Synthesis and Antimicrobial Activity of Novel Thiazole Substituted Pyrazole Derivatives

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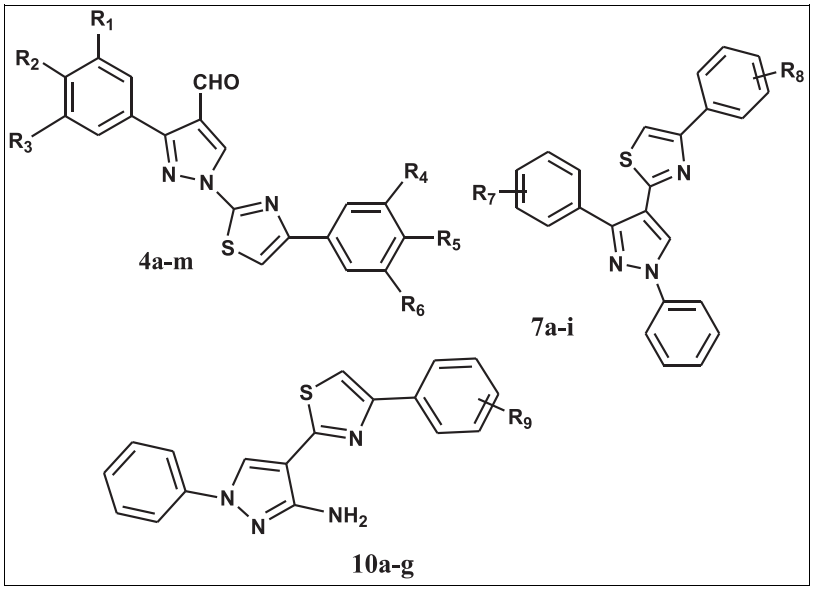

A series of new 1-[4-(2,3,4-substituted-phenyl) thiazol-2-yl]-3-(2,3,4-substituted-phenyl)-1H-pyrazole-4-carbaldehyde (**4a–m**), 4-[4-(4-substituted-phenyl) thiazol-2-yl]-3-(4-substituted-phenyl)-1-phenyl-1H-pyrazole (**7a–i**), 4-[4-(4-substituted phenyl)thiazol-2-yl]-1-phenyl-1H-pyrazol-3-amine (**10a–g**) have been synthesized by using Vilsmeier Haack formylation and Hantzsch reaction in high yield. All the synthesized compounds were tested qualitative (Zone of inhibition) and quantitative antimicrobial activities (MIC). Most of the synthesized compounds showed potent antimicrobial activity against gram positive and gram negative bacteria as well as fungi species.

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INTRODUCTION

Pyrazole and thiazole rings are privileged scaffolds for the generation of target compounds for drug discovery. The structural diversity and biological importance of pyrazoles [1–18] and thiazoles [19–34] have made them attractive targets for synthesis. Pyrazole and thiazole rings present in the same molecule could be convenient models for investigation of their biological activity. Literature revealed many syntheses of such thiazolyl-pyrazoles, which include condensation of thiocarbamoyl pyrozoles with phenacyl bromides or condensation of hydrazino thiazoles with α-cyanoacetophenones that showed anti-inflammatory activity [35,36]. Alternatively, condensation of hydrazino thiazoles with arylidine malononitriles also gave the thiazolyl-pyrazoles [37]. Another approach uses condensation of 4-(chloroacetyl)-antipyrine with thiourea [38].

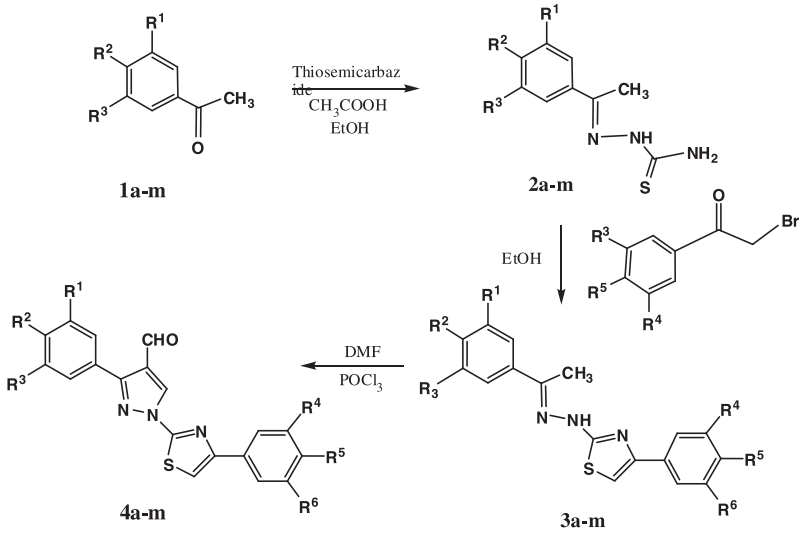
- In continuation of our effort to synthesize such compounds, that may be potent and less toxic antimicrobial agents, we report herein the synthesis of some new thiazole substituted pyrazole derivatives.

Three different series of thiazole substituted pyrazole derivatives (**4a–m**, **7a–i**, and **10a–g**) were synthesized and screened for their antimicrobial activity.

CHEMISTRY

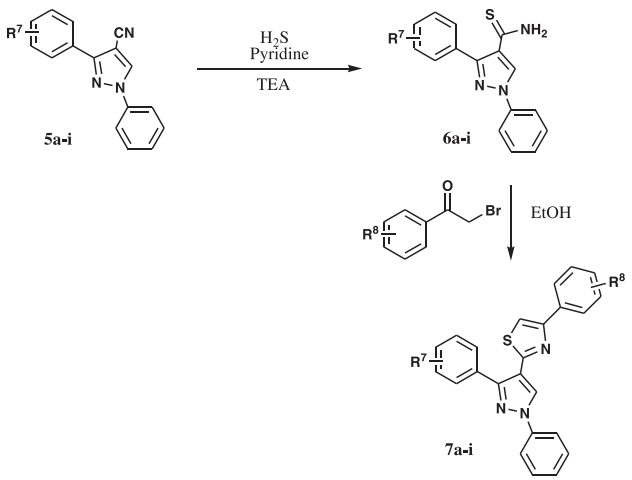
Considering the importance of pyrazole and thiazole derivatives, it was thought worthwhile to synthesize thiazole substituted pyrazole derivatives. In the present work, we report the synthesis of 1-[4-(substituted-phenyl) thiazol-2-yl]-3-(substituted-phenyl)-1H-pyrazole-4-carbaldehyde **4a–m**, 4-[4-(substituted-phenyl) thiazol-2-yl]-3-(substituted-phenyl)-1-phenyl-1H-pyrazole **7a–i**, 4-[4-(substituted phenyl)thiazol-2-yl]-1-phenyl-1H-pyrazol-3-amine **10a–g** using Vilsmeier Haack formylation and Hantzsch reaction.

Scheme 1. Synthetic pathway for the formation of compounds **4a–m**.



