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In silico strategies for the selection of chelating compounds with potential application in metal-promoted neurodegenerative diseases

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Abstract The development of new strategies to find commercial molecules with promising biochemical features is a main target in the field of biomedicine chemistry. In this work we present an in silico-based protocol that allows identifying commercial compounds with suitable metal coordinating and pharmacokinetic properties to act as metal-ion chelators in metal-promoted neurodegenerative diseases (MpND). Selection of the chelating ligands is done by combining quantum chemical calculations with the search of commercial compounds on different databases via virtual screening. Starting from different designed molecular frameworks, which mainly constitute the binding site, the virtual screening on databases facilitates the identification of different commercial molecules that enclose such scaffolds and, by imposing a set of chemical and pharmacokinetic filters, obey some drug-like requirements mandatory to deal with MpND. The quantum mechanical calculations are useful to gauge the chelating properties of the selected candidate molecules by determining the structure of metal complexes and evaluating their stability constants. With the proposed strategy, commercial compounds containing N and S donor atoms in the binding sites and capable to cross the BBB have been identified and their chelating properties analyzed.

Keywords Neurodegenerative disease · Metal-ion chelator · DFT · Virtual screening · Blood brain barrier

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Introduction

Current research in coordination chemistry is often addressed to provide knowledge on the role of metal ions, organic ligands or metal complexes in various chemistry-related areas. Within this context, the contribution of coordination chemistry to the development of bioinorganic chemistry constitutes a remarkable example. In parallel, the crucial impact of organic compounds on other sciences, and in particular biology and medicine, has led to the development of strategies for the target-oriented synthesis of designed molecules, in which some of them, involve, prior to the synthesis, the identification of the best candidate molecules by means of in silico-tools. Thus, drug discovery today is not reduced to a simple "synthesize-and-test" lottery but uses computational methods to identify and eliminate those compounds that are unlikely to survive later stages of discovery and development [1–3].

Medicinal inorganic chemistry is a young branch of bioinorganic chemistry with growing significance in both diagnosis and therapy. As reviewed, the field encompasses the role of metal ions, metal binding compounds and active metal complexes in biological and biomedical processes [4]. Neurodegenerative diseases, for instance Alzheimer's (AD), prion (PrPD) and Parkinson's (PD) disease, are due to neurological disorders of cognitive abnormalities and are the main cause of the progressive dementia among old people. The key neuropathological feature is the presence of aggregated proteins in form of toxic solid deposits in the brain. The origin of these insoluble extracellular neurotoxic deposits is still not clear and multiple factors such as pH, metal ions, protein concentration, and oxidative stress have been reported as triggering their formation [5, 6]. Nowadays growing evidence is being collected on the relationships between transition metal ions and the occurrence of neurological disorders, which can be

grouped in two generic reactions: (1) metal-protein association leading to protein aggregation; (2) metal-catalyzed protein oxidation leading to protein damage and/or generation of radical oxygen species [7, 8]. Accordingly, to date, the development of multifunctional metal-ion chelators to seek the clearance of the toxic deposits as a possible MpND treatment is attracting considerable attention [6, 9–11]. In particular, the design of chelators that act as metal-protein attenuating compounds (MPAC); i.e., that exhibit an adequate metal affinity to sequester a metal ion from the metal-protein complex but no high enough to cause the removal of metals from essential metal sites [12], is currently a very active area of research [9].

Inspired by the procedures used in drug discovery, we now present a simple method for the design and selection of chelating compounds with potential application in MpND theragnosis. The first step, essentially based on chemical knowledge and experience, leads to the selection of basic scaffolds of metal chelators and includes an assessment of their coordinative properties towards metal ions (Cu(II) and Zn(II)) via quantum chemical calculations as a first evaluation for their application in MpND. The second step makes use of several in silico tools (namely, database search and virtual filtering) in order to identify commercial compounds that enclose the selected scaffolds and fulfill the main requirements of drug design for MpND therapy. The third step involves the quantum mechanical modeling of the Cu(II)- and Zn(II)-complexes formed with the final commercial candidates to gauge their chelating properties. These tools, which comprise the management of commercial databases, substructure searching, virtual filtering, molecular descriptors calculations, as well as the use of quantum mechanics, allow the selection of a limited number of promising compounds from the huge number of possibilities present in chemical commercial libraries. Overall, the whole process of design and selection enables the finding of commercial compounds with the required biochemical and pharmacokinetic properties for their further use at no cost of time in experimental work.

Results and discussion

Design and selection of scaffold metal chelators

The departing point involves consideration of the binding features and bioinorganic chemistry of the targeted metal ions. In this work Cu(II) and Zn(II) have been chosen as probe metal ions because both of them, being essential to all living systems, readily bind to amyloid-β (Aβ) peptide, hence promoting their aggregation and the formation of toxic deposits [13–16].

