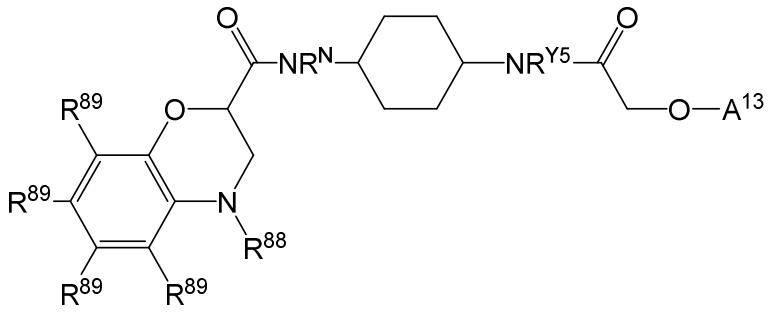
[5] syndrome), autoimmune diseases, inflammatory diseases, musculoskeletal diseases (such as myopathy), vascular diseases, ocular diseases, and genetic disorders. Aberrant protein synthesis through eIF2α phosphorylation is also characteristic of several other human genetic disorders, cystic fibrosis, amyotrophic lateral sclerosis, Huntington disease and prion disease.

SUMMARY

[6] Inhibitors of the Integrated Stress Response (ISR) pathway are described, as are methods of making and using the compounds, or salts thereof.

[5a] In one aspect, the present disclosure refers to a compound of formula (XX-I-2):



(XX-I-2)

or a pharmaceutically acceptable salt thereof,

wherein:

R^{Y5} is hydrogen or C_1-C_6 alkyl;

R^N is hydrogen or C_1-C_6 alkyl;

A^{13} is selected from the group consisting of:

C_6-C_{10} aryl optionally substituted with one or more R^{95} substituents; and

5-10 membered heteroaryl optionally substituted with one or more R^{95} substituents;

R^{95} is independently at each occurrence, halogen;

R^{88} is selected from the group consisting of hydrogen, C_1-C_6 alkyl, and C_1-C_6 haloalkyl; and

R^{89} is selected, independently at each occurrence, from the group consisting of hydrogen and halogen.

[5b] In another aspect, the present disclosure refer to a pharmaceutical composition comprising a compound as disclosed herein, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

[5c] In another aspect, the present disclosure refer to use of a compound as disclosed herein, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating a disease or disorder mediated by an integrated stress response (ISR) pathway in an individual in need thereof, , wherein the disease or disorder is selected from the group consisting of: a neurodegenerative disease, an inflammatory disease, an autoimmune disease, a metabolic syndrome, a cancer, a vascular disease, an ocular disease, a musculoskeletal disease, and a genetic disorder.

[5d] In another aspect, the present disclosure refer to a method of producing a protein, comprising contacting a eukaryotic cell comprising a nucleic acid encoding the protein with the compound or salt as disclosed herein, wherein the cell is cultured in an *in vitro* culture medium comprising the compound or salt.

[5e] In another aspect, the present disclosure refer to a method of culturing a eukaryotic cell comprising a nucleic acid encoding a protein, comprising contacting the eukaryotic cell with an *in vitro* culture medium comprising a compound or salt as disclosed herein.

[5f] In another aspect, the present disclosure refer to a method of producing a protein, comprising contacting a cell-free protein synthesis (CFPS) system comprising eukaryotic initiation factor 2 (eIF2) and a nucleic acid encoding a protein with the compound or salt as disclosed herein.

[5g] In another aspect, the present disclosure refer to an *in vitro* cell culture medium, comprising the compound or salt as disclosed herein and nutrients for cellular growth.

[5h] In another aspect, the present disclosure refer to a cell-free protein synthesis (CFPS) system comprising eukaryotic initiation factor 2 (eIF2) and a nucleic acid encoding a protein with the compound or salt as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[7] FIG. 1 shows percentage of ATF4 inhibition after induction with thapsigargin (Tg) in the presence of certain test compounds. Percentage of ATF4 inhibition was calculated as the percent reduction normalized to Tg treatment (0% inhibition) and untreated cells (100% inhibition).

[8] FIG. 2 shows protein synthesis levels in the presence of media alone or certain test compounds in unstressed cells. The levels were normalized to the media alone condition, which correspond to 100% protein synthesis.

