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Identifying Chelators for Metalloprotein Inhibitors Using a Fragment-Based Approach

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Fragment-based lead design (FBLD) has been used to identify new metal-binding groups for metalloenzyme inhibitors. When screened at 1 mM, a chelator fragment library (CFL-1.1) of 96 compounds produced hit rates ranging from 29% to 43% for five matrix metalloproteases (MMPs), 24% for anthrax lethal factor (LF), 49% for 5-lipoxygenase (5-LO), and 60% for tyrosinase (TY). The ligand efficiencies (LE) of the fragment hits are excellent, in the range of 0.4−0.8 kcal/mol. The MMP enzymes all generally elicit the same chelators as hits from CFL-1.1; however, the chelator fragments that inhibit structurally unrelated metalloenzymes (LF, 5-LO, TY) vary considerably. To develop more advanced hits, one hit from CFL-1.1, 8-hydroxyquinoline, was elaborated at four different positions around the ring system to generate new fragments. 8-Hydroxyquinoline fragments substituted at either the 5- or 7-positions gave potent hits against MMP-2, with IC_{50} values in the low micromolar range. The 8-hydroxyquinoline represents a promising new chelator scaffold for the development of MMP inhibitors that was discovered by use of a metalloprotein-focused chelator fragment library.

Introduction

Metalloenzymes represent at least a third of all proteins and utilize a wide variety of metal ion cofactors for catalytic, electron transfer, structural, or other key roles.[1,2] As a result, inhibitors of metalloenzymes are desired for mechanistic studies, as well as for applications including pesticides,[3−5] preservatives,[6] cosmetics,[7] and therapeutics. Pathogenic metalloenzyme activity is associated with many illnesses such as cancer,[8−11] inflammatory diseases,[12,13] infectious diseases,[14−19] cardiovascular diseases,[20,21] and neurodegenerative diseases.[21,22] The presence of the metal ion in these enzymes has been frequently exploited for the development of synthetic inhibitors.[18] Specifically, the metal ion cofactor can serve as an anchoring site that may be easily targeted by metal-binding groups (MBGs).[a] A range of MBGs has been used in the inhibitors of different metalloenzymes, but among the most common groups are hydroxamic acids, sulfonamides, and carboxylic acids.[23−25]

More recently, reports of matrix metalloprotease inhibitors (MMPi) with novel MBGs (often referred to as zinc-binding groups for these inhibitors, ZBGs) suggest an expanded role for the MBG in inhibitors of metalloenzymes. In particular, the MBGs of MMPi have been found to influence inhibitor potency[26−28] and selectivity.[29,30] These MBG effects have been attributed to hydrogen-bonding interactions with the protein,[31] van der Waals contacts,[30] and the affinity of the MBG for the active site metal ion.[26,28] It is possible that such interactions might be optimized by finding the best MBG for a given metalloenzyme, but to do so, a variety of MBGs would be needed to address the structural and chemical space presented by the diverse range of metalloenzyme targets. While a reasonable number of MBGs have been introduced for use in MMPi,[23,32] the study of different MBGs in inhibitors of other metalloenzymes are relatively scarce.[33−37] The limited attention given to MBGs is surprising in light of the effort put forth to systematically vary all other components of metalloenzyme inhibitors.[38]

In order to identify new chelating scaffolds for metalloenzyme inhibitors, fragment-based lead design (FBLD) has been proposed as a viable approach. FBLD is a method in which low molecular weight compounds (fragments) are screened against drug targets by use of sensitive techniques such as nuclear magnetic resonance spectroscopy or affinity mass spectrometry.[39,40] Fragments are then linked or "grown" to generate potent leads, which can be further optimized to obtain improved solubility and pharmacokinetics via traditional medicinal chemistry. FBLD has several distinct advantages over more traditional methods of lead development. Active sites are more efficiently probed by small fragments that are not limited by steric constraints or hydrogen-bonding mismatches.[39] Additionally, compounds of lower complexity can more effectively sample the available chemical diversity.[40] Leads developed by fragment methods typically have good ligand efficiencies (LE), a standard measure used to compare compound potency with molecular size by the formula $LE = -RT(\ln[IC_{50}])/HAC$ in which IC_{50} is the fragment concentration at which enzyme activity is reduced 50% and HAC is the heavy atom (or non-hydrogen atom) count.[41]

The identification of novel MBGs for metalloenzyme inhibitors is a task particularly well suited for FBLD.[42] In fact,

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[a] Abbreviations: CFL, chelator fragment library; FBLD, fragment-based lead design; HAC, heavy atom count; iNOS, inducible nitric oxide synthase; LF, lethal factor; LE, ligand efficiency; 5-LO, 5-lipoxygenase; MMP, matrix metalloproteinase; MMPi, matrix metalloproteinase inhibitor; MBGs, metal-binding groups; TY, tyrosinase; ZBG, zinc-binding group.

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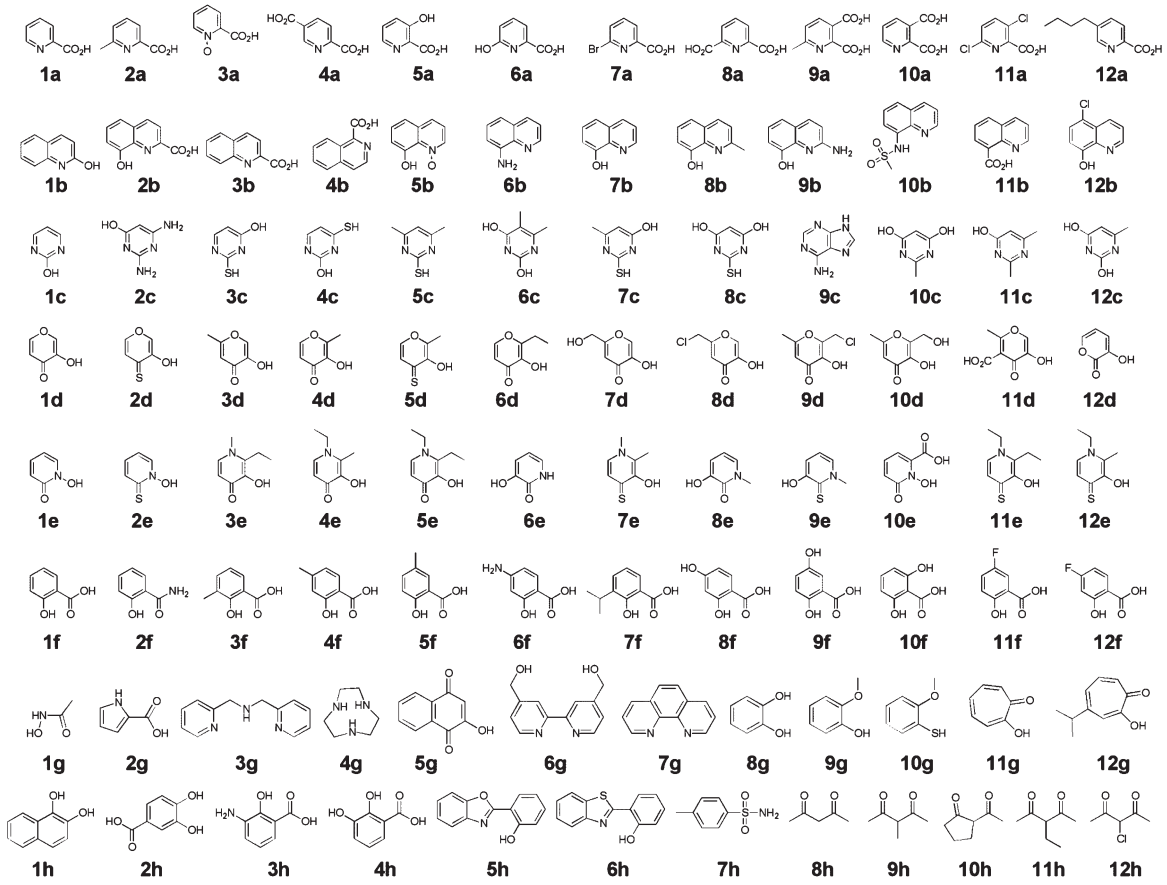

Figure 1. Structures of all fragments in CFL-1.1. The library is organized with each row corresponding to a MBG class: picolinic acids (**1a**−**12a**), quinolines (**1b**−**12b**), pyrimidines (**1c**−**12c**), hydroxypyrones (**1d**−**12d**), hydroxypyridinones (**1e**−**12e**), and salicylic acids (**1f**−**12f**). Compounds **1g**−**12g** and **1h**−**12h** are classified as miscellaneous.

many common metal chelators may be considered fragments that bind to metals with affinities in the micro- to millimolar range, which are suitable for screening in many bioassays. In addition, the binding mode of many chelators may be modeled by complexes that mimic metalloenzyme active sites or may be inferred from published crystal structures of the chelator bound to the metal of interest.[26,43−45] As a result, screening methods that produce structural binding information, which typically require large quantities of protein and time-consuming data analysis (e.g., X-ray- or NMR-based screening), may not be as essential when dealing with MBG-based library screening. Finally, chelators may function as good molecular anchors, where strongly bound fragments are unlikely to dramatically change their binding mode upon elaboration, thereby facilitating lead development.[39] Several studies have applied FBLD to the development of metalloenzyme inhibitors,[42,46−52] although in most cases the MBG was not varied as a fragment.

