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Synthesis and Structure–Activity Analysis of Novel Dihydropyridine Derivatives to Overcome Multidrug Resistance

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Abstract—The structure–activity relationships were studied on newly synthesized 1,4-dihydropyridine derivatives possessing a 1-pentyl group at the 4-position, and 3-pyridylpropylester was found to be one of the effective fragments for overcoming P-glycoprotein mediated multidrug-resistance (MDR) in cultured human cancer cells, in vitro. 3-Pyridylpropylester was also found to be one of the effective fragments for increasing the life span of P-glycoprotein overexpressing MDR P388 leukemia-bearing mice, in vivo. All compounds had weak calcium antagonistic activities, but there appeared no relationship between MDR reversing effect and calcium antagonistic activity.

Development of multidrug-resistance (MDR) in tumor cells remains one of the major clinical obstacles during antitumor chemotherapy against many malignancies. The overexpression of P-glycoprotein with a molecular weight of 170 kDa encoded by human MDR-1 gene is frequently associated with acquisition of the MDR phenotype.[1] Drug accumulation is reduced in P-glycoprotein overexpressing cells through the enhanced efflux of anticancer agents.[2]

Development of potent MDR reversal agents has long been performed in many laboratories, and 1,4-dihydropyridine derivatives such as Nifedipine and Nicardipine were known to overcome MDR.[3] However, clinical use of such calcium antagonists remains a therapeutic problem because of their strong vasodilator activity, and new drugs with no calcium antagonistic activity are required to overcome MDR in cancer patients.

Many quantitative structure–activity relationship studies on 1,4-dihydropyridines have been reported, and a substituent (*ortho* or *meta*) in the phenyl ring at the 4-position was requisite for the strong calcium antagonistic activity.[4] We previously reported that NIK-250 possessing a thiodioxene ring at the 4-position with a moderate calcium antagonistic activity could successfully reverse MDR in vivo as well as in vitro through inhibition of P-glycoprotein function.[5] Furthermore, we have reported that N276-9, which possessed an alkyl chain at the 4-position, with much lower calcium antagonistic activity was also effective to overcome MDR (Fig. 1).[6]

In this study, we further examined both MDR reversal activity and the calcium antagonistic activity of novel 1,4-dihydropyridine derivatives possessing a 1-pentyl group at the 4-position and presented their structure–activity relationships.

Chemistry

1,4-Dihydropyridine derivatives were prepared by the Hantzch reaction (Scheme 1).[7] The mixture of capronaldehyde, acetoacetic acid 3-(4-diphenylmethylpiperadyl-1-yl) propylester and 3-aminocrotonic acid 3-(3-pyridyl) propylester was refluxed in 2-propanol for several hours to give 55% of N276-16 as a pale yellow amorphous solid.

Results and Discussion

MDR reversal activity of drug sensitive newly synthesized compounds was screened in the presence of doxorubicin

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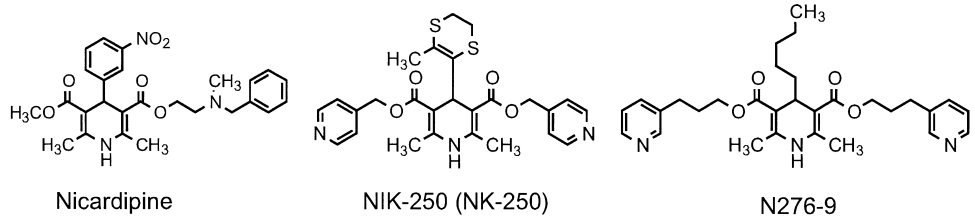
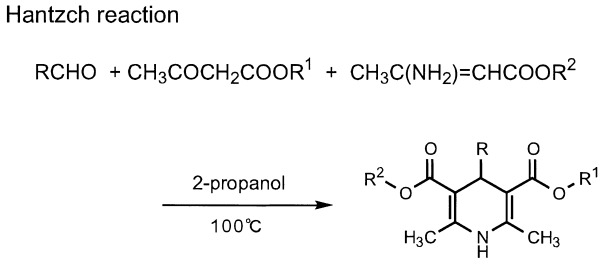


Figure 1. Structure of 1,4-dihydropyridines.



