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(54) **Titre : COMPOSES CONTENANT UN PEPTIDE**
(54) **Titre : PEPTIDE CONTAINING COMPOUNDS**

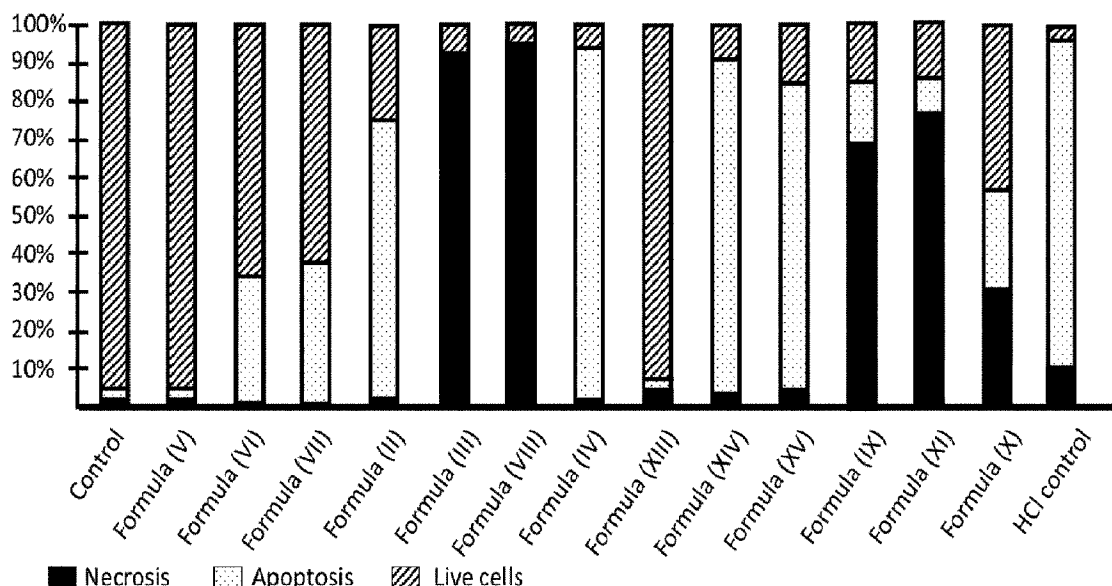


Fig. 1

(57) **Abrégé/Abstract:**

The present invention relates to the field of human therapy. In particular, the present invention relates to novel synthetic peptide-based chloride ion transporter and to compositions thereof, as well as methods of treating, reducing, inhibiting or controlling chloride ion channel related diseases.

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Abstract:

The present invention relates to the field of human therapy. In particular, the present invention relates to novel synthetic peptide-based chloride ion transporter and to compositions thereof, as well as methods of treating, reducing, inhibiting or controlling chloride ion channel related diseases.

PEPTIDE CONTAINING COMPOUNDS

FIELD OF THE INVENTION

The present invention relates to the field of human therapy. In particular, the present invention relates to novel synthetic peptide-based chloride ion transporters and to compositions thereof, as well as methods of treating, reducing, inhibiting or controlling chloride ion channel related diseases.

BACKGROUND OF THE INVENTION

Cell penetrating peptides (CPPs) or protein transduction domains (PTDs) are small peptides with less than 30 amino acid residues and of the appropriate size, charge and, polarity to pass through the cellular membrane. The main characteristics of these peptides include their ability to cross the cellular membrane using both endocytosis and energy-independent pathways, their high cellular permeability rates and their low cell toxicity and safety associated with little to no immunological response.

Currently, more than 1800 different CPPs have been reported and the vast majority of them have been experimentally tested for different applications. CPPs are classified according to the type of cargo, their physicochemical properties (cationic, hydrophobic, amphipathic), their internalization mechanism and their structural features (linearity or cyclic nature).

Cell penetrating peptides have been used for transporting various materials (peptides, proteins, DNA, RNA, etc.) through biological barriers such as plasma membranes (JP Richard, et.al., Journal of Biological Chemistry, 2003, 278, pp 585).

Despite the diversity of pathways and cell types targeted by CPP-based therapies, there are still no FDA approved CPP-conjugated drugs and several clinical trials have been discontinued to date. The problems associated with the use of CPP-conjugated drugs include: (1) in vivo stability issues due to susceptibility to proteolytic degradation; (2) immunogenicity issues; (3) poor efficiency due to the drug's failure to escape from endosomes after being internalized by cells; (4) toxicity due to the degradation of moieties; and (5) toxicity or poor efficiency due to the CPP's lack of site specificity. (L Gomes dos Reis, D Traini, Expert Opinion on Drug Delivery, 2020, 17(5), pp 647; J Habault, JL Poyet, Recent Advances in Cell Penetrating Peptide-Based Anticancer Therapies, Molecules, 2019, 24 (5), pp 927).

Intensive research efforts have been devoted to developing synthetic ion channels using artificial compounds. The main strategies are related to the synthesis of unimolecular channels or the design of self-assembled supramolecular channels. These synthetic ion transporters or ion channels could complement the impaired or lost function of cellular ion channels (N Busschaert, PA Gale, *Angewandte Chemie International Edition* 2013, 52, pp 1374) and thus be used for the treatment of channelopathies and related diseases.

Various artificial chloride transporters were developed with different molecular masses ranging from small organic molecules to supramolecular systems. These compounds either passively diffuse through the membrane with the chloride ion or form a channel in the membrane, facilitating passive ion transport. The general drawback of these compounds is their toxicity. It has been shown that some transporters increase intracellular sodium chloride concentrations, enhance cellular reactive oxygen species (ROS) levels, trigger the release of cytochrome c from the mitochondria and induce caspase activation, all of which results in apoptosis (SK Ko, et.al., *Nat Chem* 2014, 6, pp 885). On the other hand, it has been suggested that tumorous cells can be selectively killed by the utilization of artificial chloride transporters (Díaz de Greñu, et.al.; *Chemistry – A European Journal*, 2011, 17, pp 14074).

These compounds can be used in oncology as perturbing the chemical gradients within cells, triggers apoptosis, leading to the death of tumorigenic cells. Chloride transporters are effective in the case of leukemia, lymphoma, myelofibrosis, and mastocytosis (S Parikh, et.al; *Clinical Lymphoma Myeloma and Leukemia*, 2010, 10, pp 285).

Respiratory diseases such as chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis (CF), bronchiectasis, tuberculosis, and lung cancer are leading causes of death, with numbers increasing yearly. There is an unmet need for new tools that could facilitate the development of new therapies for lung-related diseases as this has been recognised as the largest therapeutic failure. The field of bioactive molecules (peptides, proteins, and nucleic acids) is an area in which potential new treatments for respiratory diseases could be developed (L Gomes dos Reis, D Traini, *Expert Opinion on Drug Delivery*, 2020, 17(5), pp 647).

CF is the most common autosomal recessive genetic disease characterized by multi-organ pathology and significantly decreased life expectancy caused by the impaired function or expression of CFTR. In CF, chloride transport is impaired due to genetic mutations of the *CFTR* gene leading to absent, or diminished function of the cystic fibrosis transmembrane conductance

regulator (CFTR) protein (BP O'Sullivan, SD Freedman, Lancet, 2009, 373, pp 1891). Recent therapeutic developments have significantly enhanced the life expectancy of patients with CF, yet the average age of death (usually caused by respiratory failure) is still 31.4 years (A Orenti, et al, ECFSPR Annual Report, 2016). In addition, 31% of the CF patients have chronic lung infections
5 caused by *Pseudomonas aeruginosa*, whereas 83% of all CF patients need pancreatic enzyme replacement therapy, resulting in a significant burden to both the patients and healthcare systems.

There is thus a significant need for the development of synthetic chloride ion transporters that could be potentially utilized in channel replacement therapy to treat diseases associated with
10 dysregulated anion transport, particularly those involving chloride ion.

Oral delivery of such therapeutic agents would be desirable, though this remains an unsolved challenge for drug formulators and drug delivery experts due to instability of the peptide-based bioactives in the GI tract, their low permeability and extremely rapid clearance (S Gupta et.al.,
15 Drug Delivery, 2013, 20, pp 237-246). Although cystic fibrosis is a systemic disease affecting – among others – the lungs, the digestive system and the reproductive system, lung infections and lung complications are the primary cause of death, accounting for up to 85% of the cases. (C Martin, et.al, Journal of Cystic Fibrosis, 2016, 15, pp 204-212). Abnormal mucus viscosity and production is known to contribute to CF pathogenesis (C Ehre et al, The International Journal of
20 Biochemistry & Cell Biology, 2014, 52, pp 136–145). Moreover, hyper-concentrated mucus with increased airway adhesion is known to induce CF-like disease in animal models (M Mall et al, Nature Medicine, 2004, 10, pp 487–493). Analysis of the bronchoalveolar lavage fluid of young CF patients indicated that abnormally viscous mucus accumulation, with increased total mucin and inflammatory factor concentrations, drives the early pathogenesis of CF disease (CR Esther et
25 al, Science Translational Medicine, 2019, 11, pp 1-11). The increase in mucus viscosity is due to the decreased cellular secretion of chloride ions, which results in an impaired fluid secretion and increased apical sodium absorption by the airway epithelial cells (H Li et.al., Current Opinion in Pharmacology, 2017, 34, pp 91-97). These alterations in ion transport ultimately result in the acidification and decreased height of the apical airway surface liquid.

30 In CF patients, ciliary movement is impaired due to these alterations and the viscous mucus layer cannot be removed from the smaller airways. This results in chronic cough and increased probability and frequency of lung infections. Hydration therapy has been demonstrated to correct CF sputum samples to near-normal viscoelasticity, reinforcing the clinical findings that
35 administration of hydrating agents yields beneficial results in patients with CF (BE Tildy and DF

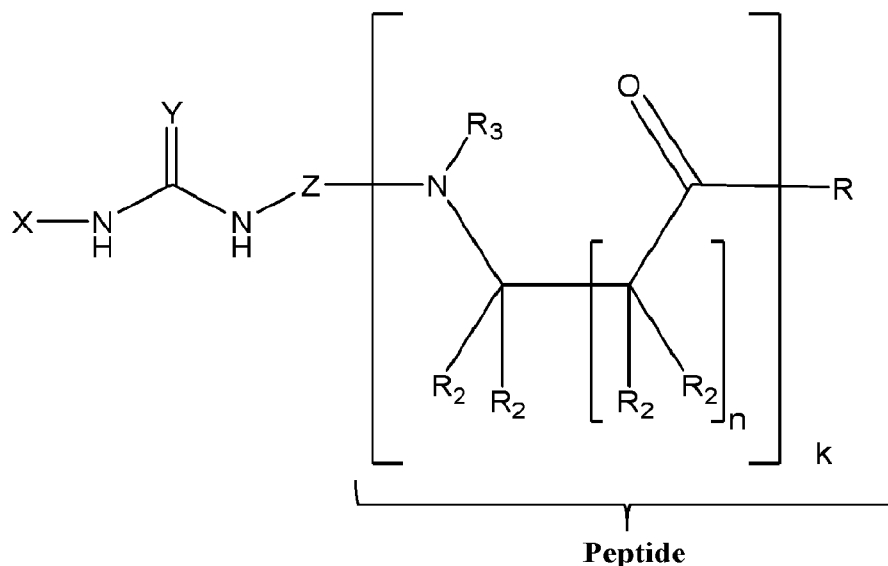
Rogers, Pharmacology, 2015, 95, pp 117-132). Therefore, a synthetic chloride ion transporter administered directly to the lungs could alleviate the symptoms associated with the highly viscous mucus layer (independent of the mutation causing the disease) by increasing the electrolyte levels of the layer and thus facilitating water transport out of the epithelial cells. Ultimately, this could lead to improved rheological properties of the mucus layer (D Schieppati, et.al, Respiratory Medicine, 2019, 153, pp 52-59). This is supported by the analysis of mucus samples, where it was shown that diluting (hydrating) mucus by a factor of 2 from 5.2% to 2.6% decreased the complex viscosity by a factor of eight (DB Hill et al, European Respiratory Journal, 2018, 52, pp 1-11).

Whilst VX (or ‘caftor’) compounds such as ivacaftor, lumacaftor, tezacaftor, elexacaftor and their combinations, as found in the marketed drug products Kalydeco, Orkambi, Symdeko and Trikafta, are effective in improving chloride ion transport, their use is limited to certain mutations of the CFTR gene. Moreover, there are numerous patients found to either not respond to or not tolerate such caftor therapies. Treatment of these patients is an unmet medical need, in which synthetic chloride ion transporters may play a major role. Also, combining these universally effective ion channel transporters with established cystic fibrosis treatments, may result in improved therapeutic outcomes.

SUMMARY OF THE INVENTION

The disclosure includes the following subject matter disclosed in the form of numbered “points”.

1. A compound of Formula (A):

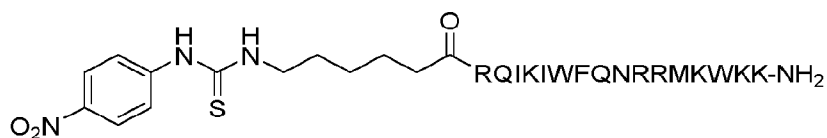


or pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof, wherein,

- 5 n= 0-10;
 k= 1-200;
 X= H, C1-10 alkyl or cycloalkyl, aryl, protecting group, C1-10 acyl, biotin, fluorescent and radioactive tracer, alkyl, cycloalkyl and acyl groups substituted with N, O, S, P, Se, Si, As or halides;
 10 Y= O, S, NH, CH₂, N-OR;
 Z= C1-10 alkyl or cycloalkyl, aryl, protecting group, C1-10 acyl, biotin, fluorescent and radioactive tracer, alkyl, cycloalkyl and acyl groups substituted with N, O, S, P, Se, Si, As or halides;
 R= H, OH, O-alkyl, NH₂, N-alkyl, SH, S-alkyl, alkyl, alkenyl, alkynyl, NH-NH₂,
 15 R₂ = H, C1-10 alkyl or cycloalkyl, aryl, these substituted with N, O, S, P, Se, Si, As or halides, and form a ring system, and glycosylated; and
 R₃ = H, C1-10 alkyl or cycloalkyl, aryl, these ideally substituted with N, O, S, P, Se, Si, As or halides, and may form ideally a ring system, and may be glycosylated, and stereoisomers including enantiomers, diastereomers, racemic
 20 mixtures, mixtures of enantiomers or combinations thereof, as well as polymorphs, tautomers, solvates, salts, esters and prodrugs thereof.

2. The compound as recited in Point 1, wherein said peptide domain comprises one or more positively charged residues.
3. The compound as recited in Points 1 or 2, wherein said peptide domain comprises arginine or lysine side-chains.
- 5 4. The compound as recited in any of Points 1 to 3, wherein said peptide domain comprises one or more cell membrane penetrating peptide (CPPs), such as cationic, amphipathic, hydrophobic or amphiphilic CPPs, preferably selected from the group consisting of PTD4, SP, pVEC, poly-arginine (arginine stretch), transportan, TAT, a translocationally active derivative or fragment of TAT and penetratin, or variants thereof, preferably selected from a CPP having

10 at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, or 95% identity to any of SEQ ID NOs: 1-16, and having cell penetrating activity, preferably selected from: residue 48-60 of TAT or penetratin, or variants thereof.
5. A compound as recited in any of Points 1 to 4, wherein said compound has Formula (I):

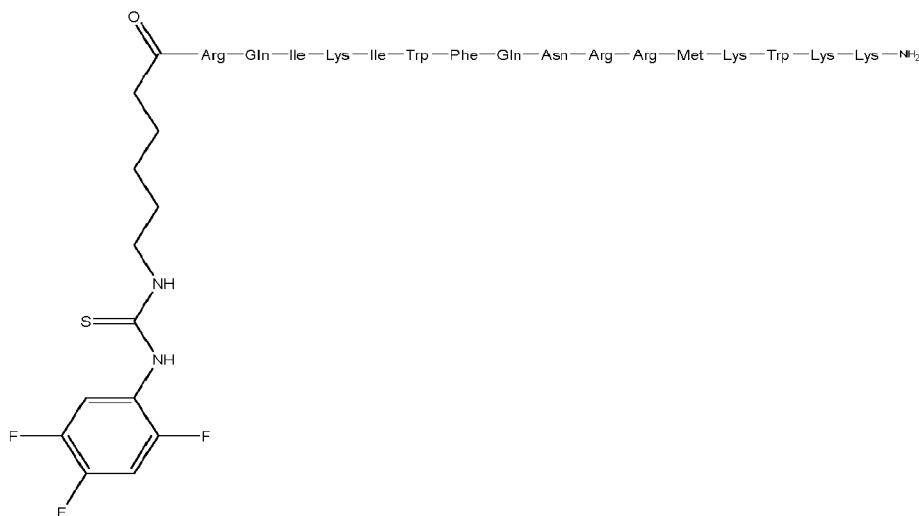


(I)

optionally wherein the molecular weight (MW) of the compound is 2539.4 Daltons.

6. A compound as recited in Point 5, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs,

20 tautomers, solvates, salts, esters, prodrugs or combinations thereof.
7. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (II):



(II)

optionally wherein the molecular weight (MW) of the compound is 2548.1 Daltons.

8. A compound as recited in Point 7, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

5 9. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (III):



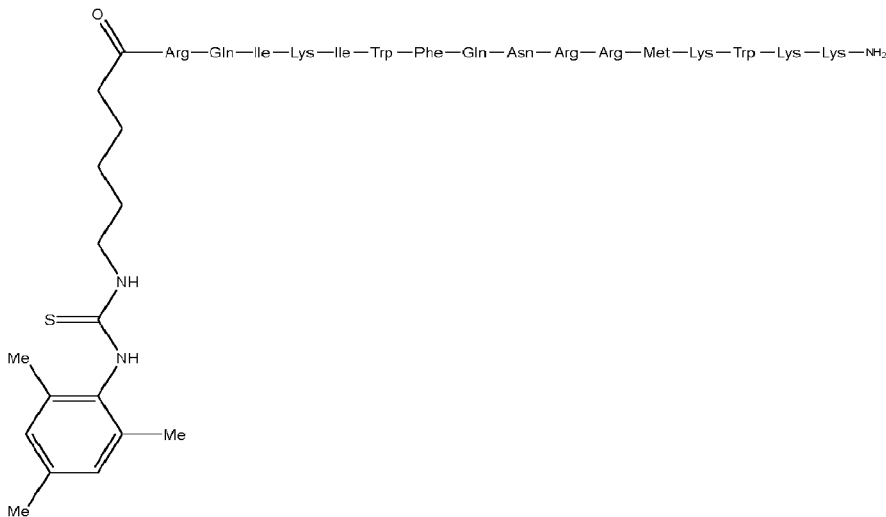
(III)

optionally wherein the molecular weight (MW) of the compound is 2548.1 Daltons.

10. A compound as recited in Point 9, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

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11. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (IV):



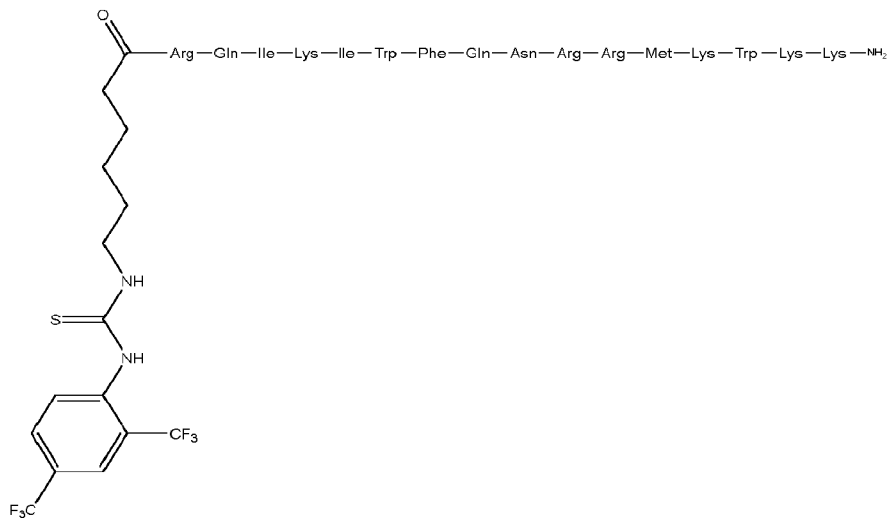
(IV)

optionally wherein the molecular weight (MW) of the compound is 2536.2 Daltons.

15

12. A compound as recited in Point 11, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

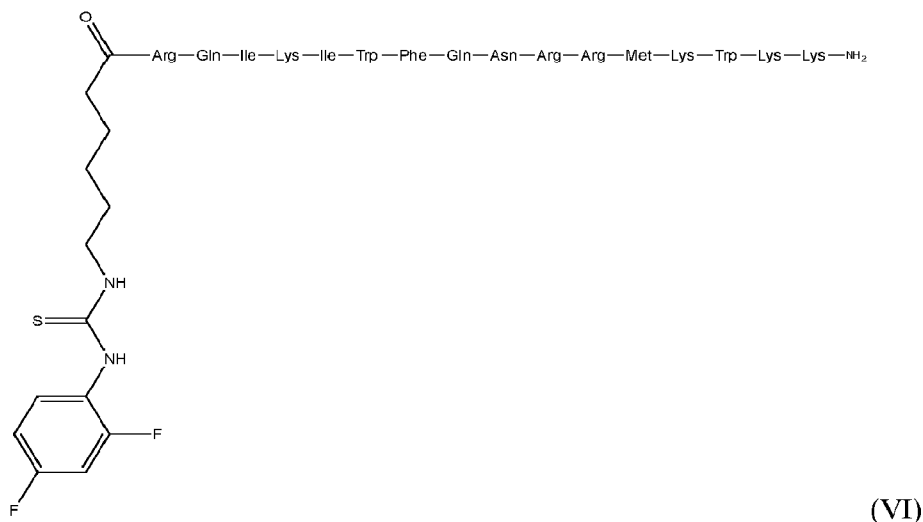
13. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (V):



optionally wherein the molecular weight (MW) of the compound is 2630.1 Daltons.

14. A compound as recited in Point 12, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

10 15. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (VI):

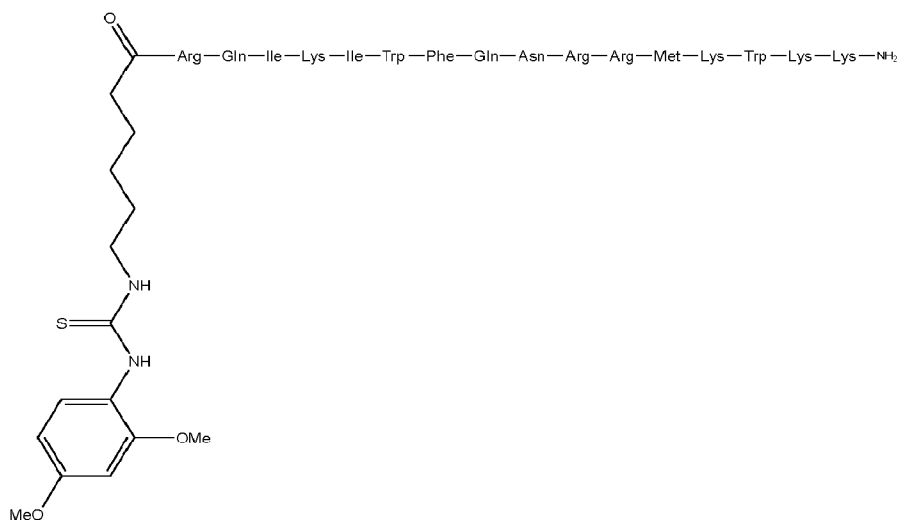


optionally wherein the molecular weight (MW) of the compound is 2530.1 Daltons.

16. A compound as recited in Point 15, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

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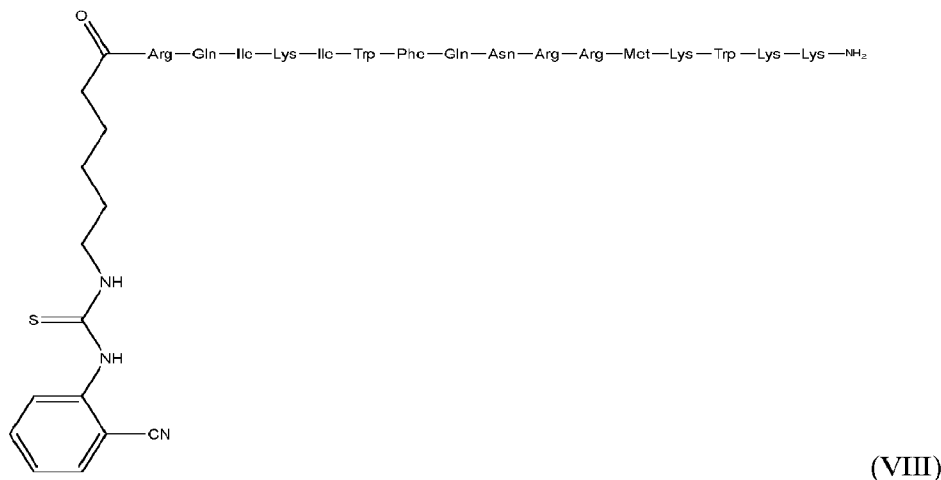
17. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (VII):



optionally wherein the molecular weight (MW) of the compound is 2554.2 Daltons.

- 5 18. A compound as recited in Point 17, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

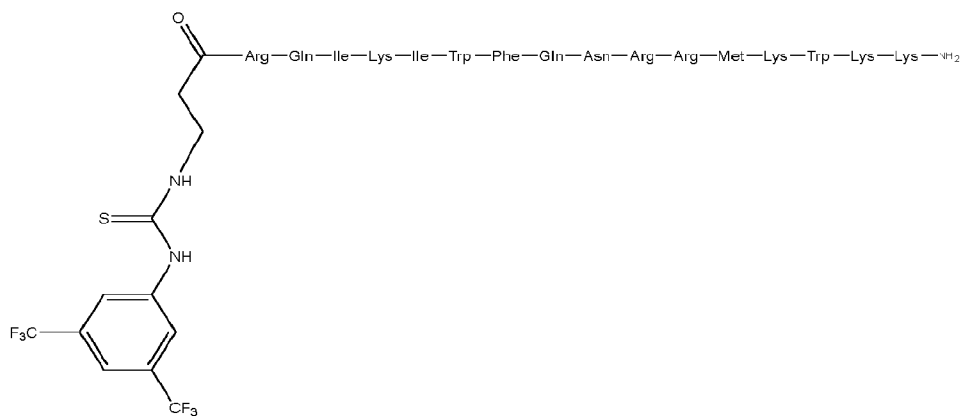
19. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (VIII):



- 10 optionally wherein the molecular weight (MW) of the compound is 2519.1 Daltons.

20. A compound as recited in Point 19, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

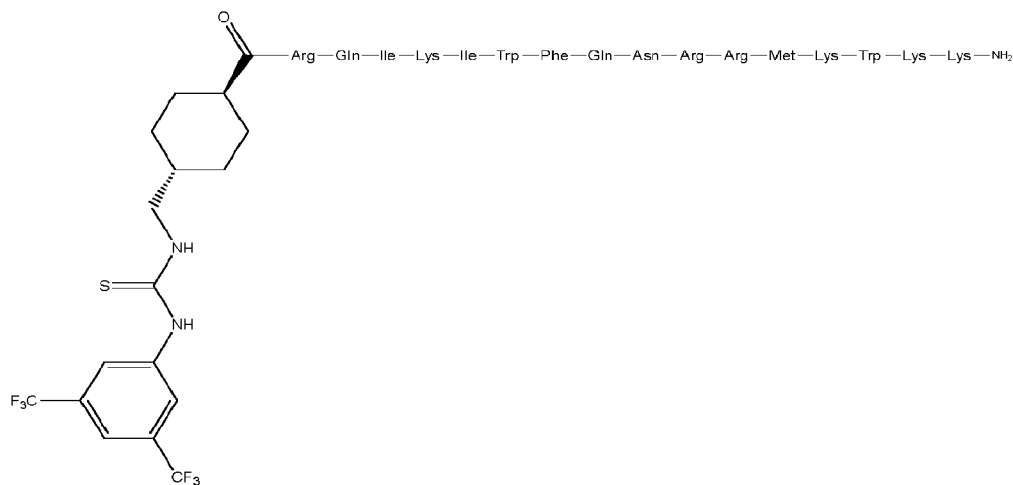
- 15 21. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (IX):



optionally wherein the molecular weight (MW) of the compound is 2588.0 Daltons.

22. A compound as recited in Point 21, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

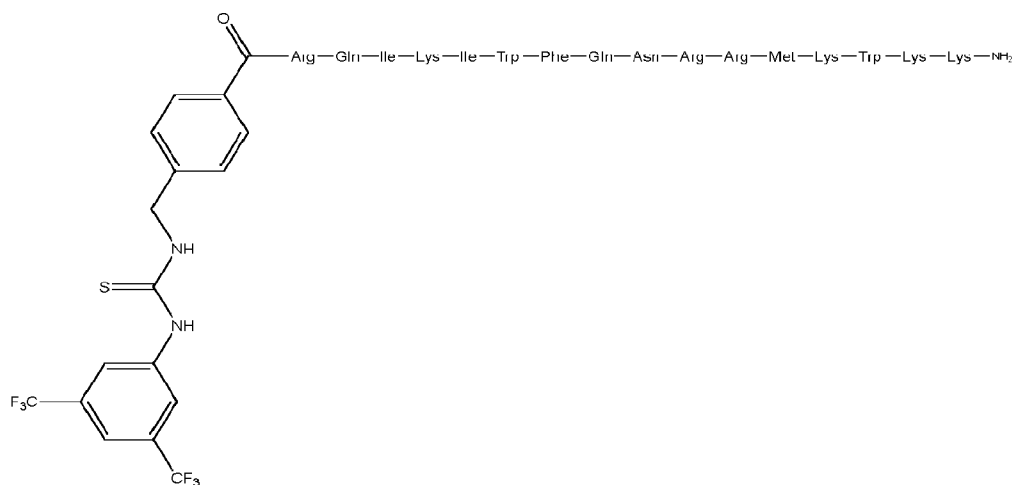
23. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (X):



optionally wherein the molecular weight (MW) of the compound is 2656.2 Daltons.

24. A compound as recited in Point 23, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

25. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (XI):

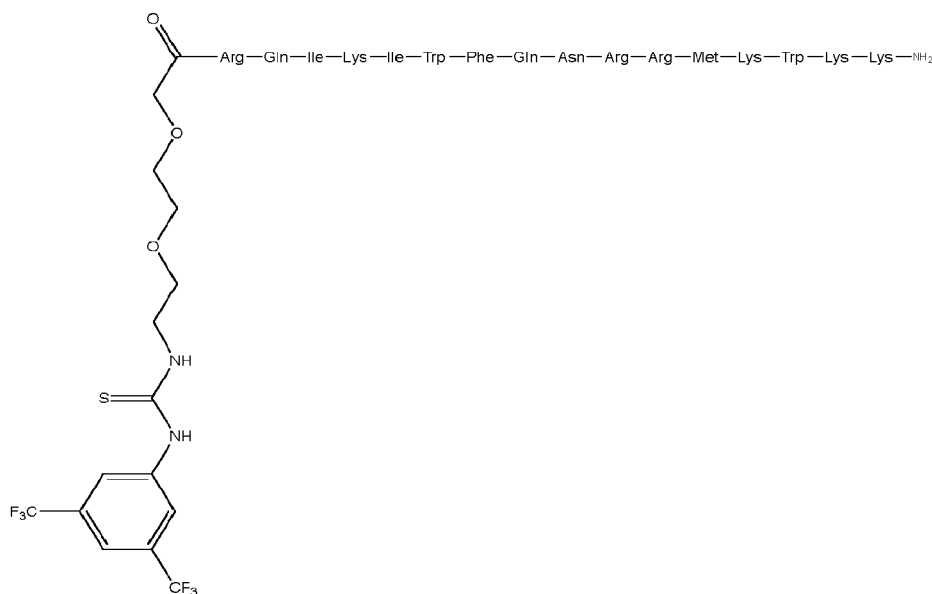


(XI)

optionally wherein the molecular weight (MW) of the compound is 2656.2 Daltons.

26. A compound as recited in Point 25, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

27. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (XII):

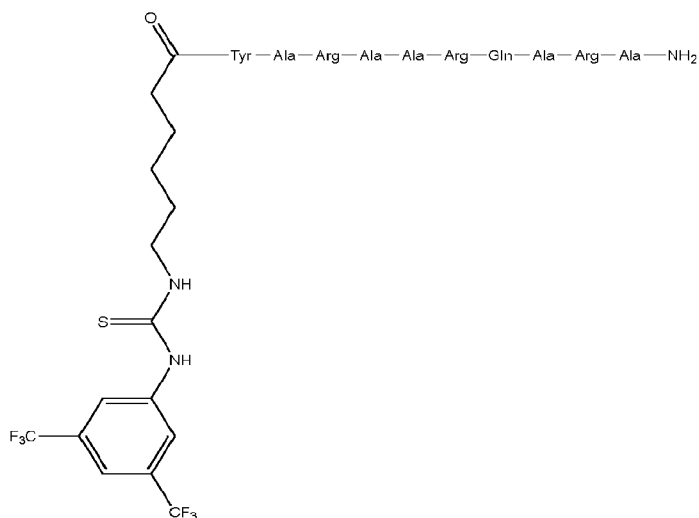


(XII)

optionally wherein the molecular weight (MW) of the compound is 2662.1 Daltons.

28. A compound as recited in Point 27, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

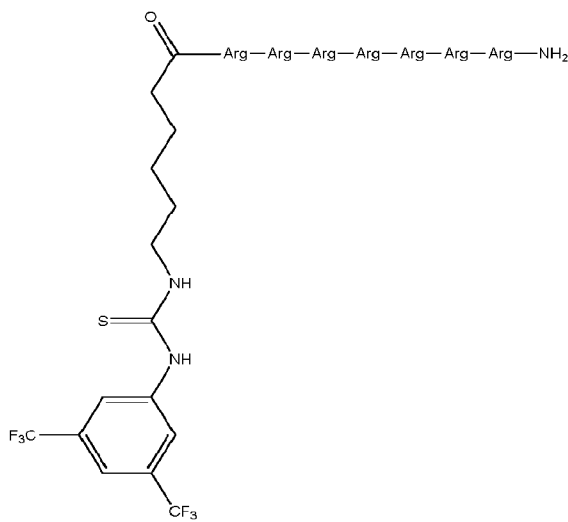
29. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (XIII):



optionally wherein the molecular weight (MW) of the compound is 1516.6 Daltons.

