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Synthesis and biological activity of novel MIF antagonists

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ABSTRACT

Two series of novel furan and indole compounds were synthesized and probed for inhibition of macrophage migration inhibitory factor (MIF) activity. Several compounds from both series inhibited the enzymatic activity of MIF at levels equal to or significantly better than ISO-1 (an early MIF inhibitor). The majority of the compounds that robustly inhibited the spontaneous secretion/release/recognition of MIF from freshly isolated human peripheral blood mononuclear cells were from the furan series (compounds **5**, **9**, **13**, **15**, and **16**). In contrast, compounds that markedly inhibited the MIF-induced production of pro-inflammatory cytokines were predominantly from the indole series (compounds **26, 29,** and **32**).

Macrophage migration inhibitory factor (MIF), a pro-inflammatory cytokine, plays a critical role in the pathogenesis of a variety of inflammatory and autoimmune diseases.[1,2] Therefore, a promising therapeutic approach to diminish pathological inflammation is to inhibit the production and/or biological activity of MIF. Given that MIF possesses enzymatic properties,[1,2] an earliest pharmacological strategy adopted to combat inflammation was to target the catalytic pocket of MIF.[1,2] Indeed, this approach has been extensively utilized by academia as well as industry to discover small molecule inhibitors of MIF enzymatic activity.[2–6] Interestingly, a recent study demonstrated that tautomerase null MIF protein is capable of binding to its receptor(s) and initiating MIF signal transduction pathway(s).[7] These results suggest that enzymatic activity of MIF is dispensable for its biological activity.[7] Accordingly, it is imperative to discover compounds that inhibit not only the enzymatic activity of MIF but also (more importantly) the biological function of MIF.[5] Previously, we have synthesized novel 1,2,4-oxadiazole, phthalimide, amide and other derivatives of ISO-1 (an early MIF inhibitor; Fig. 1) which not only suppress the enzymatic activity of MIF but also (more importantly) inhibit the MIF-induced production of interleukin-6 (IL-6) and/or interleukin-1β (IL-1β) significantly better than ISO-1.[6] In the aforementioned study,[6] we explored the structure–activity relationship of isoxazoline compounds wherein the main structural element was that of 4-hydroxy phenyl unit. With the objective of diversifying our chemistry, in this study, we replaced the 4-hydroxy phenyl unit with heterocycles such as furan and indole. The aforementioned heterocycles were chosen—in part—because of industrial interest.[8] Subsequently, we ascertained the potential of these novel compounds to inhibit MIF biological function.

The key intermediate (*E*,*Z*)-furan-3-carbaldehyde oxime (**2**), required for synthesis of different dihydro-isoxazoles, was obtained from furan-3-carbaldehyde (**1**). Compound **1** was treated with hydroxylamine hydrochloride in the presence of pyridine and ethanol (1:1) to afford **2** in 90% yield. Oxime **2** was treated with 1-fluoro-4-vinylbenzene in the presence of sodium hypochlorite in THF to yield compound **3**. With the objective of synthesizing various derivatives with aliphatic and cyclic substitutions, intermediate **2** was reacted with different alkenes [1-(trifluoromethyl)-4-vinylbenzene, 1-methoxy-4-vinylbenzene, styrene, vinylcyclohexane, but-3-en-1-ol, vinyl pivalate, dimethyl 2-methylenesuccinate, methyl methacrylate, 4-allylmorpholine, 1-vinylpyrrolidin-2-one, 2-vinylisoindoline-1,3-dione, 1-allyl-1*H*-indole] in the presence of sodium hypochlorite in THF to yield the desired products (**4–11** and **13–16**, respectively; Fig. 2). 3-(Furan-3-yl)-5-(methyl)-5-(1-methoxy-1-oxomethyl)-4,5-dihydroisoxazole (**11**) was treated

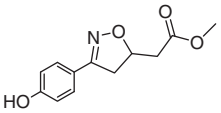

Figure 1. ISO-1: An early MIF inhibitor.

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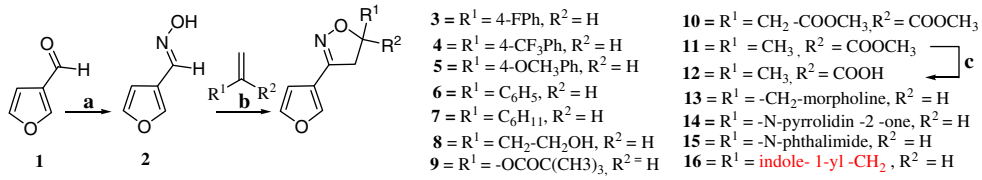

Figure 2. Synthesis of compounds **2**–**16**. Reagents and conditions: (a) $NH_2OH{\cdot}HCl/C_5H_5N/C_2H_5OH$, rt, 15–16 h, 90%; (b) different alkenes/THF, bleach, rt, 24–26 h, 10–90%; (c) compound **12** from **11**, $THF/MeOH/H_2O$, LiOH, rt, 3 h, 82%.

with lithium hydroxide monohydrate in THF, methanol and water to afford **12** (Fig. 2) in 82% yield.

