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Graphical Abstract

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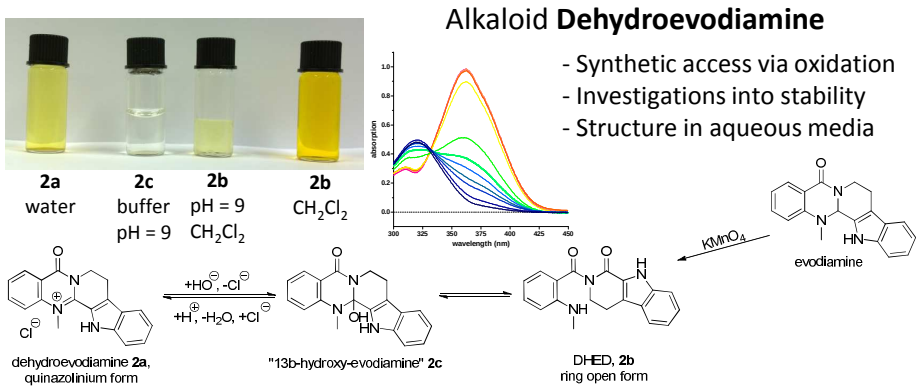

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Investigation into the stability and reactivity of the pentacyclic alkaloid dehydroevodiamine and the benz-analog thereof

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ABSTRACT

Limited synthetic approaches to obtain the biologically active alkaloid dehydroevodiamine (DHED) are known to date. Undesired demethylation in the most widely applied rout was found to be a hampering side reaction for the benz-DHED derivative leading to a quinazolinone, which represents a benz-rutaecarpine derivative. For rutaecarpine, a related plant alkaloid, many different synthetic approaches have been described. Alternative reaction procedures to obtain DHED such as methylation of rutaecarpine and oxidation of evodiamine were investigated to make DHED more easily accessible and the latter method proved to be the most successful one. Furthermore, the remarkable equilibrium between the ring-closed quinazolinium and the ring-open form of the compounds was systematically investigated by UV-Vis measurements. The ring-open form and the quinazolinium salt form the same species when incubated in buffer solution for 24 h. A better soluble form, i. e. "hydroxyevodiamine", seems to represent the biologically active form that has not yet been described.

1. Introduction

The alkaloids evodiamine, dehydroevodiamine chloride (DHED·Cl) and rutaecarpine can be isolated from the traditional Chinese medicinal plant *Evodia rutaecarpa* (Figure 1a). The plant is used against a variety of diseases like headache, abdominal pain, postpartum hemorrhage, dysmenorrhea, gastrointestinal disorders, and hypertension.[1, 2] Evodiamine improves cognition in a transgenic mouse model of Alzheimer´s disease (AD).[3] Besides this, evodiamine and derivatives thereof are anti-cancer drug candidates.[4-6] Some rutaecarpine derivatives show inhibition of acetylcholinesterase (AChE), the most important enzyme being addressed in in AD therapy.[7] Derivatives of rutaecarpine are further used against obesity, vascular pressure and platelet aggregation.[2, 8-10] The third of the above alkaloids, DHED·Cl, was first described in 1927 by Asahina and Ohta.[11] In 1960, Pachter and Suld proposed the dicarbonyl structure **2b**, which is formed from DHED·Cl after addition of base (Scheme 1b).[12] Since then, diverse and promising pharmacological properties of this alkaloid have been discovered and investigated.[13-18] Concomitantly, extraction methods from the plant were studied (most of these studies published in Chinese).[19] DHED·Cl also proved to be a one digit micromolar inhibitor of both AChE and butyrylcholinesterase (BChE) and DHED·Cl was described to improve memory deficit in Alzheimer mice and was tested against stress-induced cognitive deficits.[20-24]

The replacement of the indole ring by a benzene ring leads to benz-DHED·Cl **4a** (Figure 1b) which possesses better solubility properties compared to the alkaloid DHED and was first described in 2005.[24]

Several review articles have covered the chemistry and biological properties of compounds with quinazolinone scaffolds, in particular evodiamine and rutaecarpine.[1, 25-27] These quinazolinone structures are considered "priviledged structures" because of their versatile biological applications and of the various reactions possible for chemical modification of these scaffolds. However, for DHED up to now only five papers exist, that describe a synthetic pathway to form the pentacyclic quinazolinium core. All of them use phosphorus(V) oxychloride for activation of the indolo lactam moiety (cf. Scheme 2).[4, 6, 24, 28, 29]

We recently reported on an unexpected finding on the attempted synthesis of evodiamine.[30] The synthesis of *N*-alkylated evodiamine derivatives can be carried out by fusion of *N*-alkyl isatoic anhydride and 3,4-dihydroisoquinoline **6**. When unsubstituted isatoic anhydride **5** is used, the expected product

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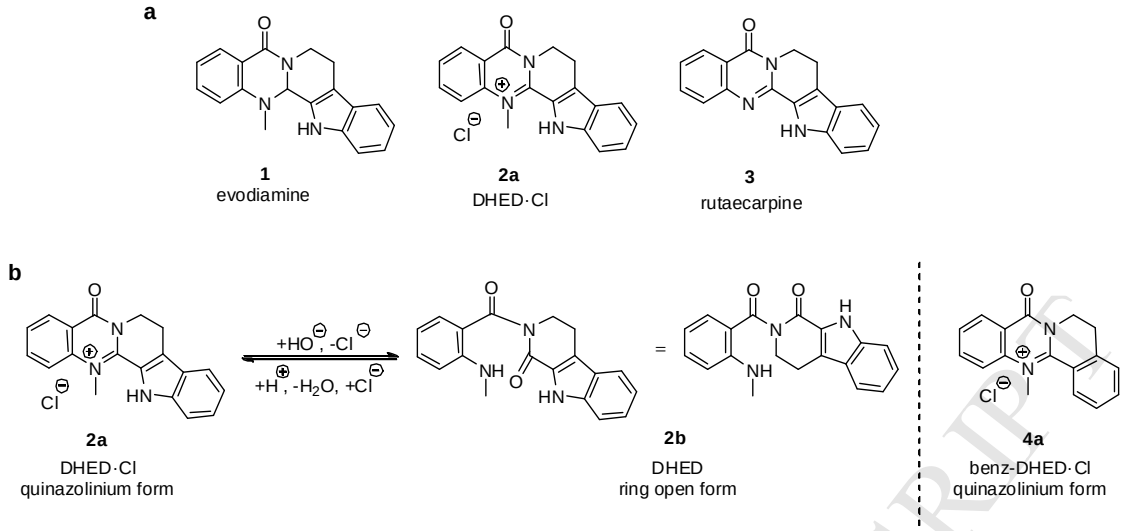
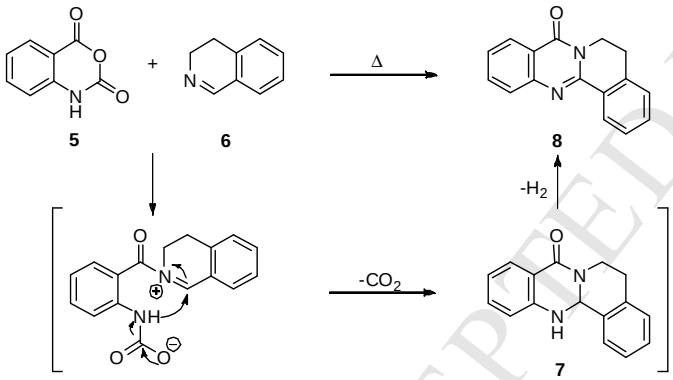


Figure 1. a) Quinazolinone alkaloids being isolated from *Evodia rutaecarpa*. **b)** Equilibrium of DHED; quinazolinium form (**2a**) postulated to occur in acidic media and the ring open form (dicarbonyl compound, **2b**) in basic media. The ring opening process is accompanied by a color change from pale yellow (**2a**) to deep orange (**2b**).[24, 28] Compound **4a** is the benz-analog of DHED and also shows pH-dependant ring opening and closing.

should be the *N*-H form of "benz-evodiamine" **7**. However, a dehydrogenation reaction occurs, which yields benz-rutaecarpine **8** instead (Scheme 1; for easier compound recognition in this context, the trem 'benz-rutaecarpine' instead of the systematic name is used to describe that the indole ring of rutaecarpine is replaced by a benzene ring).



Scheme 1. Dehydrogenation leads to the formation of benz-rutaecarpine.[30]

This is an interesting finding since this class of alkaloids and heterocycles seem to stabilize by formation of a C=N-double bond and therefore by formation of a quinazolinone structure. Especially for the synthesis of benz-DHED low yields of 20% have been described.[24] We observed that benz-rutaecarpine (i.e. demethylated DHED) is formed as a by-product and we wanted to study this in more detail. In this work, we systematically investigated whether and how a side reaction during the DHED synthesis is due to the reaction conditions applied or originates from the compound DHED itself, e.g. by spontaneous demethylation. Consequently, an alternative synthesis access to quinazolinium-salts was developed. In order to identify the form responsible for interaction with biological targets, such as AChE, for the first time the pH-dependent equilibrium of DHED and DHED·Cl was systematically investigated by UV-Vis measurements, and it turned out that at physiological pH after 24h none of the two, but a yet undescribed form is predominant. AChE and BChE inhibition data was confirmed[24] and to further characterize the biological data of DHED and especially benz-DHED, neurotoxicity and putative neuroprotective properties were evaluated on murine hippocampal cells, as well as inhibition of Aβ-plaque formation in a bacterial cell assay.

