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Synthesis, characterization and biological screening of novel 1, 4-dihydropyridine derivatives for certain pharmacological activities

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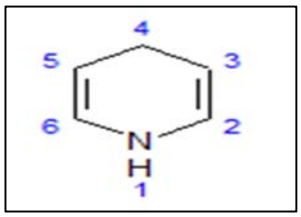
Abstract
A series of 1, 4-dihydropyridine derivatives were prepared from three compounds by the condensation reaction of ethyl acetoacetate, aromatic aldehyde and ammonium acetate at 70^0C. Derivatives of 1, 4-dihydropyridines are one of the most potent calcium antagonists. The compound exhibits various pharmacological actions which include calcium channel antagonist property, antihy pertensive [1] anti-inflammatory, antifungal, analgesic, antimicrobial, antithrombotic actions. It also shows vasodialation, anticonvulsant and stress protective effect by binding to L and N channels [2].

Keywords: Snail, Calcium channel blocker, 1, 4- Dihydropyridine**,** antihypertensive, anti-inflammatory, analgesic, antimicrobial**,** antithrombotic activity

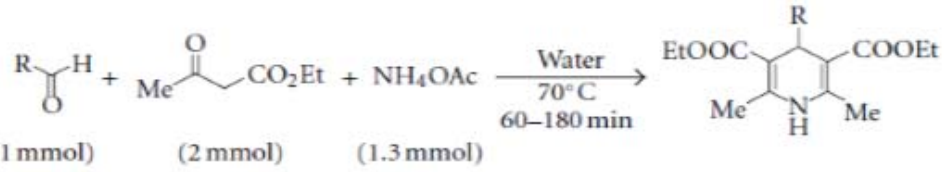
Introduction
Medicinal chemistry is a multidisciplinary subject involving organic chemistry, pharmacology, biochemistry, physiology, microbiology, toxicology & genetics. It is concerned with the design, development, identification and synthesis of compounds that can be used for prevention, treatment or cure of human or animal disease and study of their metabolism, interpretation of their mode of action at molecular level and construction of structure activity relationship (SAR) [3].
Dihydropyridines are the derivatives of pyridine which belong to an important group of heterocyclic compounds containing nitrogen in a six member ring which is saturated at the 1st and 4th position of pyridine [4].



1,4-DHP

Materials and methods
Chemistry
Nuclear Magnetic Resonance (1 H-NMR) spectra were recorded on a Bruker using CDCl3. The Chemical shift values are reported in parts per million (ppm) relative to Tetra methyl silane as internal reference. Infra-red (IR) spectra were recorded as thin films in Potassium bromide (KBr) pellets. The melting point ranges of newly synthesized compounds were determined by open glass capillary tube using digital melting point apparatus. Purity of the compound and progress of the reaction were monitored by thin layer chromatography (TLC), with detection by Ultra-violet (UV) light and/or spots were visualized by exposure to iodine vapours.

Synthesis
A series of 1, 4-dihydropyridine derivatives were prepared from three compounds by the form Condensation reaction of ethyl acetoacetate, aromatic aldehyde and ammonium acetate at 70 ^{0}C.



Substituted Aldehyde R=H,R=Ar	Ethyl acetoacetate	Ammonium acetate	1,4-Dihydropyridine derivative 70-90%

General Procedure

A mixture of an aldehyde (1 mmol), ethyl acetoacetate (2 mmol), and ammonium acetate (1.3 mmol) was vigorously stirred in water (2 ml) at 70°C. Few drops of ethanol was added to the reaction mixture, followed by crushed ice. A solid product was obtained which was filtered, washed with water, and recrystallized from aqueous ethanol [5] when the products are viscous oils they are isolated through extraction with ethylacetate.

Animals used for the study

Albino rats (wistar strain) and albino mice were used to carry out the activities. The experiments were carried out as per the guidelines of CPCSEA and approved by the Institutional Animal Ethical Committee (IAEC)

Acute toxicity study

Acute toxicity studies of the synthesized compounds were carried out using OECD [6] guideline 423. The test procedure minimizes the number of animals required to estimate the oral acute toxicity. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

Pharmacological evaluation

Antimicrobial Activity:

The anti-bacterial activity of the synthesized compounds was evaluated by agar disc diffusion method using nutrient agar medium against following micro-organisms: *Staphylococcus aureus*, *Bacillus subtilis* (Gram positive) and *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative). In the paper disc diffusion method, paper discs impregnated with test compounds (100µg/ml). The microorganism culture was spread over nutrient agar media in petri-dishes and the disc impregnated with the solution was placed on the surface of the media inoculated with the bacterial strain. The plates were incubated at 35 °C for 24 hrs. After incubation, the zone of inhibition around the disc was observed. Each testing was done in triplicate. Ciprofloxacin was used as standard drug for antibacterial activity. The results were interpreted in terms diameter (mm) of zone of inhibition [7].

