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Review

Macrophage migration inhibitory factor (MIF) in CNS diseases: Functional regulation and potential therapeutic indication

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ABSTRACT

Macrophage migration inhibitory factor (MIF) is a multifunctional protein that possesses cytokine, enzyme, and endocrine activities and acts as a chaperone-like molecule. Owing to its immune-inflammatory regulatory properties, the role of MIF has long been an attractive target in research on various autoimmune and inflammatory disorders. MIF is also widely expressed in the central nervous system (CNS), and its potential roles in CNS disorders have become a focus to elucidate the physiological and pathological effects of MIF and to explore its potential significance in the treatment of CNS diseases. Previously, the majority of work on MIF functional regulation was focused on MIF tautomerase inhibitors. However, mounting information has indicated that the functions of MIF extend far beyond its tautomerase activity. Here, we review the recent advances in understanding the complex roles of MIF in the pathogenesis of CNS disorders as well as the discovery and design of small molecules targeted to tautomerase and nuclease of MIF.

1. Introduction

Macrophage migration inhibitory factor (MIF) is a highly conserved nonglycosylated protein with a molecular weight of 12.5 kDa composed of 114 amino acids (also known as a glycosylation inhibitor), which shares 90% similarity between humans and mice. It was soon realized that MIF is a homotrimer and that another member of the MIF superfamily, the *D*-dopachrome tautomerase known as MIF2, is the only homolog of MIF at present exhibiting a similar functional spectrum to MIF. Growing evidence indicates that MIF is a pleiotropic protein that possesses properties of cytokines, enzymes, endocrine factors, chemokines, and molecular chaperones. As an endocrine factor, MIF is regulated by the hypothalamic–pituitary–adrenal cortex axis [1,2]; as an important modulator of innate immunity, MIF can be activated by various factors, such as lipopolysaccharide (LPS), IFN-γ, glucocorticoids and reactive oxidative species (ROS), which promote the secretion of itself and other proinflammatory cytokines, such as tumor necrosis factor (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β), as well as the production of inducible nitric oxide synthase (iNOS), thereby counterregulating the immunosuppressive effect of glucocorticoids (GCs); as an enzyme, MIF contains at least three catalytic centers: the well-known keto-enol tautomerase activity, thiol-protein oxidoreductase activity, and recently identified nuclease activity [1–5]. However, the relationship between the respective catalytic activity and specific biological function of MIF remains elusive.

MIF is widely expressed in almost all organs in the body, especially in the lymph, thyroid, prostate, placenta, and lung. In this regard, particular attention has been given to sepsis, rheumatoid arthritis, systemic lupus erythematosus, type 2 diabetes mellitus, cardiovascular diseases, and tumors [4]. In the central nervous system (CNS), MIF is expressed in various cells of the brain and is well documented for its roles in the regulation of neuroinflammation and neuron survival. The potential biomarker role of MIF in various CNS diseases has been proposed [6]. In addition, more functions of MIF in the brain have been elucidated recently [7,8]. It is also noted that although many small molecular MIF inhibitors have shown significant therapeutic effects in various disease-related models [1], the clinical stage of MIF selective inhibitors developed for CNS diseases treatment is still under development. The relatively slow clinical progress may be attributed to the functional complex of MIF. Two drugs, ibudilast and iguratimod, with potent MIF inhibitory activity entered clinical trials for the treatment of CNS diseases, revealing the potential value of targeting MIF in CNS diseases treatment. Therefore, we herein review the up-to-date progress of MIF in the CNS as well as the discovery and design of small-molecule MIF inhibitors for the treatment of CNS disorders.

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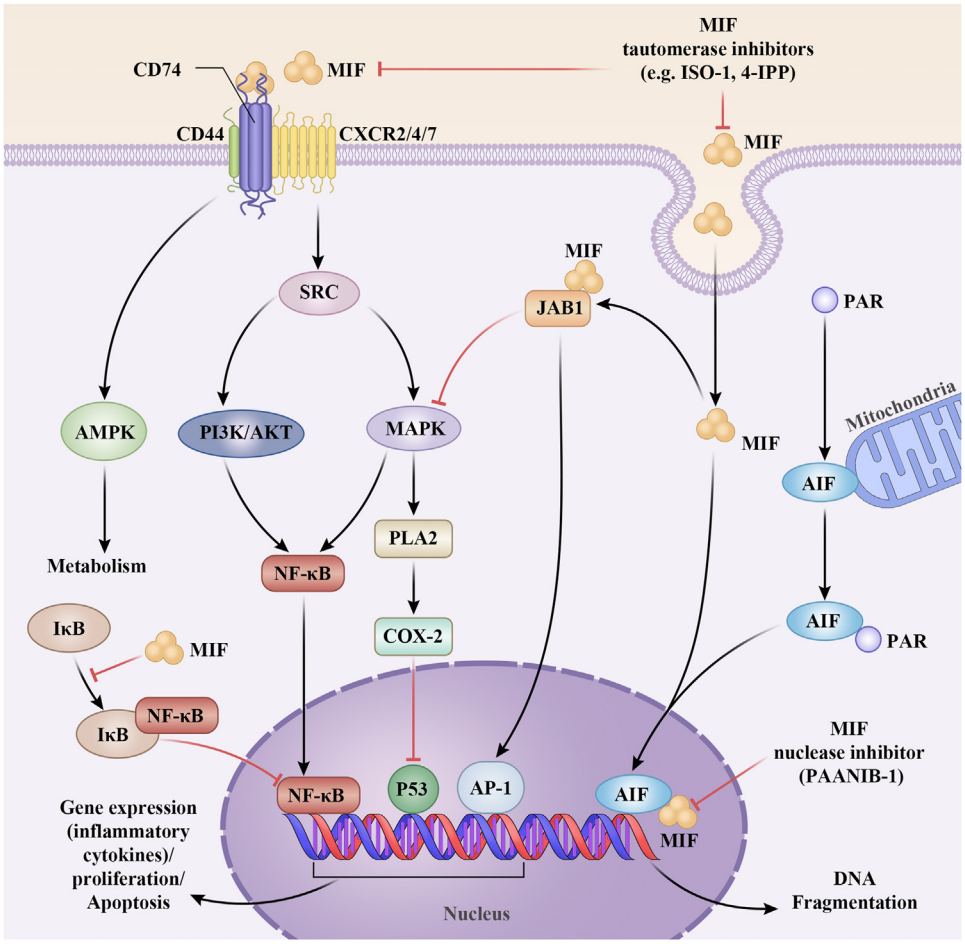

Fig. 1. MIF signaling pathway.

2. MIF in the central nervous system (CNS)

MIF is mainly expressed in the hippocampus, hypothalamus, cortex, cerebellum, and pons in rat brains [7–9], especially high expressed in mossy fibers of the dentate gyrus and dendrites of the hippocampal CA3 field. Our previous report showed that MIF is expressed in neurons, microglia, and astrocytes in the mouse brain and is mainly located in the cytoplasm [10]. Moreover, it was shown that the expression of MIF in the CNS was regulated by various factors. For instance, intracisternal injection of LPS was shown to stimulate MIF production in the brain and cerebrospinal fluid. Angiotensin II (Ang II) was reported to induce neuronal MIF expression *in vitro*. It was further revealed that increased MIF may serve as a physiological brake for the negative regulatory effects of Ang II on rat neuronal chronotropy via the type 1 receptor [11]. Consistently, brain injection of Ang II increased MIF expression in the paraventricular nucleus (PVN) of the hypothalamus, an area that plays an important regulatory role in sympathetic outflow and hypothalamus/pituitary axis activity, which in turn attenuates the increase in cell discharge and blood pressure. In addition, thrombin is also a potent inducer of MIF production from astrocytes as well as in endothelial cells [12,13]. Furthermore, hydrogen peroxide was also shown to upregulate MIF expression in rat primary motor neurons. Functional changes in MIF have been linked to pathogenesis and disease severity, and even as a potential biomarker in a number of CNS diseases [11,14,15]. On the other hand, administration of MIF recombinant protein promotes the neuroinflammatory response by stimulating microglial and astrocyte activation. We previously reported that inhibition of MIF tautomerase activity by a selective inhibitor suppressed microglial activation and protected neurons from apoptosis [15,16]. In addition to neuroinflammatory regulation, MIF was found to be expressed in neural stem/progenitor cells (NSPCs) and contribute to the proliferation of NSPCs by increasing the self-renewal of NSPCs [14], revealing the functional role of MIF in neuronal stem cell proliferation. Moreover, it was also shown that MIF may be involved in the regulation of neuroplasticity associated with learning and memory [17]. In addition, the importance of MIF in neuronal survival, including apoptosis regulation was also documented [18]. It is apparent that the functional importance of MIF in the brain is far beyond neuroinflammatory regulation and is thus worth systemically exploring.

2.1. MIF signals

MIF exerts its biological activity in an autocrine or paracrine manner, mainly through interaction with the cell surface receptor CD74 which then recruits CD44 to form a receptor complex. In addition, MIF can also bind to chemotactic receptors including CXCR2, CXCR4 and CXCR7. As shown in Fig. 1, upon receptor binding, MIF induces the activation of downstream signaling pathways including mitogen-activated protein kinase (MAPK), phosphatidylinositol 3/protein kinase B (PI3K/AKT) and NF-κB, which contribute to neuro-inflammation by inducing the production of pro-inflammatory cytokines such as IL-6 and TNF-α and regulate neuronal survival and neuroplasticity [19]. Moreover, MIF can directly enter the cytoplasm through endocytosis and bind to JUN activation domain binding protein 1 (JAB1), modulating AP-1 activity and cell proliferation [20]. Interestingly, MIF can further translocate to the nucleus by binding apoptosis-inducing factor (AIF), resulting in DNA fragmentation and cell death [3,5]. In addition, MIF showed a protective effect in ischemia/reperfusion animal models via activation of AMP-activated protein kinase (AMPK) signals [21,22].