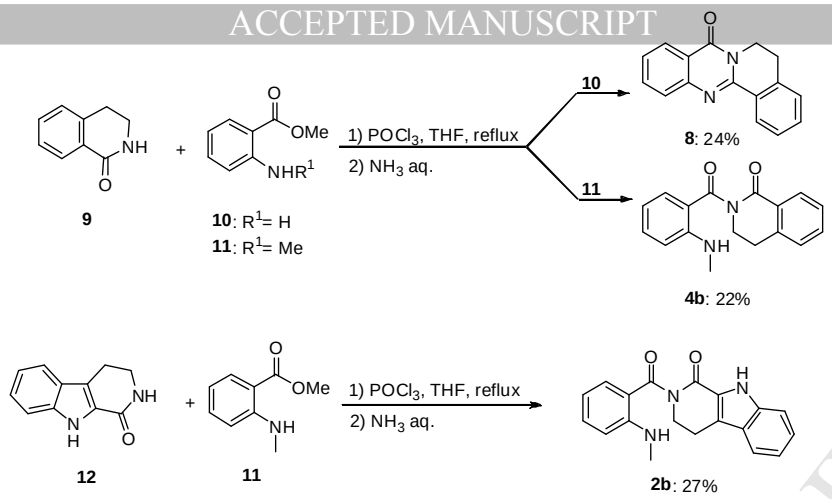
2. Results and Discussion

2.1. Synthesis and investigation into demethylation of DHED and benz-DHED

Lactams **9** and **12** were prepared in a two step synthesis with an overall yield of 51% and 75%, respectively.[24, 28, 31] To obtain anthranilic esters **10** and **11**, anthranilic acid was first converted in isatoic anhydride using triphosgene.[32] Subsequently *N*-methylation was carried out using methyl iodide according to a procedure by Beutner *et al*.[33] In the final step the methyl ester was obtained through heating in methanol with the addition of catalytic amounts of conc. sulfuric acid. The fusing reaction was performed according to the procedure described by Nakayama *et al.* (Scheme 2).[28] Firstly, the lactam was activated to the lactim chloride by heating with phosphorus(V) oxychloride in dry THF. Afterwards, the anthranilic ester was added dropwise and the mixture reacted for 41 or 96 h, respectively, to obtain compounds **4b** and **2b**. Usage of *N*-unsubstituted anthranilic ester led to formation of benz-rutaecarpine **8**.

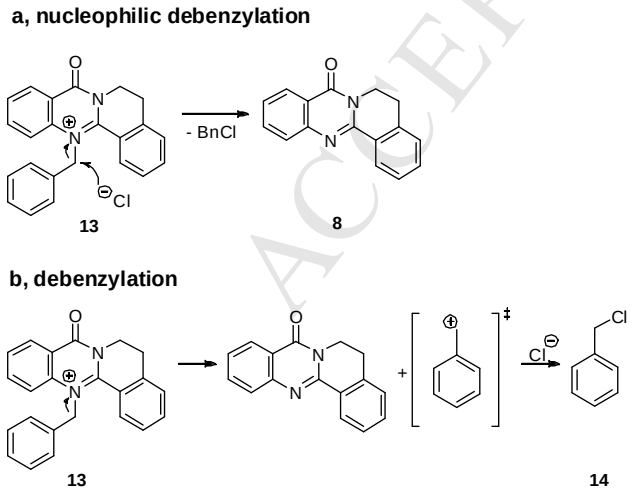
Both benz-DHED **4b** and DHED **2b** were obtained in their ringopen form through crystallization and only compound **8** required purification by column chromatography. The synthesis performed by Nakayama *et al*. led to DHED formation in 50% yield.[28] The reasons for low yields have never been reported. A similar reaction route yielding a substituted DHED compound was carried out by Dong *et al*.[4] Their procedure also used THF and 1.5 eq. of $POCl_3$ for activation. The reported overall reaction time was 3 days. Unsworth *et al.* (obtained the highest yield of 88% so far reported), and Decker used toluene as solvent, higher temperatures of 115 °C and a larger excess of 6.0 eq of $POCl_3$.[24, 29] Reported reaction times range from 1 h up to 4.5 h.

As mentioned above, we observed the loss of the *N*-methyl group by tracking the reaction mixture at certain time points with LCMS upon synthesizing benz-DHED. *N*-Benzylation of the anthranilic moiety and subjection together with



Scheme 2. Synthetic scheme to obtain the reference compounds **8** (benz-rutaecarpine), **4b** (benz-DHED) and **2b** (DHED).

benzo-lactam **9** to the above reaction conditions led to formation of benzylchloride **14** as determined by GC-MS (Scheme 3). This leads to the hypothesis that chloride ions are responsible for nucleophilic demethylation.[34] Since the benzyl group represents a good leaving group, it is also possible that the benzyl cation is formed first, because the quinazolinone might be more stable compared to the quinazolinium ion. The benzyl cation can later react with chloride ions in the reaction mixture. Therefore a second experiment was carried out by heating benz-DHED with HCl and diazabicyclooctane in an excess of 3.0 eq., as sterically hindered non nucleophilic base to complex chloride ions,. Thereby, 63% of demethylated product (benz-rutaecarpine) formed. The demethylation reaction was not observed for DHED during the reaction conditions. This accounts for a higher intrinsic stability of DHED compared to benz-DHED which might be due to electronic properties of the indole ring system. Under the reaction conditions chosen (use of $POCl_3$) formation of HCl can hardly be prevented and therefore the instability of benz-DHED seems unavoidable and limits this particular synthetic approach. Therefore the emergence of an alternative synthetic route is highly desirable and is presented below.



Scheme 3. Proposed mechanisms for debenzylation: **a**) nucleophilic debenzylation by chloride ion attack and **b**) spontaneous debenzylation yielding benz-rutaecarpine and benzylchloride during the reaction process.[34]

Stability tests were conducted to further study the heterocycles' stability at different conditions (Table 1). At first, DHED and its benz-analog in their ring open form were heated in dry toluene for 2h at reflux temperature with either 10 eq. reagent or without any reagent. This procedure was adapted from Decker and Unsworth *et al*., respectively.[24, 29] As reagents either HCl, KOH or water were chosen and added to the reaction mixture in an excess of 10.0 eq. Heating in toluene and water did not decompose the compounds and starting material was recovered. This accounts for the fact, that benz-DHED itself is stable and does not demethylate spontaneously. Under the application of excess KOH solution hydrolysis to the starting materials was observed. The compounds' behavior changed using excess of HCl in *i*-propanol. For benz-DHED, the demethylated product, benz-rutaecarpine, was formed, whereas DHED proved to be stable and salt formation was observed.

2.2. DHED´s and benz-DHED´s behavior under hydrogenation conditions

The compounds' stability under hydrogenation conditions was also investigated. Hydrogenation conditions have been applied to DHED to obtain evodiamine. In the literature DHED was reacted with $NaBH_3CN$ and H_2-PtO_2 in acetic and formic acid to yield racemic evodiamine.[12, 28] Also BH_3 in THF yielded evodiamine.[28]

The benzyl group is a widely used orthogonal protection group in the chemistry of quinazolinone-related heterocycles and can be easily removed under hydrogenation conditions without tedious purification methods.[35-38] Cleavage of the benzyl group is typically performed in methanol at room temperature using 10-mol% Pd/C catalyst.[37] Sawatzky *et al*. found that a benzyl protected evodiamine which bears a benzyloxy function in *para*-position to the anilinic nitrogen can be cleaved without decomposition of the heterocyclic scaffold. When the C=O double bond was reduced first, the scaffold is more sensitive toward hydrogenation conditions and Pd/C-catalyzed hydrogenation leads to ring-cleavage, whereas reaction in conc. HCl is suitable for debenzylation.[37]

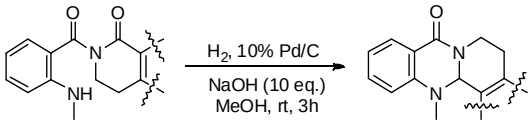
Hydrogenation conditions were applied to the alkaloids in their open form (**2b** and **4b**) and the addition of base was supposed to shift the equilibrium to the open form to prevent C=N bond reduction. Reduction of the C=N-double bond was observed even under basic conditions and yielded evodiamine and benz-evodiamine, respectively (Scheme 4). Hydrogenations without the addition of base also yielded the correspondin

Table 1. Stability test of **2b** and **4b** in toluene with addition of acid, base or water. The table shows the products formed.

starting material → (reagent; toluene, reflux, 2 h) → product

Starting material	Product formed			
	HCl in *iso*-propanol	No reagent	H_2O	KOH in *iso*-propanol
	10.0 eq.	-	10.0 eq.	10.0 eq.
Benz-DHED **4b**	**8**	**4b**	**4b**	**9** and *N*-methyl anthranilic acid
DHED **2b**	**2a**	**2b**	**2b**	**12** and *N*-methyl anthranilic acid

evodiamine core as expected. From this it can be concluded that an equilibrium between the open form and quinazolinium form exists even in the presence of base. Formation of the quinazolinium form followed by reduction leads to the corresponding dihydro-quinazolinones (evodiamine). It is concluded that DHED and its benz-analog are not stable toward hydrogenation conditions since the C=N-double bond is reduced.



