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Synthesis, Characterization and Antibacterial Activity of Two Novel Benzimidazole Compounds from Citric Acid

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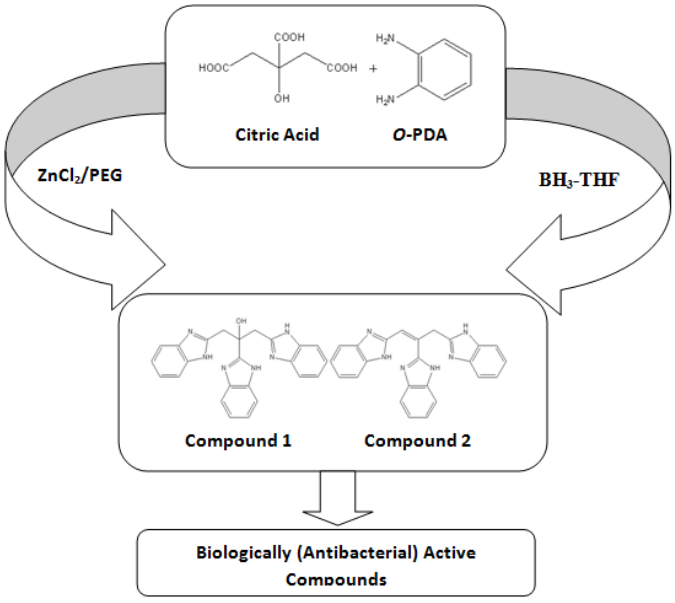
ABSTRACT

Novel benzimidazole compounds were synthesized from citric acid and o-phenylenediamine via condensation mechanism by using two different catalysts. White color compound 1 obtained with $ZnCl_2$/polyethylene glycol (PEG) and yellow color compound 2 was collected when BH_3 – THF in toluene used as catalyst. Synthesized compounds were characterized by FTIR, ^{1}H-NMR, and DART-MS techniques. Compound 1 and 2 were also evaluated for antibacterial activity through disc diffusion method and results showed that synthesized compounds 1and 2 exhibits remarkable antibacterial activity.

Graphical Abstract:

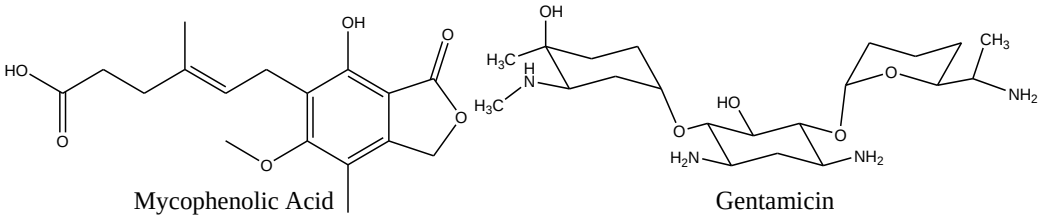


Keywords: Benzimidazole, citric acid, polyethylene glycol, antibacterial activity, disc diffusion method.

