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# Synthesis and Calcium Channel Blocking Activity of 4-Indolyl-1,4-dihydropyridines

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A series of indolyldihydropyridines has been designed, synthesized, and evaluated in order to show the effect of the indole moiety and the role of the methyl groups at the 2 and 6 positions of the dihydropyridine ring in the calcium channel blocking activity. The results seem to show that these groups constitute a critical structural requirement for the pharmacological action of these compounds. © 1997 Academic Press

## INTRODUCTION

Calcium channels play important roles in the electrical excitability, excitation–contraction coupling, stimulus secretion coupling, and other functions in many types of cells. Drugs that apparently block the movements of $Ca^{2+}$ through voltage-sensitive calcium channels are now widely used in the treatment of angina, cardiac arrhythmias, and hypertension (*1*). It has also been reported that calcium influx is connected with vascular smooth muscle contraction elicited by potassium. This influx essentially occurs through plasma membrane voltage operated $Ca^{2+}$ channels (*2*) and is blocked by $Ca^{2+}$ antagonists; among them the most potent and selective are 4-aryl-1,4-dihydropyridines (*3*). Since the discovery of the therapeutical properties of these substances, a large number of derivatives have been prepared and evaluated, resulting in the development of many useful drugs (i.e., nifedipine). The vast majority of these molecules have been prepared by Hantzsch synthesis (*4*) (or synthetic modifications), which normally implies the presence of methyl groups at the 2 and 6 positions of the dihydropyridine ring. Practically no information is available on the effect of these substituents on the pharmacological activity (*5*). Also lacking in the literature are indoles as aryl groups in these drugs. Recently we have been involved in the synthesis of 4-indolyl-1,4-dihydropyridines **1** (Scheme

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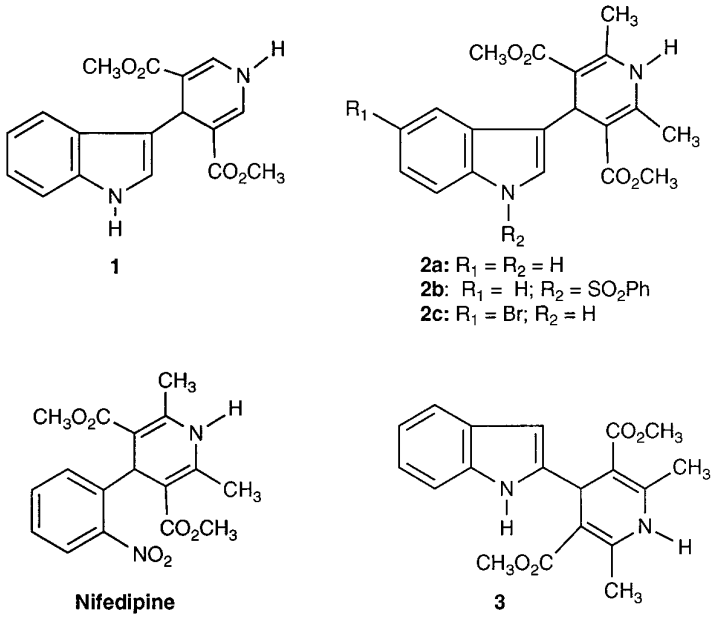


