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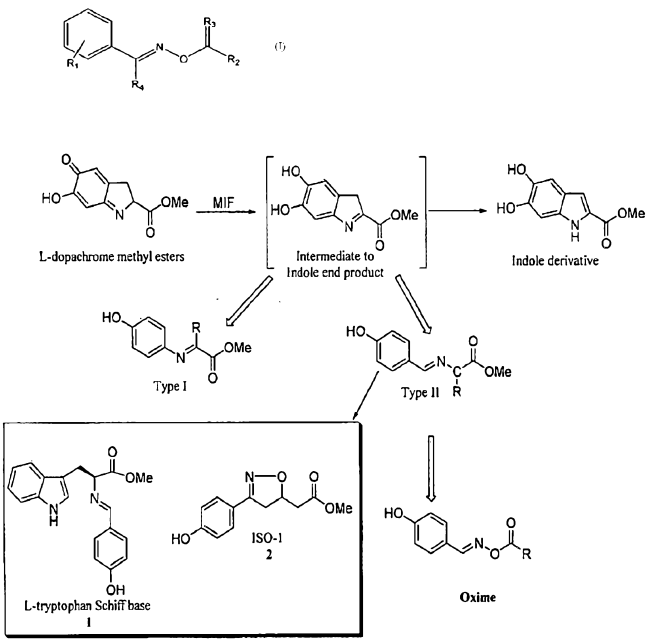

(57) Abstract: Provided are compounds of formula (I) and other compounds. Also provided are pharmaceutical compositions comprising these compounds. Additionally, methods of inhibiting macrophage migration inhibitory factor (MIF) activity in a mammal are provided, as are methods of treating or preventing inflammation in a mammal, and methods of treating a mammal having sepsis, septicemia, and/or endotoxic shock.

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OXIME DERIVATIVES AS INHIBITORS OF MACROPHAGE MIGRATION INHIBITORY FACTOR

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 60/811,258 filed June 5, 2006.

BACKGROUND OF THE INVENTION

(1) Field of the Invention

The present invention generally relates to cytokine inhibitors. More specifically, the invention is directed to inhibitors of macrophage migration inhibitory factor.

(2) Description of the Related Art

Sepsis, a potentially lethal systemic inflammatory reaction to infection, affects approximately 700,000 individuals and kills more than 215,000 people annually at a cost of $16.7 billion nationally (Martin et al., 2003). While the incidence of sepsis continues to rise (O'Brien and Abraham, 2003), to date, no small molecule therapeutic agent is currently approved by the FDA for its clinical management. Thus, severe sepsis is a common, expensive, and frequently fatal condition, with as many deaths annually as those from acute myocardial infarction (Angus et al., 2001).

Sepsis is mediated, at least in part, by soluble factors. Among these, macrophage migration inhibitory factor (MIF) has been shown to play a critical role in inflammation pathways. The biology of MIF places it in the macrophage-derived pathways of pro-inflammatory responses (Bridhuizen et al., 2001; Lue et al., 2002; Calandra, 2000; 2001). MIF was first described in the early 1960s, as a product of activated lymphocytes that inhibited the random movement or migration of cultured monocytes/macrophages (George and Vaughan, 1962; Bloom and Bennett, 1966; David, 1966). This discovery engendered significant interest, as MIF was one of the first soluble, non-immunoglobulin factors that was amenable to study *in vitro*.

MIF, produced by numerous cell types, including immune and endocrine cells, is now recognized as a pro-inflammatory counter-regulator of the anti-inflammatory activities of the glucocorticoids. *In vitro*, MIF expression abrogates the anti-inflammatory and immunosuppressive effect of glucocorticoid production on pro-inflammatory cytokines (TNF-α, IL-1, IL-2, IL-6, and IL-8) (Calandra and Bucala, 1997; Donnelly et al., 1997). In mice, administration of recombinant MIF, together with dexamethasone, completely blocks the

protective effects of dexamethasone on LPS lethality (Calandra, 1995). MIF is critically involved in the pathogenesis of a variety of inflammatory diseases. In particular, animal models of Gram-positive, Gram-negative, and polymicrobial sepsis, as well as MIF knockout models, indicate a critical role of MIF in sepsis (Calandra et al., 2000; Bozza et al., 1999; Bernhagen et al., 1993). Thus, the numerous pro-inflammatory effects of MIF together with its unique ability to override or counter-regulate the normal physiological inhibition of immune cell activation and pro-inflammatory cytokine cascades by glucocorticoids, position MIF as a critical mediator of sepsis.

In vivo studies demonstrate that MIF is an important late-acting mediator of systemic inflammation. Deletion of the MIF gene in mice conferred protection against lethal endotoxemia staphylococcal toxic shock (Bozza et al., 1999). In addition, administration of neutralizing MIF-antibody protected mice from: (a) LPS-induced lethality; (b) lethal peritonitis and septic shock induced by *E. coli* peritonitis and (c) fulminant septic shock induced by cecal ligation and puncture (CLP) in TNF-α deficient mice (Calandra, 2001; Bernhagen et al., 1993). In contrast to early mediators such as TNF-α and IL-1β, MIF release peaks and then plateaus 5 hours after the onset of CLP, thereby offering a window of opportunity for therapeutic treatment. Consequently, anti-MIF therapies are potentially more beneficial than anti-TNF-α and anti-IL-1 therapies, which have demonstrated limited benefits for patients with severe sepsis (Abraham, 1999). In contrast, administration of anti-MIF antibody 8 hours post-induction of sepsis confers significant protection in a murine CLP model of sepsis versus animals receiving control IgG. Human studies also support a role for MIF in septic shock (Beishuizen et al., 2001; Calandra et al., 2000). A correlation has been documented between the severity of injury or infection in trauma patients and MIF levels in the serum, with increased circulating levels of MIF displayed in patients with severe sepsis (6-fold) and in patients with septic shock (15-fold) (Calandra et al., 2000). Taken together, these results suggest that an MIF antagonist will prove to be a potent anti-inflammatory agent, acting both by neutralizing the direct inflammatory activity of MIF and by restoring the anti-inflammatory benefits of endogenous or administered corticosteroids.

Three-dimensional X-ray crystallographic studies have shown that MIF appears as a homotrimer (Suzuki et al., 1994; Taylor et al., 1999; Sugimoto et al., 1995; Kato et al., 1996; Lolis and Bucala, 1996; Sugimoto et al., 1996; Sun et al., 1996; Suzuki et al., 1996; Lubetsky et al., 1999; Orita et al., 2001; Lubetsky et al., 2002). MIF possesses the unusual ability to catalyze the tautomerization of D,L-dopachrome methyl esters into their corresponding indole derivatives (Rosengren et al., 1996). More recently, phenylpyruvic acid and *p*-hydroxyphenylpyruvic acid were found to be MIF substrates (Matsunaga et al., 1999a; Rosengren et al., 1997; Matsunaga et al., 1999b). The crystal structures of MIF complexed with *p*-hydroxyphenylpyruvic acid has identified an active site which lies in a hydrophobic cavity that forms between two adjacent

subunits of the homotrimer (Lubetsky et al., 1999). Proline (Pro-1 of the active site) appears to be the critical residue for enzymatic activity, since site-directed mutagenesis that substitutes a serine (P1-s) or glycine (P1-g) for Pro-1 results in mutants devoid of D-dopachrome and *p*-hydroxy-phenylpyruvic acid tautomerase activity (Lubetsky et al., 1999; Bendrat et al., 1997; Swope et al., 1998).

A correlation between tautomerase catalytic activity and MIF's cytokine activities is supported by several studies where the structure and tautomerization kinetics of homologues of human MIF from a parasitic nematode, *Brugia malayi (Bm)*, were characterized. *Bm* MIF mutant P1-g is 10-fold less active in inducing production of TNF-α and chemotactic activity of human macrophages compared to the parent *Bm* MIF and human MIF (Zang et al., 2002). In addition, the P1-g mutant is greatly impaired in its ability to stimulate superoxide generation in activated neutrophils (Swope et al., 1998). Also a P1-a mutant of MIF, which loses the tautomerase activity, loses its ability to enhance matrix metalloproteinase (MMP) mRNA levels (Onodera et al., 2000). However, a mutation in the Pro-1 region alone is not sufficient to abolish the glucocorticoid counter-regulation activity and monocyte chemotaxis inhibition (Bendrat et al., 1997; Hermanowski-Vosatka et al., 1999), and truncated MIF mutants also indicate a role for the carboxy terminus in MIF binding/activity (Kleemann et al., 2000; Mischke et al., 1997).

Activation of macrophages is an early step in inflammation and leads to an increase of pro-inflammatory cytokines, such as TNF, further resulting in tissue damage. MIF was initially, as the name suggests, shown to regulate macrophage migration. However, more recent studies have shown that a major activity of MIF is its ability to suppress the anti-inflammatory steroid response. Thus, the rationale for MIF as a therapeutic target is that blocking MIF attenuates the inflammatory cascade in sepsis and endotoxemia and improves the survival rate.

In several studies, administration of neutralizing anti-MIF antibody protects mice: (a) from LPS-induced lethality; (b) against lethal peritonitis and septic shock induced by *E. coli* peritonitis and (c) against lethal sepsis induced by cecal ligation (CLP) and puncture in TNF-α deficient mice.

The compound (*S,R*)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1) was recently designed as an inhibitor of MIF activity (PCT Publication WO 02/100332). The crystal structure of MIF complexed to ISO-1 revealed that it binds to a hydrophobic pocket. *In vitro*, ISO-1 inhibits 60% of TNF release by LPS-treated macrophages. *In vivo*, intraperitoneal administration of ISO-1 at 40 mg/kg increased the survival rate in endotoxemia and sepsis (Al-Abed et al., 2005). These results are comparable with monoclonal anti-MIF antibodies for the treatment of septic animals.

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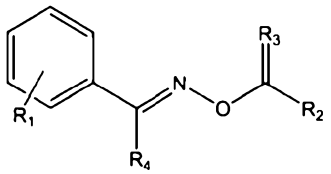
The ISO-1 structure incorporates the structure of Schiff base inhibitors of MIF enzyme activity that were designed originally to mimic the structure of dopachrome tautomerization intermediates of MIF catalysis. While ISO-1 has moderate anti-inflammatory activity, synthesis of a focused library around the ISO-1 structure alone did not significantly improve MIF inhibitor activity. Thus, new molecular scaffolds are required to identify additional MIF inhibitors.

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

SUMMARY OF THE INVENTION

According to a first aspect, the present invention provides a compound of formula I:



I , or a pharmaceutically acceptable salt, ester, or tautomer thereof, wherein

R_1 is a multiple substitution, independently OH or a halogen, wherein a first R1 is OH and a second R1 is a halogen;

R_2 comprises a ring structure in which an atom in the ring structure is bound to the carbon that is bound to R_3;

R_3 is O, $C(R_5)_2$, or S; and

R_4 is H, R_5, or a halogen, wherein

R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy.

According to a second aspect, the present invention provides apharmaceutical composition comprising the compound of the first aspect, or a pharmaceutically acceptable salt, ester, or tautomer thereof, in a pharmaceutically acceptable excipient.

According to a third apsect, the present invention provides a method of inhibiting macrophage migration inhibitory factor (MIF) activity in a mammal, the method comprising administering any one of the compounds of the first aspect or the pharmaceutical composition of the second aspect to the mammal in an amount effective to inhibit MIF activity in the mammal.

According to a fourth aspect, the present invention provides a method of treating or preventing inflammation in a mammal, the method comprising administering any one of the compounds of the first

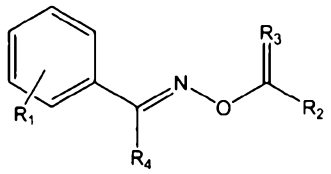
aspect or the pharmaceutical composition of the second aspect to the mammal in an amount effective to treat or prevent the inflammation in the mammal.

According to a fifth aspect, the present invention provides a method of treating a mammal having sepsis, septicemia, and/or endotoxic shock or at risk for sepsis, septicemia, and/or endotoxic shock, the method comprising administering any one of the compounds of the first aspect or the pharmaceutical composition of the second aspect to the mammal in an amount sufficient to treat or prevent the sepsis, septicemia and/or endotoxic shock.

According to a sixth aspect, the present invention provides a use of any one of the compounds of the first aspect or the pharmaceutical composition of the second aspect, for (i) inhibiting macrophage migration inhibitory factor (MIF) activity in a mammal, (ii) the preparation of a medicament for inhibiting macrophage migration inhibitory factor (MIF) activity in a mammal; (iii) treating or preventing inflammation in a mammal, (iv) the preparation of a medicament for treating or preventing inflammation in a mammal, (v) the treatment or prevention of sepsis, septicemia, and/or endotoxic shock in a mammal having or at risk for sepsis, septicemia, and/or endotoxic shock, or (vi) the preparation of a medicament for the treatment or prevention of sepsis, septicemia, and/or endotoxic shock in a mammal having or at risk for sepsis, septicemia, and/or endotoxic shock.

Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

The invention is additionally directed to a compound of formula I:



I , or a pharmaceutically acceptable salt, ester of tautomer thereof,

wherein

R_1 is a single or multiple substitution independently H, OH, R_5, $N(R_5)$, SR_5, or a holgen, wherein at least one substitution is a halogen;

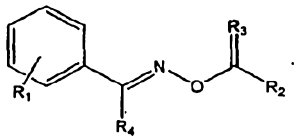
R_2 comprises a ring structure in which an atom in the ring structure is bound to the carbon that is bound to R_3;

R_3 is O, $C(R_5)_2$, or S; and

R_4 is H, R_5, or a halogen, wherein

R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy. The invention also encompasses pharmaceutical compositions comprising any of these compounds.

Also, the invention is directed to a compound of formula I:



I , or a pharmaceutically acceptable salt, ester, or tautomer thereof,

where

R_1 is a single or multiple substitution independently H, OH, R_5, $N(R_5)$, SR_5, or a halogen;

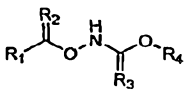
R_2 is *para*-hydroxymethylphenyl;

R_3 is O, $C(R_5)_2$, or S; and

R_4 is H, R_5, or a halogen, wherein

R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy.

