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Note

Synthesis and pharmacological screening of some 1,4-dihydropyridine and their derivatives for anticonvulsant activity

Shashikant R Pattan*[1], S S Purohit[1], V P Rasal[2], S Mallya[3], S C Marihal[3], A B Khade & M S Paschapur[2]

[1]Dept of Medicinal Chemistry, K L E S's College of Pharmacy, Belgaum 590 010, India.
[2]Dept of Pharmacology, K L E S's College of Pharmacy, Belgaum 590 010, India.
[3]Dept of Pharmacology, Goa College of Pharmacy, Panjim, Goa, 403001, India.

E-mail: shashipattan@yahoo.com

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A new series of 1,4-dihydropyridine and their derivatives have been synthesized and the structures of the compounds have been confirmed by IR and NMR. The title compounds are evaluated for anticonvulsant activity by maximal electroshock method. Some of these compounds have been found to exhibit excellent anticonvulsant activity.

Keywords: Anticonvulsant, antihypertensive, 1,4-dihydropyridine.

1,4-Dihydropyridine[1,2] are well known as calcium channel blockers and have emerged as one of the important classes of drugs for the treatment of hypertension[3]. Recently reported studies have shown that compounds possessing 1,4-dihydropyridine nucleus possess variety of biological activities including antimicrobial agents[4], myocardial infarction[5], neuroprotectant[6]. Epileptic seizures have been known to represent an occasional discharge in the nervous tissue[7], characterized by recurrent paroxysmal changes in the neurological functions caused by abnormalities in the electrical activity of the brain, infact epileptiform burst are often associated with influx of calcium ions in to nerve cells and a decrease in the extracelluar concentration of calcium precedes the onset of seizures in the many experimental models[8].

Anticonvulsant therapy however is neither universally effective nor invariably safe. Moreover blockers of voltage dependant Ca^{2+} channels display anticonvulsant activity in various models of experimental convulsions and in humans[9]. The above examples and instances demonstrate the potential use of novel 1,4-dihydropyridine derivatives as a source of valuable drug candidates for anticonvulsant activity[10]. In the present work nine new 1,4-dihydropyridine derivatives were synthesized and characterized **(Table I)** and evaluated for their anticonvulsant activity.

Experimental Section

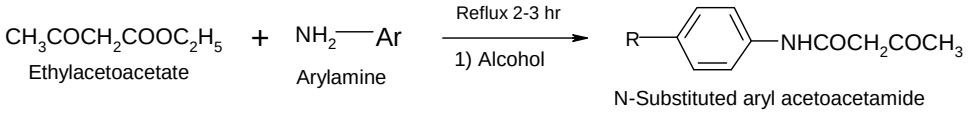
All melting points were determined in open capillary method and are uncorrected. IR spectra were recorded on Thermo Nicolet IR 200 spectrophotometer using KBr disc method. Purity of the compounds was checked on silica Gel TLC plates. ^{1}H NMR spectra (DMSO-d_6) were recorded on BRUKER amx-400 MHz using TMS as internal standard (chemical shift in δ ppm). Combustion analysis data here found to be within the limits of permissible errors.

General method for preparation of N-substituted aryl acetoacetamide 3[11,12]. An equimolar amount of ethyl acetate **1** and different aryl amine **2** were taken in a round bottom flask and dissolved in alcohol and refluxed for about 2-3 hr. The reaction mixture was cooled. The solid that separated out was filtered, washed with cold water and dried. The crude solid of anilide **3** was purified by recrystallization twice from appropriate solvent to give colourless crystals. IR (KBr): 3449 (NH), 3270 (CH-CH), 1701 (CONH), 1672 (C=O),1457 (C-N), 836, 748 cm^{-1} (CH=CH). **(Scheme I)**

General method for preparation of 1,4-dihydropyridine. N-aryl (substituted) acetoacetamide (0.01 mole) was dissolved in methanol and an appropriate aldehyde (0.05 mole) was added followed by the addition of excess of ammonia (25%). The reaction mixture was mechanically stirred for 10 min. and then heated on water bath under reflux for 10-12 hr. Methanol was removed under reduced pressure and cooled. The product thus separated was filtered and washed with methanol. It was purified by recrystallization from alcohol to give yellowish crystalline compound. IR (KBr): 3546 (OH), 3243 (CH-CH aromatic str.), 1646 (C=O), 559 (C=N), 832, 746 cm^{-1} (CH-CH def.)

