Bioorganic & Medicinal Chemistry Letters 20 (2010) 2946–2949

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Bivalent 5,8,9,13b-tetrahydro-6*H*-isoquino[1,2-*a*]isoquinolines and -isoquinolinium salts: Novel heterocyclic templates for butyrylcholinesterase inhibitors

Maria Schulze [a], Oliver Siol [b], Michael Decker [a], Jochen Lehmann [a,*]

[a] Lehrstuhl für Pharmazeutische/Medizinische Chemie, Institut für Pharmazie, Friedrich-Schiller-Universität Jena, Philosophenweg 14, D-07743 Jena, Germany
[b] Lehrstuhl für Pharmazeutische Biologie, Institut für Pharmazie, Friedrich-Schiller-Universität Jena, Semmelweisstrasse 10, D-07743 Jena, Germany

ARTICLE INFO

Article history:
Received 4 February 2010
Revised 2 March 2010
Accepted 2 March 2010
Available online 6 March 2010

Keywords:
Bivalent inhibitor
Acetylcholinesterase
Butyrylcholinesterase
Cytotoxicity

ABSTRACT

Three different types of homobivalent compounds, 5,8,9,13b-tetrahydro-6*H*-isoqino[1,2-*a*]isoquinolines bearing tertiary N-atoms, their quaternary ammonium salts and their dibenzazecine analogues, connected by alkylene spacers of various lengths were synthesized. Compared to the therapeutically used inhibitor galanthamine, some of the bivalent compounds showed much higher inhibitory activities at both cholinesterases in the Ellman test. Surprisingly, not only the quaternary salts, but also the uncharged tertiary compounds exhibited IC_{50} values at butyrylcholinesterase in the nanomolar range. Selectivity toward BChE of up to 76-fold was observed.

Cholinesterase (ChE) is one major target in the current therapy of Alzheimer's disease (AD). Clinically approved inhibitors of acetylcholinesterase (AChE) like rivastigmine, galanthamine and donepezil are the most commonly applied drugs for symptomatic treatment of cognitive deficits in AD. For the effectiveness of therapeutic intervention by ChE inhibition, the importance of a second, less specific cholinesterase in the human body, butyrylcholinesterase (BChE), is under current focus. While the treatment with selective AChE inhibitors is effective in the beginning of AD, their effectiveness decreases with progression of the disease which may be due in part to lower AChE-(10–15% of normal during AD progression), but elevated BChE-levels.[1] Positive effects of the administration of the selective BChE inhibitor cymserine on learning and β-amyloid peptide formation could be shown in rodents.[2]

Compound **1** (3-methoxy-5,8,9,13b-tetrahydro-6*H*-isoquino-[1,2-*a*]isoquinoline) was originally synthesized as a precursor for highly potent dopamine antagonists.[3]

Some structural similarities of compound **1** to galanthamine can be found (Fig. 1). We therefore considered it to be of interest, if these structures might exhibit ChE-inhibiting properties, since there are considerable efforts made to find novel structural templates for ChE inhibitors with improved therapeutic profiles.[4]

In this regard, additionally bivalent compounds should be synthesized and evaluated pharmacologically. In the last decade, the bivalent ligand/inhibitor approach by combining covalently two identical (homobivalent) or chemically related (heterobivalent) drug molecules was successfully applied in various areas of therapeutic research (including AChE and BChE inhibitors) to yield highly potent compounds with sometimes remarkably increased selectivity profiles.[5–7] Bivalent compounds of galanthamine were investigated by Guillou et al. and revealed inhibitory potencies of the homobivalent galanthamine derivatives equal to that of the univalent compound galanthamine at AChE and improved inhibition by heterodimers with galanthaminium substructure.[8] The improved enzyme inhibition of the quaternary salt is not surprising, since the natural substrate acetylcholine (ACh) itself is a quaternary ammonium salt. Hence, we used compound **2** as a potential univalent inhibitor to design homobivalent molecules with alkylene chains of various lengths as spacer. A potential problem of these compounds lies in the fact that it is unlikely for charged molecules to pass the blood–brain barrier which represents a basic requirement for drugs against neurodegenerative disorders. Since a competitive and reversible ChE inhibitor interaction with the catalytic active site (CAS) is often mediated by a protonated tertiary N-atom, we also tested the uncharged compounds for their inhibitory potency. Univalent structures are shown in Figure 1.

