In silico study of MMP inhibition†

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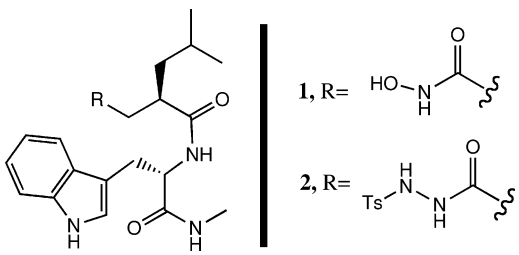
Lack of enzyme inhibition selectivity is frequently the major drawback preventing the development of enzyme inhibitors. Sulfonylhydrazides have recently been suggested to act as zinc ligands. Consequently, such derivatives potentially possess important industrial or therapeutic implications. DFT calculations (B3LYP/6–31G**+LANL2DZ theory level) of the binding modes and free energies of binding of a variety of *N*-acetyl-*N′*-sulfonylhydrazides in the presence of a Zn^{2+} ion embedded in an MMP active site model show that protonated and deprotonated sulfonylhydrazides bind the Zn^{2+} ion according to different modes. These results strongly suggest that sulfonylhydrazides can be developed as selective metalloprotease inhibitors, and the results of molecular docking computations fully support this hypothesis.

Introduction

Industrially, zinc-chelators are valuable molecules whose commercial use ranges from mineral animal food complements,[1] to fertilizers,[2] or crop protectors.[3] The electroluminescent properties of some zinc-chelators have also recently propelled them into the optoelectronic field.[4] In the therapeutic field, the zinc ion is essential in the brain,[5] zinc-chelators are physiologically relevant metal detoxifiers[6] and zinc enzymology by itself represents a specific domain since zinc is the second-most abundant transition metal in biology.[7] Therefore, chemists are actively developing zinc-chelators, recently in particular as matrix metalloprotease inhibitors (MMPIs),[8] carbonic anhydrase inhibitors,[9] or anthrax lethal factor inhibitors.[10] This latter research field is more specifically aimed at fighting bioterrorism, whereas the formers are a consequence of the involvement of carbonic anhydrase or MMPs in numerous essential physiological processes and in pathological conditions. To consider the MMP case only, specific inhibition of one (or of one homogeneous subgroup) of the 25 MMPs known in humans is now largely accepted as a promising therapeutic strategy.[11] The essentiality of MMP-inhibition specificity has then been reinforced by the discovery of the pro-tumorigenic properties of some inhibited MMPs, leading to their identification as anti-targets.[12] Specific MMP inhibition is particularly challenging because of the structural homology shared by all MMPs in the active site of which a catalytic Zn^{2+} ion coordinated to the imidazole moiety of three histidine residues is consistently localized. This zinc ion is used as an anchor by most of the synthetic MMPIs.[8b,13] Highly interestingly, selective inhibition among MMPs has recently been shown to be possible through zinc-binding modulation.[14] Such news has come as a relief since the tremendous efforts made to identify selective MMPIs by modulating the inhibitor enzyme-subpocket affinity have so far remained unrewarded. We are currently working[15] on the pharmacomodulation of galardin (**1**), an efficient but poorly selective MMPI,[16] and have recently reported that the replacement of the poorly selective hydroxamate moiety of **1** by an arylhydrazide residue was leading to much stronger inhibitors of MMP-2 *vs.* MMP-1, such as **2**.[17a] This suggests that the sulfonylhydrazide moiety could possess the zinc binding properties chased by medicinal chemists.[18]



If the transition-metal complex stability has initially been considered as simply governed by the Irving–Williams rule,[19] Zn^{2+} and heteroatom-rich ligands are now known to yield different types of complexes[20] presenting different stability parameters. Though the propensity of hydrazine to form mono- or bidentate complexes,[21] and the biological interest of metal-complexed sulfonylated sulfamides, arylsulfonyl-carbonylsulfamides, and sulfamide Schiff bases have been studied,[22] only the osmium-binding properties of sulfonylhydrazides have been reported.[23] To the best of our knowledge, the Zn^{2+}-binding properties of sulfonylhydrazides remain totally unexplored.

Herein we report the DFT study of the binding modes and free energies of binding of a series of sulfonylhydrazides, in the gas phase and in stoichiometric conditions, in the presence of an isolated Zn^{2+} ion or of a Zn^{2+} ion liganted to three 4-methylimidazole in order to mimic the three histidine imidazole moieties of the MMP active site. The study was performed using the B3LYP/6–31G**+LANL2DZ level of theory, a level which has been shown reliable for this kind of study.[24] In a second stage,

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and because our results validated the idea that sulfonylhydrazide derivatives could be useful selective MMPIs, we also evaluated the influence of different MMP active sites surrounding the protein matrix and binding cavity on the chelate formation by molecular docking.

Results and discussion

Isolated Zn^{2+} ion

We performed our calculations using **3** as the single representative of the alkylsulfonylhydrazide family since very few alkylsulfonyls are used as zinc-chelators and arylsulfonyls are particularly prone to favor metal chelation.[25] From the arylsulfonylhydrazide family, we chose **4–10** in order to evaluate the influence of the aromatic ring substituents on the zinc-binding ability of these molecules.

