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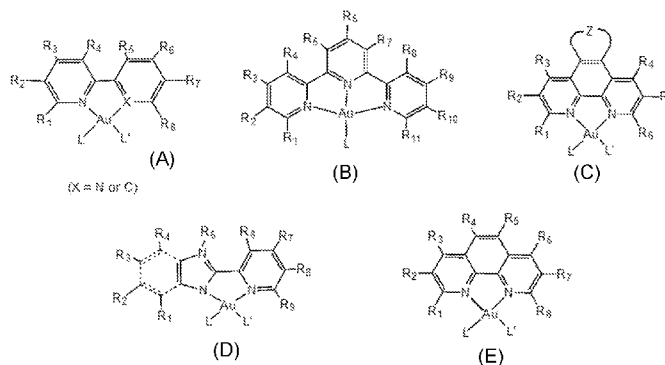
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(54) Title: GOLD(III) COMPOUNDS AS TARTRATE RESISTANT ACID PHOSPHATASE INHIBITORS, AND THERAPEUTIC USES THEREOF



(57) Abstract: The invention relates to gold (III) compounds as Tartrate Resistant Acid Phosphatase (TRAP) inhibitors, and to uses thereof, like in the treatment of pulmonary diseases like COPD and asthma. The inhibitory compounds have the general formula (A), (B), (C), (D) or (E).

Title: Gold(III) compounds as Tartrate Resistant Acid Phosphatase inhibitors, and therapeutic uses thereof.

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The invention relates to the fields of organometallic chemistry, enzymology, and medicine. In particular, it relates to gold (III) compounds as Tartrate Resistant Acid Phosphatase (TRAP) inhibitors, and to uses thereof, among others in the treatment of respiratory/pulmonary diseases like chronic obstructive pulmonary disease (COPD) and asthma.

COPD is a common but relatively unknown disease in which airways are chronically inflamed (chronic bronchitis) and lung tissue is destroyed (emphysema). Patients are characterized by varying contributions of both phenotypes. It affects around 10% of the population worldwide and is caused by cigarette smoking, air pollution and/or indoor biomass cooking. COPD is the fourth cause of mortality and morbidity in the world and is projected to be the third by 2030. Currently no effective therapy exists and transplantation is the only option for end-stage disease.

The pathogenesis of emphysema is incompletely understood but macrophages have been shown to be important in the process (6). Exposure to cigarette smoke and air pollution induces inflammation in the lungs with infiltration of macrophages and neutrophils and, after prolonged exposure, development of emphysema (7). Macrophages are considered to be the more relevant effector cells because deletion studies in mice have shown that these were indispensable to developing emphysema, while neutrophils were not (8). Macrophages can produce a host of proteolytic enzymes like matrix metalloproteins (MMPs) and cathepsins that have the ability to degrade extracellular matrix (ECM) and therefore lead to lung tissue destruction. In addition, alveolar macrophages are one of the few macrophage subsets in the body that highly express the enzyme TRAP, which in bone is involved in ECM turnover and may have a similar function in the lung (1).

Asthma is a chronic inflammation of the airways after becoming allergic to airborne allergens and affects 5-20% of the people in the Western world. Treatment consists of anti-inflammatory drugs and bronchodilators. However, a substantial group of patients with severe asthma are not treated
5 effectively with the currently available drugs and are particularly susceptible to acute worsening of their disease.

An important clinical problem for patients with lung diseases like COPD or severe asthma is their susceptibility to exacerbations. These exacerbations are an acute worsening of symptoms mostly caused by viral
10 and/or bacterial infections. Exacerbations are the major cause of morbidity and mortality in COPD and asthma. Dysfunction of alveolar macrophages has been presumed to be an important cause of impaired responses to infections (9).

A study by Capelli *et al.* (10) reported that the enzyme TRAP was
15 found to be upregulated in alveolar macrophages of smokers as compared to nonsmokers. Vuillenemot *et al.* (11) reported upregulation of TRAP gene ACP5 in an animal model of COPD.

The present inventors hypothesized that TRAP expressed by macrophages in the lung may be involved in the pathogenesis of asthma
20 and COPD. Indeed, in both diseases a higher expression of TRAP was found in lung tissue of patients as compared to controls (Figure 1A-B), suggesting TRAP may be involved in alveolar macrophage dysfunction. In addition, increased TRAP expression was found in mouse models of asthma and COPD (Figure 1C and D).

25 ACP5 encodes the enzyme tartrate-resistant acid phosphatase (TRAP; EC3.1.3.2), which belongs to the family acid phosphatases and distinguishes itself by its resistance to inhibition by tartrate. It is a metalloenzyme and contains two iron atoms at its active site. It is primarily found in lysosomes, and similar organelles, of osteoclasts, alveolar
30 macrophages, and activated macrophages and dendritic cells. Its function is

unknown but two main functions have been proposed based on the two enzymatic activities the protein harbours. Its only known substrate is the heavily phosphorylated protein osteopontin (OPN). OPN can be present extracellularly as part of lung ECM and intracellularly as a signalling molecule. One of TRAP's proposed functions is the dephosphorylation of extracellular OPN to inhibit adhesion of macrophages to ECM and promote migration of macrophages (12,13). TRAP has also been shown to promote the metastasizing behaviour of tumors, confirming the idea it may be involved in cell migration (14).

In addition, TRAP may be involved in impaired antimicrobial responses by dephosphorylating intracellular OPN. For instance, viral stimulation of TLR9 induces phosphorylation of OPN and subsequently leads to increased IFN α production to combat the virus. In patients lacking TRAP expression, IFN α levels have been found to be increased (15).

Conversely, in patients with COPD and asthma lower levels of IFN α have been found, suggesting overproduction of TRAP, and explaining their impaired responses to viral infections (16,17).

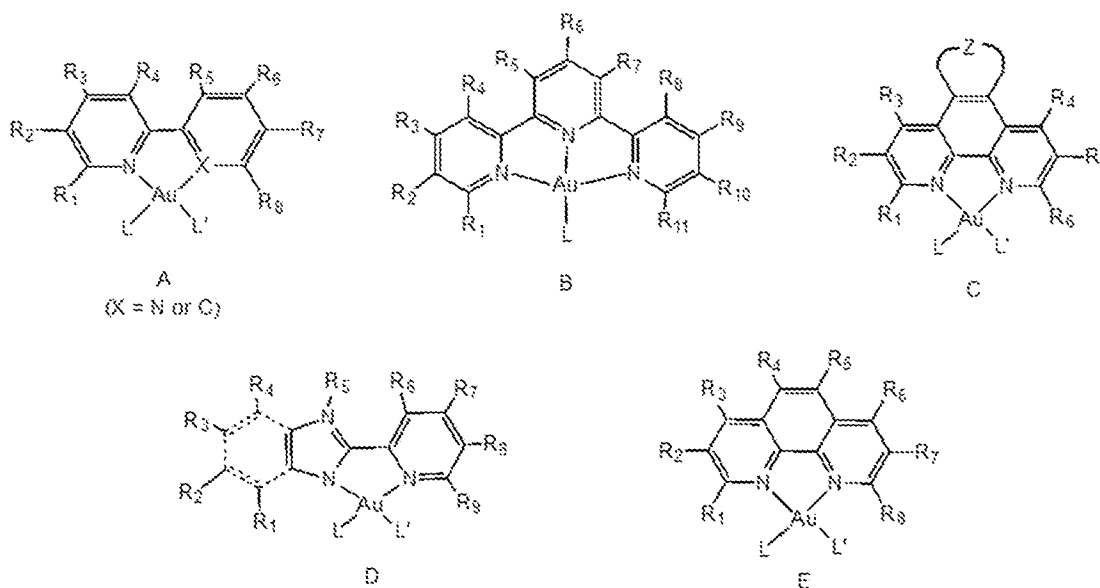
Thus, from a therapeutic point of view, ACP5/TRAP is an interesting molecular target. Unfortunately, the only known inhibitors of TRAP are the toxic metal compounds molybdate, vanadate, lead acetate, mercuric acetate and gold(III) chloride. The in arthritis therapeutically active gold(I) compounds Aurothioglucose and Aurothiomalate inhibit TRAP only at high concentration (mM range) (20). Gold chloride has been shown to be able to inhibit TRAP (Hayman *et al.*, Cell Biochem Funct 2004, 22L 275-280), but is too unstable to be used therapeutically since it is prone to undergo reduction to colloidal gold, which may also result in toxic effects. No other substances that are capable of inhibiting TRAP and suitable for therapeutic or diagnostic applications have been reported so far.

Recognizing the potential of ACP5/TRAP as therapeutic target, the present inventors therefore set out to search for novel inhibitors that

can be used *in vivo* to inhibit TRAP. In particular, they aimed at providing non-toxic inhibitory compounds showing an IC₅₀ value in the nanomolar range.

This goal was met by the surprising finding that various gold(III) coordination compounds bearing N-donor ligands are potent TRAP inhibitors *in vitro* with IC₅₀s in the nM range. Moreover, these compounds are not cytotoxic to macrophages in the concentration range showing an inhibitory effect. Interestingly, TRAP was shown to be involved in macrophage migration and this migration could be inhibited using the novel gold-based inhibitors. Lastly, it was demonstrated that inhibition of TRAP modulates the responses of macrophages to microbial compounds.

Accordingly, in one embodiment the invention provides a gold(III) coordination compound bearing N-donor ligands, the compound having the general formula A, B, C, D or E



wherein

dotted lines can be absent or present;

each of R₁ through R₁₁ is independently selected from the group consisting of H; aliphatic, heteroaliphatic, aromatic,

heteroaromatic, aliphatic-aromatic, heteroaliphatic-
heteroaromatic, cycloaliphatic, and heterocycloaliphatic groups
comprising up to four C-atoms; amines (e.g. NH_2 , aliphatic amines
– R-NH_2); halogens (e.g. chloride, iodide); moieties with hydroxyl
5 functional groups (e.g. –OH or –Y-OH); ether containing moieties of
general formula –Y-O-Y'; carbonyl containing moieties (–Y-
C(O)OH); or of amide bonds (–Y-C(O)N-Y'); sulfonamidic groups;
nitrile/nitro groups, and peptide moieties, wherein Y and Y' are
independently selected from aliphatic, heteroaliphatic, aromatic,
10 heteroaromatic, aliphatic-aromatic, heteroaliphatic-
heteroaromatic, cycloaliphatic, and heterocycloaliphatic groups
comprising up to four C-atoms;

L and L' are independently selected from the group consisting
of halogen, hydroxyl, acetate, phosphane, and thiol-bearing
15 groups (e.g. thio-sugars, cysteine and methionine groups); and

Z is a cyclic moiety selected from the group consisting of
homocyclic and heterocyclic aromatic/aliphatic moieties,
preferably 6,6-, 5,6- or 6,5-fused bi-homocyclic and bi-
heterocyclic aromatic/aliphatic moieties, wherein the
20 heterocyclic moieties may include nitrogen, oxygen and/or
sulfur atoms,

or a pharmaceutically acceptable salt or solvate thereof, for use in a method
of treating and/or alleviating the symptoms of a disease associated with
increased tartrate-resistant acid phosphatase (TRAP) activity.

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Some gold (III)-compounds for use in the present invention are known in the
art. For example, Messori *et al.* (J Med Chem 2000, 43, 3541-3548) discloses
gold complexes wherein the gold (III) center is coordinated to a polydentate

ligand with nitrogen donors, such as the compound $[\text{Au}(\text{terpy})\text{Cl}]\text{Cl}_2$ that belongs to the general formula B shown herein above. Discussed is the use of the gold complexes as cytotoxic and antitumor drug. Casini *et al.* (Dalton Trans., 2010, 39, 2239-2245) disclose the synthesis, characterization and biological properties of square planar gold(III) compounds with modified bipyridine and bipyridylamine ligands according to the general formula A. The complexes showed cytotoxicity *in vitro* towards the A2780 human ovarian carcinoma cell line and a cisplatin resistant variant thereof. WO2013/005170 relates to metal-based modulators that selectively bind to cell transmembrane proteins aquaglyceroporins AQPs, leading to its inhibition. The selective inhibition of AQP channels was accomplished among others by compounds tetracoordinated to gold (III) complexes according to the general formula A, B, C or E as shown herein above. Also disclosed is the use thereof in manufacturing pharmaceuticals, cosmetics and chemical reagents for diagnostic, treatment, prophylaxis and prevention of clinical conditions directly or indirectly related to aquaglyceroporins AQPs functions.

Hayman *et al.* (2014, Cell. Biochem. Funct. 22: 275-280) investigated whether gold (III) compounds mediate their effect on osteoclastic bone resorption by modification of TRAP activity. It was found that gold chloride was a powerful TRAP inhibitor whereas aurothioglucose or aurothiomalate had no effect.

Thus, the art fails to teach or suggest the use of the gold (III) compounds as claimed herein for the inhibition of TRAP or for the treatment of a disease associated with TRAP activity, like respiratory/ or pulmonary diseases such as COPD and asthma.

In a gold(III) compound for use according to the invention, each of R_1 through R_{11} on the N-donor ligands is independently selected from the group consisting of H; aliphatic, heteroaliphatic, aromatic, heteroaromatic,

aliphatic-aromatic, heteroaliphatic-heteroaromatic, cycloaliphatic, and heterocycloaliphatic groups comprising up to four C-atoms; amines (e.g. NH₂, aliphatic amines –R-NH₂); halogens (e.g. chloride, iodide); moieties with hydroxyl functional groups (e.g. –OH or –Y-OH); ether containing
5 moieties of general formula –Y-O-Y'; carbonyl containing moieties (-Y-CO-OH); or of amide bonds (-Y-CO-N-Y'-); sulfonamidic groups; nitrile/nitro groups, and peptide moieties, wherein Y and Y' are independently selected from aliphatic, heteroaliphatic, aromatic, heteroaromatic, aliphatic-aromatic, heteroaliphatic-heteroaromatic, cycloaliphatic, and
10 heterocycloaliphatic groups comprising up to four C-atoms.