Concerning their stereochemical preferences, Zn(II) usually shows a four coordinate tetrahedral arrangement for its ligands, while Cu(II) favours a four coordinate square planar geometry, although sometimes additional interactions with two axial ligands gives rise to octahedral coordination. According to the Pearson's theory, both Cu(II) and Zn(II) have preference for nitrogen containing ligands such as imidazole or other aromatic heterocycles. It is then fully consistent that histidine becomes a relevant residue for the binding of Cu(II) and Zn(II) in metalloproteins or when interacting with Aβ. In fact, EPR and NMR experiments on Cu^{2+}-Aβ (1–28) at physiological pH revealed that histidines (His6, His13 and His14) as well as oxygen atoms from carboxylate groups present in amino acid residues or from the carbonyl group of the peptide bond may be involved in the coordination sphere [17–23]. Moreover, Cu(II) and Zn(II) ions also bind to most oxygen donors and thiols or thioethers. Concerning sulfur-containing amino acid residues it should be noted that methionine (S-thioether) but not cysteine (S-thiol) is compatible with Cu(II) because this ion easily reacts with the thiol group causing its oxidation to disulfide. An additional requirement to design a compound able to sequester metal cations is its capacity to behave as a chelating ligand. Overall, compounds containing nitrogen, sulfur or oxygen donor atoms giving rise to five or six-membered chelate rings may be good candidates for effectively compete with the aberrant peptide for Zn(II) and Cu(II).

In order to optimize the selection of the chelating ligands from the vast number of possible molecules that enclose the above binding-sites, we followed the similarity-based principle, which establishes that structurally similar compounds are more likely to exhibit similar physicochemical and biological properties [24]. On this basis, we took into account common structural features present in drug-like molecules. Remarkably, the shapes of half of the drugs in the Comprehensive Medicinal Chemistry (CMC) database are described by the 12 most frequently occurring frameworks (reported in SI). Among them, for the sake of simplicity, we selected the basic benzene ring and two bicyclic systems, naphtalene and indene (shown in Fig. 1 as "framework ring" column). These aromatic rings, in order to exhibit metal chelating properties, must contain some of the abovementioned donor atoms. Their inclusion was based on the coordinating properties of aminobenzenethiolate ligands, and on the well-known participation of His and Met residues in zinc and copper binding. Accordingly, we designed the core scaffolds **SC1** (2-(methyl)thioaniline), **SC2** (8-(methyl)thioquinoline) and **SC3** (2-((methylthio)methyl)-1*H*-benzo[*d*]imidazole) (presented in Fig. 1 as "core scaffold" column) as essential moieties to be present in the final commercial compounds.

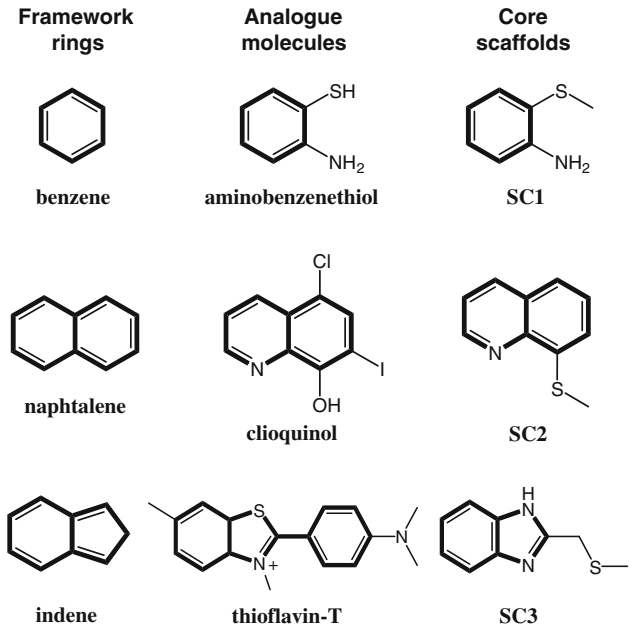

Fig. 1 Steps that lead to the design of the potential metal chelator scaffolds: "Framework rings", are common structural features present in drug-like molecules; "analogue molecules", are molecules that are employed in medicinal chemistry that enclose the framework rings; "core scaffolds", are the final designed scaffolds

As detailed in Fig. 1, it is worth noting that these scaffolds have structural features that resemble some well-known molecules involved in coordination chemistry and medicinal chemistry (shown in Fig. 1 as "analogue molecules" column). That is, scaffold **SC1** is structurally similar to aminobenzenethiol, a molecule that exhibits very strong chelating properties [25], in which the thiol group has been modified to a tioether to avoid the oxidation to disulfide. Scaffold **SC2** is analogue to clioquinol (**HCQ**), a drug that has exhibited a metal-sequestering behavior in Aβ deposits with relative success [26], in which the donor OH group has been substituted by a tioether group. Finally, scaffold **SC3** is similar to thioflavin-T, a dye molecule that plays an important role as intercalating agent in amyloid fibrils [27], in which novel features have been incorporated such as the participation of an imidazole ring and of a thioether as possible chelating groups [28]. It is worth mentioning that most of the compounds tested for MpND therapy contain N and/or O as chelating atoms [9], due to their great affinity towards metal ions such as Cu(II) and Zn(II). However, as previously mentioned, the selection of metal-protein attenuating compounds with intermediate metal affinities is an important issue. In this context, and in order to explore another series of compounds, we have considered chelators containing N and S as binding sites since their coordination to Cu(II) and Zn(II) is expected to be weaker than those containing N and O.

