Synthesis, characterization and antimicrobial activity of 2-azetidinone derivatives of benzimidazoles

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ABSTRACT

Some new 2-azetidinone derivatives possessing benzimidazole nucleus were synthesized and characterized by IR, NMR and mass spectral analysis. All synthesized compounds were screened for antimicrobial activity using cup plate method. All the compounds showed moderate to good antimicrobial activity and anti fungal activity.

Keywords: 2-azetidinone; benzimidazole; antimicrobial activity

1. INTRODUCTION

Within the wide verity of heterocycles, benzimidazole and its derivatives are found to be most promising structures investigated in the field of pharmaceutical and medicinal chemistry. Due to the unique structural features, benzimidazole is reported to shown wide range of therapeutic activities [1-3]. Benzimidazole scaffold is a useful moiety for the development of molecules of pharmaceutical or biological interest [4].

The treatment of bacterial infections still remains a challenge for scientists because of factors that include rising infectious diseases and the increasing number of multidrug-resistant microbial pathogens. So the discovery of novel and potent antibacterial as well as antifungal agent are the critical need of nowadays [5].

In recent years, there has been an increasing interest in the chemistry of 2-azetidinones because of their biological significance. Many of them have been widely investigated for several therapeutic uses. 2-azetidinones commonly known as β-lactams are still the most prescribed antibiotic agents used in medicine to treat bacterial infections and microbial diseases [6]. The β-lactam ring is the main feature of the most of the penicillins and other
antibiotics. Moreover, due to their β-lactamase inhibitory action, 2-azetidinone-based heterocycles represent an attractive target of contemporary organic synthesis [7,8]. Recently, 2-azetidinones are associated with various other biological activities such as antifungal [9], antibacterial [10,18-21], antitubercular [11], anticonvulsant [12], analgesic, anti-inflammatory [13], antiviral [14], CNS [15], cholesterol absorption [16] etc.

2. EXPERIMENTAL

All chemicals and solvents were purchased from Spectrochem Pvt Ltd., Mumbai of LR grade and were used without further purification. Melting points were taken in open capillary method and are uncorrected. IR spectra were recorded on FTIR-8400 spectrophotometer (Shimadzu, Kyoto, Japan), using DRS probe KBr pallet. 1HNMR spectra of the synthesized compounds were recorded on a Bruker-Avance-II (400 MHz) in DMSO-d6 solvent. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu, Kyoto, Japan).

2. 1. Synthetic procedure of 2-(1H-Benzimidazol-2-yl)-N-(4-hydrazinocarbonyl-phenyl)benzamide (1)

Step 1: An equimolar amount of phthalic anhydride and o-phenylenediamine were taken in RBF. Reaction mass was heated at 140-150 °C to obtained o-Benzoylene 2-1-benzimidazole. Reaction mass was Poured in chilled water and precipitates were collected. Yield 79 %

Step 2: A mixture of o-benzoylene 2-1-benzimidazole (1 mmol) and benzocaine (1 mmol) were refluxed for 4-5 hours in DMF at 150 °C. Completion of reaction was monitored by TLC. Reaction mass was Poured in chilled water and precipitates were collected as crude product. Dry in vacuo. Crystalline from DMSO to obtained analytical grade pure 2-o-(4'-carbethoxyphenyl amino carbonyl phenyl) benzimidazole. Yield 85 %

Step 3: In ethanolic solution of 2-o(4'-carbethoxyphenyl aminocarbonyl phenyl)benzimidazole (1 mmol), hydrazine hydrate (10 mmol) was added and reflux overnight. Reaction mass was cooled to RT, the precipitates were filtered and washed with chilled ethanol to collect the analytical pure 2-(1H-Benzimidazol-2-yl)-N-(4-hydrazinocarbonyl-phenyl) benzamide. Yield 80 %

2. 2. General procedure for the synthesis of (E)-2-(1H-benzo[d]imidazol-2-yl)-N-(4-(2-benzylidene hydrazine carbonyl) aryl)benzamide 2(a-l).

A mixture of (1) (1 mmol) and substituted benzaldehyde (1 mmol) in presence of catalytic amount of acetic acid was refluxed with stirring until the reaction got complete. completion of reaction was monitored by TLC. The mixture was then cooled down and poured on to crushed ice. The solid product was filtered, dried and purified by crystallization.

2. 3. General procedure for the synthesis of 2-(1H-benzo[d]imidazol-2-yl)-N-(4-(3-chloro-2-oxo-4-arylatedin-1-yl) carbamoyl) phenyl) benzamide 3(a-l).

