Bioorganic & Medicinal Chemistry Letters 21 (2011) 3317–3319

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Asymmetric synthesis and biological evaluations of (+)- and (−)-6-dimethoxymethyl-1,4-dihydropyridine-3-carboxylic acid derivatives blocking N-type calcium channels

Takashi Yamamoto *, Seiji Ohno, Seiji Niwa, Munetaka Tokumasu, Masako Hagihara, Hajime Koganei, Shin-ichi Fujita, Tomoko Takeda, Yuki Saitou, Satoshi Iwayama, Akira Takahara, Seinosuke Iwata, Masataka Shoji

Exploratory Research Laboratories, Ajinomoto Pharmaceuticals Co., Ltd, 1-1, Suzuki-cho, Kawasaki-ku, Kawasaki-shi, Kanagawa, Japan

ARTICLE INFO

Article history:
Received 26 January 2011
Revised 1 April 2011
Accepted 5 April 2011
Available online 9 April 2011

Keywords:
N-type calcium channel blocker
Structure–activity relationships
1,4-Dihydropyridine derivative
Asymmetric synthesis

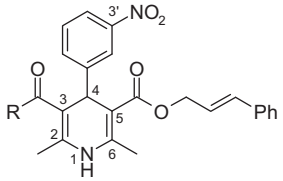
ABSTRACT

An efficient asymmetric synthesis of 1,4-dihydropyridine derivatives is described. The key step is the stereoselective Michael addition using *t*-butyl ester of L-valine as a chiral auxiliary to achieve good ee (>95% for all the tested experiments) and moderate yield. With this method, (+)-4-(3-chlorophenyl)-6-dimethoxymethyl-2-methyl-1,4-dihydropyridine-3,5-dicarboxylic acid cinnamyl ester was obtained and was characterized as a promising N-type calcium channel blocker with improved selectivity over L-type compared to its (−)- and racemic isomers.

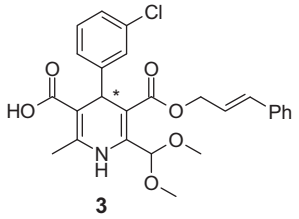
1,4-Dihydropyridines (DHPs) have been considered as a privileged pharmacophore to modulate calcium current at the voltage-dependent calcium channels (VDCCs), and widely used in clinical.[1] VDCCs mediate calcium influx into cells in response to diverse physiological changes, such as triggering various physiological events such as neuronal excitability, muscle contraction and secretion of neurotransmitters, and they are generally classified into at least five subtypes based on their pharmacological and functional properties: L- ($Ca_V1.1$–$Ca_V1.4$), P/Q- ($Ca_V2.1$), N- ($Ca_V2.2$), R- ($Ca_V2.3$) and T-type ($Ca_V3.1$–$Ca_V3.3$).[2]

Cilnidipine (**1**), one of the marketed drugs possessing a DHP pharmacophore, is a potent dual blocker for N/L-type VDCCs and is currently used for the treatment of essential hypertension in Japan and South Korea (Table 1). The N-type calcium channel distributes mainly on the endings of central and peripheral nerves to control neuronal excitability and secretion of neurotransmittersis (ex. glutamate, substance P or CGRP) in strong association with the pathological process of neuropathic pain. Thus, blockade of the N-type VDCC has been considered as a promising therapeutic target to be a novel type of analgesic. Therefore, many efforts have been made to discover efficacious small-molecule N-type VDCC blockers to date.[3]

Table 1
Activity table of dihydropyridine derivatives. In vitro inhibition against N-type (calcium influx using IMR-32 cells) and L-type (magnus method) calcium channels



