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(54) Title: DOMPERIDONE FOR USE AS ANTIVIRAL AGENT

(57) Abstract: The present invention relates to new use of domperidone. In particular it refers to its use as antiviral agent.



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Domperidone for use as antiviral agent

The invention relates to a new use of domperidone. In particular, it refers to domperidone or a pharmaceutically acceptable salt, tautomer and/or solvate thereof
5 for use as antiviral agent.

Therefore, the present invention belongs to the field of medicine.

BACKGROUND ART

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Viruses, which cause infectious disease in humans and other mammals, are a diverse group of infectious agents that differ greatly in size, shape, chemical composition, host range, and effects on hosts. A variety of antiviral agents are available for the treatment and/or prevention of diseases caused by viruses, such as hepatitis B, influenza A and
15 B and HIV, etc. However, some known viruses and the emergence of new viruses that are immune to currently available antiviral agents make new and effective antiviral agents necessary. In particular, among the known viruses, coronaviruses (CoVs) act as cross-species viruses and have the potential to spread rapidly into new host species and cause epidemic diseases. Despite the severe public health threat of
20 severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), there are currently no effective drugs available for their treatment; therefore, broad-spectrum inhibitors of emerging and endemic CoVs are urgently needed.

25 Moreover, since the emergence of a novel coronavirus (SARS-CoV-2) from Wuhan, China, in December 2019, that has caused millions of cases of human infections and huge cases of deaths globally and was declared as a pandemic by World Health Organisation, broad-spectrum inhibitors of emerging and endemic CoVs are needed. The virus spreads rapidly from the transmission among human, and the symptoms
30 caused by the virus (abbreviated "COVID-19") ranges from mild coughs and fevers to severe pneumonia and potentially life-threatening symptoms.

Waiting for the availability of new vaccines, this global emergency has triggered the search for safe and effective pharmacological approaches for eradicating the virus, or

at least reducing its effects and hindering the contagion, since, currently there is no effective therapy to treat SARS-CoV-2 infection.

Accordingly, there is a need for safe and effective antiviral agents with a wide-spectrum of anti-viral activity, especially against coronaviruses.

In view of this need, the present invention provides an already available drug that so far has not been used as antiviral and more particularly against coronaviruses infection.

10

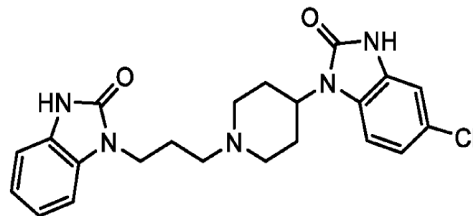
SUMMARY OF THE INVENTION

The present invention is based on the surprising finding that domperidone may be used to prevent or/and treat a virus infection.

15

The inventors have found a consistent antiviral activity of domperidone in cells infected by coronavirus, in concentrations of the drug that were non-cytotoxic.

The compound domperidone has the following formula:



20

It is a known antiemetic, gastric prokinetic agent and galactagogue, used to relieve nausea and vomiting, to increase the transit of food through the stomach, and to promote lactation by release of prolactin. This drug is readily available to the public and can be purchased from chemical companies.

25

Then, the present invention relates to domperidone or a pharmaceutically acceptable salt, tautomer and/or solvate thereof, for use in the prevention and/or the treatment of virus infection, that is, as antiviral agent.

30

In a preferred embodiment, the present invention refers to domperidone or a pharmaceutically acceptable salt, tautomer and/or solvate thereof for use in the prevention and/or the treatment of coronaviruses infection. More particularly in the prevention and/or the treatment of human coronavirus 229E infection or coronavirus
5 SARS-CoV-2 infection.

Unless otherwise stated, the compounds used in the invention are intended to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the substitution of
10 a hydrogen atom for a deuterium atom or a tritium atom, or the substitution of a carbon atom for a carbon atom enriched in ^{13}C or ^{14}C or a nitrogen atom enriched in ^{15}N fall within the scope of this invention.

The compounds used in the invention may be in crystalline form, either as free
15 compounds or as solvates (e.g.: hydrates), and it is understood that both forms fall within the scope of the present invention. Solvation methods are generally known in the state of the art. Suitable solvates are pharmaceutically acceptable solvates. In a particular embodiment, the solvate is a hydrate.

20 For its application in therapy, domperidone or a pharmaceutically acceptable salt, tautomer and/or solvate thereof will preferably be in a pharmaceutically acceptable or substantially pure form, that is, with a pharmaceutically acceptable level of purity excluding pharmaceutically acceptable excipients and not including material considered toxic at normal dosage levels. The purity levels for a compound of formula
25 (I) are preferably above 50%, more preferably above 70%, more preferably above 90%. In a preferred embodiment, they are above 95%.

The term pharmaceutically acceptable salt embraces salts with a pharmaceutically acceptable acid or base.

30 The term "pharmaceutically acceptable" relates to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic reaction or a similar unfavourable reaction, such as gastric upset, dizziness and similar side effects, when administered to a subject. Preferably, the term

"pharmaceutically acceptable" means approved by a regulatory agency of a federal or state government or collected in the US Pharmacopoeia or other generally recognised pharmacopoeia for use in animals and, more particularly, in humans.

- 5 "Tautomers" are understood to be the two isomers that differ only in the position of a functional group because between the two forms there is a chemical balance in which a migration of a group or atom occurs.

10 The term "treatment or prevention" as used herein, unless otherwise indicated, relates to reversing, alleviating and inhibiting the progress of, or preventing the disorder or condition to which it applies in such terms, one or more symptoms of such disorder or condition.

15 Other aspect of the present invention relates to a composition comprising domperidone or a pharmaceutically acceptable salt, tautomer and/or solvate thereof for use in the prevention and/or the treatment of virus infection.

20 In particular, the composition of the invention is a pharmaceutical composition. The term "pharmaceutical composition" means the composition that includes domperidone or a pharmaceutically acceptable salt, tautomer and/or solvate thereof and at least one excipient, adjuvant and/or pharmaceutically acceptable carrier.

25 The term "excipients, adjuvants and/or carriers" relates to molecular entities or substances through which the active ingredient is administered. Such pharmaceutical excipients, adjuvants or carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and similar oils, excipients, disintegrating agents, humectants or dilutes.

30 Domperidone or a pharmaceutically acceptable salt, tautomer and/or solvate thereof or the pharmaceutical composition comprising them can be administered by any suitable administration route, for example, oral, topical, parenteral or rectal administration. It is administered in a therapeutically effective amount.

In a particular embodiment, said pharmaceutical compositions may be in a pharmaceutical form of oral administration, either solid or liquid. Illustrative examples of pharmaceutical forms of oral administration include tablets, capsules, granules, solutions, suspensions, etc., and may contain conventional excipients such as binders, dilutes, disintegrating agents, lubricants, humectants, etc., and may be prepared by conventional methods. The pharmaceutical compositions may also be adapted for parenteral administration, in the form of, for example, solutions, suspensions or lyophilised, sterile products in the suitable dosage form; in this case, said pharmaceutical compositions will include suitable excipients, such as buffers, surfactants, etc. In any case, the excipients are chosen according to the pharmaceutical form of administration selected.

As used herein, the term "therapeutically effective amount" means the necessary amount of a compound for the treatment or prevention of the disease, disorder or condition to be effective. It will also depend on the subject to be treated, the severity of the illness suffered by said subject, the chosen form of administration, etc. For this reason, the doses mentioned in this invention should be considered only as guidelines for the person skilled in the art, and should be adjusted the doses according to the variables mentioned above

Domperidone or a pharmaceutically acceptable salt, tautomer and/or solvate thereof as well as the compositions containing them can be used in conjunction with other additional antiviral drugs useful in the prevention and / or treatment of diseases caused by virus infections. Said additional drugs may form part of the same composition or, alternatively, may be provided in the form of a separate composition for simultaneous or sequential administration to domperidone or a pharmaceutically acceptable salt, tautomer and/or solvate thereof.

In another aspect, the invention relates to a method for the prevention and/or treatment of virus infection in a subject, comprising the administration to said subject of a therapeutically effective amount of domperidone or a pharmaceutically acceptable salt, tautomer and/or solvate thereof.

As used in this description, the term "subject" includes any animal or human being. Preferably the subject is a human being.

Unless otherwise defined, all technical and scientific terms used herein have the same
5 meaning as commonly understood by one of ordinary skilled in the art to which this
invention belongs. Methods and materials similar or equivalent to those described
herein can be used in the practice of the present invention. Throughout the description
and claims the word "comprise" and its variations are not intended to exclude other
10 technical features, additives, components, or steps. Additional objects, advantages
and features of the invention will become apparent to those skilled in the art upon
examination of the description or may be learned by practice of the invention. The
following examples are provided by way of illustration and are not intended to be
limiting of the present invention.

15

EXAMPLES

Some examples carried out by the inventors are provided in order to illustrate the
invention.

20

Example 1. Human Coronavirus 229E-GFP infection assays

Huh7-Lunet#3 cells (hepatoma cell line; kindly provided by Dr. Thommas
Pietschmann; Twincore-Hannover) were maintained subconfluent in complete media
25 [(DMEM supplemented with 10 mM HEPES, 1X non-essential amino acids (Gibco),
100 U/ mL penicillin-streptomycin (Gibco) and 10 % Fetal Bovine Serum (FBS; heat-
inactivated at 56 °C for 30 minutes)].

Huh7-Lunet#3 cells were seeded onto 96-well plates (1×10^4 cells/well). The day after,
compound (Domperidone, MicroSource Discovery System Inc) stock solutions (10
mM in DMSO) were diluted into complete cell culture media to achieve a final
30 concentration of 100 μ M. The 100 μ M solution was serially diluted 3-fold to achieve
decreasing compound concentrations. On the other hand, 229E-GFP virus stock
(kindly provided by Dr. Volker Thiel; University of Bern) was diluted in complete media
to achieve a final concentration of 3×10^3 focus forming units (FFU)/mL. One hundred
microliters (100 μ L) of the virus dilution were mixed 1:1 with 100 μ L of the

Domperidone dilutions to achieve final compound concentrations in a range from 50 μM to 22 nM and 150 infectious units (FFU) per well in a 96 well plate. One hundred μl of the mixture was applied onto the Huh7-Lunet #3 cell monolayer in biological replicates and cells were cultured for 72 hours at 33°C in a 5% CO_2 incubator. Cells

5 were fixed in a 4% formaldehyde solution in FBS for 10 minutes at room temperature, washed twice with FBS and individual well fluorescence was measured in a SpectraMax iD3 fluorescence plate reader (Molecular Devices). Background subtraction was performed using non-infected wells and signal was normalized to the average fluorescence found in vehicle (DMSO)-treated virus-infected wells. Relative

10 infection efficiency was plotted versus compound concentration to determine the EC_{50} and EC_{90} (50% and 90% maximal effective concentration, respectively) values. Once infection efficiency had been determined, plates were stained with a 0.1% crystal violet solution in water-methanol for 30-60 minutes. Then, plates were extensively washed with water and dried before 1% SDS solution in water was added to solubilize

15 crystal violet. Absorbance was measured at 570 nm and background was subtracted from blank wells. Relative well biomass was estimated by calculating the absorbance in each well relative to the average observed in infected cells treated with DMSO. Relative biomass was plotted versus Domperidone concentration to determine the cytotoxic concentration (CC_{50}) values.

20 Data are collected in table 1.

Table 1: Domperidone Potency and cytotoxicity indexes in coronavirus 229E-GFP infection assays

Drug name	229E-GFP		
	EC_{50} (μM)	EC_{90} (μM)	CC_{50} (μM)
Domperidone	0.7	1.6	4

25 Domperidone shows efficacy to block the 90% of 229E viral infection at concentrations below the cytotoxic one. Thus, domperidone behaves as an antiviral agent against human coronavirus 229E

Example 2. SARS-CoV-2 infection assays

30 All infection experiments were performed by inoculating Vero-E6 cells (ATCC CRL-1586™) seeded onto 96-well plates (2×10^4 cells/well) with the SARS-CoV-2 strain

NL/2020 (kindly provided by Dr. R. Molenkamp, Erasmus University Medical Center Rotterdam) at low multiplicity of infection (MOI) of 0.001. Cultures were maintained at 37°C in a 5% CO₂ incubator for different lengths of time depending on the experiment. Domperidone compound was diluted from 10 mM stock solutions in complete media containing 2% FBS to achieve the indicated final concentrations.

5 Cell monolayer protection assays: Vero-E6 cell monolayers were inoculated at MOI 0.001 in the presence of 10 µM of each compound in duplicate wells. Seventy-two hours later the cells were fixed and stained using crystal violet, as described above. A wide range of compound concentrations (from 50 to 0.78 µM) from Domperidone

10 was used in subsequent experiments to determine the maximum and minimum protective concentration (PC_{max} and PC_{min}) as indicated above.

Cytotoxicity measurement by MTT assays (MTT assay: cell viability assay that infers reductive capacity of the cells via formation of formazan precipitates. Overall estimation of cell metabolic rate): Vero-E6 cell monolayers were treated with a wide

15 range of compound concentrations (from 50 to 0.78 µM) and forty-eight hours later they were subjected to MTT assays following the manufacturer's instructions. Compound concentration leading to a 50% of cytotoxicity (CC₅₀) were determined. Intracellular viral RNA quantitation: To confirm that protection of the monolayer was

20 indeed due to the ability of Domperidone to restrict virus replication, viral RNA quantitation was performed as indicated below. Vero-E6 cell monolayers were inoculated at MOI 0.001 in the presence of the indicated compound concentrations (dose µM). Forty-eight hours later cell lysates were prepared using Trizol reagent (Thermo Scientific). Viral RNA content was determined by RT-qPCR using previously

25 validated sets of primers and probes specific for the detection of the SARS-CoV-2 E gene [Corman VM et al. Euro Surveill., 2020, 25:2431] and the cellular β-actin gene, for normalization purposes. ΔCt method was used for relative quantitation of the intracellular viral RNA accumulation in compound-treated cells compared to the levels in infected cells treated with DMSO, set as 100%. Log drop for compound are

30 collected in Table 2.

