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Laboratory note

Novel inhibitors of acetyl- and butyrylcholinesterase derived from the alkaloids dehydroevodiamine and rutaecarpine

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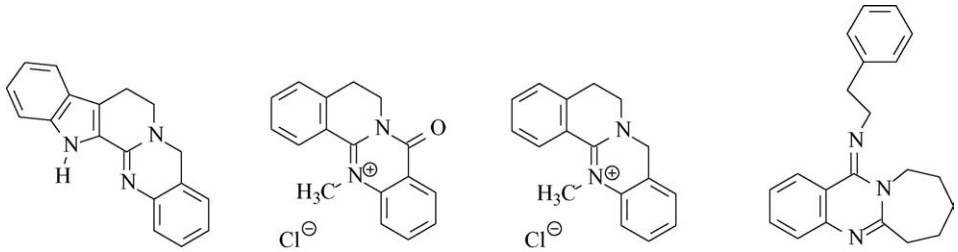
Abstract

Derived from the structures of the alkaloids rutaecarpine and dehydroevodiamine (DHED), and the long-known acetylcholinesterase (AChE) inhibitor tacrine, respectively, novel compounds were synthesised, including: 13-methyl-5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazolin-13-ium chloride (**12**), (8*Z*)-5,6-dihydro-8*H*-isoquino[1,2-*b*]quinazolin-8-imine (**13**), 5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazoline (**15a**), 13-methyl-5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazolin-13-ium chloride (**16**), 5,7,8,13-tetrahydroindolo [2′,3′:3,4]pyrido[2,1-*b*]quinazoline (**17**), and *N*-(2-phenylethyl)-*N*-[(12*Z*)-7,8,9,10-tetrahydroazepino [2,1-*b*]quinazolin-12(6*H*)-ylidene]amine (**20**), respectively. In a first step to evaluate their possible applicability for antiamnesic therapy, the inhibition of AChE and butyrylcholinesterase (BChE) were determined: compounds **13**, **15a**, **17**, and **20** are moderate or strong inhibitors of ChE, the latter two compounds show a 10-fold higher affinity to BChE. Compound **12** is a moderate inhibitor of AChE showing selectivity towards this enzyme.



Keywords: Cholinesterase inhibition; Dihydroquinazolines; Dehydroevodiamine analogues; Ellman assay

1. Introduction

Currently, cholinesterase inhibitors (ChEI) represent the treatment of choice for Alzheimer's disease (AD), as shown by clinical studies on the effects of these drugs on cognition (memory and concentration) and also on behavioural symptoms (apathy and motor agitation). Noteworthy, acetylcholinesterase (AChE) activity decreases progressively in certain brain regions from mild to severe stages of AD to reach 10–15% of normal values while butyrylcholinesterase (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 ChEI to be selective for AChE; on the contrary a good balance between AChE and BChE may result in higher efficacy [1].

It was recently reported, that the alkaloid dehydroevodiamine (DHED) (**1**) from the Chinese herb Evodia rutaecarpa Bentham shows strong antiamnesic activity in vivo, combined with a moderate AChE inhibition in vitro [2]. Its effi-

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cacy is partially attributed to AChE inhibition, but also to long-lasting facilitation of synaptic transmission due to activation of both muscarinic and NMDA receptors [3]. DHED also shows vasorelaxant activity [4]. Using our experience with both vasorelaxant compounds and biologically active indole derivatives targeting the CNS [5,6], we compared the structures of DHED (**1**) and the closely related alkaloid rutaecarpine (**2**) with the ChEI and vinylogous amidine tacrine (**3**). Hexahydroazepino[2,1-*b*]quinazolines (**4**) with AChE inhibiting properties possess a similar amidine structure (Fig. 1), which were recently described by Jaén et al. [7] (the optimised compound in terms of AChE inhibition is shown with an IC_{50} of 1.9 μM using rat brain AChE; the unsubstituted compound shows an IC_{50} of 15 μM). The work described here is a first approach to compounds, which should be ChEIs and ideally also possess antiamnesic properties due to other molecular mechanisms by combining structural elements of both classes of leads.

Therefore, by synthesising structures related to the above-mentioned lead compounds, the respective pharmacophore for the different modes of action (i.e. vasorelaxation in the brain, ChE inhibition, NMDA receptor binding) may be identified. From the chemical point of view it is important to point out that DHED exists in the form of the water-soluble quaternary salt **1** at acidic pH, whereas at alkaline pH the ring-opened dicarbonyl compound **5**, which can be extracted into organic solvents, is the stable form of DHED (Scheme 1) [8]. An important drawback of hexahydroazepino[2,1-*b*]quinazoline like **4** described by Jaén et al. is the fact, that they readily oxidise to their respective quinazolinones [9], a problem we wanted to overcome—if possible—with the compounds to be synthesised.

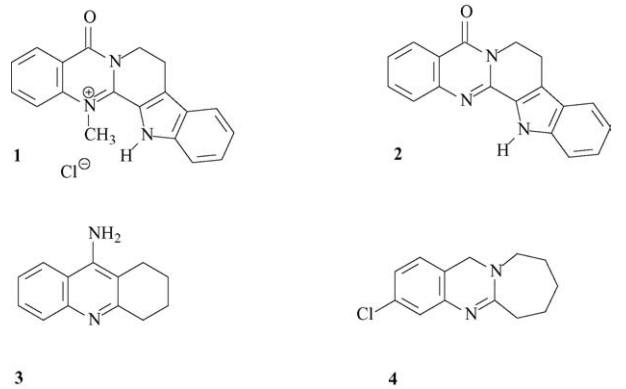
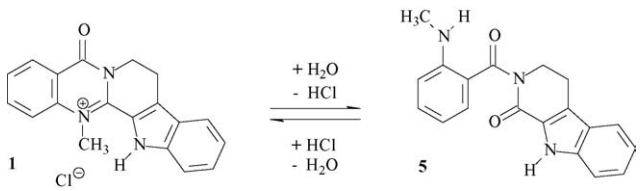


Fig. 1. Structures of DHED (**1**), rutaecarpine (**2**), tacrine (**3**), and 3-chloro-6,7,8,9,10,12-hexahydroazepino[2,1-*b*]quinazoline (**4**).



