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"Clicking" fragment leads to novel dual-binding cholinesterase inhibitors

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This work is dedicated to Prof. Pietro Traldi.

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ABSTRACT

Cholinesterase inhibitors are potent therapeutics in the treatment of Alzheimer's disease. Among them, dual binding ligands have recently gained a lot of attention. We discovered novel dual-binding cholinesterase inhibitors, using "clickable" fragments, which bind to either catalytic active site (CAS) or peripheral anionic site (PAS) of the enzyme. Copper(I)-catalyzed azide-alkyne cycloaddition allowed to effectively synthesize a series of final heterodimers, and modeling and kinetic studies confirmed their ability to bind to both CAS and PAS. A potent acetylcholinesterase inhibitor with IC_{50} = 18 nM (compound **23g**) was discovered. A target-guided approach to link fragments by the enzyme itself was tested using butyrylcholinesterase.

1. Introduction

Alzheimer's disease (AD) is the most common type of dementia among the elderly. The symptoms of the disease are serious cognitive disorders, especially a progressive amnesia. Despite the extensive research in this field, there is no effective causative treatment available so far. The most popular therapeutic strategy is based on the hypothesis that increasing the amount of acetylcholine in the synaptic gap leads to more effective use of the neurons, which slows down the progression of the disease. To achieve this goal, the use of cholinesterase inhibitors seems to be a solution [1,2]. Characteristic changes in the AD patient's brain are β-amyloid plaques (Aβ) and neurofibrillary tangles, accompanied with neurodegeneration. Significant amounts of cholinesterases have been found in both of those pathological changes [3]. It seems that cholinesterases have an impact on the formation of the plaques and tangles and additionally enhance their toxicity, activating microglia and hydrolyzing acetylcholine [4]. It therefore emerged that inhibition of the cholinesterases not only leads to a better function of the damaged cholinergic system but also prevents its further degradation. Given that those "non-classical" functions of acetylcholinesterase involve the peripheral anionic site (PAS) of the enzyme, there has been a growing interest in inhibitors targeted against this site [5]. Cholinesterase inhibitors like donepezil and galantamine are widely used in AD treatment but they exhibit numerous limitations. Therefore, the search for better inhibitors is urgent and should concentrate mainly on compounds able to bind to both the catalytic active site (CAS) and PAS of the enzyme.

While acetylcholinesterase is a well-defined pharmacological target, the role of its sister enzyme, butyrylcholinesterase, remains more obscure [5,6]. It is known that butyrylcholinesterase is also able to regulate neurotransmission by hydrolyzing acetylcholine, induces β-amyloid deposition and enhances its toxicity [4]. There is a reasonable assumption that it is the inhibition of butyrylcholinesterase, which can be more effective in the treatment of AD, as at an advanced stage of the disease, the levels of acetylcholinesterase are decreased while levels of butyrylcholinesterase remain high. Butyrylcholinesterase is therefore considered a promising drug target [7]. Apart from cholinergic functions,

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butyrylcholinesterase plays an important role in detoxification; there are also evidences of its impact on lipid metabolism and metabolic disorders [8].

Drug-discovery research suffers from problems with effective screening of a large number of potentially active compounds. One of the solutions to this problem is target-guided synthesis (TGS) [9]. In this method, two libraries of building blocks potentially able to bind to the biological target are synthesized. In the next step, a reaction between the building blocks is performed on the mixture of compounds in the presence of the target enzyme. As the enzyme is able to select the best binder, it brings them in proximity and templates the reaction to afford the most promising ligand. (Fig. 1).

TGS includes methods based on reversible reactions (dynamic combinatorial libraries), in which the presence of an enzyme shifts the equilibrium between interchanging products with preference to the best binders [10], and methods using enzyme-catalyzed irreversible reactions where the products with the highest affinity to the enzyme are produced exclusively [11]. Both methods provide an effective tool for screening of large numbers of potential ligands.

Click chemistry offers numerous advantages in drug discovery [12,13,14,15]. Kinetic target-guided synthesis (KTGS) based on the *in situ* click reaction seems to be a very promising tool thanks to its high application potential, its orthogonality, good kinetics, tolerance to physiological conditions and synthetic availability of suitable building blocks [16]. Yet, this relatively new approach has not been widely investigated so far. A full landscape of its applications in drug discovery, studying protein–protein interactions or templating coupling reactions on natural products are still to be discovered.[11,17,18]

The first enzyme used for a proof of concept of this method was acetylcholinesterase [19]. It seems to be a perfect target for an enzyme-templated coupling reaction as its active site is composed of two separate binding sites (CAS and PAS) connected by a narrow gorge rich in aromatic side chains. Sharpless and coworkers chose tacrine and phenanthridinium motifs for binding to the CAS and PAS, respectively. Building blocks with alkyl azides and alkyl acetylenes of varying chain lengths were synthesized and subjected in binary mixtures to acetylcholinesterase, leading to *in situ* assembly of bivalent femtomolar inhibitors, in which moieties binding to the CAS and PAS of the enzyme are connected with an *in situ* formed triazole linker. This strategy was then extended successfully to libraries using the *in situ* click chemistry screening approach [20]. Further docking analysis of the new inhibitors showed that the formation of the complex with acetylcholinesterase promotes significant conformational changes of the native structure of the enzyme. Therefore, the structures of these inhibitors could not have been predicted using *in silico* modeling methods [21].

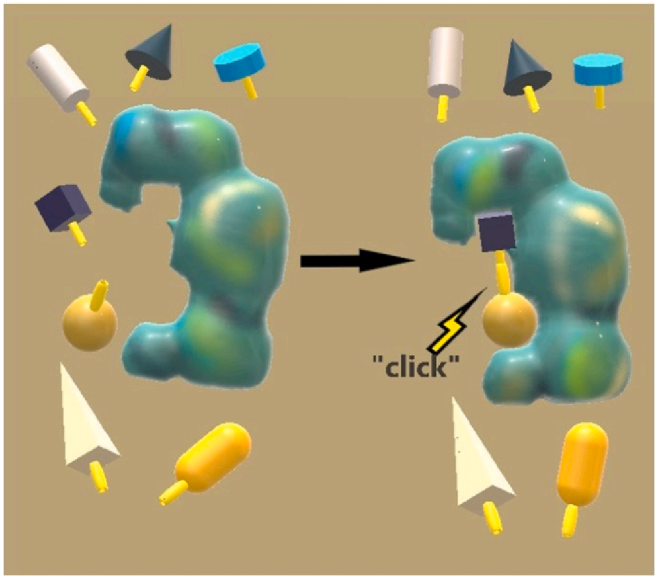


Fig. 1. Protein-templated reaction on a library of fragments.

This discovery may open promising prospects for the search for new acetylcholinesterase inhibitors. Not only because of the beauty of KTGS, in which the enzyme synthesizes its own inhibitors or the effectiveness of multicomponent screening but also because of the extremely high inhibitory activity of this new type of compounds. It was shown that the strong bivalent interaction with both binding sites of acetylcholinesterase is additionally enhanced by the presence of the triazole moiety, which apart from functioning as a linker, engages in hydrogen bonding and π-π stacking interactions with amino acid residues of the gorge of acetylcholinesterase [20]. Surprisingly, only a few "click"-based target guided syntheses of acetylcholinesterase inhibitors were reported and none of the compounds obtained was subjected to further biological testing [20,22].

Demonstrating the application of KTGS for the search for new dual binding inhibitors of butyrylcholinesterase would be of high importance for both pharmacological use and biochemical studies, providing a powerful tool to investigate the role of this ambiguous enzyme.

The aim of this study was the discovery of new dual-binding cholinesterase inhibitors with the aid of a combinatorial approach, employing "the click" reaction to generate heterodimers of high affinity towards acetyl- and butyrylcholinesterase. An additional goal was to verify if butyrylcholinesterase can act as a template in the KTGS experiments, which could open new prospects for the search of its inhibitors.

The design of new dual binding cholinesterase inhibitors was based on structures of the leading ligands of a known affinity towards cholinesterase CAS and PAS. Fragments interacting with the CAS were derived from galantamine **(1)** and cryspine A **(2)**. Fragments binding to the PAS contained an indole moiety [23], with a particular focus on melatonin **(3)** derivatives, due to its beneficial effect on neuroprotection [24] (Fig. 2). Combinatorial libraries of azides and alkynes were built in order to enable a "click" reaction between building blocks (Fig. 3). An example of the final heterodimer, expected to bind to both CAS and PAS of cholinesterases, is presented in Fig. 4.

2. Results and discussion

2.1. Chemistry

The building blocks with indole motif, designed to bind to the PAS of cholinesterases, were synthesized from tryptamine **(4)** and 5-methoxytryptamine **(5)** by reaction with the suitable carboxylic acids possessing a terminal azide group (Scheme 1).

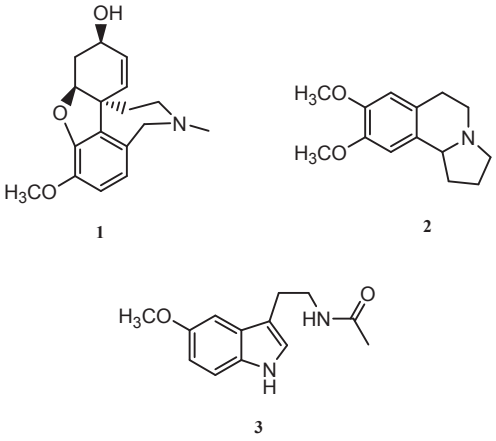


Fig. 2. Structures of the leading compounds: galantamine **1**, cryspine A **2**, melatonin **3.**

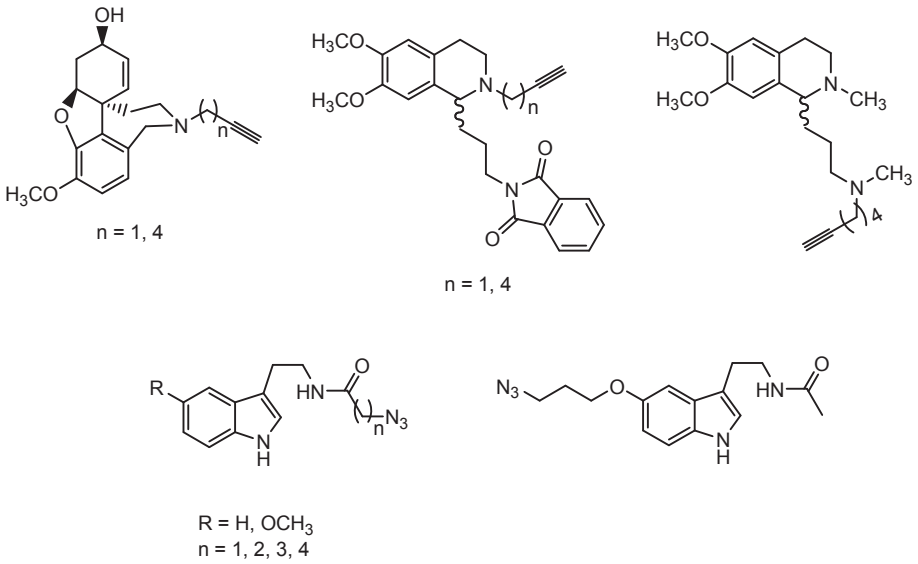


Fig. 3. Libraries of building blocks.

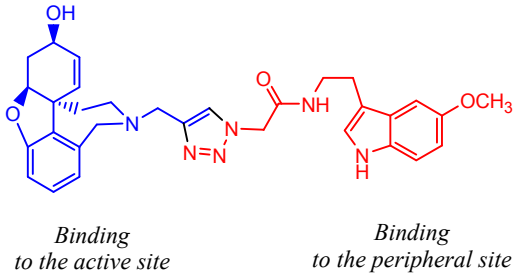
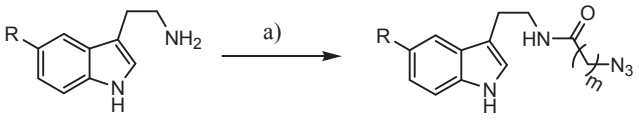


Fig. 4. Example of a potential dual binding cholinesterase inhibitor.

One indole-derived building block with the azide moiety on the other site of the molecule **10** was synthesized from *N*-acetyl-5-methoxytryptamine **(8)** (Scheme 2).

Tetrahydroisoquinoline-derived building blocks of different structures were synthesized as presented in Scheme 3. The phthalimide-protected derivative **11** was obtained according to a previously described procedure [25]. For the preliminary study of the inhibitory activity of this type of compounds, we decided to use the racemic derivatives and therefore we applied a non-enantioselective mode of the reduction of the imine bond. Surprisingly, a typical reaction with borohydride led to unwanted partial reduction of the phthalimide protecting group, resulting in compound **12**. According to our best knowledge, this effect was reported in the literature only once [26]. Replacement of borohydride with a milder cyanoborohydride allowed us to obtain amine **13**, which, in turn, was reacted with terminal haloalkynes of diverse length, giving building blocks **14a - b**.

