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ORIGINAL ARTICLE

# Synthesis, characterization and antimicrobial evaluation of some 1,3-benzothiazole-2-yl-hydrazone derivatives

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**KEYWORDS**

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**Abstract** A series of 1,3-benzothiazole-2-yl-hydrazones were synthesized and evaluated for antibacterial activity against four different bacterial species and antifungal activity against two different fungal species by disk diffusion method displaying different degree of antimicrobial activity. All the synthesized compounds were in good agreement with elemental and spectral data (FT-IR, $^1$H NMR and mass spectroscopy). *In vitro* antimicrobial activity was evaluated against the four pathogenic bacterial strains, *Bacillus subtilis*, *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas alkaligenes* and three fungal strains *Aspergillus niger, Rhizopus oryzae* and *Candida albicans*. The compounds have shown moderate activity. Compounds **3a** and **3b** were found to be most active.


## 1. 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 (Walsh, 2000; Ritter and Wong, 2001). Since in the last two decades the incidence of invasive fungal infections has risen sharply, it has become imperative to enlarge the number of antifungal drugs with more potent activity and less toxicity (Maertens and Boogaerts, 2005; Datry and Bart-Delabesse, 2006). In order to gain new insights into the complexity of the disease, robust screening methods for evaluating different natural or synthetic drugs have been carried out from the science community. In this respect, the benzothiazole constitutes an important scaffold of drugs, possessing several pharmacological functions, rendering this molecule and its derivatives powerful antitumour agents (Leong et al., 2004; Yldiz-Oren et al., 2004; Lochart et al., 2005), neurotransmission blocker (Benavides et al., 1985; Mizoule et al., 1985), antimicrobial and antifungal activities as well as used as β-amyloid imaging agents (Quiroga et al., 2002; Kok et al., 2007). Recently, structurally novel benzothiazole derivatives have been shown to

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have improved biological activity. Some of the 2-amino derivatives have been reported to possess antimicrobial activities.

Benzothiazole and its derivatives represent the types of compounds with versatility for their utilization. They contain extended π-delocalized systems which are capable of binding to DNA molecules via π–π interactions and therefore exhibit complex biological properties, for instance antitumor, antiinfective, antifungal or antihelmintic activities (Pavlovic et al., 2007).

Benzothiazole derivatives in recent years have acquired conspicuous significance due to their wide spectrum of biological activities and hence the present study was undertaken in order to synthesize some new compounds having this nucleus with the hope to enhance the biological properties of the newly designed compounds with substitution on 2 and 6 position of heterocyclic ring. Therefore, a study was initiated to explore the activity of this class of compound. The present work reports the synthesis of some new 1,3-benzothiazole-2-yl-hydrazone derivatives and their *in vitro* antibacterial and antifungal screening as a part of our program aimed at the development of new heterocyclic compounds with potential antimicrobial activities.

## 2. Experimental

The purity of the synthesized compounds were ascertained by thin layer chromatography on silica gel G in various solvent systems using iodine vapours as detecting agent. Melting points were determined by the melting point determination apparatus in open capillary tubes and are uncorrected. Elemental analyses were done using Carlo Erba 1106 CHN Analyzer. Infrared spectra were recorded on Perkin–Elmer Spectrum RXI FTIR spectrophotometer in KBr phase. Proton NMR spectra were recorded on Bruker Avance II 400 NMR Ultra Shield Spectrometer using DMSO-$d_6$ as a solvent and tetramethyl silane as internal standard. Chemical shift value is expressed in delta parts per million (ppm). All chemicals and reagents were obtained from Aldrich (USA) and Spectrochem Pvt. Ltd. (India) and were used without further purification.

### 2.1. Chemistry

6-Chloro-2-benzothiazolamine was synthesized by interaction of *p*-chloroaniline with potassium thiocyanate in the presence of bromine. 2-Benzothiazolamine was then refluxed with hydrazine hydrate to yield hydrazine derivative, 6-chloro-2-benzothiazol-2-yl-hydrazine. The final compounds (1,3-benzothiazole-2-yl-hydrazones) were synthesized by the reaction of hydrazine benzothiazole with appropriate ketones and aldehydes.

