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Synthesis Analgesic and Anti-Inflammatory Activity of Some 2-Substituted 3-Acetic Acid Benzimidazole Derivatives

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ABSTRACT

A novel series of carboxylic acid benzimidazole derivatives were synthesized and characterized by ^{1}H NMR, IR. The compounds were screened for analgesic and *In-vitro* anti-inflammatory activity. 2-((p- Chlorostyryl) 3acetic acid) 1H benzimidazole 1 at 50 mg/kg was found to be equipotent to Indomethacin. Amongst synthesized derivatives 1,2,3 and 10 are found to have an effective analgesic response whereas compounds 1,2,3,8 and 10 have potent *In-vitro* Anti-inflammatory responce showing Inhibition of protein denaturation with respect to Indomethacin. All the experimental data were statistically significant at $P<0.001$ level.

Keywords: Benzimidazole; Analgesic; *In-vitro* Anti-inflammatory, Protein denaturation.

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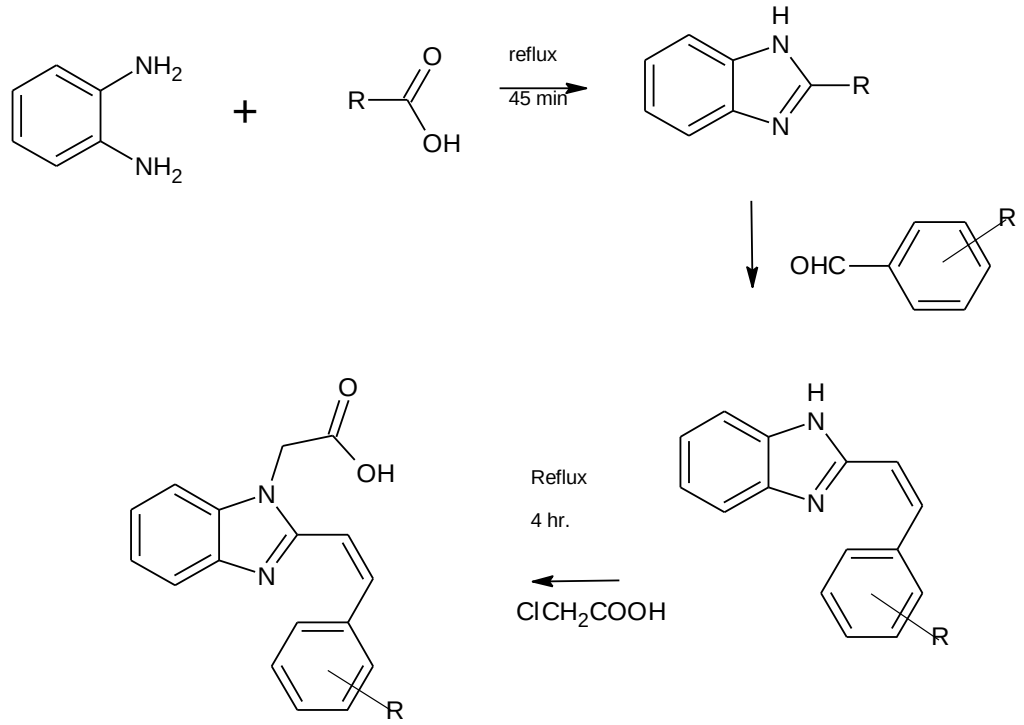
INTRODUCTION

The search for novel analgesic and anti-inflammatory agents devoid of side effects, such as irritant reactions on gastric mucosa, profound respiratory depression, nausea, constipation and physical dependence, continues to be an active area of research in medicinal chemistry. Among the various compounds developed as anti-inflammatory and analgesic agents, the 2-substituted benzimidazoles were reported to exhibit anti-inflammatory and analgesic properties. , 2-substituted benzimidazole with acetic acid group (CH_3COOH) at third position have pharmacological interest, prompts us to prepare a series of benzimidazole derivatives containing different substituents at 2nd position and acetic acid group at 3rd position and evaluate their biological activities. The drug containing benzimidazole moiety has good analgesic and antiinflammatory potential but also many limitations. Further efforts should be encouraged to ensure that novel products will be available in future. The aim of the present work is to incorporate both modules to the same molecule, with an anticipation of enhanced drug activity.