The univalent precursor **4** was synthesized according to the synthetic scheme described by Mohr et al.,[3] using (3-methoxyphenyl)ethylamine and isochroman-1-one as starting materials

* Corresponding author. Tel.: +49 3641 94 9800; fax: +49 3641 94 9802.
E-mail addresses: m.decker@qub.ac.uk (M. Decker), j.lehmann@uni-jena.de (J. Lehmann).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.011

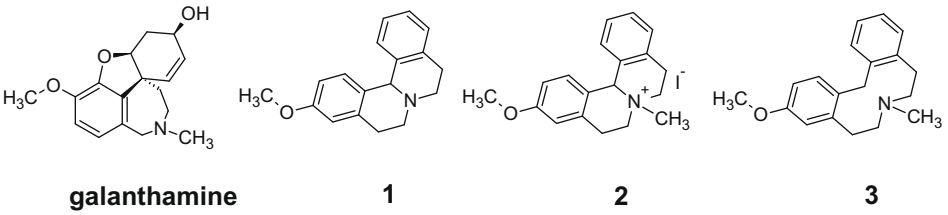


Figure 1. Tetracyclic compounds **1** and **2**, tricyclic compound **3** and the AChE inhibitor galanthamine.

(Scheme 1). Synthesis of homobivalent compounds **5a**–**f** with tertiary N-atoms was achieved in a Williamson ether synthesis by deprotonation of phenolic hydroxy groups using sodium hydride in DMF, followed by alkylation using half the molar amount of the respective dibromoalkane. Quaternization was performed with methyl iodide in acetonitrile/methanol. Compounds **7b** and **7e** were obtained under *Birch* conditions using sodium in liquid ammonia. Univalent structures were synthesized in the same manner.

We examined bivalent quaternary compounds **6a**–**f**, their non-charged precursors **5a**–**f**, the bivalent dibenzoazecines **7b**,**e** and the univalent congeners **1**, **2**, and **3** in the colorimetric Ellman assay at AChE (E.C. 3.1.1.7, type VI-S, from Electric Eel) and BChE (E.C. 3.1.1.8, from equine serum). Although there are a number of species-dependent differences between these enzymes and the human ones, the enzymes used show sufficient homology in the amino acid sequence to the human enzymes (e.g., 88% for equine serum BChE).[9]

Results of the cholinesterase assay are given in Table 1.

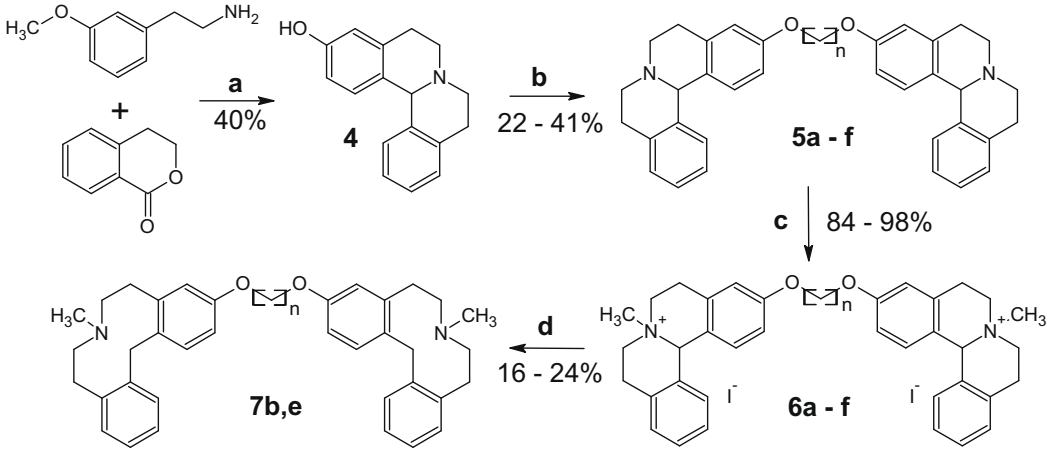
All compounds revealed higher inhibitory activities at both cholinesterases than the univalent congeners. As expected due to the similarity with the natural ligand ACh, the quaternary ammonium salts **6a**–**f** showed better potency for AChE inhibition than the uncharged molecules **5a**–**f**.

