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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NOVEL 1- PHENYL 1-*H*-PYRAZOLO[3,4-D]PYRIMIDINE DERIVATIVES

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Pyrazolo[3,4-d]Pyrimidine 1,2,4 triazoles 1,3,4 thiadiazoles thiazoles and antimicrobial activity

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ABSTRACT: Novel 1- Phenyl 1-H-Pyrazolo[3,4-d]Pyrimidine Derivatives were synthesized from the key intermediate chloro derivative (1).The conversion of chloro derivative(1) into 1,2,4 triazoles, 1,3,4 thiadiazoles and thiazoles was accomplished with the intermediacy of the ester(2) and hydrazide (3). Moreover triazole ring was annelated to the pyrazolopyrmidine core in two step reaction involving reaction with acid hydrazides followed by intramolecular cyclization in boiling glacial acetic acid Majority of the synthesized compounds showed variable antimicrobial activity on the hand tricyclic compounds(9a-b) were deprived from any antimicrobial activity against the tested micro-organisms. The hydrazine carbothioamide derivative (4) and 1,3,4 thiadiazole derivative (6) showed antimicrobial activity against *Streptococcus pneumoniae* and *Bacillis subtilis* comparable to ampicillin and a moderate antifungal activity against Asp. *Fumigates* and *C. alb*.

INTRODUCTION: Antimicrobial agents are urgently needed to combat life-threatening microbial infections. The high speed of development of bacterial resistance compared to the slow pace of new antibiotic discovery has led some experts to worn of "post –antibiotic era".[1, 2] Pyrazolo[3,4-d]pyrimidines and their annelated triazole analogues demonstrated a wide range of biological activities including anticancer, anti-inflammatory, xanthine oxidase inhibitory, and antimicrobial.[3]

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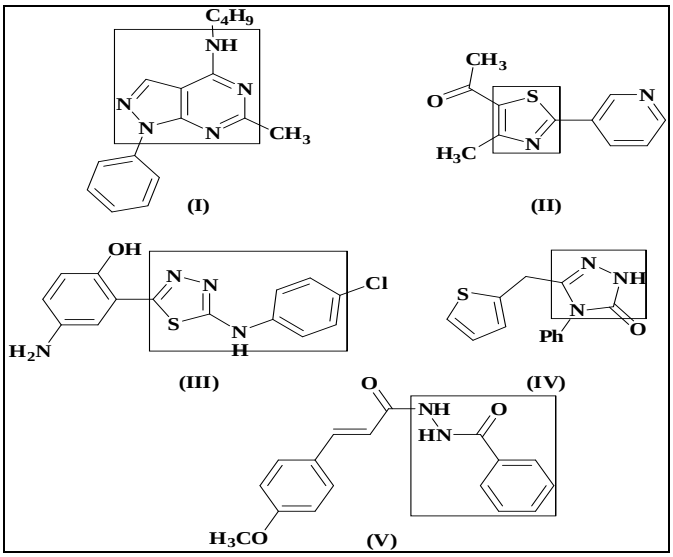
The spectrum of antimicrobial activity of these heterocycles comprises antibacterial [4-6], antifungal [7, 8], antitubercular [9,10] and antiviral activities.[11] A literature survey revealed the importance of thiazole(II)[12-16], 1,3,4-thiadiazole(III)[17,18], 1,2,4-triazoles(IV)[13-16] and N'-substituted acid hydrazides(V)[19] as promising antimicrobial agents. On the light of the above finding we anticipated that joining two or more biologically active heterocycles in a single molecule might improve antimicrobial activity.[20]

Thus the aim of the current investigation was to synthesize novel antimicrobial agents via linking pyrazolo[3,4-d]pyrimidine derivatives to 1,3,4-thiadiazole **(6)**, 1,2,4-triazole **(5)**, 1,3-thiazole **(7a,b)** and acid hydrazides **(4,8a-f)** as well as the construction of annelated pyrazolo[4,3-e][1,2,4]triazolo[4,3-c]pyrimidine derivatives