Chemistry

In this research work, the melting points of synthesized compounds were determined by open capillary tubes using paraffin bath and are uncorrected.



3a-3j

Figure 1: Protocol for Synthetic compounds.

R= p-Cl, m-NO_2, p-OCH_3, $N(CH_3)_2$, 2-OH, (3,4,5) OCH_3, CH=CH-C_6H_5, p-NO_2, C_6H_5, m-Cl.

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Thin layer chromatography is among the most useful tools for following the progress of organic chemical reactions and for assaying the purity of organic compounds. To prepare TLC, clean and dry glass plates were taken. Uniform slurry of silica gel-G in water was prepared in ratio of 1:2. The plates were prepared manually by spread plate method. These were dried first at room temperature and then kept for activation at 110°C for 1 hour. However, ethyl acetate: Benzene having proportion (1:2) was found suitable for most of the synthesized compounds. IR spectra were recorded on a JASCO FT-IR 4100 spectrophotometer, using KBr powder technique. The NMR spectra of the compounds were recorded on a Varian-NMR-mercury 300 MHz spectrophotometer from Pune University, in D_2O using DSS as an internal standard **.**

Biological Investigation

The synthesized compounds were evaluated for analgesic and *In-vitro* anti-inflammatory activities. The standard and test compounds were administered i.p. The statistical analysis was done by using One way ANOVA followed by Dunnet test.

Analgesic activity

Table 1 :- Percentage Inhibition showing Analgesic activity of 2-(substituted) 3- Acetic acid benzimidazole derivatives.

Group	Dose	No. of writhes ± SEM	Percentage Inhibition
Control	0.1ml/kg	38.5±0.56	-
Standard	15mg/kg	7.5±o.6*	80.52
3a	50mg/kg	8.16±0.8*	78.70
3b	50mg/kg	14.33±0.85*	62.78
3c	50mg/kg	12.66±1.2*	67.12
3d	50mg/kg	23.3±1.4	39.48
3e	50mg/kg	22.83±1.08	40.70
3f	50mg/kg	15.66±0.8*	59.32
3g	50mg/kg	19.3±0.97*	49.87
3h	50mg/kg	21.5±1.9	44.16
3i	50mg/kg	17.33±0.95*	54.99
3j	50mg/kg	12.66±1.2*	67.12

Male albino mice of weight range 20-25 gm were selected for study. They were grouped in different comprising of 6 animals in each group.

*p≤0.01, p≤0.05, ^{NS} Non significant, n=6
Data expressed as Mean ± SEM.
Data was analyzed by one-way ANOVA followed by Dunnett's test.

Before study food was withdrawn but animal had free access to water. Test compounds were administered orally at a dose of 50 mg/ kg body weight suspended in sterile water for injection. Standard group received Indomethacin at a dose of 15 mg/kg body weight

intraperitoneally at various pretreatment times prior to acetic acid administration. The mice are placed individually into glass beakers and after 30minutes administration of acetic acid, the mice are then observed for a period of 10 minutes and the number of writhes is recorded for each animal. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The formula for computing percent inhibition. The results are presented in Table 1.

$$\%\ inhibition = 100 - \frac{n`}{n} \times 100$$

Where, n = number of writhing in control,

n`= number of writhing in test,

In-vitro Anti-inflammatory activity

The standard drug Indomethacin and test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2M, pH7.4). Final concentration of DMF in all solution was less than

2.0%. Test solution (1ml) containing different concentration of drug was mixed with 1ml of 1%mM egg albumin solution in phosphate buffer and incubated at 27°C±1°C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60°C±1°C in water bath for 10 min. After cooling the absorbance of turbidity was measured at 660nm by UV-visible spectrophotometer. Percent inhibition of protein denaturation was calculated from control, where no drug was added. The results are presented in Table 2.