The invention is further directed to a compound of formula III



III

where

R_1 comprises a ring structure in which an atom in the ring structure is bound to the carbon that is bound to R_2;

R_2 and R_3 are independently O, $C(R_5)_2$, or S;

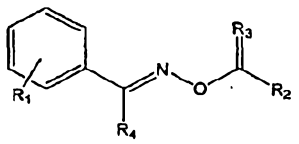
R_4 is a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy;

R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy. The invention also encompasses pharmaceutical compositions comprising any of these compounds.

Also, the invention is directed to methods of inhibiting macrophage migration inhibitory factor (MIF) activity in a mammal. The methods comprise administering any of the above-

identified pharmaceutical compositions to the mammal in an amount effective to inhibit MIF activity in the mammal.

Additionally, the invention is directed to other methods of inhibiting macrophage migration inhibitory factor (MIF) activity in a mammal. The methods comprise administering a pharmaceutical composition to the mammal in an amount effective to inhibit MIF activity in the mammal. In these methods, the pharmaceutical composition comprises a compound of formula I or formula II, or a pharmaceutically acceptable salt, ester, or tautomer thereof, in a pharmaceutically acceptable excipient, where formula I and formula II are



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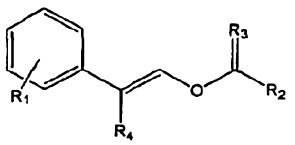


II ,

where

R_1 is a single or multiple substitution independently H, OH, R_5, $N(R_5)$, SR_5, or a halogen;

R_2 comprises a ring structure in which an atom in the ring structure is bound to the carbon that is bound to R_3;

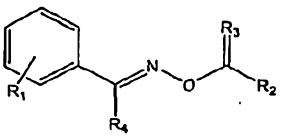
R_3 is O, $C(R_5)_2$, or S;

R_4 is H, R_5, or a halogen, where

R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C2-C6 alkoxy.

Further, the invention is directed to methods of treating or preventing inflammation in a mammal. The methods comprise administering any of the above-identified the pharmaceutical compositions to the mammal in an amount effective to treat or prevent the inflammation in the mammal.

The invention is also directed to other methods of treating or preventing inflammation in a mammal. The methods comprise administering a pharmaceutical composition to the mammal in an amount effective to treat or prevent the inflammation in the mammal, where the pharmaceutical composition comprises a compound of formula I or formula II, or a pharmaceutically acceptable salt, ester, or tautomer thereof, in a pharmaceutically acceptable excipient. Formula I and formula II are



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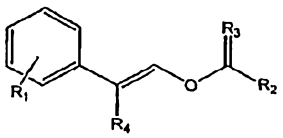


II ,

where

R_1 is a single or multiple substitution independently H, OH, R_5, $N(R_5)$, SR_5, or a halogen;

R_2 comprises a ring structure in which an atom in the ring structure is bound to the carbon that is bound to R_3;

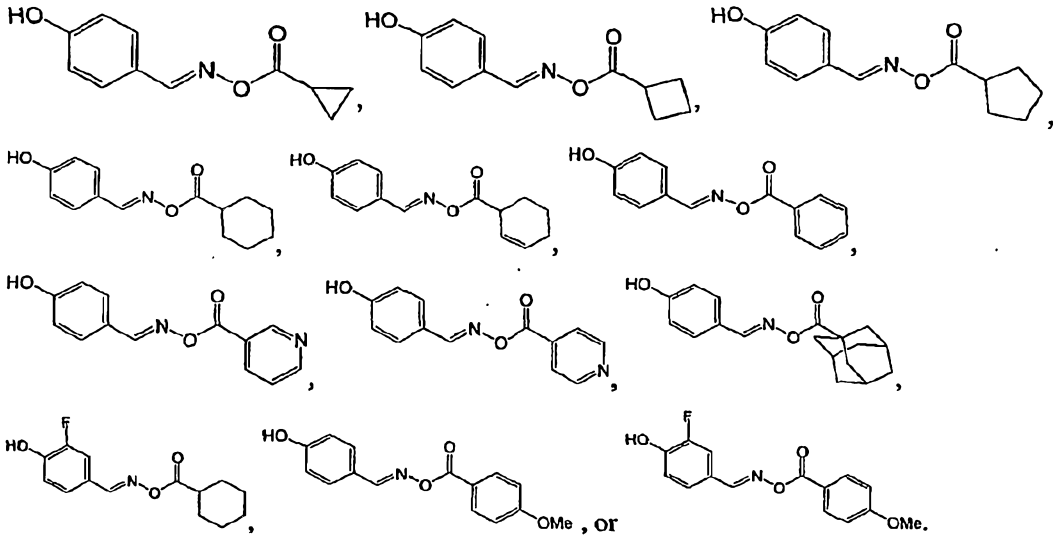
R_3 is O, $C(R_5)_2$, or S;

R_4 is H, R_5, or a halogen, where

R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy.

Additionally, the invention is directed to methods of treating a mammal having sepsis, septicemia, and/or endotoxic shock. The methods comprise administering any of the above-identified pharmaceutical compositions to the mammal in an amount sufficient to treat the sepsis, septicemia and/or endotoxic shock.

The invention is further directed to additional methods of treating a mammal having sepsis, septicemia, and/or endotoxic shock. The methods comprise administering a compound to the mammal in an amount sufficient to treat the sepsis, septicemia and/or endotoxic shock, where the compound is



BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the rationale for the synthesis of the Cyc-Oxi oxime compounds.

FIG. 2 shows three inhibitors of macrophage migration inhibitory factor (MIF).

FIG. 3 is a graph of experimental results establishing that Cyc-Oxi-11 suppresses the ability of MIF to regulate glucocorticoids in LPS-treated macrophages. Briefly, monocyte-derived macrophages from human peripheral blood were preincubated with dexamethasone (10^{-9})

or dexamethasone plus MIF (3 nM purified native MIF) and various concentrations of Cyc-Oxi-11 (0, 0.01, 0.1 and 1 mM) before the addition of 0.5 μg/ml lipopolysaccharide (LPS). TNF-α production was then measured. The data shown are mean ±SD of triplicate wells in experiments that were repeated twice.

FIG. 4 is a graph of experimental results establishing that Cyc-Oxi-11 inhibits MIF induction of TNF release from LPS-stimulated macrophages. Briefly, monocyte-derived macrophages from human peripheral blood were pre-treated with various concentrations of Cyc-Oxi-11 10 minutes prior to the addition of 0.5 μg/ml (LPS). TNF-α production was then measured. The data shown are mean ±SD of triplicate wells in experiments that were repeated twice.

FIG. 5 is a graph of the kinetics of MIF appearance in the serum post CLP surgery.

FIG. 6 is a graph showing that Cyc-Oxi-11 is protective even when given 24 h after the induction of sepsis.

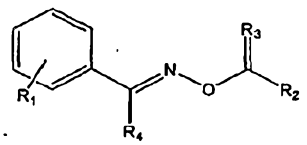
FIG. 7 shows the synthesis and activity of compounds **3a-3h**. IC_{50} represents the inhibition of MIF tautomerase activity.

FIG. 8 shows the synthesis and activity of compounds **4-5**. IC_{50} represents the inhibition of MIF tautomerase activity.

DETAILED DESCRIPTION OF THE INVENTION

As described in the Example, the inventors have identified compounds that inhibit MIF. These compounds are useful for treating or preventing inflammation in mammals.

Thus, the invention is directed to compounds of formula I:



I ,

where

R_1 is a single or multiple substitution independently H, OH, R_5, $N(R_5)$, SR_5, or a halogen;

R_2 comprises a ring structure in which an atom in the ring structure is bound to the carbon that is bound to R_3;

R_3 is O, $C(R_5)_2$, or S; and

R_4 is H, R_5, or a halogen, where

R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy.

Preferably, R_1 is H, OH or a halogen. More preferably, R_1 is OH. In the most preferred embodiments where R_1 a single substitution, R_1 is OH in the para position. Unless otherwise designated herein, the ortho, meta, or para designations of R_1 substituents of compounds of formula I designate the positions of the substituents in relation to the oxime (C=N-O-) moiety.

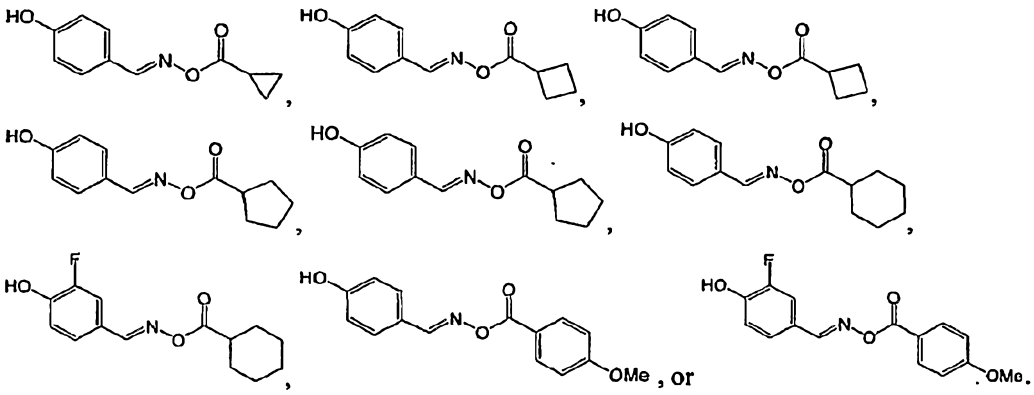
Where R_1 is multiple substitutions, one R_1 is preferably OH, most preferably in the para position, and the second substitution is preferably a halogen, more preferably fluorine. Most preferably this second substitution is a fluorine in the meta position.

It is also preferred that R_2 comprises a 3-, 4-, 5- or 6-membered alicyclic, heterocyclic or aromatic ring. When R_2 is an alicyclic ring, the most preferred rings are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexyl-2,3-ene, or 1-adamantyl. When R_2 is a heterocyclic ring, the most preferred rings are pyrimidine, pyridazine, pyrazine, pyridine, pyrazole, imidazole, pyrrole, pyran or furan rings. When R_2 is an aromatic ring, the most preferred rings are cyclobenzyl, 4-pyrimidyl, 3-pyrimidyl, or *para*-hydroxymethylphenyl (the latter as in compounds 4 and 5 of FIG. 8).

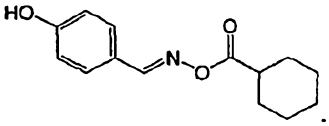
The ring structure of R_2 can comprise more than one ring, and/or be substituted or unsubstituted. When the ring structure is substituted, it is preferably substituted with at least one straight or branched C_1-C_6 alkyl, straight or branched C_1-C_6 alkenyl, straight or branched C_1-C_6 alkanoyl, straight or branched C_1-C_6 alkoxy, keto, carboxy, nitro, amino, hydroxy, halogen, cyano, diazo, thio, or hydroxyamino. More preferred substitutions on R_2 are at least one nitro, amino, hydroxyl or halogen.

Preferably, R_3 is O. It is also preferred that R_4 is H. In the most preferred compounds, R_3 is O and R_4 is H. Within those most preferred compounds, R_1 is preferably OH, most preferably in the para position. Also within the most preferred compounds where R_3 is O and R_4 is H, R_2 most preferably cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexyl-2,3-ene, cyclobenzyl, 4-pyrimidyl, 3-pyrimidyl, 1-adamantyl, or methoxyphenyl.

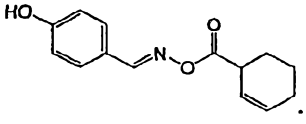
Specific preferred compounds comprise



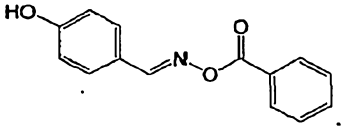
More preferably, the compound comprises, or consists of



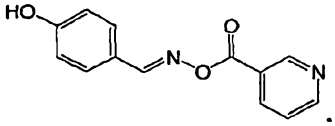
. Other preferred compounds of the present invention comprise, or consist of



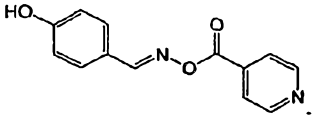
. Still other preferred compounds of the present invention comprise, or consist of



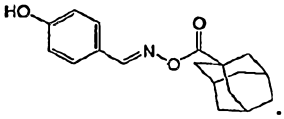
. Additional preferred compounds comprise, or consist of



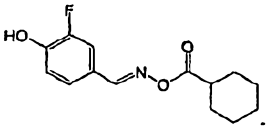
. Further preferred compounds comprise, or consist of



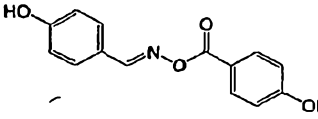
. Still additional preferred compounds comprise, or consist of



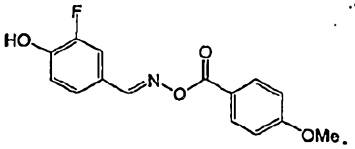
. Further additional preferred compounds comprise, or consist of



. Still further additional preferred compounds comprise, or consist of



. Additional preferred compounds comprise, or consist of



The above-described compounds are useful as inhibitors of macrophage migration inhibitory factor (MIF). Thus, the invention is also directed to pharmaceutical compositions

comprising any of the above compounds, or a pharmaceutically acceptable salt, ester, or tautomer thereof, in a pharmaceutically acceptable excipient.

By "pharmaceutically acceptable" it is meant a material that (i) is compatible with the other ingredients of the composition without rendering the composition unsuitable for its intended purpose, and (ii) is suitable for use with subjects as provided herein without undue adverse side effects (such as toxicity, irritation, and allergic response). Side effects are "undue" when their risk outweighs the benefit provided by the composition. Non-limiting examples of pharmaceutically acceptable carriers include, without limitation, any of the standard pharmaceutical carriers such as phosphate buffered saline solutions, water, emulsions such as oil/water emulsions, microemulsions, and the like.

The above-described compounds can be formulated without undue experimentation for administration to a mammal, including humans, as appropriate for the particular application. Additionally, proper dosages of the compositions can be determined without undue experimentation using standard dose-response protocols.

