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Inhibitory effect of flavonoids on human glutaminyl cyclase

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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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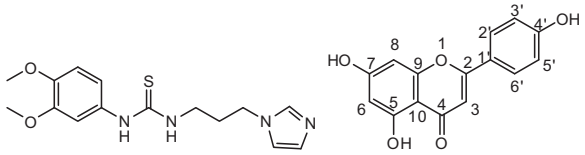


Figure 1. Structures of PBD150 (left) and apigenin (right).

(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.

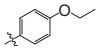
2.2. QC inhibitory activities and SAR analysis

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.

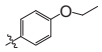
The impact of C7-OH on the activity of the apigenin derivatives was further explored. Methylation of C7-OH resulted in a total loss of potency in case of the R3-modified compounds. All of these derivatives exhibited no obvious inhibitory activities at 100 μM including **30**, **31**, **32**, **33**, **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

Table 2
R2-modified apigenin derivatives and the inhibitory activities*

Compd	R1	R2	R3	IR (%, ±SD)
10		–H	–OH	79.5 ± 4.1
11		–H	–OH	71.8 ± 1.5
12		–H	–OH	75.2 ± 8.9
13		–H	–OH	67.6 ± 5.7
14		–H	–OH	54.5 ± 1.2
15		–H	–OH	68.3 ± 1.1
16		–H	–OH	54.3 ± 5.4
17		–H	–OH	68.7 ± 2.3
18		–H	–OH	75.8 ± 2.8
19		–H	–OH	74.2 ± 3.5
20		–H	–OH	63.3 ± 2.7
21		–H	–OH	60.8 ± 3.2
22		–H	–OH	70.8 ± 1.5
23		–H	–OH	90.3 ± 4.0
24		–H	–OH	87.1 ± 5.7
25		–H	–OH	65.2 ± 1.6
26		–H	–OH	62.4 ± 2.4
27		–H	–OH	68.7 ± 3.4
28		–H	–OH	61.4 ± 2.4
29		–H	–OH	78.9 ± 1.8

* Concentration: 100 μM.

imidazole-derived chemicals including PBD150 were synthesized and tested as QC inhibitors.[13–15] The binding of imidazole with Zn^{2+} ion mainly contributed to the inhibitory activities of these compounds.[13] In view of the totally different schedule of apigenin derivatives, molecular docking was performed to evaluate the combination of apigenin derivatives and hQC.

The hQC mode reveals a typical 'open sandwich' structure. There is a hydrophobic core consisting of four parallel, two antiparallel β-sheets and the surrounding eight α-helices. The active site is characterized by a deep, tight, and angled cleft, which is defined by the hydrophobic amino acids Trp329, Val302, Asp305. Compound **1** was docked into the active site of hQC as shown in Figure 3 here.

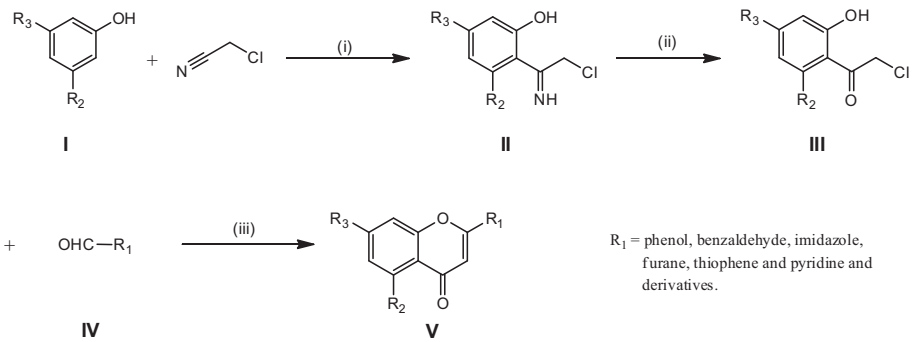
It can be seen that at the entrance of the cavity there is an open space which is not only suitable for the location of *m*-methylbenzol (R1) in compound **1** but also suitable for the location of other aromatic moieties. Thus, the modification of R1 exhibited a slightly effect on the inhibitory activities of apigenin derivatives containing C5-OH and C7-OH. And the introduction of *p*-substituents in R1 resulted in an improvement of the potency for the good match of *p*-substituents in this open space.

