Citation for final published version:


Publishers page: http://dx.doi.org/10.1039/C8MT00072G

Please note:
Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher’s version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See http://orca.cf.ac.uk/policies.html for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.
Aquaporins in cancer development: opportunities for bioinorganic chemistry to contribute novel chemical probes and therapeutic agents
Brech Aikman, Andreia de Almeida, Samuel M. Meier-Menches and Angela Casini

Aquaporins (AQPs) are membrane proteins allowing permeation of water, glycerol & hydrogen peroxide across biomembranes, and playing an important role in water homeostasis in different organs, exocrine gland secretion, urine concentration, skin moisturization, fat metabolism and neural signal transduction. Notably, a large number of studies showed that AQPs are closely associated with cancer biological functions and expressed in more than 20 human cancer cell types. Furthermore, AQPs expression is positively correlated with tumour types, grades, proliferation, migration, angiogenesis, as well as tumour-associated oedema, rendering these membrane channels attractive as both diagnostic and therapeutic targets in cancer. Recent developments in the field of AQPs modulation have identified coordination metal-based complexes as potent and selective inhibitors of aquaglyceroporins, opening new avenues in the application of inorganic compounds in medicine and chemical biology. The present review is aimed at providing an overview of AQPs structure and function, mainly in relation to cancer. In this context, the exploration of coordination metal compounds as possible inhibitors of aquaporins may open the way to novel chemical approaches to study AQPs roles in tumour growth and potentially to new drug families. Thus, we describe recent results in the field and reflect upon the potential of inorganic chemistry in providing compounds to modulate the activity of “elusive” membrane targets as the aquaporins.

Introduction
The identification of aquaporins (AQPs), highly conserved membrane protein channels that are permeated by water, revised the general view on water passage across biomembranes as being solely the result of passive diffusion across the lipid bilayer. The last decade contributed greatly to the understanding of the diverse roles of AQPs in health and disease. Particularly, the ability of AQPs to permeate not only water but also other small molecules or solutes, enables them to regulate several cell functions, which include osmotic water movement in cell volume regulation, energy metabolism, migration, adhesion and proliferation. Presently, thirteen mammalian AQP isoforms (AQP0-12) have been identified, ubiquitously distributed throughout the body. Based on structural and functional characteristics, these channel proteins are divided into two main groups: orthodox aquaporins strictly involved in water permeability (AQP0-2, AQP4, AQP5, AQP6 and AQP8) and aquaglyceroporins, facilitating transport of small uncharged solutes such as glycerol and urea, additionally to water (AQP3, AQP7, AQP9, and AQP10). Peroxiporins are a further subclass of AQPs permeable to hydrogen peroxide, and comprise both orthodox aquaporins (AQP1, AQP8) and aquaglyceroporins (AQP3 and AQP9). Finally, due to their subcellular localization, AQP11 and AQP12 are classified as S-aquaporins. The nature of selective permeability for the latter is still uncertain and they show less sequence similarity to the other isoforms. However, there are indications of AQP11 facilitating water and glycerol transport.

Pathophysiological conditions of numerous human disorders have been correlated with altered aquaporin function as a result of dys-, up- or down-regulated AQPs expression (Fig. 1). Nephrogenic diabetes insipidus and Sjögrens syndrome are just two examples of disorders linked to impaired water permeability of AQPs. The dysfunction of aquaglyceroporins’ ability to permeate glycerol, as well as their abnormal expression in certain tissues, have consequences for cell proliferation, adipocyte metabolism and epidermal water retention. Thus, aquaglyceroporins seem to play a role in metabolic disorders such as obesity, diabetes and skin diseases, e.g. atopic dermatitis. Interestingly, studies report isoforms of both orthodox aquaporins and aquaglyceroporin linked to different types of cancer, often showing a strong correlation between the level of AQP expression and the tumour grade. Notably, parasite aquaglyceroporins are also relevant to certain human diseases, such as malaria.
Since aquaporins are involved in a variety of important physiological and pathophysiological processes, they have gained increasing attention as promising targets for therapeutic intervention. However, discovering selective AQPs modulators (inhibitors) proved to be challenging. Such inhibitors would not only be useful as novel therapeutic agents but also as chemical probes to validate AQPs function in biological systems, to be used in parallel to genetic approaches (e.g. knockout animal models). After presenting a general overview on AQP structure and expression in both healthy and tumour tissue, this perspective will focus on the role of AQPs in tumorigenesis and tumour metabolism and summarize the state-of-the-art literature on the most promising AQP-targeted metal-based inhibitors highlighting their possible uses in medicine and cancer pathophysiology.

1. Aquaporin structure

Aquaporins are homo-tetrameric complexes in the cell membrane, with each monomer of ~30 kDa containing six transmembrane α-helices connected by five loops, two half helices and both N- and C-termini located on the cytoplasmic side of the membrane. Two of the loops are located intracellularly while three are extracellular domains. Each AQP monomer in the membrane creates a single narrow hourglass-shaped pore spanning the lipid bilayer (Fig. 2). Specific features of this channel are essential to the remarkable selective nature of AQPs for permeation of water and other small solutes, namely two highly conserved constriction sites acting as selectivity filters. The constriction site located near the extracellular entrance is known as the aromatic/arginine selectivity filter (ar/R SF) (Fig. 2) and the diameter of this filter determines whether small polar solutes may permeate the pore in addition to water. In orthodox aquaporins the pore’s diameter at the ar/R SF is ca. 3 Å, preventing permeation of molecules bigger than water (2.8 Å). The size of the pore is larger in aquaglyceroporins, reaching up to ~3.4 Å in diameter to allow for example glycerol permeation. Although the arginine in this constriction site is fully conserved in all mammalian AQPs isoforms, the composition of the remaining amino acids creating the ar/R SF might vary depending on the permeability for water or glycerol. Orthodox aquaporins have an ar/R SF formed by four residues, while aquaglyceroporins have an ar/R SF formed by only three residues, which most likely accounts for the different pore sizes and selectivity.

