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Novel tricyclic quinazolinimines and related tetracyclic nitrogen bridgehead compounds as cholinesterase inhibitors with selectivity towards butyrylcholinesterase

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Abstract—Tetracyclic nitrogen bridgehead compounds, dibenzodiazecines and tricyclic quinazolinimines, in which the size of the alicyclic ring system and the length of the alkyl chain between the quinazolinimine moiety and a phenyl ring connected to the imine nitrogen atom were changed systematically, were synthesized and their ability to inhibit acetyl- and butyrylcholinesterase (AChE/BChE), respectively, was evaluated. Moderate and strong inhibitors of BChE—compared to galanthamine and rivastigmine—were identified, which show mixed affinities or are moderately or highly selective towards BChE, respectively.

1. Introduction

Different forms of dementia, which involve impairment of cognitive functions, like Alzheimer's disease (AD), are characterized by reduced levels of acetylcholine (ACh) in the cortex and hippocampus. In the CNS, the cholinergic system regulates memory and learning processes, in which two enzymes degrade ACh: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). BChE appears in serum, liver, heart and CNS. 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 unchanged or even increased by 20%. Therefore, a large pool of BChE is available in glia neurons and neuritic plaques.[1] It may not be an advantage for a cholinesterase inhibitor (ChEI) to be selective for AChE; on the contrary, a good balance between AChE and BChE may result in a higher efficacy. It is likely that extracellularly diffusing ACh may come into contact with glial BChE and be effectively hydrolysed, as demonstrated by the administration of the mixed AChE/BChE inhibitor rivastigmine in AD patients, because in these patients BChE inhibition correlates significantly with cognitive improvement.[2]

The alkaloids deoxyvasicine **1** and dehydroevodiamine (DHED) **2**, which have been described as inhibitors of AChE (Fig. 1), served as lead structures.[3,4] The antiamnesic properties of dehydroevodiamine **2** can only be partially attributed to ChE inhibition, while other effects also seem to be prominent.[5]

We have recently described chemically related tri- and tetracyclic nitrogen bridgehead compounds, which are moderate or strong inhibitors of AChE and BChE (compounds **3**–**5**, Fig. 2), showing different selectivity profiles:[6] **3** is a non-selective inhibitor with IC_{50} (AChE) of 7.7 μM and IC_{50} (BChE) of 4.4 μM. **4** can be regarded as a benzodehydroevodiamine, in which the benzene ring A substitutes the indole moiety. Compound **4** is a

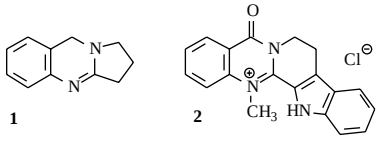

Figure 1. Alkaloids deoxyvasicine **1** and dehydroevodiamine (DHED) **2**.

Keywords: Cholinesterase inhibition; Tricyclic[2,1-*b*]quinazolinimines; Ellman's assay; Butyrylcholinesterase (BChE) selectivity.
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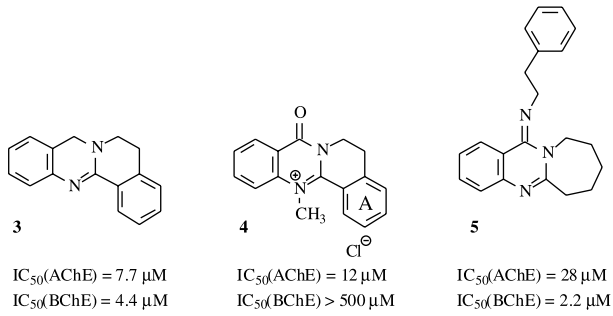


Figure 2. Structures of lead compounds 5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazoline **3**, 13-methyl-8-oxo-5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazolin-13-ium chloride (benzodehydroevodiamine) **4** and *N*-(2-phenylethyl)-*N*-(7,8,9,10-tetrahydroazepino[2,1-*b*]quinazolin-12(6*H*)-ylidene)amine **5**, respectively.

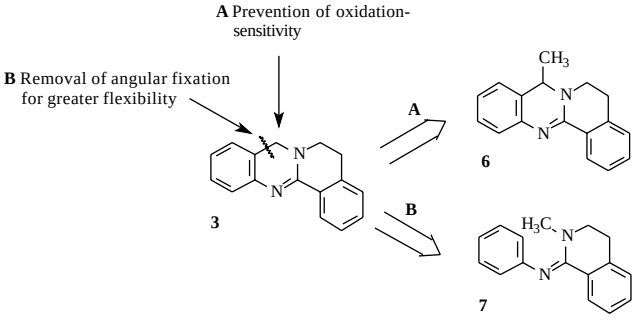


Figure 3. Structural modifications of quinazoline **3** to prevent its oxidation-sensitivity (A) and to introduce greater flexibility into the molecule (B).

selective AChE inhibitor with an IC_{50} (AChE) of 12 μM. Whereas **5** shows a 10-fold selectivity towards an BChE with IC_{50} (AChE) of 23 μM and IC_{50} (BChE) of 2.2 μM, respectively (Fig. 2).[6]

Different aims were pursued in our work: first, concerning the dihydroquinazolines deoxyvasicine **1** and compound **3**, a drawback of these substances lies in the fact that deoxyvasicine oxidizes to its corresponding quinazolinone.[3] This is also possible with compound **3**, although far less pronounced.[6] By introducing an alkyl group in position 8 of compound **3**, a new group of alkyl substituted dihydroquinazolines is produced. Using a methyl group might open a possibility to yield a compound without oxidation sensitivity, but with a similar inhibitory profile (Fig. 3).

A formal cleavage of the C–C bond in positions 8 and 8a should result in a more flexible structure, which might lead to a higher affinity or a better selectivity at the cholinesterases (Fig. 3).

Like dehydroevodiamine, benzodehydroevodiamine **4** shows a pH-dependent equilibrium between the cationic structure **4** and a non-charged dicarbonyl form, in which the C=N bond is hydrolysed at higher pH values.[6,7] Our second aim was—in order to isolate the pharmacophoric parts of benzodehydroevodiamine **4** and to find correlations between the structures of ChE inhibiting compounds of **3** and **4**—to synthesize a compound with a tertiary instead of a quaternary nitrogen (i.e., benzoevodiamine): a structure combining features of compounds **3** and **4**, but without the ability to hydrolyse reversibly at higher pH values. Removal of the carbonyl group is an additional modification step yielding a compound with a second basic nitrogen atom like in compound **3**. And finally—by a removal of the central C–N bond—a diazecine, that is, a less rigidized structure with two basic nitrogen atoms, should be synthesized and its interaction with the ChEs examined (Fig. 4).

The third aim of our work used the moderate to good affinities of the quinazolinimine **5** at the ChEs and its 10-fold selectivity towards BChE as a starting point (Fig. 5).[6] Since BChE possesses a larger void at the active site gorge,[8] either changing the size of the alicyclic ring or changing the distance between the quinazolinimine moiety and the phenyl group might result in an improved selectivity towards BChE. By investigating these two effects systematically, we wanted to find the optimum of these two parameters with respect to both affinity and selectivity.

By measuring the enzyme kinetics of the quinazolinimines, we wanted to find out what kind of inhibition these compounds exhibit, and therefore get information, if these compounds bind (exclusively) at the active site.

