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Discovery of Novel Neuronal Voltage-Dependent Calcium Channel Blockers Based on Emopamil Left Hand as a Bioactive Template

Yuichi Suzuki,[a,*] Noboru Yamamoto,[a] Yoichi Iimura,[a] Koki Kawano,[a] Teiji Kimura,[a] Satoshi Nagato,[a] Koichi Ito,[a] Makoto Komatsu,[b] Yoshihiko Norimine,[a] Manami Kimura,[a] Tetsuyuki Teramoto,[a] Yoshihisa Kaneda,[a] Takeshi Hamano,[a] Tetsuhiro Niidome[a] and Masahiro Yonaga[a]

[a]*Tsukuba Research Laboratories, Eisai Co., Ltd., 5-1-3 Tokodai, Tsukuba-shi, Ibaraki 300-2635, Japan*
[b]*Ebara Research Co., Ltd, 2-1 Honfujisawa 4-chome, Fujisawa-shi, Kanagawa 251-8502, Japan*

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Abstract—A series of novel neuronal voltage-dependent calcium channel (VDCC) blockers, with inhibitory activity at low micromolar and moderate solubility in water, was discovered by constructing and screening a focused library based on emopamil (**1**) left hand (ELH) as a bioactive template.

Cerebral ischemia causes a large increase in extracellular glutamate.[1,2] Release of glutamate from neurons requires the entry of Ca^{2+} into presynaptic terminals, which is mediated by neuronal voltage-dependent calcium channels (VDCCs). These VDCCs are classified into L, N, P, Q, R and T subtypes based on their electrophysiological and pharmacological properties.[3–5] The release of glutamate further allows Ca^{2+} to enter the neurons by activation of postsynaptic glutamate receptors.[2] Ca^{2+} can also enter postsynaptic neurons through neuronal VDCCs.[6] These events cause an excessive elevation of the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). The resulting Ca^{2+} overload leads to activation of proteases, nucleases and phospholipases, and finally to neuronal cell death.[1,7] Thus, inhibition of neuronal VDCCs is expected to reduce excessive glutamate release at the presynaptic terminals and to inhibit $[Ca^{2+}]_i$ elevation in postsynaptic neurons, thereby affording protection against neuronal cell death. Neuronal VDCC blockers are considered to be among the best candidates for neuroprotective drugs. In addition, it is expected that neuronal VDCC blockers that extensively inhibit a variety of neuronal VDCCs, such as N, P and Q subtypes, would have a greater effect than various subtype-specific VDCC blockers, which would produce only a marginal effect on the extracellular glutamate level when added alone.[4]

Various attempts to discover neuronal VDCC blockers or subtype-specific VDCC blockers have been made during the past decade; however, few non-peptide neuronal VDCC or subtype-specific VDCC blockers, which possess potent activity, water-solubility, low molecular weight and sufficient brain penetration, have been obtained so far.[8] Our strategy to search for such neuronal VDCC blockers that inhibit a variety of VDCC subtypes, including N, P and Q subtypes, does not utilize high-throughput screening, but rather involves constructing a focused library. Emopamil (**1**)[3] left hand (ELH) was chosen as a bioactive template for this focused library design. In the course of constructing and screening this focused library, we discovered a new series of VDCC blockers, which exhibit inhibitory activity for neuronal VDCCs at low micromolar in rat cortical synaptosomes assay.[9] Herein, we report the design, synthesis and structure–activity relationships (SARs) of neuronal VDCC blockers bearing ELH as a bioactive template.

The syntheses of piperazine analogues **7–9**, **18–22** and **23a–23q** are depicted in Schemes 1–5. Alkylation of phenylacetonitrile **2** with isopropyl bromide provided **3**.