Cilnidipine (**1**); R = OCH_2CH_2OMe
APJ2708 (**2**) ; R = OH



3

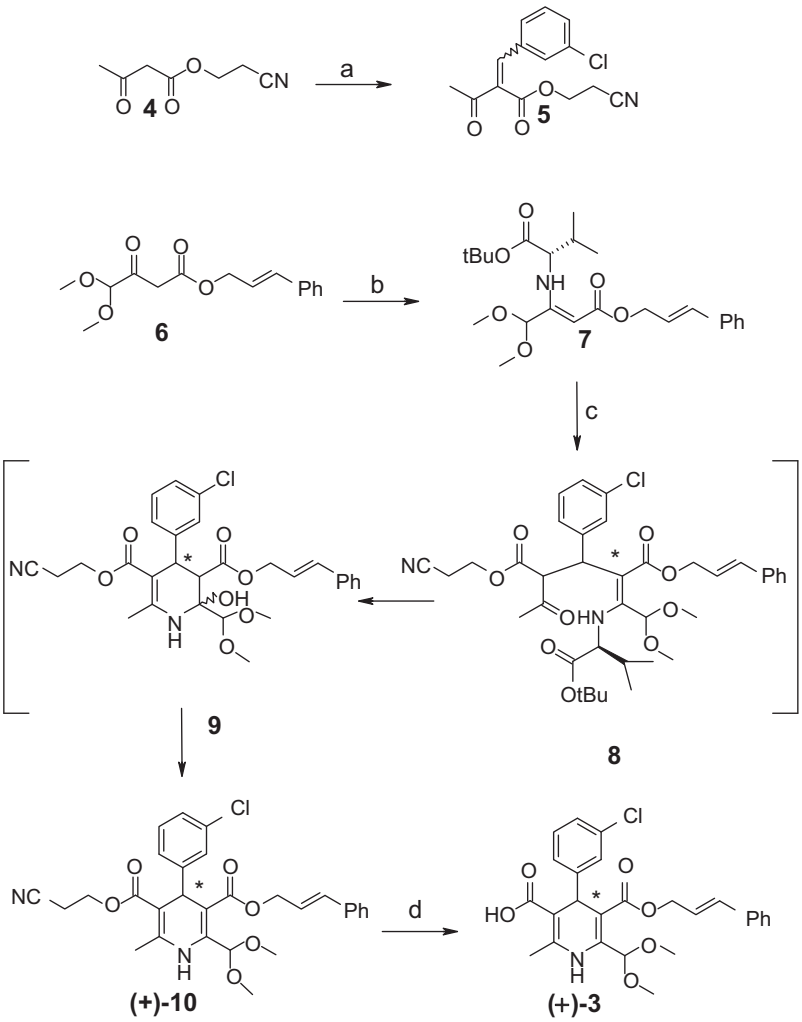
Compd	N-type IMR-32 IC_{50}, μM	L-type Magnus IC_{50}, μM	ee[a] (%)
Cilnidipine (**1**)	1.6	0.0011	Racemic
AJP2708 (**2**)	3.5	0.046	Racemic
3	3.0	8.1	Racemic
(+)-**3**	2.6	13.2	95.6[b]
(−)-**3**	2.6	1.8	97.1[c]

[a] Determined as 2-cyanoethyl ester (**10**) by chiral HPLC.
[b] Mean value of two independent experiments (96.0 and 95.2% ee).
[c] Mean value of two independent experiments (98.7 and 95.5% ee).

As previously reported,[4] we have performed a structure–activity relationship study on APJ2708 (**2**), a 3-carboxylate DHP deriva-

* Corresponding author. Tel.: +81 44 210 5826.
E-mail address: takashia_yamamoto@ajinomoto.com (T. Yamamoto).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.04.007



Scheme 1. Reagents and conditions: (a) 3-chlorobenzaldehyde, cat. piperidine, cat. AcOH, *i*-PrOH, 66.3%; (b) L-Val-*O*-*t*Bu, benzene, reflux, 61.6%; (c) (i) **5**, LDA, THF, −78 °C then −35 °C, 71.5%; (ii) 1 M HCl aq, rt; (iii) $AcONH_4$, EtOH, 50 °C, then 120 °C; (d) 1 M NaOH, MeOH, 83.7%.