2. Results and discussion

2.1. Synthesis

5,8-Dihydro-6*H*-isoquino[1,2-*b*]quinazoline **3** can be prepared by reduction of the corresponding quinazolinone under Clemmensen conditions.[6,9] In order to introduce a methyl group in position 8 a different synthetic strategy had to be applied: *o*-aminoacetophenone was reduced by sodium borohydride to 1-(2-aminophenyl)ethanol,[10] which in turn reacted after transformation into the sulfinylamino compound with hydrogen chloride to 2-(1-chloroethyl)benzenaminium chloride (a

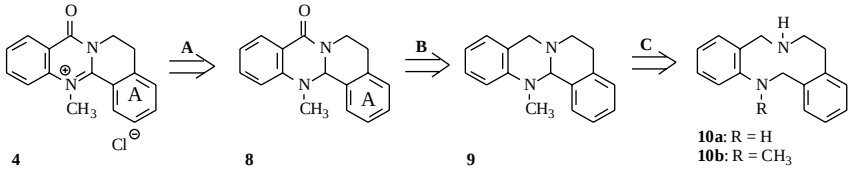


Figure 4. Structural analogues of benzodehydroevodiamine **4** for identification of the pharmacophoric parts of the molecule, that is, removal of the C=N double bond (A), removal of the carbonyl group (B) and ring-opening yielding diazecines (C).

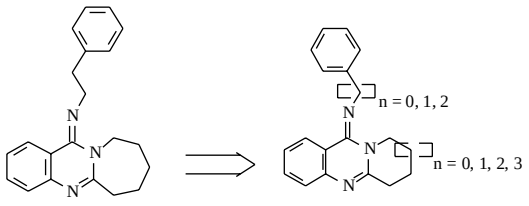


Figure 5. Structural analogues of quinazolinimine **5** concerning the ring size of the alicyclic ring system and the length of the alkylene chain connecting phenyl group and imine-*N*.

synthetic method previously applied for the preparation of α-phenylneopentyl chlorides).[11] This compound reacted with 3,4-dihydro-1(2*H*)-isoquinolinone,[12] which was activated with $POCl_3$, to compound **6** (Scheme 1).

The ring-opened compound **7** (Fig. 3) was synthesized from aniline and 2-methyl-3,4-dihydro-1(2*H*)-isoquinolinethione in the presence of mercury(II) bromide under identical conditions as described for the preparation of quinazolinimines. The quinazolinethione could be prepared from 2-methyl-3,4-dihydro-1(2*H*)-isoquinolinone using Lawesson's reagent, the previous compound prepared through cyclisation of the corresponding carbamate using a mixture of P_4O_{10} and $POCl_3$.[12]

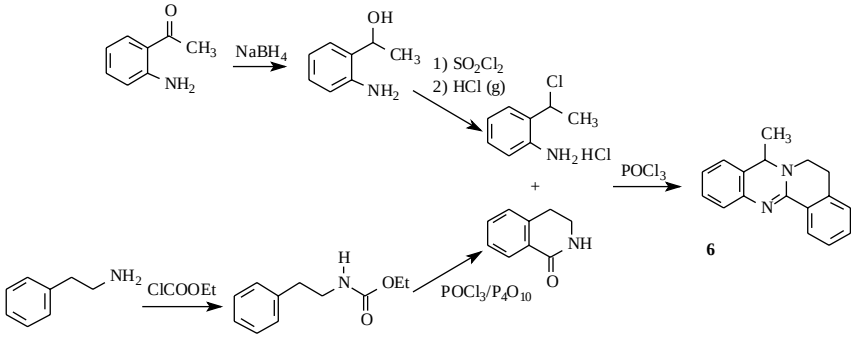
Despite the interesting pharmacological properties of this compound (see below), it proved to be fairly unstable due to amidine hydrolysis yielding the benzolactam (2-methyl-3,4-dihydro-1(2*H*)-isoquinolinone). Therefore, this class of ring-opened compounds was not further investigated.

Compound **9** was prepared by lithium aluminium hydride reduction of compound **8** (benzoevodiamine), which could be synthesized by the reaction of *N*-methylisatoic anhydride (1-methyl-2*H*-3,1-benzoxazine-2,4(1*H*)-dione) with 3,4-dihydroisoquinoline (Scheme 2).[13] For the synthesis of 3,4-dihydroisoquinoline different methods are described in the literature, oxidation of 1,2,3,4-tetrahydroisoquinoline by sulfur in dioxane turning out to be very facile (Scheme 2).[14]

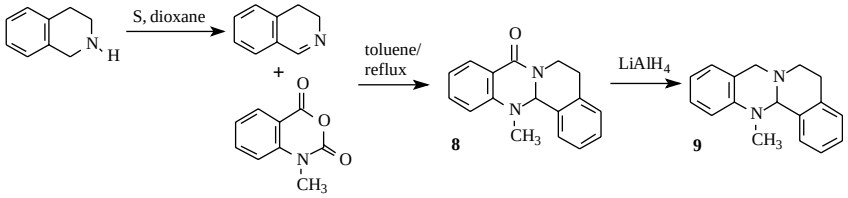
Both 14-methyl-5,6,7,8,13,14-hexahydrodibenzo[*b*,*h*][1,5]diazecine **10b** and 5,6,7,8,13,14-hexahydrodibenzo[*b*,*h*][1,5]diazecine **10a** could be prepared by reduction of compound **4** and the demethylated compound 5,6-dihydro-8*H*-isoquino[1,2-*b*]quinazoline-8-one, respectively, with borane (Scheme 3).[15] The syntheses of compound **4** and of 5,6-dihydro-8*H*-isoquino[1,2-*b*]quinazoline-8-one, respectively, have been described recently.[6,16] Interestingly, compound **10a** is also formed by methylation of compound **10b** using ethyl chloroformate and subsequent reduction of the carbamate by lithium aluminium hydride. No dimethylated compound is formed. Identical MS and NMR data proved methylation at the aromatic nitrogen atom.

The quinazolinimines were prepared according to a modified procedure for the synthesis of compound **5**, which has been described recently by us.[6] The quinazolinones were obtained by reaction of anthranilic acid with iminium ethers of lactams of different ring sizes, which were prepared by the reaction of lactams with Meerwein salt (triethyloxonium tetrafluoroborate).[17]

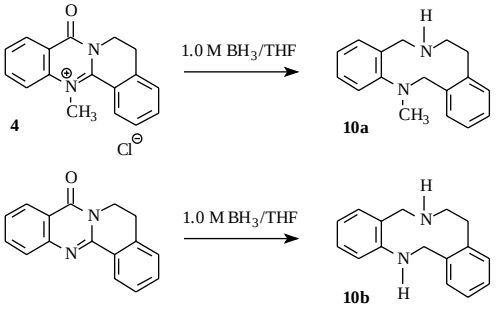
The quinazolinones were transformed with Lawesson's reagent into quinazolinethiones (**11a**–**d**).[6,9] They in turn reacted in the presence of mercury(II) bromide or silver



Scheme 1. Synthesis of 8-methyl-5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazoline **6**.



Scheme 2. Synthesis of 13-methyl-5,6,13,13a-tetrahydro-8*H*-isoquino[1,2-*b*]quinazolin-8-one **8** (benzoevodiamine) and 13-methyl-5,8,13,13a-tetrahydro-6*H*-isoquino[1,2-*b*]quinazoline **9**.



Scheme 3. Synthesis of 14-methyl-5,6,7,8,13,14-hexahydrodibenzo [*b*, *h*][1,5]diazecine **10a** and 5,6,7,8,13,14-hexahydrodibenzo [*b*,*h*][1,5] diazecine **10b**.

nitrate with aniline, benzylamine and 2-phenylethylamine, respectively, to quinazolinimines **12a–l** (Scheme 4). The use of the above-mentioned salts proved to be superior to the previously described use of mercury(II) acetate, because product purification was simplified (the use of $Hg(OAc)_2$ leads to the formation of acetamides as by-products).[6]

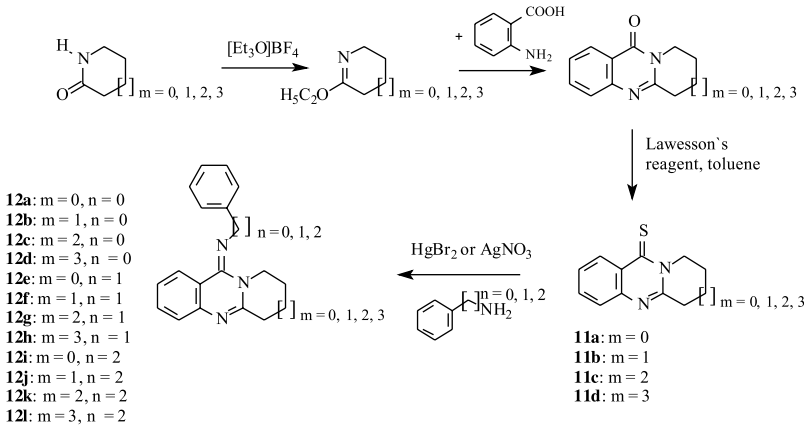
2.2. Pharmacology

Comparing compounds **3** (a quinazoline) and **6** (a quinazoline with a methyl group in position 8), it turned out that the affinities did not significantly change, but a small increase towards BChE occurred (Table 1). The methyl group did not disturb binding to the cholinesterases. But in contrast to compound **3** and the alkaloid deoxyvasicine **1**,[3] respectively, compound **6** is more stable towards air-oxygen, but its stability with respect to oxidation has to be investigated separately.