$$\%Inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} X\ 100$$

Table 2 :- IC_{50} values indicating % Inhibition of albumin denaturation

Conc.	25	50	100	150	200	250	300	350	400	450	500	IC50
Std	45.58	46.40	49.03	50.55	51.65	53./3	54.69	55.93	57.04	58.28	58.97	143.52
3a	46.40	47.37	49.44	50.55	51.79	52.34	54.28	56.07	57.04	57.77	58.42	142.17
3b	46.40	47.37	48.89	49.86	51.38	53.03	54.28	55.52	56.07	57.32	58.42	150.07
3c	45.99	46.96	49.0.3	50.41	52.20	52.90	54.97	55.66	56.76	57.45	58.28	147.88
3d	43.23	44.33	46.68	48.75	49.72	51.79	53.86	54.55	55.66	56.21	57.32	216.40
3e	42.40	43.50	45.03	46.96	48.75	49.72	51.65	55.52	53.86	55.80	57.45	258.17
3f	45.16	46.13	47.92	49.17	50.69	51.65	54.14	55.62	56.07	56.35	57.32	184.74
3g	40.74	42.12	44.33	45.16	47.37	48.06	48.75	49.72	51.24	52.20	53.31	358.65
3h	45.99	47.09	49.72	51.10	53.45	55.52	56.35	57.87	58.83	60	60.49	120.72
3i	41.85	42.95	45.58	46.40	47.65	48.75	49.72	51.51	52.62	53.45	55.66	318.68
3j	46.96	47.65	49.07	51.51	52.20	53.31	55.24	56.90	57.45	58.42	59.11	117.58

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RESULT AND DISCUSSION

Pharmacological Screening

All the synthesized compounds were subjected to analgesic activity at a 50 mg/kg, body weight dose using acetic acid induced writhing method in mice. All the synthesized compounds showed significant analgesic activity as compared to the standard Indomethacin. All the synthesized compounds were subjected to Invitro anti-inflammatory activity by using inhibition of albumin denaturation technique. The synthesized compounds has different concentration, the absorbance was measured and calculate the percentage inhibition and IC_{50} value. The results for evaluation of Analgesic and *In-vitro* Anti-inflammatory activity are presented in table 1 and 2 respectively.

Compounds 3a, 3b, 3c and 3j exhibited significant analgesicactivity with percent protection 78.70, 62.78, 67.12 and 67.12 respectively as compared to standard. Amongst all synthesized compounds could inhibit the denaturation of albumin in comparison with standard. The standard drug Indomethacin exhibited IC_{50} value are 143.52 ppm. Amongst all synthesized compounds 3a, 3b,3c,3h and 3j exhibited excellent anti-inflammatory activity with IC_{50} 142.17,150.07,147.88,120.72 and 117.58 ppm respectively as compared to standard. Compounds 3a,3b,3c,3h and 3j showed good anti-inflammatory activity as compared to standard with IC_{50} 142.17,150.07,147.88,120.72 and 117.58 ppm respectively.

Experimental Protocol

Route of synthesis

The synthesis of final compounds was carried out in three steps,

Step I. Synthesis of 2- Methyl Benzimidazole.

A mixture of o-phenyenediamine dihydrochloride (0.03Mole), 20 ml of water and (0.09Mole) of acetic acid were taken in a round bottom flask. The reaction mixture was refluxed for 45 minutes. Then the reaction mixture was poured over crushed icewith stirring. The cooled mixture was made basic by the addition of concentrated ammonia solution. The reaction was monitored by TLC precipitated product obtained was then filtered and recrystllized from ethanol.

Step II. Synthesis of 2- Substituted Benzimidazole derivatives.

The synthesized 2- Substituted Benzimidazole **(2a-2j)** were synthesized by taking a mixture of 2-methyl benzimidazole and substituted benzaldehydes into 50 ml beaker and heated on the heating mantle. When observed after 1hr the mixture becomes viscous and water elimination ceased. Finally the temperature increased to 200-210^{0}C and held for 0.5hr. The reaction was monitored with TLC the product obtained was recrystallised from ethanol.

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Step III. Synthesis of 2-Substituted 3-Acetic acid Benzimidazole derivatives.

The synthesis of acetic acid derivatives **(3a-3j)** were carried out by taking Chloroacetic acid in 20 ml of dry chloroform and 4 ml pyridine. To which an equimolar amount of 2- Substituted benzimidazole **(2a-2j)** was added and the reaction mixture was refluxed for 4hrs. The reaction was monitored by TLC after cooling the viscous residue was washed with acetone and recrystallized from ethanol.

2-((Dimethylamino styryl)-3 acetic acid)- benzimidazole.
IR(KBr) 3023.84Ar-CH, 2923.50C-H, 2865.7-OH , 1743.33C=O, 1643.05C=C, 1500.35 C=N, 1353.78C-N, 1099.23C-N , 944,701CH.

