Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Selective acetylcholinesterase inhibitors derived from muscle relaxant dantrolene

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ARTICLE INFO

Keywords:
Acetylcholinesterase
Muscle relaxant
Dantrolene
Alzheimer's disease

ABSTRACT

Dantrolene, the only therapeutic agent for malignant hyperthermia, is known to have not only a muscle relaxant effect, but also a neuroprotective effect and Alzheimer's disease improving effect. Recently, it has been reported that dantrolene has a weak inhibitory effect on acetylcholinesterase (AChE), which is a therapeutic drug target for Alzheimer's disease. Thus, we focused on developing of AChE inhibitors with benzylpiperidine/piperazine moieties that are based on the dantrolene skeleton. Several derivatives showed an inhibitory activity. Among them, *ortho*-nitro derivative **8c** showed the most potent inhibitory activity with the IC_{50} value of 34.2 nM. Furthermore, Lineweaver-Burk plot analysis indicated that **8c** is AChE-selective inhibitor, which shows only a weak inhibitory effect on butyrylcholinesterase (BuChE) and a non-competitive inhibition.

Dantrolene is a hydantoin compound that was developed in 1967 by Snyder and co-workers as a muscle relaxant.[1] Later, its application to treat malignant hyperthermia was studied in the late 1970s,[2,3] and it was approved by the FDA as a treatment for malignant hyperthermia in 1979, and it remains the only treatment to date.[4]

The effect of dantrolene on muscle relaxation and malignant hyperthermia is attributed to the suppression of Ca^{2+}-releasing channels that are called ryanodine receptors (RyRs).[5,6] There are three subtypes of RyR, i.e. skeletal-type (RyR1), cardiac-type (RyR2) and brain-type (RyR3). For these subtypes, dantrolene is known to suppress the release of Ca^{2+} from RyR1 and RyR3. RyRs are expressed in various tissues, although there are differences in their distribution among subtypes.[7,8] Therefore, it has been reported that dantrolene show improvement effect not only on malignant hyperthermia but also on various pathologies caused by the breakdown of calcium homeostasis (e.g. stroke, ischemia/reperfusion injury and neurodegenerative diseases).[9,10]

Thus, although the pharmacological effect exhibited by dantrolene has been mainly attributed to the suppression of the function of RyRs, recently it has been confirmed that it binds to molecules other than RyRs. For example, dantrolene binds to the NMDA receptor in the brain and suppresses its activation.[11] In addition to receptor proteins, inhibitory activity of acetylcholinesterase (AChE) has also been reported.[12]

We have been studying the function of muscle contraction using dantrolene derivatives and developing functional molecules by chemical modification of dantrolene. During our studies, various dantrolene derivatives were developed,[13,14] and a protein related to sugar metabolism was discovered by a photoaffinity labelled probe that suppresses skeletal muscle contraction.[13,15] In addition, we successfully developed a dantrolene derivative that suppresses mitochondrial swelling caused by Ca^{2+} overload, which is an *in vitro* model of ischemia/reperfusion injury.[16]

This time, we focused on the recent report that dantrolene shows an inhibitory effect on acetylcholinesterase.[12] Thus, more useful derivatives can be developed by appropriately modifying dantrolene. At first, AChE inhibitory activity of dantrolene was confirmed according to literature information, but the inhibitory activity could not be confirmed in our evaluation system. However, we determined that dantrolene has the ability to recognise acetylcholinesterase from the fact that dantrolene has an improvement effect on Alzheimer's disease and exhibits various neuroprotection activities. Moreover, most of the cholinesterase expressed in the brain is AChE, but it is known that the expression level of butyrylcholinesterase (BuChE), the other isozyme of cholinesterase, is higher in the blood. Since a decrease in blood cholinesterase concentration increases the risk of causing a cholinergic crisis, it is considered important to develop compounds that selectively suppress AChE over BuChE from the viewpoint of drug discovery targeting brain diseases. Thus, we decided to chemically modify dantrolene with the aim of creating a selective AChE inhibitor.

