Research Article

Synthesis, Characterization and Biological screening of novel benzimidazole derivatives for certain pharmacological activities

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Abstract

Benzimidazole derivatives are prepared by microwave synthesis method using 1, 2phenylenediamine and various carboxylic acid and their derivatives (aldehydes).All synthesized compounds were characterized by physicochemical properties, IR, NMR etc. The compounds are screened for different biological activities like antimicrobial, anti-diabetic, anti-inflammatory, anti-depressant and analgesic activities. Compounds U1 showed significant activity, compounds U1 and U5 showed significant anti-diabetic activity, compounds U2 and U3 showed significant anti-inflammatory activity, compounds U2, U3, U4 and U5 showed significant anti-depressant activity and compounds U4 and U5 showed significant analgesic activity.

Keywords: 1, 2 phenylenediamine, benzimidazole, aldehyde.

Introduction

Medicinal chemistry is a discipline at the intersection of synthetic organic chemistry, pharmacology and various other biological specialties, where they are involved with design, chemical synthesis and development of pharmaceutical agents (drugs).[1]Heterocyclic compounds are of interest in the drug design.[2]The most common heterocycles are those having five or six-membered rings with heteroatoms like nitrogen (N), oxygen (O), or sulfur (S). They are predominantly used in pharmaceuticals, agrochemicals and veterinary products. They also find applications as sanitizers, developers, antioxidants, corrosion inhibitors, copolymers and dye stuff.[3] One such compound is Benzimidazole.Benzimidazole is a heterocyclic aromatic organic compound. It is an important pharmacophore and privileged structure in medicinal chemistry. It plays a very important role with plenty of useful therapeutic activities such as antiulcer, antihypertensive, analgesic, anti-inflammatory, anti-viral, antifungals, anticancer and antihistaminic. The review of the literature showed benzimidazole derivatives to be useful pharmacological compounds in terms of various biochemical and pharmacological studies.[4]

Materials and Methods

Methodology for synthesis

Synthesis of Benzimidazole derivatives:

1. Equimolar mixture of 1, 2 Phenylenediamine (1mmol) and carboxylic acids/aldehydes (1mmol) were mixed thoroughly in an agate mortar and then placed in a little glass bottle. The mixture was irradiated in the microwave oven with 495W for 15 minutes. Cooled the solution and added 10% NaOH solution with constant stirring until the

mixture is alkaline to litmus paper. Crude benzimidazole is then filtered and washed with little ice cold water dissolved in 40 ml of hot water. 250 mg decolorized charcoal was added and digested for 15 minutes. Filtered rapidly and cooled the filtrate and resulting precipitate was filtered and dried. After the reaction was completed (monitored by TLC), the crude products were re-crystallized with 70% v/v ethanol.

Biological evaluation

Experimental protocol was approved by Institutional Animal Ethical Committee, PushpagiriCollegeof pharmacy, Thiruvalla. All compounds were tested for oral acute toxicity study as per OECD[5] guideline before evaluation of pharmacological activities.

Anti-bacterial activity

The antibacterial screening was carried out in a laminar air flow unit and all types of precautions were strictly maintained to avoid any type of contamination during the test. Ultraviolet light was switched on for half an hour before working in the laminar hood to avoid any accidental contamination. Petri dishes and other glassware were sterilized in the autoclave at 121°C temperature and at a pressure of 15 lbs./sq. inch for 15 minutes. Micropipette tips, culture media, cotton, forceps, blank disks were also sterilized.

In disc diffusion method bacterial inoculum was prepared and inoculated into the entire surface of solid agar plate with a sterile cotton- tipped swab to form an even lawn. The paper disc 6mm in diameter impregnated with diluted test drug solution(500μg/ml in ethanol) was placed on the surface of each of agar plates using a sterile pair of forceps and dried. The forceps were sterilized using flame. The plates were incubated for 3 days at 20 -25^{0}C and observed without opening them and the zone of inhibition was measured.

The newly synthesized compounds were tested for their preliminary antibacterial activity against gram positive bacteria (*Bacillus subtilis and Staphylococcus aureus*) and gram negative bacteria(*Pseudomonas aeruginosa and Escherichia coli)* by disc diffusion method using ciprofloxacin as standard.

