Inhibition of Macrophage Migration Inhibitory Factor by a Chimera of Two Allosteric Binders

Pier F. Cirillo,^{*,†,‡,§} Oluwatoyin A. Asojo,^{*,§} Uday Khire,^{||} Yashang Lee,^{†,⊥} Sara Mootien,^{†,▽} Peter Hegan,^{†,○} Alan G. Sutherland,[†] Elizabeth Peterson-Roth,[†] Michel Ledizet,[†] Raymond A. Koski,[†] and Karen G. Anthony[†]

[†]L² Diagnostics, LLC, 300 George Street, New Haven, Connecticut 06511, United States

[‡]Department of Chemistry and Chemical Engineering, University of New Haven, 300 Boston Post Road, West Haven, Connecticut 06516, United States

[§]Department of Chemistry and Biochemistry, Hampton University, 200 William R. Harvey Way, Hampton, Virginia 23668, United States

^{||}CheminPharma, LLC, 4 Research Drive, Woodbridge, Connecticut 06525, United States

Supporting Information

ABSTRACT: Human Macrophage Migration Inhibitory Factor (MIF) is a trimeric cytokine implicated in a number of inflammatory and autoimmune diseases and cancer. We previously reported that the dye p425 (Chicago Sky Blue), which bound MIF at the interface of two MIF trimers covering the tautomerase and allosteric pockets, revealed a unique strategy to block MIF's pro-inflammatory activities. Structural liabilities, including the large size, precluded p425 as a medicinal chemistry lead for drug development. We report here a rational design strategy linking only the fragment of p425 that binds over the tautomerase pocket to the core of ibudilast, a known MIF allosteric site-specific inhibitor. The chimeric compound, termed L2-4048, was shown by X-ray crystallography to bind at the allosteric and tautomerase sites as anticipated. L2-4048 retained target binding and blocked MIF's tautomerase CD74 receptor binding, and pro-inflammatory activities. Our studies lay the foundation for the design and synthesis of smaller and more drug-like compounds that retain the MIF inhibitory properties of this chimera.

KEYWORDS: MIF, CD74, inhibitor, allosteric, structure-based drug design, fragment-based drug design, X-ray

Macrophage Migration Inhibitory Factor (MIF) is a pleiotropic protein involved in the regulation of immune^{1,2} and inflammatory processes,³ as well as in the induction of apoptosis.^{4–6} MIF is prominently expressed by macrophages, T cells, and platelets, and hence it is found in tissues throughout the body.⁷ MIF has been shown to have cytokine-like activities,^{8,9} as well as to act as a chemokine,¹⁰ a hormone,¹¹ and a chaperone.¹² Recombinant MIF exists mostly as a trimer in solution and is likely recognized in this form by the CD74 receptor,^{13,14} though the oligomeric species interacting with CXCR4, CXCR2, and CXCR7 receptors are not yet fully established.^{15,16} MIF also possesses what is considered, in humans, to be vestigial enzymatic activity as a tautomerase, since no physiologic substrate has been identified to date.¹⁷ The tautomerase active site, including an N-terminal proline,¹⁸ is a cylindrical pocket located at the interface of monomer subunits, just beneath the CD74 binding region.

Because of its central and many-faceted roles, MIF has attracted considerable interest by the drug discovery community.⁷ The majority of MIF inhibitors reported to date act by binding at the tautomerase site.^{19–23} We have previously reported that the dye p425 (also known as Chicago sky blue 6B or Pontamine sky blue) (Figure 1), discovered

through a high-throughput screening effort, is a noncovalent, allosteric inhibitor of MIF.²⁴

Co-crystal structures of MIF-p425 complexes revealed that the inhibitor lies on the surface at the interface of two MIF trimers, covering the entrance to the tautomerase pocket and engaging in hydrogen bonds with Lys32, Asn109, and Asn110.²⁴ P425 inhibits not only the tautomerase activity of recombinant human MIF (*rhMIF*) but also its binding to CD74 receptor (IC₅₀ 0.81 μM), as well as its bioactivity, including dose-dependent induction of pro-inflammatory mediators such as IL-6, IL-8, and MMP3 in foreskin fibroblasts and p53-dependent apoptosis in HeLa cells.²⁴ Despite impressive MIF inhibitory activities, the large size (948.8 MW), highly charged nature brought about by four sulfonates, and the presence of two diazo linkages rendered p425 nonviable as a starting point for medicinal chemistry efforts. Therefore, we explored the possibility of creating a chimeric

