



Effects of 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 its activity, dialkyl 1,4-dihydro-2-[2-(dimethylamino) ethyl]-6-methyl-4-(1-benzyl-2-alkylthio-5-imidazolyl)-3,5-pyridinedicarboxylates (6a-f) were synthesized. The synthesis was started 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. Rabbit jejunum was used to determine the relaxant or antagonistic activity of the synthesized compounds. Some of the compounds (6c-e) inhibited the spontaneous contractile activity of jejunum, dose-dependently and completely, while high-K⁺ contracted tissues were relaxed partially.

Keywords: 2-Dimethylamino-substituted dihydropyridines; Rabbit jejunum; Calcium channel.

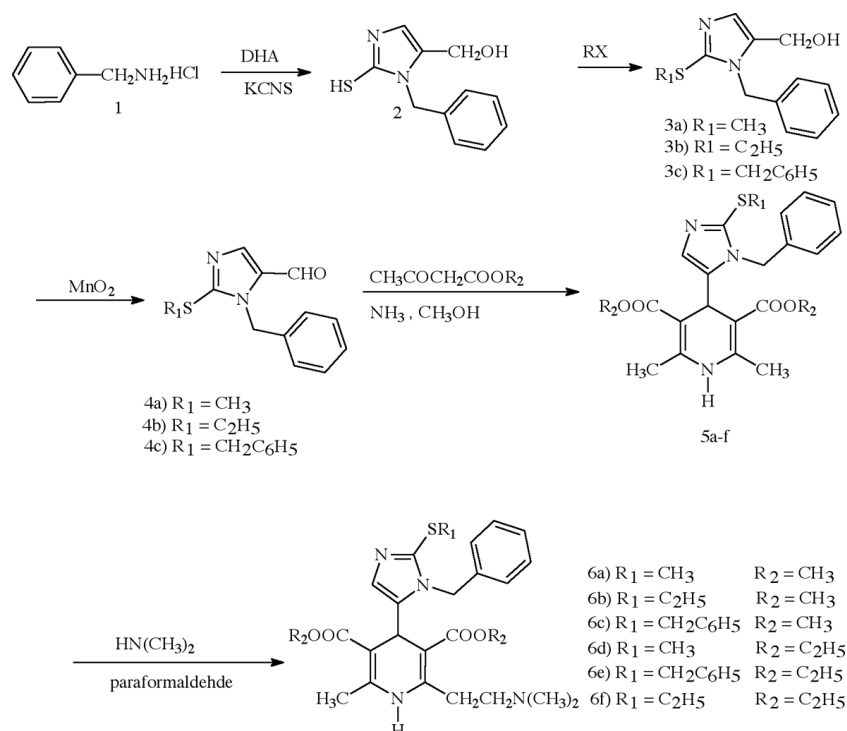
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1. Introduction

Structurally diverse groups of compounds are known to be effective as calcium antagonists. The most potent known class of antagonists are 1,4-dihydropyridines, which include the widely used nifedipine. This class of compounds has been the subject of

many structure-activity relationship studies [1]. In this study, we also synthesized several 2-alkylamino-substituted dihydropyridines, and investigated their calcium channel blocking activities. Starting from dialkyl 1,4-dihydro-2,6-dimethyl-4-(1-benzyl-2-alkylthio-5-imidazolyl)-3,5-pyridinedicarboxylates (5), whose synthesis and effects as calcium channel antagonist on guinea-pig ileum has been reported, previously [2], dialkyl 1,4-dihydro-2-[2-(dimethylamino)

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Scheme 1: 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 Hantzsch 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**).

ethyl]-6-methyl-4-(1-benzyl-2-alkylthio-5-imidazolyl)-3,5-pyridinedicarboxylates (**6**) were synthesized as illustrated in scheme 1.

Rabbit jejunum was used to determine the relaxant or antagonistic activity of the synthesized compounds (**6c-e**). This test model, particularly allows to examine the relaxant, spasmolytic, or antagonistic activities of unknown compounds, directly without the use of an agonist [3]. We report here that the synthesized compounds inhibited the spontaneous contractile activity dose-dependently and completely, while high-K⁺ contracted tissues were relaxed partially.

2. Materials and methods

2.1. 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 [4] 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**).

2.2. Synthesis of 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, 2.72 mmoles), dimethylamine hydrochloride (0.33 g; 4 mmol), paraformaldehyde (0.12 g, 4 mmol) and 0.05 ml of concentrated hydrochloric acid in ethanol (5 ml), while protected from

light, was heated at reflux for 10 h. The solvent was then evaporated and the residue was partitioned between hydrochloric acid (2 M; 30 ml) and ethyl acetate (15 ml). The aqueous phase was separated, basified with aqueous ammonia, and extracted into diethyl ether (3x30 ml). The extract was dried and the residue was chromatographed to give 0.4 g (30%) of 1,4-dihydro-2-methyl-6-[2-(dimethylamino)ethyl]-4-(1-benzyl-2-ethylthio-5-imidazolyl)-3,5-pyridinedicarboxylate dimethyl ester (**6b**) as a brown oil. IR (KBr): 1704, 1690 cm^{-1} (C=O); $^1\text{H-NMR}$ (CDCl_3): 7.65-6.92 (m, 7H, arom, H-C₄ imidazole, NH), 5.59(s, 2H, CH₂N), 5.3 (s, 1H, H-C₄ dihydropyridine), 3.6 (s, 6H, CH₃O), 3.15-2.68 (m, 6H, CH₂), 2.5 (s, 6H, NCH₃), 2.34 (s, 3H, CH₃), 1.34 (t, 3H, CH₃).

2.3. Evaluation of pharmacological activity

Male adult, healthy rabbits, ranging from 1500-1500 grams, were purchased from the animal house of Mashhad University of Medical Sciences. Animals were fasted for 12 h but had free access to water before the experiments. After cervical dislocation, jejunum was isolated, the adjacent tissues were removed, and 2-3 cm long pieces were cut. Each piece was hanged diagonally in a 10 ml organ bath filled with the Tyrode's solution (composition in mM: KCl 2.7, NaCl 136.9, MgCl₂ 1.1, NaHCO₃ 0.4, CaCl₂ 108, Glucose 5.6) and was aerated with carbogen (95% O₂ and 5% CO₂) at 37 °C. The con-

tractions were recorded with a model 7 Grass polygraph. A preload of 1.0 g was applied to each tissue. After stabilizing the tissue with 3×10^{-7} M norepinephrine, the test compounds were added in a cumulative dose-fashion. All of the compounds were dissolved in 10% DMSO and their effect on the spontaneous activity of the rabbit jejunum was determined. The experiment was repeated five times for each compound.

In a second set of experiments, calcium channel blocking activity of the compounds was studied. Eighty mM KCl was used to induce a sustained contraction. At plateau, the compounds were added in the cumulative dose-fashion. The experiments were repeated at least three times for each compounds. The Prism pad was used to present the data in graphical form; the same software also calculated I/C 50 values. Student t-test was employed and the level of significance was taken at p 0.05.

3. Results and discussion

The effects of compounds **6c**, **6d**, and **6e** in rabbit jejunum are presented in Fig1. The compound **6d** and **6e** showed a dose-dependent inhibition of jejunum movement, while compound **6c** sharply inhibited the movements after the third dose, and then followed a dose-dependent pattern. The results presented in Table 1 shows the IC₅₀ values of the test compounds, comparatively. Compound 1 is more potent in this tissue.

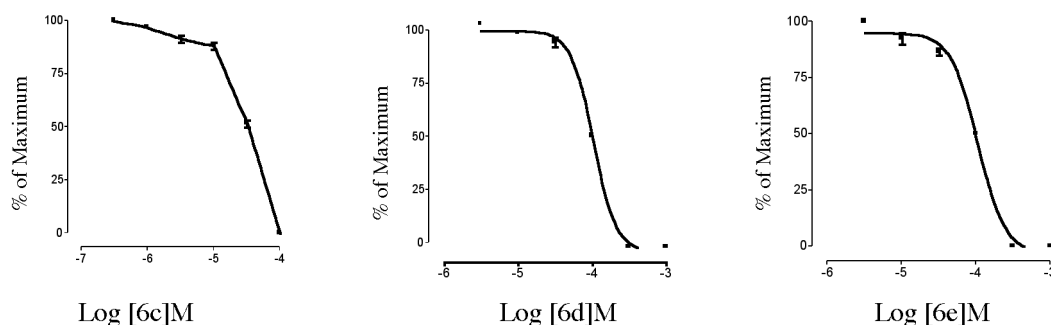


Figure 1. Effects of compounds **6c**, **6d**, **6e** on spontaneous rabbit jejunum movements.

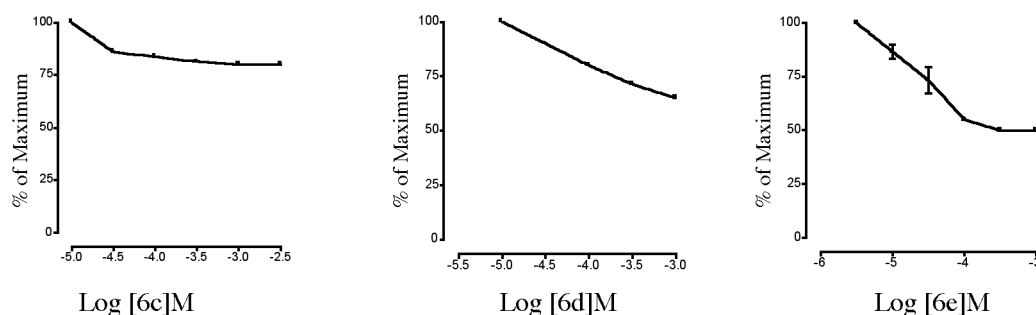


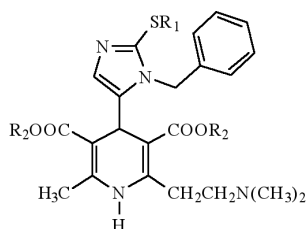
Figure 2. Effects of compounds **6c,d,e** on K^+ -contracted rabbit jejunum

The dose-dependent inhibition of spontaneous contractile activity by a test compound is a characteristic of a calcium channel antagonist [5]. To determine the calcium channel antagonistic activity of the synthesized compounds, 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 for activating the contractile element in the smooth muscle preparations [8]. Compounds which inhibit a high- K^+ induced contraction is thought to be a calcium channel antagonist [9].

As shown in Figure 1, compounds **6c** and **6d** did not relaxed the pre-contracted tis-

Table 1- IC_{50} values of 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}



sue significantly, while compound **6e** significantly relaxed the jejunum which suggest that it has partial calcium channel blocking activity.

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