Anti-diabetic activity

In- vitro by amylase inhibitory method

Porcine pancreatic amylase (PPA) was used for the preliminary screening of amylase inhibitors from the compounds. The inhibition assay was performed using the chromogenic dinitro salicylic acid method. A mixture of 500μl of test (100μg/ml) and 500μl of amylase solution prepared in 0.02M sodium phosphate buffer (pH 6.9 with 0.0006M NaCl) was incubated at 25^{0}C in a BOD incubator for 10 minutes. 500μl of pre-incubated 1% w/v

starch solution in 0.02M phosphate buffer of pH 6.9 was added to the above mixture. The reaction mixture was then incubated at 25^{0}C for 10 minutes. The reaction was stopped by adding 1.0 ml dinitro salicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 minutes and then cooled to room temperature. The reaction mixture was then diluted by adding 10ml distilled water and the absorbance was measured at 540nm. The control reaction representing 100% enzyme activity did not contain any compound. The amylase inhibitory activity was calculatedaccording to the equation

Percentage inhibition = $(A_c - A_t)/ A_c *100$

A_c - Absorbance of control
A_t- Absorbance of test

Anti inflammatory activity

In vitro protein denaturation method

A solution of 0.2 % w/v of Bovine Serum Albumin (BSA) was prepared in Tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Test drug of 100μg/ml concentration were prepared using ethanol as solvent. 50μl of each test drug was transferred to test tubes using micropipette. 5ml of 0.2% w/v BSA was added to the test tubes. The control consists of 5 ml of 0.2%w/v BSA solution and 50μl of alcohol. Diclofenac sodium 100μg/ml is used as standard. The test tubes were heated at 72^{0}C for 5 minutes and then cooled for 10 minutes. The absorbances of these solutions were determined using UV-VIS spectrophotometer at a wavelength of 660nm.

Percentage inhibition = $(A_c - A_t)/A_c *100$

A_c : absorbance of control
A_t : absorbance of test

In vivo studies - Carrageenan induced paw edema in rats

The animals were divided into seven groups of six rats in each group (control, standard and test group). Acute inflammation was induced by sub-planar injection of 0.1% w/v freshly prepared carrageenan suspension into the right hind paw of the rats. The products were suspended in distilled water and administered orally (400mg/kg) one hour before carrageenan injection. Indomethacin (10mg/kg) was given to standard group. The control group animals received vehicle CMC (1% w/v). The paw volume was measured with digitalplethysmometer at 0, 1, 2 and 3 hours after administering carrageenan. The percentage inhibition of edema was calculated for each group and compared with the control group.The percentage inhibition of edema was calculated using the formula;

% inhibition of edema = $(V_c - V_t) / V_c \times 100$

Where V_c = paw volume in test group.

V_t = paw volume in control group animal.

Anti-depressant activity

In-vivo studies

Forced Swim Test: For the forced swim test (FST), albino mice of either sex, containing six animals in each group were individually forced to swim in an open cylindrical container (diameter 20 cm, height 51.5 cm) containing 30 cm of water at 25±1°C. Treatment was given 60 minutes prior to the study. All animals were forced to swim for 6 min and the duration of immobility was observed and measured during the final 2 min interval of the test. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect.

Tail Suspension Test: For the Tail suspension Test (TST), Wistar rats of either sex containing six animals in each group. Treatment was given 60 min prior to study as described by study design. Rats were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded for 6 minutes period. Animal was considered to be immobile when it did not show any movement of the body, hanged passively and completely motionless.

Analgesics property

By Acetic acid induced writhing

Weighed and numbered the albino mice used for study. Divided the animals into control, test and standard (n=6). Administered appropriate volume of acetic acid (0.6%- 1ml/100g) IP to the control group, observe the animal. Noted the onset of writhes and record the number of writing response during a period of 10 minutes. To the test group, administered Diclofenac sodium(10mg/kg) orally. After 1 hour to the same test group administered acetic acid IP (1ml/100g). Writhing response was noted.

By Hot plate method

Weighed and numbered the animals. Mice were divided into control, test and standard. Basal reaction time was taken by observing hind paw licking or jump response in animals when placed on the hot plate maintained at a constant temperature of 55°C. Normally the animal shows such response in 6-8 seconds. A cut of period was maintained to avoid damage to the paw. Injected standard to the animal and noted down the basal reaction time for each animal on hot plate at 15 minutes, 30 minutes and 60 minutes intervals after the drug administration. Increase in the reaction time was calculated (as index of analgesia).

Results and Discussion

Table No.1- Physiochemical properties of compounds U1-U5.

Sample Code	State	Color	Molecular Formula	Molecular Weight (g/mol)	M.P (^{0}C)	Yield %w/w	R_F value	Percentage purity (in %)
U1	Solid powder	Light yellow	$C_{13}H_{10}N_4O_4$	286.2424	184	52.4	0.78	86
U2	Solid powder	Light brown	$C_{15}H_{15}N_2O$	237.2991	234	46.5	0.87	86
U3	Solid powder	Light brown	$C_{19}H_{15}N_3$	238.3415	178	64	0.89	90
U4	Solid powder	Light yellow	$C_{15}H_{14}N_2O$	238.2838	170	72.8	0.67	88
U5	Solid powder	Brown	$C_{12}H_9N_3$	195.2194	176	48.1	0.63	93

Spectral Analysis

- **2-(3, 5-dinitrophenyl)-1*H*-benzimidazole (U1)**

IR spectra- IR-(cm^{-1})1360(NO stretching), 3098 (Alkene C-H stretch), 3347 (Amine N-H stretch)

