Development of potent cholinesterase inhibitors based on a marine pharmacophore

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ABSTRACT:

The management of neurological disorders such as dementia and involves the use of cholinesterase inhibitors. These compounds can slow down the progression of these diseases and they can also be applied in the treatment of glaucoma and myasthenia gravis. The majority of the cholinesterase inhibitors in clinical use are derived from natural products and our current paper describes the use of a small marine pharmacophore to develop potent and selective cholinesterase inhibitors. Fourteen small inhibitors were designed based on recent discoveries about the inhibitory potential of a range of related marine secondary metabolites. The compounds were evaluated, in kinetic enzymatic assays, for their ability to inhibit three different cholinesterase enzymes and it was shown that compounds with a high inhibitory activity towards electric eel and human recombinant acetylcholinesterase (IC_{50} between 20-70 μM) could be prepared. It was also shown that this compound class was particularly active against butyrylcholinesterase, with IC_{50} values between 0.8-16 μM, which is an order of magnitude more potent than the clinically used positive control neostigmine. The compounds were further tested for off-target toxicity against both human umbilical vein endothelial cells and human erythrocytes and were shown to display a low mammalian cellular toxicity. Overall, the study illustrates how the brominated dipeptide marine pharmacophore can be used as a versatile natural scaffold for the design of potent, and selective cholinesterase inhibitors.

INTRODUCTION

Natural products play a central role in modern medicinal chemistry and medicine(1, 2). Approximately 32% of all approved small molecule drugs (441 out of 1394) between 1981-2019 were “direct or direct from” the natural products field(2) and approved natural product drugs can be found in almost every therapeutic class(2). Examples of areas where the available drugs are totally synthetic are for example the antihistamines, hypnotics and diuretics(2). The impact of natural products is thus dependent on the condition treated and they make particularly

crucial contributions to the areas of anti-infectives (55%) and anticancer drugs (65%)(2). Historically, the origin of the approved natural products has been terrestrial sources but with recent technological developments, the marine environment has started to significantly contribute with drug leads(3, 4). The oceans cover nearly 71% of our planet, and the marine environment represents a valuable source for providing an increasing number of bioactive compounds with molecular architectures able to bind to and modulate challenging targets(5, 6). We have just started to investigate the marine potential over the last 60 years and considering that less than 5% of the deep sea has been investigated, the ocean will continue to deliver novel and inspirational chemical scaffolds for the future(7-9). As of July 2021, 15 compounds of marine origin have been approved as drugs(4, 10) and 1407 new marine compounds were reported in 2020, mainly from fungi, sponges, cnidarians and bacteria(6).

In addition to uses for combatting infection and cancer, natural products play an important role for the management of different neurological disorders(11). Several approved drugs for Alzheimer's disease (AD) such as rivastigmine and galanthamine have been inspired by natural products(2, 12, 13). AD is the most common underlying factor of dementia and is predicted to impact 65.7 million people globally in 2030(14). An increased cholinesterase (ChE) activity is associated with the stages of AD and the only class of compounds that has shown efficacy in treating the functional and cognitive symptoms of AD is ChE inhibitors(15). These drugs do not cure AD but instead they focus on delaying the symptoms to reduce the cognitive impairment(16). The function of the ChE enzymes, namely acetylcholinesterase (AChE) that is typically located in neurons and erythrocytes, and butyrylcholinesterase (BChE) that is primarily present in liver and plasma, is to hydrolyze esters such as acetylcholine. *Via* this activity, they play a pivotal role in the termination of the acetylcholine receptor stimulation in the cholinergic synapse. In AChE, the hydrolysis of acetylcholine takes place at the bottom of the narrow gorge located 2 nm from the enzyme surface, where the esteratic site containing the catalytic triad (Ser-Glu-His) is located. The adjacent anionic site ensures that acetylcholine is correctly orientated *via* cation–π interactions with the acetylcholine quaternary ammonium group. At the rim of the gorge, there is also the peripheric anionic site, that directs the substrate towards the active site at the gorge bottom, also *via* cation– π interactions with the quaternary ammonium group of acetylcholine(12). ChE inhibitors can be designed targeting either directly the catalytic site, anionic site or the peripheral anionic site (12, 17). In this way both reversible or irreversible ChE inhibitors have been developed with the former class being used for the

management of dementia and also other conditions such as myasthenia gravis and glaucoma(12, 18-21). Many of the reversible inhibitors display an aromatic core and a nitrogen atom (often quaternary) that will engage in cation-π interaction through the aromatic gorge to facilitate an acetylcholine-like interaction with the anionic site at the bottom of the gorge(17, 22). The hydrophobic components of the successful inhibitors in use are essential for balancing the cationic charge to facilitate passage through the blood-brain barrier to reach the brain (23, 24). Cholinergic neurons are however distributed within both the peripheral and central nervous systems (CNS) and the wide applicability of cholinesterase inhibitors of ranging polarity illustrates how this class of compounds can be applied to clinical conditions both within, and outside the CNS(12).

Recently we reviewed cholinesterase inhibitors of marine origin between 1974 up to 2018 to find 185 marine ChE inhibitors reported, some with activities superior to ChE inhibitors in clinical use(12). While numerous diverse active scaffolds have been discovered over the last 50 years many of them do not display the structural features, physicochemical properties or activities needed to consider them as pharmaceutical leads(12). One group of marine secondary metabolites with global distribution and promising properties is the halogenated dipeptide motif reported from many sessile marine organisms. These compounds consist of a halogenated (generally brominated) hydrophobic end and a cationic amino acid linked *via* a constrained, often heterocyclic core to provide structural rigidity. Selected examples from both warm and cold waters include phidianidine A and B(25), barettin(26), oroidin(27), the synoxazolidinones(28, 29), ianthelline(30, 31) and the pulmonarins(32) as shown in Figure 1.

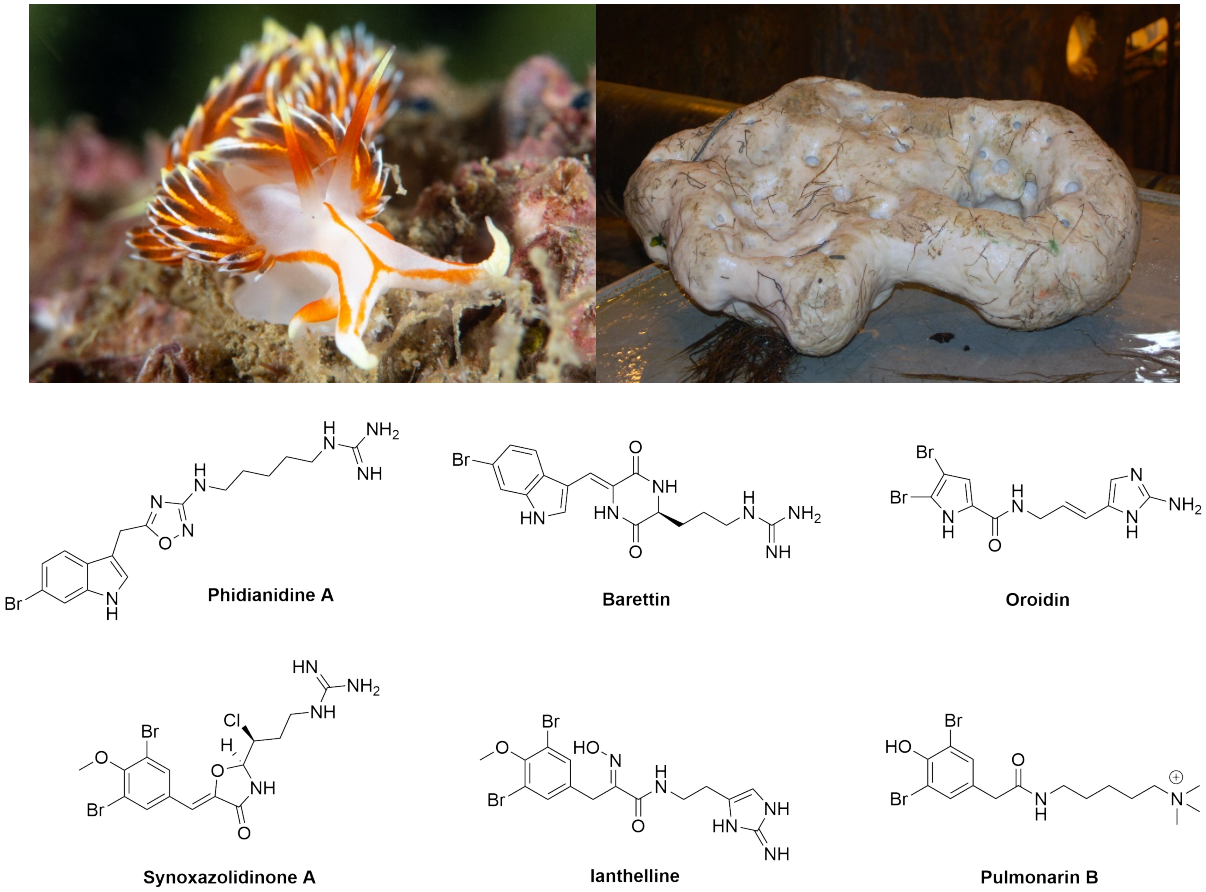


Figure 1. Several benthic marine organisms produce secondary metabolites of the brominated dipeptidic motif. Top left: The shell-less opistobranch *Phidiana militaris* (Alder & Hancock, 1864) from which phidianidine originally was isolated by Carbone and co-workers(25) can be encountered in the Indian ocean (Photo by Stewart Clarke). Top right: The large boreal deep-water sponge *Geodia baretti* (Bowerbank, 1858) is also a rich producer of barettin initially isolated by Lidgren et al(26). (Photo by Johan Svenson). Bottom: Numerous structurally related compounds have been isolated from other marine sources and they have been shown to display a range of bioactivities, such as settlement inhibition (antifouling) and cholinesterase inhibition.