Accordingly, the compositions designed for oral, lingual, sublingual, buccal and intrabuccal administration can be made without undue experimentation by means well known in the art, for example with an inert diluent or with an edible carrier. The compositions may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the pharmaceutical compositions of the present invention may be incorporated with excipients and used in the form of tablets, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gums and the like.

Tablets, pills, capsules, troches and the like may also contain binders, recipients, disintegrating agent, lubricants, sweetening agents, and flavoring agents. Some examples of binders include microcrystalline cellulose, gum tragacanth or gelatin. Examples of excipients include starch or lactose. Some examples of disintegrating agents include alginic acid, cornstarch and the like. Examples of lubricants include magnesium stearate or potassium stearate. An example of a glidant is colloidal silicon dioxide. Some examples of sweetening agents include sucrose, saccharin and the like. Examples of flavoring agents include peppermint, methyl salicylate, orange flavoring and the like. Materials used in preparing these various compositions should be pharmaceutically pure and nontoxic in the amounts used.

The compounds can easily be administered parenterally such as for example, by intravenous, intramuscular, intrathecal or subcutaneous injection. Parenteral administration can be accomplished by incorporating the compounds into a solution or suspension. Such solutions or suspensions may also include sterile diluents such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents. Parenteral

formulations may also include antibacterial agents such as for example, benzyl alcohol or methyl parabens, antioxidants such as for example, ascorbic acid or sodium bisulfite and chelating agents such as EDTA. Buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose may also be added. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

Rectal administration includes administering the compound, in a pharmaceutical composition, into the rectum or large intestine. This can be accomplished using suppositories or enemas. Suppository formulations can easily be made by methods known in the art. For example, suppository formulations can be prepared by heating glycerin to about 120° C., dissolving the composition in the glycerin, mixing the heated glycerin after which purified water may be added, and pouring the hot mixture into a suppository mold.

Transdermal administration includes percutaneous absorption of the composition through the skin. Transdermal formulations include patches (such as the well-known nicotine patch), ointments, creams, gels, salves and the like.

The present invention includes nasally administering to the mammal a therapeutically effective amount of the compound. As used herein, nasally administering or nasal administration includes administering the compound to the mucous membranes of the nasal passage or nasal cavity of the patient. As used herein, pharmaceutical compositions for nasal administration of the compound include therapeutically effective amounts of the compound prepared by well-known methods to be administered, for example, as a nasal spray, nasal drop, suspension, gel, ointment, cream or powder. Administration of the compound may also take place using a nasal tampon or nasal sponge.

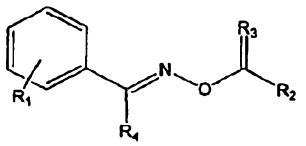
Where the compound is administered peripherally such that it must cross the blood-brain barrier, the compound is preferably formulated in a pharmaceutical composition that enhances the ability of the compound to cross the blood-brain barrier of the mammal. Such formulations are known in the art and include lipophilic compounds to promote absorption. Uptake of non-lipophilic compounds can be enhanced by combination with a lipophilic substance. Lipophilic substances that can enhance delivery of the compound across the nasal mucus include but are not limited to fatty acids (e.g., palmitic acid), gangliosides (e.g., GM-l), phospholipids (e.g., phosphatidylserine), and emulsifiers (e.g., polysorbate 80), bile salts such as sodium deoxycholate, and detergent-like substances including, for example, polysorbate 80 such as Tween™, octoxynol such as Triton™ X-100, and sodium tauro-24,25-dihydrofusidate (STDHF). See Lee et al., Biopharm., April 1988 issue:3037.

In particular embodiments of the invention, the compound is combined with micelles comprised of lipophilic substances. Such micelles can modify the permeability of the nasal

membrane to enhance absorption of the compound. Suitable lipophilic micelles include without limitation gangliosides (e.g., GM-1 ganglioside), and phospholipids (e.g., phosphatidylserine). Bile salts and their derivatives and detergent-like substances can also be included in the micelle formulation. The compound can be combined with one or several types of micelles, and can further be contained within the micelles or associated with their surface.

Alternatively, the compound can be combined with liposomes (lipid vesicles) to enhance absorption. The compound can be contained or dissolved within the liposome and/or associated with its surface. Suitable liposomes include phospholipids (e.g., phosphatidylserine) and/or gangliosides (e.g., GM-1). For methods to make phospholipid vesicles, see for example, U.S. Patent 4,921,706 to Roberts et al., and U.S. Patent 4,895,452 to Yiournas et al. Bile salts and their derivatives and detergent-like substances can also be included in the liposome formulation.

The invention is additionally directed to a compound of formula I:



I , or a pharmaceutically acceptable salt, ester, or tautomer thereof, wherein

R_1 is a single or multiple substitution independently H, OH, R_5, $N(R_5)$, SR_5, or a halogen, wherein at least one substitution is a halogen;

R_2 comprises a ring structure in which an atom in the ring structure is bound to the carbon that is bound to R_3;

R_3 is O, $C(R_5)_2$, or S; and

R_4 is H, R_5, or a halogen, wherein

R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy. The invention also encompasses pharmaceutical compositions comprising any of these compounds.

Preferably, R_1 is a multiple substitution comprising OH and a halogen. More preferably R_1 comprises OH in the para position. It is also preferred that the halogen substitution is a fluorine. More preferably, the fluorine is in the meta position.

It is also preferred that R_2 comprises a 3-, 4-, 5- or 6-membered alicyclic, heterocyclic or aromatic ring. More preferably, the ring of R_2 is alicyclic. Most preferred alicyclic rings are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexyl-2,3-ene, and 1-adamantyl.

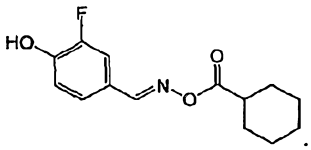
Some of the preferred rings of R_2 are heterocyclic. A preferred heterocyclic ring is *para*-hydroxymethylphenyl. Other preferred heterocyclic rings are pyrimidine, pyridazine, pyrazine, pyridine, pyrazole, imidazole, pyrrole, pyran and furan.

Others of the preferred rings of R_2 are aromatic. Most preferably the aromatic ring is cyclobenzyl, 4-pyrimidyl, or 3-pyrimidyl.

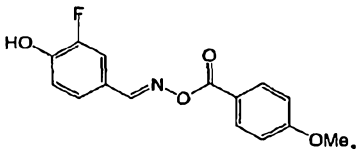
The ring structure of R_2 comprises more than one ring. Additionally, the ring structure of R_2 may be unsubstituted. Alternatively, the ring structure of R_2 is substituted with at least one straight or branched C_1-C_6 alkyl, straight or branched C_1-C_6 alkenyl, straight or branched C_1-C_6 alkanoyl, straight or branched C_1-C_6 alkoxy, keto, carboxy, nitro, amino, hydroxy, halogen, cyano, diazo, thio, or hydroxyamino. Other preferred substitutions of the ring structure of R_2 is at least one nitro, amino, hydroxyl or halogen.

Preferably with the invention compounds, R_3 is O. It is also preferred that R_4 is H. Most preferably R_3 is O and R_4 is H. With some preferred embodiments of these compounds, R_1 comprises an OH substitution and a halogen substitution. Preferably here, the OH is in the para position. More preferably here, R_2 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexyl-2,3-ene, cyclobenzyl, 4-pyrimidyl, 3-pyrimidyl, 1-adamantyl, or methoxyphenyl.

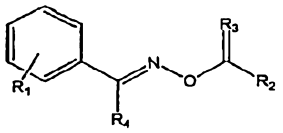
Preferably, the compound comprises, or consists of



Other preferred compounds comprise, or consist of



The invention is further directed to a compound of formula I:



I , or a pharmaceutically acceptable salt, ester, or tautomer thereof, wherein

R_1 is a single or multiple substitution independently H, OH, R_5, $N(R_5)$, SR_5, or a halogen;

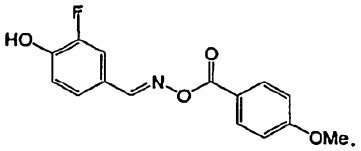
R_2 is *para*-hydroxymethylphenyl;

R_3 is O, $C(R_5)_2$, or S; and

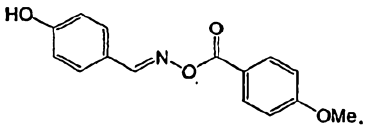
R_4 is H, R_5, or a halogen, wherein

R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy.

Preferably, R_1 is a multiple substitution comprising OH and a halogen. More preferably, OH in the para position and fluorine in the meta position. Most preferably, the compound comprises, or consists of

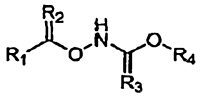


Another most preferred compound comprises, or consists of



The invention is also directed to a pharmaceutical composition comprising any the above compounds, or a pharmaceutically acceptable salt, ester, or tautomer thereof, in a pharmaceutically acceptable excipient.

The invention is further directed to a compound of formula III



III

where

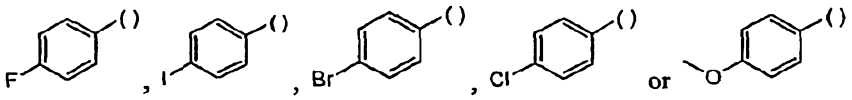
R_1 comprises a ring structure in which an atom in the ring structure is bound to the carbon that is bound to R_2;

R_2 and R_3 are independently O, $C(R_5)_2$, or S;

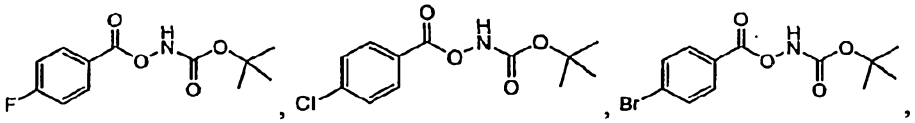
R_4 is a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy;

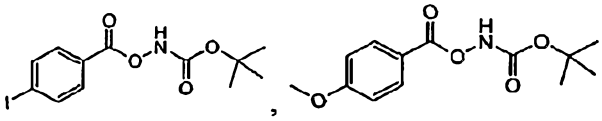
R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy. The invention also encompasses pharmaceutical compositions comprising any of these compounds.

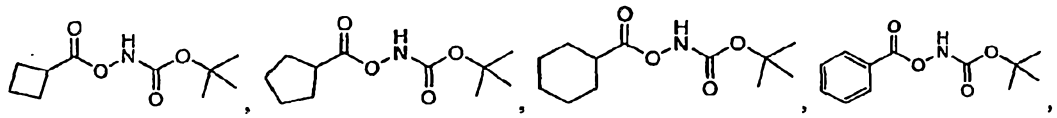
Preferably with these compounds, R_2 and R_3 are both O. Also preferably, R_4 is tert-butyl. Preferred R_1 moieties are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclobenzyl, and a substituted cyclobenzyl. Where R_1 is a substituted cyclobenzyl, it is preferably a *para*-substituted cyclobenzyl. The *para*-substituted cyclobenzyl is most preferably

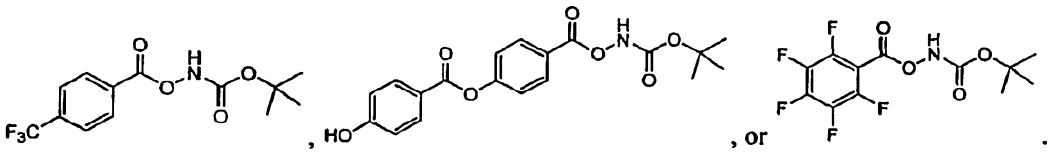


Even more preferably, the compound comprises

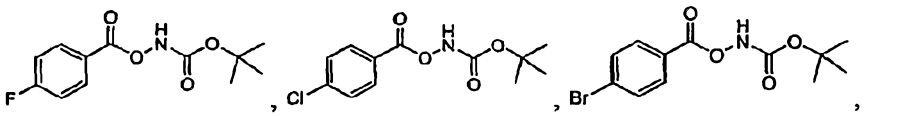


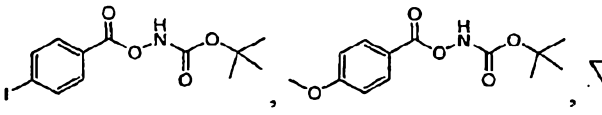


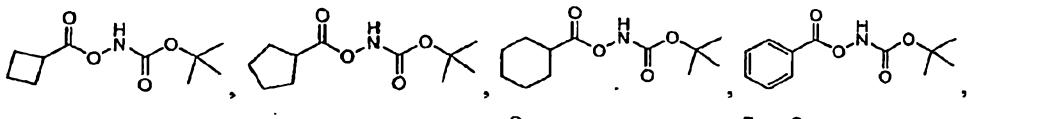


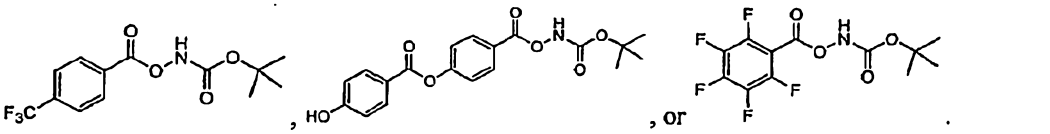


Still more preferably, the compound consists of

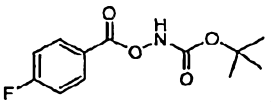




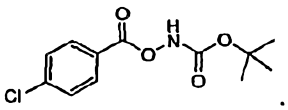




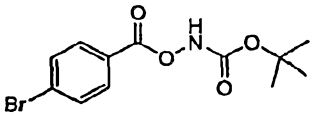
The compound most preferably comprises, or consists of



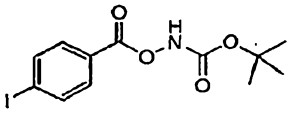
The compound can also most preferably comprise, or consist of



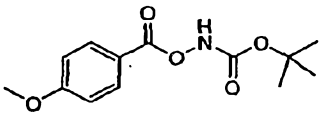
The compound can additionally most preferably comprise, or consist of



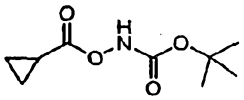
Additionally, the compound can most preferably comprise, or consist of



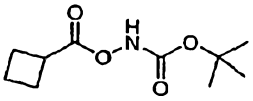
Further, the compound can most preferably comprise, or consist of



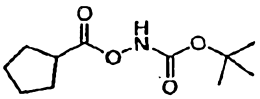
The compound can still further most preferably comprise, or consist of



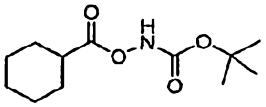
The compound can also most preferably comprise, or consist of



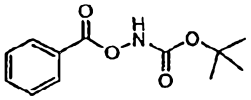
The compound can additionally preferably comprise, or consist of



Additionally, the compound can most preferably comprise, or consist of



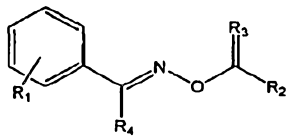
Further, the compound can most preferably comprise, or consist of



The invention also encompasses pharmaceutical composition comprising any of the above compounds, or a pharmaceutically acceptable salt, ester, or tautomer thereof,, in a pharmaceutically acceptable excipient.

