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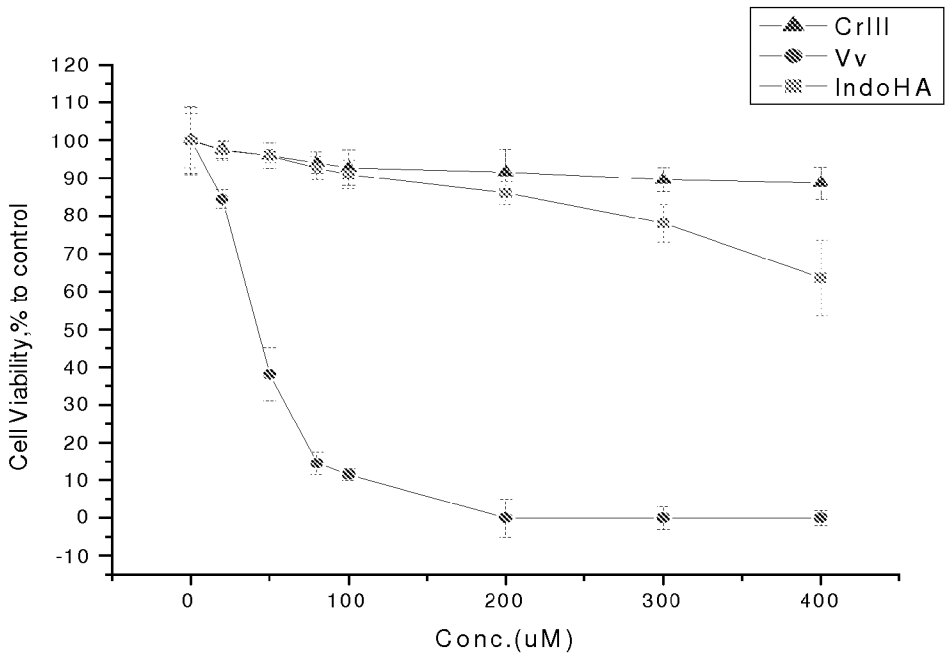
(54) Title: METAL COMPLEXES HAVING ANTI-INFLAMMATORY ACTIVITY



(57) Abstract: There are described metal complexes of anti-inflammatory ligands useful in the treatment of one or more of inflammation, cancer, diabetes, cardiovascular and other conditions.

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METAL COMPLEXES HAVING ANTI-INFLAMMATORY ACTIVITY

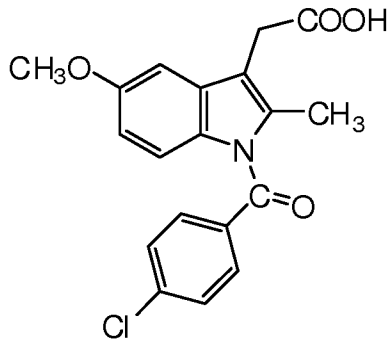
FIELD OF THE INVENTION

The invention relates to metal complexes having anti-inflammatory activity. The compounds find application in the prophylaxis and treatment of inflammation, cancer, pain, microbial infections including bacterial and viral infections, and other conditions in humans and animals.

BACKGROUND

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of a variety of inflammatory conditions in humans and animals. NSAIDs are, for example, used to treat inflammatory conditions such as rheumatoid arthritis, osteoarthritis, acute musculoskeletal disorders (such as tendonitis, sprains and strains), lower back pain (commonly referred to as lumbago), and inflammation, pain and oedema following surgical or non-surgical procedures. However, many NSAIDs cause adverse effects in humans and animals, particularly adverse gastrointestinal (GI) effects.

Indomethacin (IndoH) is a NSAID and is effective in treating inflammatory conditions in humans and animals. The structure of indomethacin is as follows:



However, indomethacin can cause severe adverse gastrointestinal effects in humans and animals, particularly when administered orally. In humans, oral administration of indomethacin can cause ulcerations in the oesophagus, stomach, duodenum and intestines, and some fatalities have been reported. In dogs, oral administration of indomethacin causes fatal gastrointestinal haemorrhaging. Other effects associated with oral administration of indomethacin include: (a) inhibition of platelet aggregation, (b) cardiovascular effects (fluid retention and peripheral oedema), (c) ocular effects (corneal deposits and retinal disturbances), (d) central nervous system effects (headaches and dizziness), (e) masking of infections due to antipyretic properties, (f) renal effects (as with other NSAIDs, there have been reports of acute interstitial nephritis with hematuria, proteinuria and, occasionally, nephrotic syndrome in patients receiving long-term administration of indomethacin). Studies have also shown that administration of indomethacin by other routes, e.g. as a suppository or by topical application (e.g. Amico-Roxas, M.; Matera, M.; Caruso, A.; Puglisi, G.; Bernardi, R.; Rinaldo, G. *Rivista Europea per le Scienze Mediche e Farmacologiche* **1982**, *4*, 199-204), also results in adverse effects.

These adverse effects have limited the use of indomethacin in the treatment of inflammation in humans and animals.

It has been reported that dinuclear metal complexes of indomethacin (i.e. complexes containing two metal coordination centres) cause less adverse gastrointestinal effects, and result in improved uptake of the drug, compared to the free indomethacin. For example, the oral administration of the dinuclear copper(II) complex of indomethacin, bis(*N,N*-dimethylformamide)tetrakis-μ-(*O,O'*-Indo)dicopper(II) ($[Cu_2(Indo)_4(DMF)_2]$, Indo is the deprotonated form of indomethacin), has been found to cause less gastrointestinal toxicity than indomethacin. The mechanism of the reduced gastrointestinal toxicity has not been elucidated, but is believed to be due to reduced interaction of the indomethacin with the COX-1 enzyme in the gastrointestinal tract. Compositions containing this complex sold under the name Cu-Algesic have been used in veterinary practice in Australia, New Zealand, South Africa and other countries. These compositions are in the form of a tablet or a paste.

Complexes of copper and dithiocarbamate have previously been studied for the treatment of melanoma. The mechanism proposed for the anti-melanoma effect of such complexes is that both the copper and the ligand affect the intracellular redox status, which makes melanoma cells more susceptible to these drugs because of the susceptibility of melanoma cells to apoptosis induced by reactive oxygen species. However, it appears that copper is not essential for the anti-melanoma effect as complexes of the redox-inactive Zn(II) and dithiocarbamate were also found to be effective (Cen, D.; Gonzalez, R. I.; Buckmeier, J. A.; Kahlon, R. S.; Tohidian, N. B.; Meyskens, F. L., Jr. *Mol. Cancer Ther.* **2002**, *1*, 197-204). It therefore seems that the presence of the dithiocarbamate ligand is necessary for the complex to exhibit anti-melanoma effects. In mouse models of melanoma no response to indomethacin was observed (Indomethacin and telomerase activity in tumor growth retardation. Lonnroth, C.; Andersson, M.; Lundholm, K. *Int. J. Oncology* **2001**, *18*, 929-937). A minimal effect of indomethacin on human melanoma lung metastases in nude mice models has been reported, although a combination of interleukin-2 (IL-2) with indomethacin led to a cure of the metastases in some cases (Cure of human melanoma lung metastases in nude mice with chronic indomethacin therapy combined with multiple rounds of IL-2: characteristics of killer cells generated in situ. Lala, P. K.; Elkashab, M.; Kerbel, R. S.; Parhar, R. S. *Int. Immunol.* **1990**, *2*, 1149-1158).

A range of human clinical trials have been conducted on the effects of indomethacin in combination therapy for the treatment of cancer (IL-1/indomethacin, IL-2/indomethacin, or IL-2/ranitidine/indomethacin). While there is some conflict as to whether there is any benefit of indomethacin in these clinical trials, the majority of the trials conclude that the benefit is marginal and that it contributes to adverse side effects such as renal toxicity (Phase II trial of interleukin-1 alpha and indomethacin in treatment of metastatic melanoma. Janik, J. E.; Miller, L. L.; Longo, D. L.; Powers, G. C.; Urba, W. J.; Kopp, W. C.; Gause, B. L.; Curti, B. D.; Fenton, R. G.; Oppenheim, J. J.; Conlon, K. C.; Holmlund, J. T.; Sznol, M.; Sharfman, W. H.; Steis, R. G.; Creekmore, S. P.; Alvord, W. G.; Beauchamp, A. E.; Smith, J. W., 2nd. *J. Natl. Cancer Inst.* **1996**, *88*, 44-49; Sustained indomethacin and ranitidine with intermittent continuous infusion of interleukin-2 in advanced malignant melanoma: a phase II study. Mertens, W. C.; Bramwell, V. H.;

Banerjee, D.; Gwadry-Sridhar, F.; Lala, P. K. *Clin. Oncol. (Roy. College Radiol. (Gt Brit.))* **1993**, *5*, 107-113; Indomethacin, ranitidine, and interleukin-2 in melanoma. Hamblin, T. J. *Lancet* **1992**, *340*, 8826; Marginal effect and interfered with treatment. Randomized trial of recombinant alpha 2b-interferon with or without indomethacin in patients with metastatic malignant melanoma. Miller, R. L.; Steis, R. G.; Clark, J. W.; Smith, J. W. 2nd; Crum, E.; McKnight, J. E.; Hawkins, M. J.; Jones, M. J.; Longo, D. L.; Urba, W. J. *Cancer Res.* **1989**, *49*, 1871-1876; Repetitive weekly cycles of interleukin-2. II. Clinical and immunologic effects of dose, schedule, and addition of indomethacin. Sosman, J. A.; Kohler, P. C.; Hank, J. A.; Moore, K. H.; Bechhofer, R.; Storer, B.; Sondel, P. M. *J. Natl. Cancer Inst.* **1988**, *80*, 1451-1461).

NSAIDs, including indomethacin and related NSAIDs, have been reported to have a chemoprotective effect against colorectal and other cancers although results from epidemiological studies have been variable (Turchanowa, L., Dauletbaev, N., Milovic, V., Stein, J. *Eur. J. Clin. Invest.* **2001**, *31*, 887-893; Collet, J.-P.; Sharpe, C.; Belzile, E.; Boivin, J.-F.; Hanley, J.; Abenhaim, L. *Brit. J. Cancer* **1999**, *81*, 62-68). It has also been reported that NSAIDs may enhance the anti-cancer activities of known anti-cancer drugs (Touhey, S.; O'Connor, R.; Plunkett, S.; Maguire, A.; Clynes, M. *Eur. J. Cancer* **2002**, *38*, 1661-1670). However, in some models of colorectal cancer, indomethacin was reported to increase mortality and metastases compared to control animals (Danzi, M.; Ferulano, G. P.; Abate, S.; Califano, G. *Carcinogenesis* **1984**, *5*, 287-289). Moreover, although other studies have reported anti-cancer activity in chemically induced colorectal cancers in rats, no such effect was found when a cultured cell line was injected into rats (Olsson, N. O.; Caignard, A.; Martin, M. S.; Martin, F. *Int. J. Immunopharmac.* **1984**, *6*, 329-334). More recent research has indicated that advanced solid tumour patients treated with indomethacin survive twice as long as do such patients who receive supportive care alone (Blanke, C. D. *Oncology (Williston Park, N.Y.)* **2002**, *16 (4 Suppl 3)*).

Cu-salicylate complexes (ie., $[Cu_2(3,5\text{-di-}iso\text{-propylsalicylate})_4L_2]$) have been shown to have a limited effect (no-statistically significant difference) in tumorogenisis in female C3H/HeNCR mice models of mammary cancer (Crispins, Jr., C. G.; Sorenson, J. R. J. *Anti-Cancer Res.* ***1992***, *12*, 1271-1273). Although these complexes had anti-cancer

activity against reticulum cell sarcoma in SJL/J mice if injected subcutaneously, they were toxic if injected via the i.p route (Crispins, Jr., C. G.; Sorenson, J. R. J. *Anti-Cancer Res.* **1988**, *8*, 77-79). In addition, the level of Cu in Cu-salicylate complexes required to have a therapeutic or strong prophylaxic effect is not appropriate for daily use in terms of potential Cu toxicity. Similarly, while IndoH has been included in topical formulations, it has not been shown to have a significant effect on either melanomas or squamous cell carcinomas when applied topically, and high concentrations of NSAIDs such as IndoH, can induce significant systemic toxicity when applied topically.

The NSAID aspirin (acetylsalicylic acid) is widely used in low dosages to prevent cardiovascular events and is generally prescribed as a standard treatment for prophylaxis of cardiac disease in high-risk patients. However, the effect of aspirin is not consistent, with a significant proportion of the population (up to 45%) being aspirin resistant ("Aspirin resistance: Definitions, mechanisms, prevalence, and clinical significance", Macchi, L., Sorel, N., Christiaens, L., Curr. Pharm. Des., 2006, 12(2), 251-258).

In recent years there have also been reports of increased risk of significant adverse side-effects associated with the long term use use of COX-2 selective NSAIDs. Data, for example, has suggested that COX-2 inhibitors such as rofecoxib, celecoxib, valecoxib and parecoxib may be associated with an increased risk of thrombotic events ("Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention", Scott, D. et al, NEJM, 2005, 352, 1071-1080; "Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery", NEJM, 2005, 352, 1081-1091; "Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial", NEJM, 2005, 352, 1092-1102). By contrast, the latest evaluation of all double blind clinical trials on non-selective NSAIDs showing some cardiovascular events has not been able to discern any significant differences between placebo controls and the NSAIDs (Salpeter, S. R.; Gregor, P.; Ormiston, T. M.; Whitlock, R.; Raina, P.; Thabane, L.; Topol, E. J. *Am. J. Med.* **2006**, *119*, 552-559).

The literature further indicates that at least some transition metals may have a role in the development of cardiovascular disease at the molecular level. In particular, both copper and zinc have been shown to accumulate in atherosclerotic plaque at a higher rate

than in surrounding vascular tissue ("Relationship of calcium, magnesium, zinc and copper concentrations in the arterial wall and serum in atherosclerosis obliterans and aneurysm", Iskra, M., Patelski, J., Majewski, W., J. Trace Elem. Med. Biol., 1997, 11(4), 248-252) suggesting that they play a role in cardiovascular pathogenesis.

Further, while copper-containing enzymes such as superoxide dismutase and ceruloplasmin are considered beneficial for the cardiovascular system, it has been suggested that free copper ions can catalyse an oxidative modification of low density lipoprotein, which is seen as a key event in atherogenesis ("The possible role of copper ions in atherogenesis: the Blue Janus", Ferns, G.A., Lamb, D.J., Taylor, A., Atherosclerosis, 1997, 133(2), 443-445). Zinc has also been implicated as a potential contributor to cardiovascular disease through its role in metal-containing proteins such as the matrix metalloproteinases, the inhibition of which has become a recent goal for cardiovascular drug development ("Matrix metalloproteinases: a therapeutic target in cardiovascular disease", Sierevogel, M.J., Pasterkamp, G., de Kleijn, D.P., Strauss, B.H., Curr. Pharm. Des., 2003, 9(13), 1033-1044). As a result, cardiovascular inflammatory related diseases have been treated by seeking to lower copper and zinc levels within the cardiovascular system or to reduce the risk of accumulation of these metals in the cardiovasculature.

SUMMARY OF THE INVENTION

The present invention relates to metal complexes having anti-inflammatory activity and their use in the prophylaxis or treatment of cancer, diseases and conditions associated with inflammation or which have an inflammatory component, pain, microbial and viral infections, and other conditions.

In a first aspect of the invention there is provided a metal complex of the following formula (1):

$$[M(L^1)_m(L^2)_n]^p \qquad (1)$$

wherein

M is a monovalent, divalent, trivalent, tetravalent, pentavalent or hexavalent metal

ion;

each L^1 is independently selected and is NH_3 or other monodentate ligand, a polydentate ligand, or a macrocyclic ligand;

each L^2 is a chelating derivative of a carboxylate or chelating amide or hydroximate NSAID, having anti-inflammatory activity, and at least one ligand L^2 is other than a salicylate or a derivative of a salicylate;

m is 0, 1, 2, 3, 4, or 5;

n is 1, 2, 3 or 4; and

p is the charge of the complex.

In another aspect of the invention there is provided a metal complex of the following formula (2):

$$[M_q(L^1)_m(L^2)_n(L^3)_r]^P \qquad (2)$$

wherein

each M is independently selected from monovalent, divalent, trivalent, tetravalent, pentavalent and hexavalent metal ions;

each L^1 is independently selected and is NH_3 or other monodentate ligand, a polydentate ligand, or a macrocyclic ligand;

each L^2 is a chelating derivative of a carboxylate or a chelating amide or hydroximate NSAID, having anti-inflammatory activity, and at least one ligand L^2 is other than a salicylate or a derivative of a salicylate;

each L^3 is independently selected and is a bridging ligand, such as an oxo, hydroxo, carboxylate (including NSAIDs), halide, or other bridging group;

m is a number from 0 to 5q;

n is a number from 1 to 2q;

p is the charge of the complex;

q is typically a number between 2 and 20 inclusive; and

r is a number from 1 to 60

Any suitable chelating derivative of a carboxylate with anti-inflammatory activity can be employed in the metal complexes of formulae (1) and (2). Typically, the chelating derivative of a carboxylate having anti-inflammatory activity is a non-steroidal anti-

inflammatory drug (NSAID). Particularly preferred examples of these chelating derivatives of carboxylate NSAIDs include derivatives of the following compounds:

Suprofen ((+)-α-methyl-4-(2-thienylcarbonyl)phenylacetic acid ("SupH"));

Tolmetin (1-methyl-5-(*p*-toluoyl)-1*H*-pyrrole-2-acetic acid ("TolH"));

Naproxen (6-methoxy-α-methyl-2-naphthaleneacetic acid ("NapH"));

Ibuprofen ((+)-α-methyl-4-(isopropylmethyl)benzeneacetic acid ("IbuH"));

Flufenamic Acid ((*N*-trifluoromethylphenyl)anthranilic acid ("FlufenH"));

Niflumic Acid ((2-(3-trifluoromethyl)phenylamino)-3-pyridinecarboxylic acid ("NifH"));

Diclofenac (2-[(2,6-dichlorophenyl)amino]phenylacetic acid ("DicH"));

Indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid ("IndoH"));

Acemetacin (1-(4-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid carboxymethyl ester ("ACMH")); and

Ketorolac ((±)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid, ("KetH") 2-amino-2-(hydroxymethyl)-1,3-propanediol).

Further suitable NSAIDs include:

Carprofen (6-chloro-**α**-methyl-9H-carbazole-2-acetic acid);

Etodolac (1,8-diethyl-1,3,4,9-tetrahydro-pyrano[3,4-b]indole-1-acetic acid);

Fentiazac (4-(4-chlorophenyl)-2-phenyl-5-thiazoleacetic acid);

Flurbiprofen (2-fluoro-**α**-methyl-[1,1'-biphenyl]-4-acetic acid);

Ketoprofen (3-benzoyl-**α**-methylbenzeneacetic acid);

Oxaprozin (4,5-diphenyl-2-oxazolepropanoic acid);

Pranoprofen (**α**-methyl-5H-[1]benzopyrano[2,3-b]pyridine-7-acetic acid);

Sulindac ((1Z)-5-fluoro-2-methyl-1-[[4-(methylsulfinyl)phenyl]methylene]-1H-indene-3-acetic acid); and

Suxibuzone (butanedioic acid, 1-[(4-butyl-3,5-dioxo-1,2-diphenyl-4-pyrazolidinyl)methyl] ester).

At least one ligand L^2 of the metal complex of formula (1) or (2) can for instance, be independently be selected from the group consisting of hydroximates, hydroxamates,

hydrazines, esters, amino acids, and peptide, sugar and amide NSAID chelating ligands (O or N bound), having anti-inflammatory activity by themselves or when hydrolysed to a carboxylic acid NSAID.

In at least some embodiments, at least one ligand L^2 will be an ester, hydroxamate, hydroximate or amide derivative of indomethacin, ibuprofen, naproxen, dichlofenac, acemetacin or ketorolac.

Other embodiments of ligand L^2 will be non-carboxylate NSAIDs that can chelate to a metal ion via an amide and other functional group or via a hydroxamate or hydroximate group.

As will be apparent to the skilled addressee, ligand L^2 can be coordinated to the metal ion via the hydroximate, hydroxamate or hydrazine group, or for example, by other groups attached to an amide or ester linkage of ligand L^2, such as sugar, amino acid, peptide, polyamine, heterocycle, and other chelate. It will also be understood that ligand L^2 can be deprotonated at substituents that include, but are not limited to, amide, carboxylic acid, alcohol and thiol functional groups to strengthen the binding of the derivative to metal one or more metal ions of the complex of formulae (1) or (2).

In at least some embodiments of metal complexes of formula (1) or (2), all or at least most of ligands L2 will be other than salicylates or derivative(s) of salicylates.

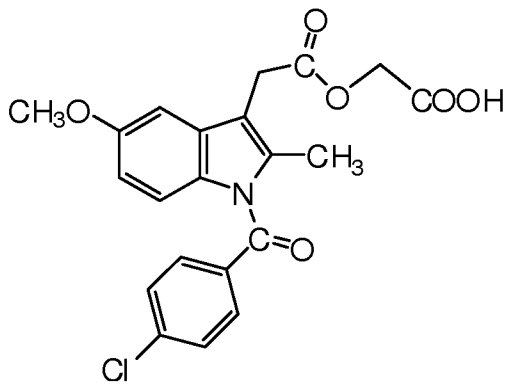
Moreover, one or more of the ancillary ligands (L^1, L^3) in the metal complexes can have anti-inflammatory, anti-diabetic, anti-microbial or anti-cancer activity, and/or be cardioprotective and/or protect against radiation-induced damage to tissues, neurodegenerative diseases and/or promote wound healing.

The metal ion of a metal complex of the invention can be copper ion or other d-block ion (e.g. zinc, titanium, vanadium, chromium, manganese, cobalt, iron, gold, platinum, ruthenium, rhodium, molybdenum and tungsten ions), or a p-block metal ion (e.g., tin, gallium or bismuth), an s-block metal ion or an f-block metal ion. In one or more forms the metal ion will be a d-block or p-block metal ion.

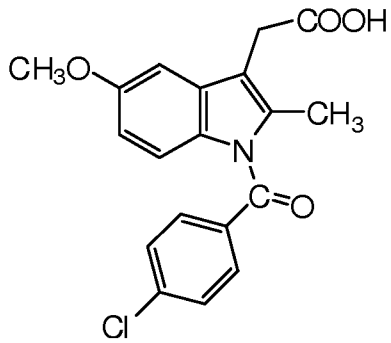
In this specification, the inclusion of the "H" at the end of an abbreviation for a carboxylate (e.g., any one of the carboxylic acid listed above) or a hydroxamate, hydroximate, or amide is used to refer to the uncharged form of the carboxylate or amide or

the parent hydroxamic acid or its monodeprotonated hydroxamate form of the doubly deprotonated hydroximate. Accordingly, the abbreviation without the "H" is used to refer to the deprotonated anionic form. For example, "IndoHAH$_2$" refers to the uncharged form of indomethacinhydroxamic acid, "IndoHAH" is used to refer to the monodeprotonated anionic form, indomethacinhydroxamate, and "IndoHA" is used to refer to the doubly deprotonated anionic form, indomethacinhydroximate. Similarly, "ACMHAH$_2$" refers to the uncharged form of acemetacinhydroxamic acid, "ACMHAH" is used to refer to the monodeprotonated anionic form, acemetacinhydroxamate, and "ACMHA" is used to refer to the doubly deprotonated anionic form, acemetacinhydroximate.

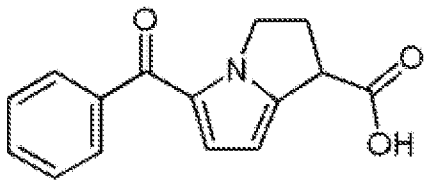
Acemetacin, 1-(4-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid carboxymethyl ester, is a glycolic acid ester of indomethacin. The structure of ACMH is shown below, as is the structure of Keterolac.



Acemetacin



Indomethacin



Ketorolac

In another aspect of the invention there is provided a pharmaceutical composition comprising a metal complex of formula (1) or formula (2), together with a pharmaceutically acceptable carrier or diluent.

In another aspect of the invention there is provided a method for prophylaxis or treatment of inflammation or a disease or condition mediated by inflammation or having an inflammatory component, comprising administering to the mammal an effective amount of a metal complex of formula (1) or formula (2).

Diseases or conditions mediated by inflammation or having an inflammatory component include diabetes, cardiovascular diseases, neurodegenerative diseases, and other conditions involving inflammation.

In another aspect of the invention there is provided a method for prophylaxis or treatment of a cancer in a mammal, comprising administering an effective amount of a metal complex of formula (1) or formula (2) to the mammal.

In another aspect of the invention there is provided a method for prophylaxis or treatment of a microbial or viral infection in a mammal, comprising administering to the mammal an effective amount of a metal complex of formula (1) or formula (2).

In another aspect of the present invention there is provided an analgesic method for prophylaxis or treatment of pain, comprising administering to the mammal an effective amount of a metal complex of formula (1) or formula (2).

In another aspect of the invention there is provided a method for promoting wound healing or inhibiting skin aging, including the prevention or treatment of wounds caused by trauma or surgery, burns, or sunburn, or ionising radiation comprising administering to a mammal in need thereof an effective amount of a metal complex of formula (1) or formula (2).

In another aspect of the present invention there is provided a method for treating damaged skin, the method comprising administering to the mammal an effective amount of a metal complex of formula (1) or formula (2).

In another aspect of the invention there is provided a method for enhancing the efficacy of radiotherapy in cancer treatment comprising administering to a mammal an effective amount of a metal complex of formula (1) or formula (2).

In another aspect of the invention there is provided use of a metal complex of formula (1) or (2) in the manufacture of a medicament for prophylaxis or treatment of inflammation or a disease or condition having an inflammatory component in a mammal.

In another aspect of the invention there is provided use of a metal complex of formula (1) or (2) in the manufacture of a medicament for prophylaxis or treatment of a cancer in a mammal.

In another aspect of the invention there is provided use of a metal complex of formula (1) or (2) in the manufacture of a medicament for prophylaxis or treatment of a microbial or viral infection in a mammal.

In another aspect of the invention there is provided use of a metal complex of formula (1) or formula (2) in the manufacture of an analgesic medicament for prophylaxis or treatment of pain in a mammal.

In another aspect of the invention there is provided use of a metal complex of formula (1) or formula (2) in the manufacture of a medicament for wound healing or inhibition and treatment of skin or tissue aging and radiation damage (both solar and ionising) in a mammal.

In another aspect of the invention there is provided use of a metal complex of formula (1) or formula (2) in the manufacture of a medicament to enhance the efficacy of radiotherapy in cancer treatment in a mammal.

One of the issues associated with the use of non-slective NSAIDs, such as indomethacin and their derivatives and complexes is gastrointestinal (GI) and renal toxicity.Metal complexes embodied by the invention may be incorporated into formulations that minimize their decomposition by biological fluids, such as gastric acid, or to change the profile of absorption of the bioactives as exemplified in International Patent Application No. PCT/AU2005/000442, to reduce GI and/or renal toxicity while substantially maintaining or enhancing efficacy of the complexes. The use of such formulations in methods of the invention is expressly encompassed.

In one or more embodiments, chelating derivatives of NSAIDs can enhance the stability of the complexes of NSAIDs. This may result in one or more of:

(i) a reduction in GI toxicity by increasing the stability of the drugs in the GI tract;

(ii) slow release forms of the NSAIDs to improve efficacy and safety profiles;

(iii) water-soluble, slow release forms of the NSAIDs for intraveneous use; and/or

(iv) the provision of chelates for delivering metal ions and other groups that are synergistic and/or enhance the efficacy of the parent NSAID in its mode of action.

Complexing a NSAID with a metal as described herein may also change the absorption profile of the NSAID.

The release of the parent carboxylate NSAID from the chelating ligand can be induced by hydrolysis of the ester, amide, hydroxamate/hydroximate or hydrazine bonds from either the complex or after the the chelating ligand has been released from the complex by: ligand substitution reactions; and/or redox catalysed substitution reactions. Once released, the NSAID derivative, the NSAID, and the metal may provide synergistic activities. For instance, the decomposition of the metal hydroximates/hydroximates can have multiple effects. As an example, a copper hydroxamate complex can exert anti-cancer, anti-inflammatory activity by a combination of independent COX-2 inhibition (by both the parent NSAID and the NSAIDHAH_2), the release of NO from the NSAIDHAH_2, matrix metalloproteinase inhibition, 5-lipoxygenase inhibition by the hydroxamic acid and apoptotic effects in cancer cells, and the effects of Cu once the complex decomposes at the site of a tumour or inflammation. The function of the Cu, in this case, is not only to provide additional biological activity but to target the organic drugs to tumours and sites of inflammation that concentrate the lipophilic complex.

