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## Abstract

Aquaporins (AQP) working as membrane channels facilitated water transport, play vital roles in various physiological progress including cell migration, energy metabolism, inflammation, etc. They are quite important drug targets, but elusive for discovery due to their undruggable properties. In this chapter, we summarized most fluently used methods for screening AQP inhibitors, including cell swelling assay, cell shrinking assay, and stopped-flow assay. And three classes of AQP inhibitors have been discussed, including metal-related inhibitors, quaternary ammonium salts, and small molecule inhibitors which further divided into four parts, sulfanilamide analogies, TGN-020, antiepileptic drugs, and others. It has been suggested that although they showed inhibition effects on AQP1, AQP3, AQP4, AQP7, or AQP9 in some researches, none of them could be

asserted as AQP inhibitors to some extent. Discovering AQP inhibitors is a big challenge, but if successful, it will be a great contribution for human health.

## Keywords

Aquaporins · Inhibitors · Water permeability · Chemical molecules · Screening

## 22.1 Introduction

As reviewed in elsewhere in this book, in mammalian, there are 13 subfamilies of aquaporins (AQP), vary from AQP0 to AQP12. From them, some channels (AQP3, 7, 9, 10) also facilitate the transport of glycerol and other small neutral solutes such as urea, carbon dioxide, and ammonia, namely aquaglyceroporins. All these aquaporins are assembled by four monomers with ~30-kDa molecular size, each monomer has a narrow aqueous pore (contained Asn-Pro-Ala (NPA) motif) flanking a narrowing (~2.8 Å in diameter for AQP1) allowing a single-file water transport driving by an osmotic gradient which participate in the regulation of physiological functions including cell migration, energy metabolism, inflammation, etc. The narrowest segment of the water channel is within the transmembrane region of the pore, which is 2.8 Å for AQP1, which is similar to the size of a single water molecule [1]. Although each channel is

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functionally independent, which means an AQP protein can be bound with four inhibitors, the narrow pore and the small molecular size bring a great challenge for identifying drugs targeting AQPs. AQPs play a crucial role in the development of diseases as previous chapters described. But up to now, most molecules under investigation target AQP1, AQP3, AQP4, AQP7, and AQP9.

In kidney, AQP1 is expressed in the epithelium of the renal proximal tubule, the thin descending limb of the loop of Henle, and the descending vasa recta. In an AQP1 knockout mouse model, severe urine concentration deficiency is observed, with increased urine output and reduced urine osmolality. AQP1 deficiency impairs the renal counter-current multiplication by reducing the permeability in the descending limb of the loop of Henle and the descending vasa recta. Besides, AQP1 has been found that overexpressed in breast cancer [2]. In an AQP1-knockout mouse model, impaired tumor growth was observed, including a reduced tumor vascularity and extensive tumor necrosis, and an enhanced survival of tumor-bearing mice was presented [3]. These studies suggest AQP1 inhibitors may have clinical indications as diuretics for the treatment of the glaucoma, cerebral edema, elevated intraocular pressure which are directly or indirectly related to abnormal fluid homeostasis and as antitumor agents [4].

AQP3 is a water- and glycerol-transporting membrane protein, expressed in the collecting duct of the kidney [5], airway epithelia, secretory glands, and skin [6, 7], that is involved in cell proliferation and migration. Previous studies show that inhibitors of AQP3 glycerol or  $H_2O_2$  transport are thought to prevent or retard skin tumor growth [8] or chronic inflammatory skin diseases including psoriasis [9]. In addition, AQP3 has a significant role in the parasite replication by inducing in human hepatocytes in response to parasite infection [10]. And glycerol mediated by AQP3 contributes to the replication of the parasite during the asexual intraerythrocytic stages [11]. So the inhibitors of AQP3 might be regarded as antiparasitic drugs.

AQP4 is expressed in perivascular end feet of astroglia in the central nervous system, which is proposed to serve physiologically as a route for the net movement of water out of the brain but in pathological conditions create vulnerability to cerebral edema, for example, after acute brain injury or stroke [12, 13]. AQP4 inhibitors are predicted to reduce brain swelling in ischemic stroke. And the glymphatic system, that is aquaporin 4 (AQP4) facilitated exchange of CSF with interstitial fluid (ISF), may provide a clearance pathway for protein species such as amyloid- $\beta$  and tau, which accumulate in the brain in Alzheimer's disease [14]. AQP4 plays an important role in the glymphatic clearance of tau from the brain, suggesting AQP4 might be a target for the treatment of Alzheimer's disease.

AQP7 expressed in adipose tissue and AQP9 expressed in liver tissue are members of aquaglyceroporins, which are involved in adipose metabolism and insulin resistance in liver [15, 16]. AQP7 deficiency leads to the decreasing of the permeability of the plasma membrane to glycerol, which causes cellular accumulation of glycerol and triglyceride as well as upregulation of glycerol kinase expression [17]. It might increase the accumulation of adipose and lead to obesity. In addition, AQP7 is now considered as a  $\beta$ -cell protein and critical regulator of intracellular glycerol content as well as insulin production and secretion [18]. There is evidence that AQP9 is related with the uptake of hepatocyte glycerol that AQP7 and AQP9 might be target for obesity or diabetes [17].

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## 22.2 Methods for Screening AQP Inhibitors

In this chapter, we are about to list main methods used for screening the AQP inhibitors. All these methods based on the measurement of the kinetics of the cell volume in response to an osmotic gradient presented as fluorescent quenching or scattering time-course or directly measurement using cell imaging.