Compound 2(a-l) (2.0 gm, 0.01 M) in dioxane (20 ml) was taken in RBF. Then chloroacetyl chloride was added slowly at room temperature with constant stirring. The reaction mixture was stirred for 8 hrs. Excess of solvent was distilled off and poured on to
ice. The product was isolated and crystallised from DMSO. Synthetic route of 2-azitidinone is showed in Scheme 1. Physical constants of newly synthesized 2-azitidinones derivatives 3a-3l are recorded in Table 1a.

![Scheme 1](image)

**Table 1a.** Physical Constant table of 2-(1H-benzo[d]imidazol-2-yl)-N-(4-((3-chloro-2-oxo-4-(aryl)azetidin-1-yl)carbamoyl)phenyl)benzamide derivatives (3a-3l).

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Compound</th>
<th>Substitution R</th>
<th>M. F.</th>
<th>M. W.</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3a</td>
<td>Phenyl</td>
<td>C_{30}H_{25}ClN_{5}O_{3}</td>
<td>535</td>
<td>59</td>
</tr>
<tr>
<td>2</td>
<td>3b</td>
<td>4-AminoPhenyl</td>
<td>C_{30}H_{25}ClN_{5}O_{3}</td>
<td>551</td>
<td>47</td>
</tr>
<tr>
<td>3</td>
<td>3c</td>
<td>2-Chlorophenyl</td>
<td>C_{30}H_{25}ClN_{5}O_{3}</td>
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<td>51</td>
</tr>
<tr>
<td>4</td>
<td>3d</td>
<td>4-Chlorophenyl</td>
<td>C_{30}H_{25}ClN_{5}O_{3}</td>
<td>569</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>3e</td>
<td>3,4-dimethyl phenyl</td>
<td>C_{32}H_{28}ClN_{5}O_{3}</td>
<td>563</td>
<td>59</td>
</tr>
<tr>
<td>6</td>
<td>3f</td>
<td>3,4-dimethoxy-5-nitro phenyl</td>
<td>C_{30}H_{25}ClN_{5}O_{3}</td>
<td>640</td>
<td>61</td>
</tr>
<tr>
<td>7</td>
<td>3g</td>
<td>2-Furyl</td>
<td>C_{28}H_{26}ClN_{5}O_{4}</td>
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<td>64</td>
</tr>
<tr>
<td>8</td>
<td>3h</td>
<td>2-Hydroxy Phenyl</td>
<td>C_{30}H_{25}ClN_{5}O_{4}</td>
<td>551</td>
<td>49</td>
</tr>
<tr>
<td>9</td>
<td>3i</td>
<td>4-Hydroxy Phenyl</td>
<td>C_{30}H_{25}ClN_{5}O_{4}</td>
<td>551</td>
<td>43</td>
</tr>
<tr>
<td>10</td>
<td>3j</td>
<td>4-N,N-Dimethyl amino Phenyl</td>
<td>C_{30}H_{25}ClN_{5}O_{4}</td>
<td>579</td>
<td>53</td>
</tr>
</tbody>
</table>
2. 4. Analytical data

2-(1H-benzo[d]imidazol-2-yl)-N-(4-((3-chloro-2-oxo-4-phenylazetidin-1-yl)carbamoyl)phenyl)benzamide (3a)

mp 238-240 °C; IR (DRS): 3260 (NH-N), 3040 (Aromatic C-H str.), 1760 (C=O str. β-lactam ring), 1640 (Amide C=O str.), 1530 (Benzimidazole C=C, C-N str.), 1340 (C-N str.), 1220 (C-O-C str), 1280 (C-H), 690 (mono substituted), 640 (C-Cl str.) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ ppm 10.90 (s, 1H), 10.14 (s, 1H), 7.98 (dd, 1H), 7.79 – 7.53 (m, 7H), 7.47 – 7.39 (m, 2H), 7.37 – 7.19 (m, 7H), 5.89 (dt, 1H), 5.38 (d, 1H), 5.08 (s, 1H). MS: m/z = 551 [M⁺]

N-(4-((2-(4-aminophenyl)-3-chloro-4-oxazetidin-1-yl)carbamoyl)phenyl)-2-(1H-benzo[d]imidazol-2-yl)benzamide (3b)

mp 248-250 °C; IR (DRS): 3400 (-NH₂), 3260 (NH-N), 3040(Aromatic C-H str.), 1760(C=O str. β-lactam ring), 1640 (Amide C=O str.), 1530 (Benzimidazole C=C, C-N str.), 1340 (C-N str.), 1220 (C-O-C str), 1280 (C-H), 840 (para substituted), 640 (C-Cl str.) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ ppm10.89 (s, 1H), 9.95 (s, 1H), 7.99 (dd, 1H), 7.80 – 7.53 (m, 7H), 7.47 – 7.39 (m, 2H), 7.24 (dd, 2H), 7.06 – 6.98 (m, 2H), 6.50 – 6.42 (m, 2H), 5.77 (dt, 1H), 5.38 (d, 1H), 5.08 (s,1H). MS: m/z = 386 [M⁺]