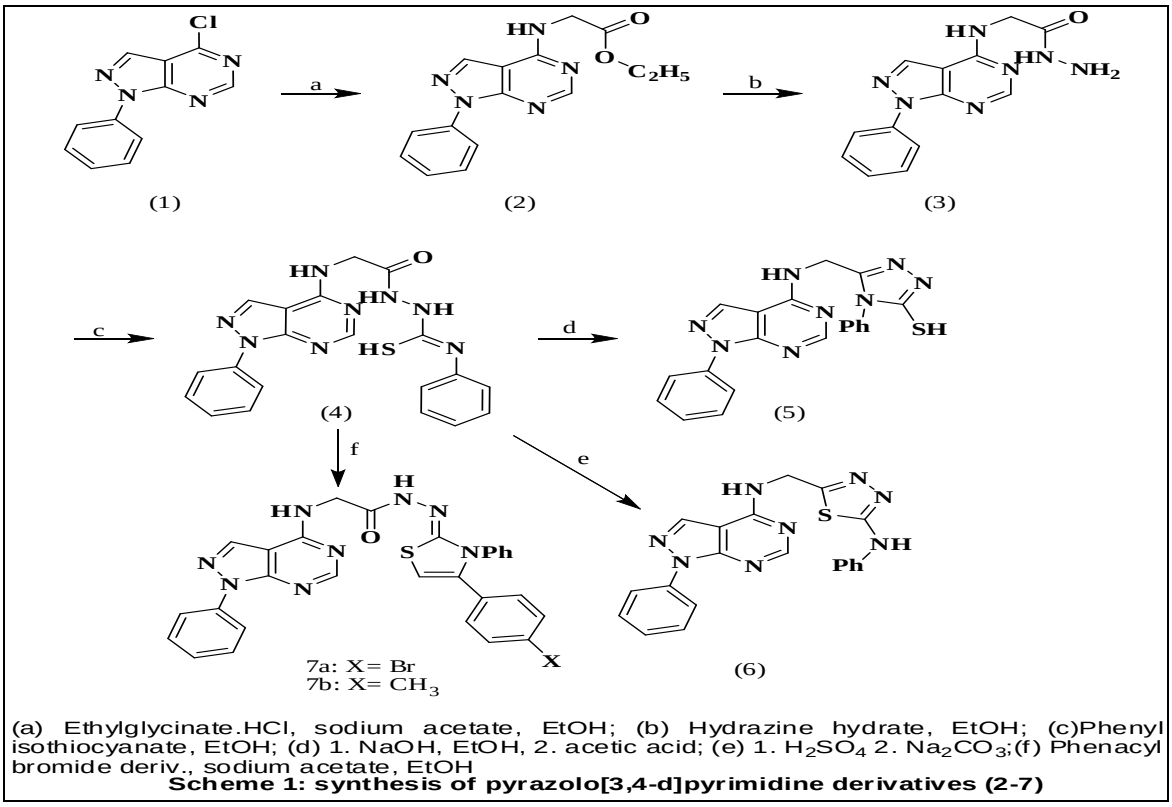
(9a,b).The newly synthesized compounds showed variable antimicrobial activity against Str. Pneumonia, *B. subtilis*, Ps. *Aeruginosa*, *E. col*, Asp. *Fumigates* and *C. alb*



RESULTS AND DISCUSSION:

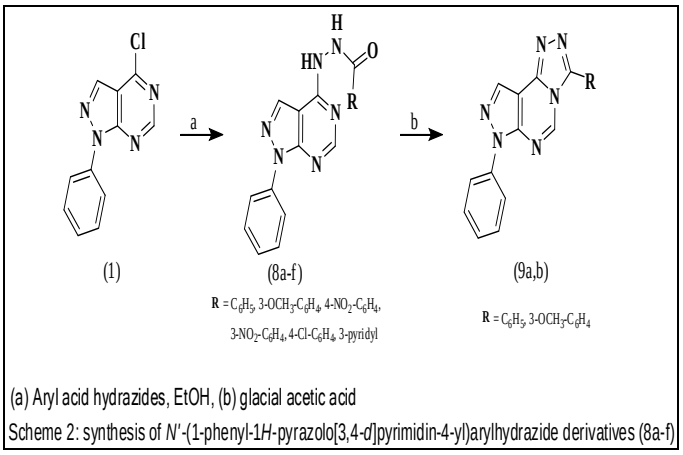
Chemistry: The key intermediate 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(1)** was synthesized from commercially available ethyl(ethoxymethylene)cyanoacetate following the reported procedure [21, 22] Novel pyrazolo[3,4-*d*]pyrimidine derivatives **[2, 3, 4, 5, 6, 7(a,b)]** were synthesized according to scheme**(1)**. 4-Chloro-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(1)** was converted into ethyl 2-[(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino]acetate **(2)** via reaction with ethyl glycinate in the presence of anhydrous sodium acetate.The ester 2 was reacted with hydrazine hydrate to afford the carbohydrazide **(3)** in 80% yield.

Condensation of carbohydrazide **(3)** with phenyl isothiocyanate resulted in formation of hydrazinecarbothioamide derivative **(4)**, which enabled the preparation of triazole **(5)**, thiadiazole **(6)** and thiazole **(7a,b)** derivatives. Cyclization of thiosemicarbazide intermediate **(4)** in ethanolic sodium hydroxide afforded the triazole-thiol derivative **(5).** Moreover, stiring of thiosemicarbazide intermediate **(4)** in conc. sulfuric acid at 25 ^{o}C resulted in the formation of 1,3,4-thiadiazole-2-amine derivative **(6)**. In addition, condensation of thiosemicarbazide derivative **(4)** with different phenacyl bromides resulted in formation of thiazole derivatives **(7a,b)** The structure of novel targets was confirmed by ^{1}HNMR, ^{13}CNMR, Mass Spectrometry and elemental analysis.**(scheme 1)**



SCHEME 1: SYNTHESIS OF PYRAZOL O [3,4-D]PYRIMIDINE DERIVATIVES(2-7)

Reaction of 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(1)** with benzoic acid hydrazide and its derivatives as well as nicotinic acid hydrazide resulted in the formation of compounds **(8a-f)** scheme **(2)**. The conversion of the substituted hydrazides **(8a-f)** into pyrazolo[4,3-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine **(9a-b)** by application of fusion at melting point was unsuccessful. This intramolecular cyclization aryl hydrazides **(8a-b)** was achieved by boiling in glacial acetic acid to afford **(9a-b)**.[23] On the other hand aryl hydrazides (8c-f) didn't undergo cyclization under the same reaction condition probably due to the presence of electron withdrawing group which might have hampered the enolization step of cyclization. The structure of novel targets was confirmed by 1HNMR, 13CNMR, Mass Spectrometry and elemental analysis. **(Scheme 2)**



(a) Aryl acid hydrazides, EtOH, (b) glacial acetic acid

Scheme 2: synthesis of *N′*-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)arylhydrazide derivatives (8a-f)

Antimicrobial Activity:

The novel pyrazolo[3,4-*d*]pyrimidine derivatives were evaluated for their *in vitro* antimicrobial activities against gram positive bacteria: *Streptococcus pneumoniae* (RCMB 010010) and *Bacillis subtilis* (RCMB 010067), gram negative bacteria: *Pseudomonas aeruginosa* (RCMB 010049) and *Escherichia coli* (RCMB 010058), and fungi: *Aspergillus fumigatus* (RCMB 02568) and *Candida albicans* (RCMB 05036) strains. Mean of inhibitory percent, minimium inhibitory concentrations (MICs, μg/mL) as well as IC50 (μg/mL) of these compounds were measured and are summarized in tables **1, 2, 3** respectively.

