Medical Journal of the
Islamic Republic of Iran

Volume 18
Number 4
Winter 1383
February 2005

EFFECTS OF NOVEL 2-ALKYLAMINO-SUBSTITUTED DIHYDROPYRIDINES ON RABBIT JEJUNUM

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ABSTRACT

In order to investigate the effects of dimethylamino substituent at position 2 of the dihydropyridine nucleus on activity, starting from dialkyl 1,4-dihydro-2,6-dimethyl-4-(1-benzyl-2-alkylthio-5-imidazolyl)-3,5-pyridinedicarboxylates (5a-f) which their synthesis and effects as calcium channel antagonist on guinea-pig ileum has been reported previously, dialkyl 1,4-dihydro-2-[2-(dimethylamino)ethyl]-6-methyl-4-(1-benzyl-2-alkylthio-5-imidazolyl)-3,5-pyridinedicarboxylates (6a-f) were synthesized.

Rabbit jejunum was used to determine the relaxant or antagonistic activity of the test compounds. The test compounds (6c-e) inhibited the spontaneous contractile activity dose-dependently and completely, while high-K+ contracted tissues were relaxed partially.

MJIRI, Vol. 18, No. 4, 361-364, 2005.

Keywords: 2-Dimethylamino-substituted dihydropyridines, Rabbit jejunum, Calcium Channel.

INTRODUCTION

Structurally diverse groups of compounds are known to be effective as calcium antagonists. The most potent class of antagonists comprises of 1,4-dihydropyridines of which the widely known agent is nifedipine. This class of compounds have been the subject of many structure-activity relationship studies.[1]

Starting from dialkyl 1,4-dihydro-2,6-dimethyl-4-(1-benzyl-2-alkylthio-5-imidazolyl)-3,5-pyridinedicarboxylates (5) which their synthesis and effects as calcium channel antagonist on guinea-pig ileum has been reported previously[2] dialkyl 1,4-dihydro-2-[2-(dimethylamino)ethyl]-6-methyl-4-(1-benzyl-2-alkylthio-5-imidazolyl)-3,5-pyridinedicarboxylates[6] were synthesized as illustrated in Fig. 1 in order to investigate the effects of dimethylamino substitute at position 2 of the dihydropyridine nucleus on activity. In brief 1-benzyl-5-hydroxymethylimidazole[2] was synthesized from benzylamine hydrochloride.[1] Reaction of 2 with alkyl halides gave corresponding substituted alkylthioimidazoles.[3] Oxidation of 3 with manganese dioxide in chloroform afforded 2-alkylthio-5-formyl-1-benzylimidazoles.[4] Symmetrical dihydropyridines (5a-f) were synthesized by classical Hantzch condensation. Then dihydropyridines (5a-f) were reacted with paraformaldehyde and dimethylamine hydrochloride to give the title dialkyl 1,4-dihydro-2-[2-(dimethylamino)ethyl]-6-methyl-4-(1-benzyl-2-alkylthio-5-imidazolyl)-3,5-pyridinedicarboxylates (6a-f).

In the present studies rabbit jejunum was used to determine the relaxant or antagonistic activity of the test compounds (6c,d ,e). This test model, particularly allows testing relaxant, spasmolytic or antagonistic activity directly without the use of agonist,[3] of unknown compounds. The test samples were added in cumulative dose-fashion to obtain dose-response curves.[4] The dose-dependent inhibition of the spontaneous contractions by the test compounds led to study the calcium channel