Anti-inflammatory Activity

***In vitro* protein denaturation method [8]**

A solution of 0.2 % w/v of Bovine Serum Albumin (BSA) was prepared in tris buffer saline and pH was adjusted to 7.4 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. The standard consist of 5ml of 0.2%w/v BSA solution and 50µl Diclofenac (100µg/ml).The test tubes were heated at 72^0C for 5 minutes and then cooled for 10 minutes. The absorbance 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

Analgesic Activity [9]

The animals were divided into three group of six animals in each group (one control,one standard and one test group). The basal reaction time was taken by observing hind paw licking or jump response in animals when placed on the hot plate maintained at constant temperature (55^0c). Standard drug (Tramadol (10mg/kg)**)** was injected to the standard group & note down the reaction time of animal on hot plate at 15m, 30m, 60m after the drug administration. Calculated the increase in the reaction time (as index of analgesia). The procedure was repeated for test group with test drug (60mg/kg) also.

Anticonvulsant Activity [10]

The animals were divided into three group of six animals in each group (one control, one standard and one test group). One group for studying the protective effects of phenytoin and other to study whether the test compound have anticonvulsant effect or not. Standard drug (Phenytoin (25mg/kg) was injected 30 min prior to the experiment. Animals were hold properly, then placed the ear electrodes and a current of 150mA for 0.2 seconds was applied. Onset and severity of convulsions along with the different stages of convulsions (tonic flexion, tonic extensor, clonic convulsions, stupor) were noted. Repeated the same with test compound (60mg/kg). The reduction in time or abolition of extensor phase was noted.

Antioxidant Activity [11]

A solution of H_2O_2 was prepared in phosphate buffer saline (P^H 7.4). 1ml of test sample and standard (Ascorbic acid) in a concentration of 100 µg\ml in ethanol were added to 2ml of H_2O_2 solution in phosphate buffer saline. Absorbance was measured in 230 nm after 10 min.

% of H202 scavenged = $(A_c - A_t)/ A_c$ X 100

Where A_c = Absorbance of control group

A_t = Absorbance of test group animal

Results and Discussion

1,4 dihydropyridine derivatives were synthesized by Hantzsch method

Physicochemical properties

Code	Molecular Formula	Molecular Weight (g/mol)	Physical State	Colour	Melting Point [0C]	Yield [% w/w]	R_F value	Percentage purity
C1	$C_{20}H_{24}N_1O_5$	358.1	solid	Off white	75^0C	76.2	0.78	86%
C2	$C_{20}H_{23}N_2O_6$	387.41	solid	orange	85^0C	70	0.9	88%
C3	$C_{22}H_{29}N_2O_4$	385.48	oil	Yellowish red	60^0C	80	0.8	85%
C4	$C_{23}H_{32}N_1O_7$	434.5	solid	yellow	84^0C	70	0.8	90%
C5	$C_{26}H_{28}N_1O_5$	434.5	solid	yellow	66^0C	85	0.8	90%

Solubility profile
Test compound is partially soluble in water, soluble in alcohol, chloroform, acetone, ethylacetate and n-Hexane.

Spectral Characterization:
Spectral Analysis of C1
IR Spectra of C1:
3506- NH stretching, 2900-CH stretching, 3445-OH stretching, 1487. 18-COO stretching.
NMR Spectra ($CDCl_3$):

δ in ppm: 7.020-7.628 (4H,Aromatic hydrogen), 2.532 -2.614 (1H, NH), 1.274-1.620 (6H, RCH), 1.274- 4. 516 (1H, OH), 1.215-1.620 (10 H, RCH_2R).