The invention is also directed to methods of inhibiting macrophage migration inhibitory factor (MIF) activity in a mammal. The methods comprise administering any of the above-identified pharmaceutical compositions to the mammal in an amount effective to inhibit MIF activity in the mammal.

Additionally, the invention is directed to other methods of inhibiting macrophage migration inhibitory factor (MIF) activity in a mammal. The methods comprise administering a pharmaceutical composition to the mammal in an amount effective to inhibit MIF activity in the mammal. In these methods, the pharmaceutical composition comprises a compound of formula I or formula II, or a pharmaceutically acceptable salt, ester, or tautomer thereof, in a pharmaceutically acceptable excipient, where formula I and formula II are



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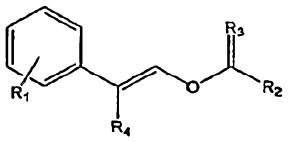


II ,

where

R_1 is a single or multiple substitution independently H, OH, R_5, $N(R_5)$, SR_5, or a halogen;

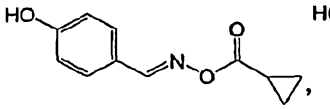
R_2 comprises a ring structure in which an atom in the ring structure is bound to the carbon that is bound to R_3;

R_3 is O, $C(R_5)_2$, or S;

R_4 is H, R_5, or a halogen, where

R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy. Preferably, the compound utilized in these methods is of formula I. It is also preferred if R_1 is OH in the para position. As with the compounds described above, R_2 preferably comprises a 3-, 4-, 5- or 6-membered alicyclic, heterocyclic or aromatic ring. More preferably, R_2 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexyl-2,3-ene, cyclobenzyl, 4-pyrimidyl, 3-pyrimidyl, 1-adamantyl, or methoxyphenyl.

Preferred specific compounds for the present methods include



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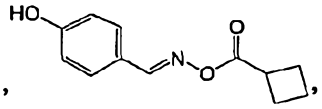

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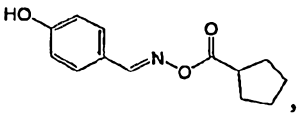

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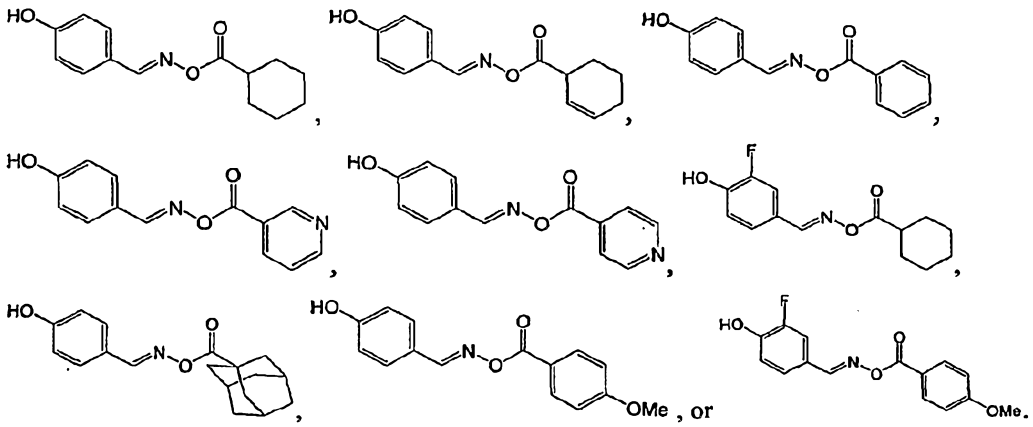
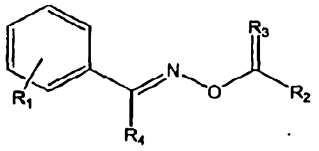


The mammal in these methods is preferably a human. The mammal also preferably has or is at risk for a condition that comprises an inflammatory cytokine cascade that is at least partially mediated by an MIF. Preferred examples of such conditions include cancer, acute respiratory distress syndrome, cytokine-mediated toxicity, psoriasis, interleukin-2 toxicity, appendicitis, peptic, gastric and duodenal ulcers, peritonitis, pancreatitis, ulcerative, pseudomembranous, acute and ischemic colitis, diverticulitis, epiglottitis, achalasia, cholangitis, cholecystitis, hepatitis, inflammatory bowel disease, Crohn's disease, enteritis, Whipple's disease, asthma, allergy, anaphylactic shock, immune complex disease, organ ischemia, reperfusion injury, organ necrosis, hay fever, sepsis, septicemia, endotoxic shock, cachexia, hyperpyrexia, eosinophilic granuloma, granulomatosis, sarcoidosis, septic abortion, epididymitis, vaginitis, prostatitis, urethritis, bronchitis, emphysema, rhinitis, cystic fibrosis, pneumonitis, pneumoconiosis, alvealitis, bronchiolitis, pharyngitis, pleurisy, sinusitis, influenza, respiratory syncytial virus infection, herpes infection, HIV infection, hepatitis B virus infection, hepatitis C virus infection, disseminated bacteremia, Dengue fever, candidiasis, malaria, filariasis, amebiasis, hydatid cysts, burns, dermatitis, dermatomyositis, sunburn, urticaria, warts, wheals, vasulitis, angiitis, endocarditis, arteritis, atherosclerosis, thrombophlebitis, pericarditis, myocarditis, myocardial ischemia, periarteritis nodosa, rheumatic fever, Alzheimer's disease, coeliac disease, congestive heart failure, meningitis, encephalitis, multiple sclerosis, cerebral infarction, cerebral embolism, Guillame-Barre syndrome, neuritis, neuralgia, spinal cord injury, paralysis, uveitis, arthritides, arthralgias, osteomyelitis, fasciitis, Paget's disease, gout, periodontal disease, rheumatoid arthritis, synovitis, myasthenia gravis, thryoiditis, systemic lupus erythematosus, Goodpasture's syndrome, Behcets's syndrome, allograft rejection, graft-versus-host disease, ankylosing spondylitis, type 1 diabetes, type 2 diabetes, Berger's disease, Retier's syndrome, or Hodgkins disease. In the most preferred embodiments, the condition is sepsis, septicemia, and/or endotoxic shock.

The invention is also directed to methods of treating or preventing inflammation in a mammal. The methods comprise administering any of the above-identified pharmaceutical compositions to the mammal in an amount effective to treat or prevent the inflammation in the mammal.

The invention is additionally directed to other methods of treating or preventing inflammation in a mammal. The methods comprise administering a pharmaceutical composition to the mammal in an amount effective to treat or prevent the inflammation in the mammal, where the pharmaceutical composition comprises a compound of formula I or formula II, or a pharmaceutically acceptable salt, ester, or tautomer thereof, in a pharmaceutically acceptable excipient. Here, formula I and formula II are



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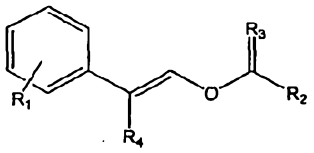


II ,

where

R_1 is a single or multiple substitution independently H, OH, R_5, $N(R_5)$, SR_5, or a halogen;

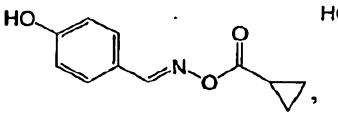
R_2 comprises a ring structure in which an atom in the ring structure is bound to the carbon that is bound to R_3;

R_3 is O, $C(R_5)_2$, or S;

R_4 is H, R_5, or a halogen, where

R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy.

As with the methods described above, the compounds in these methods are preferably of formula I. It is also preferred if R_1 of the compounds is OH in the para position. Additionally, R_2 preferably comprises a 3-, 4-, 5- or 6-membered alicyclic, heterocyclic or aromatic ring. Most preferably, R_2 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexyl-2,3-ene, cyclobenzyl, 4-pyrimidyl, 3-pyrimidyl, 1-adamantyl, or methoxyphenyl. Preferred specific compounds for these methods are



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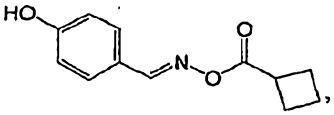

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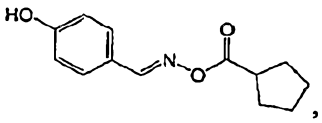

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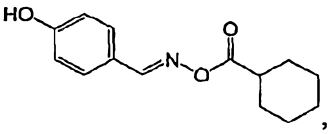

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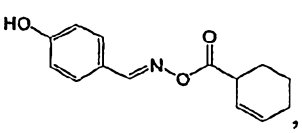

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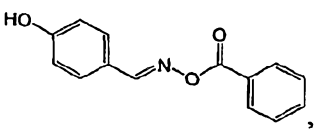

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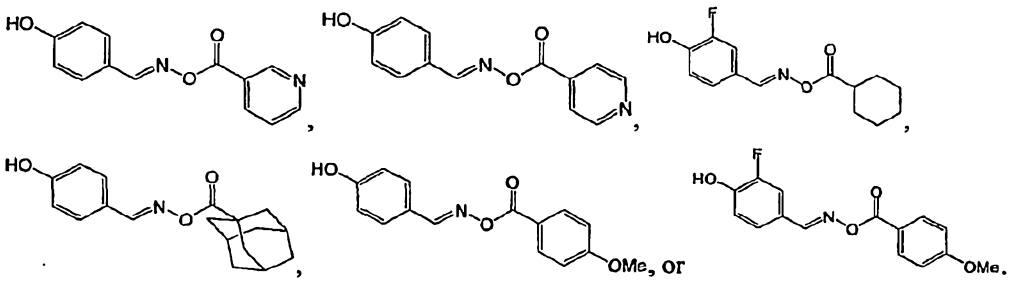


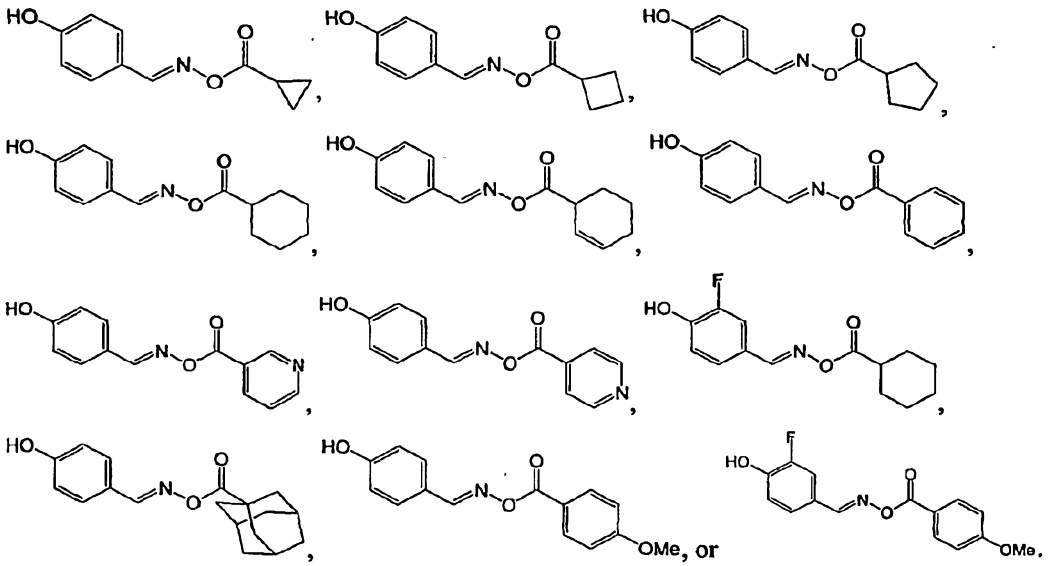
The mammal in these methods is preferably a human. The mammal also preferably has a condition that comprises an inflammatory cytokine cascade that is at least partially mediated by an MIF. Preferred examples of such conditions include cancer, acute respiratory distress syndrome, cytokine-mediated toxicity, psoriasis, interleukin-2 toxicity, appendicitis, peptic, gastric and duodenal ulcers, peritonitis, pancreatitis, ulcerative, pseudomembranous, acute and ischemic colitis, diverticulitis, epiglottitis, achalasia, cholangitis, cholecystitis, hepatitis, inflammatory bowel disease, Crohn's disease, enteritis, Whipple's disease, asthma, allergy, anaphylactic shock, immune complex disease, organ ischemia, reperfusion injury, organ necrosis, hay fever, sepsis, septicemia, endotoxic shock, cachexia, hyperpyrexia, eosinophilic granuloma, granulomatosis, sarcoidosis, septic abortion, epididymitis, vaginitis, prostatitis, urethritis, bronchitis, emphysema, rhinitis, cystic fibrosis, pneumonitis, pneumoconiosis, alvealitis, bronchiolitis, pharyngitis, pleurisy, sinusitis, influenza, respiratory syncytial virus infection, herpes infection, HIV infection, hepatitis B virus infection, hepatitis C virus infection, disseminated bacteremia, Dengue fever, candidiasis, malaria, filariasis, amebiasis, hydatid cysts, burns, dermatitis, dermatomyositis, sunburn, urticaria, warts, wheals, vasulitis, angiitis, endocarditis, arteritis, atherosclerosis, thrombophlebitis, pericarditis, myocarditis, myocardial ischemia, periarteritis nodosa, rheumatic fever, Alzheimer's disease, coeliac disease, congestive heart failure, meningitis, encephalitis, multiple sclerosis, cerebral infarction, cerebral embolism, Guillame-Barre syndrome, neuritis, neuralgia, spinal cord injury, paralysis, uveitis, arthritides, arthralgias, osteomyelitis, fasciitis, Paget's disease, gout, periodontal disease, rheumatoid arthritis, synovitis, myasthenia gravis, thryoiditis, systemic lupus erythematosus, Goodpasture's syndrome, Behcets's syndrome, allograft rejection, graft-versus-host disease, ankylosing spondylitis, Berger's disease, type 1 diabetes, type 2 diabetes, Retier's syndrome, or Hodgkins disease. In the most preferred embodiments, the mammal has or is at risk for sepsis, septicemia, and/or endotoxic shock.

