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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF SOME NOVEL DERIVATIVES OF 2-MERCAPTOBENZIMIDAZOLE AS POSSIBLE ANTIMICROBIAL AGENTS

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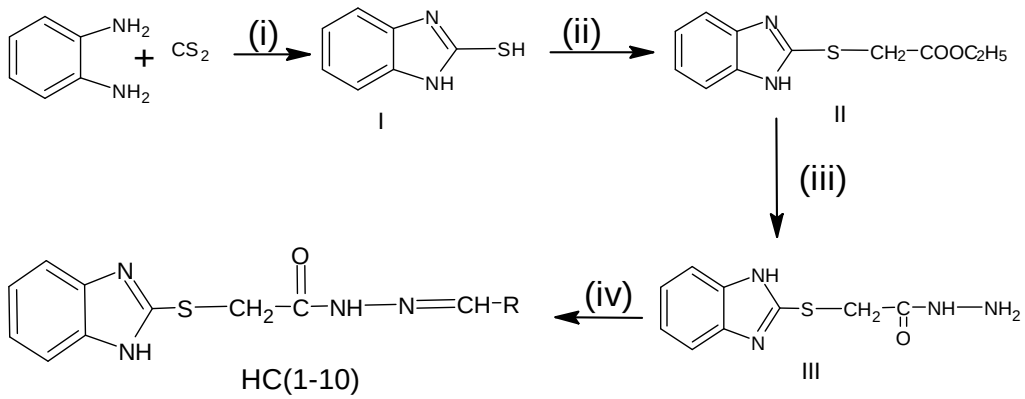
Abstract: We report a novel scaffold of 2-substituted mercaptobenzimidazole derivatives designed as a potent antimicrobial activity. Ten derivatives were synthesised, spectral characterized and evaluated for their antimicrobial and antifungal activity conducted by disc diffusion method. The synthesized derivatives HC-3, HC-5, HC-7, HC-8, HC-10 exhibited poor activity at 50 µg/ml, but at 100 µg/ml they have shown moderate activity against *S.aureus*, and moderate activity against *E.coli* whereas HC-3, HC-5, HC-7, HC-8, and HC-10, showed highest degree of inhibition at 250µg/ml and 500µg/ml against *C.albicans* when compared with standard one.

Index terms: mercaptobenzimidazole, degree of inhibition, antimicrobial and antifungal

I. INTRODUCTION:

The need of new anti-microbial agents is justified because more microorganisms are being resistance to the present drugs available in the market. Word wide researchers are trying to synthesize new drugs with better pharmacokinetic and dynamic properties with less adverse effects. The development of resistance to current anti-bacterial therapy continues to stimulate the search for more effective agents. Exclusive literature survey revealed that the benzimidazole have proved to be good bioactive molecules. They have shown diversified activities like anti-bacterial, anti-fungal, anti-inflammatory, anti-tubercular, anticonvulsant, anti-HIV, cardiac stimulant, diuretic and anticancer etc [1-8]. Therefore in view of above facts it was thought of interest to synthesize some benzimidazole derivatives. Herein, we report the synthesis, spectral characterization of some novel 2-substituted mercaptobenzimidazole derivatives and their antibacterial and antifungal evaluation.

II EXPERIMENTAL [8-13]:



Scheme 1. Synthesis of 2-substituted mercaptobenzimidazole derivatives, Reagents and conditions: (i) KOH, C_2H_5OH, (ii) Ethyl chloro acetate, KOH (iii) $NH_2NH_2.H_2O$, C_2H_5OH (iv) R-CHO, acetic acid, $ZnCl_2$.

Procure require chemicals, analytical grade solvents, reagents and materials from Sigma Aldrich and Germany, USA and E. Merck, India. Melting points (mp) were detected with open capillaries using Precision Melting point and are uncorrected. Chromatography of the synthesized intermediates and title compounds were performed on silica gel pre-coated plates (Merck: 100–200 mesh) by using chloroform and ethanol as developing solvents, and the spot were detected by UV light absorption. . IR spectra (KBr) were recorded on FTIR-8400s spectrophotometer (Shimadzu, Japan). ^{1}H NMR was obtained using a Bruker Advance-II 400 Spectrometer on 400 MHz using tetramethylsilane (TMS) as internal standard. All chemical shift values were recorded as δ (ppm), coupling constant value J is measured in hertz, the peaks are presented as s (singlet), d (doublet), t (triplet), br s (broad singlet), dd (double doublet), m (multiplet). The purity of compounds was controlled by thin layer chromatography (Merck, silica gel, HF254-361, type 60, 0.25 mm, Darmstadt, Germany).

Step-1: Preparation of 2-mercapto benzimidazole (I):-

Take a mixture of 0.1mole (10.8gm) of o-phenylenediamine, 5.65 gm (0.1mole) of potassium hydroxide and 7.67 gm (0.1mole, 6.19ml) of carbon disulfide, 100 ml of ethanol and 15 ml of H_2O are mixed well in a RBF and reaction mixture is heated with reflux condenser for 3 hrs. further add charcoal (1-1.5gm) with cautiously and the resulting mixture was further heated with reflux condenser for 10 mins, the charcoal was removed by filtration. The filtrate was heated to 60-70^{o}C, 100ml of warm water is added, and acidified with dilute acetic acid with stirring. The resulting product separated as glistening white crystals, and the mixture is placed in a refrigerator for 3 hrs to complete the crystallization. The product is collected on a Buckner funnel and dried over night at 40^{o}c. The dried product of 2-mercapto benzimidazole was collected and recrystallized with ethanol. Yield: 73% mp: 300-305^{o}C.

Step-2: Preparation ethyl (1H-benzimidazol-2-ylsulfanyl) acetate (II):-

A stirred reaction mixture containing Compound-I 4.5gm (0.03mole), ethanol 60ml and potassium hydroxide 1.68gm (0.03mole) was added and heated at 78-80^{o}C for 10-minutes. Then Ethyl Chloro Acetate (3.66ml, 0.03mole) was added in one portion, an exothermic reaction set in causing a temperature rise from 30-40^{o}C. After stirring at 25-30^{0}c for 18-hrs, the reaction mixture was added to 100gm of ice-water and stirred for

30-minutes at 0-10^{0}C. The precipitate was collected by filtration washed with water until free of chloride and air dried at 50^{0}c and recrystallized by water. Yield: 62% mp: 102-105^{0}C.

Step-3: Preparation of 2-(1H-benzimidazol-2-ylsulfanyl) acetohydrazide (III):-

The mixture of compound-II 2gm (0.004mole) and hydrazine hydrate 3ml (0.01mole) were mixed well in a RBF and heated on water bath for 10 min. then dissolved above mixture in 60 ml ethanol, mixed well in a RBF and reaction mixture is heated with reflux condenser for next 6 hrs, cooled to RT and the reaction mixture was added to 100gm of ice-water, and kept aside for the crystallization. The colorless crystals of 2-(1H-benzimidazol-2-ylsulfanyl)acetohydrazide was collected by filtration, and recrystallised from water. Yield: 60-70% mp: 180-185^{0}C.

Step-4: Preparation of Schiff bases Compound (HC 1-10):-

A equimolar mixture of compound-III (0.009 mol, 2gm) was dissolved in ethanol (10ml) and to this solution add in equimolar qty of substituted aldehydes (0.009mol, 0.917) with 4-6 drops of glacial acetic acid was added, this reaction mixture is kept under reflux for 8 hrs. After cooling to RT was added to ice cold water. Compound gets separated as solid filtered, dried and recrystallized with chloroform.

4.1 HC-1: 2-[(1*H*-benzimidazol-2-yl)sulfanyl]-*N*'-[(*Z*)-(3-methylphenyl)methylidene]acetohydrazide-
Yield: 69%. mp: 245-250^{0}C (ethanol). IR (KBr) cm^{-1}: 3340, 1330 (-NH-), 1728 (>C=O), 1612 (-C=N-), 730 (C-S-C). ^{1}H NMR (DMSO-d6, 400 MHz) δ: 2.38 (s, 1H, CH3), 4.48 (s, 2H, S-CH2), 7.01-7.97 (m, 8H, Ar-H), 8.0 (s, 1H,N=CH), 9.53 (s, 1H, NH-N), 11.38 (s, 1H, NH of benz). HRMS (EI) m/z calcd for C17H16N4OS 324, found 325.60. Anal: C (62.94), H (4.97),N (17.27).

4.2 HC-2: 2-[(1*H*-benzimidazol-2-yl)sulfanyl]-*N*'-[(*Z*)-phenylmethylidene]acetohydrazide
Yield: 62%. mp: **220-225^{0}C** (ethanol). IR (KBr) cm^{-1}: 3178 (-NH-), 1353 (-C=O), 1679, 1612 (-C=N-), 680 C-S-C ^{1}H NMR (DMSO-d6, 400 MHz) δ: 2.38 (s, **1H, CH3**), 4.5 (s, **2H, S-CH2**), **7.01-8.0** (m, **8H, Ar-H**), 8.3 (s, **1H,N=CH**), **11.7** (s, **1H, NH-N**), **12.5** (s, 1H, NH of benz). HRMS (EI) m/z calcd for $C_{16}H_{14}N_4OS$ 310. Anal: C (61.92), H (4.55),N (18.05).

4.3 **HC-3:** 2-[(1*H*-benzimidazol-2-yl)sulfanyl]-*N*'-[(*Z*)-(4-(dimethylamino)methylidene]acetohydrazide-
Yield: 71%. mp: 248-252^{0}C (ethanol). IR (KBr) cm^{-1}: 3290 (-NH-), 1670 (>C=O), 1617 (-C=N-), 670 (C-S-C). ^{1}H NMR (DMSO-d6, 400 MHz) δ: 2.9-3.1 (s, 6H, $N(CH_3)_2$), 4.80 (s, 2H, S-CH2), 6.70-7.70 (m, 8H, Ar-H), 8.0 (s, 1H,N=CH), 9.50 (s, 1H, NH-N), 11.7 (s, 1H, NH of benz). HRMS (EI) m/z calcd for $C_{18}H_{19}N_5OS$ 353 found 354.53. Anal: C (61.17), H (5.42),N (19.81).

4.4 **HC-4:** 2-[(1*H*-benzimidazol-2-yl)sulfanyl]-*N*'-[(*Z*)-(furan-2-yl)methylidene]acetohydrazide
Yield: 78%. mp: 230-235^{0}C (ethanol). IR (KBr) cm^{-1}: 3333, 1341 (-NH-), 1676 (>C=O), 1618 (-C=N-), 687 (C-S-C). ^{1}H NMR (DMSO-d6, 400 MHz) δ: 4 4.7 (s, 2H, S-CH2), 6.7 -7.70 (m, 7H, Ar-H), 8.0 (s, 1H,N=CH), 9.6 (s, 1H, NH-N), 11.9 (s, 1H, NH of benz). HRMS (EI) m/z calcd for $C_{14}H_{12}N_4O_2S$ 300. Anal: C (55.99), H (4.03),N (18.65).

4.5 **HC-5:** 2-[(1*H*-benzimidazol-2-yl)sulfanyl]-*N*'-[(*Z*)-(4-nitrophenyl) methylidene] acetohydrazide-
Yield: 63%. mp: 290-295^{0}C (ethanol), $C_{16}H_{13}N_5O_3S$ 355. Anal: C (54.08), H (3.69),N (19.71).

4.6**HC-6:**2-[(1*H*-benzimidazol-2-yl)sulfanyl]-*N*'-[(*Z*)-(2-methylphenyl)methylidene]acetohydrazide-
Yield: 71%. mp: 235-237^{0}C (ethanol), $C_{17}H_{16}N_4OS$ 324. Anal: C (62.94), H (4.67),N (17.27).

4.7 **HC-7:** 2-[(1*H*-benzimidazol-2-yl)sulfanyl]-*N*'-[(*Z*)-(4-methoxyphenyl)methylidene]acetohydrazide-

Yield: 79%. mp: 228-235^{0}C (ethanol). IR (KBr) cm^{-1}: 3340, 1330 (-NH-), 1728 (>C=O), 1612 (-C=N-), 730 (C-S-C). $C_{17}H_{16}N_4O_2S$ 340. Anal: C (59.98), H (4.74), N (16.46).

4.8 **HC-8:** 2-[(1*H*-benzimidazol-2-yl)sulfanyl]-*N*'-[(*Z*)-(2-bromophenyl)methylidene]acetohydrazide-

Yield: 63%. mp: 250-255^{0}C (ethanol). $C_{16}H_{13}BrN_4OS$ 389. Anal: C (49.37), H (3.37), N (14.36).

4.9 **HC-9:** 2-[(1*H*-benzimidazol-2-yl)sulfanyl]-*N*'-[(*Z*)-(3-phenylprop-2-en-1-ylidene]acetohydrazide-

Yield: 70%. mp: 205-208^{0}C (ethanol). $C_{18}H_{16}N_4OS$ 336. Anal: C (64.26), H (4.79), N (16.65).

4.10**HC-10:**2-[(1*H*-benzimidazol-2-yl)sulfanyl]-*N*'-[(*Z*)-(4-(dimethylaminophenylprop-2-en-1-ylidene]acetohydrazide-

Yield: 59%. mp: 253-255^{0}C (ethanol), $C_{20}H_{21}N_5OS$ 379. Anal: C (63.30), H (5.58),N (18.46).

III BIOLOGICAL EVALUVATION-

Antibacterial Activity: [14-15]

The compounds were tested in-vitro for their antibacterial activity against two microorganisms viz. *Escherichia coli* (NCTC 10418), and *Staphylococcus aureus* (NCTC 6571) which are pathogenic in human beings.

Method: Disc Agar diffusion method using Mueller-Hinton agar using *E.coli,* and *S. aures*.

Antifungal Activity: [16-17]

The compounds were tested in-vitro for their antifungal activity against *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16).

Method: Cup-Plate agar diffusion method using Sabouraud-dextrose agar using *C.albicans* and *A.niger*.

IV RESULTS & DISCUSSION

The 2- substituted mercapto benzimidazole derivatives **(HC-1 to HC-10)** were synthesised as described in scheme 1, starting with o-phenylenediamine, potassium hydroxide and carbon disulfide to obtain 2-mercapto benzimidazole **I**, treated with ethanol, potassium hydroxide was added and heated at 78-80ºC for 10-minutes then add ethyl chloro acetate to obtain ethyl (1H-benzimidazol-2-ylsulfanyl) acetate **II. C**ompound-II was trated with hydrazine to gives colorless crystals of 2-(1H-benzimidazol-2-ylsulfanyl)acetohydrazide **III**. Treatment with different substituted aldehydes gave ten dertivatives of substituted 2-mercapto benzimidazole **(HC-1 – HC-10).** All the final derivatives were tested for their antibacterial activity by using DMF as a solvent against the organisms, *S.aureus* and *E.coli*. and antifungal activity using *Candida albicans* by disc diffusion method on nutrient agar media.

The antimicrobial screening results presented on above table 1 reveals that compounds **HC-3, HC-5, HC-7, HC-8, HC-10** exhibited poor activity at 50 µg/ml, but at 100 µg/ml they have shown moderate activity against *S.aureus*, and moderate activity against *E.coli*. The compounds **HC-1, HC-2, HC-4, HC-6, HC-9** have shown the poor activity against *E. Coli* and *S.Aureus* at 50mg. but the same compounds at 100 µg/ml against same organism have shown moderate activity. and HC-**5, HC-6, HC-7, HC-8, HC-10** have shown the very good activity against *S.aureus* at 100 µg/ml when compared with the ampicillin as a standard one. The synthesized dertivatives **HC-3, HC-5, HC-7, HC-8, HC-10** have shown good anti bacterial activity due to the presence of electron donating group **OCH3, N(CH3)2 , CH3** group which is attached at 4 fourth position of the phenyl ring system and the compounds **HC-5** and **HC-8** may be due to the presence of

electron withdrawing group like **NO2, Br** are attached at the second and fourth position of the phenyl ring system.

Whereas anti-fungal activity against *Candida albicans* presented on table 2 reveled that the compounds HC-**3, HC-5, HC-7, HC-8**, and **HC-10**, Showed highest degree of inhibition at 250µg/ml and 500µg/ml against *C.albicans* when compared with Ketoconazole as a standard one. The anti fungal activity shown that **HC-3, HC-5, HC-7, HC-8, HC-10** have shown good antifungal activity it also may be due to the presence of electron donating group **OCH3, N(CH3)2 , CH3** group which is attached at 4 fourth position of the phenyl ring system and electron withdrawing group like **NO2, Br** are attached at the second and fourth position of the phenyl ring system. However the activities shown by all the compounds tested were less than that of the standard one.

Table no 1 -Anti-bacterial activity data of synthesized benzimidazole

Sr.No	compound	Concentration µg/ml	E.coli	S.Aureus
1	HC-1	50	7	9
		100	10	10
2	HC-2	50	7	7
		100	7	10
3	HC-3	50	8	9
		100	11	9
4	HC-4	50	6	7
		100	7	8
5	HC-5	50	8	8
		100	12	12
6	HC-6	50	7	11
		100	9	15
7	HC-7	50	8	12
		100	14	14
8	HC-8	50	8	9
		100	12	13
9	HC-9	50	8	8
		100	10	10
10	HC-10	50	8	12
		100	12	14
11	Ampicillin	50	24	25
		100	25	25

Zone of inhibition of synthesized compounds:

Note: 6-8 mm poor activity, 9-11 mm moderate activity, 12-15 above good.

Table no: 2-Anti-fungal activity data of synthesized benzimidazole

Sr.No	Compound	Concentration µg/ml	Candida albicans
1	HC-1	250	+
		500	-
2	HC-2	250	-
		500	-
3	HC-3	250	-
		500	-
4	HC-4	250	+
		500	-
5	HC-5	250	-
		500	-
6	HC-6	250	-
		500	-
7	HC-7	250	-
		500	-
8	HC-8	250	-
		500	-
9	HC-9	250	+
		500	-
10	HC-10	250	-
		500	-
11	Ketoconazole	250	-
		500	-

Note: (-) No growth, (+) Growth, (Keto) Ketoconazole.

V CONCLUSION

We have synthesised and spectral characterization of ten 2-substituted -mercapto benzimidazole derivatives derivatives and evaluated for their antibacterial activity and antifungal activity. Compounds HC-**5, HC-6, HC-7, HC-8, HC-10** have shown the very good activity against *S.aureus* at 100 µg/ml and HC-**3, HC-5, HC-7, HC-8**, and **HC-10**, Showed highest degree of inhibition at 250µg/ml and 500µg/ml against *C.albicans* when compared with Ketoconazole as a standard one. This study needs further evaluation for their physicochemical properties, which would be reported in future.

VI CONFLICT OF INTEREST:

None to declare

VII ACKNOWLEDGEMENT:

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