Homobivalent Quinazolinimines as Novel Nanomolar Inhibitors of Cholinesterases with Dirigible Selectivity toward Butyrylcholinesterase

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Abstract: Homobivalent dimers of quinazolinimines, which bridge the imine nitrogen atoms via a hepta- and an octamethylene spacer, with different ring sizes of the alicycles were synthesized from the corresponding quinazolinethiones. The resulting compounds show >100-fold increase of inhibitory activities compared to related monomeric compounds yielding low-nanomolar inhibitors. For heptamethylene dimers, mixed inhibition profiles were obtained, whereas for the octamethylene compounds selectivity toward butyrylcholinesterase (>180) can be achieved with an eight-membered alicycle.

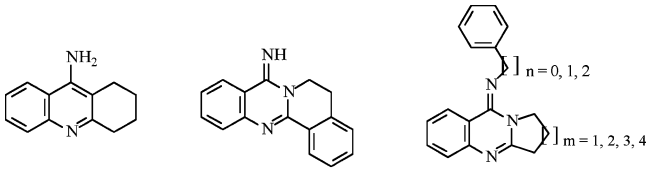
The pharmacotherapy of the most common form of dementia in elderly people, Alzheimer's disease (AD), is very limited, represented by just five FDA-approved drugs. Four of them are acetylcholinesterase inhibitors (donepezil, tacrine, rivastigmine, and galanthamine). The use of acetylcholinesterase (AChE) inhibitors (AChEIs) represents the treatment of choice for mild cases of AD.[1]

AChE also possesses a second binding site, the peripheral anionic binding site (PAS). A hydrophobic environment close to the PAS interacts with β-amyloid peptide (Aβ) and thus accelerates amyloid fibril formation.[2] This widens the application of AChEIs from purely symptomatic treatment to a possibly causative one. Recently the less substrate-specific butyrylcholinesterase (BChE) also got into the focus. BChE appears in serum, liver, heart, and the central nervous system, where it seems to play a key role in neurogenesis and cell proliferation/differentiation.[3] Its corresponding gene is amplified in some neuronal disorders, and it is involved in tumorgenesis.[3] Unlike AChE, its physiologic function is not yet completely revealed. Noteworthy, AChE activity decreases progressively in certain brain regions from mild to severe stages of AD to reach 10−15% of normal values while BChE activity is not affected or even up-regulated, making BChE very available in neuritic plaques.[4] Therefore, mixed AChE/BChE inhibition profiles may result in higher efficacy, reflected by the fact that the inhibitor rivastigmine, which is active at both enzymes, exhibits a good correlation of BChE inhibition and improvement of cognition.[5]

As a novel tool in drug development, the bivalent ligand approach has been established in which two moieties of a known ligand are covalently connected by a suitable spacer. Especially for G-protein-coupled receptors and also transporter systems, this approach proved highly successful for increasing affinities and also selectivities.[6,7]

This method has also been applied to AChE inhibitors yielding both homo- and heterobivalent inhibitors.[8] In particular, the high-activity (also toward BChE) ChE inhibitor tacrine (**1**, Chart 1), although its use is limited by its strong hepatotoxicity, served as a suitable monomer and led to the discovery of several homobivalent compounds with nanomolar and even subnanomolar activities.[9,10] Recently, on the basis of a molecular modeling approach, suitable modification of the spacer unit led to the discovery of tacrine-based heterobivalent inhibitors with nanomolar or subnanomolar activities and high BChE selectivities.[11,12] This rational approach makes use of the presence of additional binding sites present in BChE.

Chart 1. AChE Inhibitory Drug Tacrine and BChE Inhibitory Quinazolinimines **2** and **3**



1, tacrine

2: IC_{50} (AChE) =22.1 μM
IC_{50} (BChE) = 2.0 μM

3: e.g. n = 1, m = 4:
IC_{50} (AChE) = >500 μM
IC_{50} (BChE) = 1.1 μM

In the search for novel compounds and templates with ChE inhibiting properties, 5,6-dihydro-8*H*-isoquino[1,2-*b*]quinazolin-8-imine (**2**, Chart 1) was developed from the alkaloids dehydroevodiamine and deoxyvasicine and identified as a micromolar ChE inhibitor with >10-fold selectivity toward BChE.[13] SAR studies were performed, and quinazolinimines **3** were identified as inhibitors with micro- and submicromolar inhibitory activities. By variation of the distance of the phenyl ring to the heterocycle and especially by the change of the size of the alicycle, it was possible to obtain either activities at both enzymes or highly selective BChE inhibitors; BChE selectivity increased with increasing size of the alicycle, through increasing activity toward BChE and/or decreasing activity at AChE.[14] Selectivity was most probably reached by the fact that BChE possesses a larger void at the active site gorge compared to AChE.[15] This assumption is supported by the fact that kinetic measurements revealed competitive and reversible inhibition of these compounds at the active site.[14]

