Advances in Bioresearch
Adv. Biores., Vol 12 (5) September 2021: 17-22

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.12.5.1722

Advances in Bioresearch

ORIGINAL ARTICLE

Novel Benzimidazolo Pyrimidines as an Anti-Inflammatory Agent

S. S. Kadam, P. Y. Pawar
Dr. V. V. P. F's College of Pharmacy, Vilad-Ghat, Ahmadnagar-414111, Maharashtra, India.
Email – Kadam.sachin448@gmail.com

ABSTRACT
With continuation to previous work synthesis and anti-inflammatory activity of novel series of structurally related benzimidazolo pyrimidines is described. Preparation of 4-(2, 3-dihydro-1H-benzimidazol-2-yl)-1-phenylbutane-1-one by using Friedel Crafts reaction in which benzene and its derivatives reacted with cyclic anhydride such as glutaric anhydrides in presence of aluminium trichloride, resulted solid reacted with ortho-phenylenediamine in presence of sodium hydroxide to form an active hydrogen containing building block, used for synthesis of benzimidazolo pyrimidine derivatives. Twenty benzimidazolo pyrimidines derivatives was synthesized using Biginelli like reaction in which 4-(2, 3-dihydro-1H-benzimidazol-2-yl)-1-phenylbutane-1-one reacted with aldehydes and urea under acidic conditions in presence of ethanol. The acid used here was HCl. Progress of reaction was monitored by TLC. Reaction products were analysed with ^{1}H NMR and IR spectroscopy. The anti-inflammatory activity of all synthesized derivatives was performed by Carrageenan induced rat paw oedema model. Indomethacin was used as an internal standard. All synthesized derivatives has tendency to show fall in oedema.
Key Word: *Benzimidazole, Pyrimidines, Anti-inflammatory activity.*

Received 04.06.2021 Revised 22.06.2021 Accepted 19.08.2021

How to cite this article:
S. S. Kadam, P. Y. Pawar. Novel Benzimidazolo Pyrimidines as an Anti-Inflammatory Agent. Adv. Biores. Vol 12 [5] September 2021. 17-22

INTRODUCTION

Heterocyclic compounds carrying pyrimidines are of enormous importance because they represent a vital family of natural and synthetic products, several of which display valuable clinical applications and bioactivities [1,2]. Substituted pyrimidines and purines are extensively found in living things and are among the leading compounds investigated by chemists [3]. Pyrimidines represent the most abundant members of the diazine class with thymine, uracil, and cytosine being key components of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).[4]

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used to treat various ailments for over hundreds of years. As a class, these drugs possess anti-inflammatory, anti-allergy, analgesic and antipyretic activity and are widely used to treat chronic inflammatory states such as arthritis, psoriasis and asthma [5].

All of NSAIDs are approximately equivalent in terms of anti-inflammatory efficacy but also cause untoward side effects (like in gastrointestinal), in a significant fraction of treated patients and this frequently limits therapy [5].

The literature indicated that compounds having pyrimidines nucleus possess broad range of biological activities, like 5-fluorouracil as anticancer[6], idoxuridine and trifluoridine as antiviral[7], zidovudine and stavudine as antiHIV[8], trimethoprim, sulphamethiazine and sulphadiazine as antibacterial[9], sulphadoxin as antimalarial and antibacterial[10], minoxidil and prazosin as antihypertensive[11], barbiturates eg. Phenobarbitone as sedative[12], propylthiouracil as antithyroid[13] and toxoflavin as antibiotics[14].

In this study synthesis of some new series of pyrimidines derivatives coupled with benzimidazole moiety using Biginelli like reaction and tested for their anti-inflammatory activity using Carrageenan induced rat paw oedema model.

Synthetic approach based on chemical modification of some pyrimidines with the aim of improving safety profile.

MATERIAL AND METHODS

Present study is to provide a method for preparing pyrimidine-benzimidazole hybrid compound(s) of fig. 1, wherein the method comprises the steps of:

(a) Synthesis of a compound of Fig. 2.

(b) Preparing a mixture comprising compound of fig. 2, urea and substituted aldehydes.

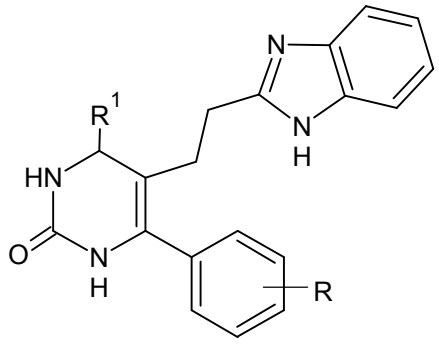

Fig. 1

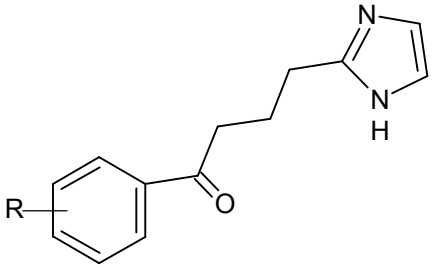

Fig. 2

(a) Synthesis of 3-(1*H*-benzimidazol-2-yl)-1-phenyl or substituted phenyl butane- 1-one (Formula II)[15]. –

process for synthesis of the compound of formula II, wherein the process comprises the steps of: (i) preparing a mixture comprising substituted benzene, glutaric anhydride and a catalyst in a solvent; (ii) heating the mixture at a temperature in the range of 75°C to 100°C for a time period in the range of 15mins to 45 min. under constant stirring; (iii) cooling the mixture and adding the water slowly to obtain a precipitated reaction mass; (iv) adding ortho-phenylenediamine to the precipitated reaction mass and then heating at a temperature in the range of 90°C to 100°C for a period in the range of 1hrs to 3 hrs to obtain a reaction mass; (v) cooling and basifying the reaction mass with a base to obtain an alkaline reaction mass; (vi) filtering the alkaline reaction mass and washing with ice cold water and drying it to obtain the compound of Formula II.

