ISSN 2321-807X

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF NOVEL PYRAZOLO[3,4-b]PYRIDINES AND THEIR SPIRO-HETEROCYCLIC DERIVATIVES

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ABSTRACT

The present work describes the synthesis of a novel series of heterocyclic moieties derived from 5-acetylpyrazolo[3,4-b]pyridine (**1**). The formation of chalcones (**2a-d**) was utilized to synthesize pyrazoline, isoxazoline and pyrimidine derivatives (**3-10**). Thiosemicarbazone and semicarbazone (**11, 17**) were utilized to synthesize other new triazolethiones, thiadiazole and selenadiazole derivatives (**11-19**). Some new spiro derivatives (**22-25**) were synthesized by the reaction of chalcone (**21**) of **1** and isatine with hydrazines, hydroxyl amines and thiourea. Also, The reaction of **1** with cyanoacetyl hydrazine gave the hydrazide-hydrazone derivative **26**, which was allowed to react with aromatic aldehydes and α-cyanocinnamonitrile to afford coumarine and substituted pyridine derivatives (**28, 29**). The structures of all the new compounds have been established on the basis of their analytical and spectral data. Twenty two of the synthesized compounds were also evaluated for their antibacterial and antifungal activity against various strains of bacteria and fungi and most are found to possess promising antimicrobial activity when compared with Chloramphenicol and Clotrimazol**e**

Keywords: Pyrazolo[3,4-b]pyridine; Chalcones; Heterocyles; Spiroindolylpyrazolopyridine; Antimicrobial

Council for Innovative Research

Peer Review Research Publishing System

Journal: Journal of Advances in Chemistry

Vol. 10, No. 7

editorjaconline@gmail.com

www.cirjac.com

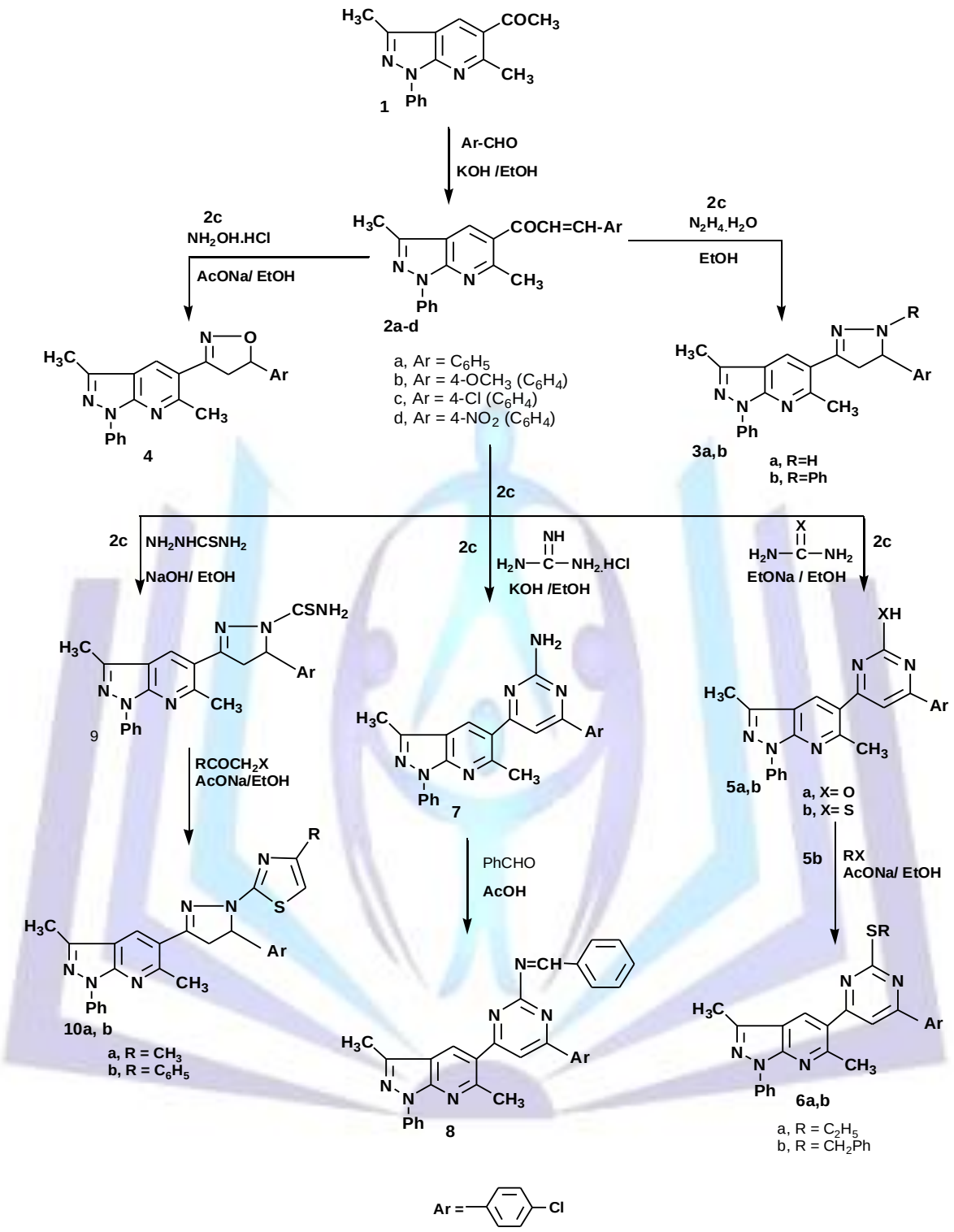
ISSN 2321-807X

INTRODUCTION

The pyrazolo[3,4-b]pyridine moieties represent important building blocks in both natural and synthetic bioactive compounds. [1] They are attractive targets in organic synthesis due to their significant biological activities such as xanthine oxidase inhibitors, cholesterol formation-inhibitor, and Anti-Alzheimer, [2,3] analgesic, [4] anxiolytic, [5] hypnotic, [6] antiviral, [7] anti-HIV, [8] corticotropin-Releasing Factor (CRF) antagonist, [9,10] antidiabetic, [11] antiarrhythmic, [12] antitumor, [13] antimalarial. [14] They also show antimicrobial and antiparasitic activities. [15-17] In addition, they are used as anti-HIV-1,[18] as a potential glucocorticoid receptor ligand for positron emission tomography (PET). [19] It was found that some derivatives of pyrazolo[3,4-b]pyridine were belived to be effective as antileishmanial agents, [20] inhibitors of erectile dysfunctions. [21] Taking all the above into consideration and in continuation of our previous work directed to synthesis of new heterocycles engaged with pyrazole nuclei, [22-25] we describe herein the utilization of the 1-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)ethanone (1) as the key intermediate in the synthesis of novel pyrazolo[3,4-b]pyridine members along with studies of the effect of some of them as antifungal and as antibacterial agents.

