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Small molecule inhibitors of macrophage migration inhibitory factor (MIF) as emerging class of therapeutics for immune disorders

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Abstract

Macrophage migration inhibitory factor (MIF) is an important cytokine for which an increasing number of functions is being described in the pathogenesis of inflammation and cancer. Nevertheless, the availability of potent and druglike MIF inhibitors that are well-characterized in relevant disease models remains limited. Highly potent and selective small molecule MIF inhibitors and validation of their use in relevant disease models will advance drug discovery. In this review we provide an overview of recent advances in the identification of MIF as a pharmacological target in the pathogenesis of inflammatory diseases and cancer. Based on that we give an overview of the current developments in the discovery and design of small molecule MIF inhibitors and define future aims in this field.

Keywords

macrophage migration inhibitory factor (MIF); inflammatory diseases; cancer; inhibitors

Introduction

Despite its discovery over 50 years ago in 1966 [1][2], the functions of the cytokine macrophage migration inhibitory factor (MIF) are still not fully elucidated. Initially, MIF was identified as a T cell-derived mediator that inhibits random movement of macrophages. Its activity was found to correlate with delayed-type hypersensitivity reactions, a prominent

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feature of several chronic diseases in humans [2]. In addition, MIF is released at sites of infection, causing macrophages to concentrate and carry out antigen processing and phagocytosis [3]. Today, MIF is recognized as a critical player in innate immune responses that play a role in multiple diseases [4][5]. Therefore, the development of small molecule MIF inhibitors that inferfere with its functions is quickly gaining importance.

The human MIF gene has been cloned and expressed for the first time in 1989 [6]. MIF is a relatively small protein that consists of 114 amino acids and has a molecular mass of 12,345 Da. Structural analysis of MIF revealed its striking similarities to bacterial enzymes from the tautomerase superfamily. Searching the human genome indicated that D-dopachrome tautomerase (D-DT) is the other gene with marked homology to MIF. Due to this similarity, D-DT is also referred to as MIF2 and an overlapping functional spectrum for MIF and D-DT has been suggested [7]. This should be taken into account in evaluation of MIF cytokine activities and in the development of small molecule MIF modulators.

MIF, a member of the tautomerase superfamily [8], is found across various organisms including bacteria, mice, plants, protozoa, helminths, molluscs, arthropods, and fish [9–11]. These tautomerase family members have similar enzyme activity involving an amino acid-terminal proline that acts as general base in keto-enol tautomerisation reactions of α-keto-carboxylates. In addition to its cytokine activity, MIF harbours keto-enol tautomerase and low-level dehalogenase activity, providing a functional link to other members of the tautomerase family [10]. MIF is a homotrimeric protein in which three monomers associate to form a symmetrical trimer (Figure 1A). Each MIF trimer has three tautomerase active sites at the interfaces of the monomer subunits. Characteristic for this family, MIF has a N-terminal proline (Pro-1), which is located within a hydrophobic pocket [12]. The residue Pro-1 was shown to be conserved between MIF and its bacterial homologues. Moreover, other invariant residues were identified as being clustered around the N-terminal proline. The evolutionary preservation of this region suggests its importance in the biological function of MIF [13]. Despite the lack of a known physiological substrate, it was shown that D-dopachrome (a stereoisomer of naturally occurring L-dopachrome), phenylpyruvate and *p*-hydroxyphenylpyruvate are accepted as substrates by MIF (Figure 1B) [14] [15]. A crystal structure of MIF in complex with *p*-hydroxyphenylpyruvate demonstrated that Pro-1 functions as a catalytic base in the tautomerase reaction [16]. It is well recognized that MIF's currently defined substrates either do not exist naturally *in vivo*, or do not exist at significant concentrations required for biological activity [17]. Nevertheless, small molecule modulators of MIF tautomerase activity may have an impact on MIF cytokine activity due to modulation of its conformation and/or ability to interact with other proteins.

Although MIF tautomerase activity may not be directly linked to its cytokine activity, it provides an opportunity for efficient screening of compound collections that could provide molecules that interfere with MIF cytokine activity. One of the best known targets to mediate MIF cytokine activity upon binding is the cluster of differentiation 74 (CD74) receptor [18] Interestingly, recent findings demonstrate that MIF binding to CD74 occurs in the vicinity of the MIF tautomerase active site, which supports the idea that MIF tautomerase inhibitors may have potential to interfere with MIF cytokine function [19]. In

this perspective, robust assays to test the ability of MIF tautomerase inhibitors to interfere with MIF cytokine functions *in vitro* and *in vivo* are highly important.

