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[Research article]

In-vivo analgesic, anti-inflammatory and central nervous system - locomotor activities of certain synthesized 2-[(1-((phenyl amino) methyl) substituted 1H-benzoimidazol-2-yl) alkyl] isoindoline-1, 3-diones.

S. Selvakumar*[2], P.C.Agarawal[1], I. Sudheer Babu[2].

[1]*Department of Pharmacy, Bundelkhand University, Jhansi, Uttar Pradesh, India.*

[2]*Department of Pharmaceutical Chemistry, Sir.C.R.Reddy College of Pharmaceutical Sciences, West Godavari (Dist), Eluru, Andrapradesh, India.*

***Corresponding Author: S.Selvakumar**

ABSTRACT

Two homologous series of 1, 2-disubstituted benzimidazoles carrying isoindolines (6a-n) were synthesized by mannich type reaction of 2-alkyl benzimidazolyl isoindoline-1,3-dione (4a-b) with different substituted aromatic primary amines (5a-g) using formaldehyde in acid as a condensing agent. The pthalic anhydride (1) and amino acids -glycine, alanine (2a-b) fused at 180° c to give 2-(1, 3-dioxoisoindolin-2-yl) carboxylic acids (3a-b). These acids undergo cyclization with 1, 2 diaminobenzene yield (4a-b). The structures of the synthesized isoindolines were confirmed by spectral analysis. The synthesized benzimidazolyl isoindoline were screened for their in-vivo antinociceptive activities. Most of the synthesized isoindolines exhibited significant analgesic and anti-inflammatory activities. Among this isoindolines 6c, 6d, 6e, 6f, 6l, 6k, 6m, 6n were showed appreciable antinociceptive potency. The isoindolines (4a-b) and (6a-n) were screened for their in-vivo central nervous system locomotor activities. Among these tested isoindolines 4a, 4b, 6a, 6b, 6e, 6f were only shows elevated central nervous system depressant potency.

Keywords: Benzimidazoles, Benzimidazolyl Isoindolines, Isoindolines, Antinociceptive, Analgesic, Anti-inflammatory, Central Nervous System Locomotor Activity

INTRODUCTION

The pharmacology of heterocyclic's especially nitrogen containing fused analogues such as benzodiazepine, benzimidazole, phenothiazine, indole, purine, quinoline, pteridine have been well-recognized for their anti-infective, antibacterial, anti-tubercular, antiviral, antifungal, antiprotozoal, anthelmintics, CNS stimulant, diuretics, antimalarial, antiviral, antineoplastics[(1)], hypnotics, antiepileptic, antihistamines, anti-inflammatory, antiarrhythmic, antihypertensive, antipsychotic activities [(2)]. Out of the above mentioned heterocyclics, benzimidazole and indole derivatives are interested due to their high affinity on the biological membranes. Literature review displayed that benzimidazole derivatives are reported to possess a antimicrobial [(3)], antiviral[(4)], antifeedant, acaricidal [(5)], antiproliferative agent [(6)], antitubercular [(7)] ,

*** Corresponding author: Dr.S.Selvakumar**
E-mail address: drselvakumar69@gmail.com

antiulcer [8] , antioxidant , anthelminitic [9] , analgesic, anti-inflammatory [10], HIV-RT inhibitor [11], central nervous system depressant [12] , anticancer [13], DNA topoisomerase inhibitors [14], antibacterial [15] and antifungal [16] activities. The literature survey reports that indoline derivatives also displayed wide range of biological activities such as antimicrobial [17], antibacterial [18], analgesic [19], anti-inflammatory [20], cyclooxygenase inhibitor [21], antihistamine [22], antioxidant [23], antiproliferative [24], neuroprotective [25], acetylcholinesterase inhibitors [26], inhibitor of human neuronal nitric oxide synthase [27]. We after following this reports, it was thought of interest to merge both of indoline and benzimidazole moieties attempted by synthesise which may give the expected pharmacological activity. So our research work involves the synthesis of some new benzimidazole carrying isoindoline heterocyclics followed by *in-vivo* analgesic, anti-inflammatory and central nervous system locomotor screening evaluation of the derivatives.

EXPERIMENTAL

Synthesis of 2-(1, 3-dioxo isoindolin-2-yl) alkyl acids (3a & 3b)

An equimolecular quantity of pthalic anhydride (1) and glycine (2a) & alanine (2b) in a glass beaker were kept in a previously heated sand bath at 180-185^{o}C. The beaker heated for five minutes with continuous stirring, melted mixture was kept aside for few minutes allow to cool, the liquid mass solidified. The white solids (3a-b) obtained were then recrystallised from ethyl alcohol.

Synthesis of 2-(1-(1-benzimidazol-2-yl) alkyl) isoindoline-1, 3-dione (4a & 4b)

The 0.1 molar quantity of (3a-b) and 0.1 molar of 1,2 diaminobenzene were taken in a round bottomed flask, refluxed in 30 ml of 4N hydrochloric acid for two hours. The reaction mixture was cooling gave a product's 2-((1-benzoimidazol-2-yl) methyl) isoindoline-1,3-dione (4a) and 2-(1-(1-benzoimidazol-2-yl) ethyl) isoindoline-1,3-dione (4b).which were filtered with ice cold water, dried and then recrystallised from ethyl alcohol.