The second selectivity filter is composed of two conserved asparagine-proline-alanine (NPA) motifs, located in the centre of the channel where the positive N-terminal ends of the two half helices meet. The formed helix dipoles moment adds to these positive ends, creating an electrostatic barrier that prevents the passage of positively charged ions through the pore. Particularly the Asn side chain amide position appears to be structurally critical to this functionality, whilst the amide nitrogens aid in directing water or solute molecules by acting as hydrogen bond donors. Molecular dynamics (MD) simulations, electron microscopy and X-ray crystallographic structures of AQPs revealed water molecules passing through the channel in a single file, orienting themselves along the local electrical field. Unlike the previously mentioned structural difference found between AQPs and aquaglyceroporins in the ar/R SF, the NPA region is conserved in most human AQ isoforms, with the exception of AQP7 (NAA-NPS), AQP11 (NPC-NPA) and AQP12 (NPT-NPA). However, all AQPs preserve the Asn residue, thus emphasizing its importance.
2. Aquaporins in normal and tumour physiology

Numerous studies observed a correlation between AQP expression levels in cancer cells and cancer malignancy. However, this observation does not imply that only one type of AQP relates to a specific cancer. In fact, multiple AQP isoforms were reportedly involved in tumours at different stages. Interestingly, some tumours also showed up-regulated expression of AQP isoforms which are usually not found in the tissue of origin. Table 1 provides an overview of the presence of different AQP isoforms in various human cancer types (studied in cells or tissue samples) and possible related functions. The involvement of aquaporins in tumours can be partially dependent on the nature of their substrate permeability, as will be explored further in the next sections. Thus, here we will summarize some of the studies that best describe the function of different AQP isoforms in cancer development.

### 2.1 Water permeation

The presence of AQPs in human tissues facilitates water permeation across the membrane, allowing fast cellular responses to changes in the osmotic gradient. Therefore, AQPs are abundantly present in tissues involved in maintaining water-based homeostasis, with a need for rapid fluid turnover such as the kidneys (AQP1, AQP2, AQP3, AQP4), the airways (AQP1, AQP3, AQP5), and the central and peripheral nerve system (AQP1, AQP4, AQP9). Notably, the ability of AQPs to transport water was proven to be involved in multiple aspects of tumour malignancy, e.g. enhanced cell migration, affecting tumour invasion, as well as oedema formation, as detailed below.

### Migration of tumour cells

The ability of tumour cells to migrate enables tissue invasion and metastasis, which are both associated with a poor prognosis. AQP isoforms 1, 4 and 5 are the main AQP isoforms shown to be involved in general cell migration. Under normal physiological circumstances, AQP1 expression is polarized on the leading edge of cell protrusions in migrating cells, which is hypothesized to enhance the process of cell movement. The increased water permeability combined with actin cleavage and ion uptake at the tip of the lamellipodia create local osmotic gradients, which drive water influx and ultimately enable lamellipodia extension and cell migration. It was suggested that AQPs facilitate the rapid changes in cell shape that take place as a migrating cell squeezes through the extracellular matrix. Such cell

<table>
<thead>
<tr>
<th>AQP isoform</th>
<th>Tissue</th>
<th>Tumour and sample type</th>
<th>Function in tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP0</td>
<td></td>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>AQP2</td>
<td>Cervical18, Bladder18, Colorectal18, Liver18, Lung18, Oesophageal18, Pancreas18, Renal18, Uterus18</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>AQP4</td>
<td>Brain32, Cervical32, Thyroid32</td>
<td>Thyroid32, Lung32</td>
<td>Grade, migration, tumour-associated oedema, adhesion, invasion and apoptosis.</td>
</tr>
<tr>
<td>AQP5</td>
<td>Breast33, Cervical33, Laryngeal33, Liver33, Lung33, Oesophageal33, Ovarian33, Pancreas33</td>
<td>Breast33, Colorectal33, Myeloblast33, Prostate33, Stomach33, Tongue33</td>
<td>Lung33, Ovarian33</td>
</tr>
<tr>
<td>AQP6</td>
<td>Ovarian73</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>AQP7</td>
<td>Thyroid74, Uterus74</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>AQP8</td>
<td>Brain75, Cervical75, Ovarian75</td>
<td>Myeloblast77</td>
<td>Migration, invasion, metastasis and anti-apoptosis, grade, proliferation.</td>
</tr>
<tr>
<td>AQP9</td>
<td>Brain76, Liver76, Ovarian76, Uterus76</td>
<td>Liver76</td>
<td>Grade, drug resistance, and energy metabolism.</td>
</tr>
<tr>
<td>AQP10</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AQP11</td>
<td>Lung81</td>
<td>Prognostic</td>
<td></td>
</tr>
<tr>
<td>AQP12</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Primary cells
volume changes are likely to require rapid water flow in and out of the cell, thus justifying the presence of AQPs water channels. In tumour cells, overexpression of AQPs could, therefore, enhance their ability for tissue invasion and metastasis. In line with this hypothesis, cancer cell lines over-expressing AQP1 demonstrate an increased ability to extravasate across blood vessels and to invade local tissue in vitro and in vivo. Similarly, over-expression of AQP1 in pulmonary adenocarcinoma tissue samples instigated a stronger capacity of cancer cell migration, invasion and metastasis. Several in vitro and in vivo assays confirmed a decreased ability of cells to migrate when the expression of AQPs is impaired. A similar effect was observed in the expression of AQP4 in astrocytes. In normal brain tissue, AQP4 is primarily localized around tight junctions of astrocytic end-feet at the cerebral microvessels, facing the blood-brain barrier, which is formed by endothelial cells. Thus, AQP4 facilitates the movement of water between blood and the brain and between the brain and cerebrospinal fluid compartments. However, in brain tumours and especially diffuse astrocytomas, AQP4 expression is greatly upregulated and intracellularly redistributed across the plasma membrane of the cancerous astrocytes, showing a completely different morphology from healthy cells. This change in phenotype also facilitates astroglial cell migration. In the comparison between wild-type and AQP4-null astrocytes, the latter displayed slow migration. Interestingly, detecting the expression levels of AQP4 to distinguish benign from malignant thyroid nodules was the claim of a recent patent. The invention is based on the discovery that AQP4 immunostaining of suspected follicular neoplasm can facilitate the distinction of benign from malignant oncocyotic lesions, and it seems useful in deciding which thyroid nodules should be dissected or removed in thyroid surgery.