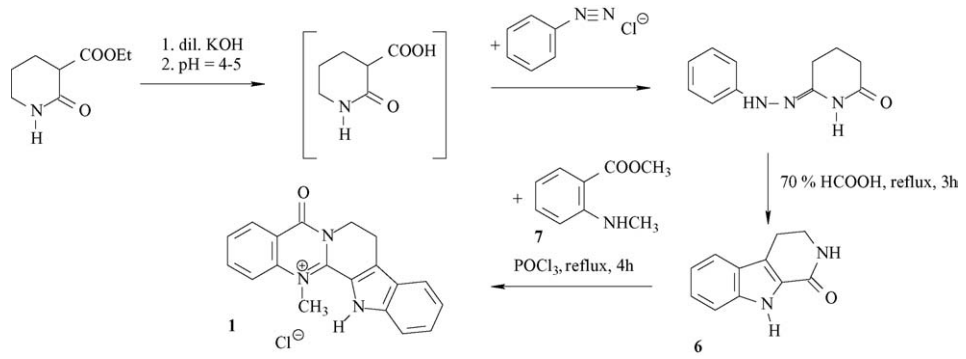
Scheme 1. pH-Dependency of the equilibrium of DHED between its dicarbonyl form **5** and the quaternary salt form **1**.

The search for new compounds was directed by several approaches: on the one hand, imines of the quinazolinones should be synthesised (both N-unsubstituted and N-substituted). An unsubstituted imine replaces the H-bond acceptor oxygen-atom by an H-bond donor. Furthermore this modification gives rise to strong bases, which can easily be solubilised in water in the form of their salts. Synthesis of substituted imines opens the way to introduction of different functionalities into the lead molecules, in an initial step we wanted to introduce a 2-phenylethyl group giving rise to a further hydrophobic moiety within the molecule. On the other hand, in order to investigate, whether the indole moiety is essential for DHED pharmacology, compounds were synthesised, in which the indole is replaced by a benzene moiety. Since a benzene moiety is less sensitive to more drastical chemical conditions, retaining activity with the respective dibenzo-compounds might give rise to facilitated modifications within the molecular structure compared to benzindolo-compounds. A further modification should be achieved by reduction of the carbonyl group (in position 5) of DHED (or its dibenzo-analogue) for combining the ChE inhibiting structural properties of DHED and a quinazoline like **4**.

2. Chemistry

Synthesis of DHED (**1**), which served both as a standard substance and a starting material for new compounds, was performed according to Pachter and Suld [8]; Lee et al. [10,11] out of methyl 2-(methylamino)benzoate and 2,3,4,9-tetrahydro-1*H*-β-carbolin-1-one (**6**) using phosphoric trichloride for activation of the lactam (Scheme 2). Rutaecarpine (**2**), which also served as a starting material for compounds, was synthesised from anthranilic acid and 2,3,4,9-tetrahydro-1*H*-β-carbolin-1-one (**6**), the latter compound was activated by conversion into its iminium ether using *Meerwein* salt (triethyloxonium tetrafluoroborate) [12]. The use of *Meerwein* salt for lactam activation proved to be better in terms of yield to the activation by phosphoric trichloride. The synthesis was also superior to a recently described synthesis of rutaecarpine [13], but the comparatively high price of starting material 3-carbethoxy-2-piperidone is a drawback.

For synthesis of dibenzo-analogues, 3,4-dihydroisoquinolin-1(2*H*)-one (**10**) (i.e. the corresponding benzolactam, which contains a benzene instead of an indole moiety) was obtained by reaction of 2-phenylethanamine (**8**) with ethyl chloroformiate [14], and subsequent cyclisation using P_2O_5 and $POCl_3$ (Scheme 3). This method—initially described for the synthesis of N-substituted benzolactams [15]—was highly superior to the use of PPA [14] in terms of yield (yield improved from <10% to >40%). 3,4-Dihydroisoquinolin-1(2*H*)-one (**10**) subsequently reacted after activation by phosphoric trichloride with the respective anthranilic acid esters to dibenzo-analogues of rutaecarpine and DHED (**11a**, **12**), respectively, therefore opening a facile approach to these compounds (Scheme 3). **11a** was previously synthesised using a



Scheme 2. Synthesis of DHED via *Japp–Klingemann* reaction according to Pachter and Suld [8]; Lee et al. [10,11].

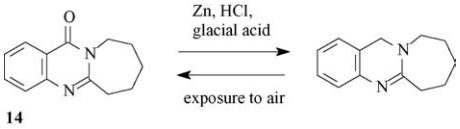
cyclocondensation reaction [16]. Yields for preparation of compounds **11** and **12** were higher, when the respective anthranilic acid esters [17] instead of the free acids were used.

5,6-Dihydro-8*H*-isoquino[1,2-*b*]quinazolin-8-imine (**13**) could be prepared out of **10** and anthranilonitrile in the presence of triethylamine in an analogous manner to the respective quinazolinones (Scheme 3).

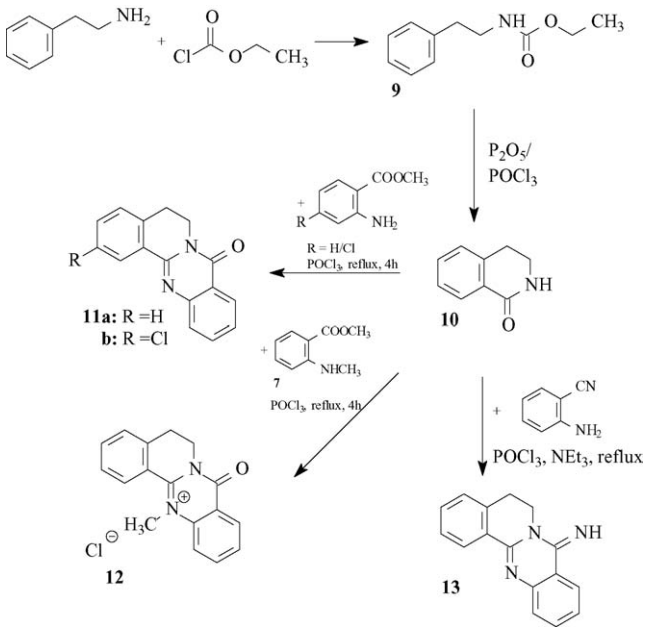
Reaction of the β-carboline **6** with anthranilonitrile to the rutaecarpine-imine either via its iminium ether or by the $POCl_3$ method gave either no product or only very low amounts of product (3% measured by GC/MS), respectively.