RESULTS AND DISCUSSION

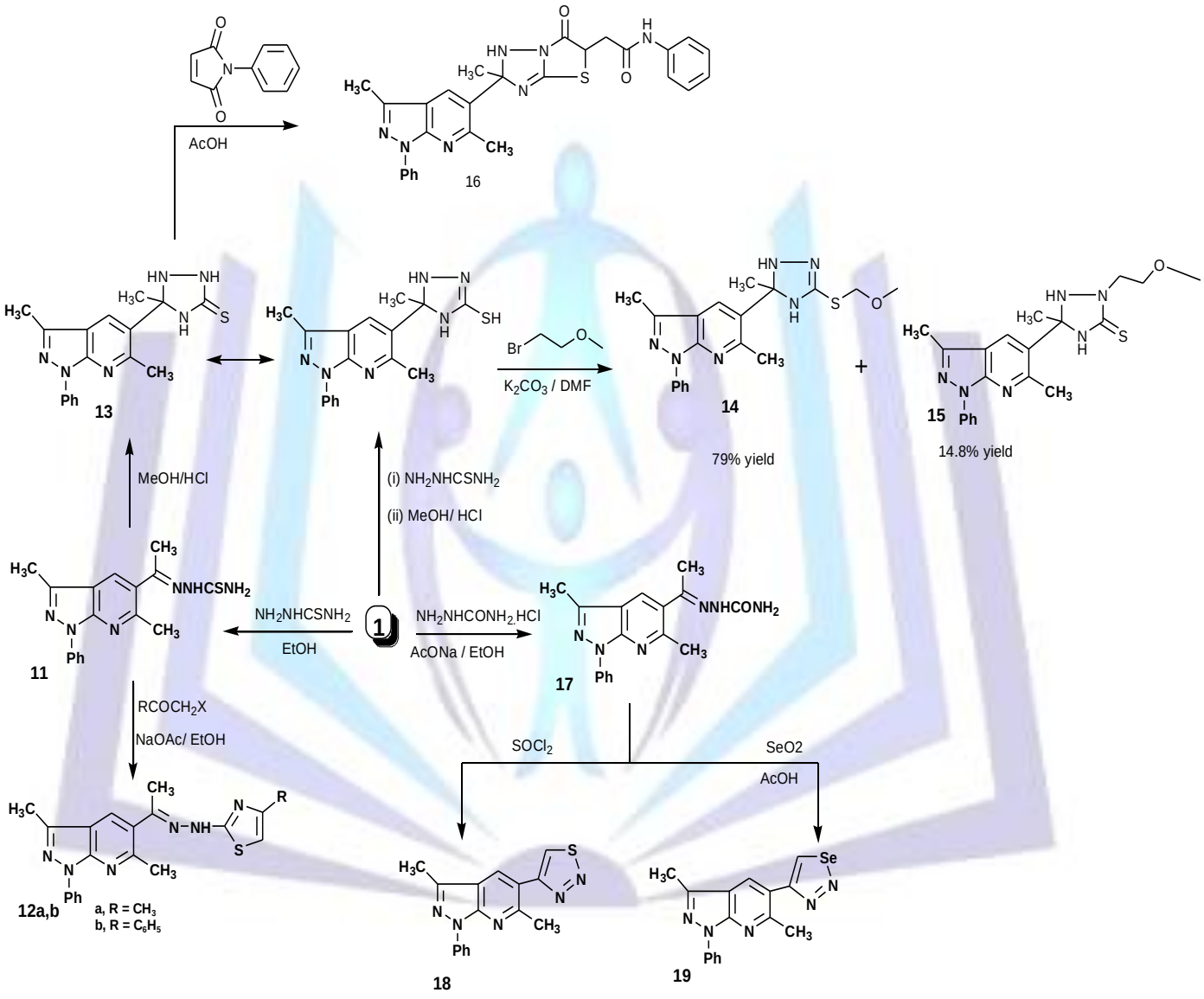
The starting compound **2a-d** were synthesized via the the Claisen-Schmidt reaction of 1-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)ethanone (**1**) [26] with different p-substituted aldehydes in ethanol and in presence of aqueous potassium hydroxide (25%). Presence of α-β-unsaturated keto function makes chalcones very prone to undergo reaction with bidentate nucleophiles to give five and six membered heterocyclic moieties. We intended to utilized this procedure to explore the formation of compounds **3-9** containing the pyrazole, isoxazole and pyrimidine rings linked on 5-position of pyrazolo[3,4-b]pyridine nucleus. Thus, cyclocondensation of chalcones **2c** (R= Cl) with hydrazine hydrate or phenyl hydrazine in dry ethanol gave the corresponding pyrazole and N-phenylpyrazole derivatives **3a** and **3b**, respectively. The ^{1}H NMR spectrum of compound **3a** and **3b** showed doublet of doublets of -CH_2 near about δ 3.12-4.15 ppm and doublet of doublets of –CH at δ 4.85-5.27 ppm, confirmed the cyclisation in pyrazoline. Also, the reaction of **2c** with hydroxylaminehydrochloride in the presence of anhydrous sodium acetate led to the formation of 5-[5-(4-chlorophenyl)-4,5-dihydro-1,2-isoxazol-3-yl]-3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine **(4).** The IR spectra of **4** clearly showed the formation of isoxazoline ring by the appearance of band at 1509 cm^{-1} for (=C=N-O). ^{1}H NMR data also confirmed the synthesis by showing a doublet at δ 3.75 ppm for 2H and a triplet at δ 4.40 ppm for 1H of isoxazoline ring. Similarly, the reaction of **2c** with urea and/or thiourea in presence of aqueous potassium hydroxide (10%) gave the corresponding pyrimidinone and thiopyrimdine derivatives **(5a, b)** respectively. S-alkylated products **(6a,b)** were obtained upon treatment of **5b** with ethyl iodide and benzyl bromide in the presence of anhydrous sodium acetate in refluxing ethanol. Moreover, treatment of **2c** with guanidine hydrochloride in dry ethanol containing sodium hydroxide solution(10%) yielded 2-Amino-4-(4-chlorophenyl)-6-(3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)pyrimidine (**7**). Heating of **7** with benzaldehyde in refluxing methanol in the presence of few drops of acetic acid resulted in the formation of the corresponding Schiff base (**8**). Condensation of chalcones with thiosemicarbazide has became an extremely popular method for making pyrazoline derivatives. Thus, treatment of **2c** with thiosemicarbazide in an ethanolic sodium hydroxide solution,yielded the pyrazole carbothioamide (**9**).The IR spectra of compound **9** showed intense bands at 3425, 3250, 1580 and 1370 cm^{-1} due to NH_2, C=N and C=S groups, respectively. In addition, the absorption band at 1120 cm^{-1} was attributed to the C-N, which also confirm the formation of desired pyrazoline ring. In the ^{1} HNMR spectra, pyrazoline protons HA and HB are germinal protons at C4 carbon appears at 3.46 and 4.0 ppm as doublet of doublets. The CH proton also appeared as doublet of doublets at 5.15 ppm due to vicinal coupling with two non-equivalent germinal protons of C4 carbon. Compound **9** underwent ready cyclization upon treatment with α-haloketones in the presence of anhydrous sodium acetate to afford 5-(4-Chlorophenyl)-4,5-dihydro-1-(4-substitutedthiazol-2-yl)-1-H-pyrazol-3-yl)-3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridines (**10a,b**) (Scheme 1). On the other hand, condensation of the target compound (**1**) in boiling ethanol with thiosemicarbazide afforded the corresponding thiosemicarbazone (**11**), [27] which was allowed to react with α-haloketones to yield the corresponding thiazolidines derivatives (**12a,b**). In addition, the thiosemicarbazone (**11**) was heated with methanolic HCl to give 5-methyl-5-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-1,2,4-triazolidine-3-thione (**13**). A further support for the structure of (**13**) was achieved by synthesis of the compound *via* another route by boiling a solution of **1** in methanolic HCl. All of spectral analyses completely identical and confirmed the formation of compound **13** by the two routes. We have observed that extensive thiol-thione tautomerism exists in compound **13**. In most cases of nucleophilic substitution of an alkyl halide on a thioamide system, sulfur atom \attack is favored**.** [28] Thus, treatment of **13** with 1-bromo-2-methoxyethane in warm DMF in the presence of potassium carbonate afforded the desired products **14** and **15**. Column chromatography of the crude material was allowed of the isolation of both isomers. The S-alkylation product **14** as a major (79% yield) and the other N-alkylation product **15** (14.8% yield). ^{1}HNMR and ^{13}CNMR spectra of the synthesized compounds provided proof of the S- and N-alkylated triazoles **14** and **15**, respectively. The CH_2 protons adjacent to the sulfur atom in compound **14** appears at 3.75 ppm and the carbon resonates at 33.6 ppm. However, CH_2 protons adjacent to the nitrogen atom in compound **15** are more download-shifted to 4.60 and the carbon resonates at 46.27 ppm. Finally, the ^{13}CNMR spectra show peak at 172 ppm corresponding to the C=S group associated with the N-



Scheme 1 Pathway for synthesis of compounds **2-10**

alkylated derivative **15**, whereas the S-alkylated derivative **14** gave peak at 152 ppm for the same carbon corresponding to C-S group. Also, compound **13** was allowed to react with N-phenyl maleimide in acetic acid to afford 2-[2-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-2-methyl-6-oxo-1,2,5,6-tetrahydro-[1,3]thiazolo[3,2-b][1,2,4]triazol-5-yl]-N-phenyl-cetamide (**16**). The IR spectra of thiazolotriazole (**16**) showed the absorption bands at 1710 and 1585 cm^{-1} corresponding to C=O and C=N, respectively. In the ^{1}H NMR spectra of compound **16**, protons of CH_2-CH fragment showed the characteristic patern of an ABX system. The chemical shifts of the protons HA, HB and HX are doublet of doublets at 2.8, 3.3and at 4.6 ppm, respectively. The acetamide NH proton appeared at a sharp singlet at 13.30 ppm. In addition, the ^{13}C CNMR spectra displayed characteristic two singlets at 173.25 and 178.85 ppm due to the acetamide carbamoyl carbon(CONH) and the thiazolidinone carbonyl carbon(CO-N-), respectively. Condensation of the target

