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Structure–activity relationship study of 1,4-dihydropyridine derivatives blocking N-type calcium channels

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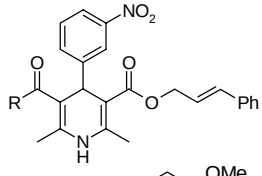
Abstract—Cilnidipine is a 1,4-dihydropyridine derived L/N-type calcium channel dual blocker possessing neuroprotective and analgesic effects which are related to its N-type calcium channel inhibitory activity. In order to find specific N-type calcium channel blockers with the least effects on cardiovascular system, we performed structure–activity relationship study on APJ2708, which is a derivative of cilnidipine, and found a promising N-type calcium channel blocker **21b** possessing analgesic effect in vivo with a 1600-fold lower activity against L-type calcium channels than that of cilnidipine.

Voltage-dependent calcium channels (VDCCs), which have been divided into five subtypes (L, Ca_V1; P/Q, $Ca_V2.1$; N, $Ca_V2.2$; R, $Ca_V2.3$; T, Ca_V3) based on their pharmacological and biophysical properties, mediate a range of cytoplasmic responses, including muscle contraction, release of neurotransmitters, calcium-dependent gene transcription, and the regulation of neuronal excitability.[1] Among them, N-type calcium channels are extensively distributed on the sympathetic nerve endings and related to the neuroprotection and neuropathic pain.[2] Several reports have suggested that a blockade or lack of N-type calcium channels can suppress the pathological processes of ischemic brain injury and pain in animal models.[3]

Cilnidipine is a 1,4-dihydropyridine derived long-acting calcium channel blocker which inhibits both L-type and N-type calcium channels,[4] and is currently used for the treatment of essential hypertension in Japan.[5] Its inhibitory effect for N-type calcium channel can be clinically observed in the reduction of white coat effect, cold pressor stress-induced platelet aggregation, urinary catecholamine excretion, and cardiac sympathetic overactivity in hypertensive patients.[6] Moreover, N-type calcium channel-blocking profile of cilnidipine may contribute to its neuroprotective action in the animal focal brain ischemia model and its intrathecal analgesic effect in rat formalin-induced pain model.[7,8]



Cilnidipine (**1**); R = O
APJ2708 (**2**) ; R = OH

Previously, we reported that APJ2708 (**2**), which is a carboxylic acid derivative of cilnidipine, has almost the same inhibitory activity against N-type calcium channels with far lower activity against L-type channels than that of cilnidipine.[7b] As a consequence of this L-type activity, APJ2708 showed a weak hemodynamic effect and needed more than 100-fold intravenously administered dose for decreasing the same amount of blood pressure as cilnidipine in vivo. We assumed that the carboxylic acid moiety of APJ2708 was the key structure for this selectivity and began our study by optimizing APJ2708 with

Keywords: N-type calcium channels blocker; Analgesic; 1,4-Dihydropyridine; Cilnidipine; Structure–activity relationship study.
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the aim of discovering N-type calcium channel blockers with low activity against L-type channels which have lesser influence on the cardiovascular systems.