The univalent tertiary compounds 3-methoxy-5,8,9,13b-tetrahydro-6*H*-isoquino-[1,2-*a*]isoquinoline (**1**) and 3-methoxy-7-methyl-5,6,7,8,9,14-hexahydrodibenzo[*d*,*g*]azecine (**3**) did not show activities at any ChE. The quaternary compound **2** is a micromolar non-selective inhibitor of both ChEs (IC_{50}(AChE) = 8.8 μM; IC_{50}(BChE) = 6.4 μM). Interestingly, the bivalent compounds showed much higher activities at both AChE and BChE. Regarding the bivalent 7-methyl-5,8,9,13b-tetrahydro-6*H*-isoquino[1,2-*a*]isoquinolinium salts **6a**–**f**, all of these compounds are submicromolar inhibitors with inhibitory potencies from IC_{50}(AChE) = 0.45 μM (compound **6a** with $n = 3$) to IC_{50}(AChE) = 0.12 μM (compound **6f** with $n = 12$). Despite the considerable differences in the lengths of the alkylene spacers (from $n = 3$ to $n = 12$), the differences in potencies are minor, not exceeding a factor of four. For each compound, activities at BChE are even higher, ranging from IC_{50}(BChE) = 0.16 μM (compound **6a** with $n = 3$) to IC_{50}(BChE) = 0.027 μM (compound **6d** with $n = 6$).

Table 1
Inhibitory activities at AChE- and BChE and resulting selectivities toward BChE

Compound	IC_{50} (AChE)[a] (μM) (pIC_{50} ± SEM)	IC_{50} (BChE)[a] (μM) (pIC_{50} ± SEM)	IC_{50}(AChE)/ IC_{50}(BChE)
Galanthamine	0.64 (6.197 ± 0.052)	8.40 (5.076 ± 0.034)	0.08
1	>10,000	>10,000	–
2	8.8 (5.057 ± 0.055)	6.4 (5.197 ± 0.020)	1.4
3	>10,000	>10,000	–
5a	4.5 (5.347 ± 0.044)	0.15 (6.835 ± 0.051)	30.8
5b	1.1 (5.971 ± 0.076	0.017 (7.768 ± 0.048)	76.2
5c	0.65 (6.511 ± 0.178)	0.017 (7.773 ± 0.036)	38.3
5d	0.69 (6.164 ± 0.158)	0.014 (7.846 ± 0.006)	48.9
5e	1.2 (5.920 ± 0.071)	0.68 (6.168 ± 0.047)	3.0
5f	4.1 (5.385 ± 0.051)	0.92 (6.035 ± 0.034)	4.2
6a	0.45 (6.343 ± 0.121)	0.16 (6.806 ± 0.033)	5.8
6b	0.39 (6.406 ± 0.076)	0.051 (7.293 ± 0.037)	8.8
6c	0.28 (6.546 ± 0.036)	0.047 (7.326 ± 0.055)	5.9
6d	0.15 (6.822 ± 0.020)	0.027 (7.570 ± 0.087)	5.6
6e	0.28 (6.557 ± 0.137)	0.11 (6.954 ± 0.034)	2.5
6f	0.12 (6.918 ± 0.055)	0.036 (7.452 ± 0.035)	3.4
7b	4.8 (5.317 ± 0.142)	0.14 (6.847 ± 0.022)	34.0
7e	2.0 (5.690 ± 0.106)	0.21 (6.683 ± 0.036)	9.9

[a] IC_{50} values are means of at least three experiments.



Scheme 1. Synthesis of the test compounds. Reagents and conditions: (a) (i) 70 °C, 7d; (ii) $POCl_3$, MeCN, 95 °C, 18 h; (iii) $NaBH_4$/MeOH, 0 °C to rt, 1 h; (iv) HBr, glacial acid, reflux, 5 h; (b) (i) NaH, dry DMF, 0 °C to rt, 1 h; (ii) dibromoalkane, dry DMF, rt, 24 h; (c) methyl iodide, MeCN/MeOH, rt, 6 h; (d) Na, NH_3 liq., −40 °C.

Despite the fact that the univalent tertiary compound **1** did not show significant activities at either AChE or BChE, the respective bivalent compounds **5a**–**f** turned out to be potent inhibitors, especially at BChE. Activities at AChE generally lay in the micro- and submicromolar range, with the lowest activity IC_{50}(AChE) = 4.5 μM (compound **5a** with $n = 3$) and the most potent compound with an IC_{50}(AChE) = 0.65 μM (compound **5c** with $n = 5$). In the contrary to the quaternary compounds **6a**–**f**, the tertiary compounds **5a**–**f** showed a tether length dependent potency with pentylene and hexylene chains as optimal spacers. This is in line with several examples from the literature, which identified these spacer lengths also as most favorable.[5,10] Most interestingly, activities at BChE are much higher ranging from IC_{50}(BChE) = 0.92 μM (compound **5f** with $n = 12$) down to IC_{50}(BChE) = 0.014 μM (compound **5d** with $n = 6$). At BChE a dependency of the activity on spacer length could be observed: Only compounds with $n = 4$, 5, and 6, respectively, showed activities from IC_{50}(BChE) = 0.014–0.017 μM, whereas $n = 3$ and also higher spacer lengths yielded compounds with 10 to 60-fold lower activities. Since the tertiary compounds **5a**–**f** exhibited lower activities at AChE and higher ones at BChE compared to quaternary compounds **6a**–**f**, BChE selectivity increased. The most selective compound **5b** ($n = 4$) showed a 76-fold selectivity towards BChE. Compounds **7b**, **e**, also with tertiary nitrogen atoms, but much less rigidized than compounds **5a**–**f**, exhibited lower activities than the other bivalent structures with the same spacer length. In general, most bivalent compounds synthesized showed similar or higher activities at AChE than the reference galanthamine, and all novel compounds were superior at BChE.

Interestingly, a relationship between spacer length and biological activity could be observed also in a cytotoxicity test on human glia cells (MG-U87) and neuronal cells (SH-SY5Y), which was performed prior to potential in vivo studies for compounds **5a**–**f** (Table 2). Therein cell viability was measured after substance administration according to previous described protocols[11] to assure innocuousness at effective concentrations in the brain. All CC_{50} values (EC_{50} equivalent of cytotoxicity tests) are at micromolar concentrations, with best ChE potency/toxicity ratio for compound **5d**. The univalent compound **1** showed much less effects on cell viability than the bivalent compounds.

There was a statistically significant relationship between spacer length and biological activity with the pentylene compound **5c** being the most cytotoxic one. In general though, much higher concentrations were necessary to observe cytotoxic effects than for AChE and particularly BChE-inhibition.

An increased activity at AChE of bivalent inhibitors could be explained by interaction of one part of the bivalent compound with the catalytic active site (CAS), where ACh is hydrolyzed, and the other part with the peripheral anionic binding site (PAS) at the outer site of the gorge.[5,12] Due to the fact that quaternary compounds **6a**–**f** and the tertiary ones **5a**–**f** do not show highly significant correlation of inhibitory potency to spacer length at AChE, these interactions with the PAS do not seem to be pronounced and highly specific with tetrahydro-6*H*-isoqino[1,2-*a*]isoquinolines and -isoquinolinium salts. Therefore docking results might be of limited value. The increase of BChE-activity is not straightforwardly explained, either. Only a very few sets of compounds have been described in which bivalency has led to pronounced increases in BChE selectivity without modifying the spacer.[6] In the huge majority of cases an increased AChE selectivity resulted from bivalency.[5] A related increase at BChE-activity and concomitant selectivity was observed with hybrid molecules from ChE-inhibiting quinazolinimines and lipoic acid.[13] With these compounds kinetic measurements were not able to prove an interaction with a second distinct binding site additional to the CAS which would result in non-competitive reversible interaction. Compound **5d** was selected for kinetic measurements at BChE, as it showed highest potencies of all investigated compounds at BChE, high BChE selectivity, and low cytotoxicity, while representing an uncharged molecule for better passage of the blood–brain barrier. The mechanism of inhibition was investigated by recording substrate–velocity curves using various substrate concentrations at different concentrations of **5d**. The resulting Lineweaver–Burk plot (i.e., reciprocal velocities vs reciprocal substrate concentrations) is shown in Figure 2:

K_m values (i.e., the negative reciprocal of the X intercept) differ, but V_{max} values (i.e., the reciprocal of the Y intercept) do not significantly change with different inhibitor concentrations at BChE. This indicates a reversible and competitive inhibition with the substrate molecule, which means that the high inhibitory activity cannot be explained by interaction of compound **5b** with a second binding site, which would lead to an altered kinetic profile.

In conclusion, we have synthesized two new kinds of bivalent compounds, each with different spacer lengths ($n = 3$ up to $n = 12$). Tertiary and quaternary bivalent structures showed greatly enhanced inhibitory potencies compared to their univalent congeners at both AChE and BChE. The charged quaternary ammonium salts **6a-f** revealed better inhibitory activities (two-digit nanomolar IC_{50} values) at AChE and BChE than galanthamine, with moderate selectivity towards BChE. To potentially gain a better penetration of the blood–brain barrier we investigated also their non-charged but protonable precursors **5a**–**f**. Compounds **5c** and **5d** showed inhibitory activities at AChE comparable to galanthamine and BChE-inhibition in the low nanomolar range. For compounds **5a**–**d** a 30-fold up to 76-fold selectivity towards BChE could be observed, what might be advantageous for the treatment in the progressed forms of Alzheimer's disease.[2,14] Inhibitory activities at cholinesterases, especially BChE, and cytotoxic effects were spacer length dependent for these substances. The bivalent ligand approach on 5,8,9,13b-tetrahydro-6*H*-isoquino[1,2-*a*]isoquinolines and -isoquinolinium salts led to compounds with greatly enhanced, submicromolar activities at both ChEs and selectivity towards BChE, introducing a novel structural template for BChE inhibitors.

