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Research Article

RAPID SYNTHESIS AND EVALUATION OF NEW 1, 4 – DIHYDROPYRIDINES AS POSSIBILE ANTI MITOTIC AGENTS

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ABSTRACT

1,4 Dihydropyridines and their derivates play vital role in biological field such as antimicrobial, anticancer, antihypertensive, antianginal, antitubercular, anticonvulsant activities. Therapeutic significance of these clinically useful drugs in treatment of tumors encouraged the development of some potent and significant compounds. A series of 4-substituted DHP derivatives (**Ia-Ie, IIa-IIe**) were synthesized and evaluated for their possible anti-mitotic activity. The structures of the synthesized compounds were confirmed on the basis of their spectral data and elemental analysis. Majority of the compounds were active in germinating onions, Bengal gram seeds as antimitotic agents and the compound IIe has shown significant antimitotic activity.

Keywords: 1, 4 DHP, germinating onions, Bengal gram seeds and antimitotic activity.

INTRODUCTION

Hantzsch, almost over 100 years ago brought out the synthesis of 1, 4-dihydropyridines (1,4-DNP) without even anticipating and visualizing their potency. The last two decades mostly have witnessed the pharmacological and therapeutic importance of a few such 1,4-dihydropyridines into the world market as successful and useful drugs in the treatment of some important cardiac ailments like angina, hypertension etc. this property is attributed mostly to their ability to block effectively the calcium channels (L-type). That's how they are called as the 'calcium channel blockers or calcium antagonists'.

Thus, 1, 4-Dihydropyridines have attracted the attention of several chemists and pharmacologists. As a result of it, several of them have been synthesized and screened for their effectiveness. Most of them vary in their nature of substituent's at 3,4 and 5 positions.

Interestingly, the 1, 4-dihydropyridine derivatives which have become drugs of medical importance are their 3,5-dicarboxylic acid esters. Further, studies and investigations by several scientists have proved these 1, 4-dihydropyridine derivatives which have become drugs of medical importance are their 3, 5-dicarboxylic acid ester. Further studies and investigations by several scientists have proved these 1, 4-dihydropyridines to exhibit not only the calcium channel blocking action or Calcium antagonism, but also to possess several other actions, such as vasodilator, bronchodilator and platelet aggregation inhibition. They also have known to exhibit cerebral anti-ischemic activity in the treatment of Alzheimer's disease, Chemo sensitizers in tumor therapy, Ant atherosclerotic,

Genoprotective, Hepatoprotective, Antidiabetic and Antiasthamatic activities[1,2,3].

Chemistry of 1, 4 – Dihydropyridines

The basic skeleton of 1, 4-DHP is a doubly unsaturated six membered cyclic system with one hetero atom and two double bonds. Owing to these double bonds and the lone pair on the N-atom, this skeleton is prone to react with electrophilic reagents. While the lone pair on N-atom afford basicity to the compounds containing this skeleton. The π electrons in double bonds facilitate the reactions with electron deficient species such as carbocations. Substitution on this skeleton shows remarkable rigid selectivity. Two equivalent and iso energetic resonance forms can be written for 1, 4-DHP. Clearly the 3 and 5 positions hold formal negative charges in this rigid forms, and more likely to react with electrophiles, when reacted with alkyl or aryl halides. 1, 4-DHP typically yields mono- or disubstituted alkyl or aryl derivatives[4,5,6].

The potency and activity of 1, 4-DHP is connected to its structure. It is possible that the basicity of the N-atom in 1, 4-DHP is critical to the function of 1, 4-DHP based drugs in their ability to bind to calcium channels. The N-atom in 1, 4-DHP is aliphatic tertiary nitrogen that is more basic than the pyridyl N-atom. The differences in basicity between these two types of N-atom are relevant when one considers the potential redox reactions that can occur in liver. For example, Bocker has studied the aromatization of 1, 4-DHP system in the metabolism of the drugs. The oxidation reaction is highly favored even under mild conditions because the product, pyridine, is aromatic. This aromatization of 1, 4-DHP is therefore an important factor in the design of animitotic drugs[7]. We considered the use of electron with drawing constituents at 3 and 5-positions because they pull the π-electrons away from the 1,4-DHP ring and thwart the aromatization[8].

