International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 1 | JAN-MAR| 2018 | 577-582
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF BENZIMIDAZOLES AND BISBENZIMIDAZOLES DERIVATIVES

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ABSTRACT

*A simple, reliable and environmental efficient has been described for the synthesis some new Benzimidazole and Bis-Benzimidazole derivatives as shown below with the objective of developing better anti-inflammatory compounds. The compounds were synthesized through one-pot reaction of aryl aldehydes with phenylenediamines using Cu (II) MCM 41 as catalyst and the compounds have achieved an excellent yield. The Cu (II) complex is recyclable and the activity is remained unchanged for three successive runs. All the new synthesized compounds were screened for anti-inflammatory at 200 mg/kg (i.p) in albino rats using carrageenan-induced rat hind paw oedema method using Indomethacin (10 mg/kg) as the standard drug. All the compounds exhibited significant activity. Compound **IIIj** has shown most promising and anti-inflammatory activity.*

KEY WORDS

Benzimidazole, Bis-Benzimidazole, Anti-inflammatory, Carrageenan

INTRODUCTION:

Inflammation is the defense mechanism to heal itself after an injury and against infections of virus and bacteria. It is the vital part of the human immune response and also repair the damaged tissues [1]. The protection response is by the production of inflammatory mediators including cyclooxygenase (COX), tumor necrosis factor (TNF). Interleukins (IL), nitric oxide (NO) etc., [2].

Benzimidazole has evolved as an important heterocyclic system due to its presence in a wide range of bioactive compounds like anti-inflammatory, anti-convulsants, analgesics, anti-histaminic, anti-ulcers, antihypertensives, antivirals, anticancer, anti-fungal, anti-inflammatory agents, and anticoagulants [3-7]. This research facilitated in development of bioactive molecules form benzimidazole was accelerated during last 10 years. Mono acyl derivative of o-phenylenediamine is readily converted into the corresponding benzimidazole which is carried out by simple heating [8]. It has been reported there are several methodologies for the preparation of benzimidazoles viz., Kelly has reported mono acyl derivative of diamine in an atmosphere of nitrogen to prevent oxidation [9], Bistrzycki has prepared the benzimidazole but at high temperature using diacyl derivatives and diamines [10]. Fischer has given simple method by heating o-phenylenediamine (OPD) with excess of mono or di-basic acid [11]. Philips has modified by refluxing OPD with mono basic acid in presence of 4N HCl (hydrochloric acid) [12].

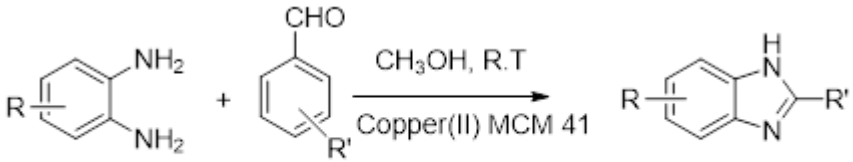
In view of promising biological activity of Benzimidazoles, a new series Benzimidazole and Bis-Benzimidazole have been synthesized as showed in **Scheme-I** and the structure of the synthesized compounds were confirmed by IR, ^{1}HNMR and Mass

analysis. In the paper, we investigate the anti-inflammatory effect of new Bezimidazole and Bis-Benzimidazole by using carrageenan induced paw oedema method.

MATERIALS AND METHODS:

Melting points were determined in open capillaries, using Toshniwal melting point apparatus, expressed in ^{o}C and are uncorrected. The IR spectra of the compounds were recorded on thermo Nicolet Nexus 670S series, FT-IR spectrometer using KBr disc.^{1}H NMR were recorded on a Avance-300 MHz instrument using TMS as an internal standard (chemical shifts in δ, ppm). Mass spectra were recorded on V.G.Autospec using EIS (+) method. Elemental analysis was performed on Perkin-Elmer series 2400 and satisfactory results ±0.4% of calculated values (C, H, N) were obtained. Copper (II) catalyst MCM 41 were purchased from sigma. The purity of the compounds was checked on silica gel-coated aluminium sheets by thin-layer chromatography (TLC).