(b) Synthesis of pyrimidine-benzimidazole hybrid (Formula I)[15]. –

Preparing a mixture comprising compound of formula II, urea, substituted aldehydes in a solvent; (c) refluxing the mixture at a temperature in the range of 78°C to 80°C for a time period in the range of 15 mins to 120 mins to obtain a reaction mixture; (d) cooling the reaction mixture at room temperature to obtain a solid mass; (e) dissolving the solid mass in a hot water and filtering to obtain a filtrate; (f) neutralising the filtrate with acid to obtain a crude pyrimidine-benzimidazole hybrid compound(s); (g) purifying the crude product to obtain pyrimidine-benzimidazole hybrid compound(s) of formula I

Scheme –

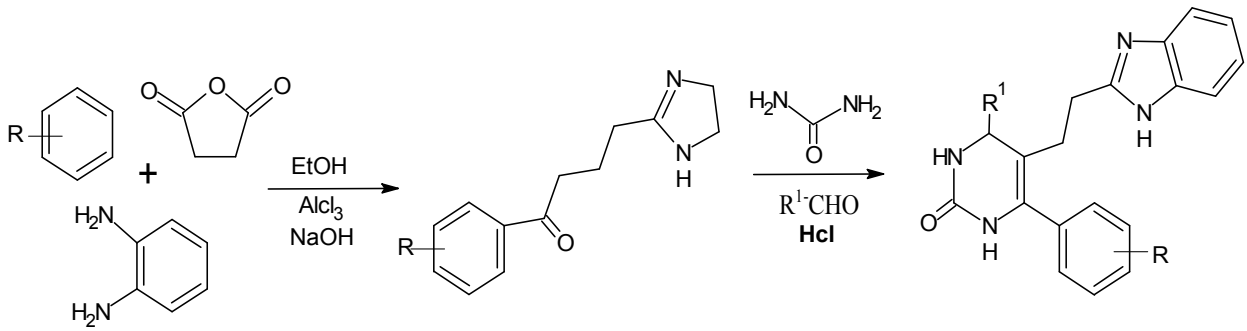

Fig. 3 Scheme synthetic route of the titled compounds.

RESULT AND DISCUSSION

Physical properties of synthesized compounds were shown in Table 1and 2–

Table 1. Physical properties of synthesized derivatives

COMPOUND CODE	R	R^1	MOLECULAR FORMULA	MOLECULAR WEIGHT GM/MOLE	MP ^0C	% YIELD	TLC BENZENE & ETHYL ACETATE (3:1)
PG1	CH3	H	C26H24N4O	408.49	139-141	74	0.353
PG2	CH3	OH	C26H24N4O2	424.49	127-129	78	0.418
PG3	CH3	M-NO2	C26H23N5O3	453.49	154-156	80	0.378
PG4	CH3	O-NO2	C26H23N5O3	453.49	131-133	69	0.389
PG5	H	H	C25H22N4O	394.46	121-123	83	0.535
PG6	H	OH	C25H22N4O2	410.46	150-152	67	0.243
PG7	H	M-NO2	C25H21N5O3	439.46	156-158	61	0.325
PG8	H	O-NO2	C25H21N5O3	439.46	153-155	64	0.410
PG9	OCH3	H	C26H24N4O2	410.46	119-121	69	0.256
PG10	OCH3	OH	C26H24N4O3	426.46	132-134	73	0.311

Table 2. Physical properties of synthesized derivatives

COMPOUND CODE	R	R^1	MOLECULAR FORMULA	MOLECULAR WEIGHT GM/MOLE	MP ^0C	% YIELD	TLC BENZENE & ETHYL ACETATE (3:1)
PG11	OCH3	M-NO2	C26H23N5O4	469.49	152-154	67	0.247
PG12	OCH3	O-NO2	C26H23N5O4	469.49	158-160	69	0.451
PG13	Cl	H	C25H21ClN4O	428.91	145-147	78	0.350
PG14	Cl	OH	C25H21ClN4O2	444.91	139-141	70	0.433
PG15	Cl	M-NO2	C25H20ClN5O3	473.91	153-155	58	0.353
PG16	Cl	O-NO2	C25H20ClN5O3	473.91	145-147	56	0.278
PG17	F	H	C25H21FN4O	412.45	146-148	64	0.250
PG18	F	OH	C25H21FN4O2	428.45	147-149	67	0.441
PG19	F	M-NO2	C25H20FN5O3	457.45	141-143	74	0.310
PG20	F	O-NO2	C25H20FN5O3	457.45	155-157	72	0.253

Chemical properties of synthesized compounds –

PG1 - IR (KBr, cm^{-1}) -N-H str. -3354, - Ar-CH.str. - 2965, -C-H methyl str. -2961, -C=O str. - 1623, - C=N str. - 1576, -C-H def.-1350,-C=C str. - 1570, -C-N str.-1426.

PG2 - IR (KBr, cm^{-1}) -OH str. - 3430, N-H str. - 3230, - Ar-CH str. - 3068,- C-H methyl str.-2966, -C=O str. - 1697, -C=C str. -1574,-C= N str. -1580, -C-N str.-1109, 1H NMR (400 MHz DMSO) 6.5-8.7 -17H (s) of Ar-H, 3.9 -1H of OH(s), 0.9-{2H(t) of CH_2}, 1.3-{2H(t) of CH_2}, 2.2-3H (s) of CH_3

PG3 - IR (KBr, cm^{-1}) N-H str. – 3280, - Ar-CH str. – 3030, C-H methyl str. -2940, -C=O str. - 1720, -NO_2 str. - 1580,-C=N str. – 1664, -C=C str. - 1550, -C-N str.-1115.

PG4 - IR (KBr, cm^{-1}) -N-H str. - 3250, - Ar-C-H.str. - 3047, - C-H methyl str. -2957, C=O str. - 1715, NO_2 str. - 1585 -, -C=N str. - 1674, - C=C str. - 1558, -C-N str.-1120.

PG5 - IR (KBr, cm^{-1}) - N-H str. - 3278, - Ar-C-H.str. – 3056,-C=O str. - 1700, -C=N str. – 1674, -C=C str. – 1570, -C-N str.-1110.

PG6 - IR (KBr, cm^{-1}) -OH str. - 3460, -N-H str. - 3245,- Ar-C-H.str. - 3030,-C-H str. - 2960,-C=O str. - 1715, -C=C str. - 1574,-C-N str.-1109, -C=N str. – 1684, 1H NMR (400 MHz DMSO) 6.5-8.7 -17H (s) of Ar-H, 3.9 -1H of OH(s), 1.6-2 H (t) of CH_2, 0.8-2 H (t) of CH_2.

PG7 - IR (KBr, cm^{-1}) -N-H str. - 3280, - Ar-C-H.str. - 3030, -C-H str. - 2940, -C=O str. - 1715, -NO_2 str. - 1581, -C=C str. - 1550, -C-N str.-1115, -C=N str. – 1674.

PG8 - IR (KBr, cm^{-1}) N-H str. - 3264, - Ar-C-H.str. - 3047, -C-H str. - 2957, -C=O str. - 1715, -NO_2 str. - 1581, -C=C str. - 1558,-C=N str. - 1664, -C-N str.-1115.

PG9 - IR (KBr, cm^{-1}) -N-H str. - 3278, - Ar-C-H str. – 3056,-C=O str. - 1715, -C=N str. - 1654, -C=C str. – 1556, -C-O str. - 1270, -C-N str.-1110.

