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ANTI-INFLAMMATORY STUDIES OF NOVEL METHYL AMINO AND HYDRAZINO BENZIMIDAZOLES ON CARRAGEENAN INDUCED PAW EDEMA IN RATS

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ABSTRACT

A series of N', N''-[1-(1H-benzimidazole-2-yl)-2-(4-substituted phenyl ethane-1, 2-diyl)] substituted aromatic amines (AK_1- AK_6) and hydrazides (AKH_6 – AKH_{11}) were synthesized by treating 2-methyl benzimidazole with different substituted aromatic aldehydes in the presence of ethanol to produce 2-[2-(4 chlorophenyl)-vinyl]-1H-benzimidazole. It was brominated to 2-[1, 2 dibromo 2-(4-substituted phenyl) ethyl]-1H-benzimidazole, which on treatment with different substituted amines and hydrazides in absolute alcohol brings forth the newer compounds. The structures attributed to the newly synthesized compounds were elucidated using IR, NMR, MASS techniques besides elemental analysis. The compounds were evaluated for their anti-inflammatory potentials by carrageenan induced paw edema bio-assay in rats. Many of the target compounds showed good anti-inflammatory activity. A most distinctive derivative, 1-[1-H-benzimidazol-2-yl]-N, N',2-tris (4-chlorophenyl)-ethane-1,2-diamine (AK_5) was identified in the present study, due to its remarkable anti-inflammatory potentials and was found to be equipotent with that of Indomethacin, observed after the introduction of p-chloro anilino group with 2- methyl benzimidazole moiety. The acute toxicity study of the compounds indicated that they were well tolerated. Therefore, such a compound would represent a fruitful matrix for the development of anti-inflammatory candidates.

KEY WORDS: Methyl amino, hydrazino benzimidazoles, anti-inflammatory potentials,

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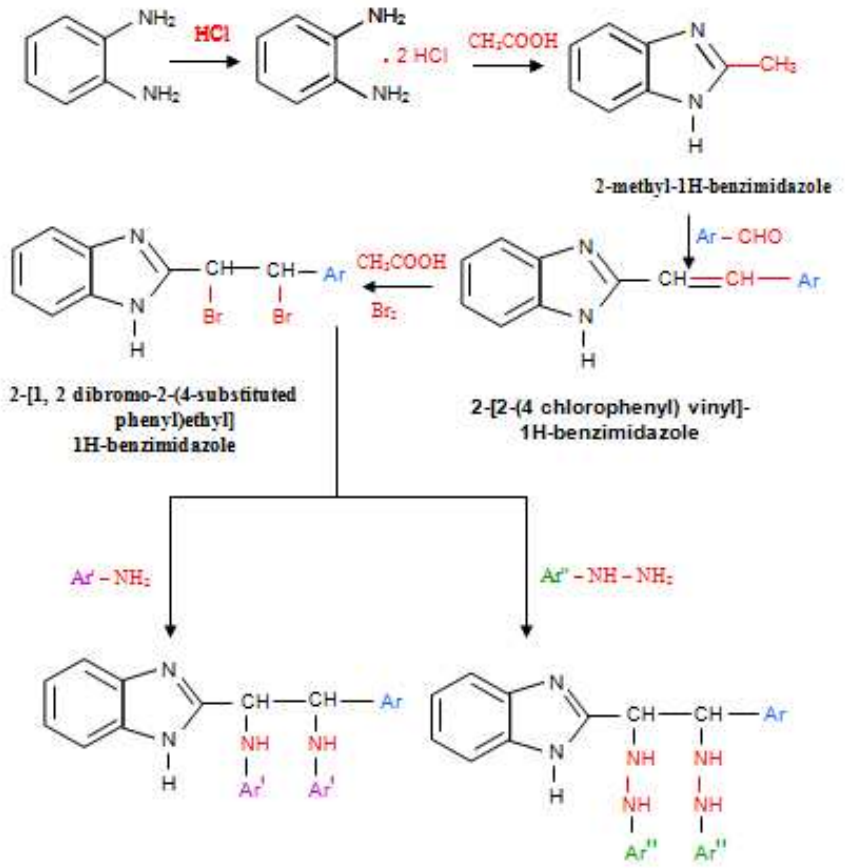
INTRODUCTION

