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Full Paper

Synthesis and antimicrobial activity of some new pyrazolo[3,4-*b*] pyrazines and related heterocycles

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Abstract

3-Methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carboxylic acid hydrazide (**5**) could be obtained in a four-step reaction starting from 6-amino-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyrazine-4-carbonitrile **1**. The latter compound was used as a key intermediate for the synthesis of several new pyrazolo[3,4-*b*]pyrazine derivatives. The structure of all the new compounds was established on the basis of their analytical and spectral analyses. The antibacterial and antifungal activities of nineteen of the synthesized compounds were evaluated against some various strains of bacteria and fungi, using streptomycin and clotrimazole as reference drugs respectively. Certain derivatives showed promising results.

Keywords: Pyrazolo[3,4-*b*]pyrazine, N-arylidene carbohydrazides, 1,3,4-oxadiazoles, 1,2,4-triazoles, 1,3,4-thiadiazoles, Antimicrobial Activity.

1. Introduction

Pyrazolo[3,4-*b*]pyrazine ring system is an interesting class of heterocycles of medicinal importance. It has been reported that some of its derivatives are used as inhibitors of protein kinases [1], blood platelet aggregation [2], bone metabolism improvers [3], anti-inflammatories [2], antifungal [4,5], antibacterial [4,6], antiparasitic [5] and anticancer agents [7,8]. On the other hand, pyrazolo[3,4-*b*]pyrazines are also used as fluorescent [9] and disperse dyes in dye chemistry [10]. Certain derivatives were reported to possess antiviral, antineoplastic and anti-fungal activities [11–13]. Keeping the above facts in mind and as a continuation of our program dealing with the development of bioactive fused pyrazines [5,13,14–18] and pyrazole-based heterocycles [19], the present work was planned to synthesize new pyrazolo[3,4-*b*]pyrazines and related heterocycles with the aim that some of them may show promising biological activities.

2. Results and Discussion

2.1. Chemistry

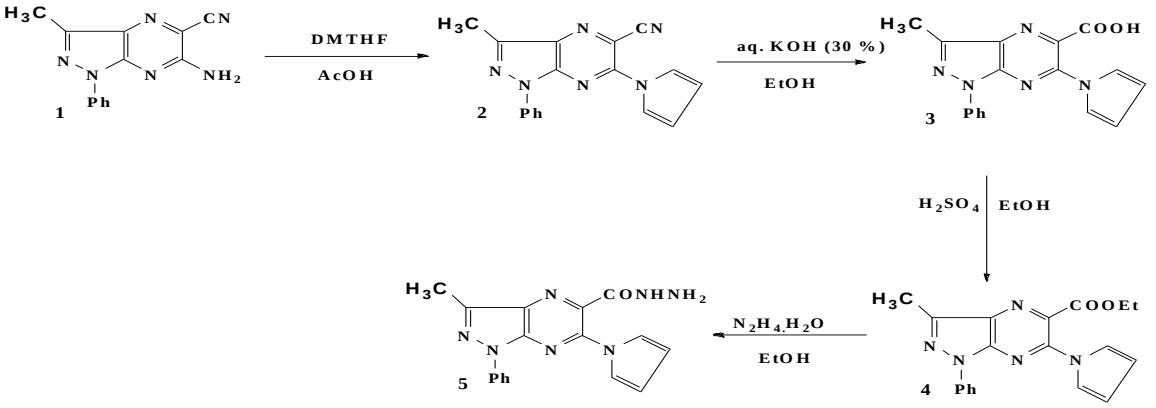
In a previous work, we have reported the synthesis of 6-amino-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbonitrile (**1**) *via* the interaction of the 5-amino-4-nitroso-2-phenyl-2*H*-pyrazol-3-ylamine with malononitrile in boiling pyridine [5]. This compound **1** when treated with 2,5-dimethoxytetrahydrofuran in boiling glacial acetic acid gave the corresponding 3-methyl-1-phenyl-6-pyrrol-1-yl-pyrazolo[3,4-*b*] pyrazine-5-carbonitrile **2.** The alkaline hydrolysis of **2** gave the carboxylic acid **3** which was esterified by refluxing in absolute ethanol in the presence of few drops of concentrated sulfuric acid to give the corresponding ethyl ester **4.** The latter compound, was readily converted into the corresponding acid hydrazide **5** (Scheme 1) upon treatment with hydrazine hydrate in refluxing ethanol. Compound **5** proved to be a versatile key intermediate for the synthesis of several pyrazolo[3,4-*b*]pyrazines. Thus, treatment of **5** with some aromatic aldehydes, or ketones, gave the corresponding hydrazones **6a-c, 7,** and **8a-c** (Scheme 2). Also the interaction **5** with some active methylene compounds such as ethyl acetoacetate and acetylacetone, gave 5-methyl-2-(3-methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbonyl)-2,4-dihydro-pyrazol-3-one and (3,5-dimethyl-pyrazol-1-yl)-(3-methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]pyrazin-5-yl)-methanone derivatives **9** and **10**

