Bioorganic & Medicinal Chemistry Letters 20 (2010) 4219–4222

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and biological evaluation of oxazole derivatives as T-type calcium channel blockers

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ARTICLE INFO

Article history:
Received 16 March 2010
Revised 11 May 2010
Accepted 12 May 2010
Available online 15 May 2010

Keywords:
T-type calcium channel
HTS assay
α_{1G} Subtype
Neuropathic pain
Oxazole

ABSTRACT

T-type calcium channel is one of therapeutic targets for the treatment of cardiovascular diseases and neuropathic pain. In this study, as a part of our ongoing efforts to develop potent T-type calcium channel blockers, we designed oxazole derivatives substituted with arylpiperazinylalkylamines. The oxazoles were synthesized in a convenient convergent synthetic method, and biologically evaluated against α_{1G} ($Ca_V3.1$) T-type calcium channel. Among total 41 oxazole compounds synthesized, the most active one was the compound **10-35** with an IC_{50} value of 0.65 μM, which is comparable with that of mibefradil.

Calcium is an essential signaling molecule with fundamental physiological roles that range from activation of calcium-dependent enzymes to the secretion of neurotransmitters and hormones.[1,2] The most abundant route of calcium entry into electrically excitable cells is by opening of voltage-gated calcium channels.[1,2] The highest organisms express several different types of voltage-gated calcium channels, which are grossly divided into two categories: high-voltage activated (HVA) and low-voltage activated (LVA) calcium channels.[3] Members of the HVA channel family include the N-, P-, Q-, L-, and R-types and typically require large membrane depolarizations to open.[3] LVA calcium channels, also known as T-types, are widely expressed in various types of neurons and can be activated by a weak depolarization near the resting membrane potential and regulate the excitability and electroresponsiveness of neurons under physiological conditions near resting states.[3]

Since mibefradil, a selective T-type calcium channel blocker, approved for the treatment of angina pectoris and hypertension (Fig. 1), was withdrawn from the market in 1998 due to unfavorable drug–drug interaction,[4,5] there have been efforts to discover novel T-type calcium channel blockers.[6] According to accumulation of new findings on T-type calcium channels, it has been reported that T-type calcium channels play crucial roles in the control of pain which are caused by hyperexcitable neurons.[7] The role of T-type calcium channels in pain has been addressed using specific genetic modulation of T-type calcium channel isoforms. Compared with wild-type mice, $Ca_V3.1$ knockout ($\alpha_{1G}^{-/-}$) mice were observed, in which, after L5 spinal nerve ligation, spontaneous pain responses were reduced and a threshold for paw withdrawal was increased in response to mechanical stimulation.[8] $Ca_V3.2$ antisense treatment resulted in major anti-nociceptive and anti-hyperalgesic effect, suggesting that $Ca_V3.2$ plays a major pronociceptive role in acute and chronic pain states.[9] Together, the results of these two studies suggest that blocking T-type calcium channels should reduce nociceptive pain and neuropathic pain. T-type calcium channel blockers such as ethosuximide and mibefradil were reported to reverse neuropathic pain in animal models.[10]

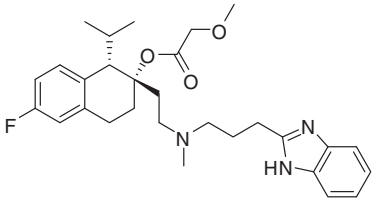

Figure 1. Structure of mibefradil.

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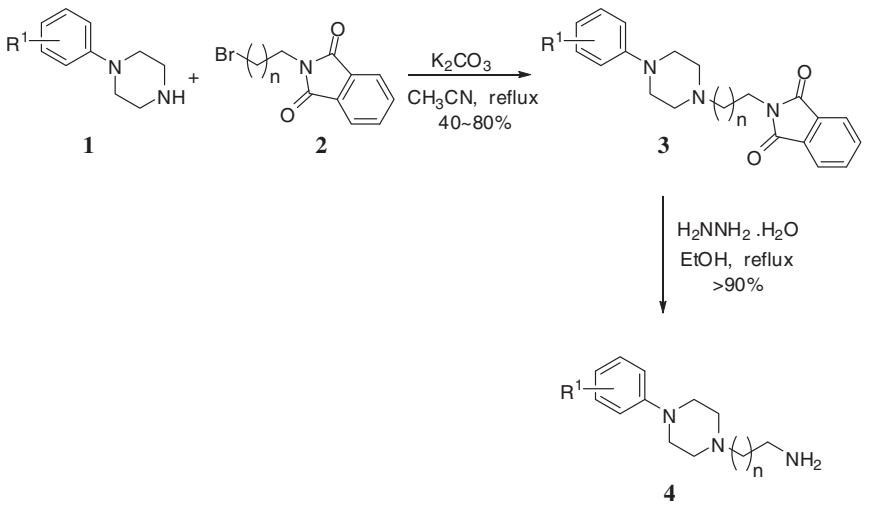
doi:10.1016/j.bmcl.2010.05.030