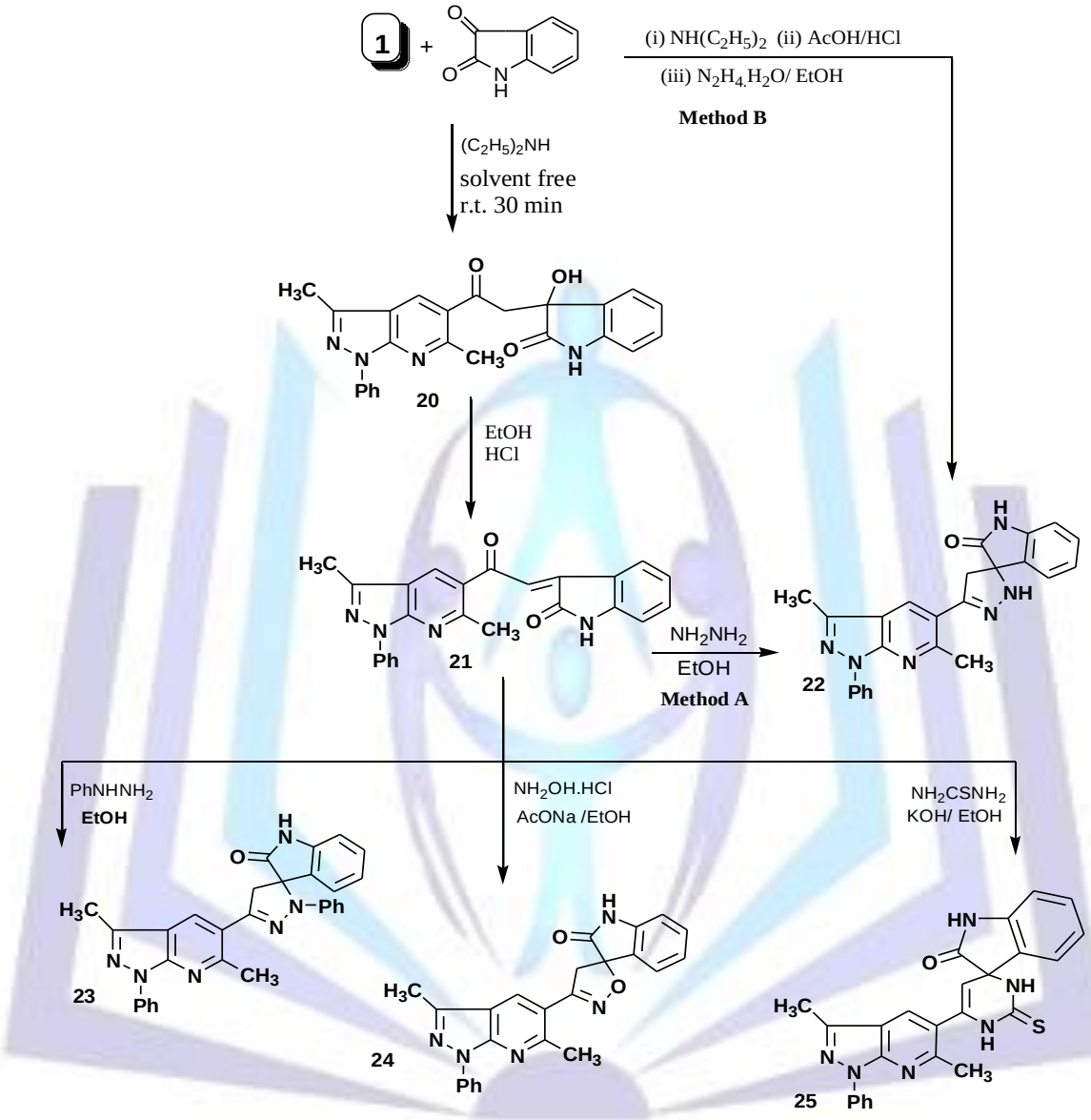
compound 4-acetyl-3-methyl-5-oxo-1-phenyl-2-pyrazoline (**1**) in boiling ethanol with semicarbazide hydrochloride afforded the corresponding semicarbazone (**17**). When the semicarbazone **17** was reacted with thionyl chloride it gave 4-(3-methyl-5-oxo-1-phenyl-2-pyrazolin-4-yl)-1,2,3-thiadiazole (**18**). On the other hand, treatment of **17** with selenium dioxide, 4-(3-methyl-5-oxo-1-phenyl-2-pyrazolin-4-yl)-1,2,3-selenadiazole (**19)** was obtained. Structure of compounds **18** and **19** was supported in the basis of elemental and spectral analysis. FT-IR spectra exhibited the absence of (NH) and (NH_2) absorption bands of the semicarbazone and revealed a characteristic absorption band at 820 cm^{-1} due to C-Se-N. ^{29} ^{1}HNMR spectra also appeared two singlets at δ 9.75 and 10.12 ppm corresponding to the CH protons of thiaziazole and selenadiazole, respectively (Scheme 2).



Scheme 2 Pathway for synthesis of compounds **11-19**

Crossed aldol condensation between 5-acetylpyrazolo[3,4-b]pyridine (1) and isatin was carried out in the presence of diethyl amine as a basic catalyst gave rise the formation of 3-hydroxy-3-(2-(3,6-Dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-yl)-2-oxoethyl)indolin-2-one (**20**). Dehydration of **20** using Ethanol-HCl mixture afforded the corresponding chalcone **21**. Nucleophilic Michael addition of hydrazine hydrate to the chalcone **21** gave spiropyrazoline derivative (**22**) (**method A)**. Moreover, spiropyrazoline (**22**) was obtained in an excellent yield via a one-pot synthesis without the isolation of the intermediate **21.** Thus, compound **1** reacted with isatin in the presence of diethylamine for 30 min at room temperature and the formed solid was treated with glacial acetic acid and hydrochloric acid for another 30 min at 80 C. Hydrazine hydrate was then added to the previous acidic solution to give the spiropyrazoline (**22**) (**method B**). The chalcone derivative (**21**) when interacted with phenylhdyrazine in ethanol as solvent, gave the required spiro N-

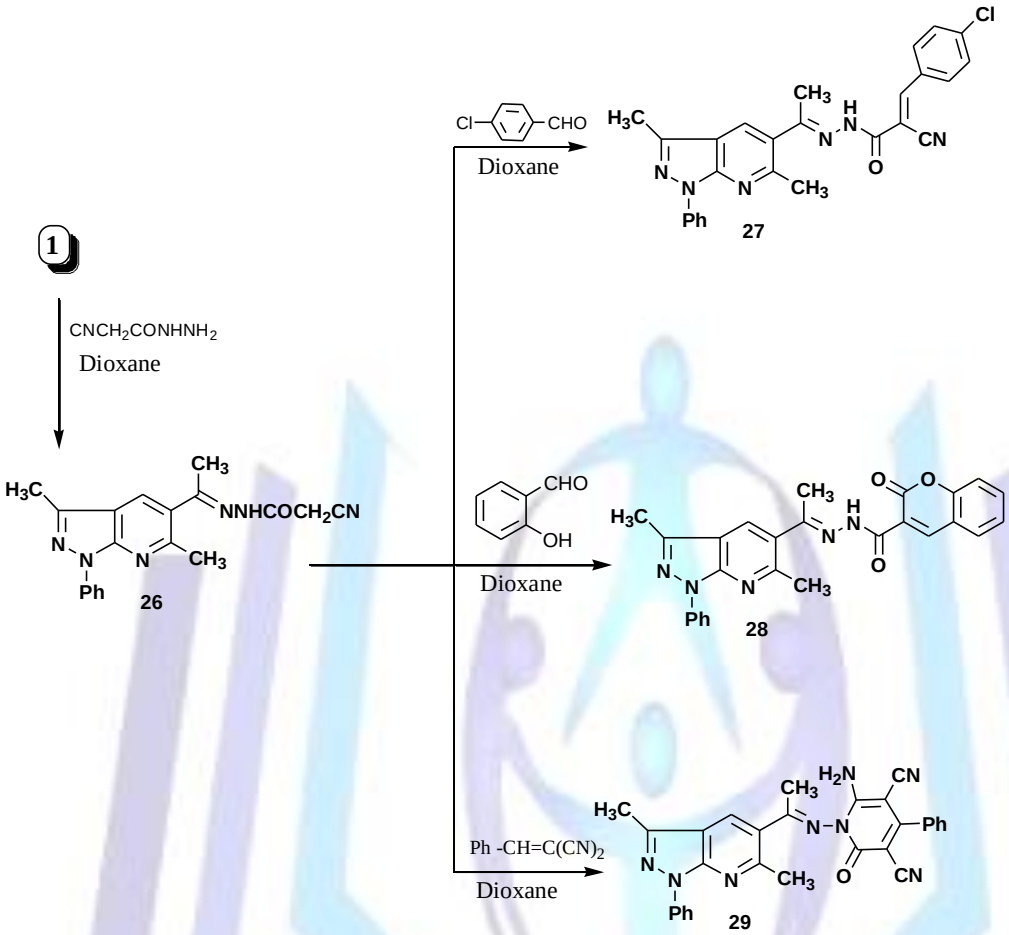
phenylpyrazoline derivative (**23**). 3′-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-4′-dihydrospiro[indol-3,5′-isoxazole]-2-(1H)-one (**24**) was obtained by the refluxing of the chalcone **21** with hydroxylamine hydrochloride in ethanol in the presence of sodium acetate as catalyst. Also, compound **21** was interacted with thiourea in ethanol as solvent and in the presence of potassium hydroxide, gave the 6′-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)- spiro[indol-3,4′ -pyrimidin-2′- (1H) thione]-2-(1H)-one (**25**) (Scheme 3).



Scheme 3 Pathway for synthesis of compounds **20-25**

^{1}HNMR spectra confirmed the formation of the chalcone **(21)** by the absence of a peak at δ 3.15 ppm corresponding to CH_2 protons in the aldol product (**20**). ^{1}HNMR spectra of the spiropyrazoline (**22**) revealed the presence of the characteristic peaks at δ 3.65, 6.30 and 8.63 ppm due to CH_2 protons of pyrazoline and NH protons of each pyrazoline and indoline moieties, respectively. The structure of **25** was indicated by spectral methods, its IR spectra revealed characteristic bands at 3340, 3210, 3180 and 1180 cm^{-1} corresponding to (NH) and (C=S), respectively. The 1 HNMR spectra of **25** showed the presence of two broad exchangeable singlets at δ 8.85 and 9.90 ppm for two NH protons of pyrimidine ring. With the aim of obtaining novel hydrazide-hydrazone with a wide spectrum of pharmaceutical applications, [30] we report herein the reaction of 5-acetylpyrazolo[3,4-b]pyridine (**1**) with cyanoacetyl hydrazine in dioxane to yield the corresponding acetohydrazide-hydrazine derivative (**26**). The structure of compound **26** was confirmed based on its analytical and spectral data. Thus, the 1H-NMR showed a singlet at δ 4.40 for the CH_2 group and a singlet (D_2O exchangeable) at δ10.95 for the NH group. Moreover, the ^{13}C- NMR spectrum showed peaks at δ 12.90, 26.80, 113.64 and 174.19 ppm corresponding to (CH_3), (CH_2), (CN) and (C=O), resoectively. Further structure elucidation of compound **26** was obtained through the study of its reactivity towards chemical reagents. Thus, reaction of **26** with p-chlorobenzaldehyde gave the corresponding benzal derivative (**27**). On the other hand, the coumarin derivative (**28**) was

obtained via the reaction of **26** with salicyladehyde . Finally, the reaction of **26** with α-cyanocinnamonitrile gave 6-amino-1-[1-(3,6- dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)ethylideneamino]-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile **(29)**. The structure of **29** was confirmed by spectral and analytical data. Thus, The IR spectra revealed the presence of absorption bands at 3452, 3368, 2229 and 2189 cm^{-1} due to NH_2 and two CN functions, respectively. (Scheme 4).



