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REVIEW ARTICLE

Glutaminyl Cyclase and Its Inhibitors

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HIGHLIGHTS

- Human glutaminyl cyclase (hQC) have two isoforms that the secreted QC (also known as sQC) and the golgi-localized QC (also known as isoQC or gQC).
- hQCs mediate the cyclization of N-terminal glutamine or glutamate residues by releasing ammonia or water.
- The secretion level of QCs increase in some diseases such as Alzheimer's (AD), Huntington's disease (HD), melanomas, thyroid carcinomas, rapid formation of atherosclerosis, septic arthritis.
- In recent years, the discovery of new drugs to inhibit QC is considered an important approach for the prevention and treatment of many physiological problems and diseases.
- It has been discovered that compounds bearing the imidazole framework have the potential to inhibit the QCs. One of the most remarkable of these agents is varoglutamstat, which is currently in phase studies.

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ABSTRACT

Human glutaminyl cyclase (hQC), which has two isoforms, is an important enzyme that catalyzes pyroglutamate modification. N-Terminal pyroglutamate (pE) modification is an important post-translational event in mammals. The pE modification catalyzed by QC is a necessary modification for the maturation and function of many proteins and peptides. However, studies have shown that the increase in the amount of QC is associated with some diseases. With the abnormal increase in the secretion of QC, Alzheimer's (AD), Huntington's disease (HD), melanomas, thyroid carcinomas, rapid formation of atherosclerosis, septic arthritis occur. With this abnormal increase, the increase in pE-amyloid beta (Aβ) and pE-chemokine ligand (CCL2) formation resulting from pE modification catalyzed by QC may cause various pathologies. Considering the consequences of abnormal secretion of QC and predisposing to the formation of diseases, it was aimed to reduce the formation of pE-modified mediators by inhibiting QC. The discovery of new drugs to inhibit QC is considered an important approach for the prevention and treatment of many physiological problems and diseases, including AD, inflammation, cancer. Therefore, it was thought that these pathological conditions could be prevented by QC inhibition, and various QC inhibitors were developed to combat it. In this review, various QC inhibitors, and their molecular structures, activities and also possible treatment options were examined. Extensive research has been done on varoglutamstat, which is in phase 2 of clinical trials, and QC inhibitors in general are summarized.

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1. Introduction

Alzheimer's disease (AD), characterized by a gradual decline in learning and memory abilities, is becoming increasingly common with an increasing elderly population. However, there is no drug that allows definitive treatment yet. It has been proven by studies that AD is triggered and started as a result of the formation of pyroglutamate-modified plaques, which are formed by a reaction catalyzed by the glutaminyl cyclase (QC) enzyme. For this reason, many studies for the treatment of AD are on the inhibition of the QC enzyme. In these studies, it is aimed to prevent and treat the development of AD by inhibiting the QC enzyme, making the modification as a result of the reaction it catalyzes, and preventing the formation of plaques that cause AD. At the same time, with the inhibition of this enzyme, it is aimed to prevent inflammation, cancer, periodontitis and related pathological conditions triggered by QC through various mechanisms [1].

1.1. Glutaminyl Cyclase Enzyme (QC)

Glutaminyl cyclase (QC), also known as glutaminyl-peptide cyclotransferase, is an enzyme that belongs to the metal-dependent aminoacyltransferase family [2]. It was first isolated from the latex of the *Carica papaya* in 1963 [3, 4]. Enzymes that catalyze the same reaction have also been identified in different organisms and isolated from rat brain, pig pituitary, bovine pituitary and spleen, human B lymphocytes, snake venom, bacterial pathogens and cone snails [5–9]. The amino acid sequences of glutaminyl cyclases identified in different species are similar, but exhibit different homology, structure and properties [5, 10–13]. For example, the amino acid sequences of glutaminyl cyclase enzymes isolated from snakes and humans are approximately 75% similar [6]. The specificities of these enzymes are related to the size and composition of the substrates [14].

Mammalian glutaminyl cyclase isoenzymes were identified in 2008 [15].There are two isoforms of glutaminyl cyclase in humans, the secreted QC (also known as sQC) and the golgi-localized QC (also known as isoQC or gQC) [15, 16]. sQC is secreted into the neuronal extracellular space by secretory granules, while isoQC is localized within the golgi complex. Secreted QC and isoQC are encoded by different genes in humans [14–16]. sQC is encoded by the QPCT gene located on chromosome 2p22.2, which contains an N-terminal signal sequence responsible for secretion, while gQC is encoded by the QPCTL gene located on chromosome 19q13.32, which contains an N-terminal sequence responsible for its retention in the golgi [15, 16]. Human QC consists of 361 amino acids, and isoQC consists of 382 amino acids. Both QC and isoQC contain catalytic domains of similar size (approximately 330 residues) and have close to 45% sequence similarity [17]. While QC is expressed more in neuronal tissues, the expression of isoQC does not show a significant difference in different tissues and organs [1].

In a study conducted on nine different mouse strains, it was observed that the highest QC/isoQC activity was in the ventral brain, followed by the cortex and hypothalamus [18]. With the removal of the gene responsible for QC, the QC activity in the mouse brain is significantly reduced in the hypothalamus and plasma, while the isoQC expression continues, so its activity is observed in peripheral organs such as the liver and spleen [19].

Human glutaminyl cyclase (hQC) is a zinc ion-dependent monomeric glycoprotein. A signal peptide containing 27 or 28 amino acid residues consists of a positively charged N-terminal region, a central hydrophobic region containing 10 amino acid residues, and a C-terminal region containing a critical nonpolar amino acid residue (Ser27 or Gly28) Figure 1 [20].

In a study, sQC and gQc structures were investigated using X-crystallography. It was observed that the secondary and tertiary structures of both QCs were quite similar to each other. Both QC and isoQC are spherical in folds with a mixed α/β structure. The structure mimics the appearance of an open sandwich with a central six-strand β layer (two of which are anti-parallel) packed between two and six α helices on opposite sides. Other parts of the protein contain helical and unstructured loops [17, 20]. The spiral and ring regions of the structure cover 42% of the total residues. Nearly half of these regions contribute to the formation of the active area. The zinc ion (Zn^{+2}) is located near Asp159, Glu202 and His330 and at the bottom of the narrow hydrophobic pocket. For the continuation of the catalytic activity, it is necessary to coordinate the zinc ion with Glu201, Trp207, Asp248 residues Figure 1 [17].

It was determined that the QC structures of other mammals and insects were highly similar to hQC. The structure of N-glycosylated QC from humans and mice is stabilized by a disulfide bond between two conserved cysteine residues at the bottom of the pocket and two surface rings close to the active site [21]. The conserved tryptophan residue Trp207 at the entrance to the active site shows a single conformation in

both N-glycosylated human and murine QCs. This situation significantly affects substrate conversion [21].

There are some important conformational changes between the sQC and gQC structures. These conformational changes affected the size of the active site of gQC compared to sQC, and due to conformational flexibility, the active site pocket of gQC was found to be slightly wider and relatively more open than sQC. The active site of sQC was confined to a narrower area [22].

IsoQC has two potential initial methionine residues, called Met I and Met II. The major differences between the QC and isoQC sequences, which are approximately 45% similar to each other, are located at the N-terminus. QC has a signal sequence that directs the protein to the secretory pathway, and QC has two N-glycosylation sites. There are no N-glycosylation sites in isoQC [23].

Although both enzymes have a similar structure and catalyze the same enzymatic reaction with similar efficiency, they assume different physiological roles because they have a different cellular distribution [16, 19, 24].

QC enzymes have a zinc dependent catalytic mechanism [14]. Consisting of 331 residues, the QC has a hydrophobic entrance and a relatively narrow binding pocket [20]. The catalytically required Zn^{+2} ion is located at the bottom of the pocket; His330 is tetrahedral coordinated by residues Asp159, Glu202 and a water molecule. In the active region of human QC crystal structures, there is a single hydrogen bond network consisting of Glu201 and Asp248 catalytic residues, with a total of five residues [25, 26]. The acidic environment in the binding pocket originates from acidic residues adjacent to the metal and can play an important role in substrate binding and catalysis by providing ideal conditions. When the substrate reaches the catalytic site, it replaces the water molecule attached to the metal, using the side chain carbonyl group to coordinate the zinc ion Figure 1 [26].