As a first step towards synthesizing indole derivatives, compound **17** was treated with 1-bromo-3-(trifluoromethyl) benzene in the presence of cesium carbonate and copper iodide in DMF at 165 °C to obtain **18**. Subsequently, compound **18** was treated with phosphoryl chloride and DMF at 165 °C to obtain **19**. The key intermediate **20** was obtained by treating **19** with hydroxylamine hydrochloride in the presence of pyridine and ethanol (1:1). The intermediate **20** was reacted with different alkenes [1-fluoro-4-vinylbenzene, 1(trifluoromethyl)-4-vinylbenzene, 1-methoxy-4-vinylbenzene, styrene, vinylcyclohexane, but-3-en-1-ol, vinyl pivalate, dimethyl 2-methylenesuccinate, methyl methacrylate, 4-allylmorpholine, 1-vinylpyrrolidin-2-one, 2-vinylisoindoline-1, 3-dione, 1-allyl-1*H*-indole] in the presence of sodium hypochlorite in THF to yield indole derivatives (**21–29** and **31–34**, respectively) (Fig. 3). Compound **30** was obtained from **29** using a hydrolysis procedure analogous to the one utilized to obtain **12** (Fig. 3). The structures of various synthesized compounds were assigned on the basis of different spectral data (experimental details are provided in the Supplementary data section).

We investigated the MIF inhibitory potential of the synthesized compounds and, in parallel, compared their activity with ISO-1. Initially, dopachrome tautomerase assays were performed. Several compounds from furan series (e.g., **5, 6, 7, 13,** and **16**) and indole series (e.g**21, 22, 23, 24, 25, 26, 27, 28, 29, 31,** and **34**) consistently inhibited the dopachrome tautomerase activity of purified recombinant human MIF at levels equal to or better than that of ISO-1 (Table 1). Interestingly, compounds from the indole series inhibited MIF enzymatic activity to a greater extent in comparison to the furan series compounds (Table 1). Furthermore, the ester in indole (**29**) series but not in furan series (**11**) elicited robust inhibition of MIF enzymatic activity as compared to its corresponding acid (**30** and **12**, respectively). The findings with indole series compounds are consistent with prior literature reports.[9] Of note, compounds in which R^1 is indol-1-yl-CH_2 (e.g., **16** and **34**) were found to be potent inhibitors of MIF enzymatic activity (Table 1).

Given our earlier observations[5], we sought to find compounds that inhibit not only the enzymatic activity of MIF but also (more importantly) the biological function of MIF. Analogous to our recent studies[6], we investigated if these compounds inhibit the purified human MIF-induced expression of TNF-α, IL-6, and IL-1β from human peripheral blood mononuclear cells (hPBMCs). Pre-treatment of MIF with compounds from furan series (e.g., **4, 6,** and **11**) or indole series (e.g., **23, 25, 26, 29, 30, 31, 32, 33,** and **34**) led to a marked suppression in the induced production of pro-inflammatory cytokines which was at times comparable to-, and at times significantly better than-, ISO-1 (Table 2). Collectively, these results provide evidence that our novel MIF antagonists not only inhibit the enzymatic activity of MIF, but also, more importantly, inhibit the biological function of MIF.

Table 1
Activity of synthesized furan and indole series compounds in MIF dopachrome tautomerase assay

R^1	R^2	Furans		Indoles	
		Compd	% Inh.	Compd	% Inh.
4-FPh	H	**3**	41**	**21**	100*
4-CF_3Ph	H	**4**	41*	**22**	51
4-OCH_3Ph	H	**5**	72**	**23**	97*
C_6H_5	H	**6**	59	**24**	71
C_6H_{11}	H	**7**	54**	**25**	100*
CH_2–CH_2OH	H	**8**	23*	**26**	91*
$OCOC(CH_3)_3$	H	**9**	30**	**27**	100*
CH_2–$COOCH_3$	$COOCH_3$	**10**	47	**28**	76*
CH_3	$COOCH_3$	**11**	22*	**29**	66
CH_3	COOH	**12**	29*	**30**	17*
–CH_2-morpholine	H	**13**	56	**31**	100*
–*N*-Pyrrolidin-2-one	H	**14**	18*	**32**	10*
-*N*-Phthalimide	H	**15**	0*	**33**	47*
Indol-1-yl-CH_2	H	**16**	100*	**34**	100**

% Inh. indicates % inhibition of MIF tautomerase activity at 100 μM. ISO-1 shows 59% inhibition at 100 μM. Results presented are representative of three separate experiments carried out at 1, 10, 30, and 100 μM.
* Indicates p <0.05 compared to ISO-1.
** Indicates p = 0.05–0.10 compared to ISO-1.