The exact origin of the compounds is ambiguous but they have been suggested to arise via the metabolism of aromatic amino acids(25). The compounds have in many cases been discovered though bioassay guided fractionation(3) and they, and their analogs, have been reported with anticancer(31), antibacterial(33), antifouling(34-37) and antioxidant(38, 39) bioactivities. Several compounds such as barettin, dehydrobarettin(40), the pulmonarins(32), and smaller brominated marine quaternary ammonium indole and phenethylamine compounds(40, 41) have recently been reported with potent ChE inhibitory properties demonstrating how this naturally occurring scaffold can generate reversible ChE inhibitors. The pharmaceutical potential of small cationic marine indoles as drug leads for neurological disorders has been reviewed by Kochanowska-Karamyan and Hamann(42) and illustrated the high activity of several small

compounds(42). In our previous studies, we observe that the larger natural compounds (the "dipeptides") display more potent activities(32, 40) than the smaller brominated indole or phenethylamine motifs which are not sufficient to cause a high ChE inhibitory activity(40, 41).

Based on these previous observations, our current study describes the design and preparation of a library of cationically substituted 6-bromoindole amides as representative simplified marine mimics with ChE inhibitory potential. The compounds were prepared for assessing the contribution from the type of basic substituent and the distance between the charge and the 6-bromoindole, which has been shown to be crucial for natural compounds(40) as shown in Figure 2.

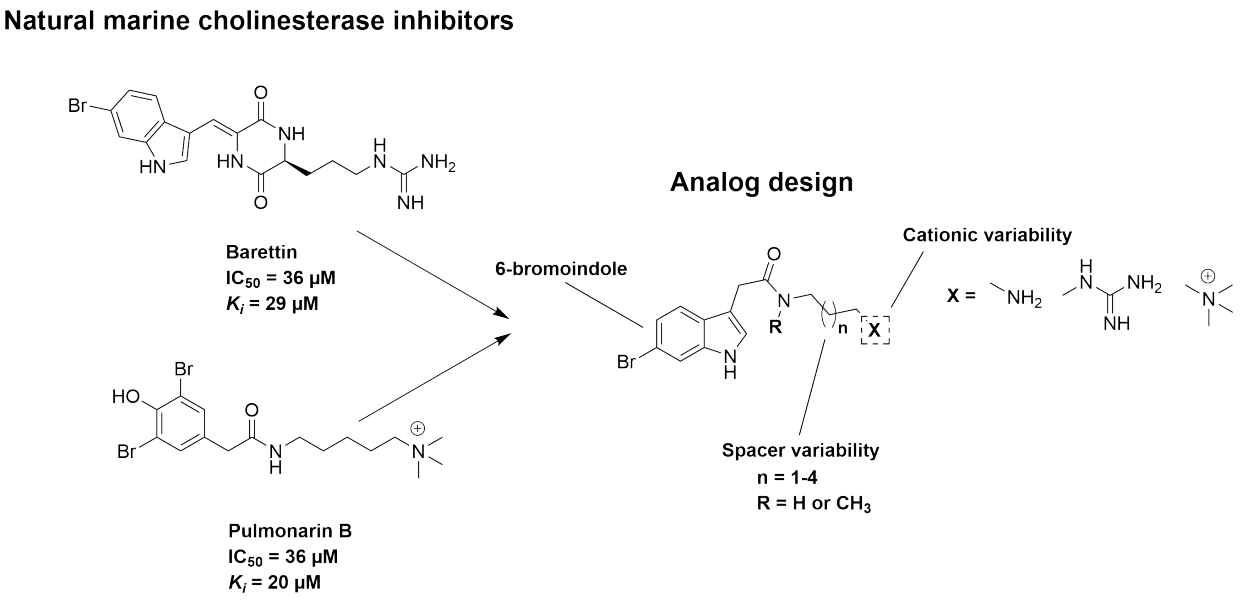


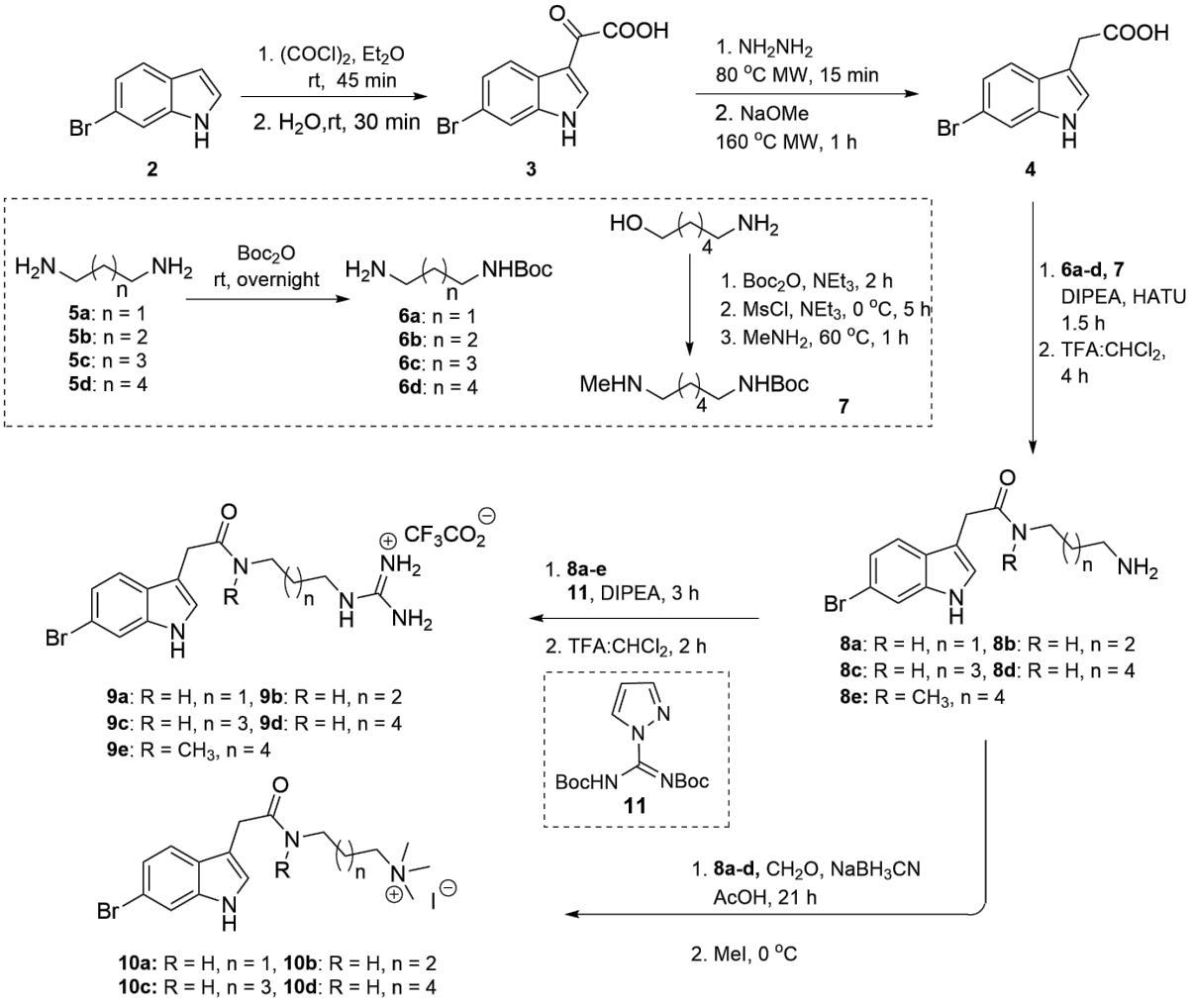
Figure 2. Rational design of the analogs from the marine brominated dipeptide pharmacoiphore prepared in the current study. Inhibitory values taken from refs(32, 40)

Fourteen compounds were prepared and evaluated as inhibitors against a series of ChE enzymes using kinetics assays(43). In addition, the potential toxicity of the prepared library was evaluated against bovine and human red blood cells and human umbilical vein endothelial cells (HUVEC).

RESULTS AND DISCUSSION

Compound design and synthesis

The compounds (**8a-10d**) were designed to span the dimensions of the reported natural inhibitors while probing the influence of charge and overall length by incorporating a variable methylene spacer (3-6 carbons). The compounds were generally prepared according to modified methods (**8** and **9** series) of Labriere et al.(36). The amine series was guanidinylated using pyrazole-1-carboxamidine guanidine to yield **9a-9e** while the quaternary compounds **10a–10d** could be generated from the free amine precursors via methylation with methyl iodide. All compounds were prepared in high yields and purity. (Supporting information)



Scheme 1. Synthetic scheme for generating the cholinesterase inhibitors from 6-bromoindole employing methodology developed by Labriere et al.(36).