In a preferred embodiment, each of R₁ through R₁₁ (also referred to herein as "R substituents" or "R groups") is independently selected from the group consisting of H; aliphatic, heteroaliphatic, aromatic, heteroaromatic, aliphatic-aromatic, heteroaliphatic-heteroaromatic, cycloaliphatic, and heterocycloaliphatic groups comprising up to four C-atoms. For example, each of R₁ through R₁₁ is independently selected from H, C₁-C₄ alkyl and C₁-C₄ alkoxy.

In another embodiment, at least one of the R substituents is selected from the group consisting of amines (e.g. NH₂, aliphatic amines –R-NH₂);
20 halogens (e.g. chloride, iodide); moieties with hydroxyl functional groups (e.g. –OH; –Y-OH); ether containing moieties of general formula –Y-O-Y'; carbonyl containing moieties (-Y-CO-OH); or of amide bonds (-Y-CO-N-Y'-); sulfonamidic groups; fluorophores; nitrile/nitro groups, and peptide moieties, wherein Y and Y' are independently selected from aliphatic,
25 heteroaliphatic, aromatic, heteroaromatic, aliphatic-aromatic, heteroaliphatic-heteroaromatic, cycloaliphatic, and heterocycloaliphatic groups comprising up to four C-atoms. In some embodiments, one or more of R₁ through R₁₁ is F, -CH₃, -CH₂CH₃, -OH, -OCH₃, or -OCH₂CH₃. In some embodiments, at least one of R₁ through R₁₁ is -CH₃ or -OCH₃.

In one embodiment, at least one of R₁ through R₁₁ is other than H, so that the (hetero)aromatic rings contain one or more substituents. The number of R substituents other than H can vary. In one embodiment, the compound contains up to 6, up to 5, or up to 4 R-groups other than H. For example, suitable compounds for use according to the invention are those having one, two, three or four R substituents selected from aliphatic, heteroaliphatic, aromatic, heteroaromatic, aliphatic-aromatic, heteroaliphatic-heteroaromatic, cycloaliphatic, and heterocycloaliphatic groups comprising up to four C-atoms; amines; halogens; moieties with hydroxyl functional groups; ether containing moieties of general formula –Y-O-Y'; carbonyl containing moieties (-Y-CO-OH); or of amide bonds (-Y-CO-N-Y'-); sulfonamidic groups; nitrile/nitro groups, and peptide moieties, while the remaining R groups are H. In another embodiment, all of the R groups are H, i.e. the gold(III) coordination compound bears unsubstituted N-donor ligands.

L and L' are independently selected from the group consisting of halogen, hydroxyl, acetate, phosphane, and thiol-bearing groups e.g. thio-sugars, cysteine and methionine groups. In a preferred embodiment of the invention, L and L' are halogen, preferably chloride or iodide.

Compounds according to any one of formula A, B, C, D or E can be screened for their efficacy using methods known in the art. For example, US 6,451,548 discloses an assay employing (recombinant) TRAP that has been activated with a cysteine protease and a phosphotyrosine or phosphoserine containing test compound. Disclosed herein below is a method using recombinant TRAP or a cell lysate as source of TRAP, and para-nitrophenylphosphate (PNPP) as enzyme substrate.

In one aspect, the invention provides a compound for use as TRAP inhibitor according to formula A, being an [Au(substituted-2,2'-bipyridine)LL']ⁿ⁺ with n = 1 or 2 or an [Au(2-phenylpyridine)LL']ⁿ⁺ with n = 0 or 1. In a preferred

aspect, the invention provides the use of a compound of formula A based on a bipyridine i.e. wherein X is N.

In an inhibitor according to formula A, all substituents R₁ through R₈ can be H. In one aspect, at least one, two, three or four of R₁ through R₈ is other
5 than H. The substituents R₁ through R₈ can be symmetric or asymmetric. Symmetrically substituted formula A compounds include those having substituents other than H at positions R₁ and R₈; R₂ and R₇; R₃ and R₆; R₄ and R₅; R₁, R₂, R₇ and R₈; R₃, R₄, R₅ and R₆, and so forth. In one embodiment, the substitutions are (only) at positions 3,3'; 4,4'; 5,5' or 6,6' of
10 the bipyridine or phenylpyridine ring. Preferably, the substituents other than H are (solely) at positions 4, 4'. For example, provided is a TRAP inhibitor according to formula A wherein X is N, and wherein the substitutions are at positions 4, 4 of the bipyridine ring.

In another embodiment, the formula A compound is asymmetrically
15 substituted. Exemplary asymmetric compounds include those having a single substituent other than H, and those having substituents other than H at positions R₁ and R₇; R₂ and R₈; R₃ and R₅; R₄ and R₆; R₁, R₂, R₆ and R₇; R₃, R₄, R₇ and R₈, and so forth. In a specific aspect, a compound of formula A is symmetric having two aliphatic or heteroaliphatic substituent at positions
20 R₃ and R₆, such as those wherein R₃ and R₆ are methoxy or ethoxy.

In one aspect, each of substituents R₁ through R₈ of a formula A inhibitor compound is independently selected from the group consisting of H, C₁-C₄ alkyl and C₁-C₄ alkoxy. An exemplary Formula A compound is
[Au(bpOMe)Cl₂][PF₆] (bpOMe = 4,4'-dimethoxy-2,2'-bipyridine) (Aubipy-O-
25 Me). Useful variants of Aubipy-O-Me include compounds having different substituents at the same positions, and compounds having one or more C₁-C₄ alkoxy substituent(s) at different positions of the bipyridine ring.

In a further embodiment, the inhibitor is a substituted-2,2',2''-terpyridine compound of formula B, wherein preferably up to four of substituents R_1 through R_{11} are other than H. In one aspect, it is an $[\text{Au}(\text{substituted-2,2',2''-terpyridine})\text{L}]^{n+}$ with $n = 2$ or 3 . An exemplary formula B compound is

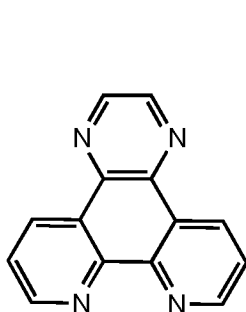
5 $[\text{Au}(2,2':6',2''\text{-terpyridine})\text{Cl}]\text{Cl}$ (Auterpy).

In a still further embodiment, the compound for use according to the invention is a polypyridyl compound of formula C. The polypyridyl moiety depicted by Z is a cyclic moiety selected from the group consisting of homocyclic and (hetero)cyclic aromatic and aliphatic moieties. The

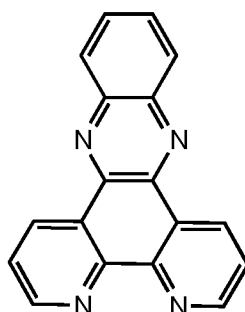
10 heterocyclic moiety may include nitrogen, oxygen and/or sulfur atoms. In one embodiment, Z is a fused ring system comprising one, two or three (hetero)cyclic moieties. Preferably, Z is a 6,6-, 5,6- or 6,5-fused bi-homocyclic or bi-heterocyclic aromatic/aliphatic moiety. Preferably, Z is a heterocyclic aromatic moiety, more preferably a heterocyclic aromatic moiety comprising

15 two nitrogen atoms. In one aspect, the compound is an $[\text{Au}(\text{polypyridyl})\text{LL'}]^{n+}$ with $n = 1$ or 2 .

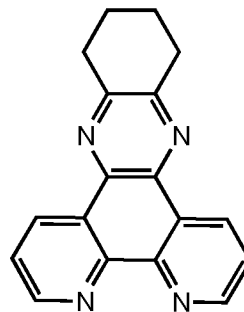
The polypyridyl scaffold can for example be based on dipyrido [3, 2-f:2', 3'-h] quinoxaline (DPQ), dipyrido [3, 2-a:2', 3'-c] phenazine (DPPZ), or dipyrido [3,2-a:2',3'-c] (6,7,8, 9-tetrahydro) phenazine (DPQC).



DPQ



DPPZ



DPQC

Accordingly, exemplary inhibitory compounds according to formula C include those wherein the polypyridyl moiety is dipyrido[3,2-f:2',3'-h]quinoxaline, dipyrido[3,2-a:2',3'-c]phenazine, or dipyrido[3,2-a:2',3'-c](6,7,8,9-tetrahydro)phenazine.

- 5 In a further embodiment, the inhibitory compound has a structure according to formula D. The dotted lines in the general formula D can be present or absent. For example, the compound may bear bidentate or tridentate nitrogen donor ligands selected from 2-(2-pyridyl)imidazole, 2-phenylimidazole, 2,6-bis(benzimidazol-2-yl)pyridine, and 1-methyl-2-[2-pyridyl]-1H-benzo[d]imidazole). Very good results were observed with
10 compounds wherein up to four of R₁ through R₉ are other than H. Preferably, at least R₅ is other than H, for example R₅ is C₁-C₄ alkyl or C₁-C₄ alkoxy. In a specific embodiment, only R₅ is other than H, for example R₅ is C₁-C₄ alkyl or C₁-C₄ alkoxy. For example, the inhibitory compound is [Au(1-methyl-2-[2-pyridyl]-1H-benzo[d]imidazole)Cl₂]Cl (AuPbImMe). A compound
15 according to formula D has not been disclosed or suggested in the art. Hence, the invention also provides a compound according to formula D and its use as medicament. Preferably, the compound is [Au(1-methyl-2-[2-pyridyl]-1H-benzo[d]imidazole)Cl₂]Cl (AuPbImMe) or functional equivalent
20 thereof. Also provided is a pharmaceutical composition comprising a compound according to formula D, and a pharmaceutical carrier, diluent or excipient.

- Still further aspects of the invention relate to inhibitory compounds according to formula E, being based on a phenantroline scaffold. Preferably,
25 the compound is an [Au(substituted-1,10-phenantroline)L₂]ⁿ⁺ with n= 1, 2 or 3. Good results can be observed with compounds wherein up to four of R₁ through R₈ are other than H.

An inhibitor compound for use in the present invention can be synthesized using methods known in the art. See for example Casini *et al.*, Dalton

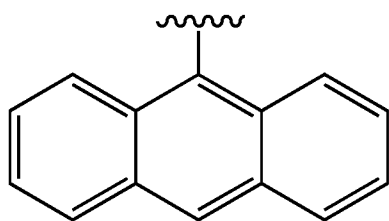
Trans. 2010, 39, 2239– 2245 or Hollis *et al.*, J. Am. Chem. Soc. 1983, 105, 4293 –4299. Compounds of formula D can be synthesized adapting the conditions from Casini *et al.*, Dalton Trans. 2010, 39, 2239– 2245, and also from Serratrice *et al.*, Inorg. Chem. 2012, 51, 3161–3171.

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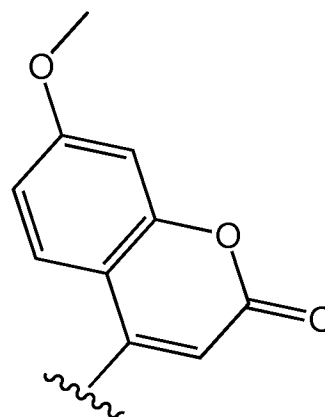
A gold (III) compound disclosed herein is suitably used as *in vitro* or *in vivo* inhibitor of tartrate-resistant acid phosphatase (TRAP). In addition, it can be modified to serve as (chemical) probe for detecting TRAP activity. For example, a probe can be a fluorescent derivative of a gold(III) compound of the invention. The probe finds its use for example in assessing the role of TRAP activity in a certain physiological process or cellular pathway. For example, the role of TRAP in the interplay between macrophages and cancer stem cells can be determined. In one aspect, the probe compound contains in at least one of the R positions a fluorophore such as an anthracenyl or coumarin moiety. Compounds containing 1,10-phenantroline are also fluorescent per se.

10
15

Specifically, one of the R groups is an anthracenyl or coumarin moiety.



R = anthracenyl moiety



R = coumarin moiety

In a further aspect, the gold (III) inhibitory compound is used, e.g. for experimental, scientific and/or drug-screening purposes, as *in vitro* inhibitor of TRAP.

In a preferred embodiment, an inhibitor compound finds it use in a method
5 of treating and/or alleviating the symptoms of a respiratory disease associated with increased tartrate-resistant acid phosphatase (TRAP) activity. Respiratory disease is a medical term that encompasses pathological conditions affecting the organs and tissues that make gas exchange possible in higher organisms, and includes conditions of the upper
10 respiratory tract, trachea, bronchi, bronchioles, alveoli, pleura and pleural cavity, and the nerves and muscles of breathing. Respiratory diseases range from mild and self-limiting, such as the common cold, to life-threatening entities like bacterial pneumonia, pulmonary embolism, acute asthma and lung cancer. The study of respiratory disease is known as pulmonology.

15 In a specific embodiment of the present invention, an inhibitor compound finds it use in a method of treating and/or alleviating the symptoms of a pulmonary disease associated with increased expression of TRAP, like asthma, COPD, smoke-induced pulmonary inflammation (e.g. cigarette smoke, wood smoke, biomass smoke, fuel combustion smoke) or (pulmonary)
20 sarcoidosis.

In a preferred embodiment, the disease is a pulmonary disease such as chronic obstructive pulmonary disease (COPD) or asthma. Without being bound by theory, upregulation of ACP5 / TRAP in pulmonary diseases like COPD assists in degradation of collagen by macrophages and, therefore,
25 aids parenchymal lung tissue destruction and assists in stimulation of macrophage migration through the lung. This enhanced migration could magnify the areas in which lung tissue can be destroyed. In addition, we propose that increased expression of ACP5/TRAP impairs antimicrobial responses in both asthma and COPD by yet unknown mechanisms, causing

enhanced disease and an increased susceptibility to exacerbations. The inventors hypothesize that inhibition of TRAP in emphysema will slow down lung tissue destruction, and in COPD and asthma it will improve responses to infections.