In this binding mode, the access to the active cavity was blocked by the benzo-γ-pyrone moiety of compound **1**. There were several direct interactions between compound **1** and QC in this hydrophobic pocket, including the π–π stack interaction between the phenolic moiety and the indole ring of Trp207, hydrogen bond between the oxygen atom at C4 carbonyl and the water molecule which was shared with amine of Gln304, and hydrogen bond between the C5-OH (R2) and the carbonyl oxygen atom of Gln304. Because of the important assistance of hydrogen bond, C5-OH was favored for the inhibitory activities of apigenin derivatives. Compared with R1-modified compounds, as a result, the R2-modified compounds exchanged C5-OH by H were found to be of medium potency.

Furthermore, the binding of C7-OH with zinc ion, which was located at the bottom of active cavity by Asp159, Glu202 and His330, might be the most important interaction between the ligand and QC. This binding was similar to the binding of imidazole of PBD150 with zinc ion in the active pocket. Supporting this, R7-modified compounds were found to be totally inactive when the hydroxyls were methylated. Therefore, C7-OH was essential for the inhibitory potency of apigenin derivatives. Difference in the binding strength of oxygen and nitrogen with the zinc ion may partly contribute to the difference in the inhibitory potency of these flavonoids and positive control. Consequently, molecular docking proved the research results and conformed the SAR of apigenin derivatives as potential hQC inhibitors (Fig. 2).

3. Conclusion

Based on the research mentioned above, it could be concluded that the effect of R1 on the inhibitory activity of apigenin derivatives is slight, but R2 (C5-OH) is favored and especially, R3 (C7-OH) is essential for the inhibitory potency. These results thus strongly implied that apigenin derivatives containing C5-OH and



Scheme 1. Reagents and conditions: (i) $ZnCl_2$, Et_2O filled with HCl gas, rt; (ii) 10% HCl/H_2O, 60 °C, 10–12 h; (iii) H_2O/EtOH (1:5, V/V), 5% NaOH, rt.

Table 3
R3-modified apigenin derivatives and the inhibitory activities*

Compd	R1	R2	R3	IR (%, ±SD)
30		–H	$-OCH_3$	–
31		–H	$-OCH_3$	–
32		–H	$-OCH_3$	–
33		–H	$-OCH_3$	–
34		–H	$-OCH_3$	–
35		–H	$-OCH_3$	–
36		–H	$-OCH_3$	–
37		–H	$-OCH_3$	–
38		–H	$-OCH_3$	–
39		–H	$-OCH_3$	–
40		–H	$-OCH_3$	–

* Concentration: 100 μM; '—': means no activity.

C7-OH may present a new class of QC inhibitors and flavonoids should be investigated deeply for the treatment of AD and other QC related diseases.

4. Materials and methods

4.1. General chemistry

The ^1H NMR (400 MHz) data was recorded on a Bruker Avance III, using $CDCl_3$ or DMSO-d_6 as solvent. The high-resolution positive ion mass spectra were obtained from a LCMS-IT-TOF (SHIMADZU). Positive control PBD150 was synthesized according to the previous report.[32] Materials were purchased from Sigma–Aldrich Co., Ltd and Sinopharm Chemical Reagent Co., Ltd. All other regents were of analytical grade and commercial.

4.2. General procedure for the preparation of compounds 1–40

Material **I** (1.0 equiv) and 2-chloroacetonitrile (1.5 equiv) were mixed in Et_2O filled with HCl gas. $ZnCl_2$ (2.0 equiv) was added and used as catalyst. The mixture was stirred at room temperature until **I** was disappeared analyzed by TLC. Then the resulting precipitate (**II**) was filtered off and reserved. Material **II** was dissolved in 10% HCl/H_2O solution, and the mixture was stirred at 60 °C for 10–12 h. When the mixture was cooled to room temperature, the resulting precipitate (**III**) was filtered off and reserved. Material **III** (1.0 equiv) and **IV** (1.0 equiv) were dissolved in the H_2O/EtOH (1:5, V/V) solution containing 5% NaOH. The mixture was stirred at room temperature until **III** and **IV** were both disappeared analyzed by TLC. EtOH was removed at reduced pressure, a large amount of water was added, then the mixture was neutralized to neutral or weak acid with 10% HCl/H_2O solution and the resulting precipitate (**V**) was filtered off, washed several times with water, purified by crystallization with EtOH or column chromatography with *n*-hexane/ethyl acetate.