Concerning AQP5, its upregulation was linked in several tumours to enhanced migration and invasive phenotypes. In fact, overexpression of this isoform has been correlated with lymph node metastases in ovarian, prostate, colon, cervical, lung and oesophageal cancers. In lung cancer cells in vitro, AQP5 overexpression was associated with increased cell migration, while AQP5 knockdown led to decrease in cell migration and invasion properties. A similar effect was observed in the migration of gastric carcinoma cells and invasion of breast cancer cells. Another in vitro study, using human glioma cells showed that increased AQP5 mRNA expression was positively correlated with proliferation rates and silencing inhibited cell proliferation, reduced migration and promoted cell apoptosis. Recently, the role of AQP5 in hepatocellular carcinoma metastasis was also evaluated. The authors showed that AQP5 was highly expressed in hepatocellular carcinoma cell lines and its downregulation inhibited the cells’ capacity of invasion and metastasis, both in vitro and in vivo.

**Tumour oedema**

Upregulation of AQPs as water channels may also contribute to oedema formation and patients with tumour oedema in the brain display an increased morbidity and mortality rate. For example, AQP1 was found upregulated in glioblastomas, enhancing the water permeability in perivascular areas like the blood-brain barrier. Based on these studies, AQP1 has the potential to be applied as a survival prognosticator in glioblastoma. Over-expression of AQP4 was also associated with tumour oedema in the brain. However, due to the upregulation of AQP4 in the peritumoral area rather than the tumour core, its involvement in migration might be even more relevant. Subsequent experiments showed that AQP4 deletion in mice increased oedema around implanted melanoma in the brain, suggesting that the increased AQP4 expression by reactive astrocytes in and around the tumour facilitate elimination of brain oedema fluid.

**2.2. Glycerol transport**

Concerning aquaglyceroporins (AQPs 3, 7, 9, 10 and 11) and their roles in physiology, as expected their expression is particularly high in tissues involved in glycerol metabolism such as adipocytes and liver, and thus, influences metabolic pathways. Circulating glycerol derives from fat lipolysis, glycerol absorbance from the...
Glycerol is an important intermediate in gluconeogenesis, lipid metabolism and in an electron shuttle system into mitochondria. The latter carries reducing equivalents from the cytosol to mitochondria for oxidative phosphorylation. Depending on the cellular energy status, metabolism can take place in the outer mitochondrial membrane.

In the body, glycerol is stored in adipose tissue in the form of triacylglycerol (TAG), which may derive from glycolysis. The TAG precursors are CoA-activated fatty acids and glycerol-3-phosphate (G-3-P), produced either from the reduction of dihydroxyacetone phosphate (DHAP) or the phosphorylation of glycerol. Adipose tissue or muscles do not express glycerol kinase (GK), the enzyme required for glycerol phosphorylation, which is why they acquire G-3-P from glycolysis. AQP7 is the primary glycerol transporter in adipose tissue and abundantly expressed in the plasma membrane of adipocytes (Fig. 3).

Under fasting conditions, glycerol released by adipose tissue upon lipolysis is taken up by the liver, most likely via AQP9 and used as a non-carbohydrate precursor to induce gluconeogenesis in hepatocytes. Thus, AQP7 seems to facilitate glycerol efflux from adipose tissue, leading to glycerol influx into hepatocytes via AQP9.

Similarly to the orthodox aquaporins, aquaglyceroporins are also involved in osmoregulation. In fact, glycerol is one of the main osmoprotective solutes in mammalian cells. In response to external gradients, cells can react by altering intracellular levels of glycerol whilst reducing or increasing membrane permeability. Like water, glycerol can cross the lipid bilayer via passive diffusion. However, this process is relatively slow and the presence of aquaglyceroporins highly increases glycerol permeability, turning glycerol permeation into a faster and more energy efficient process. In the skin, AQP3 is the most abundantly expressed isoform, in particular in keratinocytes residing in the basolateral layer of the epidermis. Under physiological conditions, water and glycerol are essential in preserving skin moisture. Glycerol acts as a humectant to prevent water evaporation, thus upholding the barrier function of the skin. In mice, deletion of AQP3 was shown to impair this barrier function by reducing elasticity and hydration, as well as showing delayed wound healing and reduced cell proliferation in the stratum corneum.

**Glycerol in cancer cell proliferation**

Overall, the experimental evidence describing increased aquaglyceroporin expression in tumours (Table 1) led to the hypothesis that glycerol contributes to tumour growth and cancer cells proliferation in two possible ways: i) as a building block in phospholipid synthesis, and/or ii) as an intermediate or regulator of ATP production. Since both pathways are essential to fast proliferating cells, such as cancerous ones, AQPs inhibition is predicted to reduce both tumour cell proliferation and migration. Below we will summarize the studies that corroborate these hypotheses on the interplay between glycerol and cancer.

Out of the four human aquaglyceroporin isoforms, AQP3 is the most studied also in relation to cancer. This isoform is widely distributed throughout the body in epithelial cells. A recent study by our group, showed that AQP3 inhibition reduced cell proliferation as a function of the AQP3 expression levels in different cell lines, including cancer cells. Furthermore, it was found that AQP3 silencing induced downregulation of several lipid synthases in gastric cancer cells *in vitro*. Thus, it was postulated that lipid synthesis impairment by AQP3 knockdown is not only the consequence of glycerol uptake decrement but also related to lipid synthesis system inhibition. The PI3K (phosphatidylinositol-4,5-

---

**Fig. 3 Aquaglyceroporins and glycerol metabolism in liver and adipose tissue.** Abbreviations: G-3-P = glycerol 3-phosphate, DHAP = dehydroxyacetone phosphate, TCA = tricarboxylic acid, FFA = free fatty acid, TAG = triacylglycerol, MAG = monoacylglycerol.
biphosphate 3-kinase) /Akt (protein kinase B) signalling pathway, which was involved in the impaired lipid and ATP production, was also inhibited after AQP3 knockdown.\textsuperscript{109}

As cancer cells divide at higher rates, they require more lipids for membrane synthesis, and more energy to sustain the vigorous proliferation and the malignant behaviour. In fact, phospholipids are used to form the various plasma membranes, but can also be catabolized to generate ATP by β-oxidation.\textsuperscript{110,111} As previously mentioned, glycerol can be metabolized to be the backbone of triglyceride (TAG), and TAG is important in maintaining cell proliferation and survival. Specifically, tumour cells can convert TAG into free fatty acid (FFA) by lipolytic processes (Fig. 4). Fatty acid oxidation (FAO) is used to generate ATP to support the cancer development.\textsuperscript{112} Thus, inhibition of lipid synthesis, via aquaglyceroporins inhibition or down-regulation, may result in material and energy supply defects.

