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REVIEW

Gold compounds as aquaporin inhibitors: new opportunities for therapy and imaging

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In recent years, gold-based compounds have been proved to hold promise in chemical biology being able to selectively inhibit proteins activities in cells and, therefore, to be exploited as either therapeutic agents or as chemical tools to detect protein/enzyme functions in living systems. In this area, we have recently described the properties of gold coordination complexes as potent and selective inhibitors of human aquaporins, protein channels involved in the transport of water and glycerol across biomembranes. Thus, we provide here an overview of the importance of aquaporins in various diseases and a detailed description of the state-of-the art progresses in the discovery of new inhibitors, including gold(III) complexes, and a description of their possible applications. Overall, the potential of coordination chemistry in providing compounds to modulate the activity of "elusive" drug targets, such as the aquaporins, will be discussed.

1. Introduction

The serendipitous discovery of the anticancer drug cisplatin (*cis*-diamminedichloroplatinum(II)) in the 1960s established medicinal inorganic chemistry as an independent and important area of drug discovery and stimulated the development of new metal complexes and inorganic compounds for the treatment of various diseases. Nowadays, the list of therapeutically prescribed metal-containing compounds includes platinum (anticancer), silver (antimicrobial), gold (antiarthritic), bismuth (antiulcer), antimony (antiprotozoal), vanadium (antidiabetic) and iron (anticancer and antimalarial) complexes [1-4]. Moreover, metal compounds as diagnostic tools have also been widely explored and are successfully applied in the clinical set for imaging of diseases [5-7]. For example, lanthanides occupy a relevant place as diagnostic agents, but also have many other medically important applications, as hypophosphatemic agents for kidney dialysis patients, for bone pain palliation and as luminescent probes in cell studies [8].

The mechanisms of biological action of metal compounds for therapy and diagnosis have been widely investigated, although, in several cases still not fully elucidated. In recent years, the general consensus on the crucial role of the interactions of metallodrugs with proteins in determining the compounds' pharmacological action, uptake and biodistribution, as well as their overall toxicity profile, resulted in an exponential increase in the number of studies.[9]

Our research in the field of anticancer metallodrugs development have focussed on the design of gold-based coordination and organometallic compounds and on the identification and validation of protein targets for some of the most promising drug candidates in our libraries.[10] Among the likely targets of gold compounds, we have recently identified the membrane protein channels aquaporins (AQPs). Notably, there is compelling evidence that these proteins are drug targets in different diseases. Thus, in this review we will provide an overview of the possible roles of aquaporins in pathological conditions and will present an update on the "state-of-the-art" in the development of modulators of AQP functions. Moreover, we will summarize the recently obtained results on gold coordination compounds as potent and selective inhibitors of these protein channels via different biophysical and computational methods.

2. Aquaporins

Aquaporins represent a family of transmembrane water channel proteins widely distributed in various tissues throughout the

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body that play a major role in transcellular and transepithelial water movement[11, 12]. Among the 13 mammalian AQPs described so far, two sub-groups are now recognized mainly determined by their transport capabilities: orthodox or classical aquaporins (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8), which are primarily water selective and facilitate water movement across cell membranes in response to osmotic gradients, and aquaglyceroporins (AQP3, AQP7, AQP9 and AQP10) which facilitate the transport of small uncharged solutes such as glycerol and urea in addition to water.[13] Although AQP6 has been proved to be a pH-sensitive chloride channel or possibly a nitrate channel and AQP8 has been found permeable to urea, they are both classified under the classical AQP group. A third sub-group named S-aquaporins (AQP11 and AQP12)[14] was defined based on their primarily subcellular location and lower sequence similarity to the other mammalian AQPs; although their permeability specificity has been difficult to establish, AQP11 was recently reported to transport both water and glycerol.[15] There is also controversial evidence that some AQPs may transport gases and ions across membranes[16]. The mammalian AQPs are expressed in various epithelia and endothelia involved in fluid transport, such as kidney tubules and glandular epithelia, as well as in other cell types such as brain glial cells, epidermis and adipocytes. A functional AQP consist of four monomers super-assembled in membranes as tetramers. There is a considerable body of information about AQP structure from electron and X-ray crystallography[12], showing AQP monomers (~30kDa) containing six membrane-spanning helical domains surrounding a narrow aqueous pore (Fig. 1).

The most remarkable feature of the aquaporin channels is their high selectivity and efficiency on water or glycerol permeation; in fact, AQPs allow water/glycerol to move freely and bidirectionally across the cell membrane, but exclude all ions such as hydroxide and hydronium ions, as well as protons[17], the latter being essential to preserve the electrochemical potential across the membrane. Although classical aquaporins are still considered mostly specific for water, AQP1-mediated permeation by small polar solutes was recently proposed, while an inverse correlation between permeability and solute hydrophobicity was found [18]. In the pore region, the water specificity is achieved by the presence of particular amino acid residues conferring size constrictions and/or charge characteristics that enable water molecules to pass through, while preventing permeation to protons or any solutes above 2.8 Å.

