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SYNTHESIS AND IN-VITRO BIOLOGICAL EVALUATION OF BEZIMIDAZOLE DERIVATIVE

Suraj Kumar Sharma*

India.

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***Corresponding Author**
Suraj Kumar Sharma
India.

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. **Objective:** Bezimidazole has a unique place in medicinal chemistry and also plays a vital role in biological processes. In the biological functions at cellular level bezimidazole plays imperative roles which lead the researchers to design a variety of its derivatives. The aim of the present study was to synthesize the novel set of *N*-substitutedbenzylidene-4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine. These compounds were screened for their anti-inflammatory, anti-bacterial and anti-fungal activities. **Method:** A novel series of *N*-substitutedbenzylidene-4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine derivatives were furnished in two steps starting from synthesis of 4-(1*H*-Benzimidazol-2-yl)benzamine from para-amino benzoic acid and ortho phenylene diamine then synthesis of 4-(1-(piperidin-1-ylmethyl)-1*H*-benzimidazol-2-yl)benzamine from 4-(1*H*-Benzimidazol-2-yl)benzamine formaldehyde and piperidine. Protein denaturation technique were performed for screening in vitro anti-inflammatory activity where as agar well diffusion technique was used for screening of anti-bacterial and anti-fungal activities respectively. **Result:** All [*N*-substitutedbenzylidene-4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine] the synthesized derivatives were characterized by Fourier-transform infrared spectroscopy (FTIR), ^{1}H nuclear magnetic resonance (NMR). The result of biological screening revealed that many of the new derivatives were endowed with improved anti-inflammatory, anti-bacterial and anti-fungal activities. **Conclusion:** Nature of the substituent played a major role in anti-inflammatory, anti-microbial and anti-fungal activities. The bezimidazole derivative

with hydroxyl, amino and alkoxy substitution exhibited potent anti-inflammatory anti-bacterial and anti-fungal activities. From the results, it was concluded that 4Hydroxy-3-methoxybenzaldehyde substituted benzylidene-4-(1-(piperidin-1-ylmethyl)-1H-benzimidazol-2-yl)benzamine] was the most active compound[3a].

KEYWORDS: Bezimidazole, Substituted Aromatic Benzaldehyde, Anti-inflammatory, Anti-bacterial, anti-fungal, Agar well diffusion technique, Protein Denaturation.

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]

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 Bruker 300 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-(1*H*-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)-1*H*-benzimidazol-2-yl)benzamine

A mixture of 4-(1*H*-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)-1*H*-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 different chemical nature than the respective parent compound.

Spectral studies

- **1 (4-(1*H*-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)-1*H*-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)-1*H*-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)-1*H*-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).