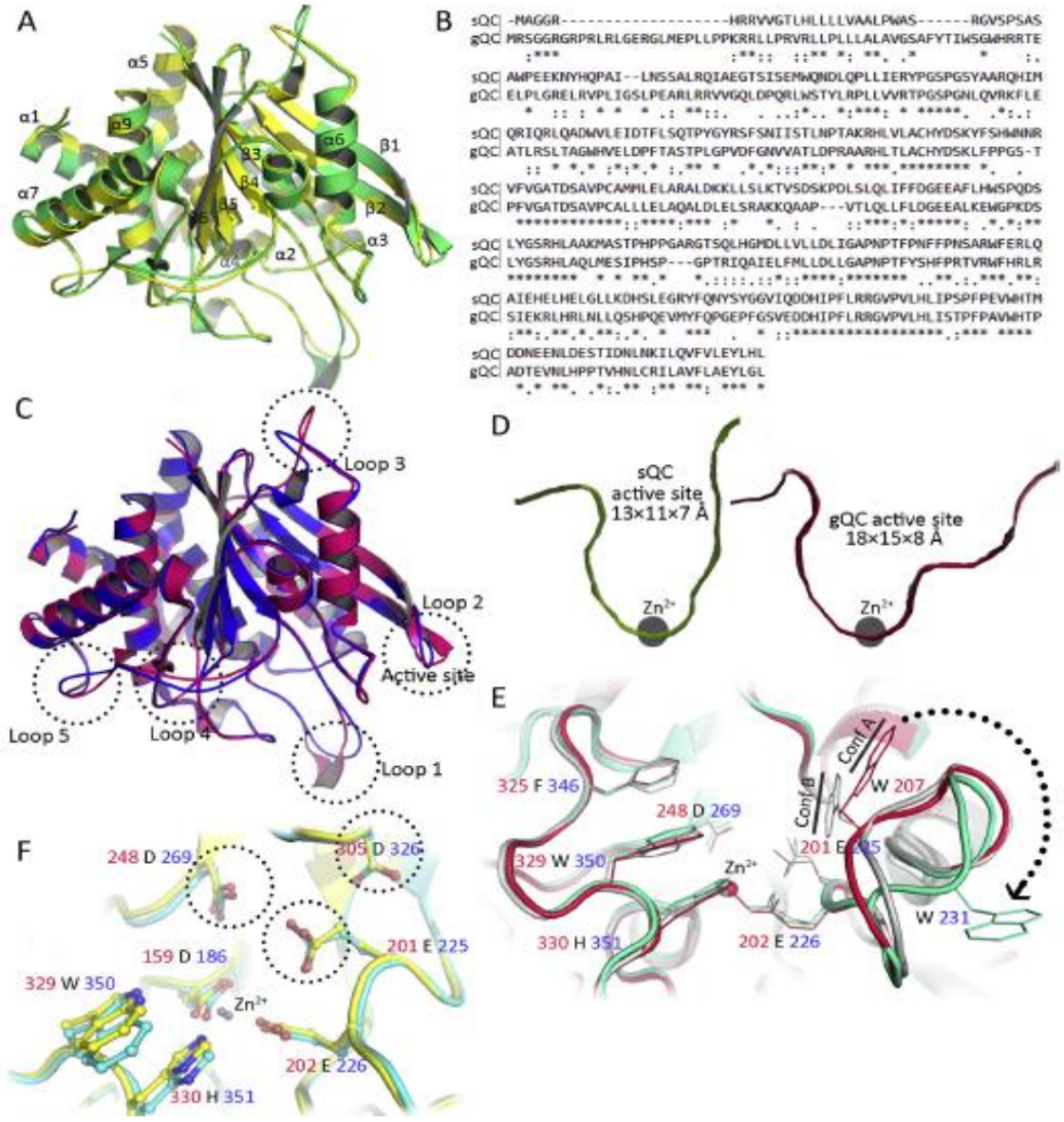


Figure 1 The structure and active sites of glutaminyl cyclase enzyme.

Various mutations were added to sQC to examine the importance of various residues and their results were evaluated [22]. The importance of the hydrogen bonding network in the active site formed between acidic residues was investigated by adding different mutations around the active site. Although the mutations did not cause a significant conformational change in the overall structure, very small structural changes in the mutation site affected the catalytic activity. It was seen that several point mutations caused the hydrogen bonding network to break down and strongly affected the steady-state catalytic activity of the enzyme. This shows that the hydrogen bond network is very important in terms of catalytic activity [26]. In the research, different mutations were added to improve the solubility of sQC and it was determined that it improved the protein solubility, but it was seen that these mutations did not have any effect on the catalytic activity and structure [27].

1.2. Functions and Effects of QC

QCs are similar to exopeptidases in terms of enzymatic reaction mechanism. hQC mediates the cyclization of N-terminal glutamine or glutamate residues by releasing ammonia or water Figure 2 [20, 28]. N-terminal glutamine and/or glutamate are cyclized to pyroglutamate in the presence of phosphate ions, facilitating synchronized proton transfers [29].

N-Terminal pyroglutamate (pE) modification is an important post-translational event in mammals. Pyroglutamate modification is essential for the maturation and/or function of peptides, hormones, cytokines, and proteins. It is also an important event to provide a suitable conformation for binding with receptors and/or to protect their N-terminus from exopeptidase-induced degradation [30, 31]. pE modification improves the function of peptides and can strengthen the activity and stability of recombinant proteins such as cellulose Cel7A [32–34].

Formerly, N-terminal pE modification, an important post-translational event, was thought to occur spontaneously. However, *in vitro* and *in vivo* studies have proven that QC catalyzes the conversion of N-terminal glutamine and glutamate to pyroglutamate [10, 29, 35].

For intramolecular cyclization to begin, the N-terminal α-amino group of the substrate must be near the γ-carbonyl carbon. The Zn^{+2} ion in the active site of QC polarizes the γ-amide group of the substrate Gln residue and stabilizes the oxyanion generated by the nucleophilic attack of α-nitrogen on the cleavable γ-carbonyl carbon. E201 and D248 of sQC are directly involved in substrate hydrolysis by promoting proton transfer from the α-amine of the substrate to the amino group on the cuttable γ-amide and providing stability for the leaving amino group on the cuttable γ-amide of the substrate. These enzymatic reactions have been confirmed by different mutation studies [22].

As a result of *in vitro* enzyme kinetic studies performed with different substrates, it has been determined that substrates containing aromatic amino acids in the optimum pH range of 7-8 have a high turnover number [2]. As a result of some studies based on gene extraction, Aβ (3-42), thyrotropin-releasing hormone (TRH), gonadotropin-releasing hormone (GnRH), gastrin, neurotensin, fertilization stimulating peptide (FPP), chemoattractant chemokine ligand (CCL2), collagen and fibronectin for QC physiological substrates have been defined [24, 36]. The excess amount of sQC in the hypothalamus, medulla and hippocampus suggested that sQC is responsible for the majority of glutaminyl cyclase activity in the brain, such as the processing of peptide hormones (TRH, GnRH, neurotensin, orexin) [22].

It provides pyroglutamate-peptide maturation of neuropeptides and hormones in the secretory pathway such as QC, TRH, GnRH, neurotensin and gastrin. Also, the chemokines CCL2 (monocyte chemoattractant protein 1, MCP-1), CX3CL1 (fractalkin), collagen and fibronectin mediate N-terminal pyroglutamate formation [5, 24, 37]. After the GnRH and TRH pro-proteins are cleaved, the pE modification takes place after the N-terminal glutamine of mature proteins is met. The functions, activities and stability of these peptides, especially TRH and MCP-1, are dependent on pE modification. Loss or change of this residue causes problems in receptor interaction, and therefore this loss or change leads to a decrease in biological activity [38, 39].

It has been proven as a result of the analysis that the distribution of QC in tissues shows higher expression in the brain and a few peripheral blood lymphocytes, and that isoQC is mainly effective on substrates in peripheral cells that do not require extensive proteolytic processing, such as CCL2, but is generally expressed ubiquitously [15, 24, 40].

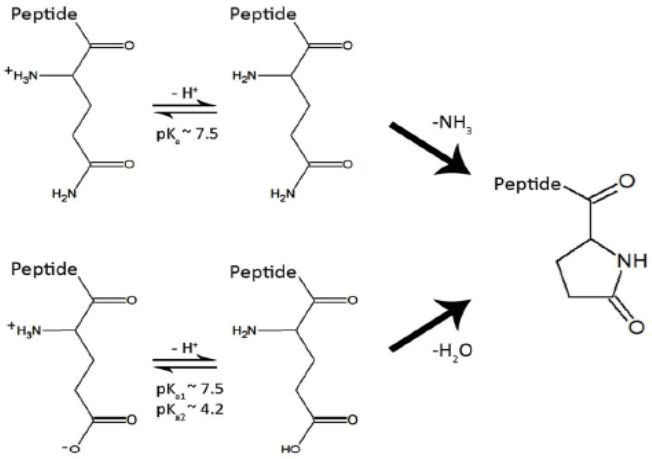


Figure 2 Catalytic activity of hQC against Glu and Gln peptides [22, 28].

Compared with non-enzymatic catalysis, the mechanism by which QC accelerates the pE modification is explained in four steps as follows:

A) Zn^{+2} polarizes the γ-amido carbonyl of the substrate based on the cation-anion interaction between Zn^{+2} and oxygen.

B) Zn^{+2} stabilizes the oxygen ions resulting from the electrophilic attack of the positively charged α-amino group.

C) A proton is transferred to the γ-amide with the help of Glu201.