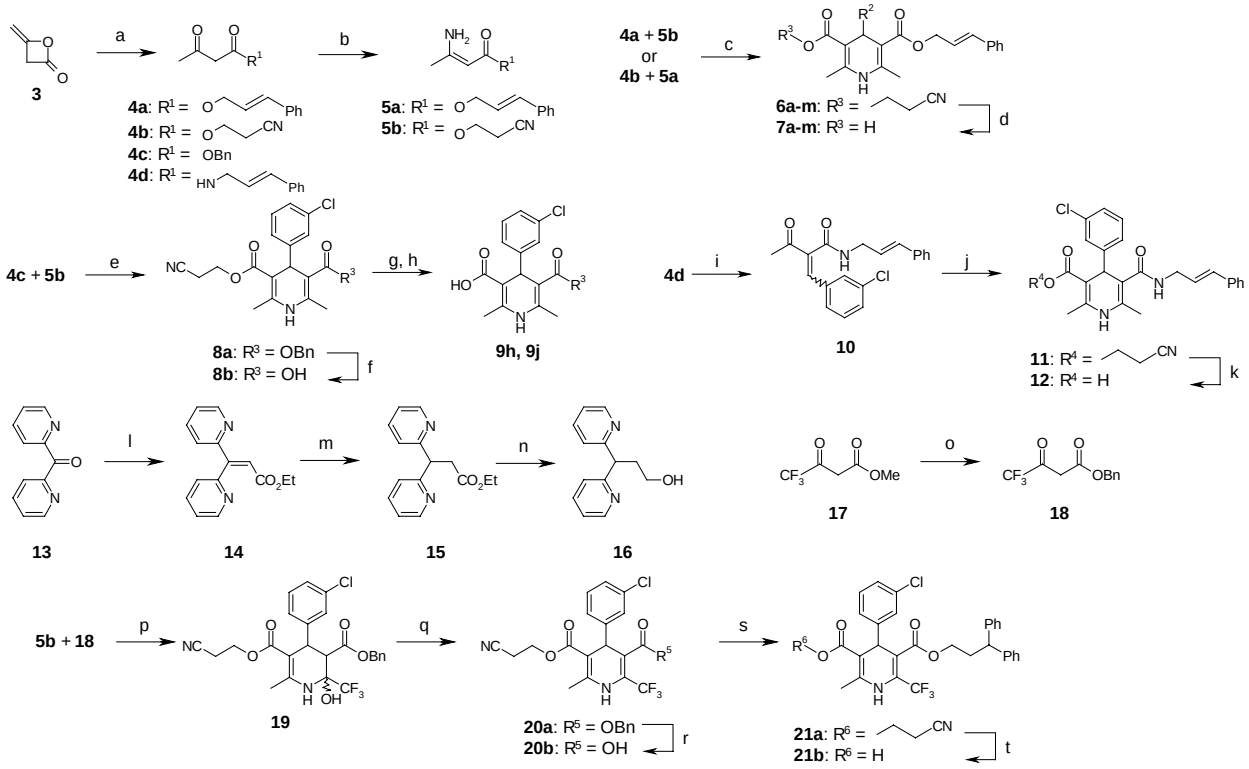
The reaction pathways used to synthesize the designed 26 compounds are described in Scheme 1.

All the 1,4-dihydropyridine ring was constructed by Hantzsch 1,4-dihydropyridine synthesis reaction.[9] The β-ketoesters (**4a**, **4b**, and **4c**) and enamines (**5a** and **5b**) were prepared from diketene **3** and the corresponding alcohol under basic conditions. The 1,4-dihydropyridine dicarboxylic acid cinnamyl ester 2-cyanoethyl ester **6a**–**m** was obtained using the three component coupling reaction of β-ketoester, enamine, and the corresponding benzaldehyde. The selective deprotection of 2-cyanoethyl ester group of **6a**–**m** was performed by the treatment with 1 M sodium hydroxide solution to give **7a**–**m**, since 2-cyanoethyl ester was removed easily via β-elimination reaction in the basic condition,[10] while cinnamyl ester was stable for hours. The 1,4-dihydropyridine dicarboxylic acid mono ester derivatives **9a**–**g** and **9i** were prepared by a similar method. The 1,4-dihydropyridine dicarboxylic acid benzyl ester 2-cyanoethyl ester **8a** was obtained using **4c** and **5b** as starting materials. Catalytic hydrogenation of **8a** yielded the 1,4-dihydropyridine dicarboxylic acid mono 2-cyanoethyl ester **8b**. The 1,4-dihydropyridine dicarboxylic acid mono ester **9h** was prepared via condensation with the corresponding alcohol in the presence of EDC followed by the removal of 2-cyanoethyl ester.

Dipyridyl-methanone **13** was reacted with triethyl phosphonoacetate under Wittig condition to give 3,3-dipyridyl acrylate **14**, which led to 3,3-dipyridyl propionate **15** by reduction using sodium borohydride in the presence of nickel chloride. Following reduction by LAH yielded 3,3-dipyridyl propanol **16**, which was condensed with **8b** to give **9j**.