H^1 NMR spectral analysis (CDCl$_3$)- in ppm: 6.830-7.284 (9H, Aromatic hydrogen), 2.976 (1H, NH)

- **4-(1*H*-benzimidazol-2-yl)-*N*, *N*-dimethylaniline (U2)**

IR spectra- IR-(cm^{-1})1500 (Benzene ring), 1402 (C-H bend alkane), 1258 (C-N Stretching)

H^1 NMR spectral analysis (CDCl$_3$)- in ppm: 6.734-8.198 (8H, Aromatic hydrogen), 2.656 (1H, NH), 1.348-1.455 (6H, R-CH)

- **2-(1*H*-benzimidazol-2-yl)-*N*-phenylaniline (U3)**

IR spectra- IR-(cm^{-1}) 1083 (C-N stretching), 1560 (Benzene ring)

H^1 NMR spectral analysis (CDCl$_3$)- in ppm: 6.734-8.198 (8H, Aromatic hydrogen), 2.656 (1H, NH), 1.348-1.455 (6H, R-CH)

- **2-(2-ethoxyphenyl)-1*H*-benzimidazole (U4)**

IR spectra- IR-(cm^{-1})1550 (Benzene ring), 1180 (C-N stretching), 1205 (C-O stretching), 740 (=C-H bending)

H^1 NMR spectral analysis (CDCl$_3$)- in ppm: 6.701-8.097 (8H, Aromatic hydrogen), 3.006-3.114 (5H, CH$_2$CH$_3$), 2.871-2.960 (1H, NH)

- **2-(pyridin-4-yl)-1H-benzimidazole (U5)**

IR spectra- IR-(cm^{-1})2920 (Hydrogen bonded salts), 1570 (Benzene ring), 1302 (-C-N- stretching), 1610 (N-H bending)

H^1 NMR spectral analysis (CDCl$_3$)- in ppm: 6.752-8.854 (8H, Aromatic hydrogen), 1.746 (1H, NH).

Anti-bacterial activity

Table No.2- Antibacterial activity of U1-U5 against Gram positive bacteria

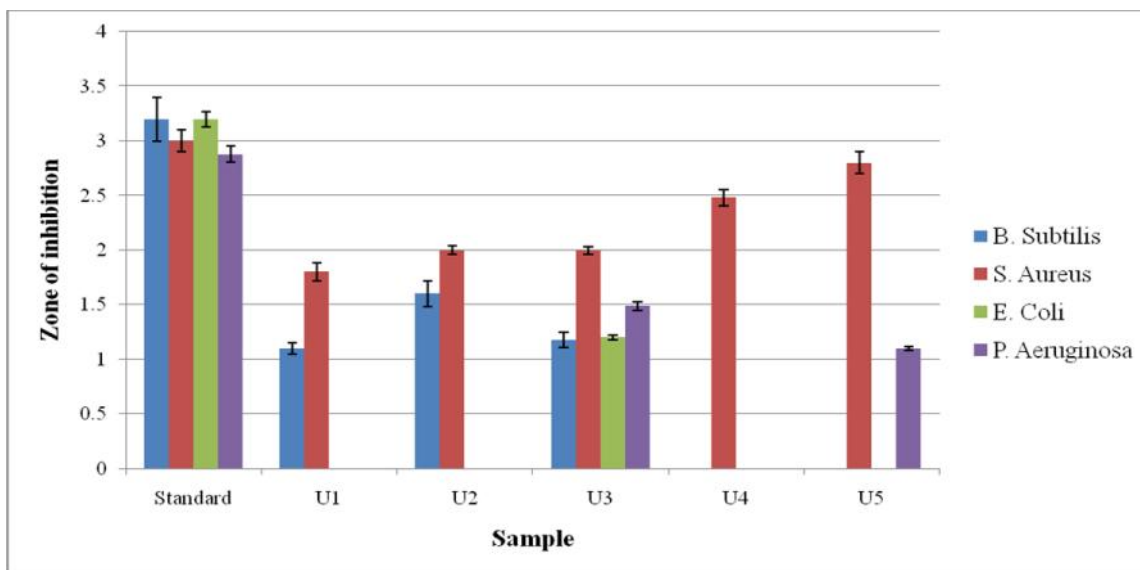| Sl.no	Sample	Zone of inhibition in cm	
		Bacillus subtilis	*Staphylococcus aureus*
1	Standard (ciprofloxacin, 10mcg)	3.2±0.11	3±0.05
2	U1	1.1±0.02	1.8±0.08
3	U2	1.6±0.07	2±0.02
4	U3	1.18±0.04	2±0.02
6	U4	Nil	2.48±0.04[**]
5	U5	Nil	2.8±0.05[**]

