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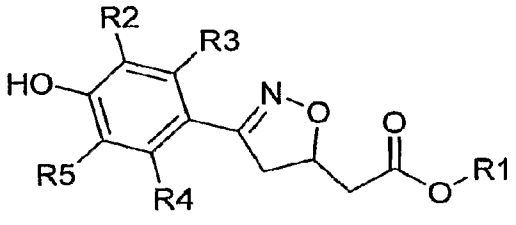
(54) Title: MODIFIED MACROPHAGE MIGRATION INHIBITORY FACTOR INHIBITORS



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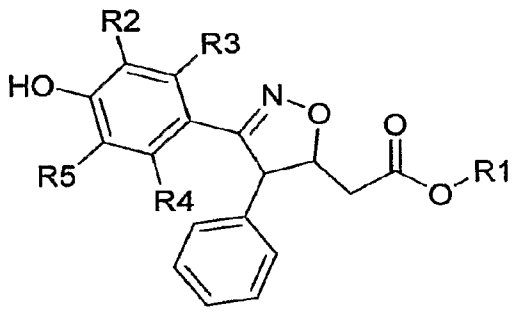


II

(57) Abstract: Provided are various compounds of Formula I (I). Also provided are various compounds of Formula II (II). Also provided are pharmaceutical compositions comprising the above 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. Further provided are methods of treating a mammal having sepsis, septicemia, and/or endotoxic shock. Also provided are methods of treating a mammal having an autoimmune disease, and methods of treating a mammal having a tumor.

WO 2007/112036 A3

MODIFIED MACROPHAGE MIGRATION INHIBITORY FACTOR INHIBITORS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 60/785,898, filed March 24, 2006.

BACKGROUND OF THE INVENTION

(1) Field of the Invention

The present invention relates to cytokine inhibitors. More specifically, the present invention identifies and characterizes several inhibitors of macrophage migration inhibitory factor.

(2) Description of the Related Art

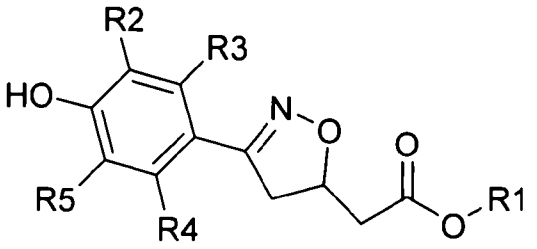
Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine, critically involved in the pathogenesis of inflammatory disorders (Calandra and Roger, 2003; Riedemann et al., 2003). Recent studies have clearly defined MIF as a critical factor in the pathophysiology of sepsis (Al-Abed et al., 2005). Abolition of MIF activity during sepsis by antibodies or ISO-1 improves cardio-circulatory efficiency and prevents the lethality associated with sepsis (Al-Abed et al., 2005; Lin et al., 2005). The specific inhibitor ISO-1, an isoxazoline, was designed to fit into the hydrophobic active site of MIF, an interaction confirmed by the crystal structure of the MIF complex with ISO-1 (FIG. l)(Lubetsky et al., 2002). Administration of ISO-1 in a clinically relevant model of sepsis confers moderate protection (80% versus 40% control). These results identify ISO-1 as the first small molecule inhibitor of MIF proinflammatory activities with therapeutic implications and indicate the potential of the MIF active site as a novel target for therapeutic interventions in human sepsis. Based on the above, identification of other isoxazolines that inhibit MIF is desired. The present invention addresses that need.

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.

SUMMARY OF THE INVENTION

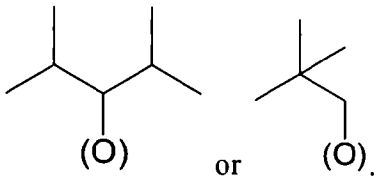
The inventor has identified and characterized several new compounds that inhibit MIF activity.

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

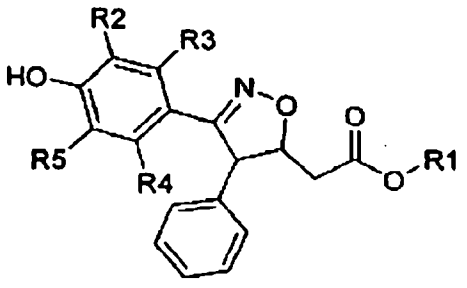


I

wherein R1 is a straight or branched C_1-C_{10} alkyl and R2, R3, R4 and R5 are independently F or H, wherein if all of R2, R3, R4 and R5 are H then R1 is



According to a second aspect, the present invention provides a compound of Formula II:



wherein Rl is a straight or branched C_1-C_{10} alkyl, and R2, R3, R4 and R5 are independently F or H, provided that not all of R2, R3, R4 and R5 are H.

According to a third aspect, the present invention provides a pharmaceutical composition comprising the compound of any one of first or second aspects, or a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable excipient.

According to a fourth aspect, the present invention provides a method of (i) treating or preventing inflammation in a mammal, (ii) treating a mammal having or at risk for sepsis, septicemia, and/or endotoxic shock, (iii) treating a mammal having or at risk for an autoimmune disease, or (iv) treating a mammal having a tumor, comprising administering the compound of the first or second aspect or the pharmaceutical composition of the third aspect to the mammal.

According to a fifth aspect, the present invention provides a use of a compound of the first or second aspect in the manufacture of a medicament for (i) treating or preventing inflammation in a mammal, (ii) treating a mammal having or at risk for sepsis, septicemia, and/or endotoxic shock, (iii) treating a mammal having or at risk for an autoimmune disease, or (iv) treating a mammal having a tumor.

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".

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a chemical pathway involving macrophage migration inhibitory factor (MIF), showing that MIF tautomerizes dopachrome methyl esters and also the structure of MIF inhibitor ISO-1.

FIG. 2 shows a chemical scheme for the synthesis of the ISO-1 -acid.

FIG. 3 shows a chemical scheme for the synthesis of stereoisomers of ISO-1 and Compound 17. The MIF inhibitory activity of the identified compounds is also provided.

FIG. 4 shows a chemical scheme for the synthesis of two diisopropylmethylester isoxazoline compounds. The MIF inhibitory activity of the identified compounds is also provided.

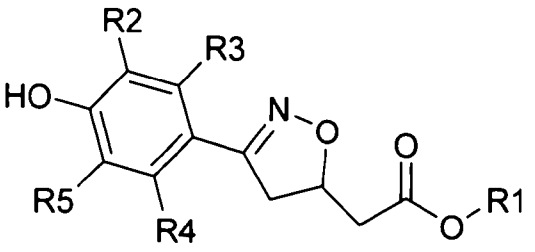
FIG. 5 is a graph of experimental results showing that ISO-63 inhibits leukocyte recruitment in an established model of acute inflammation. ** $p < 0.007$ ($n = 7$) relative to vehicle alone.

FIG. 6 is a graph of experimental results showing that ISO-63 inhibits the invasion of rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS) into Matragel.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides several new compounds that inhibit MIF activity. See Examples.

