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SYNTHESIS AND EVALUATUION OF SOME NOVEL MERCAPTOBENZIMIDAZOLE DERIVATIVES

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ABSTRACT

A new class of potentially biological active mercaptobezimidazole derivatives containing Benzimidazole moiety has been synthesized. New series of acids and aldehyde substituted benzimidazoles **A6–10** were synthesized by the Schiff's Base method. Synthesized compounds **A6–10** were confirmed by IR, ^{1}H NMR, Mass and elemental analysis and further screened for antimicrobial activity and antiprotozoal activity. The in vitro antibacterial and antifungal potential of the products were also determined using bacterial strains.(*E.coli* ATCC 25922, *S.Aureus* ATCC 29213, *P.aeruginosa* MTCC741, *C.albicans*ATCC 9025, *A.niger*ATCC 1015) The synthesized compounds exhibited significant antimicrobial & antiprotozoal activities compared to standard compounds.

Keywords: 5,6-dinitro, Schiff's Base, Antibacterial activity, Antifungal activity, Antiprotozoal activity.

INTRODUCTION

Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications[1]. This ring system is present in numerous antiparasitic, fungicidal, anithelemintic and anti-inflammatory drugs[2–5]. The first report on antibacterial activity of halogene containing benzimidazoles was published in 1964[6]. It is also known that 5,6-dinitrobenzimidazole can substitute 5,6-dimethylbenzimidazole in the vitamin B_{12} molecule in *Corynebacterium diphteriae* [7] and 2-trifluorobenzimidazoles are potent decouplers of oxidative phosphorylation in mitochondria. They are also inhibitors of photosynthesis, and some exhibit appreciable herbicidal activity[8]. Great interest is also raised by antimicrobial activity of 2- S-substituted benzimidazoles carrying additional functional groups on the benzene part of the heterocyclic core. This is because of most recent studies that revealed their anti-*Helicobacter pylori* and anti-*Mycobacterium tuberculosis* activities[9,10]. However, the antibacterial and antiprotozoal properties of this group of benzimidazoles have not been extensively studied [11]. The earliest report of their antibacterial activity appeared in 1964 [12], and more recently we have found two groups of substituted benzimidazoles, namely the 5,6-dinitro and 2-trifluoromethyl derivatives, to be promising candidates for antimicrobial drugs [13]. These evidences boosted us to carry out synthetic work for the titled compounds and evaluate their antimicrobial & antiprotozoal potential. The main objectives of studies are; To evaluate potent antimicrobial & antiprotozoal activity, To find out which functional group is responsible for antiprotozoal activity.

MATERIALS AND METHOD

All melting points were determined in open capillary tube and are uncorrected. Infrared spectra were recorded in KBr on Shimadzu 8700 spectrophotometer. The 1H NMR spectra were measured in dimethyl sulfoxide-d6 or $CDCl_3$ solutions on a Brucker 400 MHz spectrometer using TMS as an internal reference (chemical shift in d ppm). The mass spectra were recorded on LC–MS-Agilent 1100 series and API 2000 LC/MS system. Elemental analyses were performed on a Flash EA 1112 series CHNS–O analyzer. The completion of

the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminum sheets (Silica Gel 60 F254). Commercial grade solvents and reagents were used without further purification.

Synthesis of 2-Mercapto benzimidazole (A2): A mixture of 10.8 gm (0.1 mole) of o-phenylenediamine, 5.65 gm (0.1 mole) of potassium hydroxide and 7.67 gm (0.1 mole, 6.19 ml) carbon disulphide, 100 ml of 95% ethanol and 15 ml of water in 500 ml of round bottom flask were heated under reflux for 3 hr. Then added 1.15 gm of charcoal cautiously and then mixture was further heated at the reflux for 10 minutes, the charcoal was removed by filtration. The filtrate was heated to 60-70^0 C, 100 ml of warm water was added and acidified with dilute acetic acid with good stirring. The product separated as glinting white crystals, and the mixture was placed in a refrigerator for 3hr to complete crystallization. The product was collected on a Buckner funnel and dried over night at 40^{0}C. m.p. 300-302^{0}C; IR (KBr) (cm-1): 1622 cm-1 (C-N str), 3454 cm-1 (NH str), 700 cm-1 (C-S str), 1177 cm-1 (C-N str), 3180 cm-1 (C-H str aromatic); ^{1}H NMR (DMSO, 200 MHz) δ (ppm): 2.211 (1H, singlet, NH), 7.366-7.383 (3H, multiplet, benzene). m/z -158. Elemental analysis: Calculated for C, 61.62; H, 2.56; N, 8.11%; Found: C, 62.32; H, 2.67; N,84.35%.