In this report, FBLD is used to identify new MBGs for metalloenzyme inhibitors. A small chemical library of 96 metal chelators (Figure 1) was assembled and screened against five MMP family members to identify new MBGs for MMP inhibitors.[42] This library was also screened against an unrelated zinc enzyme (anthrax lethal factor, LF), a non-heme iron enzyme (5-lipoxygenase, 5-LO), a dinuclear copper enzyme (tyrosinase, TY), and a heme iron enzyme (inducible nitric oxide synthase, iNOS) in order to probe the applicability of this approach to generate new MBGs for a wide range of metalloenzymes. Finally, we demonstrate the advancement of a hit from the library by producing a focused library based on an 8-hydroxyquinoline scaffold. The findings presented show that chelators with multiple points of attachment offer more synthetic opportunities that are important for the successful development of metalloprotein inhibitors.

Results

Design and Evaluation of a Chelator Fragment Library (CFL). A fragment library consisting of metal chelators (CFL-1) was assembled as previously described.[42] All but five fragments from CFL-1 have a molecular weight < 200 amu. The fragments all have known metal-binding modes using two to four nitrogen, oxygen, and sulfur heteroatoms. CFL-1 contains a total of 96 fragments with six metal-binding classes including picolinic acids, quinolines, pyrimidines, hydroxypyrones, hydroxypyridinones, salicylic acids, and 24 miscellaneous compounds. All of the fragments are soluble up to 50 mM in DMSO and are commercially available or readily synthesized.[30,53−61] Previously reported screening of CFL-1 against MMPs identified four compounds that could not be screened in either colorimetric or fluorometric MMP assays due to problems with solubility or interference with the assay conditions.[42] These four compounds were replaced (**6b**, **10b**, **7f**, and **2h**) and a new generation of this library (CFL-1.1, Figure 1) was used for the experiments reported here.

The fragments of CFL-1.1 were screened against MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, LF, 5-LO, TY, and

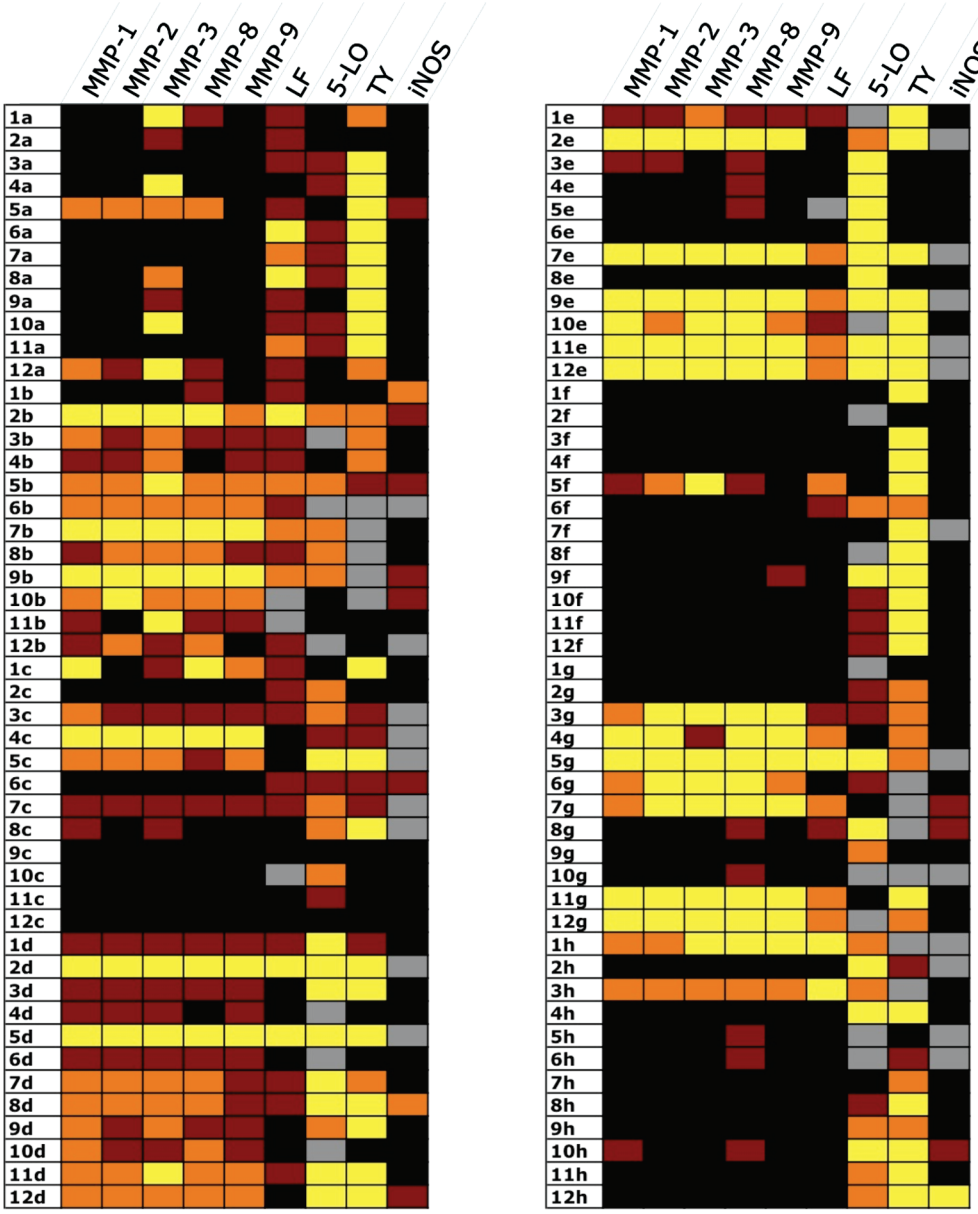

Figure 2. Thermoplot representing the results from the screens of CFL-1.1 against various metalloenzymes. Cells are color-coded by percent inhibition: black (0−25%), red (25−50%), orange (51−75%), and yellow (76−100%). Gray cells indicate compounds that interfered with the assay.

iNOS using reported assay conditions (Tables S1−S9, Supporting Information).[26,42,62,63] The results of the screens are depicted in a thermoplot (Figure 2) representing the percent inhibition of the fragments against each metalloenzyme at a fragment concentration of 1 mM. Hits were defined as fragments that inhibit enzyme activity >50% at 1 mM. Based on the screening results of CFL-1.1, the IC_{50} values of select hits against MMP-2, LF, 5-LO, and TY were determined and corresponding LEs were calculated (Table 1).

Hits against MMPs. CFL-1.1 produced a significant number of hits against MMP-1, MMP-2,[42] MMP-3, MMP-8, and MMP-9 (Figure 2). The percent of fragments identified as hits (hit rate) ranged from 29% to 43% for the MMPs screened. 3-Hydroxypicolinic acid (**5a**) decreased the activity of MMP-1, MMP-2, MMP-3, and MMP-8 by approximately 50%. Derivatives of 8-hydroxyquinoline (**2b**, **7b**, **8b**, and **9b**) strongly inhibited all five MMPs. 8-Hydroxyquinoline (**7b**) was found to have an IC_{50} value of 130 μM against MMP-2 (Table 1). Two other quinolines stood out as MMP hits: 8-hydroxyquinoline-*N*-oxide (**5b**) and the sulfonamide quinoline (**10b**).[61] Two pyrimidines (**4c** and **5c**) were MMP hits, with compound **4c** having an IC_{50} value of 121 μM against MMP-2. The sulfur-containing hydroxypyrothiones (**2d** and **5d**) completely inhibit all MMPs, while most of the hydroxypyrones (**1d**, **7d**−**11d**) showed ~50% inhibition of the MMPs as well. The IC_{50} value of hydroxypyrothione **2d** against MMP-2 was found to be 76 μM. The sulfur-containing hydroxypyridinethiones (**2e**, **7e**, **9e**, **11e**, and **12e**) also completely inhibit all the MMPs. Among the hydroxypyridinones only **1e** and **10e**, both of which are based on a 1,2-hydroxypyridinone core, showed >50% inhibition of the MMPs. In general, salicylic acids did not effectively inhibit any MMPs, while several miscellaneous compounds were found to be hits. Of particular

Table 1. Calculated IC_{50} Values, Heavy Atom Count (HAC), And Ligand Efficiency (LE) Values for Select Hits from CFL-1.1[a]

enzyme	compound	IC_{50} value (μM)	HAC	LE (kcal/mol)
MMP-2[b]	**7b**	130 ± 28	11	0.50
	4c	121 ± 37	8	0.70
	2d	76 ± 1	8	0.73
	1g	15000[64]	5	0.52
	11g	146 ± 71	9	0.61
	3h	389 ± 134	11	0.44
LF[c]	**2d**	204[65]	8	0.63
	1g	11400[65]	5	0.53
5-LO[c]	**5d**	11 ± 2	9	0.75
	9f	75 ± 5	11	0.51
TY[c]	**6a**	159 ± 28	10	0.52
	5d	3.8 ± 0.5	9	0.82
	1f	100 ± 9	10	0.55
	11g	0.4[62]	9	0.97

[a] Compound **1g** (acetohydroxamic acid) is included as a reference. [b] Ligand efficiency calculated at 37 °C. [c] Ligand efficiency calculated at 25 °C.

interest, two tropolone derivatives (**11g**, **12g**) stand out as new MBGs for MMPs, with an IC_{50} value of 146 μM (**11g**) against MMP-2.