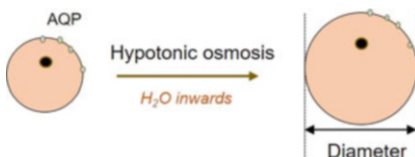
### 22.2.1 Cell Swelling Assay

Cell swelling assay is the earliest functional assay for water permeability. Tetraethyl ammonium (TEA) [19], acetazolamide [20] (AZA), and some small molecules [21, 22] were identified by this method, although it is proved to be fraught with artifact because there are many determinants of oocyte swelling later researches [17, 23, 24]. *Xenopus laevis* oocytes expressing AQP were used as tool cells. Oocytes are so big that could be easily measured by image analysis (Fig. 22.1). Water permeability and aquaporin function in *X. laevis* oocytes should only be calculated from initial osmotically induced volume changes [25].

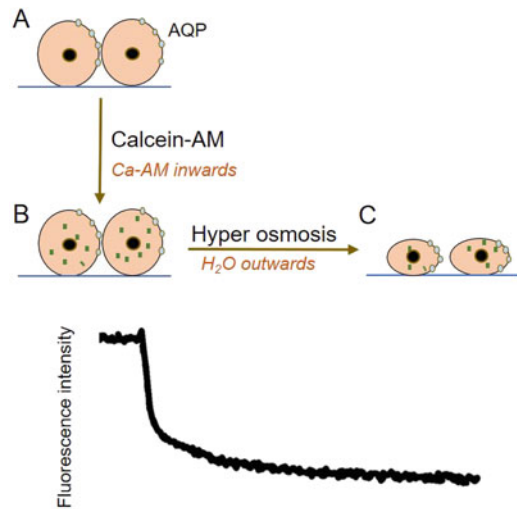
### 22.2.2 Cell Shrinking Assay

Cell shrinking assay is also regarded as fluorescence quenching assay. It is on the basis of the self-quenching of certain fluorophores such as calcein at high concentrations. It correlates cell volume with fluorescence intensity where cell shrinking should have increased the fluorescence concentration and thereby self-quenching occurs, decreased fluorescence intensity could be observed by Fluostar Optima plate reader [26]. Calcein AM is the most useful fluorescent material, which is a membrane permeable substance, releasing calcein intracellular as fluorescent material to indicate the viable cells.

The main steps could be divided into three parts (Fig. 22.2). The first part is cell culture. Cells expressing AQPs are grown as monolayer in 96-well plate or other solid supports. Cell lines



**Fig. 22.1** Cell swelling assay. AQP-overexpressed oocytes are used for imaging analysis which records the diameter representing relative increases of the cell volume induced by hypotonic osmosis by video microscopy



**Fig. 22.2** Cell shrinking assay. (a) Monolayer cells are formed. (b) Fluorescent materials (Ca-AM) are loaded. Initial fluorescence intensity is recorded. (c) Cells shrink under hypertonic buffer and the intensity decrease

cultured could be CHO cells [27, 28], MDCK [29], and other adherent cell lines that are used for transfection such as RPE cells [26]. The second part is fluorescent loaded. A fresh medium containing probenecid (an anion transporter inhibitor, to reduce the leakage of dye indicators) and Calcein AM is added, and the cells are cultured for at least 30 min. At the same time, tested compounds could be added. The last part is self-quenching. High osmotic gradients are applied, a linear dependence of fluorescence intensity is recorded, the kinetic curve is presented as Fig. 22.2. A similar method, which does not require dye loading, uses the genetically encoded, cytoplasmically expressed yellow fluorescent protein YFP-H148Q-V163S, whose fluorescence is quenched by chloride [30].

### 22.2.3 Stopped-Flow Assay

Stopped-flow assay is carried out using an apparatus in which two solutions are mixed together rapidly (in  $<1$  ms) and have an optical read-out [17]. Stopped-flow measurements can be made in plasma membrane vesicles from AQP-expressing cells, in reconstituted proteoliposomes or in small

cells such as erythrocytes. Their suspensions are prepared and then mixed with hyper osmosis medium rapidly, and cell volume reduces, thereby scattering intensity changes. Water permeability should be calculated from initial slope of scattering intensity changes induced by osmotic pressure. Besides it could assess the water outflow ability by mixed suspensions which is incubated with hyper osmosis medium previously with isotonic medium. And this technique is widely used for screening AQPs inhibitors [23, 24] and other channel inhibitors such as urea transporters [31], and it could quantitate the water permeability, but it requires specialized instruments.

## 22.3 AQPs Inhibitors

Due to the narrow Asn-Pro-Ala (NPA) motif in AQPs, the drug discovery of AQP inhibitors seems unexpectedly challenging. Here we discussed the proposed direct inhibitors of AQPs rather than molecules which affect AQP in indirect ways. Three classes of AQP inhibitors have been described: metal related inhibitors, quaternary ammonium salts, and small molecule inhibitors.

