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Synthesis, Characterization and Antibacterial Evaluation of Some Potent 2-Substituted Benzimidazole Analogues

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ABSTRACT
Present study circumspects synthesis of some novel 2-substituted analogues of Benzimidazole and their spectral characterization by means of UV, IR and ^{1}H NMR. The compounds were screened for antibacterial activity against standard strains of both Gram positive and Gram negative bacteria. Results obtained establish compounds KD1 and KD2 to be significantly responsive against different bacterial strains and as such these compounds can pave the way for development of potent antibacterial agents.

Keywords: Benzimidazoles, o-phenylenediamine, Antibacterial, Cup-plate method.

INTRODUCTION

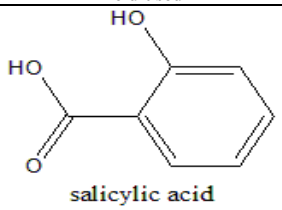
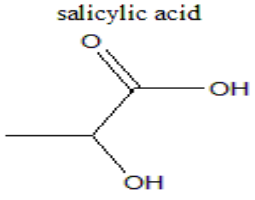
Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications, and are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio. [1-3] These derivatives are found to exhibit various biological activites such as anticancer [4], antihypertensive [5], anthelmenthic [6-8], antiprotozoal [9-10], antimicrobial [11-16], antioxidant [17-18], anti-inflammatory [19-20] and analgesic [21] activity. Different synthetic methods are reported for the synthesis of benzimidazole and its derivatives which includes processes like coupling of o-phenylenediamine with carbonyl compounds in presence of various catalysts like $ZrCl_4$, $SnCl_4$, BF_3, polyethylene glycol, ceric ammonium nitrate [22] etc. The present study utilizes the same coupling phenomenon of o-phenylenediamine with substituted organic acids in presence of ring closing agents like HCl to form 2-substituted derivatives followed by their antibacterial screening. [23-24]

MATERIALS AND METHODS

The melting points of the synthesized compounds were determined using an electric melting point apparatus by open capillary method (expressed in degree Celsius (°C)) and are uncorrected. The progress of reactions and purity of synthesized compounds were checked on silica gel-G TLC plates using various solvent combinations of different polarity. The spots were detected with iodine vapors as visualizing agent. The λ_{max} (in nm) of the synthesized compounds was recorded on Elico SL 164 UV-visible spectrophotometer using acetone as solvent. The FT-IR spectra of the synthesized compounds were recorded on a FT-IR Perkin Elmer Spectrum RX-I spectrometer using KBr disc in the range of 4000-400 cm^{-1}. The Proton NMR (^{1}H NMR) spectra were recorded in Bruker AC-F 400 FT-NMR spectrometer at a frequency of 400 MHz. Spectra were obtained in deuterated acetone (acetone-d_6) using TMS (δ 0.00 ppm) as an internal standard at room temperature. Chemical shift (δ) values are expressed in ppm relative to internal standard.

Substituted acids used for synthesis

Table. 1: Acids used for the substitution reaction to form target compounds

Compound	Acid used	R
KD1 ($C_{13}H_{10}N_2O$) Mol. Wt = 210.23	 salicylic acid	**- C_6H_5OH**
KD2 ($C_9H_{10}N_2O$) Mol. Wt = 162.19	 lactic acid	**- CH (OH)- CH_3**

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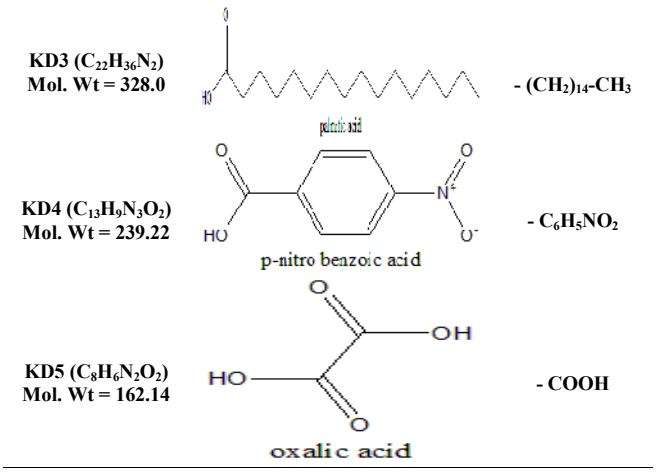