For the C7-phenolic hydroxyl methylated compounds, corresponding derivatives synthesized by the procedure mentioned above was used as starting material, and methyl iodide was used as methylating agent in the presence of K_2CO_3. Spectroscopic data of old compounds were previously reported in the literatures, and those of new compounds are listed below.

4.2.1. 5,7-Dihydroxy-2-(*m*-tolyl)-4*H*-chromen-4-one (1)

82% yield; ^1H NMR (400 MHz, DMSO): δ 10.95 (s, 1H), 10.90 (s, 1H), 7.71 (d, J = 8.2 Hz, 1H), 7.68 (s, 1H), 7.36 (t, J = 7.6 Hz, 1H), 7.22 (d, J = 7.1 Hz, 1H), 6.55 (s, 1H), 6.23 (d, J = 1.8 Hz, 1H), 6.08 (d, J = 1.8 Hz, 1H), 2.36 (s, 3H); HRMS (ESI-TOF): m/z calcd for $[C_{16}H_{12}O_4+H^+]$: 267.0657, found 267.0662.

4.2.2. 2-(4-Ethylphenyl)-5,7-dihydroxy-4*H*-chromen-4-one (2)

77% yield; ^1H NMR (400 MHz, DMSO): δ 10.96 (s, 1H), 10.92 (s, 1H), 7.81 (d, J = 8.1 Hz, 1H), 7.34 (dd, J = 21.0, 8.1 Hz, 2H), 7.19 (d, J = 8.0 Hz, 1H), 6.59 (s, 1H), 6.22 (d, J = 1.7 Hz, 1H), 6.08 (d, J = 1.7 Hz, 1H), 2.65 (m, 2H), 1.20 (t, 3H); HRMS (ESI-TOF): m/z calcd for $[C_{17}H_{14}O_4+H^+]$: 283.0965, found 283.0967.

4.2.3. 2-(4-Ethoxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one (3)

73% yield; ^1H NMR (400 MHz, DMSO): δ 10.90 (s, 1H), 10.85 (s, 1H), 7.84 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 6.58 (s, 1H), 6.21 (d, J = 1.7 Hz, 1H), 6.07 (d, J = 1.7 Hz, 1H), 4.08 (q, J = 6.9 Hz, 2H), 1.34 (t, J = 7.0 Hz, 3H); HRMS (ESI-TOF): m/z calcd for $[C_{17}H_{14}O_5+H^+]$: 297.0763, found 297.0761.

4.2.4. 2-(2,4-Dimethoxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one (4)

69% yield; the NMR and MS data of obtained compound is consistent with the reported.[33]

4.2.5. 2-(2,3-Dimethoxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one (5)

67% yield; the NMR and MS data of obtained compound is consistent with the reported.[34]

4.2.6. 2-(2-Fluorophenyl)-5,7-dihydroxy-4*H*-chromen-4-one (6)

66% yield; the NMR and MS data of obtained compound is consistent with the reported.[35]

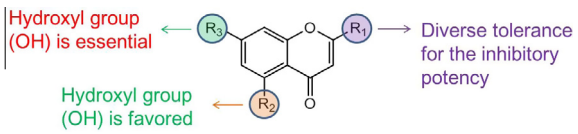


Figure 2. The structure–activity relationship of apigenin based hQC inhibitors.

Table 4
IC_{50} values of selected apigenin derivatives*

Compd	IC_{50} (±SD, μM)
1	16.1 ± 2.0
2	37.2 ± 2.4
3	14.2 ± 1.1
5	27.9 ± 1.9
6	34.6 ± 3.2
7	15.3 ± 2.1
8	19.3 ± 3.9
9	15.0 ± 3.7
11	45.2 ± 3.2
23	16.8 ± 2.6
24	26.0 ± 1.4
PBD150 **	5.3 ± 0.7

* IR ⩾80% at 100 μM.
** Positive control.

