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Synthesis and *in-vitro* screening of 3,4-dihydro-pyrimidin-2(1*H*)-one derivatives for antihypertensive and calcium channel blocking activity

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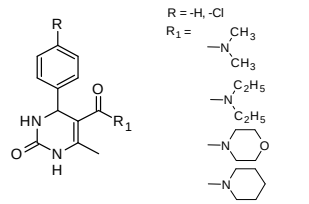
ABSTRACT

Pyrimidine plays a significant role among other heterocycles. From the literature survey, in recent years the design of 3,4-dihydropyrimidin-2(1*H*)-ones nucleus have been attracted for considerable interest because of their therapeutic and pharmacological properties. Pyrimidine nucleus was synthesized by Biginelli reaction. This product was subjected for alkaline ester hydrolysis and these derivatives on treatment with thionyl chloride and substitution by different secondary amines produced final desired compounds. The remaining compounds have been synthesized by above method. The purity of the compounds has been checked by TLC monitoring and the conformation of structure was confirmed by different spectra like UV, IR, NMR, Mass etc. The in-vitro antihypertensive and calcium channel blocking activity have been done by IC50 measurement method with nifedipine as standard.

Key words: 3,4-dihydropyrimidin-2(1*H*)-ones, Biginelli reaction, Antihypertensive and Calcium channel blocking activity, IC50, Nifedipine.

INTRODUCTION

In recent scenario heterocycles play a major role in drug synthesis. In that respect pyrimidine plays a significant role among other heterocycles. From the literature survey, in recent years 3,4-dihydropyrimidin-2(1*H*)-ones have attracted considerable interest because of their therapeutic and pharmacological properties. Several of them have been found to exhibit a wide spectrum of biological effects including antimicrobial, antitumor, antiviral, anti-inflammatory, antihypertensive, calcium channel blocker, alpha-1a adrenergic antagonist and neuropeptide antagonist (Atwal et al., 1990, Rovnyak et al., 1992, Kumar et al., 2002). Dihyropyrimidine is a bioisoster of Dihydropyridine which shows very good calcium channel blocking activity and antihypertensive activity. So in order to make effective antihypertensive drugs and having minimum of side effects it has been designed to make new derivatives of dihydropyrimidine-2-one having amide linkage. For the above purpose following compounds were planned to synthesize.



6-methyl, 3,4-dihydropyrimidin-2(1H)-one derivatives

(6a-6h) Molecular Deisgn

EXPERIMENTAL

The entire chemicals were supplied by S.D. Fine Chem. (Mumbai), Finar Chem. Ltd (Ahmedabad) and Loba Chemie Pvt. Ltd. (Mumbai). Melting points were determined by open tube capillary method and were uncorrected. Purity of compounds was checked by thin layer chromatography (TLC) on silica gel G in solvent system hexane-ethyl acetate (1:2), the spots were located under iodine vapours or UV light. IR spectra of all compounds were recorded on FT-IR 8400S Shimadzu spectrophotometer using KBr. Mass spectra were obtained using 2010EV LCMS Shimadzu instrument.

General procedure for ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro pyrimidine-5-carboxylate

0.1mole (6gm) of urea, 0.1mole (10.2ml) benzaldehyde and 0.1mole (12.6ml) of ethyl acetoacetate were taken and sufficient quantity of ethanol was added as reaction medium and refluxed at 70-80ºC temperature for 4 hrs then after cooling the reaction mixture and after addition of water the precipitate was obtained which was filtered and recrystallized from ethanol. **(4a-4b)** (Ravikumar et al., 2005, Pathak et al., 2006, Purohit et al., 2009).

General procedure of 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro pyrimidine-5-carboxylic acid

Ethyl 6-methyl- 2-oxo -4-phenyl-1 ,2,3,4- tetrahydro pyrimidine-5-carboxylate (2.60gm, 0.01mole) and 50ml of 10% alcoholic NaOH was refluxed for 1 hr. Then after cooling the reaction mixture and on acidification with conc.HCl precipitate of acid was obtained, which was filtered, washed with water and recrystallized from ethanol. **(5a-5b)** (Patil et al., 2009, Chhabria et al., 2007, Furniss et al., 1998).