Functional cytokine roles of MIF and D-DT have been described in innate and adaptive immune responses [7][13]. It has been shown that MIF stimulates the production of pro-inflammatory mediators such as tumor necrosis factor α (TNFα, interferon-γ (IFNγ), interleukins 1β, 2, 6 and 8 (IL-1β, IL-2, IL-6 and IL-8) and other effector cytokines [13]. The MIF-CD74 interaction is well known to initiate subsequent signaling cascades leading to cellular responses [18]. The biological functions of the CD74 receptor in immune diseases has recently been reviewed by Su *et al.* [20]. With respect to CD74 binding it is interesting to note that a difference has been reported between MIF and D-DT in a study by Merk *et al.* [21]. The same study indicates that MIF has a steeper dose-response ratio for macrophage migration inhibition and glucocorticoid overriding. This suggests an immune downregulatory role for D-DT in the presence of MIF. On the contrary, a recent study demonstrated that both MIF and D-DT are connected to disease progression of multiple sclerosis subjects [22]. This study and other studies as reviewed by Merk *et al.* [7] and O'Reilly *et al.* [23] indicate an overlapping activity spectrum for both cytokines.

Apart from the CD74 receptor MIF can also bind to chemokine receptors such as CXCR2, CXCR4 and CXCR7 to induce inflammatory and immune cell chemotaxis [24][25]. Given the pro-inflammatory activities, MIF is implicated in acute and chronic inflammatory diseases such as asthma, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, sepsis, diabetes, atherosclerosis and cardiovascular diseases [13]. Many studies have investigated MIF as a biomarker for various diseases that have an inflammatory component, including systemic infections and sepsis, cancer, autoimmune diseases and different metabolic disorders, suggesting its important role in these diseases [26].

It should also be noted that MIF has been demonstrated to be post-translationally modified and that these modifications affect its biological functions [27]. MIF, but not D-DT, has a CXXC motif that can be oxidized to an intramolecular disulfide-bond. Oxidised MIF has also been proposed as a biomarker for different diseases [28]. Other studies indicate that oxidized MIF is the disease-related conformational isoform that could be employed for diagnosis and therapy [29][30]. It is to be expected that MIF redox behavior interferes with binding of small molecule inhibitors. However, little is known about the structural consequences of these modifications in relation to inhibitor binding. Therefore, this represents an interesting and novel line of investigation.

Despite extensive studies on the functional role of MIF in multiple disease models, the identification and validation of the functional consequences of small molecule MIF tautomerase inhibitors is still in an early stage. The effects of such inhibitors have been investigated using the long-known standard MIF antagonist ISO-1 (Table 1) in *in vitro* assays and animal models [31]. Research on MIF antagonists is still in the preclinical stage and data on human disease are still observational [32], which indicates a need for further validation of MIF as a drug target.

In this review we aim to provide an overview of recent advances in the identification of MIF as a pharmacological target in the pathogenesis of inflammatory diseases and cancer (Figure 1C). Based on that we will provide an overview of the current developments in the disovery and design of small molecule MIF inhibitors and define future goals in this field.

The role of MIF in pathogenesis of inflammatory diseases

Acute inflammation is a protective, beneficial, and self-limiting process during innate immune responses, but chronic inflammation is maladaptive and may result in tissue injury and dysfunction. For instance, some studies have shown that MIF plays an important role in the pathology of bladder inflammation. In bladder tissue substance P, an important inflammatory mediator, increases the levels of MIF. The important role of MIF was subsequently shown by administration of an anti-MIF antibody that could decrease substance P-induced inflammatory changes in bladder [33]. MIF was also shown to play a role in worsening of lung inflammation. High levels of MIF were reported to be detrimental for survival in a mouse model of pneumococcal pneumonia. Treatment of mice with a small-molecule inhibitor of MIF, designated MIF098 (Alissa-5) (Table 2), improved survival by reducing inflammatory responses [34]. In addition, higher MIF levels were produced by alveolar macrophages in a mouse model for COPD as compared to those from healthy mice, and inhibition of MIF function by ISO-1 could block the corticosteroid-insensitive lung inflammation [35].