Table 1
The roles of MIF in AD.

Authors	Study design	Results
Popp et al. 2009 [25]	AD patients: mean age (years) = 69.77, gender (m/f) = 12/19 MCI patients: mean age (years) = 67.04, gender (m/f) = 20/8 Control: mean age (years) = 67.05, gender (m/f) = 12/7	CSF MIF increased positively with TNF-α production.
Bacher et al.2010 [26]	AD patients: mean age (years) = 66.71, gender (m/f) = 5/2 Control: mean age (years) = 66.57, gender (m/f) = 5/2 APP 23 transgenic mouse	CSF MIF increased. MIF in microglial cells was associated with Aβ plaques. ISO-1 inhibited Aβ-induced neurotoxic in SHSY and BV-2 cells.
Zhang et al. 2019 [17]	AD patients: mean age (years) = 69.4, gender (m/f) = 14/14 MCI patients: mean age (years) = 68.9, gender (m/f) = 4/6 Control: mean age (years) = 61.2, gender (m/f) = 14/16 AD patients brain: mean age (years) = 68.4, gender (m/f) = 3/2 Control brain: mean age (years) = 48.4, gender (m/f) = 4/1 APP23/MIF$^{+/-}$ mice	MIF served as a defense mechanism and a biomarker of AD. MIF upregulated both in CSF and brain tissue. MIF deficiency affected cognitive functions in AD mice. MIF colocalized and interacted with Aβ oligomers. MIF overexpression inhibited Aβ-induced cytotoxicity.
Oikonomidi et al. 2017 [32]	CI: mean age (years) = 74.16, gender (m/f) = 44/53 Control: mean age (years) = 66.38, gender (m/f) = 16/36	CSF MIF levels were higher in CI with AD which associated with higher CSF tau and p-tau and lower CSF Aβ_{1-42}, independently predicted cognitive decline.
Nasiri et al. 2020 [27]	AD patients: mean age (years) = 69.7, gender(m/f) = 10/9 Control: mean age (years) = 70.5, gender (m/f) = 5/9 6-month-old male C57BL/6 or MIF-KO mice subjected to ICV-STZ	CSF MIF increased and positively associated with tau, p-tau and negative associated with Aβ_{1-42}. ISO-1 improved the STZ-induced memory impairment in contextual by reducing cytokine production.
Flex et al. 2014 [34]	AD patients: mean age (years) = 76.6, gender (m/f) = 188/353 Control: mean age (years) = 76.7, gender (m/f) = 268/445	No significant difference in the distribution of the CRP, MIF, and TNF-gene polymorphism.
Carlred et al. 2016 [29]	18-month-old transgenic mice (3 male, 1 female) with the Arctic (E693G) and Swedish (K670N, M671L) mutations (tgArcSwe) of human APP	MIF colocalized with activated microglia (Iba1) to the Aβ positive deposits in the hippocampal region.
Li et al. 2015 [30]	APP/PS1 transgenic mice were mated with MIF$^{-/-}$ mice 3-6-month-old female WT and MIF$^{-/-}$ mice subjected to ICV-STZ Primary astrocyte were treated with high glucose to mimic STZ function	ICV-STZ increased astrocyte activation and MIF expression in the hippocampus. MIF deficiency or ISO-1 attenuated tau hyperphosphorylation and astrocyte activation.
Liang et al. 2018 [31]	4-week-old male mice received D-galactose- and AlCl3 for 90 d to induced AD model, then administrated Fufang Danshen for 14 d. SH-SY5Y cells	Fufang Danshen suppressed MIF induced apoptosis in SH-SY5Y cells by decreasing Bad levels via Akt and IKKα/β signals. Fufang Danshen downregulated the levels of MIF, Bad and INF-γ in AD mouse model.

3. MIF in CNS disorders

3.1. Alzheimer's disease (AD)

AD is the most common progressive age-related neurodegenerative disease and a leading cause of dementia. There are close to 50 million AD patients worldwide. The clinical hallmarks of AD are memory loss and cognitive dysfunction. In contrast to its high prevalence rates, the effective therapeutic approach for AD is limited, with a high failure rate in clinical trials of anti-AD drugs.

Accumulating clinical studies have revealed that MIF may be involved in the pathophysiology of AD [23,24]. In patient studies reported by Julius Popp et al. [25], the concentration of MIF was significantly increased in the cerebrospinal fluid (CSF) of AD (n = 31) and mild cognitive impairment (MCI) (n = 28) patients compared to the controls without cognitive deficits (n = 19), and they also found that the levels of MIF were positively correlated with TNF-α production. Consistently, other studies also confirmed this observation and further revealed the increased expression of MIF in the brain tissues of AD patients [17,26]. Another report revealed that MIF was increased in the CSF of early clinical stage AD patients (n = 19) compared to age-matched controls (n = 14) and was robustly correlated with tau hyperphosphorylation [27]. The formation of advanced glycation end-products (AGEs) induced by glucose/insulin dysregulation contributes to the pathology of AD. Omar Kassaar et al. [28] identified that early glycation and oxidation of MIF in the AD brain may affect MIF activity and degradation by fluorescent phenylboronate gel electrophoresis, which may imply that MIF with glucose modification and oxidation is related to hyperglycemia and innate immune system dysregulation in AD. However, the glycation and oxidation of MIF in functional regulation remain largely unknown.

Extensive studies to reveal the relationship between MIF and AD pathological development have been conducted in various experimental AD models either *in vivo* or *in vitro* [25,27]. Similar to the clinical study, increased MIF expression was also observed in brain tissues of AD animals (APP23 transgenic mice), which was shown to be associated with Aβ deposits and microglial activation [17]. It was also noted that MIF co-localizes and interacts with Aβ as well as activated microglia (Iba1) in the hippocampal region of AD model animals [29]. The correlation between MIF expression and activation of astrocytes in the brain was further confirmed in the hippocampus of mice receiving intracerebroventricular (ICV) streptozotocin (STZ), a drug that can induce cognitive impairment similar to AD. In contrast, depletion of MIF significantly attenuated tau hyperphosphorylation and astrocyte activation in ICV-STZ mice [30]. A few studies have revealed the potential significance of targeting MIF in AD treatment. Inhibition of MIF by the selective tautomerase inhibitor ISO-1 attenuated Aβ-induced toxicity [27]. Fufang Danshen (FFDS, a Chinese herbal compound) was shown to markedly improve learning and memory impairment and neuronal apoptosis and decrease the serum levels of IFN-γ in D-galactose- and AlCl3-induced AD mouse models by downregulating Bad levels stimulated by MIF [31].

However, although many data suggest that increased circulating MIF or brain regional MIF can be a biomarker for cognitive impairment in AD [17,27,32] and that targeted inhibition of MIF might be a potential therapeutic approach for the disease, the roles of MIF in AD remain controversially reported (Table 1). For instance, APP23/MIF$^{+/-}$ mice exhibited impaired learning and memory compared with APP23 AD mice [17]. MIF overexpression significantly protected neuronal cells from Aβ-induced neurotoxicity. It was also proposed that neuronal secretion of MIF may serve as a defense mechanism to compensate for decreased cognitive function in AD [17,33]. Given the complexity of MIF biological activities, instead of assessing MIF expression, it may be important to differentially study the respective function of tautomerase activity, nuclear enzymatic activity, endocrine activity, and chemokines of MIF in AD pathological development, which will provide more precise information to elucidate the role of MIF in AD either in pathological development or as a therapeutic target.

Table 2
The roles of MIF in PD.

Authors	Study design	Results
Schwarz et al. 1998 [36]	PD rat model: female Sprague-Dawley rats weighing 200 ± 250 g were injected twice of 6-OHDA into the right ascending nigrostriatal pathway and the ventral tegmental area	MIF-treated animals showed good survival of tyrosine hydroxylase positive ventral midbrain cells by reducing the number of microglial cells in the grafts.
Nicoletti et al. 2011 [37]	PD patients: mean age (years) = 61.7, gender (m/f) = 16/14 Control: mean age (years) = 57.3, gender (m/f) = 13/14	MIF serum levels increased in PD patients.
Li et al. 2019 [38]	Acute PD animal model: intraperitoneally injecting MPTP (20 mg/kg) into 7-week-old mice (n = 6) at 2-h intervals consecutively for four times Chronic PD animal model: injecting MPTP (15 mg/kg) into 7-week-old mice (n = 6) twice per week for five consecutive weeks SH-SY5Y cells	MIF increased in MPTP-induced mice as well as MPP^+ induced SH-SY5Y cells. MIF siRNA and ISO-1 showed neuroprotective effect in MPP^+ induced SY5Y cells by regulating inflammation, apoptosis and autophagy.
Cheng J et al. 2020 [39]	PD patients: n = 92 Control (age/sex-matched): n = 87 Mice with microglial deletion of Atg5 were generated by crossing the Atg5 locus floxed mice with Itgam/CD11b-Cre mice	MIF serum levels increased in PD patients. Inhibition of autophagy led to NLRP3 inflammasome activation in microglia by stimulation of the expression of MIF.
Zheng LT 2021 [15]	Microglia cells DA neurons	MIF selective inhibitor inhibited neuroinflammation and protected DA neurons.
Park et al. 2022 [5]	PD mouse models: 2 to 3-month-old C57BL/6 male and female mice with stereotaxic injection of α-syn PFF, AAV-α-syn or MPTP intoxication PAAN/MIF KO male and female mice	PAAN/MIF genetic depletion and nuclease activity mutant both alleviated neuronal loss and motor dysfunctions in PD animal model. MIF nuclease inhibitor significantly blocked α-syn PFF, AAV-α-syn overexpression or MPTP injection-induced neurodegeneration *in vivo*. MIF tautomerase inhibitors failed to inhibit MIF nuclease activity and parthanatic cell death.
Zhang et al. 2022 [16]	BV-2 cells Neurons	MIF inhibitors showed unti-neuroinflammatory and neuroprotective effects.