Synthesis of N-[(1H-benzimidazol-2-ylsulfanyl)methyl]-N-ethylethanamine (A3): An equimolarquantities (0.01 mol) of 2-Mercaptobenzimidazole and the respective compound having secondary amine i.e, diethylamine were dissolved in (30ml) methanol, a beaker under perfect ice-cold condition and stirred constantly. To this solution, formaldehyde (0.01 mol) was added slowly and heated to reflux for 3 hr. The mixture was kept overnight in the freezer. The corresponding crystals of N-[(1H-benzimidazole-2-yl sulfanyl1)methyl]-N-ethylamine obtained was recrystallised from alcohol.
M.P. 228^{0}C; IR (KBr) (cm-1): 1600 cm-1 (C=O str), 1730 cm-1 (NH str), 731 cm-1 (C-S str), 1100 cm-1 (C-N str), 3150 cm-1 (C-H str aromatic);^{1}H NMR (DMSO, 200 MHz) δ (ppm): 2.228 (1H, singlet, NH), 7.366-7.383 (3H, multiplet, benzene). m/z -238. Elemental analysis: Calculated for C, 51.62; H, 1.56; N, 8.41%; Found: C, 52.42; H, 2.17; N,8.45%.

Synthesis of ethyl (2-{[(diethylamino)methyl]sulfanyl}-1H-benzimidazol-1-yl)acetate (A4): A mixture of equimolar alkaline solution (0.5 mL, 4 N NaOH) of N-[(1H-benzimidazol-2-ylsulfanyl)methyl]-N-ethylethanamine (0.01 mol, 1.18 g) in methanol (50 mL) and ethylchloroacetate (0.01 mol, 1 mL) in methanol (30 mL) was heated gently on boiling water bath for 0.5 hr.
M.P. 180^{0}C; IR (KBr) (cm-1): 1627 cm-1 (C=O str), 1701cm-1 (NH str), 738 cm-1 (C-S str), 1177 cm-1 (C-N str), 3150 cm-1 (C-H str aromatic);^{1}H NMR (DMSO, 200 MHz) δ (ppm): 2.235 (1H, singlet, NHCOR), 7.366-7.383 (3H, multiplet, benzene). m/z -235. Elemental analysis: Calculated for C, 71.62; H, 4.66; N, 8.51%; Found: C, 70.42; H, 3.47; N,8.52%.

Synthesis of Preparation of 2-(2-{[(diethylamino)methyl]sulfanyl}-1H-benzimidazol-1-yl)acetohydrazide (A5): To a solution of above compounds (0.01 mol) dissolved in ethanol (50 mL), 99% hydrazine hydrate (1 mL) was added, and the, mixture was refluxed for 4–5 h. The reaction mixture was cooled and, the solid obtained was filtered, washed with small quantity of cold methanol.
M.P. 279^{0}C; IR (KBr) (cm-1): 2808 cm-1 (C-H str), 1621cm-1 (NH str), 1738 cm-1 (C=O str), 1218 cm-1 (C-N str), 3158 cm-1 (C-H str aromatic)^{1}H NMR (DMSO, 200 MHz) δ (ppm): 2.231 (1H, singlet, NHCOR), 7.366-7.383 (3H, multiplet, benzene). m/z -281.
Elemental analysis: Calculated for C, 66.62; H, 2.16; N, 81.41%; Found: C, 65.24; H, 2.17; N,81.33%.