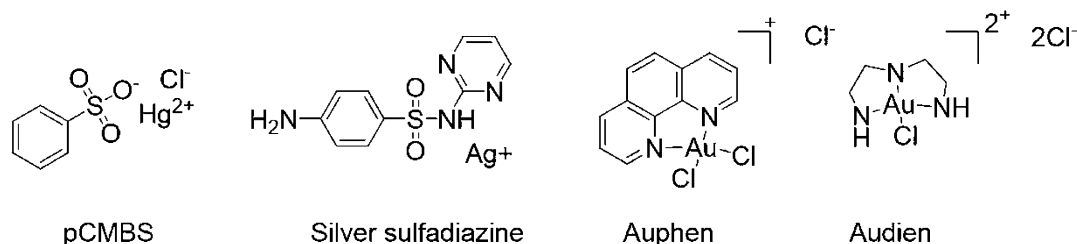
### 22.3.1 Metal-Related Inhibitors

Metal ions exist in body fluids, and these will form different bonds with biomolecules, involving in vital cellular processes. Hemoglobin is an indispensable protein in mammals and plays a role in transporting oxygen. It consists of four chains, two  $\alpha$ -chains and two  $\beta$ -chains, of which contained a cyclic heme with a ferrous atom, that would bond with oxygen. In addition, exogenous metal-containing small molecules can be effect on proteins or biomolecules targeting their metal moiety [32]. In clinic, cisplatin is widely used for the treatment of solid cancer [33], by damaging DNA of vigorously proliferating cells by forming intrastrand diadduct [34, 35] with bases in DNA chain, especially, purines.

Some metal-related compounds also showed AQP inhibition effect with lowering water

permeability. The first reported metal-related inhibitor was pCMBS (*p*-chloromercuribenzenesulfonate), which has been found that has inhibition of water permeation into erythrocytes. And  $\text{HgCl}_2$ , which has a covalent interaction with Cys189 of AQP1 in the vicinity of the conserved NPA motif in loop E, restrain the water permeability sterically [17], too. Cys189 residue is essential for the inhibition by mercury and in a mutation in the Cys189 residue of AQP1 prevents the inhibition by mercury [36]. Ag is one of the transition elements, and the diameter of  $\text{Ag}^+$  ion (2.5 Å) matches the predicted AQP channel diameter of 2.8 Å, whereas the  $\text{Hg}^{2+}$  ion is 2.2 Å which might also build interaction with sulfhydryl groups. Indeed, it has been reported that Ag as  $\text{AgNO}_3$  or silver sulfadiazine (Fig. 22.3) inhibited the water permeability of human red cell (AQP1) with high potency ( $\text{EC}_{50} = 3.9 \mu\text{M}$  or  $1.24 \mu\text{M}$ , respectively) [37]. However, these inhibitions are non-reversible, while mercury-based inhibition is reversible in the presence of mercaptoethanol, suggesting there is a different mechanism, but it is still not clear. Another common metal element,  $\text{Au}^{3+}$  with the diameter of 2.7 Å also has been reported that it has inhibitory effect on AQPs. AuPhen [32, 38] ( $[\text{Au(III)(phen)Cl}_2] \text{Cl}$ , (phen = 1,10-phenanthroline)) is a specific inhibitor against AQP3, which has inhibition effect on glycerol transport in human red blood cells (hRBC) with an  $\text{IC}_{50}$  value of 0.08 mM, while has no inhibitory effect on AQP1-mediated water permeability. In addition, Audien [39] also showed inhibition on glycerol permeability with an  $\text{IC}_{50}$  value of  $16.62 \pm 1.61 \mu\text{M}$  in hRBC. It is reported that the mechanism of Au is not the same as mercury, but does have effect on pore closure, due to protein conformational channel upon metal binding with Cys40 which was confirmed by computational modeling [39].

Although there are no direct evidences to support this assumption, it can be assumed that metal-related inhibitors might have strong toxicity due to its easily covalent bonding with Cys residues which are abundant in proteins in vitro. In addition, few researches have been done to assess these novel metal-related inhibitors as diuretics in animal models, not mention to



**Fig. 22.3** Structures of metal-related compounds

human, except for  $\text{HgCl}_2$  that was used historically only before the discovery of thiazides and loop diuretics. However, these might be applied to treat some incurable diseases such as cancer if these could be proved effective for clinic.

### 22.3.2 Proposed Quaternary Ammonium Salts

Tetraethylammonium (TEA) chloride, which is known as a blocker of voltage-gated potassium channels, was reported to reduce the water permeability of human AQP1 channels expressed in *Xenopus* oocytes reversibly [22]. TEA also inhibits water permeation through AQP2 and AQP4, whereas the water permeabilities of oocytes expressing AQP3 or AQP5 were not affected [19]. However, this effect could not be reproduced at a concentration of up to 10 mM by stopped-flow light scattering in erythrocytes, which natively express AQP1, and in epithelial cells that were stably transfected with AQP [23]. The different results occurred are due to the lower-sensitivity techniques used in earlier studies, which might have been susceptible to the secondary effects related to the distribution of ions across the plasma membrane [17].

### 22.3.3 Small Molecules

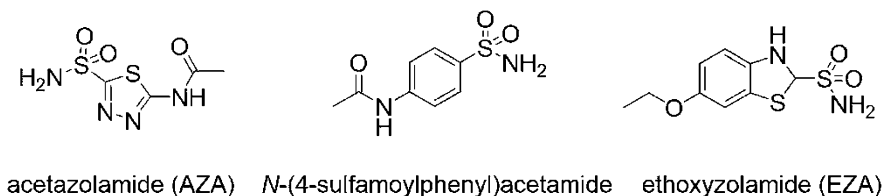
Although AQPs show as elusive drug targets, many efforts have been made.