30. A compound as recited in Point 29, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

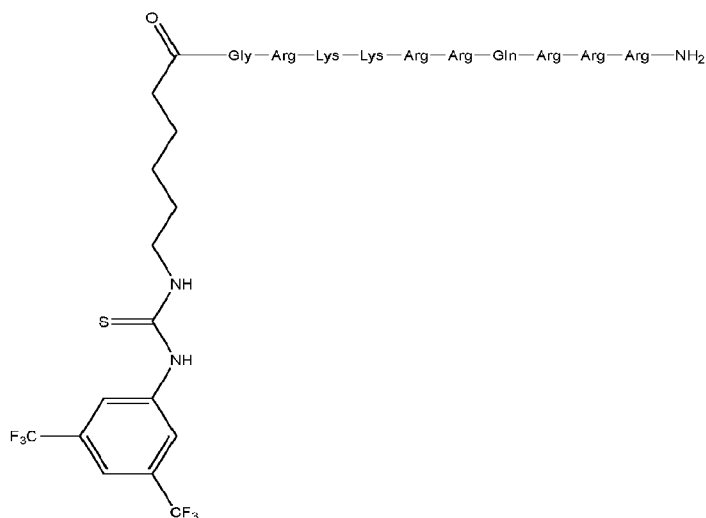
31. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (XIV):



optionally wherein the molecular weight (MW) of the compound is 1494.7 Daltons.

32. A compound as recited in Point 31, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

33. A compound as recited in any of Point 1 to 4, wherein said compound has Formula (XV):



(XV)

optionally wherein the molecular weight (MW) of the compound is 1780.0 Daltons.

34. A compound as recited in Point 33, wherein said compound is selected from pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.
35. A compound as recited in any of Points 1 to 34, wherein said compound does not induce apoptosis or necrosis in a concentration range from 100 nM to 100 μ M.
36. A compound as recited in any of Points 1 to 34, wherein said compound decreases the intracellular chloride ion concentration, when applied to HEK-293 or HeLa cells at a concentration between 100 nM and 10 μ M, optionally in a dose-dependent manner.
37. A pharmaceutical composition comprising a compound as recited in any of Points 1 to 34, preferably, wherein the pharmaceutical composition also comprises a pharmaceutically-acceptable diluent, excipient and/or carrier.
38. A pharmaceutical composition as recited in Point 37, wherein said pharmaceutical composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, ocular, otic, buccal, nasal, and topical administration; and/or formulated as a dosage form selected from the group consisting of liquid dispersions, gels, aerosols, ointments, creams, lyophilized formulations, tablets, capsules; and/or presented as a dosage form selected from the group consisting of controlled release formulations, fast melt formulations, delayed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations; and/or presented as an enema formulation, iontophoretic application, coating an implantable medical device; or combinations thereof.

39. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition according to Point 37 or 38, for use in the manufacture of a medicament for the treatment of a disease or condition.
40. A compound according to any of Points 1 to 34 or a pharmaceutical composition according to Points 37 or 38, for use in the treatment, reduction, inhibition or control of viscous sputum or mucus associated with cystic fibrosis in a human subject, wherein said compound or pharmaceutical composition increases the electrolyte content of said viscous mucus or sputum, such as chloride, optionally wherein said compound or pharmaceutical composition is administered to the lungs of said human subject by pulmonary or aerosol delivery as a solution or suspension in a liquid vehicle, or as a dry powder.
41. A compound or a pharmaceutical composition according to Point 39, wherein the medicament is for the treatment, reduction, inhibition or control of viscous sputum or mucus associated with cystic fibrosis in a human subject, wherein said medicament increases the electrolyte content of said viscous mucus or sputum, such as chloride, optionally wherein said medicament is administered to the lungs of said human subject by pulmonary or aerosol delivery as a solution or suspension in a liquid vehicle, or as a dry powder.
42. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in therapy.
43. A compound as recited in any of Points 1 to 34, or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with a chloride ion channel mutation that is involved in processes including the regulation of the membrane potential and excitability of neurons, skeletal, cardiac and smooth muscle, cell volume regulation, transepithelial ion transport, the acidification of intercellular and extracellular compartments, the cell cycle and different forms of cell death, such as apoptosis.
44. A compound as recited in any of Points 1 to 34, or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with a chloride ion channel mutation, wherein the disease is selected from: myotonia congenita Becker, Myotonia congenita Thomsen, Dystrophia myotonica 1, dystrophia myotonica 2, childhood absence epilepsy type 3, juvenile absence epilepsy, juvenile myoclonic epilepsy, epilepsy with grand mal seizures on awakening, Bartter Syndrome, Bartter syndrome with sensorineural deafness, Dent's disease, autosomal dominant osteopetrosis, autosomal recessive osteopetrosis, cystic fibrosis, idiopathic chronic pancreatitis, bronchiectasis, congenital bilateral absence of vas deferens, Best's disease, adult-onset vitelliform macular dystrophy, concentric annular macular dystrophy, cataract, juvenile myoclonic epilepsy, childhood absence epilepsy type 2,

generalized epilepsy with febrile seizures, severe myoclonic epilepsy in infancy, , insomnia and hereditary hyperekplexia.

45. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with a chloride ion channel mutation, wherein the chloride ion channel is selected from: voltage-gated chloride channel family (CLC), transmitter-gated GABA_A, glycine receptors, calcium-activated (CaCC) or calcium-dependent chloride ion channel (Ca-CIC), high (maxi) conductance chloride ion channel, cystic fibrosis transmembrane conductance regulator (CFTR), and volume-regulated channel mutations.

46. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with a voltage-sensitive chloride intracellular channel (CLIC) mutation.

47. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with a voltage-sensitive chloride intracellular channel (CLIC) mutation, wherein the voltage-sensitive chloride intracellular channel (CLIC) mutation is selected from the group of CLIC-1, CLIC-2, hCLIC-Ka (rCLIC-K1), hCLIC-Kb (rCLIC-K2); CLIC-3 to CLIC-5, and CLIC-6 and CLIC-7 chloride ion channel mutations.

48. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with a voltage-sensitive chloride intracellular channel (CLIC) mutation, wherein the disease is selected from: muscle, skeletal and bone diseases; kidney diseases, ear diseases, central nervous (CNS) diseases, eye diseases, gastrointestinal diseases, pulmonary diseases, cardiac diseases and liver diseases.

49. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with a voltage-sensitive chloride intracellular channel (CLIC) mutation, selected from: recessive and dominant myotonia, leukodystrophy, aldosteronism, diabetes insipidus, Bartter disease, Gitelman disease, CNS and retina degeneration, mental retardation, epilepsy, Dent's disease, proteinuria, impaired renal endocytosis, osteopetrosis, pulmonary hypertension, cardiac dysfunction, Alzheimer's disease, respiratory and gastrointestinal diseases, including asthma, chronic obstructive pulmonary disease, cystic fibrosis, pneumonia, colon colitis, cystic fibrosis intestinal mucous disease, ulcerative colitis, and gastrointestinal parasitic infection.

50. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with a transmitter-gated GABA_A or glycine receptor mutation.

51. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with a calcium-activated chloride ion channel mutation (CaCC or CaCIC).
52. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with a calcium-activated chloride ion channel mutation, selected from: of cystic fibroses, dry mouth and dry eye syndromes, asthma, diarrhea, hypertension, neuropathic pain and cancers.
53. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with high a (maxi) conductance chloride ion channel mutation.
54. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of diseases associated with high a (maxi) conductance chloride ion channel mutation, wherein said mutation is found in neurons, glia cells, cardiac muscle cells, lymphocytes, epithelia, macula densa cells of the kidney and human placenta syncytiotrophoblasts.
55. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with a volume-activated chloride channel mutation.
56. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a disease associated with a cystic fibrosis transmembrane conductance regulator (CFTR) mutation.
57. A compound as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 for use in the treatment of a CFTR-mediated disease selected from cystic fibrosis, asthma, smoke induced COPD, chronic bronchitis, rhinosinusitis, constipation, pancreatitis, pancreatic insufficiency, male infertility caused by congenital bilateral absence of the vas deferens (CBAVD), mild pulmonary disease, idiopathic pancreatitis, allergic bronchopulmonary aspergillosis (ABPA), liver disease, hereditary emphysema, mucopolysaccharidoses, chloride channelopathies such as myotonia congenita (Thomsen and Becker forms), Bartter's syndrome type III, Dent's disease, hyperekplexia, epilepsy, Primary Ciliary Dyskinesia (PCD), an inherited disorders of the structure and/or function of cilia, including PCD with situs inversus (also known as Kartagener syndrome), PCD without situs inversus and ciliary aplasia.
58. A method of treating, reducing, inhibiting or controlling viscous sputum or mucus associated with cystic fibrosis in a human subject, wherein said method comprises administration of a therapeutically-effective amount of a compound as recited in any of Points 1 to 34 or a

pharmaceutical composition as recited in Point 37 38 to a human subject in need thereof, wherein said method increases the electrolyte content of said viscous mucus or sputum, such as chloride, optionally wherein said compound or pharmaceutical composition is administered to the lungs of said human subject by pulmonary or aerosol delivery as a solution or suspension in a liquid vehicle, or as a dry powder.

59. A method of treating, reducing, inhibiting or controlling at least one sign or symptom of cystic fibrosis in a human subject, wherein said method comprises administration of a therapeutically effective amount of one or more compounds as recited in any of Points 1 to 34 or a pharmaceutical composition as recited in Point 37 or 38 to the human subject in need thereof, optionally in combination with one or more therapeutic agents, wherein said sign or symptom is associated with the airways or respiratory system and includes one or more of the following: abnormally viscous mucus accumulation; increased total mucin content; elevated inflammatory factor concentration; decreased cellular secretion of chloride ions; impaired fluid secretion; increased apical sodium absorption by airway epithelial cells; acidification and decreased height of the apical airway surface liquid; chronic cough; chronic lung infection, and combinations thereof.

60. A method of treating, reducing, inhibiting or controlling at least one sign or symptom associated with a chloride channelopathy in a human subject, wherein said method comprises administration of a therapeutically effective amount of one or more compounds as recited in any of Points 1 to 34, or a pharmaceutical composition or use as recited in Point 37 or 38, to a human subject in need thereof, optionally in combination with one or more therapeutic agents.

61. A compound or a pharmaceutical composition for use according to Point 39 or 42, wherein the disease or condition is associated with a chloride ion channel mutation.

62. A compound according to any one of Points 1 to 34, a pharmaceutical composition according to Point 37 or 38, a compound or pharmaceutical composition for use according to any one of Points 40 to 57 or method according to any of Points 58 and 59, wherein said compound is selected from Formula (I) to (XV), preferably selected from Formula (I), (II), (III) (IV), (V), (IX), (XI), (XIII), (XIV) and (XV), more preferably selected from Formula (III) (IV), (V), (IX), (XI), (XIII), (XIV) and (XV).

The term “identity” indicates the percentage of amino acid residues which are the same between two or more sequences, when the sequences are compared and aligned for maximum correspondence. The identity between sequences is determined over the full length of the peptides to be compared or a specified subsequence thereof, and can be determined using the local homology algorithm of Smith and Waterman (1981, Adv. Appl. Math. 2: 482), the homology

alignment algorithm of Needleman and Wunsch (1970, J. Mol. Biol. 48: 4430, the search for similarity method of Pearson and Lipman (1988, Proc. Natl. Acad. Sci., USA, 85: 2444), or computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by manual alignment and visual inspection. Other examples of algorithms that are suitable for determining percent sequence identity are the BLAST and BLAST 2.0 algorithms.

The term “peptide” as used herein, refers to a polymer of amino acid residues. The peptide may be, or may have a sequence which corresponds to, a fragment of a longer protein. The term also applies to amino acid polymers in which one or more amino acid residues is a modified residue, or a non-naturally occurring residue, such as an artificial chemical mimetic of a corresponding naturally-occurring amino acid, as well as to naturally occurring amino acid polymers.

The term “variant” as used herein means a functional variant, having the same function as the non-variant. In the context of the peptide domain or a protein, a variant may have sequence alternations compared to the non-variant sequence but retains the biological activity of the non-variant sequence. In particular, a functional variant of one of the proteins, peptides or amino acid sequences disclosed herein has at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to the reference protein, peptide or amino acid sequence, and additionally has the same function as that protein, peptide or amino acid sequence, preferably, cell-penetrating activity.

The term “poly-arginine” as used herein includes a peptide chain containing more than one amino acid residue, wherein all of the amino acid residues in the chain are arginine.

The term “pharmaceutical composition”, as used herein, means a pharmaceutical preparation suitable for administration to an intended human or animal subject for therapeutic purposes.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph showing the effect of compounds (II)-(XI) and (XIII)-(XV) on the viability of HeLa cell. The graph shows the percentage of live, apoptotic and necrotic cells in the cell population.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO: 1 is a fragment of TAT consisting of residues 48-60 of TAT.

SEQ ID NO: 2 is a fragment of TAT consisting of residues 49-57 of TAT

SEQ ID NO: 3 is a fragment of TAT consisting of residues 47-58 of TAT.

SEQ ID NO: 4 is a fragment of TAT comprising residues 48-60 of TAT.

SEQ ID NO: 5 is the amino acid sequence of penetratin.

SEQ ID NO: 6 is a variant of penetratin having W-to-F amino acid substitution at position 6 of

5 SEQ ID NO: 5, referred to herein as “penetratin variant W48F”.

SEQ ID NO: 7 is a variant of penetratin having a W-to-F amino acid substitution at position 14 of

SEQ ID NO: 5, referred to herein as “penetratin variant W56F”.

SEQ ID NO: 8 is the amino acid sequence of a variant of penetratin (SEQ ID NO: 5).

SEQ ID NO: 9 is the amino acid of transportan.

10 SEQ ID NO: 10 is the amino acid sequence of transportan-27.

SEQ ID NO: 11 is the amino acid sequence of transportan-22.

SEQ ID NO: 12 is the amino acid sequence of Pep-1.

SEQ ID NO: 13 is the amino acid sequence of Pep-2.

SEQ ID NO: 14 is the amino acid sequence of a fragment of TAT.

15 SEQ ID NO: 15 is the amino acid sequence of PTD4.

SEQ ID NO: 16 is the amino acid sequence of R7 poly-arginine.

DETAILED DESCRIPTION OF THE INVENTION

20 The present invention arises because novel synthetic chloride ion transporters have surprisingly been found, and these synthetic chloride ion transporters enable the transport of chloride ions across cell membranes. These chloride ion transporters are therefore expected to be useful for the treatment of diseases and conditions associated with chloride ion channel mutations which affect the function and/or expression of the chloride ion channels.

25 Cell-penetrating peptides are small oligopeptides typically comprising between 5 and 30 amino acid residues. They are generally positively charged and are known to possess a random conformation in aqueous environment, however in the non-polar cell membrane, they show a tendency to fold into helical conformations (C Bechara, S Sagan, FEBS Letters, 2013, 587, pp 1693). They can pass through membranes either by a direct pathway or by a vesicular mode via
30 endocytosis. Cell-penetrating peptides are known to transport various cargos ranging from small organic molecules to gene encoding DNAs (JP Richard, et.al, Journal of Biological Chemistry, 2003, 278, pp 585).

Synthetic ion transporters or ion channels could mimic the function of natural ion channels, thus
35 rendering the unmet clinical need for channel replacement therapy feasible (N Busschaert, PA

Gale, Angewandte Chemie International Edition, 2013, 52, pp 1374.). The development of lipid-bilayer chloride ion transporters for potential use in channel replacement therapy for the treatment of diseases caused by dysregulation of anion transport, such as cystic fibrosis (CF), is an area of current interest. In CF, the impaired chloride transportation is the main cause of the illness affected by the monogenic mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel (BP O'Sullivan, SD Freedman, Lancet, 2009, 373, pp 1891).

CPP-based treatments may be combined with currently used therapies in CF, as the mechanism of action of each is completely different and result in synergy. CPP therapies can enhance the effect of mucolytic drugs and airway clearance techniques, as the application of CPP may increase the hydration of mucus. Synergistic effects are found in the combined application with VX compounds as CPP-based chloride ion transport is independent from the presence of functional CFTR in the membrane.

Various synthetic chloride transporters have previously been developed, with different molecular masses ranging from small organic molecules to supramolecular systems. These compounds either passively diffuse through the membrane with the chloride ion or form a channel in the membrane, opening the way for passive ion transport (N Busschaert, PA Gale, Angewandte Chemie International Edition 2013, 52, pp 1374).

Chloride ion channels are a functionally and structurally diverse group of anion selective channels involved in processes including the regulation of the excitability of neurons, skeletal, cardiac and smooth muscle, cell volume regulation, transepithelial salt transport, the acidification of internal and extracellular compartments, the cell cycle and apoptosis.

Chloride ion channels can be classified as members of the voltage-sensitive CLIC subfamily, transmitter-gated GABA_A, and glycine receptors, calcium-activated chloride ion channels (CaClC) such as TMEM16A, high (maxi) conductance chloride ion channels, the cystic fibrosis transmembrane conductance regulator (CFTR), and volume-regulated channels.

The mammalian CLIC family contains 9 members that fall into three groups; CLIC-1, CLIC-2, hCLIC-Ka (rCLIC-K1) and hCLIC-Kb (rCLIC-K2); CLIC-3 to CLIC-5, and CLIC-6 and CLIC-7. CLIC-1 and CLIC-2 are plasma membrane chloride channels. ClC-Ka and ClC-Kb are also plasma membrane channels (largely expressed in the kidney and inner ear) when associated with barttin (BSND, Q8WZ55). The localisation of the remaining members of the CLIC family is likely

to be predominantly intracellular in vivo, although they may traffic to the plasma membrane in overexpression systems. Numerous recent reports indicate that CLIC-4, CLIC-5, CLIC-6 and CLIC-7 (and by inference CLIC-3) function as Cl^-/H^+ antiporters (secondary active transport), rather than classical Cl^- channels. The activity of CLIC-5 as a Cl^-/H^+ exchanger is important for renal endocytosis. (“Chloride channels.” British Journal of Pharmacology 2009, vol. 158, Suppl 1)

The role of CLICs in tubulogenesis and angiogenesis is well established (GR Shubha, P Neel J., S Harpreet, Frontiers in Physiology, 2020, Vol. 11, pp 96). One of the most significant pathological roles discovered for CLICs was their involvement in pulmonary hypertension (PH). PH is characterized by a loss of vasodilator influences in the pulmonary circulation, which results in pathogenic vasoconstriction, and remodeling of small intrapulmonary arteries, leading to eventual right heart failure. CLIC4 was found to be highly expressed in the pulmonary vascular endothelium of PH patients. In the heart, CLICs are extensively expressed in several types of cells. CLIC1, CLIC4, and CLIC5 were localized in adult cardiomyocytes, and further CLIC4 and CLIC5 were localized to mitochondrial membranes. CLIC5 is the first chloride channel to be identified up to the molecular level in the inner membrane of cardiac mitochondria. The first member of the CLIC family to directly implicate them in cardiac dysfunction was CLIC2. A mutation in CLIC2 (c.303C>G, p.H101Q) was found to be associated with X-linked intellectual disability (ID), atrial fibrillation, cardiomegaly, congestive heart failure (CHF), and seizures. CLIC2 is known to interact with ryanodine receptor (RyR) proteins and inhibit its activity. In blood cells, CLIC1 is known to promote platelet function, promote adhesive functions in platelets as well as endothelial cells, and is critical for vascular repair and angiogenesis. One of the major physiological roles of CLIC5 is implicated in hearing impairment. At molecular levels, CLIC5 is shown to work with cytoskeletal elements such as radixin, protein tyrosine phosphatase receptor Q, taperin, and myosin VI. These interactions are essential to stabilize membrane-cytoskeletal attachments at the base of the hair bundle. Absence of CLIC5 compromises the stability of hair bundles either by disrupting cytoskeletal filaments or by disrupting chloride ion transport in hair cells, resulting in the progressive loss of integrity of these vital structures.

Chloride intracellular ion channel proteins are known to be present in neurons and astrocytes. At the functional level, CLIC1 is characterized for its role in Alzheimer’s disease (AD) where it is present in activated microglia.

CLCA1 is a member of the CLCA (calcium-activated chloride channel regulator) family and plays an essential role in goblet cell mucus production from the respiratory tract epithelium.

CLCA1 also regulates Ca^{2+} -dependent Cl^- transport that involves the channel protein

transmembrane protein 16A (TMEM16A) and its accessory molecules. CLCA1 modulates epithelial cell chloride current and participates in the pathogenesis of mucus hypersecretory-associated respiratory and gastrointestinal diseases, including asthma, chronic obstructive pulmonary disease, cystic fibrosis, pneumonia, colon colitis, cystic fibrosis intestinal mucous disease, ulcerative colitis, and gastrointestinal parasitic infection.

The Ca^{2+} -activated chloride ion channels (CaCCs) are found in almost all species ranging from invertebrates to mammals, and the ubiquitous expression of CaCCs indicates a variety of functions important for physiology, including regulation of epithelial Cl^- secretion, excitability of neuronal and cardiac cells, smooth muscle contraction and nociception. The molecular identity of the Ca^{2+} -activated chloride ion channels (CaCCs) remained elusive until anoctamin 1 (ANO1) and anoctamin 2 (ANO2) were identified as CaCCs in 2008. ANO1 and ANO2 possess the same properties as endogenous CaCCs. These properties include anion selectivity, submicromolar sensitivity to intracellular Ca^{2+} , voltage activation at low Ca^{2+} concentrations and inhibition by the pharmacological agents niflumic acid (NFA) and 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) (J Berg, H Yang, LY Jan, J Cell Sci. 2012;125(Pt 6), pp 1367-1371).

ANO1, as a CaCC, is primarily expressed in epithelial cells, smooth muscle cells and sensory neurons. ANO2, also as a CaCC, is expressed in the olfactory sensory neurons, photoreceptor synaptic terminals, hippocampal pyramidal neurons, thalamocortical neurons, and inferior olive neurons in the brain. Other ANOs family members including ANO6 and ANO7 were onetime considered to be CaCCs¹², but evidence shows that ANOs3–7 neither generate Ca^{2+} -activated Cl^- currents nor traffic into membrane, indicating that they are endoplasmic reticulum proteins (Y Liu, Z Liu, K Wang, Acta Pharmaceutica Sinica B., 2020).

ANO1 high expression or hyperactivity can cause inflammatory diseases such as asthma and diarrhea. Outward flow of Cl^- through the activation of ANO1 in smooth muscle cells causes depolarization and smooth muscle contraction. ANO1 high expression or hyperactivity is responsible for asthma diarrhea, and hypertension. In DRG sensory neurons, activation of ANO1 by intracellular Ca^{2+} or heat causes Cl^- efflux and increases neuronal excitability for induction of neuropathic pain. The functional coupling between TRPV1 and ANO1 is also involved in nociception. In cancer cells, ANO1 upregulation promotes cell proliferation and migration, whereas ANO1 downregulation induces apoptosis through multiple signaling pathways, including EGFR/MAPK signaling pathway⁹⁸, CaMKII/MAPK signaling pathway⁵¹, TGF- β signaling pathway¹²⁰ and NF- κ B signaling pathway. Pharmacological activation of ANO1 by activators or

potentiators may serve as a therapeutic strategy for treatment of CF, dry mouth and dry eye syndromes, and inhibition of ANO1 by inhibitors may be beneficial for ANO1 related channelopathies including asthma, diarrhea, hypertension, neuropathic pain and cancers.

- 5 Maxi Cl^- channels are high conductance, anion selective, channels initially characterised in skeletal muscle and subsequently found in many cell types including neurones, glia, cardiac muscle, lymphocytes, secreting and absorbing epithelia, macula densa cells of the kidney and human placenta syncytiotrophoblasts.
- 10 CFTR, a 12TM, ABC type protein, is a cAMP-regulated epithelial cell membrane chloride ion channel involved in normal fluid transport across various epithelia. The most common mutation in CFTR (i.e. the deletion mutant, ΔF508) results in impaired trafficking of CFTR and reduces its incorporation into the plasma membrane causing cystic fibrosis. Channels carrying the ΔF508 mutation that do traffic to the plasma membrane demonstrate gating defects. In addition to acting
15 as an anion channel per se, CFTR may act as a regulator of several other conductances including inhibition of the epithelial Na channel (ENaC), calcium-activated chloride channels (CaCC) and Volume-regulated anion channel (VRAC), activation of the outwardly rectifying chloride channel (ORCC), and enhancement of the sulphonylurea sensitivity of the renal outer medullary potassium channel (ROMK2). CFTR also regulates TRPV4, which provides the Ca^{2+} signal for regulatory
20 volume decrease (RVD) in airway epithelia. The activities of CFTR and the chloride-bicarbonate exchangers SLC26A3 (DRA) and SLC26A6 (PAT1) are mutually enhanced by a physical association between the regulatory (R) domain of CFTR and the STAS domain of the SCL26 transporters, an effect facilitated by PKA-mediated phosphorylation of the R domain of CFTR. Volume activated chloride channels (VSOAC) participate in regulatory volume decrease (RVD)
25 in response to cell swelling. VRAC may also be important for several other processes including the regulation of membrane excitability, transcellular Cl^- transport, angiogenesis, cell proliferation, necrosis, apoptosis, glutamate release from astrocytes, insulin release from pancreatic β cells and resistance to the anti-cancer drug, cisplatin.
- 30 Currently more than 2000 *CFTR* gene mutations have been described, whereas only 159 mutations have been characterized in terms of disease liability (R Bolia, et al., J Paediatr Child Health, 2018, 54, pp 609). The most common mutation type in 85% of patients worldwide is the deletion of phenylalanine at position 508 (F508del), however to date mutations are classified into seven different groups according to the CFTR defect caused (K De Boeck, MD Amaral, Lancet
35 Respir Med, 2016, 4, pp 662). Class I mutations, which include frameshift, splicing or nonsense

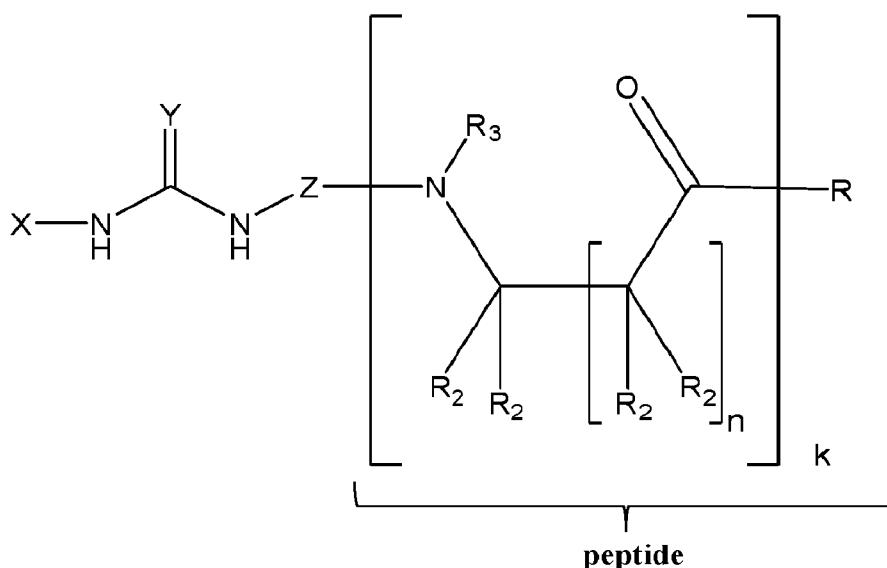
mutations that introduce premature termination codons; Class II mutations, which lead to misfolding and impaired protein biogenesis at the endoplasmic reticulum (ER); Class V mutations which result in reduced synthesis due to promoter or splicing abnormalities; and Class VI mutations that destabilize the CFTR channel in post-ER compartments and/or at the plasma membrane. Whereas Class III and IV mutations impair the gating and channel pore conductance respectively, thus selectively compromising CFTR function. In Class VII mutations, no mRNA can be detected. Current clinical treatment of CF is based on CFTR modulator therapy.

CFTR modulators include ivacaftor (Kalydeco®), lumacaftor/ivacaftor (Orkambi®), tezacaftor/ivacaftor (Symdeko®), and elexacaftor/tezacaftor/ivacaftor (Trikafta™). These drugs can increase the open state probability of CFTR and thus increase the ion efflux through the channel pore, or can promote the CFTR protein folding. Although these drugs have beneficial effects, their clinical use is restricted to limited patient populations with specific types of CFTR gene mutations. Channel replacement therapy with synthetic chloride ion transporters based on CPPs could overcome the severe limitation of current treatments, since such synthetic chloride transporters may promote chloride efflux across biological barriers even in the complete absence of CFTR protein. Therefore, chloride channel replacement therapy may provide mutation independent treatment, as the CFTR protein is not needed for chloride ion transport, and could therefore be used early in patients, whereby their patient-specific mutations do not have to be characterized prior to commencement of such therapies (as is the current standard of care). In addition, there are several patients with extremely rare mutations, which are not yet classified under the current mutation classes. CPP-based treatment may be applied in these patients without any clear restrictions.

Synthetic chloride ion transporter compounds of the present invention either passively diffuse through the membrane with the chloride ion or form a channel in the membrane, opening the way for passive ion transport.

Synthetic chloride ion transporters can be used in a mutation-independent way; thus, all patients having a disease or condition associated with a chloride ion channel mutation, such as CF patients, may be treated using the compounds and pharmaceutical compositions according to the invention.

Compounds described herein include those having the general formula A as follows:



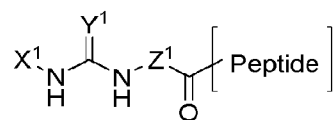
Formula (A)

wherein,

- 5 n= 0-10;
 k= 1-200;
 X= H, C1-10 alkyl or cycloalkyl, aryl, protecting group, C1-10 acyl, biotin,
 fluorescent and radioactive tracer, alkyl, cycloalkyl and acyl groups can be
 substituted with N, O, S, P, Se, Si, As or halides;
 10 Y= O, S, NH, CH₂, N-OR;
 Z= C1-10 alkyl or cycloalkyl, aryl, protecting group, C1-10 acyl, biotin,
 fluorescent and radioactive tracer, alkyl, cycloalkyl and acyl groups can be
 substituted with N, O, S, P, Se, Si, As or halides;
 R= H, OH, O-alkyl, NH₂, N-alkyl, SH, S-alkyl, alkyl, alkenyl, alkynyl, NH-NH₂;
 15 R₂ = H, C1-10 alkyl or cycloalkyl, aryl, these ideally substituted with N, O, S, P,
 Se, Si, As or halides, and may form ideally a ring system, and may be
 glycosylated, further including pharmaceutically acceptable stereoisomers,
 enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers,
 solvates, salts, esters, prodrugs or combinations thereof.
 20 R₃= H, C1-10 alkyl or cycloalkyl, aryl, these ideally substituted with N, O, S, P,
 Se, Si, As or halides, and may form ideally a ring system, and may be
 glycosylated,

further including pharmaceutically acceptable stereoisomers, enantiomers, diastereomers, racemic mixtures, polymorphs, tautomers, solvates, salts, esters, prodrugs or combinations thereof.

5 In an aspect, the invention provides a compound according to formula Ia:



Formula Ia

or a pharmaceutically acceptable salt thereof, wherein

X^1 is a C_{6-10} aryl group, optionally substituted with from 1 to 3 substituents, each
10 substituent independently selected from NO_2 , CN, halo, C_{1-4} alkyl, C_{1-4} alkoxy and C_{1-3} haloalkyl;

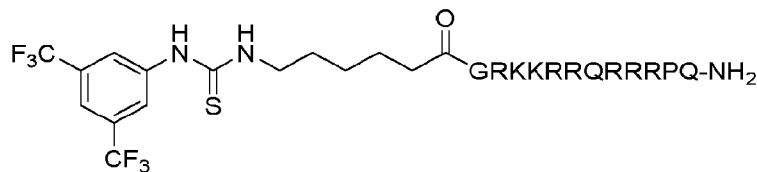
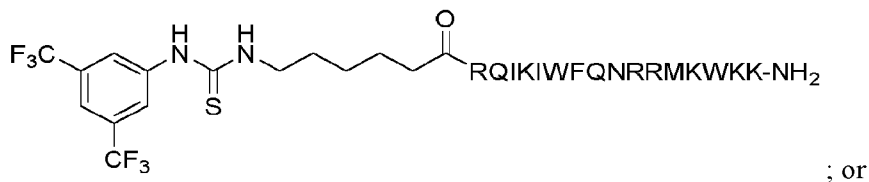
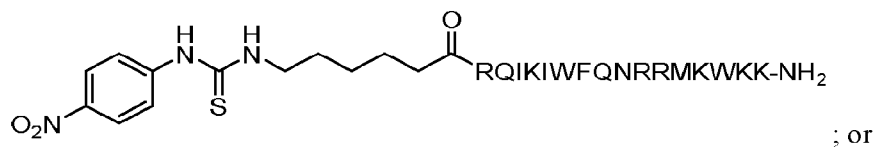
Y^1 is S or O;

Z^1 is C_{1-10} alkylene, C_{5-10} cycloalkylene, C_{2-10} heteroalkylene, or C_{6-10} aralkylene; and

the peptide domain comprises a cell penetrating peptide (CPP); and

wherein the -OH at the C-terminus of the peptide domain is optionally substituted by R^a ,
15 wherein R^a is selected from $-\text{NH}_2$, $-\text{O}-\text{C}_{1-6}$ alkyl, $-\text{NHC}_{1-6}$ alkyl, and $-\text{NH}-\text{NH}_2$;

provided the compound of formula Ia is not

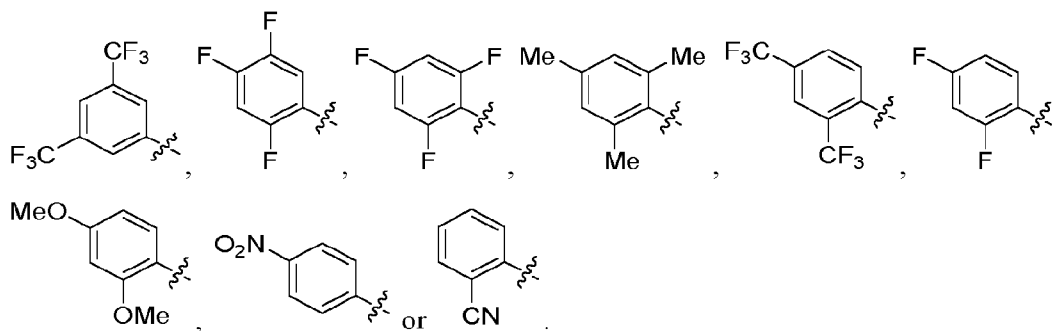


The C₆₋₁₀aryl group in X¹ may be phenyl, optionally substituted with from 1 to 3 substituents, each substituent independently selected from NO₂, CN, halo, C₁₋₄alkyl, C₁₋₄alkoxy, and C₁₋₃haloalkyl. The C₁₋₄alkyl, C₁₋₄alkoxy, and C₁₋₃haloalkyl are typically methyl, methoxy and trifluoromethyl (i.e. CF₃).