Cholinesterase inhibition

The prepared compounds were evaluated as inhibitors for electric eel AChE (eeAChE), human recombinant AChE (hAChE) and equine BChE. The standard model ChE enzyme, eeAChE was complemented with hAChE to generate more accurate human data despite significant sequence homology(44). BChE is mainly located in the blood plasma, with a broader substrate acceptance(45), and is believed to support in regulating cholinergic transmission when AChE activity is reduced(46-48). It is currently being explored as a potential therapeutic target for symptomatic treatment of AD due to its compensatory ability to hydrolyse acetylcholine and to closely aggregate with Aβ deposits(45, 49). The inhibitory potential against the selected cholinesterases was evaluated using the kinetic colorimetric assay developed by Ellman and co-workers(43). The IC_{50}-values were determined from dose-response data and Dixon plots were employed to establish the inhibition constants (Ki) and the type of inhibition for potent and moderately active inhibitors(12) as shown in Table 1 and Figures 3 and 4.

Table 1. Cholinesterase inhibitory activities of compounds **8a**-**10d**

Compound	**IC_{50} (µM)**			***K*i (µM)**		
	eeAChE	hAChE	hsBChE	eeAChE	hAChE	hsBChE
8a	>250	112.8	8.7	n.d.	84.0	2.5
8b	>250	>250	14.2	n.d.	n.d.	5.0
8c	>250	183.3	15.7	n.d.	86.0	7.0
8d	>250	127.7	11.4	n.d.	57.0	4.5
8e	172.0	136.5	4.9	n.d.	n.d.	0.9
9a	42.9	107.2	8.1	37.0	n.d.	3.7
9b	20.8	43.7	7.9	12.0	36.0	4.0
9c	30.3	30.3	7.5	20.0	14.0	6.0
9d	23.6	35.4	7.5	14.0	20.0	3.9
9e	65.1	68.9	2.5	44.0	50.0	0.4
10a	>250	>250	15.6	n.d.	n.d.	7.0
10b	>250	>250	0.8	n.d.	n.d.	0.3
10c	>250	165.3	9.8	n.d.	n.d.	10.0
10d	137.9	44.0	7.7	n.d.	14.0	4.5
Neostigmine	3.9	4.8	62.8	n.d.	n.d.	n.d.

eeAChE = electric eel acetylcholinesterase, hAChE = human recombinant acetlycholinesterase, hsBChE = horse serum butyrylcholine esterase, n.d. = not detemined. IC_{50} = concentration required to cause 50% inhibition of enzyme activity; K_i, inhibition constants were determined for compounds with IC_{50} < 50 µg/mL.

As presented in Table 1, several of the compounds displayed activity against the different ChE enzymes with varying inhibitory efficacy. Against eeAChE, the guanidine substituted compounds from series **9** exhibited a moderate inhibitory potential with IC_{50} values ranging 20 to 65 μM. This observed activity is similar to that previously reported for barettin(40) and the pulmonarins(32) and illustrates how the simplified analogs efficiently mimic the natural products. A ten-fold increase in IC_{50} in relation to the positive control neostigmine was observed for the most active inhibitors (Table 1). No clear correlation between methylene spacer length and activity was noted with **9b** and **9d** being most active. Compounds from the **8** and **10** series were inactive at the employed concentration (IC_{50} >250 μM) with the exception of **8e** and **10d** which both displayed a weak inhibitory activity. A wider inhibitory effect was seen against hAChE with more active compounds bearing amines and ammonium groups. The guanidine bearing analogs were again the most efficient inhibitors and four to six methylenes (**9b-9d**) in the spacer remained optimal in analogy to eeAChE to produce hAChE IC_{50} values between 30 and 45 μM.

BChE displays a similar catalytic function as AChE but with a slower acetylcholine hydrolysis rate(45, 50). Differences in the residues lining the active site (overall 65% amino acid sequence homology(51)) and conferring selectivity to AChE enables a wider range of substrate to reach the catalytic site of BChE(45). This is partly facilitated by the substitution of aromatic residues Tyr72, Tyr124, Tyr337, Phe295 and Phe297 in AChE by aliphatic residues Asn72, Gln124, Ala337, Leu286 and Val288 which allows larger inhibitors and substrates to enter(45, 52). This ability of BChE to accept a wider range of substrate/inhibitor molecules is clearly reflected in our results with several of the prepared compounds being potent inhibitors of BChE. The guanidine bearing inhibitors remained the most potent group with IC_{50}-values ranging 2.5 to 8.1 μM, approximately tenfold more efficient than the positive control. However, both the amine and ammonium substituted compounds were also highly active, with inhibitory potential higher than the positive control, in contrast to the eeAChE and hAChE experiments. The most potent inhibitor against BChE in our library was **10b** with an IC_{50} of 0.3 μM. The strong preferential for hsBChE over eeAChE and hAChE is shown for compounds **8c** and **9c** in Figure 3.

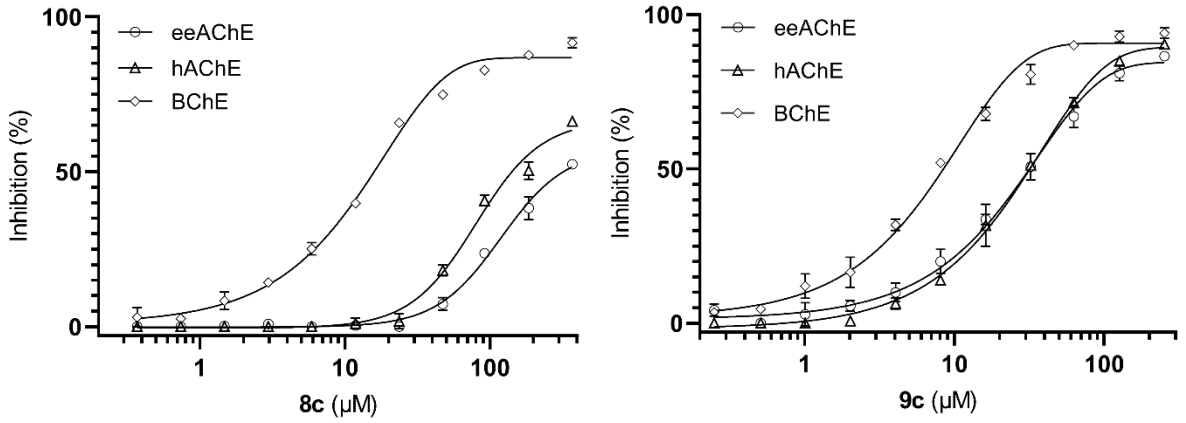


Figure 3. Dose-dependent inhibition of eeAChE, hAChE and hsBChE by compounds **8c** and **9c**. The IC_{50} values indicate a strong preferential towards hsBChE for both compounds with **9c** also being able to effectively inhibit eeAChE and hAChE.

Dixon plot analysis of the compounds revealed that they all were reversible competitive inhibitors of eeAChE, hAChE and hsBChE as shown in Figure 4 and 5. The inhibition constants were determined for most active compounds and ranged 0.3 to 10 μM against BChE which makes them more potent when be compared to the K_i-values of 14 and 48 μM for barettin and 8,9-dihydrobarettin(40).

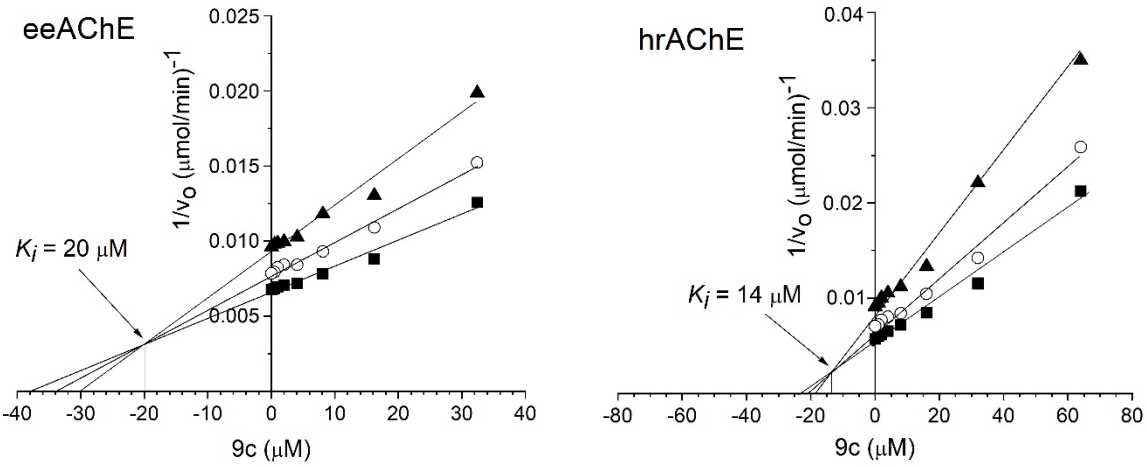


Figure 4. Dixon plots for determination of type of inhibition and inhibition constants (K_i) for compound **9c** against eeAChE) and hAChE. Acetylthiocholine chloride substrate concentrations: 0.125 mM (▲), 0.25 mM (❍), 0.5 mM (■). K_i was determined to 20 μM against eeAChE and 14 μM against hAChE.