Special Issue: Medicinal Chemistry: From Targets to Therapies

Received: July 31, 2019

Accepted: November 19, 2019

Published: November 19, 2019

Figure 1. Chemical structures of allosteric MIF inhibitors p425, ibudilast, and new chimeric compound L2-4048 (1).

molecule with more drug-like characteristics that would retain MIF binding features of p425. To this end, we devised a unique strategy to link the MIF-binding features of p425 with that of ibudilast, a clinically marketed phosphodiesterase inhibitor and a well-characterized allosteric inhibitor of MIF.²⁵ Ibudilast (AV411) and its closely related analog AV1013, bind MIF at an allosteric site adjacent to the tautomerase pocket in a region surrounded by aromatic residues such that the inhibitor binding causes a conformational change in Tyr36.²⁵ This allosteric site has independently been identified by McLean et al. through a fragment screening of inhibitors of MIF tautomerase²⁶ (for other allosteric inhibitors, see refs 7 and 27). Ibudilast inhibits MIF's tautomerase activity (k_i 30.9 μM) in a noncompetitive manner and decreases MIF-mediated chemotaxis.²⁵ Herein we report the design, synthesis, structural elucidation, and MIF inhibitory activities of L2-4048, a unique chimera of p425 and ibudilast allosteric MIF inhibitors.

Comparison of the cocrystal structures of MIF with p425 and ibudilast showed proximity, but no overlap between the two inhibitors, which suggested that a chimeric, structure-based approach utilizing binding features of both molecules would be effective. We envisioned linking only the half of p425 which occludes the tautomerase pocket with a portion of ibudilast, leading to structure 1 (L2-4048) (Figure 1). While still too large (MW 623.7 Da) and possessing two sulfonates and one diazo linkage, L2-4048 represents a step toward the discovery of a potential medicinal chemistry lead.

L2-4048 was synthesized according to Scheme 1. The *meta*-substituted propynyl nitrobenzene 2 could be synthesized in good yield according to the procedure reported by Ye et al.²⁸ Following a standard reduction, protection, and deprotection sequence, terminal alkyne 5 was achieved in moderate yield. Deprotonation to form the acetylide anion and reaction with isobutyraldehyde afforded secondary propargylic alcohol 6, which could be oxidized by bleach and TEMPO catalysis to alkynyl ketone 7. Compound 7 could be converted to the pyrazolo[1,5-*a*]pyridine system 8 by a standard procedure.²⁵ Deprotection of 8 to reveal the aniline, diazotization, and direct reaction with 4-amino-5-hydroxynaphthalene-1,3-disulfonic acid afforded L2-4048 in 9 steps from 2 and an overall yield of 3.9%.

The binding of L2-4048 to MIF was investigated by X-ray crystallography. We determined a high-resolution structure and have deposited the coordinates and structure factors in the Protein Data Bank under accession code 6PEG (Supporting Information). As we observed for p425, there is only one molecule of inhibitor bound per trimer of MIF (Figure 2A). A comparison of the three cocrystal structures of MIF with allosteric inhibitors reveals that the allosteric binding site of L2-4048 is consistently at the interface of two of the crystallographic trimers (i.e., or tightest packing trimers). Interestingly, the allosteric site involves three monomers that form an alternate trimer (Figure 2B). The network of ligand interactions includes residues from three MIF monomers, two in one trimer and one in the second trimer, as was the case with the structure of the complex with p425 (Figure 2C). L2-4048 interacts with residues in the allosteric site, forming a π -stacking interaction with Trp108 and causing the expected

Scheme 1. Synthesis of Chimeric Compound L2-4048 (1)

Figure 2. MIF-L2-4048 cocrystallization results: (A) L2-4048 (gray sticks) binds at the interface of two of the crystallographic MIF trimers (shown in ribbon). (B) Superposition of the *rh*MIF-4048 complex with both the p425-*rh*MIF complex (PDB 3U18) as well as the ternary complex of 4-hydroxyphenylpyruvate (ENO) with the allosteric analogue of ibudilast (AV1013) (PDB 3IJJ) reveals a shared allosteric site that lies in proximity to the active site where 4-hydroxyphenylpyruvate binds. (C) Stereorepresentation of the network of amino acids interacting with L2-4048.

rotation of Tyr36 as was observed for AV1013 (Figure 2C). Additionally, there is an edge-to- π interaction between the benzylic aromatic ring and Phe113. The ketone carbonyl is exposed to solvent.

