Original Article

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SYNTHESIS, SPECTRAL CHARACTERIZATION, PHARMACOLOGICAL EVALUATION OF CERTAIN 2-SUBSTITUTED BENZIMIDAZOLYL CHALCONES FOR IN-VIVO ANALGESIC, ANTI-INFLAMMATORY AND CENTRAL NERVOUS SYSTEM ACTIVITIES.

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Abstract

A series of 2-substituted benzimidazolyl chalcones were synthesized by condensation reaction of 1-(1H-benzoimidazol-2-yl)ethanone with various substituted aromatic aldehydes in presence of mild alkali. The 1-(1H-benzoimidazol-2-yl)ethanone was synthesized by oxidation of 1-(1H-benzoimidazol-2-yl)ethanol, which was synthesized by cyclisation reaction of orthophenylenediamine with 2-hydroxypropanoicacid. The yields of the synthesized benzimidazoles were ranged 52-81%. The structures of the synthesized benzimidazolyl chalcones were characterized and confirmed by FTIR,1H-NMR,13C-NMR and mass spectral data analysis. The synthesized benzimidazolyl chalcones were screened for their In-vivo analgesic, anti-inflammatory and central nervous system locomotor activities. All the synthesized benzimidazolyl chalcones showed moderate to appreciable significant analgesic activity and also some the compounds exhibited significant good anti-inflammatory properties (except 6e & 6h). Among this tested benzimidazolyl chalcones only 6d, 6f, 6g and 6h has depicted considerable central nervous system depressant activity.

Keywords: Benzimidazoles, benzimidazolyl chalcones, analgesic, anti-inflammatory & central nervous system depressant activity.

INTRODUCTION

Benzimidazole is a bicyclic heterocyclic aromatic compound and chemically named as 1,3 dideazapurine. This nucleus is important pharmacophore because structurally related to purine nucleoside bases so it interacts with most of the biological macromolecules. This moiety is also found in some natural products , such as vitamin B_{12} , marine natural product called makaluvamins etc.The literature survey revealed that the various 2- substituted 1H-benzimidazole derived compounds has displayed to possess antibacterial [1] , antitubercular [2], antifungal [3], antiviral [4], anti-allergic , central nervous system depressant ,analgesic/anti-inflammatory [5], anticancer [6], anthelminitic [7], anticonvulsant [8], antioxidant [9], anti-tumor, antidiabetic, anti asthmatic [10], spasmolytic and antiulcer [11] activities. The 1H-benzimidazole derived compounds substituted at 2 and 5 or 6 positions are also reported to possess ths various pharmacological activities such as DNA topoisomerase inhibitor [12] , acaricidal, antifeedant , antibacterial, antifungal , antiulcer, HIV-RT inhibitor [13], cysticidal, anthelminitic, histamine H_4-receptor antagonist, herbicidal, antiprotozoal [14], antioxidant, protein kinase Ck2 inhibitor and antimyobacterial [15].

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The Chalcone pharmacophore analogues are also found various natural plant products such as flavonoids, isoflavonoids, aurones, and clavicin etc. This nucleus is a integral part of various natural plant products such as terpeneoids, dicoumarol, vitamin K, digitalis glycosides. However in recent years the introduction of new synthetic chalcone derived products has been utilised instead of natural products such as warfarin for dicoumarol. The Chalcone derivatives are act as intermediate and also used as precursor for the synthesis of various novel cyanopyridines, isoxazoles, pyrazolines, pyrimidines and tetrazole heterocyclic compounds. The Chalcone analogues are also revealed for its various pharmacological properties, in the literature review such as antioxidant [16], antibacterial ,antiviral , antimicrobial , antifungal [17], antimalarial, antileishmanial, inhibiteosinophilia , antiseptical , antitrichomonal, insecticidal, antitubercular [18],analgesic/ anti-inflammatory [19], cyclooxygenase inhibitors,antiallergic, anaesthetic, antiplasmodial [20], immunosuppression ,antineoplastic, cytotoxicity, anticancer, hypotensive, antifibrogenic , anti ulcerogenic, prostaglandin binding, anxiolytic [21], trypsin inhibitor, anthelmintic , antitumor, antileukemic and antiproliferative activities. These observations prompted us to synthesize the various substituted chalcone analogues to be incorporate with benzimidazole at the 2nd position to test some better pharmacological actions such as to screen for their analgesic, anti-inflammatory and central nervous system locomotor activities.

EXPERIMENTAL

Synthesis of 1-(1H-benzoimidazol-2-yl) ethanol (3)

The lactic acid (1) and ortho-phenylene diamine (2) were weighed equimolecular quantity in a round bottom flask and refluxed with water condenser for one and half an hour. The mixture was cooled to room temperature by keeping in cooling water bath and 25% potassium hydroxide solution was slowly added until the mixture was just alkaline to litmus to yield the precipitated product. The product (3) was filtered with ice cold water, dried and the purified product (3) was recrystallised from 90% ethanol.

Synthesis of 1-(1H-benzoimidazol-2-yl)ethanone (4)

The 0.01 molar 1-(1H-benzoimidazol-2-yl) ethanol (3) was taken in a round bottom flask, to this 10ml of 50% sulphuric acid and 20% potassium dichromate mixture solutions were added. The reactant mixture was refluxed with water condenser for one hour. The oxidized reactant mixture was allowed to cool at room temperature and poured slowly, with stirring in to a beaker containing ice cold water to get the product. The product 1 - (1H-benzoimidazol-2-yl)ethanone (4) was filtered with ice cold water, dried and recrystallised from 90% ethanol.