D) Asp248, in the presence of H_2O, γ- amide improves the separation of amino [2, 41].

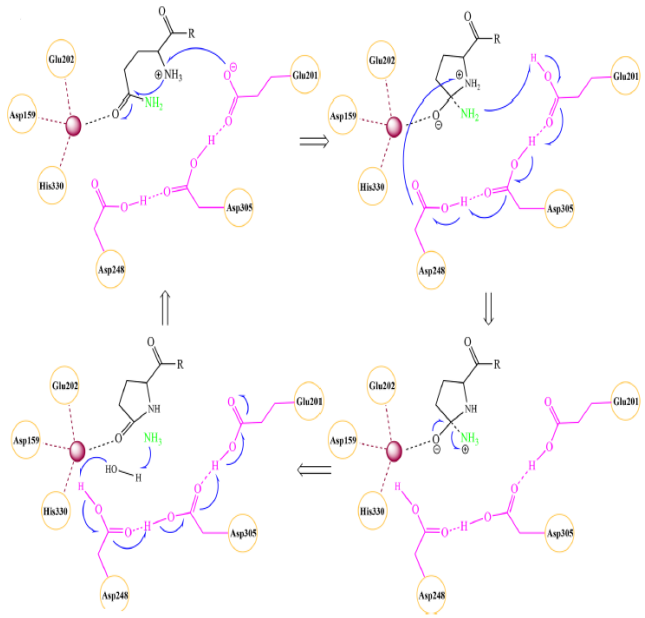


Figure 3 Catalytic activity in the QC active pocket [1]

The positively charged Zn^{+2} acts as the key agent for the transformation. The central triad formed by Glu201, Asp305, Asp248 is required for catalytic activity and the rate-limiting step of this mechanism is protein transfers Figure 3 [1]

Studies have shown that QC activity play a role on pathological events occured in AD [42] various inflammatory disorders [40] HD [43], schizophrenia [44] cancer [45] osteoporosis [46] Figure 4.

2. QC Enzyme and Diseases

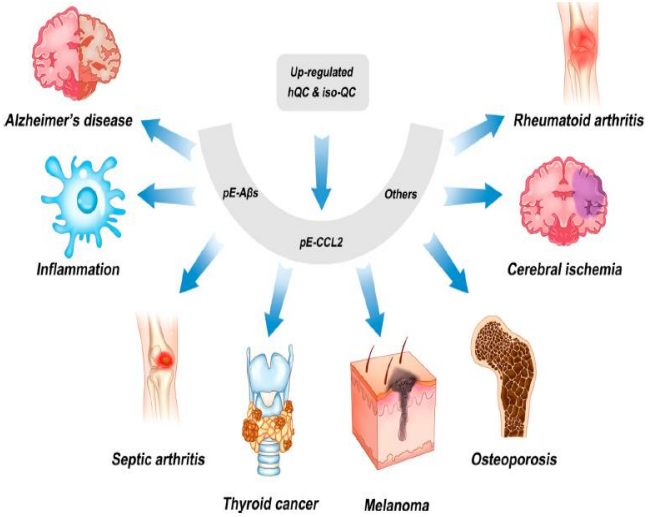


Figure 4 Upregulation of QC and various diseases to which it contributes [1].

The pE modification catalyzed by QC is a necessary modification for the maturation and function of many proteins and peptides. However, studies have shown that the increase in the amount of QC is associated with some diseases. With the abnormal increase in the secretion of QC, AD, HD, melanomas, thyroid carcinomas, rapid formation of atherosclerosis, septic arthritis occur. With this abnormal increase, the increase in pE-amyloid beta (Aβ) and pE-chemokine ligand (CCL2) formation resulting from pE modification catalyzed by QC may cause various pathologies [1].

2.1. Neurodegenerative Diseases and Alzheimer

Neurodegenerative diseases include AD, Parkinson's disease (PD), and HD. AD is the most common form of dementia [47] and the most common of other neurodegenerative diseases. It is characterized by a gradual decline in learning and memory ability. With the increasing elderly population worldwide, the incidence of AD is increasing. While research on the disease and the search for treatment continue, a treatment that can cure and stop the progression of the disease has still not been found [1].

The exact cause of AD is unknown. Some neuropathological signs such as extracellular β-amyloid (Aβ) deposits, intracellular neurofibrillary tangles, cerebral amyloidangiopathy and microglia activation can be counted among the indicators of AD [47]. Senile plaques composed of Aβ peptides were thought to be the main cause of AD [1]. However, later it was proved that pE modified Aβ's (eg pE-Aβ3-40 and pE-Aβ3-42) are the main compounds of senile plaques [48–50].

Studies have shown that the formation of Aβ plaques consisting of Aβ42 and Aβ40 is not directly related to AD symptoms and neurodegeneration. These plaques can be detected in both brains with AD and normal brains. The pathological difference between normal brain and brain with AD is that the N-cut Aβ variants are found only in brains with AD [51].

The accumulation of Aβ in senile plaques is accepted as one of the central events in AD formation [52, 53]. pE-modified Aβ's account for more than half of the total Aβ plaques in brains with AD. These plaques are not found in normal brains [1] Figure 5.

pE modification increases hydrophobicity and changes pH-dependent solubility profiles. This situation leads to changes in the biochemical and biophysical properties of Aβ [54]. Studies have shown that this strain is more neurotoxic than the full-length Aβ peptide. This strain is also more stable against degradation by aminopeptidases and has a greater tendency to aggregate [15, 24, 42, 54] Figure 6.

pE-Aβ's exhibit more neurotoxicity and contribute to the deterioration of the functions of neurons and the onset of AD [48, 55–61]. This highly toxic variant of Aβ can be detected in the early stages of amyloid deposition [49, 62]. pE-Aβ clusters accumulate mainly in the lysosomes of neurons and glia cells and increase in an age-related manner [63]. The toxicity of pE-Aβ is dependent on tau proteins and/or hyperphosphorylated tau protein. Studies have proven the effect of pE-Aβ accumulated in the frontal cortex on the severity of AD neuropathology and clinical symptoms of dementia [64, 65] Figure 6.

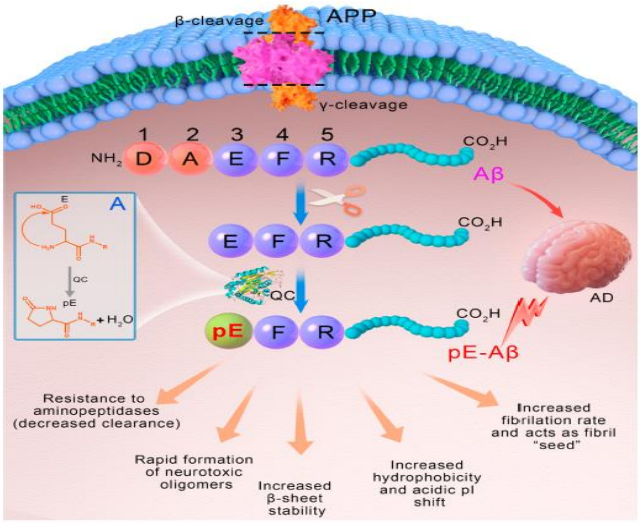


Figure 5 The formation and roles of pE Aβ in the onset of AD. A: Production of pE Aβ by upregulated QC [1].

It was determined that the levels of QC mRNA and QC protein in the peripheral blood taken from AD patients were higher than the blood taken from the control group of the same age. Based on this, it was determined that QC expression showed a positive correlation with the severity of dementia [66]. These results indicate that QC is an important target for the clinical diagnosis and treatment of AD and for the development of new anti-AD agents [1].

Inflammation also poses an important risk in the development of AD [67]. One of the inflammatory mediators, CCL2, also known as MCP-1, causes neuroinflammation by recruiting monocytes and macrophages and surrounding plaques in the brain by activated microglia and astrocytes. CCL2 exhibits its biological activity by binding with CCR2. Activated microglia and astrocytes secrete more CCL2 and inflammatory molecules. This contributes to the development and progression of AD. Therefore, reducing the level of CCL2 is considered as an important option for the treatment of AD and other inflammatory diseases [68, 69].

HD is a neurodegenerative disease characterized by involuntary and repetitive choreic movements, psychological dysfunctions and cognitive impairment caused by the progressive degeneration of cortical and striatal neurons caused by an enlarged polyglutamine pathway [43, 70, 71]. Studies have shown that aggregation and toxicity are associated with increased QC secretion in HD, and it was thought that QC inhibitors would be beneficial in slowing and healing it [43].

2.2. Inflammation

CCL2 is one of the critical mediators of neuroinflammation in neurodegenerative diseases such as AD [72, 73].

gQC is involved in CCL2 maturation and plays a dominant role in chronic inflammation [24]. During neurodegenerative diseases, monocytes and microglia are some of the most recruited immune cells at the lesion site in the central nervous system. Monocyte chemoattractant proteins (MCPs), which belong to the beta chemokine family, and especially CCL2 (MCP1) and its receptor CCR2, play a role in various neurological diseases [74]. CCL2 attracts microglia cells to the area of inflammation, surrounds senile plaques formed during AD development with immune cells and accelerates the formation of Aβ [75, 76]. This situation causes neuroinflammation and memory damage [76].

