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RESEARCH ARTICLE

Synthesis, Chemical Characterization and Antimicrobial Activity of Some Novel Benzimidazole Derivatives.

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ABSTRACT

The starting compound 2-mercapto benzimidazole (1a) / 5-methyl-2-mercapto benzimidazole (1b) was prepared from o-phenylene diamine / 4-methyl benzene-1,2-diamine, potassium hydroxide and carbon disulfide upon refluxing for 3 h in single step respectively. The 2-mercapto benzimidazole (1a) / 5-methyl-2-mercapto benzimidazole (1b) was refluxed for 60 min with potassium hydroxide, followed by chloro acetic acid and stirred for 18 h to furnish 1H-benzimidazol-2-ylthio acetic acid (2a) / 5-methyl-1H-benzimidazol-2-ylthio acetic acid (2b) respectively. The different Mannich bases ($3a_1$-$3a_{12}$) were synthesized by refluxing the appropriate substituted amines, formaldehyde with 1H-benzimidazol-2-ylthio acetic acid (2a) and 5-methyl-1H-benzimidazol-2-ylthio acetic acid (2b) in ethanolic medium for 12 h. The synthesized compounds were characterized by their physical and spectral data.

KEYWORDS: Benzimidazole, Carbon disulphide, Triazole, Tetrazole, Anti microbial activity.

INTRODUCTION:

Many important biochemical compounds and drugs of natural origin contain heterocyclic ring structures. Among carbohydrates, essential amino acids, vitamins, alkaloids, glycosides etc. the presence of heterocyclic structures in such diverse types of compounds is strongly indicates that these compounds posses different types of the pharmacological activity.

The heterocyclic substituted benzimidazole[1] have received considerable attention during last two decades as they are endowed with variety of biological activities and have wide range of therapeutic properties. A literature survey indicates that benzimidazole derivatives possess different pharmacological and biological activities, of which the most potent is anti-microbial[2], antifungal[3], anticonvulsant[4], anticancer[5] and anti-inflammatory[6-10] activities. In view of above literature survey, we thought to synthesize some new substituted benzimidazole moiety. By considering the above facts we plan to synthesize a biheterocyclic system comprising of benzimidazole nucleus and biologically important heterocyclic systems like triazoles and tetrazoles system. By considering the above facts we synthesized biheterocyclic system comprising of benzimidazole nucleus and biologically important heterocyclic systems like triazoles and tetrazoles system. We have also planned to evaluate the synthesized compounds for anti-bacterial and antifungal activity.

MATERIALS AND METHODS

SYNTHESIS OF COMPOUNDS: PREPARATION OF 2-MERCAPTO BENZIMIDAZOLE (1A):

A mixture of o-phenylenediamine (0.1 mol), potassium hydroxide (0.1 mol) and carbon disulfide (0.1 mol), 100 ml of 95% ethanol and 15 ml of water in a 500 ml round bottom flask were heated under reflux for 3 h. Then added 1-1.5 gm of charcoal cautiously and the mixture was further heated at the reflux temperature for 10 min, the charcoal was removed by filtration. The filtrate was heated to 60-70 ^{o}C, 100 ml of warm water was added and acidified with dilute acetic acid with good stirring. The product separated as glistering white crystals and the mixture is placed in a refrigerator for 3 h to complete the crystallization. The product was collected on a Buckner funnel and dried over night at 40 ^{o}C. The dried product is recrystalized by ethanol, the yield was (73%) and melting point was 300-302 ^{o}C. The Compound **(1b)** can be prepared by using 4-methyl o-phenylenediamine by same procedure as mentioned above. The yield was (75 %) and melting point was 290-292 ^{o}C.

PREPARATION OF (1*H*-BENZIMIDAZOL-2-YLTHIO) ACETIC ACID (2A):

Into a 250 ml round bottomed flask introduced a mixture containing 2-mercapto benzimidazole (0.013 mol), 20 ml of ethanol, potassium hydroxide (0.016 mol). The reaction mixture was refluxed at 78-80 ^{o}C for 1 h. After cooling the resulting solution to 30 ^{o}C added chloro acetic acid (0.012 mol) in one portion, an exothermic reaction set in causing a temperature rise from 30-40 ^{o}C. After stirring at 25-30 ^{o}C for 18 h, the reaction mixture was added to 100 gm of ice-water and stirred for 30 min at 0-10 ^{0}C. The obtained precipitate was collected by filtration, washed with water until free of chloride, air dried at 50 ^{o}C and

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recrystalized from water. The yield was (79 %) and melting point was 206-208 ^{o}C. The Compound **(2b)** was prepared by using 5-methyl- 2-mercapto benzimidazole by same procedure as mentioned above. The yield was (74 %) and melting point was 214-216 ^{o}C.

PROCEDURE FOR THE PREPARATION OF MANNICH BASES ($3A_1$):

In to 100 ml clean and dry round bottom flask introduced benzimidazolyl thio acetic acid (**2a**) (0.002 mol) dissolved in sufficient quantity of ethanol and 3-4 drops of conc. HCl was added and reaction mixture was kept for stirring with help of magnetic stirrer. To the stirring reaction mixture formaldehyde (0.002 mol) was added drop wise and stirring was continued for 10 min. Meanwhile in another 100 ml beaker 3-amino-1,2,4-triazole (0.002 mol) was dissolved in sufficient quantity of ethanol and was added into the above reaction mixture drop wise with continuous stirring, further stirring was continued for 15-20 min. After stirring the reaction mixture was refluxed for 12 h. The mixture was transferred into 100 ml beaker and allowed to cool at room temperature. The solid thus separated was filtered and dried. The obtained product **($3a_1$)** was recrystallised from ethanol. The yield was (56 %) and melting point was 256-258 ^{o}C. The other Mannich bases of this series i.e. **($3a_2$-$3a_{12}$)** were prepared by using same procedure as above and the data was given in table 1 and 2.

EXPERIMENTAL:

Melting points were determined by using Toshniwal apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel G plates using chloroform: ethyl acetate (7:3) as solvent system and U.V lamp used as a visualizing agent.

IR spectra were recorded using KBr pellets on a Shimadzu 8000 series and Jasco FT/IR 5300 Series spectrophotometer. ^{1}HNMR spectra on a Varian EM-200, Avance 200 MHz spectrophotometer using DMSO-d_6 and $CDCl_3$ as solvent and TMS as internal standard (chemical shift values expressed in ppm). Mass spectra were recorded on a Shimadzu 2010A series spectrophotometer by LC-MS method. And the results are reported in table 3

ANTIBACTERIAL ACTIVITY[11]:

The antibacterial activity of the synthesized compounds was determined by disc diffusion method. The organism chosen were *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli* and *Pseudomonas aeruginosa,* which are the representative types of gram positive and gram-negative organisms respectively. The concentration of the test compounds was 100μg/ml.ciprofloxacin was used as a standard drug. The results are reported in table 4

ANTIFUNGAL ACTIVITY[11]:

The anti-fungal activity of all compounds was determined by disc diffusion method on potato dextrose agar medium against *Aspergillus niger* and *Candida albicans*, Clotrimazole 100 μg/ml was used as a standard and DMF was used as control. And the results are reported in table 5.

RESULTS AND DISCUSSION:

From the literature survey it revealed that novel benzimidazole have been reported for number of pharmacological and biological activities and some molecules have shown significant activities and some compounds shows moderate and good activities. Here we have synthesized some novel benzimidazole analogues and screened them for their anti-bacterial and also for anti-fungal activities.

(A) ANTIBACTERIAL ACTIVITY:

The resulted synthesized compounds ($3a_1$-$3a_{12}$) were screened for antibacterial activity studies at a concentration of 100 μg/ml using DMF as a control against *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli* and *Pseudomonas aeruginosa* by disc diffusion method on agar nutrient media. Ciprofloxacin was used as standard drug for the comparison at the concentration of 100 μg/ml against Gram (+ve) and Gram (-ve) bacteria used for the study. The data in table 4 indicates that the compounds were found to possess moderate to weak activity although several benzimidazoles were reported for good antibacterial activity. The compound **$3a_1$** was active against *Pseudomonas aeruginosa*, and the compounds **$3a_1$, $3a_2$** and **$3a_3$** were active against *Bacillus subtilis,* whereas the compounds **$3a_2$** and **$3a_3$** were active against *Bacillus pumilus,* rest of the compounds showed only weak activity when compared to the standard ciprofloxacin.