Among all the possible conformations available for **3–10**, only two were found with respect to the HN_SN_CH dihedral angle (Fig. 1): a *trans* conformation, found for all eight compounds, and corresponding to an HN_SN_CH dihedral angle of −133° to −139° (−160° for **8** due to an extra $NO_2 \cdots HN_S$ hydrogen bond); and a *cis* conformation, found for all eight compounds but **3** and **8**, displaying an HN_SN_CH dihedral angle of −73° to −76°. Energetically, for each molecule, the *trans* conformation was found to be 5–6 kcal/mol more stable than the *cis*.

Then, we combined the Zn^{2+} ion and our *cis* and *trans* optimized structures. Our calculations indicated that sulfonylhydrazides behave as first-shell ligands[26] and can bind to the Zn^{2+} ion according to two different general complex types named type I and II. Finding multiple complex types was not surprising since the Zn^{2+} ion is well-known to sustain different coordination numbers. Fig. 2 shows the calculated structures. In all cases, an interaction between the Zn^{2+} ion and the sulfonamide nitrogen was observed. Such behavior reflects the expected strongest acidity of the sulfonamide proton compared to its amide counterpart. For all calculated complexes, two geometrical features can be emphasized: (1) the S–N bond is about 0.2–0.3 Å longer in the liganted form, compared to the isolated form, and (2) the chelation consistently occurs under a *cis* conformation (HN_SN_CH dihedral angle between −35° and −63°) of the sulfonylhydrazides even though the starting conformation was *trans*. Because of the large energy gap between the *cis* and *trans* unliganted molecules, it is likely that the conformational switch occurs during the chelate formation process and not prior to its formation.

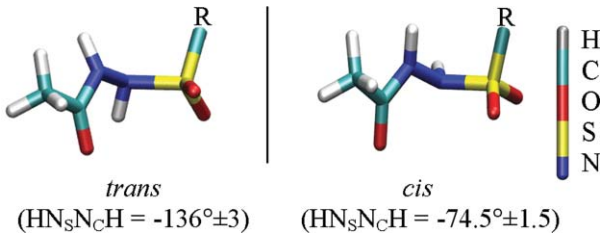

Fig. 1 Representation of the *trans* and *cis* conformations calculated for **3–10** and **4–8**, respectively.

The first complex type (Type I) results from a bidentate zinc coordination involving the oxygen of the carbonyl group and the sulfonamide nitrogen atom. This type of coordination is

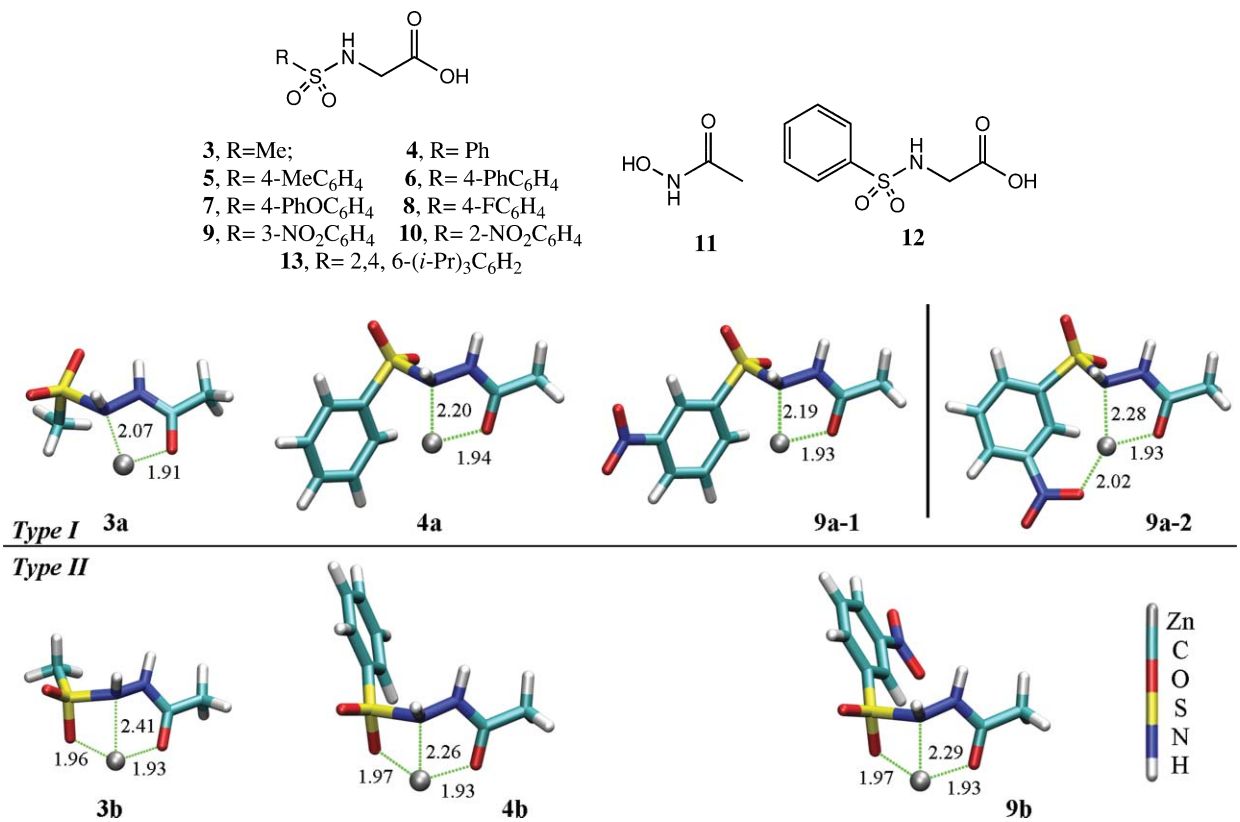

Fig. 2 B3LYP optimized type I and II structures of **3**, **4**, and **9** bound to the Zn^{2+} ion (distances in Å).