Also the reaction of benzolactam **10** with 2-(methylamino) benzonitrile in order to obtain an imine of dibenzo-DHED yielded in only 1.4% of product (GC/MS).

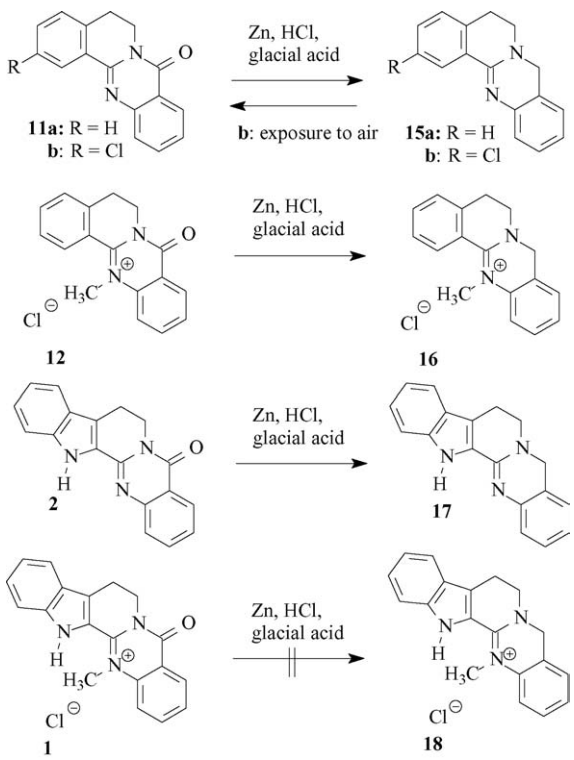
6,7,8,9,10,12-Hexahydroazepino[2,1-*b*]quinazoline—the moderate ChEI recently described by Jaén et al. [7]—readily oxidises back to the quinazolinone **14** when exposed to air (Scheme 4). We found >98% oxidation of the free base after 24 h as measured by GC/MS; therefore, it was not possible to measure enzyme inhibition with this compound. Compounds **1** (DHED), **12** (dibenzo-DHED), **2** (rutaecarpine), and **11a/b** (dibenzo-rutaecarpine and its chloro-derivate), respectively, were reduced under *Clemmensen* conditions to the respective quinazolines (Scheme 5). Reduction of DHED resulted in decomposition (probably due to reduction of the indole moiety), whereas compound **12** (dibenzo-DHED) yielded in 13-methyl-5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazolin-13-ium chloride (**16**) as expected. Noteworthy, **16** also shows a pH-dependent equilibrium between the quarternary salt and



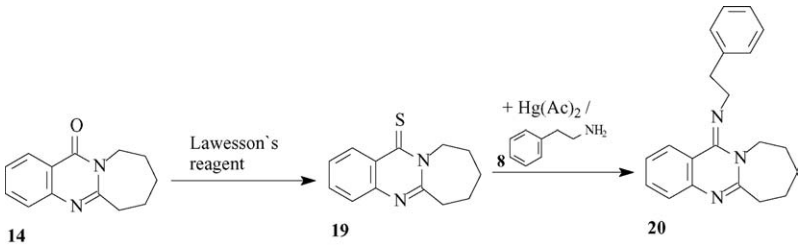
Scheme 4. Synthesis of 6,7,8,9,10,12-hexahydroazepino[2,1-*b*]quinazoline and auto oxidation to its starting material [7,9].



Scheme 3. Syntheses of the dibenzo-analogues of rutaecarpine and DHED (**11a/b**, **12**), and of 5,6-dihydro-8*H*-isoquino[1,2-*b*]quinazolin-8-imine (**13**), respectively.



Scheme 5. Reduction products of compounds **1** (DHED), **12** (dibenzo-DHED), **2** (rutaecarpine, and **11a** (dibenzo-rutaecarpine), respectively, under *Clemmensen* conditions.



Scheme 6. Synthesis of *N*-(2-phenylethyl)-*N*-[(12*Z*)-7,8,9,10-tetrahydroazepino[2,1-*b*]quinazolin-12(6*H*)-ylidene]amine (**20**).

the dicarbonyl form. This equilibrium is actually the reason for the selective reduction of the carbonyl group in compound **12** under the acidic conditions applied.

Interestingly, all reduction products obtained (**15a**, **16**, and **17**, respectively) are comparatively stable against oxygen, only the chloro-substituted compound **15b** (11-chloro-5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazoline) rapidly oxidised back to its starting material. Compounds **15a** and **17** have been previously described [16(a),18], but have not been tested for ChE inhibition. Furthermore, the procedure described here is superior to the literature methods due to its simplicity.

Another interesting compound was obtained from 7,8,9,10-tetrahydroazepino[2,1-*b*]quinazolin-12(6*H*)-one (**14**) after conversion into its quinazolinthione **19** using Lawesson's reagent (Scheme 6). Subsequent reaction with 2-phenylethanamine (**8**) could be achieved in the presence of mercury(II) acetate yielding *N*-(2-phenylethyl)-*N*-[(12*Z*)-7,8,9,10-tetrahydroazepino[2,1-*b*]quinazolin-12(6*H*)-ylidene]amine (**20**) (Scheme 6), therefore opening a possibility to couple primary amines to quinazolinones. Also the thiones of rutaecarpine and dibenzo-rutaecarpine could be coupled under these conditions with 2-phenylethanamine (**8**) (as proved by GC/MS), but the products obtained were highly unstable due to rapid hydrolysis after exposure to air yielding the quinazolinones again.