Based on these experiments, it was evident that there was no one-to-one correlation between activity observed in MIF enzymatic assay and inhibition of pro-inflammatory cytokine production seen in MIF biological assay. Indeed, compound **16** was more potent than compound **4** in inhibiting the dopachrome tautomerase activity of MIF (Table 1); however, compound **4** inhibited MIF-induced production of pro-inflammatory cytokines to a greater extent than compound **16** (Table 2). Interestingly, the *N*-pyrroli-

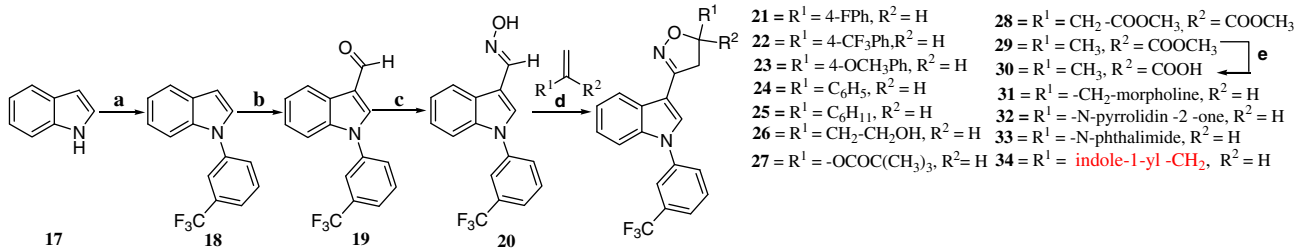

Figure 3. Synthesis of compounds **18**–**34**. Reagents and conditions: (a) 1-bromo-3-(trifluoromethyl) benzene, Cs_2CO_3, CuI, DMF, 165 °C, 3–4 h, 77%; (b) $POCl_3$, DMF, 165 °C, 2–3 h, 75%; (c) $NH_2OH{\cdot}HCl/C_5H_5N/C_2H_5OH$, rt, 15–16 h, 90%; (d) different alkenes/THF, bleach, rt, 24–26 h, 10–90%; (e) compound **30** from **29**, $THF/MeOH/H_2O$, LiOH, rt, 3 h, 78%.

Table 2
Activity of synthesized furan and indole series compounds in MIF-induced cytokine production assay in hPBMCs

R^1	R^2	Compd	Furans % Inh.			Compd	Indoles % Inh.		
			TNF-α	IL-1β	IL-6		TNF-α	IL-1β	IL-6
4-FPh	H	**3**	0*	47*	15	**21**	0*	0*	4**
4-CF_3Ph	H	**4**	76*	67*	50*	**22**	2*	15*	6**
4-OCH_3Ph	H	**5**	2.5	13	0	**23**	23	37	13
C_6H_5	H	**6**	50	31	14	**24**	0*	12**	9
C_6H_{11}	H	**7**	0*	15*	0*	**25**	27	43	19
CH_2–CH_2OH	H	**8**	11**	0*	7**	**26**	88*	88*	84*
–$OCOC(CH_3)_3$	H	**9**	0*	6*	1*	**27**	0*	15*	12
CH_2–$COOCH_3$	$COOCH_3$	**10**	4*	57**	60*	**28**	0*	23	4**
CH_3	$COOCH_3$	**11**	30	39	11	**29**	81*	89*	65*
CH_3	COOH	**12**	32	15**	7	**30**	39	54*	45*
–CH_2-morpholine	H	**13**	0*	32	49*	**31**	42	51*	36*
-*N*-Pyrrolidin-2-one	H	**14**	28	22*	49*	**32**	68*	68*	52*
-*N*-Phthalimide	H	**15**	26	12**	13	**33**	63*	43	24
Indol-1-yl-CH_2	H	**16**	30	19**	6	**34**	30	44	17

% Inh. indicates % inhibition of MIF-induced production of TNF-α, IL-1β or IL-6 at 100 μM. ISO-1 shows 35%, 34%, and 12% inhibition for TNF-α, IL-1β, and IL-6, respectively, at 100 μM. Results presented are representative of at least two experiments conducted at 10, 30, and 100 μM.
* Indicates p <0.05 compared to ISO-1,
** Indicates p = 0.05–0.10 compared to ISO-1.

din-2-one substituted compound in the indole series (compound **32**) inhibits MIF-induced production of pro-inflammatory cytokines to a greater extent than the corresponding compound in furan series (compound **14**) (Table 2). Similarly, the indole alcohol **26** is more active than corresponding compound **8** from the furan series (Table 2). In general, majority of the potent inhibitors of MIF-induced production of TNF-α, IL-6, and IL-1β were from the indole series (e.g., **26, 29,** and **32**). Within the indole series, N-substituted cyclic compounds (**32, 33**) appeared to show better inhibition of MIF biological function as compared to alkyls with cyclic substitution (**31, 34**) (Table 2).

