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Synthesis and biological evaluation of 1,4-diazepane derivatives as T-type calcium channel blockers

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ABSTRACT

We have synthesized and biologically evaluated 1,4-diazepane derivatives as T-type calcium channel blockers. In this study, we discovered compound **4s**, a potential T-type calcium channel blocker with good selectivity over hERG and N-type calcium channels. In addition, it exhibited favorable pharmacokinetic characteristics for further investigation of T-type calcium channel related diseases.

Low-voltage-activated calcium channels, also known as T-type calcium channels, play a crucial role in the regulation of neuronal excitability, in both the central and peripheral nervous systems.[1] Unlike other types of calcium channels, they comprise only a pore-forming α_1 subunit that is different from the calcium channel subtypes.[2] Three different genes encode the α_1 subunit of T-type calcium channels, termed α_{1G}, α_{1H}, and α_{1I}, respectively, each with its own distinct functional and pharmacological profile.[3] Mibefradil (Posicor, Hoffman-La Roche), the first selective T-type calcium channel blocker, was approved by the FDA for the treatment of hypertension and angina pectoris;[4] however, it was withdrawn from the US market in 1998 due to its interaction with the cytochrome P-450 3A4 enzyme which proved to be unrelated to T-type calcium channel blockage.[5] Recent studies have shown that inappropriate regulation of T-type calcium channels involves several pathophysiological diseases, such as epilepsy, pain, hypertension, congestive heart failure, and cancer.[6] Therefore, more potent and selective inhibitors are required to determine the fundamental function of T-type calcium channels in these disease states.

During the course of our program to develop a potential T-type calcium channel blocker, we have found that 1,3-dioxoisoindoline derivatives showed high potency and excellent selectivity against the T-type calcium channel over the N-type calcium channel.[7] However, further studies revealed that this particular molecular scaffold did not represent an acceptable pharmacological profile. To overcome this hurdle, we tried to find a new structural motif using the 3D ligand based pharmacophore model, which previously had been established by the hypothesis approach (HipHop) implemented in the CATALYST program.[8] As a result, we designed the 1,4-diazepane derivatives **4a–s** and **9a–s** having two hydrophobic aromatic components on both sides of the 1,4-diamines, Figure 1.

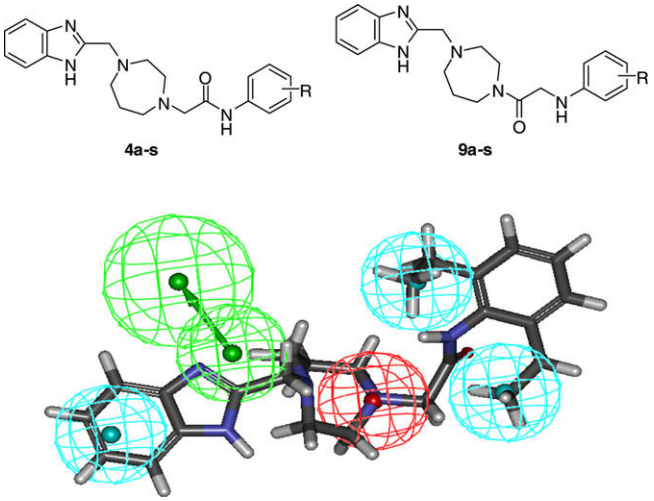

Figure 1. Designed structures of T-type calcium channel blocker and the structural mapping of in silico pharmacophore with **4a**.

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Comparing these compounds with Mibefradil, we hypothesized that the benzimidazoyl 1,4-diazepane might be acting as an inhibitory element. Thus, we planned to synthesize a series of new compounds by changing the position of the carbonyl group and replacing the substituents on the terminal phenyl group. In this study, we report our progress on the synthesis and biological evaluation of these 1,4-diazepane-based T-type calcium channel inhibitors.

