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ORIGINAL RESEARCH ARTICLE

Synthesis, Characterization and Anti-Microbial Screening of some Schiff's Bases of 2 -methyl benzimidazole derivatives.

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ABSTRACT

A variety of Schiff's bases of 2-methyl benzimidazole derivatives were synthesized from carbohydrazide (2-methyl benzimidazole 1-acetic acid hydrazide) by using ethanol, carbon disulphide, potassium hydroxide, hydrazine hydrate and aromatic aldehydes. The structures of all compounds were confirmed by spectral analysis such as IR, ^{1}HNMR and Mass. The newly synthesized compounds were screened for *in-vitro* anti-microbial activity against a variety of bacterial strains such as *Staphylococcus aureus*, *Baccillus substilis*, *Escherichia coli* and *Pseudomonas aeruginosa* including fungal strains such as *Candida albicans* and *Aspergillus niger*. The compound 2, 3, 4a, 4c and 4d shows good anti-bacterial activity and the compound 2, 3, and 4c shows good anti-fungal activity.

Key words: Carbohydrazide, Benzimidazole derivatives, Spectral analysis, anti-microbial activity.

INTRODUCTION

Anti-bacterial and anti-fungal diseases are very common all over the world. Currently used anti-microbial agents are not effective due to the resistance developed by the microbes and therefore, it is an ongoing effort to synthesize new anti-microbial agents. Although a number of drugs are available in the market, but thirst for discovering new anti-microbial drugs with better pharmacokinetic profile and lesser toxicity has become main objectives in the field of medicinal chemistry due to fast development of microbial resistance towards the existing molecules [1-2]. Benzimidazole derivatives are an important class of nitrogen containing heterocycles, which is the most promising heteroaryl moiety and has yielded many successful drugs [3-4]. Benzimidazoles are a group of molecules which have shown potential applications in a variety of pharmacological targets. It was particularly the discovery of the 5, 6-dimethyl benzimidazole units i.e. the vitamin B_{12} structure, which stimulates the interest in the research in recent times [5-6]. Benzimidazole and its derivatives were found to posses several biological activities such as anti-bacterial [7-8], anti-viral [9-10], anti-cancer [11], anti-diabetic [12], CNS depressant [13], anthelmintic [14], analgesic and anti-inflammatory [15], anti-oxidant [16] etc. with substitution at various positions. These findings led to take the synthesis of some benzimidazole derivatives and investigation of biological activity of those compounds.

MATERIALS AND METHODS

All the necessary aldehydes were obtained from Hi-media Chem.Ltd. and Lancaster Ltd. The solvents and chemicals were from Fisher, S. D. Fine Chem. Ltd. and Loba Chem. Pvt. Ltd.

Melting points of all the synthesized compounds were determined by open capillary tube method. The purity of the compounds were routinely checked by TLC using silica gel-G coated plates & spots were visualized by exposing the dry plates in iodine vapour. UV and IR were recorded at Al-Shifa College of Pharmacy, Perinthalmanna, Kerala. Mass and ^{1}HNMR were recorded at IIT, Chennai.

SCHEME -1:

I. Synthesis of 2-methyl benzimidazole.

Stannous chloride (0.008 mol) was dissolved in water (20 ml). Concentrated hydrochloric acid (30 ml) was added to the above solution and dissolved

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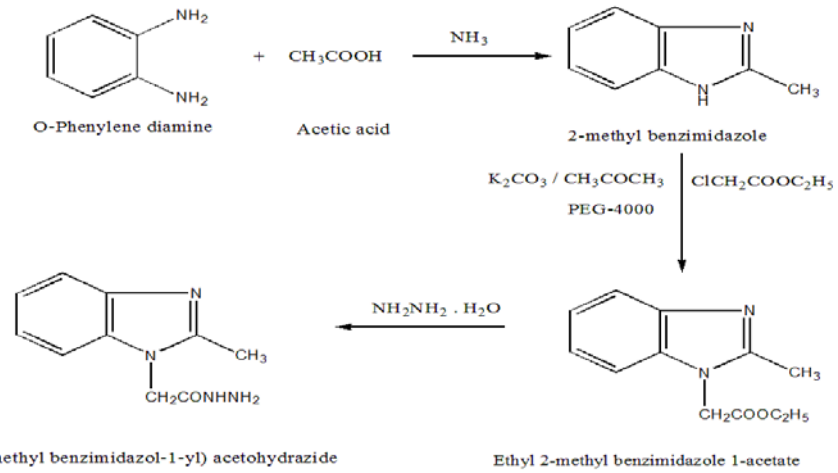
in o-phenylene diamine solution (0.0092 mol). The mixture was heated and charcoal (0.125 mol) was added and filtered. Concentrated hydrochloric acid (50 ml) was added to the hot colourless filtrate and was cooled in freezing ice and filtered. The colourless crystals of o-phenylene diamine dihydrochloride was collected and washed with small volume of concentrated hydrochloric acid and dried and characterized by chromatography.

