EVALUATION OF- 2, 6-DIMETHYL- N₃, N₅- DIPHENYL – 1, 4-DIHYDROPYRIDINE – 3,5-DICARBOHYDRAZIDE DERIVATIVES FOR IN VIVO ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES

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ABSTRACT

Novel 2,6-dimethyl-N₃, N₅-diphenyl-1,4-dihydropyridine-3,5-dicarbohydrazone (2A-2D') and its derivatives were 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'). A set of 8 new derivatives (2A-2D'), were subjected to *in vivo* anti-inflammatory and analgesic activities. Anti-inflammatory activity was performed by using Carrageenan induced paw edema method and analgesic activity was performed by Eddy's hot plate method. The obtained results were 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 class exhibited significant activity.

Keywords: *In vivo* Anti-inflammatory, Analgesic, 1, 4-Dihydropyridine, Dicarbohydrazone.

INTRODUCTION

Ever since the dawn of mankind there has been an ongoing war between mankind and the diseases. There wasn't a single day that the scientists and researchers stopped looking for better molecules for almost all the existing ailments. One of the most sought after classes of drugs are NSAIDS. Although there are a number of NSAIDS developed and available in market, rigorous search for new molecules is on wheels. This is due to variety of side effects like gastric ulceration, inhibition of platelet function, alteration in renal function, hypersensitivity etc, with the existing molecules. 1, 4-dihydropyridine is a 6 membered heterocyclic ring containing nitrogen as hetero atom. This moiety, individually as well as in combination with other moieties is known to possess a variety of pharmacological activities which include antibacterial, antifungal, anti-inflammatory, antihypertensive, anticonvulsant, anticancer, anti-diabetic activities¹⁻¹⁰. The rationale behind this work is to thoroughly study and investigate the *in vivo* anti-inflammatory and analgesic activities of 2, 6-dimethyl-N₃, N₅-diphenyl-1, 4-dihydropyridine-3, 5-dicarbohydrazone (2A-2D')¹³. These derivatives might prove to be better leads for the class of NSAIDS, with less side effects and increased therapeutic efficacy.

MATERIALS AND METHODS

Chemicals and solvents used were of reagent grade and used without further purification, were procured from Spectro Chem, Hi-Media, Merck, Sigma Aldrich and Ranbaxy. The purity of the synthesized compounds was determined by melting point using open capillary method and is uncorrected. IR (infra-red) was performed using SHIMADZU FTIR-8400S. The compounds 2A-2D' were identified by ¹H NMR (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.

Synthesis of Diethyl-2, 6-dimethyl- 1, 4-dihydropyridine-3, 5-dicarbohydrazone (1A) and diethyl-4-(4-hydroxyphenyl)-2, 6- dimethyl-1, 4-dihydropyridine-3, 5- dicarboxylate' (1A')^{11,12}

1A-1A'

Synthesis of 2, 6-dimethyl-N₃, N₅- diphenyl-1, 4-dihydropyridine-3, 5- dicarbohydrazone (2A-2D')¹³

2A-2D'

Substitutions for the derivatives are given in Table 1.

Pharmacological Activity

Anti-inflammatory activity

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 dimethyl sulfoxide (vehicle), second group received intra

peritoneal injection of indomethacin, third group to tenth group were treated with test compounds (2A-2D') intra peritoneally. After 30 minutes, 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 minutes 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^{14,15}.

$$\% \text{ edema inhibition} = 100 [1 - (V_t - V_0) \text{ Treated group} / (V_t - V_0) \text{ Control}]$$

V_0 = Initial paw volume of the rat before the administration of carrageenan (at 0 minutes).

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

Analgesic activity

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-N³, N³-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 ($55 \pm 1^\circ\text{C}$). 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 paw¹⁴⁻¹⁶.