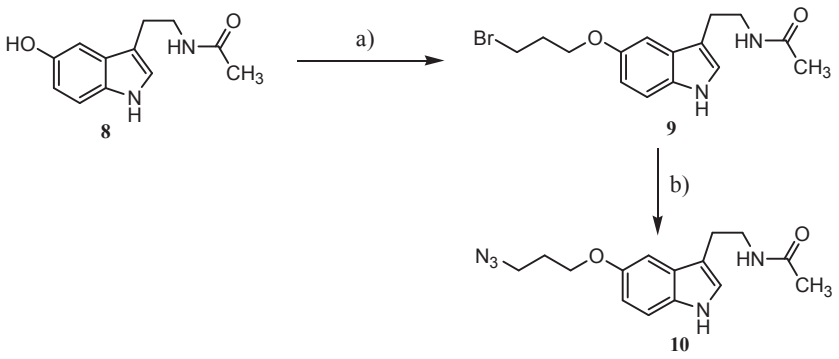
In order to obtain building block **18**, we removed the phthalimide group in compound **13** using standard conditions and tried to selectively introduce two methyl substituents on the amine functionalities, which appeared to be challenging. The primary amine, generated after deprotection, is very polar and unstable and, therefore, needs to be treated carefully and used in the next step immediately, without purification. Our first attempts to introduce two methyl groups, one on the secondary and one on the primary amine functionalities, included Boc-protection, which,



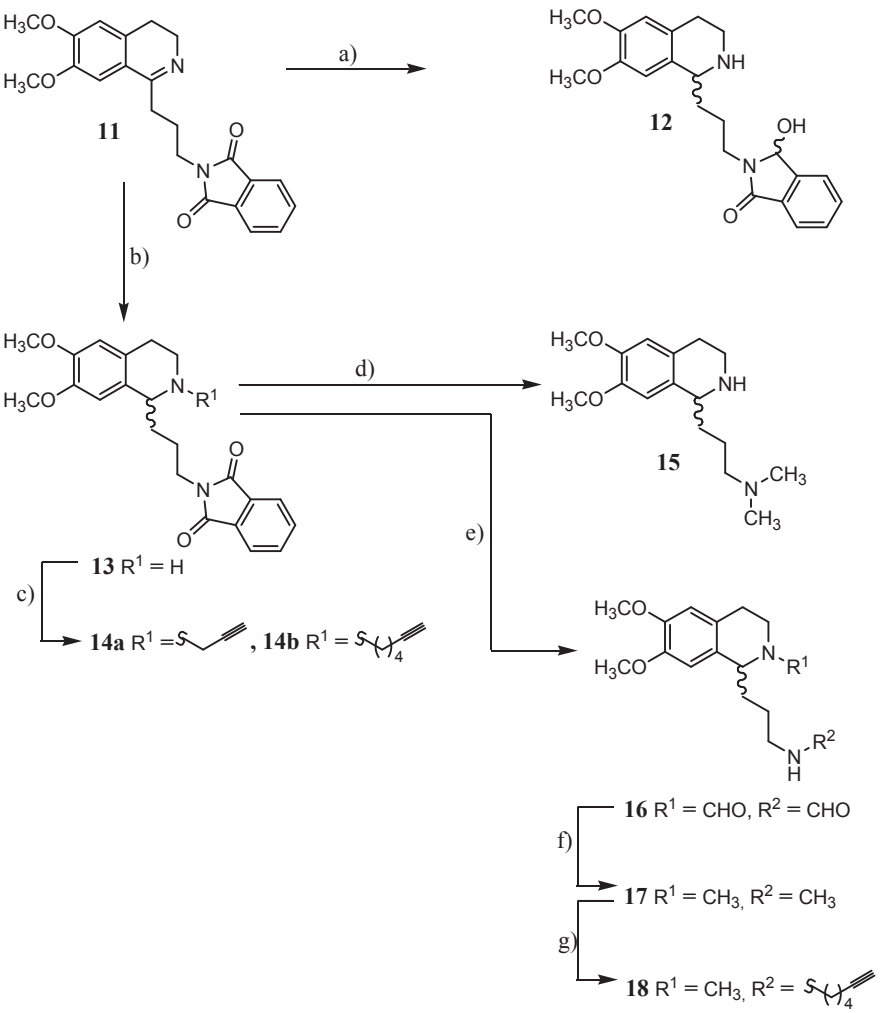
4 R = H,
5 R = OCH_3

6a R = H, m = 1
6b R = H, m = 2
6c R = H, m = 3
6d R = H, m = 4
7a R = OCH_3, m = 1
7b R = OCH_3, m = 2
7c R = OCH_3, m = 3
7d R = OCH_3, m = 4

Scheme 1. a) $N_3(CH_2)_mCOOH$, BOP, Et_3N, THF, rt, 53–93%.



Scheme 2. a) $Br(CH_2)_3Br$, NaH, EtOH, reflux, 46%, b) NaN_3, DMF, rt, 78%.



Scheme 3. a) $NaBH_4$, MeOH, rt, 75%, b) $NaBH_3CN$, MeOH, rt, 99%, c) $CH{\equiv}C(CH_2)_nX$, K_2CO_3, acetonitrile, rt, 65–73%, d) 1. Hydrazine, MeOH, reflux, 2. Boc_2O, Et_3N, DCM, rt, 3. $LiAlH_4$, THF, reflux, 62% in 3 steps, e) 1. Hydrazine, MeOH, reflux, 2. formamide, reflux, 65%, f) $LiAlH_4$, THF, reflux, 60%, g) $CH{\equiv}C(CH_2)_nX$, K_2CO_3, acetonitrile, rt, 76%.

according to the literature, usually does not lead to double-protection of the primary amines. Unfortunately, the exact structure of the resulting product was unclear. Nevertheless, we used it for the next step, in which Boc-groups were reduced to methyl groups. Analysis of the NMR spectrum of the product suggested that, surprisingly, both methyl groups were introduced on the primary amine (compound **15**). This assumption was confirmed with the crystallographic analysis of a picrate salt of compound **15A**, formed by spontaneous oxidation of **15**, during a time-consuming crystallization process (Fig. 5).

Alternatively, selective formylation followed by reduction allowed us to obtain the desired amine **17** (Scheme 3). Its reaction with haloalkyne led to building block **18** in good yield.

Galantamine-derived building blocks **20a - b** were synthesized in one step from norgalanthamine **(19)** (Scheme 4), using suitable terminal haloalkynes. Norgalantamine **(19)** was prepared from galantamine as described in the literature, by demethylation through a non-classical Polonovski reaction [27].

Strategies presented above led to a collection of building blocks: five

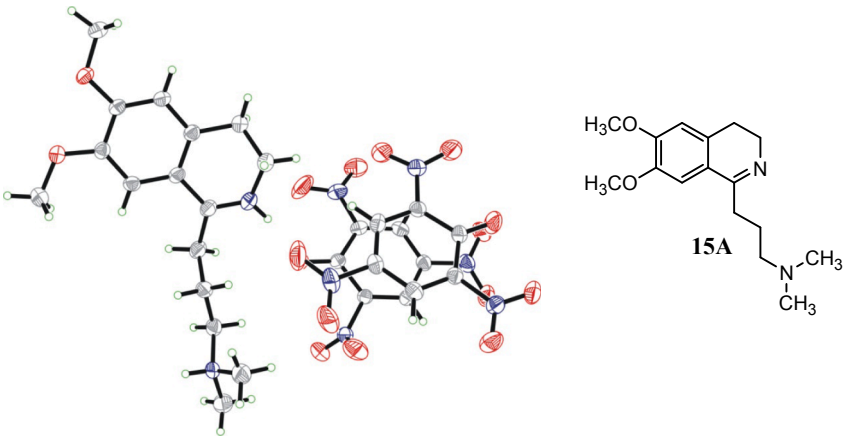
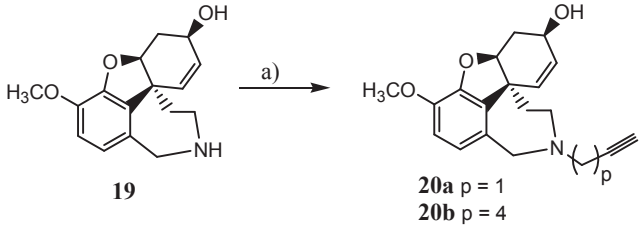
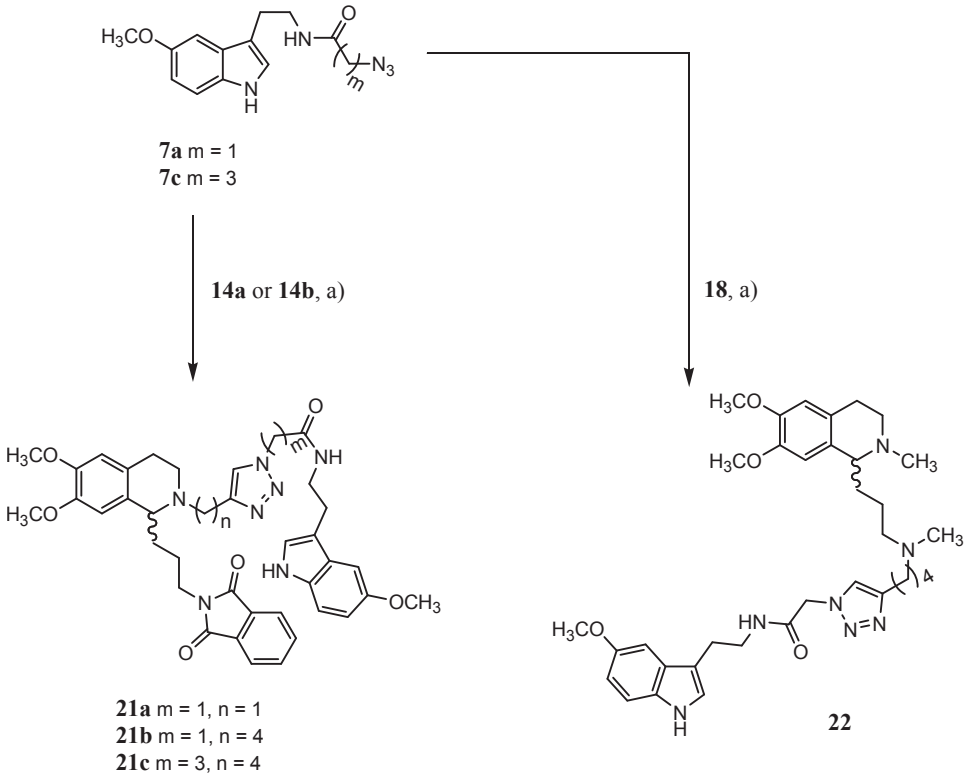


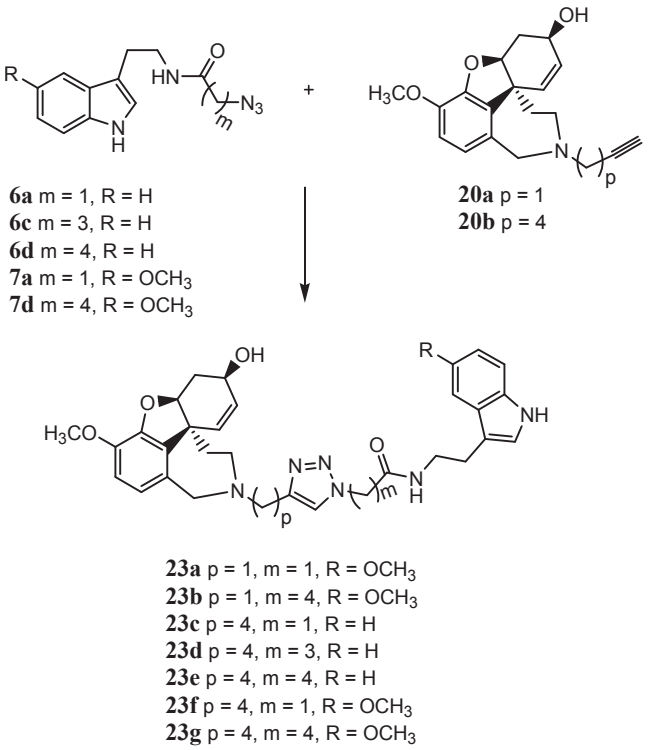
Fig. 5. Molecular structure of the picrate salt of compound **15A** (from crystallographic studies).



Scheme 4. a) $CH{\equiv}C(CH_2)_pX$, K_2CO_3, DMF, rt, 52–77%.



Scheme 5. a) $CuSO_4 \cdot 5\ H_2O$, sodium ascorbate, THF/H_2O, rt, 49–63%.



Scheme 6. a) $CuSO_4 \cdot 5\ H_2O$, sodium ascorbate, THF/H_2O, rt, 48–59%.

alkynes with tetrahydroisoquinoline or galantamine cores (compounds **14a - b**, **18**, **20a - b**) and nine tryptamine-derived azides (compounds **6a - d**, **7a - d**, **10**).

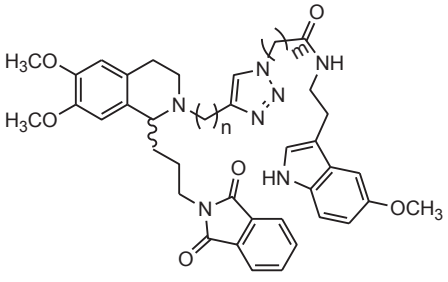
From all the possible products of cycloaddition between these building blocks, we chose a number of diverse heterodimers to be synthesized, isolated and subjected to biological evaluation. Copper-catalyzed cycloaddition reactions were performed under standard conditions and led to the final heterodimers in good yields (Schemes 5 - 6).

