SYNTHESIS AND ANTIMICROBIAL SCREENING OF NOVEL B-DIKETONES CONTAINING 2-SUBSTITUTED 2-IMIDAZOLINE MOIEITY

SHOBHITA SINGH1*, YOGESH CHANDRA JOSHI2

1Department of Chemistry, University of Rajasthan, Jaipur 302004, Rajasthan, India. Email: shobhitask@gmail.com

Received: 10 Sep 2013, Revised and Accepted: 10 Oct 2013

ABSTRACT

A series of novel β-diketones possessing 2-imidazoline moiety have been synthesized by condensation of 1-(3-Bromo-propyl)-2,4-diphenyl-4,5-dihydro-1H-imidazol(IIa), 1-(3-Bromo-propyl)-2-phenyl-3a,4,5,6,7a-hexahydro-1H-benzimidazole(IIb) and 1-(3-Bromo-propyl)-2-phenyl-1,3a,4,5,6,6a-hexahydro-cyclopentaimidazole(IIc) with different known β-diketones (IIIa-c) in the presence of sodium methoxide. All the newly synthesized compounds were characterized by elemental analysis and spectral studies. The titled compounds were screened for qualitative (inhibition zone) and quantitative antimicrobial activity (MIC) by agar well diffusion method and micro broth dilution technology respectively. The minimum inhibitory concentration represents the concentration of antimicrobial at which there is complete inhibition of growth of organism. The synthesized compounds were tested for their antibacterial activity at lower concentration against Staphylococcus aureus, Micromonaspora (gram-positive), Escherichia coli, Zymomonas mobilis (gram-negative) and antifungal activity against Fusarium culmorum, Phanerochaete chrysosporium, Penicillium chrysogenum and Alternaria solani.

Keywords: β-diketones, 2-substituted 2-imidazolines, Antibacterial and antifungal activities, Minimum inhibitory concentration (MIC), Inhibition zone (IZ), Agar well diffusion method.

INTRODUCTION

2-substituted 2-imidazolines have attracted considerable attention in recent years in the development of compound with pharmacological useful properties[1]. The importance of imidazoline units arises, because they are found in a diverse range of biological relevant compounds[2]. Imidazoline containing natural products e.g. spongotine, topsentin and nortopsentin are sought after for their antiviral and antitumor properties[3].

In organic synthesis, imidazoline units are used as synthetic intermediate in medicinal chemistry[4]. Chiral catalysis[5]. Chiral auxiliaries[6], and ligand for asymmetric catalysis[7].

Aside from their synthetic importance 2-imidazoline derivatives have been found to exhibit various pharmacological properties such as antihyperglycemic[8,9], antiinflammatory[10,11], antihypertensive [12,13], anticancer[14], antihypercholesteremic[15], antiproliferative [16] antidepressants[17], estrogen receptor agonist[18] and anticonvulsant[19] activities. In addition, the 2-substituted 2-imidazoline derivatives have been found to exhibit antidiabetic[20,21] and antiparasitic activity[22,23].

A large number of routes are known for synthesis of 2-substituted 2-imidazolines but more recently a diversifiable synthesis of imidazolines have been reported via cycloaddition of alkyl aryl and cycloalkyl substituted N-tosyl aziridines with different nitiles in the presence of Lewis acid[24,25,26][27]. The 2-substituted 2-imidazolines were treated with 1,3-di-bromo propane and subsequently condensed with various known β-diketones in the presence of sodium methoxide yielded corresponding novel β-diketones having 2-imidazoline as nucleus.

β-diketones have been widely used in organic synthesis because of their ready access, predictable reactivity and serve as precursors for the synthesis of various biologically active heterocyclic compounds[28,29] such as, diazepines, benzodiazepine pyrazoles, isoazoles, and imidazole, benzimidazole. β-diketones have also been shown to have a wide assortment of pharmacological activities like antibacterial[30], antiviral[31], systemic insecticidal[32], antioxidant[33], prophylactic antitumor[34], and breast cancer chemopreventive blocking agent[35].

![Scheme 1: Synthesis of novel β-diketones from 2,4-diphenyl 2-imidazoline derivatives](image-url)
The synthesis of heterocyclic compounds containing multi-
structure in a molecule has received considerable interest in
recent years. Encouraged by these observations and in
continuation of our research work on synthesis of β-
diketones[36] and 2-substituted 2-imidazolines[37], it prompted
us to incorporate both the bioactive molecules in a single
molecular frame to examine the additive effect towards the
antimicrobial activity.