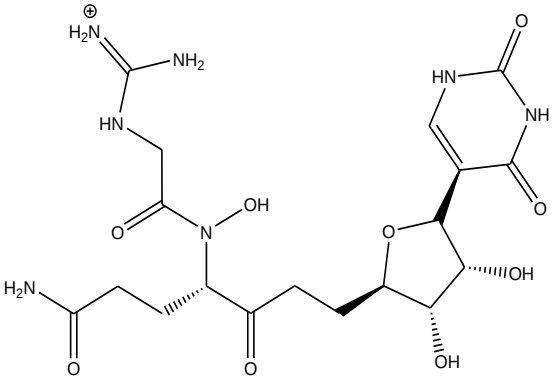
INTRODUCTION

First antibiotic (mycophenolic acid) was isolated from *Penicillium glaucum* (*P. brevicompactum*) in pure and crystalline form by Italian microbiologist Bartolomeo Gosio in 1893[1]. After that numbers of antibiotic were discovered. Recently, from 2000 to 2015, 30 new antibiotics (2 natural products, 12 natural product-derived and 16 synthetic-derived) and 2 new β-lactam/β-lactamase inhibitor combinations have been launched worldwide viz. Linezolid (2000), Telithromycin (2001), Biapenem, Ertapenem, Prulifloxacin, Pazufloxacin, Balofloxacin (2002), Daptomycin (2003), Gemifloxacin (2004), Doripenem, Tigecycline (2005), Retapamulin, Garenoxacin (2007), Ceftobiprole medocaril, Sitafloxacin (2008), Tebipenem pivoxil, Telavancin, Antofloxacin, Basifloxacin (2009), Ceftaroline fosamil (2010), Fidaxomicin (2011), Bedaquiline, Perchlozone (2012), Delamanid, Dalbavancin, Oritavancin, Tedizolid phosphate, Ceftolozane + tazobactam, Nemonoxacin, Finafloxacin (2014), Ceftazidime + avibactam, Ozenoxacin (2015). Among all of them, five were first-in-class antibiotics: linezolid, daptomycin, retapamulin, fidaxomicin and bedaquiline targets gram-positive bacteria only [2]. But research on antibiotics seems never ending because of antimicrobial resistance (AMR), emergence and reemergence of new bacterial pathogens and disease. According to a published report on antimicrobial resistance, about 700,000 people die every year from drug resistant strains of common bacterial infections, HIV, TB and malaria [3]**.** It's also more shocking to here that nearly 200,000 people die every year from multidrug-resistant and extremely drug resistant tuberculosis (TB) alone [4]. In India, antibiotic-resistant neonatal infections cause the deaths of nearly 60,000 new-borns each year [5].

These numbers are likely to be an underestimate due to poor reporting and surveillance. World health organization (WHO) published a report on global priority list of antibiotic-resistant bacteria; First priority (critical): *Acinetobacter baumannii*, carbapenem-resistant, *Pseudomonas aeruginosa*, carbapenem-resistant, *Enterobacteriaceae*, carbapenem-resistant; second priority (high): *Enterococcus faecium*, vancomycin-resistant, *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant, *Helicobacter pylori*, clarithromycin-resistant, *Campylobacter* spp., fluoroquinolone-resistant, *Salmonellae*, fluoroquinolone-resistant, *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant; third priority (medium): *Streptococcus pneumoniae*, penicillin-non-susceptible, *Haemophilus influenzae*, ampicillin-resistant, *Shigella* spp., fluoroquinolone-resistant [6]. During drug designing researchers have to attention on the structure resemblance between designed molecule and binding site in targeted bacteria. There are some major targets for antibacterial actions i.e., inhibition of cell wall synthesis, inhibition of folate synthesis, inhibition of DNA or RNA synthesis, inhibition of protein synthesis etc., [7] which should be taken in attention during antibiotics synthesis. Recently, Maffioli et al., (2017) reported the discovery of a nucleoside analog inhibitor: pseudouridimycin (PUM); that inhibits bacterial RNA polymerase (RNAP) and exhibits antibacterial activity against drug-resistant bacterial pathogens [8]. Pseudouridimycin is a natural product comprising a form amidinylated, N-hydroxylated Gly-Gln dipeptide conjugated to 6′-amino-pseudouridine. In the present work, we synthesized two novel benzimidazoles i.e., tris benzimidazole compounds from citric acid and screened them for antibacterial activity against highly pathogenic bacteria like *Staphylococcus aureus* (gram +ve), *Streptococcus pneumonia* (gram +ve), *Streptococcus pyogenes* (gram +ve), *Pseudomonas aeruginosa* (gram –ve), and *Klebsiella pneumonia* (gram –ve). Z.E. Koc et al. (2010) synthesized novel tripodal-benzimidazole from 2,4,6-tris(p-formylphenoxy)-1,3,5-triazine [9]. They screened all compounds for antibacterial activity against *salmonella* (gram –ve), *E. coli* (gram –ve), *B. subtilis* (gram +ve), *S. aeurus* (gram +ve) and found that compound 3 and 5 have better activity against *S. aeurus* and *B. subtilis* but less than standard drug Gentamicin.