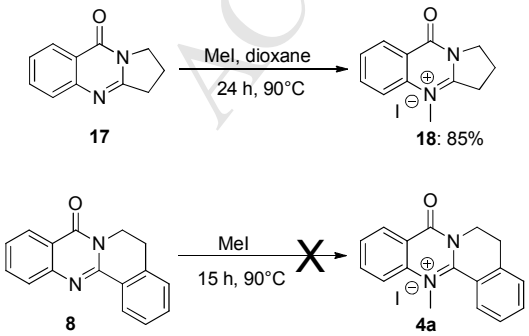
2b or **4b** **15** or **16**

Scheme 4. Application of hydrogenation conditions to **2b** and **4b** with the addition of base yielded benz-evodiamine and evodiamine as product.

2.3. Other synthetic approaches to DHED and benz-DHED

As outlined above, condensation of lactams with anthranilic esters leads to dealkylation necessitating an alternative synthetic approach to access DHED, especially benz-DHED and derivatives thereof in high yields. Two alternative reaction pathways starting from the alkaloids rutaecarpine or evodiamine are possible: 1) direct methylation of rutaecarpine or benz-rutaecarpine (Scheme 5) and 2) the oxidation of evodiamine or benz-evodiamine (Scheme 6).

Direct methylation of tricyclic quinazolinones like **17** is successful with moderate yields as shown by Darras *et al.* (Scheme 5).[32] This synthesis route fails for benz-rutaecarpine using among others neat methyliodide and dimethylsulfate and starting material is recovered (*cf.* SI for summary of reaction conditions).



17 **18**: 85%

8 **4a**

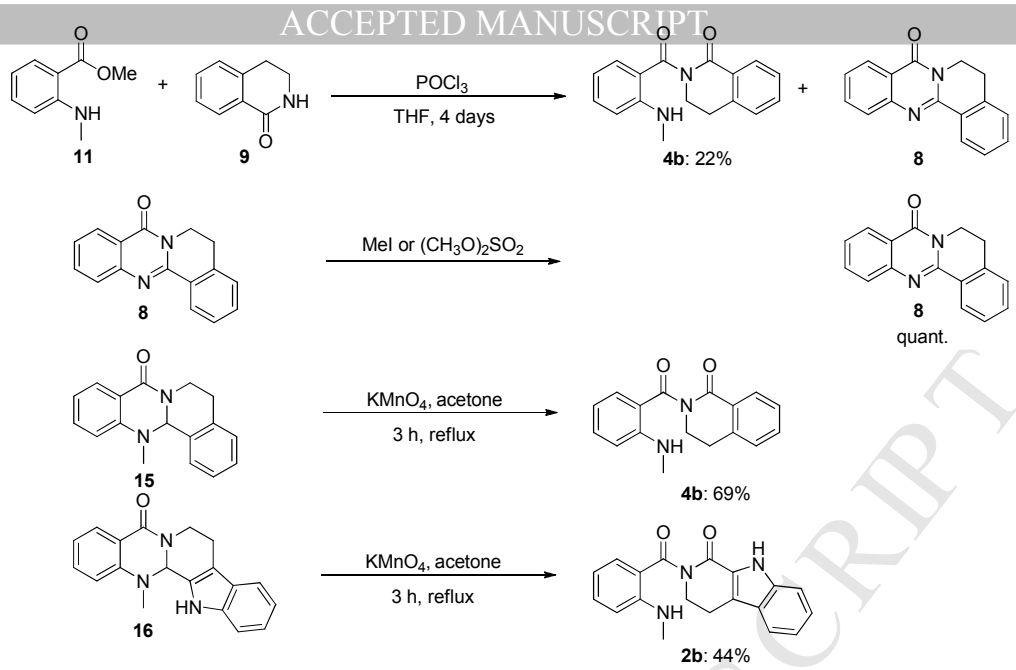
Scheme 5. Successful methylation of the tricyclic quinazolinone compound **17** (top).[32] Methylation of benz-rutaecarpine **8** failed (bottom).

This is in accordance to the demethylation results and might be caused by steric hindrance of this nitrogen atom by the extra benzene ring in this scaffold compared to the tricyclic quinazolinone **17**.

Gopinath *et al.*[39] and Danieli and Palmisano[40] reported on the oxidation of evodiamine with $KMnO_4$ and the latter also by other oxidizing agents, but without presenting any reaction conditions[39] or spectral data.[40] These early observations have not been followed up in later synthetic efforts, probably because of the limited synthetic access to evodiamine and derivatives at that time. We applied different equivalents of $KMnO_4$ (1.0 eq, 1.2 eq, 1.5 eq and 2.0 eq, respectively) and reactions were carried out in acetone under reflux for 3 h. The usage of 2.0 eq $KMnO_4$ for evodiamine gave the desired DHED compound after recrystallization from MeOH (44%, unoptimized yield). The desired benz-DHED crystallized from the crude reaction mixture (1.5 eq $KMnO_4$) and was purified by washing with petrol ether and Et_2O (69% yield). Benz-DHED is more sensitive toward oxidative conditions. When more than 2.0 eq of $KMnO_4$ are used, oxidative demethylation occurs, yielding small amounts of benz-rutaecarpine (2 %, HPLC yield). Oxidation represents a simple and high yield synthetic access to tetra- and pentacyclic quinazolinium salts from the evodiamine derivatives in a one pot reaction without time consuming purification necessity. We could show that these conditions are well applicable to the benz-analog also. Evodiamine and benz-evodiamine are synthetically readily available.[6, 28, 41-43]

2.4. Investigation into the equilibria in aqueous solution

Studies showed that DHED·Cl improves cognition in several animal models of dementia.[20-23] Besides the advantage of having a better synthesis method available which can be applied for the synthesis of structural analogs it is important to know, especially for medicinal chemists, which of the two forms (quinazolinium salt or open form) is predominant at physiological pH and therefore responsible for interaction with the target. We thus systematically investigated the equilibrium between the quinazolinium salt of DHED·Cl (yellow in water and polar organic solvents) and the ring open form (orange in organic solvents) by UV-Vis measurements (Figure 1b). To the best of our knowledge this has never been investigated before. Both the quinazolinium form (salt, from stock solution dissolved in water) and the free base (from stock solution dissolved in methanol) showed the same behavior when incubated in aqueous buffer solution with pH values ranging from 2 – 10 (Figure 2). In acidic media (pH <7) the dominance of one species with an absorption maximum of 364 nm is observed, presumably the quinazolinium form of DHED·Cl (pale yellow color).



Scheme 6. Fusion of lactam and anthranilic ester gave by-product formation (benz-rutaecarpine, **8**). The alternative reaction pathway to obtain benz-DHED via direct methylation failed; the oxidation of benz-evodiamine **15** and evodiamine **16** to benz-DHED **4b** and DHED **2b**, respectively, is an alternative reaction pathway circumventing demethylation as side reaction.

The appearance of an absorption maxima of >400 nm was expected in cuvettes with alkaline pH-value associated with the deep orange color of the ring open form **2b**.[12, 44] This observation led to the assumption that the so called "13b-hydroxy-evodiamine" (**2c**, Figure 2) is the form which might be enriched in alkaline pH >7. This structure was first proposed by Gopinath *et al*. to be the free base which was then disproved by Pachter and Suld.[12, 39] However, in this context the formation of "hydroxy-evodiamine" seems to be reasonable due to its structure presumably being more water soluble compared to the free base (Figure 2).

As can be seen in Fig. 2, there is a significant time-dependency of the occurrence of either compound **2a** or **2c** in buffer solution. It seems therefore necessary for pharmacological and biological evaluation to equilibrate the test compounds´ solution before testing and ideally to prove, e.g. by UV, that the equilibrium is reached depending on the pH applied. At pH = 8.50, at which presumably **2c** is predominant in aqueous media, a time period of 18 h is recommended based on our studies (*c.f.* Supporting Information). To get more information about the biologically active form, the IC_{50} values for DHED and benz-DHED were determined on *ee*AChE and *eq*BChE using Ellman´s assay at pH = 8 and pH = 7, respectively. Identical IC_{50} values in the one- to two-digit micromolar range were determined for both pH-values when a short incubation time (4.5min) was used. Hence under these conditions with short incubation time the quinazolinium form is presumably the predominant form, responsible for the effect on the enzyme (*c.f.* Supporting Information).