Reaction of **12** (dibenzo-DHED) with Lawesson's reagent resulted in decomposition yielding mainly the thione of dibenzo-rutaecarpine instead of the thione of dibenzo-DHED. Such a ring closure was made possible by the fact that the dicarbonyl form of dibenzo-DHE had to be used for synthesis (toluene was used as a solvent). Therefore, both carbonyl groups are converted into their thio-analogues, thus facilitating ring-closure.

3. Pharmacology

AChE and BChE inhibition was measured using the Ellman's assay as recently modified by Kapová et al. [19,20]: the respective enzymes cleave acetylthiocholine (ATC) and butyrylthiocholine (BTC) iodides. The thioles formed react in a subsequent reaction with the chromogenic compound 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) to give a yellow colour.

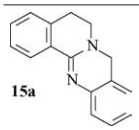
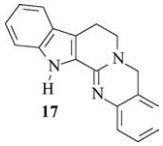
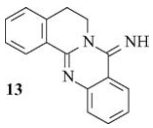
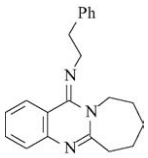
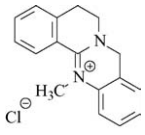
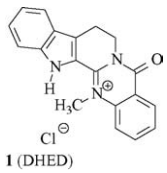
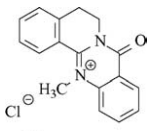
IC_{50} values of inhibition of the respective enzymes by the test compounds are given in Table 1. The ratios of the IC_{50} values at the two enzymes reflect selectivity. Inhibitions by physostigmine and DHED (**1**), respectively, were measured as standards. The compounds by Jaén at al. were too air-sensitive (oxidation to the respective insoluble quinazolinone) to get reproducible elemental analyses [7,9]; therefore it was not possible to measure these compounds reproducibly. Different aspects have to be taken into account to perform the measurements: on the one hand fairly high concentrations (i.e. 10^{-4} and 10^{-5} M) had to be used for measuring the affinities, which already exceed the solubility of some compounds. For example rutaecarpine, its dibenzo-analogue, and the quinazolinthiones were not sufficiently soluble. In our hands, DMSO—as described in the literature—could not be used for facilitated solubilisation of these compounds, because it influences AChE activity also at low concentrations (70.7% inhibition by a 10% aqueous solution in the stock aliquot); therefore ethanol was used for this purpose. On the other hand compound **17** and especially DHED showed significant absorption at 412 nm. In these cases the equilibrium absorption had to be measured prior to substrate addition, and subtraction of the respective absorption values had to be pursued. A representative curve for the inhibition of BChE by compound **20** is shown in Fig. 2.

4. Results and discussion

The amidine structure in compounds **15** and **17** is responsible for moderate to strong ChE inhibiting properties. Especially the reduction product **17** is a strong inhibitor showing some selectivity towards BChE, therefore representing a kind of a reversed binding profile of physostigmine. Compounds **13**, **17** and **20**, respectively, possess a 10-fold higher affinity to BChE, therefore being possibly advantageous for dementia therapy due to the fact, that AChE activity is drastically decreased in the brain of advanced cases of AD patients (reaching 5% AChE levels at autopsy in some regions) and a large pool of BChE still being available in glia neurons and neuritic plaques [1]. The dibenzo-analogue **15** is also quite a potent inhibitor, but with decreased selectivity.

The compounds synthesised show different selectivity profiles: reaching from higher affinities to AChE (**12**) to higher affinities to BChE (**17**). Noteworthy, the imines **13** and **20**, respectively, are moderate ChEI with a 10-fold higher affinity at BChE. DHED (**1**) and its dibenzo-analogue **12** are moderate inhibitors of AChE. DHED shows no selectivity, whereas **12** shows good selectivity towards AChE.

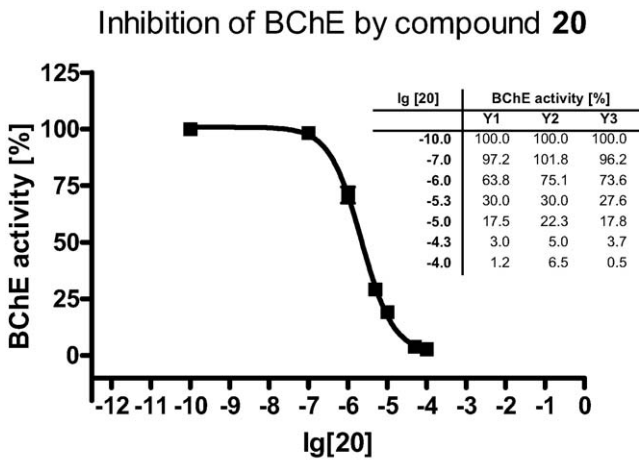
Table 1
IC_{50} values of test compounds for AChE and BChE inhibition, respectively, and resulting selectivities expressed as the ratio of IC_{50} values

Test compound	AChE inhibition [IC_{50} ($lgIC_{50}$ ± S.E.M.)]	BChE inhibition [IC_{50} ($lgIC_{50}$ ± S.E.M.)]	Selectivity [IC_{50} (BChE)/ IC_{50} (AChE)]
 **15a**	7.7 μM (–5.11 ± 0.050)	4.4 μM (–5.35 ± 0.044)	0.57
 **17**	3.4 μM (–5.47 ± 0.085)	0.5 μM (–6.34 ± 0.082)	0.15
 **13**	22.1 μM (–4.656 ± 0.065)	2.0 μM (–5.707 ± 0.044)	0.09
 **20**	27.7 μM (–4.558 ± 0.107)	2.2 μM (–5.658 ± 0.032)	0.08
 **16**	>500 μM[a]	160.2 μM (–3.795 ± 0.148)	<0.3
 **1** (DHED)	6.3 μM (–5.199 ± 0.2337) lit[2]: 37.8 μM	8.4 μM (–5.078 ± 0.0400)	1.3
 **12**	12,0 μM (–4.922 ± 0.120)	>500 μM[b]	>40
Physostigmine	0.49 μM (–6.307 ± 0.1124) lit[2]: 0.12 μM	1.17 μM (–5.934 ± 0.084)	2.4

[a] 5×10^{-5} M: 23% inhibition.
[b] 5×10^{-4} M: 77% inhibition.