AQPs share a common protein fold, with the typical six membrane-spanning helices surrounding the 20-Å-long and 3-4-Å-wide amphipathic channel (Fig. 1). Two constriction sites with distinguishing features that identify the protein subfamilies compose this channel. A first selectivity filter (SF), so called aromatic/Arginine SF (Ar/R SF), is a constricted region formed by three (in aquaglyceroporins) or four (in orthodox aquaporins) residues near the periplasmic/extracellular entrance, that determines the size of molecules allowed to pass through and provides distinguishing features that identify the subfamilies [19]. The second selectivity filter, generating an electrostatic barrier essential for proton exclusion, is composed by two conserved asparagine-proline-alanine (NPA) sequence motifs, located at the N-terminal ends of the two half-helices, at the centre of the channel (Fig. 1).

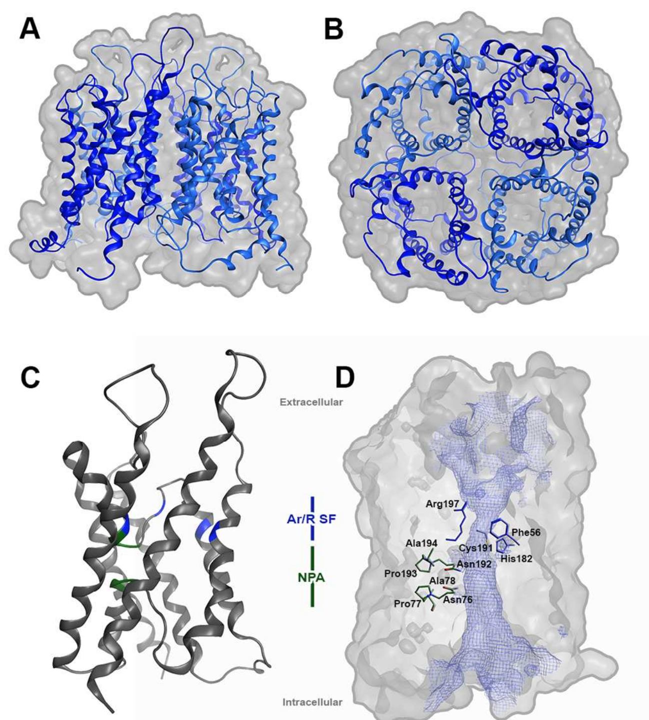

Figure 1 – Structure of AQP1 water channel (pdb 1H6I). Top panels represent the functional membrane-embedded tetramer, both in ribbon and surface representation, in lateral (A) and extracellular top (B) views. Bottom panel displays the monomeric form of the channel, displayed in ribbon (C) and surface (D), with the channel surface represented as blue mesh. The main protein regions are identified: Ar/R SF filter residues and identified cystein are coloured in blue (sulphur atom in yellow), NPA residues in green. The figures were generated with MOE software.

3. Aquaporins in disease

Much of our understanding of AQP functions in mammalian physiology has come from relatively recent phenotype analysis of mice lacking one of the AQPs[20]. These studies have confirmed the anticipated involvement of AQPs in the mechanism of urine concentration and glandular fluid secretion, and led to the discovery of unanticipated roles of AQPs in brain water balance, cell migration (angiogenesis, wound healing), cell proliferation, neural function (sensory signaling, seizures), epidermal hydration and ocular function. Specifically, the aquaglyceroporins, regulate glycerol content in epidermal, fat and other tissues, and are involved in skin hydration, cell proliferation, carcinogenesis and fat metabolism. In cancer, AQPs have proved to be highly expressed in different tumor

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types, where they are involved in tumor invasion, metastasis and growth[21]. Below we will summarize the main features of the human aquaglyceroporins characterized so far and the evidences of their possible roles in human diseases.

Aquaglyceroporins

The subclass of water channels termed aquaglyceroporins includes isoforms (AQP3, AQP7, AQP9 and AQP10), which are also able to transport glycerol and possibly urea as well as other small non-charged solutes. Their physiological roles have been more difficult to ascertain, in part because the understanding of the role of glycerol in mammals is incomplete. There is now compelling evidence for the involvement of aquaglyceroporin-mediated glycerol transport in cell proliferation, adipocyte metabolism and epidermal water retention, and their dysfunction or aberrant expression has been correlated with several pathophysiological conditions.