2.5. Radical-scavenging properties

The ORAC-assay (oxygen radical absorbance capacity) was performed to test DHED's and benz-DHED's antioxidant capacity. Antioxidants can prevent reactive oxygen species (ROS) induced neuronal cell death. Results are expressed in relation to radical scavenging properties of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (= trolox, a water soluble vitamin E analog) given in trolox equivalent (TE) unit.

DHED showed 1.44 ± 0.17 and benz-DHED 0.10 ± 0.03 trolox equivalents. This indicates that DHED possesses more pronounced radical scavenging properties, whereas benz-DHED shows much weaker radical scavenging properties. These findings are in accordance to other evodiamine or benz-evodiamine derived compounds where the indolo compounds also exhibited significantly higher trolox equivalents compared to the benz-derivatives.[32, 41] Our finding further supports the importance of the indole moiety for radical scavenging properties.

3. Conclusion

This study aimed to explore the properties of DHED and benz-DHED. UV-Vis measurements suggest that the equilibrium does not only contain the quinazolinium form and the corresponding ring open form, but presumably also the so-called "hydroxy-evodiamine" (and "hydroxy-benz-evodiamine", respectively) as water soluble form when incubated at pH >8. For medicinal chemists it is necessary to know which species is predominant at physiological pH for lead compound optimization and application of molecular docking techniques.

Besides studying these properties, new reaction pathways to DHED and especially its benz-analog were exploited. Demethylation yielding benz-rutaecarpine was clarified to be the dominant side reaction in the classical condensation of lactams with anthranilic esters resulting in low yields. All direct methylation attempts failed. This further proves the stability of the quinazolinone core in comparison to the qinazolinium structure. Oxidation of evodiamine, the latter can be obtained easily by fusion of anthranilic acid anhydride with dihydro-pyridoindole, proved to be a suitable synthetic method to give better yields for both the alkaloid DHED and the benz-DHED analog. Related synthetic methods might advantageously be explored in the future to access DHED-related compounds which were neglected by synthetic chemists up to now. Future work might focus on oxidation reactions towards evodiamine derivatives to explore the chemical space for novel DHED derivatives.

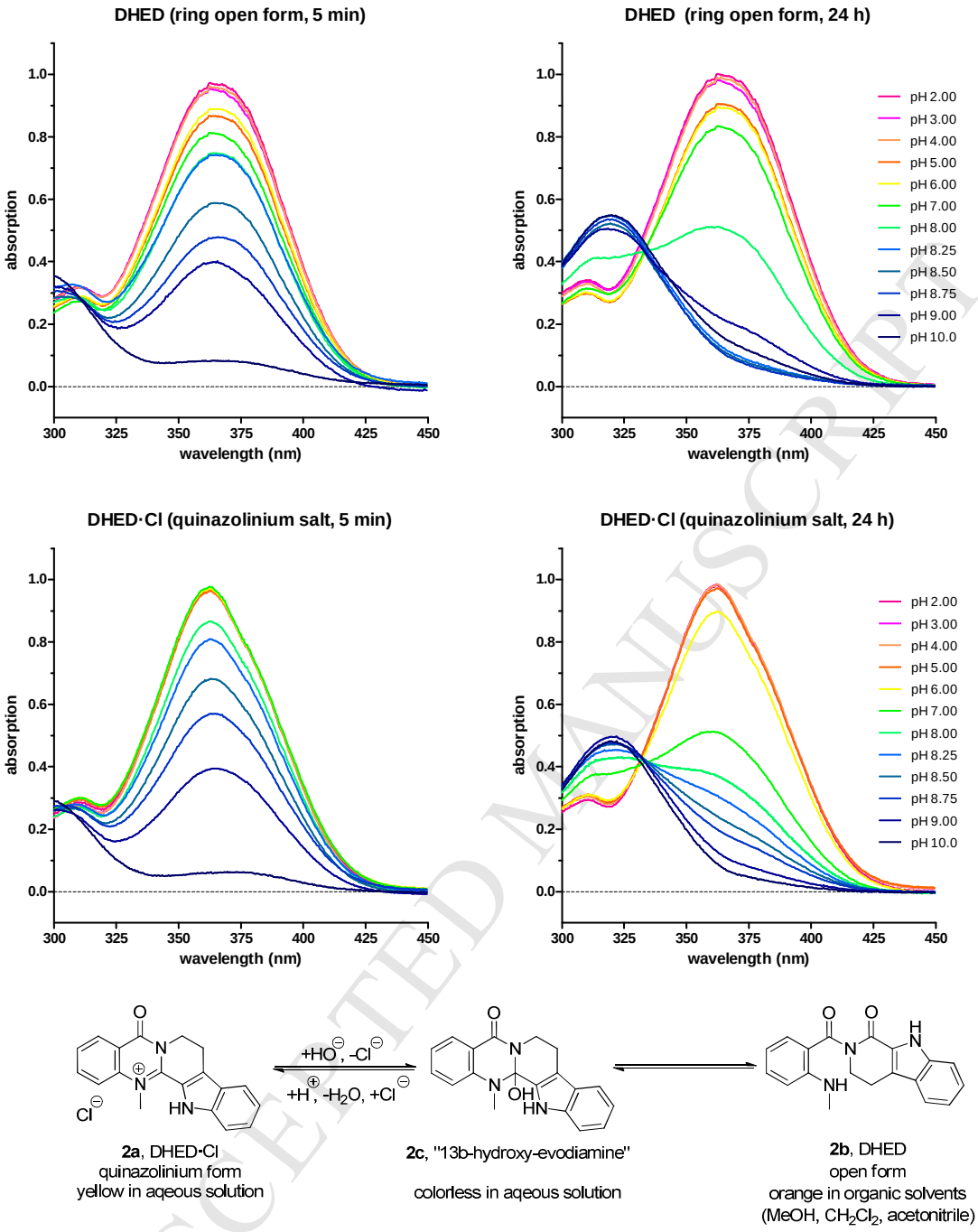


Figure 2. UV-Vis measurements of DHED as ring open form (top) and DHED as salt (bottom) in pH between 2 and 10 showing the same behavior starting from both compound forms. Therefore an equilibrium of the two first species (**2a** and **2c**) in aqueous solution is proposed and **2b** presumably can only be accessed using organic solvents.

4. Experimental section

Common reagents and solvents were obtained from commercial suppliers and used without further purification. Dry solvents like tetrahydrofuran (THF) and toluene were distilled from sodium/benzophenone under argon atmosphere. Analytical thin layer chromatography was performed using pre-coated silica gel sheets (ALUGRAM® Xtra SIL G/UV_{254}) (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Visualization of compounds was performed using UV-light (λ = 254 nm, 366 nm). Compositions of the eluent mixtures are given in volume ratios (v:v). Melting points were measured with a *Büchi* B-540 without further correction. Nuclear magnetic resonance spectra were measured on an Advance 400 (Bruker, Karlsruhe, Germany). Chemical shifts (δ) are given in ppm relative to $CDCl_3$, DMSO-d_6 or MeOD (7.26/2.50/3.31 and 77.16/39.52/49.00 für ^{1}H and ^{13}C-NMR, respectively). Denotation of rings (for **8**, **2a**, **2b**, **4a** and **4b**) was done according to *Lin et al.*[16] IR spectra were measured on a Jasco FT-IR-6100 spectrometer (Jasco, Gross-Umstadt, Germany) with a diamond-ATR unit. The position of absorption bands were given in wavelength ($\tilde{\nu}$) with the unit cm^{-1}. Intensities of absorption bands are characterized using strong (s), middle (m) and weak (w), in case the signal is broad (br). Liquid chromatography-mass

spectrometry analytic measurements were performed on an LCMS system (Shimadzu, Kyōto, Japan), consisting of a controller DGU-20A3R, a pump LC-20AB, a degasser DGU-20A, a SPD-20A UV/Vis detector and an LCMS-2020 Single Quadrupol Mass Spectrometer with ESI and DUIS Ionisation units. Stationary phase was a Synergi 4U fusion-RP (150 x 4.6 mm) column. As mobile phase, gradient MeOH/water (phase A/phase B) were used. Purifications by chromatography were carried out under atmospheric pressure with silica gel 60 (SiO_2, particle size 63 – 200 nm (Merck, Darmstadt, Germany). The solvents used for this purpose as well as for extractions were purchased in technical grade and purified by distillation prior to use. The eluent mixtures are expressed as volume fractions (20 mL).