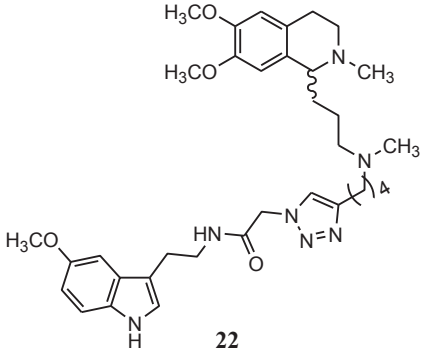
2.2. Inhibitory activity

The inhibitory activity against human acetylcholinesterase (*h*AChE) and butyrylcholinesterase (*h*BChE) derived from erythrocytes and blood serum, respectively, was determined using spectrophotometric Ellman's assay [28]. The results are presented in Tables 1 and 2 and compared to galantamine **(1)**.

Tetrahydroisoquinoline derivatives with phthaloyl substituent **21a - c** exhibit moderate inhibitory activity. Better results for butyrylcholinesterase inhibition, probably connected with a larger space in

Table 1
Inhibitory activity of the new tetrahydroisoquinoline derivatives towards human acetyl- and butyrylcholinesterase.

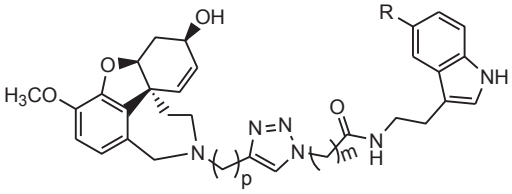




Compound	m	n	IC_{50} (μM)[a]	
			*h*AChE	*h*BChE
21a	1	1	39.9 ± 2.8	26.2 ± 2.3
21b	1	4	173.9 ± 6.9	57.2 ± 4.1
21c	3	4	158.6 ± 7.3	4.6 ± 0.4
22			2.2 ± 0.2	1.6 ± 0.1
Galantamine **1**			2.9 ± 0.3	10.6 ± 0.5

[a] Values are the mean ± SD obtained from 3 experiments.

Table 2
Inhibitory activity of the new galantamine derivatives towards human acetyl- and butyrylcholinesterase.



23a p = 1, m = 1, R = OCH_3
23b p = 1, m = 4, R = OCH_3
23c p = 4, m = 1, R = H
23d p = 4, m = 3, R = H
23e p = 4, m = 4, R = H
23f p = 4, m = 1, R = OCH_3
23g p = 4, m = 4, R = OCH_3

Compound	R	p	m	IC_{50} (μM)[a]	
				*h*AChE	*h*BChE
23a	OCH_3	1	1	5.412 ± 0.072	8.845 ± 0.117
23b	OCH_3	1	4	1.002 ± 0.085	2.017 ± 0.197
23c	H	4	1	0.138 ± 0.013	0.907 ± 0.081
23d	H	4	3	0.120 ± 0.011	0.209 ± 0.016
23e	H	4	4	0.031 ± 0.002	1.292 ± 0.119
23f	OCH_3	4	1	0.023 ± 0.002	0.554 ± 0.015
23g	OCH_3	4	4	0.018 ± 0.002	0.963 ± 0.056
Galantamine **1**				2.870 ± 0.270	10.641 ± 0.515

[a] Values are the mean ± SD obtained from 3 experiments.

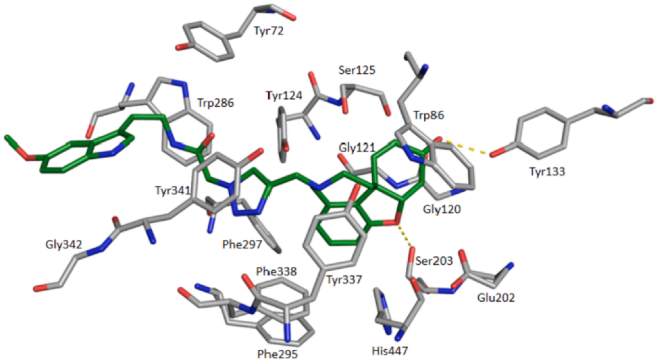


Fig. 6. Binding mode of compound **20a** in the binding pocket of AChE (PDB ID: 4EY7). Compound **20a** and the residues involved in binding are shown in stick representation, with the following color code: C: green (compound **20a**) and gray (acetylcholinesterase), O: red and N: blue. This figure was generated with PyMOL [30].

the binding pocket of this enzyme, are not surprising for such spatially demanding compounds. A less branched derivative **22** is much more effective. Its inhibitory activity in the case of acetylcholinesterase is similar to galantamine, while for butyrylcholinesterase is eight times lower.

The results obtained for galantamine-derived heterodimers are much more interesting. Apart from compound **23a** and **b**, all derivatives tested exhibit inhibitory activity in nanomolar range towards acetyl- and butyrylcholinesterase. IC_{50} values are 10–100 times lower than in the case of galantamine, which suggests not only additional interactions of the indole and triazole moieties with the binding pocket, but also a

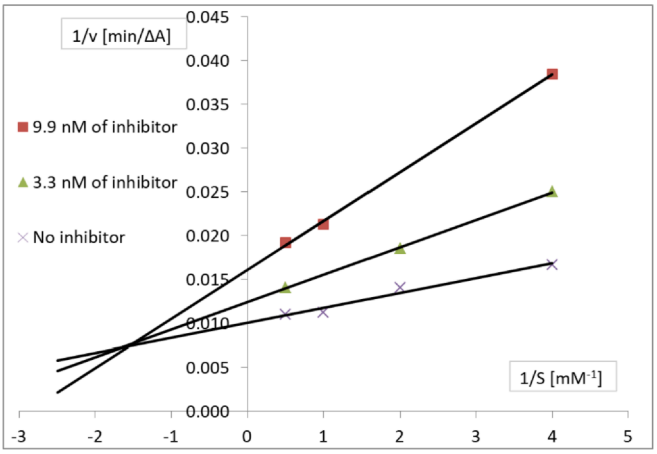


Fig. 7. Lineweaver–Burk plots of acetylcholinesterase activity with different substrate concentrations in the absence and presence of 3.3 and 9.9 nM **23d**.

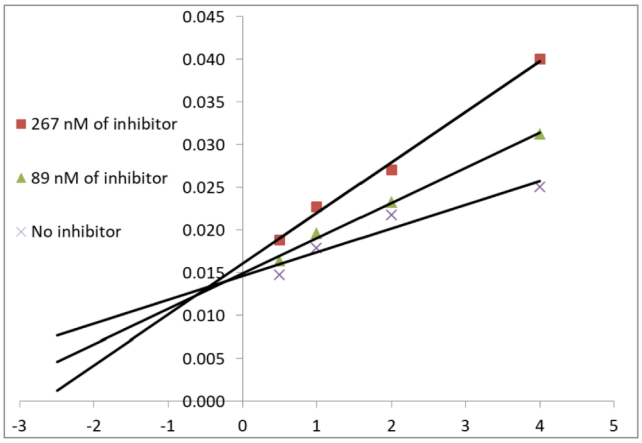


Fig. 8. Lineweaver–Burk plots of acetylcholinesterase activity with different substrate concentrations in the absence and presence of 89 and 267 nM **23d**.

synergistic effect. The fact that derivatives **23a** and **23b** are significantly less active than other similar compounds can be explained by the insufficient length of the linker (p = 1), which hinders an optimal fitting of the inhibitor to the enzyme pocket.

2.3. Modeling

The docking of the compounds in the binding pocket of AChE (PDB ID: 4EY7) was done using the software LeadIT [29].

The docking studies of compound **23a** show interaction with acetylcholinesterase, with the galantamine fragment binding to the CAS and the melatonin fragment binding to the PAS (Fig. 6).

Interacting amino acid residues (in gray):

galantamine part: Trp86, Gly120, Gly121, Tyr124, Ser125, Tyr133 (H bond), Glu202, Ser203 (H bond), Phe295, Phe297, Tyr337, Phe338, His447;

triazole: Phe297 Phe338, Tyr341;

amide linker: Tyr72, Trp286;

indole: Gly342 (most of the indole part is outside of the pocket)

2.4. Kinetic studies

As one of the most active acetyl- and butyrylcholinesterase inhibitors, compound **23d** was chosen for the kinetic analysis of the type of inhibition. For both enzymes, the reaction velocity was measured for diverse substrate concentrations in the absence of the inhibitor and after

incubation with two different concentrations of **23d**, and analyzed with Lineweaver - Burk reciprocal plots (Figs. 7 and 8).

It can be seen that the addition of **23d** induces a higher slope and lower intercept, which indicates a decreased V_{max} and increased K_m. This observation suggests a mixed type of inhibition for acetyl- and butyrylcholinesterase, which confirms the assumption that heterodimers of this type not only interact competitively with CAS but also bind to PAS.

2.5. Kinetic target-guided synthesis experiments

Having in hand a collection of strong dual binding inhibitors of butyrylcholinesterase, the aim of the further study was to investigate whether butyrylcholinesterase can function as a template in the synthesis of its own dual binding inhibitors, catalyzing the click reaction between selected fragments. We designed a combinatorial library consisting of a collection of azides (**6a - d**, **7a - d** and **10**), a selected alkyne (one of the following: **14a**, **14b**, **20a, 20b**) and butyrylcholinesterase. After 7 days of incubation at 37 °C, the libraries were analyzed using UPLC-MS technique, to verify whether any heterodimers are present. The identification of the products was based on their molecular mass as well as the retention times, in the cases where the standards were available (**21a** - **c**, **23a** - **g**). Each library was compared to a negative control, in which azides and alkynes were incubated together in the absence of butyrylcholinesterase. Also, two positive controls were prepared, to differentiate between 1,4- and 1,5-traizoles. Incubation with copper (I) allowed us to identify the 1,4-triazoles, while thermal reaction provided both 1,4- and 1,5-isomers.

First attempts to the KTGS experiment were conducted with the use of lyophilized butyrylcholinesterase from human serum. Products formed in both thermal and copper(I)-catalyzed reactions were successfully identified. Unfortunately, it was impossible to detect any heterodimeric products formed in the presence of butyrylcholinesterase. Multiple attempts were made to exclude possible flaws in the experimental setup. We optimized the concentration of methanol and DMSO used to dissolve fragments, finding a compromise between the solubility of the latter and sensitivity of the enzyme towards organic solvents. In a series of experiments, we tested various incubation times, from 3 to 14 days, and various concentrations of the fragments, from 20 to 250 μM for alkynes and from 5 to 100 μM for each azide. Alas, none of the attempts made, enabled us to confirm the formation of the products expected.

Therefore, we concentrated on the enzyme itself. The need for significant amounts of the active protein is the weakest point of the KTGS technique. For a successful experiment, almost equimolar concentrations of fragments and the enzyme are required, taking into account full preservation of the enzyme activity in the experimental conditions. To control the actual concentration of the active form of butyrylcholinesterase, we tested its activity in the initial samples as well as after 3, 6 and 9 days of incubation, using colorimetric Ellman's method. The enzyme proved to be stable, but the limited availability of human butyrylcholinesterase resulted in the inability to obtain concentrations higher than 1 μM, which appeared to be insufficient. We decided to repeat the experiments using much more available equine serum butyrylcholinesterase. It allowed us to prepare the desired libraries, in which the enzyme concentration was 10 μM. Nevertheless, none of the expected heterodimers was detected in the final samples.

The KTGS, although beautiful as a concept, has not been widely implemented in the search for new biologically active compounds. The failure of the attempts to use butyrylcholinesterase as a template in the synthesis of its own inhibitors, indicates a limited scope of applications of this technique. The need for significant amounts of the active enzyme is a strong disadvantage, which makes TGS very difficult to implement in a wide range of applications.

2.6. Conclusions

A novel type of dual-binding cholinesterase inhibitors, based on structural motifs of galantamine and tryptamine, has been discovered. The strongest inhibitors of this type exhibit IC_{50} values of 31 nM for acetylcholinesterase and 209 nM for butyrylcholinesterase. Modeling studies and kinetic experiments confirmed an optimal binding of the galantamine-tryptamine heterodimers to both CAS and PAS. Failure of the attempts to use butyrylcholinesterase as a template, which chooses from a library of fragments the best pairs and links the together, generating dual-binding ligands, shows serious limitations of the KTGS technique.

3. Experimental

3.1. Chemistry

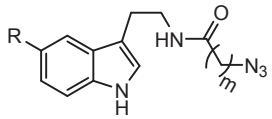
Galantamine hydrobromide was obtained from Ava Chemicals (India). 5-Methoxytryptamine was obtained from Abblis Chemicals (US). Butyrylcholinesterase Fluorescent Activity Kit was obtained from Arbor Assays (US). Other chemicals were obtained from Sigma-Aldrich, Alfa-Aesar and Across Organics. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE apparatus, operating at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR) using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in ppm. The following abbreviations were used to indicate the peak multiplicity and shape: s, singlet; d, doublet; t, triplet; quint, quintet; m, multiplet; br, broad. High-resolution mass spectra were recorded on Quatro LC AMD 604 apparatus using TOF MS ES + method. Column chromatography was performed on silica gel SiliaFlash P60 230–400 mesh ASTM (Dichrom GmbH, Germany) and aluminum oxide (Fluka, St. Louis, MO, USA) using the indicated eluents. The progress of the reactions was followed by thin-layer chromatography using plates with silica gel and aluminum oxide with methanol, chloroform methylene chloride, ethyl acetate and diethyl ether as eluents. Anhydrous magnesium sulfate was used as drying agent for the organic phases. Organic solvents were removed under reduced pressure at 40 °C.

