European Journal of Medicinal Chemistry 78 (2014) 157–166

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Fluorinated benzophenone derivatives: Balanced multipotent agents for Alzheimer's disease

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ARTICLE INFO

Article history:
Received 29 September 2013
Received in revised form
12 March 2014
Accepted 14 March 2014
Available online 16 March 2014

Keywords:
Acetylcholinesterase
Alzheimer's disease
Antioxidant activity
BACE-1
Benzophenone
Drug design
Lead identification

ABSTRACT

In an effort to develop multipotent agents against β-secretase (BACE-1) and acetylcholinesterase (AChE), able to counteract intracellular ROS formation as well, the structure of the fluorinated benzophenone **3** served as starting point for the synthesis of a small library of 3-fluoro-4-hydroxy- analogues. Among the series, derivatives **5** and **12**, carrying chemically different amino functions, showed a balanced micromolar potency against the selected targets. In particular, compound **12**, completely devoid of toxic effects, seems to be a promising lead for obtaining effective anti-AD drug candidates.

1. Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disorder that accounts for the majority of cases of dementia. It afflicts over 30 million people worldwide and these figures are expected to quadruple by 2050. At present, the only available treatments treat the symptoms of AD. Affecting neurotransmission by means of inhibitors of acetylcholinesterase (AChE), a well known molecular target involved in AD pathology, and N-methyl-D-aspartate (NMDA) receptor antagonists, makes it possible to slow down the cognitive decline associated with AD, yet showing only modest palliative clinical efficacy without affecting the disease progression or correcting the neurodegenerative process. In the last few years, this lack of an effective cure has fuelled an intense search for disease-modifying agents that, by targeting the underlying pathophysiology of AD, could control the disease process and slow down its clinical course [1]. In this scenario, the development of multitarget agents, chemical entities able to simultaneously modulate multiple biological targets significantly involved in AD neurotoxic pathway, has clearly emerged as a successful strategy [2].

A key event in AD pathogenesis is the accumulation of amyloid-β (Aβ) peptide in the brain. The $A\beta_{42}$ monomers aggregate into toxic extra-cellular oligomeric species, which, in turn, form insoluble fibrillar aggregates, that compose the core of the dense amyloid plaques [3,4]. These structures insert themselves into neuronal membranes and induce lipid peroxidation, protein oxidation and increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), together with loss of function of many antioxidant defence enzymes, thus contributing to oxidative stress and neurotoxicity [5,6]. A common concept of the amyloid cascade hypothesis is that the aggregation of $A\beta_{42}$ peptide into toxic fibrils is the main initiating event that sets off a cascade of neurobiological processes, such as neurotoxicity, oxidative damage and

Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer's disease; ABP, aminobenzylpiperidine; Aβ, amyloid-β; APP, amyloid precursor protein; BACE-1, β-secretase; CNS, central nervous system; HE, hydroxyethylene; FRET, fluorescence resonance energy transfer; PDB, protein data bank; ROS, reactive oxygen species; SAR, structure–activity relationships; *t*-BuOOH, *tert*-butylhydroperoxide.

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http://dx.doi.org/10.1016/j.ejmech.2014.03.042
0223-5234/

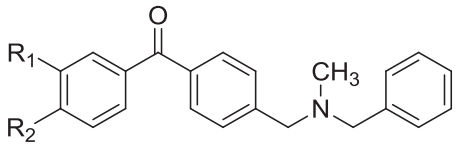
inflammation, ultimately culminating with extensive brain atrophy, neuronal dysfunction, and cognitive decline [7]. The central nervous system (CNS) accumulation of unbound transition metals, such as iron and copper, has also been considered a significant source of oxidant species [8]. Recent *in vivo* studies demonstrated the presence of Aβ in mitochondrial membranes, where it was thought to be responsible for both the disruption of the electron transport chain and the irreversible cell damage [9]. All these factors are not independent of each other, and it is plausible that, especially in the early stages of the disease process, Aβ could enter the mitochondria where it would increase the generation of ROS and induce oxidative stress. Hence, the "oxidative stress hypothesis" states that the increased production of free radicals in AD is a potential target for therapeutic strategies; as such, therapeutic modalities involving antioxidants may be an effective approach to the treatment of this neurodegenerative disease [10].

