Design and synthesis of ligands targeting MIF family enzymes and their application in medicinal chemistry

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CHAPTER 1

Introduction and outline of the thesis

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INTRODUCTION AND OUTLINE OF THE THESIS

Medicinal chemists endeavor to create and produce a pharmaceutical compound that elicits a desired effect on the human body or another living system. Those small molecules that influence biological systems causing a specific biological response are what we call “drugs.” But why should chemicals, some of which have unusually simple structures, significantly affect such a complicated construct as a human being? The explanation lies in the intricate workings of the human body, where a remarkable series of chemical reactions occur at the molecular level to maintain the body's health and functionality. If we could observe these reactions within our bodies, we would witness a splendid interplay of chemical cascades[1]. The malfunctioning of this machinery causes pathological responses, a hallmark of many common human diseases. Driven by curiosity, scientists have strived to unravel the mechanisms underlying these pathological cell responses, discovering new drug targets and, subsequently, drugs. In order to address questions in the field, this thesis integrates chemical biology and medicinal chemistry to provide a multidisciplinary approach to drug discovery.

To translate our medicinal chemistry interest into chemical biology language, we should look inside a cell. A cell signaling pathway is commonly initiated by a crucial event - a signaling molecule, also known as a ligand, binding to a receptor[2]. The signaling molecule can possess various functions that can either be exploited as target for therapeutic modulation or as starting point for drug discovery[3]. An important example of multifunctional proteins are cytokines, which have multiple roles such as: intercellular communication during immune responses,[4] stimulation of cell movement toward inflammation, infection, and trauma sites, and controlling other physiological and pathological processes[5]. Even though the interaction of cytokines can be complex, requiring a thorough understanding of their underlying biology and overlapping functions[6], targeting cytokines presents opportunities for precision medicine and new therapies for a range of immune-mediated and inflammatory illnesses.

Cytokines can exhibit unique and distinct functions, allowing for the development of therapies tailored to certain diseases or conditions based on modulation of a particular pathway or signaling cascade, while minimizing off-target effects[7]. By exploring the diversity of cytokines, we discovered one which became the focus of interest in our research group for several years. Macrophage migration inhibitory factor (MIF) is a key regulatory pleiotropic cytokine with a broad spectrum of biological functions[8]. It occupies a central role in signaling pathways involved in inflammation and has been linked to cancer pathogenesis[9]. To date, there are three members of the MIF family found in humans, among them D-dopachrome tautomerase (D-DT), D-dopachrome tautomerase-like protein (DDTL), and

MIF itself[10]. As the most studied family member, MIF was discovered in 1966 by Bloom and Bennett as a soluble factor that inhibited the random migration of macrophages. However, its cytokine-like properties and broader functions were recognized later[11]. Also, its analogy to D-dopachrome tautomerase (D-DT), and its homolog, D-dopachrome tautomerase-like protein (DDTL), was described much later. D-DT was discovered in 1993 as an enzyme that converts D-dopachrome to 5,6-dihydroxyindol and expressed in the cytoplasm of human melanoma, human liver and rat organs[12]. DDTL, which shares approximately 80% sequence homology with D-DT, was described in 2004 but nothing is known about its biological function[10]. In our attempt to broaden the knowledge about the MIF family, in keeping with the current literature, we investigated the medicinal chemistry toolbox targeting MIF and D-DT.

The MIF family of proteins share structural and genetic similarities and, presumably, functional characteristics. In addition to their cytokine-like properties, MIF family members can interact with other proteins and molecules to regulate cellular processes, including cell migration, cell survival, apoptosis, angiogenesis, and metabolism[13]. The MIF family is evolutionarily conserved across species, including certain microbial species, suggesting their importance in biological processes. Also, MIF family members exert their effects by binding to specific cell surface receptors, such as CD74[14] and CXCR2[15]. Through receptor-mediated signaling pathways, they activate intracellular signaling cascades that modulate gene expression, cellular responses, and immune cell functions. The MIF family belongs to the tautomerase superfamily of enzymes, which share a common structural beta-alpha-beta fold and catalytic tautomerase activity[16]. They use an amino-terminal proline (Pro1) with an unusually low pK(a) located within a hydrophobic pocket. The evolutionary preservation of this region suggests that MIF might exert its biological effects via enzymatic activity[17], although physiologically relevant substrates for MIF have not been identified yet. Moreover, MIF was reported to possess Cys-Ala-Leu-Cys (CALC) cysteine-based thiol-protein oxidoreductase activity. Previous studies have addressed the roles of the catalytic site residues and the C-terminus[18], while these two activities have not been directly compared. The most recent discovery that remains enigmatic for many scientists[19] was made on MIF nuclease activity[20]. While biological functions of MIF and its family members are still being elucidated, its association with the tautomerase superfamily provides insights into its evolutionary origins and potential functional relationships with other tautomerase-like enzymes. It offers the potential to target the enzymatic activity of MIF in the context of signaling pathways and cellular processes associated with the pathogenesis of MIF-related diseases.

