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Synthesis And In-Vitro Anti-Helminthic Activity Of Bezimidazole Derivative

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ABSTRACT Benzimidazoles are remarkably effective compounds, extensive biochemical and pharmacological studies have confirmed that these molecules are effective against various strains of microorganisms. This research is summarized to know about the chemistry of different derivatives of substituted benzimidazoles along with their pharmacological activities.

Keywords: Bezimidazole, Substituted Aromatic Benzaldehyde, invitro-anti-helminthic activity

I. INTRODUCTION

Benzimidazole is a heterocyclic aromatic organic compound. It is important pharmacophore and a privileged structure in medicinal chemistry[1]. This compound is bicyclic in nature which consists of the fusion of benzene and imidazole. This important group of substances has found practical applications in a number of fields: analgesic[2], anti-inflammatory[3], antibacterial[4] antifungal[5], antiviral[6], anti-helmenthic[7], anticonvulsant[8], anticancer[9], antiulcer[10], and anti-hypertensive[11].A number of methods have been reported for the synthesis of benzimidazoles and its derivatives. These methods include the coupling of ortho-phenylenediamine with carbonyl compounds [carboxylic acid, ester, acid chloride amide,] in presence of various catalysts like H_2O, HCl ,glycol, ceric ammonium nitrate In present study it reported that the synthesis of 2-alkyl & aryl substituted benzimidazoles in presence of ring closing agents and screened for anti-helmenthic activity[14]

II. MATERIALS AND METHODS

Chemicals and Reagent

The chemicals and reagents used in this work were obtained from various chemical units Avra, Oxford chemicals, SRL and SD Fine Chem. The solvents used were of LR grade and purified before their use. The silica gel G used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. Solvent systems used were n-hexane: ethyl acetate (7:3).

Instruments

Melting points were determined in open capillary tubes and are uncorrected. Progress of the reaction was monitored by TLC plates, ^{1}H NMR spectra were recorded on a Bruker300 MHz instrument in DMSO/CDC_{l3} using TMS as internal standard. Chemical shifts (δ) are expressed in ppm. IR spectra (KBr pellet) were recorded on a Perkin-Elmer BX series FTIR spectrometer.

EXPERIMENTAL SECTIONS

Synthetic Procedure

Synthesis of 4-(1H-Benzimidazol-2-yl)benzamine-: ortho-phenylenediamine (5.4 g, 0.05 mol) was dissolved in 20 ml water under heating with continuous stirring. Conc. HCl (20 ml) and p-amino benzoic acid (PABA) (6.9 g, 0.05 mol) were added to the above reaction mixture. Resulted reaction mixture was allowed to reflux for 2 hour in water bath. After completion of reaction, reaction mixture was cooled by water and then neutralized with ammonia solution. The precipitated product was separated by vacuum filtration and re-crystallized with ethanol.

Synthesis of 4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine

A mixture of 4-(1H-benzimidazol-2-yl)benzamine (2.09 g; 0.01 mol), formaldehyde (0.45 g; 0.015 mol), and piperidine (0.85 g; 0.01 mol) in ethanol (25 ml) was stirred for 2 h in magnetic stirrer. Then the resulting mixture was refluxed on water bath for 4 h. The above reaction mixture was poured on crushed ice and mixed well. The solid which obtained was filtered, dried, and re-crystallised using rectified spirit.

Synthesis of N-substitutedbenzylidene-4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine

4-(1-(Piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine (3.94 g, 0.01 mol) and different aromatic aldehyde (0.01 mol) in ethanol (50 ml) were taken in round bottomed flask. To this glacial acetic acid (1 ml) was added and refluxed for 10 h and kept aside for 24hrs. Then the solution was poured in ice cold water, stirred well and separated product was filtered. The dried product was re-crystallised using ethanol.

Identification and characterization

Synthesized compounds were indentified and characterized by the following procedure to ascertain whether all prepared compounds were of

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different chemical nature than the respective parent compound

Spectral studies

- **1 (4-(1H-Benzimidazol-2-yl) benzamine)**

- IR (KBr) **-:**3347(N-H); 1370 (Ar-NH); 3050 (CH); 1676 (C=N); 1602 (C=C).
- 1H NMR (DMSO-d6, 300 MHz, d ppm): 5.01 (S, NH2), 6.1 (d, 2H, H2, H6) amino phenyl), 7.08 (m, 2H, 5H, 6H benzimidazole), 7.46 (m, 2H, 4H, 5H benzimidazole), 7.79 (d, 2H, 5H, 5H amino phenyl), 12.46 (d NH benzimidazole,)

