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A Convenient Synthesis and Biological Activity of Novel Thieno[2,3-*c*]Pyrazole Compounds as Antimicrobial and Anti-Inflammatory Agents[1]

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Abstract—A new method for synthesizing 4-amino-3-methyl-1-phenyl-1*H*-5-substituted thieno[2,3-*c*]pyrazole was reported. The substituted groups at position 5 include carbonitrile, carboxamide, *N*-phenyl carboxamide, and benzoyl groups. The newly synthesized compounds and their derivatives were characterized by elemental analysis and spectroscopy (IR, ^{1}H NMR, and mass spectra). Furthermore, some of these synthesized compounds were screened against various pathogenic bacterial and fungal strains. The results demonstrate that most of the synthesized compounds possess a significant antibacterial activity against gram-positive and gram-negative bacteria. In addition, most of these compounds showed a remarkable anti-fungal activity. On the other hand, some of the synthesized compounds possess high anti-inflammatory activity, which was demonstrated using the carrageenan-induced rat paw edema assay.

Keywords: synthesis, reactions, thienopyrazole, thienopyrazolopyrimidine, thienopyrazoloimidazolopyrimidine

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INTRODUCTION

Pyrazole derivatives display a broad spectrum of biological activities, such as anti-inflammatory [1–5], antimicrobial [5–8], antioxidant [6], anticancer [9–11], fungicidal [12], and antiviral activities [9–11, 13, 14]. Some pyrazole derivatives were reported to possess high affinity and selectivity towards A2b adenosine receptor antagonists [15]. Particularly, aryl pyrazoles are important in medicinal and pesticidal chemistry [16]. Thieno[2,3-*c*]pyrazoles **A** are an important class of potent kinase inhibitors [17].

Literature survey on thienopyrazole revealed that most of papers focus on synthesis of the theinopyrazole substituted at position 5 similar to structure **B**. Few reports deal with *o*-bifunctionalized thienopyrazole similar to structure **A** [18–33].

In continuation of our previous work in the synthesis of heterocyclic compounds containing pyrazole moiety [34–49] we report a novel facile method of synthesis of thieno[2,3-*c*]pyrazole substituted at position 4 and 5 similar to structure **A**.

The difficulty of synthesis of such structures is caused by the fact that most syntheses are based on a starting molecule in which mercapto group is adjacent to cyano group. In the case of pyrazole, the chlorine atom is very difficult to displace with sulfur atom. We tried to achieve this using thiourea [36, 50–52] as it has been reported in other molecules but all attempts failed.

After several attempts, we displaced chlorine with sulfur in the presence of sodium borohydride and used the product in situ, without isolation, for the next reaction.

RESULTS AND DISCUSSION

5-Methyl-2-phenyl-2,4-dihydropyrazole-3-one (**I**) treated with Vilsmeier's reagent afforded 5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-carbaldehyde (**II**). When aldehyde (**II**) was allowed to react with hydroxyl amine hydrochloride in ethanol in the presence of sodium acetate, the corresponding 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde oxime (**III**) was obtained. The pyrazole aldehyde oxime (**III**) was dehydrated using acetic anhydride into the corresponding 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile derivative (**IV**).

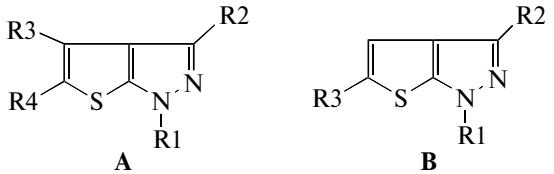

Fig. 1. The substituent positions in thieno[2,3-*c*]pyrazole.

[1] The article is published in the original.

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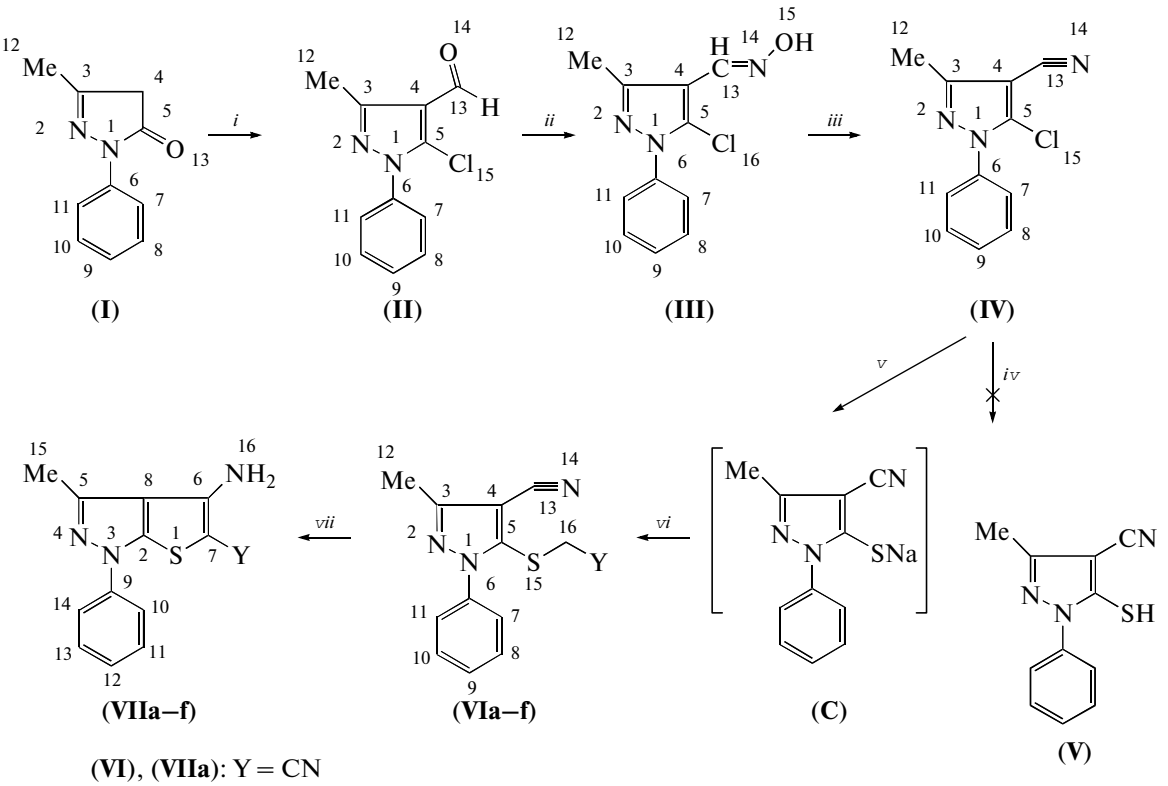