To further improve activities of quinazolinimines toward cholinesterases, the bivalent approach was applied to this novel class of ChE inhibitors. The aim was to obtain more potent inhibitors with additional strong activity at BChE or even BChE-selective compounds. Because of the fact that the quinazolinimines bind at the active site like tacrine, similar spacer lengths of the most potent tacrine dimers (a heptamethylene spacer exhibits the highest activities) were applied.[10,16]

The homobivalent inhibitors could be synthesized in a straightforward four-step synthetic pathway described in Scheme 1. Lactams with varying ring sizes (**4a**−**d**) were activated by reaction with Meerwein salt (triethyloxonium tetrafluoroborate) to give their iminium ethers (**5a**−**d**), which readily reacted with anthranilic acid (or 4-chloroanthranilic acid in the case of compound **6a**) to give the respective quinazolinones (**6a**−**e**).[14,17] The quinazolinones were transformed into yellow quinazolinethiones (**7a**−**e**) using Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide).[14,18] The quinazolinethiones were reacted with α,ω-diaminoheptane and -octane, respectively, in the presence of triethylamine and silver nitrate to give homobivalent quinazolinimines **8a**−**j**.

Another possible method to prepare dimers by connecting the two heterocyclic moieties by reacting unsubstituted imines (e.g., five-membered imine **10**, prepared by direct reaction of 2-pyrrolidinone with anthranilonitrile **9**[13,19] or eight-membered imine **11**,[13,19] Scheme 2) with α,ω-dicarbonic acids was not

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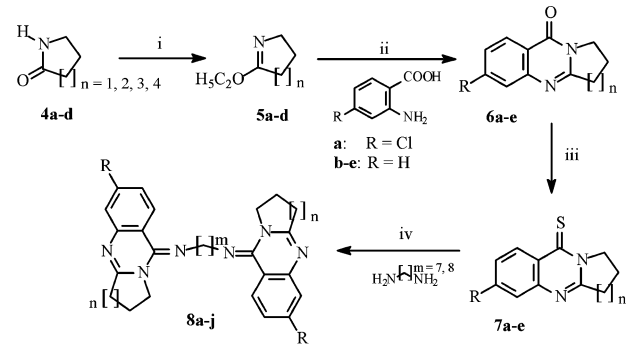
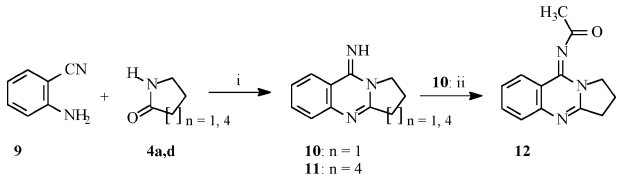
Scheme 1[a]



8a: R = Cl, n = 1, m = 7 **8f**: R = Cl, n = 1, m = 8 **6/7a**: R = Cl, n = 1
8b: R = H, n = 1, m = 7 **8g**: R = H, n = 1, m = 8 **6/7b**: R = H, n = 1
8c: R = H, n = 2, m = 7 **8h**: R = H, n = 2, m = 8 **6/7c**: R = H, n = 2
8d: R = H, n = 3, m = 7 **8i**: R = H, n = 3, m = 8 **6/7d**: R = H, n = 3
8e: R = H, n = 4, m = 7 **8j**: R = H, n = 4, m = 8 **6/7e**: R = H, n = 4

[a] Reagents: (i) (a) $[(C_2H_5)_3O]BF_4$, chloroform, room temp, 15 h; (b) cold NaOH, CH_2Cl_2 extraction; (ii) acetone, 10 °C, 2 h → 60 °C, 4 h; (iii) Lawesson's reagent, toluene, reflux, 12 h; (iv) 0.5 equiv of α,ω-diamine, $AgNO_3$, TEA, toluene, reflux, 3 h.