Assessment of the chelating properties of the scaffolds by quantum mechanical methods

Chelating properties of the previously designed scaffolds, **SC1**, **SC2** and **SC3**, towards Cu(II) and Zn(II) ions, have been evaluated by means of quantum chemical methods. In addition, and for the sake of comparison, we have considered the Cu and Zn complexes formed with clioquinol, an 8-hydroxyquinoline chelator that has been evaluated with relative success in transgenic mice [26]. This kind of calculations allows obtaining both structural features of the complexes as well as energetic data of their formation.

Zn(II) is a d^{10} metal cation and, as mentioned, its complexes usually presents a four coordination tetrahedral structure. Nevertheless, since the X-ray structure of the Zn(II) complex with clioquinol has the metal ion in a pentacoordinated environment [29], with two bidentate clioquinol and one H_2O molecules, we also performed quantum chemical calculations for the pentacoordinated complex. Results showed that in solution the tetracoordinated **[Zn(CQ)$_2$]** complex is more stable than the pentacoordinated **[Zn(CQ)$_2$(H$_2$O)]** one and thus, tetracoordination was assumed for all Zn complexes. Cu(II) is a d^9 metal cation that may show coordination numbers of 6 or 4 to form octahedral or square planar complexes, respectively. Nonetheless, since for this ion the occurrence of Jahn–Teller distortions elongates the axial bonds [30, 31], and the X-ray structure of the Cu(II) complex with clioquinol shows a square planar environment [29], in the present work, we assumed such a coordination for all Cu(II) complexes. Therefore, ML_2 species were considered for both Zn(II) and Cu(II).

The B3LYP-optimized geometries of the complexes formed between the Cu(II) and Zn(II) ions and the designed scaffolds are shown in Fig. 2 and the most relevant structural parameters are given in Table 1. As expected, Zn(II)- and Cu(II)-containing complexes adopt tetrahedral and square-planar like geometries, respectively. For Cu(II) complexes, however, noticeable distortions with respect to the strict square planar geometry are observed in some cases. As a measure of this distortion we have taken the dihedral angle,φ, defined by the two metallated ring planes. This angle ranges from 0^o for a square planar geometry to 90^o for a tetrahedral one, and its value reflects the degree of distortion from the reference geometries (tetrahedral and square-planar). It can be observed in Table 1 that the larger distortion occurs for **[Cu(SC3)$_2$]$^{2+}$**, with a φ value of 29.7°, significantly larger than that determined for the other two **[Cu(SC1)$_2$]$^{2+}$** and **[Cu(SC2)$_2$]$^{2+}$** complexes (φ = 9.9 and 0.1°, respectively). This can be attributed to the larger ligand-ligand repulsion occurring in **[Cu(SC3)$_2$]$^{2+}$** upon Cu(II) coordination in a square-planar fashion. The practically non-distorted

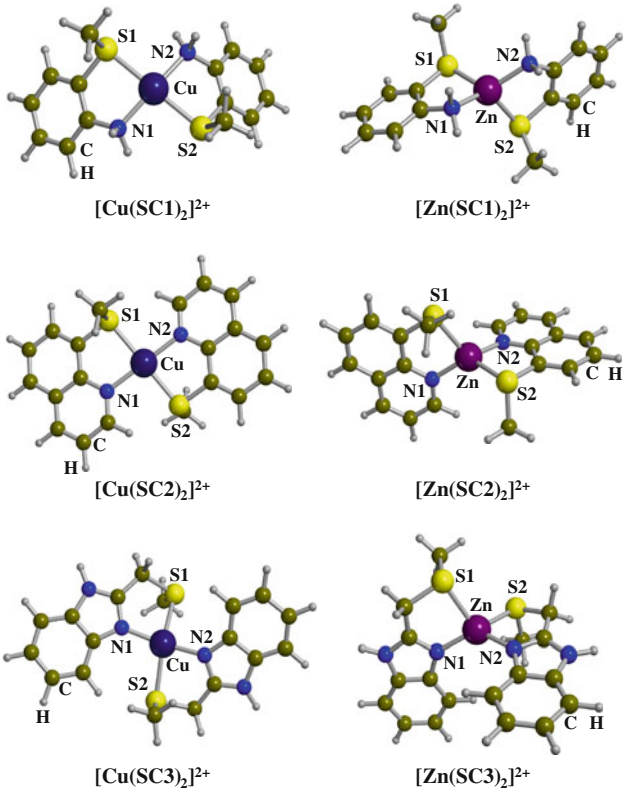

Fig. 2 B3LYP-optimized geometries of the complexes formed between the **SC1**, **SC2** and **SC3** scaffolds and the Cu(II) and Zn(II) ions

Table 1 Computed B3LYP-optimized structural parameters of the complexes formed between Cu(II) and Zn(II) ions and the scaffold ligands (**SC1**, **SC2** and **SC3**), and the candidate commercial compounds obtained via virtual screening (**VS2** and **VS3**)