![Diagram of Scheme 2](image)

**Scheme 2: Synthesis of novel β-diketones from 2-phenyl 2-imidazoline derivatives of cyclohexane**

**Scheme 3: Synthesis of novel β-diketones from 2-phenyl 2-imidazoline derivatives of cyclopentane**

**MATERIAL AND METHODS**

The melting point of the compound was determined in open capillaries and is uncorrected. The IR spectra were recorded on a Nicolet Mega-FT-IR 550 spectrometer in KBr pellets; 1H NMR and
[13]C NMR were run on model DRX 300 at 300.13Hz and 75Mz in
CDCl3 using TMS as internal standard. The mass spectra were
obtained on an LCMS instrument. Elemental analyses were done
using Perkin Elmer CHNS/O analyzer 2400. The purity of the newly
synthesized compounds was checked through TLC on aluminium
oxide 60 F254 plates (Merck) and spots were visualized by iodine
vapors or by irradiation with ultraviolet lights (254 nm).

**General procedure for synthesis of 1-(3-Bromo-propyl)-2,4-
diphenyl-4,5-dihydro-1H-imidazole/2-phenyl-3a,4,5,6,7a-
hexahydro-1H-benzoimidazole/2-phenyl-1,3a,4,5,6,6a-
hexahydro-cyclopentaimidazole (Ia-c)**

A solution of 2-substituted 2-imidazolines (Ia-c) (0.005m) and absolute alcohol was taken in round bottom flask. 1,3-di-
bromopropane (1ml, 0.01m) was then added, the reaction mixture
was refluxed for 3-4 hrs, after that it was kept overnight in
refrigerator. A solid was separated out. The reaction mixture was
filtered; the crude solid so obtained was recrystallized from
ethanol:ethylacetate mixture (2:8) afforded corresponding
compounds (Ia-c). (Scheme 1, 2 & 3)

**General procedure for synthesis novel β-Diketone derivatives**

placed freshly prepared sodium methoxide (0.54g, 0.01 mol),
acetylacetone/benzoylacetone/ dibenzoylmethane (Ila-c) (0.01
mol) and dry toluene (5ml) in a dried round-bottom flask fitted with
a guard tube and condenser. The reaction mixture was stirrer at
50°C on magnetic stirrer, until a creamy mass was obtained.
Compound 1-(3-Bromo-propyl)-2,4-diphenyl-4,5-dihydro-1H-
imidazole/2-phenyl-3a,4,5,6,7a-hexahydro-1H-benzoimidazole/2-
phenyl-1,3a,4,5,6,6a-hexahydro-cyclopentaimidazole (Ia-c) (0.01
mol) was taken in dry toluene and added drop by drop in above said
reaction solution. The reaction mixture was refluxed for 13-14 hours
at 80°C with continuous stirring. The progress of the reaction was
monitored by TLC. After completion, the reaction mixture was
cooled and toluene was removed under reduced pressure. The compound was extracted in ethylacetate. The ethylacetate was removed under reduced pressure to yield desired products (Iva-h). Purity of compounds was checked by TLC using benzene: ethanol: ammonium (7:2:1) upper layer as mobile phase. (Scheme 1, 2 & 3)

**SPECTRAL DATA**

3-[2-(2,4-Diphenyl-4,5-dihydro-imidazol-1-yl)-propyl]-pentane-2,4-dione (Iva)

Yield: 57%, m.p. 143-144°C; Anal.Calcd for C_{32}H_{24}N_{4}O_{2}: C, 76.21; H, 7.23; N, 7.73. Found: C, 76.20; H, 7.22; N, 7.70; IR νmax (KBr cm⁻¹): 3050(νAr-H), 2922(νC=H), 1613(νC=O), 1540-1630(νC=O), 1720(νC=O); ¹HNRN (300.13 MHz, CDCl₃, 6/5ppm): 7.18-7.82(m, 10H, Ar-H), 3.36(dd, 1H, J=8.7Hz, J=7.3Hz, C-6imidazoline ring), 3.41(t, 1H, C-7imidazoline ring), 3.52(t, 1H, C-imidazoline ring ), 6.31(s, 1H, -CH(CO)) 2.35(s, 6H, CH₃CO), 2.20(t, 2H, N-CH₂-CH₂-CH₂-), 1.36 (m, 2H, -CH₂-CH₂-CH₂-), 1.10(m, 2H, -CH₂-CH₂-CH₂-)[13]CNMR (75.48 MHz, CDCl₃, 6/5ppm): 21.8(CH-Cal), 28.9, 56.9, 67.5(C-5, C-4, C-2 imidazoline), 53.4(C-CO) 193.8(C-O) 125.7, 127.1, 128.5, 147.9, 160.2(Ar-Cal); LCMS(m/z): 365 [M+H⁺]

