RESEARCH ARTICLE

Synthesis and Evaluation of Benzimidazole Derivatives for Anti-tubercular and Antimicrobial Activities.

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ABSTRACT:
The present research work was aimed to synthesize some benzimidazole acetic acid derivatives and also the derivatives associated with 1,2,4 triazolone and was investigated for their biological activities. A two different series of compounds were synthesized, 4-[(2-aryl methyl- 1H-benzimidazol-1-yl) acetyl] 5 nitro-2,4 – dihydro-3H-1,2,4 triazol-3-one and 4-[(2-aryl methyl-1H-benzimidazol-1-yl)methyl] 5 nitro-2,4- dihydro-3H-1,2,4 triazol-3-one. The newly synthesized compounds have been characterized by their analytical and spectral (IR and $^{1}$H NMR, $^{13}$NMR, mass spectra) properties. All the compounds have been screened for their anti tubercular and antimicrobial activities by standard methods. Results of activities reveal that compound shown promising anti tubercular activity at both the concentration compared to standard drug streptomycin, and shown good antimicrobial activities.

KEYWORDS: 1,2,4 triazolone, benzimidazole, anti tubercular, anti bacterial, antifungal activity

INTRODUCTION:
TUBERCULOSIS is still greatest infectious cause of mortality worldwide. It is the only disease which does not require any vector for transportation from one person to another or to cross the physical boundary of the countries. Being the airborne disease with no vaccine, it is the single largest disease encountered by both developing and developed countries. Two of the common problem associated with treatment, one is serious and life threatening adverse effect of existing anti tubercular drugs such as hepatotoxicity, neuritis, depression, asthma, anorexia etc which many times forces to withdraw the treatment temporarily or change the treatment. Other one is development of resistance to due to non completion of treatment regimen by patient and hence gene mutation by organisms made its management more difficult. Another major concern is that tuberculosis is the most common HIV related opportunistic infection, and caring for patient with both the disease is major public health challenge. Such circumstances forced the scientists across the global to search newer molecules that can be used as lead compounds or the development of newer anti tubercular drugs with better and safer therapeutic effects.

Antimicrobial activity:
The antimicrobial activity of synthesized compounds was determined MIC by agar plate dilution method. The antibacterial activity was determined against gram positive organism Staphylococcus aureus, Enterococcus facalis and gram negative organism Escherichia coli, Klebsiella at 1µg/ml to 10 µg/ml concentration of sample compounds. Standard antibacterial drug Ciprofloxacin was also screened under similar 1µg/ml to 10 µg/ml concentration for comparison.

The antifungal activity was carried out against the fungi candida albican and A. Fumigratus at 1µg/ml to 10 µg/ml concentration of sample compounds. Determination of MIC carried out by agar plate dilution method. Fluconazole (1µg/ml to 10 µg/ml) was use as standard drug.

Antitubercular evaluation:
The anti tubercular screening of synthesized compound was carried out by middle brook 7H9 broth base medium against H$_{37}$ RV strain at 10 µg/ml, 25 µg/ml and 50 µg/ml. Middle brook 7H9 broth base medium was inoculated with mycobacterium tuberculosis of H$_{37}$RV strain. The inoculated medium was incubated for 37ºC for 6 weeks. At the end of 6 weeks the growth of mycobacterium tuberculosis were checked for growth.

Streptomycin (10 µg/ml, 25 µg/ml and 50 µg/ml) was used as a standard drug.
MATERIAL AND METHOD:
Melting points were determined in open capillary method and are uncorrected. IR spectra were recorded on Perkin-Elmer 1720 FT-IR using KBr pellets method. The $^1$H NMR spectra were recorded on Bruker AC 400 MHz using DMSO as solvent and tetra methyl silane as internal standard.

Proposed scheme I:
General method for preparation of phenyl acetic acid from mandelic acid:
15 gram mandelic acid (0.1 mol), 2.07 g KI, and 6 gm red phosphorous is dissolved in a solution of 70 ml phosphoric acid and 10 ml of water, and this solution is refluxed for six hours (b.p. of solution 144ºC). After the reaction mixture has cooled down, a little water is added to dissolve precipitated inorganic salts. The solution is extracted with ether and pooled extract washed with a little dilute Na$_2$SO$_4$. The ether is removed under diminished pressure and the residue is distilled (138-139ºC, 13 mm of Hg). The yield is 12.5 gm (90%) of phenyl acetic acid melting at 76ºC. 3.2 gm phosphorus could be recovered from the aqueous solution.

Synthesis of 2-benzyl 1H- benzo [d] imidazole:
Equimolar amount of 1,2 phenylenediamine and the corresponding phenyl acetic acid in 5N HCl were refluxed for 7-8 hours. The solution was cooled in an ice bath and neutralized with NaHCO$_3$. The resulting precipitate was filtered off, washed several times with water and purified by recrystallisation m.p. 182ºC.

Synthesis of ethyl 2-(2-(arylmethyl)-1H – benzo[d] imidazole-1-yl) acetate:
Ethyl chloroacetate (0.01 mol, 1.06 ml) was added to a solution of 2-methyl-1 H benzimidazole (0.01 mol, 1.32 gm) in dry acetone (20 ml). To that mixture, anhydrous K$_2$CO$_3$ (1 g) was added and the reaction mixture was refluxed for 10 hrs. Acetone was removed after completion of reaction and the residue crystallized from ethanol to give compound$^9$ m.p. 184-186ºC yield 78%.

