INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

IN VIVO ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF NOVEL DERIVATIVES OF BIS PYRAZOLIDINE-3, 5- DIONE TETHERED WITH 1, 4- DIHYDROPYRIDINE MOIETY

A. Asma Samaunnisa[1*], Riazuddin Mohammed[2], CHS. Venkataramana[1] and V. Madhavan[3]

[1]Department of Pharmaceutical Chemistry, M.S. Ramaiah College of Pharmacy, Bangalore, Karnataka, India.
[2]Department of Medical Biology, Linkoping University, Linköping, Sweden.
[3]Department of Pharmacognosy, M.S. Ramaiah College of Pharmacy, Bangalore, Karnataka, India.

ABSTRACT

A set of 8 novel 2,6-dimethyl-1,4-dihydropyridine-3,5-yl-bis[carbonyl-2- (phenyl)]pyrazolidine-3,5-diones] 3A-3D' and its derivatives were synthesized from cyclization of 2,6-dimethyl-N3,N5-diphenyl-1,4-dihydropyridine-3,5-dicarbohydrazide 2A- 2D' in the presence of diethylmalonate and acetic acid with ethanol as solvent. Derivatives 2A- 2D' were in turn synthesized from Diethyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates (1A) and Diethyl-4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates (1A'). The synthesized novel derivatives were characterized based on IR, ^{1}HNMR, Mass and Elemental analysis data. The newly synthesized derivatives 3A-3D', were subjected to *in vivo* anti-inflammatory and analgesic activities. Carrageenan induced paw edema method was used to evaluate anti-inflammatory activity and analgesic activity was performed by Eddy's hot plate method. The obtained results were thoroughly evaluated, which are tabulated and represented graphically using histogram. Activity of all the derivatives was compared with the chosen standards namely indomethacin and tramadol for anti-inflammatory and analgesic activity respectively, using dimethyl sulfoxide (DMSO) as control. Almost all the derivatives of the series 3A-3D' exhibited significant activity.

Keywords: *In vivo,* Anti-inflammatory, Analgesic, 1, 4-Dihydropyridine, Pyrazolidine-3, 5-Diones.

INTRODUCTION

Inflammation, which is a protective and defence mechanism is body's response to disturbed homeostasis caused by infection, injury or trauma resulting in systemic and local effects. The Roman writer Celsus in 1st century AD named the four famous cardinal signs of inflammation as Rubor (redness), Tumor (swelling/ edema), Calor (heat), Dolor (pain). Inflammation may be acute and chronic. Inflammatory response occurs in three distinct phases. The first phase is caused by an increase in vascular permeability resulting in exudation of fluids from the blood into the interstitial space, the second phase involves the infiltrations of leukocytes from the blood into the tissue and in third phase granuloma formation and tissue repair. Mediators of inflammation originate either from plasma or from cells. Inflammation is not one event but a series of events that occur in living mammalian tissue following injury. Inflammation involves two basic processes with some overlapping, viz. early and later inflammation response followed by healing. We have steroidal and non-steroidal anti-inflammatory agents, but none of the steroidal anti-inflammatory agents is a cure or stops the progression of arthritis or

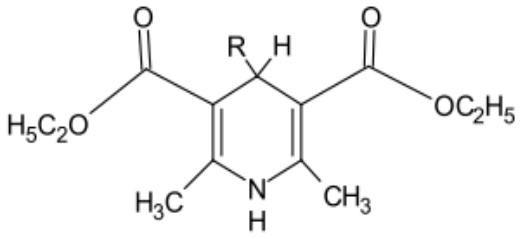
chronic inflammation. The non-steroidal anti-inflammatory agents currently in use are characterized by their ability to relieve the pain of both pathological and non-pathological inflammation associated with inflammatory disorders and to inhibit the synthesis of endogenous prostaglandins (PGs) which are the mediating factors for the inflammation process. Their mode of action is usually associated with prostaglandin synthesis[1,2]. Pyrazolidine-3, 5-diones were known since decades for their magnificent anti-inflammatory activity as well as diverse activities like anti-microbial, anti-inflammatory, antihypertensive, antineoplastic and anticonvulsant activities[3-6]. 1, 4-Dihydropyridines are endowed with anti-microbial, anti-cancer, anti- hypertensive, anti-inflammatory, anti- convulsant, anti-diabetic, anti-fungal, anti- tubercular activities[7-11]. Conventionally, Pyrazolidine-3, 5-diones which are known for their anti-inflammatory activity were ever present with their side effects. So, synthesizing the derivatives of this moiety devoid of its side effects has been an ongoing challenge for the medicinal chemists. Therefore, it is considered worthwhile to synthesize some novel pyrazolidine-3, 5-dione derivatives containing 1, 4-Dihydropyridine moiety which might possess enhanced biological activity. As of now, several approaches for the synthesis of pyrazolidine-3, 5-diones is under pipeline. This gave us the initiative to synthesize 2, 6-dimethyl-1, 4-dihydropyridine-3, 5-yl-bis [carbonyl-2- (phenyl)] pyrazolidine-3, 5-diones] 3A-3D' and its derivatives[12]. These novel derivatives were evaluated for their *in vivo* anti-inflammatory and analgesic activities.

EXPERIMENTAL

Chemicals and solvents used were of reagent grade and used without further purification, were procured from SpectroChem, Hi-Media, Merck, Sigma Aldrich and Ranbaxy. The purity of the synthesized compounds was determined by melting point using open capillary method and are uncorrected. IR (infra-red) was performed using SHIMADZU FTIR- 8400S. The compounds 3A-3D' were identified by 1HNMR (proton nuclear magnetic resonance) using amx-400 NMR, Mass using LC-MS 2010A and elemental analysis using Flash EA 1112 series Thermo finnigan. TLC was performed using Solvent system- Ethyl acetate: n-Hexane, Stationary phase- Silica Gel-G.

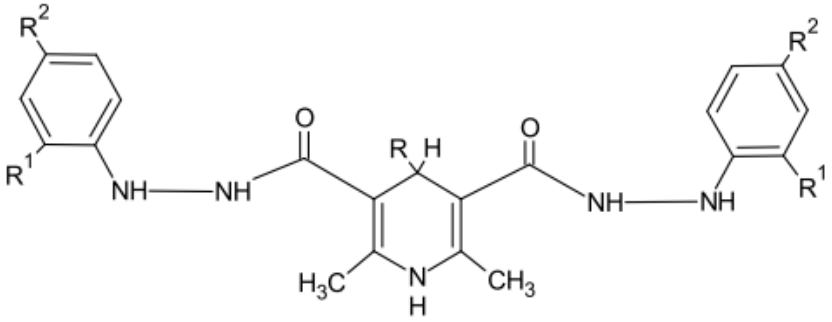
MATERIALS AND METHODS

Step1: Synthesis of Diethyl-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarbohydrazide (1A) and diethyl-4-(4-hydroxyphenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate' (1A')[13]



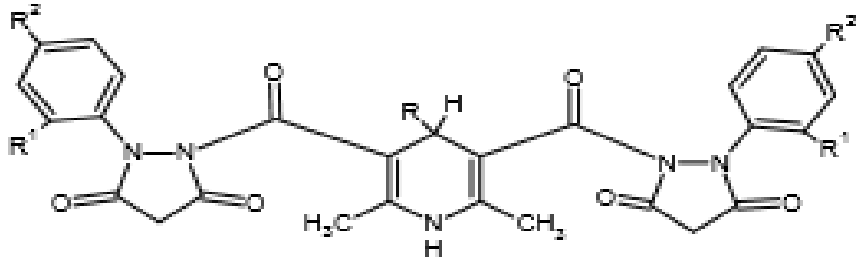
1A-1A'

Step2: Synthesis of 2, 6-dimethyl-N3, N5-diphenyl-1, 4-dihydropyridine-3, 5-dicarbohydrazide (2A-2D')[13]



2A-2D'

Step3: Synthesis of 2, 6-dimethyl-1, 4-dihydropyridine-3, 5-yl-bis [carbonyl-2-(phenyl)] pyrazolidine-3, 5- diones] (3A-3D')[12]



3A–3D'

Substitutions for the derivatives 3A-3D' are given in table 1

Table 1: set of compounds for screening

Compound	R^1	R^2	R
3A	H	H	H
3B	NO_2	NO_2	H
3C	H	Cl	H
3D	H	NO_2	H
3A'	H	H	C_6H_4OH
3B'	NO^2	NO_2	C_6H_4OH
3C'	H	Cl	C_6H_4OH
3D'	H	NO_2	C_6H_4OH

PHARMACOLOGICAL ACTIVITY

1) Anti-inflammatory activity [14, 15]

Carrageenan induced paw edema in rats

Animals were weighed and numbered. A mark was made on the hind paw (left) just beyond tibio-tarsal junction, so that the paw was dipped in the mercury up to the fixed mark. Initial paw volume (left) was noted on each rat by mercury displacement method. Animals were divided into ten groups each comprising of six rats. The first group received di-methyl sulfoxide (vehicle), second group received intraperitoneal injection of indomethacin, third group to tenth group were treated with test compounds (2A-2D') intraperitoneally. After 30 min, 0.1 ml of 1 % (w/v) carrageenan was injected in the plantar region of the left paw subcutaneously of all the groups followed by measurement of paw volume (left) of all the groups at 0, 15, 30, 60 and 120 min after carrageenan challenge. The increase in paw volume was observed and the percentage inhibition of edema exhibited by standard and test drugs was calculated by the following formula.