Fig. 1: Synthetic scheme for 2-substituted benzimidazoles

Table. 2: Antibacterial activity data

Compound	Zone of inhibition (mm)							
	Gram positive bacteria				Gram negative bacteria			
	S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
KD1	15	16	19	21	19	19	21	22
KD2	17	20	23	23	18	22	22	23
KD3	12	17	18	19	14	19	17	22
KD4	9	12	10	11	13	17	9	17
KD5	14	16	18	20	13	15	14	16
**Control	-	-	-	-	-	-	-	-
*Gentamycin	15	-	20	-	18	-	20	-

*Gentamycin (50 µg/ml) was used as positive control
**Acetone was used as negative control

General method for synthesis of 2-Substituted benzimidazoles

O-phenylene diamine (0.1 mol) and equivalent quantity of carboxylic acid (0.1 mol) was heated on a water bath at 100ºC for 1 hour. The completion of reaction was monitored by TLC. After completion of reaction, the reaction mixture was cooled and basified to a pH of 7-8 by using 10% sodium hydroxide solution. The crude benzimidazole was filtered at the pump, washed with ice cold water. The crude product was dissolved in 400 ml of boiling water and 2g of decolorizing carbon was added and digested for 15 min. The solution was filtered while hot, and the filtrate was cooled to about 10ºC. The final product was filtered, washed with 25 ml of cold water and dried at 100ºC. Pure product was obtained upon recrystallization using absolute alcohol.

Antibacterial Evaluation

The antibacterial activity of the synthesized compounds was evaluated systematically against different strains of Gram-positive bacteria like *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative bacteria like *E. coli* and *Pseudomonas aeruginosa*. The inhibition zones (in mm) of synthesized compounds were determined by cup-plate method. [25]

The sterilized medium (autoclaved at 121°C for 20 min) was inoculated using 18hr slant cultures of the test organisms and transferred into sterile Petri dishes and allowed to the media to solidify. Cups of 8 mm diameters were made on solidified media. Solutions of the synthesized compounds at a concentration of 50µg/ml and 100µg/ml were prepared in acetone. 50µl of each solution was placed in cups by means of sterile pipette. In each plate one cup was used for standard and other two for test solutions. The plates thus prepared were left for 90 min in a refrigerator for diffusion. The plates were incubated at 37°C for 24hrs and examined for inhibition zones. The experiment was performed in duplicate and the average diameter of the zones of inhibition was recorded. Gentamycin (50µg/ml) was used as standard.

RESULTS AND DISCUSSON

Physico-chemical properties and spectral data of the synthesized compounds

The yields of all the synthesized compounds were found to be satisfactory within the range of 60 to 70%. The spectral data generated upon analysis were found in accordance with the anticipated structure of the synthesized compounds.

KD1: 2-(1H-benzo[d]imidazol-2-yl)phenol)

Yield: 65%; Melting point: 250-252ºC; R_f value: 0.94; λ_{max}: 424; IR (KBr cm^{-1}): 3038 (aromatic-H stretching), 1458 (-C=C stretching), 1632 (-C=N stretching), 3191 (=C-H stretching), 1502 (-C-N stretching), 3364 (aromatic –NH bending), 3386 (Ar-OH); ^{1}H NMR (400 MHz, acetone-d_6), δ (ppm): 7.11 (m, 8H; Ar-H), 4.7 (s, 1H; OH), 3.2 (s, 1H NH).

KD2: 1-(1H-benzo[d]imidazol-2-yl)ethanol

Yield: 68%; Melting point: 176-178ºC; R_f value: 0.82; λ_{max}: 440; IR (KBr cm^{-1}): 3038 (aromatic H stretching), 1458 (-C=C stretching), 1633 (-C=N stretching), 3191 (=C-H stretching), 1502 (-C-N stretching), 3386 (aromatic –NH bending), 3364 (-CH (OH)-CH_3 stretch); ^{1}H NMR (400 MHz, acetone-d_6), δ (ppm): 5.0 (s, NH), 7.22-7.54 (m, Ar-H), 4.69 (s, 1H; broad, NH).

KD3: 2-palmitoyl-1H-benzo[d]imidazole

Yield: 67%; Melting point: 250.5ºC- 252.2ºC; R_f value: 0.93; λ_{max}: 430; IR (KBr cm^{-1}): 2955 (aromatic-H stretching), 1472 (-C=C stretching), 1638 (-C=N stretching), 2955 (=C-H stretching), 1501 (-C-N stretching), 3387 (aromatic –NH bending), 1271 (Palmitoyl group stretch); ^{1}H NMR (400 MHz, acetone-d_6), δ (ppm): 3.27 (t, 2H, CH_2), 6.71- 6.79 (m, 4H, aromatic CH).