Synthesis of 1, 2, 4 triazolin-5-one:
A mixture of semicarbazide hydrochloride (100gm, 0.90 mol), trimethyl orthoformate (305 g, 2.0 mol) and methanol (1.0 L) was stirred at 20ºC for 3 days. The solvent was the removed under reduced pressure and toluene (1.0 L) was added and the slurry concentrated further to remove residual methanol. The mixture was then cooled to 0 ºC and filtered to get 1, 2, 4- triazolin-5-one (4) (114 g, 98%) as a white solid (mp 197-199ºC)$^{10}$.

Synthesis of 3 nitro-1, 2, 4 triazol-5-one (nitration):
Nitric acid (900 ml) was added to the 150 gm of triazolone marinating the temperature between 0ºC to 5ºC. The mixture was heated to 60-70ºC with constant stirring. The reaction was exothermic and brown fumes evolved. After the reaction were chilled (0-5ºC) in ice bath, filtered and washed with water to remove excess nitric acid. Pure nitrotriazolone was obtained by crystallization from water. The yield was 80%$^{11}$.

Synthesis of 4-[(2- aryl methyl-1H-benzimidazol-1-yl) acetyl -5-nitro-2, 4-dihydro-3h-1,2,4-trizol-3-one:
Equimolar quantity of substituted benzimidazole and nitrotriazolone dissolved in alcohol reflux for 5 hours. Add one to two drops of triethylamine and again reflux for hour. Cool the reaction mixture and poured into water. The precipitate will be separated by filtration$^{12}$.

Proposed scheme II:
General procedure for mannich base condensation: A mixture of nitrotriazolone (0.01 mol) in ethanol (20 ml), formaldehyde (0.02 mol) and aromatic amine (0.02 mol) was added. To reaction mixture was refluxed for 2-6 hours, the solvent poured in ice cold water resulting solid were filtered off recrystallized using appropriate solvent$^{13}$.
**Proposed scheme-II**

![Chemical structure](image)

**Analytical data of the synthesized compounds:**

**BEN₁:** IR (KBr) cm⁻¹: 1729 (-C=O str), 2951 (-CH₂-str), 3397 (NH-str), 1636 (-C=N), 1504 (Ar-NO₂).

**BEN₂:** IR (KBr) cm⁻¹: 1733 (-C=O str), 745 (Ar-Cl str), 1506 (Ar-NO₂) 3399 (NH-str), 2992 (-CH₂-str), 1612 (-C=N).

**BEN₃:** IR (KBr) cm⁻¹: 1730 (-C=O str), 1505 (Ar-NO₂), 3390 (NH-str), 2990 (-CH₂-str), 1612 (-C=N), 3543 (-OH).

**BEN₄:** IR (KBr) cm⁻¹: 1456 (-OCH₃ str), 2987 (-CH₂-str), 1735 (-C=O str), 3416 (-NH-str), 1505 (Ar-NO₂), 1606 (-C=N).

**BFN₁:** IR (KBr) cm⁻¹: 2941 (-CH₂-str), 3405 (-NH-str), 1511 (Ar-NO₂), 1768 (-C=O str), 1637 (-C=N).

**BFN₂:** IR (KBr) cm⁻¹: 751 (Ar-Cl str), 2924 (-CH₂-str), 1504 (Ar-NO₂), 1720 (C=O str), 3420 (-NH-str), 1620 (-C=N).

**BFN₃:** IR (KBr) cm⁻¹: 3543 (-OH), 2900 (-CH₂-str), 3450 (-NH-str), 1514 (Ar-NO₂), 1735 (C=O str), 1594 (-C=N).

**BFN₄:** IR (KBr) cm⁻¹: 1450 (-OCH₃), 2921 (-CH₂-str), 3413 (-NH-str), 1512 (Ar-NO₂), 1723 (C=O str), 1614 (-C=N).

**BFN₅:** IR (KBr) cm⁻¹: 1517 (Ar-NO₂), 3433 (-NH-str), 2910 (-CH₂-str), 1697 (C=O str), 1605 (-C=N); ⋅¹H NMR- 8.34–7.55 (m, 8H, Ar), 4.13 (s, 4H, CH₂), 3.80 (s, 1H, NH).
Antimicrobial screening:  
Antibacterial activity:  
The compounds were tested in vitro for their antibacterial activity against two microorganisms viz. Escherichia coli (NCTC 10418), Klebsiella and Staphylococcus aureus (NCTC 6571), Enterococcus faecalis which are pathogenic in human beings3,4,5.

Antitubercular activity:  
The antitubercular screening was carried out by Middlebrook 7H9 agar medium against H37Rv. Strain. Middlebrook 7H9 agar medium containing different derivatives, standard drug as well as control, Middlebrook 7H9 agar medium was inoculated with Mycobacterium tuberculosis of H37Rv Strain. The inoculated bottles were incubated for 37°C for 6 weeks. At the end of 4 weeks they were checked for growth6. Results were shown in Table No 1 as:

Table No. 1: Antitubercular activity of synthesized compounds

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>Compounds</th>
<th>10µg/ml µg/ml</th>
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<th>50 µg/ml µg/ml</th>
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<td>S</td>
<td>S</td>
</tr>
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<td>S</td>
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<td>7</td>
<td>NBFN5</td>
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<tr>
<td>Standard</td>
<td>Streptomycin</td>
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REFERENCES: