In vitro Anti-inflammatory evaluation of1, 4- Di hydro pyridines

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ABSTRACT

The Novel class of 4-aryl-2, 6-dimethyl-3, 5-dicarbomethoxy/ethoxy-4-(Substituted phenyl)-1, 4-di hydro pyridine has been evaluated for the anti- Inflammatory activity in In vitro condition. The newly synthesized compounds were evaluated for in vitro anti – Inflammatory activity. This evaluation is done by using HRBC Membrane stabilization Method (UV- Absorbance, % Hemolysis at different concentration and % Protection at different concentration). The entire Novel 1-4-DHP derivative compound ***4a-4f showed*** highest *in-vitro* anti-inflammatory activity at different concentration by HRBC membrane stabilization and protection.

Keywords: 1, 4- dihydropyridine, *in-vitro* **Anti- inflammatory activity**

INTRODUCTION

Dihydropyridine derivatives, such as nifedipine, nitrendipine and nimodipine, have been found to be commercially useful molecules as calcium channel blockers. A number of dihydropyridine calcium antagonists have been introduced as potential drugs for the treatment of congestive heart failure. Further, cerebrocrast, a dihydropyridine derivative has been introduced as a neuroprotectiveagent6 . Moreover a number of dihydropyridine derivatives have been found as vasodilators, antihypertensive, bronchodilators, antiatherosclerotic, hepatoprotective, antitumour, antimutagenic, geroprotective, antidiabetic and antiplatelet aggregation agents . Dihydropyridines have attracted considerable attention in medicinal and bioorganic chemistry as potential calcium channel blockers9 and NADH models, respectively. Due to their high reactivity, dihydropyridines also constitute versatile intermediates in the synthesis of natural products. The structure activity relationships were studied on newly synthesized 1, 4- dihydropyridine derivatives possessing a 1-pentyl group at fourth position, and 3-pyridylpropylester was found to be one of the effective fragments for overcoming Pglycoprotein mediated Multidrug-resistance (MDR) in cultured human cancer cells in vitro. Heterocyclic chemistry comprises at least half of all organic chemistry research worldwide. In particular, heterocyclic structures form the basis of many pharmaceutical, agrochemical and veterinary products are classes of fused heterocyclic that are of considerable interest because of the diverse range of their biological properties, for example, anticancer, diuretic, antiinflammatory, anticonvulsant and antihypertensive activities. The in vitro toxicity of the most active diethyl dihydropyridine derivative was reported by using hemolytic assay in which the compound was found to be non-toxic to human. However the potential of dihydropyridines as antibacterial and antifungal agents has not been studied. Therefore the

current study was undertaken to synthesize different dihydropyridine derivatives and investigate their antibacterial and antifungal potential using Escherichia, Staphylococcus AureusAspergilli and Candida Albicans as model pathogens. A simple, inexpensive and efficient one-pot synthesis of 1, 4-dihydropyridine derivatives at room temperature using catalytic amount of iodine were reported with excellent product yields21. Several methods have been developed and reported for the synthesis of 1-4, dihydropyridines by using ultrasound irradiation without catalyst, microwave-assisted oxidative aromatization of Hantzsch 1, 4-Dihydropyridines using manganese dioxide Lubinu, microwave assisted synthesis, highly selective and facile synthesis using dicarboxylic acid derivatives using electro reduction, one-pot synthesis of dihydropyrimidiones catalyzed by strontium (II) triflate under solvent-free conditions, asymmetric synthesis. Yb (OTf) 3 catalyzed an efficient environmentally benign Hantzsch reaction via a four-component coupling reaction of aldehydes, dimedone, ethylacetoacetate and ammonium acetate at ambient temperature were reported. Chemistry All reagents and solvents were used as obtained from the supplier or recrystallized/ redistilled as necessary. Thin layer chromatography was performed on microscopic slides coated with silica gel G and ethanol: water (95:5) as a mobile phase. The spots were visualized by normal TLC and exposure to iodine vapour. Melting points were recorded on open capillary melting point apparatus and were uncorrected. IR spectra were recorded in KBr on SHIMADZU Fourier Transform Infrared 8400 S spectrophotometer. Mass spectra were recorded on Electron impact (EI) on a Jeol JMS-D- 300 spectrometer with the ionization potential of 70 eV. Nuclear Magnetic Resonance spectra (1 H NMR) were recorded in DMSO-d6 on Bruker advance at 400 MHz using Tetramethylsilane (TMS) as internal standard and the chemical shift (δ) were reported in parts per million (ppm). Elemental analysis data were determined using a Carlo Erba 1108 instrument or Elementar'sVario EL III micro-analyzer. All the compounds were synthesized in good to excellent yield. **Objective:**To evaluate previously synthesized derivatives of 1, 4- Dihydropyridine by HRBC membrane stabilization method for their*in-vitro*anti-inflammatory activity.

MATERIALS AND METHODS

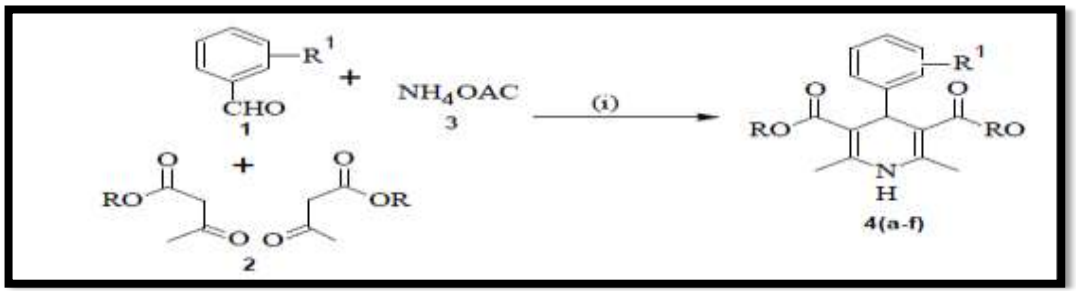
CHEMICALS: All chemicals used in the study were of analytical grade: Dextrose, Sodium citrate, Citric acid, Sodium chloride, Sodium hydroxide and Dihydrogen phosphate was purchased from SD fine chemicals, Mumbai.