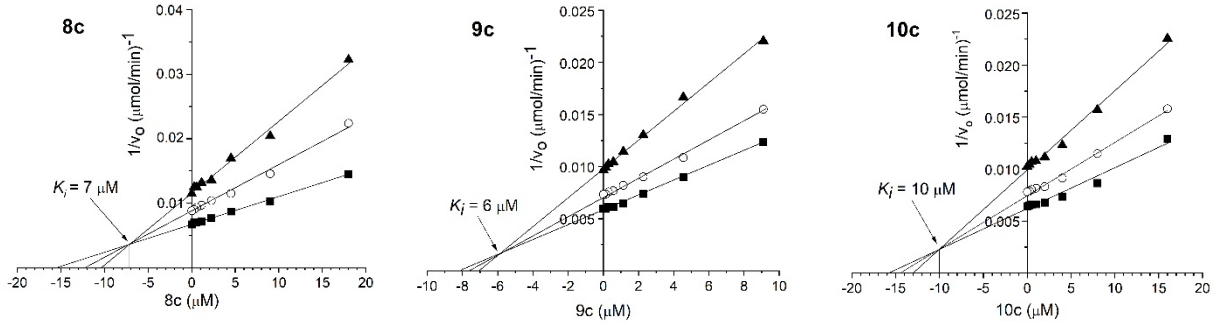


Figure 5. Dixon plots for determination of type of inhibition and inhibition constants (K_i) for compounds **8c**, **9c** and **10c** against hsBChE. Acetylthiocholine chloride substrate concentrations: 0.125 mM (▲), 0.25 mM (❍), 0.5 mM (■).

The guanidine bearing compounds displayed the highest general inhibitory potential against the three cholinesterases with compound **9c** for example displaying K_i-values of 20, 14 and 6 μM against eeAChE, hAChE and BChE, respectively. This is lower than reported for several cationic 6-bromoindoles such as the guanidine bearing barettin, 8,9-dihydrobarettin and the unsaturated quaternary ammonium-indole 6-bromoconicamin(40).

The compounds were designed to mimic a range of related marine secondary metabolites with ranging, basicity, hydrophobicity, hydrogen bonding capacity and overall length. The ability of selected compounds to inhibit marine settlement has been recently described(36) and the postulated link between settlement inhibition and ChE inhibition(12, 53) warranted a library expansion and further ChE studies. The 6-bromindole unit was kept intact and is a common, modified marine building block and our previous work has been shown to be able to access the active site of the ChE enzymes(40). Several other examples of small modified indoles have illustrated their suitability for ChE inhibition(54). Previous studies on marine analogs have illustrated a beneficial effect of a minimal length, and it is clear that the three to six methylenes evaluated here, combined with the amide linker are sufficient to allow sufficient active site interactions(40, 41). Adding methylenes to the linker brings an accompanying increase in overall hydrophobicity as presented in Table 2, but this reduction of polarity does not bring a pronounced effect on the inhibitory activity in the observed hydrophobicity range.

Table 2. Physicochemical properties of compounds **8a**-**10d**

Compound	Mw (g/mol)	Log *P*[a]	Solvent excluded volume (Å^3)	Retention time (min)
8a	309.05	2.31	229.23	11.77
8b	323.06	2.73	246.48	11.93
8c	337.08	3.15	263.75	12.20
8d	351.09	3.57	281.01	12.68
8e	365.11	3.80	296.04	13.63
9a	351.08	2.31	259.21	12.28
9b	365.09	2.73	276.46	12.47
9c	379.11	3.15	293.72	12.95
9d	393.12	3.57	310.25	13.43
9e	407.13	3.80	326.00	14.28
10a	352.10	2.31	283.48	12.28
10b	366.12	2.73	300.72	12.67
10c	380.13	3.15	318.00	13.02
10d	394.15	3.57	335.26	13.47

[a]Calculated using ChemBio3D Ultra 14.0 disregarding the substituted and ionized nitrogen atom hence only the contribution from the substituted 6-bromoindole

Nevertheless, *N*-methylation of the amide nitrogen (**8e** and **9e**) does generate the most potent hsBChE inhibitors of the two series. While the theoretical effect on Log *P* and the solvent excluded volume (Table 2) is in line with adding an extra methylene, the experimental retention time suggest that both **8e** and **9e** are significantly more hydrophobic than their non-methylated counterparts **8d** and **9d** and this may result from the formation of solution conformers and loss of hydrogen bonding capacity not accounted for in the modelling of the physicochemical properties.

The main difference between the compounds is the choice of basic group. All the compounds can be regarded as basic and charged in a physiological context and this is clear when analysing the retention times which illustrates a dependence on the size and solvent excluded volume rather than the type of basic group. The constant positive charge of quaternary compounds have been shown to yield superior AChE inhibitors compared to secondary amines(41) but the guanidine group have also been shown to generate good binding to the active site illustrating that the natural quaternary group of acetylcholine is not pivotal for activity(40). The pK_a of the primary amines (the "**8** series") and the guanidines (the "**9** series") are 10.5, 12.5 but this difference in base strength does not generate a big change in overall polarity(55). The more significant difference between the basic groups lies in the ability of the guanidine group to

engage in multiple hydrogen bonding interactions and this have been shown to have implications for many bioactive compounds(56-58). It is likely this increased hydrogen bonding capacity that enables the guanidine bearing compounds to more efficiently access and interact with the site of inhibition in eeAChe and hAChE while the differences in binding site composition in BChE(45) allows the amines and quaternary group a similar access.

While all the current compounds are classified as reversible and competitive inhibitors (indicating binding inside the active gorge) from the Dixon plot analysis, the exact binding site of the compounds is unclear. Similar compounds have also been described as reversible, non-competitive inhibitors (indicating binding to the peripheral anionic site)(32). Inhibitors bearing quaternary nitrogen and/or aromatic rings can bind both to the anionic and hydrophobic binding sites depending on their concentration(59) and these residues are abundant both at the peripheral anionic site and inside the gorge. The natural ligand acetylcholine can also bind to both sites of AChE, depending on its concentration(60).

In our study, neostigmine was employed as positive control and the observed ten-fold reduction in efficiency of neostigmine against hsBChE compared to eeAChE and hAChE is consistent with literature data(12, 61). Several of the most active inhibitors displayed submicromolar IC_{50}-values against hsBChE while the experimental IC_{50} for neostigmine was recorded at 62.8 μM. The observed inhibitory range clearly represents an inhibitory efficiency that is in the clinically relevant realm as the IC_{50}-values of approved AChE inhibitors range has been reported to 0.02 to 40 μM as summarised in Moodie et al.(12). It has further been observed in many studies that the absolute values for ChE inhibitors and their controls can vary significantly between studies due to handling, enzyme source and concentration employed(12) and it is thus paramount to always compare to a relevant positive control to fully understand the inhibitory potential of the tested compounds. The potential of the investigated compounds is further increased by the fact that natural barettin and its analogs are efficient antioxidants(38, 39) suggesting that there is potential to tailor this activity into this scaffold to generate multi-target directed ligands (MTDLs) for AD treatment(62). Natural products have been heralded as a particularly promising source for MTDL design (62, 63) and our results could be used to accelerate this development.

Cytotoxicity and hemolysis

The potential of the compounds from a drug development perspective hinges on many factors and safety is essential. To study the off-target toxicity of the compounds they were evaluated against both HUVEC as well as bovine and human erythrocytes as shown in Figures 6 and 7.

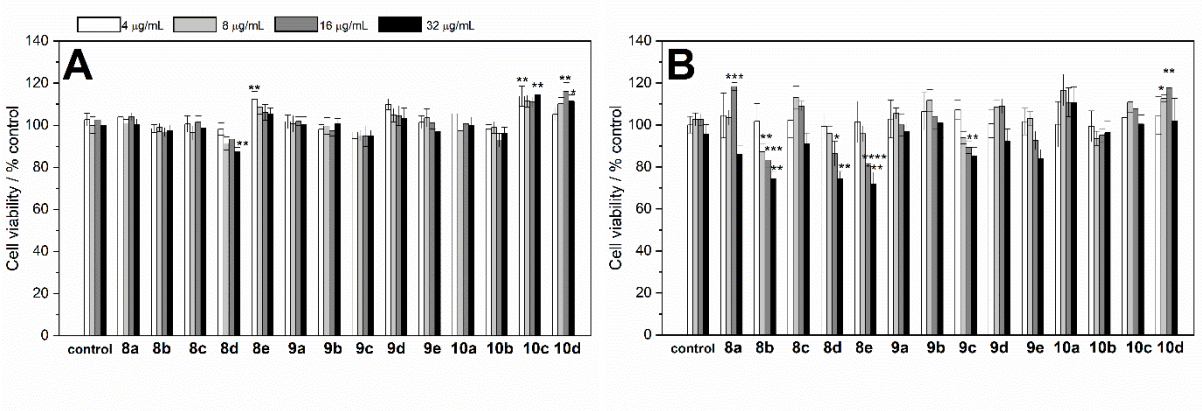


Figure 6. Effects of compounds **8a-10d** on cell survival of mammalian HUVEC cells after 1h (A) and 24h (B) of treatment. Data are mean ± SEM of three independent experiments (*p < 0.05; **p < 0.01; *** p < 0.001; **** p < 0,0001 vs. control including appropriate dilution of methanol).

