IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Synthesis Characterization And Screening Of Anti-Inflammatory Activity Of Benzimidazole Derivatives

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ABSTRACT: Benzimidazole derivatives were synthesized in two steps. All synthesized Benzimidazole derivatives were characterized for Molecular formula, Physical state, Color, Melting point, Yield, Solubility and R_f - value and structure of synthesized Benzimidazole derivatives were elucidated using spectrogram data as well as physical method e.g. FT-IR, and ^1H NMR. Moreover, synthesized compounds characterized by the TLC and melting point. In addition, by visual inspection synthesized compounds also characterized. Novel synthesized Benzimidazole derivatives were evaluated *in- vitro* for anti-inflammatory activity by kit available in market estimate the inhibition of Cox-1 and Cox-2 activity. Aspirin was used as standard drug; aspirin is a well known non selective Cox- inhibitor drug. Novel synthesized Benzimidazole derivatives BIZ-4 was exhibited excellent anti-inflammatory activity against COX-1 & Cox-2 both with IC_{50} value less than <1mM. BIZ-1, BIZ-2, BIZ-3, BIZ-5 compound were possessed poor to moderate activity against both COX-1 & Cox-2. It can be concluded from anti-inflammatory study of Novel synthesized Benzimidazole derivatives that inhibition was increased with concentration of samples increased.

KEYWORD: Benzimidazole, Solubility, Anti-inflammatory, FT-IR, and NMR.

INTRODUCTION

Inflammation conditions that effect people millions of in their life span. Prostaglandins are produced within the body's cell by the enzyme cyclooxygenase[1] (COX-1, COX-2). Both enzyme produce prostaglandin that promote inflammation, pain, fever, the prostaglandin that protect the stomach and support platelet and blood clotting also are reduced[2]. NSAID are the common choice for the treatment of a number of inflammatory diseases associated with a number of pathogenic conditions[3]. The major side effects of NSAIDs are their gastrointestinal ulcerogenic activity. COX-2 in contrast is induced in inflammatory cells in response[4]. NSAIDs can cause ulcer in the stomach and promote bleeding[5]. NSAID increase the risk potentially fatal, stomach and intestinal adverse reaction for example, bleeding, ulcers and perforation of stomach or

intestines. COX-2 inhibitors cause less bleeding and fewer ulcer than other NSAID drug Rofecoxib, Valdecoxib[6].

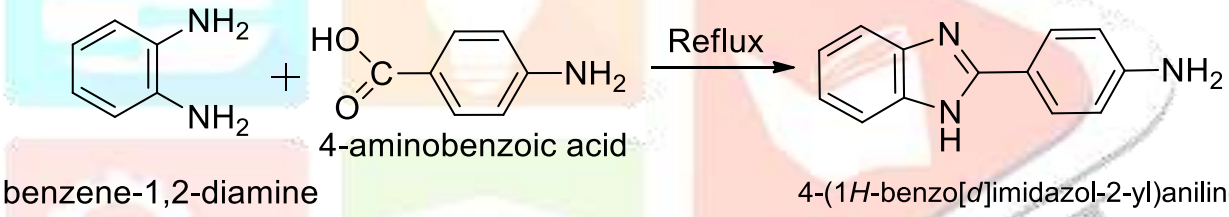
Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibit a range of biological activities[7]. This ring system is involves in numerous activities e.g. antimicrobial, antifungal, anti-tuberculosis, anti-aggregatory, vasodialating, antithrombic, antidepressant, anticonvulsant, anti-inflammatory, antihypertensive, lipid lowering activity, anti-oxidant activity[8,9,10]. Benzimidazoles possess the anti-inflammatory potential[11]. Considering all the above facts we are synthesizing Benzimidazole derivatives and screened for their anti-inflammatory having lesser side effect.

MATERIALS AND METHODS

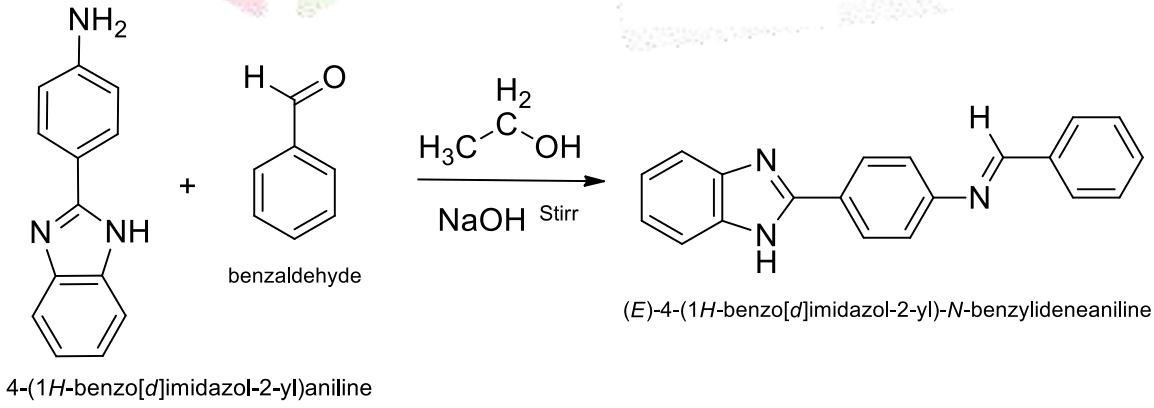
Benzene-1,2-diamine, 4-aminobenzoic acid, Benzaldehyde were purchased from Himedia (Mumbai). 4-Methyl benzaldehyde, 4-Nitro benzaldehyde, 4-Chloro benzaldehyde, 4- Methoxy benzaldehyde were purchased from Sigma Aldrich Co. Other solvents and reagents were belongs to L.R. grade.

Methods of Synthesis of Benzimidazole Derivatives

(a) Synthesis of Benzimidazole (4-(1H-benzo[d]imidazol-2-yl)aniline) [BIZ-0]: Benzimimdazoles were synthesized by stirring Benzene-1,2-diamine (O-Phenylene diamine) (15 mmol) and substituted 4-amiinobenzoic acid (30 mmol) in 4N HCl (40ml) with reflux for 5 hours. Mixtures were cooled at room temperature then add NaOH (Solid) to neutralize the solution. The obtained solids were filtered, washed with cold water, dried in using vacuum and recrystallized with methanol obtained product was 4-(1H-benzo[d]imidazol-2-yl)aniline.



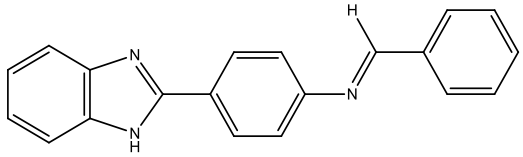
(b) Synthesis of targeted Benzimidazole Derivatives: Benzimidazole synthesized in first step 4-(1H-benzo[d]imidazol-2-yl)aniline (10mmol) was taken in a beaker with 2M NaOH (10ml) and to it was added a solution of Benzaldehyde (10mmol) in methanol (50ml) drop wise. The mixture was stir by a magnetic bead on a hot plate at 65-70 ^{O}C for three hours. After cooling, the mixture was filtered and evaporated under reduced pressure. The product obtained, washed with ethanol and dried.



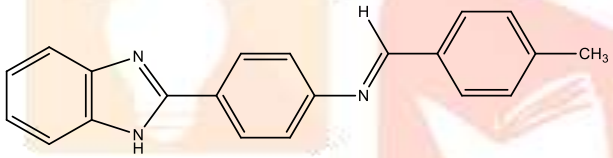
RESULTS AND DISCUSSION

Characterization and Structural Elucidation of Novel Synthesized Benzimidazole

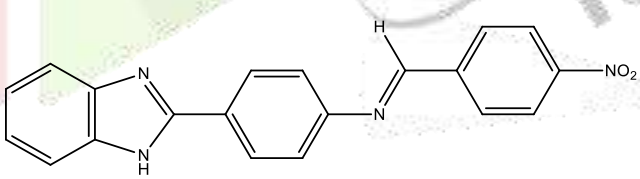
(A) (E)-4-(1H-benzo[d]imidazol-2-yl)-N-benzylideneaniline (BIZ-1): $C_{20}H_{15}N_3$; Yellowish, Crystalline solid; Melting Point (153-155^{O}C), Yield (53.10%,); **FT-IR (KBr):** cm^{-1} 1681 N=C str. (imine), 1655 C=N str. (Ar), 1608 C=C str. 1540 C-C str. (Ar), 1489 C-H str., 1325 C-H str. **^{1}H NMR ($CDCL_3$, 400 MHz):** δ 8.66 (s, 1H, CH- imine), 7.83(d, 2H, *J* = 6.08, CH- Ar.), 7.79(d, 2H, CH-Ar), 7.65(d, 2H, CH-Ar), 7.59(d, 2H, *J* = 3.12, CH-Ar.), 7.52(t, 3H, *J* = 3.12, 3.4, CH-Ar), 7.22(t, 2H, *J* = 3.12, 3.4, CH-Ar.), 5.01(s, 1H, NH- imidazol ring), ppm.



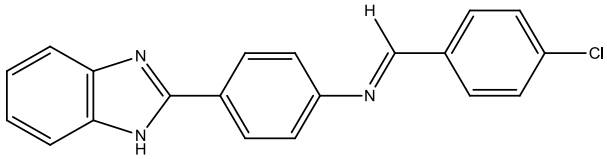
(B) (E)-4-(1H-benzo[d]imidazol-2-yl)-N-(4methylbenzylidene) aniline (BIZ-2): $C_{21}H_{17}N_3$; Colorless, Crystalline solid; Melting Point (149-151 ^{O}C), Yield (66.41%,); **FT-IR (KBr):** cm^{-1} 2822 C-H str. (alkane), 1679 N=C str. (imine), 1634 C=N str. (Ar), 1534 C-C str. (Ar). **^{1}H NMR ($CDCl_3$, 400 MHz):** δ 8.66 (s, 1H, CH- imine), 7.79(d, 2H, CH-Ar), 7.71(d, 2H, *J* = 6.08, CH- Ar.), 7.65(d, 2H, CH-Ar), 7.59(d, 2H, *J* = 3.12, CH-Ar.), 7.28(d, 2H, *J* = 3.12, CH-Ar), 7.22(t, 2H, *J* = 3.12, 3.4, CH-Ar.), 5.01(s, 1H, NH- imidazol ring), 2.34(s, 3H, -CH_3.), ppm.



(C) (E)-4-(1H-benzo[d]imidazol-2-yl)-N-(4-nitrobenzylidene) aniline (BIZ-3): $C_{20}H_{14}N_4O_2$; Pale Yellow, Crystalline solid; Melting Point (185-187 ^{O}C), Yield (71.03 %,); **FT-IR (KBr):** cm^{-1} 1678 N=C str. (imine), 1647 C=N str.(Ar), 1618 C=C (Alkene), 1552 C-C str. (Ar), 1435 C-H str., 1313 C-H str., 1221 N-O str. **^{1}H NMR ($CDCl_3$, 400 MHz):** δ 8.66 (s, 1H, CH- imine), 8.33 (d, 2H, CH-Ar), 8.09 (d, 2H, *J* = 6.08, CH- Ar.), 7.79(d, 2H, CH-Ar), 7.65(d, 2H, *J* = 3.12, CH-Ar.), 7.59(d, 2H, *J* = 3.12, CH-Ar), 7.22(t, 2H, *J* = 3.12, 3.4, CH-Ar.), 5.01(s, 1H, NH- imidazol ring), ppm.



(D) (E)-4-(1H-benzo[d]imidazol-2-yl)-N-(4-chlorobenzylidene) aniline (BIZ-4): $C_{20}H_{14}ClN_3$; off White, Crystalline solid; Melting Point (164-166 ^{O}C), Yield (67.52 %,); **FT-IR (KBr):**cm^{-1} 1686 N=C str. (imine), 1655 C=N str.(Ar), 1610 C=C (Alkene).1550 C-C str. (Ar), 1441 C-H str., 1351 C-N str. **^{1}H NMR ($CDCl_3$, 400 MHz):** δ 8.66 (s, 1H, CH- imine), 7.79(d, 2H, CH-Ar), 7.77(d, 2H, *J* = 6.08, CH- Ar.), 7.65(d, 2H, CH-Ar), 7.59(d, 2H, *J* = 3.12, CH-Ar.), 7.52(d, 2H, *J* = 3.12, CH-Ar), 7.22(t, 2H, *J* = 3.12, 3.4, CH-Ar.), 5.01(s, 1H, NH- imidazol ring), ppm.