Access to the tautomerase pocket is blocked by L2-4048 in the same fashion as with p425. The sulfonate ion *ortho*- to the amine is able to interact electrostatically with the charged ammonium side chain of Lys32 (2.7 Å distance, Figure 3).

Figure 3. View of the tautomerase active site of the complex of MIF with L2-4048 (orange). Key amino acid side chains that are in close proximity with the inhibitor are highlighted: Lys 32 (green), Ile64 (blue), and Ala103 (aqua). Pro1 from the active site is evident in the background between Ile64 and Lys32.

Probably because of a high desolvation penalty, this extra charged interaction does not in any way improve binding over ibudilast alone. A similar finding involving the engagement of this same Lys32 was reported by Jorgensen et al.²⁹ The other sulfonate ion is surrounded by a shell of water molecules, just as in the p425 structure. The portion of the naphthalene bearing the diazo and hydroxyl groups is involved in hydrophobic interactions with Ile64 and Ala103 (Figure 3).

Key MIF residues that control activation of CD74, identified by functional analyses of alanine mutants, include Tyr36, Lys66, Asn109, Ile64, and Trp108.²³ Figure 4 shows a comparison of our binding mode in relation to the binding of AV1013 in the allosteric site only, and the binding of the McLean et al. quinolone,²⁶ one of the few MIF inhibitors that occupies both the allosteric site and the tautomerase pocket. By not only occupying the allosteric pocket resulting in the conformational change of Tyr36, but also extending over the surface of MIF, our inhibitor may provide advantages over the other inhibitors whose binding sometimes results in agonistic rather than inhibitory activity with respect to CD74 or other proteins.³⁰

MIF binding efficiency and select activity data of L2-4048 compared to p425 are summarized in Table 1. Surface Plasmon Resonance revealed a K_d of binding very similar to that of p425, and approximately 6.4- to 10-fold lower affinity than ibudilast, which was 1.4 μ M in this assay. The Binding Efficiency Index ($BEI = pk_d/MW$)³¹ for L2-4048 is approximately 25% higher than for p425.

When viewing L2-4048 as an ibudilast analog, the addition of the naphthyl-diazo-phenyl groups has not improved binding affinity. Furthermore, compared with p425, L2-4048 displays a

Figure 4. Comparison of MIF allosteric pocket ligands from superimposing of cocrystal structures of MIF complexed with AV1013 (blue, PDB 3IJG), the McLean et al. quinolone (ref 26) (green, PDB 3LST), which extends into the tautomerase active site, and L2-4048 (orange, PDB 6PEG).

Table 1. Binding Affinity and Inhibition of MIF-Mediated Activities by p425 and L2-4048 (See Supporting Information)

Compound	p425	L2-4048
MW (Da)	904.9	623.7
SPR Biacore K_d (μ M)	9.04 ± 0.83	6.86 ± 0.77
Tautomerase IC_{50} (μ M)	0.37 ± 0.32	7.59 ± 0.21
CD74 binding IC_{50} (μ M)	0.40 ± 0.04	0.93 ± 0.71
IL-8 induction IC_{50} (μ M)	3.90 ± 0.22	4.42 ± 0.85

20-fold lower potency in the tautomerase assay and 2-fold less in inhibiting recombinant MIF–CD74 interaction. The interference with the MIF–CD74 interaction, rather than the blocking of the vestigial enzymatic activity, should have the highest biological relevance and should therefore be most predictive for results in cellular assays. Indeed, L2-4048 attenuates MIF's pro-inflammatory activity to a similar extent as p425 as revealed by decreased IL-8 secretion in MIF-stimulated foreskin fibroblasts. Thus, while L2-4048 maintains a fairly consistent level of activity across the various assays, the outlier in Table 1 is the tautomerase IC_{50} for p425. The reasons for this are unclear at present, though it has been suggested that p425 binds potently only as an aggregate.³² An evaluation of the propensity for L2-4048 to form aggregates in solution is therefore ongoing.

Details from the MIF–L2-4048 cocrystal structure will be used in future efforts to find suitable replacements of the diazo and sulfonate moieties. Furthermore, our analysis provides a new starting point for the potential discovery of chimeric molecules of ibudilast with tautomerase-site inhibitors, as in the McLean et al. quinolone, if a way to bypass the Phe113 side chain is found. These efforts will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmchemlett.9b00351>.