An abnormal increase in the amount of isoQC triggers inflammatory reactions by modifying the CCL2 pE [18, 24, 40, 77]. CCL2 generally induces chemotaxis of monocytes and microglia by binding with CC chemokine receptor type 2 (CCR2) [78, 79]. The CCL2-CCR2 pathway has an important role in acute and chronic inflammatory disorders, diseases such as atherosclerosis, fibrosis and AD [80–85]. Considering its role in the immune system, the CCL2-CCR2 pathway is an important potential target in the treatment of inflammatory diseases [68, 86].

2.3. Cancer

Abnormally increased QC/ isoQC may also be the main cause of thyroid carcinomas. When the QC expression in the tissues of patients with goiter was examined, it was determined that it showed a positive correlation with the CCL2 level. It was detected as pE-CCL2 in the supernatant of thyroid cancer cells [45]. With the increase in the functional activity of CCL2, monocytes/macrophages collect in the areas of inflammation, triggering the development of thyroid carcinomas [1].

CD47 is a transmembrane protein ligand for myeloid cells that interacts with transmembrane signal regulatory protein α (SIRPα) expressed on the surface of myeloid cells (macrophages and antigen presenting cells). It can be expressed as an immune checkpoint [1, 87].

CD47 is overexpressed in various cancers and plays an important role in the development of these cancers. CD47 binds to signal regulatory protein α (SIRPα), creating a "don't eat me" signal. As a result, cancer cells are spared from phagocytosis and antigen presentation [47, 88]. *In vitro*, pE modification occurs by isoQC catalysed by Gln19 at the N-terminal of CD47, which contributes to the binding of CD47 to SIRPα [89, 90].

CD47 expression is increased in cells of various disorders such as atherosclerosis, fibrotic diseases, infectious diseases. At the same time, CD47 expression is increased in tumor cells [88]. Therefore, binding of CD47 to SIRPα reduces both innate and adaptive immune response, helping tumor cells to escape immune surveillance [91]. When the crystallographic analysis of the CD47-SIRPα complex was performed, the presence of N-Terminal pE at the binding interface was detected [92] , and this modification, catalyzed by the isoQC enzyme, proved to be necessary for effective interaction [89, 90].

Reduction of isoQC activity can be achieved by genetic deletion or pharmacological inhibition using QC/isoQC inhibitors. This led to disruption of CD47-SIRPα complex formation by inhibiting post-translational pE modification. As a result, with the disappearance of the "don't eat me" signal, phagocytosis occurred and cancer cells were cleared [89, 90, 93]. Therefore, the isoQC enzyme is considered as a potential target for the regulation of the formation of the CD47-SIRPα complex and for cancer immunotherapy [47].

2.4. Periodontitis and Related Disorders

As a result of the studies, it has been determined that periodontitis and rheumatoid arthritis (RA) are related to each other in terms of many clinical and pathological features such as bone and tissue destruction, high-level inflammatory formation. In studies, it has been determined that there is a strong relationship between *Porphyromonas gingivalis (P. gingivalis), Tannerella forsythia (T. forsythia)* and *Prevotella intermedia (P. intermedia)* bacteria and periodontal diseases. The pE residue was detected at the N-terminal ends of the proteins secreted by these pathogens. This indicates the presence and expression of bacterial QC (BacQC) enzymes. *P. gingivalis* and *P. gingivalis* QC enzyme (PgQC) are also associated with RA [94]. *P. gingivalis* has also been associated with different diseases such as AD and cancer [95].

As a result of a study, it has been proven that the expression of PgQC and human QCs is high in chronic periodontitis and RA patients and has an important role in the maintenance of chronic inflammation [96]. Therefore, QCs obtained from oral pathogens are considered as important targets for the development of small molecule inhibitors for the treatment and prophylaxis of RA, AD and periodontitis-related diseases [97].

3. Discovery of QC Inhibitors

Only four drugs, including acetylcholinesterase (AchE) inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists, are used for the clinical treatment of AD, a chronic neurodegenerative disease. These drugs relieve some symptoms for a limited time. Existing drugs have no effect on stopping or slowing the progression of AD [98].

Owing to the "amyloid hypothesis", which suggests that the accumulation of neurotoxic amyloid-β (Aβ) oligomers in the brain plays a key role in the pathogenesis of AD, it has become a promising target for the treatment of AD [53]. Studies for the last 20 years have been conducted to reduce the levels of Aβ monomers, oligomers, aggregates and plaques in AD brains by inhibiting Aβ-producing enzymes, preventing aggregation, and increasing the clearance of Aβ from the brain, but as a result of these studies, sufficient efficacy could not be achieved in clinical trials [99–101].

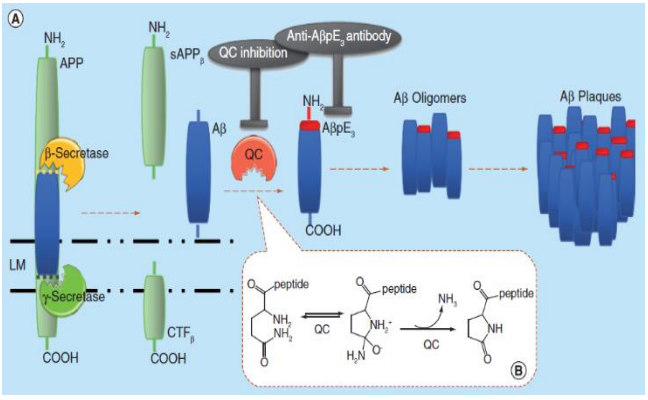


Figure 6 Scheme of Aβ formation and the role of QC in pyroglutamate-amyloid-β production and subsequent Aβ oligomerization and aggregation [102].

Studies have shown that the accumulation of Aβ peptides begins decades before any symptoms of AD appear. Considering all these, antiamyloid treatments are considered a reasonable approach for patients with early-stage AD [103].

Considering the consequences of abnormal secretion of QC and predisposing to the formation of diseases, it was aimed to reduce the formation of pE-modified mediators by inhibiting QC. Discovery of new drugs to inhibit QC for the prevention and treatment of many physiological problems and diseases including AD, inflammation, cancer may be an important approach. Many compounds developed in the past years have been identified as QC inhibitors [22, 102].

3.1. sQC Inhibitors

Previous studies and the structures and binding modes of the inhibitors studied have suggested that most sQC inhibitors are composed of four major pharmacophores. These pharmacophores are:

A metal bonding group (MBG),

An alkyl chain with different substitutions (links the side domains of the inhibitor),

An aromatic heterocyclic group located in one of the flanking regions (opposite MBG),

A region that mimics the binding interactions of the guanidine moiety of the Arg of Aβ3E42 (known as Phe-Arg mimetics).

For a potential sQC inhibitor should have three of these four pharmacophores to achieve the required activity, at least and in particular first and third. Except for these properties that an inhibitor must have, all properties help the inhibitor to develop its inhibitory potency [104–109].

The activity of sQC inhibitors is mainly driven by the coordination between MBG and Zn^{+2} ion located in the active site of sQC. Many heterocyclic compounds known to coordinate with the Zn^{+2} ion were tested against sQC. As a result of the researches, it has been revealed that only imidazole, benzimidazole and triazole-based compounds are potential sQC inhibitors [14] Figure 7.

3.2. Imidazole-Based Inhibitors and Their Hybrids

In a study conducted in 1987, it was determined that the post-translational conversion of a glutaminyl-peptide (Gln-His-Pro NH_2) to pyroglutamyl-peptide (PiroGlu-His-Pro-NH_2 (TRH)) was catalyzed by QC [110]. Later, it was determined that the activity of hQC was inhibited by 1,10-phenanthroline and dipicolinic acid depending on time. Thus, imidazole and imidazole derivatives have been identified as competitive QC inhibitors. These metal chelators inhibit the activity, which is reversed by the addition of Zn^{+2}, and the addition of other metal ions such as Co^{+2}, Mn^{+2} partially reactivates the activity. As a result of these studies, it was determined that hQC is a metal-dependent enzyme, inhibited by imidazole derivatives depending on pH, and the kinetic parameter of the QC-catalyzed transformation is based on Km [14].

Most bidentate heterocyclic compounds have the ability to bond with metal ions to form stable coordinated complexes. Many phenanthroline-type heterocyclic compounds, including five- and six-membered ring systems, have been studied for the discovery of new QC inhibitors. As a result of these investigations, imidazole and its derivatives were found to be more effective on QC inhibition compared to other compounds [14].