Compound **16** does not show significant affinity towards AChE, and only low affinity towards BChE. This is actually quite surprising, because this compound combines the structures of the ChEIs **15** and **12**, respectively. Since it has not been described yet, which form of DHED (quarternary salt or dicarbonyl compound) interacts with the enzyme, the reason for the low affinity of **16** remains unclear, but its inhibition profile gives rise to the assumption, that the mode of action of ChE inhibition of the quarternary compounds might be different from the ones of the amidines.

Because of the fact, that DHED's antiamnesic properties are only partially attributed to AChE inhibition [3], the compounds described here are currently evaluated in suitable in vivo models and with respect to their affinity to the NMDA receptor.



lg [20]	BChE activity [%]		
	Y1	Y2	Y3
-10.0	100.0	100.0	100.0
-7.0	97.2	101.8	96.2
-6.0	63.8	75.1	73.6
-5.3	30.0	30.0	27.6
-5.0	17.5	22.3	17.8
-4.3	3.0	5.0	3.7
-4.0	1.2	6.5	0.5

Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	1.141
TOP	100.9
LOGEC50	-5.658
HILLSLOPE	-1.073
EC50	2.1985e-006
Std. Error	
BOTTOM	1.644
TOP	1.419
LOGEC50	0.03154
HILLSLOPE	0.07302
95% Confidence Intervals	
BOTTOM	-2.329 to 4.610
TOP	97.88 to 103.9
LOGEC50	-5.724 to -5.591
HILLSLOPE	-1.227 to -0.9189
EC50	1.8861e-006 to 2.5626e-006
Goodness of Fit	
Degrees of Freedom	17
R^2	0.9953
Absolute Sum of Squares	153.4
Sy.x	3.004
Data	
Number of X values	7
Number of Y replicates	3
Total number of values	21
Number of missing values	0

Fig. 2. Inhibition of BChE by compound **20** (analysed with Graph-Pad Prism™).

5. Conclusion

In these first studies compounds with high (**17**) or moderate (**12**, **13**, **15**, **20**) affinities towards ChE were synthesised, which excel the naturally occurring lead structures as well as preceding synthetic leads. Compound **17** reaches affinities comparable to the well-established drug physostigmine. Some selectivity towards BChE could be reached with compounds **17**, **13** and **20**. Therefore the strategy to combine structural elements of known inhibitors with features of naturally occurring molecules to improve affinities proved successful in this preliminary work.

A first step to find new structures for AChE/BChE inhibiting and potentially antiamnesic compounds derived from alkaloids has been made; no SAR has been performed yet. None of our leads has been optimised yet in terms of the substitution pattern (apart from a chloro-substitution of compound **15**, which led to an oxidation-sensitive compound), or—with respect to compound **20**, which contains an additional hydrophobic moiety—in terms of the size of the alkyl-chain connected to the aromatic moiety or the aromatic moiety itself. Stability of imine-compounds regarding hydrolysis and of the amidines regarding oxidation after exposure to air, respectively, seems to be fairly hard to predict with the available data and has to be investigated thoroughly.

6. Experimental protocols

6.1. General

Melting points (m.p.) are uncorrected and were measured in open capillary tubes, using a Gallenkamp m.p. 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). MS data were determined by GC/MS, using a Hewlett Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific). For detection iodine vapour or UV light (254 nm), respectively, was used. Silica gel column chromatography utilised silica gel 60 63–200 μm (Baker). UV-measurements were performed on a Jasco V-570 UV/VIS/NIR spectrophotometer.

6.2. Chemistry

7,8,9,10-Tetrahydroazepino[2,1-*b*]quinazolin-12(6*H*)-one (**14**) was prepared as described by Petersen and Tietze [12].

2,3,4,9-Tetrahydro-1*H*-β-carbolin-1-one (**6**) was prepared according to the procedure described by Lee et al. [10], DHED (**1**) out of **6** according to Huang and Lee [11], and rutaecarpine (**2**) out of **6** according to Petersen and Tietze [12], respectively.

*6.2.1. Ethyl 2-phenylethylcarbamate (**9**)*

To a solution of 43.63 g (0.36 mol) of 2-phenylethanamine (**8**) in 400 mL of dry diethyl ether a solution of 19.53 g (0.18 mol) ethyl chloroformiate in 60 mL of diethyl ether was added dropwise under ice-cooling. A white precipitate (the hydrochloride of the amine) formed immediately. After vigorous stirring for 2 h at r.t., the solution was filtered, and the ether removed in vacuo. The resulting oil solidified on standing.

Compound **9**: white crystals (33.4 g, 96% yield). M.p. 38 °C (lit [14]. M.p. 37–39 °C).

*6.2.2. 3,4-Dihydroisoquinolin-1(2*H*)-one (**10**)*

To a solution of 14.22 g (74 mmol) ethyl 2-phenylethylcarbamate (**9**) in 74 mL of phosphoric trichloride 21.8 g (147 mmol) P_2O_5 were added. The solution was heated for

2 h under reflux. After 10 min the mixture solidified, but under further heating a clear solution was formed again. Excess phosphoric trichloride was removed in vacuo, and the resulting residue was quenched with ice-water (CAUTION: highly exothermic reaction). The solution was neutralised with $NaHCO_3$, and extracted three times with diethylether. The combined organic phases were washed twice with water, dried with sodium sulphate, and the solvent removed in vacuo.

