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Research Article

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Synthesis, characterization and evaluation of analgesic activity of some 5-nitro benzimidazole derivatives

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ABSTRACT

In literature benzimidazole derivatives are reported to possess variety of pharmacological activities of which analgesic activity is one of the important. Moreover benzimidazole derivatives have found their clinical application as analgesic agent. Orthophenylenediamine and 4-choro orthophenylenediamine were condensed with three varieties of substituted benzoic acid to get six benzimidazole derivatives, of which nitration were carried out to obtain their corresponding 5-nitro derivatives. The synthesized compounds were characterized by IR and ^{1}H-NMR spectroscopy. Determination of melting point, R_f value and solubility profile for all compounds were carried out. The synthesized compounds were evaluated for their analgesic activity by the tail flick method in mice. Maximum of the compounds exerted interesting profile of analgesic activity the dose 200 mg/kg b.w.

Keywords: 5-nitro benzimidazole, analgesic activity, tail flick method.

INTRODUCTION

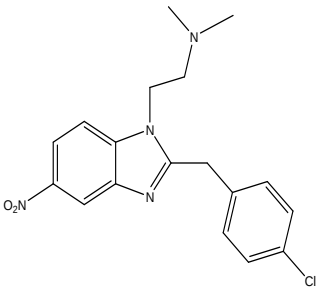
International Association for the Study of Pain (IASP) has defined pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It can be defined as characteristic neurophysiological sensation which arises from noxious stimuli. Analgesics are the agents which relieve or decrease pain sensation by increasing threshold to painful stimuli without causing loss of consciousness. The cause of the pain may be physiologic, inflammation and neuropathic. Pain can be classified into two types: Integumental pain which is superficial and related to skin muscle and joints and visceral pain which is deep seated and related to heart, kidney, stomach, gall bladder etc.

Analgesics are the drugs which decrease the pain sensation. There are 2 types of analgesic agents: Opioid analgesics and Non steroidal anti-inflammatory agents (NSAIDs).Opiod analgesics are mainly used to treat the visceral pain. They cause respiratory depression, CNS depression, drug dependence. But opoid analgesics lack anti-inflammatory, antipyretic or uricosuric action. NSAIDs on the otherhand are mainly used to treat integumental pain. The main physiological peripheral receptors are sensitized by pro-inflammatory autocoids like prostaglandin, 5-HT, histamine, bradykinin, interleukin etc. These drugs are most effective against pain associated with inflammation.

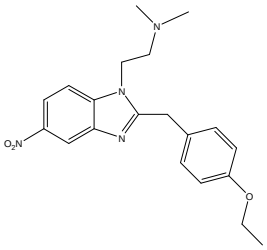
When a tissue is injured, prostaglandin synthesis increases in that tissue. The prostaglandins have 2 major actions: They are the mediators of inflammation as well as they sensitize the pain receptors at the nerve endings by lowering the threshold of response to painful stimuli. Moreover allows the other mediators (histamine, bradykinin, 5-HT etc.) which cause inflammation to intensify activation of the sensory neurons. Thus, a drug that prevents synthesis of prostaglandins will be effective in treating pain due to inflammation. The mechanism of action involves the inhibition of cyclooxygenases enzymes in the arachidonic acid cascade for synthesis of prostaglandins.

The literature survey reveals benzimidazole derivatives are reported to possess wide range biological activities like antimicrobial[1,2], antimycobacterial[3], antiprotozoal[4], antiviral[5], analgesic[6-8], anti-inflammatory[7-10], anticonvulsant[11], anticancer[12], antihypertensive[13], antioxidant[14]etc.

Clinically approved analgesic drugs such as etonitazine, clonitazine are benzimidazole derivatives[15].