General Method for Preparation of 2(2{[((diethylamino) 5-substituted aryl 1, 3,4oxadiazol) methyl]sulfanyl}-1H-benzimidazole (A6-10): An equimolar mixture of compound 4 (0.001mol) and substituted carboxylic acid in phosphoryl chloride was refluxed for 10–16 h. Then reaction mixture was cooled, poured into ice-cold water and neutralized with 20% $NaHCO_3$ solution.[13]
A6: M.P. 365^{0}C; IR (KBr) (cm-1): 2998 cm-1 (C-H str), 1654cm-1 (NH str), 1721 cm-1 (C=O str), 1216 cm-1 (C-N str), 3155 cm-1 (C-H str aromatic) ^{1}H NMR (DMSO, 200 MHz) δ (ppm): 2.244 (1H, singlet, NHCOR), 7.366 (3H, multiplet, benzene). m/z –365. Elemental analysis: Calculated forC, 61.32; H, 2.33; N, 81.66%; Found: C, 61.24; H, 2.67; N,81.33%

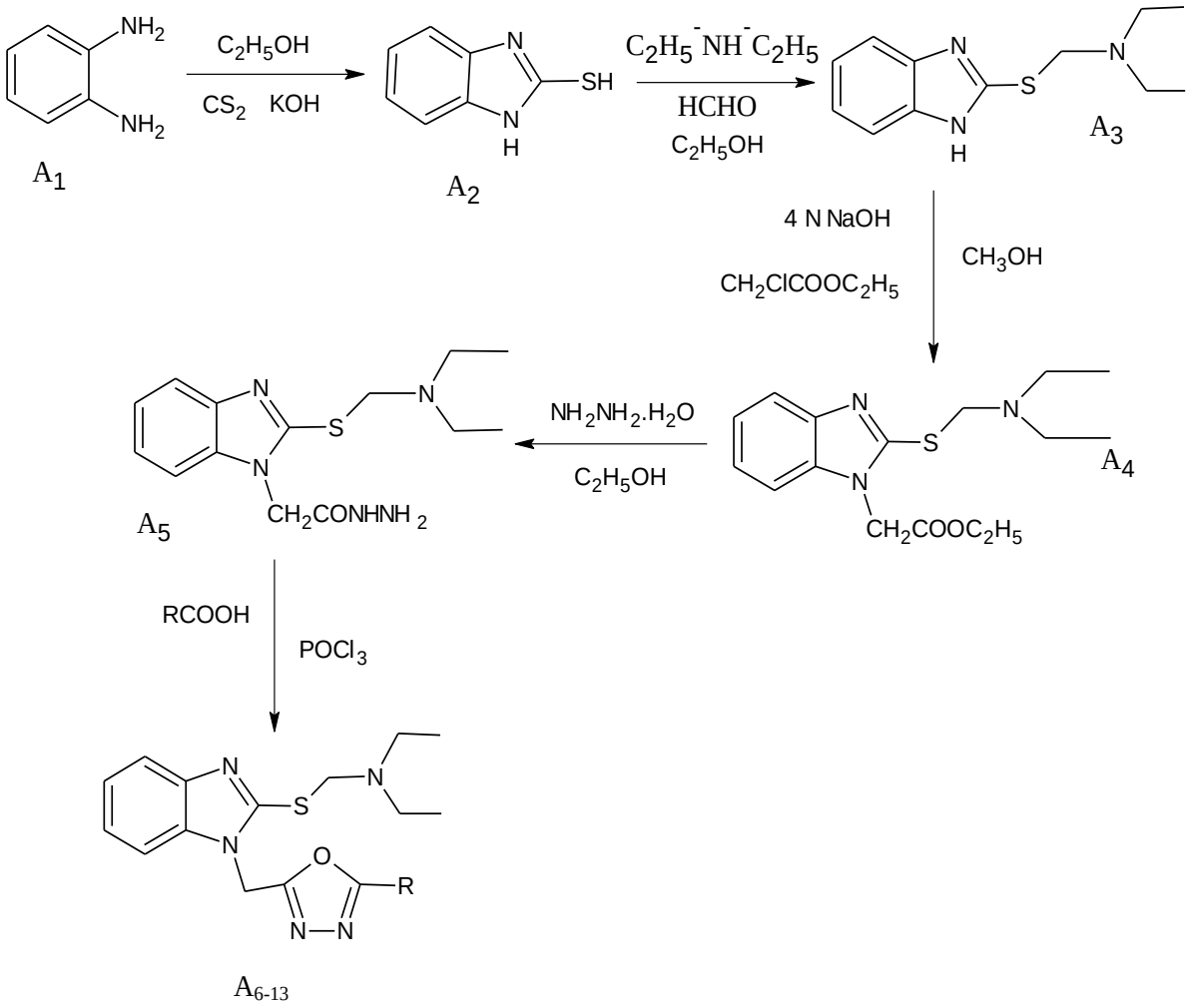
A7: M.P. 221^{0}C; IR (KBr) (cm-1): 2658 cm-1 (C-H str), 1443cm-1 (NH str), 1521 cm-1 (C=O str), 1266 cm-1 (C-N str), 3765 cm-1 (C-H str aromatic) ^{1}H NMR (DMSO, 200 MHz) δ (ppm): 2.112 (1H, singlet, NHCOR), 7.543 (3H, multiplet, benzene). m/z –471. Elemental analysis: Calculated for C, 61.32; H, 2.33; N, 81.66%; Found: C, 68.24; H, 2.77; N,82.23%.