Scheme 2[a]



[a] Reagents: (i) $POCl_3$, TEA, chloroform, reflux, 24 h; (ii) 2 equiv of acetic anhydride, room temperature, 5 h.

further developed because an acetylated imine (**12**) showed strongly decreased activities probably due to a loss of basicity of the imine-N (Scheme 2, Table 1).

n-Butylquinazolinimines with a five-membered alicycle (**13a**,**b**) were prepared for comparison from excess *n*-butylamine and **7a** and **7b** (Scheme 3)[14] and can be regarded as the exact "monomers" of the symmetrical dimeric **8f** and **8g** (Table 1).

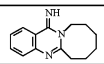
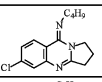
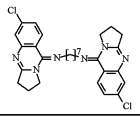
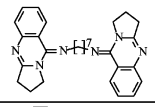
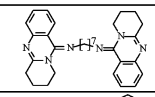
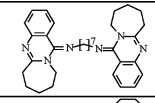
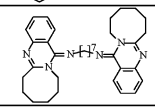
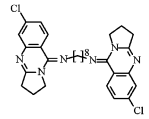
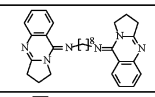
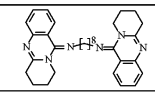
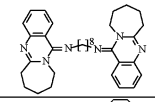
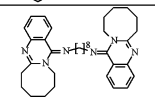
The compounds synthsized were tested for inhibition of AChE (E.C. 3.1.1.7, type VI-S, from Electric Eel) and BChE (E.C. 3.1.1.8, from equine serum), respectively, using the Ellman assay.[13,20] This colorimetric assay is based on in situ generated chromophores formed after enzymatic cleavage of acetyl- and butyrylthiocholine and reaction of the resulting thiocholine with Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid).[20] Selectivity is expressed as the ratio IC_{50}(AChE)/IC_{50}(BChE); higher values therefore reflect higher BChE selectivity. Inhibitory activities are presented in Table 1.

The imine-N-unsubstituted compounds **10** and **11** are micromolar ChE inhibitors; the eight-membered-ring **11** only shows a very moderate BChE selectivity (of about a factor of 5).

This is in strong contrast to the compounds previously described that bear a phenyl ring at the imine-N like **3**, which are highly BChE selective.[14] Therefore, N-substitution with phenyl, benzyl, and phenylethyl groups, respectively, and a concomitant larger alicycle size lead to BChE selectivity. Substitution with a butyl group (**13b**) only leads to a small increase in inhibitory activity without any change in the selectivity profiles. Similar to tacrine, 4-chloro substitution (**13a**) further increases activity at both enzymes by a factor of 2. Acetylation of the five-membered-ring quinazolinimine (**12**) leads to a dramatic decrease in activity by a factor of 10 for AChE and approximately 30 for BChE.

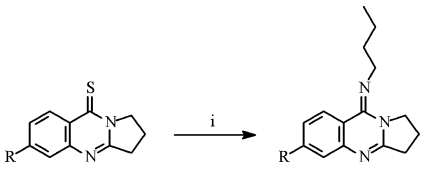
The bivalent inhibitors connected by a heptamethylene spacer (i.e., **8a**−**e**) show a dramatic increase, by a factor of 100, in inhibitory activity at both enzymes compared with the monomeric compounds **13a**,**b** (and also **10**). The bivalent heptamethylene quinazolinimines do not exhibit selectivity toward one of the cholinesterases, a fact that might make them valuable compounds for a potential treatment of AD because of the importance of BChE in the later stages of AD.[4,5] Interestingly, the size of the alicycle does not significantly influence activities or selectivities. Compounds **8a**−**e** show more or less the same inhibition with the highest activity for the seven-membered-ring dimer **8d**, a compound with an IC_{50} of 40 nM at AChE