Each value represent Mean ± SEM, n = 3, [**]=p < 0.01 vs. standard (One way ANOVA followed by Dunnett's test)

Table No.3- Antibacterial activity of U1-U5 against Gram negative bacteria

Sl.no	Sample	Zone of inhibition in cm	
		Escherichia coli	*Pseudomonas aeruginosa*
1	Standard (ciprofloxacin, 10mcg)	3.2±0.04	2.88±0.04
2	U1	Nil	Nil
3	U2	Nil	Nil
4	U3	1.2±0.01	1.49±0.02
6	U4	Nil	Nil
5	U5	Nil	1.1±0.01

Each value represent Mean ± SEM, n = 3, [**]=p < 0.01 vs. standard (One way ANOVA followed by Dunnett's test)

Fig No.1- Antibacterial activity of U1-U5 by measuring the zone of inhibition in cm.

Anti-diabetic activity

In vitro by   amylase inhibitory method for compounds U1-U5

Table No.4- *In vitro* by   amylase inhibitory method for compounds U1-U5

Sl.no	Sample	Absorbance	Percentage of inhibition
1	U1	0.35±0.004[**]	65.48%
2	U2	0.77±0.004	24.06%
3	U3	0.95±0.03	6.3%
4	U4	0.96±0.03	5.32%
5	U5	0.462±0.006[**]	54.63%
6	Control	1.014±0.007	-

Each value represent Mean ± SEM, n = 3, [**]=$p < 0.01$ vs. control (One way ANOVA followed by Dunnett's test)

Fig No.2- Anti-diabetic activity of compounds U1-U5 by   - amylase inhibitory method
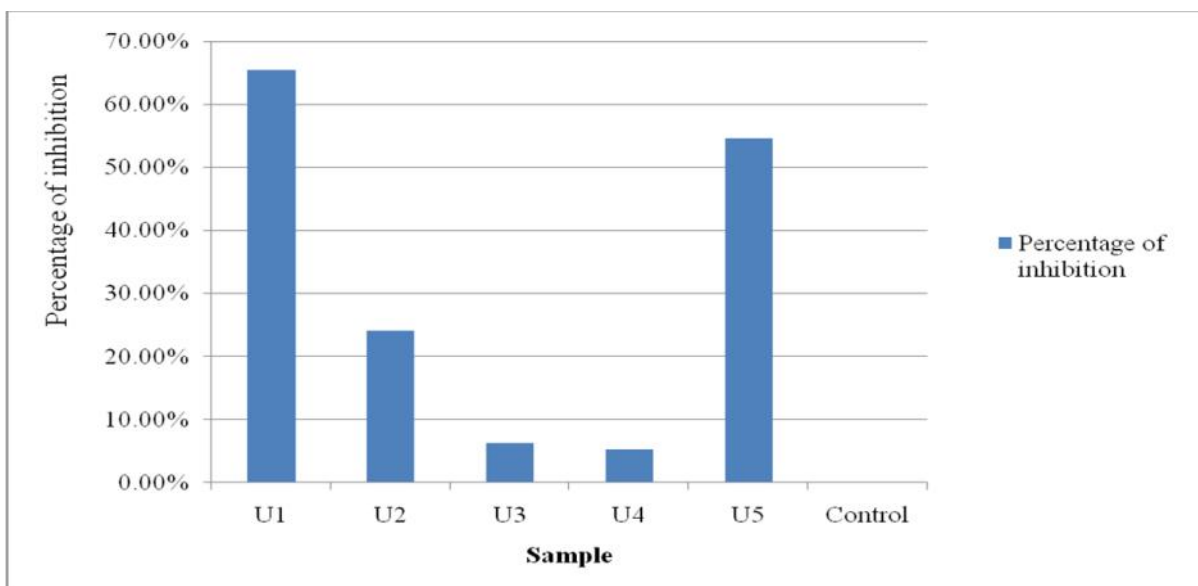
Anti-inflammatory activity

In vitro **anti-inflammatory activity by Protein denaturation method**

Table No.5- Percentage inhibition of protein denaturation by U1-U5

| Sl.no.	Sample	Absorbance at 660nm	Percentage inhibition compared to control
1	U1	1.628±0.004	6.7%
2	U2	0.902±0.005[**]	48.36%
3	U3	1.044±0.004[**]	40.24%
4	U4	1.352±0.002	22.5%
5	U5	1.671±0.001	4.3%
6	Control	1.747±0.001	-
7	Standard (Diclofenac sodium 100µg/ml)	0.669±0.002[**]	61.7%

Each value represent Mean ± SEM, n = 3, [**]=p < 0.01 vs. control (One way ANOVA followed by Dunnett's test)