PG10 - IR (KBr, cm^{-1}) -OH str. - 3430, - NH str. - 3230, - Ar-C-H str. - 3068, -C-H str. - 2966, -C=O str. – 1707, C=N str. – 1644, -C=C str.-1574, -C-O str. - 1280, -C-N str. – 1109, 1H NMR (400 MHz DMSO) 6.5-8.7 -17H (s) of Ar-H, 4.1- 3H of OCH_3(s), 3.7 -1H of OH(s), 1.6-2H (t) of CH_2, 0.9-2H (t) of CH_2

PG11 - IR (KBr, cm^{-1}) - NH.str. - 3280, - Ar-C-H str. - 3068, -C-H str. - 2940, -C=O str. - 1720, -C=N str. – 1640, -C=C str.-1550, -NO_2 str.- 1328, -C-O str. - 1278, -C-N str. - 1115.
PG12 - IR (KBr, cm^{-1}) - NH str. - 3264, - Ar-C-H str. -3047, -C-H str. - 2957, -C=O str. - 1722, C=N str. – 1648, -C=C str.-1558, -NO_2 str.- 1571, -C-O str. - 1275, -C-N str. - 1110.
PG13 - IR (KBr, cm^{-1}) -N-H str. - 3278, - Ar-C-H str. - 3056, -C=O str. - 1715, -C=N str. – 1580, -C=C str.-1556, -C-N str.- 1110, -C-Cl str. - 660.
PG14 - IR (KBr, cm^{-1}) -OH str. - 3430, -N-H str. - 3230, - Ar-C-H str. - 3068, -C=O str. - 1697, -C=C str. - 1574, -C-O str,- 1280, -C-N str.- 1109, -C-Cl str.- 662, 1H NMR (400 MHz DMSO) 6.6-8.8 -17H (s) of Ar-H, 3.8 -1H of OH(s), 1.3-2H (t) of CH_2, 0.8-2H (t) of CH_2.
PG15 - IR (KBr, cm^{-1}) -N-H str. - 3280, - Ar-C-H str. - 3030,-C=O str. - 1720, -C=C str.- 1550, -NO_2 str.- 1325, -C-N str.- 1115, -C-Cl str.- 672.
PG16 - IR (KBr, cm^{-1}) -N-H str. - 3264, - Ar-CH.str. - 3047,-C=O str. - 1715, -C=C str.- 1558, - NO_2 str.- 1328, -C-N str.- 1115, -C-Cl str. 666.
PG17 - IR (KBr, cm^{-1}) -N-H str. - 3269, - Ar-C-H str. - 3057, -C-H str. - 2968, -C=O str. - 1717, -C=C str.- 1561, -C-N str.- 1142, -C-F str.- 713.
PG18 - IR (KBr, cm^{-1}) -O-H str. - 3580, -N-H str. - 3350,- Ar-C-H str. - 3047, -C-H str. - 2937, -C=O str. - 1725, -C=C str.- 1558, -C-O str. - 1275, -C-N str.- 1120, -C-F str.- 718.
PG19 - IR (KBr, cm^{-1}) -N-H str. – 3258,- Ar-C-H str. - 3067, -C-H str. - 2957, -C=O str. - 1741, -C=C str.- 1561, -NO_2 str.- 1325, -C-N str.- 1122, -C-F str.- 710, 1H NMR (400 MHz DMSO) 6.5-8.7 -17H (s) of Ar-H, 1.3-2H (t) of CH_2, 1.3-2H (t) of CH_2.
PG20 - IR (KBr, cm^{-1}) -N-H str. - 3257, - Ar-C-H str. - 3027, -C-H str. - 2970, -C=O str. - 1715, -C=C str.- 1556, -NO_2 str.- 1332, -C-N str.- 1115, -C-F str.- 715.

Pharmacological evaluation

Animals -

Albino rats of either sex weighing 100–150 g were obtained from, Laxmi Biofarms Pvt. Ltd. (CPCSEA. 127) Alephata, Pune, India. All the animals were housed under standard ambient conditions of temperature (22 ± 3°C) and relative humidity of 50 ± 5%. A 12:12 h light:dark cycle was maintained. All the animals were allowed to have free access to water and standard laboratory animal diet 24 h prior to pharmacological studies. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC).

Anti-inflammatory activity [16]

Albino rats of either sex (100-150 g) were divided into 3 different groups, containing six animals each. Individual weight of animals determined before the test substance is administered.

Animals were fasted for 12 h before experiment and only water was allowed. While the first group was a control one and received vehicle (Tween 80 in propylene glycol (10% v/v), 0.5 ml per rat), the second group received Indomethacin (50 mg/kg). The entire remaining group received the test compounds at the 50 mg/kg dose orally. All the suspensions for oral dose were prepared in the vehicle mentioned above and administered in a constant volume of 0.5 ml per rat.

Table 2 Anti-inflammatory activity of synthesized compounds

Group	Dose	Carrageenan Induced Paw oedema					
		1Hr		3 Hr		5 Hr	
		EV	EI	EV	EI	EV	EI
Control	Saline	0.98	-	1.07	-	0.87	-
Indomethacin	50 mg/kg	0.55	43.87	0.25	76.63	0.38	56.32
PG1		0.65	33.67	0.45	57.94	0.50	42.52
PG2		0.66	32.65	0.43	59.81	0.48	44.82
PG3		0.80	18.36	0.55	45.79	0.65	25.28
PG4		0.58	40.81	0.28	73.83	0.41	52.87
PG5	50 mg/kg	0.66	32.65	0.43	59.81	0.48	44.82
PG6		0.64	34.69	0.44	58.87	0.47	45.97
PG7		0.63	35.71	0.43	59.81	0.49	43.67
PG8		0.78	20.40	0.57	46.72	0.63	27.58
PG9		0.64	34.69	0.44	58.87	0.47	45.97
PG10		0.57	41.83	0.29	72.89	0.40	54.02

One hr. after the administration of the test compound and Indomethacin 0.1 ml 1% w/v suspension of carrageenan was injected in to the subplanater of left paw of control and test animals. Immediately, the paw volume was measured using plethysmometer (initial paw volume) there after the paw volume was measured after one, three and five hour. The difference between initial and subsequent readings gave the edema volume for the corresponding time. Percentage inhibition was calculated.
The results for present study as shown in following table 2 and 3 -

Table 3 Anti-inflammatory activity of synthesized compounds

Group	Dose	Carrageenan Induced Paw oedema					
		1Hr		3 Hr		5 Hr	
		EV	EI	EV	EI	EV	EI
Control	Saline	0.98	-	1.07	-	0.87	-
Indomethacin	50 mg/kg	0.55	43.87	0.25	76.63	0.38	56.32
PG11		0.56	33.67	0.30	71.96	0.40	54.02
PG12		0.65	33.67	0.45	57.94	0.50	42.52
PG13		0.76	22.44	0.54	49.53	0.61	29.88
PG14		0.56	33.67	0.30	71.96	0.40	54.02
PG15	50 mg/kg	0.65	33.67	0.44	58.87	0.51	41.37
PG16		0.62	36.73	0.45	57.98	0.50	42.52
PG17		0.63	35.71	0.43	59.81	0.49	43.67
PG18		0.65	33.67	0.44	58.87	0.51	41.37
PG19		0.79	19.38	0.53	50.46	0.63	27.58
PG20		0.76	22.44	0.54	49.53	0.61	29.88