In general, the fragments from CFL-1.1 did not show specificity among the MMPs, that is, fragments found as hits were potent against all of the MMPs tested. One notable exception was the activity of several picolinic acids against MMP-3. In general, the picolinic acids were not active against the MMPs, but MMP-3 was inhibited by six picolinic acid derivatives (**1a**, **4a**, **5a**, **8a**, **10a**, and **12a**). Of these, only **5a** and **12a** were found to show any significant activity against other MMPs. Compound **1a**, which does not come up as a hit against MMP-1, MMP-2, MMP-8, or MMP-9, was previously reported to have an IC_{50} value of 181 μM against MMP-3.[28] To date, the origin of the selectivity of the picolinic acids for MMP-3 is unclear. Nonetheless, this finding validates the robust nature of this library screening approach and suggests that appropriate selection of the chelator fragment in combination with a suitable backbone group, may be able to augment the specificity of an MMPi.

Hits against Other Metalloenzymes. Screening of CFL-1.1 against LF at 1 mM yielded 22 hits, giving a hit rate of 24%. Four compounds from CFL-1.1 were not screened because they were found to precipitate or interfere with the assay (**10b**, **11b**, **10c**, **5e**). In contrast to the MMPs, several of the picolinic acids (**1a**−**3a**, **5a**, **9a**−**11a**) inhibited LF at close to 50%. Three picolinic acids (**6a**−**8a**) inhibit LF >70% and are all substituted at the 6-position, unlike the picolinic acid hits against MMP-3. Quinoline compounds **2b**, **5b**, **7b**, and **9b** were hits against LF. The hydroxypyrothiones (**2d** and **5d**) completely inhibit LF at 1 mM; however, the hydroxypyridinethiones (**7e**, **9e**, **11e**, and **12e**) only inhibit LF ~50%. Fragment **2d** was previously reported to have an IC_{50} value of 204 μM against LF,[65] again validating the screening results performed here. In addition, several miscellaneous compounds were found to be hits against LF (**4g**, **5g**, **7g**, **11g**, **12g**, **1h**, and **3h**).

The hit rate of CFL-1.1 against the non-heme iron protein 5-LO was 49%. Several of the 8-hydroxyquinoline derivatives (**2b**, **7b**−**9b**) including 8-hydroxyquinoline-*N*-oxide (**5b**) hit 5-LO. Unlike the zinc-dependent enzymes, a number of pyrimidine derivatives (**2c**, **3c**, **5c**, **7c**, **8c**, and **10c**) hit 5-LO at 1 mM. Of these compounds, **3c**, **5c**, **7c**, and **8c** each have a thiol at the 2-position. All of the tested hydroxypyrones (**1d**−**3d**, **5d**, **7d**−**9d**, **11d**, **12d**) and hydroxypyridinones (**2e**−**9e**, **11e**, **12e**) inhibit 5-LO >70%. The IC_{50} value of **5d** against 5-LO was found to be 11 μM. Unlike the zinc-dependent MMPs and LF that were more effectively inhibited by the sulfur (thione) derivatives of the hydroxypyrones (**1d**−**12d**) and hydroxypyridinones (**1e**−**12e**), 5-LO was inhibited by both O,O and O,S chelators of these compound classes (compare **1d** and **2d**; **8e** and **9e**). Consistent with our findings, fragment **1e** has appeared in the literature as part of 5-LO inhibitors and dual inhibitors of 5-LO and COX.[66−68] Furthermore, two salicylic acid derivatives (**6f**, **9f**) were identified as 5-LO hits with compound **9f** having an IC_{50} value of 75 μM against 5-LO. Among the miscellaneous fragments, catechol derivatives (**8g**, **1h**−**4h**) and acetylacetone derivatives (**8h**−**12h**) also inhibit 5-LO.

CFL-1.1 has a very high hit rate of 60% against the copper-dependent TY. Several of the chelators identified from the screening of CFL-1.1 have been previously reported as inhibitors of TY including tropolone (**11g**, IC_{50} = 0.4 μM),[62] kojic acid (**7d**, IC_{50} = 23 μM),[62] 1,2-hydroxypyridinone (**1e**, IC_{50} = 1.2 μM)[69] and L-mimosine,[70] an amino acid derivative of 3,4-hydroxypyridinones (**3e**−**5e**). The IC_{50} values of maltol (**4d**) and several other hydroxypyridinones (**3e**, **4e**, **6e**, and **8e**) against TY have also been reported.[71] All of the aforementioned fragments and structurally related compounds were confirmed as TY hits from CFL-1.1 (Figure 2). Novel hits against TY from CFL-1.1 included thiopyrones (**2d** and **5d**) and thiopyridinones (**2e**, **7e**, **9e**, **11e**, and **12e**). Compound **5d** was found to have an IC_{50} value of 3.8 μM against TY (Table 1). Similar to the MMPs, the pyrones and pyridinones with O,S donor atoms are more potent against TY than the corresponding compounds with O,O donor atoms (compare **1d** and **2d**, **4d** and **5d**, **8e** and **9e**, **4e** and **12e**; Figure 2). However, like 5-LO, many O,O donor atom chelators (**3d**, **8d**, **9d**, **11d**, **1e**, and **10e**) did show some inhibition against TY. Other new hits included the picolinic acids (**1a**−**12a**), with all but one fragment (**2a**) inhibiting TY >75%. Quinoline fragments containing carboxylic acids (**2b**−**4b**) were found to be hits against TY. Also, salicylic acids (**1f**, **3f**−**5f**, and **7f**−**12f**) were very effective at inhibiting TY activity, showing more than 90% inhibition, with salicylic acid (**1f**) having an IC_{50} value of 100 μM against TY (Table 1). A wide variety of miscellaneous compounds were also found to be hits against TY (**4h**, **7h**−**12h**).

The hit rate of CFL-1.1 against the heme iron protein iNOS at 1 mM was 4% (3 out of 73 fragments). Several compounds were excluded from screening based on insolubility in the assay buffer or incompatibility with the assay reagents. There are only six compounds (**5a**, **1b**, **6c**, **8d**, **12d**, and **12h**) that inhibit iNOS greater than 40%, and these fragments cover nearly all the metal-binding classes represented in CFL-1.1. Overall, CFL-1.1 did not reveal any good fragment hits against iNOS.

8-Hydroxyquinoline Sublibrary Synthesis and Screening. In order to demonstrate (a) an ability to further develop novel hits from CFL-1.1 and (b) the versatility offered by new chelator scaffolds, a sublibrary of fragments was developed based on a single hit from CFL-1.1. The 8-hydroxyquinoline scaffold (**7b**) was selected for development into a small sublibrary because it was found to be a potent hit against the MMPs in our screening of CFL-1.1. To the best of our knowledge, hydroxyquinolines have seen very limited use as a MBG in MMP inhibitors.[23,24,31,72,73] A focused library of

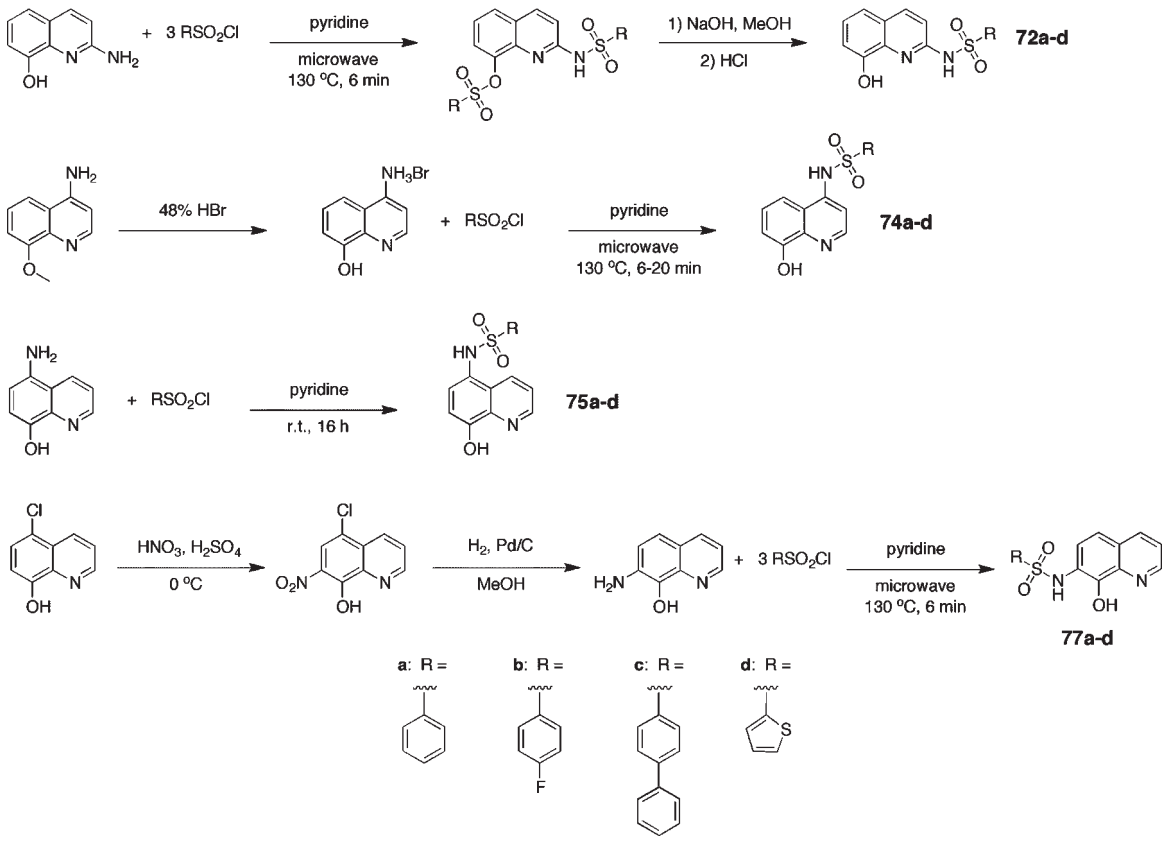

Figure 3. Synthesis of the components of the 8-hydroxyquinoline sublibrary. Substituents were appended to four different positions (2-, 4-, 5-, and 7-positions) around the hydroxyquinoline ring.