Fig No.3- Percentage of Inhibition of Protein Denaturation by U1-U5
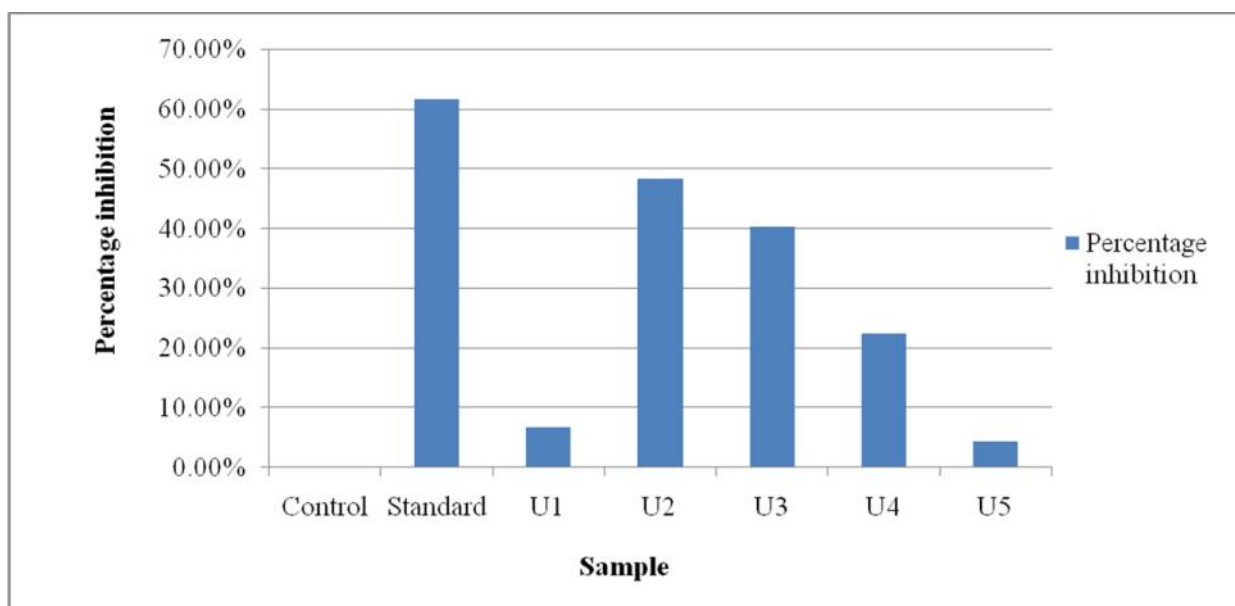
In-vivo **anti-inflammatory studies by carrageenan induced paw edema in rats**

Table No.6- Percentage inhibition of compounds U1-U5 using carrageenan induced paw edema.

Sl No.	Sample	Percentage inhibition compared to control				
		Zeroth hour	After 1 hour	After 2 hour	After 3 hour	After 4 hours
1.	U1	0%	2%	12%	16%	16%
2.	U2	1.8%	9.4%	24.5%	35.8%	47%
3.	U3	3.8%	9.6%	15.3%	30.7%	53.8%
4.	U4	5.8%	7.8%	17.6%	21.5%	25%
5.	U5	0%	9.4%	11%	16%	22.6%
6.	Standard (Indomethacin)	3.8%	23%	26%	40%	63%
7.	Control (CMC)	-	-	-	-	-