Synthesis of various 2-chalcone substituted1H-benzimidazoles (6a-j)

Weighed 0.01 molar 1-(1H-benzoimidazol-2-yl) ethanone (4) and 10ml 30% potassium hydroxide solution were taken in a round bottom flask to this add 0.012 molar of following various aromatic aldehydes (5a-j)namely,para-anisaldehyde(5a, (R_3 & R_2 = H, R_1=OCH_3, n=1)), benzaldehyde (5b, (R_1, R_2 &R_3 =H, n=1)), 3-phenyl acrylaldehyde (5c, (R_1,R_2&R_3=H, n=2)), 2-chloro benzaldehyde (5d, (R_3=Cl,R_2 & R_1=H, n=1)), 4-(dimethylamino) benzaldehyde (5e, (R_3 & R_2=H, R_1=$N(CH_3)_2$, n=1)), 4-fluorobenzaldehyde (5f, (R_3 &R_2=H, R_1=F,n=1)), 2-nitro benzaldehyde (5g,(R_3=NO_2,R_2&R_1=H, n=1)),4-nitro benzaldehyde (5h, (R_3 & R_2=H, R_1= NO_2, n=1)), 2-hydroxybenzaldehyde (5i, (R_3=OH, R_2&R_1=H, n=1)) and 4-hydroxy 3-methoxybenzaldehyde (5j, (R_3 =H, R_2=OCH_3& R_1= OH, n=1)) separately, with stirring then reflux the mixture for 2 hours with water condenser, allow to cool to the room temperature and pour the mixture in to a beaker containing ice cold water, with continuous stirring to get a product. The precipitated benzimidazolyl Chalcones (6a-j) was collected by filtration with ice cold water, washed, dried and recrystallised from 90%ethanol.

The purity of the synthesized2-substituted benzimidazolyl chalcones were checked by Thin Layer Chromatography using silica gel-60 F_{254} aluminium sheets using chloroform: ethanol (8:2) mixture as eluent solvent and spots were identified in a ultra violet chamber (Table-1). The benzimidazolyl chalcones were also identified by their melting point and Rf values. The chemical structures of all synthesized 2-substituted benzimidazolyl Chalcones