Compound **8**: white crystals (4.56 g, 41% yield). M.p. 70 °C (lit [21]. M.p. 73 °C). ^{1}H NMR (250 MHz, DMSO-d_6) δ 2.89 (2H, t, J = 6 Hz, CH_2CH$_2$N), 3.36 (2H, t, J = 6 Hz, CH$_2$NH), 7.20–7.47 (3H, m, arom.), 7.86 (1H, d, J = 7 Hz, C(8)*H*), 7.95 (1H, bs, N*H*) ppm. IR (KBr): 3340, 1663, 1337, 1060, 750, 701 cm^{-1}.

*6.2.3. General procedure for the preparation of 5,6-Dihydro-8*H*-isoquino[1,2-*b*]quinazolin-8-one (**11a**) and 11-chloro-5,6-dihydro-8*H*-isoquino[1,2-*b*]-quinazolin-8-one (**11b**)*

To a solution of 2.0 g (13.59 mmol) of 3,4-dihydroisoquinolin-1(2*H*)-one (**10**) in 40 mL of hot toluene 16 mL of phosphoric trichloride were added dropwise. The mixture was heated under reflux in a nitrogen atmosphere. After 30 min 2.44 g (16.14 mmol) methyl anthranilate were added (prepared according to [17]), and the mixture was heated for further 4 h under reflux. After cooling, toluene and excess $POCl_3$ were removed under reduced pressure. To the residue 80 ml of 20% aqueous K_2CO_3 solution were given, followed by 100 mL of CH_2Cl_2, the organic layer was separated, and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic phases were washed twice with brine, dried with Na_2SO_4, the solvent removed under reduced pressure, and the residue purified in the manner described.

Compound **11a**: white solid (2.0 g, 59% yield). Purification by column chromatography with EtOAc/MeOH (1:1) as eluent. M.p. 158 °C (lit [16]. M.p. 163–164 °C). ^{1}H NMR (250 MHz, DMSO-d_6) δ 3.12 (2H, t, J = 7.5 Hz, CH_2CH$_2$N), 4.30 (2H, t, J = 7.5 Hz, CH$_2$N), 7.40–7.60 (4H, m, arom.), 7.73–7.88 (2H, m, arom.), 8.2 (1H, d, J = 7 Hz, C(1)H), 8.37 (1H, d, J = 7 Hz, C(9)H) ppm. EI-MS *m/z* 247, 233, 218, 190, 128, 110, 102. IR (KBr): 3460, 2356, 1675, 1591, 1560, 1470, 1398, 1150, 1102, 762, 738, 620 cm^{-1}. Anal. ($C_{16}H_{12}N_2O$): C, H, N.

Compound **11b**: off-white solid (2.13 g, 56% yield). Purification by recrystallisation from acetone. M.p. 154 °C. ^{1}H NMR (250 MHz, DMSO-d_6) δ 3.12 (2H, t, J = 6.5 °Hz, CH_2CH$_2$N), 4.28 (2H, t, J = 6.5 Hz, CH$_2$N), 7.41–7.58 (4H, m, arom.), 7.78 (1H, s, C(12)H), 8.2 (1H, d, J = 8.5 Hz, C(9)H), 8.36 (1H, d, J = 2.5 Hz, C(1)H) ppm. EI-MS *m/z* 281, 267, 246, 218, 190, 128, 109. IR (KBr): 3470, 1677, 1561, 1477, 1340, 922, 890, 767 cm^{-1}. Anal. ($C_{16}H_{11}ClN_2O\cdot0.5H_2O$): C, H, N.

*6.2.4. 13-Methyl-5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazolin-13-ium chloride/2-[2-(methylamino)benzyl]-3,4-dihydroisoquinolin-1(2*H*)-one (salt/free base, **12**)*

To a solution of 2.56 g (17.2 mmol) of 3,4-dihydroisoquinolin-1(2*H*)-one (**10**) in 40 mL of hot toluene 20 mL of phosphoric trichloride were added dropwise. The mixture was heated under reflux under nitrogen atmosphere. After 30 min 3.37 g (20.4 mmol) methyl 2-(methylamino)benzoate (**7**) were added (prepared according to [17]), and the mixture was heated for further 4 h under reflux. After 2 h a second, dark phase separated. After cooling, toluene and excess $POCl_3$ were removed under reduced pressure. The residue was treated with concentrated hydrochloric acid (10 mL) and 200 mL of hot water. The suspension was filtered, and the aqueous phase was alkalised with ammonia and extracted three times with diethylether. The combined organic phases were dried with Na_2SO_4, and the solvent removed in vacuo. The residue was purified by column chromatography with EtOAc/MeOH (1:1) as eluent to give 910 mg (20% yield) of a yellow solid. The salt could be obtained by removing the water of the acidic aqueous solution, followed by column chromatography, but this was a more time-consuming procedure.

Compound **12**: M.p. 99 °C (free base). ^{1}H NMR (250 MHz, DMSO-d_6) salt: δ 3.19 (2H, t, J = 6 Hz, CH_2CH$_2$N), 4.24 (5H, bs, CH$_3$, CH$_2$N), 7.68–7.88 (4H, m, arom.), 8.20 (2H, m, arom.), 8.2 (1H, d, J = 7.8 Hz, C(1)H), ppm. Free base: 2.81 (3H, d, J = 5 Hz, CH$_3$), 3.16 (2H, t, J = 12.5 Hz, CH_2CH$_2$N), 3.90 (2H, t, J = 12.5 Hz, CH$_2$N), 6.51 (1H, t, J = 10 Hz, C(5)H), 6.70 (1H, J = 10 Hz, C(3)H), 7.05 (1H, d, J = 5 Hz, NH), 7.32 (4H, m, arom.), 7.47 (1H, t, J = 5 Hz, C(6)H), 7.90 (1H, d, J = 5 Hz, C(7)H) ppm. EI-MS (free base) *m/z* 280, 247, 207, 148, 133, 105. IR (free base, KBr): 3400, 1675, 1385, 1307, 1235, 906, 741, 653 cm^{-1}. Anal. ($C_{17}H_{16}N_2O_2\cdot0.5H_2O$, free base): C, H, N.

