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(54) **Title**: ANTI-METHANOGENIC COMPOSITIONS AND USES THEREOF

(57) **Abstract**: The present invention relates to a method for reducing the symptoms of constipation, irritable bowel syndrome, discomfort from gaseous distention, and/ or obesity in humans. More particularly, it relates to the use of a nitrooxy organic molecule to reduce the symptoms, and/or treat constipation, irritable bowel syndrome, discomfort from gaseous distention, and obesity.

ANTI-METHANOGENIC COMPOSITIONS AND USES THEREOF

The present invention relates to a method for reducing the symptoms of constipation, irritable bowel syndrome, and/ or obesity in humans. More particularly, it relates to the use of a nitrooxy organic molecule to reduce the symptoms, and/or treat constipation, irritable bowel syndrome, discomfort from gaseous distention, and obesity.

The present inventors surprisingly found that the compounds specified herein after, have a great potential for use reducing the symptoms and or treating patients suffering from constipation, irritable bowel syndrome, discomfort from gaseous distention, and/or obesity. Said compounds are also more stable than those described in the prior art, safer for the humans using it, they can be produced at industrial scale at a cost compatible with the pharmaceutical industry, and they are active at very low concentration.

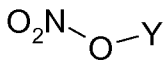
Several recent studies suggest a link between intestinal methane production and gastrointestinal symptoms and disorders such as (chronic) constipation and constipation predominant irritable bowel syndrome (IBS-C), discomfort from gaseous distention as well as metabolic diseases like obesity.

Methane (CH_4) production in humans is due to methanogenic archaea in the intestine. The dominant methanogen inhabiting the human gut is the archaea Methanobrevibacter smithii (M. smithii), followed by Methanosphaera stadtmanae (M. stadtmanae).

In order to treat constipation, irritable bowel syndrome, discomfort from gaseous distention, and/or obesity there is thus an ongoing need for safe and effective approaches for the long term suppression of enteric methanogenesis and/or excessive methane production by effectively reducing and/ or inhibiting the methane production in M. smithii and/ or M. stadtmanae.

Surprisingly it has now been found that specific nitrooxy compounds are able to significantly reduce the methane production in M. smithii and M. stadtmanae and thus are particular suitable for reducing the symptoms and/ or treating constipation, irritable bowel syndrome, discomfort from gaseous distention, and/ or obesity in patients in need thereof.

Thus, in a first embodiment, the present invention relates to a method for reducing and/or treating the symptoms of constipation, irritable bowel syndrome, and/or obesity in human, said method comprising applying an effective amount of at least one nitrooxy compound of formula (I),



formula (I)

wherein Y has the molecular formula $C_aH_bO_dN_eS_g$ wherein

a is an integer between 1 and 25, preferably between 1 and 10
b is an integer between 2 and 51, preferably between 2 and 21
d is an integer between 0 and 10, preferably between 0 and 10
e is an integer between 0 and 5, preferably between 0 and 3
g is an integer between 0 and 3, preferably between 0 and 1,

or a pharmaceutically acceptable salt thereof to a human in need thereof.

In all embodiments the term ‘an integer between X and Y’ is inclusive and also contains the X and the Y value.

The residue Y may be straight chained, branched, cyclic, bicyclic, aromatic as well as heteroaromatic and may contain one or more –OH, CN, -COOH, $-NH_2$, $-ONO_2$ group(s) and/ or one or more –NH-, -OC(=O)-, -(O=)CO-, $-SO_2-$, -C(=O)-, -C(=O)NH-, -O- or -N= structural elements at any position. Exemplary compounds of formula (I) are illustrated in table 1.

It is well understood to a person skilled in the art, that the nitrooxy group(s) in formula (I) are bound to one of the carbon atom(s) of Y.

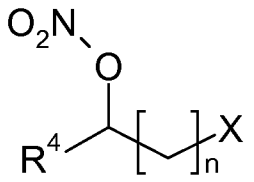
In a preferred embodiment, the compounds of formula (I) contain 1 to 4 nitrooxy groups, preferably 1, 2 or 4 nitrooxy groups.

In all embodiments of the present invention, preferred compounds of formula (I) are compounds, wherein a is comprised between 1 and 10, preferably between 3 and 8.

In all embodiments of the present invention, particular preferred compounds of formula (I) are compounds, wherein g is 0 or 1.

In all embodiments of the present invention, it is to be understood that compounds of formula (I) can be in any isomeric form.