3.2. Parkinson's disease (PD)

PD is the second most common neurodegenerative disease and affects approximately 1% of the elderly population. The hallmarks of PD are abnormal aggregation of α-synuclein (α-syn) and progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). There is no cure for the disease, although currently available treatments can relieve symptoms.

In the early 1990s, MIF was found to catalyze the conversion of 3,4-dihydroxyphenylaminechrome and norepinephrinechrome to indoledihydroxy derivatives, precursors of neuromelanin, which implies that MIF may participate in catecholamine metabolism regulation and thus may elicit protective effects on dopaminergic neurons by modulating catecholamine-related neuronal death [35]. In support of this hypothesis, Schwarz et al. [36] found that MIF-treated animals showed better graft survival than control animals in a 6-hydroxydopamine-induced PD rat model. These data suggest that MIF activity may be beneficial for PD. However, a clinical study by Alessandra Nicoletti et al. [37] showed that serum MIF levels were elevated in PD patients (PD patients: 5939 ± 2623 pg/ml, n = 30 vs. healthy controls: 4006 ± 1687 pg/ml, n = 27) in 2011. Consistently, MIF was also found to be increased in MPTP-induced PD mice as well as MPP^+-induced dopaminergic death in SH-SY5Y cell lines, and knockdown of MIF by siRNA showed a neuroprotective effect in MPP^+-induced SY5Y cells by regulating inflammation, apoptosis and autophagy [38]. We previously showed that a selective MIF tautomerase inhibitor suppressed microglial activation to produce neuroprotection [15,16]. Thus, it appears that the role of MIF in PD remains debated [6]. However, very recently, the nuclease activity of MIF in PD pathogenesis and its potential therapeutic role have been reported [5]. The nuclease activity of MIF can cleave genomic DNA into 20-50 kb fragments and then induce parthanatos, as reported by Dawson in 2016 [3]. MIF is thus also called parthanatos-associated apoptosis-inducing factor nuclease (PAAN), a member of the PD-D/E(X)K nuclease-mediated cell death family. The most recent study by the group revealed that the effect of PAAN/MIF on PD was mainly related to its nuclease activity but not tautomerase activity [5]. They found that DA neuron damage induced by pathological α-syn was dependent on the nuclease activity of PAAN/MIF. Genetic depletion of PAAN/MIF or nuclease activity mutation both alleviated DA neuronal loss and motor dysfunctions in the α-syn preformed fibril (PFF) mouse model of sporadic PD. This finding may provide an explanation for the controversial data on the role of MIF in PD pathogenesis, which may also reveal a potential new pathway for PD drug discovery (Table 2).

3.3. Major depressive disorder (MDD)

MDD is a common and multifactorial psychiatric disease that affects a large proportion of the worldwide population. The exact etiology of MDD remains unclear. However, it is generally believed that interactions between genetic and environmental components may be the key factors. The pathological changes of MDD involve abnormal monoamine transmission and the alteration in the hypothalamus-pituitary-adrenal (HPA) axis. In addition, neuroinflammation and impaired neurogenesis are also charted as pathological alterations in MDD. Current drug treatment is mainly targeted to the modulation of monoamine neurotransmitters. However, nearly 40% of MDD patients do not respond to the current anti-depressant drugs. There is still an unmet need to develop new and effective antidepressant drugs. MIF is known to participate in regulating HPA activity and the inflammatory response. Clinical studies from depressive patients showed that the plasma level of MIF in patients with Beck Depression Inventory-II (BDI-II) ≥ 14 was 40% higher than that of patients with less severe depressive symptoms (BDI-II < 14), and the increase in MIF was correlated with a blunted response to acute stress and lower morning cortisol [40]. Consistently, Musil et al. [41] also found that the baseline levels of MIF were significantly elevated in MDD patients (n = 32) compared with healthy controls (n = 20). This difference remained statistically significant after three and five weeks of treatment with reboxetine and add-on celecoxib. Interestingly, the basal level of TGF-β was significantly lower in the depressed patients than in the healthy controls. However, sCD14 did not differ at any time between the depression patients and the healthy controls. TGF-β can induce both regulatory/inhibitory and proinflammatory T cells, depending on whether proinflammatory cytokines such as IL-6 are present. It was conceivable that the change in MIF was associated somewhat with TGF-β, since there is close interplay between MIF cytokines and TGF-β signaling pathway components. In contrast, there is little evidence to depict the relevance between MIF and sCD14. This result is supported by a study [42] in which stroke patients with major depression had significantly higher plasma MIF levels than those patients without depression. In addition to a potential biomarker role, these results also im-

Table 3
Summary of MIF in MDD.

Authors	Study design	Results
Edwards et al. 2010 [40]	Participants: mean age (years) = 19.8, gender(m/f) = 62/64 then divided into two groups High-depressive patients: n = 28, BDI-II ≥ 14 Low-depressive patients: n = 84, BDI-II < 14	Elevated plasma MIF in high depressive patients than the low depressive patients.
Xu et al. 2018 [42]	Stroke patients: mean age (years) = 64, gender (m/f) = 181/152 Stroke with major depression: n = 95	Elevated plasma MIF in stroke patients with major depression than those in depression-free stroke patients.
Swoboda et al. 2022 [43]	MDD with drug treatment: mean age (years) = 46, gender (m/f) = 34/32 MDD without drug: mean age (years) = 47, gender (m/f) = 27/37 Remitted patients: mean age (years) = 49, gender (m/f) = 11/28 Healthy Control: mean age (years) = 42, gender (m/f) = 30/31	No significant group differences in MIF. No support for MIF as a biomarker for the diagnosis or monitoring of MDD.
Musil et al. 2011 [41]	Depressive patients were divided into celecoxib group: mean age (years) = 44.8, gender (m/f) = 11/7 and placebo group: mean age (years) = 44.3, gender (m/f) = 5/9 Control: mean age (years) = 40.0, gender(m/f)=15/5	Increased MIF levels but reduced TGF-β in depressive patients. Increased MIF levels in the celecoxib group compared placebo group, but have no statistically significant differences.
Conboy et al. 2011 [45]	MIF KO mice treatment by chronic stress, stress hormones administration, and fluoxetine	MIF knockout reduced neurogenesis, increased anxiety, depression, and impaired memory. ISO-1 reduced neurogenesis.
Gellén et al. 2017 [48]	Clomipramine-induced depression in female rat pups	Dramatic changes in MIF expression in brain, particularly in astrocytes.

Table 4
The role of MIF in schizophrenia.

Authors	Study design	Results
Schwarz et al. 2014 [49]	Schizophrenia patients: mean age (years) = 30, gender (m/f) = 118/62 Matched control: mean age (years) = 33, gender (m/f) = 232/166	MIF level in serum was significantly elevated in schizophrenia patients.
Chan et al. 2015 [51]	Schizophrenia patients: 127 first-onset drug-naive Control: n = 204	MIF serum level was increased in schizophrenia patients.
Okazaki et al. 2018 [50]	ELISA [86 patients: mean age (years) = 54.3, gender (m/f) = 46/40 vs. 51 controls: mean age (years) = 48.4, gender (m/f) = 23/28] Western blotting [18 patients: mean age (years) = 60.1, gender (m/f) = 9/9 vs. 22 controls: mean age (years) = 56.0, gender (m/f) = 17/5] TaqMan SNP genotyping assay [1483 patients: mean age (years) = 46.4, gender (m/f) = 796/687 vs. 1454 controls: mean age (years) = 46.6, gender (m/f) = 709/745]	Serum MIF levels were higher in schizophrenia patients and positively correlated with antipsychotic doses. MIF levels had no change in the postmortem brain of schizophrenia patients. MIF promoter polymorphisms were involved in schizophrenia.
Zhong et al. 2021 [54]	First-episode schizophrenia (FES) patients: mean age (years) = 24.56, gender (m/f) = 54/34 Control: mean age (years) = 23.92, gender (m/f) = 49/39	FES patients with metabolic syndrome showed higher serum levels of MIF, Hcy, and hs-CRP than healthy controls.
Cui et al. 2018 [52]	Drug-naïve and first-episode schizophrenia patients: mean age(years) = 27.95, gender (m/f) = 30/30 8-week-old WT and MIF$^{-/-}$ female mice	Olanzapine increased MIF expression, BMI, circulating insulin and triglyceride. MIF deficiency protected mice from olanzapine-induced insulin resistance.
Okazaki et al. 2021 [53]	Primary astrocytes	Clozapine increased MIF mRNA and protein expression in a time-dependent manner as well as acetylation of H3K27 in MIF promoter.

plied that MIF might be a pathway to elucidate the relationship among the pathological development, therapeutic response of MDD and neuroimmunity, as well as the HPA axis [41]. However, a very recent report by Swoboda [43] suggested that there was no clear evidence to support MIF as a biomarker for the diagnosis or monitoring of MDD, although their data revealed a potential sex difference in the role of MIF in depression.