AQP3 is expressed in a wide variety of organs such as the kidney and urinary tract, digestive tract, respiratory tract, skin, and eye.[22] Phenotype analysis of AQP3-null mice revealed an important role of AQP3 in kidney and in epidermal physiology, leading to defects in urine-concentrating function, [23] reduced skin hydration and elasticity and impaired skin wound healing.[22] Interestingly, AQP3 is highly expressed in skin tumors and AQP3 deficiency or blockage inhibits cell proliferation and reduces tumor growth in mice, which may result from impaired glycerol uptake by tumor cells.[24] AQP3 was also found to have high expression levels in tumors from different origin, such esophageal and oral squamous cell carcinoma,[25] ovarian [26] and cervical[27] cancer. Liu et al investigated AQP3 expression in normal and neoplastic lung tissues and found AQP3 expression was related to tumor differentiation and clinical stage in lung adenocarcinomas.[28] pointing to a novel function for AQP3 expression in high-grade tumors. The involvement of AQP3 expression and functional activity in cell proliferation was further demonstrated using a human epidermoid carcinoma cell line[29], suggesting that the modulation of AQP3 expression or function could be explored for cancer therapeutics.

AQP7 is widely expressed in testis,[30] kidney, pancreas and adipose tissue,[31] where its role in glycerol release/uptake in adipocytes has been correlated with obesity onset.[32] In adipocytes, triglycerides are hydrolyzed to glycerol and free fatty acids, which are released into the circulation. Thus, glycerol is taken up by the liver for hepatic glucose synthesis. While for sometime controversy on the precise cellular localization of AQP7 existed,[31] its simultaneous expression both in adipocytes and in the capillary endothelia of mice adipose tissue was detected and its role as a water and glycerol channel was correlated with adipocyte lipid accumulation.[33] AQP7 has also been investigated in human subjects and studies revealed a correlation of AQP7 down-regulation with obesity[34] or with reduced plasma glycerol during exercise.[35] Additional investigations in mice and human-AQP7 are needed to better define AQP7 role on obesity and diabetes.

AQP9 is expressed in various organs, including testis, brain, leukocytes, epididymis, spleen and liver [36] where it was proposed to play a role in glycerol uptake for gluconeogenesis during fasting.[37, 38] In hepatocytes it is expressed in the sinusoidal (basolateral) plasma membrane. Because AQP9 is also highly permeable to glycerol and urea, it may provide an entry route for glycerol and an exit route for urea and a number of other solutes produced within hepatocytes.[39] Indeed, *in vivo* studies have demonstrated that in rats fasted for 96 hours, expression of AQP9 in liver increases 20-fold, suggesting that during starvation, the liver takes up glycerol for gluconeogenesis and does it with the involvement of AQP9.[38] In addition, studies in AQP9-null mice suggested an impairment of liver glycerol uptake, which may result in an improvement of the diabetic state,[31, 40] but further studies are necessary to determine the exact contribution of AQP9 in metabolic disorders such as diabetes and obesity.

Human AQP10 is found mainly in the gastrointestinal tract, but was also detected in teeth, muscle and gingiva [31]. Recently, *in vivo* studies on healthy volunteers have demonstrated the presence of AQP10 (together with AQP3) in the stratum corneum.[41] Interestingly, AQP10 was reported to be a pseudogene in mice[42], rendering impossible the generation of AQP10-null mice for the identification of disease phenotypes. Thus, the functional significance of this aquaglyceroporin is still obscure.

In addition to mammalian pathophysiology, aquaglyceroporins seem to be relevant in non-mammalian cell survival. This is the case of AQP3 and AQP9 expressed in human and mice red blood cells respectively, facilitating glycerol delivery to the cells infected with the malaria parasite *Plasmodium falciparum*. Since glycerol can be used by the malaria parasite for lipid biogenesis and as an energy source, inhibition of AQP3 and AQP9 may be exploited in the search for antimalarial agents.[43] In addition, *P. falciparum* has its own aquaglyceroporin (PfAQP) whose X-ray structure has been reported and which contains NLA and NPS residue patterns instead of the NPA motifs of other AQPs.[44]

Interestingly, a number of aquaglyceroporins were identified in parasitic protozoa (*Plasmodium*, *Trypanosoma brucei*, *Leishmania*, *Toxoplasma* and *Cryptosporifium*) and are known to permeate water, glycerol, urea and several other polyols.[45] Parasitic AQPs are important for glycerol recruitment during the organism's reproductive blood stage and, thus, modulation of those AQPs involved in parasite life-cycle regulation or reproduction may represent a strategy for novel therapeutics. Moreover, parasitic AQPs appear to be the major entry routes of uptake of cytotoxic compounds (hydroxyurea, dihydroxyacetone, and the hydroxide of trivalent metalloids arsenic and antimony) able to kill protozoan parasites, and AQP deletion renders the parasites more resistant to treatments.[46] Thus, the use of parasitic AQPs as a vehicle for toxic substances may also be a further pathway for research. Interestingly, since aquaporins are able to transport solutes bilaterally across the cellular membranes they can not only import glycerol for metabolism, but, due to their ability to

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transport other solutes, may also be important to export small-molecule toxins, important for pathogenicity.[45]