An interesting change in the selectivity profile occurred with the ring-opened compound **7**. Affinity towards BChE slightly increased, but its affinity towards AChE decreased of a factor of 15. Since amidine **7** is labile towards hydrolysis at higher pH values, the IC_{50} values given in Table 1 might be determined under partial hydrolysis of **7** and are therefore to be considered for estimation only. This property of compound **7** limits its application.

Concerning compound **8** (benzoevodiamine), it turned out that it neither inhibits AChE nor BChE at 10^{-5} M; therefore, the quaternary nitrogen atom of compound **4** (and the ability of this compound to form a dicarbonyl form) seems to be essential for its AChE inhibiting properties. Compound **9**, which—in contrast to compound **8**—lacks the carbonyl group and therefore possesses two basic tertiary nitrogen atoms in the form of a nitrogen bridgehead compound is a moderate inhibitor of BChE, but shows only very weak affinity towards AChE. An interesting observation can be made regarding compounds **10a** and **b**, respectively. Whereas the tricyclic diazecine **10a** shows only moderate affinity towards both ChEs (roughly 50% inhibition at 10^{-4} M), the diazecine **10b** with two secondary nitrogen atoms is a moderate inhibitor at both enzymes with a threefold higher affinity at BChE. This means that the secondary nitrogen atom in vicinity of the benzene ring increases affinities.

The pharmacological profiles of the quinazolinimines **12a–l** are of special interest (Table 1). All of these compounds show micromolar or higher affinities to BChE with at least a 10-fold lower affinity towards AChE. Some of these compounds (i.e., **12c**, **d** and **h**) show more than 200-fold selectivity towards BChE. Two general trends can be observed: on the one hand, an increasing ring size lowers the affinity towards AChE, whereas the BChE affinity stays the same or—especially in the case of anilino-derivatives—is even increased. These properties could be expected, because BChE possesses a larger void at the active site gorge.[8] An increasing distance of the phenyl ring from the quinazolinimine-heterocycle generally increased affinity towards AChE. In a less pronounced manner, this also applies for BChE: the six-membered ring quinazolinimine phenylethyl-compound **12j** possesses a more than 100-fold higher affinity as that of the respective anilino-compound **12b**, whereas the eight-membered ring quinazolinimine phenylethyl-compound **12l** possesses only a threefold higher affinity than the respective anilino-compound **12d**. Taken together, the best selectivity for BChE was reached for compound **12h**, a seven-membered quinazolinimine with a benzyl-group. The most active com-



Scheme 4. Synthesis of quinazolinimines **12a–l**.

Table 1. IC_{50} values ($pIC_{50} = -\lg IC_{50}$ in brackets) of test compounds for AChE and BChE inhibition, respectively, and resulting selectivities expressed as the ratio of IC_{50} values

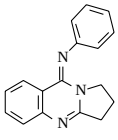
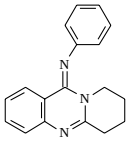
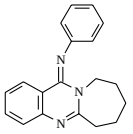
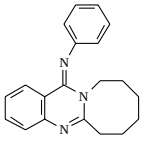
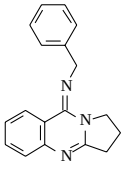
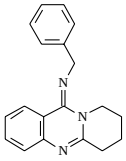
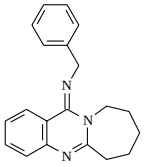
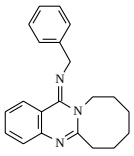
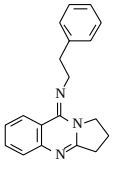
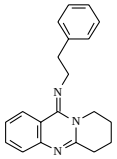
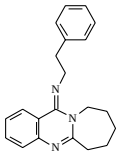
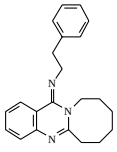
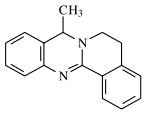
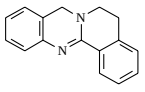
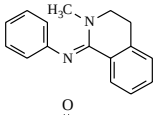
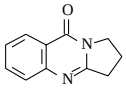
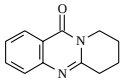
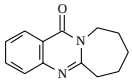
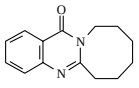
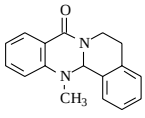
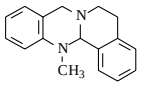
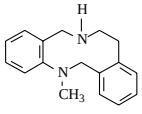
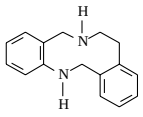
Test compound	AchE inhibition, μM [IC_{50} (pIC_{50} ± SEM)]	BChE inhibition, μM [IC_{50} (pIC_{50} ± SEM)]	Selectivity [IC_{50} (BchE)/IC_{50} (AChE)]
Rivastigmine	>500[a,b] (lit.:[1] 48)	3.9[a] (lit.:[1] 54) (5.409 ± 0.050)	<0.008
Galanthamine	0.64 (lit.:[1] 0.8) (6.197 ± 0.052)	8.4 (lit.:[1] 7.3) (5.076 ± 0.034)	13.1
 **12a**	>500	19.5 (4.709 ± 0.206)	<0.039
 **12b**	98.6 (4.006 ± 0.823)	22.3 (4.651 ± 0.142)	0.226
 **12c**	>500	4.1 (5.392 ± 0.160)	<0.008
 **12d**	>500	2.3 (5.630 ± 0.173)	<0.005
 **12e**	13.9 (4.856 ± 0.063)	1.87 (5.728 ± 0.028)	0.135
 **12f**	25.5 (4.594 ± 0.112)	1.4 (5.844 ± 0.088)	0.055
 **12g**	86.6[c] (4.063)	1.5 (5.831 ± 0.089)	0.017
 **12h**	>500	1.1 (5.942 ± 0.165)	<0.002
 **12i**	7.3 (5.139 ± 0.065)	0.62 (6.210 ± 0.034)	0.085

Table 1 (*continued*)

Test compound	AchE inhibition, μM [IC_{50} (pIC_{50} ± SEM)]	BChE inhibition, μM [IC_{50} (pIC_{50} ± SEM)]	Selectivity [IC_{50} (BchE)/IC_{50} (AChE)]
 **12j**	14.9 (4.828 ± 0.315)	0.144 (6.84 ± 0.031)	0.01
 **12k**	27.7[6] (4.558 ± 0.107)	2.2[6] (5.658 ± 0.032)	0.08[6]
 **12l**	11.5 (4.940 ± 0.092)	0.62 (6.207 ± 0.048)	0.05
 **6**	8.5 (5.069 ± 0.092)	2.9 (5.53 ± 0.039)	0.34
 **3**	7.7[6] (5.11 ± 0.050)	4.4[6] (5.35 ± 0.044)	0.57[6]
 HCl **7**	118.6[i] (3.926 ± 0.263)	2.0[i] (5.696 ± 0.057)	0.017
	82.5 (4.084 ± 0.066)	25.1 (4.600 ± 0.058)	0.30
	38.6 (4.414 ± 0.213)	>500	>12.95
	279[d] (3.554)	>500	>1.79
	430[e] (3.366)	>500	>1.16
 **8**	>500[f]	>500[f]	
 **9**	>500[e]	11.9 (4.924 ± 0.084)	<0.024
 **10a**	>500[g]	>500[h]	