INSTRUMENT: Reference standard Diclofenac was obtained from Cipla Ltd, Bangalore. Shimadzu 1701 UV-Visible spectrophotometer was used for the estimation of invitroanti-inflammatory activity.[1]

In vitro anti-inflammatory activity by HRBC Membrane stabilization Method:

The anti-inflammatory activity of synthesized derivatives was determined by HRBC membranestabilization method. Blood was collected from healthyvolunteers. The collected blood was mixed with equalvolume of (2% dextrose, 0.8% sodium citrate, 0.05% citricacid& 0.42% sodium chloride in water). The blood wascentrifuged at 300 rpm and packed cells were washed withisosaline (0.85%, pH 7.2) & 10% v/v suspension was madewith isosaline.The assay mixture contained the drug (concentrationas mentioned in Table 2). 1 mL phosphate buffer (0.15 M,pH 7.4), 2 mL of hyposaline (0.36%) % 0.5 mL of HRBCsuspension.Diclofenac was used as the reference drug.Instead of hyposaline, 2 mL of distilled water was usedas control. All the assay mixtures were collected at 37 for 30 min and centrifuged. The hemoglobin content inthe supernatant solution was estimated using colorimeterat 560 nm. The percentage heamolysis was calculatedby assuming the heamolysis produced in the presenceof distilled water as 100%. The percentage of HRBCmembrane stabilization or protection was calculated using the followingformula.

General Reaction for the Synthesis of1-4-Dihydropyridines:



Scheme I: *4 (a-f) Reagents and Condition: 1) Substituted Benzaldehyde 2) Beta Ketoesters 3) Ammonium Acetate i)Room Temp.* ***(Previously reported)***

Table 2:U.V Absorbance of sample and Standard.

Compound No.	R1	R2	Absorbance			
			25	**50**	**100**	**200**
4a	3-OCH3, 4-OH	-OCH3	1.1	0.99	0.96	0.90
4b	3-OCH3, 4-OH	-OC2H5	1.2	1.00	0.94	0.94
4c	4-OH	-OCH3	1.2	0.90	0.92	0.92
4d	4-OH	-OC2H5	1.2	0.90	0.92	0.92
4e	3,4-OCH3	-OC2H5	1.2	0.99	0.99	0.94
4f	3,4,5-OCH3	-OC2H5	1.2	0.98	1.00	1.00
Control			1.2	1.2	1.2	1.2
Std	**Diclofenac Sod.**		0.48	0.432	0.348	0.096

Table 3: % Heamolysis at different concentration µg/ml of synthesized drug

Compound No.	R	R2	% Heamolysis at different concentration µg/ml of synthesized drug			
			25	**50**	**100**	**200**
4a	3-OCH3, 4-OH	-OCH3	8.34	17.5	20	25
4b	3-OCH3, 4-OH	-OC2H5	Nil	16.67	21.67	21.67
4c	4-OH	-OCH3	Nil	25	23.34	23.34

4d	4-OH	-OC2H5	Nil	25	23.34	23.34
4e	3,4-OCH3	-OC2H5	Nil	17.5	17.5	21.67
4f	3,4,5-OCH3	-OC2H5	Nil	18.34	16.67	16.67
Control			100	100	100	100
Std	**Diclofenac Sod.**		**60**	**64**	**71**	**92**

Table 4: ***In vitro*** **anti-inflammatory activity** ***(% Protection at different concentration µg/ml of synthesized drug)***

Compound No.	**R1**	**R2**	**% Protection at different concentration µg/ml of synthesized drug**			
			25	**50**	**100**	**200**
4a	3-OCH3, 4-OH	-OCH3	91.66	82.50	80	75
4b	3-OCH3, 4-OH	-OC2H5	Nil	83.33	78.33	78.33
4c	4-OH	-OCH3	Nil	75	76.66	76.66
4d	4-OH	-OC2H5	Nil	75	76.66	76.66
4e	3,4-OCH3	-OC2H5	Nil	82.5	82.5	78.33
4f	3,4,5-OCH3	-OC2H5	Nil	81.66	83.33	83.33
Control			100	100	100	100
Std	**Diclofenac Sod.**		40	36	29	08

DISCUSSION

The previously synthesized compounds **(*4a-4f*)** evaluated for their *in vitro* anti-inflammatory activity by using HRBC Membrane stabilization method in which diclofenac sodium was used in three graded dose 25, 50 &100 µg/mlas standard drug. The presence of 2-chlorophenyl group in the 4c and 4f comparatively showed good binding ability to the binding site. Among the Synthesize compounds compound ***4a & 4f***showed minimal *in vitro* anti-inflammatoryactivity.

CONCLUSION

Novel 1-4-DHP derivatives were previously synthesized and characterized by physicochemical and spectral analysis. Novel 1-4-DHPderivativewere screened *in-vitro* anti-inflammatory activity by HRBC Membrane stabilization method. The entireNovel 1-4-DHP derivative compound ***4a-4fshowed*** highest *in-vitro* anti-inflammatory activity at different concentration by HRBC membrane stabilization and protection.

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Page No. 935

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