The catalytic site of AChE has a narrow and deep pocket structure,

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https://doi.org/10.1016/j.bmcl.2019.126888
Received 31 October 2019; Received in revised form 3 December 2019; Accepted 3 December 2019

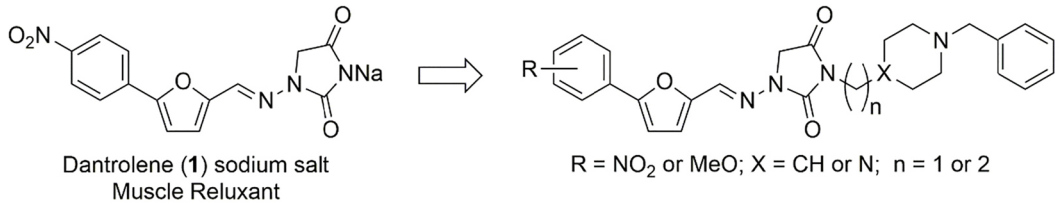
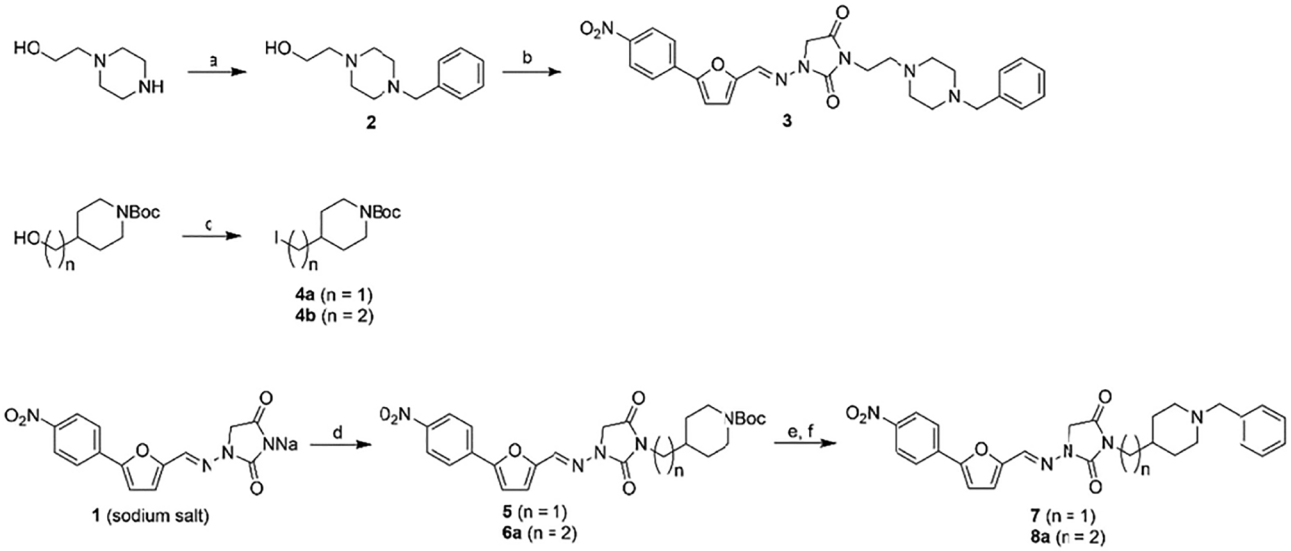
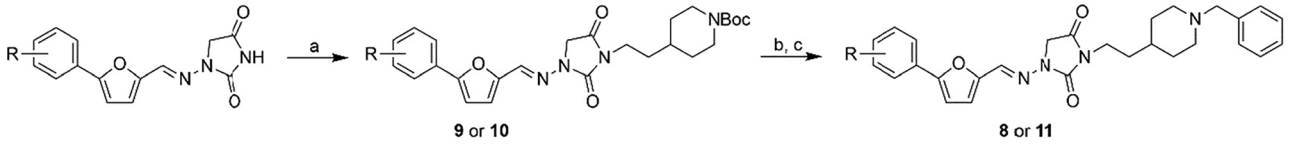


Fig. 1. Design of AChE inhibitors derived from muscle relaxant dantrolene.