(*continued on next page*)

Table 1 (*continued*)

Test compound	AchE inhibition, μM [IC_{50} (pIC_{50} ± SEM)]	BChE inhibition, μM [IC_{50} (pIC_{50} ± SEM)]	Selectivity [IC_{50} (BchE)/IC_{50} (AChE)]
 **10b**	50.1 (4.300 ± 0.86)	15.6 (4.806 ± 0.219)	0.311

[a] IC_{50} values are time-dependent.[18]
[b] 10^{-3} M: 55% inhibition.
[c] 10^{-5} M: 28% inhibition.
[d] 10^{-4} M: 55% inhibition.
[e] 10^{-4} M: 50% inhibition.
[f] No inhibition at 10^{-5} M.
[g] $10^{-4.3}$ M: 26% inhibition.
[h] $10^{-4.3}$ M: 33% inhibition.
[i] Some hydrolysis might take place under assay conditions (values for affinity estimation only).

pound is **12j** with an IC_{50} (BChE) = 0.14 μM and a selectivity of 1/100 towards BChE.

The quinazolinimines show some lability towards hydrolysis, by which the respective quinazolinones are formed. For this reason also the quinazolinones were tested for their ability to inhibit the cholinesterases, in order to prove, that the effects measured are solely due to the quinazilinimines. The six-, seven- and eight-membered quinazolinones show no activity to BChE at a concentration of 10^{-5} M, but are weak inhibitors of AChE. The six- and five-membered compounds show IC_{50} values of 39 and 83 μM, respectively. Interestingly, the five-membered quinazolinone is also a moderate inhibitor of BChE (IC_{50} = 25 μM), but by far not in the range of the respective quinazolinimines.

Compound **12l** was selected for kinetic measurements, because it shows high affinities towards both cholinesterases and chemically represents the quinazolinimines synthesized and tested. The mechanism of inhibition was analyzed by recording substrate–velocity curves in the absence and in the presence of different concentrations of **12l**. Substrate concentration was varied between 25 and 450 μM. For BChE 0.1, 0.2, 0.8 and 1.0 μM concentrations, respectively, of compound **12l** were applied, for AChE 10 and 15 μM of **12l** were used. Fig. 6 shows the substrate–velocity curves for BChE and the resulting Lineweaver–Burk plots, that is, reciprocal rates versus reciprocal substrate concentrations for the different inhibitor concentrations. K_m values (i.e., the negative reciprocal of the X intercept) differ, but in contrast the V_{max} value, (i.e., the reciprocal of the Y intercept) is not changed with different inhibitor concentrations. Similar results were obtained for AChE (Fig. 7). Fewer plots were recorded for AChE, because at higher concentrations **12l** is not sufficiently soluble anymore. The Lineweaver–Burk plot for BChE clearly shows reversible and competitive inhibition by the quinazolinimine, meaning that these compounds compete for the same active site as the substrate butyrylcholine. For AChE the results are not that unequivocal due to the limited concentrations of inhibitor applicable, but seem to show the same type of inhibition.

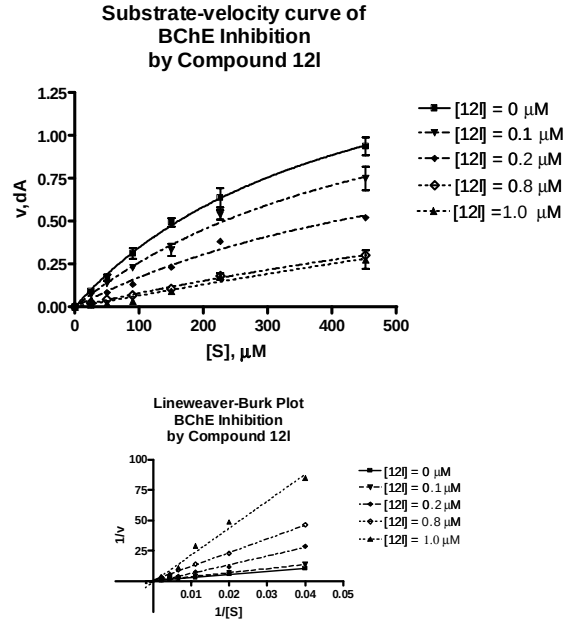


Figure 6. Substrate–velocity curve of BChE activity with different substrate concentrations (25–450 μM) in the absence and presence of 0.1, 0.2, 0.8 and 1.0 μM compound **12l** and resulting Lineweaver–Burk plots.

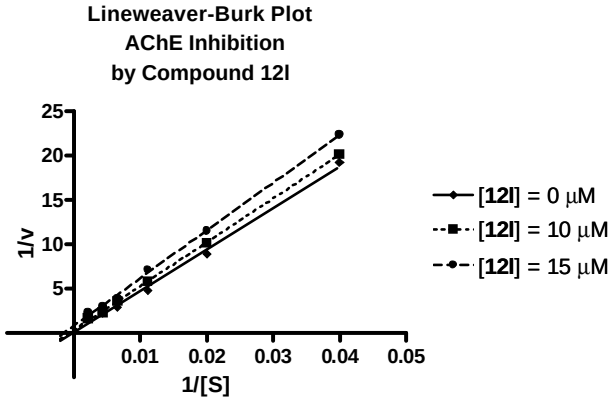


Figure 7. Lineweaver–Burk plots of AChE activity over a range of substrate concentrations (25–450 μM) in the absence and in the presence of 10 and 15 μM **12l**, respectively.

3. Conclusion

Concerning the different aims of our project, several conclusions can be drawn: first, by introducing a methyl group in position 8 of the quinazoline **3** it was possible to synthesize a compound (**6**) with almost the same affinities as the parent compound, but with a less pronounced sensitivity for oxidation to the quinazolinone.

Second, a ring-opened amidine **7** structurally related to compound **3** was synthesized, which shows 60-fold selectivity towards BChE with higher affinities at BChE than the established drugs galanthamine and rivastigmine, respectively. But this compound turned out to be labile to hydrolysis, therefore this class of compounds has not been further investigated.

Third, in order to find out pharmacophoric similarities between the AChE inhibiting compound **4** and the unselective inhibitor **3**, different compounds were synthesized and evaluated pharmacologically, that contain structural features of both compounds: compound **8** containing the amide group of compound **4** but with a tertiary nitrogen atom does not show any activity at the ChEs, but removal of the carbonyl function creates the moderate BChE inhibitor **9**. Cleavage of the central C–N bond yields the diazecine **10a**, which represents a less constraint compound that is an unselective and only a very weak inhibitor of both ChEs. Interestingly, the demethylated compound **10b** with two hydrogen donating nitrogen atoms again represents a moderate inhibitor with threefold selectivity towards BChE.

Finally, SARs concerning the quinazolinimines **12a**–**l** have been performed, in which the distance between the phenyl ring and the heterocycle was shortened and in which the size of the alicyclic ring was changed systematically, in order to increase affinity and especially to improve selectivity towards BChE, since BChE possesses a larger void at the active site of the enzyme. This strategy was highly successful, because compounds with both micromolar and submicromolar affinities towards BChE were identified with 100-fold and higher selectivities. The compounds synthesized show similar or higher affinities than galanthamine and especially the mixed inhibitor rivastigmine with highly improved selectivity profiles (rivastigmine is a very slow inhibitor of ChEs, therefore IC_{50} values are highly time-dependent).[18] But also a number of potent mixed inhibitors have been found. Compound **12l** representing the quinazolinimines synthesized was chosen for kinetic measurements. A Lineweaver–Burk plot for BChE revealed a competitive and reversible binding mode for the quinazolinimines.