tive of **1,** to find an effective and selective N-type calcium channel inhibitor with the least influence on cardiovascular system. As a result of the structural optimization, 4-(3-chlorophenyl)-6-dimethylacetal DHP derivative **3** was identified as an promising N-type VDCC blocker showing significant analgesic potency with more than 170-fold lower activity for L-type channel compared to that of **2**.[4d]

Here, when DHP derivative has non-identical ester groups in the 3- and 5-positions, the molecule is chiral, with the carbon at 4 position as the stereogenic center, which sometimes has critical effect on its biological activities; for example, *S*-configuration is crucial for potent inhibitory activity for L-type VDCC.[5] Thus, the separation of two enantiomers of racemic DHP derivative **3** could be an effective strategy to identify a compound with the improved selectivity for the N-type VDCC over the L-type channels.

Several methods for synthesis of chiral DHPs have been reported, including asymmetric induction,[6] resolution of racemic mixture,[7] or chemoenzymatic method.[8] Asymmetric Michael addition using α-methylbenzylamine as chiral inducer was also reported by Ashworth et al.,[9] but their procedure suffered from low d.e. (about 70%). We hypothesized that steric hindrance of the α-methylbenzylamine was not enough to achieve sufficient stereoselectivity, and applied novel approach to synthesis of highly enantioselective isomer of compound **3**. Our synthetic route was shown in the Scheme 1. The key of this method is using *t*-butyl ester of L-valine, which is easily available and its bulky isopropyl group plays an effective role as a driving force for the highly enantiomeric purity (>95% ee in all the tested experiments) of the obtained chiral DHPs.

The experiments were performed as follows.[10] 2-Benzylidene-3-oxo-butyric acid 2-cyanoethyl ester (**5**) was prepared using Knoevenagel condensation of 3-oxo-butyric acid 2-cyanoethyl ester (**4**) with the 3-chlorobenzaldehyde. The 4,4-dimethoxy-3-oxo-butyric acid cinnamyl ester (**6**) and L-valine *t*-butyl ester was refluxed in benzene to afford enamine **7**, which was treated with LDA at −78 °C followed by slow addition of **5** to conduct key stereoselective step. The obtained crude **8** was dissolved in ethanol, and then ammonium acetate was added to yield the mixture of DHP diester (+)-**10** and its hydrated intermediate **9**, which was heated to convert into (+)-**10** by a single spot. The obtained (+)-**10** showed 96.0% ee based on the HPLC analysis using Daicel chiralcel OD-H column. 1 M sodium hydroxide solution was treated to the desired chiral DHP dicarboxylic acid mono cinnamyl ester (+)-**3** by 44.0% overall yield from **6**.[11,12] The corresponding enantiomer (−)-**3** was also prepared with the same procedure using D-valine *t*-butyl ester.

The obtained (+)- and (−)-**3** were characterized using IMR-32 human neuroblastoma cells for N-type VDCC[13] and rat thoracic aorta ring for L-type VDCC.[14,15] As found in Table 1, two enantiomers showed different inhibitory activities for L-type VDCC: (−)-**3** was 7 times more potent than (+)-**3** (1.8 and 13.2 μM, respectively). For the N-type VDCC, these enantiomers both showed

potent activities with no detectable difference (2.6 μM), implying that the chirarity at the C4 position affects only on the binding at the L-type VDCC, but not at the N-type channels.

In summary, novel asymmetric Michael addition was performed using L- or D-valine *t*-butyl ester as a chiral auxiliary for the preparation of enantioenriched 6-dimethoxymethyl-1,4-dihydropyridine-3-carboxylic acid DHP derivatives. Among the two synthesized enantiomers, (+)-**3** was found as an effective blocker for N-type VDCC with the improved selectivity over L-type channel, compared to its (−)- and racemic isomers. Thus, (+)-**3** could be considered as an interesting research tool to seek for a promising drug candidate to control severe to moderate pain states.