3-[2-(2,4-Diphenyl-4,5-dihydro-imidazol-1-yl)-propyl]-1,3-diphenyl-propane-1,3-dione (Ivb)

Yield: 53%, m.p. 136-137°C; Anal.Calcd for C_{38}H_{28}N_{4}O_{2}: C, 81.45; H, 6.21; N, 5.76. Found: 81.43; H, 6.19; N, 5.75; IR νmax (KBr cm⁻¹): 3030(νAr-H), 2926(νC=H), 1610(νC=O), 1470-1615(νC=O), 1725(νC=O); ¹HNRN (300.13 MHz, CDCl₃, 6/5ppm): 7.20-7.94(m, 20H, Ar-H), 3.45(dd, 1H, J=8.6Hz, J=7.5Hz, C-6imidazoline ring), 3.51(t, 1H, C-7imidazoline ring) 3.14(t, 1H, C-6imidazoline ring), 6.80(s, 1H, -CH₂), 2.34(t, 2H, N-CH₂-CH₂-CH₂-), 1.39(m, 2H, -CH₂-CH₂-CH₂-), 1.18(m, 2H, -CH₂-CH₂-CH₂-)[13]CNMR (75.48 MHz, CDCl₃, 6/5ppm) 28.9, 56.9, 67.5(C-5,C-4,2-imidazoline) 52.9(C-CO) 197.6(C-O) 121-129(Ar-Cal) 125.7, 127.1, 128.5, 147.9, 160.2(Ar-Cal); LCMS(m/z): 487 [M+H⁺]

1-Phenyl-2-[3-(2-phenyl-3a,4,5,6,7,7a-hexahydro-benzimidazol-1-yl)-propyl]-pentane-2,4-dione (Ivg)

Yield: 35%, m.p. 115-116°C; Anal.Calcd for C_{20}H_{24}N_{4}O_{2}: C, 75.79; H, 8.03; N, 8.58. Found: C, 75.79; H, 8.01; N, 8.56; IR νmax (KBr cm⁻¹): 3055(νAr-H), 2928(νC=H), 1610(νC=O), 1462-1620(νC=O), 1720(νC=O); ¹HNRN (300.13 MHz, CDCl₃, 6/5ppm): 27.0-7.94(m, 30H, Ar-H), 3.45(dd, 1H, J=8.6Hz, J=7.5Hz, C-6imidazoline ring), 3.51(t, 1H, C-7imidazoline ring) 3.14(t, 1H, C-6imidazoline ring), 6.80(s, 1H, -CH₂), 2.34(s, 6H, CH₃CO), 2.34(t, 2H, N-CH₂-CH₂-CH₂-), 2.36(s, 3H, CH₃CO), 1.37(m, 2H, -CH₂-CH₂-CH₂-), 1.12(m, 2H, -CH₂-CH₂-CH₂-)[13]CNMR (75.48 MHz, CDCl₃, 6/5ppm): 21.4(CH-Cal) 28.9, 56.9, 67.5(C-5,C-4,2-imidazoline) 55.6(C-Cal) 193.80(CO) 197.6(C-O) 123-129(Ar-Cal) 125.7, 127.1, 128.5, 147.9, 160.2(Ar-Cal); LCMS(m/z): 425 [M+H⁺]

3-[2-(2-phenyl-3a,4,5,6,7,7a-hexahydro-benzimidazol-1-yl)-pentane-2,4-dione (Ivd)