4. Experimental protocols

4.1. General

Melting points are uncorrected and were measured in open capillary tubes, using a Gallenkamp melting point apparatus. ^{1}H NMR spectral data were obtained from a Bruker Advance 250 spectrometer (250 MHz). Elemental analyses were performed on a Hereaus Vario EL apparatus. TLC was performed on silica gel F254 plates (Merck). For detection iodine vapour or UV light (254 nm), respectively, was used. MS data were determined by GC/MS, using a Hewlett Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific). Silica gel column chromatography utilized silica gel 60 63–200 μm (Baker). UV measurements were performed on a Jasco V-570 UV/vis/NIR spectrophotometer.

4.2. Chemistry

4.2.1. General procedure for the preparation of thiolactams and quinazolinethiones. A solution of 5.0 mmol of the respective lactam or quinazolinone in 50 mL of dried toluene was stirred under reflux with 1.0 g (2.5 mmol) of Lawesson's reagent. After 4 h, the reaction mixture was cooled to rt, the toluene removed under reduced pressure and the residue was column chromatographed using CH_2Cl_2 as eluent.

4.2.1.1. 2-Methyl-3,4-dihydro-1(2*H*)-isoquinolinethione. Yellow crystals. Mp 98 °C (lit.[19] mp 98 °C). EI-MS *m*/*z* 177, 162, 144, 134, 115, 103, 89, 77. Other spectroscopic data are in accordance with the literature data.[19]

2,3-Dihydropyrrolo[2,1-*b*]quinazoline-9(1*H*)-thione (**11a**) was prepared as described above; the spectroscopic data are in accordance with literature data.[20] The preparation and spectroscopic data of 7,8,9,10-tetrahydroazepino[2,1-*b*]quinazoline-12(6*H*)-thione (**11c**) have been described recently.[6]

4.2.1.2. 6,7,8,9-Tetrahydro-11*H*-pyrido[2,1-*b*]quinazoline-11-thione (11b). Yellow crystals. 570 mg (53% yield). Mp 132 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.85–2.06 (4H, m, $CH_2CH_2CH_2CH_2$), 2.99–3.04 (2H, t, J = 6.25 Hz, CH_2C), 4.5–4.55 (2H, t, J = 6.25 Hz, CH_2N), 7.35–7.42 (1H, t, J = 7.5 Hz, C(3)H), 7.5–7.54 (1H, d, J = 7.5 Hz, C(4)H), 7.62–7.69 (1H, t, J = 7.5 Hz, C(2)H), 8.69–8.73 (1H, d, J = 7.5 Hz, C(1)H) ppm. IR (KBr): 3441, 1950, 1584, 1552, 1471, 1376, 1306, 1205, 980, 768 cm^{-1}. EI-MS *m*/*z* 216, 201, 183, 176, 169, 155, 129, 102, 77. Anal. ($C_{12}H_{12}N_2S$): C, H, N, S.

4.2.1.3. 6,7,8,9,10,11-Hexahydro-13*H*-azocino[2,1-*b*]quinazoline-13-thione (11d). Yellow crystals. 785 mg (74% yield). Mp 103 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.25–2.05 (8H, m, $CH_2(CH_2)_4CH_2$), 3.14–3.19 (2H, m, CH_2C), 4.99 (2H, br s, CH_2N), 7.43–7.5 (1H, t, J = 7.5 Hz, C(3)H), 7.6–7.64 (1H, d, J = 7.5 Hz, C(4)H), 7.69–7.76 (1H, t, J = 7.5 Hz, C(2)H), 8.76–8.8 (1H, d, J = 7.5 Hz, C(1)H) ppm. IR (KBr): 3448, 2925, 1581, 1552, 1360, 1181, 1082, 1021, 773 cm^{-1}. EI-MS *m*/*z* 244, 215, 203, 189, 176, 155, 129, 102, 77. Anal. ($C_{14}H_{16}N_2S$): C, H, N, S.

4.2.2. General procedure for the preparation of quinazolinimines. To a solution of 2 mmol of the respective quinazolinethione **11** in 15 mL of toluene were added 6 mmol aniline, benzylamine or 2-phenylethylamine,

respectively. The mixture was heated under reflux under vigorous stirring. To the stirred solution, 1.44 g (4 mmol) of mercury(II) bromide or—if explicitly mentioned in the description of the compound below—680 mg (4 mmol) of silver nitrate was added. Using silver nitrate the suspension darkened immediately. The mixture was heated for 4 h (2 h when using silver nitrate). After cooling, the precipitate was filtered off, the toluene was removed under reduced pressure and the residue was column chromatographed using CH_2Cl_2/ethyl acetate, 2:1 as eluent.

4.2.2.1. *N*-(2,3-Dihydropyrrolo[2,1-*b*]quinazolin-9(1*H*)-ylidene)aniline (12a). Silver nitrate was used for coupling. White powder. 161 mg (31% yield). Mp 164 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.09–2.21 (2H, qui, J = 7.5 Hz, $CH_2CH_2CH_2$), 3.0–3.06 (2H, t, J = 7.5 Hz, CH_2C), 4.03 (2H, br s, CH_2N), 6.78–6.94 (3H, m, 3× CH-phenyl), 6.94–7.0 (1H, t, J = 7.25 Hz, C(2)H) 7.24–7.42 (5H, m, 2× CH-phenyl, C(1,3,4)H) ppm. IR (KBr): 3422, 2968, 1644, 1466, 1403, 1257, 1024, 770, 694, 662 cm^{-1}. EI-MS *m*/*z* 260, 245, 233, 218, 184, 159, 129, 77. Anal. ($C_{17}H_{15}N_3$): C, H, N.

4.2.2.2. *N*-Benzyl-*N*-(2,3-dihydropyrrolo[2,1-*b*]quinazolin-9(1*H*)-ylidene)amine (12e). Silver nitrate was used for coupling. White powder. 259 mg (47% yield). Mp 99 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.03–2.15 (2H, qui, J = 7.5 Hz, $CH_2CH_2CH_2$), 2.93–2.99 (2H, t, J = 7.5 Hz, CH_2C), 4.0–4.05 (2H, t, J = 7.5 Hz, CH_2N), 5.05 (2H, s, CH_2-benzyl), 7.1–7.44 (8H, m, CH-phenyl, C(1,2,3)H), 8.05–8.08 (1H, d, J = 7.5 Hz, C(4)H) ppm. IR (KBr): 3450, 2958, 1635, 1603, 1410, 1287, 1268, 1030, 802, 761, 736, 698, 660 cm^{-1}. EI-MS *m*/*z* 275, 246, 198, 184, 170, 142, 129, 102, 91. Anal. ($C_{18}H_{17}N_3$): C, H, N.

4.2.2.3. *N*-(2,3-Dihydropyrrolo[2,1-*b*]quinazolin-9(1*H*)-ylidene)-*N*-(2-phenylethyl)amine (12i). Silver nitrate was used for coupling. Smeary pale yellow solid. 139 mg (24% yield). Mp 70 °C (continuously melting). ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.02–2.14 (2H, qui, J = 7.5 Hz, $CH_2CH_2CH_2$), 2.92–3.01 (4H, m, CH_2C, CH_2-phenyl), 3.94–4.14 (4H, m, CH_2CH_2-phenyl, CH_2N), 7.12–7.25 (6H, m, C*H*-phenyl, C(7)H), 7.43–7.44 (2H, m, C(8,6)H), 8.0–8.03 (1H, d, J = 8 Hz, C(5)H) ppm. IR (KBr): 3433, 2920, 1638, 1600, 1410, 1290, 1030, 761, 704, 666 cm^{-1}. EI-MS *m*/*z* 289, 259, 198, 183, 169, 142, 102, 91, 77. Anal. ($C_{19}H_{19}N_3$× 1/2EtOAc): C, H, N.