Values are expressed as mean ± SEM (n=6), EV – Oedema volume, EI – Oedema inhibition
*Significant at p<0.05, **highly significant at p<0.01,***very highly significant at p<0.001

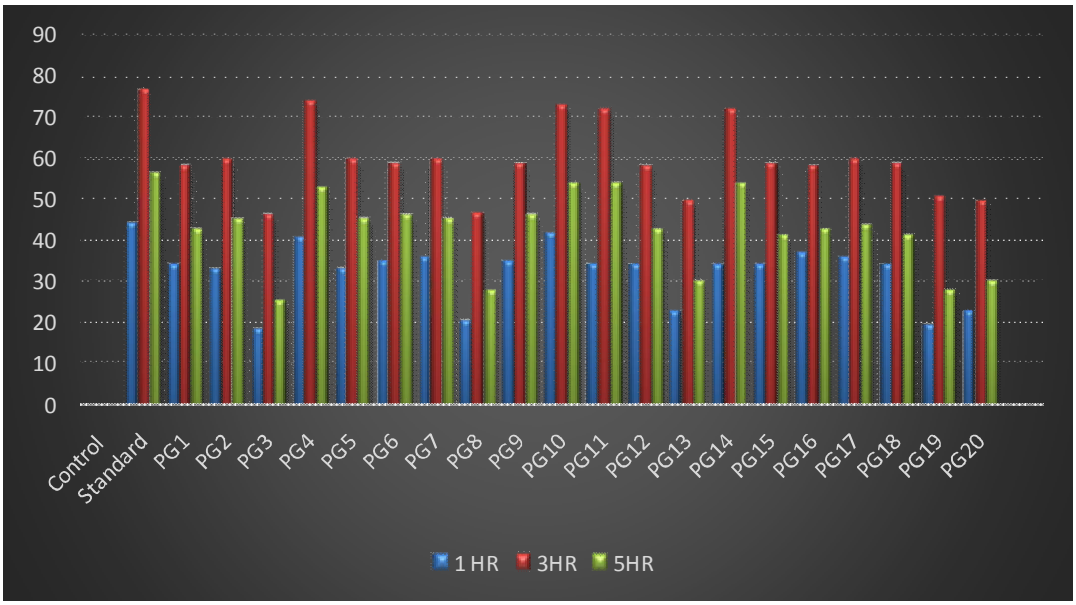

Fig. 4 Graphical representation for anti-inflammatory activity

CONCLUSION

Twenty benzimidazole coupled pyrimidines derivatives were synthesized and screened for anti-inflammatory activity. Structures of all synthesized compounds were characterized by IR, 1H NMR spectroscopy. It was interesting to note that all derivatives showed anti-inflammatory effect. Out of twenty derivatives PG4, PG10, PG11 and PG14 showed very highly significant fall in oedema. PG1, PG2, PG5, PG6, PG7, PG9, PG12, PG15, PG16, PG17, PG18 showed highly significant fall in oedema.

ACKNOWLEDGEMENT
The authors are also thankful to Dr. S. L. Jadhav Principal and Dr. D. D. Gaikwad CEO, Vishal Institute of Pharmaceutical Education and Research, Ale (M.S.) India for providing laboratory facility.

CONFLICT OF INTEREST
Authors do not have any conflict of interest.

REFERENCES
1. Kontogiorgis C.A., Hadjipavlou-Litina D.J. (2002). Non steroidal anti-inflammatory and anti-allergy agents. Current Med. Chem., 9, 89.
2. Borik R.M., Fawzy N.M., Abu-Bakr S.M. & Aly M.S. (2018). Design, Synthesis, anticancer evaluation and docking studies of novel heterocyclic derivatives obtained via reactions involving curcumin. Molecules., 23, 1398.
3. Clercq E. D., Guangdi L., (2016). Approved Antiviral Drugs over the Past 50 Years, Clinical Microbiology Reviews., 29 (3), 695-747.
4. Eswaramma S., Rao K. S., (2017). Synthesis of dual responsive carbohydrate polymer based IPN microbeads for controlled release of anti-HIV drug. Carbohydrate Polymers., 156, 125-134.
5. Atatreh N., Youssef A.M., Ghattas M.A.,(2019). Anti-inflamatory drug approach: Synthesis and biological evaluation of novel prrazolo [3,4-d] pyrimidines compounds. Bioorg. Chem., 86, 393-400.
6. Vila J., Morales J.M., Delpierre C.B., (2020). Current landscape in the discovery of novel antibacterial agents. Clinical Microbio. and infection, 26, (5), 596-603.
7. Ramsay L.E., Parnell L. and Waller P.C., (1987). Comparison of Nifedipine, Prazosin and Hydralazine added to treatment of hypertensive patients uncontrolled by Thiazide diuretic plus beta-blocker. Postgraduate Medical Journal,63, 99-103.
8. Michael C.S., & Riskin B.J., (1991). The clinical use of barbiturates in neurological disorders. Drugs,42, 365–378.
9. Elias A.N., Nanda V.S., Pandian R., (2004). Serum TNF-α in psoriasis after treatment with propylthiouracil, an antithyroid thioureylene, BMC Dermatology,4, 1471-1487.
10. [10]. Florez, L., Scherlach, K., Gaube, P.(2017). Antibiotic-producing symbionts dynamically transition between plant pathogenicity and insect-defensive mutualism. Nature Communication, 8,15172.
11. Rashid H., Martines M.A.U., Duarte A.P.,(2021). Research developments in the syntheses, anti-inflammatory activities and structure–activity relationships of pyrimidines, Royal society of chemistry Adv., 11, 6060.
12. Mokale S. N., Shinde S.S., Elgire R. D., Sangshetti J.N., Shinde D.B.,(2010). Synthesis and anti-inflammatory activity of some3-(4,6-disubstituted-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-yl) propanoic acid derivatives. Bio-org. and Med. Letters.,20,(15),1845-1854.
13. Mohamed M.S., Awad S.M.,and Sayed A.I.,(2010). Synthesis of some pyrimidines derivatives as anti-microbial and anti-inflammatory agents. Molecules., 15, 1882-1890.
14. Sondhi, S. M., Singh, N., Johar, M., Kumar A., (2005). Synthesis, anti-inflammatory and analgesic activities evaluation of some mono, bi, tricyclic pyrimidine derivatives. Bioorg. Med. Chem., 13, 6158–6166.
15. Furniss B.S., Hannaford A.J., Smith P. W. G., Tatchell A. R., (2008). Vogel's textbook of practical organic chemistry, Fifth edition, 1015, 1162-63.
16. Winter C. A., Risley E. A., Nuss G. W., (1962). Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proceedings of the society for Expt. Bio. & Medicine., 544-547.