Mean inhibitory percents, MICs and IC50s of the compounds against the pathogenic gram positive bacteria, gram negative bacteria and fungal strains were determined using micro-broth assay using Ampicillin, Gentamicin and Amphotericin B as reference standards for gram positive bacteria, gram negative bacteria and fungi respectively.. The majority of the compounds, except 7-phenyl-3-(substituted) - 7*H* -pyrazolo [4,3-*e*][1,2,4]triazolo [4,3-c]-pyrimidine**(9a,b)** demonstrated good antimicrobial activity. Thiadiazole derivative **(6)** shows better activity against *Streptococcus Pnemounia* and equal activity against *Bacillus Subtilis* compared to ampicillin and slightly lower activity against *Aspergillus fumigatus* and *Candida albicans* compared to amphotericin B and against *Escherichia coli* compared to gentamicin.

Antimicrobial activity of the intermediate thiosemicarbazide derivative **(4)** lies between that of its cyclized forms; thiadiazole **(6)** and triazole **(5)**. It is noticed that cyclization of thiosemicarbazide derivative **(4)** with phenacyl bromide derivatives resulted in compounds **(7a,7b)** of lower antimicrobial activity than compound **(4)**. Antimicrobial activity results showed that substitution with p-bromo **(7a)** gives higher activity than substitution with p-methyl **(7b)**. Incorporation of aryl hydrazide derivatives into the 4-position of pyrazolo[3,4-*d*]pyrimidine resulted in the formation of compounds of varied antimicrobial activity according to the substituent on the phenyl of the carbohydrazide group and the rank order of antimicrobial activity for compounds **(8a-f)** was **8c > 8b > 8f > 8a > 8d > 8e**.

Experimental

Chemistry:

General: Melting points (°C) were determined with a Gallenkamp melting point apparatus (London, UK), and are uncorrected**.** Infrared spectral analyses were carried out by Shimadzu FT 8000 spectrometer and expressed in wave number (cm^{-1}), using KBr disc. ^{1}HNMR spectra were recorded on Varian Mercury-500 (500 MHz) (Palo Alto, CA, USA), Varian Gemini-300BB (300 MHz) (Foster City, CA, USA),and Bruker 400 MHz AV III (400MHz) (Biocity's, CA, USA) using dimethyl sulfoxide (DMSO)-*d6* as a solvent and tetramethylsilane (TMS) as internal standard (chemical shift in δ, ppm). ^{13}CNMR spectra were recorded on Varian Gemini-300BB (100 MHz)

(Foster City, CA, USA) using dimethyl sulfoxide (DMSO)-*d6* as a solvent and tetramethylsilane (TMS) as internal standard (chemical shift in δ, ppm). Mass spectra were determined using a GC/MS Shimadzu QP1000EX (Shimadzu corporation, Tokyo, Japan) with ionization energy 70 eV. Elemental Analyses were determined using the Heraeus (Hanay, Germany) Vario EL-III (Elementar) CHNS analyzer (Hanay, Germany) and automatic Elemental Analyzer CHN model 2400 Perkin Elemer (USA). All reactions were monitored by thin-layer chromatography (TLC) using Silica gel 60 GF245 (E-Merck-Germany) and were visualized by iodine vapors or by UV-lamp at wavelength λ 254nm .All reagents were purchased from Sigma-Aldrich and Acros and were used without purification unless otherwise indicated.

Ethyl 2-[(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino]acetate (2):

To a solution of 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(III)** (10 gm, 43.4 mmol) in ethanol (40 mL), ethyl glycinate hydrochloride (6 gm, 43.4 mmol) was added in the presence of fused sodium acetate (7.13 gm, 86.8 mmol) . The reaction mixture was refluxed for 18 hours then cooled, then the white precipitate formed was filtered, washed with water, dried and purified by crystallization from petroleum ether to give the title compound (73%), m.p.= 167-169 °C. **^{1}HNMR** (400 MHz, DMSO-d_6) δ: 1.21 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3), 4.14 (q, *J* = 6.1 Hz, *J* = 12.3 Hz, 2H, OCH_2CH_3), 4.31 (d, *J* = 6 Hz, 2H, NH-CH_2-COO), 7.34 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.56 (t, *J* = 8 Hz, 2H, Ar-H), 8.19 (d, *J* = 7.6 Hz, 2H, Ar-H), 8.39 (s, 1H, Ar pyrazole C3-H), 8.45 (s, 1H, pyrimidine C6-H), 8.92 (s, 1H, NH-CH_2-$CO_2C_2H_5$, exchangeable) ppm; Analysis for $C_{15}H_{15}N_5O_2$ (297.31): Calcd: C, 60.60; H, 5.09; N, 23.56,Found: C, 60.84; H, 5.32; N, 23.92%.