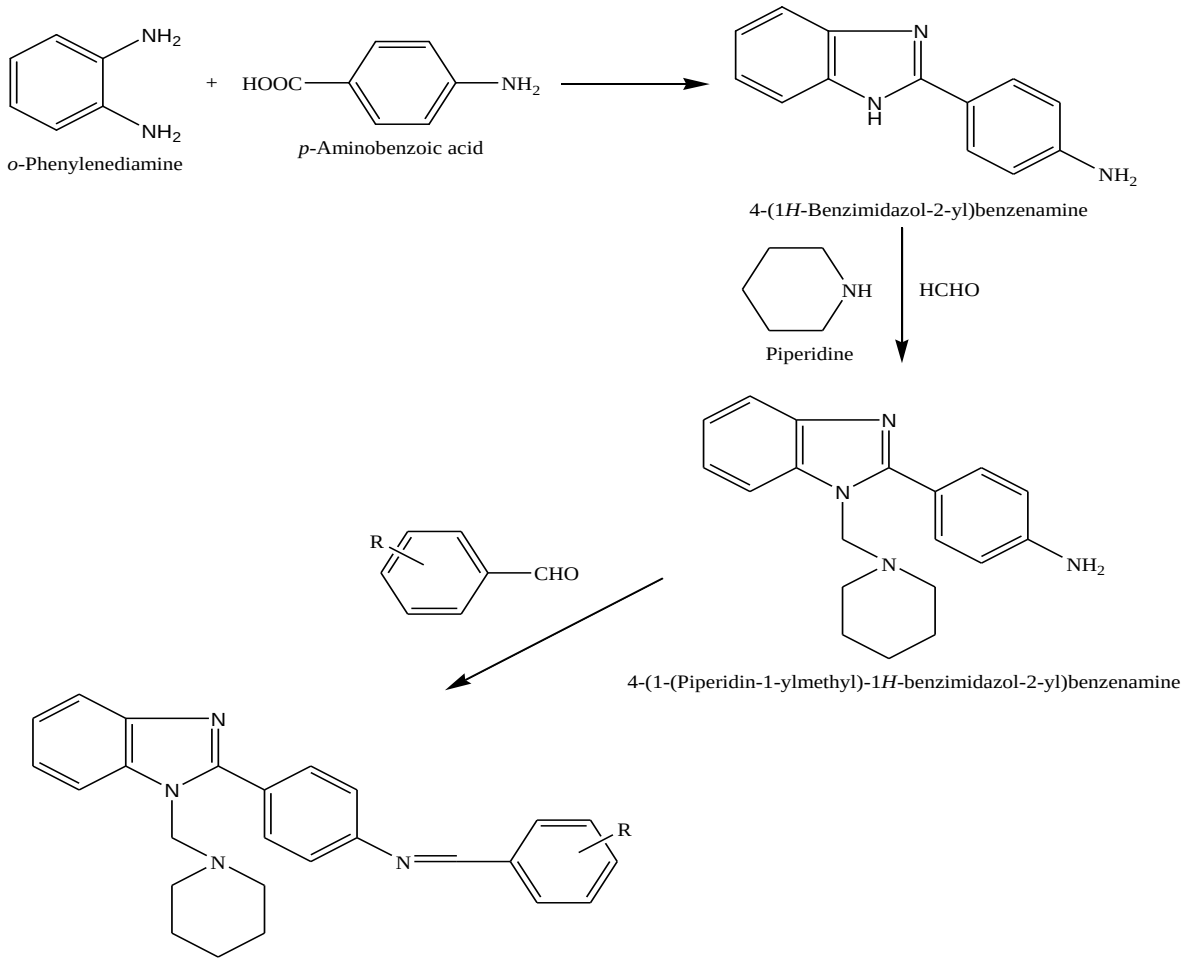
➢ **(3c)4-methoxybenzal4-(1-(piperidin-1-ylmethyl)-1*H*-benzimidazol-2-yl)benzamine**

- 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)-1*H*-benzimidazol-2-yl)benzamine**

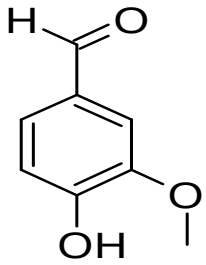
- IR(KBr)-3394(NH), 3015(CH), 1289(Ar-NH), 2973,2867(N-CH_2), 1601(C=C), 1673(C=N).
- 1-H NMR(DMSO-d6, 300 MHz, d ppm): 1.280(t,1H,2H,3H,4H piperidine) 2.3(t, Ar-CH_3) 3.01(S, NH2 amino phenyl), 2.9[m Ar-N(CH_3)$_2$] 3.03 (d, 2H,H3,6H amino phenyl), 3.05 (d, 2H, 3H, 5H amino phenyl),7.08 (m,2H,5H,6H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

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

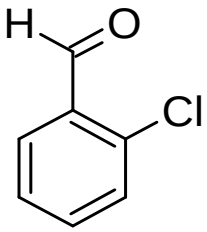


N-Substitutedbenzylidene-4-(1-(piperidin-1-ylmethyl)-1*H*-benzimidazol-2-yl)benzenamine