Aβ is generated by the proteolytic processing of a larger membrane-bound precursor protein, known as the amyloid precursor protein (APP), upon sequential cleavage by two aspartyl proteases, β-secretase (also known as β-site APP cleaving enzyme-1, or BACE-1) and γ-secretase. It has been demonstrated that a variety of stress factors, including hypoxia, ischaemic injury and inflammation, can induce BACE-1 expression in experimental models of sporadic AD [11–14]. In particular, recent studies support the hypothesis that oxidative stress, secretase function and Aβ production are strictly interrelated events and suggest that inhibition of BACE-1 may have a synergic therapeutic effect with antioxidant compounds [10,11,15]. In this context, novel multipotent agents against BACE-1 and oxidative stress have gained attention for their potential as effective anti-AD drug candidates. The first generation of BACE-1 inhibitors focused on compounds with a peptide or peptidomimetic structure, designed as transition-state (TS) mimetics, and different substrate models were designed to closely interact with the BACE-1 catalytic aspartic acids (Asp 32 and Asp 228, catalytic dyad) [16] such as OM99-2, a 8-aminoacid residue hydroxyethylene (HE)-based analogue that spans the P4 to P4′ binding pockets of BACE-1, showing a noteworthy hydrogen bonding network within the active site, together with a direct interaction among the OH of the HE function and the catalytic dyad. Notwithstanding its nanomolar inhibitory potency, this compound showed suboptimal *in vivo* pharmacokinetics and low brain penetration [17]. Generally, the majority of the early BACE-1 inhibitors were characterized by complex, high molecular weight structural motifs, lacking drug-like properties [18]. Since the first X-ray crystal structures of BACE-1 were reported, intensive efforts have focused on the development of potent enzyme inhibitors that possess ideal properties such as oral bioavailability and a good pharmacokinetic profile [17]. The identification of small molecule inhibitors of BACE-1 with CNS permeability represents an important and difficult challenge since, in order to mediate brain Aβ lowering, inhibitors ought to be able to cross the blood–brain barrier (BBB) [19]. A 3-D pharmacophore map of BACE-1 has also been proposed, to guide the design and optimization of inhibitors [20]. Currently, several crystal structures of the catalytic domain of BACE-1, alone or in complex with an inhibitor, have been deposited in the Protein Data Bank (PDB). The structural information that emerged from these studies was employed for structure-based drug discovery projects, leading to the identification of several classes of non-peptidic BACE-1 inhibitors with novel core templates and with improved pharmacokinetics profile [16,18,21].

2. Design

In a drug discovery effort to obtain single multitarget small molecules as anti-AD drug candidates, we further investigated the potential of the benzophenone core structure to hit several targets involved in AD. This scaffold has indeed proved to be a privileged structure, a versatile pharmacophore nucleus, that could be exploited through suitable modifications to provide ligands for an array of biological targets [22,23]. Benzophenone has recently been employed by the authors as a starting point for obtaining compounds able to modulate the actions of AChE [24,25]. Following a previous research project aimed at identifying new chemical entities able to inhibit both BACE-1 and AChE enzymes [26], our internal benzophenone-based collection of AChE inhibitors, bearing a *N,N′*-benzylmethylamine function to target the catalytic binding site of the enzyme, was screened against BACE-1. Given that the cyclic amines proved to be suitable for specific hydrogen bonding with the catalytic aspartic acid, this tertiary amine function could hold promise for interacting with the enzyme, improving the compound solubility as well. Among the tested compounds, derivatives **1**–**4**, with AChE inhibition values ranging from sub-micro to low micromolar (Table 1), showed a promising trend of BACE-1 inhibition, that made it possible to gain insight into the benzophenone substitution pattern essential for this enzyme. Compound **1**, with a 3,4-dimethoxy benzophenone nucleus, endowed with sub-micromolar AChE inhibitory potency [24], when tested at 5 μM concentration showed low BACE-1 inhibition (20%). Removal of the methoxy group in position 3 (compound **2**) led to a notable decrease in potency. Interestingly compound **3**, with a fluorine atom instead of the 3-methoxy substituent, was identified as a weak BACE-1 inhibitor (10.72% of inhibition at 3.38 μM concentration). Notwithstanding its poor activity against BACE-1, compound **3** might be interesting from a pharmacokinetic perspective, since the presence of a fluorine substituent on an aromatic ring could impart a variety of properties, including enhanced binding interactions, metabolic stability, and selective reactivity [27]. For these reasons, **3** could be regarded as a hit compound to be further modified to obtain more potent analogues, and given that the strongly electron withdrawing effect of the fluorine atom markedly influences the acidity of neighbouring functional groups, we then synthesized the corresponding des-methyl analogue **5** (Table 2). Gratifyingly, this compound was able to potently inhibit BACE-1, with an IC_{50} value in the low micromolar range, providing a five-

Table 1
*h*BACE-1 and *h*AChE inhibition profiles of compounds **1**–**4**.



Cmpd	R_1	R_2	*h*BACE-1 inhibition (%)[a,b]	*h*AChE inhibition[b,c] IC_{50} (μM) ± SEM
1	OCH_3	OCH_3	20 (at 5 μM)	0.46 ± 0.04
2	H	OCH_3	n.i.[d]	1.82 ± 0.08
3	F	OCH_3	10.72 (at 3.38 μM)	1.57 ± 0.08
4	H	OH	n.i.[d]	2.10 ± 0.09

[a] % inhibition of BACE-1 activity at the reported concentration of the tested compounds.
[b] Values are mean of two independent measurements, each performed in triplicate.
[c] See Refs. [24,25]. IC_{50} values represent the concentration of inhibitor required to decrease enzyme activity by 50% IC_{50} values were determined by following Ellman's method.
[d] n.i.: not inhibiting up to 4 μM.