References and notes

1. (a) Safak, C.; Simsek, R. *Mini-Rev. Med. Chem.* **2006**, *6*, 747; (b) Furukawa, T.; Nukada, T.; Namiki, Y.; Miyashita, Y.; Hatsuno, K.; Ueno, Y.; Yamakawa, T.; Isshiki, T. *Eur. J. Pharmacol.* **2009**, *613*, 100.
2. Yaksh, T. L. *J. Pain* **2006**, *7*, S13.
3. (a) Yamamoto, T.; Takahara, A. *Curr. Top. Med. Chem.* **2009**, *9*, 377; (b) Barrow, J. C.; Duffy, J. L. *Annu. Rev. Med. Chem.* **2010**, *45*, 2; (c) Pajouhesh, H.; Feng, Z. P.; Ding, Y.; Zhang, L.; Pajouhesh, H.; Morrison, J. L.; Belardetti, F.; Tringham, E.; Simonson, E.; Vanderah, T. W.; Porreca, F.; Zamponi, G. W.; Mitscher, L. A.; Snutch, T. P. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1378.
4. (a) Yamamoto, T.; Niwa, S.; Ohno, S.; Onishi, T.; Matsueda, H.; Koganei, H.; Uneyama, H.; Fujita, S.; Takeda, T.; Kito, M.; Ono, Y.; Saitou, Y.; Takahara, A.; Iwata, S.; Yamamoto, H.; Shoji, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 798; (b) Yamamoto, T.; Niwa, S.; Iwayama, S.; Koganei, H.; Fujita, S.; Takeda, T.; Kito, M.; Ono, Y.; Saitou, Y.; Takahara, A.; Iwata, S.; Yamamoto, H.; Shoji, M. *Bioorg. Med. Chem.* **2006**, *14*, 5333; (c) Yamamoto, T.; Niwa, S.; Ohno, S.; Tokumasu, M.; Masuzawa, Y.; Nakanishi, C.; Nakajo, A.; Onishi, T.; Koganei, H.; Fujita, S.; Takeda, T.; Kito, M.; Ono, Y.; Saitou, Y.; Takahara, A.; Iwata, S.; Shoji, M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4813; (d) Yamamoto, T.; Niwa, S.; Ohno, S.; Tokumasu, M.; Masuzawa, Y.; Nakanishi, C.; Nakajo, A.; Onishi, T.; Koganei, H.; Fujita, S.; Takeda, T.; Kito, M.; Ono, Y.; Saitou, Y.; Takahara, A.; Iwata, S.; Shoji, M. *Drugs Future* **2008**, *33*(Suppl. A), P150.
5. Arhancet, G. B.; Woodard, S. S.; Dietz, J. D.; Garland, D. J.; Wagner, G. M.; Iyanar, K.; Collins, J. T.; Blinn, J. R.; Numann, R. E.; Hu, X.; Huang, H. C. *J. Med. Chem.* **2010**, *53*, 4300.
6. Martin, N.; Martinez-Grau, A.; Seoane, C.; Marco, J. L.; Albert, A.; Cano, F. H. *Tetrahedron: Asymmetry* **1995**, *6*, 877.
7. See the following reference and those cited within: Zhang, B. L.; He, W.; Shi, X.; Huan, M. L.; Huang, Q. J.; Zhou, S. Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 805. and references herein.
8. Kosugi, Y.; Hori, M.; Tatsuo, N. *Heterocycles* **1994**, *39*, 591.
9. Ashworth, I.; Hopes, P.; Levin, D.; Patel, I.; Salloo, R. *Tetrahedron Lett.* **2002**, *43*, 4931.
10. The synthesis of (+)-**3** was conducted as follows:
    Step1. *Synthesis of 2-Acetyl-3-(3-chlorophenyl)-acrylic acid (2-cyanoethyl) ester (**5**)*: 3-Oxo-butenoic acid 2-cyanoethyl ester (**4**) 3.00 g (19.3 mmol), 3-chlorobenzaldehyde 2.72 g (19.3 mmol), acetic adid 79 mg (0.97 mmol) and piperidine 58 mg (0.97 mmol) was stirred in 2-propanol (50 mL) for overnight. The solvent was evaporated under reduced pressure and the residue was purified by the silica gel chromatography (hexane/EtOAc, 3:1) to obtain the title compound as an yellow oil (3.55 g, 12.8 mmol, 66.3%). ^{1}H NMR ($CDCl_3$) δ: 2.05 (3H, s), 2.63 (2H, t, J = 6.6 Hz), 3.92 (2H, t, J = 6.6 Hz), 7.00–7.88 (5H, m). MS (ESI, m/z) 278 $(M+H)^+$.