respectively (Scheme 2). On the other hand, compounds containing 1,3,4-oxadizole ring are known to exhibit antimicrobial [20, 21], analgesic [22] and tublin inhibitor [23] properties. Accordingly, it would be advantageious to incorporate such a hetrocyclic ring in our pyrazolopyrazine ring system. Thus, cyclization of carbohydrazide **5** with CS_2 in the presence of dry pyridine afforded the 5-(3-methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-yl)-[1,3,4]oxadiazole-2-thiol (**11**), which was easily S-alkylated with methyl iodide and/ or phenacyl bromide in ethanol in the presence of anhydrous sodium acetate to give the thioethers **12a,b** respectively (Scheme 3). Also, the reaction of **5** with substituted benzoic acid in boiling $POCl_3$, yielded 5-(2-aryl-1,3,4-oxadiazol-5-yl)-3-methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]-pyrazine **(13a,b).** On the other hand, the pyrazolopyrazinyl thiosemicarbazide **14** was easily obtained by the reaction of the carbohydrazide **5** with phenyl isothiocyanate in boiling ethanol. Compound **14** underwent two different cyclization reactions depending on the reaction conditions. Thus, heating of **14** in an aqueous NaOH solution, yielded, 5-(3-Methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-yl)-4-phenyl-*4H*-[1,2,4]triazole-3-thiol **(15)** . Whereas, treatment of **14** with concentrated H_2SO_4 at room temperature, led to the formation of 2-phenylamino-1,3,4-thiadiazole derivative **16** (Scheme 3)

