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

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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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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**.

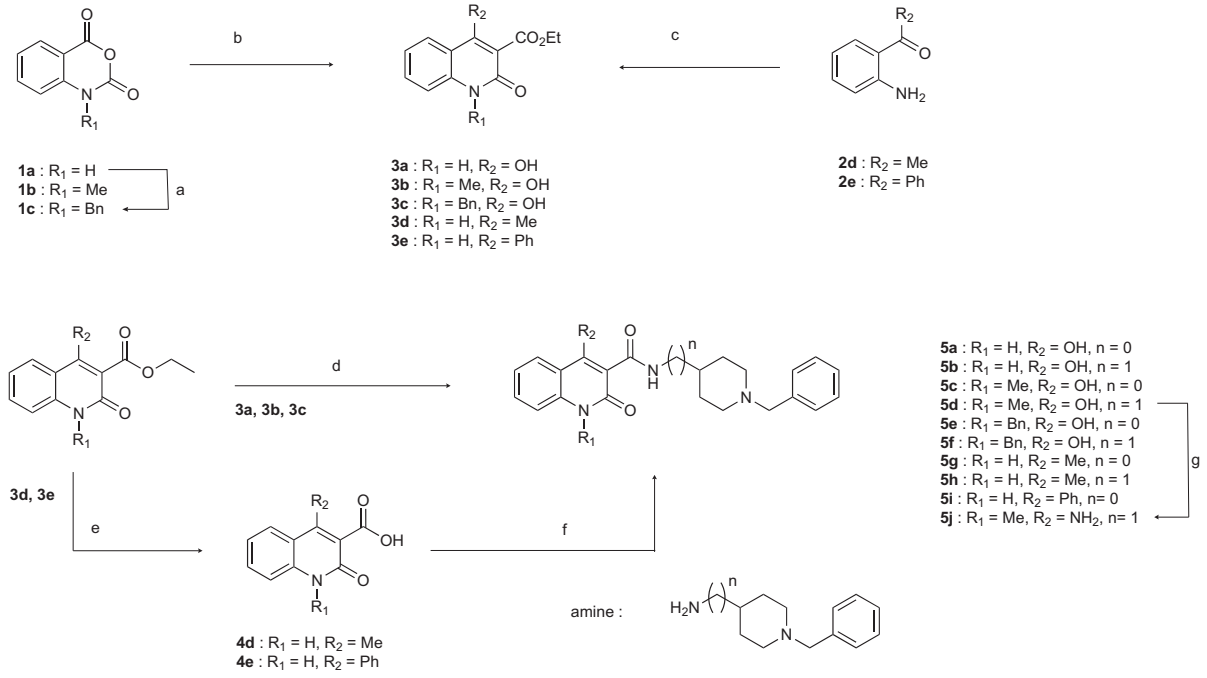
Compounds **8a–d** and **9a–d** were prepared according to the route outlined in Scheme 2. Methoxylated derivatives of quinolone (**7**) were prepared starting from 4,5-dimethoxyanthranilate (**6a–c**) or N-substituted anthranilate with methoxybenzyl (**6d**). Then benzylpiperidine moiety was coupled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