*Corresponding author. Tel.: +81-298-47-7391; fax: +81-298-47-4012; e-mail: y5-suzuki@hhc.eisai.co.jp

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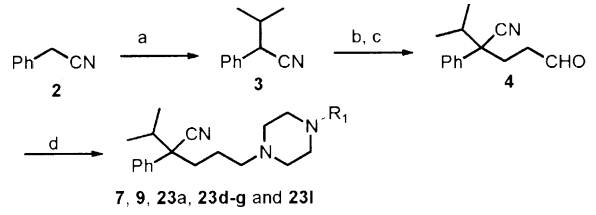
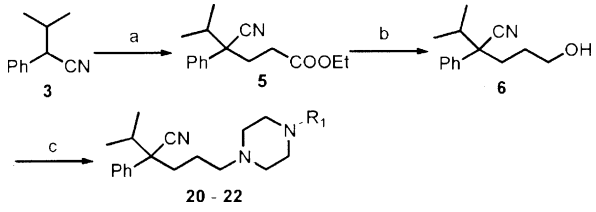
The racemic aldehyde **4** was prepared through alkylation of **3** with 2-(2-bromoethyl)-1,3-dioxolane, followed by hydrolysis. The aldehyde **4** was used to reductively alkylate a variety of piperazines, affording **7**, **9**, **23a**, **23d–g** and **23l** (Scheme 1).[10] The racemic alcohol **6** was generated via Michael reaction of **3**, followed by reduction of **5** with lithium aluminum hydride. The alcohol **6** was coupled with a variety of piperazines to yield **20–22** (Scheme 2). Compound **7** was debenzylated by catalytic hydrogenation to furnish the piperazine **8** which was coupled with alkyl halides to give **18** and **19** (Scheme 3). Compounds **23b–c**, **23h** and **23o–q** were prepared by Mitsunobu reaction (Scheme 4), and **23j–k** were produced by *O*-alkylation of the chloride **10** (Scheme 4). Compound **23i** was synthesized by aromatic nucleophilic substitution (Scheme 4). Catalytic hydrogenation of **23l** produced **23m**, which was converted to **23n** by reductive amination as shown in Scheme 5. Piperazine intermediates (**12a–c**, **14**, **16** and **17**) were prepared as shown in Scheme 6. Piperazine intermediates **12a–c** were prepared through Mitsunobu reaction between formyl-protected 1-piperazineethanol[11] **11** and the requisite phenols followed by hydrolysis. The 4-nitrophenyl intermediate **14** was generated by aromatic nucleophilic substitution of 4-fluoronitrobenzene. 1-Benzylpiperazine **15** was coupled with substituted phenoxyethyl bromide, followed by removal of the benzyl protecting group to provide **16** and **17**. All analogues were prepared as racemic compounds.

Inhibitory effects of piperazine analogues on neuronal VDCCs were evaluated by employing rat cortical synaptosomes.[12] The anticonvulsant effect of a moderately active compound was evaluated in an audiogenic seizure model using DBA/2 mice to confirm in vivo activity and permeability into the brain.[13]

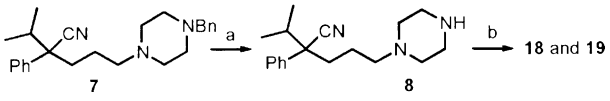
Nifedipine and amlodipine are dihydropyridine derivatives.[14] It is generally accepted that dihydropyridines are specific blockers of the L-type Ca^{2+} channel.[14] Nifedipine is also specific toward L-type Ca^{2+} channel.[14] However, amlodipine acts on both L- and N-type Ca^{2+} channels despite the minor structural difference from nifedipine.[14] This result suggests the possibility of modifying an L-type Ca^{2+} channel blocker to shift its activity towards neuronal VDCCs. Compound **1**, which is an L-type Ca^{2+} channel blocker,[3] was selected as a template to modify because 3-methyl-2-phenylbutanenitrile (ELH) was expected to be suitable template to construct a focused library aimed at neuronal VDCC blockers, due to its low molecular weight. We were also encouraged to pursue analogues of **1**, as this compound already shows a slight activity against neuronal VDCCs (**1**; $IC_{50} = 28\,\mu M$). A piperazine moiety was attached to



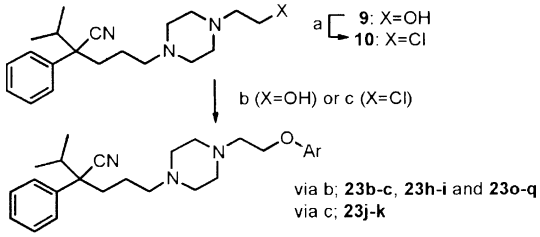
Scheme 1. Reagent and reaction conditions: (a) 50% KOH aq, 2-bromopropane, DMSO; (b) $NaNH_2$, 2-(2-bromoethyl)-1,3-dioxolane, THF reflux; (c) 2 N HCl–acetone; (d) $NaB(OAc)_3H$, AcOH, 1,2-dichloroethane, piperazine derivatives.