(B) ANTIFUNGAL ACTIVITY:

All the synthesized compounds were screened for antifungal activity studies at a concentration of 100 μg/ml using DMF as a control against *Aspergillus niger* and *Candida albicans* on potato dextrose agar media. Clotrimazole 100 μg/ml is used as standard. The data in table 5 indicates that compound **$3a_4$** shown significant activity against *Candida albicans* rest of the compounds exhibited weak activity against *Candida albicans.* The

compounds **$3a_2$** and **$3a_{12}$** were also shown significant activity against *Aspergillus niger* and rest of the compounds showed weak activity against *Aspergillus niger.*

CONCLUSION:

From the anti-bacterial screening it was found that the compounds showed moderate to weak activity. The compounds were found to possess good activity against some of the organisms used for the study, rest of the compounds were found to exhibit weak activities when compared to standard Ciprofloxacin, which produced maximum zone of Inhibition.

In antifungal activity screening, when compared to standard Clotrimazole, the synthesized compound showed minimum antifungal activity against *Candida albicans*. The above results establish the fact that the substituted benzimidazole can be studied further to search for new antibacterial compounds.

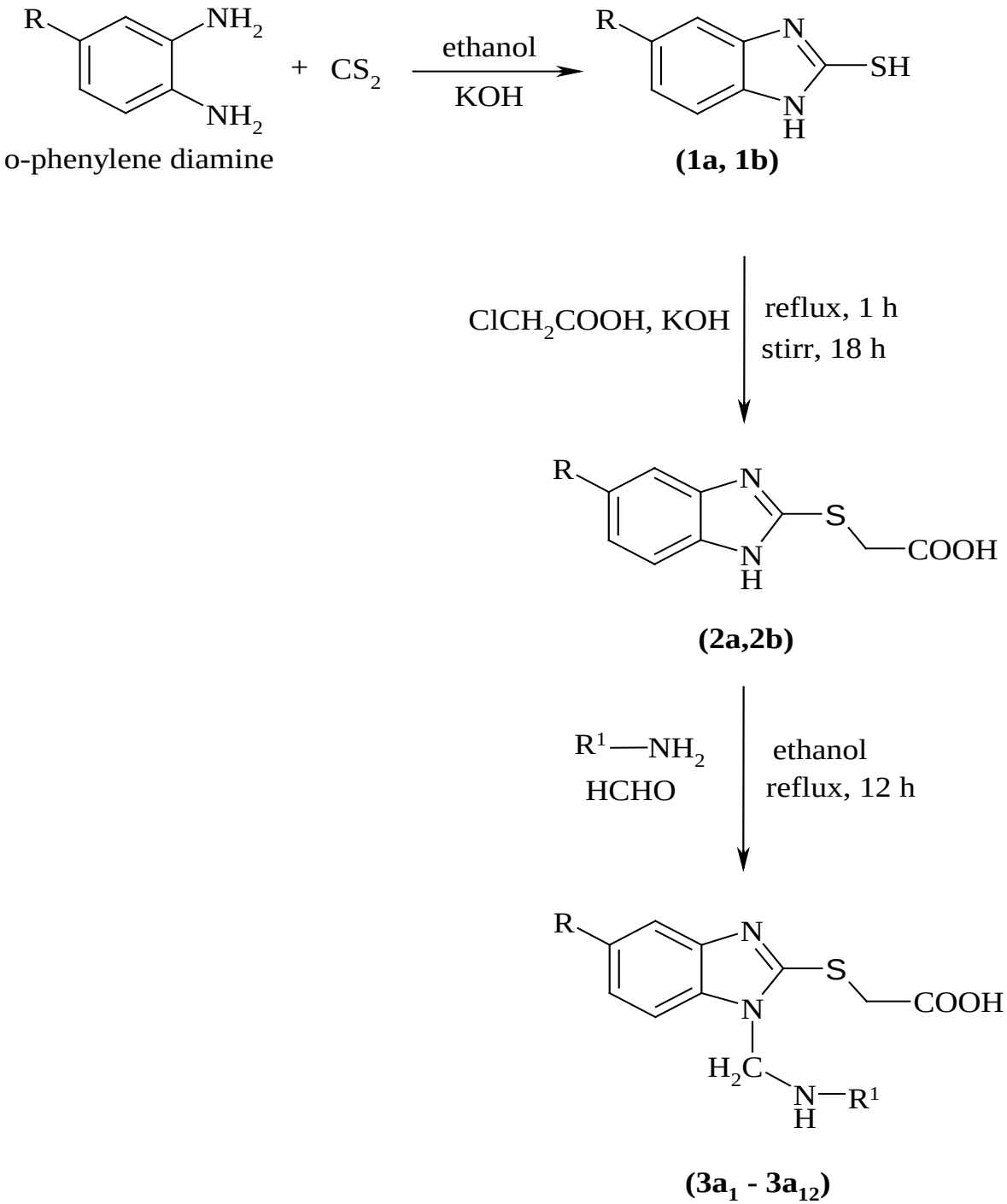
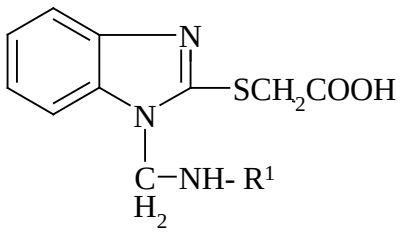