A large number of substitutions have been incorporated in benzimidazoles to produce therapeutically interesting drug candidates and these hybrid molecules exert remarkable biological and pharmacological potentials. In view of the various reports, benzothiazenyl, sulphono, methoxy indolo, benzothiazolyl carbonyl, thiadiazinyl, diphenyl aryl tetra hydro, aryl pyrazolyl, aryl pyrimidinyl benzimidazoles have received significant attention for their anthelmintic[1,2], anti-viral[3], anti-microbial[4], CNS depressant[5], analgesic[6], anti-inflammatory[7] and anti-fungal potentials[8]. Moreover 5, 6-dimethyl benzimidazole is an important moiety in vitamin B_{12} structure used in treating megaloblastic anemia. Other important biologically active compounds include mebendazole and thiabandazole which are good against nematode infections. Inflammation is a complex biological response of vascular tissues to harmful stimuli and initiates the healing process. The treatment of inflammatory conditions still remains as an important challenge. A number of anti-inflammatory agents have been discovered and many of them have disappeared due to their side effects and lack of specificity. Therefore a potent inhibitor of inflammation does not really exist and intensive investigation seems to be necessary. Owing to the versatility of benzimidazoles, we have synthesized N', N''-[1-(1H-benzimidazole-2-yl)-2-(4-substituted phenyl ethane-1, 2-diyl)] substituted aromatic amines and hydrazides and evaluated them for their anti-inflammatory activity by carrageenan induced paw edema bio-assay and also analyzed for acute toxicity.

MATERIALS AND METHODS

Laboratory grade solvents, reagents and chemicals were procured from Loba chemie, Mumbai. Melting points of the synthesized compounds were determined by a open tube capillary method and were uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel G plates using Benzene-Chloroform-Methanol (5:3:2) as the solvent system and the spots were determined either under ultra violet light or through the exposure to iodine vapors. The IR spectra were recorded on Jasco FT/IR-410 spectrophotometer, KBr Press, Shimadzhu at SRIPMS, Coimbatore. ^{1}H NMR spectra were recorded on Bruker (AMX-400) in DMSO (D_6) using tetra methyl silane (TMS) as the internal standard at IISC, Bangalore. Chemical shifts (d) were reported in parts per million (ppm) downfield from TMS. Mass spectra (LCMS - 2010 with HPLC UV detector, Shimadzu) were recorded at JSS College of Pharmacy, Ooty. Spectral data were consistent with the assigned structures. The elemental analysis was carried out at IICT, Hyderabad (C, H, N) were found to be within the range of ±0.4% of theoretical values. The Pharmacological experiments were done at Periyar college of Pharmaceutical Sciences, Trichy, Tamilnadu and approved by Institute Animal Ethical Committee, with register No: 412.

Scheme



N',N''-[1- (1H- benzoimidazole- 2-yl)-2-(4-substituted phenyl ethane-1,2- diyl)] substituted aromatic amino and hydrazide derivatives
Ar = 4-chloro phenyl, 4-nitro Phenyl, 4-fluoro phenyl, 4-methyl phenyl

Ar' = 4-carboxy phenyl, 3-hydroxy4-carboxy phenyl, 4-chloro phenyl,4-fluro phenyl, 4- methyl phenyl

Ar'' = pyridine-4-carbonyl , phenyl, benzoyl

METHOD OF SYNTHESIS

1. Preparation of 2-methyl benzimidazole
O-phenylenediamine (0.77 mole, 14g) was dissolved in con.hydrochloric acid (1g) and heated with 1-2 g of decolorizing carbon. Con. hydrochloric acid (50 ml) was added to the hot colourless filtrate, cooled in a freezing mixture of ice and the colourless crystals of o-phenylenediamine dihydrochloride separately. Prepared dihydrochloride (4.38g, 0.03 moles) was refluxed with acetic acid (5.48 g, 0.09 moles) for 45 mins. Then this mixture was cooled. Then conc. ammonia solution was gradually added to the cooled mixture and the precipitated product was recrystallized from aqueous ethanol (10%). Yield: 85%; mp 175°C; R_f 0.6; IR (cm^-1): 1590.95(C=N str), 1425 (C=C Aro str), 856.24 (CH Aro bend); ^{1}H NMR (400 MHz, DMSO- d_6, ppm): 7.2-8.6 (5H, Ar), 12.2(NH).