4.2.2.4. *N*-(6,7,8,9-Tetrahydro-11*H*-pyrido[2,1-*b*]quinazolin-11-ylidene)aniline (12b). White powder. 140 mg (25% yield). Mp 113 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.8–1.99 (4H, m, $CH_2CH_2CH_2CH_2$), 2.84–2.89 (2H, t, J = 6.25 Hz, CH_2C), 3.93–3.98 (2H, t, J = 6.25 Hz, CH_2N), 6.71–6.81 (3H, m, 3× CH-phenyl), 6.91–6.98 (1H, t, J = 7.25 Hz, C(2)H) 7.12–7.36 (5H, m, 2× CH-phenyl, C(1,3,4)H) ppm. IR (KBr): 3447, 2941, 1629, 1586, 1482, 1398, 1263, 1100, 769, 700, 666 cm^{-1}. EI-MS *m*/*z* 274, 260, 246, 184, 172, 159, 77. Anal. ($C_{18}H_{17}N_3$): C, H, N.

4.2.2.5. *N*-Benzyl-*N*-(6,7,8,9-tetrahydro-11*H*-pyrido[2,1-*b*]quinazolin-11-ylidene)amine (12f). White powder. 150 mg (26% yield). Mp 96 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.83–2.05 (4H, m, $CH_2CH_2CH_2CH_2$), 2.86–2.91 (2H, t, J = 6.25 Hz, CH_2C), 3.98–4.03 (2H, t, J = 6.25 Hz, CH_2N), 5.13 (2H, s, CH_2-benzyl), 7.22–7.52 (8H, m, CH-phenyl, C(1,2,3)H), 8.03–8.06 (1H, d, J = 7.5 Hz, C(4)H) ppm. IR (KBr): 3459, 2930, 1632, 1584, 1473, 1261, 758, 732, 698, 641 cm^{-1}. EI-MS *m*/*z* 289, 260, 212, 198, 184, 155, 91. Anal. ($C_{19}H_{19}N_3$): C, H, N.

4.2.2.6. *N*-(2-Phenylethyl)-*N*-(6,7,8,9-tetrahydro-11*H*-pyrido[2,1-*b*]quinazolin-11-ylidene)amine (12j). Smeary white solid. 160 mg (26% yield). Mp 52 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.85–1.98 (4H, m, $CH_2CH_2CH_2CH_2$), 2.82–2.87 (2H, t, J = 7.5 Hz, CH_2C), 3.02–3.08 (2H, t, J = 7 Hz, CH_2-phenyl), 3.98–4.03 (2H, t, J = 7 Hz, CH_2CH_2-phenyl), 4.07–4.13 (2H, t, J = 7.5 Hz, CH_2N), 7.15–7.32 (6H, m, CH-phenyl, C(2)H), 7.44–7.48 (2H, m, C(1,3)H), 7.97–8.0 (1H, d, J = 8.5 Hz, C(4)H) ppm. IR (KBr): 3442, 2945, 1629, 1579, 1483, 1402, 1246, 1176, 763, 700, 668 cm^{-1}. EI-MS *m*/*z* 303, 281, 212, 199, 184, 155, 129, 102. Anal. ($C_{20}H_{21}N_3$): C, H, N.

4.2.2.7. *N*-(7,8,9,10-Tetrahydroazepino[2,1-*b*]quinazolin-12(6*H*)-ylidene)aniline (12c). White powder. 220 mg (39% yield). Mp 152 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.85–1.9 (6H, m, $CH_2(CH_2)_3CH_2$), 3.0–3.04 (2H, m, CH_2C), 4.45 (2H, br s, CH_2N), 6.8–7.02 (3H, m, 3× CH-phenyl), 7.04–7.05 (1H, t, J = 7.25 Hz, C(2)H), 7.13–7.43 (5H, m, 2× CH-phenyl, C(1,3,4)H) ppm. IR (KBr): 3421, 2920, 1638, 1587, 1264, 1135, 980, 900, 771, 703 cm^{-1}. EI-MS *m*/*z* 289, 260, 234, 186, 172, 159, 102, 77. Anal. ($C_{19}H_{19}N_3$): C, H, N.

4.2.2.8. *N*-Benzyl-*N*-(7,8,9,10-tetrahydroazepino[2,1-*b*]quinazolin-12(6*H*)-ylidene)amine (12g). White powder. 316 mg (62% yield). Mp 95 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.84–1.9 (6H, m, $CH_2(CH_2)_3CH_2$), 2.93–2.98 (2H, m, CH_2C), 4.38 (2H, br s, CH_2N), 5.1 (2H, s, CH_2-benzyl), 7.2–7.53 (8H, m, CH-phenyl, C(1,2,3)H), 7.96–7.99 (1H, d, J = 7.5 Hz, C(4)H) ppm. IR (KBr): 3503, 2927, 1632, 1584, 1410, 1141, 761, 729, 698, 672, 641 cm^{-1}. EI-MS *m*/*z* 303, 274, 260, 249, 212, 198, 183, 170, 155, 142, 129, 102, 91, 77. Anal. ($C_{20}H_{21}N_3$): C, H, N.

4.2.2.9. *N*-(6,7,8,9,10,11-Hexahydro-13*H*-azocino[2,1-*b*]quinazolin-13-ylidene)aniline (12d). White powder. 320 mg (53% yield). Mp 149 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.2–2.2 (8H, m, $CH_2(CH_2)_4CH_2$), 2.96–3.01 (2H, m, CH_2C), 4.42 (2H, br s, CH_2N), 6.79–6.89 (3H, m, 3× CH-phenyl), 7.04–7.05 (1H, t, J = 7.25 Hz, C(2)H), 7.23–7.49 (5H, m, 2× CH-phenyl, C(1,3,4)H) ppm. IR (KBr): 3448, 2915, 2850, 1649, 1582, 1471, 1408, 1246, 1150, 773, 701, 666 cm^{-1}. EI-MS *m*/*z* 303,

274, 260, 248, 235, 172, 159, 129, 77. Anal. ($C_{20}H_{21}N_3$): C, H, N.

4.2.2.10. *N*-Benzyl-*N*-(6,7,8,9,10,11-hexahydro-13*H*-azocino[2,1-*b*]quinazolin-13-ylidene)amine (12h). Silver nitrate was used for coupling. White powder. 510 mg (81% yield). Mp 105 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.15–1.96 (8H, m, $CH_2(CH_2)_4CH_2$), 2.81–2.86 (2H, m, CH_2C), 4.32 (2H, br s, CH_2N), 5.06 (2H, s, benzyl-CH_2), 7.1–8.0 (8H, m, CH-phenyl, C(1,2,3)H), 8.16–8.2 (1H, d, J = 7.5 Hz, C(4)H) ppm. IR (KBr): 3450, 2917, 2855, 1632, 1576, 1408, 1356, 1238, 1137, 762, 729, 696, 669, 640 cm^{-1}. EI-MS *m*/*z* 317, 288, 276, 262, 249, 226, 183, 155, 129, 102, 91, 77. Anal. ($C_{21}H_{23}N_3$): C, H, N.

4.2.2.11. *N*-(2-Phenylethyl)-*N*-(6,7,8,9,10,11-hexahydro-13*H*-azocino[2,1-*b*]quinazolin-13-ylidene)amine (12l). Smeary white solid. 264 mg (40% yield). Mp 55 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.24–1.98 (8H, m, $CH_2(CH_2)_4CH_2$), 2.85–2.9 (2H, m, CH_2C), 3.01–3.06 (2H, t, J = 7 Hz, CH_2-phenyl), 4.14–4.17 (2H, t, J = 7 Hz, CH_2CH_2-phenyl), 4.27 (2H, br s, CH_2N), 7.15–7.48 (8H, m, CH-phenyl, C(1,2,3)H), 8.01–8.04 (1H, d, J = 7.5 Hz, C(4)H) ppm. IR (KBr): 3442, 2922, 2855, 1629, 1579, 1443, 1358, 1238, 1140, 761, 700, 668, 640 cm^{-1}. EI-MS *m*/*z* 331, 302, 276, 263, 240, 227, 184, 171, 159, 129, 102, 91, 77. Anal. ($C_{22}H_{25}N_3$): C, H, N.