EXPERIMENTAL PROCEDURE

1. Synthesis of 4-alkyl 3, 5-bis carboethoxy 2, 6-dimethyl 1,4-dihydropyridines (I):

Ethyl aceetoacetate (0.02 moles) and an appropriate aliphatic aldehyde (0.01 mole) were taken into a beaker (250ml) and dissolved in minimum quantity of solvent methanol (5-10ml). An appropriate aryl amine (0.01 mole) was added while wtirring. A funnel was hanged in the beaker and covered with a watch-glass and then the reaction mixture was subjected to the microwave irradiation at 400 Watt each in a domestic LG little chef microwave oven for 2-4 min. the solvent was removed, and the residue was cooled and triturated with crushed ice. The resultant produced was filtered, washed with small portions of cold water and dried. It was purified by recrystallization from hot methanol[9,10].

Nomenclature

Ia – *3,5-bis carboethoxy -2,6-dimethyl 1,4-dihydropyridine*

Ib – *4-Methyl -3, 5-bis carboethoxy -2, 6-dimethyl 1, 4-dihydropyridine*

Ic – *4-Ethyl -3, 5-bis carboethoxy -2, 6-dimethyl 1, 4-dihydropyridine*

Id – *4-Propyl -3, 5-bis carboethoxy -2, 6-dimethyl 1, 4-dihydropyridine*

Ie - *4-Butyl -3, 5-bis carboethoxy -2, 6-dimethyl 1, 4-dihydropyridine*

Characterization

*Compound **Ia***

Mol. Forml: $C_{13}H_{24}NO_4$; Mol wt. 258; Solubility: Methanol; M.p 180°C; R_f : 0.4 (Hexane; Ethyl acetate).

*Compound **Ib***

Mol. Forml: $C_{14}H_{26}NO_4$; Mol wt. 272; Solubility: Methanol; M.p 118°C; R_f : 0.44 (Hexane; Ethyl acetate); ^{1}H NMR-(CD_3OD) δppm: 0.8 (s, 3H, CH_3), 1.3-1.5 (t, 6H,2CH_3 of 2$COOCH_2$ CH_3), 2.56 (s, 6H, CH_3 at 2nd & 6th position), 2.78-2.85(m, H at C_4), 4.3-4.4 (q, 4H CH_2 of 2 $COOCH_2CH_3$), 8.70(s, H, NH).

*Compound **Ic***

$C_{15}H_{28}NO_4$; Mol wt. 286; Solubility: Methanol; M.p 110°C; R_f : 0.5 (Hexane; Ethyl acetate →3:2); ^{1}H NMR-(CD_3OD) δppm: 0.60-0.75(t, 3H, CH_3 of C_2H_5), 1.25-1.40 (m, 6H, 2CH_3 of 2$COOCH_2CH_3$), 2.50(s, 6H, 2CH_3 at 2nd & 6th position), 3.2-3.3 (t, H at C_4), 4.3-4.4 (q 4H, 2CH_2 of 2$COOCH_2CH_3$), 8.75(s, H-NH DHP), 1.45-154 9m, 2H, CH_2 of C_2H_5).

*Compound **Id***

$C_{16}H_{30}NO_4$; Mol wt. 300; Solubility: Methanol; M.p 122°C; R_f : 0.6 (Hexane; Ethyl acetate →3:2); ^{1}H NMR-(CD_3OD) δppm: 0.92(t, 3H, CH_3 of C_3H_7), 1.3-1.5(q, 6h, 2CH_3 of 2$COOCH_2CH_3$), 1.6-1.7 (m, 2H, CH_2 of C_3H_7), 2.26(s, 6H, 2CH_3 at 2nd & 6th position, 3.1-3.3 (t, H at C_4), 4.2-4.4 (m, 4H, 2CH_2 of 2$COOCH_2CH_3$), 8.75(s, H-NH).

Compound ***Ie:*** $C_{17}H_{32}NO_4$; Mol wt. 314; Solubility: Methanol; M.p 143°C; R_f : 0.65 (Hexane; Ethyl acetate →3:2); ^{1}H NMR-(CD_3OD) δppm: 0.92(t, 3H, CH_3 of C_4H_9), 1.3-1.5(m, 6H, $2CH_3$ of $2COOCH_2CH_3$), 1.6-1.7(t, 2H, CH_2 of C_4H_9), 2.26(s, 6H, $2CH_3$ at 2nd & 6th position), 3.1-3.3(t, H at C_4), 4.2-4.4 (m, 4 H, $2CH_2$ of $2COOCH_2CH_3$), 8.75(s, H-NH).

2. Synthesis of 4-alkyl 3,5-biscarbamoyl 2,6-dimethyl 1,4-dihydropyridine (II)

N-Acetoacetylation of Aniline:

Acetoacetanilide have been prepared from a reaction of ethylacetoacetate with appropriate aromatic primary amine. The acetoacetylation of aniline is carried out by the microwave – induced methods. This method has been found to vary in their reaction times and percentage yield of the product.