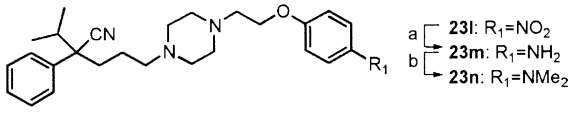
Scheme 2. Reagent and reaction conditions: (a) potassium *tert*-butoxide, ethyl acrylate, THF; (b) lithium aluminum hydride, THF; (c) MsCl, triethylamine, MeCN then NaI, H_2O, piperazine derivatives.



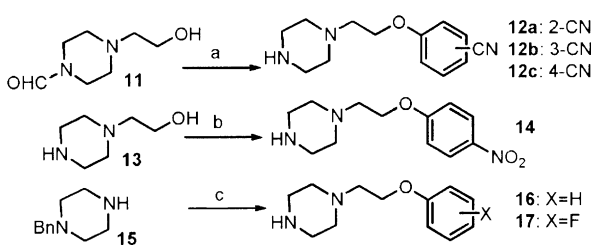
Scheme 3. Reagent and reaction conditions: (a) H_2, $Pd(OH)_2/C$, MeOH; (b) alkyl halides, triethylamine, MeCN.



Scheme 4. Reagent and reaction conditions: (a) $SOCl_2$, CH_2Cl_2; (b) substituted phenols, Ph_3P, diethyl azodicarboxylate or NaH, 2-bromopyridine for **23i**; (c) substituted phenols, NaH, DMSO.



Scheme 5. Reagent and reaction conditions: (a) H_2, Pd/C, MeOH; (b) aq HCHO, $NaBH_3CN$, AcOH, MeCN.



Scheme 6. Reagent and reaction conditions: (a) (i) 2-, 3- or 4-cyanophenol, Ph_3P, diethyl azodicarboxylate; (ii) 4 N HCl–MeOH; (b) potassium *tert*-butoxide, 4-fluoronitrobenzene; (c) substituted phenoxyethyl bromide, triethylamine, MeCN; H_2, $Pd(OH)_2/C$, MeOH.

ELH with a view to ensuring aqueous solubility by adding another basic amine. This approach is summarized in Figure 1.

Our next course of action was to improve the in vitro potency by way of adding a variety of optional moieties to piperazine. An initial SAR study was started with the replacement of R_1 (Table 1). The results of these modifications led us to identify compounds **21**, **22** and **23a** with low micromolar inhibitory activities for the neuronal VDCCs (Table 1). As regards introduction of lipophilic substituents (Table 2), less lipophilic compounds were preferable and **23a** (ClogP; **21**: 4.37, **22**: 4.75, **23a**: 4.32) was selected for further SAR study. Modification of substituents on the phenyl ring was first investigated. The SARs are summarized in Table 2. Introduction of a lipophilic group into the phenyl moiety, either an electron-withdrawing group or an electron-donating group, improved the blocking activity towards the neuronal VDCCs. Compound **23h**, which has a Cl substituent on the phenyl moiety, displayed increased VDCC blocking activity in the synaptosomes assay (**23h**, $IC_{50} = 3.4\,\mu M$). In contrast, introduction of a hydrophilic group was detrimental. Compound **23m** showed decreased VDCC blocking activity in the synaptosomes assay (**23m**, $IC_{50} = 13\,\mu M$).

A fluorine atom and a cyano group were each used as a probe to explore the activity of three regioisomers on the phenyl ring (**23b–d**, **23e–g**, Table 2). In the case of the fluorine atom, the trend of neuronal VDCC blocking potency was: **23d** (*para*) > **23c** (*meta*) > **23b** (*ortho*) (Table 2: $IC_{50} = 5.9$, 6.2 and 9.3 μM, respectively) with the *para* fluoro analogue (**23d**) being the most active of the three. In the case of the cyano group, however, the trend of potency was: **23e** (*ortho*) < **23g** (*para*) < **23f** (*meta*) (Table 2: $IC_{50} = 16$, 13 and 7.7 μM, respectively). Though both fluorine and cyano groups are electron-withdrawing, they exhibited significantly different SARs. We reasoned that a lipophilic group could favor potent activity for neuronal VDCCs blockade, while a hydrophilic group could be less favorable, as exemplified by 4-*N*,*N*-dimethylamino (**23n**) and 4-amino (**23m**) substituents. To test this hypothesis, naphthyl (**23q**) and pyridyl derivatives (**23i–k**) were prepared as a representative lipophilic and hydrophilic groups, respectively. In accordance with our hypothesis, **23q** exhibited potent activity ($IC_{50} = 2.5\,\mu M$), while **23i–k** had essentially lost the activity ($IC_{50} > 16\,\mu M$). This result suggested that polar functionality is poorly tolerated. In general, lipophilic compounds tended to show high potency with relatively little dependence of the activity on substituent ('flat' SAR; IC_{50} values ranged from 2.5 to 9.6 μM).