2. General procedure for preparation of 2-[2-(4 chlorophenyl) vinyl]-1H-benzimidazole

2-methyl benzimidazole (0.01 mole, 1.76 g) was dissolved in ethanol and sodium hydroxide solution (30 ml, 10%) was added and then cooled. (0.01 mole) Different substituted aromatic aldehydes dissolved in a minimum quantity of ethanol was added and stirred for 5 hrs and left overnight. The solid separates on addition of Con. hydrochloric acid drop wise. It was recrystallized from aqueous ethanol (10%). Yield: 89%; mp 144°C; R_f 0.58; IR (cm^{-1}): 3095(C-H Aro str), 3329(NH str), 1595(C=N str), 1650 (CH=CH str); ^{1}H NMR (400 MHz, DMSO - d_6, ppm): 7.1-8.5 (16H, Ar), 12.6 (1H,NH)), 8.28 (CH=CH), 8.15 (CH=CH).

3. General procedure for the synthesis of 2-[1, 2 dibromo-2-(4-substituted phenyl) ethyl] 1H-benzimidazole

The arylidine compound (0.01 mole, 2.98 g) was dissolved in glacial acetic acid (10 ml) and bromine (0.03 mole, 6 ml). Then it was stirred for 3 hrs and left overnight. Crushed ice was added to the reaction mixture and the intermediate obtained was filtered, dried and recrystallized from aqueous ethanol (50%). Yield: 89%; mp 244°C; R_f 0.64; IR (cm^{-1}): 3095(C-H Aro str), 3329(NH str), 1595(C=N str), 1650 (CH=CH str); ^{1}H NMR (400 MHz, DMSO - d_6, ppm): 7.0-8.4 (16H, Ar), 12.6 (1H, NH).

4. General procedure for the synthesis of N', N"-[1- (1H- benzoimidazole- 2-yl)-2-(4-substituted phenyl ethane-1,2- diyl)] substituted aromatic amino and hydrazide derivatives

Aryl substituted dibromo compound (0.01 mole, 4.15g) was added with (0.015 mole) sodium pellet in 50 ml of absolute alcohol. Different substituted amines and hydrazides (0.01 moles) were added and refluxed for 24 hrs. The volume of the reaction mixture was concentrated to half and the mixture was poured on the crushed ice, filtered and recrystallized in aqueous ethanol (50%).

4.1. 4, 4'-[1-(1H-benzimidazol-2yl)-2-(4-chlorophenyl) ethane -1, 2-diyl] bis (azanediyl) dibenzoic acid (AK_1)

Solid, yield: 68%; mp. 208-209 °C; R_f: 0.64; UV (λ_{max}) 284 nm in ethanol: IR (KBr, V_{max} cm^{-1}): 3231.31 (OH), 1683.55 (C=O), 1591.95 (C=N), 1425.14 (C=C Ar), 1090.55 (C-Cl Ar), 850.45 (CH Ar bend) , ^{1}H NMR (400 MHz, DMSO - d_6, ppm): 3.5-3.8 (2H, NH), 4.2-4.6 (2H,CH), 7.1-8.4 (16H, Ar), 12.2(1H,NH), 13.2 (2H,COOH) , ^{13} C NMR (DMSO - δ ppm) : 64 (C,attached to NH &chlorophenyl), 66 (C,attached to benzimidazole & NH), 112-152 (imidazole C), 172 (C, COOH); Mass : (m/z): 528 (M+). Anal. Calcd. For C_{29} H_{23} N_4 O_4 Cl : C, 66.14; H, 4.36; N, 10.63. Found: C, 66.08; H, 4.32; N, 10.61 %.

4.2. 4, 4'-[1-(1H-benzimidazol-2yl)-2-(4-chlorophenyl) ethane -1, 2-diyl] bis (azanediyl) bis (2-hydroxybenzoicacid) (AK_2)