Carboxylic acids with terminal azide group were obtained from suitable terminal halogenocarboxylic acids using sodium azide [31].

Phthalimide-protected derivative **11** was obtained as described in the literature [25].

Norgalantamine **19** was obtained from galantamine hydrobromide as described in the literature [27].

3.1.1. Synthesis of tryptamine-derived azides



6a R = H, m = 1
6b R = H, m = 2
6c R = H, m = 3
6d R = H, m = 4
7a R = OCH_3, m = 1
7b R = OCH_3, m = 2
7c R = OCH_3, m = 3
7d R = OCH_3, m = 4

General procedure for the synthesis of azides 6a - d and 7a - d

Tryptamine **4** (for azides **6a - d**) or 5-methoxytryptamine **5** (for azides **7a - d**) (1 mmol) was dissolved in dry THF (5 mL). A suitable carboxylic acid with terminal azide group (1.2 mmol), BOP (1.1 mmol) and triethylamine (1.1 mmol) were added step by step. The reaction was left to stir at room temperature for 24 h, under inert atmosphere. The

solvent was evaporated, and the residue was dissolved in diethyl ether (15 mL). The solution was washed with water and brine, then dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under reduced pressure, and the product was purified by column chromatography (SiO_2, diethyl ether).

6a was obtained as colorless oil in 60% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.25 (s, 1H, $NH_{indolyl}$), 7.61 (d, $J = 7.8$ Hz, 1H, H_{aromat}), 7.39 (d, $J = 7.8$ Hz, 1H, H_{aromat}), 7.22 (td, $J_1 = 6.9$ Hz, $J_2 = 1.2$ Hz, 1H, H_{aromat}), 7.15 (td, $J_1 = 6.9$ Hz, $J_2 = 0.9$ Hz, 1H, H_{aromat}), 7.03 (d, $J = 2.1$ Hz, 1H, H_{aromat}), 6.44 (br.s, 1H, CONH), 3.91 (s, 2H, CH_2N_3), 3.64 (q, $J = 6.6$ Hz, 2H, CH_2NH), 3.00 (t, $J = 6.9$ Hz, 2H, CH_2CH_2NH).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 166.7, 136.5, 127.2, 122.3, 122.1, 119.6, 118.6, 112.5, 111.4, 52.7, 39.8, 25.2.

HR MS ES(+) (*m/z*) calculated for $C_{12}H_{13}N_5ONa$ ($[M + Na]^+$) 266.1018, found 266.1019.

6b was obtained as colorless oil in 86% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.30 (s, 1H, $NH_{indolyl}$), 7.59 (d, $J = 7.8$ Hz, 1H, H_{aromat}), 7.37 (d, $J = 7.8$ Hz, 1H, H_{aromat}), 7.20 (td, $J_1 = 6.9$ Hz, $J_2 = 1.2$ Hz, 1H, H_{aromat}), 7.12 (td, $J_1 = 6.9$ Hz, $J_2 = 0.9$ Hz, 1H, H_{aromat}), 7.02 (d, $J = 2.4$ Hz, 1H, H_{aromat}), 5.80 (br.s, 1H, CONH), 3.60 (m, 4H, CH_2N_3, CH_2NH), 3.01 (t, $J = 6.9$ Hz, 2H, $CH_2CH_2N_3$), 2.31 (t, $J = 6.3$ Hz, 2H, $CH_2CH_2N_3$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 170.0, 136.5, 127.4, 122.3, 122.3, 119.6, 118.7, 112.8, 111.5, 47.5, 40.0, 36.0, 25.3.

HR MS ES(+) (*m/z*) calculated for $C_{13}H_{15}N_5ONa$ ($[M + Na]^+$) 280.1174, found 280.1176.

6c was obtained as colorless oil in 76% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.25 (s, 1H, $NH_{indolyl}$), 7.59 (d, $J = 7.8$ Hz, 1H, H_{aromat}), 7.37 (d, $J = 7.8$ Hz, 1H, H_{aromat}), 7.20 (td, $J_1 = 6.9$ Hz, $J_2 = 1.2$ Hz, 1H, H_{aromat}), 7.12 (td, $J_1 = 6.9$ Hz, $J_2 = 0.9$ Hz, 1H, H_{aromat}), 7.01 (d, $J = 2.4$ Hz, 1H, H_{aromat}), 5.63 (br.s, 1H, CONH), 3.60 (q, $J = 6.6$ Hz, 2H, CH_2NH), 3.27 (t, $J = 6.6$ Hz, 2H, CH_2CH_2NH), 2.96 (t, $J = 6.3$ Hz, 2H, CH_2N_3), 2.16 (t, $J = 7.2$ Hz, 2H, CH_2CONH), 1.86 (quint, $J = 6.9$ Hz, 2H, CH_2 CH_2 N_3).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 172.0, 136.5, 127.4, 122.4, 122.2, 119.62, 118.8, 112. 9, 111.4, 50.8, 39.9, 33.4, 25.4, 24.9.

HR MS ES(+) (*m/z*) calculated for $C_{14}H_{17}N_5ONa$ ($[M + Na]^+$) 294.1331, found 294.1329.

6d was obtained as colorless oil in 53% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.47 (s, 1H, $NH_{indolyl}$), 7.59 (d, $J = 7.8$ Hz, 1H, H_{aromat}), 7.37 (d, $J = 7.8$ Hz, 1H, H_{aromat}), 7.20 (td, $J_1 = 6.9$ Hz, $J_2 = 1.2$ Hz, 1H, H_{aromat}), 7.12 (td, $J_1 = 6.9$ Hz, $J_2 = 0.9$ Hz, 1H, H_{aromat}), 7.00 (d, $J = 2.1$ Hz, 1H, H_{aromat}), 5.68 (br.s, 1H, CONH), 3.59 (q, $J = 6.6$ Hz, 2H, CH_2NH), 3.22 (t, $J = 6.6$ Hz, 2H, CH_2CH_2NH), 2.97 (t, $J = 6.3$ Hz, 2H, CH_2N_3), 2.11 (t, $J = 7.2$ Hz, 2H, CH_2CONH), 1.72–1.49 (m, 4H, $(CH_2)_2CH_2N_3$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 172.6, 136.5, 127.4, 122.2, 122.2, 119.5, 118.7, 112.8, 111.4, 51.2, 39.9, 36.0, 28.4, 25.3, 22.9.

HR MS ES(+) (*m/z*) calculated for $C_{15}H_{19}N_5ONa$ ($[M + Na]^+$) 308.1487, found 308.1491.

7a was obtained as colorless oil in 69% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.17 (s, 1H, $NH_{indolyl}$), 7.26 (d, $J = 9.0$ Hz, 1H, H_{aromat}), 7.04 (d, $J = 2.4$ Hz, 1H, H_{aromat}), 7.01 (d, $J = 2.4$ Hz, 1H, H_{aromat}), 6.88 (dd, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz, 1H, H_{aromat}), 6.45 (br.s, 1H, CONH), 3.92 (s, 2H, CH_2N_3), 3.87 (s, 3H, OCH_3), 3.62 (q, $J = 6.6$ Hz, 2H, CH_2NH), 2.96 (t, $J = 6.9$ Hz, 2H, CH_2CH_2NH).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 166.7, 154.1, 131.6, 127.6, 122.9, 112.5, 112.2, 112.1, 100.5, 56.0, 52.7, 39.6, 25.2.

HR MS ES(+) (*m/z*) calculated for $C_{13}H_{15}N_5O_2Na$ ($[M + Na]^+$) 296.1123, found 296.1127.

7b was obtained as colorless oil in 66% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.30 (s, 1H, $NH_{indolyl}$), 7.25 (d, $J = 9.0$ Hz, 1H, H_{aromat}), 7.02 (d, $J = 2.4$ Hz, 1H, H_{aromat}), 7.98 (d, $J = 2.7$ Hz, 1H, H_{aromat}), 6.86 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.4$ Hz, 1H, H_{aromat}), 5.85 (br.s, 1H, CONH), 3.84 (s, 3H, OCH_3), 3.57 (m, 4H, CH_2N_3, CH_2NH), 2.93 (t, $J = 6.9$ Hz, 2H, $CH_2CH_2N_3$), 2.31 (t, $J = 6.3$ Hz, 2H, $CH_2CH_2N_3$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 170.0, 154.1, 131.7, 127.8, 123.1, 112.41, 112.4, 112.2, 100.6, 56.1, 47.5, 39.9, 35.9, 25.2.

HR MS ES(+) (*m/z*) calculated for $C_{14}H_{17}N_5O_2Na$ ($[M + Na]^+$) 310.1280, found 310.1282.

7c was obtained as colorless oil in 93% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.14 (s, 1H, $NH_{indolyl}$), 7.26 (d, $J = 8.7$ Hz, 1H, H_{aromat}), 7.04 (d, $J = 2.4$ Hz, 1H, H_{aromat}), 7.01 (d, $J = 2.4$ Hz, 1H, H_{aromat}), 6.87 (dd, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz, 1H, H_{aromat}), 5.61 (br.s, 1H, CONH), 3.86 (s, 3H, OCH_3), 3.59 (q, $J = 6.3$ Hz, 2H, CH_2NH), 3.29 (t, $J = 6.6$ Hz, 2H, CH_2CH_2NH), 2.94 (t, $J = 6.3$ Hz, 2H, CH_2N_3), 2.18 (t, $J = 7.2$ Hz, 2H, CH_2CONH), 1,88 (quint, $J = 6.9$ Hz, 2H, $CH_2CH_2N_3$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 171.8, 154.2, 131.7, 127.8, 123.0, 112.6, 112.5, 112.2, 100.6, 56.1, 50.9, 39.7, 33.4, 25.4, 24.9.

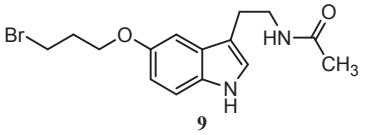
HR MS ES(+) (*m/z*) calculated for $C_{15}H_{19}N_5O_2Na$ ($[M + Na]^+$) 324.1436, found 324.1434.

7d was obtained as colorless oil in 63% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.06 (s, 1H, $NH_{indolyl}$), 7.26 (d, $J = 8.7$ Hz, 1H, H_{aromat}), 7.03 (d, $J = 2.4$ Hz, 1H, H_{aromat}), 7.01 (d, $J = 2.4$ Hz, 1H, H_{aromat}), 6.88 (dd, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz, 1H, H_{aromat}), 5.54 (br.s, 1H, CONH), 3.86 (s, 3H, OCH_3), 3.60 (q, $J = 6.6$ Hz, 2H, CH_2NH), 3.25 (t, $J = 6.6$ Hz, 2H, CH_2CH_2NH), 2.94 (t, $J = 6.3$ Hz, 2H, CH_2N_3), 2.13 (t, $J = 7.2$ Hz, 2H, CH_2CONH), 1.75–1.51 (m, 4H, $(CH_2)_2CH_2N_3$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 172.4, 154.2, 131.7, 127.9, 122.9, 112.8, 112.6, 112.1, 100.7, 56.1, 51.3, 39.7, 36.2, 28.5, 25.4, 22.9.

HR MS ES(+) (*m/z*) calculated for $C_{16}H_{21}N_5O_2Na$ ($[M + Na]^+$) 338.1593, found 338.1589.

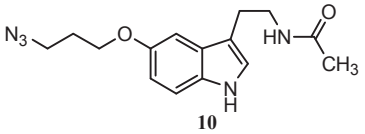


9

N-Acetyl-5-methoxytryptamine **8** (0.4 mmol, 85 mg) was dissolved in ethanol (10 mL), and NaH (1 eq, 10 mg) was added. The reaction was stirred for 30 min. 1,3-dibromopropane (3 eq, 234 mg) was added, and the reaction mixture was stirred at 60 °C under inert atmosphere for 3 h. The solvent was evaporated, and the product was purified by column chromatography (Al_2O_3, 0–1% methanol in dichloromethane), giving **9** (60 mg, 46%) as colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.98 (s, 1H, $NH_{indolyl}$), 7.28 (d, $J = 8.7$ Hz, 1H, H_{aromat}), 7.06 (d, $J = 2.4$ Hz, 1H, H_{aromat}), 7.03 (d, $J = 2.7$ Hz, 1H, H_{aromat}), 6.88 (dd, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz, 1H, H_{aromat}), 5.52 (br.s, 1H, CONH), 4.15 (t, 2H, $J = 6.0$ Hz, CH_2O), 3.65 (t, 2H, $J = 6.3$ Hz, $BrCH_2$), 3.59 (q, $J = 6.3$ Hz, 2H, CH_2NH), 2.94 (t, $J = 6.6$ Hz, 2H, CH_2CH_2NH), 2.34 (p, $J = 6.3$ Hz, 2H, $BrCH_2CH_2CH_2O$), 1.94 (s, 3H, CH_3CONH).