Clonitazine



Etonitazine

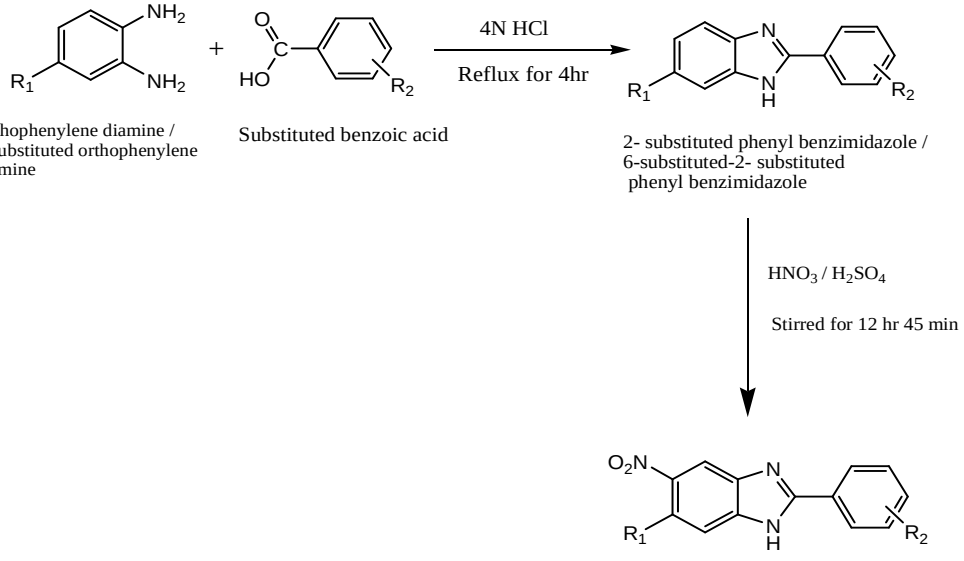
It has been reported that nitro heterocyclic compounds have diverse biological activities, anti-inflammatory, vasodialatory, sedative, hypnotic, narcotic activity etc.[16]

By considering all these facts it was planned to synthesize six 5-nitro benzimidazole derivatives (T1-T6) which were evaluated for their analgesic activity by tail flick method in mice. The compounds were characterized by IR and ^{1}H NMR spectroscopy.

EXPERIMENTAL SECTION

The reagents and chemicals used were of laboratory grade and obtained from Oxford Laboratory Reagent, Alfa Aesar, SDFCL, NR Chem, FAIZ Chemicals Agencies and Changshu Yangyuan Chemical. The melting points of the six synthesized compounds were determined by open capillary tube method and are uncorrected. The completion of the reaction was monitored by Thin Layer Chromatography (TLC) on pre-coated silica gel (HF254-200 mesh) aluminum plates from E-merk using ethyl acetate: n-hexane (4:1) as mobile phase. Spots were detected under the UV chamber. The IR spectra of the compounds were recorded with potassium bromide pellets (KBr) technique on. The ^{1}H-NMR spectra of the synthesized compounds were recorded at 400 MHz at BRUKER NMR spectrophotometer in DMSO (with TMS for ^{1}H-NMR as internal reference). The test animals were obtained under the norms from the animal house of Anurag Pharmacy College.

Synthesis:



Orthophenylene diamine / 4-substituted orthophenylene diamine

Substituted benzoic acid

2- substituted phenyl benzimidazole / 6-substituted-2- substituted phenyl benzimidazole

5-nitro-2- substituted phenyl benzimidazole / 5-nitro-6-nitro-2- substituted phenyl benzimidazole

R_1 = -H, -Cl
R_2 = 2-Cl- , 3-NO_2- , 4- NO_2-

Compound 1: 2-(2-chlorophenyl)-5-nitro -1H-benzo[d] imidazole; MF- $C_{13}H_8ClN_3O_2$; Mp- 160-162^{0}C; R_f value-0.90; Freely soluble in DMF, DMSO; Yield- 58.0%; I R (KBr) ν_{max} (cm^{-1}) : 3042.39 (C-C str.aromatic), 2828.02 (N-H str. aromatic), 1599.27 (C=C str. aromatic), 1528.36.(NO_2 str. sym), 1345.03 (NO_2 str. assym.), C-Cl str.(710.93); ^{1}H NMR (DMSO) δ ppm: 7.22-8.68(7H, aromatic H), 5.20(1H, N-H).

Compound 2: 5-nitro-2-(3-nitrophenyl)-1H-benzo[d]imidazole; MF- $C_{13}H_8N_4O_4$; Mp- 155-157^{0}C; R_f value-0.70; Freely soluble in DMF, DMSO; Yield- 62.32%; I R (KBr) ν_{max} (cm^{-1}) : 3101.30 (C-C str.aromatic), 2796.37 (N-H str. aromatic), 1605.93 (C=C str. aromatic), 1527.06 (NO_2 str. sym.), 1346.37 (NO_2 str. Assym.); ^{1}H NMR (DMSO) δ ppm: 7.63-8.81 (4H, aromatic H), 5.23(1H, N-H).