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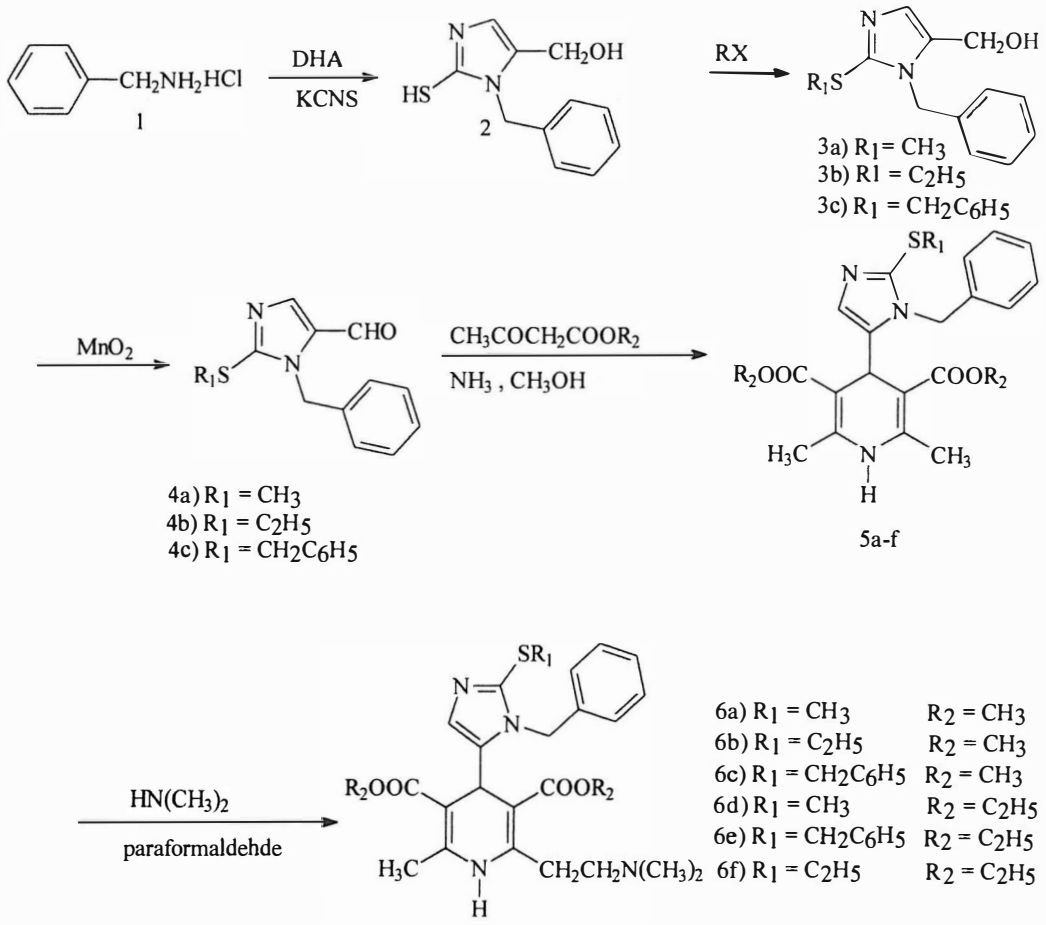

Fig. 1. Steps for synthesis of title dihydropyridines.

blocking (CCBs) activity.[5] The administration of high-K^+induced contraction of the smooth muscles by virtue of calcium influx into the cells from the extracellular spaces.[6] Calcium channel blockers tend to inhibit these induced contractions as a result of their inhibitory action at the calcium channel.[7]

So, pre-contracted rabbit jejunum was treated by the test compound in cumulative dose-fashion to obtain a dose-dependent inhibitory curve.[4] The test compounds inhibited the spontaneous contractile activity dose-dependently and completely, while high-K^+ contracted tissues were relaxed partially.

MATERIAL AND METHODS

Chemistry

Melting points were determined using the capillary apparatus with a system of Gallenkamp. 1H-NMR spectra were run on a Bruker AC-80 spectrometer. Infrared spectra were recorded on a FT-IR Perkin-Elmer Paragon 1000 spectrophotometer.

Compounds 2 to 5 were synthesized as described previously.[2] The developed procedure[10] is exemplified with the obtaining of 1,4-dihydro-2-methyl-6-[2-(dimethylamino)ethyl]-4-(1-benzyl-2-ethylthio-5-imidazolyl)-3,5-pyridinedicarboxylate dimethyl ester (6b).

1,4-dihydro-2-methyl-6-[2-(dimethylamino)ethyl]-4-(1-benzyl-2-ethylthio-5-imidazolyl)-3,5-pyridinedicarboxylate dimethyl ester (6b)

A solution of 5b (1.2g, 2.72mmoles), dimethylamine hydrochloride (0.33g, 4mmoles), paraformaldehyde (0.12g, 4mmoles) and 0.05ml of concentrated hydrochloric acid in ethanol (5mL) while protected from light was heated at reflux for 10h. The solvent was then evaporated and the residue was partitioned between hydrochloric acid (2M, 30mL) and ethyl acetate (15mL). The aqueous phase was separated, basified with aqueous ammonia , and extracted into diethyl ether (3 x 30mL). The extract was dried and evaporated and the residue was chromatographed to give 0.4g (30%) of 1,4-dihydro-2-methyl-6-[2-(dimethylamino)ethyl]-4-(1-benzyl-2-

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ethylthio-5-imidazolyl)-3,5-pyridine-dicarboxylate dimethyl ester (6b) as brown oil. IR (KBr) : 1704, 1690cm^{-1} (C=O); $^{-1}$H-NMR ($CDCl_5$): 7.65-6.92 (m, 7H, arom, H-C_4 imidazole, NH), 5.59(s, 2H, CH_2N), 5.3 (s, 1H, H-C_4 dihydropyridine), 3.6 (s, 6H, CH3O), 3.15-2.68 (m, 6H, CH_2), 2.5 (s, 6H, NCH_3), 2.34 (s, 3H, CH_3), 1.34 (t, 3H, CH_3).

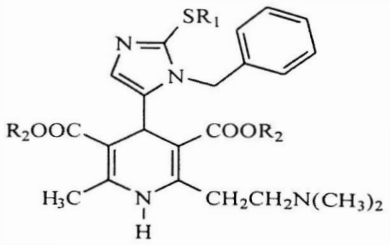
Evaluation of pharmacological activity

Male adult, healthy rabbits ranging from 1500±150 grams were purchased from the local market. Animals were fasted for 12 hrs but had free access to water before the day of the experiment. After cervical dislocation the jejunum was isolated, the adjacent tissues were removed, and 2-3 cm long pieces were cut and each piece was hanged diagonally in 10 mL organ bath. Tyrode's solution (composition in mM: KCl 2.7, NaCl 136.9, $MgCl_2$ 1.1, $NaHCO_3$ 0.4, $CaCl_2$ 108, Glucose5.6) was filled in the bath and aerated with carbogen (95% O_2 and 5% CO_2) at 37°C (thermostatically controlled). One end of the tissue was attached to a hook in the bath and the other one to force displacement transducer (FT.03) which was attached to a Polygraph, Grass model 7. A preload of 1.0 gram was applied to each tissue. After stabilizing the tissue with 3.10^{-7} M norepinephrine, the test compounds were added in cumulative dose-fashion. All these compounds were dissolved in DMSO. Five replicates of each compound were prepared to determine the effects on spontaneous activity of rabbit jejunum.

In the second set of experiments, calcium channel blocking activity was studied. 80 mM KCl was used to induce sustained contraction. At plateau the compounds were added in cumulative dose-fashion. Three replicates of each compound were prepared.

The prism pad was used to present the data in graphical form; the same software also calculated IC50 values. Student's t-test was employed and the level of significance was taken at $p<0.05$.

Table I. IC_{50} values of the test compounds 6c,d, e on spontaneous rabbit jejunum.



