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CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF SUBSTITUTED BENZIMIDAZOLE DERIVATIVES

Minakshi Verma[1], Amita Tilak[1], Ranjana Sharma[1], Ashish Kumar Gupta[2]
[1]Department of Pharmacy, GSVM Medical College, Kanpur
[2] Department of Pharmacy, VBS Purvanchal University, Jaunpur

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Corresponding author: Minakshi Verma
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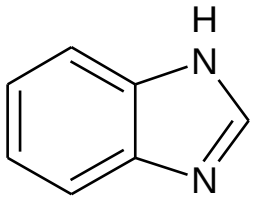
Abstract
A blend of 32.4 g (0.1 mole) of ortho phenylenediamine (OPD), 19.5g (0.1 mol) of Potassium hydroxide (KOH) and 26 g (0.1 mole) of carbon disulphide (CS2), 300 ml of 95% ethanol and water were taken in round base flagon and reflux give coupling prouct benzimidazole 2-thiol (1) was isolated as flicker white precious stone. 10.75ml of concentrated nitric corrosive and equivalent amount of concentrated sulphuric corrosive (1:1) was included The nitration item 5-nitro benzimidazole 2-thiol (2) was shaped as yellowish pale shading, at that point S-alkylated subordinates framed from 5-Niro benzimidazole 2-thiol utilizing acetonitrile (20 ml) containing 2 ml (14 mmol) DBU followed by the expansion of 0.72 g (5 mmol) of 2-diethylaminoethylchloride (3i), 4-aminobenzylchloride (3ii), 4-Hydroxybenzylchloride (3iii), 4-nitro 2-Hydroxybenzylchloride (3iv) give The arrangement was mixed for the time being at room temperature and the dissolvable vanished. Water (5 ml) was added to the buildup and the blend was carried to pH 7 with CH3COOH. The accelerate framed was sifted and solidified from H2O to give last item (3a), (3b), (3c), (3d) individually.
Keywords: Benzimidazole, Anti-microbial, S-Alkylation benzimidazole, 2- mercapto benzimidazole.

Introduction

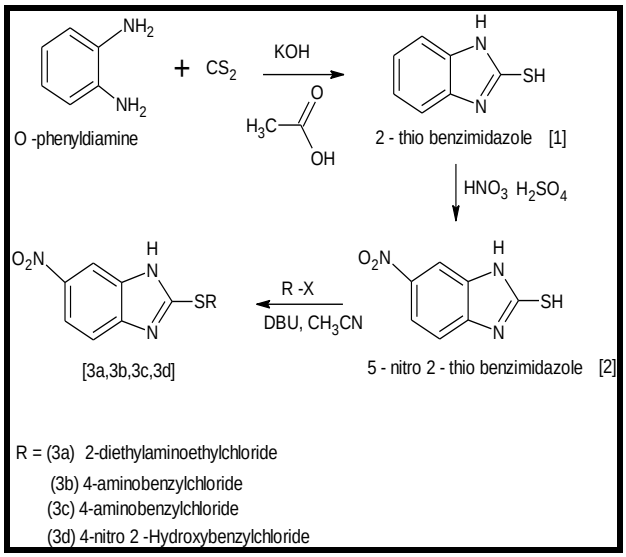
Benzimidazole is a heterocyclic sweet-smelling natural compound. A crystalline compound benzimidazole (C7H6N2) is utilized in natural amalgamation and represses the development of certain smaller scale organism.1 it is a significant pharmacophore and a special structure in restorative science. This compound is bicyclic in nature which comprises of the combination of benzene and imidazole. This day is a moiety of decision which has numerous pharmacological properties. The dissolving purpose of benzimidazole is 170ºC. It will be noticed that the presentation of a substituent into the 1-position when all is said in done brings down the liquefying point. This gives off an impression of being because of the way that benzimidazoles containing hydrogen in the 1-position are related. Benzimidazoles with the imide nitrogen (i.e., hydrogen in the 1-position) are typically progressively dissolvable in polar solvents and less dissolvable in natural solvents. On the other hand, the presentation of polar groupings into the particle builds dissolvability in polar solvents; along these lines, 2-aminobenzimidazole is dissolvable in water. Benzimidazoles are feebly fundamental, being to some degree less essential than the imidazoles.2 The overall basicity of the benzimidazole ring has been resolved likewise by a backhanded subjective technique including examination of the range of a cyanine color containing the benzimidazole ring as a constituent with cyanine colors got from other fundamental substances. The strategy includes an estimation of the deviation in λmax, which is thought to be because of the basicity of the heterocyclic structure. Benzimidazoles are likewise adequately acidic to be commonly dissolvable in watery salt and structure N-metallic mixes. The acidic properties of the benzimidazoles, similar to those of the imidazoles, appear to be because of adjustment of the particle by reverberation.[3]