In experimental studies, MIF-deficient (knockout) mice exhibit increased depressive-like behavior [44], and other experimental studies have shown that MIF expression is important for hippocampal neurogenesis [45]. They found that MIF knockout reduced neurogenesis and induced cell proliferation. Those mice showed increased anxiety and depression behaviors as well as impaired hippocampus-dependent memory.

It seems that there is a controversial role for MIF: both anti- and pro-depressive roles [44,46]. Notably, the observed increase in MIF levels was determined in the plasma of MDD patients (Table 3), and the antidepressant effect of MIF was mainly investigated in the brains of mice and rats. There is very limited information regarding MIF expression in the brains of MDD patients. The plasma MIF changes may represent the collective results of disease, whereas experimental studies have focused more on the pathogenesis or development of MDD. Finally, a study showed that the MIF-promoted effect of IL-6 on depression depended on the concentrations of IL-6 and MIF. High concentrations may be antidepressant, while low concentrations are pro-depressive [47].

3.4. Schizophrenia

Accumulating clinical data indicate a potential role of MIF in schizophrenia [46]. Serum MIF levels were reported to be significantly increased in schizophrenia patients, compared with healthy controls (Table 4). MIF content in serum was further found to be positively correlated with age of onset, antipsychotic dose, and severity of disease [46,49-51]. For instance, Satoshi Okazakia [50] found that serum MIF levels were significantly higher in schizophrenia patients (n = 86) than in the control group (n = 51) and positively correlated with antipsychotic doses. They further showed that serum MIF was higher in the earlier age of onset patients (≥ 40 ng/mL) than in the matched groups

(< 40 ng/mL). It was also reported that first-episode schizophrenia patients with metabolic syndrome exhibited higher levels of serum MIF, homocysteine, and high-sensitivity C-reactive protein than patients without metabolic syndrome. In addition, MIF promotor polymorphisms were observed to be associated with schizophrenia [50].

Anti-schizophrenia drugs cause metabolic sequelae in 30%-60% of patients, which has been a challenge in clinical practice. Clinical studies have shown that elevated MIF was accordingly accompanied by an increase in body mass index (BMI), circulating insulin, and triglycerides in schizophrenic patients who received olanzapine monotherapy in relation to drug-naïve patients [52]. MIF knockdown protected mice from olanzapine-induced insulin resistance, which is in agreement with the observed role of MIF in type 2 diabetes. They further found that administration of anti-MIF antibodies significantly inhibited olanzapine-induced food intake and subsequent body weight gain without influencing the peripheral level of MIF [52]. Further study demonstrated that the clozapine-induced increase in MIF expression was mainly related to histone H3 at lysine 27 residue (H3K27) acetylation in astrocytes [53]. They confirmed that common antipsychotics, especially clozapine, upregulated both the mRNA and protein expression of MIF. Moreover, the acetylation of H3K27 in the MIF promoter was also upregulated by treatment with clozapine in primarily cultured astrocytes. These data therefore suggested that MIF contributes to metabolic side effects induced by atypical antipsychotic therapy, and targeting MIF may provide a potential approach for schizophrenia treatment.

3.5. Ischemic stroke

Ischemic stroke is one of the main causes of death and disability. Ischemic stroke is mainly due to brain blood supply obstruction, which leads to brain tissue hypoxia and glucose deficiency, eventually causing tissue function injury.

The role of MIF in the pathological development of ischemic stroke has been extensively studied but remains controversial. Human MIF promoter activity is markedly increased under hypoxia, and there is a functional hypoxia-inducible factor 1α (HIF1α)-binding site in the region between +8 and +35 bp in the MIF promoter [55,56]. Elevated plasma MIF content was repeatedly reported in various clinical studies in ischemic stroke patients [42,57-59]. It was shown that serum MIF levels were elevated in acute-stage acute ischemic stroke (AIS) patients (n = 146) but not in chronic-stage patients in relation to matched controls (n = 45) [57]. MIF levels were positively associated with infarct volume and National Institutes of Health Stroke Scale (NIHSS) score [57] and were independently related to clinical severity and poor outcome (n = 289) [58]. Moreover, elevated plasma levels of MIF increased the risk of developing post-stroke depression in the next three months in Chinese AIS patients (n = 333) [42].

There were also studies showing elevated expression of MIF mRNA and protein in peripheral blood mononuclear cells (PBMCs) of stroke patients (n = 102), which was positively correlated with disease severity [55]. Moreover, the expression of CD74 and MIF were both significantly increased in PBMCs of ischemic stroke patients (n = 20) and strongly correlated with infarct size and neurological outcomes [60]. While clinical studies support the potential biomarker role of MIF in stroke, experimental studies have produced conflicting results. Increased MIF expression in the ischemic boundary zone of rats or decreased MIF in the infarction area were reported in rats subjected to transient distal middle cerebral artery ligation (MCAl) [61]. Overexpression of MIF reduced the cell death induced by oxygen-glucose deprivation (OGD) [56]. MIF-knockout mice exhibited caspase-3 activation, neuronal loss, and increased infarct development via NF-κB signaling under hypoxia [61]. Further study demonstrated that brain-derived nerve factor (BDNF) may be a key modulator mediating the neuroprotective effect of MIF against stroke [62,63]. Specifically, Chang et al. [64] demonstrated that early exercise [initiated 48 hours after middle cerebral artery occlusion (MCAO)] could improve motor dysfunction and neuronal recovery after cerebral ischemia/reperfusion (I/R) in rats by increasing the levels of MIF and BDNF. Administration of recombinant MIF increased human neuroblastoma cell survival by stimulating the expression of BDNF, microtubule-associated protein 2 (MAP2) and Bcl2 while decreasing the expression of caspase-3 and Bax in the OGD model. The MIF treatment-mediated changes were reversed by the MIF tautomerase inhibitor ISO-1 [62], revealing that the tautomerase activity of MIF may be involved in the effect. Similarly, in a mouse model of MCAO, intracerebroventricular administration of MIF also significantly improved the performance on the rotarod test as well as with lower total infarct volume, which may be associated with the higher levels of BDNF and MAP2 [63]. Jung C et al. [65] confirmed the protective effect of MIF and further elucidated the optimal administration conditions of MIF in an *in vitro* stroke model. They found that 60 ng/mL MIF showed the best protection regardless of administration time. Mechanistically, the neuroprotective effects of MIF were found to be associated with histone deacetylase 6 (HDAC6) inhibition [66]. Genetic ablation or pharmacological inhibition of HDAC6 reduced MIF acetylation after ischemic stroke. Administration of an HDAC6 inhibitor or aspirin protects against cortical neuronal death after ischemia by promoting MIF acetylation on the K78 residue, which impairs MIF translocation to the nucleus by suppressing the interaction between MIF and apoptosis-inducing factor (AIF). Treatment with baicalein, a natural flavonoid that possesses neuroprotective effects by activating the PI3K/AKT pathway, significantly decreased cerebral infarct volume and neurological scores by attenuating cytokine release, PARP-1 activation, AIF and MIF nuclear translocation in cerebral ischemic/reperfusion rats [67]. In addition, MIF was recognized as the target of microRNA-493 (miR-493) in ischemic stroke in a rat model [68]. The expression of miR-493 was decreased in the ischemic boundary zone of the MCAO-induced ischemic stroke rat model as well as in rat brain microvascular endothelial cells (RBMECs) in response to OGD. It was further shown that miR-493 expression was negatively correlated with MIF expression. Inhibiting miR-493 by injection of antagomir-493 could increase ischemic boundary zone capillary density and ameliorate focal infarct volume as well as neurologic deficits in rats subjected to MCAO. Knockdown of MIF abolished the protective effect of miR-493 inhibition on angiogenesis, whereas recombinant MIF treatment rescued the protective effect.

It was also noted that in the first week, increased MIF was observed in neurons, astrocytes and microglia of the peri-infarct region following ischemic stroke. However, there were no differences in GFAP immunoreactivity, the number of $CD74^+$ cells or spleen weight between wild-type and MIF-KO mice when subjected to MCAO at this stage of stroke. This suggests that MIF appeared not to be involved in the inflammatory/immune response during the first 7 days following experimental stroke [69]. In addition, female MIF KO mice also showed significantly larger infarction volumes than wild-type females and ovariectomized female mice. Meanwhile, it was also found that female MIF KO mice displayed more microglial activation [70]. These results suggested that the neuroprotective effect of MIF in the early stage of stroke was mainly related to its modulation of neuronal activity rather than inflammatory regulation, and a potential sex difference for MIF in stroke may also exist.

In contrast to the neuroprotection of MIF, there is also some evidence indicating that MIF exerts a deleterious influence on stroke. Inaˊcio et al. [71] found that MIF promoted neuronal death and aggravated neurologic deficits in MCAO mice. Other reports employing MIF knockout mice found that MIF deficiency decreased infarct volume and promoted recovery of neurologic function in MCAO mice with an increase in galectin-3 immunoreactivity without alteration of the levels of IL-1β and CD86 in the brain. In addition, Danshen is a famous Chinese herb for the treatment of tissue injuries and neuroprotection, and it was reported that inhibition of MIF expression may be involved in its protective effects. Another study demonstrated that MIF administra-

Table 5
The role of MIF in stroke.