Condensation of Acetoacetanilide with different Aliphatic Aldehyde, and Ammonium acetate:

Acetoacetanilide should subjected to a three component condensation reaction involving itself, an appropriate aliphatic aldehyde and ammonium acetate by microwave irradiation method, while monitoring the progress of the reaction by TLC technique.

Nomenclature

IIa – *3, 5-bis carbamoyl -2, 6-dimethyl 1, 4-dihydropyridine*

IIb – *4-Methyl -3, 5-bis carbamoyl -2, 6-dimethyl 1, 4-dihydropyridine*

IIc – *4-Ethyl -3, 5-bis carbamoyl -2, 6-dimethyl 1, 4-dihydropyridine*

IId – *4-Propyl -3, 5-bis carbamoyl -2, 6-dimethyl 1, 4-dihydropyridine*

IIe – *4- Butyl -3, 5-bis carbamoyl -2, 6-dimethyl 1, 4-dihydropyridine*

Compound ***II_a***

$C_{21}H_{21}O_2N_2$; Mol wt. 347; Solubility: Methanol; M.p 123-125°C; R_F: 0.7 (Hexane; Ethyl acetate →4:3).

Compound ***IIb***

$C_{22}H_{23}O_2N_3$; Mol wt. 361; Solubility: Methanol; M.p 130-132°C; R_f : 0.74 (Hexane; Ethyl acetate →4:3); ^{1}H NMR-(CD_3OD) δppm: 0.82(d, 3H, CH_3), 2.56(s. 6H, CH_3), 6.5-6.7(m,5H, C_6H_5), 7.2-7.3(m, 5H, C_6H_5), 8.75(s, H, -NH Dihydropyridines), 10.78(s, H, -NH Carbamoyl).

Compound ***IIc***

$C_{23}H_{25}O_2N_3$; Mol wt. 375; Solubility: Methanol; M.p 110-112°C; R_f: 0.8 (Hexane; Ethyl acetate →4:3); ^{1}H NMR-(CD_3OD) δppm: 0.82(t, 2H, CH_2 of Ethyl group), 2.26(s, 6H, CH_3 Group at 2nd & 6th position). 3.1-3.3 (t, H), 6.6-6.9 (t, 5H, C_6H_5), 7.2-7.4 (m, 5H, C_6H_5), 8.75 (s, -H, -NH Dihydropyridines), 10.78 (s, H, -NH, Carbamoyl).

Compound ***IId***

$C_{24}H_{27}O_2N_3$; Mol wt. 389; Solubility: Methanol; m.p 126-128°C; R_f : 0.9 (Hexane; Ethylacetate →4:3); ^{1}H NMR-(CD_3OD) δppm: 0.6-0.9(t, 3H, CH_3 of Propyl), 1.1-1.2(m, 2H, CH_2 of Propyl), 1.4-1.5(m, 2H, CH_2 of Propyl), 2.26(s, 6H, CH_3 groups at 2nd & 6th position), 3.1-3.4(t, H), 6.81(t, 5H, C_6H_5), 7.3-7.4(m, 5H, C_6H_5), 8.75(s, -H, -NH DHP), 10.78(s, -H, -NH Carbamoyl).

Compound ***IIe***

$C_{25}H_{29}O_2N_3$; Mol wt. 403; Solubility: Methanol; m.p 143-148°C; R_f : 0.94 (Hexane; Ethylacetate →4:3); ^{1}H NMR-(CD_3OD) δppm: 0.6-0.9(t, 3H, CH_3 of Butyl), 1.1-1.2(m, 2H, CH_2 of Butyl), 1.29-1.31(m, 4H, $2CH_2$ of Butyl), 2.26(s, 6H, CH_3 groups at 2nd & 6th position), 3.1-3.4 (t, H), 6.81(t, 5H, C_6H_5), 7.3-7.4(m, 5H, C_6H_5), 8.75(s, -H, -NH DHP), 10.78(s, -H, -NH Carbamoyl)

PHARMACOLOGICAL EVALUATION FOR ANTIMITOTIC ACTIVITY

1. Using Germinating Bengal gram seeds

A Bengal gram seed of a good quality was taken and soaken overnight with water to hasten the germination process. The next day, the seeds were distributed in a group of 10 each in Petri dishes on moistened filter paper. Drug solutions were prepared in 1% DMSO at concentrations ranging from 125-1000µg/ml and added to the filter paper the Petri dishes. One Petri dish served as DMSO control and one served as Paclitaxol (Positive) control. The seeds were allowed to germinate for 7days and care was taken to moisten the filter paper with control and drug solutions for every 24hours. The length of the radicals was measured in cm at the end of 7th day and % mean values of the DMSO (control) treated and %inhibition in growth is calculated. The values are plotted on a graph[11,12].