The higher metabolic activity of certain tissues or cells (eg., cancer cells) can also be employed to increase the rate of ester and amide hydrolysis of metal complexes of ester, amino acids, peptide and sugar ligands and the like as described herein, as a way of targeting disease states. Moreover, inert oxidation states of metals (e.g., Pt(IV), Ru(III), Co(III)) may selectively target hypoxic sites associated with certain solid tumours and ischemia associated with heart attacks, strokes and other cardiovascular conditions. Thus,

in at least some forms, the metal ions, co-ligands and metal oxidation states can be utilized to optimise the rate of release and/or hydrolysis of the NSAID-derivative to minimise side-effects such as GI and renal toxicities, and/or to provide sufficient stability to target the disease site before the bioactives of the complex are released.

All publications mentioned in this specification are herein incorporated by reference. Any discussion of documents, acts, materials, devices, articles or the like that has been included in this specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed anywhere in the world before the priority date of this application.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers, integers or steps.

The features and advantages of the present invention will become further apparent from the following detailed description of preferred embodiments and the accompanying drawings.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

Figure 1 is a graph showing the effect of $[V^{V}O(IndoHAH)(IndoHA)]$, $[Cr^{III}(IndoHAH)_3]$ and $IndoHAH_2$ suspended in MCT oil on the viability of A549 cells, where the concentration range refers to the equivalent concentration of IndoHA and not those of the vanadium or chromium complex.

Figure 2 is a graph of gastric damage (mm^2) for active ingredients (Samples P, Q and R) dosed by means of oral gavage with a composition of MCT organogel and at Indomethacin Equivalence treat rate of 2 mg kg^{-1} bw.

Figure 3 is a graph of the **a**nti-inflammatory effect on rat paw oedema ($\%\Delta\ mm^3$) of active ingredients (Samples P, Q and R) dosed by means of oral gavage with a

composition of MCT organogel and at an Indomethacin Equivalence treat rate of 2 mg kg^{-1} bw.

Figure 4 is a graph showing the effect of ACM, CuACM and ZnACM suspended in MCT oil on the viability of A549 cancer cells, where the concentration range refers to the equivalent concentration of Indo and not the zinc or copper complexes.

Figure 5 is a graph showing the effect of $[V^{V}O(IndoHAH)(IndoHA)].2MeOH$ and $IndoHAH_2$ suspended in MCT oil on the viability of A549 cells, where the concentration range refers to the equivalent concentration of IndoHA and not the copper complex;

Figure 6 is a graph showing the effect of $[V^{V}O(IndoHAH)(IndoHA)].2MeOH$ and $IndoHAH_2$ suspended in MCT oil on the viability of A549 cells, where the concentration range refers to the equivalent concentration of IndoHA and not the copper complex

Figure 7 is a graph showing the ability of the vanadium(V) complex $[V^{V}O(IndoHAH)_2(OMe)]$ (4 mg oxametacin equivalents per kg of body weight) to decrease blood sugar levels in diabetic rats.

DEFINITIONS

In this specification, the abbreviation "Im" refers to imidazole.

In this specification the term "halo" refers to fluoro, chloro, bromo or iodo.

In this specification the term "alkyl" used either alone or in a compound word such as "arylalkyl", refers to a straight chain, branched or mono- or polycyclic alkyl. Examples of straight chain and branched alkyl include methyl, ethyl, propyl, *iso*-propyl, butyl, *iso*-butyl, *sec*-butyl, *tert*-butyl, amyl, *iso*-amyl, *sec*-amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl. Examples of cyclic alkyl include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

In this specification, the term "cycloalkyl" refers to a saturated monocyclic or polycyclic alkyl having 3 to 12 carbons.

In this specification, the term "alkenyl" refers to a straight chain, branched or cyclic alkenyl with one or more double bonds. Preferably, the alkenyl is a C_2 to C_{20}

alkenyl, more preferably C_2 to C_6 alkenyl. Examples of alkenyl include vinyl, allyl, 1-methylvinyl, butenyl, *iso*-butenyl, 3-methyl-2-butenyl, 1-pentenyl, cyclopentenyl, 1-methylcyclopentenyl, 1-hexenyl, 3-hexenyl, cyclohexenyl, 1-heptenyl, 3-heptenyl, 1-octenyl, cyclooctenyl, 1-nonenyl, 2-nonenyl, 3-nonenyl, 1-decenyl, 3-decenyl, 1,3-butadienyl, 1,4-pentadienyl, 1,3-cyclopentadienyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl, 1,3-cycloheptadienyl, 1,3,5-cycloheptatrienyl and 1,3,5,7-cyclooctatetraenyl.

In this specification, the term "alkynyl" refers to a straight chain, branched or cyclic alkynyl with one or more triple bonds, preferably a C_2 to C_{20} alkynyl, more preferably a C_2 to C_6 alkynyl.

In this specification, the term "aryl" used either alone or in compound words such as "arylalkyl", refers to a radical of a single, polynuclear, conjugated or fused aromatic hydrocarbon or aromatic heterocyclic ring system. Examples of aryl include phenyl, naphthyl and furyl. When the aryl comprises a heterocyclic aromatic ring system, the aromatic heterocyclic ring system may contain 1 to 4 heteroatoms independently selected from N, O and S and may contain up to 9 carbon atoms in the ring.

In this specification, the term "arylalkyl" refers to an alkyl substituted with an aryl group. An example of arylalkyl is benzyl.

In this specification, the term "bidentate ligand" refers to a ligand having two co-ordination bonds to a metal atom. Bidentate ligands include unsymmetric bidentate ligands with one weaker and one relatively stronger bond to the metal atom. In this specification, the term "monodentate ligand" refers to a ligand having a single co-ordination bond with a metal atom.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

Metal complexes in one or more embodiments of the invention find application in the prophylaxis or treatment of various diseases and conditions including inflammation, diseases and disorders characterised, mediated or involving inflammatory components such

as cardiovascular, neurodegenerative and diabetic conditions, pain, cancer, microbial and viral infections, the treatment of wounds, burns, skin damage, radiation damage (eg., solar and ionising radiation damage), and slowing/inhibition of skin aging.

The present inventors have also surprisingly found that metal complexes of the invention having anti-inflammatory activity have application in preventing or treating cancers including carcinomas such as lung cancers, and may have fewer side-effects and/or be more effective in preventing or treating the cancers in terms of efficacy and/or safety than the anti-inflammatory ligand(s) in the complex alone. For example, the present inventors have found that complexes of a metal and derivatives of indomethacin (such as hydroximates or hydroxamates) may be more effective in preventing or treating carcinomas, than its derivative alone.

While studies have indicated that IndoH itself has some anti-cancer activity in carcinomas (especially colorectal cancers), which is believed to be due to a range of effects including inhibition of the COX enzymes that are upregulated in cancer cells (Vane, J. R.; Bakhle, Y. S.; Botting, R. M. *Annu. Rev. Pharmacol. Toxicol.* **1998**, *38*, 97-120) and reduction of angiogenesis, it is surprising that a complex of $IndoHAH_2$ and a metal such as vanadium(IV) is much more cytotoxic to cancer cells than $IndoHAH_2$.

Without wishing to be bound by theory, it is the hypothesis of the inventors that copper complexes formed *in vivo* from the chelation of copper by the copper chelators may themselves have a strong anti-cancer effect rather than the anti-cancer effect being due solely to the removal of copper in addition to the reduction in angiogenesis when Cu is sequestered. More particularly, the efficacy of the complexes is considered to be due to a combination of both the ligand (e.g, hydroximate, hydroxamate, ester or amide derivative) and the metal atom(s). Complexes of a metal and a carboxylate, or hydroximate, hydroxamate, ester or amide derivatives thereof having anti-inflammatory activity, such as vanadium complexes with NSAID hydroxamic acids and copper complexes with NSAID hydroxamic acids, have not previously been shown to be useful in the general prophylaxis or treatment of carcinomas.

The inventors have also surprisingly found that oral doses of inert complexes, such as Cr(III) complexes of NSAID hydroxamic acids may have low GI toxicity, but greater anti-inflammatory efficacy than the the parent NSAID hydroxamic acid.

In addition, the inventors have discovered that complexation of chelating derivatives of NSAIDs to inert metal amine complexes, such as those of Co(III) and Ru(III) can result in complexes that are sufficiently stable and soluble in saline phosphate buffer to be used for intraveneous delivery for the treatment of various conditions.

Examples of carboxylic acids having anti-inflammatory activity that can be utilised as precursers of hydroxamate, hydroximate, hydrazine, ester and amide chelating ligands for metal complexes embodied by the invention include the following.

Suprofen = (+)-α-methyl-4-(2-thienyl-carbonyl)phenylacetic acid (SupH);

Tolmentin = 1-methyl-5-(*p*-toluoyl)-1*H*-pyrrole-2-acetic acid (TolH);

DMSO = dimethylsulfoxide;

Naproxen = 6-methoxy-α-methyl-2-naphthaleneacetic acid (NapH);

Ibuprofen = (+)-α-methyl-4-(isopropylmethyl)benzeneacetic acid (IbuH);

Metronidazole = 2-methyl-5-nitrobenzimidazole

Flufenamic Acid = (*N*-trifluoromethylphenyl)anthranilic acid (FlufenH);

Niflumic Acid = 2-((3-trifluoromethyl)phenylamino)-3-pyridinecarboxylic acid (NifH);

Indomethacin = 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid (IndoH); and

Diclofenac = 2-[(2,6-dichlorophenyl)amino]phenylacetic acid (DicH).

Acemetacin (1-(4-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid carboxymethyl ester ("ACMH")); and

Ketorolac ((±)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid, ("KetH") 2-amino-2-(hydroxymethyl)-1,3-propanediol).

Further suitable NSAIDs include:

Carprofen (6-chloro-**α**-methyl-9H-carbazole-2-acetic acid);

Etodolac (1,8-diethyl-1,3,4,9-tetrahydro-pyrano[3,4-b]indole-1-acetic acid);

Fentiazac (4-(4-chlorophenyl)-2-phenyl-5-thiazoleacetic acid);

Flurbiprofen (2-fluoro-α-methyl-[1,1'-biphenyl]-4-acetic acid);

Ketoprofen (3-benzoyl-α-methylbenzeneacetic acid);

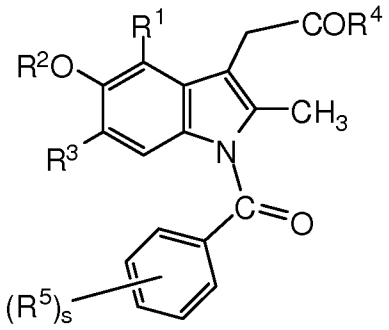
Oxaprozin (4,5-diphenyl-2-oxazolepropanoic acid);

Pranoprofen (α-methyl-5H-[1]benzopyrano[2,3-b]pyridine-7-acetic acid);

Sulindac ((1Z)-5-fluoro-2-methyl-1-[[4-(methylsulfinyl)phenyl]methylene]-1H-indene-3-acetic acid); and

Suxibuzone (butanedioic acid, 1-[(4-butyl-3,5-dioxo-1,2-diphenyl-4-pyrazolidinyl)methyl] ester).

The chelating derivative of a carboxylate having anti-inflammatory activity in a metal complex of formula (1) or (2) can also for instance be a ligand of formula (3) or (4)

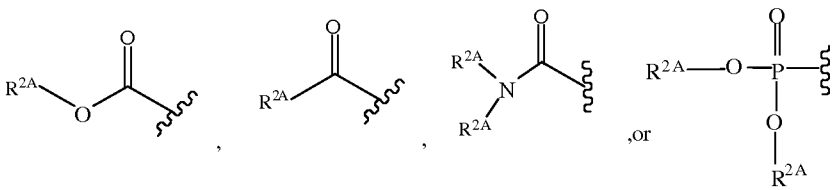


(3)

wherein:

R^1 is H or halo (i.e. Cl, F, Br or I);

R^2 is H; a C_1 to C_6 alkyl, an alkenyl or an alkynyl, where the C_1 to C_6 alkyl, alkenyl or alkynyl may be optionally substituted; or



wherein each R^{2A} is independently selected from the group consisting of H, C_1 to C_6 alkyl, alkenyl, alkynyl, aryl, cycloalkyl and arylalkyl, where the C_1 to C_6 alkyl, alkenyl,

alkynyl, aryl, cycloalkyl or arylalkyl may be optionally substituted;

R^3 is H or halo;

COR^4 is a carboxylate group or its deprotonated form; or

R^4 is $NR^{4A}OH$, $NR4^AN(R^{4A})2$, $NR^{4A}N=R^{4A}$, $NR^{4A}R^{4B}$, or OR^{4C};

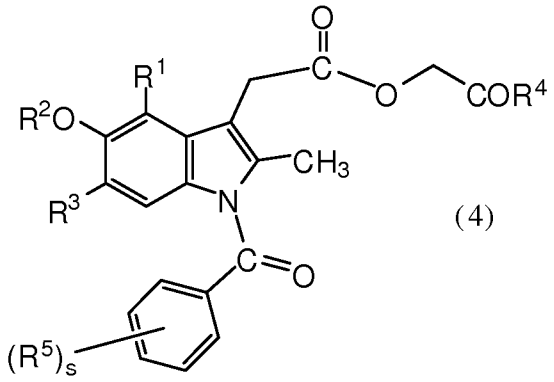
R^{4A} is H, or an aliphatic, aryl or heterocyclic group optionally substituted with one or more functional groups forming a co-ordination bond of the metal complex;

R^{4B} is a substituent group optionally forming at least one co-ordination bond of the metal complex;

R^{4C} is a substituent group forming at least two co-ordination bonds in the metal complex; and

each R^5 is independently selected from the group consisting of halo, $-CH_3$, -CN, $-OCH_3$, $-SCH_3$ and $-CH_2CH_3$, where the $-CH_3$, $-OCH_3$, $-SCH_3$ or $-CH_2CH_3$ may be optionally substituted; and

s is 1, 2, 3,4 or 5.

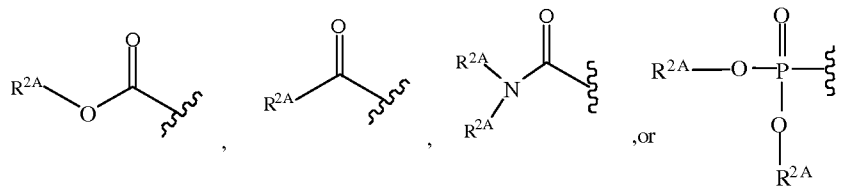


(4)

wherein:

R^1 is H or halo (i.e. Cl, F, Br or I);

R^2 is H; a C_1 to C_6 alkyl, an alkenyl or an alkynyl, where the C_1 to C_6 alkyl, alkenyl or alkynyl may be optionally substituted; or



wherein each R^{2A} is independently selected from the group consisting of H, C_1 to C_6 alkyl, alkenyl, alkynyl, aryl, cycloalkyl and arylalkyl, where the C_1 to C_6 alkyl, alkenyl, alkynyl, aryl, cycloalkyl or arylalkyl may be optionally substituted;

R^3 is H or halo;

COR^4 is a carboxylate group or its deprotonated form; or

R^4 is $NR^{4A}OH$, $NR4^AN(R^{4A})2$, $NR^{4A}N=R^{4A}$, $NR^{4A}R^{4B}$, or OR^{4B};

R^{4A} is H, or an aliphatic or aryl group optionally substituted with one or more functional groups forming a co-ordination bond of the metal complex;

R^{4B} is a substituent group optionally forming at least one co-ordination bond of the metal complex;

R^{4C} is a substituent group forming at least two co-ordination bonds in the metal complex; and

each R^5 is independently selected from the group consisting of halo, $-CH_3$, -CN, $-OCH_3$, $-SCH_3$ and $-CH_2CH_3$, where the $-CH_3$, $-OCH_3$, $-SCH_3$ or $-CH_2CH_3$ may be optionally substituted; and

s is 1, 2, 3, 4 or 5.

When R^2 of the ligand of formula (3) or (4) is a C_1 to C_6 alkyl, an alkenyl or an alkynyl, the C_1 to C_6 alkyl, alkenyl or alkynyl may be substituted with one or more substituents. The one or more substituents may, for example, be independently selected from the group consisting of halo, -OH, -COOH and $-NH_2$.

When R^{2A} is a C_1 to C_6 alkyl, an alkenyl, an alkynyl, an aryl, a cycloalkyl or an arylalkyl, the C_1 to C_6 alkyl, alkenyl, alkynyl, aryl, cycloalkyl or arylalkyl may be substituted with one or more substituents. The one or more substituents may, for example, be independently selected from the group consisting of halo, –OH, -COOH and $-NH_2$.

When R^5 is $-CH_3$, $-OCH_3$, $-SCH_3$ or $-CH_2CH_3$, the $-CH_3$, $-OCH_3$, $-SCH_3$ or $-CH_2CH_3$ may be substituted with one or more substituents. The one or more substituents

may, for example, be independently selected from the group consisting of halo, -OH, -COOH and $-NH_2$.

In some embodiments, R^{4A} can be an aliphatic or aromatic group substituted with one or more heteroatoms or heterocyclic groups (eg., 1-6 heteroatoms selected from O, N and S).

In some embodiments R^{4B} can be a substituent derived from an amino acid, a peptide, a sugar, an acyclic aliphatic amine, or a cyclic amine, hydroxy acid, a polydentate heterocyclic or combinations thereof. It will also be understood that the derivative can contain one or more of a deprotonated at amide, carboxylic acid, alcohol, thiol group(s) or the like.

In some embodiments R^{4C} can be a substituent derived from an amino acid, a peptide, a sugar, an acyclic aliphatic amine, or a cyclic amine, hydroxy acid, a polydentate heterocyclic or combinations thereof. Further, it will also be understood that the derivative can contain one or more deprotonated amide, carboxylic acid, alcohol, thiol group(s) or the like.

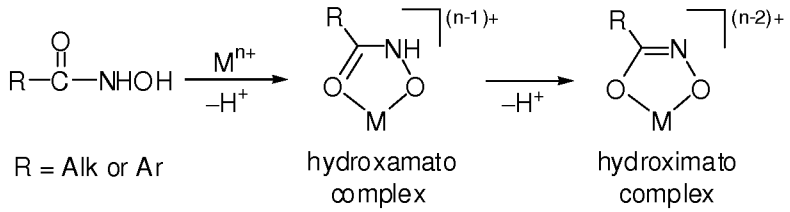
Further derivatives of carboxylic acids that can be employed in metal complexes embodied by the invention include hydroxamic acid derivatives of carboxylates with anti-inflammatory activity, which form hydroxamato or hydroximato complexes with the metal; hydrazine derivatives, esters of carboxylates having anti-inflammatory activity, such as sugar derivatives and those of amino acids and peptides; and amide derivatives of carboxylates having anti-inflammatory activity, such as amino acids, peptides amines, and sugars that form chelates of deprotonated amides with the metal.

A hydroxamic acid having anti-inflammatory activity can form hydroxamato or hydroximato complexes with a metal ion in the complex. An amide having anti-inflammatory activity can form chelates of deprotonated amides or amide monodentate complexes with a metal ion in the complex.

Hydroxamic acids having anti-inflammatory activity that can be utilised in the complexes used in the method of the invention include carboxylates linked by a linker to a hydroxamate group (eg., International Patent Application No. PCT/US03/019228 (WO 04/000215)). Hydroxamic acid derivatives of carboxylates with anti-inflammatory

activity, such as indomethacin, ibuprofen, naproxen, dichlofenac, acemetacin or ketorolac are particularly preferred.

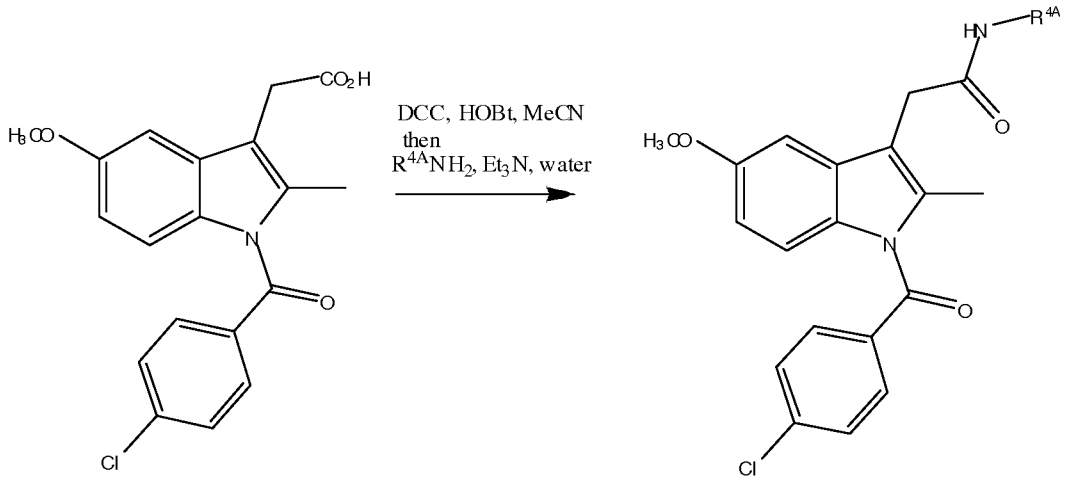
The equilibrium between a hydroxamato and the hydroximato complex may change with pH as shown below in Scheme 1. As such, the terms hydroxamate and hydroximate in the context of the present invention are interchangeable. In Scheme 1, RCO_2H is the parent carboxylic acid with anti-inflammatory activity.



Scheme 1

Such hydroxamic acids can also be substituted on the N, in which case only the hydroxamato coordination mode is possible.

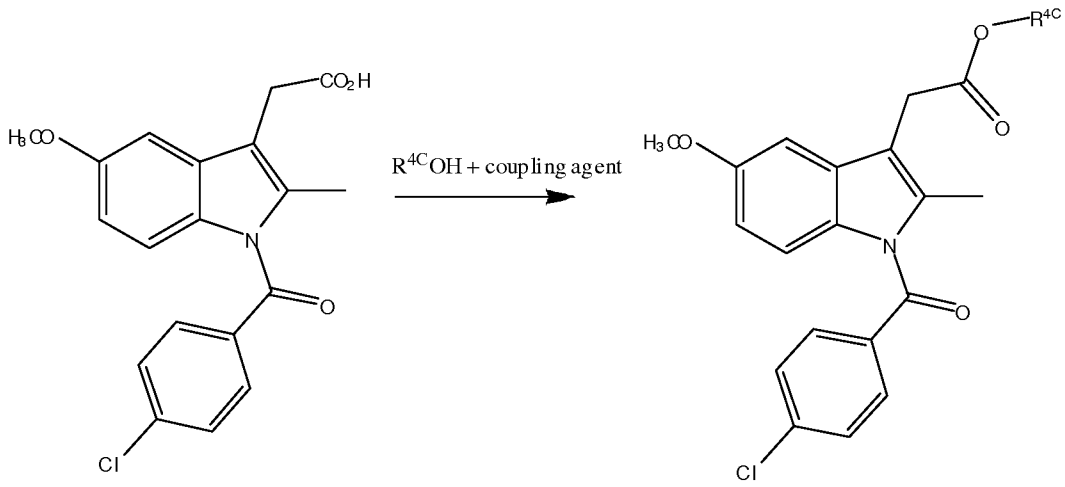
Amino acid, peptide or other amide derivatives of carboxylic acids having anti-inflammatory activity can be prepared as described in International Patent Application No. WO 95/04030, or by modifications thereof. See, for instance, the indomethacin examples below (Scheme 2). In this example, R can contain one or more functional groups that act as other donor groups to form a metal chelate. Suitable coupling reactions include those with amino acids to form a mixed amide/carboxylate donor set, or more complex donor bidentate sets with amino acids containing metal binding side-chains, e.g., cysteine, serine, methionine, histidine, tyrosine, and the like. Other suitable R groups include amino sugar derivatives and glycoproteins that can both target tumour cells and act as metal chelates. The coupling reaction can also involve short chain peptides, which act as chelating ligands, or other groups to give metal chelators with anti-inflammatory activities, as described in WO 95/04030.



Scheme 2

The group forming the amide linkage can be an amino acid (natural or artificial), any natural or artificial peptide (including glycopeptides), a amino sugar derivative, a polydentate amine, amino alcohol, amino thiol, or other polydentate amine ligand containing one or more donor groups including heterocycles.

Ester derivatives of carboxylic acids having anti-inflammatory activites can be prepared by a variety of ester coupling reactions. See, for instance, the indomethacin example shown below (Scheme 3). In this example, R can be an alkyl or aryl group containing a substituent which can act as a polydentate ligand, e.g., amino and alcohol groups (eg., prepared from a polydentate aminoalcohol), with serine, tyrosine or short-chain peptides containing serine or tyrosine. Other suitable chelating groups that can be coupled and may also target tumours, include sugars and glycoproteins.



Scheme 3

Metal complexes embodied by the invention may also be prepared by methods outlined in Example 1 below. In at least some forms, the complexes contain hydroximate, hydroxamate, amide, or ester derivatives of indomethacin, ibuprofen, naproxen, dichlofenac, acemetacin or ketorolac as ligands as described above. In metal complexes embodied by the invention, the functional groups of the ligands may themselves bind to the metal ion, and/or other ligating groups that are linked by these functionalities can bind to the metal.

In particularly preferred embodiments, the metal complex may be a complex of the formula (1):

$$[M(L^1)_m(L^2)_n]^p \qquad (1)$$

wherein

M is idependently chosen from a monovalent, divalent, trivalent, tetravalent, pentavalent or hexavalent metal ion;

each L^1 is independently selected and is NH_3 or other monodentate ligand, a polydentate ligand, or a macrocyclic ligand;

each L^2 is independently a chelating derivative of a carboxylate such as a hydroximate, hydroxamate, hydrazine, ester, amino acid, or a peptide, sugar or amide

NSAID chelating ligand (O or N bound), or a chelating amide or hydroximate NSAID, having anti-inflammatory activity, and at least one ligand L^2 is other than a salicylate or a derivative of a salicylate;

m is 0, 1, 2, 3, 4, or 5;

n is 1, 2, 3 or 4; and

p is the charge of the complex.

In other embodiments, a metal complex as described herein can be a metal complex of the following formula (2):

$$[M_q(L^1)_m(L^2)_n(L^3)_r]^p \qquad (2)$$

wherein

each M is independently selected from monovalent, divalent, trivalent, tetravalent, pentavalent and hexavalent metal ions;

each L^1 is independently selected and is NH_3 or other monodentate ligand, a polydentate ligand, or a macrocyclic ligand;

each L^2 is indendently a chelating derivative of a carboxylate such as hydroximate, hydroxamate, ester, amino acid, or peptide, sugar or amide NSAID chelating ligand (O or N bound), or a chelating amide or hydroximate NSAID, having anti-inflammatory activity, and at least one ligand L^2 is other than a salicylate or a derivative of a salicylate;

each L^3 is independently selected and is a bridging ligand;

m is a number from 0 to 5q;

n is a number from 1 to 2q;

p is the charge of the complex;

q is typically a number between 2 and 20 inclusive; and

r is a number from 1 to 60.

It will be understood that all individual values and ranges of values m, n, q and r in formula (2) are expressly encompassed. For instance, q can be 2, 3, 4, 5, etc., r can be 1, 2, 3, 4, 5, 6, etc. In some embodiments, q will be a number in a range from 2 to 15, more preferably in a range of from 2 to 10 and most preferably, in a range of from 2 to 4, and

wherein r is in a range of from 1 to 20, more preferably in a range of from 1 to 15 and most preferably, in a range of from 1 to 10.

The metal ion of a metal complex embodied by the invention can be a d-block, f-block, p-block or s-block metal ion. Typically, the metal M will be a divalent, trivalent, tetravalent, pentavalent or hexavalent d-block metal, preferably, Co(II), Cu(II), Fe(II), Mn(II), Ni(II), Pt(II), Ru(II), Zn(II), Au(III), Co(III), Cr(III), Fe(III), Mn(III), Ru(III), Mn(IV), Mo(IV), Pt(IV), Ru(IV), Ti(IV), V(IV), Mo(V), V(V), W(V), Mo(VI), W(VI) or a trivalent or tetravalent p-block metal such as Ga(III), Bi(III) or Sn(IV).

