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(54) Title: AMINE DERIVATIVES AS IL-15 ACTIVITY INHIBITORS

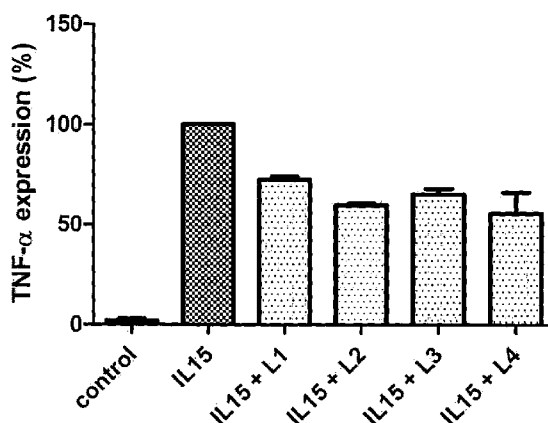


Fig. 2. Effect of the compounds L1, L2, L3 and L4 (200 μ M) on IL-15-induced TNF- α synthesis in

(57) Abstract: The present invention relates to the benzoic acid derivatives of the formula (I), as described in the claims, their tau-tomers, isomers, pharmaceutically acceptable salts and solvates, for the use in the treatment of diseases related to interleukin 15 overproduction.

944

Amine derivatives as IL-15 activity inhibitors

5 Field of the invention

The present invention relates to the specific amine derivatives for use in the treatment of the diseases related to interleukin 15 overproduction. The compounds of the formula (I) or (II), as described hereinafter, binding to interleukin 15 effectively inhibit its binding to the specific IL-15R α receptor, and hence they can be used in the treatment of the inflammatory related diseases, such as rheumatoid arthritis.

Background of the invention

Interleukin 15 (IL-15) is the cytokine exerting pleiotropic activity towards immune system cells as well as other cell types. IL-15 exhibits broad spectrum bioactivity, therefore it is placed at the top of the pro-inflammatory cytokines cascade. The impairment of the mechanisms regulating the expression of the IL-15 results in the overproduction of this cytokine and contributes directly to the development of such pathologies as inflammatory processes, autoimmune diseases, infections and neoplastic changes. IL-15 is considered a crucial cytokine in the etiology of rheumatoid arthritis (McInnes I.B. et al., Nat. Med. 2, 175-82 (1996); McInnes I.B. et al., Nat. Med. 3, 189-95 (1007); McInnes I.B. et al., Immunol Today 19, 75-9 (1998), psoriasis (Villadsen L.S. et al., J. Clin. Invest. 112, 1571-80 (2003), inflammatory bowel disease (Kirman I, Nielsen O.H., Am. J. Gastroenterol 91, 1789-1794 (1996); Sakai T. et al., Gastroenterology 114, 1237-1243 (1998)), sarcoidosis (Agostini C.T.L. et al., J. Immunol. 157, 910-8 (1996)) and T-cell leukemias (Dobbeling U. et al., Blood 92, 252-8 (1998)). Much attention is given to IL-15, on account of this cytokine's participation in transplant rejection (Baan C.C. et al., Transplant Proc. 31, 2726-8 (1999); Lewis E.C. et al., Cytokine 34, 106-13 (2006); Shi R. et al., Transpl. Immunol. 12, 103-8 (2004); Ferrari-Lacraz S. et al., Transplantation 82, 1510-7 (2006); Zheng X.X. et al., Transplantation 81, 109-16 (2006)).

Significant involvement of IL-15 in the development of the pathogeneses of the aforementioned afflictions suggests that targeting this cytokine pathway can reduce or eliminate the occurrence of IL-15 related disorders. This strategy has been proved lately by a number of biological assays. Reduction of the IL-15-mediated biological effects using the soluble receptor IL-15R α (Liew F.Y., McInnes I.B., Ann. Rheum. Dis. 61 Supl. 2, ii100-2 (2002); Ruchatz H. et al., J Immunol. 160, 5664-60 (1998); Smith X.G. et al., J. Immunol. 165, 3444-50 (2000); Wei X et al., J. Immunol. 167, 277-82 (2001)), antibodies inhibiting receptor IL-2/IL-15R β (Morris J.C., Proc. Natl. Acad. Sci. USA 103, 401-6 (2001); Tinubu S.A. et al., J. Immunol. 153, 4330-8 (1994), antibodies inhibiting IL-15

(Villadsen L.S. et al. J. Clin. Invest. 112, 1571-80 (2003) or modified IL-15 molecule of competitive antagonist activity (Ferrari-Lacraz S. et al., J. Immunol. 173, 5818-26 (2004); Kim Y.S. et al., J. Immunol. 160, 5742-8 (1998)) always resulted in the alleviation of disease symptoms. Experimental therapies demonstrated decreased collagen-induced rheumatoid arthritis incidence in mice (Ruchatz H. et al., J. Immunol. 160, 5664-60 (1998); Ferrari-Lacraz S. et al., J. Immunol. 173, 5818-26 (2004); Kim Y.S. et al., J. Immunol. 160, 5742-8 (1998)) and primates (Liew F.Y., McInnes I.B., Ann. Rheum. Dis. 61 Supl. 2, ii100-2 (2002)), attenuation of psoriasis symptoms in the mice model of the disease (Villadsen L.S. et al., J. Clin. Invest. 112, 1571-80 (2003)), reduction of carrageenan-induced inflammation in mice (Wei X et al., J. Immunol. 167, 277-82 (2001)) and, also in mice, prolonged survival of heart allotransplants (Smith X.S. et al., J. Immunol. 165, 3444-50 (2000); Tinubu S.A. et al., J. Immunol. 153, 4330-8 (1994) and islets of Langerhans (Ferrari-Lacraz S. et al., J. Immunol. 173, 5818-26 (2004)). Currently adopted strategies, based on inhibiting the IL-15, seem to be effective, but none of them has been approved for clinical use so far. The most advanced and promising, from the medical application point of view, are the trials aimed at the inhibition of the IL-15 activity by anti-IL-15 human antibodies (HuMax-IL15, AMG-714) (Baslund B. et al., Arthritis Rheum 52, 2686-92 (2005)). However, the matter of serious concern is the effect of 'reverse signaling' (Budagian V. et al., J. Biol. Chem. (2004)). It is assumed that the antibody HuMax-IL15 and IL-15 complex can itself induce a cell response, thus limiting the therapeutic effectiveness of this antibody directed toward IL-15 neutralization (Budagian V. et al. Cytokine Growth Factor Rev. 17, 259-80 (2006).