Synthesis of 2-alkylisoindole-1, 3-dione analogues of 1-substituted benzimidazoles (6a-n)

The 0.1 molar quantity of (4a-b) was taken in a round bottomed flask, with 0.2 molar 35% formaldehyde mixed in acetic acid and to this 0.2 molar quantity of following primary aromatic amines (5a-g) was added namely aniline (5a), para-aminobenzoicacid (5b), anthranillic acid (5c),sulphanilic acid (5d), sulphonamide (5e), ortho-aminophenol (5f) and para-aminophenol (5g), and the mixture was refluxed for 4 hours. The flask mixtures were cooled and poured in to a beaker containing ice cold water with stirring. The precipitated products 2-((1-((phenylamino) methyl)-1-benzoimidazol-2-yl) methyl) isoindoline-1,3-dione (6a),2-(1-(1-((phenylamino) methyl)-1-benzoimidazol-2-yl) ethyl) isoindoline-1,3-dione (6b) 4-((2-((1,3-dioxoisoindolin-2-yl) methyl)-1-benzoimidazol-1-yl) methylamino) benzoicacid (6c), 4-((2-(1-(1,3-dioxoisoindolin-2-yl) ethyl)-1-benzoimidazol-1-yl) methylamino) benzoic acid (6d), 2-((2-((1,3-dioxoisoindolin-2-yl) methyl)-1-benzoimidazol-1-yl) methylamino) benzoic acid (6e), 2-((2-(1-(1,3-dioxoisoindolin-2-yl) ethyl)-1-benzoimidazol-1-yl) methyl amino) benzoicacid (6f), 4-((2-((1,3-dioxoisoindolin-2-yl) methyl)-1-benzo imidazol-1-yl) methylamino) benzene sulfonicacid (6g), 4-((2-(1-(1,3-dioxoisoindolin-2-yl)ethyl)-1-benzo imidazol-1-yl) methylamino) benzene sulfonicacid (6h), 4-((2-((1,3-dioxoisoindolin-2-yl) methyl)-1-benzoimidazol-1-yl) methylamino) benzene sulfonamide (6i), 4-((2-(1-(1,3-dioxo isoindolin-2-yl)ethyl)-1-benzoimidazol-1-yl) methylamino) benzene sulfonamide (6j), 2-((1-((2-hydroxy phenylamino)methyl)-1-benzoimidazol-2-yl) methyl) isoindoline-1,3-dione (6k), 2-(1-(1-((2-hydroxyphenylamino)methyl)-1-benzoimidazol-2-yl) ethyl) isoindoline-1,3-dione (6l), 2-((1-((4-hydroxyphenylamino)methyl)-1-benzoimidazol-2-yl) methyl) isoindoline-1,3-dione(6m),2-(1-(1-((4-hydroxyphenylamino)methyl)-1-benzoimidazol-2-yl)ethyl)isoindoline -1,3-dione (6n) were filtered, kept overnight in a freezer, dried and recrystallised from ethyl alcohol. The purity of the synthesized benzimidazolyl isoindolines were identified by melting point determination and thin layer chromatography using coated silica gel-60 F 254 aluminium

sheets using chloroform: ethanol (8:2) as a solvent eluent followed by visualized in a ultra violet chamber (Table-1).

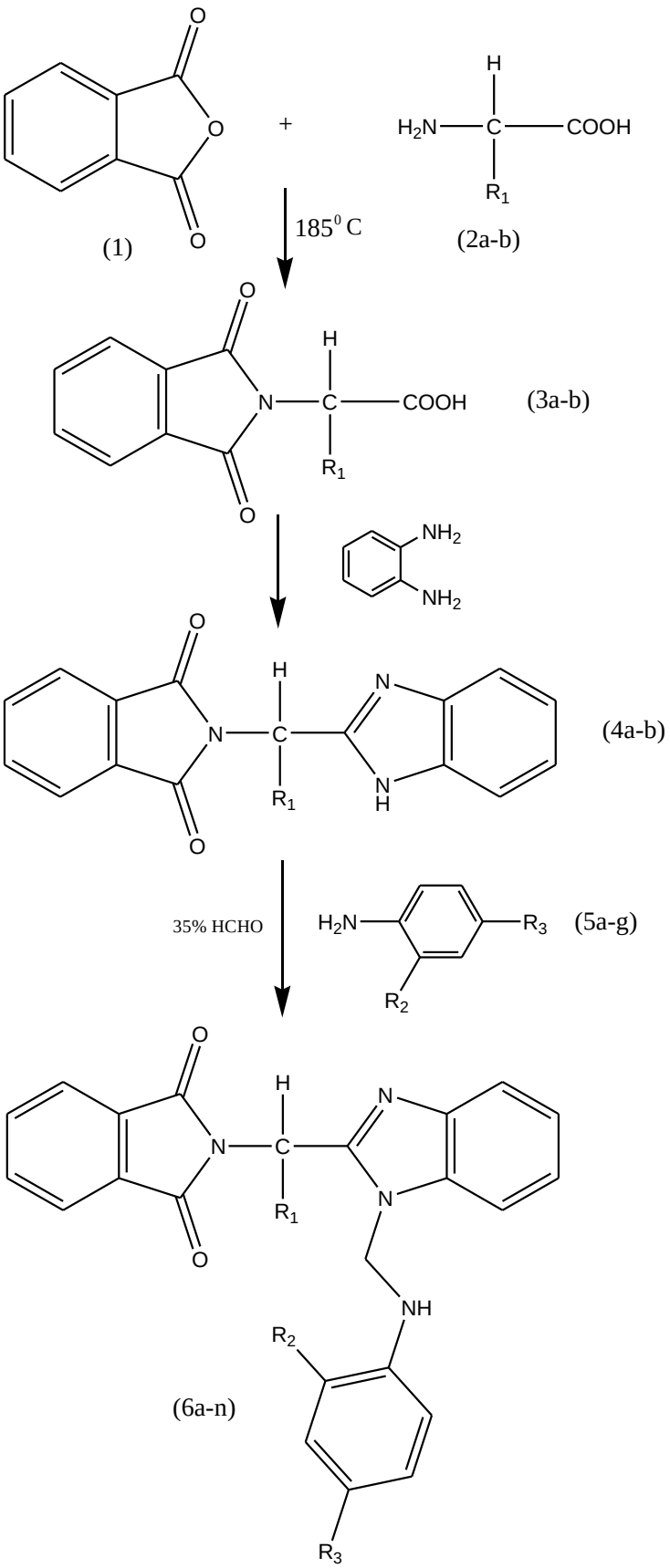
Table-1 Physical analysis of synthesised benzimidazolyl isoindolines