(E) (E)-4-(1H-benzo[d]imidazol-2-yl)-N-(4-methoxybenzylidene) aniline (BIZ-5): $C_{21}H_{17}N_3O$; colorless, Crystalline solid; Melting Point (186-188 ^{O}C), Yield (72.37 %,); **FT-IR (KBr):** cm^{-1} 2826 C-H str.(alkane),

1647 C=N str.(Ar), 1602 C=C (Ar), 1547 C-C str. (Ar), 1439 C-H str., 1307 C-N str.. **^{1}H NMR (MeOD, 400 MHz):** δ 8.66 (s, 1H, CH- imine), 7.84(d, 2H, *J* = 6.08, CH- Ar.), 7.79(d, 2H, CH-Ar), 7.65(d, 2H, CH-Ar), 7.59(d, 2H, *J* = 3.12, CH-Ar.), 7.22(t, 2H, *J* = 3.12, 3.4, CH-Ar.), 7.08(d, 2H, *J* = 3.12, CH-Ar), 5.01(s, 1H, NH- imidazol ring), 3.83(s, 3H, -CH_3.), ppm.

***In-vitro* Anti-Inflammatory Evaluation by COX- Inhibition assay**

(A) COX- 1 Inhibition Assay:

Table No. 5: Effect of Novel Synthesized Benzimidazoles on COX-1

S. No.	Code of Sample	% Inhibition in different Concentrations			IC $_{50}$ Value (mM)
		0.1mM	1mM	10mM	
1	**BIZ-1**	28.83 ± 0.63	38.83 ± 0.21	51.23 ± 0.56	<10
2	**BIZ-2**	30.65 ± 0.54	46.76 ± 0.63	52.53 ± 1.65	< 10
3	**BIZ-3**	26.74 ± 1.11	38.85 ± 1.74	57.84 ± 1.62	< 10
4	**BIZ-4**	37.75 ± 1.42	**53.45 ± 1.22**	67.43 ± 1.11	< 1
5	**BIZ-5**	29.86 ± 0.35	43.26 ± 1.91	58.43 ± 1.16	**< 10**
6	**Aspirin**	45.34 ± 1.03	74.28 ± 1.35	91.12 ± 1.52	< 1