In addition, our unpublished results demonstrate significant angiogenic activity of IL-15. The participation of IL-15 in angiogenesis *in vivo* has already been reported (Angiolillo A.L. et al., Biochem. Biophys. Res. Commun. 233, 231-7 (1997); Kuniyasu H. et al., Pathobiology 69, 86-95 (2001)), but our recent outcomes have shown the IL-15-induced proliferation and migration of endothelial cells. The identification of another angiogenic factor was not surprising, because its presence had already been detected in rheumatoid arthritis, where the impaired mechanism of angiogenesis is considered to be the main pathogenesis of this illness.

Rheumatoid arthritis (RA) is the most common systemic disease of connective tissue which affects on average about 1% of the world's population. Recent estimates showed higher prevalence in women than in men (3:1). The highest prevalence of RA has been reported in the 30-60 age range. According to the estimates, about 30% of people afflicted with RA suffer from severe symptoms and within several years end up with a disability. The average life span of people suffering from RA is about 10 years shorter, according to the statistical data. It is thought that the onset of RA results from the complex combination of many factors, such as genetic predisposition, impaired innate and acquired immune response and environmental components. Pharmacological treatment of RA, currently available on the medical market, does not remove the cause of the disease. Non-steroidal

anti-inflammatory drugs (NSAIDs) are medications which as well as having pain-relieving (analgesic) effects have the effect of reducing the inflammation when used over a period of time. They suppresses the symptoms of the disease, but do not stop the progress of the illness.

Among the disease-modifying antirheumatic drugs (DMARDs), the first line treatment in RA is methotrexate. The other synthetic DMARDs are leflunomide, sulfasalazine, hydroxychloroquine, D-penicillamine, gold salts, azathioprine, cyclosporine and cyclophosphamide. However, even in patients responding to the treatment, the disease progresses and diminished medical efficacy is observed after long term treatment.

The other class of DMARDs are biological. Among the drugs available on the pharmaceutical market, the inhibitors of the tumor necrosis factor (TNF) could be mentioned:

- infliximab (chimeric anti-TNF monoclonal antibody);
- etanercept (fusion protein, consisting of the extracellular receptor domain p75 for TNF and the Fc fragment of the human antibody IgG1);
- adalimumab (human anti-TNF monoclonal antibody),

as well as the inhibitors of other proteins:

- anakinra (IL-1 receptor antagonist);
- abatacept, fusion protein composed of the Fc region of the immunoglobulin IgG1 fused to the extracellular domain of CTLA-4. By binding co-stimulating molecules B7-1 and B7-2 located on the antigen-presenting cells it inhibits the co-stimulatory signal transduction by CD28 on T cells;
- rituximab, chimeric monoclonal antibody against the protein CD20 primary found on the surface of mature B cells acting by eliminating B cells.

Tocilizumab, humanized monoclonal antibody against the interleukin-6 receptor, has been approved for the European pharmaceutical market as the first IL-6 inhibitor.

Positive results of phase II clinical trials carried out by Amgen company with AMG-714 (previously HuMax-IL15) - human monoclonal antibody that targets IL-15, were disclosed in 2004 (McInnes, I., et al.). However, to date there is no available information about the phase III clinical trials.

The introduction of biologic medical products into rheumatoid arthritis therapy is considered big progress in the RA treatment, but these biologics (usually used in combination with methotrexate of cytostatic and immunosuppressive properties) are only effective in limiting the disease symptoms and delaying joint degradation in about 30% of treated patients.

Due to the limited therapeutic effects and high production costs of currently available biopharmaceuticals, the demand for an efficacious anti-RA drug of new generation still remains valid. Ongoing research is aimed at discovering new molecular mechanisms for targeted therapies.

Peptides of modified sequences mimicking IL-15 (revealed, among others, in WO 2006/029578 and WO 2010/037351) have been proposed as potential pharmaceuticals in the rheumatoid arthritis treatment. These peptides are supposed to bind to the receptor IL-15R α subunit, which should result in the inhibition of the T cells proliferation, TNF- α induction and expression of IL-8 and IL-6. To date the efficacy of these new compounds has not been proved in clinical studies.

A need to design a new drug based on a small synthetic molecule inspired the authors of the present invention to alter the concept of the treatment of the IL-15 overexpression related diseases. This approach relates to the inhibition of the IL-15 biological activity by a compound selectively binding to IL-15R α receptor. According to the current state of knowledge and our own unpublished results, the strategy, while implemented, should reduce the pro-inflammatory cascade as well as angiogenesis caused by the pro-inflammatory cytokine IL-15. The effect of additive synergism is likely to be a so far unrecognized mechanism of biologicals inhibiting IL-15 biological activity.

Phenylpyrazole anilide derivatives, disclosed by Ushio H. et al. in Letters in Drug Design Discovery, 5, 292-296 (2008), are the only small chemical molecules of potential application in rheumatoid arthritis which interfere with the interaction of the receptor IL-15R α and its ligand (IL-15). The compound Y-320 belonging to the aforementioned group of compounds with proven high bioavailability and *in vitro* activity inhibits the IL-15-induced T cells activity.

The present invention relates to the discovery of a compound which will fulfill the criteria of Lipinski's rules for drug candidates. The compound is expected to block the interaction between IL-15 and its specific IL-15R α receptor by binding to the IL-15. Thus, it could be expected the compound will selectively and effectively inhibit the biological activity of this cytokine.