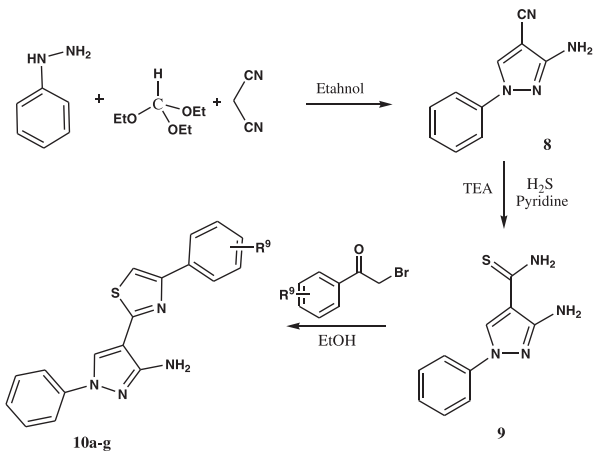
Compound	R^1	R^2	R^3	R^4	R^5	R^6	Yield(%)	m.p. (°C)
4a	H	H	H	H	Br	H	74	205-207
4b	CF_3	H	CF_3	F	OMe	H	63	214-216
4c	H	Br	H	H	NO_2	H	65	>300
4d	CF_3	H	CF_3	NO_2	H	H	63	235-237
4e	H	NO_2	H	F	OMe	H	68	240-242
4f	H	NO_2	H	H	Br	H	66	>300
4g	H	NO_2	H	CF_3	H	CF_3	72	224-226
4h	CF_3	H	CF_3	CF_3	H	CF_3	67	222-224
4i	H	Br	H	H	Br	H	62	>300
4j	CF_3	H	CF_3	H	Br	H	66	278-280
4k	CF_3	H	CF_3	H	Cl	H	77	281-283
4l	H	Br	H	H	Cl	H	68	244-246
4m	H	Cl	H	H	NO_2	H	64	260-262

Scheme 2. Synthetic pathway for the formation of compounds **7a–i**.



Compound	R^7	R^8	Yield (%)	mp (°C)
7a	F	NO_2	65	232-234
7b	F	Cl	66	197-199
7c	F	H	64	188-190
7d	F	Br	67	208-210
7e	F	F	60	193-195
7f	F	CH_3	75	212-214
7g	Br	CH_3	63	223-225
7h	Br	NO_2	67	> 300
7i	Br	H	73	215-217

Scheme 3. Synthetic pathway for the formation of compounds **10a–g**.



Compound	R^9	Yield	mp (°C)
10a	F	71	195-197
10b	Cl	67	202-204
10c	Br	70	192-194
10d	CH_3	65	189-191
10e	H	68	186-188
10f	$4NO_2$	71	228-230
10g	$3NO_2$	66	217-219

Compounds **4a–m**, were synthesized (Scheme 1) from the substituted acetophenone **1a–m**, which were converted into their corresponding thiosemicarbazones **2a–m**. The thiosemicarbazones **2a–m** upon Hantzsch reaction with phenacyl bromide gave thiazoles **3a–m**. The thiazoles **3a–m** upon Vilsmeier Haack formylation gave 1-[4-(substituted-phenyl) thiazol-2-yl]-3-(substituted-phenyl)-1H-pyrazole-4-carbaldehyde (**4a–m**). Compounds **7a–i**, were synthesized (Scheme 2) from the key intermediate nitrile pyrazoles **5a–i**, which in turn were prepared by the known literature method [18]. The reaction of nitrile pyrazole **5a–i** with hydrogen sulfide gas in pyridine and catalytic amount of triethyl amine gave corresponding thioamides **6a–i**. These thioamides **6a–i** upon Hantzsch reaction with phenacyl bromide gave 4-[4-(substituted-phenyl) thiazol-2-yl]-3-(substituted-phenyl)-1-phenyl-1H-pyrazole **7a–i**. Compounds **10a–g** were synthesized as delineated in Scheme 3. Reaction of phenyl hydrazine, triethyl orthoformate, and malonyl nitrile gave 3-amino-4-cyno pyrazole **8a–g** [39]. Compounds **8a–g** were converted into corresponding thioamides **9a–g** as in the case of nitrile pyrazole **5a–i**. These thioamides **9a–g** upon Hantzsch reaction with phenacyl bromide gave 4-[4-(substituted phenyl) thiazol-2-yl]-1-phenyl-1H-pyrazol-3-amine **10a–g**.

BIOLOGICAL RESULTS AND DISCUSSION

All the synthesized compounds were tested for antibacterial activity against bacteria *Bacillus subtilis* (2250), *Staphylococcus aureus* (2079), *Escherichia coli* (2109), and *Pseudomonas aeruginosa* (2036) and antifungal activity against two fungi *Candida albicans* (3471) and *Aspergillus niger* (545). To evaluate the activity of the synthesized compounds, the zone of inhibition (128 µg/mL in dimethyl sulfoxide (DMSO)) and minimum inhibitory concentrations (MICs; at 128, 64, 32, 16, 8, 4, 2, and 1 ug/mL in DMSO) were determined using agar diffusion method [40–42]. Known antibiotic Chloramphenicol

Table 1

Antimicrobial screening of synthesized compounds **4a–10g** (zone diameter of growth inhibition in mm).

	Microorganisms					
Compounds[a]	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Candida. albicans*
4a	22.1	-	20.25	-	-	-
4b	-	17.62	-	18.53	18.23	17.56
4c	-	18.84	-	20.4	-	-
4d	18.22	20.4	-	-	-	-
4e	24.41	-	16.7	-	-	19.09
4f	-	-	12.51	-	-	15.02
4g	-	12.79	-	16.44	-	-
4h	-	-	14.22	-	13.63	-
4i	-	13.18	-	-	-	-
4j	19.38	-	-	-	-	17.2
4k	-	-	-	-	11.81	-
4l	-	17.86	-	18.64	14.3	12.51
4m	-	18.42	-	-	-	13.48
7a	24.68	-	-	18.94	19.32	-
7b	19.03	17.58	18.29	18.81	15.21	19.35
7c	27.13	-	-	-	17.33	18.02
7d	-	-	14.36	-	17.52	20.08
7e	-	21.12	24.14		-	15.74
7f	18.32	-	-	-	18.20	17.44
7g	26.68			19.94	18.36	-
7h	-	18.68	-	14.38	-	-
7i	21.58	-	22.38	-	-	15.24
10a	19.48	-	-	-	16.32	-
10b	22.25	-	14.38	-	12.81	-
10c	-	17.54	-	-	-	15.74
10d	-	15.84	-	-	11.25	15.38
10e	-	-	-	-	12.50	-
10f		-	-	21.54		-
10g	-	-	15.38	-	13.32	-
Nystatin	NA	NA	NA	NA	20.32	22.03
Chloramphenicol	31.4	27.55	29.24	23.87	NA	NA

[a]Chloromphenicol (128 µg/disk) and [a]Nystatin (128 µg/disk) were used as reference; synthesized compounds (128 µg/disk); NA, not applicable; -, inactive.

(the reference for antibacterial drugs) and Nystatin (the reference for antifungal drug) were used for comparison. The zone of inhibition and MIC against micro organisms tested is reported in Tables 1 and 2, respectively. As shown in Table 1 most of the compounds were active against gram positive and gram negative bacteria as well as both the fungi species.