The first small-molecule inhibitor of MIF, **ISO-1**, was discovered in 2005[21]. It contains an isoxazoline ring bearing a 4-hydroxyphenyl fragment and has been shown to inhibit the tautomerase activity of MIF competitively. Nowadays, it is the most studied ligand, which has become a prototype for the future design of MIF inhibitors. **4-CPPC** is the first

selective and reversible competitive inhibitor reported for D-DT, introducing carboxylic acid substitution as an important pharmacophore[22]. The study demonstrated that **4-CPPC** binds to the active site (which is not clearly identified yet) of D-DT and induces a major conformational change of the C-terminal region, a behavior that principally differs from that of MIF inhibitors and could potentially impact cellular function[23]. The idea of creating inhibitors that bind in a unique region on MIF[24] inducing its conformational changes[25] revealed a new way to study protein activity through alterations in its overall shape, flexibility, and dynamics. These changes can impact the binding affinity of MIF for its ligands, substrates, or interacting proteins regulating their biological interactions and downstream signaling pathways. Allosteric inhibitors of MIF are underrepresented, while for D-DT such inhibitors are not known until now. In this thesis, we will fill the gap broadening the knowledge about MIF family modulators. The overall aim of the thesis is to address several aspects of the design and synthesis of ligands targeting MIF family enzymes and their application in medicinal chemistry.

In **Chapter 2** we discuss one of the approaches to indirectly modulate protein expression and activity. Here we refer to epigenetics, which concerns heritable variations in gene expression without altering the DNA sequence[26]. We summarize the current literature on epigenetic regulation in MIF gene expression and *MIF*-induced signaling in association with inflammatory diseases and cancer. The current literature indicates that histone deacetylase inhibitors (HDACis) impair transcription of the *MIF* gene and have a strong impact on *in vivo* and *in vitro* MIF protein expression. Non-coding RNAs, such as long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) can also contribute to the regulation of MIF expression. Gaining a more advanced understanding of the specific epigenetic mechanisms involved in the regulation of *MIF* gene expression and MIF-induced signaling can open new insights into the diagnosis and treatment of human cancers.

The versatile functions of MIF proteins gained another dimension upon the identification of MIF as a protein that harbours nuclease enzyme activity by *Wang et al*.[20] These authors described that this activity causes DNA fragmentation in parthanatos, which is a type of a caspase independent cell death. In our attempt to capitalize on these findings we failed to unambiguously confirm MIF nuclease activity along the lines described by *Wang et al*.[20] Nevertheless, we were able to identify small molecule modulators of MIF function in parthanatos in **Chapter 3**. Intriguingly, we were able to discover new MIF tautomerase inhibitors from a triazole-based compound collection that bind to an allosteric MIF binding site. These allosteric MIF inhibitors prevented binding of MIF to AIF in the model of parthanatic cell death in contrast to orthosteric MIF tautomerase inhibitors. This discovery provides perspectives for the discovery of novel therapeutic strategies targeting MIF in various pathological conditions.

New modalities to target protein function are gaining importance in medicinal chemistry. The discovery of Proteolysis Targeting Chimera (PROTAC) strategy in 2001[27] provides unique opportunities to exploit natural proteosomal degradation of “undruggable” targets. Although their development and optimization can present some challenges[28], the advantages of this method are discussed in **Chapter 4**. Here we describe the development of a first in class D-DT directed PROTAC, which might have a potential to study diseases caused by overexpression of D-DT. To construct these complex bifunctional molecules, we found a facile way to synthesize a carboxylate labelled pomalidomide building blocks, which proved to be low yielding using traditional synthetic methods[29]. This method became a core of the synthetic pathway toward final PROTACs, which, combined with 'click' chemistry[30] provided the final molecule within three synthetic steps. The initial experiments on A549 cell line indicate that optimized PROTAC has the potential to degrade D-DT in cancer cells, eliminating both enzymatic and non-enzymatic functions of the protein, which makes it a powerful tool for chemical biology studies.