4.1. Experimental procedures to target compounds

*4.1.1. 2-(2-(Methylamino)benzoyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-one (**2b**)*

To a stirred solution of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-one (190 mg, 1.01 mmol, 1.0 eq) in dry THF (15 mL), $POCl_3$ (0.13 mL, 1.42 mmol, 1.4 eq) was added and the mixture was stirred under argon atmosphere for 3 h at 60 °C. Then, N-methylanthranilate (280 mg, 1.69 mmol, 1.7 eq) was added, the temperature increased to 75 °C and the mixture stirred for 96 h. The solution was cooled to rt, CH_2Cl_2 and 2M NaOH were added (pH = 13). The water layer was removed and the remaining orange colored organic layer was acidified with 2M HCl (pH = 2). By this the color disappeared and water was added. The organic phase was removed and 2M NaOH was added to the water phase. The water phase was extracted three times with CH_2Cl_2 (3 x 30 mL) and dried over Na_2SO_4. The solvent was evaporated and the remaining brownish oil was kept in the freezer. Upon warming it to rt light yellowish crystals formed. The crystals were filtered off and washed tree times with diethyl ether. A yellow solid was formed during washing. This was dried and then washed as above. This yielded an orange solid (89.4 mg, 0.28 mmol, 27%). R_f = 0.20 (SiO_2, 100% ethyl acetate). Mp = 170.7 – 173.0 °C. ^{1}H-NMR (400 MHz, $CDCl_3$, 300 K): δ = 10.12 (s, 1H, N*H*$_{indole}$), 7.63 – 7.61 (m, 1H, Ar-*H*$_{E\text{-}ring}$), 7.44 (dd, J = 8.0, 1.6 Hz, 1H, Ar-*H*$_{A\text{-}ring}$), 7.38 – 7.34 (m, 1H, Ar-*H*$_{A\text{-}ring}$), 7.30 – 7.23 (m, 1H, Ar-*H*$_{E\text{-}ring}$), 7.19 (d, J = 5.1 Hz, 1H, N*H*CH$_3$) 7.17 – 7.13 (m, 1H, Ar-*H*$_{E\text{-}ring}$), 7.09 (d, J = 8.3 Hz, 1H, Ar-*H*$_{E\text{-}ring}$), 6.77 (dd, J = 8.6, 1.0 Hz, 1H, Ar-H$_{A\text{-}ring}$), 6.53 – 6.49 (m, 1H, Ar-*H*$_{A\text{-}ring}$), 4.19 (t, J = 6.5 Hz, 2H, NC*H*$_2$CH$_2$), 3.20 (t, J = 6.5 Hz, 2H, NCH$_2$C*H*$_2$), 2.95 (d, J = 4.8 Hz, 3H, C*H*$_3$) ppm. ^{13}C{1H}-NMR (101 MHz, $CDCl_3$, 300 K): δ = 175.98 (s, *C*=O), 162.27 (s, *C*=O), 151.15 (s, *C*$_{quart.}$), 151.05 (s, *C*$_{quart.}$), 138.70 (s, *C*$_{quart.}$), 134.66 (s, Ar-*C*), 132.50 (s, Ar-*C*), 126.24 (s, Ar-*C*), 124.83 (s, *C*$_{quart.}$), 122.94 (s, *C*$_{quart.}$), 120.71 (s, Ar-*C*), 120.67 (s, Ar-*C*), 115.90 (s, *C*$_{quart.}$), 114.73 (s, Ar-*C*), 113.16 (s, Ar-*C*), 111.22 (s, Ar-*C*), 47.64 (s, NCH$_2$CH$_2$), 29.89 (s, *C*H$_3$), 21.29 (s, NCH$_2$*C*H$_2$) ppm. IR: ν = 3403w, 3264m, 2811w, 2371w, 2357w, 2349w, 1662s, 1605m, 1574m, 1551m, 1511m, 1487m, 1424w, 1395m, 1368w, 1321m, 1287m, 1265w, 1243w, 1231m, 1202m, 1174m, 1128w, 1107w, 1093w, 1067w, 1047m, 1003w, 993w, 980w, 946m, 892m, 851w, 803w, 776w, 768m, 747s, 740s, 712m, 676w, 659m cm^{-1}. HPLC: Synergi 4U fusion-RP (15 x 0.46 cm), water/methanol (30-90%), 0.1% formic acid, 1.00 mL/min, 20 °C, t_R= 4.537 min, purity= 95.30%. Mass: calc. for $[M+H]^+$ ($C_{19}H_{18}N_3O_2$) requires m/z: 320.14 (open), 302.13 (closed); found: 302.20. Spectral data is in accordance with literature data.[28]

*4.1.2. 14-Methyl-5-oxo-5,7,8,13-tetrahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-14-ium chloride (**2a**)*

Compound **2b** was dissolved in CH_2Cl_2 and 1 M HCl in *iso*-propanol (8.0 eq) was added. The mixture discolored immediately and was stirred for 15 min at rt prior to removal of the solvent *in vacuo*. This yielded the quinazolinium form **2a** in quantitative yield and no further purification was necessary. R_f = 0.29 (tailing) (SiO_2, 100% ethyl acetate). Mp = 204 – 206 °C. ^{1}H-NMR (400 MHz, MeOD, 300 K): δ = 8.54 – 8.44 (m, 1H, Ar-*H*$_{E\text{-}ring}$), 8.19 – 8.09 (m, 2H, Ar-*H*$_{E\text{-}ring}$), 7.90 (dt, *J* = 8.3, 1.0 Hz, 1H, Ar-*H*$_{A\text{-}ring}$), 7.83 (ddd, *J* = 8.1, 6.9, 1.3 Hz, 1H, Ar-*H*$_{E\text{-}ring}$), 7.74 – 7.67 (m, 1H, Ar-*H*$_{A\text{-}ring}$), 7.60 (ddd, *J* = 8.4, 6.9, 1.1 Hz, 1H, Ar-*H*$_{A\text{-}ring}$), 7.35 (ddd, *J* = 8.1, 6.9, 0.9 Hz, 1H, Ar-*H*$_{A\text{-}ring}$), 4.69 – 4.58 (m, 2H, NC*H*$_2$CH$_2$), 4.49 (s, 3H, *C*H$_3$), 3.50 – 3.40 (m, 2H, NCH$_2$*C*H$_2$) ppm. (NH missing in MeOD) ^{13}C{^{1}H}-NMR (101 MHz, MeOD, 300 K): δ = 159.54 (s, *C*=O), 151.75 (s, *C*=N), 143.60 (s, *C*$_{quart.}$), 141.36 (s, *C*$_{quart.}$), 137.90 (s, Ar-*C*), 133.08 (s, *C*$_{quart.}$), 130.67 (s, Ar-*C*), 129.86 (s, Ar-*C*), 129.45 (s, Ar-*C*), 125.15 (s, *C*$_{quart.}$), 123.35 (s, Ar-*C*), 122.57 (s, Ar-*C*), 121.11 (s, *C*$_{quart.}$), 120.42 (s, *C*$_{quart.}$), 119.28 (s, Ar-*C*), 114.41 (s, Ar-*C*), 43.46 (s, NCH$_2$CH$_2$), 41.45 (s, *C*H$_3$), 20.03 (s, NCH$_2$*C*H$_2$) ppm. IR: ν = 3287 – 2308 brw, 1701s, 1608s, 1542s, 1498s, 1455m, 1511m, 1384w, 1365w, 1335s, 1278m, 1255m, 1236w, 1206m, 1167w, 1122w, 1103m, 1049w, 1008w, 966w, 936w, 851w, 768m, 751s, 718w, 675m cm^{-1}. HPLC: Synergi 4U fusion-RP (15 x 0.46 cm), water/methanol (30-95%), 0.1% formic acid, 1.00 mL/min, 20 °C, t_R= 4.599 min, purity= 94.99%. Mass: calc. for $[M]^+$ ($C_{19}H_{16}N_3O$) requires m/z: 302.13; found: 302.15.