(VI), **(VIIa)**: Y = CN
(VI), **(VIIb)**: Y = $CONH_2$
(VI), **(VIIc)**: Y = CONHPh
(VI), **(VIId)**: Y = CONHPhMe-*p*
(VI), **(VIIe)**: Y = CONHPhOMe-*p*
(VI), **(VIIf)**: Y = CONHPhCl-*p*

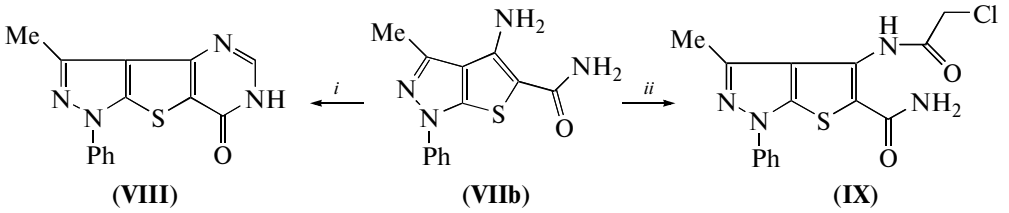
Regents: *i* = $POCl_3$/DMF; *ii* = $H_2NOH.HCl$/AcONa/EtOH; *iii* = Ac_2O; *iv* = H_2NCSNH_2/EtOH; *v* = S_8/$NaBH_4$/EtOH; *vi* = YCH_2X/EtOH/AcONa; *vii* = EtOH/EtONa

Scheme 1. Synthesis of 4-amino-3-methyl-1-phenyl-5-substituted 1*H*-thieno[2,3-*c*]pyrazoles (**VIIa–f**).

The attempt to synthesize thieno[2,3-*c*]pyrazole through converting chloropyrazole carbonitrile (**IV**) into mercaptopyrazole (**V**) using thiourea in ethanol, as with other moieties, followed by reacting with α-halogenated compounds, failed. This forced us to search for another method to synthesize thienopyrazole (**VII**). The desired result was achieved by the reaction of elemental sulfur with chloropyrazole in the presence of sodium borohydride through reduction of sulfur in ethanol to afford not isolated intermediate sodium salt **C**, which was used in situ in the next reaction with α-halogenated compound to afford *S*-alkylated mercaptopyrazole carbonitrile (**VIa–f**). Compounds (**VIa–f**) underwent Thorpe-Ziegler cyclization upon heating in ethanolic sodium ethoxide solution to afford thienopyrazole (**VIIa–f**). Conversion of (**VIb**) to (**VIIb**) was proven by spectral analysis, ^1H NMR revealed the disappearance of the signal at 3.30, characteristic for $-CH_2-$ group in compound (**VI**), and appearance of the signal at 6.90, characteristic for NH_2. Also, the IR of (**VIIb**) showed disappearance of the band characteristic for CN group at 2220 cm^{-1} in compound (**VIb**) and appearance of bands characteristic for NH_2 group. Mass spectrum of compound (**VIb**) showed molecular ion peak at 272.14 and showed the base peak at 255.77, which means that the molecular ion lost one molecule of ammonia to give the base peak.

When aminocarboxamide compound (**VIIb**) was allowed to react with triethyl orthoformate in presence of catalytic amount of acetic acid, 3-methyl-1-pyrazolothienopyrimidinone (**VIII**) was obtained.

On the other hand, the reaction of (**VIIb**) with chloroacetyl chloride in dioxane followed by neutralization with sodium carbonate solution afforded the chloroacetyl amino compound (**IX**).



Reagents: *i* = $HC(OEt)_3$/AcOH, *ii* = $ClCH_2COCl$/dioxane

Scheme 2. Reaction of aminothieno[2,3-*c*]pyrazole carboxamide (**VIIb**) with triethylorthoformate and chloroacetyl chloride.

Antimicrobial Activity

Some of the synthesized compounds were chosen and screened in vitro for their antimicrobial activity against some strains of bacteria and fungi. Antifungal and antibacterial activities of tested compounds were evaluated by the reported method using 0.005% (50 μg/mL) concentration of selected compounds in DMSO as a solvent. The inhibition zone (mm) for the antifungal activity was compared with clotrimazole as a reference. In the case of antibacterial activity, concentration of tested compounds was 2% and the inhibition zone in mm was compared with a series of antibiotics according to the sensitivity of each type of bacteria to the most effective antibiotic for it as a reference.

Some tested compounds showed high antibacterial activity (Table 1). Aminocarbonitrile compound (**VIIa**) show low antibacterial activity compared with other tested compounds. We found that aminocarboxamide compound (**VIIb**) showed high antibacterial activity against some strains of gram-negative bacteria, such as *Esherichia coli* and *Klebsiella pneumoniae*, with values almost the same as those of the corresponding reference antibiotics (nitrofuratoin and imipenem, respectively). Also, compound (**VIIb**) showed moderate activity against most of the tested strains of gram-positive bacteria and against *Pseudomonas aeruginosa*, which is a gram-negative bacterium. The antibacterial activities of 4-amino-*N*-(4-chlorophenyl)-3-methyl-1-phenyl-1*H*-thieno[2,3-*c*]pyrazole-5-carboxamide compound (**VIIe**) and 4-amino-*N*-(4-chlorophenyl)-3-methyl-1-phenyl-1*H*-thieno[2,3-*c*]pyrazole-5-carboxamide compound (**VIIf**) were high compared with other tested compounds, which did not affect any strain. Chloroacetylation of aminocaboxamide (**VIIb**) afforded chloroacetylamino derivative (**IX**) showing high antibacterial activity against gram-positive bacteria, such as *Staphylococcus aureus* and *Clostridium difficile*. Also, compound (**IX**) showed moderate activity against Streptococcus strains, which are gram-positive bacteria, whereas it showed low antibacterial activities against all tested strains of gram-negative bacteria.

