Bioorganic & Medicinal Chemistry Letters 27 (2017) 2443–2449

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Cytochrome P450 binding studies of novel tacrine derivatives: Predicting the risk of hepatotoxicity

Alanna McEneny-King, Wesseem Osman, Andrea N. Edginton, Praveen P.N. Rao *

School of Pharmacy, Health Sciences Campus, University of Waterloo, 200 University Avenue West, Waterloo, Ontario N2L 3G1, Canada

ARTICLE INFO

Article history:
Received 27 February 2017
Revised 30 March 2017
Accepted 1 April 2017
Available online 3 April 2017

Keywords:
Cholinesterase inhibitors
Tacrine
Hepatotoxicity
Cytochrome P450
CYP1A2
CYP3A4
Molecular docking
3,4-Dimethoxybenzylamino

ABSTRACT

The 1,2,3,4-tetrahydroacridine derivative tacrine was the first drug approved to treat Alzheimer's disease (AD). It is known to act as a potent cholinesterase inhibitor. However, tacrine was removed from the market due to its hepatotoxicity concerns as it undergoes metabolism to toxic quinonemethide species through the cytochrome P450 enzyme CYP1A2. Despite these challenges, tacrine serves as a useful template in the development of novel multi-targeting anti-AD agents. In this regard, we sought to evaluate the risk of hepatotoxicity in a series of C9 substituted tacrine derivatives that exhibit cholinesterase inhibition properties. The hepatotoxic potential of tacrine derivatives was evaluated using recombinant cytochrome (CYP) P450 CYP1A2 and CYP3A4 enzymes. Molecular docking studies were conducted to predict their binding modes and potential risk of forming hepatotoxic metabolites. Tacrine derivatives compound **1** (*N*-(3,4-dimethoxybenzyl)-1,2,3,4-tetrahydroacridin-9-amine) and **2** (6-chloro-*N*-(3,4-dimethoxybenzyl)-1,2,3,4-tetrahydroacridin-9-amine) which possess a C9 3,4-dimethoxybenzylamino substituent exhibited weak binding to CYP1A2 enzyme (**1**, IC_{50} = 33.0 μM; **2**, IC_{50} = 8.5 μM) compared to tacrine (CYP1A2 IC_{50} = 1.5 μM). Modeling studies show that the presence of a bulky 3,4-dimethoxybenzylamino C9 substituent prevents the orientation of the 1,2,3,4-tetrahydroacridine ring close to the heme-iron center of CYP1A2 thereby reducing the risk of forming hepatotoxic species.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and is currently the most common cause of cognitive dysfunction in the elderly population. As per the cholinergic hypothesis of AD, the symptoms of AD are related to a decline in acetylcholine (ACh) levels.[1] Based on this, the strategy of inhibiting the cholinesterase enzymes responsible for ACh degradation, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), proved more successful and are currently the backbone of AD pharmacotherapy.[2] Tacrine (Cognex®, Fig. 1) was the first cholinesterase inhibitor (ChEI) to be approved for the management of AD symptoms in 1993. It is a potent inhibitor of both AChE and BuChE.[3] Tacrine (1,2,3,4-tetrahydroacridin-9-amine) has since been removed from the market due to adverse effects, including hepatotoxicity in a significant percentage of patients.[4] Literature data indicates that around half of patients on tacrine had alanine aminotransferase (ALT) levels above the upper limit of normal and approximately one-quarter of patients ALT levels were more than three times the upper limit of normal.[5] Patients are usually asymptomatic and ALT levels return to normal about one month after tacrine cessation. Unfortunately, there are no reliable predictors of hepatotoxicity. Circulating tacrine:metabolite ratios, sex, smoking status, concomitant drug use, and pre-existing renal/hepatic disease have all been investigated but no significant covariates were identified.[6]