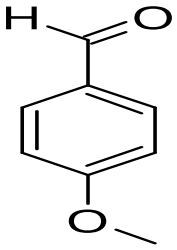
R=



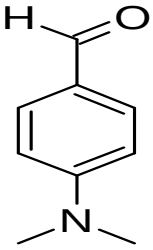
3a



3b



3c



3d

In-vitro anti-inflammatory screening

Protein Denaturation method[15]**-** The synthesized compounds 3a to 3d were screened for anti-inflammatory activity by using inhibition of albumin denaturation technique which was studied according to Muzushima and Kabayashi with slight modification. The 5ml of reaction mixture was comprised of 0.2ml of eggs albumin, 2.8ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentration(100µg\ml, 200µg\ml,500µg\ml) of test drug. Similar volume of double distilled water served a control. Then the mixture was incubated at 37 °C in incubator for about 15mins and then heated at 70 °C for 5mins. After cooling, their absorbance was measured at 660nm by using pure blank. Diclofenac sodium (standard drug) was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated by the formula. Percent inhibition = [vt/vc]×100 Where,

VT= absorbance of test, VC=absorbance of control.

In Vitro-Anti-Bacterial Activity

Diffusion disc technique[16,17] -The synthesized compounds 3a to 3d were screened against bacteria to know their antimicrobial activity. Compounds were screened for antibacterial activity against *Bacillus subtilis* (Gram +ve) and *Escherichia coli* (Gram -ve) strains were used. The fresh culture of bacteria are obtained by inoculating bacteria into peptone water liquid media and incubated at 37 ± 2˚ C for 18 – 24 hours. This culture mixed with nutrient agar media (20%) and poured into petridish by following aseptic techniques. After solidification of the media five bores are made at equal distance by using sterile steel cork borer (8 mm diameter). Into these cups different concentrations of standard drugs and synthesized compounds are introduced. Dimethyl formamide was used as a control. After introduction of standard drugs and synthesized compounds, the plates were placed in a refrigerator at 80-100 C for proper diffusion of drugs into the media. After two hours of cold incubation, the petriplates are transferred to incubator and maintained at 370±20C for 18-24

hours. After the incubation period, the petriplates were observed for zone of inhibition by using vernier scale. The results evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drugs. The results are the mean value of zone of inhibition measured in millimeters.

The standard drugs and synthesized compounds were dissolved in minimum quantity of dimethylformamide (DMF) and made up the volume with distilled water to get 50g/ml and 100g/ml concentrations. Procaine penicillin used against *Bacillus subtilis* and streptomycin were used against *Escherichia coli,* standard drugs for antibacterial activity. The pH was adjusted to 7.4 ± .1 at 250C temperature.

In-Vitro-Antifungal activity

Agar well diffusion method[18,19] - The following fungal species are used to assess the antifungal activity of compounds, they are *Candida albicans*, *Aspergillus niger* these were obtained from Fungal Culture Collection Laboratory, Department of Microbiology, GITAM Institute of Pharmacy. GITAM Deemed to be University. Media for fungal cultures.

[Sabourad's Dextrose Agar Medium (SDA)]

Peptone 10.00 g, Dextrose 40.00 g, Agar 20.00 g, Distilled water 1000 ml

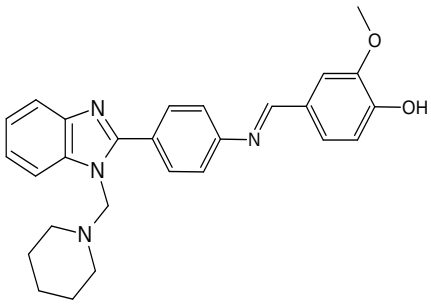
The antifungal activity of synthesized compounds 3a to 3d was determined by agar well diffusion method. The culture plates inoculated with test organisms were allowed to solidify and punched with sterile cork borer (7.0 mm diameter) to make open wells. The open wells were filled with 0.05 mL or 50 µL of the test compounds. The test was carried out on SDA Plates and incubated at 22-30c, respectively for 72 hrs. The test organisms were sub-cultured using potato-dextrose-agar medium. The tubes containing sterilized medium were inoculated with test fungi and after incubation at 37C for 48 hours, they were stored at 0-4 c in refrigerator. The zones of inhibition were measured and recorded.