Fig No.4- Percentage inhibition of U1-U5 and Indomethacin in anti-inflammatory activity in rats using Digital Plethysmometer
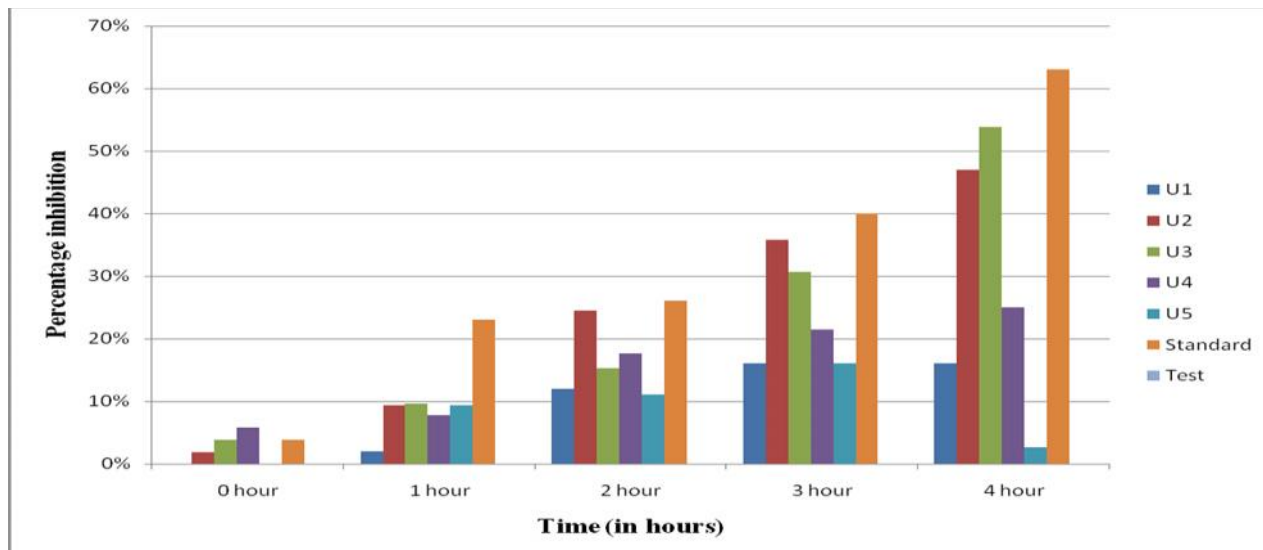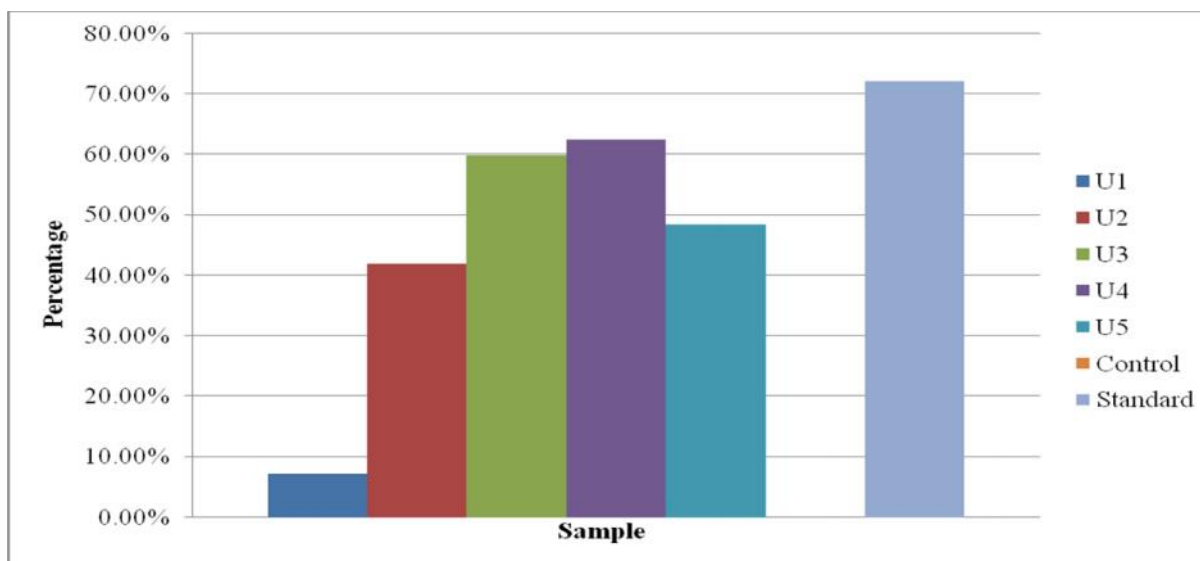

Anti-depressant activity

Table No.7- Anti-depressant activity of U1-U5 by Forced swim test.

Sl no.	Sample	Duration of immobility	Percentage immobility compared to control
1	U1	68.83±0.90	7.3%
2	U2	43.16±0.87[**]	41.93%
3	U3	29.83±1.01[**]	59.86%
4	U4	27.83±0.98[**]	62.55%
5	U5	38.33±1.22[**]	48.43%
6	Control	74.33±1.22	-
7	Standard (Clomipramine 20 mg/kg)	20.66±1.05[**]	72.20%

Each value represent Mean ± SEM, n = 6, [**]=p < 0.01 vs. Control (One way ANOVA followed by Dunnett's test)

Fig No.5- Effect of U1-U5 and clomipramine in anti-depressant activity in rats by Forced swim test.


By Tail suspension test

Table No.8- Anti-depressant activity of U1-U5 by Tail suspension test

Sl no.	Sample	Duration of immobility	Percentage immobility
1	U1	242±2.31	2.81%
2	U2	173.33±2.29**	30.38%
3	U3	142.16±2.4**	42.90%
4	U4	124.33±1.89**	50.20%
5	U5	154.16±3.07**	38.08%
6	Control	249±3.48	-
7	Standard (clomipramine 20 mg/kg)	86.83±3.34**	65.12%

Each value represent Mean ± SEM, n = 6, **=$p < 0.01$ vs. control (One way ANOVA followed by Dunnett's 't' test)