2-[(1-Phenyl-1*H*-pyrazolo[3,4 -*d*] pyrimidin – 4 - yl) amino]acetohydrazide (3)

To a solution of ethyl 2-[(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-*yl*)amino]acetate **(2)** (5 gm, 16.8mmol) in ethanol (20mL), hydrazine hydrate (5mL, 102mmol) was added then the mixture was refluxed for 6 hours. After cooling, the formed precipitate was filtered, washed with water, dried then recrystallized from ethanol, to afford the title compound (80%), m.p.= 138-140 °C. **^{1}HNMR** (400 MHz, DMSO-d_6) δ: 4.15 (d, *J* = 5.92 Hz, 2H, CH_2) 4.51 (s, 2H, NH_2), 7.35 (t, *J* = 7.36 Hz, 1H, Ar-H), 7.55 (t, *J* = 8.24, 2H, Ar-H), 8.19 (d, J = 7.72 Hz, 2H, Ar-H), 8.37 (s, 1H, pyrazole C3-H), 8.45 (s, 1H, pyrimidine C6-H), 8.77 (s, 1H, C-NH-CH_2), 9.25 (s, 1H, CO-NH-NH_2) ppm. **^{13}CNMR** (100 MHz, DMSO-d_6) δ: 57.21, 102.08, 120.21, 120.99, 126.22, 129.02, 129.21, 134.30, 138.87, 152.73, 155.82, 156.66, 168.10 ppm; Analysis for $C_{13}H_{13}N_7O$ (283.29): **Calcd:** C, 55.12; H, 4.63; N, 34.61, **Found:** C, 55.35; H, 4.44; N, 33.92%

***N*-Phenyl-2-{2-[(1-phenyl - 1*H* - pyrazolo [3,4-*d*] pyrimidin-4-yl)amino] acetyl} hydrazine carbo thioamide (4):**

To a solution of 2-[(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino]acetohydrazide **(3)** (0.5gm, 1.8 mmol) in ethanol (10 mL) equivalent amount of phenyl isothiocyanate (0.25gm, 1.8 mmol) was added. The reaction mixture was refluxed for 15 hours. The separated solid was filtered, washed with ethanol and crystallized from ethanol\DMF, affording the title compound (76%), mp=154°C. **^{1}HNMR** (500 MHz, DMSO-d_6) δ: 4.61 (s, 2H, CH_2), 7.32-7.40 (m, 6H, Ar-H), 7.50 (d, *J* = 6.9 Hz, 2H, Ar-H), 8.13 (d, *J* = 7.65 Hz, 2H, Ar-H), 8.20 (s, 1H, pyrazole C3-H), 8.30 (s, 1H, pyrimidine C6-H), 8.82 (s, 3H, NH), 13.89 (s, 1H, SH, exchangeable) ppm; Analysis for$C_{20}H_{18}N_8OS$ **(**418.47): **Calcd:** C, 57.40; H, 4.34; N, 26.78, **Found:** C, 57.46; H, 4.41; N, 26.63%.

4-Phenyl – 5 - {[(1-phenyl - 1*H* – pyrazolo [3,4-*d*] pyrimidin-4-yl)amino]methyl}-4*H*-1,2,4-triazole-3-thiol (5)

To a solution of *N*-phenyl-2-{2-[(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl) amino] acetyl} hydrazinecarbothioamide **(4)** (0.1gm, 0.25 mmol) in ethanol (10mL), sodium hydroxide (0.1gm, 2.5 mmol) was added. The reaction mixture was refluxed for 6 hours. The solution is neutralized using glacial acetic acid and the separated solid was filtered, washed with water and crystallized from Chloroform/Petroleum ether to obtain the title compound (63%), mp=260-262 °C, **IR: ν =** 3261 (NH), 3049 (C-H aromatic), 2929 (C-H aliphatic), 1597 (C-C aromatic), 1302 (C-N aromatic amine).**^{1}HNMR** (400 MHz, DMSO-d_6) δ: 4.65 (d, *J* = 5.24 Hz , 2H, CH_2), 7.33-7.45 (m, 6H, Ar-H),

7.55 (t, *J* = 8.16 Hz, 2H, Ar-H), 8.16 (d, *J* = 7.72 Hz, 2H, Ar-H), 8.25 (s, 1H, pyrazole C3-H), 8.33 (s, 1H, pyrimidine C6-H), 8.83 (s, 1H, NH), 13.52 (s, 1H, SH) ppm. **^{13}CNMR** (100 MHz, DMSO-d_6) δ: 48.22, 102.83, 120.26, 121.00, 126.24, 128.09, 129.16, 130.54, 133.33, 138.76, 145.25, 146.85, 149.71, 153.24, 157.10, 168.20 ppm; Analysis for $C_{20}H_{16}N_8S$ (400.46) **Calcd;** C, 59.98; H, 4.03; N, 27.98**, Found;** C, 60.12; H, 3.89; N, 28.22%.

***N*-Phenyl-5-{[(1-phenyl - 1*H* - pyrazolo [3,4-*d*] pyrimidin-4-yl)amino]methyl}-1,3,4-thiadiazol-2-amine (6):**

Compound **(4)** (0.1gm, 0.25 mmol) was dissolved in sulphuric acid (5mL). The mixture is stirred at room temperature for 24 hours. The mixture is poured on ice then neutralized with 10 M sodium carbonate**.** The separated solid was filtered, washed with water and crystallized from chloroform/ petrolium ether, to obtain the title compound (75 %), mp = 224-227 °C. **IR: ν =** 3399 (NH), 3275 (NH), 3049 (C-H aromatic), 2928 (C-H aliphatic), 1596 (C-C aromatic), 1304 (C-N aromatic amine).