### 2.2. General method

The title compounds were prepared in following steps:

#### 2.2.1. Synthesis of 6-chloro-2-benzothiazolamine (*1*)

*p*-Chloroaniline (0.01 mol) and potassium thiocyanate (0.08 mol) were dissolved in glacial acetic acid (20 mL), cooled and stirred for 15 min at 2–4 °C. Cold bromine solution (0.01 mol, 1.6 mL in 6 mL acetic acid) was added drop wise. Stirring was continued for 2 h and then at room temperature for 10 h. Separated hydrochloride salt was filtered off, washed with acetic acid, dissolved in hot water and neutralized with aqueous ammonia solution (25%) The resulting precipitate was filtered off, washed with water and recrystallized from ethanol.

#### 2.2.2. Synthesis of 6-chloro-2-benzothiazol-2-yl-hydrazine (*2*)

With stirring to hydrazine hydrate (99%, 6 mL) solution conc. HCl (6 mL) was added drop wise at 5–10 °C temperature. To it ethylene glycol (24 mL) and compound (**1**) (0.03 mol) were added in portion and refluxed for 3 h. Reaction mixture cooled to room temperature and was poured to crushed ice to afford a solid which was filtered and recrystallized from ethanol to yield compound **2**.

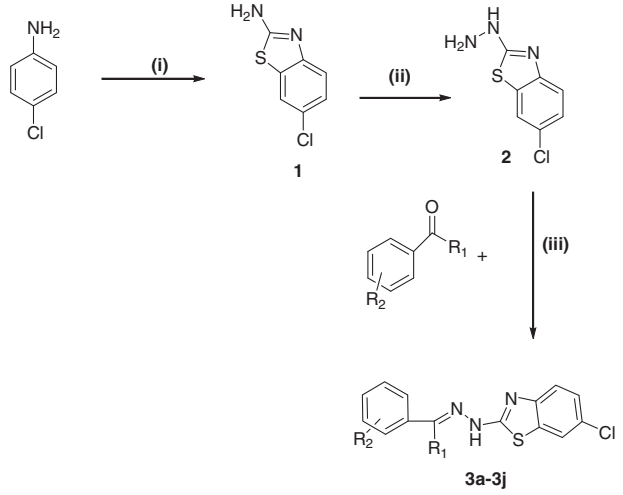
#### 2.2.3. Synthesis of 1-(4-substituted phenyl) ethanone (6-chloro-1,3-benzothiazol-2-yl) hydrazone (*3a–3b*)

The solution of compound (**2**, 1.5 mmol) in glacial acetic acid (5 mL) and ethanol (20 mL) was heated to boiling and refluxed with appropriate aromatic ketones (2.2 mmol) for 5 h. The reaction mixture was cooled to room temperature and kept overnight. The solid separated was collected out, washed with methanol, dried and recrystallized from ethanol to get the pure final compound.

By adopting similar type of procedures, 10 compounds were synthesized. Physical and analytical data of synthesized compounds is given in Table 1. Synthetic pathway for preparation of title compounds is shown in Scheme 1.

**Table 1** Physical data of synthesized compounds.

| Compound | $R_1$ | $R_2$ | Colour | Melting point (°C) | Yield (%) | $R_f$ value |
|---|---|---|---|---|---|---|
| **1** | – | – | Yellowish | 160–163 | 81 | 0.66 (benzene/acetone 8:2) |
| **2** | – | – | Light grey | 220–223 | 83 | 0.66 (benzene/acetone 8:2) |
| **3a** | $CH_3$ | $NO_2$ | Yellowish | 280–283 | 74 | 0.79 ($CHCl_3$/$CH_3OH$ 9.5:0.5) |
| **3b** | $CH_3$ | Br | Gray | 220–225 | 78 | 0.76 (benzene/acetone 9:1) |
| **3c** | $CH_3$ | $OCH_3$ | Light brown | 230–235 | 75 | 0.71 (benzene/acetone 8:2) |
| **3d** | $CH_3$ | Cl | Light brown | 213–215 | 78 | 0.82 (benzene/acetone 9:1) |
| **3e** | $CH_3$ | OH | Brown | 225–228 | 81 | 0.79 (benzene/acetone 9:1) |
| **3f** | $CH_3$ | $NH_2$ | Yellowish white | 234–238 | 76 | 0.78 ($CHCl_3$/$CH_3OH$ 8.5:1.5) |
| **3g** | $C_6H_5$ | H | Brown | 190–193 | 73 | 0.74 ($CHCl_3$/$CH_3OH$ 8.5:1.5) |
| **3h** | $CH_3$ | H | White | 180–183 | 74 | 0.81 ($CHCl_3$/$CH_3OH$ 9.5:0.5) |
| **3i** | H | F | Creamish brown | 230–233 | 76 | 0.83 (Benzene/$CHCl_3$ 8:2) |
| **3j** | H | $CH_3$ | Light gray | 220–224 | 78 | 0.76 (benzene/acetone 8:2) |