Synthetic procedures; analytical data for intermediates 3–8 and final L2-4048 (1); biophysical assays; in vitro

cellular activity studies; crystallization; and structure determination data (PDF)

■ Accession Codes

The crystal structure for the complex of L2-4048 with MIF was deposited in the RSCB Protein Data Bank with PDB ID 6PEG.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: pcirillo@newhaven.edu.

*E-mail: oluwatoyin.asojo@hamptonu.edu and asojo@bcm.edu, previously at National School of Tropical Medicine, Baylor College of Medicine, 1102 Bates Avenue, Houston, Texas 77030.

ORCID

Pier F. Cirillo: 0000-0002-1458-5674

Oluwatoyin A. Asojo: 0000-0002-4043-2700

Present Addresses

[†](Y.L.) Boehringer Ingelheim Corp., Old Ridgebury Rd., Ridgefield, CT 06877, U.S.A.

[‡](S.M.) Azitra Inc., 400 Farmington Ave., Farmington, CT 06032, U.S.A.

[○](P.H.) Arvinas, 395 Winchester Ave, New Haven, CT 06511, U.S.A.

Notes

The authors declare the following competing financial interest(s): The authors are submitting a patent application on compound L2-4048 and analogs derived from it. The authors claim no other conflicts of interest.

■ ACKNOWLEDGMENTS

The authors wish to thank Dr. Sukyeong Lee at the Baylor College of Medicine for access to the X-ray facility. This work was supported by NIH grant R44 AR067569 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

■ ABBREVIATIONS

BEI, Binding Efficiency Index; Boc, *tert*-butoxycarbonyl; CD74, Cluster of Differentiation 74; CXCR2, Chemokine Receptor 2; CXCR4, Chemokine Receptor 4; CXCR7, Chemokine Receptor 7; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCM, dichloromethane; ENO, 4-hydroxyphenylpyruvate enol form; HPP, 4-hydroxyphenylpyruvate; IL-8, Interleukin-8; MIF, Macrophage Migration Inhibitory Factor; SPR, Surface Plasmon Resonance; TBAF, tetrabutylammonium fluoride; TEMPO, 2,2,6,6-tetramethylpiperidin-1-yloxy; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

■ REFERENCES

- (1) Calandra, T.; Froidevaux, C.; Martin, C.; Roger, T. Macrophage Migration Inhibitory Factor and Host Innate Immune Defenses Against Bacterial Sepsis. *J. Infect. Dis.* **2003**, *187* (Suppl. 2), S385–S390.
- (2) Calandra, T.; Roger, T. Macrophage Migration Inhibitory Factor: a Regulator of Innate Immunity. *Nat. Rev. Immunol.* **2003**, *3*, 791–800.
- (3) Kasama, T.; Ohtsuka, K.; Sato, M.; Takahashi, R.; Wakabayashi, K.; Kobayashi, K. Macrophage Migration Inhibitory Factor: a Multifunctional Cytokine in Rheumatic Diseases. *Arthritis* **2010**, *2010*, 1.
- (4) Fallica, J.; Varela, L.; Johnston, L.; Kim, B.; Serebreni, L.; Wang, L.; Damarla, M.; Kolb, T. M.; Hassoun, P. M.; Damico, R.

Macrophage Migration Inhibitory Factor: a Novel Inhibitor of Apoptosis Signal-Regulating Kinase 1-p38-Xanthine Oxidoreductase-Dependent Cigarette Smoke-Induced Apoptosis. *Am. J. Respir. Cell Mol. Biol.* **2016**, *54* (4), 504–514.

(5) Guo, Y.; Hou, J.; Luo, Y.; Wang, D. Functional Disruption of Macrophage Migration Inhibitory Factor Suppresses Proliferation of Human H460 Lung Cancer Cells by Caspase-Dependent Apoptosis. *Cancer Cell Int.* **2013**, *13*, 28.

(6) Kim, J. Y.; Kwok, S.-K.; Hur, K.-H.; Kim, H.-J.; Kim, N.-S.; Yoo, S.-A.; Kim, W.-U.; Cho, C.-S. Up-Regulated Macrophage Migration Inhibitory Factor Protects Apoptosis of Dermal Fibroblasts in Patients with Systemic Sclerosis. *Clin. Exp. Immunol.* **2008**, *152* (2), 328–335.

(7) Trivedi-Parmar, V.; Jorgensen, W. L. Advances and Insights for Small Molecule Inhibition of Macrophage Migration Inhibitory Factor. *J. Med. Chem.* **2018**, *61*, 8104–8119.