Compound 3: 5-nitro-2-(4-nitrophenyl)-1H-benzo[d]imidazole; MF- $C_{13}H_8N_4O_4$; Mp- 148-150^{0}C; R_f value-0.84; Freely soluble in DMF, DMSO; Yield- 53.22%, I R (KBr) ν_{max} (cm^{-1}) : 3097.64 (C-C str.aromatic), 2757.46 (N-H str. aromatic), 1608.71 (C=C str. aromatic), 1547.95 (NO_2 str. sym.), 1342.13 (NO_2 str. Assym.); ^{1}H NMR (DMSO) δ ppm: 7.31-8.81(7H, aromatic H), 5.32(1H, N-H).

Compound 4: 6-chloro-2-(2-chlorophenyl) -5-nitro-1H-benzo[d] imidazole; MF- $C_{13}H_7C_{l2}N_3O_2$; Mp- 201-203^{0}C; R_f value-0.78; Freely soluble in DMF, DMSO; Yield- 64.25%; I R (KBr) ν_{max} (cm^{-1}) : 3039.36 (C-C str.aromatic), 2788.93 (N-H str. aromatic), 1610.92 (C=C str. aromatic), 1576.29 (NO_2 str. sym.), 1345.03 (NO_2 srt. assym.), 711.87 (C-Cl str.); ^{1}H NMR (DMSO) δ ppm: 7.38-8.60(6H, aromatic H); 5.22(1H, N-H).

Compound 5: 6-chloro-5-nitro-2-(3-nitrophenyl)-1H-benzo[d] imidazole; MF- $C_{13}H_7ClN_4O_4$; Mp- 183-185^{0}C; R_f value-0.65; Freely soluble in DMF, DMSO; Yield- 55.08%; I R (KBr) ν_{max} (cm^{-1}) : 3146.29 (C-C str.aromatic), 2615.93 (N-H str. aromatic), 1582.40 (C=C str. aromatic), 1537.26 (NO_2 str. sym.), 1346.37 (NO_2 srt. assym.), 733.09 (C-Cl str.); ^{1}H NMR (DMSO) δ ppm: 7.95-8.92(6H aromatic H); 5.38(1H, N-H).

Compound 6: 6-chloro-5-nitro-2-(4-nitrophenyl)-1H-benzo [d] imidazole; MF- $C_{13}H_7ClN_4O_4$; Mp- 176-178^{0}C; R_f value-0.74; Freely soluble in DMF, DMSO; Yield- 66.12%; I R (KBr) ν_{max} (cm^{-1}) : 3145.52 (C-C str.aromatic), 2799.46 (N-H str. aromatic), 1606.97 (C=C str. aromatic), 1547.95 (NO_2 str. sym.), 1358.09 (NO_2 str. assym.), 705.92 (C-Cl str.); ^{1}H NMR (DMSO) δ ppm: 7.62-8.73(4H, aromatic H), 5.37(1H,N-H).

Procedure Followed For Synthesis:

Preparation of 2-Substituted Benzimidazoles[17]

Orthophenylenediamine / 4-chloro orthophenylenediamine (0.01 mole) was condensed with various benzoic acids (0.01mole) in 50 ml 4 N HCl. The mixture was refluxed for about 4hr at 80^0 C. The solution was cooled and was poured into ice cold water and was neutralized with concentrated ammonia solution. The precipitate obtained was filtered through suction and dried.

Compounds were recrystallized from water: ethanol (8:2).

Preparation of 5-nitro benzimidazole deriavative[18]

10.75 ml of concentrated nitric acid was placed in three necked flask and equal quantity of sulphuric acid (1:1) was added slowly. The mixture was kept in ice cold water. Then the product of the first step was mixed in portions during ½ hr under room temperature. After that it was stirred continuously for 12 hr 45 min and the reaction mixture was poured slowly over crushed ice with stirring. The precipitated product was filtered out, washed with cold water and dried. The product was recrystallized from ethanol.

Pharmacological Evaluation:

Acute oral toxicity studies

In the present study the acute oral toxicity of the synthesized compounds were performed by acute toxic class method (OECD guideline 423). Animals were observed individually after dosing at least once during the first 30 min; periodically during the first 24 hr with special attention given during the first 4 hr and daily thereafter, for a total of 14 days. As no mortality was observed with the administered dose, a dose 200 mg/Kg b.w. was selected for the further pharmacological evaluation.