Scheme:Synthesis of 2- disubstituted benzimidazolyl chalcones

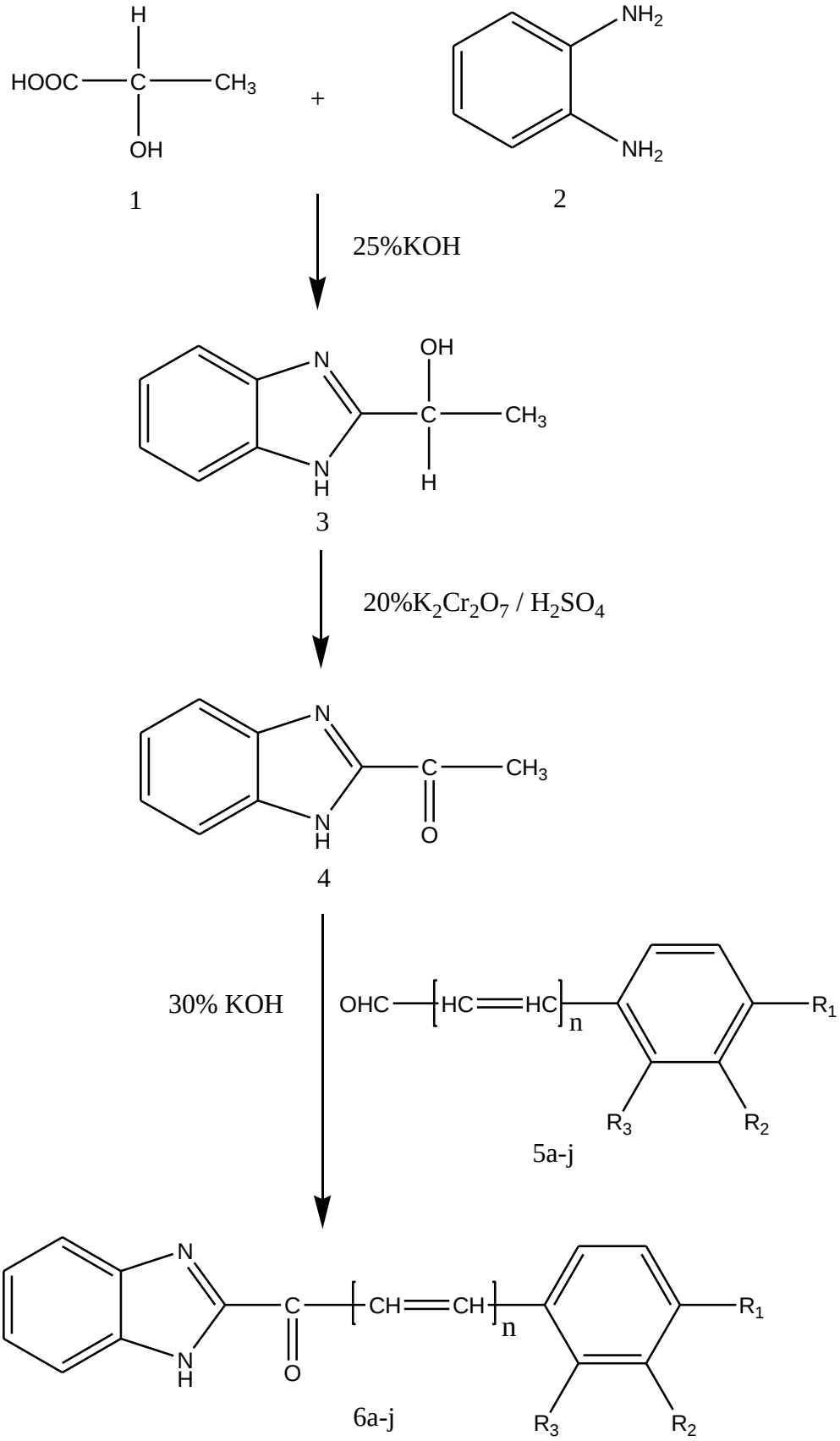

Table -1
Physical analysis of synthesised 2-substituted benzimidazolyl Chalcones

Compounds	Molecular formula	R_1	R_2	R_3	n	Melting Point °C	R_f value	Yield %
6a	$C_{17}H_{14}N_2O_2$	OCH_3	H	H	1	203–204	0.60	65
6b	$C_{16}H_{12}N_2O$	H	H	H	1	183–184	0.54	62
6c	$C_{18}H_{14}N_2O$	H	H	H	2	195–196	0.57	66
6d	$C_{16}H_{11}ClN_2O$	H	H	Cl	1	210–211	0.62	72
6e	$C_{18}H_{17}N_3O$	$N(CH_3)_2$	H	H	1	214–215	0.65	75
6f	$C_{16}H_{11}FN_2O$	F	H	H	1	187–188	0.68	81
6g	$C_{16}H_{11}N_3O_3$	H	H	NO_2	1	222–223	0.43	52
6h	$C_{16}H_{11}N_3O_3$	NO_2	H	H	1	236–237	0.41	58
6i	$C_{16}H_{12}N_2O_2$	H	H	OH	1	190–191	0.84	55
6j	$C_{17}H_{14}N_2O_3$	OH	OCH_3	H	1	229–230	0.80	60

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

Spectral data

1-(1H-benzo[d]imidazol-2-yl)-3-(4-methoxy phenyl) propenone (compound 6a)

IR (ν in cm^{-1})

3440.1-3413.1 (N–H str), 3034.0 (=C–H str), 2886.5 (C–H str,CH_3), 1707.9 (C=O str), 1659.8 (C=C str, Aliphatic), 1603.0-1507.4 (C=C str, Aryl), 1342.5-1134.1 (C–N str), 1250.8-1054.1 (C–O str, OCH_3).

^{1}HNMR (δ in ppm)

8.195 (S,1H,N–H), 7.898-7.248 (m,4H,Ar-H), 7.304-7.424 (d,2H,-CH=CH-phenyl), 6.719-6.723 (d,2H,O=C–CH=CH) , 6.752-7.219 (m,4H,P-substituted phenyl), 3.868(S,3H,OCH_3).

^{13}CNMR (δ in ppm)

188.46(C=O), 115.31 – 123.57(fused phenyl), 138.66(fused C), 142.05(C=N), 122.61 (O=C–CH=CH), 146.37(–CH=CH–phenyl) , 114.17-127.45-130.35 (P-substituted phenyl), 159.36(C–O, Ar–OCH_3), 55.44 (OCH_3).

MS (m/z,%): 93.15 (41), 105.39 (32) ,117.51 (100), 133.19 (12),145.74 (36), 161.60 (61), 188.21 (17), 202.83 (15), 218.76 (12), 247.51 (20), 263.70 (0.09), 278.36 (9).

1-(1H-benzo[d]imidazol-2-yl)-3-Phenyl Propenone (compound 6b)

IR (ν in cm^{-1})

3389.0-3337.9 (N–H str), 3062.1-2997.6 (=C–H str), 1703.3 (C=O str), 1660.7 (C=C str, Aliphatic), 1598.3-1444.7 (C=C str, Aryl), 1317.4-1132.2 (C-N str).

^{1}HNMR (δ in ppm)

8.274(S,1H,N–H), 7.830-7.247(m,4H,Ar-H), 7.314-7.453 (d,2H,-CH=CH-phenyl), 6.761-6.765(d,2H,O=C–CH=CH) , 7.048-7.256(m,5H, Phenyl).

^{13}CNMR (δ in ppm)

188.61(C=O), 115.23 -123.40(fused phenyl), 138.40(fused C), 141.92(C=N), 121.93(O=C–CH=CH), 146.18(–CH=CH–phenyl), 126.37- 129.59-134.75(phenyl).

MS (m/z,%)

92.10 (28), 105.08 (63), 117.31 (100), 131.25 (33), 145.20 (20), 159.39 (25), 171.22 (59), 194.75 (11), 218.52 (13), 248.27 (10).

1-(1H-benzo[d]imidazol-2-yl)-5-Phenyl Pentadienone (compound 6c).

IR (ν in cm^{-1})

3379.4 (N–H str), 3063.0-2997.0 (=C–H str), 1676.2 (C=O str), 1659.8 (C=C str, Aliphatic), 1594.2-1450.5 (C=C str, Aryl), 1319.3-1172.7 (C–N str).

^{1}HNMR (δ in ppm)

8.072(S,1H,N–H), 7.899-7.259(m,4H,Ar-H),6.351-6.398(d,2H,O=C–CH=CH), 7.357-7.351(d,2H,O=C–CH=CH), 6.760-6.732(d,2H,-CH=CH-phenyl), 6.498-

6.525(d,2H,-CH=CH-phenyl), 7.043-7.338(m,5H, Phenyl).

^{13}CNMR (δ in ppm)

187.75(C=O),115.52– 123.21(fused phenyl), 137.35(fused C), 142.20(C=N), 122.97(O=C–CH=CH), 143.04(–CH=CH–phenyl) , 120.32(=CH–CH=), 126.83(–CH=CH–), 126.38-129.43-135.75 (phenyl).

MS (m/z,%): 93.12 (27),103.18 (24),117.11 (100), 129.37(45), 145.59 (22), 157.49 (65), 172.83 (14), 184.70 (32), 198.21 (28), 218.33 (11), 247.52 (15), 274.39 (13).

1-(1H-benzo[d]imidazol-2-yl)-3-(2-Chloro Phenyl) Propenone (compound 6d)

IR (ν in cm^{-1}): 3374.5 (N–H str), 3062.1 (=C–H str), 1674.2 (C=O str), 1663.6 (C=C str, Aliphatic), 1594.2-1515.1 (C=C str, Aryl), 1337.6-1169.8 (C–N str), 1053.1 (C–Cl str).

^{1}HNMR (δ in ppm): 8.242(S,1H,N–H), 7.869-7.244(m,4H,Ar-H), 7.403-7.396(d,2H,-CH=CH-phenyl), 6.753-6.766 (d,2H,O=C–CH=CH) , 7.069-7.285(m,4H,o-substituted phenyl).