Authors	Study design	Results
Wang et al. 2009 [55]	AIS patients: mean age (years) = 68.61, gender (m/f) = 61/41 Control: mean age (years) = 68.12, gender (m/f) = 32/25 12-week-old male and female Wistar rats subjected to MCAO	MIF protein and mRNA were increased in stroke patients and rat models, and positively correlated to the disease severity.
Zis et al. 2015 [56]	Human ischemic brain HEK293 cells Primary cortical neurons of rat brain subjected to OGD	MIF was increased in human ischemic brain. MIF promoter activity was upregulated under hypoxia. MIF overexpression reduced OGD-induced cell death.
Yang et al. 2017 [60]	AIS patients: mean age (years) = 66.60, gender (m/f) = 13/7 Control: mean age (years) = 63.71, gender (m/f) = 8/6	MIF and CD74 levels were increased in PBMCs of ischemic stroke patients.
Li et al. 2017 [57]	First episode of AIS patients within 24 h of symptom onset: mean age (years) = 65.35, gender (m/f) = 83/63 Control: mean age (years) = 66.33, gender (m/f) = 26/19	MIF serum levels were increased and positively associated with infarct volume, and NIHSS score.
Liu et al. 2018 [59]	AIS patients: n=39 Control: n=14 13-15-week-old male Wistar rats and SHRs subjected to MCAO ARBECs subjected to OGD	MIF blood levels were upregulated both in stroke patients and MCAO rats. MIF showed a deleterious influence on stroke by destroying the tight junction of blood-brain barrier both *in vitro* and *in vivo*. ISO-1 showed a neuroprotective effect in stroke.
Xu et al. 2018 [42]	AIS patients: mean age (years) = 64, gender (m/f) = 181/152	Elevated plasma levels of MIF were associated with an increased risk of post-stroke depression.
Wang et al. 2019 [58]	AIS patients: mean age (years) = 61, gender (m/f) = 155/134	MIF serum levels were upregulated and independently related to clinical severity and poor outcome.
Inácio et al. 2011 [69]	8-36-week-old WT and $MIF^{-/-}$ male mice subjected to MCAO	MIF deletion did not affect the inflammatory/immune response during the first week after the stroke.
Zhang et al. 2014 [61]	10-12-week-old WT and $MIF^{-/-}$ male mice subjected to transient distal MCAl Cell of HEK293, BV-2 and human neuroblastoma N2A	MIF knockout resulted in caspase-3 activation, neuronal loss, and increased infarct development via NF-kB signaling. Overexpression of MIF reduced the cell death induced by H_2O_2.
Turtzo et al. 2013 [70]	8-10-week-old male and female WT and $MIF^{-/-}$ mice subjected to MCAO	Female MIF KO mice showed larger strokes compared to WT females as well as in ovariectomized female mice.
Bae et al. 2020 [62]	SH-SY5Y cells subjected to OGD/R MIF recombinant and ISO-1	MIF increased the levels of BDNF, Bcl2, and MAP2, and decreased the levels of Caspase-3 and Bax under OGD, which could be reversed by ISO-1.
Chang et al. 2019 [64]	8-week-old male Sprague-Dawley rats subjected to MCAO	Exercise could improve motor dysfunction and neuronal recovery after I/R in rats by increasing the levels of MIF and BDNF.
Kim et al. 2022 [63]	10-12-week-old adult male c57/BL6 mice subjected to MCAO	ICV of MIF exhibited better performance on the rotarod test and lower total infarct volume by increasing the levels of BDNF and MAP2.
Jung et al. 2021 [65]	SH-SY5Y cells subjected to OGD/R	60 ng/mL of MIF showed the best protective effect but have no significant difference in administration time.
Hu et al. 2022 [66]	2-3-month-old male HDAC6 mutant mice and MIF K78Q mice subjected to photothrombotic ischemia	HDAC6 inhibitor or aspirin protected cortical neuronal death by promoting MIF acetylation on the K78 residue.
Li et al. 2020 [67]	Male rats subjected to MCAO SH-SY5Y cells subjected to OGD	Baicalein decreased cerebral infarct volume and neurological scores by inhibiting MIF.
Li et al. 2016 [68]	Male rats subjected to MCAO RBMECs subjected to OGD/R	Protective effect of miR-493 inhibition in angiogenesis was attenuated by knocking down MIF after MCAO.
Inácio et al. 2011 [71]	8-36-week-old WT and $MIF^{-/-}$ male mice subjected to MCAO Primary neuronal cells subjected to OGD	MIF-KO mice decreased infarct volume and promoted recovery of neurologic function after MCAO.

tion to MCAO mice enlarged the infarction volume by disrupting tight junctions of the blood-brain barrier (BBB) in adult rat brain endothelial cells, although MIF treatment did not produce neuronal toxicity in primary culture [59]. They found that inhibition of MIF by ISO-1 had a strong neuroprotective effect.

Taken together, the potential clinical importance of MIF as a biomarker for stroke has been accepted. While most studies have confirmed the protective role of MIF in stroke (Table 5), there are also a few reports revealing a controversial result. The details regarding this difference are not clear and may be associated with the differences in the model employed and the design of the experiments.

3.6. Multiple sclerosis (MS)

MS is a CNS autoimmune disease that affects more than 2 million patients worldwide and is characterized by demyelination and axon loss, although the pathogenesis remains elusive. The potential role of MIF in MS has attracted extensive attention. Elevation of plasma MIF expression has been repeatedly reported in MS and is considered a biomarker for exacerbation of disease progression [8]. For instance, Gil Benedek et al. [72] showed that plasma MIF was significantly elevated in MS subjects compared with healthy controls when combining males and females in all phases of MS. Interestingly, MIF was found to be higher in progressive male MS subjects than in clinically isolated syndrome (CIS) and relapsing-remitting MS subjects but not in female subjects, suggesting a potential sex difference [72]. An analysis comparing MIF in CSF between relapsed and remitted MS patients separately also revealed an increased MIF concentration in relapsed subjects [73]. Another study evaluated the transcriptomic expression of MIF, *D*-dopachrome tautomerase, their receptors CD74 and CD44, and MIF coreceptors CXCR2, CXCR4, and CXCR7 in the peripheral blood of patients with CIS and found that MIF, DDT, and CD44 are overexpressed in $CD4^+$ T cells but not in $CD8^+$ T cells, B cells, or monocytes from patients with CIS compared to healthy controls. The author speculated that MIF may participate in the pathogenetic process as a cytokine and chemokine and recruit peripheric inflammatory cells into the blood-brain barrier [74]. These data further illustrated the potential biomarker role of MIF in MS progression. Interestingly, although Hjæresen et al. [75] recently reported that MIF can be a good prognostic marker for disease progression of MS conducted in Danish and Swedish patient studies, they found that MIF was decreased in newly diagnosed CIS and relapsed and remitted MS patients but was high in patients with secondary progressive MS. They found that MIF is mainly localized in neurons and astrocytes, but to our surprise, there were no microglia in MS human brains. They may implicate the immune-modulating role of MIF that may not necessarily be involved in the later stage of MS [74].

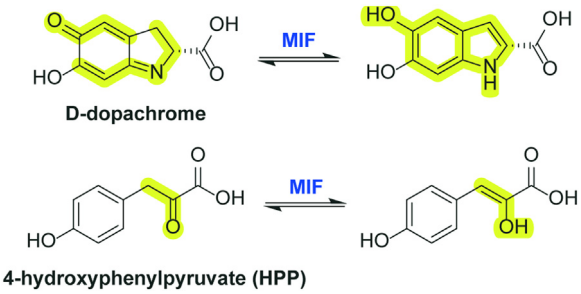

Fig. 2. Keto-enol tautomerisation reactions catalyzed by MIF.

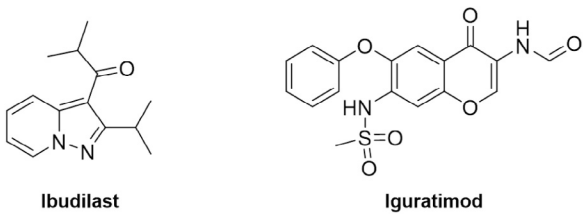

Fig. 3. Old drugs repurposed as potent MIF inhibitors.

Experimental autoimmune encephalomyelitis (EAE) is the most widely used animal model that is believed to mimic some key features of MS, such as inflammation, demyelination, remyelination, and gliosis. An early report showed that anti-MIF Ab treatment of SJL mice with acute EAE improved disease severity and accelerated recovery [76]. It has also been demonstrated that MIF knockout mice are resistant to EAE induction, as manifested by lower scores and less macrophage and $CD4^+$ T-cell infiltration in the spinal cord and cerebellum. Glucocorticoids (GCs) are used as standard treatment for acute attacks of MS; however, GCs eventually lose efficacy and do not prevent disease progression. MIF may play a key role in the resistance of pathogenic CD4(+) T cells to GC treatment in EAE mice [77]. MIF promotes neuroinflammation and contributes to the pathophysiology of MS. Stereotaxic microinjection of MIF into the naïve spinal cord of MIF KO mice to create a milieu for activation of resident microglia and/or macrophages could restore EAE-mediated inflammatory pathology, suggesting the role of MIF in maintaining inflammation [78]. It was also noted that mice genetically deficient in either MIF-1 or MIF-2 each resulted in an approximately 25% reduction in moderate EAE compared to wild-type mice. However, mice deficient in both MIF-1 and MIF-2 KO did not induce a greater reduction in EAE severity, suggesting that the two MIF homologs likely affect the same pathogenic pathways [79].

