Bioorganic & Medicinal Chemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Quinolone–benzylpiperidine derivatives as novel acetylcholinesterase inhibitor and antioxidant hybrids for Alzheimer Disease

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ARTICLE INFO

Article history:
Received 16 December 2013
Revised 20 February 2014
Accepted 24 February 2014
Available online xxxx

Keywords:
Acetylcholinesterase
Antioxidant
Hybrid
Quinolone
N-Benzylpiperidine

ABSTRACT

Design, synthesis and evaluation of new acetylcholinesterase inhibitors by combining quinolinecarboxamide to a benzylpiperidine moiety are described. Then, a series of hybrids have been developed by introducing radical scavengers. Molecular modeling was performed and structure activity relationships are discussed. Among the series, most potent compounds show effective AchE inhibitions, high selectivities over butyrylcholinesterase and high radical scavenging activities. On the basis of this work, the ability of quinolone derivatives to serve in the design of *N*-benzylpiperidine linked multipotent molecules for the treatment of Alzheimer Disease has been established.

1. Introduction

Alzheimer's Disease (AD) is the major cause of dementia affecting approximately 10% of the population over the age of 65-year-old and its incidence rises exponentially with age.[1] This incurable neurodegenerative disease is characterized by progressive failure of thought, memory and language. The primary therapeutic approach to address cognitive loss associated with AD is based on acetylcholinesterase (AChE).[2] This symptomatic treatment led to the suggestion that AD is associated with an impairment in cholinergic transmission.[3]

The AD pathogenesis involves numerous pathway and several theories. The widely accepted amyloid theory states that an accumulation of β-amyloid peptides, a main component of the senile plaques, initiates the pathogenic cascade.[4] Then, takes place inflammation, neuronal dysfunctions and imbalance of kinase and phosphatase activities causing the characteristic neurofibrillary tangles. Another theory suggests that neurofibrillary tangles occur prior to the senile plaques.[5] In any case, oxidative stress is a main feature of neuroinflammation and leads to neuronal damages.[6] Beyond a symptomatic effect, clinical evidences accumulated during the last decade suggest a neuroprotective effect of currently marketed anti-Alzheimer AChE inhibitors.[7] In order to improve AChE inhibitors as a pharmacological instrument to retard progressive neurodegeneration, scientists are engaged in the development of multipotent compounds.[8] As oxidative stress plays a central role in AD pathogenesis, it is a key target to retard AD's progression[9] and multitargeted drugs have been designed to combine AChE inhibition and antioxidant properties.[10]

The multitarget approach is based on the unique structural properties of AChE and the interaction of the enzyme with the inhibitors.[11] The structure of AChE reveals two main binding sites: the catalytic binding site, comprising the Ser-His-Glu catalytic triad, and the peripheral anionic binding site, connected by a deep and hydrophobic gorge. Dual binding site inhibitors bind to both the catalytic and the peripheral site. They are designed by assembling a heterocyclic ring to an *N*-benzylpiperidine or to a tacrine through a linker of appropriate length. Heterocyclic ring interacts with the peripheral anionic site while the second moiety binds to the gorge and the catalytic site.[12] In hybrid design, the moiety that scavenges Reactive Oxygen Species (ROS) generally interacts with the peripheral anionic site[13] which is implicated in other biological processes.[14]

The quinolone structures include motifs exhibiting a wide variety of biological activities[15] One of which is ROS scavenging ability.[16] Quinolone scaffold has already been efficiently used for

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http://dx.doi.org/10.1016/j.bmc.2014.02.046
0968-0896/

design of dual AChE inhibitor.[17] However quinolones have never been associated to a benzylpiperidine moiety for this purpose. Moreover, such derivatives of coumarin, the oxygen analog of quinolone, are efficient and widely studied AChE inhibitors.[18] We introduce here new acetylcholinesterase inhibitors, whose structures include a quinolone and a benzylpiperidine moiety. To assess their potential in hybrids design a series of compounds displaying simultaneously acetylcholinesterase inhibition and antioxidant activity have been developed. Inhibitions of AChE and BuChE have been determined using Ellman's method and the antioxidant properties evaluated by radical scavenging activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and anion superoxide. In adition, molecular modelling study was performed to determine the binding mode of the best inhibitor to the AChE.

2. Results and discussion

2.1. Synthetic pathways

The synthesis of compounds **5a–j** has been approached as shown in Scheme 1. The quinolone moiety (**3**) was easily prepared by treatment of isatoic anhydride (**1**) or aminophenone (**2**) with diethylmalonate and then the benzylpiperidine moiety was attached trough amidification.

N-benzylisatoic anhydride **1c** was obtained by N-alkylation of isatoic anhydride **1a** with benzyl bromide.[19] Quinolones **3a–c** were prepared from isatoic anhydride and diethyl malonate using NaH in DMF as previously described.[20] Quinolones **3d** and **3e** were obtained by reaction of diethyl malonate and respectively 2′-aminoacetophenone and 2-aminobenzophenone under reflux with DBU. Amides **5a–f** (R_2 = OH) were obtained directly by the reaction of esters **3a–c** with 4-amino-1-benzylpiperidine (n = 0) or 2-(1-benzyl-4-piperidinyl)methylamine (n = 1) in refluxing xylene. For amides **5g–h** (R_2 = Me) and **5i** (R_2 = Ph), we had to proceed through an acyl chloride. First, **3d** and **3e** were saponified by NaOH in an ethanol–water mixture under reflux. Carboxylic acids **4d** and **4e** were converted into acyl chlorides with oxalyl chloride and a catalytic amount of DMF in CH_2Cl_2. Acyl chlorides were directly engaged in amidification with 4-amino-1-benzylpiperidine or 2-(1-benzyl-4-piperidinyl)methylamine in presence of Et_3N as base in CH_2Cl_2 to afford **5g–i**. The difference can be explained by an intramolecular H-bond between hydroxyl substituent in position 4 (R_2 = OH) and the ester function in position 3 that presumably provides transamidification. In addition **5d** was chlorinated by $POCl_3$, then the 4-chloro substituted derivative was reacted with NH_3 in ethanol to give **5j**.

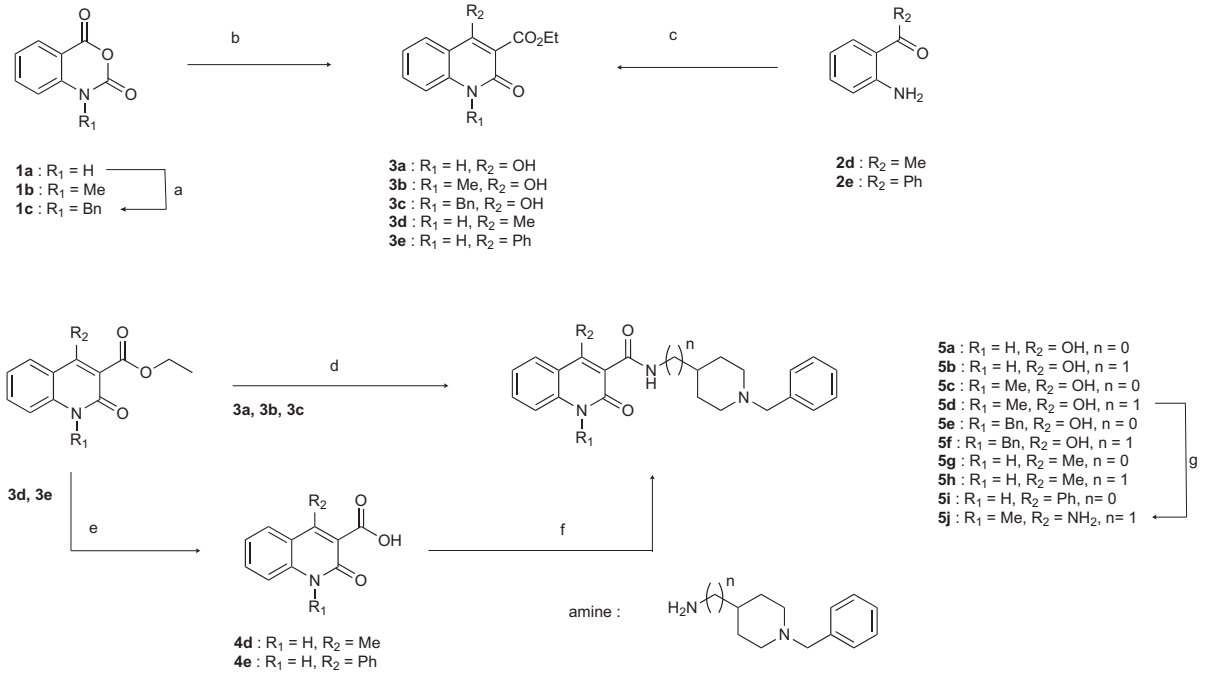
Compounds **8a–d** and **9a–d** were prepared according to the route outlined in Scheme 2. Methoxylated derivatives of quinolone (**7**) were prepared starting from 4,5-dimethoxyanthranilate (**6a–c**) or N-substituted anthranilate with methoxybenzyl (**6d**). Then benzylpiperidine moiety was coupled through amidification. The hybrid compounds were obtained by demethylation.

Methyl 2-aminobenzoate and methyl 2-amino-4,5-dimethoxybenzoate were both used unchanged and modified by methylation or benzylation. Methylation was conducted by Chan–Lam coupling[21] with stoichiometric amount of copper(II), methylboronic acid and pyridine under reflux in dioxane to give **6b**. Benzylation was mediated by reductive amination using sodium cyanoborohydride as reducing agent and conducted to **6c** and **6d** respectively. In this series, quinolones were prepared in 2 steps. Ethyl malonyl chloride was reacting with **6a–d** in presence of Et_3N in CH_2Cl_2, amide intermediates were succinctly purified and involved in cyclization by NaOMe in MeOH to afford **7a–d** with moderate to good yield depending on steric hindrance. Then **7a–d** were reacted with 2-(1-benzyl-4-piperidinyl)methylamine in refluxing xylene to give **8a–d**. Lastly methyl groups were removed by BBr_3 in CH_2Cl_2 to give **9a–b**.