(Fig 1: Structure of Benzimidazole)

An antibacterial is a compound or substance that kills or slows down the growth of bacteria.[4] The term is often used synonymously with the term *antibiotic(s)*; today, however, with increased knowledge of the causative agents of various infectious diseases, antibiotic(s) has come to denote a broader range of antimicrobial compounds, including antifungal and other compounds.[13] Imidazole and Benzimidazole Synthesis is a comprehensive survey of the known methods of syntheses and ring modification. It brings together the multitude of synthesis of the imidazole ring in a systemic way interms of specific bond formation, and recommends the most attractive synthetic approaches.

Materials & Methods: ***synthetic scheme***



Synthetic Procedure

Step 1: Synthesis of 2-thio benzimidazole: Coupling Reaction

A mixture of 32.4 g (0.1 mole) of Ortho phenylenediamine (OPD), 19.5g (0.1 mol) of Potassium hydroxide (KOH) and 26 g (0.1 mole) of carbon disulphide (CS_2), 300 ml of 95% ethanol and 45 ml of water were taken in round bottom flask and reflux for 3 hour Norit was then added cautiously & after mixture had been heated for 10 minutes. Norit(Charcoal) was removed by filtration. A yellowish filtrate of benzimidazole 2-thiolate was collected in conical flask. Filtrate of benzimidazole 2-thiolate was heated at 60-70 0C, mix 300ml of water followed by 25 ml of glacial acetic acid with efficient stirring. The product benzimidazole 2-thiol was separated as glisten white crystal. Then it was placed in refrigerator for 3 hours to complete crystallization. Crystaline solid product precipitated out. The precipitate was collected by suction (Buchner funnel) and repeatedly washed with small portions of water and recrystallised using Ethanol to yield 1H-benzimidazole-2–thio (1).[5]

Step 2: Synthesis of 5-Nitro benzimidazole :Nitration Reaction

10.75ml of concentrated nitric acid was placed in three necked flask and equal quantity of concentrated sulphuric acid (1:1) was added slowly. The mixture was kept in the ice cold water then compound No. 1 (6.72gm) was mixed in portions during ½ hour under room temperature. After stirred continuously for 12 hours 45 minutes and then the reaction mixture was poured slowly over crushed ice with stirring. The precipitated product was filtered out and washes with cold water. The final product was formed as yellowish pale.

Step 3: Synthesis of S-Alkylation 5-Niro benzimidazole: Dehydrogenation reaction.

3a:N,N-diethyl-2-[(6-nitro-1H-benzimidazol-2-yl) sulfanyl]ethanamine:

0.6 g (4 mmol) of 5-nitro 2-thiobenzimidazole was dissolved in acetonitrile (20 ml) containing 2 ml (14 mmol) DBU followed by the addition of 0.72 g (5 mmol) of 2-diethylaminoethylchloride hydrochloride. The solution was stirred overnight at room temperature and the solvent evaporated. Water (5 ml) was added to the residue and the mixture was brought to pH 7 with CH_3COOH, the precipitate formed was filtered and crystallized from H_2O to give final Product 3a and 3b-4-{[(6-nitro-1H-benzimid azol-2-yl)sulfanyl] methyl}aniline, 3c-4-{[(6-n itro-1H-benzimidazol-2-yl)sulfanyl]methyl}ph enol and 3d-5-amino-2-{[(6-nitro-1H-bezimid azol-2 yl) sulf anyl]methyl}phenol.