Complex	Structural parameters				
	Cu-N1	Cu-N2	Cu-S1	Cu-S2	φ
$[Cu(SC1)_2]^{2+}$	2.042	2.042	2.413	2.413	9.9
$[Cu(SC2)_2]^{2+}$	2.043	2.043	2.393	2.393	0.1
$[Cu(SC3)_2]^{2+}$	1.978	1.970	2.461	2.464	29.7
$[Cu(VS2)_2]^{2+}$	2.044	2.044	2.394	2.394	0.1
$[Cu(VS3)_2]^{2+}$	1.984	1.975	2.477	2.463	25.1
Complex	Structural parameters				
	Zn-N1	Zn-N2	Zn-S1	Zn-S2	φ
$[Zn(SC1)_2]^{2+}$	2.087	2.087	2.384	2.384	85.5
$[Zn(SC2)_2]^{2+}$	2.024	2.027	2.402	2.401	87.2
$[Zn(SC3)_2]^{2+}$	1.987	1.987	2.481	2.477	83.9
$[Zn(VS2)_2]^{2+}$	2.031	2.034	2.395	2.396	86.7
$[Zn(VS3)_2]^{2+}$	1.993	1.991	2.465	2.468	85.2

φ refers to the torsional angle between the planes defined by the metallated rings. Bond lengths in Å, angles in degrees

Table 2 Computed reaction free energies at $T = 298$ K, ΔG_{sol} (in kcal mol^{-1}), and log β_2 of the formation of the complexes according to the reaction $[M(H_2O)_4]^{2+} + 2L \rightarrow [M(L)_2]^{2+} + 4H_2O$ for SC1 SC2 and SC3 ligands and $[M(H_2O)_4]^{2+} + 2HCQ \rightarrow [M(CQ)_2] + 4H_2O + 2H^+$ for clioquinol (HCQ), in water ($\varepsilon = 78.4$) and ethanol ($\varepsilon = 24.5$)

Complex	Water ΔG_{sol}	log β_2	Ethanol ΔG_{sol}	log β_2	Exp. log β_2
$[Cu(SC1)_2]^{2+}$	−16.9	12.4	−18.7	13.7	9.7 (0.04)
$[Cu(SC2)_2]^{2+}$	−25.9	19.0	−27.2	19.9	
$[Cu(SC3)_2]^{2+}$	−22.5	16.5	−25.3	18.6	18.4 (0.06)
$[Cu(VS2)_2]^{2+}$	−22.1	16.2	−23.6	17.3	
$[Cu(VS3)_2]^{2+}$	−19.5	14.3	−22.3	16.4	
$[Cu(CQ)_2]$[a]	−27.0	19.8	−34.6	25.4	21.91 (3)[a]
$[Zn(SC1)_2]^{2+}$	−5.8	4.2	−8.4	6.1	9.8 (0.09)
$[Zn(SC2)_2]^{2+}$	−16.2	11.8	−20.3	14.9	
$[Zn(SC3)_2]^{2+}$	−15.1	11.1	−20.0	14.7	17.6 (0.017)
$[Zn(VS2)_2]^{2+}$	−12.2	8.9	−15.0	11.0	
$[Zn(VS3)_2]^{2+}$	−10.6	7.8	−14.0	10.3	
$[Zn(CQ)_2]$[a]	−18.4	13.5	−25.1	18.4	20.23 (1)[a]

Experimental log β_2 stability constants are also added

[a] From Ref. [41]

geometries given in the Zn(II)-containing complexes indicates a low ligand-ligand repulsion when adopting a tetrahedral geometry. In order to determine the relative chelating ability of the different scaffolds, we have computed the reaction free energy of the complex formation in solution (ΔG_{sol}) at $T = 298$ K; i.e., we have considered the following reaction $[M(H_2O)_4]^{2+} + 2L \rightarrow [ML_2]^{2+} + 4H_2O$, hereafter referred as R1. Among the different strategies developed in quantum chemistry to account for solvent effects, the polarizable continuum model is often employed due to its good balance between accuracy and simplicity. This method represents the solvent as a continuous medium which is characterized by its dielectric constant (ε). Since according to experimental requirements, formation of these complexes proceed in a water/ethanol (1:1) solution, the energetics of R1 has been computed considering the species immersed in a continuum dielectric of either water or ethanol ($\varepsilon = 78.4$ and 24.5, respectively), as a first approximation to account for solvent effects. The computed ΔG_{sol} values are summarized in Table 2, together with an estimation of the stability constants log β_2. From these data the following conclusions can be obtained: (1) all computed free reaction energies are negative, so that the formation of all the complexes does indeed occur; (2) **SC1** scaffold is the one which exhibits the less affinity towards Cu(II) and Zn(II) ions, as it gives the less negative ΔG_{sol} values; (3) for Cu(II), **SC2** is the ligand that forms

the most stable complexes, although it is immediately followed by **SC3**, whereas for Zn(II) both scaffolds exhibit similar (and strong) chelating properties; (4) chelation is more favorable for Cu(II) than for Zn(II); i.e., the former complexes exhibit more negative ΔG_{sol} values, (5) reactions in ethanol are more favorable than in water, differences being around 3–5 kcal mol^{-1}. Additionally, the larger distortion from a square planar geometry observed in $\mathbf{[Cu(SC3)_2]^{2+}}$ (see φ in Table 1) causes ΔG_{sol} to be less negative than for $\mathbf{[Cu(SC2)_2]^{2+}}$ mainly due to solvent effects, thereby decreasing its stability constant.