Analgesic activity

The analgesic activity of the synthesized compounds was evaluated by tail-flick method. Swiss Albino mice (n=6) were chosen by random sampling technique for the study. Diclofenac sodium at the dose of 10 mg/kg (p.o.) was administered as standard drug for comparison. The test compounds were administered by the oral routes at the dose level of 200 mg/kg b.w. The mice were hold in position by a proper restrainer with the tail extending out and the tail (up to 6 cm) was taken and dipped in a beaker. In that beaker water should be maintained at 56 ± 4^{0}C. The time in sec taken by the mice to withdraw their tail completely out of water was taken these are the reaction time. The

observation was carried out at 0, 60,120 and 180 min after the administration of our synthesized compounds. A cut off point of 15 sec was observed to avoid the tail damage. The percentage analgesic activity was easily calculated by the below mentioned formula and the results are presented in table I.

PAA = [(B-A)/B] X 100%

Where,
B - Reaction time in sec after treatment
A -Reaction time in sec before treatment
PAA - Percentage analgesic activity

RESULTS

Most of the synthesized compounds had shown interesting analgesic activity on tail flick method. Highest analgesic activity was observed at 180 min for five out of six synthesized compounds, observations obtained at the 180 min had been selected as the basis of discussion. When compared with standard drug diclofenac (PAA-76.70) the compounds **T2** (PAA-71.13) exhibited comparable analgesic activity. When compared with standard drug diclofenac (PAA-76.70) the compounds **T2** (PAA-71.13) exhibited comparable analgesic activity. Form the synthesized compounds **T4** (PAA-48.24) exhibited lowest analgesic activity. The order of analgesic activity of the synthesized compounds was found to be (at 180 min).

T2> T6 > T1 > T5> T3 >T4

Table I: Analgesic activity of the synthesized compounds and standard at 200 mg/kg b.w.

Compounds	Dose (mg/kg)	0 min	120 min		180 min		240 min	
		MEAN ±SEM	MEAN ±SEM	%	MEAN ± SEM	%	MEAN ± SEM	%
Control	-	2.33±0.21	2.66±0.21	-	2.83±0.16	-	3.00±0.21	-
Diclofenac sodium	10	2.33±0.21	6.00±0.36***	61.16	10.00±0.57***	76.70	9.50±0.42***	75.47
T1	200	2.15±0.30	4.00±0.36^{ns}	46.25	5.83±0.60***	63.12	4.83±0.47^{ns}	55.48
T2	200	2.50±0.22	5.00±0.63**	50.00	8.66±0.42***	71.13	6.33±0.76***	60.50
T3	200	2.33±0.21	3.66±0.33^{ns}	36.33	4.72±0.42^{ns}	50.63	6.16±0.60***	62.17
T4	200	2.50±0.22	4.50±0.42*	44.44	4.83±0.70*	48.24	4.16±0.30^{ns}	39.90
T5	200	2.50±0.34	4.16±0.60^{ns}	39.90	6.16±0.40***	59.41	3.83±0.30^{ns}	34.72
T6	200	2.83±0.16	4.50±0.56*	37.11	7.33±0.42***	61.39	5.16±0.54*	45.15

*Significant differences with respect to control was evaluated by (ANOVA), Dunnet's t test *P<0.05, **P<0.01, ***P<0.01, ns (Non significant), % (Percentage protection)*

DISCUSSION

It appears reasonable to suggest that the presence of 3-nitrophenyl group at 2nd position, nitro group at 5th position of the benzimidazole ring resulted in better analgesic activity among the six synthesized derivatives. Moreover these compounds may be more effective as they may act as the donor of nitric oxide moiety. Nitric oxide donating NSAIDs are found to be more effective as nitric oxide-donating NSAIDs (NONSAIDs) which are capable of generating the radical biomediator and gastroprotective agent NO. NO contributes to the modulation of several key physiological functions in the digestive system having the ability to increase the mucosal blood flow, resulting in enhanced mucosal resistance to ulceration, prevents adherence of leukocytes to the vascular endothelium, modulates gastroduodenal secretion of mucus and bicarbonate[20].

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