MIF also acts as an essential mediator of host immunity against various bacterial infections, however, its persistent or recurrent expression during chronic inflammatory disease stages can lead to loss of function and mortality. The involvement of MIF in the enhancement of biofilm formation by *Pseudomonas aeruginosa* was shown by the use of SCD-19, a small molecule inhibitor targeting MIF tautomerase activity. Application of this inhibitor resulted in lower bacterial burden in a mouse model of this infection as compared to untreated mice [36]. As another example, MIF promotor polymorphisms resulting in high MIF levels in cerebrospinal fluid of patients with streptococcal meningitis correlated with systemic complications and death [37]. Moreover, the authors of this study showed a reduction in bacterial load in a mouse model of pneumococcal pneumonia and sepsis after treatment with an anti-MIF antibody. From these studies it becomes clear that MIF plays a role in biofilm formation and mortality during bacterial infections, and it provides initial evidence that targeting MIF with small molecule inhibitors has potential to interfere with such pathological conditions.

A relationship of MIF with autoimmune inflammatory disease has been observed in experimental autoimmune myocarditis. Early treatment of this disease with an anti-MIF antibody markedly delayed the onset of, and significantly reduced the severity of, this disease in rats [38]. The importance of MIF in the autoimmune inflammatory process has also been demonstrated for rheumatoid arthritis. It was observed that treatment with an anti-MIF antibody before immunization with type II collagen leads to delayed onset of arthritis in a mouse model of collagen-induced arthritis [39]. Altogether, these studies indicated that MIF correlates with disease severity in autoimmune inflammatory diseases and that treatment with anti-MIF antibodies has beneficial effects.

The role of MIF in pathogenesis of cancer

Inflammation and immunity play key roles in the onset and progression of cancer. Persistent or recurrent inflammation is related to development of cancer. In contrast, anti-cancer immune responses counteract the development and progression of cancer. From this perspective, the functional output of MIF as a key cytokine of the immune system can be connected to various aspects of oncology [40].

High serum levels of MIF are seen in cancer patients and tissues and MIF has consequently been proposed as a biomarker. In addition, high MIF levels correlate with poor prognosis in various carcinomas. Besides being involved in angiogenesis and thus indirectly in promoting tumour growth, the interaction of MIF with its receptors has been shown to initiate cancer promoting signal transduction pathways. For example, binding of MIF to CD74 can lead to stimulation of the ERK1/2 but also PI3K/AKT pathways and binding of MIF to the CXCR4 receptor was suggested to induce metastases [41].

Involvement of MIF in the development of prostate cancer was shown by studies with an androgen-independent prostate cancer cell line. In these studies, inhibition of MIF by ISO-1, anti-MIF antibody, or MIF siRNA resulted in decreased cell proliferation. ISO-1 significantly decreased tumor volume and tumor angiogenesis [42]. In another study with a prostate cancer cell line, treatment with anti-MIF antibodies was reported to reduce cell growth and in a xenograft mouse model of prostate cancer, anti-MIF antibodies were shown to limit tumor growth [43].

The involvement of MIF in the development of bladder cancer has also been described. MIF was reported to promote *in vitro* and *in vivo* bladder cancer progression via increasing cell proliferation and angiogenesis. The orally available MIF inhibitors, CPSI-2705 and -1306 (Table 2), have been shown to effectively decrease the growth and progression of bladder cancer *in vivo* [44].

A study on the role of MIF in the development of colon cancer in patients reported a positive correlation between MIF serum concentrations and colorectal cancer severity [45], thus indicating a potential use of MIF as biomarker. The same study demonstrated in a mouse model with colon carcinoma cell transplants that treatment with MIFinhibitor ISO-1 or anti-MIF antibodies resulted in significant reduction in the tumor burden, thus indicating a potential use of MIF directed therapeutics in cancer.

Furthermore, MIF has been indicated to be involved in the progression of lung cancer. Blocking the hydrophobic pocket that harbours MIF tautomerase activity, by a small molecule inhibitor of the isocoumarin class, SCD-19 (Table 2), significantly attenuated lung cancer growth [46].

Taken together, these studies demonstrate a positive correlation between MIF and the progression of cancer. Application of small molecule MIF inhibitors or anti-MIF antibodies attenuated cancer growth, thus indicating the potential of anti-MIF therapeutics in cancer [47][48][49].