Scheme 4 Pathway for synthesis of compounds **26-29**

Antimicrobial Activity

The antimicrobial activity of **22** selected 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. [31] The screening tests were carried out in triplicate and the results were expressed as a mean of three determinations. Chloramphenicol and Clotrimazole were used as standards. Data are represented as % inhibition with reference to standards in (Table1). The results obtained from table 1 revealed that a comparison of the antimicrobial activity of the chalcone derivatives (**2a-d**), indicated that the para-chloro derivative **2c** induced more activity against all of the tested microorganisms (76 - ›100% growth inhibition). The 5-isoxazolinyl derivative **4** was more potent than the 5-pyrazolinyl derivative **3a** and showed a good antifungal activity only against *C. albicans* (89% growth inhibition) and *A. flavus* (79% growth inhibition). The 5- pyrimidinylthione compound **5b** (R= CH_2Ph) showed a remarkable antimicrobial activity towards all of gram-positive bacteria (›100% growth inhibition against *S.aureus*) and also exhibited an excellent antifungal activity against *C. albicans* (›100% growth inhibition). Formation of Schiff base of 5-aminopyrimidinyl derivative with benzaldehyde compound **8** showed only ›100% growth inhibition against *C. albicans* which was comparable to clotrimazole .The N-thiazolylpyrazolinyl derivative **10a**(R= CH_3) showed a remarkable antibacterial activity against gram-negative bacteria species *E.coli* (›100% growth inhibition) and *P. aeruoginosa* (83% growth inhibition) and also exhibited a strong antifungal activity against *C. albicans* (›100% growth inhibition). While, its phenyl isomer **10b** (R= Ph) was inactive towards most of the tested microorganisms. Similarly, the N-phenyl thiazolylhydrazine derivative **12b** (R=Ph) did not show any activity against all of tested bacteria and fungi species. Whereas, it's N-methyl derivative **12a** (R=CH_3) showed an excellent antimicrobial activity against all of tested bacteria and fungi species except *P. aeruoginosa* (95% growth inhibition against *E.coli*). Building up a new 1,2,4-triazolidine thione compound **13** showed ›100% growth inhibition activity against all of tested bacteria and fungi species except *P. aeruoginosa*. The S-alkylating product compound **14** exihibited only 86% growth inhibition against *E.coli*. The creation of a novel thiazolotriazole ring compound **16**, enhanced the antimicrobial activity.

ISSN 2321-807X

Table 1: Antimicrobial activity of some pyrazolo[3,4-b]pyridine derivatives

Compounds	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.aeruoginosa*	*C.albicans*	*A. flavus*
2a	-	10(40)	9 (43)	-	8 (30)	-
2b	-	8(32)	-	-	-	-
2c	17(85)	19(76)	-	16(89)	34(›100)	35(92)
2d	-	-	-	-	6(22)	-
3a	-	-	-	-	7(26)	-
4	-	-	-	-	24(89)	30(79)
5a	-	-	-	-	9 (33)	-
5b	26(›100)	21(84)	-	-	31(›100)	-
8	-	-	11(52)	-	29(›100)	23(61)
10a	-	-	25(›100)	15(83)	30(›100)	26(68)
10b	-	-	-	-	-	12(32)
12a	18(90)	20(80)	20(95)	-	23(85)	30(79)
12b	-	-	-	-	-	-
13	25(›100)	24(96)	28(›100)	-	32(›100)	41(›100)
14	-	-	18(86)	-	-	-
16	14(70)	22(88)	-	-	31(›100)	36(95)
18	22(›100)	19(76)	24(›100)	-	-	32(84)
19	-	-	-	-	29(›100)	40(›100)
22	8(40)	-	-	-	11(41)	10(26)
24	-	-	-	-	-	-
25	-	-	28(›100)	16(89)	30(›100)	-
28	-	-	-	-	-	-
Chloramphenicol	20(100)	25(100)	21(100)	18(100)	-	-
Clotrimazole	-	-	-	-	27(100)	38(100)

*The percentage zone of inhibition was calculated against the bacterial and fungal strains. Solvent and negative control: DMSO (dimethylsulfoxide)- No inhibition observed

Thus, compound **16** showed a significant antibacterial activity against gram-positive only (88% growth inhibition against *B.cereus*) and also exhibited an excellent antifungal activity (›100% growth inhibition against *C. albicans).* On the other hand, the comparison of the percentage of growth inhibition of the thiadiazole and selenadiazole **18** and **19** respectively, demonstrated that thiadiazole derivative **18** is more potent against all of tested bacteria and fungi species except *P. aeruoginosa and C. albicans* (›100% growth inhibition against *S.aureus and E.coli*), while, the selenadiazole **19** showed only an excellent antifungal activity (›100% growth inhibition against *C. albicans and A. flavus*). Among all of the spiroindolyl derivatives **22**, **24** and **25** only, the spiroindolylpyrimidinethione **25** showed a significant antibacterial activity against gram-negative species (›100% growth inhibition against *E.coli*) and also exhibited ›100% growth inhibition against *C. albicans*. With respect to the formation of coumarin compound **28**, it was devoid of growth inhibition against all of the tested microorganisms.

CONCLUSION

The present study research study reports the convienent synthesis, reactions and antimicrobial activity of a new series of pyrazolo[3,4-b]pyridines carrying biologically active heterocyclic entities. Their Screening results revealed that most of the tested compounds showed a remarkable to an excellent activities and might be helpful in the future development of pyrazolopyridines as analogues as a novel antimicrobial agents.

EXPERIMENTAL

Melting points are uncorrected and determined using a Gallenkamp melting point apparatus. IR spectra were recorded on a Pye-Unicam SP 3-100 spectrophotometer using the KBr wafer technique. ^{1}H NMR spectra were recorded on a Varian EM-390 90 MHz spectrometer and on GNM-LA (400 MHz) in DMSO-d_6 as solvent and TMS as internal standard. Chemical shifts are expressed in ppm. ^{13}C NMR spectra were measured on a Varian EM-200, 100 MHZ spectrometer. Mass spectra were determined on a JEOL JMS-600 spectrometer. Elemental analyses were carried out at the Microanalytical Unit at the at Assiut University (Egypt). Compounds **1** was prepared according to literature procedure. [26] Compounds **2a**, **11** and **17** were prepared were prepared as previously reported.[27]

General procedure for the synthesis of 1-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)-3-substitutedphenylprop-2-en-1-one (2a-d).

A mixture of 1 (0.002 mol), appropriate aromatic aldehydes (0.00 2 mol) and potassium hydroxide solution (5 mL, 25%) in ethanol (25 mL) was stirred overnight. The product was filtered off, washed with water, dried and crystallized from ethanol.

1-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (2b)

A yellow crystals, 56%, mp 134-136ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3062 (CH- aromatic), 2985, 2930 (CH- aliphatic), 1682 (C=O), 1585 (C=N), 1439 (C=C), 1070 (C-O); ^{1}H NMR δ 2.40 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 6.75 (s, 1H, CH-ethylenic), 7.50-8.35 (m, 12H, 9H-aromatic + 1H, CH-ethylenic + 1H, CH-pyridine), Anal. Calcd for $C_{24}H_{21}N_3O_2$(383.44): C, 75.18; H, 5.52; N,10.96. Found: C, 75.51; H, 5.88; N, 11.25%.

1-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)-3-(4-chlorophenyl)prop-2-en-1-one (2c)

A yellow crystals, 83%, mp 188-190ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3080(CH- aromatic), 2980, 2866(CH- aliphatic), 1690 (C=O), 1570 (C=N), 1490 (C=C); ^{1}H NMR δ 2.45 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 6.63 (s, 1H, CH-ethylenic), 7.50-8.30 (m, 12H,10H-aromatic + 1H, CH-ethylenic + 1H, CH-pyridine), Anal. Calcd. for $C_{23}H_{18}ClN_3O$(387.86): C, 71.22; H, 4.68; Cl, 9.14; N, 10.83. Found: C, 71.48; H, 5.02; Cl, 9.37; N, 11.11%.

1-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)-3-(4-nitrophenyl)prop-2-en-1-one (2d)

A yellow crystals, 69%, mp 208-210ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3097 (CH- aromatic), 2953, 2880 (CH- aliphatic), 1678 (C=O), 1565 (C=N), 1469 (C=C); ^{1}H NMR δ 2.40 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 6.48 (s, 1H, CH-ethylenic), 7.40-8.55 (m, 12H,10H-aromatic + 1H, CH-ethylenic + 1H, CH-pyridine), Anal. Calcd. for $C_{23}H_{18}N_4O_3$(398.41): C, 69.34; H, 4.55; N, 14.06. Found: C, 69.59; H, 4.84; N, 14.30%.

General procedure for the synthesis of 5-[5-(4-chloro phenyl)-3,6-dimethyl--4,5-dihydro-1H (phenyl)-pyrazol-3-yl]-1-phenyl-1H-pyrazolo[3,4-b]pyridine (3a,b)

A mixture of chalcone 2c (0.001 mol), hydrazine hydrate or phenyl hydrazine (0.001mol) was heated under reflux for 4 h, in 20 mL ethanol then cooled and the residual material was filtered off and recrystallized from ethanol.