Advances in Bioresearch
Adv. Biores., Vol 12 (5) September 2021: 17-22

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.12.5.1722

Advances in Bioresearch

ORIGINAL ARTICLE

Novel Benzimidazolo Pyrimidines as an Anti-Inflammatory Agent

S. S. Kadam, P. Y. Pawar
Dr. V. V. P. F's College of Pharmacy, Vilad-Ghat, Ahmadnagar-414111, Maharashtra, India.
Email – Kadam.sachin448@gmail.com

ABSTRACT
With continuation to previous work synthesis and anti-inflammatory activity of novel series of structurally related benzimidazolo pyrimidines is described. Preparation of 4-(2, 3-dihydro-1H-benzimidazol-2-yl)-1-phenylbutane-1-one by using Friedel Crafts reaction in which benzene and its derivatives reacted with cyclic anhydride such as glutaric anhydrides in presence of aluminium trichloride, resulted solid reacted with ortho-phenylenediamine in presence of sodium hydroxide to form an active hydrogen containing building block, used for synthesis of benzimidazolo pyrimidine derivatives. Twenty benzimidazolo pyrimidines derivatives was synthesized using Biginelli like reaction in which 4-(2, 3-dihydro-1H-benzimidazol-2-yl)-1-phenylbutane-1-one reacted with aldehydes and urea under acidic conditions in presence of ethanol. The acid used here was HCl. Progress of reaction was monitored by TLC. Reaction products were analysed with ^{1}H NMR and IR spectroscopy. The anti-inflammatory activity of all synthesized derivatives was performed by Carrageenan induced rat paw oedema model. Indomethacin was used as an internal standard. All synthesized derivatives has tendency to show fall in oedema.
Key Word: *Benzimidazole, Pyrimidines, Anti-inflammatory activity.*

Received 04.06.2021 Revised 22.06.2021 Accepted 19.08.2021

How to cite this article:
S. S. Kadam, P. Y. Pawar. Novel Benzimidazolo Pyrimidines as an Anti-Inflammatory Agent. Adv. Biores. Vol 12 [5] September 2021. 17-22

INTRODUCTION

Heterocyclic compounds carrying pyrimidines are of enormous importance because they represent a vital family of natural and synthetic products, several of which display valuable clinical applications and bioactivities [1,2]. Substituted pyrimidines and purines are extensively found in living things and are among the leading compounds investigated by chemists [3]. Pyrimidines represent the most abundant members of the diazine class with thymine, uracil, and cytosine being key components of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).[4]

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used to treat various ailments for over hundreds of years. As a class, these drugs possess anti-inflammatory, anti-allergy, analgesic and antipyretic activity and are widely used to treat chronic inflammatory states such as arthritis, psoriasis and asthma [5].

All of NSAIDs are approximately equivalent in terms of anti-inflammatory efficacy but also cause untoward side effects (like in gastrointestinal), in a significant fraction of treated patients and this frequently limits therapy [5].

The literature indicated that compounds having pyrimidines nucleus possess broad range of biological activities, like 5-fluorouracil as anticancer[6], idoxuridine and trifluoridine as antiviral[7], zidovudine and stavudine as antiHIV[8], trimethoprim, sulphamethiazine and sulphadiazine as antibacterial[9], sulphadoxin as antimalarial and antibacterial[10], minoxidil and prazosin as antihypertensive[11], barbiturates eg. Phenobarbitone as sedative[12], propylthiouracil as antithyroid[13] and toxoflavin as antibiotics[14].

In this study synthesis of some new series of pyrimidines derivatives coupled with benzimidazole moiety using Biginelli like reaction and tested for their anti-inflammatory activity using Carrageenan induced rat paw oedema model.

Synthetic approach based on chemical modification of some pyrimidines with the aim of improving safety profile.

MATERIAL AND METHODS

Present study is to provide a method for preparing pyrimidine-benzimidazole hybrid compound(s) of fig. 1, wherein the method comprises the steps of:

(a) Synthesis of a compound of Fig. 2.

(b) Preparing a mixture comprising compound of fig. 2, urea and substituted aldehydes.

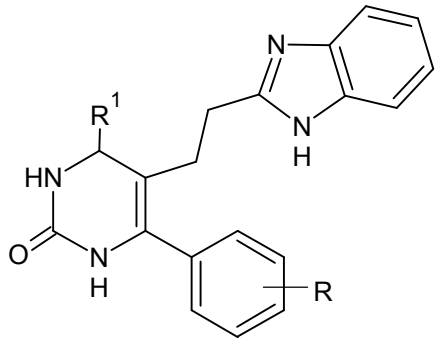

Fig. 1

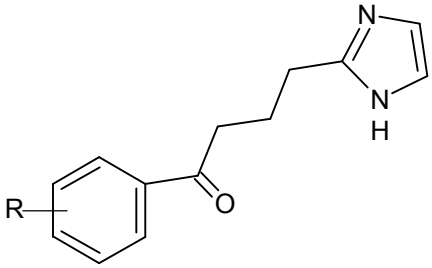

Fig. 2

(a) Synthesis of 3-(1*H*-benzimidazol-2-yl)-1-phenyl or substituted phenyl butane- 1-one (Formula II)[15]. –

process for synthesis of the compound of formula II, wherein the process comprises the steps of: (i) preparing a mixture comprising substituted benzene, glutaric anhydride and a catalyst in a solvent; (ii) heating the mixture at a temperature in the range of 75°C to 100°C for a time period in the range of 15mins to 45 min. under constant stirring; (iii) cooling the mixture and adding the water slowly to obtain a precipitated reaction mass; (iv) adding ortho-phenylenediamine to the precipitated reaction mass and then heating at a temperature in the range of 90°C to 100°C for a period in the range of 1hrs to 3 hrs to obtain a reaction mass; (v) cooling and basifying the reaction mass with a base to obtain an alkaline reaction mass; (vi) filtering the alkaline reaction mass and washing with ice cold water and drying it to obtain the compound of Formula II.