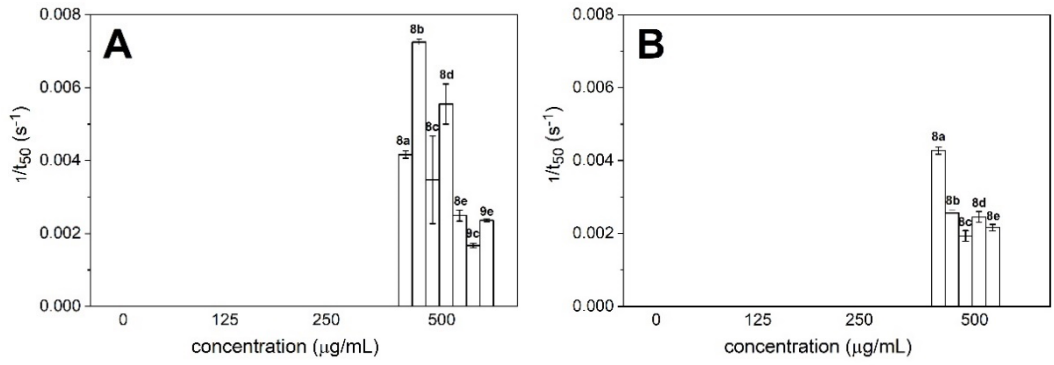


Figure 7. Reciprocal values of the half-times (t_{50}) of hemolysis of bovine (**A**) and human red blood cells (**B**), caused by the compounds **8a-d**, **9c** and **9e**. Each point represents the mean of three measurements with the corresponding standard error.

The majority of the tested compounds did not exhibit any effect on the viability of the of the HUVEC cells after either 1 or 24 h incubation. Of the tested compounds, only **8d** displayed a significant reduction in cell viability (90% viability) after 1 h against the mammalian HUVEC cells at 32 μg/mL (91 μM). After 24 h, reduction in cell viability for compound **8b**, **8e** and **9c** were also observed in addition. The largest reduction after 24 h was observed for **8e** with 75%

remaining viability at 32 μg/mL (88 μM). No hemolytic activity was observed for any compound at concentrations <250 μg/mL (614-806 μM) and most compounds remained inactive also at >500 μg/mL (1.23-1.62 mM) . The amine containing compounds, together with **9c** and **9e,** displayed some hemolysis at 500 μg/mL (1.23-1.62 mM) towards the human erythrocytes while only the amino compounds affecting the bovine erythrocytes likely due to the differences in membrane lipid composition(64). Both the HUVEC cells and the erythrocytes identified the same compounds as potentially toxic at elevated concentrations with good consistency between the assays. The majority of the evaluated compounds were however non-toxic at the employed concentrations supporting therapeutic indexes well in excess of 100 in relation to the IC_{50} towards hsBChE highlighting the potential to develop potent ChE inhibitors with a good safety profile using the investigated marine pharmacophore.

CONCLUSIONS

In the current study, fourteen small inhibitors, designed based on recent discoveries about the inhibitory potential of related marine secondary metabolites have been evaluated as inhibitors for a range of ChE enzymes. It was shown that the simplified marine scaffold could be used to generate potent inhibitors with activities tenfold those of the positive control neostigmine against hsBChE. The ability to form multiple hydrogen bonding interactions via incorporation of a guanidine instead of an amine or a quaternary ammonium group is key for a general inhibitory activity across the three different enzymes evaluated. Cellular toxicity studies against HUVEC cells, human and bovine erythrocytes illustrate a low general toxicity and a wide therapeutic index. BChE is emerging as potential drug target for neurological disorders and by being a mainly circulatory enzyme with a wider substrate acceptance it is expected that a larger freedom in terms of molecular design is possible for the generation of future inhibitors. Our compounds presented here are more potent than the established positive control neostigmine against BChE and they reveal structure activity relationships enabling further optimisation. The employed scaffold, inspired by natural marine settlement inhibitors with numerous bioactivities, is well suited for additional improvement via hydrophobic modifications(37) into MTDL candidates for further development.

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EXPERIMENTAL SECTION

Chemicals and Equipment. All reagents and solvents were purchased commercially and used directly without any further purification. IR spectra were obtained on an Agilent Technologies Cary 630 FTIR 318 spectrometer. ^{1}H and ^{13}C-NMR spectra were recorded at ambient

temperature at a frequency of 400 and 101 MHz, respectively on a Bruker spectrometer. The chemical shifts are reported in ppm and are referenced to the relevant solvent peak: $CDCl_3$ at δ_H 7.26 and δ_C 77.16; CD_3OD at δ_H 3.31 and δ_C 49.0; $(CD_3)_2SO$ at δ_H 2.50 and δ_C 39.5 ppm. High-resolution mass spectra (HRMS) were recorded using MeOH solution on LTQ Orbitrap XL in either positive or negative electrospray ionization (ESI) modes. Microwave reactions were performed in 20 mL vials using a Discover SP from CEM using an Anton Parr Monowave 300 instrument TLC was performed on Merck silica gel 60 F254 plates, using UV light at 254 nm, and PMA staining followed by heating for detection. Flash column chromatography was performed by using the indicated solvent system and silica gel (40–63 mm).

Synthesis

Intermediates and final compounds **8a-8e**, **9a-9d** and **10a** were prepared according to published procedures with matching spectral data (Supporting information)(36). All compounds are >95% pure by HPLC analysis The generation and characterization of novel compounds are described below.

*2-(6-Bromo-1H-indol-3-yl)-N-(6-guanidinohexyl)-N-methylacetamide trifluoroacetate (**9e**).* To a solution of crude **8e** (0.049 g, 0.14 mmol, 1.0 eq.) and *N*,*N'*-Di-Boc-1*H*-pyrazole-1-carboxamidine (0.73 g, 0.28 mmol, 2.0 eq.) in THF (10 mL) was added DIPEA (0.05 mL, 0.28 mmol, 2.0 eq.). The mixture was stirred at rt for 3 h, quenched with H_2O (20 mL), extracted with diethyl ether (3 × 20 mL), washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The diboc-intermediate compound (confirmed by HRMS) was used without further purification and dissolved in TFA:CH_2Cl_2 (1:1, 10 mL) and the reaction mixture was stirred at room temperature for 2 h, quenched with H_2O, extracted with CH_2Cl_2 (2 × 10 mL), washed with H_2O (2 × 10 mL) and the solvent was removed under reduced pressure to afford **9e** as brown oil (19 mg, 33 %). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.63 (dd, J = 4.9, 1.9 Hz, 1H), 7.18 (dd, J = 8.6, 3.1 Hz, 1H), 7.13 – 7.01 (m, 2H), 3.72 (d, J = 0.9 Hz, 2H), 3.30 (td, J = 7.4, 2.5 Hz, 2H), 3.02 – 2.96 (m, 2H), 2.97 (d, J = 11.6 Hz, 3H), 1.49 – 1.28 (m, 4H), 1.23 – 1.02 (m, 4H). ^{13}C NMR (101 MHz, Methanol-d_4) δ: mixture:172.5, 157.2, 135.4, 128.9, 124.7, 123.9, 121.0, 112.6, 111.7, 107.5, 54.4, 40.9, 34.9, 32.5, 31.1, 28.3, 26.4, 25.7. FTMS *m*/*z* 408.1406 $[M+H]^+$ (calcd for $C_{18}H_{27}{}^{79}BrN_5O^+$, 408.1393). HPLC purity: 95.0%.

*4-(2-(6-Bromo-1H-indol-3-yl)acetamido)-N,N,N-trimethylbutan-1-aminium iodide, (**10b**).* To a 0 °C solution of **8b** (100 mg, 0.31 mmol, 1.0 eq.) and glacial acetic acid (71 µL, 1.24 mmol,

4.0 eq.) in MeOH (10 mL) under argon atmosphere, was added sodium cyanoborohydride (39 mg, 0.62 mmol, 2.0 eq.). A solution of formaldehyde (37%) (55 µL, 0.74 mmol, 2.4 eq.) in MeOH (2 mL) was carefully added dropwise over 10 min, and then stirred at rt for overnight (21h), quenched with Na_2CO_3 (2 M) until pH 8-9 and concentrated under reduced pressure. The residue was taken up in $CHCl_3$, washed with brine and dried over Na_2SO_4, filtered and concentrated to obtain the dimethylated product (108 mg, 99%). ^{1}H NMR (400 MHz, MeOD) δ 7.92 (s, 1H), 7.74 (d, *J* = 1.9 Hz, 1H), 7.29 (d, *J* = 8.6 Hz, 1H), 7.25 – 7.17 (m, 2H), 3.61 (s, 2H), 3.21 (t, *J* = 6.4 Hz, 2H), 2.34 – 2.22 (m, 2H), 2.16 (s, 6H), 1.46 (pd, *J* = 8.8, 7.3, 3.2 Hz, 4H). ^{13}C NMR (101 MHz, MeOD) δ 173.10, 125.07, 123.88, 120.71, 112.63, 111.74, 108.12, 58.75, 47.39, 47.18, 46.97, 43.82, 38.81, 32.55, 26.91, 23.94.To a 0 °C solution of the dimethylated product (97 mg, 0.28 mmol) in $CHCl_3$ (5 mL) was added iodomethane (52 µl, 0.84 mmol, 3 eq.) in portions (× 3). The precipitate was isolated, co-evaporated with MeOH (× 3) to afford the product **10b** as a light-yellow solid (115 mg, 83 %). ^{1}H NMR (400 MHz, MeOD) δ 7.80 (d, *J* = 1.9 Hz, 1H), 7.34 – 7.27 (m, 2H), 7.23 (dd, *J* = 8.6, 2.0 Hz, 1H), 3.65 (s, 2H), 3.28 (td, *J* = 7.7, 7.1, 3.6 Hz, 4H), 2.96 (s, 9H), 1.70 – 1.53 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 135.39, 125.40, 123.94, 120.83, 112.87, 108.25, 65.86, 52.13, 52.08, 52.05, 47.40, 47.19, 46.97, 37.66, 32.72, 25.85, 19.52. FTMS *m/z* 366.1176 $[M]^+$ (calcd for $C_{17}H_{25}{}^{79}BrN_3O^+$, 366.1176). HPLC purity: 98.8%