Careful analysis of the MICs in Table 2 provides some lead molecules with good antibacterial and antifungal activity. Of the compounds **4a–10g** tested, compounds with electron-withdrawing F, Cl, Br, CF_3, and NO_2 at the phenyl ring expressed a moderate to good activity against most of the tested pathogens, they inhibited the Gram-negative and Gram-positive pathogens equally. Compounds **4a** to **10g** required about 32–128 μg/mL against Gram-positive and Gram-negative bacteria as well as both the fungi species, whereas **4e** required 32 μg/mL, **4j**, **7a**, **7e** and **7g** required 64 μg/mL, and **4a**, **4d**, **7b**, **7f**, **7i**, **10a**, **10b** required 128 μg/mL against *S. aureus*. Also compounds **4b**, **4c**, **4d**, **4g**, **4i**, **4l**, **4m**, **7b**, **7e**, **7h**, **10c**, and **10d** registered their MIC at 128 μg/mL against *E. coli*. However, the CF_3 substituent did not enhance the activity. Introduction of the F, Cl, Br, and NO_2 substituent on the phenyl ring showed an improvement in its activity. Compounds **7b**, **7i** and **4a**, **4e**, **4f**, **4h**, **7d**, **7e**, **10b**, **10f**, **10g** showed MIC at 64 and 128 μg/mL, respectively against *B. subtilis* (they are four and eightfold less potent than Chloramphenicol). Compounds **4b**, **4c**, **4g**, **4l**, **7a**, **7b**, **7g**, and **7h** registered MIC at 128 μg/mL against *P. aeruginusa*.

Table 2 also describes the MIC of synthesized compounds for their antifungal activity. The compound **4a** with unsubstituted phenyl ring did not exhibit activity even at 128 μg/mL. However, the introduction of F, Cl, Br, and NO_2 substituents exhibited moderate to good activity against *C. albicans* and *A. niger*, whereas, except the **4a**, **4c**, **4d**, **4g**, **4i**, **7h**, and **10f**, all other compounds showed

Table 2

Antimicrobial screening of synthesized compounds **4a–10g** MIC in μg/mL.

	Microorganisms					
Compounds[a]	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Candida albicans*
4a	128	-	128	-	-	-
4b	-	128	-	128	32	64
4c	-	128	-	128	-	-
4d	128	128	-	-	-	-
4e	32	-	128	-	-	64
4f	-	-	128	-	-	128
4g	-	128	-	128	-	-
4h	-	-	128	-	128	-
4i	-	128	-	-	-	-
4j	64	-	-	-	-	64
4k	-	-	-	-	128	64
4l	-	128	-	128	128	128
4m	-	128	-	-	-	128
7a	64	-	-	128	32	-
7b	128	128	64	128	128	32
7c	64	-	-	-	64	64
7d	-	-	128	-	64	64
7e	-	128	128		32	32
7f	128	-	-	-	32	64
7g	64	-	-	128	32	-
7h	-	128	-	128	-	-
7i	128	-	64	-	-	128
10a	128	-	-	-	64	-
10b	128	-	128	-	128	-
10c	-	128	-	-	-	128
10d	-	128	-	-	128	128
10e	-	-	-	-	128	-
10f	-	-	128	-	-	-
10g	-	-	128	-	128	-
Nystatin	NA	NA	NA	NA	16	16
Chloramphenicol	16	16	16	16	NA	NA

[a]Chloromphenicol (μg/mL) and [a]Nystatin (μg/mL) were used as reference; NA, not applicable; -, inactive.

moderate to good activity against *A. niger* and *C. albican*. Of the halo-substituted compounds **4b**, **7a**, **7e**, **7f**, and **7g** registered a good activity against *A. niger* at 32 μg/mL, also compounds **7d**, **7e**, and **10a** recorded moderate activity at 64 μg/mL, which is fourfold lower than standard Nystatin. In addition, compounds **7b** and **7f** registered the MIC at 32 μg/mL against *C. albicans*. Introduction of the halo substituent on the phenyl ring moderately inhibited the growth of *A. niger* and *C. albican*.

CONCLUSION

In conclusion, a series of new compounds **4a–m**, **7a–i**, and **10a–g** were synthesized. The pharmacological studies were undertaken to evaluate the effect of substituents and position of thiazole on pyrazole ring for their antimicrobial activities. Most of the synthesized compounds exhibited moderate to good activity towards Gram-positive and Gram-negative bacteria as well as both the fungi species. The enhancement in antibacterial and antifungal activity can be attributed to the presence of pharmacologically active F, Cl, Br, and NO_2 groups irrespective of their position in the molecule.

EXPERIMENTAL

Melting points were determined in capillary tubes in silicon oil bath using a Veego melting point apparatus and are uncorrected. ^{1}H (300 MHz) NMR and ^{13}C (75 MHz) NMR spectra were recorded on Varian mercury XL-300. Chemical shifts are reported from internal tetramethylsilane standard and are given in δ units. The solvent for NMR spectra was $CDCl_3$ and DMSO-d_6. Infrared spectra were taken on Shimadzu FTIR-408 in KBr. The mass spectra were recorded on Shimadzu GC-MS QP 2010A mass spectrometer with an ionization potential of 70 eV. Elemental analysis was performed on a Hosli CH-analyzer.

General procedure for the synthesis of (E)-1-[1-(substituted phenyl) ethylidene] thiosemicarbazide (2a–m). A mixture of substituted acetophenone **1a–m** (1 mol), thiosemicarbazide (1 mol), and acetic acid (1 mL) in ethanol (20 mL) was refluxed for 30 min. After the completion of the reaction, as monitored on TLC, the reaction mixture was cooled at room temperature. The product was filtered, washed with water, dried, and recrystallized from ethanol.

General procedure for the synthesis of (E)-2-[4-(substituted phenyl) thiazol-2-yl] -1-[(1-(substituted phenyl) ethylidene] hydrazine (3a–m). A mixture of (E)-1-[1-(4-substituted phenyl) ethylidene] thiosemicarbazide **2a–m** (1 mol) and substituted phenacyl bromide (1 mol) in absolute ethanol (25 mL) was refluxed for 1 h, on completion of reaction, reaction mixture was cooled to room temperature. The product separated out was filtered, washed with ethanol, dried, and recrystallized from appropriate solvent.

General procedure for the synthesis of 1-[4-(substituted-phenyl) thiazol-2-yl]-3-(substituted-phenyl)-1H-pyrazole-4-carbaldehyde (4a–m). To a well stirred and cooled (0°C) DMF solution, $POCl_3$ was added drop wise during 1 h. After complete addition of $POCl_3$, the reaction mixture was further stirred at 0°C for 1 h. To this well stirred and cooled reaction mixture, a solution of **3a–m** in anhydrous DMF was added dropwise during 1 h; after complete addition, reaction mixture was heated at 65–70°C for 2 h. The reaction mixture was poured into crushed ice and left overnight in a refrigerator, during which the product separates out as solid mass. The product was filtered, washed with Na_2CO_3 (5%, 30 mL), water, and recrystallized from DMF-ethanol mixture. The yields of **4a–m** for this step are reported along with the spectral data given in the succeeding text.

General procedure for the synthesis of 3-(substituted phenyl)-1-phenyl-1H-pyrazole-4-carbothioamide (6a–i). Carbonitrile **5a–i** (1 mol) and triethylamine (1 mL) were taken in pyridine (25 mL), and excess of hydrogen sulfide gas was passed through this solution for 2h. The reaction mixture was poured in ice cold water, the product separated out was filtered, washed with water and recrystallized from ethanol.