Results summarized are the mean values of n = 3 ± S.D

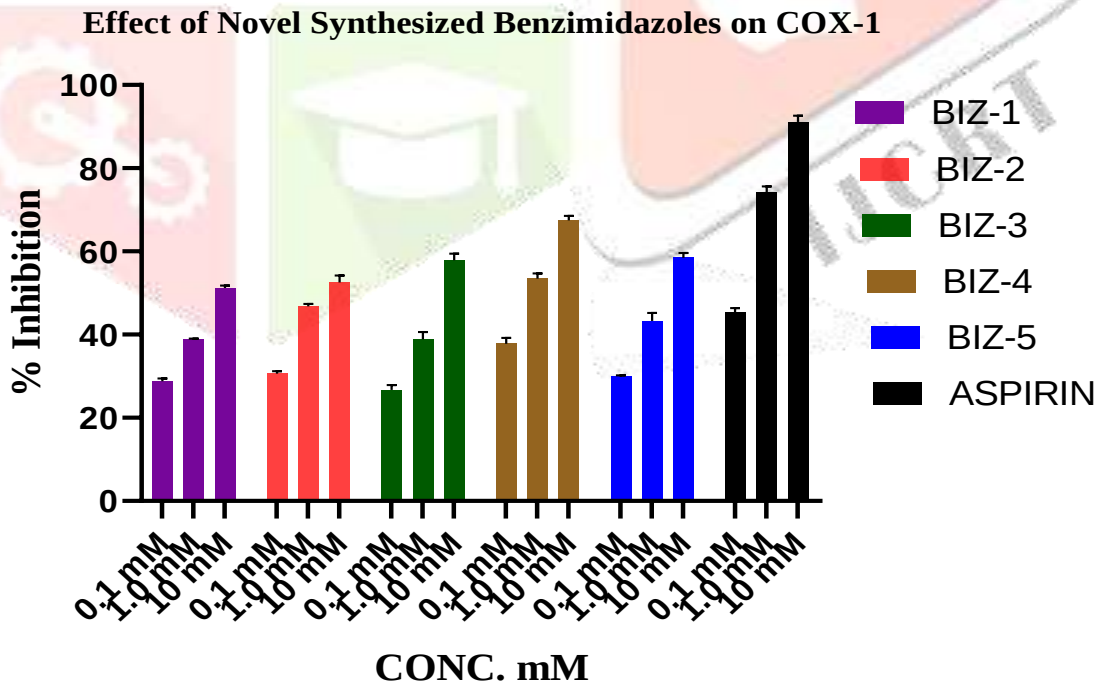


Figure 14: Effect of Novel Synthesized Benzimidazoles on COX-1

(B) COX- 2 Inhibition Assay:

Table No. 6: Effect of Novel Synthesized Benzimidazoles on COX-2

S. No.	Code of Sample	% Inhibition in different Concentrations			IC $_{50}$ Value
		0.1mM	1mM	10mM	
1	**BIZ-1**	19.24 ± 0.43	32.53 ± 1.62	45.74 ± 0.75	> 10
2	**BIZ-2**	20.54 ± 1.19	32.36 ± 0.85	49.69 ± 0.64	> 10
3	**BIZ-3**	24.37 ± 0.35	35.27 ± 0.91	75.74 ± 0.95	< 10
4	**BIZ-4**	38.74 ± 1.34	**57.32 ± 1.48**	72.78 ± 0.85	< 1
5	**BIZ-5**	30.87 ± 0.93	45.86 ± 0.24	68.86 ± 0.83	**< 1**
6	**Aspirin**	48.55 ± 1.73	58.50 ± 1.13	91.27 ± 0.22	< 1