In the drug discovery process, production time and synthetic feasibility have always been key factors. Multicomponent reaction (MCR) chemistry can significantly accelerate the synthesis of new molecules and allows a convenient coverage of a larger chemical space, thus alleviating some of the challenges associated with traditional synthetic methods. In **Chapter 5** we describe the miniaturization and acceleration of an unprecedented Ugi-3-component reaction, giving access to the iminopyrrolidine-2-carboxylic acid derivatives. The described moiety shares key pharmacophoric and structural features of several biologically relevant compounds such as ectoine[31] and linarinic acid[32] which are traditionally synthesized using multistep synthetic protocols. This new reaction creates opportunities to access this type of compounds, and it proceeds generally stereoselectively, in good yields, and tolerates various functional groups. The created pipeline enables efficient access to complex scaffolds and rapid exploration of chemical space, which is in high demand in synthetic and medicinal chemistry. Finally, in **Chapter 6**, there is a summary of the main thesis findings and an outlook towards discussed areas and inspiration for future scientists in the field.

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REFERENCES

1. Patrick GL. An introduction to medicinal chemistry. Oxford university press; 2023 Mar 16.

2. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular Biology of the Cell. 4th edition 2002. Garland Science.

3. Teixeira CS, Sousa SF. Current status of the use of multifunctional enzymes as anti-cancer drug targets. Pharmaceutics. 2021 Dec 21;14(1):10.

4. Dinarello CA. Historical insights into cytokines. European journal of immunology. 2007 Nov;37(S1):S34-45.

5. Zhang JM, An J. Cytokines, inflammation and pain. International anesthesiology clinics. 2007;45(2):27.

6. Megha KB, Joseph X, Akhil V, Mohanan PV. Cascade of immune mechanism and consequences of inflammatory disorders. Phytomedicine. 2021 Oct 1;91:153712.

7. Deckers J, Anbergen T, Hokke AM, de Dreu A, Schrijver DP, de Bruin K, Toner YC, Beldman TJ, Spangler JB, de Greef TF, Grisoni F. Engineering cytokine therapeutics. Nature Reviews Bioengineering. 2023 Apr;1(4):286-303.

8. Grieb G, Merk M, Bernhagen J, Bucala R. Macrophage migration inhibitory factor (MIF): a promising biomarker. Drug news & perspectives. 2010 May;23(4):257.

9. O'Reilly C, Doroudian M, Mawhinney L, Donnelly SC. Targeting MIF in cancer: therapeutic strategies, current developments, and future opportunities. Medicinal research reviews. 2016 Apr;36(3):440-60.

10. Florez-Sampedro L, Brandsma CA, de Vries M, Timens W, Bults R, Vermeulen CJ, van den Berge M, Obeidat ME, Joubert P, Nickle DC, Poelarends GJ. Genetic regulation of gene expression of MIF family members in lung tissue. Scientific reports. 2020 Oct 12;10(1):16980.

11. Bacher M, Metz CN, Calandra T, Mayer K, Chesney J, Lohoff M, Gemsa D, Donnelly T, Bucala R. An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. Proceedings of the National Academy of Sciences. 1996 Jul 23;93(15):7849-54.

12. Odh G, Hindemith A, Rosengren AM, Rosengren E, Rorsman H. Isolation of a

new tautomerase monitored by the conversion of D-dopachrome to 5, 6-dihydroxyindole. Biochemical and biophysical research communications. 1993 Dec 15;197(2):619-24.

13. Song S, Xiao Z, Dekker FJ, Poelarends GJ, Melgert BN. Macrophage migration inhibitory factor family proteins are multitasking cytokines in tissue injury. Cellular and Molecular Life Sciences. 2022 Feb;79(2):105.

14. Szczęśniak P, Henke T, Fröhlich S, Plessmann U, Urlaub H, Leng L, Bucala R, Grosse R, Meinhardt A, Klug J. Extracellular MIF, but not its homologue D-DT, promotes fibroblast motility independently of its receptor complex CD74/CD44. Journal of cell science. 2021 Feb 1;134(3):jcs217356.

15. Schindler L, Zwissler L, Krammer C, Hendgen-Cotta U, Rassaf T, Hampton MB, Dickerhof N, Bernhagen J. Macrophage migration inhibitory factor inhibits neutrophil apoptosis by inducing cytokine release from mononuclear cells. Journal of Leukocyte Biology. 2021 Nov;110(5):893-905.

16. Poelarends GJ, Veetil VP, Whitman CP. The chemical versatility of the β–α–β fold: Catalytic promiscuity and divergent evolution in the tautomerase superfamily. Cellular and molecular life sciences. 2008 Nov;65:3606-18.