% edema inhibition = 100 [1-(Vt-Vo) Treated group / (Vt-Vo) Control]

V0= Initial paw volume of the rat before the administration of carrageenan (at 0 minutes).

Vt= Final paw volume of the rat after the administration of carrageenan (at 120 minutes).

2) Analgesic activity[14,15,16]

Analgesic activity was carried out by Eddy's hot plate method. Animals were divided into ten groups each comprising of six mice. The mice were treated with di-methyl sulfoxide (control), Tramadol (standard) and 2, 6-dimethyl-N^3, N^5-diphenyl-1, 4-dihydropyridine-3, 5-dicarbohydrazides (2A-2D') half an hour prior to analgesic screening.

Eddy's hot plate method is used for screening of central analgesic property. The mice were placed on analgesiometer which is eddy's hot plate kept and maintained at constant temperature (55±1^{o}C). The time of response, that is the time taken by the animal to lick its hind paw or jump after placing it on the hot plate, is recorded as the reaction of painful stimuli. A cut off period of 15 sec was considered to avoid the damage to the paw.

RESULTS AND DISCUSSION

The derivatives under study 3A-3D' were synthesized by the cyclization of 2, 6-dimethyl-N3, N5-diphenyl-1, 4-dihydropyridine-3, 5-dicarbohydrazide (2A- 2D'), in the presence of diethylmalonate and acetic acid with ethanol as solvent. Derivatives 2A-2D' were

synthesized from 1A-1A' by condensation in the presence of respective phenyl hydrazine's. 1A-1A' are in turn synthesized using conventional Hantzsch method by condensation of ethyl acetoacetate with an aldehyde in the presence of ammonia. All the synthesized compounds are considerably pure and the purity of these compounds was assessed using TLC. The structures were confirmed by ^{1}HNMR, Mass, IR and Elemental analysis data.

The newly synthesized derivatives 3A-3D' were screened for their *invivo* anti-inflammatory as well as analgesic activities by using carrageenan induced paw edema in rats and Eddy's hot plate method respectively.

Among the derivatives screened for anti-inflammatory activity, 3A, 3B, 3D, 3A' and 3B' exhibited significantly good activity which is more than 50% of the standard drug, 3D' has shown moderate activity, whereas other derivatives of the class namely 3C and 3C' exhibited low activity when compared with the standard indomethacin as well as with the other derivatives of this class. Results are summarized in table2 and figure1.

All the derivatives which are screened for analgesic activity has shown significant activity in comparison with the standard tramadol. Derivatives 3A, 3D, 3A', 3C' has shown significantly good activity and the derivatives 3B, 3C', 3D' has shown moderate activity whereas the derivatives 3C, 3B' has shown lower activity when compared to other derivatives of the class as well as standard. Results are summarized in table3 and figure 2.

CONCLUSION

The newly synthesized derivatives 3A-3D' served their rationale of synthesizing better anti-inflammatory and analgesic molecules. All the derivatives 3A-3D' have shown considerably good anti-inflammatory and analgesic activities. Both the activities are exhibited significantly high. Further study on these derivatives might provide a better insight into their pharmacological activities which might even throw a light on hidden potential of these candidates.

Statistical Analysis

The experimental data were expressed as mean±SEM. The data were analyzed using ANOVA and Tukey-Kramer multiple comparison test. The results were considered statistically significant if $P<0.05$.

ACKNOWLEDGEMENT

I am grateful to express my sincere thanks to the Gokula Education Foundation (GEF medical), the Management and staff of M.S. Ramaiah College of Pharmacy, Bangalore for providing all the facilities and encouragement for carrying out the work. I am also thankful to Indian Institute of Science (IISC), Bangalore for their analytical services (Spectral data). I sincerely thank Mr. Riazuddin Mohammed, Department of Medical biology, Linköping University, Linköping, Sweden for animal studies.