In order to check the robustness of these results, the stability constants of the corresponding ML_2 (L = **SC1** and **SC3**) complexes have been determined by UV–vis titration experiments (details of the experimental procedure are given in the "Experimental Section"). Results are reported in Table 2 and indicate that: (1) Cu(II) exhibits stronger affinity towards all ligands than Zn(II) and (2) for both metal ions, the stability constants follow the order: **CQ** > **SC3** > **SC1**. Both trends are in perfect agreement with those provided by quantum chemical calculations, which validates the present strategy. In summary, quantum chemical calculations allows determining that among the three N,S chelators chosen (**SC1**, **SC2** and **SC3**) the last two are indeed the scaffolds that form more stable complexes with Cu(II) and Zn(II); i.e., are the best chelators. Thus, in the next step, the search for commercial compounds via virtual screening will be focused on compounds that enclose scaffolds **SC2** and **SC3**.

Search of commercial compounds enclosing the SC2 and SC3 scaffolds by virtual screening methods

Identifying potential lead molecules is day by day a more automated process. Molecule screening from different databases is currently an in silico cost-effective technique widely used in drug discovery processes [1, 3, 32]. This method provides a conduit for researchers to reach a rapid identification of potential active molecules so that experimental studies can be initiated with only one bioactive lead.

The first step in any virtual screening protocol is the removal of undesirable compounds from a commercial database to be searched. The Instant J Chem tool provides the ability to filter compounds based on a number of criteria (functional groups or molecular frameworks, molecular weight or pharmacokinetic properties). In our case, in order to identify a commercial metal chelating compound with potential application in MpND, we resorted to a virtual screening technique involving two main steps: (1) searching through the selected commercial database for structures that enclose **SC2** and **SC3**; (2) filtering the resultant list of molecules in such a way that the selected commercial compounds satisfy a set of chemical and pharmacokinetic requirements mandatory to deal with MpND. Table 3 reports the number of commercial compounds that pass the subsequent filters described below.

Table 3 Number of commercial compounds that enclose SC2 and SC3 and fulfill the subsequent constraints imposed

Filters	SC2	SC3
Enclosing scaffolds	430	160
Lipinski's rule and MW $\leq$ 450	320	150
log BB	99	40
Lead likeness	41	17
Visual inspection and basic coordination chemistry	4	8

One of the largest collections of molecules is assembled by emolecules®. It contains about 8 million commercial compounds from more than 150 suppliers. Among them, the first step of the virtual screening resulted in 430 and 160 molecules that contain **SC2** and **SC3**, respectively. These commercial compounds exhibit different drug-like properties as well as different degrees of lipophilicity, this latter one being a crucial property for crossing the blood brain barrier (BBB). Thus, not all these molecules are able to be used in MpND and a second set of successive filtering must be performed in order to obtain molecules that fulfill drug-like criteria.

The first filtering stage is inspired in the Lipinski's rule of five [1], which is commonly used to determine if a chemical compound with a certain pharmacological activity has properties that would make it a likely orally active drug in humans. This rule is based on the observation that most medication drugs are relatively small and lipophilic molecules. Thus, in analogy to these rules, molecules with a molecular weight (MW), less than 450 (although strictly Lipinski's rule suggests MW < 500), with a calculated logarithm of the octanol–water partition coefficient (so-called clogP) less than 5, and with a number of hydrogen-bond donor and acceptor atoms less than 5 and 10, respectively, should ensure brain permeation [3, 33]. Applying Lipinski's rule to the commercial compounds that enclose **SC2** and **SC3** reduces the number of candidates to 320 and 150, respectively. Compounds that satisfy the previous requirements have been newly filtered by calculating its log BB, which is a common measure of the degree of BBB permeation that has shown a very good predictive ability. It is stated that only those molecules with log BB ≥ -0.3 are able to cross the BBB [34]. Log BB can be computed by means of the Clark's equation [3], log BB $= -0.0148$TPSA $+ 0.152c\log P + 0.139$, which involves the above-defined clogP variable and the topological polar surface area (TPSA) [35, 36]. TPSA descriptor is defined as the sum of surfaces of polar atoms in a molecule and

provides good correlation with experimental transport through membranes. In particular, it has been successfully applied to predict BBB crossing [37]. In the present case, imposing this new filter significantly reduces the number of candidates, the number of compounds enclosing **SC2** and **SC3** becoming now 99 and 40, respectively.

Compounds obtained can be further filtered by considering the lead-likeness concept [32], which includes additional restrictions as compared to Lipinski's rules [1]. The drug-like filter permits larger and more highly functionalized molecules, while the lead-filter only allows smaller molecules, which are more suitable as starting points for an optimization program. We have taken into account additional computational criteria already reported in order to select lead-like compounds. These are: MW $\leq$ 460, $-4 \leq c\log P \leq 4.2$, $-4 \leq \log D_{7.4}$ (Log P at pH 7.4) ≤ 4, number of rotable bonds ≤ 10, number of rings ≤ 4, H donors ≤ 5 and H acceptors ≤ 9 [32, 38]. However, due to the fact that some of them have already been taken into account (e.g. $c\log P$ involved in log BB and the modified MW in Lipinski's rules) we have only incorporated the new criteria.