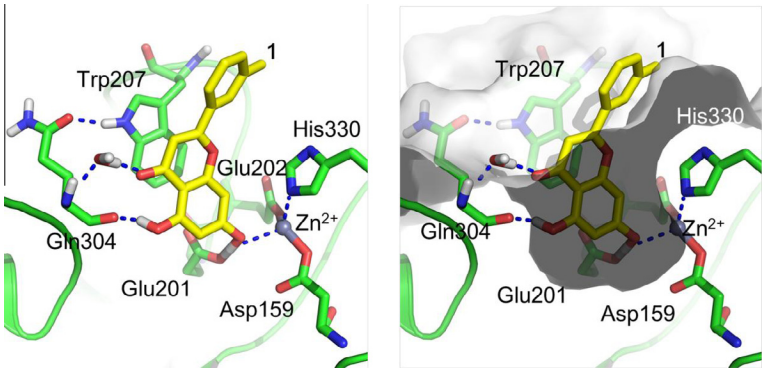


Figure 3. Possible binding mode for compound **1** at the active site of hQC. Left: the capped stick mode. Right: a close-up view of molecular surface of the active site. Compound **1** and some active site residues are colored yellow and green, respectively, and the zinc ions are shown as gray balls.

4.2.7. 5,7-Dihydroxy-2-(thiophen-2-yl)-4*H*-chromen-4-one (7)

80% yield; the NMR and MS data of obtained compound is consistent with the reported.[36]

4.2.8. 5,7-Dihydroxy-2-(3-methylthiophen-2-yl)-4*H*-chromen-4-one (8)

79% yield; ^{1}H NMR (400 MHz, DMSO): δ 10.95 (s, 1H), 10.88 (s, 1H), 7.75 (d, J = 5.1 Hz, 1H), 7.04 (d, J = 5.1 Hz, 1H), 6.83 (s, 1H), 6.17 (d, J = 1.7 Hz, 1H), 6.08 (d, J = 1.7 Hz, 1H), 2.37 (s, 3H); HRMS (ESI-TOF): m/z calcd for [$C_{14}H_{10}O_4S$+H^+]: 273.0222, found 273.0234.

4.2.9. 5,7-Dihydroxy-2-(5-methylthiophen-2-yl)-4*H*-chromen-4-one (9)

78% yield; ^{1}H NMR (400 MHz, DMSO): δ 10.90 (s, 1H), 10.86 (s, 1H), 7.40 (d, J = 3.5 Hz, 1H), 6.90 (s, 1H), 6.89 (d, J = 2.8 Hz, 1H), 6.16 (d, J = 1.5 Hz, 1H), 6.06 (d, J = 1.5 Hz, 1H), 2.51 (s, 3H); HRMS (ESI-TOF): m/z calcd for [$C_{14}H_{10}O_4S$+H^+]: 273.0222, found 273.0229.

4.2.10. 2-(4-Ethylphenyl)-7-hydroxy-4*H*-chromen-4-one (10)

78% yield; the NMR and MS data of obtained compound is consistent with the reported.[37]

4.2.11. 7-Hydroxy-2-(4-hydroxyphenyl)-4*H*-chromen-4-one (11)

75% yield; the NMR and MS data of obtained compound is consistent with the reported.[38]

4.2.12. 7-Hydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (12)

76% yield; the NMR and MS data of obtained compound is consistent with the reported.[38]

4.2.13. 2-(4-Ethoxyphenyl)-7-hydroxy-4*H*-chromen-4-one (13)

75% yield; the NMR and MS data of obtained compound is consistent with the reported.[39]

4.2.14. 7-Hydroxy-2-(2-methoxyphenyl)-4*H*-chromen-4-one (14)

69% yield; the NMR and MS data of obtained compound is consistent with the reported.[40]

4.2.15. 2-(3,4-Dimethoxyphenyl)-7-hydroxy-4*H*-chromen-4-one (15)

71% yield; the NMR and MS data of obtained compound is consistent with the reported.[41]

4.2.16. 2-(2,3-Dimethoxyphenyl)-7-hydroxy-4*H*-chromen-4-one (16)

67% yield; ^{1}H NMR (400 MHz, DMSO): δ 11.24 (s, 1H), 7.80–7.75 (m, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.21 (t, J = 8.0 Hz, 1H), 7.15 (d, J = 8.2 Hz, 1H), 6.96 (s, 1H), 6.78 (d, J = 1.8 Hz, 1H), 6.72 (dd, J = 8.5, 1.9 Hz, 1H), 3.84 (s, 3H), 3.81 (s, 3H); HRMS (ESI-TOF): m/z calcd for [$C_{17}H_{14}O_5$+H^+]: 297.0763, found 297.0773.

4.2.17. 7-Hydroxy-2-(3,4,5-trimethoxyphenyl)-4*H*-chromen-4-one (17)

63% yield; the NMR and MS data of obtained compound is consistent with the reported.[41]

4.2.18. 7-Hydroxy-2-(4-(methylthio)phenyl)-4*H*-chromen-4-one (18)

81% yield; ^{1}H NMR (400 MHz, DMSO): δ 7.89 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 8.4 Hz, 1H), 7.36 (d, J = 8.6 Hz, 2H), 6.78 (d, J = 1.9 Hz, 1H), 6.77 (s, 1H), 6.71 (dd, J = 8.4, 2.0 Hz, 1H), 2.53 (s, 3H); HRMS (ESI-TOF): m/z calcd for [$C_{16}H_{12}O_3S$+H^+]: 283.0429, found 283.0422.

4.2.19. 2-(4-Fluorophenyl)-7-hydroxy-4*H*-chromen-4-one (19)

78% yield; the NMR and MS data of obtained compound is consistent with the reported.[42]

4.2.20. 2-(3-Fluorophenyl)-7-hydroxy-4*H*-chromen-4-one (20)

77% yield; ^{1}H NMR (400 MHz, DMSO): δ 7.78 (dd, J = 11.2, 4.6 Hz, 2H), 7.64 (d, J = 8.5 Hz, 1H), 7.54 (td, J = 8.2, 6.2 Hz, 1H), 7.28 (td, J = 8.2, 2.2 Hz, 1H), 6.84 (d, J = 1.9 Hz, 1H), 6.82 (s, 1H), 6.74 (dd, J = 8.5, 2.0 Hz, 1H); HRMS (ESI-TOF): m/z calcd for [$C_{15}H_9O_3F$+H^+]: 255.0457, found 255.0464.

4.2.21. 2-(2-Fluorophenyl)-7-hydroxy-4*H*-chromen-4-one (21)

63% yield; the NMR and MS data of obtained compound is consistent with the reported.[43]

4.2.22. 7-Hydroxy-2-(2-(trifluoromethyl)phenyl)-4*H*-chromen-4-one (22)

71% yield; ^{1}H NMR (400 MHz, DMSO): δ 11.39 (s, 1H), 8.36 (d, J = 7.9 Hz, 1H), 7.86 (dd, J = 14.6, 7.9 Hz, 2H), 7.68 (d, J = 8.5 Hz, 1H), 7.64 (t, J = 7.7 Hz, 1H), 6.83–6.79 (m, 2H), 6.75 (d, J = 2.0 Hz, 1H); HRMS (ESI-TOF): m/z calcd for [$C_{16}H_9O_3F_3$+H^+]: 305.0426, found 305.0439.

4.2.23. 2-(4-(Dimethylamino)phenyl)-7-hydroxy-4*H*-chromen-4-one (23)

64% yield; the NMR and MS data of obtained compound is consistent with the reported.[37]

4.2.24. 7-Hydroxy-2-(pyridin-4-yl)-4*H*-chromen-4-one (24)

72% yield; the NMR and MS data of obtained compound is consistent with the reported.[44]

4.2.25. 7-Hydroxy-2-(pyridin-3-yl)-4*H*-chromen-4-one (25)

71% yield; the NMR and MS data of obtained compound is consistent with the reported.[39]

4.2.26. 7-Hydroxy-2-(pyridin-2-yl)-4*H*-chromen-4-one (26)

71% yield; ^{1}H NMR (400 MHz, DMSO): δ 8.70 (d, J = 4.0 Hz, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.94 (dd, J = 10.8, 4.6 Hz, 1H), 7.65 (d, J = 8.5 Hz, 1H), 7.40 (dd, J = 7.0, 5.3 Hz, 1H), 6.78 (d, J = 1.7 Hz, 1H), 6.71 (dd, J = 8.5, 1.8 Hz, 1H), 6.67 (s, 1H); HRMS (ESI-TOF): m/z calcd for [$C_{14}H_9NO_3$+H^+]: 240.0655, found 240.0658.

4.2.27. 2-(Furan-2-yl)-7-hydroxy-4*H*-chromen-4-one (27)

75% yield; the NMR and MS data of obtained compound is consistent with the reported.[45]

4.2.28. 2-(Furan-3-yl)-7-hydroxy-4*H*-chromen-4-one (28)

78% yield; ^{1}H NMR (400 MHz, DMSO): δ 11.19 (s, 1H), 8.27(s, 1H), 7.84(s, 1H), 7.60 (d, J = 8.5 Hz, 1H), 7.01 (d, J = 1.6 Hz, 1H), 6.79 (d, J = 2.0 Hz, 2H), 6.71 (dd, J = 8.4, 1.9 Hz, 1H); HRMS (ESI-TOF): m/z calcd for [$C_{13}H_8O_4$+H^+]: 229.0495, found 229.0494.

4.2.29. 7-Hydroxy-2-(5-methylfuran-2-yl)-4*H*-chromen-4-one (29)

73% yield; ^{1}H NMR (400 MHz, DMSO): δ 7.56 (d, J = 9.2 Hz, 1H), 7.09 (s, 1H), 7.01 (d, J = 3.4 Hz, 1H), 6.39 (d, J = 3.4 Hz, 1H), 6.21 (dd, J = 9.2, 1.4 Hz, 1H), 5.98 (s, 1H), 2.37 (s, 3H); HRMS (ESI-TOF): m/z calcd for [$C_{14}H_{10}O_4$+H^+]: 241.0501, found 241.0511.

4.2.30. 7-Methoxy-2-(3-methylthiophen-2-yl)-4*H*-chromen-4-one (30)

95% yield; ^{1}H NMR (400 MHz, CDCl3): δ 7.70 (d, J = 8.5 Hz, 1H), 7.52 (d, J = 5.1 Hz, 1H), 7.17 (d, J = 0.7 Hz, 1H), 6.95 (d, J = 5.1 Hz, 1H), 6.79 (d, J = 2.0 Hz, 1H), 6.76 (dd, J = 8.6, 2.1 Hz, 1H), 3.93 (s, 3H), 2.44 (s, 3H); HRMS (ESI-TOF): m/z calcd for [$C_{15}H_{12}O_3S$+H^+]: 273.0585, found 273.0582.

4.2.31. 7-Methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (31)

96% yield; the NMR and MS data of obtained compound is consistent with the reported.[46]

4.2.32. 7-Methoxy-2-(2-methoxyphenyl)-4*H*-chromen-4-one (32)

94% yield; the NMR and MS data of obtained compound is consistent with the reported.[47]

4.2.33. 2-(3,4-Dimethoxyphenyl)-7-methoxy-4*H*-chromen-4-one (33)

97% yield; the NMR and MS data of obtained compound is consistent with the reported.[47]

4.2.34. 7-Methoxy-2-(3,4,5-trimethoxyphenyl)-4*H*-chromen-4-one (34)

95% yield; the NMR and MS data of obtained compound is consistent with the reported.[48]

4.2.35. 2-(4-Fluorophenyl)-7-methoxy-4*H*-chromen-4-one (35)

97% yield; the NMR and MS data of obtained compound is consistent with the reported.[49]

4.2.36. 2-(3-Fluorophenyl)-7-methoxy-4*H*-chromen-4-one (36)

97% yield; the NMR and MS data of obtained compound is consistent with the reported.[50]

4.2.37. 7-Methoxy-2-(2-(trifluoromethyl)phenyl)-4*H*-chromen-4-one (37)

91% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (d, J = 8.0 Hz, 1H), 7.74 (dd, J = 8.1, 5.7 Hz, 2H), 7.65 (dd, J = 12.5, 4.8 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.12 (d, J = 1.7 Hz, 1H), 6.78 (dd, J = 8.5, 2.1 Hz, 1H), 6.75 (d, J = 2.0 Hz, 1H), 3.93 (s,3H); HRMS (ESI-TOF): m/z calcd for [$C_{17}H_{11}O_3F_3$+H^+]: 321.0739, found321.0742.