Solid, yield: 72%; mp. 170-171°C; R_f: 0.61; UV (λ_{max}) 286 nm in ethanol: IR (KBr, V_{max} cm^{-1}) : 3066.35 (NH), 2886.98 (CH), 1689.11 (C=O), 1587.32 (C=N), 1085.66 (C-Cl Ar), 1011.44 (CN), 852.14 (CH Ar bend) , ^{1}H NMR (400 MHz, DMSO - d_6, ppm): 3.3-3.6 (2H, NH), 3.9-4.2 (2H,CH), 4.5-4.7 (2H,OH), 7.0-8.4 (14H, Ar), 12.2-12.3(1H,NH), 13.0-13.2 (2H,COOH) , ^{13} C NMR (DMSO - δ ppm) : 65 (C,attached to NH &chlorophenyl), 67 (C,attached to benzimidazole & NH), 104-120, 141, 151 (imidazole C), 168 (C, COOH), 159(C Ar, with OH); Mass : (m/z): 560 (M+). Anal. Calcd. For C_{29} H_{23} N_4 O_6 Cl: C, 62.25; H, 4.11; N,10.01. Found: C, 62.30; H, 4.08; N, 10.03 %.

4.3. 4, 4'-[1-(1H-benzimidazol-2yl)-2-(4-nitrophenyl) ethane -1, 2-diyl] bis (azanediyl) dibenzoicacid (AK_3)

Solid, yield: 82%; mp. 158-159°C; R_f :0.69; UV (λ_{max}) 287 nm in ethanol: IR (KBr, V_{max} cm^{-1}) : 3066.35 (NH), 2886.98 (CH), 1689.11 (C=O), 1587.32 (C=N), 1085.66 (C-Cl Ar), 1011.44 (CN), 852.14 (CH Ar bend) , ^{1}H NMR (400 MHz, DMSO - d_6, ppm): 3.3-3.6 (2H, NH),

3.9-4.2 (2H,CH), 4.5-4.7 (2H,OH), 7.0-8.4 (14H, Ar), 12.0-12.3 (1H,NH), 13.0-13.2 (2H,COOH) , ^{13} C NMR (DMSO - δ ppm) : 65 (C,attached to NH &chlorophenyl), 67 (C,attached to benzimidazole & NH), 104-120, 141, 151 (imidazole C), 168 (C, COOH), 159(C Ar, with OH); Mass : (m/z): 655 (M+). Anal. Calcd. For C_{29} H_{23} N_5 O_6 : C, 53.19; H,3.52; N,11.20. Found: C, 53.23; H, 3.56; N, 11.23 %.

4.4. 4, 4'-[1-(1H-benzimidazol-2yl)-2-(4-nitrophenyl) ethane -1, 2-diyl] bis (azanediyl) bis (2-hydroxybenzoicacid) (AK_4)

Solid, yield: 73%; mp. 172-173°C; R_f: 0.68 ; UV (λ_{max}) 259 nm in ethanol: IR (KBr, V_{max} cm^{-1}) : 37470.1(OH), 2836.77(CH), 1692.19 (C=N), 1587.13(C=O), 1409.71 (C=C), 1349.93 (nitro Ar), 1101.15(CN), 798.38(Ar bending), ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.7-3.9 (2H, NH), 4.5 (2H,CH), 6.6-7.9 (16H,Ar); Mass : (m/z): 726 (M+). Anal. Calcd. For C_{29} H_{23} N_5 O_8 : C, 47.99; H, 3.17; N, 9.65. Found: C, 47.92; H, 3.19; N, 9.63 %.

4.5. 1-[1-H-benzimidazol-2-yl]-N, N', 2-tris (4-chlorophenyl) ethane-1, 2-diamine (AK_5)

Solid, yield: 68%; mp. 185-186°C; R_f: 0.66; UV (λ_{max}) 279 nm in ethanol: IR (KBr, V_{max} cm^{-1}) : 3477.03 (NH), 2825.2 (CH), 1594.84 (C=N), 1587.32 (C=N), 1482.99 (C=C), 1091.51 (C-Cl), 852.38 (CH Ar bend) , ^{1}H NMR (400 MHz, DMSO - d_6, δ ppm): 3.7-3.9 (2H, NH), 4.5 (2H,CH), 6.6-7.9 (16H, Ar), Mass : (m/z): 509 (M+). Anal. Calcd. For C_{27} H_2 N_4 Cl_3: C, 63.79; H, 4.14; N, 11.62. Found: C, 11.62; H, 4.08; N, 11.66 %.

