin B were used as reference drugs and were also obtained from the same source.

3.2.2. Method

3.2.2.1. Preparation of bacterial suspensions. Suspension of the above-mentioned micro-organisms was prepared by inoculating fresh stock cultures into separate broth tubes, each containing 7 ml of nutrient broth (*pepton*, 0.3%) beef extract (0.3%). The inoculated tubes were incubated at 37 °C for 24 h.

3.2.2.2. Preparation of solutions of the tested compounds and reference drugs. Solutions of the tested compounds and reference drugs were prepared by dissolving 0.5 g of the compound in 10 ml DMF.

3.2.2.3. Agar diffusion test.[33–36] Talls of nutrient agar were melted and poured each in an empty sterile petridishes (100 × 15 mm) and left for 24 h. A specific culture of each organism was spread with a dry sterile swab on the surface of the previously prepared plates. Sterile discs of 9.6 mm diameter were impregnated with solutions of the tested compound, left to dry and were then placed on the surface of the inoculated plate. Discs of antimicrobial standard were put in the centre of the plate agar and incubated at 37 °C for 24 h. After incubation, the plates were examined visually and the zone of inhibition was measured. The test was repeated five times for each compound.

3.2.2.4. Determination of MIC. Determination of the MIC of compound **3** against *A. fumigatus* was achieved using 96-well microbioassay system.[37] Briefly, a range of concentrations (10–100 μg/ml) of the compound were made using Czapeck medium containing sucrose (20 g/l), KH_2PO_4 (1 g/l), $NaNO_2$ (2 g/l), $MgSO_4{\cdot}7\,H_2O$ (0.5 g/l), $FeSO_4{\cdot}7H_2O$ (0.01 g/l) and KCl (0.5 g/l).

Medium (180 μl) containing appropriate concentrations of the compound was added to the microwells. Each concentration is repeated in triplicate. Medium controls (medium without compound and medium with the same range of concentration of fluconazole) were also included. The inoculum was obtained from 48 h old fungal cultures grown on Sabouraud agar. Each microwell was inoculated with 20 μl of conidial suspension to obtain a final concentration of 5×10^3 conidia of *A. F.* The plates were incubated at 23 °C for 72 h, and growth was observed every 24 h. Growth was then evaluated by measuring the absorbance of each well at 620 nm using a microplate photometer. The experiment was repeated three times.

3.2.3. Statistical analysis. Data are expressed as mean ± SE. Differences between control and treated tubes were tested using one-way ANOVA followed by multiple comparisons by Duncan's multiple rang test. A probability value less than 0.05 was considered statistically significant.

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