Yield: 4%, m.p. 73-74°C; Anal.Calcd for C_{24}H_{28}N_{4}O_{2}: C, 74.08; H, 8.29; N, 8.23. Found: C, 74.06; H, 8.27; N, 8.21; IR νmax (KBr cm⁻¹): 3035(νAr-H), 2922(νC=H), 1614(νC=O), 1460-1629(νC=O), 1712(νC=O); ¹HNRN (300.13 MHz, CDCl₃, 6/5ppm): 7.26-7.76(m, 5H, Ar-H), 3.33(t, 2H, C₆,C₇imidazoline ring), 4.70(s, 1H, -CHCO), 2.97(t, 2H, N-CH₂-CH₂-CH₂-), 2.37(s, 3H, CH₃CO), 1.88-1.52(m, 8H, cyclohexene ring), 1.34(m, 2H, -CH₂-CH₂-CH₂-), 0.83(m, 2H, -CH₂-CH₂-CH₂-)[13]CNMR (75.48 MHz, CDCl₃, 6/5ppm): 17.9, 26.1, 28.3(cyclohexene ring), 21.8(CH-Cal), 53.4(CO) 67.8(C-5 & C-4 imidazoline), 71.8(C-2 imidazoline), 121-129(Ar-Cal) 125.7, 127.3, 128.3, 129.9, 163(νAr-Cal), 198(νO-Cal); LCMS(m/z): 341 [M+H⁺]

Screening for antimicrobial activity

All the synthesized heterocyclic compounds (Iva-h) were screened for their antimicrobial activity by agar well diffusion method[38,39]at concentration of 100µg/mL. The bacterial strains used were Escherichia coli MTCC 448, Zymomonas mobilis MTCC 88 (gram-negative) and Staphylococcus aureus MTCC 3160, Micromonaspora MTCC 3296 (gram-positive) and fungal strains namely Fusarium culmorum MTCC 349, Planochaeta chrysosporium MTCC 787, Penicillium chrysogenum MTCC 161 and Alternaria solani MTCC 2101. Ampicillin and Fluconazole were used as standard drugs for comparison of the antibacterial and antifungal activity, respectively.

Agar well-diffusion method

The agar well-diffusion method was followed to determine the antimicrobial activity. The medium was sterilized by autoclaving at 120°C (15 lb/in²). Briefly, 100µL of broth culture containing test strain was added to 30 mL of nutrient agar (NA) medium (for antibacterial activity) and to 25 mL of potato dextrose agar (PDA) medium (for antifungal activity) at 37°C. Mixed well and then poured aseptically into a 15 cm sterile glass petri plate. The medium was allowed to solidify, and 8 mm wells were dug with a sterile metallic borer. Then, a DMSO solution of the test sample (1mL) at 1 mg/mL was added to the respective wells. DMSO served as a negative control. The standard antimicrobial drugs (1mg/mL)
Ampicillin (for bacterial assay) and (1mg/mL) Fluconazole (for fungal assay) were used as positive control. The newly synthesized compounds were added in well at concentration of 100µg/ml for antibacterial and antifungal activity assay. Triplicate plates of each microorganism strain were prepared and were incubated aerobically at 37±2°C for 24 h for antibacterial and 28 ± 2°C for 48 h for antifungal activity respectively. The antimicrobial activity was determined by measuring the diameter of zone showing complete inhibition (mm), thereby the zones were precisely measured with the aid of a Vernier Caliper (precision 0.1mm). The growth inhibition was calculated with reference to the positive control. Zone of inhibition were measured in (mm) against various strains and detailed data is illustrated in table 1 and 2.

**Determination of minimum inhibitory concentration**

Minimum inhibitory concentration (MIC) was performed by serial dilution technique using 96-well microtitre plates[40]. The synthesized compounds were dissolved in broth LB medium (100µl) with bacterial inoculum (1.0 X 10^5CFU per well) to attain the final concentration of 1mg/ml. The microplates were incubated at 480C for 24 h. The concentrations with no visible growth were taken as MIC[41]. Similarly potato dextrose agar (PDA) was used to investigate MIC for fungal cultures. For the purpose a modified microdilution technique, using 96-well microtitre plates. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween80(v/v). The spore suspension was adjusted with sterile saline to concentration of approximately 1.0-10^7 in a final volume of 100µl per well. The microplates were incubated for 72h at 280C. The values of MICs of the tested compounds are presented in table 1 & 2.

**RESULT AND DISCUSSION**

The desired compounds (IVa-h) were synthesized as outlined in the scheme I & II. Compounds (IVa-h) were synthesized by reacting 2-substituted 2-imidazolidone (Ia-c) with 1,3-dibromopropane using absolute ethanol in refluxing to give 1-(3-Bromo-propyl)-2,4-diphenyl-4,5-dihydro-1H-imidazole/2-phenyl-3a,4,5,6,7,7a-hexahydro-1H-benzoimidazole/2-phenyl-1,3a,4,5,6a hexahydro cyclopentaimidazole (IIa-c) and subsequently condense with different known 1,3-diketones (IIa-c) in presence of sodium methoxide. The structures suggested for all newly synthesized β-Diketones are in good agreement with their elemental and spectral data. Selected diagnostic bands of IR spectra of (IVa-h) showed useful information about structure of the title compounds. Further evidence for the formation of novel β-diketones was obtained by recording the mass spectra which showed characteristic molecular ion peaks that are in conformity with molecular formula.