The present invention is thus directed to compounds of Formula I:

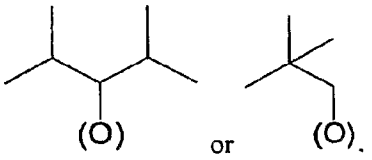


I

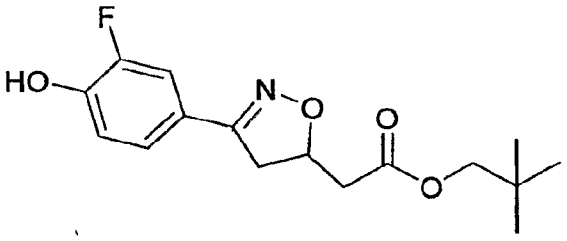
where Rl is a straight or branched C_1-C_{10} alkyl and R2, R3, R4 and R5 are independently an F or H, wherein if all of R2, R3, R4 and R5 is H then Rl is not CH_3. Preferably, only one of R2, R3, R4 and R5 is F. More preferably, R2 is F.

With some preferred compounds, all of R2, R3, R4 and R5 are H. With other preferred compounds, R2 is F.

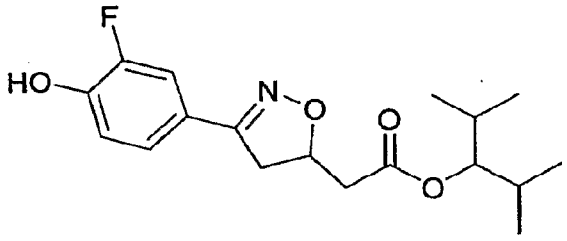
In additional preferred compounds, R1 is



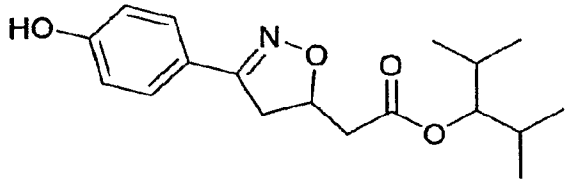
A more preferred compound is compound 17, having the formula



An additional preferred compound is ISO-63, having the formula

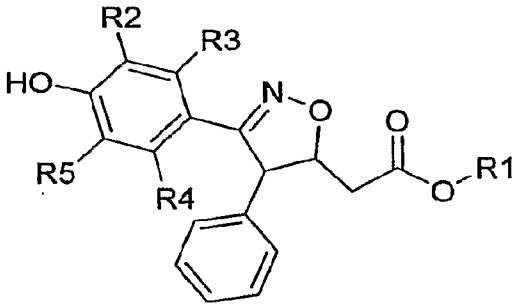


Still another preferred compound is ISO-60, having the formula



In the most preferred embodiments, the compound is an (R) isomer.

An important discovery related to this invention is that addition of one or more fluorine moieties on the aromatic ring of isoxazoline MIF inhibitors improves the inhibitory activity. See Example 1. Thus, other isoxazoline MIF inhibitors, such as those disclosed in U.S. Patent Application Publication No. 2005-0250826 A1, would be expected to be improved by addition of one or more fluorine moieties on the aromatic ring. Thus, the invention is also directed to a compound of Formula II:



II

where R1 is a straight or branched C_1-C_{10} alkyl, and R2, R3, R4 and R5 are independently F or H, provided that not all of R2, R3, R4 and R5 are H. Preferably, only one of R2, R3, R4 and R5 is F. More preferably, R2 is F. When R2 is F, it is preferred that R3, R4 and R5 are H.

The invention is also directed to pharmaceutical compositions comprising any of the above compounds, or a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier.

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 compounds can also be prepared for nasal administration. As used herein, nasal administration includes administering the compound to the mucous membranes of the nasal passage or nasal cavity of the patient. 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.

The compounds of the invention may be administered per se (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine, the salts should be both pharmacologically and pharmaceutically acceptable, but non-pharmaceutically acceptable salts

may conveniently be used to prepare the free active compound or pharmaceutically acceptable salts thereof. Pharmacologically and pharmaceutically acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicyclic, p-toluenesulfonic, tartaric, citric, methanesulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzenesulphonic. Also, pharmaceutically acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

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

These methods can be used on any mammal. Preferably, the mammal is a human. It is also preferred that 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. Non-limiting examples of such conditions include proliferative vascular disease, 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, 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, Berger's disease, Retier's syndrome and Hodgkins disease. A preferred such condition is sepsis, septicemia, and/or endotoxic shock.

MIF has been shown to play an important role in autoimmune disease. See, e.g., Cvetjovic et al., 2005. The present methods would thus be useful in treatment of autoimmune disease. Thus, in some aspect of these methods, the mammal has or is at risk for an autoimmune disease. Non-limiting examples of such autoimmune diseases are multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, graft versus host disease, autoimmune pulmonary inflammation, autoimmune encephalomyelitis, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, Crohn's disease, scleroderma, psoriasis, Sjögren's syndrome and autoimmune inflammatory eye disease.

MIF also is known to promote tumor invasion and metastasis. See, e.g., Sun et al., 2005. The present methods would therefore be useful for treatment of a mammal that has a tumor.

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

For these methods, the mammal is preferably a human. The mammal can have, or be at risk for, a disease involving inflammation, for example proliferative vascular disease, 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, alveolitis, 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, Berger's disease, Retier's syndrome, or Hodgkins disease.

Preferably, the mammal has sepsis, septicemia, and/or endotoxic shock, or is at risk for sepsis, septicemia, and/or endotoxic shock.

These methods can include the administration of a second anti-inflammatory agent to the mammal. Examples of such second anti-inflammatory agents are NSAIDs, salicylates, COX inhibitors, COX-2 inhibitors, and steroids. 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 VIII, an activated protein C, or an α2A-adrenergic antagonist.

The present invention is also directed to methods of treating a mammal having sepsis, septicemia, and/or endotoxic shock. The methods comprise administering the above pharmaceutical composition to the mammal in an amount effective to treat the sepsis, septicemia and/or endotoxic shock.

The invention is further directed to methods of treating a mammal having an autoimmune disease. The methods comprise administering the above pharmaceutical composition to the mammal in an amount effective to treat the autoimmune disease. Examples of such autoimmune diseases include multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, graft versus host disease, autoimmune pulmonary inflammation, autoimmune encephalomyelitis, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, Crohn's disease, scleroderma, psoriasis, Sjögren's syndrome and autoimmune inflammatory eye disease.

Additionally, the present invention is directed to methods of treating a mammal having a tumor, the method comprising administering the above pharmaceutical composition to the mammal in an amount effective to treat the tumor.

These compounds can be expected to be effectively administered orally. Thus, in any of the above methods, the pharmaceutical composition can be administered orally. Alternatively, the pharmaceutical composition can be administered parenterally.

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. Critical Modifications of the ISO-1 Scaffold Improve its Potent Inhibition of Macrophage Migration Inhibitory Factor (MIF) activity

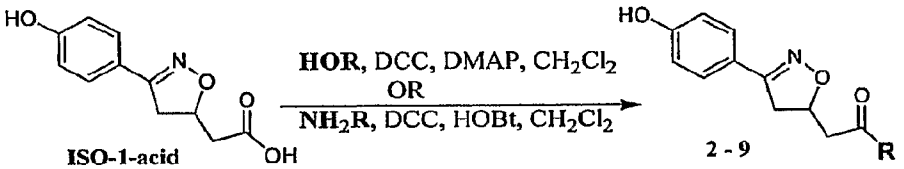
Example Summary

Based on the scaffold of (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1), an inhibitor of the proinflammatory cytokine MIF, two critical modifications and chiral resolution have significantly improved the potency of the inhibition. (R)-17 is 20-fold more potent than ISO-1 and inhibits MIF tautomerase activity with an IC50 of 550 nM.

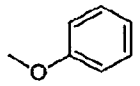
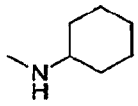
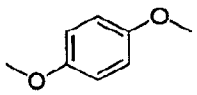
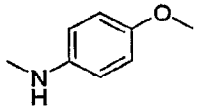
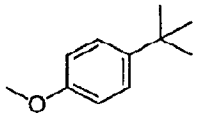
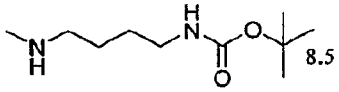
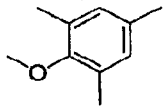
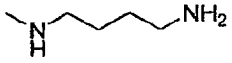
Results and Discussion

To improve the potency of ISO-1, the structure-activity relationship of ISO-1 was explored. Previously, critical functional groups were identified within the ISO-1 scaffold as evident by the loss of its MIF inhibitory effect upon methylation of the *para*-hydroxyl functional group, oxidation of 4,5-dihydro-isoxazole to isoxazole or reduction of methyl ester to alcohol (Lubetsky et al., 2002). Herein, it was discovered that mono-fluorination onto the ortho position of the phenolic group of ISO-1 improved the inhibition of MIF activity up to 41% (FIG. 3). Also, the alkyl tail of ISO-1 was investigated with various ester and amide analogues. The new synthetic route provides the precursor (ISO-1-acid) in large scale (FIG. 2). Esterification and amide formation between the ISO-1-acid and alcohol (or amine) was accomplished using a standard DCC coupling protocol (DCC, DMAP or HOBt) (Table 1).

Table 1. Synthesis of Compounds 2 - 9.

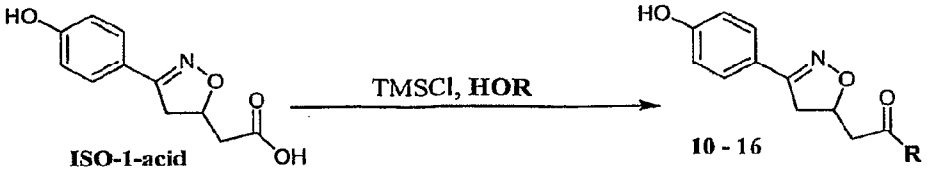


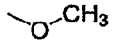
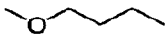
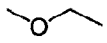
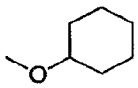
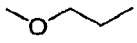
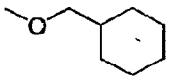
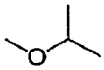
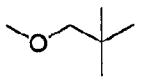
ISO-1-acid 2 - 9

Compd	R	IC_{50}* (uM)	Compd	R	IC_{50}* (uM)
2		10	6		24
3		8.5	7		26
4		10	8		8.5
5		20	9		>100

As summarized in Tables 1 and 2, we found that the ester analogues are more potent inhibitors than the amide counterpart (e.g. compounds **14** (IC_{50} = 2.5 μM) vs. **6** (IC_{50} = 24 μM) and **3** (IC_{50} = 8.5 μM) vs. **7** (IC_{50} = 26 μM)). We further investigated the influence of the bulkiness of the ester group on the potency of inhibiting MIF activity. The esterification process of ISO-1-acid was accomplished with TMSCl using the following alcohols: ethanol, **10**; 1-propanol, **11**; 2-propanol, **12**; 1-butanol, **13**; cyclohexanol, **14**; cyclohexylmethanol, **15** and neopentylalcohol, **16** (Table 2). As shown in Table 3, the most bulky alcohol (compound **16**, neopentyl ester analogue) shows a superior inhibition activity (IC_{50} = 1.5 μM) which is about ten times more potent than ISO-1.

Table 2. Synthesis of Compounds **10 - 16.**



Compd	R	IC_{50}* (uM)	Compd	R	IC_{50}* (uM)
ISO-1		11	13		3
10		12	14		2.5
11		4	15		5
12		6	16		1.5

The crystal structure analysis of MIF/ISO-1 complex predicts that the (R)-isomer of ISO-1 fits into the electron density better than (S)-isomer, suggesting that the (R)-isomer may bind with higher affinity to the MIF active site (Dios et al., 2002). Previously, the importance of the absolute configuration of the amino acid Schiff bases was shown for the inhibitory effect of MIF (Lubetsky et al., 2002). Hence, resolution of the optical active ISO-1 and determination of the inhibitory activity was needed for each isomer. Chiral resolution was accomplished as previously described through fractional crystallization of the diastereomeric cinchonidine salts of methylated ISO-1-acid (FIG. 3)(Wityak et al., 1997). The (S)-configuration salts were found to be less soluble and were crystallized out from the chiral mixture. The (S) and (R)-configuration salts were then acidified by 1N HCl, demethylated with BBr_3 and esterified with TMSCl in methanol to yield (S)-ISO-1 (90% ee) and (R)-ISO-1 (90% ee). Both isomers were tested for activity in an MIF dopachrome tautomerase assay. (R)-ISO-1 inhibited MIF tautomerase activity with an IC_{50} of about 7 μM, but (S)-ISO-1 was 50% less active with an IC_{50} of about 13 μM (FIG. 3).

In order to obtain the most potent, specific small molecule MIF inhibitor, three critical steps need to be integrated into the ISO-1 scaffold: (1) fluorination of the phenolic group of ISO-1, preferably mono-fluorination onto the ortho-position of the phenolic group; (2) a bulkier functional group replacing the methyl ester of ISO-1, such as a neopentyl ester; and (3) chiral

resolution to obtain an (R)-isomer. Thus, compound **17** (FIG. 3) was optimized to be a potent inhibitor of MIF activity with an IC_{50} of 750 nM. After the classical chiral resolution, (R)-**17** inhibited MIF tautomerase activity with an IC_{50} of 550 nM, while the (S)-**17** was 50% less active with an IC_{50} of about 1.1 μM (FIG. 3). Compound (R)-**17** is 20 times more potent than the parent compound ISO-1.

After two critical modifications on ISO-1 scaffold, we have improved the inhibition of MIF tautomerase activity to a nano-molar concentration (550 nM), which is about 20 times more potent than ISO-1.

Materials and Methods

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 (50 mM potassium phosphate, pH 7.2). Then *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.

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 I_2 vapor. NMR spectra were preformed on a Jeol Eclipse 270 spectrometer at 270 MHz for ^1H NMR spectra and 67.5 MHz for the ^{13}C NMR spectra. Coupling constants are reported in Hertz (Hz), and chemical shifts are reported in parts per million (ppm) relative to 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 or positive-ion mode.

Preparation of 4-Methoxybenzaldehyde Oximė. To a solution of 4-methoxybenzaldehyde (5.0 g, 36.8 mmol) in methanol (300 mL) was added hydroxylamine hydrochloride (7.6 g, 110.4 mmol) and 2N NaOH (37 mL, 73.6 mmol). The mixture was stirred at room temperature for 6 hours. The mixture was neutralized to pH 4 by using 1N HCl. Excess methanol was removed in vacuo to precipitate out the oxime. The precipitations were filtered and washed with water. The product was dried under vacuum and yield a white solid (4.9g, 88%): ^1H NMR (270 MHz, acetone-d_6) δ 8.07 (s, 1H), 7.55 (d, $J = 8.2$ Hz, 2H), 6.95 (d, $J = 8.2$ Hz, 2H), 3.82 (s, 3H).

Preparation of ISO-1-acid. To a solution of 4-methoxybenzaldoxime (4g, 26 mmol) in anhydrous DMF (500 mL) was added NCS (5.2g, 39 mmol). The reaction mixture was stirred for 5 hours at RT affording the chloro ovime. To this solution, vinylacetic acid (6.6 mL, 78 mmol) was added, followed by the dropwise addition of triethylamine (5.5 mL, 39 mmol) in DMF (50 mL). The reaction mixture was stirred under N_2 at RT for 48 hours. The solvent was removed *in vacuo* and the residue was taken up in EtOAc. The EtOAc solution was washed with 0.5N HCl, water, brine, and dried with anhydrous $MgSO_4$. The final solution was concentrated *in vacuo* and dried under vacuum pump to afford **1** in quantitative yield. To a solution of **1** (40 – 50 mM in dry dichloromethane) was treated with an excess (8 – 10 equivalence) of boron tribromide (1M solution in dichloromethane, Aldrich cat #: 211222) at 0 °C under N_2. The reaction mixture was allowed to reach room temperature over 5 – 6 hrs and then quenched with aqueous saturated $NaHCO_3$ (caution: BBr_3 reacts violently with water!!!). The mixture was stirred for ½ hr and then diluted with water and CH_2Cl_2. The organic layer was separated from the aqueous layer and discarded. The aqueous portion was neutralized with 1N HCl to pH 4 and extracted with EtOAc. The combined EtOAc solution was washed with brine and dried with anhydrous $MgSO_4$ to afford ISO-1-acid as pale yellow powder in good yield (75%). ^{1}H NMR (300 MHz, acetone-d_6) δ 10.65 (br, 1H), 8.75 (s, 1H), 7.52 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 5.01 (m, 1H), 3.50 (m, 1H), 3.05 (m, 1H), 2.68 (m, 2H); ESI-MS *m/z* 220 (M^-).

General DCC Coupling Procedure for Formation of Esters or Amides. To a solution of ISO-1-acid (100 mM in dry dichloromethane) was treated with 1.1 equivalences DCC, 0.2 equivalences DMAP and 1.5 equivalences alcohols (or 0.2 equivalences HOBt and 1.5 equivalences amines). The mixture was stirred for 8 hrs at RT. The formed white precipitate was filtered off and washed with CH_2Cl_2 and the filtrate was evaporated to dryness. The residue was purified on silica gel (hexane/EtOAc/MeOH 4/3/1) to give the esters or amides as a white solid. Compound **2** (65% yield): ^{1}H NMR (300 MHz, acetone-d_6) δ 8.75 (br, 1H), 7.54 (d, J = 8.7 Hz, 2H), 7.38 (m, 2H), 7.22 (m, 1H), 7.11 (m, 2H), 6.86 (d, J = 8.7 Hz, 2H), 5.10 (m, 1H), 3.54 (m, 1H), 3.27 (m, 1H), 2.96 (m, 2H); ESI-MS *m/z* 296 (M^-). Compound **3** (60% yield): ^{1}H NMR (300 MHz, acetone-d_6) δ 8.78 (s, 1H), 7.52 (d, J = 8.7 Hz, 2H), 7.02 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 5.10 (m, 1H), 3.76 (s, 3H), 3.53 (m, 1H), 3.25 (m, 1H), 2.76 (m, 2H); ESI-MS *m/z* 326 (M^-). Compound **4** (40% yield): ^{1}H NMR (300 MHz, acetone-d_6) δ 8.75 (br, 1H), 7.55 (d, J = 8.7 Hz, 2H), 7.39 (d, J = 8.7 Hz, 2H), 7.03 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 5.11 (m, 1H), 3.55 (m, 1H), 3.23 (m, 1H), 2.95 (m, 2H), 1.30 (s, 9H); ESI-MS *m/z* 352 (M^-). Compound **5** (30% yield): ^{1}H NMR (300 MHz, acetone-d_6) δ 8.75 (s, 1H), 7.72 (d, J = 8.7 Hz, 2H), 7.17 (d, J = 8.7 Hz, 2H), 6.86 (s, 2H), 5.10 (m, 1H), 3.55 (m, 1H), 3.25 (m, 1H), 2.96 (m, 2H), 2.05 (s, 9H); ESI-MS *m/z* 338 (M^-). Compound **6** (80% yield): ^{1}H NMR (300 MHz,

acetone-d_6) δ 8.79 (s, 1H), 7.52 (d, J = 8.7 Hz, 2H), 7.00 (br, 1H), 6.85 (d, J = 8.7 Hz, 2H), 4.94 (m, 1H), 3.64 (m, 1H), 3.42 (m, 1H), 3.10 (m, 1H), 2.52 (m, 1H), 2.37 (m, 1H) 1.90 – 1.00 (m, 10H); ESI-MS *m/z* 301 (M^-). Compound 7 (88% yield): ^{1}H NMR (300 MHz, acetone-d_6) δ 9.10 (br, 1H), 8.80 (br, 1H), 7.53 (m, 4H), 6.84 (m, 4H), 5.06 (m, 1H), 3.72 (s, 3H), 3.49 (m, 1H), 3.17 (m, 1H), 2.73(m, 1H), 2.60 (m, 1H); ESI-MS *m/z* 325 (M^-). Compound **8** (95% yield) ^{1}H NMR (270 MHz, acetone-d_6) δ 7.52 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 6.07 (br, 1H), 5.02 (m, 1H), 3.46 (m, 1H), 3.13 (m, 5H), 2.56 (m, 2H), 1.51 (m, 4H), 1.38 (s, 9H); ESI-MS *m/z* 414 ($M+Na^+$). Compound **9** (90% yield) ^{1}H NMR (270 MHz, acetone-d_6) δ 8.63 (br, 1H), 7.52 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.04 (m, 1H), 3.84 (m, 2H), 3.28 (m, 3H), 2.58 (m, 3H), 1.86 (m, 2H), 1.65 (m, 2H); ESI-MS *m/z* 292 (M^+).