S no	Indolines	Molecular formula	R_1	R_2	R_3	Melting Point ^oC	R_f value	Yield %
1	4a	$C_{16}H_{11}N_3O_2$	H	-	-	186-187	0.76	78
2	4b	$C_{17}H_{13}N_3O_2$	CH_3	-	-	192-193	0.72	75
3	6a	$C_{23}H_{18}N_4O_2$	H	H	H	197-198	0.45	63
4	6b	$C_{24}H_{20}N_4O_2$	CH_3	H	H	201-202	0.42	61
5	6c	$C_{24}H_{18}N_4O_4$	H	H	COOH	253-254	0.78	67
6	6d	$C_{25}H_{20}N_4O_4$	CH_3	H	COOH	257-258	0.73	64
7	6e	$C_{24}H_{18}N_4O_4$	H	COOH	H	208-209	0.75	52
8	6f	$C_{25}H_{20}N_4O_4$	CH_3	COOH	H	213-214	0.70	48
9	6g	$C_{23}H_{18}N_4O_5S$	H	H	SO_3H	277-278	0.59	65
10	6h	$C_{24}H_{20}N_4O_5S$	CH_3	H	SO_3H	282-283	0.56	62
11	6i	$C_{23}H_{19}N_5O_4S$	H	H	SO_2NH_2	225-226	0.51	69
12	6j	$C_{24}H_{21}N_5O_4S$	CH_3	H	SO_2NH_2	233-234	0.48	66
13	6k	$C_{23}H_{18}N_4O_3$	H	OH	H	237-238	0.85	44
14	6l	$C_{24}H_{20}N_4O_3$	CH_3	OH	H	242-243	0.79	40
15	6m	$C_{23}H_{18}N_4O_3$	H	H	OH	246-247	0.87	57
16	6n	$C_{24}H_{20}N_4O_3$	CH_3	H	OH	251-252	0.81	54

Solvent system (TLC): chloroform: 90% ethanol (8:2)

Benzimidazolyl isoindoline

SCHEME



Analytical and spectral data

2-(1-(1H-benzo imidazol-2-yl) ethyl) isoindoline-1, 3-dione (compound 4b)

IR (ν in cm^{-1})

3408.33-3334.07 (N–H,str), 3063.06-3028.34 (Aromatic,C–H,str), 2983.98-2948.29 (CH_3,str), 2885.60- 2828.70(CH_2,str), 1767.82 (N–C=O,str), 1681.02 (C=N,str) , 1593.25-1496.81(Aromatic, C=C), 1453.41-1394.58 (C–H,ben), 1317.43-1293.31 (C–N,str).

^{1}HNMR (δ in ppm)

1.382–1.390 (d,2H, N– CH–C<u>H</u>$_3$–Ar), 4.165–4.223 (q,4H, N–C<u>H</u>–CH$_3$–Ar), 7.249–7.783 (m,4H,Ar-H,Benzimidazole), 7.625–8.102 (m,4H,Ar-H, Isoindoline), 8.524 (S,1H,N–H).

^{13}CNMR (δ in ppm)

18.32 (N– CH–<u>C</u>H$_3$–Ar), 45.51 (N– <u>C</u>H–CH$_3$–Ar), 115.39-123.43 (Ar-C,Benzimidazole), 138.40 (fused C), 141.95 (C=N), 127.78-132.89 (Ar-C, Isoindoline), 168.12 (C=O).

MS (m/z, %)

77.05 (76), 91.08 (36), 106.06 (14), 117.25 (100), 132.07 (24), 145.02 (60), 164.10 (15), 172.36 (28), 188.12 (42), 210.27 (16), 222.10 (21), 235.08 (11), 250.55 (13), 264.74 (22), 275.26 (10), 291.10 (6),

4-((2-((1, 3-dioxoisoindolin-2-yl) methyl)-1H-benzoimidazol-1-yl) methylamino) benzoicacid (compound 6c)

IR (ν in cm^{-1})

3460.41 -2540.33(O–H,str), 3383.26-3364.93 (N–H,str), 3114.18-3025.45 (Aromatic,C–H,str), 2982.05- 2887.53 (CH_2,str), 1774.57-1722.49 (N–C=O,str), 1706.46 (HO–C=O,str), 1695.49 (C=N,str) ,1600.97-1550.25 (Aromatic, C=C), 1410.98-1386.86 (C–H,ben), 1324.18-1300.07 (C–N,str), 1210.37-1203.62 (C–O,str).

^{1}HNMR (δ in ppm)

3.056 (S, 1H, Ph–N<u>H</u>– CH$_2$), 4.126 (S, 2H, N– C<u>H</u>$_2$–Ar), 4.743–4.758 (d,2H,NH–C<u>H</u>$_2$–Ar),7.230 –7.839 (m,4H,Ar-H,Benzimidazole), 7.638–8.178 (m,4H,Ar-H, Isoindoline), 6.768–7.582 (m,4H, Subs phenyl), 10.540 (S, 1H, COOH).

^{13}CNMR (δ in ppm)

35.37 (N– <u>C</u>H$_2$–Ar), 64.65 (N– <u>C</u>H$_2$–NH), 115.61-124.63 (Ar-C,Benzimidazole), 134.38-138.54 (fused C), 142.82 (C=N), 152.63 (<u>Ar</u>–NH), 127.85-133.14 (Ar-C, Isoindoline), 114.42-117.67-131.13 (subs phenyl),169.48(C=O), 171.77(COOH)

MS (m/z, %)

77.85 (58), 103.42 (45), 117.20 (100), 121.44 (24), 130.22 (52), 147.45 (33), 189.46 (28), 205.49 (31), 236.21 (40), 260.00 (20), 276.00 (25), 305.20 (22), 329.37 (10), 354.46 (19), 381.24 (6), 409.35 (11), 426.22 (7).