5-[5-(4-chlorophenyl)-3,6-dimethyl--4,5-dihydro-1H-pyrazol-3-yl]-1-phenyl-1H-pyrazolo[3,4-b]pyridine (3a)

A yellow crystals, 66%, mp 159-161ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3100 (NH), 3020 (CH- aromatic), 2980 (CH- aliphatic), 1530 (C=N), 1440(C=C); ^{1}H NMR δ 2.48 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 3.12(dd, J_1=4.75 Hz, J_2= 7.70, 1H, CH_2-pyrazoline), 4.21(dd, J_1=3.80 Hz, J_2= 8.20, 1H, CH_2-pyrazoline), 4.85 (dd, J_1=1.60 Hz, J_2= 8.90, 1H, CH-pyrazoline) 7.70-8.45 (m, 11H, 10H-aromatic + 1H, CH-pyridine), 8.80 (s, 1H, NH); MS m/z (%): 401.46 (M^+, 100); Anal. Calcd. for $C_{23}H_{20}ClN_5$ (401.89): C, 68.74; H, 5.02; Cl, 8.82; N, 17.43. Found: C, 69.03; H, 5.38; Cl, 9.13; N, 17.70%.

5-[5-(4-Chlorophenyl)-3,6-dimethyl--4,5-dihydro-1-phenylpyrazol-3-yl]-1-phenyl-1H-pyrazolo[3,4-b]pyridine (3b)

A yellow crystals, 71%, mp 223-225ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3066 (CH- aromatic), 2969 (CH- aloiphatic), 1546 (C=N), 1481 (C=C); ^{1}H NMR δ 2.55 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 3.25 (dd, J_1=5.30 Hz, J_2= 2.70, 1H, CH_2-pyrazoline), 4.15 (dd, J_1= 3.60 Hz, J_2= 1.80, 1H, CH_2-pyrazoline), 5.27(dd, J_1=8.40 Hz, J_2= 1.20, 1H, CH-pyrazoline) 7.65-8.55 (m, 15H, 14H-aromatic + 1H, CH-pyridine); Anal. Calcd. for $C_{29}H_{24}ClN_5$ (477.99): C, 72.87; H, 5.06; Cl, 7.42; N, 14.65. Found: C, 73.12; H, 5.33; Cl, 7.65; N, 14.84%.

5-[5-(4-chlorophenyl)-4,5-dihydro-1,2-isoxazol-3-yl]-3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine (4)

A mixture of chalcone 2c (0.38 g, 0.001 mol), hydroxylamine hydrochloride (0.07g, 0.001 mol) and anhydrous sodium acetate (1 g) was refluxed in ethanol (20 mL) for 6h, then allowed to cool and poured into cold water. The precipitated product thus formed was collected by filtration, dried and recrystallized from methanol to give compound 4 as yellow

crystals, 68%, mp 165-167ºC. FT-IR (KBr) v_{max}/ cm^{-1}: 3078 (CH- aromatic), 2965 (CH- aliphatic), 1620 (C=N), 1540 (C=C), 1509 (=C=N-O); ^{1}H NMR δ 2.37 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 3.15 (dd, J_1=5.30 Hz, J_2= 2.70, 1H, CH_2- isoxazol), 3.78 (dd, J_1= 3.60 Hz, J_2= 1.80, 1H, CH_2- isoxazol), 5.15 (dd, J_1=8.40 Hz, J_2= 1.20, 1H, CH- isoxazol) 7.65-8.55 (m, 15H, 14H-aromatic + 1H, CH-pyridine); Anal. Calcd. for $C_{29}H_{24}ClN_5$ (477.99): C, 72.87; H, 5.06; Cl, 7.42; N, 14.65. Found: C, 73.12; H, 5.33; Cl, 7.65; N, 14.84%.

General procedure for the synthesis of 4-(4-chlorophenyl)-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)pyrimidin-2-ol (thiol) (5a,b)

A mixture of chalcone 2c (0.02 mol), urea or thiourea (0.02 mol) were dissolved in ethanolic sodium hydroxide (5%, 10 mL) was stirred about 2-3 hours with a magnetic stirrer. This was then poured into 400 ml of cold water with continuous stirring for an hour and then kept in refrigerator for 24 hours. The precipitate obtained was filtered, washed and recrystallized ethanol.

4-(4-chlorophenyl)-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)pyrimidin-2-ol (5a)

A pale brown crystals, 71%, mp 236-238ºC. FT-IR (KBr) v_{max}/ cm^{-1}: 3460 (OH), 3060 (CH- aromatic), 2982 (CH- aliphatic), 1606 (C=N), 1472 (C=C); ^{1}H NMR δ 2.61 (s, 3H, CH_3), 2.74 (s, 3H, CH_3), 3.20 (s, 1H, OH), 7.25 (s, 1H, CH-pyrimidine), 7.55-8.60 (m, 10H, 9H-aromatic + 1H, CH-pyridine); Anal. Calcd. for $C_{24}H_{18}ClN_5O$ (427.89): C, 67.37; H, 4.24; Cl, 8.29; N, 16.37. Found: C, 67.71; H, 4.48; Cl, 8.52; N, 16.39%.

4-(4-chlorophenyl)-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)pyrimidin-2-thiol (5b)

A pale brown crystals, 79%, mp 293-295ºC. FT-IR (KBr) v_{max}/ cm^{-1}: 3372, 3267 (NH), 3061 (CH- aromatic), 2995 (CH-aliphatic), 1626 (C=N), 1375 (C=S); ^{1}H NMR δ 2.55 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 3.40 (s, 1H, SH), 7.50-8.54 (m, 11H, 9H-aromatic + 1H, CH-pyridine + 1H, CH- pyrimidine); Anal. Calcd. for $C_{24}H_{18}ClN_5S$ (443.95): C, 64.93; H, 4.09; Cl, 7.99; N, 15.78; S, 7.22%. Found: C, 65.28; H, 4.24; Cl, 8.30; N, 16.07; S, 7.53%.

General procedure for the synthesis of 2-substituted thio-4-(4-chlorophenyl)-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)pyrimidine (6a,b)

A mixture of 5b (0.01 mol), ethyl iodide or benzyl bromide (0.01 mol) in ethanol (40 mL) was refluxed in the presence of anhydrous sodium acetate (0.9 g, 0.011 mol) for 4 h. The solid product separated from the hot mixture was filtered off, washed with water and recrystallized from ethanol

4-(4-Chlorophenyl)-2-ethylthio-4-(4-chlorophenyl)-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)pyrimidine (6a)

A pale yellow crystal, 68%, mp 167-169ºC. FT-IR (KBr) v_{max}/ cm^{-1}: 3088 (CH- aromatic), 2975 (CH-aliphatic), 1620 (C=N), 1498 (C=C), 1266 (C-S); ^{1}H NMR δ 1.25 (t, 3H, SCH_2-$\underline{CH_3}$), 2.45 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 4.00 (q, 2H, $S\underline{CH_2}CH_3$), 7.40 (s, 1H, CH-pyrimidine), 7.60-8.75 (m, 10H, 9H-aromatic + 1H, CH-pyridine); Anal. Calcd. for $C_{26}H_{22}ClN_5S$ (472.13): C, 66.16; H, 4.70; Cl, 5.71; N, 14.84; S, 6.79. Found: C, 66.48; H, 5.06; Cl, 5.82; N, 15.13; S, 7.12%.

2-Benzylthio-4-(4-chlorophenyl)-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)pyrimidine (6b)

A pale yellow crystals, 61%, mp 157-159ºC. FT-IR (KBr) v_{max}/ cm^{-1}: 3090 (CH- aromatic), 2874 (CH- aliphatic), 1615 (C=N), 1398 (C=C), 1244 (C-S); ^{1}H NMR δ 2.40 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 4.15 (s, 2H, $S\underline{CH_2}Ph$), 7.35 (s, 1H, CH-pyrimidine), 7.55-8.60 (m, 16H, 15H-aromatic + 1H, CH-pyridine); Anal. Calcd. for $C_{31}H_{24}ClN_5S$ (534.07): C, 69.72; H, 4.53; Cl, 6.64; N, 13.11; S, 6.00. Found: C, 66.93; H, 5.00; Cl, 6.77; N, 13.39; S, 6.21%.

***2*-Amino-4-(4-chlorophenyl)-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)pyrimidine (7)**

A mixture of chalcone 2c (0.76 g, 0.002 mol), guanidine hydrochloride (0.19 g, 0.002 mol) and ethanolic sodium hydroxide (10%, 10mL) was refluxed for 2–3 h. After cooling, the solid formed was filtered off, air dried and recrystallized from absolute ethanol to give compound 7 as brown crystals, 71%, mp 305-307ºC. FT-IR (KBr) v_{max}/ cm^{-1}: 3320, 3290 (NH_2), 3035 (CH- aromatic), 2880 (CH- aliphatic), 1627 (C=N), 1526 (C=C); ^{1}H NMR δ 2.54 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 6.95 (s, 1H, CH-pyrimidine), 7.55-8.45 (m, 10H, 9H-aromatic + 1H, CH-pyridine), 9.45 (s, 2H, NH_2, D_2O exchangeable); Anal. Calcd. for $C_{24}H_{19}ClN_6$ (426.9): C, 67.52; H, 4.49; Cl, 8.30; N, 19.69. Found: C, 67.83; H, 4.79; Cl, 8.60; N, 19.77%.

2-(4-Chlorobenzylideneamino)-4-(4-chlorophenyl)-6-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)pyrimidine (8)

A mixture of compound 7 (0.43 g, 0.001 mol) and benzaldehyde (0.11 g, 0.001 mol) was stirred under reflux in methanol (30 ml) in the presence of a few drops of glacial acetic acid 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 dioxane to give

compound 8 as brown crystals, 68%, mp 263-265ºC. FT-IR (KBr) v_{max}/ cm^{-1}: 3018(CH- aromatic), 2890 (CH- aliphatic), 1633 (C=N), 1525 (C=C); ^{1}H NMR δ 2.48(s, 3H, CH_3), 2.70 (s, 3H, CH_3), 7.35-8.35 (m, 17H, 14H-aromatic + 1H, CH-pyridine + 1H, CH-pyrimidine + 1H, CH=N); Anal. Calcd. for $C_{31}H_{23}ClN_6$ (515.01): C, 72.30; H, 4.50; Cl, 6.88; N, 16.32. Found: C, 72.58; H, 4.90; Cl, 7.10; N, 16.62%.