- **2 [4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine]**

- IR (KBr): 3345(NH), 3050(CH), 1374(Ar-NH), 1676(C=N), 1602(C=C)
- 1H NMR-1H NMR (DMSO-d6, 300 MHz, d ppm): 1.239 (t,1H,2H,3H,4H piperidine) 5.54 (S, NH2 amino phenyl), 6.62 (d, 2H, 2H, 6H amino phenyl), 7.0 (m, 2H, 5H, 6H benzimidazole), 7.41 (m, 2H, H4, H7 benzimidazole), 7.80 (d, 2H, 3H, 5H amino phenyl), 12.50 (d NH benzimidazole),

- **(3a)(4-Hydroxy-3-methoxybenzal)4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine]**

- IR(KBr): 3584(Ar-OH), 3357(NH), 3010(CH), 1270(Ar-NH), 1670(C=N), 1601(C=C),1171(C-O),1270(C-O-C)
- 1HNMR (DMSO-d6, 300 MHz, d ppm): 1.244(t,1H,2H,3H,4H piperidine) 2.3(t, Ar-CH_3) 3.74(s, O-H) 3.8(s,Ar-OCH_3) 3.89(s, C-O-C ether) 5.58 (S, NH2 amino phenyl), 6.63 (d,2H,H2,H6 amino phenyl),6.9(s,Ar-OCH_3) 7.08 (m, 2H, H5, 6H benzimidazole), 7.46 (m, 2H, H4, 7H benzimidazole), 7.71 (d, 2H, 3H, 5H amino phenyl), 12.42 (d NH benzimidazole),

- **(3b)2-chloro benzal4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine**

- IR(KBr)-3399(NH), 3015(CH), 1290(Ar-NH), 1676(C=N), 1601(C=C)
- 1-H NMR(DMSO-d6, 300 MHz, d ppm): 1.239(t,1H,2H,3H,4H piperidine) 2.3(t, Ar-CH_3) 2.5 (Ar-Cl)5.58 (S, NH2 amino phenyl), 6.63 (d, 2H, H2, 6H amino phenyl), 7.08 (m, 2H, H5, H6 benzimidazole), 7.46 (m, 2H, 4H, 7H benzimidazole), 7.79 (d, 2H, 3H, 5H amino phenyl), 12.46 (NH benzimidazole),

- **(3c)4-methoxybenzal4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine**

- IR (KBr)-3373(NH), 3109(CH), 1285(Ar-NH), 1602(C=C),1672(C=N), 1250(C-O-C), 1172(C-O)
- 1-H NMR(DMSO-d6,300MHz,d ppm):1.254(t,1H,2H,3H,4H piperidine) 2.3(t, Ar-CH_3) 3.71(s,Ar-OCH_3) 4.37 (S, NH2 amino phenyl), 6.63 (d, 1H, H2,H6 amino phenyl),6.88 (s,Ar-OCH_3) 7.08 (m, 2H, H5, H6 benzimidazole), 7.46 (m, 2H, H4, H7 benzimidazole), 7.90 (d, 2H, H3, H5 amino phenyl), 12.70 (NH benzimidazole)

- **(3d) 4-dimethyl amino benzal4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine**

- IR(KBr)-3394(NH), 3015(CH), 1289(Ar-NH), 2973,2867(N-CH_2), 1601(C=C), 1673(C=N)
- 1-H NMR(DMSO-d6, 300 MHz, d ppm): 1.280(t,1H,2H,3H,4H piperidine) 2.3(t, Ar-CH_3) 3.01(S, NH2 amino phenyl), 2.9[m Ar-$N(CH_3)_2$] 3.03 (d, 2H,H3,6H amino phenyl), 3.05 (d, 2H, 3H, 5H amino phenyl),7.08 (m,2H,5H,6H benzimidazole), 7.46 (m, 2H, H4, 7H benzimidazole),12.58(d NH benzimidazole),

Scheme-1synthesis of N-substitutedbenzylidene-4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine

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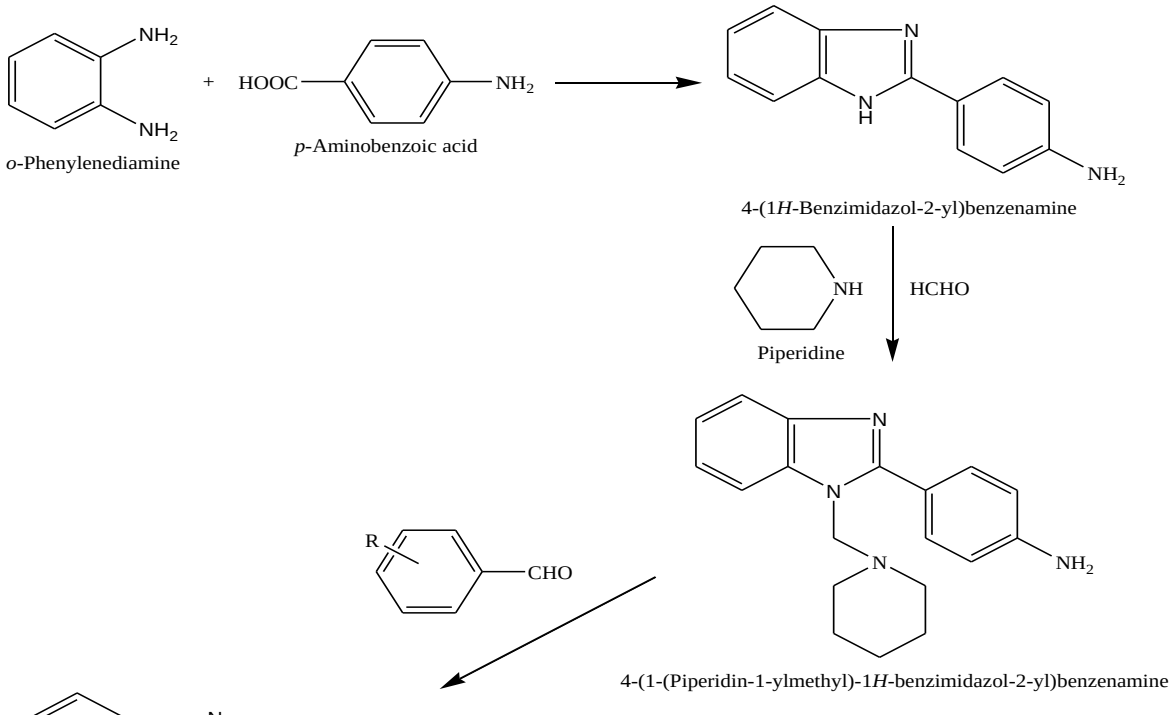

o-Phenylenediamine

p-Aminobenzoic acid

4-(1*H*-Benzimidazol-2-yl)benzenamine

Piperidine

4-(1-(Piperidin-1-ylmethyl)-1*H*-benzimidazol-2-yl)benzenamine

N-Substitutedbenzylidene-4-(1-(piperidin-1-ylmethyl)-1*H*-benzimidazol-2-yl)benzenamine

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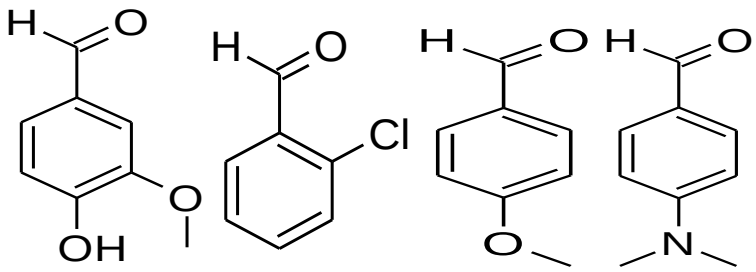

3a 3b 3c 3d

III. RESULTS

The title compounds **3a-3d**were synthesized as per the protocol shown in Scheme 1. In the present work, by substituting different aromatic aldehydes at the C-26 position of 4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine , a sequence of novel bezimidazoles derivatives **3a-3d** were synthesized. Presence of particular groups was identified from IR spectra by means of some characteristic absorption bands. The IR spectrum of bezimidazoles showed characteristic intense absorption bands at 3347(N-H); 1370 (Ar-NH); 3050 (CH); 1676 (C=N); 1602 (C=C)). The formation of benzimidazole was confirmed from the absorption bands of IR spectra. The absorption band at 3347 indicates NH Stretch of the benzimidazole ring. Further, it can also be confirmed from the ^{1}H NMR spectral data. A

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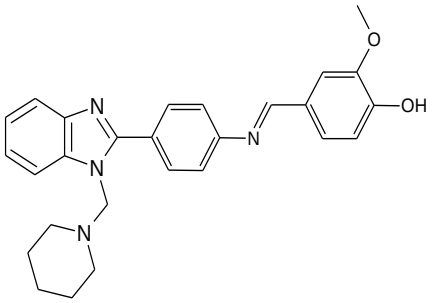
strong peak at **δ 5.01** ppm integrating for N-H proton,**δ 61**.shows amino aryls moiety and,**δ 7.46(s)** confirm bezimidazole. The spectrum also revealed a triplet at **δ 1.254** (t piperidine) ppm for the proton of the bezimidazolering..Thestructure of title compounds **3a–3d** were further confirmed by the appearance of various other peaks in NMR spectroscopy corresponding to the assigned structure.