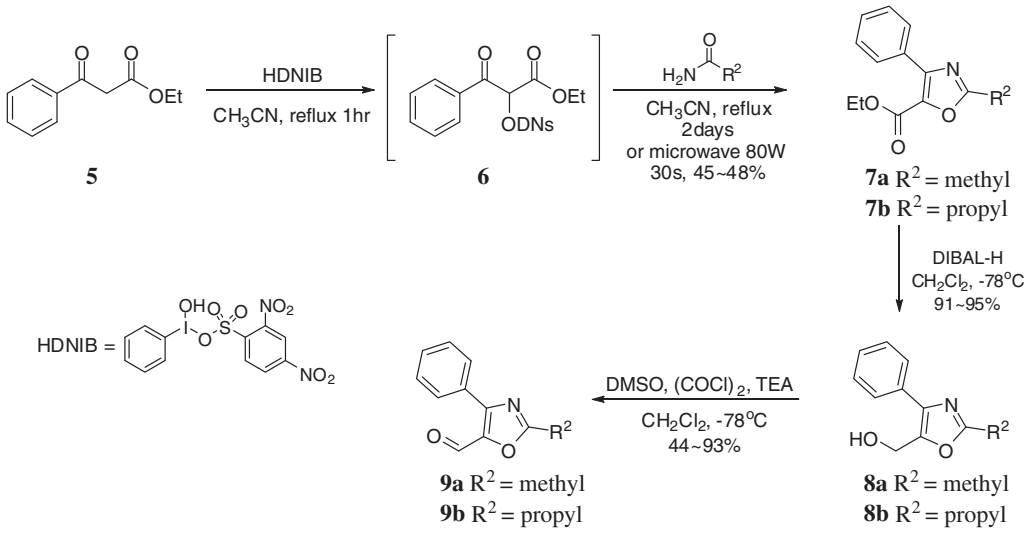
Previously, we reported isoxazole derivatives substituted with arylpiperazinylalkylamines as T-type calcium channel blockers.[6e] Their activities depended on the substituents on isoxazoles, aryl moieties of arylpiperazine, and alkyl chains. In this study, to obtain better pharmacological activities against T-type calcium channel, we introduced an oxazole group instead of the isoxazole group and more diverse aryl moieties at arylpiperazines. By using an oxazole scaffold, structurally unaccessible positions of the isoxazole ring can be substituted with various functional groups, which would allow extensive structure–activity relationship study. Herein, we report the synthesis and T-type calcium channel inhibitory activities of oxazole derivatives with arylpiperazinylalkylamines substituents.

The strategy to synthesize the designed oxazole derivatives is a convergent synthesis, where the separately prepared arylpiperazinylalkylamine part (Scheme 1) and the oxazole part (Scheme 2) were combined through reductive amination to afford the desired oxazole derivatives (Scheme 3).

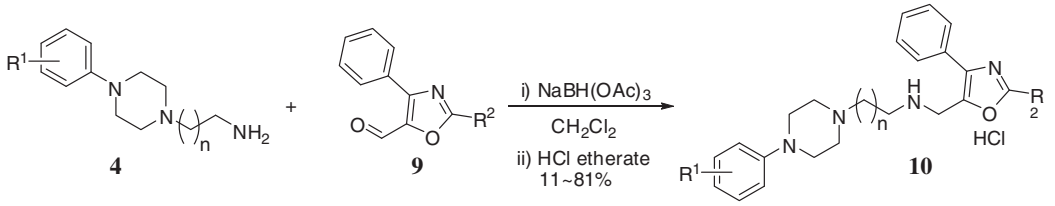
Arylpiperazinylalkylamines **4** were synthesized starting from arylpiperazines **1** in two steps. Various arylpiperazines underwent alkylation reaction with *N*-(2-bromoethyl)phthalimide ($n = 1$) or *N*-(3-bromopropyl)phthalimide ($n = 2$) to afford compounds **3** in



Scheme 1. Synthesis of arylpiperazinylalkylamines.



Scheme 2. Synthesis of oxazole parts.



Scheme 3. Convergent synthesis of desired oxazole derivatives **10**.

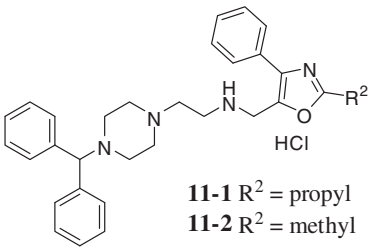
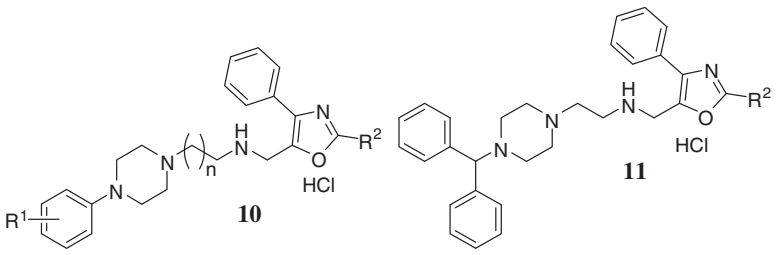


Figure 2. Benzhydrylpiperazine derivatives **11**.

40–80% yields. The compounds **3** were converted to the corresponding amines **4** by treatment with hydrazine in refluxing EtOH in over 90% yields (Scheme 1).

The oxazole parts, oxazole aldehydes **9**, were synthesized starting from ethyl benzoylacetate **5** in four steps. The benzoylacetate **5** was treated with hydroxyl(2,4-dinitrobenzensulfonyloxy)iodobenzene (HDNIB), which was easily prepared from diacetoxyiodobenzene and 2,4-dinitrobenzensulfonic acid, to afford an intermediate **6** installed with a good leaving group, 2,4-dinitrobenzenesulfonate (ODNs) (Scheme 2).[11] The intermediate **6** was condensed with acetamide in acetonitrile under reflux conditions to give one oxazole **7a** with R^2 as methyl group in 45% yield, while reaction with butyramide under microwave irradiation afforded the other oxazole **7b** with R^2 as propyl group in 48% yield. The oxazoles **7** underwent reduction by treating with DIBAL-H to afford the corresponding alcohols **8a** and **8b** in 91–95% yields. Swern oxidation of the alcohols **8** provided the corresponding aldehydes **9a** and **9b** in 44–93% yields.

Table 1
Inhibitory activities of oxazole derivatives **10** and **11** against α_{1G} ($Ca_V3.1$) T-type calcium channel



Entry	Compd	R^1	R^2		%Inhbition at 10 μM	IC_{50} in μM
1	**10-1**	H	Propyl	1	54.26	10.25 ± 0.91
2	**10-2**	2-F	Propyl	1	46.49	—[b]
3	**10-3**	3-F	Propyl	1	62.34	3.35 ± 0.26
4	**10-4**	4-F	Propyl	1	54.08	7.08 ± 0.29
5	**10-5**	2-Cl	Propyl	1	60.07	4.37 ± 0.30
6	**10-6**	3-Cl	Propyl	1	51.24	1.45 ± 0.9[b]
7	**10-7**	4-Cl	Propyl	1	55.84	2.82 ± 0.04
8	**10-8**	2-CH_3	Propyl	1	51.34	4.30 ± 0.41
9	**10-9**	3-CH_3	Propyl	1	43.13	—[b]
10	**10-10**	4-CH_3	Propyl	1	48.06	—[b]
11	**10-11**	2-OCH_3	Propyl	1	39.73	—[b]
12	**10-12**	3-OCH_3	Propyl	1	40.17	—[b]
13	**10-13**	4-OCH_3	Propyl	1	51.49	9.20 ± 0.46
14	**10-14**	2-CF_3	Propyl	1	54.62	2.10 ± 0.09
15	**10-15**	3-CF_3	Propyl	1	56.30	1.94 ± 0.12
16	**10-16**	4-CF_3	Propyl	1	58.70	1.30 ± 0.08
17	**10-17**	2,3-$DiCH_3$	Propyl	1	66.68	1.71 ± 0.16
18	**10-18**	2,4-$DiCH_3$	Propyl	1	64.28	2.22 ± 0.11
19	**10-19**	3,4-$DiCH_3$	Propyl	1	67.87	2.22 ± 0.27
20	**10-20**	2-Pyridyl[a]	Propyl	1	48.13	—[b]
21	**10-21**	H	Propyl	2	43.69	—[b]
22	**10-22**	2-F	Propyl	2	54.41	16.13 ± 3.48
23	**10-23**	3-F	Propyl	2	68.94	3.03 ± 0.22
24	**10-24**	4-F	Propyl	2	62.53	3.21 ± 0.20
25	**10-25**	2-Cl	Propyl	2	64.72	2.80 ± 0.39
26	**10-26**	3-Cl	Propyl	2	65.88	1.75 ± 0.04
27	**10-27**	4-Cl	Propyl	2	63.25	1.31 ± 0.05
28	**10-28**	2-CH_3	Propyl	2	54.45	8.75 ± 5.4
29	**10-29**	3-CH_3	Propyl	2	47.78	1.19 ± 0.6
30	**10-30**	4-CH_3	Propyl	2	53.92	130.01 ± 10.4
31	**10-31**	2-OCH_3	Propyl	2	41.03	—[b]
32	**10-32**	3-OCH_3	Propyl	2	36.35	—[b]
33	**10-33**	4-OCH_3	Propyl	2	42.13	—[b]
34	**10-34**	2-CF_3	Propyl	2	44.99	—[b]
35	**10-35**	3-CF_3	Propyl	2	51.63	0.65 ± 0.3
36	**10-36**	4-CF_3	Propyl	2	51.62	7.25 ± 3.7
37	**10-37**	2,3-$DiCH_3$	Propyl	2	55.24	1.78 ± 0.14
38	**10-38**	3,4-$DiCH_3$	Propyl	2	54.59	3.02 ± 0.18
39	**10-39**	4-Cl	Methyl	2	38.08	—[b]
40	**11-1**	—[b]	Propyl	—[b]	71.85	7.47 ± 1.69
41	**11-2**	—[b]	Methyl	—[b]	57.32	—[b]
42		Mibefradil			80.00	0.83 ± 0.19

[a] Pyridyl instead of R^1-phenyl.
[b] Not available or determined.

Arylpiperazinylalkylamines **4** and the oxazole aldehydes **9** were combined by reductive amination to afford compounds **10** in 11–81% yields (Scheme 3). Instead of R^1-substituted phenyl, benzhydryl group was also introduced with as same procedures as shown here to afford compounds **11** in Figure 2.

A total of 41 oxazole derivatives **10** and **11** were biologically evaluated against α_{1G} ($Ca_V3.1$) T-type calcium channel in HEK293 cells which stably express both T-type calcium channel $Ca_V3.1$ and potassium channel Kir2.1.[12] All the synthesized compounds were screened by fluorescence-based high-throughput screening (HTS) FDSS6000 assay,[13] and the %-inhibitions of Ca^{2+} current measured at 10 μM concentration of the oxazole derivatives are summarized in Table 1. Among those, compounds with over 50% inhibition of Ca^{2+} current in FDSS assay were selected for patch-clamp assays using single cells to evaluate IC_{50} values.[14,15]

In general, the %-inhibition results from the fluorescence-based HTS FDSS6000 assay do not directly correlate with the IC_{50} values obtained from patch-clamp assays. In this study, we also observed that the two compounds **10-23** and **10-19** with the highest %-inhibitions show inhibitory activities with moderate IC_{50} values of 3.03 μM and 2.22 μM, respectively. On the other hand, two compounds **10-35** and **10-16** with the most potent IC_{50} values have low inhibition of T-type calcium channel activity (51.63% and 58.70% inhibitions, respectively). Nevertheless, the filtering process using FDSS6000 assay is necessary because compounds active in both fluorescence-based and electrophysiological methods could have more possibility to be eventually effective in in vivo system. The %-inhibition results were used as primary screening tool to filter compounds for further laborious patch-clamp assays.

A total of 28 compounds were selected to obtain IC_{50} values against T-type calcium channel, especially α_{1G} subtype. The IC_{50} values are between 0.65 μM and 130.01 μM as shown in Table 1. There is no huge difference in inhibitory activities according to the chain length ($n = 1$ or 2). However, the electronic nature of the R^1 substituents exerted subtle effects on the activities of the compounds. The compounds with electron-withdrawing groups such as F, Cl, and CF_3 are more potent than those with electron-donating groups such as OCH_3. The position of the substituent also affects the biological activity. Generally, oxazole analogues with electron-withdrawing R^1 substituents at *meta* position show good inhibitory activities against T-type calcium channel. The compound **10-3** with *meta*-fluoro (3-F) substituent shows better inhibitory activity than **10-2** and **10-4** with *ortho*- and *para*-fluoro groups, respectively. By the same token, compounds **10-6**, **10-23**, and **10-35** are more potent than the corresponding regioisomers. Among those, the compound **10-35** with *meta*-CF_3 (3-CF_3) shows best inhibitory activity against α_{1G} ($Ca_V3.1$) T-type calcium channel with an IC_{50} value of 0.65 μM which is comparable to that of mibefradil.[16] In the case of compounds **10-15** and **10-26** with *meta*-substituents, their activities were only slightly lower than those of compounds **10-16** and **10-27** with *para*-substituents. On the other hand, it is noteworthy that the dimethyl substituted compounds **10-17**, **10-18**, **10-19**, **10-37**, and **10-38** show potent activities with IC_{50} values of 1.78–3.02 μM, regardless of the substitution patterns. The compound **11-1** with a benzhydrylpiperazine group shows the highest %-inhibition with 71.85%, but shows only moderate activity with an IC_{50} value of 7.47 μM. When R^2 substituents are changed from a propyl group to a methyl group, activities are reduced (entries 39 and 41 in Table 1).

Previously, we reported isoxazole derivatives as T-type calcium channel blockers,[6e] in which compounds with 3-CF_3–phenylarylpiperazinylalkylamine substituent showed the most potent inhibitory activity against T-type calcium channel. In this oxazole series, the most active compound **10-35** also has R^1 substituent as 3-CF_3. For this reason, it is fair to assume that the aromatic *meta*-trifluoromethyl substituent may play an important role in inhibitory activity of oxazoles as well as isoxazole derivatives.

In summary, oxazole derivatives with arylpiperazinylalkylamines were designed, synthesized, and biologically evaluated against α_{1G} ($Ca_V3.1$) T-type calcium channel. Among total 41 oxazole compounds synthesized, the most active one was the compound **10-35** with an IC_{50} value of 0.65 μM, which is comparable to that of mibefradil. The compound **10-35** possesses 3-CF_3 as R^1 substituent, which correlates well to the results of previous isoxazole series. Further evaluations of the compound **10-35** such as selectivity for other calcium channels, pharmacokinetics and neuronal analgesic effect are in progress.

Acknowledgement

This work was supported by Korea Institute of Science and Technology.

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15. *Two methods are available:* One is a manual patch-clamp assay (EPC 10®, HEKA, Germany) and the other is an automated patch-clamp assay (NPC®-16 patchliner, Nanion Technologies, Germany). Either of the patch-clamp assays was used to obtain IC_{50} values of the selected oxazole derivatives, irrespective of kinds of the compounds, because both IC_{50}s of mibefradil from two methods was practically same with IC_{50} values of 0.86 μM and 0.83 μM, respectively.
16. *Spectral data:* Free form of **10-35**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (t, $J = 7.45$ Hz, 3H), 1.70–1.89 (m, 4H), 2.48 (t, $J = 7.07$ Hz, 2H), 2.59 (br t, $J = 5.1$ Hz, 4H), 2.72–2.79 (m, 4H), 3.19 (br t, $J = 5.1$ Hz, 4H), 4.02 (s, 2H), 6.98–7.13 (m, 3H), 7.23–7.36 (m, 2H), 7.38–7.45 (m, 2H), 7.65 (dd, $J = 8.3$, 1.3 Hz, 2H); ^{13}C NMR (100 M Hz, $CDCl_3$) δ 13.7, 20.6, 26.0, 30.1, 43.7, 78.2, 78.5, 53.0, 57.1, 112.1, 115.9, 118.7, 124.3 (q, $J = 272.4$ Hz), 127.1, 127.7, 128.7, 129.5, 131.4 (q, $J = 31.7$ Hz), 131.8, 136.5, 144.4, 151.3, 163.7; HCl salt form of **10-35**: ^{1}H NMR (400 MHz, MeOD) δ 1.06 (t, $J = 7.45$ Hz, 3H), 1.89 (sxt, $J = 7.43$ Hz, 2H), 2.21–2.36 (m, 2H), 2.88 (t, $J = 7.45$ Hz, 2H), 3.24–3.34 (m, 8H), 3.62–3.75 (m, 2H), 3.84–4.00 (m, 2H), 4.61 (s, 2H), 7.20 (d, $J = 7.58$ Hz, 1H), 7.25–7.31 (m, 2H), 7.42–7.47 (m, 2H), 7.52 (t, $J = 7.33$ Hz, 2H), 7.71 (d, $J = 7.33$ Hz, 2H); HRMS (FAB, M+1) calcd for $C_{27}H_{34}F_3N_4O$ 487.2679, found 487.2683.