17. Dios A, Mitchell RA, Aljabari B, Lubetsky J, O'Connor K, Liao H, Senter PD, Manogue KR, Lolis E, Metz C, Bucala R. Inhibition of MIF bioactivity by rational design of pharmacological inhibitors of MIF tautomerase activity. Journal of medicinal chemistry. 2002 Jun 6;45(12):2410-6.

18. Kleemann R, Rorsman H, Rosengren E, Mischke R, Mai NT, Bernhagen J. Dissection of the enzymatic and immunologic functions of macrophage migration inhibitory factor: Full immunologic activity of N-terminally truncated mutants. European journal of biochemistry. 2000 Dec;267(24):7183-93.

19. Harirchian MH, Zahednasab H, Karampoor S. The enigmatic role of macrophage inhibitory factor (MIF) in the pathogenesis of multiple sclerosis (MS). Journal of the Neurological Sciences. 2022 Sep 15;440.

20. Wang Y, An R, Umanah GK, Park H, Nambiar K, Eacker SM, Kim B, Bao L, Harraz MM, Chang C, Chen R. A nuclease that mediates cell death induced by DNA damage and poly (ADP-ribose) polymerase-1. Science. 2016 Oct 7;354(6308):aad6872.

21. Al-Abed Y, Dabideen D, Aljabari B, Valster A, Messmer D, Ochani M, Tanovic M,

Ochani K, Bacher M, Nicoletti F, Metz C. ISO-1 binding to the tautomerase active site of MIF inhibits its pro-inflammatory activity and increases survival in severe sepsis. Journal of Biological Chemistry. 2005 Nov 4;280(44):36541-4.

22. Pantouris G, Bucala R, Lolis EJ. Structural plasticity in the C-terminal region of macrophage migration inhibitory factor-2 is associated with an induced fit mechanism for a selective inhibitor. Biochemistry. 2018 May 30;57(26):3599-605.

23. Bernhagen J. Separating cytokine twins with a small molecule. Journal of Biological Chemistry. 2019 Dec 6;294(49):18532-3.

24. Cho Y, Crichlow GV, Vermeire JJ, Leng L, Du X, Hodsdon ME, Bucala R, Cappello M, Gross M, Gaeta F, Johnson K. Allosteric inhibition of macrophage migration inhibitory factor revealed by ibudilast. Proceedings of the National Academy of Sciences. 2010 Jun 22;107(25):11313-8.

25. Bai F, Asojo OA, Cirillo P, Ciustea M, Ledizet M, Aristoff PA, Leng L, Koski RA, Powell TJ, Bucala R, Anthony KG. A novel allosteric inhibitor of macrophage migration inhibitory factor (MIF). Journal of Biological Chemistry. 2012 Aug 31;287(36):30653-63.

26. Gibney ER, Nolan CM. Epigenetics and gene expression. Heredity. 2010 Jul;105(1):4-13.

27. Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies RJ. Protacs: Chimeric molecules that target proteins to the Skp1–Cullin–F box complex for ubiquitination and degradation. Proceedings of the National Academy of Sciences. 2001 Jul 17;98(15):8554-9.

28. Békés M, Langley DR, Crews CM. PROTAC targeted protein degraders: the past is prologue. Nature Reviews Drug Discovery. 2022 Mar;21(3):181-200.

29. Liu Z, Hu M, Yang Y, Du C, Zhou H, Liu C, Chen Y, Fan L, Ma H, Gong Y, Xie Y. An overview of PROTACs: a promising drug discovery paradigm. Molecular Biomedicine. 2022 Dec 20;3(1):46.

30. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. A stepwise huisgen cycloaddition process: copper (I)-catalyzed regioselective "ligation" of azides and terminal alkynes. Angewandte Chemie. 2002 Jul 15;114(14):2708-11.

31. Ng HS, Wan PK, Kondo A, Chang JS, Lan JC. Production and recovery of ectoine: a review of current state and future prospects. Processes. 2023 Jan 20;11(2):339.

32. Tian Y, Ma C, Feng L, Zhang L, Hao F, Pan L, Cheng M. Synthesis and Biological Evaluation of (−)-Linarinic Acid Derivatives as Neuroprotective Agents against OGD-Induced Cell Damage. Archiv der Pharmazie. 2012 Jun;345(6):423-30.

“You’re a scientist,” he said. “Your job is to question things — to search for answers. But sometimes — and I know this for a fact — there just aren’t any.”

Bonnie Garmus,
Lessons in Chemistry