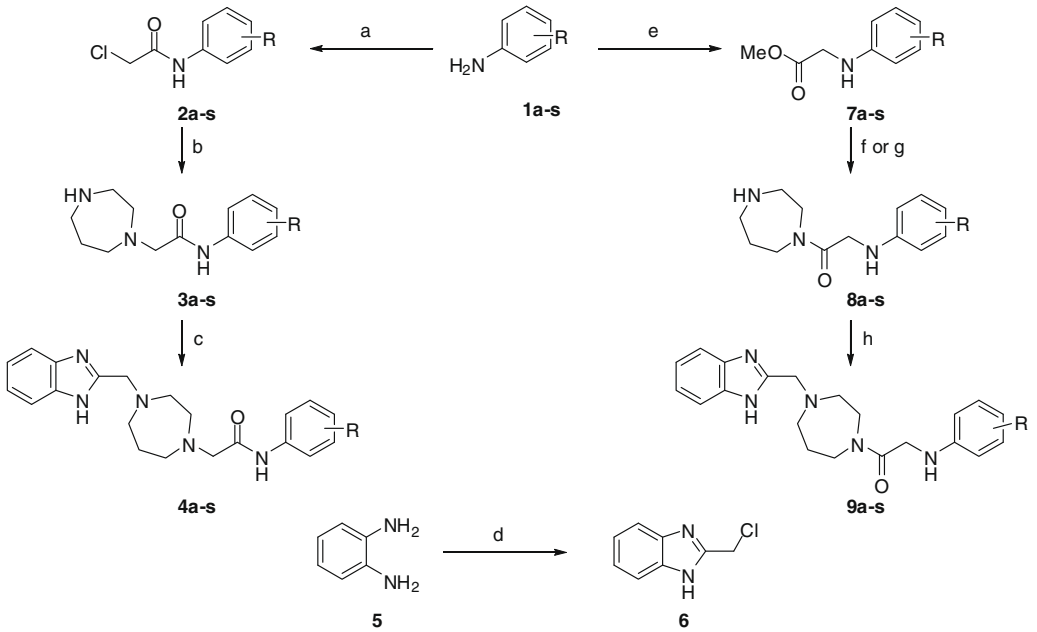
The synthesis of 1,4-diazepane derivatives is illustrated in Scheme 1. The anilines **1a–s** were reacted with chloroacetyl chloride to give the chloro amides **2a–s**, which were subjected to S_N2 displacement with 1,4-diazepane to afford the amines **3a–s**. The first series of target compounds **4a–s**[9] were obtained by coupling the amines **3a–s** with 2-chloromethyl benzimidazole **6**, prepared from condensation of the diamine **5** with chloroacetic acid in the presence of 5 N hydrochloric acid.

The second set of compounds, **9a–s**, was prepared following a reaction sequence similar to the first one. Alkylation of methyl bromoacetate with the anilines **1a–s** provided the esters **7a–s**. The 1,4-diazepanes **8a–s** were generated by microwave-assisted amide formation[10] of **7a–s** with either the free or the *N*-Boc-protected 1,4-diazepane. In the latter case, the additional removal of the Boc protective group was required to produce the corresponding amines. Finally, the treatment of **8a–s** with 2-chloromethyl benzimidazole **6** under basic conditions furnished the final diazepane derivatives **9a–s**.

The preliminary T-type calcium channel blocking activity of the synthesized compounds against the T-type calcium channel subtype α_{1G} exogenously expressed in HEK293 cells was evaluated using the FDSS6000 HTS system.[11] Table 1 summarizes the in vitro potency of **4a–s** and **9a–s**. Most of the compounds in the series **4a–s** exhibited greater than 40% inhibitory activity against the α_{1G} calcium channel at 10 μM concentration whereas the **9a–s** series showed relatively low activity except for **9a** and **9q**, indicating that the position of the carbonyl group is very important for the inhibition. The substituent on the phenyl ring had little effect. Next, we investigated the IC_{50} values of selected compounds using the whole-cell patch-clamp method[12] and the results are shown in Table 2. Among those tested, compound **4s,** bearing the 4-trifluoromethyl group on the terminal phenyl ring, turned out to be the best with an IC_{50} value of 1.23 μM. Additionally, we found that compound **4s** was highly selective for the T-type over the N-type channel and the hERG channel. For comparison purposes, we also evaluated the inhibitory activity of Mibefradil against those channels and found that it inhibited the T-type, hERG, and L-type channels with IC_{50} values of 1.34, 1.40 and 1.34 μM, respectively. These results indicated that in terms of selectivity, compound **4s** is far superior to Mibefradil and in terms of potency, it is just as active. Compound **9q**, which had the highest % of inhibition value in the FDSS assay, was tested with regard to the T-type and hERG channels. However, it gave a disappointingly lower efficacy and, in particular, poor selectivity. As a result, we discontinued investigation of this compound.