**Scheme 1** Reagents and conditions: (i) $CH_3COOH$, KSCN, $BR_2$, stirring 10 h; (ii) $NH_2NH_2{\cdot}H_2O$, ethylene glycol, reflux 3 h; (iii) ethanol, reflux 5 h.

*2.3. Spectral data*

*2.3.1. 6-Chloro-2-benzothiazolamine (**1**)*

IR (KBr, $cm^{-1}$): 3417 (NH), 3086 (CH-Ar), 1512 (C═N), 1263, 1280 (C–N), 1082 (C–Cl), 615 (C–S–C). $^1H$ NMR (300 MHz, DMSO-$d_6$, $\delta$ ppm): 7.12 (m, 7H, Ar-H), 7.10 (s, 1H, NHN–, $D_2O$ exchangeable). Anal. Calcd. for $C_9H_8ClNS$: C, 52.32; H, 3.29; N, 7.63. Found: C, 51.87; H, 3.30; N, 7.60.

*2.3.2. 6-Chloro-2-benzothiazol-2-yl-hydrazine (**2**)*

IR (KBr, $cm^{-1}$): 3484 (NH), 3084 (CH-Ar), 1548 (C═N), 1257 (C–N), 1112 (C–Cl), 604 (C–S–C). $^1H$ NMR (300 MHz, DMSO-$d_6$, $\delta$ ppm): 7.87 (m, 7H, Ar-H), 4.0 (s, 1H, NHN–, $D_2O$ exchangeable). Anal. Calcd. for $C_8H_7ClN_2S$: C, 48.36; H, 3.55; N, 14.10. Found: C, 48.87; H, 3.36; N, 15.6.

*2.3.3. 1-(4-Nitrophenyl)ethanone(6-chloro-1,3-benzothiazol-2-yl) hydrazone (**3a**)*

IR (KBr, $cm^{-1}$): 3321 (NH), 3119 (CH-Ar), 2879, 2962 (CH-Aliph.), 1554 (C═N), 1452, 1336 (Ar C–$NO_2$), 1257 (C–N), 964 (C–Cl), 719 (C–S–C). $^1H$ NMR (300 MHz, DMSO-$d_6$, $\delta$ ppm): 0.9 (s, 3H, $CH_3$), 8.14–7.16 (m, 7H, Ar-H), 7.13 (s, 1H, NHN–, $D_2O$ exchangeable). Anal. Calcd. for $C_{15}H_{11}ClN_4O_2S$: C, 51.95; H, 3.20; N, 16.6. Found: C, 51.87; H, 3.30; N, 15.6.

*2.3.4. 1-(4-Bromophenyl)ethanone(6-chloro-1,3-benzothiazol-2-yl) hydrazone (**3b**)*

IR (KBr, $cm^{-1}$): 3253 (NH), 3082 (CH-Ar), 2951, 2845 (CH-Aliph.), 1558 (C═N), 1263 (C–N), 1005 (C–Cl), 663 (C–S–C), 559 (C–Br), $^1H$ NMR (300 MHz, DMSO-$d_6$, $\delta$ ppm): 1.2 (s, 3H, $CH_3$), 8.04–7.16 (m, 7H, Ar-H), 7.56 (s, 1H, NHN–, $D_2O$ exchangeable). Anal. Calcd. for $C_{15}H_{11}BrClN_3S$: C, 47.32; H, 2.91; N, 11.4. Found: C, 47.45; H, 3.10; N, 11.2.