KD4: 2-(4-nitrophenyl)-1H-benzo[d]imidazole

Yield: 60%; Melting point: 280-282ºC; R_f value: 0.925; λ_{max}: 430; IR (KBr cm^{-1}): 3027 (aromatic-H stretching), 1460 (-C=C stretching), 1633 (-C=N stretching), 3027 (=C-H stretching), 1501 (-C-N stretching), 3364 (aromatic –NH bending), 1522 (Ar-NO_2 group stretch); ^{1}H NMR (400 MHz, acetone-d_6), δ (ppm): 8.19 (m, 2H; Ar-H- C3′ & C5′), 7.91 (m, 2H; Ar-H- C2′&C6′), 7.88 (m, 2H; Ar-H- C4 & C7), 6.83 (m, 2H; Ar-H- C5 & C6), 4.69 (s, 1H; broad, NH).

KD5: 4-(1H-benzo[d]imidazol-2-yl)benzoic acid

Yield: 70%; Melting point: 171-172ºC; R_f value: 0.86; λ_{max}: 445; IR (KBr cm^{-1}): 3046 (aromatic-H stretching), 1472 (-C=C stretching), 1614 (-C=N stretching), 3046 (=C-H stretching), 1501 (-C-N stretching), 3440 (aromatic –NH bending), 1614 (>C=O stretch); ^{1}H NMR (400 MHz, acetone-d_6), δ (ppm): 4.7 (1H, -NH), 7.18 (1H, -COOH), 6.71, 6.78 (2H, Ar).

Antibacterial activity data of the synthesized compounds

Antibacterial screening of the synthesized compounds against different strains of Gram positive and Gram negative bacteria show compounds KD2 and KD1 exhibiting marked inhibition of both Gram positive and negative strains whereas compounds KD5 and KD3 too showed considerable amount of activity. Compound KD4 showed the least activity amongst the series.

Stressing on the structural influence on the activity of the synthesized novel analogues, it can be observed that the hydroxyl group (-OH) present in both KD2 (from lactic acid) and KD1 (from salicylic acid) may have a vital role in the activity of the compounds. Whereas a nitro group (-NO_2) in KD4 (from p-nitro benzoic acid) may possibly diminish the inhibitory activity to a considerable extent. It is evident from the research work that this series of synthesized and screened compounds along with further explored ones from the same series of 2-substituted benzimidazole may pave the way for development of some very potent antibacterial agents.