(8) Bloom, J.; Sun, S.; Al-Abed, Y. MIF, a Controversial Cytokine: a Review of Structural Features, Challenges and Opportunities for Drug Development. *Expert Opin. Ther. Targets* **2016**, *20* (12), 1463–1475.

(9) Bernhagen, J.; Calandra, T.; Mitchell, R. A.; Martin, S. B.; Tracey, K. J.; Voelter, W.; Manogue, K. R.; Cerami, A.; Bucala, R. MIF is a Pituitary-Derived Cytokine that Potentiates Lethal Endotoxemia. *Nature* **1993**, *365*, 756–759.

(10) Bernhagen, J.; Krohn, R.; Lue, H.; Gregory, J. L.; Zernecke, A.; Koenen, R. R.; Dewor, M.; Georgiev, E.; Schober, A.; Leng, L.; Kooistra, T.; Fingerle-Rowson, G.; Ghezzi, P.; Kleemann, R.; McColl, S. R.; Bucala, R.; Hickey, M. J.; Weber, C. MIF is a Noncognate Ligand of CXC Chemokine Receptors in Inflammatory and Atherogenic Cell Recruitment. *Nat. Med.* **2007**, *13*, 587–596.

(11) Bucala, R. Identification of MIF as a New Pituitary Hormone and a Macrophage Cytokine and its Role in Endotoxic Shock. *Immunol. Lett.* **1994**, *43*, 23–26.

(12) Cherepkova, O. A.; Lyutova, E. M.; Eronina, T. B.; Gurvits, B. Y. Chaperone-Like Activity of Macrophage Migration Inhibitory Factor. *Int. J. Biochem. Cell Biol.* **2006**, *38*, 43–55.

(13) Leng, M.; Metz, C. N.; Fang, Y.; Xu, J.; Donnelly, S.; Baugh, J.; Delohery, T.; Chen, Y.; Mitchell, R. A.; Bucala, R. MIF Signal Transduction Initiated by Binding to CD74. *J. Exp. Med.* **2003**, *197*, 1467–1476.

(14) Shi, X.; Leng, L.; Wang, T.; Wang, W.; Du, X.; Li, J.; McDonald, C.; Chen, Z.; Murphy, J. W.; Lolis, E. J.; Noble, P.; Knudson, W.; Bucala, R. CD44 Is the Signaling Component of the Macrophage Migration Inhibitory Factor-CD74 Receptor Complex. *Immunity* **2006**, *25*, 595–606.

(15) Alampour-Rajabi, S.; El Bounkari, O.; Rot, A.; Muller-Newen, G.; Bachelier, F.; Gawaz, M.; Weber, C.; Schober, A.; Bernhagen, J. MIF Interacts with CXCR7 to Promote Receptor Internalization, ERK1/2 and ZAP70 Signaling, and Lymphocyte Chemotaxis. *FASEB J.* **2015**, *29*, 4497–4511.

(16) Schwartz, V.; Lue, H.; Kraemer, S.; Korbil, J.; Krohn, R.; Ohl, K.; Bucala, R.; Weber, C.; Bernhagen, J. A Functional Heteromeric MIF Receptor Formed by CD74 and CXCR4. *FEBS Lett.* **2009**, *583*, 2749–2757.

(17) Rosengren, E.; Bucala, R.; Aman, P.; Jacobsson, L.; Odh, G.; Metz, C. N.; Rorsman, H. The Immunomodulatory Mediator Macrophage Migration Inhibitory Factor (MIF) Catalyzes a Tautomerase Reaction. *Mol. Med.* **1996**, *2*, 143–149.

(18) Stivers, J. T.; Abeygunawardana, C.; Mildvan, A. S.; Hajipour, G.; Whitman, C. P. 4-Oxalocrotonate Tautomerase: pH-Dependence of Catalysis and pKa Values of Active Site Residues. *Biochemistry* **1996**, *35*, 814–823.

(19) Vujicic, M.; Nikolic, I.; Krajnovic, T.; Cheng, K.-F.; VanPatten, S.; He, M.; Stosic-Grujicic, S.; Stojanovic, E.; Al-Abed, Y.; Saksida, T. Novel Inhibitors of Macrophage Migration Inhibitory Factor Prevent Cytokine-Induced Beta-Cell Death. *Eur. J. Pharmacol.* **2014**, *740*, 683–689.