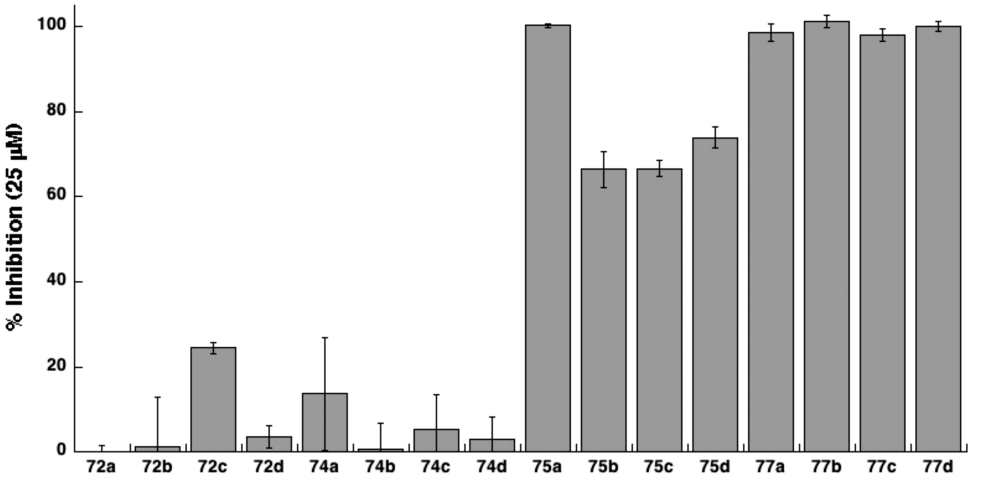

Figure 4. Results of hydroxyquinoline sublibrary screen against MMP-2 at 25 μM. Only compounds with substituents at the 5- and 7-positions gave significant inhibition activity.

16 fragments based on 8-hydroxyquinoline was prepared by derivatizing this scaffold around the ring at positions 2, 4, 5, and 7. These four positions were selected in order to place substituents thoroughly around the ring system, as well as for the synthetic accessibility of these particular sites.[74] At each of the four positions, an amine group was installed that was coupled with one of four different sulfonyl chlorides to generate sulfonamide analogues (Figure 3). Substituents were loosely selected based on efficacy of previously reported MMP inhibitors.[47,61]

The 8-hydroxyquinoline-focused library was initially screened against MMP-2 (as a representative metalloprotein target) at a concentration of 25 μM; the results are shown in Figure 4. The assay results clearly show that the derivatives substituted at the 2- and 4-positions (**72a**−**d** and **74a**−**d**) are ineffective against MMP-2 (only **72c** has an IC_{50} value of 48 ± 1 μM). In contrast, the compounds substituted at the 5- and 7-positions (**75a**−**d** and **77a**−**d**) all showed >65% inhibition of MMP-2, regardless of the specific R-group. The IC_{50} values of these compounds against MMP-2 were found to be in the low micromolar range (Table 2). The most potent hit from this sublibrary, **77b**, has an IC_{50} value of 3 μM, representing a >40-fold improvement over fragment **7b**. This improvement clearly demonstrates that hits from

Table 2. Calculated IC_{50} Values, Heavy Atom Count (HAC), and Ligand Efficiency (LE) for **75a**−**d** and **77a**−**d** Fragments

compound	IC_{50} value (μM)	HAC	LE (kcal/mol) (37 °C)
75a	8.8 ± 0.6	21	0.34
75b	7.2 ± 1.0	22	0.33
75c	10.8 ± 0.4	27	0.26
75d	8.4 ± 0.3	20	0.36
77a	5.6 ± 1.9	21	0.36
77b	3.0 ± 0.5	22	0.36
77c	3.9 ± 0.4	27	0.28
77d	4.3 ± 0.2	20	0.38

CFL-1.1 can be readily improved by derivatization in a focused library. In addition, it is important to note that effective inhibition in this focused library is highly dependent on the position of the substituent with only two positions giving potent derivatives. Furthermore, the position of the substituent appears to be more important than the nature of the substituent at this stage of fragment growth; that is, none of the backbone substituents results in effective MMP-2 inhibition at all positions on the hydroxyquinoline ring. This shows both the versatility and promise presented by identifying new chelator leads from a fragment library; one hit fragment can generate more than one subsequent focused fragment (in this case both **75a**−**d** and **77a**−**d** fragments).

Discussion

Fragments identified as hits against the MMPs from screening CFL-1.1 included hydroxypyrothiones (**2d** and **5d**), hydroxypyridinethiones (**2e**, **7e**, and **9e**), and nitrogen-based ligands (**1a**, **3g**, and **4g**). Many of these compounds had been identified in early studies,[26,28,64] demonstrating that the CFL approach was valid and could readily identify active fragments. Consistent with prior reports, the O,S donor ligands (**2d**, **5d**, **2e**, **7e**, and **9e**) are more potent against MMPs than their structural analogs with O,O donor atoms (**1d**, **4d**, **1e**, and **8e**).[26,64] New MMP hits from CFL-1.1 include picolinic acids (**5a**, **12a**), quinolines (**2b**, **3b**, **5b**−**10b**, **12b**), pyrimidines (**1c**, **3c**−**5c**), hydroxypyrones (**7d**−**12d**), hydroxypyridinones (**10e**−**12e**), salicylic acid (**5f**), hydroxyquinone (**5g**), a bipyridine derivative (**6g**), tropolones (**11g**, **12g**), naphthalene diol (**1h**), and a benzoic acid derivative (**3h**). By comparing the inhibition of MMPs across a chelator class, information about binding mode can be inferred. The most potent quinolines (**2b**, **7b**−**9b**) are all substituted with a hydroxyl group at the 8-position. The similarities in structures and activities of these compounds support a conserved binding mode with the quinoline nitrogen and the hydroxyl oxygen atoms binding to the zinc(II) ion.

Perhaps the most important result from the screens of CFL-1.1 against the five MMPs is the commonality of hits across the enzyme family. Compounds that inhibit one MMP tend to inhibit all of the MMPs tested. Conversely, the set of fragments that fail to inhibit at 1 mM are the same for all five MMPs. This can be readily recognized in the thermoplot (Figure 2) by comparing the inhibition of hits (**9b**, **4c**, **2d**, **12e**, and **11g**) and "non-hits" (**6a**, **6c**, **8e**, **1f**, and **8h**) across all five MMPs. Because the zinc(II) active site is highly conserved across MMP family members, few differences in chelator potency are expected among different MMP enzymes. However, some recent reports have indicated that in combination with an appropriate backbone group, the selection of a given chelator can augment the selectivity of an MMPi.[30]

While the screening results of CFL-1.1 are largely the same across the different MMPs, MMP-3 did show some variability. Fifteen fragments were found to be significantly more potent for MMP-3 than the other MMPs. This is most clearly seen with the picolinic acids, where seven of these compounds (**1a**, **2a**, **4a**, **8a**, **9a**, **10a**, **12a**) show improved inhibition of MMP-3 relative to the other MMPs. In particular, **1a**, **4a**, **10a**, and **12a** inhibit MMP-3 >80% at 1 mM but are not potent against other MMPs. Unlike most other MMPs, which operate best at pH 7.4, MMP-3 activity is optimal at pH 6, which may be one factor that contributes to the difference in potency of the picolinic acids against this particular MMP.[75]

In order to evaluate the use of CFL-1.1 against other metalloenzymes, the library was screened against LF, 5-LO, TY, and iNOS. All of these enzymes possess metal ions at their active sites and were chosen for their functional diversity and biomedical importance. The fragments that hit each enzyme vary significantly, which suggests that choosing specific MBG scaffolds can be used as a starting point for developing metalloenzyme-selective inhibitors. The LF hits of CFL-1.1 most closely align with the MMP hits, a fact that reflects that they are both zinc(II)-dependent proteases. Even with their similarities, the thermoplot (Figure 2) shows LF hits that are not hits against the MMPs. For example, several picolinic acids that do not inhibit MMPs (**6a**−**8a**, **11a**) reduce LF activity moderately at 1 mM. The different coordination environment of the zinc(II) ion in these enzymes, as well as divergence in the immediate active site environment between LF and MMPs, likely contribute to the ability of picolinic acids to selectively inhibit LF.[76−79]

The hits against the non-heme iron enzyme 5-LO are very different from the hits against LF or the MMPs. Numerous pyrimidines (**2c**, **3c**, **5c**, **7c**, **8c**, **10c**) are 5-LO hits, and essentially all hydroxypyrones and hydroxypyridinones effectively inhibit 5-LO. Unlike the zinc(II)-dependent enzymes, which show a strong preference for O,S donor ligands among the hydroxypyrones and hydroxypyridinones, 5-LO shows no pronounced preference, which likely reflects the greater affinity of the iron(III) ion (which is generated in the 5-LO catalytic cycle) for hard Lewis basic O,O donor ligands. For the dinuclear copper enzyme TY, the most distinguishing hits are the picolinic acids (**1a**−**12a**) and the salicylic acids (**1f**−**12f**), the latter of which were found to be generally ineffective against all the other metalloenzymes studied. It is our contention that the differences between the hits found for 5-LO, and TY when compared with the MMPs and LF are significant and support the use of CFLs in the discovery and development of metalloprotein-selective inhibitors. These varying preferences for MBGs likely arise from both the ligand affinities for the different metal ions and differences in the shape and size of the metal active sites. The high degree of variability among metalloenzyme hits supports the notion that metal-binding fragments may be optimized for a given metalloenzyme, which represents an area of study that has not been given substantial attention in the development of metalloenzyme inhibitors.