4. Targeted MIF drugs for CNS diseases

Currently, the majority of MIF inhibitors are developed to selectively inhibit keto-enol tautomerisation reactions (Fig. 2) toward MIF substrates *D*-dopachrome and 4-hydroxyphenylpyruvate (HPP). Although the MIF tautomerase active site might not be directly linked to the pocket of its cytokine function, modulation of tautomerase activity indeed interferes with MIF cytokine activity, thereby providing a valuable opportunity for the screening of efficient MIF inhibitors by various approaches. Herein, we summarize the currently identified MIF tautomerase inhibitors and a newly identified nuclease inhibitor for CNS treatment and briefly discuss their structure-activity relationship (SAR).

4.1. Marketed Drugs as MIF inhibitors by Drug Repurpose

4.1.1. Ibudilast

Repurposing or repositioning marketed drugs with proven good safety and pharmacokinetic profiles for novel indications is an attractive, economical, and feasible approach to drug discovery. Ibudilast (KC-404; AV-411; MN-166, Fig. 3) is an orally available anti-inflammatory drug that functions as a nonselective phosphodiesterase (PDE)-3,4,10 inhibitor [80], which was marketed for the treatment of bronchial asthma for over 40 years in Japan and, more recently, for post-stroke dizziness and allergic conjunctivitis. In 2010, ibudilast was identified as a noncompetitive inhibitor of MIF tautomerase activity with dissociation constant (K_d) and inhibition constant (K_i) values of 55.1 and 30.9 μM, respectively [80]. Ibudilast dose-dependently inhibited MIF-induced PBMC chemotactic activity through the CXCR2 receptor. Significant inhibition was achieved even at concentrations as low as 10 nM. Subsequent NMR and X-ray studies suggested that ibudilast is bound to MIF at its allosteric site adjacent to the tautomerase active site [80].

Ibudilast has a broader range of pharmacological activities [80]. In the CNS, it has been shown to inhibit inflammation and provide neuronal protection. For instance, the drug was shown to inhibit the production of proinflammatory cytokines (IL-6, IL-1β, TNF-α, leukotriene B4, NO) in activated glial cells via blockade of Toll-like receptor 4 (TLR-4), while it stimulates anti-inflammatory cytokines such as IL-10 and IL-4. The neuroprotection of ibudilast was attributed to either induction of various neurotrophic factors, including glial cell-derived neurotrophic factor, nerve growth factor, and neurotrophin 4, or inhibition of neuroinflammation. Moreover, ibudilast was recently identified as an autophagy enhancer that increases the clearance of disease-linked TAR DNA binding protein (TDP-43) and superoxide dismutase 1 (SOD1) protein aggregates through transcription factor EB (TFEB)-mediated autophagy and lysosomal biogenesis, indicating its potential for the treatment of several neurodegenerative disorders [81]. Additionally, ibudilast has good blood–brain barrier permeability and an acceptable safety profile and efficacy in oral administration.

Earlier studies suggest that ibudilast may improve survival outcomes and slow disease progression in patients with amyotrophic lateral sclerosis (ALS). In 2020, ibudilast advanced into phase 2b/3 with a 12-month double-blind clinical trial followed by a 6-month open-label extension phase for evaluation of its efficacy, safety, and tolerability on function, muscle strength, quality of life, and survival of patients diagnosed with ALS in 2020 (NCT04057898, Table 6). Ibudilast was also shown in randomized controlled trials to reduce ventral striatum activation in response to visual alcohol cues (NCT03489850) and decrease alcohol cue-elicited functional connectivity between the ventral striatum seed and reward-processing regions, including the orbitofrontal and anterior cingulate cortices [82]. Most recently, a new randomized, double-blind placebo-controlled study of ibudilast was launched that involved 6 weeks of ibudilast treatment designed to reduce or stop alcohol drinking (NCT05414240). Moreover, ibudilast can attenuate methamphetamine-induced peripheral pro-inflammatory effects in patients with methamphetamine use disorder [83] and thus is being investigated for the treatment of methamphetamine addiction in a phase 2 clinical trial (NCT03341078). Additionally, ibudilast was reported to sensitize glioblastoma to temozolomide and improve survival times by targeting MIF [84]. A combination treatment of ibudilast and temozolomide is currently being evaluated in patients with newly diagnosed and recurrent glioblastoma in a phase 1/2 clinical trial (NCT03782415). Collectively, ibudilast emerges as a promising drug candidate for treating neurological conditions, including ALS, neuroblastoma, and alcohol and drug abuse disorder [82,83].

4.1.2. Iguratimod

Iguratimod (T-614, Fig. 3) is a clinically utilized chromene derivative approved for the treatment of rheumatoid arthritis only in Japan and China by inhibiting COX-2 or modulating NF-κB activation. Iguratimod was identified as an MIF-specific inhibitor with an IC_{50} value of 6.81 μM in a dopachrome tautomerase assay and attenuated a systemic inflammatory response in a murine endotoxemia model in an MIF-dependent fashion, as reported by Joshua Bloom and colleagues in 2016 [85]. However, iguratimod actually had weak anti-inflammatory activity, exhibit-

Table 6
MIF inhibitors in ongoing human clinical trials.

Name	Highest clinic trial	Conditions	NCT Number	Study Start
Ibudilast	Phase 2b/3	ALS	NCT04057898	2020
(KC-404; AV-411;	Recruiting			
MN-166)	Phase 2	Alcohol Use Disorder (AUD)	NCT05414240	2023
	Recruiting		NCT03594435	2018
	Phase 2	Methamphetamine Addiction	NCT03341078	2019
	Recruiting			
	Phase 1/2	Newly Diagnosed and Recurrent	NCT03782415	2018
	Recruiting	Glioblastoma		
Iguratimod	Phase 4	Primary Sjögren's Syndrome	NCT04981145	2022
(T-614)	Recruiting			
	Phase 4	Rheumatoid Arthritis	NCT05626348	2021
	Recruiting		NCT04927000	2021
			NCT04928066	2020
	Phase 2/3	Hand Osteoarthritis	NCT05216757	2022
	Not yet recruiting			
	Phase 2	Steroid-resistant/Relapse Immune	NCT05302024	2022
	Not yet recruiting	Thrombocytopenia	NCT05281068	2021
	Recruiting			
	Not Applicable	Diffuse cutaneous systemic sclerosis	NCT04515706	2021
	Not yet recruiting			

Table 7
The mechanism of action of MIF inhibitors.

MIF inhibitors	MIF inhibitory activity	Mechanism of action	Potential Binding sites
Ibudilast	K_d = 55.1 μM	MIF tautomerase inhibitor	
	K_i = 30.9 μM	Anti-inflammation Neuroprotection	
Iguratimod	IC_{50} = 6.81 μM	MIF tautomerase inhibitor	
		Anti-inflammation	
		Immunomodulation	
		Osteoprotection	
		Anti-pulmonary fibrosis	
ISO-1	IC_{50} = 7-100 μM	MIF tautomerase inhibitor	LYS32, ILE64,
		Anti-inflammation	ASN97, MET2, PRO1,
		Neuroprotection	TYR36,
Z-312	IC_{50} = 0.55 μM	Covalent or slow, tight-binding MIF inhibitor	
		Anti-inflammation	
Z-590	IC_{50} = 40.54 μM	MIF tautomerase inhibitor	
		Anti-neuroinflammation	
		Neuroprotection	
11	IC_{50} = 11.95 μM	MIF tautomerase inhibitor	
		Anti-neuroinflammation	
4-IPP	IC_{50} = 0.2-0.5 μM	Covalent MIF inhibitor	PRO1, TYR36, HIS62,
		Anti-inflammation	VAL106, PHE113,
			TYR95, ASN97
PAANIB-1	IC_{50} = 0.28 μM	MIF nuclease inhibitor	LEU61
		Prevention of	ASN72
		neurodegeneration	

ing poor suppression of many cytokines, including MCP-1 and IL-8, by using their *in vitro* LPS-driven assays [85]. In particular, it barely suppressed TNF-α release at concentrations up to 50 μM in monocytes. Nevertheless, iguratimod has significant synergistic effects with glucocorticoids in suppressing inflammation *in vitro* and *in vivo* [85]. In a mouse model of multiple sclerosis, combination therapy with dexamethasone and iguratimod is more efficacious in slowing disease progression than either drug alone. Hence, iguratimod with steroid-sparing therapeutic potential may be repurposed in other MIF-relevant diseases, including multiple sclerosis. Moreover, iguratimod has the potential to regulate the immune balance, promote the differentiation of osteoblasts, inhibit osteoclastogenesis, and decrease pulmonary fibrosis (Table 7) [86]. In recent years, iguratimod has been applied to the treatment of a variety of inflammatory diseases, such as Sjögren's syndrome, hand osteoarthritis, immune thrombocytopenia, ankylosing spondylitis and systemic lupus erythematosus [86]. In addition, iguratimod was found to block dermal fibroblast activation and skin fibrosis by regulating the TGF-β1/Smad signaling pathway in experimental systemic sclerosis models [87], and is thus also being clinically evaluated for potential use in patients with diffuse cutaneous systemic sclerosis (Table 6, NCT04515706).