These methods can further comprise administering a second anti-inflammatory agent to the mammal. Nonlimiting examples of the second anti-inflammatory agent is an NSAID, a salicylate, a COX inhibitor, a COX-2 inhibitor, or a steroid. Preferably, the mammal has or is at

risk for sepsis, septicemia, and/or endotoxic shock and the second treatment is administration of a muscarinic agonist, an adrenomedullin, an adrenomedullin binding protein, a milk fat globule epidermal growth factor factor VIII, an activated protein C, or an α_{2A}-adrenergic antagonist.

The invention is also directed to a method of treating a mammal having sepsis, septicemia, and/or endotoxic shock. The method comprises administering any of the above pharmaceutical compositions to the mammal in an amount sufficient to treat the sepsis, septicemia and/or endotoxic shock.

The invention is further directed to other methods of treating a mammal having sepsis, septicemia, and/or endotoxic shock. The methods comprise administering a compound to the mammal in an amount sufficient to treat the sepsis, septicemia and/or endotoxic shock, where the compound is



Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims, which follow the examples.

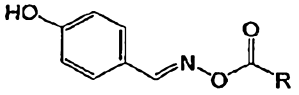
Example 1. Oxime inhibitors of macrophage migration inhibitory factor.

One route for the design of inhibitors of MIF pro-inflammatory activity has focused on interfering with the MIF tautomerase active site to inhibit tautomerase activity. In this regard, disruption of the active site by insertion of an alanine between Pro-1 and Met-2 (pam) abolishes the tautomerase catalytic activity and the resultant mutant is defective in the *in vitro*

glucocorticoid counter-regulatory activity of MIF (Lubetsky et al., 2002). Also, a P450-dependent metabolite of acetaminophen, N-acetyl-*p*-benzoquinone imine (NAPQI) covalently binds to MIF at its enzymatic site and inactivates MIF cytokine activity in a number of *in vitro* bioassays, including interference with the anti-inflammatory effect of dexamethasone, suggesting a role of the active site in mediating MIF bioactivity (Senter et al., 2002). Unfortunately, the toxicity of NAPQI prevents its use as a systemic MIF antagonist. Therefore, it was hypothesized that compounds mimicking the indole product of MIF's tautomerase catalysis could bind in the active site and be effective inhibitors. To achieve this goal, Schiff base adducts were synthesized by coupling amino acid methyl ester with p-hydroxybenzaldehyde to furnish an amino acid Schiff base-type compound, Type II. The most potent inhibitor was found to be tryptophan Schiff base with an IC_{50} of 1.65 μM (Dios et al., 2002). Due to the slow rate of hydrolysis of tryptophan Schiff base in aqueous medium, additional phenylimine scaffolds were explored as potential MIF antagonists. Several representative phenylimine compounds were tested for dopachrome tautomerase inhibitory activity and it was concluded that the isoxazolines represent an attractive scaffold for further attention (Lubetsky et al., 2002). The lead inhibitor of MIF tautomerase and proinflammatory activity in this series is (*S,R*)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1). The crystal structure of MIF complexed to ISO-1 revealed binding in the active site similar to *p*-hydroxy-phenylpyruvic acid. Further study of MIF bound with its inhibitor showed that active site occupation is associated with inhibition of MIF pro-inflammatory properties *in vivo* and *in vitro*, further establishing a role for the catalytic active site of MIF in inflammatory activities. These prior studies provided a molecular basis for the rational design of a new class of compounds resulting in the production of Cyc-Oxi-11, which in confirmatory testing is 30-fold more potent than ISO-1 in MIF tautomerase inhibition. In addition, Cyc-Oxi-11 lacks obvious toxicity at high doses, both *in vitro* and *in vivo*. Cyc-Oxi-11 and related compounds were evaluated as possible therapeutic agents for the treatment of sepsis.

Design of Cyc-Oxi-11. ISO-1, the pharmacological inhibitor of MIF, neutralizes exogenous and endogenous MIF in *in vitro* and *in vivo* models. This indicates the successful application of chemical approaches to antagonize MIF biological activities. Although ISO-1 has significant anti-inflammatory activity, development of better inhibitors was desired. Synthesis of a focused library centered on the ISO-1 structure did not significantly improve the activity (data not shown). However, ISO-1 was integrated with two other scaffolds (FIG. 1) to design a new scaffold, an example of which is Cyc-Oxi-11 (= OXIM-11) (FIG. 2), which, along with its fluoridated derivative (Example 2) is a more potent inhibitor of MIF activity than any previously described compound. Cyc-Oxi-11 is one of 29 oxime derivatives that were synthesized around the scaffold and is 30-fold more potent inhibitor of MIF proinflammatory activity *in vitro* than

ISO-1. Representative structures of the novel oxime scaffold are presented in Table 1, with their IC_{50} of inhibiting MIF dopachrome tautomerase activity. Since toxicity is a concern with respect to the proposed therapeutic use of any novel compound, preliminary acute toxicity screening of Cyc-Oxi-11 was conducted. No evidence of toxicity in intraperitoneal injection was found with doses up to 100 mg/Kg (data not shown). In preliminary studies, the antibacterial effect of Cyc-Oxi-11 was also tested and was found to be negative.



Cyc-Oxi

R	IC_{50}(μM)	R	IC_{50}
CH_3-	85.0		7
	5.5		38
	12.0		18
	4.8		
	3.4		
	1.3		
	3.0		

Table 1. Selected structures of Cyc-Oxi (OXIM) scaffold. IC_{50} represents the inhibition of MIF tautomerase activity

Cyc-Oxi-11 binding to the MIF active site down-regulates MIF glucocorticoid-regulating activity on LPS-activated monocytes. As shown with the study of ISO-1 above, the more potent neutralization of MIF proinflammatory activity *in vitro* is associated with enhanced inhibitory effect on MIF tautomerase activity. This association is further borne out in the new class of Cyc-Oxi agents. As shown in FIG. 3, Cyc-Oxi-11 significantly inhibited MIF-dependent interference with glucocorticoids from LPS-stimulated macrophages and Cyc-Oxi-11 is one of the most potent inhibitor of MIF tautomerase and proinflammatory activity with an IC_{50} of ~1.3 μM in both assays (30-fold more potent than ISO-1). Cyc-Oxi-11 inhibits TNF release *in vitro*.

Cyc-Oxi-11 inhibits MIF proinflammatory activity *in vitro*. It has been shown that the macrophage is an abundant source of MIF (Calandra et al., 1994), which is released after LPS stimulation. This led to an examination of the autocrine and paracrine activity of secreted MIF *in vitro*. Previous studies showed that neutralization of MIF using antibodies blocked TNF secretion by LPS-stimulated macrophages. Here, it was determined whether Cyc-Oxi-11 neutralization of secreted MIF from LPS-stimulated human macrophages is able to inhibit MIF activity to mediate TNF release. As shown in FIG. 4, Cyc-Oxi-11, in a dose-dependent manner, inhibits TNF release by LPS-stimulated human macrophages similarly to anti-MIF antibody treatment.

Small molecules that bind at the catalytic site of MIF abrogate the inflammatory and glucocorticoid regulatory functions of the molecule. The rational design of inhibitors has resulted in the identification of Cyc-Oxi-11 and certain related OXIM scaffold compounds as the most potent inhibitor of MIF activity yet designed. Since the preliminary acute toxicity screening found no evidence of toxicity at doses up to 100 mg/kg this molecule shows potential as a therapeutic agent for reducing the devastating morbidity and mortality associated with sepsis, as well as other MIF-mediated diseases and conditions.

The activity of Cyc-Oxi-11 to prevent death by sepsis was next evaluated. The kinetics of MIF appearance in the serum post CLP surgery was determined by collecting blood after 6, 12, 24, 36 and 48 hours post CLP and then analyzing the serum by Western Blot to determine the circulating MIF levels. Five mice were tested per time point. As shown in FIG. 5, serum MIF starts to increase after 12 hours and peaks at about 36 hours. $*P < 0.05$.

Other mice were then injected intraperitoneally with Cyc-Oxi-11 (0.1 mg/mouse/day) or vehicle 24 hours after CLP (n=13). An additional two injections were given, on day 2 and day 3. Thirteen mice were tested. The results are shown in FIG. 6. Cyc-Oxi-11 treatment 24 hours after onset of sepsis considerably reduced the deaths caused by CLP ($P < 0.001$)

Example 2. Fluorination of OXIM-11 (Cyc-Oxi-11) Improves its Potent Inhibition of Macrophage Migration Inhibitory Factor Activity

Example Summary

The synthesis of a series of halogenated (E)- 4-hydroxybenzaldehyde O-cyclohexanecarbonyloxime (OXIM-11, FIG. 2) as potent and specific inhibitors of MIF tautomerase activity is described. Only mono-fluorination of the 4-hydroxy bearing phenyl ring of the OXIM scaffold improves the potency of the inhibitors, up to 63% compared to the parent compounds.

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that plays a critical role in the pathogenesis of inflammatory diseases. MIF, a homotrimer (Sun et al., 1996;

Sugimoto et al., 1996), possesses the unique ability to catalyze the tautomerization of non-physiological substrates such as D or L-dopachrome methyl ester into their corresponding indole derivatives (Rosengren et al., 1996). Blocking this active site using either mutagenesis or small molecules inhibits MIF biological activity in sepsis (Beishuizen et al., 2001; Lue et al., 2002; Calandra et al., 2002; Calendra et al., 2000), EAN and type 1 diabetes (Cvetkovic et al., 2005).

Recently, we further modified the target molecule to give the most potent inhibitor of MIF, (*E*)-4-hydroxybenzaldehyde *O*-cyclohexanecarbonyloxime (OXIM-11, cyc-oxi-11)(FIG. 2; compound **3a** in FIG. 7). As established here, the molecule with cyclohexyl group (**3a**)(FIG. 7) and 4-Methoxyphenyl group (**4**, FIG. 8) (Scheme 2) have the most inhibition activity. **3a** inhibits the dopachrome tautomerase activity with an IC_{50} of 1.3 μM, and **4** inhibits the dopachrome tautomerase activity, with an IC_{50} of 1.1 μM.

This Example describes the influence of ortho-halogenation in respect to the hydroxyl group on the potency to inhibit MIF tautomerase activity. Thus, the synthesis of a series of halogenated (E)- 4-hydroxybenzaldehyde O-cyclohexanecarbonyloxime (OXIM-11, **3a**), a potent and specific inhibitor of the MIF tautomerase activity is described. The mono-fluorination ortho to the hydroxyl improves the inhibition of MIF bioactivity up to 63%.

The halogenated 4-hydroxybenzaldehydes **1b-1h** (FIG. 7) were either commercially available or prepared according to the procedure described in literature (Lawrence et al., 2003). The oximes **2a-2h** (FIG. 7) were synthesized in excellent yields by condensation of hydroxylamine with the aldehydes **1a-1h** in basic alcoholic solvent. The final compounds **3a-3h** (FIG. 7) were synthesized in good yields by condensation of oximes **2a-2h** with cyclohexanecarboxylic acid chloride in dry dichloromethane in present of pyridine from 0 °C to room temperature overnight (Scheme 1) (See Supplemental Material below). Compound **4** and **5** were prepared as the similar method as the compound **3** (FIG. 8). The final compounds **3a-3h, 4** and **5** reported here were fully characterized by ^{1}H NMR, ^{13}C NMR and ESI-MS (See Supplemental Material below).

The crystal structure of MIF complexed to ISO-1 or OXIM-11 revealed that the phenolic group has a critical role in binding within the active site of MIF in both substrate and inhibitors. Compounds bearing halogens in an ortho position of the phenolic group were thus evaluated to determine whether the halogen enhances the hydrogen bond of the phenol for the additional binding within the active site of MIF. The candidate compounds **3a-3h** were synthesized, and the inhibition of MIF dopachrome tautomerase activity is presented in FIG. 7.

The (*E*)-4-hydroxybenzaldehyde *O*-cyclohexanecarbonyloxime (OXIM-11, **3a**), inhibits MIF dopachrome tautomerase activity with an IC_{50} of 1.3 μM. Mono-fluorination on the ortho position of the phenolic group, compound **3b**, improves the inhibition of the dopachrome

tautomerase activity by 35%, to an IC_{50} of 0.9 μM. Besides the steric effect, the strong electronegativity of the fluorine substituent polarizes the phenolic ring and enhances the OH hydrogen bond accepting ability that corresponds to the observed the most potency of compound **3b**. Difluoro analogue **3c** and tetrafluoro analogue **3d** were considerably less potent than **3b** because of the electrostatic repulsion of the fluorine groups (Malamas et al., 2004). For example, the 2,6-difluoro analogue **3c** is most likely to have repulsion with the amide group of Asn-97. However, the other halogenated compounds bearing chlorine or bromine or iodine, compound **3e-3h**, have reduced activity (FIG. 7). This finding is not surprising because the hydrogen bonds between the side-chain of Asn-97 and hydroxyl group are the key interaction within the MIF active site (Lubetsky et al., 1999). Introducing bulky halogens such as chlorine, bromine and iodine ortho to the hydroxyl group significantly alter the size of the molecule, and result in noticeably decreased ligand binding. Also, the intramolecular hydrogen bonds between OH and the halogens reduce the OH hydrogen bond donating ability as evidenced by increasing the acidity of OH in proton NMR analysis (FIG. 7) (Himo et al., 2000). Therefore, fluorine on the ortho position of the phenolic group on compound **3b** has a critical and specific role for additional binding within the active site of MIF. The enhancement on the dopachrome tautomerase activity with mono-fluorination was also observed with (*E*)- 3-fluoro-4-hydroxybenzaldehyde *O*-4'-methoxyphenyl carbonyloxime (**5**). That analog has a 63% improvement in the dopachrome tautomerase activity over the parent compound (**4**) (FIG. 8).