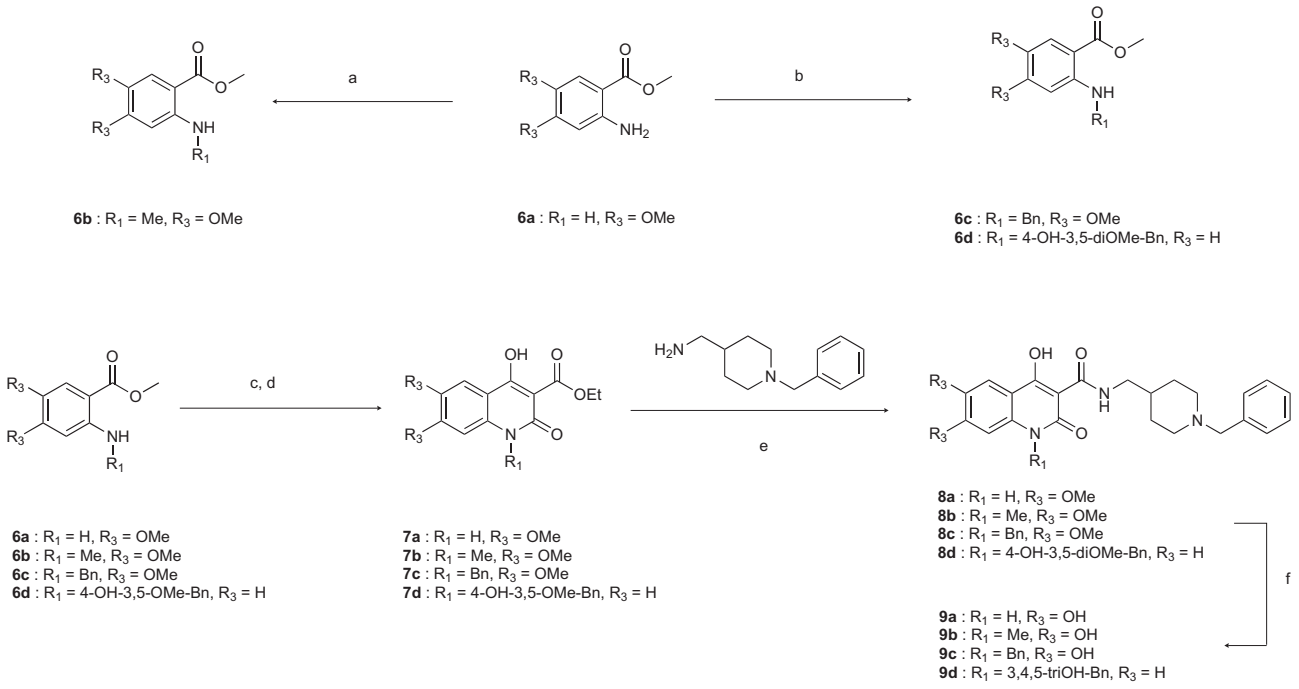
2.2. In vitro analysis of compounds 5a–j, 8a–d and 9a–d

2.2.1. Cholinesterase assays

All newly synthesized compounds were tested for their inhibitory activities toward AChE using Ellman's method.[22] Butyrylcholinesterase inhibitory activities were also assessed to explore

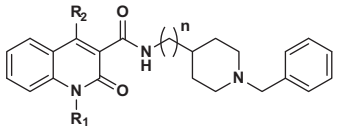


Scheme 1. Reagents and conditions: (a) BnBr, DIPEA, DMA, 90 °C, 3 h, 95%; (b) diethyl malonate, NaH, DMF, reflux, 5 h, 64–71%; (c) diethyl malonate, DBU, reflux, overnight, 56–89%; (d) amine, xylene, reflux, 3 h, 56–99%; (e) NaOH, EtOH, H_2O, reflux, overnight, 87–100%; (f) (i) oxalyl chloride, DMF_{cat}, CH_2Cl_2, 0 °C to rt, 1 h; (ii) amine, Et_3N, CH_2Cl_2, 0 °C to rt, 3 h, 57–72%; (g) $POCl_3$, 1 h then NH_3, EtOH, overnight, 55%.



Scheme 2. Reagents and conditions: (a) $MeB(OH)_2$, $Cu(OAc)_2$, Py, dioxane, reflux, 24 h, 66%; (b)ArCHO, $NaBH_3CN$, $AcOH_{cat}$, MeOH, rt, overnight, 41–61%; (c) ethyl malonyl chloride, Et_3N, CH_2Cl_2, 0 °C to rt, 3 h; (d) NaOMe, MeOH, rt, overnight, 82–98% for 2 steps; (e) xylene, reflux, 3 h, 82–98%; (f) BBr_3, CH_2Cl_2, −30 °C to rt, overnight, 55–87%.

Table 1
Cholinesterase activities and selectivities of compounds **5a–j**



Compounds	R_1	R_2	n	IC_{50} (μM) ± SE[a]		Selectivity
				AChE	BuChE	
5a	H	OH	0	2.27 ± 0.65	>10	> 4
5b	H	OH	1	1.41 ± 0.17	6.02 ± 1.74	4
5c	Me	OH	0	1.52 ± 0.13	>10	>6
5d	Me	OH	1	0.37 ± 0.05	4.26 ± 0.54	11
5e	Bn	OH	0	0.39 ± 0.10	>10	>25
5f	Bn	OH	1	0.49 ± 0.11	>10	>20
5g	H	Me	0	2.46 ± 0.60	>10	>4
5h	H	Me	1	0.47 ± 0.10	>10	>21
5i	H	Ph	0	>10	>10	—
5j	Me	NH_2	1	0.38 ± 0.07	1.14 ± 0.17	3
Donepezil				0.033 ± 0.012	1.82 ± 0.09	55
Tacrine				0.065 ± 0.009	0.0033 ± 0.0004	0.05

[a] AChE from electric eel and BuChE from equine serum were used. IC_{50} values represent the inhibitor concentrations required to decrease enzyme activity by 50%. Results are presented as the average ± Standard Error of three independent assays.

selectivity toward both enzymes. Tacrine and donepezil were taken as reference compounds (Table 1).

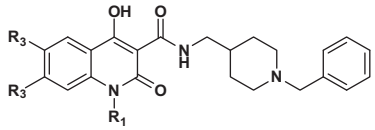
For the first time *N*-benzylpiperidinyl-dihydroquinoline-3-carboxamides were evaluated as AChE inhibitors. Somes informations could be gleaned from these results. AChE inhibition increases when *N*-quinoline is substituted (R_1, **5c**, **5e** > **5a**). Modifying the chain length between the quinolone and the benzylpiperidine moiety affects the affinity. Thus compounds with a methylene linkage (n = 1: **5b**, **5d** and **5h**) between the *N*-amide and the piperidine ring generaly present a higher activity than compounds without the spacer (n = 0: respectively **5a**, **5c** and **5g**). Results concerning residues in position 4 (R_2) were also instructive. A small group is required as phenyl group cancel out activity (**5i**). An amino group has similar activity than a hydroxyl group (**5j** = , **5d**). However, presence of a methyl group in position 4 retains activity but reverses the methylene linkage finding (**5g** > **5h**).All compounds showed selectivity toward AChE over BuChE with ratios from 3 to above 25. These results were within the range of the reference compounds we tested.

These data oriented the choice of the scaffold for the design of AChE inhibitors—antioxidant hybrids. Since the best results were obtained from compounds with a methylene linkage (n = 1) and a hydoxyl substituent in position 4 (R_2 = OH), we selected this moiety as model for hybrid design. As *N*-quinoline substitution increases the activity, we prepared *N*-methyl, *N*-benzyl and *N*-phenolic derivatives.

On one hand, we prepared molecules bearing 6,7-dimethoxy and 6,7-dihydroxy moieties on the quinolone ring (Table 2, R_3 = OMe, OH). On the other hand, we prepared some compounds with phenolic and polyphenolic moieties to replace the *N*-benzyl substituent (Table 2, R_1 = Sy, SyOH). Compound **8a** (R_1 = H, R_3 = OMe) has a good AChE inhibition with an IC_{50} of 0.11 μM and a high selectivity for AChE as no BuChE inhibition was observed at 60 μM. Product **9a** (R_1 = H, R_3 = OH) representing the O-demethylated analog retains activity range. No BuChE inhibition was observed at 10 μM. Compound **8b** (R_1 = Me, R_3 = OMe) and **9c** (R_1 = Bn, R_3 = OH) could not be dissolved in the conditions of cholinesterase assay, the hydrochlorides were prepared. Unfortunately compound **8b** (R_1 = Me, R_3 = OMe) and the catechol **9b** (R_1 = Me, R_3 = OH) have unsatisfactory activity on AChE. Compound **8c** (R_1 = Bn, R_3 = OMe) presents moderate inhibition of AChE while **9c** (R_1 = Bn, R_3 = OH) was ineffective in cholinesterase assays. Compound **8d** (R_1 = Sy) shows moderate AChE inhibition but no selectivity. Moreover O-demethylation into triphenolic **9d** (R_1 = SyOH) clearly affects the inhibitory activity.

Hydroxy, or even better, methoxy substituents on the quinolone ring (R_3 = OH, OMe) appear to improve selectivity toward AChE over BuChE. Compounds **8a** and **9a** did not show any inhibition of butyrylcholinesterase activity under 60 μM and 10 μM

Table 2
Cholinesterase activities and selectivities of compounds **8a–d** and **9a–d**



Compounds	R_1	R_3	IC_{50} (μM) ± SE[a]		Selectivity
			AChE	BuChE	
8a	H	OMe	0.11 ± 0.03 h[b]: 0.23 ± 0.03	>60	>545
9a	H	OH	0.48 ± 0.14 h[b]: 0.98 ± 0.35	>10	>20
8b·HCl	Me	OMe	2.93 ± 0.63	>10	>3
9b	Me	OH	2.02 ± 0.41	>10	5
8c	Bn	OMe	0.27 ± 0.07	>60	>222
9c·HCl	Bn	OH	>10	>10	-
8d	Sy[c]	H	0.56 ± 0.15	0.33 ± 0.26	0.6
9d	SyOH[c]	H	3.89 ± 0.50	>10	>2
Donepezil			0.033 ± 0.012	1.82 ± 0.09	55
Tacrine			0.065 ± 0.009	0.0033 ± 0.0004	0.55

[a] AChE from electric eel and BuChE from equine serum were used. IC_{50} values represent the required inhibitor concentrations to decrease enzyme activity by 50%. Results are presented as the average ± Standard Error of three independent assays.
[b] AChE from human erythrocytes was used.
[c] Sy = 4-OH-3,5-diOMe-Bn and SyOH = 3,4,5-triOH-Bn.

respectively. However, compound **8d** (R_1 = Sy, R_3 = H) is slightly selective for butyrylcholinesterase.

To sum up, **8a** and **9a** are the most potent compounds in terms of inhibition and selectivity within the series. To further explore their potenties, we tested their activity on human cholinesterase and observed that inhibitory activities are close but lower with an IC_{50} of 0.23 μM and 0.98 μM for **8a** and **9a** respectively. In addtiton, based on the low solubility of *N*-substituted derivatives, we can say that the NH quinoline unsubstitution of **8a** and **9a** improves solubility.

2.2.2. Antioxidant assays

Each compound was tested for radical scavenging activity. The effect of the synthesized compounds with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was first investigated.[23] In the absence of phenolic substituents, no radical scavenging activity was observed. However all phenolic derivatives interacted with DPPH with EC_{50} values ranging from 12.2 to 107 μM (Table 3).

Table 3
Antioxidant activities of compounds **9a–d**

Compounds	R_1	R_3	EC_{50} (μM) ± SE[a]	
			DPPH	$O_2^{\cdot-}$
9a	H	OH	12.2 ± 0.4	138.8 ± 6.6
9b	Me	OH	21.7 ± 0.6	40% ± 2%[b]
9c	Bn	OH	20.8 ± 0.7	Insoluble
8d	Sy[c]	H	90.5 ± 6.3	33% ± 3%[b]
9d	SyOH[c]	H	107.0 ± 7.3	140.4 ± 0.7
Quercetine			10.3 ± 0.3	49.9 ± 7.9
Curcumine			26.6 ± 0.9	nd[d]

[a] EC_{50} values represent the required compound concentrations to reduce radical activity by 50%. Results are presented as the average ± Standard Error of three independent assays.
[b] Since this experiment was limited by the solubility of compounds in aqueous solution, two results are expressed by the rate of radicals being scavenged at the concentration of 100 μM. Data are expressed as the average of three independent measurements (SE = Standard Error).
[c] Sy = 4-OH-3,5-diOMe-Bn and SyOH = 3,4,5-triOH-Bn.
[d] Not determined.