Scheme 1. Synthesis of 1,4-dihydropyridines.

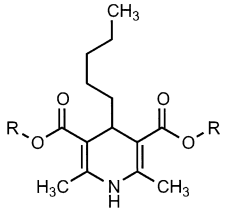
(DXR) using parental KB cells and their MDR counterpart KB/VJ300 cells in vitro. Combined with vincristine (VCR), antitumor activity of these compounds was screened by mice carrying P388 leukemia cells in vivo.[5]

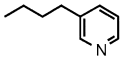
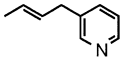
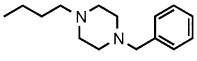
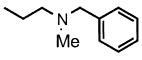
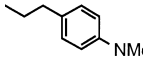
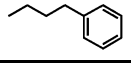
We first synthesized the symmetric 1,4-dihydropyridines possessing a 1-pentyl group at the 4-position to determine the structure–activity relationships for MDR reversing effect in vitro (Table 1). Ford et al. reported that the compound possessing one or more tertiary amino groups showed a strong MDR reversing effect, and a cyclic amine was more effective than a noncyclic amine.[8] Our results that TD-52 had no effect and N276-13 was more effective than TD-46 was consistent with Ford's review.[8] By contrast, TD-65 showed almost no such biological effect.

We next determined MDR reversing effect in vivo, and N276-9 was found to be the most active compound (Table 1). We thus replaced one of the 3-pyridylpropylesters and examined their MDR reversing effects in vitro and in vivo (Table 2). N276-16 showed the most effective activity among these active compounds, and we further screened some N276-16 derivatives possessing 3-(4-diphenylmethylpiperazyl-1-yl) propylester (Table 3).

Examination of MDR reversal activities in vitro of TD-65, TD-71 and TD-144 (Tables 1–3) indicated that 2-(4-

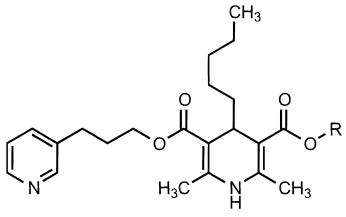
Table 1. MDR reversing activities of 3,5-symmetric 1,4-dihydropyridines

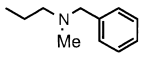
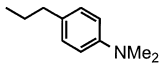
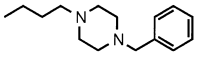
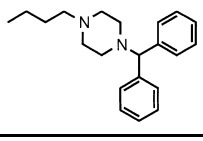


Compound no.	R	Resistance index[a]	Antitumor activity (%)[b]
N276-9		2.0	161
TD-90		1.8	89
N276-13		1.1	127
TD-46		3.9	128
TD-65		11.2	No data
TD-52		22.7	No data

[a]Resistance index was determined by the IC_{50} of DXR with test compounds (1 μg/mL) in KB/VJ300 cells dividing by the IC_{50} of DXR without test compounds in KB cells.
[b]Antitumor activity was determined by the survival days of mice treated by VCR (0.1 mg/kg, ip) with test compounds (100 mg/kg, ip) dividing by the survival days of mice treated by VCR (0.1 mg/kg, ip) alone.

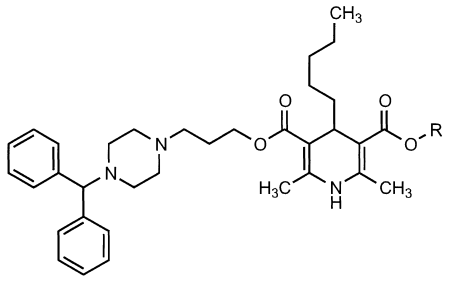
Table 2. MDR reversing activities of 3-substituted 1,4-dihydropyridines

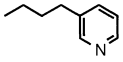
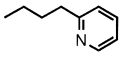
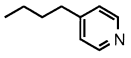
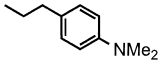


Compound no.	R	Resistance index[a]	Antitumor activity (%)[b]
TD-48		1.4	144
TD-71		1.8	125
TD-85		1.0	150
N276-16		1.1	143 (30 mg/kg)

[a]Resistance index was determined by the IC_{50} of DXR with test compounds (1 μg/mL) in KB/VJ300 cells dividing by the IC_{50} of DXR without test compounds in KB cells.
[b]Antitumor activity was determined by the survival days of mice treated by VCR (0.1 mg/kg, ip) with test compounds (100 mg/kg, ip) dividing by the survival days of mice treated by VCR (0.1 mg/kg, ip) alone.

Table 3. MDR reversing activities of N276-16 derivatives



Compound no.	R	Resistance index[a]	Antitumor activity (%)[b]
N276-16		1.1	143
TD-130		1.7	120
TD-136		1.0	No data
TD-144		23.5	No data
N276-9		2.0	116

[a]Resistance index was determined by the IC_{50} of DXR with test compounds (1 μg/mL) in KB/VJ300 cells dividing by the IC_{50} of DXR without test compounds in KB cells.
[b]Antitumor activity was determined by the survival days of mice treated by VCR (0.1 mg/kg, ip) with test compounds (100 mg/kg, ip) dividing by the survival days of mice treated by VCR (0.1 mg/kg, ip) alone.

Table 4. Calcium antagonistic activities of 1,4-dihydropyridines

Compound no.	IC_{50} (μM)
N276-9	46.0
N276-13	230
TD-46	115
TD-48	135
TD-71	45.0
TD-85	33.0
N276-16	175
NK-250	8.7
Nicardipine	1.4

N,*N*-dimethylaminophenyl) ethyl and 3-(4-diphenylmethylpiperazyl-1-yl) propyl groups were much less effective than pyridylpropyl groups. TD-130 indicated that the nitrogen atom at the 2-position must be sterically hindered and less effective than 3- or 4-pyridylpropylester (Table 3). TD-136 possessing 4-pyridylpropylester was toxic for mice without VCR, and we thought that 3-pyridylpropylester is one of the effective substituents to overcome MDR in vitro and in vivo.

We evaluated calcium antagonistic activities of the selected compounds by the inhibitory effect on high potassium depolarization-induced contracture in isolated rat aorta, and found that all of the compounds have weak activities (Table 4). There appears to be no close relationship between calcium antagonistic activity and MDR reversing activity.

Some compounds such as N276-13 and N276-16 with weak calcium antagonistic activities showed effective MDR reducing activities both in vitro and in vivo. In particular, N276-16 was expected to be the most suitable compound to overcome MDR.

References

1. Kohno, K.; Kikuchi, J.; Sato, S.; Takano, H.; Saburi, Y.; Asoh, K.; Kuwano, M. *Jpn. J. Cancer Res.* **1988**, *79*, 1238.
2. Hasegawa, S.; Abe, T.; Naito, S.; Kotoh, S.; Kumazawa, J.; Hipfner, D. R.; Deeley, R. G.; Cole, S. P. C.; Kuwano, M. *Br. J. Cancer* **1995**, *71*, 907.
3. Tsuruo, T.; Iida, H.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* **1983**, *43*, 2267.
4. Janis, R. A.; Triggle, D. J. *J. Med. Chem.* **1983**, *26*, 775.
5. Kiue, A.; Sano, T.; Suzuki, K.; Inada, H.; Okumura, M.; Kikuchi, J.; Sato, S.; Kohno, K.; Kuwano, M. *Cancer Res.* **1990**, *50*, 310.
6. Tanabe, H.; Tasaka, S.; Ohmori, H.; Gomi, N.; Sasaki, Y.; Machida, T.; Iino, M.; Kiue, A.; Naito, S.; Kuwano, M. *Bioorg. Med. Chem.* **1998**, *6*, 2219.
7. Hantzch, A. *Justus Liebigs Ann. Chem.* **1882**, *215*, 1.
8. Ford, J. M.; Halt, W. N. *Pharmacol. Rev.* **1990**, *42*, 3.