Mycophenolic Acid Gentamicin

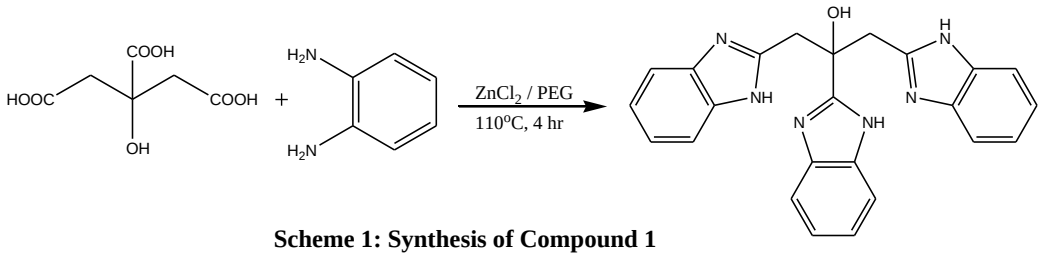


Pseudouridimycine (PUM)

MATERIALS AND METHODS

All chemicals and solvents were purchased from Sigma Aldrich and used without any further purification. FTIR data obtained from Agilent carry 630 FTIR spectrometer, ^{1}H-NMR data was collected from Bruker avance 400, and molecular weight of compounds obtained from Agilent 6520 Q-Tof through DART Mass Spectrometry technique.

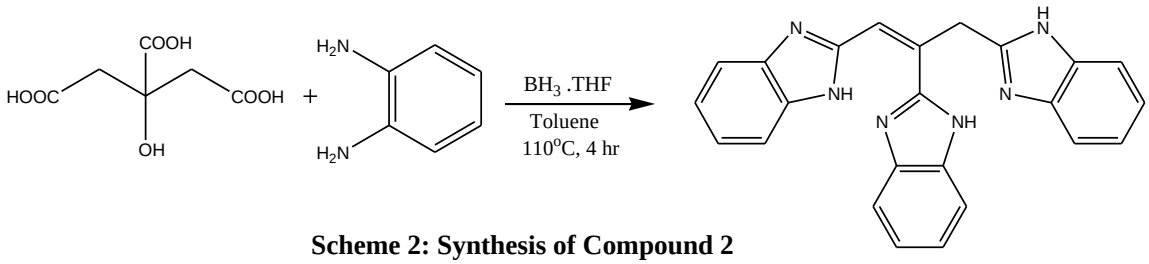
Compound 1: 1, 2, 3-tri (1H-benzo[d]imidazol-2-yl)propan-2-ol: Citric acid (1gram, 0.005mol) and *o*-phenylenediamine (1.58g, 0.0146mol) were mixed and taken together in a round bottom flask in the ratio of 1:3. Anhydrous $ZnCl_2$ (2.1g, 0.015mol) used as catalyst in sufficient amount of polyethylene glycol (PEG). Reaction mixture heated at 110^{o}c for 4 h. After completion of reaction, cooled reaction mixture was diluted several times with water to wash out the polyethylene glycol (scheme 1). White color solid obtained after washing with 82% of isolated yield (0.82gram). FTIR (cm^{-1}):3449, 3013, 2829, 1599, 1138, 811, 750**;** DART MS calculated for $C_{24}H_{20}N_6O$ $[M+3H]^+$ is 411. Due to solubility problem, ^{1}H-NMR and ^{13}C-NMR data still awaited.



Scheme 1: Synthesis of Compound 1

Compound 2: (2-(2, 3-di (1H-benzo[d]imidazol-2-yl)prop-1-enyl)-1H-benzo[d]imidazole): Citric acid (1g, 0.005mol) and *o*-phenylenediamine (1.58g, 0.0146mol) were finely grinded, mixed and taken together in a round bottom flask in the ratio of 1:3. Reaction carried out in presence of Lewis acid catalyst in the ratio of 3:1 of BH_3-THF(0.33mol) with respect to citric acid in sufficient amount of toluene. Reaction mixture heated together on a reaction synthesizer at 110^{o}c for 4 h. After reaction completion, progress of reaction was checked by thin layer chromatography. Cooled reaction mixture was washed several times

with chloroform (scheme 2). Yellow color solid obtained after washing. Reaction proceeds with 89% of isolated yield (0.89gram). FTIR(cm^{-1}): 3072; 2871; 2603; 1649; 1540; 1222; 1088; 837;728,462;^{1}H NMR(dmso-d_6): 8.74; 7.59; 7.22; 6.34; 3.22, DART-MS for $C_{14}H_{18}N_6$ $[M+H]^+$is 391.

