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Synthesis and biological evaluation of quinazolinone-based hydrazones with potential use in Alzheimer's disease

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ABSTRACT

Discovering multifunctional agents for the treatment of Alzheimer's disease (AD) is an attractive therapeutic approach. BACE1 (β-site amyloid precursor protein cleaving enzyme 1) inhibitors may play a pivotal role in treating AD. Therefore, the discovery of novel non-peptide BACE1 inhibitors with desirable blood brain barrier permeability is a favorable approach for treatment. Moreover, the antioxidant potential of a drug could serve as an added value for designing dual-acting therapeutic agents. Here, we report the design, synthesis and biological evaluation of quinazolinone-hydrazone derivatives as new multi-target candidates for the treatment of AD. The compounds were investigated for their *in vitro* BACE1 inhibitory potential using a FRET-based enzymatic assay and also screened for antioxidant activity using DPPH. Among them, compound **4h** bearing a 2,3-dichlorophenyl moiety showed the highest activity with an IC_{50} value of 3.7 μM against BACE1. In addition, compound **4i** with a 2,4-dihydroxyphenyl scaffold demonstrated moderate BACE1 inhibitory activity (IC_{50} = 27.6 μM) with a significant antioxidant effect (IC_{50} = 8.4 μM). Furthermore, docking studies revealed strong interaction between compound **4h** and the key residues of BACE1 active site. These results demonstrate that quinazolinone-hydrazone derivatives represent a valuable scaffold for the discovery of novel non-peptidic BACE1 inhibitors.

1. Introduction

Alzheimer's disease (AD) causes memory loss, cognitive dysfunction and dementia. AD is a multifactorial neurodegenerative disease associated with several pathophysiological factors such as amyloid-β (Aβ) deposits, tau protein aggregation, oxidative stress, and decreased acetylcholine levels [1]. The accumulation of Aβ peptides results in extracellular and intracellular formation of neurofibrillary tangles in the brain and leads to neuronal death. A group of proteases known as secretases are major contributors to the generation of Aβ (usually containing 40–42 amino acids), via the catalytic cleavage of amyloid precursor protein (APP) [2]. The amyloid hypothesis suggests that β-secretase (β-site APP-cleaving enzyme 1, BACE1) first cleaves APP at the N-terminus to yield a membrane-bound C-terminal fragment called C99, followed by the subsequent cleavage by γ–secretase to generate the C-terminus Aβ peptides in various lengths, including Aβ40-42. Based on BACE1's key role in the pathogenesis of AD, BACE1 inhibitors that decrease the production of all forms of Aβ are potential drug candidates for treating AD [3,4]. Early attempts to design BACE1 inhibitors were focused on transition state peptidomimetic analogs. Gosh et al. reported the first generation of two potent peptide inhibitors; OM99-2 (K_i = 1.6 nM) and OM00-3 (K_i = 0.3 nM) [5,6]. However, as CNS drugs, the unfavorable physicochemical characteristics of peptidic compounds such as their low blood brain barrier (BBB) crossing, poor oral bioavailability and susceptibility to P-glycoprotein (Pgp) transport created a demand for non-peptide BACE1 inhibitors [7,8].

In this regard, virtual screening of a large library of compounds led to the discovery of 2-aminoquinazoline derivatives as potential inhibitors of BACE1 [9–11]. Further structure-based optimization by Baxter and coworkers resulted in compound **1** (Fig. 1) as a potent BACE1 inhibitor. However, the selectivity of this compound was modest compared to other proteases like renin and cathepsin

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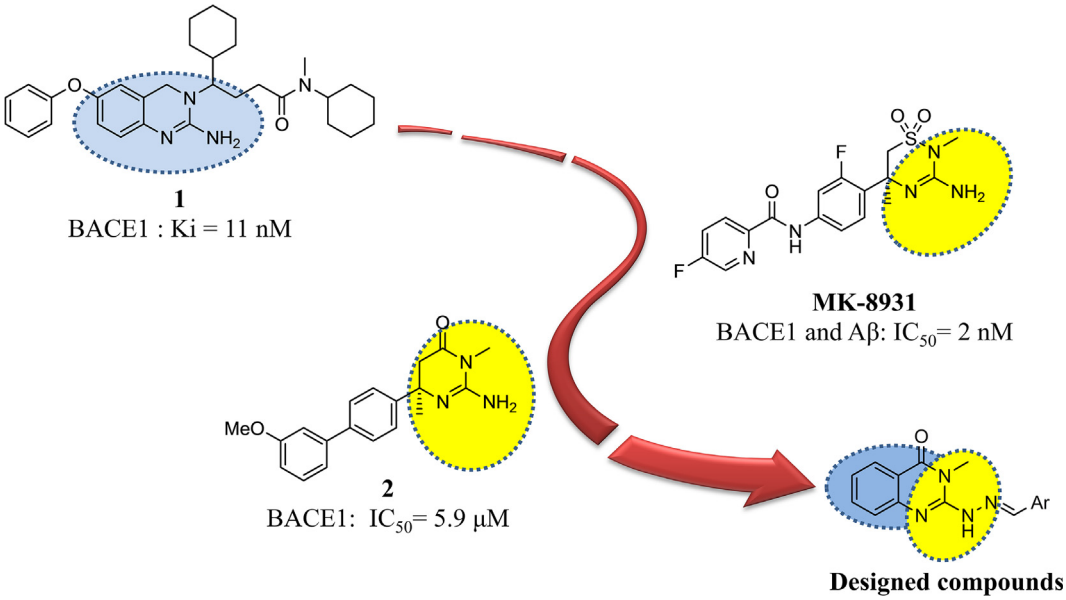

Fig. 1. Design strategy of target compounds with 3-methylquinazolin-4(3*H*)-one-hydrazone structure as BACE1 inhibitors.