highly similar to the coordination depicted for *N*-tosylglycine,[27] strongly suggesting that *N*′-acetyl-*N*-sulfonylhydrazides and *N*-α-sulfonylamino-acids share similar metal binding properties. For the aromatic ring containing derivatives **4–10**, our calculations indicated also an extra π-coordination between the zinc and the aromatic system. The strength of that coordination was evaluated by measuring the distance between the zinc ion and the aromatic ring centroid, and the angle between the distance segment and the aromatic ring plane. In each case a distance of 3.04 ± 0.05 Å and an angle of 41.5° ±1.6 were obtained (for **9a-2** the distance and angle were 3.52 Å and 37.5°, respectively, since an additional coordination with NO_2 occurred). Interestingly, NO_2-substituted **9** and **10** behaved differently, reflecting the importance of the presence of additional chelating atoms on the aromatic moiety. For the *m*-NO_2-substituted derivative **9**, in addition to the pure type I coordination mode (**9a-1**), a form with an extra coordination involving the oxygen of the NO_2 group was also found (**9a-2**). This modified pattern resulted in a slight weakening of the NH–Zn interaction (2.28 *vs.* 2.19 Å) and the strong reduction of the π-stacking interaction (3.52 *vs.* 3.04 Å) to accommodate the bending of the molecule. The CO–Zn coordination in **9a-1** remained strong and unaffected (1.93 Å), so it appears that this coordination is driving the chelate formation. Finally, we explored the *o*-NO_2 substituted derivative **10**. In that case, no minimum was found for a type I chelate and only a form involving the SO_2 group was obtained (type II, *vide infra*).

The second complex type calculated (Type II) results from a triple interaction between the Zn^{2+} ion and the ligand. This leads to a penicillin-like skeleton due to interactions involving, in addition to the *N*-sulfonamide–Zn interaction, two equally coordinated oxygen atoms: one from the acetyl function and one from the sulfonyl function. The bond length between these oxygen atoms and the zinc is of 1.94 ± 0.01 Å and 1.98 ± 0.01 Å, respectively. For compounds **3–9**, the coordination formed by the sulfonamide nitrogen atom that acts as a Lewis base has a bond length between 2.12 and 2.41 Å indicating a weaker coordination, compared to the two O–Zn interactions. However, this coordination appeared to be strengthened by electron donating groups on the aromatic ring (the bond length increases to 2.25 and 2.30 Å for **8b** and **9b**; respectively). This neatly suggests that in the type II coordination mode, the nature of the aromatic ring substituents can also influence the ligand coordination strength. As in the 2-nitrophenylsulfonyl amino acid series,[27] *ortho*-substitution with a NO_2 group dramatically modifies the chelation mode of the *N*-acetyl-*N*′-sulfonylhydrazides. Indeed, for **10** two modified type II forms (**10b-1** and **-2**) were calculated. Both of them incorporate a tetradentate zinc. Compared to the chelate of type II, the extra coordination concerns the Zn^{2+} ion and the NO_2 group. In this new pattern the strength of the coordination induced by the acetyl group was unchanged whereas the N–Zn and Zn–O (O of SO_2) distances were moderately increased, reflecting a weaker interaction that was counterbalanced by a strong Zn–O–NO chelation (2.0 ± 0.06 Å). Involvement of the *o*-NO_2 aromatic substituent in lead or zinc coordination by *N*-2-(nitrophenylsulfonyl)-glycine has already been experimentally observed.[28] In terms of geometry, chelates **10b-1** and **-2** can be seen as differing by the orientation of the SO_2 group. As a consequence of the chelation, **10b-1** and **-2** can be seen as the *Rs* and *Ss* derivatives, respectively (Fig. 3).

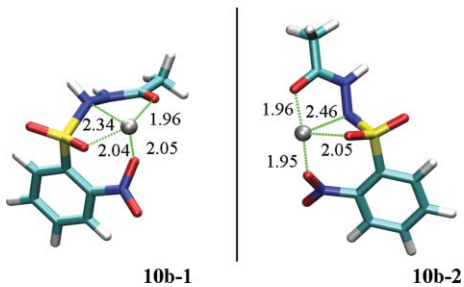

Fig. 3 B3LYP optimized structures of **10** bound to the Zn^{2+} ion (distances in Å).