Biological Evaluation[7]

Anti-microbial activity

All the compounds synthesize in the present investigation will screen for their anti-bacterial activity by Cup plate Method. Antibacterial activities will test on nutrient medium against, Staphylococcus aureus, and Escherchia coli which are representative types of gram positive and gram negative organisms respectively. The antibacterial activities of the compounds will assess by disc-diffusion method.

Preparation of nutrient agar media

The nutrient agar media will prepare by using the following ingredients.

Peptone (Bacteriological)-20 gm, Beef extract (Bacteriological)-5 gm

Sodium chloride-5 gm, Agar-20 gm, Distilled water up to1000 ml

Weigh quantities of peptone and beef extract will dissolve in distilled water by gentle warming and then specified amount of agar will dissolve by heating on water bath. Then the pH of the solution will adjust to7.2 to7.4 by adding the sodium chloride and the volume of the final solution will make up to 1000 ml with distilled water. Then it will transfer in to a suitable container, plugged with non-adsorbent cotton and the media will sterilize by in autoclave at 121^0C for 20 minutes at 15 lbs pressure.**Preparation of test solutions**10 mg of the compound will dissolve in 10 ml of DMF. From this 1 ml

of solution was taking and dilute up to 10 ml with DMF. Now the concentration of the test solution wills 100 μg/ml. From the stock solution 1ml of solution will take and dilute with 1ml of DMF now the concentration is 50μg/ml.

Preparation of standard antibiotic solution Amoxicillin will use as standard antibiotics for comparison and solutions will prepare by using sterile water, as they were water-soluble. The solutions are dilute by using sterile water so that the concentrations of the solutions will 100 μg/ml and 50 μg/ml.

Preparation of Discs: Discs of 6-7 mm in diameter will punch from NO: 1 Whattmann filter paper with sterile cork borer of same size. These discs will sterilize by keeping in oven at 140^0C for 60 minutes. Then standard and test solutions will add to each disc and discs will air-dry.

Method of Testing: The sterilize media will cool to 45^0C with gentle shaking to bring about uniform cooling and then inoculate with 18-24 hrs old culture under aseptic conditions, mixwell by gentle shaking. This will pour in to sterile Petri dishes (properly labeled) and allow the medium to set. After solidification all the Petri dishes will transfer to laminar flow unit. Then the discs which were previously prepared will carefully kept on the solidified media by using sterilized forceps. These Petri dishes will kept as it is for one-hour diffusion at room temperature and then for incubation at 37^0C for 24 hours in an incubator. The extent diameter of inhibition after 24 hours will measure as the zone of inhibition in millimeters.

Anti-Fungal Activity

Broth dilution method will use for screening Antifungal activity as described below.

Broth double dilution method

The broth double dilution method will use to evaluate the minimal inhibitory concentration (MIC) of the test compounds the classical method yields accurate, precise and quantitative results for the amount of antimicrobial agent that is needed to inhibit growth of microorganisms. Determination of minimum inhibitory concentration (MIC) by broth double dilution method. MIC of the entire test (synthesized compounds) was determined using the said method. following controls was also incorporated :-

Drug control- Ketoconazole as reference standard was used.

Solvent control – DMF and DMSO was used as solvent controls.

Sabourauds Dextrose Broth (SDB) and Malt extract Glucose Yeast extract peptone broth (MGYP) was used as nutrient medium for growth of microorganism and MIC determination for C.albicans.