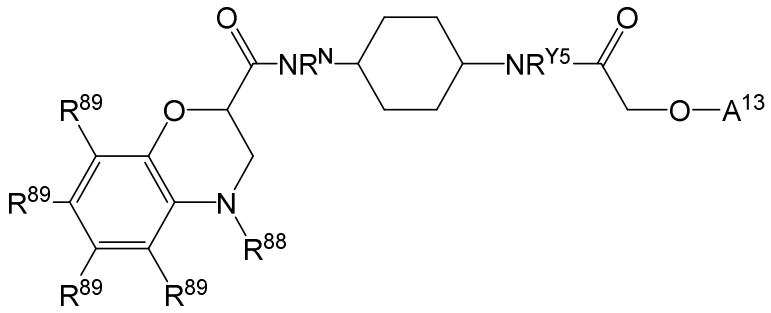
[9] FIG. 3A and FIG. 3B show percent recovery of protein synthesis in a stressed cell in the presence of one of several test compounds at a concentration of 100 nM (FIG. 3A) or 1 μM (FIG. 3B). The levels were normalized to the media alone and to Tg alone conditions, which correspond to 100% and 0% respectively. FIG. 3C shows percent ATF4 inhibition for select compounds against percent recovery of protein synthesis.

[10] FIG. 4A shows representative levels of ATF4 expression and β-actin expression (control) in SH-SY5Y cells after being treated with cell culture media conditioned using wild-type CHO cells (wtCM) or 7PA2 CHO cells (7PA2CM).

[11] FIG. 4B shows percent inhibition of ATF4 expression in SH-5Y5Y cells after incubation with conditioned media from 7PA2 CHO cells and one of several test compounds. The levels were normalized to the media alone and to conditioned media from 7PA2 CHO cells alone, which correspond to 100% and 0% respectively.

CLAIMS

1. A compound of formula (XX-I-2):



(XX-I-2)

or a pharmaceutically acceptable salt thereof,

wherein:

R^{Y5} is hydrogen or C_1-C_6 alkyl;

R^N is hydrogen or C_1-C_6 alkyl;

A^{13} is selected from the group consisting of:

C_6-C_{10} aryl optionally substituted with one or more R^{95} substituents; and

5-10 membered heteroaryl optionally substituted with one or more R^{95} substituents;

R^{95} is, independently at each occurrence, halogen;

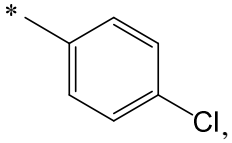
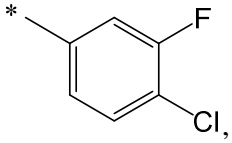
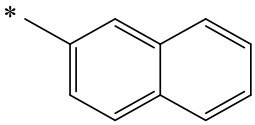
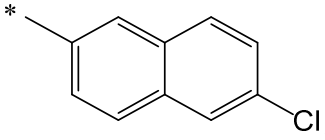
R^{88} is selected from the group consisting of hydrogen, C_1-C_6 alkyl, and C_1-C_6 haloalkyl; and

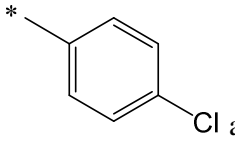
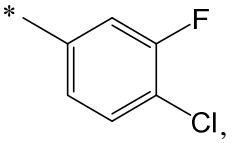
R^{89} is selected, independently at each occurrence, from the group consisting of hydrogen and halogen.

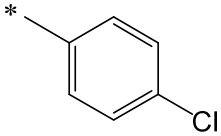
2. The compound of claim 1, or a or a pharmaceutically acceptable salt thereof, wherein R^{Y5} is hydrogen.

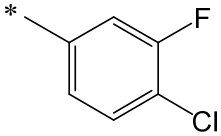
3. The compound of claim 1 or 2, or a pharmaceutically acceptable salt thereof, wherein R^N is hydrogen.

4. The compound of any one of claims 1-3, or a pharmaceutically acceptable salt thereof, wherein A^{13} is C_6-C_{10} aryl optionally substituted with one or more R^{95} substituents.