Hydroxy and methoxy groups on the quinolone ring or the *N*-benzyl substituent were introduced in order to target a radical scavenging activity. We used catechol moieties for their well known radical scavenging activities and a moiety derived from syringaldehyde, a natural phenolic compound showing potent anti-oxidant activity.[24]

The best activities were obtained when the dihydroxy moiety was on the quinolone ring (Table 3, R_3 = OH, **9a**, **9b**, **9c**). Recently it was reported[16a,b] that quinolone and iso-quinoline alkaloids with only one hydroxyl showed lower antioxidant activity than **9a–c**. Thus the catechol moiety is crucial for improving radical scavenging activity. Hydroxyl substituents must be on the quinolone ring to significantly affect the radical scavenging activity. Thus it is likely that the 3,4-insaturation of the quinolone ring is important for antioxidant activity.[25] By comparison, syringaldehyde derivative **8d** (R_1 = Sy) and its polyhydroxylated analogue **9d** (R_1 = SyOH) are less active. These derivatives have no hydroxyl substituent on the quinolone ring and their antioxidant activity depends only on the presence of the phenolic functional groups on the *N*-benzyl substituent (R_1). Moreover, since **9b** (R_1 = Me) and **9c** (R_1 = Bn) have slightly lower antioxidant activity than **9a** (R_1 = H), hydrogen atom on the *N*-quinolone appears to be involved in high scavenging activity.

The superoxide anion ($O_2^{\cdot-}$) is the first product of the univalent reduction of oxygen and is generated in many biological processes. Hence, the superoxide anion scavenging activity of hybrid compounds was explored using the non-enzymatic phenazine methosulfate NADH-system.[26] All phenolic derivatives were found to have moderate activity. But **9a** is again the most active product. Unlike the DPPH test, in this assay the position of the hydroxyl did not affect the anion superoxide scavenging activity (**9a** ≈ **9d**).

2.2.3. Structure activities relationships summary

In term of AChE inhibition, compounds with methylene linkage between the quinolone-caboxamide and the benzylpiperidine ring generally present higher activity than equivalent compounds without the spacer (**5b** > **5a**, **5d** > **5c**, **5h** > **5g**). In this case, a hydroxyl in position 4 seems to be required. Unlike the first series, in the hybrid design *N*-quinolone substituents clearly reduced solubility and best inhibitions were obtained with *N*-unsubstituted quinolone derivatives.

In term of radical scavenging activity the best activities were obtained when a catechol moiety was on the quinolone ring (Table 3, R_3 = OH, **9a**, **9b**, **9c**) and were improved by an unsubstituted *N*-quinolone. EC_{50} (DPPH) of compound **9a** was found to be similar as quercetin and two fold higher than curcumin.

Among these series compounds **8a** and **9a** were the most potent in term of AChE inhibition and radical scavenging activity. The inhibitory activity was verified on human AChE. These two compounds showed close but lower inhibitory activity. Structure–activities relationships for compounds **9a** are summarized in Figure 1.

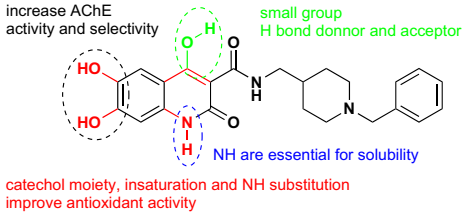


Figure 1. Structure–activities relationships of compound **9a**.

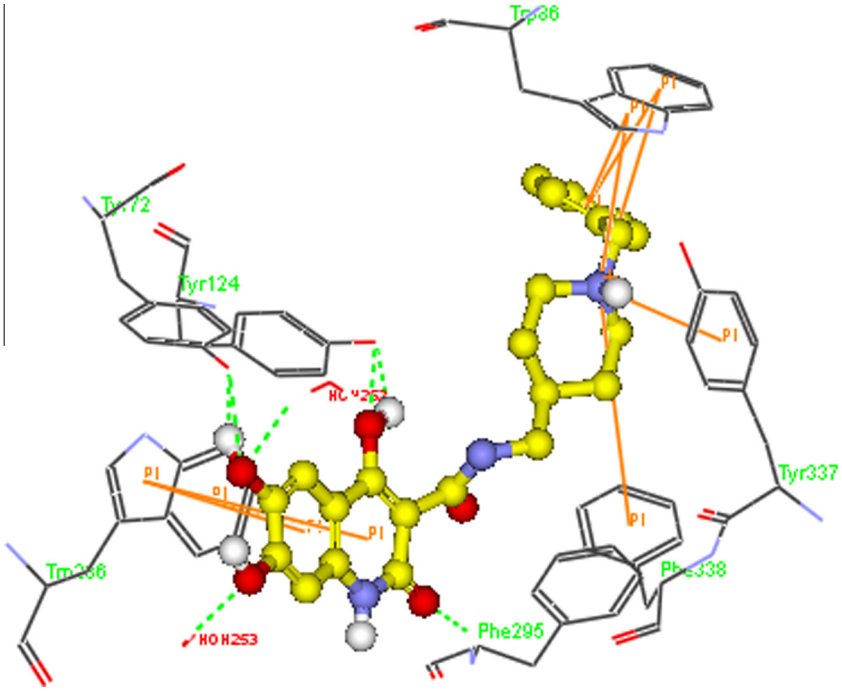


Figure 2. Compound **9a** (balls and sticks in yellow) in the binding site of hAChE (lines in grey) from 4ey7. Amino-acids and hydrogens have been removed to highlight the interactions. The peripheral anionic site is represented by Trp 286 and Tyr 72, the gorge is represented by Phe338 and Tyr337 and the catalytic pocket is represented by Trp86. π interactions are in orange solid lines and Hydrogen Bonding interactions are in green dotted lines.

2.3. Molecular modeling studies of compounds 9a

We performed molecular docking simulation for **9a** to *Torpedo californica* AChE[27] (*t*AChE) and *Human* AChE[28] (*h*AChE) using the CDocker program[29] implemented in Discovery studio 2.5 software.

The position of compound **9a** in the binding site of *h*AChE is shown in Figure 2. Compound **9a** binds in the same way to *t*AChE and *h*AChE. Hereinafter, aminoacids and distances of the bindings in AChE are indicated in brackets. The phenyl moiety displays a weak π-stacking interaction in the central catalytic pocket represented by Trp 86 (4.06–5.22 Å). Piperidinium is snaking along the gorge, making cation-π interactions[30] with aromatic rings in Tyr337 (3.83 Å) and even less in Phe338 (6.21 Å). The quinolone moiety is linked to the peripheral anionic site by π-stacking interactions with Trp286 (4.06–5.71 Å). These data are in agreement with the literature.[27,28] Compounds **9a** displays two other interactions: an H-bond between the hydroxyl group in position 4 (R_2 = OH) of the quinolone and a hydroxyl of the Tyr124 (2.32–3.05 Å) and another one between the carbonyle in position 2 of the quinolone and a NH of the Phe295 (1.97 Å). In addition, unlike compound **8a** (data not shown) the hydroxyl in position 6 (R_3 = OH) of the quinolone displays an H-Bond donor interaction in AChE with a hydroxyl of the Tyr72 (2.01–2.64 Å). Docking simulations clearly demonstrate that the radical scavenger moiety is placed by the peripheral anionic site.

3. Conclusions

We have designed synthesized and evaluated two series of quinolone-3-carboxamides linked to benzylpiperidine. All compounds except one are selective toward AChE versus BuChE, provide micromolar range inhibition and among them nine derivatives were found to inhibit AChE enzyme in submicromolar range. All phenolic compounds of the hybrid series have shown moderate to high radical scavenging activities. Compounds **8a** and **9a** are the most potent. Compound **8a** is an effective inhibitor of AChE and hybrid **9a** shows high radical scavenging activity without altering the inhibition of AChE. The catechol moiety of **9a** provides antioxidant activity and is also involved in an additional interaction with AChE. Our work established the ability of quinolone derivatives to serve in the design of multipotent hit molecules for the treatment of AD. New generation of quinolones derivatives are currently under investigation.

4. Experimental section

4.1. Synthesis

Starting materials reagents and analytical grade solvents were purchased from Sigma–Aldrich and Acros. All reaction were routinely checked by TLC using Merck Kieselgel 60 F_{254} aluminium plates and visualized by UV light. IR spectra were performed on a Perkin Elmer Spectrum65 with UATR and principal absorptions are given in cm^{-1}. Weak absorptions, noted (w), are probably due to intramolecular hydrogen bonds or to the propensity of 2(1*H*)-quinolinones to form dimers. Broad signal are noted (b). ^1H and ^{13}C NMR spectra were recorded in the specified deuterated solvent at 300 MHz and 75 MHz on a Brucker AC 300 instrument. Chemical shifts are expressed in parts per million (δ) relative to the solvent signal and the couplings constants *J* are given in Hertz (Hz). The following abbreviations were used: s (singlet), d (doublet), t (triplet), q (quadruplet), dd (doublet of doublet), m (multiplet), bs (broad signal). Elemental analysis were carried out at the Service Central d'Analyses, ISA—UMR CNRS 5280—69 360 Solaize. ESI-MS analyses were carried out at the Service Commun d'Analyse, ICMR—UMR CNRS 6229—51 100 Reims.

4.1.1. Typical procedure for preparation of compounds 3a–e

To a suspension of NaH (1 equiv) in DMF at 0 °C was slowly added diethyl malonate (10 equiv) and a solution of the corresponding isatoic anhydride (1 equiv) in DMF. Then the reaction mixture was heated to reflux. After completion of the reaction

(TLC monitoring) the crude mixture was quenched with water and acidified with concentrated hydrochloric acid. The resulting solid was filtered, washed with water and diethylether and then dried in vacuo to give the desired compound.

4.1.1.1. Ethyl 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylate 3a. According to the general procedure, the reaction was carried out with NaH (0.408 g, 17 mmol), diethyl malonate (27.2 g, 170 mmol) and isatoic anhydride (2.8 g, 17 mmol). The compound was obtained as a white solid (2.81 g, 71%). IR (ATR) γ cm^{-1}: 3406, 3193, 1658, 1604. ^1H NMR (DMSO D_6), δ ppm: 11.47 (s, 1H), 7.94 (d, J = 8.1 Hz, 1H), 7.62 (t, J = 7.2 Hz, 1H), 7.27 (d, J = 8.1 Hz, 1H), 7.20 (t, J = 7.5 Hz, 1H), 4.34 (q, J = 7.2 Hz, 2H), 1.31 (t, J = 7.2 Hz, 3H). Anal. Calcd For $C_{12}H_{11}NO_4$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.72; H, 4.78; N, 6.06.