Examples of L^1 ligands useful in complexes of formulae (1) and (2) include: monodentate ligands such as halos, aqua, hydroxo, oxo, CO, NO, amines, alcohols, amides, sulfoxides, *N*-heterocylces, *O*-heterocycles, and *S*-heterocycles; polydentate acyclic ligands include amines, amino acids, peptides, alcohol sugars, hydroxyacids, polycarboxylates, *N*-heterocylces, *O*-heterocycles, and *S*-heterocycles, and other functional groups that can form co-ordinate bonds with a metal ion, and combinations thereof; polydentate macrocyclic ligands include amines, crown ethers, macrocyclic, thioethers, macrocyclic peptides and amides and ligands with combinations of these and other metal binding substituents.

Examples of L^2 ligands useful in complexes of formulae (1) and (2) include those of formulae (3) or (4) and R^6COR^4, where R^6COOH is a carboxylic acid NSAID and R^4 is selected from $NR^{4A}OH$, $NR^{4A}N(R^{4A})_2$, $NR^{4A}N{=}R^{4A}$, $NR^{4A}R^{4B}$, OR^{4C} and deprotonated forms thereof, wherein R^4 is as described in ligands of formula (3) or (4) above.

Examples of L^3 ligands useful in complexes of formula (2) include oxo, hydroxo, carboxylate (including carboxylate NSAIDs), halo and other bridging groups.

Examples of suitable aliphatic and aromatic groups include substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl and heterocyclic groups. Examples of heterocyclic groups include heterocycles comprising one or more N, O and/or S atoms. In some embodiments, the heterocycle is optionally substituted. The heterocycle can for example be selected from the group consisting of isoquinolyl, quinolyl, piperidinyl, pyridinyl, 2-methylpyridinyl, imadazoyl, pyranyl, pyrrolyl, pyrimidinyl, indolyl, purinyl and quinolizinyl.

Examples of complexes of formula (1) include: $[M(L^2)_n(NR^7R^8R^9)_{m)}]^p$ wherein each L^2 is independently a bidentate derivative of a NSAID, each $(NR^7R^8R^9)$ is independently a monodentate amine ligand or a polydentate amine ligand (eg., a cyclic amine), n is 1 or 2, m is 1, 2, 3, or 4, and M is selected from Ru(II), Co(III), Cr(III), Ir(III), Os(III), Rh(III), Ru(III) and Pt(IV); $[M(L^2)(NR^7R^8R^9)_m]^p$ where L^2 is a tridentate derivative of a NSAID, each $(NR^7R^8R^9)$ is independently a monodentate amine ligand or a polydentate amine ligand (eg., a cyclic amine), m is 1, 2 or 3 and M is selected from Ru(II), Co(III), Cr(III), Ir(III), Os(III), Rh(III), Ru(III) and Pt(IV); $[M(L^2)(NR^7R^8R^9)_m]^p$ wherein L^2 is a tetradentate derivative of an NSAID, $(NR^7R^8R^9)$ is a monodentate amine ligand or a bidentate amine ligand, m = 1 or 2, and M is selected from Ru(II), Co(III), Cr(III), Ir(III), Os(III), Rh(III), Ru(III) and Pt(IV); $[M(L^2)(NR^7R^8R^9)]^p$ where L^2 is a pentadentate derivative of a NSAID, $(NR^7R^8R^9)$ is a monodentate amine ligand, and M is selected from Ru(II), Co(III), Cr(III), Ir(III), Os(III), Rh(III), Ru(III) and Pt(IV); $[M(L^2)_3]^p$ where L^2 is a bidentate derivative of a NSAID, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Mn(III), Os(III), Rh(III), Ru(III), Bi(III) and Pt(IV); $[M(L^2)_2]^p$ where L^2 is a tridentate derivative of a NSAID, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Ru(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Mn(III), Os(III), Rh(III), Ru(III), Bi(III) and Pt(IV); $[M(L^2)]^p$ where L^2 is a sexidentate derivative of a NSAID, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Ru(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Mn(III), Os(III), Rh(III), Ru(III), Bi(III), and Pt(IV); $[M(L^2)_n(OH_t)_{(6-2n)}]^p$ wherein each L^2 is independently a bidentate derivative of a NSAID, t is independently selected from 0, 1 or 2, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Os(III), Rh(III), Ru(III), Pt(IV), Ti(IV), V(IV), Mn(IV), V(V), Mo(V), W(V), Mo(VI), and W(VI); $[M(L^2)(OH_t)_3]^p$ where L^2 is a tridentate derivative of a NSAID, t is independently selected from 0, 1 or 2, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Os(III), Rh(III), Ru(III), Pt(IV), Ti(IV), V(IV), Mn(IV), V(V), Mo(V), W(V), Mo(VI) and W(VI); $[M(L^2)(OH_t)_2]^p$ where L^2 is a tetradentate derivative of a NSAID, t is independently selected from 0, 1 or 2, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Os(III), Rh(III), Ru(III), Pt(IV), Ti(IV), V(IV), Mn(IV), V(V), Mo(V), W(V), Mo(VI) and W(VI);

$[M(L^2)(OH_t)]^p$ where L^2 is a pentadentate derivative of a NSAID, t is 0, 1 or 2, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Os(III), Rh(III), Ru(III), Pt(IV), Ti(IV), V(IV), Mn(IV), V(V), Mo(V), W(V), Mo(VI) and W(VI); $[M(L^2)_3]^p$ where each L^2 is independently selected from bidentate derivatives of NSAIDs, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Os(III), Rh(III), Ru(III) and Pt(IV); $[M(L^2)(NR^7R^8R^9)_2]^p$ where L^2 is a bidentate derivative of a NSAID, $(NR^7R^8R^9)$ is independently selected from monodentate amine ligands or is a bidentate amine ligand, and M is selected from Cu(II), Pd(II), Pt(II) and Au(III); $[M(L^2)(NR^7R^8R^9)]^p$ wherein L^2 is a tridentate derivative of a NSAID, $(NR^7R^8R^9)$ is a monodentate amine ligand, and M is selected from Cu(II), Pd(II), Pt(II) and Au(III); $[M(L^2)_2]^p$ wherein L^2 is a bidentate derivative of a NSAID, and M is selected from Cu(II), Ni(II), Pd(II), Pt(II) and Au(III); and five-coordinate, $[V(O)(L^1)_{(m-1)}(L^2)_n]^p$; andwherein R^7, R^8 and R^9 are independently H or an optionally substituted aliphatic, heterocyclic or aromatic group.

Specific examples of metal complexes of formula (1) include [Cu(IndoHAH)(OH)], $[Co(en)_2(IndoHAH)]Cl_2$, $[Co(en)_2(IndoHAH)](CF_3SO_3)_2$, $[V^VO(IndoHAH)(IndoHA)]\cdot 2MeOH\cdot 1.5H_2O$, $[V^VO(IndoHAH)_2(OMe)]$ $[Cr(IndoHAH)_2(OH_2)_2](NO_3)\cdot H_2O$, [Cu(Indo-Gly)(Im)$_2$] and $[Ga(IndoHAH)_2(OH_2)_2]Cl$ (eg., see Example 1 below).

Examples of formula (2) include: $[M_2(NR^7R^8R^9)_{m'}(L^1)_{m''}(L^2)_n(L^3)_r]^p$, where each $(NR^7R^8R^9)$ is independently selected from monodentate or polydentate amine ligands, m' = 0, 1, 2, 3, 4, 5, 6, 7, or 8, each L^1 is independently a monodentate ligand (**such as** aqua, CO, NO, heterocycle, sulfoxide and amide and/or a chelating amino acid, peptide, heterocycle, or hydroxyacid), m" = 0, 1, 2, 3, 4, 5, 6, 7, or 8, each L^2 is independently a chelating derivative of a NSAID, n = 1, 2, 3, or 4, each L^3 is independently selected from oxo, hydroxo, carboxylate and halo ligands, r = 1, 2 or 3.and each M is independently selected from Fe(II), Mn(II), Ru(II), Co(III), Cr(III), Fe(III), Ir(III), Os(III), Rh(III), Ru(III), Ru(IV), Os(IV) and Pt(IV).

Inflammatory diseases and conditions that can be treated in accordance with one or more embodiments of the invention include primary arthritis (osteoarthritis, rheumatoid

arthritis, septic arthritis, gout and pseudogout, juvenile arthritis, Still's disease, ankylosing spondylitis), secondary arthritis caused by other diseases (systemic lupus erythematosus, Henoch-Schönlein purpura, psoriatic arthritis, reactive arthritis (Reiter's syndrome), hemochromatosis, hepatitis, Wegener's granulomatosis (and many other vasculitis syndromes), familial Mediterranean fever, hyperimmunoglobulinemia D and periodic fever syndrome, and TNF-alpha receptor associated periodic fever syndrome), bronchitis, bursitis, scoliosis, muscle and joint injury, colitis (ulcerative colitis, Crohn's colitis, diversion colitis, ischemic colitis, infectious colitis, chemical colitis and atypical colitis, and pseudomembranous colitis), conjunctivitis, dermatitis, epicondylitis, tendonitis.

Diseases and conditions with an inflammatory component that can be treated in accordance with one or more embodiments of the invention include psoriasis, rosacea,and neurodegenerative, cardiovascular and diabetes related diseases and conditions. Inflammation is an important component of the formation of arterial plaques and acute inflammation follows strokes and heart attacks, due to the involvement of reactive oxygen species. Similar oxidative damage is associated with the onset and progression of neurodegenerative diseases and diabetes. See for example, Dragomir, E.; Simionescu, M.. Monocyte Chemoattractant Protein-1 - a major contributor to the inflammatory process associated with diabetes. *Arch. Physiol. Biochem.* (2006), 112, 239-244; Kadiu, I.; Glanzer, J. G.; Kipnis, J.; Gendelman, H. E.; Thomas, M. P. Mononuclear phagocytes in the pathogenesis of neurodegenerative diseases. Neurotoxicity Res. (2005), 8, 25-50.

Cardiovascular diseases and conditions that can be treated in accordance with one or more embodiments of the invention include acute and chronic cardiovascular inflammation including as a result of surgery or other trauma, cardiovascular disease, angina pectoris, arteritis, atheroma, atherosclerosis, arteriosclerosis, congestive heart failure, coronary heart disease, cardiomyopathy, myocardial infarction, stroke, ischeamic conditions, ischaemic cardiomyopathy, patent ductus arteriosus, high blood pressure, pulmonary hypertension peripheral artery disease, coronary artery disease, coronary artery spasm, pericarditis and strokes.

Diabetes related diseases and conditions that can be treated include Type I diabetes mellitus, Type II diabetes mellitus, gestational diabetes mellitus (GDM), insulin-dependent

diabetes, non-insulin dependent diabetes, juvenile onset diabetes, late onset diabetes, maturity-onset diabetes of the young (MODY), insulin sensitive diabetes, insulin deficient diabetes, carbohydrate intolerance, and diabetes associated with another disease or condition (eg., such as polycystic ovary disease or acanthosis nigricans), and non-resistant forms of diabetes observed following pancreatic surgery and for instance, following trauma to the pancreas (eg., as a result of injury). Pre-diabetic conditions leading to the above can also be treated with metal complexes embodied by the invention.

Neurodegenerative conditions that can be treated include dementia, Lewy body disease, Parkinsons diseases, Alzheimers disease, amyloid plaque deposition diseases, multiple sclerosis, demyelination diseases, and motor neurone diseases.

Carcinomas that can be treated include lesions and tumours of the epithelium. The lesion can, for example, be a skin lesion such as basal cell carcinoma, squamous cell carcinoma or melanoma. The carcinoma can be selected from other cancers of the epithelium, such as lung cancer, cancer of the oesophagus, colon cancer, colorectal cancer, breast cancer, lung cancer, and other cancers of the epithelial tissues such as epithelial cancers of the tongue, salivary glands, gums and other areas of the mouth, oropharynx, nasopharynx, hypopharynx, oesophagus, pancreas, stomach, small intestine, duodenum, gall bladder, pancreas, larynx, trachea, uterus, cervix, ovary, vagina, vulva, prostate, testes, penis, bladder, kidney, thyroid, eye, and mestastic cancers thereof. However, it will be understood that use of metal complexes embodied by the invention is not limited to epithelial cancers and metal complexes of formula (1), (2) or (3) also have application in the prophylaxis or treatment of non-epithelial cancers. The application of metal complexes in the treatment of carcinoma is further described in Applicant's co-pending International Patent Application No. PCT/AU2006/000403 the contents of which is incorporated herein by cross-reference in its entirety.

Examples of non-carcinoma cancers which can be treated in accordance with one or more embodiments of the invention include leukemias (chronic myeloid, acute myeloid, chronic lymphocytic, acute lymphoblastic and hairy cell), Non-Hodgkin lymphoma, Hodgkin lymphoma, multiple myeloma, sarcomas, lymphomas, Kaposi's sarcomas (classic, endemic or African, AIDS-related, transplant-related), primary bone cancers (osteosarcoma,

Ewing's sarcoma, chondrosarcoma, spindle cell sarcoma, chordoma, angiosarcoma), soft tissue sarcomas (dermatofibrosarcoma, desmoid tumor, desmoplastic small round cell tumor, extraskeletal chondrosarcoma, extraskeletal osteosarcoma, fibrosarcoma, hemangiopericytoma, hemangiosarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, malignant fibrous histiocytoma, neurofibrosarcoma, rhabdomyosarcoma, synovial sarcoma), Askin's Tumor, malignant hemangioendothelioma, malignant schwannoma, mesothelioma, gliomas (ependymomas, astrocytomas, oligodendrogliomas, and mixed gliomas, such as oligoastrocytomas), choriocarcinoma, germ cell tumours (dysgerminoma and nondysgerminomatous ovarian tumours, teratoma or seminoma testicular cancers), sex cord-stromal tumours (granulosa stromal cell tumours, Sertoli- or Sertoli-Leydig cell tumours, lipid cell tumors and gynandroblastomas). The treatment of cancers with metal complexes incorporating ligands having anti-inflammatory activity is further described in the Applicant's co-pending International Patent Application filed 26 March 2007 entitled "Combination therapy for treatment of cancer" the contents of which are incorporated herein by cross-reference in its entirety.

Analgesic applications of embodiments of metal complexes of the invention include treatment of post-operative pain, pain caused by bone cancer, arthritic pain, muscle pain, period pain, severe headaches, and pain associated with inflammatory diseases and conditions, trauma and infection.

Metal complexes as described herein can be taken orally, intraveneously (as many are water soluble) or by direct application to the site of infection (see example). Many infections are also associated with hypoxia. H and, hence, complexes that release the active under conditions of hypoxia can deliver the ligand/metaldrug selectively to the site of infection. Microbial pathogens that can be treated by one or more embodiments of the invention include bacterial, fungal and yeast pathogens which cause systemic, mucosal, oral, nasal, oropharyngeal, nasalpharyngeal, pharyngeal, digestive tract, vaginal, respiratory tract, urinary tract, kidney, eye and skin infections, including *Chlamydia* species, *Haemophilus influenzae* species, Non-typable *Haemophilus influenzae* (NTHi) species, *Pseudomonas* species, *Streptococcus* species, *Staphylococcus* species, *E. coli* species, *Mycoplasma* species and *Helicobacter* species amongst others. Examples of

bacterial pathogens include *P. aeruginosa*, *Non-typeable H. influenzae* (NTHi), *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, *Helicobacter pylori*, Haemophilus influenzae type b (Hib), *Staphylococcus aureus*, *Staphylococcus albus*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Moraxella catarrhalis*, *Streptococcus pyrogenes*, *Chlostridium diptheriae*, *M. tuberculosis* and *M. genitalium*. Fungal pathogens include *Aspergillus* species. Yeast pathogens include for instance *Saccharomyces* species and the candidiasis causing agent *Candida albicans*.

Indomethacin for instance has been reported to have an anti-microbial effect on *H. pylori*. These bacteria have been identified as a causative agent of at least some gastric cancers. Observations by the present inventors indicate that metal complexes embodied by the invention such as copper indomethacin can have a stronger anti-bacterial effect on gut bacteria than indomethacin alone and it will be understood that one or more methods embodied by the invention extend to combination therapy with other chemotherapeutic agents and drugs for the prophylaxis of *H. pylori* infections and gastric cancers involving a microbial component, as well as other microbial infections such as those exemplified above. Any conventionally known agents or drugs commonly used for the prophylaxis or treatment of such bacterial, fungal and other microbial infections can be used in such combination therapy.

Viral infections that may be treated by one or more embodiments of metal complexes of the invention include retroviruses such as Human Immunodeficiency virus (eg., HIV-1, HIV-2), DNA viruses such as Epstein-Barr virus (EBV), Human papillomavirus (HPV), Hepatitis B virus and Hepatitis C virus, Human T-cell lymphotropic virus, Kaposi's sarcoma associated herpes virus, herpes simplex viruses (HSV-1, HSV-2), varicella-zoster virus, vaccinia virus, SV40 virus, respiratory syncytial virus (RSV), parainfluenza viruses (PIV), human metapneumovirus, positive-stranded RNA viruses such as rhinoviruses, polioviruse, rubella virus and equine encephalitis viruses, and further RNA viruses including influenza virus, (eg influenza A and influenza B viruses), measles virus and mumps virus.

Treatment with type -2 selective and non-selective cyclooxygenase inhibitors for instance improves T-cell proliferation in HIV-infected patients on anti-retrovral therapy

(Johansson CC, et al., AIDS 18(6): 951-952 Apr. **2004**). Hence, the invention further extends to combination therapy of metal complexes embodied by the present invention with anti-viral drugs and/or treatments. Any conventionally known anti-viral drug may be employed including Acyclovir (acylguanosine), Arildone and WIN drugs which inhibit viral uncoating, Pleconaril, Amantadine, Rimantadine, nucleoside analogue drugs, further DNA polymerase inhibitors such as Ganciclovir, Azidothymidine (AZT), and adenosine arabinoside, dideoxyinosine, iodo-deoxyuridine, trifluorothymidine, Nevirapine, pyridinone derivatives, Efavirenz, RNA synthesis inhibitors, RNA cleavage enzymes and protease inhibitors.

Surprisingly, embodiments of metal complexes described herein including those with Cu may also promote angiogenesis and so have application in wound healing, treating tissue damage, inhibiting skin aging, and promoting angiogenesis in skin and other tissues, including hypoxic and ischemic tissues. For instance, Ru and amino acid complexes containing Co can strongly promote angiogenesis by the release of Co, such that any of the complexes of formulae (1)-(3) with at least one L^2 = Co can promote angiogenesis, as can Co complexes, when they are reduced to Co(II) under hypoxic conditions that will also release the NSAID or NSAID derivative (Li Volti, G.; Sacerdoti, D.; Sangras, B.; Vanella, A.; Mezentsev, A.; Scapagnini, G.; Falck, J. R.; Abraham, N. G. Carbon monoxide signaling in promoting angiogenesis in human microvessel endothelial cells. *Antioxidants & Redox Signaling* (2005), 7, 704-710; Tanaka, T.; Kojima, I.; Ohse, T.; Ingelfinger, J. R.; Adler, S.; Fujita, T.; Nangaku, M. Cobalt promotes angiogenesis via hypoxia-inducible factor and protects tubulointerstitium in the remnant kidney model. *Lab. Invest.* (2005), 85, 1292-1307).

Moreover, the anti-microbial action of embodiments of metal complexes of the invention can promote healing of wounds and have application in the treatment and prevention of skin conditions that have a microbial component. The presence of metal ions such as Zn in certain complexes can also promote skin repair**.** Further to this, metal complexes described herein may provide a means of improved delivery and release of ligands that inhibit COX-2 and 5-LO (lipoxygenase) enzymes which have synergistic effects on reducing skin damage, such as that arising from sunburn and other burns. Thus,

metal complexes embodied by the invention can be used to deliver NSAIDs with beneficial effects in wound and tissue repair as well as the metal of the complex to areas of hypoxia, particularly in areas of bacterial infection often associated with slow healing wounds that are difficult to treat systemically and topically because of poor vascularisation. Metal complexes of Co(III) and Ru(III) are particularly preferred for wound healing, tissue repair and anti-skin aging applications.

While studies have indicated that IndoH itself has some anti-cancer activity in carcinomas believed to be due to a range of effects including inhibition of the COX enzymes which are upregulated in cancer cells (Vane, J. R.; Bakhle, Y. S.; Botting, R. M. *Annu. Rev. Pharmacol. Toxicol.* **1998**, *38*, 97-120) and a reduction of angiogenesis, the inventors have surprising found that metal complexes of indomethacin (Indo) can be much more effective in preventing or treating carcinomas than indomethacin as a result of the promotion of angiogenesis.

Without wishing to be bound by theory, promotion of angiogenesis is believed to contribute to inhibition of skin aging by facilitating the regeneration and neovascularization of tissue, facilitating the transport of nutrients and oxygen to tissue, and/or generally promoting blood flow to tissue, particularly after tissue inflammation, or exposure of skin tissue to injury or insult. The inhibition of skin aging can manifest itself in one or more of increased or maintenance or vascularity of the skin, the maintenance or enhancement of elasticity of the skin, delayed deterioration of elasticity of the skin, decreased or delayed formation of creases or fine or deep wrinkles in the skin, decreased or delayed thinning of skin, the inhibition of loss of underlying fat from the skin, the inhibition of the development of transparency of skin, and inhibition of other visual markers associated with skin aging such as the formation or keratosis, dryness, and cracking of the skin.

Angiogenesis in wound healing can be assessed by measuring the extent of vessel growth at the site of wounds as described in Erpek, S.; Kilic, N.; Kozaci, D.; Dikicioglu, E.; Kavak, T. *Revue De Medecine Veterinaire* 2006, *157*, 185-192). Any suitable conventionally known protocol for assessing aging of the skin can be used to score the efficacy of metal complexes described herein. Skin damage that leads to aging effects can for example be assessed by examination of the erythema reducing capacity of a metal

complex as described herein in animals or humans exposed to the complex, Grundmann, J. U.; Bockelmann, R.; Bonnekoh, B.; Gollnick, H. P. M, *Photochem. Photobiol.* **2001**, *74*, 587-592). In particular, examination of the skin for biochemical markers of aging such as the induction of heme oxygenase, and the depletion of IFN-γ and IL-12 from the epidermis can provide short term information. Aging and skin damage from UV exposure in hairless mice can for instance be assessed histologically by examining changes in epidermal hyperplasia and dermal mast cell numbers, pronounced focal elastotic deposits, degraded dermal collagen and deposition of glycosaminoglycans in the lower dermis (Tyrell, R. M.; Reeve, V. E. *Prog. Biophys. Mol. Biol.* **2006**, *92*, 86-91; Reeve, V. E.; Widyarini, S.; Domanski, D.; Chew, E.; Barnes, K. *Photochem. Photobiol.* **2005**, *81,* 1548-1553).

The treatment of skin damage is to be taken in the broadest sense to encompass the treatment of any skin damage responsive to the application of a metal complex as described herein and is not limited to skin damage arising from inflammation and microbial infections (or having a microbial component), trauma, burns (including radiation burns) and skin conditions.

Wound or tissue repair encompassed by one or more methods embodied by the invention include repair following cuts and abrasions, photodamage or tissue insult resulting from exposure to ultraviolet radiation including erythema, burns, non-healing skin ulcers including diabetic, venous stasis, and pressure ulcers, and tissue damage caused by surgery or as a result of injury or trauma. The treatment of burns includes burns arising from exposure of tissue to excessive heat as well as from ultra-violet radiation (eg., sunburn) , and ionizing radiation as may result from cancer radiation therapy for the treatment of cancer, neoplastic disease or other disease or condition.

For wound healing, treating burns, inhibiting skin aging and the like, metal complexes embodied by the invention can be applied topically to the tissue to be treated although for internal treatment, the metal complexes can be administered systemically. For general use in inhibiting skin aging, the metal complex can be topically applied on a daily basis to areas of the skin exposed to ultraviolet radiation such as the face, neck, arms, shoulders and legs while undertaking normal daily or leisure activities such as sunbaking. This also applies to methods of the invention for prophylaxis or treatment of carcinomas and other cancers. In

either instance, the metal complex can be formulated in a sunscreen or cosmetic composition. Suitable sunscreen and cosmetic formulations are for example described in the Applicant's co-pending Interntaional Patent Application No. PCT/IB2006/002423 the contents of which is incorporated herein by reference in its entirety.

As used herein, the term "effective amount" means an amount to treat or provide a prophylactic, therapeutic or chemopreventative effect. The specific "effective amount" will vary with factors such as the disease or condition for which the metal complex is being administered, the composition in which the metal complex is being administered, the route of administration, the age and physical condition of the human or animal, the type of animal being treated and the duration of the treatment, the nature of concurrent therapy (if any). The dosage administered and route of administration will be at the discretion of the attending, clinician or veterinarian and will be determined in accordance with accepted medical or veterinary principles. For instance, a low dosage may initially be administered which is subsequently increased at each administration following evaluation of the response of the subject. Likewise, the frequency of administration may be determined in the same way, that is, by continuously monitoring the response of the subject and modifying the interval between dosages.

The metal complex can be co-administered in combination with one or more chemotherapeutic agents conventionally used in the treatment of the particular disease, condition, or infection at hand. By "co-administered" is meant simultaneous administration in the same formulation or a plurality of formulations by the same or different routes, or sequential administration by the same or different routes. By "sequential" administration is meant one is administered one after the other. The interval between the administration of the metal complex may be relatively short and can for instance be seconds or minutes, or longer periods of times such as hours or even a day or more. The metal complex may be administered before or following the chemotherapeutic agent(s).

A composition embodied by the invention will typically further comprise a pharmaceutically acceptable carrier and be formulated to minimise dissociation of the metal complex to enhance the stability of the complex and shelf life of the formulation. Carrier formulations for enhancing stability of the complex are for instance described in the

co-pending International Patent Application No. PCT/AU2005/000442 and co-pending International Patent Application No. PCT/AU2006/000403 of the Applicant, the contents of both of which are incorporated herein by cross-reference in their entirety.

The metal complex may be dissolved in the composition or may be present in the composition as a solid. The solid complex may be in the form of a crystal containing solvents of crystallisation and/or waters of crystallisation. When the complex is charged, the complex will be associated with a counter ion.

The complex will generally be administered in the form of a composition comprising the complex together with a pharmaceutically acceptable carrier.

As used herein, a "pharmaceutically acceptable carrier" is a pharmaceutically acceptable solvent, suspending agent or vehicle for delivering the complex to a human or animal. The carrier may be liquid or solid and is selected with the intended manner of administration in mind. The carrier is "pharmaceutically acceptable" in the sense of being not biologically or otherwise undesirable, i.e., the carrier may be administered to a human or animal along with the complex without causing any or a substantial adverse reaction. For instance, the carrier may be a solvent or dispersion medium containing one or more of physiological saline, ethanol, polyol (e.g. glycerol, propylene glycol, liquid polyethylene glycol and the like), vegetable oils and mixtures thereof.

The pharmaceutical compositions can be formulated as described in International Application No. PCT/AU2005/000442 filed 30 March 2005, the contents of which is incorporated herein by cross-reference in its entirety. As described in PCT/AU2005/000442, a formulation having a colloidal structure or which forms a colloidal structure post administration is particularly desirable for administration of metal complexes. Examples of suitable compositions having a colloidal structure or which form a colloidal structure upon, or following administration, are exemplified in PCT/AU2005/00042 and any suitable such formulations for the selected mode of administration may be utilised in methods embodied by the present invention. Formation of the colloidal structure can for instance occur when the composition contacts an aqueous biological fluid in the human or animal body, for example, on contact with an aqueous fluid in the digestive tract.

A composition has a colloidal structure if it comprises a colloidal system. A colloidal system is a system in which particles of a colloidal size of any nature (eg., solid as liquid or gas) are dispersed in a colloidal phase of a different composition or state. In particularly preferred embodiments, the composition comprises micelles in an aqueous carrier or is an oil-in-water emulsion, or forms micelles or an oil-in-water emulsion when the composition is administered to a human or animal body.

Without wishing to be bound by theory, it is believed the colloidal structure protects the metal complex from interaction with acids or other compounds which would otherwise interact with the complex to cause the complex to dissociate. It is also believed the colloidal structure reduces the extent to which some compounds present in the composition are able to interact with the complex, e.g. during storage of the composition, that may cause the complex to dissociate. Similarly, when such a composition is administered to a subject, the colloidal structure may limit the extent to which some compounds that come into contact with the composition after it is administered are able to interact with the complex and which cause the complex to dissociate before it is absorbed. For example, for compositions administered orally, the colloidal structure may limit the extent to which compounds present in stomach acid are able to interact with the complex to cause the complex to dissociate before it is absorbed through the gastrointestinal tract.