2-(1-(1-((2-hydroxyphenylamino) methyl)-1H-benzoimidazol-2-yl) ethyl) isoindoline-1,3-dione (compound 6l)

IR (ν in cm^{-1})

3565.53-3306.10 (O–H,str), 3408.33-3375.54 (N–H,str), 3062.10-3024.48 (Aromatic,C–H,str), 2981.08-2950.22 (CH_3,str), 2883.68-2840.28 (CH_2,str), 1768.78-1762.03 (N–C=O,str), 1653.05-1624.12 (C=N,str) , 1594.22-1496.81 (Aromatic, C=C), 1457.27-1396.51 (C–H,ben), 1317.43-1268.24 (C–N,str), 1212.30-1139.97 (C–O,str).

^{1}HNMR (δ in ppm)

1.460–1.493 (d,2H, N– CH–C<u>H</u>$_3$–Ar),3.064 (S, 1H, Ph–N<u>H</u>– CH$_2$), 4.122–4.200 (q,4H, N–C<u>H</u>–CH$_3$–Ar), 4.773–4.855 (d,2H,NH–C<u>H</u>$_2$–Ar), 5.154 (S, 1H, OH), 7.252–7.784 (m,4H,Ar-H,Benzimidazole), 7.635–8.173 (m,4H,Ar-H, Isoindoline), 6.254–7.512 (m,4H, Subs phenyl).

^{13}CNMR (δ in ppm)

18.44 (N– CH–<u>C</u>H$_3$–Ar), 39.65 (N– <u>C</u>H–CH$_3$–Ar), 64.50 (N– <u>C</u>H$_2$–NH), 114.41-118.77-131.63 (subs phenyl), 115.22-123.52 (Ar-C,Benzimidazole), 127.36-132.92 (Ar-C, Isoindoline), 134.20-138.52 (fused C), 142.12 (C=N), 143.30, 145.05, 168.53 (C=O)

MS (m/z, %)

77.05 (76), 91.08 (49), 103.06 (8), 117.06 (100), 132.07 (25), 144.67 (59), 150.05 (7), 162.04 (21), 179.07 (6), 193.12 (14), 221.11(40), 235.13 (19), 253.08 (51), 261.16 (38), 281.12 (13), 294.02 (33), 312.10 (26), 327.04 (47), 337.10 (8), 357.43 (5), 378.74 (31), 392.41 (18), 412.64 (5).

PHARMACOLOGICAL STUDIES

Animals

The selected Albino mice and Albino rats of wistar strain either sex were procured from the animal house. The animals were maintained in polypropylene cages in standard environmental conditions at 25^0c-30^0c in a 12 hours light/dark cycle. The animals were fed with standard rodent laboratory pellet diet and water libitum. The experiments carried out according the protocols with guidelines duly approved by the Institutional Ethical Committee (Registration no-23/243 Dated 10.08.11). Ethical Committee clearance approval was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Committee for Purpose of Control and Supervision of Experiments on Animals).The animals were acclimatized for one week under laboratory conditions.

Analgesic activity

The *in-vivo* analgesic activity was performed by writhing reflex syndrome test [(28)] using mice of either sex with a weighing between 20 and 25 grams were used. The each group contains five animals were adopted for control, standard and tested mice. The control group treated with 1% CMC in water. The tested concentrations of isoindolines and standard drug diclofenac prepared at a doses level of 10 mg/ kg, 20 mg/ kg and 30 mg/ kg were suspended in 1% carboxy methyl cellulose in water. The tested isoindolines and standard drug administered orally by intragastric tube 1 hour prior to intraperitoneal injection of 0.1ml of 3% v/v aqueous acetic acid in water for injection . The number of writhing reflex movement is observed and recorded for each mice a period of 15 minutes. The number of writhing reflex induced in tested isoindolines groups and standard group were compared with those in control group. The formula for calculating percentage protection is: average writhing reflex of the control group minus writhing reflex of the tested isoindolines groups divided by writhing reflex of the control group times 100%. The analgesic activity was expressed as the time periods with the maximum percentage of protection is considered as a peak time and was analysed statistically. These results are displayed in table no.2.

Table-2 *In vivo* analgesic activity of synthesized benzimidazolyl isoindolines (4a-b & 6a-n) in by writhing reflex syndrome method

S.No	Group	No of writhings			Percentage protection		
		10 mg/kg	20 mg/kg	30 mg/kg	10 mg/kg	20 mg/kg	30 mg/kg
1	4a	30.0±0.63	27.8±0.86 [a]	24.8±1.06 [a]	08.5	15.24	24.39
2	4b	29.4±0.67 [a]	26.4±0.40 [a]	24.2±0.37 [a]	10.3	19.51	26.21
3	6a	28.0±0.83 [a]	25.4±0.74 [a]	22.8±0.73 [a]	14.60	22.56	30.48
4	6b	27.2±0.48 [a]	24.8±0.96 [a]	21.8±0.58 [a]	17.0	24.39	33.53
5	6c	24.8±0.58 [a]	17.8±0.66 [a]	13.6±0.67 [a]	24.39	45.73	58.53
6	6d	24.8±0.66 [a]	17.2±0.73 [a]	12.6±0.87 [a]	24.39	47.56	61.58
7	6e	23.8±0.48 [a]	16.2±0.80 [a]	12.0±0.70 [a]	27.43	50.60	63.41
8	6f	23.4±0.67 [a]	15.4±0.50 [a]	10.8±0.48 [a]	28.65	53.04	67.07
9	6g	26.0±0.31 [a]	22.0±0.77 [a]	20.0±0.89 [a]	20.73	32.92	39.02
10	6h	25.4±0.74 [a]	21.6±0.67 [a]	18.8±0.80 [a]	22.56	34.14	42.68
11	6i	26.2±0.58 [a]	24.0±0.70 [a]	21.2±0.37 [a]	20.12	26.82	35.36
12	6j	26.0±0.54 [a]	23.0±0.31 [a]	20.4±0.87 [a]	20.73	29.87	37.80
13	6k	25.0±0.44 [a]	19.2±0.91 [a]	15.6±0.67 [a]	23.78	41.46	52.43
14	6l	24.8±0.73 [a]	18.6±0.74 [a]	14.6±0.81 [a]	24.39	43.29	55.48
15	6m	25.4±0.50 [a]	20.8±0.80 [a]	18.4±0.92 [a]	22.56	36.58	43.90
16	6n	25.2± 0.37 [a]	20.2± 0.58 [a]	17.2± 0.73 [a]	23.17	38.41	47.56
17	STD	21.4±0.60 [a]	12.8±0.58 [a]	09.0± 0.31 [a]	34.75	60.97	72.56
18	CL	32.8±.58			-		