One thing the aforementioned proteins (MMPs, LF, 5-LO, TY) have in common is that they are all inhibited by a high percentage of fragments from CFL-1.1. In contrast, few fragments from CFL-1.1 inhibit iNOS, which is consistent with the fact that fragments chosen for CFL-1.1 are designed to bind metals through two or more donor atoms. CFL-1.1 was designed specifically for metalloenzymes with more than one adjacent, vacant coordination site on the metal ion

(i.e., most fragments provide two or more donor atoms for binding). Because iNOS is a heme enzyme, only one axial binding site is accessible on the iron ion, rendering most of the fragments of CFL-1.1 ineffective due to an inability to chelate the heme center ion. The ability to target some metalloenzymes, but not others, by rational selection of the library fragments again illustrates the ability to tailor these libraries to specific classes of metalloenzyme targets.

Examination of the LE values reveals that the hits from the screen of CFL-1.1 are very high-quality fragments for FBLD. LE values account for both ligand size and potency and are a good way to compare the quality of initial hits. Generally a LE of > 0.3 kcal/mol is considered promising for a fragment hit.[41] Table 1 shows the ligand efficiencies of a number of chelator hits against MMP-2, LF, 5-LO, and TY. Compound **1g** (acetohydroxamic acid) represents the fragment equivalent of the most common MBG for many metalloenzyme inhibitors including MMPs and LF.[24,26,31,65] The LE of **1g** against MMP-2 is excellent, at 0.52 kcal/mol, but other hits from CFL-1.1 far exceed this value. For example, compounds **4c** and **2d** demonstrate extraordinary LE values of 0.70 and 0.73 kcal/mol against MMP-2, respectively. Similarly, fragment **2d** has a higher LE for LF when compared with **1g** (Table 1). Several of the chelators identified as 5-LO and TY hits also show very high LE values, demonstrating the usefulness of CFL-1.1 for identifying scaffolds for elaboration into potent metalloprotein inhibitors.

The ability to elaborate these MBG fragments into more advanced hits is essential for inhibitor development. Unlike hydroxamic acids, many of the chelators from CFL-1.1 may be modified at more than one position due to their cyclic structures. As a proof-of-concept, derivatives of **7b**, which was a potent hit against MMP-2 (Table 2), were synthesized with substituents at one of four different positions around the 8-hydroxyquinoline ring. As seen in Figure 4, the positioning of the backbone has a pronounced effect on the potency of the inhibitor. The fragments with substituents on the 5- and 7-positions of 8-hydroxyquinoline inhibit MMP-2 with micromolar IC_{50} values, while derivatives with backbones at the 2-position or 4-position show little efficacy when screened at 25 μM. Interestingly, the structural differences between these backbones do not affect inhibitor potency as significantly as the position of the groups on the MBG. It is likely that differences in the relative orientation of the fragment in the active site, imposed by the metal−ligand interactions, contribute to the observed differences in activity. The synthetic flexibility offered by chelators with multiple sites of derivitzation (such as **7b**) may be used to probe productive binding pockets surrounding the metal ion. In addition, having multiple sites of substitution demonstrates that one hit from CFL-1.1 can actually produce multiple sublibraries and advanced hits of different connectivity (e.g., 5- and 7-substituted fragments). Sublibraries can also be developed with fragments bearing more than one substituent on these cycle scaffolds.

Conclusions

This work describes the development and evaluation of a fragment library based on metal chelators. A diverse collection of ligands is presented in order to offer alternatives to the common metal-binding motifs found in most metalloenzyme inhibitors. CFL-1.1 produced high hit rates against MMPs, LF, 5-LO, and TY, but not against the heme-dependent iNOS. Many of the chelators identified as new MBGs may be elaborated at multiple positions, as demonstrated by the preparation of a small, focused library based on an 8-hydroxyquinoline fragment. This focused library showed that substitution at either the 5- or 7-positions of the 8-hydroxyquinoline gave potent leads against MMP-2, while derivatives at the 2- and 4-positions did not. Overall this study shows the value of chelator libraries in FBLD against metalloprotein targets and should open up a wide variety of new scaffolds from which new inhibitors can be devised.

Experimental Section

General. Unless otherwise noted, starting materials were purchased from commercial suppliers (Sigma-Aldrich, ChemBridge, Acros Organics) and were used without further purification. All commercial materials were listed as 95% purity or greater and were used without further purification. The purity of all synthesized compounds was determined to be ≥95% by either elemental analysis or HPLC. Flash silica gel chromatography was performed using Merck silica gel 40−63 μm mesh. ^{1}H NMR spectra were recorded on one of several Varian FT-NMR spectrometers, property of the Department of Chemistry and Biochemistry, University of California, San Diego. Mass spectrometry was performed at the Small Molecule Mass Spectrometry Facility in the Department of Chemistry and Biochemistry, University of California, San Diego. Microwave reactions were performed in 10 mL vials using a CEM Discover S-class microwave reactor. For all assays, IC_{50} values were obtained for select fragments that showed ≥50% inhibition. IC_{50} values of hits were obtained by preparing serial dilutions of DMSO stock solutions for each compound. IC_{50} values were calculated from the plotted dose−response curve using GraphPad Prism 5 software.

Chelator Fragment Library 1.1 (CFL-1.1): Synthesis and Characterization. CFL-1.1 was assembled as previously described for CFL-1 with the following exceptions:[42] four compounds in CFL-1 were replaced with those shown in Figure 1 (**6b**, **10b**, **7f**, and **2h**). Compounds **6b**, **7f**, and **2h** from CFL-1.1 were obtained from commercial suppliers, while compound **10b** was synthesized as described below. Compounds were stored as solids or in DMSO stock solutions at a concentration of 50 mM.

Fragment 10b. 8-Aminoquinoline (200 mg, 1.39 mmol) and methanesulfonyl chloride (162 μL, 2.08 mmol) were dissolved in 3 mL of pyridine. The solution was irradiated in a microwave synthesizer for 3 min at 130 °C.[61] The reaction mixture was poured over 10 mL of H_2O, and the precipitate was isolated by vacuum filtration. Yield: 210 mg (68%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.34 (s, br, 1H), 8.90 (dd, J = 4.0, 1.7 Hz, 1H), 8.40 (dd, J = 8.6, 1.7 Hz, 1H), 7.70 (m, 2H), 7.62 (q, J = 4 Hz, 1H), 7.57 (t, J = 8 Hz, 1H), 3.12 (s, 3H). ESI-MS(+): m/z 223.22 [M + H]$^+$. ^{13}C NMR (125 MHz, DMSO-d_6): δ 149.9, 139.3, 137.1, 134.7, 128.7, 127.3, 123.3, 122.9, 117.2. Anal. Calcd for $C_{10}H_{10}N_2O_2S$: C, 54.04; H, 4.53; N, 12.60. Found: C, 53.70; H, 4.37; N, 12.50.

***N*-(8-Hydroxyquinolin-2-yl)benzenesulfonamide (72a).** 2-Amino-8-hydroxyquinoline (100 mg, 0.624 mmol) and benzenesulfonyl chloride (236 μL, 1.87 mmol) were dissolved in 3 mL of pyridine. The reaction mixture was irradiated in a microwave synthesizer at 130 °C for 6 min. The reaction was quenched with 10 mL of water and then extracted with 10 mL of CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and dried to yield a white powder. This white powder is the intermediate 2-(phenylsulfonamido)quinolin-8-yl benzenesulfonate, which contains a sulfonamide moiety at the 2-position as well as a sulfonate ester group at the 8-position of the ring. Therefore, to remove the sulfonate ester, the intermediate was dissolved in 2−3 mL of MeOH, followed by addition of 3 mL of 2 M NaOH dropwise, after which the mixture was heated to reflux for 4−6 h under N_2. After heating, the solution was allowed to cool to room temperature, and the MeOH was removed under vacuum.

To the aqueous solution was added 1 M HCl dropwise until a precipitate formed. The precipitate was filtered, rinsed with water, and purified by flash chromatography on silica with 0−3% MeOH in CH_2Cl_2 as the eluant. A white solid was isolated. Yield: 40 mg (21%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.12 (s, br, 1H), 11.04 (s, br, 1H), 8.18 (d, J = 9.2 Hz, 1H), 7.89 (s, br, 2H), 7.59−7.53 (m, 3H), 7.29−7.22 (m, 2H), 7.12 (d, J = 6.4 Hz, 1H), 7.06 (d, J = 10 Hz, 1H). ESI-MS (+): m/z 301.26 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6): δ 143.3, 141.9, 132.6, 132.6, 126.3, 125.3, 118.7, 115.4. Anal. Calcd for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33. Found: C, 60.23; H, 4.23; N, 9.39.