General Procedure:



1 2 3

Scheme-I

SYNTHESIS OF BENZIMIDAZOLE (3):

A mixture of substituted aldehyde (0.106 g, 1.0 mmol), OPD (0.108 g, 1.0 mmol) and catalyst (5 mol %) in methanol (6 mL) was stirred at room temperature for appropriate time. On completion of the reaction, as indicated by TLC, the catalyst was separated by filtration. The reaction mixture was separated by filtration and recrystallised from methanol to yield benzimidazole and Bis-benzimidazole [13]. All produced were characterized by FT-IR, ^{1}H NMR and Mass spectral data.

Spectral data:

1. 2,2'-bis(4,5-dichlorophenyl)-1H-5,5' bibenzimidazole (IIIi):

Mol. F: $C_{26}H_{14}Cl_4N_4$; M. P: 312 ^{o}C. FT-IR (KBr, ν cm^{-1}): 3109 (N-H), 2810 (Ar-H), 1306 (Ar-Cl), 1471 (C=C), 802,698 (Ar-Cl). ^{1}H NMR (250 MHz, DMSO-δ_6): δ ppm = 7.32 – 7.75 (m, ArH, 8H), 8.41 (s, NH, 2H). MS (EI, 70 eV): m/z (%) = 448 (M+, 100%)

2. 2,2'-bis(4-nitrophenyl)-1H-5,5'-bibenzimidazole (IIIj):

Mol. F: $C_{26}H_{16}N_6O_4$; M. P: 328 ^{o}C. FT-IR (KBr, ν cm^{-1}): 3062 (N-H), 2862 (Ar-H), 1340 (-NO_2), 1446 (C=C), 964,764 (Ar-NO_2). ^{1}H NMR (250 MHz, DMSO-δ_6): δ ppm = 7.59 – 7.70 (m, ArH, 8H), 8.25 (s, NH, 2H). MS (EI, 70 eV): m/z (%) = 475.12 (M+, 100%)

3.2,2'-bis(4-chlorophenyl)-1H-5,5'-bibenzimidazole (IIIk):

Mol. F: $C_{26}H_{16}Cl_2N_4$; M. P: 356 ^{o}C. FT-IR (KBr, ν cm^{-1}): 3045 (N-H), 2825 (Ar-H), 1324 (-Cl), 1489 (C=C), 927,725 (Ar-Cl). ^{1}H NMR (250 MHz, DMSO-δ_6): δ ppm = 7.24 – 7.50 (m, ArH, 8H), 8.45 (s, NH, 2H). MS (EI, 70 eV): m/z (%) = 454.2 (M+, 100%)

4. 2,2'-dip-tolyl-1H-5,5'-bibenzimidazole (IIIl):

Mol. F: $C_{28}H_{12}N_4$; M. P: 375^{o}C. FT-IR (KBr, ν cm^{-1}): 3018 (N-H), 2914 (Ar-H), 1624 (-CH_3), 1286 (C=C), 823,727 (Ar-H). ^{1}H NMR (250 MHz, DMSO-δ_6): δ ppm = 2.37 (m, -2CH_3, 6H), 7.38 – 7.83 (m, ArH, 8H) 8.12 (s, NH, 2H). MS (EI, 70 eV): m/z (%) = 414.08 (M+, 100%).