RESULTS AND DISCUSSION

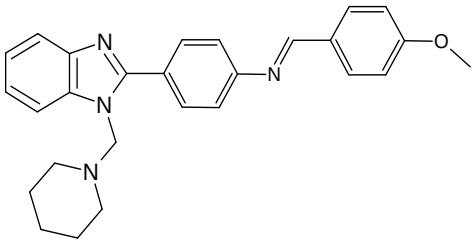
The title compounds **3a-3d** were synthesized as per the protocol shown in Scheme 1. In the present work, by substituting different aromatic aldehydes at the C-26 position of 4-(1-(piperidin-1-ylmethyl)-1*H*-benzimidazol-2-yl)benzamine, a sequence of novel bezimidazoles derivatives **3a-3d** were synthesized. 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 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 bezimidazole ring. The structure of title compounds **3a–3d** were further confirmed by the appearance of various other peaks in NMR spectroscopy corresponding to the assigned structure.

In-vitro anti-inflammatory screening by Protein denaturation method- In-vitro anti-inflammatory activity of test compounds was evaluated egg albumin denaturation method.[20] The anti-inflammatory activity results (Table 1) revealed that all the test compounds showed better activity when compared to that of standard drug. From the results, it was observed that the compounds **3a** and **3c** exhibited good activity when compared to that of standard drug. It may be due to the presence of O-H group on the phenyl ring attached at para position. The compound **3c** showed more activity in compare to standard drug. The presence of O-CH_3 and O-H group attached to the aromatic phenyl ring may contribute to its activity. Rest of the compounds showed moderate anti-inflammatory activity.



3a



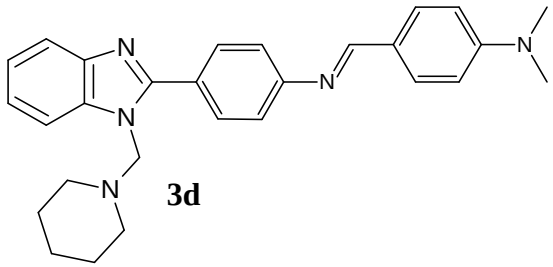
3c

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured.[21] Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of synthezied drug inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 90.89% was observed at 500µg/ml. Diclofenac was used as a standard anti inflammation drug and it

showed the maximum inhibition 89.23% at the concentration of 500µg/ml compared with control.

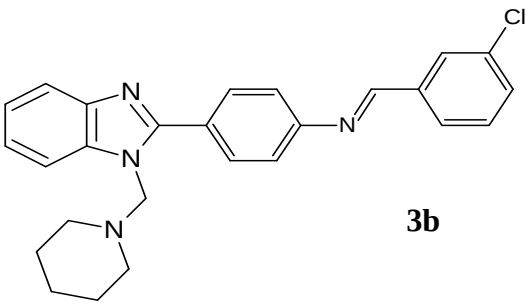
- **Anti-bacterial activity by Diffusion disc technique**

The synthesized compounds **3a,3d** were screened for antibacterial activity against *bacillus substillis* and *E. coli* by agar diffusion technique using procaine penicillin and streptomycin as reference repectively.[22] The diameter of the zone of inhibition exhibited by the compounds was measured. It was found that compounds **3c and 3d** were the most active against *bacillus subtillis* gram (+ve) at a concentration of 100µg/ml. The compounds **3b 3c** and **3d** screened against *E.coli* and it shows maximum activity at 100 µg/ml.



3d

- **Anti-fungal activity by Agar well diffusion method-** The data reveal that the compounds **3a, 3c** have excellent antifungal activity against the test fungi and nearly equal to the standard drug. Compounds **3b, 3d** have showed moderate activity. The compounds **3a** showed better activity than the compounds **3c**, because O-H group is present along with O-CH_3 but in 3c only ethoxy (O-CH_3) group is present that may be reason for good antifungal activity.+



3b

Physical characterization (table 1).

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

In vitro anti inflammatory activity (table 2).