*4.1.3. 2-(2-(Methylamino)benzoyl)-3,4-dihydroisoquinolin-1(2H)-one (**4b**)*

3,4-Dihydroisoquinolin-1(2*H*)-one (170 mg, 1.18 mmol, 1.0 eq) was dissolved in dry THF (12 mL) and $POCl_3$ (0.18 mL, 1.75 mmol, 1.5 eq) added at 60 °C. The mixture was stirred under Ar atmosphere for 90 min. Methyl 2-(methylamino)benzoate (300 mg, 1.79 mmol, 1.5 eq) was dissolved in dry THF (3.5 mL) and added dropwise to the reaction mixture over 5 min. The temperature was increased to 75 °C and the solution stirred for 41 h. The solution was cooled to rt and 2M HCl and CH_2Cl_2 were added until the aqeous phase reached pH = 2. The organic layer was separated and the aqueous layer was basified using 2M NaOH to pH = 9. By this a color change from colorless to yellow was observed. The water phase was extracted three times with CH_2Cl_2 (3 x 50 mL) and dried over Na_2SO_4. The solvent was evaporated. The formed crystals were filtered off and washed three times with petrol ether until the organic phase remained colorless. This yielded light-yellowish crystals (71.7 mg, 0.26 mmol, 22%). R_f = 0.23-0.72 (tailing) (SiO_2, methanol/dichloromethane/NH_3 (25% aq-solution) 10:1:0.1%). Mp = 136.5 – 137.0 °C. ^{1}H-NMR (400 MHz, $CDCl_3$, 300 K): δ = 8.13 (dd, *J* = 7.8, 1.4 Hz, 1H, C(NH)-C*H*$_{A\text{-}ring}$), 7.52 (td, *J* = 7.5, 1.4 Hz, 1H, Ar-*H*$_{D\text{-}ring}$), 7.43 (dd, *J* = 8.0, 1.6 Hz, 1H, Ar-*H*$_{D\text{-}ring}$), 7.39 – 7.35 (m, 2H, Ar-*H*$_{A\text{-}ring}$), 7.32 – 7.27 (m, 2H, N*H* and Ar-*H*$_{D\text{-}ring}$), 6.73 (dd, *J* = 8.5, 1.0 Hz, 1H, C$_{quart.}$-C*H*$_{A\text{-}ring}$), 6.55 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1H, Ar-*H*$_{D\text{-}ring}$), 4.01 – 3.98 (m, 2H, NC*H*$_2$CH$_2$), 3.18 (t, *J* = 6.2 Hz, 2H, NCH$_2$C*H*$_2$), 2.94 (d, *J* = 4.6 Hz, 3H, C*H*$_3$) ppm. ^{13}C{^{1}H}-NMR (101 MHz, $CDCl_3$, 300 K): δ = 176.61 (s, *C*=O), 165.51 (s, *C*=O), 151.37 (s, *C*$_{quart.}$), 139.92 (s, *C*$_{quart.}$), 134.87 (s, Ar-*C*$_{A\text{-}ring}$), 133.32 (s, Ar-*C*$_{D\text{-}ring}$), 132.64 (Ar-*C*$_{D\text{-}ring}$), 129.76 (s, C(NH)-*C*H$_{A\text{-}ring}$), 128.69 (s, *C*$_{quart.}$), 127.63 (s, Ar-*C*$_{D\text{-}ring}$), 127.44 (s, Ar-*C*$_{A\text{-}ring}$), 115.21 (s, *C*$_{quart.}$), 114.64 (s, Ar-*C*$_{D\text{-}ring}$), 111.41 (s, Ar-*C*$_{A\text{-}ring}$), 45.11 (s, N*C*H$_2$CH$_2$), 29.82 (s, *C*H$_3$), 28.65 (s, NCH$_2$*C*H$_2$) ppm. IR: ν = 3370m, 2898w, 2822w, 2372w, 2350w, 1688s, 1640s, 1603m, 1569m, 1516m, 1470m, 1422m, 1374m, 1335m, 1303s, 1265s, 1223s, 1176m, 1140s, 1089m, 1043m, 1006m, 960m, 905m, 848m, 797m, 786m, 742s, 716m, 691m, 653m cm^{-1}. HPLC: Synergi 4U fusion-

RP (15 x 0.46 cm), water/methanol (10-80%), 1.00 mL/min, 20 °C, t_R= 4.916 min, purity >99.99%. Mass: calc. for $[M+H]^+$ ($C_{17}H_{17}N_2O_2$) requires m/z: 281.13 (open), 263.12 (closed); found: 263.15. Spectral data is in accordance with literature data.[24]

*4.1.4. 13-Methyl-8-oxo-6,8-dihydro-5H-isoquinolino[1,2-b]quinazolin-13-ium chloride (**4a**)*

Compound **4b** was dissolved in CH_2Cl_2 and 1 M HCl in *iso*-propanol (8.0 eq) was added. The mixture discolored immediately and was stirred for 15 min at rt prior to removal of the solvent *in vacuo*. This yielded the quinazolinium form **4a** in quantitative yield and no further purification was necessary. R_f= 0.11 – 0.39 (tailing) (SiO_2, methanol/dichloromethane/NH_3 (25% aq-solution) 10:1:0.1%). Mp = 237 – 239 °C (decomposition). ^{1}H-NMR (400 MHz, MeOD, 300 K): δ = 8.53 – 8.45 (m, 1H, Ar-*H*), 8.23 – 8.12 (m, 3H, Ar-*H*), 7.91 – 7.82 (m, 2H, Ar-*H*), 7.70 – 7.67 (m, 2H, Ar-*H*), 4.42 (t, *J* = 6.2 Hz, 2H, NCH_2CH$_2$), 4.35 (s, 3H, CH_3), 3.26 (t, *J* = 6.3 Hz, 2H, NCH$_2$CH_2) ppm. ^{13}C{^{1}H}-NMR (101 MHz, MeOD, 300 K): δ = 159.92 (s, *C*=O), 159.05 (s, *C*=N), 144.27 (s, $C_{quart.}$), 141.67 (s, $C_{quart.}$), 138.12 (s, Ar-*C*), 137.32 (s, Ar-*C*), 133.48 (s, Ar-*C*), 130.66 (s, Ar-*C*), 129.84 (s, Ar-*C*), 129.32 (s, Ar-*C*), 128.63 (s, Ar-*C*), 124.12 (s, $C_{quart.}$), 120.44 (s, Ar-*C*), 120.22 (s, $C_{quart.}$), 64.75 (s, Ar-*C*), 45.08 (s, *C*H$_3$), 42.85 (s, NCH$_2$CH$_2$), 28.03 (s, NCH$_2$*C*H$_2$) ppm. IR: ν = 3353brw, 1693s, 1615s, 1600s, 1578w, 1545s, 1490s, 1461m, 1422s, 1334m, 1307m, 1282s, 1248m, 1213w, 1149m, 1102m, 1040w, 1000m, 977w, 953w, 903w, 815w, 791w, 763s, 745m, 687m, 666w cm^{-1}. HPLC: Synergi 4U fusion-RP (15 x 0.46 cm), water/methanol (30-90%), 1.00 mL/min, 20 °C, t_R= 2.454 min, purity= 98.27%. Mass: calc. for $[M]^+$ ($C_{17}H_{15}N_2O$) requires m/z: 263.12; found: 263.10.

*4.1.5. 5H-Isoquinolino[1,2-b]quinazolin-8(6H)-one (**8**)*

3,4-Dihydroisoquinolin-1(2*H*)-one (190 mg, 1.28 mmol, 1.0 eq) was dissolved in dry THF (15 mL) and $POCl_3$ (0.15 mL, 1.54 mmol, 1.2 eq) added at 60 °C. The mixture was stirred under Ar atmosphere for 40 min. Methyl 2-aminobenzoate (340 mg, 2.25 mmol, 1.8 eq) was dissolved in dry THF (3 mL) and added dropwise to the reaction mixture over 5 min. The temperature was increased to 75 °C and the solution stirred for 113 h. The solution was cooled to rt and 25% NH_3 solution (6 mL) was added until the aqeous phase reached pH = 9. The yellow mixture was stirred vigorously for 30 min. The mixture was extracted with CH_2Cl_2 (4 x 50 mL), washed with brine and the combined organic layers were dried over Na_2SO_4. Evaporation of solvent yielded 0.38 g of crude product. Parts of the crude product (220 mg) were purified by column chromatography (SiO_2, 30 x 2 cm, petrolether/ethyl acetate 2:1, F 8-15) to yield off-white crystals (84.7 mg, 0.302 mmol, 24%). R_f= 0.50 (SiO_2, petrolether/ethyl acetate 2:1). Mp = 158.0 – 158.7 °C. ^{1}H-NMR (400 MHz, $CDCl_3$, 300 K): δ = 8.56 – 8.41 (m, 1H, Ar-$H_{D\text{-ring}}$), 8.42 – 8.24 (m, 1H, Ar-$H_{A\text{-ring}}$), 7.86 – 7.63 (m, 2H, Ar-$H_{A\text{-ring}}$), 7.54 – 7.41 (m, 3H, Ar-$H_{D\text{-ring}}$ (2H) and Ar-$H_{A\text{-ring}}$ (1H)), 7.33 – 7.25 (m, 1H, Ar-$H_{D\text{-ring}}$), 4.47 – 4.37 (m, 2H, NCH_2CH$_2$), 3.11 (t, *J* = 6.4 Hz, 2H, NCH$_2$CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$, 300 K): δ = 161.85 (s, *C*=O), 149.51 (s, $C_{quart.}$), 147.97 (s, *C*=N), 137.19 (s, $C_{quart.}$), 134.35 (Ar-$C_{A\text{-ring}}$), 131.84 (Ar-$C_{A\text{-ring}}$), 129.74 (s, $C_{quart.}$), 128.18 (Ar-$C_{D\text{-ring}}$), 127.77 (2 Ar-$C_{A\text{-ring}}$), 127.64 (Ar-$C_{D\text{-ring}}$), 127.01 (Ar-$C_{D\text{-ring}}$), 126.66 (Ar-$C_{D\text{-ring}}$), 120.91 (s, $C_{quart.}$), 39.76 (s, NCH_2CH$_2$), 27.63 (s, NCH$_2$CH_2) ppm. IR: ν = 3070w, 3031w, 2928w, 2901w, 2850w, 2359w, 2120w, 1921w, 1668s, 1608m, 1589s, 1557s, 1470s, 1457s, 1395s, 1334s, 1308m, 1265m, 1253m, 1173m, 1149s, 1108m, 1065w, 1030w, 1013w, 980m, 958w, 947m, 905m, 876m, 840m, 795w, 760s, 737s, 705s, 691s, 669m cm^{-1}. HPLC: Synergi 4U fusion-RP (15 x 0.46 cm), water/methanol (30-90%), 1.00 mL/min, 20 °C, t_R= 9.917 min, purity >99.99%. Mass: calc. for $[M+H]^+$ ($C_{16}H_{13}N_2O$) requires m/z: 249.10; found: 249.05. Spectral data is in accordance with literature data.[24]