#### 22.3.3.1 Sulfanilamide Analogies

Sulfanilamide analogies (Fig. 22.4) which were reported as AQP inhibitors could be divided into two types, namely arylsulfonamide which derived from CA (carbonic anhydrase, CA) inhibitors and sulfamoyl benzoic analogies which developed from bumetanide as loop diuretics.

AZA is used as a carbonic anhydrase inhibitor. Previous studies showed that there was a big similarity between AQP1 and some carbonic anhydrase isoenzymes in the tissue distribution and even the subcellular localization, suggesting that the potential relationship between the two proteins in structures or functions [40]. In an AQP1-cRNA inject oocyte model [40] and HEK293 cells transfected with pEGFP/AQP1 model, AZA inhibited the osmotic water permeability, and surface plasmon resonance (SPR) study proved this inhibition might function through direct binding between AZA and AQP1 [20]. However, other conflicting data from the same group showed acetazolamide-inhibited AQP1 protein expression [41]. What is more, in erythrocytes or AQP1-expressing epithelial cells, no inhibition of AQP1 water transport at concentrations of AZA up to 2 mM was observed [23]. AZA might be an AQP1 downregulator.

Later, due to the sequence homology between AQP1 and AQP4, AZA was tested using *Xenopus* oocytes expressing AQP4 (hAQP4b), and it was found to have an  $\text{IC}_{50}$  against AQP4 of 0.9  $\mu\text{M}$  with a maximum inhibition of 85% [42]. Besides, they explored additional pan-CA inhibitors, namely *N*-(4-sulfamoylphenyl) acetamide and ethoxazolamide (EZA), which showed potential inhibition against AQP4 in different extent in *Xenopus* oocyte model [42]. Also, the



**Fig. 22.4** Structures of sulfonamides

virtual docking studies showed that the sulfonamide interacts with the guanidyl group of Arg216 as well as with the carbonyl group of Gly209. It remains that the sulfonamide moiety might be essential for AQP4 “inhibition,” but these might be artifacts that compounds might affect cell size or shape, cell volume regulation, nonaquaporin ion, or solute transporters [24]. It can not be concluded the inhibition only rely on the *Xenopus* oocyte expression system.

AQPs are membrane channels, which have similarities with other ion channels. With this opinion, various channels and transporter blockers have been screened for AQP inhibition. From them, some loop diuretics, mainly, bumetanide and furosemide [43] showed modest inhibition effect on AQP-mediated osmotic swelling in *Xenopus* oocytes. And though computational docking and structure–function relationship (SAR) study, sulfamoyl benzoic scaffold was supposed to be an important pharmacophore element, so based on the core structure, series of compounds were developed, AqB013 showed block effect on water permeability facilitated by AQP1 and AQP4 with IC<sub>50</sub> values of approximately 20 μM and 50 μM, respectively [43].

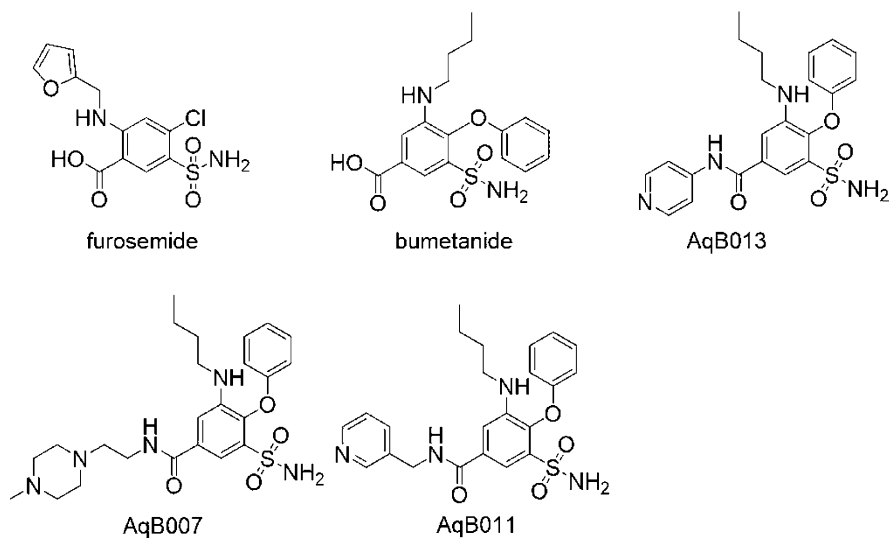
Besides, bumetanide derivatives AqB007 and AqB011 (Fig. 22.5) were proved as selective blockers to inhibit AQP1 ion conductance with no effect on water channel activity, and AqB011 was the most potent blocker with an IC<sub>50</sub> value of 14 μM by two-electrode voltage clamp and optical osmotic swelling assays [21]. Except for AqB007 and AqB011, AqB050 (the chemical structure was not found) was regarded as a selective inhibitor of AQP1 by effecting ion conductance (A. Yool et al. manuscript in preparation), and it only showed significant decrease in cell

proliferation in AQP1-high cells, while no statistically difference in AQP1-low cells [44]. Although the data in vitro presented antimalignant mesothelioma potential, but in a xenograft mouse model, AqB050 had no biologically significant effect on growth of established tumor [44]. It needs to be clarified why “AQP1 inhibitor,” which derived from loop diuretics, has the same bioactivity with that caused by AQP1 knockdown, but showed no antitumor effect in vivo.