On the other hand, compounds (**VIIe**) and (**VIIf**) showed high antifungal activity against *Candida albicans*, *Trichophyton rubrum*, and *Aspergillus flavus*, while compound (**VIIb**) showed the highest antifungal activity against *Trichophyton rubrum* (Table 2). However, compounds (**VIIa**), (**VIIb**), and (**VIIe**) showed moderate activity against some species of fungi, while compound (**IX**) did not affect any of fungi species tested. Compound (**VIIa**) was inactive with respect to most of the tested species of fungi.

Anti-Inflammatory Activity

The results of anti-inflammatory activity assessment for some of the synthesized compounds are presented in Figs. 2 and 3.

Table 1. Antibacterial activity (inhibition zone, mm)

Compound / Bacteria strain	(VIIa)	(VIIb)	(VIIe)	(VIIf)	(IX)	Reference antibacterial agent	Ref	
							value	sensitivity
Staphylococcus aureus (+)	R (8)	I (14)	S (15)	S(17)	S (16)	Levofoxacin	S	23
Streptococcus pneumoniae (+)	R (7)	I (13)	S(16)	S (16)	I (13)	Ciprofloxacin	S	28
Clostridium difficile (+)	R (8)	I (14)	S(17)	S (15)	S (21)	Ofloxacin	S	22
Esherichia coli (−)	R (7)	S (21)	S (20)	S (20)	I (10)	Nitrofuratoin	S	21
Klebsiella pneumoniae (−)	R (7)	S (22)	S (22)	S (18)	I (9)	Imipenem	S	23
Pseudomonas aeruginosa (−)	R (8)	I (14)	S (19)	S (20)	I (9)	Clindamycin	S	20

The amount added in each pore, 50 mL. S means sensitive, I means intermediate sensitivity, and R means resistant. (+) indicates gram-positive bacteria and (−), gram-negative ones.

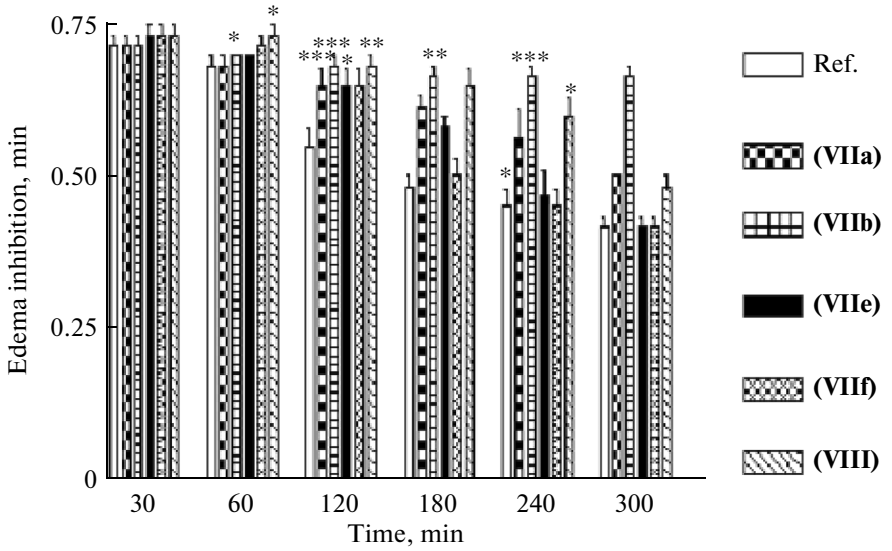


Fig. 2. Paw edema inhibition by compounds (**VIIa**), (**VIIb**), (**VIIe**), (**VIIf**), (**VIII**), and the reference compound, indomethacin.

The inflammatory response is represented as the percentage of the increase in paw swelling upon the anti-inflammatory effect of tested compounds (Fig. 2) and as the percentage of paw edema inhibition (Fig. 3) calculated for each group at each time point using the following ratio:

$$(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{treated}} \times 100/(V_t - V_0)_{\text{control}},$$

where V_t is the average volume of paw swelling at a given time point and V_0 is the average volume just after carrageenan injection.

From the previous results we found that some of the tested compounds showed high anti-inflammatory activity compared with indomethacin. For example, a significant difference exists between indomethacin and aminocarboxamide (**VIIb**) with the P value less than 0.05, which means that compound (**VIIb**) did not affect the inflammation in rats after 1 h of treatment. After 2 h, most compounds showed significant differences from indomethacin. Effect of compound (**VIIf**) was the closest to that of indomethacin. After 3 h, aminomethoxyphenyl carboxamide (**VIIe**) and aminochlorophenyl carboxamide (**VIIf**) show no significant difference from indomethacin, while the effect of amino carboxamide compound (**VIIb**) was significantly different ($P < 0.01$). After 5 h, aminomethoxyphenyl carboxamide (**VIIe**) and aminochlorophenyl carboxamide (**VIIf**) persisted in showing no significant difference from indomethacin, which means they are potent anti-inflammatory agents.

In this work we synthesized bifunctional thieno[2,3-*c*]pyrazole compounds by incorporating sulfur metal instead of chlorine in 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile compound (**IV**) by using sulfur metal in the presence of sodium borohydride followed by Thorpe–Ziegler cyclization. The novel synthesized compounds showed high antimicrobial and anti-inflammatory activities.

EXPERIMENTAL

All melting points are corrected and measured on a Fisher-John apparatus. IR spectra (KBr, ν, cm^{-1}) were recorded with a Perkin-Elmer 1430 Spectrophotometer. ^1H NMR and ^{13}C NMR spectra (δ, ppm; J, Hz) were obtained on a Varian EM-390 MHz (90 MHz) and Joel (400 MHz) spectrometers in $CDCl_3$, DMSO-d_6,

Table 2. Antifungal activity (inhibition zone, mm)

Compound / Fungal strains	(VIIa)	(VIIb)	(VIIe)	(VIIf)	(IX)	Ref	
						value	sensitivity
Candida albicans	I (10)	I (14)	I (14)	S (16)	R (9)	S	23
Trichophyton rubrum	I (13)	S (25)	S (15)	S (18)	R (9)	S	28
Aspergillus flavus	R (0)	R (0)	I (13)	I (12)	R (8)	S	22
Fusarium oxysporum	I (10)	R (7)	R (8)	R (8)	R (8)	S	21
Scopulariopsis brevicaulis	R (0)	R (0)	R (0)	R (7)	R (0)	S	23
Geotrichum candidum	R (0)	R (8)	R (9)	R (0)	R (0)	S	20

The amount added in each pore, 50 mL. Ref = clotrimazole S means sensitive, I means intermediate sensitivity, and R means resistant.