4.2.3. 8-Methyl-5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazoline (6). To a solution of 1 g (7.3 mmol) of 1-(2-aminophenyl)ethanol[10] in 20 mL of toluene were added dropwise 5 mL of thionyl chloride. A solid was formed. The mixture was heated for 24 h under reflux, during which time the solid dissolved again. After cooling, toluene and excess thionyl chloride were removed under reduced pressure. Without further characterization the formed 1-[2-(sulfinylamino)phenyl]ethanol was dissolved in 50 mL of dried diethyl ether and dry HCl was passed for 2 h through the solution.[11] The precipitated 2-(1-chloroethyl)benzenaminium chloride was washed with dry ether and was used without further characterization for the next step.

To a solution of 515 mg (3.5 mmol) of 3,4-dihydroisoquinolin-1(2*H*)-one in 10 mL of boiling toluene 4 mL of phosphoric trichloride were added and the mixture was refluxed. After 30 min, 675 mg (3.5 mmol) of 2-(1-chloroethyl)benzenaminium chloride was added and the mixture was refluxed for additional 4 h, during which time a small second phase separated. To the cooled mixture 15 mL of concentrated hydrochloric acid and a large quantity of hot water were added. After cooling, the solution was extracted twice with diethyl ether. The organic layers were discarded. The aqueous phase with alkalized with ammonia and extracted three times with diethyl ether. The combined organic phases of the second extraction were dried with sodium sulfate, and the solvent removed under reduced pressure. The residue was purified by column chromatography using ethyl acetate/methanol, 1:1 as eluent.

Colourless oil. 120 mg (16% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.35–1.38 (3H, d, J = 8 Hz, CH_3), 2.97–3.06 (2H, m, CH_2C), 3.33–3.44 (1H, m, CH_2CH_2), 3.56–3.71 (1H, m, CH_2CH_2), 4.58–4.67 (1H, q, J = 8 Hz, C*H*), 6.94–7.39 (7H, m, CH-arom), 8.34–8.38 (1H, m, C(1)H) ppm. IR (KBr): 3423, 2923, 1670, 1548, 1475, 769, 739 cm^{-1}. EI-MS *m*/*z* 248 (7), 233 (100), 218 (24), 190, 124, 116 (25), 102, 89, 77. Anal. ($C_{17}H_{16}N_2$): C, H, N.

4.2.4. *N*-(2-Methyl-3,4-dihydroisoquinolin-1(2*H*)-ylidene)aniline (hydrochloride) (7). This compound was prepared analogously to the quinazolinimines (see Section 4.2.2). Equimolar amounts of 2-methyl-3,4-dihydro-1(2*H*)-isoquinolinethione (6 mmol, for preparation, see Section 4.2.1) and mercury(II) acetate were used. Directly after preparation of the amidine and its purification by column chromatography (EtOAc/MeOH, 1:1) 6 M MeOH·HCl were added to form the corresponding hydrochloride.

Colourless oil. 324 mg (15% yield). ^{1}H NMR (hydrochloride; 250 MHz, DMSO-d_6): δ 3.10–3.14 (2H, t, J = 6.25 Hz, CH_2C), 3.14 (3H, s, CH_3), 3.76–3.81 (2H, t, J = 6.25 Hz, CH_2N), 7.2–7.85 (9H, m, CH-arom), 11.06 (1H, br s, NH) ppm. IR (hydrochloride; KBr): 3395, 3050, 2898, 2594, 1625, 1444, 1256, 1074, 765, 692, 479 cm^{-1}. EI-MS *m*/*z* 235, 219, 192, 165, 145, 132, 116, 89, 77. Anal. (free base: $C_{16}H_{16}N_2$): C, H, N.

4.2.5. 13-Methyl-5,6,13,13a-tetrahydro-8*H*-isoquino[1,2-*b*]quinazolin-8-one (benzoevodiamine, 8). A solution of 3 g (22.9 mmol) of *N*-methylisatoic anhydride (1-methyl-2*H*-3,1-benzoxazine-2,4(1*H*)-dione) and 4.45 g (25.2 mmol) of 3,4-dihydroisoquinoline[14] in 50 mL toluene was refluxed for 24 h. After cooling, the solvent was removed under reduced pressure and the residue was column chromatographed using *n*-hexane/ethyl acetate, 2:1 as eluent.

Light yellow crystals. 2.72 g (45% yield). Mp 138 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.85–2.87 (1H, m, CH_2C), 2.96 (3H, s, CH_3), 2.95–3.06 (1H, m, CH_2C), 3.24–3.32 (1H, m, CH_2N), 4.37–4.45 (1H, m, CH_2N), 5.91 (1H, s, CH), 6.85–6.88 (1H, t, J = 6.75 Hz, C(10)*H*), 6.97–7.0 (1H, d, J = 8 Hz, C(12)*H*), 7.14–7.23 (4H, m, C(1,2,3,4)*H*), 7.4–7.43 (1H, t, J = 6 Hz, C(11)*H*), 7.67–7.7 (1H, d, J = 6 Hz, C(9)*H*) ppm. IR (KBr): 3462, 2863, 1651, 1606, 1416, 1401, 1299, 1283, 1169, 1141, 929, 783 cm^{-1}. EI-MS *m*/*z* 263, 235, 219, 190, 160, 146, 133, 105, 92, 77. Anal. ($C_{17}H_{16}N_2O$): C, H, N.

4.2.6. 13-Methyl-5,8,13,13a-tetrahydro-6*H*-isoquino[1,2-*b*]quinazoline (9). To a suspension of 200 mg (5.3 mmol) of lithium aluminium hydride in 20 mL of dried THF was slowly added a solution of 1 g (3.8 mmol) of 13-methyl-5,6,13,13a-tetrahydro-8*H*-isoquino[1,2-*b*]quinazolin-8-one (**8**) in 40 mL of dried THF. After completion of addition, the mixture was refluxed for 3 h and afterwards stirring was continued at rt overnight. Excess hydride was destroyed by a careful addition of diluted sodium hydroxide solution. The organic phase was separated and the aqueous phase was extracted twice with diethyl ether. The combined organic phas-

es were dried over sodium sulfate, and the solvents were removed under reduced pressure. The residue was column chromatographed using methanol/ethyl acetate, 1:1 as eluent.

Colourless oil. 660 mg (72% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.64 (3H, s, CH_3), 2.73–2.93 (2H, m, CH_2CH_2N), 3.06–3.35 (2H, m, CH_2CH_2N), 3.89–3.96 (1H, d, J = 15.5 Hz, $NCH_2C_{aromat.}$), 4.08–4.14 (1H, d, J = 15.5 Hz, $NCH_2C_{aromat.}$), 4.97 (1H, s, CH), 6.83–7.06 (3H, m, C(9, 10, 12)*H*), 7.15–7.3 (4H, m, C(2, 3, 4, 11)*H*), 7.38–7.42 (1H, m, C(1)*H*) ppm. IR (KBr): 3399, 2918, 2802, 1606, 1492, 1369, 1118, 935, 746 cm^{-1}. EI-MS *m*/*z* 250, 235, 220, 145, 132, 120, 103, 91, 77. Anal. ($C_{17}H_{18}N_2$): C, H, N.

4.2.7. 14-Methyl-5,6,7,8,13,14-hexahydrodibenzo[*b*,*h*]-[1,5]diazecine (10a). To a suspension of 200 mg (0.67 mmol) of 13-methyl-8-oxo-5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazolin-13-ium chloride[6] in 10 mL of dried THF was added 6.4 mL of 1.0 M borane solution in THF under N_2-atmosphere. The solid got into solution under evolution of hydrogen while stirring 2 h at rt. The solution was refluxed for additional 3 h. The mixture was cooled to rt and 5 mL of water was carefully added drop-by-drop to the stirred mixture. After the addition of 2 mL of 50% sodium hydroxide solution, stirring was continued for 1 h at rt. The mixture was extracted three times with 30 mL of dichloromethane. The combined extracts were dried over sodium sulfate and were evaporated under reduced pressure to give the crude product, which was purified by column chromatography using *n*-hexane/ethyl acetate, 1/1 as eluent.