Up to this point, we have identified 41 and 17 commercial compounds enclosing **SC2** and **SC3**, respectively, that satisfy the previous constraints imposed (reported in the SI). Nonetheless, as a final stage, it is highly recommended to submit these resulting candidates to a visual inspection, especially to rule out those compounds that exhibit unwanted functionalities, as it is not desirable to waste resources removing them in the optimization stage. Some of the candidates contain halide atoms so that they can definitely be excluded since this kind of compounds are generally prone to solvolysis or hydrolysis and are normally reactive towards biological nucleophiles. Along this same line, other candidates contain sulphonyl groups which can also be ruled out since these groups may present unfavorable pharmacokinetic properties [39, 40]. On the other hand, there are some candidates that carry other donor atoms, in addition to the S and N enclosed in the scaffolds. These molecules, although not exhibiting any undesired reactivity with biological components, present competitive metal binding sites, thus rendering the metal coordination a more complex problem. Because of that, in order to simplify the metal binding to the ligand candidates, all these molecules have been excluded, thereby ensuring the metal-N/S coordination.

The set of commercial compounds enclosing **SC2** and **SC3** resulting from this last filter, 4 and 8, respectively, are very similar to the respective scaffolds (up to a point that **SC2** is indeed one possible candidate) and mainly differ in the aliphatic, allyl and aromatic side chains attached to the S atom. Among all of them, final selected candidates, **VS2** (8-(allylthio)quinoline) and **VS3** (2-((allylthio)methyl)-1*H*-benzo[*d*]imidazole), shown in Fig. 3, are the simplest ones. The main structural difference between **SC2/SC3** and **VS2/VS3** is that the methyl group attached to the S atom in the former set is substituted by a $CH_2CH{=}CH_2$ allyl group in the latter one.

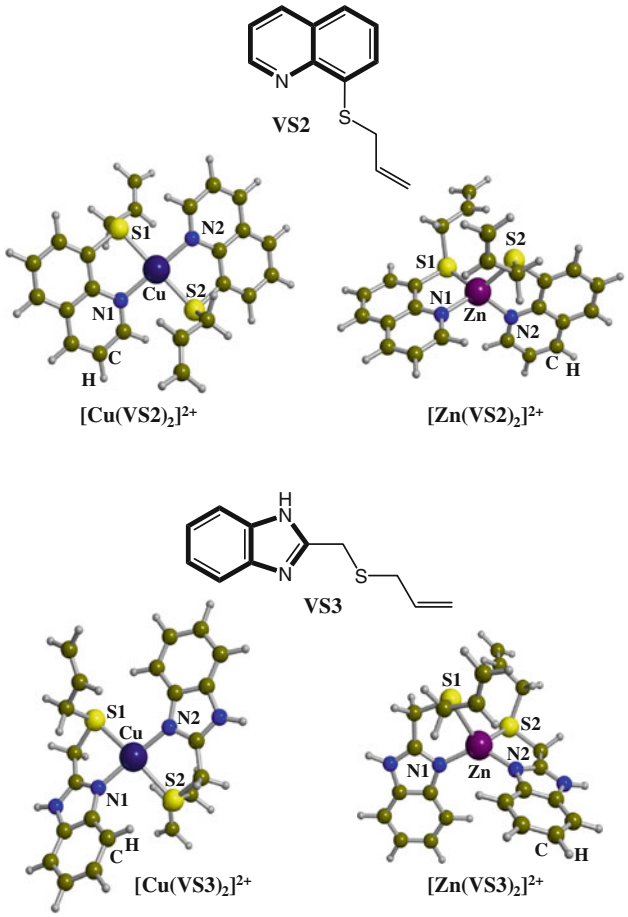

Fig. 3 Commercial compounds (**VS2** and **VS3**) obtained through database virtual screening that fulfill all the imposed filters and the B3LYP-optimized geometries of the Cu(II)–Zn(II)-complexes

In order to evaluate the chelating properties of **VS2** and **VS3**, their Cu(II) and Zn(II) complexes have been studied by means of quantum chemical calculations. The B3LYP-optimized complexes are presented in Fig. 3 and the structural parameters summarized in Table 1. As expected due to the structural similarity between the scaffolds and the commercial compounds, the **VS2** and **VS3** metal complexes present similar optimized parameters compared to the **SC2** and **SC3** ones, respectively. Moreover, ΔG_{sol} values indicate that complex formation is also a favorable process for both metal ions and ligands, although computed values are somewhat less negative than those obtained for the respective scaffolds. This is probably due to the fact that **VS2** and **VS3** are larger ligands, which introduce larger ligand-ligand repulsions in the complex. As a result,

values of log β_2 for Cu(II) and Zn(II) with **VS2** and **VS3** are smaller than to the corresponding scaffolds. Overall, the metal–ligand affinity depends on various factors, such as the ligand-ligand repulsion, which in turn may influence the coordination environment, or the chelating ability of the basic sites. Along this latter line, quantum chemical calculations could help tuning the ligand chelating properties by introducing functional groups that exert electronic effects (i.e., electron donor/withdrawing), thereby modulating the stability constants to the desired needs.

The same in silico-based strategy was already adopted by some of us [41] in order to identify multifunctional commercial compounds based on the 2-phenyl-1*H*-indene molecular ring, and containing N, O and S atoms, for application in AD. The three final molecules selected in that search were experimentally assayed, the results indicating that they can behave as efficient Cu(II) and Zn(II) chelators. In particular **HBX** (2(-2-hydroxyphenil)benzoxazole) was found to be a very good chelator with log β_2 values of 23 and 22 for Cu(II) and Zn(II), respectively, somewhat larger than those determined for clioquinol (21 and 20, respectively). Present ligands show smaller stability constants (16–17 for Cu(II) and 10–11 for Zn(II)) but differences between the two metal ions are larger, which may be useful to preferentially sequester Cu(II) over Zn(II). Moreover, smaller stability constants may be more adequate for ligands that should act as metal-protein attenuating compounds [12].

Conclusions

This work presents an in silico strategy to identify commercial compounds that exhibit suitable pharmacokinetic and coordination properties towards Cu(II) and Zn(II) to be used as potential metal-chelators in neurodegenerative diseases.

The strategy presented entails the following successive steps:

1. Designing core scaffolds that include the proper binding sites.
2. Evaluating the chelating properties of the scaffold by means of quantum mechanical calculations on the Cu(II)- and Zn(II)-scaffold complexes.
3. Searching commercial compounds that enclose the designed scaffolds through database virtual screening and filtering these molecules according to a set of drug-like requirements mandatory to deal with MpND.
4. Determining the chelating properties (log β_2) of the final candidates by quantum chemical methods.

Adopting this procedure, three different scaffolds (**SC1**, **SC2** and **SC3**) enclosing N and S donor atoms were initially designed, and the binding properties evaluated by quantum chemical calculations. Results indicated that, among them, **SC2** (8-(methyl)thioquinoline) and **SC3** (2-((methylthio)methyl)-1*H*-benzo[*d*]imidazole) exhibit the best chelating properties towards Cu(II) and Zn(II), and accordingly, the subsequent virtual screening was focused on identifying commercial compounds that enclosed these two scaffolds. The large number of compounds identified with this screening was then submitted to chemical and pharmacokinetic filtering subsets. These filters are based on: (1) the Lipinski's rule of five, which evaluates drug-likeness; i.e., determines if a certain chemical compound has properties that would make it a likely active drug in humans, (2) the ability to cross the BBB, determined by calculating the log BB through the Clark's equation, (3) the lead-likeness, which satisfies Lipinski's rule of thumb but includes additional selection criteria that have been recognized in effective leads, and (4) a visual inspection of the final candidates, in order to avoid carrying unwanted functionalities. At the end of this stage, several commercial compounds were identified, and the chelating properties of the simplest ones, **VS2** (8-(allylthio)quinoline) and **VS3** (2-((allylthio)methyl)-1*H*-benzo[*d*]imidazole), evaluated by computing the stability constants (log β_2) through quantum mechanical calculations, which indeed indicated the suitability of the candidates to be possible metal chelators with potential applications in MpND theragnosis.

Overall, combination of in silico virtual screening methods with quantum chemical calculations in solution appears to be a promising protocol to design new metal chelating ligands with the desired properties for application in neurodegenerative diseases. Quantum chemical calculations, whose reliability has been confirmed by comparing the computed stability constants in solution (log β_2) with those obtained experimentally by UV–Vis titration, provide information on the electronic and molecular structure of the coordinating complexes, as well as on the metal binding affinity. Virtual screening enables us to identify commercial compounds that enclose the desired molecular framework and include the proper pharmacokinetic properties for their potential use in metal promoted neurodegenerative diseases.

Experimental section

Virtual screening

Database and substructure (scaffold) searching: eMolecules, Inc. is a free openly source chemical structure search engine (http://www.emolecules.com). It contains about 8 million of commercial compounds from more than 150 suppliers. The web interface supports both structure search

and text search (Chemical name, CAS number, SMILES). For structure search, chemists can draw the molecular structure into ChemWriter, a Java application, implemented by emolecules interface [42]. In the searching process, there are two basic categories (exact structure or substructure search) that consist of finding all the compounds in the database that have the given structure of a substructure. First of all, each scaffold (or substructure) is drawn in ChemWriter and the resultant molecules are presented in a table containing 2D chemical structure, molecular properties such as MW and purchasing information. The second step consists of filtering the resultant search based on the presence of a sulphonyl group or a N,S-heterocyclic ring as part of the binding site in its structure. Hence, those structures without a sulphonyl group or a N,S-heterocyclic ring are rejected. All the resultant data is downloaded as SDF for further analysis.

Filtering subsets: The list of resultant molecules obtained in the previous steps (subsets) is uploaded in Instant Jchem v.5.3.1 (IJC) software [38]. This program is able to visualize, calculate chemical descriptors and manage resultant data in order to filter large amounts of molecules. An initial filtering stage was used to remove those molecules not satisfying the following ADME (absorption, distribution, metabolism and elimination) properties, according to criteria inspired by Lipinski's rule of five: molecular weight (MW) between 200 and 450 Daltons, clogP less than (or equal to) 5, number of hydrogen-bond acceptors (HBA) less than (or equal to) 10, hydrogen-bond donors less than (or equal to) 5. In a second filtering stage the log BB of each molecule was determined according to the Clark's equation: $\log BB = -0.0148\ TPSA + 0.152 clogP + 0.139$. For this purpose, it was necessary to calculate the topological Surface Area (TPSA). Exceptuating clogP, all software applications used above are parts of ChemAxon's Jchem software suite (100% Java) [38]. Physicochemical calculations are provided by ChemAxon's prediction plugins [38]. Hardware and software requirements are as follows: any system running Java Runtime Environment 1.4 or above. ClogP was calculated using the cLogP v.4.0 program (http://biobyte.com.index.html) developed by Hansch and Leo [43].

Quantum mechanical calculations

All calculations were performed using the Gaussian03 set of programs [44]. Molecular geometries of both the Cu^{2+}- and Zn^{2+}-complexes and of the free chelating ligands were optimized using the non-local hybrid three-parameter B3LYP density functional method [45, 46], a robust and well tested functional that has been successfully used in a great variety of systems including open shell Cu(II) complexes with saturated coordination environments and similar spin density distribution. For Cu and Zn we have employed the Watcher's primitive set (14s9p5d) [47], supplemented with one s, two p, and one d diffuse functions, and one f polarization function [48], the final basis set being (15s11p6d1f)/[10s7p4d1f]. For C, N, O, S, and H the standard 6-31 ++G(d,p) basis set has been employed. Thermodynamic corrections have been obtained assuming an ideal gas, unscaled harmonic vibrational frequencies, and the rigid rotor approximation by standard statistical methods [49]. Solvation effects were modeled through single-point energy calculations at the same level of theory, with water and ethanol as solvents, using the self-consistent field polarizable continuum model, COSMO [50, 51]. In order to improve the thermodynamic calculations of the $[M(H_2O)_4]^{2+} + 2L \rightarrow [ML_2]^{2+} + 4H_2O$ reaction in solution, we have considered the experimental free energy values for the solvation of water ($\Delta G_{Solv(H2O)}$), −6.31 kcal mol^{-1} [52], when considering water as solvent, and −7.56 kcal mol^{-1} for ethanol as solvent. For clioquinol (HCQ) the generic reaction for the formation of the complexes is $[M(H_2O)_4]^{2+} + 2HCQ \rightarrow [M(CQ)_2] + 4H_2O + 2H^+$, since HCQ deprotonates upon coordination. In this case, the reaction energy in solution, ΔG_{Solv}, is computed assuming that the solvation energies of H^+ ($\Delta G_{Solv(H+)}$) in water and in ethanol are −265.9 [53] and −263.5 kcal mol^{-1} [54], respectively, for the ethanol case adopting the strategy followed by Shroff et al. [55]. Since these reactions occur in solution, the entropy obtained in gas phase was converted from 1 atm to 1 M by subtracting the $R \ln(V_1/V_2)$ cal K^{-1} mol^{-1} term to account for the volume change between the two states at 298 K [56]. Also the terms of RT ln(55.6) and RT ln(17.6) were added to the respective $\Delta G_{Wat(H_2O)}$ and $\Delta G_{Eth(H_2O)}$ terms, since liquid water and ethanol concentrations are 55.6 and 17.6 M, respectively. Further details are available in SI.

UV–vis determination of stability constants

Since compounds **SC1** and **SC3** are slightly soluble in water, ethanolic 0.25 and 0.5 mM stock solutions were prepared, respectively. In each titration, the solvent composition in the vessel was 50% (v/v) ethanol/water. For each compound, the acidity constant was determined by titrating a 0.10 and 0.012 mM solution of the compound, respectively, with microadditions of 0.5 M NaOH standard solution, recording the UV–vis spectrum at each titration point. At least 30 spectra were taken in the pH range 2–11. The data were analyzed by importing both the absorbance and pH values into the pHab2000 computer program [57], which calculates acidity constants by means of a linear least-squares curve-fitting analysis. The stability constants for the Cu(II) and Zn(II) complexes with **SC1** and **SC3** were also determined by UV–vis pH titrations under the same conditions as outlined above. The ligand-to-metal

molar ratios used in the experiments were 1:1 and 2:1 for each ligand with M = Cu(II) or Zn(II). For each titration, at least 30 spectra were recorded in the pH range 2–12. As with the p*K*a values, the stability constants were calculated with the pHab2000 computer program. Speciation diagrams for the free ligands and the corresponding complexes [M = Cu(II) or Zn(II); L = **SC1** and **SC3**] were calculated using the program HySS2009 [58].

Electronic Supplementary Information

The most frequently occurring frameworks present in drug-like molecules, commercial compounds obtained previous to visual inspection, details related to the thermodynamic cycle employed to obtain ΔG_{sol} of reaction R1, and solution speciation diagrams for SC1 and SC3 at protonation and with Cu(II) and Zn(II).

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