In terms of binding Gibbs free energy at 298° K (Table 1), and for comparison purposes, we first used acetohydroxamic acid (AHA, **11**) since it is a well-known zinc chelator.[29] Calculated free binding energy of the acetohydroxamic acid–Zn^{2+} complex was found to be 159.9 kcal/mol lower than its separated constitutive species (Table 1). The bidentate type I chelate formed by **1** was found to be in the same energetic range (–174.7 kcal/mol) whereas aromatic-containing sulfonylhydrazides showed an even greater stabilization

Table 1 Binding energy (ΔE) and binding Gibbs free energy (ΔG°) at 298° K in kcal/mol for **1–8** and **11**. Numbers between brackets do not include ZPE corrections

	ΔE		ΔG°		
Ligand	Type I (a)	Type II (b)	Type I (a)	Type II (b)	$\Delta\Delta G^{0a}$
3	–182.9 (–183.4)	–209.9 (–210.9)	–174.7	–200.5	–25.8
4	–219.3 (–220.5)	–225.3 (–226.5)	–209.0	–215.5	–6.5
5	–224.0 (–225.1)	–230.0 (–231.1)	–213.4	–219.7	–6.3
6	–228.3 (–229.2)	–234.9 (–235.5)	–218.2	–226.0	–7.8
7	–226.7 (–227.6)	–234.6 (–235.6)	–216.3	–224.4	–8.1
8	–212.8 (–213.9)	–222.5 (–223.7)	–202.8	–212.8	–10.0
	a-1 –223.2 (–224.3)		**a-1** –212.1		+10.2
9	**a-2** –200.1 (–200.7)	–211.8 (–212.7)		–201.9	
			a-2 –190.3		–11.6
		b-1 –242.8 (–244.2)		**b-1**-232.4	—
10	—	**b-2** –233.1 (–234.3)	—	**b-2** –222.5	—
11	–168.0 (–169.0)			–159.9	—

[a] $\Delta\Delta G^0 = \Delta G^\circ_{II} - \Delta G^\circ_{I}$.

likely due to the additional π-interaction. Type II complexes (tridentate) were found to be even lower in energy than their corresponding type I analogues (Table 1). Consequently, binding of the sulfonylhydrazides to Zn^{2+} in the bi- or tridentate mode induces a noticeable stabilization. Between the bi- and tridentate mode, the larger energetic difference ($\Delta\Delta G^0$) was observed for the alkylsulfonylhydrazide **3** (−25.8 kcal/mol). In the aromatic series, a $\Delta\Delta G^0$ of −6 to −12 kcal/mol was calculated. Concerning the NO_2 series, for compound **9**, the multidentate chelate **9a-1** including the NO_2 group was found to be 21.8 kcal/mol lower in energy than the pure type I form **9a-2**. The larger stabilization energy of −232.4 kcal/mol was obtained for **10b-1** in which the most favorable type II chelation is intensified by the NO_2 chelation.

Having demonstrated the zinc-binding potential of arylsulfonylhydrazides and some of the binding-governing parameters, we then decided to evaluate their zinc-binding ability in an enzyme active site model composed of a Zn^{2+} ion liganted to three 4-methylimidazole.

Imidazole-liganted Zn^{2+} ion

Prior to carrying out our calculations, we considered it necessary to validate our computational model. For the Zn(4-methylimidazole)$_3$ model, that was achieved by comparing our geometry parameters calculated for $[Zn(4\text{-methylimidazole})_3H_2O]^{2+}$ with reported data for $[Zn(imidazole)_3H_2O]^{2+}$ at the B3LYP/6–31G* level of theory.[30] A good agreement was observed in terms of bond length (2.134 Å *vs.* 2.091 Å for the Zn–O distance) and angle (108.0°, 103.1°, 100.3° *vs.* 110.0°, 102.1°, 100.4° for O–Zn–N angles). Then we validated the coordination mode aspect using **1**, acetylhydroxamic acid (AHA, **11**), and finally *N*-phenylsulfonylglycine (**12**) as a representative of the well studied arylsulfonamide MMPI family.[31] In each case, our calculations yielded the currently accepted coordination form as the most energetically favorable complex.

Then, we began our calculations using **3** again as a representative of the alkylsulfonylhydrazide family and **4–10** and **13**[32] as members of the arylsulfonylhydrazide type. For all the evaluated compounds but **3**, a single mode of binding corresponding to a tridentate form was obtained (Fig. 4). For **3**, an alternative and almost similar in energy state corresponding to a monodentate chelate involving the carbonyl oxygen and the Zn^{2+} ion (Zn–O distance 2.06 Å) was identified in addition to the tridentate mode of binding. Moreover, the calculated mode of binding shared by **3–11** and **13** displayed a *cis* HN_S-N_CH geometry totally reminiscent of the type II binding mode calculated for the isolated Zn^{2+} ion. However, close examination of different complexes showed that, conversely to type II, in the presence of the three imidazole groups, the

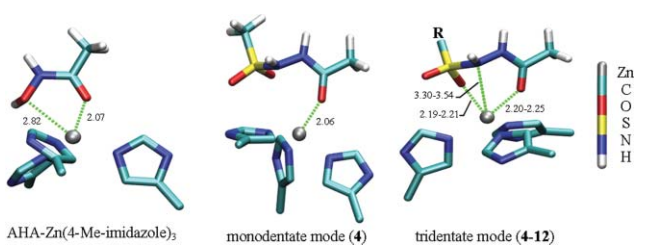

Fig. 4 B3LYP optimized structures of **3–10** and **13** liganted to the Zn(4-Me-imidazole)$_3$ [(4-MI)$_3$Zn)] complex (distances in Å).

