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Synthesis and antiprotozoal activity of nitro and halogeno substituted some novel mercaptobenzimidazole derivatives

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ABSTRACT

The earliest report of Benzimidazole derivatives antibacterial activity appeared in 1964, 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. In this paper we present new data on the antimicrobial and antiprotozoal activities of 5,6-dinitro and 2-dialkylaminosubstituted benzimidazoles.

INTRODUCTION

Benzimidazole derivatives are of wide interest because of their diverse biological activityand clinical applications [1]. This ring system is present in numerous antiparasitic, fungicidal, anithelemintic and anti-inflammatory drugs [2–5]. Also, some benzimidazole nucleosides, particularly 5,6-dichlorobenzimidazole-1-_-D-ribofuranoside (DRB) and its 2-substituted derivatives show activity against human cytomegalovirus [6]. It is also known that 5,6-dinitrobenzimidazole can substitute 5,6-dimethylbenzimidazole in the vitamin B12 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]. Most recently, antiprotozoal activity of substituted 2-trifluorobenzimidazoles has been reported [9], consistent with several earlier studies on the anti-giardial activity of various benzimidazole derivatives [10, 11]. However, the general antimicrobial activity of benzimidazole derivatives has not been extensively investigated. 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]. In this paper we present new data on the antimicrobial and antiprotozoal activities of 5,6-dinitro and 2-dialkylaminosubstituted benzimidazoles.

MATERIALS AND METHODS

Experimental

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 a Jeol SX-102 instrument.

Synthesis of 2-Mercapto benzimidazole (A2) –

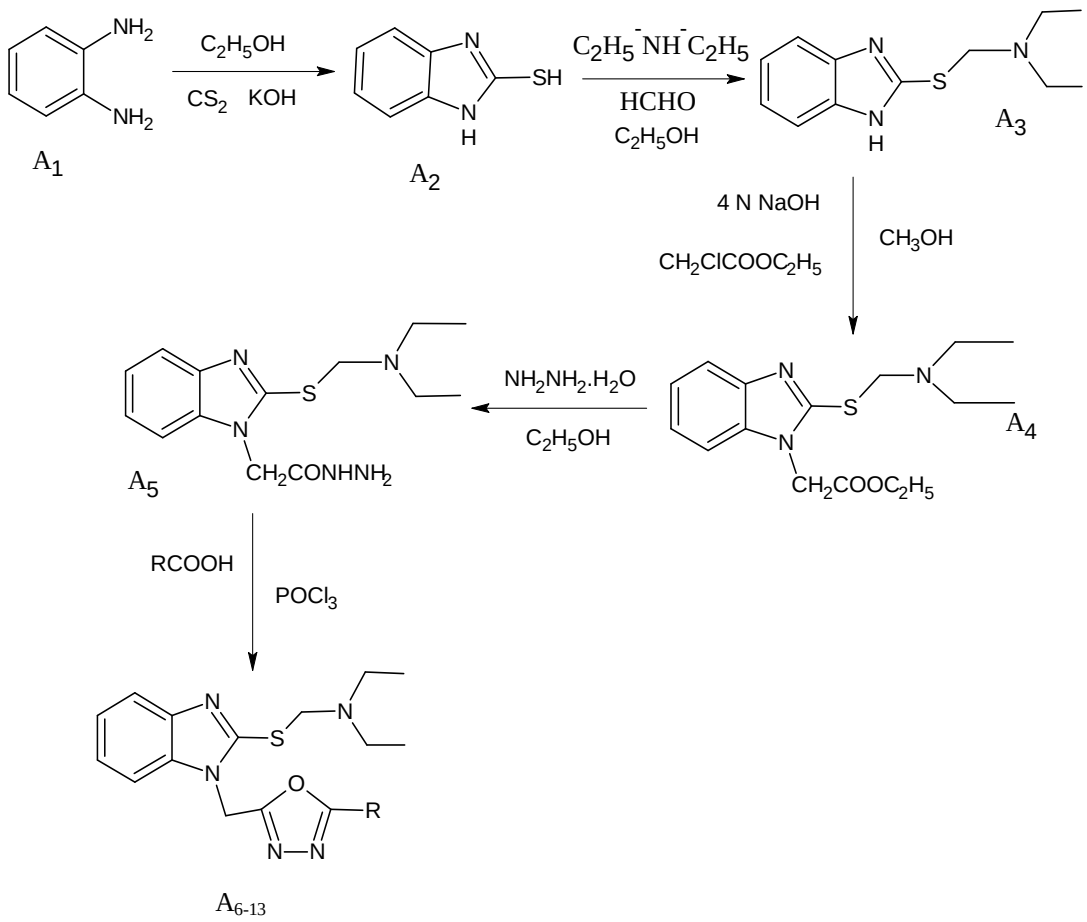
A mixture of 10.8 gm (0.1 mole) of o-phenylenediamine, 5.65 gm (0.1 mole) of potassium hydroxide and 7.67 gm (0.1 mole, 6.19 ml) carbon disulphide, 100 ml of 95% ethanol and 15 ml of water in 500 ml of round bottom flask were heated under reflux for 3 hr. Then added 1.15 gm of charcoal cautiously and then mixture was further heated at the reflux for 10 minutes, the charcoal was removed by filtartion. 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 seperated as glisting white crystals, and the mixture was placed in a refrigarator for 3hr to complete crystallization. The product was collected on a Buckner funnel and dried over night at 40^0C. The dried product was recrystalized by ethanol, the yield was 98% and melting point is 300-302^0C.

Synthesis of N-[(1H-benzimidazol-2-ylsulfanyl)methyl]-N-ethylethanamine (A3)-
Equimolar quqntities (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 cotent was kept overnight in the freezer. The corresponding crystals of N-[(1H-benzimidazole-2-yl sulfanyl1)methyl]-N-ethylamine obtained was recrystallised from alcohol.

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) ofN-[(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 h. The solid thus obtained on cooling was recrystallized from chloroform to give 3. Yield 76%,mp 178–180^0C.

Scheme-



(R as shown in Table 1)

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, to give step 5 respectively.

General Method for Preparation of 2(2{[((diethylamino) 5-substituted aryl 1, 3,4oxadiazol) methyl]sulfanyl}-1H-benzimidazole (A6-13)-

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. The resultant solid was filtered, washed with water and recrystallized from ethanol to give the title compounds.

Spectral data of some of the synthesized compounds

Synthesis of 2-Mercapto benzimidazole (A2)–

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). 1H NMR (DMSO, 200 MHz) δ (ppm): 2.211 (1H, singlet, NH), 7.366-7.383 (3H, multiplet, benzene). m/z -158.

Synthesis of N-[(1H-benzimidazol-2-ylsulfanyl) methyl]-N-ethylethanamine (A3)-

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). 1H NMR (DMSO, 200 MHz) δ (ppm): 2.228 (1H, singlet, NH), 7.366-7.383 (3H, multiplet, benzene). m/z -238.

Synthesis of ethyl (2-{[(diethylamino) methyl]sulfanyl}-1H-benzimidazol-1-yl)acetate (A4)-

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). 1H NMR (DMSO, 200 MHz) δ (ppm): 2.235 (1H, singlet, NHCOR), 7.366-7.383 (3H, multiplet, benzene). m/z -235.

Synthesis of Preparation of 2-(2-{[(diethylamino) methyl]sulfanyl}-1H-benzimidazol-1-yl)acetohydrazide (A5)-

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). 1H NMR (DMSO, 200 MHz) δ (ppm): 2.231 (1H, singlet, NHCOR), 7.366-7.383 (3H, multiplet, benzene). m/z -281.

2-{5-[(2-{[(ethylamino) methyl] sulfanyl}-1*H*-benzimidazol-1-yl) methyl]-1, 3, 4-oxadiazol-2-yl}-4, 6-dinitrophenol (A7)-

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.

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 methodwere 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 asinhibitors 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.

RESULTS AND DISCUSSION

The in vitro Antiprotozoal activity of the derivatives -methyl-1-[(5-substituted-1,3,4-oxadiazol-2-yl)methyl]-1H-benzimidazole A6-13 is characterized in Table 1. With thin layerchromatography and spectral analysis like IR and NMR spectra. Some of the synthesized compounds tested were endowed with a medium activity against Nyctotherus cordiformis.

Of these, 2-{5-[(2-{[(ethylamino)methyl]sulfanyl}-1*H*-benzimidazol-1-yl)methyl]-1,3,4-oxadiazol-2-yl}-4,6-dinitrophenol and 2-{5-[(2-{[(ethylamino)methyl]sulfanyl}-1*H*-benzimidazol-1-yl)methoxy]-1,3,4-oxadiazol-2-yl}-2,4-dichlorophenol.**A7** and **A10**were found to be most potent. The compounds with parasubstitution 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 which is shown in Table. 2.

Table 1. Physical and analytical data of compounds A6-13

Sr No.	R	Yield (%)	Melting Point. (^{0}C)	Molecular Formula	Molecular Wt
1	-C_6H_5	72	160-162	$C_{19}H_{19}N_5OS$	365.45
2	-$C_6H_3N_2O_5$	80	140-142.	$C_{19}H_{17}N_7O_6S$	471.44
3	-C_8H_7	78	134-138.	$C_{21}H_{21}N_5OS$	391.48
4	-C_6H_4Cl	75	154-156.	$C_{19}H_{18}ClN_5OS$	399.89
5	-$C_7H_5Cl_2O$	81	110-112.	$C_{20}H_{19}Cl_2N_5O_2S$	464.36
6	- C_6H_6N	84	188-190.	$C_{19}H_{20}N_6OS$	380.46
7	-C_7H_7	67	120-122.	$C_{20}H_{21}N_5OS$	379.47
8	- C_6H_6N	81	150-152.	$C_{19}H_{20}N_6OS$	380.46

Table 2. Antiprotozoal effect of synthesized compound against fresh water protozoa

Compounds	Observation of protozoa after 2 min for sensitivity/resistance		Total no of protozoa counted			
	No.of motile/ resistant organisms	No. of non-motile Sensitive organisms	*Paramecium caudatum*	*Vorticella campanula*	*Opalina ranarum*	*Nyctotherus cordiformis*
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

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. The pharmacological study was undertaken to evaluate the effects of substituents on the antiprotozoal activity. All the synthesized compounds exhibited betterantiprotozoal activity towards *Paramecium caudatum*,*Vorticella campanula*, some of the synthesized compounds showed good antiprotozoal activity. From Tables 2, it can be inferred that as the number of carbon atomincreases 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 parasubstitution 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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