In conclusion, the mono-fluorination onto the ortho position to the hydroxyl group has a critical impact on ligand binding within the MIF active site.

Supplementary material

MIF tautomerase activity was measured by UV-Visible recording spectrophotometry (SHIMADZU, UV1600U). A fresh stock solution of L-dopachrome methyl ester was prepared at 2.4 mM through oxidation of L-3,4-dihydroxyphenylalanine methyl ester with sodium periodate. 1 μL of MIF solution (800-900 ng/mL) and 1 μL of a DMSO solution with various concentrations of the enzymatic inhibitor were added into a plastic cuvette (10 mm, 1.5 mL) containing 0.7 mL assay buffer (1X potassium phosphate, pH 7.2). The L-dopachrome methyl ester solution (0.3 mL) was added to the assay buffer mixture. Activity was determined at room temperature and the spectrometric measurements were made at $\lambda = 475$ nm for 20 seconds by monitoring the rate of decolorization of L-dopachrome methyl ester in comparison to a standard solution.

General procedure for the synthesis of halogenated (E)- 4-hydroxybenzaldehyde O-cyclohexanecarbonyloxime (3a-3h). A mixture of halogenated 4-hydroxybenzaldehyde oxime (**2a-2h**, 12.8 mmol) and cyclohexanecarboxylic acid chloride (13.4 mmol) in 70 mL dry dichloromethane was cooled to 0 °C. To this suspension added pyridine (12.8 mmol) dropwise,

which resulted in a pale yellow solution. The solution was stirred at 0 °C for 10 min and then was allowed to warm to room temperature for 18 hr. The mixture was diluted with CH_2Cl_2 and water and the layers were separated. The aqueous portion was washed with CH_2Cl_2, and the combined organic fraction was washed with saturated NaCl and dried over $MgSO_4$. Filtration and evaporation *in vacuo* afforded a white solid, which was purified by flash chromatography (40% EtOAc/hexanes). Crystallization from EtOAc/hexanes afforded the desired white solid product (**3a-3h**).

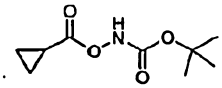
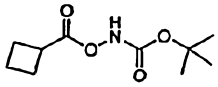
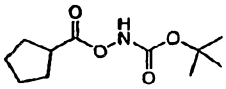
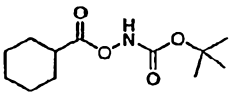
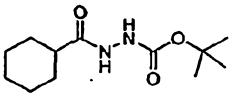
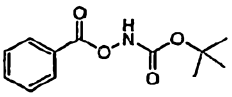
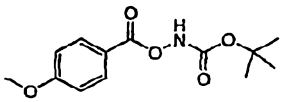
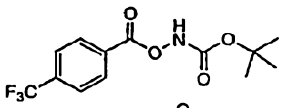
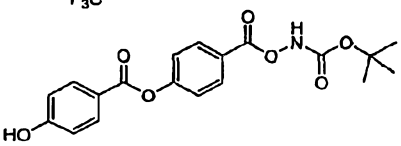
Analytical data for compounds **3a-3h**. All solvents were HPLC-grade from Fisher Scientific. Silica gel (Selecto Scientific, 32-63 μm average particle size) was used for flash column chromatography (FCC). Aluminum-backed Silica Gel 60 with a 254 nm fluorescent indicator TLC plates were used. Spots on TLC plates were visualized under a short wavelength UV lamp or stained with I2 vapor. NMR spectra were preformed on a Jeol Eclipse 270 spectrometer at 270 MHz for ^{1}H NMR spectra and 67.5 MHz for ^{13}C NMR spectra. Coupling constants are reported in Hertz (Hz), and chemical shifts are reported in parts per million (ppm) relative to the deuterated solvent peak. The coupling constants (J) are measured in Hertz (Hz) and assigned as s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). Low-resolution mass spectra were acquired using Thermofinnigan LCQ DecaXPplus quadrupole ion trap MS with negative-ion mode.

Analytical data of some selected compounds. Compound **3a**: isolated as white solid product (38%). ^{1}H NMR (270 MHz, acetone-d6) δ 9.04 (br, 1H), 8.42 (s, 1H), 7.65 (d, J = 8.7 Hz, 2H), 6.94 (d, J = 8.7 Hz, 2H), 2.46 (m, 1H), 1.2-2.0 (m, 10H); ^{13}C NMR (67.5 MHz, acetone-d6) δ 172.23, 160.56, 155.92, 130.06, 122.23, 115.88, 41.67, 25.19; ESI-MS m/z 246 (M^-). Compound **3b**: isolated as white solid product (40%). ^{1}H NMR (270 MHz, acetone-d6) δ 9.37 (br, 1H), 8.44 (s, 1H), 7.55 (m, 1H), 7.45 (m, 1H), 7.10 (m, 1H); 2.46 (m, 1H), 1.2-2.0 (m, 10H); ^{13}C NMR (67.5 MHz, acetone-d6) δ 172.10, 155.16, 153.29, 149.72, 148.22, 125.75, 123.07, 118.18, 115.20, 41.61, 25.17; ESI-MS m/z 264 (M^-). Compound **3c**: isolated as white solid product (35%). ^{1}H NMR (270 MHz, acetone-d6) δ 10.79 (br, 1H), 8.16 (s, 1H), 7.40 (d, J = 8.4Hz, 2H), 2.76 (m, 1H), 1.2-2.0 (m, 10H); ESI-MS m/z 282 (M^-). Compound **3d**: isolated as white solid product (45%). ^{1}H NMR (270 MHz, acetone-d6) δ 11.34 (br, 1H), 8.22 (s, 1H), 2.85 (m, 1H), 1.2-2.0 (m, 10H); ^{13}C NMR (67.5 MHz, acetone-d6) δ 171.50, 146.54, 142.89, 139.25, 138.02, 129.32, 110.44, 42.16, 24.84; ESI-MS m/z 321 (M^-). Compound **3e**: isolated as white solid product (40%). ^{1}H NMR (270 MHz, acetone-d6) δ 9.55 (br, 1H), 8.50 (s, 1H), 7.85 (d, J = 2.0 Hz, 1H), 7.67 (dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, 1H), 7.18 (d, J = 8.5 Hz, 1H), 2.55 (m, 1H), 1.2-2.0 (m, 10H); ESI-MS m/z 280 (M^-). Compound **3f**: isolated as white solid product (39%). ^{1}H NMR

(270 MHz, acetone-d6) δ 9.66 (br, 1H), 8.44 (s, 1H), 7.94 (d, J = 2.0 Hz, 1H), 7.66 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 2.47 (m, 1H), 1.2-2.0 (m, 10H); ^{13}C NMR (67.5 MHz, acetone-d6) δ 172.12, 156.93, 154.75, 133.09, 128.87, 124.01, 116.82, 110.04, 41.62, 25.18. Compound **3g**: isolated as white solid product (37%). ^{1}H NMR (270 MHz, acetone-d_6) δ 10.82 (br, 1H), 8.15 (s, 1H), 7.93 (s, 2H), 2.73 (m, 1H), 1.2-2.0 (m, 10H). Compound **3h**: isolated as white solid product (40%). ^{1}H NMR (270 MHz, acetone-d_6) δ 10.74 (br, 1H), 8.13 (s, 2H), 8.10 (s, 1H), 2.71 (m, 1H), 1.2-2.0 (m, 10H). Compound **4**: isolated as white solid product (40%). ^{1}H NMR (300 MHz, acetone-d_6) δ 9.05 (br, 1H), 8.60 (s, 1H), 8.02 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.7 Hz, 2H), 7.05 (d, J = 8.7 Hz, 2H), 6.93 (d, J = 8.7 Hz, 2H), 3.87 (s, 3H); ESI-MS *m/z* 270 (M^-). Compound **5**: isolated as white solid product (40%). ^{1}H NMR (300 MHz, acetone-d_6) δ 9.37 (br, 1H), 8.62 (s, 1H), 8.02 (d, J = 8.4 Hz, 2H), 7.60 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.06 (m, 3H), 3.87 (s, 3H); ESI-MS *m/z* 288 (M^-).

Example 3. Additional compounds inhibiting MIF.

Additional compounds were produced and tested for MIF inhibitory activity *in vitro* by the methods described in the above examples. Table 2 provides the results of those experiments.

Compound	Structure	IC_{50} (μM)
BKII-91		1.9
BKII-92		0.37
BKII-93		0.7
BKII-85		19.5
BKII-90		60
BKII-98		0.23
BKII-99		1.2
BKIII-9W		69
BKIII-6Y		50

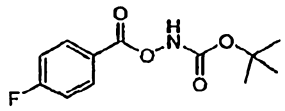
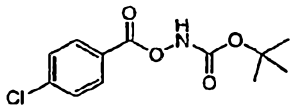
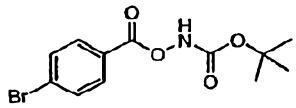
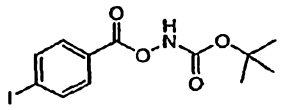
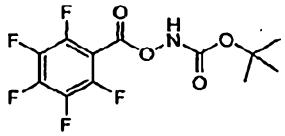
Compound	Structure	IC_{50} (μM)
BKIII-8W		0.27
BKIII-8Y		0.87
BKIII-11Y		1.30
BKIII-9Y		1.7
BKIII-6W		118

Table 2. Additional MIF inhibitors.

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In view of the above, it will be seen that the several advantages of the invention are achieved and other advantages attained.

As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

All references cited in this specification are hereby incorporated by reference. The discussion of the references herein is intended merely to summarize the assertions made by the authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.

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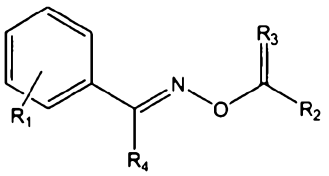
What is claimed is:

1 . A compound of formula I:



I , or a pharmaceutically acceptable salt, ester, or tautomer thereof, wherein

R_1 is a multiple substitution, independently OH or a halogen, wherein a first R1 is OH and a second R1 is a halogen;

R_2 comprises a ring structure in which an atom in the ring structure is bound to the carbon that is bound to R_3;

R_3 is O, $C(R_5)_2$, or S; and

R_4 is H, R_5, or a halogen, wherein

R_5 is independently H, a straight or branched C_2-C_6 alkyl, a straight or branched C_2-C_6 alkenyl, a straight or branched C_2-C_6 alkanoyl, or a straight or branched C_2-C_6 alkoxy.

2. The compound of claim 1, wherein R_1 comprises OH in the para position.

3. The compound of any one of claims 1-2, wherein the halogen substitution is a fluorine.

4. The compound of claim 3, wherein the fluorine is in the meta position.

5. The compound of any one of claims 1-4, wherein R_2 comprises a 3-, 4-, 5- or 6-membered alicyclic, heterocyclic or aromatic ring.

6. The compound of claim 5, wherein the ring of R_2 is alicyclic.

7. The compound of claim 6, wherein the alicyclic ring is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexyl-2,3-ene, or 1-adamantyl.

8. The compound of claim 5, wherein the ring of R_2 is heterocyclic.

9. The compound of claim 8, wherein the heterocyclic ring is pyrimidine, pyridazine, pyrazine, pyridine, pyrazole, imidazole, pyrrole, pyran or furan.

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10. The compound of claim 5, wherein the ring of R_2 is aromatic.

11. The compound of claim 10, wherein the ring of R_2 is *para*-methoxyphenyl or para-hydroxymethylphenyl.

12. The compound of claim 10, wherein the aromatic ring is benzyl, 4-pyrimidyl, or 3-pyrimidyl.

13. The compound of claim 5, wherein the ring structure of R_2 comprises more than one ring.

14. The compound of any one of claims 1-13, wherein the ring structure of R_2 is unsubstituted.

15. The compound of any one of claims 1-13, wherein the ring structure of R_2 is substituted with at least one straight or branched C_1-C_6 alkyl, straight or branched C_1-C_6 alkenyl, straight or branched C_1-C_6 alkanoyl, straight or branched C_1-C_6 alkoxy, keto, carboxy, nitro, amino, hydroxy, halogen, cyano, diazo, thio, or hydroxyamino.

16. The compound of claim 15, wherein the ring structure of R_2 is substituted with at least one nitro, amino, hydroxyl or halogen.

17. The compound of any one of claims 1-16, wherein R_3 is O.

18. The compound of any one of claims 1-17, wherein R_4 is H.

19. The compound of claim 1 having formula I, wherein R_3 is O and R_4 is H.

20. The compound of claim 19, wherein R_1 comprises an OH substitution and a halogen substitution.

21. The compound of claim 20, wherein the OH is in the para position.

22. The compound of any one of claims 19-21, wherein R_2 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexyl-2,3-ene, benzyl, 4-pyrimidyl, 3-pyrimidyl, 1-adamantyl, or methoxyphenyl.

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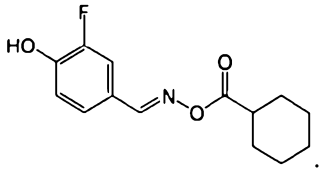
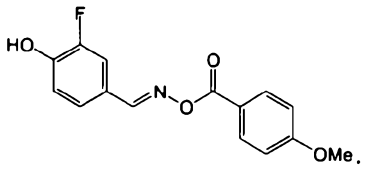
23. The compound of claim 1, comprising or consisting of



24. The compound of claim 1, comprising or consisting of



25. A pharmaceutical composition comprising the compound of any one of claims 1-24, or a pharmaceutically acceptable salt, ester, or tautomer thereof, in a pharmaceutically acceptable excipient.

26. A method of inhibiting macrophage migration inhibitory factor (MIF) activity in a mammal, the method comprising administering any one of the compounds of claims 1-24 or the pharmaceutical composition of claim 25 to the mammal in an amount effective to inhibit MIF activity in the mammal.