The above compound (0.036 mol) was dissolved in water (20 ml). Glacial acetic acid (4 ml) was added and this mixture was heated under reflux for 45 min and the cooled reaction mixture was converted to distinctly basic by gradual addition of concentrated ammonia solution. The precipitate was collected and the product was crystallized from 10 % aqueous ethanol.

Scheme-1: Synthesis of 2-(2-methyl benzimidazole-1-yl) acetohydrazide



II. Synthesis of Ethyl 2-methyl benzimidazole-1-acetate.

To a mixture of 2-methyl benzimidazole (0.01 mol), polyethylene glycol-4000 (5 ml) and anhydrous potassium carbonate (0.0072 mol) in dry acetone (30 ml), ethyl chloroacetate (1 ml) drop wise was added at room temperature for 20-30 min. The reaction mixture was further stirred at room temperature for 10-11 hr. The inorganic solid was filtered off and the filtrate was concentrated in vacuum. The semisolid obtained was dissolved in water and was extracted with ethyl acetate. The ethyl acetate was removed under vacuum and the solid obtained was recrystallized by ethanol.

III. Synthesis of 2-(2-methyl benzimidazol-1-yl) acetohydrazide. [1]

To a solution of the above compound (0.01 mol) dissolved in dry methanol (50 ml), 98 % hydrazine hydrate (1ml) was added and the mixture was refluxed for 3 hr. The reaction mixture was cooled and the solid obtained was filtered, washed with small quantity of cold methanol and recrystallized with methanol. The purity of the compound was confirmed by getting a single spot in TLC.

SCHEME -2:

I. Synthesis of 2-[(2-methyl benzimidazol-1-yl) methyl] oxadiazole-5-thiol. [2]

To a mixture of carbohydrazide **[1]** (0.0106 mol) in ethanol (200 ml), a solution of potassium hydroxide (0.0149 mol) in ethanol (20 ml) was added, followed by carbon disulphide (20 ml). The reaction mixture was heated under reflux for 8 hr. Then it was concentrated, acidified with dilute hydrochloric acid and the resulting solid was collected, washed with water and recrystallized with ethanol. The purity of the compound was confirmed by getting a single spot in TLC.

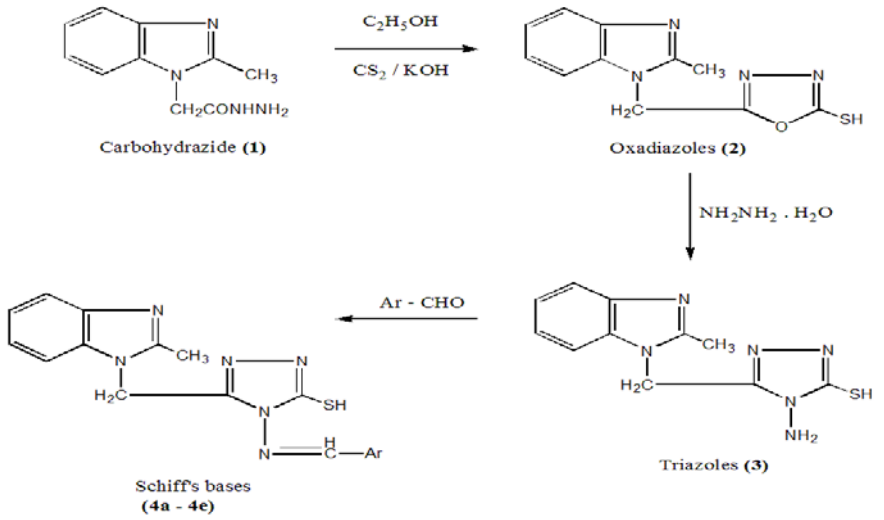
II. Synthesis of 4-amino-5-[(2-methyl benzimidazol-1-yl) methyl]-4*H*-1,2,4-triazole-3-thiol. [3]

A mixture of compound **[2] (**0.0121 mol) and 99 % hydrazine hydrate (2 ml) in absolute ethanol (20 ml) was refluxed for 6 hr. The solvent and the excess hydrazine hydrate were removed under reduced pressure. The residue was washed with ether and then recrystallized with ethanol. The purity of the compound was confirmed by getting a single spot in TLC.

