strong motivation for the development of new therapeutics that have alternate mechanisms of actions compared to the compounds in routine clinical use, and metal complexes can also be used to diagnose multidrug resistance (see **Chapter 3.26**).[11,12] There have been particular recent advances in the use of protein-binding gold compounds, an early inorganic medicinal success story that has not yet fully delivered on initial promise.[13–15] There is also considerable potential in the anticancer applications of ruthenium compounds (see **Chapter 3.25**).[16]

The chelator design can be of paramount importance in controlling the specificity of binding, the compound stability in the biological medium or *in vivo*, and can also be used to target particular tissues or locations in the body.[17] Macrocyclic ligands have found particularly widespread use for *in vivo* applications due to high complex stability and also represent a useful way of controlling geometry at additional exchangeable ligand sites.[18] Other key areas that are covered in this chapter include the recognition of particular proteins by designed molecules and the interaction of metal complexes with peptides.[19,20]

We have largely excluded studies of the interactions with prion proteins as, despite its importance, the main interest of this research is in the 'free' metal ion binding (see **Chapter 3.09**).[21] Other biological targets such as DNA, saccharides, and binding of compounds through conjugated groups, that is, complexes peripherally substituted with sugars, have also been excluded (see **Chapters 3.24** and **3.25**).[22] There is a considerable volume of literature on the attachment of metal complexes to biomolecules via conjugation reactions which is not relevant unless coordination bonds are formed or further insight is offered into a combination of coordination and noncovalent interactions.[23]