General procedure for the synthesis of 4-[4-(substituted-phenyl) thiazol-2-yl]-3-(4-substituted-phenyl)-1-phenyl-1H-pyrazole (7a–i). A mixture of **6a–i** (1 mol) and substituted phenacyl bromide (1 mol) in absolute ethanol (25 mL) was refluxed for 1 h. On completion of reaction, the reaction mixture was cooled to room temperature, the product thus separated was filtered, washed with ethanol, dried, and recrystallized from ethanol: DMF.

General procedure for the synthesis of 3-amino-1-phenyl-1H- pyrazole-4-carbonitrile (8). A mixture of malononitrile (1.5 mol) and triethylorthoformate (1 mol) was added slowly to the solution of phenyl hydrazine (1 mol) in absolute ethanol with stirring. After complete addition, the solution was heated to gentle boiling for 2 h. The solution was kept overnight in a refrigerator. The product was filtered, washed with water, and recrystallized from hot water.

General procedure for the synthesis of 3-amino-1-phenyl-1H-pyrazole-4-carbothioamide (9). Carbonitrile (1 mol) **8** and triethylamine (1 mL) were dissolved in pyridine (25 mL), and excess of hydrogen sulfide gas was passed through this solution for 2 h. The reaction mixture was poured in ice cold water to obtain a solid product that was filtered, washed with water, and recrystallized from ethanol.

General procedure for the synthesis of 4-[4-(substituted phenyl) thiazol-2-yl)-1-phenyl-1H-pyrazol-3-amine (10a–g). A mixture of 3-amino-1-phenyl-1H-pyrazole-4-carbothioamide **9** (1 mol) and substituted phenacyl bromide (1 mol) in absolute ethanol (25 mL) was refluxed for 1 h. After completion of reaction, as monitored on TLC, reaction mixture was cooled to room temperature. The product thus separated was filtered, washed with ethanol, dried, and recrystallized in ethanol.

1-[4-(4-Bromophenyl)thiazol-2-yl]-3-phenyl-1H-pyrazole-4-carbaldehyde (4a). Yield: (74%); mp: 205–207°C; IR (KBr, cm^{-1}) 1689 (CHO); ^{1}H NMR ($CDCl_3$): δ 7.48–7.55 (m, 5H, Ar–H); 7.80 (d, J = 8.5 Hz, 2H); 7.66 (d, J = 8.5 Hz, 2H); 10.11 (s, 1H, CHO); 7.30 (s, 1H, pyrazole); 9.10 (s, 1H, thiazole); ^{13}C NMR ($CDCl_3$): δ 104; 110; 121; 123; 128; 129; 132; 137; 139; 142; 148; 152; 158; 182; *Anal*. Calcd for $C_{19}H_{12}BrN_3OS$: C, 55.62; H, 2.95; N, 10.24; Found: C, 55.27; H, 3.08; N, 10.32; ms: *m/z* 408.99 (M^+), 409.99 (M + 1), 410.99.

3-[3,5-Bis(trifluoromethyl)phenyl]-1-[4-(3-fluoro-4-ethoxyphenyl) thiazol-2-yl]-1H-pyrazole-4-carbaldehyde (4b). Yield: (63%); mp: 214–216°C; IR (KBr, cm^{-1}) 1690 (CHO); ^{1}H NMR ($CDCl_3$): δ 8.54 (s, 2H); 7.98 (s, 1H); 10.11 (s, 1H, CHO); 7.30

(s, 1H, pyrazole); 9.10 (s, 1H, thiazole); 7.68 (d, $J = 8.5$ Hz, 1H); 7.64 (d, $J = 8.5$ Hz, 1H); 7.05 (d, $J = 8.5$ Hz, 1H); 3.96 (s, 3H); *Anal*. Calcd for $C_{22}H_{12}F_7N_3O_2S$: C, 51.27; H, 2.35; N, 8.15; Found: C, 51.53; H, 2.45; N, 8.72; ms: *m/z* 515.05 (M^+), 516.06 (M + 1), 517.05.

3-(4-Bromophenyl)-1-[4-(4-nitrophenyl) thiazol-2-yl]-1H-pyrazole-4-carbaldehyde (4c). Yield: (65%); mp: >300°C; IR (KBr, cm^{-1}) 1685 (CHO); ^{1}H NMR ($CDCl_3$): δ 7.81 (d, $J = 8.5$ Hz, 2H); 7.61 (d, $J = 8.5$ Hz, 2H); 10.14 (s, 1H, CHO); 7.43 (S, 1H, pyrazole); 9.115 (s, 1H, thiazole); 8.22 (d, $J = 8.5$ Hz, 2H); 8.38 (d, $J = 8.5$ Hz, 2H); ^{13}C NMR: δ 104; 110; 121;123; 128; 129; 132; 137; 139; 142; 148; 152; 158; 182 (CHO); *Anal*. Calcd for $C_{19}H_{11}BrN_4O_3S$: C, 50.12; H, 2.44; N, 12.31; Found: C, 50.12; H, 2.44; N, 12.3; ms: *m/z*: 455.97[M^+], 453.97[M + 1], 456.97.

3-[3,5-Bis(trifluoromethyl)phenyl]-1-[4-(3-nitrophenyl)thiazol-2-yl]-1H-pyrazole-4-carbaldehyde (4d). Yield: (63%); mp: 235–237°C; IR (KBr, cm^{-1}) 1690 (CHO); ^{1}H NMR ($CDCl_3$): δ 8.549 (s, 2H); 8.018 (s, 1H); 10.14 (s, 1H, CHO); 7.60 (s, 1H, pyrazole); 9.162 (s, 1H, thiazole); 8.81 (s, 1H); 8.27 (d, $J = 8.5$ Hz, 1H); 8.24 (dd, $J = 8.5$ Hz, 1H); 7.67 (dd, $J = 8.5$ Hz and $J = 2$ Hz, 1H); *Anal*. Calcd for $C_{21}H_{10}F_6N_4O_3S$: C, 49.23; H, 1.97; N, 10.93; Found: C, 49.32; H, 1.91; N, 10.98; ms: *m/z* 512.04 [M^+], 513.04 [M + 1], 514.

1-[4-(3-Fluoro-4-methoxyphenyl) thiazol-2-yl]-3-(4-nitrophenyl)-1H-pyrazole-4-carbaldehyde (4e). Yield: (68%); mp: 240–242°C; IR (KBr, cm^{-1}) 1690 (CHO); ^{1}H NMR ($CDCl_3$): δ 8.34 (d, $J = 8.5$ Hz, 2H); 8.22 (d, $J = 8.5$ Hz, 2H,); 10.06 (s, 1H, CHO); 8.02 (s, 1H, pyrazole); 9.54 (s, 1H, thiazole); 7.82 (d, $J = 8$ Hz, 1H); 7.23 (d, $J = 8$ Hz, 1H); 7.78 (d, 1H); 3.89 (s, 3H); *Anal*. Calcd for $C_{20}H_{13}FN_4O_4S$: C, 43.57; H, 2.38; N, 10.16; Found: C, 43.41; H, 2.46; N, 10.28; ms: *m/z* 550.97 [M^+], 551.97 [M + 1].

1-[4-(4-Bromophenyl) thiazol-2-yl]-3-(4-nitrophenyl)-1H-pyrazole-4-carbaldehyde (4f). Yield: (66%); mp: >300°C; IR (KBr, cm^{-1}): 1685 (CHO); ^{1}H NMR ($CDCl_3$): δ 8.16 (d, $J = 8.5$ Hz, 2H); 8.33 (d, $J = 8.5$ Hz, 2H); 10.15 (s, 1H, CHO); 7.52 (s, 1H, pyrazole); 9.15 (s, 1H, thiazole); 7.78 (d, $J = 8.5$ Hz, 2H); 7.62 (d, $J = 8.5$ Hz, 2H); *Anal*. Calcd for $C_{19}H_{11}BrN_4O_3S$: C, 50.12; H, 2.44; N, 12.31; Found: C, 49.96; H, 2.78; N, 12.45; ms: *m/z* 512.04 (M^+), 513.04 (M + 1), 514.03.

1-[4-{3,5-Bis(trifluoromethyl)phenyl} thiazol-2-yl]-3-(4-nitrophenyl)-1H-pyrazole-4-carbaldehyde (4g). Yield: (72%); mp: 224–226°C; IR (KBr, cm^{-1}) 1687 (CHO); ^{1}H NMR ($CDCl_3$): δ 8.16 (d, $J = 8.5$ Hz, 2H); 8.33 (d, $J = 8.5$ Hz, 2H); 10.15 (s, 1H, CHO); 7.52 (s, 1H, pyrazole); 9.15 (s, 1H, thiazole); 7.87 (s, 1H); 8.66 (s, 2H); *Anal*. Calcd for $C_{21}H_{10}F_6N_4O_3S$: C, 49.23; H, 1.97; N, 10.93; Found: C, 49.32; H, 1.89; N, 10.83; ms: *m/z* 512.04 [M^+], 513.04 [M + 1], 514.03.

3-[3,5-Bis(trifluoromethyl) phenyl]-1-[4-{3,5-zis(trifluoromethyl) phenyl} thiazol-2-yl]-1H-pyrazole-4-carbaldehyde (4h). Yield: (66%); mp: 222–224°C; IR (KBr, cm^{-1}) 1685 (CHO); ^{1}H NMR ($CDCl_3$): δ 8.54 (s, 2H); 7.96 (s, 1Hs); 10.11 (s, 1H, CHO); 7.43 (s, 1H, pyrazole); 9.10 (s, 1H, thiazole); 8.61 (s, 2H); 7.77 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 104; 110; 123; 124 (CF_3); 129; 131; 133; 137; 142; 152; 158; 182; *Anal*. Calcd for $C_{23}H_9F_{12}N_3OS$: C, 45.78; H, 1.50; N, 6.96; Found: C, 45.63; H, 1.67; N, 6.84; ms: *m/z* 603.03 (M^+), 604.03 (M + 1), 605.02.

3-(4-Bromophenyl)-1- [4-(4-bromophenyl)thiazol-2-yl]-1H-pyrazole-4-carbaldehyde (4i). Yield: (62%); mp: >300°C; IR (KBr, cm^{-1}) 1690 (CHO); ^{1}H NMR ($CDCl_3$): δ 7.80 (d, 4H, $J = 8.5$ Hz); 7.53–7.59 (d, $J = 8.5$ Hz, 4H); 10.11 (s, 1H, CHO); 7.42 (s, 1H, pyrazole); 9.10 (s, 1H, thiazole); *Anal*. Calcd for $C_{19}H_{11}Br_2N_3OS$: C, 46.65; H, 2.27; N, 8.59; Found: C, 46.51; H, 2.39; N, 8.62; ms: *m/z* 488.90 (M^+), 489.89 (M + 1), 490.85.

3-[3,5-Bis(trifluoromethyl)phenyl]-1-[4-(4-bromophenyl)thiazol-2-yl]-1H-pyrazole-4-carbaldehyde (4j). Yield: (66%); mp: 278–280°C; IR (KBr, cm^{-1}) 1690 (CHO); ^{1}H NMR ($CDCl_3$): δ 8.53 (s, 2H); 7.98 (s, 1H); 10.11 (s, 1H, CHO); 7.42 (s, 1H, pyrazole); 9.10 (s, 1H, thiazole); 7.80 (d, $J = 8.5$ Hz, 2H); 7.59 (d, $J = 8.5$ Hz, 2H); ^{13}C NMR: δ 104; 110; 123; 124; 129; 131; 132; 133; 137; 142; 158; 152; 182; *Anal*. Calcd for $C_{21}H_{10}BrF_6N_3OS$: C, 46.17; H, 1.85; N, 7.69; Found: C, 46.38; H, 1.77; N, 7.58; ms: *m/z* 544.96 [M^+], 545.96 [M + 1].

3-[3,5-Bis(trifluoromethyl)phenyl]-1-[4-(4-chlorophenyl)thiazol-2-yl]-1H-pyrazole-4-carbaldehyde (4k). Yield: (77%); mp: 281–283°C; IR (KBr, cm^{-1}) 1685 (CHO); ^{1}H NMR ($CDCl_3$): δ 8.53 (s, 2H); 7.98 (s, 1H); 10.11(s, 1H, CHO); 7.41 (s, 1H, pyrazole); 9.10 (s, 1H, thiazole); 7.86 (d, $J = 8.5$ Hz, 2H); 7.44 (d, $J = 8.5$ Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 104; 110; 123; 124; 128; 129; 131; 133; 134; 137; 142; 152; 158; 182; *Anal*. Calcd for $C_{21}H_{10}ClF_6N_3OS$: C, 50.26; H, 2.01; N, 8.37; Found: C, 50.44; H, 2.08; N, 8.45; ms: *m/z* 501.01 [M^+], 502.02 [M + 1], 503.01.

3-(4-Bromophenyl)-1-[4-(4-chlorophenyl)thiazol-2-yl]-1H-pyrazole-4-carbaldehyde (4l). Yield: (68%); mp: 244–246°C; IR (KBr, cm^{-1}) 1682 (CHO); ^{1}H NMR ($CDCl_3$): δ 7.81 (d, $J = 8.5$ Hz, 2H); 7.66 (d, $J = 8.5$ Hz, 2H); 10.09 (s, 1H, CHO); 7.60 (s, 1H, pyrazole); 9.12 (s, 1H, thiazole); 7.88 (d, $J = 8.5$ Hz, 2H); 7.46 (d, $J = 8.5$ Hz, 2H); *Anal*. Calcd for $C_{19}H_{11}BrClN_3OS$: C, 51.31; H, 2.49; N, 9.45; Found: C, 51.18; H, 2.85; N, 9.72; ms: *m/z* 444.95 [M^+], 446.09 [M + 1].

3-(4-Chlorophenyl)-1-[4-(4-nitrophenyl) thiazol-2-yl]-1H-pyrazole-4-carbaldehyde (4m). Yield: (64%); mp: 210–212°C; IR (KBr, cm^{-1}) 1688 (CHO); ^{1}H NMR ($CDCl_3$): δ 7.87 (d, $J = 8.5$ Hz, 2H); 7.50 (d, $J = 8.5$ Hz, 2H); 10.09 (s, 1H, CHO); 7.60 (s, 1H, pyrazole); 9.06 (s, 1H, thiazole); 8.09 (d, $J = 8.5$ Hz, 2H); 8.33 (d, $J = 8.5$ Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 104; 110; 121; 128; 129; 131; 134; 137; 139; 142; 148; 152; 158; 182; *Anal*. Calcd for $C_{19}H_{11}ClN_4O_3S$: C, 55.55; H, 2.70; N, 13.64; Found: C, 55.58; H, 2.75; N, 13.60; ms: *m/z* 410.02 [M^+], 411.03 [M + 1], 412.02.

3-(4-Fluorophenyl)-4-[4-(4-nitrophenyl)thiazol-2-yl]-1-phenyl-1H-pyrazole (7a). Yield: (65%); mp: 232–234°C; ^{1}H NMR ($CDCl_3$): δ 7.82 (d, $J = 8.5$ Hz, 2H); 7.59 (d, $J = 8.5$ Hz, 2H); 8.43 (d, $J = 8.5$ Hz, 2H); 7.92 (d, $J = 8.5$ Hz, 2H); 7.50 (m, 5H); 8.10 (s, 1H, thiazole); 7.71 (s, 1H, pyrazole); ^{13}C NMR ($CDCl_3$): δ 106; 110; 116; 120; 126; 128; 129; 131; 139; 145; 148; 152; 154; 162; *Anal*. Calcd for $C_{24}H_{15}FN_4O_2S$: C, 65.15; H, 3.42; N, 12.66; Found: C, 65.31; H, 3.72; N, 12.38; ms: *m/z* 442.09 (M^+), 443.09 (M + 1), 444.09.

4-[4-(4-Chlorophenyl)thiazol-2-yl]-3-(4-fluorophenyl)-1-phenyl-1H-pyrazole (7b). Yield: (66%); mp: 190–192°C; ^{1}H NMR ($CDCl_3$): δ 7.85 (d, $J = 8$ Hz, 2H); 7.44 (d, $J = 8$ Hz, 2H); 7.82 (d, $J = 8.5$ Hz, 2H), 7.38 (d, $J = 8.5$ Hz, 2H); 7.18 (m, 2H), 7.54 (m, 3H); 9.25(s, 1H, thiazole); 7.75 (1H, s, pyrazole); ^{13}C NMR ($CDCl_3$): δ 106; 110; 116; 120; 126; 128; 129; 131; 134; 139; 145; 152; 154; 163; *Anal*. Calcd for $C_{24}H_{15}ClFN_3S$: C, 66.74; H, 3.50; N, 9.73; Found: C, 66.66; H, 3.57; N, 9.62; ms: *m/z* 431.07 [M^+], 432.07 [M + 1], 433.06, 434.07.

3-(4-Fluorophenyl)-1-phenyl-4- (4-phenylthiazol-2-yl)-1H-pyrazole (7c). Yield: (64%); mp: 185–187°C; ^{1}H NMR ($CDCl_3$): δ 7.82 (d, $J = 8.5$ Hz, 2H); 7.59 (d, $J = 8.5$ Hz, 2H); 7.97 (d, $J = 8.5$ Hz, 2H); 7.55 (d, $J = 8.5$ Hz, 5H); 7.5 (m, 5H,); 8.38 (s, 1H, thiazole); 7.74 (s, 1H, pyrazole); ^{13}C NMR ($CDCl_3$): δ 106; 110; 116; 120; 126; 127; 128; 129; 131; 133; 139; 145; 152; 154;

162; *Anal*. Calcd for $C_{24}H_{16}FN_3S$: C, 72.52; H, 4.06; N, 10.57; Found: C, 72.48; H, 4.13; N, 10.63; ms: *m/z* 397.10 [M^+], 398.1 [M + 1], 399.2, 400.1.

4-[4-(4-Bromophenyl)thiazol-2-yl-3-(4-fluorophenyl)-1-phenyl-1H-pyrazole (7d). Yield: (67%); mp: 208–210°C; ^{1}H NMR ($CDCl_3$): δ 7.83 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H); 7.79 (d, *J* = 8.5 Hz, 2H); 7.56 (d, *J* = 8.5 Hz, 2H); 7.17 (m, 2H); 7.5 (m, 3H); 7.85 (s, 1H, thiazole); 7.49 (s, 1H, pyrazole); ^{13}C NMR ($CDCl_3$): δ 106; 110; 116; 120; 123; 126; 128; 129; 131; 132; 139; 145; 152; 154; 162; *Anal*. Calcd for $C_{24}H_{15}BFrN_3S$: C, 60.51; H, 3.17; N, 8.82; Found: C, 60.57; H, 3.23; N, 8.83; ms: *m/z* 477.01 [M^+], 478.0 [M + 1], 479, 480, 477, 476, 398, 205.

3-(4-Fluorophenyl)-4-[4-(4-fluorophenyl)thiazol-2-yl]-1-phenyl-1H-pyrazole (7e). Yield: (60%); mp: 193–195°C; ^{1}H NMR ($CDCl_3$): δ 7.92 (d, *J* = 8.5 Hz, 2H); 7.84 (d, *J* = 8.5 Hz, 2H); 7.89 (d, *J* = 8.5 Hz, 2H); 7.77 (d, *J* = 8.5 Hz, 2H); 7.15 (m, 2H); 7.5 (m, 3H); 8.10 (s, 1H, thiazole); 7.74 (s, 1H, pyrazole); ^{13}C NMR ($CDCl_3$): δ 106; 110; 116; 120; 126; 128; 129; 131; 139; 145; 152; 154; 162; *Anal*. Calcd for $C_{24}H_{15}F_2N_3S$: C, 69.38; H, 3.64; N, 10.11; Found: C, 69.81; H, 3.27; N, 10.74; ms: *m/z* 415.1 (M^+), 416.1 (M + 1), 417.1, 418.1.

3-(4-Fluorophenyl)-1-phenyl-4-(4-p-tolylthiazol-2-yl)-1H-pyrazole (7f). Yield: (75%); mp: 212–214°C; ^{1}H NMR ($CDCl_3$): δ 7.92 (d, *J* = 8.5 Hz, 2H); 7.84 (d, *J* = 8.5 Hz, 2H); 7.63 (d, *J* = 8.5 Hz, 2H); 7.53 (d, *J* = 8.5 Hz, 2H); 7.46 (m, 5H); 8.37 (s, 1H, thiazole); 7.74 (s, 1H, pyrazole); *Anal*. Calcd for $C_{25}H_{18}FN_3S$: C, 72.97; H, 4.41; N, 10.21; Found: C, 72.22; H, 4.71; N, 10.36; ms: *m/z* 411.12 (M^+), 412.12 (M + 1), 413.12.

3-(4-Bromophenyl)-1-phenyl-4-(4-p-tolylthiazol-2-yl)-1H-pyrazole (7g). Yield: (63%); mp: 223–225°C; ^{1}H NMR ($CDCl_3$): δ 7.97 (d, *J* = 8 Hz, 2H); 7.74 (d, *J* = 8.5 Hz, 2H); 7.63 (d, *J* = 8.5 Hz, 2H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.46 (m, 5H), 8.37 (s, 1H, thiazole); 7.74 (s, 1H, pyrazole); *Anal*. Calcd for $C_{25}H_{18}BrN_3S$: C, 63.56; H, 3.84; N, 8.90; Found: C, 63.57; H, 3.94; N, 8.83; ms: *m/z* 471.9 [M^+], 472.04 [M + 1], 420.9, 348.8, 304.8, 255.9, 194.6, 148.6, 101.7.

3-(4-Bromophenyl)-4-[4-(4-nitrophenyl)thiazol-2-yl]-1-phenyl-1H-pyrazole (7h). Yield: (67%); mp: >300°C; ^{1}H NMR ($CDCl_3$): δ 8.12 (d, *J* = 8.5 Hz, 2H); 7.73 (d, *J* = 8.5 Hz, 2H); 8.43 (d, *J* = 8.5 Hz, 2H); 7.92 (d, *J* = 8.5 Hz, 2H); 7.50 (m, 5H); 8.10 (s, 1H, thiazole); 7.71 (s, 1H, pyrazole); *Anal*. Calcd for $C_{24}H_{15}BrN_4O_2S$: C, 57.27; H, 3.00; N, 11.13; Found: C, 57.35; H, 3.07; N, 11.18; ms: *m/z* 501.0 [M^+], 502.9 [M + 1], 503.9, 504.9, 476.1, 433.1, 318.9, 255.8, 176.7, 120.5, 88.0.

3-(4-Bromophenyl)-1-phenyl-4-(4-phenylthiazol-2-yl)-1H-pyrazole (7i). Yield: (73%); mp: 215–217°C; ^{1}H NMR ($CDCl_3$): δ 7.98 (d, *J* = 8.50 Hz, 2H), 7.74 (d, *J* = 8.5 Hz, 2H); 7.97 (d, *J* = 8 Hz, 2H); 7.55 (d, *J* = 8.5 Hz, 5H); 7.5 (m, 5H); 8.38 (s, 1H, thiazole); 7.74 (s, 1H, pyrazole); *Anal*. Calcd for $C_{24}H_{16}BrN_3S$: C, 62.89; H, 3.52; N, 9.17; Found: C, 62.74; H, 3.58; N, 9.25; ms: *m/z* 458.8 [M^+], 459.9 [M + 1], 457.0, 413.0, 323.6, 284.3, 238.6, 197.5, 88.5.

4-[4-(4-Fluorophenyl)thiazol-2-yl]-1-phenyl-1H-pyrazol-3-amine (10a). Yield: (71%); mp: 197–199°C; IR (KBr, cm^{-1}) 3476, 3335 (NH_2); ^{1}H NMR ($CDCl_3$): δ 7.48–7.63 (m, 5H); 7.55 (d, *J* = 8.5 Hz, 2H); 7.90 (d, *J* = 8.5 Hz, 2H); 7.85 (s, 1H, thiazole); 7.45 (s, 1H, pyrazole); 5.71 (bs, 2H, NH_2); ^{13}C NMR ($CDCl_3$): δ 94; 110; 116; 120; 126; 127; 128; 129; 133; 139; 150; 152; 154; 162; *Anal*. Calcd for $C_{18}H_{13}FN_4S$: C, 64.27; H, 3.90; N, 16.66; Found: C, 64.89; H, 3.52; N, 16.17; ms: *m/z* 336.1 [M^+], 337.1 [M + 1], 338.0, 322.9, 274.9, 241.8, 195.6, 152.6, 133.5, 78.7.

4-[4-(4-Chlorophenyl)thiazol-2-yl]-1-phenyl-1H-pyrazol-3-amine (10b). Yield: (67%); mp: 202–204°C; IR (KBr, cm^{-1}) 3487, 3336 (NH_2); ^{1}H NMR ($CDCl_3$): δ 7.48–7.63 (m, 5H); 7.62 (d, *J* = 8.5 Hz, 2H); 7.85 (d, *J* = 8.5 Hz, 2H); 7.86 (s, 1H, thiazole); 7.47 (s, 1H, pyrazole); 5.71 (bs, 2H, NH_2); ^{13}C NMR: δ 110; 120; 126; 128; 129; 131; 134; 139; 150; 152; 154; *Anal*. Calcd for $C_{18}H_{13}ClN_4S$: C, 61.27; H, 3.71; N, 15.88; Found: C, 61.69; H, 3.62; N, 15.57; ms: *m/z* 351.9 [M^+], 352.7 [M + 1], 353.8 [M + 2], 350.6 [M-1], 337.6, 324.8, 296.8, 254.7, 215.5, 174.4, 146.4, 112.3, 78.7.

4-[4-(4-Bromophenyl)thiazol-2-yl]-1-phenyl-1H-pyrazol-3-amine (10c). Yield: (70%); mp: 192–194°C; IR (KBr, cm^{-1}) 3480, 3335 (NH_2); ^{1}H NMR ($CDCl_3$): δ 7.48–7.63 (m, 5H); 7.55 (d, *J* = 8.5 Hz, 2H); 7.90 (d, *J* = 8.5 Hz, 2H); 7.89 (s, 1H, thiazole); 7.47 (s, 1H, pyrazole); 5.69 (bs, 2H, NH_2); ^{13}C NMR ($CDCl_3$): δ 110; 120; 123; 126; 128; 129; 132; 139; 150; 152; 154; *Anal*. Calcd for $C_{18}H_{13}BrN_4S$: C, 54.42; H, 3.30; N, 14.10; Found: C, 54.89; H, 3.77; N, 14.59; ms: *m/z* 395.8 [M^+], 396.7 [M + 1], 397.5, 366.7, 360.9, 324.7, 282.8, 268.8, 255.8, 148.5, 78.7.

1-Phenyl-4-(4-p-tolylthiazol-2-yl)-1H-pyrazol-3-amine Compound (10d). Yield: (65%); mp: 188–190°C; IR (KBr, cm^{-1}) 3476, 3338 (NH_2); ^{1}H NMR ($CDCl_3$): δ 7.48–7.63 (m, 5H); 7.53 (d, *J* = 8.5 Hz, 2H); 7.63 (d, *J* = 8.5 Hz, 2H); 7.85 (s, 1H, thiazole); 7.46 (s, 1H, pyrazole); 5.67(bs, 2H, NH_2); *Anal*. Calcd for, $C_{19}H_{16}N_4S$: C, 68.65; H, 4.85; N, 16.85; Found: C, 68.37; H, 4.78; N, 16.25; ms: *m/z* 332.1 [M^+], 333.2 [M + 1], 331.2, 318.8, 270.9, 256.8, 191.6, 129.5.78.6, 15.0.

1-Phenyl-4-(4-phenylthiazol-2-yl)-1H-pyrazol-3-amine (10e). Yield: (68%); mp: 186–188°C; IR (KBr, cm^{-1}) 3480, 3330 (NH_2); ^{1}H NMR ($CDCl_3$): δ 7.48–7.70 (m, 10H); 7.89 (s, 1H, thiazole);7.44 (s, 1H, pyrazole); 5.65 (bs, 2H, NH_2); ^{13}C NMR ($CDCl_3$): δ 94; 110; 120; 126; 127; 128; 129; 133; 139; 150; 152; 154; *Anal*. Calcd for $C_{18}H_{14}N_4S$: C, 67.90; H, 4.43; N, 17.60; Found: C, 67.57; H, 4.72; N, 17.24; ms: *m/z* 317.8[M^+], 318.7[M + 1], 319.1, 303.9, 255.9, 241.8, 22.6, 176.6, 132.6, 114.5.