Advantageous compounds of formula (I) according to the present invention are compounds of formula (II),



formula (II),

wherein

n is comprised between 0 and 12, preferably comprised between 0 and 6 and, wherein, if n ≠ 0, the carbon chain is a linear, a cyclic, or branched aliphatic carbon chain which may be non-substituted or substituted with up to 3 hydroxyl-, alkoxy-, amino-, alkylamino-, dialkylamino- or nitrooxy groups, or an alkenyl, or an alkynyl carbon chain mono- or polyunsaturated and in any isomeric form,

R^4 is independently, hydrogen or a saturated straight, cyclic or branched chain of an alkyl or alkenyl group containing 1 to 12, preferably 1 to 6 carbon atoms,

X is hydrogen, R^5, $R^5{\equiv}N$, $-OR^5$, $-OCOR^5$, $-NR^5R^6$, $-ONO_2$, $-COOR^5$, $-CONR^5R^6$, $-NHSO_2R^5$, or $-SO_2NHR^5$, preferably

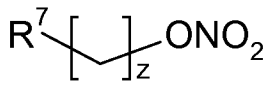
R^5 and R^6 are independently, hydrogen, C_1-C_{12} straight, branched or cyclic alkyl chain, non substituted or substituted with up to 3 hydroxyl-, alkoxy-, amino-,

alkylamino-, dialkylamino- or nitrooxy groups, alkenyl, or alkynyl carbon chain which may be mono or polyunsaturated, and in any isomeric form.

It is to be understood in the above definition of compounds of formula (II) that when n > 2, the carbon chain can be linear or branched at any position along the carbon chain. In addition, the carbon chain can be branched by multiple branches at different positions along the carbon chain. Moreover, when n > 3, the aliphatic carbon chain may form a cyclic moiety. This cyclic moiety can carry the nitrooxy moiety at any position (2, 3, 4), and it can also be branched at multiple positions by any aliphatic groups. The branched aliphatic groups are preferably, methyl, ethyl or propyl. Moreover, the carbon chain may be further substituted with up to 3 hydroxyl-, alkoxy-, amino-, alkylamino-, dialkylamino- or nitrooxy groups.

In the above definition of derivatives of the formula (II) a preferred alkyl group is methyl, ethyl, propyl, isopropyl, butyl, sec. butyl, isobutyl, pentyl, neopentyl, hexyl, cyclohexyl, and 2-ethyl-hexyl and octyl. Furthermore any alkyl or alkenyl group containing three or more carbon atoms can be straight chain, branched, or cyclic. In addition for the straight chain or branched C_2-C_{10}-alkenylene group, this is understood to encompass alkenylene groups with one or (from C_4) more double bonds; examples of such alkenylene groups are those of the formulae -CH=CH-, -CH=CH-CH_2-, -CH=CH-$(CH_2)_3$- and -$(CH=CH)_2$-.

Further advantageous nitrooxy compounds of formula (I) in all embodiments of the present invention are compounds of formula (III)



formula (III),

wherein

z is an integer between 1 and 10,

R^7 is H, C_1-C_6 alkyl, phenyl, -OH, $-NH_2$, -CN, -COOH, $-O(C=O)R^8$, $-NHC(=O)R^8$, SO_2NHR^8, or $-ONO_2$, and

R^8 is C_1-C_6 alkyl, phenyl, pyridyl such as preferably 2-pyridyl

with the proviso that when z is > 3 the hydrocarbon chain may be interrupted by –O– or -NH-.

Particular preferred compounds of formula (I) in all embodiments of the present invention are compounds of formula (III) wherein
z is an integer between 3 and 9
R^7 is OH, COOH or $-ONO_2$,
with the proviso that if z is 4 the hydrocarbon chain may be interrupted by -NH- such as in particular the compounds of formula (IV) $R7-(CH_2)_2-NH-(CH_2)_2-ONO_2$ (IV).

In a further embodiment, the present invention relates to a method for the reduction of the symptoms and/ or the treatment of constipation, irritable bowel syndrome, discomfort from gaseous distention, and/or obesity in human, said method comprising applying an effective amount of at least one nitrooxy compound of formula (I) or a pharmaceutically acceptable salt thereof to a patient in need thereof and optionally appreciating the effect.

In yet a further embodiment the invention relates to a composition further comprising a pharmaceutically acceptable carrier comprising at least one nitrooxy compound of formula (I) or a pharmaceutically acceptable salt thereof for the reduction of the symptoms and/ or the treatment of constipation, irritable bowel syndrome, discomfort from gaseous distention, and/or obesity in humans. Preferably the composition is a pharmaceutical composition, more preferably an oral pharmaceutical composition.

Furthermore, the present invention relates to the use of at least one nitrooxy compound of formula (I) or a pharmaceutically acceptable salt thereof for the reduction of the symptoms and/ or the treatment of constipation, irritable bowel syndrome, discomfort from gaseous distention, and/or obesity in humans.

