

From Sensors to Silencers: Quinoline- and Benzimidazole-Sulfonamides as Inhibitors for Zinc Proteases

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This study combines the areas of metal ion sensors and Fragment-Based Drug Discovery (FBDD)¹ to generate a novel class of matrix metalloproteinase inhibitor (MMPi) lead fragments. Zinc is an essential element for humans and plays an important role in a wide range of biological processes, prompting the development of a large number of Zn(II)-selective small molecule sensors as tools to better understand the biological trafficking of this metal ion.² Among the most widely used Zn(II) fluorescent sensors are those based on the 8-sulfonamidoquinoline core (part of the Zinquin family, Figure 1),³ which show a selective turn-on fluorescent response for Zn(II) over other biologically relevant metal ions such as Mn(II), Fe(II), and Cu(II). A new class of fluorescent sensors based on the structurally related 2-(2'-benzene-sulfonamidophenyl) benzimidazole chelating group, which have the advantage of displaying a ratiometric fluorescent response, have also been described.^{4,5} Based on the high affinity and selectivity of these molecules for Zn(II) and their capacity to form complexes with zinc-dependent metalloproteins,⁶ it is surprising that these scaffolds have not yet been purposely applied for the development of metalloenzyme inhibitors.⁷ Herein, zinc-binding moieties from these sensors are used to generate libraries that produce semiselective MMPi hits. These findings show that combining the extensive work in the area of small molecule metal ion sensors with the FBDD approach to drug development is a powerful combination for the discovery of novel metalloprotein inhibitors.

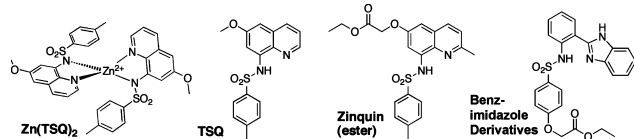
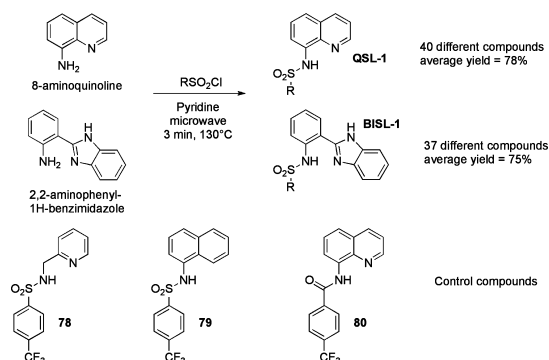


Figure 1. Structure of chelating-sulfonamide zinc sensors.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases which have been identified as therapeutic targets for diseases such as multiple sclerosis, arthritis, cardiovascular disease, and cancer.⁸ Most MMPi are comprised of a zinc-binding group (ZBG, most commonly a hydroxamic acid) and a peptidomimetic “backbone”. The ZBG binds the active site metal ion, while the backbone engages in noncovalent interactions with the protein surfaces.⁹ A major challenge in this area, due to structural similarities between the different MMPs, is to obtain selective MMPi. The fragment libraries reported here show that the two different core scaffolds show some degree of selective MMP inhibition, which may be useful in combination with backbone substituents to generate even greater MMP selectivity.¹⁰

A fragment library based on the 8-sulfonamidoquinoline ZBG was prepared (Quinoline Sulfonamide Library 1 = QSL-1, compounds **1–40**) by combining 8-aminoquinoline with sulfonyl chlorides using microwave irradiation. The typical procedure for this coupling requires long reaction times; in contrast, when performed using a microwave at 130 °C in pyridine, the coupling was complete within 3 min with generally good yields (average yield = 78% for 40 compounds). Using the same procedure, a second library based on the 2-sulfonamidophenylbenzimidazole ZBG (Benzimidazole Sulfonamide Library 1 = BISL-1, compounds **41–77**) was generated (Scheme 1).

Scheme 1. Synthesis of Two Sulfonamide Libraries Using an Efficient Microwave Procedure (Top); Control Compounds (Bottom)



The two libraries, QSL-1 and BISL-1, were first screened against MMP-2, -3, and -9 at a concentration of 50 μ M and fragments that produced >50% inhibition were categorized as a hit. The assay results clearly showed that both fragment libraries gave hits that were generally more potent for MMP-2 over MMP-9 and -3. Out of the 40 fragments in QSL-1, 25 were hits against MMP-2, 11 against MMP-3, and only 3 against MMP-9. For BISL-1, 27 compounds were hits against MMP-2, 2 against MMP-3, and only 1 against MMP-9. Considering that the majority of sulfonamide backbones for these two libraries were identical (35 out of 40), the results indicate that the QSL versus BISL scaffold plays a role in the semiselective MMP inhibition by these fragments.