The toxicity observed in patients on tacrine has been attributed to certain metabolites produced when tacrine undergoes oxidative hydroxylation by the hepatic enzyme cytochrome P450 1A2 (CYP1A2). In tacrine, the addition of the hydroxyl group via oxidative metabolism by CYP1A2 can occur at the C1, C2, C4 and C7 positions, and subsequent rearrangement to a reactive 7-quinonemethide and 1,7-quinonemethide intermediates (Fig. 2). These species are able to bind irreversibly to liver cells and cause hepatocellular necrosis in a mechanism similar to that of acetaminophen.[7] The mechanism of tacrine hepatotoxicity was further elucidated by Madden and coworkers through incubations with glutathione.[8] Glutathione is a nucleophilic scavenger and plays an important role in protecting the liver against a number of electrophilic hepatotoxins by forming detoxified conjugates. Incubations with a physiologically relevant concentration of glutathione eliminated tacrine cytotoxicity and reduced formation of protein-reactive metabolites, confirming that an electrophilic tacrine metabolite appears to be involved in its toxicity.[8]

* Corresponding author.
E-mail address: praopera@uwaterloo.ca (P.P.N. Rao).

http://dx.doi.org/10.1016/j.bmcl.2017.04.006
0960-894X/

Fig. 1. Chemical structure of tacrine.

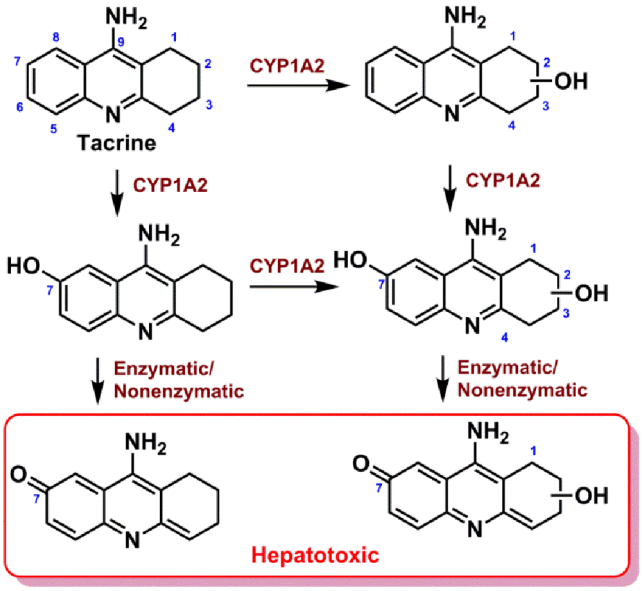

Fig. 2. CYP1A2 mediated metabolism of tacrine to form hepatotoxic species.

Today, the ChEIs still represent the mainstay of AD pharmacotherapy represented by marketed drugs – donepezil, galantamine and rivastigmine.[2] However, these compounds only treat the symptoms of mild to moderate AD rather than preventing or reversing the disease progression, emphasizing the need to target multiple pathways if therapy is to be successful. One of the other hypotheses related to AD etiology is the amyloid cascade hypothesis, which suggests that the characteristic amyloid-β (Aβ) plaques observed in the brains of AD patients are not just a marker of the disease but are, in fact, the principal driver of AD pathology due to their neurotoxicity.[9] Since the current class of agents used in AD therapy typically target only one hypotheses associated with AD pathophysiology, they are not efficacious in the long term. In this regard, new anti-AD agents are being developed to target multiple pathological features of AD. A common strategy for the development of multifunctional compounds is the use of existing AD treatments as a scaffold. Despite its toxicity issues, tacrine remains a popular scaffold as a starting point for the design of multifunctional compounds with improved safety and enlarged biological profiles. In addition to inhibiting cholinesterase activity and Aβ aggregation, tacrine hybrids have also been designed to target reactive oxygen species, neuronal calcium channels, vasorelaxation and metal chelation but hepatotoxicity is not often investigated.[10–13] The toxicity observed in tacrine has been successfully reversed in some derivatives by either blocking the C7 site with a methoxy group to prevent the formation of quinone-type toxic metabolites, or by attaching an antioxidant moiety to counter oxidative stress.[14,15] However, the hepatotoxicity aspect of tacrine derivatives remains unexplored in the majority of cases.