Table 1: Physical data of Benzimidazole and Bis-Benzimidazole

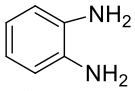
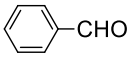
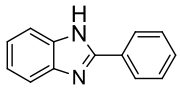
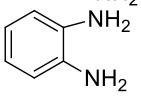
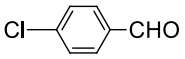
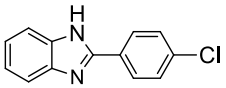
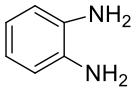
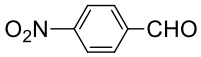
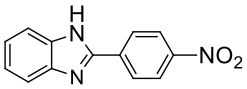
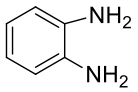
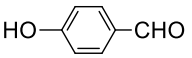
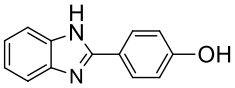
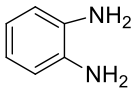
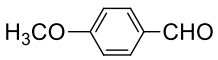
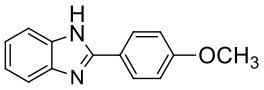
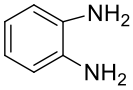
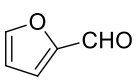
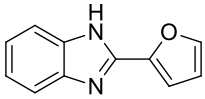
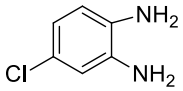
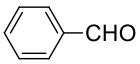
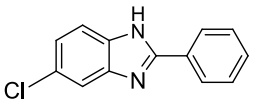
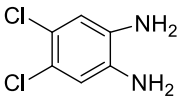
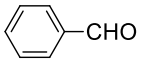
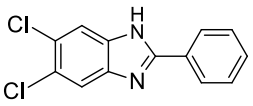
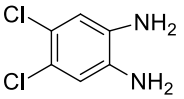
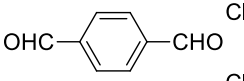
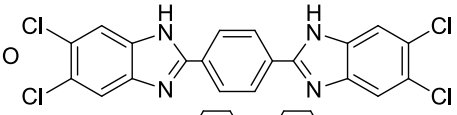
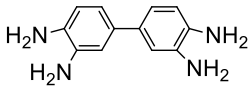
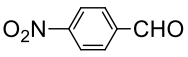
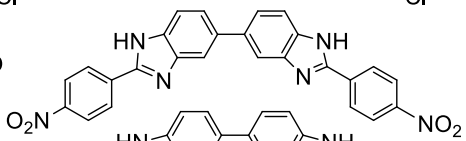
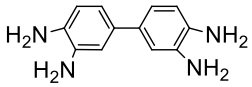
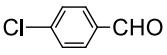
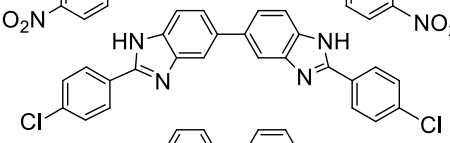
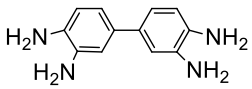
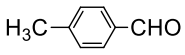
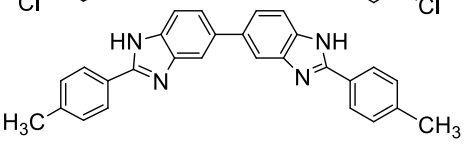
Entry	Diamine	Aldehyde	Product (B)1	Time (h)	Yield (%)
IIIa				4	96
IIIb				4	98
IIIc				6	86
IIId				5	90
IIIe				6	90
IIIf				6	93
IIIg				5	96
IIIh				5	95
IIIi				7	80
IIIj				8	82
IIIk				6	84
IIIl				6	88

Table 2: Optimization of catalyst

Sl. No.	Amount of Cu (II) MCM 41 Catalyst	Time (h)	Yield (%)
1	5	2	90
2	10	2	98
3	15	2	98.5

Table 3: Recycle of catalyst

Sl. No	No. of cycle	Yield (%)
Entry 1	1	98
Entry 2	2	97
Entry 3	3	96

Pharmacological screening:

Animals

The animals were procured from Mahaveer Enterprises, Hyderabad, India. Male Wister rats (160-210 gm) and Albino mice (25-30 gm) were used for assessing anti-inflammatory and analgesic activity respectively. The animals were acclimatized for a period of 14 days prior to performing the experiments and maintained under standard husbandry conditions and had free access to food and water *ad libitum.* The animals were divided into different groups each consist of six animals were fasted overnight prior to the experiments. The protocol of the present study was reviewed and approved by the Institutional Animal Ethical Committee (IAEC).

