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Synthesis of Some New 1-(6-Amino-1,3-Benzothiazol-2-YL)-3-(Substitutedphenyl)-1*H*-Pyrazole-4-Carbaldehyde Derivatives with Anti-Microbial Activity.

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ABSTRACT

A new series of 1-(6-amino-1,3-benzothiazol-2-yl)-3-(substitutedphenyl)-1*H*-pyrazole-4-carbaldehyde, **5a-g**, were synthesized. All the newly synthesized compounds were screened for their *in vitro* antibacterial activity, against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* and for antifungal activity against *Aspergillus niger* and *Candida albicans*. Compounds **5b**, **5f**, **5d**, **5a** showed excellent *in vitro* antibacterial activity and antifungal activity than the standard drugs. All the compounds were characterized by IR, ^{1}HNMR, LCMS mass and C, H, N analyses.

Keywords: Antibacterial, Antifungal, 1,3-benzothiazol-2-yl

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INTRODUCTION

Among heterocycles, pyrazole derivatives are reported to possess important pharmacological activities like antibacterial (1), antifungal (2), anti-inflammatory (3), etc. The benzothiazole derivatives are also possess biological activities like antimicrobial (4), anti-inflammatory (5), anticancer (6), antidiabetic (7), and antiviral (8). In continuation of work on the synthesis of novel heterocycles systems, we report herein the synthesis and antimicrobial assessment of 1-(6-amino-1,3-benzothiazol-2-yl)-3-(substitutedphenyl)-1*H*-pyrazole-4-carbaldehyde derivatives, **5a-g**.

EXPERIMENTAL

All the newly synthesized compounds gave moderate to good yields. The homogeneity of synthesized compounds was ascertained by thin layer chromatography (TLC) on silica gel G (Merck) coated plates by using (benzene: acetone, 8:2) as a solvent system. The visualization was done by using iodine vaporus and UV light chamber. The chemicals and solvents used for experimental work were commercially procured from CDH, E. Merck, S.D. fine chem. and Qualigens. The silica gel G used for analytical chromatography was obtained from E. Merck. Melting points were determined in open glass capillary tubes in a Hicon melting apparatus and are uncorrected. IR spectra were recorded in KBr pellets on JASCO FT-IR 410 spectrophotometer. The ^{1}HNMR spectra were recorded downfield on VNMRS-500 "Agilent-NMR" using (TMS) tetra methyl silane as an internal standard. The chemical shift are reported in ppm δ scale. LCMS Mass spectra were recorded on MASPEC low resolution mass spectrometer at an ionization potential of 70eV and are expressed as (m/z).

For the synthesis of new benzothiazole derivatives, initially, p-amino aniline **(1)** was treated with thiocyanogen generated from bromine and alkaline thiocyanate in acetic acid medium led to the formation of 1,3-benzothiazole-2,6-diamine **(2)**. Compound **(2)** was treated with hydrazine hydrate in the presence of ethylene glycol to give 2-hydrazinyl-1,3-benzothiazol-6-amine **(3)**. The compound **(3)** on reaction with appropriate acetophenones and glacial acetic acid in the presence of absolute ethanol to give 6-amino-1,3-benzothiazol-2-yl-hydrazones derivatives**(4a-g)**.The hydrazones **(4a-g)** were added in mixture of Vilsmeier-Haack reagent (DMF/$POCl_3$) in an open Erlenmeyer flask and irradiated in microwave oven for 45-120 sec to get final compounds 3-(2-substitutedphenyl)-1-(6-amino-1,3-benzothiazol-2-yl)-1*H*-pyrazole-4-carbaldehyde derivatives **(5a-g)** in good to moderate yields **(Scheme 1)**. The physical constants of the synthesized compounds are given in Table 1.

Synthesis of 1,3-benzothiazole-2,6-diamine **(2)** (m.p. 166-168ºC) was carried out by standard procedure (9).

General method of synthesis of 2-hydrazinyl-1,3-benzothiazol-6-amine **(3).**

Conc. HCl (6 ml) was added drop wise with stirring to hydrazine hydrate (99 %, 6 ml) at 5-10 ºC. To it ethylene glycol (24 ml) and compound **(2)** (0.03 mol) were added in portions and refluxed for 3hrs. On cooling white solid separate out, which was filtered, washed with water and recrystallized from ethanol. Purity of compound was checked by TLC using silica gel-G coated plates by using toluene: ethyl acetate: formic acid (5:4:1, v/v/vv) as solvent system and visualization in UV light.

Yield: 82 %, m.p. 174-176ºC, IR (KBr, cm^{-1}): 3370 (NH), 3015 (CH-Ar), 1468 (C=N), 697 (C-S-C, benzothiazole). ^{1}H NMR ($CDCl_3$, δ ppm): 4.58 (s, 2H of NH_2), 5.02 (s, 2H of NH_2, D_2O exchangeable), 7.23-7.13(t, 1H of Ar-H), 7.35-7.25 (q, 1H of Ar-H), 7.62-7.52 (d, 1H of Ar-H), 9.05 (s, 1H of NH). MS (m/z): 180 [M^+], Anal. Calcd. for $C_7H_8N_4S$: C, 46.65; H, 4.47; N, 31.09%; found: C, 46.63; H, 4.45; N, 31.02%

General method of synthesis of 2-{(2*E*)-2-[1-(substitutedphenyl)ethylidene]hydrazinyl}-1,3-benzothiazol-6-amine **(4a-g)**

A mixture of 2-hydrazinyl-1,3-benzothiazol-6-amine **3** (1.5 mmole) and substituted acetophenone (2.2 mmole) was taken in a flask containing absolute ethanol (60 ml) and glacial acetic acid (4-5 drops). The solution was then refluxed for 5-12 hrs on water bath. On cooling solid separated out, which was filtered, washed with little water and recrystallized from absolute alcohol to get hydrazone **4**. The other compounds of this series were synthesized similarly.

2-{(2*E*)-2-[1-(4-bromophenyl)ethylidene]hydrazinyl}-1,3-benzothiazol-6-amine **(4a)**

IR (KBr, cm^{-1}): 3094 (CH-Ar), 3250 (NH str.), 1617 (C=N str.), 685 (C-S-C), 1120 (C-N), 563 (C-Br), ^{1}H NMR ($CDCl_3$, δ ppm): 2.33 (s, 3H, CH_3-C=N-, D_2O exchangeable), 4.86 (s, 2H, C_6-NH_2), 4.73 (s, 1H, NH), 6.98-7.12 (m, 3H, Ar-H). MS (m/z): 360 (M^+), Analysis. Calcd. for $C_{15}H_{13}BrN_4S$: C 49.87, H 3.63, N 15.50%; found: C 49.82, H 3.58, N 15.45%.

2-{(2*E*)-2-[1-(4-chlorophenyl)ethylidene]hydrazinyl}-1,3-benzothiazol-6-amine **(4b)**

IR (KBr, cm^{-1}): 3082 (CH-Ar), 3182 (NH str), 1552 (C=N), 1262 (C-N), 684 (C-S-C), 804 (C-Cl),^{1}H NMR ($CDCl_3$, δ ppm): 4.34 (s, 1H, NH, D_2O exchangeable), 2.31 (s, 3H, CH_3-C=N-), 7.95-7.92 (m, 3H, Ar-H, *J* = 10 Hz), 3.18 (s, 2H, Ar-NH_2), MS (m/z): 316 (M^+), Analysis. Calcd. for $C_{15}H_{13}ClN_4S$: C 56.87, H 4.14, N 17.68%; found: C 56.84, H 4.10, N 17.66%.

4-{(1*E*)-1-[2-(6-amino-1,3-benzothiazol-2-yl)hydrazinylidene]ethyl}phenol **(4c)**

IR (KBr, cm^{-1}): 3274 (NH), 1640 (C=N),1058 (C-N), 612 (C-S-C), 3410 (OH), ^{1}H NMR ($CDCl_3$, δ ppm): 4.40 (s, 1H, NH, D_2O exchangeable), 2.35 (s, 3H, CH_3-C=N-), 7.82-8.10 (m, 3H, Ar-H, *J* = 9.5 Hz) 10.2 (s, 1H, Ar-OH) 3.92 (s, 2H, Ar-NH_2), MS (m/z): 298 (M^+), Analysis. Calcd. for $C_{15}H_{14}N_4SO$: C 60.38, H 4.73, N 18.78%; found: C 60.34, H 4.70, N 18.73%.

2-{(2*E*)-2-[1-(4-aminophenyl)ethylidene]hydrazinyl}-1,3-benzothiazol-6-amine **(4d)**

IR (KBr, cm^{-1}): 3420 (NH_2), 3268 (NH str), 1572 (C=N), 1162 (C-N), 657 (C-S-C), ^{1}H NMR ($CDCl_3$, δ ppm): 4.32 (s, 1H, NH, D_2O exchangeable), 2.36 (s, 3H, CH_3-C=N-), 7.68-7.80 (m, 3H, Ar-H, *J* = 9 Hz), 4.34 (s, 4H, NH_2), MS (m/z): 297 (M^+), Analysis. Calcd. for $C_{15}H_{15}N_5S$: C 60.58, H 5.08, N 23.55%; found: C 60.53, H 5.04, N 23.50%.

2-{(2*E*)-2-[1-(4-methoxyphenyl)ethylidene]hydrazinyl}-1,3-benzothiazol-6-amine **(4e)**

IR (KBr, cm^{-1}): 3268 (NH), 1682 (C=N),1224 (C-N), 694 (C-S-C), ^{1}H NMR ($CDCl_3$, δ ppm): 4.46 (s, 1H, NH, D_2O exchangeable), 2.42 (s, 3H, CH_3-C=N-), 7.62-7.72 (m, 3H, Ar-H, *J* = 10 Hz), 3.84 (s, 3H, Ar-OCH_3), MS (m/z): 312 (M^+), Analysis. Calcd. for $C_{16}H_{16}N_4SO$: C 61.52, H 5.16, N 17.93%; found: C 61.48, H 5.14, N 17.90%.

2-{(2*E*)-2-[1-(4-fluorophenyl)ethylidene]hydrazinyl}-1,3-benzothiazol-6-amine **(4f)**

IR (KBr, cm^{-1}): 3325 (NH), 1610 (C=N),1065 (C-N), 675 (C-S-C), 1310 (C-F), ^{1}H NMR ($CDCl_3$, δ ppm): 4.26 (s, 1H, NH, D_2O exchangeable), 2.25 (s, 3H, CH_3-C=N-), 7.77-8.20 (m, 3H, Ar-H, *J* = 9 Hz), MS (m/z): 300 (M^+), Analysis. Calcd. for $C_{15}H_{13}FN_4S$: C 59.98, H 4.36, N 18.65%; found: C 59.94, H 4.30, N 18.64%.

2-{(2*E*)-2-[1-(4-nitrophenyl)ethylidene]hydrazinyl}-1,3-benzothiazol-6-amine **(4g)**

IR (KBr, cm^{-1}): 3368 (NH), 1632 (C=N), 1144 (C-N), 590 (C-S-C), 1320 (C-NO_2), ^{1}H NMR ($CDCl_3$, δ ppm): 4.35 (s, 1H, NH, D_2O exchangeable), 2.32 (s, 3H, CH_3-C=N), 7.80-8.10 (m, 3H, Ar-H, *J* = 9 Hz), MS (m/z): 327 (M^+), Analysis. Calcd. for $C_{15}H_{13}N_5O_2S$: C 55.03, H 4.00, N 21.39%; found: C 55.00, H 3.98, N 21.36%.

General method of synthesis of 1-(6-amino-1,3-benzothiazol-2-yl)-3-(substitutedphenyl)-1*H*-pyrazole-4-carbaldehyde **(5a-g)**

To the Vilsmeier-Haack reagent prepared from DMF (10 ml) and $POCl_3$ (1.2 ml, 12 mmol), and compound **4** (4 mmol) were added separately and the reaction mixture was irradiated in microwave oven for 45-120 s. After completion of the reaction reaction mixture was poured into ice cold water. The solid that separated on neutralization with $NaHCO_3$ was filtered, washed with water and recrystallized from $CHCl_3$-EtOH to get final compound **5**. The other compounds of this series were synthesized similarly.

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1-(6-amino-1,3-benzothiazol-2-yl)-3-(4-bromophenyl)-1*H*-pyrazole-4-carbaldehyde **(5a)**

IR (KBr, cm^{-1}): 1628 (C=O), 2774, 2875 (CH-Ar), ^1H NMR ($CDCl_3$, δ ppm): 9.04 (s, 1H, pyrazole, D_2O exchangeable), 9.96 (s, 1H, CHO), 7.44 -7.34(m, 3H, ArH), 7.94 (s, 2H, ArH), 8.14-8.04(m, 2H, ArH), MS (m/z): 397 (M^+), Analysis. Calcd. for $C_{17}H_{11}BrN_4OS$: C 51.14, H 2.78, N 14.03%; found: C 51.11, H 2.76, N 14.00%.

1-(6-amino-1,3-benzothiazol-2-yl)-3-(4-chlorophenyl)-1*H*-pyrazole-4-carbaldehyde **(5b)**

IR (KBr, cm^{-1}): 1725 (C=O), 2783, 2868 (CH-Ar); ^1H NMR ($CDCl_3$, δ ppm): 9.04 (s, 1H, pyrazole, D_2O exchangeable), 9.92 (s, 1H, CHO), 7.54-7.50 (m, 3H, ArH), 7.92 (s, 2H, ArH), 8.16 -8.13(m, 2H, ArH, MS (m/z): 354 (M^+), Analysis. Calcd. for $C_{17}H_{11}ClN_4OS$: C 57.55, H 3.12, N 15.79%; found: C 57.53, H 3.10, N 15.75%.

1-(6-amino-1,3-benzothiazol-2-yl)-3-(4-hydroxyphenyl)-1*H*-pyrazole-4-carbaldehyde **(5c)**

IR (KBr, cm^{-1}): 1690 (C=O), 2768, 2880 (CH-Ar), ^1H NMR ($CDCl_3$, δ ppm): 9.16(s, 1H, pyrazole, D_2O exchangeable), 9.98 (s, 1H, CHO), 7.62-7.52 (m, 3H, ArH), 7.83 (s, 2H, ArH), 8.26-8.10 (m, 2H, ArH), 10.2 (Ar-OH), MS (m/z): 336 (M^+), Analysis. Calcd. for $C_{17}H_{12}N_4O_2S$: C 60.70, H 3.60, N 16.66%; found: C 60.68, H 3.58, N 16.63%.

1-(6-amino-1,3-benzothiazol-2-yl)-3-(4-aminophenyl)-1*H*-pyrazole-4-carbaldehyde **(5d)**

IR (KBr, cm^{-1}): 1678 (C=O), 2785, 2892 (C-H), ^1H NMR ($CDCl_3$, δ ppm): 9.10 (s, 1H, pyrazole, D_2O exchangeable), 9.91 (s, 1H, CHO), 7.77-7.68 (m, 3H, ArH), 7.91 (s, 2H, ArH), 7.76-7.66 (m, 2H, ArH), MS (m/z): 335 (M^+), Analysis. Calcd. for $C_{17}H_{13}N_5OS$: C 60.88, H 3.91, N 20.88%; found: C 60.85, H 3.88, N 20.85%.

1-(6-amino-1,3-benzothiazol-2-yl)-3-(4-methoxyphenyl)-1*H*-pyrazole-4-carbaldehyde **(5e)**

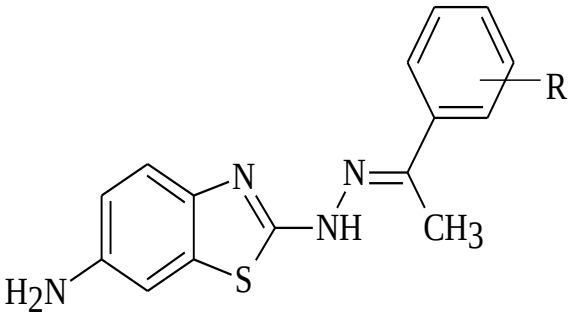
IR (KBr, cm^{-1}): 1712 (C=O), 2775, 2876 (C-H), ^1H NMR ($CDCl_3$, δ ppm): 9.08 (s, 1H, pyrazole, D_2O exchangeable), 9.95 (s, 1H, CHO), 7.72-7.68 (m, 3H, ArH, *J* = 9 Hz), 7.98 (s, 2H, ArH), 7.98-7.86 (m, 2H, ArH), 3.86 (s, 3H, OCH_3), MS (m/z): 350 (M^+), Analysis. Calcd. for $C_{18}H_{14}N_4O_2S$: C 61.70, H 4.03, N 15.99%; found: C 61.67, H 3.99, N 15.96%.

1-(6-amino-1,3-benzothiazol-2-yl)-3-(4-fluorophenyl)-1*H*-pyrazole-4-carbaldehyde **(5f)**

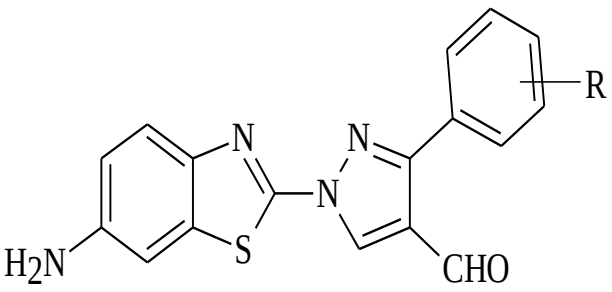
IR (KBr, cm^{-1}): 1708 (C=O), 2769, 2803 (C-H), ^1H NMR ($CDCl_3$, δ ppm): 9.04 (s, 1H, pyrazole, D_2O exchangeable), 9.82 (s, 1H, CHO), 7.77-7.66 (m, 3H, ArH, *J* = 10 Hz), 7.94 (s, 2H, ArH), 7.84-7.71 (m, 2H, ArH), ^1H NMR ($CDCl_3$, δ ppm): MS (m/z): 338 (M^+), Analysis. Calcd. for $C_{17}H_{11}FN_4OS$: C 60.34, H 3.28, N 16.56%; found: C 60.33, H 3.26, N 16.53%.

1-(6-amino-1,3-benzothiazol-2-yl)-3-(4-nitrophenyl)-1*H*-pyrazole-4-carbaldehyde **(5g)**

IR (KBr, cm^{-1}): 1716 (C=O), 2712, 2874 (CH-Ar), ^1H NMR ($CDCl_3$, δ ppm): 9.12 (s, 1H, pyrazole, D_2O exchangeable), 9.86 (s, 1H, CHO), 7.52-7.40 (m, 3H, ArH, *J* = 10 Hz), 7.96 (s, 2H, ArH), 8.20-7.98 (m, 2H, ArH), MS (m/z): 365 (M^+), Analysis. Calcd. for $C_{17}H_{11}N_5O_3S$: C 55.88, H 3.03, N 19.17%; found: C 55.85, H 2.99, N 19.13%.



(4a-g)



(5a-g)

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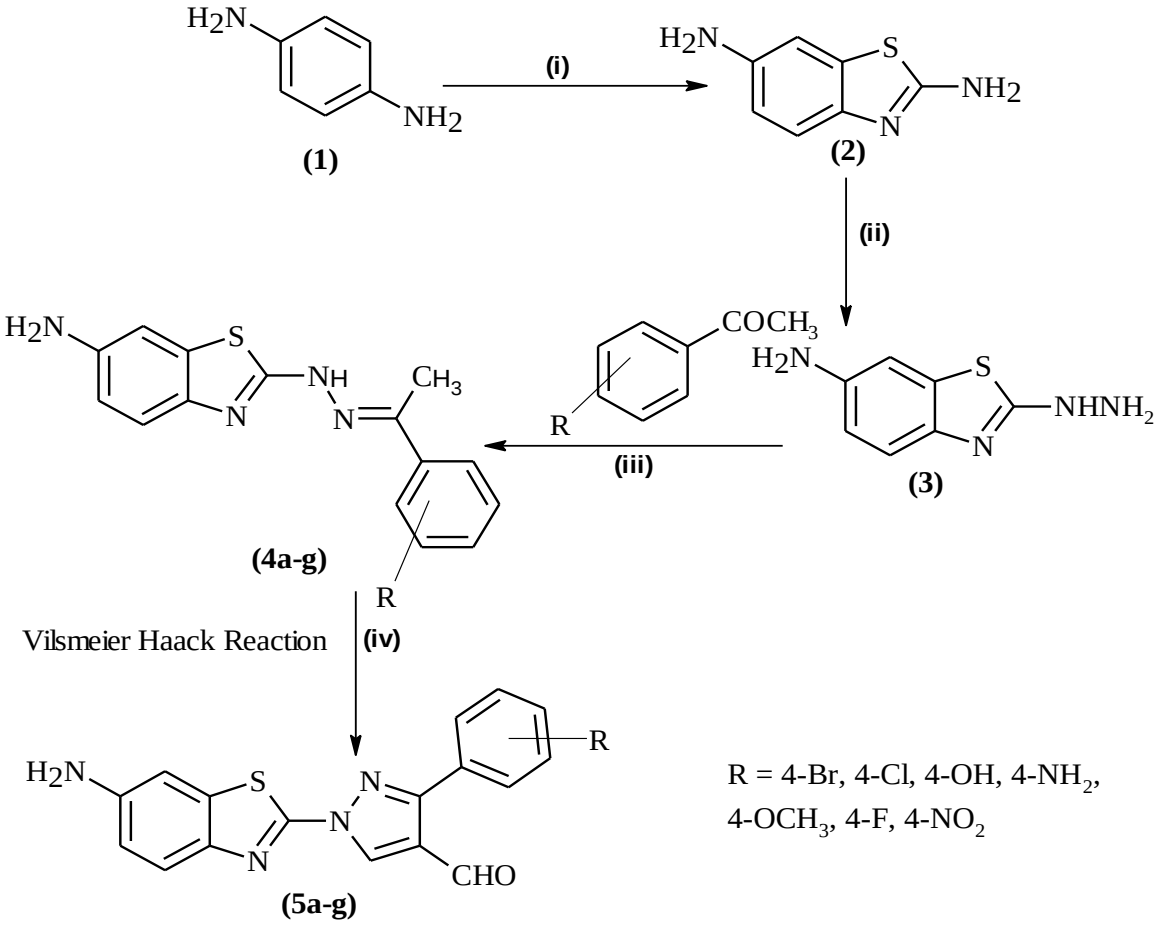
Table 1: Physical data of the synthesized compounds (4a-g, 5a-g).

Compd.	R	Mol. Formula	Yield (%)	M.p. (°C)	Mol. Weight	R_f[a]
4a	4-Br	$C_{15}H_{13}BrN_4S$	78	198-201	361.23	0.92
4b	4-Cl	$C_{15}H_{13}ClN_4S$	81	188-190	316.82	0.82
4c	4-OH	$C_{15}H_{14}N_4OS$	74	175-177	298.3	0.84
4d	4-NH_2	$C_{15}H_{15}N_5S$	68	210-212	297.34	1.2
4e	4-OCH_3	$C_{16}H_{16}N_4OS$	70	220-222	312.34	0.82
4f	4-F	$C_{15}H_{13}FN_4S$	73	167-170	300.35	0.77
4g	4-NO_2	$C_{15}H_{13}N_5O_2S$	82	178-180	327.38	1.2
5a	4-Br	$C_{17}H_{11}BrN_4OS$	67	205-207	399.26	0.94
5b	4-Cl	$C_{17}H_{11}ClN_4OS$	65	218-220	354.81	0.85
5c	4-OH	$C_{17}H_{12}N_4O_2S$	70	165-167	336.36	0.75
5d	4-NH_2	$C_{17}H_{13}N_5OS$	72	182-184	335.36	0.68
5e	4-OCH_3	$C_{18}H_{14}N_4O_2S$	75	194-196	350.34	1.1
5f	4-F	$C_{17}H_{11}FN_4OS$	76	212-214	338.38	0.88
5g	4-NO_2	$C_{17}H_{11}N_5O_3S$	82	162-164	365.36	0.72

Elemental analysis was found to be within ± 0.4% of theoretical values.
[a]Eluents used in TLC were benzene : acetone (8:2, v/v) for all compounds.



Scheme 1: Reagents and Conditions (i) Glacial acetic acid, KSCN, Br_2, Stirring 10 h (ii) $NH_2.NH_2.H_2O$, ethylene glycol, reflux for 3 h (iii) ethanol, reflux 5 h. (iv) DMF/$POCl_3$, MWI

Antimicrobial activity

All the synthesized compounds **5a-g** were screened for their *in vitro* antibacterial activity against Gram positive bacteria [*Staphylococcus aureus* (ATCC-25923)], Gram-negative bacteria [*Escherichia coli* (ATCC-

25922), *Pseudomonas aeruginosa* (ATCC-27853)] strains and antifungal activity against *Aspergillus niger* (MTCC-281) and *Candida albicans* (ATCC 2099) by cup plate method and agar diffusion method. Norfloxacin and ketoconazole were used as the reference drugs. The test compounds and standards were evaluated for 100 µg/mL concentration. DMF (N,N-dimethylformamide) was used as solvent and control. Data are represented as % inhibition with reference to standards in Table 2.

Table 2: Antibacterial and Antifungal activity of compounds 5a-g.

Compd.	Diameter of zone of inhibition (mm)					% inhibition with reference drug				
	S. aureus	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albicans*	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albicans*
5a	21	16	20	15	16	84	66	77	75	89
5b	23	20	22	17	14	92	83	85	85	78
5c	16	13	14	13	15	64	54	54	65	83
5d	20	14	17	15	15	80	58	65	75	83
5e	17	12	15	14	13	68	50	58	70	72
5f	22	18	21	16	15	88	75	81	80	83
5g	18	12	16	14	14	72	50	62	70	78
Norfloxacin	25	24	26	-	-	100	100	100		
Ketoconazole				20	18				100	100

RESULTS

Various 2-[3-(4-substitutedphenyl)-4-formyl-pyrazol-1-yl]-6-amino benzothiazoles derivatives **(5a-g)** have been prepared in fairly good yields by **Scheme 1**. The structures of synthesized compounds have been confirmed by their elemental analysis, IR and ^{1}HNMR spectra. The FT-IR spectra exhibited a strong characteristic band in the region 1695-1735 cm^{-1} due to C=O (str.), and a weak band in the region 2732-2795 cm^{-1} due to C-H (str.) of the aldehyde group. The ^{1}HNMR spectra showed two sharp singlets at δ 9.05 and δ 9.95 confirmed the presence of C_5-H of the pyrazole ring and C-H of the C_4-aldehyde group respectively. The synthesized compounds were evaluated for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal activity against *Aspergillus niger* and *Candida albicans.* Compounds **5b**, **5f**, **5d**, **5a** showed excellent antimicrobial activity while the other compounds showed moderate activity. Compounds **5b**, **5f**, **5d**, **5a** were also showed good antifungal activity.

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