MS EI(+)(*m/z*): 339 $[M + H]^+$



10

Compound **9** (0.09 mmol, 30 mg) was dissolved in DMF (1 mL). Sodium azide (2 eq, 12 mg) was added. The reaction mixture was refluxed under inert atmosphere overnight. It was allowed to reach room temperature, brine was added, and the solution was extracted three times with diethyl ether. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and filtered. The product was purified by column chromatography (SiO_2, ethyl acetate), giving **10** (21

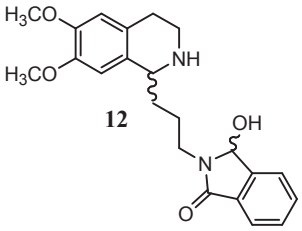
mg, 78%) as colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.37 (s, 1H, $NH_{indolyl}$), 7.24 (d, J = 8.7 Hz, 1H, H_{aromat}), 7.05 (d, J = 2.4 Hz, 1H, H_{aromat}), 6.98 (d, J = 2.1 Hz, 1H, H_{aromat}), 6.88 (dd, J_1 = 8.7 Hz, J_2 = 2.4 Hz, 1H, H_{aromat}), 5.68 (br.s, 1H, CONH), 4.08 (t, 2H, J = 6.0 Hz, CH_2O), 3.60–3-50 (m, 4H, N_3CH_2, CH_2NH), 2.92 (t, J = 6.6 Hz, 2H, CH_2CH_2NH), 2.05 (p, J = 6.3 Hz, 2H, $N_3CH_2CH_2CH_2O$), 1.92 (s, 3H, CH_3CONH).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 170.4, 153.1, 131.9, 127.8, 123.1, 112.8, 112.6, 112.2, 101.9, 65.6, 48.5, 39.9, 29.1, 25.4, 23.5.

HR MS ES(+) (m/z) calculated for $C_{15}H_{19}N_5O_2Na$ ([M + Na]$^+$) 324.1436, found 324.1434.

3.1.2. Synthesis of tetrahydroisoquinoline-derived alkynes

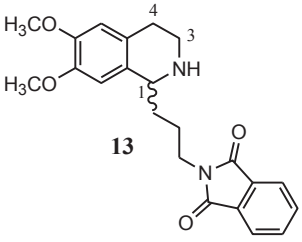


12

Compound **11** (40 mg, 0.1 mmol) was dissolved in methanol (2 mL). Sodium borohydride (1.5 eq, 6 mg) was added. The reaction mixture was stirred in an inert atmosphere overnight. After that time, brine was slowly added and the mixture was extracted 3 times with DCM. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and filtered. The product was purified by column chromatography (SiO_2, 0–2% methanol in chloroform), giving **12** (30 mg, 75% yield) as colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm) (for 1 diastereoisomer): 7.71 (d, J = 7.5, 1H, $H_{benz.phthaloyl}$), 7.57–7.50 (m, 2H, $2H_{benz.phthaloyl}$), 7.44–7.41 (m, 1H, $H_{benz.phthaloyl}$), 7.53 (s, 1H, H_{benz}), 7.48 (s, 1H, H_{benz}), 7.58 (s, 1H, $CHOH_{phthaloyl}$), 3.81 (s, 6H, $3OCH_3$), 3.72 (m, 2H, $CH_{2phthaloyl}$), 3.51 (m, 1H, H-1), 2.99–2.83 (m, 1H, H-4a), 2.69 (m, 1H, H-4b), 2.54 (m, 2H, H-3a, H-3b), 2.01–1.63 (m, 4H, $2CH_2$).

MS EI(+)(m/z): 383 [M + H]$^+$

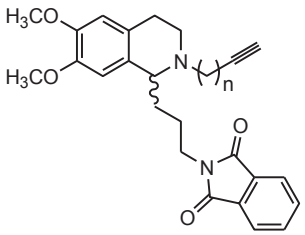


13

Compound **11** (1.0 g, 2.6 mmol) was dissolved in methanol (20 mL). Sodium cyanoborohydride (2 eq, 328 mg) was added. The reaction mixture was stirred in an inert atmosphere overnight. Aqueous solution of NH_4SO_4 (10 mL) was slowly added and the mixture was stirred for additional 15 min. After that time, 20% $NH_{3aq.}$ Solution was added to reach pH = 5. The resulting solution was extracted 3 times with DCM. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and filtered. The product was purified by column chromatography (SiO_2, 0–2% methanol in chloroform), giving **13** (0.99 g, 99% yield) as colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.85–7.82 (m, 2H, $2H_{phthaloyl}$), 7.73–7.69 (m, 2H, $2H_{phthaloyl}$), 6.60 (s, 1H, H_{benz}), 6.54 (s, 1H, H_{benz}), 3.98–3.94 (m, 1H, H-1), 3.83 (s, 6H, $2OCH_3$), 3.80–3.72 (m, 2H, $CH_{2phthaloyl}$), 3.21–3.15 (m, 1H, H-4a), 2.98–2.92 (m, 1H, H-4b), 2.74–2.61 (m, 2H, H-3a, H-3b), 1.88–1.73 (m, 4H, $2CH_2$).

MS EI(+)(m/z): 381 [M + H]$^+$



14a n = 1
14b n = 4

General procedure for the synthesis of alkynes 14a - b

Compound **13** (26 mg, 0.10 mmol) was dissolved in acetonitrile (2 mL). 1-Bromopropyne (1.1 eq) for **14a** or 1-iodohexyne (1.1 eq) for **14b** and K_2CO_3 (2 eq, 26 mg) were added, and the reaction mixture was stirred in an inert atmosphere for 1 h. After that time, brine was added and the mixture was extracted 3 times with chloroform. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and filtered. The product was purified by column chromatography (Al_2O_3, 0–2% methanol in chloroform).

14a was obtained as colorless oil in 65% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.83–7.80 (m, 2H, $2H_{phthaloyl}$), 7.72–7.68 (m, 2H, $2H_{phthaloyl}$), 6.57 (s, 1H, H_{benz}), 6.52 (s, 1H, H_{benz}), 3.85 (t, J = 5.1 Hz, 1H, H-1), 3.83 (s, 3H, $2OCH_3$), 3.79 (s, 3H, $2OCH_3$), 3.71–3.66 (m, 2H, $CH_{2phthaloyl}$), 3.46 (qd, J_1 = 16.8 Hz, J_2 = 2.4 Hz, 1H, $NCH_2C{\equiv}$) 3.10–3.02 (m, 1H, H-4a), 2.96–2.88 (m, 1H, H-4b), 2.67 (m, 2H, H-3a, H-3b), 2.19 (t, J = 2.4 Hz, 1H, ≡CH), 1.81–1.61 (m, 4H, $2CH_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 168.6, 147.5, 147.4, 134.0, 132.3, 129.4, 126.9, 123.2, 111.3, 110.2, 80.2, 72.6, 58.8, 56.0, 55.9, 45.7, 43.2, 38.1, 32.3, 25.8, 24.4.

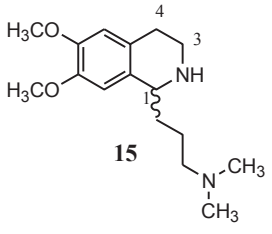
HR MS ES(+) (m/z) calculated for $C_{25}H_{27}N_2O_4$ ([M + H]$^+$) 419.1971, found 419.2002.

14b was obtained as colorless oil in 73% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.85–7.82 (m, 2H, $2H_{phthaloyl}$), 7.72–7.69 (m, 2H, $2H_{phthaloyl}$), 6.54 (s, 1H, H_{benz}), 6.52 (s, 1H, H_{benz}), 3.82 (s, 3H, $2OCH_3$), 3.82 (s, 3H, $2OCH_3$), 3.80–3.66 (m, 2H, $CH_{2phthaloyl}$), 3.55–3.51 (m, 2H, H-1), 3.20–3.10 (m, 1H, H-4a), 2.84–2.72 (m, 2H, H-4b, H-3a), 2.44–2.35 (m, 2H, H-3b), 2.52 (t, J = 6.6 Hz, 2H, NCH_2), 2.18 (m, 2H, $CH_2C{\equiv}CH$), 1.91 (t, J = 2.4 Hz, ≡CH), 1.87–1.48 (m, 8H, $4CH_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 168.6, 147.4, 147.3, 134.0, 132.3, 130.5, 126.6, 123.3, 111.5, 110.8, 84.7, 68.4, 60.4, 56.11, 55.9, 52.9, 43.5, 38.1, 33.5, 27.3, 26.4, 25.7, 23.6, 18.5.

HR MS ES(+) (m/z) calculated for $C_{28}H_{32}N_2O_4Na$ ([M + Na]$^+$) 483.2260, found 483.2246.



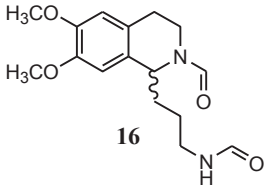
15

Compound **13** (0.5 mmol, 190 mg) was dissolved in methanol (5 mL) and hydrazine monohydrate (1.1 eq, 26 mg) was added. The reaction mixture was stirred in an inert atmosphere for 1 h. After that time, methanol was removed under reduced pressure. The residue was suspended in chloroform, white precipitate was filtered off and the solvent was removed under reduced pressure. The residue was dissolved in DCM (5 mL). Boc_2O (2.5 eq, 273 mg) and Et_3N (2.5 eq, 126 mg) were added and the reaction mixture was stirred in an inert atmosphere overnight.

After that time, brine was slowly added and the mixture was extracted 3 times with DCM. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and filtered. The product was purified by column chromatography (SiO_2, 10% ethyl acetate in cyclohexane). The resulting product was dissolved in dry THF (5 mL). Under an inert atmosphere, $LiAlH_4$ (1 mL 2.4 M solution in THF) was slowly added. The reaction mixture was refluxed for 1 h and after that time it was allowed to reach room temperature and cooled down to 0 °C in a water–ice bath. Saturated solution of Rochelle salt was added (20 mL) and the mixture was stirred for 2 h. The water phase was extracted 4 times with chloroform. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and filtered. The product was purified by column chromatography (Al_2O_3, 0–4% methanol in chloroform), giving **15** (86 mg, 62% in 3 steps) as colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 6.61 (s, 1H, H_{benz}), 6.55 (s, 1H, H_{benz}), 3.91 (m, 1H, H-1), 3.25–3.17 (m, 1H, H-4a), 3.00–2.91 (m, 1H, H-4b), 2.79–2.61 (m, 2H, H-3a, H-3b), 2.31 (m, 2H, NCH_2), 2.22 (s, 6H, $2NCH_3$), 1.89–1.50 (m, 5H, $2CH_2$, NH).

MS EI(+)(*m/z*): 279 $[M + H]^+$



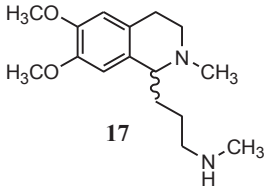
16

Compound **13** (2.4 mmol, 0.92 g) was dissolved in methanol (20 mL), and hydrazine monohydrate (1.1 eq, 132 mg) was added. The reaction mixture was stirred in an inert atmosphere for 1 h. After that time, methanol was removed under reduced pressure. The residue was suspended in chloroform, white precipitate was filtered off and the solvent was removed under reduced pressure. The residue was dissolved in formamide (20 mL), and the reaction mixture was stirred at 80 °C for 3 h. After that time, the reaction was allowed to reach room temperature, water was added, and the solution was extracted 3 times with diethyl ether. The combined organic layers were washed 3 times with brine, dried over anhydrous $MgSO_4$ and filtered. The product was purified by column chromatography (SiO_2, 0–4% methanol in chloroform), giving **16** (0.48 g, 65%) as colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.22 (s, 1H, CHO), 8.20 (s, 1H, CHO); 6,59 (s, 1H, H_{benz}), 6.55 (s, 1H, H_{benz}), 5.36 (t, $J = 7.2$ Hz, CHN), 4.46 (m, 1H, NH), 3.86 (s, 3H, OCH_3), 3.84 ((s, 3H, OCH_3), 3.73–3.27 (m, 4H, $2CH_2$), 3.09–2.63 (m, 2H, CH_2), 1.84 (m, 2H, CH_2) 1.64 (m, 2H, CH_2).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 162.1, 161.5, 147.9, 147.5, 128.3, 124.6, 111.5, 110.0, 56.2, 56.0, 50.4, 38.2, 34.4, 33.7, 27.1, 25.8.

MS EI(+)(*m/z*): 307 $[M + H]^+$



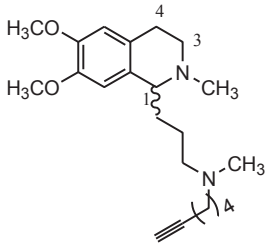
17

Compound **16** (240 mg, 0.78 mmol) was dissolved in dry THF (20 mL). Under an inert atmosphere, $LiAlH_4$ (6 eq, 2 mL 2.4 M solution in THF) was slowly added. The reaction mixture was refluxed for 1 h and after that time it was allowed to reach room temperature and cooled down to 0 °C in a water–ice bath. Saturated solution of Rochelle salt was added (20 mL), and the mixture was stirred for 2 h. The water phase was extracted 4 times with chloroform. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and filtered. The product was purified by column chromatography (Al_2O_3, 0–4% methanol in chloroform), giving **17** (105 g, 60%) as colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 6.56 (s, 1H, H_{benz}), 6.53 (s, 1H, H_{benz}), 3.83 (s, 6H, 2 OCH_3), 3.40 (t, $J = 5.4$ Hz, 1H, H-1), 3.09 (m, 1H, H-4a), 2.76–2.53 (m, 6H, H-4b, H-3a, H-3b, CH_2N, NH), 2.43 (s, 3H, NCH_3), 2.39 (s, 3H, NCH_3), 1.84–1.72 (m, 2H, CH_2), 1.67–1.39 (m, 2H, CH_2).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 147.4, 147.3, 129.9, 126.7, 111.3, 110.2, 63.4, 56.1, 55.9, 52.3, 48.3. 42.8, 36.3, 32.8, 25.8, 25.7.