(b) Synthesis of pyrimidine-benzimidazole hybrid (Formula I)[15]. –

Preparing a mixture comprising compound of formula II, urea, substituted aldehydes in a solvent; (c) refluxing the mixture at a temperature in the range of 78°C to 80°C for a time period in the range of 15 mins to 120 mins to obtain a reaction mixture; (d) cooling the reaction mixture at room temperature to obtain a solid mass; (e) dissolving the solid mass in a hot water and filtering to obtain a filtrate; (f) neutralising the filtrate with acid to obtain a crude pyrimidine-benzimidazole hybrid compound(s); (g) purifying the crude product to obtain pyrimidine-benzimidazole hybrid compound(s) of formula I

Scheme –

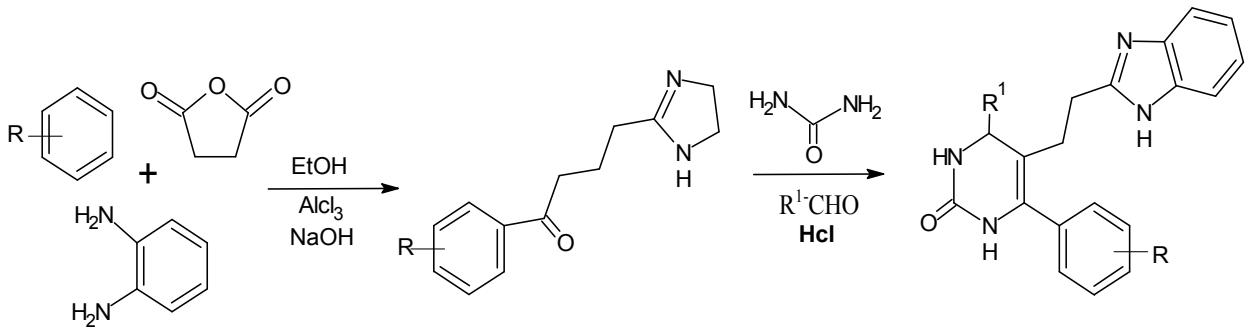

Fig. 3 Scheme synthetic route of the titled compounds.

RESULT AND DISCUSSION

Physical properties of synthesized compounds were shown in Table 1and 2–

Table 1. Physical properties of synthesized derivatives

COMPOUND CODE	R	R^1	MOLECULAR FORMULA	MOLECULAR WEIGHT GM/MOLE	MP ^0C	% YIELD	TLC BENZENE & ETHYL ACETATE (3:1)
PG1	CH3	H	C26H24N4O	408.49	139-141	74	0.353
PG2	CH3	OH	C26H24N4O2	424.49	127-129	78	0.418
PG3	CH3	M-NO2	C26H23N5O3	453.49	154-156	80	0.378
PG4	CH3	O-NO2	C26H23N5O3	453.49	131-133	69	0.389
PG5	H	H	C25H22N4O	394.46	121-123	83	0.535
PG6	H	OH	C25H22N4O2	410.46	150-152	67	0.243
PG7	H	M-NO2	C25H21N5O3	439.46	156-158	61	0.325
PG8	H	O-NO2	C25H21N5O3	439.46	153-155	64	0.410
PG9	OCH3	H	C26H24N4O2	410.46	119-121	69	0.256
PG10	OCH3	OH	C26H24N4O3	426.46	132-134	73	0.311

Table 2. Physical properties of synthesized derivatives

COMPOUND CODE	R	R^1	MOLECULAR FORMULA	MOLECULAR WEIGHT GM/MOLE	MP ^0C	% YIELD	TLC BENZENE & ETHYL ACETATE (3:1)
PG11	OCH3	M-NO2	C26H23N5O4	469.49	152-154	67	0.247
PG12	OCH3	O-NO2	C26H23N5O4	469.49	158-160	69	0.451
PG13	Cl	H	C25H21ClN4O	428.91	145-147	78	0.350
PG14	Cl	OH	C25H21ClN4O2	444.91	139-141	70	0.433
PG15	Cl	M-NO2	C25H20ClN5O3	473.91	153-155	58	0.353
PG16	Cl	O-NO2	C25H20ClN5O3	473.91	145-147	56	0.278
PG17	F	H	C25H21FN4O	412.45	146-148	64	0.250
PG18	F	OH	C25H21FN4O2	428.45	147-149	67	0.441
PG19	F	M-NO2	C25H20FN5O3	457.45	141-143	74	0.310
PG20	F	O-NO2	C25H20FN5O3	457.45	155-157	72	0.253

Chemical properties of synthesized compounds –

PG1 - IR (KBr, cm^{-1}) -N-H str. -3354, - Ar-CH.str. - 2965, -C-H methyl str. -2961, -C=O str. - 1623, - C=N str. - 1576, -C-H def.-1350,-C=C str. - 1570, -C-N str.-1426.

PG2 - IR (KBr, cm^{-1}) -OH str. - 3430, N-H str. - 3230, - Ar-CH str. - 3068,- C-H methyl str.-2966, -C=O str. - 1697, -C=C str. -1574,-C= N str. -1580, -C-N str.-1109, 1H NMR (400 MHz DMSO) 6.5-8.7 -17H (s) of Ar-H, 3.9 -1H of OH(s), 0.9-{2H(t) of CH_2}, 1.3-{2H(t) of CH_2}, 2.2-3H (s) of CH_3

PG3 - IR (KBr, cm^{-1}) N-H str. – 3280, - Ar-CH str. – 3030, C-H methyl str. -2940, -C=O str. - 1720, -NO_2 str. - 1580,-C=N str. – 1664, -C=C str. - 1550, -C-N str.-1115.

PG4 - IR (KBr, cm^{-1}) -N-H str. - 3250, - Ar-C-H.str. - 3047, - C-H methyl str. -2957, C=O str. - 1715, NO_2 str. - 1585 -, -C=N str. - 1674, - C=C str. - 1558, -C-N str.-1120.

PG5 - IR (KBr, cm^{-1}) - N-H str. - 3278, - Ar-C-H.str. – 3056,-C=O str. - 1700, -C=N str. – 1674, -C=C str. – 1570, -C-N str.-1110.

PG6 - IR (KBr, cm^{-1}) -OH str. - 3460, -N-H str. - 3245,- Ar-C-H.str. - 3030,-C-H str. - 2960,-C=O str. - 1715, -C=C str. - 1574,-C-N str.-1109, -C=N str. – 1684, 1H NMR (400 MHz DMSO) 6.5-8.7 -17H (s) of Ar-H, 3.9 -1H of OH(s), 1.6-2 H (t) of CH_2, 0.8-2 H (t) of CH_2.

PG7 - IR (KBr, cm^{-1}) -N-H str. - 3280, - Ar-C-H.str. - 3030, -C-H str. - 2940, -C=O str. - 1715, -NO_2 str. - 1581, -C=C str. - 1550, -C-N str.-1115, -C=N str. – 1674.

PG8 - IR (KBr, cm^{-1}) N-H str. - 3264, - Ar-C-H.str. - 3047, -C-H str. - 2957, -C=O str. - 1715, -NO_2 str. - 1581, -C=C str. - 1558,-C=N str. - 1664, -C-N str.-1115.

PG9 - IR (KBr, cm^{-1}) -N-H str. - 3278, - Ar-C-H str. – 3056,-C=O str. - 1715, -C=N str. - 1654, -C=C str. – 1556, -C-O str. - 1270, -C-N str.-1110.