4.1.1.2. Ethyl 4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate 3b. According to the general procedure, the reaction was carried out with NaH (0.408 g, 17 mmol), diethyl malonate (27.2 g, 170 mmol) and methylisatoic anhydride (3 g, 17 mmol). The compound was obtained as a white solid (2.98 g, 71%). IR (ATR) γ cm^{-1}: 3200–3000 (w), 2996, 2977, 1657, 1624, 1593, 1561. ^1H NMR (DMSO D_6), δ ppm: 13.02 (bs, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.72 (dd, J = 8.0 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.28 (t, J = 8.0 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 3.52 (s, 3H), 1.30 (t, J = 7.1 Hz, 3H). ^{13}C NMR (DMSO D_6), δ ppm: 169.7, 166.1, 158.9, 140.8, 134.1, 124.8, 122.1, 115.2, 114.7, 101.2, 61.7, 29.2, 14.3. Anal. Calcd for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.09; H, 5.24; N, 5.76.

4.1.1.3. Ethyl 1-benzyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylate 3c. According to the general procedure, the reaction was carried out with NaH (0.24 g, 10 mmol), diethyl malonate (16 g, 100 mmol) and methylisatoic anhydride (2.53 g, 10 mmol). The compound was obtained as a white solid (2.07 g, 64%). IR (ATR) γ cm^{-1}: 3250–3100 (w), 2986, 2937, 1669, 1622, 1560. ^1H NMR ($CDCl_3$), δ ppm: 14.36 (s, 1H), 8.20 (d, J = 7.8 Hz, 1H), 7.53 (t, J = 7.4 Hz, 1H), 7.31–7.18 (m, 7H), 5.51 (s, 2H), 4.52 (q, J = 7.1 Hz, 2H), 1.49 (t, J = 7.1 Hz, 3H). ^{13}C NMR (DMSO D_6), δ ppm: 171.9, 166.6, 158.2, 136.2, 134.4, 134.2, 128.6, 127.0, 126.3, 126.2, 121.9, 116.2, 115.0, 114.8, 62.3, 45.6, 14.1. Anal. Calcd for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33. Found: C, 70.69; H, 5.41; N, 4.22.

4.1.2. Ethyl 4-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate 3d

A mixture of 1-(2-aminophenyl)ethanone (2 g, 16.9 mmol), diethyl malonate (3.76 g, 23.5 mmol) and DBU (719 mg, 4.73 mmol) was heated to reflux overnight. After cooling, the residue was recristallized in ethyl acetate. The compound was washed by ethyl acetate and dichloromethane and was obtained as a white solid (2.21 g, 56%). IR (ATR) γ cm^{-1}: 3312 (w), 3001, 2981, 1728, 1647, 1608, 1561, 1505. ^1H NMR (DMSO D_6), δ ppm: 11.99 (s, 1H), 7.80 (d, J = 7.7 Hz, 1H), 7.57 (t, J = 7.7 Hz, 1H), 7.33 (d, J = 7.7 Hz, 1H), 7.25 (t, J = 7.7 Hz, 1H), 4.31 (q, J = 7.1 Hz, 2H), 2.39 (s, 3H), 1.29 (t, J = 7.1 Hz, 3H). ^{13}C NMR (DMSO D_6), δ ppm: 166.0, 158.4, 144.2, 137.9, 131.1, 126.4, 125.3, 122.1, 118.4, 115.4, 60.9, 15.6, 13.9. Anal. Calcd for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.66; H, 5.64; N, 6.11.

4.1.3. Ethyl 2-oxo-4-phenyl-1,2-dihydroquinoline-3-carboxylate 3e

A mixture of (2-aminophenyl)(phenyl)methanone (500 mg, 2.54 mmol), diethyl malonate (568 mg, 3.55 mmol) and DBU (54 mg, 0.354 mmol) was heated to reflux overnight. After cooling, the residue was dissolved in ethyl acetate, washed with a solution of HCl 1 N, a satured solution of $NaHCO_3$ and then brine and dried over sodium sulfate. The organic layer was filtred and solvent was evaporated under vacuum. The compound was obtained as a white solid (662 mg, 89%). IR (ATR) γ cm^{-1}: 3200–3050 (w), 2981, 2935, 2836, 1729, 1644, 1557. ^1H NMR (DMSO D_6), δ ppm: 12.28 (s, 1H), 7.63–7.54 (m, 4H), 7.41 (d, J = 8.2 Hz, 1H), 7.36–7.28 (m, 2H), 7.20–7.10 (m, 2H), 3.95 (q, J = 7.0 Hz, 2H), 0.85 (t, J = 7.0 Hz, 3H). ^{13}C NMR (DMSO D_6), δ ppm: 164.9, 158.4, 148.3, 138.5, 133.9, 131.4, 128.8, 128.4, 128.3, 127.1, 126.9, 122.4, 118.1, 115.6, 60.5, 13.4. Anal. Calcd For $C_{18}H_{15}NO_3$: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.60; H, 5.21; N, 4.62.

4.1.4. 4-Methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid 4d

To a solution of ethyl 4-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate **3d** (1.5 g, 6.49 mmol) in ethanol was added a solution of KOH 4 N (6.5 mL, 26 mmol) and the mixture was heated to reflux overnight. After cooling, the reaction mixture was diluted with water and washed with ethyl acetate. The aqueous layer was acidified and extracted with ethyl acetate. Crude mixture was dried over magnesium sulfate and concentrated in vacuo to give the compound (1.15 g, 87%) as a white solid. IR (ATR) γ cm^{-1}: 3250–2600 (l), 3136 (w), 3041, 2940, 2885, 1697, 1625, 1598, 1538. ^1H NMR (DMSO D_6), δ ppm: 13.69 (bs, 1H), 12.31 (bs, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.60 (t, J = 7.8 Hz, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.29 (t, J = 7.8 Hz, 1H), 2.56 (s, 3H). ^{13}C NMR (DMSO D_6), δ ppm: 166.8, 159.8, 146.5, 137.6, 131.4, 125.6, 124.9, 122.4, 119.0, 115.6, 15.9. Anal. Calcd for $C_{11}H_9NO_3$: C, 65.02; H, 4.46; N, 6.89. Found: C, 64.82; H, 4.66; N, 6.99.

4.1.5. 2-Oxo-4-phenyl-1,2-dihydroquinoline-3-carboxylic acid 4e

To a solution of ethyl 2-oxo-4-phenyl-1,2-dihydroquinoline-3-carboxylate **3e** (1.172 g, 4 mmol) in ethanol was added a solution of KOH 4 N (4 mL, 16 mmol) and the mixture was heated to reflux overnight. After cooling, reaction mixture was diluted with water and washed with ethyl acetate. Aqueous layer was acidified and extracted with ethyl acetate. Crude mixture was dried over magnesium sulfate and concentrated in vacuo to give the compound (1.06 g, 100%) as a white solid. IR (ATR) γ cm^{-1}: 3220 (w), 3200-2200 (l), 3001, 2940, 2835, 1695, 1646, 1577, 1555. ^1H NMR (DMSO D_6), δ ppm: 13.06 (bs, 1H), 12.31 (bs, 1H), 7.62–7.47 (m, 4H), 7.42 (d, J = 8.1 Hz, 1H), 7.38–7.30 (m, 2H), 7.15 (t, J = 7.5 Hz, 1H), 7.07 (d, J = 8.1 Hz, 1H). ^{13}C NMR (DMSO D_6), δ ppm: 166.1, 159.0, 147.5, 138.2, 134.3, 131.1, 128.5, 128.4, 128.2, 127.1, 126.9, 122.2, 118.6, 115.5. Anal. Calcd for $C_{16}H_{11}NO_3$: C, 72.45; H, 4.18; N, 5.28. Found: C, 72.33; H, 4.29; N, 5.44.

4.1.6. Typical procedure for preparation of compounds 5a-i

*Method A: Compounds **5a–f***: Quinolinone ester **3a–c** (1 equiv) was added to a solution of the amine (1.1 equiv) in xylene and the mixture was refluxed. After completion of the reaction (TLC monitoring), solvent was removed in vacuo and products were obtained by precipitation, column chromatography and/or recrystallization.

*Method B: Compounds **5g–i***: To a solution of acid **4d–e** (1 equiv) at 0 °C in dry dichloromethane, was added oxalyl chloride (1.2 equiv) and dry *N,N*-dimethylformamide (0.2 equiv). After 30 min, the reaction mixture was allowed to warm up at rt. After completion of this step (TLC monitoring) the solvent was evaporated. The residue was diluted with dichloromethane and added to a solution of 4-Amino-1-benzylpiperidine (n = 0) or 2-(1-benzyl-4-piperidinyl)methylamine (n = 1) (1 equiv) and triethylamine (4 equiv) at 0 °C in dry dichloromethane. After 3 h at rt, reaction mixture was washed with $NaHCO_3$ 5% and brine. The organic layer was dried over sodium sulfate, filtered and concentred in vacuo. Pure product was obtained by recrystallization.

4.1.6.1. *N*-(1-Benzylpiperidin-4-yl)-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide 5a. The compound was prepared according to method A from ethyl 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylate **3a** (0.4 g, 1.7 mmol) and 1-benzylpiperidin-4-amine (355 mg, 1.87 mmol) for 3 h to reflux. Compound precipitated after cooling and was filtered and washed by diethyl ether. The product was obtained as a white solid (359 mg, 56%). IR (ATR) γ cm^{-1}: 3250, 3300–3000 (w), 3100–3000 (w), 2922, 2803, 2765, 1636, 1603, 1568. ^1H NMR (DMSO D_6), δ ppm: 11.28, (bs, 1H), 10.43 (s, 2H), 7.96 (d, J = 7.6 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.43–7.17 (m, 7H), 4.00–3.71 (m, 1H), 3.48 (s, 2H), 2.70 (m, 2H), 2.17 (t, J = 10.0 Hz, 2H), 2.00–1.80 (m, 2H), 1.54 (m, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 172.5, 169.8, 162.8, 138.6, 138.4, 133.6, 128.6, 128.1, 126.8, 124.0, 122.2, 115.9, 115.8, 95.4, 62.0, 51.2, 45.6, 31.2. Anal. Calcd for $C_{22}H_{23}N_3O_3$: C, 70.01; H, 6.14; N, 11.13. Found: C, 69.68; H, 6.20; N, 10.72.

4.1.6.2. *N*-((1-Benzylpiperidin-4-yl)methyl)-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide 5b. The compound was prepared according to method A from ethyl 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylate **3a** (250 mg, 1.07 mmol) and (1-benzylpiperidin-4-yl)methanamine (241 mg, 1.18 mmol) for 3 h to reflux. Compound precipitated after cooling and was filtered and washed by diethyl ether. The product was obtained as a white solid (300 mg, 79%). IR (ATR) γ cm^{-1}: 3673, 3246, 3300–3000 (w), 3100–3000 (w), 2991, 2936, 2905, 2805, 2754, 1634, 1562. ^1H NMR (DMSO D_6), δ ppm: 11.85 (bs, 1H), 10.41 (s, 2H), 7.96 (d, J = 7.9 Hz, 1H), 7.67 (t, J = 7.9 Hz, 1H), 7.36 (d, J = 7.9 Hz, 1H), 7.29–7.43 (m, 6H) 3.43 (s, 2H), 3. 29 (m, 2H), 2.81 (m, 2H), 1.90 (t, J = 11.2 Hz, 2H), 1.63 (m, 3H), 1.24 (m, 2H). ^{13}C NMR (DMSO D_6) δ ppm: 171.1, 162.8, 162.2, 139.1, 139.2, 134.1, 129.2, 128.6, 127.2, 124.5, 122.8, 116.4, 116.3, 97.4, 62.8, 53.3, 44.2, 36.1, 30.0. Anal. Calcd for $C_{23}H_{25}N_3O_3$: C, 70.57; H, 6.44; N, 10.73. Found: C, 70.34; H, 6.47; N, 10.66.

4.1.6.3. *N*-(1-Benzylpiperidin-4-yl)-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxamide 5c. The compound was prepared according to method A from ethyl 4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate **3b** (247 mg, 1 mmol) and 1-benzylpiperidin-4-amine (209 mg, 1.1 mmol) during 3 h of reflux. Compound precipitated after cooling and was filtered and washed by diethyl ether as a white solid (246 mg, 63%). IR (ATR) γ cm^{-1}: 3250–3000 (w), 3100–3000 (w), 3061, 2936, 2800, 2756, 1638, 1555. ^1H NMR (DMSO D_6), δ ppm: 10.51 (s, 1H), 10.49 (s, 1H), 8.09 (d, J = 7.7 Hz, 1H), 7.80 (t, J = 7.7 Hz, 1H), 7.62 (d, J = 7.7 Hz, 1H), 7.47–7.12 (m, 6H), 3.95–3.79 (m, 1H), 3.63 (s, 3H), 3.49 (s, 2H), 2.70 (m, 2H), 2.20 (t, J = 9.8 Hz, 2H), 2.00–1.80 (m, 2H), 1.56 (m, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 169.7, 165.1, 156.2, 139.5, 138.4, 134.0, 128.6, 128.0, 126.7, 124.4, 122.4, 115.2, 113.8, 95.7, 62.0, 51.0, 45.6, 31.1, 28.9. Anal. Calcd for $C_{23}H_{25}N_3O_3$: C, 70.57; H, 6.44; N, 10.73. Found: C, 70.21; H, 6.47; N, 10.61.

4.1.6.4. *N*-((1-Benzylpiperidin-4-yl)methyl)-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxamide 5d. Title compound was prepared according to method A from ethyl 4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate **3b** (250 mg, 1.01 mmol) and (1-benzylpiperidin-4-yl)methanamine (226 mg, 1.11 mmol) during 3 h of reflux. Solvent was removed in vacuo and compound was obtained by column chromatography (dichloromethane–methanol: 1–4% gradient) as a white solid (258 mg, 63%). IR (ATR) γ cm^{-1}: 3670, 3250–3000 (w), 3100–3000 (w), 2938, 2911, 2794, 2750, 1627, 1558, 1503. ^1H NMR (DMSO D_6), δ ppm: 10.45 (s, 2H), 8.08 (d, J = 7.7 Hz, 1H), 7.80 (t, J = 7.7 Hz, 1H), 7.62 (d, J = 7.7 Hz, 1H), 7.37 (t, J = 7.7 Hz, 1H), 7.31–7.15 (m, 5H), 3.62 (s, 3H), 3.44 (s, 2H), 3.31 (m, 2H), 2.82 (m, 2H), 1.92 (m, 2H), 1.62 (m, 3H), 1.26 (m, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 170.5, 164.3, 161.7, 139.5, 138.4, 134.1, 128.6, 128.0, 126.7, 124.4, 122.4, 115.4, 115.3, 95.7, 62.2, 52.7, 43.7, 35.4, 29.4, 29.0. Anal. Calcd for $C_{24}H_{27}N_3O_3$: C, 71.09; H, 6.71; N, 10.36. Found: C, 71.36; H, 6.88; N, 10.24.

4.1.6.5. 1-Benzyl-*N*-(1-benzylpiperidin-4-yl)-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide 5e. Title compound was prepared according to method A from ethyl 1-benzyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylate **3c** (161 mg, 0.5 mmol) and 1-benzylpiperidin-4-amine (104.5 mg, 0.55 mmol) during 3 h of reflux. Solvent was removed in vacuo and compound was obtained by column chromatography (dichloromethane–methanol: 1–3% gradient) as a white solid (229 mg, 98%). IR (ATR) γ cm^{-1}: 3196, 3100–3000(w), 2937, 2910, 2790, 1627, 1560. ^1H NMR (DMSO D_6), δ ppm: 17.53 (s, 1H), 10.30 (bs, 1H), 8.13 (dd, J = 7.8 Hz, 1H), 7.69 (t, J = 7.8 Hz, 1H), 7.45 (d, J = 8.6 Hz, 1H), 7.40–7.14 (m, 11H), 5.55 (s, 2H), 3.96–3.84 (m, 1H), 3.48 (s, 2H), 2.71 (m, 2H), 2.27–2.11 (m, 2H), 1.92 (d, J = 9.8 Hz, 2H), 1.58 (m, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 171.6, 169.7, 162.1, 138.9, 138.3, 136.4, 134.1, 128.7, 128.6, 128.0, 126.9, 126.7, 126.1, 124.6, 122.5, 115.7, 115.1, 95.6, 61.9, 51.1, 45.8, 44.5, 31.0. Anal. Calcd for $C_{29}H_{29}N_3O_3$: C, 74.50; H, 6.25; N, 8.99. Found: C, 74.23; H, 6.39; N, 8.78.

4.1.6.6. 1-Benzyl-*N*-((1-benzylpiperidin-4-yl)methyl)-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide 5f. Title compound was prepared according to method A from ethyl 1-benzyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylate **3c** (209 mg, 0.65 mmol) and (1-benzylpiperidin-4-yl)methanamine (146 mg, 0.71 mmol) during 3 h of reflux. Solvent was removed in vacuo and compound was obtained by column chromatography (ethyl acetate–cyclohexane: 1–1) as a white solid (250 mg, 80%). IR (ATR) γ cm^{-1}: 3184, 3100–3000 (w), 2929, 2802, 2760, 1624, 1585, 1554. ^1H NMR (DMSO D_6) δ ppm: 17.62 (s, 1H), 10.40 (bs, 1H), 8.12 (d, J = 7.9 Hz, 1H), 7.69 (t, J = 7.9 Hz, 1H), 7.45 (d, J = 8.6 Hz, 1H), 7.40–7.19 (m, 11H), 5.55 (s, 2H), 3.46 (s, 2H), 3.33 (d, 2H), 2.83 (d, J = 10.7 Hz, 2H), 1.95 (t, J = 11.7 Hz, 2H), 1.66 (m, 3H), 1.14–1.36 (m, 2H). ^{13}C NMR (DMSO D_6) δ ppm: 172.3, 172.2, 171.1, 139.5, 138.8, 137.1, 134.7, 129.2, 129.1, 128.6, 127.5, 127.3, 126.8, 125.2, 123.2, 121.6, 116.2, 116.1, 62.7, 53.2, 45.1, 44.4, 35.9, 29.9. Anal. Calcd for $C_{30}H_{31}N_3O_3$: C, 74.82; H, 6.49; N, 8.73. Found: C, 74.54; H, 6.56; N, 8.64.

4.1.6.7. *N*-(1-Benzylpiperidin-4-yl)-4-methyl-2-oxo-1,2-dihydroquinoline-3-carboxamide 5g. According to method B from 4-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid **4d** (142 mg, 0.7 mmol), oxalyl chloride (107 mg, 0.84 mmol), dimethylformamide (11 μL) in dry dichloromethane (5 mL), and then 1-benzylpiperidin-4-amine (133 mg, 0.7 mmol), triethylamine (283 mg, 2.8 mmol) in dry dichloromethane (5 mL). Title compound was obtained by recrystallization in diethyl ether as a white solid (190 mg, 72%). IR (ATR) γ cm^{-1}: 3306, 3100–3000 (w), 2950, 2850, 2811, 1663, 1630, 1562, 1525. ^1H NMR (DMSO D_6), δ ppm: 11.78 (s, 1H), 8.21 (br d, J = 7.5 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.39–7.15 (m, 7H), 3.82–3.62 (m, 1H), 3.46 (s, 2H), 2.78 (d, J = 11.5 Hz, 2H), 2.36 (s, 3H), 2.06 (t, J = 10.5 Hz, 2H), 1.90–1.73 (m, 2H), 1.56–1.37 (m, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 164.6, 159.2, 142.8, 138.5, 137.8, 130.3, 129.9, 128.5, 128.0, 126.6, 125.0, 121.7, 119.0, 115.1, 62.0, 51.7, 46.0, 31.3, 15.4. Anal. Calcd for $C_{23}H_{25}N_3O_2$: C, 73.57; H, 6.71; N, 11.19. Found: C, 72.79; H, 6.78; N, 11.07.

4.1.6.8. *N*-((1-Benzylpiperidin-4-yl)methyl)-4-methyl-2-oxo-1,2-dihydroquinoline-3-carboxamide 5h. According to method B from 4-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid

4d (142 mg, 0.7 mmol), oxalyl chloride (107 mg, 0.84 mmol), dimethylformamide (11 μL) in dry dichloromethane (5 mL), and then (1-benzylpiperidin-4-yl)methanamine (143 mg, 0.7 mmol), triethylamine (283 mg, 2.8 mmol) in dry dichloromethane (5 mL). Title compound was obtained by recrystallization in diethyl ether as a white solid (158 mg, 58%). IR (ATR) γ cm^{-1}: 3334, 3100–3000 (w), 2939, 2915, 2801, 2765, 1641, 1535, 1504. ^1H NMR (DMSO D_6), δ ppm: 11.76 (s, 1H), 8.26 (bt, J = 4.9 Hz, 1H), 7.77 (d, J = 7.7 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.37–7.16 (m, 7H), 3.46 (s, 2H), 3.11 (t, J = 6.0 Hz, 2H), 2.81 (d, J = 11.35 Hz, 2H), 2.36 (s, 3H), 2.02–1.89 (m, 2H), 1.84–1.62 (m, 2H), 1.60–1.42 (m, 1H), 1.31–1.10 (m, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 165.4, 159.3, 142.9, 138.4, 137.7, 130.3, 130.0, 128.6, 127.9, 126.6, 125.0, 121.8, 119.0, 115.1, 62.2, 52.7, 44.1, 35.5, 29.5, 15.6. Anal. Calcd for $C_{24}H_{27}N_3O_2$: C, 74.01; H, 6.99; N, 10.79. Found: C, 73.63; H, 7.16; N, 10.77.

4.1.6.9. *N*-(1-Benzylpiperidin-4-yl)-2-oxo-4-phenyl-1,2-dihydroquinoline-3-carboxamide 5i.