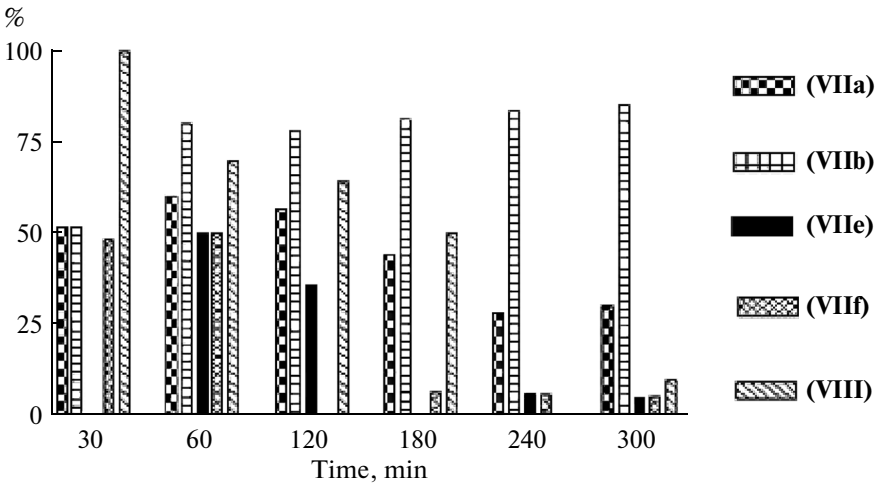


Fig. 3. Paw edema inhibition by compounds (**VIIa**), (**VIIb**), (**VIIe**), (**VIIf**), and (**XIII**), % to the reference compound (indomethacin).

and CF_3CO_2D using Me_4Si as internal standard. Mass spectra were measured on a Joel-JMS 600 spectrometer. Analytical data were obtained on Elemental Analyze system GmbH-VarioEL V.3 microanalyzer in the central lab of Assiut University. Compounds (**I**)–(**IV**) were synthesized according to literature procedure [53].

*3-Methyl-1-Phenyl-5-Substituted Thiopyrazole-4-Carbonitrile (**VIa–f**). General Procedure*

Sodium borohydride (4 g, 0.105 mol) was added to a suspension of finely powered sulfur (4 g, 0.125 mol) in absolute ethanol (60 mL) in an ice bathin small portions till all sulfur powder dissolved, then chlorocyanopyrazole (**IV**) (10 g, 46 mmol) was added to the reaction mixture with stirring in ice bath for 1 h. The reaction mixture was refluxed for 4 h followed by cooling. The α-halogenated alkylating agent (46 mmol) was added to the mixture. The reaction mixture was left overnight with stirring. The solid precipitate which formed on cold was filtered off, dried, and recrystallized from the proper solvent.

5-(Cyanomethylthio)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile (VIa) was obtained by the reaction of chlorocyanopyrazole (**IV**) with chloroacetonitrile as described above. The solid precipitate was recrystallized from ethanol as white crystals. Yield 10 g (85%); mp 78–80°C. IR spectrum: 3035 (CH aromatic), 2985–2925 (CH aliphatic), 2275, 2227 (2CN). ^{1}H NMR spectrum (90 MHz, DMSO-d_6): 2.85 (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 7.60–7.40 (m, 5H, ArH). EI-MS: *m/z* 254.40 [M^+]. Found: C, 61.23; H, 4.20; N, 21.98; S, 12.75%. Calcd.: $C_{13}H_{10}NO_4S$ (254.32): C, 61.40; H, 3.96; N, 22.03; S, 12.61%.

2-(4-Cyano-3-methyl-1-phenyl-1*H*-pyrazol-5-ylthio)-acetamide (VIb) was obtained by the reaction with chloroacetamide as described above. The solid precipitate which formed by stirring was filtered off, dried, and recrystallized from ethanol as white crystals. Yield 10 g (80%); mp 144–146°C. IR spectrum: 3450, 3300 (NH_2), 3050 (CH aromatic), 2920, 2850 (CH aliphatic), 2220 (CN), 1660 (CO amide), 1590 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): 2.35 (s, 3H, CH_3), 3.30 (s, 2H, CH_2), 7.15 (s, 2H, NH_2 disappeared by D_2O), 7.30–7.70 (m, 5H, ArH). EI-MS: *m/z* 272.14 [M^+]. Found: C, 57.26; H, 4.50; N, 20.60; S, 12.00%. Calcd.: $C_{13}H_{12}N_4OS$ (272.33): C, 57.34; H, 4.44; N, 20.57; S, 11.77%.

2-(4-Cyano-3-methyl-1-phenyl-1*H*-pyrazol-5-ylthio)-*N*-phenylacetamide (VIc) was obtained by the reaction with 2-chloro-*N*-phenylacetamide. The solid precipitate formed was filtered off, dried, and recrystallized from ethanol as white crystals. Yield: 11 g (69%); mp 128–130°C. IR spectrum: 3160 (NH), 3057 (CH aromatic), 2920, 2885 (CH aliphatic), 2227 (CN), 1654 (CO). ^{1}H NMR (400 MHz, DMSO-d_6): 2.56 (s, 3H, CH_3), 4.03 (s, 2H, CH_2), 7.08–7.52 (m, 9H, ArH), 10.15(s, H ,NH). Found: C, 65.72; H, 4.55; N, 15.95; S, 9.40%. Calcd.: $C_{19}H_{16}N_4OS$ (348.43): C, 65.50; H, 4.63; N, 16.08; S, 9.20%.

2-(4-Cyano-3-methyl-1-phenyl-1*H*-pyrazol-5-ylthio)-*N*-(*p*-tolyl)-acetamide (VId) was obtained by the reaction with 2-chloro-*N*-(*p*-tolyl)-acetamide. The solid precipitate formed was filtered off, dried, and recrystallized from ethanol as white crystals. Yield: 10.5 g (63%); mp 126–128°C. IR spectrum: 3130 (NH), 3070 (CH aromatic), 2905, 2885 (CH aliphatic), 2220 (CN), 1660 (CO). ^{1}H NMR (400 MHz, DMSO-d_6): 2.31 (s, 3H, CH_3-Ph), 2.56 (s, 3H, CH_3), 4.03 (s, 2H, CH_2), 7.21–7.52 (m, 9H, ArH), 9.21(s, H, NH). Found: C, 66.05; H, 4.95; N, 15.35; S, 8.65%. Calcd.:

$C_{20}H_{18}N_4OS$ (362.46): C, 66.28; H, 5.01; N,15.46; S, 8.85%.