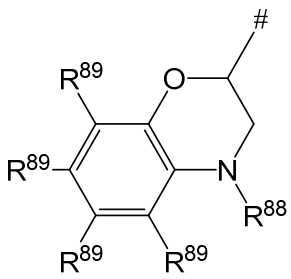
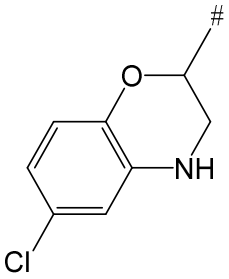
5. The compound of any one of claims 1-4, or a pharmaceutically acceptable salt thereof, wherein A^{13} is selected from the group consisting of , , , and , wherein the * represents the attachment point to the remainder of the molecule.

6. The compound of any one of claims 1-5, or a pharmaceutically acceptable salt thereof, wherein A^{13} is selected from the group consisting of  and , wherein the * represents the attachment point to the remainder of the molecule.

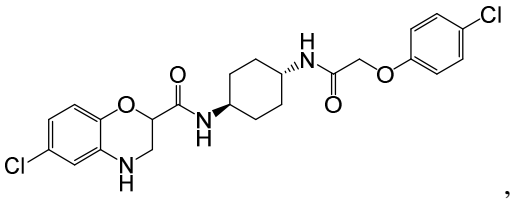
7. The compound of any one of claims 1-6, or a pharmaceutically acceptable salt thereof, wherein A^{13} is , wherein the * represents the attachment point to the remainder of the molecule.

8. The compound of any one of claims 1-7, or a pharmaceutically acceptable salt thereof, wherein A^{13} is , wherein the * represents the attachment point to the remainder of the molecule.

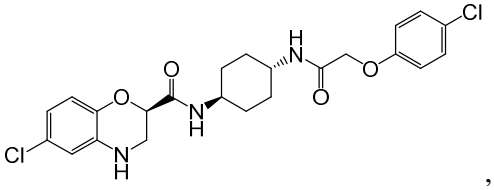
9. The compound of any one of claims 1-8, or a pharmaceutically acceptable salt thereof,

wherein the moiety  is , wherein # represents the attachment point to the remainder of the molecule.

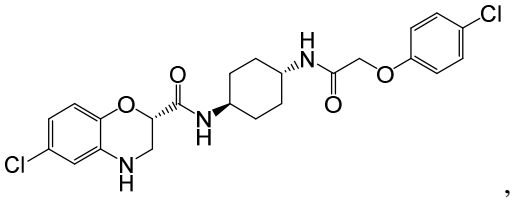
10. The compound of any one of claims 1-9, or a pharmaceutically acceptable salt thereof,

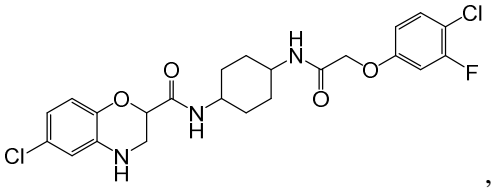
wherein the compound is , or a pharmaceutically acceptable salt thereof.

11. The compound of any one of claims 1-10, or a pharmaceutically acceptable salt thereof,

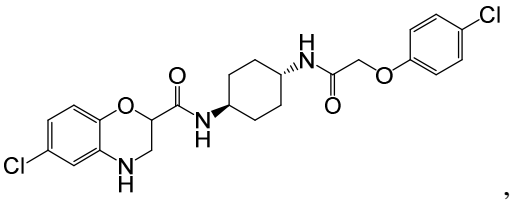
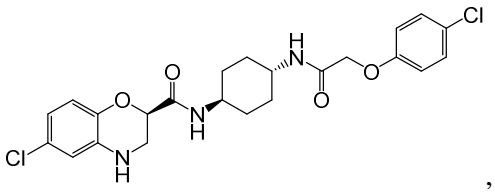
wherein the compound is , or a pharmaceutically acceptable salt thereof.

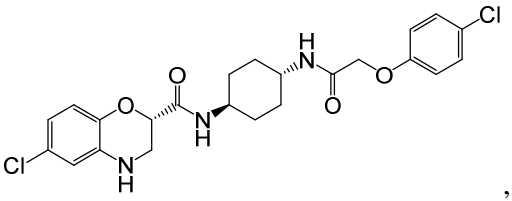
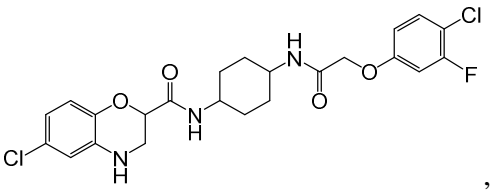
12. The compound of any one of claims 1-10 or a pharmaceutically acceptable salt thereof,

wherein the compound is , or a pharmaceutically acceptable salt thereof.