Results summarized are the mean values of n = 3 ± S.D

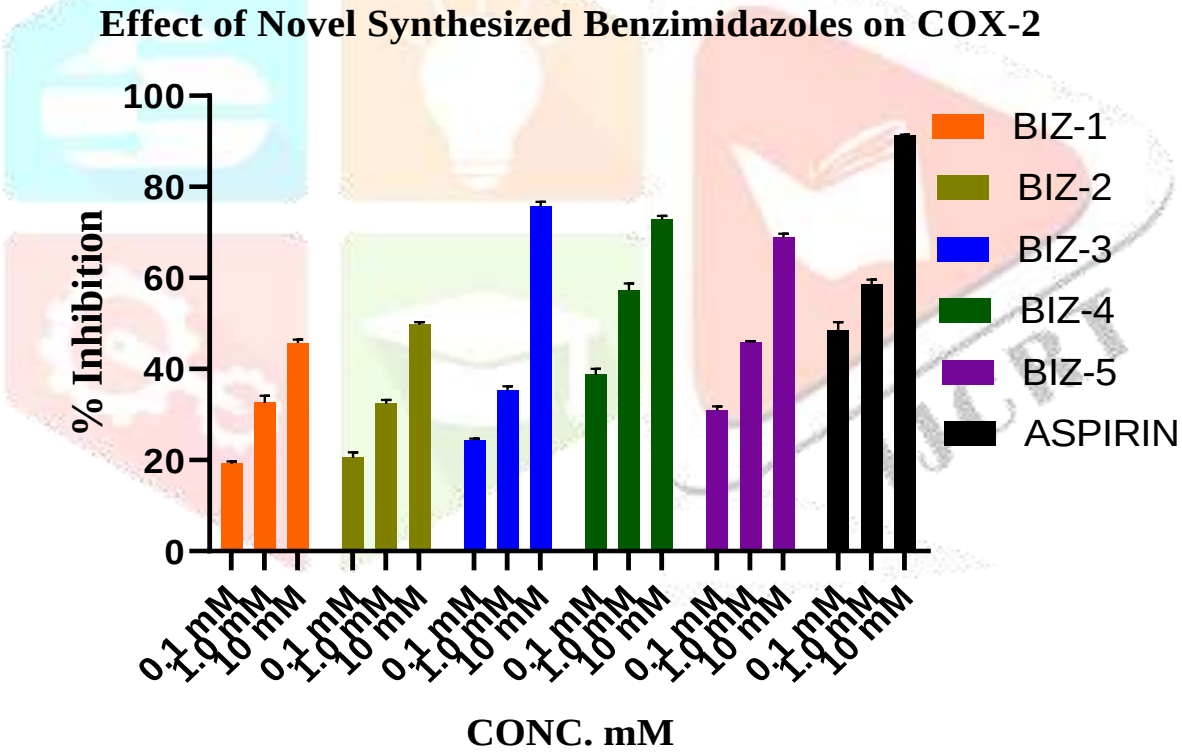


Figure 15: Effect of Novel Synthesized Benzimidazoles on COX-2

Discussion

7.3.1 Discussion on synthetic compounds:

Benzimidazole derivatives were synthesized in two steps. In the first step Benzimidazoles were synthesized by stirring O-Phenylene diamine (15 mmol) and substituted 4-aminobenzoic acid (30 mmol) in 4N HCl (40ml) with reflux for 5 hours. In second step, substituted Benzaldehydes was reacted with 4-(1H-benzo[d]imidazol-2-yl)aniline prepared in first step, gives different type of novel benzimidazole derivatives by the string onn hot plate in presence of 2M NaOH (10ml) and ethanol. In the second step aldehyde (-CHO) was reacted with amine (NH_2) group formed imine (–N=C-) bond.

All synthesized Benzimidazole derivatives were characterized for Molecular formula, Physical state, Color, Melting point, Yield, Solubility and R_f - value and structure of synthesized Benzimidazole derivatives were elucidated using spectrogram data as well as physical method e.g. Elemental analysis, FT-IR, ^{1}H NMR and Mass.

Appearance of absorption bands in derivatives at 1681 N=C str. (imine), which wass present in all Benzimidazole in FT-IR spectrum clearly indicated. These facts were further supported by ^{1}H NMR spectrum, presence of peak at 8.66 (s, 1H, CH- imine), 7.22(t, 2H, CH-Ar.), 5.01(s, 1H, NH- imidazol ring) ppm constantly in every Benzimidazole derivative, corresponding to their structure. Also molecular mass were found in Mass spectroscopy. Moreover, synthesized compounds characterized by the TLC and melting point. In addition, by visual inspection synthesized compounds also characterized.

7.4.2 Discussion on biological activity of synthesized compounds:

Novel synthesized Benzimidazole derivatives were evaluated *in- vitro* for anti-inflammatory activity by kit available in market estimate the inhibition of COX-1 and COX-2 activity. Aspirin was used as standard drug; aspirin is a well known non selective COX- inhibitor drug. Novel synthesized Benzimidazole derivatives BIZ-4 was exhibited excellent anti-inflammatory activity against COX-1 & COX-2 both with IC_{50} value less than <1mM. BIZ-1, BIZ-2, BIZ-3, BIZ-5 compound were possessed poor to moderate activity against both COX-1 & COX-2.

It can be concluded on the basis of structures of compounds that when electron withdrawing groups added on structure e.g. Cl more biologically active against both COX-1 & COX-2. Also it can be concluded from anti-inflammatory study of Novel synthesized Benzimidazole derivatives that inhibition was increased with concentration of samples increased.

CONCLUSION

Benzimidazole derivatives are interesting groups of heterocyclic compounds exhibiting diverse pharmacological activities. They have wide range of applications starting from antimicrobial, anti-inflammatory, analgesic, antitubercular, anticancer to antidepressant and antihypertensive activities. Reported structure based drug design too gives an emphasis on Benzimidazole moiety. We thought that these models as such for synthesis give good opportunities to look for discovering ideal lead for anti-inflammatory activity. On the basis of these synthesized some new Benzimidazole derivatives then carried out test for their anti-inflammatory action. Further design may prove an alternative and very useful and fruitful in the discovery of new anti-inflammatory activity in comparison to Aspirin as standard.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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