According to method B from 2-oxo-4-phenyl-1,2-dihydroquinoline-3-carboxylic acid **4e** (185.5 mg, 0.7 mmol), oxalyl chloride (107 mg, 0.84 mmol), dimethylformamide (11 μL) in dry dichloromethane (5 mL), and then 1-benzylpiperidin-4-amine (133 mg, 0.7 mmol), triethylamine (283 mg, 2.8 mmol) in dry dichloromethane (5 mL). Title compound was obtained by recrystallization in a dichloromethane–diethyl ether mixture as a white solid (176 mg, 57%). IR (ATR) γ cm^{-1}: 3277, 3200–3000 (w), 3058, 2940, 2800, 2755, 1665, 1634, 1541. ^1H NMR (DMSO D_6), δ ppm: 12.02 (s, 1H), 7.95 (d, J = 7.4 Hz, 1H), 7.59–7.15 (m, 12H), 7.10 (t, J = 7.5 Hz, 1H), 7.03 (d, J = 7.5 Hz, 1H), 3.46–3.35 (m, 1H), 3.30 (s, 2H), 2.57 (d, J = 11.5 Hz, 2H), 1.88 (t, J = 10.5 Hz, 2H), 1.36 (d, J = 10.5 Hz, 2H), 1.20–1.06 (m, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 163.4, 159.2, 146.7, 138.5, 138.2, 134.4, 130.4, 128.7, 128.5, 128.1, 128.0, 127.9, 127.8, 127.7, 126.6, 121.8, 118.8, 115.2, 61.9, 51.4, 45.5, 30.8. Anal. Calcd for $C_{28}H_{27}N_3O_2$: C, 76.86; H, 6.22; N, 9.60. Found: C, 76.38; H, 6.28; N, 9.59.

4.1.7. 4-Amino-*N*-((1-benzylpiperidin-4-yl)methyl)-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxamide 5j

A solution of *N*-((1-benzylpiperidin-4-yl)methyl)-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxamide **5d** (150 mg, 0.37 mmol) in phosphorous oxychloride (2 mL) was stirred at room temperature for 1 h. The mixture was poured into saturated $NaHCO_3$ and the resulting precipitate was collected and engaged in reaction without further purification. The precipitates and ammonia were heated overnight in ethanol to 100 °C in a sealed tube. The solvent was evaporated and the residue was purified by column chromatography (dichloromethane–methanol: 9–1) and appeared as a white solid (81 mg, 55%). IR (ATR) γ cm^{-1}: 3324, 3400–2900, 2918, 2851, 1727, 1633, 1620, 1568, 1547. ^1H NMR (DMSO D_6), δ ppm: 8.45 (t, J = 7.8 Hz, 1H), 7.88 (dd, J = 7.8, 1.4 Hz, 1H), 7.63 (dt, J = 7.8, 1.4 Hz, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.32–7.20 (m, 6H), 3.47 (s, 3H), 3.43 (s, 2H), 3.33 (s, 2H), 2.79 (m, 2H), 1.89 (t, J = 10.8 Hz, 2H), 1.63 (m, 3H), 1.26–1.18 (m, 2H), 1.07 (s, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 168.9, 166.9, 156.8, 141.1, 134.2, 132.4, 129.1, 128.6, 127.2, 124.1, 122.6, 116.5, 111.7, 85.7, 62.8, 53.2, 48.3, 36.3, 29.9, 28.3. Anal. Calcd for $C_{24}H_{28}N_4O_2$: C, 71.26; H, 6.98; N, 13.85. Found: C, 70.96; H, 6.99; N, 13.95.

4.1.8. Methyl 4,5-dimethoxy-2-(methylamino)benzoate 6b

To a solution of methyl 2-amino-4,5-dimethoxybenzoate (1.02 g, 4.84 mmol) in dioxane was added copper-(II) acetate (2.2 g, 12.1 mmol) and pyridine (956 mg, 12.1 mmol). The mixture was stired 15 min., methyl boronic acid (688 mg, 12.1 mmol) was added and the reaction was refluxed 24 h. After cooling, the mixture was filtered through Celite® and solvent was evaporated under vacuum. The compound was obtained by column chromatography (cyclohexane–dichloromethane: 7–1) and appeared as a white solid (720 mg, 66%). IR (ATR) γ cm^{-1}: 3374, 2996, 2960, 2935, 2917, 2850, 1661, 1579, 1519. ^1H NMR (DMSO D_6), δ ppm: 7.58 (bs, 1H), 7.37 (s, 1H), 6.13 (s, 1H), 3.92 (s, 3H), 3.82 (s, 6H), 2.91 (s, 3H). ^{13}C NMR (DMSO D_6), δ ppm: 168.0, 156.0, 149.5, 139.0, 114.1, 100.0, 94.8, 56.6, 55.9, 51.6, 30.0. Anal. Calcd for $C_{11}H_{15}NO_4$: C, 58.66; H, 6.71; N, 6.22. Found: C, 58.76; H, 6.69; N, 6.24.

4.1.9. Methyl 2-(benzylamino)-4,5-dimethoxybenzoate 6c

To a solution of methyl 2-amino-4,5-dimethoxybenzoate (469 mg, 2.2 mmol) in a dichloromethane–methanol (1–1), benzaldehyde (279 mg, 2.2 mmol) and acetic acid (0.5 mL) were added. The solution was stirred at room temperature for 1 h and $NaBH_3CN$ (330 mg, 5.24 mmol) was added. After overnight reaction, water was added and the crude product was extracted with ethyl acetate and washed with brine. Organic layers were dried over magnesium sulfate and concentrated in vacuo. The compound was obtained by recrystallization in cyclohexane to give the title compound (404 mg, 61%) as a white solid. IR (ATR) γ cm^{-1}: 3343, 3013, 2961, 2835, 1673, 1620, 1582, 1518. ^1H NMR (DMSO D_6) δ ppm: 8.01 (bs, 1H), 7.44-7.22 (m, 6H), 6.28 (s, 1H), 4.47 (s, 2H), 3.76 (s, 3H), 3.70 (s, 3H), 3.64 (s, 3H). ^{13}C NMR (DMSO D_6) δ ppm: 168.6, 155.1, 148.2, 139.3, 139.0, 128.7, 127.2, 127.1, 113.5, 101.1, 95.1, 56.5, 55.6, 51.3, 47.5. Anal. Calcd for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.86; H, 6.49; N, 4.67.

4.1.10. Methyl 2-(4-hydroxy-3,5-dimethoxybenzylamino) benzoate 6d

To a solution of methyl anthranilate (1.02 g, 6.62 mmol) in dichloromethane–methanol (1–1), syringaldehyde (1.00 g, 6.62 mmol) and acetic acid (2 mL) were added. The solution was stirred at room temperature for 1 h and $NaBH_3CN$ (105 mg, 26.5 mmol) was added. After overnight reaction, water was added and the crude product was extracted with dichloromethane and washed with brine. Organic layer was dried over magnesium sulfate and concentrated in vacuo. The compound was obtained by column chromatography (hexane–dichloromethane: 2–1) to give the title compound (610 mg, 41%) as a white solid. IR (ATR) γ cm^{-1}: 3361, 2930, 2845, 1680, 1610, 1578, 1511. ^1H NMR (DMSO D_6) δ ppm: 8.08 (s, 1H), 7.92 (d, J = 7.9 Hz, 1H), 7.31 (t, J = 7.8 Hz, 1H), 6.77–6.60 (m, 2H), 6.59 (s, 2H), 5.48 (s, 1H), 4.35 (s, 2H), 3.86 (s, 9H). ^{13}C NMR (DMSO D_6) δ ppm: 169.1, 151.0, 147.2, 134.6, 133.7, 131.6, 130.0, 115.0, 111.8, 110.2, 103.8, 56.3, 51.5, 47.4. Anal. Calcd for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.03; N, 4.41. Found: C, 64.42; H, 6.09; N, 4.44.

4.1.11. Typical procedure for preparation of compounds 7a–d

A solution of ethyl malonyl chloride (1.1 equiv) in dry dichloromethane (distilled on P_2O_5) was slowly added to a solution of aminobenzoate **6a–d** (1 equiv) and triethylamine (3 equiv) at 0 °C under nitrogen atmosphere in dry dichloromethane. After 30 min the reaction mixture was allowed to warm up at rt. After completion of the reaction, (1–3 h, TLC monitoring: a drop of the reaction mixture is diluted in methanol to form an ester which is compared to ethyl malonic acid), the mixture was washed with $NaHCO_3$ 5% and brine. The organic layer is dried over sodium sulfate, filtered and concentrated in vacuo.

This residue was engaged without further purification into the next step.

The residue was diluted in dry methanol (distilled on Na^0), freshly prepared NaOMe (2.2 equiv) was added and the reaction was stirred at rt overnight. The mixture was diluted in water and acidified with a solution of hydrochloric acid below pH 2. The

product precipitated and/or was extracted with dichloromethane. The organic layer was washed with brine, dried over magnesium sulfate and concentrated in vacuo. The compound was obtained by carefull column chromatography.

4.1.11.1. Methyl 4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylate 7a. According to the general procedure (4.1.11.) from ethyl malonyl chloride (433 mg, 2.11 mmol); methyl 4,5-dimethoxy-2-aminobenzoate (593 mg, 1.92 mmol); triethylamine (582 mg, 5.76 mmol) for 3 h at rt. Then NaOMe (228 mg, 4.22 mmol) in methanol overnight. The title compound was obtained by column chromatography (dichloromethane–methanol: 9–1) as a white solid (350 mg, 65%). IR (ATR) γ cm^{-1}: 3200–3000 (w), 2957, 2902, 2830, 1668, 1632, 1610, 1513. ^1H NMR (DMSO D_6), δ ppm: 13.68 (s, 1H), 11.36 (s, 1H), 7.26 (s, 1H), 6.78 (s, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H). ^{13}C NMR (DMSO D_6), δ ppm: 172.4, 170.3, 159.5, 155.5, 145.4, 137.3, 105.7, 104.3, 97.7, 97.2, 56.3, 56.1, 52.8. Anal. Calcd for $C_{13}H_{13}NO_6$: C, 55.91; H, 4.69; N, 5.02. Found: 55.41; H, 4.86; N, 5.09.

4.1.11.2. Ethyl 4-hydroxy-6,7-dimethoxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate 7b. According to the general procedure (4.1.11.) from ethyl malonyl chloride (433 mg, 2.89 mmol); **6b** (593 mg, 2.63 mmol); triethylamine (799 mg, 7.91 mmol) for 3 h at rt. Then NaOMe (312 mg, 5.78 mmol) in methanol overnight. The title compound was obtained by column chromatography (dichloromethane–methanol: 9–1) as a white solid (577 mg, 65%). IR (ATR) γ cm^{-1}: 3200–3000 (w), 3002, 2965, 2935, 2830, 1643, 1595, 1567, 1516. ^1H NMR (DMSO D_6), δ ppm: 13.38 (bs, 1H), 7.39 (s, 1H), 6.92 (s, 1H), 4.33 (q, J = 7.1 Hz, 2H), 3.84 (s, 3H), 3.83 (s, 3H), 3.55 (s, 3H), 1.30 (t, J = 7.1 Hz, 3H). ^{13}C NMR (DMSO D_6), δ ppm: 171.3, 167.7, 158.9, 155.4, 145.3, 138.0, 106.9, 105.5, 98.5, 98.8, 61.6, 56.6, 56.3, 29.6, 14.5. Anal. Calcd for $C_{15}H_{17}NO_6$: C, 58.63; H, 5.58; N, 4.56. Found: C, 58.43; H, 5.66; N, 4.59.