4.6. N, N',-[1-(1H-benzimidazol-2-yl)-2-(4-chlorophenyl) ethane-1, 2-diyl]di isonicotino hydrazide (AKH_6)

Solid, yield: 62%; mp. 197-198°C; R_f: 0.59; UV (λ_{max}) 284 nm in ethanol: IR (KBr, V_{max} cm^{-1}) : 3415.31 (NH), 2843.52 (CH), 1684.52(C=O), 1591.95 (C=N), 1424.17(C=C), 1090.55(C-Cl Ar) , 1015.34(CN), 851.42 (CH Ar bend), ^{1}H NMR (400 MHz, DMSO - d_6, δ ppm): 1.9-2.1 (2H, NH), 4.2-4.4 (2H,CH), 6.5-7.8 (16H, Ar), 7.9-8.1 (2H,NH), 11.3-11.7 (1H,NH imidazole) , ^{13}C NMR (DMSO - ppm) : 62 (CH, attached to NH &chlorophenyl), 63 (CH,attached to benzimidazole & NH), 115-141,150 (imidazole C), 167; Mass : (m/z): 528 (M+). Anal. Calcd. For C_{27} H_{23} N_8 O_2 Cl: C, 61.53; H, 4.36; N, 21.27. Found: C, 61.56; H, 4.38; N, 21.29 %.

4.7. 2-[2-(4-chlorophenyl)-1,2-bis(2-phenylhydrazinyl-2-(4-tolyl)ethyl]-1H benzimidazole (AKH_7)

Solid, yield:80%; mp. 137–138°C; R_f: 0.69; UV (λ_{max}) 267 nm in ethanol: IR (KBr, V_{max} cm^{-1}) : 3401.1 (NH), 2811.7 (CH), 1898.77(C=N), 1359.57 (C=C), 997.02(CN), 1091.51(C-Cl Ar) , 845.6 (CH Ar bend), ^{1}H NMR (400 MHz, DMSO - d_6, δ ppm): 1.7-2.1 (2H, NH), 3.9-4.0 (2H,CH), 7.1-8.3 (18H, Ar), 12.18 (1H,NH imidazole) , ^{13}C NMR (DMSO - ppm) : 62 (CH, attached to NH & chlorophenyl), 65 (CH,attached to benzimidazole & NH), 112-141, (imidazole C), 167; Mass : (m/z): 470 (M+). Anal. Calcd. For C_{27} H_{25} N_6 Cl : C, 59.08; H, 5.33; N, 17.91. Found: C, 59.11; H, 5.29; N, 17.89 %.

4.8. 4, 4'-[1-(1H-benzimidazol-2yl)-2-(4-nitrophenyl) ethane -1, 2-diyl] bis (azanediyl) dibenzoicacid (AKH_8)

Solid, yield: 68% mp. 198-199°C; R_f: 0.58 ; UV (λ_{max}) 259 nm in ethanol: IR (KBr, V_{max} cm^{-1}) : 3465.46 (NH), 2805.92 (CH), 1600.63.77(C=N), 1365.35 (C=C), 1016.3(CH of methyl) , 1015.34 (CN), 851.42 (CH Ar bend), ^{1}H NMR (400 MHz, DMSO - d_6, δ ppm): 1.8-2.0 (2H, NH), 3.8-4.1 (2H,CH), 7.2-8.4 (18H, Ar), 12.69 (1H,NH imidazole) , Mass : (m/z): 297 (M+). Anal. Calcd. For C_{16} H_{20} N_6: C, 64.86; H, 6.76; N, 28.37. Found: C, 63.81; H, 6.71; N, 28.39 %.

4.9. N, N',-[1-(1H-benzimidazol-2-yl)-2-(4-nitrophenyl) ethane-1,2-diyl]di isonicotino hydrazide (AKH_9)

Solid, yield: 78%; mp. 156-157°C; R_f: 0.59 ; UV (λ_{max}) 287 nm in ethanol: IR (KBr, V_{max} cm^{-1}) : 3515.31 (NH), 2843.52 (CH), 1684.52(C=O), 1591.95 (C=N), 1424.17(C=C Ar) , 1329.56(Ar. Nitro bend), 851.48(CH Ar bend), ^{1}H NMR (400 MHz, DMSO - d_6, δ ppm): 1.7-2.1 (2H, NH), 3.9-4.0 (2H,CH), 7.1-8.3 (18H, Ar), 12.18 (1H,NH imidazole) ; Mass : (m/z): 539 (M+). Anal. Calcd. For $C_{27}H_{23}N_9O_4$: C, 60.27; H, 4.27; N, 23.44. Found: C, 60.21; H, 4.24; N, 23.42 %.