General TMSCl Esterification Procedure. To a solution of ISO-1-acid (50 mg. 0.23 mmol) in a 3 mL alcohol (ethanol, **10**; 1-propanol, **11**; 2-propanol, **12**; 1-butanol, **13**; cyclohexanol, **14**; cyclohexylmethanol, **15** and neopentylalcohol, **16**) was added 0.1 mL TMSCl. The mixture was stirred for 2 hrs at RT (for **14**, **15** and **16**: 3 hrs at 50 °C). The mixture was evaporated to dryness and the residue was subjected to purification on silica gel (hexane/EtOAc 4/3) to afford white solid or pale yellow oil in quantitative yield. Compound **10**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.74 (s, 1H), 7.52 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 4.97 (m, 1H), 4.10 (q, 2H), 3.51 (m, 1H), 3.12 (m, 1H), 2.66 (m, 2H) 1.19 (t, 3H); ESI-MS *m/z* 248 (M^-). Compound **11**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.75 (s, 1H), 7.51 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 4.98 (m, 1H), 4.01 (t, 2H), 3.51 (m, 1H), 3.15 (m, 1H), 2.66 (m, 2H) 1.60 (m, 2H), 0.89 (t, 3H); ESI-MS *m/z* 262 (M^-). Compound **12**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.74 (s, 1H), 7.51 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 4.97 (m, 2H), 4.72 (m, 1H), 3.51 (m, 1H), 3.12 (m, 1H), 2.63 (m, 2H) 1.18 (d, j = 6.3 Hz, 6H); ESI-MS *m/z* 262 (M^-). Compound **13**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.78 (s, 1H), 7.52 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 4.99 (m, 1H), 4.05 (t, 2H), 3.51 (m, 1H), 3.12 (m, 1H), 2.68 (m, 2H) 1.10 – 1.60 (m, 4H), 0.88 (t, 3H); ESI-MS *m/z* 276 (M^-). Compound **14**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.84 (br, 1H), 7.52 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 4.98 (m, 1H), 4.72 (m, 1H), 3.51 (m, 1H), 3.15 (m, 1H), 2.66 (m, 2H) 1.90 – 1.20 (m, 10H); ESI-MS *m/z* 302 (M^-). Compound **15**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.78 (s, 1H), 7.55 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 5.02 (m, 1H), 3.90 (d, J = 6.7 Hz, 2H), 3.51 (m, 1H), 3.15 (m, 1H), 2.72 (m, 2H) 1.80 – 0.90 (m, 11H); ESI-MS *m/z* 302 (M^-). Compound **16**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.79 (s, 1H), 7.54 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 5.05 (m, 1H), 3.82 (m, 2H), 3.52 (m, 1H), 3.18 (m, 1H), 2.75 (m, 2H) 0.96 (s, 9H); ESI-MS *m/z* 292 (M^+).

Classical Resolution of acid **1** via Crystallation of the Cinchonidine Salts. **(R,S)-1** (1.3g, 5.5 mmol) was dissolved in hot acetone (25 mL), cinchonidine (1.61g, 5.5 mmol) was added, and

the solution was cool to RT and allowed to stand at –20 °C overnight. The resulting white solid was filtered to give **(S)-1** salts. The filtrate was concentrated in vacuo to afford **(R)-1** salts. To a solution of **(R)-1** salts or **(S)-1** salts (200 mg, 0.4 mmol) in chloroform (3 mL) was added 1N HCl in ether (3 mL, 3 mmol). The resulting white precipitate was filtered off and the filtrate was concentrated in vacuo to give **(R)-1** or **(S)-1** in quantitative yield.

Example 2. Effect of isoxazoline MIF inhibitors on leukocyte recruitment in response to acute inflammation.

Air pouches were made according to standard procedures (Garcia-Ramallo et al., 2002) on Swiss Webster male mice (25-30 g) by injecting sterile air s.c. on day 0 (6 ml) and day 3 (3 ml). On day 6, animals were treated with a single i.p. injection with either vehicle (350 µl of 20% DMSO), ISO-1 (40 mg/kg), or ISO-63 (40 mg/kg). After 15 min, the animals were challenged by injecting 1 ml 1% carrageenan (in PBS) into the air pouch cavity. Five hr after carrageenan injection the animals were sacrificed, the pouches washed with PBS, exudate collected, and the total number of infiltrating cells quantitated.

FIG. 5 summarizes the results of this assay. The ISO-63 treatment, but not the ISO-1 treatment, caused a significant reduction in leukocyte recruitment in response to acute inflammation.

Example 3. Effect of isoxazoline MIF inhibitors on invasion of rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS) into Matrigel.

Invasion was assayed by measuring cell invasion through Matrigel Invasion Chambers (Becton Dickinson, MA). One day after treatment of RA-FLS with 25 µM ISO-1 or 100 nM ISO-63, 4 x 10^4 cells were placed in the upper chamber in serum-free medium. 500 µl of whole medium containing 10% FBS and 10% human serum and was added to the bottom chamber. After 24 hours of incubation at 37 °C, cells on the upper surface of the filter were wiped off with a Q-tip and the filter was fixed in 4% formaldehyde/PBS. After staining with crystal violet, migrated cells were counted using an inverted microscope.

FIG. 6 summarizes the results of this assay. The ISO-63 treatment reduced cell invasion to a much greater extent than the ISO-1 treatment.

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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.

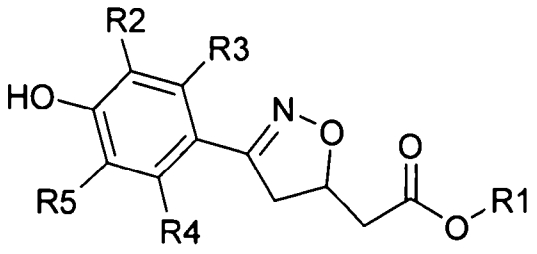
As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

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.

2007230948 06 Jun 2012

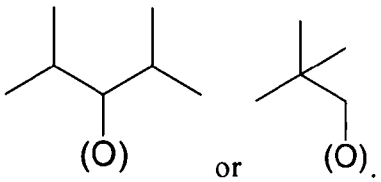
CLAIMS

1. A compound of Formula I:



I

wherein R1 is a straight or branched C_1-C_{10} alkyl and R2, R3, R4 and R5 are independently F or H, wherein if all of R2, R3, R4 and R5 are H then R1 is

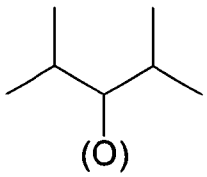


2. The compound of claim 1, wherein only one of R2, R3, R4 and R5 is F.

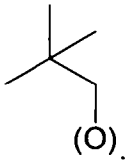
3. The compound of claim 2, wherein R2 is F.

4. The compound of claim 1, wherein all of R2, R3, R4 and R5 is H.

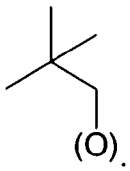
5. The compound of claim 1, wherein R1 is



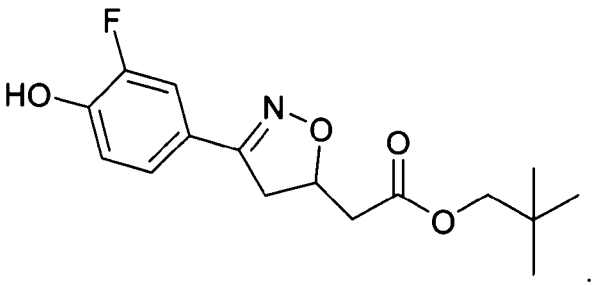
6. The compound of claim 1, wherein R1 is



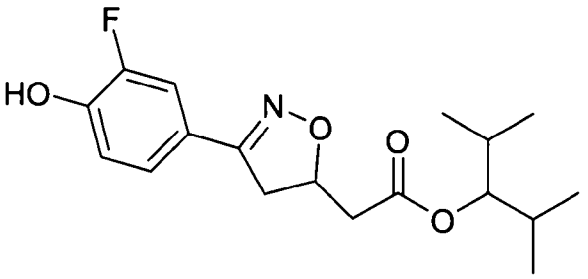
7. The compound of claim 4, wherein R1 is



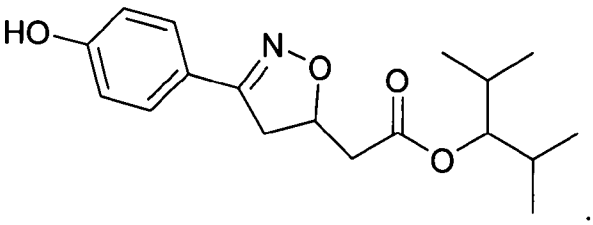
8. The compound of claim 1, wherein the compound is compound 17, having the formula