2. Using germinating Onions (Allium cepa)

Onions *(Allium cepa)* of a good quality were taken and hasten the germination process. The next day the bulbs were distributed in a group of two each in Petri dishes on moistened filter paper. Drug solutions were prepared in 1% DMSO at concentrations ranging from 125-1000µg/ml and added to the filter paper in the Petri dishes. One Petri dish served as solvent control, and one served as Paclitaxol (Positive)

control. The bulbs were allowed to germinate for 7days and care was taken to moisten the filter paper with control and extracts every 24hours. The length of the radicals was measured in cm at the end of 7th day and percentage mean values of the control treated and percentage inhibition in growth is calculated the values are plotted on a graph.

RESULTS

All the 1, 4 Dihydropyridines derivatives were synthesized according to the standard procedure as mentioned in the scheme. In all the cases completion of reactions were confirmed by TLC & characterized with the help of spectral data (IR, ^{1}NMR). All the derivatives were purified by column chromatography. Melting points of the compounds were measured using open capillary tube.

Antimitotic activity of the derivatives showing good cytotoxic activity was done on germinating Allium cepa and Bengal gram seeds derivatives Ib, Ic, Id, Ie, IIa, IIb, IIc, IId tested for activity. Paclitaxol 10µg/ml was taken as positive control. The compounds were solubilized in solvent. Solvent was taken as control. The values of percentage inhibition of growth at various concentrations are shown in *fig. 1-4.*

Among all the 1, 4-dihydropyridine derivatives carbamoyl derivatives shown better antimitotic activity than carboethoxy derivatives.

Among all the carboethoxy derivatives of 1, 4-dihydropyridine, 4-butyl 3, 5-bis carboethoxy 2, 6-dimethyl 1, 4-dihydropyridine have shown better activity.

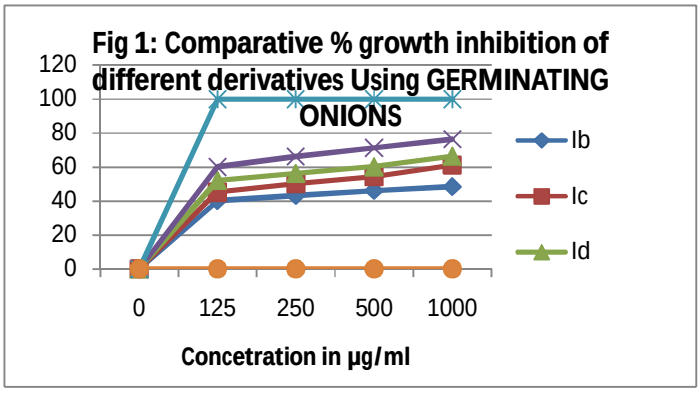
Among all the 1, 4-dihydropyridine derivatives 4-Butyl 3, 5- Biscarbamoyl 2, 6-dimethyl – 1,4-DHP shown significant activity.

DISCUSSION

1, 4 – Dihydropyridines were prepared by Hantzsch synthesis exclusively by microwave irradiation method. In 1882 Hantzch reported the first synthesized of 1, 4 – DHP. The classical method for the synthesis of 1,4 – Dihydropyridines is a one-pot condensation of an aldehydropyridines is a one-pot condensation of an aldehyde with ethyl acetoacetate, and ammonia either in acetic acid or refluxing in alcohol. However, the yields of 1,4-dihydropyridines obtained by this method are generally low. Recently the solvent free synthesis of 1,4-DHP was reported. However this method required more time for the synthesis especially when electron withdrawing group present on aromatic ring. With these observation and continuous research for the synthesis of 1, 4 – Dihydropyridines derivatives we have proposed an efficient and versatile method for the preparation of 1,4 – Dihydropyridines that provides scope for further improvement towards milder reaction conditions and improved yields.

CONCLUSION

In the present study all the proposed carboethoxy (Ia, Ib, Ic, Id, Ie) and Carbamoyl (IIb, IIc, IId, IIe) derivatives of 1, 4-dihydropyridines were synthesized and their antimitotic activity was determined. Compounds IIa, IIb, IIc, IId demonstrated good antimitotic activity when compared to that of respective parent compound and the control. Out of all the derivatives Carbamoyl derivative IIe demonstrated significant activity when compared to the all other derivatives and control.