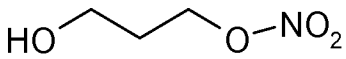
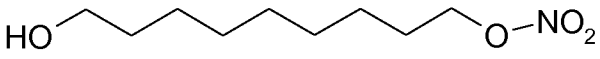
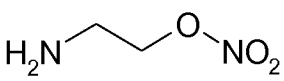
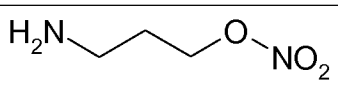
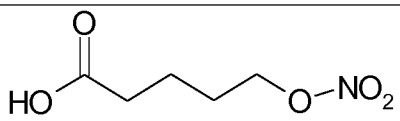
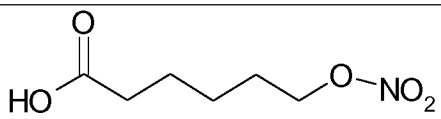
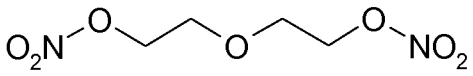
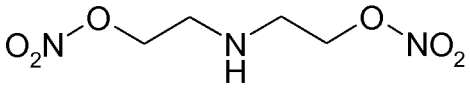
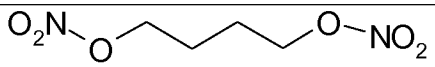
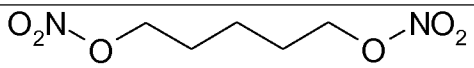
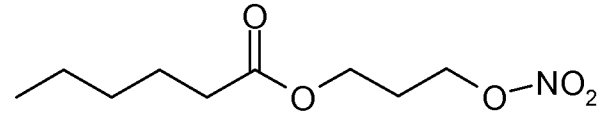
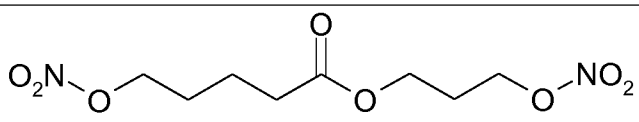
Furthermore, the invention relates to the treatment of symptoms and diseases linked to an excessive methane production in humans such as in particular constipation, irritable bowel syndrome, discomfort from gaseous distention, and/ or obesity.

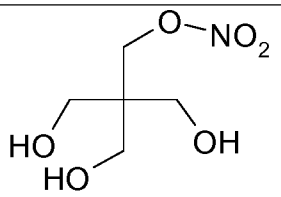
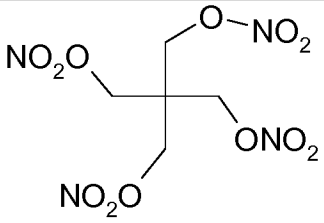
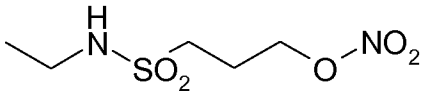
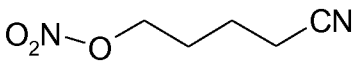
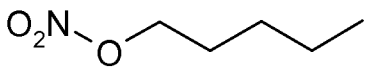
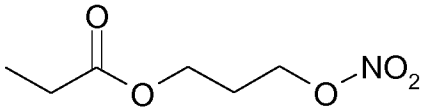
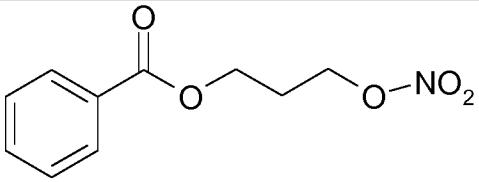
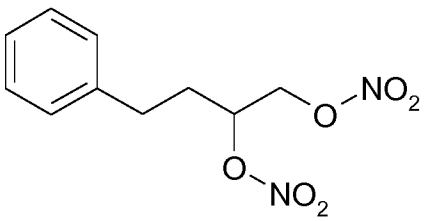
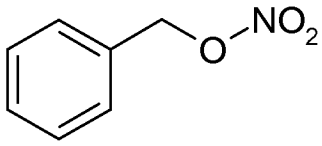
In another embodiment the invention relates to a method for the reduction of the symptoms and/ or the treatment of constipation, irritable bowel syndrome, discomfort from gaseous distention, and/or obesity in human by reducing and/ or inhibiting the methane production in M. smithii and/ or M. stadtmanae in their natural habitat such as in particular in the human gut, said method comprising applying an effective amount of at least one nitrooxy compound of formula (I) with all the definitions and preferences given herein or a pharmaceutically acceptable salt thereof to a patient in need thereof and optionally appreciating the effect.