The IC₅₀ values against MMP-2, -3, -8, and -9 for three of the best fragments from each library were determined. For comparison purposes, fragments with the same sulfonamide groups were selected from QSL-1 and BISL-1 (Table 1). For QSL-1, the potency against different MMPs varies substantially depending on the sulfonamide moiety. In the case of small substituents, such as thiophene or *p*-trifluoromethylphenyl, there is a preference toward MMP-2 and -8 (Table 1, fragments **2** and **3**). However,







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in the case of a larger substituent (Table 1, fragment **1**), broad-spectrum activity is observed. This is likely due to the strong interaction of the biphenyl substituent with the deep S1' pocket of these MMPs. For BISL-1, a greater preference for MMP-2 (Table 1, fragments **42** and **43**) is obtained. This is best illustrated by the *p*-trifluoromethylphenyl derivatives (Table 1, fragments **3** and **43**), where both chelating sulfonamides inhibit MMP-2 over MMP-3 and -9, but the benzimidazole compound (Table 1, fragment **43**) shows reduced activity against MMP-8. This fragment is uniquely potent against MMP-2 when compared with the other MMPs tested and is particularly notable for discriminating between the gelatinases (MMP-2 and -9).¹¹ This finding clearly demonstrates that the core scaffold has a significant effect on the selectivity of these lead compounds.

Table 1. IC₅₀ Values (μM) of Select Fragments against Four MMPs

No.	Structure	MMP-2	MMP-3	MMP-8	MMP-9
1		5.2 (± 0.1)	20.0 (± 1.8)	16.5 (± 2.6)	31.1 (± 0.2)
41		>100	87.0 (± 1.8)	>100	>100
2		16.4 (± 0.4)	>100	32.8 (± 2.8)	>100
42		37.2 (± 6.0)	>100	85.1 (± 6.0)	>100
3		1.6 (± 0.1)	>100	3.3 (± 0.5)	>100
43		6.9 (± 1.1)	>100	>100	>100

Evidence that these chelating sulfonamide fragments were binding to the active site Zn(II) ion was provided by three control compounds with structural similarities to fragment **3**. Each of these control compounds (Scheme 1, **78–80**) has a reduced metal-binding capacity due to either (i) lacking the two nitrogen donor atoms required to achieve chelation (**79**) or (ii) having an insufficiently acidic N–H group (**78**, **80**) such that metal binding cannot compete with ligand protonation. At a concentration of 500 μM all three control compounds showed <30% inhibition of MMP-2 (>200-fold loss of activity vs **3**), indicating that the QSL-1 and BISL-1 libraries inhibit MMPs by Zn(II) ion coordination. In addition, extended X-ray adsorption fine structure (EXAFS) spectroscopy of fragment **3** with an MMP indicate binding to the active site Zn(II) ion (Figure S6), providing direct evidence that these fragments bind to the MMP metal ion.

To better understand the activity of these fragments, computational docking studies were performed on fragments **3** and **43** (Table 1, fragments **3** and **43**) in MMP-2, -3, -8, and -9 using the Glide software package (Schrödinger, Supporting Information). The 100 best poses against each MMP were filtered based on distance/geometry between the ZBG and the active site Zn(II) ion (Supporting Information).³ This filtering procedure ensured that only structures with geometric parameters that produce reasonable metal chelation are evaluated.

Fragment **3** generated acceptable poses for all four MMPs; however, the lowest energy poses for MMP-3 and -9 were >3.5 kcal/mol higher ($\Delta E_{ele+vdw}$, Table S1) than those for MMP-2 and -8, consistent with the selectivity for this fragment for the latter MMPs (Table 1). Acceptable poses for fragment **43** were readily obtained with MMP-2, but not with the other MMPs examined. Structures of fragment **43** in MMP-3, -8, and -9 were all >3.4 kcal/

mol higher in energy than the conformation observed with MMP-2. Again, these calculations are consistent with the experimental data showing that fragment **43** is selective for MMP-2. The best poses of fragments **3** and **43** in the active site of MMP-2 are shown in Figure 2. Interestingly, both fragments have the sulfonamide moiety pointed toward the unprimed side of the active site.¹¹ In spite of the intrinsic limitations of classical approaches to accurately describe systems involving metal ions, the docking analysis was otherwise wholly consistent with the experimental findings summarized (Table 1) and provides some initial insight into the binding of these fragments. Because the electrostatic component is the most important contribution to the calculated $\Delta E_{ele+vdw}$, our findings also suggest that, despite the homology among MMPs, differences in the electrostatic environment around the Zn(II) ion (Figure S5) may have a significant effect on inhibitor-receptor recognition.

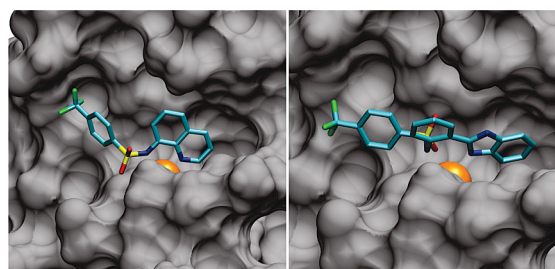


Figure 2. Model structure of the complex formed between MMP-2 (gray surface, zinc shown as orange sphere) and fragments **3** (left) and **43** (right).

In summary, we have used known scaffolds from fluorescent Zn(II) sensors as ZBGs in the design of two focused fragment libraries. Most of the fragments exhibited a preference for MMP-2, which was generally consistent with computational analysis. One fragment (Table 1, fragment **43**) shows low micromolar activity against MMP-2 and no significant activity against MMP-3, -8, and -9. Ongoing efforts to elaborate these new scaffolds are expected to produce more potent, semiselective MMPi.

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Supporting Information Available: Experimental details, Figures S1–S6, and Tables S1–S2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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