4.2.38. 7-Methoxy-2-(pyridin-3-yl)-4*H*-chromen-4-one (38)

93% yield; the NMR and MS data of obtained compound is consistent with the reported.[51]

4.2.39. 2-(Furan-2-yl)-7-methoxy-4*H*-chromen-4-one (39)

95% yield; the NMR and MS data of obtained compound is consistent with the reported.[52]

4.2.40. 2-(Furan-3-yl)-7-methoxy-4*H*-chromen-4-one (40)

97% yield; ^{1}H NMR (400 MHz, DMSO): δ 8.29(s, 1H), 7.87 (s, 1H) 7.67 (d, J = 8.6 Hz, 1H), 7.12 (d, J = 1.9 Hz, 1H), 7.03 (s, 1H), 6.88–6.82 (m, 2H), 3.93 (s, 1H); HRMS (ESI-TOF): m/z calcd for [$C_{14}H_{10}O_4$+H^+]: 243.0652, found 243.0654.

4.3. In vitro inhibition studies on hQC

4.3.1. Preparation of hQC

Cloning, expression and large scale preparation of hQC were performed according to the previously reports.[28–30] Generally, the gene of hQC was inserted via the *BamH*I and *Xho*I restriction sites into *Escherichia coli* expression vector pET32a (Invitrogen) with additionally introduction of an N-terminal His6-tag (primer pair Cs/Cas). Primers, 5-3: ACCT*GGATCC*GCTTCTGCTTGGCCGG, BamHI; 5-3: TATC*CTCGAG*TTACAGGTGCAGGTATTC, XhoI (Takara). *E. coli* strain DH5α was used for all cloning procedures. The cDNA was verified by sequencing (Samgon). Plasmid DNA was amplified, purified, and linearized. hQC was heterologously expressed in *E. coli* BL21(DE3) using Fernbach flasks at room temperature overnight and expression induced by addition of 0.2 mM IPTG (isopropyl β-D-1-thiogalactopyranoside). Cells were disrupted with 1 mg/mL lysozyme and a freeze-thaw cycle. The purification of hQC protein followed two chromatographic steps: Ni^{2+}-IMAC (immobilized metal affinity chromatography), and molecular sieve chromatography. QC-containing fractions were pooled and purity was analyzed by SDS–PAGE (15%, sodium dodecyl sulfate polyacrylamide gel electrophoresis) and Coomassie Blue staining. The purified hQC enzyme was stored at −80 °C without glycerol.

4.3.2. hQC inhibition studies

The hQC activity was assayed essentially by spectrophotometric assessment as described elsewhere.[31] Briefly, the assay reactions (200 μL) consisted of varying concentrations (0–4 mM) of freshly prepared H-Gln-Gln-H, 30 units/ml glutamic acid dehydrogenase, 0.5 mM NADH/H^+, and 15 mM α-ketoglutaric acid in 0.05 M Tris–HCl, pH 8.0. Reactions were started by the addition of hQC and were monitored by recording the decrease in absorbance at 340 nm for 15 min. In the screening, the assay reaction composition was the same as described above, except for the addition of different concentrations of the apigenin derivatives in the solution of hQC. The inhibition rate (IR) was calculated according to the formula: IR (%) = (Vc − Vs)/Vc, Vc means the reaction velocity of control, Vs means the reaction velocity of samples. The IC_{50} values were determined graphically from log concentration versus % of inhibition curves. All experiments were performed in triplicate.

4.3.3. Statistical analysis

The results are expressed as the mean ± SD of at least three independent experiments.

4.4. Molecular docking study

Crystal structures of hQC with PDB code 2AFX were used for docking study. The Molecular Operating Environment (MOE, vers. 2011.10, Chemical Computing Group, Montreal, Canada) and GOLD (vers. 5.0, CCDC Software Ltd, Cambridge, U.K.) were used to compute the ligand–protein interactions. The cocrystallized 1-benzylimidazole was removed from the system. The active site was defined using the zinc ion as the center.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.03.064.

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