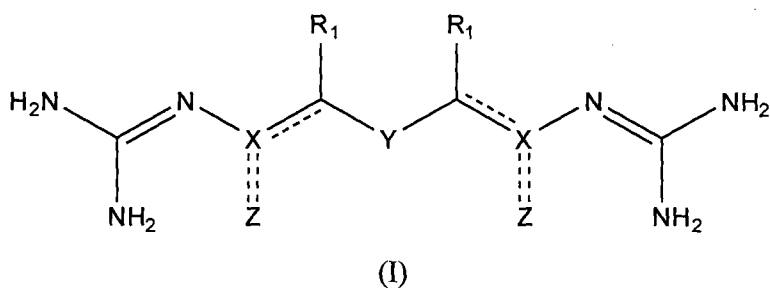
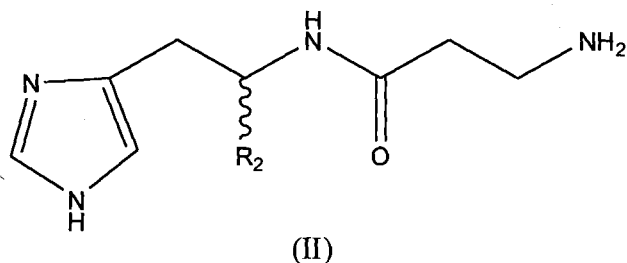
Summary of the invention

Identification of a chemical compound which will fit in IL-15R α receptor was facilitated due to evaluation of the receptor domain structure, which is of a key importance while binding to IL-15 (Wei X. et al., J. Immunol. 167, 277-82 (2001)). The search for small chemical molecules to fit in IL-15 receptor domain was carried out on the basis of the model of [IL-15R α – IL-15] complex structure, elaborated on the basis of the crystal structure of the complex fragment (2Z3Q code of Protein Data Bank). The assignment of receptor binding sites pharmacophores, considering specific interactions with IL-15, was enabled due to determination of the 3D structure of [IL-15R α – IL-15] complex. It was followed by search of small molecules data base ZINC, embracing about 20 million chemical compounds, to find the molecules, which can meet the structure requirements for the pharmacophores. The screening included more than 10 thousand compounds fulfilling the

established criteria. The calculations were also carried out to predict theoretically binding affinity and strength of the selected compounds and the receptor active site. Molecular docking of the chosen molecules was performed using GLIDE software. While docking, IL-15 binding site of the receptor remained rigid, but the molecule was able to undergo conformation changes due to modifications of rotating chemical bonds. Additional docking carried out for the pre-selected 500 molecules, allowed induction fitting in of the two interacting partners (protein receptor and small molecule compound).

Using described above screening methods, the group of compounds has been selected, which blocking IL-15 inhibit effectively binding IL-15 to its specific receptor IL-15R α . This biological activity of the selected compounds has been proved *in vitro* studies.

The present invention provides the compounds of the formula (I) or (II)



wherein

X represents C or N,

Y represents CH₂ or NH,

R₁ represents H or CH₃,

R₂ represents H for COOH,

----- represents single or double bond,

===== represents double bond or lack of bond,

providing that

when ----- is single bond, than ===== is double bond and Z represents O,

and their tautomers, isomers, pharmaceutically accepted salts and solvates,

for the use in the treatment of the diseases related to interleukin 15 overproduction.

The *in vitro* biological activity studies of the abovementioned compounds demonstrated the efficacious inhibition of the IL-15-induced cell proliferation and TNF- α and IL-17 expression by the amine derivatives of the formula (I) and (II).

5 The amine derivatives of the formula (I) or (II) could be used to inhibit IL-15 overproduction due to inhibition of IL-15 binding to its specific IL-15R α receptor.

In particular, the compounds of the formula (I) or (II) can be used in prevention and therapeutic treatment of the diseases from the group including rheumatoid arthritis, psoriasis, inflammatory bowel disease, sarcoidosis, T-cell leukemias or transplant rejection.

10 Most preferably, the amine derivatives of the formula (I) or (II) can be used in treatment of rheumatoid arthritis.

The amine derivatives of the formula (I) or (II) according to the present invention can be administered to the individual in therapeutically effective doses to an individual in need of such a treatment, in particular to human.

15 Although the administration of the compounds of the formula (I) or (II) *per se* is considered, in general, they will be used in the pharmaceutical preparations, suitable for particular routes of administration.

Another embodiment of the present invention relates to the use of the compounds of the formula (I) or (II), their tautomers, isomers, pharmaceutically acceptable salts or solvates for manufacturing
20 pharmaceutical preparations used in prevention or treatment of the diseases, such as rheumatoid arthritis, psoriasis, inflammatory bowel disease, sarcoidosis, T-cell leukemias or transplant rejection.

Next embodiment of the present invention relates to the pharmaceutical preparation consisting of therapeutically effective dose of the compound of the formula (I) or (II), or its tautomer, isomer,
25 pharmaceutically acceptable salt or solvate as the active ingredient and pharmaceutically acceptable carriers and/or excipients.

Description of the invention

Enamines of the formula (I) are susceptible to tautomerisation, forming appropriate imines or
30 remaining in imine-enamine equilibrium. Different types of conjugations can occur, due to the presence of additional nitrogen atoms or carbonyl groups in the side chain of the molecule.

In the compounds of the formula (II) stereogenic center can be present, therefore these compounds can exist as different optical isomers or the mixtures thereof at different ratios, for example, as the racemic mixtures.

35 All the tautomers, isomers and mixtures thereof are the subject matter of the present invention. Single optical isomers can be obtained according to the methods known to those who are skilled in the art, like for example, chiral HPLC, enzymatic or chiral auxiliary separation, or they can be

obtained synthetically under stereocontrolled conditions.

In one embodiment, the compounds to be used according to the present invention are aminoimine derivatives of the formula (I),

wherein

5 X represents C,

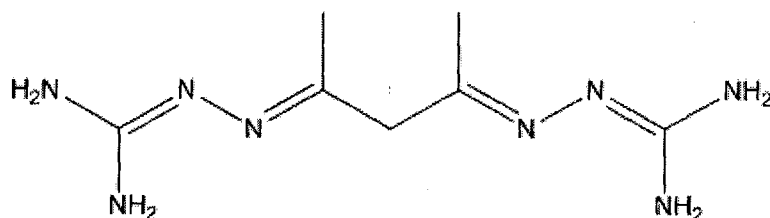
Y represents NH,

R₁ represents H,

-----represents single bond,

=====represents lack of bond, which is the compound

10



at any possible tautomeric form.