2-((m- nitro styryl) 3- acetic acid) benzimidazole.
IR (KBr) 3019.98Ar-CH , 2969.84C-H , 2865.85-OH , 1739.48C=O, 1643.05C=C, 1496.49C=N , 1438.64Ar-NO2, 1353.78C-N, 944.49C-H , 3073.98 C=C-H .

2-((vinyl styryl) 3- acetic acid) benzimidazole.
IR (KBr) 3019.98Ar-CH , 2965.98C-H , 2865.7-OH , 1739.48C=O, 1638.02C=C, 1496.49 C=N, 1434.78C-H , 1319.67C-N , 957 C-H .

2-((3,4,5 trimethoxy styryl) 3-acetic acid benzimidazole.
IR (KBr) 3023.84Ar-CH , 2865.7C-H , 2742.28 OH, 1735.62C=O, 1639.2C=C
1496.49 C=N, 1434.76C-H, 1319.67C-N, 755.959798.385C-H, 1214.93Ar-O-R.
1H NMR (D2O): δ 3.7((s) 3H), δ 3.8((s) 6H),δ 5.0((s) 2H),δ 6.5((d) 1H), δ 6.7 ((d)1H), δ 7.0((s) 2H), δ 7.3((t) 1H) δ 7.5((t) 1H), δ 8.0((d) 2H).

2-((p- nitro styryl) 3- acetic acid) benzimidazole.
IR (KBr) 3019.98Ar-CH , 2969.84C-H , 2865.85-OH , 1739.48C=O, 1643.05C=C, 1496.49C=N , 1520.45Ar-NO2, 1353.78C-N, 944.49C-H , 3073.98 C=C-H .

2-((m-chloro styryl) 3- acetic acid) benzimidazole.
IR (KBr) 3023.84 Ar-CH , 2969.84C-H , 2865.7 -OH , 1731.76 C=O, 1639.20C=C, 1496.49C=N , 813.81Ar-Cl, 732.61C-H , 1315.21 C-N, 2969.84
-CH.

2-((p-chloro styryl) 3- acetic acid) benzimidazole.
IR (KBr) 3058.55 Ar-CH , 2955.45C-H , 2795.95 -OH , 1751.67 C=O, 1693.91C=C, 1554.34C=N , 840.81Ar-Cl, 735.55C-H , 1361.50 C-N, 2960.54
-CH.

2-((styryl) 3- acetic acid) benzimidazole.
IR (KBr) 3029.85Ar-CH , 2945.56 C-H , 2896.21-OH , 1725.20C=O, 1640.20C=C, 1501.02 C=N ,1437.21C-H , 1391.65C-N ,755 C-H .

2-((m-hydroxy styryl) 3- acetic acid) benzimidazole.
IR (KBr) 3023.23 Ar-CH , 2865.7C-H , 2796.05 -OH , 1690.12 C=O, 1665.20C=C, 1502.05C=N , 3235.7Ar-OH, 735.8C-H , 1312.25 C-N, 3001.23
-CH.

SUMMARY AND CONCLUDING REMARKS

The title compounds 2-substituted 3 acetic acid derivatives **(3a-3j)** were successfully synthesized from 2-substituted benzimidazoles and chloroacetic acid in the presence of catalytic amount of pyridine in dry chloroform. Amongst all synthesized compounds **3a,3b,3c** and **3j** exhibited significant analgesic activity with percent protection **78.70, 62.78, 67.12** and **67.12** respectively as compared to standard. The standard drug Indomethacin exhibited IC_{50} value are **143.52 ppm.** Amongst all synthesized compounds **3a,3b,3c**,**3h,3j** exhibited excellent anti-inflammatory activity with IC_{50} **142.17,150.07,147.88,120.72 and 117.58 ppm** respectively as compared to standard. 2-substituted 3 acetic acid benzimidazole have shown significant analgesic activity on acetic acid induced writhing model indicating predominantly inhibition of peripheral pain mechanism. 2-substituted 3 acetic acid benzimidazole also shown Anti-inflammatory activity on *In-Vitro* anti-inflammatory model indicating the Inhibition of protein denaturation. The result of the research work proves that 2-substituted 3 acetic acid benzimidazole can lead to a potential drug candidate in future. Research can be further done with a hope to develop the lead molecule and get a better drug candidate.

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