*2.3.5. 1-(4-Methoxyphenyl)ethanone(6-chloro-1,3-benzothiazol-2-yl) hydrazone (**3c**)*

IR (KBr, $cm^{-1}$): 3207 (NH), 3070 (CH-Ar), 2953, 2835 (CH-Aliph.), 1550 (C═N), 1255 (C–N), 1035 (C–Cl), 713 (C–S–C). $^1H$ NMR (300 MHz, DMSO-$d_6$, $\delta$ ppm): 1.07 (s, 3H, $CH_3$), 8.06–7.08, 6.87 (m, 7H, Ar-H), 3.754 (s, 1H, Ar-OH), 7.08 (s, 1H, NHN–, $D_2O$ exchangeable). Anal. Calcd. for $C_{16}H_{14}ClN_3OS$: C, 57.91; H, 4.25; N, 12.66. Found: C, 57.45; H, 4.10; N, 12.24.

*2.3.6. 1-(4-Chlorophenyl)ethanone(6-chloro-1,3-benzothiazol-2-yl) hydrazone (**3d**)*

IR (KBr, $cm^{-1}$): 3314 (NH), 3084 (CH-Ar), 2949 (CH-Aliph.), 1548 (C═N), 1257 (C–N), 1112 (C–Cl), 688 (C–S–C). $^1H$ NMR (300 MHz, DMSO-$d_6$, $\delta$ ppm): 0.86 (s, 3H, $CH_3$), 8.04–7.16 (m, 7H, Ar-H), 7.13 (s, 1H, NHN–, $D_2O$ exchangeable). Anal. Calcd. for $C_{15}H_{11}C_{l2}N_3S$: C, 53.54; H, 3.43; N, 12.66. Found: C, 53.45; H, 3.10; N, 12.24.

*2.3.7. 1-(4-Hydroxyphenyl)ethanone(6-chloro-1,3-benzothiazol-2-yl) hydrazone (**3e**)*

IR (KBr, $cm^{-1}$): 3309 (OH), 3042 (CH-Ar), 2981, 2805 (CH-Aliph.), 1558 (C═N), 1267 (C–N), 1114 (N–N), 1068 (C–Cl), 578 (C–S–C). $^1H$ NMR (300 MHz, DMSO-$d_6$, $\delta$ ppm): 0.91 (s, 3H, $CH_3$), 8.34–7.45 (m, 7H, Ar-H), 5.0 (s, 1H, Ar-OH), 7.12 (s, 1H, NHN–, $D_2O$ exchangeable). Anal. Calcd. for $C_{15}H_{12}ClN_3OS$: C, 56.69; H, 3.81; N, 13.22. Found: C, 57.36; H, 3.27; N, 12.24.

*2.3.8. 1-(4-Aminophenyl)ethanone(6-chloro-1,3-benzothiazol-2-yl) hydrazone (**3f**)*

IR (KBr, $cm^{-1}$): 3489–3308 (NH), 3090 (CH-Ar), 2962 (CH-Aliph.), 1558 (C═N), 1294 (C–N), 1064 (C–Cl), 808 (C–S–C). $^1H$ NMR (300 MHz, DMSO-$d_6$, $\delta$ ppm): 0.97 (s, 3H, $CH_3$), 7.934–7.302 (m, 7H, Ar-H), 3.186 (s, 2H, Ar C–NH), 7.17 (s, 1H, NHN–, $D_2O$ exchangeable). Anal. Calcd. for $C_{15}H_{13}ClN_4S$: C, 56.87; H, 4.14; N, 17.68. Found: C, 56.35; H, 4.54; N, 16.24.

*2.3.9. Diphenylmethanone(6-chloro-1,3-benzothiazol-2-yl)hydrazone (**3g**)*

IR (KBr, $cm^{-1}$): 3301 (NH), 3016 (CH-Ar), 1494 (C═N), 1288 (C–N), 1016 (C–Cl), 653 (C–S–C). $^1H$ NMR (300 MHz, DMSO-$d_6$, $\delta$ ppm): 8.17–6.7 (m, 7H, Ar-H), 7.0 (s, 1H, NHN–, $D_2O$ exchangeable). Anal. Calcd. for $C_{20}H_{14}ClN_3S$: C, 66.02; H, 3.88; N, 11.55. Found: C, 66.35; H, 4.54; N, 12.24.