Biological activity:

Anti-inflammatory activity using Carrageenan induced rat hind paw edema method

The normal paw volumes of all the rats were measured initially and were divided into seven groups of six animals each and were treated with the vehicle as control (0.5 % sodium CMC), standard ibuprofen (50 mg/kg) and all the test compounds (**IIIa-IIII**) in sodium CMC; 0.1 % w/v, 50 and 100 mg/kg b.w.). Carrageenan (0.1 ml of a 1% suspension in saline) was injected into the sub plantar region of the right hind paw of each rat. The vehicle, Standard and test compounds were administered 30 min prior to the injection of Carrageenan. The swelling (paw volumes) produced after injection of the phlogistic agent was measured in all the rats at 1, 2, 3 and 4 hr after Carrageenan treatment by using plethysmometer**.** A significant reduction in the paw volume compared to vehicle treated control animals was considered an inflammatory response [14-16].

$$\% \text{ Inhibition} = [(V_T - V_0)\text{ control} - (V_T - V_0)\text{ treated groups}] / (V_T - V_0)\text{ control} * 100$$

V_0 = paw volume of the rat before administration of Carrageenan

V_T = paw volume of the rat after administration of Carrageenan at different time intervals

RESULTS AND DISCUSSION:

The synthesis of the Copper complex (II) MCM 41 was accomplished using literature methodology and previously reported procedure was used catalyst for the synthesis of Benzimidazole and Bis-Benzimidazole. The synthesis of benzimidazole were carried out by reacting OPD with substituted benzaldehydes using copper as catalyst. All the test compounds were purified with column chromatography and the reactions are monitored with TLC. The physical constants were shown in **Table-1**. The compounds were prepared by the methodologies outlined in **Scheme-I.**

The IR spectra of all compounds IIIa-l show absorption bands at around 3100-3018 (-NH) and 3080- 3030 cm^{-1} (=C-H). The ^1H NMR spectra of compounds shows 8.45-8.12 ppm for –NH and δ7.10- 7.85 ppm for Aromatic protons. For compound 2, 2'-dip-tolyl-1H-5,5'-bibenzimidazole (IIII) the methyl protons have shown at δ 2.45 ppm. All the compounds were recorded for mass spectra and have shown molecular ion as base peak.

Optimisation and Re-use of catalyst:

Initially, the amount of catalyst used is optimized by gradually increasing the concentration of Cu (II) MCM 41. From the **Table-2** it was relieved that at 15% mole of catalyst the reaction have achieved very good yields [17].

From an environment view, it is desirable to minimize the organic waste due transformation. In this context, the catalyst was recycled for subsequent runs. The recyclability of the catalyst was determined by reaction between benzaldehydes and OPD in presence of Cu (II) MCM 41 catalyst in ethanol. The catalyst was separated after the reaction and the recycled catalyst was used for further runs, it was found that its catalyst activity does

not show any significant decrease even after 3 runs and with good yields **(Table 3)** [18].

***Anti-inflammatory activity*:**

The anti-inflammatory activity of the eight Benzimidazole and Bis-Benzimidazole **IIIb-d, IIIh-l** compounds were studied at dose of 200 mg/kg (b.w) using carrageenan-induced paw edema model in rats and the results were presented in **Table 4** and % inhibition of paw edema were shown in **Figure 1**. From these data it appears that all compounds reduced paw oedema in comparison to the control group at 1, 2, 4 and 6 hrs post carrageenan injection significantly which are comparable with standard Indomethacin (10 mg/kg)