4. AQP inhibitors

The identification of AQP modulators has turned out to be unexpectedly challenging. Four classes of AQP-targeted small molecules have been described so far: i) cysteine-reactive heavy metal inhibitors; ii) small-molecules that are reported to inhibit water conductance; iii) small-molecules targeting the interaction between AQP4 and the neuromyelitis optica (NMO) autoantibody; and iv) agents that act as chemical chaperones to facilitate the cellular processing of nephrogenic diabetes insipidus (NDI)-causing AQP2 mutants.[47]

Following this categorization, the chemical compounds that modulate (inhibiting) AQP mediated water flux include heavy metals (e.g. $HgCl_2$, silver sulfadiazine) [48-51], and inorganic salts (e.g. $ZnCl_2$, $NiCl_2$), [52] quaternary ammonium salts [53-55], as well as sulfonamides, [56] all acting as inhibitors of orthodox water channels. Unfortunately, all these compounds are not suitable for therapeutic applications or to study AQP function in biological systems mostly due to their toxic side effects and lack of selectivity. In 2012, Beitz and de Groot et al reported on organic inhibitors of hAQP1 identified by virtual screening tested in a *Xenopus* oocyte swelling assay,[57] but up to now no validation of their selectivity has been reported.

Concerning aquaglyceroporin modulators, only a few small molecule inhibitors of AQP9 water permeability were identified (Fig. 2) although it must be noted that effects on glycerol transport has not been evaluated for most of these compounds made exception for HTS13286 [37, 58]. Moreover, as the compounds' solubility in aqueous solution is very limited, they are currently not suitable for *in vivo* experiments. Overall, independent verification of AQP inhibition by these compounds is still awaited.

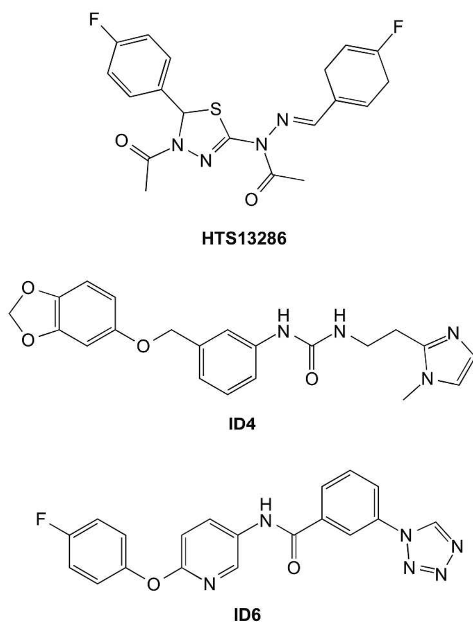

Figure 2 – Small-molecule inhibitors of aquaglyceroporin-9 isoform.

A few examples are present in the literature concerning AQP-based diagnostics. These include the use of AQP antibodies, and there is one prominent example of an AQP antibody-based diagnostic test where AQP4 has been implicated as a marker of the central inflammatory demyelinating disease NMO, or Devic's disease[59]. Alternatively, one established example is the assay of AQP2 immunoreactive protein in urine to distinguish among various etiologies of nephrogenic diabetes insipidus (NDI) [60]. However, no small-molecule diagnostic agents for AQP functions have been described so far.

Inhibition of human AQP3 by coordination gold(III) complexes

The limited number of AQP modulators available in the literature, prompted us to explore the properties of coordination metal complexes as possible inhibitors. Thus, we reported on the potent and selective inhibition of AQP3 by a water-soluble gold(III) coordination compound, $[Au(phen)Cl_2]Cl$ (phen = 1,10-phenatroline, Auphen) (Fig. 3). Notably, Auphen inhibited glycerol transport in human red blood cells (hRBC), with an IC_{50} = 0.8 ± 0.08 μM, while having only a modest inhibitory effect on water permeability[61]. hRBC are know to express large amount of AQP1 and AQP3 accountable for membrane permeability to water and glycerol, respectively[62]. The selectivity of the compound towards AQP3 was confirmed in transfected PC12 cell lines with overexpression of either AQP1 or AQP3.

Inspired by these initial promising results, we investigated other gold-based compounds as possible AQP3 inhibitors in order to achieve basic structure-activity relationships, fundamental for drug design. Thus, we selected a series of square planar gold(III) complexes containing functionalised bipyridine or terpyridine ligands of general formula $[Au(N^{\wedge}N)Cl_x][PF_6]$ [where N^N = 2,2'-bipyridine, 4,4'-dimethyl-2,2'-bipyridine, and 4,4'-diamino-2,2'-bipyridine] (Fig. 3).[63] Moreover, the 1,10-phenantroline derivatives of Pt(II) and Cu(II) were also included in our investigation to compare the effects of metal substitution on the AQP3 inhibition potency. The effects of the compounds on both water and glycerol permeation were also tested on hRBC.