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REFERENCES

1. Ansari KF, Lal C. Synthesis, physicochemical properties and anti-microbial activity of some new benzimidazole derivatives. Eur. J. Med. Chem. 2009; 44: 4028-4033.
2. Grocer H, Kus C, Boykin DW, Yildiz S, Altanlar N. Synthesis and Anti-fungal Properties of Some Benzimidazole Derivatives. Bioorg. Med. Chem. 2002; 10: 2589-2596.
3. Kazimierczuk Z, Upcroft JA, Upcroft P, Gorska A, Starosciak B, Laudy A. Synthesis and antiproto-zoal activity of some 2-(trifluoromethyl)-1H-benzimidazole bioisosteres. Acta Biochim. Pol. 2002; 49: 185-195.
4. Starcevic K, Kraji M, Ester K, Sabol I, grce M, Pavelic K, Karminskizamola G. Synthesis, antiviral and antitumor activity of 2-substituted-5-amidino-benzimidazoles. Bioorg Med Chem. 2007; 15(13): 4419.
5. Kubo K, Inada Y, Kohara Y, Sugiura Y, Ojima M, Itoh K, Furukawa Y, Nishikawa YK, Naka T. Nonpeptide angiotensin II receptor antagonists. Synthesis and biological activity of benzimidazoles. J Med. Chem. 1993; 36(12): 1772-84.
6. Dubay R, Abuzar S, Sharma S, Chatterjee RK, Katiyar JC. Synthesis and anthelmintic activity of 5(6)-(benzimidazol-2-ylcarbamoyl) and (4-substituted piperazin-1-yl)benzimidazoles. J Med Chem. 1985; 28(11): 1748-50.
7. Mavrova AT, Denkova PS, Tsenov YA, Anichina KK, Vutchev DL. Synthesis and antitrichinellosis activity of some bis(benzimidazol-2-yl)amines. Bioorg Med Chem. 2007; 15(18): 6291-97.
8. Ravina E , Sanchez-Alonso R, Fueyo J, Baltar MP, Bos J, Iglesias R, Sanmartin ML. Synthesis and potential anthelmintic activity of methyl-5-(4-salicyloyl-piperazin-1-yl)-benzimidazole-2-carbamates. Arzneimittelforschung. 1993; 43(6): 689-94.
9. Navarette Vazquez G, Cedilla R, Hernandez Campos A, Yepez A, Hernandez luis F, Valdez J, Morels R, Cortes R , Hernandez M, Castillo R. Synthesis and antiparasitic activity of 2-(trifluoromethyl)-benzimidazole derivatives. Bioorg Med Chem Lett. 2001; 11: 187-90.
10. Katiyar SK, Gordon VR, Mc Laughlin GL, Edlind TD. Antiprotozoal activities of benzimidazoles and correlations with beta-tubulin sequence. Antimicrob Agents Chemother. 1994; 38(9): 2086-90.
11. Goker H, Kus C, Boykin DW, Yildiz S, Altanlar N. Synthesis of some new 2-substituted-phenyl-1H-benzimidazole-5-carbonitriles and their potent activity against Candida species. Bioorg Med Chem. 2002; 10(8):2589-96.
12. Goker H, Ozden S, Yildiz S, Boykin DW. Synthesis and potent antibacterial activity against MRSA of some novel 1,2-disubstituted-1H-benzimidazole-N-alkylated-5-carboxamidines. Eur J Med Chem. 2005; 40(10): 1062-69.
13. Desai KG, Desai KR. Green route for the heterocyclization of 2-mercaptobenzimidazole into beta-lactum segment derivatives containing -CONH- bridge with benzimidazole: Screening in vitro antimicrobial activity with various microorganisms. Bioorg Med Chem. 2006; 14(24): 8271-79.
14. Kazimierczuk Z, Upcroft JA, Upcroft P, Gorska A, Starosciak B, Laudy A. Synthesis, antiprotozoal and antibacterial activity of nitro- and halogeno-substituted benzimidazole derivatives. Acta Biochim Pol. 2002; 49(1): 185-95.
15. Mohammad BG, Hussien MA, Abdel-Alim AA, Hashem M. Synthesis and antimicrobial activity of some new 1-alkyl-2-alkylthio-1,2,4-triazolobenzimidazole derivatives. Arch Pharm Res. 2006; 29(1): 26-33.
16. Pawar NS, Dalal DS, Shimpi SR, Mahulikar PP. Studies of antimicrobial activity of N-alkyl and N-acyl 2-(4-thiazolyl)-1H-benzimidazoles. Eur J Pharm Sci. 2004; 21: 115-18.
17. Kus C, Ayhan-Kilcigil G, Can Eke B, Iscan N. Synthesis and antioxidant properties of some novel benzimidazole derivatives on lipid peroxidation in the rat liver. Arch Pharm Res. 2004; 27(2): 156-63.
18. Ates-Alagoz A, Kus C, Coban T. Synthesis and antioxidant properties of novel benzimidazoles containing substituted indole or 1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene fragments. J Enzyme Inhib Med Chem. 2005; 20(4): 325-31.
19. Lazer ES, Matteo MR, Possanza GJ. Benzimidazole derivatives with atypical antiinflammatory activity. J Med Chem. 1987; 30(4): 726-29.
20. Lackner TE, Clissold SP. Bifonazole. A review of its antimicrobial activity and therapeutic use in superficial mycoses. Drugs. 1989; 38(2): 204-25.
21. Ito K, Kagaya H, Fukuda E, Yoshino K, Nose T. Pharmacological studies of a new non-steroidal antiinflammatory drug: 2-(5-ethylpyridin-2-yl)benzimidazole (KB-1043) Arzneimittelforschung. 1982; 32(1) :49-55.
22. Grimmet M R in (eds) Katritzky A R C W Rees. Hetero.chem. 1984; 457
23. Furniss BS, Hannaford AJ, Peter WG, Smith Tetchell AR. Vogel's Text book of Practical Organic Chemistry, 1989.
24. Ansari KF, Lal C. Synthesis and Biological Activity of Some Heterocyclic Compounds containing Benzimidazole and β-lactam Moiety. J Chem Sci. 2009; 121(6):1017-1025,
25. Hawkey PM, Lewis DA. Medical bacteriology-a practical approach, Oxford University press, 1994.