Table 2: Domperidone Potency and cytotoxicity indexes in coronavirus SARS-CoV-2 infection assays

PC MAX (μM)	PC MIN (μM)	HIGHEST NON-TOXIC CONCENTRATION (MTT)	DOSE (μM) TESTED in VIRAL LOAD	VIRAL LOAD MOI 0.001 (% OF CONTROL)	LOG DROP	Approximate EC ₉₀ (μM)	MTT CC ₅₀ (μM)
25	12.5	12.5	25	0.0	4.89	8.00	25
			12.5	0.0	4.33		25
			6.25	16.9	0.76		25
			3.125	41.1	0.38		25

PC Max: Maximum concentration of compound that protects cells from the cytopathic effect induced by the virus.

PC Min: Minimum concentration of compound that protects cells from the cytopathic effect induced by the virus.

5

MTT CC₅₀: Concentration of compound that causes a 50% reduction in the MTT assay

EC₉₀: Effective concentration 90: Compound dose that reduces viral load by 1 order of magnitude.

10

According to these results, Domperidone presents antiviral activity in SARS-CoV-2 at non-cytotoxic concentrations (6.25-12 μM) showing potential to be used as treatment for COVID-19.

CLAIMS

- 5 1. Domperidone, a pharmaceutically acceptable salt, tautomer and/or solvate thereof or a composition comprising it for use in the prevention and/or the treatment of coronavirus infection.
- 10 2. Domperidone, a pharmaceutically acceptable salt, tautomer and/or solvate thereof or a composition comprising it for use, according to claim 1, in the prevention and/or the treatment of human coronavirus 229E infection.
- 15 3. Domperidone, a pharmaceutically acceptable salt, tautomer and/or solvate thereof or a composition comprising it for use, according to claim 1, in the prevention and/or the treatment of human coronavirus SARS-CoV-2 infection.
- 20 4. Domperidone, a pharmaceutically acceptable salt, tautomer and/or solvate thereof or a composition comprising it for use, according to any of previous claims, in combination with another antiviral agent.
- 25 5. Domperidone, a pharmaceutically acceptable salt, tautomer and/or solvate thereof or a composition comprising it for use, according to any of previous claims, wherein its administration is oral, topical, parenteral or rectal.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/069682

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/454 A61P31/14
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 A61P

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, 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.
A	CN 106 580 985 A (TANGSHAN LUANMU BIOLOGY TECH CO LTD) 26 April 2017 (2017-04-26) the whole machine translation in particular abstract Embodiments ----- -/--	1-5

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

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search 23 September 2021	Date of mailing of the international search report 08/10/2021
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Hornich-Paraf, E
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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2021/069682

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>US 2017/027975 A1 (FRIEMAN MATTHEW [US] ET AL) 2 February 2017 (2017-02-02) in particular 'Summary of the invention' 'Brief description of Drawings' paragraph [0029] Examples paragraphs [0047] - [0050] paragraph [0070] table 2 'Discussion' Claims claim 4</p>	1-5
A	<p>----- WO 2010/112497 A1 (ESTEVE LABOR DR [ES]; SABATE ELIAS DAVID [ES] ET AL.) 7 October 2010 (2010-10-07) the whole document in particular page 5, line 13 - line 22 page 8, line 14 - line 24 page 14, line 29 - page 15, line 12 page 11, line 28 - page 12, line 9 page 17, line 16 - page 19, line 15 examples 2, 3 Claims</p>	1-5
A	<p>----- Wikipedia: "https://web.archive.org/web/20200602005953/https://en.wikipedia.org/wiki/Dopamine_antagonist", 15 April 2020 (2020-04-15), XP055768228, Retrieved from the Internet: URL:https://web.archive.org/web/20200602005953/https://en.wikipedia.org/wiki/Dopamine_antagonist [retrieved on 2021-01-25] in particular 'First-generation antipsychotics (typical)' 'Dopamine antagonists used to treat nausea and vomiting' 'Domperidone' 'Chlorpromazine' 'Fluphenazine'</p>	1-5

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2021/069682

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