Notably, the gold(III) complexes were the most effective inhibitors of glycerol permeability via AQP3, with an IC_{50} in the low μM range, and comparable in potency to Auphen. Moreover, AQP3 inhibition resulted to be practically irreversible and only excess of the reducing agent 2-mercaptoethanol (EtSH) allowed restoring of glycerol transport.

Within the metal-phenantroline series, the AQP3 inhibition potency decreased drastically in the order Auphen > Cuphen >> Ptphen. Interestingly, no inhibition effect was achieved when incubating the cells with Au(I) compounds, therefore, demonstrating the necessity of Au(III)-based scaffolds to achieve protein binding and blockage of the channel. At this point, our mechanistic hypothesis was based on the possibility

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for Auphen and analogues to bind to certain amino acid residues in the channel close to the SF domains, thus acting as a "cork" preventing the passage of glycerol.

To further investigate the mechanisms of AQP inhibition by gold compounds, we undertook molecular modelling studies. Initially, a homology model of human AQP3 was built and compared to the structure of human AQP1, allowing the identification and characterization of protein binding pockets[64].

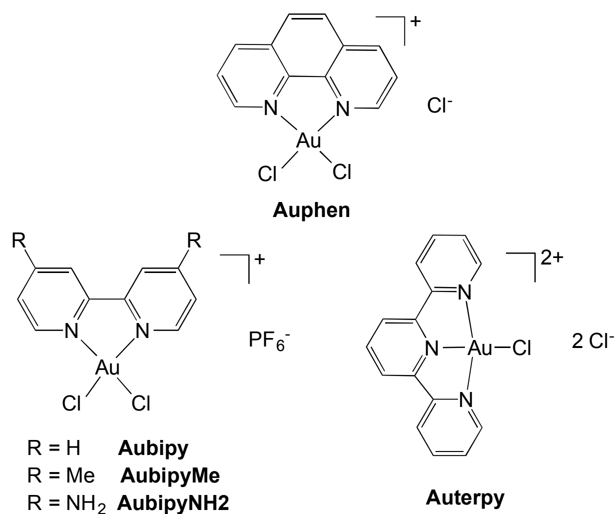

Figure 3 – Gold(III) coordination compounds as inhibitors of aquaglyceroporins.

Subsequently, the study of non-covalent binding of Auphen to AQP1 and AQP3 by docking approaches allowed to ascertain the possibility for the gold complex to reach the SF domain of AQP3 more efficiently than in the case of AQP1, allowing the compounds to bind at protein sites closer to the constriction pore of the AQP3. Indeed, it is known that the AQP1 channel cross-section size is the major determinant of selectivity for larger amphipathic molecules such as glycerol [19] and these same steric restrictions might also apply to gold(III) complexes.

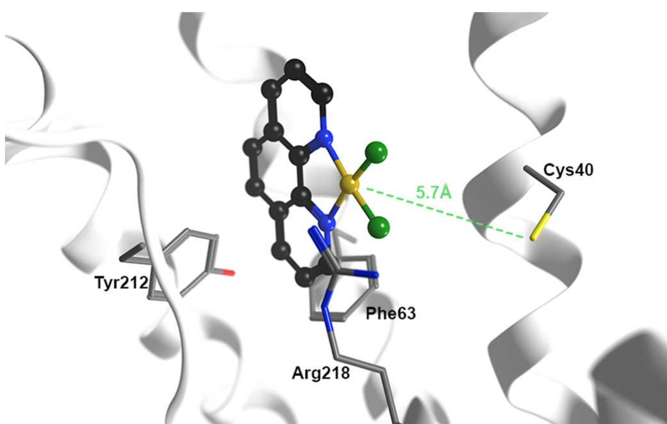

Figure 4 – Molecular docking of the gold(III) complex Auphen (in black ball-and-stick representation) in the homology model of hAQP3 (grey cartoon representation) generated with MOE. Side chains of the residues composing the Ar/R SF, as well as the proposed binding site, Cys40, are represented in sticks. The gold atom is represented in gold yellow while the chlorides are represented in green. Other atoms are represented in their element colour.