PG10 - IR (KBr, cm^{-1}) -OH str. - 3430, - NH str. - 3230, - Ar-C-H str. - 3068, -C-H str. - 2966, -C=O str. – 1707, C=N str. – 1644, -C=C str.-1574, -C-O str. - 1280, -C-N str. – 1109, 1H NMR (400 MHz DMSO) 6.5-8.7 -17H (s) of Ar-H, 4.1- 3H of OCH_3(s), 3.7 -1H of OH(s), 1.6-2H (t) of CH_2, 0.9-2H (t) of CH_2

PG11 - IR (KBr, cm^{-1}) - NH.str. - 3280, - Ar-C-H str. - 3068, -C-H str. - 2940, -C=O str. - 1720, -C=N str. – 1640, -C=C str.-1550, -NO_2 str.- 1328, -C-O str. - 1278, -C-N str. - 1115.
PG12 - IR (KBr, cm^{-1}) - NH str. - 3264, - Ar-C-H str. -3047, -C-H str. - 2957, -C=O str. - 1722, C=N str. – 1648, -C=C str.-1558, -NO_2 str.- 1571, -C-O str. - 1275, -C-N str. - 1110.
PG13 - IR (KBr, cm^{-1}) -N-H str. - 3278, - Ar-C-H str. - 3056, -C=O str. - 1715, -C=N str. – 1580, -C=C str.-1556, -C-N str.- 1110, -C-Cl str. - 660.
PG14 - IR (KBr, cm^{-1}) -OH str. - 3430, -N-H str. - 3230, - Ar-C-H str. - 3068, -C=O str. - 1697, -C=C str. - 1574, -C-O str,- 1280, -C-N str.- 1109, -C-Cl str.- 662, 1H NMR (400 MHz DMSO) 6.6-8.8 -17H (s) of Ar-H, 3.8 -1H of OH(s), 1.3-2H (t) of CH_2, 0.8-2H (t) of CH_2.
PG15 - IR (KBr, cm^{-1}) -N-H str. - 3280, - Ar-C-H str. - 3030,-C=O str. - 1720, -C=C str.- 1550, -NO_2 str.- 1325, -C-N str.- 1115, -C-Cl str.- 672.
PG16 - IR (KBr, cm^{-1}) -N-H str. - 3264, - Ar-CH.str. - 3047,-C=O str. - 1715, -C=C str.- 1558, - NO_2 str.- 1328, -C-N str.- 1115, -C-Cl str. 666.
PG17 - IR (KBr, cm^{-1}) -N-H str. - 3269, - Ar-C-H str. - 3057, -C-H str. - 2968, -C=O str. - 1717, -C=C str.- 1561, -C-N str.- 1142, -C-F str.- 713.
PG18 - IR (KBr, cm^{-1}) -O-H str. - 3580, -N-H str. - 3350,- Ar-C-H str. - 3047, -C-H str. - 2937, -C=O str. - 1725, -C=C str.- 1558, -C-O str. - 1275, -C-N str.- 1120, -C-F str.- 718.
PG19 - IR (KBr, cm^{-1}) -N-H str. – 3258,- Ar-C-H str. - 3067, -C-H str. - 2957, -C=O str. - 1741, -C=C str.- 1561, -NO_2 str.- 1325, -C-N str.- 1122, -C-F str.- 710, 1H NMR (400 MHz DMSO) 6.5-8.7 -17H (s) of Ar-H, 1.3-2H (t) of CH_2, 1.3-2H (t) of CH_2.
PG20 - IR (KBr, cm^{-1}) -N-H str. - 3257, - Ar-C-H str. - 3027, -C-H str. - 2970, -C=O str. - 1715, -C=C str.- 1556, -NO_2 str.- 1332, -C-N str.- 1115, -C-F str.- 715.

Pharmacological evaluation

Animals -

Albino rats of either sex weighing 100–150 g were obtained from, Laxmi Biofarms Pvt. Ltd. (CPCSEA. 127) Alephata, Pune, India. All the animals were housed under standard ambient conditions of temperature (22 ± 3°C) and relative humidity of 50 ± 5%. A 12:12 h light:dark cycle was maintained. All the animals were allowed to have free access to water and standard laboratory animal diet 24 h prior to pharmacological studies. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC).

Anti-inflammatory activity [16]

Albino rats of either sex (100-150 g) were divided into 3 different groups, containing six animals each. Individual weight of animals determined before the test substance is administered.

Animals were fasted for 12 h before experiment and only water was allowed. While the first group was a control one and received vehicle (Tween 80 in propylene glycol (10% v/v), 0.5 ml per rat), the second group received Indomethacin (50 mg/kg). The entire remaining group received the test compounds at the 50 mg/kg dose orally. All the suspensions for oral dose were prepared in the vehicle mentioned above and administered in a constant volume of 0.5 ml per rat.

Table 2 Anti-inflammatory activity of synthesized compounds

Group	Dose	Carrageenan Induced Paw oedema					
		1Hr		3 Hr		5 Hr	
		EV	EI	EV	EI	EV	EI
Control	Saline	0.98	-	1.07	-	0.87	-
Indomethacin	50 mg/kg	0.55	43.87	0.25	76.63	0.38	56.32
PG1		0.65	33.67	0.45	57.94	0.50	42.52
PG2		0.66	32.65	0.43	59.81	0.48	44.82
PG3		0.80	18.36	0.55	45.79	0.65	25.28
PG4		0.58	40.81	0.28	73.83	0.41	52.87
PG5	50 mg/kg	0.66	32.65	0.43	59.81	0.48	44.82
PG6		0.64	34.69	0.44	58.87	0.47	45.97
PG7		0.63	35.71	0.43	59.81	0.49	43.67
PG8		0.78	20.40	0.57	46.72	0.63	27.58
PG9		0.64	34.69	0.44	58.87	0.47	45.97
PG10		0.57	41.83	0.29	72.89	0.40	54.02

One hr. after the administration of the test compound and Indomethacin 0.1 ml 1% w/v suspension of carrageenan was injected in to the subplanater of left paw of control and test animals. Immediately, the paw volume was measured using plethysmometer (initial paw volume) there after the paw volume was measured after one, three and five hour. The difference between initial and subsequent readings gave the edema volume for the corresponding time. Percentage inhibition was calculated.
The results for present study as shown in following table 2 and 3 -

Table 3 Anti-inflammatory activity of synthesized compounds

Group	Dose	Carrageenan Induced Paw oedema					
		1Hr		3 Hr		5 Hr	
		EV	EI	EV	EI	EV	EI
Control	Saline	0.98	-	1.07	-	0.87	-
Indomethacin	50 mg/kg	0.55	43.87	0.25	76.63	0.38	56.32
PG11		0.56	33.67	0.30	71.96	0.40	54.02
PG12		0.65	33.67	0.45	57.94	0.50	42.52
PG13		0.76	22.44	0.54	49.53	0.61	29.88
PG14		0.56	33.67	0.30	71.96	0.40	54.02
PG15	50 mg/kg	0.65	33.67	0.44	58.87	0.51	41.37
PG16		0.62	36.73	0.45	57.98	0.50	42.52
PG17		0.63	35.71	0.43	59.81	0.49	43.67
PG18		0.65	33.67	0.44	58.87	0.51	41.37
PG19		0.79	19.38	0.53	50.46	0.63	27.58
PG20		0.76	22.44	0.54	49.53	0.61	29.88