4.10. 2-[2-(4-nitrophenyl)-1, 2-bis (2-phenylhydrazinyl-ethyl]-1H-benzimidazole (AKH_{10})

Solid, yield:66%; mp. 163-164°C; R_f: 0.56; UV (λ_{max}) 268 nm in ethanol: IR (KBr, V_{max} cm^{-1}) : 3338.74 (NH), 3102.13 (CH Ar), 1585.2 (C=N), 1362.36 (C=C), 786.85 (CH Ar bend), ^{1}H NMR (400 MHz, DMSO - d_6, δ ppm): 2.1 (2H, NH), 3.6-3.8 (2H,NH), 4.0(1H,CH), 4.6(1H,CH) , 6.6-8.4(18H, Ar); ^{13}C NMR (DMSO - ppm) : 63 (CH, attached to NH &chlorophenyl), 66 (CH,attached to benzimidazole & NH), 115-141, (imidazole C), 167; Mass : (m/z): 480 (M+). Anal. Calcd. For $C_{15}H_{17}N_6Cl$: C, 67.64; H,5.21; N, 20.45. Found: C, 67.59; H, 5.18; N, 20.47%.

4.11. 2-[2-(4-chlorophenyl)-1, 2-dihydrazinyl ethyl]-1H-benzimidazole (AKH_{11})

Solid, yield:58%; mp. 219 -220°C; R_f: 0.51 ; UV (λ_{max}) 271 nm in ethanol: IR (KBr, V_{max} cm^{-1}) : 3436.33 (NH), 2832.92 (CH), 1596.77(C=N), 1359.57 (C=C), 856.24 (CH Ar bend), ^{1}H NMR (400 MHz, DMSO - d_6, δ ppm): 1.7-2.0 (2H, NH), 3.7-4.0 (2H,CH), 7.1-8.3 (18H, Ar), 12.18 (1H,NH imidazole) , ^{13}C NMR (DMSO - ppm) : 61 (CH, attached to NH &chlorophenyl), 64 (CH,attached to benzimidazole & NH), 111-141, (imidazole C), 167; Mass : (m/z): 318 (M+). Anal. Calcd. For $C_{15}H_{17}N_6Cl$: C, 56.81; H, 5.36; N, 26.51. Found: C, 56.76; H, 5.33; N, 26.49 %.

PHARMACOLOGICAL STUDIES

Anti- inflammatory screening - Carrageenan induced hind paw edema bioassay

Carrageenans are complex group of polysaccharides made of repeating galactose related monomers. Cardinal signs of inflammation immediately follow on subcutaneous injection resulting in edema, hyperalgesia and erythema due to the action of pro-inflammatory agents like bradykinin, histamine, tachykinin, complement, reactive oxygen and nitrogen species. Such agents can be generated *in situ* at the site of injection. The inflammatory response is usually quantified by increase in paw size (edema) which is maximum around 5 hours of post carrageenan injection. The NSAID like indomethacin can be used to modulate this condition. This model therefore has a vital role in novel drug development. Carrageenan induced hind paw edema bioassay was performed as per method described by Winter et al., 1962[9]. Albino rats of either sex weighing 150 - 200gms were divided into twelve groups. Each groups having six animals. Hind paw edema was induced in the animals by injecting 1% w/v carrageenan solution. Group-1 served as a control (0.1 ml of 1% w/v carrageenan solution in DMSO was injected into the subplantar tissue of the left hind paw of the rat and the right hind paw). Group-2 and group-3 received oral administration of 100 mg /kg and 200 mg/kg of synthesized compound-AKH_6 respectively. Group-4 and group-5 received oral administration of 100 mg /kg and 200 mg/kg of synthesized compound-AKH_2 respectively. Group-6 and group-7 received oral administration of 100 mg /kg and 200 mg/kg of synthesized compound-AKH_7 respectively. Group-8 and group-9 received oral administration of 100 mg /kg and 200 mg/kg of synthesized compound-AKH_8 respectively. Group-10 and group-11 received oral administration of 100 mg /kg and 200 mg/kg of synthesized compound-AKH_5 respectively. Group-12 received oral administration of 10

mg/kg indomethacin (standard drug). All synthesized compounds and standard drug were administered one hour prior to carrageenan injection. Synthesized compounds were perfomed in Periyar College of Pharmaceutical Sciences for Girls, Trichy, Tamilnadu. Before performing the animal experiments, ethical clearance was obtained from Institutional Animal Ethics (CPCSEA Reg No. 412). The volume of the mercury displaced in the plethysmograph was measured at the end of 0, 60, 120, 180 and 240 minutes. The % increase in paw edema of the treated group was compared with that of the control and the inhibitory effect of the drugs were studied**.** The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation. Students'*t*' test was performed for statistical analysis. The '*P'* values less than 0.01 were considered significant.