    Step 2. *Synthesis of 3-(tert-butoxycarbonyl-2-methylpropylamino)-4,4-dimethoxybutenoic acid cinnamyl ester (**7**)*: 3-Oxo-4,4-dimethoxybutenoic acid cinnamyl ester (**6**) 16.24 g (58.4 mmol) and L-Val-*O*-*t*Bu 10.12 g (58.4 mmol) was heated in benzene (60 mL) at reflux for 6 h while the water by-product was collected in a Dean–Stark trap. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography (hexane/EtOAc, 9:1) to obtain the title compound (15.58 g, 35.9 mmol, 61.6%). $[\alpha]_D^{25}$ = +95.52 (c 1.00, MeOH). ^{1}H NMR ($CDCl_3$) δ: 0.98–1.02 (6H, m), 1.46 (9H, s), 2.11–2.26 (1H, m), 3.33 (6H, s), 4.25 (1H, dd, J = 4.5, 10.8 Hz), 4.61 (1H, s), 4.76 (2H, m), 4.89 (1H, s), 6.32 (1H, m), 6.65 (1H, d, J = 15.9 Hz), 7.20–7.31 (5H, m), 8.48 (1H, d, J = 9.0 Hz). MS (ESI, m/z) 434 $(M+H)^+$.
    Step 3. *Synthesis of (+)-4-(3-chlorophenyl)-6-dimethoxymethyl-2-methyl-1,4-dihydropyridine-3,5-dicarboxylic acid cinnamyl ester 5-(2-cyanoethyl) ester ((+)-**10**)*: 567 mg of **7** (1.31 mmol) was dissolved in THF (50 mL) and 2 M solution of LDA in THF (0.678 mL) was slowly added to the obtained solution at −78 °C, and then stirred for 2 h 30 min. 364 mg of **5** (1.31 mmol) in THF (32 mL) was added to the reaction mixture, stirred at −78 °C for 3 h and then at −35 °C for overnight. 1 M HClaq (3 mL) was slowly added to the obtained mixture at −35 °C followed by additional 1 M HCl aq (3 mL) at rt. After stirred for 6 h, the reaction mixture was extracted three times with EtOAc, the organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The obtained residue was dissolved in ethanol (40 mL) and ammonium acetate 537 mg (6.55 mmol) was added to the obtained solution. After stirring for overnight at 50 °C, the solvent was evaporated under reduced pressure. After extracting three times with EtOAc from water, the organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The residue was heated at 120 °C for 2 h 30 min, and then purified by silica gel chromatography (hexane/EtOAc, 10:1) to obtain the title compound (502 mg, 0.94 mmol, 71.5%). Optical purity: 96.0% ee (analytical HPLC system: HITACHI L-6200 system; Column: Daicel chiralcel OD-H, 25 cm × 0.46 cm ID, Daicel Chemical Industries Inc.; Solvent: *n*-hexane/EtOH = 92:8; Flow rate: 1.0 mL/min; Detect: 254 nm), $[\alpha]_D^{25}$ = +43.59 (c 1.28, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 2.39 (3H, s), 2.61 (2H, t, J = 6.3 Hz), 3.44 (3H, s), 3.47 (3H, s), 4.19–4.32 (2H, m), 4.66–4.83 (2H, m), 5.05 (1H, s), 6.04 (1H, s), 6.24 (1H, dt, J = 9.3, 16.2 Hz), 6.56 (1H, d, J = 16.2 Hz), 6.83 (1H, br s), 7.10–7.39 (9H, m). MS (ESI, m/z) 535 $(M-H)^-$.