5 Accordingly, also provided is a pharmaceutical composition formulated for respiratory or pulmonary administration and/or inhalation, comprising one or more TRAP inhibitory compounds as disclosed herein. The inhalable formulation is a solution, suspension, emulsion, colloidal dispersion, or dry powder, wherein the formulation is suitable for administration to the lungs
10 of a mammal.

In a specific aspect, the TRAP inhibitor compound is formulated for use or contained in a medical device called a metered dose inhaler (MDI) or a dry powder inhaler (DPI). A metered dose inhaler is a handheld device that delivers a specific amount of medication in aerosol form. The MDI consists
15 of a pressurized canister inside a plastic case, with a mouthpiece attached. Its portability makes it easy to use anywhere, anytime. MDIs use a chemical propellant to push medication out of the inhaler. In a preferred embodiment, the compound is in the form of a dry powder and suitable for use in a dry powder inhaler, which is also a handheld device. A DPI delivers medication
20 to the lungs as you inhale through it. It doesn't contain propellants or other ingredients.

To give the most effective dry powder aerosol, therefore, the particles should be large while in the inhaler, but small when in the respiratory tract. In an attempt to achieve that situation, one type of dry powder for use in dry
25 powder inhalers may include carrier particles to which the fine active particles comprising the TRAP inhibitor adhere whilst in the inhaler device, but which are dispersed from the surfaces of the carrier particles on inhalation into the respiratory tract to give a fine suspension. In a specific aspect of the invention, the TRAP inhibitory compound is administered by

the multidose breath-actuated dry powder inhaler (DPI) known under the tradename Novolizer.

- A further embodiment of the invention relates to a method of treating and/or alleviating in a subject the symptoms of a respiratory disease associated with increased tartrate-resistant acid phosphatase (TRAP) activity, comprising administering to the subject an effective amount of a TRAP inhibitor as herein disclosed. In a specific aspect, the method comprises administering at least one compound selected from the group consisting of [Au(bpOMe)Cl₂][PF₆] (bpOMe = 4,4'-dimethoxy-2,2'-bipyridine) (Aubipy-O-Me); an [Au(substituted-2,2',2''-terpyridine)L]ⁿ⁺ with n = 2 or 3; [Au(2,2':6',2''-terpyridine)Cl]Cl (Auterpy); a compound of formula C, being an [Au(polypyridyl)LL']ⁿ⁺ with n = 1 or 2.; or a compound of formula C, based on dipyrido[3,2-f:2',3'-h]quinoxaline, dipyrido[3,2-a:2',3'-c]phenazine, or dipyrido[3,2-a:2',3'-c](6,7,8,9-tetrahydro)phenazine.
- 15 In another embodiment, the method comprises administering a compound of formula D, bearing bidentate or tridentate nitrogen donor ligands selected from 2-(2-pyridyl)imidazole, 2-phenylimidazole, 2,6-bis(benzimidazol-2-yl)pyridine, and 1-methyl-2-[2-pyridyl]-1H-benzo[d]imidazole; [Au(1-methyl-2-[2-pyridyl]-1H-benzo[d]imidazole)Cl₂]Cl (AuPbImMe); or a compound of
- 20 formula E, being an [Au(substituted-1,10-phenantroline)L₂]ⁿ⁺ with n= 1, 2 or 3.

Exemplary diseases to be treated include those mentioned herein above, in particular respiratory diseases associated with increased expression of TRAP such as asthma, COPD, smoke-induced inflammation and sarcoidosis.

25

Also provided herein is a method of inhibiting TRAP activity in a mammal, comprising administering to the mammal an inhalable formulation

described herein. In one aspect, the mammal has at least one symptom of a TRAP-dependent or TRAP-mediated (respiratory) disease or condition.

LEGEND TO THE FIGURES

5

Figure 1:

A: Numbers of macrophages showing TRAP activity are higher in parenchymal lung tissue of patients with fatal asthma as compared to controls dying of nonpulmonary causes.

10 **B:** TRAP gene ACP5 is a major upregulated gene in lung tissue of COPD patients compared to non-COPD controls.

C: Numbers of macrophages showing TRAP activity are higher in parenchymal lung tissue of mice exposed to house dust mite to induce experimental asthma than in healthy control mice.

15 **D:** Numbers of macrophages showing TRAP activity are higher in parenchymal lung tissue of mice exposed to cigarette smoke to induce experimental COPD than in control mice exposed to air.

Figure 2:

20 Structures of exemplary compounds showing TRAP inhibitory activity.

Figure 3:

Recombinant human TRAP was used to assess the TRAP inhibitory capacity of the gold (III) compounds Aubipy-O-Me, Auterpy, Auoxo1 and

25 Aurothiomalate as compared to NaAuCl₄ (see also figure 4). TRAP can catalyze the formation of p-nitrophenyl (PNP) from the colorimetric substrate p-nitrophenyl phosphate, which can be measured photospectrometrically. Increasing concentrations of the gold compounds were used to study their inhibiting potential of this conversion. NaAuCl₄
30 and Aubipy-O-Me were the most potent inhibitors (n=3).

Figure 4:

Cytotoxicity of the gold compounds of the gold (III) compounds Aubipy-O-Me, Auterpy, Auoxo1 and Aurothiomalate as compared to NaAuCl₄ was
5 measured by a proliferation assay using 3H-thymidin.

Figure 5:

Cell lysates of murine alveolar macrophages (MPI, kind gift of G. Fejer) were used to assess the TRAP inhibitory capacity of our gold compounds
10 Aubipy-O-Me, AuPblmMe, (dipyNH)AuCl₂ and (py^b-H)AuCl₂ as compared to NaAuCl₄. PNP formation was assessed in the presence of tartrate to eliminate the contribution of other phosphatases to the dephosphorylation of PNPP. NaAuCl₄, Aubipy-O-Me and AuPblmMe were the most potent inhibitors (n=3).

15

Figure 6:

Pooled tissue lysate of lung tissue obtained from human COPD patients was used to assess the TRAP inhibitory capacity of the gold compound Aubipy-O-Me as compared to NaAuCl₄. PNP formation was determined in the
20 presence of tartrate to eliminate the contribution of other phosphatases to the dephosphorylation of PNPP.

Figure 7:

A. In a transwell set-up, RAW 264.7 macrophages were allowed to
25 migrate over an osteopontin-coated membrane with or without RANKL stimulation (200ng/ml). RANKL-stimulated macrophages migrated significantly more through an osteopontin-coated membrane as compared to vehicle-stimulated macrophages.

B. The effect of RANKL-induced TRAP expression on migration behaviour was specific for osteopontin, as no effects of RANKL or Aubipy-O-Me were seen on macrophages cultured on collagen-coated membranes.

The number of cells that had migrated from the insert to the bottom well
5 was calculated relative to the number of unstimulated cells that had migrated. Each experiment was performed in quadruplicate and performed 6 times.

C. Live cell tracking of macrophages in an osteopontin-coated well revealed that macrophage migratory behavior was higher in the presence of
10 RANKL (200ng/ml) as compared to the vehicle-treated control and AubipyOMe inhibited this migration. Differences were tested with a Kruskal-Wallis test and a Dunn's post test, *P<0.05.

Figure 8:

15 Human monocyte-derived macrophages (n=3) have higher expression of several pro-inflammatory genes when stimulated with 100 ng/ml lipopolysaccharide (LPS) as compared to control macrophages. Inhibition of TRAP during this LPS stimulation with 300 nM or 1 μ M Aubipy-O-Me lowered the expression of these pro-inflammatory genes.

EXPERIMENTAL SECTION

Materials and methods

5 Cell culture

The Murine RAW 267.4 macrophage cell line was obtained from American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, *Penicillin/Streptavidin* (Invitrogen, Carlsbad, CA) and
10 L-glutamine (Invitrogen, Carlsbad, CA) at 37°C under 5% CO₂ and humidified conditions.

Gold inhibitors

The following compounds were tested for their ability to inhibit TRAP
15 activity: [AuCl₄]⁻, Authiomalate, AubipyOMe, Auterpy, Auoxo1, AuPbImMe, (dipyNH)AuCl₂ and (py^b-H)AuCl₂, wherein dipyNH means dipyridin-2-ylamine and py^b means benzyldipyrine. AubipyOMe and (dipyNH)AuCl₂ were prepared and characterized using the procedure described by Casini *et al.* (Dalton Trans. 2010 Mar 7;39(9):2239-45). Auoxo1
20 was prepared according to reported procedure by Casini *et al.* (J Med Chem. 2006 Sep 7;49(18):5524-31). (py^b-H)AuCl₂ was prepared following the procedure reported by Cinellu *et al.* (J. Chem. Soc., Dalton Trans., 1996, 4217–4225). Auterpy was synthesized according to Hollis *et al.* (J. Am. Chem. SOC. 1983, 105, 4293-4299). [AuCl₄]⁻ and Authiomalate are
25 commercially available from Sigma-Aldrich. AuPbImMe was synthesized according to conditions adapted from Casini *et al.* (Dalton Trans. 2010, 39, 2239– 2245).

TRAP activity assay

TRAP activity was assessed by incubating either recombinant TRAP (1.25ng/ml, R&D, Minneapolis, USA) or cell lysate (1:2 ratio) with L para-Nitrophenylphosphate (PNPP) solution [100 mM PNPP, 200 mM sodium citrate, 200 mM sodium chloride, 80 mM sodium tartrate (L+), pH 4.5]. Cell lysate was prepared by collecting cells directly from a culture flask in acetate buffer (pH 5.0) at a density of 500.000 cells/ml and sonication of cells. Subsequently, recombinant TRAP or cell lysate was incubated with increasing concentrations of AubipyOMe (range 0-40 μ M). Samples were incubated with PNPP solution at a 1:1 ratio up to an hour at 37°C. The reaction was stopped using 1M NaOH and the absorption at 410 nm was measured using a spectrophotometer. The inhibitory effect was calculated from the ratio of absorbance between the treated and untreated cells. The IC50 value was calculated as the concentration of inhibitor caused a 50% reduction in TRAP activity. Data was presented as mean $\pm$ SEM and consisted of at least three independent experiments.

Proliferation assay

Cytotoxicity of the compounds was assessed using 3H-thymidin. In short, RAW macrophages were grown in 96-well plates (Corning Incorporated, NY) in a medium volume of 100 μ l, at a density of 5000 cells/well and grown for 48h. Cells were exposed to various concentrations of inhibitor were prepared from a freshly made stock solution (10mM) in DMSO and diluted in medium including relevant negative controls (range 31-3934nM). After 28h hours, tritium thymidine (0.25uCi/ml) (Perkin Elmer, Waltham, USA) was added for 10 hours, after which excess of thymidine was washed away. Cells were subsequently incubated with 100 μ l of 10.5% TCA solution at 4°C for 30 min and lysed in 100 μ l of 1M NaOH. The lysate was transferred to a scintillation vial with 4 ml of scintillation fluid (Perkin Elmer). Samples were measured using the LS 6500 Multi-purpose Scintillation Counter (Perkin Elmer).

Macrophage motility

Motility of RAW macrophages was assessed by either live cell tracking using a confocal microscope or a Corning transwell culturing system (Sigma-Aldrich, St. Louis, USA). For the live cell tracking experiments, RAW cells
5 were plated on osteopontin (OPN, 10µg/ml) coated Lab-tek chamber slides (Nunc, Hatfield, USA) at a density of 7500 cells/well. Cells were stimulated with RANKL (200ng/ml, kindly provided by dr. R.H. Cool, University of Groningen, The Netherlands.) for two days to increase TRAP expression, followed by CFSE labeling (Invitrogen, Life Technologies Europe BV,
10 Bleiswijk, The Netherlands), over night tracking in the presence of 1% zymosan solution (Sigma-Aldrich, St. Louise, USA), and presence or absence of 128 nM AubipyOMe. Video material was analyzed using Imaris software.

In addition to live cell imaging, migration ability was assessed using a transwell culturing system with inserts (Sigma-Aldrich, St. Louis,
15 USA) coated with osteopontin (R&D, Minneapolis, USA) or collagen (Advanced Biomatrix, Delta, Canada) to determine macrophage migration over the membrane. Briefly, RAW macrophages, pre-stimulated with or without 200ng/ml RANKL for 72h to increase TRAP expression, were seeded with 250,000 cells/well on 8 µm pore inserts coated with 10µg/ml
20 osteopontin or collagen. Cells were cultured for 16h in the presence or absence of 128 nM AubipyOMe. The next day, total cell numbers in the lower compartment, including dead cells, were counted. The number of cells migrated was calculated relative to cells not stimulated with RANKL.

25 Generation of monocyte-derived macrophages

Monocytes were isolated from buffy coats of healthy blood donors (NHS Blood and Transplant, UK) by Lymphoprep (Axis-Shield, Oslo, Norway) density gradient centrifugation followed by plastic adherence in IMDM (Lonza, Basel, Switzerland) containing 10% human pool serum, 1%
30 penicillin/streptomycin (Invitrogen, San Diego, CA), and 0.25% ciprofloxacin

(Bayer, Leverkusen, Germany). PBMCs were incubated, and nonadherent cells were removed after 2 h incubation by extensive washing with PBS. Subsequently, adherent monocytes were detached using PBS containing 10 mM EDTA at room temperature and plated for macrophage maturation at a density of 1×10^5 cells/cm² in IMDM containing 10% human pool serum, 1% penicillin/streptomycin, and 0.25% ciprofloxacin.

TRAP-dependent macrophage responses to lipopolysaccharide (LPS)

For microarray analysis LPS -responses, MDMs were cultured for 7 days. At day 7, LPS (100 ng/ml; *Salmonella minnesota* R595; Alexis Biochemicals, Lausen, Switzerland), and 300 nM, or 1 μ M Aubipy-O-Me were added 6 h prior to harvesting the cells ($n = 3$). Control cells received vehicle.

RNA extraction and analysis

RNA was extracted with RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. After extraction, the sample was incubated with Turbo DNase at 37 °C for 30 minutes and subsequently re-purified using RNeasy clean-up protocol. Expression of the following genes were subsequently analyzed as compared to the expression of β -actin: Interferon-regulatory factor-1 (IRF1), Serpin Peptidase Inhibitor, Clade G (C1 Inhibitor), Member 1 (SERPING1), Apolipoprotein L3 (APOL3), interleukin-6 (IL6), tumor necrosis factor alpha (TNF), Chemokine (C-C motif) ligand 20 (CCL20), Interleukin-12 p40 (IL12B), indoleamine 2,3-dioxygenase 1 (IDO1), and Chemokine (C-C Motif) Ligand 4 (CCL4).

Animals

Male A/JOLA^{Hsd} mice and male and female BALB/c mice (8-10 weeks old) were obtained from Harlan (Horst, The Netherlands). Animals were kept in a temperature-controlled room with a 12h dark/light cycle and with

permanent access to food and water. All animal experiments were approved by the Institutional Animal Care and Use Committee. The animal experiment was performed in the animal facility of the University of Groningen according to strict governmental and international guidelines on animal experimentation.

Smoke-induced lung inflammation in mice

To model COPD, we exposed male A/JOLA-Hsd mice nose-only to mainstream cigarette smoke for 9 months in an experimental setup as described before by us (Van der Strate, 2006). In short, mice were exposed daily to mainstream smoke from four 2R1 Reference Cigarettes (University of Kentucky, KY). This protocol was continued for 5 days/week for 9 months. Control mice were sham-exposed to room air under similar conditions, following the same duration of exposure as the smoke-exposed group. The method used to check the delivery of total particulate matter by the smoking equipment has previously been described by Griffith and Hancock. The smoking equipment was calibrated before every smoking session to ensure accurate and standardized smoke exposure. Carboxyhaemoglobin levels were measured to determine actual smoke exposure.

20

Allergic lung inflammation

To model asthma, we exposed male and female BALB/c mice intranasally to whole body house dust mite (HDM) extract (*Dermatophagoides pteronyssinus*, Greer laboratories, Lenoir, USA) in 40 µl phosphate-buffered saline (PBS) according to a protocol we have described before (Draijer med inflame 2013). In short, mice were exposed to HDM extract under isoflurane anesthesia: one time to a high dose of HDM (100 µg) in the first week, 5 times to a low dose (10 µg) in the second week and were sacrificed on day 21. Control animals (n=8) were exposed to 40 µl PBS according to this same schedule. Mice were sacrificed on day 24, three days after the last HDM

30

exposure and the right lung was inflated with 0.5 ml 50% Tissue-Tek® O.C.T.[™] compound (Sakura, Finetek Europe B.V., Zoeterwoude, The Netherlands) in PBS and formalin-fixed for histological analyses. Other parameters of allergic lung inflammation of these animals are described in
5 detail by Draijer et al {Draijer 2013}.

Enzyme histochemistry

TRAP activity was measured via enzyme histochemistry. The staining was performed on 3µm sections of mouse lung tissue imbedded in paraffin.
10 Deparaffinated, rehydrated sections were incubated over night in a zinc-buffer solution (0.1M Tris, pH 7.4 complemented with 30 mM calcium acetate, 23 mM zinc acetate and 37 mM zinc chloride), following pre-incubation in a 0.2 M acetate buffer (0.2 M sodium acetate, 50 mM L(+) tartaric acid, pH 5.0). Slides were then incubated for 2 hours at 37°C with
15 the reaction solution (0.2 M acetate buffer containing 0.5 mg/ml Naphtol AS-MX phosphate (Sigma-Aldrich, St. Louis, USA) and 1.1 mg/ml fast red TR salt (Sigma-Aldrich, St. Louis, USA). A hematoxylin counterstaining was applied and stained sections were imbedded in DEPEX mounting medium (VWR, Murarrie, Australia). The number of positive cells (#/mm²) was
20 counted manually with the aid of ImageScope software.

Patient material

COPD

Gene expression data of TRAP was obtained from 311 patients with COPD
25 and 270 non-COPD controls who were part of the Lung eQTL dataset from three academic sites. Details of this population as well as a detailed description of the whole genome mRNA profiling has been previously published by Brandsma et al. {Thorax 2015}. Data was corrected for the following potential confounders: age, gender, pack-years and smoking
30 status. Lung tissue samples were collected from patients undergoing lung

tumor resection or lung transplantation. In case of tumor resections, macroscopically normal lung tissue was taken far distant from the tumor and histology of all samples was checked for abnormalities using standard haematoxylin and eosin staining. Lung samples were obtained in accordance
5 with local ethical guidelines.

Asthma

Post mortem lung tissues from subjects with fatal asthma or death from nonpulmonary causes (controls) were retrieved from the Department of
10 Pathology of São Paulo University (São Paulo, Brazil). A detailed clinical and demographic description of this population has been previously published by Mauad et al. { Rev Panam Salud Publica 2008; 23: 418–423}. For this study we investigated the presence of TRAP activity in paraffin-embedded peripheral lung tissue samples of 10 asthma patients and 10
15 controls as described below.

EXAMPLE 1: Gold(III) compounds inhibit TRAP activity

20 The inhibitory capacity of four different Gold(III) coordination compounds on TRAP was assessed by using recombinant TRAP in combination with increasing concentrations of candidate inhibitors and these were compared to the positive control NaAuCl₄ (figure 3). Two out of four compounds were able to reduce TRAP activity within the used concentration range, namely
25 Aubipy-O-Me and Auterpy. Aubipy-O-Me was almost as potent as positive control NaAuCl₄ in inhibiting TRAP activity. Its IC₅₀ value was 476 nM as compared to 280 nM for NaAuCl₄ (table 1). Notably, the lowest concentration of Aubipy-O-Me already gave a significant reduction of TRAP activity compared to vehicle. The inhibitory effect of Aubipy-O-Me further
30 increased in a dose-dependent manner and the highest concentration

Aubipy-O-Me reduced TRAP activity significantly. Auterpy was less potent than Aubipy-O-Me and had an IC₅₀ value of 1643 nM.

Cytotoxicity of these gold compounds was measured by a proliferation assay using 3H-thymidin (figure 4). None of the compounds except for Auterpy
5 showed severe cytotoxicity in the concentration range needed for successful inhibition of TRAP activity. The IC₅₀ value for inhibiting cell proliferation was lower than the value for inhibiting TRAP (1024 nM for proliferation inhibition versus 1643 nM for inhibition of TRAP), rendering Auterpy unsuitable for use of TRAP inhibition in cells (table 1 and 2).

10

EXAMPLE 2: Inhibition of cell-derived TRAP

The inhibitory capacity of three other different Gold(III) coordination compounds on TRAP in comparison to Aubipy-O-Me and NaAuCl₄ was
15 assessed by using cell lysates of high TRAP-expressing macrophages in combination with increasing concentrations of the candidate inhibitors (figure 5). AuPblmMe was equally effective as Aubipy-O-Me in this system, while the other two, (py^b-H)AuCl₂ and (dipyNH)AuCl₂, were less potent. In this more complex cell mixture Aubipy-O-Me was less effective in
20 inhibiting cell-derived TRAP as compared to using recombinant TRAP. The IC₅₀ value increased 6-fold to 3 μM (table 3).

To test whether the Gold (III) compounds could also inhibit TRAP in lung tissue of COPD patients, pooled lung tissue lysates of COPD patients were incubated with increasing concentrations of Aubipy-O-Me or NaAuCl₄ (see figure 6). Aubipy-O-Me could inhibit human TRAP in lung tissue of COPD patients with an IC₅₀ of 8 μM. Tables 1-3 herein below summarize the IC₅₀ data obtained.

Table 1. IC₅₀ of recombinant TRAP inhibition.

Recombinant TRAP inhibition	IC₅₀ (nM)
Authiomalate	No inhibition found
Auterpy	1643
Auoxo1	No inhibition found
Aubipy-O-Me	476
[AuCl ₄] ⁻	280

Table 2. IC₅₀ of the cytotoxicity measured with proliferation

Cytotoxicity Proliferation	IC₅₀ (nM)
Authiomalate	No toxicity found
Auterpy	1024
Auoxo1	No toxicity found
AubipyOMe	7494
[AuCl ₄] ⁻	No toxicity found

Table 3. IC₅₀ of cell-derived TRAP inhibition.

TRAP isolated from cells	IC₅₀ (nM)
[AuCl ₄] ⁻	470
AubipyOMe	2951
AuPblMe	3559
(dipyNH)AuCl ₂	4093
(py ^b -H)AuCl ₂	10396

EXAMPLE 3: Macrophage motility depends on TRAP activity and is inhibited by AubipyOMe

5 Macrophage motility is suggested to be TRAP-dependent. Macrophages may require TRAP activity to detach and enable cell movement when attached to an osteopontin surface. Therefore, macrophages were seeded on osteopontin-coated surfaces when macrophage motility was assessed using live tracking of macrophages by confocal microscopy or using a transwell-incubation
10 system in the absence or presence of the TRAP inhibitor AubipyOMe. Videos of live-tracked macrophages (movies not shown, but representative tracking plots are shown in figure 7C)), indicated little movement of macrophages incubated with the vehicle, but a significant amount of movement in the presence of RANKL, a cytokine that induces the
15 expression of TRAP in these macrophages. Following incubation with the inhibitor, macrophages' movement is less, suggesting that TRAP activity is necessary for macrophage movement. These results were quantified in a transwell-migration assay, in which more RANKL-stimulated macrophages migrated through an osteopontin-coated membrane compared to vehicle-
20 stimulated cells (figure 7A), while this migration was suppressed in the presence of Aubipy-O-Me. This effect was specific for an osteopontin-coating, because migration through a coating of collagen was not affected by RANKL stimulation and/or Aubipy-O-Me incubation (figure 7B).

25 **EXAMPLE 4: Human macrophage responses to the bacterial compound lipopolysaccharide (LPS) are altered when co-incubated with AubipyOMe.**

Macrophage responses to micro-organisms such as bacteria and viruses is suggested to be influenced by TRAP. For instance intracellular TRAP was
30 shown to inhibit production of interferon alpha (15). To investigate if TRAP

influences the responses to Gram-negative bacteria, human macrophages were stimulated with LPS in the presence or absence of 2 concentrations of Aubipy-O-Me. LPS clearly induced the expression of genes associated with inflammation such as IRF1, SERPING1, APOL3, IL6, TNF, CCL20, IL12B, IDO1, and CCL4 (figure 8). Aubipy-O-Me was found to inhibit these responses.

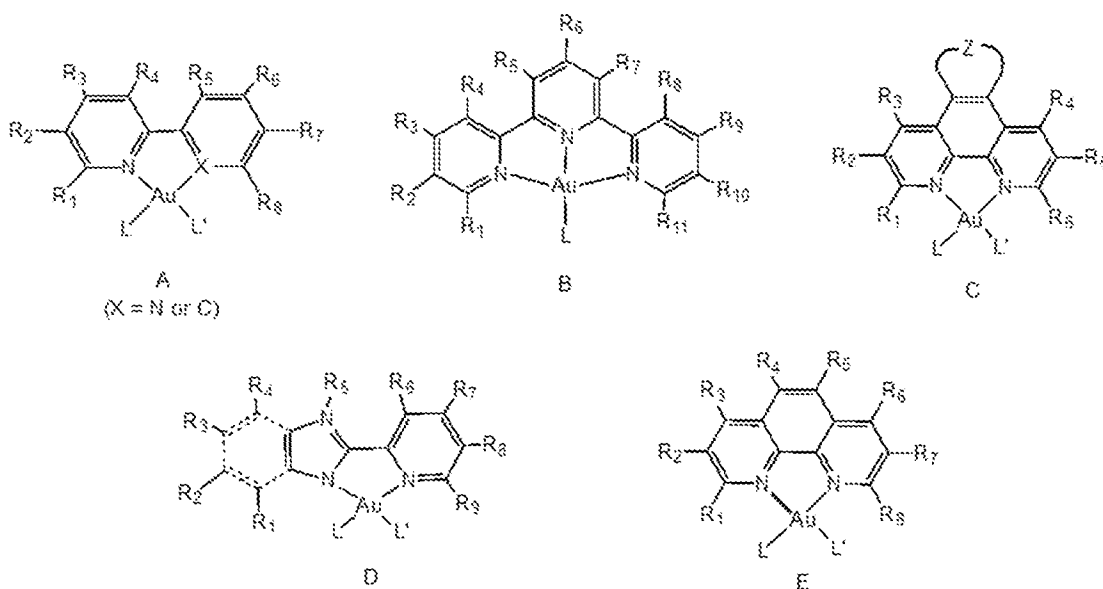
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Claims

1. A gold(III) coordination compound bearing N-donor ligands, the compound having the general formula A, B, C, D or E

5



wherein

- each of R₁ through R₁₁ is independently selected from the group consisting of H; aliphatic, heteroaliphatic, aromatic, heteroaromatic, aliphatic-aromatic, heteroaliphatic-heteroaromatic, cycloaliphatic, and heterocycloaliphatic groups comprising up to four C-atoms; amines (e.g. NH₂, aliphatic amines); halogens (e.g. chloride, iodide); moieties with hydroxyl functional groups (e.g. -OH or -Y-OH); ether containing moieties of general formula -Y-O-Y'; carbonyl containing moieties (-Y-CO-OH); or of amide bonds (-Y-CO-N-Y'-); sulfonamidic groups; fluorophores; nitrile/nitro groups, and peptide moieties, wherein Y and Y' are independently selected

from aliphatic, heteroaliphatic, aromatic, heteroaromatic, aliphatic-aromatic, heteroaliphatic-heteroaromatic, cycloaliphatic, and heterocycloaliphatic groups comprising up to four C-atoms;

5 L and L' are independently selected from the group consisting of halogen, hydroxyl, acetate, phosphane, and thiol-bearing groups (e.g. thio-sugars, cysteine and methionine groups); and

Z is a cyclic moiety selected from the group consisting of homocyclic and heterocyclic aromatic/aliphatic moieties,
10 wherein the heterocyclic moieties may include nitrogen, oxygen and/or sulfur atoms;

dotted lines can be absent or present;

or a pharmaceutically acceptable salt or solvate thereof, for use in a method of treating and/or alleviating the symptoms of a respiratory disease
15 associated with increased tartrate-resistant acid phosphatase (TRAP) activity.