^{1}HNMR (400 MHz, DMSO-d_6) δ: 4.65 (s, 1H, NH-Ph), 4.77 (d, *J* = 4.6 Hz, 2H, CH_2), 7.09 (d, *J* = 5.48 Hz, 1H, Ar-H), 7.33 - 7.48 (m, 4H, Ar-H), 7.58 (t, *J* = 8.28 Hz, 3H, Ar-H), 8.14 - 8.30 (m, 2H, Ar-H), 8.33 (s, 1H, pyrazole C3-H), 8.36 (s, 1H, pyrimidine C6-H), 9.01 (s, 1H, $NHCH_2$) ppm. **^{13}CNMR** (100 MHz, DMSO-d_6) δ: 52.33, 102.32, 117.32, 119.22, 121.23, 128.09, 128.34, 128.47, 129.81, 132.98, 134.15, 139.37, 148.22, 153.23, 155.59, 156.51 ppm; Analysis for $C_{20}H_{16}N_8S$ **(**400.46): **Calcd:** C, 59.98; H, 4.03; N, 27.98**, Found:** C, 60.31; H, 4.11; N, 28.15%

***N'*-[4-(4-Substituted-phenyl)-3-phenylthiazol-2 (3*H*)-ylidene]-2-[(1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-yl)amino]acetohydrazide (7a,b):**

To a solution of Compound **(4)** (0.1gm, 0.25 mmol) in ethanol (10mL) equivalent amount of p-substituted phenacyl bromide (0.25 mmol) was added in the presence of anhydrous sodium acetate (0.041gm, 0.5mmol). The reaction mixture was refluxed for 4 hours. The separated solid was filtered, washed with ethanol and crystallized from chloroform/petrolium ether (60-80°C) to afford the title compounds.

Compound (7a): X=Br [m.p.= 292-294 °C, **(**86%)].

^{1}HNMR (300 MHz, DMSO-d_6) δ: 4.74 (d, *J* = 4.8 Hz, 2H, CH_2), 6.92 (s, 1H, thiazol C5-H), 7.30-7.55 (m, 7 Ar-H), 7.68 (d, *J* = 8.7 Hz, 2 Ar-H), 7.79 (d, *J* = 8.1 Hz, 2 Ar-H), 8.15 (t, *J* = 7.8 Hz, 3 Ar-H), 8.18 (s, 1H, pyrazole C3-H), 8.29 (s, 1H, pyrimidine C6-H), 8.79 (s, 2H, 2 NH) ppm. **^{13}CNMR** (100 MHz, DMSO-*d*6) δ: 58.6, 98.03, 102.34, 121.21, 122.46, 123.72, 126.96, 127.84, 128.78, 129.71 130.01, 131.24, 132.72, 133.93, 134.13, 139.35, 142.36, 147.98, 148.2, 150.17, 154.47, 156.49, 170.04 ppm; Analysis for $C_{28}H_{21}BrN_8OS$ (597.49): Calcd: C, 56.29; H, 3.54; N, 18.75, Found: C, 56.56; H, 3.67; N, 18.46 %.

Compound (7b): X=CH_3 [m.p. = 284-287 °C, **(**82%)].

IR: ν = 3261 (NH), 3037 (C-H aromatic), 2917 (C-H aliphatic), 1677 (C=O), 1599 (C-C aromatic), 1304 (C-N aromatic amine).**^{1}HNMR** (400 MHz, DMSO-d_6) δ: 2.39 (s, 3H, CH_3), 4.78 (d, *J* = 5.28 Hz, 2H, CH_2), 7.32 (s, 1H, thiazol C5-H), 7.35 (d, *J*= 8.32 Hz, 3H, Ar-H), 7.44-7.56 (m, 7H, Ar-H), 7.90 (d, *J* = 8.2 Hz, 2H, Ar-H), 8.16 (d, *J* =8.64 Hz, 2H, Ar-H), 8.21 (s, 1H, pyrazole C3-H), 8.33 (s, 1H, pyrimidine C5-H), 8.9 (s, 2H, 2 NH) ppm. **^{13}CNMR** (100 MHz, DMSO-d_6) δ: 18.82, 56.89 , 102.06, 105.18, 120.21, 121.00, 122.38, 127.16, 129.21, 129.41, 129.61, 129.80, 132.60, 132.76, 138.81, 139.10, 144.26, 147.21, 148.22, 150.2, 153.10, 156.3, 170.45 ppm; Analysis for $C_{29}H_{24}N_8OS$ (532.62): Calcd: C, 65.40; H, 4.54; N, 21.04, Found: C, 65.31; H, 4.42; N, 20.84 %.

***N'*-(1-Phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl) arylhydrazide derivatives (8a-f):**

To a solution of 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(1)** (1gm, 4.3 mmol) in ethanol (40mL), different acid hydrazide derivatives (4.3 mmol) were added in the presence of fused sodium acetate (0.7gm, 8.7mmol). The reaction mixture was refluxed for 4 hours. After cooling, the formed crystals were filtered, washed with water, dried and recrystallized from DMF/petroleum ether (60/80 °C) to afford the target compounds

Compound (8a): R=C_6H_5- [m.p.= 273-277 °C, **(**82%)].