Table 2 Binding energy (ΔE) and binding Gibbs free energy (ΔG°) at 298° K in kcal/mol for **3–11** and **13**

Ligand	ΔE	ΔG°	ΔΔG[a]
3	−12.1 (−10.9)	−6.6	2.6
	−12.3[b] (−11.5)[b]	−7.2[b]	2.0[b]
4	−15.7 (−14.9)	−9.0	0.2
5	−17.4 (−16.5)	−10.3	−0.9
6	−16.0 (−15.1)	−9.4	−0.2
7	−16.8 (−15.9)	−9.8	−0.6
8	−13.3 (−12.3)	−7.2	2.0
9	−7.5 (−6.4)	−1.5	7.7
10	−12.9 (−12.3)	−5.6	3.6
11	−12.4 (−11.5)	−9.2	—
13	−15.3 (−14.2)	−9.0	0.2

[a] ΔG° calculated *vs* **11**. [b] Monodentate mode.

sulfamide nitrogen atom was now localized in the zinc second-shell[25] (bond length of ~3.35 Å) whereas the two oxygen atoms acted as first-shell ligands (bond length <2.25 Å). Identical results were obtained when a type I conformation was used as the initial step for the DFT calculation. Interestingly, all chelates resulting from arylsulfonylhydrazide **4–10** presented very similar geometric features with H–N–N–H, C–N–N–S, and N–N–C–S dihedral angles of ~−48°, −90°, and −53°, respectively (ESI).† Even the two bulky *i*-Pr groups placed on each side of the ipso-carbon in **13** did not modify the geometry of the chelate.

Formation of the tridentate chelates generally resulted in a gain of ~15 kcal/mol, compared to Zn(4-methylimidazole)$_3$H$_2$O, (Table 2) suggesting that, conversely to the results observed for the isolated Zn^{2+} ion, the aromatic substituents have little, if any, influence on the binding properties of the arylsulfonylhydrazides when the zinc is liganted to the three imidazoles. The energy gain for **3** and **10** was calculated to be only ~12 kcal/mol. Therefore, the hypothesis that **3** could bind to the zinc under two distinct forms, or as an equilibrium between two distinct forms, in the enzyme active site should not be discarded.

Some Zn-chelator enzyme inhibitors have been shown to actually act as deprotonated species into the enzyme active-site.[18a,30] Consequently, we also decided to evaluate the ligand properties of a selection of sulfonylhydrazides (**3**, **4**, **7**, and **10**) deprotonated on the sulfamide nitrogen whose pK is influenced by the aromatic ring substituent.[23] Comparison of our calculated bond length and angle with the literature values reported for *O*-deprotonated acetohydroxamic acid[30] and X-ray structures[33] allowed us to validate our calculation theory level in the anionic form. The initial state conformation is known to be of critical importance for calculation. Although MMP inhibition has been shown to result from several successive events,[34] the intimate sequence of the events leading to substrate deprotonation is unknown. Consequently, we performed two sets of calculations. The first started from the tridentate complex conformation previously calculated and from which the sulfamide proton was removed. The second started from a monodentate form in which the sulfamide nitrogen anion was directly bound to the Zn^{2+} ion. Our first set of calculations afforded tridentate complexes reminiscent of those observed with the protonated species as the most stable conformation. For compounds **3**, **4**, and **7** the anion-zinc chelation was found to be stronger than in the protonated series as shown by the shorter N–Zn distance (2.4 to 3.1 Å) (Fig. 5). Unexpectedly, in the case of **10**

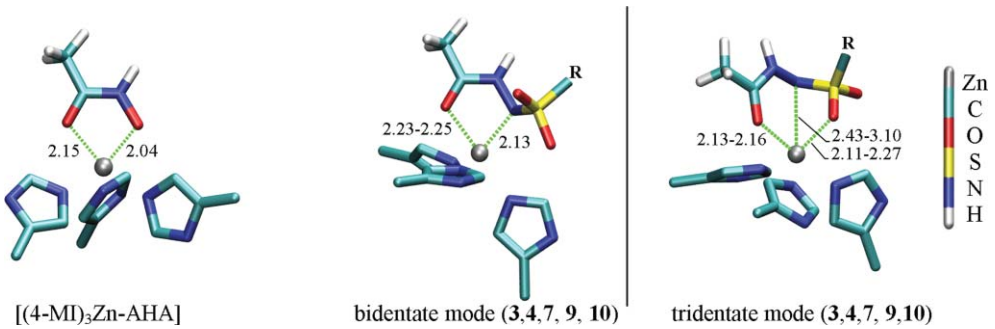

Fig. 5 B3LYP optimized structures of the anion of **3**, **4**, and **7**, **9**, **10**, and **11** liganted to the Zn(4-Me-imidazole)$_3$ complex (distances in Å).

Table 3 Binding energy (ΔE) and binding Gibbs free energy (ΔG°) at 298° K in kcal/mol for deprotonated **3**, **4**, **7**, **9–11**. Numbers between brackets do not include ZPE corrections

Ligand	Mode	ΔE	ΔG°	ΔΔG[a]
3	*bidentate*	−172.1 (−171.8)	−166.4	33.1
	tridentate	−167.0 (−166.8)	−161.4	38.1
4	*bidentate*	−166.5 (−166.3)	−160.7	38.8
	tridentate	−162.8 (−162.2)	−157.5	42.0
7	*bidentate*[b]	−161.8 (−161.7)	−153.2	46.3
	tridentate	−158.5 (−158.1)	−152.2	47.3
9	*bidentate*	−153.4 (−153.0)	−147.9	51.6
	tridentate	−149.5 (−149.2)	−143.4	56.1
10	*bidentate*	−155.0 (−154.8)	−148.8	50.7
	tridentate[c]	−154.9 (−155.1)	−146.7	52.8
11	*bidentate*	−203.0 (−202.9)	−199.5	—

[a] $\Delta\Delta G^0$ calculated *vs* **11**. [b] Due to a flat potential energy surface, we could not remove a very small imaginary frequency (2i). [c] Pseudo tridentate (Zn–N longer than 3 Å).

this bond was found to be longer (3.7 Å), confirming the reduced zinc-binding ability of **10** in its protonated or deprotonated form. When we performed our calculations from the N–Zn monodentate initial state, only the type I bidentate conformation calculated during our isolated zinc study, and similar to that proposed for sulfonylated aminoacid hydroxamate anions,[35] was found.

