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9-Substituted acridine derivatives as acetylcholinesterase and butyrylcholinesterase inhibitors possessing antioxidant activity for Alzheimer's disease treatment

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ABSTRACT

We investigated the inhibitory activity of 4 groups of novel acridine derivatives against acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and carboxylesterase (CaE) using the methods of enzyme kinetics and molecular docking. Antioxidant activity of the compounds was determined using the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical decolorization assay as their ability to scavenge free radicals. Analysis of the esterase profiles and antiradical activities of the acridine derivatives showed that 9-aryl(heteroaryl)-*N*-methyl-9,10-dihydroacridines have a high radical-scavenging activity but low potency as AChE and BChE inhibitors, whereas 9-aryl(heteroaryl)-*N*-methyl-acridinium tetrafluoroborates effectively inhibit cholinesterases but do not exhibit antiradical activity. In contrast, a group of derivatives of 9-heterocyclic amino-*N*-methyl-9,10-dihydroacridine has been found that combine effective inhibition of AChE and BChE with rather high radical-scavenging activity. The results of molecular docking well explain the observed features in the efficacy, selectivity, and mechanism of cholinesterase inhibition by the acridine derivatives. Thus, in a series of acridine derivatives we have found compounds possessing dual properties of effective and selective cholinesterase inhibition together with free radical scavenging, which makes promising the use of the acridine scaffold to create multifunctional drugs for the therapy of neurodegenerative diseases.

1. Introduction

Acridines are considered privileged scaffolds in drug discovery for protozoan and neurodegenerative diseases.[1] Acridine derivatives have a broad spectrum of therapeutic applications as antibacterial,[2] antimalarial,[3] antileishmanial and antitrypanosomal,[4] antiviral,[5] anticancer[6] and antiprion[7–9] agents. They have also been reported to have anti-inflammatory, anti-diabetic[10,11] and anti-Alzheimer activity.[12–14] Recently they demonstrated an anti-TDP-43 aggregation effect in ALS disease models.[15] Acridine derivatives are optimal starting points for the design of novel hybrid and dimeric multitarget lead and drug candidates.[1]

Alzheimer's disease (AD), the most widely encountered type of dementia in older people, is a multifactorial and fatal neurodegenerative disorder, which is characterized by an inexorable decline in cognitive function and memory that progresses to the complete degradation of personality. AD involves degeneration of cholinergic neurons and diminishing cholinergic transmission.[16] Anticholinesterase drugs are used to compensate for deficiency of the neurotransmitter acetylcholine.[17] They replenish the acetylcholine deficit in the brain by inhibiting cholinesterases, thereby increasing the duration of acetylcholine action on postsynaptic receptors, thus enhancing cholinergic transmission. In a normal brain, acetylcholine is predominantly (80%) hydrolyzed by acetylcholinesterase (AChE, EC 3.1.1.7), whereas butyrylcholinesterase (BChE, EC

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3.1.1.8) plays a supplementary role. However, with progression of AD, the AChE activity decreases, whereas the activity of BChE gradually increases.[18,19] This phenomenon enhances the significance of BChE as an additional therapeutic target for reducing the cholinergic deficiency inherent in AD.[20–22]

Currently, AD therapy is mainly founded on cholinesterase inhibitors, which are able to increase acetylcholine levels in cholinergic synapses. To date, the number of approved drugs is limited to only three cholinesterase inhibitors (rivastigmine, donepezil, and galantamine), and the *N*-methyl-D-aspartate (NMDA) receptor antagonist, memantine.[23,24]

The multifactorial nature of AD is commonly recognized, implying the involvement a number of neurobiological targets in the development of this neurodegenerative disease. In this context, the concept of multitarget drugs having an integrated action on a number of biological targets involved in pathogenesis of the disease currently appears to be highly promising in the design of drugs for treating AD.[25,26]

Oxidative stress leading to oxidative damage of cell membranes, mitochondria, lipids and proteins, may be one of the possible causes of neuronal death.[27] Oxidative stress is characterized as an imbalance between biochemical processes leading to the production of reactive oxygen species (ROS) and their removal.[28] The efficiency of the brain's antioxidant system gradually declines with age, and this decline is more pronounced in AD patients. This fact substantiates the use of antioxidants in AD therapy,[29] and the development of cholinesterase inhibitors with attendant antioxidant properties is a modern trend in research directed toward efficient therapy of AD.[30–32]