*6.2.5. (8Z)-5,6-Dihydro-8*H*-isoquino[1,2-*b*]quinazolin-8-imine (**13**)*

To a solution of 3.7 g (25 mmol) of 3,4-dihydroisoquinolin-1(2*H*)-one (**10**) in 70 mL of chloroform 2.75 mL of phosphoric trichloride (30 mmol) were added drop wise. After 1 h of stirring at r.t., 6.25 mL of triethylamine were added, followed by a solution of 2.54 g (21.5 mmol) of anthranilonitrile in chloroform. The mixture was stirred at r.t. overnight, followed by heating to reflux for 24 h during which time a fine crystalline precipitate was formed. The mixture was treated with 125 mL of 15% aqueous K_2CO_3 solution, the organic layer was separated, the aqueous phase extracted with chloroform, and the combined organic layers dried over potassium carbonate. The chloroform was removed under reduced pressure, and the solid formed was recrystallised from acetone.

Compound **13**: white crystals (2.15 g, 41% yield). M.p. 138 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 3.13 (2H, t, J = 6.5 Hz, CH_2CH$_2$N), 4.44 (2H, t, J = 6.5 Hz, CH$_2$N), 7.22–7.67 (4H, m, arom.), 7.85 (1H, d, J = 8 Hz, C(1)H), 8.45 (1H, d, J = 8 Hz, C(12)H) ppm. EI-MS *m/z* 246, 218, 190, 128, 116, 102. IR (KBr): 3280, 1621, 1591, 1584, 1482, 1398, 1325, 1144, 997, 867, 774, 704 cm^{-1}. Anal. ($C_{16}H_{13}N_3$): C, H, N.

*6.2.6. General procedure for the preparation of quinazolines (5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazoline (**15a/b**)/2-[2-(methylamino)benzyl]-3,4-dihydroisoquinolin-1(2*H*)-one (free base of **16**)/5,7,8,13-tetrahydroindolo[2′,3′:3,4]pyrido[2,1-*b*]quinazoline (**17**))*

A solution (in the case of rutaecarpine (**2**) a suspension) of 1.5 mmol of the respective quinazolinone (i.e. **2**, **11a/b**, **12**) in 10 mL of glacial acetic acid was stirred at 60 °C with 4.7 g of zinc dust. Concentrated hydrochloric acid (4 mL) was added dropwise, and the mixture was kept at this temperature for 1.5 h. The zinc was filtered off, and the solution was concentrated under reduced pressure. Aqueous sodium hydroxide solution (25%) was added till the precipitated zinc hydroxide started to get into solution, and the mixture was extracted three times with tetrahydrofuran (yields are much higher using THF than using diethylether for example).

*6.2.6.1. 5,8-Dihydro-6*H*-isoquino[1,2-*b*]quinazoline (**15**).* 5,6-Dihydro-8*H*-isoquino[1,2-b]quinazolin-8-one (**11**) as starting material. White powder. M.p. 237 °C (hydrochloride). 0.15 g (40% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.07 (2H, t, J = 7 Hz, CH_2CH$_2$N), 3.41 (2H, t, J = 7 Hz, CH$_2$CH_2N), 4.59 (2H, s, benzyl-CH$_2$), 6.99–7.41 (7H, m, arom.), 8.4 (1H, d, J = 6.8 Hz, C(1)H) ppm. IR (hydrochloride, KBr): 3388, 1717, 1639, 1560, 1500, 1446, 1385, 765, 683 cm^{-1}. EI-MS *m/z* 233, 218, 204, 132, 116. Anal. ($C_{16}H_{15}ClN_2$, hydrochloride): C, H, N.

Spectroscopic data is in accordance with the data available in the literature [16(a)].

*6.2.6.2. 5,7,8,13-Tetrahydroindolo[2′,3′:3,4]pyrido[2,1-b]quinazoline (**17**).* Rutaecarpine (**2**) as starting material. Light beige powder. M.p. 220 °C. 0.12 g (30% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.05 (2H, t, J = 7.5 Hz, CH_2CH$_2$N), 3.44 (2H, t, J = 7.5 Hz, CH$_2$CH_2N), 4.47 (2H, s, benzyl-CH$_2$), 6.87–7.2 (7H, m, arom.), 7.41 (1H, d, J = 8.8 Hz, C(9)H), 11.63 (1H, bs, indole-NH) ppm. IR (KBr): 3436, 3088, 1621, 1591, 1548, 1488, 1325, 759, 741 cm^{-1}. EI-MS *m/z* 272, 257, 243, 217, 135. Anal. ($C_{18}H_{15}N_3$·MeOH): C, H, N.

Spectroscopic data is in accordance with the data available in the literature [18].

*6.2.6.3. 2-[2-(Methylamino)benzyl]-3,4-dihydroisoquinolin-1(2*H*)-one (free base of **16**).* 13-Methyl-5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazolin-13-ium chloride (**12**) as starting material. Light yellow oil. 0.12 g (25% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.8 (3H, s, CH$_3$), 2.92 (2H, t, J = 5.5 Hz, CH_2CH$_2$N), 3.44 (2H, bs, CH$_2$CH_2N), 4.78 (2H, s, benzyl-CH$_2$), 5.22 (1H, bs, NH), 6.71 (2H, d, J = 7 Hz, C(6)H, C(5)H.), 7.15–7.22 (5H, m, arom.) 7.27 (1H, d, J = 8 Hz, C(8)H) ppm. IR (KBr): 3412, 2920, 1627, 1518, 1428, 1241, 1042, 930, 753 cm^{-1}. EI-MS *m/z* 266, 249, 161, 132, 106, 77. Anal. ($C_{17}H_{18}N_2O$·1/2H_2O): C, H, N.

*6.2.7. General procedure for the preparation of quinazolinthiones (7,8,9,10-tetrahydroazepino[2,1-b]-quinazoline-12(6*H*)-thione (**19**)/5,6-dihydro-8*H*-isoquino-[1,2-b]quinazoline-8-thione/8,13-dihydroindolo-[2′,3′:3,4]pyrido[2,1-b]quinazoline-5(7*H*)-thione)*

A solution (rutaecarpine (**2**) got into solution in the heat) of 5.0 mmol of the respective quinazolinone (i.e. **2**, **11a**, **14**) in 50 mL of dried toluene was stirred under reflux with 1.0 g (2.5 mmol) Lawesson's reagent. After 4 h the reaction mixture was cooled to r.t., the toluene removed under reduced pressure, and the residue column chromatographed using $CHCl_3$ as eluent.