The mapping of the extracellular surface allowed establishing the possible gold binding sites and their exposure to inhibitors binding in both aquaporin isoforms. It must be noted that Au(III) ions are prone to interact with sulphur-donor groups of proteins such as the thiolate of cysteine or the thioether of methionine residues. In AQP1 none of the cysteine, methionine or histidine residues present in the protein sequence appear to be accessible for gold binding, whereas in AQP3 the thiol group of Cys40 is projected towards the extracellular space approaching the channel pore. Therefore, we proposed this residue as a likely candidate for binding to gold(III) complexes via a direct Au-thiol bond (Fig. 4). These results were confirmed by site directed mutagenesis studies, in which Cys40 was replaced by a serine residue (Ser40).[29]

Inhibition of human AQP7 by gold complexes

Following our promising results on AQP3 inhibition by Auphen, we tested the compound as a possible inhibitor of another aquaglyceroporin isoform, namely AQP7 expressed in adipocytes. For this purpose, both water and glycerol permeability were assessed in murine adipocytes, containing the murine isoform (mAQP7) by fluorescence microscopy. Moreover, to study the effect of the compound in the human protein isoform (hAQP7), the murine adipocytes were further engineered to overexpress hAQP7.[33]
Treatment with 15 μM Auphen induced a significant effect in decreasing both water and glycerol permeability through AQP7 (both murine and human). Specifically, water permeability was decreased of 37% and 63%, and glycerol transport was reduced by 74% and 79% for control and hAQP7-overexpessing adipocytes, respectively.[65]

In order to investigate the mechanism of inhibition of Auphen, and since no human glycerol channel has a resolved structure yet, we built a homology model of hAQP7 using the crystal structure of *E. coli*'s glycerol facilitator (GlpF) as a reference structure.[66] In the model we were able to identify the main distinctive features of aquaporins, namely the six transmembrane helices and the conserved regions Ar/R SF and NPA motif (Fig. 5).

According to our proposed mechanism of action of Auphen for hAQP3 inhibition, Cys40 is the crucial residue for binding.[64] Taking again into account coordination chemistry rules according to which gold ions have high affinity for "soft" nucleophiles such as sulphur atoms of cysteine and methionine residues, we looked for residues of this type inside the hAQP7 channel. Interestingly, the composition of hAQP7 and hAQP3 in terms of sulphur-donors is very different. In fact, while in hAQP3 Cys40 is located near the Ar/R SF, hAQP7 has no cysteines inside the channel. Instead, in hAQP7 three

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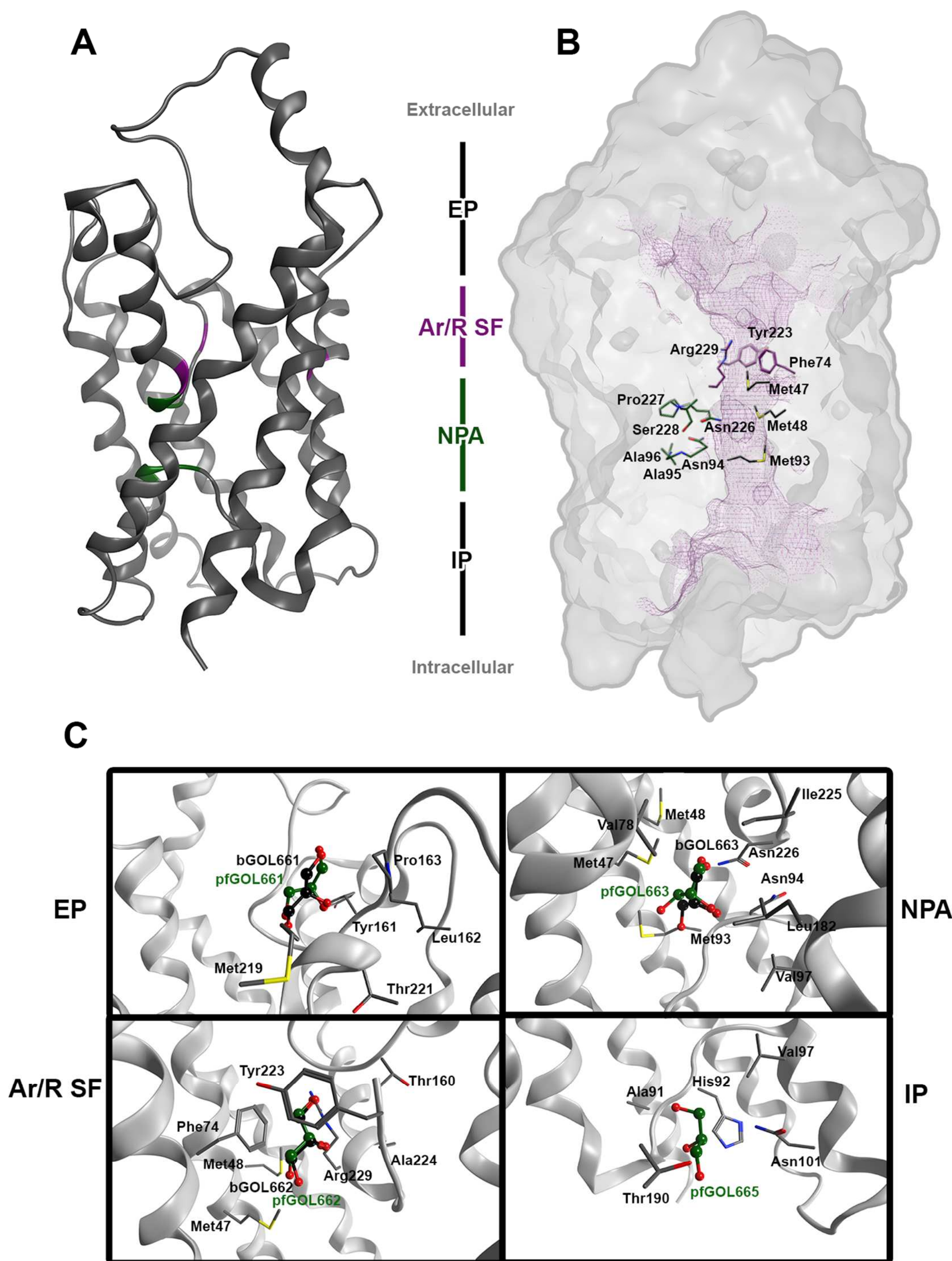

Figure 5 – Homology model of hAQP7 in cartoon (A) and surface (B) representation generated with MOE software. The main protein regions are identified as extracellular pocket (EP), aromatic/arginine selectivity filter (Ar/R SF), NPA motif and intracellular pocket (IP). Ar/R SF residues are coloured in purple, NPA residues in green and the identified methionines in black, with the sulphur atoms in yellow. In the right panel (B), the channel surface is shown in purple mesh. (C) Representation of glycerol molecules from bGlpF (pdb 1FX8, represented in black) and pfAQP (pdb 3C02, represented in green) superposed inside of the channel of hAQP7 homology model generated with MOE. The glycerol (GOL) molecules are labelled according to the provenience (b: bacterial; pf: *P. falciparum*) and to the original numbering found in the respective pdf files. The four glycerol pockets are identified as extracellular pocket (EP), Ar/R SF, NPA region and intracellular pocket (IP).

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methionine residues (Met47, Met48 and Met93) are present between the two conserved Ar/R SF and NPA regions.

As a second step, in order to investigate the importance of such residues for glycerol transport and to discriminate among them to select the likely one(s) for gold binding and efficient hAQP7 inhibition, we studied the interactions of glycerol molecules in our model. Therefore, we used both hAQP7 and hAQP3 homology models superposed with the structures of bGlp[67] and the *P. falciparum* isoform (pfAQP),[44] the latter structures containing glycerol molecules inside the channel. This approach is very useful to identify glycerol binding pockets, as well as side-chains that could be crucial for its transport.

Thus, we were able to identify 4 glycerol binding-pockets: two of them were within the conserved regions Ar/R SF and NPA, while other two were located in the intra- and extracellular regions, respectively (Fig. 5). Moreover, we were also able to highlight differences between hAQP7 and hAQP3, regarding channel composition: in spite of the very similar structure and general scaffold, the two isoforms have different composition concerning the type of residues lining the channel, as well as different channel shape and size. Such distinctive features can certainly account for differences in selectivity towards various small-molecule inhibitors and be the key determinant in the “smart” design of selective drugs, targeting different aquaglyceroporins.

Concerning hAQP7, looking at the interactions between the various glycerol molecules and residues in the channel, we were able to predict interactions between the side-chains of Met47 and Met93, suggesting that binding of Auphen at these sites may efficiently disrupt glycerol transport (Figure 5).

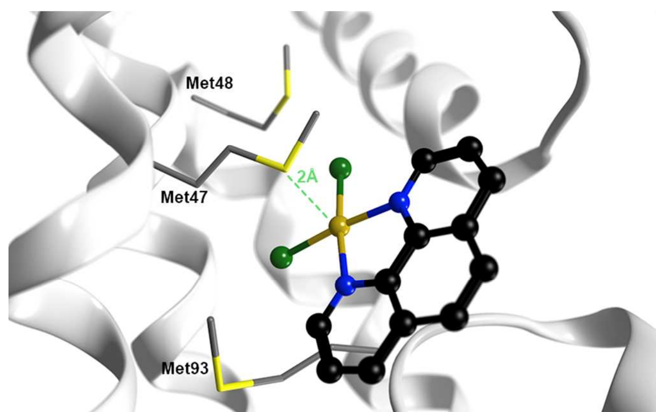

Figure 6 – Non-covalent molecular docking of Auphen (black ball and stick representation) inside of the NPA pocket of hAQP7 (grey cartoon representation). Side-chains of Met47, Met48 and Met93 are presented in grey sticks. Sulphur atoms are shown in bright yellow, chlorides in green, nitrogen atoms in blue and gold atom in gold yellow.

Furthermore, to be able to assess possible binding of the gold(III) complex to the sulphur of one of the two methionines we used a molecular docking approach. Unfortunately, it is not possible to simulate a covalent bond to a metal using molecular docking tools, as this type of metal ion is not parameterized. Instead, we limited our study to the investigation of non-covalent binding poses of Auphen in the intracellular region, where the two Met47 and Met93 are accessible for gold binding. Thus, we could conclude that it is possible for Auphen to reach the NPA pocket and bind to the sulphur of Met47, while stabilizing interactions between the phenantroline ligand and other amino acid side-chains within the pocket may also occur; the latter favouring the approach of the compound from the intracellular space (Figure 6).