In an earlier study, we had observed that an isoxazole series compound (a fluorinated analog of ISO-1, ISO-F) and a substituted quinoline series compound containing the furan moiety (7E) docked differentially in the active site of MIF.[5] Thus, given our findings in the MIF-induced cytokine production assay (Table 2), it would be of interest to ascertain the docking pattern of furan and indole series compounds. In particular, it would be interesting to determine whether the docking pattern is critically dependent on the heterocyclic core or on the aliphatic and cyclic substitutions. It is well-established that MIF can bind to CD74, CXCR2, and CXCR4.[10] However, the identity of the MIF receptor(s) which is (are) critically involved in MIF-induced production of pro-inflammatory cytokines is currently unknown. Preliminary investigations in our laboratory suggest that CD74 is not the principal MIF receptor mediating MIF-induced production of pro-inflammatory cytokines in hPBMCs (Dagia et al., manuscript in preparation). It would, thus, be of interest to ascertain if the furan and indole series compounds affect the MIF binding to CXCR2 and/or CXCR4.

The synthesized compounds were also evaluated for their potential to inhibit the secretion/release/recognition of MIF from hPBMCs. Several compounds from the furan series (e.g., **5, 9, 13, 15,** and **16**) inhibited the secretion/release/recognition of MIF from hPBMCs at levels equal to or better than ISO-1 (Table 3). Interestingly, none of the synthesized compounds from indole series potently inhibited the secretion/release/recognition of MIF from hPBMCs.

In the aforementioned assay, it was found that the acid from the indole (**30**) series showed relatively more (albeit marginal) MIF inhibition than the corresponding ester (**29**). Of note, these observations are in direct contrast to our findings in the dopachrome tautomerase assay (Table 1). The secretion of MIF from THP-1 cells is known to occur via a non-classical pathway.[7] Reagents modulating the ABC transporter, but not protein synthesis inhibitors such as cycloheximide, are known to inhibit the secretion of MIF.[7] Whether ISO-1 and **5**, **9**, **13**, **15,** and **16** work in a similar manner and inhibit the secretion of MIF by affecting the ABC transporter is currently unknown and warrants further investigation. However, the data generated from our recent study[5] and earlier studies[11] are consistent with the hypothesis that the inhibitory activity of these aforementioned compounds in cell-based ELISA assays is, at least in part, due to these compounds binding directly to MIF and preventing its recognition. Since several of these compounds (e.g., **5**, **9**, **13**, **15**, and **16**) showed equivalent or better MIF inhibition potential in cell-based ELISA assays (in comparison to ISO-1), it would be of interest to determine their ability to interact with MIF in comparison to ISO-1. These studies are currently ongoing in our laboratory. Most importantly, it would be of interest to assess the efficacy of one or more of these MIF inhibitors (e.g., **4**, **26**, **29**, and **32**) in a MIF-dependent model of experimental inflammation.

Table 3
Activity of synthesized furan and indole series compounds in MIF ELISA assays using hPBMCs

R^1	R^2	Furans		Indoles	
		Compd	% Inh.	Compd	% Inh.
4-FPh	H	**3**	51*	**21**	0*
4-CF_3Ph	H	**4**	60*	**22**	5*
4-OCH_3Ph	H	**5**	82**	**23**	1*
C_6H_5	H	**6**	68*	**24**	2*
C_6H_{11}	H	**7**	35*	**25**	22*
CH_2-CH_2OH	H	**8**	58*	**26**	0*
–$OCOC(CH_3)_3$	H	**9**	86	**27**	5*
CH_2–$COOCH_3$	$COOCH_3$	**10**	0*	**28**	10*
CH_3	$COOCH_3$	**11**	0*	**29**	0*
CH_3	COOH	**12**	21*	**30**	14*
–CH_2-morpholine	H	**13**	78**	**31**	0*
-*N*-Pyrrolidin-2-one	H	**14**	36*	**32**	0*
-*N*-Phthalimide	H	**15**	83	**33**	0*
Indol-1-yl-CH_2	H	**16**	95*	**34**	8*

% Inh. indicates % inhibition of secretion/release/recognition of MIF at 100 μM. ISO-1 shows 87% inhibition at 100 μM. Results presented are representative of at least two experiments carried out at 3, 10, 30, and 100 μM.
* Indicates p <0.05 compared to ISO-1.
** Indicates p = 0.05–0.10 compared to ISO-1.

In conclusion, we have synthesized novel furan and indole compounds which inhibit the biological activity of MIF.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.127.

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