^{1}HNMR (300 MHz, DMSO-d_6) δ: 7.3 (t, *J* = 7.8 Hz,21H, Ar-H), 7.4-7.8 (m, 4H, Ar-H), 8.00 (d, *J* = 7.2 Hz, 2H, Ar-H), 8.15 (d, *J* = 7.5 Hz, 2H, Ar-H), 8.45 (s, 1H, pyrazole C3-H), 8.81 (s, 1H, pyrimidine C6-H), 10.19 (s, 1H, NH, exchangeable) , 11.13 (s, 1H, NH, exchangeable) ppm. **^{13}CNMR** (100 MHz, DMSO-d_6) δ: 96.20, 120.91, 126.35, 126.43, 127.49, 127.56, 129.09, 132.11, 133.50, 138.59, 144.01, 151.89, 156.49, 166.34 ppm; Analysis for $C_{18}H_{14}N_6O$ (330.34) Calcd: C, 65.44; H, 4.27; N, 25.44, Found: C, 65.32; H, 4.11; N, 25.27 %.

Compound (8b): R=3-OCH_3-C_6H_4- [m.p.= 286-290 °C, **(**85%)].
^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.33 (s, 3H, O-CH3), 7.22 - 8.38 (m, 9H, Ar-H), 8.46 (s, 1H, pyrazole C3-H), 8.54 (s, 1H, pyrimidine C6-H), 10.23 (s, 1H, NH, exchangeabale), 11.19 (s, 1H, NH, exchangeabale) ppm. **^{13}CNMR** (100 MHz, DMSO-d_6) δ: 55.40, 103.30, 112.70, 118.05, 119.77, 120.95, 126.38, 126.41, 126.47, 129.22, 129.99, 133.54, 138.60, 155.94, 156.48, 159.40, 166.19 ppm. **MS analysis:** m/z (rel. intensity)= 360 (M^+, 6.3), 342 (99.9), 341(99.9), 312 (99.9), 76 (99.9). Analysis for $C_{19}H_{16}N_6O_2$ (360.37) Calcd: C, 63.32; H, 4.48; N, 23.32, Found: C, 63.66; H, 4.65; N, 23.16 %.

Compound (8c): R=4-NO_2-C_6H_4- [m.p.= 279-282 °C, **(**85%)].
^{1}HNMR (300 MHz, DMSO-d_6) δ: 7.35-8.42 (m, 9H, Ar-H) 8.48 (s, 1H, pyrazole C3-H), 8.89 (s, 1H, pyrimidine C6-H), 11.18 (s, 1H, NH exchangeable), 11.53 (s, 1H, NH exchangeable) ppm. **^{13}CNMR** (100 MHz, DMSO-d_6) δ: 101.25, 120.93, 122.29, 126.37, 126.56, 129.10, 130.57, 133.61, 133.92, 134.65, 138.60, 147.96, 156.34, 162.35, 166.23 ppm. **MS analysis:** m/z (rel. intensity)= 375 (M^+, 99.9), 357 (99.9), 225 (99.9), 195 (55.6), 150 (99.9), 77 (99.9), 77 (55.4); Analysis for $C_{18}H_{13}N_7O_3$ (375.34) Calcd: C, 57.60; H, 3.49; N, 26.12, Found: C, 57.36; H, 3.34; N, 25.94 %.

Compound (8d): R=3-NO_2-C_6H_4- [m.p.= 290-293 °C, **(**84%)].
^{1}HNMR (300 MHz, DMSO-d_6) δ: 7.34-8.48 (m, 9H, Ar-H), 8.50 (s, 1H, pyrazole C3-H), 8.82 (s, 1H, pyrimidine C6-H), 10.45 (s, 1H, NH exchangeable), 11.35 (s, 1H, NH exchangeable) ppm.; Analysis for $C_{18}H_{13}N_7O_3$ (375.34) Calcd: C, 57.60; H, 3.49; N, 26.12, Found: C, 57.42; H, 3.31; N, 26.09 %.

Compound (8e): R=4-Cl-C_6H_4- [m.p.= 296-299 °C, **(**89%)].
^{1}HNMR (300 MHz, DMSO-d_6) δ: 7.5 (t, *J* = 8.1, 1H, Ar-H), 7.6 (m, 4H, Ar-H), 8.14 (d, *J* = 7.8 Hz, 2H, Ar-H), 8.28 (d, *J* = 7.8 Hz, 2H, Ar-H), 8.51(s, 1H, pyrazole C3-H), 8.81 (s, 1H, pyrimidine C6-H), 9.7 (s, 2H, 2 NH, exchangeable) ppm; Analysis for $C_{18}H_{13}ClN_6O$ (364.79) Calcd: C, 59.27; H, 3.59; N, 23.04, Found: C, 59.44; H, 3.75; N, 23.28 %.

Compound (8f): R=3-pyridyl- [m.p.= 230-233 °C, **(**77%)].
^{1}HNMR (300 MHz, DMSO-d_6) δ: 7.36 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.50-7.65 (m, 3H, Ar-H), 8.18 (d, *J* = 7.8 Hz, 2H, Ar-H), 8.33(d, *J* = 7.8 Hz, 2H, Ar-H), 8.47 (s, 1H, pyridine C2-H), 8.82 (d, *J* = 4.5 Hz, 1H, pyrazole C3-H), 9.15 (s, 1H, pyrimidine C6-H), 10.54 (s, 1H, NH, exchangeable), 10.97 (s, 1H, NH, exchangeable) ppm. **^{13}CNMR** (100 MHz, DMSO-d_6) δ: 101.94**,** 120.95, 123.88, 126.40, 129.23, 130.52, 133.58, 135.39, 138.61, 148.47, 148.59, 152.92, 156.52, 165.23, 186.63 ppm; Analysis for $C_{17}H_{13}N_7O$ (331.33) Calcd: C, 61.62; H, 3.95; N, 29.59, Found: C, 61.53; H, 3.84; N, 29.31 %.

7-Phenyl-3-(substituted)-7*H*-pyrazolo[4,3-*e*] [1,2,4] triazolo[4,3-c]-pyrimidine (9a,b):
A solution of compound **(**8a) or compound (8b) (1 gm) in galacial acetic acid, was refluxed for 18 hours. The solution was allowed to evaporate in rotary evaporator and the residue was poured on ice. The precipitated compound is filtered, washed with water, and recrystalized from Chloroform/ petroleum ether to afford the target compounds.

Compound (9a): R=C_6H_5- [m.p= above 300 °C, (44%)]:
^{1}HNMR (300 MHz, DMSO-d_6) δ: 7.45-7.68 (m, 6H, Ar-H), 8.14 (d, *J* = 7.5 Hz, 2H, Ar-H), 8.26-8.30 (m, 2H, Ar-H), 8.85 (s, 1H, pyrazole C3-H), 9.77 (s, 1H, pyrimidine C6-H) ppm; Analysis for $C_{18}H_{12}N_6$ (312.33) Calcd: C, 69.22; H, 3.87; N, 26.91, Found: C, 69.45; H, 3.55; N, 26.78 %.

Compound (9b): R=3-OCH_3-C_6H_4- [m.p= above 300 °C, (52%)]:

^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.84 (s, 3H, O-CH_3), 7.13 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.46-7.62 (q, *J* = 7.5 Hz, 15.6 Hz, 3H, Ar-H), 7.64 (t, *J* = 8.1 Hz, 2H, Ar-H), 7.86 (d, *J* = 8.1 Hz, 1H, Ar-H), 8.14 (d, *J* = 8.4 Hz, 2H, Ar-H), 8.84 (s, 1H, pyrazole C3-H), 9.75 (s, 1H, pyrimidine C6-H) ppm. **MS analysis:** m/z (rel. intensity)= 342 (M^+, 99.9), 341(99.9), 312 (99.9), 76 (99.9); Analysis for $C_{19}H_{14}N_6O$ (342.35) Calcd: C, 66.66; H, 4.12; N, 24.55, Found: C, 66.52; H, 4.28; N, 24.29 %.

Antimicrobial Activity:

Determination of inhibitory % produced on a range of clinically pathogenic microorganisms using (125 µg) concentration of tested samples:

Gram positive bacteria: *Streptococcus pneumoniae* (RCMB 010010) and *Bacillis subtilis* (RCMB 010067), gram negative bacteria: *Pseudomonas aeruginosa* (RCMB 010049) and *Escherichia coli* (RCMB 010058), and fungi: *Aspergillus fumigatus* (RCMB 02568) and *Candida albicans* (RCMB 05036) strains were used in this study. The microbial suspensions equivalent to the turbidity of 0.5 McFarland (10^8 CFU/ml) standard were prepared from a fresh subculture of tested bacteria in Mueller Hinton Broth (MHB) and tested fungi in Sabouraud Dextrose Broth (SDB) then this suspension was diluted to 10^6 CFU/ml using MHB for bacteria and Sabourand dextrose Broth (SDB) for tested fungi. The adjusted microbial inoculum (100µl)were added to each well of sterile 96-well flat-bottomed microtiter plate containing the tested concentration of tested samples (100µl/well). As a result, last inoculum concentration of 5 X 10^5 CFU/ml was obtained in each well.

Three wells containing microbial suspension with no sample using DMSO employed for dissolving the tested compound (Growth control) and two wells containing only media (background control) were included in this plate. Optical densities were measured after 24 hrs at 37 °C for bacteria and after 48hrs at 28°C for fungi using a multi-detection microplate reader (sun Rise - Tecan, USA) at 600nm. Ampicillin, Gentamicin and Amphotericin B were used as standards for Gram positive bacteria, Gram negative bacteria and fungi respectively.

TABLE 1: THE INHIBITORY PERCENTAGE OF THE TESTED COMPOUNDS WAS ILLUSTRATED IN

M.O.	Fungi		Gram positive bacteria		Gram negative bacteria	
Tested Samples	*Asp. fumigatus* (RCMB 02568)	C. albicans (RCMB 05036)	*Str. pneumoniae* (RCMB 010010)	*B. subtilis* (RCMB 010067)	*Ps. aeruginosa* (RCMB 010043)	*E. coli* (RCMB 010052)
Standards	Amphotericin B		Ampicillin		Gentamicin	
	90.31 ± 0.58	95.21 ± 0.44	86.32 ± 0.58	99.62 ± 0.63	72.21 ± 0.58	75.42 ± 0.58
2	NA	NA	NA	NA	NA	NA
3	6.43 ± 0.44	NA	8.14 ± 0.37	15.3 ± 0.25	4.28 ± 0.25	9.25 ± 0.37
4	80.35±0.44	71.32±0.37	86.32±0.58	89.14±0.25	NA	76.31±0.25
5	43.2±0.44	35.9±0.63	53.6±0.44	76.2±0.25	NA	57.8±0.58
6	84.32±0.44	72.58±0.44	92.21±0.37	97.31±0.25	NA	73.44±0.44
7a	49.67±0.58	38.26±0.37	85.65±0.44	72.44±0.37	NA	60.32±0.25
7b	24.21±0.37	31.24±0.63	41.21±0.63	53.93±0.25	NA	13.66±0.25
8a	48.31±0.44	38.92±0.37	50.27±0.28	51.46±0.37	29.93±0.58	52.14±0.24
8b	68.32±0.44	57.94±0.37	76.23±0.28	87.4±0.37	52.94±0.58	66.87±0.24
8c	71.34±0.58	62.14±0.63	74.94±0.25	88.17±0.37	54.63±0.44	67.26±0.37
8d	46.23±0.44	36.12±0.37	49.65±0.58	50.94±0.25	25.27±0.58	47.13±0.25
8e	14.36±0.37	13.17±0.44	16.37±0.58	17.26±0.44	NA	16.38±0.44
8f	62.74±0.25	53.16±0.44	75.28±0.63	85.58±0.58	53.43±0.44	66.95±0.37
9a	NA	NA	NA	NA	NA	NA
9b	NA	NA	NA	NA	NA	NA