2-(4-Cyano-3-methyl-1-phenyl-1*H*-pyrazol-5-ylthio)-*N*-(*p*-anisyl)-acetamide (VIe) was obtained by the reaction with 2-chloro-*N*-(*p*-anisyl)-acetamide. The solid precipitate formed was filtered off, dried, and recrystallized from ethanol as white crystals. Yield: 13 g (74%); mp 148–150°C. IR spectrum: 3304 (NH), 3035 (CH aromatic), 2976, 2870 (CH aliphatic), 2229 (CN), 1655 (CO). ^{1}H NMR (400 MHz, DMSO-d_6): 2.52 (s, 3H, CH_3), 3.70 (s, 2H, CH_2), 7.30–8.00 (m, 9H, ArH), 9.15 (s, 1H, NH). Found: C, 63.55; H, 4.70; N, 14.77; S, 8.32%. Calcd.: $C_{20}H_{18}N_4OS$ (378.46): C, 63.47; H, 4.79; N,14.80; S, 8.47%.

2-(4-Cyano-3-methyl-1-phenyl-1*H*-pyrazol-5-ylthio)-*N*-(*p*-chlorophenyl)-acetamide (VIf) was obtained by the reaction with 2-chloro-*N*-(4-chlorophenyl)-acetamide. The solid precipitate formed was filtered off, dried, and recrystallized from ethanol as white crystals. Yield: 13 g (74%); mp 132–134°C. IR spectrum: 3304 (NH), 3035 (CH aromatic), 2976, 2870 (CH aliphatic), 2229 (CN), 1655 (CO). ^{1}H NMR (400 MHz, DMSO-d_6): 2.32 (s, 3H, CH_3), 3.70 (s, 2H, CH_2), 7.33–7.99 (m, 9H, ArH), 10.28 (s, 1H, NH). Found: C, 59.83; H, 3.90; Cl, 9.20; N, 14.70; S, 8.50%. Calcd.: $C_{19}H_{15}ClN_4OS$ (382.87): C, 59.60; H, 3.95; Cl,9.26; N,14.63; S, 8.37%.

*4-Amino-3-Methyl-5-Substituted-1-Phenyl-1H-Thieno[2,3-c]-5-Carbonitrilepyrazole (**VIIa–f**). General Procedure*

A mixture of a substituted pyrazole carbonitrile compound (**VIa–f**) (4 g, 0.016 mol) in absolute ethanol (20 mL) and sodium ethoxide solution (2.5 mL) was gently refluxed for 10 min. The solid precipitate which formed on hot was filtered off, dried, and recrystallized from the mixture of ethanol–dioxane, 2 : 1.

4-Amino-3-methyl-1-phenyl-1*H*-thieno[2,3-*c*]pyrazole-5-carbonitrile (VIIa) was obtained from compound (**VIa**) as white crystals in 75% yield. mp 198–200°C; IR spectrum: 3456, 3359, 3200 (NH_2), 3045 (CH aromatic), 2950, 2890 (CH aliphatic), 2184 (CN); ^{1}H NMR (400 MHz, DMSO-d_6): 3.37 (s, 3H, CH_3), 6.93 (s, 2H, NH_2), 7.26–7.56 (m, 5H, ArH). EI-MS: *m/z* 254.33 [M^+]. Found: C, 61.50; H, 4.03; N, 21.99; S, 12.65%. Calcd.: $C_{13}H_{10}N_4S$ (254.32): C, 61.40; H, 3.96; N, 22.03; S, 12.61%.

4-Amino-3-methyl-1-phenyl-1*H*-thieno[2,3-c]pyrazole-5-carboxamide(VIIb) was obtained from compound (**VIb**) as white crystals in 70% yield. mp 214–216°C; IR spectrum: 3400, 3305, 3190 ($2NH_2$), 3050 (CH aromatic), 2910 (CH aliphatic), 1635 (CO amide), 1580 (C=N); ^{1}H NMR (90 MHz, DMSO-d_6): 2.60 (s, 3H, CH_3), 6.90 (s, 2H, NH_2 amide), 7.0 (s, 2H, NH_2), 7.30–7.70 (m, 5H, ArH). EI-MS: *m/z* 271.60 [$M^+ - 1$]. Found: C, 57.44; H, 4.55; N, 20.47; S, 11.65%. Calcd.: $C_{13}H_{12}N_4OS$ (272.33): C, 57.34; H, 4.44; N, 20.57; S, 11.77%.

4-Amino-3-methyl-*N*,1-diphenyl-1*H*-thieno[2,3-*c*]pyrazole-5-carboxamide (VIIc) was obtained from compound (**VIc**) as white crystals in 68% yield. mp 202–204°C; IR spectrum: 3304, 3201 (NH_2), 3135 (NH), 3050 (CH aromatic), 2007, 2887 (CH aliphatic), 1686 (CO); ^{1}H NMR (400 MHz, DMSO-d_6): 2.63 (s, 3H, CH_3), 6.64 (s, 2H, NH_2), 7.10–8.03 (m, 9H, ArH), 8.89 (s, H, NH). Found: C, 65.35; H, 4.55; N, 15.95; S, 9.40%. Calcd.: $C_{19}H_{16}N_4OS$ (348.43): C, 65.50; H, 4.63; N,16.08; S, 9.20%.

4-Amino-3-methyl-1-phenyl-*N*-*p*-tolyl-1*H*-thieno[2,3-*c*]pyrazole-5-carboxamide (VIId) was obtained from compound (**VId**) as white crystals in 70% yield. mp 208–210°C; IR spectrum: 3290, 3215 (NH_2), 3130 (NH), 3070 (CH aromatic), 2905, 2885 (CH aliphatic), 1655 (CO); ^{1}H NMR (400 MHz, DMSO-d_6): 2.30 (s, 3H, CH_3-Ph), 2.63 (s,3H,CH_3), 6.64 (s, 2H, NH_2), 7.30–7.58 (m, 9H, ArH), 8.84 (s, H, NH). EI-MS: *m/z* 361.20 [M^+]. Found: C, 66.07; H, 4.95; N, 15.35; S, 8.65%. Calcd.: $C_{20}H_{18}N_4OS$ (362.46): C, 66.28; H, 5.01; N, 15.46; S, 8.85%.