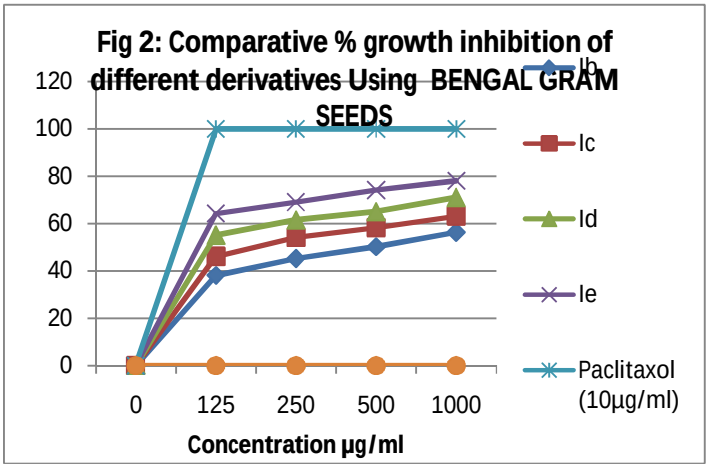


Fig 2: Comparative % growth inhibition of different derivatives Using BENGAL GRAM SEEDS
120
100
80
60
40
20
0
0
125
250
500
1000
Concentration µg/ml
Ib
Ic
Id
Ie
Paclitaxol (10µg/ml)

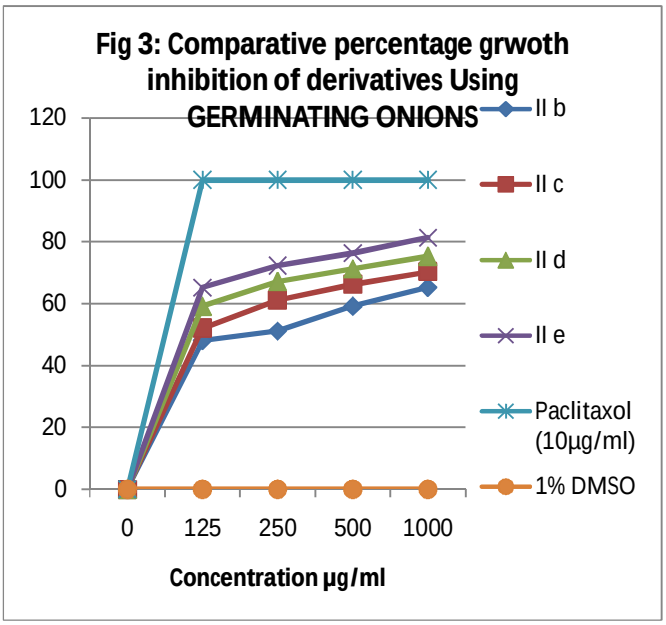


Fig 3: Comparative percentage grwoth inhibition of derivatives Using GERMINATING ONIONS
120
100
80
60
40
20
0
0
125
250
500
1000
Concentration µg/ml
II b
II c
II d
II e
Paclitaxol (10µg/ml)
1% DMSO

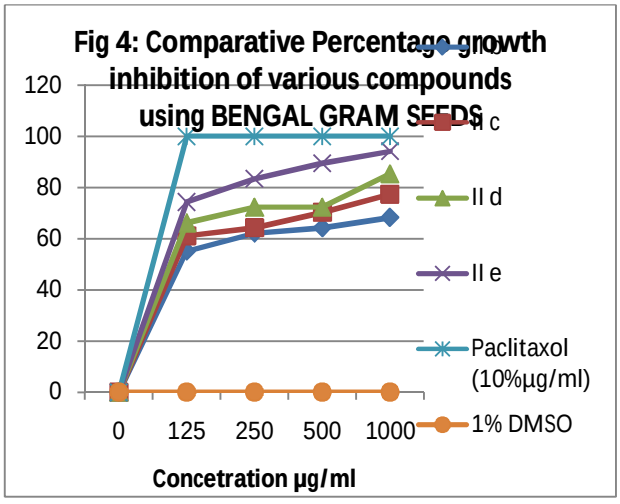


Fig 4: Comparative Percentage grwoth inhibition of various compounds using BENGAL GRAM SEEDS
120
100
80
60
40
20
0
0
125
250
500
1000
Concetration µg/ml
II c
II d
II e
Paclitaxol (10%µg/ml)
1% DMSO

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