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Synthesis and Evaluation of New 4-Aryl/Heteroaryl-2, 6-dimethyl-3,5-bis-N-(arylcarbamoyl)-1, 4-dihydropyridines as Pharmacodynamic Agents - Part-I

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Seven new 4-aryl/heteroaryl-2, 6-dimethyl-3, 5-bis-N-(2-methyl phenyl) carbamoyl-1, 4-dihydropyridines were prepared through one-pot synthesis starting from (2-methylphenyl) acetoacetamide using appropriate aromatic aldehydes and liquid ammonia. These compounds were purified and characterised by their analytical and spectral data. Pharmacological screening of the new 1,4-dihydropyridines was carried out for CNS depresant (anticonvulsant and analgesic) and cardiovascular (inotropic and blood pressure) activities by standard methods. Quite a few of them were found to be active.

VOLTAGE-GATED ion channels are a family of integral membrane proteins that conductions (Na^+, K^+, Ca^+) selectively and effectively across the cell membrane. Their activity is regulated by activator and inhibitor drug molecules that bind to specific sites on the channel proteins[1,2]. Among the voltage-gated ion channels, one of the most widely studied is the L-type calcium channel which has separate binding sites for the three major chemical classes of clinically used calcium channel antagonist drugs, 1,4-dihydropyridines (Nifedipine), phenylalkylamines (Verapamil) and benzothiazepines (Diltiazen)[3].

Calcium channel blockers are increasingly becoming drugs of primary line of treatment. These agents reduce vascular resistance by blocking calcium entry through slow calcium channel but also decrease ventricular contractility by the same mechanism. Although felodipine and several other DHP derivatives have been reported to have enhanced vascular selectivity[4,5], calcium channel blockers have a limited role in the treatment of heart failure[6-9].

STRUCTURE activity relationship studies of 1,4-dihydropyridines[10] revealed the following: 1) Good activity is generally observed with those dihydropyridines having a cyclic substituent in the 4-position. The most active compounds were the 4-ortho-substituted phenyl derivatives. Activity usually decreases as ortho-substitute is moved to the meta and even more so when moved to the para-position on the phenyl ring.

2) Activity is generally independant of the electronegativity of the substituent on the phenyl ring.
3) Substitution on the nitrogen usually decreases potency.
4) Of the substituents studied at the 2,6-positions, 2,6-dimethyl substitution is the best; and
5) Replacement of 3,5-carboxy groups by other electron-withdrawing substituents produces a marked decrease in activity, but, of these the 3,5-diacetyl compounds generally were the most active. There is a little difference in potency between the methyl and ethyl esters.

Recent literature[11] revealed that dihydropyridines represented by nifedipine found to potentiate the morphine-induced analgesia measured in the hot-plate, but not in the tail-flick test. In another experiment, nifedipine also produced a significant degree of antagonism of the 5-HT response. The anticonvulsant activity of a voltage-dependent calcium channel antagonist, nifedipine was also investigated on chemically induced-convulsions. It antagonised the convulsions (ED_{50} : 6.5 mg/kg)[12]. Quite recently[13],

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a few of the 1,4-dihydropyridine derivatives are also reported to possess antiasthmatic activity by reducing *in vitro* lipoperoxidation and *in vivo* experimental hyper-reactivity and cell in filtration.

In view of such an enormous and wide Pharmacological importance of 1,4-dihydropyridines, it has been felt worthwhile to synthesize some new analogs possessing some important structural features as envisaged in SAR studies with a view to evaluate them for their varied pharmacological activities. As a first step in this direction, the synthesis of a series of new 1,4-dihydropyridines containing methyl groups at 2,6-position and a methylphenyl substituent as a carboxamido group at 3,5-positions with a variable 4-substituent has been taken-up. An attempt, has been made to synthesize the title compounds by one-pot method as outline in Scheme-1.

For this purpose, the required N-(2-methylphenyl) acetoacetamide (I) has been prepared by the condensation of o-toluidine with ethyl acetoacetate in alcohol. The same has been subjected to a reaction with an appropriate aromatic aldehyde (II) and excess of ammonia. A single product has been obtained in each of such reaction and characterized as the corresponding 4-aryl/heteroaryl-2, 6-dimethyl-3, 5-bis-N-(2-methylphenyl) carbamoyl-1,4-dihydropyridine (V), on the basis of their analytical and spectral (IR and PMR) data.

IR spectrum (KBr) exhibited characteristic absorption bands at (in cm^{-1}) : 3200 (dihydropyridine NH), 3350 (amide-NH), 1685 (amido-C=0), 2980 (C-H of CH_3) 1580 (C=C).

PMR spectrum (in $CDCl_3$) showed characteristic absorption peaks at (in δ, ppm): 1.98 (s, 6H, two CH_3 2.5); 2.20 (s, 6H, two Ar-CH_3), 4.79 (s, 1H, C_4-H), 5.54 (br, 1H, D_2O exchangeable-NH), 6.8 to 7.9 (m, 13H, Ar-H) and 8.6 (s, 2H, D_2O exchangeable; two CO-NH).

EXPERIMENTAL

All the melting points were determined in open capillaries using Toshniwal melting point apparatus and are uncorrected. Purity of the compounds was checked on TLC (neutral alumina) plates. IR spectra of the compounds were recorded on Perkin-Elmer-282 Infra-red spectrophotometer as KBr pellets and PMR spectra on Jeol FX-900 spectrophotometer using TMS as an internal standard.

N-(2-Methylphenyl) acetoacetamide was obtained by a simple condensation of ethyl acetoacetate with o-toluidine, in alcohol and identified by its physical and analytical data.

Synthesis of 4-Aryl/heteroaryl-2,6-dimethyl-3,5-bis-N-(2-methyl-phenyl)carbamoyl-1,4-dihydropyridines (V) - General procedure:

N-(2-Methylphenyl) acetoacetamide (I; 0.05 mole) was dissolved in alcohol (25 ml) and an appropriate aromatic/heteroaromatic aldehyde (II; 0.025 mole) was added followed by the addition of excess of ammonia (25%). The reaction mixture was stirred for 10 min and then heated under reflux for 10-12 h on a water-bath. Alcohol was removed from the reaction mixture to the possible extent and cooled. The resultant product was filtered, washed with small portions of alcohol and dried. It was purified by recrystallization from hot alcohol.

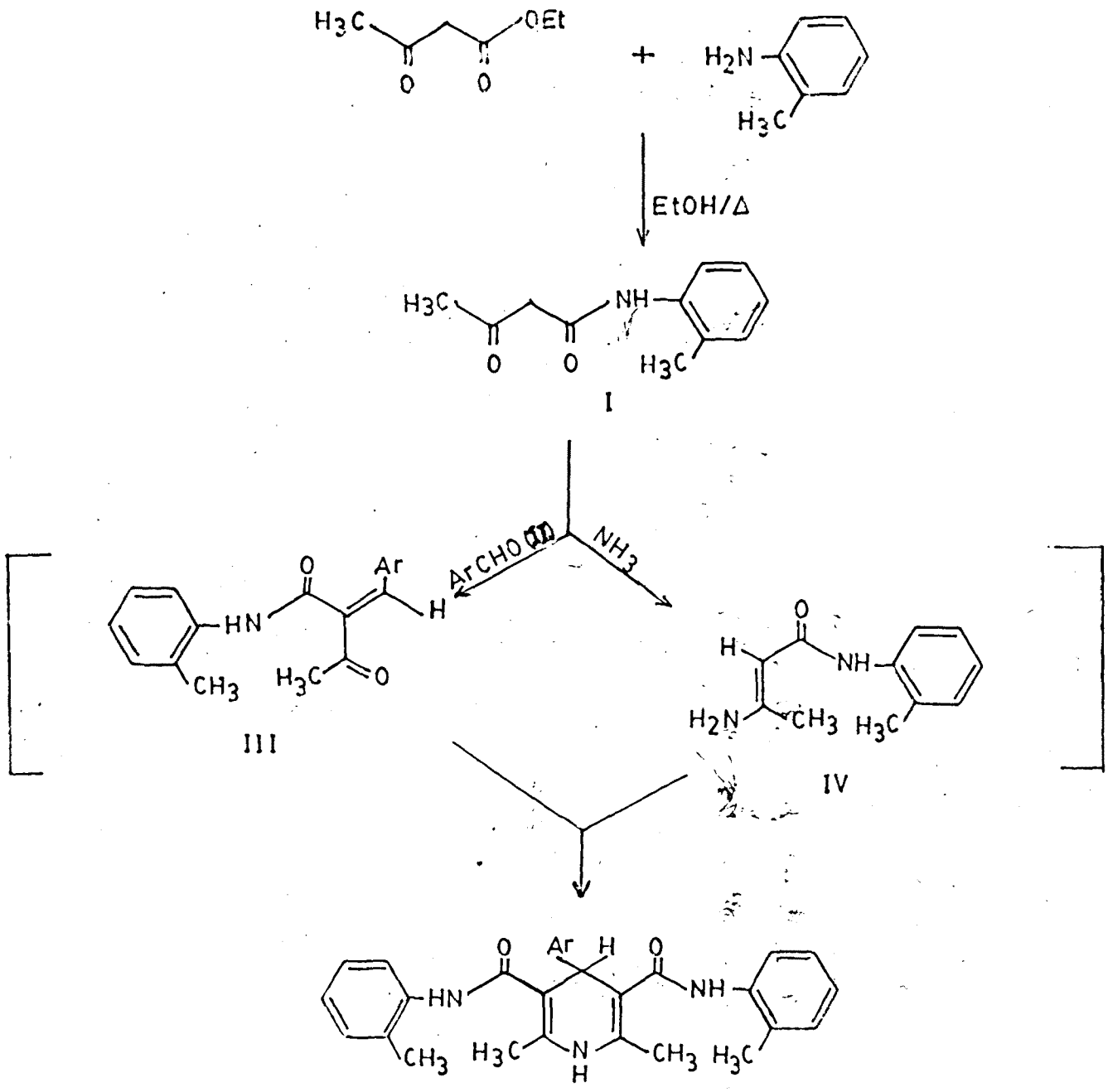
Following this procedure seven different dihydropyridines were prepared and characterised. Their physical and analytical data are presented in Table-1.

PHARMACOLOGICAL STUDIES

Acute Toxicity studies (LD_{50} values) : All the seven new 1,4-dihydropyridines were subjected to acute toxicity studies by the method described by Turner[14] using albino mice (six in each group). Graded doses of the test compounds as 1% Tween-80 solution were administered intraperitoneally and the results are presented in Table-2.

Potentiation of Pentobarbitone Sodium-induced Sleep: Since the gross bahavioural studies of the compounds indicated them to cause CNS depression, their effect on pentobarbitone sodium-induced sleep was studied in albino mice (six in each group) by the method suggested by Seth et al[15] at a dose of 5 mg/kg (bw) i.p. as 1% Tween-80 solution. The percent increase in sleeping time was determined and recorded in Table-2.

Anticonvulsant Activity: Anticonvulsant activity of the new 1,4-dihydropyridines was assayed at a dose of 5 mg/kg (bw), i.p. by the method of Toman *et al*[16] in albino mice (six in each group using the electroconvulsiometer. The average response time (in sec., Stupor) is noted and presented in Table-2.



SCHEME - 1

Analgesic Activity: On noting that these new 1,4-dihydropyridines are CNS depressant, their analgesic activity was assayed in albino mice (six in each group) adopting Eddy's hot-plate analgesiometer method[17]. Their average response time (in sec) was recorded at 0, 15, 30, 60 and 120 min and the last two values are presented in Table-2.

Cardiovascular Activity: In view of the structural features, the test compounds were evaluated for the following actions on the Cardiovascular system.

a) Effect on Isolated Perfused Frog's Heart: It was studied using the conventional method described by Burn[18], at a dose of 10 and 50 mcg of each of the compounds. Since

Table 1: Physical and analytical data of 4_Aryl/Heteroaryl-2,6-di-methyl-3,5-bis-N-(2-methylphenyl) carbamoyl-1,4-dihydropyridines(V)

Sl. No.	Compd* No.	Substituent Ar	m.p. (°C)	Mol. formula	% Nitrogen** Found (calcd)
1.	Va	Phenyl	234	$C_{29}H_{29}N_3O_2$	9.20(9.31)
2.	Vb	2-Nitrophenyl	218	$C_{29}H_{28}N_4O_4$	11.38(11.29)
3.	Vc	2-Hydroxyphenyl	224	$C_{29}H_{29}N_3O_3$	9.12(8.99)
4.	Vd	3,4-dimethoxyphenyl	118	$C_{31}H_{33}N_3O_4$	8.00(8.20)
5.	Ve	4-Hydroxy-3-methoxyphenyl	220	$C_{30}H_{31}N_3O_4$	8.58(8.45)
6.	Vf	4-N, N-Dimethylaminophenyl	108	$C_{31}H_{34}N_4O_2$	11.41(11.33)
7.	Vg	2-Furyl	208	$C_{27}H_{27}N_3O_3$	9.30(9.52)

* Yield ranged from 56% to 82%.

** Satisfactory C&H analyses were also recorded.

Table 2. Pharmacological Activities of 4-Aryl/Hetero-Aryl-2,6-dimethyl-3,5-bis-N-(2-methylphenyl)-1,4-dihydropyridines(V)

Compd.	% potentiation of sleeping time	Analgesic Activity average response time(sec) 1h	2h	Anticonvulsent Activity average response time (in sec)	% Reduction in B.P.	LD_{50} i.p (mg)
Va	115.0	13.0	12.6	118.0	15	25.0
Vb	75.2	5.9	6.0	119.2	20	20.7
Vc	62.9	8.1	8.5	118.0	27	21.0
Vd	96.0	5.5	6.4	102.0	15	28.4
Ve	73.8	6.2	5.9	120.0	10	38.0
Vf	98.7	6.1	5.8	118.0	15	41.5
Vg	48.0	11.0	11.2	106.0	10	34.5
Control	—	4.6	4.5	126.0	00	—
Morphine Sulphate	—	15.0	15.0	94.6	—	—
Nifedipine	78.9	10.1	9.6	110.4	39	—

observations revealed no significant effect, the results are not shown.

b) Effect on Blood Pressure of Anesthetized dog: It was carried out as per the methods suggested by Burn[18] and

Harris[19]. Each of the compound was administered in the form of its solution (1% in Tween-80) through the femoral vein and the blood pressure (in mm/Hg) before and after the administration was observed and only the differences were shown in Table-2. Three dogs were used for each group.

RESULTS AND DISCUSSION

Persual of Table-2 indicates that the compound Vf with p-N, N-dimethyl aminophenyl group is relatively less toxic and compounds Ve and Vg with 4-hydroxy-3-methoxyphenyl and 2-furfuryl are next in the order. Whereas, compounds Vb and Vc with 2-nitrophenyl and 2-hydroxyphenyl group, respectively were found to be relatively more toxic. Gross behaviour studies indicated the compounds to be CNS depressants.

Results show all the seven compounds to increase the pento-barbitone-induced sleeping time. The sleep potentiating effect of compound Va with a phenyl group was found to be the maximum and compound Vf being the next. Compounds Vg and Vd were found to protect the test animals against convulsions to the same extent as that of nifedifine. Results of analgesic activity by hot-plate method reveal compounds Va (phenyl) and Vg (2-furyl) to be moderately potent when compound with morphine sulphate employed as the standard. The test compounds did not show any significant effect on isolated perfused frogs heart.

Data showing the effect of the compounds on carotid blood pressure in anaesthetized dogs as presented in Table-2 indicate that all the seven compounds lower the carotid blood pressure.

The effect is highest in the compounds Vb and Vc having 2-Nitro-phenyl and 2-Hydroxyphenyl groups.

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