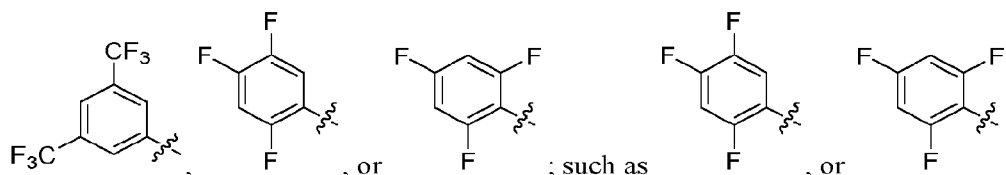
In preferred embodiments, the C₆₋₁₀aryl group in X¹ (e.g. phenyl) is substituted. There may be three substituents. There may be two substituents. There may be one substituent. The substituent(s) may in some embodiments be independently selected from CN, halo, C₁₋₄alkyl, C₁₋₄alkoxy, and C₁₋₃haloalkyl. The C₁₋₄alkyl, C₁₋₄alkoxy, and C₁₋₃haloalkyl are typically methyl, methoxy and trifluoromethyl (i.e. CF₃). The substituent(s) may be independently selected from CN, halo (e.g. F), methyl, methoxy and CF₃.

In some exemplary embodiments, the substituents at X¹ are each halo, e.g. F, such as in the case of bis- or tris-fluoro substituted phenyl. In some exemplary embodiments, the substituents are each C₁₋₄alkyl, e.g. methyl, such as in the case of tris-methyl substituted phenyl. In some exemplary embodiments, the substituents are each C₁₋₄alkoxy, e.g. methoxy, such as in the case of bis-methoxy substituted phenyl. In some exemplary embodiments, the substituents are each C₁₋₃haloalkyl, e.g. trifluoromethyl, such as in the case of bis-trifluoromethyl substituted phenyl. Where the substituent is or includes CN, the CN may be the sole substituent, e.g. in the case of mono-cyano substituted phenyl.

X¹ may be



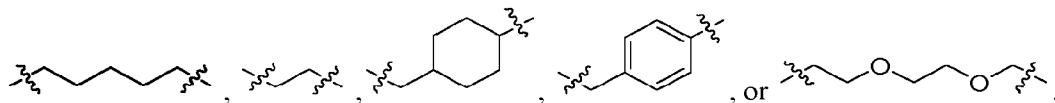
X¹ may be



Y^1 is preferably S.

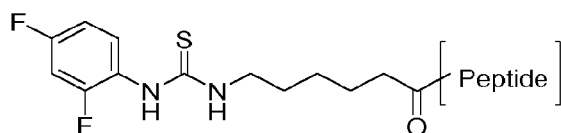
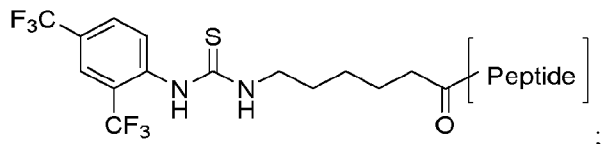
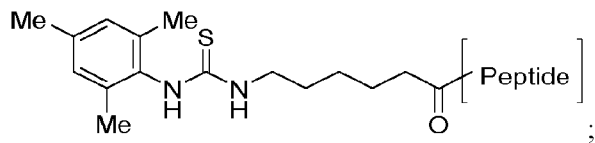
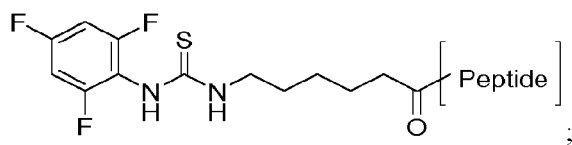
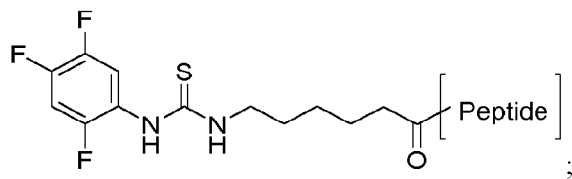
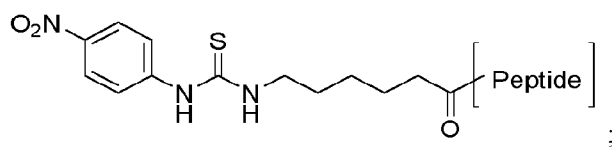
Z^1 may be C_{2-6} alkylene, for example C_{2-5} alkylene. It may be C_2 alkylene. It may be C_5 alkylene. The alkylene is preferably linear (unbranched) alkylene, e.g. wherein C_2 alkylene is ethylene, and C_5 alkylene is n-pentylene.

5 Z^1 may be

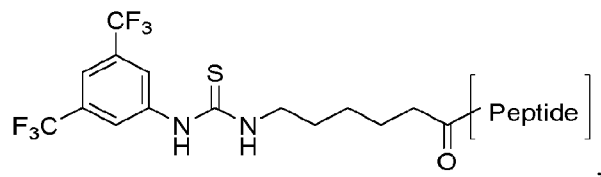
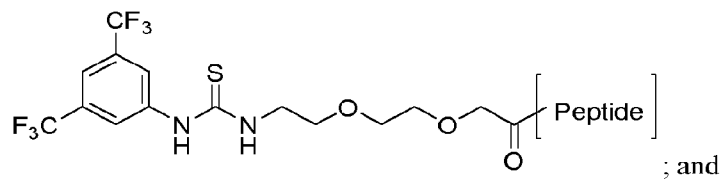
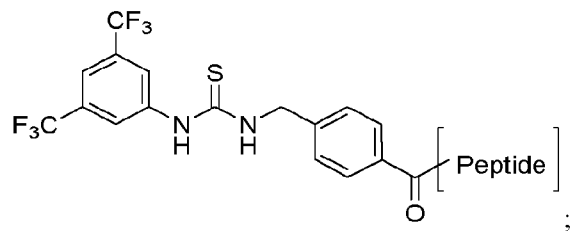
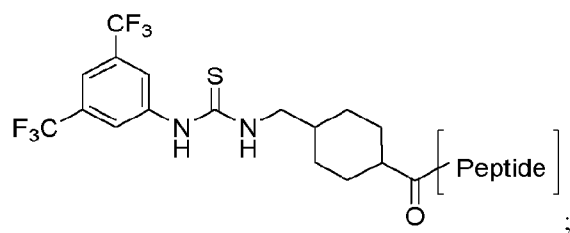
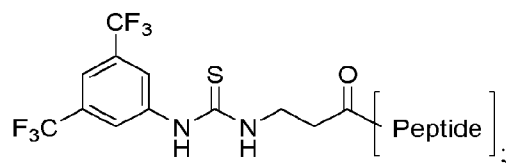
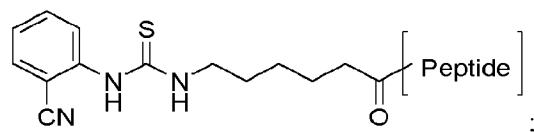
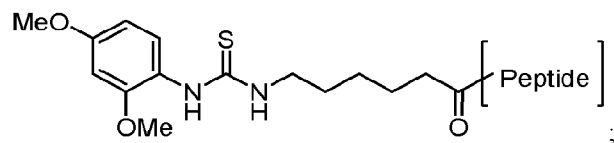


Z^1 may for instance be .

10 The disclosure also provides a compound of Formula Ia selected from:



15



or a pharmaceutically acceptable salt thereof,

wherein the peptide domain is as defined above for formula Ia and

wherein the -OH at the C-terminus of the peptide domain is optionally substituted by R^a , wherein R^a is selected from -NH₂, -O-C₁₋₆alkyl, -NHC₁₋₆alkyl, and -NH-NH₂, as described above, preferably wherein R^a is -NH₂.

The CPP may be a cationic, amphipathic, hydrophobic or amphiphilic CPP. The CPP may comprise PTD4, SP, pVEC, poly-arginine, transportan, TAT, a translocationally active derivative or fragment of TAT, penetratin, herpes simplex virus protein VP22 or a translocationally-active homologue thereof, or a variant thereof having at least 70% identity thereto.

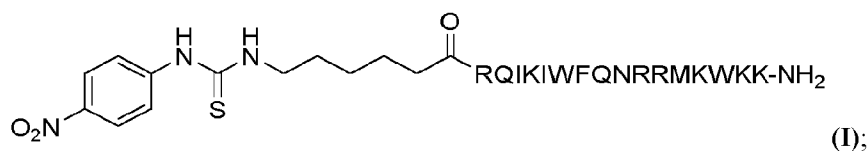
- 5 The CPP may have at least 70% sequence identity to one of SEQ ID NOs: 1-16.

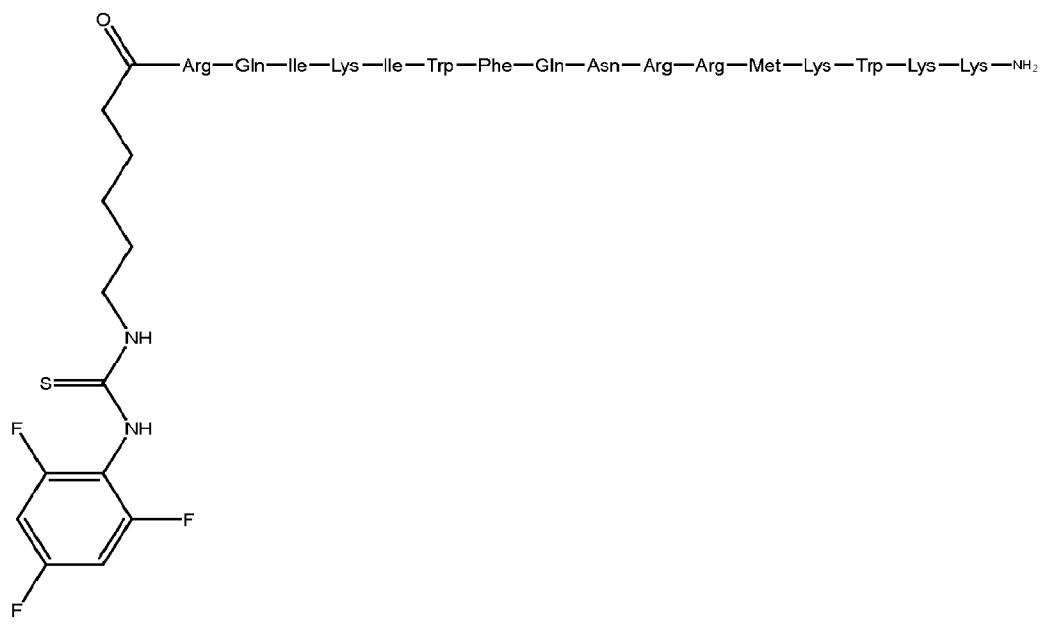
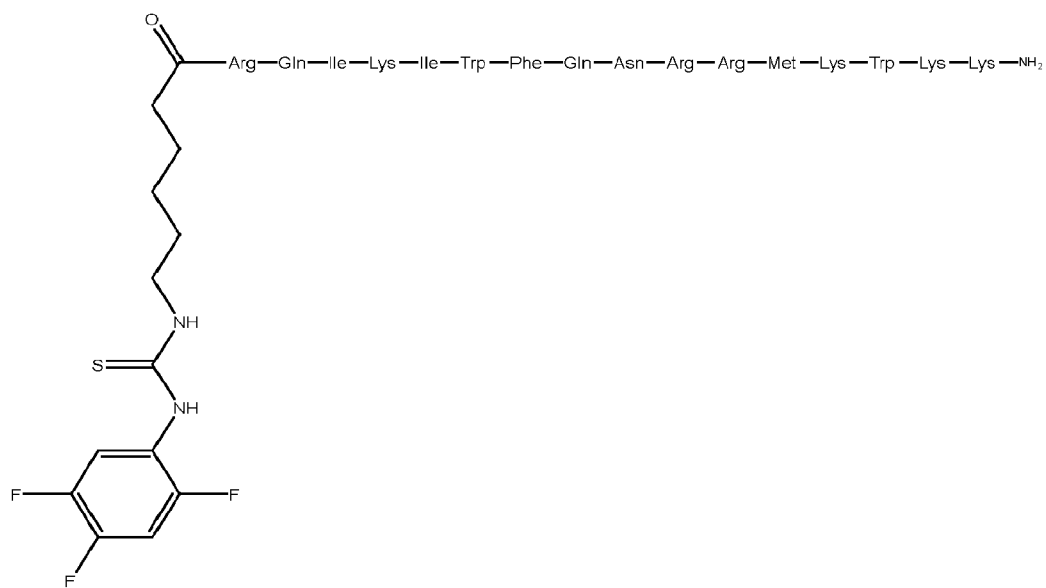
The CPP may comprise one of SEQ ID NOs: 1-8 and 14-16, preferably one of SEQ ID NOs: 5, 14, 15 and 16.

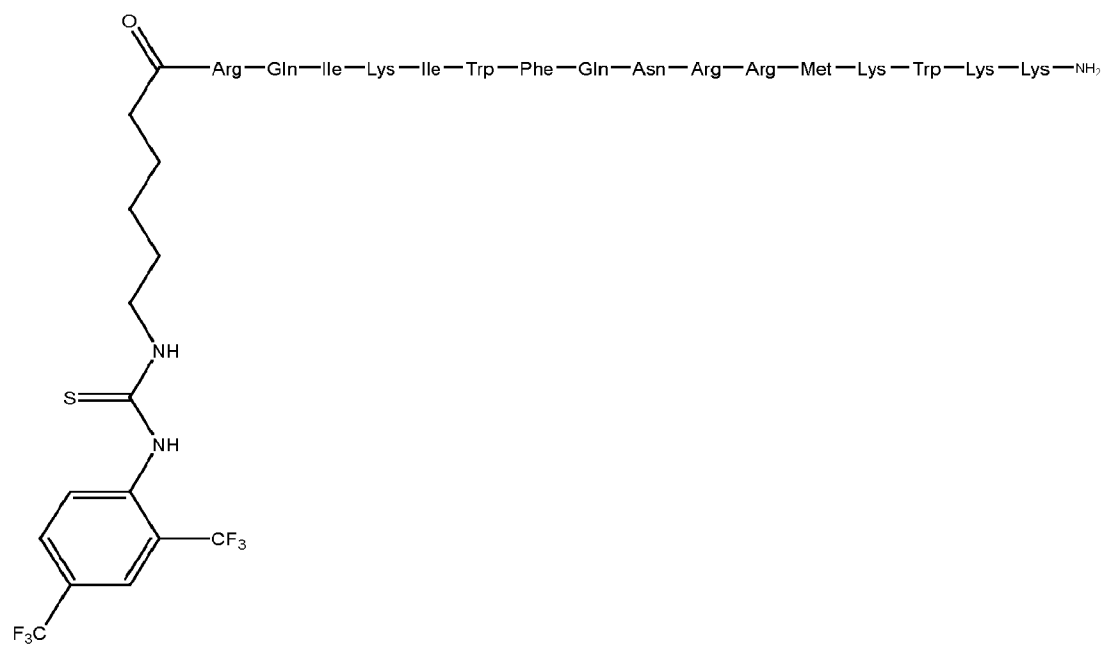
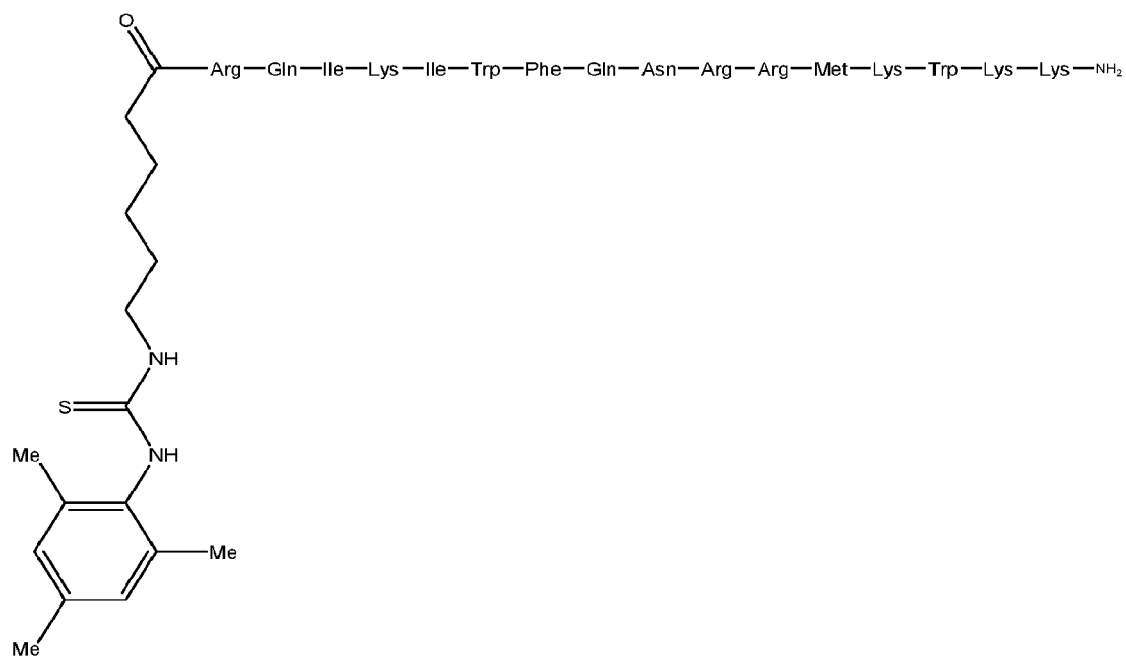
- In compounds of formula Ia, and any embodiment such as those described above, the -OH at the C-terminus of the peptide domain may be substituted by R^a, wherein R^a is selected from -NH₂, -O-C₁₋₆alkyl, -NHC₁₋₆alkyl, and -NH-NH₂. Substitution by R^a in this context is intended to refer to the -OH part of the carboxylic acid end of the peptide being notionally replaced by the R^a moiety. In the case of R^a being -NH₂ for instance, the C-terminus of the peptide would end in a carboxamide moiety, i.e. -C(=O)-NH₂, instead of a conventional carboxylic acid moiety. It is intended that CPPs substituted in this way have cell-penetrating activity.

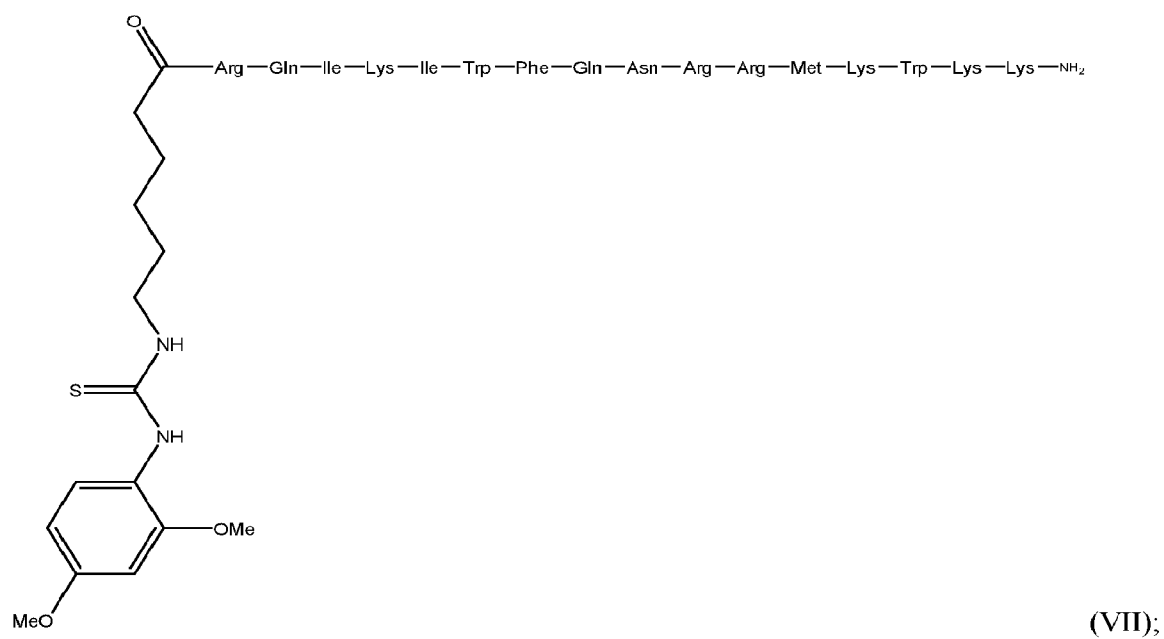
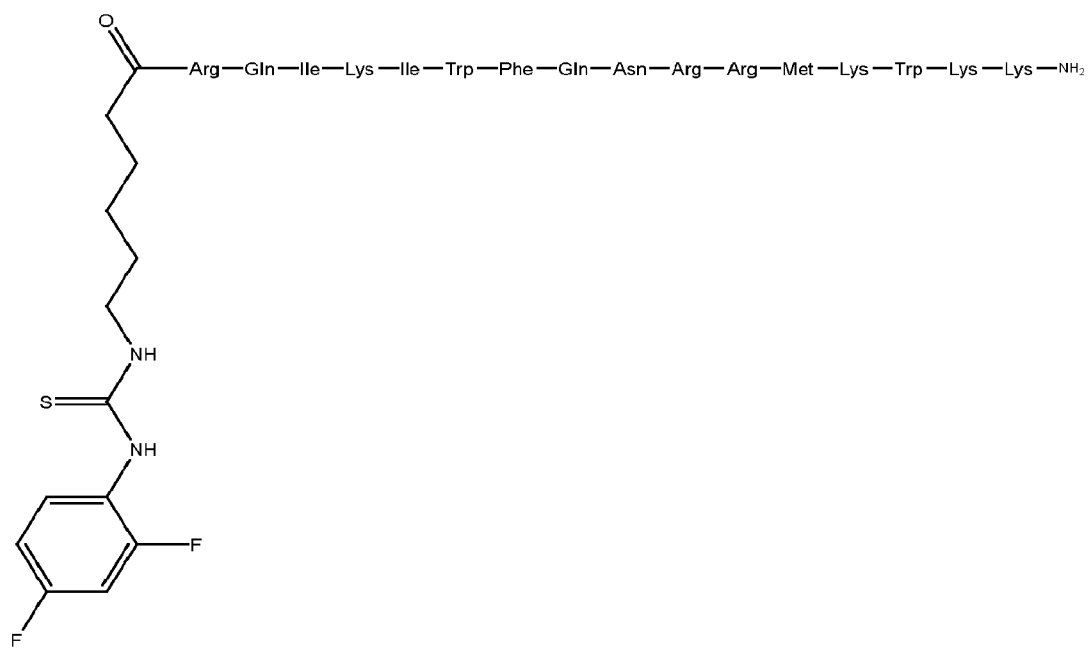
- 15 In typical embodiments, the -OH at the C-terminus of the peptide domain is substituted by R^a, preferably wherein R^a is -NH₂.

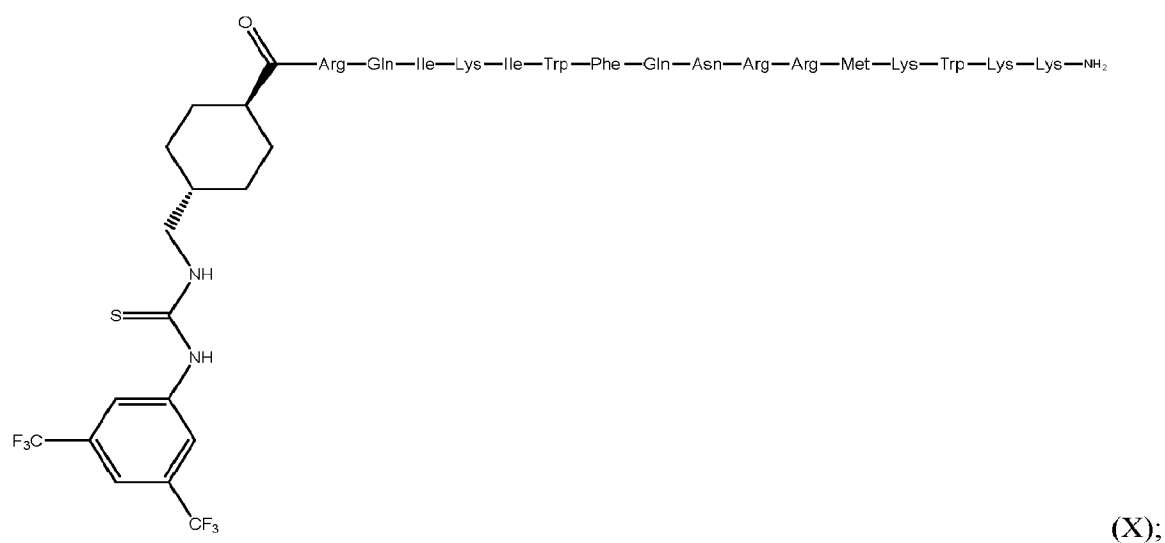
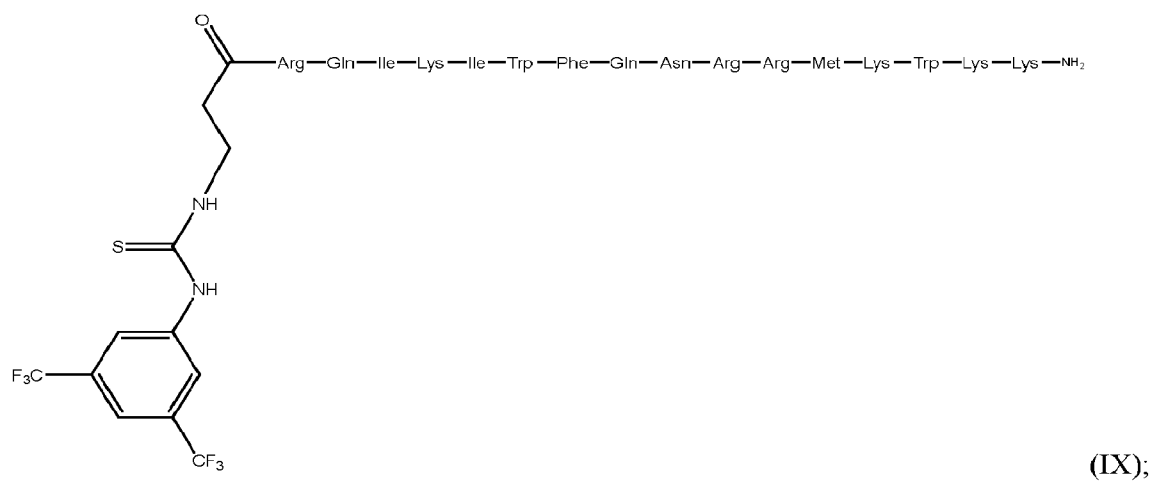
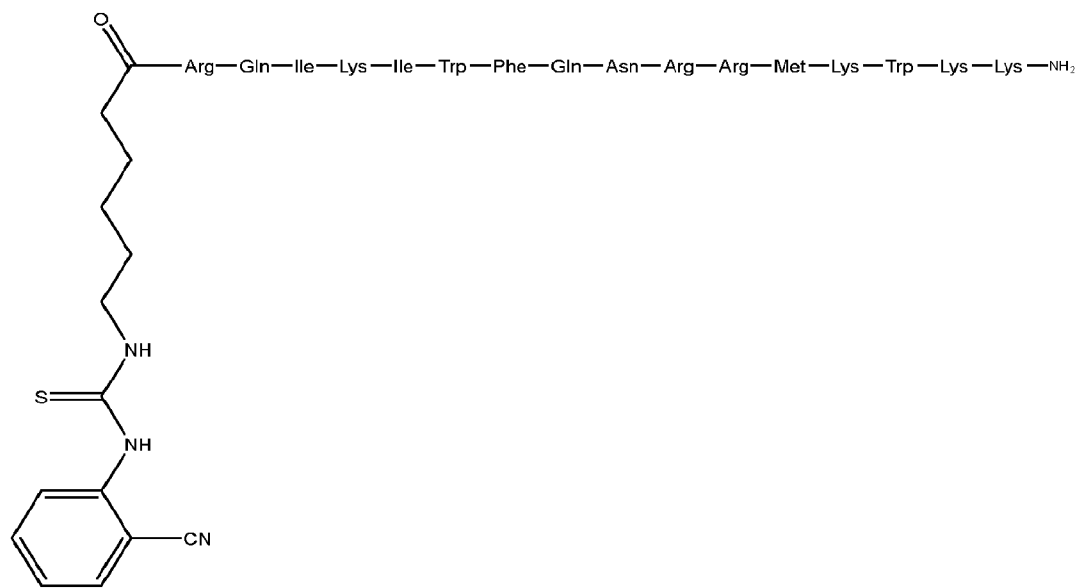
The compound of Formula Ia may be selected from:

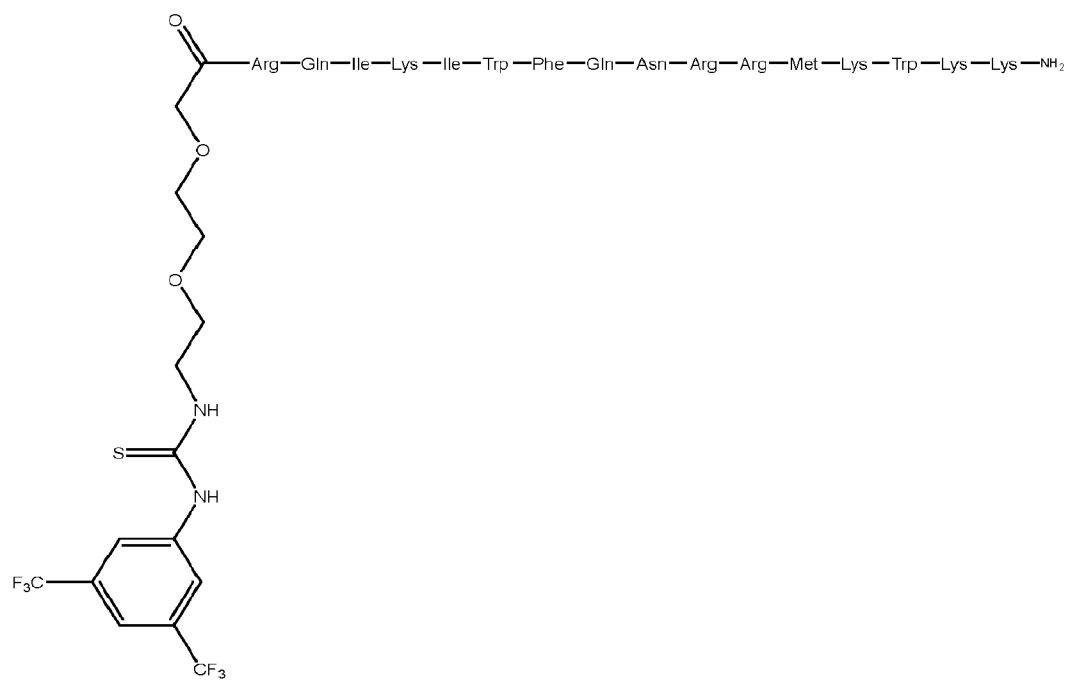
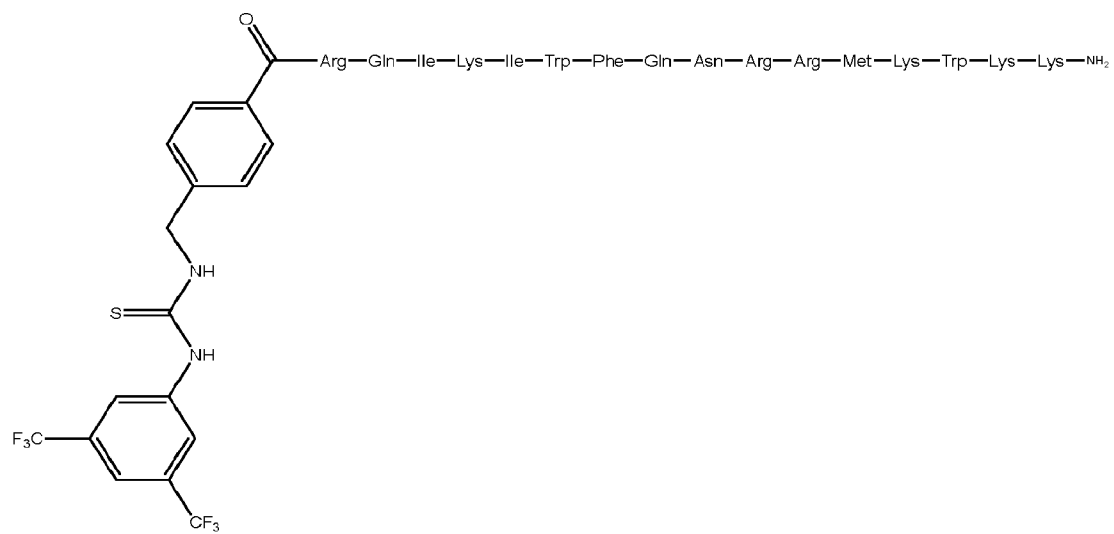