A8: M.P.. 221^{0}C; IR (KBr) (cm-1): 2886 cm-1 (C-H str), 1554 cm-1 (NH str), 1521 cm-1 (C=O str), 1266 cm-1 (C-N str), 3765 cm-1 (C-H str aromatic) ^{1}H NMR (DMSO, 200 MHz) δ (ppm): 2.33 (1H, singlet, NHCOR), 7.583 (3H, multiplet, benzene). m/z –319. Elemental analysis: Calculated for C, 51.32; H, 4.33; N, 99.66%; Found: C, 51.24; H, 4.77; N, 99.23%.

A9: M.P. 154^{0}C;IR (KBr) (cm-1): 2765 cm-1 (C-H str), 1676cm-1 (NH str), 1731 cm-1 (C=O str), 1326 cm-1 (C-N str), 3355 cm-1 (C-H str aromatic) ^{1}H NMR (DMSO, 200 MHz) δ (ppm): 2.144 (1H, singlet, NHCOR), 7.366-7.324 (3H, multiplet, benzene). m/z –399. Elemental analysis: Calculated for C, 61.23; H, 2.55; N, 81.99%; Found: C, 61.24; H, 2.67; N,81.98%.

A10: M.P. 112^{0}C; IR (KBr) (cm-1): 2435 cm-1 (C-H str), 1656cm-1 (NH str), 1721 cm-1 (C=O str), 1356 cm-1 (C-N str), 3755 cm-1 (C-H str aromatic) ^{1}H NMR (DMSO, 200 MHz) δ (ppm): 2.1774 (1H, singlet, NHCOR), 7.546 (3H, multiplet, benzene). m/z –464. Elemental analysis: Calculated for; C, 61.43; H, 2.65; N, 82.33%; Found: C, 61.44; H, 2.67; N, 81.98%.

A11: M.P. 158 ^{0}C; IR (KBr) (cm-1): 2802 cm-1 (C-H str), 780 cm-1 (C-S str), 1738 cm-1 (C=O str), 1218 cm-1 (C-N str), 3078 cm-1 (C-H str aromatic). 1H NMR (DMSO, 200 MHz) δ (ppm): 2.1 (multiple, 1H), 12.122 (1H, singlet, COOH), 7.031-7.683 (10H, multiplet, benzene). m/z -471. Elemental analysis: Calculated for; C, 61.33; H, 2.65; N, 88.79%; Found: C, 61.44; H, 2.67; N, 86.78%.



(R as shown in Table 1)

Table No: 1 It represents substituents attached with its molecular formula & wt.

R	Yield (%)	Molecular Formula	Molecular Wt
$-C_6H_5$	72	$C_{19}H_{19}N_5OS$	365.45
$-C_6H_3N_2O_5$	80	$C_{19}H_{17}N_7O_6S$	471.44
$-C_8H_7$	78	$C_{21}H_{21}N_5OS$	391.48
$-C_6H_4Cl$	75	$C_{19}H_{18}ClN_5OS$	399.89
$-C_7H_5Cl_2O$	81	$C_{20}H_{19}Cl_2N_5O_2S$	464.36
$- C_6H_6N$	84	$C_{19}H_{20}N_6OS$	380.46
$-C_7H_7$	67	$C_{20}H_{21}N_5OS$	379.47
$- C_6H_6N$	81	$C_{19}H_{20}N_6OS$	380.46