27. The method of claim 26, wherein the mammal has or is at risk for a condition that comprises an inflammatory cytokine cascade that is at least partially mediated by an MIF.

28. The method of claim 27, wherein the condition is cancer, acute respiratory distress syndrome, cytokine-mediated toxicity, psoriasis, interleukin-2 toxicity, appendicitis, peptic, gastric and duodenal ulcers, peritonitis, pancreatitis, ulcerative, pseudomembranous, acute and ischemic colitis, diverticulitis, epiglottitis, achalasia, cholangitis, cholecystitis, hepatitis, inflammatory bowel disease, Crohn's disease, enteritis, Whipple's disease, asthma, allergy, anaphylactic shock, immune complex disease, organ ischemia, reperfusion injury, organ necrosis, hay fever, sepsis, septicemia, endotoxic shock, cachexia, hyperpyrexia, eosinophilic granuloma, granulomatosis, sarcoidosis, septic abortion, epididymitis, vaginitis, prostatitis, urethritis, bronchitis, emphysema, rhinitis, cystic fibrosis, pneumonitis, pneumoconiosis, alvealitis, bronchiolitis, pharyngitis, pleurisy, sinusitis, influenza, respiratory syncytial virus infection, herpes infection, HIV infection, hepatitis B virus infection, hepatitis C virus infection, disseminated bacteremia, Dengue fever, candidiasis, malaria, filariasis, amebiasis, hydatid cysts, burns, dermatitis, dermatomyositis, sunburn, urticaria, warts, wheals, vasulitis, angiitis, endocarditis, arteritis, atherosclerosis, thrombophlebitis, pericarditis, myocarditis, myocardial ischemia, periarteritis nodosa, rheumatic fever, Alzheimer's disease, coeliac disease, congestive heart failure, meningitis, encephalitis, multiple sclerosis, cerebral infarction, cerebral embolism, Guillame-Barre

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syndrome, neuritis, neuralgia, spinal cord injury, paralysis, uveitis, arthritides, arthralgias, osteomyelitis, fasciitis, Paget's disease, gout, periodontal disease, rheumatoid arthritis, synovitis, myasthenia gravis, thryoiditis, systemic lupus erythematosus, Goodpasture's syndrome, Behcets's syndrome, allograft rejection, graft-versus-host disease, ankylosing spondylitis, Berger's disease, type 1 diabetes, type 2 diabetes, Retier's syndrome, or Hodgkins disease.

29. The method of claim 28, wherein the condition is sepsis, septicemia, and/or endotoxic shock.

30. A method of treating or preventing inflammation in a mammal, the method comprising administering any one of the compounds of claims 1-24 or the pharmaceutical composition of claim 25 to the mammal in an amount effective to treat or prevent the inflammation in the mammal.

31. The method of claim 30, wherein the mammal has cancer, acute respiratory distress syndrome, cytokine-mediated toxicity, psoriasis, interleukin-2 toxicity, appendicitis, peptic, gastric and duodenal ulcers, peritonitis, pancreatitis, ulcerative, pseudomembranous, acute and ischemic colitis, diverticulitis, epiglottitis, achalasia, cholangitis, cholecystitis, hepatitis, inflammatory bowel disease, Crohn's disease, enteritis, Whipple's disease, asthma, allergy, anaphylactic shock, immune complex disease, organ ischemia, reperfusion injury, organ necrosis, hay fever, sepsis, septicemia, endotoxic shock, cachexia, hyperpyrexia, eosinophilic granuloma, granulomatosis, sarcoidosis, septic abortion, epididymitis, vaginitis, prostatitis, urethritis, bronchitis, emphysema, rhinitis, cystic fibrosis, pneumonitis, pneumoconiosis, alvealitis, bronchiolitis, pharyngitis, pleurisy, sinusitis, influenza, respiratory syncytial virus infection, herpes infection, HIV infection, hepatitis B virus infection, hepatitis C virus infection, disseminated bacteremia, Dengue fever, candidiasis, malaria, filariasis, amebiasis, hydatid cysts, burns, dermatitis, dermatomyositis, sunburn, urticaria, warts, wheals, vasulitis, angiitis, endocarditis, arteritis, atherosclerosis, thrombophlebitis, pericarditis, myocarditis, myocardial ischemia, periarteritis nodosa, rheumatic fever, Alzheimer's disease, coeliac disease, congestive heart failure, meningitis, encephalitis, multiple sclerosis, cerebral infarction, cerebral embolism, Guillame-Barre syndrome, neuritis, neuralgia, spinal cord injury, paralysis, uveitis, arthritides, arthralgias, osteomyelitis, fasciitis, Paget's disease, gout, periodontal disease, rheumatoid arthritis, synovitis, myasthenia gravis, thryoiditis, systemic lupus erythematosus, Goodpasture's syndrome, Behcets's syndrome, allograft rejection, graft-versus-host disease, ankylosing spondylitis, Berger's disease, type 1 diabetes, type 2 diabetes, Retier's syndrome, or Hodgkins disease.

32. The method of claim 31, wherein the mammal has sepsis, septicemia, and/or endotoxic shock or is at risk for sepsis, septicemia, and/or endotoxic shock.

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33. A method of treating a mammal having sepsis, septicemia, and/or endotoxic shock or at risk for sepsis, septicemia, and/or endotoxic shock, the method comprising administering any one of the compounds of claims 1-24 or the pharmaceutical composition of claim 25 to the mammal in an amount sufficient to treat or prevent the sepsis, septicemia and/or endotoxic shock.

34. The method of any one of claims 26-33, wherein the mammal is a human.

35. Use of any one of the compounds of claims 1-24 or the pharmaceutical composition of claim 25, for (i) inhibiting macrophage migration inhibitory factor (MIF) activity in a mammal, (ii) the preparation of a medicament for inhibiting macrophage migration inhibitory factor (MIF) activity in a mammal; (iii) treating or preventing inflammation in a mammal, (iv) the preparation of a medicament for treating or preventing inflammation in a mammal, (v) the treatment or prevention of sepsis, septicemia, and/or endotoxic shock in a mammal having or at risk for sepsis, septicemia, and/or endotoxic shock, or (vi) the preparation of a medicament for the treatment or prevention of sepsis, septicemia, and/or endotoxic shock in a mammal having or at risk for sepsis, septicemia, and/or endotoxic shock.

36. The use of claim 35, wherein the mammal has cancer, acute respiratory distress syndrome, cytokine-mediated toxicity, psoriasis, interleukin-2 toxicity, appendicitis, peptic, gastric and duodenal ulcers, peritonitis, pancreatitis, ulcerative, pseudomembranous, acute and ischemic colitis, diverticulitis, epiglottitis, achalasia, cholangitis, cholecystitis, hepatitis, inflammatory bowel disease, Crohn's disease, enteritis, Whipple's disease, asthma, allergy, anaphylactic shock, immune complex disease, organ ischemia, reperfusion injury, organ necrosis, hay fever, sepsis, septicemia, endotoxic shock, cachexia, hyperpyrexia, eosinophilic granuloma, granulomatosis, sarcoidosis, septic abortion, epididymitis, vaginitis, prostatitis, urethritis, bronchitis, emphysema, rhinitis, cystic fibrosis, pneumonitis, pneumoconiosis, alvealitis, bronchiolitis, pharyngitis, pleurisy, sinusitis, influenza, respiratory syncytial virus infection, herpes infection, HIV infection, hepatitis B virus infection, hepatitis C virus infection, disseminated bacteremia, Dengue fever, candidiasis, malaria, filariasis, amebiasis, hydatid cysts, burns, dermatitis, dermatomyositis, sunburn, urticaria, warts, wheals, vasulitis, angiitis, endocarditis, arteritis, atherosclerosis, thrombophlebitis, pericarditis, myocarditis, myocardial ischemia, periarteritis nodosa, rheumatic fever, Alzheimer's disease, coeliac disease, congestive heart failure, meningitis, encephalitis, multiple sclerosis, cerebral infarction, cerebral embolism, Guillame-Barre syndrome, neuritis, neuralgia, spinal cord injury, paralysis, uveitis, arthritides, arthralgias, osteomyelitis, fasciitis, Paget's disease, gout, periodontal disease, rheumatoid arthritis, synovitis, myasthenia gravis, thryoiditis, systemic lupus erythematosus, Goodpasture's syndrome, Behcets's syndrome, allograft rejection, graft-versus-host

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disease, ankylosing spondylitis, Berger's disease, type 1 diabetes, type 2 diabetes, Retier's syndrome, or Hodgkins disease.

37. A compound according to claim 1; a pharmaceutical composition according to claim 25; a method according to any one of claims 26, 30 or 33; or use according to claim 35, substantially as herein described with reference to any one or more of the examples but excluding comparative examples.

FIG. 1

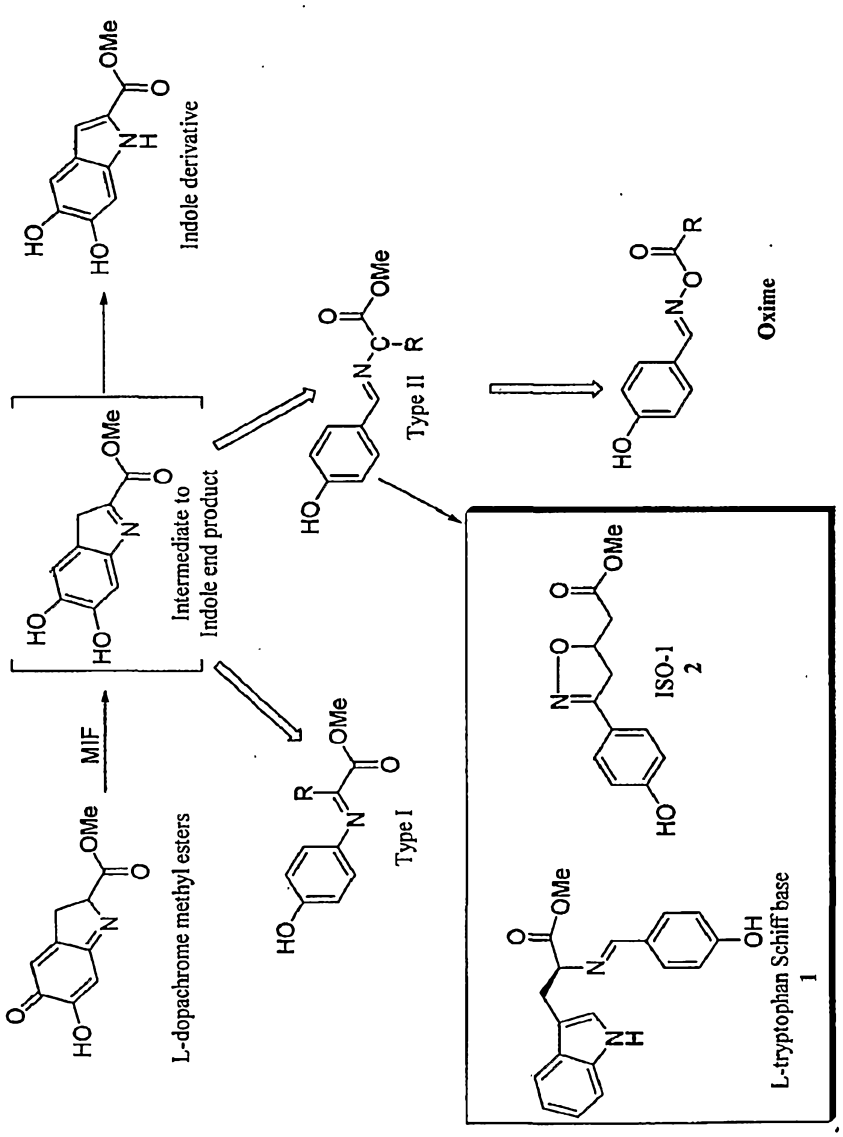
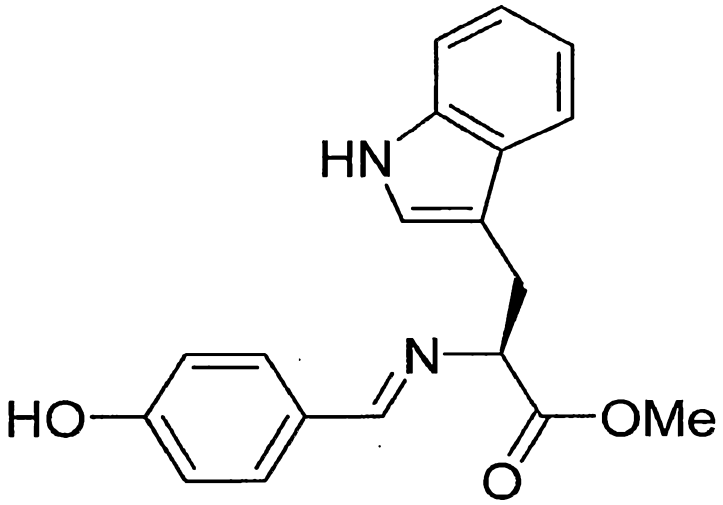
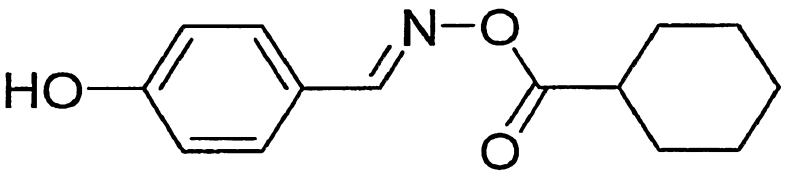

L-dopachrome methyl esters
MIF
Intermediate to
Indole end product
Indole derivative
Type I
Type II
Oxime
ISO-1
2
L-tryptophan Schiff base
1

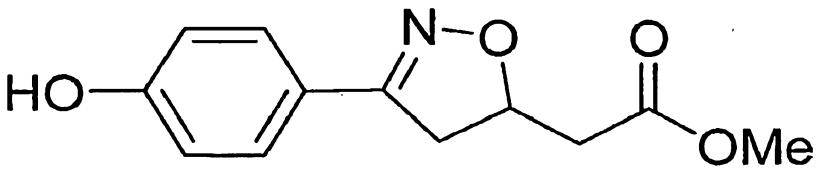
FIG. 2



L-Tryp Schiff Base



OXIM-11 (= cyc-oxi-11)