2-(1H-benzo[d]imidazol-2-yl)-N-(4-((3-chloro-2-(2-chlorophenyl)-4-oxazetidin-1-yl)carbamoyl)phenyl)benzamide (3c)

mp 295-298 °C; IR (DRS): 3260 (NH-N), 3040 (Aromatic C-H str.), 1760 (C=O str. β-lactam ring), 1640 (Amide C=O str.), 1530 (Benzimidazole C=C, C-N str.), 1340 (C-N str.), 1220 (C-O-C str), 1280 (C-H), 770 (1,2-di sub.), 640 (C-Cl str.) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ ppm 10.87 (s, 1H), 10.23 (s, 1H), 7.99 (dd, 1H), 7.79 – 7.53 (m, 7H), 7.51 – 7.39 (m, 3H), 7.24 (dd, 2H), 7.20 – 7.08 (m, 2H), 7.05 – 6.96 (m, 1H), 6.08 (dd, 1H), 5.42 (d, 1H) 5.08 (s, 1H). MS: m/z = 569 [M⁺]

2-(1H-benzo[d]imidazol-2-yl)-N-(4-((3-chloro-2-(4-chlorophenyl)-4-oxazetidin-1-yl)carbamoyl)phenyl)benzamide (3d)

mp 264-266 °C; IR (DRS): 3260 (NH-N), 3040 (Aromatic C-H str.), 1760 (C=O str. β-lactam ring), 1640 (Amide C=O str.), 1530 (Benzimidazole C=C, C-N str.), 1340 (C-N str.), 1220 (C-O-C str), 1280 (C-H), 840 (1,4-di sub.), 640 (C-Cl str.) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ ppm 10.87 (s, 1H), 10.01 (s, 1H), 7.99 (dd, 1H), 7.79 – 7.53 (m, 7H), 7.47 – 7.37 (m, 4H), 7.30 – 7.19 (m, 4H), 5.93 (dt, 1H), 5.39 (d, 1H), 5.08 (s, 1H). MS: m/z = 569 [M⁺]
(C-N str.), 1220 (C-O-C str), 1280 (C-H), 640 (C-Cl str.) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) ppm 10.90 (s, 1H), 10.08 (s, 1H), 7.98 (dd, 1H), 7.79 – 7.53 (m, 7H), 7.47 – 7.39 (m, 2H), 7.24 (dd, 2H), 7.05 – 6.89 (m, 3H), 5.88 (dt, 1H), 5.39 (d, 1H), 5.08 (s, 1H), 2.29 (s, 3H), 2.23 (s, 3H). MS: \(m/z = 563\) [M]\(^+\)

**2-(1H-benzo[d]imidazol-2-yl)-N-((4-((3-chloro-2-(3,4-dimethoxy-5-nitrophenyl)-4-oxoazetidin-1-yl)carbamoyl)phenyl)benzamide (3f)**

mp 179-181 °C; IR (DRS): 3260 (NH-N), 3040 (Aromatic C-H str.), 1760 (C=O str. \(\beta\)-lactam ring), 1640 (Amide C=O str.), 1550 (-NO\(_2\)), 1530 (Benzimidazole C=C, C-N str.), 1340 (C-N str.), 1220 (C-O-C str), 1280 (C-H), 640 (C-Cl str.) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) ppm 10.86 (s, 1H), 10.24 (s, 1H), 8.00 (dd, 1H), 7.79 – 7.53 (m, 8H), 7.47 – 7.35 (m, 3H), 7.24 (dd, 2H), 5.87 (dt, 1H), 5.39 (d,1H), 5.08 (s,1H), 3.85 (s, 6H). MS: \(m/z = 640\) [M]\(^+\).

**2-(1H-benzo[d]imidazol-2-yl)-N-((4-((3-chloro-2-(furan-2-yl)-4-oxoazetidin-1-yl)carbamoyl)phenyl)benzamide (3g)**

mp 201-203 °C; IR (DRS): 3260 (NH-N), 3040 (Aromatic C-H str.), 1760 (C=O str. \(\beta\)-lactam ring), 1640 (Amide C=O str.), 1530 (Benzimidazole C=C, C-N str.), 1340 (C-N str.), 1220 (C-O-C str), 1280 (C-H), 640 (C-Cl str.) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) ppm 10.74 (s, 1H), 9.65 (s, 1H), 7.81 (dd, 2H), 7.76 – 7.52 (m, 7H), 7.47 – 7.39 (m, 2H), 7.24 (dd, 2H), 6.71 (dd, 1H), 6.39 (t, 1H), 6.09 (d, 1H), 5.08 (s, 1H ), 5.46 (d,1H). MS: \(m/z = 525\) [M]\(^+\).