General procedure of preparation of 6-methyl-4-phenyl-3,4-dihdropyrimidin-2(2H)-one derivatives

6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro pyrimidine-5-carboxylic acid (2.3gm, 0.01mole) and add 15ml of thionyl chloride were refluxed for 30mins. Unreacted thionyl chloride was removed by heating reaction mixture on water bath. The acid chloride product was treated with 3 to 4 times of different amines and ethanol as reaction medium and stirred for 5 hrs. It was then added into cold water to get the precipitate, which was filtered and recrystallized from alcohol. **(6a-6h)** (Heda et al., 2009, Mishra et al., 2008, Singh et al., 2008).

Biological evalution

Pharmacological evaluation is a crucial thing to ensure the activity of the compounds. In this era, the prevalence of heart diseases has increased to a great extent. Antihypertensive agents are among the most commonly used to treat the variety of heart diseases. Literature review revealed that substituted dihydropyrimidine containing compounds show different biological activities. These compounds are also evaluated for their antihypertensive activity, calcium channel blocking activity.

Scheme of Synthesis

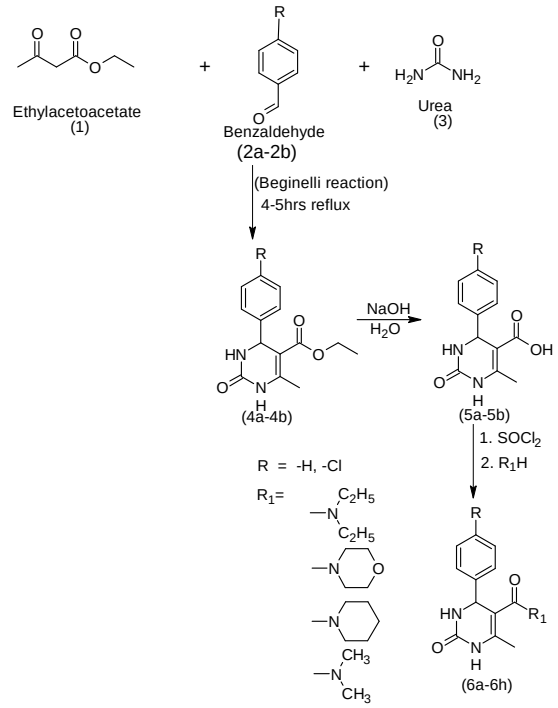


Table-1: Physical Characteristics of Synthesized Compounds.

Compound code	R	R_1	Molecular Formula	Molecular Weight (g/mol)	Melting Point (°C)	Yield (% w/w)	R_f Value
4a	H	$-OC_2H_5$	$C_{14}H_{16}N_2O_3$	260.28	198-202	80.00	0.57
4b	Cl	$-OC_2H_5$	$C_{14}H_{15}N_2O_3Cl$	294.73	210-214	73.00	0.60
5a	H	-OH	$C_{12}H_{12}N_2O_3$	232.23	198-202	49.00	0.33
5b	Cl	-OH	$C_{12}H_{11}N_2O_3Cl$	266.68	166-170	42.00	0.36
6a	H	$-N(CH_3)_2$	$C_{16}H_{17}N_3O_2$	259.30	176-180	73.78	0.65
6b	H	$-N(C_2H_5)_2$	$C_{16}H_{21}N_3O_2$	287.35	180-184	70.54	0.62
6c	H	-(4-morpholinyl)	$C_{17}H_{21}N_3O_2$	301.34	170-174	65.00	0.55
6d	H	-piperidinyl	$C_{14}H_{16}N_2O_3$	299.36	168-172	68.50	0.53
6e	Cl	$-N(CH_3)_2$	$C_{16}H_{16}N_3O_2Cl$	294.9	190-194	67.00	0.64
6f	Cl	$-N(C_2H_5)_2$	$C_{16}H_{20}N_3O_2Cl$	321.80	194-198	65.00	0.60
6g	Cl	-(4-morpholinyl)	$C_{16}H_{18}N_3O_3Cl$	335.78	176-180	55.00	0.58
6h	Cl	-piperidinyl	$C_{17}H_{20}N_3O_2Cl$	333.81	198-202	58.00	0.58