III. Synthesis of Schiff's bases of 2-methyl benzimidazole derivatives. [4a - 4e]

To a solution of the above compound **[3]** (0.0105 mol) in absolute ethanol (30 ml), the appropriate aromatic aldehydes, namely, p-chlorobenzaldehyde, p-hydroxybenzaldehyde, o-nitrobenzaldehyde (0.012 mol) etc. were added separately. The reaction mixture was refluxed for 4 hr. The formed solid after cooling was filtered off and recrystallized with ethanol. The purity of the compound was confirmed by getting a single spot in TLC.

Scheme-2: Synthesis of Schiff's bases of 2-methyl benzimidazole derivatives.



Compound code	Ar
4a	2-nitrobenzaldehyde
4b	3-nitrbenzaldehyde
4c	4-chlorobenzaldehyde
4d	4-hydroxybenzaldehyde
4e	4-hydroxy 3-methoxybenzaldehyde

SPECTRAL DATA OF THE SYNTHESIZED COMPOUNDS

Compound-[1]: 2-(2-methyl benzimidazol-1-yl) acetohydrazide:- IR (in KBr) cm^{-1}: 1693.43 (C=O; str.), 1646.76 (C=N; str.), 1515.57 (N-H; bend.), 1462.21 (Ar C=C; str.), 757.94 (Ar C-H; bend.), 1367.50 (C-H; bend.). **^{1}HNMR** (in DMSO-d_6) δ, ppm: 2.46 (s, 3H, CH_3), 4.62 (d, 2H, CH_2), 8.0 (t, 1H, CONH), 2.0 (d, 2H, NH_2), 7.26-7.7(m, 4H, Ar-H). **Mass** (m/z): 204 (M^+), 72, 102, 117, 130,147.

Compound-[2]: 2-[(2-methyl benzimidazole-1-yl) methyl]oxadiazole-5-thiol:- IR (in KBr) cm^{-1}: 2908.83 (C-H; str.), 1490.01 (Ar C=C; str.), 1275.10 & 1196.58 (C-N; vib.), 1066.04 (C-O; str.), 1445.33 & 962.21 (C-H; bend.), 785.16 (Ar C-H; bend.). **^{1}HNMR** (in DMSO-d_6) δ, ppm: 4.98 (s, 2H, CH_2), 2.41 (s, 3H, CH_3), 3.01 (s, 1H, SH), 7.26-7.0 (m, 4H, Ar-H). **Mass** (m/z): 214 (M^+), 104, 118, 134, 146

Compound-[3]:4-amino-5-[(2-methyl benzimidazol-1-yl)methyl]-4*H*-1,2,4-triazole-3-thiol:- IR (in KBr) cm^{-1}: 3123.76 (N-H; bend.), 1576.84 & 1475.36 (Ar C=C; str.), 1276.79 & 1154.17 (C-N; vib.), 2873.47 (C-H; str.), 873.97 (Ar C-H; bend.).**^{1}HNMR** (in DMSO-d_6) δ, ppm: 2.43 (s, 3H, CH_3), 4.99 (s, 2H, CH_2), 2.0 (s, 2H, NH_2), 3.0 (s, 1H, SH), 7.2-7.6 (m, 4H, Ar-H). **Mass** (m/z): 260 (M^+), 121, 151

Compound-[4a]: 4-(2-nitrobenzylideneamino)-5-[(2-methyl-1*H*-benzimidazol-1-yl)methyl]-4*H*-1,2,4-triazole-3-thiol:- IR (in KBr) cm^{-1} : 1687.41 (C=N; str.), 1509.92 (N-H; bend.), 1264.99 & 1135.07 (C-N; vib.), 1445.95, 1393.29 & 997.57 (C-H; bend). **^{1}HNMR** (in DMSO-d_6) δ, ppm: 8.10 (s, 1H, N=CH), 2.42 (s, 3H, CH_3), 4.98 (s, 2H, N-CH_2), 3.02 (s, 1H, SH), 7.25-8.62 (m, 8H, Ar-H). **Mass** (m/z): 394 (M^+), 102, 119, 149.