Compound	R_1	R_2	IC_{50}
6c	$CH_2C_6H_5$	CH_3	3.0×10^{-5}
6D	CH_3	C_2H_5	1.0×10^{-4}
6E	$CH_2C_6H_5$	C_2H_5	1.0×10^{-4}

RESULTS AND DISCUSSION

The effects of compounds 6c,d ,e in rabbit jejunum are presented in Fig1. The compound 6d,e showed dose-dependent inhibition while compound 6c inhibited sharply after the 3rd dose and then followed a dose-dependent pattern. Table I reports IC50 values of the test compounds comparatively; compound 1 is more potent in this tissue. The dose-dependent inhibition of spontaneous contractile activity by a test compound is a characteristic of a calcium channel antagonist.[8] So the results of the spontaneous tissue led to determine the calcium channel antagonistic activity. For this purpose, high-K^+ was used to induce sustained contraction in the tissues. A high-K^+ is known to cause the contraction in the smooth muscles due to entry of Ca^{++} into the cells through the voltage-dependent calcium channels (VDCs).[6,7] The cytoplasmic calcium $[Ca^{++}]_i$ is responsible to activate the contractile element in the smooth muscle preparations.[9] The test compound inhibiting high-K^+

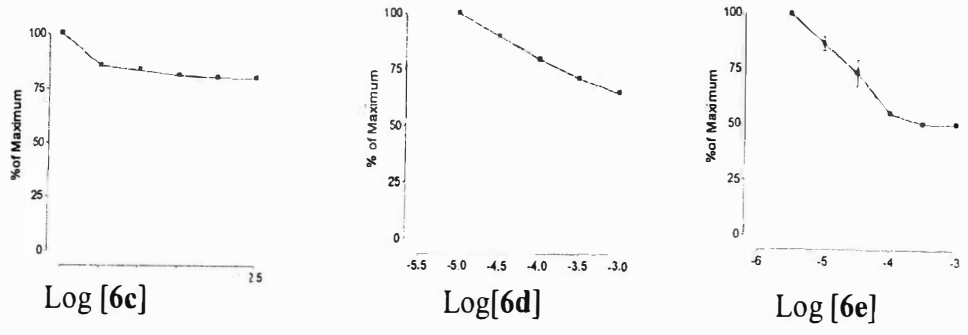


Fig. 2. Effect of compounds 6 c,d,e on spontaneous rabbit jejunum movements.

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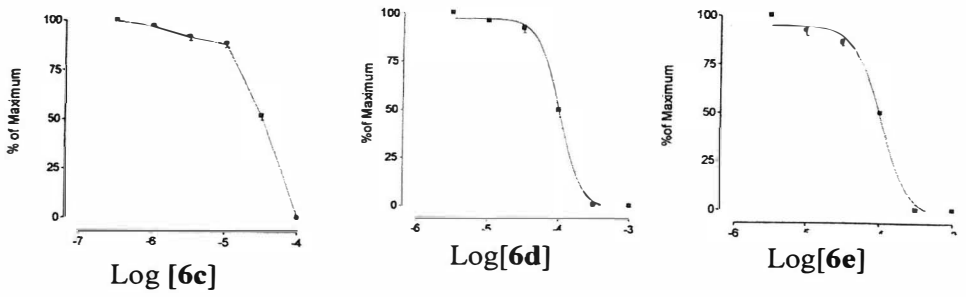

Fig. 3. Effect of compounds 6 c,d,e on K^+ -contracted rabbit jejunum.

induced contraction is thought to be a Ca^{++} antagonist.[5]

In the present studies compound 6c, d relaxed the pre-contracted tissue insignificantly, while compound 6e showed partial calcium channel blocking activity (Fig. 3).

Consequently, the tested compounds are likely to be K^+-channel openers, on the basis of the complete and dose-dependent inhibition of spontaneous contractile activity and partial relaxation of high-K^+ induced contraction. Further experiments are needed to explore the K^+-channel opener activity.

ACKNOWLEDGEMENT

The work was partially supported by a grant from the Research Council of Medical Sciences, University of Mashhad.

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