Similarly, for compositions administered by other routes, the colloidal structure may limit the extent to which compounds that come into contact with the composition after it is administered, e.g. strong chelators of Cu(II), such as peptides, or reductants of Cu(II), such as thiol-containing biomolecules, are able to interact with the complex to cause the complex to dissociate. As indicated above, some compositions may not have a colloidal structure but will be formulated such that when administered to a human or animal body by the intended route of administration, a colloidal structure is formed. For example, in some embodiments, the composition is immiscible with water, and is thus immiscible with aqueous biological fluids whereby a colloidal system is thereby formed.

Preferably, the colloidal structure is maintained for a sufficient time after administration of the composition for the majority, for example more than 70%, 80% or 90%, of the metal complex, to be absorbed by the body as a metal complex.

Oils that may be utilized in compositions include pharmaceutically acceptable vegetable or mineral oils. Suitable oils include, but are not limited to: triglycerides, particularly medium chain triglycerides, combinations of medium chain and long-chain triglycerides, combinations of triglycerides with fish oil; vegetable oils, such as, soya oil, safflower oil and sunflower oils; isopropyl myristate; and paraffins. Such oils are suitable for use in compositions for oral, injectable, or topical administration.

When the composition comprises micelles in an aqueous carrier, the composition will typically further comprise one or more surfactants for formation of the micelles. Any surfactants may be used that are capable of forming micelles in the aqueous carrier, are pharmaceutically acceptable when administered by the intended route of administration, and which substantially do not interact with the metal carboxylate complex to cause dissociation from the metal when the composition is stored in the absence of light. Suitable surfactants for use in compositions for oral or topical administration include, but are not limited to, the sorbitan fatty acid ester group of surfactants. Such surfactants comprise mono-, tri-, or partial esters of fatty acids such as oleic, lauric, palmic and stearic acids, and include sorbitan trioleate (Span 85), sorbitan monooleate (Span 80), sorbitan tristearate (Span 65), sorbitan monostearate (Span 60), sorbitan monopalmitate (Span 40), and sorbitan monolaurate (Span 20).

Other suitable surfactants include the macrogol (polyoxyethylene) esters and ethers. These surfactants include, but are not limited to, the caster oil polyoxyethylene group of surfactants, such as Termul 1284 and caster oil ethoxylate. Additional surfactants in this class include the Polyoxyethylene Sorbitan Fatty Acid Esters group of surfactants, including polyoxyethylene (20) sorbitan monolaurate (Tween 20), polyoxyethylene (4) sorbitan monolaurate(Tween 21), and
polyoxyethylene (20) sorbitan monooleate (Tween 80).

A composition as described herein may can optionally further comprise one or more solvents or solubilising components for increasing the solubility of the metal carboxylate complex in the composition. The solvent may, for example, be tetraglycol (IUPAC name: 2-[2-[(tetrahydro-2-furanyl)methoxy]ethoxy]ethanol; other names: 2-[2-(tetrahydrofurfuryloxy)ethoxy]ethanol; tetrahydrofurfuryldiethyleneglycol ether) or other

glycofurols (also known as tetrahydrofurfurylpolyethyleneglycol ethers), polyethylene glycols, glycerol, propylene glycol, or other pharmaceutically acceptable glycol. An example of a solubilising component is a polyvinylacohol/povidone mixture. The composition may also further comprise a thickener such as Aerosil 200, clay or another inorganic filler.

Preferably, such compositions contain more than 80%, preferably more than 90%, and more preferably more than 95%, of the total amount of hydroxamate, hydroximate, ester, or amide derivative having anti-inflammatory activity present in the composition as part of a metal complex. Preferably, also less than 10% of the hydroxamate, hydroximate or amide derivative complexed with the metal dissociates from the metal when the composition is stored for 12 months in the absence of light at room temperature (18 °C to 25 °C). The amount of the hydoxamate, hydroximate, ester or amide remaining bound to the metal complex can be readily determined by a person skilled in the art using known methods such as EPR spectroscopy for complexes that give EPR signals or using more specialized experiments involving X-ray absorption spectroscopy for all complexes (e.g., XAFS Studies of Anti-inflammatory Dinuclear and Mononuclear Zn(II) Complexes of Indomethacin. Zhou, Q.; Hambley, T. W.; Kennedy, B. J.; Lay, P. A. *Inorg. Chem.* **2003**, *42*, 8557-8566; Determination of the Structures of Antiinflammatory Copper(II) Dimers of Indomethacin by Multiple-Scattering Analyses of X-ray Absorption Fine Structure Weder, J.E.; Hambley, T. W.; Kennedy, B. J.; Lay, P. A.; Foran, G. J.; Rich, A. M. *Inorg. Chem.* **2001**, *40*, 1295-1302; Three-Dimensional Structure Determination using Multiple-Scattering Analysis of XAFS: Applications to Metalloproteins and Coordination Chemistry. Levina, A.; Armstrong, R. S.; Lay, P. A. *Coord. Chem. Rev.* **2005**, *249*, 141-160).

In other embodiments, such as intraveneous injections, the complex is dissolved in isotonic saline solution immediately before it is injected.

More generally, the metal complex can be dissolved in the composition or can be present in the composition as a solid. The solid complex may be in the form of a crystal containing solvents of crystallisation and/or waters of crystallisation. When the complex is charged, the complex will be associated with a counter ion.

The composition for use in the method of the invention may be suitable for oral, rectal, nasal, topical (including buccal and sublingual), ophthalmological, vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration, or for administration respiratoraly, intratrachaely, nasopharanyngealy, intraoccularly, intrathecally, intranasally, by infusion, or via IV group patch and by implant. With respect to intravenous administration, particularly suitable routes are via injection into blood vessels which supply a tumour, tissues or particular organs to be treated. Agents may also be delivered into cavities such as for example the pleural or peritoneal cavity, or be injected directly into tumour tissue. The composition may conveniently be presented in unit dosage form and may be prepared by methods well known in the art of pharmacy. Such methods include the step of bringing into association the complex with the carrier. Typically, the carrier comprises two or more ingredients. In general, the composition of the present invention is prepared by uniformly and intimately bringing into association the complex with the carrier, and then, if necessary, shaping the product. The complex and the one or more components making up the carrier may be mixed in any order. However, it is preferred that the components are mixed in a manner that minimises the amount of the complex that dissociates during the preparation of the composition.

A composition for oral administration can be in the form of a viscous paste, a tablet, a capsule, a chewable composition, or any other form suitable for oral administration. The composition can also be encapsulated in a hard or soft capsule (e.g. gelatine) by techniques known in the art. Moreover, the metal complex may be provided in the form of ingestible tablets, buccal tablets, troches, elixirs, suspensions or syrups. Slow release formulations and formulations for facilitating passage through the environment of the stomach to the small intestines are also well known to the skilled addressee and are expressly encompassed by the invention.

A composition for oral use can also comprise one or more agents selected from the group of sweetening agents such as sucrose, lactose or saccharin, disintegrating agents such as corn starch, potato starch or alginic acid, lubricants such as magnesium stearate, flavouring agents, colouring agents and preserving agents e.g. such as sorbic acid, in order to produce pharmaceutically elegant and palatable preparations.

A chewable composition can, for example, comprise the metal complex, one or more flavourants, a base formulation, one or more preservatives, one or more pH modifiers, one or more desiccants and one or more fillers. As an example, for a chewable composition the base may comprise pre-gel starch, gelatine, flour and water. The composition may also comprise other components including phosphoric acid, salt, sugar, sorbitol and/or glycerol, sorbic acid and/or potassium sorbate, benzoic acid, propionic acid and maltodextrin. A chewable composition for an animal such as a dog for example, can comprise the complex, meat emulsion, an acidulate (e.g. phosphoric acid), one or more antifungal agents (e.g. benzoic acid and sorbic acid), sugar or sugar alcohol, and salt.

A composition for topical application can comprise the complex in a conventional oil-in-water emulsion, water-in-oil emulsion, or water-immiscible pharmaceutical carrier suitable for topical application. Such carriers include for example, lacrilube, cetomacrogol cream BP, wool fat ointment BP or emulsifying ointment BP. Such carriers are typically in the form of an emulsion or are immiscible with water.

An example of a composition for topical application to skin is a composition comprising 0.5-2% w/w of the complex in an emulsifying cream with chlorocresol (4-chloro-3-methylphenol) as a preservative, the emulsifying cream comprising:

cetomacrogol emulsifying wax	15 g
liquid paraffin	10 g
white soft paraffin	10 g
chlorocresol	0.1 g
propylene glycol	5 mL
purified and cooled water	to 100 g

Another example of a topical composition for application to skin is a composition comprising 2% w/w of the complex in wool fat. This composition is immiscible with water.

Compositions for parenteral administration include compositions in the form of sterile aqueous or non-aqueous suspensions and emulsions. A composition embodied by the invention can also include one or more pharmaceutically active components in addition to

the complex that have anti-cancer activity or other therapeutic activity. Such active components include conventionally used anti-inflammatory drugs, and conventionally used metal and non-metal based chemotherapeutic and anti-cancer agents such as those identified above.

Typically, the metal complex constitutes about 0.005% to about 20% by weight of the composition, preferably about 0.005% to about 6% by weight of the composition, more preferably about 0.01% to about 3% by weight of the composition. For treatment of the skin (eg., skin carcinoma, treatment of burns etc), a topically acceptable composition of the invention can comprise the metal complex in an amount of about 0.1% by weight of the composition or less.

The dosage of a metal complex embodied by the invention will depend on a number of factors including whether the complex is to be administered for prophylactic or therapeutic use, the disease or condition for which the active is intended to be administered, the severity of the condition, the age of the individual, and related factors including weight and general health of the individual as may be determined in accordance with accepted medical principles. For instance, a low dosage may initially be given which is subsequently increased or descreased at each administration following evaluation of the individual's response. Similarly, the frequency of administration can be determined in the same way that is, by continuously monitoring the individual's response between each dosage and if necessary, increasing the frequency of administration or alternatively, reducing the frequency of administration.

For oral, intravenous injection or other form of systemic administration, a metal complex as described herein will normally be administered at a dosage up to about 0.5-4 mg/kg body weight and preferably, in a range of from about 0.1 mg/kg to about 10 mg/kg body weight per day, depending on the condition being treated and the nature of the complex. More preferably, the metal complex will be administered at a dosage in a range of from 0.5 mg/kg to about 4 mg/kg body weight, and most preferably, in a range of from 1 mg/kg to about 3 mg/kg body weight. Typical oral or suppository doses will be in the range of 1 mg/kg to 4 mg/kg; sunscreen compositions and topical compositions for the prophylaxis of skin damage and aging, the metal complex will typically be dosed in a range

of 0.01-0.05% w/w topical carriers, but for more localised topical application to skin carcinomas, for wound healing or the treatment of pain and inflammation, topical formulations will typically be administered as more concentrated 0.25-2% w/w formulations, such that the maximum dosage fall with the ranges indicated above. Injection directly into cancerous lesions can have concentrations as high as 30% w/w, whereby a volume of the formulation equivalent to the volume of the lesion is injected.

Suitable pharmaceutically acceptable carriers and formulations useful in the present invention may for instance be found in handbooks and texts well known to the skilled addressee, such as "Remington: The Science and Practice of Pharmacy (Mack Publishing Co., 1995)" and subsequent update versions thereof, the contents of which is incorporated herein in its entirety by reference.

The mammalian subject may be a human or an animal. The animal can, for example, be a companion animal such as a dog or cat, or a domestic animal such as a horse, pony, donkey, mule, camel, llama, alpaca, pig, cow or sheep, or a zoo animal. Suitable animals include members of the Orders *Primates, Rodentia, Lagomorpha, Cetacea, Carnivora, Perissodactyla* and *Artiodactyla.* Typically, the subject will be a primate and more usually, a human being.

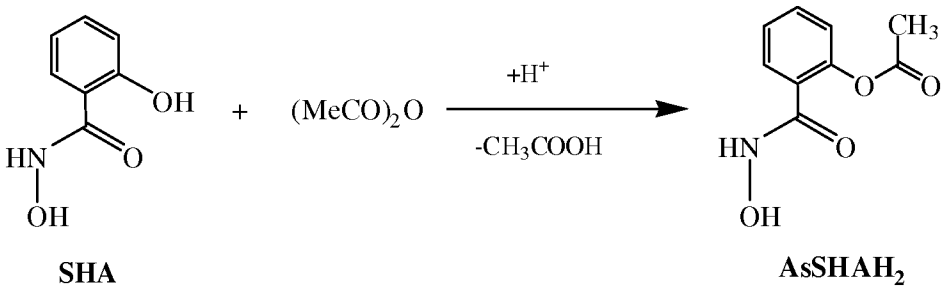
A number of embodiments of the present invention will now be described below by reference to the following non-limiting examples.

EXAMPLE 1: Preparation of compounds

1.1.1 Metal hydroxamate complexes

Exemplary preparative methods for the synthesis of several metal hydroxamate compounds are shown below.

Acetylsalicylhydroxamic Acid ($AcSHAH_2$) and its copper complex



SHA **AsSHAH$_2$**

Scheme 4: Synthetic route for AcSHAH$_2$

Salicylhydroxamic acid (SHAH$_2$) (7.65 g, 50 mmol) was mixed with acetic anhydride (9.5 ml, 100 mmol). The solution was acidified with H_3PO_4 (1 ml) and was stirred in a water bath at 60 °C for 30 min. Distilled water (5 ml) was added to the solution in order to decompose the unreacted acetic anhydride, and the resulting solution were stirred at room temperature until the vapor from the solution gave no acid reaction towards litmus paper. Finally, the reaction mixture was mixed with distilled water (50 ml) and AcSHAH$_2$ precipitated as a white powder solid (68.2%). Anal. Calcd. for $C_9H_9NO_4$: C, 55.39; H, 4.65; N, 7.18. Found: C, 55.23; H, 4.61; N, 7.06%. NMR (acetone-d_6): δ 7.82 (dd, 1H, Ph-*H*), 8.82 (bm, 1H, -N*H*OH), 7.67 (m, 4H, -C_6H_4Cl), 7.15-6.66 (m, 3H, -$C_6H_3OCH_3$), 3.77 (s, 3H, -OCH_3), 3.37 (s, 2H, O=CCH_2), 2.50 (s, 3H, CCH_3).

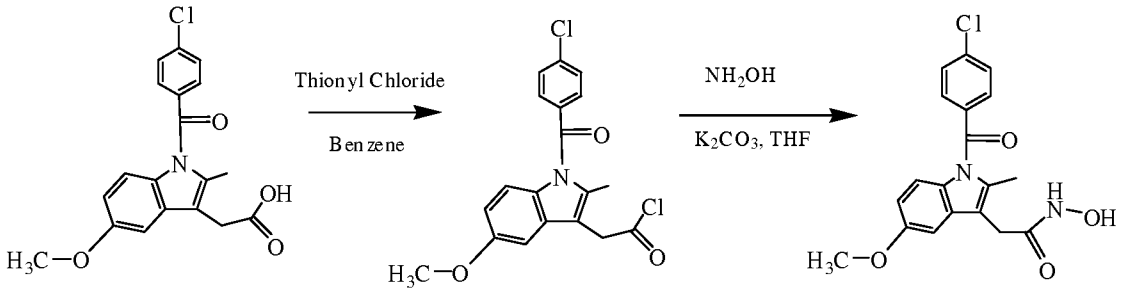
The ligand was characterized by elemental analysis and infrared spectrometry. The IR spectrum of AcSHAH$_2$ gave two absorption bands centred at 3322 and 3272 cm^{-1}, ascribed to the (OH) and (NH) stretching vibrations, respectively. In the ^{1}H-n.m.r spectrum of AcSHAH$_2$, the phenolic OH resonance of SHA at δ 12.44 ppm disappeared and a new singlet appeared at d 2.25 ppm for CH_3. Acetylation of SHA influenced the resonances of the hydroxamic OH and NH which are shifted from 11.52 to 10.74 ppm and 9.35 to 10.41 ppm, respectively. In the IR spectrum, extensive coupling occurs for several vibrations making qualitative deductions difficult. However, AcSHA shows two bands centered at 3322 and 3272 cm^{-1}, ascribed to the m(OH) and m(NH) stretching vibrations, respectively. The appearance of a sharp and strong peak located at ca. 1787 cm^{-1} in the spectrum of

AcSHA clearly indicates the presence of the acetyl carbonyl, in addition to a peak due to the hydroxamic carbonyl at ca. 1640 cm^{-1}.

Copper complex of AcSHA—[Cu(AcSHAH)(OH)]

$Cu(OAc)_2.H_2O$ (1.0 g, 5 mmol) was added to a solution of $AcSHAH_2$ (1.77 g, 10 mmol) in EtOAc (20 mL). The resulting green complex was filtered, washed with EtOAc and dried in *vacuo*. Anal. Calcd. for $C_9H_9CuNO_5$: C, 39.35; H, 3.30; Cu, 23.13; N, 5.10; Found: C, 41.45; H, 3.13; Cu, 23.17; N, 5.08%.

IndoHAH$_2$ and its Copper Complex



Scheme 5: Synthetic routine for $IndoHAH_2$

A mixture of indomethacin (7.14 g, 20 mmol) and excess $SOCl_2$ (4.5 mL) in dry benzene (150 mL) was refluxed for 3 h in a 100 mL round-bottom flask fitted with a condenser and drying tube (using $CaSO_4$). The reaction was clarified by refluxing. Excess $SOCl_2$, SO_2, and HCl were removed under partial vacuum at 0°C. The indomethacin acid chloride remaining was then reconstituted in anhydrous distilled tetrahydrofuran (THF). (See Meyer, G.A., Lostritto, R.T., and Johnson, J.F., *J. Appl. Polymer Sci.* **1991**, *42*, 2247-2253 and Davaran, S. and Entezami, A. A., *J. Controlled Release* **1997**, *47*, 41-49.)

A mixture of hydroxylamine hydrochloride (1.39 g, 0.02 mmol) and potassium carbonate (2.764 g, 0.02 mmol) was dissolved in water (4 mL), diethyl ether (200 mL) was added, and the resulting suspension was stirred for 10 min, then the suspension of

indomethacin acid chloride (prepared above) in THF (120 mL) was added and the reaction mixture was allowed to come to room temperature (RT) and additionally stirred for 48 h. The suspension was then filtered and washed with additional anhydrous ether and then re-suspended in boiling THF (300 mL) and allowed to recrystallize at RT. The white crystals were collected by filtration and washed twice with diethyl ether to offer 5.6 g white product. Anal Calcd for $C_{19}H_{17}ClN_2O_4$: C, 61.21; H, 4.60; Cl, 9.51; N, 7.51. Found: C, 60.68; H, 4.77; Cl, 9.06; N, 7.07%. NMR (DMSO-d_6): δ 10.65 (s, 1H, NO*H*), 8.82 (bm, 1H, -N*H*OH), 7.67 (m, 4H, -C_6H_4Cl), 7.15-6.66 (m, 3H, -$C_6H_3OCH_3$), 3.77 (s, 3H, -OCH_3), 3.37 (s, 2H, O=CCH_2-), 2.50 (s, 3H, CCH_3).

Copper complex of IndoHAH - [Cu(IndoHAH)(OH)]

IndoHA (0.932 g, 2.5 mmol) in ethanol (15 mL) was added to $Cu(OAc)_2.H_2O$ (0.25 g, 1.25 mmol) in ethanol (15 mL) and the mixture was left to stir overnight. The resulting green complex was filtrated, washed with ethanol and dried in *vacuo*. Anal. Calcd. for $C_{19}H_{17}ClCuN_2O_5$: C, 50.45; H, 3.79; N, 6.19; Cl, 7.84; Cu, 14.05. Found: C, 50.96; H, 3.52; N, 6.03; Cl, 8.05; Cu, 13.59. The microanalysis indicated that the complex was either a dimer with two OH^- bridges or $IndoHAH^-$ bridges, or a polymer with these bridging ligands.

Vanadium(V) complex of IndoHA(H)

$VOSO_4 \cdot 5H_2O$ (50.6 mg, 0.200 mmol) and $IndoHAH_2$ (149 mg, 0.400 mmol) were dissolved in methanol (MeOH, HPLC grade, 5.0 mL). The colour of the solution immediately turned dark-red. This solution was added to ice-cold H_2O (Milli-Q grade, 50 mL), which led to the formation of a fine brown precipitate. The precipitate was isolated by centrifugation (5 min at 4000 *g*) and dissolved in a minimal volume of MeOH (~20 mL). The resultant solution (which was slightly cloudy) was filtered through a small-pore (No. 4) glass filter under vacuum. The volume of the filtrate was reduced by ~2/3 under reduced pressure (at 40 °C), which led to the formation of a red precipitate. After cooling the solution to ~0 °C, the precipitate was isolated on a glass filter (No. 4) under vacuum, and

dried overnight under vacuum over silica gel. Yield, 48.9 mg (54.3% as calculated for $[V^{V}O(IndoHAH)(IndoHA)]\cdot 2MeOH\cdot 1.5H_2O$.

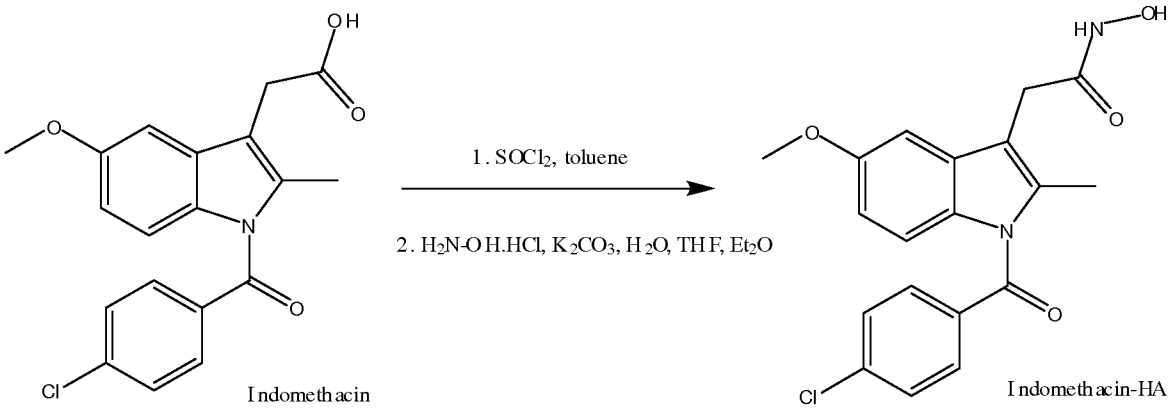
The most intense signal in the electrospray mass spectrum (solution in MeOH, ~1 mg mL^{-1}): *m/z* -807.1 (corresponds to $[V^{V}O(L)_2]^-$, according to the isotope distribution pattern). Characteristic signals in the IR spectrum (solid mixture with KBr, diffuse reflectance mode): 1686 cm^{-1} (s) (C=O of the hydroxamato group); ~3300 cm^{-1} (br) and 1590 cm^{-1} (m) (-NH of the hydroxamato group); 992 cm^{-1} (m) (V=O). EPR spectrum (X-band, 22 °C, solution in acetone, ~10 mg mL^{-1}): no signals (i.e., the product is V(V) rather than V(IV) complex). Calculated for $[V^{V}O(IndoHAH)(IndoHA)]\cdot 2MeOH\cdot 1.5H_2O$ ($C_{40}H_{42}Cl_2N_4O_{12.5}V$): C, 53.34%; H, 4.70%; N, 6.22%. Found: C, 54.05%; H, 4.46%; N, 6.22%.

The colour of the complex is strongly solvent-dependent: the solid compound is brown, solutions in methanol are orange-red, and solutions in tetrahydrofuran are dark-purple. Thus, it is most likely that the complex is six-coordinate, with a molecule of solvent as a ligand.

Vanadium(V) complex of IndoHA(H), $[VO(IndoHAH)_2(OMe)]$ or $[VO(IndoHA)(IndoHAH)(MeOH)]$

It is not possible to determine whether there is a second deprotonation of one of the hydroxamic acids or a deprotonation of the MeOH since both complexes have five identical microanalysis and mass spectra.

Synthesis of IndoHA



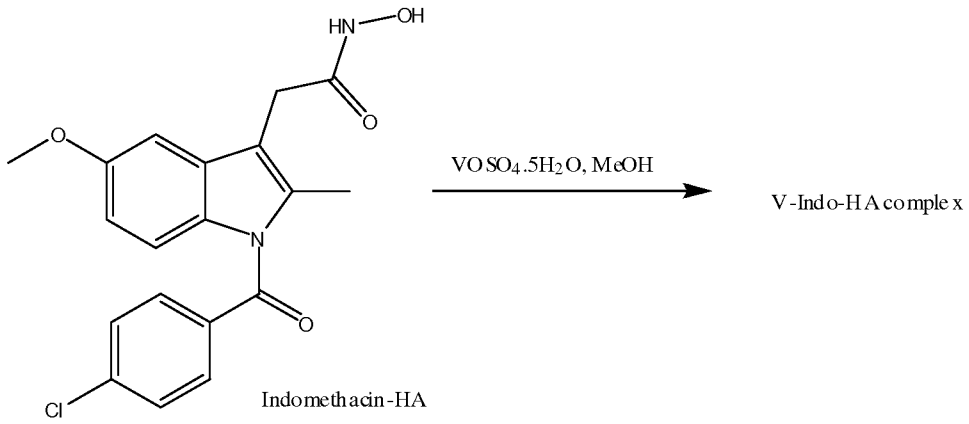
Scheme 6

To a stirred suspension of indomethacin (0.50 g, 1.40 mmol) in toluene (5 mL) was added thionyl chloride (1 mL, excess) and the mixture was heated to 80 °C. The solid dissolved and the batch became darker in colour, turning green and then brown. After 1.5 hr, TLC (quench into methanol) showed that the reaction was complete. The majority of the solvent and acidic vapours ($SOCl_2$, HCl, SO_2) were removed in the fume hood under a stream of nitrogen. The remainder was then removed via rotary evaporator to give a dark green oil / foam. This was redissolved in THF (3 mL).

To a second vessel was charged NH_2-OH.HCl (196 mg, 2.80 mmol) and K_2CO_3 (0.39 g, 2.80 mmol), followed by water (0.75 mL) and diethyl ether (12.5 mL) to give a mixture of two liquid phases (solids all dissolved). To this was added the THF solution of the acid chloride, over 2 minutes. A pale brown precipitate formed and the reaction was left to stir at room temperature. After 2 hr, TLC indicated that reaction was complete.

Sat. $NaHCO_3$ (aq) (20 mL) and EtOAc (20 mL) were then added, these phases were separated, and the organic phase was washed with further sat. $NaHCO_3$ (aq) (20 mL). The combined aqueous phase was back-extracted with EtOAc (20 mL). The combined organic portions were washed with water (20 mL), dried ($MgSO_4$) and concentrated in vacuo to give an orange / yellow solid. This was recrystallised from EtOAc / hexane to give a tan coloured solid (0.28 g, 54%).

The vanadium (V) complex was then synthesised using Indo-HA as follows.



Scheme 7

Indo-HA (300 mg, 0.81 mmol) was stirred in methanol (20 mL) to give a yellow slurry. To this was added a (pale blue) solution of $VOSO_4.5H_2O$ (107 mg, 0.40 mmol) in methanol (10 mL). The batch immediately turned dark red and almost all the solid dissolved. More methanol (2 mL) was added to rinse in the vanadium solution. A small amount of solid remained out of solution, but after 20 minutes this had dissolved to give a complete dissolution. After 2 hr, the batch had formed a solid dark red precipitate. Oxygen was bubbled through the batch for 1 minute to ensure complete oxidation from V(IV) to V(V). There was no visual change with the oxygen bubbling. The batch was concentrated to reduce the volume to around half the original volume, prior to being cooled to 0 °C and then filtered. The orange / red solid obtained was dried under high vacuum to give 251 mg. Anal. Calcd for $C_{39}H_{35}N_4Cl_2O_{10}V$: C, 55.66; H, 4.19; N, 6.66; Cl, 8.43; O, 19.01; V, 6.05. Found: C, 55.69; H, 4.22; N, 6.72, Cl, 8.53; O, 19.05; V, 6.08.

Chromium(III) complex of IndoHAH

$Cr(NO_3)_3 \cdot 9H_2O$ (40.0 mg, 0.100 mmol) and IndoHA (149 mg, 0.400 mmol) were dissolved with stirring in MeOH (HPLC grade, 5.0 mL), and triethylorthoformate (2.0 mL; a water-withdrawing agent) was added. The solution was gently refluxed (~60 °C) for 4 h (which led to a colour change from blue to dark-green), then left at 22 °C overnight. The volume of the solution was reduced to ~1 mL under a stream of N_2. The concentrated solution was applied to a column (1.5×15 cm) of Sephadex LH-20, and eluted with MeOH. The fast-moving grey-green fraction was collected, evaporated to dryness under reduced pressure (40 °C), and dried over silica gel under vacuum overnight. A smaller second fraction (yellow-green) was also collected, but it did not show the expected signals in the electrospray mass spectra (ESMS), and was not further analysed. Yield of the grey-green compound, 66.4 mg (72.6% based on the $[Cr(LH)_2(OH_2)_2](NO_3) \cdot H_2O$ structure, MW = 913.6 Da, where LH_2 = IndoHA).