Fig No.6- Effect of U1-U5 and Clomipramine in anti-depressant activity in rats by Tail suspension test.
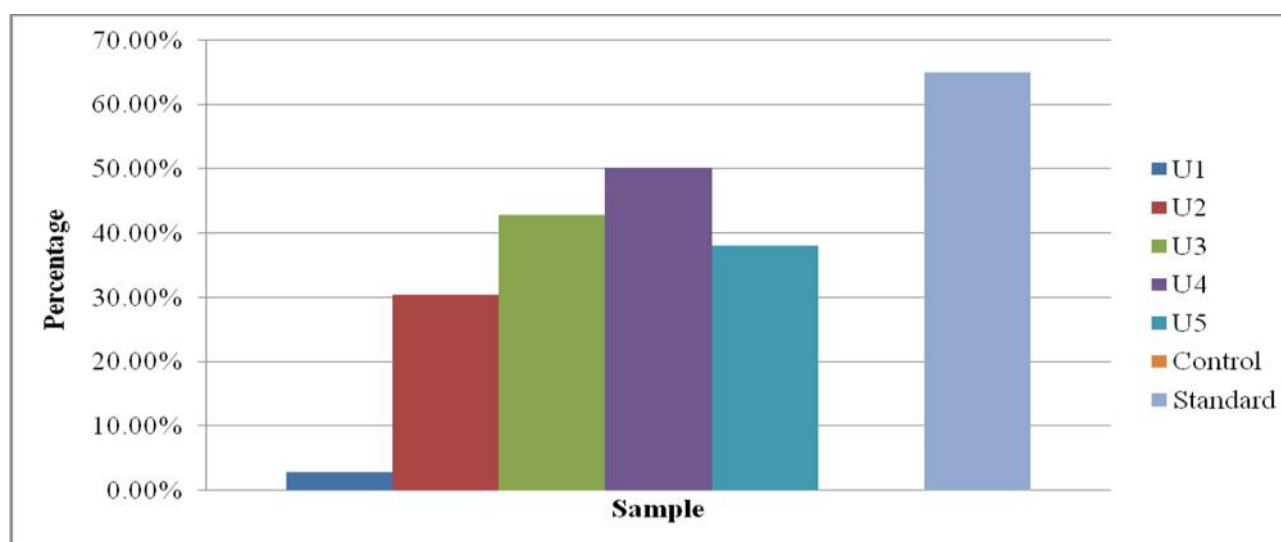
Analgesic property

**By Acetic acid induced writhing method.**

Table No.9-Analgesic property of U1-U5 using Acetic acid induced writhing.

Sl.no	Sample	No. of Writhing	Percentage inhibition
1	U1	63±0.98	14.08%
2	U2	54±0.71	26.36%
3	U3	59.66±0.98	19.54%
4	U4	30±0.8**	59.08%
5	U5	27.16±0.47**	63.18%
6	Control	73.33±0.76	-
7	Standard (Diclofenac sodium 10 mg/kg))	14.66±1.17**	80%

Each value represent Mean ± SEM, n = 6, **=$p < 0.01$ vs. control (One way ANOVA followed by Dunnett's test)

**Fig No.7- Effect of U1-U5 and Diclofenac sodium in analgesic activity using Acetic acid induced writhing.**
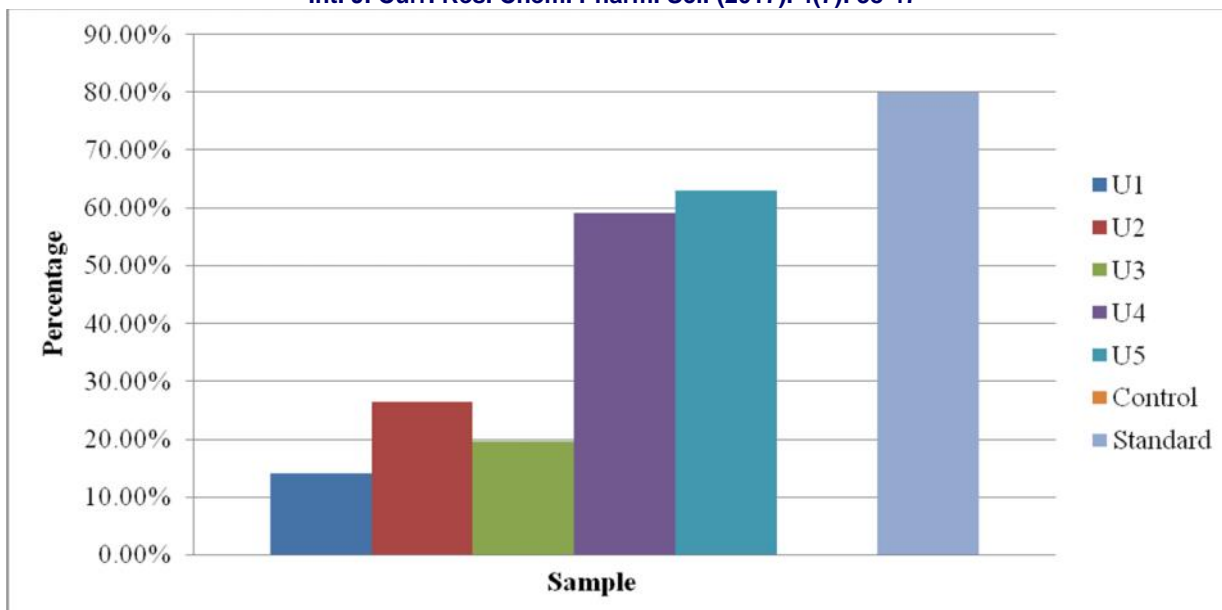
By Eddy's Hot Plate method

Table No.10 - Analgesic property of U1-U5 using Eddy's hot plate method.