D, and was found to be a Pgp substrate [10]. Further optimization to improve the pharmacokinetic properties resulted in iminopyrimidinone derivative **2** with improved BBB penetration [12]. The cocrystal structure of compound **2** in the BACE1 active site suggested that the guanidine is involved in H-bond interactions with two key catalytic residues, Asp32 and Asp228 and also the methyl substituent plays an important role in avoiding unfavorable steric interactions. While no BACE1 inhibitors have been FDA approved to date, small molecule inhibitors from aminothiadiazine derivatives (such as MK-8931) with high potency against BACE1 have entered into human clinical trials, owing to their favorable pharmacokinetics and suitable brain penetration [13].

To find new multi-target anti-AD agents [14,15], we hybridized the key fragments of the 2-aminiquinazoline **1**, MK-8931 and the iminopyrimidinone **2** skeleton, which led to the design of novel 3-methylquinazolin-4(3*H*)-one hydrazones **4**–**6** as potential non-peptide BACE1 inhibitors (Fig. 1). The introduction of an imine linker in the designed molecules **4**–**6** could facilitate H-bond interactions between the backbone and the catalytic aspartate, and provide suitable linkage for the attachment of a wide range of hetero/aromatic pendant groups. On the other hand, recent studies demonstrated the antioxidant potential of the quinazoline skeleton [16,17]. Accordingly, we introduced different aryl and heteroaryl pendants that favored ligand-active site interactions and offered antioxidant potential of the target compounds. The target compounds were evaluated for their BACE1 inhibitory and antioxidant activities to investigate their potential use for treating AD.

2. Materials and methods

2.1. Chemistry

Melting points were determined with a Thermo Scientific Electrothermal digital apparatus (Thermo Fisher Scientific Inc.). ^{1}H NMR (300 or 500 MHz) and ^{13}C NMR (100 or 125 MHz) spectra were recorded on a Bruker 300 or 500 Fourier transform spectrometer; the chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane. Infrared (IR) spectra were recorded on Perkin Elmer Spectrum RXI FTIR spectrophotomer in KBr phase. Electron impact mass spectra were recorded on a Varian Mat 311-A70 eV instrument (Varian, Fort Collins, USA). Chemicals used were supplied from Sigma-Aldrich, Fluka and Merck chemical companies. TLC was performed on the glass-backed silica gel sheets (Silica Gel 60 GF254) and visualized under UV light (254 nm).

2.1.1. Synthesis of 2-mercapto-3-methylquinazolin-4(3H)-one (*2*)

Anthranilic acid (**1**, 20 mmol) and triethylamine (2 mL) were dissolved in EtOH (10 mL) and methyl isothiocyanate (30 mmol) was added to the mixture. The reaction mixture was heated under reflux for 2 h. After cooling, the precipitate was filtered and washed 3 times with cold ethanol, dried and recrystallized from ethanol. Light cream solid; Yield 81%; IR (KBr, cm^{-1}) ν_{max}: 3029 (C—H, aromatic), 1683 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.97 (d, 1H, J = 7.8 Hz, quinazolinone-H), 7.73 (t, 1H, J = 7.8 Hz, quinazolinone-H), 7.4–7.30 (m, 2H, quinazolinone-H), 3.65 (s, 3H, N-CH$_3$); MS (EI) *m/z* (%): 192 (M$^+$, 100), 159 (17), 119 (38).

2.1.2. Synthesis of 2-hydrazinyl-3-methylquinazolin-4(3H)-one (*3*)

A mixture of compound **2** (1 mmol) and hydrazine hydrate (1 mL, 32 mmol) in BuOH (20 mL) was refluxed for 20 h. After cooling, the precipitated solid was separated and recrystallized from diethyl ether to give pure compound **3**. White solid; Yield 52%; ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 8.08 (d, 1H, J = 6.0 Hz, quinazolinone-H), 7.60 (t, 1H, J = 6.0 Hz, quinazolinone-H), 7.42 (d, 1H, J = 6.0 Hz, 1H, quinazolinone-H), 7.16 (t, 1H, J = 6.0 Hz, quinazolinone-H), 6.43 (s, 1H, NH), 4.54 (s, 2H, NH$_2$), 3.10 (s, 3H, N-CH$_3$); MS *m/z* (%): 191 ([M+1]$^+$, 69), 190 (M$^+$,13), 175 (46), 159 (5.5), 144 (100).

2.1.3. General procedure for the synthesis of hydrazones *4–6*

To a solution of approperiate aryl aldehyde or isatin (1 mmol) in absolute ethanol (5 mL) was added few drops of acetic acid and stirred at room temperature. Then, 2-hydrazinyl-3-methylquinazolin-4(3*H*)-one (**3**, 0.19 g, 1 mmol) dissolved in absolute ethanol (5 mL) was added to the stirred reaction solution and the mixture was refluxed overnight. After completion of the reaction as indicated by TLC, the reaction mixture was allowed to cool in room temperature. The precipitated product was filtered and recrystallized from methanol.

*2.1.3.1. 3-Methyl-2-(2-(2-nitrobenzylidene)hydrazinyl)quinazolin-4(3H)-one (**4a**).* Yellow solid; Yield 70%; mp: 220–222 °C; ^{1}H NMR