All the compounds were dissolved in DMF and standard dissolve in DMSO.

All the compounds were serially diluted.

Test compounds were dissolved in sterile DMF and Ketoconazole was dissolved in the sterile DMSO.

The test compounds and standard drug solution was diluted using Sabourauds Dextrose Broth (SDB) and Malt extract Glucose Yeast extract peptone broth (MGYP) so as to get required concentration.

To serially diluted solution, test organisms were added using saline solutions or broth.

Then the plates were incubated at 37^0C for 48 hrs.The growth of microorganism in the test compound solutions and control drug was seen after incubation.

Methods Used For Screening:

In screening the test compounds was dissolved in DMF, so as to give 800mg/ml which was then serially diluted. Ketoconazole used as standard, was dissolved in sterile DMSO. DMF, DMSO was also test as control.

Result & Discussion

All the synthesized compounds were off white, light yellow to brown colored crystalline solids. All the compounds are soluble in methanol and water, partially in chloroform, ethanol. The melting point of the compounds was in the range of 120ºC to 310ºC. IR spectra of all compounds were recorded on Shimadzu FT-IR 8400S spectrophotometer using KBr. All the synthesized compounds have shown characteristic stretching and bending in desired range. Mass spectra were obtained using. All the spectra were taken by direct infusion mass with ESI and APCI in positive and negative mode ionization ranging from 100-500 m/e. All the compounds possess a molecular ion M+, M+1 peak. The ^{1}H NMR spectra of some of the compounds were studied in d6- DMSO on a Bruker II Avance 400 MHz NMR spectrometer. All the compounds show characteristic chemical shift from TMS in terms of δ ppm. δ value obtained in the desired range which signifies the presence of aromatic ring.

Table 1: Physical characteristic of synthesized compounds

Compound Code	Molecular formula	% yield	Melting point In ºC	Rf value
3a	$C_{13}H_{18}N_4O_2S$	67	160	0.57
3b	$C_{14}H_{12}N_4O_2S$	53	192	0.45
3c	$C_{14}H_{11}N_3O_2S$	75	205	0.62
3d	$C_{14}H_{10}N_4O_5S$	71.5	189	0.52

Table 2: Spectral characteristics of synthesized compounds

Compound Code	IR (cm^{-1})	Mass (m/e)	^{1}H-NMR
3a	- C-H(2910), - N-H(3352), -NO_2(1570)	294.12 (Base) 295.12[M+] 296.11[M+1]	3.03(t,3H-CH_2) 2.76(t,3H-CH_2) 1.04(s,6 H,-CH_3), 2.40(s,4 H,-CH_2),
3b	C-H(2890), -NH_2 (3380), -NO_2(1545),	300.07 (Base) 301.07 [M+1] 302.06[M+2]	**NA**
3c	-N-H(3319), -OH (3457), -NO_2(1553)	301.05(Base) 301.32 [M+] 302.06[M+1] 303.05 [M+2]	**NA**
3d	-C-H(2910), -OH(3580), -NO_2(1542)	346.04(Base) 346.32 [M+] 347.04[M+1]	**NA**

Table 3: Results of antimicrobial activity of the tested compounds

Sr.No	Compound	Concentration µg/ml	*E.coli*	*S.Aureus*	*c.albicans*
1	**3a**	50	9	10	-
		100	11	10	09
2	**3b**	50	9	11	-
		100	12	11	10
3	**3c**	50	9	10	-
		100	13	14	15
4	**3d**	50	16	13	-
		100	18	17	10
STD	Amoxicillin	50	24	25	-
		100	25	25	-
STD	ketaconazole	100	-	-	21

Conclusion

From the above data of anti-microbial activity was found Compound 3d and 3c is near to potent derivatives, as anti-bacterial and anti-fungal respectively to most potent Amoxicilline for anti-bacterial and ketaconazole for anti-fungal activity. Further modification of compound possible for optimum activity.

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