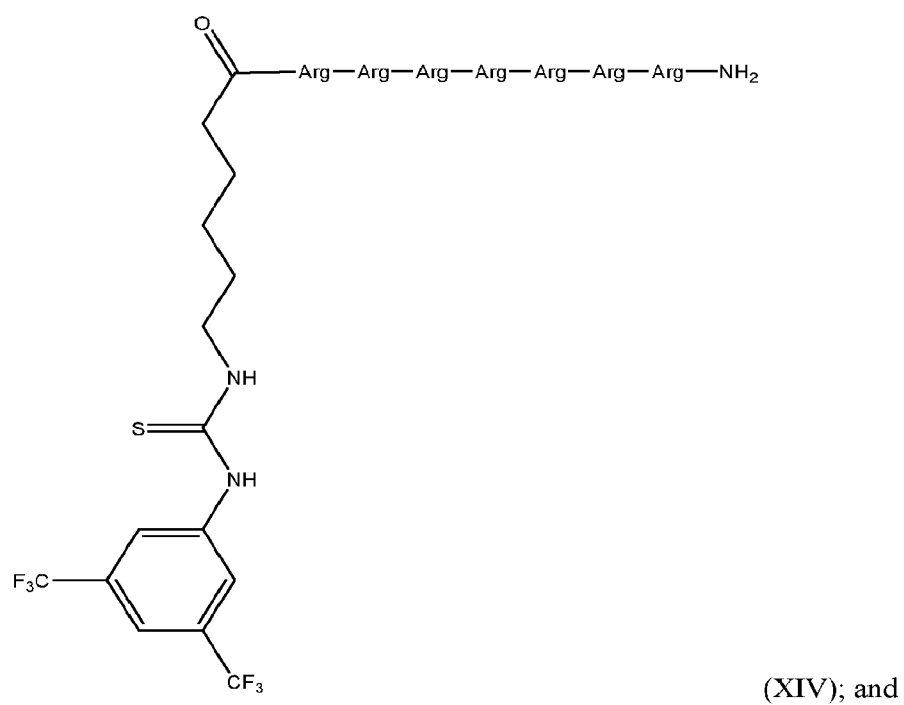
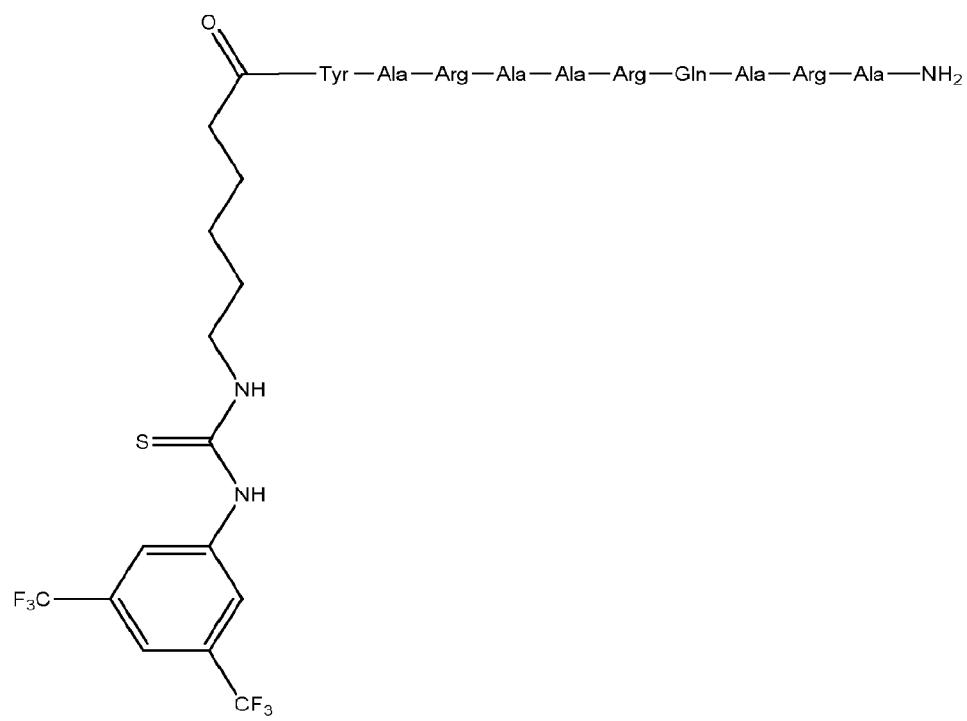


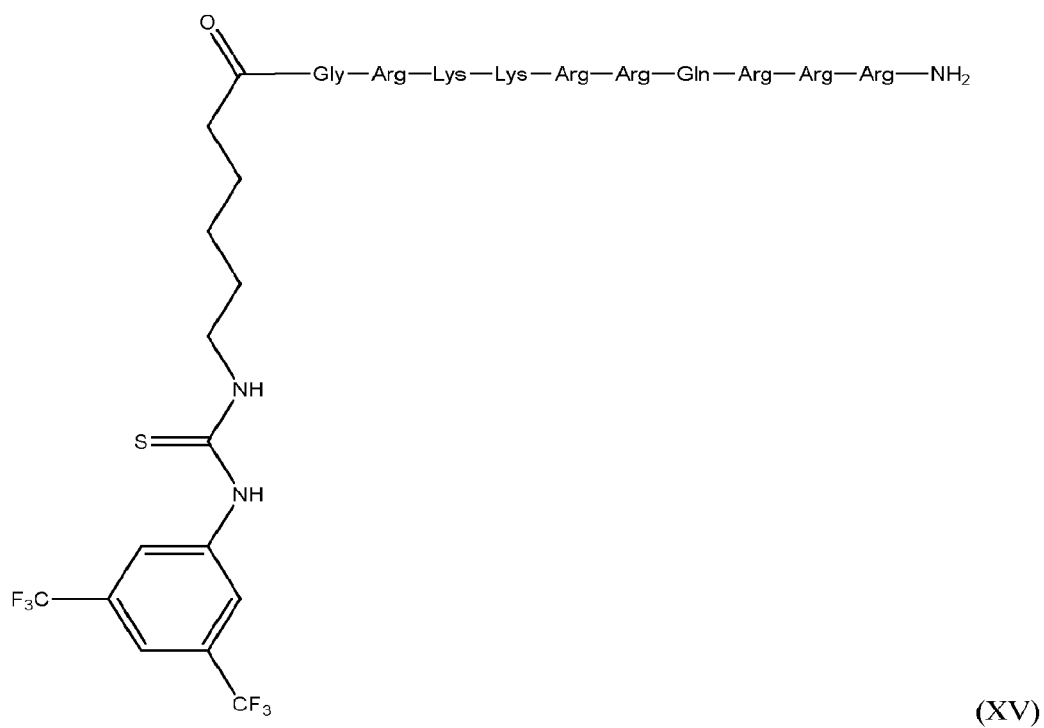






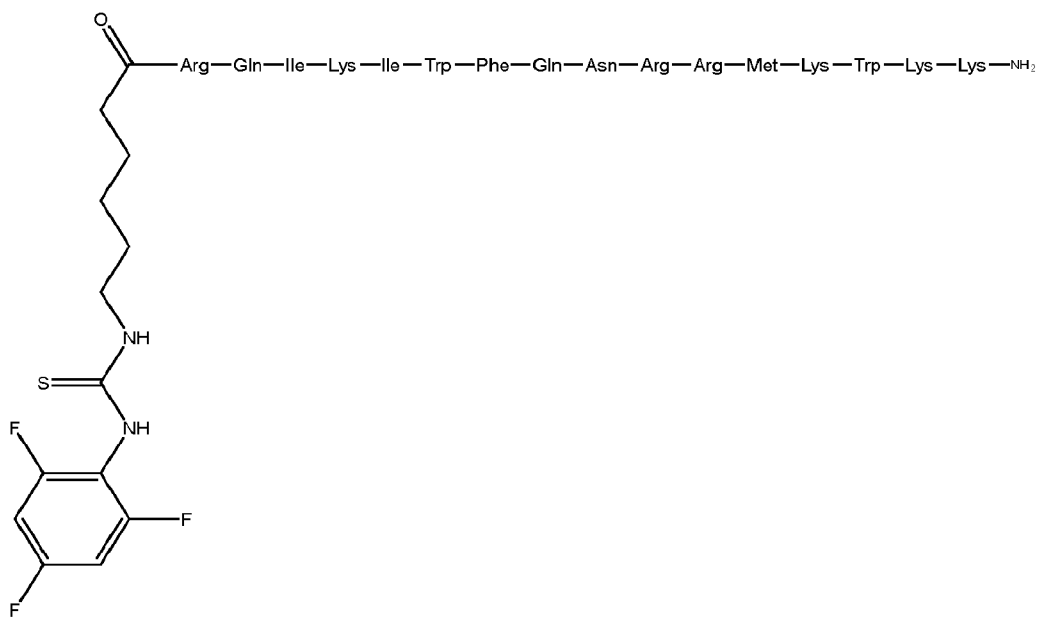


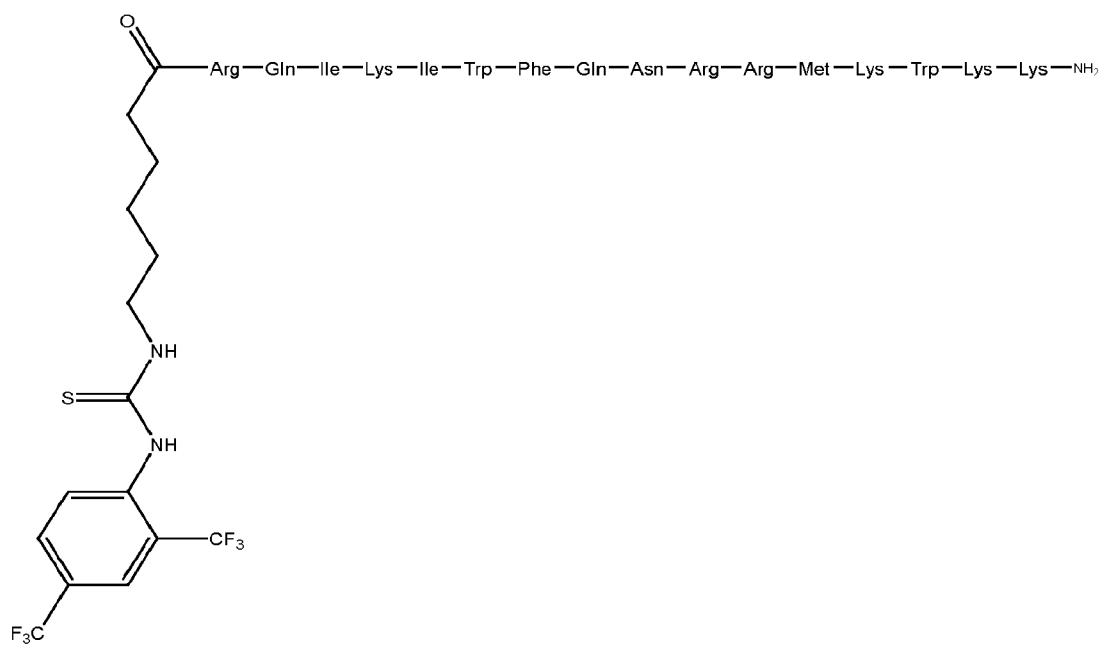
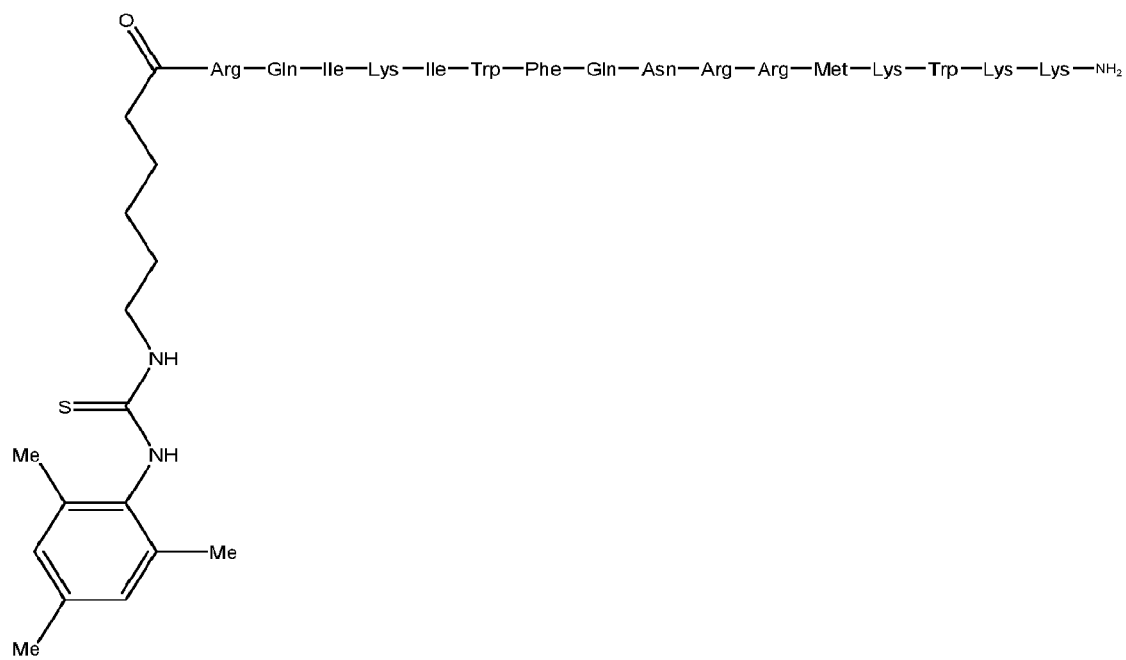


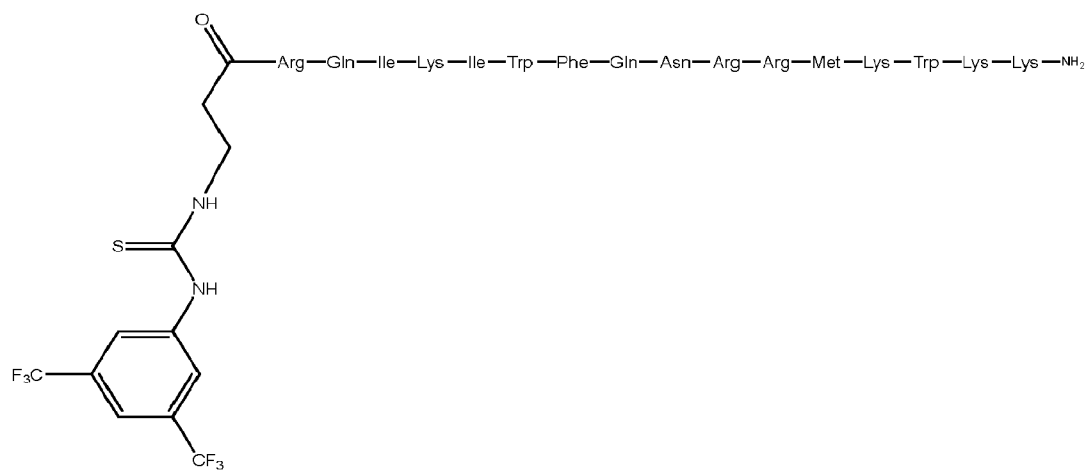


or pharmaceutically acceptable salts thereof.

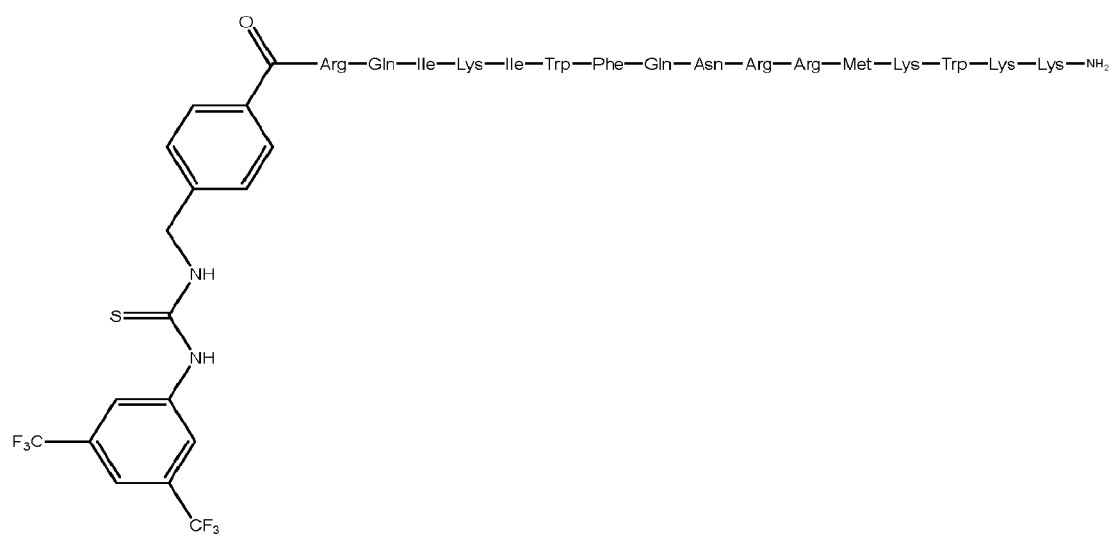
Preferably, the compound of formula Ia is selected from:



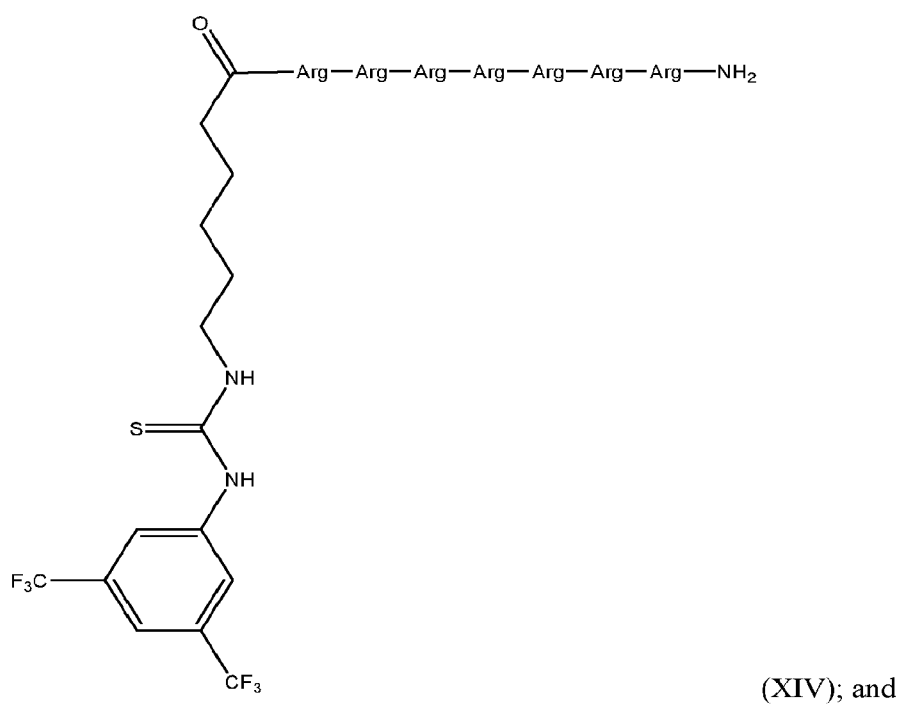
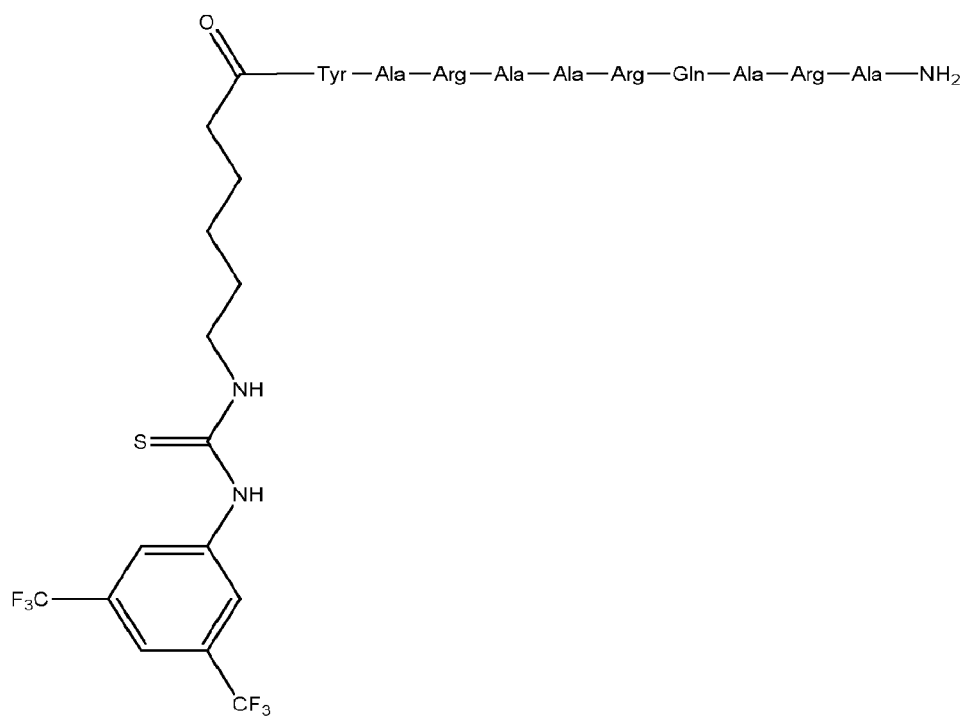


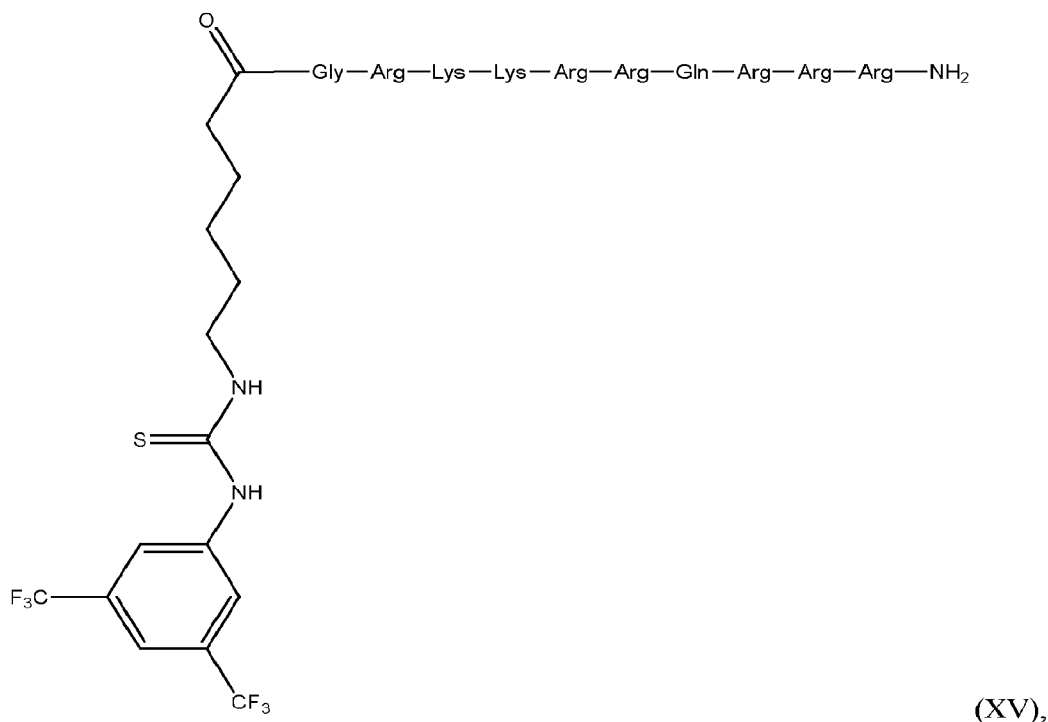


(IX);



(XI);





or pharmaceutically acceptable salts thereof.

In such structures, it will be appreciated that the -NH_2 depicted at the C-terminus end of the compounds is intended to depict the notional substitution of the -OH part of the carboxylic acid end of the peptide by an NH_2 moiety (i.e. R^a is NH_2). In other words, these compounds terminate in a carboxamide at the C-terminus, not a carboxylic acid.

In an aspect is provided a pharmaceutical composition comprising a compound or pharmaceutically acceptable salt thereof as defined herein, e.g. according to formula Ia or any embodiment thereof.

In an aspect is provided a compound or pharmaceutically acceptable salt thereof as defined herein, e.g. according to formula Ia or any embodiment thereof, or a pharmaceutical composition thereof, for use in the treatment of a disease or condition.

In an aspect is provided the use of a compound or pharmaceutically acceptable salt thereof as defined herein, e.g. according to formula Ia or any embodiment thereof, or a pharmaceutical composition thereof, for the manufacture of a medicament for the treatment of a disease or condition.

In an aspect is provided a method of treatment of a disease or condition in a subject, wherein the method comprises administering a compound or a pharmaceutically acceptable salt thereof as defined herein, e.g. according to formula Ia or any embodiment thereof, or a pharmaceutical composition thereof, to a subject in need thereof.

5

The disease or condition may be associated with a chloride ion channel mutation or is a channelopathy. The chloride ion channel mutation may be selected from a voltage-sensitive CLIC, transmitter-gated GABA_A, glycine receptors, calcium-activated chloride ion channel (CaCC), high (maxi) conductance chloride ion channel, cystic fibrosis transmembrane conductance regulator (CFTR) and a volume-regulated channel mutation.

10

The disease or condition (e.g. associated with the chloride ion channel mutation) may be selected from myotonia congenita Becker, Myotonia congenita Thomsen, Dystrophia myotonica 1, dystrophia myotonica 2, childhood absence epilepsy type 3, juvenile absence epilepsy, juvenile myoclonic epilepsy, epilepsy with grand mal seizures on awakening, Bartter Syndrome, Bartter syndrome with sensorineural deafness, Dent's disease, autosomal dominant osteopetrosis, autosomal recessive osteopetrosis, cystic fibrosis, idiopathic chronic pancreatitis, bronchiectasis, congenital bilateral absence of vas deferens, Best's disease, adult-onset vitelliform macular dystrophy, concentric annular macular dystrophy, cataract, juvenile myoclonic epilepsy, childhood absence epilepsy type 2, generalized epilepsy with febrile seizures, severe myoclonic epilepsy in infancy, insomnia, hereditary hyperekplexia, recessive and dominant myotonia, leukodystrophy, aldosteronism, diabetes insipidus, Giltelman disease, CNS and retina degeneration, mental retardation, epilepsy, proteinuria, impaired renal endocytosis, osteopetrosis, pulmonary hypertension, cardiac dysfunction, Alzheimer's disease, respiratory and gastrointestinal diseases, including asthma, chronic obstructive pulmonary disease, cystic fibrosis, pneumonia, colon colitis, cystic fibrosis intestinal mucous disease, ulcerative colitis, and gastrointestinal parasitic infection, dry mouth and dry eye syndromes, diarrhea, hypertension, neuropathic pain, cancers, smoke induced COPD, chronic bronchitis, rhinosinusitis, constipation, pancreatitis, pancreatic insufficiency, male infertility caused by congenital bilateral absence of the vas deferens (CBAVD), mild pulmonary disease, idiopathic pancreatitis, allergic bronchopulmonary aspergillosis (ABPA), liver disease, hereditary emphysema, mucopolysaccharidoses, Bartter's syndrome type III, Primary Ciliary Dyskinesia (PCD), an inherited disorders of the structure and/or function of cilia, including PCD with situs inversus (also known as Kartagener syndrome), PCD without situs inversus and ciliary aplasia.

35

In an aspect is provided a compound or pharmaceutically acceptable salt thereof as defined herein, e.g. according to formula Ia or any embodiment thereof, or a pharmaceutical composition thereof, for use in a method of treating a respiratory disease, preferably COPD, asthma, bronchiectasis, pneumonia or cystic fibrosis.

5

Also provided is a kit comprising a compound or pharmaceutically acceptable salt thereof as defined herein, e.g. according to formula Ia or any embodiment thereof, or a pharmaceutical composition thereof.

10 In some embodiments of the invention, the peptide domain(s) of the compounds described herein comprises one or more positively charged residues.

In some embodiments of the invention, said peptide domain(s) of the compounds described herein contains at least one arginine or lysine side-chain.

15

In some embodiments of the invention, said peptide domain(s) of the compounds described herein comprises a cell penetrating peptide (CPP), preferably a cationic, amphipathic, hydrophobic or amphiphilic CPP. CPPs have cell-penetrating activity.

20 In some embodiments of the invention, said peptide domain(s) comprises one or more cell penetrating peptides selected from the group consisting of PTD4, SP, pVEC, poly-arginine (arginine stretch), transportan, TAT, a translocationally active derivative or fragment of TAT, penetratin, herpes simplex virus protein VP22 or a translocationally-active homologue thereof from a different herpes virus, or a variant thereof having at least having at least 70%, 71%, 72%,
25 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity thereto. In some embodiments, the poly-arginine comprises or consists of 2, 3, 4, 5, 6, 7, 8, 9, or 10 consecutive arginine residues. In some embodiments, the poly-arginine consists of 6 or 7 consecutive arginine residues. In some embodiments, the peptide domain comprises PTD4,
30 translocationally active fragment of TAT (i.e. which has cell penetrating activity), poly-arginine or penetratin.

In some embodiments of the invention, said peptide domain(s) of the compounds described herein comprises a cell penetrating peptide (CPP) selected from one or more of the following:

- a) HIV-TAT protein or a translocationally active derivative or fragment thereof, such as residue 48 to 60 of TAT: GRKKRRQRRRPPQ (SEQ ID NO:1),
- b) the TAT 49-57 peptide: RKKRRQRRR (SEQ ID NO: 2),
- c) YGRKKRRQRRRP (SEQ ID NO: 3) (a longer peptide containing TAT49-57 – it is TAT residues 47-58),
- d) GRKKRRQRRRPPQ (SEQ ID NO: 4) (a longer peptide containing TAT49-57 – it is TAT residues 48-60),
- e) penetratin having the sequence RQIKIWFQNRRMKWKK (SEQ ID NO:5),
- f) penetratin variant W48F having the sequence RQIKIFFQNRRMKWKK (SEQ ID NO: 6),
- g) penetratin variant W56F having the sequence RQIKIWFQNRRMKFKK (SEQ ID NO: 7),
- h) penetratin variant having the sequence RQIKIWFQNRRMKFKK (SEQ ID NO: 8),
- i) transportan having the sequence GWTLNSAGYLLGKINLKALAALAKKIL (SEQ ID NO: 9),
- j) transportan-27 having the sequence GWYLNNSAGYLLGK(e-Cys)INLKALAALAKKIL (SEQ ID NO: 10),
- k) transportan-22 having the sequence GWYLNNSAGYLLGK(e-Cys)INLKALAAL (SEQ ID NO: 11),
- l) herpes simplex virus protein VP22 or a translocationally-active homologue thereof from a different herpes virus such as MDV protein UL49,
- m) Pep-1, having the sequence KETWWETWWTEWSQPKKKKRKV (SEQ ID NO: 12),
- n) Pep-2, having the sequence KETWFETWFTTEWSQPKKKKRKV (SEQ ID NO: 13),
- o) A fragment of TAT having the sequence GRKKRRQRRR (SEQ ID NO: 14),
- p) PTD4 having the sequence YARAAARQARA (SEQ ID NO: 15),
- q) R7 poly-arginine having the sequence RRRRRRRR (SEQ ID NO: 16), and
- r) a CPP having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to one of a)-q) above and having cell penetrating activity.

In some embodiments, said peptide domain(s) of the compounds described herein may comprise or consist of a fragment of TAT having the amino acid sequence of SEQ ID NO: 1, or a variant thereof having at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81 %, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 1 and having cell penetrating activity; or comprise or consist of penetratin having the amino acid sequence of SEQ ID NO: 5, or a variant thereof having at least

70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 5 and having cell penetrating activity.

- 5 In some embodiments, said peptide domain(s) of the compounds described herein may comprise or consist of the amino acid sequence of any one of SEQ ID NOs: 2 to 4 or 6 to 16, or a sequence which has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to any one of SEQ ID NOs: 2 to 4 or 6 to 16, and having cell penetrating activity.
- 10 In some embodiments, the peptide domain comprises or consists of a sequence which has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to one of SEQ ID NOs: 1, 2, 3, 5-7, 9 and 12-16. In some embodiments, the peptide domain comprises or consists of a sequence which at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to one of SEQ ID NOs: 1-8 and 14-16. In some embodiments, the peptide domain
- 15 comprises or consists of a sequence which has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to one of SEQ ID NOs: 5-8 and 14-16, preferably to one of SEQ ID NOs: 5, 14, 15 and 16. In some embodiments, the peptide domain comprises or consists of one of SEQ ID NOs: 1, 2, 3, 5-7, 9 and 12-16 or to one of SEQ ID NOs: 1-8 and 14-16, preferably to one of SEQ ID NOs: 5, 14, 15 and 16, and more preferably SEQ ID NO: 5.

20

As discussed above, any of the CPPs disclosed herein may have a C-terminal which is optionally substituted by R^a as in Formula Ia, wherein the CPP has cell-penetrating activity when R^a is present. In some embodiments, the C-terminal of the CPP is substituted with R^a. In preferred embodiments, R^a is NH₂ (i.e. the amino acid at the C-terminal terminates in carboxamide moiety).

- 25 The C-terminal of the peptide domain may be substituted in this manner even when the peptide domain consists of one of the proteins and peptides described above.

In some embodiments of the invention, the compounds described herein do not induce apoptosis or necrosis in a concentration range from 100 nM to 100 μM.

30

In some embodiments of the invention, the compounds of the present invention are amphipathic.

In some embodiments of the invention, the compounds of the present invention decrease the intracellular (Cl⁻) chloride ion concentration in a dose-dependent manner, optionally when applied

- 35 into or onto an epithelial surface, optionally in a concentration range from 0.1nM to 10mM, 1nM

to 1mM, 100nM to 20μM, 100nM to 15μM, 1μM to 20μM, 1μM to 15μM, 100 nM to 10 μM or 1μM to 10μM when applied to HeLa cells.

In some embodiments, the application of said compound to a *Xenopus laevis* oocyte increases the current between the oocyte internal and external part using a two-electrodes voltage clamp setup perfusing the external part with Cl⁻ free buffer at a concentration between 0.1nM to 10mM, 1nM to 1mM, 100nM to 20μM, 100nM to 15μM, 1μM to 20μM, 1μM to 15μM, 100 nM to 10 μM or 1μM to 10μM, optionally in a dose-dependent manner.

Pharmaceutically acceptable salts

In some embodiments of the invention, said salts can be prepared from any compound of the invention having functionality capable of forming salts, for example a base functionality. Pharmaceutically acceptable salts can be prepared with organic or inorganic acids. Compounds of the invention that contain one or more basic functional groups are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable organic and inorganic acids. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed.

Examples of pharmaceutically acceptable salts include but are not limited to sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caprate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, terephthalate, sulfonate, xylene sulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, β-hydroxybutyrate, glycolate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate, adipate, alginate, mucate, aspartate, benzenesulfonate, butyrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, glucoheptanoate, glycerophosphate, hemisulfate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, palmoate, pectinate, persulfate, picrate, pivalate, salicylate, thiocyanate, tosylate, arginate and undecanoate. See, for example, Berge et al. "Pharmaceutical Salts", J. Pharm. Sci. 66:1-19 (1977) and the like.

Acids commonly employed to form pharmaceutically acceptable salts include but are not limited to inorganic acids such as hydrogen bisulfide, hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid and phosphoric acid, as well as organic acids such as paratoluenesulfonic acid, salicylic acid, tartaric acid, bitartaric acid, ascorbic acid, maleic acid, besylic acid, fumaric acid, gluconic acid, glucuronic acid, formic acid, glutamic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, lactic acid, oxalic acid, para-bromophenylsulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid and acetic acid.

Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

In some embodiments, pharmaceutically acceptable acid addition salts include those formed with mineral acids such as hydrochloric acid and hydrobromic acid, and especially those formed with organic acids such as trifluoroacetic acid and maleic acid.

In some embodiments, there is provided a pharmaceutical composition comprising a compound or pharmaceutically acceptable salt of the invention, which may be any of the compounds or corresponding salts disclosed herein. Such pharmaceutical compositions may also comprise a pharmaceutically-acceptable diluent, carrier and/or excipient. In some embodiments, the pharmaceutical composition comprises more than one compound of the invention. In some embodiments, the pharmaceutical composition further comprises one or more additional active ingredients and/or adjuvants. In certain embodiments the pharmaceutical composition may further comprise one or more ingredients therapeutically effective for the same disease indication.

Solvates & hydrates

The compounds of this disclosure may exist in unsolvated or solvated forms. The term “solvate” includes molecular complexes comprising a given compound and one or more pharmaceutically acceptable solvent molecules such as water or C₁₋₆ alcohols, *e.g.* ethanol. The term “hydrate” means a “solvate” where the solvent is water.

Amorphous & crystalline forms

The compounds of the invention may exist in solid states from amorphous through to crystalline forms. All such solid forms are included within the invention.

Isomeric forms

Compounds of the invention may exist in one or more geometrical, optical, enantiomeric, diastereomeric and tautomeric forms, including but not limited to *cis*- and *trans*-forms, *E*- and *Z*-forms, *R*-, *S*- and *meso*-forms, keto- and enol-forms. All such isomeric forms are included within the invention. The isomeric forms may be in isomerically pure or enriched form, as well as in mixtures of isomers (*e.g.* racemic or diastereomeric mixtures).

Isotopic labeling

The disclosure includes pharmaceutically acceptable isotopically-labelled compounds of the invention wherein one or more atoms are replaced by atoms having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Isotopically-labelled compounds can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described herein using an appropriate isotopically-labelled reagent in place of the non-labelled reagent previously employed.

Chemical group definitions:

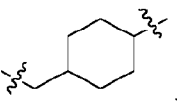
“Aryl” as mentioned herein is intended to include monocyclic or polycyclic fused ring aromatic groups (depending on the number of carbon atoms present). Preferred is phenyl.

The term “alkyl” is intended to include straight (*i.e.* linear) or branched, saturated, acyclic hydrocarbyl groups. Methyl is preferred.

The term “halogen” (or “halo”) includes fluorine, chlorine, bromine and iodine. In preferred examples, halo is F.

The term “alkylene” includes divalent, straight or branched, saturated, acyclic hydrocarbyl groups. In typical embodiments the alkylene is straight (*i.e.* linear / not branched). Examples include ethylene (C_2 -alkylene) and n-pentylene (a C_5 -alkylene).

The term “cycloalkylene” includes divalent, saturated, hydrocarbyl groups containing at least 1 saturated carbocyclic group. The cycloalkylene group may consist solely of a saturated carbocyclic group, or may contain additional non-cyclic carbon atoms in the chain, provided at least 1 saturated

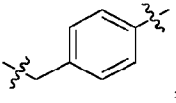
carbocyclic group is present, such as in the case of .

The term “heteroalkylene” includes alkylene groups in which up to three carbon atoms, in one embodiment up to two carbon atoms, in another embodiment one carbon atom, are each notionally replaced independently by O or N, typically O, provided at least one of the alkyl carbon atoms

remains. Examples of heteroalkylene groups thus include ethers. In exemplary compounds of the invention, the heteroalkylene group, if present, is typically linked to the remaining parts of the compound via carbon atoms. Linear (non-branched) heteroalkylene groups are preferred.

By way of clarification, in relation to the above mentioned heteroatom containing groups, where a numerical of carbon atoms is given, for instance C₂₋₁₀heteroalkylene, what is intended is a group based on C₂₋₁₀alkylene in which one or more of the 2-10 chain carbon atoms is replaced by O or N. Accordingly, a C₂₋₁₀heteroalkylene group, for example, will contain less than 2-10 chain carbon atoms.

The term “aralkylene” refers to an alkylene group notionally substituted with an aryl group in the

chain. Examples include , wherein a phenyl group has been notionally substituted into the alkylene carbon chain (this structure is an example of a C₇-aralkylene). In the absence of any specific indication, the alkylene and aryl moieties of the aralkylene may be spatially orientated in the compound from left to right in either order, e.g., -aryl-alkylenc- or -alkylenc-aryl-.

The compounds of the invention are useful for the treatment of diseases or conditions associated with malfunctions in chloride ion channels, and with mutations in genes encoding chloride ion channels (i.e. channelopathies) which lead to abnormal, deficient or absent chloride ion channel function. In particular, Tables 1 and 2 show that the compounds of the invention have chloride ion transporter function.

The present disclosure provides compounds of the invention or a pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or pharmaceutically acceptable salts thereof, for use in therapy. The present disclosure also provide a pharmaceutical composition of the invention, comprising a compound of the invention, preferably selected from compounds (I) to (XV), or a pharmaceutically acceptable salt thereof, for use in therapy.

The present disclosure also provides the use of a compound of the invention or a pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or pharmaceutically acceptable salts thereof, for use in the manufacture of a medicament for use in therapy. The present disclosure also provides the use of a pharmaceutical composition of the invention, comprising a compound of the invention, preferably selected from compounds (I) to (XV), or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for use in therapy.

In some embodiments of the invention, there is provided a method of treating a disease associated with a chloride ion channel mutation, preferably wherein the chloride ion channel is involved in processes including the regulation of the excitability of neurons, skeletal, cardiac and smooth muscle, cell volume regulation, transepithelial salt transport, the acidification of internal and extracellular compartments, the cell cycle and apoptosis, preferably by administering a therapeutically effective amount of a compound or pharmaceutical composition according to the invention described herein, such as compound (I) to (XV) or variant thereof or a pharmaceutical composition comprising any of compounds (I) to (XV), to a human subject in need thereof. In some embodiments, the compound of the invention, preferably selected from compounds (I) to (XV) or pharmaceutically acceptable salts thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or pharmaceutically acceptable salts thereof, is for use in the treatment of a disease associated with a chloride ion channel mutation that is involved in a process including the regulation of the excitability of neurons, skeletal, cardiac and smooth muscle, cell volume regulation, transepithelial salt transport, the acidification of internal and extracellular compartments, the cell cycle and apoptosis. Also provided is use of the compound of the invention or a pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or pharmaceutically acceptable salts thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or pharmaceutically acceptable salts thereof, in the manufacture of a medicament for the treatment of a disease associated with a chloride ion channel mutation that is involved in a process including the regulation of the excitability of neurons, skeletal, cardiac and smooth muscle, cell volume regulation, transepithelial salt transport, the acidification of internal and extracellular compartments, the cell cycle and apoptosis. The disease associated with a chloride ion channel mutation may be selected from the group of myotonia congenita Becker, Myotonia congenita Thomsen, Dystrophia myotonica 1, dystrophia myotonica 2, childhood absence epilepsy type 3, juvenile absence epilepsy, juvenile myoclonic epilepsy, epilepsy with grand mal seizures on awakening, Bartter Syndrome, Bartter syndrome with sensorineurial deafness, Dent's disease, autosomal dominant osteopetrosis, autosomal recessive osteopetrosis, cystic fibrosis, idiopathic chronic pancreatitis, bronchiectasis, congenital bilateral absence of vas deferens, Best's disease, adult-onset vitelliform macular dystrophy, concentric annular macular dystrophy, cataract, juvenile myoclonic epilepsy, childhood absence epilepsy type 2, generalized epilepsy with febrile seizures plus, severe myoclonic epilepsy in infancy, insomnia and hereditary hyperekplexia.

In some embodiments of the invention, the chloride ion channel mutation is selected from the group of a voltage-sensitive CLIC, transmitter-gated GABA_A, glycine receptors, calcium-

activated chloride ion channel (CaCC), high (maxi) conductance chloride ion channel, cystic fibrosis transmembrane conductance regulator (CFTR), and a volume-regulated channel mutation.

In some embodiments of the invention, there is provided a method of treating of a disease associated with a voltage-sensitive chloride intracellular channel (CLIC) mutation, preferably comprising administering a therapeutically effective amount of a compound according to the invention described herein, preferably one of compounds (I) to (XV) or a variant thereof, or a pharmaceutical composition according to the invention described herein, preferably comprising any of compounds (I) to (XV) or variant thereof, to a human subject in need thereof. In some embodiments, the invention provides a compound of the invention or a pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, for use in the treatment of a disease associated with a voltage-sensitive chloride intracellular channel (CLIC) mutation. Also provided is use of a compound of the invention or a pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a disease associated with a voltage-sensitive chloride intracellular channel (CLIC) mutation. The voltage-sensitive chloride intracellular channel (CLIC) mutation may be selected from the group of CLIC-1, CLIC-2, hCLIC-Ka (rCLIC-K1), hCLIC-Kb (rCLIC-K2); CLIC-3 to CLIC-5, and CLIC-6 and CLIC-7 chloride ion channel mutations. In some embodiments of the invention, the voltage-sensitive chloride intracellular channel (CLIC) associated disease is selected from the group of muscle, skeletal and bone diseases, kidney diseases, ear diseases, central nervous (CNS) diseases, eye diseases, gastrointestinal diseases, pulmonary diseases, cardiac diseases and liver diseases. In some embodiments of the invention, the voltage-sensitive chloride intracellular channel (CLIC) associated disease is selected from the group of recessive and dominant myotonia, leukodystrophy, aldosteronism, diabetes insipidus, Bartter disease, Gitelman disease, CNS and retina degeneration, mental retardation, epilepsy, Dent's disease, proteinuria, impaired renal endocytosis, osteopetrosis, pulmonary hypertension, cardiac dysfunction, Alzheimer's disease, associated respiratory and gastrointestinal diseases, including asthma, chronic obstructive pulmonary disease, cystic fibrosis, pneumonia, colon colitis, cystic fibrosis intestinal mucous disease, ulcerative colitis, and gastrointestinal parasitic infection.

In some embodiments of the invention, there is provided a method of treating of a disease associated with a transmitter-gated GABA_A and glycine receptor mutation. In some embodiments, there is provided a compound of the invention or pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, for use in the treatment of a disease associated with a transmitter-gated GABA_A and glycine receptor mutation. Also provided is the use of a compound of the invention or pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or pharmaceutically acceptable salts thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a disease associated with a transmitter-gated GABA_A and glycine receptor mutation.

In some embodiments of the invention, there is provided a method of treating a disease associated with a calcium-activated chloride ion channel mutation, preferably by administering a therapeutically effective amount of a compound or pharmaceutically acceptable salt thereof according to the invention described herein, such as compound (I) to (XV) or variant thereof, or a pharmaceutical composition of the invention, to a human subject in need thereof. In some embodiments, the compound of the invention or a pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or pharmaceutically acceptable salts thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or pharmaceutically acceptable salts thereof, is for use in the treatment of a disease associated with a calcium-activated chloride ion channel mutation. Also provided is use of the compound of the invention or a pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or pharmaceutically acceptable salts thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a disease associated with a calcium-activated chloride ion channel mutation. In some embodiments, the calcium-activated chloride ion channel associated disease is selected from the group of cystic fibroses, dry mouth and dry eye syndromes, asthma, diarrhea, hypertension, neuropathic pain and cancers.

In some embodiments of the invention, there is provided a method of treating a disease associated with a high (maxi) conductance chloride ion channel mutation, preferably by administering a therapeutically effective amount of a compound according to the invention described herein or

pharmaceutically acceptable salt thereof, such as compound (I) to (XV) or pharmaceutically acceptable salt thereof or variant thereof, or a pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or pharmaceutically acceptable salt thereof, to a human subject in need thereof. In some embodiments, the compound of the invention or a pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, is for use in the treatment of a disease associated with a high (maxi) conductance chloride ion channel mutation. Also disclosed is use of the compound of the invention or a pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a disease associated with a high (maxi) conductance chloride ion channel mutation. In some embodiments of the invention, the mutation is found in neurons cells, glia cells, cardiac muscle cells, lymphocytes, epithelia, macula densa cells of the kidney and human placenta syncytiotrophoblasts.

In some embodiments of the invention, there is provided a method of treating a disease associated with a volume-activated chloride channel mutation, preferably by administering a therapeutically effective amount of a compound according to the invention described herein or a pharmaceutically acceptable salt thereof, such as compound (I) to (XV) or pharmaceutically acceptable salt thereof, or variant thereof, or a pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, to a human subject in need thereof. In some embodiments, the compound of the invention or a pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, is for use in the treatment of a disease associated with a volume-activated chloride channel mutation. Also disclosed is use of the compound of the invention or a pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a disease associated with a volume-activated chloride channel mutation.

In some embodiments of the invention, there is provided a method of treating a disease associated with a cystic fibrosis transmembrane conductance regulator (CFTR) mutation, preferably by administering a therapeutically effective amount of a compound according to the invention described herein, such as compound (I) to (XV) or pharmaceutically acceptable salt thereof or variant thereof, or a pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or pharmaceutically acceptable salt thereof, to a human subject in need thereof. In some embodiments of the invention, the compounds of the invention or pharmaceutically acceptable salts thereof, preferably selected from compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, is for use in the treatment of a disease associated with a cystic fibrosis transmembrane conductance regulator (CFTR) mutation. Described is use of the compound of the invention or pharmaceutically acceptable salt thereof, preferably selected from compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a disease associated with a cystic fibrosis transmembrane conductance regulator (CFTR) mutation. In some embodiments, the compound and the pharmaceutical composition of the present invention are for use in the treatment of CFTR-mediated diseases selected from cystic fibrosis, asthma, smoke induced COPD, chronic bronchitis, rhinosinusitis, constipation, pancreatitis, pancreatic insufficiency, male infertility caused by congenital bilateral absence of the vas deferens (CBAVD), mild pulmonary disease, idiopathic pancreatitis, allergic bronchopulmonary aspergillosis (ABPA), liver disease, hereditary emphysema, hereditary hemochromatosis, coagulation-fibrinolysis deficiencies, such as protein C deficiency, Type 1 hereditary angioedema, lipid processing deficiencies, such as familial hypercholesterolemia, Type 1 chylomicronemia, abetalipoproteinemia, lysosomal storage diseases, such as I-cell disease/pseudo-Hurler, mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, polyendocrinopathy/hyperinsulemia, Diabetes mellitus, Laron dwarfism, myeloperoxidase deficiency, primary hypoparathyroidism, melanoma, glycanosis CDG type 1, congenital hyperthyroidism, osteogenesis imperfecta, hereditary hypofibrinogenemia, ACT deficiency, Diabetes insipidus (DI), neurophyseal DI, neprogenic DI, Charcot-Marie Tooth syndrome, Perlizaeus-Merzbacher disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, progressive supranuclear palsy, Pick's disease, several polyglutamine neurological disorders such as Huntington's, spinocerebellar ataxia type I, spinal and bulbar muscular atrophy, dentatorubal pallidolusian, and myotonic dystrophy,

as well as spongiform encephalopathies, such as hereditary Creutzfeldt-Jakob disease (due to prion protein processing defect), Fabry disease, Straussler-Scheinker syndrome, COPD, dry-eye disease, or Sjogren's disease, Osteoporosis, Osteopenia, bone healing and bone growth (including bone repair, bone regeneration, reducing bone resorption and increasing bone deposition), Gorham's Syndrome; chloride channelopathies such as myotonia congenita (Thomsen and Becker forms), Bartter's syndrome type III, Dent's disease, hyperekplexia, epilepsy, Gitelman, lysosomal storage disease, Angelman syndrome, and Primary Ciliary Dyskinesia (PCD), an inherited disorders of the structure and/or function of cilia, including PCD with situs inversus (also known as Kartagener syndrome), PCD without situs inversus and ciliary aplasia.

In some embodiments of the invention, the compound, pharmaceutically acceptable salt thereof, or the pharmaceutical composition of the present invention may be used in the treatment of cystic fibrosis patients presenting with one or more CFTR mutations, including Class I (e.g. G542X, W1282X, R553X, Glu831X), Class II (e.g. F508del, N1303K, I507del), Class III (e.g. G551D, S549N, V520F), Class IV (e.g. R117H, D1152H, R374P), or Class V mutations (e.g. 3849+10kbC>T, 2789+5G>A, A455E). The CF patient may present as a homozygotes or heterozygotes for any such CFTR mutation, e.g. F508del homozygote.

In some embodiments of the invention, the compound, pharmaceutically acceptable salt thereof, or the pharmaceutical composition of the present invention may be used in the treatment of channelopathies, which are a heterogeneous group of disorders resulting from the dysfunction of ion channels located in the membranes of all cells and many cellular organelles, including diseases of the respiratory system (e.g., cystic fibrosis) and the urinary system (e.g., Bartter syndrome). The compound, pharmaceutically acceptable salt or the pharmaceutical composition may be administered in combination with one or more therapeutic agents.

The compounds of the invention may be prepared by elongating the peptide chain on a suitable gel resin such as TentaGel (R) RAM resin with a Rink amide linker. The coupling is preferably performed in two steps, namely, by dissolving Fmoc protected amino acid, the uronium coupling agent O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) and N,N-diisopropylethylamine (DIPEA) in N,N-dimethylformamide (DMF) as solvent, under three hours of shaking in the first step and then the second coupling is performed with amino acid, HATU and DIPEA; then the resin is washed with DMF, methanol and DCM, and the washing is preferably followed by a deprotection step using 2% DBU and 2% piperidine in DMF in two steps with 15 and 5 minutes reaction times. To exemplify the preparation of a compound of the

invention containing a thiourea, i.e. wherein Y or Y¹ is S, after the coupling of the amino acids, the thiourea element is created, whereby the free N-terminus is reacted with specific isothiocyanates under alkaline conditions in DMF. After the completion of the sequence and thiourea construct the cleavage was carried out with TFA/water/dl-dithiothreitol (DTT)/TIS at 0°C for 1 h.

In some embodiments of the invention, there is provided a method of treating a chloride ion channelopathy in a subject in need thereof, wherein said method comprises administration of a therapeutically effective amount of one or more compounds disclosed herein, to the subject, optionally in combination with one or more therapeutic agents, preferably by administering a therapeutically effective amount of compound (I) to (XV) or variant thereof, to a human subject in need thereof.

The disclosure provides one or more compounds disclosed herein, or pharmaceutically acceptable salt thereof for use in a method of treating a chloride ion channelopathy in a subject in need thereof. Also provided is use of one or more compounds disclosed herein, or pharmaceutically acceptable salt thereof in the manufacture of a medicament for treating a chloride ion channelopathy in a subject in need thereof. Additional therapeutic agents may optionally be administered as part of the treatment. The present disclosure also thus provides a combination comprising a compound or pharmaceutically acceptable salt thereof and one or more additional therapeutic agents for use in a method of treating a chloride ion channelopathy in a subject in need thereof, wherein said method comprises administration of a therapeutically effective amount of one or more of the compounds or pharmaceutically acceptable salts thereof and one or more additional therapeutic agents, to the subject in need thereof.

In some embodiments of the invention, there is provided a method of treating a CFTR-mediated disease selected from cystic fibrosis, asthma, COPD, smoke induced COPD, and chronic bronchitis fibrosis in a subject in need thereof, wherein said method comprises administering of a therapeutically effective amount of one or more compounds or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition disclosed herein to a human subject in need thereof. In some embodiments, the compound, or pharmaceutically acceptable salt thereof, or the pharmaceutical composition of the invention is for use in the treatment of a CFTR-mediated disease selected from cystic fibrosis, asthma, COPD, smoke induced COPD, and chronic bronchitis fibrosis. The compound or the pharmaceutical composition may be administered in combination with one or more therapeutic agents. The present disclosure also thus provides a

combination comprising one or more compounds or pharmaceutically acceptable salts thereof and one or more additional therapeutic agents for use in a method of treating a CFTR-mediated disease selected from cystic fibrosis, asthma, COPD, smoke induced COPD, and chronic bronchitis fibrosis in a subject in need thereof, wherein said method comprises administration of a therapeutically effective amount of the one or more compounds or pharmaceutically acceptable salts thereof and the one or more additional therapeutic agents, to the subject in need thereof. In some embodiments, the CFTR-mediated disease is cystic fibrosis. In some embodiments, the compound, or the compound in the pharmaceutical composition, is any of compounds (I) to (XV), or pharmaceutically acceptable salt thereof or a variant thereof.

In some embodiments of the invention, there is provided a method of treating cystic fibrosis in a human subject in need thereof, wherein said method comprises administration of a therapeutically effective amount of one or more compounds, pharmaceutically acceptable salts or a pharmaceutical composition disclosed herein, to the human subject, optionally in combination with one or more therapeutic agents, preferably by administering a therapeutically effective amount of compound (I) to (XV), salt or variant thereof, or a pharmaceutical composition comprising any of compounds (I) to (XV) or a variant thereof, to a human subject in need thereof. The disclosure thus provides one or more compounds or a pharmaceutical composition disclosed herein, optionally in combination with one or more therapeutic agents, preferably by administering a therapeutically effective amount of compound (I) to (XV) or pharmaceutically acceptable salt thereof or variant thereof, or a pharmaceutical composition comprising any of compounds (I) to (XV) or a pharmaceutically acceptable salt thereof or variant thereof, for use in a method of treating cystic fibrosis in a human subject in need thereof. The present disclosure also thus provides a combination comprising a one or more compounds or pharmaceutically acceptable salts thereof and one or more additional therapeutic agents for use in a method of treating a cystic fibrosis, wherein the method comprises administration of a therapeutically effective amount of the one or more compounds or pharmaceutically acceptable salt thereof and the one or more additional therapeutic agents, to the subject in need thereof.

In some embodiments of the invention, there is provided a method of treating, reducing, inhibiting or controlling cystic fibrosis in a subject, wherein said method comprises simultaneously, separately or sequentially administering to the subject, (i) one or more therapeutic agents, and, (ii) a therapeutically effective amount of one or more compounds disclosed herein or a pharmaceutical composition disclosed herein, preferably by administering a therapeutically effective amount of compound (I) to (XV) or variant thereof, or a pharmaceutical composition

comprising any of compounds (I) to (XV) or a variant thereof, to a human subject in need thereof. The present disclosure also thus provides a combination comprising a one or more compounds or pharmaceutically acceptable salts thereof and one or more additional therapeutic agents for use in a method of treating a cystic fibrosis, wherein the method comprises administration of a therapeutically effective amount of the one or more compounds or pharmaceutically acceptable salt thereof and the one or more additional therapeutic agents, to the subject in need thereof.

In some embodiments of the invention, there is provided a method of treating, reducing, inhibiting or controlling at least one sign or symptom of cystic fibrosis in a subject, wherein said method comprises administration of a therapeutically effective amount of one or more compounds disclosed herein or a pharmaceutical composition disclosed herein, to the human subject, optionally in combination with one or more therapeutic agents, wherein said sign or symptom is associated with the airways or respiratory system and includes one or more of the following: abnormally viscous mucus accumulation; increased total mucin content; elevated inflammatory factor concentration; decreased cellular secretion of chloride ions; impaired fluid secretion; increased apical sodium absorption by airway epithelial cells; acidification and decreased height of the apical airway surface liquid; chronic cough; chronic lung infection, and combinations thereof. Preferably said method involves administering a therapeutically effective amount of a compound according to the invention described herein, such as compound (I) to (XV) or variant thereof, or a pharmaceutical composition comprising any of compounds (I) to (XV) or a variant thereof, to a human subject in need thereof.

In some embodiments of the invention, there is provided a method of treating, reducing, inhibiting or controlling at least one sign or symptom of cystic fibrosis in a subject, wherein said method comprises simultaneously, separately or sequentially administering to the subject, (i) one or more therapeutic agents, and, (ii) a therapeutically effective amount of one or more compounds disclosed herein or a pharmaceutical composition disclosed herein, wherein said sign or symptom is associated with the airways or respiratory system and includes one or more of the following: abnormally viscous mucus accumulation; increased total mucin content; elevated inflammatory factor concentration; decreased cellular secretion of chloride ions; impaired fluid secretion; increased apical sodium absorption by airway epithelial cells; acidification and decreased height of the apical airway surface liquid; chronic cough; chronic lung infection, and combinations thereof. Preferably said method involves administering a therapeutically effective amount of a compound according to the invention described herein, such as compound (I) to (XV) or variant

thereof, or a pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a variant thereof, to a human subject in need thereof.

In some embodiments of the invention, there is provided a compound, pharmaceutically acceptable salt, or a pharmaceutical composition of the invention for use in the treatment, reduction, inhibition or control of viscous sputum or mucus associated with cystic fibrosis in a human subject, wherein said compound or pharmaceutical composition increases the electrolyte content of said viscous mucus or sputum, such as chloride, optionally wherein said compound or pharmaceutical composition is administered to the lungs of said human subject by pulmonary or aerosol delivery as a solution or suspension in a liquid vehicle, or as a dry powder. Preferably said compound is one of compounds (I) to (XV) or a variant thereof, or said pharmaceutical composition comprises compound (I) to (XV) or variant thereof.

In some embodiments of the invention, there is provided a method of treating, reducing, inhibiting or controlling viscous sputum or mucus associated with cystic fibrosis in a human subject, wherein said method comprises administration of a compound or pharmaceutical composition according to the invention described herein, wherein said method increases the electrolyte content of said viscous mucus or sputum, such as chloride, optionally wherein said compound or pharmaceutical composition is administered to the lungs of said human subject by pulmonary or aerosol delivery as a solution or suspension in a liquid vehicle, or as a dry powder. Preferably said method involves administering a therapeutically effective amount of a compound according to the invention described herein, such as compound (I) to (XIV) or variant thereof, or a pharmaceutical composition of the invention, preferably comprising any of compounds (I) to (XV) or a variant thereof, to a human subject in need thereof.

In some embodiments of the invention, there is provided a complex comprising a cell-penetrating peptide (CPP) covalently linked via an X-NH-C(=Y)-NH- moiety at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities.

In some embodiments of the invention, there is provided a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group, preferably wherein said complex is amphipathic.

In some embodiments of the invention, there is provided a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities selected from the group consisting of an acetyl, an aromatic, a fatty acid, a cholesterol, a poly-ethylene glycol, a nuclear localization signal, nuclear export signal, an antibody, a polysaccharide and a targeting molecule, via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group.

In some embodiments of the invention, there is provided a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities selected in the group consisting of an acetyl, an aromatic, a fatty acid, a cholesterol, a poly-ethylene glycol, a nuclear localization signal, nuclear export signal, an antibody, a polysaccharide and a targeting molecule, via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group for use in the treatment of channelopathies, which are a heterogeneous group of disorders resulting from the dysfunction of ion channels located in the membranes of all cells and many cellular organelles, including diseases of the respiratory system (e.g., cystic fibrosis) and the urinary system (e.g., Bartter syndrome).

In some embodiments of the invention, there is provided a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group, preferably wherein said complex is amphipathic, for use in the manufacture of a medicament, e.g. for the treatment of a disease or condition.

In some embodiments of the invention, is provided a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group, preferably wherein said complex is amphipathic, for use in the treatment of disease or condition, preferably a chloride ion channelopathy.

In some embodiments of the invention, there is provided a method of treating a chloride ion channelopathy in a subject in need thereof, wherein said method comprises administration of a therapeutically effective amount of a complex comprising a cell-penetrating peptide (CPP)

covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities selected in the group consisting of an acetyl, an aromatic, a fatty acid, a cholesterol, a poly-ethylene glycol, a nuclear localization signal, nuclear export signal, an antibody, a polysaccharide and a targeting molecule, via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group, to a human subject in need thereof. In some embodiments, there is provided a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities selected in the group consisting of an acetyl, an aromatic, a fatty acid, a cholesterol, a poly-ethylene glycol, a nuclear localization signal, nuclear export signal, an antibody, a polysaccharide and a targeting molecule, via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group for use in treating a chloride ion channelopathy in a subject in need thereof.

In some embodiments of the invention, there is provided a method of treating a CFTR-mediated disease selected from cystic fibrosis, asthma, COPD, smoke induced COPD, and chronic bronchitis fibrosis in a subject in need thereof, wherein said method comprises administration of a therapeutically effective amount of a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities selected in the group consisting of an acetyl, an aromatic, a fatty acid, a cholesterol, a poly-ethylene glycol, a nuclear localization signal, nuclear export signal, an antibody, a polysaccharide and a targeting molecule, via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group, optionally in combination with one or more therapeutic agents, to a human subject in need thereof. In some embodiments, there is provided a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities selected in the group consisting of an acetyl, an aromatic, a fatty acid, a cholesterol, a poly-ethylene glycol, a nuclear localization signal, nuclear export signal, an antibody, a polysaccharide and a targeting molecule, via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group for use in the treatment of a CFTR-mediated disease selected from cystic fibrosis, asthma, COPD, smoke induced COPD, and chronic bronchitis fibrosis, optionally wherein the treatment comprises administering the complex in combination with one or more therapeutic agents.

In some embodiments of the invention, there is provided a method of treating cystic fibrosis in a human subject in need thereof, wherein said method comprises administration of a therapeutically

effective amount of a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities selected in the group consisting of an acetyl, an aromatic, a fatty acid, a cholesterol, a poly-ethylene glycol, a nuclear localization signal, nuclear export signal, an antibody, a polysaccharide and a targeting molecule, via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group, to a human subject in need thereof. In some embodiments, there is provided a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities selected in the group consisting of an acetyl, an aromatic, a fatty acid, a cholesterol, a poly-ethylene glycol, a nuclear localization signal, nuclear export signal, an antibody, a polysaccharide and a targeting molecule, via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group for use in the treatment of cystic fibrosis.

In some embodiments of the invention, there is provided a method of treating, reducing, inhibiting or controlling cystic fibrosis in a subject, wherein said method comprises simultaneously, separately or sequentially administering to the subject, (i) one or more therapeutic agents, and, (ii) a therapeutically effective amount of a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group, preferably wherein said complex is amphipathic, to a human subject in need thereof.

In some embodiments of the invention, there is provided a method of treating, reducing, inhibiting or controlling at least one sign or symptom of cystic fibrosis in a subject, wherein said method comprises administration of a therapeutically effective amount a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group, preferably wherein said complex is amphipathic, to the human subject, optionally in combination with one or more therapeutic agents, wherein said sign or symptom is associated with the airways or respiratory system and includes one or more of the following: abnormally viscous mucus accumulation; increased total mucin content; elevated inflammatory factor concentration; decreased cellular secretion of chloride ions; impaired fluid secretion; increased apical sodium absorption by airway

epithelial cells; acidification and decreased height of the apical airway surface liquid; chronic cough; chronic lung infection, and combinations thereof.

In some embodiments of the invention, there is provided a method of treating, reducing, inhibiting or controlling at least one sign or symptom of cystic fibrosis in a subject, wherein said method comprises simultaneously, separately or sequentially administering to the subject, (i) one or more therapeutic agents, and, (ii) a therapeutically effective amount of one or more compounds disclosed herein, wherein said sign or symptom is associated with the airways or respiratory system and includes one or more of the following: abnormally viscous mucus accumulation; increased total mucin content; elevated inflammatory factor concentration; decreased cellular secretion of chloride ions; impaired fluid secretion; increased apical sodium absorption by airway epithelial cells; acidification and decreased height of the apical airway surface liquid; chronic cough; chronic lung infection, and combinations thereof. Preferably said method involves administering a therapeutically effective amount of a one or more complexes comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group, preferably wherein said complex is amphipathic, to a human subject in need thereof.

In some embodiments of the invention, there is provided a pharmaceutical composition for use in the treatment, reduction, inhibition or control of viscous sputum or mucus associated with cystic fibrosis in a human subject, wherein said pharmaceutical composition increases the electrolyte content of said viscous mucus or sputum, such as chloride, optionally wherein said pharmaceutical composition is administered to the lungs of said human subject by pulmonary or aerosol delivery as a solution or suspension in a liquid vehicle, or as a dry powder. Preferably said composition comprises a complex comprising a cell-penetrating peptide (CPP) covalently linked at the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group, preferably wherein said complex is amphipathic.

In some embodiments of the invention, there is provided a method of treating, reducing, inhibiting or controlling viscous sputum or mucus associated with cystic fibrosis in a human subject, wherein said method comprises administration of a compound or pharmaceutical composition according to the invention described herein, wherein said method increases the electrolyte content of said viscous mucus or sputum, such as chloride, optionally wherein said compound or

pharmaceutical composition is administered to the lungs of said human subject by pulmonary or aerosol delivery as a solution or suspension in a liquid vehicle, or as a dry powder. Preferably said method involves administering a therapeutically effective amount of a complex comprising a cell-penetrating peptide (CPP) covalently linked to the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group, preferably wherein said complex is amphipathic, to a human subject in need thereof.

The invention also provides a complex comprising a cell-penetrating peptide (CPP) covalently linked to the N-terminal end of the amino acid sequence of said CPP to one or several chemical entities via a linker preferably selected from a thiourea, (thio)ureido, (hydroxy)guanidino, ethylenediamine or acylthiourea group, for use in the treatment of a disease associated with a chloride ion channel mutation, wherein the disease is selected from: myotonia congenita Becker, Myotonia congenita Thomsen, Dystrophia myotonica 1, dystrophia myotonica 2, childhood absence epilepsy type 3, juvenile absence epilepsy, juvenile myoclonic epilepsy, epilepsy with grand mal seizures on awakening, Bartter Syndrome, Bartter syndrome with sensorineural deafness, Dent's disease, autosomal dominant osteopetrosis, autosomal recessive osteopetrosis, cystic fibrosis, idiopathic chronic pancreatitis, bronchiectasis, congenital bilateral absence of vas deferens, Best's disease, adult-onset vitelliform macular dystrophy, concentric annular macular dystrophy, cataract, juvenile myoclonic epilepsy, childhood absence epilepsy type 2, generalized epilepsy with febrile seizures plus, severe myoclonic epilepsy in infancy, insomnia and hereditary hyperekplexia.

In some embodiments, there is provided a compound, pharmaceutical composition or complex for use in the manufacture of a medicament for the treatment of a disease or condition. The compound or pharmaceutically acceptable salt thereof, pharmaceutical composition or complex is any of the compounds, pharmaceutical compositions and complexes disclosed herein. The disease or condition may be any of the diseases or conditions disclosed herein. The medicament may be in any of the dosage forms disclosed herein, and/or may be administered by any route disclosed herein. The treatment may comprise administration of the medicament in combination with one or more therapeutic agents, as detailed herein.

The treatment disclosed herein may also include reducing, inhibiting or controlling the disease or condition, preferably treating, reducing, inhibiting or controlling at least one sign or symptom of the disease or condition. For example, the treatment of cystic fibrosis includes reducing, inhibiting

or controlling cystic fibrosis in a subject, preferably treating, reducing, inhibiting or controlling at least one sign or symptom of cystic fibrosis in a subject. When the disease is cystic fibrosis, the sign or symptom may be associated with the airways or respiratory system and may include one or more of the following: abnormally viscous mucus accumulation; increased total mucin content; elevated inflammatory factor concentration; decreased cellular secretion of chloride ions; impaired fluid secretion; increased apical sodium absorption by airway epithelial cells; acidification and decreased height of the apical airway surface liquid; chronic cough; chronic lung infection, and combinations thereof.

The treatment disclosed herein may comprise administering the compound, pharmaceutical composition or complex of the invention in combination with one or more therapeutic agents. The compound, pharmaceutical composition or complex of the invention may be administered simultaneously or sequentially with the one or more therapeutic agents. When the compound, pharmaceutical composition or complex is administered simultaneously with the one or more therapeutic agents, the compound, pharmaceutical composition or complex may be administered separately from, or in a single composition with, the one or more therapeutic agents. When the compound, pharmaceutical composition or complex is administered simultaneously with, but separately from, the one or more therapeutic agents, the compound, pharmaceutical composition or the complex and the one or more therapeutic agents are administered one after the other, in either order.

A therapeutically effective amount, as disclosed herein, may be between about 1 ng and about 5 g, preferably between about 10 ng and 1 g.

In some embodiments, there is provided a kit comprising a compound, pharmaceutical composition or complex of the invention. In some embodiments, the kit also comprises one or more therapeutic agents. In these embodiments, each of the compound, pharmaceutical composition or complex, and the one or more therapeutic agents, may be provided in separate vials or compartments. In some embodiments, the kit further comprises instructions for the administration of the compound, pharmaceutical composition or complex, and optionally the one or more therapeutic agents.

EXAMPLES

EXAMPLE 1

Synthesis of Formulae (I)-(XV)

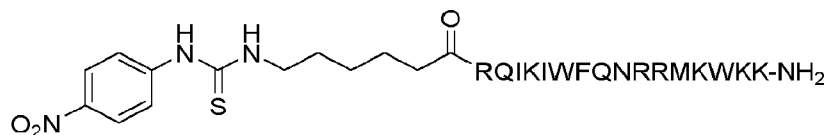
With Fmoc chemistry the peptide chain was elongated on TentaGel R RAM resin (0.19 mmol/g) (E Bayer, Angew. Chem. Int. Ed., 1991, 30, pp 113.) with a Rink amide linker on a 0.4 mmol scale manually. The coupling was performed in two steps. In the first step 3 equivalents of Fmoc protected amino acid, 3 equivalents of the uronium coupling agent O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) (LA Carpino, Am. Chem. Soc., 1993, 115, pp 4379.) and 6 equivalents N,N-diisopropylethylamine (DIPEA) was used in N,N-dimethylformamide (DMF) as solvent with three hours of shaking. The second coupling was performed with 1 equivalent amino acid, 1 equivalent HATU and 2 equivalents of DIPEA. After the coupling steps, the resin was washed 3 times with DMF, once with methanol and 3 times with DCM. By these coupling conditions no truncated sequences was observed. The deprotection step was performed with 2% DBU and 2% piperidine in DMF in two steps with 15 and 5 minutes reaction times.

The resin was washed with the same solvents as described previously. After the coupling of the amino acids, the thiourea element was installed. In this regard, the linker moiety between peptide and thiourea group in compounds of the invention was also installed using analogous amide coupling chemistry. The free N-terminus was then reacted with specific isothiocyanates under alkaline conditions in DMF. The isothiocyanates used to prepare example compounds I-XV were commercially obtained. For instance, to install the 3,5-bis(trifluoromethyl)phenyl moiety in the compound according to formula IX, the free N-terminus was reacted with 3,5-bis(trifluoromethyl)phenyl isothiocyanate (98% strength obtained from Sigma-Aldrich). After the completion of the sequence and thiourea construct, the cleavage of the compound from the resin was carried out with TFA/water/dl-dithiothreitol (DTT)/TIS at 0°C for 1 h. The cleavage has been performed with TFA/water/DL-dithiothreitol (DTT)/TIS (90/5/2.5/2.5) at 0 °C for 1 h. The purification was performed by reverse-phase HPLC, using a Phenomenex Luna C18 100 Å 10 µm column (10 mm x 250 mm).¹¹⁷ The HPLC apparatus was made by JASCO and the solvent system used was as follows: 0.1% TFA in water; 0.1% TFA, 80% acetonitrile in water; linear gradient was used during 60 min, at a flow rate of 4.0 mL min⁻¹, with detection at 206 nm. The fractions purity was determined by analytical HPLC using a JASCO HPLC system with a Phenomenex Luna C18 100 Å 5 µm column (4.6 mm x 250 mm) and the pure fractions were pooled and lyophilized. The purified peptides were characterized by mass spectrometry.

For HCl salt preparation, the TFA salts of the peptides were dissolved in distilled water at 5 mg/mL concentration. Then using continuous stirring Amberlite IRA 410 chloride anion exchange resin was added to the solution at 20x excess compared to the amount of TFA salts and

was stirred for 1 hour at 300 rpm. After 1 hour the suspensions were filtered through a porosity 3 glass filter. The remained resin was then washed with 0.1 N HCl and distilled water. The filtrates were then lyophilized in order to obtain solid HCl salt.

5 Formula (I):



The molecular weight (MW) of the compound is 2537.4 Da; retention time is 12.8 min and its chromatographic properties: Gradient: 5->80% 25 min., A eluent: 0.1% TFA water, B eluent:
 10 0.1% TFA 80% ACN 20% water (Column: Phenomenex Luna C18(2) 5um, 100A, 250*4.6 mm).

Formula (II):



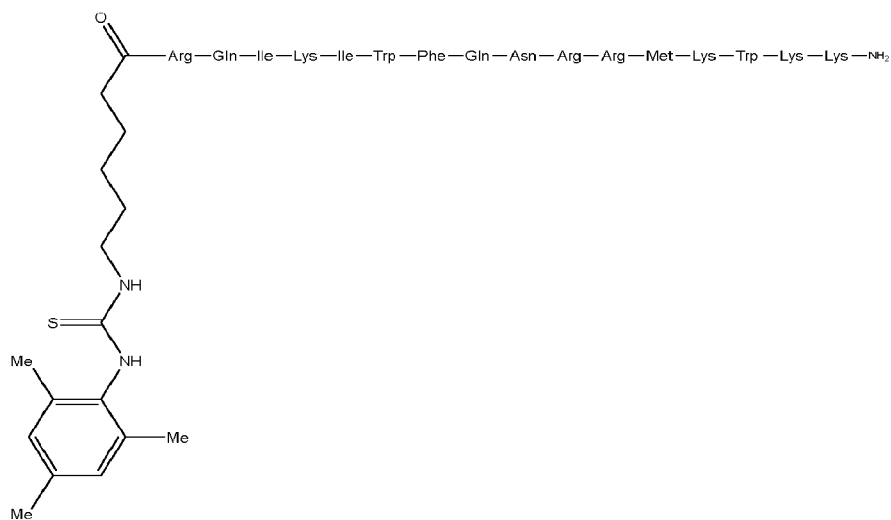
15 The molecular weight (MW) of the compound is 2548.1 Da;

Formula (III):



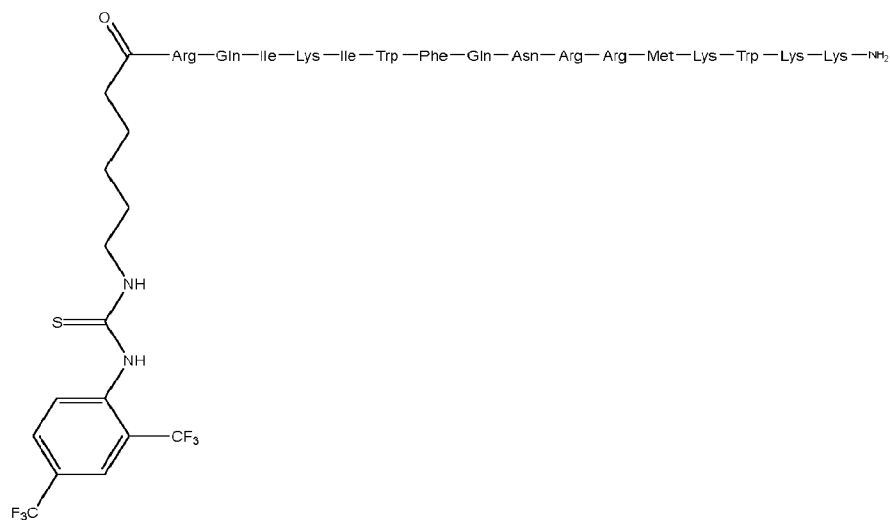
The molecular weight (MW) of the compound is 2548.1 Da;

5 Formula (IV):



The molecular weight (MW) of the compound is 2536.2 Da;

10 Formula (V):



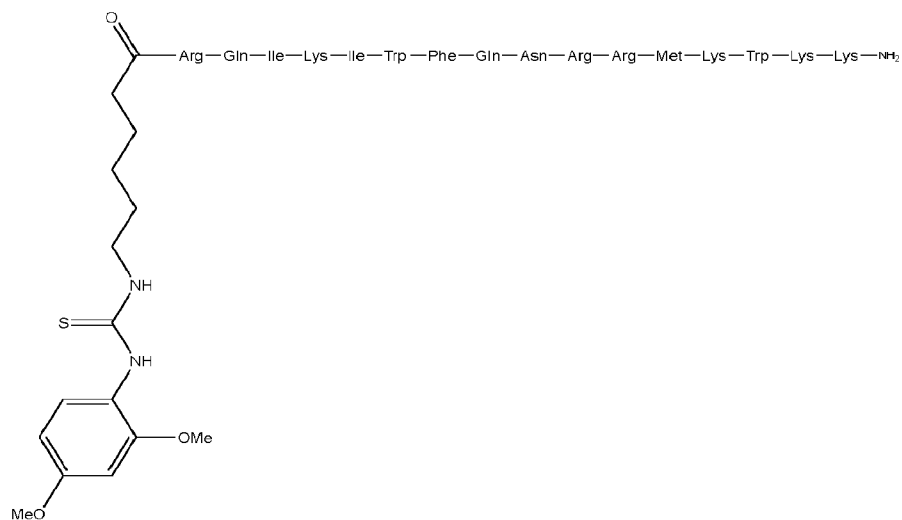
The molecular weight (MW) of the compound is 2630.1 Da;

5 Formula (VI):



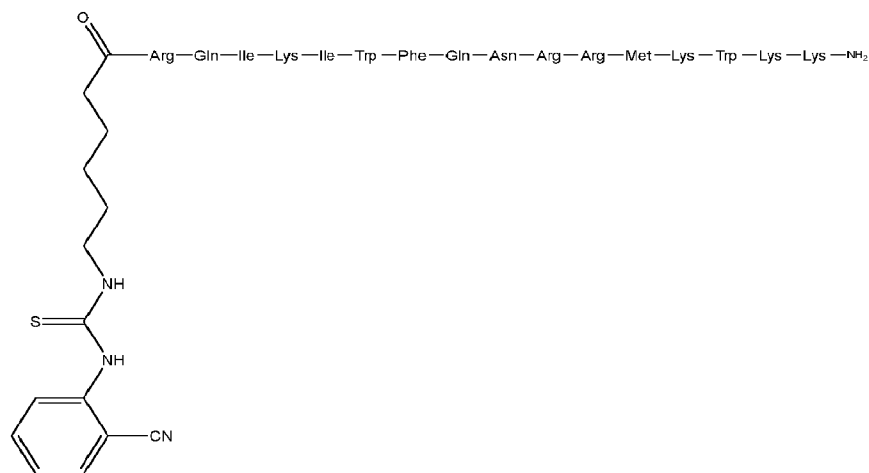
The molecular weight (MW) of the compound is 2530.1 Da;

10 Formula (VII):



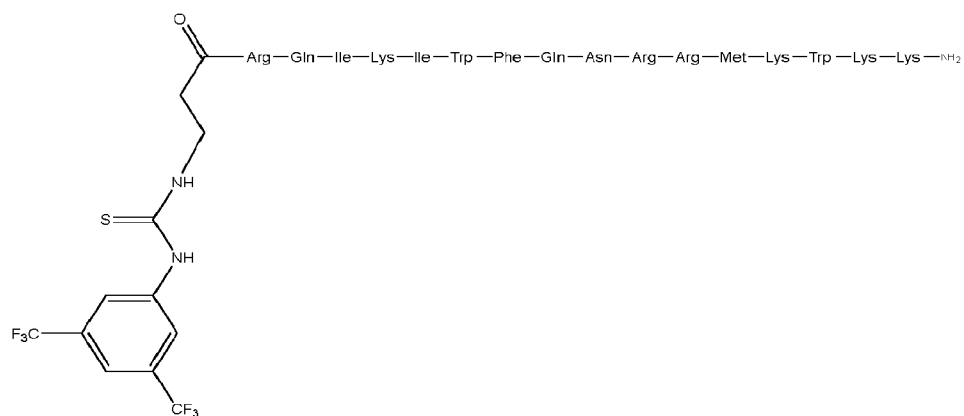
The molecular weight (MW) of the compound is 2554.2 Da;

5 Formula (VIII):



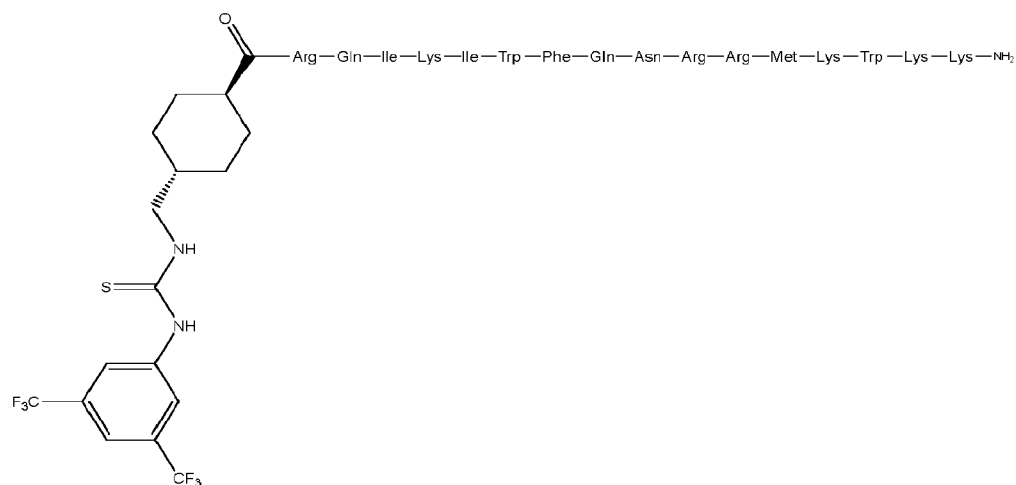
The molecular weight (MW) of the compound is 2519.1 Da;

10 Formula (IX):



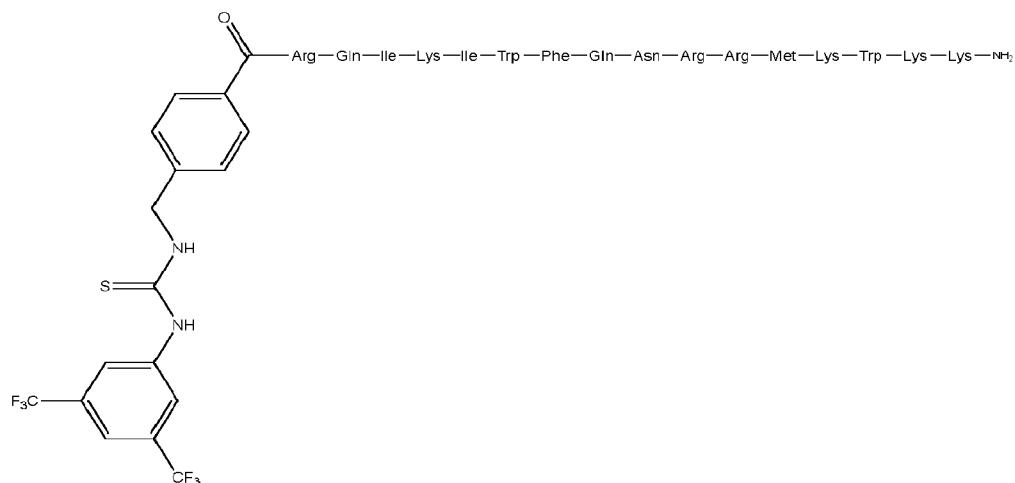
The molecular weight (MW) of the compound is 2588.0 Da;

5 Formula (X):



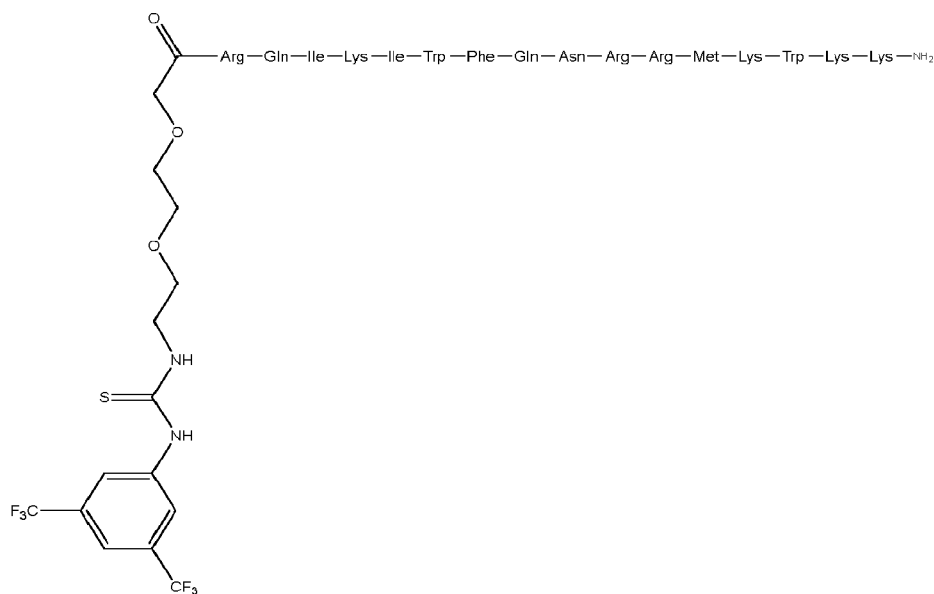
The molecular weight (MW) of the compound is 2656.2 Da;

10 Formula (XI):



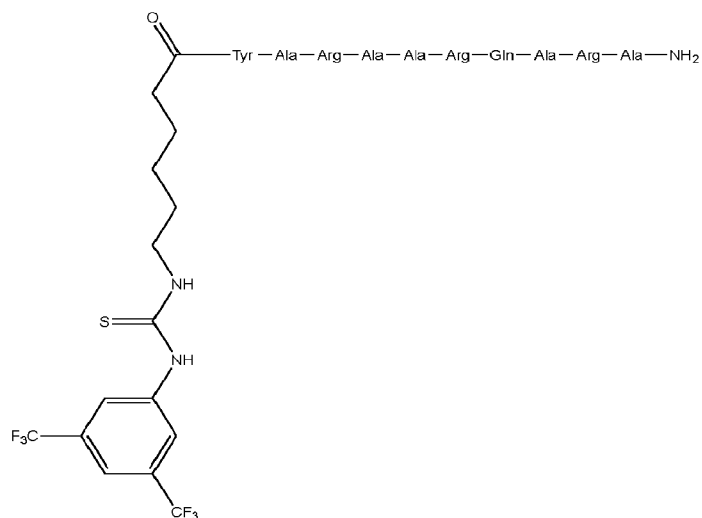
The molecular weight (MW) of the compound is 2650.1 Da;

5 Formula (XII):



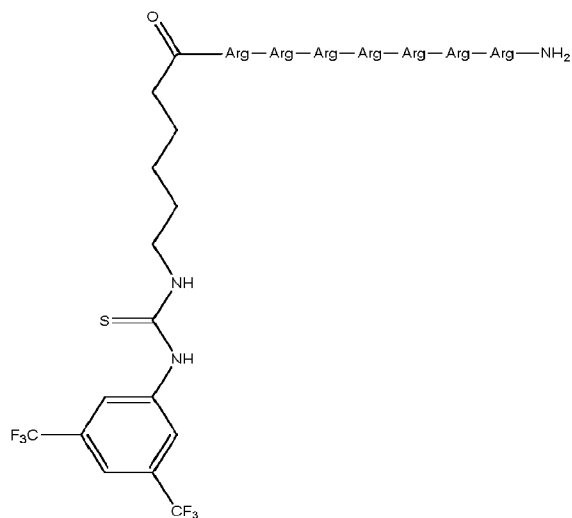
The molecular weight (MW) of the compound is 2662.1 Da;

10 Formula (XIII):



The molecular weight (MW) of the compound is 1516.6 Da;

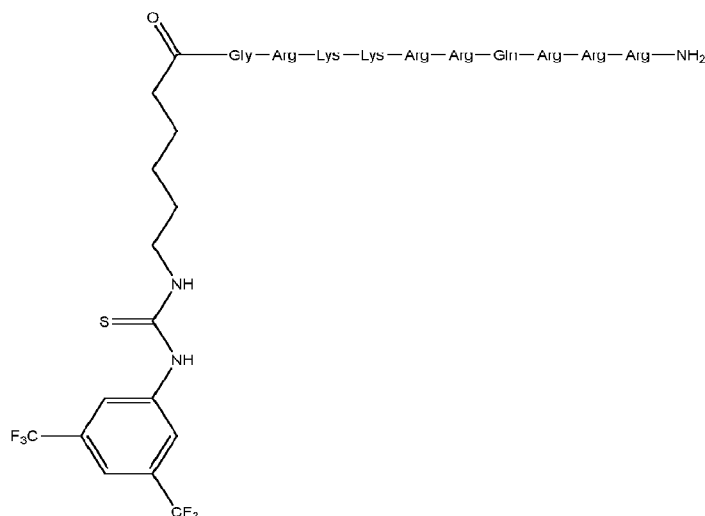
Formula (XIV):



5

The molecular weight (MW) of the compound is 1494.7 Da;

Formula (XV):



The molecular weight (MW) of the compound is 1780.0 Da.

Example 2

5

Effect of compounds of Formula (I) – Formula (XV) on cell fate

To investigate the effect of compounds according to the invention on cell fate, an apoptosis/necrosis detection kit (blue, green, red) (Abcam; Cat No.: ab176749) was used to simultaneously monitor apoptotic, necrotic, and healthy cells. The phosphoserine (PS) sensor used in this kit had green fluorescence (Ex/Em = 490/525 nm) upon binding to membrane PS. Necrosis has been characterized as passive, accidental cell death resulting from environmental perturbations with uncontrolled release of inflammatory cellular contents. Loss of plasma membrane integrity, as demonstrated by the ability of a membrane-impermeable 7-Aminoactinomycin D (7-AAD) (Ex/Em = 546/647 nm) to label the nucleus, represents a straightforward approach to demonstrate late-stage apoptosis and necrosis. In addition, this kit also provided a live cell cytoplasm labeling dye, CytoCalcein Violet 450 (Ex/Em = 405/450 nm), for labeling living cell cytoplasm. This kit has been optimized to simultaneously detect cell apoptosis, necrosis and healthy cells (blue). The images were captured by confocal microscope.

HeLa cells were grown on a CELLview Slide (Greiner Bio-One; Cat. No.: 543079) in a humidified incubator at 37°C. When the cells reach optimal confluency, they were washed 2x with pre-warmed Hepes. The compounds were applied to the wells at a concentration of 100 µM in 37°C standard Hepes. The cells were co-incubated with the molecules for 30 min in a humidified incubator at 37°C. After incubation, the cells are washed 3x with warm Hepes. Apoptosis/Necrosis detection kit components were mixed according to the manufacturers protocol, then added to the wells. The cells were incubated with the components for 30 minutes in

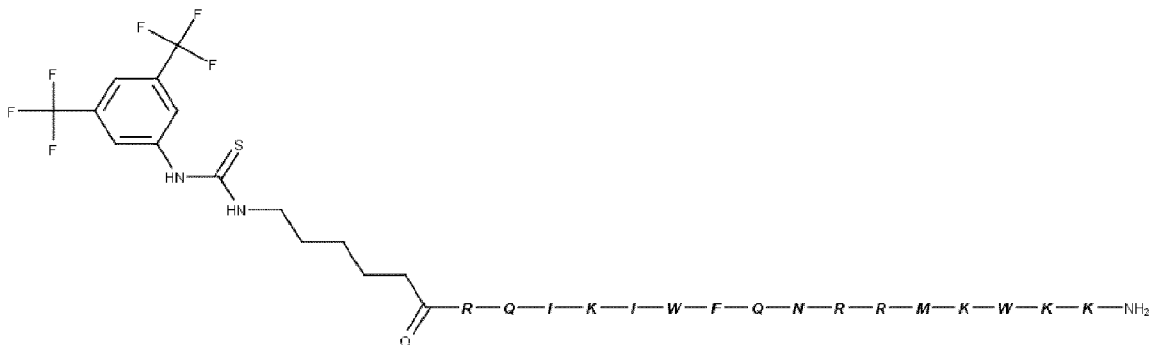
a humidified incubator at 37°C. After incubation, the cells were washed from the reaction mix 3x in -pre-warmed Hepes, then immediately visualized at a Zeiss LSM 880 confocal microscope.

The results of the experiment are shown in Figure 1. Formulae (V), (VI), (VII) and (XIII) had the least effect on cell viability, i.e. these compounds resulted in the greatest percentage of live cells.

Regulation of ion channels or transporters expressed or added in/to Xenopus laevis oocytes

Two-Electrode Voltage-Clamp (TEVC) recording in *Xenopus laevis* oocytes provides a powerful method to investigate the functions and regulation of ion channels or transporters expressed or added in/to *Xenopus laevis* oocytes. The plasma membrane of the oocyte is impaled by two microelectrodes, one for voltage sensing and the other one for current injection.

Solutions used for the experiments were 3 M KCl (internal), Cl⁻ free (external: in bath, then perfusion of cells with 5ml/cell). Compounds were dissolved in 93 mM Na-aspartate, 5 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, 50 µg/ml Pen.-Strept, pH 7.4. to make 10 µM solutions. The negative control was penetratin, while the positive control was Formula XVI (which has been identified in PCT application no. PCT/IB2020/061590 to have chloride ion transporter function):



(XVI).

For each tested compound, the signal (uA) from each tested compound was normalized against the positive control, and is shown as a percentage of the signal from the positive control in Table 1 below (Oocyte % = percentage of the signal (uA) – baseline (negative control) compared to positive control was determined). If the oocyte% was higher than 100%, then the test compound was considered as having a higher chloride ion transport capability compared to the positive control. If the oocyte % was less than 80%, then the test compound was considered to have a

lower chloride ion transport capability than the positive control (it should be noted that these test compounds still have chloride ion transporter functionality). The results are shown in Table 1.

Table 1: results of Two-Electrode Voltage-Clamp (TEVC) recording

Formula	Oocyte%
II	53.00
III	44.00
IV	25.60
V	80.00
VI	43.00
VII	28.00
VIII	33.60
IX	54.80
X	47.30
XI	65.30
XIV	2.80
XV	1.20

5

Example 3

Effect of Formula (I) – Formula (XV) on the intracellular Cl⁻ level in 2D HeLa cells in extracellular Cl⁻ free media

10

To assess the biological activity of the tested peptides, changes of intracellular Cl^- level were measured by loading HeLa cells with $5\mu\text{M}$ N-(Ethoxycarbonylmethyl)-6-Methoxyquinolinium Bromide (MQAE; ThermoFischer; Catalog number: E3101) for 30 min in the presence of 0.05% Pluronic F-127. Cells were bathed with Cl^- free external solution and treated with $10\mu\text{M}$ of each compound at 37°C at a perfusion rate of 2-3 ml/min. Region of interests (ROIs) were determined by the xcellence softver (Olympus) and changes of Cl^- were determined by exciting the cells with an MT20 light source equipped with a 340/11 nm excitation filter. Excitation and emission wavelengths were separated by a 400 nm beam splitter and the emitted light was captured by a Hamamatsu ORCA- ER CCD camera. One measurement per second was obtained. During further analysis, the fluorescence signals were normalized to the initial fluorescence intensity (F_1/F_0) and expressed as normalized MQAE fluorescence. The maximal fluorescent intensity changes were calculated, and the average maximal fluorescent intensity for three repeats is shown in Table 1. Notably, an increase of normalised fluorescent intensity represents a decrease in the intracellular Cl^- concentration.

Compounds of Formulae (II), (III), (IV), (V), (VI), (VII), (VIII), (IX), (X), (XI), (XIII), (XIV) and (XV) were tested and displayed different characteristics in the intracellular Cl^- measurements. (Table 2). A high average value indicates a low chloride ion concentration inside the cell, so indicates that the compound in question is effective as a chloride ion transporter.

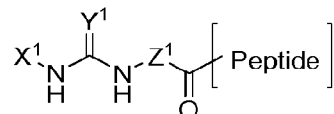
Table 2 - Results of the intracellular chloride ion measurements that shows the average maximal responses to each compound (AVE: average; SEM: standard error of means)

Formula	Cl- transport - Max responses (MQAE) AVE	Cl- transport - Max responses (MQAE) SEM
I		
II	0.0029	0.0004
III	0.0221	0.0025
IV	0.0699	0.0075
V	0.0378	0.0022
VI	0.0069	0.0005

VII	0.0035	0.0003
VIII	-0.0042	0.0003
IX	0.0100	0.0013
X	0.0352	0.0024
XI	0.0023	0.0006
XIII	0.0355	0.0012
XIV	0.0461	0.0040
XV	0.0512	0.0033

CLAIMS:

1. A compound according to formula Ia:



Formula Ia

or a pharmaceutically acceptable salt thereof, wherein

X¹ is a C₆₋₁₀aryl group, optionally substituted with from 1 to 3 substituents, each substituent independently selected from NO₂, CN, halo, C₁₋₄alkyl, C₁₋₄alkoxy and C₁₋₃haloalkyl;

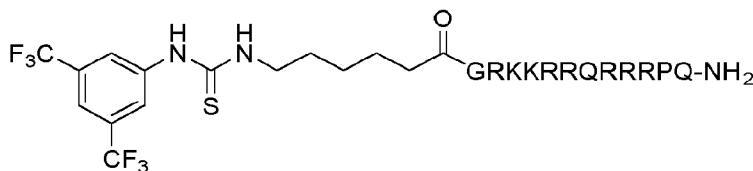
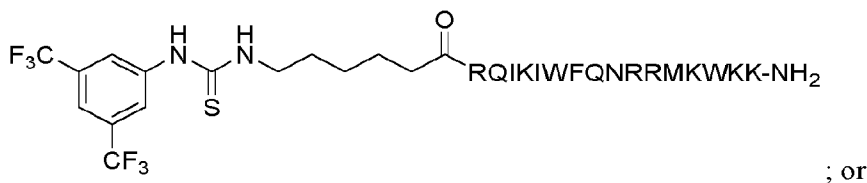
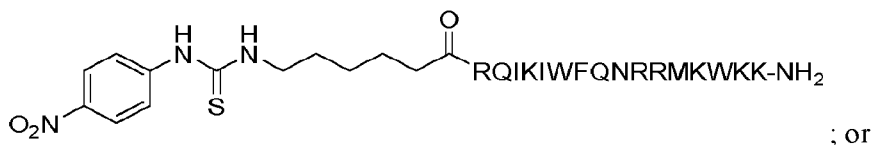
Y¹ is S or O;

Z¹ is C₁₋₁₀alkylene, C₅₋₁₀cycloalkylene, C₂₋₁₀heteroalkylene, or C₆₋₁₀aralkylene; and

the peptide domain comprises a cell penetrating peptide (CPP); and

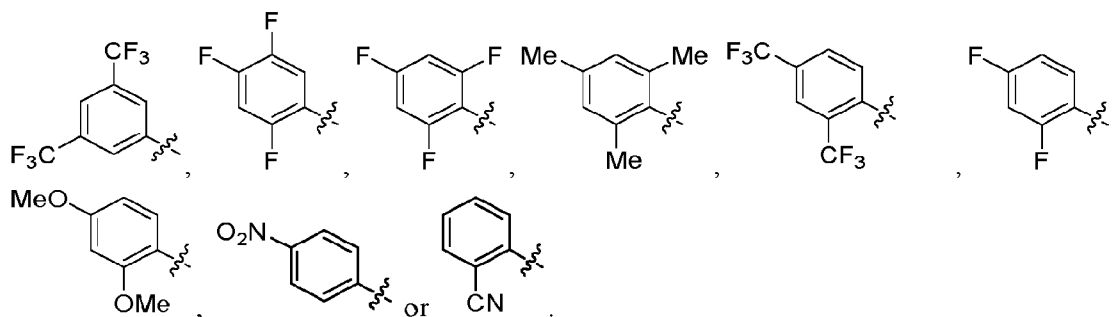
wherein the -OH at the C-terminus of the peptide domain is optionally substituted by R^a, wherein R^a is selected from -NH₂, -O-C₁₋₆alkyl, -NHC₁₋₆alkyl, and -NH-NH₂;

provided the compound of formula Ia is not

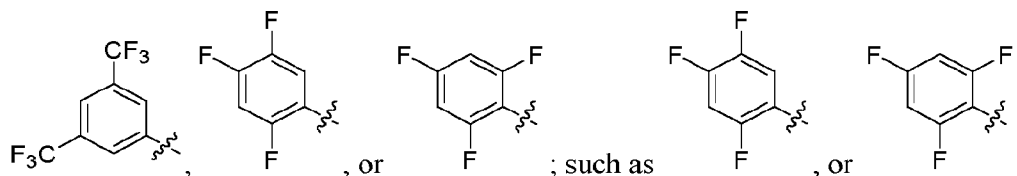


2. The compound or pharmaceutically acceptable salt thereof according to claim 1, wherein the C₆₋₁₀aryl group in X¹ is phenyl, optionally substituted with from 1 to 3 substituents, each substituent independently selected from NO₂, CN, halo, C₁₋₄alkyl, C₁₋₄alkoxy, and C₁₋₃haloalkyl.
3. The compound or pharmaceutically acceptable salt thereof according to any previous claim, wherein X¹ is substituted with from 1 to 3 substituents, each substituent independently selected from CN, halo, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₃haloalkyl.