4.1.11.3. Ethyl 1-benzyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylate 7c. According to the general procedure (4.1.11.) from ethyl malonyl chloride (157 mg, 1.05 mmol); **6c** (301 mg, 0.95 mmol); triethylamine (288 mg, 2.85 mmol) for 3 h at rt. Then NaOMe (103 mg, 1.9 mmol) in methanol overnight. The compound was obtained by column chromatography (dichloromethane–ethyl acetate: 9–1) as a white solid (240 mg, 66%). IR (ATR) γ cm^{-1}: 3100–3000 (w), 2946, 2838, 1623, 1598, 1567. ^1H NMR ($CDCl_3$), δ ppm: 14.37 (s, 1H), 7.50 (s, 1H), 7.34–7.20 (m, 5H), 6.61 (s, 1H), 5.51 (s, 2H), 4.52 (q, J = 7.1 Hz, 2H), 3.93 (s, 3H), 3.75 (s, 3H), 1.49 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$), δ ppm: 173.1, 171.3, 160.0, 155.0, 145.1, 137.4, 136.8, 128.8, 127.3, 126.6, 107.7, 105.3, 97.9, 96.2, 62.2, 56.2, 56.0, 46.0, 14.3. Anal. Calcd for $C_{21}H_{21}NO_6$: C, 65.79; H, 5.52; N, 3.65. Found: C, 65.27; H, 5.62; N, 3.62.

4.1.11.4. Ethyl 4-hydroxy-1-(4-hydroxy-3,5-dimethoxybenzyl)-2-oxo-1,2-dihydroquinoline-3-carboxylate 7d. According to the general procedure (4.1.11.) from ethyl malonyl chloride (1.07 g, 7.11 mmol); methyl 2-(4-hydroxy-3,5-dimethoxybenzylamino)benzoate **6d** (600 mg, 5.93 mmol); triethylamine (1.80 g, 17.79 mmol) for 4 h at rt. Then NaOMe (312 mg, 5.78 mmol) in methanol overnight. The compound was obtained by column chromatography (dichloromethane) as a white solid (925 mg, 46%). IR (ATR) γ cm^{-1}: 3361, 3100–3000 (w), 2981, 2939, 1615, 1592, 1560, 1520. ^1H NMR ($CDCl_3$), δ ppm: 14.35 (s, 1H), 8.20 (d, J = 7.0 Hz, 1H), 7.55 (t, J = 7.0 Hz, 1H), 7.39–7.03 (m, 3H), 6.42 (s, 2H), 5.42 (s, 2H), 4.53 (q, J = 6.8 Hz, 2H), 3.80 (s, 6H), 1.49 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$), δ ppm: 172.7, 171.9, 159.6, 147.4, 141.0, 134.2, 127.7, 125.7, 122.0, 115.1, 115.0, 103.7, 97.9, 62.3, 56.4, 45.8, 14.2. Anal. Calcd for $C_{21}H_{21}NO_7$: C, 63.15; H, 5.30; N, 3.51. Found: C, 62.92; H, 5.24; N, 3.54.

4.1.12. Preparation of compounds 8a–d

Conpounds were prepared according to method A of amidification (4.1.6).

4.1.12.1. *N*-((1-Benzylpiperidin-4-yl)methyl)-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide 8a. Title compound was prepared from **7a** (338 mg, 1.15 mmol) and (1-benzylpiperidin-4-yl)methanamine (259 mg, 1.27 mmol) for 3 h to reflux in xylene. After cooling to room temperature the product precipitated, and was washed by diethyl ether to give a white solid (447 mg, 86%). IR (ATR) γ cm^{-1}: 3237, 3161, 3200–3000 (w), 2915, 2799, 2935, 1639, 1511. ^1H NMR ($CDCl_3$), δ ppm: 17.14 (s, 1H), 10.14 (t, J = 5.7 Hz, 1H), 9.80 (s, 1H), 7.43 (s, 1H), 7.35–7.20 (m, 5H), 6.55 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 3.49 (s, 2H), 3.42–3.25 (m, 2H), 2.90 (d, J = 11.3 Hz, 2H), 1.96 (t, J = 11.0 Hz, 2H), 1.76 (d, J = 12.5 Hz, 2H), 1.53–1.63 (m, 1H), 1.43–1.28 (m, 2H). ^{13}C NMR ($CDCl_3$), δ ppm: 172.9, 171.1, 163.7, 155.0, 146.2, 138.5, 134.3, 129.0, 128.1, 126.9, 108.5, 104.7, 97.3, 95.2, 63.3, 56.2, 56.1, 53.3, 44.6, 36.3, 30.2. Anal. Calcd for $C_{25}H_{29}N_3O_5$: C, 66.50; H, 6.47; N, 9.31. Found: C, 65.91; H, 6.44; N, 9.51.

4.1.12.2. *N*-((1-Benzylpiperidin-4-yl)methyl)-4-hydroxy-6,7-dimethoxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxamide 8b. Title compound was prepared from **7b** (152 mg, 0.519 mmol) and (1-benzylpiperidin-4-yl)methanamine (127 mg, 0.622 mmol) for 3 h to reflux in xylene. Solvent was removed in vacuo and title compound was obtained as a white solid (198 mg, 82%) by column chromatography (dichloromethane–methanol: 1–4% gradient). IR (ATR) γ cm^{-1}: 3191 (w), 3200–3000 (w), 3000, 2922, 2795, 2753, 1637, 1574, 1525. ^1H NMR ($CDCl_3$), δ ppm: 17.16 (s, 1H), 10.43 (s, 1H), 7.56 (s, 1H), 7.37–7.17 (m, 5H), 6.74 (s, 1H), 4.03 (s, 3H), 3.98 (s, 3H), 3.68 (s, 3H), 3.50 (s, 2H), 3.42–3.30 (m, 2H), 2.91 (d, J = 11.1 Hz, 2H), 1.98 (t, J = 11.2 Hz, 2H), 1.77 (d, J = 11.9 Hz, 2H), 1.64–1.53 (m, 1H), 1.50–1.29 (m, 2H). ^{13}C NMR ($CDCl_3$), δ ppm: 171.3, 171.1, 162.6, 154.6, 145.5, 138.5, 136.1, 129.1, 128.1, 126.8, 109.1, 105.3, 96.7, 95.5, 63.4, 56.2 (2C), 53.3, 44.5, 36.0, 30.1, 29.3. Anal. Calcd for $C_{26}H_{31}N_3O_5$: C, 67.08; H, 6.71; N, 9.03. Found: C, 67.29; H, 6.77; N, 8.95.

4.1.12.3. 1-Benzyl-*N*-((1-benzylpiperidin-4-yl)methyl)-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide 8c. According to method A of amidification (4.1.4.) from **7c** (158 mg, 0.4 mmol) and (1-benzylpiperidin-4-yl)methanamine (93 mg, 0.44 mmol) for 4 h to reflux in xylene. The solvent was removed in vacuo and the compound was obtained as a white solid (196 mg, 91%) by crystallization from pentane. IR (ATR) γ cm^{-1}: 3186, 3200–3000 (w), 3002, 2924, 2791, 2746, 1628, 1569, 1517. ^1H NMR (DMSO D_6), δ ppm: 17.39 (bs, 1H), 10.45 (bs, 1H), 7.39 (s, 1H); 7.28 (m, 10H), 6.89 (s, 1H), 5.57 (s, 2H), 3.81 (s, 3H), 3.74 (s, 3H), 3.42 (s, 2H), 3.29 (m, 2H), 2.80 (d, J = 10.0 Hz, 2H), 1.90 (t, J = 10.0 Hz, 2H), 1.65 (d, J = 12.0 Hz, 3H), 1.26 (m, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 171.3, 172.2, 162.4, 154.9, 145.7, 139.1, 137.4, 135.7, 129.3, 129.2, 128.6, 128.5, 127.6, 127.2, 108.5, 104.9, 99.2, 94.8, 62.9, 56.4, 56.1, 53.3, 45.2, 44.4, 36.1, 30.1. Anal. Calcd for $C_{32}H_{35}N_3O_5$: C, 70.96; H, 6.51; N, 7.76. Found: C, 70.53; H, 6.60; N, 7.67.

4.1.12.4. *N*-((1-Benzylpiperidin-4-yl)methyl)-4-hydroxy-1-(4-hydroxy-3,5-dimethoxybenzyl)-2-oxo-1,2-dihydroquinoline-3-carboxamide 8d. According to method A of amidification (4.1.4.) from **7d** (117 mg, 0.293 mmol) and (1-benzylpiperidin-4-yl)methanamine (90 mg, 0.44 mmol) for 4 h of reflux in xylene. The solvent was removed in vacuo and the title compound was obtained as a white solid (157 mg, 96%) by column chromatography

(dichloromethane–methanol: 8%). IR (ATR) γ cm^{-1}: 3221, 3400–3000, 2924, 2803, 1628, 1561, 1517. ^1H NMR (DMSO D_6), δ ppm: 17.57 (bs, 1H), 10.47 (bs, 1H), 8.33 (s, 1H), 8.09 (d, J = 7.8 Hz, 1H), 7.71 (t, J = 7.8 Hz, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.40–7.18 (m, 6H), 6.53 (s, 2H), 5.41 (s, 2H), 3.65 (s, 6H), 3.43 (s, 2H), 3.36–3.28 (m, 2H), 2.80 (d, J = 10.2 Hz, 2H), 1.90 (t, J = 11.1 Hz, 2H), 1.70–1.50 (m, 3H), 1.35–1.14 (m, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 172.1, 171.2, 162.7, 148.6, 139.6, 139.0, 135.3, 134.6, 129.2, 128.6, 127.3, 127.1, 125.1, 123.1, 116.4, 107.9, 104.9, 96.2, 62.8, 56.5, 53.2, 45.3, 44.4, 35.9, 30.0. Anal. Calcd for $C_{32}H_{35}N_3O_6$: C, 68.92; H, 6.33; N, 7.54. Found: C, 68.56; H, 6.29; N, 7.56.

4.1.13. Typical procedure for the preparation of 9a–d

To a solution of the appropriate methoxy derivative **8a–d** (1 equiv) in dry dichloromethane at −30 °C was slowly added boron tribromide (1 M in dichloromethane, 3 equiv per methoxy function) under nitrogen atmosphere. The mixture was allowed slowly to warm up to room temperature and stirred overnight. The reaction was quenched by careful addition of brine and product was extracted by ethyl acetate. Organic layers were evaporated under reduce pressure and the product was obtained by column chromatography and/or crystallization.

4.1.13.1. *N*-((1-Benzylpiperidin-4-yl)methyl)-4,6,7-trihydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide 9a.

According to the typical procedure from **8a** (91 mg, 0.202 mmol) and and BBr_3 1 M in dichloromethane (1.22 mL, 1.22 mmol). The compound was obtained by column chromatography (dichloromethane–methanol: 1–5%) and recrystallization from acetone as a white solid (58 mg, 68%). IR (ATR) γ cm^{-1}: 3227, 3600–2800 (l), 2972, 2689, 1659, 1615, 1549. ^1H NMR (DMSO D_6), δ ppm: 16.66 (s, 1H), 11.48 (s, 2H), 11.15–11.00 (m, 1H), 10.56–10.42 (m, 1H), 7.49–7.35 (m, 5H), 6.91 (s, 1H), 6.59 (s, 1H), 4.29–4.15 (m, 2H), 3.31–3.20 (m, 2H), 3.14–3.02 (m, 1H), 2.99–2.79 (m, 2H), 1.86–1.40 (m, 6H). ^{13}C NMR (DMSO D_6), δ ppm: 171.8, 171.7, 162.8, 153.6, 143.1, 136.0, 134.8, 131.8, 130.0, 129.3, 107.7, 106.8, 101.3, 94.2, 59.8, 51.8, 43.0, 34.0, 27.0. Anal. Calcd for $C_{23}H_{25}N_3O_5$: C, 65.24; H, 5.95; N, 9.92. Found: C, 64.94; H, 5.91; N, 9.86%. HRMS (ESI, $(M+H)^+$) calcd 424, 1794, found 424, 1798.