3.22.2 Carbonic Anhydrase Inhibitors

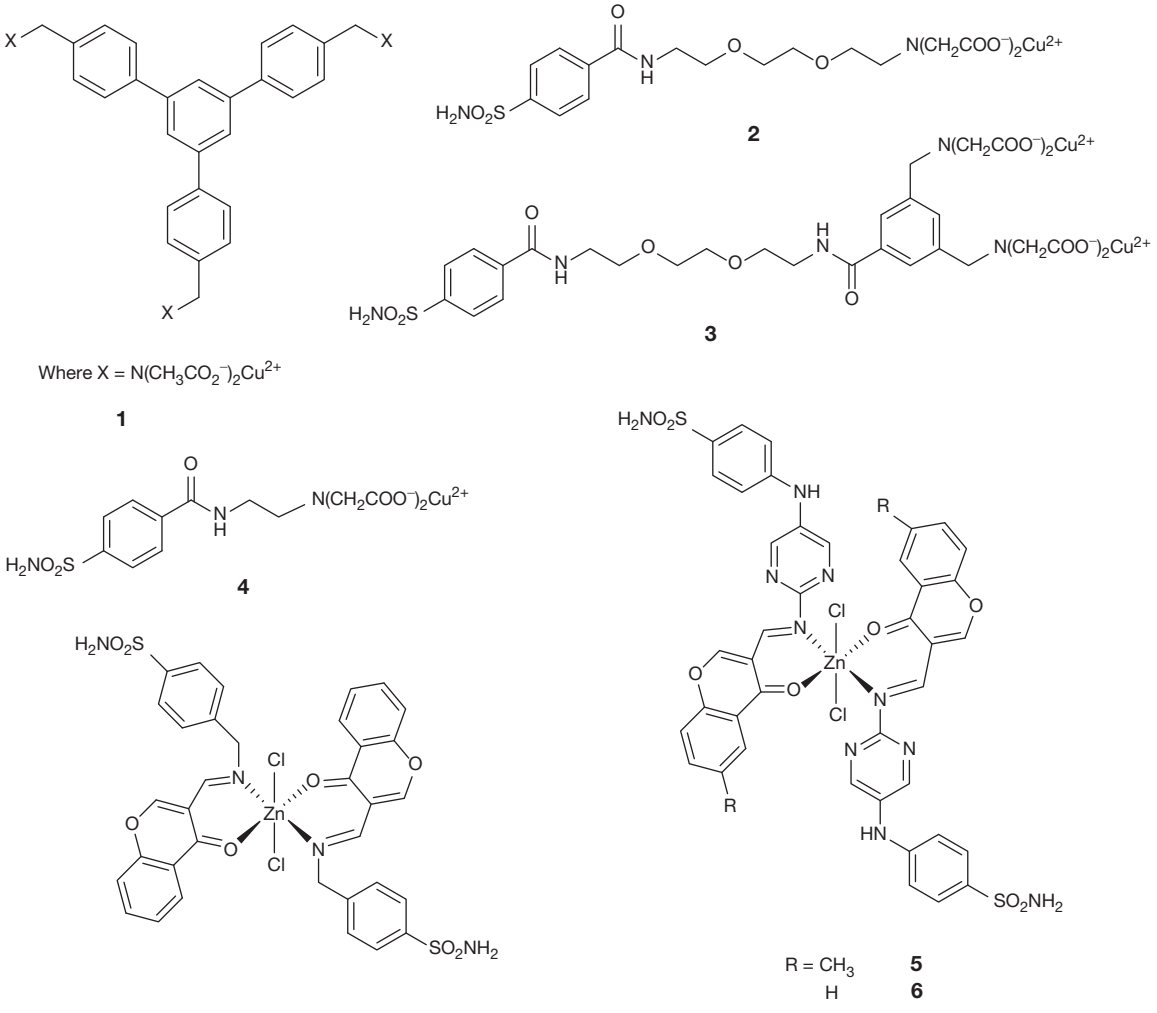
Carbonic anhydrases (CAs) are zinc(II)-containing metalloenzymes which catalyze the interconversion of carbon dioxide and hydrogencarbonate (see **Chapter 3.08**). They are also involved in a number of biosynthetic reactions including gluconeogenesis, lipogenesis, and amino acid and pyrimidine nucleotide biosynthesis.[24] Twelve catalytically active CA isozymes have been described in humans, some of which are implicated in pathologic disease states such as cancer, glaucoma, and epilepsy. There have been a number of different approaches taken to bind molecules to CAs via coordination interactions. The enzyme is an obvious target for binding of organic molecules to the zinc(II) center to inhibit enzyme activity as it contains an exchangeable site where the substrate binds. Aryl sulfonamides are highly effective inhibitors that are in clinical use and further research development. However, other approaches can be taken to bind metal complexes to CAs using coordination interactions. CAs have unique surface histidine patterns which can be exploited to target and bind ligands specifically and selectively allowing recognition of CA and potentially different isozymes. Mallik and coworkers designed a tris–copper(II) complex to selectively bind to a CA (bovine erythrocyte) based on its surface histidine pattern, with iminodiacetic acid as the copper(II) complex unit.[25] Their complexes displayed selective binding to CA against two other proteins (chicken egg albumin and chicken egg lysozome) with different surface histidine patterns using mono- and bis-analogous ligands as controls. When the pattern of copper(II) ions on a complex matched the surface pattern of histidines of the protein, strong and selective binding was achieved ($K = \sim 3 \times 10^5\ M^{-1}$), see compound **1**. They extended the comparison to include myoglobin as a control and investigate the impact of having four copper(II) iminodiacetate units underlining the required match of the binding group arrangement to the surface for strong binding.[26] The concept of protein recognition with metal complexes based on the surface histidine pattern of the protein has not been widely exploited but was further investigated.

The same group went on to develop this concept further by converting a weak inhibitor of hCA-II, benzenesulfonamide, into a potent inhibitor by attachment of a metal chelating tether group to give a two-prong binding group.[27,28] hCA-II was selected as it is the best-studied CA and is also one of the most catalytically efficient enzymes. A copper(II)-containing tether group attached to a benzenesulfonamide active site inhibitor by a triethylene glycol spacer inhibitor **2** was found to enhance binding affinity by 40-fold to hCA-II in comparison to the analogous copper(II) free compound. Similarly conjugate **3** with two copper(II) prongs demonstrated an 800-fold increase in potency over the benzenesulfonamide control. The improved potency is attributed to the combined interaction with the active site and surface-exposed histidine residues. A further aim is to employ this strategy to general application and the production of isozyme-specific inhibitors as potential drugs.