Figure No. 1: SCHEME



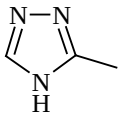
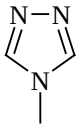
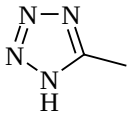
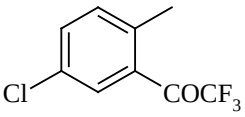
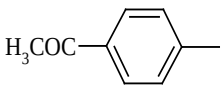
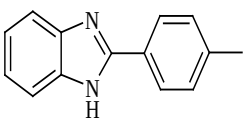
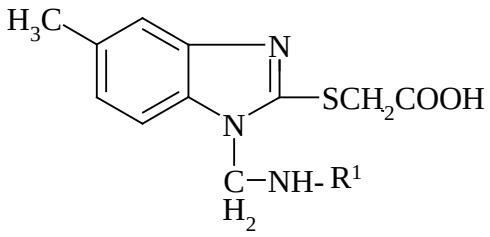
Compound Code	R^1	Molecular Formula	Molecular Weight	Melting Point (°C)	Yield %
$3a_1$		$C_{12}H_{12}N_6O_2S$	304	256 - 258	56
$3a_2$		$C_{12}H_{12}N_6O_2S$	304	224 - 226	48
$3a_3$		$C_{11}H_{11}N_7O_2S$	305	234 -236	54
$3a_4$		$C_{18}H_{13}N_3O_3SClF_3$	444	276 -278	57
$3a_5$		$C_{18}H_{17}N_3O_3S$	355	203-205	49
$3a_6$		$C_{23}H_{19}N_5O_2S$	429	268-270	54

Table No. 1: Physical characterization of the synthesized compounds ($3a_1$-$3a_6$).