S no	Compound	Concentration (µ/ml)	% Inhibition
01	Blank	_	_
02	Standard (diclofenac)	100	30.22%
		200	64.6%
		500	89.23%
03	3a	100	22.3%
		200	62.8%
		500	80.15%
		200	29.22%
04	3b	100	12.63%
		200	20.78%
		500	31.33%
05	3c	100	30.69%
		200	65.36%
		500	90.89%
06	3d	100	12.45%
		200	30.22%
		500	61.09%

Anti-bacterial screening (table 3).

S.no	Compound	Concentration µg/ml	Zone of inhibition (activity index) In mm)	
			Bacillus substilis (gram +ve)	Escherichia coli (gram –ve)
01	Procaine penicillin	50	10	_
		100	20	_
02	Streptomycin	50		10
		100		20
03	3a	50	12(0.06)	10(0.65)
		100	13(0.70)	13(0.08)
04	3b	50	12(0.60)	15(0.70)
		100	12(0.70)	14(0.60)
05	3c	50	14(0.75)	12(0.60)
		100	13(0.70)	14(0.70)
06	3d	50	12(0.60)	14(0.35)
		100	14(0.75)	12.(0.75)

In vitro antifungal activity (table 4)

S no	Compound	Concentration µg/ml	Zone of inhibition (activity index) In mm)	
			Candida albicans	Aspergillus niger
01	Standard (nystatin)	10	15	18
02	3a	10	12	16
03	3b	10	6	4
04	3c	10	10	12
05	3d	10	4	5

CONCLUSION

In summary, a series of novel bezimidazole derivatives **3a, 3d** were synthesized and characterized by FTIR, ^{1}H-NMR. These derivatives were evaluated for their anti-inflammatory, anti-bacterial and anti-fungal activities. In general, hydroxyl, alkoxy and amino-group substituted compounds exhibited potent anti-inflammatory, anti-bacterial and anti-fungal activities. From the study, it was concluded that in this series nature of the substituent played a major role anti-inflammatory activity, anti-microbial activity and anti-fungal activity than its position. Among several tested compounds, **3d** amine substituted benzaldehyde showed better anti-inflammatory activity which was more potent than reference standard diclofenac, where as **3a,3c,3d** are hydroxyl substituted benzaldehyde derivative that show better anti-bacterial and anti-fungal activities Hence, theses analogue could be developed as a new class anti-inflammatory agent and anti-microbial agent.

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AUTHOR'S CONTRIBUTIONS

Suraj kumar Sharma: Performed the experiments, Dr. S. Raja : Conceived the idea, study design and finalized the manuscript and Assisted in experimental work and helped in the preparation of manuscript.

CONFLICTS OF INTERESTS

There are no conflicts of interest.

REFERENCE

1. Babita Goswami, V.U.; Bobesh, K.A.; Soni, V.; Devi, P.B.; Pulla, V.K.; Suryadevara, P.; Chennubhotla, K.S.; Kulkarni, P.; Yogeeswari, P., Pharmacological activities of benzimidazole derivatives- Overview, IJSID, 2012; 2(1): 121-136.
2. Manna K, Aggarwal Y. Microwave assisted synthesis of new indophenazine 1, 3,trisubstituted pyrrazoline derivatives of benzofuran and their antimicrobial activity. Bioorg. Med. Chem. Lett, 2009; 19: 2688-2692.
3. Ramanpreet et al. Benzimidazole derivatives-An overview, IJRPC 2011; 1(3).
4. Shukla, Synthesis and Biological Screening of benzimidazole derivatives, IJPSR, 2012; 3(3): 922-927.
5. Tupe AP*, Pawar PY, Mane BY, and Magar SD, Synthesis Analgesic and Anti-Inflammatory Activity of Some 2-Substituted 3-Acetic Acid Benzimidazole Derivatives, RJPBCS, April – June 2013; 4(2): 928.
6. Sanahanbi N.* and T. Sivakumar, Synthesis of Some Schiff's Bases of 2-Methyl Benzimidazole Derivatives and Screening of Analgesic and Anti-Inflammatory Activities, CODEN(USA): AJBPAD, 2013; 2(4).
7. Marriappan G Synthesis and evaluation of Mannich bases of benzimidazole derivatives, Indian Journal of Chemistry, September 2011; v50B: 1216-1219.
8. Sandeep Waghulde1, Ganesh Lonsane4, Varsha Borwandkar3, Sachin Laddha2, Synthesis and anti-inflammatory activity of novel 2-(Substituted alkyl or aryl pyridynyl) benzimidazole derivatives.