Each average value represents as the mean ± SEM (n=5). Significance level [a] $P<0.001$ as compared with the respective control. STD-Diclofenac , CL-Control

Anti- inflammatory activity

The *in-vivo* anti-inflammatory activities of the synthesized isoindolines were evaluated by carrageenan induced rat hind paw oedema method [29]. The Wister albino rats of either sex range of weighing 180-220 grams were selected for experiment. The rats were divided into control, standard and tested synthesized isoindolines groups, each group consisting of six rats. The standard drug diclofenac and tested isoindolines were prepared as a suspension of 1% sodium carboxy methyl cellose in water for injection. The one group was administered with 1% CMC suspension which served as control. The three standard groups were treated with doses of 10 mg/kg, 20 mg/kg and 30 mg/kg 1% CMC suspensions of diclofenac separately and other tested isoindolines groups were treated with same doses of 10 mg/kg, 20 mg/kg and 30 mg/kg in about 0.3ml of 1% CMC suspension of tested isoindolines compounds. The tested isoindolines and standard drug were administered orally by intragastric (stomach) tube 1 hour before the induction of inflammation. An acute inflammation was induced by a subcutaneous injection of an irritant 0.1ml of 1% carrageenen solution in to the sub-planter surface region of left hind paw before that animals were lightly anaesthetized with diethyl ether and chloroform. The rats oedema paw volume were measured using plethysmographically, immediately after carrageenan injection and then after at hourly intervals for up to about 5 hours. An oedema is expressed as a increase in rat paw volume with respect to 1% CMC control. The any significant variation increase or decrease in the rats paw oedema volume compared to the control group up to 5 hours were considered as anti-inflammatory response. The difference of average values between tested isoindolines groups and control group is calculated for every 1 hour intervals and the values were analysed statistically. The percentage inhibition of edema inflammation were calculated by the formula : average mean edema volume of the control group minus average mean edema volume of the tested group divided by average mean edema volume of the control group times 100%.All the values were expressed as mean ± SEM. The data were statistically analyzed by one-way ANOVA followed by Dunnett's test. P values < 0.05 were considered as significant. These results are displayed in table no.3.

Table-3 *In vivo* anti-inflammatory activity of synthesized benzimidazolyl isoindolines (4a-b & 6a-n) by carrageenan induced rat hind paw oedema method

Groups	Dose (mg/kg)	Rat Paw edema volume				% protection		
		After 0 hr	After 1 hr	After 3 hr	After 5 hr	After 1 hr	After 3 hr	After 5 hr
	10	0.83±0.033	1.43±0.033	1.80±0.051	2.13±0.042	2.0	5.2	4.4
4a	20	0.80±0.051	1.36±0.033	1.70±0.068	2.00±0.073	6.8	10.5	10.3
	30	0.83±0.033	1.33±0.042	1.63±0.033*	1.90±0.044*	8.9	14.2	14.7
	10	0.80±0.051	1.43±0.033	1.73±0.042	2.06±0.060	2.0	8.9	7.6
4b	20	0.83±0.061	1.33±0.042	1.66±0.042	1.96±0.061	8.9	12.6	12.1
	30	0.83±0.033	1.30±0.044	1.60± 0.051**	1.90±0.044*	10.9	15.7	14.7
	10	0.80±0.051	1.33±0.042	1.70±0.068	2.03±0.080	8.9	10.5	8.9
6a	20	0.80±0.073	1.26±0.042	1.66±0.061	1.93±0.084	13.6	12.6	13.4
	30	0.80±0.051	1.23±0.033*	1.56±0.061***	1.83±0.061**	15.7	17.8	17.9
	10	0.80±0.051	1.33±0.042	1.70±0.044	2.00±0.073	8.9	10.5	10.3
6b	20	0.83±0.061	1.26±0.042	1.60±0.051*	1.86±0.084*	13.6	15.7	16.5
	30	0.80±0.051	1.20±0.051**	1.53±0.042***	1.80±0.073***	17.8	19.4	19.2
	10	0.83±0.061	1.26±0.042*	1.50±0.044***	1.66±0.084***	13.6	21.0	25.5
6c	20	0.80±0.073	1.20±0.051**	1.40±0.073***	1.43±0.061***	17.8	26.3	35.8
	30	0.83±0.061	1.16±0.033***	1.23±0.033***	1.30±0.068***	20.5	35.2	41.7
	10	0.80±0.051	1.26±0.042*	1.50±0.068***	1.63±0.080***	13.6	21.0	26.9