Spectral Analysis of C2
IR Spectra:
3458- NH stretching, 2800-CH stretching, 1645-COO stretching, 1560- NO_2 stretching, 1720-CO stretching of aldehyde.
NMR Spectra ($CDCl_3$):

δ in ppm: 7.284-8.342 (4H,Aromatic hydrogen), 2.374-2.450 (1H, NH), 1.215-1.250 (6H, RCH), 1.215-1.250 (10 H, RCH_2R).

Spectral Analysis of C3
IR Spectra:
3430- NH stretching, 2580-CH stretching, 1645-COOstretching, 2810-N-CH_3 stretching.
NMR Spectra ($CDCl_3$):

δ in ppm: 7.139-7.312 (4H,Aromatic hydrogen), 2.352 (1H, NH), 1.220 -1.256 (12H, RCH), 1.220-1.256 (10 H, RCH_2R).

Spectral Analysis of C4
IR Spectra:
3453-NH stretching, 2800-CH stretching, 1645-COO stretching, 2830- OCH_3 stretching
NMR Spectra ($CDCl_3$):

δ in ppm: 6.533-7.284 (2H,Aromatic hydrogen), 2.365 -2.438 (1H, NH), 1.253-1.89 (6H, RCH), 1.253-1.289 (10 H, RCH_2R), 3.815-3.908 (9H, R-OCH_3)

Spectral Analysis of C5
IR Spectra:
3441-NH stretching, 2800- CH stretching, 1700-COO stretching, 1169- C-O stretching in ring.
NMR Spectra ($CDCl_3$):

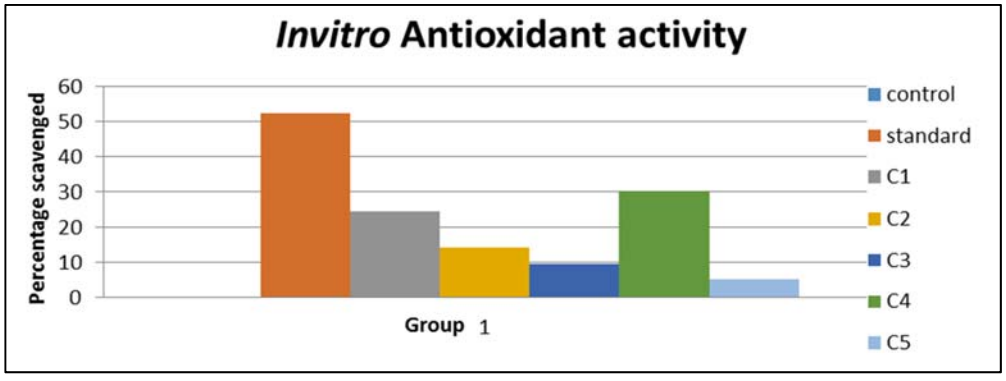
δ in ppm: 7.205-7.441 (8H,Aromatic hydrogen), 2.344(1H, NH), 1.223-1.258 (6H, RCH), 1.223-1.258 (10 H, RCH_2R)

Acute Toxicity Study
The compounds showed toxic effects at a dose of 2000mg/kg. The safe dose of the drug was found to be 300mg/kg.

Screening for Biological Activities
1. ***In vitro* Antioxidant Activity (Hydrogen peroxide scavenging method)**

Sample	Absorbance	Percentage of H_2O_2 scavenged
Control	0.753±0.113	-
Standard	0.358±0.045**	52.45**
C1	0.553±0.147**	24.59**
C2	0.592±0.112	14.4
C3	0.681±0.124	9.56
C4	0.525±0.115**	30.27**
C5	0.713±0.134	5.31

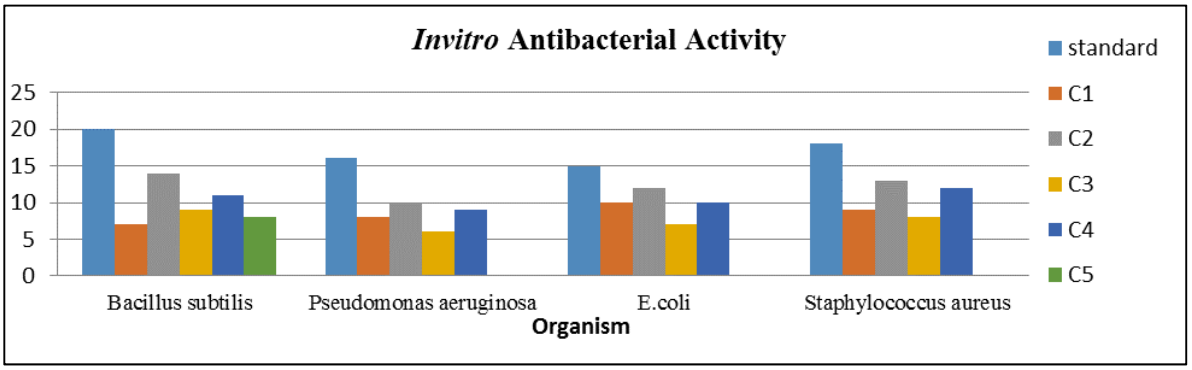
Each value represent Mean ± SEM, n = 3, p <0.01