9. K F Ansari and C Lal*, Synthesis and biological activity of some heterocyclic compounds containing benzimidazole and beta - lactam moiety, J. Chem. Sci, November 2009; 121(6): 1017–1025.
10. Hamdan S. Al-Ebaisat., Synthesis and Biological Activities of Some Benzimidazoles Derivatives, J. Appl. Sci. Environ. Manage, Sept 2011; 15(3): 451–454.
11. Uday Kalidhar, Amandeep Kaur, An Overview on Some Benzimidazole and Sulfonamide Derivatives with Anti-Microbial Activity, RJPBCS, October – December 2011; 2(4): 1116.
12. Bauer, R.W.; Kirby, M.D.K.; Sherris, J.C.; Turek, M. Antibiotic susceptibility testing by standard single disc diffusion method Text book of practicle organic chemistry, 4th edition, 1162-1163.
13. Wagner EC, Millett WH. Benzimidazole. Organic Syntheses Coll, 1943; 2: 65.
14. Hawkey PM, Lewis DA. United Kingdom: Oxford University Press; 1994. Medical bacteriology-A practical approach; pp. 181–94 Satish gupta's short text book of medical microbiology, 9th edition.
15. Panneer Selvam, Radhika, S. Janagaraj, A. Siva Kumar, Research in Biotechnology, synthesis of novel 2-substituted benzimidazole derivatives as potential anti microbial agents of, 2011: 50-57.
16. Simone Budow, Mariola Kozlowska, Agata Gorska, Zygmunt Kazimierczuk, Henning Eickmeier, Paolo La Colla, Gilles Gosselin, and Frank Seela, Synthesis of substituted benzimidazoles and anti viral activity and also synthesis of nucleosides ARKIVOC, 2009; 225-250.
17. Angela Rao, Alba Chimirri, Erik De Clercq, Anna Maria Monforte Pietro Monforte, Christophe Pannecouque, Maria Zappala, Synthesis and anti-HIV activity of 1,2substitutedbenzimidazoles.Elsevier Il Farmaco, 2002; 57: 819/823.
18. John M. Gardiner, Colin R. Loyns, Synthesis of Novel 1, 4- and 1,7substituted2Mercapto- and 2-Methylmercapto- Benzimidazoles: Acyclic Analogues of the HIV-l RT Inhibitor, Elsevier Science Ltd Temhedron, 1995; 51(42): Il515-11530.
19. Yalpnl, i & enl, E $enerl, A Akm, N Up-ti.irk, synthesis and the structure-activity relationships of some substituted benzoxazoles, oxazolo(4,5=b)pyridines, benzothiazoles and benzimidazoles as antimicrobial agents, Elsevier, Paris Eur JMed Chem, 1992; 27: 4: 406.

20. Basavaraja HS, Nagamani JE, Vijay Kumar M, Padmashali B. Antimicrobial evaluation of novel substituted pyrimidinopyrazoles and pyrimidinotriazoles. J Adv Pharm Technol Res, 2010; 1: 236.
21. Bruno O, Bondavalli F, Ranise A, Schenone P, Losasso C, Cilenti L, et al. 3,5-Diphenyl-1*H*-pyrazole derivatives. V: 1-Acetyl-4-hydroxy-3, 5-diphenyl-2-pyrazoline esters, 4-hydroxy-3,5-diphenyl-1*H*-pyrazole esters and *N*- substituted-4-(3-amino-2-hydroxy-1-propoxy)- 1-methyl-3,5- diphenyl-1*H*-pyrazoles with antiarrhythmic, sedative and platelet antiaggregating activities. Farmaco, 1990; 45: 147–66.
22. Cottineau B, Toto P, Marot C, Pipaud A, Chenault J. Synthesis and hypoglycemic evaluation of substituted pyrazole-4-carboxylic acids. Bioorg Med Chem Lett, 2002; 12: 2105–8.