13. The compound of any one of claims 1-9, or a pharmaceutically acceptable salt thereof, wherein the compound is , or a pharmaceutically acceptable salt thereof.

14. A compound selected from the group consisting of:

, ,

, and , or a pharmaceutically acceptable salt thereof.

15. A pharmaceutical composition comprising a compound of any of the preceding claims, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

16. Use of a compound according to any one of claims 1 to 14, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition according to claim 15, in the manufacture of a medicament for treating a disease or disorder mediated by an integrated stress response (ISR) pathway in an individual in need thereof, wherein the disease or disorder is selected from the group consisting of: a neurodegenerative disease, an inflammatory disease, an autoimmune disease, a metabolic syndrome, a cancer, a vascular disease, an ocular disease, a musculoskeletal disease, and a genetic disorder.

17. The use of claim 16, wherein the compound, the pharmaceutically acceptable salt, or the pharmaceutical composition is to be administered in combination with a therapeutically effective amount of one or more additional anti-cancer agents.

18. The use of claim 16, wherein the disease or disorder is mediated by phosphorylation of eIF2α and/or the guanine nucleotide exchange factor (GEF) activity of eIF2B.

19. The use of any one of claims 16-18, wherein the disease or disorder is mediated by a decrease in protein synthesis.

20. The use of any one of claims 16-19, wherein the disease or disorder is mediated by the expression of ATF4, CHOP or BACE-1.

21. The use of any one of claims 16-20, wherein the disease is vanishing white matter disease, childhood ataxia with CNS hypomyelination, intellectual disability syndrome, Alzheimer's disease, prion disease, Creutzfeldt-Jakob disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) disease, cognitive impairment, frontotemporal dementia (FTD), traumatic brain injury, postoperative cognitive dysfunction (PCD), neuro-otological syndromes, hearing loss, Huntington's disease, stroke, chronic traumatic encephalopathy, spinal cord injury, dementias or cognitive impairment, arthritis, psoriatic arthritis, psoriasis, juvenile idiopathic arthritis, asthma, allergic asthma, bronchial asthma, tuberculosis, chronic airway disorder, cystic fibrosis, glomerulonephritis, membranous nephropathy, sarcoidosis, vasculitis, ichthyosis, transplant rejection, interstitial cystitis, atopic dermatitis or inflammatory bowel disease, Crohn's disease, ulcerative colitis, celiac disease, systemic lupus erythematosus, type 1 diabetes, multiple sclerosis, rheumatoid arthritis, alcoholic liver steatosis, obesity, glucose intolerance, insulin resistance, hyperglycemia, fatty liver, dyslipidemia, hyperlipidemia, type 2 diabetes, pancreatic cancer, breast cancer, kidney cancer, bladder cancer, prostate cancer, testicular cancer, urothelial cancer, endometrial cancer, ovarian cancer, cervical cancer, renal cancer, esophageal cancer, gastrointestinal stromal tumor (GIST), multiple myeloma, cancer of secretory cells, thyroid cancer, gastrointestinal carcinoma, chronic myeloid leukemia, hepatocellular carcinoma, colon cancer, melanoma, malignant glioma, glioblastoma, glioblastoma multiforme, astrocytoma, dysplastic gangliocytoma of the cerebellum, Ewing's sarcoma, rhabdomyosarcoma, ependymoma, medulloblastoma, ductal adenocarcinoma, adenosquamous carcinoma, nephroblastoma, acinar cell carcinoma, lung cancer, non-Hodgkin's lymphoma, Burkitt's lymphoma, chronic lymphocytic leukemia, monoclonal gammopathy of undetermined

significance (MGUS), plasmocytoma, lymphoplasmacytic lymphoma, acute lymphoblastic leukemia, Pelizaeus-Merzbacher disease, atherosclerosis, abdominal aortic aneurism, carotid artery disease, deep vein thrombosis, Buerger's disease, chronic venous hypertension, vascular calcification, telangiectasia or lymphoedema, glaucoma, age-related macular degeneration, inflammatory retinal disease, retinal vascular disease, diabetic retinopathy, uveitis, rosacea, Sjogren´s syndrome or neovascularization in proliferative retinopathy, hyperhomocysteinemia, skeletal muscle atrophy, myopathy, muscular dystrophy, muscular wasting, sarcopenia, Duchenne muscular dystrophy (DMD), Becker's disease, myotonic dystrophy, X-linked dilated cardiomyopathy, spinal muscular atrophy (SMA), Down syndrome, MEHMO syndrome, metaphyseal chondrodysplasia, Schmid type (MCDS), depression, or social behavior impairment.