In the next embodiment, the compounds to be used according to the present invention are aminoimine derivatives of the formula (I), wherein:

15 X represents N,

Y represents CH₂,

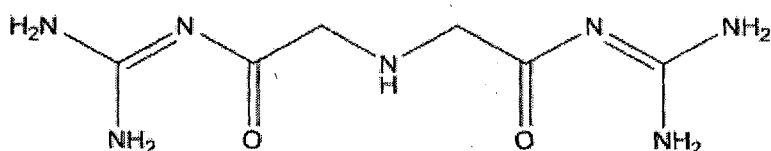
R₁ represents CH₃,

-----represents single bond,

=====represents double bond, and

20

Z represents O, which is the compound

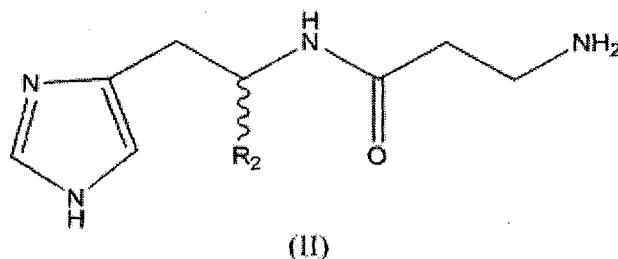


at any possible tautomeric form.

25

In the another embodiment, the compounds to be used according to the present invention are the substituted β -alanine derivatives of the formula (II),

8



wherein:

R_2 represents H or CH_3 , and their optical isomers, in particular β -alanyl-D-histidine and β -alanylhistamine

The compounds according to the present invention, exerting the highest biological activity are the derivatives of the formula (I), selected among 2,2'-imino-bis[(N-(aminoiminomethyl)acetamide] and 2,2'-((1*E*,3*E*)-1,3-dimethyl-1,3-propane-1,3-diylidene)-bis-carbaimidoylhydrazine as well as the derivatives of the formula (II) selected among β -alanyl-D-histidine and β -alanylhistamine.

The compounds of the formula (I) and (II) are commercially available or they can be obtained synthetically, according to the methods described for the similar compounds.

The compounds of the formula (I) and (II) can be obtained and/or used as pharmaceutically acceptable salts with acids. The wording 'pharmaceutically acceptable salts' represents salts formed with pharmaceutically acceptable inorganic and organic acids. They can be selected from the group comprising, for example, hydrochloric acid, hydrobromic acid, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, adipic, ascorbic, salicylic, succinic, tartaric, acetic, citric, formic, benzoic, malic, p-toluenesulfonic, methanesulfonic, naphthalene-2-sulfonic, benzenesulfonic acid and others. Most preferably, hydrochlorides are the salts of the derivatives of the formula (I) and (II).

As it was said before, the derivatives of the formula (I) or (II) can be used *per se* or in a combination with the other active ingredients at effective therapeutic doses, in treatment of the diseases or disorders, which have been diagnosed or they are likely to be related to IL-15 overproduction.

The term "treatment" refers to suppression of state, disorder or disease, that means inhibition, reduction or delay of the disease development, recurrence of the illness or at least one of its symptoms, or recovery, which means, regression of state, disorder or disease or at least one of its symptoms.

'Therapeutically effective dose' refers to an amount of the compound, which is sufficient to produce therapeutic response when administered to the individual to cure state, disorder or disease.

'Therapeutically effective dose' will vary, depending on the nature of the selected compound and a route of administration, kind of disease and its status, age, body weight, physical state, susceptibility to treatment and it can be recommended by the medical doctor on the basis of his own experience

and results of the clinical trials.

Therapeutic dose of the derivatives of the formula (I) or (II) can be administered as a single dose or divided doses given in the certain intervals of time, for example as two, three, four or more daily doses.

5 To produce particular pharmaceutical form, pharmaceutical preparation, except for active ingredient, may also contain known and pharmaceutically acceptable carriers and/or excipients, which are inert and do not interact with the active ingredient.

10 Pharmaceutical composition, according to the invention, may be formulated in any suitable pharmaceutical form for systemic administration, for example oral administration, such as tablets and capsules, starch capsules, coated tablets or enteral tablets; as powders or granules; as solution, suspension or emulsion. Tablets and capsules for oral administration may contain excipients routinely used in pharmaceutical practice, such as binders, diluents, disintegrants or lubricants. The tablets may be coated by any method known in the art. Liquid pharmaceutical compositions for oral administration may be manufactured as, for example, aqueous or oily suspensions, solutions, 15 emulsions, syrups and elixirs or they may be produced as dry substances for preparation of solutions or suspensions *ex tempore* with water or other suitable diluent. The liquid pharmaceutical preparations may contain, routinely used in pharmaceutical practice excipients, such as dispersing and emulsifying agents, non-aqueous carriers (they may comprise eatable oils) or preservatives. The selection and amount of the excipients depends on pharmaceutical dosage form and route of drug administration. Pharmaceutical composition may be formulated into any suitable pharmaceutical form, by any method known in the art, using any pharmaceutically acceptable carriers, diluents, fillers and other excipients. 20

Pharmaceutical preparation for oral administration may be formulated, in particular, as capsules. In such a case, the active substance is combined with the carrier and the resulting 25 composition is used to fill in the gelatin shells. The gelatin capsules can be manufactured as having soft or hard gelatin shells, depending on the composition of gel mass used in a production process. Soft capsules' gelatin mass consists of plasticizers, such as glycerol, sorbitol; preservatives, such as benzoic acid, its salts and alkyl hydroxybenzoates; colorants and flavors. The capsules' filling may be used as oily solution, suspension or emulsion. The suitable diluents embrace, for example, castor oil, 30 coconut oil, olive oil, palm oil, corn oil, arachis oil, synthetic and natural triglycerides of fatty acids, unsaturated medium-chain fatty acids, modified long-chain fatty acids, glycol esters, polyethylene glycols and others. Suitable excipients comprise tensides, for example, lecitin, mono- and diglycerides, fatty acids esters with polyoxoethylene sorbitane.

35 Pharmaceutical preparation suitable for parenteral administration, for example, for intramuscular, subcutaneous or intravenous administration, may be used as ready suspension, lyophilizate, suspension *ex tempore* or concentrate for intravenous infusions. These preparations may be formulated as unit dosage forms in ampoules, initially filled syringes, low capacity infusions, or in multi-dosage containers, consisting of preservatives and carriers, diluents, stabilizers and/or

dispersing agents. The carriers suitable for intravenous administration of pharmaceutical preparation comprise, for example, sterile aqueous solutions, such as the solution of physiological salt, the solutions of carbohydrates, for example, glucose, mannitol, dextrose, lactose and aqueous buffer solutions, for example, phosphate buffers. The pharmaceutical composition may also contain other
5 excipients, routinely used to maintain isoosmoticity, antioxidants, preservatives and the others. Alternatively, the active ingredient may be used as a powder obtained due to isolation of the solid compound under septic conditions, or by lyophilisation from the solvent, for preparation of suspensions *ex tempore* in a suitable diluent, for example sterile, deprived of pyrogenic substances water.