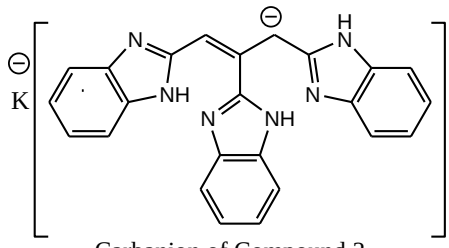


Scheme 2: Synthesis of Compound 2

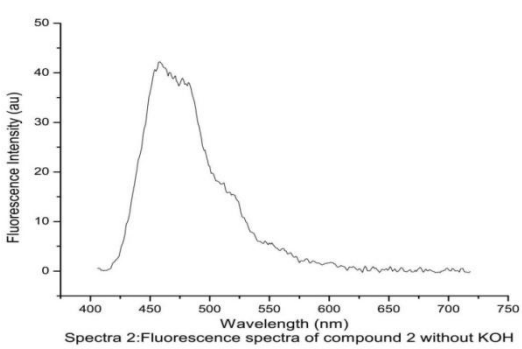
RESULTS AND DISCUSSION

Compound 2 is soluble in dimethyl sulfoxide (DMSO) solvent to a greater extent. When 1ml of 0.01M KOH added in 10 mL solution of compound 2 in DMSO it shows fluorescence (a family of ubiquitous luminescence) and color of solution turns yellow to greenish and this fluorescence behavior of compound confirm by fluorescence emission spectra of compound 2 in DMSO solvent (spectra 1and 2).
Fluorescence absorption maximum wavelength of compound 2 is 458nm and fluorescent intensity is 40au. Change in fluorescence absorption wavelength observed on adding KOH, wavelength shifts towards longer wavelength 483nm (bathochromic shift) and fluorescence intensity increased 40au to 80au. It shows significant change in fluorescence behavior of compound 2.

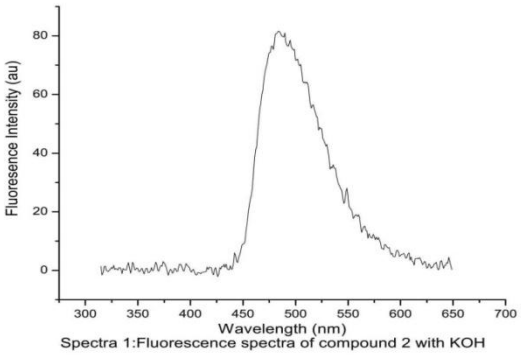
It was assumed that the color change of compound 2 on adding KOH solution was due to the formation of a stable carbanion. Main characteristics of fluorophore (Fluorescence compound) are maximum excitation and emission wavelength, extinction coefficient ($Mol^{-1}cm^{-1}$), quantum yield, lifetime, and stoke shift. Fluorescence occurs when an electron of a molecule, or atom relaxes to its ground state(S_0) by emitting a photon from an excited singlet state (S_1)[10]. Fluorescence used as a non-destructive way of analysis or tracking of biological molecules in the life science.