Table 2
Cell viability measured in a photometric MTT assay

Compound	CC_{50} (μM) glia cells	CC_{50} (μM) neuronal cells
1	774.9 ± 22.1	171.1 ± 41.70
5a	26.92 ± 1.93	8.94 ± 1.93
5b	19.51 ± 3.00	6.35 ± 1.30
5c	8.70 ± 2.94	—
5d	20.73 ± 1.13	7.40 ± 0.22
5e	41.97 ± 6.86	19.24 ± 1.85
5f	46.63 ± 7.98	—

CC_{50} values are means of at least three experiments, each performed in triplicate ± SD.

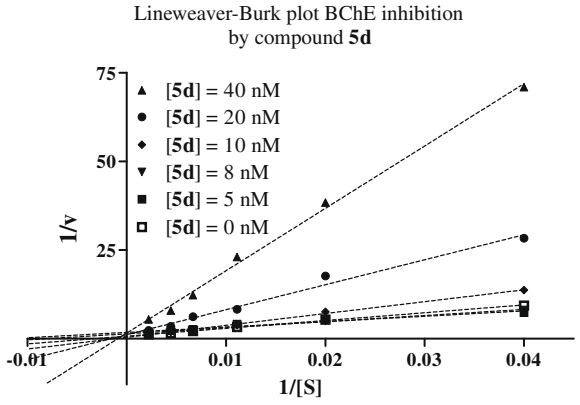


Figure 2. Lineweaver–Burk plot resulting from substrate–velocity curves at different concentrations of compound **5d**.

Acknowledgment

The authors would like to thank Petra Wiecha for the reliable technical assistance in performing the cholinesterase assay.

Supplementary data

Supplementary data (syntheses and spectral data of target compounds, substrate–velocity curves and description of pharmacological assays) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.011.

References and notes

1. Giacobini, E.; DeSarno, P.; Clark, B.; McIlhany, M. *Prog. Brain Res.* **1989**, *79*, 335.
2. Greig, N. H.; Utsuki, T.; Ingram, D. K.; Wang, Y.; Pepeu, G.; Scali, C.; Yu, Q. S.; Mamczarz, J.; Holloway, H. W.; Giordano, T.; Chen, D.; Furukawa, K.; Sambamurti, K.; Brossi, A.; Lahiri, D. K. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17213.
3. Mohr, P.; Decker, M.; Enzensperger, C.; Lehmann, J. *J. Med. Chem.* **2006**, *49*, 2110.
4. Loizzo, M. R.; Tundis, R.; Menichini, F.; Menichini, F. *Curr. Med. Chem.* **2008**, *15*, 1209.
5. Du, D. M.; Carlier, P. R. *Curr. Pharm. Des.* **2004**, *10*, 3141.
6. Decker, M. *J. Med. Chem.* **2006**, *49*, 5411.
7. Peng, X.; Neumeyer, J. L. *Curr. Top. Med. Chem.* **2007**, *7*, 363.
8. Guillou, C.; Mary, A.; Renko, D. Z.; Gras, E.; Thal, C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 637.
9. Savini, L.; Gaeta, G.; Fattorusso, C.; Catalanotti, B.; Campiani, G.; Chiasserini, L.; Pellerano, C.; Novellino, E.; McKissic, D.; Saxena, A. S. *J. Med. Chem.* **2003**, *46*, 1.
10. Holzgrabe, U.; Kapkova, P.; Alptuzun, V.; Scheiber, J.; Kugelmann, E. *Expert Opin. Ther. Targets* **2007**, *11*, 161.
11. Schulze, M.; Siol, O.; Meise, W.; Lehmann, J.; Enzensperger, C. *Arch. Pharm.* **2010**, accepted.
12. Pang, Y. P.; Quiram, P.; Jelacic, T.; Hong, F.; Brimijoin, S. *J. Biol. Chem.* **1996**, *271*, 23646.
13. Decker, M.; Kraus, B.; Heilmann, J. *Bioorg. Med. Chem.* **2008**, *16*, 4252.
14. O'Brien, K. K.; Saxby, B. K.; Ballard, C. G.; Grace, J.; Harrington, F.; Ford, G. A.; O'Brien, J. T.; Swan, A. G.; Fairbairn, A. F.; Wesnes, K.; del Ser, T.; Edwardson, J. A.; Morris, C. M.; McKeith, I. G. *Pharmacogenetics* **2003**, *13*, 231.