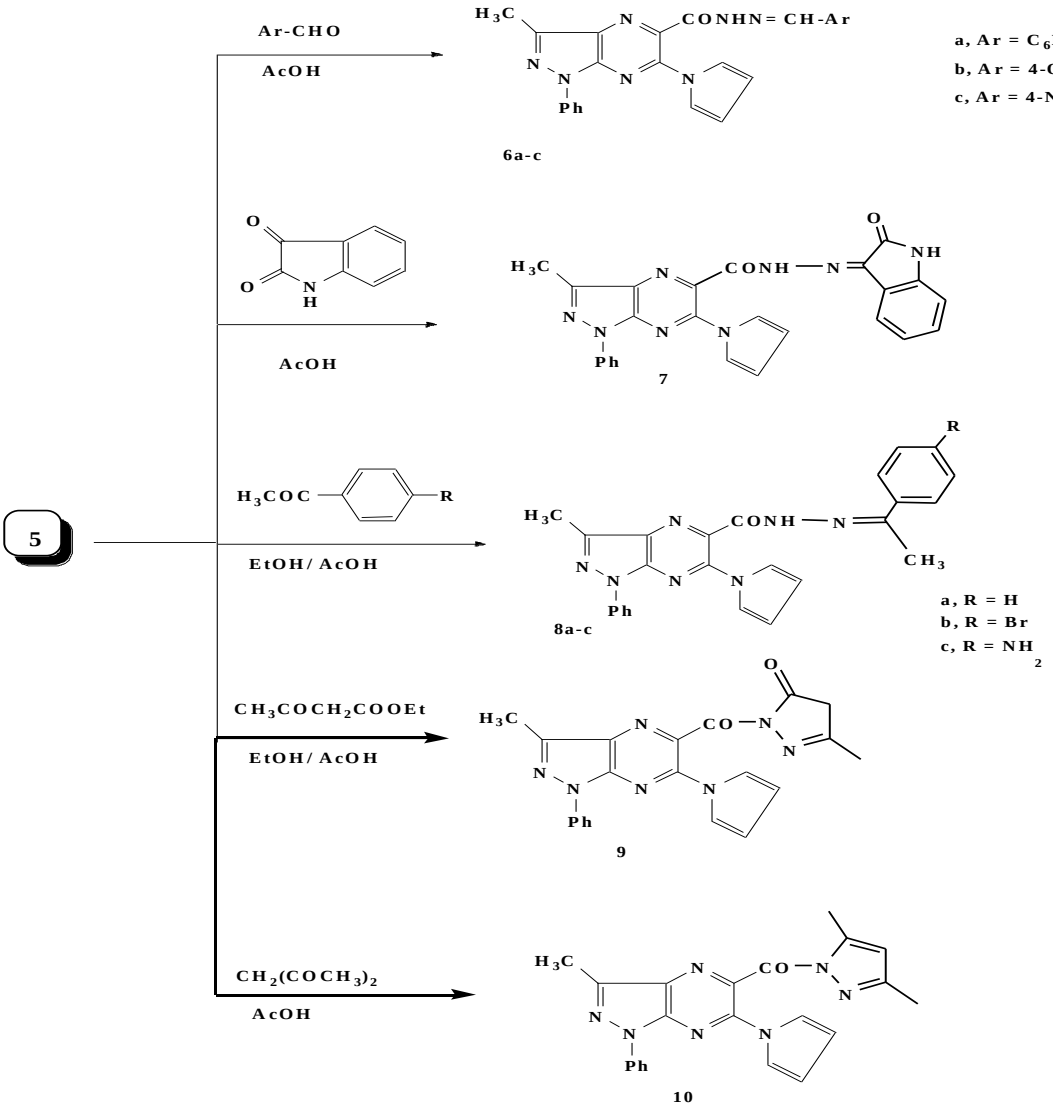
2.2. Antimicrobial Activity

Using agar well-diffusion method [24], all the synthesized compounds **2–16** were screened against two Gram-positive bacteria; *Staphylococcus aureus* (AUMC B.54) and *Bacillus cereus* (AUMC B.52) and two Gram-negative bacteria; *Escherichia coli*(AUMC B.53) and *Pseudomonas aeruginosa* (AUMC B.73) and also against two fungal strains: *Candida albicans* (AUMC No. 418) and *Aspergillus flavus* (AUMC No. 1276). The results depicted in **Table 1** showed that a good number of the compounds under evaluation exhibited good to excellent antibacterial activity against both Gram +ve and Gram -ve bacteria. Compound **16** showed antibacterial activity against *S. aureus* higher than that of the reference drug streptomycin itself. It showed also very high activity against *B. cereus and P. aeruginosa* and high activity against *E. coli* in comparison with streptomycin. Also, three derivatives showed higher antifungal activity than the reference drug clotrimazole.

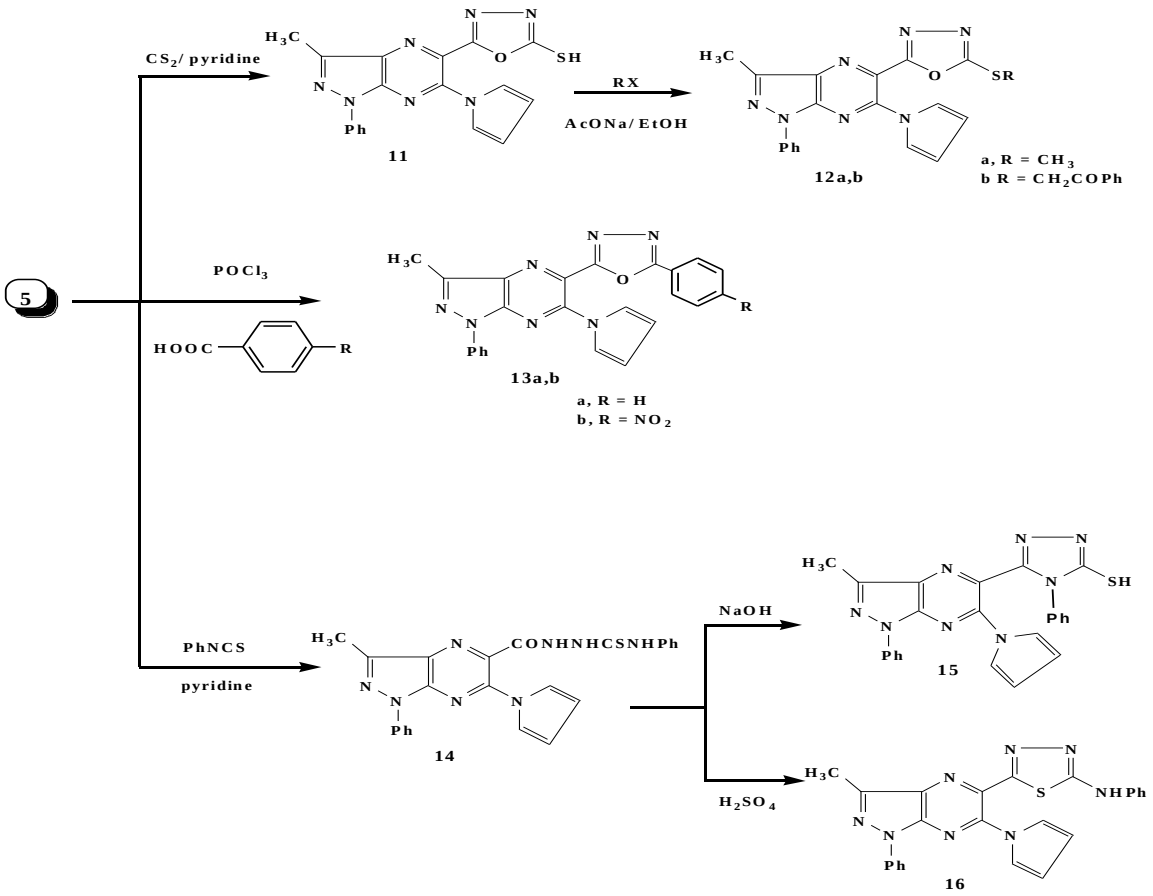
Compound **16** showed high antifungal activity against both strains under test *C. Albicans* and *A. Flavus*, however its activity against the latter fungus was higher than the reference drug clotrimazole. The nitro derivative **6c** , showed higher activity, in comparison with the other two arylidene carbohydrazides **6a,b** against all bacterial strains under test, whether Gram +ve or Gram -ve, but showed no antifungal activity. It is of interest to note that, three derivatives showed higher antifungal activity than the reference drug clotrimazole. Compound **8b** displayed higher activity against *A. flavus* than the that of clotrimazole. Also it displayed activity against Gram +ve bacteria but no activity against Gram -ve ones**.** Similarly, **13b** showed high activity against Gram +ve bacteria and no activity against Gram -ve bacteria meanwhile, it exhibited an antifungal activity against *C. albicans* higher than that of clotrimazole itself.