*6.2.7.1. 7,8,9,10-Tetrahydroazepino[2,1-b]quinazoline-12(6*H*)-thione (**19**).* 7,8,9,10-Tetrahydroazepino[2,1-*b*]quinazolin-12(6*H*)-one (**14**) as starting material. Deep yellow crystals. 1.02 g (89% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.09 (6H, bs, (CH_2)$_3$CH$_2$N), 3.25 (2H, s, CH$_2$(CH$_2$)$_4$N), 5.09 (2H, s, CH$_2$N), 7.48 (1H, t, J = 1.3 Hz, C(3)H), 7.60–7.74 (2H, m, C(4)H, C(2)H), 8.78 (1H, d, J = 7 Hz, C(1)H) ppm. EI-MS *m/z* 230, 215, 201, 176, 155, 102. Other spectroscopic data is in accordance with literature data [22].

*6.2.7.2. 5,6-Dihydro-8*H*-isoquino[1,2-b]quinazoline-8-thione.* 5,6-Dihydro-8H-isoquino[1,2-*b*]quinazoline-8-one (**11a**) as starting material. Deep yellow crystals. M.p. 169 °C. 1.13 g (86% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.18 (2H, t, J = 6.5 Hz, CH_2CH$_2$N), 5.04 (2H, t, J = 6.5 Hz, CH$_2$N), 7.29–7.52 (4H, m, arom.), 8.48 (1H, d, J = 8 Hz, C(12)H), 8.84 (1H, d, J = 8 Hz, C(9)H) ppm. IR (KBr): 3448, 1591, 1548, 1476, 1325, 1186, 939, 771, 741 cm^{-1}. EI-MS *m/z* 264, 249, 231, 218, 128. Anal. ($C_{16}H_{12}N_2S$): C, H, N, S.

*6.2.7.3. 8,13-Dihydroindolo[2′,3′:3,4]pyrido[2,1-b]quinazoline-5(7*H*)-thione.* Rutaecarpine (**2**) as starting material. Deep yellow crystals. M.p. 196 °C. 1.29 g (85% yield). ^{1}H NMR (250 MHz, DMSO-d_6) δ 3.27 (2H, t, J = 7.5 Hz, CH_2CH$_2$N), 5.11 (2H, t, J = 7.5 Hz, CH$_2$N), 7.11 (1H, t, J = 7.5 Hz, C(11)H), 7.86 (1H, t, J = 7.5 Hz, C(2)H), 7.50–7.89 (4H, m, arom.) 7.86 (1H, t, J = 7.5 Hz, C(1)H), 8.68 (1H, d, J = 10 Hz, C(4)H), 11.92 (1H, s, indole-NH) ppm. IR (KBr): 3448, 1627, 1597, 1470, 1319, 1217, 939, 765 cm^{-1}. EI-MS *m/z* 302, 286, 270, 257, 167, 151. Anal. ($C_{18}H_{13}N_3S$): C, H, N, S.

*6.2.8. N-(2-Phenylethyl)-N-[(12Z)-7,8,9,10-tetrahydroazepino[2,1-b]quinazolin-12(6*H*)-ylidene]amine (**20**)*

To a solution of 460 mg (2 mmol) 7,8,9,10-tetrahydroazepino[2,1-*b*]quinazolin-12(6*H*)-one (**14**) in 15 mL of toluene were added 726 mg (6 mmol) of 2-phenylethanamine (**8**). Under vigorous stirring 640 mg (2 mmol) mercury(II) acetate were added. The suspension darkened immediately. The mixture was heated under reflux for 2 h. After cooling, the dark precipitate was filtered off, the toluene was removed under reduced pressure, and the residue was column chromatographed using chloroform as eluent.

Compound **20**: yellow oil (0.37 g, 58% yield). ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.79–1.90 (6H, b, (CH_2)$_3$CH$_2$N),

2.90–2.99 (4H, m, CH_2(CH_2)$_4$N, benzyl-CH_2), 3.67 (2H, m, (CH_2)$_3$CH_2N), 4.41 (2H, m, Ph-C$H_2$$CH_2$), 7.21–7.33 (5H, m, phenyl-arom.), 7.75 (1H, t, J = 6.5 Hz, C(2)H), 8.02 (2H, m, C(1,3)H), 8.22 (1H, d, J = 7.5 Hz, C(4)H) ppm. EI-MS *m/z* 318, 226, 213, 197, 185, 155. IR (KBr): 3421, 2932, 1621, 1593, 1477, 1400, 977, 774, 703 cm^{-1}. Anal. ($C_{21}H_{23}N_3$·3/4H_2O): C, H, N.

6.3. 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). DTNB (Ellman's reagent), ATC and BTC iodides were obtained from Fluka (Buchs, Switzerland).

The assay was performed as described in the following procedure [19,20]: stock solutions of the test compounds were prepared in ethanol, 100 μL of which gave a final concentration of 10-3 M when diluted to the final volume of 3.32 mL. The highest concentration of the test compounds applied in the assay was 10-4 M (10 % EtOH in the strock solution did not influence enzyme activity). In order to obtain an inhibition curve, at least five different concentrations (normally 10^{-4}–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) were dissolved in 100 mL of water and adjusted with NaOH to pH = 8.0 ± 0.1. Enzyme solutions were prepared to give 2.5 units mL^{-1} in 1.4 mL aliquots. Furthermore, 0.01 M DTNB solution, and 0.075 M ATC and BTC solutions, respectively, were used. A cuvette containing 3.0 mL of 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 were 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 of water replaced the enzyme solution.

The inhibition curve was obtained by plotting the percentage enzyme activity (100% for the reference) versus logarithm of test compound concentration (Fig. 2).

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