Table 2: Anti-inflammatory activity of the derivatives 3A-3D' using Carrageenan induced paw edema in rats

Carrageenan induced rat paw edema				
Samples	**Volume (V_o)**	**Maximum Displacement (V_t)**	**Percentage increase in paw volume (%)**	**Percentage inhibition of edema (%)**
Control	0.4371±0.0016	0.7336±0.0019	67.83	-
Standard	0.3891± 0.0018	0.4597±0.0025	18.14***	76.18
3A	0.4132±0.0011	0.5451±0.0002	31.92***	55.52
3B	0.3787±0.0020	0.5140±0.0013	35.72***	54.37
3C	0.3717±0.0009	0.5729±0.0023	54.12**	32.15
3D	0.3894±0.0017	0.5226±0.0014	34.20***	55.08
3A'	0.4010±0.0021	0.5360±0.0014	33.66***	54.47
3B'	0.3748±0.0011	0.5094±0.0021	35.91***	54.61
3C'	0.4654±0.0012	0.6664±0.0017	43.18***	32.21
3D'	0.4872±0.0017	0.6885±0.0020	41.31***	42.11

All values are expressed as mean±SEM (n=6).
*P < 0.05, **P < 0.01, ***P < 0.0001 significant compared to control.

Table 3: Analgesic activity of the derivatives 3A-3D' using Eddy's hot plate method

Eddy's hot plate method		
S.No	**Sample**	**Response time (sec)**
1	Control	3.98±0.4364
2	Standard	14.55±0.4671***
3	3A	13.01±0.5014***
4	3B	12.89±0.5891**
5	3C	12.05±0.5798*
6	3D	13.17±0.5005***
7	3A'	13.41±0.6211***
8	3B'	12.45±0.5514*
9	3C'	13.32±0.5805***
10	3D'	12.97±0.6519**

All values are expressed as mean±SEM (n=6). *P < 0.05, **P < 0.01, ***P < 0.0001 significant compared to control.

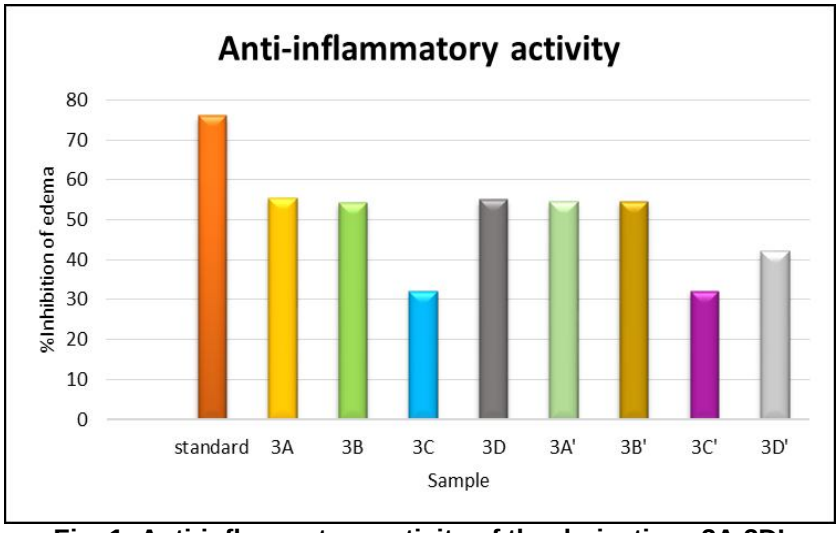


Fig. 1: Anti-inflammatory activity of the derivatives 3A-3D' using Carrageenan induced paw edema in rats

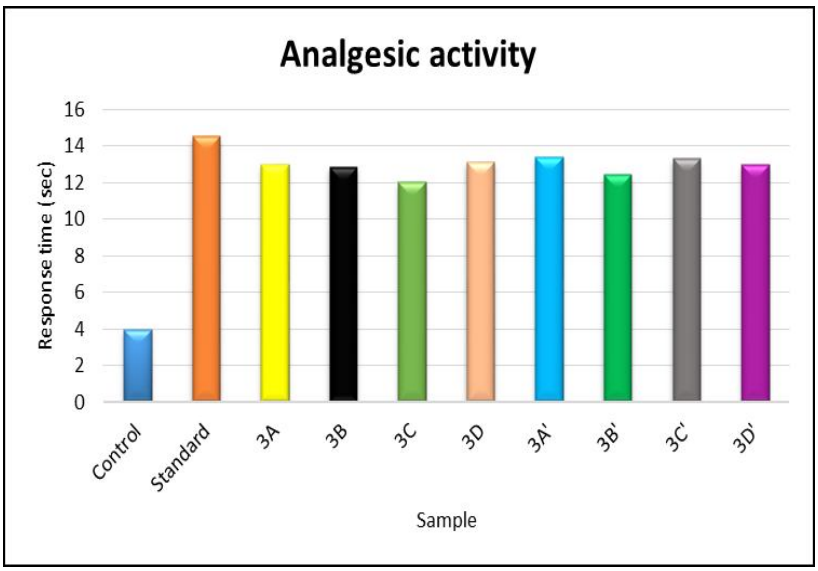


Fig. 2: Analgesic activity of the derivatives 3A-3D' using Eddy's hot plate method