10 The derivatives of the formula (I) and (II) have demonstrated biological activity *in vitro* studies. When efficacy of the compounds is being proved *in vivo* experiments in the animal models of the diseases, these derivatives can be potential drug candidates used in protection and treatment of the diseases and inflammatory processes, which are related to IL-15 overproduction, such as rheumatoid arthritis, psoriasis, inflammatory bowel disease, sarcoidosis, T-cell leukemias or
15 transplant rejection.

Biological studies

20 *in vitro* Studies

All the experiments were carried out on peripheral blood mononuclear cells (PBMC) from healthy donors. The cells were isolated under standard protocol regarding density gradient centrifugation. According to the general protocol, 6 ml of blood was layered on 3 ml of Lymphoprep (Axis-shield, Norway), then centrifuged at 800 x g for 15 min. The layer of PBMC was collected, the
25 cells were washed twice with phosphate buffered saline (PBS) (BIOMED-LUBLIN, Poland) and suspended in medium RPMI1640 (Gibco, Great Britain), containing 10 mM HEPES (Sigma, USA), 10% fetal calf serum (BIOMED-LUBLIN, Poland) and antibiotic (streptomycin sulfate, sodium penicillate G, amphotericin B, PAA, Austria).

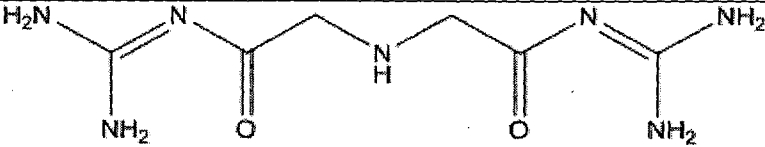
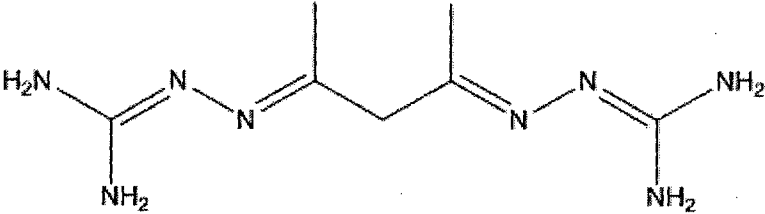
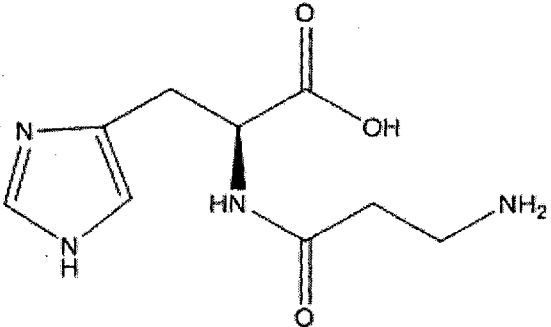
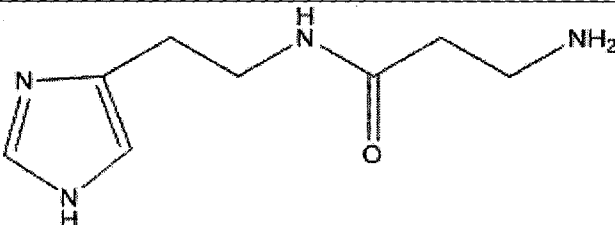
Efficacy tests of the compounds initially selected in the screening stage, evaluating fit in the
30 IL-15R α receptor active site, using IL-15R α - IL-15 complex as the model, were performed. The tested compounds were commercially available or synthesized following known synthetic procedures.

In all the *in vitro* experiments PBMC stimulated with IL-15 were used as the control.

The compounds of the formula (I) or (II) are commercially available or have been synthesized
35 according to the generally known procedures.

The compounds of the general formula (I) and (II) showing the highest inhibitory activity are collected in the Table 1.

Table 1. Chemical structures and names of the compounds.

Compound	Chemical structure
L1	
L2	
L3	
L4	

Chemical structures of the compounds, characterized by different stereoisometry, substituents and functional groups present, were determined by means of spectroscopic methods: ^1H and ^{13}C NMR (proton and carbon nuclear magnetic resonance), IR (infra-red spectroscopy), GC/MS (gas chromatography-mass spectrometry), HR MS (high resolution mass spectrometry) as well as microanalysis. The compounds were purified by crystallization or silica-gel chromatography to obtain the final products of purity not lower than 95%. Compound's purity was determined by HPLC (high performance liquid chromatography) analyses. The final products were stored in vials at 0 – 5°C. *In vitro* experiments were carried out using freshly prepared stock solutions in water, ethanol or DMSO, depending on the polarity and physico-chemical properties of the tested compounds.

Example 1

Effect of the tested compounds on interleukin 15-induced PBMC proliferation.

5 PBMC proliferation was evaluated using commercially available BrdU Cell Proliferation Assay (Calbiochem, Merck, Germany). PBMC were seeded in a 96-well plate (25×10^3 cells in 200 μ l culture medium/well). Next day the cells were treated with the tested compounds at final concentrations: 20 μ M, 50 μ M, 100 μ M and 200 μ M. After 30 min. incubation with the compounds, the cells were stimulated with IL-15 at final concentration 5 ng/ml, incubated for 4 days, for last 24 h to the culture medium bromodeoxyuridine (BrdU) at concentration recommended by the manufacturer was added. After completion of the incubation the cells were centrifuged (10 min., 160xg) and fixed. Further experimental steps were performed according to the manufacturer's protocol.

10 Effect on interleukin 15-induced PBMC proliferation exerted by the compound L4 at different concentrations (20 μ M, 50 μ M, 100 μ M i 200 μ M) is illustrated in diagram in Fig. 1.

15 The results are presented as OD values (absorbance) measured at 450 nm against reference of 540 nm.

The derivative L4 shows concentration dependent inhibition on interleukin 15-induced PBMC proliferation.

20 Example 2

Effect of the tested compounds on IL-15-induced TNF- α synthesis in PBMC

Evaluation of TNF- α synthesis in IL-15-stimulated PBMC was performed using ELISA method and commercially available tests (R&D, USA). The experiment was carried out strictly according to the manufacturer's recommendations.