In this study, we investigated if chemical modification alters affinity for CYP450 enzymes and to evaluate the risk of hepatotoxicity in a novel class of tacrine derivatives reported by our group recently.[16] These compounds were previously assessed for their ability to inhibit cholinesterase activity and self-induced Aβ aggregation. The four most promising derivatives (Fig. 3, **1**–**4**) from this series were investigated for their inhibition potential toward CYP1A2 and CYP3A4 enzymes using in vitro assays. The results were correlated with molecular modeling studies to predict the major sites of metabolism and the likelihood of these compounds to cause hepatotoxicity. These studies reveal that C9 3,4-dimethoxybenzylamino substituted tacrine derivatives are unlikely to form hepatotoxic metabolites.

The ability of tacrine derivatives **1**–**4** to inhibit CYP1A2 activity was evaluated at various concentrations (0.01, 1, 10, and 20 μM) using a fluorescence assay that is based on the potential of test compounds to prevent the formation of a fluorescence probe in the presence of CYP1A2.[17] The reference agent, α-naphthoflavone which is a known CYP1A2 inhibitor, is a potent competitive inhibitor of CYP1A2 enzyme.[18] We used it to compare the CYP1A2 inhibition profile of tacrine derivatives **1**–**4**. Furthermore, α-naphthoflavone is not a substrate for CYP1A2 and is reported to undergo negligible metabolism through CYP1A2.[18] Therefore, it serves as a suitable reference standard to determine if tacrine derivatives **1**–**4** would act as inhibitors or substrates of CYP1A2. In contrast, tacrine itself is known to undergo metabolism by CYP1A2 and is considered as a substrate.[19] α-Naphthoflavone exhibited strong (>80%) inhibition of CYP1A2 (Fig. 4) at concentrations above 1 μM, with an IC_{50} of 0.34 μM (Table 1). Tacrine reached similar levels of inhibition (~75%) at concentrations of 10 and 20 μM, while displaying weaker inhibition at low concentrations (14% and 45% at 0.01 and 1 μM, IC_{50} = 1.5 μM). Among the tacrine derivatives investigated, compound **4** proved to be the most potent inhibitor of CYP1A2, displaying an inhibition profile similar to that of α-naphthoflavone (>80% inhibition at

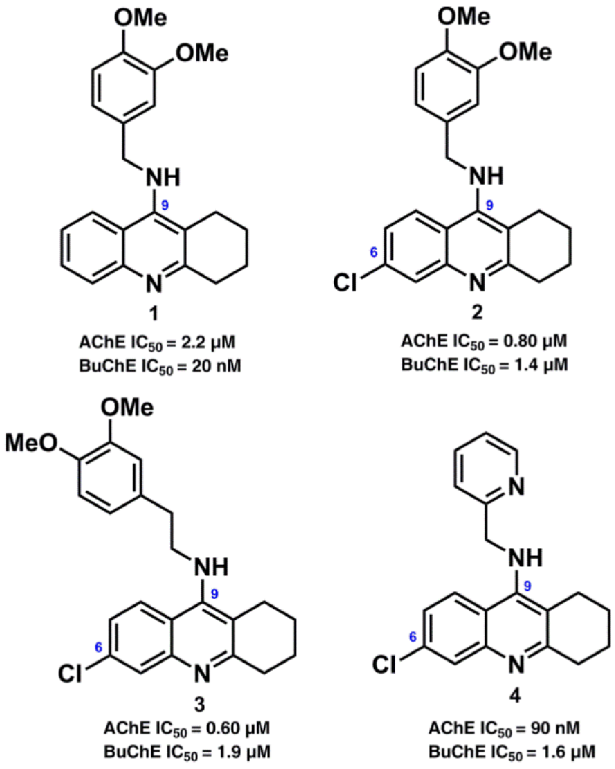

Fig. 3. Chemical structures of tacrine derivatives (**1**–**4**).