REFERENCES

1. Tripathi KD. Essentials of Medical Pharmacology. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi. 2003; 5th Ed: 150-167.
2. Mohan H. Textbook of Pathology. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi. 2003; 4th Ed: 123-124.
3. Kutterer KM, Davis JM, Singh G, Yang Y, Hu W, Severin A, Rasmussen BA, Krishnamurthy G, Failli A and Katz AH. 4-Alkyl and 4, 4'-dialkyl- 1, 2-bis(4- chlorophenyl) pyrazolidine-3,5-dione derivatives as new inhibitors of bacterial cell wall biosynthesis. Bioorg Med Chem. 2005;15:2527-2531.
4. Gilbert AM, Failli A, Jay S, Yang Y, Severin A, Singh G, Hu W, Keeney D, Peterson PJ and Katz Al. Pyrazolidine-3,5-diones and 5- hydroxy-1H-pyrazol-3(2H)- ones, Inhibitors of UDP-N-acetylenolpyruvyl Glucosamine Reductase. J Med Chem. 2006;49:6027-6036.
5. Cauvin C, Le Bourdonnec B, Norberg B, Hénichart JP and Durant F. Pyrazolidine-3, 5-dione angiotensin-II receptor antagonists. Acta Cryst. 2001;C57:1330- 1332.
6. Kornet MJ, Thorstenson JH and Lubawy WC. Anticonvulsant Activity of 1-Alkyl-4-substituted 3, 5-pyrazolidinediones. J Pharmceutical Sciences. 1974;63:1090-1093.
7. Anil CK, Arya P, Chandrani M, Pankaj K, Yogesh Y and Ajendra SK. Microwave-assisted synthesis of antimicrobial dihydropyridines and tetrahydropyrimidin-2-ones: Novel compounds against aspergillosis. Bioorg and Med Chem. 2006;14:973-981.
8. Ashraf AH, Ibrahim TM, Khaled AM, Lehmann J, Tinsley HN and Gary BD. Design, synthesis and biological evaluation of novel pyridine derivatives as anticancer agents and phosphodiesterase 3 inhibitors. Bioorg and med chem. 2009;17:5974-5982.
9. Pattan SR, Bhat AR, Taranill AD, Purohit SS and Reddy VVK. Synthesis of new 1, 4-dihydropyridine derivatives as antihypertensive agents. Indian J Heterocyclic Chem. 2005; 15: 65-66.
10. Sushma D, Archana, Sukhjinder S, Munirajam S and Nitin K. Synthesis and antiinflammatory activity of some 2-amino pyridines. Indian J Heterocyclic Chem. 2007;16:411-412.
11. Asma Samaunnisa A. Design, synthesis and Biological evaluation of novel bis pyrazolidine dione derivatives containing pyridine moiety. 2011.
12. Asma samaunnisa A, Venkataramana CHS and Madhavan V. Synthesis, characterization and biological evaluation of novel derivatives of bis pyrazolidine-3,5-dione tethered with 1,4-dihydropyridine moiety. CIOP. 2013;2(2):36-42.
13. Asma Samaunnisa A, Venkataramana CHS and Madhavan V. Synthesis, characterization and biological evaluation of novel N3, N5-diphenyl-1,4-dihydropyridine-3,5-dicarbohydrazide derivatives. International Journal of Research in Pharmacy and Chemistry. 2013;3(1):160-7
14. Gerhard Vogel H. Drug discovery and evaluation, pharmacological assays. 2nd ed. Springer; 2006;696.
15. Fahmy HH and Soliman GA. Synthesis of new salicylamide derivatives with evaluation of their anti-inflammatory, analgesic and antipyretic activities. Archibes of Pharmacal Research. 2001;24(3):180-189.
16. Asati KC, Srivastava SK and Srivastava SD. Synthesis of 5-arylidene-2-aryl-3-(benzotriazloacetamidyl)-1,3-thiazololidin-4- ones as analgesic and antimicrobial agents. Indian Journal of Chemistry. 2006;45(B):526-531.