The β-ketoamide **4d** was obtained by similar a condition as that described for β-ketoesters. The 1,4-dihydropyridine dicarboxylic acid 2-cyanoethyl ester cinnamyl amide **11** was synthesized via two-step reactions, in which **4d** and benzaldehyde were condensed under Knoevenagel condition followed by 1,4-dihydropyridine ring construction using **5b**. The treatment of 1 N sodium hydroxide solution yielded the desired 1,4-dihydropyridine dicarboxylic acid mono cinnamyl amide **12**.

The synthesis of 2-trifluoromethyl 1,4-dihydropyridine derivative **21b** required a different method since the dehydration of aminal **19** did not proceed under similar conditions as those used for 2-methyl 1,4-dihydropyridine derivatives. Compound **19** was isolated and subsequent phosphorus oxychloride/pyridine adsorbed silica gel[11] treatment was applied for the dehydration. Following hydrogenation, etherification and removal of 2-cyanoethyl ester yielded the desired compound **21b**.



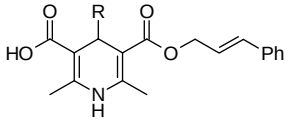
Scheme 1. Reagents: (a) alcohol or amine, Et_3N, toluene; (b) $AcONH_4$, *i*-PrOH; (c) R_2CHO, *i*-PrOH; (d) 1 M NaOH, MeOH; (e) 3-chlorobenzaldehyde, *i*-PrOH; (f) H_2, Pd/C, EtOH; (g) alcohol, EDC, Et_3N, CH_2Cl_2; (h) 1 M NaOH, MeOH; (i) 3-chlorobenzaldehyde, *i*-PrOH; (j) **5b**, *i*-PrOH; (k) 1 M NaOH, MeOH; (l) triethyl phosphonoacetate, NaH, EtOH; (m) $NaBH_4$, $NiCl_2{\cdot}6H_2O$, EtOH; (n) LAH, THF; (o) BnOH, toluene; (p) 3-chlorobenzaldehyde, *i*-PrOH; (q) $POCl_3$, pyridine, silica gel, 1,2-dichloroethane; (r) H_2, Pd/C, EtOH; (s) 3,3-diphenylpropanol, EDC, DMAP, Et_3N, CH_2Cl_2; (t) 1 M NaOH, MeOH.

The lead compound, APJ2708 (**2**), showed two times lower activity against N-type calcium channels (IC_{50} = 3.5 μM; IMR-32 assay[12]) than that of cilnidipine (IC_{50} = 1.6 μM), while its L-type activity was more than 40-fold lower (IC_{50} = 0.046 μM; Magnus method[13]). We ascribed this significant decrease in L-type activity to the presence of carboxyl group in **2**. Therefore, we started the optimizations by changing the substituent on the benzene ring bound to 4-position of the 1,4-dihydropyridine ring (Table 1).

First, the modification of nitro group at 3-position of benzene ring was investigated. The derivatives possessing 2- or 4-nitro group had a significantly lower activity against N-type calcium channels (**7a**; IC_{50} = 37 μM, **7b**; 21 μM) than that of cilnidipine. The derivative with a phenyl group (**7c**) resulted in increased activity against N-type channel (IC_{50} = 2.0 μM). However, the derivatives with a heterocyclic ring (**7k**, **7l**, and **7m**) showed lower activities for N-type channels (IC_{50} = 23, 2.9, and 11 μM, respectively) than that of **7c**. Therefore, the optimization was next focused on 3-position of the benzene ring. The introduction of an electron-donating methyl group (**7f**) showed a slightly improved activity for N-type (IC_{50} = 1.8 μM) with 70-fold lower activity for L-type (IC_{50} = 0.076 μM). As for the electron-withdrawing substituents, methoxycarbonyl (**7d**) and carboxylic acid (**7e**) derivatives resulted in lower activities for N-type channels (IC_{50} = 4.6 and 37 μM, respectively). On the other hand, the derivatives with halogen atom revealed to have improved activity against N-type calcium channels (IC_{50} = 1.3 μM; **7g**, 1.0 μM; **7h**, 0.79 μM; **7i** and 1.7 μM; **7j**, respectively) than that of **7c**. Among them, **7h** was most promising since it had high selectivity for N-type channels. The selectivity of 3-methyl derivative **7f** was also high, which suggested that there was no influence of electronic effect of the substituent on the benzene ring. However, we chose **7h** as the best since it had more potent activity against N-type calcium channels and it may have more potential as a second lead compound.