ISO-1

FIG. 3

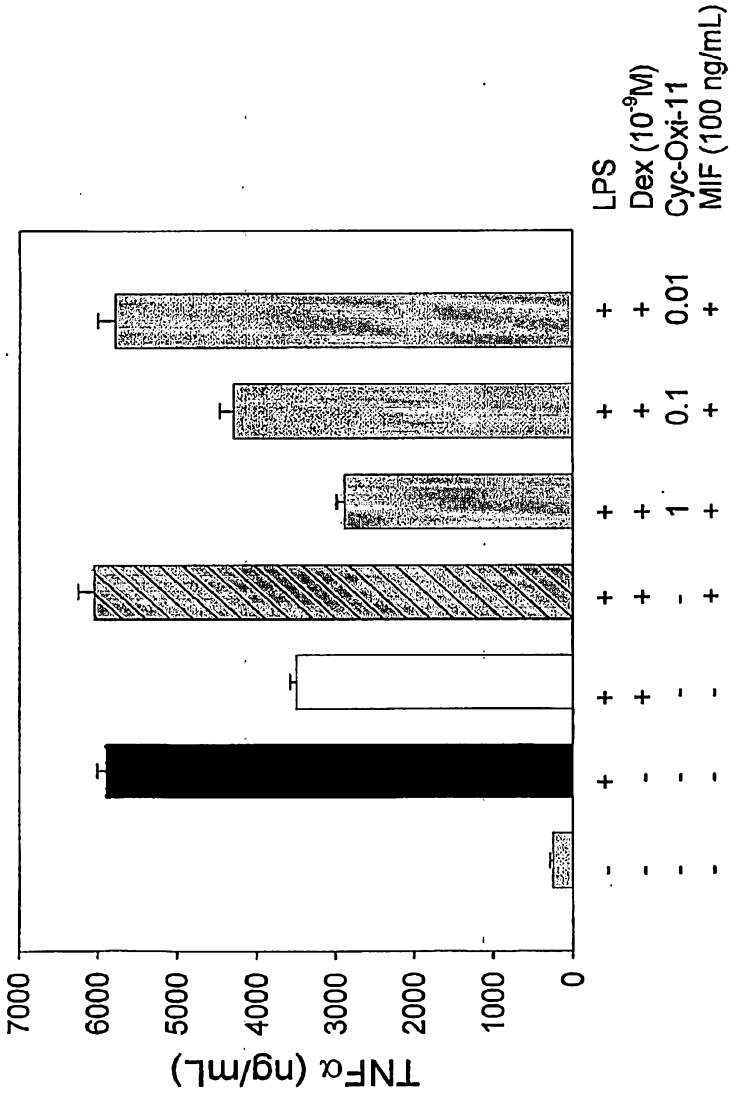

TNFα (ng/mL)
7000
6000
5000
4000
3000
2000
1000
0
LPS
Dex (10^{-9}M)
Cyc-Oxi-11
MIF (100 ng/mL)
0.1
0.01
1

FIG. 4

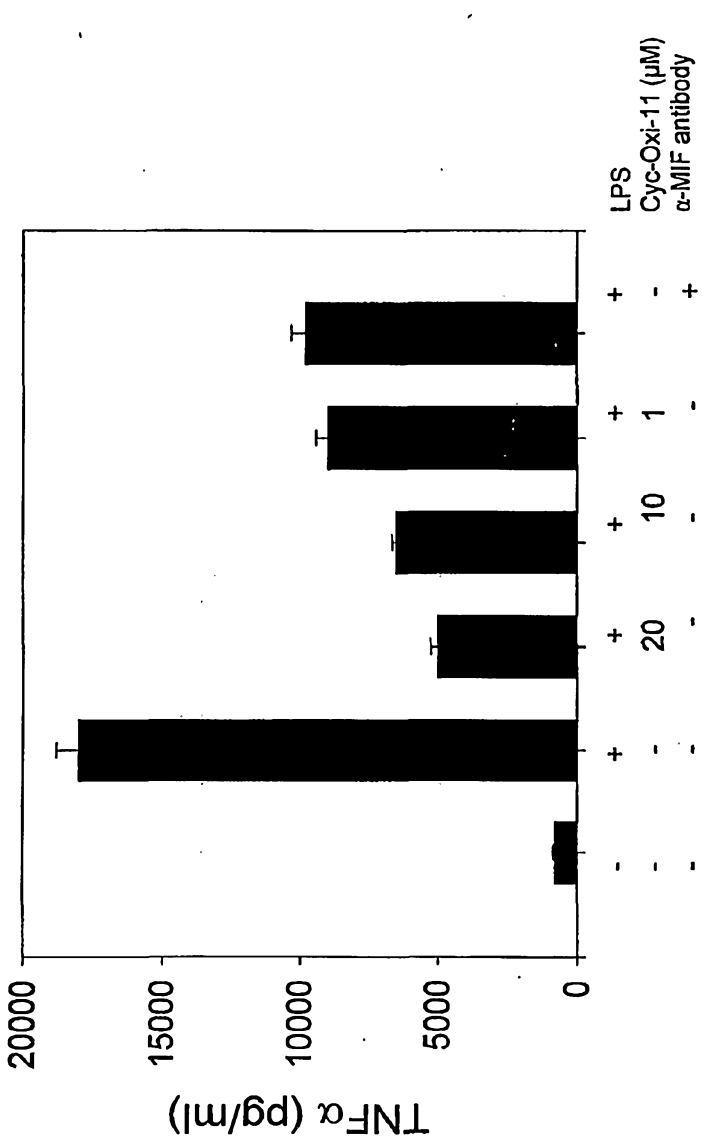

TNFα (pg/ml)
20000
15000
10000
5000
0
LPS
Cyc-Oxi-11 (μM)
α-MIF antibody
- + + + + +
- - 20 10 1 -
- - - - - +

FIG. 5

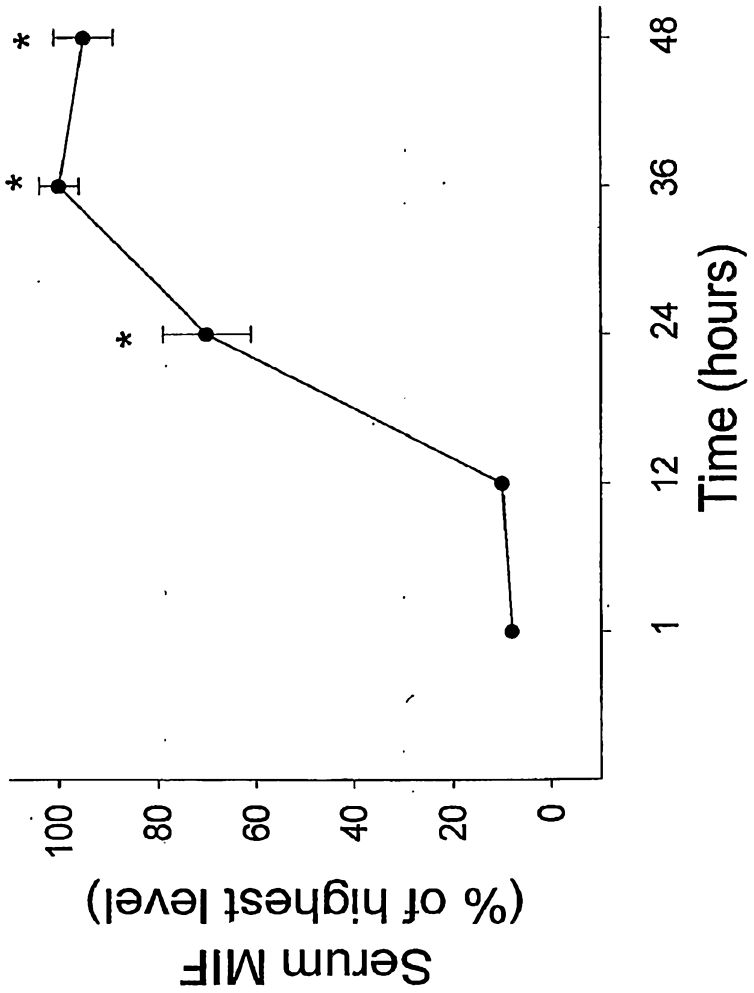

Serum MIF
(% of highest level)
100
80
60
40
20
0
1
12
24
36
48
Time (hours)
*
*
*

FIG. 6

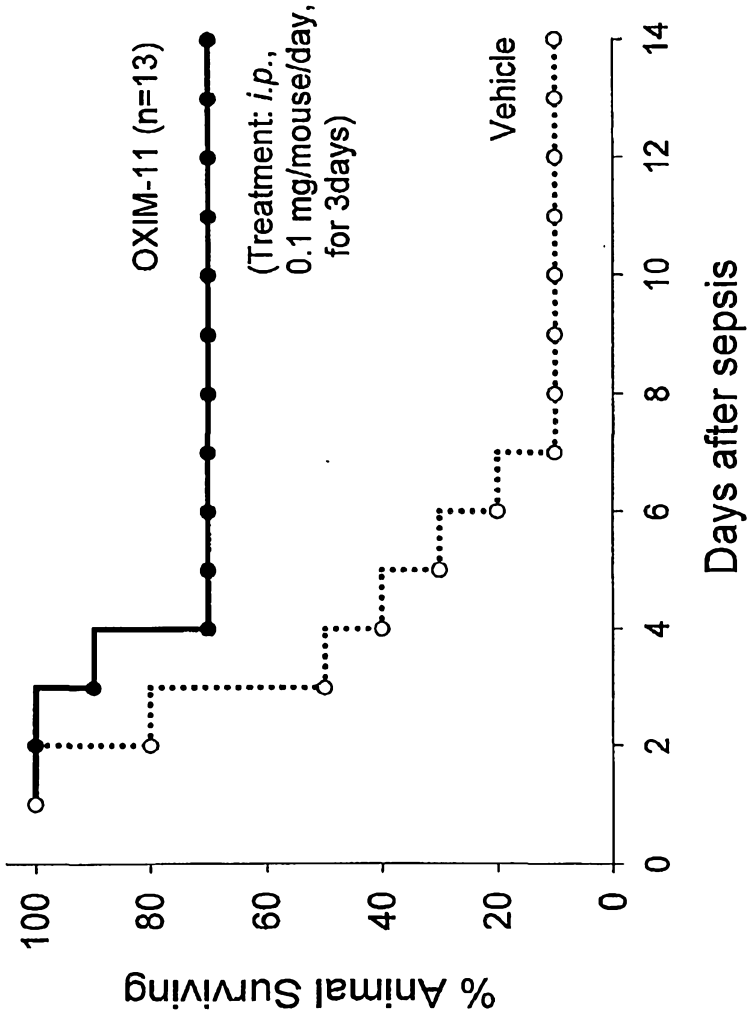
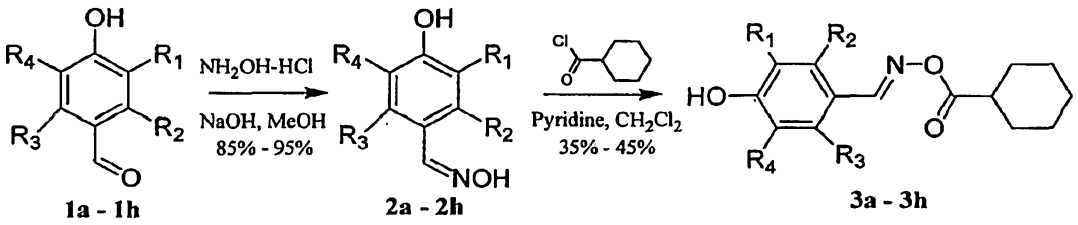

% Animal Surviving
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Days after sepsis
OXIM-11 (n=13)
(Treatment: i.p., 0.1 mg/mouse/day, for 3days)
Vehicle

FIG. 7

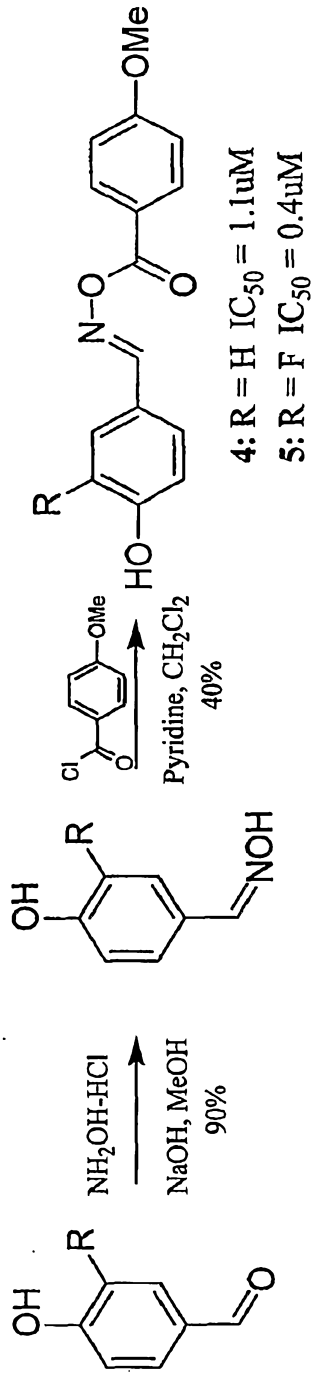


Compound	R_1	R_2	R_3	R_4	IC_{50}[a] (uM)	OH Chemical Shift[b] (ppm)
3a	H	H	H	H	1.3	9.05
3b	F	H	H	H	0.9	9.37
3c	F	H	H	F	80	10.79
3d	F	F	F	F	55	11.34
3e	Cl	H	H	H	15	9.55
3f	Br	H	H	H	40	9.66
3g	Br	H	H	Br	55	10.82
3h	I	H	H	I	50	10.74

[a]From ref. [11]

[b]Compounds dissolve in acetone-d_6 (100mM), the chemical shifts are reported in parts per million (ppm) relative to deuterated solvent peak.

FIG. 8



4: R = H IC_{50} = 1.1uM

5: R = F IC_{50} = 0.4uM