2. Compound for use according to claim 1, wherein L and L' are halogen, preferably chloride or iodide.

3. Compound for use according to any one of the preceding claims,
20 wherein each of R₁ through R₁₁ is independently selected from the group consisting of H, C₁-C₄ alkyl and C₁-C₄ alkoxy.

4. Compound for use according to any one of the preceding claims according to formula A, being an [Au(substituted-2,2'-bipyridine)LL']ⁿ⁺ with n = 1 or 2 or an [Au(2-phenylpyridine)LL']ⁿ⁺ with n = 0 or 1.

5. Compound for use according to any one of the preceding claims according to formula A, wherein the substitutions are (only) at positions 6,6'; 3,3'; 4,4'; or 5,5' of the bipyridine or phenylpyridine ring.
6. Compound for use according to claim 4 or 5, wherein X is N,
5 preferably with the substitutions of the bipyridine ring at positions 4, 4'.
7. Compound for use according to any one of claims 4-6, wherein each of R₁ through R₇ is independently selected from the group consisting of H, C₁-C₄ alkyl and C₁-C₄ alkoxy.
8. Compound for use according to claim 7, being [Au(bpOMe)Cl₂][PF₆]
10 (bpOMe = 4,4'-dimethoxy-2,2'-bipyridine) (Aubipy-O-Me).
9. Compound for use according to any one of claims 1-3 of formula B, preferably wherein up to four of R₁ through R₁₁ are other than H.
10. Compound for use according to claim 9, being an [Au(substituted-2,2',2''-terpyridine)L]ⁿ⁺ with n = 2 or 3;
11. Compound for use according to claim 9, being [Au(2,2':6',2''-terpyridine)Cl]Cl (Auterpy).
15
12. Compound for use according to any one of claims 1-3 of formula C, being an [Au(polypyridyl)LL']ⁿ⁺ with n = 1 or 2.
13. Compound for use according to any one of claims 1-3 of formula C,
20 based on dipyrido[3,2-f:2',3'-h]quinoxaline, dipyrido[3,2-a:2',3'-c]phenazine, or dipyrido[3,2-a:2',3'-c](6,7,8,9-tetrahydro)phenazine.
14. Compound for use according to any one of claims 1-3 of formula D, bearing bidentate or tridentate nitrogen donor ligands selected from 2-(2-pyridyl)imidazole, 2-phenylimidazole, 2,6-bis(benzimidazol-2-yl)pyridine,
25 and 1-methyl-2-[2-pyridyl]-1H-benzo[d]imidazole).

15. Compound for use according to any one of claims 1-3 of formula D, wherein up to four of R₁ through R₉ are other than H.
16. Compound for use according to claim 15, being [Au(1-methyl-2-[2-pyridyl]-1H-benzo[d]imidazole)Cl₂]Cl (AuPbImMe).
- 5 17. Compound for use according to any one of claims 1-3 of formula E, being an [Au(substituted-1,10-phenantroline)L₂]ⁿ⁺ with n= 1, 2 or 3
18. Compound for use according to any one of the preceding claims, wherein said disease is selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), smoke-induced
10 inflammation and sarcoidosis.
19. Compound for use according to claim 18, wherein said disease is COPD or asthma.
20. Compound as recited in any one of claims 1 to 17, for use as *in vitro* inhibitor of tartrate-resistant phosphatase (TRAP).
- 15 21. A pharmaceutical composition formulated for pulmonary administration / inhalation, comprising one or more compounds as recited in any one of claims 1-17.
22. Compound according to formula D as defined in any of claims 1-3, 14 and 15.
- 20 23. Compound according to claim 22, for use as medicament.
24. Pharmaceutical composition comprising a compound according to claim 22, and a pharmaceutical carrier, diluent or excipient.
25. A medical device comprising a TRAP-inhibitory compound as recited in any one of claims 1 to 17.

26. Medical device according to claim 25, being a metered dose inhaler (MDI) or a dry powder inhaler (DPI).

27. Medical device according to claim 25 or 26, wherein said TRAP-inhibitory compound is present in the form of a dry powder.