*2.3.10. 1-Phenylethanone(6-chloro-1,3-benzothiazol-2-yl)hydrazone (**3h**)*

IR (KBr, $cm^{-1}$): 3306 (NH), 3090 (CH-Ar), 2947 (CH-Aliph.), 1550 (C═N), 1257 (C–N), 1107 (C–Cl). $^1H$ NMR (300 MHz, DMSO-$d_6$, $\delta$ ppm): 1.15 (s, 3H, $CH_3$), 7.86–6.7 (m, 7H, Ar-H), 7.11 (s, 1H, NHN–, $D_2O$ exchangeable). Anal. Calcd. for $C_{15}H_{12}ClN_3S$: C, 59.70; H, 4.01; N, 13.92. Found: C, 59.35; H, 4.54; N, 12.24.

*2.3.11. 4-Fluorobenzaldehyde(6-chloro-1,3-benzothiazol-2-yl) hydrazone (**3i**)*

IR (KBr, $cm^{-1}$): 3309 (NH), 3039 (CH-Ar), 2949 (CH-Aliph.), 1570 (C═N), 1267 (C–N), 1124 (C–F), 1089 (C–Cl), 580 (C–S–C). $^1H$ NMR (300 MHz, DMSO-$d_6$, $\delta$ ppm): 8.027 (s, 1H, Ar CH═N), 7.962–7.059 (m, 7H, Ar-H), 3.947 (s, 1H, NHN–, $D_2O$ exchangeable). Anal. Calcd. for $C_{14}H_9ClFN_3S$: C, 54.99; H, 2.97; N, 13.74. Found: C, 54.35; H, 2.54; N, 12.87.

**Table 2** *In vitro* antibacterial activity of the title compounds (**3a–j**).

| Compound | Conc. (μg/mL) | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | *E. coli* [MTCC 43] | *B. subtilis* [MTCC 441] | *K. pneumoniae* [MTCC 432] | *P. alkaligenes* [MTCC 493] |
| **3a** | 50 | – | 18.5 | – | 9.0 |
| | 100 | – | 19.0 | – | 9.0 |
| **3b** | 50 | – | 11.2 | – | 7.0 |
| | 100 | – | 23.2 | – | 10.0 |
| **3c** | 50 | – | – | 9.1 | 8.0 |
| | 100 | 8.0 | – | 10.9 | 11.0 |
| **3d** | 50 | – | 7.0 | 9.0 | 8.0 |
| | 100 | – | 7.0 | 9.0 | 12.0 |
| **3e** | 50 | – | 7.0 | 8.0 | 7.0 |
| | 100 | – | 7.5 | 9.0 | 8.0 |
| **3f** | 50 | – | 7.0 | 8.0 | – |
| | 100 | – | 8.5 | 9.0 | – |
| **3g** | 50 | – | 7.0 | – | 8.0 |
| | 100 | – | 7.5 | – | 8.0 |
| **3h** | 50 | – | 7.0 | – | 8.0 |
| | 100 | – | 7.5 | – | 8.0 |
| **3i** | 50 | – | 7.0 | 9.0 | – |
| | 100 | – | 9.0 | 9.0 | – |
| **3j** | 50 | – | 9.0 | 8.0 | – |
| | 100 | – | 13.0 | 9.0 | – |
| Control (DMF) | 50 | – | – | – | – |
| | 100 | – | – | – | – |
| Norfloxacin (standard drug) | 50 | 16 | 24 | 18 | 19 |
| | 100 | 18 | 25 | 20 | 20 |

**Table 3** *In vitro* antifungal activity of the title compounds[a] (**3a–j**).

| Compound | Zone of inhibition (in mm) | | |
|---|---|---|---|
| | *C. albicans* [MTCC-183] | *R. oryzae* [MTCC-262] | *A. niger* [MTCC-554] |
| **3a** | ++ | ++ | + |
| **3b** | ++ | + | + |
| **3c** | + | + | – |
| **3d** | – | ± | ± |
| **3e** | – | – | – |
| **3f** | – | – | – |
| **3g** | – | – | – |
| **3h** | – | – | – |
| **3i** | ± | – | – |
| **3j** | ± | – | ± |
| Ketoconazole (standard drug) | ++ | ++ | ++ |
| DMF (control) | – | – | – |

++: no growth, no fungal colony (highly active).
+: 0–20% growth, 1–2 fungal colony (active).
±: 20–40 growth, 2–4 fungal colony (moderately active).
–: >40% growth, more than four fungal colonies (no activity).
[a] Concentration: 100 μg/mL.