The second main hypothesis for the role of AQP3 in tumour cell migration and proliferation relates to the fact that its overexpression provides the cell with higher glycerol permeability leading to higher ATP content,\textsuperscript{113} required for a greater demand for biosynthesis. This idea of a direct correlation between glycerol and ATP production was developed in 2008, when Hara-Chikuma and Verkman observed a remarkable resistance to skin tumorigenesis in AQP3-deficient mice.\textsuperscript{59} Their data suggested that glycerol permeability via AQP3 is required for epidermal cell proliferation and tumorigenesis, as the cellular glycerol levels were positively correlated with cellular ATP content.\textsuperscript{59} In detail, a reduction of glycerol in epidermal cells, its metabolite G-3-P, and ATP were observed in AQP3-deficiency without impairment of mitochondrial function.\textsuperscript{59} Glycerol supplementation corrected the reduced proliferation and ATP content under AQP3 deficiency. Thus, it was suggested that glycerol could be a key regulator of cellular ATP, which could justify the overexpression of AQP3 in some cancers.

However, this hypothesis needs some careful reflection on the biochemical pathways possibly linking glycerol to ATP synthesis, particularly in relation to cancer cell metabolism. In general, tumours display a deregulated metabolism to meet the high demand for nutrients used to support cell proliferation and survival, while maintaining a balanced redox status.\textsuperscript{114} Thus, in cancer, glucose and glutamine are often the main energy sources.\textsuperscript{115,116} In the early 20s, Warburg theorized that impaired mitochondrial respiration would drive tumorigenesis and cause an increased consumption of glucose, reflected in high lactate production, \textit{i.e.} aerobic fermentation, named the \textit{Warburg effect}.\textsuperscript{117} However, current research challenges this interpretation postulating that tumours retain their capacity to perform oxidative phosphorylation, even within the hypoxic environment, and metabolic changes are rather an effect of various malignant cellular transformations than a cause of tumorigenesis.\textsuperscript{114,118}

Proliferating cancer cells have an increased need for precursors and intermediates for biosynthesis and reducing equivalents, which the catabolism of glucose and glutamine can provide.\textsuperscript{119} Oxidation of carbon skeletons, produced from glucose, allows the cells to capture the electrons in the form of reduced nicotinamide adenine dinucleotide (NADH), which are then introduced into the mitochondrial electron transport chain via the malate-aspartate shuttle. This process contributes to cellular respiration and ATP generation.\textsuperscript{118} Proliferating tumour cells seem to prefer converting excess pyruvate into lactate, which contributes to maintaining the cytoplasmic level of the NAD⁺/NADH ratio to promote a continued glucose metabolism.\textsuperscript{118,119}

In this context, tumours overexpressing aquaglyceroporins are thought to use glycerol as an intermediate for pyruvate through the glycerol 3-phosphate shuttle (G-3-P shuttle) leading to ATP production (Fig. 4). The G-3-P shuttle is a complex process used to transfer electrons from cytosolic NADH to the mitochondrial electron transport chain (Fig. 4).\textsuperscript{120} In this shuttle, the enzyme called cytoplasmic glycerol-3-phosphate dehydrogenase 1 (cGPDH) converts dihydroxyacetone phosphate to G-3-P by oxidizing one molecule of NADH to NAD⁺, whilst the mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH) converts G-3-P to DHAP by reducing flavine adenine dinucleotide (FAD⁺)\textsuperscript{120} This system regenerates cytosolic oxidized nicotinamide adenine dinucleotide (NAD⁺) and supports oxidative phosphorylation in mitochondria, thus production of adenosine triphosphate (ATP). In humans, the G-3-P shuttle functions mainly in the brain and skeletal muscle.\textsuperscript{101}

In proliferating cells, glycerol phosphorylation is catalysed by Glycerol Kinase (GK), converting glycerol to G-3-P.\textsuperscript{100} The G-3-P can either be utilized in the biosynthesis of phospholipids or as an important structural component of cellular membranes or enter the glycolytic pathway, when converted to DHAP by mGPDH.\textsuperscript{100,101,121} However, the latter is less likely to occur, as proliferating cells interconvert DHAP to G-3-P in order to re-oxidize cytosolic NADH generated from glycolysis, transferring reducing equivalents into the electron transport chain. The enzyme mGPDH shows an increased activity in prostate cancer cells, melanoma and breast cancer,
thought to uphold glycolysis and facilitating the oxidation of NADH.\textsuperscript{122}

\textbf{Fig. 4} Possible model of aquaglyceroporin involvement in glycerol metabolism of proliferating tumour cells. Abbreviations: ATP = adenine triphosphate, DHAP = dehydroxyacetone phosphate, \textit{FAD}^+ = flavine adenine dinucleotide, \textit{FADH}_2 = hydroquinone form of flavine adenine dinucleotide, \textit{FFA} = free fatty acids, \textit{cGPDH} and \textit{mGPDH} = cytosolic and mitochondrial, respectively, \textit{glycerol-3-phosphate dehydrogenase}, \textit{G1Pa} = glucose-1-phosphate:adenylyltransferase, \textit{G-3-P} = glycerol-3-phosphate, \textit{GK} = glycerol kinase, \textit{TCA} = tricarboxylic acid, \textit{NAD}^+ = oxidized nicotinamide adenine dinucleotide, NADH = reduced nicotinamide adenine dinucleotide.

It is important to recognize that cancer cell metabolism is continuously adapting and reprogramming to optimize the use of available nutrients. Overall, although glycerol is more likely to contribute to tumour cell growth as a precursor for phospholipids, its interplay in the generation of ATP cannot be ruled out, and this aspect would warrant further investigation.

Despite AQP3 being the most studied isoform in relation to cancer, all other aquaglyceroporins have been shown to be expressed in cancer tissue, with an established correlation to several types of cancers (Table 1).

For example, immunostaining of AQP7 in epithelial ovarian cancer showed expression in the plasma membranes of benign tumour cells, while being located in the nuclear membrane of borderline and malignant cells.\textsuperscript{80} Interestingly, despite the selective nuclear staining in malignant tumour tissue, western blot analysis of protein levels showed that AQP7 is significantly higher in malignant and borderline tumour than in benign tumour and normal ovarian tissue. This indicates that translocation and expression of this isoform may be crucial for ovarian carcinogenesis.