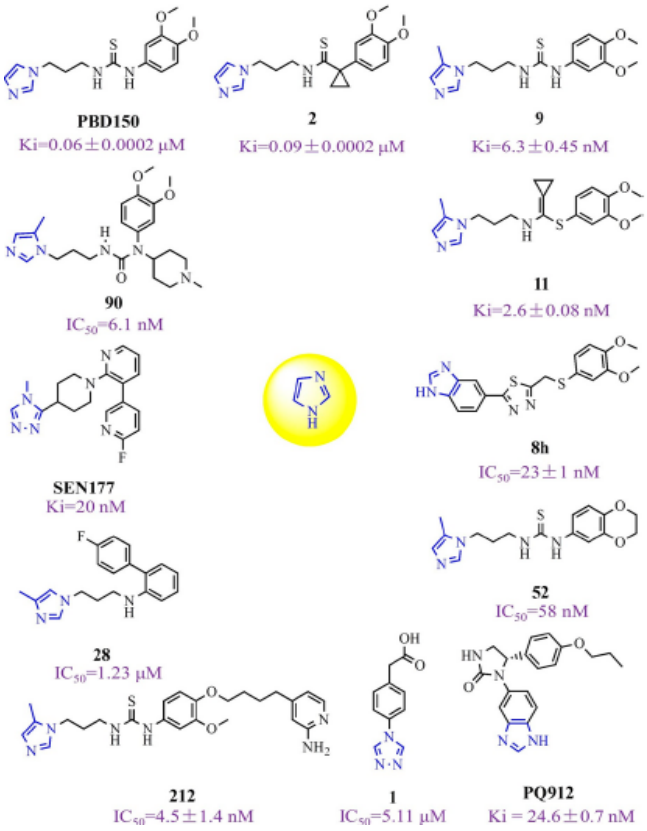


Figure 7 Imidazole and triazole-based QC inhibitors [1].

As a result of a structure-activity relationship (SAR) analysis, it was determined that imidazole is an important motif that acts by binding to Zn^{+2} in the lower part of the pocket and inhibits QC activity [105].

Modifications of the imidazole moiety significantly affect the inhibitory potency. In the studies, methylation and benzylation of the nitrogen atom at position 1 of imidazole resulted in a 3- and 14-fold increase in potency, respectively. Addition of phenylalanine at the same position, phenylation at the same position, and methylation of the carbon atom at position 2 significantly reduced the inhibitory potency [105].

Imidazol-1-ylalkyl thiourea was developed as the basic structure of thiourea derivatives designed using a ligand-based optimization approach Figure 8. 1-(3-(1H-imidazol-1-yl)propyl)-3-(3,4-dimethoxyphenyl)) thiourea, designated PBD150, has proven to be a potent inhibitor. As a result of the analysis, it was found that Zn^{+2} can be coordinated by imidazole. It has been determined that the donor nitrogen of thiourea forms the basic hydrogen bond. It has been found that the phenyl portion of the inhibitor conforms to the hydrophobic pocket and methoxy substitutions on the aromatic portion have a great effect on inhibitory binding [105]. As a result of the studies, it has been determined that imidazole-propyl-thioamides (molecule number 2 shown in the figure) are a potential inhibitor class with the same structure [105].

The QC homology model has been developed for the development of new QC inhibitors. Replacement of thiourea with cyanoguanidine or nitrovinyldiamine was not found to have a significant effect on potency. However, replacing 3,4-dimethoxyphenyl with saturated carbocyclic substitutions resulted in a slight improvement in potency due to a different binding mode. Combining thiourea with thioxopyrimidine increased the potency in the presence of the preferred cyclic saturated structure Figure 9. As a result of the analysis, 5-methylimidazole thioureas, cyanoguanidines, nitrovinyldiamines, and thioxopyrinidines were found to be more effective in inhibitory activity than 4-methylimidazole analogs. With the addition of methyl at position 5, compounds 9 and 11 in the figure showed 10 and 35 times higher potency, respectively, than PBD150 [104]. Binding of 5-methylimidazole with Zn^{+2} showed higher inhibitory activity than other parts of these compounds [1].

8h, in which benzimidazole attached to 1,3,4-oxadiazole was determined as the metal binding moiety and shown in the figure among the compounds prepared in this way, showed a strong QC inhibitory activity [111]. Considering the binding mode of the preferred substrate with QC and the structure of PBD150, half of the compound number 52 is obtained by incorporating heterocyclic rings into the aromatic moiety region in N-aryl-N-(5-methyl-1H-imidazol-1-yl)propyl thioureas. the maximum inhibitory concentration (IC_{50}) value was found to be twice that of PBD150 [107].

The compound shown as PQ912 in the figure [(S)-1-(1H-benzo[d]imidazol-5-yl)-5-(4-propoxyphenyl)imidazolidin-2-one)], unlike the other structures mentioned, is imidazolidin-2- benzimidazole, given as the Zn^{+2} binding moiety at position 1 of on, is a new QC inhibitor developed close to o-substituted 4-propoxyphenyl at position 5. PQ912 has the distinction of being the first QC inhibitor to pass a clinical trial against AD [112–114].

Diphenyl conjugated imidazole derivatives (DPCI) were prepared and a diphenyl moiety was synthesized by adding one more phenyl ring to the phenyl portion and an imidazole functional linker to the *ortho* position of this ring to reduce flexibility and increase blood brain barrier penetration. For example, the structure 28, available in the figure, exhibited a remarkable QC inhibitory activity [115]. Preservation of chelation between the imidazole ring in the lower part of the pocket and Zn^{+2} facilitated the passage through the blood-brain barrier and increased potency [1].

Compound 212 in the figure was prepared by adding different parts based on the structure of PBD150 and reduced Aβ accumulation and restored cognitive functions in AD mice [106].

The SEN177 compound, consisting of a triazine ring as the hQC binding part, was designed and showed a significant QC inhibitory activity [28].

N-substituted thiourea/urea analogs of compound 9 shown in the figure were prepared by incorporating different alkyl groups, aromatic and heteroaromatic functional groups. As a result of SAR analysis, it was seen that the ZE conformation of the compound in the active site is very important for QC

inhibitory activity. Compound 90 shown in the figure showed promising efficacy [116].

The piperidine-4-carboxamide derivative, developed through pharmacophore-assisted high-throughput virtual screening, is a novel structure for the development of QC inhibitors based on its unique binding pattern at the active site of the QC [117].

As a result of all these studies, for QC inhibitor potency:

1. A Zn^{+2} binding motif to bind to the active zinc ion at the bottom of the pocket,

2. A large hydrophobic residue is required to occupy the penultimate position for N-Terminal glutamine. These two rules should be observed in the development of new QC inhibitors [1].

It is aimed to improve potency and specificity using a ligand-based optimization approach. Imidazole-based inhibitors have been extensively investigated against sQC, and further screening of alkyl imidazoles has identified a thiourea analog as a key construct for the identification of all imidazole-based inhibitors. The Ki value (activity) of this thiourea analog was found to be 1.24 ± 0.03 μM and various modifications made on the compound were expressed as x, y and z [22]. Substitution of the methyl group on the imidazole ring at the X position enhanced the inhibitory activity. The methyl group at the 5-position was thought to be directed to a small subpocket of the active site, promoting an additional hydrophobic interaction for the inhibitor, resulting in greater improvement in activity compared to the methyl group at the 4-position. However, it is thought that the substitution of bulkier hydrophobic groups at this position may cause destabilization of the interactions due to steric conflicts with the side chains of the active site residues. The volume of the lower pocket is not sufficient for larger substitutions [22].

The y region, which is an important place for the substitution of chemical moieties such as thiourea, urea, benzothiazole, thioamides, cyanoguanidine, thioxypyramidine, and nitrovinyldiamine, was investigated and it was determined that the y region mimics the hydrogen bond donor of the substrate matching the C-terminal amide nitrogen [104, 105, 107].

The three-carbon alkyl chain linking MBG and the pharmacophore II was conserved in all the inhibitors designed.

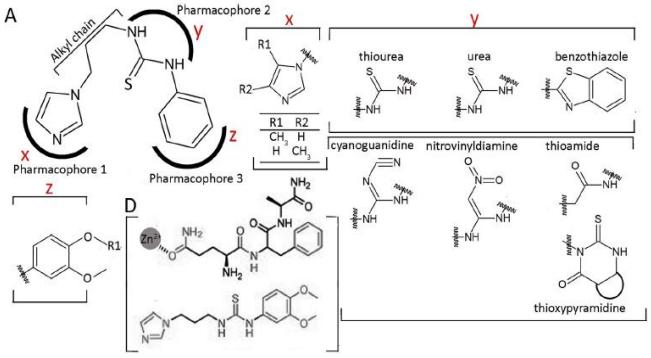


Figure 8 Basic structure of thiourea analogues. The regions where modifications were made are marked as x, y and z [22].