25 PBMC were seeded in a 24-well plate (2×10^6 cells in 1ml of culture medium/well), treated with the tested compounds and IL-15 (5 ng/ml), which was pre-incubated for 30 min. with the tested compounds. After 48 h incubation, culture medium was collected from each well to determine TNF- α concentration, the cells were harvested and lysed, in cell lysates concentration of total protein was measured. The obtained values of TNF- α concentration were calculated on 1 mg of protein. The results are expressed as percentage change of concentration of TNF- α synthesized in PBMC in regard to IL-15-stimulated control cells.

The Effect of the selected compounds L1, L2, L3 i L4 at concentration 200 μ M on IL-15-induced TNF- α synthesis in PBMC is depicted in Fig. 2.

35 The tested compounds significantly inhibit IL-15-induced TNF- α synthesis in PBMC in comparison with the control cells stimulated with IL-15 alone.

Example 3

Effect of the tested compounds on IL-15-induced IL-17 synthesis in PBMC

Evaluation of IL-17 synthesis in IL-15-stimulated PBMC was performed using ELISA method and commercially available tests (R&D, USA). The experiment was carried out strictly according to the manufacturer's recommendations.

PBMC were seeded in a 24-well plate (2×10^6 cells in 1ml of culture medium/well), treated with inhibitors, and IL-15 (5 ng/ml), which were pre-incubated for 30 min. with the tested compounds. After 48 h incubation culture medium was collected from each well to determine IL-17 concentration, the cells were harvested and lysed, in cell lysates concentration of total protein was measured. The obtained values of IL-17 concentration were calculated on 1 mg of protein. The results are expressed as percentage change of concentration of IL-17 synthesized in PBMC in regard to stimulated with IL-15 control cells.

The Effect of the selected compounds L1, L2, L3 i L4 at concentration 200 μ M on IL-15-induced IL-17 synthesis in PBMC is depicted in Fig. 3.

All the tested compounds significantly reduce IL-15-induced IL-17 synthesis in PBMC in comparison with the control cells stimulated with IL-15 alone.

The derivatives L2 and L4 showed the highest inhibitory activity.

Discussion

1. Screening for the selected compounds efficacy of inhibition of IL-15-induced cell proliferation and cytotoxicity evaluation.

Increased cells proliferation is one of the characteristic responses to IL-15 stimulation. Selective blocking of IL-15 specific receptor IL-15R α results in inhibition of the cytokine biological activity. As the consequence, IL-15-induced increased cells proliferation does not occur. Effect of the selected compounds on proliferation of peripheral blood mononuclear cells (PBMC) isolated from blood of healthy donors was evaluated in the assay using fluorescent CFSE dye (Molecular Dynamics, Great Britain) and in bromodeoxyuridine incorporation test (BrdU) (BrdU Cell Proliferation Assay, Calbiochem, USA). PBMC population consists of several cell types, these are lymphocytes and monocytes, which are characterized by the expression of IL-15 specific IL-15R α receptor that explains their extensive proliferation in response to interleukin 15 stimulation. Inhibition of cells proliferation in the presence of the tested compounds can be also the visible sign of cells death caused by cytotoxic or apoptotic activity of the analyzed derivatives. Effect of the selected benzoic acid derivatives on the cells viability was tested by two methods:

a. Measurement of lactate dehydrogenase (LDH) concentration in culture medium and cultured cells (CytoTox 96 Non-Radioactive Cytotoxicity Assay, Promega, USA). LDH is the cytosolic enzyme, which under physiological conditions is not released from the cells. However, when cell membrane

damage or cell death occurs, LDH enzyme is released into the matrix. Increased LDH activity in culture medium correlates with increased number of dead cells resulting from cytotoxic effect of the tested compounds. LDH activity measured in cell lysates is used for assessment of number of living cells;

- 5 b. Cytofluorometric assay with annexin V and propidin iodide for apoptosis and necrosis assessment. Annexin V shows high affinity towards phosphatidilserine (PS), which is transferred to the outer leaflet of the plasma membrane in apoptosis. The appearance of PS on the cell surface is an indicator of initial or intermediate stage of cell apoptosis. Intact plasma membrane is not permeable for propidin iodide, but when the outer membrane loses its integrity, which occurs in necrosis, propidin iodide
10 enters the cytosol and stains the necrotic cells.

Only the compounds, which inhibit IL-15-induced PBMC proliferation and do not exert apoptotic effect, were chosen in the next research step. Each compound was tested twice on PBMC cells isolated from blood of different donors.

- 15 2. Efficacy assessment of the selected derivatives according to the invention, inhibiting IL-15-induced TNF- α and IL-17 production.

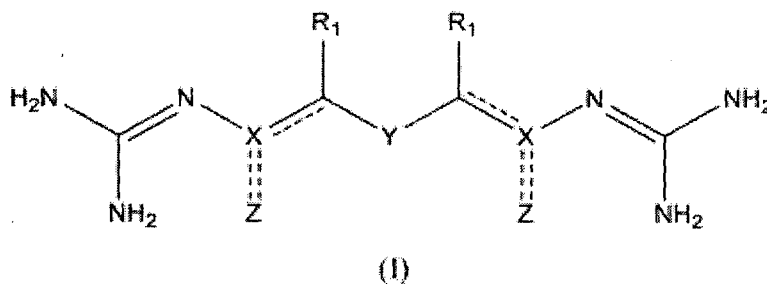
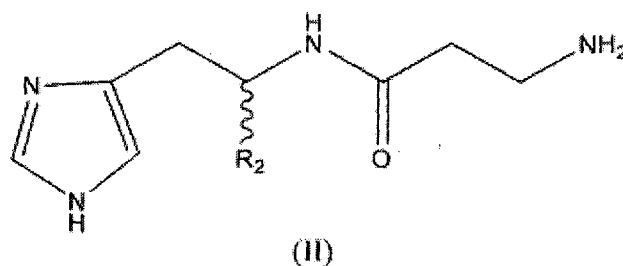
- Peripheral blood mononuclear cells (PBMC) respond to IL-15 stimulation not only by increased proliferation, but also synthesizing many pro-inflammatory cytokines, among others, for example, TNF- α and IL-17. Inhibition of IL-15 biological activity should result in reduced proliferation and
20 decreased synthesis of TNF- α and IL-17.