AQP9 has been shown to be in human glioblastoma, most glioma cells showing high AQP9 protein expression on the cell surface.\textsuperscript{123} Other authors have shown that AQP9 mRNA is present in specific subpopulations of glioma cells and leukocytes infiltrating the tumour tissue.\textsuperscript{124} Moreover, work by Fossdal et al. revealed that AQP9 mRNA and protein expression is increased in glioblastoma stem cells.\textsuperscript{125} Expression of this isoform in glioblastoma may indicate a role in glioma-associated lactic acidosis, by facilitating glycerol and lactate excretion, and/or being involved in the energy metabolism of glioma cells. This example highlights the crucial role of AQPs not only in glycerol uptake but also in regulating its efflux (Fig. 4). In epithelial ovarian cancer, AQP9 was shown to be localized in the basolateral membranes of both benign and borderline tumour cells, while it was widely distributed throughout membranes of malignant cells.\textsuperscript{80} Moreover, protein expression analysis by western blot revealed that there is a significant difference between tumour types as follows: ovarian malignant > borderline > benign/normal tissue. Interestingly, AQP9 was shown to be higher expressed in mucous than serous ovarian tumours and also correlated with tumour grade: higher expression in undifferentiated (grade 3) than well differentiated (grade 1/2) tumours.\textsuperscript{78,80} Therefore, increased AQP9 expression may be related to poor prognosis, highlighting once more the importance of understanding the role of aquaglyceroporins and glycerol in carcinogenesis.

Interestingly, AQP9 was shown to be down-regulated in hepatocellular carcinoma.\textsuperscript{81} Moreover, its overexpression suppressed cell invasion, both \textit{in vitro} and xenograft tumour growth, as well as hepatoma cell invasion by inhibiting epithelial-to-mesenchymal transition. This fact clearly shows that the role of aquaglyceroporins in cancer development may differ according to specific tissue types and the tissue's requirement of either glycerol uptake or efflux.

Little is known about the role and expression of AQP10 and 11 in cancer. One study, that used microarray data to investigate prognostic values of AQP mRNA expression in human ovarian cancer, showed that AQP3, 10 and 11 are correlated with improved overall survival in ovarian cancer patients.\textsuperscript{126} However, nothing is known about the protein expression of AQP10 and 11 in cancer cells or tissues.
Finally, while the correlation of aquaglyceroporins with several types of cancers is established (Table 1), as anticipated in the previous examples, the need of tumours for glycerol could be dependent of their type and stage of differentiation. Thus, metastatic and non-metastatic cancers may also have different aquaglyceroporin expression, even within the same tissue origin. For example, using high-throughput technology with microarray datasets, it was shown that, in melanoma, the transition to an increased malignant phenotype was correlated to a reduced expression of AQP3 compared to normal skin and benign nevi.\textsuperscript{127} Thus, the requirement for glycerol might decrease, as the focus shifts from tumour proliferation to migration.

The possible role of AQP3 downregulation as an indicator for the invasive character was also observed in prostate tumours.\textsuperscript{127} These findings are supported by Jain. et al., who examined consumption and release of 219 metabolites in the medium of the 60 cell lines of the NCI-60 screen.\textsuperscript{115} Their results suggest increased consumption of glycerol in some non-metastatic over metastatic cancer cell lines.

Overall, it is important to note that glycerol requirements may vary with the specific tissue of origin, malignancy, differentiation and metastatic properties, making aquaporins very challenging to study. Furthermore, due to AQPs bi-directionality of permeation, tumours' need to control glycerol uptake or efflux may lead to decreased expression of particular isoforms.

### 2.3 Hydrogen peroxide transport

Similarly to water, H$_2$O$_2$ was originally thought to freely cross the cell membrane by means of passive diffusion. However, experimental studies suggested rapid diffusion through the lipid bilayer was likely facilitated by membrane protein like AQP$_5$.\textsuperscript{128-130} Currently, there is evidence for H$_2$O$_2$ permeation by AQP3,\textsuperscript{131} AQP8,\textsuperscript{132} and AQP9,\textsuperscript{133} while hydrogen peroxide permeation of AQP1 is still controversial.\textsuperscript{110,131} Since isoforms from both orthodox AQPs and aquaglyceroporins have hydrogen peroxide as a substrate, they are also referred to as peroxiporins. AQP3 and AQP9 can both be found in the plasma membrane, whereas AQP8, in addition to its plasma membrane localization, is also present in the inner membrane of mitochondria, which are a major source of reactive oxygen species (ROS) in animal and plants.\textsuperscript{134} For example, Marchissio et al. examined whether knockdown of mitochondrial AQP8 (mtAQP8) in human hepatoma cells using siRNA had an effect on mitochondrial H$_2$O$_2$ efflux.\textsuperscript{135} The authors found that, compared to wild-type mitochondria, isolated mtAQP8 knockdown mitochondria had a lower H$_2$O$_2$ efflux ability, while the cells suffered from reduced viability. Hydrogen peroxide is part of the ROS that comprise superoxide, superoxide radicals and hydroxyl radicals. In healthy cells, ROS play a significant role in maintaining homeostasis, where intra- and extracellular concentrations of ROS are controlled using scavenging systems to balance ROS generation and elimination.\textsuperscript{136} ROS are generated as a by-product of aerobic metabolism, with nicotinamide adenine dinucleotide phosphate oxidases (NOX) and the mitochondrial electron transport chain (mETC) as the main sources.\textsuperscript{137} Scavenging enzymes and antioxidant agents that limit ROS accumulation are superoxide dismutase (SODs), catalase, glutathione peroxidases, the thioredoxin system, heme-oxygenase and nicotinamide adenine dinucleotide phosphate (NADPH)/nicotinamide adenine dinucleotide (NADP$^+$).\textsuperscript{137,138} Depending on their concentration, ROS are involved in physiological or pathological changes and can activate signalling pathways to stimulate cell proliferation, differentiation, migration, apoptosis, adaption to hypoxia, immune function, and other processes.\textsuperscript{139} Alterations in cells' ability to transport ROS can, therefore, have a profound effect on their viability.

Increased ROS levels, particularly hydrogen peroxide, are found in pathological conditions such as cancer.\textsuperscript{137,140,141} Specifically, hydrogen peroxide plays a role in (mitochondrial) NOX and ERK (extracellular-regulated kinase)-PI3/Akt. Both are activated by receptor tyrosine kinase (RTK) mediated cell signalling, and involved in cell survival, protein synthesis, proliferation and mTORC2 induced cell migration (Fig. 5).\textsuperscript{142}
Interestingly, several authors have previously reported an association between peroxiporins and ERK, NOX, mTOR or PI3K/Akt, both in healthy\(^{143}\) as cancerous cells\(^{144,145}\). Involvement of AQP3 in the ERK pathways, for example, was demonstrated in vitro using human epidermal keratinocytes, where inhibition of ERK phosphorylation via ARNT (aryl hydrocarbon receptor nuclear translocator) activation led to both a downregulated expression of AQP3 and inhibited cell proliferation.\(^{144}\) The correlation between the peroxiporins and these pathways, linked to their ability to facilitate \(\text{H}_2\text{O}_2\) transport, was not uncovered until recently. Hara-Chikuma et al. unexpectedly found that AQP3 was expressed not only by keratinocytes but also by skin-infiltrating T-cells and regulates their trafficking in cutaneous immune reactions.\(^{146}\) Specifically, it was demonstrated the necessity of AQP3 mediated \(\text{H}_2\text{O}_2\) permeation in chemokine-dependent T-lymphocyte migration in mice, where knocked-down expression of AQP3 proved to impair movement of the cells during immune response.\(^{146}\) According to the obtained results, hydrogen peroxide is suspected to be involved in a signalling cascade resulting in the actin polymerization needed for cell movement, showing that cell migration may be at least partially dependent on \(\text{H}_2\text{O}_2\). Recently, AQP3 has been shown to mediate hydrogen peroxide-dependent responses to environmental stress in colonic epithelia.\(^{147}\) Thus, the increased membrane permeability to \(\text{H}_2\text{O}_2\) in AQP3-expressing colonic endothelial cell allowed them to respond to external \(\text{H}_2\text{O}_2\) at concentrations relevant for cellular signalling processes (1–100 \(\mu\text{M}\)), such as migration and pathogen recognition.\(^{147}\)

Modulated expression of AQPs in cancer cells can, therefore, be related not only to their ability to transport water or glycerol, but also \(\text{H}_2\text{O}_2\). Hara-Chikuma et al. demonstrated that AQP3 permeated hydrogen peroxide is necessary for keratinocyte migration and proliferation, suggesting this to be the same processes which have been implicated in cutaneous wound healing and tumorigenesis.\(^{148}\) It is most likely that the elevated concentrations of intracellular peroxiporin permeated \(\text{H}_2\text{O}_2\) in cancer cells may result in downstream signalling events. The exact nature of this association is currently widely investigated for its role in cancer cell regulation of proliferation, survival, differentiation, invasion and metastasis.\(^{58,77,81,86,132,149}\)

For example, AQP3 expression in tumour cells was correlated with NOX2 and epidermal growth factor receptor (EGFR) dependent cancer progression via hydrogen peroxide permeation. Knockdown of AQP3 reduced intracellular \(\text{H}_2\text{O}_2\) concentrations, which impaired EGF-induced ERK and Akt activation and ultimately decreased tumour growth and migration.\(^{80}\) Another study focusing on breast cancer cells indicated a role for AQP3-mediated \(\text{H}_2\text{O}_2\) uptake, produced by NOX2, in cell migration. The AQP3-transported \(\text{H}_2\text{O}_2\) activated the PI3K/Akt pathway by oxidizing PTEN (phosphatase and tensin homolog), showing overexpression of AQP3 promoted migration of breast cancer cells in association with elevated \(\text{H}_2\text{O}_2\) both in vitro as in vivo.\(^{58}\) AQP8-transported \(\text{H}_2\text{O}_2\) produced by NOX upon vascular endothelial growth factor (VEGF) stimulation, seems to act in a similar way, by maintaining activation of Akt through oxidation of PTEN in leukaemia cell lines.\(^{77}\) Remarkably, AQP9 downregulated expression was correlated with hepatocellular carcinoma cells migration. Over-expression of AQP9 resulted in decreased levels of PI3K/Akt and suppressed cell invasion in vitro and xenograft tumour growth in vivo.\(^{81}\)

These studies on the potential role of AQPs in \(\text{H}_2\text{O}_2\) permeation in relation to cancer are relatively recent, and their possible importance as new therapeutic targets or biomarkers requires further investigation. This is illustrated by pharmacological ascorbate, a promising treatment in cancer therapy, that unfortunately shows a large variation in susceptibility.\(^{150,151}\) After intravenous administration, the ascorbate readily generates extracellular \(\text{H}_2\text{O}_2\), which permeates the cancer cell membrane and
accumulate intracellularly, ultimately inducing cell death due to excessive ROS.152 The latest findings by Erudaitius et al. suggested that the success of pharmacological ascorbate treatment of pancreatic cancer may depend on peroxiporin (AQP3) expression.153 Besides AQP3, AQP8 and AQP9, other isoforms are still explored as potential peroxiporins. AQP5, for example, is an interesting candidate due to its association with ERG and PTEN in prostate cancer,154 with EGFR/ERK in human glioma cell lines,91 and in hepatocellular carcinoma metastasis with NF-κB82. Interestingly, a recent study on rat AQP5 showed its ability to facilitate H₂O₂ membrane diffusion in yeast.155 Thus, more research should be conducted to elucidate AQP5's possible role as peroxiporin in human cancers.

3. Aquaporin Inhibitors

As highlighted above, there is strong evidence for AQPs as drug targets in different diseases, including cancer. Moreover, analysis of the involvement of AQPs in the life cycle of disease-causing organisms (e.g. malaria parasites) suggests additional opportunities for pharmacological intervention in the treatment of human diseases. However, the identification of AQPs modulators (inhibitors) for both therapeutic and diagnostic applications has turned out to be extremely challenging. So far, four classes of AQP-targeted small molecules have been described: (i) metal-based inhibitors; (ii) small-molecules that are reported to inhibit water conductance (e.g., sulfonamides); (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.156 Despite numerous studies, for several of the small organic compounds to date no validation of their selectivity has been reported. Thus, in the following chapters, we will focus on the most promising metal-based AQPs inhibitors and their possible mechanisms of action described at a molecular level.