Compound-[4b]: 4-(3-nitrobenzylideneamino)-5-[(2-methyl-1*H*-benzimidazol-1-yl)methyl-4*H*-1,2, 4-triazole-3-thiol:- IR(in KBr) cm^{-1}: 1527.03 (Ar N-O; str.), 1344.18 & 1192.27 (C-N; vib.), 2873.06 (C-H; str.), 923 (C-H; bend.), 723.45 (Ar C-H; bend.). **^{1}HNMR** (in DMSO-d_6) δ, ppm: 2.42 (s, 1H, CH_3), 4.97 (s, 2H, CH_2), 3.01 (s, 1H, SH), 8.12 (s, 1H, N=CH), 7.26-8.61 (m, 8H, Ar-H). **Mass** (m/z): 393 (M^+), 69, 105, 121, 135.

Compound-[4c]:4-(4-chlorobenzylideneamino)-5-[(2-methyl-1*H*-benzimidazol-1-yl)methyl]-4*H*-1,2,4-triazole-3-thiol:- IR (in KBr) cm^{-1}: 1675.16 (C=C; str.), 1281.01, 1167.79 & 1118.60 (C-N; vib.), 755.84 (C-Cl; str.), 1416.74, 845.16 & 926.51 (C-H; bend.). **^{1}HNMR** (in DMSO-d_6) δ, ppm: 2.40 (s, 3H, CH_3), 4.99 (s, 2H, CH_2), 3.0 (s,

1H, SH), 8.11 (s, 1H, N=CH), 7.2-7.7 (m, 8H, Ar-H). **Mass** (m/z): 382.1 (M^+), 112, 140.

Compound-[4d]:4-[(3-mercapto-5-[(2-methyl-1*H*-benzimidazol-1-yl)methyl]-4*H*-1,2,4-triazol-4-ylimino)methyl]phenol:- IR (in KBr) cm^{-1}: 1591.82 (Ar C=C; str.), 1663.93 (Ar C=N; str.), 1380.93 & 1314.95 (phenol C-O; str.) 1211.77 (phenol O-H; bend.), 1109.19 (C-N; vib.), 826.72 (Ar C-H; bend.), 2878.41 (C-H; str.). **^{1}HNMR** (in DMSO-d_6) δ, ppm: 2.14 (s, 3H, CH_3), 4.96 (s, 2H, CH_2), 3.03 (S, 1H, SH), 8.14 (s, 1H, N=CH), 5.0 (s, 1H, OH), 6.8-7.7 (m, 8H, Ar-H). **Mass** (m/z): 366 (M^+), 103,119.

Compound-[4e]: 4-[(3-mercapto-5-[(2-methyl-1*H*-benzimidazol-1-y1)methyl]-4*H*-1,2,4-triazol-4-ylimino)methyl]-2-methoxyphenol:- IR (in KBr) cm^{-1} : 1666.08 (C=N; str.), 1592.67 (Ar C=C; str.), 1373.47 (C-N; vib.), 1204.38 (Phenol O-H; bend.), 1427.96 (C-H; bend.), 810.37 & 725.27 (Ar C-H; bend.). **^{1}HNMR** (in DMSO-d_6) δ, ppm: 2.44(s, 3H, CH_3), 4.98 (s, 2H, CH_2), 3.1 (s, 1H, SH), 8.16 (s, 1H, N=CH), 5.12 (s, 1H, OH), 3.73 (s, 3H, OCH_3), 6.83-7.78 (m, 7H, Ar-H). **Mass** (m/z): 394 (M^+), 121, 135, 150.

IN-VITRO ANTI-MICROBIAL EVALUATION OF THE SYNTHESIZED COMPOUNDS [7-8].

The anti-microbial activity was determined using Kirby-Bauer method (KB method) by measuring the zone of inhibition in mm. All the newly synthesized compounds (1-4e) were screened for anti-bacterial activity against gram positive bacteria such as *Staphylococcus aureus*, *Bacillus substilis* and gram negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. Anti-fungal activity was tested against *Candida albicans* and *Aspergillus niger*. Concentration of 500 µg/disc and 250 µg/disc were used for all the tested compounds and the results were compared with the standard drug, ciprofloxacin (10 µg/disc) for anti-bacterial activity and fluconazole (10 µg/disc) for anti-fungal activity. DMSO was used as the vehicle for both anti-bacterial and anti-fungal activity. The results were interpreted as per KB method.