4-Amino-3-methyl-1-phenyl-*N*-*p*-anisyl-1*H*-thieno[2,3-*c*]pyrazole-5-carboxamide (VIIe) was obtained from compound (**VIe**) as white crystals in 65% yield. mp 208–210°C; IR spectrum: 3429, 3329, 3275 ($2NH_2$, NH), 3030 (CH aromatic), 2860 (CH aliphatic), 1625 (CO); ^{1}H NMR (400 MHz, DMSO-d_6): 2.48 (s, 3H, CH_3), 3.71 (s, 3H, OCH3), 7.15 (s, 2H, NH_2), 7.30–7.70 (m, 9H, ArH), 9.15 (s, H, NH). Found: C, 63.55; H, 4.70; N, 14.73; S; 8.37%. Calcd.: $C_{20}H_{18}N_4O_2S$ (378.46): C, 63.47; H, 4.79; N, 14.80; S, 8.47%.

4-Amino-3-methyl-1-phenyl-*N*-*p*-chlorophenyl-1*H*-thieno[2,3-*c*]pyrazole-5-carboxamide (VIIf) was obtained from compound (**VIf**) as white crystals in 70% yield. mp 204–206°C; IR spectrum: 3429, 3336, 3275 ($2NH_2$, NH), 3050 (CH aromatic), 2880 (CH aliphatic), 1640 (CO); ^{1}H NMR (400 MHz, DMSO-d_6): 2.49 (s, 3H, CH_3), 7.25 (s, 2H, NH_2), 7.31–7.71 (m, 9H, ArH), 9.37 (s, H, NH). EI-MS: *m/z* 381.30 [$M^+ - 1$]. Found: C, 59.71; H, 4.05; Cl, 9.04; N, 14.55; S, 8.32%. Calcd.: $C_{19}H_{15}NClN_4OS$ (382.87): C, 59.60; H, 3.95; Cl, 9.26; N, 14.63; S, 8.37%.

3-Methyl-1-phenylpyrazolo[3',4':5,4]thieno[3,2-*d*]pyrimidin-7(6*H*)-one (VIII). To a refluxed mixture of amino carboxamide (**VIIb**) (2 g, 7 mmol) and triethyl orthoformate (6 mL, 36 mmol), few drops of glacial acetic acid was added. The reaction mixture was heated under reflux for 30 min. The solid precipitate which is formed on hot was filtered off, dried, and recrystal-

ized from dioxane as white needles. Yield: 1.8 g (86%); mp 350–352°C; IR spectrum: 3380 (NH broad), 3050 (CH aromatic), 3000, 2900 (CH aliphatic), 1665 (CO amide), 1590 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): 2.50 (s, 3H, CH_3), 7.30–7.80 (m, 5H, ArH), 8.30 (s, 1H, CH pyrimidine), 10.50 (s, 1H, NH), Found: C, 59.44; H, 3.60; N, 19.60 ; S, 11.00%. Calcd.: $C_{14}H_{10}N_4OS$ (282.33) C; 59.56, H; 3.57, N; 19.84 ,S; 11.36%.

4-(2-Chloro-acetylamino)-3-methyl-1-phenyl-1*H*-thieno[2,3-*c*]pyrazole-5-carboxamide (IX). A solution of compound (**VIIb**) (2 g, 7 mmol) and chloroacetyl chloride (0.8 mL, 0.9 mmol) in dioxane (30 mL) was heated on water bath for 3 hours. The solid product obtained by cooling and pouring on diluted sodium carbonate solution was filtered off, dried, and recrystallized from ethanol into smoke white crystals. Yield: 2.1 g (86%); mp 258–260°C; IR spectrum: 3380, 3300, 3180 (NH, NH_2), 3050 (CH aromatic), 2920, 2850 (CH aliphatic), 1690, 1650 (2C=O amide); ^{1}H NMR (400 MHz, DMSO-d_6)*:* 2.40 (s, 3H, CH_3), 4.50 (s, 2H, CH_2), 7.30–7.80 (m, 7H, ArH + NH_2), 10.50 (s, 1H, NH). Found: C; 51.52, H; 3.90, Cl; 10.00; N, 15.95; S, 9.00%. Calcd.: $C_{15}H_{13}ClN_4O_2S$ (348.81): C; 51.65, H; 3.76; Cl; 10.16 N; 16.06; S, 9.19%.

Procedure of Antimicrobial Activity

The fungal species were previously isolated from cases of human dermatophytosis [54]. The fungi were grown in sterilized 9-cm Perti dishes containing Sabouraud's dextrose agar (SDA) supplemented with 0.05% chloramphenicol to suppress bacterial contamination [55]. From these cultures, agar discs (10 mm diam.) containing spores and hyphae were transferred aseptically to screw-topped vials containing 20 mL sterile distilled water. After thorough shaking, 1 mL samples of the spore suspension were pipetted into sterile Perti dishes, followed by the addition of 15 mL liquefied SDA medium, which was then left to solidify. The tested compounds and tolnaftate were dissolved in DMSO to give 2.0% concentration. Antifungal and antibacterial activities were determined according to the method reported by Bauer et al. [56] using 3-mm diameter filter paper discs (Watmann no. 3) loaded with 10 mL of the solution under investigation (200 mL/disc, 2.0%). The discs were placed on the surface of the fungal cultures, which were incubated at 30°C. The diameter of the inhibition zone around each disc was measured. The method [56] was used for determining antibacterial activity too.

Procedure of Anti-Inflammatory Activity

Anti-inflammatory activity was measured using carrageenan-induced rat paw edema assay [57, 58]. Edema was induced by subplantar injection of 100 μL of 1% freshly prepared solution of carrageenan in distilled water into the right-hind paws of each rat of all the groups. Animals of group A/B/C, were treated with the single dose of tested compound, group D was treated with indomethacin. Paw thickness were measured just before the carrageenan injection, that is, at "0 hour" and then at 30 min, 1, 2, 3, 4, and 5 h after carrageenan injection. Increase in paw thickness was measured as the difference in paw thickness at "0 hour" and paw thickness at relevant time point.

Statistical Analysis

The results were analyzed by one way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison test as a post-Test. These analyses were carried out using computer Prism program for windows, version 3.0 (Graph pad software, Inc., San Diego, CA, US). The significant differences between groups were accepted at $p < 0.05$*, 0.01**, or 0.001***, and the data are expressed as mean ± standard error (SE).

REFERENCES

1. Sauzem, P.D., Machado, P., Rubin, M.A., Sant'Anna, G.D.S., Faber, H.B., de Souza, A.H., Mello, C.F., Beck, P., Burrow, R.A., Bonacorso, H.G., Zanatta, N., and Martins, M.A.P., *Eur. J. Med. Chem.*, 2008, vol. 43, pp. 1237–1247.
2. Amir, M. and Shikha, K., *Eur. J. Med. Chem.,* 2004, vol. 39, pp. 535–545.
3. Palaska, E., Sahin, G., Kelicen, P., Durlu, N.T., and Altinok, G., *Il Farmaco*, 2002, vol. 57, pp. 101–107.
4. Palaska, E., Sahin, G., Kelicen, P., Demirdamar, R., and Altinok, G., *Arzneim.-Forsch./Drug Res.*, 2001, vol. 51, 478–484 (Chem. Abstr., 2001, vol. 135, p. 326954).
5. Bekhit, A.A., Ashour, H.M.A., Abdel Ghany, Y.S., Bekhit, A.A., and Baraka, A., *Eur. J. Med. Chem.*, 2008, vol. 43, pp. 456–463.
6. Padmaja, A., Payani, T., Dinneswara, R.G., and Padmavathi, V., *Eur. J. Med. Chem.*, 2009, vol. 44, pp. 4557–4566.
7. Ragavan, R.V., Vijayakumar, V., and Kumari, N.S., *Eur. J. Med. Chem.*, 2010, vol. 45, pp 1173–1180.
8. El-Sayed, W.A., Flefel, E.M., and Morsy, E.M.H., *Der Pharma Chemica*, 2012, vol. 4, pp. 23–32.
9. Riyadh, S.M., Farghaly, T.A., Abdallah, M. A., Abdalla, M.M., and Abd El-Aziz, M.R., *Eur. J. Med. Chem.*, 2010, vol. 45, pp. 1042–1050.
10. El-borai, M.A., Rizk, H.F., Abd-Aal, M.F., and El-Deeb, I.Y., *Eur. J. Med. Chem.*, 2012, vol. 48, pp. 92–96.
11. Vujasinovic, I., Paravic-Radicevic, A., Mlinaric-Majerski, K., Brajsa, K., and Bertosa, B., *Bioorg. Med. Chem.*, 2012, vol.20, pp. 2101–2110.
12. Yang, X.-D., *J. Chem. Res.*, 2008, pp.489–491.
13. Rashad, A.E., Hegab, M.I., Abdel-Megeid, R.E., Fathalla, N., and Abdel-Megeid, F.M.E., *Eur. J. Med. Chem.*, 2009, vol. 44, pp. 3285–3292.

14. Genin, M.J., Biles, C., Keiser, B.J., Poppe, S.M., Swaney, S.M., Tarplay, W.G., Yagi, Y., and Romero, D.L., *J. Med. Chem.*, 2000, vol. 43, pp. 1034–1040.
15. Elzein, E., Kalla, R., Li, X., Perry, T., Parkhill, E., Palle, V., Varkhedkar, V., Gimbel, A., Zeng, D., Lustig, D., Leung, D., and Zablocki, J., *Bioorg. Med. Chem. Lett.*, 2006, vol. 16, pp. 302–306.
16. John, W.L., Patera, R.M., Plummer, M.J., Halling, B.P., and Yuhas, D.A., *Pest. Sci.*, 1994, vol. 42, pp. 29–36.
17. Chandanshive, J.Z., Bonini B.F., Gentili, D. Fochi, M., Bernardi, L., and Franchini, M.C., *Eur. J. Org. Chem.*, 2010, vol. 33, pp. 6440–6447.
18. Zanze, I.A., Darczak, D., Sarris, K., Phelan, K.M., Huth, J.R., Song, D., Johnson, E.F., Jia, Y., and Djuric, S.W., *Bioorg. Med. Chem. Lett.*, 2006, vol. 16, pp. 96–99.
19. McLean, L.R., Zhang, Y., Zaidi, N., Bi, X., Wang, R., Dharanipragada, R., Jurcak, J. G., Gillespy, T.A., Zhao, Z., Musick, K.Y., Choi, Y.M., Barrague, M., Peppard, J., Smicker, M., Duguid, M., Parkar, A., Fordham, J., and Kominos, D., *Bioorg. Med. Chem. Lett.*, 2012, vol. 22, pp. 3296–3300.
20. Kvitko, I.Ya., *Chem. Heterocycl. Compd.,* 1969, vol. 5, pp. 567–567.
21. Elgemeie, G.H., Zaghary, W.A., Nasr, T.M., and Amin, K.M., *J. Carbohydr. Chem.*, 2008, vol. 27, pp. 345–356.
22. Zanze, I.A. and Hajduk, P.J., *Drug Discovery Today,* 2009, vol. 14, pp. 291–297.
23. Jurcak, J.G., Barrague, M., Gillespy, T.A., Edwards, M.L., Musick, K.Y., Weintraub, P.M., Du, Y., Dharanipragada, R.M., and Parkar, A.A., European Patent 1682553B1, 2005.
24. Hidekazu, I., Hidenobu, M., and Yasuhiro, H., European Patent 2433943A1, 2006.
25. Aggarwal, R., Kumar, V., Kumar, R., and Singh, S.P., *Beilstein J. Org. Chem.*, 2011, vol. 7, pp. 179–197.
26. El-Saraf, G.A., El-Sayed, A.M., and El-Saghier, A.M.M., *Heteroatom. Chemistry*, 2003, vol. 14, pp. 211–217.
27. Fancellie, D., Moll, J.,, Pulici, M., Quartieri, F., and Bandiera, T., US Patent 7943654 B2, 2011.
28. Elgemeie, G.H., Amin, K.M., El-Badry, O.M., Hassan, G.S., Farag, A.B., Velazequez, C., and El-Kadi, A.O., *Am. J. Sci.*, 2012, vol. 8, pp. 1071–1076.
29. Dodiya, D.K., Trivedl, A.R., Jarsania, S.H., Vaghasia, Sh.J., and Shah, V.H., *J. Serb. Chem. Soc.*, 2008, vol. 7, pp. 683–690.
30. Wang, Z.W., Ren, J., Chen, H.S., and Li, Z.M., *Chinese Chemical Lett.*, 1999, vol. 10 (3), pp. 189–190.
31. Eller, G.A., Vilkauskaite, G., Iauskiene, E. A., Kus, A.S., and Holzer, W., *Synth. Commun.*, 2011, vol. 41, pp. 541–547.
32. Bindi, S., Fancelli, D., Alli, C., Berta, D., Bertrand, J.A., Cameron, A.D., Cappella, P., Carpinelli, P., Cervi, G., Croci, V., D'Anello, M., Forte, B., Giorgini, L. M., Marsiglio A., Moll, J., Pesenti, E., Pittalà V., Pulici, M., Sirtori, F. R., Roletto, F., Soncini, Ch., Storici, P., Varasi, M., Volpi, D., Zugnoni, P., and Vianello, P., *Bioorg. Med. Chem.*, 2010, vol. 18, pp. 7113–7120.
33. Swords, R., Mahalingama, D., O'Dwyer, M., Santocanale, C., Kelly, K., Carew, J., and Giles, F., *Eur. J. Cancer*, 2010, vol. 46, pp. 33–40.
34. El-Emary, T.I., Kamal El-Dean, A.M., and El-Kashef, H.S., *Il Farmaco*, 1998, vol. 53, no. 6, pp. 383–388.
35. Kamal El-Dean, A.M. and Radwan, S.M., *Die Pharmazie*, 1998, vol. 53, no. 12, pp. 839–843.
36. Ibrahim, S.A., Hammam, A.M., Kamal El-Dean, A.M., Mohamed, A.A., and Rageh, N.M., *Can. J. Appl. Spectrosc.*, 1993, vol.38. 1, pp. 1–6.
37. Kamal El-Dean, A.M., Mohamed, A A., Geies, A.A., and Atalla, A.A., *Bull. Fac. Sci. Assiut Univ.*, 1991, vol. 20, pp. 15–21.
38. Kamal El-Dean, A. M., Radwan, Sh. M., and Zaki, R.M., *Eur. J. Med. Chem.*, 2011, vol. 46, no. 2, pp. 567–578.
39. Kamal, El-Dean, A.M., and Abdel-Moneam, M.E., *Phosphorus Sulfur Silicon Relat. Elem.*, 2002, vol. 177, no. 12, pp. 2745–2751.
40. Geies, A. A., Kamal El-Dean, A.M., and Abdel Monem, M.I., *Zeitschrift fur Naturforschung B*, 1992, vol. 47, no. 10, pp. 1438–1440.
41. Kamal El-Dean, A.M., Geies, A.A., and Mohamed, T.A., *Ind. J. Chem. Sect. B*, 1991, vol. 30, no. 9, pp. 878–882.
42. Kamal El-Dean, A.M., and El-Kashef, H.S., *Die Pharmazie*, 1996, vol. 51, no. 3, pp. 155–158.
43. El-khawaga, A.M., Kamal El-Dean, A.M., Radwan, Sh.M., and Ahmed, M.M., *Bull. Korean Chem. Soc.*, 2009, vol. 30, no. 3, pp. 561–566.
44. Kamal El-Dean, A.M., El-khawaga, A.M., Radwan, Sh.M., and Ahmed, M. M., *Phosphorus Sulfur Silicon Relat. Elem.*, 2009, vol.184, no. 8, pp. 2034–2048.
45. Kamal El-Dean, A.M., and Geies A.A., *J. Chem. Res., Synop.*, 1997, pp. 352–353.
46. Kamal El-Dean, A.M., Geies, A.A., and Moustafa, O.S., *Afinidad*, 1998, vol. 55, no. 476, pp. 295–298.
47. Kamal El-Dean, A.M., Atalla, A.A., and Gaber, A.M., *Bull. Fac. Sci., Assiut University*, 1990, vol. 19, no. 1b, pp. 23–33.
48. Kamal El-Dean, A.M., Zaki, R.M., Geies, A.A., Radwan, Sh.M., and Tolba, M.S., *Russ. J. Bioorg. Chem.*, 2013, vol. 39, no. 5, pp. 553–564.
49. Kamal El-Dean, A.M., Atalla, A.A., Mohamed, T.A., and Geies, A.A., *Z. Naturforsch., B: Chem. Sci.*, 1991, vol. 46, no. 4, pp. 541–546.
50. Kamal El-Dean, A.M., Radwan, Sh.M., and Zaki, R.M., *J. Chin. Chem. Soc.*, 2008, vol. 55, no. 6, pp. 1–10.
51. Badr, M.Z.A., Kamal El-Dean, A.M., Moustafa, O.S., and Zaki, R.M., *J. Chem. Res.*, 2006, vol. 11, pp. 748–752.
52. Geies, A.A., Kamal El-Dean, A.M., and Moustafa, O.S., *Monatshefte fur Chemie,* 1996, vol. 127, pp. 1263–1272.
53. Haider, N., Farghaly, A., Al-Mekhlafi, N., and El-Kashef, H., *J. Chem. Res.*, 2005, vol. 2005, no. 12, pp. 761–765.
54. Moubasher, A.H., El-Naghy, M.A., Maghazy, S.M., and El-Gendy, Z., *Korean J. Mycol.*, 1993, vol. 21, pp. 77–84.
55. Al-Doory, Y., *Laboratory Medical Mycology*, Philadelphia: Lea and Febiger, 1980.
56. Bauer, A.W., Kibry, M.M., and Truck, J.C.M., *Am. J. Clin. Pathol.,* 1966, vol.45, pp. 493–496.
57. Winter, C.A., Risley, E.A., and Nuss, G.W., *Proc. Soc. Exper. Biol. Med.*, 1962, vol. 111, pp. 544–547.
58. Adeyemi, O.O., Okpo, S.O., and Ogunti, O.O., *Fitoterapia*, 2002, vol. 73, no. 5, pp. 375–380.