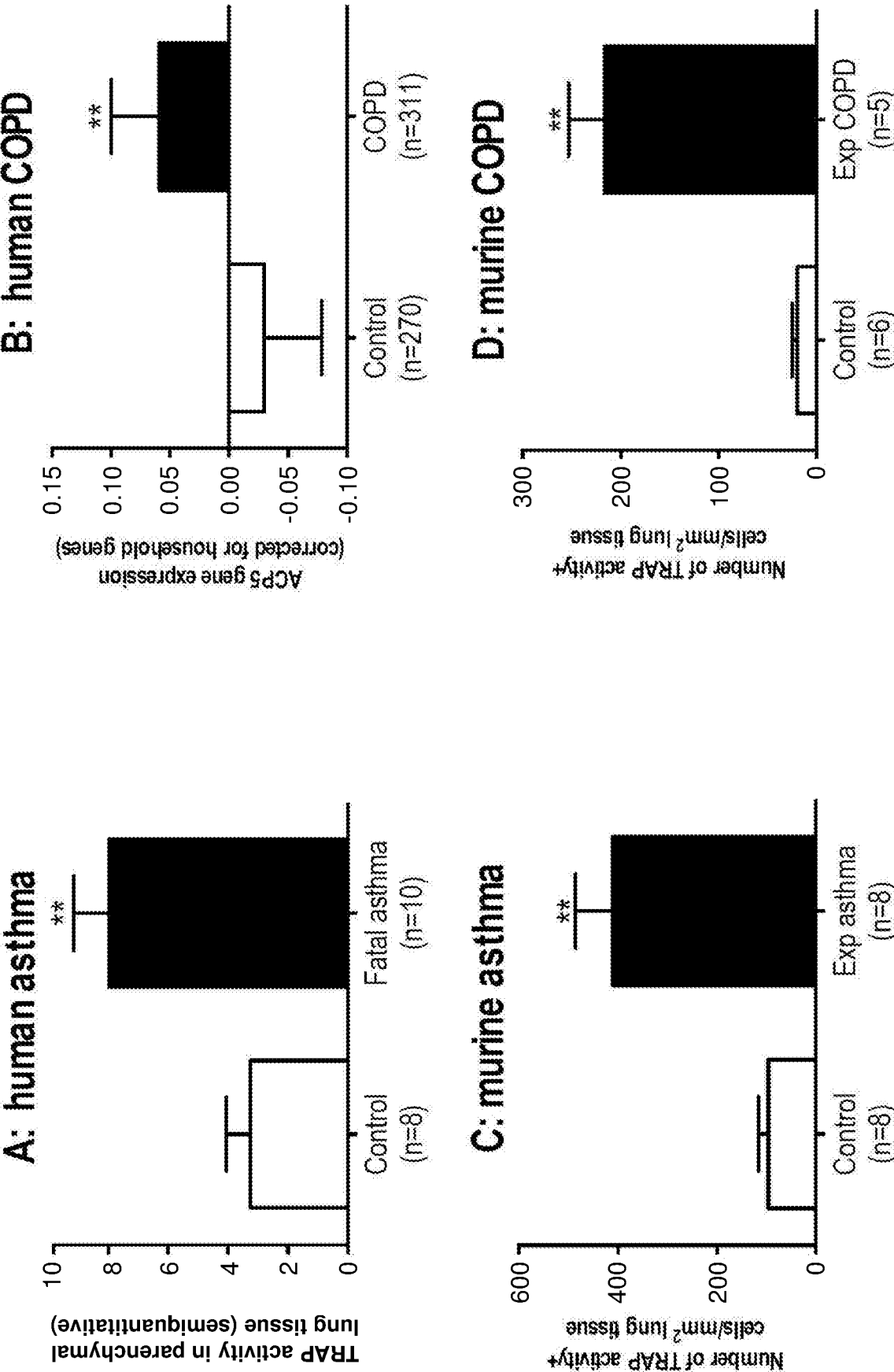


Fig. 1

2/12

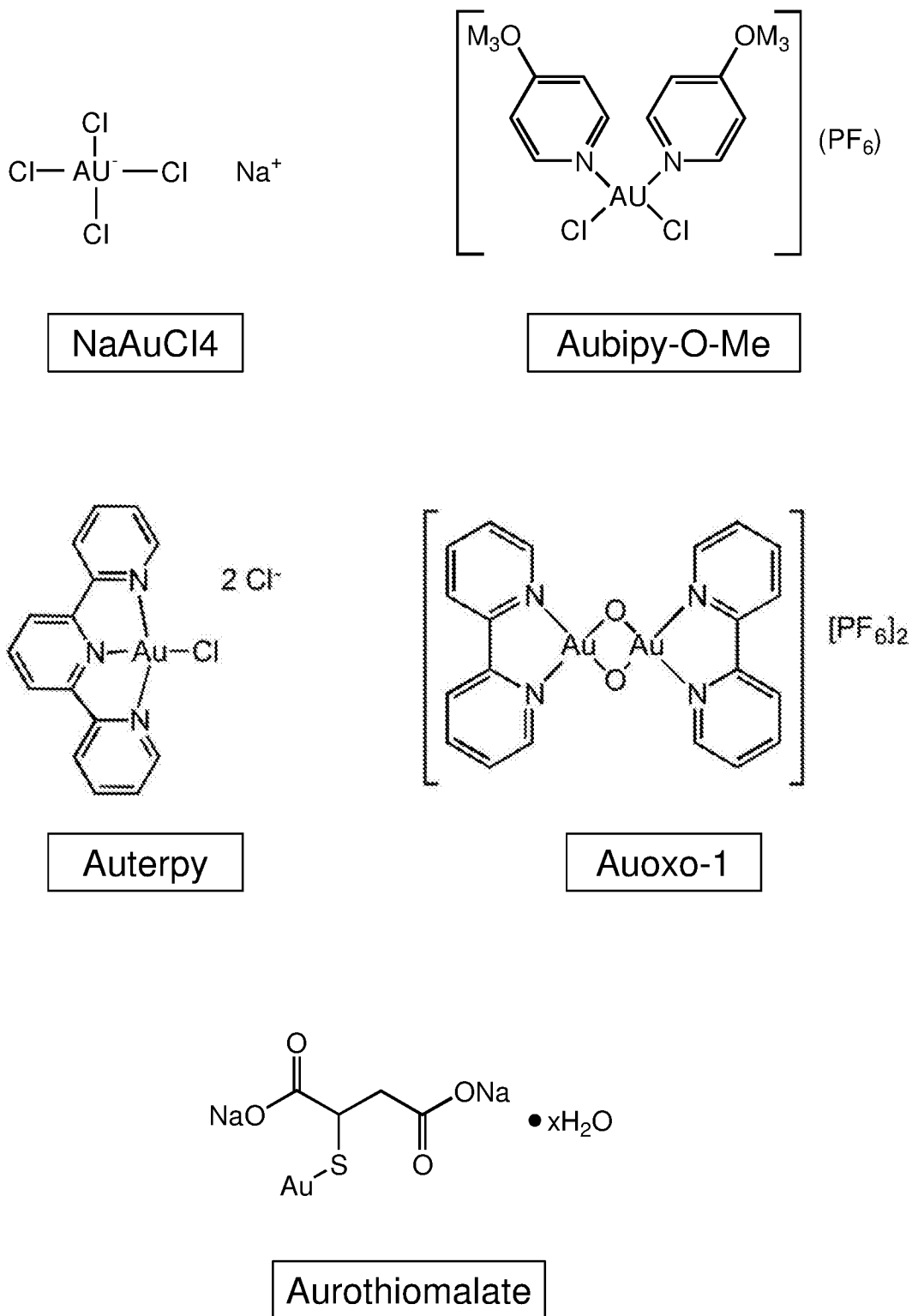


Fig. 2

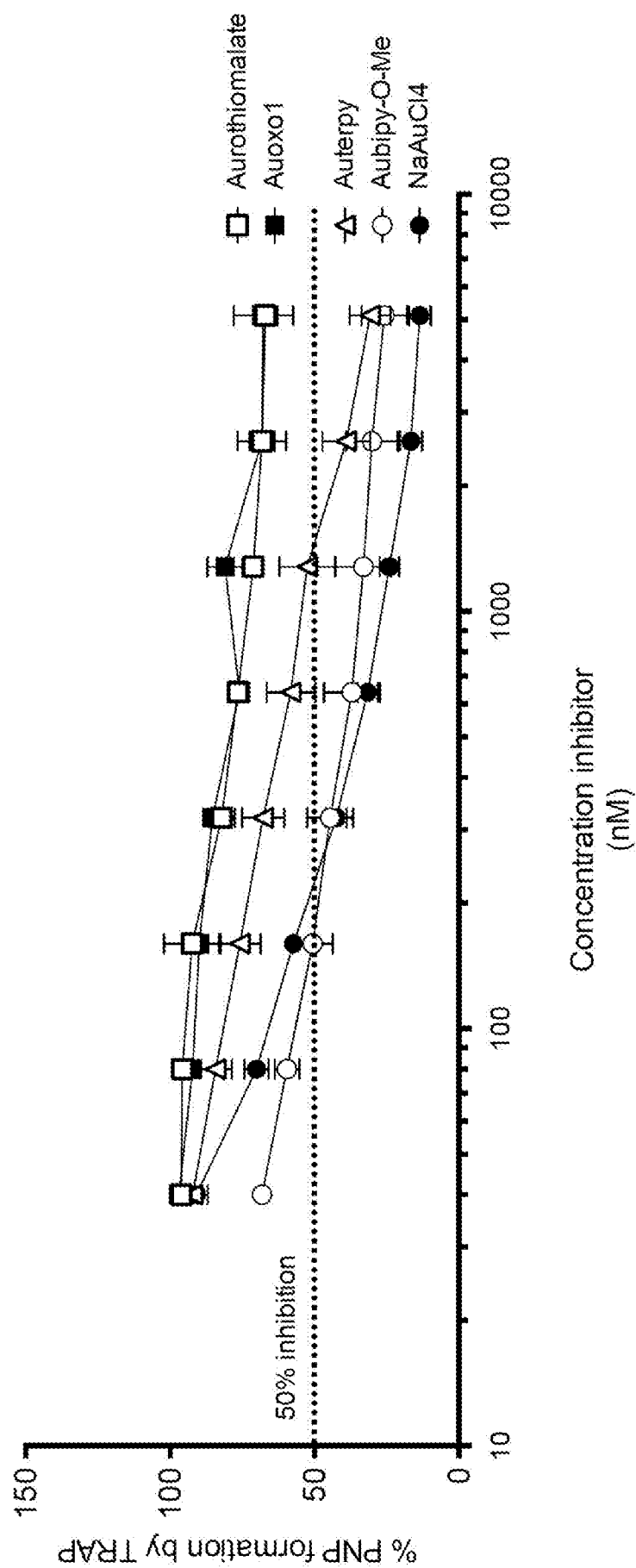


Fig. 3

4/12

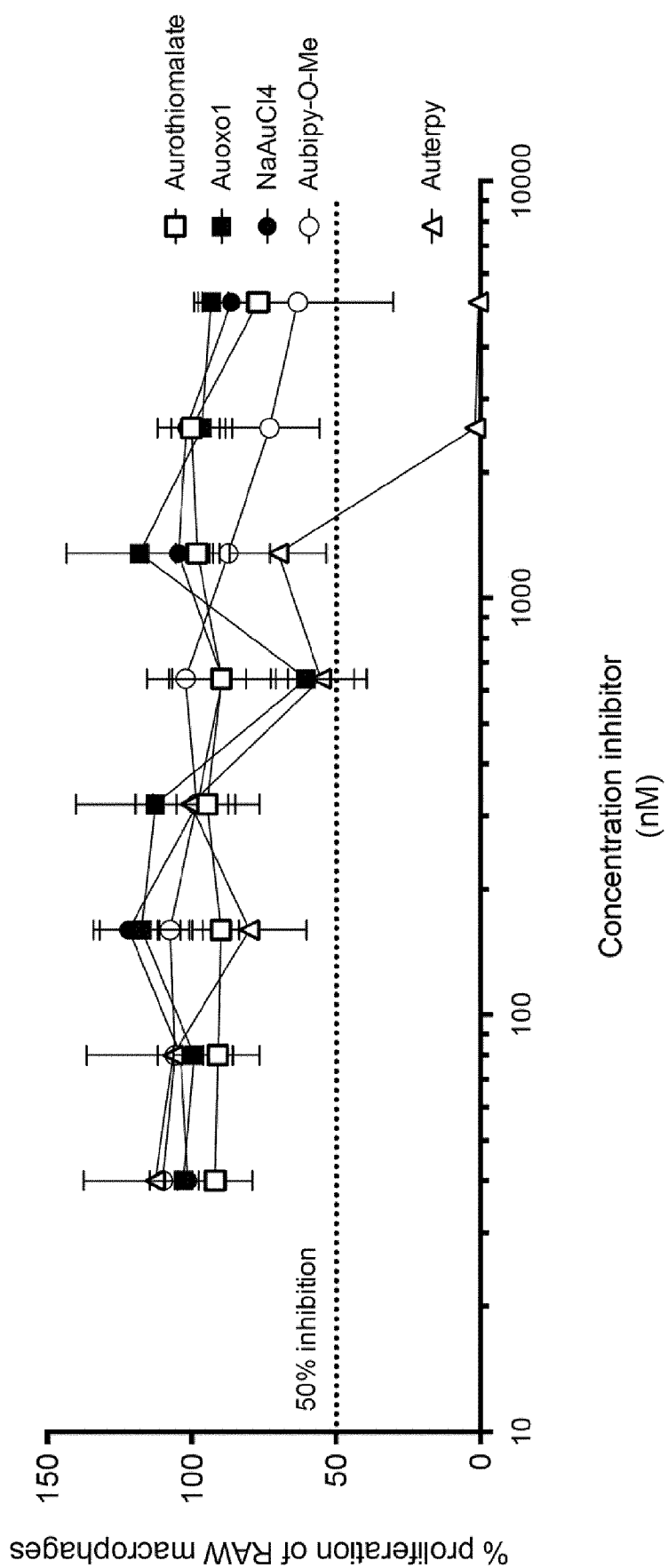


Fig. 4

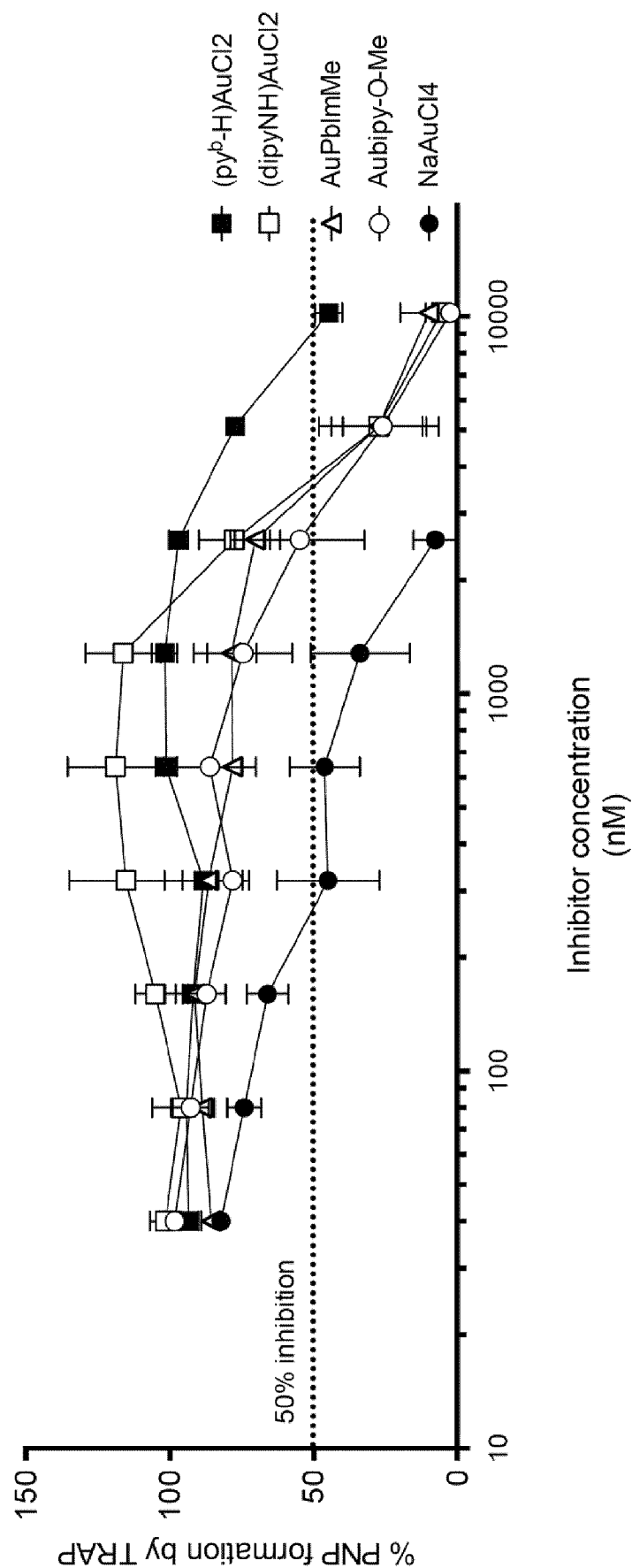


Fig. 5

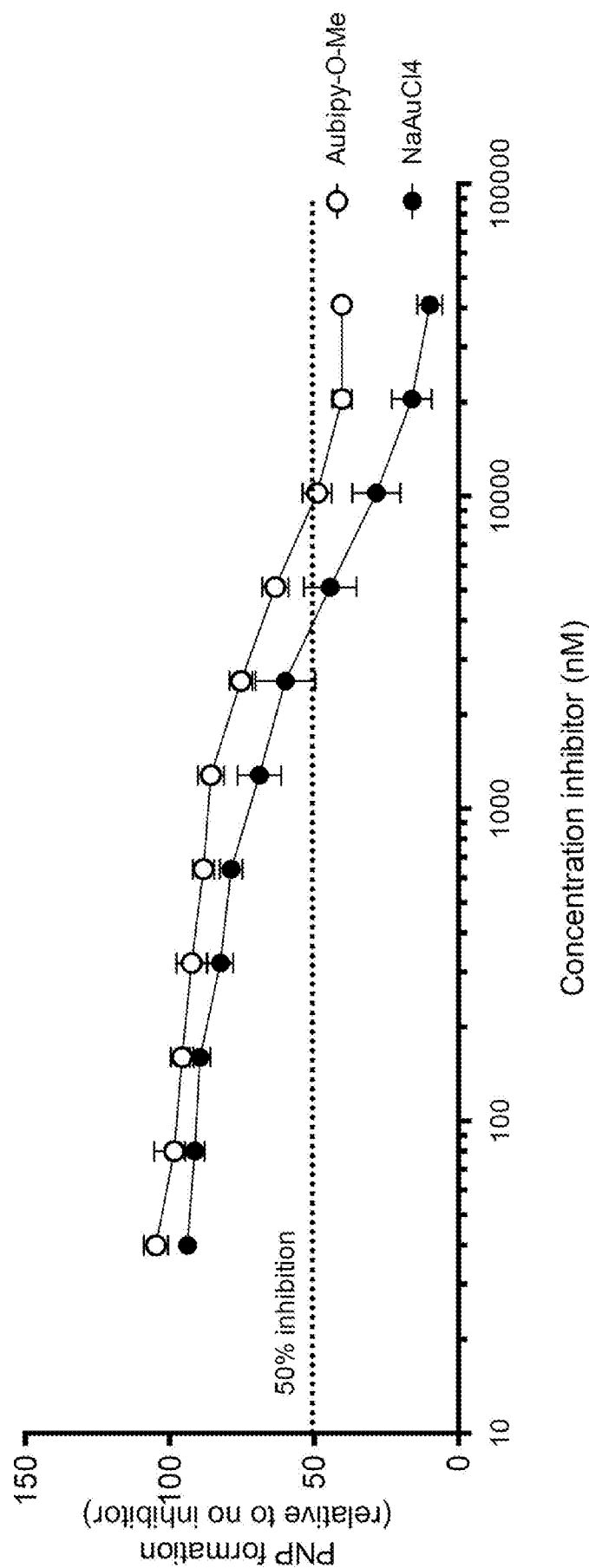


Fig. 6

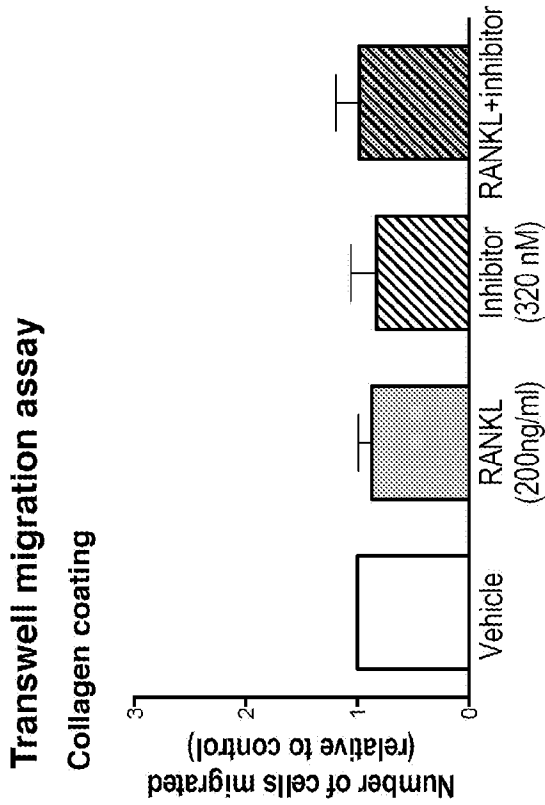