New sQC inhibitors were synthesized by bioisosterically replacing thiourea and thioamide at Y position with thioguanidine, thioxypyramidine and nitrovinyldiamine groups [104]. As a result of the studies, it was determined that some compounds containing cyoguanidine and nitrovinyldiamine were as strong as the parent compound (Figures E and F). The thio group in the main compounds is an important part in terms of inhibitory activity [22]. Some of the compounds containing methyl substituted (substituted) thioxypyramidine at the 5-position of the imidazole group are equally active as parent compounds (Figure G). Compounds with methyl substitutions at the 4-position were shown to exhibit 10-fold better inhibitory activity compared to 5-position substitutions (Figures H and K) [104].

In studies, a significant increase in activity was observed as a result of modifications in the pharmacophore IV. Groups such as terminal amine, amide, guanidine, cyclic amine and nitrogen-containing heterocyclic rings are attached to one of the methoxy groups *via* the alkyl chain. As a result of the studies, it was thought that the length of the alkyl chain is important in terms of inhibitory activity [106, 108, 109] Although the substitutions in the pharmacophore IV have improved the potency, the elongation of the alkyl chain can cause off-target interactions as it gives high flexibility to the molecules [22].

In another study, the thiocarbamide moiety was replaced with a phenyl ring. Thus, new inhibitors were synthesized that reduce the flexibility of the inhibitors, increase the hydrophobicity and support the blood-brain barrier penetration. IC_{50} values were found to be lower than the parent compound [115]. The compound shown in Figure R is considered a promising inhibitor in this class [22].

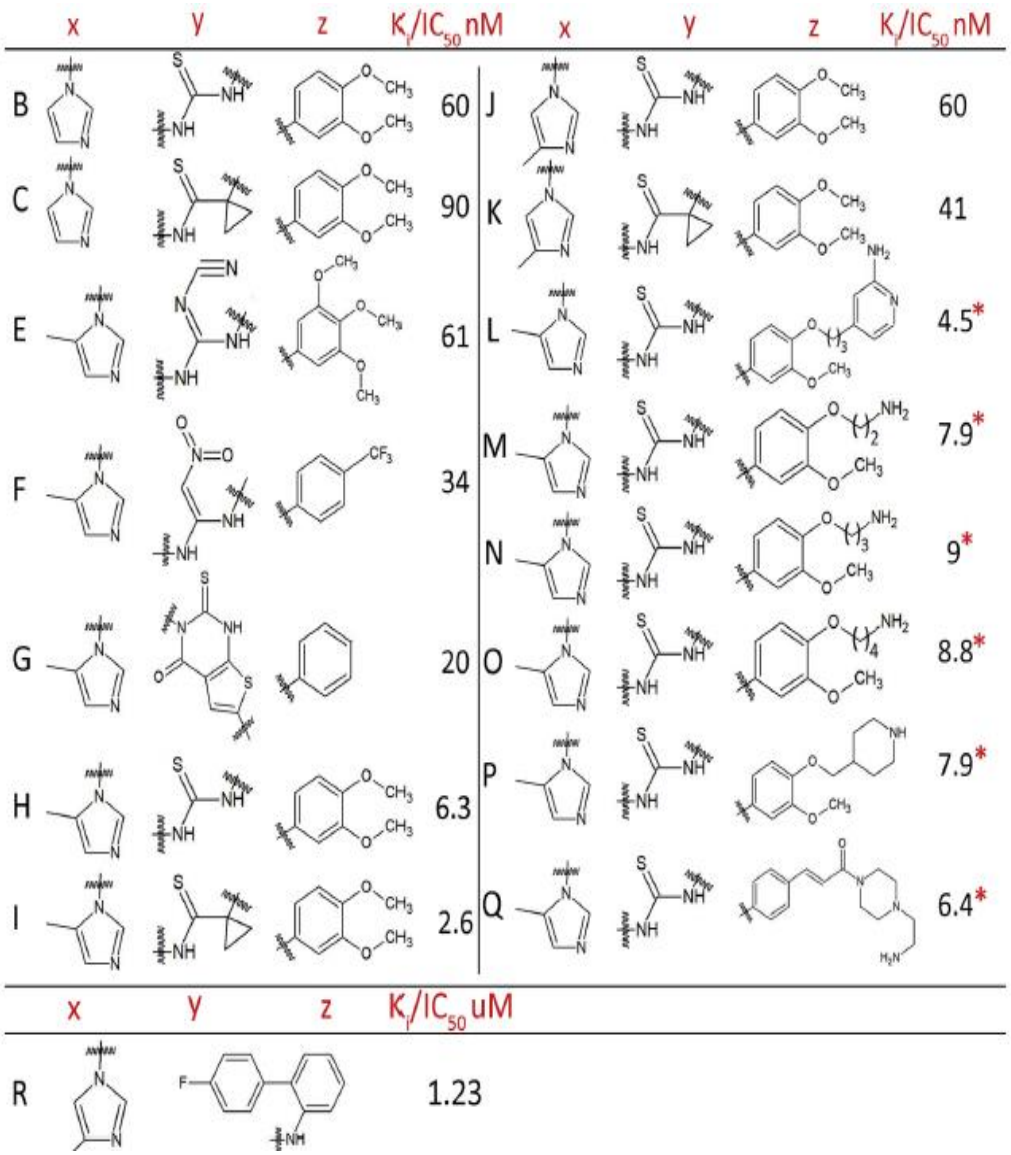
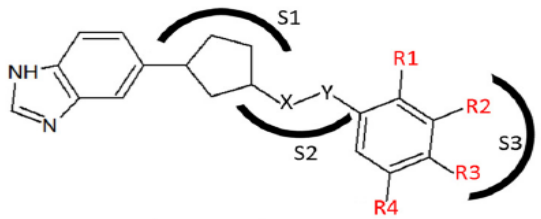


Figure 9 The functional groups substituted at each position are shown. B to R: Some of the selected imidazole-based inhibitors are shown, with a structural comparison of one of the substrate and known sQC inhibitor PBD150 shown in D. IC_{50} values are marked with * and the rest are Ki values [22] .

3.3. Benzimidazole-based Inhibitors

A



B

Sl no	S1	S2		S3				K_i in µM
		X	Y	R1	R2	R3	R4	
1	1,3,4 thiadiazole	CH_2	S	OCH_3	OCH_3	H	H	0.023
2	1,3,4 thiadiazole	CH_2	SO_2	OCH_3	OCH_3	H	H	0.080
3	1,3,4 thiadiazole	CH_2	CH_2	H	OCH_3	OCH_3	H	0.107

Figure 10 Framework of benzimidazole-based inhibitors [22].

(A). In benzimidazole-based inhibitors, the alkyl chain and the pharmacophore II (Substrate mimicry site) part are absent Figure 10). As shown in the figure above, various substitutions at the S1 and S3 positions affected the activity. At the S2 position, the length of the alkyl chain linking the heterocyclic group and the terminal phenyl group has increased. At the same time, amino, methylene, ether, sulfur, sulfone and ethylene groups are substituted at the S2 position. Various hydrophobic substitutions of varying size and character have been made to the S3 position, including benzyl, flat aliphatic residues (B). As a result of the studies, it was thought that the aromatic ring at the S3 position supports arrangement (stacking) and docking interactions. Substitution of methoxy groups at different positions promoted inhibition in most cases [22].

3.4. Methyl Triazole-based Inhibitors

Studies have identified methyl triazole-based inhibitors against sQC [43] Figure 11).

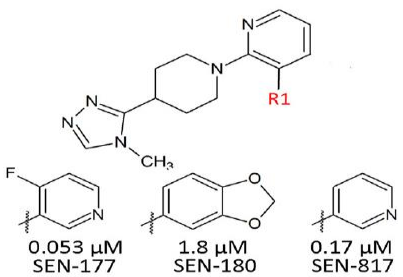


Figure 11 Triazole-based QC inhibitors [22].

As the basic structure is shown in the figure, these inhibitors are defined as SEN-177, SEN-180 and SEN-817. The IC_{50} profile of these inhibitors suggested that the inhibitors are more selective against gQC than sQC [22].

3.5. Natural Products and Derivatives

With a reverse metabolomics approach, studies have suggested that sulfolipids from microalgae are potential inhibitors of sQC. Three sQC inhibitors from the sulfolipid family (sQC) Figure 12) were determined by the preparation of 24 methanol extracts of various algae species such as *Scenedesmus rubescens, Scenedesmus producto-capitatus, Scenedesmus accuminatus, Scenedesmus pectinatusi, Tetradesmus wisconsinensis* and *Eustigmatos magnus*, and evaluating the inhibitory activity Figure 12) [118]. It is thought that sulfolipids will be a new structure in which the sulfonate group can act as an important pharmacophore group that binds to Zn^{+2} [1].

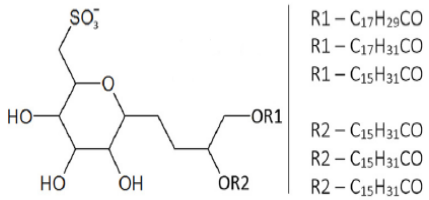


Figure 12 Basic structure of sulfolipid [22].