4.1.13.2. *N*-((1-Benzylpiperidin-4-yl)methyl)-4,6,7-trihydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxamide 9b.

According to the typical procedure from **8b** (52.3 mg, 0.11 mmol) and BBr_3 1 M in dichloromethane (0.67 mL, 0.67 mmol). The compound was obtained as a white solid (42 mg, 87%) by column chromatography (dichloromethane–methanol: 15%). IR (ATR) γ cm^{-1}: 3327, 3203, 3500–3000 (l), 2954, 2732, 1646, 1564, 1529. ^1H NMR (DMSO D_6), δ ppm: 16.97 (s, 1H), 10.52 (t, J = 5.9 Hz, 1H), 10.43 (s, 1H), 9.68 (s, 1H), 7.59–7.38 (m, 5H), 7.36 (s, 1H), 6.90 (s, 1H), 4.26 (s, 2H), 3.52 (s, 3H), 3.43–3.33 (m, 2H), 3.07–2.80 (m, 2H), 1.92–1.69 (m, 5H), 1.58–1.33 (m, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 171.0, 170.0, 161.3, 153.1, 142.2, 135.8, 135.2, 131.2, 129.4, 128.7, 108.1, 107.0, 101.1, 93.6, 55.4, 54.8, 51.3, 42.6, 33.4, 28.8. HRMS (ESI, $(M+H)^+$) calc 438.2029, found 438.2030.

4.1.13.3. 1-benzyl-*N*-((1-benzylpiperidin-4-yl)methyl)-4,6,7-trihydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide 9c.

According to the typical procedure from **8c** (52.3 mg, 0.11 mmol) and BBr_3 1 M in dichloromethane (0.67 mL, 0.67 mmol). The compound was obtained as a white solid (42 mg, 87%) by column chromatography (dichloromethane–methanol 15%). IR (ATR) γ cm^{-1}: 3600–2800 (l), 3390, 3064, 2937, 1626, 1555. ^1H NMR (DMSO D_6), δ ppm: 11.49 (bs, 1H), 11.30 (bs, 1H), 10.53 (bs, 2H), 7.70–7.10 (m, 11H), 6.76 (s, 1H), 5.41 (s, 2H), 4.29 (s, 2H), 3.92–3.60 (m, 4H), 3.54–3.22 (m, 2H), 1.98–1.74 (m, 3H), 1.58–1.32 (m, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 171.7, 171.1, 162.2, 153.6, 143.0, 137.2, 136.4, 135.1, 131.9, 131.8, 130.0, 129.3, 129.1, 126.8, 108.8, 107.9, 102.3, 94.0, 59.7, 51.8, 51.7, 45.3, 33.9, 27.1. HRMS (ESI, $(M+H)^+$) calc 514.2342, found 514.2328.

4.1.13.4. *N*-((1-benzylpiperidin-4-yl)methyl)-4-hydroxy-2-oxoide 9d.

According to the typical procedure **8d** (42 mg, 0.075 mmol) and BBr_3 1 M in dichloromethane (0.45 mL, 0.45 mmol). The compound was obtained obtained as a white solid (18 mg, 45%) by column chromatography (dichloromethane–methanol: 20%). IR (ATR) γ cm^{-1}: 3500–2800 (l), 3220, 2955, 2919, 2851, 1727, 1626, 1562. ^1H NMR (DMSO D_6), δ ppm: 17.56 (s, 1H), 10.44 (bs, 1H), 8.78 (s, 2H), 8.11 (d, J = 8.1 Hz, 1H), 8.03 (s, 1H), 7.71 (t, J = 8.1 Hz, 1H), 7.45 (d, J = 8.1 Hz, 1H), 7.39–7.26 (m, 6H), 6.09 (s, 2H), 5.30 (bs, 2H), 3.46 (s, 2H), 3.36–3.28 (m, 2H), 2.98–2.78 (m, 2H), 1.94–1.82 (m, 2H), 1.78–1.58 (m, 3H), 1.38–1.20 (m, 2H). ^{13}C NMR (DMSO D_6), δ ppm: 172.2, 171.2, 167.2, 146.8, 139.7, 134.8, 134.6, 128.8 128.7, 128.6, 127.8, 127.0, 125.1, 125.0, 116.5, 116.0, 105.6, 96.2, 67.7, 53.2, 45.4, 45.0, 31.0, 29.4. HRMS (ESI, $(M+H)^+$) calc 530.2271, found 530.2267.

4.2. In vitro assays

4.2.1. Inhibition of AChE and BuChE

The method of Ellman et al. was followed.[22] Human erythrocyte AChE, *Torpedo califonica* AChE and equine BChE were obtained from Sigma–Aldrich. Stock solutions were prepared by dissolving lyophilized enzymes in phosphate buffer solution (pH = 8.0). Solutions of tested compounds were prepared starting from 10 mM stock solutions in DMSO diluted with aqueous assay medium to a final content of organic solvent always under 1%. Five concentrations of each compound were used in order to obtain inhibition of AChE and BuChE comprised between 20 and 80%.

The assay solution in a total volume of 3 mL consisted of 0.1 M phosphate buffer solution pH = 8.0 and contained 5,5′-dithio-bis(2-nitrobenzoic acid), DTNB (2625 μL, 0.35 mM final concentration), sample (3 μL, 0.01–10 μM final concentration), AChE (29 μL, 0.035 u.i./mL final concentration) or BuChE (60 μL, 0.05 u.i./mL final concentration) and substrate (acetylthiocholine iodide, ATCh or butyrylthiocholine iodide, BTCh respectively, 105 μL, 0.35 mM final concentration). Increasing concentration of tested compounds were added to the assay solution and pre-incubated for 10 min at room temperature with the enzyme followed by the addition of substrate and absorbance was measured after 15 min. Assay were done with a blank containing all components except the enzyme in order to account for non enzymatic reactions and one sample where only inhibitor was replaced by the buffer solution was always present to yield the 100% of ChE activity (control). Absorbance values were recorded at 412 nm in quadruplicate and the values were averaged. The pourcentage inhibition was calculated as $[(A_{control} - A_{blank}) - (A_{sample} - A_{blank})/(A_{control} - A_{blank})] \times 100$.

Bovine Serum Albumin (BSA) is usually added up to 0.5% of the total reaction volume to reduce the coating of target enzyme during the incubation. In our assay, no obvious effect from BSA on the activity of the compounds was found. This indicates that the new compounds selectively inhibit the target enzymes but do not interact with BSA.

The concentration of compound which determined 50% inhibition of the AChE activity (IC_{50}) was calculated using a sigmoidal hill slope model.

4.2.2. DPPH free radical scavenging activity

Assay for the scavenging of stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was done as previously reported.[23] A freshly prepared solution of DPPH (0.15 mM, 2.7 mL) in methanol was added to a methanol solution of tested compounds or reference

compounds (quercetin and curcumin) at different concentrations. The concentrations of the tested compounds were used in order to obtain activities comprised between 20% and 80%. The mixture was stirred at room temperature in the dark for 2 h and the absorbance was measured in quadruplicate at 517 nm. Radical Scavenging Activity (RSA%) was calculated as $[(A_{control} - A_{sample})/A_{control}] \times 100$ where $A_{control}$ represents absorbance of control without test sample and A_{sample} represents absorbance in the presence of the test sample. The scavenging activity was expressed as the EC_{50}, the concentration of compounds required for scavenging 50% of the DPPH radicals in the solution. EC_{50} values were calculated from linear fits.

4.2.3. Superoxide anion scavenging assay

The technic of Robak and Gryglewski.[26] based on the reactions of NADH, phenazine methosulfate (PMS), molecular oxygen and nitroblue tetrazolium (NBT) was used to evaluate the superoxide anion scavenging. The NADH/PMS/O_2/NBT system involves the intermediate formation of the superoxide anion radical from the interaction of reduced PMS with O_2; the superoxide anion radical then reduces NBT to the highly coloured formazan. The reaction was followed by measuring the absorbance of the formazan at 578 nm. The incubation mixture contained in 2 mL of 16 mM Tris–HCl buffer at pH = 8, NADH (400 μL, 78 μM final concentration), nitroblue tetrazolium (400 μL, 50 μM final concentration), PMS (400 μL, 10 μM final concentration) and compounds to be tested at various concentrations (from 12.5 to 200 μM). Controls were realized by replacing PMS by the buffer and quercetine was used as reference for the assay. Radical Scavenging Activity (RSA%) was calculated as $[(A_{control} - A_{sample})/A_{controle}] \times 100$ where $A_{control}$ represents absorbance of control and A_{sample} represents absorbance in the presence of PMS. The scavenging activity was expressed as the EC_{50}, the concentration of compounds required for scavenging 50% of the superoxide anion radicals in the solution. EC_{50} values were calculated from linear fits.

4.3. Docking study

The hAChE and tAChE three-dimensional structures were obtained from Protein Data Bank (PDB), accession code 4ey7[28] and 1eve[27] respectively. The CDocker program[29] implement in Discovery studio 2.5 software was used. Inhibitors and unnecessary water molecules were removed, hydrogens were added and CHARMm forcefield was applyed. The crystal structure was defined as the receptor and the active site was defined as a sphere within 8 Å reach of the inhibitor. The docking was performed using the default parameters. Ligands were ionized by a pH-based method: ionization state are predicted for a pH range of 5.5–9.5 using estimated pK_a values included in drugbank database. For each ligand a generation of 3D coordinates was realized by calculation using CATALYST algorithm. Random ligand conformations were generated using high-temperature molecular dynamics. Ten conformations were generated from equilibrium and minimization of the starting ligand structure. 1000 steps of molecular dynamics and a targeted temperature of 1000 K was used to generate random starting conformations. Then ligand conformers were translated into the binding site sphere. Candidates poses were then created using random rigid body rotation (with a maximum of 800 rotations and a 300 kcal mol^{-1} energy threshold) followed by simulated annealing (with 2000 heating phase steps, a target temperature of 700 K, 5000 steps of cooling phase and a cooling targeted temperature of 300 K). CHARMm forcefield was used to determine the CDocker score used to sort the poses of each input ligand. The ten best poses are saved and the result can be considered as convergent when all poses are about in the same position. Donepezil (E2020) was used to validate our docking model, the prediction agreed with the crystallographic results with 1.34 and 1.59 Å all-atom root-mean-square deviations for *h*AChE and *t*AChE respectively. The docking (CDOCKER)energy including the intramolecular energy for the ligand and the ligand–protein interactions energy of compd **9a** and donepezil in *h*AChE were 52.86 kcal mol^{-1} and 33.95 kcal mol^{-1} respectively.

Acknowledgments

This work was supported by a grant from the CONSEIL REGIONAL DE FRANCHE COMTE grant N° 2011-07304. Authors thank Dr. F. Chérioux for fruitfull discussions.

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Please cite this article in press as: Pudlo, M.; et al. *Bioorg. Med. Chem.* (2014), http://dx.doi.org/10.1016/j.bmc.2014.02.046