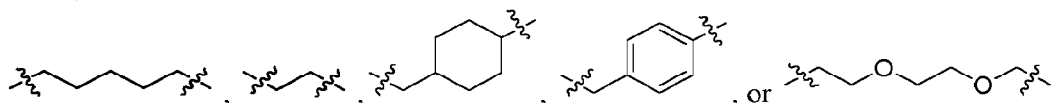
4. The compound or pharmaceutically acceptable salt thereof according to any previous claim, wherein X^1 is substituted with from 1 to 3 substituents, each substituent independently selected from CN, halo, methyl, methoxy and CF_3 .
5. The compound or pharmaceutically acceptable salt thereof according to any previous claim, wherein X^1 is



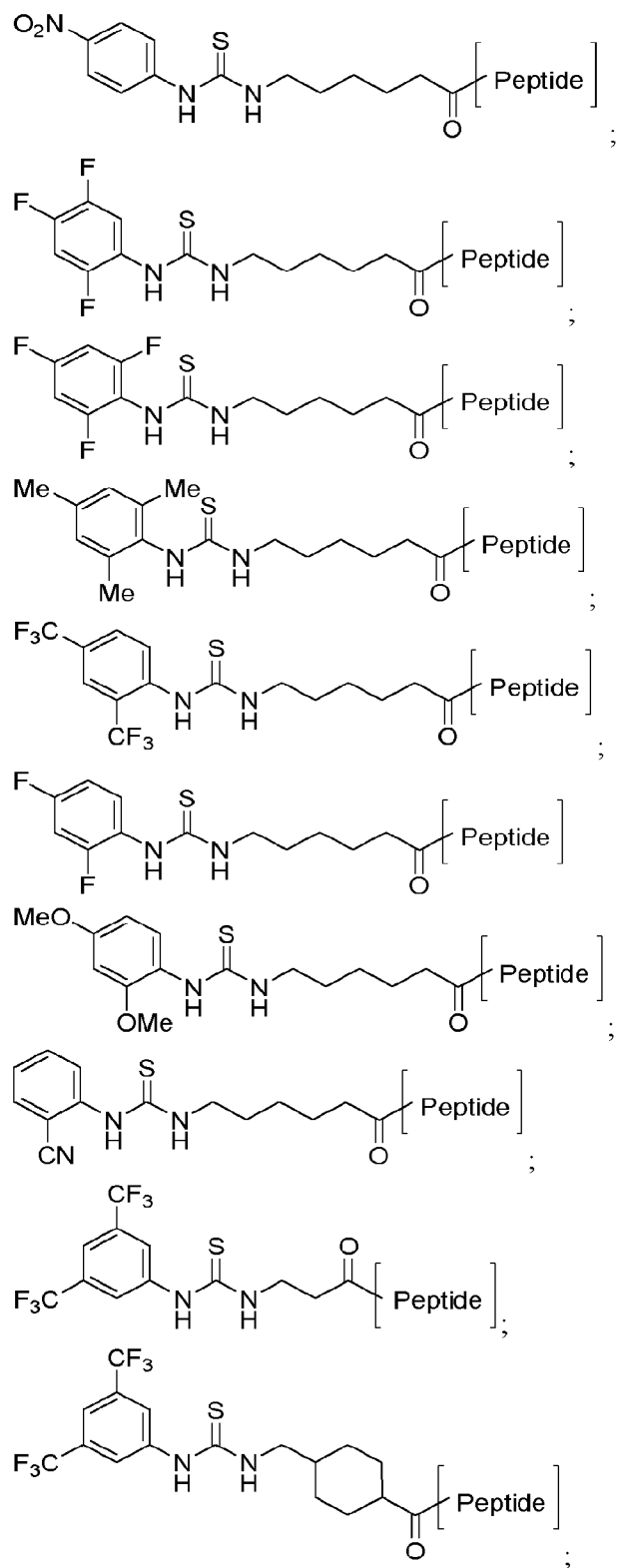
6. The compound or pharmaceutically acceptable salt thereof according to claim 5, wherein X^1 is

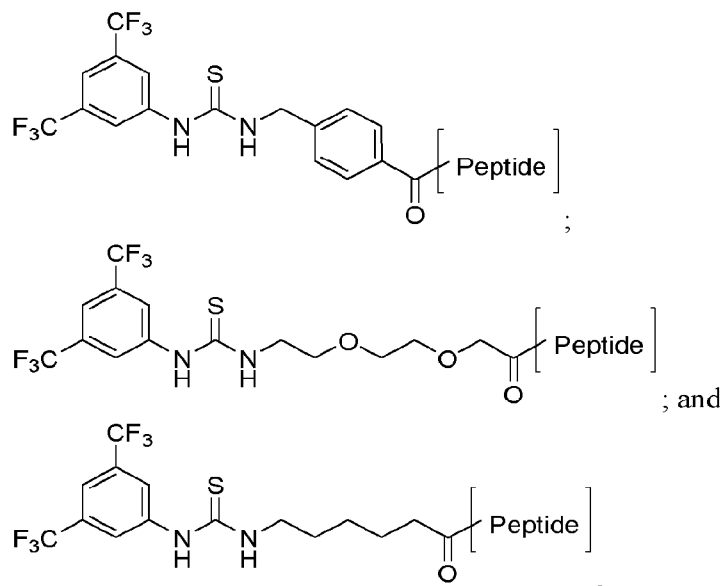


7. The compound or pharmaceutically acceptable salt thereof according to any previous claim, wherein Y^1 is S.
8. The compound or pharmaceutically acceptable salt thereof according to any previous claim, wherein Z^1 is C_{2-6} alkylene, for example C_{2-5} alkylene, such as C_2 alkylene or C_5 alkylene, preferably C_5 alkylene.
9. The compound or pharmaceutically acceptable salt thereof according to any one of claims 1 to 7, wherein Z^1 is



10. The compound or pharmaceutically acceptable salt thereof according to claim 8 or 9, wherein Z^1 is
11. The compound or pharmaceutically acceptable salt thereof according to claim 1 wherein the compound of formula Ia is selected from:





wherein the peptide domain is as defined in claim 1 and

wherein the -OH at the C-terminus of the peptide domain is optionally substituted by R^a , wherein R^a is selected from $-\text{NH}_2$, $-\text{O}-\text{C}_{1-6}\text{alkyl}$, $-\text{NHC}_{1-6}\text{alkyl}$, and $-\text{NH}-\text{NH}_2$.

12. The compound or pharmaceutically acceptable salt thereof according to any previous claim, wherein the CPP is a cationic, amphipathic, hydrophobic or amphiphilic CPP.

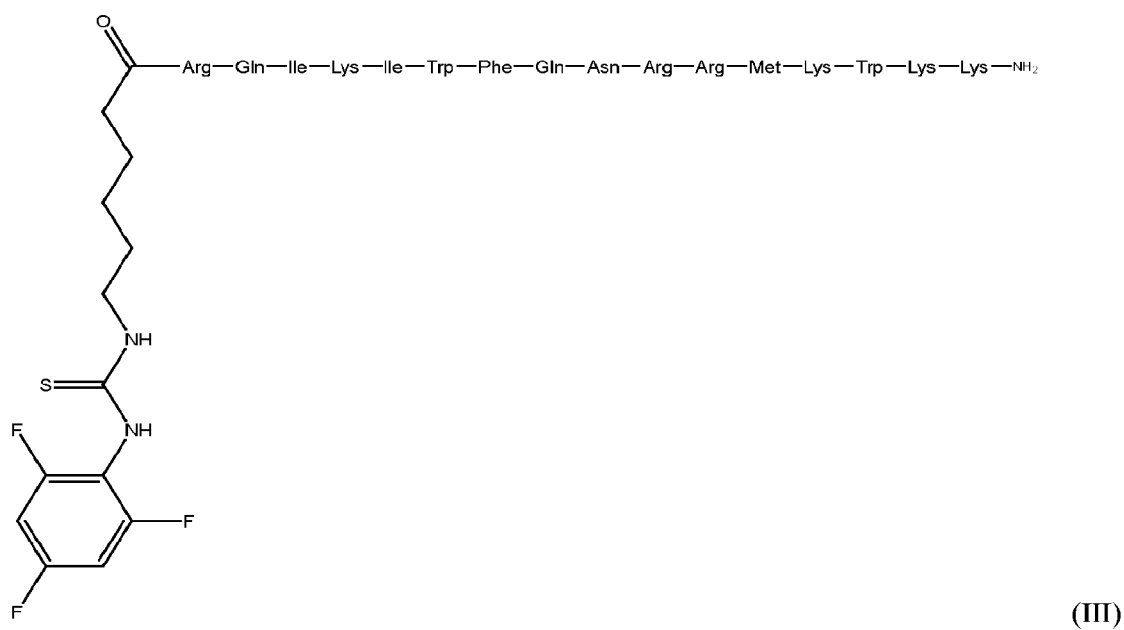
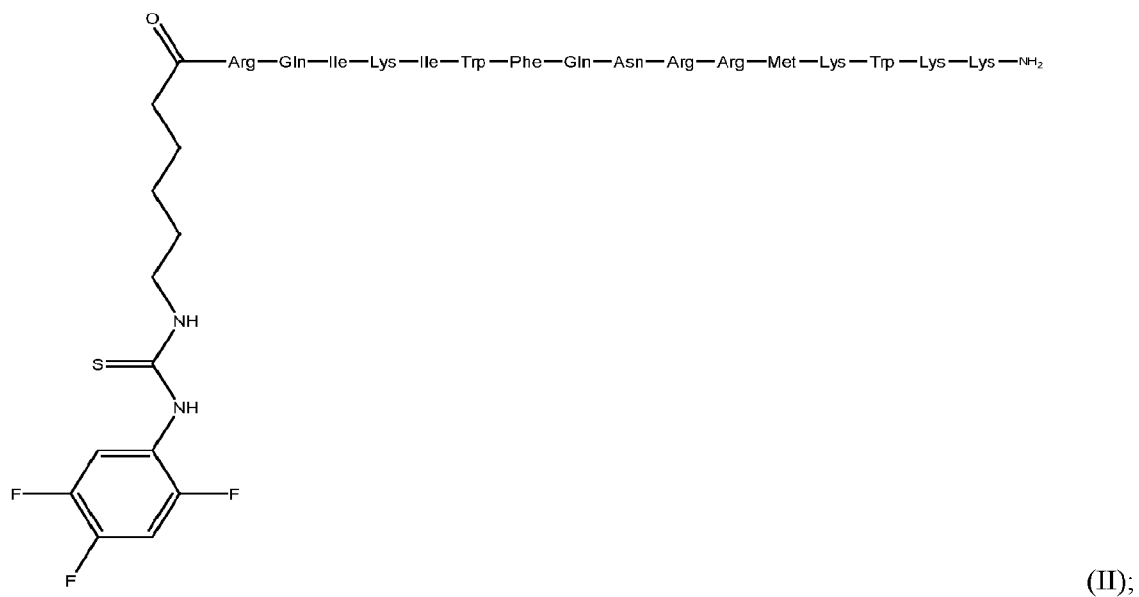
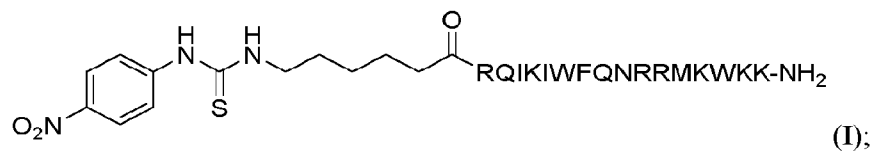
13. The compound or pharmaceutically acceptable salt thereof according to any previous claim, wherein the CPP comprises PTD4, SP, pVEC, poly-arginine, transportan, TAT, a translocationally active derivative or fragment of TAT, penetratin, herpes simplex virus protein VP22 or a translocationally-active homologue thereof, or a variant thereof having at least 70% identity thereto.

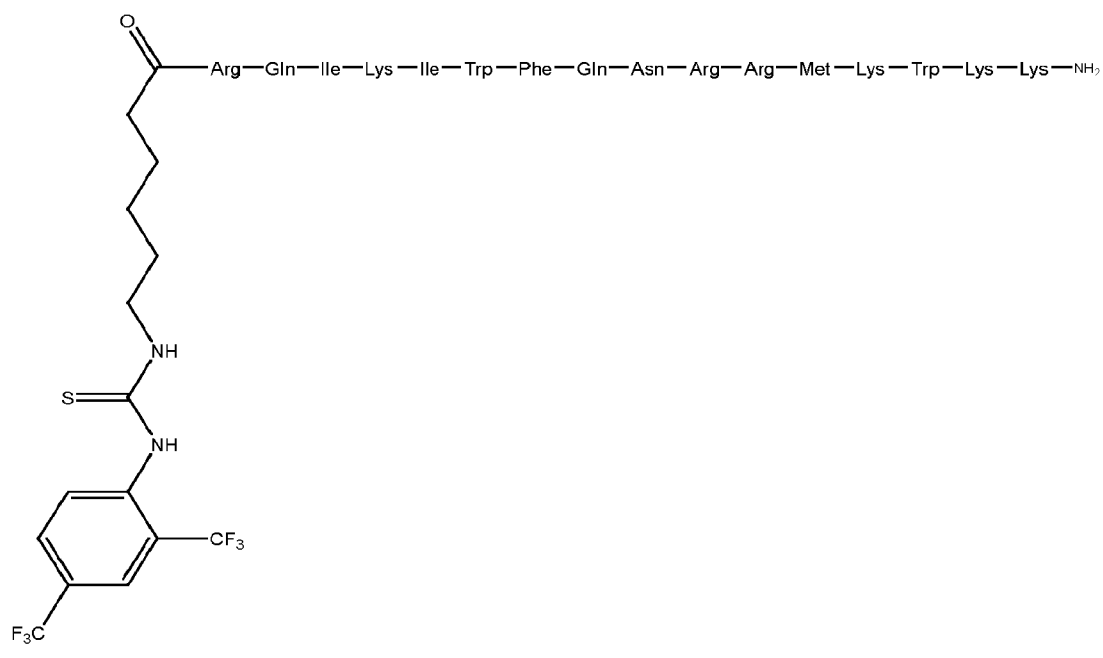
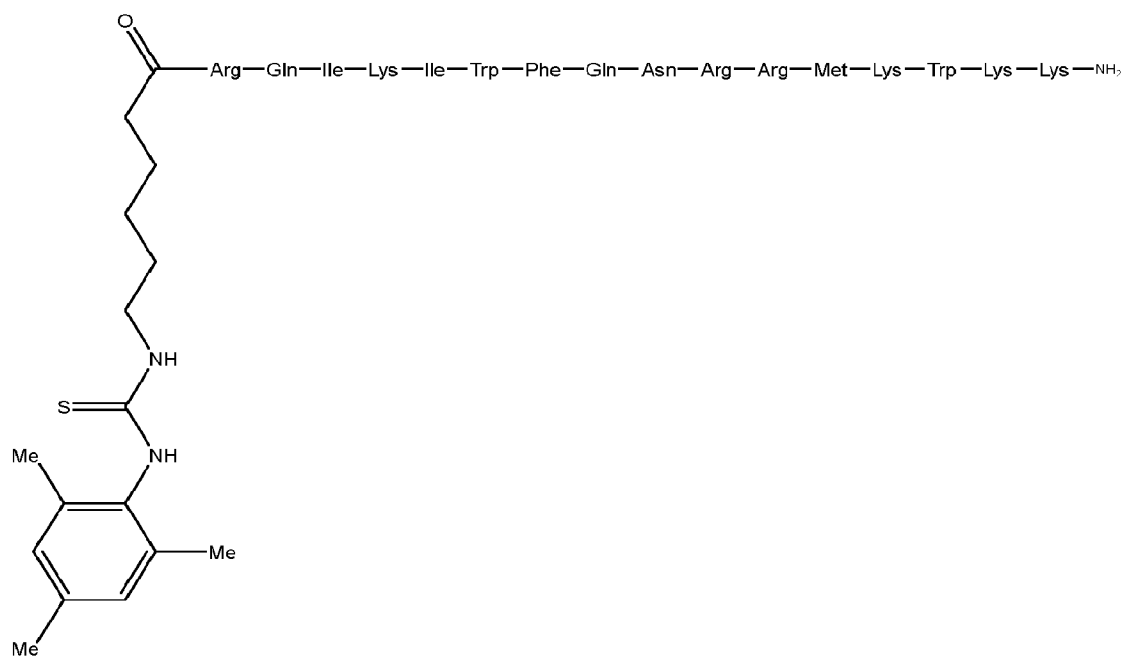
14. The compound or pharmaceutically acceptable salt thereof according to claim 13, wherein the CPP has at least 70% sequence identity to one of SEQ ID NOs: 1-16.

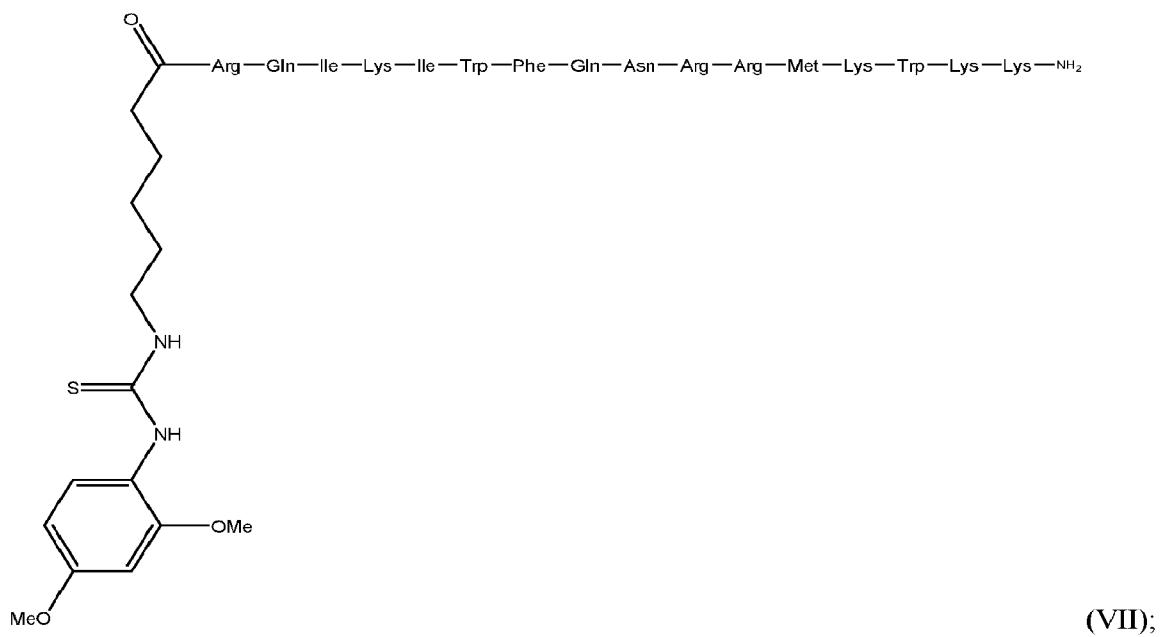
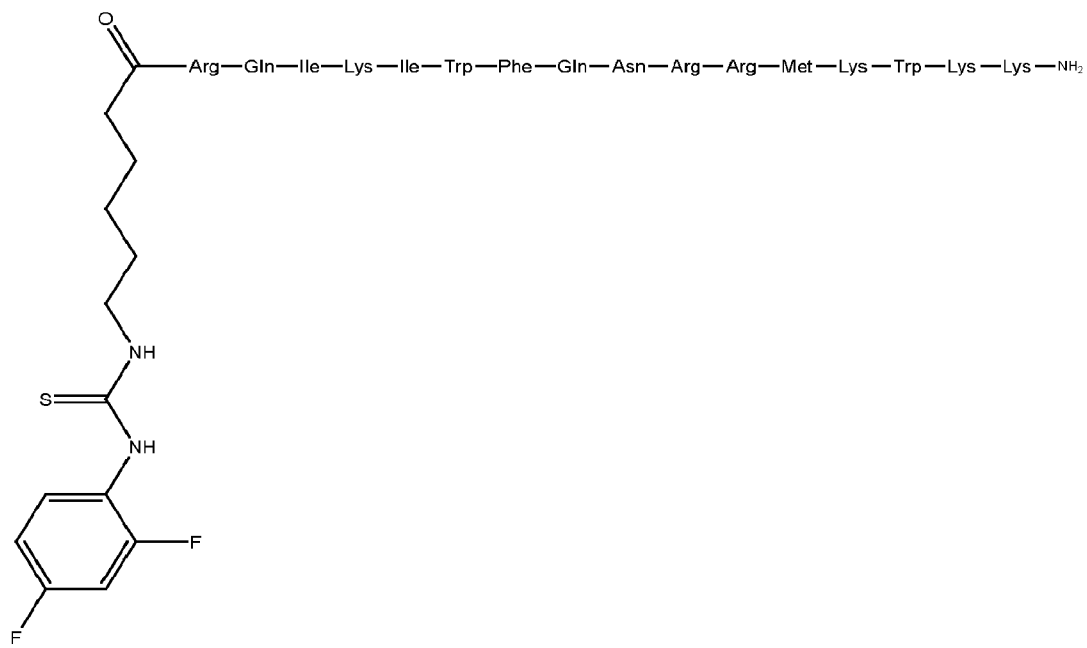
15. The compound or pharmaceutically acceptable salt thereof according to claim 14, wherein the CPP comprises one of SEQ ID NOs: 1-8 and 14-16, preferably one of SEQ ID NOs: 5, 14, 15 and 16.

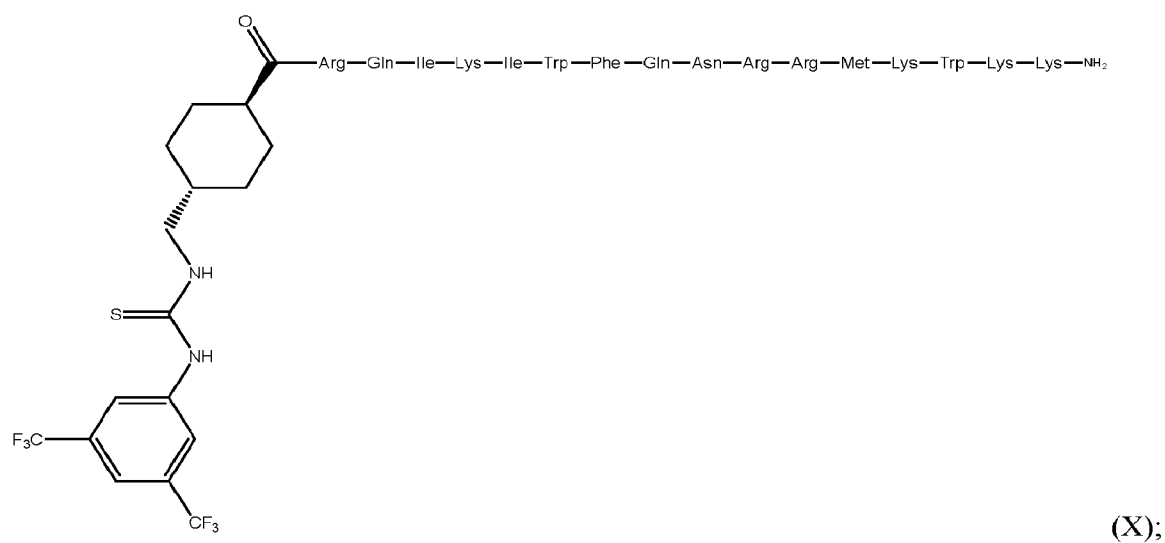
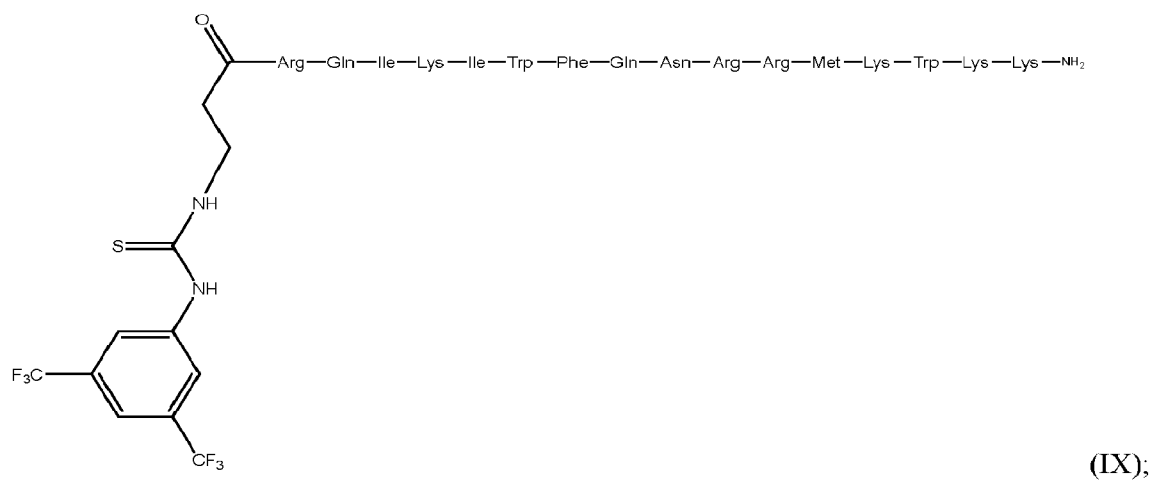
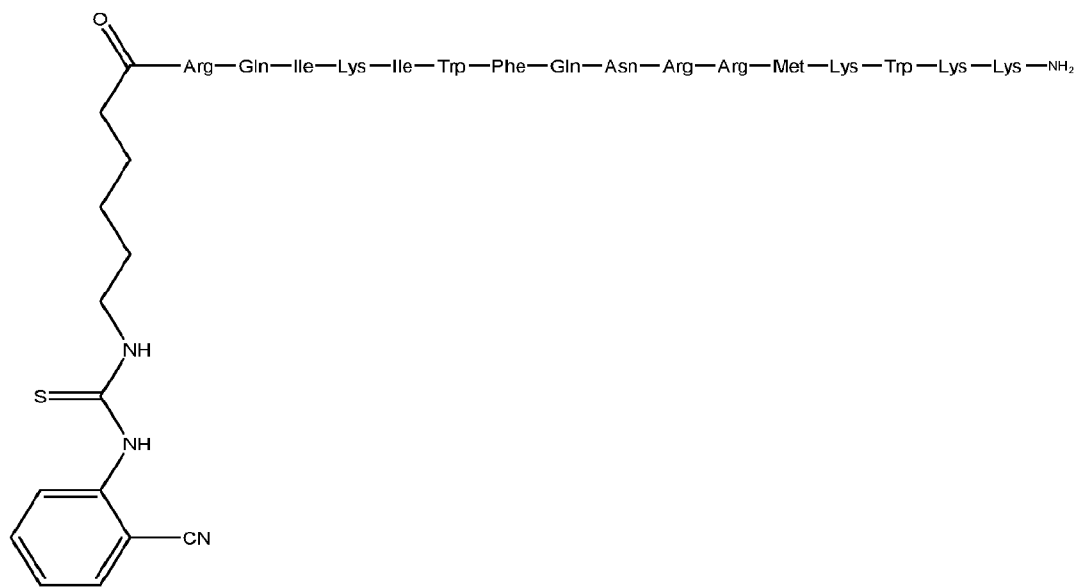
16. The compound or pharmaceutically acceptable salt thereof according to any previous claim, wherein the -OH at the C-terminus of the peptide domain is substituted by R^a , preferably wherein R^a is $-\text{NH}_2$.

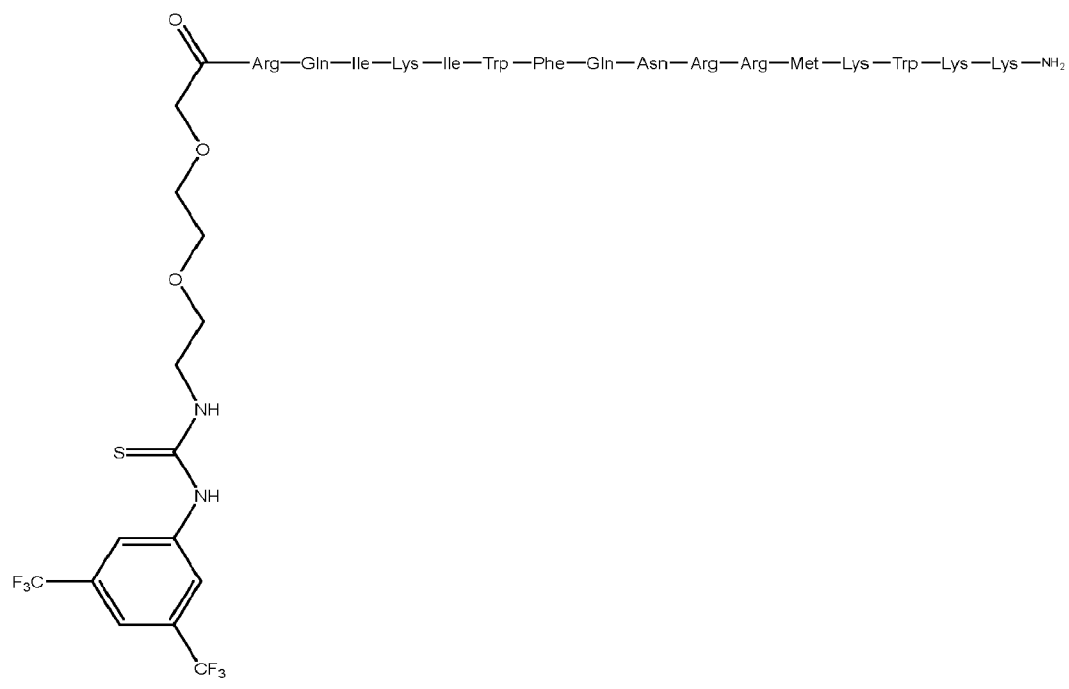
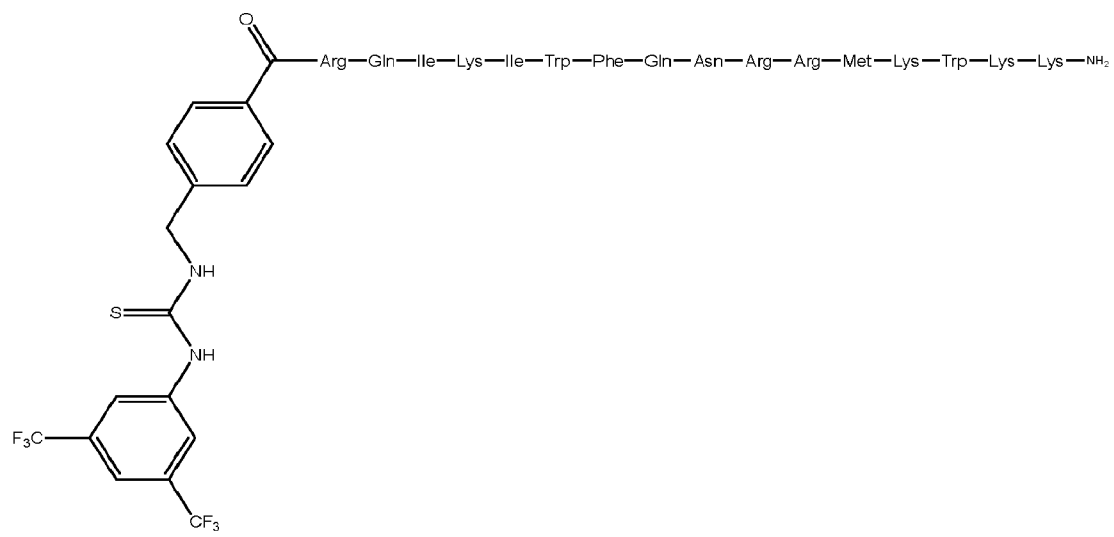
17. The compound or pharmaceutically acceptable salt thereof according to any of the preceding claims, wherein the compound of formula Ia is selected from:

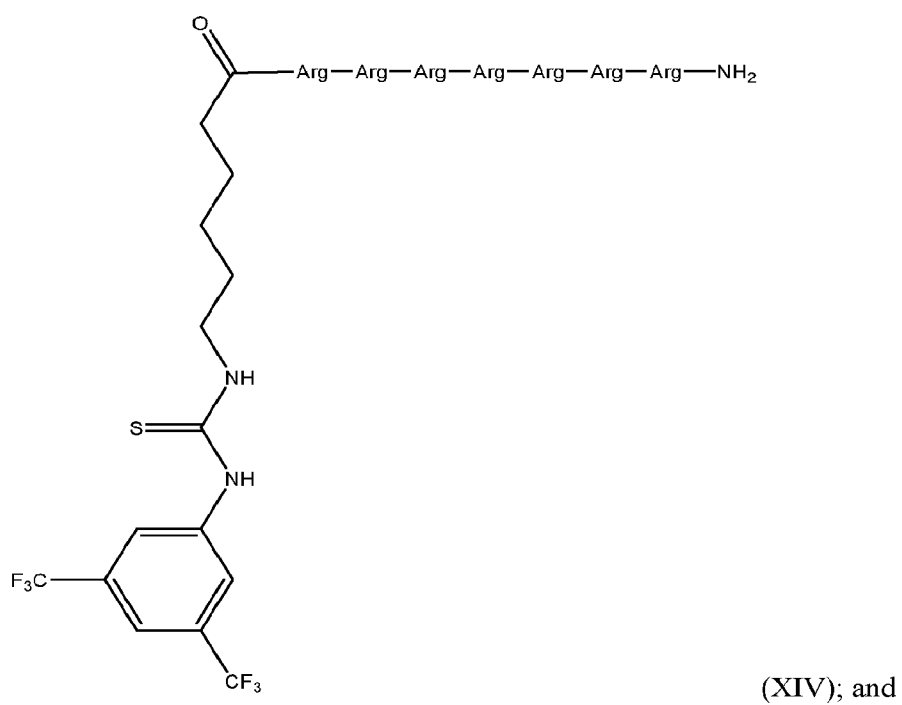
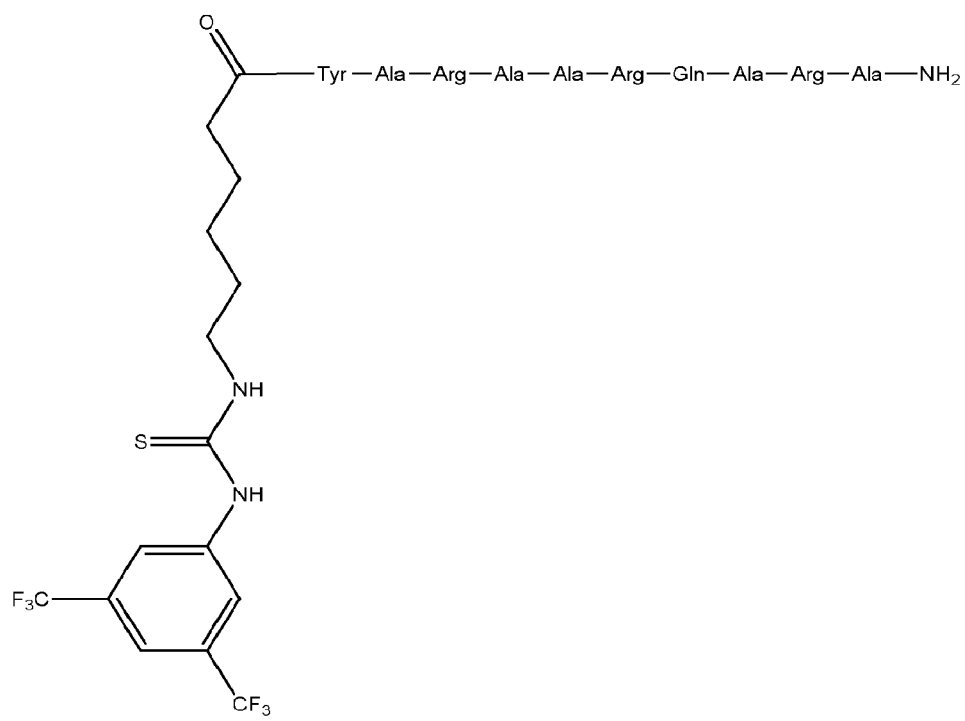


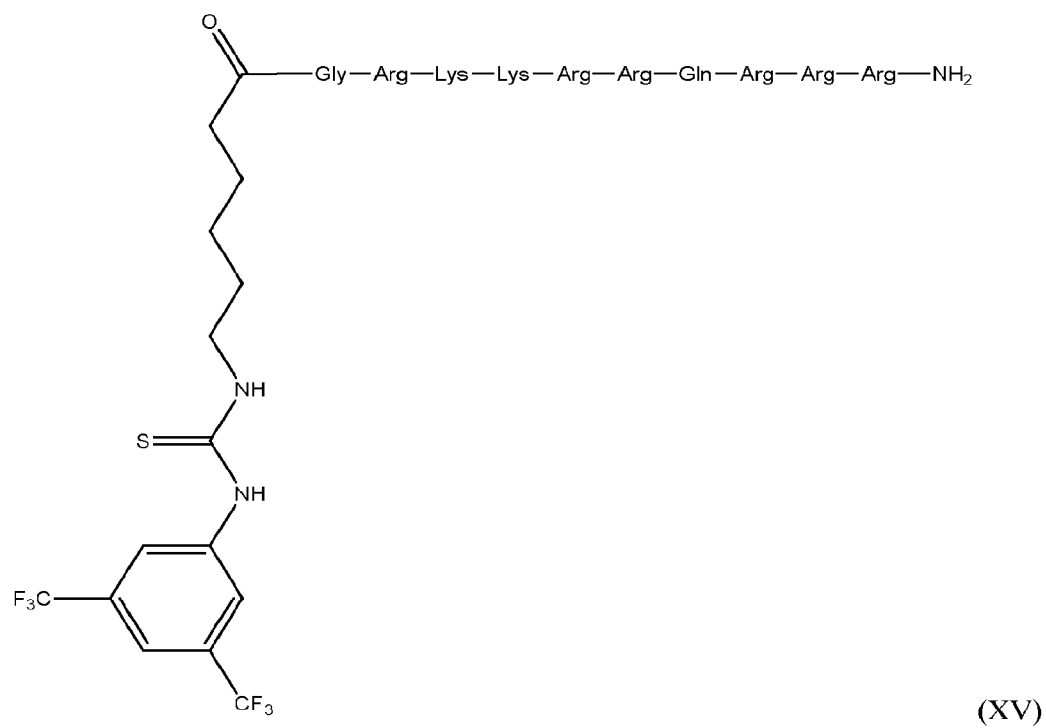






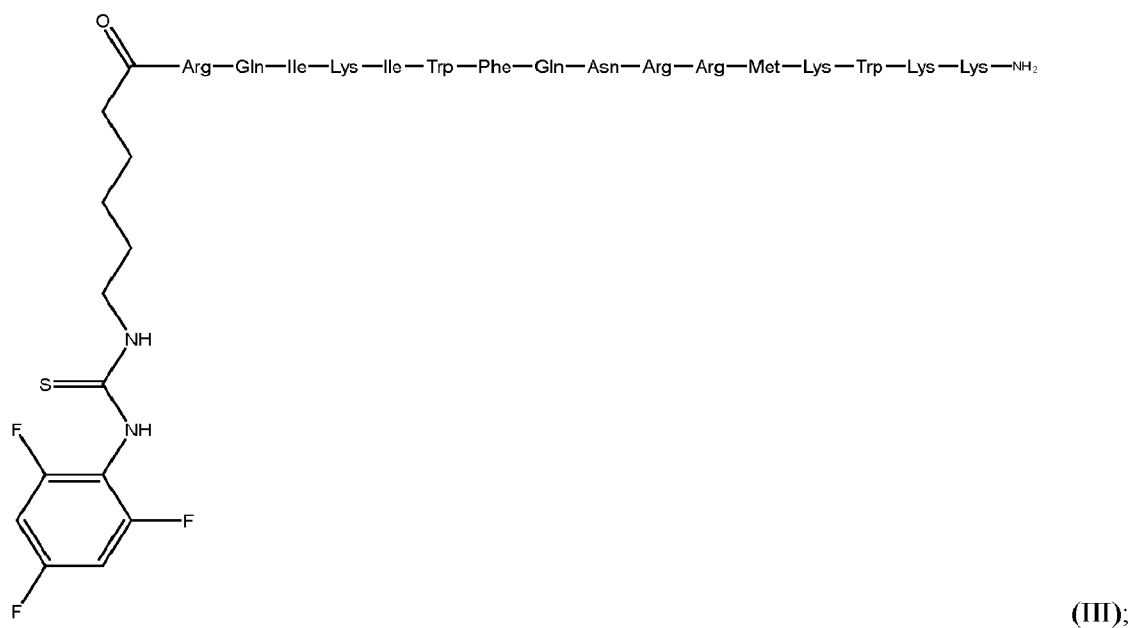


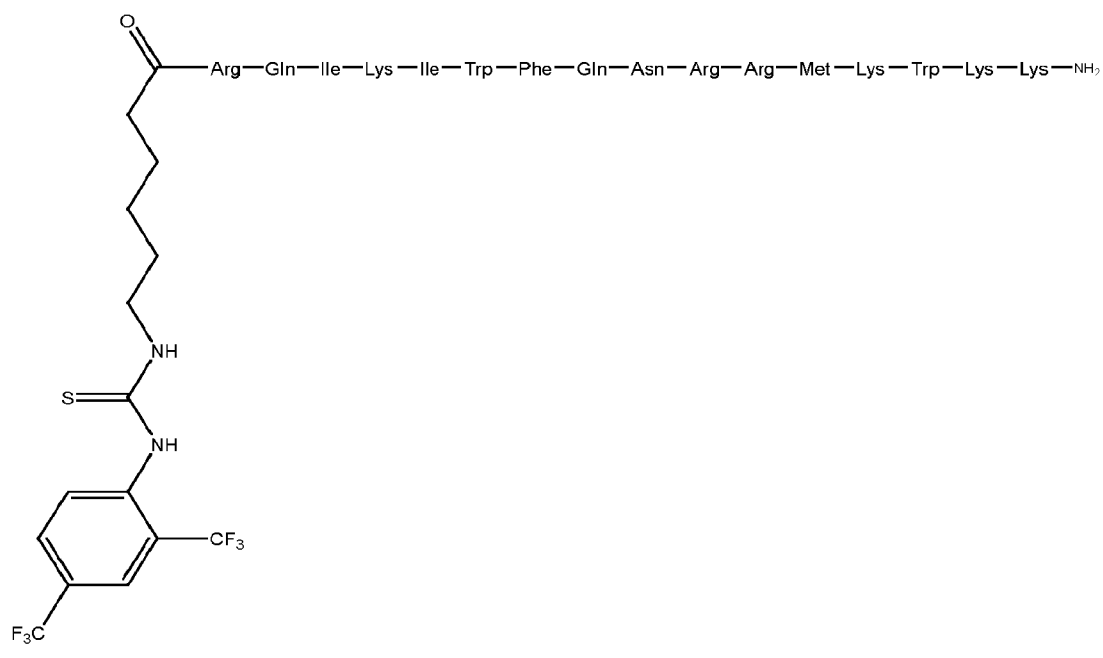
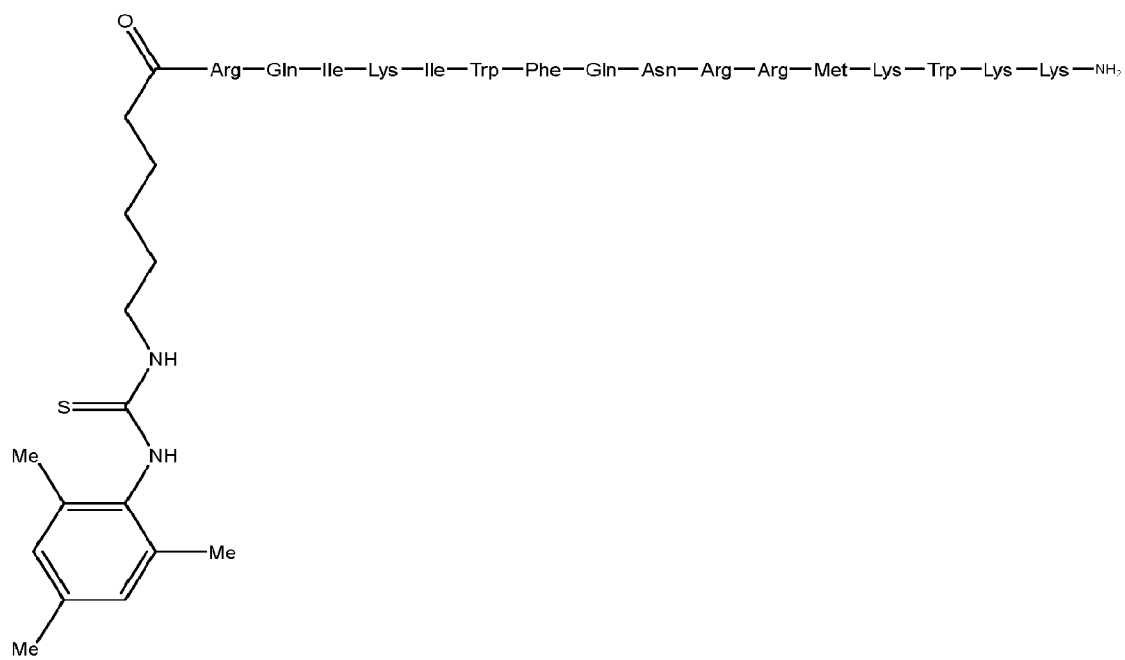


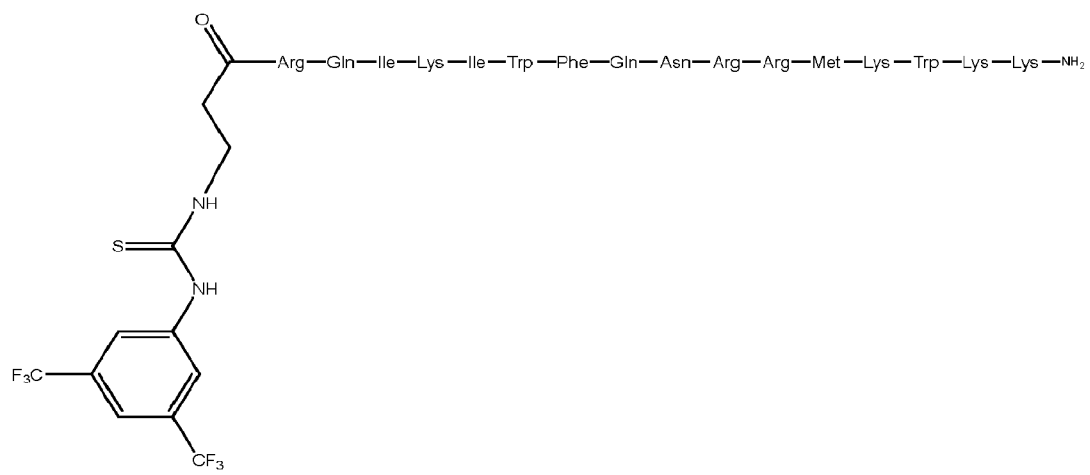


or pharmaceutically acceptable salts thereof.

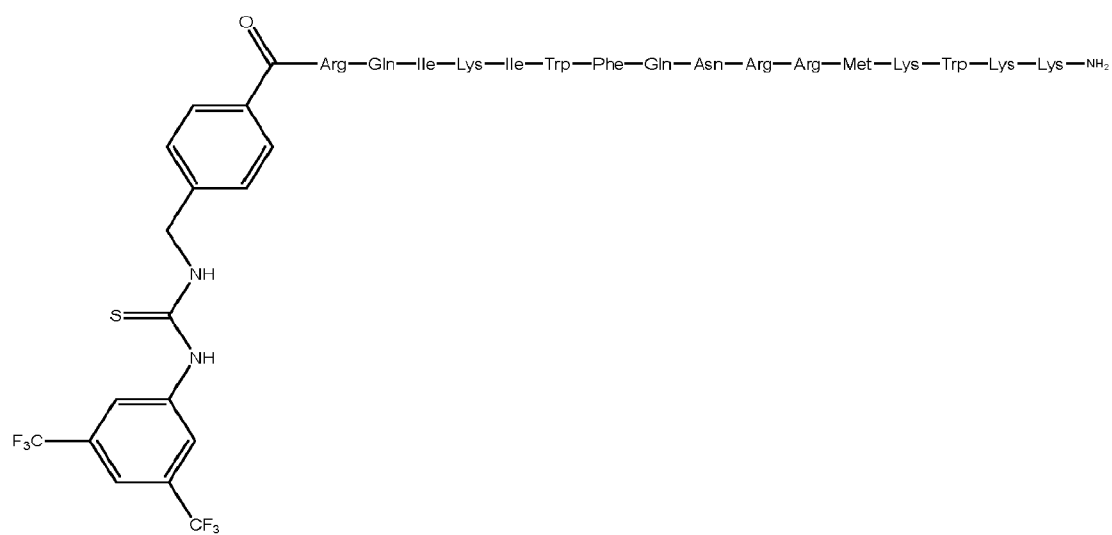
18. The compound or pharmaceutically acceptable salt according to claim 17, wherein the compound of formula Ia is selected from:



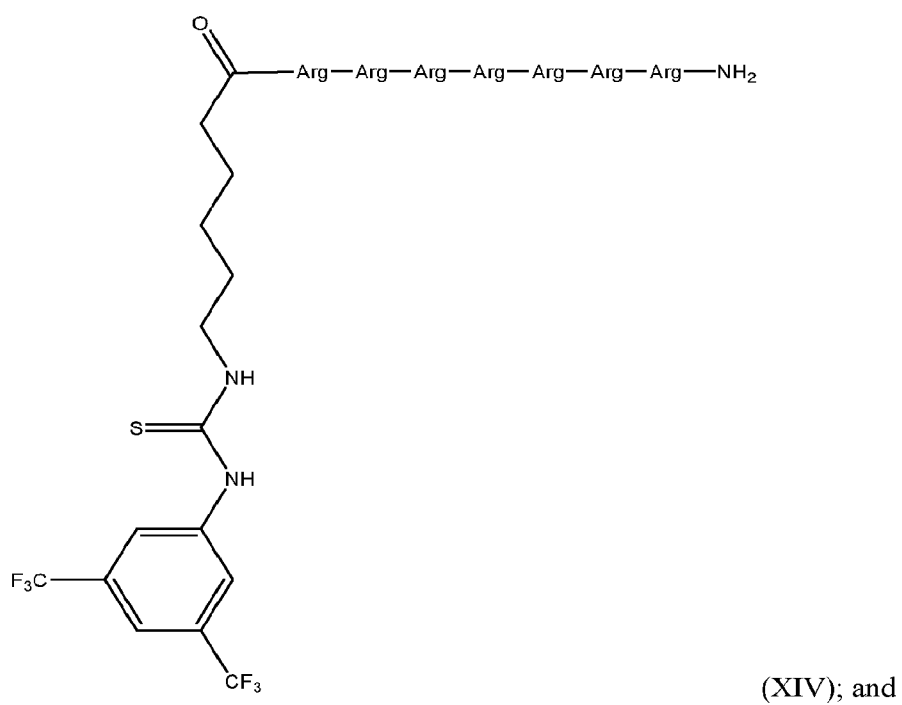
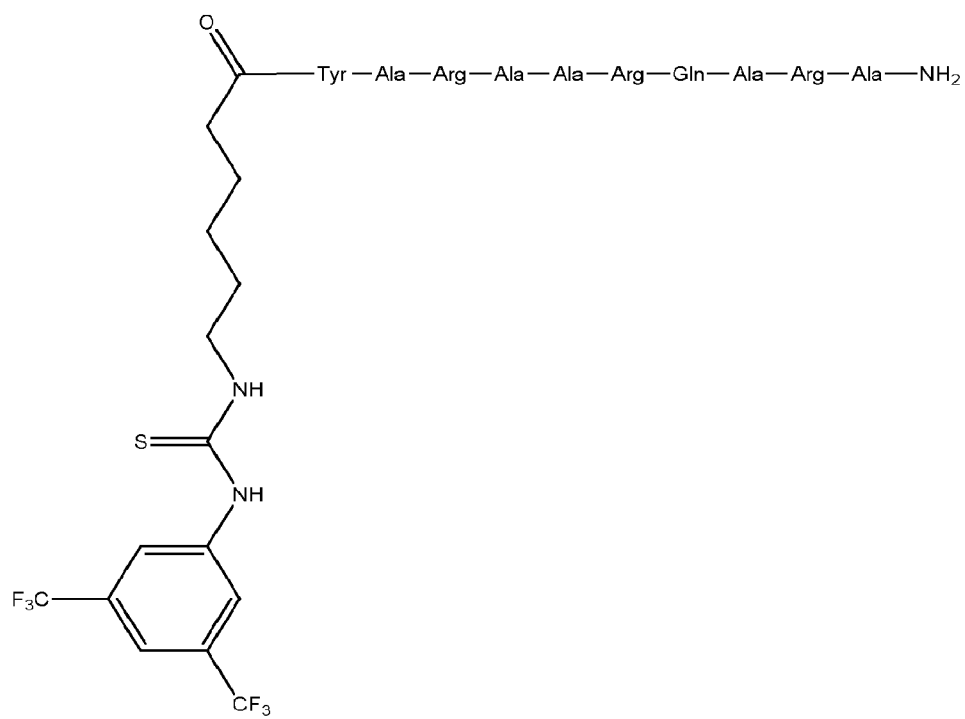


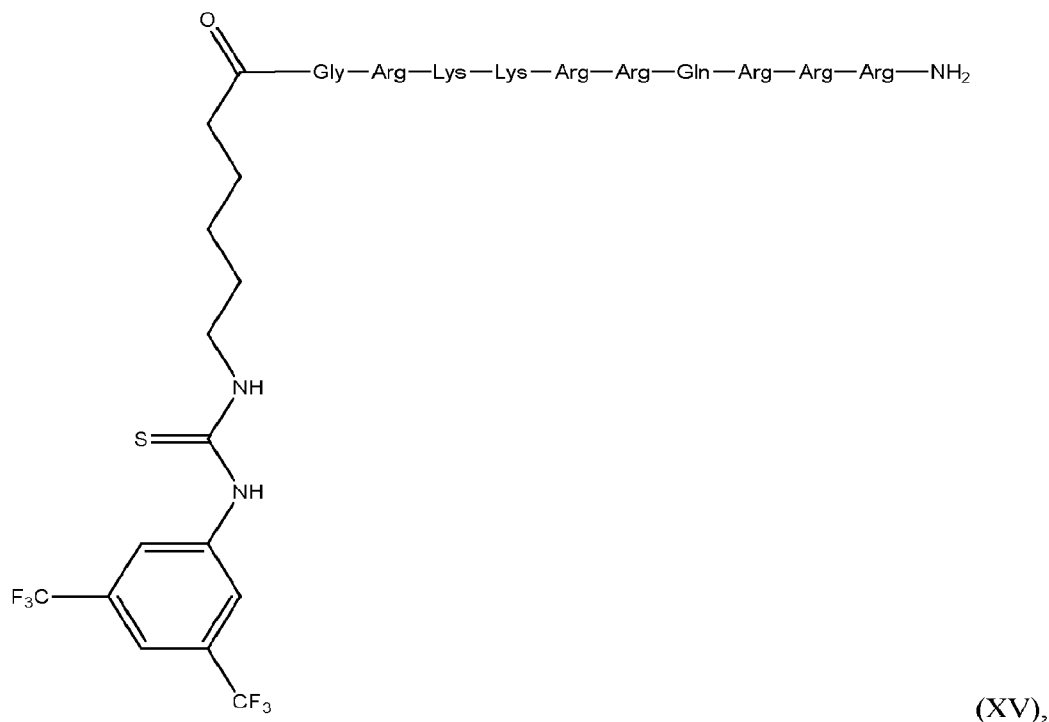


(IX);



(XI);





or pharmaceutically acceptable salts thereof.

19. A pharmaceutical composition comprising a compound or pharmaceutically acceptable salt thereof as defined according to any of claims 1-18.

20. A compound or pharmaceutically acceptable salt thereof as defined in any of claims 1-18, or a pharmaceutical composition as defined in claim 19, for use in the treatment of a disease or condition.

21. Use of a compound or pharmaceutically acceptable salt thereof as defined in any of claims 1-18, or a pharmaceutical composition as defined in claim 19, for the a manufacture of a medicament for the treatment of a disease or condition.

22. A method of treatment of a disease or condition in a subject, wherein the method comprises administering a compound or a pharmaceutically acceptable salt thereof as defined in any of claims 1-18, or a pharmaceutical composition as defined in claim 19, to a subject in need thereof.

23. The compound, or pharmaceutically acceptable salt thereof, or a pharmaceutical composition for use according to claim 20, the use according to claim 21, or method of treatment

according to claim 22, wherein the disease or condition is associated with a chloride ion channel mutation or is a channelopathy.

24. The compound or pharmaceutically acceptable salt thereof, or a pharmaceutical composition for use according to claim 23, the use according to claim 23, or the method of treatment according to claim 23, wherein the chloride ion channel mutation is selected from a voltage-sensitive CLIC, transmitter-gated GABAA, glycine receptors, calcium-activated chloride ion channel (CaCC), high (maxi) conductance chloride ion channel, cystic fibrosis transmembrane conductance regulator (CFTR) and a volume-regulated channel mutation.

25. The compound or pharmaceutically acceptable salt thereof, or a pharmaceutical composition for use according to claim 24, the use according to claim 24, or the method of treatment according to claim 24, wherein the disease or condition is selected from myotonia congenita Becker, Myotonia congenita Thomsen, Dystrophia myotonica 1, dystrophia myotonica 2, childhood absence epilepsy type 3, juvenile absence epilepsy, juvenile myoclonic epilepsy, epilepsy with grand mal seizures on awakening, Bartter Syndrome, Bartter syndrome with sensorineural deafness, Dent's disease, autosomal dominant osteopetrosis, autosomal recessive osteopetrosis, cystic fibrosis, idiopathic chronic pancreatitis, bronchiectasis, congenital bilateral absence of vas deferens, Best's disease, adult-onset vitelliform macular dystrophy, concentric annular macular dystrophy, cataract, juvenile myoclonic epilepsy, childhood absence epilepsy type 2, generalized epilepsy with febrile seizures, severe myoclonic epilepsy in infancy, insomnia, hereditary hyperekplexia, recessive and dominant myotonia, leukodystrophy, aldosteronism, diabetes insipidus, Giltman disease, CNS and retina degeneration, mental retardation, epilepsy, proteinuria, impaired renal endocytosis, osteopetrosis, pulmonary hypertension, cardiac dysfunction, Alzheimer's disease, respiratory and gastrointestinal diseases, including asthma, chronic obstructive pulmonary disease, cystic fibrosis, pneumonia, colon colitis, cystic fibrosis intestinal mucous disease, ulcerative colitis, and gastrointestinal parasitic infection, dry mouth and dry eye syndromes, diarrhea, hypertension, neuropathic pain, cancers, smoke induced COPD, chronic bronchitis, rhinosinusitis, constipation, pancreatitis, pancreatic insufficiency, male infertility caused by congenital bilateral absence of the vas deferens (CBAVD), mild pulmonary disease, idiopathic pancreatitis, allergic bronchopulmonary aspergillosis (ABPA), liver disease, hereditary emphysema, mucopolysaccharidoses, Bartter's syndrome type III, Primary Ciliary Dyskinesia (PCD), an inherited disorders of the structure and/or function of cilia, including PCD with situs inversus (also known as Kartagener syndrome), PCD without situs inversus and ciliary aplasia.

26. A compound or pharmaceutically acceptable salt thereof as defined in any of claims 1-18, or a pharmaceutical composition as defined in claim 19, for use in a method of treating a respiratory disease, preferably cystic fibrosis.

27. A kit comprising a compound or pharmaceutically acceptable salt thereof as defined in any of claims 1-18, or a pharmaceutical composition as defined in claim 19.

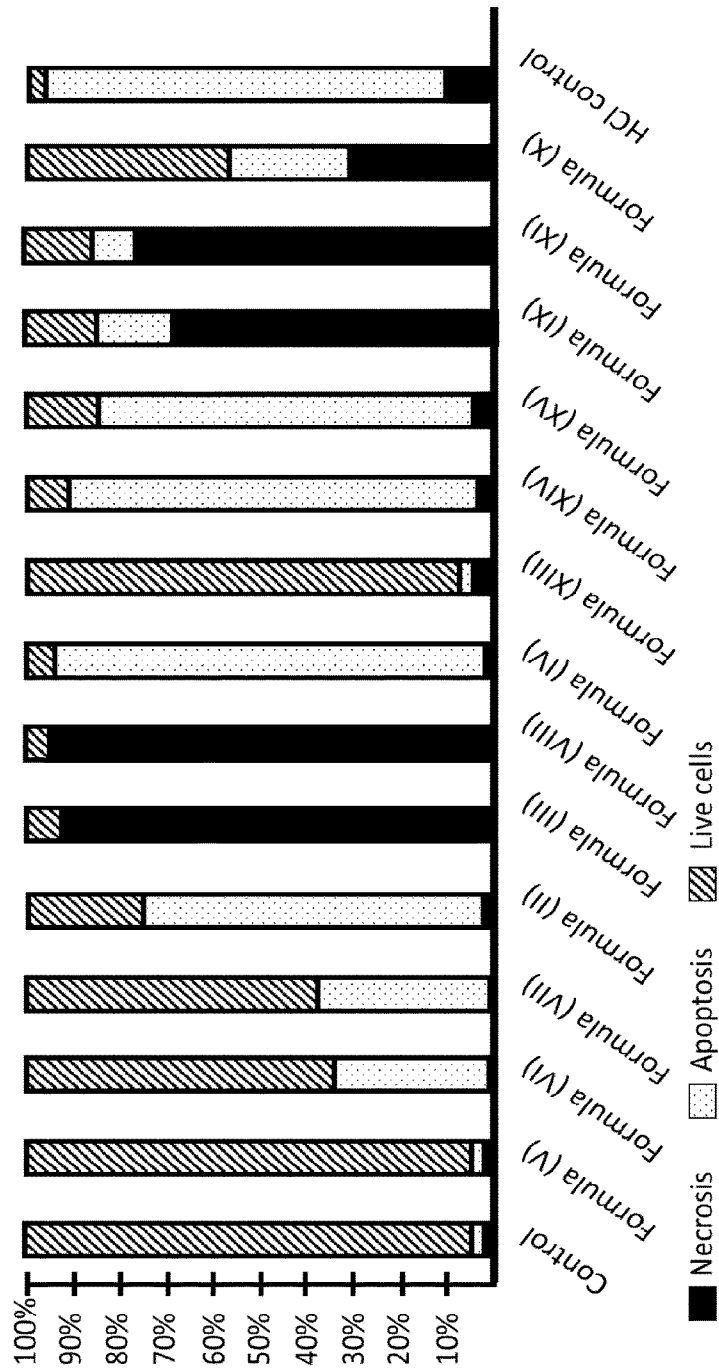


Fig. 1

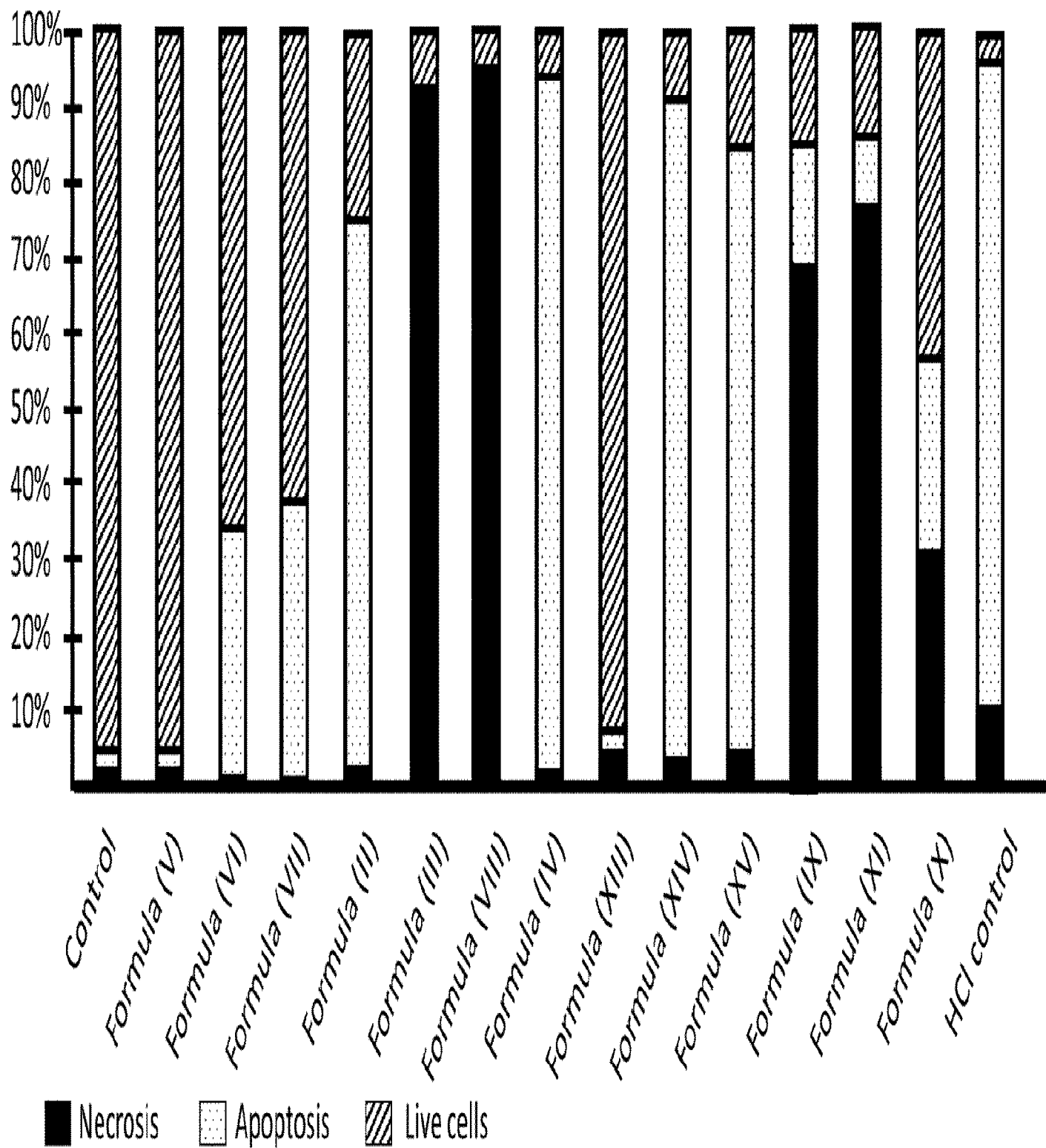


Fig. 1