5-(4-Chlorophenyl)-4,5-dihydro-3-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-5-yl)pyrazole-1-carbothioamide (9)

A mixture chalcone 2c (0.76 g, 0.002 mol) and thiosemicarbazide (0.18 g, 0.001 mol) in and ethanolic sodium hydroxide (25%, 10 mL) was refluxed for 3 h.The reaction mixture was allowed to cool to room temperature, whereby a solid formed that was filtered off and crystallized from DMF-H_2O to give compound 9 as yellow crystals, 70%, mp 202-204ºC. FT-IR (KBr) v_{max}/ cm^{-1}: 3425, 3250 (NH_2), 3057 (CH- aromatic), 2927 (CH- aliphatic), 1580 (C=N), 1460 (C=C), 1370 (C=S), 1120 (C-N); ^{1}H NMR δ 2.52 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 3.46(dd, J_1=4.59 Hz, J_2= 8.25, 1H, CH_2-pyrazoline), 4.0(dd, J_1=4.68 Hz, J_2= 8.45, 1H, CH_2-pyrazoline), 5.15 (dd, J_1=1.68 Hz, J_2= 8.85, 1H, CH-pyrazoline), 6.70 (s, 1H, NH_2); 7.45-8.30 (m, 10H, 9H-aromatic + 1H, CH-pyridine); Anal. Calcd. for $C_{24}H_{21}ClN_6S$ (460.98): C, 62.53; H, 4.59; Cl, 7.69; N, 18.23; S, 6.96. Found: C, 62.82; H, 5.01; Cl, 8.01; N, 18.47; S, 7.16%; MS m/z (%): 460.42 (M^+, 76).

General procedure for the synthesis of 5-(4-Chlorophenyl)-4,5-dihydro-1-(4-substitutedthiazol-2-yl)-1-H-pyrazol-3-yl)-3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridines (10a,b)

A mixture of 9 (0.01 mol), chloroacetone or phenacyl bromide (0.01 mol) in ethanol (50 mL) was refluxed in the presence of anhydrous sodium acetate (0.9 g, 0.011 mol) for 4 h. The solid product separated from the hot mixture was filtered off, washed with water and recrystallized from the proper solvent.

5-(4-Chlorophenyl)-4,5-dihydro-1-(4-methylthiazol-2-yl)-1-H-pyrazol-3-yl)-3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine (10a).

A yellow crystals (EtOH), 70%, mp 325-327ºC. FT-IR (KBr) v_{max}/ cm^{-1}: 3066 (CH- aromatic), 2947 (CH- aliphatic), 1626 (C=N), 1562 (C=C);^{1}H NMR δ 2.55 (s, 3H, CH_3), 2.77 (s, 3H, CH_3), 3.19(dd, J_1=5.22 Hz, J_2= 7.35, 1H, CH_2-pyrazoline), 3.65 (dd, J_1=3.75 Hz, J_2= 9.31, 1H, CH_2-pyrazoline), 4.75 (dd, J_1=1.45 Hz, J_2= 8.52, 1H, CH-pyrazoline), 6.45 (s, 1H, CH-thiazole); 7.30- 8.47 (m, 10H, 9H-aromatic + 1H, CH-pyridine). Anal. Calcd. for $C_{27}H_{23}ClN_6S$ (499.03): C, 64.98; H, 4.65; Cl, 7.10; N, 16.84; S, 6.43. Found: C, 65.12; H, 4.98; Cl, 7.38; N, 17.09; S, 6.49%.

5-(4-Chlorophenyl)-4,5-dihydro-1-(4-phenylthiazol-2-yl)-1-H-pyrazol-3-yl)-3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridines (10b)

A yellow crystals (Dioxane),71%, mp 217-219ºC. FT-IR (KBr) v_{max}/ cm^{-1}: 3051 (CH- aromatic), 2962 (CH- aliphatic), 1637 (C=N), 1581(C=C); ^{1}H NMR δ 2.59 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 3.08 (dd, J_1=5.17 Hz, J_2= 8.12, 1H, CH_2-pyrazoline), 3.81 (dd, J_1=3.90 Hz, J_2= 9.65, 1H, CH_2-pyrazoline), 5.38 (dd, J_1=0.87 Hz, J_2= 6.88, 1H, CH-pyrazoline), 6.20 (s, 1H, CH-thiazole); 7.10- 8.55 (m, 15H, 14H-aromatic + 1H, CH-pyridine). Anal. Calcd. for $C_{32}H_{25}ClN_6S$ (561.1): C, 68.50; H, 4.49; Cl, 6.32; N, 14.98; S, 5.71. Found: C, 68.83; H, 4.67; Cl, 6.59; N, 15.27; S, 5.95%.

General procedure for the synthesis of 1-(1-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)ethylidene)-2-(4-substitutedthiazol-2-yl)hydrazine (12a,b)

A mixture of thiosemicarbazone 11 (0.001 mol), chloroacetone or phenacyl bromide (0.001 mol) in ethanol (30 mL) was refluxed in the presence of anhydrous sodium acetate (0.9 g, 0.011 mol) for 4 h. The solid product separated from the hot mixture was filtered off, washed with water and recrystallized from the proper solvent.

1-(1-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)ethylidene)-2-(4-methylthiazol-2-yl)hydrazine (12a)

A yellow crystals (MeOH), 71%, mp 292-294ºC. FT-IR (KBr) v_{max}/ cm^{-1}: 3290 (NH), 3060 (CH- aromatic), 2887 (CH-aliphatic), 1629 (C=N), 1567 (C=C); ^{1}H NMR δ 1.25 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 3.08(s, 3H, CH_3), 6.25 (s, 1H, CH-thiazole); 6.95- 8.45 (m, 6H, 5H-aromatic + 1H, CH-pyridine), 9.35 (s, 1H, NH, D_2O-exchangeable). Anal. Calcd. for $C_{20}H_{20}N_6S$ (376.48): C, 63.81; H, 5.35; N, 22.32; S, 8.52. Found: C, 63.96; H, 5.68; N, 22.42; S, 8.83%.

1-(1-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)ethylidene)-2-(4-phenylthiazol-2-yl)hydrazine (12b)

A yellow crystals (Acetone/H_2O), 67%, mp 225-227ºC. FT-IR (KBr) v_{max}/ cm^{-1}: 3325 (NH), 3048 (CH- aromatic), 2966 (CH- aliphatic), 1634 (C=N), 1536 (C=C); ^{1}H NMR δ 0.95 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 6.70 (s, 1H, CH-thiazole); 7.37- 8.35 (m, 11H, 10H-aromatic + 1H, CH-pyridine), 9.15 (s, 1H, NH, D_2O-exchangeable). Anal. Calcd. for $C_{25}H_{22}N_6S$ (438.55): C, 68.47; H, 5.06; N, 19.16; S, 7.32. Found: C, 68.69; H, 5.41; N, 19.36; S, 7.57%.

5-Methyl-5-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-1,2,4-triazolidine-3-thione (13)

Method A

A mixture of 11 (0.34 g, 0.001 mol) in methanol (20 mL) and hydrochloric acid (1.5 mL) was heated on the water-bath for 30 minutes. The solid obtained after cooling was filtered off, washed with water till acid-free then crystallized from methanol to give compound 13as yellow crystals, 62%, mp 145-147ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3360, 3345 and 3220 (NH), 3050 (CH- aromatic stretch), 2980(CH- aliphatic stretch), 2720 (C=S), 1575 (C=N); ^{1}H NMR δ 1.55 (s, 3H, CH_3), 2.0 (s, 1H, NH), 2.15 (s, 1H, NH), 2.25 (s, 1H, NH), 2.50 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 7.35- 8.20 (m, 6H, 5H-aromatic + 1H, CH-pyridine), Anal. Calcd. for $C_{17}H_{18}N_6S$ (338.47): C, 60.33; H, 5.36; N,24.83; S, 9.47. Found: C, 60.71; H, 5.59; N, 25.12; S, 9.68%; MS: *m/z* 338.19(M^+,68).

Method B

A mixture 1 (0.26 g, 0.001 mol) and thiosemicarbazide (0.09g, 0.001 mole) in methanol (20 mL) and hydrochloric acid (1.5 mL) was heated on the water-bath for 2 h. The solid obtained after cooling was filtered off, washed with water till acid-free then crystallized from methanol to give compound 13. All of analytical and spectral data are completely identical as described in method A.

General procedure for the synthesis of 14 and 15

A mixture of 13 (0.01 mol), 1-bromo-2-methoxyethane (0.01 mol) and K_2CO_3 (1.50 g) in 20 mL DMF was stirred at 75–80°C overnight. Then, 30 mL water was added and the resulting mixture was extracted with $CHCl_3$. The organic layer wasseparated, washed with water (4x30 mL), and dried over Na_2SO_4. After solvent evaporation, the residue was purified by column chromatography on silica gel using $CHCl_3$–CH_3COOCH_3 (70:30) as an eluent to afford **14** and **15**.