The *m/z* values for the most intense signals in ESMS (solution in MeOH, ~1 mg mL^{-1}) were as follows: +1165.9 (rel. abund. 100%; $[Cr(LH)_2]^+ \cdot LH_2$ or $[Cr(LH)_3] \cdot H^+$); +857.5(99%; $[Cr(LH)_2]^+ \cdot 2MeOH$); +843.4 (98%; $[Cr(LH)_2]^+ \cdot MeOH \cdot H_2O$); +829.3 (85%; $[Cr(LH)_2]^+ \cdot 2H_2O$); +811.2 (32%; $[Cr(LH)_2]^+ \cdot H_2O$); +794.0 (56%; $[Cr(LH)_2]^+$); +1960.8 (60%; $2[Cr(LH_2)]^+ \cdot LH^-$); –855.0 (100%; $[Cr(LH)_2(OMe)_2]^-$); –917.9 (62%; $[Cr(L)(LH)] \cdot NO_3^-$); –792.2 (55%; $[CrL_2]^-$); and –1649.8 (30%; $[Cr(LH)_2(OMe)_2]^- \cdot [Cr(L)(LH)]$). All the assignments are in agreement with the predicted isotope distribution patterns.

Characteristic signals in the IR spectrum (solid mixture with KBr, diffuse reflectance mode): 1686(s) (C=O of the hydroxamato group); ~3300(br) and 1595(m) ($\tilde{N}H$ of the hydroxamato group); ~3000(br) (H_2O). Calculated for $[Cr(LH)_2(OH_2)_2](NO_3) \cdot H_2O$ ($C_{38}H_{40}Cl_2N_5O_{14}Cr$): C, 49.95%; H, 4.41%; N, 7.66%. Found: C, 50.86%; H, 4.29%; N, 7.21%.

Synthesis and characterisation of a Ga(III)-IndoHAH complex

An acidic aqueous solution of Ga(III) (0.64 M) was prepared by partial dissolution of a piece of metallic Ga (99.99%, Fluka) in aqueous HCl (~5 M, ultra-pure, Merck), and the amount of dissolved Ga was determined by the mass difference. A portion of this solution (5.0 mM) was evaporated to dryness at 100 °C, and the residue was dried under vacuum overnight and dissolved in anhydrous MeOH (5.0 mL), giving a solution of $GaCl_3$ (0.64 M) in MeOH. This solution (0.78 mL, 0.050 mmol Ga) was added to a solution of IndoHA (55.8 mg, 0.15 mmol) in MeOH (~2 mL), followed by the addition of a solution of KOH in MeOH (1.5 mL of 0.10 M solution, 0.15 mmol KOH). The resultant solution was left at room temperature (RT) for 6 h (during which time a small amount of white precipitate formed), then filtered through a 0.20-μm membrane filter, and the filtrate was evaporated to dryness under a stream of N_2. The yellow oily residue was suspended in H_2O (~5 mL), leading to a yellow-white amorphous solid. This solid was separated by vacuum filtration, washed with H_2O, and dried under vacuum (yield of the dry solid, 45.5 mg). Repeated syntheses were carried out as described above, but either without the addition of KOH, or with the addition of excess KOH (0.45 mmol). All three syntheses led to yellow-white amorphous solids, which gave identical IR spectra. Therefore, only one product (from the above-described synthesis) was further characterised.

A comparison of ^{1}H NMR spectra of $IndoHAH_2$ and the Ga(III)-IndoHAH complex (in d_6-DMSO) showed that all the signals of the parent ligand were split into two for the complex, but all the resultant signals were shifted compared with that of the ligand (i.e., there was no unreacted ligand left). Only one highly polar proton signal (~12 ppm), corresponding to the hydroxamato group, was observed in the spectrum of Ga(III)-IndoHAH, instead of two such signals for IndoHA (~9 and ~11 ppm). Thus, the NMR data indicate the formation of two Ga(III)-IndoHAH complexes (or geometric isomers) with singly deprotonated IndoHAH ligands. These results are consistent with the IR data, showing that the C=O signal at 1649 cm^{-1} for $IndoHAH_2$ was replaced with two signals at 1684 and 1591 cm^{-1}. The main ESMS signal for Ga(III)-IndoHAH (~1 mM solution in DMF) was that at m/z = +883.7 (^{69}Ga), corresponding to $[Ga(LH)_2]^+ \cdot DMF$ (where LH_2 = $IndoHAH_2$). The Ga content in the complex, determined spectrophotometrically with 4-(2-

pyridylazo)resorcine (PAR) after digestion of the complex with concentrated HNO_3, was 8.0 and 8.7% (for two parallel samples,). An acidic aqueous solution of Ga(III) (0.64 M, see above) was used as a standard. The Ga content in the complex is close to that expected for $[Ga(LH)_2(Cl)(OH_2)]$ (FW = 865.4, 8.0% Ga) or $[Ga(LH)_2(OH_2)_2]Cl$ (FW = 883.4, 7.9% Ga), but not to that for $[Ga(LH)_3]$ (FW = 1183.0, 5.9% Ga). In summary, the data indicate the formation of a bis-ligated Ga(III) hydroxamato complex, $[Ga(LH)_2(OH_2)_2]^+$

Cobalt complexes of IndoHAH

***Cis*-$[Co(en)_2(OSO_2CF_3)_2](CF_3SO_3)$**

Nitrogen was bubbled through a solution of *trans*-$[Co(en)_2Cl_2]Cl$ (8.0 g) in anhydrous CF_3SO_3H (36 mL) as it was heated at 90-100 °C for 3 h. After the solution was cooled, diethyl ether (0.15 L) was added slowly with vigorous stirring. The solid was filtered, triturated on the frit with ether (~60 mL) and dried in air. The product was ground in a mortar, boiled in chloroform (~80 mL) for 10 min to remove traces of CF_3SO_3H, filtered, washed with ether, and dried in vacuum over P_2O_5 to give a free-flowing purple powder (27 g, 94% yield). ^{1}HNMR (300 MHz): δ 2.90 (3 H, *trans* NH_3), 3.16 (12 H, *cis* NH_3).

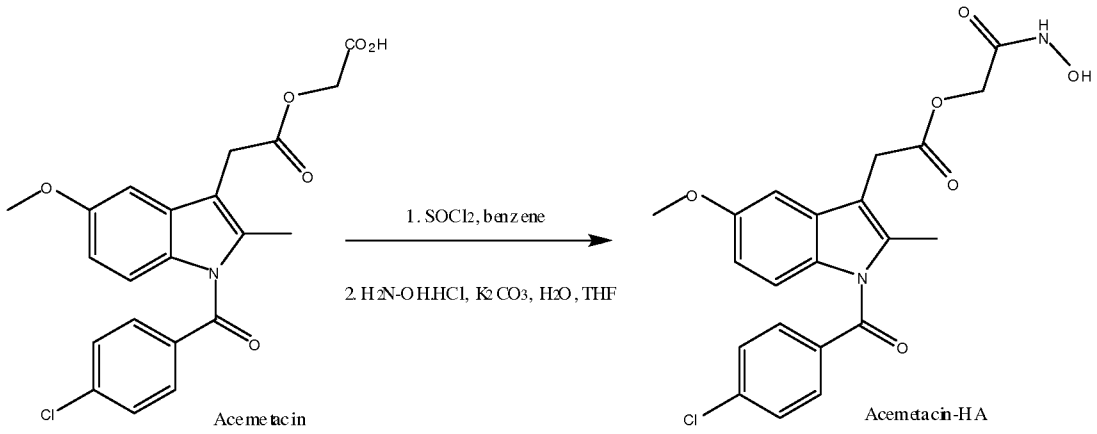
$[Co(en)_2(IndoHAH)](CF_3SO_3)_2$

To a solution of *cis*-$[Co(en)_2(OSO_2CF_3)_2](CF_3SO_3)$ (1.25 g, 2 mmol) in DMSO (10 mL) was slowly added a solution of oxametacin (0.802 g, 2.2mmol) in DMSO (10 mL) and the resulting mixture was heated at 80 °C overnight. After cooling, the orange solution was slowly added to ice-cooled diethyl ether (400 mL) with vigorous stirring, the oily compounds solidified after decantation, stirring and sonication. To remove impurities, the orange coloured compound was dissolved in the minimum volume of ethanol, filtered, and the filtrate was slowly added to ice-cold diethyl ether with vigorous stirring. The precipitate obtained was dried in a vacuum desiccator over P_2O_5. ^{1}H NMR (DMSO-d_6): δ 11.84(s), 7.69-6.72(m), 5.02-4.17 (m), 3.79(s), 3.64(s), 3.32(s), 2.45(t), 2.26(s). ^{13}C NMR (DMSO-d_6): 13.46, 23.80, 42.84, 42.98, 44.81, 45.07, 55.50, 102.01, 111.18, 111.53, 114.54, 122.79, 129.04, 130.37, 130.54, 131.25, 133.99, 136.15, 137.82, 155.60, 167.88, 168.59.

$[Co(en)_2(IndoHAH)]Cl_2$

$[Co(en)_2(IndoHAH)](CF_3SO_3)_2$ was dissolved in minimum volume of acetone (HPLC grade), and was added to two equivalent of $Et_4NCl{\cdot}5H_2O$ with stirring, after stirring 10 min, a pink coloured solid precipitated out from the solution. The solid was removed by filtration, and re-dissolved in water, filtered and the insoluble solids discarded. The filtrate was then slowly added to ice-cold diethyl ether with vigorous stirring. The precipitate was filtered, washed with diethyl ether, and dried in desiccator under vacuum. ^1H NMR (DMSO-d_6): δ 11.84(s, broad), 7.72-6.72(m, Ph-*H*), 5.05-4.39 (m), 3.79(d), 3.65(s), 3.33(s), 3.21(tetra), 2.25(s), 1.16. ^{13}C NMR (DMSO-d_6): 7.41, 13.85, 24.20, 43.26, 43.37, 45.17, 45.43, 51.77, 55.89, 102.40, 111.57, 111.99, 114.92, 129.42, 130.75, 130.94, 131.64, 134.39, 136.50, 138.18, 155.98, 168.26, 168.91.

Acemetacin-hydroxamic acid



Scheme 8

To a suspension of acemetacin (1.40 g, 3.37 mmol) in benzene (14 ml) was added thionyl chloride (1 ml, excess). The batch was stirred and heated to reflux for 30 minutes. After this time the reflux condenser was exchanged for a distillation kit and the batch was distilled to remove HCl, SO, SO_2, and most of the benzene. The residue was then concentrated via rotary evaporator to obtain a pale orange solid, and the solid was redissolved in THF (15 ml).

Hydroxylamine HCl (0.47 g, 6.74 mmol) and potassium carbonate (0.93 g, 6.74 mmol) were dissolved in water (2 ml), diethyl ether (35 ml) was then added and the mixture was stirred. To this was added the acid chloride solution described above. After 16 hrs, EtOAc (40 ml) and sat. $NaHCO_3$ (aq) (40 ml) were added, and the two phases were separated. The organic phase was further washed with sat. $NaHCO_3$ (aq) (3 x 20 ml), dried ($MgSO_4$), and concentrated in vacuo to afford a yellow foam: 1.15 g obtained. This was recrystallised from EtOAc / hexane to afford 1.02 g as a pale yellow solid, 70% molar yield.

m/z (negative electrospray): 429 (L^- for AcHA ^{35}Cl), 100%, and 431 (L^- for AcHA ^{37}Cl), 34%.

δH (300 MHz, DMSO-d_6) 10.73 (br s, 0.70 H), 10.67 (br s, 0.09 H), 10.22 (br s, 0.16 H), 9.22 (br s, 0.16 H), 8.97 (br s, 0.70 H), 8.84 (br s, 0.09 H), 7.70-7.63 (4H, m, $C_6\underline{H}_4Cl$), 7.08 (1H, d, *J* 2.4 Hz), 6.94 (1H, d, *J* 9.0 Hz), 6.72 (1H, dd, *J* 9.0, 2.4 Hz), 4.47 (2H, s, $OC\underline{H}_2$), 3.87 (2H, s, $O{=}CC\underline{H}_2$), 3.78 (3H, s, $OC\underline{H}_3$), and 2.22 (3H, s, $CC\underline{H}_3$).

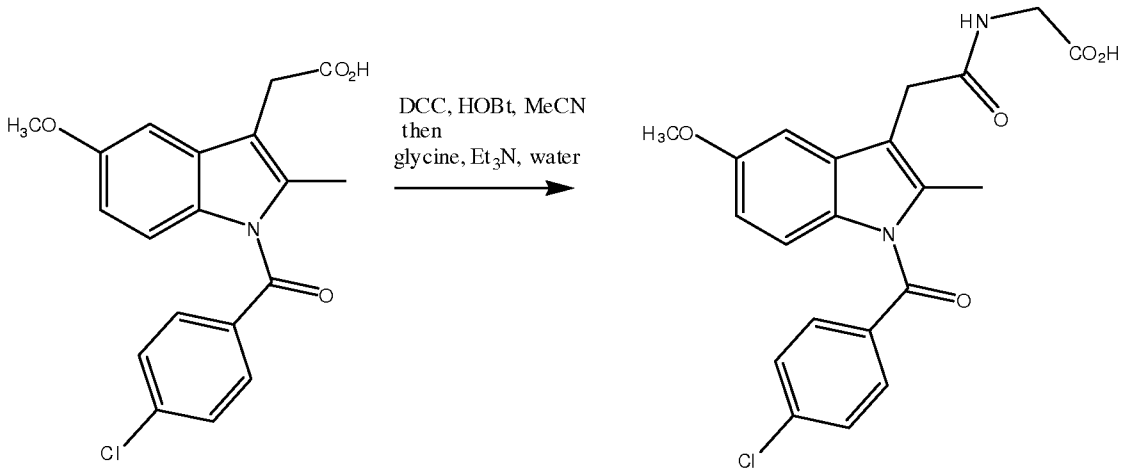
Analogous reactions to those described above can be used to make other metal acemetacin-hydroxamic acid complexes. Similarly, complexes of metals with other NSAID hydroxamic acid ligands can be prepared using these techniques.

1.1.4 Metal amine complexes with amide derivatives of carboxylates

Preparation of Indomethacin-glycine adduct

The method is based on that described in International Patent Application No. WO 95/04030, with the modification that 1-hydroxybenzotriazole (HOBt) was used in place of hydroxysuccinimide (HOSu). To a mixture of IndoH (3.97 g, 11.10 mmol) and HOBt (1.65 g, 12.21 mmol) was added acetonitrile (100 ml) and the resulting yellow suspension was stirred at room temperature. To this slurry was added N,N'-dicyclohexylcarbodiimide (DCC) (2.52 g, 12.21 mmol). After a few minutes, the mixture became thick. Stirring was continued at room temperature. After 1 hr, a solution of glycine (0.92 g, 12.21 mmol) in water (10 ml) and triethylamine (1.7 ml, 1.23 g, 12.21 mmol) was added dropwise. Additional water (20 ml) was added to help improve the mobility of the

mixture. After stirring at room temperature for 20 hours, water (100 ml) was added, and the mixture was acidified using dil. HCl (aq). EtOAc (30 ml) was then added and the batch was filtered to remove solid urea by-product. The aqueous and organic phases were separated and the aqueous was again extracted with EtOAc (30 ml). The combined organic phases were dried ($MgSO_4$) and concentrated in vacuo to give a yellow foam. This crude product was purified by column chromatography using silica and a gradient eluent system of DCM:MeOH (98:2, increasing the methanol content gradually to 3%, 5% then 10%). The fractions containing clean product were combined and concentrated in vacuo to give a pale yellow solid: 2.75 g (60%). Electrospray mass spec (negative ion): 413 (L^- for ^{35}Cl), 100%, and 415 (L^- for ^{37}Cl), 40%.



Scheme 9

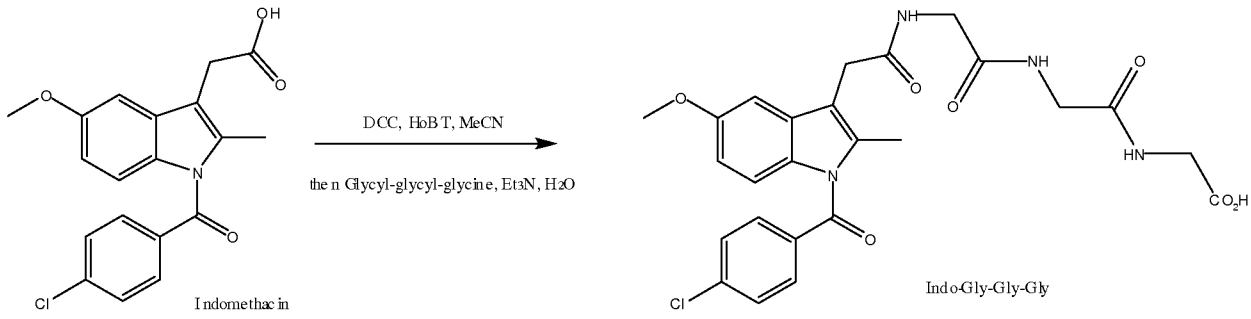
[Cu(Indo-Gly)(Im)$_2$]

$Cu(OAc)_2 \cdot H_2O$ (0.108 g, 0.5408 mmol) in methanol (8 mL) and water (1 mL) was sonicated for 0.5 h to facilitate dissolution. Indomethacin-glycine (0.464 g, 1.118 mmol) and imidazole (0.076 g, 1.118 mmol) in methanol (25 mL) was stirred until it dissolved. The solution of Cu^{2+} was added dropwise to the solution of indomethacin-glycine and imidazole at ~30 °C with stirring and the mixture changed colour from green to dark blue. The solid was formed after 30 min, filtered and washed with methanol (3 mL) once, and dried under N_2 at room temperature. The yield was 0.26 g (46.8 %). EPR spectroscopy confirmed that this compound is a monomer. Electrospray mass spectroscopy of [Cu(Indo-

$Gly)_2(Im)_2]$ in DMF shows the presence of the $[Cu(Indo\text{-}Gly)]^+$ (475.9) and [Cu(Indo-Gly)(Imidazole)]$^+$ (544.0). From the IR spectra typical peaks of ligands of Indomethacin-glycine and imidazole are observed.

Vanadium and chromium complexes of this ligand are prepared by similar methods as those described for IndoHA, but electrospray mass spectrometry indicates that the complexes are polymeric rather than monomeric.

Indomethacin-glycyl-glycyl-glycine

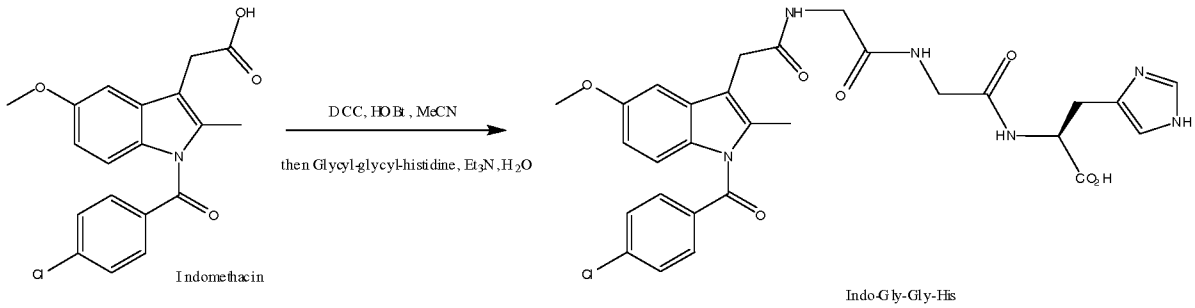


Scheme 10

To a mixture of indomethacin (75 mg, 0.21 mmol) and HOBt (31 mg, 0.23 mmol) was added acetonitrile (5 ml) and the resulting yellow suspension was stirred at room temperature. To this slurry was added N,N'-dicyclohexylcarbodiimide (DCC) (47 mg, 0.23 mmol) and the mixture was left to stir at room temperature. After 1 hour, a solution of glycyl-glycyl-glycine (52 mg, 0.27 mmol) in water (1 ml) and triethylamine (0.04 ml, 27 mg, 0.27 mmol) was added dropwise. The batch was stirred overnight at room temperature.

After 16 hrs, water (50 ml) was added and the mixture was extracted twice with EtOAc (50 ml, then 15 ml). The combined organic extracts were washed with brine (20 ml) and then concentrated in vacuo to give a pale yellow solid: 0.36 g obtained. The crude product was recrystallised from MeCN to give a white solid, 0.17 g. This is > 100% yield and NMR confirmed that the product was contaminated with DCC-urea.

Indomethacin-Gly-Gly-His



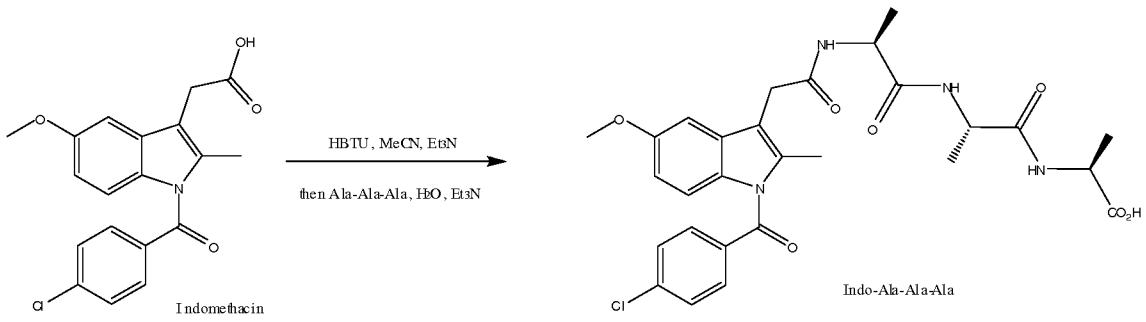
Scheme 11

To a stirred suspension of Indomethacin (93 mg, 0.26 mmol) and HOBt (39 mg, 0.29 mmol, 1.1 eq) in acetonitrile (5 ml) at room temperature was added DCC (60 mg, 0.29 mmol, 1.1 eq). The DCC was washed in with additional acetonitrile (2 ml) and all the solid dissolved. The solution was stirred at room temperature for 2 hrs and a precipitate formed during this time.

A solution of Gly-Gly-His (84 mg, 0.31 mmol, 1.2 eq) in water (1 ml) and triethylamine (0.04 ml, 31 mg, 0.31 mmol, 1.2 eq) was added dropwise to the batch. The reaction was stirred at room temperature for 16 hrs. The batch was filtered to remove by-product DCC-urea, and the filter-cake was washed with EtOAc (2 x 4 ml). However, the product Indo-Gly-Gly-His was out of solution at the end of the reaction and was isolated on the filter-cake. After drying, this solid weighed 164 mg; the expected amount of DCC-urea was 65 mg. NMR confirmed that the solid was a combination of Indo-Gly-Gly-His and DCC-urea. By NMR, the purity was 88% Indo-Gly-Gly-His (by weight).

δH (300MHz, DMSO-d6) 8.33-8.29 (1H, m, one of CONH), 8.21-8.14 (2H, m, two of CONH), 7.70-7.62 (4H, m, C_6H_4Cl), 7.57 (1H, s, one of Histidine-CH), 7.14 (1H, d, *J*2.4 Hz, one of $C_6H_3OCH_3$), 6.93 (1H, d, *J*9.0 Hz, one of $C_6H_3OCH_3$), 6.69 (1H, dd, *J*9.0, 2.5 Hz, one of $C_6H_3OCH_3$), 3.79-3.69 (8H, m, CH_3O, 2 x CH_2NH, and NHC^*HCH_2), 3.59 (2H, s, indole-CH_2), 2.96-2.80 (2H, m, C^*HCH_2), and 2.23 (3H, s, indole-CH_3). [peaks for DCC-urea were also observed: 5.57 (d, *J*8.0 Hz), and 1.80-0.97 (m)].

Indomethacin-Ala-Ala-Ala



Scheme 12

To a stirred suspension of Indomethacin (200 mg, 0.56 mmol) in acetonitrile (10 ml) at room temperature was added triethylamine (0.09 ml, 62 mg, 0.62 mmol, 1.1 eq) and the solid all dissolved to give a yellow solution. HBTU (*O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate) (235 mg, 0.62 mmol, 1.1 eq) was added portionwise. This solid all dissolved initially but a precipitate formed during the next five minutes.

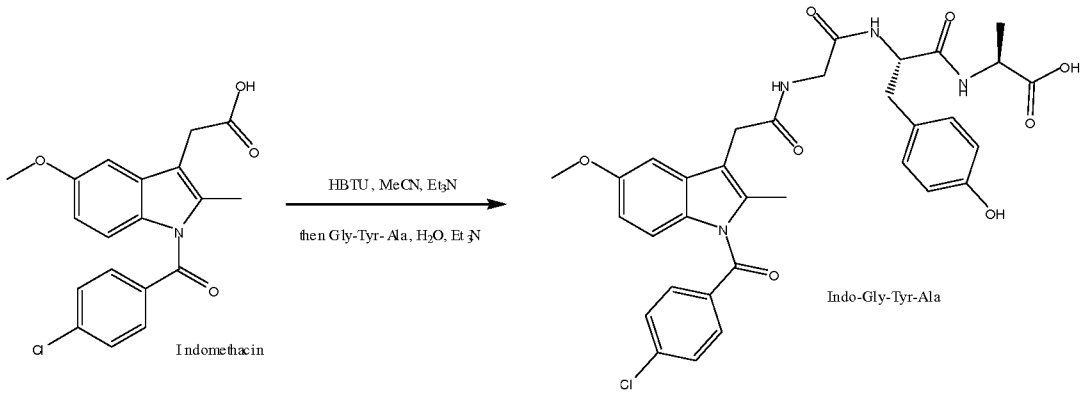
After 1.5 hrs, a solution of Ala-Ala-Ala (143 mg, 0.62 mmol, 1.1 eq) in water (4 ml) and triethylamine (0.09 ml, 62 mg, 0.62 mmol, 1.1 eq) was added dropwise, and the batch was stirred overnight at room temperature.

After 16 hrs, TLC confirmed that all the Indomethacin had been consumed (silica, DCM : MeOH, 90 : 10). Water (50 ml) and EtOAc (50 ml) were added and the aqueous phase was adjusted to pH 4-5 using dil. HCl (aq). Separation was poor, but the two phases did eventually separate. The aqueous was extracted with further EtOAc (25 ml). The combined EtOAc phases were washed with sat. brine (50 ml) and the brine was then re-extracted with EtOAc (25 ml). The combined EtOAc phases were then concentrated in vacuo to give a pale yellow solid (0.74 g obtained).

The crude product was slurried in a mixture of IPA (60 ml) and water (5 ml) and heated to reflux. The material did not dissolve so the hot mixture was filtered to isolate the off-white solid, 0.25 g obtained after drying (78% yield).

NMR confirmed the correct structure and indicated that the material was of high purity. δH (300MHz, DMSO-d6) 12.75-12.35 (0.8H, br s, CO_2H), 8.34 (1H, d, *J*7.5 Hz, one of NH), 8.05 (1H, d, *J*7.2 Hz, one of NH), 7.99 (1H, d, *J*8.1 Hz, one of NH), 7.69-7.62 (4H, m, C_6H_4Cl), 7.15 (1H, d, *J*2.4 Hz, one of $C_6H_3OCH_3$), 6.94 (1H, d, *J*9.0 Hz, one of $C_6H_3OCH_3$), 6.69 (1H, dd, *J*9.0, 2.5 Hz, one of $C_6H_3OCH_3$), 4.33-4.14 (3H, m, 3 x NHC^*HCH_3), 3.76 (3H, s, CH_3O), 3.56 (2H, br s, indole-CH_2), 2.22 (3H, s, indole-CH_3), and 1.25-1.19 (9H, m, 3 x NHC^*HCH_3).

Indomethacin-Gly-Tyr-Ala



Scheme 13

To a stirred suspension of Indomethacin (226 mg, 0.63 mmol) in acetonitrile (6 ml) at room temperature was added triethylamine (0.1 ml, 70 mg, 0.70 mmol, 1.1 eq) and the solid all dissolved to give a bright yellow solution. HBTU (*O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate) (268 mg, 0.70 mmol, 1.1 eq) was added portionwise. This solid all dissolved. Traces of HBTU were washed in using acetonitrile (2 ml).