AnthelminticActivity

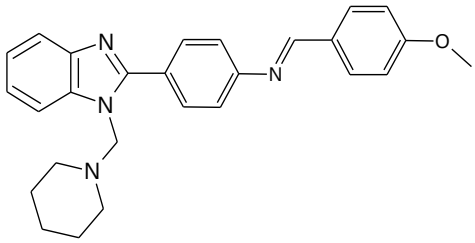
Pheretimaposthuma(Indian adult earth worms) of nearlyequalsize(6cms±1)wereselectedrandomlyfort hepresentstudy.The worms were acclimatized to the laboratoryconditions before experimentation. The earth worms weredividedinto four groups of sixearthwormsineach.Albendazole suspension in the concentration of 10 mg / mlserved as a standard and poured into petri dishes. The testextract were prepared in the concentrations of 5 mg / ml, 10mg / ml, 15 mg / ml, 20 mg / ml, 25 mg / ml, 30 mg / ml.Normalsaline servedascontrol.Six earthwormsnearlyequal size 6 cms ± 1 were taken for each concentration andplaced in petri dishes at room temperature. The time takenfor complete paralysis and death were recorded. The meanparalysis time and mean lethal time for each sample wascalculated.Thetimetakenfortheworms tobebeco memotionlesswasnotedasparalysistimeandtoascertai ndeath,eachwormwasfrequentlyappliedwithexternal stimuliwhich stimulates or induce movements in the earthworm, ifalive

Table no-1

Treatment	Concentrationused(mg/ml)	Timetakenforparalysis(min) X±S.D	Timetakenfordeath(min)X± S.D
Control	---	---	---
Standard(Albendazole)	10 mg/ml	17±1.571*	39±1.932*
3a	5mg/ml	76±3.303*	99.33±0.402*
3b	10 mg/ml	65.33± 2.883**	90.67± 3.921**
3c	15 mg/ml	56±2.017**	85.00± 5.310**
3d	20 mg/ml	4.33±1.498**	69.83± 2.496**



3a



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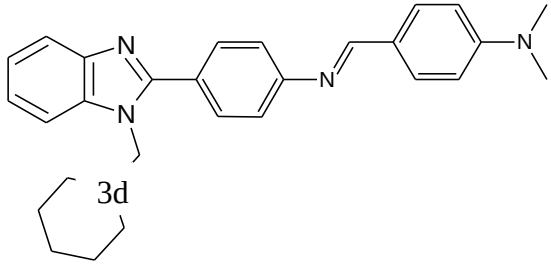

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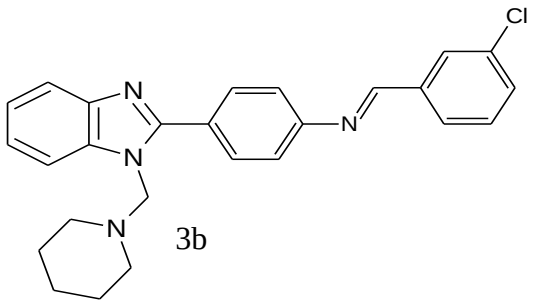

3b

IV. DISCUSSION

Based on theearlierstudieson activities of bezimidazole derivativesusedtoevaluate theanthelminticactivity and showed the effect in a dose dependent manner.Themean± SEM values (statisticalanalysis) were calculatedforeachderivatives

Theresultsofanthelminticactivityonearthworm Phertimaprosthumawas given in Table 1, reveal that,thedifferentderivatives has shown paralysis and death of earth worms and it wascomparedwithalbendazoleasreferencedrug.

V. CONCLUSION

In summary, a series of novel bezimidazole derivatives **3a,3b,3c, 3d** were synthesized and characterized by FTIR, ^{1}H-NMR. These derivatives were evaluated for their anti-helminthic activity . In general, hydroxyl, alkoxy and amino-group substituted compounds exhibited potent anti-helminthic activity. From the study, it was concluded that in this series nature of the substituent played a major role in anti-helminthic activity than its position. Among several tested compounds, **3d** amine substituted benzaldehyde showed better anti-helminthic activity which was more potent than reference standardalbendazole,

Physical characterization (table 2)

S.no	Compound	M.P (c)	Time (hr)	Yield	Molecular weight	Molecular Formula	Nature	Soluble in	Insoluble in
01	3a	90-100	10	84%	440.5368	$C_{28}H_{31}N_5$	Dark red crystal	Ethanol, methanol, acetone	Ethylacetate, Benzene, Cyclohexane, distilled water
02	3b	150-160	10	72%	428.9565	$C_{26}H_{25}ClN_4$	Yellow crystal	Ethanol, methanol, acetone	Ethylacetate, Benzene, Cyclohexane, distilled water
03	3c	120-125	10	89%	424.5372	$C_{27}H_{28}N_4O$	Yellowish brown crystal	Ethanol, methanol, acetone	Ethylacetate, Benzene, Cyclohexane,distilled water
04	3d	110-115	10	78%	437.5792	$C_{28}H_{31}N_5$	Brown crystal	Ethanol, methanol, acetone	Ethylacetate, Benzene, Cyclohexane, distilled water

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