^{13}CNMR (δ in ppm): 188.51(C=O), 115.55 – 123.49 (fused phenyl), 138.70(fused C), 142.62(C=N), 121.87(O=C–CH=CH), 146.03(–CH=CH–phenyl), 127.00-128.48-135.95 (O-substituted phenyl), 133.56(C–Cl).

MS (m/z,%): 91.46 (38), 104.61 (22), 117.40 (100), 131.15 (34), 145.20 (28), 165.38 (57), 172.69 (23), 192.58 (17), 206.74 (20), 217.37 (0.08), 247.18(12), 282.54 (0.06).

1-(1H-benzo[d]imidazol-2-yl)-3-(4-(Dimethyl amino) Phenyl) Propenone (compound 6e)

IR (ν in cm^{-1}): 3466.2-3365.9 (N–H str), 3064.9-3026.4(=C–H str), 2905.8-2809.4 (C–H str, CH_3), 1677.1(C=O str), 1660.7 (C=C str, Aliphatic), 1594.2-1491.0 (C=C str, Aryl), 1323.2-1166.0 (C–N str).

^{1}HNMR (δ in ppm): 8.325(S,1H,N–H), 7.894-7.279(m,4H,Ar-H), 7.305-7.474 (d,2H,-CH=CH-phenyl), 6.719-6.748(d,2H,O=C–CH=CH) , 6.769-7.154(m,4H,P-substituted phenyl), 3.074(S,6H,-N–$(CH_3)_2$).

^{13}CNMR (δ in ppm): 188.72(C=O), 115.12 – 123.32(fused phenyl), 138.48(fused C), 141.82(C=N), 121.83(O=C–CH=CH), 145.98(–CH=CH–phenyl) , 111.60-124.90-126.45 (P-substituted phenyl), 152.40(Ar–N), 40.20(CH_3).

MS (m/z,%): 93.44 (58), 104.74 (41), 117.20 (100), 130.19 (18), 146.52 (25), 158.27 (12), 172.03 (21), 186.62 (72), 200.01 (27), 215.83 (47), 247.30 (14), 276.41 (16), 291.38(0.09).

1-(1H-benzo[d]imidazol-2-yl)-3-(4-Fluoro Phenyl) Propenone (compound 6f)

IR (ν in cm^{-1}): 3391.9 (N–H str), 3064.9(=C–H str), 1677.1 (C=O str), 1659.8 (C=C str, aliphatic), 1595.1-1494.8 (C=C str, Aryl), 1319.3-1157.3 (C–N str), 1213.2 (C-F str).

^{1}HNMR (δ in ppm): 8.233(S,1H,N–H), 7.844-7.298 (m,4H,Ar-H), 7.396-7.411(d,2H,-CH=CH-phenyl), 6.710-6.729(d,2H,O=C–CH=CH) , 7.251-7.101(m,4H,P-substituted phenyl).

^{13}CNMR (δ in ppm): 188.37(C=O), 115.46–123.38(fused phenyl), 138.67(fused C), 142.26(C=N), 121.70(O=C–CH=CH), 145.99(–CH=CH–phenyl), 115.97-127.77-130.61, 132.92, (P-substituted phenyl),163.57(Ar–F).

MS (m/z,%): 92.08 (39), 103.06 (24), 117.31 (100), 122.07 (62), 131.84 (26), 145.77(18), 149.46 (42), 159.53 (23), 172.18 (33), 177.17 (49), 190.12 (73), 217.73 (16), 247.43 (21), 266.16 (11).

1-(1H-benzo[d]imidazol-2-yl)-3-(2-nitrophenyl) Propenone (compound 6g)

IR (ν in cm^{-1}): 3440.1-3357.2 (N–H str), 3062.1 (=C–H str), 1672.3 (C=O str), 1666.5 (C=C str, Aliphatic), 1593.2-1519.9 (C=C str, Aryl), 1572.0 (NO_2 str),1341.5-1319.3 (C–N str).

^{1}HNMR (δ in ppm): 8.264(S,1H,N–H), 7.893-7.279 (m,4H,Ar-H), 7.365-7.450 (d,2H,-CH=CH-phenyl), 6.766-6.792 (d,2H,O=C–CH=CH) , 6.510-7.634(m,4H,O-substituted phenyl).

^{13}CNMR (δ in ppm): 188.71 (C=O), 115.47–123.47(fused phenyl), 138.44(fused C), 142.65(C=N), 122.22(O=C–CH=CH), 145.63(–CH=CH–phenyl), 151.96(Ar–NO_2), 120.28- 128.95-134.73 (o-substituted phenyl).

MS (m/z,%): 93.50 (52), 107.38 (76), 117.00 (100), 132.40 (14), 146.51 (11), 159.72 (28), 175.39 (15), 187.33 (46), 203.06 (35), 216.10 (17), 247.01 (0.08), 275.38 (12), 293.13 (0.08).

1-(1H-benzo[d]imidazol-2-yl)-3-(4-nitrophenyl) Propenone (compound 6h)

IR (ν in cm^{-1}): 3418.9-3376.5 (N–H str), 3064.9 (=C–H str), 1702.8 (C=O str), 1664.6 (C=C str, Aliphatic), 1597.3-1484.2 (C=C str, Aryl), 1553.7(NO_2 str), 1320.3-1170.8 (C–N str).

^{1}HNMR (δ in ppm): 8.353(S,1H,N–H), 7.828-7.202 (m,4H,Ar-H), 7.351-7.493(d,2H,-CH=CH-phenyl), 6.742-6.769(d,2H,O=C–CH=CH) , 6.625-7.651(m,4H,P-substituted phenyl).

^{13}CNMR (δ in ppm): 188.43(C=O), 115.54–123.65(fused phenyl), 138.74(fused C), 142.54(C=N), 122.54(O=C–CH=CH), 146.31(–CH=CH–phenyl), 154.42(Ar–NO_2), 120.31- 128.04-136.94 (P-substituted phenyl).

MS (m/z,%): 93.05 (58), 107.26(82), 117.62(100), 132.25 (25), 148.51 (15), 160.15 (32), 176.29 (12), 187.02 (28), 201.19(51), 217.38 (22), 247.49 (11), 277.16 (13), 293.37 (0.09).