6d	20	0.83±0.033	1.20±0.051**	1.33±0.042***	1.40±0.073***	17.8	30.0	37.2
	30	0.80±0.051	1.13±0.042***	1.20±0.073***	1.26±0.042***	22.6	36.8	43.4
	10	0.80±0.051	1.26±0.042*	1.46±0.042***	1.60±0.051***	13.6	23.1	28.2
6 e	20	0.83±0.033	1.16±0.033***	1.23±0.061***	1.36±0.080***	20.5	35.2	39.0
	30	0.80±0.051	1.06±0.042***	1.16±0.033***	1.23±0.080***	27.3	38.9	44.8
	10	0.83±0.033	1.23±0.033**	1.43±0.033***	1.56±0.061***	15.7	24.7	30.0
6f	20	0.83±0.033	1.10±0.044***	1.20±0.051***	1.30±0.085***	24.6	36.8	41.7
	30	0.80±0.051	1.00±0.051***	1.13±0.066***	1.20±0.073***	31.5	40.5	46.1
	10	0.80±0.073	1.33±0.042	1.66±0.061	1.86±0.042**	8.9	12.6	16.5
6g	20	0.80±0.051	1.26±0.042	1.56±0.061**	1.70±0.085***	13.6	17.8	23.7
	30	0.83±0.061	1.16±0.033***	1.46±0.066***	1.60±0.051***	20.5	23.1	28.2
	10	0.83±0.033	1.33±0.042	1.63±0.061*	1.83±0.061***	8.9	14.2	17.9
6h	20	0.83±0.033	1.23±0.033*	1.53±0.066***	1.63±0.080***	15.7	19.4	26.9
	30	0.80±0.073	1.20±0.051**	1.43±0.033***	1.53±0.066***	17.8	24.7	31.3
	10	0.80±0.073	1.33±0.042	1.63±0.033*	1.93 ±0.042*	8.9	14.2	13.4
6i	20	0.83±0.033	1.30±0.044	1.53±0.042***	1.80±0.073**	10.9	19.4	19.2
	30	0.83±0.033	1.26±0.042	1.46±0.042***	1.73±0.084***	13.6	23.1	22.4
	10	0.80±0.073	1.33±0.042	1.63±0.061*	1.90±0.044*	8.9	14.2	14.7
6j	20	0.83±0.033	1.26±0.042	1.50±0.044***	1.76±0.095**	13.6	21.0	21.0
	30	0.83±0.033	1.20±0.051**	1.43±0.061***	1.66±0.084***	17.8	24.7	25.5
	10	0.83±0.033	1.30±0.044	1.56 ±0.033***	1.73 ±0.084***	10.9	17.8	22.4
6k	20	0.83±0.033	1.20±0.051**	1.46±0.066***	1.53±0.084***	17.8	23.1	31.3
	30	0.80±0.051	1.10±0.044***	1.23±0.061***	1.40±0.073***	24.6	35.2	37.2
	10	0.83±0.033	1.26±0.042	1.60±0.073**	1.70±0.044***	13.6	15.7	23.7
6l	20	0.83±0.033	1.16±0.033***	1.36±0.033***	1.46±0.066***	20.5	28.4	34.5
	30	0.80±0.073	1.06± 0.042***	1.26± 0.042***	1.33±0.084***	27.3	33.6	40.3
	10	0.83±0.033	1.30 ±0.044	1.63±0.033*	1.80 ±0.073***	10.9	14.2	19.2
6m	20	0.83±0.061	1.23±0.033*	1.46±0.042***	1.60±0.089***	15.7	23.1	28.2
	30	0.80±0.073	1.13±0.042***	1.40±0.051***	1.50±0.085***	22.6	26.3	32.7
	10	0.80±0.051	1.30±0.044	1.60±0.051**	1.76±0.033***	10.9	15.7	21.0
6n	20	0.80±0.051	1.20±0.051**	1.43±0.061***	1.56±0.061***	17.8	24.7	30.0
	30	0.83±0.033	1.16± 0.033***	1.36±0.033***	1.43±0.061***	20.5	28.4	35.8
	10	0.80±0.073	1.26±0.042*	1.43±0.061***	1.50±0.068***	13.6	24.7	32.7
STD	20	0.80±0.051	1.10±0.044***	1.16±0.061***	1.23±0.061***	24.6	38.9	44.8
	30	0.83±0.061	0.96±0.033***	1.03±0.033***	1.06±0.042***	34.2	45.7	52.4
CL		0.80±0.051	1.46±0.042	1.90±0.044	2.23±0.033	-	-	-

Each value represents the mean ± SEM (n=6). Significance levels *P<0.5, **P<0.01 and ***P<0.001 as compared with the respective control. STD-Diclofenac, CL-Control.

Evaluation for Central nervous system-locomotor activity

The central nervous system stimulant or depressant locomotor activity of isoindolines was evaluated by using digital actophotometer [30], Adult albino mice of either sex weighing 20-28 g were divided into control, standards and test isoindoline groups of five animals in to each group and numbered. The 0.25ml of 1% CMC suspension vehicle was administered for five

days once daily before starting the experiment. The animals were fasted for six hours before experiment and they were allowed to adapt to the activity cage environment for at least five minutes. The activity cage was calibrated prior to experimentation. The mouse was placed individually in the digital activity cage and the basal activity counts of each mouse were noted for15 minutes 2 days before to start experiment. A count is recorded when the beam of light falling on the photoelectric cell of actophotometer which connected in circuit with a counter, is cut off by mice. This test involves placing a mouse separately in an activity cage which enables movement of the mice across a light beam to be recorded as a locomotion count. The tested isoindoline compounds were administered orally by intragastric (stomach) tube at a doses of 10 mg/kg, 20 mg/kg & 30 mg/kg body weight in the form of suspension in 0.25ml of 1% CMC while two other groups received diazepam at a dose of 5mg/kg and caffeine at a dose of 10mg/kg body weight as a standard drugs, also given in the form of suspension in 0.25ml of 1% CMC.The control group mice received with 0.25ml suspension of 1% CMC in water. The locomotor behaviour was monitored after 60 minutes of administration of drugs, the actophotometer counts were measured for a period of 15 minutes. The difference in the number of counts for each group was recorded. The mean score for standard and test isoindoline groups were compared the results with control group. The percentage increase or decrease in locomotor activity was then calculated. These results are displayed in table no.4

Table 4 In *vivo* Central nervous system- locomotor activity of synthesized benzimidazolyl isoindolines (4a-b & 6a-n) by using actophotometer method