The selected derivatives at different concentrations were used to assess their Effect on IL-15-related cytokine synthesis. The biological activity of the compounds was evaluated measuring TNF- α and IL-17 concentrations in culture medium collected after incubation completion, using immunoenzymatic tests ELISA.

- 25 The results obtained in these test prove inhibition of cytokine synthesis and they are another evidence for biological activity of the tested compounds.

Claims

1. The amine derivatives of the formula (I) or (II)



wherein

X represents C or N.

Y represents CH₂ or NH,

10 R_1 represents H or CH_3 ,

R₂ represents H or COOH,

==== represents single or double bond,

====represents double bond or no bond,

providing

15 when is single bond, than is double bond and Z represents O,

and their tautomers, isomers, pharmaceutically acceptable salt and solvates,

for the use in the treatment of the diseases related to interleukin 15 overproduction.

20 2. The amine derivatives for use according to claim 1, wherein in the formula (I):

X represents C,

Y represents NH,

R_1 represents H,

-----represents single bond,

=====represents no bond.

3. The amine derivatives for use according to claim 1, wherein in the formula (I):

X represents N,

Y represents CH₂,

R₁ represents CH₃,

-----represents single bond,

=====represents double bond, and

Z represents O.

4. The amine derivatives for use according to claim 1, selected from the group comprising:

2,2'-Imino-bis[(N-(aminoiminomethyl))acetamide], and

2,2'-((1*E*,3*E*)-1,3-Dimethyl-1,3-propane-1,3-diylidene)-biscarbaminidoylhydrazine.

5. The amine derivatives for use according to claim 1, selected from the group comprising:

β-alanyl-D-histidine and β-alanylhistamine.

6. The amine derivatives for use according to claim 1, wherein the compound of the formula (I) or (II) binds to IL-15, thus inhibits binding the cytokine to its specific IL-15Rα receptor.

7. The amine derivatives for use according to claim 1, wherein the compound of the formula (I) or (II) is to be used in the prevention and the therapeutic treatment of rheumatoid arthritis, psoriasis, inflammatory bowel disease, sarcoidosis, T-cell leukemias or transplant rejection.

8. The amine derivatives for use according to claim 1, wherein the compound of the formula (I) or (II) is to be used in the treatment of rheumatoid arthritis.

9. The amine derivatives for use according to claim 1, wherein the compound of the formula (I) or (II) is to be administered in a therapeutically effective dose to an individual in need of such a treatment.

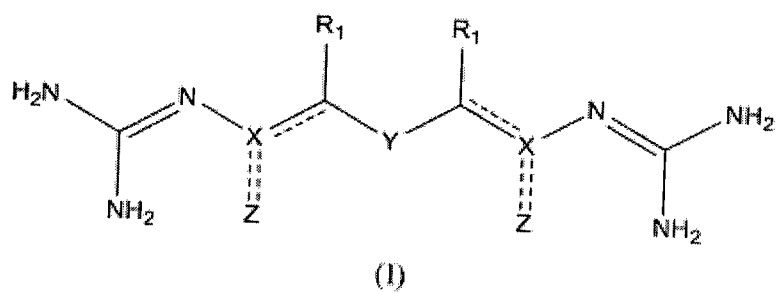
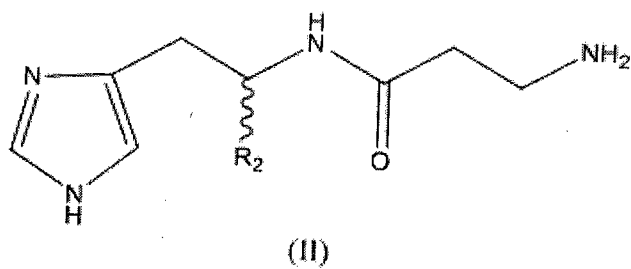
10. The amine derivatives for use according to claim 1, wherein the compound of the formula (I) or (II) is to be administered as the active ingredient of the pharmaceutical preparation or a single dosage form.

11. The use of the amine derivatives of the formula (I) or (II), their tautomers, isomers, pharmaceutically acceptable salt or solvates according to claim 1, for manufacturing of the pharmaceutical preparation to be used in prevention or treatment of the diseases listed in claim 7.

- 5 12. The pharmaceutical preparation containing therapeutically effective dose of the amine derivative of the formula (I) or (II), its isomer, tautomer, pharmaceutically accepted salt or solvates according to claim 1, as the active ingredient together with the pharmaceutically acceptable carriers and/or excipients.

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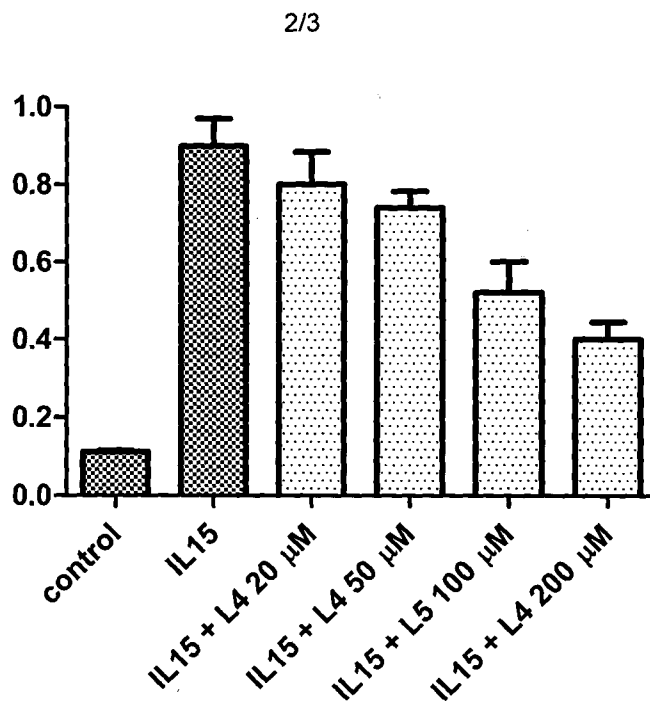


Fig. 1. Effect of the compound L4 (20 μ M, 50 μ M, 100 μ M and 200 μ M) on IL-15-stimulated PBMC proliferation.