Fig. 7B

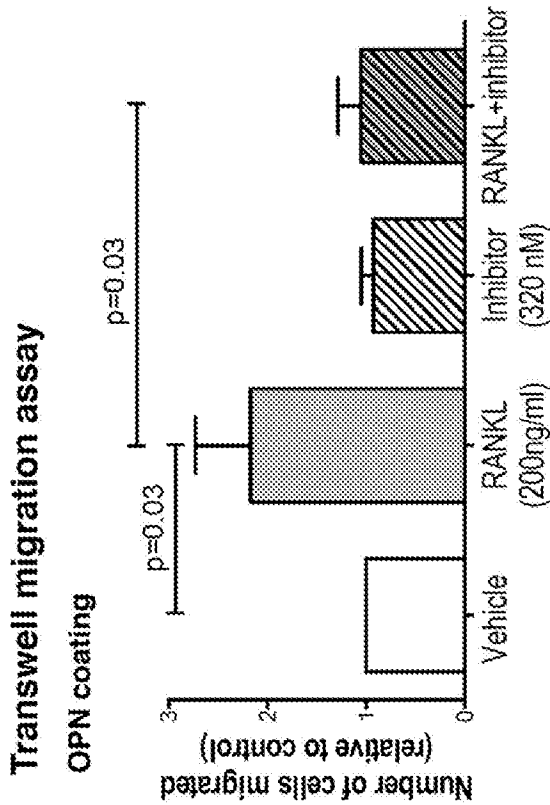


Fig. 7A

Macrophage motility

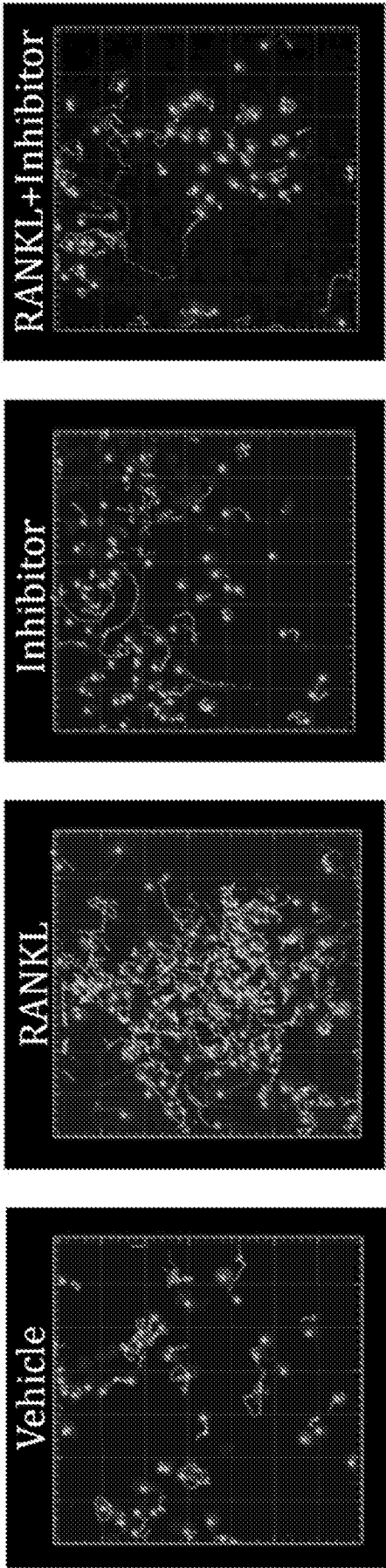


Fig. 7C

8/12

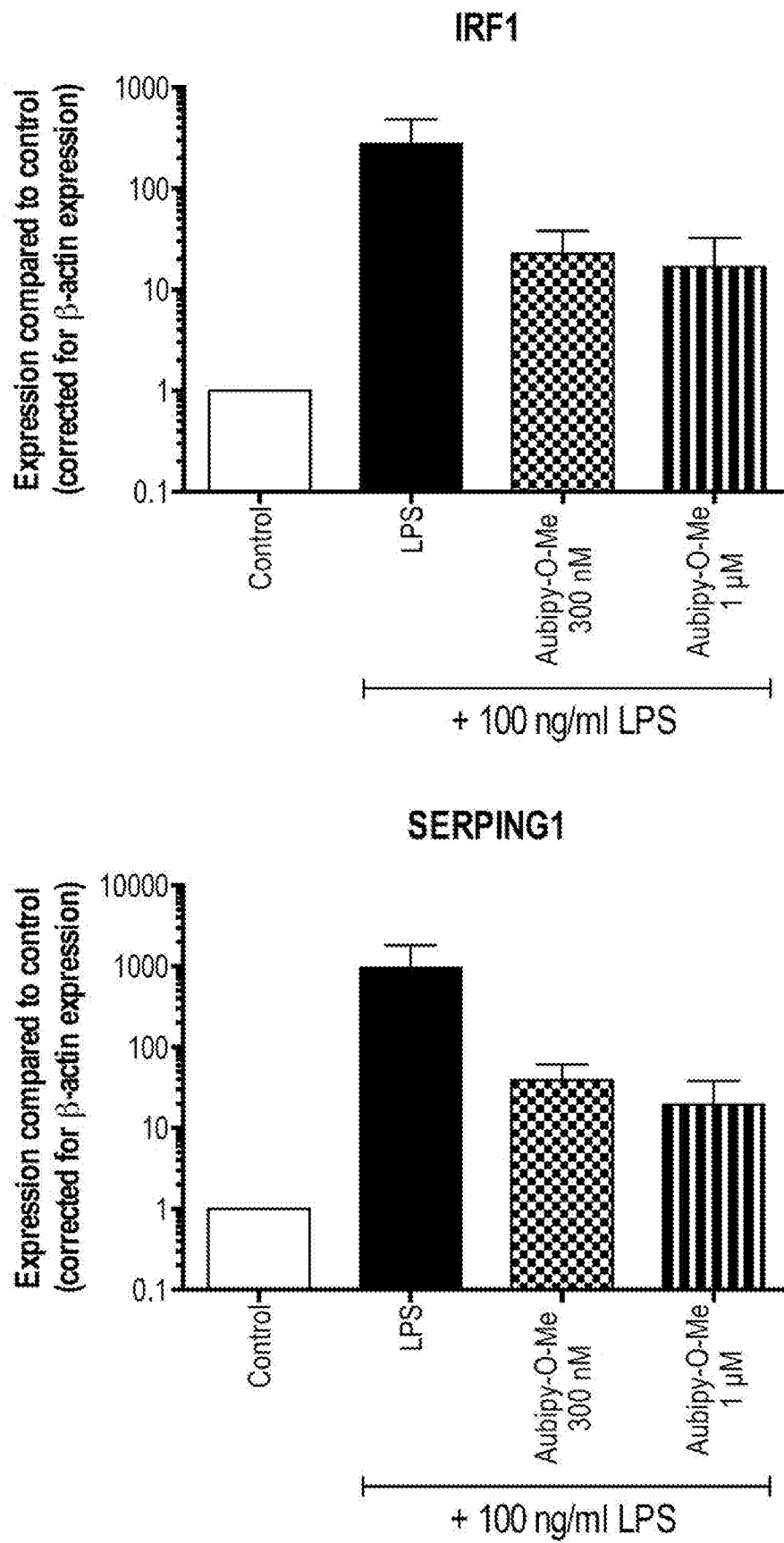


Fig. 8

9/12

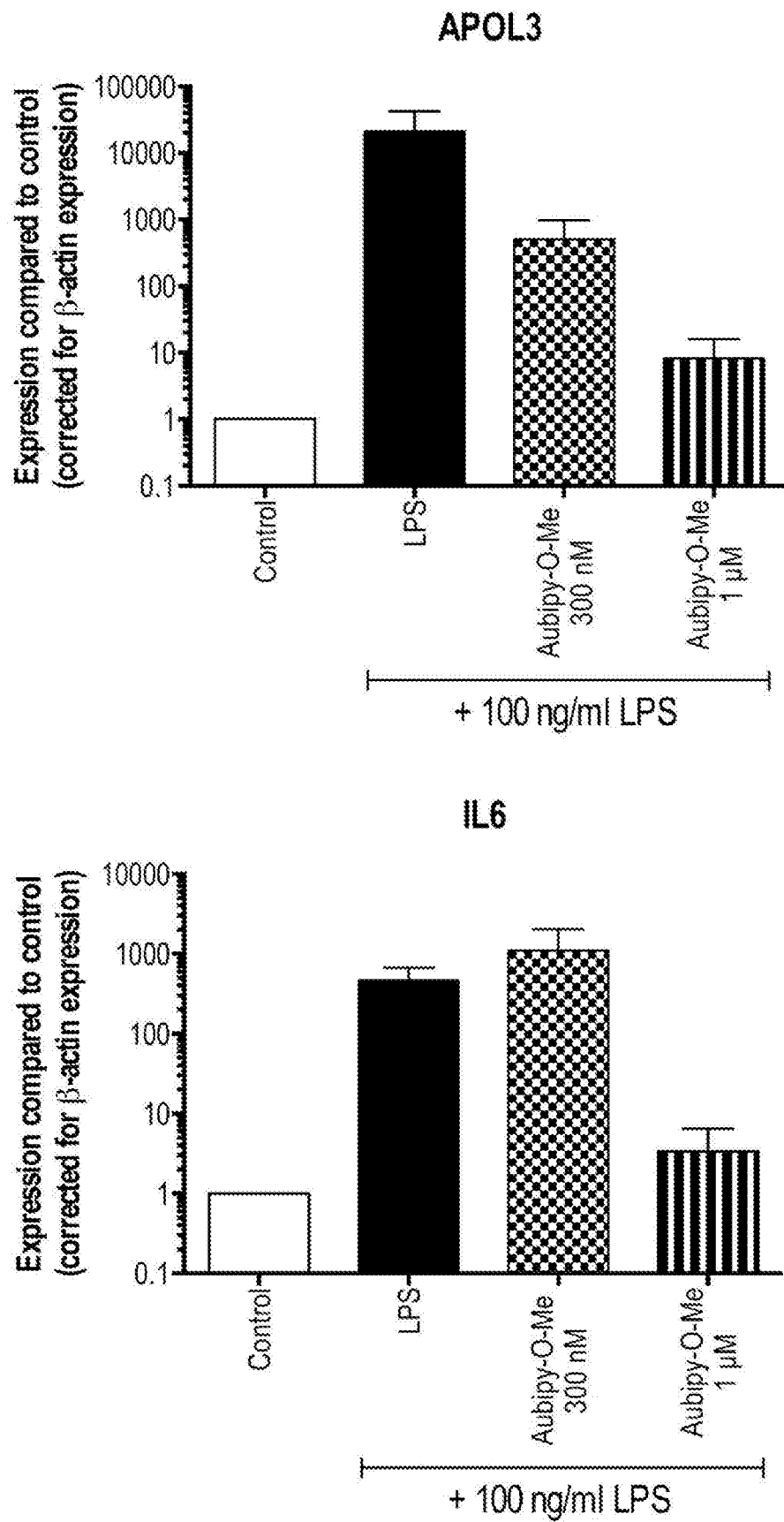


Fig. 8, contd.

10/12

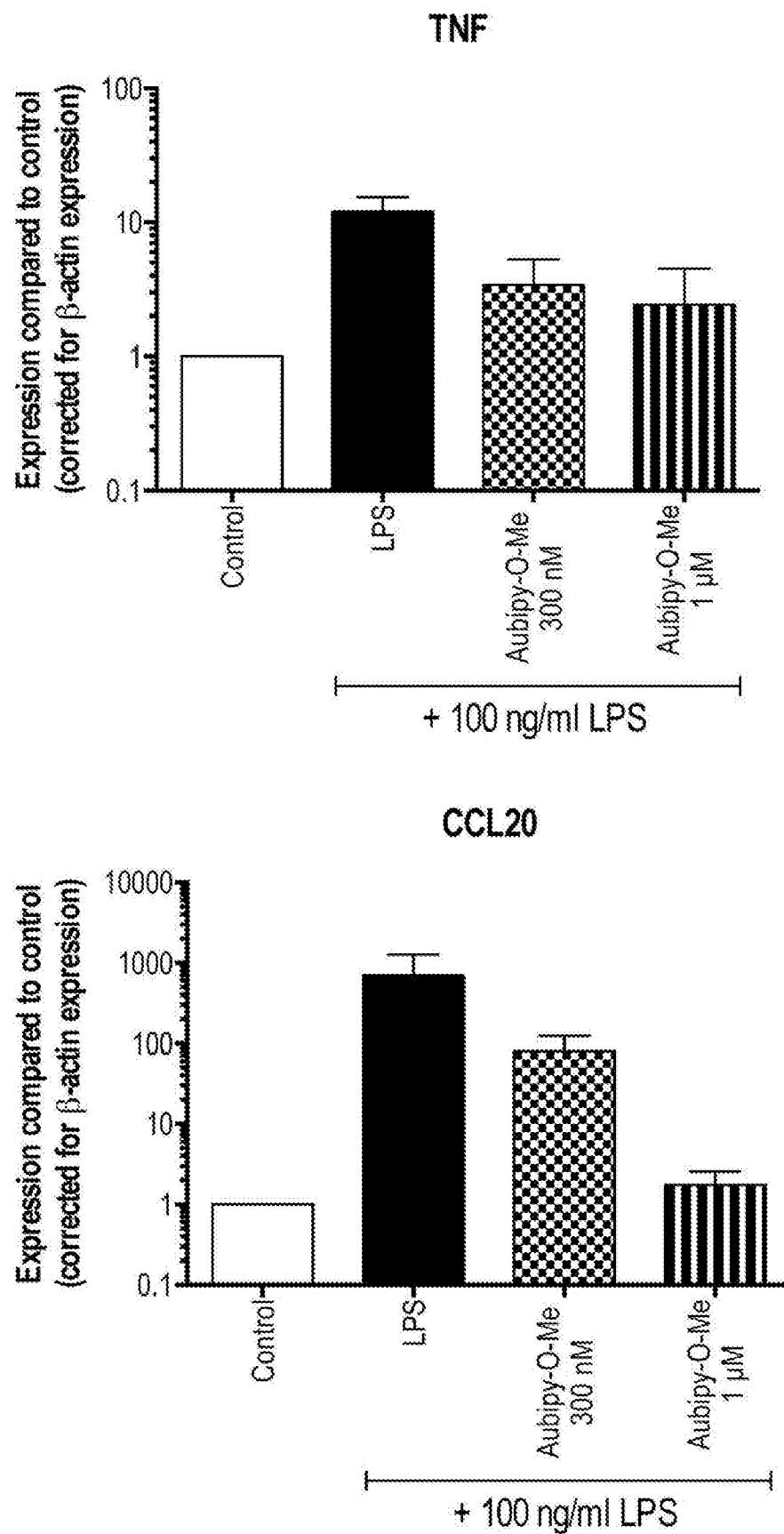


Fig. 8, contd.