In order to determine the potential of compound **4s** as a lead compound for therapeutic targets, we subjected it to a pharmacokinetic profile assay. The pharmacokinetic data for **4s** after intravenous and oral administration in rats are presented in Table 3. Based on our analysis of its pharmacokinetic parameters, we discovered that compound **4s** was absorbed acceptably, eliminated relatively slowly, and possessed a good oral bioavailability of 43%. The considerably high value of the mean volume of distribution suggests that it tends to bind to tissue components or plasma proteins. Although the brain to plasma ratio in oral administration was only moderate, the significant brain penetration characteristic of **4s** (B/P ratio = 0.44) via intravenous administration indicated that the compound could be a viable candidate for treatment of CNS disorders.

In summary, we have synthesized and evaluated two series of 1,4-diazepane derivatives **4a–s**/**9a–s** as potential T-type calcium channel blockers. Using the FDSS HTS system, we rapidly screened the title compounds and selected several having high potency. On comparing the biological activities of Mibefradil, we identified the potent and highly selective T-type calcium channel blocker **4s**, which displays an excellent pharmacokinetic profile in rats. These results suggest that the 1,4-diazepane analogue **4s** will be a potential therapeutic candidate for the treatment of various neurological diseases related to the T-type calcium channel without cardiovascular side effects.



Scheme 1. Reagents and conditions: (a) chloroacetyl chloride, CH_2Cl_2, 0–23 °C, 79–99%; (b) 1,4-diazepane, CH_2Cl_2, 0 °C, 75–85%; (c) iPr_2NEt, **6**, DMF, 60 °C; (d) chloroacetic acid, 5 N HCl, 110–130 °C; (e) methyl bromoacetate, iPr_2NEt, DMF, 60 °C, 36–99%; (f) 1,4-diazepane, TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene), THF, μW, 75 °C, 27–64%; (g) (i) *N*-Boc-1,4-diazepane, TBD, THF, μW, 75 °C, 34–99%; (ii) TFA, CH_2Cl_2, or 1 N HCl, MeOH, 29–77%; (h) iPr_2NEt, DMF, 70 °C, 32–86%.

Table 1
In vitro T-type calcium channel blocking activity of 1,4-diazepane derivatives using FDSS6000 HTS systems[a]

Entry	Compounds	R	HEK293 cell % inhibition (10 μM)	
			4	**9**
1	**4a/9a**	2,6-Diethyl-	57.40	62.37
2	**4b/9b**	2,4-Dimethyl-	47.64	21.14
3	**4c/9c**	3,4-Dimethyl-	56.22	16.48
4	**4d/9d**	2,6-Dimethyl-	39.78	29.48
5	**4e/9e**	2-F–	32.35	22.31
6	**4f/9f**	3-F–	46.35	7.52
7	**4g/9g**	4-F–	46.64	12.77
8	**4h/9h**	2-Cl–	56.76	32.22
9	**4i/9i**	3-Cl–	41.66	40.87
10	**4j/9j**	4-Cl–	51.29	36.85
11	**4k/9k**	2-CH_3–	51.83	7.04
12	**4l/9l**	3-CH_3–	54.79	8.48
13	**4m/9m**	4-CH_3–	44.75	16.36
14	**4/n/9n**	2-CH_3O–	59.15	8.29
15	**4o/9o**	3-CH_3O–	46.07	−1.54
16	**4p/9p**	4-CH_3O–	32.36	0.92
17	**4q/9q**	2-CF_3–	41.68	66.00
18	**4r/9r**	3-CF_3–	60.07	27.29
19	**4s/9s**	4-CF_3–	60.73	28.19
Mibefradil			78.92	

[a] % inhibition value was obtained at 10 μM.

Table 2
Inhibitory activity of selected compounds against T-type calcium, hERG, and N-type calcium channels

Compounds	T-type (α_{1G}) IC_{50}, μM[a]	hERG IC_{50}, μM[a]	N-type (α_{1B}) IC_{50}, μM[a]
4a	2.08 ± 0.22		
4c	2.39 ± 0.21		
4h	1.47 ± 0.16		
4n	12.60 ± 0.87		
4s	1.23 ± 0.04	4.97 ± 1.90	28.71 ± 8.71
9a	4.00 ± 1.31		
9q	3.17 ± 0.84	1.32 ± 0.17	
Mibefradil	1.34 ± 0.49	1.40 ± 0.29	1.34 ± 0.02

[a] IC_{50} value(±SD) was obtained from a dose–response curve.

Table 3
Mean pharmacokinetic parameters in rat plasma following intravenous ($n = 4$) and oral ($n = 3$) administration of **4s**

	Intravenous	Oral
C_{max} (μg/ml)	—	2.284 (±0.6358)
T_{max} (min)	—	120 (60–120)[a]
$T_{1/2}$ (min)	340.5 (±106.1)	260.3 (±81.65)
V_{dss} (ml/kg)	2325 (±731.1)	—
B/P ratio	0.4382 (±0.2306)	0.1048 (±0.03623)
F (%)	—	43.42%

Values are presented as mean (standard deviation in parentheses). C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}; V_{dss}, apparent volume of distribution at steady state; F, bioavailability.

[a] Median (range) for T_{max}.

Acknowledgment

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9. *Spectral data of compound* **4s**: ^1H NMR ($CDCl_3$, 400 MHz) δ 9.44 (br s, 1H), 7.67 (d, 2H, J = 8.50 Hz), 7.54 (q, 4H, J = 2.69 Hz), 7.24–7.21 (m, 2H), 3.97 (s, 2H), 3.25 (s, 2H), 2.83–2.79 (m, 8H), 1.86–1.82 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.5, 152.2, 140.6, 126.3, 125.8, 125.4, 122.6, 119.1, 63.5, 62.3, 60.4, 56.5, 55.8, 54.7, 29.7, 27.9. LRMS (m/z): [M+H$^+$] calcd for $C_{22}H_{25}F_3N_5O$ 432.5, Found 432.2.
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11. *Experimental procedure for the FDSS6000 assay*: HEK293 cells which express both stable a_{1G} and Kir2.1 subunits were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL), geneticin (500 μg/mL), and puromycin (1 μg/mL) at 37 °C in a humid atmosphere of 5% CO_2 and 95% air. Cells were seeded into 96-well black wall clear bottom plates at a density of 4×10^4 cells/well and were used the next day for the high-throughput screening (HTS) FDSS6000 assay. For the FDSS6000 assay, cells were incubated for 60 min at room temperature with 5 μM fluo3/AM and 0.001% Pluronic F-127 in a Hepes-

buffered solution composed of (in mM): 115 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 1.8 $CaCl_2$, 20 Hepes, and 13.8 glucose (pH 7.4). During the fluorescence-based FDSS6000 assay, in which a_{1G} T-type Ca^{2+} channels were activated using a high concentration of KCl (70 mM) in 10 mM $CaCl_2$ containing a Hepes-buffered solution, an increase in $[Ca^{2+}]_i$ by KCl-induced depolarization was detected. Throughout the entire procedure, cells were washed in a BIO-TEK 96-well washer. All data were collected and analyzed using FDSS6000 and related software (Hamamatsu, Japan).

12. *Experimental procedure for the patch-clamp test (electro-physiological recording)*: For the recording of a_{1G} T-type Ca^{2+} currents, the standard whole-cell patch-clamp method was utilized. Briefly, borosilicate glass electrodes with a resistance of 3–4 MX were pulled and filled with the internal solution containing (in mM): 130 KCl, 11 EGTA, 5 Mg–ATP, and 10 Hepes (pH 7.4). The external solution contained (in mM): 140 NaCl, 2 $CaCl_2$, 10 Hepes, and 10 glucose (pH 7.4). a_{1G} T-type Ca^{2+} currents were evoked every 15 s by a 50 ms depolarizing voltage step from −100 mV to −30 mV. The molar concentrations of test compounds required to produce 50% inhibition of peak currents (IC_{50}) were determined from fitting raw data into dose–response curves. The current recordings were obtained using an EPC-9 amplifier and Pulse/Pulsefit software program (HEKA, Germany). For more details, see: Rhim, H.; Lee, Y. S.; Park, S. J.; Chung, B. Y.; Lee, J. Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 283.