Comparison of the energy level of the bi- and tridentate conformations calculated in the anion series indicated that the bidentate conformation is more favorable (4 to 5 kcal/mol) (Table 3). For **10**, the bi- and tridentate conformations of almost similar energy level precluded the identification of an unambiguous chelated form.

Finally, we applied our model to calculate the energetic gain for galardin (**1**). Compared to **11**, this gain was found to be −32.8 kcal/mol confirming that hydroxamate derivatives are stronger Zn^{2+}-chelators than sulfonylhydrazides. However, the energy values found for these latter being consistent with an enzyme inhibition, we decided to investigate the possibility of a selective MMP inhibition by **3**, **5–7**, and **10** and determined their interactions with the MMP-1, -2, -9, -12, and -14 active site and surrounding pockets using molecular docking.

MMP inhibition and molecular docking

Docking studies were performed using the Autodock 4.1 software.[36] The residue numbering employed for each MMP is that used in the Swissprot database.

MMPs can be discriminated as a function of their S'_1 specificity pocket. Therefore, we selected MMP-1; MMP-12, and -14; and MMP-2, and -9; as representatives of the shallow, deep, and intermediate S'_1 pocket family, respectively. Crystallographic data of each evaluated MMP were obtained from the Protein Data Bank (see experimental). The docking pose of each compound was evaluated using the Autodock-integrated free energy scoring function. Docking studies with **3**, **5–7**, and **10** furnished a realistic, and lowest energy, model for each sulfonylhydrazide docked at the surface of the five evaluated MMPs. For K_i calculations, top poses with a RMSD value less than 2.0 Å were selected after clustering. Those calculated values can be used as a reflection of the biological activity and as an essential discriminator of selectivity.[37] In our case, sulfonylhydrazides could be partitioned into three groups (Table 4). Compound **3**, the representative of the alkylsulfonylhydrazide family, had calculated K_is in the μM range (13–70 μM), whereas calculated K_is of **5** and **10** were found in the high nM range (36–2190 nM), and those of **6** and **7** in the low nM range (0.8–42 nM).

Analysis of the MMP-docked sulfonylhydrazides showed that aromatic-containing compounds **5–7** and **10** adopted a similar position and orientation with the enzyme active site (Fig. 6).

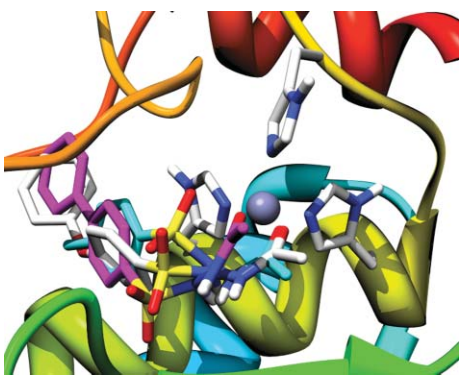

Fig. 6 Molecular docking of compounds **5–7** with MMP-12 (**5** cyan, **6** white and **7** purple, Zn^{2+} ion green sphere).

In each case, the *N,N′*-disubstituted hydrazide moiety was localized in the S_1 subsite and chelated the Zn^{2+} ion. Highly interestingly, whereas our previous docking studies have shown that the SO_2 substituting aromatic moiety of galardin-derived sulfonylhydrazides is localized in the enzyme S_2 subsite,[17b] the

Table 4 Calculated K_is (nM) and type of chelation for compounds **3**, **5–7** and **10** *vs.* selected MMPs

Ligands	MMP-1	MMP-2	MMP-9	MMP-12	MMP-14
3	25040	70350	13210	3320	67360
	Type II	*Type II*	*Type II*	*Type II*	*Type I*
5	2190	991	210	97	683
	Type II	*Type I*	*Type II*	*Type II*	*Monodentate*
10	1030	851	49	36	597
	Monodentate	*Type I*	*Type I*	*Type I*	*Type I*
6	31	11	1.7	0.8	8
	Type II	*Monodentate*	*Monodentate*	*Monodentate*	*Monodentate*
7	42	11	5	2	9
	Type II	*Type II*	*Type II*	*Monodentate*	*Monodentate*