Carbanion of Compound 2



Spectra 2:Fluorescence spectra of compound 2 without KOH



Spectra 1:Fluorescence spectra of compound 2 with KOH

Antibacterial Activity of Synthesized Compounds: Disc diffusion method was used for the evaluation of antibacterial activity. Both compounds were screened against five pathogenic bacterial strains viz. *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655, *Streptococcus pyogenes* MTCC 442, *Pseudomonas aeruginosa* MTCC 2474, and *Klebsiella pneumoniae* MTCC 4030. But due to low solubility in DMSO of compound 1, salt form ($C_{24}H_{20}N_6O.nHCl$) of compound 1 was used. Then dried compounds were dissolved in dimethyl sulfoxide (DMSO) with a concentration of 0.1 gmL^{-1}. Filter paper discs (6 mm in diameter) were impregnated with 30 µL of compound solution and placed on cation-adjusted Mueller Hinton agar plates, which were inoculated with test organisms according to the standard protocol [11]. The plates were incubated at 37°C for 24 h in BOD incubator and the diameter of the zone of inhibition was measured in millimeter surrounding the disc. Filter paper discs containing DMSO without any test compounds served as a negative control and no inhibition was observed. Additionally, for comparative purposes, broad spectrum antibiotic (Erythromycin) (15 mgL^{-1}, 30µL) was used as positive control. Each sample was assayed in triplicate and the mean±SD values were observed. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest millimeter (mm) as observed from the clear zones surrounding the filter paper disc. Results are shown in Table 1.

Determination of Minimum Inhibitory Concentrations (MICs): Two-fold serial dilution method was used to determine the minimum inhibitory concentrations (MICs) against selected bacterial organisms [12]. Compound 1 and compound 2 diluted by double fold (2:2) with nutrient broth in a series of six test tubes. Concentration of 50, 25, 12.5, 6.25, 3.12 and 1.56 $mgmL^{-1}$ of compound were prepared separately and dissolved in 1 mL of DMSO. An aliquot of 1 mL of bacterial suspension (1.5×10^6) was inoculated into each tube. Control tubes were inoculated with same quantity of sterile distilled water. All tubes were incubated at 37°C for 24 h. The lowest concentration that did not permit any visible growth when compared with control was considered as the minimum inhibitory concentration. The MICs was considered as the lowest concentration that could not produce a single bacterial colony. The contents of all tubes that showed no visible growth were cultured on Mueller-Hinton agar, incubated at 37 °C for 24 h. Result of determined MIC values are shown in Table 2.

Table 1. The inhibition zones (MIZ) diameters of synthesized compound against pathogens

Bacteria	Diameters of the inhibition zone (mm)		Positive control	Negative control
	Compound 1	Compound 2	Erythromycin	DMSO
S. aureus	20.0±0.51	22.6±0.39	25.0±1.54	0
S. pyogenes	11.3±0.94	10.6±1.43	20.3±0.67	0
S. pneumoniae	15.3±0.24	12.0±1.27	20.6±0.43	0
P. aeruginosa	17.0±1.53	14.3±0.63	21.3±1.32	0
K. pneumoniae	16.6±0.42	13.0±0.86	20.3±0.32	0

Table 2. Minimum Inhibitory Concentration (MICs) values of Compound 1, 2 & Erythromycin

S. No.	Bacteria	MICs Value (mg/ml) of Compound 1	MICs Value (mg/ml) of Compound 2	MICs Value (mg/ml) of Erythromycin
1	*S. aureus*	3.12	3.12	1.57
2	*S. pyogenes*	6.25	12.5	3.12
3	*S. pneumoniae*	3.12	6.25	1.57
4	*P. aeruginosa*	12.5	12.5	6.25
5	*K. pneumoniae*	25	25	3.12

APPLICATIONS

This work is an attempt to investigate the antibacterial activity of tris-benzimidazole compounds synthesized from citric acid and it will be helpful in research on antibiotics discoveries.

CONCLUSIONS

There is health crisis situation created by drug resistance bacterial pathogens and this drives the new discoveries in medicinal chemistry regarding bacterial infections. Present work was an attempt to investigate antibacterial activity of benzimidazole derivatives synthesized from citric acid against pathogenic bacteria. From results, it can be assumed that the synthesized compounds are new in the area of antibacterial research, after some necessary structural modification. Compound 2 containing conjugated system of pi-electrons, exhibits florescence property in salt form, is also promising in fluorescence chemistry. Synthesized compounds play an important role in metal complex synthesis and can be used as multi dentate ligands.

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