Flavonoids have shown pharmacological properties such as anti-oxidation and anti-inflammation. In studies, it was determined that apigenin Figure 13) showed an inhibition rate of 75% against sQC at a concentration of 100 μM [119]. Therefore, various apigenin derivatives were synthesized and tested against sQC. Substitutions are shown in E. Apigenin and synthesized analogues lack several important pharmacophoric properties. Most of the synthesized inhibitors were found to have very weak interactions compared to imidazole-based compounds. Considering these results, it was understood that pharmacophoric interactions have a great importance on inhibitory activity. The IC_{50} values of some promising apigenin derivatives range from 16 μM to 37 μM [22].

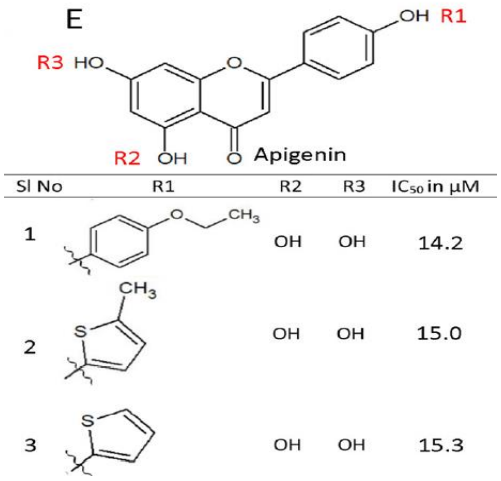


Figure 13 Apigenin structures and synthetic analogs [22].

pE AB aggregation and cytotoxicity, *in vitro* and *in vivo* QC activity were significantly reduced by oleuropein aglycone (OLE) Figure 14), a natural phenol found in extra virgin olive oil. It has been determined by *in vivo* studies that it improves memory and behavioral performance [120].

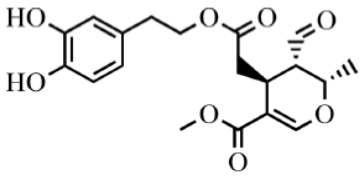


Figure 14 Oleuropein aglycone (OLE) [1].

3.6. Varoglutamstat (PQ912)

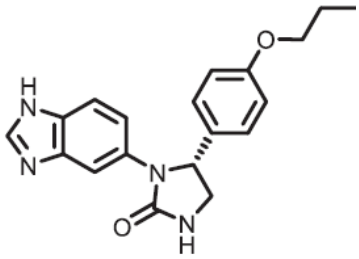


Figure 15 Benzimidazole-based QC inhibitor PQ912 [98]

Varoglutamstat (formerly PQ912) Figure 15) is a small molecule that inhibits the activity of QC to reduce the level of pyroglutamate-A-beta (pGluAB42). PQ912 is the first small molecule to enter clinical trial. Phase 1 and phase 2 studies have shown that PQ912 has a positive effect on cognitive function and reverses AD-induced changes in theta and alpha rhythms [112, 114, 121].

Studies have shown that pGluAB42 is a special form of amyloid that is highly synaptotoxic and plays an important role in the development of AD [122].

Varoglutamstat was evaluated in a phase 2b, placebo-controlled, double-blind, randomized clinical trial on safety and tolerability, efficacy on cognition, brain activity, and biomarkers of AD. Results are expected in early 2023 [122].

In human autopsy studies in AD patients, pGluAB42 was found to constitute 10-50% of the total amyloid load. Therefore, it is thought that pGluAB42 plays an important role in the development of AD [48, 123]. pGluAB42 has become a therapeutic target in the treatment of AD [122]. At the same time, it was thought that Donanemab, a monoclonal antibody targeting pGluAB42, may have an effect on treatment as a result of studies [124].

The small molecule QC inhibitor varoglutamstat inhibits QC activity and reduces the level of pGluAB42. Up to 14 days of treatment was evaluated in healthy young and elderly subjects treated with up to 1800 mg of varoglutamstat (BID) twice daily and was evaluated in a large phase 1 study. The study found that varoglutamstat was well tolerated with few and mild adverse effects (AE) and showed clear pharmacodynamic effects in terms of QC inhibition in plasma and cerebrospinal fluid (CSF) [112].

In a phase 2a, randomized, double-blind, placebo-controlled, biomarker-confirmed clinical trial in AD patients (n=120), volunteers were treated with varoglutamstat at doses of 800 mg for 12 weeks to further evaluate the effects on safety and biomarkers [114]. Varoglutamstat has demonstrated an acceptable safety and tolerability profile at lower doses and slower titration. A significant improvement in working memory, reduction in synaptotoxicity, and lower neurogranin levels were seen in the varoglutamstat treatment group. It has also been found to provide improvements in various other experimental biomarkers [114]. Post-hoc results supported the enhancement of synaptoplasticity with varoglutamstat treatment in a network analysis [122].

Trials with varoglutamstat, a phase 2b trial with endpoints in terms of favorable benefit-risk ratio, biological effect on QC inhibition, reduced synaptic toxicity, cognitive function, biomarkers, and long-term safety and tolerability, resulted in beneficial safety data suggesting a clinical effect. and tolerability profile, providing justification for designing the state-of-the-art product [122]. Studies with varoglutamstat have shown a beneficial safety and tolerable profile. This allowed to design a reasonable phase 2 study with appropriate benefit-risk ratio, QC enzyme inhibition effect, reduced synaptic toxicity, predictive data on clinical effect, results on cognitive function, biomarkers, long-term safety and tolerability [122].

Changes in biochemical biomarkers are important to detect biological/pharmacodynamic effect in phase 2b AD trials. In the SAPHIR study [114] YKL-40, a neuroinflammation marker in AD [125] , was shown to decrease by approximately 5% from baseline levels within 12 weeks when varoglutamstat was used. In addition, the synaptic biomarker neurogranin was reduced by approximately 4% [122].

Although clinical trials have not yet been concluded, based on ongoing studies, minor QC activity reductions by varoglutamstat are expected to prevent the formation of the neurotoxic pyro-GluAB. Including the dose-finding phase in the data analysis allows for longer follow-up of treated individuals and provides information on the long-term treatment effect with regard to both safety and efficacy data without affecting the duration of outcome [122].

Studies show that pyroglutamate-modified Aβ (pGlu3-Aβ; AβN3pG) peptides play a very important role in the development and progression of AD. Approaches targeting pGlu3-Aβ with QC inhibition (Varoglutamstat) or monoclonal antibodies (Donanemab) are currently under clinical investigation [126].

One study evaluated combination therapy of Varoglutamstat (PQ912) and a pGlu3-Aβ-specific antibody (m6) in transgenic mice. While single treatments at subtherapeutic doses showed moderate (16-41%) but statistically insignificant reductions in Aβ42 and pGlu-Aβ42 in mouse brain, the combination of both treatments resulted in significant reductions in Aβ of 45-65%. The mechanisms of action of varoglutamstat (PQ912) and monoclonal antibody differ. While PQ912 inhibits the formation of pGlu3-Aβ in different compartments, the antibody is able to clear existing pGlu3-Aβ residues. Study results show that the combination of the small molecule Varoglutamstat and a monoclonal antibody directed against pE3Aβ may allow reduction of individual compound doses while maintaining the therapeutic effect [126].

Several monoclonal antibodies to Aβ are available for the development of AD therapy. Recent promising results from clinical studies with Lecanemab (BAN2401) targeting large soluble Aβ protofibrils or Donanemab for pGlu3-Aβ and accelerated approval of Aducanumab provide clear support for the amyloid hypothesis. Therefore, targeting Aβ is seen as a reasonable treatment development approach for AD (Sevigny et al 2016, Budd et al 2017, Cummings et al 2021, Selkoe et al 2016).

Donanemab has recently demonstrated significant removal of amyloid burden and cognitive stabilization in a Phase 2 clinical trial [124, 127]. It is also the first antibody in clinical development to specifically bind pyroglutamate-modified amyloid peptides (pGlu3-AB, AβN3pG). The basic rationale for targeting pGlu-Aβ is based on the observation of a specific toxicity of these molecular species of Aβ [54, 64, 128, 129].

Varoglutamstat, a first-class QC inhibitor, has been proven to be safe and has an effect on AD in clinical phase 1 and 2 studies [112, 114]. While inhibition of QC suppresses pGlu formation, targeting with antibodies aims to block the clearance and/or aggregation of pGlu-Aβ after formation [130]. In a study, considering the different mechanisms of action of these two treatments for the same purpose, a combination therapy was targeted to examine whether the effects of the two compounds could be combined. A study in a mouse model of AD overexpressing human amyloid precursor protein containing human QC found that the reduction in pGlu3-Aβ42 as well as Aβ42 was greater for the combination treatment. It is thought that the combination of the two treatment strategies for the treatment of AD may have an additive effect [126].

4. Other Studies

In the study carried out by Manh *et al.* in 2021, potent QC inhibitors with subnanomolar IC_{50} values up to 290 times higher than varoglutamstat (PQ912), which is in phase 2, were determined [98].