**2-(1H-benzo[d]imidazol-2-yl)-N-((4-((3-chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl)carbamoyl)phenyl)benzamide (3h)**

mp 218-220 °C; IR (DRS): 3400 (-OH), 3260 (NH-N), 3040 (Aromatic C-H str.), 1760 (C=O str. \(\beta\)-lactam ring), 1640 (Amide C=O str.), 1530 (Benzimidazole C=C, C-N str.), 1340 (C-N str.), 1220 (C-O-C str), 1280 (C-H), 760(1,2-di.sub.), 640 (C-Cl str.) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) ppm 10.90 (s, 1H), 8.21 (s, 1H), 7.92 (dd, 1H), 7.73 – 7.52 (m, 7H), 7.47 – 7.39 (m, 2H), 7.24 (dd, 2H), 7.13 – 6.89 (m, 4H), 6.38 (dd, 1H), 5.66 (d, 1H), 5.23 (dd, 1H), 5.08 (s,1H). MS: \(m/z = 551\) [M]\(^+\).

**2-(1H-benzo[d]imidazol-2-yl)-N-((4-((3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)carbamoyl)phenyl)benzamide (3i)**

mp 236-238 °C; IR (DRS): 3400 (-OH), 3260 (NH-N), 3040 (Aromatic C-H str.), 1760 (C=O str. \(\beta\)-lactam ring), 1640 (Amide C=O str.), 1530 (Benzimidazole C=C, C-N str.), 1340 (C-N str.), 1220 (C-O-C str), 1280 (C-H), 840 (1,4-di.sub.), 640 (C-Cl str.) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) ppm 10.90 (s, 1H), 10.14 (s, 1H), 7.98 (dd, 1H), 7.79 – 7.53 (m, 7H), 7.47 – 7.39 (m, 2H), 7.37 – 7.19 (m, 7H), 5.89 (dt, 1H), 5.38 (d, 1H), 5.35 (s, 1H), 5.08 (s, 1H). MS: \(m/z = 551\) [M]\(^+\).

**2-(1H-benzo[d]imidazol-2-yl)-N-((4-((3-chloro-2-(4-(dimethylamino)phenyl)-4-oxoazetidin-1-yl)carbamoyl)phenyl)benzamide (3j)**

mp 259-261 °C; IR (DRS): 3260 (NH-N), 3040 (Aromatic C-H str.), 2930 (sp\(^3\)-CH\(_3\) str.) 1760 (C=O str. \(\beta\)-lactam ring), 1640 (Amide C=O str.), 1530 (Benzimidazole C=C, C-N str.), 1340 (C-N str.), 1220 ( C-O-C str), 1280 (C-H), 840 (1,4-i sub.), 640 (C-Cl str.) cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) ppm 10.87 (s, 1H), 10.01 (s, 1H), 7.99 (dd, \(J = 7.4, 2.0\) Hz, 85
1H), 7.79 – 7.53 (m, 7H), 7.47 – 7.39 (m, 2H), 7.24 (dd, 2H), 7.04 – 6.95 (m, 2H), 6.61 – 6.53 (m, 2H), 5.83 (dt, 1H), 5.38 (d, 1H), 5.08 (s, 1H), 3.02 (s, 6H). MS: \( m/z = 579 \) [M]+.

2-(1H-benzo[d]imidazol-3-yl)-N-(4-((3-chloro-2-(3,5-dibromo-2-hydroxyphenyl))-4-azetidin-1-yl)carbamoyl)phenyl)benzamide (3k)

mp 195-197 °C; IR (DRS): 3430 (-OH), 3260 (NH-N), 3040 (Aromatic C-H str.), 1760 (C=O str. β-lactam ring), 1640 (Amide C=O str.), 1530 (Benzimidazole C=C, C-N str.), 1340 (C-N str.), 1220 (C-O-C str), 1280 (C-H), 640 (C-Cl str.) cm⁻¹; \(^1\)H NMR (400 MHz, DMSO-d₆): δ ppm 10.90 (s, 1H), 10.21 (s, 1H), 7.98 (dd, 1H), 7.79 – 7.53 (m, 9H), 7.47 – 7.39 (m, 2H), 7.24 (dd, 2H), 7.14 (dd, 1H), 5.90 (dd, 1H), 5.53 (d, 1H), 5.08 (s, 1H). MS: \( m/z = 709 \) [M]+.