Sl. No.	Sample	Basal reaction before drug administration	Pain perception after drug administration		
			15 minutes	30 minutes	60 minutes
1.	U1	1.41±0.07	2.73±0.13	3.01±0.04	2.21±0.06
2.	U2	2.06±0.09	2.1±0.05	1.7±0.09	1.6±0.05
3.	U3	1.18±0.07	1.5±0.05	1.03±0.08	1.3±0.09
4.	U4	1.32±0.01	1.56±0.01	2.10±0.02	1.82±0.02
5.	U5	1.79±0.01	1.72±0.01	1.90±0.01	2.47±0.04
6.	Control	1.98±0.09	2.45±0.07	3.08±0.09	2.11±0.06
7.	Standard (Tramadol 10 mg/kg)	3.45±0.09[**]	7±0.08[**]	8.23±0.38[**]	9.21±0.12[**]

Each value represent Mean ± SEM, n = 6, [**]=$p < 0.01$ vs. control (One way ANOVA followed by Dunnett's test)

Figure No.8- Effect of U1-U5 and Tramadol in analgesic activity using Eddy's Hot plate method.
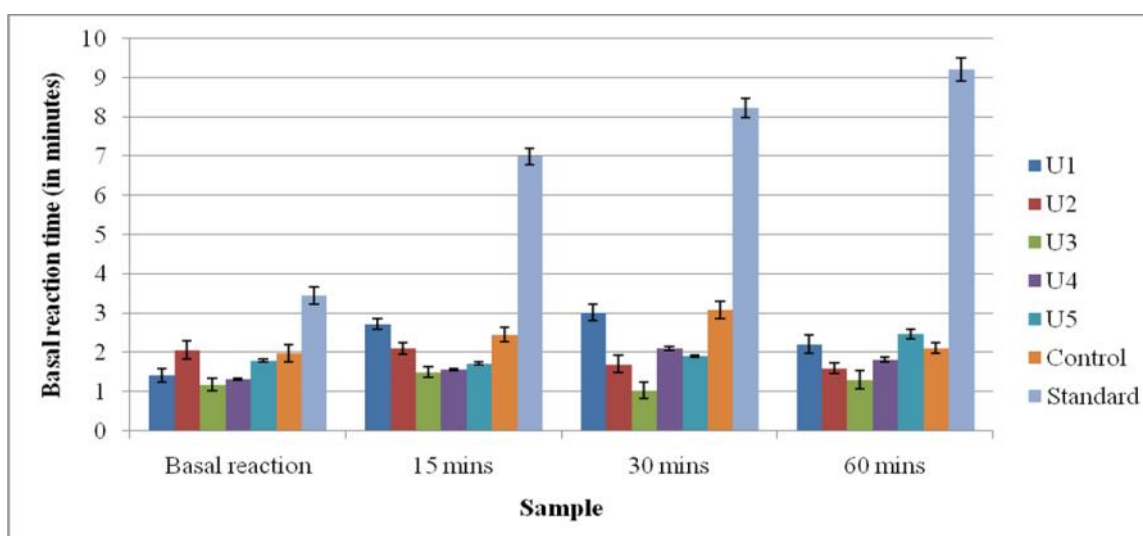
Conclusion

The objective of the study was to synthesize benzimidazole derivatives (U1-U5). All the synthesized

compounds were characterized by their physicochemical properties like melting point, $R_f$value, solubility IR spectral and NMR.Preliminarypharmacological screening was performed, which includes acute toxicity testing (LD50) and dose was selected. Compound U3 shows maximum activity for anti-bacterial activity.Compound U1 shows maximum activity for anti-diabetic activity.Compound U2 shows maximum activity for anti-inflammatory activity.Compound U4 shows maximum activity for anti-depressant activity.Compound U5 shows maximum activity for analgesic activity and also compounds U1-U5 did not show any activity for centrally acing analgesic activity. All the synthesis was carried out through microwave synthesis method which is more efficient and eco-friendly than the conventional method. The activity of various compounds varies according to the substituents attached to the nucleus and hence it becomes easy to produce compounds possessing specific activities. It was further concluded that benzimidazole nucleus possess potential for further investigation in future.

Acknowledgments

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