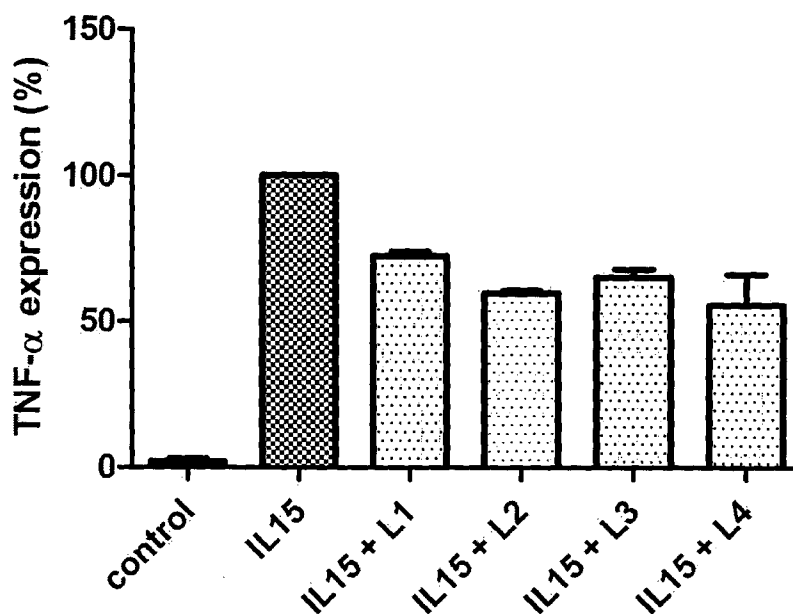
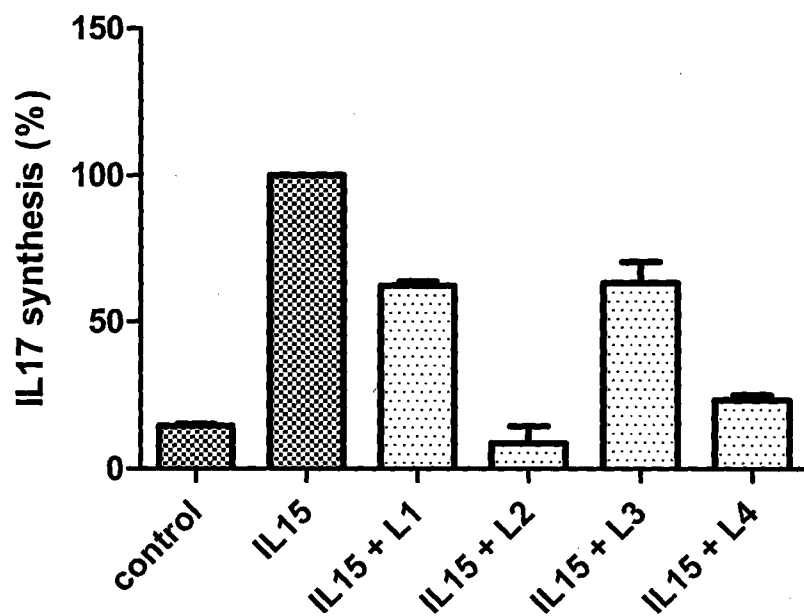


Fig. 2. Effect of the compounds L1, L2, L3 and L4 (200 μ M) on IL-15-induced TNF- α synthesis in

PBMC.

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Fig. 3. Effect of the compounds L1, L2, L3 and L4 (200 µM) on IL-15-induced IL-17 synthesis in PBMC.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/000896

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/00 A61K31/155 A61K31/16 A61K31/417 A61K31/4172
ADD. A61P29/00

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, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2005 120007 A (TOKYOTO IGAKU KENKYU KIKO; YUHABU KK) 12 May 2005 (2005-05-12) 0009; 0011; 0032; 0058; claim 3 -----	1,6-12
X	KATARÍNA BAUEROVÁ ET AL: "Utilization of adjuvant arthritis model for evaluation of new approaches in rheumatoid arthritis therapy focused on regulation of immune processes and oxidative stress", INTERDISCIPLINARY TOXICOLOGY, vol. 4, no. 1, 1 January 2011 (2011-01-01), XP055136934, ISSN: 1337-6853, DOI: 10.2478/v10102-011-0007-9 abstract; discussion ----- -/--	1,6-12



Further documents are listed in the continuation of Box C.



See patent family annex.

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"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

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"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

1 September 2014

Date of mailing of the international search report

05/09/2014

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/000896

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	ODASHIMA M ET AL: "Zinc l-carnosine protects colonic mucosal injury through induction of heat shock protein 72 and suppression of NF-kappaB activation", LIFE SCIENCES, PERGAMON PRESS, OXFORD, GB, vol. 79, no. 24, 10 November 2006 (2006-11-10), pages 2245-2250, XP028051007, ISSN: 0024-3205, DOI: 10.1016/J.LFS.2006.07.032 [retrieved on 2006-11-10] title, abstract; Fig 7, p. 2248, last of col. 1	1,6,7, 9-12
X	JP 2008 031066 A (INTER CYTO NANO SCIENCE CO LTD; HAMARI CHEMICALS LTD) 14 February 2008 (2008-02-14) 0051; examples; claim 7;	1,5-12
X	DE 43 28 871 A1 (BEIERSDORF AG [DE]) 2 March 1995 (1995-03-02) last of p. 2; p. 2, l. 52-55; p. 2, l. 21-26; p. 4, l. 31-35	1,5-12
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X	DAVE C ET AL: "Studies on the structure-activity relationship among aliphatic and aromatic bisguanyldhydrazones and some related compounds", CHEMICO-BIOLOGICAL INTERACTIONS, ELSEVIER SCIENCE IRLAND, IR, vol. 16, no. 1, 1 January 1977 (1977-01-01), pages 57-68, XP025889213, ISSN: 0009-2797, DOI: 10.1016/0009-2797(77)90153-3 [retrieved on 1977-01-01] table I, compound VI; p. 64. second full	11,12
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/000896

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CORTI A ET AL: "SPECIFIC INHIBITION OF THE ENZYMIC DECARBOXYLATION OF S-ADENOSYLMETHIONINE BY METHYLGLYOXAL BIS(GUANYLHYDRAZONE) AND RELATED SUBSTANCES", BIOCHEMICAL JOURNAL, PORTLAND PRESS LTD, GB, vol. 139, no. 2, 1 January 1974 (1974-01-01), pages 351-357, XP008014009, ISSN: 0264-6021 table 4, 4th compound	11,12
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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Form PCT/ISA/210 (patent family annex) (April 2005)