4.2. ISO-1

(*S,R*)-3- (4-Hydrophenyl)-4,5-dihydro-5-isoazole acetic acid methyl ester (ISO-1, Fig. 4a), discovered in 2002, is the most frequently used small molecule MIF tautomerase competitive inhibitor. As shown in Fig. 4b, X-ray crystallography of ISO-1 bound to MIF revealed that MIF is a toroid-shaped, trimeric protein with a total of 342 amino acid residues. The phenolic ring of ISO-1, as a common MIF motif, formed an important hydrogen bond with ASN97 in the deep pocket of the active site, while the isoxazoline and ester moieties generated the remaining hydrogen-bond interactions with LYS32 and ILE64 (Fig. 4b).

The isoxazoline inhibitor ISO-1, a stable analog of the tautomerase substrate dopachrome, covalently binds to the tautomerase activity site of MIF with a wide range of reported IC_{50} values from 7 μM to >100 μM due to the assays used and different systems [88]. ISO-1 is known to inhibit inflammation and protect cells (Fig. 1). However, ISO-1 had no impact on insulin biosynthesis, indicating that the recognition site between MIF and insulin differed from the one between MIF and its tautomerase inhibitor ISO-1 [89]. It was reported that pharmacological inhibition of MIF by ISO-1 (i.p., 7 mg/kg) administration for 14 days to mimic post-

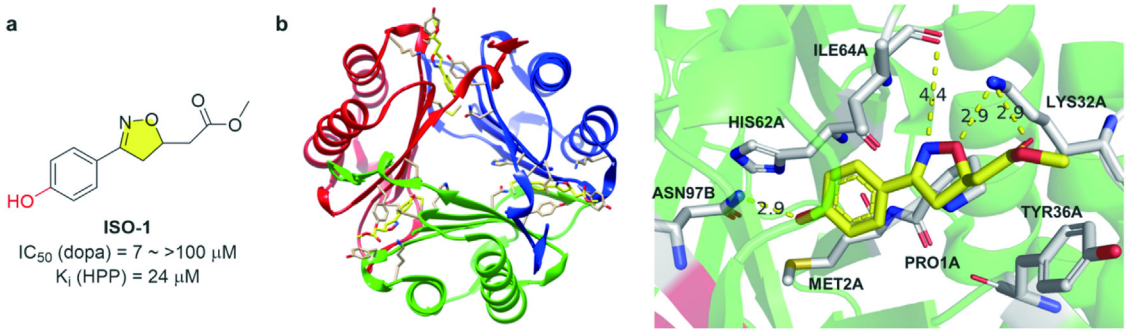

Fig. 4. ISO-1 bound to MIF. (a) Chemical structure of ISO-1. (b) The X-ray cocrystal structure of ISO-1 with MIF (PDB code: 1LJT). The inhibitor occupies all three active sites (left) and forms hydrogen bonds with the active site residues LYS32, ILE64, and ASN97B (right).

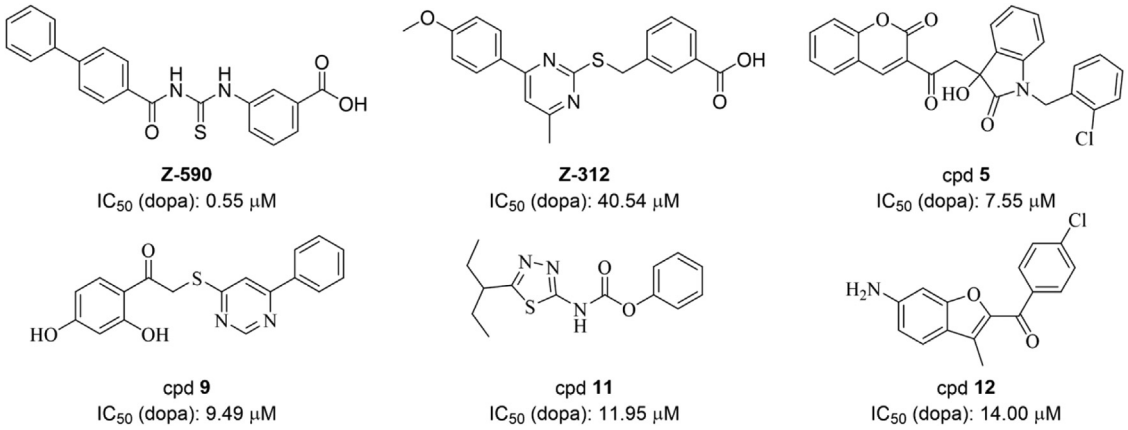

Fig. 5. Anti-neuroinflammatory MIF inhibitors by virtual screening.

natal MIF deletion modulated hippocampal neurogenesis *in vivo*, which has been linked to affective behaviors such as anxiety and depression, as well as cognitive functions such as learning and memory [45]. Subsequent studies found that ISO-1 could abolish Aβ-mediated neurotoxicity in both the murine microglial BV-2 cell line and neuroblastoma SHSY cells [26]. Moreover, intraperitoneal injections of ISO-1 (daily, 20 mg/kg) for 4 weeks following STZ injection significantly improved STZ-induced cognitive impairment in contextual memory performance by reducing the production of cytokines [27]. ISO-1 also showed a neuroprotective effect in MPP^+-induced SY5Y cells by regulating apoptosis and autophagy [38]. Furthermore, the neuroprotective effect of ISO-1 was found to be associated with inhibiting LDH activity in cortical neurons after oxygen glucose deprivation [71]. Additionally, ISO-1 administration (40 mg/kg, i.p.) markedly improved the phenotypes in mouse myelin oligodendrocyte glycoprotein (MOG)35-55-induced experimental MS [90]. Altogether, ISO-1 is not only a valuable chemical tool to validate MIF functions in the CNS but also has therapeutic potential for CNS diseases.

4.3. MIF inhibitors Z-312, Z-590 and 11 for neuroinflammatory-related diseases

In 2014, our team identified ten chemically diverse compounds, including Z-590 and Z-312 (Fig. 5), as potent MIF inhibitors in the micromolar range by structure-based virtual screening. Among these, Z-590 exhibited the most potent inhibitory activity with an IC_{50} value less than 1 μM (0.55 μM), which is 26-fold more potent than the prototypical MIF inhibitor ISO-1 (IC_{50} = 14.41 ± 1.59 μM) [91]. Z-590 with an acylthiourea functionality may be a covalent inhibitor or slow, tight-binding inhibitor based on its effect of incubation time on the initial velocity of the MIF tautomerase reaction [92]. We found that Z-590 not only significantly inhibited NO production in LPS-stimulated RAW 264.7 cells but also was more potent than ISO-1 (213% vs. 128%) in suppressing MIF glucocorticoid overriding activity [91]. Z-590 was more potent at inhibiting MIF-induced cell migration and ERK phosphorylation than ISO-1 in RAW 264.7 cells [88]. Given its good MIF tautomerase inhibitory activity and effective inhibition of MIF biological properties *in vitro*, Z-590 was later investigated for its effect on lipopolysaccharide (LPS)-activated microglial cell activation [10]. As expected, Z-590 significantly decreased the production of LPS-induced nitric oxide (NO), TNF-α, interleukin (IL)-6, IL-1β, cyclooxygenase (COX-2), iNOS and ROS by inhibiting the MAPK signaling pathway in BV-2 microglial cells. Moreover, the anti-inflammatory effect of Z-590 was found to be dependent on MIF, as evidenced by using MIF knockdown BV-2 cells via siRNA transfection. Furthermore, Z-590 attenuated the neurotoxicity of activated microglia toward HT-22 hippocampal cells in a microglia-conditioned medium system, suggesting its potent neuroprotection for microglia-mediated neuroinflammation. Additionally, Z-590 also significantly suppressed paw edema, serum TNF-α level, IL-6 level, and spleen index, as well as markedly ameliorated joint inflammation and articular cartilage damage in an adjuvant-induced arthritis (AIA) rat model [93], which suggest a potential therapeutic treatment for rheumatoid arthritis.

Another new benzoic acid derivative, Z-312 [91], was recently explored for its effects on LPS-induced microglial activation and neuroinflammation *in vitro* and *in vivo* by our team [15]. Although Z-312 was 2.8-fold less potent in the inhibition of MIF dopachrome tautomerase activity than the reference compound ISO-1 (IC_{50}40.54 vs. 14.41 μM) [91], Z-312 significantly inhibited NO secretion with no cytotoxicity at the tested concentration and decreased the production of proinflammatory factors, including proinflammatory cytokines (TNF-α, IL-6, IL-1β, PGE2) and synthetic enzymes (iNOS and COX-2), in LPS-stimulated mi-

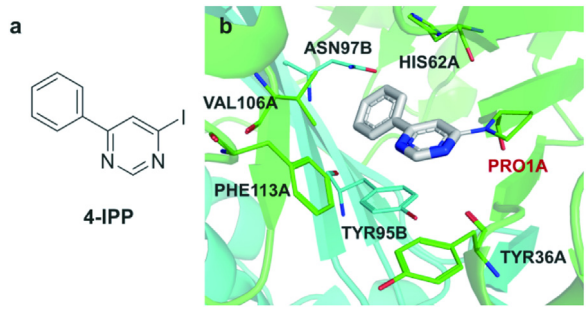

Fig. 6. 4-IPP bound to MIF. (a) Chemical structure of the covalent MIF inhibitor 4-IPP. (b) The X-ray cocrystal structure of 4-IPP with MIF (PDB code: 3B9S).

croglial cells [15]. Moreover, Z-312 inhibited LPS-induced NF-κB activation in microglial cells and suppressed MAPK signaling pathways in BV-2 cells. Additionally, Z-312 markedly protected MIF against proteolysis in the drug-affinity responsive target stability assay, indicating that Z-312 directly interacted with its target protein MIF. Z-312 also protected HT-22 neuroblastoma cells from activated microglia–induced cytotoxicity, suggesting its potential *in vitro* neuroprotective activity. Moreover, in an LPS-induced PD mouse model, Z-312 (20 mg/kg, i.p.) exhibited an *in vivo* neuroprotective effect against dopaminergic neuronal loss and microglial activation in male C57BL/6 mice [15].

