Bioorganic & Medicinal Chemistry 24 (2016) 2280–2286

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Inhibitory effect of flavonoids on human glutaminyl cyclase

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ARTICLE INFO

Article history:
Received 8 March 2016
Revised 30 March 2016
Accepted 30 March 2016
Available online 1 April 2016

Keywords:
Glutaminyl cyclase
Flavonoids
Synthesis
Enzyme inhibitor
Alzheimer's disease

ABSTRACT

Glutaminyl cyclase (QC) plays an important role in the pathogenesis of Alzheimer's disease (AD) and can be a potential target for the development of novel anti-AD agents. However, the study of QC inhibitors are still less. Here, phenol-4′ (R1-), C5-OH (R2-) and C7-OH (R3-) modified apigenin derivatives were synthesized as a new class of human QC (hQC) inhibitors. The efficacy investigation of these compounds was performed by spectrophotometric assessment and the structure–activity relationship (SAR) was evaluated. Molecular docking was also carried out to analyze the binding mode of the synthesized flavonoid to the active site of hQC.

1. Introduction

Glutaminyl cyclase (QC, EC 2.3.2.5) is one kind of acyltransferases, which catalyzes intramolecular cyclization of N-terminal glutamine residues to pyroglutamic acid (pGlu) with the concomitant liberation of ammonia.[1] This post-translational formation of pGlu is an important process for the maturation of various bioactive neuropeptides, hormones, cytokines and for their biological activity, because the pGlu is required to protect the N termini from exopeptidase degradation and/or to develop the proper conformation. QC is abundant in mammalian secretory tissue such as secretory glands or brain tissue including hippocampus and cortex. Recently, the described ability of human QC (hQC) to convert the N-terminal glutamate of β-amyloids (Aβs) into respective pGlu-modified Aβs (pE-Aβs) suggests a potential involvement of hQC in the initiation of the formation of neurotoxic plaques in Alzheimer's disease (AD).[2]

AD, a progressive neurodegenerative disorder, is the most common cause of dementia among elderly people, and these are no effective therapeutic modality for the prevention, halting or reversal of AD currently.[3,4] The extracellular plaques composed of neurotoxic Aβ have been supposed to be involved in the onset of AD.[5] However, recent evidence indicates that pE-Aβs exhibit an increased neurotoxicity, hydrophobicity, accelerated aggregation kinetics, and resistance to degradation of aminopeptidases as compared to native Aβs.[6,7] pE-Aβs are really the main components of the plaques in the AD brains (more than 60%).[8] The formation of pE-Aβs is likely to be a crucial event in the progress of the disease.

Furthermore, it is demonstrated that the expression of QC is characteristically up-regulated in the early stage of AD and the hallmark of the inhibition of QC is the prevention of the formation of pE-Aβs and plaques.[9,10] The application of QC inhibitors as a new therapeutic strategy has been proved to be effective in different transgenic animal models, even in Phase I clinical trial in AD patients.[9,11,12] Unfortunately, only a few of imidazole derivatives were reported as QC inhibitors so far, for instance PBD150 (Fig. 1, left).[13–15]

Flavonoids exhibit plenty of desired pharmacological effects including antioxidation and antiinflammation.[16–19] According to the new reports, the consumption of flavonoid-rich foods is associated with lower incidence of dementia in human beings.[20,21] Cholinesterase inhibition and anti-amyloidogenic effects of flavonoids have been recognized.[22,23] Yet, the inhibitory effect of flavonoids on QC has not been investigated.

Interestingly, apigenin (Fig. 1, right) was founded to be effective in the inhibition of hQC in our research (not published). We hypothesize that flavonoids may be a new class of QC inhibitors. Then, three series of apigenin derivatives, phenol-4′ (R1), C5-OH

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http://dx.doi.org/10.1016/j.bmc.2016.03.064
0968-0896/

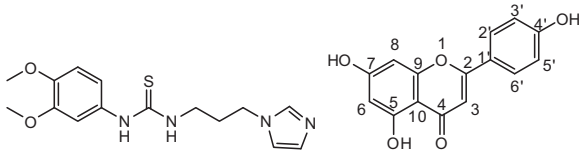


Figure 1. Structures of PBD150 (left) and apigenin (right).

(R2) and C7-OH (R3) modified, were synthesized in the present study. The evaluation of the potency of these inhibitors and the analysis of structure–activity relationship (SAR) were performed. The results of molecular docking provided further insights into the interaction between flavonoids and hQC.

2. Results and discussion

2.1. Chemistry

The presence of phenolic hydroxyl is important for the maintenance of the pharmacological effects of flavonoids. Phenol-4′ (R1), C5-OH (R2) and C7-OH (R3) modified apigenin derivatives were synthesized here to access the influence of these phenolic hydroxyls on the inhibitory activities. The preparation of R1-modified compounds **1**–**9** (Table 1) and R2-modified compounds **10**–**29** (Table 2) was conducted according to Scheme 1. These apigenin derivatives were generated starting from the commercially available chemicals **I** by Hosech reaction and followed by the Baker–Venkataraman rearrangement with chemicals **IV** to give an overall yields of 60–81%.[24–26] R3-modified chemicals **30**–**40** (Table 3) were obtained from the reaction of the corresponding R2-modified compounds with methyl iodide as methylating agent (scheme was not shown here) in high yields (91–98%, but **38** in 50%). All the apigenin derivatives were yellow/red powder or crystals.

The benzo-γ-pyrone skeletons of apigenin derivatives were not changed, as hQC prefers substrates with an aromatic ring besides the zinc ion in the active site.[27] The backbone is also needed for flavonoids to exhibit the pharmacological activities.

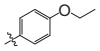
2.2. QC inhibitory activities and SAR analysis

In this study, recombinant hQC was expressed in *Escherichia coli* cells and used as the source of QC for our assays in vitro according to the previous reports.[28–30] The efficacy of these apigenin derivatives were evaluated using spectrophotometric assessment as described by Schilling et al.[31] IC_{50} values were determined from the inhibitory dose response curves.

The R1-modified chemicals were found to be of high potency (Table 1). Compounds containing alkyl groups on R1, **1** & **2**, exhibited an increased potency as compared to apigenin. Alkylation of *p*-phenolic hydroxyl on R1 (**3**) improved the inhibitory activity of the compound. The exchange of the phenyl motif by thiophene also resulted in an improvement of the inhibitory activities, such as **7**, **8** and **9**. The hydrophobicity of R1, the number and/or position of the alkoxy groups all exhibited a slightly impact on the inhibitory potency, including **3**, **4** and **5**, but the inhibitory activities of R1-modified apigenin derivatives in the presence of C5-OH and C7-OH has been proved overall.

The influence of C5-OH on the activity was found to be more pronounced in the case of R2-modified apigenin derivatives (Table 2). The replacement of C5-OH by H resulted in a moderate drop of potency in general. The inhibitory activities of these compounds decreased nearly half of the activities of the derivatives containing C5-OH, such as **2**, **3**, **5**, **6** versus **10**, **13**, **16**, **21**, respectively. It was denoted that the potency of some compounds (**23** and **24**) was the same as that of derivatives **1**–**9**. However, chemicals in the absence of C5-OH exhibited a decreased inhibitory potency obviously. So, C5-OH would be favored for the potential inhibitory activities of apigenin derivatives. The position of substituents on R1 affected the inhibitory activities of these compounds slightly, such as **12**, **15**, **19**, **24** versus **14**, **16**, **21**, **26** respectively. It could be seen that the introduction of *para*-substituents on R1 led to an increased potency. Moreover, the inhibitory activity of the compounds was negatively correlated with the hydrophobicity of R1. This tendency was opposite from the tendency in the R1-modified apigenin derivatives mentioned above.

Table 1
R1-modified apigenin derivatives and the inhibitory activities*

Compd	R1	R2	R3	IR (%, ±SD)
Apigenin		–OH	–OH	75.2 ± 2.3
1		–OH	–OH	93.0 ± 2.0
2		–OH	–OH	85.2 ± 3.6
3		–OH	–OH	91.5 ± 3.2
4		–OH	–OH	71.7 ± 2.6
5		–OH	–OH	87.7 ± 2.0
6		–OH	–OH	84.2 ± 1.9
7		–OH	–OH	92.6 ± 1.1
8		–OH	–OH	91.6 ± 2.8
9		–OH	–OH	92.4 ± 1.4

* Concentration: 100 μM; IR: inhibitory rate.

The impact of C7-OH on the activity of the apigenin derivatives was further explored. Methylation of C7-OH resulted in a total loss of potency in case of the R3-modified compounds. All of these derivatives exhibited no obvious inhibitory activities at 100 μM including **30**, **31**, **32**, **33**, **34**, **35**, **36**, **37**, **38**, **39**, **40** (Table 3) compared with compounds **8**, **12**, **14**, **15**, **17**, **19**, **20**, **22**, **25**, **27**, **28**, respectively. Therefore, C7-OH was found to be crucial for the inhibitory activities of apigenin derivatives and for the binding of flavonoids at the active site of hQC. According to all these results, the structure–activity relationship of apigenin-based hQC inhibitors could be shown in Figure 2.

Then, IC_{50} values (Table 4) of these apigenin derivatives (IR ⩾80% at 100 μM) were determined from the inhibitory dose response curves. Generally, these selected derivatives exhibited IC_{50} values ranging from 14.2 to 45.2 μM and most of the compounds contained C5-OH and C7-OH. The IC_{50} value of **11** was almost 3-fold weaker than that of **3**, which was turned out to be the most potent compound. However, the IC_{50} value of **3** was 3-fold lower than that of PBD150. Based on the difference in the inhibitory potency between apigenin derivatives and positive control, there may be different inhibitory mechanisms and/or different binding strength for the synthesized flavonoids because of their particular structures.

2.3. Molecular docking

It is suggested that hQC contains one zinc ion at the bottom of the active site. According to these findings, a limited number of