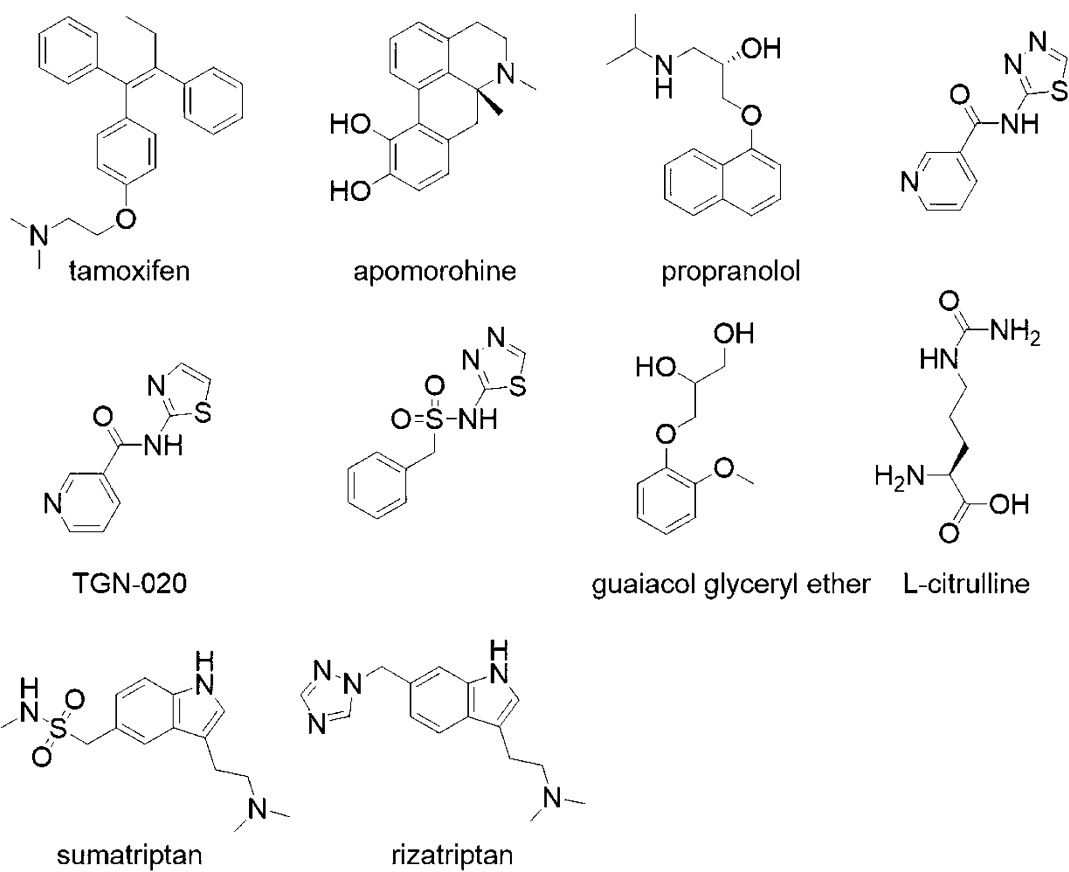
And the inhibition on AQP1 needs to be confirmed using alternative functional assays that are less prone to artifacts [17]. In addition, Verkman’s group has retested the inhibition against AQP1 of AqB013 by stopped-flow light scattering in human and rat erythrocytes that natively express AQP1, in hemoglobin-free membrane vesicles from rat and human erythrocytes, and in plasma membrane vesicles isolated from AQP1-transfected Chinese hamster ovary cell cultures, and it showed no significant inhibition on AQP1 water permeability [24], which is more convincing. And in a MCAO mouse model [45] and a spinal cord injury rat model [46], bumetanide-treated group had a significant attenuation of AQP4 protein expression, which reminds bumetanide might be an AQP1 downregulator, too.

### 22.3.3.2 TGN-020

Eighteen compounds were identified based on conserved physicochemical features of previously discovered compounds in silico, and more than half (10 compounds) of the compounds (structures are showed in Fig. 22.6) showed AQP4 inhibition in *Xenopus* oocytes transfected to express AQP4 model [47, 48]. From them, three compounds including TGN-020



**Fig. 22.5** Structures of bumetanide derivatives



**Fig. 22.6** Structures of 10 compounds which might have AQP4 inhibition



(2-(nicotinamoyl)-1,3,4-thiadiazole), sumatriptan (5-HT<sub>1B</sub>/1D agonist), and rizatriptan (5-HT<sub>1B</sub>/1D agonist) had strong AQP4 inhibition with IC<sub>50</sub> values of 3, 11, and 2  $\mu$ M, respectively [47]. And docking model showed TGN-020 directly blocked the pore of water transport.

The effects of TGN-020 on regional cerebral blood flow (rCBF) were examined in wild-type (WT) and AQP-4 knockout (KO) mice in vivo [49]. And TGN-020 increased regional cerebral blood flow but showed no effect on KO mice, suggesting that the TGN-020 worked on AQP4. In the diabetic retina model, TGN-020 suppressed the expression of AQP4 and GFAP [50]. And in another unilateral middle cerebral artery occlusion (MCAO) model, TGN-020 also showed downregulating effect on AQP4 in the SON [51]. So it is more exactly to define TGN-020 as an AQP4 modulator rather than an AQP4 inhibitor.