The optimization of cinnamyl moiety of **7h** was also investigated (Table 2). The activity of cinnamyl amide derivative **12** was tested but its activity against N-type channels was decreased (IC_{50} = 4.5 μM). Changing the benzene ring to the cyclohexyl ring (**9a**) resulted in increased N-type calcium channel activity (IC_{50} = 0.83 μM), but its activity against L-type channel was also high (IC_{50} = 0.0037 μM). Introduction of an electron-withdrawing chlorine atom at 4-position (**9b**) increased L-type activity (IC_{50} = 0.0058 μM) with a

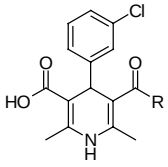
Table 1. In vitro results of dihydropyridine derivatives

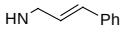
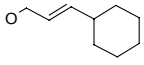
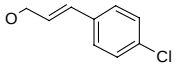
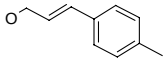
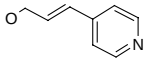
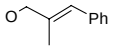
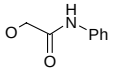
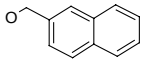
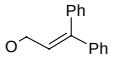
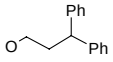
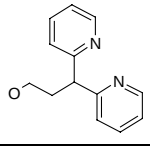


Compound	R	N-type IMR-32 IC_{50} (μM)	L-type Magnus IC_{50} (μM)
Cilnidipine (**1**)		1.6	0.0011
2	3-NO_2-Ph	3.5	0.046
7a	2-NO_2-Ph	37	0.031
7b	4-NO_2-Ph	21	1.6
7c	Ph	2.0	0.0090
7d	3-CO_2Me-Ph	4.6	4.4
7e	3-CO_2H-Ph	37	>10
7f	3-Me-Ph	1.8	0.076
7g	3-F-Ph	1.3	0.020
7h	3-Cl-Ph	1.0	0.020
7i	3-Br-Ph	0.79	0.010
7j	3-I-Ph	1.7	0.013
7k	3-Pyridyl	24	0.19
7l	3-Thienyl	2.9	0.044
7m	3-Furyl	11	0.13

The inhibition against N-type (calcium influx using IMR-32 cells) and L-type (magnus method) calcium channels.

Table 2. In vitro results of dihydropyridine derivatives



Compound	R	N-type IMR-32 IC_{50} (μM)	L-type Magnus IC_{50} (μM)
12		4.5	0.85
9a		0.83	0.0037
9b		1.5	0.0058
9c		0.89	0.0068
9d		6.2	0.32
9e		0.68	0.0028
9f		28	0.050
9g		1.0	0.014
9h		3.9	0.20
9i		1.1	0.36
9j		10	1.6

The inhibition against N-type (calcium influx using IMR-32 cells) and L-type (Magnus method) calcium channels.

slightly lower N-type activity (IC_{50} = 1.5 μM). The electron-donating methyl group at 4-position showed improved potency for N-type calcium channels (IC_{50} = 0.89 μM), but its activity against L-type was also increased (IC_{50} = 0.0068 μM). The inhibitory activity for N-type calcium channels of 4-pyridyl derivative **9d** was lower than that of cilnidipine.

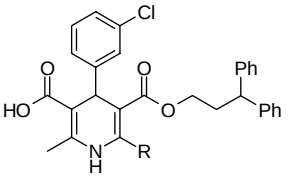
The introduction of a β-methyl group (**9e**) or a naphthyl group (**9g**) resulted in increased activities against L-type channels (IC_{50} = 0.0028 and 0.014 μM, respectively). The amide (**9f**) derivatives showed lower activity against N-type calcium channel. The diphenylmethyl derivative **9i** had an almost equivalent potency for N-type (IC_{50} = 1.1 μM) with that of **7h** with considerably decreased activity against L-type calcium channels (IC_{50} = 0.36 μM), which was more than 320-fold lower than that of cilnidipine. However, the N-type activity of its diphenylmethylidene (**9h**) and bipyridylmethyl (**9j**) derivatives for N-type channels was decreased.