3.1 AQPs inhibition by metal ions

In normal physiology, AQPs can be regulated by various factors, including protein phosphorylation, pH and metal ions. The latter are mainly divalent cations, such as first-row transition metals, some of them being physiologically relevant. For example, Zelenina et al. have investigated the effects of Ni²⁺ (NiCl₂) on the water permeability of AQP3 in human lung epithelial cells, showing that it is capable of decreasing permeability to only 30%, at a concentration of 1 mM after 1 min of incubation.157 The inhibition was shown to be reversible and Ni²⁺ did not have any effect on AQP4 or AQP5.

Later, the same authors studied the effects of Ni²⁺ (as NiCl₂) and Cu²⁺ (as CuSO₄) on glycerol permeability and found that both ions reduce AQP3 permeability at a concentration of 1 mM in HEPES buffer.158 Interestingly, Pb²⁺ and Zn²⁺ ions had no effect on AQP3 permeability. Furthermore, AQP7, another aquaglyceroporin, was insensitive to copper.

Zelenina et al. have also investigated which amino acid residues are involved in the inhibitory mechanism of both Ni²⁺ and Cu²⁺ ions by site-directed mutagenesis, and suggested serine, histidine and tryptophan residues as possible binding sites.157,158 Interestingly, Ser152 was identified as a common determinant of both Ni²⁺/Cu²⁺ and pH sensitivity. All these residues are in the extracellular loops of the AQP3 monomers, and loop movement was observed in the “gating” mechanism of several AQPs and appears to be a crucial feature in channel closure.159–161 For example, histidine residues in such loops can “tune” the pH sensitivity of AQP3 towards certain pH values, as suggested by molecular modelling.162

Among the benchmark AQPs inhibitors, the mercurial compounds pCMBS (p-chloromercurybenzene sulphonate) and HgCl₂ have been widely applied in in vitro biochemical assays to study AQPs function, despite their scarce selectivity and extreme toxicity. The mechanism of inhibition involves direct binding of Hg²⁺ ions to AQPs via modification of cysteine residues based on the classical hard soft acids and bases (HSAB) theory. In order to confirm such mechanism, several studies were performed on Cys-mutated isoforms of human AQP1. For example, Xenopus oocytes were transfected with Cys-mutated AQP1 isoforms and the effects of mercury inhibition were evaluated.163 From all cysteine residues in AQP1, only one was shown to confer sensitivity to the mercurial salt HgCl₂, namely Cys189. When this cysteine is mutated to either serine or glycine, water permeability of the oocytes was slightly decreased, indicating that this residue may be of importance for water transport. Moreover, cells expressing the Cys189Ser mutant lost sensitivity to HgCl₂.

The current literature provides two mechanisms of inhibition of AQPs by mercury: the first is simple occlusion of the water pore by the mercury ions found in the vicinity of the cysteine residues lining the pore; the second is conformational change (collapse of the water pore) at the selectivity filter (namely, the (ar/R) SF) region, induced by mercury bound to a cysteine residue nearby.

The first hypothesis was formulated after the atomic-resolution structure of human AQP1 was solved, where Cys189 was shown to...
be positioned inside the channel, just above the ar/R SF. Therefore, it was hypothesized that Hg$^{2+}$ binding to this site was likely to prevent passage of water molecules via steric effects.

The second mechanism of AQP inhibition by mercurial compounds was first proposed in an *in silico* study on the basis of molecular dynamics (MD) simulations of the bovine aquaporin AQP1 (bAQP1). bAQP1 contains a cysteine residue (Cys191) in the ar/R SF region, which may bind Hg$^{2+}$ similarly to hAQP1. According to the MD simulations of both free AQP1 and Hg-bound AQP1, the energy barrier for Hg-AQP1 is much higher than that of free AQP1 at the ar/R SF. Moreover, calculations show that mercury binding induces a collapse of the orientation of amino acid residues at the ar/R SF and the constriction of the space between Arg197 and His182.

Later on, our group investigated the molecular mechanism of inhibition of human AQP3 by Hg$^{2+}$, using MD approaches. In support of the second mechanistic hypothesis, we observed important protein conformational changes upon binding of metal ions to Cys40 leading to a collapse of the ar/R SF, and subsequent blockage of water permeation.

### 3.2 Gold complexes as inhibitors of aquaglyceroporins

Recently, coordination gold(III) compounds have been identified as selective aquaglyceroporin inhibitors. In details, we have reported for the first time on the potent and selective inhibition of human AQP3 by the water-soluble gold(III) compound [Au(phen)Cl$_2$]Cl (phen = 1,10-phenanthroline, *Auphen*, Fig. 6). Interestingly, Auphen inhibited glycerol transport in human red blood cells (hRBC), with an IC$_{50}$ = 0.8 ± 0.08 μM, whilst having no inhibitory effect on water permeability mediated by the orthodox water channel AQP1.

![Structure of the gold(III) complexes](image)

In a further study, our group examined Auphen’s capacity of inhibiting cell proliferation in various cell lines with different levels of AQP3 expression. Our results showed a direct correlation between AQP3 expression levels and the inhibition of cell growth by the gold(III) compound. Functional studies also demonstrated AQP3 inhibition in the cell lines where proliferation was affected by treatment with the gold compound.

Using molecular modelling approaches, we investigated the non-covalent binding of *Auphen* to AQPs at a molecular level and found that its isoform selectivity is due to the accessibility to Cys40, whose thiol group is a likely candidate for direct binding to Au(III) complexes. The involvement of this residue in the inhibition mechanism was further confirmed by site-directed mutagenesis studies in a subsequent study. Additional results on other Au(III) compounds with different N^N ligand scaffolds allowed us to establish preliminary structure-activity relationships: the most effective compounds were those featuring at least one positive charge, one ligand that could be exchanged to allow metal coordination to protein residues, and aromatic ligands.

Unexpectedly, Au(I) complexes did not show any AQPs inhibition properties, implying that the hard-soft acid-base (HSAB) theory is not sufficient to predict the affinity for a metal compound for a certain protein binding site. Quantum mechanics/molecular mechanics (QM/MM) calculations suggested that the ligand moiety may play a major role in orienting the selectivity towards a certain isoform, stabilizing the position of the inhibitor in the extracellular binding pocket.