Acute toxicity study

Each animal, at the commencement of its dosing, was between 8 and 12 weeks old and their weight variation was within ± 20% of the mean weight of any previously dosed animals. The temperature in the experimental animal room was 22ºC (± 3ºC) and the relative humidity was between 50-60%. These animals were fed with pellet diet manufactured by Amrut laboratory, Animal Feed Company, Sangli, Maharashtra and drinking water ad libitum. They were kept in 12 hrs/12 hrs light/dark cycle and maintained for at least 5 d prior to dosing to allow for acclimatization to the laboratory conditions. The animal experimental protocol has been approved by our Institutional Animal Ethics Committee vide reference no: 412. Acute toxicity study for the newer synthesized compounds was performed as per method described by Lingaraju et al., 2011[10]. A total of 5 animals were used which received a single oral dose of 2000 mg/ kg of the synthesized compounds. Animals were kept overnight fasting prior to drug administration. After the administration of the synthesized compounds, food was withheld for further 3-4 hours. Animals were observed individually at least once during the first 30 minutes after dosing periodically during the first 24 hours and up to 14 days after drug administration.

Table 1
Anti-inflammatory activity of synthetic compounds against Carrageenan induced Paw Edema in Rats

Treatment	% Increase in paw volume Mean ± S.E. (n=6) Post insult time of assay in minutes					% inhibition in paw volume
	0	60	120	180	240	
Control (0.5 ml/ kg)	37.81±1.53	68.42 ±3.24	95.73±7.35	107.95±8.09	110.16±9.45	-
AKH_6 100 mg /kg	29.35 ±1.94	59.38±4.2	63.92±5.21	72.83±6.42 *	74.71±6.21 *	32.53
AKH_6 200 mg / kg	30.68±2.15	53.81±3.74	60.39±4.91	69.47±5.01 *	71.28±5.92 *	35.64
AK_2 100 mg /kg	31.13±1.94	41.19±3.21	56.83 ±5.1	71.59±6.27 *	73.48±6.48 *	33.68
AK_2 200 mg / kg	31.33±2.71	40.47±3.72	52.85±4.15	65.48±4.95 **	68.72±5.12 *	39.34
AKH_7 (100 mg / kg)	27.82± 1.38	45.63±2.96	62.94±4.76	73.69±6.26 *	69.47±5.21 *	31.73

AKH_7 (200 mg / kg)	29.62±1.27	39.64±2.04	57.83±4.72	68.14±5.53 *	71.29±5.9 *	36.87
AKH_8 100 mg /kg	32.63 ±2.15	62.54±4.92	68.43±5.73	70.93±6.54*	72.26±6.79 *	34.29
AKH_8 200 mg / kg	30.71 ±2.34	58.49 4.25	62.92±4.95	67.19±4.58**	70.09±6.23 **	37.75
AK_5 (100 mg / kg)	28.72 ±1.86	49.32 ±4.5	72.4 ±6.9	66.87±6.12**	61.36±5.5 **	38.05
AK_5 (200 mg / kg)	30.25 ±2.07	47.62±4.2	61.54 ±5.4	59.93±4.7 **	56.26±5.1 **	44.48
Indomethacin (10 mg / kg)	26.7 ±0.93	35.79±1.63	39.2 ±2.25	54.3±4.21 **	57.32±4.02**	49.69