5-[5-(2-Methoxy-ethylsulfanyl)-3-methyl-3,4-dihydro-2H-[1,2,4]triazol-3-yl)-3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine (14)

A yellow crystals, 79%, mp 176-178ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3405 and 3325 (NH), 3048 (CH- aromatic), 2966 (CH- aliphatic), 1611 (C=N) 1589 (C=C), 1226 (C-O); ^{1}H NMR δ: 1.35 (s, 3H, CH_3), 2.2 (s, 1H, NH), 2.40 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 3.30 (s, 3H, OCH_3), 3.75 (t, 2H, S-CH_2), 4.15(t,2H, CH_2-O), 7.20- 8.15 (m, 6H, 5H-aromatic + 1H, CH-pyridine), 8.30 (s, 1H, NH, D_2O-exchangeable); ^{13}C-NMR (DMSO-d6) δ:12.30 (CH_3), 14.25(CH_3), 30.85 (CH_3), 33.60(S-CH_2), 50.72(C), 54.26(O-CH_3), 63.8 (CH_2), 104.12(C), 117.81(2CH), 124.30(CH), 125.0(CH), 130.45(2CH), 135.16(CH), 137.77(C), 139.23(C), 150.24(C), 152.0(C-S), 155.71(C); Anal. Calcd. for $C_{20}H_{24}N_6OS$ (396.51): C, 60.58; H, 6.10; N, 21.20; S, 8.09%. Found: C, 60.93; H, 6.39; N, 21.42; S, 8.31%. MS: *m/z* 396.88(M^+,100)

5-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-2-(2-methoxy-ethyl)-5-methyl-[1,2,4]triazolidine-3-thione (15)

A yellow crystals, 14.8%, mp 192-194ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3390 and 3345 (N-H stretching), 3005 (C-H aromatic stretching), 2970 (C-H aliphatic stretching), 1600(C=C stretching), 1215(C-O, stretching): ^{1}H NMR δ: 1.30 (s, 3H, CH_3), 2.2 (s, 1H, NH), 2.30 (s, 1H, NH), 2.55 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 3.85 (s, 3H, OCH3), 4.60 (t, 2H, N-CH_2), 4.75 (t,2H, CH_2-O), 7.30- 8.25 (m, 6H, 5H-aromatic + 1H, CH-pyridine); ^{13}C-NMR (DMSO-d6) δ:11.78 (CH_3), 13.95(CH_3), 30.48 (CH_3), 43.34(O-CH_3), 46.27(N-CH_2), 50.61(C), 60.44 (CH_2), 105.23(C), 115.71(2CH), 124.63(CH), 125.11(CH), 129.64 (2CH), 133.33(CH), 137.81(C), 139.90(C), 151.11(C), 152.47(C), 172.0(C=S); Anal. Calcd. for $C_{20}H_{24}N_6OS$ (396.51): C, 60.58; H, 6.10; N, 21.20; S, 8.09%. Found: C, 60.76; H, 6.42; N, 21.58; S, 8.28%.

2-[2-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-2-methyl-6-oxo-1,2,5,6-tetrahydro-[1,3]thiazolo[3,2-b][1,2,4]triazol-5-yl]-N-phenyl-acetamide (16)

A mixture of 13 (0.66 g,, 0.002 mol) and N-phenylmaleimide (0.35 g, 0.02 mol) was refluxed for 2 h in glacial acetic (10 mL). After cooling to r. t., the reaction mixture was poured into 50 mL of water. The precipitated colorless powder was filtered off, washed with methanol and recrystallized from ethanol to give compound 16 as yellow crystals, 65%, mp 183-185ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3395, 3350 (NH), 3088 (CH- aromatic), 2987(CH- aliphatic), 1710 (C=O), 1690 (C=O), 1585 (C=N); ^{1}H NMR δ 1.40 (s, 3H, CH_3), 2.15 (s, 1H, NH), 2.55 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 2.80 (dd, J_1=3.66 Hz, J_2= 8.70, 1H, CH_2), 3.30 (dd, J_1= 4.35 Hz, J_2= 1.85, 1H, CH_2), 4.60 (dd, J_1=3.50 Hz, J_2= 0.98, 1H, CH- thiazole), 7.40- 8.25 (m, 11H, 10H-aromatic + 1H, CH-pyridine), 13.30 (s, 1H, NH); ^{13}C-NMR (DMSO-d6) δ:12.55(CH_3), 15.70(CH_3), 29.11(CH_3), 38.65 (CH_2), 48.45(CH cyclic), 69.28(C), 106.23(C), 118.09 (2CH), 120.35(2CH), 123.15 (CH), 124.98(CH), 128.66 (2CH), 129.64 (2CH), 130.45 (C), 137.07(C), 140.33(C), 141.72 (C), 150.51(C), 152.18(C), 159.36(C), 164.11 (C), 173.25(C=O cyclic), 178.85 (C=O); Anal. Calcd. for $C_{27}H_{25}N_7O_2S$ (511.60): C, 63.39; H, 4.93; N,19.16; S, 6.27. Found: C, 63.66; H, 5.16; N, 19.38; S, 6.60%; MS: *m/z* 511.39(M^+,73).

3,6-Dimethyl-1-phenyl-5-(1,2,3-thiadiazol-4-yl)-1H-pyrazolo[3,4-b]pyridine (18)

An excess amount of thionyl chloride (10 mL) was stirred at room temperature and the semicarbazone 17 (0.97 g, 0.003 mol) was added in several portions. The mixtures were stirred at r.t. overnight until no more hydrogen chloride was produced, the product was washed with diethyl ether, dried, crystallized from benzene / pet.ether 40-60°C to afford brown powder of 18, 83%, mp 130-132ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3075(CH- aromatic), 2880(CH- aliphatic), 1590 ((C=N), 1475((C=C); ^{1}H NMR δ 2.38 (s, 3H, CH_3), 2.55(s, 3H, CH_3), 7.20- 8.40 (m, 6H, 5H-aromatic + 1H, CH-pyridine), 9.75(s,

1H, CH-thiadiazole); Anal. Calcd. for $C_{16}H_{13}N_5S$ (307.37): C, 62.52; H, 4.26; N,22.78; S, 10.43. Found: C, 62.81; H, 4.57; N, 23.08; S, 10.62%; MS: *m/z* 307.13 (M^+, 73).

3,6-Dimethyl-1-phenyl-5-(1,2,3-selenadiazol-4-yl)-1H-pyrazolo[3,4-b]pyridine (19)

The semicarbazone derivative 17 (0.97 g, 0.003 mol) was dissolved in glacial acetic acid (25 mL). To the hot solution, selenium dioxide powder (0.55 g, 0.005 mol) was added and the reaction mixture was stirred for 24 h. The mixture was filtered and the filtrates poured into ice water and extracted with $CHCl_3$ (3×50 mL). The combined organic layers were washed with a saturated sodium carbonate solution, dried over anhydrous sodium sulfate. After evaporation of the solvent the residue was crystallized from toluene to give brown powder of 19, 74%, mp 112-114ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3007(CH- aromatic), 2985(CH- aliphatic), 1595 (C=N), 1480((C=C), 820 (C-Se-N); ^{1}H NMR δ 2.45 (s, 3H, CH_3), 2.61(s, 3H, CH_3), 7.33- 8.29 (m, 6H, 5H-aromatic + 1H, CH-pyridine), 10.12(s, 1H, CH-selenadiazole); Anal. Calcd. for $C_{16}H_{13}N_5Se$ (354.27): C, 54.24; H, 3.70; N,19.77%. Found: C, 54.60; H, 3.92; N, 20.11; MS: *m/z* 354.19 (M^+, 56).

3-Hydroxy-3-(2-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-2-oxoethyl)indolin-2-one (20).

To a solid homogenous mixture of 1 (0.52 g, 0.002 mol) and isatine (0.3 g, 0.002 mol), 10 drops of dimethylamine was added and the mixture stirred for 15-30 minutes and a colorless solid formed was recrystallized from ethanol to give buff powder of 20, 61%, mp 191-193ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3500 (OH), 3410 (NH), 3080(CH- aromatic), 2970(CH aliphatic), 1705(C=O), 1630(C=O) 1580(C=N), 1465(C=C); ^{1}H NMR δ 2.20 (s, 1H, OH), 2.50 (s, 3H, CH_3), 2.70(s, 3H, CH_3), 3.15 (s, 2H, CH_2), 7.35- 8.40 (m, 10H, 9H-aromatic + 1H, CH-pyridine), 8.60 (s, 1H, NH); Anal. Calcd. for $C_{24}H_{20}N_4O_3$ (412.44): C, 69.89; H, 4.89; N,13.58%. Found: C, 70.27; H, 4.95; N, 13.83.

3-(2-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-2-oxoethylidene)indolin-2-one (21).

A mixture of 20 (0.4g, 0.001 mol), ethanol 25 mL and 40 mL of dilute HCl solution (25%), was allowed to stand overnight, fine orange needles were formed and recrystallized from ethanol to give buff powder of 21, 69%, mp 210-212ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3395 (NH), 3087(CH-aromatic), 2990(CH- aliphatic), 1675(C=O), 1630(C=O) , 1565(C=N), 1480(C=C) ; ^{1}H NMR δ 2.55 (s, 3H, CH_3), 2 .68(s, 3H, CH_3), 7.28- 8.55 (m, 11H, 10H-aromatic + 1H, CH-pyridine), 8.70 (s, 1H, NH); Anal. Calcd. for $C_{24}H_{18}N_4O_2$ (394.43): C, 73.08; H, 4.60; N,14.20%. Found: C, 73.31; H, 4.93; N, 14.54%.

5-′(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-2′ , 4′-dihydrospiro[indol-3,3′-pyrazol]-2-(1H)-one (22).