22. A method of producing a protein, comprising contacting a eukaryotic cell comprising a nucleic acid encoding the protein with the compound or salt of any one of claims 1-14, wherein the cell is cultured in an *in vitro* culture medium comprising the compound or salt.

23. A method of culturing a eukaryotic cell comprising a nucleic acid encoding a protein, comprising contacting the eukaryotic cell with an *in vitro* culture medium comprising a compound or salt of any one of claims 1-14.

24. The method of claim 22 or 23, wherein the nucleic acid encoding the protein is a recombinant nucleic acid.

25. The method of any one of claims 22-24, wherein the cell is a human embryonic kidney (HEK) cell or a Chinese hamster ovary (CHO) cell.

26. The method of any one of claims 22-24, wherein the cell is a yeast cell, a wheat germ cell, an insect cell, a rabbit reticulocyte, a cervical cancer cell, a baby hamster kidney cell, a murine myeloma cell, an HT-1080 cell, a PER.C6 cell, a plant cell, a hybridoma cell, or a human blood derived leukocyte

27. A method of producing a protein, comprising contacting a cell-free protein synthesis (CFPS) system comprising eukaryotic initiation factor 2 (eIF2) and a nucleic acid encoding a protein with the compound or salt of any one of claims 1-14.

28. The method of any one of claims 22-27, wherein the protein is an antibody or a fragment thereof.

29. The method of any one of claims 22-27, wherein the protein is a recombinant protein, an enzyme, an allergenic peptide, a cytokine, a peptide, a hormone, erythropoietin (EPO), an interferon, a granulocyte-colony stimulating factor (G-CSF), an anticoagulant, or a clotting factor.

30. The method of any one of claims 22-29, comprising purifying the protein.

31. An *in vitro* cell culture medium, comprising the compound or salt of any one of claims 1-14 and nutrients for cellular growth.

32. The cell culture medium of claim 31, comprising a eukaryotic cell comprising a nucleic acid encoding a protein.

33. The cell culture medium of claim 31 or 32, further comprising a compound for inducing protein expression.

34. The cell culture medium of any one of claims 31-33, wherein the nucleic acid encoding the protein is a recombinant nucleic acid.

35. The cell culture medium of any one of claims 31-34, wherein the protein is an antibody or a fragment thereof.

36. The cell culture medium of any one of claims 31-34, wherein the protein is a recombinant protein, an enzyme, an allergenic peptide, a cytokine, a peptide, a hormone,

erythropoietin (EPO), an interferon, a granulocyte-colony stimulating factor (G-CSF), an anticoagulant, or a clotting factor.

37. The cell culture medium of any one of claims 31-36, wherein the eukaryotic cell is a human embryonic kidney (HEK) cell or a Chinese hamster ovary (CHO) cell.

38. The cell culture medium of any one of claims 31-36, wherein the cell is a yeast cell, a wheat germ cell, an insect cell, a rabbit reticulocyte, a cervical cancer cell, a baby hamster kidney cell, a murine myeloma cell, an HT-1080 cell, a PER.C6 cell, a plant cell, a hybridoma cell, or a human blood derived leukocyte

39. A cell-free protein synthesis (CFPS) system comprising eukaryotic initiation factor 2 (eIF2) and a nucleic acid encoding a protein with the compound or salt of any one of claims 1-14.

40. The CFPS system of claim 39, comprising a eukaryotic cell extract comprising eIF2.

41. The CFPS system of claim 39 or 40, further comprising eIF2B.

42. The CFPS system of any one of claims 39-41, wherein the protein is an antibody or a fragment thereof.

43. The CFPS system of any one of claims 39-42, wherein the protein is a recombinant protein, an enzyme, an allergenic peptide, a cytokine, a peptide, a hormone, erythropoietin (EPO), an interferon, a granulocyte-colony stimulating factor (G-CSF), an anticoagulant, or a clotting factor.