It is well known that compounds of the acridine family are able to inhibit AChE and BChE.[33–35] Tacrine (9-amino-1,2,3,4-tetrahydroacridine), a potent reversible inhibitor of AChE and BChE, was the first drug approved by the FDA for treatment of Alzheimer's disease.[36] However, it was withdrawn from clinical use because of its hepatotoxicity.[37] Nevertheless, there is a continuing interest in the tacrine template to design new hybrid molecules that might be safer and more effective AD drugs than tacrine.[38–41] Although the precise mechanism of hepatotoxicity of tacrine has not been elucidated, oxidative stress appears to be involved to some degree. Therefore, derivatization of acridine-based anticholinesterase compounds in a manner that confers antioxidant activity would be expected to ameliorate this toxic concern.

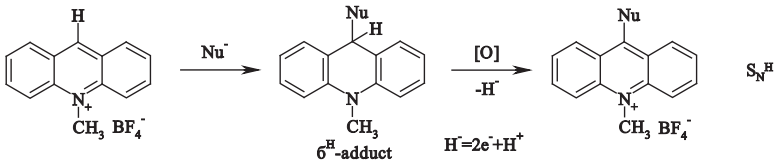
The aim of the present study was to investigate the inhibitory properties of novel acridine derivatives against the key enzymes of the cholinergic nervous system AChE and BChE, using both kinetic and computational molecular modeling methods, as well to assess the ability of compounds to scavenge free radicals using the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. In addition, we have determined the inhibitory activity of the synthesized acridines toward carboxylesterase (CaE, EC 3.1.1.1), a serine hydrolase structurally related to cholinesterases that catalyzes the hydrolysis of many therapeutically important agents bearing ester and other hydrolysable groups.[42,43] The ability of anticholinesterase compounds used for AD therapy to inhibit CaE could lead to undesirable drug-drug interactions.[44] Consequently, in this investigation we were seeking to find compounds with anticholinesterase and antiradical activity but lacking anti-CaE activity.

2. Results and discussion

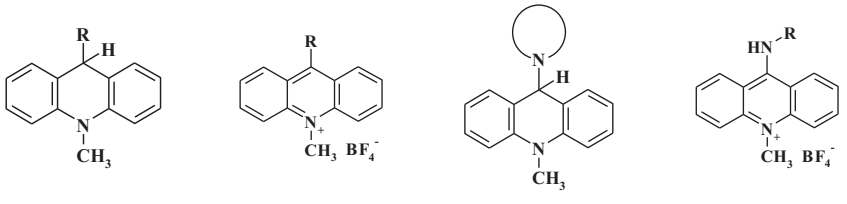
2.1. Synthesis of acridine derivatives

Acridine derivatives can be obtained using three main approaches. The first one is based on construction of the acridine ring system through the reaction of the corresponding carboxylic acid with diphenylamine in the presence of $ZnCl_2$ at elevated temperatures (200–260°C)[45,46] The second approach entails functionalization of acridine using metal-catalyzed cross-coupling reactions.[47] However, these two methods have some disadvantages, such as the necessity to incorporate good leaving groups, the formation of byproducts due to side reactions of organometallic reagents, and some difficulties in eliminating catalysts and auxiliary ligands. Fortunately, there is a third approach involving metal-free methods for direct C–H functionalization of acridines, based on nucleophilic aromatic substitution of hydrogen, the so-called S_N^H reactions.[48–54]

A mechanism that is commonly accepted for the S_N^H reactions involves two steps. The first one is addition of a nucleophile to an aromatic ring, thus leading to the formation of σ^H-adducts. Oxidative aromatization of σ^H-adducts is realized at the second step by action of an outer-sphere oxidant (Scheme 1). The ability of σ^H-adducts to undergo aromatization into S_N^H products varies greatly: from very unstable and hardly spectroscopically detectable σ^H-adducts to rather stable compounds, derived from the reactions of *N*-methylacridinium salts. In the latter case, dihydroacridines can be easily isolated for studying their biological activity. Moreover, it is possible to carry out aromatization of dihydroacridines in order to estimate how these structural changes affect their biological properties.



Scheme 1. Mechanism of S_N^H reactions.



1a-j **2a-m** **3a-f** **4a,b**

Fig. 1. Structures of the studied acridine derivatives.