2-(1H-benzo[d]imidazol-3-yl)-N-(4-((3-chloro-2-(3-hydroxy-4-methoxyphenyl))-4-oxo azetidin-1-yl)carbamoyl)phenyl)benzamide(3l)

mp 249-151 °C; IR (DRS): 3430 (-OH), 3260 (NH-N), 3040 (Aromatic C-H str.), 1760 (C=O str. β-lactam ring), 1640 (Amide C=O str.), 1530 (Benzimidazole C=C, C-N str.), 1340 (C-N str.), 1220 (C-O-C str), 1280 (C-H), 640 (C-Cl str.) cm⁻¹; \(^1\)H NMR (400 MHz, DMSO-d₆): δ ppm 10.89 (s, 1H), 9.91 (s, 1H), 7.99 (dd, 1H), 7.78 – 7.53 (m, 7H), 7.47 – 7.39 (m, 2H), 7.24 (dd, 2H), 7.16 (s, 1H), 6.81 – 6.73 (m, 2H), 6.69 (dt, 1H), 5.82 (dt, 1H), 5.38 (d, 1H), 5.08 (s, 1H), 3.02 (s, 1H). MS: \( m/z = 582 \) [M]+.

3. ANTI MICROBIAL ACTIVITY

All the glass apparatus used were sterilized before use. The Cup plate method was used to determine the minimum inhibitory concentration (MIC) of the synthesized compounds. [17] Zone of inhibition was measured in millimetre.

Bacterial strain of Staphylococcus aureus, Bacillus megaterium, Escherichia coli, Pseudomonas fluorescens and fungal strains of Aspergillus flavus, Candida albicans were used in the present study.

DMSO was used as the control solvent for the compounds. A blank test was carried out to check the antimicrobial activity of DMSO. Ampicillin, norfloxacin and Chloramphenicol were used as the standard drugs for antibacterial activity. Greseofulvin was used as the standard drug for antifungal activity.

The synthesized 2-azetidinone 3a–3l were screened for their antimicrobial activity by the cup plate method to evaluate the minimum inhibitory concentration Table1b.

All of the precursors 3(a–l) of the title compounds showed antibacterial activity in the range of 10-18 mm for Staphylococcus aureus, 10-17 mm for Bacillus megaterium, 10-18 mm for Escherichia coli, and 10-14 mm for Pseudomonas fluorescens.

It was observed that compound 2-chloro phenyl (3C) and 2-hydroxy phenyl (3h) against bacterial strains have found to be moderately active as compared to ampicillin.

All the compounds are not found effective as compared to Norfloxacin and Chloramphenicol.

Against fungal pathogen all compound have shown moderate activity as compared to griseofulvin.
Table 1b. Anti microbial activity of 2-(1H-benzo[d]imidazol-2-yl)-N-(4-((3-chloro-2-oxo-4-(aryl)azetidin-1-yl)carbamoyl)phenyl)benzamide derivatives (3a-3l).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Antibacterial Activity (Zone of inhibition in m.m.)</th>
<th>Antifungal activity (Zone of inhibition in m.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram +ve Bacteria</td>
<td>Gram –ve Bacteria</td>
</tr>
<tr>
<td></td>
<td>S.aureus</td>
<td>B.megateriun</td>
</tr>
<tr>
<td>3a</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>3b</td>
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</tr>
<tr>
<td>3l</td>
<td>12</td>
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</tr>
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Antibacterial Activity (Zone of inhibition in m.m.)

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<tr>
<th>Standard Drugs</th>
<th>S.aureus</th>
<th>B.megateriun</th>
<th>E.coli</th>
<th>P.fluorescences</th>
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</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>-</td>
<td>16</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>-</td>
<td>18</td>
<td>-</td>
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<tr>
<td>Norfloxacin</td>
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<td>27</td>
<td>-</td>
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</table>

Antifungal activity (Zone of inhibition in m.m.)

<table>
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<tr>
<th>Standard Drug</th>
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<tbody>
<tr>
<td>Greseofulvin</td>
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<td>25</td>
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</table>

4. CONCLUSION

In conclusion, a new series of compound 3(a–I) were synthesized. Synthesized compounds screened for their biological study. The investigation of antimicrobial (antibacterial and antifungal) activities data revealed that the compound 3c and 3h displayed good activity, the compound 3l showed moderate activity and rested compounds showed less activity compared with standard drugs.

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