*4.1.6. 13-Methyl-13,13a-dihydro-5H-isoquinolino[1,2-b]quinazolin-8(6H)-one (**15**)*

The synthesis was carried out according to the procedure used by F. H. Darras, *et al*. with 1-Methyl-1H-benzo[d][1,3]oxazine-2,4-dione (0.32 g, 1.15 mmol, 1.0 eq), 3,4-dihydroisoquinoline (0.21 g, 1.18 mmol, 1.02 eq) in dry toluene (10 mL). This yielded the title compound as pale yellow solid (3.93 mmol, 87%).[32] R_f= 0.39 (SiO_2, PE/EA 3:1). Mp = 128.2 – 129.6 °C. ^{1}H-NMR (400 MHz, $CDCl_3$, 300 K): δ = 8.07 – 8.04 (m, 1H, Ar-$H_{D\text{-ring}}$), 7.48 – 7.39 (m, 1H, Ar-$H_{A\text{-ring}}$), 7.42 – 7.37 (m, 1H, Ar-$H_{A\text{-ring}}$), 7.31 – 7.29 (m, 2H, Ar-$H_{A\text{-ring}}$ and Ar-$H_{D\text{-ring}}$), 7.23 – 7.21 (m, 1H, Ar-$H_{D\text{-ring}}$), 7.13 – 7.05 (m, 2H, Ar-$H_{A\text{-ring}}$ and Ar-$H_{D\text{-ring}}$), 5.76 (s, 1H, NC*H*N), 4.69 – 4.64 (m, 1H, NC*H*HCH$_2$), 3.28 – 3.21 (m, 1H, NCH*H*CH$_2$), 3.08 – 2.97 (m, 1H, NCH$_2$C*H*H), 2.88 – 2.82 (m, 1H, NCH$_2$CH*H*), 2.59 (s, 3H, CH_3) ppm. ^{13}C{^{1}H}-NMR (101 MHz, $CDCl_3$, 300 K): δ = 164.39 (s, *C*=O), 150.55 (s, $C_{quart.}$), 137.15 (s, $C_{quart.}$), 133.20 (s, Ar-$C_{A\text{-ring}}$), 132.49 (s, $C_{quart.}$), 128.99 (s, Ar-$C_{D\text{-ring}}$), 128.84 (s, Ar-$C_{A\text{-ring}}$), 128.51 (s, Ar-$C_{D\text{-ring}}$), 128.06 (s, Ar-$C_{A\text{-ring}}$ or Ar-$C_{D\text{-ring}}$), 127.04 (s, Ar-$C_{A\text{-ring}}$ or Ar-$C_{D\text{-ring}}$), 122.35 (s, Ar-$C_{A\text{-ring}}$ or Ar-$C_{D\text{-ring}}$), 122.06 (s, $C_{quart.}$), 119.87 (s, Ar-$C_{A\text{-ring}}$ or Ar-$C_{D\text{-ring}}$), 72.06 (s, N*C*HN), 39.36 (s, NCH_2CH$_2$), 36.45 (s, *C*H$_3$), 28.62 (s, NCH$_2$CH_2), ppm. IR: ν = 2865w, 1649s, 1601m, 1465m, 1451m, 1418m, 1402m, 1364w, 1341m, 1302m, 1285m, 1240w, 1167m, 1144m, 1119m, 1076m, 1051w, 1031m, 955m, 928m, 905w, 876w, 858w, 807w, 796w, 781s, 761s, 702s, 661w, 651w cm^{-1}. HPLC: Synergi 4U fusion-RP (15 x 0.46 cm), water/methanol (30-90%), 0.1% formic acid, 1.00 mL/min, 20 °C, t_R= 9.870 min, purity >99.99%. Mass: calc. for $[M+H]^+$ ($C_{17}H_{17}N_2O$) requires m/z: 265.13; found: 265.10. Spectral data is in accordance with the literature.[32]

*4.1.7. 14-Methyl-7,8,13b,14-tetrahydroindolo[2',3':3,4]pyrido[2,1-b]quinazolin-5(13H)-one (**16**)*

The synthesis was carried out according to the procedure used by G. Huang, *et al*. with 1-methyl-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (0.82 g, 4.61 mmol, 1.0 eq), 4,9-dihydro-3*H*-pyrido[3,4-*b*]indole (0.80 g, 4.70 mmol, 1.02 eq) in dry CH_2Cl_2 (15 mL) yielding the title compound as beige solid (1.37 g, 4.52 mmol, 98%).[41] R_f= 0.70 (SiO_2, PE/EA 1:1). Mp = 254 – 256 °C (decomposition). ^{1}H-NMR (400 MHz, DMSO-d_6, 300 K): δ = 11.05 (s, 1H, N*H*), 7.79 (dd, *J* = 7.8, 1.6 Hz, 1H, Ar-*H*), 7.50 – 7.45 (m, 2H, Ar-*H*), 7.38 – 7.35 (m, 1H, Ar-*H*), 7.14 – 6.87 (m, 4H, Ar-*H*), 6.12 (d, *J* = 1.5 Hz, 1H, C$_{tert.}$-*H*), 4.65 – 4.61 (m, 1H, NC*H*HCH$_2$), 3.24 – 3.17 (m, 1H, NCH*H*CH$_2$), 2.97 – 2.89 (m, 1H, NCH$_2$C*H*H), 2.88 (s, 3H, CH_3), 2.84 – 2.75 (m, 1H, NCH$_2$CH*H*) ppm. ^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, 300 K): δ = 164.28 (s, *C*=O), 148.78 (s, $C_{quart.}$), 136.50 (s, $C_{quart.}$), 133.48 (s, Ar-*C*), 130.63 (s, $C_{quart.}$), 128.01 (s, Ar-*C*), 125.98 (s, $C_{quart.}$), 121.88 (s, Ar-*C*), 120.29 (s, Ar-*C*), 119.24 (s, $C_{quart.}$), 118.93 (s, Ar-*C*), 118.24 (s, Ar-*C*), 117.46 (s, Ar-*C*), 111.68 (s, Ar-*C*), 111.53 (s, $C_{quart.}$), 69.80 ($C_{tert.}$-H), 40.92 (NCH_2CH$_2$), 36.47 (*C*H$_3$), 19.51 (NCH$_2$CH_2) ppm. IR: ν = 3211w, 2943w, 2914w, 2845w, 1627s, 1604s, 1508m, 1494w, 1472w, 1447m, 1406w, 1389m, 1343w, 1323w, 1308m, 1280m, 1262m, 1227m, 1201w, 1164m, 1145w, 1129w, 1109w, 1028w, 1011w, 941w, 879w, 844w, 745s, 733s, 689m cm^{-1}. HPLC: Synergi 4U fusion-RP (15 x 0.46 cm), water/methanol (30-90%), 0.1% formic acid, 1.00 mL/min, 20 °C, t_R= 9.965 min, purity= 94.44%. Mass: calc. for $[M+H]^+$ ($C_{19}H_{18}N_3O$) requires m/z: 304.14; found: 304.10. Spectral data is in accordance with the literature.[43]

4.2. ORAC-assay

The antioxidant activity was determined by the oxygen radical absorbance capacity-fluorescein (ORAC-FL) assay.[32,41] The ORAC assay measures antioxidant scavenging activity against peroxyl radicals, their formation induced by 2,20-azobis(2 amidinopropane) dihydrochloride (AAPH) at 37°C.

The reaction was carried out in 75 mM phosphate buffer (pH 7.4) and the final reaction mixture was 200 mL. Antioxidant (20 mL) and fluorescein (120 mL, 300 nM final concentration) were placed in the wells of a 96 well plate and the mixture was incubated for 15 min at 37 °C. Then AAPH (Sigma, Steinheim Germany) solution (60 mL; 12 mM final concentration) was added rapidly. The plate was immediately placed into a SpectraFluor Plus plate reader (Tecan, Crailsheim, Germany) and fluorescence measured every 60 s for 90 min with excitation at 485 nm and emission at 535 nm. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Sigma, Steinheim, Germany) was used as standard (1-8 µM, final concentration). A blank (FL + AAPH) using phosphate buffer instead of antioxidant and Trolox calibration were carried out in each assay. The samples were measured at different concentrations (1-5 µM). All reaction mixtures were prepared fourfold and at least four independent runs were performed for each sample. Fluorescence measurements were normalized to the curve of the blank (without antioxidant). From the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as:

$$AUC = 1 + \sum_{i=1}^{i=90} f_i/f_0 \qquad (1)$$

Where f_0 is the initial fluorescence at 0 min and f_i is the fluorescence at time i. The net AUC for a sample was calculated as follows:

Net AUC = AUC antioxidant –AUC blank (2)

The ORAC-FL values were calculated:

[(AUC Sample _ AUC blank) / (AUC Trolox - AUC blank)]x [(concentration of Trolox/concentration of sample)] (3)

and expressed as Trolox equivalents by using the standard curve calculated for each assay. Final results were in µM of Trolox equivalent/µM of compound.