4-Fluoro-*N*-(8-hydroxyquinolin-2-yl)benzenesulfonamide (72b). Compound **72b** was synthesized according to the procedure described for **72a** starting from 2-amino-8-hydroxyquinoline (100 mg, 0.624 mmol) and 4-fluorobenzene-1-sulfonyl chloride (364 mg, 1.87 mmol). Purification of this compound did not require column chromatography. Yield: 74 mg (37%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.17 (s, br, 1H), 11.05 (s, br, 1H), 8.19 (d, J = 9.2 Hz, 1H), 7.95 (s, br, 2H), 7.38 (t, J = 8.8 Hz, 2H), 7.30−7.23 (m, 2H), 7.13 (d, J = 7.2 Hz, 1H), 7.08 (d, J = 9.6 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 165.3, 163.3, 129.2, 125.4, 118.7, 116.6, 116.4. ESI-MS (+): m/z 319.24 $[M + H]^+$. Anal. Calcd for $C_{15}H_{11}FN_2O_3S$: C, 56.60; H, 3.48; N, 8.80. Found: C, 56.99; H, 3.82; N, 8.74.

***N*-(8-Hydroxyquinolin-2-yl)-[1,1′-biphenyl]-4-sulfonamide (72c).** Compound **72c** was synthesized according to the procedure described for **72a** starting from 2-amino-8-hydroxyquinoline (100 mg, 0.624 mmol) and [1,1′-biphenyl]-4-sulfonyl chloride (473 mg, 1.87 mmol). Yield: 26 mg (11%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.19 (s, br, 1H), 11.07 (s, br, 1H), 8.19 (d, J = 9.2 Hz, 1H), 7.95 (d, J = 5.6 Hz, 2H), 7.83 (d, J = 8 Hz, 2H), 7.69 (d, J = 7.6 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.41 (t, J = 6.8 Hz, 1H), 7.30−7.22 (m, 2H), 7.13 (d, J = 7.6 Hz, 1H), 7.08 (d, J = 10 Hz, 1H). ESI-MS (+): m/z 377.28 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6): δ 139.1, 129.5, 128.8, 127.7, 127.5, 125.3, 118.7, 118.7. Anal. Calcd for $C_{21}H_{16}N_2O_3S \cdot 0.46\,CH_3OH$: C, 65.89; H, 4.60; N, 7.16. Found: C, 65.92; H, 4.67; N, 7.45.

***N*-(8-Hydroxyquinolin-2-yl)thiophene-2-sulfonamide (72d).** Compound **72d** was synthesized according to the procedure described for **72a** starting from 2-amino-8-hydroxyquinoline (100 mg, 0.624 mmol) and thiophene-2-sulfonyl chloride (342 mg, 1.87 mmol). Yield: 30 mg (16%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.17 (d, J = 9.2 Hz, 1H), 7.72 (m, 2H), 7.29 (m, 2H), 7.15 (dd, J = 5.6, 2.8 Hz, 1H), 7.10 (m, 2H). ESI-MS (+): m/z 307.30 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6) δ 148.8, 142.5, 132.1, 130.7, 127.8, 125.5, 118.7, 115.7. HPLC: 98.39% pure.

4-Amino-8-hydroxyquinoline·HBr. 4-Amino-8-hydroxyquinoline was prepared according to a literature procedure starting from 4-amino-8-methoxyquinoline (100 mg, 0.57 mmol).[80] Yield: 130 mg (84%). ^{1}H NMR (400 MHz, D_2O): δ 7.77 (d, J = 6.4 Hz, 1H), 7.10 (d, J = 6.4 Hz, 2H), 6.93 (dd, J = 6.8, 2.4 Hz, 1H), 6.40 (d, J = 7.2 Hz, 1H). ESI-MS(+): m/z 161.33 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6) δ 149.9, 139.3, 137.1, 134.7, 128.7, 127.3, 123.3, 122.9, 117.2.

***N*-(8-Hydroxyquinolin-4-yl)benzenesulfonamide (74a).** 4-Amino-8-hydroxyquinoline·HBr (50 mg, 0.21 mmol) and benzenesulfonyl chloride (29 μL, 0.23 mmol) were dissolved in 3 mL of pyridine. The reaction mixture was irradiated in a microwave synthesizer at 130 °C for 6 min. The reaction was quenched with 10 mL of water and then extracted with 10 mL of CH_2Cl_2. The organic layer was dried with $MgSO_4$, filtered, and dried to yield a white solid. Yield: 16 mg (25%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.05 (d, J = 6 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.82 (d, J = 8.4 Hz, 2H), 7.57 (q, J = 6.4 Hz, 2H), 7.42 (t, J = 8 Hz, 2H), 7.35 (t, J = 8 Hz, 1H), 6.53 (d, J = 5.6 Hz, 1H). ESI-MS(+): m/z 301.05 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.6, 163.4, 160.6, 150.4, 148.8, 134.9, 134.5, 129.7, 128.7, 122.4, 119.8, 103.4. Anal. Calcd for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33. Found: C, 59.73; H, 4.08; N, 9.32.

4-Fluoro-*N*-(8-hydroxyquinolin-4-yl)benzenesulfonamide (74b). Compound **74b** was synthesized according to the procedure described for **74a** starting from 4-amino-8-hydroxyquinoline·HBr (100 mg, 0.41 mmol) and 4-fluorobenzene-1-sulfonyl chloride (126 mg, 0.65 mmol). Synthesis of this compound required 20 min of microwave irradiation. A white solid was isolated. Yield: 52 mg (40%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.49 (s, 1H), 8.04, (t, J = 9.2 Hz, 1H), 7.87−7.84 (m, 2H), 7.59 (d, J = 6.9 Hz, 1H), 7.40 (t, J = 7.4 Hz, 1H), 7.16 (t, J = 6.9 Hz, 2H), 6.58 (d, J = 3.4 Hz, 1H). ESI-MS(+): m/z 319.02 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.48, 164.46, 160.9, 131.9, 131.8, 131.8, 127.9, 127.8, 122.2, 116.7, 116.5, 114.4, 114.2, 102.9. Anal. Calcd for $C_{15}H_{11}FN_2O_3S \cdot 0.73CH_3OH \cdot 0.14H_2O$: C, 54.84; H, 4.89; N, 8.13. Found: C, 55.36; H, 4.02; N, 7.75.

***N*-(8-Hydroxyquinolin-4-yl)-[1,1′-biphenyl]-4-sulfonamide (74c).** Compound **74c** was synthesized according to the procedure described for **74a** starting from 4-amino-8-hydroxyquinoline·HBr (100 mg, 0.41 mmol) and [1,1′-biphenyl]-4-sulfonyl chloride (157 mg, 0.62 mmol). Synthesis of this compound required 10 min of microwave irradiation. A white solid was isolated. Yield: 30 mg (20%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (s, 1H), 7.00 (d, J = 8.6 Hz, 2H), 7.69 (d, J = 6.9 Hz, 1H), 7.61 (d, J = 8.0 Hz, 2H), 7.55 (t, J = 8.5 Hz, 3H), 7.47 (t, J = 6.3 Hz, 2H), 7.40 (t, J = 7.4 Hz, 1H) 7.32 (t, J = 8.0 Hz, 1H), 6.49 (s, 1H). ESI-MS(+): m/z 377.09 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6): δ 146.2, 138.5, 134.7, 129.6, 129.4, 129.3, 128.6, 127.7, 127.6, 127.6, 127.5, 122.3, 103.3. Anal. Calcd for $C_{21}H_{16}N_2O_3S \cdot 0.92\,CH_3OH$: C, 64.86; H, 4.89; N, 6.90. Found: C, 64.68; H, 4.59; N, 6.49.

***N*-(8-Hydroxyquinolin-4-yl)-thiophene-2-sulfonamide (74d).** Compound **74d** was synthesized according to the procedure described for **74a** starting from 4-amino-8-hydroxyquinoline·HBr (100 mg, 0.41 mmol) and thiophene-2-sulfonyl chloride (112 mg, 0.61 mmol). A white solid was isolated. Yield: 54 mg (44%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.09 (d, J = 5.2 Hz, 1H), 8.01 (d, J = 8.6 Hz, 1H), 7.79 (d, J = 5.2 Hz, 1H), 7.61 (d, J = 3.4 Hz, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.38 (t, J = 8.0 Hz, 1H), 7.02 (t, J = 8.6 Hz, 1H), 6.55 (d, J = 5.8 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 151.6, 150.6, 144.9, 142.0, 136.4, 136.0, 134.4, 127.8, 122.5, 122.1, 121.6, 120.1, 103.0. ESI-MS(+): m/z 307.03 $[M + H]^+$. Anal. Calcd for $C_{13}H_{10}N_2O_3S_2$: C, 50.97; H, 3.29; N, 9.14. Found: C, 50.68; H, 3.64; N, 9.10.