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Small molecule inhibitors of MIF

Because of the essential involvement of MIF in the progression of numerous disorders with an inflammatory component, it is not surprising that attempts were made to find MIF directed therapeutics. One line of development is the application of biologicals, such as anti-MIF antibodies, as novel therapeutics. This approach has been used in many proof of concept studies [38] [39] [33] [42] [43] [45]. One clinical trial with an anti-MIF antibody has been reported, however no results have been revealed yet [50]. The other line of development is to generate MIF-binding small molecules with the aim to interfere with MIF functions. To develop MIF inhibitors, many studies resort to evaluating interference with MIF tautomerase activity. In this perspective, the evaluation of MIF tautomerase inhibitors for their interference with MIF cytokine functions in relevant disease models is highly important. Compared with biologicals, small molecule MIF inhibitors offer advantages such as lower manufacturing costs, non-immunogenic reaction and the possibility of oral administration. Therefore, this route of exploration gained tremendous interest. Here, we summarize the currently identified MIF tautomerase inhibitors and discuss their structure-activity relationships.

Inhibitors containing a chromen-4-one scaffold were identified in 2001 and their K_i values range between 0.04 and 7.4 μM. The most potent compound of this class is Orita-13 with a K_i of 0.04 μM [51]. However, a later investigation reported K_i values in the range of 13-22 μM for Orita-13 [52]. The phenol functionality in Orita-13 is also found in the MIF tautomerase substrates D-dopachrome and *p*-hydroxyphenylpyruvate and proved to be a succesfull design motif for MIF inhibitors (Table 1).

Isoxazolines as MIF inhibitors

The most frequently used reference inhibitor for MIF tautomerase activity is ISO-1, which was discovered in 2002. This inhibitor of the isoxazoline class was reported to inhibit MIF tautomerase activity in a dose-dependent manner. It binds at the same position as the substrate *p*-hydroxyphenylpyruvate with an IC_{50} of about 7 μM [53]. A later study described a K_i of 24 μM for inhibition of MIF tautomerase activity [52]. A MIF-CD74 binding study reported a maximum of 40% inhibition at 10 μM (Table 1) [54]. Further studies showed that inhibition of MIF by ISO-1 in a mouse model significantly reduced prostate cancer [42], colon cancer [45], and blocked melanoma cell growth [55]. Another study in a mouse model showed that this MIF inhibitor blocks corticosteroid-insensitive lung inflammation [35]. In addition, ISO-1 was also reported to inhibit MIF activity in a mouse model of type 1 diabetes and to result in the delayed onset of this disease [56]. Altogether, ISO-1 is a valuable compound that is widely used as a reference inhibitor in the initial validation of small molecule MIF tautomerase inhibitors as potential therapeutics in diseases with an inflammatory component.

Another small molecule MIF tautomerase inhibitor with an isoxazoline scaffold is CPSI-1306 (Table 2). This inhibitor lacks the characteristic phenol functionality, which is advantageous for applications *in vivo*. Phenol functionalities are generally considered to be non-druglike because they are prone to phase II bioconjugation reactions thus resulting in quick inactivation and excretion in *in vivo* experiments. Oral administration of this inhibitor

resulted in less severe symptoms in a mouse model for multiple sclerosis as compared to untreated mice [57]. Further small molecules with the same scaffold were synthesized and evaluated for MIF-inhibitory activity. The IC_{50} of the most active compound, Alam-4b (Table 1), was 7.3 μM in a MIF tautomerase assay and this compound was shown to be nontoxic in a cell viability assay [58]. Subsequently, in 2014, another small molecule inhibitor of isoxazoline class, ISO-66 (Table 1), was reported. Its IC_{50} in the MIF tautomerase assay was 1.5 μM and long-term administration of ISO-66 in a mouse model of colon cancer or melanoma was shown to be nontoxic and to decrease tumor burden significantly [59]. Thus, studies on MIF inhibitors with an isoxazoline scaffold demonstrate that the development and application of small molecule MIF inhibitors has potential to provide novel therapeutics.