The reversibility of AQP3 inhibition was also studied by pre-treating hRBC with the gold compounds for 30 min at r.t. and, subsequently, washing the cells with either excess of the thiol containing reducing agent β-mercaptoethanol, the sulphur donor L-Cys, or the N-donor His. In all cases, treatment with the competitor molecules led to an almost complete recovery of glycerol permeability, ruling out possible oxidative modification of amino acid residues by the Au(III) complexes.

When the 1,10-phenanthroline derivatives of Pt(II) and Cu(II) were also included in the investigation to compare the effects of metal substitution on the AQP3 inhibition potency, remarkably, the inhibition potency decreased drastically in the order: *Auphen* > Cuphen (IC$_{50}$ = 81.9 ± 4.1 μM) >> Ptphen (IC$_{50}$ > 200 μM).
Recently, MD studies were conducted for the first time on another potent and selective inhibitor - the compound \([\text{Au}(1\text{-methyl-2-}(\text{pyridin-2-yl)-benzimidazole})\text{Cl}_2\text{PF}_6]\) (\(\text{Au}1\), Fig. 6) - bound to Cys40 of AQP3 (Fig. 7).

The obtained results showed that protein conformational changes upon metal binding to Cys40, and not direct steric blockage of the channel by the metal compound, are mostly responsible for the observed inhibition of water and glycerol permeation (Fig. 8). These findings are in line with the above-mentioned studies on AQPs inhibition by mercury. Furthermore, binding of \(\text{Au}1\) in one monomer also affects substrate permeability in an adjacent one, thus, altering the overall extracellular distribution of hydrophobic/hydrophilic surfaces of the tetramer, which, in turn, orients the approach of the substrates to the pore. Moreover, in this study, a correlation between the affinity of the \(\text{Au}1\) complex towards Cys binding and AQP3 inhibition was highlighted, while no influence of the different oxidative character of the metal complexes was observed.\(^{168}\)

Further investigation of another selective AQP3 inhibitor, the gold(III) cationic complex \([\text{Au}(\text{bipy})\text{Cl}_2\text{PF}_6]\) (bipy = 2,2'-bipyridine, \(\text{Aubipy}\), Fig. 6), bound to the protein channel, has been performed by means of a multi-level theoretical workflow that includes QM, MD and QM/MM approaches.\(^{169}\) In this study, three key aspects for AQP3 inhibition by gold compounds have emerged: i) speciation of the gold(III) complex prior protein binding (formation of aquo-complexes), ii) stability of non-covalent adducts between the compound aromatic ligand and the extracellular pore side, and iii) conformational changes induced within the pore by the coordinative binding of \(\text{Au}1\) ions leading to pore closure, in line with the above-mentioned study on \(\text{Auphen}\).

Notably, in this latter study, we suggest that the presence of thiol binding sites in AQP3 is necessary but not sufficient to determine the selectivity of \(\text{Aubipy}\). Instead, the formation of stable non-covalent \(\text{Aubipy}-\text{AQP3}\) adducts is required to compensate the thermodynamic and kinetic barriers associated with the formation of the final covalent \(\text{Au}-\text{Cys}\) adduct. In detail, the overall \(\text{Aubipy}-\text{AQP3}\) binding process may be described as depicted in Fig. 9.

Overall, these results have important implications for future inhibitors’ design, showing that amino acid residues other than those lining the pore could be targeted, provided that their modification leads to the necessary conformational changes to achieve channel closure. In addition, careful fine-tuning of the interactions between the metal complex and specific residues at the pore extracellular entrance, leading to the formation of the first non-covalent adduct, may be achieved by different substituents on the ligand bound to \(\text{Au}1\), as well as by the use of different aromatic scaffolds (e.g., C=N-cyclometallated and benzimidazole ligands). In this way, one may increase the selectivity of the compound for inhibition of AQP3 compared with other aquaglyceroporin isoforms.

Selectivity of aquaglyceroporin inhibition is essential to deliver a targeted therapy, dependent on the isoform of interest. In fact, \(\text{Auphen}\) was also observed to inhibit AQP7 although with lower
potency and different mechanism of inhibition, as indicated by permeability studies and molecular modelling approaches, respectively. Specifically, whilst Cys residues corresponding to Cys40 in AQP3 are not available, several Met side chains are accessible, either on the extracellular or the intracellular side of human AQP7, for binding to Au(III) complexes.

The fact that the inhibition mechanism of the same compound with two different AQPs may be different, indicates that it is possible to take advantage of even small structural differences between the isoforms to optimize the inhibitor’s chemical scaffold and, thus, to achieve selectivity.

4. Conclusions and Perspectives

Clinical and preclinical studies evidence that AQP expression is increased in a number of cancers. Therefore, in recent years, biological functions and signalling pathways of AQPs in cancer have been intensively investigated in a condition of AQP depletion, using genetic approaches. AQPs are also involved in the carcinogenesis and pathogenesis of tumour-associated oedema, tumour cell proliferation and migration. The exact nature of this correlation is still the subject of discussion, emphasizing the need to develop targeted modulators to study the mechanism of these potential therapeutic targets. In fact, numerous in vivo and in vitro studies have shown several attractive opportunities for AQP-targeted therapy. Furthermore, some of the aquaglyceroporins have also been postulated to be responsible for the uptake of inorganic chemotherapeutics, such as the anti-leukemic arsenic trioxide.

Within this framework, the possible contribution of bio-inorganic chemistry is crucial, in that, taking advantage of the promising studies on gold(III) compounds as selective aquaglyceroporins inhibitors, and in combining highly integrated investigational approaches, new metal-based complexes may be optimized to target specific AQPs isoforms. For example, we consider copper complexes as alternative promising candidates to gold compounds, and ongoing studies in our lab aim at designing new ligand systems able to favour the binding of Cu(II) and Cu(I) ions to AQPs. Thus, the design and use of either coordination or organometallic metal compounds may unravel unexpected roles of AQPs in the molecular mechanisms of diseases and provide new tools in chemotherapy and imaging.

Finally, inorganic chemistry offers important advantages with respect to organic chemistry. For example, metal complexes constitute an ideal drug design platform, where not only the geometric properties of the molecule can be easily varied, but also offering the possibility of “fine-tuning” the reactivity of the compounds via appropriate ligands’ choice, maintaining the AQPs inhibition activity while reducing the side-effects.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Authors acknowledge Cardiff University for funding.

References

11. G. Benga, On the definition, nomenclature and


99. L. Reshef, Y. Olswang, H. Cassuto, B. Blum, C. M. Croniger,