***N*-(8-Hydroxyquinolin-5-yl)benzenesulfonamide (75a).** 5-Amino-8-hydroxyquinoline·2HCl (100 mg, 0.429 mmol) and benzenesulfonyl chloride (81 μL, 0.644 mmol) were dissolved in 3 mL of pyridine. The reaction was allowed to stir overnight under N_2. The reaction was quenched with 10 mL of water and extracted with 10 mL of CH_2Cl_2. The organic layer was dried over $MgSO_4$ and purified by flash silica chromatography in 0−3% methanol in CH_2Cl_2. Yield: 22 mg (17%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.98 (s, 2H), 8.80 (d, J = 4 Hz, 1H), 8.26 (d, J = 8.4 Hz, 1H), 7.59 (d, J = 8 Hz, 3H), 7.51−7.45 (m, 3H), 6.94 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 152.7, 148.2, 139.4, 138.4, 132.7, 132.1, 129.1, 126.8, 126.5, 126.4, 122.4, 121.6, 110.5. ESI-MS (+): m/z 301.19 $[M + H]^+$. Anal. Calcd for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33. Found: C, 59.88; H, 4.40; N, 9.70.

4-Fluoro-*N*-(8-hydroxyquinolin-5-yl)benzenesulfonamide (75b). Compound **75b** was synthesized according to the procedure described for **75a** starting from 5-amino-8-hydroxyquinoline·2HCl (100 mg, 0.429 mmol) and 4-fluorobenzenesulfonyl chloride (125 mg, 0.644 mmol). Yield: 24 mg (21%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s, br, 2H), 8.81 (dd, J = 4, 1.6 Hz, 1H), 8.26 (dd, J = 8.8, 1.6 Hz, 1H), 7.63 (dd, J = 8.8, 5.2 Hz, 2H), 7.49 (dd, J = 8.4, 4 Hz, 1H), 7.34 (t, J = 8.8 Hz, 2H), 6.95 (s, 2H). ESI-MS (+): m/z 319.24 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.2, 152.8, 148.3, 148.3, 138.4, 135.8, 132.1, 129.9, 129.8, 126.7, 126.4, 122.2, 121.7, 116.35, 116.2, 110.5. Anal. Calcd for $C_{15}H_{11}FN_2O_3S \cdot 0.29CH_3OH$: C, 56.05; H, 3.74; N, 8.55. Found: C, 55.88; H, 3.77; N, 8.91.

***N*-(8-Hydroxyquinolin-5-yl)-[1,1′-biphenyl]-4-sulfonamide (75c).** Compound **75c** was synthesized according to the procedure described for **75a** starting from 5-amino-8-hydroxyquinoline·2HCl (100 mg, 0.429 mmol) and [1,1′-biphenyl]-4-sulfonyl chloride (163 mg,

0.644 mmol). Yield: 60 mg (37%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s, br, 1H), 9.99 (s, br, 1H), 8.79 (dd, J = 4, 1.6 Hz, 1H), 8.31 (dd, J = 8.4, 1.2 Hz, 1H), 7.79 (d, J = 8.4 Hz, 2H), 7.68 (dd, J = 13.2, 7.2 Hz, 4H), 7.50−7.43 (m, 4H), 6.98 (q, J = 8 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 152.7, 148.2, 144.0, 138.4, 138.3, 132.2, 129.1, 128.6, 127.5, 127.2, 127.0, 126.5, 126.4, 122.4, 122.4, 121.6, 110.5. ESI-MS (+): m/z 377.16 $[M + H]^+$. Anal. Calcd for $C_{21}H_{16}N_2O_3S$: C, 67.00; H, 4.28; N, 7.45. Found: C, 66.77; H, 4.66; N, 7.45.

***N*-(8-Hydroxyquinolin-5-yl)thiophene-2-sulfonamide (75d).** Compound **75d** was synthesized according to the procedure described for **75a** starting from 5-amino-8-hydroxyquinoline·2HCl (100 mg, 0.429 mmol) and thiophene-2-sulfonyl chloride (118 mg, 0.644 mmol). Yield: 21 mg (16%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.77 (dd, J = 4, 1.6 Hz, 1H), 8.33 (dd, J = 8.4, 1.6 Hz, 1H), 7.71 (dd, J = 4.8, 1.6 Hz, 1H), 7.43 (dd, J = 8.4, 4 Hz, 1H), 7.33 (dd, J = 4, 1.6 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.03 (dd, J = 5.2, 3.6 Hz, 1H), 6.98 (d, J = 8 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 153.3, 148.7, 140.3, 138.8, 133.7, 132.8, 132.4, 128.1, 127.2, 126.9, 122.6, 122.2, 110.9. ESI-MS (+): m/z 307.21 $[M + H]^+$. Anal. Calcd for $C_{13}H_{10}N_2O_3S_2$: C, 50.97; H, 3.29; N, 9.14. Found: C, 50.82; H, 3.60; N, 9.48.

5-Chloro-8-hydroxy-7-nitroquinoline. 5-Chloro-8-hydroxy-7-nitroquinoline was prepared according to a literature procedure.[74] Yield: 1.6 g (66%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.10 (d, J = 4.4 Hz, 1H), 8.61 (d, J = 9.2 Hz, 1H), 8.21 (s, 1H), 7.95 (dd, J = 8.4, 4.4 Hz, 1H). ESI-MS(−): m/z 223.35 $[M - H]^-$. ^{13}C NMR (125 MHz, DMSO-d_6): δ 150.9, 150.5, 140.4, 134.2, 132.8, 129.0, 126.4, 122.4, 118.4.

7-Amino-8-hydroxyquinoline. 7-Amino-8-hydroxyquinoline was prepared according to a literature procedure.[74] Yield: 406 mg (95%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.16 (s, br, 1H), 8.77 (d, J = 7.5 Hz, 1H), 8.71 (d, J = 5.7 Hz, 1H), 8.34 (d, J = 3 Hz, 1H), 7.66 (d, J = 8.7 Hz, 1H), 7.48 (t, J = 5.4 Hz, 1H), 7.39 (d, J = 8.7 Hz, 1H). ESI-MS(+): m/z 161.32 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6) δ 145.3, 142.7, 131.0, 123.2, 122.5, 122.2, 121.2, 117.1, 115.8.

***N*-(8-Hydroxyquinolin-7-yl)benzenesulfonamide (77a).** Compound **77a** was synthesized according to the procedure described for **74a** starting from 7-amino-8-hydroxyquinoline (100 mg, 0.625 mmol) and benzenesulfonyl chloride (78 μL, 0.618 mmol). The product was purified by flash silica chromatography in 0−3% MeOH in CH_2Cl_2 followed by recrystallization in EtOH. Yield: 11 mg (6%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.81 (s, br, 2H), 8.76 (s, 1H), 8.24 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 6.8 Hz, 2H), 7.56−7.51 (m, 1H), 7.47 (d, J = 7.6 Hz, 4H), 7.31 (dd, J = 8.8, 2.8 Hz, 1H). ESI-MS (+): m/z 301.19 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6): δ 148.9, 146.2, 141.2, 138.8, 136.5, 133.0, 129.4, 127.0, 126.8, 125.2, 121.8, 121.1, 117.5. Anal. Calcd for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33. Found: C, 59.97; H, 4.34; N, 9.20.

4-Fluoro-*N*-(8-hydroxyquinolin-7-yl)benzenesulfonamide (77b). Compound **77b** was synthesized according to the procedure described for **74a** starting from 7-amino-8-hydroxyquinoline (100 mg, 0.625 mmol) and 4-fluorobenzene-1-sulfonyl chloride (120 mg, 0.618 mmol). The product was purified by recrystallization in EtOH. Yield: 52 mg (26%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.84 (s, br, 1H), 8.78 (dd, J = 3.6, 0.9 Hz, 1H), 8.27 (dd, J = 8.4, 1.8 Hz, 1H), 7.77 (dd, J = 9, 5.7 Hz, 2H), 7.51−7.46 (m, 2H), 7.36−7.28 (m, 3H). ESI-MS (+): m/z 319.03 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6): δ 148.9, 146.7, 138.8, 137.5, 136.5, 130.1, 130.0, 127.0, 125.9, 121.9, 120.8, 117.6, 116.6, 116.4. Anal. Calcd for $C_{15}H_{11}FN_2O_3S \cdot 0.29H_2O$: C, 55.74; H, 3.60; N, 8.67. Found: C, 55.54; H, 4.02; N, 9.11.

***N*-(8-Hydroxyquinolin-7-yl)-[1,1′-biphenyl]-4-sulfonamide (77c).** Compound **77c** was synthesized according to the procedure described for **74a** starting from 7-amino-8-hydroxyquinoline (100 mg, 0.625 mmol) and [1,1′-biphenyl]-4-sulfonyl chloride (156 mg, 0.618 mmol). The product was purified by flash silica chromatography in 0−3% MeOH in CH_2Cl_2. Yield: 30 mg (13%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.93 (s, br, 2H), 8.81 (m, 1H), 8.29 (dd, J = 8.4, 2 Hz, 1H), 7.83 (q, J = 8 Hz, 4H), 7.71 (d, J = 6.4 Hz, 2H), 7.57 (dd, J = 8.8, 3.6 Hz, 1H), 7.51−7.47 (m, 3H), 7.44 (dd, J = 8, 3.2 Hz, 1H), 7.38 (dd, J = 8.8, 3.2 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 148.5, 145.7, 143.9, 139.6, 138.4, 138.3, 136.0, 129.1, 128.5, 127.3, 127.1, 127.0, 126.3, 124.8, 121.3, 120.7, 117.1. ESI-MS (+): m/z 377.10 $[M + H]^+$. Anal. Calcd for $C_{21}H_{16}N_2O_3S$: C, 67.00; H, 4.28; N, 7.45. Found: C, 66.78; H, 4.68; N, 7.28.