11/12

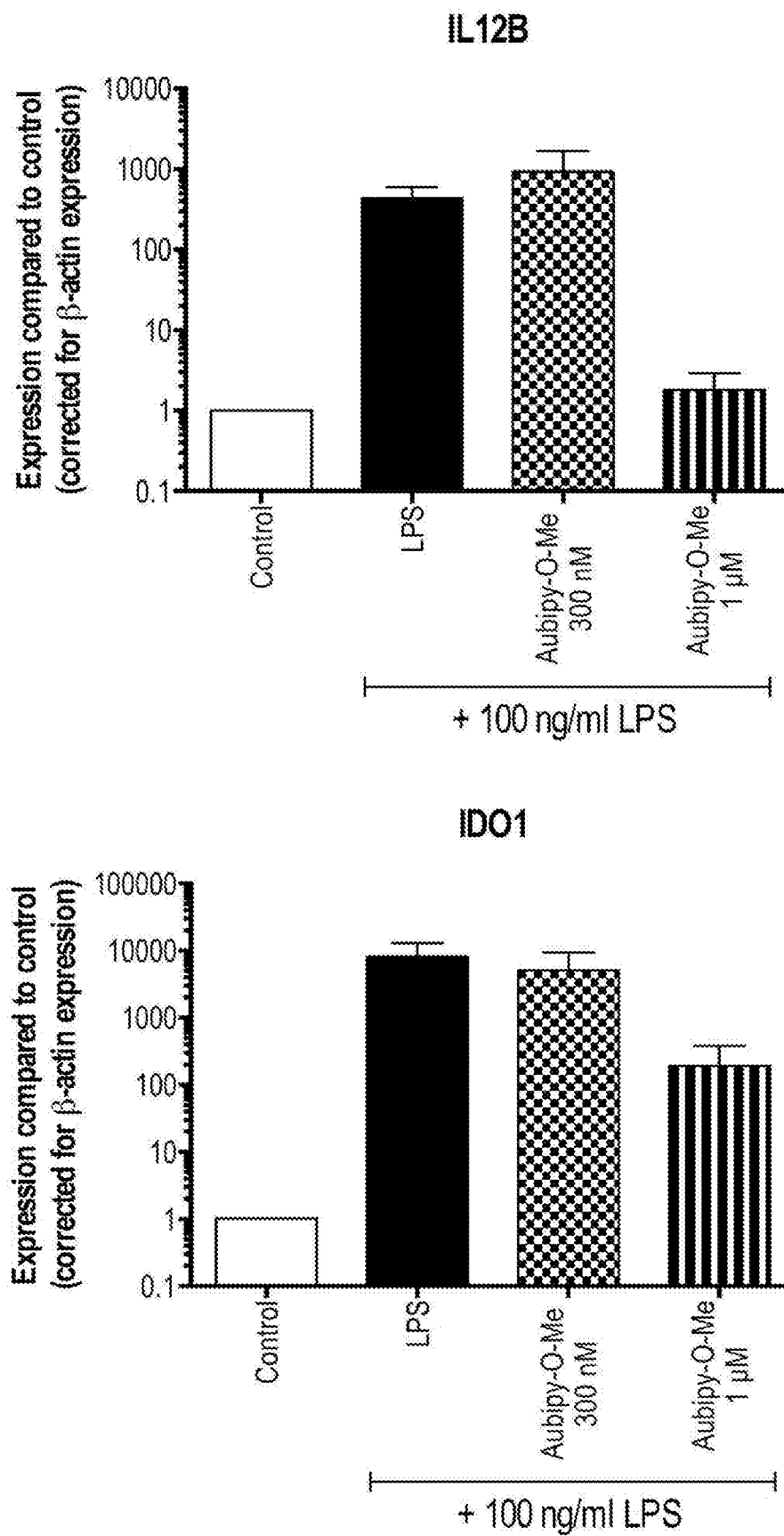


Fig. 8, contd.

12/12

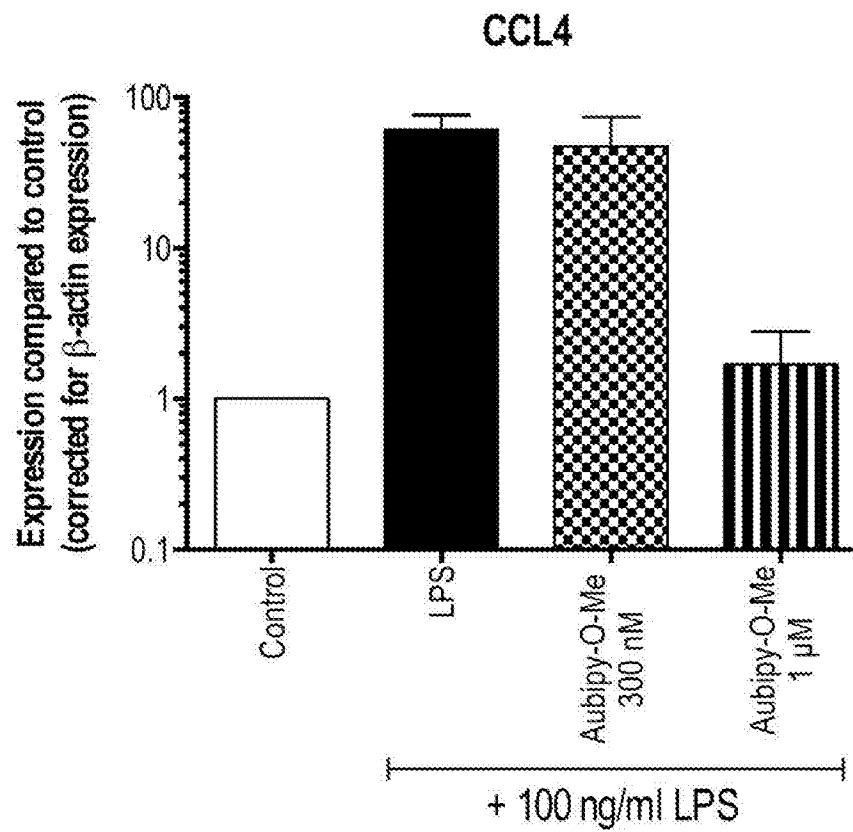


Fig. 8, contd.

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2016/050666

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/44 A61K31/4439 A61K31/444 A61K31/4745 A61P11/00
A61P31/12 A61P35/00

ADD.

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)

A61K

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)

EPO-Internal, CHEM ABS Data, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/005170 A2 (UNIV LISBOA [PT]; TAVARES REBELO DE SOVERAL RODRIGUES MARIA DA GRACA []) 10 January 2013 (2013-01-10) cited in the application claims 1-17 figure 1; compound auphen page 19, last paragraph - page 25 ----- -/--	1-27

☒ Further documents are listed in the continuation of Box C.

☒ 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

12 December 2016

Date of mailing of the international search report

16/12/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Authorized officer

Renard, Delphine

INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2016/050666

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANGELA CASINI ET AL: "Chemistry, antiproliferative properties, tumor selectivity, and molecular mechanisms of novel gold(III) compounds for cancer treatment: a systematic study", JBIC JOURNAL OF BIOLOGICAL INORGANIC CHEMISTRY, SPRINGER, BERLIN, DE, vol. 14, no. 7, 20 June 2009 (2009-06-20), pages 1139-1149, XP019742548, ISSN: 1432-1327, DOI: 10.1007/S00775-009-0558-9 figure 1; compounds C1-3 table 2 "in vitro antitumor activity and tumor selectivity"; page 1144 - page 1146 -----	1-7, 9-11,17, 20,21, 25-27
X	YANLI WANG ET AL: "Interaction of the Human Prion Protein PrP106-126 with Metal Complexes: Potential Therapeutic Agents Against Prion Disease", CHEMISTRY - A EUROPEAN JOURNAL., vol. 16, no. 45, 3 December 2010 (2010-12-03), pages 13339-13342, XP055294670, WEINHEIM, DE ISSN: 0947-6539, DOI: 10.1002/chem.201002207 compound B and D page 13340, right-hand column, last paragraph page 13341, right-hand column, last paragraph -----	1-7,17, 20,21, 25-27
X	AMANI VAHID ET AL: "Synthesis and characterization of a series of gold(III) complexes with the 4,4'-dimethyl-2,2'-bipyridine ligand: Counterion influence on the cytotoxicity of gold(III) c", POLYHEDRON, vol. 79, 9 May 2014 (2014-05-09), pages 104-115, XP028879659, ISSN: 0277-5387, DOI: 10.1016/J.POLY.2014.04.064 figure scheme 1 3.6 in vitro anticancer properties; table 6 ----- -/--	1-7,20, 21,25-27

INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2016/050666

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANGELA CASINI ET AL: "Synthesis, characterisation and biological properties of gold(iii) compounds with modified bipyridine and bipyridylamine ligands", DALTON TRANSACTIONS: THE INTERNATIONAL JOURNAL FOR INORGANIC, ORGANOMETALLIC AND BIOINORGANIC CHEMISTRY, vol. 39, no. 9, 1 January 2010 (2010-01-01), page 2239, XP055294487, GB ISSN: 1477-9226, DOI: 10.1039/b921019a table 2; compounds 1-4 abstract -----	1-8,20, 21,25-27
X	DAMING FAN ET AL: "Chemical and biological studies of the dichloro(2-phenylpyridine) gold(III) complex and its derivatives", DALTON TRANSACTIONS: THE INTERNATIONAL JOURNAL FOR INORGANIC, ORGANOMETALLIC AND BIOINORGANIC CHEMISTRY, no. 13, 1 January 2003 (2003-01-01), pages 2680-2685, XP055294643, GB ISSN: 1477-9226, DOI: 10.1039/B303297C compounds 1-2 abstract table 5 -----	1-4,7, 20,21, 25-27
X	M. ARSENIJEVIC ET AL.: "Cytotoxicity of gold(III) Complexes on A549 Human Lung CarcinomaEpithelial Cell Line", MEDICINAL CHEMISTRY, vol. 8, 2012, pages 2-8, XP002760676, figure 1; compound [Au(bipy)Cl₂]⁺ 4. discussion; figures 4-5 -----	1-7,20, 21,25-27
X	MORORE MPHAHLELE ET AL: "Modification of HIV-1 reverse transcriptase and integrase activity by gold(III) complexes in direct biochemical assays", BIOORGANIC & MEDICINAL CHEMISTRY, PERGAMON, GB, vol. 20, no. 1, 25 October 2011 (2011-10-25), pages 401-407, XP028354070, ISSN: 0968-0896, DOI: 10.1016/J.BMC.2011.10.072 [retrieved on 2011-11-03] table 2; compounds 1-2 abstract ----- -/--	1,3-7, 20,21, 25-27

INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2016/050666

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PENGFEI SHI ET AL: "DNA binding properties of novel cytotoxic gold(III) complexes of terpyridine ligands: the impact of steric and electrostatic effects", JBIC JOURNAL OF BIOLOGICAL INORGANIC CHEMISTRY, SPRINGER, BERLIN, DE, vol. 11, no. 6, 9 June 2006 (2006-06-09), pages 745-752, XP019431101, ISSN: 1432-1327, DOI: 10.1007/S00775-006-0120-Y figure 1; compounds 1-4 abstract concluding remarks; page 751 -----	1-3,9, 10,20, 21,25-27
X	PALANICHAMY K ET AL: "Synthesis, characterization, and aqueous chemistry of cytotoxic Au(III) polypyridyl complexes", INORGANICA CHIMICA ACTA, ELSEVIER BV, NL, vol. 359, no. 1, 1 January 2006 (2006-01-01), pages 44-52, XP028069035, ISSN: 0020-1693, DOI: 10.1016/J.ICA.2005.08.030 [retrieved on 2006-01-01] abstract table 3; compounds 1-4 -----	1-3,12, 13,20, 21,25-27
X	MARIA SERRATRICE ET AL: "Synthesis, Structural Characterization, Solution Behavior, and in Vitro Antiproliferative Properties of a Series of Gold Complexes with 2-(2'-Pyridyl)benzimidazole as Ligand: Comparisons of Gold(III) versus Gold(I) and Mononuclear versus Binuclear Derivatives", INORGANIC CHEMISTRY, vol. 51, no. 5, 5 March 2012 (2012-03-05), pages 3161-3171, XP055294488, EASTON, US ISSN: 0020-1669, DOI: 10.1021/ic202639t figure chart I; table 3; compounds 1-2 abstract antiproliferative properties of study compounds; page 3166 - page 3167 ----- -/--	1-3, 14-16, 20-27

INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2016/050666

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SERRATRICE MARIA ET AL: "Cytotoxic gold compounds: synthesis, biological characterization and investigation of their inhibition properties of the zinc finger protein PARP-1", DALTON TRANSACTIONS: THE INTERNATIONAL JOURNAL FOR INORGANIC, ORGANOMETALLIC AND BIOINORGANIC CHEMISTRY; [6015A], ROYAL SOCIETY OF CHEMISTRY, GB, vol. 41, no. 11, 1 January 2012 (2012-01-01), pages 3287-3293, XP009191321, ISSN: 1477-9226 abstract chart 1 : first compound of the second line; compound 1a(auIII) table 1 Synthesis of 1aAu(III); page 3290 -----	1-3,14, 15,20-27
X	US 2015/125550 A1 (EICHLER JACK F [US] ET AL) 7 May 2015 (2015-05-07) claims 1-22 table 3 3.3 glutathione stability and in vitro antitumor activity paragraph [0033] examples 1-2 -----	1-3,17, 20,21, 25-27
A	Alison R Hayman ET AL: "Tartrate-resistant acid phosphatase: a potential target for therapeutic gold", cell biochemistry and function Cell Biochem Funct, 21 June 2014 (2014-06-21), pages 275-280, XP055294729, DOI: 10.1027/cbf.1133 Retrieved from the Internet: URL:http://onlinelibrary.wiley.com/store/10.1002/cbf.1133/asset/1133ftp.pdf?v=1&t=iroq94t7&s=20a4aa035117c3573ac87ed72e393b482637c854 [retrieved on 2016-08-10] the whole document -----	1-27

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/NL2016/050666

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013005170 A2	10-01-2013	PT 105786 A WO 2013005170 A2	04-01-2013 10-01-2013
US 2015125550 A1	07-05-2015	NONE	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL2016/050666

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.:
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:
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:

see additional sheet

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 an 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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-27

Present set of claims

1.1. claims: 1-20, 22-24

Gold (III) derivatives of formulae A-E for use in the treatment of the symptoms of respiratory disorders such as asthma, COPD, smoked-induced inflammation and sarcoidosis

1.2. claims: 21, 25-27

Pharmaceutical composition and medical device comprising the gold (III) derivatives of formulae A-E