aromatic moiety of **5–7** and **10** was consistently localized in the S'_1 enzyme subsite, a position stabilized by van der Waals interactions with His_{218}-Glu_{219}, Ala_{182}-Leu_{181} and with the amino acids located at the entrance of the S'_1 subsite: Pro_{238}-Thr_{239}-Tyr_{240}-Lys_{241} and Val_{235} (MMP-12 numbering). A similar S'_1 pocket filling by arylsulfonyl groups has already been reported for efficient small and nonpeptide MMPIs such as Prinomastat[37] or CGS 27023A,[38,39] and *N*-arylsulfonylglycine hydroxamic acid derivatives.[40] The *N*-acetyl moiety of **5–7** and **10** was found to point toward the outer part of the active site in each case suggesting that our selected bulky substituents could be accommodated by the enzyme. For compound **3**, whose nitrogen atoms are mono-substituted with a mesyl or acetyl group, two different and poorly stabilized docked structures were obtained. In the case of MMP-14, the methyl of the mesyl residue of **3** was localized at the entrance of the S'_1 pocket whereas this place was occupied by the methyl of the acetyl moiety for MMP-1, -2, -9, -12, and -14 (not shown). That the four aromatic-containing molecules could satisfyingly fill the S'_1 pocket of MMP-1, -2, -9, -12, and -14 evidenced their potential use as MMPIs but could appear deceptive in term of selectivity. So we decided to examine more closely the zinc-sulfonylhydrazide chelate structure. The distances between the Zn^{2+} ion and the sulfonylhydrazide atoms of **5** and **7** indicated a strong type II (DFT study) chelate stabilized by H-bonds between the hydrazide NHs and Ala_{182} and Glu_{219} (MMP-1), Ala_{165} and Glu_{202} (MMP-2), and Ala_{189} and Glu_{402} (MMP-9) (shallow and intermediate S'_1 pocket); leading us to anticipate a low specificity in term of inhibition (Fig. 7).

Docked in the active site of MMP-12 and -14, **5** and **7** behaved differently. In the active site of MMP-12, compound **5** formed a type II chelate whereas a monodentate complex reminiscent of that calculated during our DFT study was obtained for MMP-14 as well as for **7** in the active site of MMP-12 or -14 (deep S'_1 pocket). H-bonds between the SO_2 group and $NHLeu_{181}$; and between $Ala_{182}CO$ and NH-Ac stabilized this complex for MMP-12 ($NHAla_{200}$ and $Ala_{200}CO$ for MMP-14). Inside the S'_1 pocket, van der Waals interactions were found with the aromatic moiety of **7** and residues Tyr_{240}, Lys_{241}, Val_{235} and Thr_{215} (Fig. 8).

When **10** was docked in the MMP-1 active site (shallow S'_1 pocket), the Zn^{2+} ion appeared chelated by the lone pair of the acetyl and nitro oxygen atom according to the model also calculated by our DFT study. For the intermediate and deep S'_1 pocket series (MMP-2 and -9, and MMP-12, -14; respectively) the bidentate complex formed between the Zn^{2+} ion and the SO_2 and CO oxygen atoms and stabilized by H-bonds between the nitro group and $NHAla_{182}$ (MMP-12 numbering) led us to anticipate a low inhibition selectivity. In the case of **6**, four different chelates

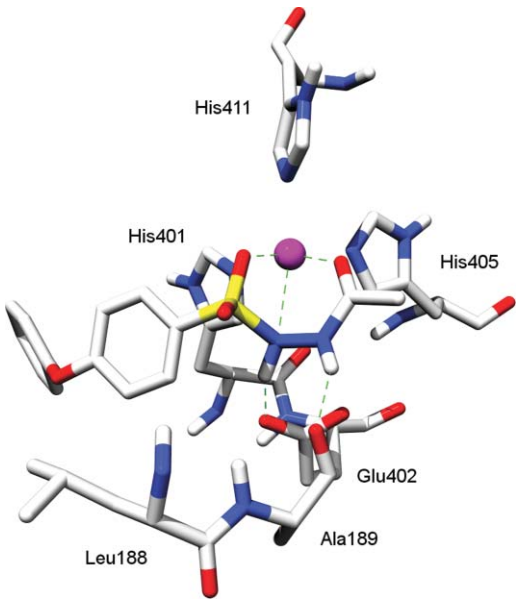

Fig. 7 Top pose of compound **7** with MMP-9.

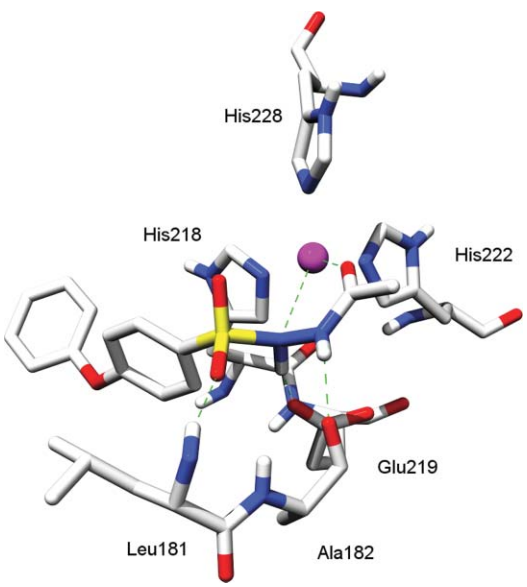

Fig. 8 Top pose of compound **7** with MMP-12.

were obtained. For the shallow S'_1 pocket series (MMP-1) a type I chelate was observed, stabilized by a network of three H-bonds: between SO_2 and $HNAla_{182}$; $Ala_{182}CO$ and NHAc; and SO_2NH and $Glu_{219}CO$ (Fig. 9).