9. The compound of claim 1, wherein the compound is ISO-63, having the formula



10. The compound of claim 1, wherein the compound is ISO-60, having the formula

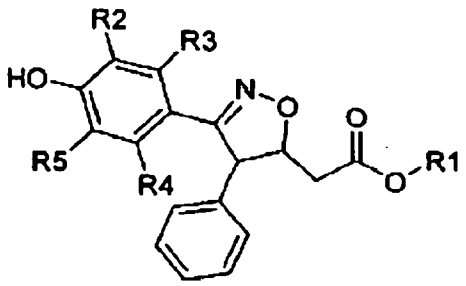


11. The compound of any one of claims 1-10, wherein the compound is an (R) isomer.

12. The compound of any one of claims 1-10, wherein the compound is an (S) isomer.

13. The compound of any one of claims 1-4, 11 or 12, wherein R1 is a C_3-C_{10} alkyl.

14. A compound of Formula II:



wherein R1 is a straight or branched C_1-C_{10} alkyl, and R2, R3, R4 and R5 are independently F or H, provided that not all of R2, R3, R4 and R5 are H.

15. The compound of claim 14, wherein only one of R2, R3, R4 and R5 is F.

16. The compound of claim 14 or 15, wherein R2 is F.

17. A pharmaceutical composition comprising the compound of any one of claims 1-16, or a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable excipient.

18. A method of (i) treating or preventing inflammation in a mammal, (ii) treating a mammal having or at risk for sepsis, septicemia, and/or endotoxic shock, (iii) treating a mammal having or at risk for an autoimmune disease, or (iv) treating a mammal having a tumor, comprising administering the compound of any one of claims 1-16 or the pharmaceutical composition of claim 17 to the mammal.

19. The method of claim 18, 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, or the mammal has or is at risk for an autoimmune disease.

20. The method of claim 19, wherein the condition is proliferative vascular disease, 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, 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, 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, Berger's disease, Retier's syndrome, or Hodgkins disease, and wherein the autoimmune disease is multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, graft versus host disease, autoimmune pulmonary inflammation, autoimmune encephalomyelitis, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, Crohn's disease, scleroderma, psoriasis, Sjögren's syndrome or autoimmune inflammatory eye disease.

21. The method of any one of claims 18-20, further comprising administering a second anti-inflammatory agent to the mammal.

22. The method of claim 21, wherein the second anti-inflammatory agent is an NSAID, a salicylate, a COX inhibitor, a COX-2 inhibitor, or a steroid.

23. The method of claim 21, wherein 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 VIII, an activated protein C, or an α2A-adrenergic antagonist.

24. Use of a compound of any one of claims 1-16 in the manufacture of a medicament for (i) treating or preventing inflammation in a mammal, (ii) treating a mammal having or at risk for sepsis, septicemia, and/or endotoxic shock, (iii) treating a mammal having or at risk for an autoimmune disease, or (iv) treating a mammal having a tumor.

25. The use of claim 24, 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, or the mammal has or is at risk for an autoimmune disease.

26. The use of claim 25, wherein the condition is proliferative vascular disease, 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, 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, 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

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rejection, graft-versus-host disease, ankylosing spondylitis, Berger's disease, type 1 diabetes, type 2 diabetes, Berger's disease, Retier's syndrome, or Hodgkins disease, and wherein the autoimmune disease is multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, graft versus host disease, autoimmune pulmonary inflammation, autoimmune encephalomyelitis, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, Crohn's disease, scleroderma, psoriasis, Sjögren's syndrome or autoimmune inflammatory eye disease.

27. The use of any one of claims 24-26, wherein the medicament is for administration in conjunction with a second anti-inflammatory agent.

28. The use of claim 27, wherein the second anti-inflammatory agent is an NSAID, a salicylate, a COX inhibitor, a COX-2 inhibitor, or a steroid.

29. The use of claim 27, wherein 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 VIII, an activated protein C, or an α2A-adrenergic antagonist.

30. A compound according to claim 1; or a pharmaceutical composition according to claim 17; or a method according to claim 18; or a use according to claim 24, substantially as herein described with reference to any one of the embodiments of the invention illustrated in the accompanying drawings and/or examples.