Scheme 1. Reagents and conditions: (a) BnBr, Et_3N, MeCN, rt, 52%; (b) **1**, DEAD, PPh_3, THF, 60 °C, 5.7%; (c) I_2, PPh_3, imidazole, THF, rt, 97% (n = 1), 88% (n = 2); (d) **4a** or **4b**, DMF, rt, 52% (n = 1), 96% (n = 2); (e) 4.0 M HCl (1,4-dioxane soln), CH_2Cl_2, rt; (f) BnBr, K_2CO_3, DMF, rt, 75% (**7**, 2 steps), 55% (**8a**, 2 steps).



Scheme 2. Reagents and conditions: (a) **4b**, K_2CO_3, DMF, rt, 82%-quant.; (b) 4.0 M HCl (1,4-dioxane soln), CH_2Cl_2, rt; (c) BnBr, K_2CO_3, DMF, rt, 38–63% (2 steps).

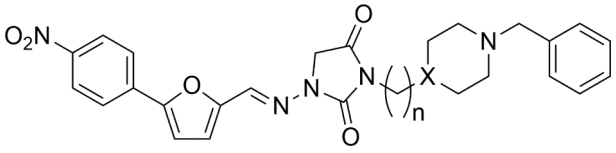
and benzylpiperidine inhibitors represented by donepezil are known to form a cation-π interaction with Phe330 at the catalytic site when the piperidine ring nitrogen is cationised.[17,18] Therefore, a compound in which this benzylpiperidine unit and a piperazine unit of a six-membered ring species capable of cationization were introduced into the hydantoin portion of dantrolene was designed (Fig. 1).

The syntheses of piperidine/piperazine moieties introduced at hydantoin of dantrolene and dantrolene derivatives are outlined in Schemes 1 and 2, respectively. Briefly, commercially available 1-(2-hydroxyethyl)piperazine was benzylated with benzyl bromide followed by the introduction of a benzylpiperazine unit into hydantoin under Mitsunobu reaction condition to give **3**. The preparation of dantrolene derivatives with benzylpiperidine moiety was carried out using dantrolene sodium salt and 1-[(*tert*-butoxycarbonyl)-4-iodomethyl]piperidine (**4a**)[19] or 1-[(*tert*-butoxycarbonyl)-4-(2-iodoethyl)]piperidine (**4b**)[20] prepared from the corresponding alcohol to obtain Boc protected compounds **5** and **6a**. Then, the deprotection of Boc group under acidic condition followed by benzylation with benzyl bromide to give dantrolene derivatives with benzylpiperidine moiety **7** and **8a**.

The effects of dantrolene sodium salt, three synthesized compounds (i.e. **3**, **7** and **8a**), and Tacrine hydrochloride (positive control) on AChE activity were determined by Ellman method[21] in sodium phosphate buffer (pH 8.0) in the absence or presence of the abovementioned compounds. First, we measured the enzyme inhibition rate of compounds at a fixed concentration of 1.0 μM against AChE, as summarized in Table 1. Dantrolene sodium salt and dantrolene derivative with *N*-benzylpiperidine moiety connected by a methylene bridge **7** did not show the AChE inhibitory activity, whereas derivatives with *N*-benzylpiperazine (**3**) and *N*-benzylpiperidine (**8a**) moieties connected by an ethylene bridge exhibited a significant AChE inhibitory activity. Tacrine hyrdochloride, a positive control compound, also significantly suppressed AChE activity.

On the basis of the abovementioned result, we synthesized dantrolene derivatives, which are based on **8a** with nitro (**8b** and **8c**) or

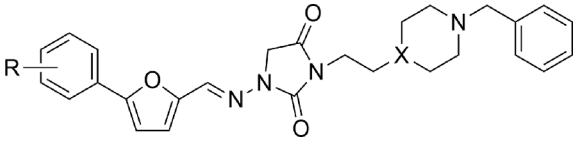
Table 1
Effect of dantrolene sodium salt and its derivatives **3**, **7** and **8a** on AChE inhibition.



Compound	X	n	Inhibition rate (%)[a]
Dantrolene (**1**)[b]	–	–	N.I.[c]
Tacrine·HCl	–	–	70.8[d]
3	N	2	67.9
7	CH	1	N.I.[c]
8a	CH	2	91.3

[a] At 1.0 μM.
[b] Sodium salt.
[c] No inhibition.
[d] At 0.10 μM.

Table 2
Effect of dantrolene derivatives **3**, **8a-c** and **11a-c** on AChE and BuChE inhibition.



Compound	R	X	IC_{50} value for AChE (nM)	Inhibition rate for BuChE (%)[a]
3	4-NO_2	N	201	N.I.[b]
8a	4-NO_2	CH	46.4	N.I.[b]
8b	3-NO_2	CH	290	N.I.[b]
8c	2-NO_2	CH	34.2	14.6 (4.47 μM)[c]
11a	4-MeO	CH	108	4.6
11b	3-MeO	CH	47.0	3.8
11c	2-MeO	CH	90.4	1.6
Tacrine·HCl	–	–	106[d]	64.5[e]

[a] At 1.0 μM.
[b] No inhibition.
[c] IC_{50} value in parenthesis.
[d] Literature value in Ref. 22.
[e] At 20 nM.

methoxy (**11a–c**) groups to examine the effect of substituents on the phenyl group of **8a** on AChE inhibition. At the same time, the inhibitory effect of the compounds on BuChE was performed, and the selectivity for the cholinesterase isozyme was also evaluated. Syntheses of these compounds were carried out in the same manner as the synthesis of **8a**, as summarized in Scheme 2.

As shown in Table 2, all synthesized compounds showed moderate to potent AChE inhibition activity. Compound **8b** (with an electron withdrawing group in the *meta* position) and compounds **11a** and **11b** (with an electron donating group in the *ortho* or *para* position) showed lower activity than that of **8a** and the same or slightly stronger activity compared to that of piperazine compound **3**. In contrast, **8c** (with an electron withdrawing group in the *ortho* position) and **11b** (with an electron donating group in the *meta* position) exhibited the same or more potent activity than **8a**. Especially, **8c** indicated the most potent

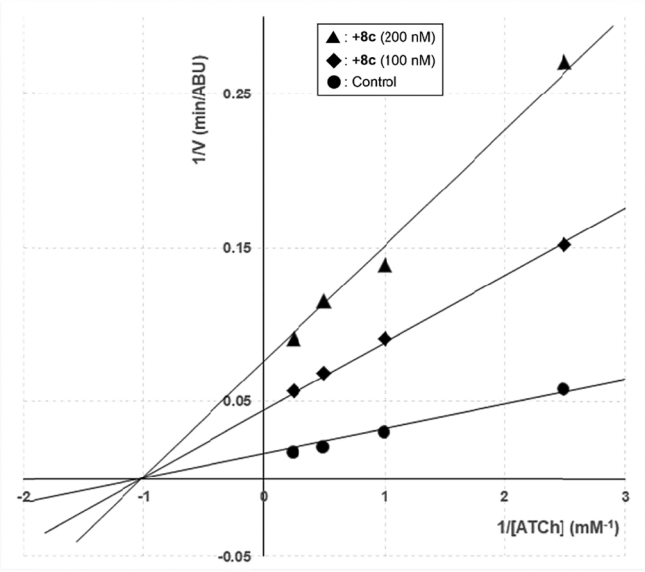


Fig. 2. Lineweaver–Burk plot analysis for AChE using **8c**. [ATCh]: concentration of acetylthiocholine, substrate as AChE. ABU: absorbance of 5-mercapto-2-nitrobenzoic acid which generate from 5,5′-dithiobis(2-nitrobenzoic acid) as color reagent of Ellman method. The change in absorbance (ABU) per minute was taken as *V*.

AChE inhibition activity among the synthesized compounds with the IC_{50} value of 34.2 nM. Concerning BuChE inhibition activity, all compounds revealed that weak or no inhibition activity in the presence of 1.0 μM inhibitor concentration. Even **8c**, which showed the strongest inhibitory activity, had an inhibition rate of 14.6% in the presence of 1.0 μM, and its IC_{50} value was 4.47 μM. From the above results, the selectivity of **8c** for acetylcholinesterase is estimated to be at least about 130 times.

Finally, the inhibition mechanism of **8c**, which showed the strongest inhibitory activity, was analyzed. In the Lineweaver-Burk plot analysis using **8c** and AChE, the drawn line intersected on the horizontal axis, indicated that the inhibition mode of **8c** is non-competitive (Fig. 2).

In addition, when a docking study was performed using **8c** and AChE by Molegro Virtual Docker 6.0,[23] the nitrophenyl group was accommodated in the pocket consisting of His287, Trp286, and Tyr72 existing near the entrance of the ligand binding site of AChE (Fig. 3a). This result suggested that the distance between the nitro group of **8c** and the hydroxyl group of Tyr72 is close, and an efficient interaction is formed. On the other hand, the part corresponding to the AChE pocket of BuChE is covered by Ala277 (Fig. 3b). Therefore, it is speculated that the interaction formed in this pocket affects the AChE/BuChE selectivity shown by **8c**.

In summary, we successfully developed novel AChE inhibitors that are based on muscle relaxant dantrolene. Compound with nitro group at the *ortho* position **8c** exhibited the most potent and selective AChE inhibitory activity with the IC_{50} value of 34.2 nM. In addition, Lineweaver-Burk plot analysis revealed that the inhibition mode of **8c** was non-competitive. Further structural development and structure-activity relationship studies are currently underway.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

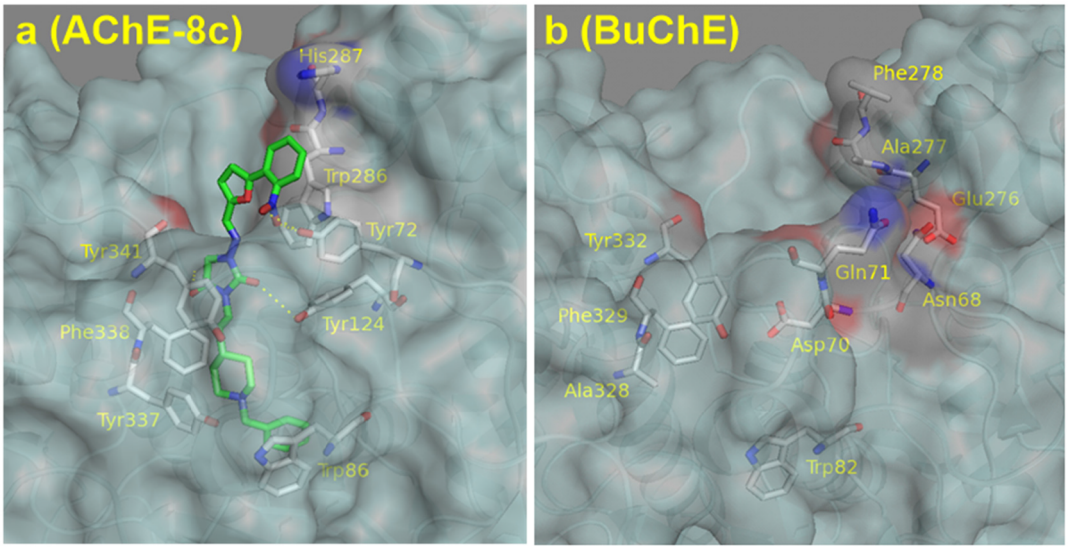


Fig. 3. (a) Proposed binding mode of **8c** (green stick) to AChE catalytic site (PDB: 4EY7); (b) crystal structure of BuChE catalytic site (PDB: 4BDS). The molecular mode of docked **8c** with AChE was displayed using PyMOL (The PyMOL Molecular Graphics System, Version 0.99 Schrödinger, LLC).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.126888.

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