1-(1H-benzo[d]imidazol-2-yl)-3-(2-hydroxyphenyl) propenone (compound 6i)

IR (ν in cm^{-1}): 3419.9 (O–H str), 3388.0 (N–H str), 3095.3 (=C–H str), 1657.8 (C=O str), 1643.4-1635.6 (C=C str, Aliphatic), 1592.2-1476.5 (C=C str, Aryl), 1345.3-1139.9 (C–N str),1247.9- 1209.4 (C–O str,OH).

^{1}HNMR (δ in ppm): 8.157(S,1H,N–H), 7.874-7.293 (m,4H,Ar-H), 7.324-7.448(d,2H,-CH=CH-phenyl), 6.721-6.740 (d,2H,O=C–CH=CH), 6.681-7.267 (m,4H,O-substituted phenyl), 4.871 (S,1H,ortho substituted–OH).

^{13}CNMR (δ in ppm): 188.51(C=O), 115.43–123.15(fused phenyl), 138.30(fused C), 142.23(C=N), 122.811(O=C–CH=CH), 146.33(–CH=CH–phenyl), 157.56(Ar–OH), 119.88-127.83- 129.91- 130.11 (O-substituted phenyl).

MS (m/z,%): 93.06 (47), 105.52 (28), 117.04 (100), 131.51 (23), 147.45 (42), 158.20 (25), 174.31(62), 188.59 (33), 217.81 (11), 247.10 (17), 264.52 (12).

1-(1H-benzo[d]imidazol-2-yl)-3-(4-hydroxy-3-methoxyphenyl) propenone (compound 6j)

IR (ν in cm^{-1}): 3418.9 (O–H str) , 3395.7 (N–H str), 3064.0 (=C–H str), 2929.9- 2848.9(C–H str,OCH_3), 1675.2 (C=O str), 1661.7-1632.8 (C=C str, Aliphatic), 1594.2-1505.4 (C=C str, Aryl), 1343.4-1321.2 (C–N str), 1172.7-1056.0 (C–O str,OCH_3), 1270.1-1211.3 (C–O str,OH).

^{1}HNMR (δ in ppm): 8.023(S,1H,N–H), 7.831-7.245 (m,4H,Ar-H), 7.392-7.406(d,2H,-CH=CH-phenyl), 6.740-6.713(d,2H,O=C–CH=CH) , 6.679-7.197(m,4H,P-substituted phenyl), 4.926 (S,1H,OH), 3.758(S,3H,OCH_3).

^{13}CNMR (δ in ppm): 188.60(C=O), 115.21–123.19(fused phenyl), 138.39(fused C), 141.86(C=N), 122.0(O=C–CH=CH), 146.55(–CH=CH–phenyl), 143.57(Ar–OH),157.38 (Ar–OCH_3),55.92-57.04 (OCH_3), 120.30-129.68-131.67-134.73 (substituted phenyl).

MS (m/z,%): 92.55 (52), 104.07(38), 117.19 (100), 130.72 (15), 146.29 (21), 162.42 (11), 177.39 (46), 189.83(13), 203.27 (31), 218.14 (71), 247.62 (9), 279.17 (12), 294.34 (0.09).

MATERIALS AND METHODS

Animals

The Albino mice and Albino rats of wister strain either sex were selected and procured from the animal house. The animals were maintained in polypropylene cages were housed in groups of six per cage in standard environmental conditions at the temperature of 25^{0}c-30^{0}c in a 12 hours light/dark cycle. The animals were fed with standard rodent laboratory pellet diet and water libitum. However, pellet diet was withdrawn six hours before and during the experiments .The In-vivo screening tested according the protocols with guidelines duly approved by the Institutional Ethical Committee. 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).

PHARMACOLOGICAL STUDIES

ANALGESIC ACTIVITY

The *in-vivo* analgesic activity was performed by writhing reflex syndrome test [22] 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 benzimidazoles 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 benzimidazoles 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 benzimidazole 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 benzimidazoles 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.

ANTI- INFLAMMATORY ACTIVITY

The *in-vivo* anti-inflammatory activities of the synthesized benzimidazoles were evaluated by carrageenan induced rat hind paw oedema method [23]. 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 benzimidazoles groups, each group consisting of six rats. The standard drug diclofenac and tested benzimidazoles 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 benzimidazoles 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 benzimidazoles compounds. The tested benzimidazoles 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 diethylether 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 benzimidazoles 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%..

CNS - LOCOMOTOR ACTIVITY

In-vivo central nervous system stimulant or depressant activity of the synthesized benzimidazoles for its locomotor activity was evaluated by using digital actophotometer [24]. The albino mice of either sex weighing 20-28 g were seperated into control, standards and test groups of five mice in to each group. The 0.3ml of 1% CMC suspension was given orally for five days once daily before starting the experiment. The mice were fasted for six hours before experiment and they were allowed to adapt to the actophotometer cage environment for few minutes. The mice were placed individually in the digital actophotometer which count the movement of the mice across a light beam to be measured as locomotion, were noted for 15 minutes. The tested benzimidazoles were administered orally by intragastric (stomach) tube at doses of 10 mg/kg, 20 mg/kg and 30 mg/kg body weight of mouse in the form of suspension in 0.3ml of 1% CMC. While two standard groups administered with diazepam at a dose of 5mg/kg and caffeine at a dose of 10mg/kg, these also administered in the form of suspension in 0.3ml of 1% CMC. The control group mice received only with 0.3ml suspension of 1% CMC in a drinking water. The locomotor behaviour was observed after 1 hour of administration of the benzimidazoles and standard drugs. The numbers of counts for each group were

recorded for a period of 15 minutes. The mean average scores for standard and benzimidazole groups were compared the results with control group. The percentage central nervous system stimulant or depressant activities were then calculated.