After 2.5 hrs, a white precipitate had formed. A solution of Gly-Tyr-Ala (215 mg, 0.70 mmol, 1.1 eq) in water (6 ml) and triethylamine (0.1 ml, 70 mg, 0.70 mmol, 1.1 eq) was added dropwise and washed in with more water (1 ml). Most of the solid dissolved and the batch was stirred overnight at room temperature. After 16 hrs, all solid had dissolved to

give a pale yellow solution. TLC confirmed that all the Indomethacin had been consumed (silica, DCM : MeOH, 90 : 10). Water (25 ml) and EtOAc (40 ml) were added and the aqueous phase was adjusted to pH 4 using dil. HCl (aq). Separation was poor, but the two phases did eventually separate with gentle heating. The aqueous had to be readjusted to pH 4 using dil. HCl (aq). This aqueous was then extracted with further EtOAc (25 ml). The aqueous was again adjusted to pH 3-4 using dil. HCl (aq) and extracted with EtOAc (25 ml). The combined EtOAc phases were washed with water (25 ml) then sat. brine (25 ml), then concentrated in vacuo to give a pale yellow foam (0.67 g obtained).

Recrystallisation: EtOAc (15 ml) was added and the mixture was heated to reflux. However, the solid did not dissolve. The batch was allowed to cool to room temperature and then filtered to afford a yellow solid which was dried under vacuum at 40°C to give 0.36 g. This was shown by NMR to be the correct product but 92% purity.

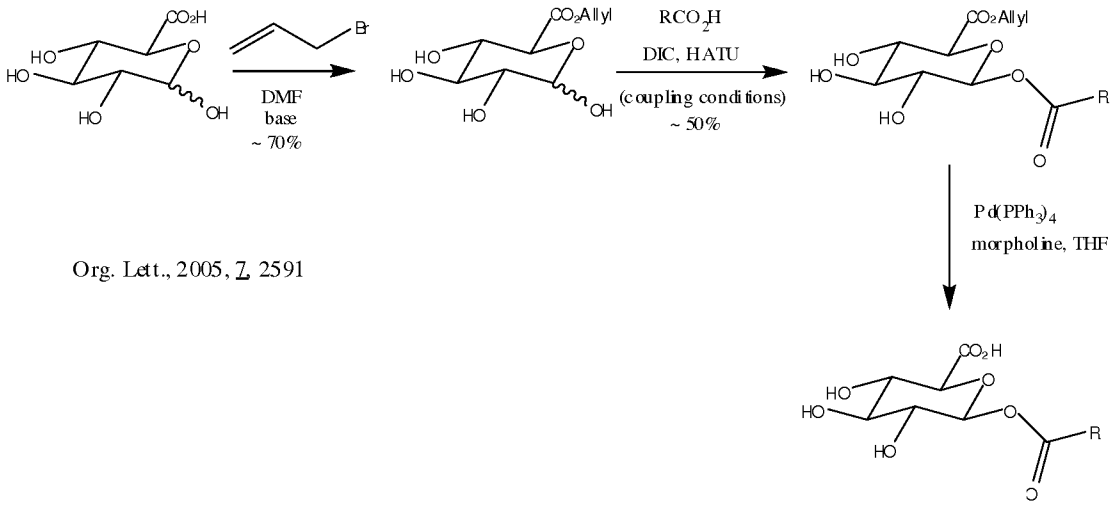
Yield 0.36 g, 92% purity = 0.33 g, 100% purity, i.e. 80% yield.

δH (300MHz, DMSO-d6) 12.75-12.40 (0.8H, br s, CO_2H), 9.23 (1H, s, phenolic OH), 8.34 (1H, d, *J*7.3 Hz, one of NH), 8.28-8.24 (1H, m (pseudo t), one of NH), 8.00 (1H, d, *J*8.5 Hz, one of NH), 7.69-7.63 (4H, m, C_6H_4Cl), 7.15 (1H, d, *J*2.4 Hz, one of $C_6H_3OCH_3$), 7.01 (2H, d, *J*8.4 Hz, two of C_6H_4OH), 6.93 (1H, d, *J*9.0 Hz, one of $C_6H_3OCH_3$), 6.68 (1H, dd, *J*9.0, 2.5 Hz, one of $C_6H_3OCH_3$), 6.62 (2H, d, *J*8.4 Hz, two of C_6H_4OH), 4.49-4.41 (1H, m, NHC^*HCH_3), 4.23-4.14 (1H, m, NHC^*HCH_2Ar), 3.75 (3H, s, CH_3O), 3.79-3.71 and 3.58-3.51 (2H, m, $NHCH_2$), 3.55 (2H, s, indole-CH_2), 2.94-2.85 and 2.64-2.55 (2H, m, C^*HCH_2Ar), 2.21 (3H, s, indole-CH_3), and 1.27 (3H, d, *J*7.3 Hz, C^*HCH_3).

When mixed with metal ions, these peptide derivatives of NSAIDs can bind via combinations of the deprotonated amides, side-chains of the peptides and/or terminal carboxylates to produce a wide variety of complexes with virtually all of the metallic elements of the periodic table.

Sugar derivatives as ligands

These ligands can be prepared by the following reaction schemes and can coordinate to metal ions via the diol functions.



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Scheme 14

The sugar groups and functionalised sugars bind to many different metal ions on mixing, usually via deprotoned alcohol groups of the sugars.

EXAMPLE 2 Cytotoxicity Studies of the NSAIDs on the A549 (non-small lung) cancer cells

2.1 Experimental

2.1.1 Medium Preparation for A549 cancer cells

Advanced DMEM medium was used in all the cell culture work. The medium did not contain certain components needed to facilitate cell growth. Therefore, antibiotics-actimycotic (0.5 mL), (100 U m^{-1} penicillin, 100 µg mL^{-1} streptomycin and 0.25 µg mL^{-1} amphotericin B), 200 mM glutamine solution (0.5 mL) and fetal calf serum (2 %, 0.8 mL) were added to the medium (40 mL) before proceeding with any cell work. All of the above components were obtained from Gibco Industries Inc. (Langley, OK, USA). All other reagents used in the cell work were obtained from Sigma (St. Louis, MO, USA).

2.1.2 Thawing of frozen A549 cancer cells

Frozen cells were stored in liquid nitrogen. The cells were rapidly warmed in a 37 °C water bath for approximately 5 min. The cell suspension was then transferred to a 10-mL centrifuge tube with 9 mL of medium and centrifuged for 3 min at 2000 rpm. The medium was removed from the resultant pellet and fresh medium (1 mL) was added to resuspend the cells, then transfer the cells to a 10-cm plate with fresh medium (10 mL) added to it. Cells were incubated at 37 °C in a humidified atmosphere containing 5 % CO_2 for 3 days.

2.1.3 Subculturing of A549 cells

The medium was removed from the cells and the cell layer was washed with phosphate buffer solution (PBS, 10 mL) prior to trypsination with 0.25 % trypsin EDTA solution (4 mL). Cells were then incubated for 6 min at 37 °C, after which medium with serum (5 mL) was added to inactivate the trypsin. The cell suspension was then collected into a centrifuge tube and the mixture was centrifuged at 2000 rpm for 3 min. The medium was subsequently removed from the cell pellet and fresh medium (1 mL) was added to resuspend the cells. The cell suspension (0.58 mL) was transferred from the total cell suspension to a centrifuge tube. Further, medium (3 mL) was added to the centrifuge tube and the cells were counted using a haemocytometer.

2.1.4 Seeding of A549 cells for cytotoxicity experiments

The cell suspension (100 µL per well) was transferred to four sets of ninety six-well plates with each well having approximately the same amount of cells ($1x10^4$ cells/well/100 µL). The plates were incubated overnight at 37 °C prior to the addition of the test compound.

2.2 Sample preparation for $IndoHAH_2$ (oxametacin), $[V^{V}O(IndoHAH)(IndoHA)].2MeOH$, and $[Cr(IndoHAH)_2(OH_2)_2](NO_3)\cdot H_2O$

$IndoHAH_2$ (oxametacin), $[V^{V}O(IndoHAH)_2]$, and $[Cr(LH)_2(OH_2)_2](NO_3)\cdot H_2O$ were tested over a range of concentrations (20-400 μM). After all the samples were finely dispersed in medium chain triglycerides (MCT) oil with sonication, the sample suspension (100 μL) was added to plastic vials with subsequent addition of medium (100 μL). The vials were vigorously vortexed to obtain a homogenous suspension. The whole procedure was kept sterile by rinsing every single pasteur pipettes with ethanol (70%) and flicking it dry. Blank assays with the MCT oil only had no effects on the cells.

2.2.3 Treatment of A549 cells with $IndoHAH_2$ (oxametacin), $[V^{V}O(IndoHAH)(IndoHA)].2MeOH$ and $[Cr(LH)_2(OH_2)_2](NO_3)\cdot H_2O$

The medium was removed from all the wells via a vacuum pump. The first and the last row of wells were left without addition of the test compound and were used as control wells. To the rest of the wells, appropriate concentrations of the test compound (20-400 μM) in complete medium were added into the wells. After treatment, the plates were incubated at 37 °C for 3 days.

2.2.4 Quantification of A549 cancer cells

For the MTT assay, the medium was removed from the plates, MTT (1 mg/mL) was added to all the wells and the cells were further incubated for approximately 4 h at 37 °C to allow sufficient time for it to interact with the cells. The medium was then carefully discarded and the cellular contents were extracted using DMSO (100 μL per well).

For the crystal violet blue assay, the medium was removed from the plates and the cells were washed twice with saline (0.9% NaCl). To all the wells, crystal violet blue dye (1 mL in 5% PBS) was added and the mixture was left for ~ 1 min to stain the cells. The dye was then removed and the cells were extracted using a solution of propan-2-ol (1 mL).

Absorption at 595 nm was determined using an ELISA plate reader. The percent survival was determined by the intensity of the absorbance obtained, which correlated to the

amount of cells present in each well. The negative control wells were arbitrarily assigned as 100% survival. The MTT assay provides a measure only for viable cells.

Statistical analysis of data of all the cell work after quantification with the plate reader was achieved using Origin 6.1 software (Microcal Inc., 1999). Comparison of test compound cytotoxicities was analyzed using a two-tailed *t* test.

2.3 Cytotoxicity results

By contrast to *in vitro* experiments with Indo and metal complexes, complexation of IndoHA with a metal ion had a dramatic effect on the cell viability and the V(V) complex had a very significant effect on cell viability (Figure 1). The inert Cr(III) complex is less reactive than the parent complex but activity is expected to increase with ease of release of the ligand.

2.4 Discussion

Of particular interest is the relatively high activity of the V-IndoHA complex. It is considered that this lipophilic complex will transfer both the ligand and the metal inside the cells, where they are likely to exert their activities separately: the ligand by the many processes discussed above and the V by phosphatase activity.

EXAMPLE 3 Treatment of inflammation by oral administration

3.1 Methodology

3.1.1 Test compositions

The composition containing the metal complexes in medium chain triglyceride (MCT) organogel paste and/or an aqueous 2% w/w CMC solution were freshly prepared for each experiment. The organogel paste is described in PCT International Patent Application No. PCT/AU2005/000442.

3.1.2 Animals

Sprague-Dawley rats (weighing 200-250 g) used for these studies were supplied by the laboratory animal services at The University of Sydney, Sydney Australia. Animals were housed in polypropylene cages and allowed free access to standard laboratory rat chow (Purina Rat Chow, Ralston Purina, St Louis MO) and tap water. Animals were housed in the animal care facility of the Faculty of Pharmacy at ambient temperature and humidity with a 12-h light-dark cycle. The experimental animal protocols were approved by the Animal Ethics Committee of the University of Sydney.

3.1.3 *In Vivo* anti-inflammatory activity and gastric toxicity

Groups of four rats (unless otherwise stated) were used for all studies. All doses were calculated as molar equivalents of IndoH. Rats were allowed free access to food and water except for gastric toxicity studies, when they were fasted for 24 h but still with free access to water. For gavage administration, rats were dosed orally with the copper complex (at relevant molar equivalents of IndoH) in either 2% w/w aqueous CMC solution or MCT organogel. The treatment groups were compared to a control cohort dosed with either neat 2% w/w aqueous CMC solution or neat MCT organogel.

Inflammation was induced (1 h after dosing by gavage with an injection of carrageenan (0.1 mL, 1% w/v in isotonic saline) into the plantar region of the hind paw. Paw volume was measured prior to dosing and at 3 h after carrageenan injection by immersing the left hind paw (to the lateral malleus) into a vessel filled with water and measuring the volume of water displaced as decribed in International Patent Application No. PCT/AU2005/000442 filed 30 March 2005, the contents of which is incorporated herein by cross-reference in its entirety. Immediately after paw volume measurements, 24 h-fasted animals were euthanased and the stomach was excised and opened by incision along the greater curvature. The stomach was rinsed and examined to determine the extent of macroscopic gastric toxicity, which is reported as the summation of the area of macroscopic ulcerations (mm^2).

The calculation of the power of the experiment to compare two treatment groups with a *P*-value threshold of 0.05 was determined using the GraphPad StatMate program (*GraphPad Instat*; version 3.01 for WIN95/NT, GraphPad Software Inc., 1998).

3.1.4 *In Vivo* small intestinal toxicity

Groups of four rats (unless otherwise stated) were used for all studies and were treated similarly as described above, except that they were allowed free access to food and water during the assay. At 24 h after dosing, the entire small intestine was excised and flushed with water to expel the intestinal contents. The intestine was examined from 10 cm distal to the ligament of Treitz to the ileocecal junction, and the toxicity is reported as the summation of the area of macroscopic ulcerations (mm^2).

3.1.5 Statistical analysis

The Student *t* test was used to compare mean values between two groups and repeated measures ANOVA followed by Bonferroni correction for comparisons was used to compare mean values between more than two groups. Data are expressed as the mean ± SEM. All reported *P* values are two-sided, and $P<0.05$ was considered statistically significant.

3.2 Results and Discussion

3.2.1 Experiment 1: Acute small intestine ulceration and inhibition of Carrageenan-induced paw edema for oxametacin and metal derivatives

Oral administration at an Indomethacin Equivalence (EI) treat dose of 2 mg kg^{-1} bw of Oxametacin (Sample P), Cr-Oxametacin (Sample Q) and V-O-Oxametacin (Sample R) did not result in any significant gastric ulceration. However, there was a significant ($P<0.01$ (**)) anti-inflammatory effect as a result of the administration of Cr-Oxametacin compared to the control cohort. The small intestine and anti-inflammatory effects of the compositions are shown in Figures 2 and 3, respectively.

3.2.2 Discussion

At IE treat dose of 2 mg kg bw^{-1}, the MCT organogel compositions of Oxametacin, Cr-Oxametacin and V-O-Oxametacin were non-irritating to the gastric mucosa with only the Cr-Oxametacin complex exhibiting significant anti-inflammatory activity. This demonstrates that a slow release oxametacin complex can have low GI with enhanced efficacy of the parent hydroamic acid.

3.3 Experiment 2: Acute GI ulceration and inhibition of Carrageenan-induced paw edema: Comparison of aspirin hydroxamic acid and a complex with other metal NSAIDs

Oral administration at an Indomethacin Equivalence (EI) treatment dose of 10 mg kg^{-1} bw of ACM, $[Cu(ACM)_2(H_2O)_2]$ $[Zn_2(ACM)_4(H_2O)_2]$, AcSHAS and [Cu(AcSHAH)(OH)] did not result in any significant ($P<0.05$) anti-inflammatory effect for AcSAHA or [Cu(AcSAHA)(OH)]. However, $[Cu(ACM)_2(H_2O)_2]$ and $[Zn_2(ACM)_4(H_2O)_2]$, were significantly anti-inflammatory at $P< 0.01$(**) compared to the control cohort. ACM was significantly more anti-inflammatory than [Cu(AcSHAH)(OH)] ($P < 0.05$(*), $[Cu(ACM)_2(H_2O)_2]$ and $[Zn_2(ACM)_4(H_2O)_2]$ were more anti-inflammatory ($P < 0.05$(*)) than either AcSAHA and [Cu(AcSHAH)(OH)], respectively. $[Zn_2(ACM)_4(H_2O)_2]$ was significantly more anti-inflammatory ($P < 0.01$(**)) than [Cu(AcSAHA)(OH)]. Whilst there was no statistically significant difference in intestinal ulceration between the treatments of ACM, $[Zn_2(ACM)_4(H_2O)_2]$, AcSHAS and [Cu(AcSHAH)(OH)] compared to control cohort ($P<0.05$), there was significant small intestine ulceration ($P<0.05$) compared to control with respect to $[Cu(ACM)_2(H_2O)_2]$ treatment. However, as the standard error was large for $[Cu(ACM)_2(H_2O)_2]$, all treatments were found to be comparable with respect to potential small intestine ulceration ($P>0.05$). There was no signifincat gastric ulceration ($P<0.05$) compared to control with respect to $[Zn_2(ACM)_4(H_2O)_2]$, AcSHAS and [Cu(AcSHAH)(OH)]. There was, however, significant gastric ulceration with respect to ACM ($P< 0.05$ (*)) and $[Cu(ACM)_2(H_2O)_2]$ ($P< 0.01$ (**)) compared to the control cohort. Between the treatments, $[Zn_2(ACM)_4(H_2O)_2]$,

AcSHA and [Cu(AcSHAH)(OH)] resulted in significantly less gastric ulcerations ($P<0.05$) than $[Cu(ACM)_2(H_2O)_2]$.

When provided in a polyethylene glycol (PEG) formulation the GI toxicity was reduced significantly such that there was no significant gastric (in 24-hr fasted animals) or small intestinal toxicity of $[V(IndoHAH)_2(OMe)]$ at 4 mg/kg of oxametacin equivalents in the formulation.

3.3.1 Discussion

At IE of 10 mg/kg bw, AcHAHA and its copper complex were not efficacious and accordingly did not cause gastric or small intestine ulceration. Thus, the metal complexes reported in the literature have no therapeutic value compared to the chelating NSAIDs of the present invention.

EXAMPLE 4 Intravenous injection toxicity of $[Co(en)_2(Oxametacin)]Cl_2.4H_2O$.

This study was conducted to demonstrate the efficacy and safety of the water-soluble complex $[Co(en)_2(Oxametacin)]Cl_2.4H_2O$ of formula (1) for intraveneous injection. Indomethacin is normally dosed intravenously at 0.1 to 0.2 mg/kg bw per day in humans. The high solubility of the Co complex allowed its intraveneous toxicity to be examined at much higher levels.

4.1 Experimental

Freshly prepared solutions containing the cobalt complex (active ingredient) in sterile saline was prepared by dissolving the cobalt complex (without heat) in the saline. The resultant clear solution was filtered with a sterile 0.22 micron filter and used immediately. The i.v push was given over approximately 10 s. All other experimental procedures are as described in Example 3. The animals were monitored for toxicity over 24 hr.

4.2 Results

One death occurred at the time of the tail vein intravenous injection (i.v.i) in the 3.2 mg/kg bw treatment group ($n = 2$), which is 20-30 times higher than the normal

intravenous dose in humans for IndoH, indicating an LD_{50} = 3.2 mg/kg bw for this study. Nil small intestinal damage was observed in any of the treatment groups. Severe tail vein necrosis (+++) was observed in one of two rats in the 0.8 mg/kg bw treatment group at 24 hours post treatment. Minimal tail necrosis (+) in one or two rats in the 1.6 mg/kg bw treatment group at 24 hours post treatment. Necrosis as a result of trauma at the injection site cannot be discounted as a dose-response was not observed. Because of the rapid onset, the death in the highest treatment group (3.2 mg/kg bw) was attributed to the drug, but a change in isotonicity cannot be discounted.

4.3 Discussion

A typical i.v. dose of IndoH administered to infants for closure of the ductus aorta is 0.1 to 0.2 mg kg^{-1} bw. High doses of IndoH have adverse effect on the central nervous system (CNS). Such effects include malaise and listlessness, drowsiness, hearing disturbances and in rare cases convulsions and coma. Based on these studies, a No Observed Adverse Effect Level (NOAEL) of ~1 mg kg^{-1} bw and LD50 of 3.3 mg/kg bw is proposed for i.v. administration of $[Co(en)_2(Oxametacin)]Cl_2$.

EXAMPLE 5 Comparison of the fficacy and toxicity of oxametacin ($IndoHAH_2$) at 4 mg/kg BW, $[Ga(IndoHAH)_2(OH_2)_2]Cl$ at 5.4 mg/kg BW (indomethacin equivalent doses) by oral gavage in MCT organogel

This study was conducted to investigate the efficacy and the gastric ulceration potential of oxametacin ($IndoHAH_2$), $[Ga(IndoHAH)_2(OH_2)_2]Cl$ and $[Cu_2(Indo)_4(H_2O)_2]$ dosed by means of a single dose oral gavage at indomethacin (IndoH) equivalent (I.E.) 4 mg/kg bw, 3.6 mg/kg bw and 1 mg/kg bw, respectively.

The recommended daily treatment dose of IndoH in humans is approximately 1 mg/kg bw administered in divided doses. Oxametacin in typically dosed orally at 2-3 mg/kg.

5.1 Indomethacin equivalence

All evaluations of the efficacy and toxicity of the metal complexes were investigated using composition containing the active ingredient (A.I) administered as molar

IndoH equivalents. Accordingly, the complex can be described in terms of indomethacin equivalence (IE) or equimolar amount of Indo. The IE is the weight in mg of the Active Ingredient (A.I.). The IE can be calculated from the Molecular Weight (MW) of the A.I. and IndoH by the relationship:

$$IE = \frac{\text{MW active ingredient x n}}{\text{MW of indomethacin x n}}$$

where n equals 2 for the Ga complex.

Table 1: Table of Indomethacin (IndoH) Equivalence

1 mg IndoH = 1.21 mg $[Ga(IndoHA)_2(OH_2)_2]Cl$
1 mg IndoH = 1.04 mg Oxametacin ($IndoHAH_2$)

5.2 Experimental

5.2.1 Efficacy and toxicity

The efficacy and safety of the test formulations is described below in a series of *in vivo* studies for the assessment of the test composition as an anti-inflammatory agent and for its ability to induce acute gastric ulceration.

5.2.2 Samples

The test formulations, transparent Medium Chain triglyceride (MCT) oily gels, were formulated at the University of Sydney, Australia. The test formulation was tested for the ability to inhibit inflammation in an inflammatory model, the carrageenan-induced paw oedema model, and were also tested in a gastric ulceration model as described below.

5.2.3 Animals

Sprague-Dawley rats (average weight 380-450 g) used for these studies were supplied by the laboratory animal services at The University of Sydney. Animals were housed in polypropylene cages and allowed free access to standard laboratory rat chow (Purina Rat Chow, Ralston Purina, St Louis MO) and tap water. Animals were housed in the animal care facility at The University of Sydney, at ambient temperature and humidity with a 12-h light-dark cycle. The experimental animal protocols were approved by the Animal Ethics Committee of The University of Sydney.

5.2.4 In Vivo anti-inflammatory activity and gastric toxicity

Groups of 4 rats were used for all studies. Rats were allowed free access to food and water except for gastric toxicity studies, when they were fasted for 24 h but still with free access to water. For gavage administration, rats were dosed orally with the test formulation. The treatment groups were compared to a control cohort (n = 4). Inflammation was induced (1 h after dosing by gavage with an injection of carrageenan (0.1 mL, 1% w/v in isotonic saline) into the plantar region of the hind paw. Paw volume was measured prior to dosing and at 3 h after carrageenan injection by immersing the right hind paw (to the lateral malleus) into a vessel filled with water and sitting on a top pan balance tared to zero. According to Archimedes principle, the volume of water displaced by the paw was readily determined by reading the increased weight registered by the balance (i.e., submersing a paw of 1 c.c. volume registers an increase of 1 gm on the balance). The change in the measured parameter (ΔP) for paw volume (Δmm^3) at n = 3 hours after carrageenan injection is given by:

$$\Delta P = P_{t=n} - P_{t=0} \qquad \text{(I)}$$

The percent inhibition (% inhibition) at 3 hr in the measured parameter (P) due to the treatment is given as the difference between the % increase in the value of P in the control group and the treatment group at n = 3 hr, with the % increase in the value of P given by:

$$[(P_{t=n} - P_{n=0}) \div P_{n=0}] \cdot 100 \qquad \text{(II)}$$

Immediately after paw volume measurements, 24-hr-fasted animals were euthanased and the stomach was excised and opened by incision along the greater curvature. The stomach was rinsed and examined to determine the extent of macroscopic gastric lesions, which is reported as the summation of the area of macroscopic ulcerations (mm^2).

5.3 Results

5.3.1 Acute gastric ulceration

Nil gastric ulceration (0, 0, 0, 0 mm^2) was observed in the control, Oxametacin (IndoHAH_2) I.E. 4 mg/kg bw and [Cu_2(Indo)$_4$(OH_2)$_2$] I.E. 1 mg/kg bw groups. Minimal gastric ulceration (0, 0, 0, 2 mm^2) was observed in the [Ga(IndoHA)$_2$(OH_2)$_2$]Cl 3.6 mg/kg bw treatment group.

5.3.2 Inhibition of Carrageenan-induced paw edema

45 (±9)%, 53 (±12)% and 34 (±12)% Mean Inhibition (±sem) in paw oedema (mm^3) was observed in the IndoHAH_2 IE 4 mg/kg bw, [Ga(IndoHA)$_2$(OH_2)$_2$]Cl IE 3.6 mg/kg bw and [Cu_2(Indo)$_4$(OH_2)$_2$] I.E. 1 mg/kg bw treatment groups, respectively. No statistically significant difference in % mean inhibition ($p > 0.05$) was found between the treatment groups, although the Ga complex appears to be slightly more efficacious.

5.4 Discussion

A single dose oral gavage of the IndoHAH_2-based A.I.s in MCT organogel at I.E. treatment dose of approximately 4 mg/kg bw resulted in an effective anti-inflammatory response compared to controls with nil to minimal gastric ulceration. No difference in anti-inflammatory response or gastric ulceration potential, however, was observed between IndoHA and the Ga complex of IndoHA in the rat model. At approximately 3.6 times the I.E. daily treatment dose of IndoH, a single oral dose of the Ga-complex of IndoHAH was anti-inflammatory and had no GI toxicity.

5.5 Conclusion

In the rat model, a single oral dose of the Ga complex of IndoHA administered at close to the recommended oral dose of $IndoHAH_2$ was found to be efficacious and non-ulcerogenic to the stomach. The results indicate that it is somewhat more efficacious than the free ligand. Since both Ga and IndoHA have anti-cancer effects (observations by inventors), and the complex has a strong anti-inflammatory effect, the low toxicity of the complex makes it an attractive compound for chemotherapeutic treatment in other areas such as cancer.

EXAMPLE 6 Cytotoxicity studies of the NSAIDs on A549 (non-small lung), A2780 (Pt-resitant ovarian) and Hep-2 cancer cells

6.1 Experimental

The cytotoxicity of metal complexes on cancer cell lines A549, A2780 and Hep-2 were assessed using MTT and crystal violet blue assays.

6.1.1 Medium Preparation for A549, A2780 and Hep-2 cell lines

Advanced DMEM medium was used in all the cell culture work. The medium did not contain certain components needed to facilitate cell growth. Therefore, for the A549 cells, antibiotics-actimycotic (0.5 mL), (100 U m^{-1} penicillin, 100 µg mL^{-1} streptomycin and 0.25 µg mL^{-1} amphotericin B), 200 mM glutamine solution (0.5 mL) and fetal calf serum (2 %, 0.8 mL) were added to the medium (40 mL) before proceeding with any cell work. For the Hep-2 and A2780 cells, Advanced DMEM medium (40 mL) was supplemented with antibiotics-actimycotic (0.5 mL), (100 U m^{-1} penicilin, 100 µg mL^{-1} streptomycin and 0.25 µg mL^{-1} amphotericin B), 200 mM glutamine solution (1.0 mL) and fetal calf serum (5 %, 4 mL). All of the above components were obtained from Gibco Industries Inc. (Langley, OK, USA). All other reagents used in the cell work were obtained from Sigma (St. Louis, MO, USA).

6.1.2 Thawing of frozen A549, A2780 and Hep-2 cancer cells

Frozen cells were stored in liquid nitrogen. The cells were rapidly warmed in a 37 °C water bath for approximately 5 min. The cell suspension was then transferred to a 10-mL centrifuge tube with 9 mL of medium and centrifuged for 3 min at 2000 rpm. The medium was removed from the resultant pellet and fresh medium (1 mL) was added to resuspend the cells, then transfer the cells to a 10-cm plate with fresh medium (10 mL) added to it. Cells were incubated at 37 °C in a humidified atmosphere containing 5 % CO_2 for 3 days.

