International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

Biological evaluation of some new 1,3-benzothiazole-2-yl derivatives containing pyrazole moiety

Deepa Chauhan[1]*, A. A.Siddiqui[2], Rajkumari Kataria[3], Dr. Robin Singh[4]

1*Sunder deep Pharmacy College, NH-24, Ghaziabad-201001(U.P.)-India.
2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard University, NewDelhi-110 062-India.
3ITS Paramedical College (Pharmacy), 16km-stone, Muradnagar, Ghaziabad-201206 (U.P.)-India.
4Indian Pharmacopoeia Commission, Sector-23, Ghaziabad-201001(U.P.)-India

***Corresponding author e-mail: deepachauhan608@gmail.com**

ABSTRACT

A series of 2-[3-(substituted phenyl)-4-formylpyrazol-1-yl]-6-nitro benzothiazole derivatives **(5a-g)** have been synthesized through Vilsmeier-Haack reaction on hydrazones **(4a-g)** of appropriate substituted aromatic ketones with 6-nitro-benzothiazol-2-yl hydrazine under microwave irradiation in fairly good yields. Structural assignments of the synthesized compounds were based on their IR, ^{1}HNMR, mass spectral studies and elemental analysis. Those compounds were also screened for their *in vitro* antibacterial against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and antifungal activity against *Aspergillus niger* and *Candida albicans*. Some of the compounds exhibited promising antibacterial and antifungal activities as compared to reference drugs Norfloxacin and ketoconazole.

Keywords: 1,3-Benzothiazole, Antibacterial activity, Antifungal activity, Formylated pyrazole

INTRODUCTION

The rapid emergence of multidrug resistant pathogenic bacteria has become a serious health threat worldwide. It has been postulated that the development of resistance to known antibiotics could be overcome by identifying new drug targets via genomics, improving existing antibiotics and by identifying new antibacterial agents with novel structure and mode of action.

It is an established fact that benzothiazole derivatives contain extended p-delocalized systems which are capable of binding to DNA molecules via p–p interactions and therefore exhibited various biological properties like antitumor, antimicrobial and anthelmintic activities [1]. Benzothiazoles comprise a novel class of therapeutic compounds found to possess a number of biological activities such as anticancer [2-3], anti-inflammatory [4], antimicrobial [5-6], antidiabetic [7], antiviral [8] and antileishmanial [9]. Therefore from the above facts we herein report the synthesis of some new formylated pyrazolyl benzothiazole derivatives with the hope of finding of good antibacterial and antifungal compounds as the need of emergence.

MATERIALS AND METHODS

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 different solvent systems. 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 (KBr) 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. The elemental analyses (C, H, and N) of all compounds were performed and the measured values agreed within calculated ones.

Experimental

Synthesis of 6-nitro-2-benzothiazolamine (2)

General Procedure: A mixture of p-nitro aniline (0.01 mol) and potassium thiocyanate (0.01 mol) in 150 ml glacial acetic acid (10 %) was cooled and stirred mechanically for 30 minutes at 2-4 ºC. To this solution bromine (0.01 mol, 1.6 ml in 6 ml glacial acetic acid) was added drop wise at such a rate to keep the temperature of the solution below 10ºC throughout the addition. After all the bromine was added (105 min), stirring was continued for an additional 6 hrs at room temperature. The precipitate of hydrochloric salt of benzothiazole was filtered, washed with acetic acid and dried. Separated hydrochloric salt was dissolved in hot water and neutralized with aqueous ammonia solution (25 %), filtered, washed with water, dried and recrystallized with benzene to obtain 6-nitro-2-benzothiazolamine **2**.

Yield: 70 %; m. p. 232-235 °C; IR (KBr, cm^{-1}): 3312 (NH), 3080 (CH-Ar), 1520 (C=N), 1272 (C-N), 604 (C-S-C), 1320 (C-NO_2); ^{1}HNMR (300 MHz, DMSO-d_6, δ ppm): 7.78-7.70 (m, 3H, Ar-H), 7.12 (s, 2H, NH_2, D_2O exchangeable); MS (m/z): 195 [M^+]; Anal. Calcd. for $C_7H_5N_3O_2S$: C, 43.08 ; H, 2.58; N, 21.53. Found: C, 42.98; H, 2.45; N, 21.45.

Synthesis of 6-nitrobenzothiazol-2-yl-hydrazine (3)

General Procedure: 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 **(3)**.

Yield: 80 %; m. p. 210-212 °C; IR (KBr, cm^{-1}): 3240 (NH), 3105 (CH-Ar), 1567 (C=N),1150 (C-N), 696 (C-S-C), 1296 (C-NO_2); ^{1}HNMR (300 MHz, DMSO-d_6, δ ppm): 4.92 (s, 2H, NH_2), 9.10 (s, 1H, -NHN, D_2O exchangeable), 7.15-6.97 (m, 3H, Ar-H); MS (m/z): 210 [M^+]; Anal. Calcd. for $C_7H_6N_4O_2S$: C, 40.00; H, 2.88; N, 26.64. Found: C, 39.90; H, 2.78; N, 26.44.

Synthesis of 6-nitrobenzothiazol-2-yl-hydrazones (4a-g)

General Procedure: A mixture of 6-nitrobenzothiazole-2-yl hydrazine **3** (1.5 mmol) and appropriate aromatic substituted ketones (2.2 mmol) in absolute ethanol (60 ml) containing glacial acetic acid (4-5 drops) was taken and refluxed for 5-13 hrs on water bath. (Till a different spot on TLC may appear). On cooling solid separated out, which was filtered, washed with little water and recrystallized from absolute alcohol to get hydrazones (**4a-g)**.

6-nitro-2-(-2-[1-(4-chlorophenyl)ethylidene] hydrazinyl)-1,3-benzothiazole (4a)

Yield: 60 %; m. p. 185-187 °C; IR (KBr, cm^{-1}): 3344 (NH), 1612 (C=N),1154 (C-N), 676 (C-S-C), 1293 (C-NO_2); ^{1}HNMR (300 MHz, DMSO-d_6, δ ppm): 4.40 (s, 1H, NH), 2.41 (s, 3H, CH_3-C=N- , D_2O exchangeable), 6.94-7.22 (m, 3H, Ar-H); MS (m/z): 346 [M^+]; Anal. Calcd. for $C_{15}H_{11}ClN_4SO_2$: C, 51.95; H, 3.20; N, 16.16. Found: C, 51.85; H, 3.08; N, 16.02

2-(-2-[1-(4-bromophenyl)ethylidene]hydrazinyl)-6-nitro-1,3-benzothiazole (4b)

Yield: 58 %; m. p. 148-150 °C; IR (KBr, cm^{-1}): 3258 (NH), 1588 (C=N),1260 (C-N), 685 (C-S-C), 596 (C-Br); ^{1}HNMR (300 MHz, DMSO-d_6, δ ppm): 4.32 (s, 1H, NH, D_2O exchangeable), 2.32 (s, 3H, CH_3-C=N-), 7.85-7.92 (m, 3H, Ar-H); MS (m/z): 390[M^+]; Anal. Calcd. for $C_{15}H_{11}BrN_4SO_2$: C, 46.05; H, 3.82; N, 14.32. Found: C, 45.94; H, 3.70; N, 14.22

6-nitro-2-(-2-[1-(4-nitrophenyl)ethylidene] hydrazinyl)-1,3-benzothiazole (4c)

Yield: 62 %; m. p. 182-184°C; IR (KBr, cm^{-1}): 3368 (NH), 1632 (C=N), 1144 (C-N), 590 (C-S-C), 1320 (C-NO_2); ^{1}HNMR (300 MHz, DMSO-d_6, 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); MS (m/z): 357 [M^+]; Anal. Calcd. for $C_{15}H_{11}N_5SO_4$: C, 50.42; H, 3.10; N, 19.60. Found: C, 50.38 H, 3.03; N, 19.56

4-(1-[2-(6-nitro-1,3-benzothiazol-2-yl) hydrazinylidene]ethyl phenol (4d)

Yield: 45 %; m. p. 194-196 °C; IR (KBr, cm^{-1}): 3274 (NH), 1640 (C=N),1058 (C-N), 612 (C-S-C), 3410 (OH); ^{1}HNMR (300 MHz, DMSO-d_6, 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) 10.3 (s, 1H, Ar-OH); MS (m/z): 328 [M^+]; Anal. Calcd. for

$C_{15}H_{12}N_4O_3S$: C, 54.87; H, 3.68; N, 17.04. Found: C, 54.68; H, 3.48; N, 16.97

6-nitro-2-(-2-[1-(4-methoxyphenyl) ethylidene]hydrazinyl)-1,3-benzothiazole (4e)
Yield: 53 %; m. p. 138-140 °C; IR (KBr, cm^{-1}): 3268 (NH), 1682 (C=N),1224 (C-N), 694 (C-S-C); ^{1}HNMR (300 MHz, DMSO-d_6, 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), 3.84 (s, 3H, OCH_3); MS (m/z): 342 [M^+]; Anal. Calcd. for $C_{16}H_{14}N_4O_3S$: C, 56.14; H, 4.12; N, 16.36. Found: C, 56.02; H, 4.04; N, 16.24

6-nitro-2-(-2-[1-(4-fluorophenyl)ethylidene] hydrazinyl)-1,3-benzothiazole (4f)
Yield: 46 %; m. p. 210-212 °C; IR (KBr, cm^{-1}): 3325 (NH), 1610 (C=N),1065 (C-N), 675 (C-S-C), 1230 (C-F); ^{1}HNMR (300 MHz, DMSO-d_6, 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); MS (m/z): 330 [M^+]; Anal. Calcd. for $C_{15}H_{11}FN_4SO_2$: C, 54.54; H, 3.36; N, 16.96. Found: C, 54.42; H, 3.19; N, 16.83

4-(1-[2-(6-nitro-1,3-benzothiazol-2-yl) hydrazinylidene]ethyl)aniline (4g)
Yield: 64 %; m. p. 200-202 °C; IR (KBr, cm^{-1}): 3420 (NH_2), 3268 (NH), 1572 (C=N), 1162 (C-N), 657 (C-S-C); ^{1}HNMR (300 MHz, DMSO-d_6, 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), 3.24 (s, 2H, NH_2); MS (m/z): 327 [M^+]; Anal. Calcd. for $C_{15}H_{13}N_5SO_2$: C, 55.03; H, 4.00; N, 21.39. Found: C, 54.89; H, 3.92; N, 21.20

Synthesis of 2-[3-(4-substitutedphenyl)-4-formyl-pyrazol-1-yl]-6-nitro benzothiazoles (5a-g)
General Procedure: To the Vilsmeier-Haack reagent prepared from DMF (10 ml) and $POCl_3$ (1.2 ml, 12 mmol), hydrazones **4a-g** (4 mmol) were added and the reaction mixtures were irradiated in microwave oven for 45-120 s. After completion of the reaction each 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 compounds **5a-g**.

2-[3-(4-chlorophenyl)-4-formyl-pyrazol-1-yl]-6-nitro benzothiazole (5a)
Yield: 51 %; m. p. 190-193 °C; IR (KBr, cm^{-1}): 1725 (C=O), 2783, 2868 (CH-Ar); ^{1}HNMR (300 MHz, DMSO-d_6, δ ppm): 9.04 (s, 1H, pyrazole, D_2O exchangeable), 9.94 (s, 1H, CHO), 7.54-7.50 (m, 3H, Ar-H), 7.92 (s, 2H, Ar-H), 8.16 -8.13(m, 2H, Ar-H); MS (m/z): 384 [M^+]; Anal. Calcd. for $C_{17}H_9ClN_4O_3S$: C, 53.06; H, 2.36; N, 14.56 Found: C, 52.90; H, 2.24; N, 14.48

2-[3-(4-bromphenyl)-4-formyl-pyrazol-1-yl]-6-nitro benzothiazole (5b)
Yield: 43 %; m. p. 167-170 °C; IR (KBr, cm^{-1}): 1628 (C=O), 2775, 2875 (CH-Ar); ^{1}HNMR (300 MHz, DMSO-d_6, δ ppm): 9.04 (s, 1H, pyrazole, D_2O exchangeable), 9.96 (s, 1H, CHO), 7.44 -7.34(m, 3H, Ar-H), 7.94 (s, 2H, ArH), 8.14-8.04(m, 2H, Ar-H); MS (m/z): 427 [M^+]; Anal. Calcd. for $C_{17}H_9BrN_4O_3S$: C, 47.57; H, 2.11; N, 13.05. Found: C, 47.44; H, 2.08; N, 12.88

2-[3-(4-nitrophenyl)-4-formyl-pyrazol-1-yl]-6-nitro benzothiazole (5c)
Yield: 52 %; m. p. 245-247 °C; IR (KBr, cm^{-1}): 1716 (C=O), 2712, 2874 (CH-Ar); ^{1}HNMR (300 MHz, DMSO-d_6, δ ppm): 9.12 (s, 1H, pyrazole, D_2O exchangeable), 9.86 (s, 1H, CHO), 7.52-7.40 (m, 3H, ArH), 7.96 (s, 2H, Ar-H), 8.20-7.98 (m, 2H, Ar-H); MS (m/z): 395 [M^+]; Anal. Calcd. for $C_{17}H_9N_5SO_5$: C, 51.65; H, 2.29; N, 17.70. Found: C, 51.53; H, 2.15; N, 17.57

2-[3-(4-hydroxyphenyl)-4-formyl-pyrazol-1-yl]-6-nitro benzothiazole (5d)
Yield: 56 %; m. p. 196-198 °C; IR (KBr, cm^{-1}): 1690 (C=O), 2768, 2880 (CH-Ar), 3380 (OH); ^{1}HNMR (300 MHz, DMSO-d_6, 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, Ar-H), 8.26-8.10 (m, 2H, Ar-H), 10.2 (s, 1H, Ar-OH); MS (m/z): 365 [M^+]; Anal. Calcd. for $C_{17}H_{10}N_4SO_4$: C, 55.72; H, 2.75; N, 15.28. Found: C, 55.66; H, 2.62; N, 15.08

2-[3-(4-methoxyphenyl)-4-formyl-pyrazol-1-yl]-6-nitro benzothiazole (5e)
Yield: 46 %; m. p. 288-290 °C; IR (KBr, cm^{-1}): 1712 (C=O), 2775, 2876 (CH-Ar); ^{1}HNMR (300 MHz, DMSO-d_6, δ ppm): 9.08 (s, 1H, pyrazole, D_2O exchangeable), 9.95 (s, 1H, CHO), 7.72-7.68 (m, 3H, Ar-H), 7.98 (s, 2H, Ar-H), 7.98-7.86 (m, 2H, Ar-H), 3.8 (s, 3H, OCH_3); MS (m/z): 380 [M^+]; Anal. Calcd. for $C_{18}H_{12}N_4SO_4$: C, 56.84; H, 3.18; N, 14.73 Found: C, 56.72; H, 3.04; N, 14.69

2-[3-(4-fluorophenyl)-4-formyl-pyrazol-1-yl]-6-nitro benzothiazole (5f)
Yield: 50 %; m. p. 257-259 °C; IR (KBr, cm^{-1}): 1708 (C=O), 2769, 2803 (CH-Ar); ^{1}HNMR (300 MHz, DMSO-d_6, δ ppm): 9.04 (s, 1H, pyrazole, D_2O exchangeable), 9.80 (s, 1H, CHO), 7.77-7.66 (m, 3H, ArH), 7.94 (s, 2H, Ar-H), 7.84-7.71 (m, 2H, Ar-H); MS (m/z): 368 [M^+]; Anal. Calcd. for $C_{17}H_9FN_4O_3S$:

C, 55.43; H, 2.46; N, 15.21. Found: C, 55.40; H, 2.36; N, 15.12

2-[3-(4-aminophenyl)-4-formyl-pyrazol-1-yl]-6-nitro benzothiazole (5g)

Yield: 42 %; m. p. 176-178 °C; IR (KBr, cm^{-1}): 1688 (C=O), 2785, 2892 (CH-Ar); ^{1}HNMR (300 MHz, DMSO-d_6, δ ppm): 9.10 (s, 1H, pyrazole, D_2O exchangeable), 9.93 (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): 365 [M^+]; Anal. Calcd. for $C_{17}H_{11}N_5O_3S$: C, 55.88; H, 3.03; N, 19.16. Found: C, 55.75; H, 2.96; N, 19.02

RESULTS AND DISCUSSION

Antibacterial activity: *In vitro* antibacterial activity of the synthesized compounds **(5a-g)** was examined against Gram positive bacteria [*Staphylococcus aureus* (ATCC-25923)] and Gram-negative bacteria [*Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-27853) and *Klebsiella pneumoniae* (MTCC-432)] by measuring zone of inhibition. The antibacterial activity was performed by disc diffusion method at the concentration level of 100 µg/ml. Norfloxacin was used as standard drug at a concentration of 100 µg/ml. Nutrient agar was used as culture media and DMSO was used as solvent control. The results of antibacterial activity are shown in **Table 1**.

Antifungal activity: *In vitro* antifungal activity of the synthesized compounds **(5a-g)** was examined against *Aspergillus niger* (MTCC-281) and *Candida albicans* (ATCC 2099) by measuring zone of inhibition. The antifungal activity was performed by disc diffusion method at the concentration level of 100 µg/ml. Ketoconazole was used as the reference drug for antifungal activity at the concentration level of 100 µg/ml. Sabouraud dextrose agar was used as culture media and DMSO was used as solvent control. The results of antifungal activity are shown in the **Table 2**.

Table 1. Antibacterial activity of the synthesized compounds (5a-g)

Compound	Zone of Inhibition (mm)			
	S. aureus	***E. coli***	***P. aeruginosa***	***K. pneumoniae***
5a	15	14	14	14
5b	18	14	16	18
5c	19	16	17	18
5d	10	10	10	11
5e	11	11	12	12
5f	9	10	10	11
5g	11	10	11	11
Norfloxacin	20	17	18	19
DMF (Control)	-	-	-	-

Table 2. Antifungal activity of the synthesized compounds (5a-g)

Compound	Zone of Inhibition (mm)	
	Aspergillus niger	***Candida albicans***
5a	13	14
5b	15	14
5c	15	16
5d	10	10
5e	10	11
5f	12	11
5g	11	12
Ketoconazole	16	17
DMF (Control)	-	-

Some new 2-[3-(4-substitutedphenyl)-4-formyl-pyrazol-1-yl]-6-nitro 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 1690-1720 cm^{-1} due to C=O (str.), and a weak band in the region 2730-2785 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.

CONCLUSION

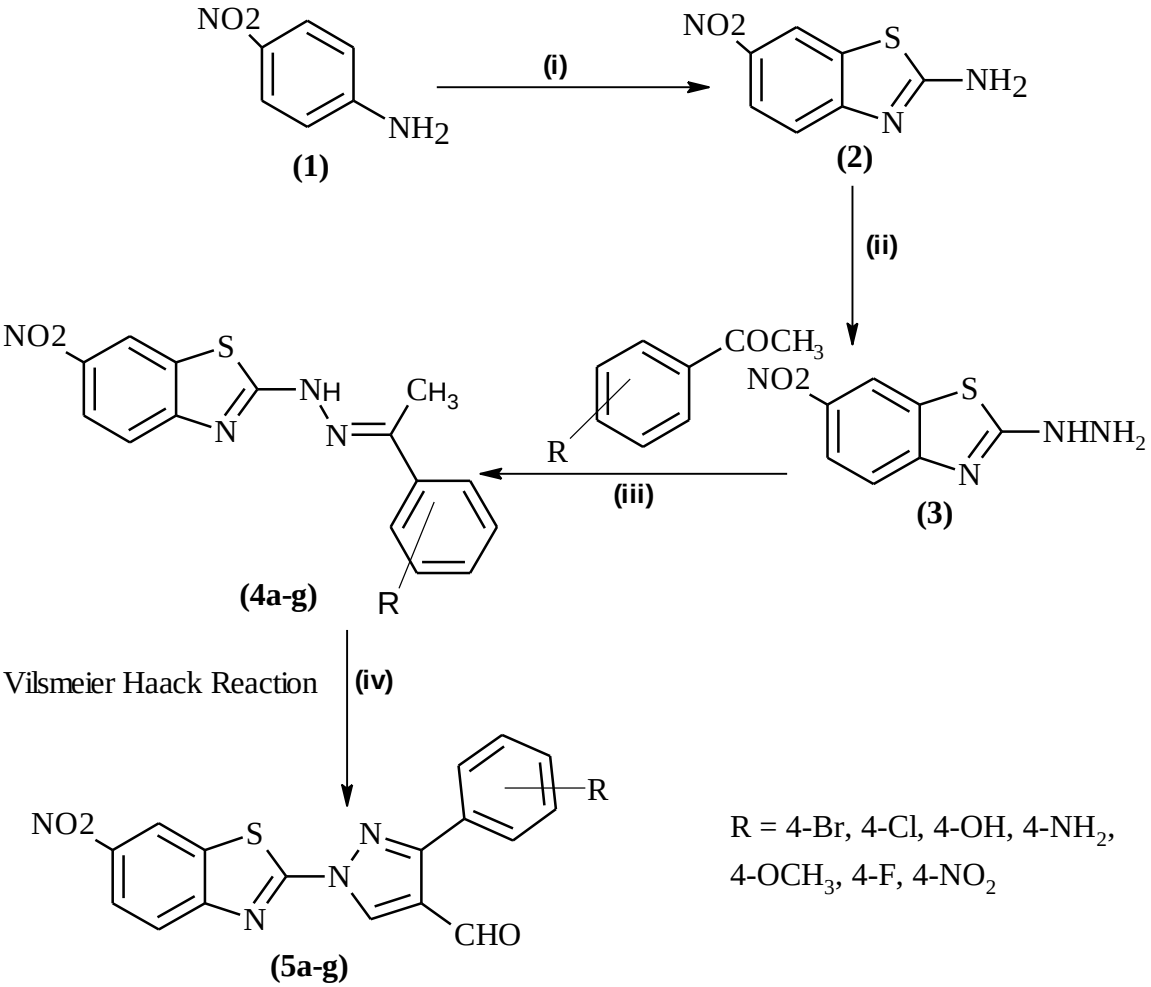
The synthesized compounds were evaluated for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and antifungal activity against *Aspergillus niger* and *Candida albicans.* Compounds **5c**, **5b**, **5a**, showed excellent antimicrobial activity while the other compounds showed moderate activity. Compounds **5c**, **5b**, **5a** were also showed good activity against *Aspergillus niger* and *Candida albicans*. It is concluded from the above synthetic procedures and antimicrobial activity of compounds that presence of electron withdrawing groups at the para position of phenyl ring exhibited good antimicrobial activity among all of the rest of compounds.

ACKNOWLEDGEMENT

The authors are thankful to Jamia Hamdard and IPC, Ghaziabad for providing facilities for spectral analysis of compounds.

REFERENCES

1. Pavlovic G, Soldin Z, Popovic Z, Kulenovic VT. Polyhedron, 2007; 26: 5162–70.
2. Caputo P, Calabro CS, Micale N. Med Chem Res, 2012; 21: 2644-51.
3. Kamal A, Reddy KS, Khan MN. Bioorg Med Chem, 2010; 18(13): 4747-61.
4. Shafi S, Alam MM, Mulakayala N. E J Med Chem, 2012; 49: 324-33.
5. Amir M, Javed SA, Hassan ZM. Med Chem Res, 2012; 21(7): 1261-70.
6. Asundaria ST, Patel KC. Pharm Chem Lett, 2012; 45(12): 725-31.
7. Patil VS, Nandra KP, Ghosh S. E J Med Chem, 2013; 59: 304-9.
8. Nagarajan SR, De Crescenzo GA, Bioorg and Med Chem, 2003; 11(22): 4769-77.
9. Delmas F, Avellaneda A, Giorgio CD. E J Med Chem, 2004; 39(9): 685-90.



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