Among the compounds tested in this study, the following cyclopentylmethyl derivative compound Figure 16) exhibited the strongest *in vitro* activity among N-alkyl/aryl-substituted urea compounds with an IC_{50} value of 0.1 nM [98].

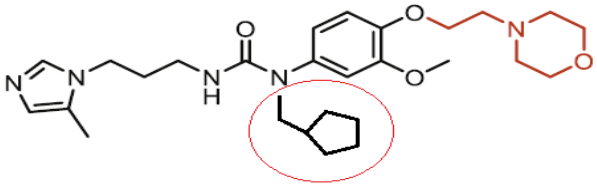


Figure 16 QC inhibitor having a cyclopentylmethyl group discovered by Manh *et al.* [98]

When the crystal structure of human QC in complex with the above compound was examined, it was determined that a strong binding occurred at the active site and it was supported that the compound showed strong *in vitro* and *in vivo* activity for the specific inhibition of QC [98].

Furthermore, among the compounds tested in this study, the following benzimidazole derivative compound among the N-alkyl/aryl-substituted urea compounds Figure 17) exhibited the most promising *in vivo* efficacy, selectivity and drug-friendly profile [98].

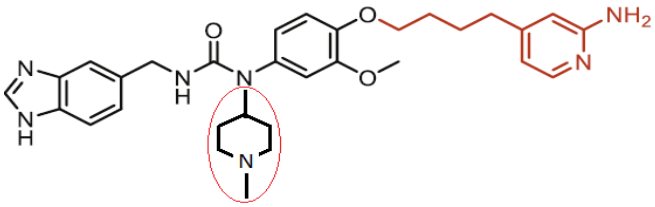


Figure 17 Benzimidazole derivative QC inhibitor carrying 1-methyl-4-piperidinyl group discovered by Manh *et al.* [98]

The above compound significantly reduced the concentration of pyroform Aβ and total Aβ in the brain of an animal model of AD [98].

The accumulation of Aβ peptides begins decades before any symptoms of the disease appear [103]. It has been determined that many types of Aβ fibrils and oligomers are neurotoxic [131, 132]. The application based on the removal of Aβ from the brain, called Aβ clearance therapy, constitutes a reasonable treatment option to reduce cognitive and functional decline in AD patients. For this application, Aβ antibodies and QC inhibitors such as aducanumab, donanemab form the basis of antiamyloid therapy for early stage patients with AD. It is thought that targeting specific forms of Aβ species can provide an effective treatment for AD with monotherapy or multiple treatment options [98].

In studies on AD, it was thought that the pyroglutamate form of Aβ, AβN3pE, caused high neuronal and glial toxicities. However, its contribution to the formation of hyperphosphorylated tau suggests that there may be a link between the two neurotoxic types in AD. In a recent clinical trial, patients exhibiting AD symptoms were treated with a monoclonal antibody, donanemab, with significant improvements in cognition and daily function. Therefore, AβN3pE has been supported as an important therapeutic target for therapy [133].

New classes of potent QC inhibitors designed using bioisostere-based [134] , pharmacophore-based [106, 109] and structure-based approaches (Hoang et al 2019) have recently been investigated. Among these, the following representative inhibitors showed strong QC inhibition [98] Figure 18) Figure 19).

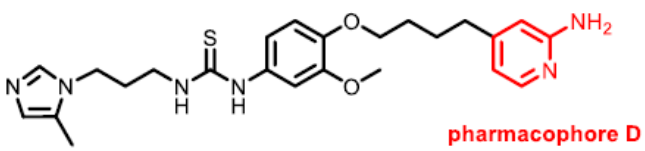


Figure 18 QC inhibitor pharmacophore found by Manh *et al.* [98].

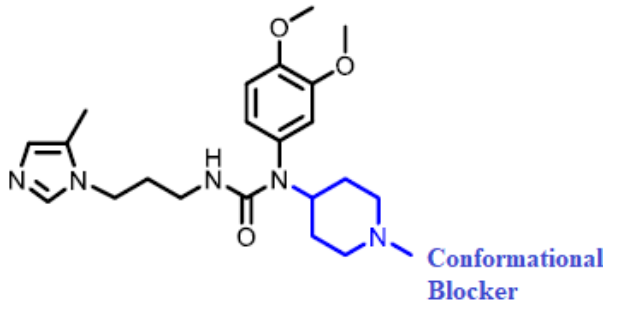


Figure 19 QC inhibitor conformational blocker found by Manh *et al.* [98].

These compounds significantly reduced AβN3pE and total Aβ concentrations in the brain, as well as improved cognitive functions in an animal model of AD. With the combination of the above two prototype compounds, the following prototypes of the following new QC inhibitor designs were obtained [98] Figure 20).

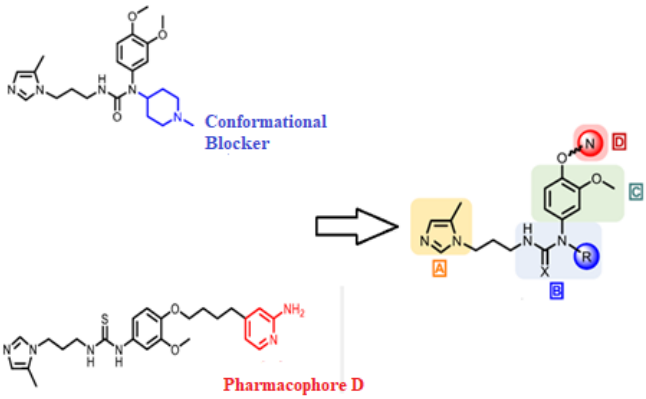


Figure 20 Combination of QC inhibitor pharmacophore D and conformational blocker by Manh *et al.* (Zinc binding groups: Pharmacophore A, Hydrogen binding donors: Pharmacophore B, Phenyl groups: Pharmacophore C are the main pharmacophores of this structure. In addition, as the Argmimetic group: Pharmacophore D was included) [98].

In this study conducted by Manh *et al.* in 2021, a series of N-substituted N-(4-aminoalkylphenyl) thiourea/urea analogues were synthesized as a new scaffolds. Structure activity relationships (SARs) of these structures as hQC inhibitors have been investigated. The potent inhibitors selected were also measured for their ability to reduce the formation of AβN3pE-42 in acute and transgenic mouse models of AD. At the same time, their ability to improve cognition and behavior was examined and evaluated *in vivo* [98]

5. Conclusion

Studies on AD and its treatment have so far found that Aβ plaques contribute to the development of AD. Therefore, it was thought that inhibition of QC enzyme, which catalyzes the formation of pyroglutamate-modified Aβ plaques, could prevent the formation of pE-modified plaques and thus AD development.

As a result of some studies, many QC enzyme inhibitors have been developed, and *in vitro* and *in vivo* studies have shown that some of them show high activity. As a result of the studies, it was determined that only imidazole,

benzimidazole and triazole-based compounds are potential sQC inhibitors.

As a result of structure-activity analysis, it was understood that imidazole acts by binding to zinc ion and is an important structure in inhibiting QC activity. It has been determined that the modifications of the imidazole structure significantly affect the potency. Thiourea derivatives such as the designed compound PBD150 have proven to be potent inhibitors. Imidazole-based inhibitors have been extensively investigated against sQC and some of the compounds have been considered as promising inhibitors. The benzimidazole derivative compound PQ912, also known as varoglutamstat, is an effective QC inhibitor currently in phase 2 in clinical trials. Triazole derivatives have been investigated in some studies and triazole derivatives are thought to be more selective against gQC than sQC.

As a result of the investigation of the QC inhibitory potentials of natural products and their derivatives, it was thought that the sulfonate group in sulpholipids could be an important pharmacophore group that binds to the zinc ion. Oleuropein (OLE) aglycone has been proven by *in vivo* studies to improve memory and behavioral performance.

Varoglutamstat is the first small molecule to enter clinical trial. It has been proven by studies that the small molecule QC inhibitor varoglutamstat inhibits QC activity and reduces the level of Aβ plaques. In phase 1 and phase 2 studies, it was found that PQ912 had a positive effect on cognitive function and reversed AD-induced changes in theta and alpha rhythms. Phase 2b results of varoglutamstat are expected in 2023.

Studies with varoglutamstat have shown that it is effective and safe with a favorable benefit-risk ratio, potent QC inhibitory activity, reduced synaptic toxicity, and long-term safety and tolerability.

As a result of the studies, it has been determined that treatment options based on the combination of QC inhibitors and monoclonal antibodies are more effective on the development and treatment of AD. It is thought that this combination will allow for the reduction of individual compound doses while maintaining the therapeutic effect. In the study of Manh *et al.* in 2021, potent QC inhibitors with subnanomolar IC_{50} values up to 290 times higher than varoglutamstat were discovered.

Declaration of Conflict of Interest

Authors declare that they have no conflict of interest with any person, institution, or company.

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