6.1.3 Subculturing of A549, A2780 and Hep-2 cells

The medium was removed from the cells and the cell layer was washed with phosphate buffer solution (PBS, 10 mL) prior to trypsination with 0.25 % trypsin EDTA solution (4 mL). Cells were then incubated for 6 min at 37 °C, after which medium with serum (5 mL) was added to inactivate the trypsin. The cell suspension was then collected into a centrifuge tube and the mixture was centrifuged at 2000 rpm for 3 min. The medium was subsequently removed from the cell pellet and fresh medium (1 mL, A549, and 1 mL Hep-2) was added to resuspend the cells. The cell suspension (0.58 mL, A549, 0.24 mL, A2780 and 1.6 mL, Hep-2) was transferred from the total cell suspension to a centrifuge tube. Further, medium (3 mL, for A549, A2780 and Hep-2) was added to the centrifuge tube and the cells were counted using a haemocytometer.

6.1.4 Seeding of A549, A2780 and Hep-2 cells for cytotoxicity experiments

The cell suspension (100 µL per well) was transferred to four sets of ninety six-well plates with each well having approximately the same amount of cells ($1x10^4$ cells/well/100 µL for A549 and Hep-2 and $1x10^5$ cells/well/100 µL for A2780). The plates were incubated overnight at 37 °C prior to the addition of the test compound.

6.2 Sample preparation for indomethacin and $[Cu_2(Indo)_4(OH_2)_2]$, $[Zn(Indo)(OH_2)_2]$, ACMH, $[Cu(ACM)_2(OH_2)_2]$, $[Zn(ACM)_2(OH_2)_2]$, aspirin, $[Cu(asp)_2(3\text{-pic})_2]$, $IndoHAH_2$ (oxametacin), $[V^VO(IndoHAH)_2]$, ibuprofen and $[Cu(ibup)_2(2\text{-Meim})_2]$

Indomethacin and $[Cu_2(Indo)_4(OH_2)_2]$ were tested over the concentration range 10-300 μM, while ACMH, $[Cu(ACM)_2(OH_2)_2]$, $[Zn(ACM)_2(OH_2)_2]$, aspirin, $IndoHAH_2$ (oxametacin), $[V^VO(IndoHAH)_2]$, ibuprofen, $[Cu(asp)_2(3\text{-pic})_2]$ and $[Cu(ibup)_2(2\text{-Mim})_2]$ were tested over the concentration range 20-400 μM. After all the samples were finely dispersed in medium chain triglycerides (MCT) oil with sonication, the sample suspension (100 μL) was added to plastic vials with subsequent addition of medium (100 μL). The vials were vigorously vortexed to obtain a homogenous suspension. Blank assays with the MCT oil only had no effects on the cells.

6.2.1 Treatment of A549 and A2780 cells with indomethacin and $[Cu_2(Indo)_4(OH_2)_2]$

The medium was removed from all the wells via a vacuum pump. A number of wells were left without addition of the test compound and were used as control wells. Appropriate concentrations of the test compound (10-300 μM) in complete medium were added to the remainder of the wells. After treatment, the plates were incubated at 37 °C for 3 days.

6.2.2 Treatment of A549 and Hep-2 cells with $[Zn(Indo)_2(OH_2)_2]$

The medium was removed from all the wells via a vacuum pump. The first and the last row of wells were left without addition of the test compound and were used as control wells. To the remainder of the wells appropriate concentrations of the test compound (20-400 μM) in complete medium were added into the wells. After treatment, the plates were incubated at 37 °C for 3 days.

6.2.3 Treatment of A549 cells with ACMH, $[Cu(ACM)_2(OH_2)_2]$, $[Zn(ACM)_2(OH_2)_2]$, aspirin, $[Cu(asp)_2(3\text{-pic})_2]$, $IndoHAH_2$ (oxametacin), $[V^VO(IndoHAH)_2]$, ibuprofen and $[Cu(ibup)_2(2\text{-Mim})_2]$

The medium was removed from all the wells via a vacuum pump. The first and the last row of wells were left without addition of the test compound and were used as control wells. To the rest of the wells, appropriate concentrations of the test compound (20-400 μM) in complete medium were added into the wells. After treatment, the plates were incubated at 37 °C for 3 days.

6.2.4 Quantification of A549, A2780 and Hep-2 cancer cells

For the MTT assay, the medium was removed from the plates, MTT (1 mg/mL) was added to all the wells and the cells were further incubated for approximately 4 h at 37 °C to allow sufficient time for it to interact with the cells. The medium was then carefully discarded and the cellular contents were extracted using DMSO (100 μL per well).

For crystal violet blue assays, the medium was removed from the plates and the cells were washed twice with saline (0.9% NaCl). To all the wells, crystal violet blue dye (1 mL in 5% PBS) was added and the mixture was left for ~ 1 min to stain the cells. The dye was then removed and the cells were extracted using a solution of propan-2-ol (1 mL).

Absorption at 595 nm was determined using an ELISA plate reader. The percent survival was determined by the intensity of the absorbance obtained, which correlated to the amount of cells present in each well. The negative control wells were arbitrarily assigned as 100% survival. The MTT assay provides a measure only for viable cells.

Statistical analysis of data of all the cell work after quantification with the plate reader was achieved using Origin 6.1 software (Microcal Inc., 1999). Comparison of test compound cytotoxicities was analyzed using a two-tailed *t* test.

6.3 Cytotoxicity results

The cytotoxicity of the drugs and cell lines tested according to the above protocol is presented in the following table and discussion. Data are expressed as the mean ± SEM.

All reported *P* values are two-sided, and $P<0.05$ was considered statistically significant. Although not statistically significant, the results are consistent with those obtained for other cancer cell lines where the dinuclear complex is more active than IndoH against both the A549 and A2780 cell lines. Complexation of ACM to either Cu(II) or Zn(II) decreased the LC_{50} values in the A549 cell lines (Figure 4). Unlike IndoH and ACMH, none of the parent NSAIDs, aspirin, ibuprofen, $AcSHAH_2$, or $IndoHAH_2$ had significant effects on the cell viability for A549 even up to 400 μM. While the Cu complexes of aspirin and ibuprofen did effect cell viability, the LC_{50} values were the same (~300 μM) in both cases and probably reflected normal Cu toxicity, without any specific effect of the complex. No significant effect on cell viability of the Cu complex of AcSHAH was observed.

Table 2: LC_{50} values for IndoH and $[Cu_2(Indo)_4(OH_2)_2]$ in A549 and A2780 cells

Complexes	LC_{50} values ±S.E.M
IndoH	53.4 μM ±3.8 (A549) 56.1 μM ±5.1 (A2780)
Cu(II)-Indo	49.2 μM ± 5.8 (A549) 45.2 μM ± 4.9 (A2780)

By contrast, complexation of IndoHA with a metal ion had a dramatic effect on the cell viability and the V(V) complex had the greatest effect on cell viability of all of the metal complexes (Figure 5).

The Zn Indo complex was also compared against two cell lines Hep-2 and A549 and it was shown to have an equivalent effect on cell viability across the two cell lines (Figure 6).

6.4 Discussion

These results indicate that CuIndo is more effective than Indo against a broad range of cancers if applied topically or injected directly into the lesion or tissue containing a lesion (a practice which has been shown to be more effective in chemotherapy for a variety of external and internal lesions and has been increasingly accepted as a preferred mode of treatment). Similarly, the Zn and Cu ACM complexes appear to be more effective than ACM alone.

The V-IndoHA complex was found to have the highest activity. It is though that this lipophilic complex will effect transfer of both the ligand and the metal into cells, where they are likely to exert their activities separately: the ligand by Cox inhibition and the V by phosphatase activity. Given that the CuIndo complexes are much more active as anti-cancer agents *in vivo* than in *in vitro* cell assays, it is likely that the V(V) complex is even more potent *in vivo*.

EXAMPLE 7: Cytotoxicity of NSAIDs and their metal complexes in Leukemia cell lines

7.1 Experimental

The following cell lines were used in this study: HL-60 (ATCC No. CCL-240, human acute promyelocytic leukemia) and MOLT-3 (ATCC No. CRL-1552, human acute lymphoblastic leukemia).

To evaluate the cytoxicity of the test NSAIDs and metal complexes a standard MTT assay was employed. The cells were incubated with the applicable test NSAID or metal complex for 48 h, adjusted for non-adherent cells (e.g., the formed formazan crystal was solubilised by overnight incubation with a detergent solution. Stock solutions of all the compounds were prepared in DMSO.

The LC_{50} values presented below are the averages and standard deviations of four replicas from the same culture plate. The following designations are used: IndoH is indomethacin; $IndoHAH_2$ is indomethacin hydroxamic acid (oxamethacin); Co(III)-Indo is

$[Co(NH_3)_5(Indo)]Cl_2$.acetone, FW = 629.85); V(V)-IndoHA is $[VO(L)(LH)]\cdot 2MeOH\cdot 1.5H_2O$ (FW = 897.0, LH_2 = IndoHA); Keto is ketoralac (FW = 376.4 for the Tris salt, Sigma); and Cu(II)-Keto is $[Cu_2L_4(OH_2)_2].2H_2O$, where LH = Keto (FW = 304.1 calculated for 1 mol of Keto).

Compound	Cell Line	LC_{50}, □M
IndoH	HL-60	285 ± 29
IndoH	MOLT-3	396 ± 9
Co(III)-Indo	HL-60	367 ± 41
Co(III)-Indo	MOLT-3	137± 9
$IndoHAH_2$	HL-60	8.0 ± 0.5
$IndoHAH_2$	MOLT-3	11 ± 1
V(V)-IndoHA	HL-60	2.5 ± 0.3
V(V)-IndoHA	MOLT-3	3.8 ± 0.5
Keto	HL-60	263 ± 42
Keto	MOLT-3	170 ± 30
Cu(II)-Keto	HL-60	377 ± 20
Cu(II)-Keto	MOLT-3	320 ± 50

7.2 Results and discussion

It is apparent from the results that $IndoHAH_2$ is much more cytotoxic than IndoH. As is the case for the carcinomas, the V(V)-IndoHA complex is even more cytotoxic than the parent ligand indicating this comkpound is suitable for animal/human cancer treatment studies. The low activity of the ketoralac ligand and complex may be correlated with the low solubility of these in the cell suspension. It is considered likely that water-soluble forms will be far more active.

The IC_{50} for the CoIndo complex is a factor of three lower than for IndoH for treatment of MOLT-3 cells (even though it is an inert metal complex). The Co(III)

oxamethacin complex (and related complexes) have high water solubility, high stability for intravenous use, and the active may be released on reduction once the complex is absorbed by the leukemia cells.

EXAMPLE 8 Effect on blood sugar levels in a diabetic rat model following treatment with [$V^{V}O(IndoHAH)_2(OMe)$]

8.1 Experimental

8.1.1 Induction of diabetes and measurement of blood sugar level (BSL)

24 age-matched male Sprague Dawley rats weighing ~400 mg were obtained from Animal Laboratory Supplies (Perth, Australia). Animals were housed two rats per cage and except where specified allowed *ad libitum* access to food and water. Diabetes was induced in a subgroup of 12 animals with a single dose of streptozocin (STZ) (*i.p* 60 mg/kg). Diabetes was confirmed in 10 animals 3 days later by measurement of BSL at a level of greater than 15 mmol/L using a glucometer. Treatment protocols were initiated 7 days after administration of STZ.

Diabetic animals were randomly divided into drug treatment (DRx: n = 5) and placebo groups (DP; n = 5), and drug was administered as a single gavage dose (4 mg/kg of oxametacin equivalent, delivered as the vanadium complex in an MCT organogel) between 7 and 9 am in the morning. After 7 days of treatment, tail vein blood was obtained immediately before administration and at 2, 4, 6, 8 and 18 hr following administration of the vanadium complex. Blood sugar level was measured by glucometer (Ames).

8.2 Results and discussion

The BSL of diabetic animals treated with either drug (black circles) or placebo (open circles) are shown in Figure 7. As can be seen, treatment of diabetic animals with the vanadium complex induced a 6-10-mmol/l decrease in blood glucose level when compared with the placebo treated group. This decrease was maintained for a further 6 hr and had returned to baseline levels by 24 hr. Treatment of non-diabetic control animals had no

blood sugar lowering effect over a similar time course (data not shown). These data indicate that the drug has insulin-like action in that it can lower blood glucose levels in diabetic animals at much lower concentrations of vanadium than is normally administered to observe such effects. The results also illustrate the potential role for the drug in simultaneously treating diabetes and inflammatory conditions in the one dose (either alone or in combination therapy with drug(s) conventionally used for the treatment of diabetes) for humans and animals suffering from this condition.

Although the invention has been described with reference to a number of examples, it will be appreciated by those skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. All such variations and/or modifications are to be considered within the scope of the present invention the nature of which is to be determined from the foregoing description. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS

1. A metal complex of the following formula (1):

$$[M(L^1)_m(L^2)_n]^p \qquad (1)$$

wherein

M is a monovalent, divalent, trivalent, tetravalent, pentavalent or hexavalent metal ion;

each L^1 is independently selected and is NH_3 or other monodentate ligand, a polydentate ligand, or a macrocyclic ligand;

each L^2 is independently a chelating derivative of a carboxylate or a chelating amide or hydroximate NSAID, having anti-inflammatory activity, and at least one ligand L^2 is other than a salicylate or a derivative of a salicylate;

m is 0, 1, 2, 3, 4, or 5;

n is 1, 2, 3 or 4; and

p is the charge of the complex.

2. A metal complex according to claim 1, wherein each L^2 is independently a chelating derivative of a carboxylate having anti-inflammatory activity.

3. A metal complex according to claims 1 wherein at least one ligand L^2 is independently selected from the group consisting of hydroximates, hydroxamates, hydrazines, esters, amino acids, and peptide, sugar and amide NSAID chelating ligands (O or N bound).

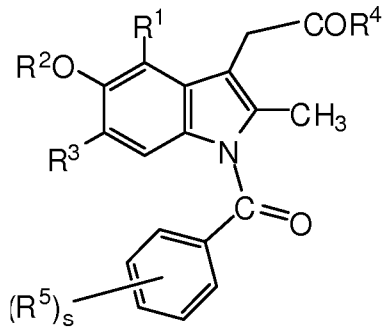
4. A complex according to claims 1 to 3 wherein each L^2 is independently a chelating derivative of a carboxylic acid selected from the group consisting of suprofen, tolmetin, naproxen, ibuprofen, flufenamic acid, niflumic acid , diclofenac, indomethacin, acemetacin, and ketorolac.

5. A complex according to claims 1 to 3 wherein each L^2 is a independently a chelating derivative of a carboxylic acid selected from the group consisting of carprofen, etodolac, fentiazac, flurbiprofen, ketoprofen, oxaprozin, pranoprofen, sulindac or suxibuzone.

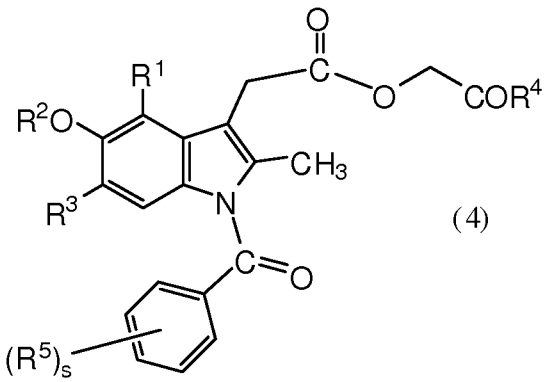
6. A complex according to claims 1 and 3 where the NSAID chelates a metal ion of the complex via one or more amide groups and one or more other functional groups.

7. A complex according to claims 1 and 3 where the NSAID is a hydroxamic acid.

8. A complex according to claim 1 wherein L^2 is independently a chelating derivative ligand of formula (3) or (4) as follows:



(3)

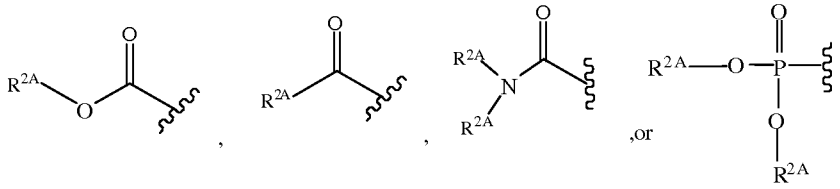


(4)

wherein:

R^1 is H or halo;

R^2 is H; a C_1 to C_6 alkyl, an alkenyl or an alkynyl, where the C_1 to C_6 alkyl, alkenyl or alkynyl may be optionally substituted; or



wherein each R^{2A} is independently selected from the group consisting of H, C_1 to

C_6 alkyl, alkenyl, alkynyl, aryl, cycloalkyl and arylalkyl, where the C_1 to C_6 alkyl, alkenyl, alkynyl, aryl, cycloalkyl or arylalkyl may be optionally substituted;

R^3 is H or halo; and

COR^4 is a carboxylate group or its deprotonated form; or

R^4 is $NR^{4A}OH$, $NR4^AN(R^{4A})_2$, $NR^{4A}N=R^{4A}$, $NR^{4A}R^{4B}$, or OR^{4C};

R^{4A} is H, or an aliphatic, aryl or heterocyclic group optionally substituted with one or more functional groups forming a co-ordination bond of the metal complex;

R^{4B} is a substituent group optionally forming at least one co-ordination bond of the metal complex;

R^{4C} is a substituent group forming at least two co-ordination bonds of the metal complex; and

each R^5 is independently selected from the group consisting of halo, $–CH_3$, –CN, $–OCH_3$, $–SCH_3$ and $–CH_2CH_3$, where the $–CH_3$, $–OCH_3$, $–SCH_3$ or $–CH_2CH_3$ may be optionally substituted; and

s is 1,2, 3, 4 or 5.

9. A complex according to claim 8 wherein when R^2 is a C_1 to C_6 alkyl, an alkenyl or an alkynyl, the C_1 to C_6 alkyl, alkenyl or alkynyl is optionally substituted with one or more substituents independently selected from the group consisting of halo, -OH, -COOH and $–NH_2$.

10. A complex according to claim 8 or 9 wherein when R^{2A} is a C_1 to C_6 alkyl, an alkenyl, an alkynyl, an aryl, a cycloalkyl or an arylalkyl, the C_1 to C_6 alkyl, alkenyl, alkynyl, aryl, cycloalkyl or arylalkyl is optionally substituted with one or more substituents independently selected from the group consisting of halo, –OH, -COOH and $-NH_2$.

11. A complex according to any one of claims 8 to 10 wherein when R^5 is $–CH_3$, $-OCH_3$, $-SCH_3$ or $–CH_2CH_3$, the $–CH_3$, $-OCH_3$, $-SCH_3$ or $–CH_2CH_3$ is optionally substituted with one or more substituents independently selected from the group consisting of halo, -OH, -COOH and $–NH_2$.

12. A complex according to any one of claims 8 to 11 wherein R^{4A} is an aliphatic, heterocylic or aromatic group substituted with one or more heteroatoms or heterocyclic groups.

13. A complex according to any one of claims 8 to 12 wherein R^{4B} is a substituent derived from the group consisting of an amino acid, amino alcohol, hydroxy acid, a peptide, a sugar, an acyclic aliphatic amine, and a cyclic amine.

14. A complex according to any one of the claimes 8 to 11 where R^{4C} is a substituent forming at least two co-ordination bonds in the metal complex and is derived from an amino acid, amino alcohol, hydroxy acid, a peptide, or a sugar.

15. A complex according to claims 1 or 2 wherein at least one ligand L^2 is a hydrazine derivative of a carboxylic acid with anti-inflammatory activity.

16. A complex according to claim 1 or 2 wherein at least one ligand L^2 is an amino acid derivative with an ester or amide linkage to a NSAID with anti-inflammatory activity.

17. A complex according to claims 1 or 2 wherein at least one ligand L^2 is a peptide derivative with an ester or amide linkage to a NSAID with anti-inflammatory activity.

18. A complex according to claims 1 or 2 wherein at least one ligand L^2 is a sugar derivative with an ester or amide linkage to a NSAID with anti-inflammatory activity.

19. A complex according to claims 1 or 2 wherein at least one L^2 is a polydentate amine derivative of a NSAID having anti-inflammatory activity.

20. A complex according to any one of claims 1 to 19 wherein the metal complex has one or more L^1 ligands of which at least one is NH_3.

21. A complex according to any one of claims 1 to 19 wherein the complex has one or more L^1 ligands at least one of which is $NH_2CH_2CH_2NH_2$.

22. A complex according to any one of claims 1 to 19 wherein the complex has one or more L^1 ligands at least one of which is independently selected from the group consisting of OH_2, OH^-, $^-OXH_3$ and O^{2-}.

23. A complex according to any one of claims 1 to 23 wherein one or more of the ligands L^1 have anti-inflammatory, anti-diabetic, anti-microbial or anti-cancer activity.

24. A complex according to any one of claims 1 to 23 wherein M is a d-block, f-block, p-block or s-block metal ion.

25. A complex according to claim 24 wherein M is a d-block or p-block metal ion.

26. A complex according to claim 24 wherein M is a metal ion selected from the group consisting of Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, W, Ru, Pt, Ag, Au, Ga, Sn and Bi.

27. A complex according to claim 26 wherein the metal ion is selected from the group consisting of V, Fe, Co, Cu, Zn, Ru, or Pt..

28. A complex according to claim 1 selected from the group consisting of $[M(L^2)_n(NR^7R^8R^9)_{m)}]^p$ wherein each L^2 is independently a bidentate derivative of a NSAID, each $(NR^7R^8R^9)$ is independently a monodentate amine ligand or a polydentate amine ligand (eg., a cyclic amine), n is 1 or 2, m is 1, 2, 3, or 4, and M is selected from Ru(II), Co(III), Cr(III), Ir(III), Os(III), Rh(III), Ru(III) and Pt(IV); $[M(L^2)(NR^7R^8R^9)_m]^p$ where L^2 is a tridentate derivative of a NSAID, each $(NR^7R^8R^9)$ is independently a monodentate amine ligand or a polydentate amine ligand (eg., a cyclic amine), m is 1, 2 or 3 and M is selected from Ru(II), Co(III), Cr(III), Ir(III), Os(III), Rh(III), Ru(III) and Pt(IV); $[M(L^2)(NR^7R^8R^9)_m]^p$ wherein L^2 is a tetradentate derivative of an NSAID, $(NR^7R^8R^9)$ is a monodentate amine ligand or a bidentate amine ligand, m = 1 or 2, and M is selected from Ru(II), Co(III), Cr(III), Ir(III), Os(III), Rh(III), Ru(III) and Pt(IV); $[M(L^2)(NR^7R^8R^9)]^p$ where L^2 is a pentadentate derivative of a NSAID, $(NR^7R^8R^9)$ is a monodentate amine ligand, and M is selected from Ru(II), Co(III), Cr(III), Ir(III), Os(III), Rh(III), Ru(III) and Pt(IV); $[M(L^2)_3]^p$ where L^2 is a bidentate derivative of a NSAID, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Mn(III), Os(III), Rh(III), Ru(III), Bi(III) and Pt(IV); $[M(L^2)_2]^p$ where L^2 is a tridentate derivative of a NSAID, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Ru(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Mn(III), Os(III), Rh(III), Ru(III), Bi(III) and Pt(IV); $[M(L^2)]^p$ where L^2 is a sexidentate derivative of a NSAID, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Ru(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Mn(III), Os(III), Rh(III), Ru(III), Bi(III), and Pt(IV); $[M(L^2)_n(OH_t)_{(6-2n)}]^p$ wherein each L^2 is independently a bidentate derivative of a NSAID, t is independently selected from 0, 1 or 2, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Os(III), Rh(III), Ru(III), Pt(IV), Ti(IV), V(IV), Mn(IV), V(V), Mo(V), W(V), Mo(VI), and W(VI); $[M(L^2)(OH_t)_3]^p$ where L^2 is a tridentate derivative of a NSAID, t is independently selected from 0, 1 or 2, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Os(III), Rh(III), Ru(III), Pt(IV), Ti(IV), V(IV), Mn(IV), V(V), Mo(V), W(V), Mo(VI) and W(VI); $[M(L^2)(OH_t)_2]^p$ where L^2 is a tetradentate derivative of a

NSAID, t is independently selected from 0, 1 or 2, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Os(III), Rh(III), Ru(III), Pt(IV), Ti(IV), V(IV), Mn(IV), V(V), Mo(V), W(V), Mo(VI) and W(VI); $[M(L^2)(OH_t)]^p$ where L^2 is a pentadentate derivative of a NSAID, t is 0, 1 or 2, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Os(III), Rh(III), Ru(III), Pt(IV), Ti(IV), V(IV), Mn(IV), V(V), Mo(V), W(V), Mo(VI) and W(VI); $[M(L^2)_3]^p$ where each L^2 is independently selected from bidentate derivatives of NSAIDs, and M is selected from Fe(II), Mn(II), Cu(II), Zn(II), Co(III), Cr(III), Fe(III), Ga(III), Ir(III), Os(III), Rh(III), Ru(III) and Pt(IV); $[M(L^2)(NR^7R^8R^9)_2]^p$ where L^2 is a bidentate derivative of a NSAID, $(NR^7R^8R^9)$ is independently selected from monodentate amine ligands or is a bidentate amine ligand, and M is selected from Cu(II), Pd(II), Pt(II) and Au(III); $[M(L^2)(NR^7R^8R^9)]^p$ wherein L^2 is a tridentate derivative of a NSAID, $(NR^7R^8R^9)$ is a monodentate amine ligand, and M is selected from Cu(II), Pd(II), Pt(II) and Au(III); $[M(L^2)_2]^p$ wherein L^2 is a bidentate derivative of a NSAID, and M is selected from Cu(II), Ni(II), Pd(II), Pt(II) and Au(III); and five-coordinate, $[V(O)(L^1)_{(m-1)}(L^2)_n]^p$; and wherein R^7, R^8 and R^9 are independently H or an optionally substituted aliphatic, heterocyclic or aromatic group.

29. A complex according to claim 1 wherein the complex is a complex selected from the group consisting of [Cu(IndoHAH)(OH)], $[Co(en)_2(IndoHAH)]Cl_2$, $[Co(en)_2(IndoHAH)](CF_3SO_3)_2$, $[V^VO(IndoHAH)(IndoHA)]\cdot 2MeOH\cdot 1.5H_2O$, $[V^VO(IndoHAH)_2(OMe)]$, $[Cr(IndoHAH)_2(OH_2)_2](NO_3)\cdot H_2O$, [Cu(Indo-Gly)$(Im)_2$] and $[Ga(IndoHAH)_2(OH_2)_2]Cl$, wherein IndoHAH is a mono-deprotonated hydroxamic acid of indomethacin, IndoHA is a doubly deprotonated hydroxamic acid of indomethacin, Indo-Gly is a glycine derivative of indomethacin linked by an amide to Indo; and Im is imidazole.

30. A metal complex of the following formula (2):

$$[M_q(L^1)_m(L^2)_n(L^3)_r]^p \qquad (2)$$

wherein

each M is independently selected from a monovalent, divalent, trivalent, tetravalent, pentavalent and hexavalent metal ions;

each L^1 is independently selected and is NH_3 or other monodentate ligand, a polydentate ligand, or a macrocyclic ligand;

each L^2 is independently a chelating derivative of a carboxylate,or a chelating amide or hydroximate NSAID, having anti-inflammatory activity, and is other than a salicylate or a derivative of a salicylate;

each L^3 is independently a bridging ligand;

m is a number from 0 to 5q;

n is a number from 1 to 2q;

p is the charge of the complex;

q is a number between 2 and 20 inclusive; and

r is a number from 1 to 60.

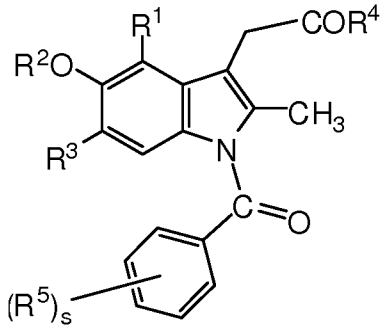
31. A metal complex according to claim 30 wherein the ligand L^2 is independently selected from the group consisting of hydroximates, hydroxamates, hydrazines, esters, amino acids, and peptide, sugar and amide NSAID chelating ligands (O or N bound).

32. A complex according to claim 30 or 31 wherein L^2 is a independently a chelating derivative of a carboxylic acid selected from the group consisting of suprofen, tolmetin, naproxen, ibuprofen, flufenamic acid, niflumic acid , diclofenac, indomethacin, acemetacin, and ketorolac.