    Step 4. *Synthesis of (+)-4-(3-chlorophenyl)-6-dimethoxymethyl-2-methyl-1,4-dihydropyridine-3,5-dicarboxylic acid cinnamyl ester ((+)-**3**)*: 64.6 mg of (+)-**10** (0.121 mmol) was dissolved in MeOH (10 mL) and 1 M NaOH (0.13 mL) was added to the obtained solution. After stirring for 4 h at rt, 1 M HCl aq (0.14 mL) was added and the solvent was removed under reduced pressure. After extracting three times with EtOAc, the organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The residue was purified by the silica gel chromatography (hexane/EtOAc, 10:1 to 2:1) to obtain the title compound (49.0 mg, 0.10 mmol, 83.7%). Purity: 98.9% (analytical HPLC system: HITACHI L-6200 system; C-18 reversed-phase column: YMC-Pack ODS AM, 15 cm × 0.46 cm ID, YMC Co., Ltd; Solvent: water/acetonitrile = 50:50 to 80:20 in 30 min; Flow rate: 1.0 mL/min; Detect: 254 nm), $[\alpha]_D^{25}$ = +7.69 (c 0.858, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 2.38 (3H, s), 3.43 (3H, s), 3.47 (3H, s), 4.65–4.83 (2H, m), 5.06 (1H, s), 6.04 (1H, s), 6.23 (1H, dt, J = 6.0, 15.9 Hz), 6.54 (1H, d, J = 15.9 Hz), 6.86 (1H, br s), 7.07–7.38 (9H, m). HR-MS (FAB, m/z) calcd 482.1370 $(M-H)^-$ observed 482.1363.
11. The X-ray crystal structural study of a derivative of compound **3** (4-(3,4-dichloro-5-methoxyphenyl)-6-dimethoxymethyl-2-methyl-1,4-dihydropyridine-3,5-dicarboxilic acid cinnamyl ester) showed that the enantiomer synthesized using L-Val-*O*-*t*Bu is as a (*R*)-isomer (unpublished data).
12. Due to peak broadening in chiral HPLC conditions, the optical purify of nether (+)- nor (−)-3 could be directly determined. However, a derivative of compound **3** (4-(3-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid cinnamyl ester) was obtained with 96.0% ee using same synthetic procedure (unpublished data), implying that the hydrolysis step induces negligible racemization at the C-4 position.
13. Takahara, A.; Fujita, S.; Moki, K.; Ono, Y.; Koganei, H.; Iwayama, S.; Yamamoto, H. *Hypertens. Res.* **2003**, *26*, 743.
14. Feng, P. C.; Haynes, L. J.; Magnus, K. E. *Nature* **1961**, *191*, 1108.
15. As reported in the reference (Takahara, A.; Koganei, H.; Takeda, T.; Iwata, S. *Eur. J. Pharmacol.* **2002**, *434*, 43.), cilnidipine (compound **1**) can inhibit both the sympathetic N-type and vascular L-type calcium channels in antihypertensive doses (3 μg/kg, iv administration), suggesting that its ratio of L-type/N-type blocking activity is about 1. Based on the information, the IMR-32 assay system for this series of compounds can be estimated to be about 1000 times less sensitive than our assay in Magnus method using rat thoracic aorta ring for detecting each calcium channel blocking action. Therefore, the IC_{50} values of the method against L-type VDCC could not be simply compared with the IC_{50} values of the cell-based IMR-32 method against N-type channel.