To confirm the structural rationale motivating the design of two-prong inhibitors, Christianson and Srivastava managed to produce x-ray quality crystals of a series of copper(II) coordinated benzenesulfonamide two-prong inhibitors with hCA-I and hCA-II allowing structural determination.[24] The ionized NH-group of each benzenesulfonamide molecule coordinates to the active site zinc(II) ion and the copper(II) ion of the iminodiacetic acid–Cu(II) prongs coordinates to histidine residues as anticipated, see **Figure 1**. The tightest bound inhibitor **4** was shown to bind to His200 in hCA-I and His64 in hCA-II.

A series of octahedrally coordinated zinc(II)-coordinated Schiff base sulfonamides have been prepared and evaluated as inhibitors of four physiologically relevant CA isoforms, I, II, IX, and XII, by Supuran and coworkers. Except for sulfaguanidine-derived compounds which were inactive, all zinc(II)-containing compounds showed increased inhibition potency in comparison to their analogous zinc-free derivatives against all the CA isoforms. Complex **5** was the most potent inhibitor of hCA-I ($K_i = 10$ nM) and complex **7** for hCA-II ($K_i = 1.9$ nM). For hCA-IX and hCA-XII, complex **6** showed the greatest degree of inhibition ($K_i = 6.3$ and 5.9 nM, respectively).[29] Their previous investigations have attributed this increased potency to the interaction of the zinc(II) ion with His64 at the active site.[30]

Supuran and coworkers also investigated the inhibiting properties of photochromic cis-1,2-alpha-dithienylethene-based compounds incorporating either one or two benzenesulfonamide and copper(II)-iminodiacetic acid units along with bis-ethyleneglycol-methyl ether moieties, in both their open- and closed-ring forms against five physiologically



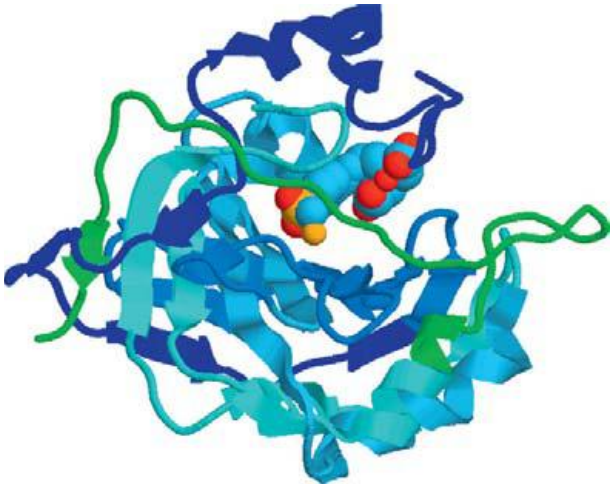


Figure 1 A copper(II) diaminoacetate anchored inhibitor BR30 binding to the hCAII zinc(II) and a histidine.

relevant CA isoforms (I, II, IX, XII, and XIV).[31] They obtained varied results when investigating the inhibition properties and concluded that in this case the two-prong approach may not be effective for the design of isozyme-selective CA inhibitors.

Two series of aromatic sulfonamide copper(II) derivatives incorporating metal chelating moieties, diethylenetriamine-pentaacetic acid (DTPA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), and 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA), were also reported by Supuran. These derivatives showed modest inhibition of CA-I and CA-II (K_i = 66–2130 and 27–360 nM, respectively) but were excellent inhibitors of the tumor-associated isoform CA-IX (K_i = 4.1–110 nM).[32] The copper(II) complexes have higher potency than the free sulfonamides, and there is the potential to use radioactive copper isotopes (such as ^{64}Cu or ^{67}Cu) in this type of CA inhibitor for diagnostic/therapeutic applications.