### 22.3.3.3 Antiepileptic Drugs

Cause of their pan-CA isozyme inhibitions and similarity in physiochemical properties with AZA and EZA, 14 antiepileptic drugs (AEDs), such as topiramate (TPM) and zonisamide (ZNS) were tested using virtual docking experiments in silico, and nine of them were investigated functionally in vitro in *Xenopus* oocyte expressing system [52]. Seven of the candidates were found to inhibit AQP4 function, then four compounds including topiramate (TPM), zonisamide (ZNS), phenytoin (PHT), and lamotrigine (LTG) (Fig. 22.7) were then selected for a dose-dependent study. The IC<sub>50</sub> values were 10, 3.3, 9.8, and 8.1  $\mu$ M, respectively. And the correlation studies suggested that AEDS with a docking energy >50 kcal/mol might have inhibitory effect on AQP4. However, due to the use of a nonstandard algorithm and no computational details (such as search space and energy minimization criteria), it is difficult to assess the merit of the reported binding computations.

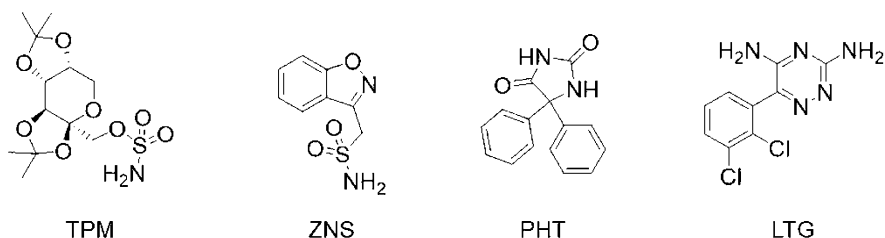
Despite its association with elevated seizure threshold following chemical convulsants, it is predicted that AQP4 deficiency could reduce sound- and light-evoked potentials and increased threshold and prolonged duration of induced

seizures [53]. In short, AQP4 inhibition would likely worsen rather than prevent seizures. With this doubt, Yang et al. [54] retested reported AEDs with AQP4 inhibition in FRT cell plasma membrane vesicles measured by stopped-flow light scattering, in AQP4-expressing FRT cell monolayers and in brain glial cells, none of these showed inhibitory effect on AQP4-mediated water permeability. None of them have AQP1 inhibitions, too.

### 22.3.3.4 Other Compounds

A total of 3575 compounds including 418 FDA-approved drugs were screened by calcein-loaded cells using an automated fluorescence microplate reader-based assay [55]. Four molecules of National Cancer Institute's chemical library (NSC164914, NSC670229, NSC168597, NSC301460, Fig. 22.8) were identified that affected both AQP4- and AQP1-mediated water permeability with IC<sub>50</sub> values varying from 20 to 50  $\mu$ M. Nevertheless, in another report [24], these 4 compounds showed no AQP1 or AQP4 inhibition by stopped-flow scattering analysis. Interestingly, these two literatures came to different conclusions although they adopted the same assessment method by stopped-flow light-scattering measurement in erythrocytes from adult Wistar rats. Artificial or objective factors might be affected, but some points could be confirmed that NSC 168597 and NSC 164914 as organolead and organotin molecule, respectively, were reported to be neurotoxins [56] and would cause erythrocyte lysis [57]. As to NSC 301460 which belongs to aminolipopeptide antibiotics [58] is isolated from a marine sponge-derived fungus *Trichoderma* sp., whose mechanism is to damage bacterial cell membranes by forming pores [24, 59]. Large volume of NSC 301460 chemical structure makes it impossible to block the water pore in AQPs directly. Another report [60] identified two more compounds from NSC derived from NSC 670229 as novel hAQP1 inhibitors by yeast freeze-thaw assay and stopped-flow water permeability assay. However, yeast freeze-thaw assay could not exclude high toxicity compounds affecting yeast viability and stopped-flow spectrometer. And in this chapter