Mobile phase: (Hexane: Ethyl acetate 1:2)

Measurement of Antihypertensive activity

Purpose and rationale

There are various *in-vivo* and *in-vitro* methods are available for evaluation of antihypertensive activity. Antihypertensive activity was performed by *in-vitro* method in which effect of test compounds on blood pressure is measured. (Naik et al., 2007).

Procedure

Heparin at the dose of 2000 IU/ kg by I.V. route has been administered to rats of either sex. Rats of either sex have been anaesthetized with Pentothal sodium 80 mg/kg given by intraperitoneally. Blood pressure transducer was calibrated initially by using of mercury manometer. For each rat, the carotid artery was cannulated and attached to blood pressure transducer to record the initial arterial blood pressure which will be calibrated initially by using of mercury manometer. In the similar way on the opposite

side, the jugular vein was cannulated to administer 0.3ml heparinised saline for checking normal flow of fluid in the vein then the different doses of test samples were used to measure the effect on blood pressure by inhibition of adrenaline response. (Humle et al., 2008).

Calcium antagonism in the isolated rat ileum

Purpose and rationale

Contraction of ileum was induced by adding potassium chloride & calcium chloride to the organ bath containing slightly modified Tyrode solution (NaCl=8.0gm/l, KCl=0.2gm/l, $CaCl_2$=0.18gm/l, NaH_2PO_4=0.1gm/l, $MgCl_2$=0.1gm/l, Glucose=1.0gm/l, $NaHCO_3$=1.0gm/l). Test drugs with calcium channel blocking activity have a relaxing effect (Mohamed et al., 2007).

Procedure

The assembly was being set up and arrangement was made for experiment. The animal kept for overnight fasting was stunned by a sharp blow on head the head and sacrificed by cutting neck blood vessels. The abdominal cavity was quickly opened and a piece of ileum was isolated. It was placed in a petridish containing tyroide solution maintained at 37˚C. The mesentery of ileum was removed and the interior contend was washed by blowing Tyrode solution (NaCl=8.0gm/l, KCl=0.2gm/l, $CaCl_2$=0.18gm/l, NaH_2PO_4=0.1gm/l, $MgCl_2$=0.1gm/l, Glucose=1.0gm/l, $NaHCO_3$=1.0gm/l) with help of pipette. The tissue was mounted in mammalian organ bath and connected to isotonic frontal writing lever. The tissue was allowed to stabilize for 30min. The responses of acetylcholine were taken till the maximum effect was obtained. The normal Tyrode solution was changed with Tyrode containing test solution. The responses of acetylcholine were taken with same dose and continued till maximum effect obtained.

The percentage of relaxation from the test-drug, pre-contracted level was calculated for each concentration of test compound. An *IC*50 was calculated by linear regression analysis:

$$y = 96.18x + 1.372$$

If y = 50%, then x = 0.5ml dose

IC_{50} of nifedipine = dose for 50% inhibition* conc. ÷ bath capacity = (0.5ml) × (1000µg/ml) ÷ 25ml = 20µg/ml

CONCLUSION

All the eight synthesized compounds (6a-6h) were screened for antihypertensive and calcium channel blocking activity. Nifedipine was used as standard reference drug for screening of antihypertensive and calcium channel blocker because nifedipine and test compounds both have similar bioisosteric nucleus. In the test samples (dihydropyrimidine ring) there are two nitrogen (N) atoms which is bioisosteric with (CH) and one methyl group (CH_3) which is bioisosteric with ketone (C=O) of nifedipine (dihydropyridine ring).

Table-2: Spectral data of synthesized compounds

Compound code	UV (λmax, nm)	IR (υ, cm^{-1})	Mass (m/z)	NMR (δ, ppm)
4a	285	3290 (-NH), 1691, 1680 (-C=O) 1427 (-CH_3 deformation), 1280 (C-O)	260.9 [M^+]	
5a	256	3184 (-NH), 1680 (-C=O) 1400 (-CH_3 deformation), 1244 (C-O), 3000-3400 (OH)	232 [M^+], 218 [M-CH_3]	
6a	387	3221 (-NH), 1691, 1680 (-C=O) 1427 (-CH_3 deformation), 1552 (C=C Ph), 1280 (C-O)	259 [M^+]	
6b	380	3269 (-NH), 1677, 1690 (-C=O) 1440 (-CH_3 deformation), 1552 (C=C Ph)	288.9 [M^+]	7.2-8.2 (m, 6H, Ar**H**), 5.4 (d, 1H, N**H**), 5.9 (s, 1H, N**H**), 1.15 (t, 6H, C**H**$_3$), 4.0 (q, 4H, N**H**), 2.3(s, 3H, C**H**$_3$)
6c	389	3100 (-NH), 1689 (-C=O)	301.4 [M^+]	
6d	366	3240 (-NH), 1677, 1652 (-C=O) 1427 (-CH_3 deformation), 1552 (C=C Ph)	299.3 [M^+]	
4b	284	C-Cl (825.48), C=O (1647, 1700), -CH_3 deformation (1489), C-O (1226), NH (3200)	---	
5b	274	C-Cl (825.48), C=O (1677), -CH_3 deformation (1489), C-O (1234), NH (3226), OH(broad) (3200-3300)	267 [M^+]	
6e	282	C-Cl (825.33), C=O (1685, 1634), -CH_3 deformation (1488), C-N (1226), NH (3190, 3224)	294.8 [M^{+1}]	7.3-7.8 (m, 5H, Ar**H**), 6.8 (d, 1H, N**H**), 6.9 (s, 1H, N**H**), 1.2 (s, 3H, C**H**$_3$), 2.9 (s, 6H, C**H**$_3$)
6f	270	C-Cl (829.33), C=O (1667, 1647), -CH_3 deformation (1488), C-N (1226), NH (3139)	322.8 [M^{+1}]	
6g	272	C-Cl (829.33), C=O (1674), -CH_3 deformation (1474), C-N (1234), NH (3097, 3217)	335.7 [M^+]	
6h	280	C-Cl (825), C=O (1647, 1700),-CH_3 deformation (1488), C-O (1226), NH (3251)	333.8 [M^+]	7.4-7.8 (m, 5H, Ar**H**), 6.2 (s, 2H, N**H**), 1.2 (s, 3H, C**H**$_3$), 1.4-2.4 (m, 10H, C**H**$_2$)

The ester (-COO-) linkage of nifedipine has been replaced by amide (-CONH-) linkage in the test compounds.

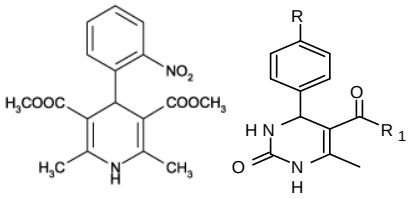


Table: 3 Screening of Antihypertensive activity

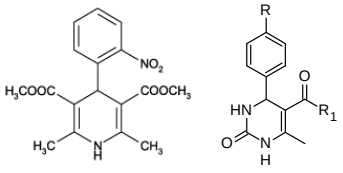
Compound code	Dose (ml)	Control (mm Hg) (H)	Test (mm Hg) (h)	% Inhibition in blood pressure
Nifedipine	0.3	29.17	20.00	31.44
	0.3	28.34	20.84	26.46
6a	0.3	29.17	24.17	17.14
	0.3	30.00	23.34	22.20
6b	0.3	27.50	21.67	21.20
	0.3	29.17	22.50	22.87
6c	0.3	29.17	20.00	31.44
	0.3	30.00	21.67	27.77
6d	0.3	29.17	25.00	14.30
	0.3	29.17	25.84	11.16
6e	0.3	29.17	24.17	17.14
	0.3	29.17	23.84	18.27
6f	0.3	28.34	22.50	20.61
	0.3	27.50	24.17	12.10
6g	0.3	28.34	22.50	20.61
	0.3	28.34	24.17	14.71
6h	0.3	28.34	25.00	11.79
	0.3	27.50	22.50	17.14

Table: 4 Screening of Calcium Channel Blocking activity of Nifedipine

Compound	Dose (ml)	Control (cm) (H)	Test (cm) (h)	% Inhibition	IC_{50} (µg/ml)
Nifedipine	0.1	3.4	3.0	11.76	20
	0.2	3.4	2.7	20.58	
	0.3	3.4	2.3	32.35	
	0.4	3.3	2.1	35.29	
	0.5	3.3	1.7	48.48	
	0.6	3.3	1.2	61.76	

RESULTS AND DISCUSSION

All the eight synthesized compounds (6a-6h) were screened for antihypertensive and calcium channel blocking activity. Nifedipine was used as standard reference drug for screening of antihypertensive and calcium channel blocker because nifedipine and test compounds both have similar bioisosteric nucleus. In the test samples (dihydropyrimidine ring) there are two nitrogen (N) atoms which is bioisosteric with (CH) and one methyl group (CH_3) which is bioisosteric with ketone (C=O) of nifedipine (dihydropyridine ring). The ester (-COO-) linkage of nifedipine has been replaced by amide (-CONH-) linkage in the test compounds.

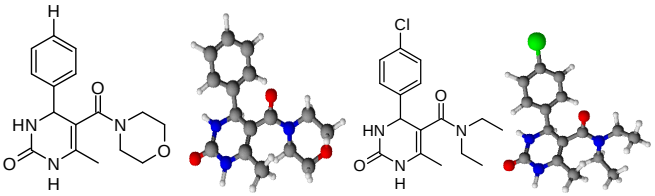


Nifedipine Synthesized molecule

Table: 5 Screening of Calcium Channel Blocking activity

Compound code	Dose (ml)	Control (cm) (H)	Test (cm) (h)	% Inhibition	IC_{50}
6a	0.1	3.3	3.0	9.09	22
	0.3	3.3	2.3	30.30	
	0.5	3.4	1.9	44.12	
6b	0.1	3.4	3.1	8.82	36.54
	0.3	3.4	2.5	26.47	
	0.5	3.3	2.4	27.72	
6c	0.1	3.3	3.1	6.06	21.06
	0.3	3.3	2.4	27.27	
	0.5	3.3	1.9	42.43	
6d	0.1	3.3	3.0	9.09	22
	0.3	3.4	2.1	38.23	
	0.5	3.3	2.0	41.18	
6e	0.1	3.4	3.0	11.76	21.10
	0.3	3.4	2.8	17.65	
	0.5	3.3	1.7	48.48	
6f	0.1	3.4	2.8	17.64	19.76
	0.3	3.4	2.2	35.29	
	0.5	3.4	1.7	50.00	
6g	0.1	3.4	2.5	26.47	28.99
	0.3	3.3	2.3	30.30	
	0.5	3.3	1.9	42.42	
6h	0.1	3.4	3.0	11.76	24.26
	0.3	3.4	2.4	29.41	
	0.5	3.4	2.0	41.18	

Compound **6c** was found to have better antihypertensive activity and compound **6f** found to have better calcium channel blocker activity.



6c **3D structure** **6f** **3D structure**

6c: 6-methyl-5-(morpholin-4-ylcarbonyl)-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-one (logP=0.8).

6f: 4-(4-chlorophenyl)-*N*,*N*-diethyl-6-methyl-2-oxo-1,2, 3,4-tetrahydropyrimidine-5-carboxamide (logP=2.80).

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