*2.3.12. 4-Methylbenzaldehyde(6-chloro-1,3-benzothiazol-2-yl) hydrazone (**3j**)*

IR (KBr, $cm^{-1}$): 3169 (NH), 3028 (CH-Ar), 2949 (CH-Aliph.), 1570 (C═N), 1267 (C–N), 1072 (C–Cl), 582 (C–S–C). $^1$H NMR (300 MHz, DMSO-$d_6$, $\delta$ ppm): 2.25 (s, 3H, $CH_3$), 8.17–7.1 (m, 7H, Ar-H), 4.0 (s, 1H, NHN–, $D_2O$ exchangeable). Anal. Calcd. for $C_{15}H_{12}ClN_3S$: C, 59.70; H, 4.01; N, 13.92. Found: C, 59.98; H, 4.76; N, 12.87.

## 3. Antimicrobial evaluation

Antimicrobial screening of the 1,3-benzothiazole-2-yl-hydrazones was done following the disc diffusion technique (Grover and Moore, 1962; Barry, 1976; Tripathi and Mishra, 2007). All the compounds (**3a–3j**) were screened for their *in vitro* antibacterial activity against *Bacillus subtilis* [MTCC 441], *Escherichia coli* [MTCC 43], *Klebsiella pneumoniae* [MTCC 432], and *Pseudomonas alkaligenes* [MTCC 493] at 50 and 100 μg/mL with Norfloxacin as the standard drug. Antifungal activity was conducted against *Aspergillus niger, Rhizopus oryzae* and *Candida albicans* at 100 μg/mL using Ketoconazole as the standard drug. The test organisms were first cultured in nutrient broth and incubated for 24 h at 37 °C and then freshly prepared bacterial cells and fungal spores were spread onto the Muller Hinton agar plates and Potato Dextrose Agar medium, respectively, in a laminar flow cabinet. The test compounds which were previously dissolved in DMF were then soaked onto sterile disks of Whatman filter paper (5 mm diameter). The disks were then placed onto the surface of the previously prepared inoculated plates and incubated. The zone of inhibition was recorded in mm after incubation of plates for 24 h (antibacterial) and 72 h (antifungal) at 37 °C as shown in Tables 2 and 3.

## 4. Results and discussion

The synthesis of 1-(4-substituted phenyl) ethanone (6-chloro-1,3-benzothiazol-2-yl) hydrazone (**3a–j**) was accomplished as

presented in Scheme 1. It involves the cyclization of aromatic amine and formation of compound **1**, through KSCN then involves the reaction of hydrazine hydrate with compound **1** in the presence of ethylene glycol and finally formation of the compounds (**3a–j**) through reaction of substituted ketones in the presence of ethanol and compound **2** by refluxing for 6 h. Synthesized compounds were characterized by elemental analysis, FT-IR, and $^1$H NMR spectrum. The FT-IR spectrum exhibited characteristic bands for NH, CH-Ar and C═N at 3484–3306, 3090–3016 and 1568–1512 $cm^{-1}$. The $^1$H NMR spectrum showed singlet at $\delta$ 1.146–0.905 confirmed N═C–$CH_3$, multiplets ranging from $\delta$ 8.12–6.86, confirmed aromatic protons and singlet ranges at $\delta$ 7.17–6.057 confirmed the presence of N*H*N–, respectively, which were $D_2O$ exchangeable. The synthesized compounds were evaluated for their anti-bacterial activity against *B. subtilis*, *E. coli, K. pneumoniae* and *P. alkaligenes* and anti-fungal activity against *A. niger, C. albicans*, and *R. oryzae*. Compounds **3a** and **3b** showed maximum activity (18–23 mm) zone size against *B. subtilis* due to substitution of electron donating group (i.e. $CH_3$) on $R_1$ position and electron withdrawing group (i.e. $NO_2$, Br) on $R_2$ position while all other compounds showed moderate activity. Compound **3a** and **3b** were also found to have good activity against *A. niger* and *C. albicans*.

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