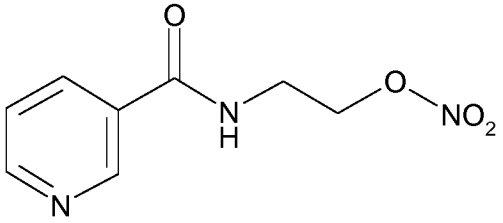
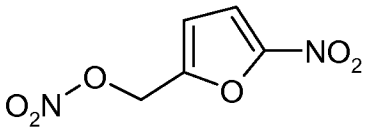
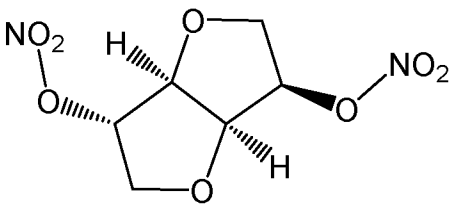
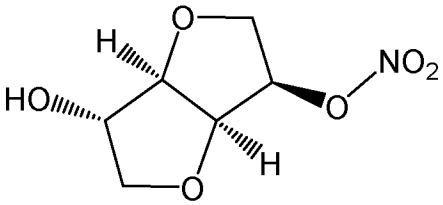
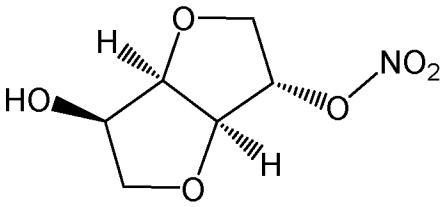
In a further embodiment, the present invention also relates to a composition further comprising a pharmaceutically acceptable carrier comprising at least one nitrooxy compound of formula (I) or a pharmaceutically acceptable salt thereof for the reduction of the symptoms and/ or the treatment of constipation, irritable bowel syndrome, discomfort from gaseous distention, and/or obesity in human by reducing/ inhibiting the growth of M. smithii and/ or M. stadtmanae in their natural habitat such as in particular in the human gut.

In all embodiments of the present invention it is particular advantageous to select the nitrooxy compound of formula (I) from the group consisting of 3-nitrooxypropanol, 9-nitrooxynonanol, 2-aminoethyl nitrate, 3-aminopropyl nitrate, 5-nitroxy pentanoic acid, 6-nitroxy hexanoic acid, bis-(2-nitrooxyethyl) ether, bis(2-hydroxyethyl)amine dinitrate, 1,3-bis-nitrooxypropane, 1,4-bis-nitrooxybutane, 1,5-bis-nitrooxypentane, 3-nitrooxy-propyl hexanoate, 3-nitrooxy-propyl 5-nitrooxy-hexanoate, 2-(hydroxymethyl)-2-(nitrooxymethyl)-1,3-propanediol, 3-bis(nitryloxy)-2,2-bis(nitryloxy-methyl)propan, N-ethyl-3-nitrooxy-propionic sulfonyl amide, 5-nitrooxy-pentanenitrile, 5-nitrooxy-pentane, 3-nitrooxy-propyl propionate, 3-nitrooxy-propyl benzoate, rac-4-phenylbutane-1,2-diyl dinitrate, benzylnitrate, N-[2-(nitrooxy)ethyl]-3-pyridinecarboxamide, 2-nitro-5-nitrooxymethyl-furan, 1,4:3,6-dianhydro-2,5-di-O-nitro-D-glucitol, 1,4:3,6-Dianhydro-D-glucitol 5-mononitrate and 1,4:3,6-Dianhydro-D-glucitol 2-mononitrate, and as outlined in table 1.

Table 1: Particular advantageous nitrooxy compounds for the purposes of the present invention

#	Molecular structure	Chemical name
1		3-Nitrooxypropanol
2		9-Nitrooxynonanol
3		2-aminoethyl nitrate
4		3-aminopropyl nitrate
5		5-nitroxy pentanoic acid
6		6-nitroxy hexanoic acid
7		Bis-(2-nitrooxyethyl) ether
8		Bis(2-hydroxyethyl)amine dinitrate
9		1,3-bis-Nitrooxypropane
10		1,4-bis-Nitrooxybutane
11		1,5-bis-Nitrooxypentane
12		3-Nitrooxy-propyl hexanoate
13		3-Nitrooxy-propyl 5-nitrooxy-hexanoate

14		2-(Hydroxymethyl)-2-(nitrooxymethyl)-1,3-propanediol
15		1,3-Bis(nitryloxy)-2,2-bis(nitryloxy-methyl)propan
16		N-Ethyl-3-nitrooxy-propionic sulfonyl amide
17		5-Nitrooxy-pentanenitrile
18		5-Nitrooxy-pentane
19		3-Nitrooxy-propyl propionate
20		3-Nitrooxy-propyl benzoate
21		rac-4-Phenylbutane-1,2-diyl dinitrate
22		Benzylnitrate

23		N-[2-(Nitrooxy)ethyl]-3-pyridinecarboxamide
24		2-Nitro-5-nitrooxymethyl-furan
25		1,4:3,6-dianhydro-2,5-di-O-nitro-D-glucitol
26		1,4:3,6-Dianhydro-D-glucitol 5-mononitrate (Isosorbit-5-mononitrate)
27		1,4:3,6-Dianhydro-D-glucitol 2-mononitrate Isosorbit-2-mononitrate