(20) Dziedzic, P.; Cisneros, J. A.; Robertson, M. J.; Hare, A. A.; Danford, N. E.; Baxter, R. H. G.; Jorgensen, W. L. Design, Synthesis, and Protein Crystallography of Biaryltriazoles as Potent Tautomerase

Inhibitors of Macrophage Migration Inhibitory Factor. *J. Am. Chem. Soc.* **2015**, *137*, 2996–3003.

(21) Garai, J.; Lorand, T. Macrophage Migration Inhibitory Factor (MIF) Tautomerase Inhibitors as Potential Novel Antiinflammatory Agents: Current Developments. *Curr. Med. Chem.* **2009**, *16* (9), 1091–1114.

(22) Orita, M.; Yamamoto, S.; Katayama, N.; Fujita, S. Macrophage Migration Inhibitory Factor and the Discovery of Tautomerase Inhibitors. *Curr. Pharm. Des.* **2002**, *8* (14), 1297–1317.

(23) Pantouris, G.; Syed, M. A.; Fan, C.; Rajasekaran, D.; Cho, T. Y.; Rosenberg, E. M., Jr.; Bucala, R.; Bhandari, V.; Lolis, E. J. An Analysis of MIF Structural Features that Control Functional Activation of CD74. *Chem. Biol.* **2015**, *22*, 1197–1205.

(24) Bai, F.; Asojo, O. A.; Cirillo, P.; Ciustea, M.; Ledizet, M.; Aristoff, P. A.; Leng, L.; Koski, R. A.; Powell, T. J.; Bucala, R.; Anthony, K. G. A Novel Allosteric Inhibitor of Macrophage Migration Inhibitory Factor (MIF). *J. Biol. Chem.* **2012**, *287* (36), 30653–30663.

(25) Cho, Y.; Crichlow, G. V.; Vermeire, J. J.; Leng, L.; Du, X.; Hodsdon, M. E.; Bucala, R.; Cappello, M.; Gross, M.; Gaeta, F.; Johnson, K.; Lolis, E. J. Allosteric Inhibition of Macrophage Migration Inhibitory Factor Revealed by Ibudilast. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (25), 11313–11318.

(26) McLean, L. R.; Zhang, Y.; Li, H.; Choi, Y.-M.; Han, Z.; Vaz, R. J.; Li, Y. Fragment Screening of Inhibitors for MIF Tautomerase Reveals a Cryptic Surface Binding Site. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1821–1824.

(27) Ouertatani-Sakouhi, H.; El-Turk, F.; Fauvet, B.; Cho, M.-K.; Pinar Karpinar, D.; Le Roy, D.; Dewor, M.; Roger, T.; Bernhagen, J.; Calandra, T.; Zweckstetter, M.; Lashuel, H. A. Identification and Characterization of Novel Classes of Macrophage Migration Inhibitory Factor (MIF) Inhibitors with Distinct Mechanisms of Action. *J. Biol. Chem.* **2010**, *285*, 26581–26598.

(28) Ye, F.; Ma, X.; Xiao, Q.; Li, H.; Zhang, Y.; Wang, J. C(sp³)-C(sp³) Bond Formation Through Cu-Catalyzed Cross-Coupling of N-Tosylhydrazones and Trialkylsilylalkynes. *J. Am. Chem. Soc.* **2012**, *134*, 5742–5745.

(29) Dawson, T. K.; Dziedzic, P.; Robertson, M. J.; Cisneros, J. A.; Krimmer, S. G.; Newton, A. S.; Tirado-Rives, J.; Jorgensen, W. L. Adding a Hydrogen-Bond May Not Help: Naphthyridones vs Quinoline Inhibitors of Macrophage Migration Inhibitory Factor. *ACS Med. Chem. Lett.* **2017**, *8* (12), 1287–1291.

(30) Jorgensen, W. J.; Gandavadi, S.; Du, X.; Hare, A. A.; Trofimov, A.; Leng, L.; Bucala, R. Receptor Agonists of Macrophage Migration Inhibitory Factor. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7033–7036.

(31) Abad-Zapatero, C.; Metz, J. T. Ligand Efficiency Indices as Guideposts for Drug Discovery. *Drug Discovery Today* **2005**, *10* (7), 464–469.

(32) Cisneros, J. A.; Robertson, M. J.; Valhondo, M.; Jorgensen, W. L. A Fluorescence Polarization Assay for Binding to Macrophage Migration Inhibitory Factor and Crystal Structures for Complexes of Two Potent Inhibitors. *J. Am. Chem. Soc.* **2016**, *138*, 8630–8638.