Group	Average number of movements in 15 minutes					
	10 mg/kg	Percentage	20 mg/kg	Percentage	30 mg/kg	Percentage
4a	134.4±1.03***	87.7	109.6±1.60***	71.5	105.0±2.28***	68.5
4b	133.6±1.12***	87.2	103.0±1.41***	67.2	100.8±2.31***	65.7
6a	129.0±1.09***	84.2	101.0±1.58***	65.9	100.2±2.17***	65.4
6b	126.2±0.96***	82.3	96.02±1.88***	62.7	93.6±2.20***	61.0
6c	146.0±1.04**	95.3	126.4±1.28***	82.5	120.0±2.58***	78.3
6d	143.0±1.14***	93.3	117.0±1.78***	76.3	111.8±2.72***	72.9
6e	128.0±1.18***	83.5	99.2±2.08***	64.7	94.6±3.10***	61.7
6f	121.6±1.03***	79.3	92.6±1.56***	60.4	89.6±3.02***	58.4
6g	143.6±1.12***	93.7	117.6±1.93***	76.7	115.2±2.99***	75.1
6h	140.4±1.07***	91.6	112.4±1.32***	73.3	108.2±2.59***	70.6
6i	145.0±1.09***	94.6	131.0±1.78***	85.5	128.4±2.24***	83.8
6j	144.4±0.81***	94.2	126.0±1.70***	82.2	120.8±2.78***	78.8
6k	152.2±0.86	99.3	146.6±2.20	95.6	144.8±3.33	94.5
6l	150.0±0.94	97.9	143.2±1.93*	93.4	134.0±2.82***	84.4
6m	152.8±1.06	99.7	148.2±2.05	96.7	147.2±3.15	96.0
6n	150.4± 1.16	98.1	141.8± 1.65**	92.5	139.0± 2.91*	90.7
CE	208.4±1.63***	136.0	Not tested	–	Not tested	–
DM	073.2±1.39*** (5 mg/kg)	47.7	Not tested	–	Not tested	–
CL	153.2±1.02				Nil	

Each value represents the mean ± SEM (n=5). Significance levels *P<0.5, **P<0.01 and ***P<0.001 as compared with the respective control.

CE–Caffeine DM–Diazepam CL–control

RESULTS AND DISCUSSION

In this study, two series of various substituted benzimidazolyl isoindoline derivatives were prepared and analytical structural elucidation was carried out by IR, ^{1}HNMR, ^{13}CNMR, mass spectral studies for conforming the molecular formula of all the synthesized isoindolines. They were evaluated for their antinociceptive properties. It revealed that all the synthesized benzimidazolyl isoindolines at different tested concentrations 10mg/kg, 20mg/kg and 30mg/kg were showed significant analgesic activity (except 4a at 10mg) against control when compared with that of the standard drug diclofenac. . It has been observed that 6d, 6e,6f were showed potent analgesic activity. The isoindolines 6c,6k,6l exerted good and 6g, 6h,6m,6n were possess moderate analgesic activities. Meanwhile the benzimidazolyl isoindolines 6b, 6i, 6j were exhibited less and 6a, 4a, 4b were showed weak analgesic activity when compared with that of the standard drug diclofenac. Data shows that all isoindolines were given significant activity at higher 30mg/kg concentration. The isoindolines 4a, 4b, 6a were showed non-significant results at low 10mg/kg and 20mg/kg concentrations. In comparision with diclofenac, isoindolines 6c,6d,6e,6f,6l were shows better activity. Among this, isoindolines 6h,6k,6m,6n were exhibited good and isoindolines 6g,6i,6j displayed moderate anti-inflammatory activities. On the other hand, isoindolines 4a, 4b, 6a, 6b were endowed the weak anti-inflammatory activity. It was noticed that, anti-inflammatory activities increases when increasing concentration of isoindolines.

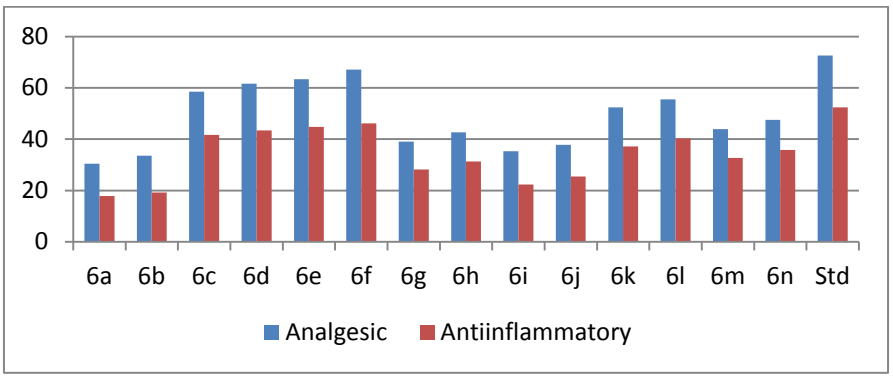


Fig. No.1. Graphical representation of the analgesic and anti-inflammatory activity of the synthesized benzimidazolyl isoindoline compounds

Standard and Test Dose = 30 mg/kg in each group of animal.

When structure activity relationship studies are concerned, benzimidazolyl isoindolines bearing carboxylic acid, hydroxy, sulfonic acid and sulfonamide substituted analogues has shown better analgesic and anti-inflammatory activity than unsubstituted analogues. In the series, acid and hydroxy substituent analogues are found to exhibit excellent antinociceptive activity than sulfonic acid and sulfonamide substituted analogues. Among this, polar electron withdrawing carboxylic acid substituted analogues (6c,6d,6e,6f) were marked greater antinociceptive potency than polar electron donating hydroxy substituted isoindolines (6k,6l,6m,6n). Meanwhile, acidic sulfonic acid analogues (6g, 6h) are displayed higher antinociceptive activity than sulfonamide substituted analogues (6i,6j). Moreover, 1-phenyl substituted benzimida zolyl isoindolines (6a, 6b) are shows more antinociceptive properties than unsubstituted benzimidazolyl isoindolines (4a, 4b). The results also shows that the 2-methyl substituted benzimidazolyl isoindolines (4a,6a,6c,6e,6g,6i,6k,6m) have comparably less antinociceptive activity than 2-ethyl substituted benzimidazolyl isoindolines (4b,6b,6d,6f,6h,6j,6l,6n). So it is possible to say that non polar electron donating alkyl group is necessary to increase anti-nociceptive property. In the two series of 1- substituted benzimidazoles , the ortho carboxylic acid and ortho hydroxyl substituted isoindolines (6e,6f&6k,6l) produced excellent antinociceptive activity than that of para carboxylic acid and para hydroxyl substituted isoindolines (6c,6d & 6m,6n).