1,2,3-triazoles as MIF inhibitors

In 2010, 1,2,3-triazole derivatives were reported as inhibitors of MIF. The most potent compounds, Jorgensen-3g and Jorgensen-3h (Table 1), showed IC_{50} values of about 1 μM for MIF tautomerase activity and MIF-CD74 binding [60]. Subsequently, in 2015, improvements were made by synthesis of several optimized biaryltriazoles. This provided potent compounds with a phenolic hydroxyl group that bind to the MIF tautomerase active site. Neverthless, some compounds had limited water solubility. The activity of this class of compounds was further improved by the addition of a fluorine atom adjacent to the phenolic hydroxyl group to enhance the hydrogen bond interaction with residue Asn-97 of MIF. This yielded the most potent compound, Dziedzic-3bb (Table 1), having a K_i value of 0.057 μM and a solubility that is in the normal range for orally avialable drugs [61].

The synthesis of fluorescently-labeled MIF inhibitors with a biaryltriazole scaffold was described in 2016. These inhibitors were used in a fluorescence polarization assay to assess the direct binding of inhibitors to the active site of MIF. The two most potent inhibitors, denoted Cisneros-3i and Cisneros-3j, were reported to have K_i's of 0.057 and 0.034 μM in the tautomerase assay and K_d's of 0.071 and 0.063 μM in the fluorescence polarization-based binding assay, respectively (Table 1) [62].

Covalent MIF inhibitors

The specific reactivity of the proline in the MIF active site provides opportunities to develop covalent inhibitors. In 2008, a compound of the phenyl-pyrimidine class, 4-IPP (Table 2), was reported to inactivate the MIF catalytic function by dehalogenation and formation of a covalent bond between C-4 of pyrimidine and the N-terminal nitrogen of Pro-1 in the MIF tautomerase active site. This compound also inferferes with the biological functions of MIF as it was reported to irreversibly inhibit lung adenocarcinoma cell migration and anchorage-independent growth [63]. Later on, it was described that 4-IPP inhibits the growth of thyroid cancer cells by inducing apoptosis and mitotic cell death [64]. In 2009, isothiocyanates were discovered as irreversible MIF tautomerase inhibitors. The isothiocyanate BITC (Table 2) was shown to covalently modify the Pro-1 residue in the MIF active site. This drastically alters the MIF tertiary structure and results in loss of its tautomerase activity and in inhibition of MIF binding to CD74 [65].

The Woodward's reagent K is a classical heterocyclic electrophile with a specific reactivity [66][67]. Taking advantage of the specific reactivity of Woodward's Reagent K, covalent MIF inhibitors were developed. These inhibitors were shown to react with the active site Pro-1 of MIF and were applied for covalent labeling of MIF that proved to be selective. The covalent inhibitors were used as probes for labeling and imaging of MIF activities in living cells [68]. These examples demonstrate that it is possible to develop covalent active site-directed inhibitors of MIF that bind with a reasonable level of selectivity. Such inhibitors have great potential for labeling and imaging of enzyme activity *in vitro* and *in vivo*. In contrast to their application in imaging, covalent inhibitors are not preferred in pharmacotherapy due to concerns about their off-target effects.

Other type of MIF inhibitors

Apart from the isoxazolines and 1,2,3-triazoles, other types of reversible MIF inhibitors were also developed. In 2006, MIF inhibitors with a benzoxazinone scaffold were described and patented. The most potent compound, NVS-2, was reported to have an IC_{50} of 0.020 μM (Table 2) [69]. A later assay by Cisneros *et al.* reported a similar value for the K_i of 0.027 μM in the tautomerase assay and a K_d of 0.055 μM in the fluorescence polarization-based binding assay [62]. Subsequently, in 2010, substituted benzoxazol-2-ones were discovered as MIF antagonists (Table 2). One potent inhibitor from this class, MIF098 (Alissa-5), showed noncovalent inhibition in the MIF tautomerase assay, with an IC_{50} of around 0.010 μM. This inhibitor was further reported to attenuate MIF-dependent ERK phosphorylation in human synovial fibroblasts, which demonstrates possible use of MIF inhibition as therapy in rheumatoid arthritis [70].

In 2012, an allosteric MIF tautomerase inhibitor p425 was identified in a high-throughput screening of a library consisting of 230,000 small molecules. However, this compound is a sulfonated azo compound (also known as pontamine sky blue), which has poor druglike properties [71]. Another study, in 2016, reported inhibitor K664-1 (Table 2) with a pyrimidazole scaffold as a novel MIF inhibitor with an IC_{50} of 0.16 μM in the MIF tautomerase assay [72]. This inhibitor provided protection to β-cells from cytokine-triggered apoptosis in a mouse model, which demonstrates its potential for the prevention of diabetes progression [73].