Finally, we introduced the trifluoromethyl moiety at the 6-position of the 1,4-dihydropyridine ring and found that the derivative **21b** had a 1600-fold lower activity against L-type channels (IC_{50} = 1.8 μM) with an almost equivalent activity for N-type (IC_{50} = 1.7 μM) to cilnidipine (Table 3). This considerable decrease of L-type activity might be ascribed to the inductive effect of trifluoromethyl moiety which increases the acidity of carboxylic acid at 3-position of the 1,4-dihydropyridine ring.

Subsequently, the oral analgesic effect of **21b** was tested in vivo using formalin-induced pain model.[14] As can be seen in Table 4, **21b** showed equivalent oral activity to cilnidipine. This result indicated that **21b** was an orally effective N-type calcium channel blocker. Moreover, this result showed that the activity for N-type calcium channels is more important than that for L-type to exert the analgesic efficacy.

In conclusion, the SAR study on 4,5 and 6-position of series of 1,4-dihydropyridine derivatives starting from APJ2708 led to the discovery of novel N-type calcium channel blockers with less effects on cardiovascular system than those of cilnidipine and APJ2708. Among them, promising 4-(3-chloro-phenyl)-2-methyl-6-trifluoromethyl-1,4-dihydropyridine-3,5-dicarboxylic acid 5-(3,3-diphenylpropyl) ester **21b**, which had an almost equipotent activity against N-type calcium channels but 1600-fold lower activity for L-type than that of cilnidipine, showed equivalent oral analgesic activity to cilnidipine. This result reemphasized the importance of inhibitory activity against N-type channels for the treatment of neuropathic pain. Further SAR studies are under progress and will be reported elsewhere.

Table 3. In vitro results of dihydropyridine derivatives



Compound	R	N-type IMR-32 IC_{50} (μM)	L-type Magnus IC_{50} (μM)
9i	Me	1.1	0.36
21b	CF_3	1.7	1.8

The inhibition against N-type (calcium influx using IMR-32 cells) and L-type (Magnus method) calcium channels.

Table 4. Inhibitory activities of compounds in phase II pain responses following footpad injection of formalin in the rat

Compound	Rat formalin test (30 mg/kg, po)	
	Inhibition %	*n*
Cilnidipine	46	5
21b	46	5

Inhibition % is given by the control and test compound.

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13. Inhibitory activity against L-type calcium channels was estimated from the effects on high K^+-induced contraction in rat thoracic aorta ring as follows: male SD rats (7 weeks old) were used. The thoracic aorta was removed, cleared of adhering periadventitial fat, and cut into rings of 3 mm width. Removal of the endothelium was achieved by gently rubbing the luminal surface. The ring was mounted in an organ bath filled with warmed (37 °C), oxygenated (95% O_2/5% CO_2) Tyrode's solution (pH 7.4). The ring was equilibrated under resting tension of 2*g* for 1 h. Then the ring was incubated in high K^+ solution and then in Tyrode's solution for 45 min each. The solution was replaced with high K^+ solution again. After attaining the maximum contraction reaction, the test compound was cumulatively added at intervals of 90 min to attain concentrations of 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M. The inhibitory rate of the test compound against the maximum contraction response was employed as the index of the antagonistic activity on L-type calcium channels. Importantly, we have confirmed that the current IMR model is about 1000 times less sensitive than the Magnus or in vivo model for detecting each calcium channel-blocking action, as described in our previous report (Takahara, A.; Fujita, S.; Moki, K.; Ono, Y.; Koganei, H.; Iwayama, S.; Yamamoto, H. *Hypertens. Res.* **2003**, *26*, 743). Therefore, the IC_{50} values of the method against L-type calcium channel could not be simply compared with the IC_{50} values of the cell-based IMR-32 method against N-type calcium channels.
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