Method A

A mixture of 21 (0.4 g, 0.001 mol) and hydrazine hydrate (0.20 mL) in ethanol (25 mL) was heated under reflux for 4 h, then left to cool, the residual material was filtered off and recrystallized from ethanol to afford yellow crystals of 22, 71%, mp 211-213ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3420, 3365 (NH), 3069(CH- aromatic), 2877(CH- aliphatic) , 1646(C=O), 1570(C=N), 1473(C=C); ^{1}H NMR δ 2.60 (s, 3H, CH_3), 2 .70(s, 3H, CH_3), 3.60 (s, 2H, CH_2), 6.30 (s, 1H, NH), 7.35- 8.48 (m, 10H, 9H-aromatic + 1H, CH-pyridine), 8.63(s, 1H, NH); Anal. Calcd. for $C_{24}H_{20}N_6O$ (408.46): C, 70.57; H, 4.94; N,20.58%. Found: C, 70.84; H, 5.11; N, 20.79%. MS: *m/z* 408.30 (M^+, 82).

Method B.

To a mixture of 1 (0.52 g, 0.002 mol) and isatin (0.3 g, 0.002 mol) and, dimethylamine (5 drops) was added and the mixture was stirred for 30 min at room temperature. To the formed solid, glacial acetic acid (10 mL) and concentrated hydrochloric acid (3 drops) were added and the reaction mixture was heated at 80 ºC for 30 min. Hydrazine hydrate (0.02 mol) was added to the previous reaction mixture and heating was continued at 80 ºC for 1 h to give spiro compound 22. The product was identical with that formed by method A.

5-′(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-2′ -phenyl-4′-dihydrospiro[indol-3,3′-pyrazol]-2-(1H)-one (23)

A mixture of 21 (0.4 g, 0.001 mol) and phenyl hydrazine (0.5 mL) in ethanol (25 mL) was heated under reflux for 7 h, then left to cool, the residual material was filtered off and recrystallized from ethanol to afford yellow crystals of 23, 63%, mp 320-322ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3087(CH-aromatic), 2889(CH- aliphatic), 1650(C=O), 1565(C=N), 1482(C=C); ^{1}H NMR δ 2.64 (s, 3H, CH_3), 2.75(s, 3H, CH_3), 3.49 (s, 2H, CH_2), 7.25- 8.51 (m, 15H, 14H-aromatic + 1H, CH-pyridine), 8.60 (s, 1H, NH); Anal. Calcd. for $C_{30}H_{24}N_6O$ (484.55): C, 74.36; H, 4.99; N,17.34%. Found: C, 70.55; H, 5.23; N, 17.70%.

3′-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)- 4′-dihydrospiro[indol-3,5′-isoxazole]-2-(1H)-one (24).

A mixture of compound 21 (0.4 g, 0.001 mol), hydroxylamine hydrochloride (0.14 g, 0.002 mol) and anhydrous sodium acetate (0.085 g, 0.003 mol) in absolute ethanol (10 mL) was refluxed for 6 h. After cooling, the reaction mixture was poured onto ice-water (50 mL). The solid that formed was filtered off, air dried and recrystallized from absolute ethanol to afford white crystals of 24, 66%, mp 273-275ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3090(CH- aromatic), 2880(CH- aliphatic), 1655(C=O) , 1560(C=N), 1460(C=C) 1250 (C-O-N); ^{1}H NMR δ 2.66 (s, 3H, CH_3), 2.79(s, 3H, CH_3), 3.25 (s, 2H, CH_2), 7.25- 8.45 (m, 10H, 9H-aromatic + 1H, CH-pyridine), 8.65 (s, 1H, NH); Anal. Calcd. for $C_{24}H_{19}N_5O_2$ (409.44): C, 70.40; H, 4.68; N,17.10%. Found: C, 70.73; H, 4.92; N, 17.50%.

6′-(3,6-Dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)- spiro[indol-3,4′ -pyrimidin-2′- (1H) thione]-2-(1H)-one (25).

A mixture of chalcone 21(0.4 g, 0.001 mol), thiourea (0.076 g, 0.001 mol) and potassium hydroxide (1.0 g) in ethanol (40 ml) was refluxed on a boiling water bath for one hour. The reaction mixture was left overnight and then concentrated under reduced pressure. The solid residue was collected, washed with water and recrystallized from ethanol to yield yellow crystals of 25, 68%, mp 172-174ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3340, 3210, 3180 (NH), 3085(CH- aromatic), 2890(CH-aliphatic), 1645(C=O) , 1555(C=N), 1490(C=C) 1180 (C=S); ^{1}H NMR δ 2.55 (s, 3H, CH_3), 2.75(s, 3H, CH_3), 6.20 (s, 1H, CH-pyrimidine), 7.15- 8.35 (m, 10H, 9H-aromatic + 1H, CH-pyridine), 8.65(s, 1H, NH), 8.85 (s,br, 1H, NH), 9.90 (s, br, 1H, NH); Anal. Calcd. for $C_{25}H_{20}N_6OS$ (452.53): C, 66.35; H, 4.45; N,18.57; S, 7.09%. Found: C, 66.59; H, 4.78; N, 18.88; S, 7.32%.

2-Cyano-N'-[1-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-ethylidene]acetocarbohydrazide (26)

To a solution of cyanoacetylhydrazine (0.99 g, 0.001 mol) in 1,4-dioxane (20 mL), compound 1 (0.26 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 4 h then poured onto a beaker containing an ice/water mixture. The formed solid product was collected by filtration and recrystallized from ethanol to give white crystals 0f 26, 68%, mp 209-211ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3365(NH), 3090(CH-aromatic), 2880(CH-aliphatic), 2245 (CN), 1679(C=O), 1570(C=N), 1475(C=C); ^{1}H NMR δ 1.90 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.78(s, 3H, CH_3), 4.40 (s, 2H, CH_2),7.26- 8.30 (m, 6H, 5H-aromatic + 1H, CH-pyridine); Anal. Calcd. for $C_{19}H_{18}N_6O$ (346.39): C, 65.88; H, 5.24; N, 24.26%. Found: C, 66.13; H, 5.49; N, 24.60%; ^{13}C-NMR (DMSO-d6) δ:11.95 (CH_3), 12.80(CH_3), 15.60(CH_3), 26.80(CH_2), 105.79(C), 113.64 (CN), 118.50(2CH), 124.91(C), 126.11(C), 128.76(2CH), 132.81(C), 136.34(CH), 138.13(C), 150.41(C), 156.37(C), 158.22(C), 174.19 (C=O); MS: *m/z* 346.54 (M^+, 62).

3-(4-Chlorophenyl)-2-cyano-N'-[1-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-ethylidene]acrylo-hydrazide (27)

To a solution of 26 (0.35 g, 0.001 mol) in 1,4-dioxane (20 mL), p-chlorobenzaldehyde (0.14 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 6 h then poured onto a beaker containing an ice/water mixture. The formed solid product was collected by filtration and and recrystallized from ethanol to give white crystals of 27, 60%, mp 103-105ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3345(NH), 3087(CH- aromatic), 2969(CH- aliphatic), 2238 (CN), 1660(C=O), 1620(C=N), 1498(C=C); ^{1}H NMR δ 1.65 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.65(s, 3H, CH_3), 7.17- 8.33 (m, 11H, 9H-aromatic + 1H, CH-pyridine + 1 H, C=CH); Anal. Calcd. for $C_{26}H_{21}ClN_6O$ (468.94):C, 66.59; H, 4.51;Cl, 7.56; N, 17.92%. Found: C, 66.81; H, 4.79; Cl, 7.83; N, 18.16%.

2-Oxo-N'-[1-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-ethylidene]-2H-chromene-3-carbohydrazide (28)

To a solution of 26 (0.35 g, 0.001 mol) in 1,4-dioxane (20 mL), salicylaldehyde (0.12 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 5 h then poured onto a beaker containing an ice/water mixture. The formed solid product was collected by filtration and and recrystallized from ethanol to give white crystals of 28, 64%, mp 216-218ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3368(NH), 3080(CH-aromatic), 2943(CH- aliphatic), 1670(C=O), 1650(C=N), 1490(C=C); ^{1}H NMR δ 1.83 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.66(s, 3H, CH_3), 7.20- 8.35 (m, 11H, 10H-aromatic + 1H, CH-pyridine), 8.72 (s, 1H, CH-Coumarin); Anal. Calcd. for $C_{26}H_{21}N_5O_3$ (451.48):C, 69.17; H, 4.69; N, 15.51%. Found: C, 69.33; H, 4.75; N, 15.77%.

4-Amino-1-[1-(3,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-yl)-ethylideneamino]-2oxo-4-phenyl-1,2-dihydro pyridine-3,5-dicarbonitrile (29)

To a solution of 26 (0.35 g, 0.001 mol) in 1,4-dioxane (20 mL), 2-benzylidenemalononitrile (0.15 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 6 h then poured onto a beaker containing an ice/water mixture. The formed solid product was collected by filtration and and recrystallized from ethanol to give pale yellow crystals of 29, 77%, mp 324-326ºC. FT-IR (KBr) ν_{max}/ cm^{-1}: 3452, 3368(NH_2), 3095(CH- aromatic), 2971(CH- aliphatic),2229, 2189 (2CN), 1665(C=O), 1641(C=N), 1488(C=C); ^{1}H NMR δ 2.11 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.64(s, 3H, CH_3), 3.87 (s, 2H, NH_2), 7.27- 8.48 (m, 11H, 10H-aromatic + 1H, CH-pyridine); Anal. Calcd. for $C_{29}H_{22}N_8O$ (498.54):C, 69.87; H, 4.45; N, 22.48%. Found: C, 70.19; H, 4.58; N, 22.65%.

ANTIMICROBIAL ACTIVITY

The antimicrobial activity of 22 selected 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. [31] 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. Chloramphenicol and Clotrimazole were used as standards. Data are represented as % inhibition with reference to standards in (Table1).

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