Table-2

In-vivo analgesic activity of synthesized 2-substituted benzimidazolyl chalcones (6a-j) by writhing reflex syndrome method

S.No	Groups	Average No. of writhing reflex up to 15 minutes			Percentage protection		
		10 mg/kg	20 mg/kg	30 mg/kg	10 mg/kg	20 mg/kg	30 mg/kg
1	6a	25.0±0.632***	19.8±0.663***	19.2±0.583***	23.78	39.63	41.46
2	6b	28.4±0.894***	24.6±0.600***	23.8±0.480***	13.41	25.00	27.43
3	6c	28.8±0.489**	24.8±0.583***	24.4±0.400***	12.19	24.39	25.60
4	6d	23.0±0.447***	17.2±0.800***	16.0±0.547***	29.87	47.56	51.21
5	6e	25.4±0.748***	20.8±0.734***	19.8±0.583***	22.56	36.58	39.63
6	6f	24.4±0.509***	17.8±0.489***	17.0±0.632***	25.60	45.73	48.17
7	6g	26.8±0.583***	22.0±0.707***	21.4±0.600***	18.29	32.92	34.75
8	6h	27.6±0.678***	23.8±0.860***	22.8±0.480***	15.85	27.43	30.48
9	6i	22.4±0.748***	16.2±0.734***	15.6±0.678***	31.70	50.60	52.43
10	6j	24.0±0.707***	18.4±0.509***	17.2±0.734***	26.82	43.90	47.56
11	STD	21.4±0.600***	12.8±0.583***	09.0±0.447***	34.75	60.97	72.56
12	CTL		32.8±.583			-	

Each average value represents as the mean ± SEM (n=5). Significance level **P<0.01 and ***P<0.001 as compared with the respective control. STD-Diclofenac , CTL-Control

Table-3

Anti-inflammatory activity of the Compounds (6a-j) by carrageenan induced rat paw edema method

Groups	Dose (mg/kg)	Rat paw edema volume average value				Percentage protection		
		After 0 hour	After 1 hour	After 3 hours	After 5 hours	After 1 hour	After 3 hours	After 5 hours
	10	0.80±0.051	1.40±0.051	1.76±0.033	2.06±0.042	4.10	7.36	7.62
6a	20	0.80±0.073	1.26±0.042*	1.66±0.021**	1.86±0.042***	13.69	12.63	16.59
	30	0.83±0.061	1.23±0.033**	1.50±0.068***	1.63±0.080***	15.75	21.05	26.90
	10	0.80±0.073	1.43±0.061	1.86±0.042	2.20±0.051	2.05	2.10	1.34
6b	20	0.83±0.033	1.33±0.042	1.73±0.042	2.03±0.061	8.90	8.94	8.96
	30	0.80±0.051	1.36±0.061	1.60±0.051**	1.80±0.051***	6.84	15.78	19.28
	10	0.83±0.033	1.46±0.042	1.86±0.042	2.23±0.033	-	2.10	-
6c	20	0.80±0.051	1.40±0.051	1.73±0.042	2.06±0.060	4.10	8.94	7.62
	30	0.83±0.033	1.36±0.033	1.66±0.061*	1.83±0.061**	6.84	12.63	17.93
	10	0.83±0.033	1.33±0.066	1.63±0.033***	2.00±0.051**	8.90	14.21	10.31
6d	20	0.83±0.033	1.20±0.051**	1.43±0.033***	1.60±0.073***	17.80	24.73	28.25
	30	0.83±0.033	1.06±0.042***	1.26±0.042***	1.43±0.061***	27.39	33.68	35.87
	10	0.80±0.051	1.43±0.033	1.83±0.033	2.13±0.042	2.05	3.68	4.48
6e	20	0.83±0.061	1.40±0.051	1.80±0.051	2.10±0.044	4.10	5.26	5.82
	30	0.80±0.073	1.30±0.044	1.80±0.051	2.06±0.042	10.95	5.26	7.62
	10	0.83±0.033	1.36±0.061	1.70±0.044*	2.00±0.051**	6.84	10.52	10.31

6f	20	0.83±0.061	1.23±0.033**	1.56±0.033***	1.63±0.061***	15.75	17.89	26.90
	30	0.80±0.073	1.16±0.033***	1.36±0.061***	1.50±0.085***	20.54	28.42	32.73
	10	0.83±0.033	1.46±0.066	1.90±0.044	2.23±0.033	-	-	-
6g	20	0.80±0.051	1.43±0.033	1.83±0.033	2.13±0.042	2.05	3.68	4.48
	30	0.80±0.073	1.40±0.051	1.76±0.033	2.00±0.073	4.10	7.36	10.31
	10	0.80±0.051	1.46±0.042	1.90±0.044	2.23±0.033	-	-	-
6h	20	0.83±0.033	1.46±0.042	1.86±0.042	2.13±0.042	-	2.10	4.48
	30	0.83±0.061	1.43±0.033	1.73±0.042	2.06±0.060	2.05	8.94	7.62
	10	0.83±0.033	1.33±0.066	1.63±0.033***	1.66±0.021***	8.90	14.21	25.56
6i	20	0.80±0.073	1.16±0.033***	1.36±0.033***	1.50±0.068***	20.54	28.42	32.73
	30	0.80±0.051	1.03±0.033***	1.20±0.051***	1.40±0.073***	29.45	36.84	37.21
	10	0.80±0.051	1.36±0.061	1.70±0.044*	2.03±0.033*	6.84	10.52	8.96
6j	20	0.83±0.061	1.23±0.033**	1.53±0.042***	1.66±0.042***	15.75	19.47	25.56
	30	0.83±0.033	1.16±0.061***	1.33±0.066***	1.53±0.084***	20.54	30.00	31.39
	10	0.80±0.073	1.26±0.042*	1.43±0.061***	1.50±0.068***	13.69	24.73	32.73
STD	20	0.80±0.051	1.10±0.044***	1.16±0.061***	1.23±0.061***	24.65	38.94	44.84
	30	0.83±0.061	0.96±0.033***	1.03±0.033***	1.06±0.042***	34.24	45.78	52.46
CTL		0.80±0.051	1.46±0.042	1.90±0.044	2.23±0.033	-	-	-

Each paw edema average 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 , CTL-Control

Table-4

In-vivo central nervous system –locomotor activity of synthesized 2-substituted benzimidazolyl Chalcones (6a-j) by using digital actophotometer .