Most preferred, in all embodiments of the present invention, is the use of 3-nitrooxypropanol (CAS-No: 100502-66-7), 1,4:3,6-dianhydro-D-glucitol 5-mononitrate (Isosorbit-5-mononitrat, CAS-No: 16051-77-7), 1,4:3,6-dianhydro-D-glucitol 2-mononitrate (Isosorbit-2-mononitrate, CAS 16106-20-0), 1,4:3,6-dianhydro-2,5-di-O-nitro-D-glucitol (Isosorbitdinitrate, CAS-No: 87-33-2), 1,3-bis(nitryloxy)-2,2-bis(nitryloxy-methyl)-propan (Pentaerythrityltetranitrat, Nitropenta, CAS-No: 78-11-5), N-[2-(nitrooxy)ethyl]-3-pyridinecarboxamide (Nicorandil, CAS-No: 65141-46-0), 2-aminoethyl nitrate (Nilatil, CAS-No: 13445-63-1), bis(2-hydroxyethyl)amine dinitrate (CAS-No 20830-49-3), 1,4-bis-nitrooxybutane (CAS 3457-91-8), 6-nitroxy hexanoic acid (CAS 74754-55-5), 3-nitrooxy-propyl hexanoate (CAS 1383661-90-2), benzylnitrate (CAS 15285-42-4), 9-nitrooxynonanol and N-[2-(nitrooxy)ethyl]-3-pyridinecarboxamide (CAS 65141-

46-0), such as even more in particular 3-nitrooxypropanol, 1,4:3,6-dianhydro-D-glucitol 5-mononitrate (Isosorbit-5-mononitrate), 1,4:3,6-Dianhydro-D-glucitol 2-mononitrate (Isosorbit-2-mononitrate), 1,4-bis-nitrooxybutane, 6-nitroxy hexanoic acid, 3-nitrooxy-propyl hexanoate, benzylnitrate, 9-nitrooxynonanol, N-[2-(nitrooxy)ethyl]-3-pyridinecarboxamide.

The compounds according to the present invention are known and either commercially available or can be prepared in analogy to the processes as e.g. disclosed in WO2012/084629.

If the compounds of formula (I) can be converted into a salt (e.g. as they carry a $-NH_2$, -NH- or an acid group), this can be done according to a conventional method in the art, for example, by treating the respective compound with an organic or inorganic acid or a base in a solvent such as ethers, lower alcohols, ethyl acetate, hexane or the like at approximately room temperature.

Suitable acids/ bases which release a pharmaceutically acceptable ion, i.e. an ion that is not toxic are well known to a person skilled in the art. Examples of suitable ions encompass ammonium and alkyl ammonium, lithium, sodium, potassium, magnesium, calcium, chloride as well as acetate ions.

In all embodiments of the present invention preferably only one nitrooxy compound as defined by formula (I) with all the definitions and preferences as given herein is used/ administered.

The term '*Irritable bowel syndrome (IBS)*' refers to a functional gastrointestinal disorder that results in abdominal pain and/or discomfort, along with changes in bowel habits. IBS is classified into four subtypes based on a person's stool consistency: constipation-associated IBS (IBS-C); diarrhea-associated IBS (IBS-D); mixed (or alternating) IBS (IBS-M or IBS-A); and unsubtyped (or unspecified) IBS (IBS-U). In a preferred embodiment, the IBS to be treated according to the present invention is IBS-C.

The term '*reduction of the symptoms'* as used herein refers to a reduction of any signs and symptoms for the comfort and well-being of the patient.

The term '*reduction of the symptoms of obesity*' includes any weight loss of a person desiring such weight loss, if considered purely cosmetic as well as due to medical reasons.

The term '*effective amount*' as used herein refers to an amount necessary to obtain the desired physiological effect, respectively a perceivable decrease in the symptoms to be treated. The physiological effect may be achieved by a single dose or by repeated doses. The dosage administered may, of course, vary depending upon known factors such as the physiological characteristics of the particular composition and its mode and route of administration; the type, age, health and weight of the recipient; the nature and extend of symptoms; the kind of concurrent treatment and can be adjusted by a person skilled in the art.

Generally, the term '*effective amount*' as used herein refers to amounts of at least 0.1 mg, preferably to amounts in the range of 1 mg to 1000 mg, most preferably to amounts in the range of 1 mg to 500 mg.

In all embodiments of the present invention, the at least one nitrooxy compound of formula (I) or a pharmaceutically acceptable salt thereof is preferably administered via a pharmaceutical composition, more preferably via an oral pharmaceutical composition further comprising a pharmaceutically acceptable carrier either in the form of a single dose or by multiple doses.

The dosages of the at least one nitrooxy compound of formula (I) or a pharmaceutically acceptable salt thereof via a pharmaceutical composition can be determined by the expert in the field with normal preclinical and clinical trials, or with the usual considerations regarding the formulation of pharmaceutical composition.