FIG. 1

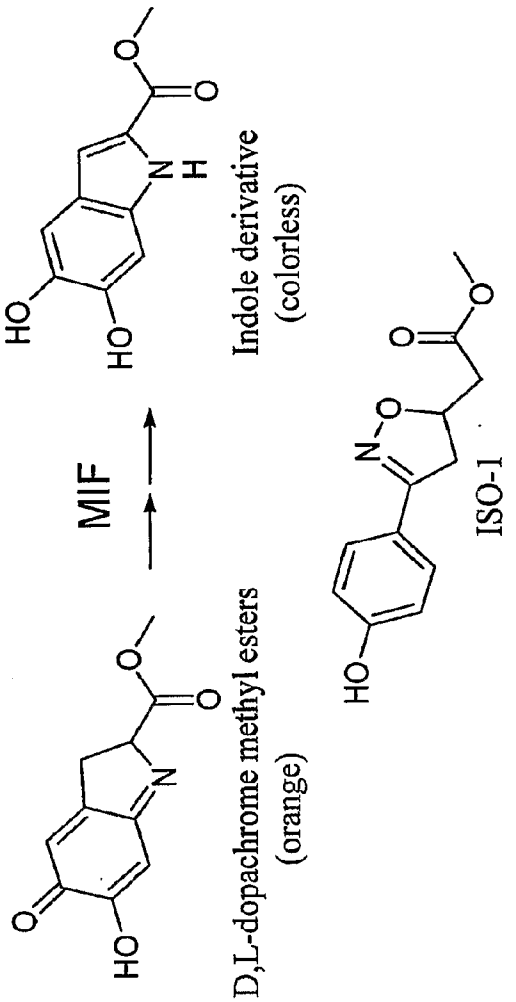

MIF
D,L-dopachrome methyl esters
(orange)
Indole derivative
(colorless)
ISO-1

FIG. 2

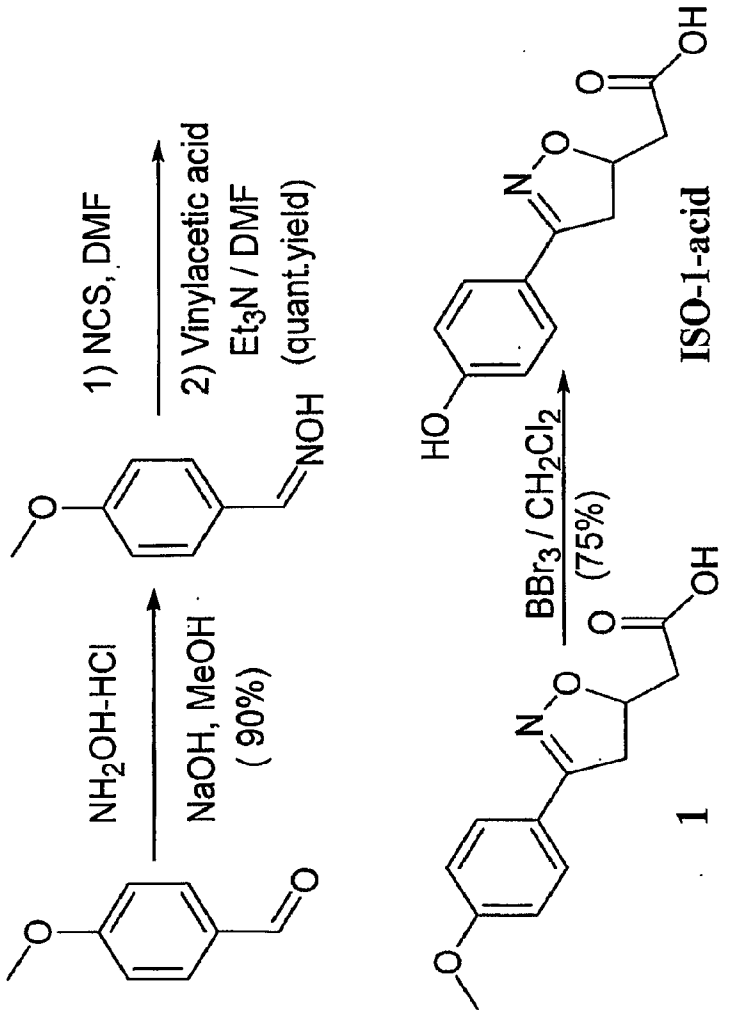

NH2OH-HCl
NaOH, MeOH
(90%)
1) NCS, DMF
2) Vinylacetic acid
Et3N / DMF
(quant.yield)
NOH
BBr3 / CH2Cl2
(75%)
HO
OH
ISO-1-acid
1

FIG. 3

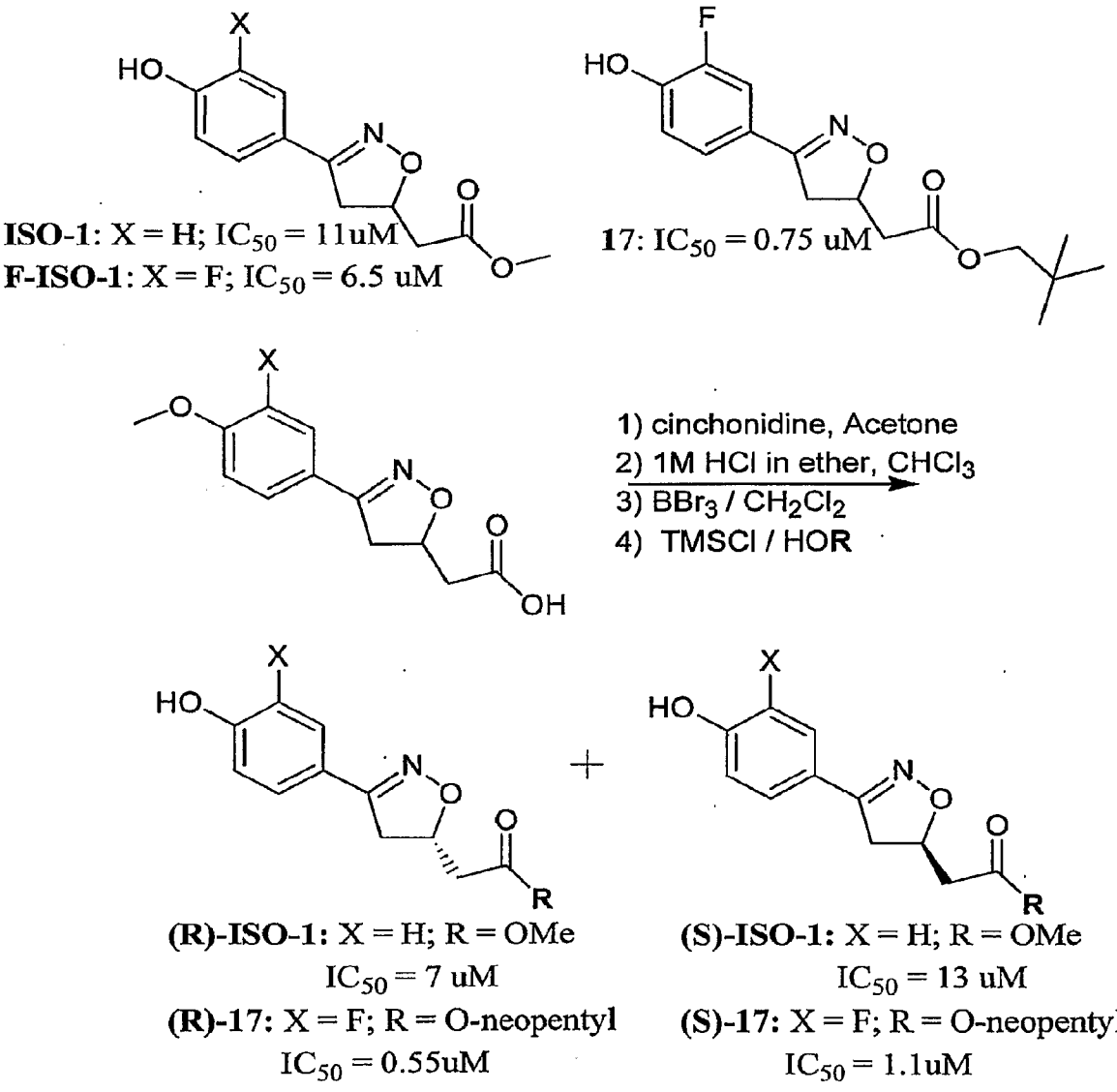

X
HO
N
O
O
O
ISO-1: X = H; IC50 = 11uM
F-ISO-1: X = F; IC50 = 6.5 uM
F
HO
17: IC50 = 0.75 uM
1) cinchonidine, Acetone
2) 1M HCl in ether, CHCl3
3) BBr3 / CH2Cl2
4) TMSCl / HOR
OH
R
(R)-ISO-1: X = H; R = OMe
IC50 = 7 uM
(R)-17: X = F; R = O-neopentyl
IC50 = 0.55uM
(S)-ISO-1: X = H; R = OMe
IC50 = 13 uM
(S)-17: X = F; R = O-neopentyl
IC50 = 1.1uM