MS EI(+)(*m/z*): 279 $[M + H]^+$



18

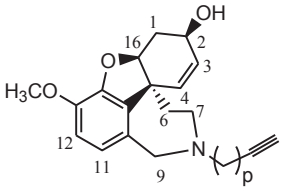
Compound **17** (26 mg, 0.10 mmol) was dissolved in acetonitrile (2 mL). 1-Iodohexyne (1.1 eq, 33 μL) and K_2CO_3 (2 eq, 26 mg) were added, and the reaction mixture was stirred in an inert atmosphere for 1 h. After that time, brine was added and the mixture was extracted 3 times with chloroform. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and filtered. The product was purified by column chromatography (Al_2O_3, 0–2% methanol in chloroform), giving **18** (29 mg, 76% yield) as colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 6,58 (s, 1H, H_{benz}), 6.54 (s, 1H, H_{benz}), 3.83 (s, 6H, 2 OCH_3), 3.38 (t, $J = 5.3$ Hz, 1H, H-1), 3.09 (m, 1H, H-4a), 2.67 (m, 3H, H-4b, H-3a, H-3b), 2.43 (s, 3H, NCH_3), 2.30 (m, 4H, $2NCH_2$), 2.22–2.16 (m, 2H, $CH_2C\equiv$), 2.15 (s, 3H, NCH_3), 1.93 (t, $J = 2.7$ Hz, 1H, ≡CH), 1.78–1.71 (m, 2H, CH_2), 1.61–1.36 (m, 6H, $3CH_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 147.3, 147.2, 130.3, 126.9, 111.4, 110.2, 84.6, 68.4, 63.4, 58.1, 57.3, 56.1, 55.9, 48.6, 43.0, 42.3, 32.8, 26.6, 26.5, 25.9, 23.2, 18.5.

MS EI(+)(*m/z*): 359 $[M + H]^+$

3.1.3. Synthesis of galantamine-derived alkynes



20a p = 1
20b p = 4

General procedure for the synthesis of alkynes 20a-b

Norgalantamine **19** (0.1 mmol, 27 mg) was dissolved in DMF (1 mL). 3-Bromopropyne (1.5 eq) for **20a** or 6-iodohex-1-yne (1.5 eq) for **20b** and potassium carbonate (2 eq) were added. The reaction mixture was stirred for 2 h. After this time DMF was evaporated under reduced pressure, the residue was dissolved in chloroform (10 mL), and the solution was washed three times with brine. The organic phase was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under reduced pressure and the product was purified by column chromatography (SiO_2, 0–7% methanol in chloroform).

20a was obtained as colorless oil in 52% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 6.66 (d, $J = 8.1$ Hz, 2H, H-12, H-11), 6.08 (d, $J = 10.2$ Hz, 1H, H-4), 6.00 (dd, $J_1 = 10.2$ Hz, $J_2 = 3.9$ Hz, 1H, H-3), 4.60 (br s, 1H, H-16), 4.14 (br t, $J = 4.8$ Hz, 1H, H-2), 4.10 (d, $J = 15.3$ Hz, 1H, H-9a), 3.85 (d, $J = 15.9$ Hz, 1H, H-9b), 3.83 (s, 3H, OCH_3), 3.43 (s, 2H, $NCH_2C\equiv$), 3.32 (td, $J_1 = 12.9$ Hz, $J_2 = 2.1$ Hz, 1H, H-

7a), 3.17 (dt, $J_1 = 14.4$ Hz, $J_2 = 3.3$ Hz, 1H, H-7b), 2.68 (dm, $J = 15.6$ Hz, 1H, H-1a), 2.40 (br. s, OH), 2.28 (t, $J = 2.7$ Hz, 1H, ≡CH), 2.05 (m, 2H, H-1b, H-6a), 1.65 (dm, 1H, $J = 13.8$ Hz, H-6b).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 146.0, 144.4, 133.2, 128.8, 127.9, 127.0, 122.3, 111.4, 88.9, 79.5, 73.1, 62.2, 58.5, 56.1, 51.8, 48.3, 44.5, 34.7, 30.1.

HR MS ES(+) (*m/z*) calculated for $C_{19}H_{22}NO_3$ ($[M + H]^+$) 312.1600, found 312.1602.

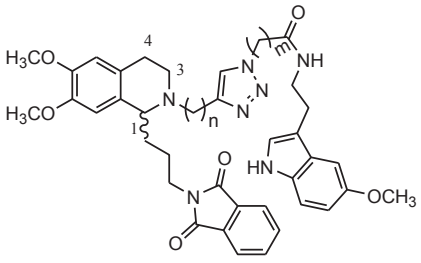
20b was obtained as colorless oil in 77% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 6.66 (d, $J = 8.1$ Hz, 1H, H-12), 6.61 (d, $J = 8.1$ Hz, 1H, H-11), 6.09 (d, $J = 10.5$ Hz, 1H, H-4), 6.00 (dd, $J_1 = 10.2$ Hz, $J_2 = 4.8$ Hz, 1H, H-3), 4.60 (br s, 1H, H-16), 4.14 (br t, $J = 4.8$ Hz, 1H, H-2), 4.13 (d, $J = 15.3$ Hz, 1H, H-9a), 3.83 (s, 3H, OCH_3), 3.81 (d, $J = 15.9$ Hz, 1H, H-9b), 3.35 (td, $J_1 = 12.9$ Hz, $J_2 = 1.8$ Hz, 1H, H-7a), 3.17 (dt, $J_1 = 15.0$ Hz, $J_2 = 3.0$ Hz, 1H, H-7b), 2.70 (dm, $J = 15.6$ Hz, 1H, H-1a), 2.52 (m, 2H, NCH_2), 2.19 (m, 2H, $CH_2C{\equiv}CH$), 2.05 (m, 3H, H-1b, 2H-6), 1.95 (t, $J = 2.7$ Hz, 1H, ≡CH), 1.73 (br. s, OH), 1.62–1.48 (m, 4H, $2CH_2$).

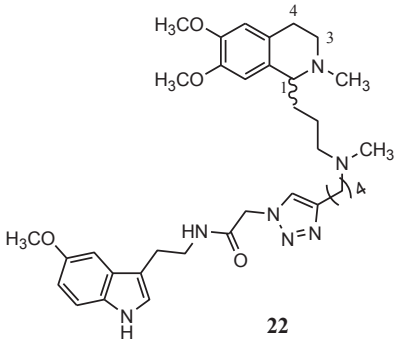
^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 145.9, 144.2, 133.3, 129.6, 127.7, 127.1, 122.2, 111.3, 88.9, 84.5, 68.6, 62.3, 57.8, 56.0, 51.7, 48.6, 44.4, 33.1, 30.1, 26.5, 26.3, 18.5. 166.7, 154.2, 131.6, 127.6, 122.9, 112.5, 112.2, 112.1, 100.5, 56.0, 52.7, 39.6, 25.2.

HR MS ES(+) (*m/z*) calculated for $C_{22}H_{27}NO_3Na$ ($[M + Na]^+$) 376.1889, found 376.1902.

3.1.4. Synthesis of tetrahydroisoquinoline-derived heterodimers



21a m = 1, n = 1
21b m = 1, n = 4
21c m = 3, n = 4



22

General procedure for the synthesis of heterodimers 21a - c and 22

A tetrahydroisoquinoline-derived alkyne (0.05 mmol) and a tryptamine-derived azide (0.05 mmol) were dissolved in THF (4 mL). $CuSO_4{\cdot}5H_2O$ (0.15 eq) and sodium ascorbate (0.45 eq) dissolved in water (1 mL) were added to the solution, and the reaction mixture was stirred for 48 h·THF was removed under reduced pressure, brine (10 mL) was added, and the water solution was extracted three times with chloroform. The combined organic phases were washed with brine, dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under reduced pressure and the product was purified by column chromatography (Al_2O_3, chloroform).

21a was obtained as colorless oil in 58% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.38 (s, 1H, $NH_{indolyl}$), 7.78 (m, 1H, $H_{phthaloyl}$), 7.68 (s, 1H, $H_{triazole}$), 7.67 (m, 1H, $H_{phthaloyl}$), 7.19 (d, $J = 9.0$ Hz, 1H, $H_{indolyl}$), 6.93 (d, $J = 2.4$ Hz, 1H, $H_{indolyl}$), 6.85 (d, $J = 2.4$ Hz, 1H, $H_{indolyl}$), 6.81 (dd, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz, 1H, $H_{indolyl}$), 6.54 (s, 1H, H_{benz}), 6.51 (s, 1H, H_{benz}), 6.27 (br. t, 1H, $J = 5.7$ Hz, CONH), 5.00 (s, 2H, triazoleCH_2CO), 3.82 (m, 12H, H-1, NCH_2triazole, $3OCH_3$), 3.66 (m, 2H, $CH_{2phthaloyl}$), 3.53 (q, $J = 6.6$ Hz, 2H, CH_2NH), 3.12 (m, 1H, H-4a), 2.87 (t, $J = 6.6$ Hz, 2H, CH_2CH_2NH), 2.76 (m, 2H, H-4b, H-3a), 2.48 (m, 1H, H-3b), 1.85–1.67 (m, 4H, $2CH_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 168.5, 165.3, 153.9, 147.4, 147.4, 147.1, 133.9, 132.0, 131.5, 129.5, 127.4, 126.1, 124.5, 123.2, 123.1, 112.3, 112.4, 111.6, 111.4, 110.5, 100.3, 59.8, 55.9, 55.9, 55.8, 53.4, 53.1, 48.7, 43.7, 43.7, 39.5, 37.9, 33.0, 25.0, 24.8, 23.8.

HR MS ES(+) (*m/z*) calculated for $C_{38}H_{41}N_7O_6Na$ ($[M + Na]^+$) 714.3016, found 714.3010.

21b was obtained as colorless oil in 49% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.70 (s, 1H, $NH_{indolyl}$), 7.78 (m, 1H, $H_{phthaloyl}$), 7.68 (m, 1H, $H_{phthaloyl}$), 7.26 (s, 1H, $H_{triazole}$), 7.16 (d, $J = 9.0$ Hz, 1H, $H_{indolyl}$), 6.93 (d, $J = 2.4$ Hz, 1H, $H_{indolyl}$), 6.82 (d, $J = 2.4$ Hz, 1H, $H_{indolyl}$), 6.80 (dd, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz, 1H, $H_{indolyl}$), 6.54 (s, 1H, H_{benz}), 6.53 (s, 1H, H_{benz}), 6.09 (br. t, 1H, $J = 5.7$ Hz, CONH), 4.95 (s, 2H, triazoleCH_2CO), 3.83 (m, 10*H*, H-1, $3OCH_3$), 3.69 (m, 2H, $CH_{2phthaloyl}$), 3.55 (m, 3H, CH_2NH, H-4a), 2.87 (t, $J = 6.6$ Hz, 2H, CH_2CH_2NH), 2.78 (m, 2H, H-4b, H-3a), 2.68 (t, $J = 7.2$ Hz, 2H, CH_2triazole), 2.56 (t, $J = 6.6$ Hz, 2H, NCH_2), 2.44 (m, 1H, H-3b), 1.83–1.56 (m, 8H, $4CH_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 168.6, 165.5, 154.0, 148.9, 147.5, 147.3, 134.0, 132.1, 131.6, 130.1, 127.6, 126.4, 123.2, 123.2, 122.5, 112.3, 112.2, 111.7, 111.5, 110.8, 100.4, 60.3, 56.1, 56.0, 55.9, 53.1, 43.7, 39.7, 38.07, 33.2, 27.6, 27.1, 25.8, 25.5, 24.8, 23.8.

HR MS ES(+) (*m/z*) calculated for $C_{41}H_{47}N_7O_6Na$ ($[M + Na]^+$) 756.3486, found 756.3499.

21c was obtained as colorless oil in 59% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.42 (s, 1H, $NH_{indolyl}$), 7.78 (m, 1H, $H_{phthaloyl}$), 7.70 (m, 1H, $H_{phthaloyl}$), 7.23 (s, 1H, $H_{triazole}$), 7.21 (d, $J = 9.0$ Hz, 1H, $H_{indolyl}$), 7.03 (d, $J = 2.4$ Hz, 1H, $H_{indolyl}$), 7.00 (d, $J = 2.4$ Hz, 1H, $H_{indolyl}$), 6.82 (dd, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz, 1H, $H_{indolyl}$), 6.54 (s, 1H, H_{benz}), 6.52 (s, 1H, H_{benz}), 5.92 (br. t, 1H, $J = 5.7$ Hz, CONH), 4.30 (t, $J = 6.3$ Hz, 2H, triazoleCH_2), 3.83 (m, 10*H*, H-4, $3OCH_3$), 3.70 (m, 2H, $CH_{2phthaloyl}$), 3.58 (m, 3H, CH_2NH, H-1a), 2.93 (t, $J = 6.6$ Hz, 2H, CH_2CH_2NH), 2.77 (m, 2H, H-1b, H-2a), 2.69 (t, $J = 7.2$ Hz, 2H, CH_2triazole), 2.54 (t, $J = 6.6$ Hz, 2H, NCH_2), 2.41 (m, 1H, H-2b), 2.09 (m, 2H, CH_2CO), 1.85–1.52 (m, 10*H*, $5CH_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 171.5, 168.6, 154.1, 148.4, 147.3, 147.3, 134.0, 132.2, 131.7, 130.4, 127.8, 126.6, 123.2, 123.2, 121.0, 112.4, 112.4, 112.1, 111.5, 110.8, 100.6, 60.4, 56.0, 56.0, 55.9, 53.5, 49.0, 43.6, 39.6, 38.1, 33.4, 32.8, 27.8, 27.3, 26.3, 25.7, 25.7, 25.3, 23.7.

HR MS ES(+) (*m/z*) calculated for $C_{43}H_{51}N_7O_6Na$ ($[M + Na]^+$) 784.3799, found 784.3775.

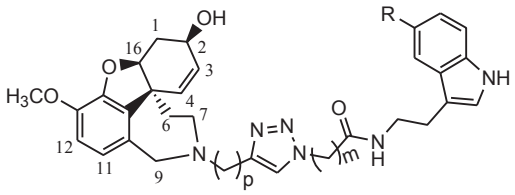
22 was obtained as colorless oil in 63% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 10.05 (s, 1H, $NH_{indolyl}$), 7.19 (d, J = 8.7 Hz, 1H, $H_{indolyl}$), 6.98 (s, 1H, $H_{indolyl}$), 6.96 (d, J = 2.7 Hz, 1H, $H_{indolyl}$), 6.83 (dd, J_1 = 8.7 Hz, J_2 = 2.4 Hz, 1H, $H_{indolyl}$), 6.67 (d, J = 2.1 Hz, 1H, $H_{indolyl}$), 6.54 (s, 1H, H_{benzyl}); 6.52 (s, 1H, H_{benzyl}), 5.69 (t, J = 5.4 Hz, 1H, CONH), 4.92 (s, 2H, triazoleCH_2CO), 3.85 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.65 (m, 1H, NCH), 3.54 (q, J = 6.3 Hz, 2H, CH_2NH), 3.38 (t, J = 5.1 Hz, 1H, H-1a), 3.06 (m, 1H, H-1b), 2.89 (t, J = 6.6 Hz, 2H, CH_2CH_2NH), 2.66 (m, 4H, H-2a, H-2b, CH_2triazole), 2.41 (m, 7H, $2NCH_2$, NCH_3), 2.28 (s, 3H, NCH_3), 1.81–1.43 (m, 8H, $4CH_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 165.5, 153.9, 148.8, 147.3, 131.9, 130.0, 127.6, 126.9, 123.3, 122.3, 112.3, 112.3, 111.5, 111.3, 110.2, 100.3, 63. 3, 58.2, 57.4, 56.1, 55.9, 53.1, 48.4, 43.0, 42.2, 39.6, 33.0, 27.3, 26.8, 25.7, 25.4, 24.6, 22.7.

HR MS ES(+) (m/z) calculated for $C_{35}H_{50}N_7O_4$ ($[M + H]^+$) 632.3924, found 632.3932.

3.1.5. Synthesis of galantamine-derived heterodimers



23a p = 1, m = 1, R = OCH_3
23b p = 1, m = 4, R = OCH_3
23c p = 4, m = 1, R = H
23d p = 4, m = 3, R = H
23e p = 4, m = 4, R = H
23f p = 4, m = 1, R = OCH_3
23g p = 4, m = 4, R = OCH_3

General procedure for the synthesis of heterdimers 23a - g

A galantamine-derived alkyne (0.05 mmol) and a tryptamine-derived azide (0.05 mmol) were dissolved in THF (4 mL). $CuSO_4{\cdot}5H_2O$ (0.15 eq) and sodium ascorbate (0.45 eq) dissolved in water (1 mL) were added to the solution, and the reaction mixture was stirred for 48 h·THF was removed under reduced pressure, brine (10 mL) was added, and the water solution was extracted three timed with chloroform. The combined organic phases were washed with brine, dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under reduced pressure and the product was purified by column chromatography (Al_2O_3, chloroform).

23a was obtained as colorless oil in 56% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.40 (s, 1H, $NH_{indolyl}$), 7.45 (s, 1H, $H_{triazole}$), 7.18 (d, J = 9.0 Hz, 1H, $H_{indolyl}$), 6.95 (d, J = 2.4 Hz, 1H, $H_{indolyl}$), 6.83 (m, 2H, $2H_{indolyl}$), 6.64 (d, J = 8.4 Hz, 1H, H-12), 6.55 (d, J = 8.1 Hz, 1H, H-11), 6.45 (br. t, 1H, J = 5.7 Hz, CONH), 6.06 (d, J = 10.2 Hz, 1H, H-4), 6.00 (dd, J_1 = 10.5 Hz, J_2 = 4.2 Hz, 1H, H-3), 4.94 (s, 2H, triazoleCH_2CO), 4.62 (s, 1H, H-16), 4.12 (m, 2H, H-2, H-9a), 3.83 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.76 (s, 2H, NCH_2triazole), 3.55 (q, J = 6.6 Hz, 2H, CH_2NH), 3.78 (d, J = 16.8 Hz, 1H, H-9b), 3.32 (t, J = 12.9 Hz, 1H, H-7a), 3.15 (d, J_1 = 15.0 Hz, 1H, H-7b), 2.89 (t, J = 6.6 Hz, 2H, CH_2CH_2NH), 2.68 (dm, J = 15.9 Hz, 1H, H-1a), 2.37 (br. s, OH), 2.05 (td, J_1 = 13.5 Hz, J_2 = 2.7 Hz, 1H, H-1b), (dm, J = 15.6 Hz, 1H, H-6a), 1.54 (d, J = 13.8 Hz, 1H, H-6b).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 165.2, 154.1, 146.1, 146.0, 144.4, 133.3, 131.7, 128.8, 127.9, 127.6, 126.9, 124.3, 123.3, 122.3, 112.4, 112.2, 111.7, 111.5, 100.5, 88.8, 62.1, 58.2, 56.1, 53.2, 51.7, 48.4, 47.7, 39.6, 33.7, 30.0, 24.9.

HR MS ES(+) (m/z) calculated for $C_{32}H_{37}N_6O_5$ ($[M + H]^+$) 585.2825, found 585.2798.

23b was obtained as colorless oil in 58% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.45 (s, 1H, $NH_{indolyl}$), 7.43 (s, 1H, $H_{triazole}$), 7.23 (d, J = 9.0 Hz, 1H, $H_{indolyl}$), 7.01 (d, J = 2.4 Hz, 1H, $H_{indolyl}$), 6.88 (s, 1H, $H_{indolyl}$), 6.86 (dd, J_1 = 8.7 Hz, J_2 = 2.4 Hz, 1H, $H_{indolyl}$), 6.64 (d, J = 8.1 Hz, 1H, H-12), 6.55 (d, J = 8.1 Hz, 1H, H-11), 6.07 (d, J = 10.8 Hz, 1H, H-4), 5.99 (dd, J_1 = 10.2 Hz, J_2 = 4.2 Hz, 1H, H-3), 5.49 (br. t, 1H, J = 5.7 Hz, CONH), 4.64 (s, 1H, H-16), 4.31 (t, 1H, J = 6.9 Hz, triazoleCH_2), 4.14 (m, 2H, H-2, H-9a), 3.87 (s, 2H, NCH_2triazole), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.58 (q, J = 6.3 Hz, 2H, CH_2NH), 3.78 (m, 2H, H-9b, H-7a), 2.95 (d, J_1 = 15.0 Hz, 1H, H-7b), 2.94 (t, J = 6.6 Hz, 2H, CH_2CH_2NH), 2.67 (dm, J = 15.9 Hz, 1H, H-1a), 2.41 (br. s, OH), 2.14 (m, 1H, H-1b), 2.13 (t, J = 7.3 Hz, 2H, $COCH_2$), 2.00 (dm, J = 15.6 Hz, 1H, H-6a), 1.61 (m, 5H, H-6b, $2CH_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 172.1, 154.3, 146.2, 145.4, 144.7, 132.3, 131.7, 128.1, 127.8, 127.0, 126.9, 124.3, 123.3, 122.6, 112.5, 112.2, 111.7, 111.5, 100.7, 89.0, 62.2, 59.2, 58.4, 56.1, 56.1, 51.2, 48.8, 48.1, 39.4, 37.5, 30.0, 30.0, 29.7, 25.3, 22.8.

HR MS ES(+) (m/z) calculated for $C_{35}H_{43}N_6O_5$ ($[M + H]^+$) 627.3295, found 627.3282.

23c was obtained as colorless oil in 68% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 9.44 (s, 1H, $NH_{indolyl}$), 7.59 (d, J = 7.8 Hz, 1H, $H_{indolyl}$), 7.28 (d, J = 7.8 Hz, 1H, $H_{indolyl}$), 7.18 (td, J_1 = 9.9 Hz, J_2 = 1.2 Hz, 1H, $H_{indolyl}$), 7.09 (td, J_1 = 6.9 Hz, J_2 = 1.2 Hz, 1H, $H_{indolyl}$), 7.08 (s, 1H, $H_{triazole}$), 6.76 (d, J = 2.4 Hz, 1H, $H_{indolyl}$), 6.69 (d, J = 8.4 Hz, 1H, H-12), 6.64 (d, J = 8.4 Hz, 1H, H-11), 6.08 (d, J = 10.2 Hz, 1H, H-4), 6.00 (dd, J_1 = 10.2 Hz, J_2 = 4.2 Hz, 1H, H-3), 5.88 (t, 1H, J = 5.1 Hz, CONH), 4.89 (s, 2H, triazoleCH_2CO), 4.60 (br s, 1H, H-16), 4.18 (m, 2H, H-2, H-9a), 3.88 (d, J = 15.3 Hz, 1H, H-9b), 3.84 (s, 3H, OCH_3), 3.55 (q, J = 6.3 Hz, 2H, CH_2NH), 3.42 (td, J_1 = 12.9 Hz, J_2 = 1.8 Hz, 1H, H-7a), 3.26 (dt, J_1 = 15.0 Hz, J_2 = 3.0 Hz, 1H, H-7b), 3.92 (t, J = 6.6 Hz, 2H, CH_2CH_2NH), 2.68 (t, J = 6.9 Hz, 3H, CH_2triazole, H-1a), 2.58 (m, 2H, NCH_2), 2.05 (m, 2H, H-1b, H-6a), 1.86 (br. s, OH), 1.69–1.52 (m, 5H, H-6b, $2CH_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 165.4, 148.6, 146.0, 144.4, 136.7, 133.3, 128.7, 128.0, 127.9, 127.2, 126.8, 122.5, 122.3, 122.2, 119.4, 118.6, 111.9, 111.5, 111.4, 88.8, 62.1, 57.6, 56.1, 53.1, 51.7, 51.0, 48.5, 39.7, 32.8, 30.1, 27.1, 26.7, 25.4, 24.7.

HR MS ES(+) (m/z) calculated for $C_{34}H_{41}N_6O_4$ ($[M + H]^+$) 597.3189, found 597.3206.

23d was obtained as colorless oil in 48% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.66 (s, 1H, $NH_{indolyl}$), 7.58 (d, J = 8.1 Hz, 1H, $H_{indolyl}$), 7.34 (d, J = 7.1 Hz, 1H, $H_{indolyl}$), 7.18 (td, J_1 = 9.9 Hz, J_2 = 1.2 Hz, 1H, $H_{indolyl}$), 7.16 (s, 1H, $H_{triazole}$), 7.10 (td, J_1 = 6.9 Hz, J_2 = 1.2 Hz, 1H, $H_{indolyl}$), 7.05 (d, J = 2.4 Hz, 1H, $H_{indolyl}$), 6.66 (d, J = 8.1 Hz, 1H, H-12), 6.59 (d, J = 8.1 Hz, 1H, H-11), 6.07 (d, J = 10.2 Hz, 1H, H-4), 6.00 (dd, J_1 = 10.2 Hz, J_2 = 3.9 Hz, 1H, H-3), 5.79 (t, 1H, J = 5.7 Hz, CONH), 4.59 (br s, 1H, H-16), 4.27 (t, 2H, J = 6.6 Hz, triazoleCH_2), 4.12 (m, 2H, H-2, H-9a), 3.80 (d, J = 15.6 Hz, 1H, H-9b), 3.82 (s, 3H, OCH_3), 3.59 (q, J = 6.6 Hz, 2H, CH_2NH), 3.34 (td, J_1 = 12.9 Hz, J_2 = 1.8 Hz, 1H, H-7a), 3.15 (dt, J_1 = 15.0 Hz, J_2 = 3.0 Hz, 1H, H-7b), 2.97 (t, J = 6.6 Hz, 2H, CH_2CH_2NH), 2.68 (m, 3H, CH_2triazole, H-1a), 2.48 (m, 2H, NCH_2), 2.19–1.93 (m, 5H, H-1b, H-6a, $COCH_2$, OH), 1.67–1.47 (m, 7H, H-6b, $3CH_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 171.4, 148.2, 145.9, 144.3, 136.6, 133.3, 129.1, 127.8, 127.4, 127.0, 122.5, 122.2, 122.2 121.0, 119.5, 118.8, 112.7, 111.5, 111.3, 88.8, 62.2, 57.8, 56.0, 51.7, 49.0, 48.5, 39.7, 32.9, 32.7, 30.1, 27.2, 26.9, 26.2, 25.5, 25.3.