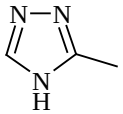
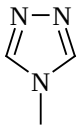
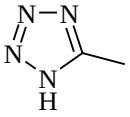
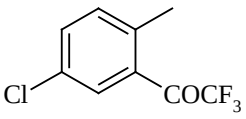
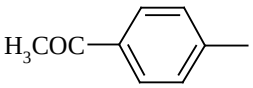
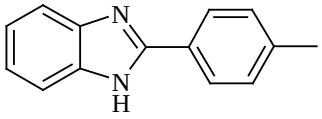
Compound Code	R^1	Molecular Formula	Molecular Weight	Melting Point (^oC)	Yield %
$3a_7$		$C_{13}H_{14}N_6O_2S$	318	182 - 184	52
$3a_8$		$C_{13}H_{14}N_6O_2S$	318	132 - 134	40
$3a_9$		$C_{12}H_{13}N_7O_2S$	319	174 -176	55
$3a_{10}$		$C_{19}H_{15}N_3O_3SClF_3$	458	252 -254	51
$3a_{11}$		$C_{19}H_{19}N_3O_3S$	369	183-185	50
$3a_{12}$		$C_{24}H_{21}N_5O_2S$	443	185-187	48

Table No. 2: Physical characterization of the synthesized compounds ($3a_7$-$3a_{12}$).

Sr. No	Compound	Mass spectra	IR spectra(KBr)	NMR Spectra (in DMSO)
1	2a	Mol wt.208 m/z = 209	3153(OH),3144(NH), 2980&2917(CH=CH), 1675(C=O)	4.1(sCH_2 of CH_2COOH),7.0-7.4(mArH),12.5(sOH of COOH),12.7(sH of NH)
2	2b	Mol wt. 222 m/z = 223	3114(NH),2980 & 2917(Ar-CH),1680(C=O)	2.3(dH of CH_3),4.1(sH of CH_2COOH),6.9-7.3(mH of Ar) 12.4(sH of NH)
3	$3a_1$	Mol wt. 304 m/z = 305	3180(NH),2950 &2870(Ar-CH=CH),1678(C=O)	3.9-4.0(dH of CH_2COOH), 4.8(dH of N-CH_2-NH), 6.4-7.3(H of Ar,NH),7.6(sH of NH), 9.6(sH of OH)
4	$3a_7$	Mol wt. 318 m/z = 319	3115(NH), 2950 & 2820(Ar CH=CH),1732(C=O), 1604(CH=N)	2.3-2.4(dH of CH_3), 4.2(dH of CH_2COOH), 5.6(mH of N-CH_2-NH), 6.9-7.4(mH of Ar-H,NH) 8.1-8.3(sH of NH), 12.4(sH of OH)

Table No. 3: Spectral data of representative synthesized compounds

Sample Code	*Inhibition of zone diameter in mm			
	B.subtilis	***B.pumilus***	***E.coli***	***P.aeruginosa***
	100 μg/ml	100 μg/ml	100 μg/ml	100 μg/ml
$3a_1$	**17**	**12**	8	**16**
$3a_2$	**19**	**17**	12	10
$3a_3$	**18**	**19**	11	14
$3a_4$	9	6	10	12
$3a_5$	8	7	9	10
$3a_6$	12	9	5	13
$3a_7$	5	10	8	12
$3a_8$	7	6	6	14
$3a_9$	10	6	13	9
$3a_{10}$	9	7	5	8
$3a_{11}$	6	8	8	8
$3a_{12}$	8	8	7	9
Standard Ciprofloxacin	28	30	32	30
DMF	-	-	-	-

*(n=3) Where: '-'denotes no activity, 8-12 mm poor activity, 13-17 mm moderate activity, 18-20 and above good activity

Table No. 4: Anti-bacterial activity of synthesized compounds ($3a_1$-$3a_{12}$).

Sample Code	Inhibition zone diameter in mm	
	A.niger	*C.albicans*
	100 μg/ml	100 μg/ml
$3a_1$	6	8
$3a_2$	13	12
$3a_3$	7	8
$3a_4$	10	14
$3a_5$	7	6
$3a_6$	8	7
$3a_7$	6	5
$3a_8$	7	6
$3a_9$	5	9
$3a_{10}$	6	8
$3a_{11}$	7	8
$3a_{12}$	13	8
Standard Clotrimazole	26	28
DMF	-	-
Where " NI" denote no activity, 06 – 08 mm poor activity, 08 – 12 mm moderate activity, 12-15 mm good activity.		

Table No 5: Antifungal activity of synthesized compounds ($3a_1$-$3a_{12}$).

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