Table 1. AChE and BChE Inhibition Results[a]

compound	AChE IC_{50}, nM (pIC_{50}±SEM)	BChE IC_{50}, nM (pIC_{50}±SEM)	Selectivity IC_{50}(AChE)/ IC_{50}(BChE)
Galantamine	640 (6.197 ± 0.052)	8400 (5.076 ± 0.033)	0.08
10 	13200 (4.881 ± 0.061)	7200 (6.145 ± 0.031)	1.8
11[19] 	14600 (4.836 ± 0.083)	3000 (5.517 ± 0.038)	4.9
12 	115000 (3.941 ± 0.044)	204000 (3.369 ± 0.157)	0.6
13a 	3900 (5.406 ± 0.085)	2300 (5.639 ± 0.083)	1.7
13b 	9500 (5.023 ± 0.083)	5500 (5.262 ± 0.049)	1.7
8a 	59 (7.226 ± 0.138)	61 (7.216 ± 0.036)	1.0
8b 	79 (7.104 ± 0.02956)	88 (7.056 ± 0.104)	0.9
8c 	77 (7.114 ± 0.173)	8.5 (8.069 ± 0.101)	9
8d 	40 (7.397 ± 0.079)	24 (7.612 ± 0.079)	1.6
8e 	65 (7.185 ± 0.096)	27 (7.573 ± 0.133)	2.4
8f 	50.2 (7.299 ± 0.029)	45.4 (7.343 ± 0.105)	1.1
8g 	58 (7.236 ± 0.054)	4.8 (8.318 ± 0.170)	12
8h 	37 (7.433 ± 0.176)	7.6 (8.121 ± 0.017)	4.9
8i 	1277 (5.894 ± 0.132)	13 (7.888 ± 0.110)	98
8j 	14400 (4.842 ± 0.059)	76 (7.120 ± 0.082)	189

[a] Values are the mean of at least three independent determinations.

Scheme 3[a]



7a: R = Cl, n = 1
7b: R = H, n = 1

13a: R = Cl, n = 1
13b: R = H, n = 1

[a] Reagents: (i) 3 equiv of *n*-butylamine, $AgNO_3$, TEA, toluene, reflux, 2 h.

and 24 nM at BChE. Therefore, **8d** is 16 times more potent at AChE than the drug galantamine and 350 times more potent at BChE. In contrast to the N-butylimines, 4-chloro substitution only leads to a small increase (1.5-fold) in activity.

In contrast to the bis-tacrine compounds,[10,16] the octamethylene-bridged quinazolinimines showed even higher activities than the heptamethylene-bridged compounds. Apart from the chloro-substituted dimer **8f**, all of the octamethylene dimers showed BChE selectivity (from 5-fold to almost 200-fold). This is on one hand due to a higher activity at BChE, which is highest for the smaller ring sizes, especially the compounds with five- and six-membered alicycles, i.e., **8g** and **8h**, which reach low-nanomolar activities. On the other hand, activity at AChE decreases with increasing size of the alicyclus; for the seven-membered-ring dimer **8i** 100-fold selectivity is reached, and for the eight-membered-ring dimer **8j** selectivity is 190-fold. But because of the high nanomolar activities, **8i** is still almost as potent as galantamine at AChE, whereas **8i** is 650 times more potent at BChE than galantamine. The chloro-substituted **8f** is as potent as the unsubstituted **8g** at AChE, whereas activity at BChE is 10-fold lower.

The structure−activity relationships between tacrine and quinazolinimines differ in two major points: chloro substitution of the bivalent **8** does not (significantly) improve activity, and optimal distance in terms of activity and selectivity toward BChE is achieved by eight and not seven methylene groups, as for bis-tacrines.[10,16]

In summary, the application of the bivalent ligand approach to quinazolinimines as a novel class of cholinesterase inhibitors proved highly successful. Inhibitory activities increased by a factor of 100 for the heptamethylene-bridged compounds and by a factor of 1000 for octamethylene-bridged inhibitors (at BChE). Heptamethylene-bridged compounds are inhibitors exhibiting similar activities at both enzymes with two-digit nanomolar activities, whereas octamethylene-bridged inhibitors exhibit increasing selectivity toward BChE with increasing size of the alicycle, reaching a selectivity of approximately 190 for the eight-membered-ring dimer. Small ring sizes show the lowest selectivity but the highest activity.

All bivalent inhibitors synthesized greatly exceed the activity of the established drug galantamine at BChE and, apart from the most BChE-selective compounds **8i**,**j**, also at AChE.

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Supporting Information Available: Synthetic procedures, analytical characterization for **7a**, **8a**−**j**, **10**−**12**, **13a**,**b**, and pharmacological procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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