Author contributions

M. D. was responsible for supervision and development of the whole project. S. W. performed the chemical syntheses, stability tests and UV-Vis measurements. A. E. under the supervision of R. S. conducted the Aβ-inhibition assay.

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Supplementary data

Supplementary data (methylation conditions, UV-Vis for benz-DHED, experimental data for intermediates, neurotoxicity and Aβ-inhibition assays) associated with this article can be found in the online version, at http://dx.doi.org...

References and notes

1. Lee, S. H.; Son, J. K.; Jeong, B. S.; Jeong, T. C.; Chang, H. W.; Lee, E. S.; Jahng, Y. *Molecules* **2008**, *13*, 272-300.
2. Wang, G. J.; Wu, X. C.; Chen, C. F.; Lin, L. C.; Huang, Y. T.; Shan, J.; Pang, P. K. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 1237-1244.
3. Yuan, S. M.; Gao, K.; Wang, D. M.; Quan, X. Z.; Liu, J. N.; Ma, C. M.; Qin, C.; Zhang, L. F. *Acta Pharmacol. Sin.* **2011**, *32*, 295-302.
4. Dong, G.; Wang, S.; Miao, Z.; Yao, J.; Zhang, Y.; Guo, Z.; Zhang, W.; Sheng, C. *J. Med. Chem.* **2012**, *55*, 7593-7613.
5. Jiang, J.; Hu, C. *Molecules* **2009**, *14*, 1852-1859.
6. Wang, S.; Fang, K.; Dong, G.; Chen, S.; Liu, N.; Miao, Z.; Yao, J.; Li, J.; Zhang, W.; Sheng, C. *J. Med. Chem.* **2015**, *58*, 6678-6696.
7. Wang, B.; Mai, Y. C.; Li, Y.; Hou, J. Q.; Huang, S. L.; Ou, T. M.; Tan, J. H.; An, L. K.; Li, D.; Gu, L. Q.; Huang, Z. S. *Eur. J. Med. Chem.* **2010**, *45*, 1415-1423.
8. Chen, Z.; Hu, G.; Li, D.; Chen, J.; Li, Y.; Zhou, H.; Xie, Y. *Bioorg. Med. Chem.* **2009**, *17*, 2351-2359.
9. Kim, S. J.; Lee, S. J.; Lee, S.; Chae, S.; Han, M. D.; Mar, W.; Nam, K. W. *Biochem. Biophys. Res. Commun.* **2009**, *389*, 437-442.
10. Sheen, W. S.; Tsai, I. L.; Teng, C. M.; Ko, F. N.; Chen, I. S. *Planta Med.* **1996**, *62*, 175-176.
11. Asahina, Y.; Ohta, T. *J. Pharm. Soc. Japan* **1926**, *530*, 293.
12. Pachter, I. J.; Suld, G. *J. Org. Chem.* **1960**, *25*, 1680-1683.
13. Noh, E. J.; Ahn, K. S.; Shin, E. M.; Jung, S. H.; Kim, Y. S. *Life Sci.* **2006**, *79*, 695-701.
14. Peng, J.-H.; Zhang, C.-E.; Wei, W.; Hong, X.-P.; Pan, X.-P.; Wang, J.-Z. *Neuropharmacology* **2007**, *52*, 1521-1527.
15. Loh, S. H.; Tsai, Y. T.; Lee, C. Y.; Chang, C. Y.; Tsai, C. S.; Cheng, T. H.; Lin, C. I. *J. Ethnopharmacol.* **2014**, *153*, 753-762.
16. Lin, L. C.; Li, S. H.; Wu, Y. T.; Kuo, K. L.; Tsai, T. H. *J. Agric. Food Chem.* **2012**, *60*, 1595-1604.
17. Wong, K. K. *Planta Med.* **1996**, *62*, 246-249.
18. Ahn, S.-H.; Jeon, S.-H.; Tsuruo, T.; Shim, C.-K.; Chung, S.-J. *J. Pharm. Sci.* **2004**, *93*, 283-292.
19. Zhao, Y.; Zhao, Y.; Zhou, X.; Gong, X. *Pharmacogn Mag.* **2014**, *10*, 374-383.
20. Park, C. H.; Kim, S.-H.; Choi, W.; Lee, C.-J.; Kim, J. S.; Kang, S. S.; Suh, Y.-H. *Planta Med.* **1996**, *62*, 405-409.
21. Park, C. H.; Lee, Y.-J.; Lee, S. H.; Choi, S. H.; Kim, H.-S.; Jeong, S.-J.; Kim, S. S.; Suh, Y. H. *J. Neurochem.* **2000**, *74*, 244-253.
22. Park, E.-J.; Suh, Y.-H.; Kim, J.-Y.; Choi, S.; Lee, C.-J. *NeuroReport* **2003**, *14*, 399-403.
23. Kim, H. J.; Shin, K. Y.; Chang, K.-A.; Ahn, S.; Choi, H. S.; Kim, H.-S.; Suh, Y.-H. *Korean J. Physiol. Pharmacol.* **2014**, *18*, 55-59.
24. Decker, M. *Eur. J. Med. Chem.* **2005**, *40*, 305-313.
25. Son, J.-K.; Chang, H. W.; Jahng, Y. *Molecules* **2015**, *20*, 10800-10821.
26. Khan, I.; Ibrar, A.; Abbas, N.; Saeed, A. *Eur. J. Med. Chem.* **2014**, *76*, 193-244.
27. Khan, I.; Ibrar, A.; Ahmed, W.; Saeed, A. *Eur. J. Med. Chem.* **2015**, *90*, 124-169.
28. Nakayama, A.; Kogure, N.; Kitajima, M.; Takayama, H. *Heterocycles* **2008**, *76*, 861-865.
29. Unsworth, W. P.; Kitsiou, C.; Taylor, R. J. K. *Org. Lett.* **2013**, *15*, 3302-3305.
30. Huang, G.; Roos, D.; Stadtmüller, P.; Decker, M. *Tetrahedron Lett.* **2014**, *55*, 3607-3609.
31. Whittaker, N. *J. Chem. Soc. C.* **1969**, 85-89.

32. Darras, F. H.; Kling, B.; Heilmann, J.; Decker, M. *ACS Med. Chem. Lett.* **2012**, *3*, 914-919.
33. Beutner, G. L.; Kuethe, J. T.; Yasuda, N. *J. Org. Chem.* **2007**, *72*, 7058-7061.
34. Walport, L. J.; Hopkinson, R. J.; Schofield, C. J. *Curr. Opin. Chem. Biol.* **2012**, *16*, 525-534.
35. Martinot, T. A.; Austad, B. C.; Côté, A.; Depew, K. M.; Genov, D.; Greinier, L.; Helble, J.; Lescarbeau, A.; Nair, S.; Trudeau, M.; White, P.; Yu, L.-C. *Org. Process Res. Dev.* **2015**, *19*, 1693-1702.
36. Shaik, A. A.; Nishat, S.; Andreana, P. R. *Org. Lett.* **2015**, *17*, 2582-2585.
37. Swatzky, E.; Bukowczan, J.; Decker, M. *Tetrahedron Lett.* **2014**, *55*, 2973-2976.
38. Darras, F. H.; Pockes, S.; Huang, G.; Wehle, S.; Strasser, A.; Wittmann, H. J.; Nimczick, M.; Sotriffer, C. A.; Decker, M. *ACS Chem. Neurosci.* **2014**, *5*, 225-242.
39. Gopinath, K. W.; Govindachari, T. R.; Ramadas Rao, U. *Tetrahedron* **1960**, *8*, 293-295.
40. Danieli, B.; Palmisano, G. *Heterocycles* **1978**, *9*, 803-806.
41. Huang, G.; Kling, B.; Darras, F. H.; Heilmann, J.; Decker, M. *Eur. J. Med. Chem.* **2014**, *81*, 15-21.
42. Decker, M.; Krauth, F.; Lehmann, J. *Bioorg. Med. Chem.* **2006**, *14*, 1966-1977.
43. Unsworth, W. P.; Kitsiou, C.; Taylor, R. J. K. *Org. Lett.* **2013**, *15*, 258-261.
44. Bléger, D.; Schwarz, J.; Brouwer, A. M.; Hecht, S. *J. Am. Chem. Soc.* **2012**, *134*, 20597-20600.