HR MS ES(+) (m/z) calculated for $C_{36}H_{45}N_6O_4$ ($[M + H]^+$) 625.3502, found 625.3560.

23e was obtained as colorless oil in 55% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.68 (s, 1H, $NH_{indolyl}$), 7.57 (d, J = 7.8 Hz, 1H, $H_{indolyl}$), 7.34 (d, J = 8.1 Hz, 1H, $H_{indolyl}$), 7.20 (s, 1H, $H_{triazole}$), 7.18 (td, J_1 = 8.1 Hz, J_2 = 1.2 Hz, 1H, $H_{indolyl}$), 7.09 (td, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H, $H_{indolyl}$), 6.96 (d, J = 2.4 Hz, 1H, $H_{indolyl}$), 6.65 (d, J = 8.4 Hz, 1H, H-12), 6.58 (d, J = 8.1 Hz, 1H, H-11), 6.07 (d, J = 10.2 Hz, 1H, H-4), 5.98 (dd, J_1 = 10.2 Hz, J_2 = 4.2 Hz, 1H, H-3), 5.64 (t, 1H, J = 5.4 Hz, CONH), 4.58 (br s, 1H, H-16), 4.24 (t, 2H, J = 7.2 Hz,

triazoleC$\underline{H}_2$), 4.11 (m, 2H, H-2, H-9a), 3.80 (d, $J = 15.6$ Hz, 1H, H-9b), 3.82 (s, 3H, OC$\underline{H}_3$), 3.57 (q, $J = 6.6$ Hz, 2H, C$\underline{H}_2$NH), 3.33 (td, $J_1 = 12.9$ Hz, $J_2 = 1.8$ Hz, 1H, H-7a), 3.14 (dt, $J_1 = 15.0$ Hz, $J_2 = 3.0$ Hz, 1H, H-7b), 2.95 (t, $J = 6.6$ Hz, 2H, C$\underline{H}_2$CH$_2$NH), 2.69 (m, 3H, C$\underline{H}_2$triazole, H-1a), 2.53 (m, 2H, NC$\underline{H}_2$), 2.41 (br.s, 1H, O$\underline{H}$), 2.09 (t, $J = 7.2$ Hz, 2H, COC$\underline{H}_2$), 2.06 (m, 1H, H-1b), 1.98 (m, 1H, H-6a), 1.71–1.51 (m, 9H, H-6b, 4C$\underline{H}_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 172.2, 148.2, 145.9, 144.2, 136.6, 133.3, 129.3, 127.7, 127.4, 127.0, 125.6, 122.5, 122.2 120.8, 119.4, 118.7, 112.7, 111.5, 111.3, 88.8, 62.2, 57.8, 56.0, 51.7, 49.9, 48.5, 39.7, 35.8, 33.0, 30.4, 30.1, 29.7, 27.3, 27.0, 25.6, 25.3, 22.7.

HR MS ES(+) (*m/z*) calculated for $C_{37}H_{47}N_6O_4$ ($[M + H]^+$) 639.3659, found 639.3601.

23f was obtained as colorless oil in 53% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 9.30 (s, 1H, N$\underline{H}_{indolyl}$), 7.16 (d, $J = 9.0$ Hz, 1H, H$_{indolyl}$), 7.10 (s, 1H, H$_{triazole}$), 6.96 (d, $J = 2.1$ Hz, 1H, H$_{indolyl}$), 6.85 (dd, $J_1 = 8.4$ Hz, $J_2 = 2.4$ Hz, 1H, H$_{indolyl}$), 6.73 (d, $J = 2.1$ Hz, 1H, H$_{indolyl}$), 6.66 (d, $J = 7.2$ Hz, 1H, H-12), 6.65 (d, $J = 8.1$ Hz, 1H, H-11), 6.08 (d, $J = 10.2$ Hz, 1H, H-4), 6.00 (dd, $J_1 = 10.2$ Hz, $J_2 = 4.2$ Hz, 1H, H-3), 5.81 (br. t, 1H, $J = 6.0$ Hz, CON$\underline{H}$), 4.92 (s, 2H, triazoleC$\underline{H}_2$CO), 4.61 (s, 1H, H-16), 4.18 (m, 2H, H-2, H-9a), 3.91 (d, $J = 16.8$ Hz, 1H, H-9b), 3.85 (s, 3H, OC$\underline{H}_3$), 3.84 (s, 3H, OC$\underline{H}_3$), 3.56 (q, $J = 6.6$ Hz, 2H, C$\underline{H}_2$NH), 3.32 (t, $J = 12.9$ Hz, 1H, H-7a), 3.15 (d, $J_1 = 15.0$ Hz, 1H, H-7b), 2.89 (t, $J = 6.6$ Hz, 2H, C$\underline{H}_2$CH$_2$NH), 2.69 (m, 3H, H-1a, C$\underline{H}_2$triazole), 2.59 (m, 2H, NC$\underline{H}_2$), 2.36 (br. s, O$\underline{H}$), 2.0 5 (m, 2H, H-1b, H-6a), 1.64 (m, 5H, H-6b, 2C$\underline{H}_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 165.4, 154.1, 147.3, 146.0, 144.5, 131.8, 128.0, 127.6, 127.9, 127.6, 126.8, 124.3, 123.1, 122.3, 112.5, 112.2, 111.6, 111.4, 100.4, 88.9, 62.2, 58.2, 56.1, 53.2, 51.8, 48.5, 47.7, 39.6, 32.8, 30.1, 27.2, 26.7, 25.4, 24.7.

HR MS ES(+) (*m/z*) calculated for $C_{35}H_{43}N_6O_5$ ($[M + H]^+$) 627.3295, found 627.3282.

23 g was obtained as colorless oil in 59% yield.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.44 (s, 1H, N$\underline{H}_{indolyl}$), 7.26 (d, $J = 9.0$ Hz, 1H, H$_{indolyl}$), 7.21 (s, 1H, H$_{triazole}$), 7.01 (d, $J = 2.4$ Hz, 1H, H$_{indolyl}$), 6.97 (d, $J = 2.4$ Hz, 1H, H$_{indolyl}$), 6.87 (dd, $J_1 = 8.4$ Hz, $J_2 = 2.1$ Hz, 1H, H$_{indolyl}$), 6.68 (d, $J = 8.1$ Hz, 1H, H-12), 6.59 (d, $J = 8.1$ Hz, 1H, H-11), 6.09 (d, $J = 10.2$ Hz, 1H, H-4), 6.00 (dd, $J_1 = 10.6$ Hz, $J_2 = 4.5$ Hz, 1H, H-3), 5.80 (br. t, 1H, $J = 5.4$ Hz, CON$\underline{H}$), 4.59 (s, 1H, H-16), 4.26 (t, $J = 7.2$ Hz, 2H, triazoleC$\underline{H}_2$), 4.13 (m, 2H, H-2, H-9a), 3.79 (d, $J = 18.9$ Hz, 1H, H-9b), 3.85 (s, 3H, OC$\underline{H}_3$), 3.83 (s, 3H, OC$\underline{H}_3$), 3.58 (q, $J = 6.6$ Hz, 2H, C$\underline{H}_2$NH), 3.33 (t, $J = 12.9$ Hz, 1H, H-7a), 3.15 (d, $J_1 = 15.0$ Hz, 1H, H-7b), 2.93 (t, $J = 6.6$ Hz, 2H, C$\underline{H}_2$CH$_2$NH), 2.69 (m, 3H, C$\underline{H}_2$triazole, H-1a), 2.53 (m, 2H, NC$\underline{H}_2$), 2.41 (br.s, 1H, O$\underline{H}$), 2.08 (t, $J = 4.2$ Hz, 2H, COC$\underline{H}_2$), 2.06 (m, 1H, H-1b), 1.98 (m, 1H, H-6a), 1.67–1.47 (m, 9H, H-6b, 4C$\underline{H}_2$).

^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 172.2, 154.1, 148.2, 145.9, 144.2, 133.3, 131.7, 129.5, 127.8, 127.7, 127.1, 123.3, 122.2, 120.8, 112.4, 112.4, 112.2, 111.3, 100.6, 88.9, 62.2, 57.8, 56.1, 56.0, 51.7, 51.3, 49.9, 48.5, 39.5, 33.0, 30.1, 29.8, 27.2, 27.1, 25.7, 25.3, 22.7.

HR MS ES(+) (*m/z*) calculated for $C_{38}H_{49}N_6O_5$ ($[M + H]^+$) 669.3764, found 669.3732.

3.2. Inhibitory activity

Samples of *h*AChE and *h*BChE were derived from human whole red blood cells and plasma, respectively, according to the known procedure [32]. 10% EDTA buffer was added to freshly collected blood (20 μL of buffer:1 mL of blood). Blood was centrifuged for 10 min (10000 g, 4 °C). The plasma was diluted 1:125 with the 0.1 M Na_3PO_4 buffer (pH 7.4). Erythrocytes were washed three times with isotonic saline, lysed in 9 volumes of the 0.1 M Na_3PO_4 buffer (pH 7.4) containing 0.5% Triton-X and diluted with additional 19 volumes of buffer.

Stock solutions of test compounds were prepared in Tween 80/EtOH 3:1 (v/v). Final dilutions were prepared in the 0.1 M Na_3PO_4 buffer (pH 8.0). Samples of enzymes were preincubated with increasing concentrations of the test compound ranging from 1 nM to 100 mM (30 min, room temperature) and then incubated with their respective substrates (0.5 mM) and DTNB (25 min, 37 °C). The production of a yellow anion was measured by a spectrophotometer. Each tested compound was analyzed in triplicate on three separate occasions.

The enzyme activity at each concentration of the tested compound was expressed as a percentage of the activity in the absence of the compound and plotted as a function of its log concentration. The inhibitory activity was calculated as IC_{50} values.

3.3. Kinetic studies

The kinetic measurements were performed in a similar manner, with substrate concentrations 0.25, 0.5, 1 and 2 mM, DTNB concentration 1.2 mM, and **23d** concentrations 3.3 and 9.9 nM for AChE and 89 and 267 nM for BChE. V_{max} and K_m values of the Michaelis–Menten kinetics were calculated by nonlinear regression from substrate–velocity curves. Linear regression was used for calculating the Lineweaver–Burk plots.

3.4. KTGS experiments

All the experiments were conducted in ammonium-citrate buffer (total volume 100 μL, 2 mM ammonium citrate, 100 mM NaCl, pH 7.4).

A stock solution containing a mixture of azides **6a - d**, **7a - d** and **10** was prepared by adding 10 μL of 100 mM methanolic solution of each azide to 110 μL of ammonium-citrate buffer.

Alkynes **14a-b** were dissolved in DMSO to give 100 mM solutions, which were subsequently diluted 4 times with ammonium-citrate buffer.

Alkynes **20a-b** were dissolved in methanol to give 100 mM solutions, which were subsequently diluted 4 times with ammonium-citrate buffer.

3.5. Protein-templated reaction

The activity of each butyrylcholinesterase sample as well as the stability of the enzyme in the experiment conditions were determined using butyrylcholinesterase activity assay kit (Arbor assays).

Butyrylcholinesterase (form equine serum, 0.001 μmol) was dissolved in ammonium-citrate buffer (80 μL). A stock solution of azides (10 μL) and a solution of a selected alkyne (10 μL) were added. The reaction mixture was shortly vortexed and allowed to stand at 37 °C for 7 days.

Negative control

A stock solution of azides (10 μL) and a solution of a selected alkyne (10 μL) were added to ammonium-citrate buffer (80 μL). The reaction mixture was shortly vortexed and allowed to stand at 37 °C for 7 days.

Positive control a - CuCAAC

A stock solution of azides (10 μL), a solution of a selected alkyne (10 μL), aqueous copper sulfate (10 μL, 2 mM stock solution) and aqueous sodium ascorbate (10 μL, 6 mM stock solution) were added to ammonium-citrate buffer (80 μL). The reaction mixture was shortly vortexed and allowed to stand at room temperature for 7 days.

Positive control b - huisgen cycloaddition

A stock solution of azides (10 μL) and a solution of a selected alkyne (10 μL) were added to ammonium-citrate buffer (80 μL). The reaction mixture was shortly vortexed and heated to 80 °C for 7 days.

Final concentrations: Butyrylcholinesterase: 10 μM; azides: 50 mM each; alkyne: 250 μmol; aqueous copper sulfate: 200 μM; sodium ascorbate: 600 μM; methanol: 0.7%.

UPLC measurements: UPLC was performed using a Waters Acquity UPLC system coupled to a Synapt G2 HDMS. All analyses were performed using a reversed phase UPLC column (Acquity C18 Column, 1.7 μm, 2.1 mm × 100 mm), column temperature 30 °C, flow rate 0.25 mL/

min. Positive-ion mass spectra were acquired using ES ionization.

Eluent A: acetonitrile + 0.1% of formic acid.

Eluent B: water + 0.1% of formic acid.

The library components were eluted with a gradient from 95% A → 5% A over 20 min, followed by 5% A over 3 min.

Positive-ion mass spectra were acquired using ES ionization.

The cycloaddition products were identified by molecular weight and retention times.

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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.

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