RESULTS AND DISCUSSION

The derivatives 1A-1A' were synthesized using conventional Hantzsch method by condensation of ethyl acetoacetate with

an aldehyde in the presence of ammonia and 2A-2D' were synthesized by condensation in the presence of respective phenyl hydrazines. Purity of these compounds was assessed using TLC and the structures were confirmed by ¹HNMR, Mass, IR and Elemental analysis data. The newly synthesized derivatives 2A-2D' were screened for their *in vivo* 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, 2A, 2C and 2D exhibited good activity, 2B' and 2C' has shown moderate activity, whereas other derivatives of the class namely 2B, 2A', and 2D' exhibited considerably low activity when compared with the standard indomethacin. Results summarized in Table 2 and Figure 1. All the derivatives which are screened for analgesic activity have shown significant activity in comparison with the standard tramadol. Derivatives 2A, 2B, 2A', 2C' has shown significantly good activity and the derivative 2D has shown moderate activity whereas the derivatives 2C, 2B', 2D' has shown lower activity when compared to other derivatives of the class as well as standard. The derivatives exhibited considerably good anti-inflammatory and analgesic activities. Results summarized in Table 3 and Figure 2.

Table 1: Substitutions of the Synthesized Derivatives

Compound	R ¹	R ²	R
1A	-	-	H
1A'	-	-	C ₆ H ₄ OH
2A	H	H	H
2B	NO ₂	NO ₂	H
2C	H	Cl	H
2D	H	NO ₂	H
2A'	H	H	C ₆ H ₄ OH
2B'	NO ₂	NO ₂	C ₆ H ₄ OH
2C'	H	Cl	C ₆ H ₄ OH
2D'	H	NO ₂	C ₆ H ₄ OH

Table 2: Anti-inflammatory Activity using Carrageenan induced Paw edema in Rats

Carrageenan induced rat paw edema				
Samples	Volume (V ₀)	Maximum Displacement (V _t)	Percentage increase in paw volume (%)	Percentage inhibition of edema (%)
Control	0.4272 ± 0.0004	0.7034 ± 0.00128	62	-
Standard	0.3793 ± 0.0003	0.4511 ± 0.00018	19***	74
2A	0.4022 ± 0.0002	0.6106 ± 0.0002	52***	24
2B	0.3894 ± 0.00017	0.6239 ± 0.0002	62**	15
2C	0.3807 ± 0.0002	0.5884 ± 0.00018	54***	25
2D	0.3906 ± 0.00025	0.5819 ± 0.00015	49***	31
2A'	0.3676 ± 0.00016	0.6013 ± 0.00016	64*	15
2B'	0.3847 ± 0.00016	0.6167 ± 0.00014	61**	16
2C'	0.4361 ± 0.00019	0.6607 ± 0.0002	51***	19
2D'	0.3744 ± 0.00015	0.6122 ± 0.00022	65*	14

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 using Eddy's hot plate Method

Eddy's hot plate method		
S. No	Sample	Response time (sec)
1	Control	3.17 ± 0.4172
2	Standard	14.05 ± 0.4517***
3	2A	13.31 ± 0.5103***
4	2B	13.07 ± 0.6015***
5	2C	12.43 ± 0.5819**
6	2D	12.98 ± 0.4197**
7	2A'	13.52 ± 0.7123***
8	2B'	12.15 ± 0.5411*
9	2C'	13.55 ± 0.6121***
10	2D'	12.57 ± 0.7012***

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

Figure 1: Anti-inflammatory Activity using Carrageenan induced Paw edema in Rats

Figure 2: Analgesic Activity using Eddy's hot plate Method

Statistical Analysis

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

CONCLUSION

These derivatives were synthesized with a rationale of developing lead molecules with better therapeutic efficacy. All the derivatives 2A-2D' have shown considerably good anti-inflammatory and analgesic activities. Analgesic activity is more when compared to the anti-inflammatory activity. Further study on these derivatives might provide a better insight into their pharmacological activities.

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