Antibacterial activity: The antibacterial activity of synthesized compounds was evaluated by the agar well diffusion method. All the bacterial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a bacterial suspension of approximately 1.5×10^8 *cfu/ml*. 10 *mL* of nutrient agar medium was poured into each Petri plate and plates were swabbed with 100 μLinocula of the test microorganisms and kept for 10 to 15 *min* for adsorption. Using sterile cork borer of 8 to 10*mm* diameter, wells were bored into the seeded agar plates and these were loaded with a 100 μLvolume with concentration of 2.0 *mg* $mL^{-1}$of each compounds reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37ºC for 24 hrs. Antibacterial activity of each organotin complex was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi Antibiotic zone scale). DMSO was used as a negative control whereas Ciprofloxacin was used as positive control. This procedure was performed in three replicate plates for each organism.

Sr.No	**Compound**	**Zone of inhibition diameter in (mm)**		
		***E.coli* ATCC 25922**	***S.Aureus*ATCC 29213**	***P.aeruginosa*MTCC741**
1	A6	25	20	40
2	A7	37	28	24
3	A8	22	20	17
4	A9	30	32	29
5	A10	27	24	25
6	A11	28	22	20
	Standard	47	46	47
	Control	-	-	-

Table 2. Antibacterial activity of compounds **A6-A11**
Note: - 15-20 mm poor activity, 20-25 mm moderate activity, above 25 good activity.
Standard (S) = Ciprofloxacin Control (C) = DMF

Determination of minimal inhibitory concentration: The synthesized compounds were dissolved in DMF to prepare a stock solution of 1 *mg/ml* conc. with this stock solution different dilutions 800 μg to 5 $\mu g/ml$ were prepared. The ciprofloxacin was also prepared in DMF to obtain a conc. of 800 $\mu g/ml$ to 5 μg/ml. The sterile test tube containing 1 ml of sterile media were added with 1 ml of different serially diluted test samples. To these tubes 0.1 ml of normal saline solution suspended with respective microorganisms were inoculated and incubated at 37 ± 2^0C for 18 to 24 hrs. The growths in the tubes were observed visually for turbidity and inhibition was determined by lowest concentrations of sample that prevented the development of turbidity. The procedure was repeated to confirm the MIC.

Sr. No.	Compound code	*S.aureus* ATCC 29213	*E.coli* ATCC 25922	*Pseudomonas* aeruginosa MTCC 741
1	A6	200 μg/ml	50 μg/ml	200 μg/ml
2	A7	50 μg/ml	100 μg/ml	50 μg/ml
3	A8	100 μg/ml	200 μg/ml	100 μg/ml
4	A9	100 μg/ml	200 μg/ml	100 μg/ml
5	A10	200 μg/ml	400 μg/ml	50 μg/ml
6	A11	100 μg/ml	200 μg/ml	100 μg/ml

Table 3. Antibacterial activity of compounds **A6-A11** by minimum inhibitory concentration method

Antifungal activity: The antifungal activity of synthesized compound was evaluated by poisoned food technique. The molds were grown on potato-dextrose-agar medium at 25^{o}C for 7 days and used as inoculate. The 15 *mL* of molten potato-dextrose-agar medium (45^{o}C) was poisoned by the addition of 100 *μL*volume of each compounds having concentration of 2.0 *mg* mL^{-1}reconstituted in the DMSO, poured into a sterile Petri plate and allowed it to solidify at room temperature. The solidified poisoned agar plates were inoculated at the center with fungal plugs (8mm diameter) obtained from the colony margins and incubated at 25^{o}C for 7 days. DMSO was used as the negative control whereas Ketoconazole was used as the positive control. The diameter of the zone of inhibition was read with zone reader (HiAntibiotic zone scale). Diameter of fungal colonies was measured and expressed as percent mycelial inhibition by applying the formula: Percent inhibition of myelial growth = (dc-dt) / dc × 100; dc = average diameter of fungal colony in negative control sets; dt = average diameter fungal colony in experimental sets. The experiments were performed in triplicate in order to minimize the errors.

Sr.No	Compound	Zone of inhibition diameter in (mm)	
		*C.albicans*ATCC 9025	*A.niger*ATCC 1015
1	A6	24	22
2	A7	20	28
3	A8	28	38
4	A9	32	36
5	A10	24	30
6	A11	28	36
	Standard	33	44
	Control	-	-

Table 4. Antifungal activity of compounds **A6-11**
Note: - 0-15 mm poor activity, 15-25 mm moderate activity, above 25 good activity.
Standard(S) = Ketoconazole Control (C) = DMF

Antiprotozoal evaluation: The in vitro antiprotozoal activity was performed against *Paramecium caudatum* and *Vorticella campanula*, *Rectal ciliates* were *Opalina ranarum*, *Nyctotherus cordiformis*. To evaluate the activity of synthesized compounds against protozoa microscopic count method were determined. Known Antiprotozoal like Albendazole and Metronidazole were used for comparison. By comparing the antimicrobial activity of the synthesized compounds, it was found that the tested compounds are more effective against the protozoa. [14] It is believed that the strong lipophilic character

of the molecule plays an essential role for antiprotozoal activity. These 2-methyl-1-[(5-substituted-1,3,4-oxadiazol-2-yl)methyl]-1H-benzimidazole may act *via*reduction of the nitro group *via* ferredoxin in the same way metronidazole acts, but not as inhibitors of tubulin polymerization as albendazole does, since the metronidazole-resistantline was not susceptible to these compounds. This is what is expected of drugs with a similar mechanism of action to metronidazole.

Preparation of the culture media for free-living protozoa: Undefined complex medium was used to culture protozoa. In this method, few leaves of submerged weeds from a pond were collected and kept in a 1-liter jar having distilled water. It was covered and allowed to rot. Within a few days large numbers of protozoa appeared. In order to grow them, hay infusion was prepared by autoclaving hay in tap water and then the supernatant was collected. A few grains of wheat were added to it and were kept undisturbed for four days, in order to get bacterial growth that serves as a source for protozoal nutrition. Then about 5 ml of the inoculum was transferred to the infusion and incubated for two days. This was used for testing the antiprotozoal activity.

Antiprotozoal test: It was made by *microscopic count method*. 1 ml of aqueous solution of acetonic extract was added to 4ml of protozoal inoculum, to get a final concentration of 4 mg/ml. After two minutes, 0.02 ml was transferred onto a glass slide. In control experiment, only 1 ml of distilled water, instead of aqueous extract, was added to the 4ml of inoculum. Both the test and control samples were examined under a compound microscope and motile and non-motile organisms were counted. Non-motile organisms were considered as non-viable due to its susceptibility towards the extract and motile were considered as resistant to the extracts. Tests were repeated four times and the average number of motile/non-motile organisms was recorded.

Compounds	Observation of protozoa after 2 min		Total no of protozoa counted			
			Paramecium caudatum	*Vorticella campanula*	*Opalina ranarum*	*Nyctotherus cordiformis*
	No.of motile/ resistant organisms	No.of non-motile Sensitive organisms				
A6	0	All	6±1	4 ±2	5±1	3±2
A7	0	All	8 ± 2	7 ±1	9±2	7±2
A8	0	All	5±2	3 ±1	6±1	5±1
A9	0	All	3±1	6 ±2	4±2	7±2
A10	0	All	7±2	9 ± 1	9±1	5±1
A11	0	All	4±1	4 ±1	7±1	3±2
A12	0	All	7±2	5 ±2	3±2	5±1
A13	0	All	3±1	3 ±1	4±2	3±1
Std (Metronidazole)	All	0	10 ± 1	12 ± 2	12±1	10±1

Table5. Antiprotozoal effect of synthesized compound against fresh water protozoa

RESULT AND DISCUSSION

All the test compounds were evaluated for antibacterial activity against *S.aureus* ATCC 29213, *E.coli* ATCC 25922, *Pseudomonas aeruginosa* MTCC 741 following the agar diffusion method of assay using ciprofloxacin as the reference drug. The compounds **A6, A7** exhibited good activity against *S. aureus* ATCC 29213, *P.aeruginosa* MTCC 741 and *E.coli* ATCC 25922 and **A8, A9, A10** exhibited Good activity against *A.niger* ATCC 1015, *C.albicans* ATCC 9025 and other have shown moderate activity against *S.aureus* ATCC 29213, *E.coli* ATCC 25922, *P.aeruginosa* MTCC 741, *A.niger* ATCC 1015, *C.albicans* ATCC 9025.

For minimum inhibitory concentration (MIC) method **A6, A7** were found moderately active while, **A8, A9** and **A10** were found to have an average activity compared with standard. Test compounds were found to be more sensitive towards *S.aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 and **A6** was found moderately active, while **A8** and **A9** are found to have an average activity compared with standard. Test compounds were found to be more sensitive towards *Aspergillus niger* ATCC 1015 and *Candida*

albicans ATCC 9025. The compound **A7** and **A10** were found to be most potent. The compounds with para substitution on the benzene ring of **A9** and **A12** also showed better activity against *Paramecium caudatum*. For *Opalina ranarum*, the tested compounds showed low to moderate antiprotozoal activity

CONCLUSION

In conclusion, several substituted 2-substituted-1-[{(5-substituted alkyl/aryl)-1,3,4-oxadiazol-2-yl} methyl]-1H-benzimidazoles A(6-13) were synthesized and screened for various activites like antibacterial, antifungal and antiprotozoal activity. The para substitution on benzene nucleus at oxadiazole moiety supports the chemotherapeutic activity. While 5,6-dinitro and 4,6-dichloro benzimidazoles with oxadiazole heterocyclic ring have potent antimicrobial activity. The obtained results revealed that the nature of substituent and substitution pattern on the benzene ring may have a considerable impact on the antibacterial, antifungal and antiprotozoal activities of the synthesized compounds have particular importance, a nitro group has a considerable impact on antibacterial and antifungal activity. Also chloro and nitro group shows considerable effect on antiprotozoal activity.

All the synthesized compounds exhibited better antiprotozoal activity towards *Paramecium caudatum, Vorticella campanula*, some of the synthesized compounds showed good antiprotozoal activity. From Tables 4, it can be inferred that as the number of carbon atom increases in side chain at 2-position of oxadiazole heterocyclic ring causes an increase in the intensity of the activity against *Paramecium caudatum, Vorticella campanula* , Opalina ranarum and also the para substitution on benzene nucleus at oxadiazole moiety supports the chemotherapeutic activity. While 5,6-dinitro and 4,6-dichlorobenzimidazoles with oxadiazole heterocyclic ring have potent antiprotozoal activity.

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REFERENCES

1. Sheehan, D.J., Hitchcock, C. A. & Sibley, C.M. Clin. Microbiol. Rev, 1999; 12: 40–79.
2. Habib, N.S., Soliman, R., Ashour, F.A. & el-Taiebi, M. Pharmazie, 1997; 52:746–749.
3. Tuncbilek, M., Goker, H., Ertan, R., Eryigit, R., Kendi, E. & Altanlar E. Arch. Pharm, 1997; 330:372–376.
4. Pedini, M., Alunni Bistochi, G., Ricci, A., Bastianini, L. & Lepri, E. Farmaco,1994; 49:823–827.
5. Lackner, T.E. & Clissold, S.P. (1989) Drugs, 1989; 38:204–225.
6. Bishop, B.C., Chelton, E.T.J., Jones, A.S., Biochem. Pharmacol, 1964; 13:751–754.
7. Pawelkiewicz, J. & Zodrow, K. Acta Microbiol. Polon, 1957;6:9–15.
8. 8.Burton, D.E., Lambie, A.J., Ludgate, J.C., Newbold, G.T., Percival, A. & Saggers, D.T. Nature 1965; 208:1166–1170.
9. Klimesova, V., Koci, J., Waisser, K., Kaustova, J, Farmaco 2002; 57:259– 265.
10. Kuhler, T.C., Swanson, M., Christenson, B., Klintenberg, A.C., Lamm, B., Fagerhag, J., Gatti, R., Olwegard-Halvarsson, M., Shcherbuchin, V., Elebring, T., Sjostrom, J.E. J. Med. Chem, 2002; 45:4282–4299.
11. Bishop, B.C., Chelton, E.T.J. & Jones, A.S.Biochem. Pharmacol, 1964; 13:751–754.
12. Stefanska, J.Z., Gralewska, R., Starosciak, B.J. & Kazimierczuk, Z.Pharmazie.1999; 54: 879–884.
13. Priyal Jain, Vaibhav Jain, Abhishek K Jain, Pradeep K Singour. Int. J. of Drug Design and Discovery, 2011;2 (4):633-636.
14. Shibumon G. and Benny P.J In. Journal of Pharmaceutical & Biological Archives, 2010; 1(4):385-388.