FIG. 4

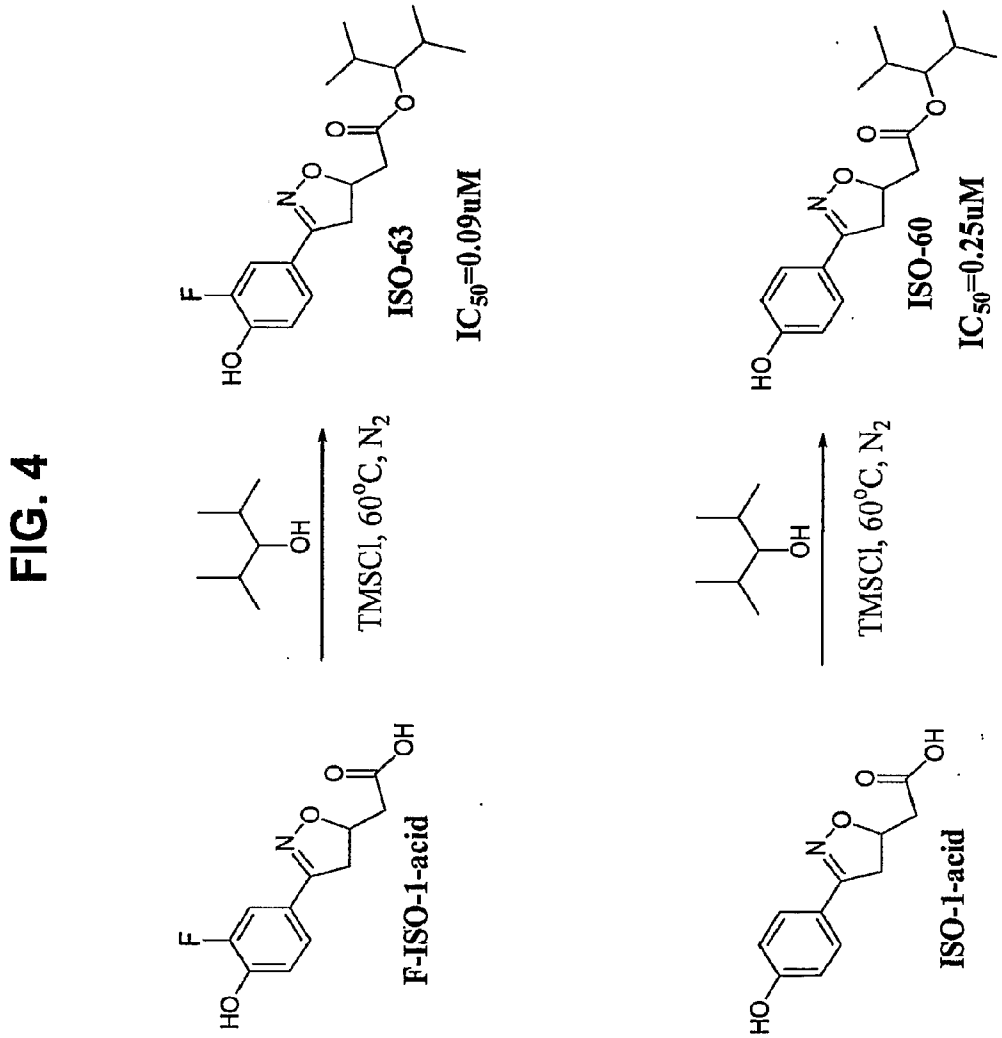

F-ISO-1-acid
TMSCl, 60°C, N2
ISO-63
IC50=0.09uM
ISO-1-acid
TMSCl, 60°C, N2
ISO-60
IC50=0.25uM

FIG. 5

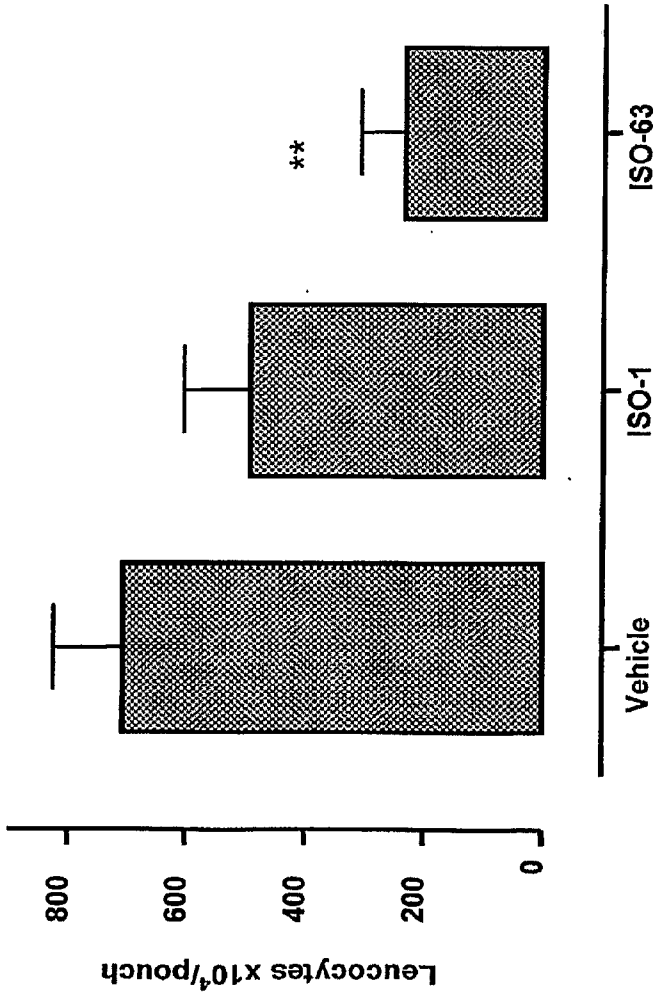
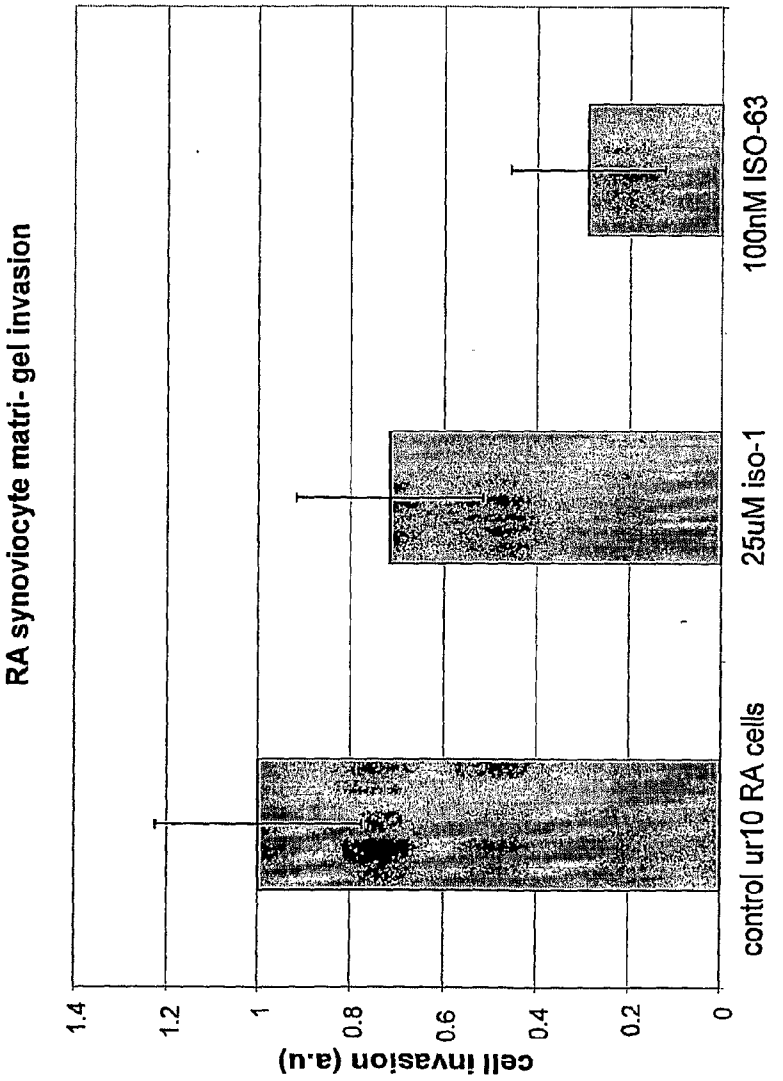


FIG. 6


RA synoviocyte matri- gel invasion
cell invasion (a.u)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
control ur10 RA cells
25uM iso-1
100nM ISO-63