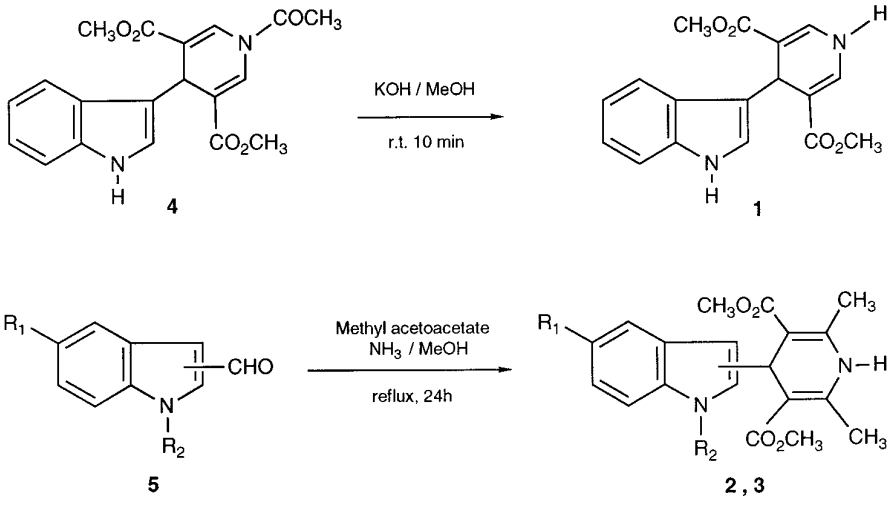

SCHEME 1

1) through a two-step sequence consisting of the addition of indoles to *N*-acylpyridinium salts followed by subsequent deprotection (*6*). Thus it seemed interesting to test the compounds and to compare their activity in front of the classical Hantzsch 4-phenyl-2,6-dimethyl-1,4-dihydropyridines.

## RESULTS AND DISCUSSION

Following our interest in the chemistry of *N*-alkyl-4-indolyldihydropyridines (*7*), we were able to prepare the *N*–H analogs by a two-step sequence involving the addition of indoles to *N*-acylpyridinium salts followed by mild deprotection of the amide function (*6*). Unfortunately, this procedure yielded dihydropyridine **4** in very low yield. The addition of a more powerful indole nucleophile (the lithium cuprate) was found to be useful for increasing the yield of this process. Treatment of *N*-acyldihydropyridine **4** with 1% KOH in methanol afforded the target compound **1** (Scheme 2). The 2-methylindole analog of **1** was prepared according to our published procedure (*6*). Reference compounds **2** (*8*) and **3** were prepared by Hantzsch synthesis by treatment of the corresponding indolecarboxyaldehyde, ammonia, and methyl acetoacetate. The compounds thus prepared showed espectroscopical and analytical data in agreement with their structures. They were stored under inert atmosphere ($N_2$ or Ar), protected from light at $-20°C$.

Potassium (80 mM) causes a biphasic contraction of rat vas deferens, and there




SCHEME 2

is a phasic response due to a burst of action potentials, followed by the tonic response during which the fast twitch response is inhibited and tension is maintained at a lower level. As these responses are blocked by calcium free conditions, it has been deduced that potassium mainly utilizes extracellular calcium for contraction (*9*). Therefore, it is well established that KCl-induced contractions are an appropriate model for investigating drugs that affect voltage-dependent calcium channels. For this reason, it was of interest to use this experimental model to study the putative blocking of calcium channels by the above-mentioned indole derivatives. In the isolated rat thoracic aorta, KCl induces a slow contraction that leads to an equilibrium after 20 min of depolarization. For this reason, all these measures were made at that time. Nifedipine (from 1 nM to 0.1 $\mu$M), **2a** (from 0.7 to 5 $\mu$M), **3** (from

TABLE 1
$IC_{50}$ Values Obtained for Nifedipine and Different Compounds in KCl-Induced Contractions in Rat Aorta Rings

| Compound | $IC_{50}$ ($\mu$M) | SE |
|---|---|---|
| Nifedipine | 2.6 E-3 | 0.1 E-3 |
| 2a | 2.65 | 0.19 |
| 2b | 3.65 | 0.53 |
| 3 | 12.19 | 2.16 |

*Note.* Data are mean ± SE from $n$ = 6–12 preparations.

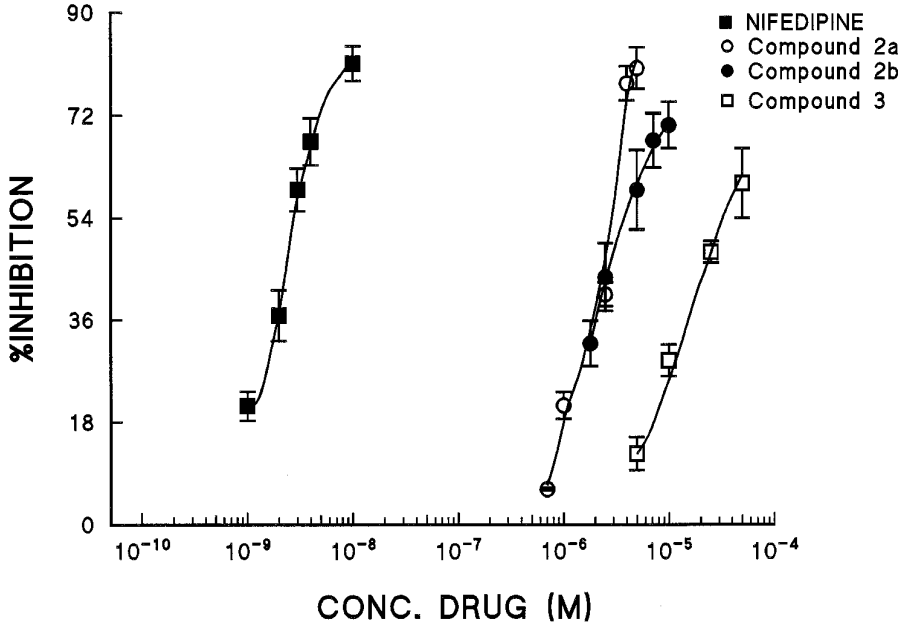



FIG. 1. Concentration–response curve of nifedipine and different indole derivatives on KCl-induced contractions in rat aortic rings.

5 to 50 $\mu$M), and **2b** (from 1.8 to 10 $\mu$M) inhibit in a concentration-dependent way the effect of KCl-induced contractions in isolated rat aorta, whereas the other compounds tested (**1, 4,** and the 2-methylindole analog of **1**) did not, or did so only at high concentrations (higher than 10 mM). The potency observed for these new derivatives according to their $IC_{50}$ values was **2a** > **2b** > **3** (Table 1 and Fig. 1).

Regarding the effect on KCl-induced contractions in rat vas deferens, nifedipidine (from 0.5 nM to 2 $\mu$M) reduces both the phasic and the tonic responses to KCl, being more selective for the second response as can be seen from their $IC_{50}$ values (Tables 2 and 3, Figs. 2 and 3). Similarly, **2a** (from 50 nM to 5 $\mu$M), **3** (from 5 to 50 $\mu$M), **2b** (from 50 nM to 25 $\mu$M), and **2c** (3 to 15 $\mu$M) inhibit, in a concentration-dependent manner, depolarizing responses induced in rat vas deferens (Table 2). The order of potency was **2a** > **2c** ≅ **2b** > **3,** as occurs in aortic rings. The compounds that showed no activity in aortic rings were unable to inhibit KCl-induced concentrations in rat vas deferens.

With respect to the recently described neuroprotection associated with $Ca^{2+}$ antagonists (*10*), dihydropyridines **1** and **2a** were able to inhibit the production of reactive oxygen species (ROS) by 35% at the maximum concentration used (100 $\mu$M). This antioxidant effect, although not directly related with the calcium channel blockage, could be considered as an additional mechanism of neuroprotection in ischemic brain damage. These experiments also complement the very scarce infor-

TABLE 2
$IC_{50}$ Values Obtained for Nifedipine and Different Compounds in KCl-Induced Phasic Contractions in Rat Vas Deferens

| Compound | $IC_{50}$ ($\mu$M) | SE |
|---|---|---|
| Nifedipine | 0.05 | 0.01 |
| 2a | 0.71 | 0.22 |
| 2b | 8.04 | 1.10 |
| 2c | 11.59 | 1.78 |
| 3 | 8.04 | 1.10 |

*Note.* Data are mean ± SE from $n$ = 6–12 preparations.

mation about dihydropyridines like **1,** without methyl groups at the 2 and 6 positions (*3, 5*).

## SUMMARY

To our knowledge, the results presented here clearly show that the presence of methyl groups at the 2 and 6 positions of the dihydropyridine ring is needed for effective blockage of calcium channels, because compounds lacking this structural feature are inactive. The tested 4-indolyl-1,4-dihydropyridines showed the same potency as nifedipine but lower efficacy in blocking the KCl-contractions in rat aorta and vas deferens. The inhibition of ROS production was also similar to that of nifedipine, being related to the antioxidant properties of dihydropyridines, regardless of their substitution pattern.

TABLE 3
$IC_{50}$ Values Obtained for Nifedipine and Different Compounds in KCl-Induced Tonic Contractions in Rat Vas Deferens

| Compound | $IC_{50}$ ($\mu$M) | SE |
|---|---|---|
| Nifedipine | 0.02 | 0.005 |
| 2a | 0.52 | 0.14 |
| 2b | 5.07 | 0.99 |
| 2c | 4.73 | 1.44 |
| 3 | 10.08 | 1.36 |

*Note.* Data are mean ± SE from $n$ = 6–12 preparations.