Table 1 and 2 summarize in vitro antimicrobial activity data of the novel β-Diketones containing 2-substituted 2-imidazolidone moiety (IVa-h). Among the synthesized compounds in the series, the compound IVe showed good inhibition response against *Staphylococcus aureus* with MIC value of 18.0µg/ml. Remaining compounds were not so effective against the test bacterial strain. Compound IVe and IVf exhibited maximum activity against *Micromonospora* with MIC values 23.0µg/ml and 23.5µg/ml respectively. But compound IVa and IVb showed weak activity against same pathogen. Compound IVc had effective inhibition against *Zymomonas mobilis* with MIC value of 22.5µg/ml. Rest of the compounds showed moderate inhibition except IVb and IVh which were poorly effective against the test pathogen. Compound IVf showed highest inhibition against *Escherichia coli* with MIC value of 20.0µg/ml. While remaining all compounds were also effective against same pathogen as compared to the standard drug Ampicillin.

The antifungal data point out that compound IVa, IVd and IVg had effective inhibition against *Penicillium chrysogenum* with MIC value of 21.5, 22.0 and 22.5µg/ml respectively. Rest of the compounds was not so effective against the test pathogen. Compound IVd and IVh exhibited maximum inhibition against *Alternaria solani* with the same MIC value 20.0µg/ml. Remaining compounds showed moderate inhibition except IVc and IVf which were poorly effective against the same pathogen. Compound IVf showed moderate to good activity against *Fusarium culmorum* with 18.5µg/ml MIC value while rest of the compounds in the series did not show significant activity against this fungus. Compound IVd amongst all synthesized compound in the series showed maximum inhibition against *Phanerochaete chrysosporium* with MIC value of 18.0µg/ml. Remaining compounds possessed promising activity against same pathogen as compared to the standard drug Fluconazole.

**CONCLUSION**

In conclusion we have approached a direct and efficient route to novel β-diketones containing 2-imidazoline as nucleus from the reaction of 2-substituted 2-imidazoline derivatives with known 1,3-diketones in good yields. The new molecular framework has shown broad spectrum antimicrobial activity. The result reveals that novel β-diketones were significantly effective against both Gram-positive and Gram-negative organisms as well as against fungal strains when compared to the standard positive control.

**ACKNOWLEDGEMENT**

Authors are thankful to Head, Department of Chemistry, University of Rajasthan, Jaipur for providing necessary laboratory facilities. One of us, Shobhita Singh is thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, for the award of senior research fellowship.

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**Table 1: Zone of inhibition (in mm) of 100µg/ml concentration of novel β-diketones (IVa-h) against bacteria strains.**

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>Staphylococcus aureus</em> MTCC 3160 Iz(AI)=MIC</th>
<th><em>Micromonospora</em> MTCC 3296 Iz(AI)=MIC</th>
<th><em>Zymomonas mobilis</em> MTCC 88 Iz(AI)=MIC</th>
<th><em>Escherichia coli</em> MTCC 448 Iz(AI)=MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVa</td>
<td>10(0.34)23.0</td>
<td>10(0.37)27.5</td>
<td>13(0.52)25.5</td>
<td>11(0.5)27.5</td>
</tr>
<tr>
<td>IVb</td>
<td>12(0.41)21.5</td>
<td>11(0.40)27.0</td>
<td>9(0.36)29.5</td>
<td>13(0.6)25.5</td>
</tr>
<tr>
<td>IVc</td>
<td>14(0.48)19.5</td>
<td>15(0.55)25.5</td>
<td>16(0.6)22.5</td>
<td>12(0.5)24.5</td>
</tr>
<tr>
<td>IVd</td>
<td>11(0.37)22.5</td>
<td>13(0.49)26.5</td>
<td>12(0.48)27.0</td>
<td>14(0.6)26.5</td>
</tr>
<tr>
<td>IVe</td>
<td>16(0.55)18.0</td>
<td>19(0.70)25.0</td>
<td>15(0.6)23.5</td>
<td>15(0.7)23.0</td>
</tr>
<tr>
<td>IVf</td>
<td>12(0.41)21.5</td>
<td>18(0.6)23.5</td>
<td>14(0.5)25.0</td>
<td>20(0.95)20.0</td>
</tr>
<tr>
<td>IVg</td>
<td>13(0.44)20.0</td>
<td>16(0.59)25.0</td>
<td>12(0.48)27.0</td>
<td>12(0.5)24.5</td>
</tr>
<tr>
<td>IVh</td>
<td>10(0.34)23.0</td>
<td>17(0.62)24.5</td>
<td>10(0.40)28.0</td>
<td>16(0.7)22.0</td>
</tr>
</tbody>
</table>

Ampicillin | 29(15) | 27(21.5) | 25(20.0) | 21(18.5) |

IZ = Inhibition area (zone) excluding diameter of disc. AI = Activity Index = Inhibition area of sample/Inhibition area of standard. MIC = Minimum inhibitory concentration measured in µg/ml.
Table 2: Zone of inhibition (in mm) of 100µg/ml concentration of novel β-diketones (IVA-h) against fungal strains

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>Penicillium chrysogenum</em> MTCC 161</th>
<th><em>Alternaria solani</em> MTCC 2101</th>
<th><em>Fusarium. culmorum</em> MTCC 349</th>
<th><em>Phanerochaete. chrysosporium</em> MTCC 787</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IZ (in mm)</td>
<td>IZ (AI) (in mm)</td>
<td>IZ (AI) (in mm)</td>
<td>IZ (AI) (in mm)</td>
</tr>
<tr>
<td>IVA</td>
<td>15(0.60)21.5</td>
<td>10(0.50)24.0</td>
<td>10(0.40)21.5</td>
<td>10(0.55)21.5</td>
</tr>
<tr>
<td>IVB</td>
<td>8(0.32)24.5</td>
<td>12(0.60)22.0</td>
<td>11(0.44)20.5</td>
<td>11(0.61)20.0</td>
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<tr>
<td>IVC</td>
<td>10(0.40)24.0</td>
<td>8(0.40)25.5</td>
<td>10(0.40)21.5</td>
<td>12(0.66)19.5</td>
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<td>IVD</td>
<td>14(0.56)22.0</td>
<td>15(0.75)20.0</td>
<td>12(0.49)20.5</td>
<td>13(0.72)18.0</td>
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<tr>
<td>IVE</td>
<td>8(0.32)24.5</td>
<td>11(0.55)23.5</td>
<td>9(0.36)23.0</td>
<td>9(0.50)22.0</td>
</tr>
<tr>
<td>IVF</td>
<td>10(0.40)24.0</td>
<td>9(0.45)25.0</td>
<td>15(0.60)18.5</td>
<td>11(0.61)20.6</td>
</tr>
<tr>
<td>IVG</td>
<td>13(0.52)22.5</td>
<td>13(0.65)22.5</td>
<td>9(0.36)19.0</td>
<td>10(0.55)21.5</td>
</tr>
<tr>
<td>IVA</td>
<td>10(0.40)24.0</td>
<td>15(0.75)20.0</td>
<td>7(0.28)23.0</td>
<td>9(0.50)22.0</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>25(19.0)</td>
<td>20(18.5)</td>
<td>25(17.0)</td>
<td>18(16.5)</td>
</tr>
</tbody>
</table>

IZ = Inhibition area (zone) excluding diameter of disc. AI = Activity Index = Inhibition area of sample/Inhibition area of standard. MIC= Minimum inhibitory concentration measured in µg/ml.

**Fig. 1:** Zone of inhibition by compounds (IVA-h) over grown bacterial cultures (Nutrient Agar) after 24hrs at 100µg/ml concentration by agar well diffusion method using Ampicillin as standard drug.

Picture 1 & 2 show screening effect against *Staphylococcus aureus* MTCC 3160; 3 & 4 against *Micromonospora* MTCC 3296 (gram +ve); 5 & 6 against *Zymomonas mobilis* MTCC 88 and 7 & 8 against *Escherichia coli* MTCC 448 (gram -ve).

**Fig. 2:** Zone of inhibition by compounds (IVA-h) over grown fungal cultures (Potato Dextrose Agar) after 48hrs at 100µg/ml concentration by agar well diffusion method using Fluconazole as standard drug.

Picture 1 & 2 show screening effect against *Penicillium chrysogenum* MTCC 161; 3 & 4 against *Alternaria solani* MTCC 2101; 5 & 6 against *Fusarium culmorum* MTCC 349 and 7 & 8 against *Phanerochaete chrysosporium* MTCC 787.
REFERENCES