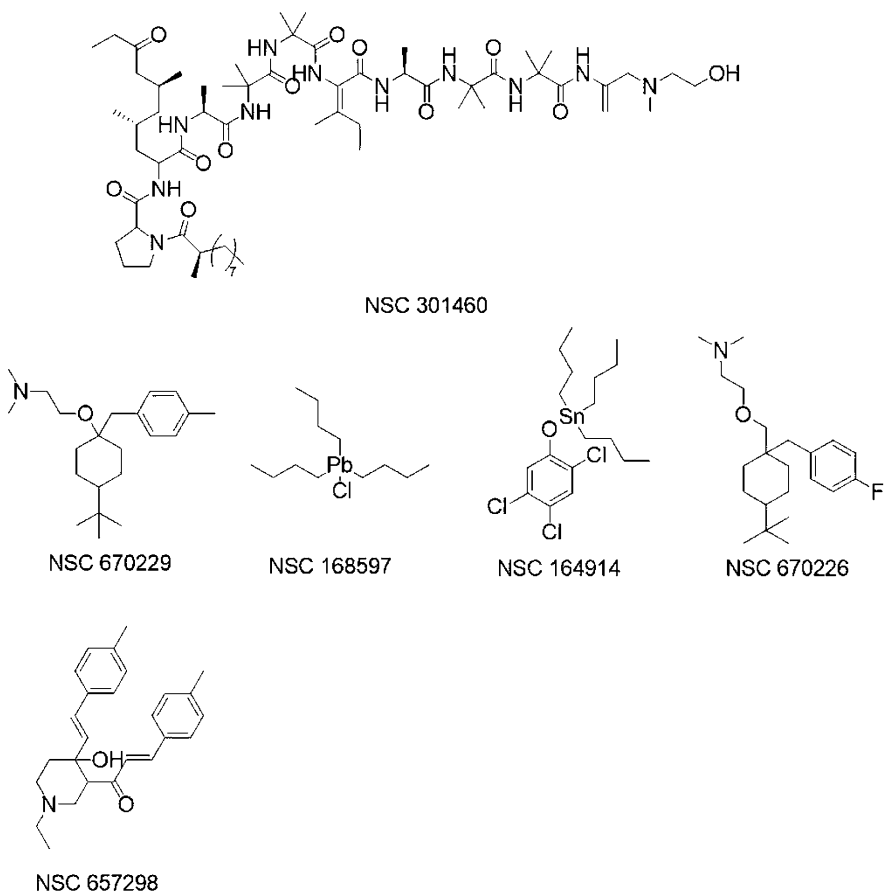


**Fig. 22.7** Structures of four compounds of AEDs

somehow, interpretation of possible inhibitory effects was confounded by the multiexponential kinetics of the light-scattering data [24].

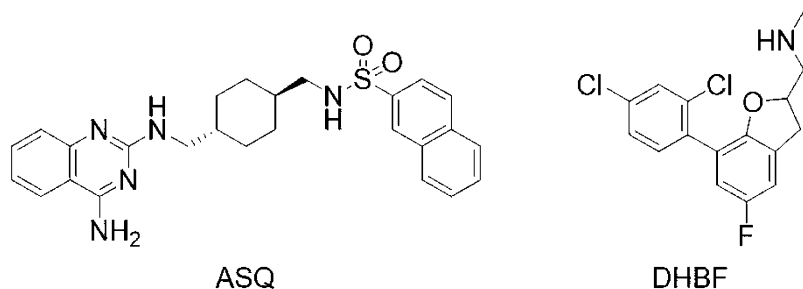
Novartis Co. also participated in drug discovery of AQP1 inhibitors on account for the function of AQP1 by mediating water permeability into the lens. Approximately 6000 drug-like molecules from AICON's collection were

selected for screen using a high-throughput assay based on fluorescence quenching assay in CHO cells overexpressed AQP1 [61]. Two classes of compounds belonging to aromatic sulfonamide (ASQ) and dihydrobenzofuran (DHBH) (Fig. 22.9) showed IC<sub>50</sub> values of hAQP1 in the range of 3–30 μM in primary screening assay. In addition, two lead compounds have AQP1

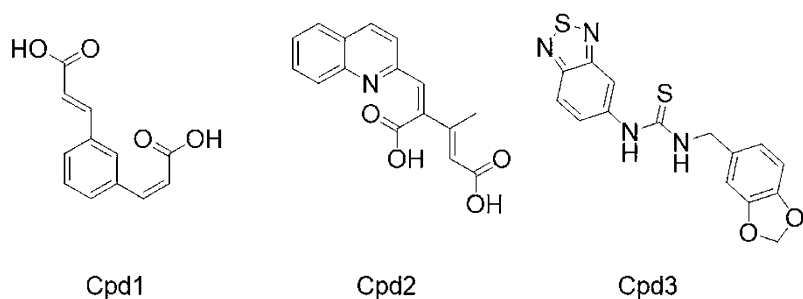


**Fig. 22.8** Structures of NSC compounds

**Fig. 22.9** Structures of ASQ and DHBH



**Fig. 22.10** Structures of Cpd1, Cpd2, and Cpd3 identified by molecular docking

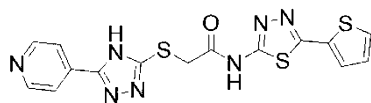


inhibition in *Xenopus* oocyte-swelling assay and stopped-flow assay [61]. Nevertheless, in another research [24], lead compounds showed variable activities in *Xenopus* oocyte, erythrocyte ghost, and AQP1 proteoliposome assays due to the erythrocyte crenation and aggregation which is the main reason to induce the potential artifacts in light-scattering assays.

Molecular docking is the efficient method for discovering compounds which has interactions with the target protein. Scientists have tried to apply this method into primary screening for AQP inhibitors followed by functional assays. AEDs were discovered as AQP4 inhibitors by the computational method [52], even though they showed less AQP inhibition in a follow-up research [23]. But we could not deny the computational method and efforts have been made to optimize the algorithm to improve the precision. The novel compounds (Fig. 22.10) were identified by molecular docking against the hAQP1 and experimentally tested the activity on AQP1 inhibition in a *Xenopus* oocyte swelling assay [62]. Subsequent molecular dynamics simulations suggested a new binding mode that strongly involves the ar/R selectivity filter and

Lys36, a residue that is not conserved among the hAQP family. Although none of the molecules showed an inhibitory effect in a red blood cell assay, the inhibition of oocyte swelling of these compounds could be abolished by mutating Lys36 to alanine. It suggested that the observed reduction of water flux is hAQP1-dependent and not triggered by an indirect effect, but there is an obvious discrepancy between results obtained from *Xenopus* oocyte and erythrocyte.