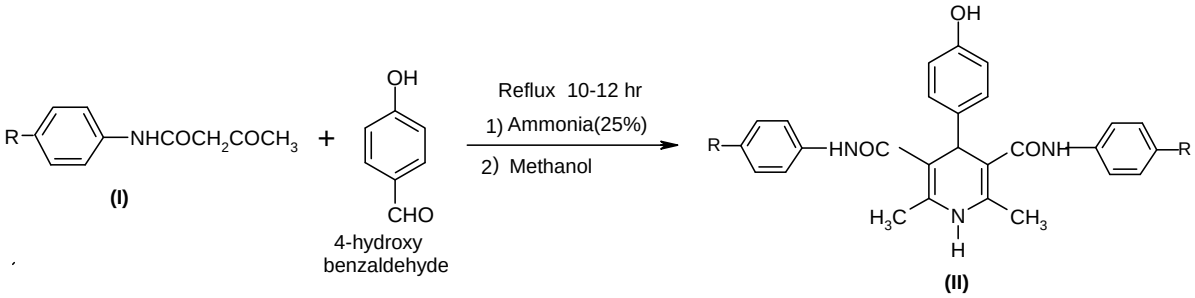
General method for preparation of 1,4-dihydro-2,6-dimethyl- 4-{4-[3-(piperidine / morpholine / 2-aminopyrazine/1-amino-4-methylpiperazine)-2-hydroxypropoxy]-phynyl}-pyridine-3,5-carbamoyl

STEP-I



(I)

STEP-II



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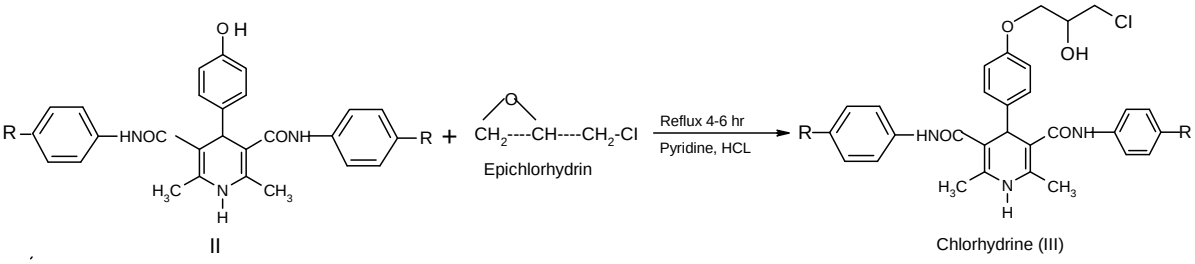

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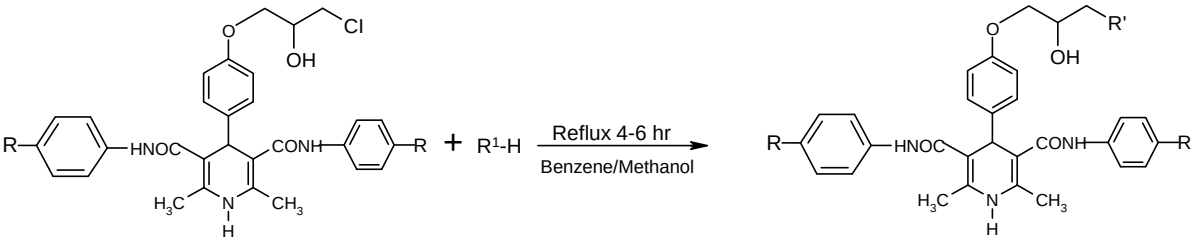


Scheme I

C_1 - C_9. A mixture of 1, 4-dihydropyridine (**4,** 0.01 mole) and 1-chloro-2,3-epoxypropane (25 mL) was refluxed on a water bath in the presence of basic catalyst, pyridine, for about 4-6 hr. The crude epoxide **5** separated was added into an equal volume of chloroform and excess of conc. HCl (10 mL) and a mixture was stirred for 30 min. The chloroform layer that separated out was washed with small amount of ice cold water to remove the excess of HCl. The organic layer was dried (Na_2SO_4) and the resulting chlorhydrine **6** compound was refluxed with 2-aminopyrazine/1-amino-4-methylpiperazine **7** in benzene about 6 hr. The resulting solution was concentrated under reduced pressure, the mixture was then poured into ice cold water, when solid separated out, it was filtered and recrystallized from ethanol to afford brownish crystals. The physical constants are recorded in **Table I**.

C_1: IR (KBr): 3450 (N-H), 3239 (O-H amide), 2947 (C-H ar.), 1777 (C=O), 821 (C-N), 1596 cm^{-1} (NO_2) ; ^{1}H NMR: δ 6.5-7.8 (12H, m, Ar.CH), 1.9 (6H, 2 CH_3), 2.7 (4 H, 2 CH_2 scuccinamide), 2.5 (1 H, O-H), 8.0 (2 H, 2 CONH amide), 3.76 (2H, O-CH_2), 3.67 (2H, N-CH_2), 9.9 (1H, NH).