Values are expressed as mean ± SEM (n=6), EV – Oedema volume, EI – Oedema inhibition
*Significant at p<0.05, **highly significant at p<0.01,***very highly significant at p<0.001

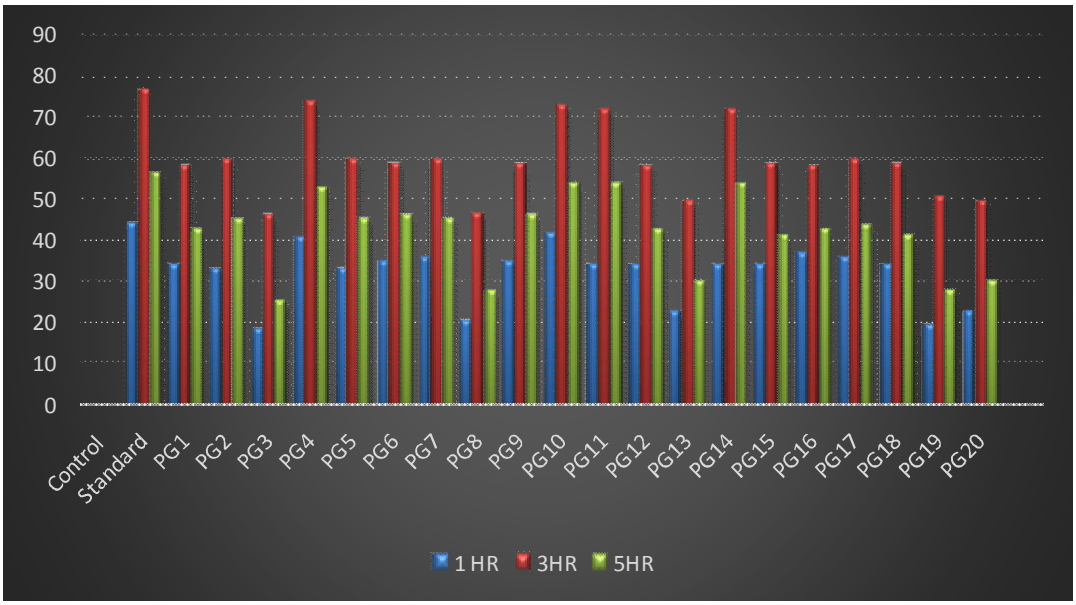

Fig. 4 Graphical representation for anti-inflammatory activity

CONCLUSION

Twenty benzimidazole coupled pyrimidines derivatives were synthesized and screened for anti-inflammatory activity. Structures of all synthesized compounds were characterized by IR, 1H NMR spectroscopy. It was interesting to note that all derivatives showed anti-inflammatory effect. Out of twenty derivatives PG4, PG10, PG11 and PG14 showed very highly significant fall in oedema. PG1, PG2, PG5, PG6, PG7, PG9, PG12, PG15, PG16, PG17, PG18 showed highly significant fall in oedema.

ACKNOWLEDGEMENT
The authors are also thankful to Dr. S. L. Jadhav Principal and Dr. D. D. Gaikwad CEO, Vishal Institute of Pharmaceutical Education and Research, Ale (M.S.) India for providing laboratory facility.

CONFLICT OF INTEREST
Authors do not have any conflict of interest.

REFERENCES
1. Kontogiorgis C.A., Hadjipavlou-Litina D.J. (2002). Non steroidal anti-inflammatory and anti-allergy agents. Current Med. Chem., 9, 89.
2. Borik R.M., Fawzy N.M., Abu-Bakr S.M. & Aly M.S. (2018). Design, Synthesis, anticancer evaluation and docking studies of novel heterocyclic derivatives obtained via reactions involving curcumin. Molecules., 23, 1398.
3. Clercq E. D., Guangdi L., (2016). Approved Antiviral Drugs over the Past 50 Years, Clinical Microbiology Reviews., 29 (3), 695-747.
4. Eswaramma S., Rao K. S., (2017). Synthesis of dual responsive carbohydrate polymer based IPN microbeads for controlled release of anti-HIV drug. Carbohydrate Polymers., 156, 125-134.
5. Atatreh N., Youssef A.M., Ghattas M.A.,(2019). Anti-inflamatory drug approach: Synthesis and biological evaluation of novel prrazolo [3,4-d] pyrimidines compounds. Bioorg. Chem., 86, 393-400.
6. Vila J., Morales J.M., Delpierre C.B., (2020). Current landscape in the discovery of novel antibacterial agents. Clinical Microbio. and infection, 26, (5), 596-603.
7. Ramsay L.E., Parnell L. and Waller P.C., (1987). Comparison of Nifedipine, Prazosin and Hydralazine added to treatment of hypertensive patients uncontrolled by Thiazide diuretic plus beta-blocker. Postgraduate Medical Journal,63, 99-103.
8. Michael C.S., & Riskin B.J., (1991). The clinical use of barbiturates in neurological disorders. Drugs,42, 365–378.
9. Elias A.N., Nanda V.S., Pandian R., (2004). Serum TNF-α in psoriasis after treatment with propylthiouracil, an antithyroid thioureylene, BMC Dermatology,4, 1471-1487.
10. [10]. Florez, L., Scherlach, K., Gaube, P.(2017). Antibiotic-producing symbionts dynamically transition between plant pathogenicity and insect-defensive mutualism. Nature Communication, 8,15172.
11. Rashid H., Martines M.A.U., Duarte A.P.,(2021). Research developments in the syntheses, anti-inflammatory activities and structure–activity relationships of pyrimidines, Royal society of chemistry Adv., 11, 6060.
12. Mokale S. N., Shinde S.S., Elgire R. D., Sangshetti J.N., Shinde D.B.,(2010). Synthesis and anti-inflammatory activity of some3-(4,6-disubstituted-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-yl) propanoic acid derivatives. Bio-org. and Med. Letters.,20,(15),1845-1854.
13. Mohamed M.S., Awad S.M.,and Sayed A.I.,(2010). Synthesis of some pyrimidines derivatives as anti-microbial and anti-inflammatory agents. Molecules., 15, 1882-1890.
14. Sondhi, S. M., Singh, N., Johar, M., Kumar A., (2005). Synthesis, anti-inflammatory and analgesic activities evaluation of some mono, bi, tricyclic pyrimidine derivatives. Bioorg. Med. Chem., 13, 6158–6166.
15. Furniss B.S., Hannaford A.J., Smith P. W. G., Tatchell A. R., (2008). Vogel's textbook of practical organic chemistry, Fifth edition, 1015, 1162-63.
16. Winter C. A., Risley E. A., Nuss G. W., (1962). Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proceedings of the society for Expt. Bio. & Medicine., 544-547.