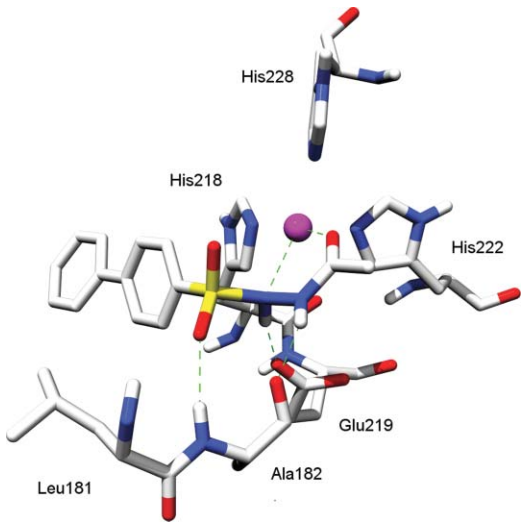

Fig. 9 Top pose of compound **6** with MMP-1.

In the intermediate series (MMP-2 and -9), the SO_2–Zn interaction was lost, resulting in a monodentate complex stabilized by a H-bond between SO_2 and $NHAla_{165}$ for MMP-12 ($NHLeu_{188}$ for MMP-12). In the deep S'_1 pocket series (MMP-12, and -14) a monodentate chelate was observed for MMP-12 and -14 due to H-bonds between NHLeu and SO_2 and AlaCO and NHAc (Fig. 10). Therefore, selective MMP inhibition can be expected for **6**.

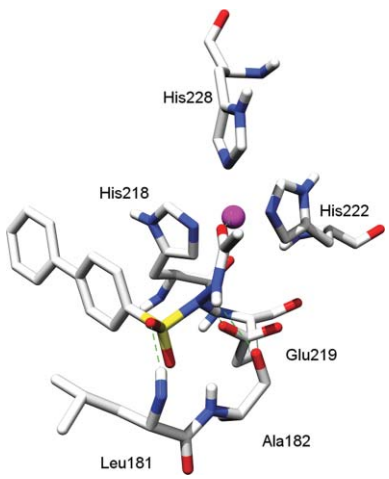

Fig. 10 Top pose of compound **6** with MMP-12.

In an attempt to correlate our calculations and the MMP inhibitor character of our molecules, we rapidly synthesized the already known **3–5**[41] to evaluate their MMP inhibitory activity. Unfortunately, the poor water solubility of **3–5** precluded the accurate determination of their enzyme inhibitory properties, and even though some enzyme inhibition was persistently observed, these experiments mainly showed that pharmacomodulation on the acetyl end was necessary to furnish water solubility.

Biological implications and conclusion

Our results indicate that the sulfonylhydrazide moiety can bind to the Zn^{2+} ion and form mono-, bi-, or tridentate chelates. Whereas the zinc coordination by the sulfonamide nitrogen could have been anticipated, the participation of the SO_2 group oxygen atom, which brings an unarguable gain in energy, was unexpected. When combined with the enzyme, each aromatic-containing ligand filled the S'_1 selectivity pocket. However, only **6** seems to be a promising derivative. Therefore, selective MMP inhibition by using sulfonylhydrazides seems an achievable goal. Such a hypothesis is fully consistent with our theoretical results[17c] that predict a reduced influence of the arylsulfonylhydrazide aromatic substituents for MMP-2 and can explain the selectivity of inhibition that we have observed in the MMP series.[17a,b] Because galardin contains a Leu residue at the P'_1 site, some of the deceptive results obtained with sulfonylhydrazide-containing galardin analogues[17b] could result from a competitive process for the S'_1 subsite by the sulfonylhydrazide and *i*-butyl moieties of the same molecule. Our results clearly demonstrate the potential of sulfonylhydrazides to become selective MMPIs and we are currently working on the synthesis of water soluble compounds.

Experimental

Experimental details of the computational method

HF-DFT calculations were performed using the Gaussian03 package.[42] All structures were fully optimized by using the B3LYP hybrid functional.[43–46] Two basis sets have been used: the LANL2DZ basis set[47–49] for the Zn atom and the 6–31G** basis set[50–53] for the other atoms. Thermochemical corrections were obtained from harmonic frequency analysis of the optimized structures for standard conditions. Derivative **3** was first built up *in silico* without any metal atom. Other derivatives were built from **3** by replacing the methyl group by the necessary substituent. The zinc ion was then placed at a reasonable distance from the sulfonamide nitrogen and each evaluated structure was optimized.

Experimental details of the docking

Docking studies were carried out using the program Autodock 4.1.[54] The structures of MMP-1, -2, -9, -12, and -13 were obtained from a Protein Data Bank file (code 966C.pdb, 1QIB.pdb, 1GKC.pdb, 3F17.pdb, 1BQQ.pdb; respectively)[55] and were treated as described before.[17b] Ligand structures optimized during the DFT study were docked to MMPs. All the flexible torsions, except the amide bonds, were allowed to rotate during the docking stage. Each docking experiment was performed with two runs constituted of a series of 250 simulations. Each docking simulation was carried out with an initial population of 250 individuals, a maximum number of 5,000,000 energy evaluations and a maximum number of 50,000 generations. The pseudo-Solis and Wets modification methods were used with default parameters. Docked conformations of the ligands were clustered with a root mean square deviation (RMSD) cut-off of 0.5 Å.

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