Table I — Characterization data of compounds **(C_1-C_9)**

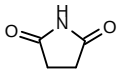
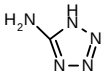
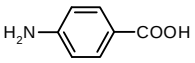
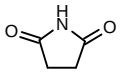
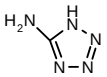
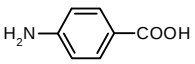
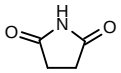
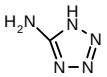
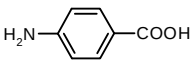
Compd..	R	R^1	Molecular formula	Mol. Wt.	m.p. °C	Yield %	Rf Value	Elemental analysis C	H	N
C_1	p-NO_2		$C_{34}H_{32}N_6O_{10}$	684	236	30	0.61	59.65 (59.40	4.71 5.00	12.27 12.45)
C_2	p-NO_2		$C_{31}H_{30}N_{10}O_8$	670	239	25	0.52	55.52 (55.15	4.51 4.61	20.89 20.49)
C_3	p-NO_2		$C_{37}H_{34}N_6O_{10}$	722	195	20	0.55	61.49 (61.79	4.74 4.52	11.63 11.80)
C_4	m-NO_2		$C_{34}H_{32}N_6O_{10}$	684	229	25	0.54	59.65 (59.45	4.71 4.95	12.27 12.35)
C_5	m-NO_2		$C_{31}H_{30}N_{10}O_8$	670	199	35	0.49	55.52 (55.42	4.51 4.33	20.89 20.48)
C_6	m-NO_2		$C_{37}H_{34}N_6O_{10}$	722	209	27	0.49	61.49 (61.91	4.74 4.61	11.63 11.21)
C_7	m-Cl		$C_{34}H_{32}Cl_2N_4O_6$	663	216	30	0.52	61.54 (61.81	4.86 4.61	8.44 8.25)
C_8	p-Cl		$C_{31}H_{30}Cl_2N_8O_4$	649	228	30	0.42	57.32 (57.65	4.66 4.98	17.25 17.91)
C_9	p-Cl		$C_{37}H_{34}Cl_2N_4O_6$	701	229	25	0.61	63.34 (63.95	4.88 4.61	7.99 7.65)

Table II — Anticonvulsant activity

Groups	Time (sec) in various phases of convulsions (Mean±SEM) Flexion	Extension	Clonus	Stupor	Recovery
Control	8.000±0.2582	12.33±0.6667	4.833 ±0.4014	23.83±0.7923	287.5±1.784
Standard	6.500±0.3416*	6.500±0.3416**	3.833 ±0.6009***	4.167±0.4773**	99.67±1.745**
C_1	4.167±0.4014**	8.333±0.3333**	3.500 ±0.4282***	6.167±1.014**	118.2±4.269**
C_2	2.667±0.2108**	8.167±0.3073**	4.833 ±0.3073***	5.333±0.4944**	76.83±1.493**
C_3	2.833±0.4014**	8.667±0.4944**	5.167 ±0.3073***	2.667±0.3333**	105.7±1.358**
C_4	3.167±0.4773**	8.667±0.5578***	3.667 ±0.6667***	3.167±0.4773**	84.17±2.868**
C_5	3.167±0.4773**	8.833±0.6009**	3.667 ±0.6667***	3.167±0.4773**	83.17±2.182**
C_6	3.500±0.4282**	8.333±0.4944**	3.833 ±0.6009***	3.667±0.4944**	82.67±3.007**
C_7	3.167±0.4773**	8.833±0.6009**	3.667 ±0.4216***	3.667±0.4944**	81.67±2.996**
C_8	3.000±0.2582**	9.000±0.5774**	3.500 ±0.4282***	2.833±0.4014**	127.3±1.453**
C_9	3.500±0.5627**	8.500±0.5627**	4.333 ±0.6667***	3.667±0.6667**	84.67±3.106**

Note: *P<0.05, **P<0.01, ***P>0.05.

C_2 : IR (KBr): 3479 (N-H), 2932 (C-H Ar.), 3283 (O-H), 1594 (C=N), 1282 (C-N), 1060 (C-O), 1511 (NO_2), 994 cm^{-1} (C-H Ar.); ^1H NMR: δ 7.0-7.8 (12H, m, Ar. CH), 1.2 (6H, 2 CH_3), 4.0 (1H, C-NH 5-amino-tetrazole), 2.5 (1H, O-H), 8.1 (2H, 2 CONH amide), 4.1 (2H, O-CH_2), 3.8 (2H, N-CH_2), 9.9 (1H, NH).

C_3: IR (KBr): 3363 (O-H), 3460 (N-H), 2968 (C-H ar), 1441 (C-O), 3229 (O-H carboxylic), 1515 (NO_2), 1672 (CONH amide), 1127 cm^{-1} (C-H def Ar.) ; ^1H NMR: δ 7.0-7.9 (16H,m. Ar. CH), 2.7 (6H, 2 CH_3), 3.9 (C-NH, PABA), 2.1 (1H, O-H), 8.1 (2H, 2 CONH amide), 3.7 (2H, O-CH_2), 3.6 (2H,N-CH_2), 9.9 (1H, NH).

C_4: IR (KBr): 3076 (N-H), 3163 (O-H), 2955 (C-H Ar), 1710 (C=O), 1294 (C-N), 1415 (C=C), 1773 (CONH amide), 1587 (NO_2 Ar.), 935, 1241 cm^{-1} (C-H def Ar.); ^1H NMR: δ 7.0-7.5 (12H,m, Ar. CH), 1.8 (6H, 2 CH_3), 2.5 (4H, 2 CH_2 succinamide), 2.9 (1H, OH), 7.8 (2 H, 2 CONH amide), 4.2 (2H, O-CH_2), 3.7 (2H,N-CH_2), 9.9 (1H, NH).

C_5: IR (KBr): 3191 (N-H), 3441 (O-H), 3084 (C-H Ar), 1492 (NO_2), 1551 (C=C), 1671 (CONH amide), 992, 1092 cm^{-1} (C-H Ar.)

C_6: IR (KBr): 3367 (N-H), 3462 (O-H), 2880 (C-H Ar), 1524 (NO_2), 1673 (CONH amide), 1424 (C-O), 1257 cm^{-1} (C-H Ar.)

C_7: IR (KBr): 3184 (N-H), 3367 (O-H), 2951 (C-H Ar), 1709 (C=O), 1597 (C=C), 732 (C-Cl), 1773 (CONH amide), 1225, 1193 (C-H def Ar.) ^1H NMR: δ 6.6-7.4 (12H,m, Ar. CH), 1.4 (6H, 2 CH_3), 1.8 (4H, 2 CH_2 succinamide), 2.75 (1H, OH), 7.9 (2H, 2 CONH amide), 4.2 (2H, O-CH_2), 3.8 (2H,N-CH_2), 9.9 (1H, NH).

C_8: IR (KBr): 3334 (O-H), 3282 (N-H), 2932 (C-H Ar), 1679 (CONH amide), 1513 (C=C), 723 (C-Cl), 1445 (C-N), 1649 (C=N), 827, 994 (C-H def. Ar.); ^1H NMR: δ 6.9-7.6 (12H, Ar. CH), 1.6 (6H, 2 CH_3), 3.9 (1H, C-NH 5-aminotetrazole), 2.6 (1H, OH), 7.9 (2H, 2 CONH amide), 4.7 (2H, O-CH_2), 4.3 (2H,N-CH_2), 9.9 (1H, NH).

C_9: IR (KBr): 3275 (N-H), 3372 (O-H), 2663 (C-H ar), 1680 (CONH amide), 1526 (C=C), 774, 669 (C-Cl ar.), 1423 (C-O), 839, 1109 cm^{-1} (C-H def. Ar.); ^1H NMR: δ 7.0-7.8 (16H, Ar. CH), 4.2 (1H, C-NH PABA), 1.2 (1H, OH), 7.9 (2H, 2 × CONH amide), 3.7 (2H, O-CH_2), 3.4 (2H,N-CH_2), 9.9 (1H, NH).

Anticonvulsant activity[13]

The Maximal Electroshock (MES) method was used to induce the convulsions. The stimulus was applied via corneal or ear clip electrodes and the current shock of 150 mA for 0.25 seconds (since animals used were rats) applied and the convulsions were observed. The convulsions of animals of different groups in different stages were compared. **(Table II)**.

Result and Discussion

The title compounds were assessed for their anticonvulsant activity by subjecting the animals (rats) to Maximal Electroshock (MES) test and the convulsions shown by an animal in different stages viz flexion, extension, clonus, stupor and recovery. Convulsions of animals in different groups were compared.

All the compounds synthesized **(C_1-C_9)**, showed promising anticonvulsant activity. Compounds **C_1**, **C_4** and **C_8** have shown the maximum anticonvulsant activity when compared with the standard drug. The results were calculated by subjecting the animals (mice) to Maximal Electroshock (MES) test and there was reduction in time of different phases of convulsions in test groups compared to control group.

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