In 2016, compound T-614 (also known as iguratimod) was found to selectively inhibit MIF *in vitro* and *in vivo*. The compound has synergic effects with glucocorticoids to slow disease progression in a mouse model of multiple sclerosis. The IC_{50} of that compound in the MIF tautomerase assay was 6.81 μM (Table 2) [72]. Recently, novel types of MIF inhibitors were discovered using substitution-oriented screening (SOS). Inspired by the known chromen-4-one inhibitor Orita-13, a focused collection of compounds with a chromene scaffold was screened for MIF binding. In this study, inhibitors **10** and **17** (denoted Kok-10 and Kok-17, Table 2) provided IC_{50} values in the low micromolar range (18 and 6.2 μM, respectively) in the MIF tautomerase assay. The binding proved to be reversible and the enzyme kinetics suggested no direct interaction of these compounds with the substrate binding pocket [74].

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Future perspective of inhibitor development

Altogether it can be concluded that a diverse array of structures can be employed to develop MIF inhibitors that interact with MIF tautomerase activity via direct competition, via allosteric modulation of substrate binding, or via covalent binding. This provides a valuable starting points to design novel structural motifs that can be employed to interfere with MIF cytokine functions. Then, inhibitors of the enzymatic activity of MIF should also be evaluated in assays for binding to its cellular receptors such as CD74, CXCR2, CXCR4 and CXCR7 and/or in disease models.Within this context it is important to note that a study by Cisneros *et al.* in 2016 demonstrated that the reported IC_{50}'s of MIF tautomerase inhibitors were often not reproducible [52]. Most inhibitors were shown to be less potent than previously reported. As pointed out, for covalent or slow-tight binding inhibitors the IC_{50}'s are time dependent. Therefore, it is important to evaluate the reversibility of binding by recovery of enzyme activity in, for example, dilution experiments [75], which is too often neglected. Another complicating factor is the enzyme kinetics of the MIF tautomerase activity for its substrate *p*-hydroxyphenylpyruvate (4-HPP) that provides a sigmoidal curve, which cannot be fitted to a simple one-to-one binding model. Thus, the Michaelis-menten constant K_m cannot be derived easily and one needs to resort to $K_{half,app}$ [74] or $[S]_{0.5}$ [16]. This issue complicates the calculation of the equilibrium constant for inhibition (K_i) from IC_{50} values. Therefore, we argue to include enzyme dilution experiments and enzyme kinetic studies, or direct binding assays, if IC_{50} values are reported in order to provide a more complete analysis of MIF binding.

Conclusive remarks

MIF has been described to play a key role in the pathogenesis of inflammatory diseases and cancer. Small molecule inhibitors of MIF have been developed and used in studies to investigate the biological role of MIF. Inhibitor ISO-1 is widely used as a reference compound for MIF inhibition in mouse models of lung inflammation, prostate cancer, colon cancer, melanoma and diabetes. Other small molecule inhibitors also provided positive effects in various disease models. Altogether this indicates the potential of MIF inhibitors for development of novel therapeutics for diseases with an inflammatory component.

It is commonly presumed that MIF inhibitors identified in a MIF tautomerase assay have potential to interfere with MIF cytokine functions. Following this line of argumentation several classes of MIF tautomerase inhibitors have been identified. The isoxazolines and the 1,2,3-triazoles are important classes of inhibitors from which potent MIF inhibitors were identified. Also covalent inhibitors that react with the active site Pro-1 of MIF have been identified and in one case used for activity-based labeling of MIF in living cells. Over time an increasing number of MIF inhibitors has been described, thus providing more insight in structure-activity relationships for MIF binding. A complicating factor in the analysis of MIF inhibitors proved to be covalent or slow-tight binding behavior that results in overestimation of the inhibitors potency. Also the sigmoidal enzyme kinetics for MIF tautomerase activity complicates analysis of MIF binding. We argue that anticipation of these issues is needed for successful further development of the field.

Ultimately, the identification of potent MIF inhibitors with favorable properties for drug discovery programs will enable the identification of novel therapeutics that target MIF functions in diseases with an inflammatory component. Furthermore, attention should be given toward the MIF structural homolog D-DT, which has been demonstrated to have an overlapping functional spectrum of action. This suggests that the combined or separate therapeutic targeting of D-DT and MIF could have additional advantages.