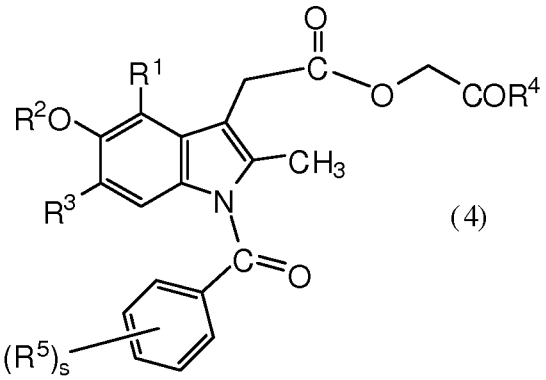
33. A complex according to claim 30 or 31 wherein L^2 is a independently a chelating derivative of a carboxylic acid selected from the group consisting of carprofen, etodolac, fentiazac, flurbiprofen, ketoprofen, oxaprozin, pranoprofen, sulindac or suxibuzone.

34. A complex according to claim 30 or 31 where the NSAID chelates a metal ion of the metal complex via one or more amide groups and one or more other functional groups.

35. A complex according to claim 30 wherein L^2 is independently a chelating derivative of formula (3) or (4) as follows:



(3)

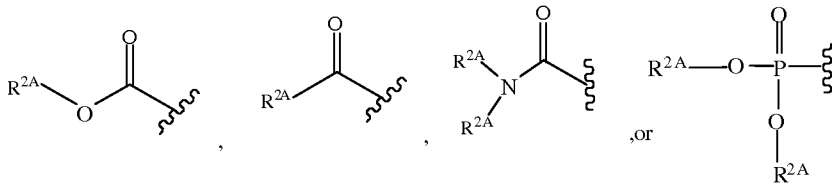


(4)

wherein:

R^1 is H or halo;

R^2 is H; a C_1 to C_6 alkyl, an alkenyl or an alkynyl, where the C_1 to C_6 alkyl, alkenyl or alkynyl may be optionally substituted; or



wherein each R^{2A} is independently selected from the group consisting of H, C_1 to C_6 alkyl, alkenyl, alkynyl, aryl, cycloalkyl and arylalkyl, where the C_1 to C_6 alkyl, alkenyl, alkynyl, aryl, cycloalkyl or arylalkyl may be optionally substituted;

R^3 is H or halo; and

COR^4 is a carboxylate group or its deprotonated form; or

R^4 is $NR^{4A}OH$, $NR4^AN(R^{4A})2$, $NR^{4A}N{=}R^{4A}$, $NR^{4A}R^{4B}$, or OR^{4B};

R^{4A} is H, or an aliphatic, aryl or heterocyclic group optionally substituted with one or more functional groups forming a co-ordination bond of the metal complex;

R^{4B} is a substituent group optionally forming at least one co-ordination bond of the metal complex;

R^{4C} is a substituent group forming at least two co-ordination bonds of the metal complex; and

each R^5 is independently selected from the group consisting of halo, -CH_3, -CN, -OCH_3, -SCH_3 and –CH_2CH_3, where the –CH_3, -OCH_3, -SCH_3 or –CH_2CH_3 may be optionally substituted; and

s is 1,2 3,4 or 5.

36. A complex according to claim 35 wherein when R^2 is a C_1 to C_6 alkyl, an alkenyl or an alkynyl, the C_1 to C_6 alkyl, alkenyl or alkynyl is optionally substituted with one or more substituents independently selected from the group consisting of halo, -OH, -COOH and –NH_2.

37. A complex according to claim 35 or 36 wherein when R^{2A} is a C_1 to C_6 alkyl, an alkenyl, an alkynyl, an aryl, a cycloalkyl or an arylalkyl, the C_1 to C_6 alkyl, alkenyl, alkynyl, aryl, cycloalkyl or arylalkyl is optionally substituted with one or more substituents independently selected from the group consisting of halo, –OH, -COOH and -NH_2.

38. A complex according to any one of claims 35 to 37 wherein when R^5 is –CH_3, -OCH_3, -SCH_3 or –CH_2CH_3, the –CH_3, -OCH_3, -SCH_3 or –CH_2CH_3 is optionally substituted with one or more substituents independently selected from the group consisting of halo, -OH, -COOH and –NH_2.

39. A complex according to any one of claims 35 to 38 wherein R^{4A} is an aliphatic, heterocylic or aromatic group substituted with one or more heteroatoms or heterocyclic groups.

40. A complex according to any one of claims 35 to 39 wherein R^{4B} is a substituent derived from the group consisting of an amino acid, amino alcohol, hydroxy acid, a peptide, a sugar, an acyclic aliphatic amine, and a cyclic amine.

41. A complex according to any one of the claimes 35 to 40 wherein R^{4C} is a substituent forming at least two co-ordination bonds in the metal complex and is derived from an amino acid, amino alcohol, hydroxy acid, a peptide, or a sugar.

42. A complex according to claim 30 wherein at least one ligand L^2 is a hydrazine derivative of a carboxylic acid with anti-inflammatory activity.

43. A complex according to claim 30 wherein at least one ligand L^2 is an amino acid derivative with an ester or amide linkage to a NSAID with anti-inflammatory activity.

44. A complex according to claim 30 wherein at least one ligand L^2 is a peptide derivative with an ester or amide linkage to a NSAID with anti-inflammatory activity.

45. A complex according to claim 30 wherein at least one ligand L^2 is a sugar derivative with an ester or amide linkage to a NSAID with anti-inflammatory activity.

46. A complex according to claim 30 wherein at least one ligand L^2 is a polydentate amine derivative of a NSAID.

47. A complex according to any one of claims 30 to 46 wherein the metal complex has one or more L^1 ligands at least one of which is NH_3.

48. A complex according to any one of claims 30 to 46 wherein the metal complex has one or more L^1 ligands of which at least one is $NH_2CH_2CH_2NH_2$.

49. A complex according to any one of claims 30 to 49 wherein one or more of ligands L^1 and L^3 has anti-inflammatory, anti-diabetic, anti-microbial or anti-cancer activity.

50. A complex according to any one of claims 30 to 44 wherein each L^3 is a bridging ligand independently selected from the group consisting of oxo, hydroxo, carboxylate and halo ligands.

51. A complex according to any one of claims 30 to 60 wherein metal ion M is independently selected from the group of monovalent, divalent, trivalent, tetravalent, pentavalent and hexavalent d-block metal ions and trivalent or tetravalent p block metal ions.

52. A complex according to claim 51 wherein M is a metal ion selected from the group consisting of Ga(III), Bi(III) and Sn(IV).

53. A complex according to claim 51 wherein each metal of the complex is selected from the group consisting of Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, W, Ru, Pt, Ag, Au, Ga,

Sn and Bi.

54. A complex according to claim 30 wherein the complex is $[M_2(NR^7R^8R^9)_{m-}(L^1)_{m''}(L^2)_n(L^3)_r]^p$, where each $(NR^7R^8R^9)$ is independently selected from monodentate or polydentate amine ligands, m' = 0, 1, 2, 3, 4, 5, 6, 7, or 8, each L^1 is independently a monodentate ligand, m" = 0, 1, 2, 3, 4, 5, 6, 7, or 8, each L^2 is independently a chelating derivative of a NSAID, n = 1, 2, 3, or 4, each L^3 is independently selected from oxo, hydroxo, carboxylate and halo ligands, r = 1, 2 or 3.and each M is independently selected from Fe(II), Mn(II), Ru(II), Co(III), Cr(III), Fe(III), Ir(III), Os(III), Rh(III), Ru(III), Ru(IV), Os(IV) and Pt(IV).

55. A pharmaceutical composition comprising a metal complex as defined in any one of claims 1 to 54 together with a pharmaceutically acceptable carrier or diluent.

56. A method for prophylaxis or treatment of inflammation or a disease or condition mediated by inflammation or having an inflammatory component, comprising administering to the mammal an effective amount of a metal complex as defined in any one of claims 1 to 54.

57. A method for prophylaxis or treatment of a cancer in a mammal, comprising administering to the mammal an effective amount of a metal complex as defined in any one of claims 1 to 54.

58. A method for prophylaxis or treatment of a microbial or viral infection in a mammal, comprising administering to the mammal an effective amount of a metal complex as defined in any one of claims 1 to 54.

59. A method for prophylaxis or treatment of pain in a mammal, comprising administering to the mammal an effective amount of a metal complex as defined in any one of claims 1 to 54.

60. A method for promoting wound healing, inhibiting skin aging, or for the prophylaxis or treatment of radiation-induced skin or organ damage comprising administering to a mammal in need thereof an effective amount of a metal complex as defined in any one of claims 1 to 54.

61. A method for treating damaged skin of a mammal, comprising administering to the mammal an effective amount of a metal complex as defined in any one of claims 1 to 54.

62. A method for prophylaxis or treatment or diabetes or prediabetic conditions of a mammal, comprising administering to the mammal an effective amount of a metal complex as defined in any one of the claims 1 to 54.

63. A method for protecting against ionising radiation induced skin or organ damage during radiotherapy or other sources of ionising radiation in a mammal, comprising administering to the mammal an effective amount of a metal complex as defined in any one of claims 1 to 54.

64. A method for enhancing the effectiveness of radiotherapy in cancer treatment, comprising administering to the mammal in need thereof an effective amount of a metal complex as defined in any one of claims 1 to 54.

FIGURE 1

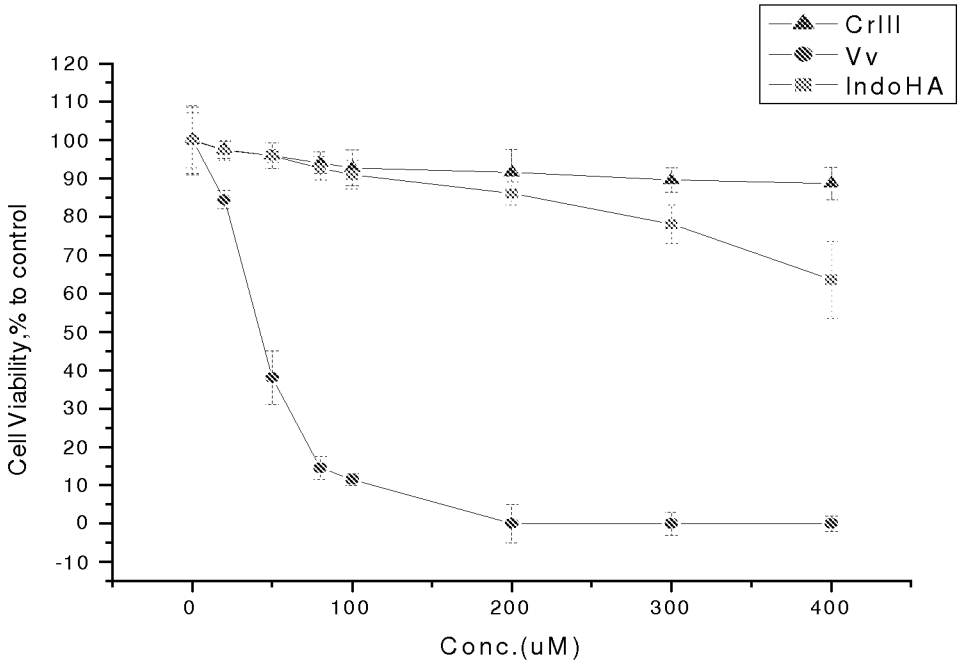


FIGURE 2

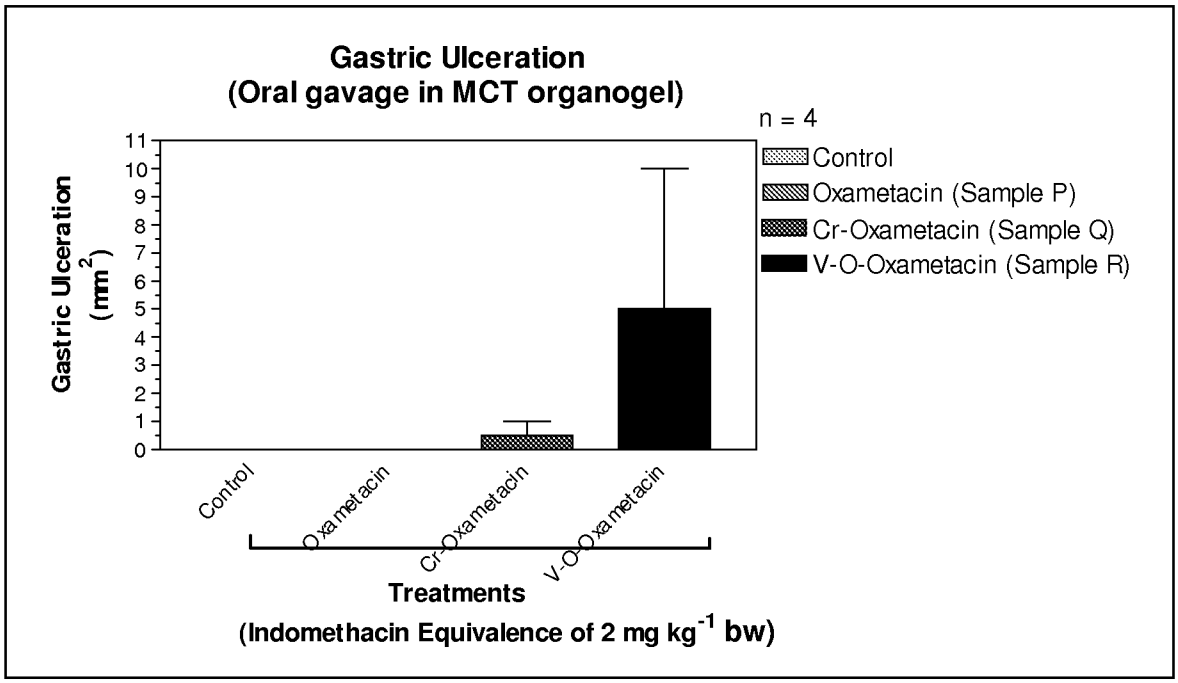


FIGURE 3

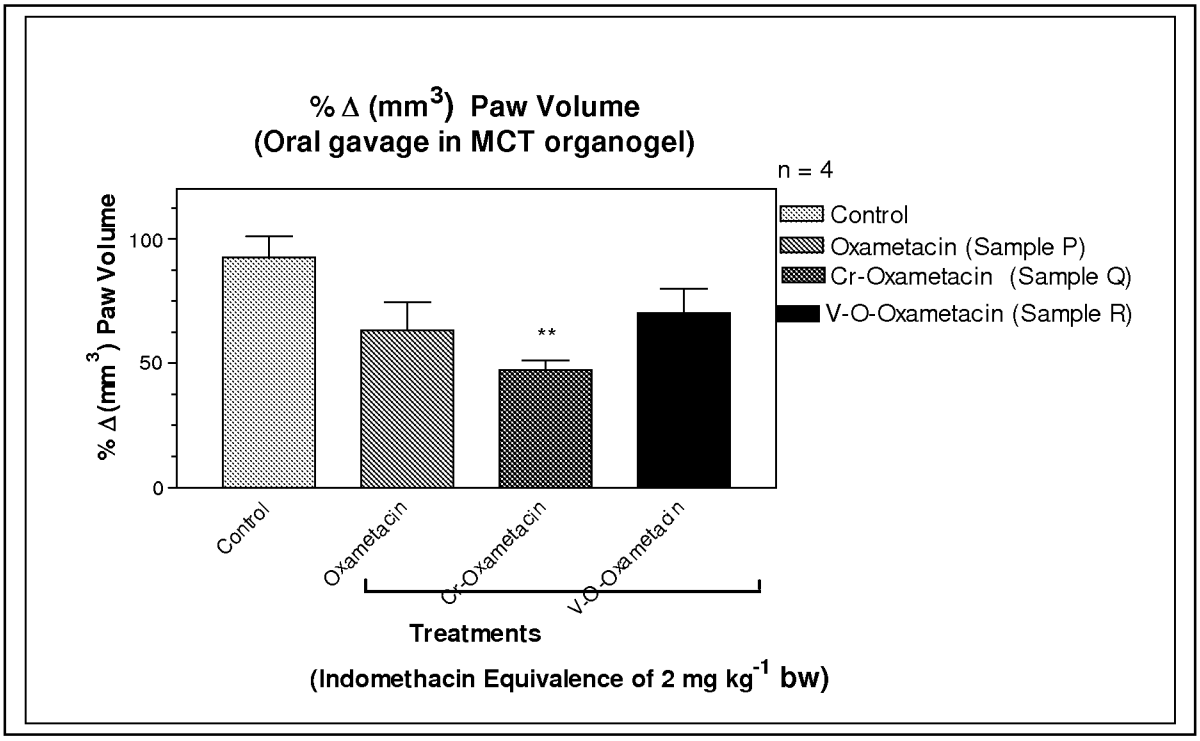

% Δ (mm3) Paw Volume
(Oral gavage in MCT organogel)
n = 4
Control
Oxametacin (Sample P)
Cr-Oxametacin (Sample Q)
V-O-Oxametacin (Sample R)
% Δ (mm3) Paw Volume
100
50
0
**
Control
Oxametacin
Cr-Oxametacin
V-O-Oxametacin
Treatments
(Indomethacin Equivalence of 2 mg kg-1 bw)

FIGURE 4

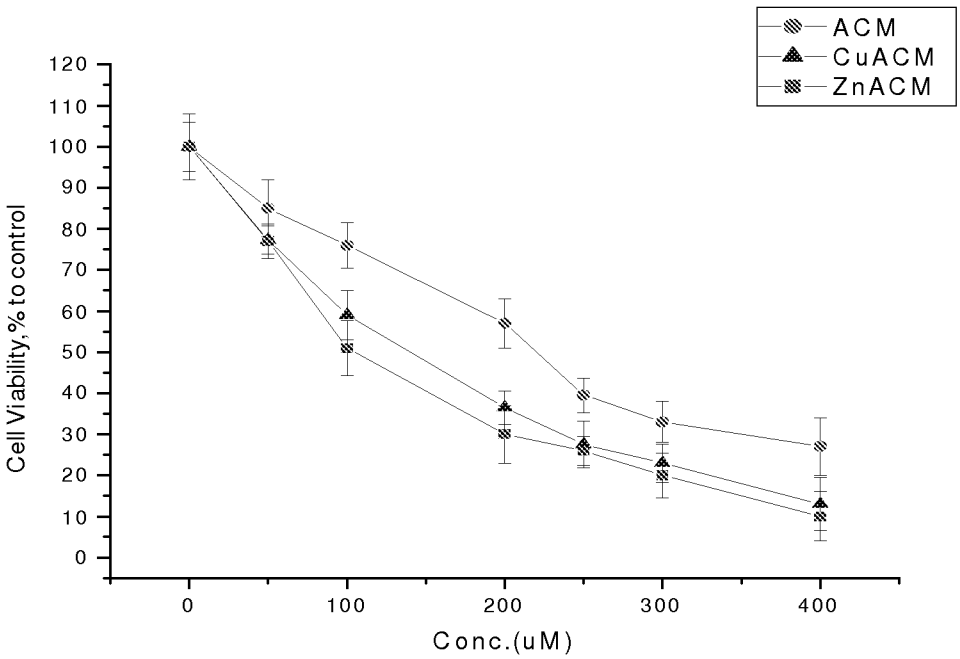


FIGURE 5

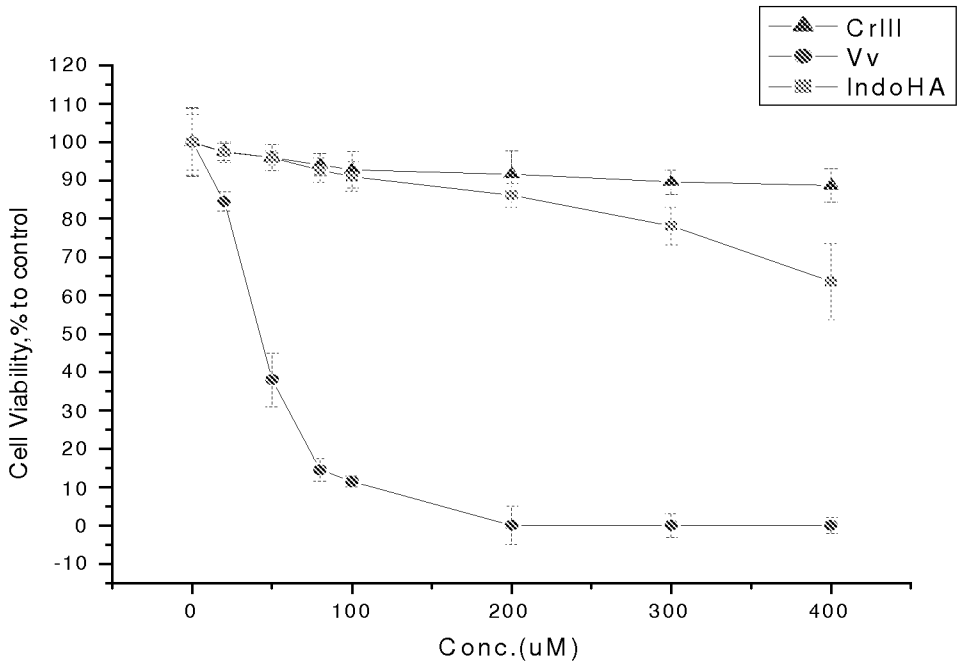


FIGURE 6

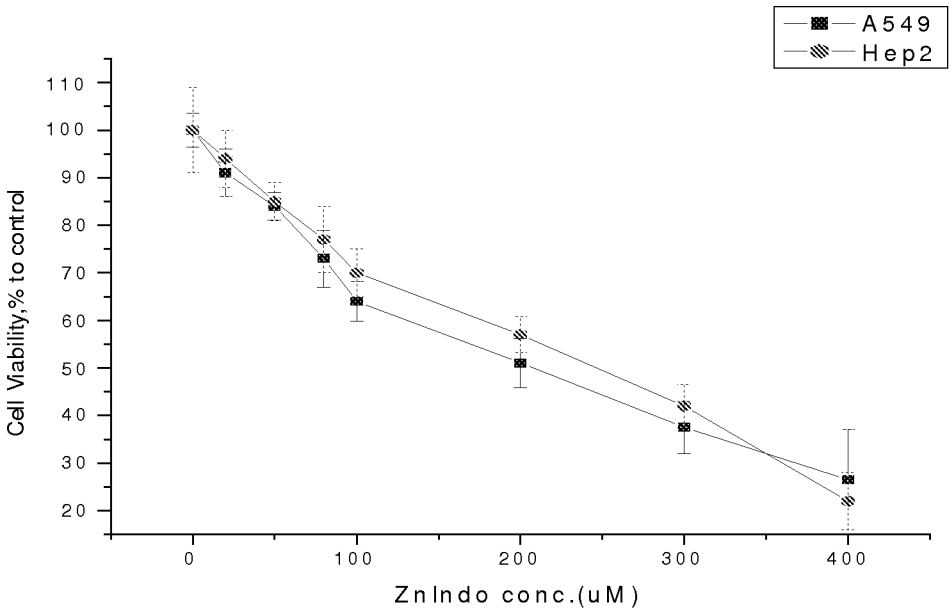
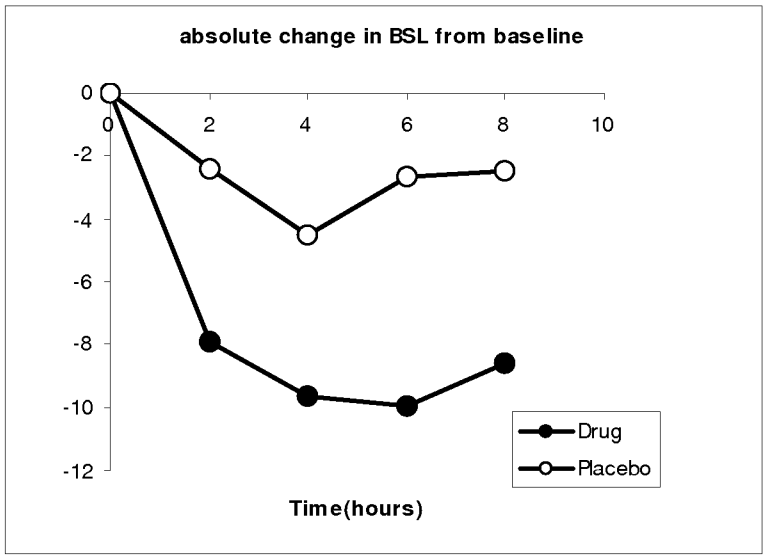


FIGURE 7



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2007/000375

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61P 29/00 (2006.01) ***A61K 31/555*** (2006.01)
A61K 31/19 (2006.01) ***C07D 209/28*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Database: STN; File: CA, WPIDS; Keyword: indomethacin, acemetacin, NSAID, metal etc., chelate etc.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/094809 A1 (THE UNIVERSITY OF SYDNEY) 13 October 2005. Whole document, particularly pages 7-10 and the examples.	1, 2, 4-6, 8-11, 20-28, 30-38, 47-64
X	US 5,466,824 A1 (BIOCHEMICAL VETERINARY RESEARCH PTY) 14 November 1995 *Whole document, particularly columns 3 and 4 and examples.*	1, 2, 4-6, 8-11, 20-28, 30-38, 47-64
X	Lörinc et al, "Mono-, di- and polymeric copper(II) complexes with diclofenic acid (NSAID drug), structures, spectral and magnetic properties", Monograph Series of the International Conferences on Coordination Chemistry (2005), vol.7, pp.176-186 Whole document, particularly the abstract, pages 178-179.	1, 2, 4, 22-27, 30, 32, 34, 49, 51-64

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 May 2007	29 MAY 2007
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **LOREN DYER** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : (02) 6283 3110

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2007/000375

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dendrinou-Samara et al, "Anti-inflammatory drugs interacting with Zn(II), Cd(II) and Pt(II) metal irons", Journal of Inorganic Biochemistry, vol.71 (1998), pp.171-179 Whole document	1-11, 20-28, 30-38, 47-64
X	Amer et al, "Spectrophotometric study of etodolac complexes with copper(II) and iron(II)", Journal of AOAC International, vol.88 (2005), no.6, pp.1637-1643 Whole document, particularly figure 7.	1, 2, 5, 23-28
X	Weder et al, "Copper complexes of non-steroidal anti-inflammatory drugs: an opportunity yet to be realised", Coordination Chemistry Reviews, vol.232 (2002), pp.95-126 Whole document, particularly table 1, tables 3-5, section 4 and 5.	1-6, 8-13, 16, 19, 20-28, 30-38, 47-64
X	Dillon et al, "Copper and zinc complexes as antiinflammatory drugs", Metal Ions in Biological Systems, vol.41 (2004), pp.253-277 Whole document, particularly tables 1 and 2.	1-6, 8-13, 16, 19, 20-28, 30-38, 47-64
X	Cini, "Anti-inflammatory compounds as ligands in metal complexes as revealed in x-ray structural studies", Comments on Modern Chemistry, vol.22 (2000), iss.3-4, pp.151-186 Whole document, particularly table II.1, scheme 1.1, pages 153, 180.	1-11, 20-28, 30-38, 47-64
X	WO 2005/002293 A2 (VANDERBILT UNIVERSITY) 6 January 2005 Whole document, particularly pages 6-11, 37 and 59, figure 10, compound 14 and 15.	1-6, 8, 9, 12-14, 16, 19, 22-28, 30-44, 50-64
X	WO 2004/000215 A2 (MEDINOX, INC) 31 December 2003 Whole document, particularly paragraphs 9, 13, 15, 24, 84-85, scheme 5.	1-11, 20-37, 47-64

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2007/000375

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: **1-64 in part**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
See additional sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: II

Claims 1-64 relate to compounds of various general formulas which define a metal chelate of a very large and ill-defined range of compounds with anti-inflammatory activity. This concept encompasses a large number of known compounds and as such is not considered to be limited to the inventive concept. Furthermore, the breadth of the claims and the large number of variable definitions and groupings claimed act to disguise the inventive concept. A search over their entire scope of the claims is thus infeasible. As such, the search has been restricted to more closely reflect the examples- metal chelates of indomethacin or acemetacin and their derivatives specifically, or NSAIDs in general.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2007/000375

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2005094809	AU	2005229023	CA	2561380	EP	1734945
		GB	2430369	WO	2006099684		
WO	2005002293	AU	2004253159	CA	2530408	EP	1638612
		US	2005002859				
US	5466824	AU	56663/90	CA	2058754	EP	0473655
		NO	914565	NZ	233776	US	5310936
		WO	9014337				
WO	2004000215	AU	2003279188	US	6620813	US	2004077691

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX