Bioorganic & Medicinal Chemistry Letters 23 (2013) 3162–3165

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

NO-donating tacrine derivatives as potential butyrylcholinesterase inhibitors with vasorelaxation activity

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ARTICLE INFO

Article history:
Received 26 January 2013
Revised 24 March 2013
Accepted 3 April 2013
Available online 10 April 2013

Keywords:
Alzheimer's disease
Butyrylcholinesterase inhibitors
Tacrine
Nitric oxide
Vasodilator properties

ABSTRACT

To search for potent anti-Alzheimer's disease (AD) agents with multifunctional effects, 12 NO-donating tacrine–flurbiprofen hybrid compounds (**2a–l**) were synthesized and biologically evaluated. It was found that all the new target compounds showed selective butyrylcholinesterase (BuChE) inhibitory activity in vitro comparable or higher than tacrine and the tacrine–flurbiprofen hybrid compounds **1a–c**, and released moderate amount of NO in vitro. The kinetic study suggests that one of the most active and highest BuChE selective compounds **2d** may not only compete with the substrate for the same catalytic active site (CAS) but also interact with a second binding site. Furthermore, **2d** and **2l** exhibited significant vascular relaxation effect, which is beneficial for the treatment of AD. All the results suggest that **2d** and **2l** might be promising lead compounds for further research.

Alzheimer's disease (AD) is an age-related chronic, neurodegenerative disorder, manifested as loss of cholinergic neurons and reduced levels of neurotransmitter acetylcholine (ACh) in the brain.[1] To improve cholinergic function, the strategy of restoring the ACh level has been investigated.[2] It is well-known that two cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are responsible for ACh hydrolysis. In normal brains, AChE accounts for the majority of cholinesterase activity and is thus initially considered as the most important target for the treatment of AD.[3,4] In fact, most currently prescribed AD drugs are AChE inhibitors (AChEI), such as tacrine and rivastigmine.[5] However, AChEIs can only halt the progression of AD for a while instead of preventing or reversing it.[6,7] In comparison, BuChE plays a minor role in the healthy brain, whereas its level and activity progressively increases in the AD patient brains,[8,9] which suggests BuChE should also be taken as a crucial target. Recently, positive effects of the administration of the selective BuChE inhibitor (BuChEI) cymserine on learning and improving cognitive performance have already been shown in rodents.[10]

In addition to the cholinergic strategy, increasing evidence suggests that nitric oxide (NO), a free radical gas, may be beneficial for the treatment of AD by increasing blood supply[11] and regulating the cerebral circulation.[12] Consequently, to search for potent NO-donating BuChEI with multifunctional effects is of great interest.

In this study, we conjugated nitrate, a NO donor moiety, via alkylene side chain, to the tacrine–flurbiprofen hybrid compounds

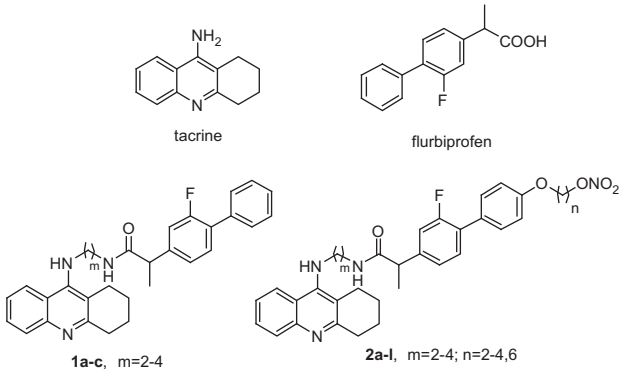

Figure 1. Structures of tacrine, flurbiprofen, compounds **1a–c**, and the target compounds **2a–l**.

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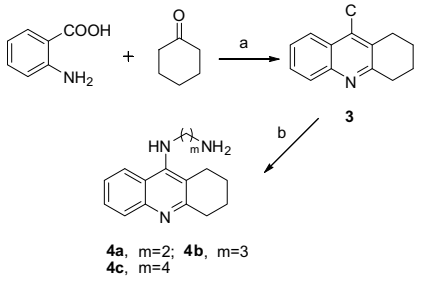
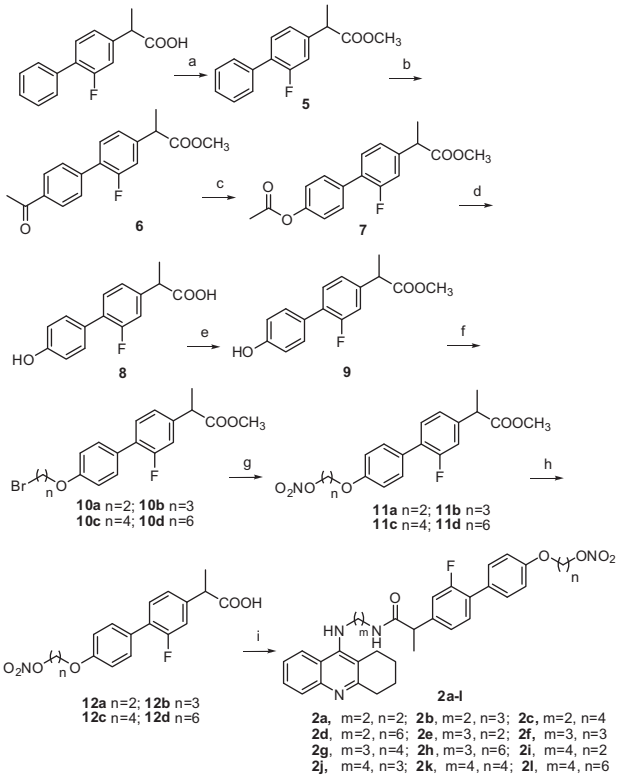
http://dx.doi.org/10.1016/j.bmcl.2013.04.008

(**1a–c**, Fig. 1), which were previously prepared by our group as potential BuChEIs.[13] Herein we report the synthesis and biological evaluation of the target compounds (**2a–l**).

The synthesis of **2a–l** is outlined in Schemes 1 and 2. The intermediate acridine **3** was synthesized starting from anthranilic acid and cyclohexanone according to a previously reported protocol.[14] Subsequently, various alkylenediamines were introduced to **3** by substitution giving the aminoalkylamino acridines **4a–c** (Scheme 1). To synthesize the other key intermediates **12a–d**, the carboxyl group of flurbiprofen was methylated to generate ester **5**, followed by the Friedel–Crafts acylation and the Baeyer–Villiger oxidation rearrangement to yield methyl ester **7**, which was hydrolyzed and acetylated to produce 4′-OH flurbiprofen **8**. Methylation of **8** resulted in ester **9**, which was treated with the corresponding dibromoalkanes to give the halogenated intermediates **10a–d**, and



Scheme 1. Synthetic route of **4a–c**. Reagents and conditions: (a) $POCl_3$, reflux, 3 h; (b) pentanol, $NH_2(CH_2)_mNH_2$, reflux, 18 h.



Scheme 2. Synthetic route of **2a–l**. Reagents an conditions: (a) CH_3OH, H_2SO_4, reflux, 3 h; (b) CH_3COCl, $AlCl_3$, anhydrous CH_2Cl_2, reflux, 12 h; (c) mCPBA, CH_2Cl_2, room temperature, 48 h; (d) NaOH, THF/CH_3OH/H_2O, room temperature, 6 h; (e) CH_3OH, H_2SO_4, reflux, 3 h; (f) $Br(CH_2)_nBr$, DMF, K_2CO_3, 65 °C, 6 h; (g) $AgNO_3$, CH_3CN, 60 °C, 6 h; (h) LiOH, THF/CH_3OH/H_2O, room temperature, 24 h; (i) DCC, DMAP, anhydrous CH_2Cl_2, room temperature, 24 h.

subsequent treatment of $AgNO_3$ in dry CH_3CN offered nitrates **11a–d**. The nitrates were deprotected under basic conditions to yield acids **12a–d**, which were coupled with **4a–c** in the presence of DCC/DMAP to provide the target compounds **2a–l** (Scheme 2).

Target compounds **2a–l** were tested for in vitro inhibition of AChE from electrophorus electricus (eeAChE) and BuChE from equine serum, respectively, using the Ellman assay[15] (Table 1). It was observed that all the compounds showed comparable or better BuChE inhibitory activity (IC_{50}s range from 3.9–13.9 nM) than tacrine and **1a–c**. Interestingly, it was found that the length of linker connecting the phenol and nitrate moieties influenced the selectivity of compounds. Generally, when the length of the linker increased, the AChE inhibitory activity decreased, leading to enhanced BuChE selectivity. The best results were obtained with **2d** and **2l**, which contain a six-carbon chain linker, and have IC_{50} values of 4309.5 and 1456.4 nM against AChE and IC_{50} ratios are 567 and 373, respectively.

To gain insights into the binding mode of this new family of compounds on BuChE, a kinetic study was carried out with one of the most promising compound **2d**. The mechanism was

Table 1
Inhibition of AChE and BuChE (IC_{50} values), selectivity ratios and NO release (presented as nitrite)

Compd	IC_{50} (nM) ± SEM[a]		Selectivity ratio[b]	Nitrite[c] (μg/ml)
	AChE	BuChE		
1a	714.9 ± 251.7	58.7 ± 7.3	12.2	ND[d]
1b	344.8 ± 45.5	13.9 ± 3.4	24.8	ND
1c	193.1 ± 8.5	11.0 ± 1.6	17.6	ND
2a	182.7 ± 25.2	9.9 ± 0.7	18.5	0.209 ± 0.012
2b	340.9 ± 89.1	10.0 ± 0.4	34.1	0.302 ± 0.039
2c	1345.4 ± 990.3	10.1 ± 0.8	133.2	0.456 ± 0.014
2d	4309.5 ± 1801.0	7.6 ± 0.5	567.0	0.424 ± 0.039
2e	112.4 ± 7.8	8.1 ± 1.2	13.9	0.219 ± 0.009
2f	188.6 ± 71.9	8.6 ± 1.1	21.9	0.308 ± 0.051
2g	356.5 ± 40.3	7.9 ± 1.0	45.1	0.366 ± 0.051
2h	1010.8 ± 315.1	8.1 ± 1.6	124.8	0.353 ± 0.058
2i	227.3 ± 25.3	13.9 ± 1.5	16.4	0.268 ± 0.017
2j	261.2 ± 59.0	10.6 ± 0.6	24.6	0.347 ± 0.045
2k	641.2 ± 128.2	7.7 ± 0.7	83.3	0.321 ± 0.039
2l	1456.4 ± 226.9	3.9 ± 0.3	373.4	0.321 ± 0.058
Tacrine	69.8 ± 11.1	10.6 ± 1.1	6.6	0.011 ± 0.004
ISMN	ND	ND	ND	0.412 ± 0.013

[a] Data is the mean of at least three determinations.
[b] Selectivity ratio = (IC50 of AChE)/(IC50 of BuChE).
[c] All values are the mean ± SEM.
[d] ND means not determined.

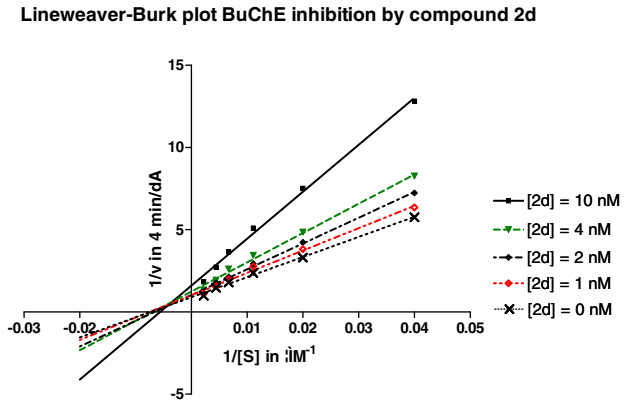


Figure 2. Linewear–Burk plots resulting from sub-velocity curves of BuChE activity with different substrate concentrations (25–450 μM) in the absence and presence of **2d** (1, 2, 4, 10 nM).

analyzed by Lineweaver–Burk reciprocal plots, which were reciprocal rates versus reciprocal substrate concentrations for different concentrations of **2d**. The results indicated both increased slopes (decreased Vmax) and intercepts (higher Km) at increasing concentration of **2d** (Fig. 2), suggesting a mixed-type inhibition of **2d**, which may not only compete with the substrate for the same catalytic active site (CAS), but also interact with a second binding site.

To investigate the location of the second binding site for the enzyme and compound **2d**, a molecular docking study using Libdock within Discovery Studio (DS, Accelrys) was performed. The results (Fig. 3) showed that the tacrine scaffold of **2d** could insert into the CAS surrounded by residue His438, Tyr440, Ser198 and Ile69. At the mouth of the gorge, the benzene ring of flurbiprofen moiety in **2d** showed strong parallel π–π stacking against the benzene ring of Tyr332, which was considered as the key residue in the second binding site. Additionally, the nitrate group in **2d** could form hydrogen bonds with Ala277, Glu276 and Asn68, which can enhance the intermolecular recognition between **2d** and BuChE.

The ability of compounds **2a–l** to release NO was measured using the Griess reaction[16] by quantifying the nitrite produced from the oxidative reaction of NO, oxygen and water (Table 1). The results showed that all the target compounds generated higher levels of nitrite than tacrine and similar levels (0.209–0.456 μg/ml) compared to the positive control isosorbide mononitrate (ISMN, 0.412 μg/ml).

To evaluate the vasorelaxation effect, the two active compounds **2d** and **2l** were tested in an ex vivo organ bath (coronar arteries from rat) using vascular relaxation assay, in which tacrine was employed as the negative control. As shown in Table 2, both **2d** (21.9%) and **2l** (31.3%) exhibited significant blood vessel relaxation activity in comparison with tacrine (3.4%). These results suggest that NO release from the compounds might be closely associated with their vascular relaxation activity, leading to potential anti-AD activity.

Table 2
Relaxation activity (%) of tacrine, **2d** and **2l** at 100 μM

Compounds	Relaxation activity[a] (%)
Tacrine	3.4 ± 3.7
2d	21.9 ± 7.0
2l	31.3 ± 5.7

[a] All values are the mean ± SEM.

In conclusion, a series of NO-donating tacrine–flurbiprofen hybrid compounds have been designed and synthesized as novel potential BuChEIs. All of the compounds showed comparable or higher BuChE inhibitory activity compared to tacrine and the parent compounds **1a–c**. The inhibitory mechanism of one of the most active and highest selective compound **2d** was analyzed by Lineweaver–Burk reciprocal plots, suggesting a dual site binding mode for BuChE. All the compounds could release moderate amount of NO in vitro, and compounds **2d** and **2l** displayed significant vascular relaxation activity as compared to tacrine, which is beneficial for the treatment of AD. All the results suggest that the novel NO-donating tacrine–flurbiprofen hybrids **2d** and **2l** might be promising lead compounds for further research.

Acknowledgments

The authors thank the financial support from National Science Foundation of China (NSFC, No. 30973608) and the Natural Science Foundation of Jiangsu Province of China (No. BK2009296).

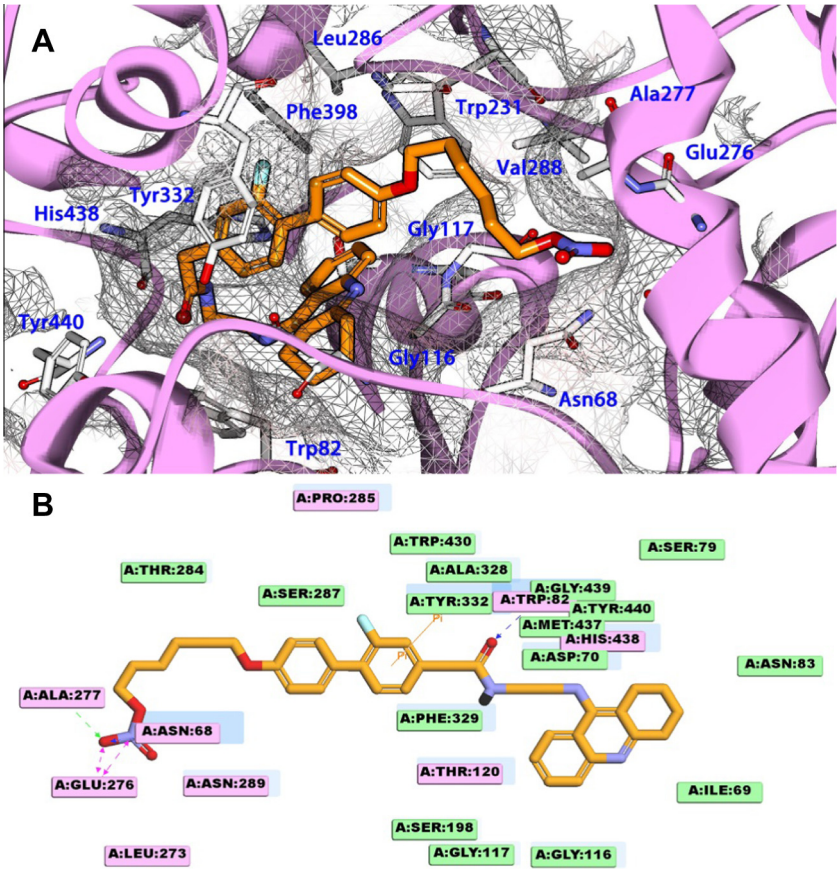


Figure 3. Representation of the binding mode of compound **2d** with BuChE (PDB id: 1POM), compound **2d** and the key residues of BuChE are shown as stick form.

Supplementary data

Supplementary data (syntheses and spectra data of intermediates and all target compounds **2a–l**, and protocols of pharmacological assays) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.008.

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