Notably, our modelling analysis points out to different mechanism of inhibition for Auphen in the case of AQP3 and AQP7. Specifically, while the compound’s binding domain is located in the extracellular entrance of the channel in hAQP3, in hAQP7 Auphen binds in the intracellular side. Thus, while in the case of hAQP3, the binding of Auphen from the extracellular side doesn’t imply its cell uptake, internalization of the compound by the cells is necessary prior binding and inhibition in hAQP7. These differences are crucial to design gold compounds highly selective for a specific isoform, i.e. to achieve selectivity for hAQP7 a more lipophilic and membrane permeable drug could be imagined.

Effects of gold compounds on cell proliferation

Concerning the above mentioned possible roles of aquaglyceroporins, specifically AQP3, in cancer progressions, we also recently analysed in depth Auphen’s capacity of inhibiting cell proliferation in different cell lines (cancerous and non-cancerous) with different levels of AQP3 expression, and we investigated the possible correlation of the observed antiproliferative activities with the AQP3 inhibition properties of Auphen in the selected cells.[29]

To this end, various mammalian cell lines differing in AQP3 expression level were used: no expression (PC12), moderate (NIH/3T3) or high (A431, epidermoid carcinoma) endogenous expression, cells stably expressing AQP3 (PC12-AQP3), and HEK293T cells transiently transfected (HEK-AQP3) for AQP3 expression. Auphen reduced approximately 50% the proliferation in A431 and PC12-AQP3, 15% in HEK-AQP3 and had no effect in wt-PC12 and NIH/3T3. Dose response curves with the A431 tumoral cell line gave an EC_{50} of 1.99 ± 0.47 μM. Silencing AQP3 expression in the same A431 cell line alleviated Auphen effect on cell proliferation with a subsequent increase in the EC_{50}. The effect on cell proliferation was confirmed by detecting a strong arrest in the S-G2/M phases of the cell cycle of cells treated with Auphen.

Additionally, functional studies allowed correlating the inhibition of cell proliferation with the impairment of AQP3 activity. In fact, evaluation of glycerol permeability of cells differing in AQP3 expression showed 50% inhibition of glycerol uptake in A431 treated cells, demonstrating that Auphen anti-proliferative effect correlates with its ability to block the AQP3 channel. Overall, these results anticipate a

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targeted therapeutic effect on carcinomas and other cancer types with large AQP3 expression and provide a rational for the use of gold compounds as anticancer agents.

Conclusions and Perspectives

In conclusion, in view of the broad expression profile and the wide range of pathologies in which aquaporins are implicated, transferring knowledge of AQP structure and physiological function to the clinic is , and there is great translational potential in aquaporin-based therapeutics and diagnostics. AQP-based modulator drugs are predicted to be of broad potential utility in the treatment of several diseases including cancer and others mentioned above, as well as diagnostic agents. Moreover, analysis of AQP involvement in the life-cycle of pathogenic protozoan parasites suggests additional opportunities for pharmacological intervention in the treatment of human diseases.

In spite of the numerous studies already available, further (patho)physiological investigation is needed to elucidate the functional role of AQP channels in cellular behaviours.
Therefore, the necessity of selective modulators (inhibitors) of AQP channels is impellent, that could be used as either chemical probes to detect AQP function in biological systems, or as innovative therapeutic agents in a variety of disease states.

In this context, inorganic coordination chemistry offers important advantages with respect to organic chemistry. Specifically, metal complexes offer an ideal drug design platform. For example, the geometric properties of the metal coordination can be used as scaffold to accurately position ligands to obtain a three dimensional structure that has high specificity for a protein target. Most importantly, the possibility of "fine-tuning" the reactivity of gold complexes via the use of selected ligands, maintaining their biological activity while reducing their side-effects, is particularly attractive and makes them extremely different from sulphur-reactive heavy metal inorganic salts. Gold ions may also be used as a glue to link different ligands acting in a synergistic fashion, and allowing the compounds to be detected in cells for imaging applications (e.g. by fluorescence microscopy). This latter feature is particularly appealing with respect to the possibility to track the localization of AQPs in cells via the use of fluorescent inhibitors.

Certainly, chemical design of innovative and highly selective inhibitors should be supported by structural information about the target AQP isoform. When the latter is lacking, computational methods are essential tools to obtain structure-activity relationship, and several homology models have recently been proposed for different aquaglyceroporin isoforms. The use of such models is recommended to achieve a deeper understanding of the key structural features involved in water/glycerol transport, and helpful to improve AQP-targeted drug design.

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