Preferably, the dosage of the one nitrooxy compound of formula (I) or a pharmaceutically acceptable salt thereof for humans (usually determined for a

70kg person) is at least 0.1mg. Preferably the daily dosage should be in the range of from about 1 mg/day to about 1000 mg/day, more preferably from about 1 mg/day to about 500 mg/day.

The term '*a pharmaceutically acceptable carrier*' refers to additives and adjuvants, excipients or diluents which are conventionally used in pharmaceutical compositions and which are e.g. described in Remington's Pharmaceutical Sciences, supra, a standard reference text in this field. Examples of such pharmaceutically acceptable carriers are both inorganic and organic carrier materials, suitable for oral/parenteral/injectable/ rectal administration and include water, gelatin, gum arabic, lactose, starch, magnesium stearate, talc, vegetable oils, and the like.

The pharmaceutical composition may further comprise conventional pharmaceutical additives and adjuvants, excipients or diluents, including, but not limited to, water, gelatin of any origin, vegetable gums, ligninsulfonate, talc, sugars, starch, gum arabic, vegetable oils, polyalkylene glycols, flavoring agents, preservatives, stabilizers, emulsifying agents, buffers, lubricants, colorants, wetting agents, fillers, and the like.

The dosages and ratios of the individual components in a pharmaceutical composition can be determined by the expert in the field with normal preclinical and clinical trials, or with the usual considerations regarding the formulation of pharmaceutical composition.

In a preferred embodiment the at least one nitrooxy compound of formula (I) or a pharmaceutically acceptable salt thereof is administered via a pharmaceutical composition either in the form of a single dose or by multiple doses in an amount of at least 0.01 mg/ kg bodyweight/ day, preferably in an amount of 0.01-10 mg/ kg body weight/ day, most preferably in an amount of 0.01-5 mg/ kg body weight / day.

The compositions according to the present invention may be in any galenic form that is suitable for administering to the human body, more in particular in any form

that is conventional for oral administration, e.g. in solid form, for example as (additives/supplements for) food, food premixes, fortified food, tablets, pills, granules, dragées, capsules, and effervescent formulations such as powders and tablets, or in liquid form, for instance in the form of solutions, emulsions or suspensions, for example as beverages, pastes and oily suspensions. The pastes may be filled into hard or soft shell capsules, whereby the capsules feature e.g. a matrix of (fish, swine, poultry, cow) gelatin, plant proteins or ligninsulfonate. Examples for other application forms are forms for transdermal, rectal, parenteral, topical or injectable administration.

The pharmaceutical compositions according to the present invention are preferably in the form of controlled (delayed) release formulations as well as in a form which is able to pass the stomach and release the at least one nitrooxy compound only in the gut such as formulations which comprise a modified-release coating that is substantially stable in gastric fluid or formulations which comprise a modified-release coating that is degraded by a microbial enzyme present in the gut flora which can be easily be prepared by a person skilled in the art.

The present invention also relates to such pharmaceutical formulations comprising at least on compound of formula (I) with all the definitions and preferences as given herein, which further comprise a modified-release coating that is substantially stable in gastric fluid as well as to pharmaceutical formulations comprising at least on compound of formula (I) with all the definitions and preferences as given herein, which further comprise a modified-release coating that is degraded by a microbial enzyme present in the gut flora.

Preferably, the pharmaceutical formulation comprising a modified-release coating release at least 60% of the nitrooxy compound after the stomach and into one or more regions of the intestinal tract. In certain embodiments, the formulation releases the nitrooxy compounds in the small intestine, including one or more of the duodenum, jejunum, and ileum. In other embodiments, the formulation releases the nitrooxy compounds in the large intestine (eg, one or more of the cecum, ascending, transverse, descending or sigmoid portions of the colon, and rectum).

In a particular advantageous embodiment the compositions and formulations according to the present invention are in the form of a tablet, a pill, a granule, a dragées, a capsule or an effervescent formulation which are in particular intended for oral application.

The invention will now be elucidated by way of the following examples, without however being limited thereto.

Example 1: Influence of 3-Nitrooxypropanol (3-NOP) on the growth/ methane production M. smithii and M. stadtmanae

The methanogens outlined in table 2 were grown in 5 mL of the respective culture medium at 37°C in 15-mL Hungate tubes sealed with butyl rubber septum and aluminum seals. The 8 mL gas phase was 80% H_2/20% CO_2 at 2 x 10^5 Pa pressure. In the case of *M. stadtmanae* H_2 and methanol (100 mM) was the energy source. Methanogens growth was followed by CH_4 production using a flame ionization-detector GC (0.5 ml of gas from headspace, manual injection in the GC (HP Hewlett 5890, Packard Series II, Waldbronn, Germany) using a 1 mL Sample-Lock® syringe (Hamilton, Nevada, USA). The concentration of CH_4 was determined using a standard curve generated by injecting different volumes of 99.9 % pure CH_4 prior to and after the injection of samples.

Three consecutive 5 mL batches of incubations were conducted. In each batch the following treatments were used in triplicate: (i) blanks, only media; (ii) controls, cultures in the absence of inhibitor; (iii) 3-NOP added to a final concentration of 0.5 µM, 5 µM, 50 µM, 100 µM, 250 µM and 500 µM. 3-NOP was added on day 2 after culture inoculation with 0.5 mL of an actively growing culture. The growth was measured over 12 days every 2 days coinciding with new pressurization of headspace to 2 bar with 80:20 H_2:CO_2 gas mixture.

Results: Table 3 shows the lowest concentration of 3-NOP able to significantly decrease the growth/ methane production (>50%) in the respective archaea already after 2 days, which decrease was also maintained over the whole measurement period of 12 days.

Table 2: Methanogens

Strain	Methanogens	Culture medium	Source*
861	*Methanobrevibacter smithii*	119	DSMZ
3091	*Methanosphaera stadtmanae*	322	DSMZ

*Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany

Table 3 Results

#	Archaea	Concentration 3-NOP
1	*Methanobrevibacter smithii*	2.5 µM
2	*Methanosphaera stadtmanae*	5 µM

Example 2: Influence of various other nitrooxy compounds on the growth/ methane production of M. smithii

The experimental set up as outlined in example 1 above was applied to the testing of various further nitrooxy compounds shown in table 4 at concentrations of 2.5 µM, 25 µM, 250 µM.

Results: Table 4 shows the decrease (in %) of the growth/ methane production by the respective nitrooxy compound at the concentration level of 25 µM in M. smithii after 2 days, which decrease was maintained over the whole measurement period of 9.

Table 4 Results

#	Nitrooxy compound	Decrease (over 9d)
3	1,4-Bis-nitrooxybutane	41%
4	9-Nitrooxynonanol	43%
5	6-Nitroxyhexanoic acid	36%
6	3-Nitrooxy-propyl hexanoate	25%
7	Isosorbit-2-mononitrate	41%
8	Isosorbit-5-mononitrate	41%
9	Benzylnitrate	55%

10	N-[2-(Nitrooxy)ethyl]-3-pyridinecarboxamide	39%

Example 3: Enteric-coated tablets for oral application

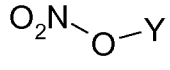
With the compounds according to the present invention such as in particular with the compounds outlined in table 1, enteric coated tablets for oral application can be prepared according to standard procedures in the art as illustrated in Table 5.

Table 5: Enteric-coated tablets

Common Name	**wt.-%**
Nitrooxy compound as listed in table 1	12
Cellulose, microcrystalline	ad 100
Copovidone (Kollidon® VA64 Fine)	6
Silicon dioxide	2
Magnesium stearate	1
Crospovidone (Kollidon® CL)	5
Enteric polymer, pH 5.5 Poly(methacrylic acid-co-ethylacrylate) 1:1 (EUDRAGIT®L 30D-55 + PlasAcryl™ HTP20)	13

Claims

1. A method for reducing and/or treating the symptoms of constipation, irritable bowel syndrome, discomfort from gaseous distention, and/or obesity in a human, said method comprising applying an effective amount of at least one nitrooxy compound of formula (I),



formula (I)

wherein Y has the molecular formula $C_aH_bO_dN_eS_g$, wherein

a is an integer between 1 and 25, preferably between 1 and 10

b is an integer between 2 and 51, preferably between 2 and 21

d is an integer between 0 and 10, preferably between 0 and 10

e is an integer between 0 and 5, preferably between 0 and 3

g is an integer between 0 and 3, preferably between 0 and 1,

or a pharmaceutically acceptable salt thereof to a human in need thereof.

2. The method according to claim 1, wherein the at least one organic molecule substituted at any position with at least one nitrooxy group is selected from the group consisting of 3-nitrooxypropanol, 9-nitrooxynonanol, 2-aminoethyl nitrate, 3-aminopropyl nitrate, 5-nitroxy pentanoic acid, 6-nitroxyhexanoic acid, bis-(2-nitrooxyethyl) ether, bis(2-hydroxyethyl)amine dinitrate, 1,3-bis-nitrooxypropane, 1,4-bis-nitrooxybutane, 1,5-bis-nitrooxypentane, 3-nitrooxy-propyl hexanoate, 3-nitrooxy-propyl 5-nitrooxy-hexanoate, 2-(hydroxymethyl)-2-(nitrooxymethyl)-1,3-propanediol, 3-bis(nitryloxy)-2,2-bis(nitryloxy-methyl)propan, N-ethyl-3-nitrooxy-propionic sulfonyl amide, 5-nitrooxy-pentanenitrile, 5-nitrooxy-pentane, 3-nitrooxy-propyl propionate, 3-nitrooxy-propyl benzoate, rac-4-phenylbutane-1,2-diyl dinitrate, benzylnitrate, N-[2-(nitrooxy)ethyl]-3-pyridinecarboxamide, 2-nitro-5-nitrooxymethyl-furan, 1,4:3,6-dianhydro-2,5-di-O-nitro-D-glucitol, 1,4:3,6-dianhydro-D-glucitol 5-mononitrate and 1,4:3,6-dianhydro-D-glucitol 2-mononitrate.