2. ***In-vitro* Antibacterial Activity**

S. No	Sample	Zone of inhibition in mm			
		Bacillus subtilis	*Pseudomonas aeruginosa*	*E. Coli*	*Staphylococcus Aureus*
1	Standard(Ciprofloxacin)	20±0.022 **	16±0.058**	15±0.017**	18±0.059 **
2	C1	7±0.122	8±0.184	10±0.155	9±0.158
3	C2	14±0.121**	10±0.173**	12±0.147**	13±0.157**
4	C3	9±0.198	6±0.125	7±0.148	8±0.178
5	C4	11±0.195**	9±0.143**	10±0.187*	12±0.189**
6	C5	8±0.187	-	.-	-

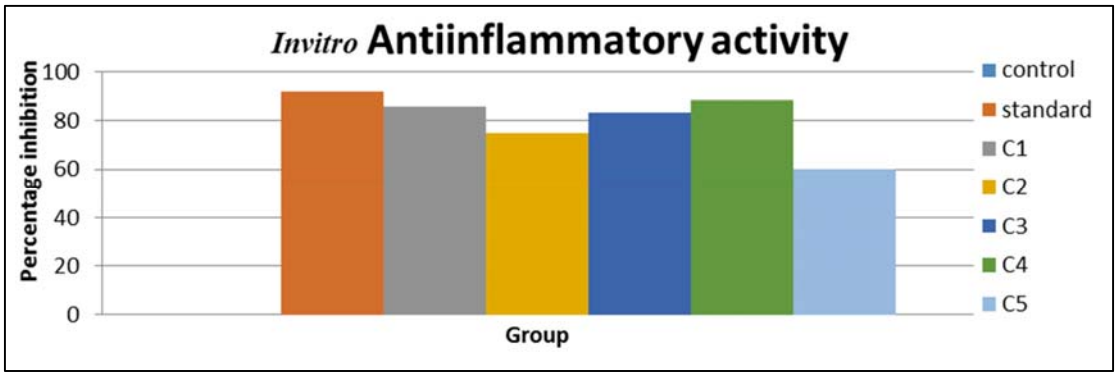
Each value represent Mean ± SEM, n = 3, p < 0.01



***In vitro*- Anti-inflammatory activity**:

S. No.	Sample	Absorbance	Percentage.. of inhibition
1	Control	1.747±0.234	-
2	Standard	0.1399±0.021**	92%
3	C1	0.1924±0.116**	85.9%
4	C2	0.4321±0.147	75%
5	C3	0.2367±0.078	83.4%
6	C4	0.1882±0.063**	88.2%
7	C5	0.6826±0.183	60%

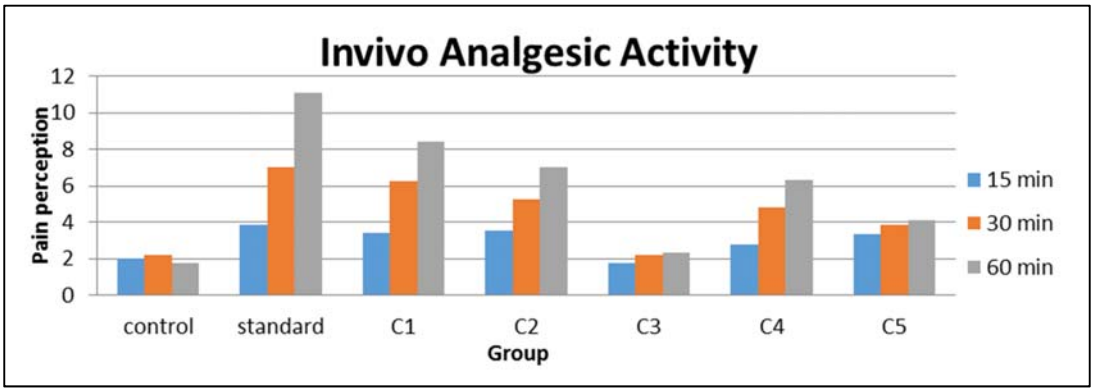
Each value represent Mean ± SEM, n = 3, p <0.01