*5-(2-(6-Bromo-1H-indol-3-yl)acetamido)-N,N,N-trimethylpentan-1-aminium iodide, (**10c**).* To a 0 °C solution of **8c** (100 mg, 0.30 mmol, 1.0 eq.) and glacial acetic acid (70 µL, 1.18 mmol, 4.0 eq.) in MeOH (10 mL) under argon atmosphere, was added sodium cyanoborohydride (38 mg, 0.60 mmol, 2.0 eq.). A solution of formaldehyde (37%) (54 µL, 0.72 mmol, 2.4 eq.) in MeOH (2 mL) was carefully added dropwise over 10 min, and then stirred at rt for overnight, quenched with Na_2CO_3 (2 M) until pH 8-9 and concentrated under reduced pressure. The residue was taken up in $CHCl_3$, washed with brine and dried over Na_2SO_4, filtered, and concentrated to obtain the dimethylated product. (103 mg, 94%). ^{1}H NMR (400 MHz, MeOD) δ 7.92 (s, 1H), 7.74 (d, *J* = 1.9 Hz, 1H), 7.29 (d, *J* = 8.6 Hz, 1H), 7.26 – 7.15 (m, 2H), 3.61 (s, 2H), 3.20 (t, *J* = 6.8 Hz, 2H), 2.22 (s, 8H), 1.57 – 1.38 (m, 4H), 1.24 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (101 MHz, MeOD) δ 135.39, 125.11, 123.87, 120.77, 112.64, 111.69, 108.14, 59.09, 47.39, 47.18, 46.97, 43.92, 38.92, 32.58, 28.83, 26.42, 24.28.

To a 0 °C solution of the dimethylated product (90 mg, 0.25 mmol) in $CHCl_3$ (5 mL) was added iodomethane (47 µl, 0.75 mmol, 3 eq.) in portions (× 3). The precipitate was isolated, co-evaporated with MeOH (× 3) to afford the product **10c** as a light-yellow solid (104 mg, 82 %).

^{1}H NMR (400 MHz, MeOD) δ 7.76 (d, *J* = 1.9 Hz, 1H), 7.31 (d, *J* = 8.6 Hz, 1H), 7.26 – 7.18 (m, 2H), 3.61 (s, 2H), 3.23 (t, *J* = 6.7 Hz, 2H), 3.19 – 3.10 (m, 2H), 3.05 (s, 9H), 1.71 (td, *J* = 12.1, 10.0, 6.2 Hz, 2H), 1.56 (p, *J* = 7.0 Hz, 2H), 1.28 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, MeOD) δ 125.30, 123.87, 120.88, 112.80, 66.34, 52.22, 52.18, 52.14, 47.39, 47.18, 46.97, 38.10, 32.65, 28.29, 22.77, 21.82. FTMS *m/z* 380.1331 $[M]^+$ (calcd for $C_{18}H_{27}{}^{79}BrN_3O^+$, 380.1332). HPLC purity: 98.7%

*6-(2-(6-Bromo-1H-indol-3-yl)acetamido)-N,N,N-trimethylhexan-1-aminium iodide, (**10d**).* To a 0 °C solution of **8d** (100 mg, 0.28 mmol, 1.0 eq.) and glacial acetic acid (69 µL, 1.28 mmol, 4.0 eq.) in MeOH (10 mL) under argon atmosphere, was added sodium cyanoborohydride (35 mg, 0.56 mmol, 2.0 eq.). A solution of formaldehyde (37%) (53 µL, 0.67 mmol, 2.4 eq.) in MeOH (2 mL) was carefully added dropwise over 10 min, and then stirred at rt for overnight, quenched with Na_2CO_3 (2 M) until pH 8-9 and concentrated under reduced pressure. The residue was taken up in $CHCl_3$, washed with brine and dried over Na_2SO_4, filtered, and concentrated to obtain the dimethylated product. (104 mg, 98%). ^{1}H NMR (400 MHz, MeOD) δ 7.92 (s, 1H), 7.73 (d, *J* = 1.8 Hz, 1H), 7.29 (d, *J* = 8.6 Hz, 1H), 7.26 – 7.14 (m, 2H), 3.61 (s, 2H), 3.20 (d, *J* = 6.8 Hz, 2H), 2.31 – 2.16 (m, 8H), 1.56 – 1.37 (m, 4H), 1.28 (dq, *J* = 7.4, 4.5, 3.5 Hz, 4H). ^{13}C NMR (101 MHz, MeOD) δ 173.06, 135.38, 128.90, 125.10, 123.87, 120.76, 112.63, 111.70, 78.46, 59.18, 47.40, 47.18, 46.97, 43.95, 39.01, 32.57, 28.95, 26.82, 26.73, 26.39. To a 0 °C solution of the dimethylated product (90 mg, 0.24 mmol) in $CHCl_3$ (5 mL) was added iodomethane (46 µl, 0.72 mmol) in portions (× 3). The precipitate was isolated, co-evaporated with MeOH (× 3) to afford the product **10d** as a pale-yellow solid (107 mg, 86 %). ^{1}H NMR (400 MHz, MeOD) δ 7.76 (d, *J* = 1.9 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 7.27 – 7.17 (m, 2H), 3.63 (s, 2H), 3.30 – 3.13 (m, 4H), 3.09 (s, 9H), 1.75 – 1.63 (m, 2H), 1.53 (dt, *J* = 6.9, 3.8 Hz, 2H), 1.41 – 1.26 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 125.3, 123.8, 120.9, 112.8, 66.3, 52.2, 52.2, 52.1, 47.4, 47.2, 47.0, 38.7, 32.6, 28.7, 25.8, 25.4, 22.4. FTMS *m/z* 394.1491 $[M]^+$ (calcd for $C_{19}H_{29}{}^{79}BrN_3O^+$, 394.1489). HPLC purity: 96.2%.

Cholinesterase Inhibition

The activities of electric eel acetylcholinesterase (eeAChE), human recombinant AChE (hAChE) or horse serum butyrylcholinesterase (hsBChE) (all Sigma-Aldrich, St. Louis, Missouri, USA), each dissolved in 100 mM potassium phosphate buffer (pH 7.4), were measured by the Ellman method(43) adapted for microtiter plates(65). Stock solutions of the

tested compounds (2 mg/mL) were prepared in 100% methanol (MeOH), gradually diluted in 100 mM potassium phosphate buffer (pH 7.4), and 25 μL of each dilution was added to the wells of the microtiter plate. Acetylthiocholine chloride (1 mM) and 5,5′-dithiobis-2-nitrobenzoic acid (0.5 mM), dissolved in the same buffer, were added (100 μL) to all the wells, followed by the addition of eeAChE, hAChE or hsBChE (50 μL). The final concentration of all the assayed enzymes was 0.0075 U/mL. Kinetics were followed spectrophotometrically at 405 nm and 25 °C for 5 min using a microplate kinetic reader (Dynex Technologies Inc, Chantilly, Virginia, USA). Blank reactions without the inhibitors were performed in the presence of the appropriate dilution of MeOH in 100 mM potassium phosphate buffer (pH 7.4). At the end of the experiments, the concentrations of compounds causing 50% inhibition of cholinesterase activity (IC_{50}) were determined. To determine the inhibition constants (K_i), the kinetics were monitored using three different final substrate concentrations (0.125, 0.25, 0.5 mM). Each measurement was repeated at least three times. Data were analysed using OriginPro software (OriginPro 2020, OriginLab Corporation, Northampton, Massachusetts, USA).

Cytotoxicity

Human umbilical vein endothelial cells (HUVEC) were grown in modified Eagle medium with glutamine (Gibco, Invitrogen, UK), 100 μg/mL penicillin-streptomycin (Gibco, Invitrogen, UK), and 10 % foetal bovine serum (Gibco, Invitrogen, UK) in a CO_2 incubator (5% CO_2, 95 % air, 95 % relative humidity) at 37 °C. For the *in vitro* cytotoxicity assay, the cells were plated in sterile 96-well microtiter plates (TPP, Switzerland) at 5×10^3 cells/mL per well. Cells were left to attach and after 24 h, different compounds **8a-10d** at different concentrations (4, 8, 16 and 32 μg/mL) in growth medium with serum and penicillin-streptomycin was added. Compound stock solutions were prepared in methanol and therefore appropriate dilutions of methanol in growth medium were used as controls. After 1 h or 24 h of incubation under cell growth conditions, the cytotoxicity was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test in which in viable cells water-soluble diphenyl tetrazolium bromide is converted to insoluble formazan. 100 μL of MTT (0.5 mg/mL in phosphate buffered saline) (Merck, Germany) was added to each well and after 3h the content was carefully removed. 100 μL of dimethyl sulfoxide (Fisher Scientific, UK) was added and the absorbance was measured at 570 nm using microplate reader Cytation 3 (BioTek, USA). The amount of the formed soluble formazan, which corresponds to the absorption, is proportional to the number of viable cells. The viability (%) was calculated as the ratio between treated vs. control cells. Results represent means ± SEM from three independent experiments.

The differences were analysed using Student's *t*-tests on two populations, with at least $p < 0.05$ considered significant.

Measurement of Hemolytic Activity

Haemolytic activity was measured by a turbidimetric method as described previously(66). Bovine or human erythrocytes were centrifuged from freshly collected citrated blood and washed three times with 140 mM NaCl, 20 mM Tris-HCl buffer, pH 8.0. Typically, 100 µL of different concentrations (twelve concentrations, serially diluted 1:2, the highest final concentration was 500 µg/mL) of tested compounds were combined with 100 µL of erythrocyte suspension with an initial absorbance (at 650 nm) of 0.5. The decrease of absorbance was monitored for 20 min using a Kinetic Microplate Reader (Dynex Technologies Inc, Chantilly, Virginia, USA) in order to define the time needed for 50% haemolysis (t_{50}). All experiments were performed at 25 °C and repeated three times.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information, ^{1}H and ^{13}C NMR spectra and additional experimental details is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest

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ABBREVIATIONS

AD, Alzheimer's disease; ChE, cholinesterase; CNS, central nervous system; eeAChE, electric eel acetylcholine esterase; hAChE, human acetylcholine esterase; hsBChE horse serum acetylcholine esterase; HUVEC, human umbilical vein endothelial cells; *K*i, inhibition constants; μM, micromolar; MTDLs, multi-target directed ligands MTDLs; NMR, nuclear magnetic resonance; HRMS, high-resolution mass spectra; MeOH, methanol; TFA, trifluoroacetic acid; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide);

REFERENCES

1. Butler MS. Natural products to drugs: natural product-derived compounds in clinical trials. Natural Product Reports. (2008) 25:475-516 DOI: 10.1039/B514294F.

2. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Nearly F our Decades from 01/1981 to 09/2019. Journal of Natural Products. (2020) 83:770-803 DOI: 10.1021/acs.jnatprod.9b01285.
3. Svenson J. MabCent: Arctic marine bioprospecting in Norway. Phytochemistry Reviews. (2013) 12:567-78.
4. Banerjee P, Mandhare A, Bagalkote V. Marine natural products as source of new drugs: an updated patent review (July 2018-July 2021). Expert Opinion on Therapeutic Patents. (2022) 32:317-63 DOI: 10.1080/13543776.2022.2012150.
5. Bauer RA, Wurst JM, Tan DS. Expanding the range of 'druggable' targets with natural product-based libraries: an academic perspective. Current Opinion in Chemical Biology. (2010) 14:308-14 DOI: https://doi.org/10.1016/j.cbpa.2010.02.001.
6. Carroll AR, Copp BR, Davis RA, Keyzers RA, Prinsep MR. Marine natural products. Natural product reports. (2022).
7. Jiménez C. Marine Natural Products in Medicinal Chemistry. ACS Medicinal Chemistry Letters. (2018) 9:959-61 DOI: 10.1021/acsmedchemlett.8b00368.
8. Newman DJ, Cragg GM. Drugs and Drug Candidates from Marine Sources: An Assessment of the Current "State of Play". Planta Med. (2016) 82:775-89.
9. Nelson A, Karageorgis G. Natural product-informed exploration of chemical space to enable bioactive molecular discovery. RSC Medicinal Chemistry. (2021) 12:353-62 DOI: 10.1039/D0MD00376J.
10. Wu AC, Jelielek KK, Le HQ, Butt M, Newman DJ, Glaser KB, et al. The 2021 Marine Pharmacology and Pharmaceuticals Pipeline. The FASEB Journal. (2022) 36 DOI: https://doi.org/10.1096/fasebj.2022.36.S1.L7586.
11. Ayaz M, Ullah F, Sadiq A, Kim MO, Ali T. Editorial: Natural Products-Based Drugs: Potential Therapeutics Against Alzheimer's Disease and Other Neurological Disorders. Frontiers in Pharmacology. (2019) 10 DOI: 10.3389/fphar.2019.01417.
12. Moodie LW, Sepčić K, Turk T, Frangež R, Svenson J. Natural cholinesterase inhibitors from marine organisms. Natural Product Reports. (2019) 36:1053-92.
13. Sramek JJ, Frackiewicz EJ, Cutler NR. Review of the acetylcholinesterase inhibitor galanthamine. Expert Opinion on Investigational Drugs. (2000) 9:2393-402 DOI: 10.1517/13543784.9.10.2393.
14. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: A systematic review and metaanalysis. Alzheimer's & Dementia. (2013) 9:63-75.e2 DOI: https://doi.org/10.1016/j.jalz.2012.11.007.

15. Mehta M, Adem A, Sabbagh M. New acetylcholinesterase inhibitors for Alzheimer's disease. International Journal of Alzheimer's disease. (2012) 2012.
16. Deardorff WJ, Feen E, Grossberg GT. The Use of Cholinesterase Inhibitors Across All Stages of Alzheimer's Disease. Drugs & Aging. (2015) 32:537-47 DOI: 10.1007/s40266-015-0273-x.
17. Sussman JL, Harel M, Frolow F, Oefner C, Goldman A, Toker L, et al. Atomic Structure of Acetylcholinesterase from Torpedo californica: A Prototypic Acetylcholine-Binding Protein. Science. (1991) 253:872-9 DOI: doi:10.1126/science.1678899.
18. Ibach B, Haen E. Acetylcholinesterase Inhibition in Alzheimer's Disease. Current Pharmaceutical Design. (2004) 10:231-51 DOI: 10.2174/1381612043386509.
19. Talesa VN. Acetylcholinesterase in Alzheimer's disease. Mechanisms of Ageing and Development. (2001) 122:1961-9 DOI: https://doi.org/10.1016/S0047-6374(01)00309-8.
20. Weinreb RN, Leung CKS, Crowston JG, Medeiros FA, Friedman DS, Wiggs JL, et al. Primary open-angle glaucoma. Nature Reviews Disease Primers. (2016) 2:16067 DOI: 10.1038/nrdp.2016.67.
21. Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. Muscle & Nerve. (2008) 37:141-9 DOI: https://doi.org/10.1002/mus.20950.
22. Silman I, Sussman JL. Acetylcholinesterase: How is structure related to function? Chemico-biological interactions. (2008) 175:3-10 DOI: https://doi.org/10.1016/j.cbi.2008.05.035.
23. Banks WA. Characteristics of compounds that cross the blood-brain barrier. BMC Neurology. (2009) 9:S3 DOI: 10.1186/1471-2377-9-S1-S3.
24. Neuwelt E, Abbott NJ, Abrey L, Banks WA, Blakley B, Davis T, et al. Strategies to advance translational research into brain barriers. The Lancet Neurology. (2008) 7:84-96 DOI: https://doi.org/10.1016/S1474-4422(07)70326-5.
25. Carbone M, Li Y, Irace C, Mollo E, Castelluccio F, Di Pascale A, et al. Structure and Cytotoxicity of Phidianidines A and B: First Finding of 1,2,4-Oxadiazole System in a Marine Natural Product. Organic Letters. (2011) 13:2516-9 DOI: 10.1021/ol200234r.
26. Lidgren G, Bohlin L, Bergman J. Studies of swedish marine organisms VII. A novel biologically active indole alkaloid from the sponge geodia baretti. Tetrahedron Letters. (1986) 27:3283-4 DOI: https://doi.org/10.1016/S0040-4039(00)84776-0.

27. Forenza S, Minale L, Riccio R, Fattorusso E. New bromo-pyrrole derivatives from the sponge Agelas oroides. Journal of the Chemical Society D: Chemical Communications. (1971):1129-30 DOI: 10.1039/C29710001129.
28. Tadesse M, Strøm MB, Svenson J, Jaspars M, Milne BF, Tørfoss V, et al. Synoxazolidinones A and B: novel bioactive alkaloids from the ascidian Synoicum pulmonaria. Organic letters. (2010) 12:4752-5.
29. Tadesse M, Svenson J, Jaspars M, Strøm MB, Abdelrahman MH, Andersen JH, et al. Synoxazolidinone C; a bicyclic member of the synoxazolidinone family with antibacterial and anticancer activities. Tetrahedron Letters. (2011) 52:1804-6.
30. Litaudon M, Guyot M. Ianthelline, un nouveau derive de lfl dibromo-3, 5 tyrosine, isole de l'eponge ianthella ardis (bahamas). Tetrahedron Letters. (1986) 27:4455-6.
31. Hanssen KØ, Andersen JH, Stiberg T, Engh RA, Svenson J, Genevière A-M, et al. Antitumoral and mechanistic studies of ianthelline isolated from the Arctic sponge Stryphnus fortis. Anticancer Research. (2012) 32:4287-97.
32. Tadesse M, Svenson J, Sepčić K, Trembleau L, Engqvist M, Andersen JH, et al. Isolation and synthesis of pulmonarins A and B, acetylcholinesterase inhibitors from the colonial ascidian Synoicum pulmonaria. Journal of Natural Products. (2014) 77:364-9.
33. Labriere C, Kondori N, Caous JS, Boomgaren M, Sandholm K, Ekdahl KN, et al. Development and evaluation of cationic amphiphilic antimicrobial 2,5-diketopiperazines. Journal of peptide science : an official publication of the European Peptide Society. (2018) 24:e3090 DOI: 10.1002/psc.3090.
34. Trepos R, Cervin G, Hellio C, Pavia H, Stensen W, Stensvåg K, et al. Antifouling compounds from the sub-arctic ascidian Synoicum pulmonaria: Synoxazolidinones A and C, pulmonarins A and B, and synthetic analogues. Journal of Natural Products. (2014) 77:2105-13.
35. Hanssen KØ, Cervin G, Trepos R, Petitbois J, Haug T, Hansen E, et al. The bromotyrosine derivative ianthelline isolated from the arctic marine sponge Stryphnus fortis inhibits marine micro-and macrobiofouling. Marine Biotechnology. (2014) 16:684-94.
36. Labriere C, Elumalai V, Staffansson J, Cervin G, Le Norcy T, Denardou H, et al. Phidianidine A and Synthetic Analogues as Naturally Inspired Marine Antifoulants. Journal of Natural Products. (2020) DOI: 10.1021/acs.jnatprod.0c00881.

37. Labriere C, Cervin G, Pavia H, Hansen JH, Svenson J. Structure–Activity Relationship Probing of the Natural Marine Antifoulant Barettin. Marine Biotechnology. (2021) 23:904-16 DOI: 10.1007/s10126-021-10074-z.
38. Lind KF, Hansen E, Østerud B, Eilertsen K-E, Bayer A, Engqvist M, et al. Antioxidant and anti-inflammatory activities of barettin. Marine Drugs (2013) 11:2655-66.
39. Labriere C, Andersen JH, Albrigtsen M, Hansen JH, Svenson J. Heterocyclic cellular lipid peroxidation inhibitors inspired by the marine antioxidant barettin. Bioorganic Chemistry. (2019) 84:106-14.
40. Olsen EK, Hansen E, Moodie LW, Isaksson J, Sepčić K, Cergolj M, et al. Marine AChE inhibitors isolated from Geodia barretti: natural compounds and their synthetic analogs. Organic Biomolecular Chemistry (2016) 14:1629-40.
41. Moodie LW, Žužek MC, Frangež R, Andersen JH, Hansen E, Olsen EK, et al. Synthetic analogs of stryphnusin isolated from the marine sponge Stryphnus fortis inhibit acetylcholinesterase with no effect on muscle function or neuromuscular transmission. Organic Biomolecular Chemistry. (2016) 14:11220-9 DOI: 10.1039/c6ob02120d.
42. Kochanowska-Karamyan AJ, Hamann MT. Marine Indole Alkaloids: Potential New Drug Leads for the Control of Depression and Anxiety. Chemical Reviews. (2010) 110:4489-97 DOI: 10.1021/cr900211p.
43. Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical pharmacology. (1961) 7:88-95.
44. Orhan IE. Nature: A Substantial Source of Auspicious Substances with Acetylcholinesterase Inhibitory Action. Current Neuropharmacology. (2013) 11:379-87.
45. Ha ZY, Mathew S, Yeong KY. Butyrylcholinesterase: A Multifaceted Pharmacological Target and Tool. Current Protein and Peptide Science. (2020) 21:99-109 DOI: 10.2174/1389203720666191107094949.
46. Pezzementi L, Chatonnet A. Evolution of cholinesterases in the animal kingdom. Chemico-biological interactions. (2010) 187:27-33 DOI: https://doi.org/10.1016/j.cbi.2010.03.043.
47. Li B, Stribley JA, Ticu A, Xie W, Schopfer LM, Hammond P, et al. Abundant Tissue Butyrylcholinesterase and Its Possible Function in the Acetylcholinesterase Knockout Mouse. Journal of Neurochemistry. (2000) 75:1320-31 DOI: https://doi.org/10.1046/j.1471-4159.2000.751320.x.

48. Darvesh S, Hopkins DA, Geula C. Neurobiology of butyrylcholinesterase. Nature Reviews Neuroscience. (2003) 4:131-8 DOI: 10.1038/nrn1035.

49. Li Q, Chen Y, Xing S, Liao Q, Xiong B, Wang Y, et al. Highly Potent and Selective Butyrylcholinesterase Inhibitors for Cognitive Improvement and Neuroprotection. Journal of medicinal chemistry. (2021) 64:6856-76 DOI: 10.1021/acs.jmedchem.1c00167.

50. Mendel B, Rudney H. Studies on cholinesterase: 1. Cholinesterase and pseudo-cholinesterase. Biochem J. (1943) 37:59-63 DOI: 10.1042/bj0370059.

51. Çokuğraş AN. Butyrylcholinesterase: structure and physiological importance. Turk J Biochem. (2003) 28:54-61.

52. Radic Z, Pickering NA, Vellom DC, Camp S, Taylor P. Three distinct domains in the cholinesterase molecule confer selectivity for acetyl- and butyrylcholinesterase inhibitors. Biochemistry. (1993) 32:12074-84 DOI: 10.1021/bi00096a018.

53. Arabshahi HJ, Trobec T, Foulon V, Hellio C, Frangež R, Sepčić K, et al. Using Virtual AChE Homology Screening to Identify Small Molecules With the Ability to Inhibit Marine Biofouling. Frontiers in Marine Science. (2021) 8 DOI: 10.3389/fmars.2021.762287.

54. Khorana N, Changwichit K, Ingkaninan K, Utsintong M. Prospective acetylcholinesterase inhibitory activity of indole and its analogs. Bioorganic & Medicinal Chemistry Letters. (2012) 22:2885-8 DOI: https://doi.org/10.1016/j.bmcl.2012.02.057.

55. Svenson J, Karstad R, Flaten GE, Brandsdal BO, Brandl M, Svendsen JS. Altered activity and physicochemical properties of short cationic antimicrobial peptides by incorporation of arginine analogues. Molecular pharmaceutics. (2009) 6:996-1005 DOI: 10.1021/mp900057k.

56. Svendsen JSM, Grant TM, Rennison D, Brimble MA, Svenson J. Very short and stable lactoferricin-derived antimicrobial peptides: design principles and potential uses. Accounts of chemical research. (2019) 52:749-59 DOI: 10.1021/acs.accounts.8b00624.

57. Cotton FA, Day VW, Hazen EE, Larsen S. Structure of methylguanidinium dihydrogen orthophosphate. Model compound for arginine-phosphate hydrogen bonding. Journal of the American Chemical Society. (1973) 95:4834-40 DOI: 10.1021/ja00796a012.

58. Meloni BP, Mastaglia FL, Knuckey NW. Cationic Arginine-Rich Peptides (CARPs): A Novel Class of Neuroprotective Agents With a Multimodal Mechanism of Action. Front Neurol. (2020) 11:108 DOI: 10.3389/fneur.2020.00108.

59. Robaire B, Kato G. Effects of Edrophonium, Eserine, Decamethonium, d-Tubocurarine, and Gallamine on the Kinetics of Membrane-Bound and Solubilized Eel Acetylcholinesterase. Molecular Pharmacology. (1975) 11:722-34.

60. Radić Z, Reiner E, Taylor P. Role of the peripheral anionic site on acetylcholinesterase: inhibition by substrates and coumarin derivatives. Molecular Pharmacology. (1991) 39:98-104.

61. Soukup O, Winder M, Kumar Killi U, Wsol V, Jun D, Kuca K, et al. Acetylcholinesterase Inhibitors and Drugs Acting on Muscarinic Receptors- Potential Crosstalk of Cholinergic Mechanisms During Pharmacological Treatment. Current Neuropharmacology. (2017) 15:637-53.

62. Rossi M, Freschi M, de Camargo Nascente L, Salerno A, de Melo Viana Teixeira S, Nachon F, et al. Sustainable Drug Discovery of Multi-Target-Directed Ligands for Alzheimer's Disease. Journal of medicinal chemistry. (2021) 64:4972-90 DOI: 10.1021/acs.jmedchem.1c00048.

63. Agis-Torres A, Sollhuber M, Fernandez M, Sanchez-Montero JM. Multi-Target-Directed Ligands and other Therapeutic Strategies in the Search of a Real Solution for Alzheimer’s Disease. Current Neuropharmacology. (2014) 12:2-36.

64. Greco I, Molchanova N, Holmedal E, Jenssen H, Hummel BD, Watts JL, et al. Correlation between hemolytic activity, cytotoxicity and systemic in vivo toxicity of synthetic antimicrobial peptides. Scientific Reports. (2020) 10:1-13 DOI: 10.1038/s41598-020-69995-9.

65. Ristovski S, Uzelac M, Kljun J, Lipec T, Uršič M, Zemljič Jokhadar Š, et al. Organoruthenium Prodrugs as a New Class of Cholinesterase and Glutathione-S-Transferase Inhibitors. ChemMedChem. (2018) 13:2166-76 DOI: https://doi.org/10.1002/cmdc.201800432.

66. Sepčić K, Berne S, Potrich C, Turk T, Maček P, Menestrina G. Interaction of ostreolysin, a cytolytic protein from the edible mushroom Pleurotus ostreatus, with lipid membranes and modulation by lysophospholipids. European Journal of Biochemistry. (2003) 270:1199-210 DOI: https://doi.org/10.1046/j.1432-1033.2003.03480.x.

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