Besides the continuous efforts made in discovering AQP1 inhibitor and AQP4 inhibitors, it is also worthy to mention that a compound, namely HTS13286 from a 1920 small molecules library stands out by its stronger selective inhibition of AQP9 with an  $IC_{50}$  value of 0.15  $\mu$ M by a CHO mAQP9 cells shrinking assay [27]. And HTS13286 also affected mAQP9 solute permeability, including glycerol and urea, which has the same effect with AQP9 gene deletion on glycerol gluconeogenesis in perfused mouse livers. In a word, a glycerol-specific increase in glucose output in wild-type livers was suppressed by HTS13286 and absent in AQP9<sup>-/-</sup> livers. However, due to its low solubility, it is not suitable for



HTS13286

**Fig. 22.11** Structure of HTS13286

experiment *in vivo*, yet. For 10 years, it has been still no new developments for AQP9 inhibitors and no further progress for this series of HTS 13286 (Fig. 22.11), which remind us there is a long way and more exactly a tough way to go.

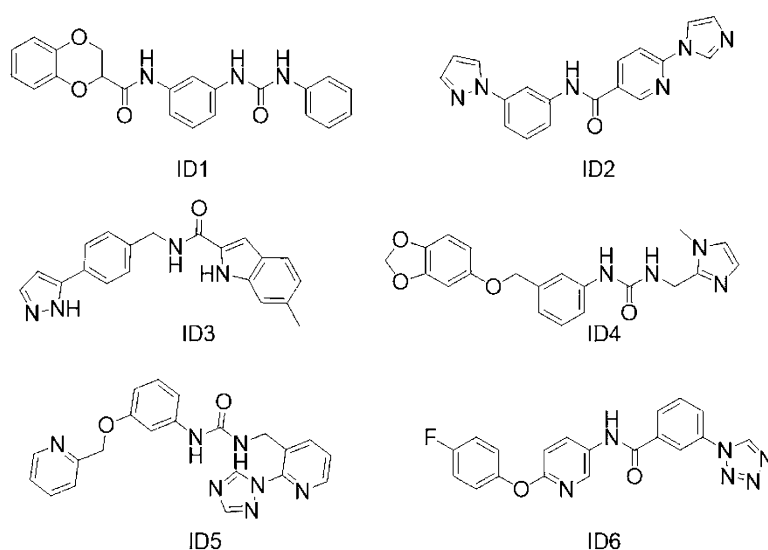
An optimization was applied in molecular docking for screening both channel-binding compounds and channel-blocking compounds [63]. Thirty active compounds with the 105 compounds that were top-ranked by virtual screen were identified by CHO-hAQP9 cell shrinking assay. Nine of the 30 compounds produced an  $IC_{50}$  values of less than 50  $\mu M$  (the structures of best six compounds were presented in Fig. 22.12). It is worth noting that they found hAQP9 F180V mutant cells presented reduced water permeability. We hope drug discovery in AQP inhibitors could be benefitted from advanced technology *in silico*.

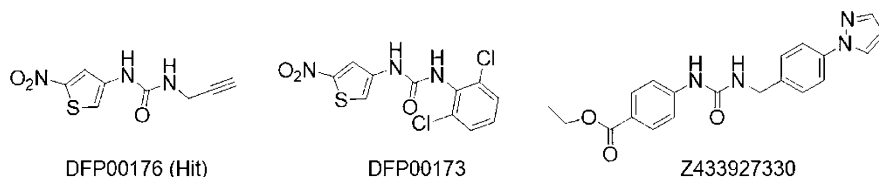
AQP3 as an aquaglyceroporin is known to conduct water, glycerol, as well as  $H_2O_2$ . Its inhibitors have potential for treating disorders of

water retention. DFP00176 was defined as hit compound by CHO cells expressing mAQP3 shrinking assays from a library of 7360 drug-like small molecules [28]. Then SAR study was operated among 12 commercially available structurally compounds, DFP00173 that possesses a urea linker, 2,6-dichlorophenyl in the right-hand site, and Z433927330 that possesses a methylurea linker were selected for specificity test for AQPs (Fig. 22.13). It was found that DFP00173 has inhibition against mouse and human AQP3 with  $IC_{50}$  of 0.1–0.4  $\mu M$ , but low efficacy toward mouse AQP7 and AQP9 while Z433927330, a partial AQP3 inhibitor ( $IC_{50}$ , 0.7–0.9  $\mu M$ ), also has potent and efficacious inhibition against mouse AQP7 water permeability ( $IC_{50}$ , 0.2  $\mu M$ ) [28]. These two compounds could be tools for investigating the functions of aquaglyceroporins, and we are looking forward to the further research *in vivo*.

## 22.4 Summary and Prospect

From the point of the view, it is still no effective AQP inhibitors except for metal-related compounds whose toxicity could not be denied. AQPs play important roles in various physiological activities, including proliferation and

**Fig. 22.12** Structures of the best six compounds screened by CHO-hAQP9 cell assay



**Fig. 22.13** Structures of DFP00173, DFP00176, and Z433927330

migration in tumor disease, brain edema, and other water-retention disorders, which makes them important drug targets. With the rapid development of artificial intelligence and computer-aided drug discovery, new approach might be applied, and we believe novel AQP inhibitors could be expected. In addition, further study on DFP00173 is worth waiting for.

**Acknowledgments** The work was supported by the National Natural Science Foundation of China grants 81974083, 82273999, 81620108029, 81330074, the Beijing Natural Science Foundation grant 7212151, the grant from the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences 2022-JKCS-15, and FWNR-2022-0019; RFBR-20-015-00147a.

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