White crystals. 90 mg (54% yield). Mp 90 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.7–2.74 (2H, t, J = 5 Hz, CH_2CH_2N), 2.81 (3H, s, CH_3), 2.86–2.91 (2H, t, J = 5 Hz, CH_2CH_2N), 3.63 (2H, s, CH_2NCH_3), 3.69 (2H, s, CH_2NH), 6.12 (1H, br s, NH), 6.63–6.72 (2H, m, C(9, 12)*H*), 7.02–7.17 (5H, m, C(1, 2, 3, 4, 10)*H*), 7.24–7.3 (1H, t, J = 7.5 Hz, C(11)*H*) ppm. IR (KBr): 3465, 2914, 1606, 1508, 1448, 1274, 1081, 752 cm^{-1}. EI-MS *m*/*z* 252, 220, 193, 147, 132, 120, 91, 77. Anal. ($C_{17}H_{20}N_2O$): C, H, N.

4.2.8. 5,6,7,8,13,14-Hexahydrodibenzo[*b*,*h*][1,5]diazecine (10b). This compound was prepared in the same manner as described for compound **10a**. As starting material 248 mg (1 mmol) of 5,6-dihydro-8*H*-isoquino[1,2-*b*]quinazolin-8-one[6,16] and 10 mL of 1.0 M borane solution in THF were used.

Ethyl acetate/*n*-hexane, 2:1 was used as eluent.

Colourless oil. 76 mg (32% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.72–2.77 (2H, t, J = 6.5 Hz, CH_2CH_2N), 2.88–2.93 (2H, t, J = 6.5 Hz, CH_2CH_2N), 3.62 (2H, s, $C_{arom.}CH_2NHC_{arom.}$), 3.71 (2H, s, $C_{arom.}CH_2NHC_{aliphat.}$), 4.7 (2H, br s, 2× NH), 6.65–6.77 (2H, m, C(9, 12)*H*), 7.0–7.17 (6H, m, $CH_{arom.}$) ppm. IR (KBr): 3428, 2803, 1615, 1495, 1460, 1289, 1085, 935, 748 cm^{-1}. EI-MS *m*/*z* 239, 233, 220, 206, 146, 132, 117, 106, 77. Anal. ($C_{16}H_{18}N_2$): C, H, N.

4.3. Pharmacology

AChE (E.C.3.1.1.7, Type VI-S, from Electric Eel) and BChE (E.C.3.1.1.8, from equine serum) were purchased from Sigma–Aldrich (Steinheim, Germany). 5,5′-Dithiobis(2-nitrobenzoic acid) (Ellman's reagent, DTNB), acetylthiocholine (ATC) and butyrylthiocholine iodides (BTC) were obtained from Fluka (Buchs, Switzerland).

As references for ChE inhibition the established drugs galanthamine and rivastigmine were used. Rivastigmine hydrogentartrate was obtained from Novartis Pharma AG, Basel, Switzerland. Galanthamine hydrobromide was obtained from JANSSEN-CILAG GmbH, Neuss, Germany.

The assay was performed as described in the following procedure:[21] a stock solution of the test compound was prepared in ethanol, and the highest concentration of the test compounds applied in the assay was 10^{-4} M (final concentration). In order to obtain an inhibition curve, at least five different concentrations (normally in the range of 10^{-4} to 10^{-9} M) of the test compound were measured at 25 °C at 412 nm, each concentration in triplicate.

For buffer preparation, 1.36 g of potassium dihydrogen phosphate (10 mmol) was dissolved in 100 mL water and adjusted with NaOH to pH 8.0 ± 0.1. Enzyme solutions were prepared to give 2.5 units/mL in 1.4 mL aliquots. Furthermore, 0.01 M DTNB solution, 0.075 M ATC and BTC solutions, respectively, were used. A cuvette containing 3.0 mL phosphate buffer, 100 μL of the respective enzyme and 100 μL of the test compound solution was allowed to stand for 5 min, then 100 μL of DTNB was added, and the reaction was started by addition of 20 μL of the substrate solution (ATC/BTC). The solution was mixed immediately, and exactly 2.5 min after substrate addition the absorption was measured. For the reference value, 100 μL of water replaced the test compound solution. For determining the blank value, additionally 100 μL water replaced the enzyme solution.

The inhibition curve was obtained by plotting percentage enzyme activity (100% for the reference) versus logarithm of test compound concentration.

For the kinetic measurements the following substrate concentrations were used: 0, 25, 50, 90, 150, 266 and 452 μM. In contrast to the above-described affinity measurements, the reaction time was extended to 4 min before measurement of the absorption. V_{max} and K_m values, respectively, of the Michaelis–Menten kinetics were calculated by non-linear regression out of the substrate velocity curves. Linear regression was used for calculating the Lineweaver–Burk plots.

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References and notes

1. Giacobini, E. In *Cognitive Enhancing Drugs*; Buccafusco, J. J., Ed.; Birkhäuser: Basel–Boston–Berlin, 2004; pp 11–36.
2. Giacobini, E.; Spiegel, R.; Enz, A.; Veroff, A. E.; Cutler, R. E. *J. Neural Transm.* **2002**, *109*, 1053.
3. Tulyaganov, N.; Sadritdinov, F.; Suleimanova, G. A. *Farmakol. i Toksikol.* **1986**, *49*, 37.
4. Park, C. H.; Kim, S.-H.; Choi, W.; Lee, Y.-L.; Kim, J. S.; Kang, S. S.; Suh, Y.-H. *Planta Med.* **1996**, *62*, 405.
5. Park, E. J.; Suh, Y.-H.; Kim, J.-Y.; Choi, S.; Lee, C.-J. *NeuroReport* **2003**, *14*, 399.
6. Decker, M. *Eur. J. Med. Chem.* **2005**, *40*, 305.
7. Pachter, I. J.; Suld, G. *J. Org. Chem.* **1960**, *25*, 1680.
8. Saxena, A.; Redman, A. M. G.; Jiang, X.; Lockridge, O.; Doctor, B. P. *Biochemistry* **1997**, *36*, 14642, and references therein.
9. Jaén, J. C.; Gregor, V. E.; Lee, C.; Davies, R.; Emmerling, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 737.
10. Nieminen, T. E. A.; Hase, T. A. *Tetrahedron Lett.* **1987**, *28*, 4725.
11. Zieger, H. E.; Bright, D. A.; Haubenstock, H. *J. Org. Chem.* **1986**, *51*, 1180.
12. Wang, X.-J.; Tan, J.; Grozinger, K. *Tetrahedron Lett.* **1998**, *39*, 6609.
13. Houlihan, W. J.; Manning, R. E. U.S. Patent 3,530,129, 1970.
14. Takacs, K.; Ajzert, I. K.; Marmarosi, T.; Fleischer, J.; Rimai, J.; Ecseri Puskas, M. W.O. Patent 8800586, 1988.
15. Takeuchi, H.; Matsushita, Y.; Eguchi, S. *J. Org. Chem.* **1991**, *56*, 1535.
16. Kanmacher, I.; Stambach, J. F.; Jung, L. *Heterocycles* **1990**, *31*, 2131.
17. Petersen, S.; Tietze, E. *Liebigs Ann. Chem.* **1959**, *623*, 166.
18. Ogura, H.; Kosasa, T.; Kuriya, Y.; Yamanishi, Y. *Methods Find. Exp. Clin. Pharmacol.* **2000**, *22*, 609.
19. Ajzert, K. I.; Takacs, K. *Liebigs Ann. Chem.* **1987**, *12*, 1061.
20. Jen, T.; Dienel, B.; Dowalo, F.; Van Hoeven, H.; Bender, P.; Loev, B. *J. Med. Chem.* **1973**, *16*, 633.
21. Ellman, G. L.; Coutney, K. D.; Anders, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.