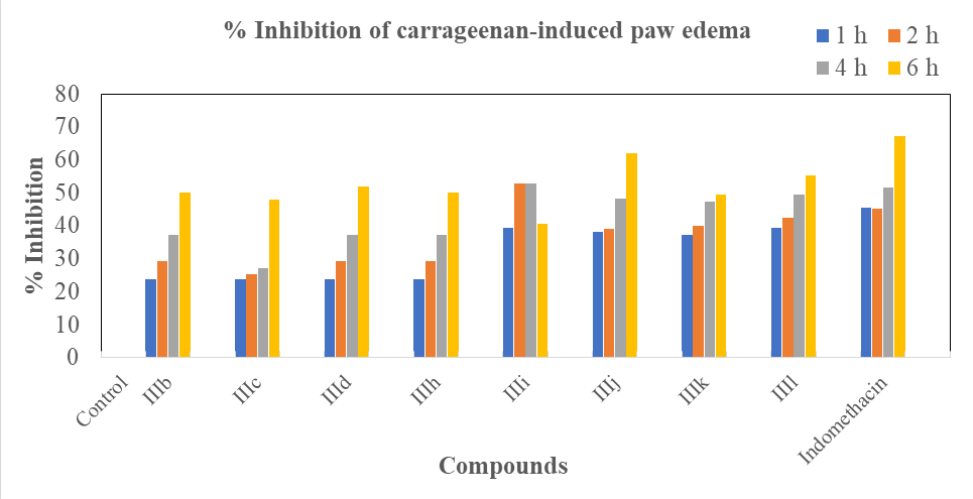


Figure 1: % Inhibition of the synthesized compounds using paw oedema method in rats

Table 4: Anti-inflammatory of the synthesized compounds using paw oedema method in rats

Sl. No	Compounds	Paw edema volume in ml (200 mg/kg)			
		1 h	2 h	4 h	6 h
1	Control	0.42 ± 0.06	0.51 ± 0.03	0.62 ± 0.04	0.68 ± 0.06
2	Indomethacin (10 mg/kg)	0.23 ± 0.06 (45.5)	0.28 ± 0.05 (45.09)	0.3 ± 0.11 (51.61)	0.29 ± 0.1 (67.35)
3	IIIb	0.31 ± 0.08 (23.80)	0.35 ± 0.13 (29.41)	0.38 ± 0.07 (37.09)	0.31 ± 0.15 (50)
4	IIIc	0.30 ± 0.12 (23.7)	0.34 ± 0.11 (25.4)	0.37 ± 0.03 (27.10)	0.32 ± 0.11 (48)
5	IIIe	0.31 ± 0.11 (23.79)	0.33 ± 0.09 (29.31)	0.36 ± 0.03 (37.09)	0.31 ± 0.12 (52)
6	IIIf	0.32± 0.12 (23.80)	0.36 ± 0.10 (29.41)	0.39 ± 0.08 (37.09)	0.34± 0.12 (50)
7	IIIg	0.25 ± 0.05 (39.4)	0.24 ± 0.07 (52.9)	0.29 ± 0.01 (52.8)	0.31 ± 0.09 (40.5)
8	IIIi	0.26 ± 0.02 (38.09)	0.31 ± 0.07 (39.21)	0.32± 0.06 (48.38)	0.27 ± 0.11 (61.87)
9	IIIj	0.28±0.13 (37.19)	0.33 ± 0.08 (40.10)	0.34 ± 0.08 (47.24)	0.29 ± 0.10 (49.58)
10	IIIh	0.29 ± 0.11 (39.26)	0.31 ± 0.06 (42.52)	0.32 ± 0.07 (49.33)	0.27 ± 0.12 (55.29)

Among the tested compounds, **IIIb, IIId, IIIj,** and **IIIl** were considered to have potent anti-inflammatory activity and was comparable with standard anti-inflammatory drug, indomethacin. It was found that, **IIIj** revealed slightly enhanced anti-inflammatory properties than those of the corresponding derivatives with the percentage inhibition of 61.87 %. The results indicated that the introduction of electron-withdrawing groups resulted in increased activity. Presence of halogens on the aryl ring results in increased activity and Bis-

Benzimidazole have shown more potent activity than Benzimidazole derivatives.

CONCLUSION:

The above results define the synthesis of Bis-Benzimidazole using copper as catalyst and these derivatives were screened for anti-inflammatory activity using carrageenan induced paw edema method. From current preliminary investigations that there is a need for further advanced studies, at minimum on the few of the test compounds which are found to be superior. The prospect that these compounds would be choosy COX-2 inhibitors will be explored in our future studies.

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