***N*-(8-Hydroxyquinolin-7-yl)thiophene-2-sulfonamide (77d).** Compound **77d** was synthesized according to the procedure described for **74a** starting from 7-amino-8-hydroxyquinoline (100 mg, 0.625 mmol) and thiophene-2-sulfonyl chloride (113 mg, 0.618 mmol). The product was purified by flash silica chromatography in 0−3% MeOH in CH_2Cl_2 followed by recrystallization in EtOH. Yield: 27 mg (14%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.97 (s, br, 1H), 8.80 (d, J = 4.4 Hz, 1H), 8.28 (d, J = 8 Hz, 1H), 7.83 (d, J = 3.6 Hz, 1H), 7.49 (d, J = 9.2 Hz, 4H), 7.35 (d, J = 8.8 Hz, 1H), 7.06 (t, J = 4.4 Hz, 1H). ESI-MS (+): m/z 306.98 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO-d_6): δ 148.5, 146.5, 141.1, 138.4, 136.1, 132.9, 131.8, 127.4, 126.6, 125.4, 121.5, 120.2, 117.1. Anal. Calcd for $C_{15}H_{12}N_2O_3S$: C, 49.85; H, 4.06; N, 8.39. Found: C, 49.38; H, 4.24; N, 8.05.

Fluorometric Screening against MMPs. CFL-1.1 was screened against MMP-1, -2, -3, -8, and -9 at a concentration of 1 mM for each fragment. The assay was carried out in white NUNC 96-well plates as previously described.[64] Each well contained a total volume of 90 μL including buffer (50 mM HEPES, 10 mM $CaCl_2$, 0.05% Brij-35, pH 7.5), human recombinant MMP (ENZO Life Sciences; 15.3 U of MMP-1, 1.16 U of MMP-2, 2 U of MMP-3, 1.84 U of MMP-8, or 0.9 U of MMP-9), and the fragment solution (1 mM final concentration). After a 30 min incubation period at 37 °C, the reaction was initiated by the addition of 10 μL of the fluorogenic MMP substrate (4 μM final concentration, Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2·AcOH, ENZO Life Sciences). Fluorescence measurements were recorded using a Bio-Tek Flx 800 fluorescence plate reader every minute for 20 min with excitation and emission wavelengths at 320 and 400 nm, respectively. The rate of fluorescence increase was compared for samples versus negative controls (no inhibitor, arbitrarily set as 100% activity). Eighteen compounds from CFL-1.1 were omitted from the screening due to excessive background fluorescence that interfered with the assay readings. A separate buffer (50 mM MES, 10 mM $CaCl_2$, 0.05% Brij-35, pH 6.0) was used for all experiments with MMP-3. The 8-hydroxyquinoline sublibrary was screened against MMP-2 using the procedure described above with a final fragment concentration in each well of 25 μM.

Colorimetric Screening against MMPs. Due to excessive background fluorescence, 18 compounds from CFL-1.1 were screened in a colorimetric assay against MMP-1, -2, -3, -8, and -9 at a concentration of 1 mM for each fragment. The assay was carried out in clear Costar 96-well, half-area, flat-bottom assay plates. Each well contained a total volume of 90 μL including buffer (50 mM HEPES, 10 mM $CaCl_2$, 0.05% Brij-35, 1 mM DTNB, pH 7.5), human recombinant MMP (ENZO Life Sciences), and the fragment solution (1 mM final concentration). After a 30 min incubation period at 37 °C, the reaction was initiated by the addition of 10 μL of chromogenic MMP substrate (500 μM final concentration, Ac-Pro-Leu-Gly-[2-mercapto-4-methyl-pentanoyl]-Leu-Gly-OC_2H_5, ENZO Life Sciences). Absorbance was monitored at 405 nm using a Bio-Tek ELx 808 colorimetric plate reader, and measurements were recorded every minute for 20 min. The rate of absorbance increase was compared for samples versus negative controls (no inhibitor, arbitrarily set as 100% activity). A separate buffer (50 mM MES, 10 mM $CaCl_2$, 0.05% Brij-35, 1 mM DNTB, pH 6.0) was used for all experiments with MMP-3.

Screening against LF. CFL-1.1 was screened against LF at a concentration of 1 mM for each fragment. The assay was carried

out in white NUNC 96-well plates as previously described.[42] Each well contained a volume of 90 μL including buffer (20 mM HEPES, pH 7.4), recombinant LF (10 nM final concentration, List Biological Laboratories), and the fragment solution (1 mM final concentration). After a 20 min incubation period at 25 °C, the reaction was initiated by the addition of 10 μL of fluorogenic LF substrate (2 μM final concentration, MAPKKide DABCYL/FITC, List Biological Laboratories). Fluorescence measurements were recorded using a Bio-Tek Flx 800 fluorescence plate reader every minute for 20 min with excitation and emission wavelengths at 485 and 528 nm, respectively. The rate of fluorescence increase was compared for samples versus negative controls (no inhibitor, arbitrarily set as 100% activity). During this screen, four compounds (**10b**, **11b**, **10c**, **5e**) were excluded from the assay due to precipitation or interference with the assay.

Screening against 5-LO. CFL-1.1 was screened against 5-LO at a concentration of 1 mM for each fragment. The assay was performed according to a literature procedure at room temperature.[63] Each well contained a volume of 80 μL including buffer (50 mM Tris, 2 mM EDTA, 2 mM $CaCl_2$, pH 7.5), human recombinant 5-LO (0.2 U, Cayman Chemicals), reporter dye (2′,7′-dichlorofluorescin diacetate; H2DCFDA, 10 μM, Invitrogen), fragment solution (1 mM), arachidonic acid (AA, 3 μM, Fischer Scientific), and adenosine triphosphate (ATP, 10 μM, Sigma-Aldrich). H2DCFDA and 5-LO were incubated for 5 min prior to the addition of the fragment solution. This was followed by a second incubation for 10 min. The reaction was initiated by the addition of a substrate solution containing AA and ATP. The reaction was monitored using a Bio-Tek Flx 800 fluorescence plate reader. Fluorescence measurements were recorded every minute for 20 min with excitation and emission wavelengths at 485 and 528 nm, respectively. The rate of fluorescence increase was compared for samples versus negative controls (no inhibitor, arbitrarily set as 100% activity). The 5-LO screen of CFL-1.1 resulted in nine compounds (**3b**, **4d**, **6d**, **10d**, **10e**, **8f**, **1g**, **10g**, and **12g**) that significantly increased the fluorescence of the assay relative to the control with no inhibitor. The origin of this interference was not identified, and these compounds were excluded from further examination with respect to 5-LO.

Screening against TY. CFL-1.1 was screened against TY at a concentration of 1 mM for each fragment. The assay was performed according to a literature procedure at room temperature.[62] Each well contained a volume of 100 μL including buffer (50 nM phosphate, pH 6.8), mushroom TY (30 U, Sigma-Aldrich), fragment solution (1 mM), and L-dopamine (0.5 mM, Sigma-Aldrich). Mushroom TY and the fragment solution were incubated for 10 min. A background absorbance reading at 475 nm was recorded using a Bio-Tek ELx 808 colorimetric plate reader. L-Dopamine was added to initiate the reaction, which was allowed to proceed for 10 min before a second absorbance reading at 475 nm was taken. After subtracting the background absorbance, the remaining absorbance of the negative controls (no inhibitor) was arbitrarily set as 100% activity. The ratio of absorbance between inhibitor and control wells was defined as percent TY activity. Several compounds from CFL-1.1 (**6b**−**10b**, **6g**−**9g**, and **3h**) interfered with the TY assay, resulting in an increase in absorbance relative to the negative control. The origin of this interference was not identified, and these compounds were excluded from further examination with respect to TY.

Screening against iNOS. CFL-1.1 was screened against iNOS at a concentration of 1 mM for each fragment. iNOS assays were performed at 37 °C using a commercially available colorimetric assay kit purchased from Calbiochem. Murine recombinant iNOS protein (0.1 U, Calbiochem), substrate L-arginine (80 μmol, Sigma-Aldrich), and inhibitor fragments (80 μmol) were incubated in assay buffer (Calbiochem, Catalog No. 482702) at a total volume of 60 μL for 5 min. A nitrate reductase solution (prepared as directed by supplier) was added (10 μL) followed by 10 μL of a freshly made stock of 1 mM NADPH to initiate the reaction. After 40 min incubation, the reaction was stopped by heat inactivation of the iNOS (incubation for 30 s in boiling water). In order to destroy excess NADPH, 10 μL of a lactate dehydrogenase (LDH) solution and 10 μL of an LDH cofactor solution were added followed by 20 min incubation. Following this incubation, a background reading of absorbance was taken at 540 nm using a Bio-Tek ELx 808 colorimetric plate reader. Griess Reagent 1 (1% sulfanilamide in 5% phosphoric acid) was added (50 μL) to each well followed by 50 μL of Griess Reagent 2 (0.1% *N*-(1-naphthyl)ethylenediamine·2HCl in water). The reagents were allowed to develop for 10 min prior to collecting a second absorbance measurement at 540 nm. After subtracting the background absorbance, the remaining absorbance of the negative controls (no inhibitor) was arbitrarily set as 100% activity. The ratio of absorbance between inhibitor and control wells was defined as percent iNOS activity.

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Supporting Information Available: Tables of percent inhibition of CFL-1.1 fragments against the tested proteins. This material is available free of charge via the Internet at http://pubs.acs.org.

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