***Invivo* Analgesic Activity**

S. No	Sample	Basal. Reaction before.. drug administration (sec)	Pain perception After drug administration (sec)		
			After 15minute	After 30 minute	After 60 minute
1	C1	3.6 ±0.014**	5±0.0142**	6±0.014**	5±0.013**
2	C2	4.1±0.017	4±0.0187	3.9±0.0176	4±0.017
3	C3	3.1±0.099**	4.1±0.024**	3.9±0.023**	4.5±0.022**
4	C4	4.1±0.022**	4.5±0.098**	4±0.098**	5.2±0.097**
5	C5	3±0.028	4.8±0.028	4.2±0.027	4±0.026
6	Control	2.2±0.0135	2±0.0134	3±0.0135	2±0.0134
7	standard	5±0.021**	7.5±0.021**	12±0.0198**	9.2±0.0231**

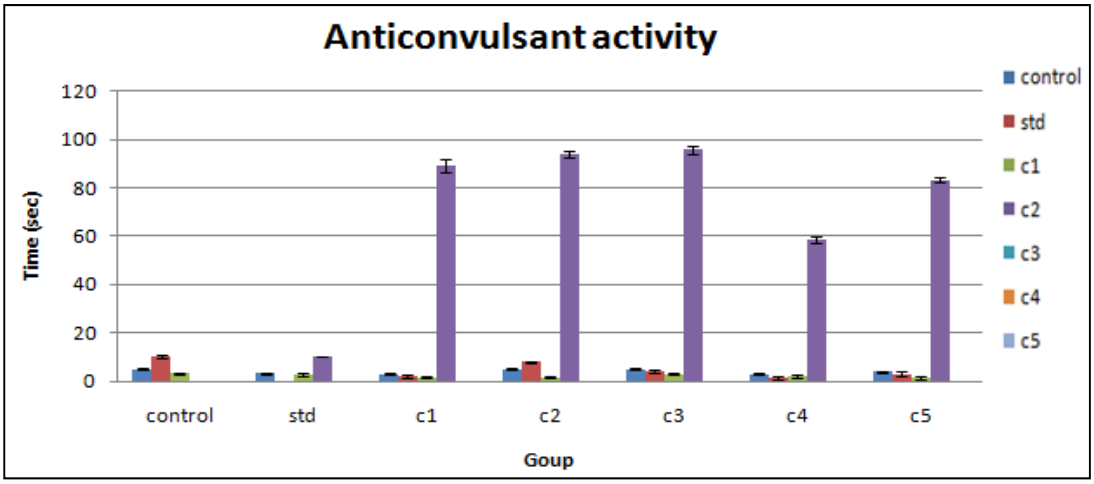
Each value represent Mean ± SEM, n = 6, p <0.01



***In vivo* Anticonvulsant Activity**

		Group			
S. No		**Tonic flexion**	**Tonic extensor**	**Clonic**	**Stupor**
1	Control	5±.028	10±.033	3±.036	-
2	Standard	3±.019	-	2±.033	10±.035
3	C1	4±.036	3±.021	2±.036	90±033
4	C2	6±.034	9±.0210	2±.034	95±.036
5	C3	6±.036	5±.047	4±.056	110±.022
6	C4	4±033**	2±.028**	3±.033**	60±.035**
7	C5	5±.045	4±.043	2±.035	85±.044

Each value represent Mean ± SEM, n = 6, p <0.01



Conclusion

Antibacerial activity: C2>C4>C1>C3>C5
Anti-inflammatory activity: C4>C1>C3>C2>C5
Analgesic activity: C1>C4>C3>C5>C2
Antioxidant activity: C4>C1>C2>C3>C5
Anticonvulsant activity: C4>C1>C5>C3>C2

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