4-[4-(4-Nitrophenyl)thiazol-2-yl]-1-phenyl-1H-pyrazol-3-amine (10f). Yield: (71%); mp: 220–222°C; IR (KBr, cm^{-1}) 3484, 3340 (NH_2); ^{1}H NMR ($CDCl_3$): δ 7.48–7.63 (m, 5H); 7.97 (d, *J* = 8.5 Hz, 2H); 8.32 (d, *J* = 8.5 Hz, 2H); 7.87 (s, 1H, thiazole); 7.45 (s, 1H, pyrazole); 5.69 (bs, 2H, NH_2); ^{13}C NMR ($CDCl_3$): δ 94; 110; 120; 121; 126; 128; 129; 139; 148; 150; 152; 154; *Anal*. Calcd for $C_{18}H_{13}N_5O_2S$: C, 59.49; H, 3.61; N, 19.27; Found: C, 59.89; H, 3.73; N, 19.57; ms: *m/z* 362.8 [M^+], 363.8 [M + 1], 361.6, 317.8, 255.9, 148.5, 78.7.

4-[4-(3-Nitrophenyl)thiazol-2-yl]-1-phenyl-1H-pyrazol-3-amine (10 g). Yield: (66%); mp: 218–220°C; IR (KBr, cm^{-1}) 3478, 3335 (NH_2); ^{1}H NMR ($CDCl_3$): δ 7.48–7.63 (m, 5H); 8.81 (s, 1H,); 8.27 (d, *J* = 8.5 Hz, 1H); 8.24 (dd, *J* = 8.5 and 2 Hz, 1H); 7.67 (dd, *J* = 8.5 and 2 Hz, 1H); 7.84 (s, 1H, thiazole); 7.45 (s, 1H, pyrazole); 5.71(bs, 2H, NH_2); *Anal*. Calcd for $C_{18}H_{13}N_5O_2S$: C, 59.49; H, 3.61; N, 19.27; Found: C, 59.89; H, 3.73; N, 19.57; ms: *m/z* 362.7 [M^+], 363.8 [M + 1], 361.7, 338.8, 317.8, 296.7, 255.5, 148.6, 78.6.

Antimicrobial activity. For antibacterial activity, solid medium used for the study was Muller–Hinton agar (MHA; Hi media) of the following composition, beef infusion 300 g/L, casein acid hydrolysate 17.5 g/L, starch 1.5 g/mL, agar–agar 17 g/L, and sterilized distilled water 1000 mL adjusted to pH 7.4. soyabean casein digest agar (SCDA; casein enzymatic hydrolysate 17.0 g/L, papain digest of soyabean 3.0 g/L, NaCL 5.0 g/L, dipotassium phosphate 2.5 g/L, and distilled water 1000 mL adjusted to pH 7.3), was used for biological assays.

For antifungal activity, solid medium used for the study was potato dextrose agar (Hi media) of the following composition: potato 250 g, dextrose 10 g, agar–agar 20 g, and sterile distilled water 100 mL adjusted to pH 7.3.

Test microorganisms. All the synthesized compounds were screened for their *in vitro* antibacterial activity against the standard strains *B. subtilis* (2250), *S. aureus* (2079), *E. coli* (2109), and *P. aeruginosa* (2036) and for their antifungal activity against *C. albicans* (3471) and *A. niger* (545). All the strains were obtained from microbial type culture collection (MTCC) at the NCIM, Pune, India.

Primary screening. The antibacterial activity of all the newly synthesized compounds was carried out by the agar-well diffusion assay technique [40,41]. Bacterial cultures (24-h-old) of all test microorganisms were used as inoculums, which were adjusted to 0.5 McFarland standards, that is, 1.5×10^8 CFU/mL. The stock solutions of all test compounds (128 μg/mL) were prepared by dissolving 128 μg of the test compound in DMSO (1 mL). Chloramphenicol and DMSO were used as positive and negative controls, respectively.

Twenty milliliter of molten and cooled MHA and 320 μL of each test bacterial culture were mixed (separate flasks were used for each bacterial culture) and poured in sterilized and labeled Petri plates. The wells of 6 mm were punched in the solidified Petri plates, aseptically. Fifty microliters from stock solutions of all compounds as well as controls was added to each well of labeled Petri plates and incubated at 35°C for 24 h. The diameter of the zone of growth inhibition around each well was measured after incubation by using vernier caliper.

For the antifungal activity, sliced potatoes were taken with 500 mL of distilled water in a pan and boiled for half an hour till a spoon when placed on a slice can pierce into it. Filtered it while hot and broth was again taken in a pan with rest of the distilled water. Dextrose dissolved in distilled water and weighed agar was added to the broth and heated it to boil. The medium thus obtained was sterilized in pressure cooker for 30 min. Sterilized medium (15 mL each) was pipette out into flat Petri plates. When it solidified, 15 mL of warm seeded agar was applied over it. The seeded agar was made by cooling the medium to 40°C and then adding spore suspension to seeded medium. The spores were obtained from 10 days culture of *C. albicans* and *A. niger* species. The final inoculums size was adjusted to 1×10^6 spore mL^{-1}. Nystatin and DMSO were used as positive and negative controls, respectively.

Before the solidification of agar, the plate was tilted to ensure that coverage should be even. These Petri plates were then put into the refrigerator upside down to prevent condensation of moisture. Concentration, 128 μg/mL, of the synthesized compounds were prepared by dissolving the required quantity of compounds in DMSO, sterilized Whatmann filter paper number 541 disks were prepared by cutting 6 mm diameter with cork borer and were spread individually with needle and planted upon the chilled seeded medium. The culture plates were then incubated for 24 to 72 h at 37°C, and inhibition zone around each disk was measured from the center of the disks. The diameter of growth inhibition zone was calculated by vernier caliper.

Minimum inhibitory concentration. MIC of compounds against Gram-positive and Gram-negative test bacteria was determined by the method of the National Committee for Clinical and Laboratory Standards [42]. All the test cultures were streaked on SCDA and incubated overnight at 37°C. Turbidity of all the bacterial cultures was adjusted to 0.5 McFarland standards by preparing bacterial suspension of 3–5 well isolated colonies of the same morphological type selected from an agar plate culture. The cultures were further diluted 10-fold to obtain an inoculums size of 1.2×10^7 CFU/mL. Stock solutions of 4 mg/mL of each compound were prepared in DMSO and were appropriately diluted to obtain a final concentration of 128, 64, 32, 16, 8, 4, 2, and 1 μg/mL. Standard antibiotic Chloramphenicol was also diluted to obtain a final concentration in the same manner. Three hundred and twenty microliters of each dilution was added to 20 mL molten and cooled MHA (separate flasks were taken for each dilution). After thorough mixing, the medium was poured in sterilized Petri plates. The test bacterial cultures were spotted in a predefined pattern by ascetically transferring 5 mL of each bacterial culture on the surface of solidified agar plates and incubated at 35°C for 24 h.

For antifungal activity, the MICs of synthesized compounds **4a–10 g** were determined in the range of concentrations from 128 to 1 μg/mL. The standardized micro broth dilution methods were used according to the guidelines of Clinical and Laboratory Standards Institute (formerly National Committee for Clinical and Laboratory Standards) [42]. Table 2 summarizes the minimum concentration of each derivative necessary to completely inhibit (MIC_{90}) the growth of two standardized opportunistic pathogenic fungi including *C. albicans and A. Niger*.

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