MEAN ± SEM * P < 0.01 Vs Control, ** P < 0.001 Vs control

RESULTS AND DISCUSSION

As outlined in the scheme, the synthesis of eleven novel compounds, N', N''-[1-(1H-benzimidazole-2-yl)-2-(4-substituted phenyl ethane-1, 2-diyl) substituted aromatic amines (**AK_1- AK_6**) and hydrazides (**AKH_6 – AKH_{11}**) have been reported as part of our research work in developing novel anti-inflammatory agents. Initially, a characteristic reaction occurs when o-phenylenediamine dihydrochloride was reacted with different substituted aromatic aldehydes where in aldol condensation takes place. The methyl group in benzimidazole is highly active due to the presence of -C=N- group and the intermediate (1), 2-[2-(4-chloro phenyl) vinyl]-1H benzimidazole was formed. Then bromination was performed by simple addition reaction to produce the intermediate (2) which is, 2-[1, 2 dibromo-2-(4-substituted phenyl) ethyl1H-benzimidazole. Finally by nucleophilic substitution, different aromatic amines and hydrazides were added in the presence of ethanol. The substitution follows SN_1 mechanism which led to the formation of N', N''-[1-(1H-benzimidazole-2-yl)-2-(4-substituted phenyl ethane-1, 2-diyl) substituted aromatic amino and hydrazide derivatives. The structures attributed to the newly synthesized compounds were elucidated using IR, ^{1}H NMR, ^{13C} NMR and MASS techniques besides elemental analysis. The synthesized compounds gave M+ peak in reasonable intensities. According to the results from elemental analysis, carbon, hydrogen and nitrogen are found within the range of ±0.4% of theoretical values. Anti-inflammatory screening was carried out by carrageenan induced hind paw edema method using Indomethacin as the standard. Many of the target compounds showed anti-inflammatory activity. The results illustrate that N, N',-[1-(1H-benzimidazol-2-yl)-2-(4-chlorophenyl)-ethane-1,2-diyl]di isonicotino hydrazide (AKH_6), 4, 4'-[1-(1H-benzimidazol-2yl)-2-(4-chlorophenyl)-ethane -1, 2-diyl] bis (azanediyl) bis (2-hydroxybenzoicacid) (AK_2), 2-[2-(4-chlorophenyl)-1,2-bis(2-phenylhydrazinyl-2-(4-tolyl)ethyl]-1H-benzimidazole (AKH_7) and 4, 4'-[1-(1H-benzimidazol-2yl)-2-(4-nitrophenyl) ethane -1, 2-diyl] bis (azanediyl) di benzoic acid (AKH_8) showed % inhibition of 32. 53, 33.68, 31.73 and 34.29 respectively at a dose of 100 mg/kg and 35.64, 39.34, 36.87 and 37.75 respectively at a dose of 200 mg/kg body weight, which was a significant data (Table 1). At a dose of 100 mg/ kg, 1-[1-H-benzimidazol-2-yl]-N, N', 2-tris (4-chlorophenyl)-ethane-1, 2-diamine (AK_5) exhibited 38.05 % of inhibition of edema and at a dose of 200 mg/kg showed 44.48% inhibition which is significant to the standard drug, Indomethacin. Acute toxicity studies were performed. The results indicate that there was neither mortality nor any signs of clinical abnormality in the tested animals and the compounds up to a dose of 2000 mg/ kg

were reported to be non-lethal. The LD_{50} of the synthesized compounds falls under class 4 values as per the OECD guide lines.

CONCLUSION

To sum up, N', N''-[1-(1H-benzimidazole-2-yl)-2-(4-substituted phenyl ethane-1, 2-diyl) substituted aromatic amines and hydrazides have been synthesized and assessed for their anti-inflammatory potentials by Carrageenan induced hind paw edema bioassay in albino rats. 1-[1-H-benzimidazol-2-yl]-N,N', 2-tris (4-chlorophenyl)-ethane-1, 2-diamine (AK_5) possesses significant anti-inflammatory potentials by inhibiting edema at the order of 38.05% and 44.48% at a dose of 100 mg/ kg and 200 mg/ kg respectively, thus proving its equipotency with the standard drug, indomethacin. According to the SAR observed, the introduction of p-chloro aniline with 2-methyl benzimidazole has a major role in producing significant anti-inflammatory potentials. Acute toxicity study indicates that, the compounds up to a dose of 2000 mg/ kg are non – lethal. At the outset, from the research work carried out, 1-[1-H-benzimidazol-2-yl]-N, N', 2-tris (4-chlorophenyl) ethane-1, 2-diamine was identified as a lead compound and chronic toxicity studies could be tried for the development of a novel anti-inflammatory agent with lesser adverse effects when compared with the existing NSAIDS.

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