3. The method according to claim 1, wherein the at least one organic molecule substituted at any position with at least one nitrooxy group is selected from the group consisting of 3-nitrooxypropanol, 1,4:3,6-dianhydro-D-glucitol 5-mononitrate, 1,4:3,6-dianhydro-D-glucitol 2-mononitrate, 1,4:3,6-dianhydro-2,5-di-O-nitro-D-glucitol, 1,3-bis(nitryloxy)-2,2-bis(nitryloxy-methyl)-propan, N-[2-(nitrooxy)ethyl]-3-pyridinecarboxamide, 2-aminoethyl nitrate, bis(2-hydroxyethyl)amine dinitrate, 1,4-bis-nitrooxybutane, 6-nitroxy hexanoic acid, 3-nitrooxy-propyl hexanoate, benzylnitrate, 9-nitrooxynonanol and N-[2-(nitrooxy)ethyl]-3-pyridinecarboxamide.

4. The method according to claim 1, wherein the at least one organic molecule substituted at any position with at least one nitrooxy group is selected from the group consisting of 3-nitrooxypropanol, 1,4:3,6-dianhydro-D-glucitol 5-mononitrate (Isosorbit-5-mononitrate), 1,4:3,6-dianhydro-D-glucitol 2-mononitrate (Isosorbit-2-mononitrate), 1,4-bis-nitrooxybutane, 6-nitroxy hexanoic acid, 3-nitrooxy-propyl hexanoate, benzylnitrate, 9-nitrooxynonanol, N-[2-(nitrooxy)ethyl]-3-pyridinecarboxamide.

5. The method according to any one of claims 1 to 4, wherein the effective amount is selected in the range of 1 mg to 1000 mg, preferably in the range of 1 mg to 500 mg.

6. The method according to any one of claims 1 to 5, wherein the at least one organic molecule substituted at any position with at least one nitrooxy group or a pharmaceutically acceptable salt thereof is administered via a pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

7. The method according to claim 6, wherein the pharmaceutical composition is an oral composition.

8. The method according to claim 7, wherein the oral pharmaceutical composition is in the form of a tablet, a pill, a granule, a dragée, a capsule or an effervescent formulation.

9. The method according to any one of claims 6 to 8, wherein the at least one nitrooxy compound of formula (I) or a pharmaceutically acceptable salt thereof is administered via the pharmaceutical composition either in the form of a single dose or by multiple doses in an amount of at least 0.01 mg/ kg bodyweight/ day, preferably in an amount of 0.01-10 mg/ kg body weight/ day, most preferably in an amount of 0.01-5 mg/ kg body weight / day.

10. The method according to any one of claims 6 to 9, wherein the pharmaceutical composition is in the form of a controlled (delayed) release formulation and/ or in a form which is able to pass the stomach to release the nitrooxy compounds in the gut

11. A pharmaceutical formulation comprising at least one nitrooxy compound of formula (I) or a pharmaceutically acceptable salt thereof as defined in anyone of the preceding claims, characterized in that the formulation comprises a modified-release coating that is substantially stable in gastric fluids.

12. A pharmaceutical formulation comprising at least one nitrooxy compound of formula (I) or a pharmaceutically acceptable salt thereof as defined in anyone of the preceding claims, characterized in that the formulation comprises at least one a modified-release coating that is degraded by a microbial enzyme present in the gut flora

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/052851

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/21 A61K31/216 A61K31/22 A61K31/235 A61K31/275
A61K31/34 A61K31/4406 A61K9/28 A61P1/00 A61P1/10
A61P3/04

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, 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.
X	WO 99/16436 A1 (RHODES JOHN [GB]; EVANS BRIAN KENNETH [GB]) 8 April 1999 (1999-04-08) abstract page 1, line 1 - line 20 page 4, line 12 - page 5, line 24 page 8, line 9 - line 26 page 9, line 21 - page 11, line 23; claims -----	1-12
X	WO 2006/127591 A2 (NITROMED INC [US]; GARVEY DAVID S [US]) 30 November 2006 (2006-11-30) abstract page 72, line 16 - line 23 page 73, line 26 - page 74, line 3 page 75, line 21 - line 22; claims 1-3,6,7 ----- -/--	1-12

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"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

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 April 2017	13/04/2017

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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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