Scheme 1


5
Ar-CHO
AcOH
6a-c
a, Ar = C6H5
b, Ar = 4-OMe-C6H4
c, Ar = 4-NO2-C6H4
AcOH
7
H3COC
R
EtOH/AcOH
8a-c
a, R = H
b, R = Br
c, R = NH2
CH3COCH2COOEt
EtOH/AcOH
9
CH2(COCH3)2
AcOH
10

Scheme 2



Scheme 3

Compound	Diameter of zone of inhibition (mm) / % inhibition with reference to standard					
	Gram-positive bacteria		Gram- nagative bacteria		Fungi	
	S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. flavus*
2	-	9.5(32)	-	-	-	-
3	11.3(37)	-	-	-	-	12.3(48)
4	15.6(51)	-	-	-	-	-
5	10.9(36)	-	-	-	-	10.6(41)
6a	15.3(50)	-	14.9(56)	-	-	-
6b	13.2(43)	11.3(38)	-	-	-	-
6c	29.6(97)	23.7(80)	17.4(65)	20.3(83)	-	-
7	9.8(32)	-	-	-	13.3(66)	-

8a	-	11.1(37)	-	-	9.7(48)	-
8b	25.2(83)	26.6(90)	-	-	-	26.2(›100)
8c	13.4(44)	-	-	-	-	12.3(48)
9	-	-	-	14.6(60)	-	13.1(51)
11	22.1(73)	15.4(52)	19.6(73)	-	-	-
12a	29.1(96)	23.5(79)	21.5(81)	20.7(85)	-	14.5(57)
12b	12.6(41)	14.6(49)	-	-	-	-
13a	12.6(41)	-	-	-	12.8(63)	-
13b	20.3(67)	26.2(88)	-	-	20.9(›100)	-
15	-	-	-	-	18.8 (93)	-
16	31.3(›100)	28.8(97)	16.9(63)	22.1(91)	13.6(67)	26.2(›100)
Streptomycin	30.4(100)	29.7(100)	26.7(100)	24.4(100)	-	-
Clotrimazole	-	-	-	-	20.2(100)	25.6(100)

3. Experimental

3.1. Materials and Methods

Melting points were determined on a Gallankamp apparatus and are uncorrected. The IR spectra were recorded on a Shimadzu 470 IR-spectrophotometer (KBr; ν cm^{-1}). The ^1H NMR spectra were taken on a Varian EM- 390, 90 MHz spectrometer or on a Jeol LA 400 MHz FT-NMR spectrometer. Starting precursor, 6-Amino-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyrazin-5-carbonitrile (**1**) was prepared according to a reported method prepared in our earlier publication [5].

3-Methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbonitrile (2)

A mixture of **1** (2.50 g, 0.01 mol) and 2,5-dimethoxytetrahydrofuran (1.26 g, 0.01 mol) in glacial acetic acid (20 mL) was refluxed for 12 h. After cooling, the resultant solid product was collected by filtration and washed with water, and the crude product recrystallized from ethanol, gave 3.16 g of gray needles (87% yield), mp 155-157 ºC; IR (ν cm^{-1}): 2224 (C≡N). ^1H NMR ($CDCl_3$): (400 MHz): δ = 7.41-8.07 (5H, m, Ar-H), 6.90 (2H, m, 2,5-H of pyrrolyl), 6.35 (2H, m, 3,4-H of pyrrolyl), 2.64 (3H, s, CH_3). Anal. Calcd. For $C_{17}H_{12}N_6$ (300.32): C, 67.99; H, 4.03; N, 27.98. Found: C, 68.25; H, 4.32; N, 28.15%.

3-Methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carboxylic acid (3)

A mixture of **2** (3.0 g, 0.01 mol), aqueous KOH solution (10 %, 25 ml) and ethanol (25 ml) was refluxed for 12 h. After cooling, the resultant solid product was acidified with dil. HCl and the solid formed was collected by filtration and washed with water, and the crude product recrystallized from acetone-water to give 2.85 g of yellow crystals (89% yield), mp 200-202 ºC; IR (ν cm^{-1}): 2924,

broad (OH), 1670 (CO) cm^{-1}. Anal. Calcd. For $C_{17}H_{13}N_5O_2$ (319.32): C, 63.94; H, 4.10; N, 21.93. Found: C, 64.28; H, 4.39; N, 21.79 %.

Ethyl-3-methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carboxylate (4)

A solution of **3** (3.2 g, 0.01 mol) in absolute ethanol (30 ml) and conc. H_2SO_4 (3 ml) was refluxed for 7 h. After cooling, pour into ice cold water and neutralized with aq. Na_2CO_3. The solid formed was collected by filtration and washed with water, and the crude product recrystallized from ethanol to give 3.09 g of yellow crystals (88% yield), mp. 148-150 ºC. IR (ν cm^{-1}): 1676 (CO). ^{1}H NMR ($CDCl_3$): (400 MHz): δ = 7.42-8.16 (5H, m, Ar-H), 7.18 (2H, m, 2,5-H of pyrrolyl), 6.65 (2H, m, 3,4-H of pyrrolyl), 4.30-4.60 (2H, q, OCH_2), 2.66 (3H, s, CH_3), 1.3-1.6 (3H, t, CH_3 of ester). Anal. Calcd. For $C_{19}H_{17}N_5O_2$ (347.37): C, 65.69; H, 4.93; N, 20.16. Found: C, 66.02; H, 5.12; N, 20.45%.

3-Methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-b]pyrazine-5-carbohydrazide (5)

A mixture of compound **4** (1.7 g, 0.005 mol) and hydrazine hydrate (10 mL, 85% solution) was refluxed in absolute ethanol (20 mL) for 9 h. After cooling, the resultant solid product was collected by filtration and washed with water, and the crude product recrystallized from ethanol to give 1.33 g of gray white needles (83% yield), mp 258-260 ºC; IR (ν cm^{-1}): 3388, 3317 (NH_2), 3180 (NH), 1694 (CO); ^{1}H NMR ($CDCl_3$): (90 MHz): δ = 9.75 (br, 1H, NH), 7.41-8.26 (5H, m, Ar-H), 6.85 (2H, m, 2,5-H of pyrrolyl), 6.60 (2H, m, 3,4-H of pyrrolyl), 6.40 (br, 2H, NH_2), 2.70 (s, 3H, CH_3) . MS: m/z = 333.12 (M+, 62 %). Anal. Calcd. For $C_{17}H_{15}N_7O$ (333.35): C, 61.25; H, 4.54; N, 29.41. Found: C, 61.59; H, 4.82; N, 29.69%.

N-Arylidene-3-methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbo-hydrazide (6a-c)

A mixture of **5** (0.66 g, 0.002 mol) the appropriate aromatic aldehyde (0.002 mol) was stirred under reflux in ethanol (30 ml) in the presence of a few drops of piperidine for 5 h. The reaction mixture was allowed to cool to room temperature, poured into water, whereby a solid formed that was filtered off and crystallized from ethanol.

N-Benzylidene-3-methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbohydrazide (6a):

Yellow crystals, m.p. 182-184°C, yield (0.59 g, 71%). IR (ν cm^{-1}): 3245 (NH), 1670 (CO). Anal. Calcd. For $C_{24}H_{19}N_7O$ (421.25): C,

68.40; H, 4.54; N, 23.26. Found: C, 68.59; H, 4.73; N, 23.13 %.

N-(4-Methoxybenzylidene)-3-methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbohydrazide (6b):

Yellow crystals, m.p. 250-252°C, yield (0.65 g, 73%). IR (ν cm^{-1}): 3311 (NH), 1678 (CO). ^1H NMR ($CDCl_3$): (90 MHz): δ = 10.25 (s, 1H, NH), 7.44-8.30 (9H, m, Ar-H), 6.70 (2H, m, 2,5-H of pyrrolyl), 6.66 (2H, m, 3,4-H of pyrrolyl), 3.45 (s, 3H, OCH_3) 2.77 (s, 3H, CH_3). Anal. Calcd. For $C_{25}H_{21}N_7O_2$ (451.48): C, 66.51; H, 4.69; N, 21.72. Found: C, 68.75; H, 4.92; N, 21.88 %.

N-(4-Nitrobenzylidene)-3-methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*] pyrazine-5-carbohydrazide (6c):

Yellow crystals, m.p. 272-274°C, yield (0.69 g, 75%). IR (ν cm^{-1}): 3356 (NH), 1658 (CO). Anal. Calcd. For $C_{24}H_{18}N_8O_3$ (466.45): C, 61.80; H, 3.89; N, 24.02. Found: C, 62.08; H, 4.13; N, 24.37 %.

3-Methyl-(2-oxo-1,2-dihydro-indol-3-ylidene)-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbohydrazide (7)

A stirred mixture of 5 (0.66 g, 0.002 mol) and isatin (0.29 g, 0.002 mmol) in EtOH (15 ml) acidified with 4 drops of glacial acetic acid was refluxed for 10. The reaction mixture was then concentrated, cooled and filtered. The filtrate was either crystallized from ethanol to give 0.74 g of yellow needles (84% yield), mp 301-303 ºC; IR (ν cm^{-1}): 3281 (NH), 1702, 1643 (2CO). MS: m/z = 462.32 (M+, 27.9 %). Anal. Calcd. For $C_{25}H_{18}N_8O_2$ (462.46): C, 64.93; H, 3.92; N, 24.23. Found: C, 65.17; H, 4.25; N, 24.56%.

3-Methyl-1-phenyl-N'-(1-arylethylidene)-6-(1*H*-pyrrol-1-yl)-1*H*-pyrazolo[3,4-*b*] pyrazine-5-carbohydrazide (8a-c).

To a solution of compound **5** (0.66 g, 0.002 mol) in 1,4-dioxane (20 mL), appropriate acetophenone (0.002 mol) was added. The reaction mixture was heated under reflux for 12h, then it was left to cool overnight and the formed solid product was collected by filtration and recrystallized from dioxane.

3-Methyl-1-phenyl-N'-(1-phenylethylidene)-6-(pyrrol-1-yl)-1*H*-pyrazolo[3,4-*b*] pyrazine-5-carbohydrazide (8a)

Yellow crystals, m.p. 186-188°C, yield (0.69 g, 80%). IR (ν cm^{-1}): 3337 (NH), 1670 (CO). Anal. Calcd. For $C_{25}H_{21}N_7O$ (435.48): C, 68.95; H, 4.86; N, 22.51. Found: C, 69.24; H, 4.93; N, 22.78 %

3-Methyl-1-phenyl-N'-1-(4-bromophenyl) ethylidene-6-(pyrrol-1-yl)-1*H*-pyrazolo [3,4-*b*]pyrazine-5-carbohydrazide (8b)

Yellow crystals, m.p. 211-213°C, yield (0.63 g, 63%). IR (ν cm^{-1}): 3380 (NH), 1666 (CO). Anal. Calcd. For $C_{25}H_{20}BrN_7O$ (514.38): C, 58.38; H, 3.92; Br, 15.53, N, 19.06. Found: C, 58.63; H, 4.19; Br, 15.67; N, 19.27 %

N'-1-(4-Aminophenyl)ethylidene-3-Methyl-1-phenyl-6-(pyrrol-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-5-carbohydrazide (8c)

Yellow crystals, m.p. 321-323°C, yield (0.58 g, 65%). IR (ν cm^{-1}): 3426, 3380, 3343 (NH, NH_2), 1663 (CO) . Anal. Calcd. For $C_{25}H_{22}N_8$ (450.50): C, 66.65; H, 4.92; N, 24.87. Found: C, 66.96; H, 5.22; N, 25.19 %.

5-Methyl-2-(3-methyl-1-phenyl-6-pyrrol-1-yl-*1H*-pyrazolo[3,4-*b*]pyrazine-5-carbonyl)-2,4-dihydro-pyrazol-3-one (9)

A mixture of **5** (0.66 g, 0.002 mol) and ethyl acetoacetate (0.26 g, 0.002 mol) in ethanol (25 mL) and acetic acid (1 mL) was heated under reflux for 8 h. The reaction mixture was then poured into cold water; the solid precipitate that formed was filtered off, washed with water and crystallized from ethanol to give of yellow crystals, m.p 200-202 ºC, yield (0.52 g, 65 %); IR (ν cm^{-1}): 1675, 1656 (2 CO); ^{1}H NMR ($CDCl_3$): (90 MHz): δ = 7.44-8.30 (5H, m, Ar-H), 6.95 (2H, m, 2,5-H of pyrrolyl), 6.78 (2H, m, 3,4-H of pyrrolyl), 2.88 (s, 3H, CH_3), 2.68 (s, 2H, CH_2), 2.35 (s, 3H, CH_3). Anal. Calcd. For $C_{21}H_{17}N_7O_2$ (399.41): C, 63.15; H, 4.29; N, 24.55. Found: C, 63.38; H, 4.52; N, 24.41%.

(3,5-Dimethyl-pyrazol-1-yl)-(3-methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*] pyrazin-5-yl)-methanone (10)

A mixture of compound **5** (0.66 g, 0.002 mol) and acetylacetone (0.2 g, 0.002 mol) in ethanol (20 mL) was heated under reflux for 5 h. The reaction mixture was then poured into cold water; the solid precipitate that formed was filtered off, washed with water and crystallized from ethanol to give of yellow crystals, mp 185-185 ºC, (0.62 g, 79%); IR (ν cm^{-1}): 1700 (CO); ^{1}H NMR (DMSO-d6): (400 MHz): δ = 6.96-8.13 (5H, m, Ar-H), 7.15 (2H, m, 2,5-H of pyrrolyl), 6.66 (2H, m, 3,4-H of pyrrolyl), 6.15 (s, 1H, CH-pyrazole), 2.74 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.40 (s, 3H, CH_3). Anal. Calcd. For $C_{22}H_{19}N_7O$ (397.43): C, 66.49; H, 4.82; N, 24.67. Found: C, 66.74; H, 5.02; N, 24.93%.

5-(3-Methyl-1-phenyl-6-(pyrrol-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-5-yl)-[1,3,4] oxadiazole-2-thiol (11)

A mixture of compound **5** (1.32 g, 0.004 mol) and carbon disulfide (8 mL) in pyridine (20 mL) was heated on a water bath for 20 h at 50 ºC. The reaction mixture was then poured into an open dish and a little cold ethanol was added; the yellow product that formed was filtered off, dried and crystallized from dioxane to give of yellow needles, m.p 295-297 ºC, yield (1.15 g, 77%); IR (ν cm^{-1}): 2758 (SH). MS: m/z = 375.22 (M+, 43.5 %). Anal. Calcd. For $C_{18}H_{13}N_7OS$ (375.41): C, 57.59; H, 3.49; N, 26.12; S, 8.54. Found: C, 57.82; H, 3.78; N, 26.44; S, 8.91 %.

2-Substitutedthio-5-(3-methyl-1-phenyl-6-(pyrrol-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-5-yl)-1,3,4-oxadiazole (12a,b)

A mixture of **11** (1.87g, 0.005 mol), methyl iodide and/or phenacyl bromide (0.005 mol) and 2 g sodium acetate in ethanol (30 mL) was refluxed for 3 h, and then allowed to cool. The solid product was collected, washed with water several times, dried, and recrystallized from ethanol.

2-Methylthio-5-(3-methyl-1-phenyl-6-(pyrrol-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-5-yl)-1,3,4-oxadiazole (12a)

Yellow crystals. m.p. 215-217°C, yield (1.60 g, 84 %). IR (ν cm^{-1}): 2988 (CH-aliphatic), 1625 (C=N). ^{1}H NMR (DMSO-d6): (90 MHz): δ = 7.55 -8.13 (5H, m, Ar-H), 7.08 (2H, m, 2,5-H of pyrrolyl), 6.23 (2H, m, 3,4-H of pyrrolyl), 2.95 (s, 3H, S-CH_3), 2.68 (s, 3H, CH_3). Anal. Calcd. For $C_{19}H_{15}N_7OS$ (389.43): C, 58.60; H, 3.88; N, 25.18; S, 8.23. Found: C, 58.98; H, 4.15; N, 25.33; S, 8.49 %

2-Benzoylmethylthio-5-(3-methyl-1-phenyl-6-(pyrrol-1-yl)-1*H*-pyrazolo[3,4-*b*] pyrazine-5-yl)-1,3,4-oxadiazole (12b)

Yellow crystals. m.p. 178-180°C, yield (1.80 g, 75 %). IR (ν cm^{-1}): 1682 (CO). ^{1}H NMR (DMSO-d6): (400 MHz): δ = 7.25 -8.19 (10H, m, Ar-H), 7.15 (2H, m, 2,5-H of pyrrolyl), 6.55 (2H, m, 3,4-H of pyrrolyl), 4.25 (s, 2H, S-CH_2), 2.71 (s, 3H, CH_3). Anal. Calcd. For $C_{26}H_{19}N_7O_2S$ (493.54): C, 63.27; H, 3.88; N, 19.87; S, 6.50. Found: C, 63.57; H, 4.20; N, 19.69; S, 6.33 %.

5-(2-Aryl-1,3,4-oxadiazol-5-yl)-3-methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*] -pyrazine (13a,b)

An equimolar mixture of **5** (0.004 mol) and appropriate aromatic acids (0.004 mole) was refluxed in the presence of $POCl_3$ (10 mL) for 3 h. The reaction mixture was cooled and poured to crushed ice followed by treatment

with a solution of Na_2CO_3. The solid product obtained was filtered and recrystallized from DMF.

5-(2-phenyl-1,3,4-oxadiazol-5-yl)-3-methyl -1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,-*b*] -pyrazine (13a)

Brown crystals. m.p. 198-200°C, yield (1.80 g, 75 %). IR: 3090 (CH-aromatic), 1620 (C=N) cm^{-1}. MS: m/z = 419.17 (M+, 37 %). Anal. Calcd. For $C_{24}H_{17}N_7O$ (419.44): C, 68.72; H, 4.09; N, 23.38. Found: C, 68.98; H, 4.41; N, 23.69 %.

5-[2-(4-nitrophenyl)-1,3,4-oxadiazol-5-yl]-3-methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*]-pyrazine (13b)

Yellow crystals. m.p. 245-247°C, yield (1.80 g, 75 %). IR (ν cm^{-1}): 3120 (CH-aromatic), 1627 (C=N). Anal. Calcd. For $C_{24}H_{16}N_8O_3$ (464.44): C, 62.07; H, 3.47; N, 24.13. Found: C, 62.29; H, 3.79; N, 24.48 %.

N-Phenyl,N'-(methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-b]pyrazin-5-oyl) thiosemicarbazide (14)

A mixture of **5** (1.32 g, 0.004 mol) and phenylisothiocynate (0.54 g, 0.004 mol) in ethanol (15 mL) was heated under reflux for 3 h. The reaction mixture was allowed to cool at room temperature; the solid precipitate that formed was filtered off, and crystallized from dioxane to give yellow crystals, m.p. 266-268 ºC, yield (1.25 g, 67%). IR (ν cm^{-1}): 3378, 3210, 3162 (NH), 1683 (CO). ^1H NMR (DMSO-d6): (90 MHz): δ = 9.85 (br. s, 1H, NH, exchangeable with D_2O), 9.30 (br. s, 1H, NH, exchangeable with D_2O), 7.40 -8.23 (10H, m, Ar-H), 7.15 (2H, m, 2,5-H of pyrrolyl), 7.00 (br. s, 1H, NH, exchangeable with D_2O), 6.60 (2H, m, 3,4-H of pyrrolyl), 2.68 (s, 3H, CH_3). Anal. Calcd. For $C_{24}H_{20}N_8OS$ (468.53): C, 61.52; H, 4.30; N, 23.92; S, 6.84. Found: C, 61.87; H, 4.55; N, 24.23; S, 7.11 %.

5-(3-Methyl-1-phenyl-6-(pyrrol-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazin-5-yl)-4-phenyl-*4H*-[1,2,4]triazole-3-thiol (15)

A suspension of **14** (0.468 g, 0.001 mol) in an aqueous solution of NaOH (20 mL 2N) was heated under reflux for 3 h. The reaction mixture was then poured into cold water and acidified with HCl. The solid precipitate that separated was filtered, washed with water, dried and crystallized from dioxane to give 0.35 g of yellow crystals (77%), mp 310-312 ºC; IR: (ν cm^{-1}) 3459 (NH), 1178 (C=S). Anal. Calcd. For $C_{24}H_{18}N_8S$ (450.52): C, 63.98; H, 4.03; N, 24.87; S, 7.12. Found: C, 64.16; H, 4.22; N, 25.02; S, 7.33 %.

5-(3-Methyl-1-phenyl-6-(pyrrol-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazin-5-yl)-2-phenyl-amino[1,3,4]thiadiazole (16)

A mixture of **14** (0.468 g, 0.001 mol) and H_2SO_4 (10 mL) was stirred at room temperature for 3 h. The reaction mixture was then poured into ice, the solid precipitate that separated was collected and crystallized from dioxane to give 0.37 of yellow crystals (78%), mp 275-277 ºC; IR (ν cm^{-1}): 3318 (NH) Anal. Calcd. For $C_{24}H_{18}N_8S$ (450.52): C, 63.98; H, 4.03; N, 24.87; S, 7.12. Found: C, 64.29; H, 4.31; N, 25.12; S, 7.44 %.

Antimicrobial Activity

The antimicrobial activity of the synthesized compounds was screened against bacterial strains *Staphylococcus aureus* (AUMC B.54), *Bacillus cereus* (AUMC B.52) as gram-positive bacteria and *Escherichia coli* (AUMC B.53), *Pseudomonas aeruginosa* (AUMC B.73) as gram-negative bacteria and fungal strains *Candida albicans* (AUMC No.214), *Aspergillus flavus* (AUMC No.1276) using the agar well-diffusion method. all microbial strains were kindly provided by the Assiut University Mycological Centre (AUMC). To prepare inocula for bioassay, bacterial strains were individually cultured for 48 h in 100 mL conical flasks containing 30 mL nutrient broth medium. Fungi were grown for 7 days in 100 mL conicals containing 30 mL Sabouraud's dextrose broth. Bioassay was done in 10 cm sterile plastic Petri plates in which microbial suspension (1 mL/plate) and 15 mL appropriate agar medium (15 mL/plate) were poured. Nutrient agar and Sabouraud's dextrose agar were respectively used for bacteria and fungi. After solidification of the media, 5 mm diameter cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer. Chemical compounds dissolved in DMSO at 2%w/v (=20 mg/mL) were pipetted in the cavities. The screening tests were carried out in triplicate and the results were expressed as a mean of three determinations. *Streptomycin* and *Clotrimazole* were used as standards. Data are represented as % inhibition with reference to standards in (Table1).

4. Conclusion

We have described a simple and efficient method for the synthesis of 3-Methyl-1-phenyl-6-pyrrol-1-yl-1*H*-pyrazolo[3,4-*b*] pyrazine-5-carboxylic acid hydrazide (**5**). This compound was used as a starting material for the construction of a new series of pyrazolo[3,4-b]pyrazines **6-16**. Compound **16** proved to be the best one which showed high activity against all of microorganisms used. It showed higher activity against *S. aur*eus and *A. fla*vus more

than the reference drugs streptomycin and clotrimazole, respectively.

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