RESULTS AND DISCUSSION

All the eight different derivatives of benzimidazole were synthesized through different schemes. The 2-methyl benzimidazole was first converted into the corresponding carbohydrazide. The above carbohydrazide are converted into oxadiazoles and triazoles and allowed to get converted into Schiff's bases with different aromatic aldehydes. All the synthesized compounds were analyzed by spectral data for confirmation of the formation and purity of the compounds.

All the newly synthesized Schiff's bases of 2-methyl benzimidazole derivatives **[1 - 4e]** were screened for *in-vitro* anti-bacterial and anti-fungal activity. The compound 2, 3, 4a, 4c and 4d shows good activity against all the tested anti-microbial organisms.

Table 1: Characterization data of the synthesized derivative compounds.

Compound code	Mol. formula	Mol. Weight	Melting point	Recrystallization solvent	% Yield	R_f Value
1	$C_{10}H_{12}N_4O$	204	261	Methanol	73	0.56
2	$C_{11}H_{10}N_4O$	214	250	Acetic acid / water	68	0.73
3	$C_{11}H_{12}N_6S$	260	259	Chloroform / petroleum ether	67	0.62
4a	$C_{18}H_{15}N_7O_2S$	393	241	Acetic acid / water	69	0.64
4b	$C_{18}H_{15}N_7O_2S$	393	242	Acetic acid / water	68	0.67
4c	$C_{18}H_{15}N_6SCl$	382.5	232	Ethanol	61	0.82
4d	$C_{18}H_{16}N_6OS$	364	230	Ethanol	62	0.80
4e	$C_{19}H_{18}N_6O_2S$	394	233	Ethanol	60	0.83

Table 2: *In-vitro* screening of anti-bacterial activity of the synthesized compounds by Agar diffusion method.

Compound code	Zone of inhibition of the synthesized compounds (mm)							
	Staphylococcus aureus (gram +ve)		*Baccillus substilis* (gram +ve)		*Escherichia coli* (gram –ve)		*Pseudomonas aeruginosa* (gram–ve)	
	500 µg/disc	250 µg/disc	500 µg/disc	250 µg/disc	500 µg/disc	250 µg/disc	500 µg/disc	250 µg/disc
1	-	-	12	10	-	-	-	-
2	-	-	20	19	18	11	15	12
3	19	17	23	19	17	10	-	-
4a	21	18	-	-	18	10	16	13
4b	18	12	-	-	09	11	18	16
4c	20	16	12	11	-	-	10	12
4d	11	13	10	11	11	10	08	12
4e	08	15	18	14	-	-	12	14
Blank (DMF)	-	-	-	-	-	-	-	-
Standard ciprofloxacin (10 µg/disc)	26	26	25	25	23	23	24	24

(-) indicates no zone of inhibition

Table 3: *In-vitro* screening of anti-fungal activity of synthesized compounds by Agar diffusion method.

Compound code	Zone of inhibition of the synthesized compounds (mm)			
	Candida albicans		*Aspergillus niger*	
	500 µg/disc	250 µg/disc	500 µg/disc	250 µg/disc
1	8	10	-	-
2	11	16	18	9
3	19	16	15	14
4a	-	-	12	13
4b	18	13	-	-
4c	19	11	9	12
4d	-	-	20	19
4e	-	-	-	-
Blank (DMF)	-	-	-	-
Standard fluconazole (10 µg/disc)	22	22	23	23

(-) indicates no zone of inhibition

CONCLUSION

All the synthesized compounds were characterized by IR, ^{1}HNMR and Mass spectral properties. The synthesized compounds were screened for *in-vitro* anti-bacterial and anti-fungal activity. The compound 2, 3, 4a, 4c and 4d shows good activity against all the anti-bacterial organisms tested such as *S. aureus, B. substilis, E. coli and P. aeruginosa* .The compound 2, 3 and 4c shows good anti-fungal activity against *C. albicans* and *A. niger*. All the tested compounds exhibited moderate to good anti-bacterial activity against both gram positive and gram negative bacteria. A few of the compounds exhibited anti-fungal activity near to standard.

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