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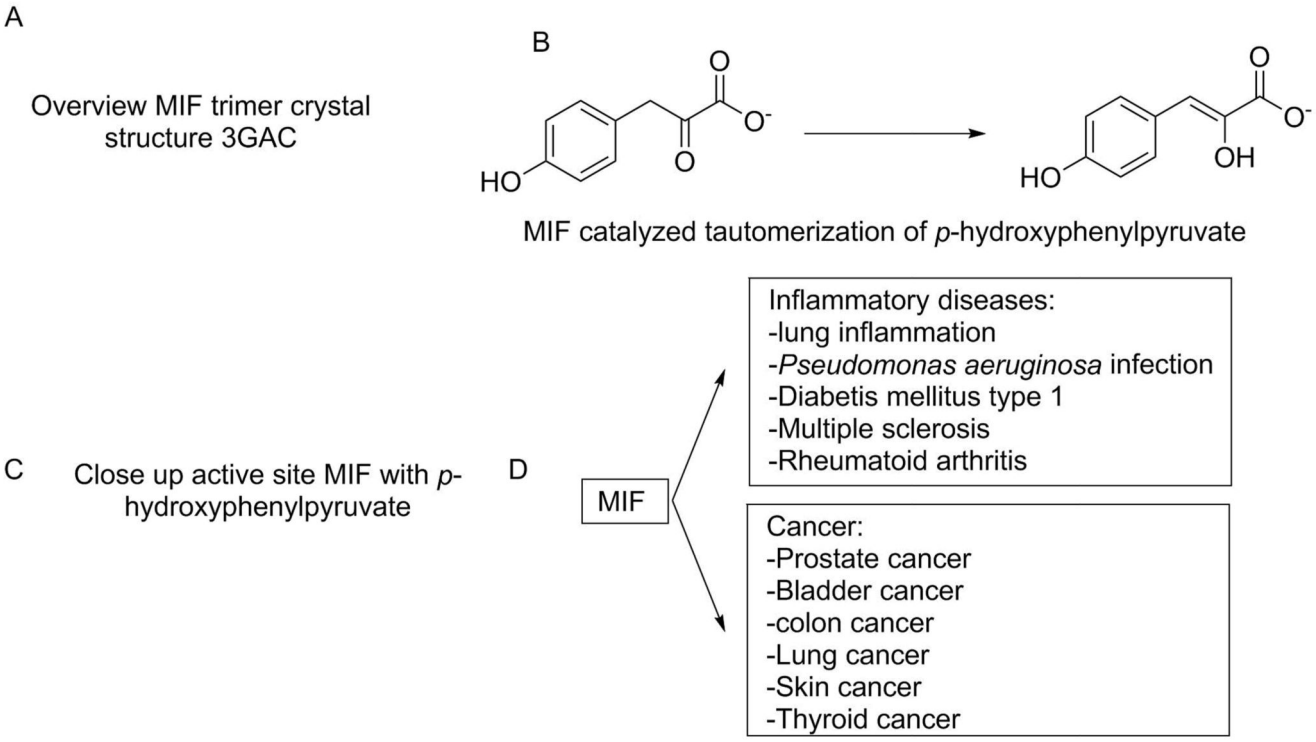

Figure 1.

A. Crystal structure of MIF, showing that three monomers associate to form a symmetrical homotrimer (PDB 1CA7), **B.** MIF catalyses the tautomerisation of α-keto-carboxylates, **C.** Diseases for which a role of MIF has been described.