The central nervous system locomotor activity of the isoindolines data noticed that most

of the isoindolines were shows varying degrees of reducing locomotor activities against control group was observed at 20mg/kg and 30mg/kg concentrations. The most of the isoindolines produce significant depressant activity at the all tested 10mg/kg , 20mg/kg, 30mg/kg concentrations but only 6k, 6m not produced significant decrease of locomotor activity were observed at all the tested concentrations . In this context , the isoindolines 6b,6e,6f were only expressed higher depressant activity and isoindolines 6a,4a,4b also gave considerably good depressant activity when compared to standard CNS depressant drug diazepam. Among this isoindolines 6c, 6d, 6g, 6h, 6j were displayed comparably lower depressant activity. However, isoindolines 6i, 6k,6l,6m,6n exhibited bad central nervous system depressant properties than the compared other isoindolines. All the synthesized isoindolines were fails to show central nervous system stimulant activity when compared to standard CNS stimulant drug caffeine.

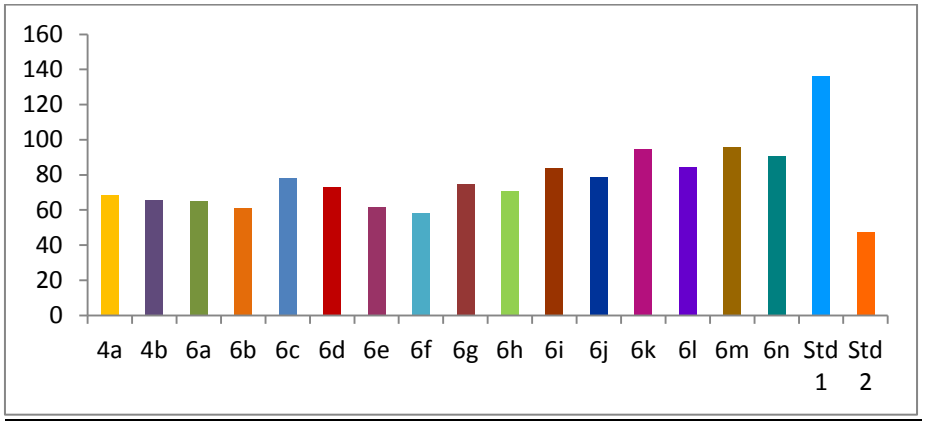


Fig. No. 2. Graphical representation of the CNS locomotor movement of the Scheme 1 synthesized compounds

Standard stimulant Dose =10 mg/kg, Standard depressant Dose =>5mg/kg and Test Dose = 30 mg/kg in each group of animals

.

When Structure activity relationship studies are concerned, 1-ortho phenyl carboxylic acid substituted (6e, 6f) and 1- phenyl substituted benzimidazolyl isoindolines (6a, 6b) were only displays good depressant activity. The unsubstituted benzimidazolyl isoindolines (4a, 4b) also displays considerable depressant properties than other tested isoindolines . A close examination of table 4 shows that the 2-ethyl substituted benzimidazolyl isoindolines were marked more depressant activity than 2-methyl substituted benzimidazolyl isoindolines .The 1-para phenyl sulfonic acid substituted (6g,6h) and 1-para phenyl acid substituted benzimidazolyl isoindolines (6c,6d) were depicted more appreciable depressant activity than 1- para phenyl sulfonamide substituted benzimidazolyl isoindolines (6i,6j). Anyway, the 1-para phenyl sulfonic acid substituted (6g,6h) analogues were more potent than 1-para phenyl carboxlic acid substituted (6c,6d) analogues. The 1- phenyl hydroxy group substituted (6k, 6m, 6n) analogues were exerting very weak depressant activity.

CONCLUSION

In summary, we have described the synthesis of two series of 1,2-substituted benzimidazoles carrying isoindolines with their spectral studies. The SAR studies revealed that 1-phenyl carboxylic acid (6c, 6d, 6e, 6f) and 1-phenyl hydroxyl (6k, 6l, 6m and 6n) substituted benzimidazolyl isoindolines were expressed higher In-vivo analgesic and anti-inflammatory activity then other groups. Among this the carboxylic acid substituted isoindolines analogues were more potent then hydroxy substituted analogues. Anyway, all the tested isoindolines were gave considerably significant antinociceptive activities at higher concentration at 30mg/kg. Moreover, the 1-phenyl ortho substituted and 2-ethyl substituted

benzimidazolyl isoindolines were depicted more analgesic, anti-inflammatory then the corresponding 1-phenyl para substituted and 2-methyl substituted analogue

The results proves that 1-phenyl substituted (6a,6b) and that 1-phenyl carboxylic acid substituted (6f,6e) benzimidazolyl isoindolines were produced good central nervous system depressant activity but the 1-phenyl hydroxyl substituted (6k, 6l, 6m and 6n) analogues were fails to give considerable central nervous system depressant activity. Moreover, the 1-phenyl ortho substituted and 2-ethyl substituted benzimidazolyl isoindolines were depicted more central nervous system depressant activity then the corresponding 1-phenyl para substituted and 2-methyl substituted analogues. All the tested benzimidazolyl isoindolines were fails to exert central nervous system stimulant activity.

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