	Average number of cut-off movements in 15 minutes					
Groups	10 mg/kg	Percentage	20 mg/kg	Percentage	30 mg/kg	Percentage
6a	152.6±1.030	99.60	149.8±1.828	97.78	132.6±2.112***	86.55
6b	150.2±0.969	98.04	148.8±1.685	97.12	128.2±2.267***	83.68
6c	150.6±0.812	98.30	148.0±2.074	96.60	121.8±2.154***	79.50
6d	146.2±1.068**	95.43	126.0±1.924***	82.24	85.8±2.354***	56.00
6e	152.2±1.114	99.34	150.0±1.581	97.91	135.2±1.985***	88.25
6f	147.2±1.020*	96.08	126.8±1.200***	82.76	90.0±2.429***	58.74
6g	144.2±0.969***	94.12	118.6±1.806***	77.41	78.6±3.076***	51.30
6h	148.2±1.068	96.73	131.2±1.594***	85.63	93.0±2.646***	60.70
6i	152.0±1.049	99.21	149.2±2.035	97.38	143.4±3.295	93.60
6j	153.0±1.095	99.86	152.4±1.568	99.47	149.2±2.478	97.38
A	208.4±1.63***	136.03	Not tested	--	Not tested	--
B	073.2±1.393***	47.78	Not tested	--	Not tested	--
CTL	153.2±1.020				Nil	

Each cut-off average 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.

A-Caffeine (10 mg/kg) B-Diazepam (less than 5 mg/kg)

RESULTS AND DISCUSSION

Our study was based on the synthesis of various 2-substituted benzimidazolyl Chalcones derivatives and evaluated for their analgesic, anti-inflammatory and central nervous system locomotor activity. The results of the *in-vivo* analgesic activity are displayed in Table-2 & Figure-1. It is revealed that all the synthesized benzimidazoles at different tested concentrations 10mg/kg, 20mg/kg and 30mg/kg were showed significant analgesic property against control, and it is observed that activity steeply increases when increasing concentration of the benzimidazoles. It has been displayed that benzimidazoles 6d & 6i were exhibited excellent and 6a,6f & 6j were showed good analgesic potency when those were compared with the standard drug diclofenac. The benzimidazoles 6g, 6e are exerted moderate and 6b, 6c&6h are possess weak analgesic activities. The anti-inflammatory screening data is noticed in Table-3 & Figure-1. It shows that some benzimidazoles are given significant activity at 20mg/kg and 30mg/kg concentrations. In comparison with diclofenac, benzimidazoles 6d, 6f, 6i, 6j are exhibited good and 6a showed considerable anti-inflammatory activities. On the other hand, benzimidazoles 6b,6c have lowest and 6e,6g,6h are showed bad anti-inflammatory activity. It was noticed that, anti-inflammatoryactivities increases when increasing concentration of tested benzimidazoles (except 6e & 6h).

When ***structure activity relationship studies*** are concerned 2-substituted, benzimidazolyl chalcones bearing –OH,–OCH_3 or –$N(CH_3)_2$ such a electron donating substituted analogues and electron withdrawing substituents such as NO_2, F or Cl has shows more analgesic activity than unsubstituted benzimidazoles. In the tested series,polar electron donating substituents such as –OH and –OCH_3 groups are found to exhibit the more analgesic activity than nonpolar electron donating substituent like–$N(CH_3)_2$group. Meanwhile, the electronegative F, Cl substituted analogues are also displayed good analgesic properties than bulky NO_2 substituted benzimidazoles.The tested ortho substituted benzimidazoles were exerted more potent analgesic properties than para substituted benzimidazoles . The results showed that F, Cl, –OH and–OCH_3 substituted benzimidazoles have appreciable anti-inflammatory properties but the bulky –$N(CH_3)_2$ and NO_2 substituted benzimidazoles exerted bad anti-inflammatory activity than the unsubstituted analogues. Moreover, the orthoOH , ortho Cl substituted benzimidazoles (6d,6i) produced excellant antinociceptiveactivity than that of 4–OH and 4 or 3–OCH_3 substituted benzimidazoles (6a,6j). Amoung this 4–F substituted benzimidazole (6f) marked equal antinociceptive potency to the 4–OH substituted benzimidazole (6j). Moreover extendedly conjugated benzimidazole (6c) slighty less antinociceptive activity than parent analogue (6b).

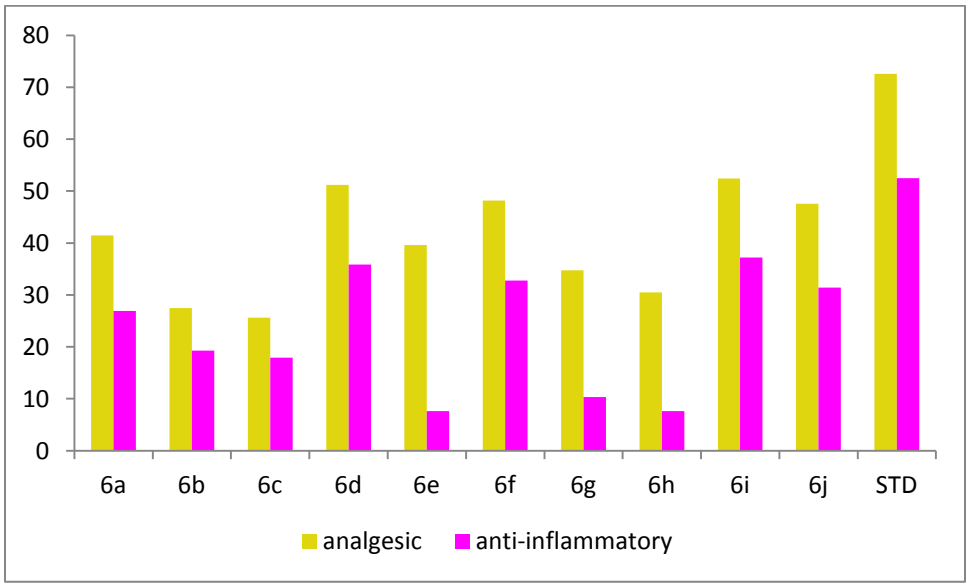

Fig. No.1. Graphical representation of the analgesic and anti-inflammatory percentage activity of the synthesized Compounds (6a-j), Standard and Test Dose = 30 mg/kg in each group of animals.