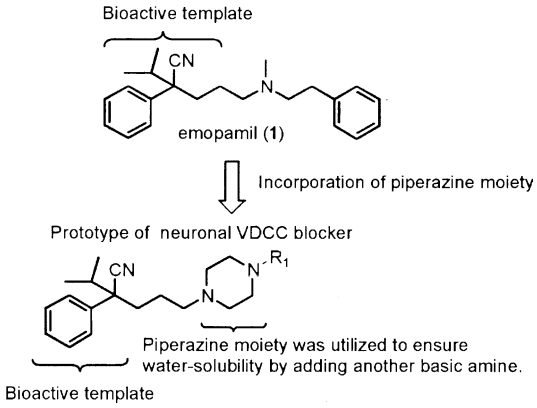
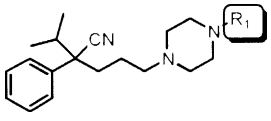
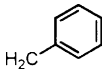
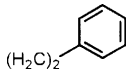
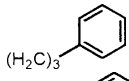
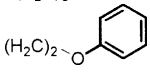


Figure 1. Library design applying ELH as a bioactive template for neuronal VDCC blocker.

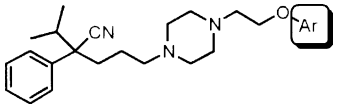
Table 1. Neuronal VDCC inhibitory activity of substituted piperazine analogues (**1**, **7–9**, **18–22** and **23a**)



Compd	R1	Synaptosomes[a] IC_{50} (μM)
Emopamil (**1**)		28
8	H	16 <
9	$(CH_2)_2OH$	16 <
18	H_2C–	16 <
19	$(H_2C)_4$-≡	16 <
20		16 <
7		12
21		7.7
22		4.5
23a		8.9

[a]See ref 12.

Table 2. Neuronal VDCC inhibitory activity of substituted piperazine analogues (**23a–q**)



Compd	Ar	Synaptosomes[a] IC_{50} (μM)
23a	Ph	8.9
23b	2-F-Ph	9.3
23c	3-F-Ph	6.2
23d	4-F-Ph	5.9
23e	2-CN-Ph	16
23f	3-CN-Ph	7.7
23g	4-CN-Ph	13
23h	4-Cl-Ph	3.4
23i	2-Pyridyl	16 <
23j	3-Pyridyl	16 <
23k	4-Pyridyl	16 <
23l	4-NO_2-Ph	7.5
23m	4-NH_2-Ph	13
23n	4-*N*,*N*-Dimethylamino-Ph	16 <
23o	4-MeO-Ph	9.6
23p	3,4-di-F-Ph	5.2
23q	1-Napthyl	2.5

[a]See ref 12.

Compound **23d**, which has a good balance of lipophilicity and aqueous solubility, was chosen as a representative potent compound. The anticonvulsant effect of **23d** was evaluated in the audiogenic seizure model, and clear antiseizure activity was observed (100% protection from the tonic phase at 10 mg/kg, iv).

In conclusion, ELH has been demonstrated to be a viable bioactive template for the preparation of a focused library aimed at neuronal VDCC blockers. Several analogues displayed higher affinity than **1** for neuronal VDCCs. Inhibitory activity for the neuronal calcium channels turned out to be closely associated with the balance between the lipophilicity and polarity. The representative compound **23d** exhibited an anticonvulsant effect in an audiogenic seizure model using DBA/2 mice and is considered to be a candidate neuroprotective agent. SARs for **23d** will be discussed elsewhere.

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