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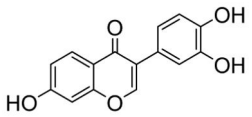
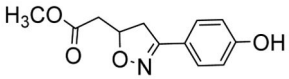
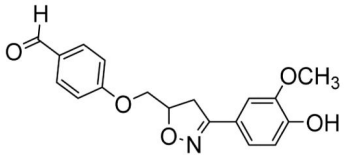
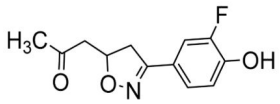
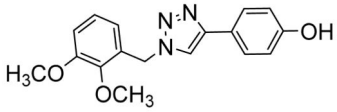
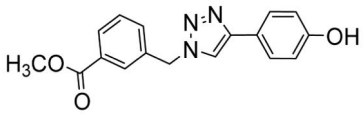
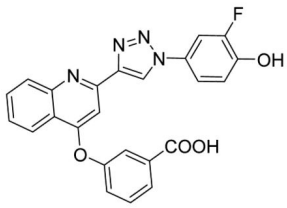
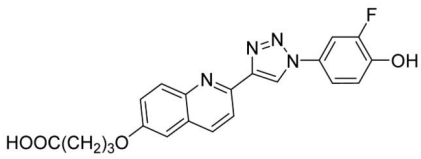
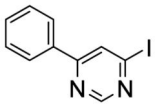
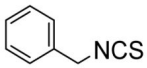
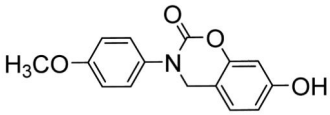
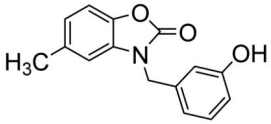
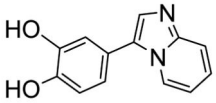
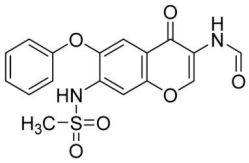
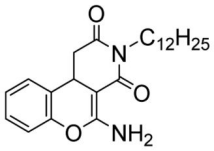
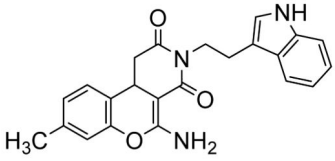
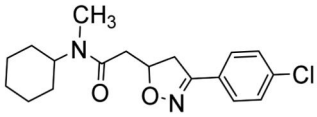
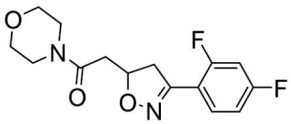
Table 1

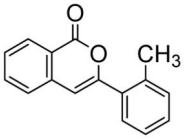
MIF inhibitors with a phenol functionality as the key structural element that presumably binds to the active site residue Asn-97 of MIF.

Class	Compound	Structure	Activity, μM Reference(s)
chromen-4-one	Orita-13		K_i = 0.04 [39] (TA), K_i = 17 [49] (TA), K_i = 13-22 [40] (TA)
isoxazoline	ISO-1		IC_{50} = 7 [41] (TA), IC_{50} = 24 [40] (TA); Max. 40% inhibition [42] (BA)
	Alam-4b		IC_{50} = 7.3 [46] (TA)
	ISO-66		IC_{50} = 1.5 [47] (TA)
1,2,3-triazole	Jorgensen-3g		IC_{50} = 0.75 [48] (TA); IC_{50} = 0.9 [48] (BA)
	Jorgensen-3h		IC_{50} = 1 [48] (BA)
	Dziedzic-3bb (Cisneros-3i)		Dziedzic-3bb: K_i = 0.057 [49] (TA) Cisneros-3i: K_i = 0.057 [50] (TA); K_d = 0.071 [50] (BA)
	Cisneros-3j		K_i = 0.034 [50] (TA); K_d = 0.063 [50] (BA)

TA, tautomerase assay; BA, binding assay MIF-CD74

Table 2
Covalent MIF inhibitors and MIF inhibitors with other structures

Class	Compound	Structure	Activity, μM Reference(s)
phenyl-pyrimidine	4-IPP		IC_{50} = 0.2-0.5 [51] (TA)
isothiocyanate	BITC		IC_{50} = 0.79 [53] (TA)
benzoxazinone	NVS-2		IC_{50} = 0.020 [55] (TA); K_i = 0.027 [50] (TA); Kd = 0.055 [50] (BA)
benzoxazol-2-one	MIF098 (Alissa-5)		IC_{50} = 0.01 [56] (TA)
pyrimidazole	K664-1		IC_{50} = 0.11 [59] (TA); K_i = 45 [40] (TA), K_i = 0.16 [58] (TA)
chromene	T-614		IC_{50} = 6.81 [58] (TA)
	Kok-10		IC_{50} = 18 [60] (TA)
	Kok-17		IC_{50} = 6.2 [60] (TA)
isoxazoline	CPSI-2705		2-10-fold more potent than ISO-1 [35] (TA)
	CPSI-1306		100-fold more potent than ISO-1 [35] (TA)

Class	Compound	Structure	Activity, μM Reference(s)
isocoumarin	SCD-19		100% inhibition at concentration of 100 μM [37] (TA)

TA, tautomerase assay; BA, binding assay MIF-CD74k

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