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Short communication

Synthesis and *in vitro* evaluation of novel rhodanine derivatives as potential cholinesterase inhibitors

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ABSTRACT

Based on a broad spectrum of biological activities of rhodanines, we synthesized aromatic amides and esters of 2-(4-oxo-2-thioxothiazolidin-3-yl)acetic acid (rhodanine-3-acetic acid) *via* carbodiimide- or PCl_3-mediated coupling. Both esters and amides were investigated for their *in vitro* inhibitory potency and selectivity against acetylcholinesterase (AChE) from electric eel and butyrylcholinesterase (BChE) from equine serum using Ellman's spectrophotometric method. The derivatives exhibited mostly a moderate activity against both cholinesterases. IC_{50} values for AChE were in a closer concentration range of 24.05–86.85 μM when compared to BChE inhibition (7.92–227.19 μM). The esters caused the more efficient inhibition of AChE than amides and parent acid. The esterification and amidation of the rhodanine-3-acetic acid increased inhibition of BChE, even up to 26 times. Derivatives of 4-nitroaniline/phenol showed the activity superior to other substituents (H, Cl, CH_3, OCH_3, CF_3). Rhodanines produced a balanced inhibition of both cholinesterases. Seven derivatives produced the more potent inhibition of AChE than rivastigmine, a clinically used drug; additional three compounds were comparable. Two amides exceeded inhibitory potency of rivastigmine towards BChE. Importantly, this is the first evidence that rhodanine-based compounds are able to inhibit BChE.

1. Introduction

Rhodanine (2-thioxothiazolidin-4-one) has become an interesting important heterocyclic moiety in medicinal chemistry owing to its wide spectrum of biological activities through different mechanisms of action. Due to various possibilities of chemical modification, rhodanine-based compounds were considered as a privileged scaffold in drug discovery [1].

Rhodanines have been reported as, e.g., potential antibacterial [2–5], antifungal [2,6,7], antiviral [8], anticancer [9] and anti-inflammatory [10] agents. They are considered to be useful for the treatment and prevention of diabetes-related complications [11]. Also many enzymes have been identified as their potential molecular targets. Additionally, they can potentially be used in the treatment of obesity, neurologic and psychiatric disorders, cystic fibrosis, etc. [1]. Generally, rhodanines were found to be non-mutagenic [12].

However, they have gained a reputation as promiscuous, pan assay interference compounds (PAINS) and aggregators that can interact non-specifically with target proteins as well as undergo Michael 1,4-conjugative addition with various nucleophiles in the case of 5-arylmethylidenerhodanines [13]. The 5-benzylidene substitution was also reported to complicate the investigation in cell-based assays as well as *in vivo* because they can react with glutathione and other compounds with free thiol groups within cells [1,13].

The prevalence of dementias has been increased globally as well as health-care expenses for their treatment. Alzheimer's disease (AD) represents the most frequent cause characterized by a progressive deterioration in cognition, in particular the memory domain, function and behaviour. The incidence rate for dementia increases exponentially with age [14].

The exact cause of the AD is still uncertain, but several hypotheses have been proposed. Based on cholinergic hypothesis, deficiency of acetylcholine (ACh) in brain was observed either due to decreased production or amplified acetylcholinesterase (AChE) activity. This decreased level of the neurotransmitter causes impairment of the cholinergic neurotransmission and the cholinergic augmentation will improve the symptoms of AD [15]. Principal physiological function of AChE (EC 3.1.1.7) is the hydrolysis of ACh,

Abbreviations: ACh, acetylcholine; AD, Alzheimer's disease; AChE, acetylcholinesterase; BBB, blood brain barrier; BChE, butyrylcholinesterase; ChE, cholinesterases; CNS, central nervous system.

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resulting in the termination of the nerve impulse. Also butyrylcholinesterase (also called pseudocholinesterase or plasma cholinesterase; BChE; EC 3.1.1.8) terminates the action of ACh [15,16].

It is necessary to identify novel drugs that prevent, delay the onset, slow the progression, or improve the symptoms of AD. Today, only four cholinesterase inhibitors (tacrine, donepezil, rivastigmine, galantamine) and an *N*-methyl-D-aspartate receptor antagonist (memantine) are approved. No new treatments have been marketed for the treatment of AD since 2003. Unfortunately, many failures have occurred [17]. Several rhodanine derivatives have been reported as potentially useful agents for the treatment of Alzheimer's disease: inhibitors of glycogen synthase kinase-3 [18,19], tau aggregation [20,21] or AChE [10] inhibitors.

2-(4-Oxo-2-thioxothiazolidin-3-yl)acetic acid (rhodanine-3-acetic acid) represents one of the frequent thiazolidine/rhodanine-based fragments. However, a majority of reported rhodanine-3-acetic acid derivatives have been based on Knoevenagel reaction with aromatic aldehydes to form 5-arylidenerhodanines. The compounds with substituted carboxyl functional group are rarer.

As a part of our ongoing screening and search for novel cholinesterases inhibitors [22–24] we designed and synthesized a series of aromatic rhodanine-3-acetic acid esters and amides. To avoid possible non-specific reactivity as Michael acceptors, rhodanine-3-acetic acid was not reacted with aldehydes and only carboxyl group was modified. To the best of our knowledge, rhodanine-3-acetic acid (2-(4-oxo-2-thioxothiazolidin-3-yl)acetic acid) and its derivatives with unsubstituted carbon 5 in the thiazolidine ring have not been reported in the literature to date as inhibitors of neither AChE nor BChE.

An ideal potential cholinesterase inhibitor should combine a powerful enzyme inhibition (IC_{50} values in submicromolar range), a lack of cytotoxicity for mammalian cells and optimal physicochemical properties for drug-likeness and crossing the blood brain barrier (BBB). From a theoretical point of view, dual inhibition of both cholinesterases may be beneficial.

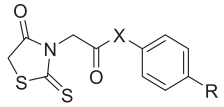
2. Results and discussion

2.1. Chemistry

We have chosen several substituents with different electronic effects as a substitution pattern for aniline and phenol used for the derivatization of the 2-(4-oxo-2-thioxothiazolidin-3-yl)acetic acid **1** (Table 1): H (no effect), Cl (−I and +M effects), CF_3 (−I effect), CH_3 (+I effect), OCH_3 (+M and −I effects) and NO_2 (−I and −M effects).

Amides of rhodanine-3-acetic acid **2** were obtained by two synthetic procedures. Method A, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC)-mediated coupling in the presence of 1-hydroxybenzotriazole (HOBt; Scheme 1) was successful for five amides providing yields within the range of 76–95%. Reaction of 4-(trifluoromethyl)aniline provides the lowest but still acceptable yield (76%) due to strong electron-withdrawing properties of the CF_3 moiety. However, this method failed in the case of 4-nitroaniline. Other additional attempts with different reaction conditions, changing carbodiimides, additives, using 1,1′-carbonyldiimidazole and mixed anhydrides methods led also to an unsatisfactory outcome. Then, we applied PCl_3-mediated reaction in dry pyridine (Method B; Scheme 2; yield of **2f** was 78%) according to Rijkers et al. [25], which is useful for very weak nucleophiles like 4-nitroaniline.

Table 1
IC_{50} values of rhodanine derivatives **1–3** for acetylcholinesterase and butyrylcholinesterase.

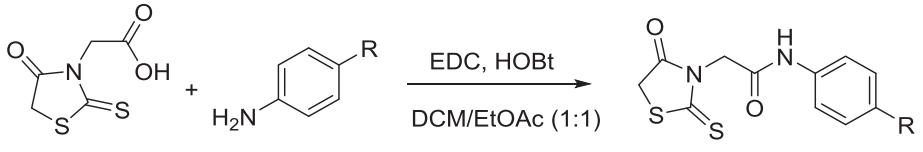


Code	X	R	IC_{50} for AChE [μM]	IC_{50} for BChE [μM]	Selectivity to AChE
1	- (free acid)		54.08 ± 1.34	205.44 ± 25.97	3.8
2a	NH	H	63.06 ± 2.86	61.06 ± 0.08	1.0
2b	NH	Cl	56.76 ± 1.47	26.57 ± 0.29	0.5
2c	NH	CF_3	86.85 ± 5.12	227.19 ± 11.56	2.6
2d	NH	CH_3	58.46 ± 0.63	77.69 ± 0.82	1.3
2e	NH	OCH_3	66.70 ± 1.77	65.37 ± 0.31	1.0
2f	NH	NO_2	30.82 ± 0.72	**7.92 ± 0.75**	0.3
3a	O	H	45.34 ± 0.62	76.80 ± 0.96	1.7
3b	O	Cl	41.53 ± 0.38	50.57 ± 0.97	1.2
3c	O	CF_3	37.79 ± 1.46	54.85 ± 1.10	1.5
3d	O	CH_3	44.52 ± 0.76	52.29 ± 0.50	1.2
3e	O	OCH_3	41.45 ± 0.04	58.03 ± 0.98	1.4
3f	O	NO_2	**24.05 ± 0.28**	51.00 ± 0.53	2.1
Rivastigmine			56.10 ± 1.41	38.40 ± 1.97	0.7
Galantamine			1.54 ± 0.02	2.77 ± 0.15	1.8

AChE and BChE inhibition is expressed as the mean ± SD (n = 3 experiments). Selectivity to AChE: IC50 for BChE/IC50 for AChE. The best results for each enzyme are shown in bold.

Esters **3** were obtained by Steglich-type esterification employing EDC and DMAP (Scheme 3). The general yields ranged from 66% (4-(trifluoromethyl)aniline derivative **3c**) up to 79%.

Compounds were characterized by melting points, IR and NMR spectra; their purity was checked by thin-layer chromatography and elemental analysis. ^{1}H NMR spectra of the amides **2** contained singlets attributed to the amidic N—H proton in the ranges of 10.17–10.48 ppm (**2b**, **2d**, **2e**) and 8.78–8.82 ppm in the case of aniline derivatives with electron-withdrawing substituents (**2c**, **2f**). Methylene group connecting heterocyclic nitrogen (N-3) and carboxamide produced a sharp singlet in the range of 4.66–4.87 ppm. The singlets of thiazolidine —CH_2— hydrogens were observed at 4.14–4.40 ppm. ^{13}C NMR spectra contain signals of two C=O carbon atoms (amidic, heterocyclic) in the downfield region of 163.12–174.44 ppm. The chemical shift of C=S carbon is in the range of 200.89–203.57 ppm. N-3 connected with —CH_2— group showed signals around 47 ppm and thiazolidine —CH_2— around 36 ppm. In ^{1}H NMR spectra of the esters **3**, hydrogens of —CH_2— linker between N-3 and —C(=O)O— group were observed as singlets in the range of 4.93–4.99 ppm. The chemical shift of thiazolidine —CH_2— hydrogens singlets was 4.10–4.14 ppm. ^{13}C NMR spectra contain signals of two C=O carbons atoms (C=O ester,



1 **2**

Scheme 1. Synthesis of amides **2** (EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; HOBt = 1-hydroxybenzotriazole; DCM = dichloromethane; EtOAc = ethyl acetate; R = H, Cl, CF_3, CH_3, OCH_3, NO_2).