N.A.: no activity, ***Asp. fumigatus:*** *Aspergillus fumigatus,* ***C. albicans:*** *Candida albicans,* ***Str. pneumoniae:*** *Streptococcus pneumoniae,* ***B. subtilis:*** *Bacillis subtilis,* ***Ps. aeruginosa:*** *Pseudomonas aeruginosa,* ***E. coli:*** *Escherichia coli*

Tab. 1 Mean of inhibitory % ± standard derivation produced on a range of clinically pathogenic microorganisms using (125 µg) concentration of tested samples

Determination of minimum inhibitory concentration (MIC) and inhibitory concentration 50 (IC50):

For the determination of MIC of tested samples by the micro-broth assay, the percentage of growth at each sample concentration was calculated with the following equation: % growth = [(OD600 of wells containing the sample/OD600 of the sample-free well) X 100] after subtraction of background ODs (ODs of microorganism-free wells) [24].

TABLE 2: THE MIC OF THE TESTED COMPOUNDS WERE ILLUSTRATED IN

M.O.	Fungi		Gram positive bacteria		Gram negative bacteria	
Tested Samples	*Asp. fumigatus* (RCMB 02568)	C. albicans (RCMB 05036)	*Str. pneumoniae* (RCMB 010010)	*B. subtilis* (RCMB 010067)	*Ps. aeruginosa* (RCMB 010043)	*E. coli* (RCMB 010052)
Standards	Amphotericin B		Ampicillin		Gentamicin	
	0.12	**0.06**	**0.12**	**0.03**	**0.98**	**0.49**
2	NA	NA	NA	NA	NA	NA
3	>100	NA	>100	>100	>100	>100
4	0.49	0.98	0.12	0.06	NA	0.98
5	15.63	31.2	15.63	0.98	NA	3.9
6	0.24	0.98	0.06	0.03	NA	0.98
7a	7.81	31.2	3.9	0.98	NA	3.9
7b	>100	>100	31.2	7.81	NA	>100
8a	15.63	31.2	15.63	7.8	>100	7.81
8b	1.95	7.81	0.49	0.12	15.63	1.95
8c	0.98	3.9	0.98	0.12	15.63	1.95
8d	15.63	31.2	31.26	7.81	>100	15.63
8e	>100	>100	>100	>100	NA	>100
8f	3.9	15.63	0.49	0.12	15.63	1.95
9a	NA	NA	NA	NA	NA	NA
9b	NA	NA	NA	NA	NA	NA

N.A.: no activity, ***Asp. fumigatus:*** *Aspergillus fumigatus,* ***C. albicans:****Candida albicans,* ***Str. pneumoniae:*** *Streptococcus pneumoniae,* ***B. subtilis:****Bacillis subtilis,* ***Ps. aeruginosa:****Pseudomonas aeruginosa,****E. coli:*** *Escherichia coli.*

Tab. 2 Antimicrobial Activity as MIC (µg/ml) of tested samples against tested microorganism.

TABLE 3: IC50 FOR EACH COMPOUND WERE MEASURED DURING MEASURING THE MIC, THE VALUES OF IC50 WERE ILLUSTRATED IN

M.O.	Fungi		Gram positive bacteria		Gram negative bacteria	
Tested Samples	*Asp. fumigatus* (RCMB 02568)	C. albicans (RCMB 05036)	*Str. pneumoniae* (RCMB 010010)	*B. subtilis* (RCMB 010067)	*Ps. aeruginosa* (RCMB 010043)	*E. coli* (RCMB 010052)
Standards	Amphotericin B		Ampicillin		Gentamicin	
	0.92	**0.21**	**0.42**	**0.14**	**2.15**	**1.24**
2	NA	NA	NA	NA	NA	NA
3	>100	>100	>100	>100	>100	>100
4	0.49	0.98	0.12	0.06	NA	0.98
5	58.32	>100	58.32	3.21	NA	15.21
6	0.91	3.14	0.24	0.14	NA	0.98
7a	30.24	>100	14.68	3.24	NA	14.92
7b	>100	>100	>100	7.52	NA	>100
8a	60.12	>100	59.63	32.15	>100	31.86
8b	6.89	7.21	1.92	0.38	60.21	6.95
8c	2.64	3.01	2.31	0.41	59.21	7.21
8d	60.12	>100	>100	31.12	>100	59.21
8e	>100	>100	>100	>100	NA	>100
8f	14.92	58.32	1.75	0.41	60.21	6.95
9a	NA	NA	NA	NA	NA	NA
9b	NA	NA	NA	NA	NA	NA

N.A.: no activity, ***Asp. fumigatus:*** *Aspergillus fumigatus,* ***C. albicans:****Candida albicans,* ***Str. pneumoniae:*** *Streptococcus pneumoniae,* ***B. subtilis:*** *Bacillis subtilis,* ***Ps. aeruginosa:*** *Pseudomonas aeruginosa,* ***E. coli:*** *Escherichia coli.*

Tab. 3 Antimicrobial Activity as IC50 (µg/ml) of tested samples against tested microorganism

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