Most recently, our group [16] discovered twelve new compounds with better MIF inhibitory activity than ISO-1 by an integrated virtual screening strategy. All of the compounds were predicted to cross the BBB by Qikprop with logBB values of −3.0 to 1.2. For compounds **5, 9, 11**, and **12** (Fig. 5), the IC_{50} values were as follows: 7.55 μM, 9.49 μM, 11.95 μM and 14.00 μM in the dopachrome tautomerase assay. Biological activity assays measuring NO release in LPS-primed BV-2 microglial cells exhibited good potency with IC_{50} values of 35.40, 28.32, 23.84, and 27.80 μM, whereas ISO-1 was 3-fold less potent (IC_{50} = 100.03 ± 2.00 μM). These four compounds were verified to have direct interactions with MIF using drug affinity responsive target stability (DARTS), and their anti-inflammatory effects were dependent on MIF. Compound **11** was then chosen for further characterization *in vitro*. Compound **11** was found to be a competitive inhibitor with a K_i value of 7.949 ± 0.8378 μM. It significantly inhibited the counterregulation of MIF on glucocorticoid-mediated immunosuppression in a dose-dependent manner in LPS-activated primary microglial cells and markedly decreased LPS-induced production of proinflammatory cytokines, including IL-6, TNF-α, iNOS, COX-2, and IL-1β, in BV-2 microglial cells by suppressing the activation of nuclear factor kappa B (NF-κB) and MAPKs. Additionally, compound **11** attenuated the neurotoxicity of microglial CM toward HT-22 neuroblastoma cells [16]. The potential applications of newly developed MIF inhibitors in neuroinflammatory CNS diseases are under investigation.

4.4. 4-IPP as a covalent MIF inhibitor

4-Iodo-6-phenylpyrimidine (4-IPP, Fig. 6) was discovered as a suicide substrate for MIF by a unique virtual screening strategy in 2008. LC–MS analysis and cocrystallization studies of MIF revealed that the phenyl-pyrimidine derivative 4-IPP bound covalently and irreversibly to MIF at the nitrogen of the catalytic NH_2-terminal PRO1A in the MIF tautomerase active site by deiodination at the 4-position of 4-IPP (Fig. 6b). Additionally, 4-IPP interacted with MIF through van der Waals interactions of the hydrophobic binding pocket, including residues TYR36A, HIS62A, VAL106A, PHE113A, TYR95B, and ASN97B. Moreover, although 4-IPP (IC_{50}: 0.2-0.5 μM) inhibited MIF dopachrome tautomerase activity with 10-fold less potency than ISO-1 in whole cell lysates, 4-IPP is approximately 5-10-fold more potent than ISO-1 for blocking MIF-dependent catalysis, lung adenocarcinoma cell migration,

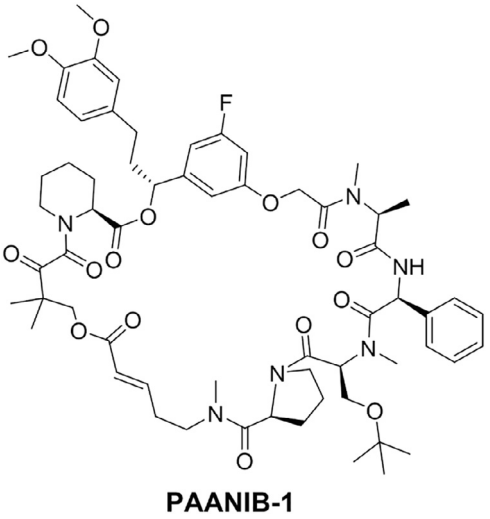

Fig. 7. Chemical structure of the MIF nuclease inhibitor PAANIB-1.

and anchorage-independent growth. The potential indication of 4-IPP in spinal cord injury was also investigated in an experimental rat model, and treatment with 8 μl 4-IPP (100 mM) in lesion sites markedly decreased the expression of CCL5, COX2, and mPGES-1 and the production of PGE_2 in astrocytes through activation of the CD74 receptor [94,95]. There was also a study showing that intrathecal injection of 4-IPP attenuated the expression of CNS injury-induced cholesterol-25-hydroxylase (CH25H) and *cyp27a1* mRNA in astrocytes [96]. All these results suggest that 4-IPP provides a novel mechanism and a potential therapeutic strategy for the repair of injured CNS.

4.5. MIF nuclease inhibitor PAANIB-1

In addition to modulation of tautomerase and cytokine activity, MIF was reported to have distinct nuclease activity (Fig. 1), which was involved in neuronal survival, including dopaminergic survival, in PD [3,5] (see more details in Section 2). A subsequent HTS assay was developed by the Dawson group to screen the rapafucin library containing 45,000 macrocyclic compounds in the search for MIF nuclease inhibitors. Among them, 4 individual compounds prominently inhibited nuclease activity, and neither inhibited MIF oxidoreductase activity nor its tautomerase activity. Subsequent structural optimizations identified PAANIB-1 (Fig. 7) as a first-in-class, potent, and selective MIF nuclease inhibitor with good bioavailability and brain permeability. PAANIB-1 did not disrupt the interaction of MIF with its substrate, and may directly bind to residues LEU61 and ASN72 that are distant from the nuclease active site, GLU21, to inhibit MIF nuclease activity without affecting tautomerase or cytokine activity. All these results indicate that MIF nuclease activity is quite distinct from its tautomerase activity, oxidoreductase activity, and cytokine activity.

PAANIB-1 was found to protect α-syn-PFF-induced synaptic dysfunction, reduction in NAD^+, and cell death, whereas MIF tautomerase inhibitors ISO-1, p-425, or Ibudilast failed to inhibit MIF nuclease activity [5]. Moreover, oral administration of PAANIB-1 (5 mg/kg) starting 1 month after the intrastriatal α-syn PFF injection resulted in significantly less loss of DA neurons, indicating the protective effect of the drug against neurodegeneration in PD. They further demonstrated that PAANIB-1 (doses of 5 and 15 mg/kg) also significantly prevented neurodegeneration in an α-synucleinopathy model induced by injection of AAV-human α-syn into the substantia nigra or in a typical chemical lesion animal model induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Collectively, these recent findings revealed that selectively targeting the nuclease activity of MIF may be a promising novel strategy for PD disease-modifying treatments.

5. Summary & perspectives

Since the discovery of MIF and its early misclassification as a T-cell-derived proinflammatory cytokine in 1966, the scientific community has made progress in understanding the functions and regulation of MIF in physiological and pathophysiological conditions. In addition to the immunomodulatory role in neuroinflammation [15,16], the regulation of neuroprotection and neuroplasticity has also attracted more attention. Moreover, the recently reported importance of the nuclease activity of MIF in dopaminergic survival may provide a novel angle for fully elucidating the functions and implications of MIF in the brain [5]. MIF has been regarded as a promising and potential biomarker for some CNS diseases. Extensive preclinical studies have also revealed a promising therapeutic role for a few neuropsychiatric disorders and neurodegenerative diseases. Some of them have already advanced to later stages of clinical trials.

In past decades, success in the discovery of a large and diverse catalog of MIF inhibitors can be attributed to the synergy of three strategies: drug repurposing, virtual screening, and high-throughput screening. Some of those drugs have progressed to various clinical stages in peripheral diseases. For CNS diseases, although a few compounds have been tested in experimental disease models in the CNS, only a limited number of candidate drugs have been processed into clinical trials for brain disorders, including MS, drug abuse, and ALS (Table 6). The gap may be attributed to the limitations of the BBB or the pharmacokinetic profile of compounds. However, it may rely more on understanding the functions and regulation of MIF in the brain. Similar to the recently identified nuclease activity of MIF as a promising target for PD [5], a full understanding of its function and regulation may shed light on the therapeutic potential of targeting MIF in CNS diseases. On the other hand, efforts to discover potent MIF tautomerase inhibitors have mainly focused on screening exercises without hit-to-lead optimization. In particular, further medicinal chemistry efforts combined with consideration of BBB permeability and safety and metabolic profiles in the brain to optimize the currently available MIF inhibitors with enhanced potency and selectivity are also highly anticipated to pave the way for the development of new CNS medications by selectively targeting MIF.

Nuclease activity is responsible for large-scale DNA fragmentation and thus parthanatic cell death. Given the importance of parthanatos in pathological development in a wide range of CNS disorders, the newly identified MIF nuclease inhibitor PAANIB-1 was proven to prevent neurodegeneration in multiple PD models [5]. MIF nuclease inhibitors may expand the scope of the clinical utility to other forms of neurodegeneration except for PD and deserve further study. At present, only rapamycin-inspired macrocycles have been identified as MIF nuclease inhibitors, and their co-crystallized structures binding to MIF remain to be elucidated. The rapid development of structural biology and computational methods and tools will facilitate the efficient identification of more potent and selective nuclease MIF inhibitors by using structure-based drug design and virtual screening once the MIF nuclease pocket is confirmed.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work.

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