The central nervous system locomotor activities of the benzimidazoles were evaluated by using digital actophotometer and the results are shown in Table-4 & Figure-2. The data noticed that most of the benzimidazoles are shows varying degrees of reducing locomotor activities against control group. All the synthesized benzimidazoles were fails to show central nervous system stimulant activity and also most of the tested benzimidazoles does not produce significant depressant activity at the tested 10mg/kg &20mg/kg low concentrations but a all the tested benzimidazoles produced significant decrease of locomotor activity count were observed at 30mg/kg high concentration (except compounds 6i and 6j). Amoung this, the benzimidazoles 6d,6f,6g,6h were only expressed higher depressant property when compared to standard drug diazepam and benzimidazoles 6b,6c revealed mild depressant properties.

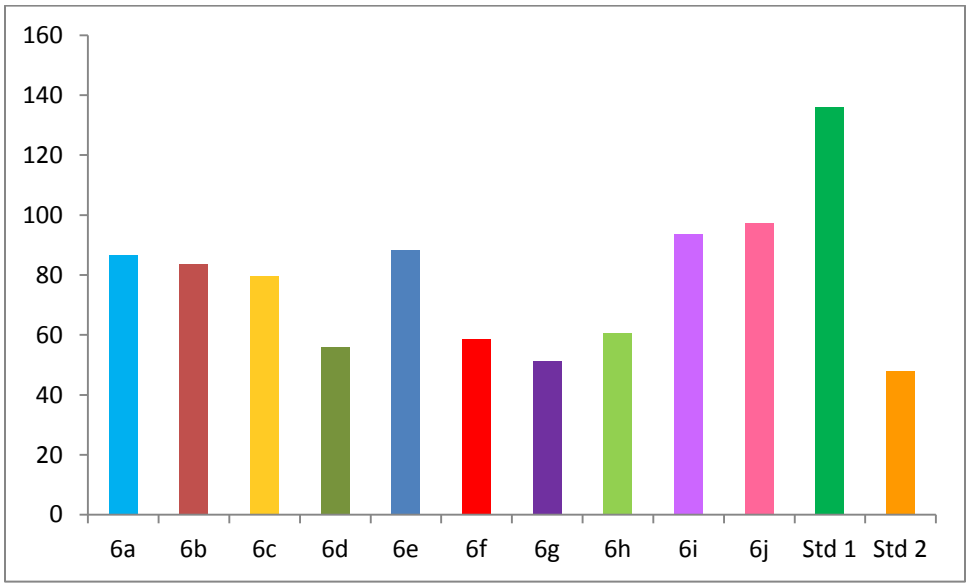

Fig. No.2. Graphical representation of the CNS locomotor movement of the synthesized compounds (6a-j) compared with stimulant (Std1) and depressant (Std2) standards

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

So the structure activity relationship studies proves that electron withdrawing substituents such as NO_2, F or Cl functional groups must be necessary to improve central nervous system depressant activity. Furthermore the benzimidazoles 6d & 6g possess less number of cut-offs than 6f & 6h. This results also revealed that bulky electron withdrawing NO_2 group in a 2^{nd} position substituted benzimidazole (6g) produced better depressant activity than 2^{nd} position Cl substituted benzimidazole (6d) but the 4–F substituted benzimidazole (6f) elicits more central nervous depressant system activity than bulky 4–NO_2 substituted analogue. Meanwhile the non-polar electron donating –$N(CH_3)_2$ or–OCH_3 substituted analogues (6e & 6a) showed more number of cut-offs and polar electron donating OH substituted benzimidazoles (6i & 6j) high number cut-offs values when compared to the unsubstituted benzimidazole analogues (6b & 6C). The extended conjugate benzimidazolyl chalcone (6c) shows slighty elevated the locomotor depressant activity than parent analogue (6b).

CONCLUSION

In summary, we have described the synthesis, series of ten various 2-substituted benzimidazolyl chalcones. The structures of the synthesized heterocyclic analogues were verified by FTIR,^{1}H-NMR, ^{13}C-NMR mass spectral

data[25] and physical analysis.The *in-vivo* analgesic activity data indicates that benzimidazoles 6d, 6f, 6i and 6j have higher activity than other tested groups. The benzimidazoles 6a, 6d, 6f, 6i and 6j analogues exhibited good anti-inflammatory activity. Amoung this benzimidazoles 6d and 6i only exert high antinociceptive potency, so the results proves that ortho electron donating OH group substituted analogue and ortho electron withdrawing Cl substituted benzimidazole have given potent antinociceptive properties. On the other hand bulky electron donating $-N(CH_3)_2$ and electro negative NO_2 substituted benzimidazoles drastically reduce the anti-inflammatory activity than unsubstituted analogues .

The central nervous system activity reports exhibiting that, the electron withdrawing group in 2nd position substituted benzimidazolyl chalcones (6d,6g) produce more central nervous system depressant activity than that of 4th position substituted analogues (6f,6h). The polar OH group analogue fails to produce considerable CNS depressant activity. Finally SAR studies concluded that 2nd substituted benzimidazole analogues exhibits an elevated analgesic and anti-inflammatory activitythan the corresponding 4th substituted and unsubstitued analogues. In fact, the electron withdrawing substituted benzimidazoles only depicted central nervous system depressant activity. Moreover the extended conjugated analogue (6c) have exerted less analgesic and anti-inflammatoryactivity but displayed comparablely more CNS depressant activity than the benzimidazolyl chalcone (6b).

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