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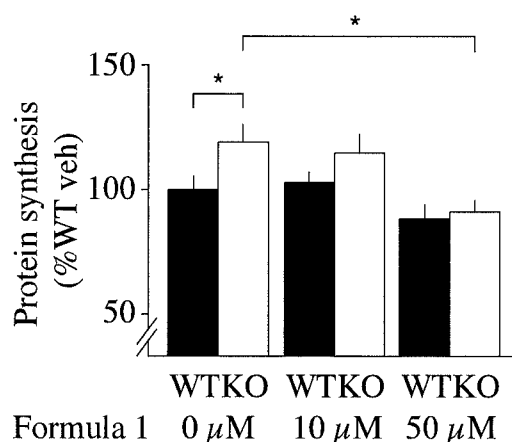
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(54) Title: METHODS OF TREATING SEIZURE DISORDERS

Figure 2A



(57) Abstract: Seizure disorders are treated by Ras inhibitors. In an embodiment, the Ras inhibitor includes a farnesyl transferase inhibitor. In another embodiment, the farnesyl transferase inhibitor includes a 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor. In another embodiment, the 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor is not a Ras inhibitor. Subjects having fragile X syndrome, autism, Angelman syndrome, Costello syndrome, cardio facio cutaneous syndrome, neurofibromatosis type I, Noonan syndrome and Coffin-Lowry syndrome that have seizure disorders can be treated.

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METHODS OF TREATING SEIZURE DISORDERS

RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 61/405,446, filed on October 21, 2010. The entire teachings of the above
5 application are incorporated herein by reference.

GOVERNMENT SUPPORT

This invention was made with government support under HD046943 and R21MH090452 awarded by the National Institutes of Health. The government has certain rights in the invention.

10 BACKGROUND OF THE INVENTION

Seizure disorders, including epilepsy, can affect daily activities of adults and children. Seizures can develop in early childhood or adolescence. Seizure disorders can be associated with abnormal electrical activity in the brain resulting in temporary loss of consciousness, body convulsions, unusual movements and staring
15 spells, all of which affect daily activities and the health of the affected individual. Current treatments for seizure disorders, including seizure disorders in individuals with autism, fragile X syndrome, Angelman syndrome, Costello syndrome, cardio facio cutaneous syndrome (CFC), neurofibromatosis type I, Noonan syndrome and Coffin-Lowry syndrome, can include drug therapy, such as anticonvulsant drugs.
20 However, such treatments can result in unwanted side-effects and may not treat the underlying cause of the seizure disorder.

SUMMARY OF THE INVENTION

The invention is generally directed to methods of treating a seizure disorder in an individual by administering compositions that include a Ras inhibitor and/or an
25 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor.

In an embodiment, the invention is a method of treating a subject having a seizure disorder, comprising the step of administering a composition that includes at least one Ras inhibitor.

In another embodiment, the invention is a method of treating a subject
5 having a seizure disorder, comprising the step of administering a composition that includes at least one 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor.

The methods of the invention have the advantage of normalizing synaptic signaling in the central nervous system to thereby treat an underlying cause of the seizure disorder.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1I depict changes in protein synthesis and ERK1/2 activation in hippocampal slices.

Figures 1A-1C show that protein synthesis is excessive in the Fmr1 KO.

Figures 1D-1F show that the MEK1/2-ERK1/2 inhibitor U0126 corrects
15 excessive protein synthesis and reduces ERK1/2 activation in the Fmr1 KO.

Figures 1G-1I shows that LOVASTATIN (Formula I, "lovastn") in the lactone form can correct protein synthesis and reduce ERK1/2 activation in the Fmr1 KO.

Figures 2A-2G show changes in the presence of LOVASTATIN (Formula I,
20 "lovastn") in the acid form.

Figures 2A-C show that Formula I corrects protein synthesis in Fmr1 KO hippocampal slices. The significant normalization of protein synthesis by Formula I is shown as a summary bar graph (Figure 2A) and yoked WT-KO slices with vehicle (Figure 2B) and with 50 μ M Formula I (Figure 2C).

Figures 2D and 2E show that Formula I inhibits Ras activation in
25 hippocampal slices.

Figures 2F and 2G show that Formula I downregulates ERK1/2 in hippocampal slices. Error bars = s.e.m.

Figures 3A-3L show that Formula I (LOVASTATIN, "lov") significantly
30 reduces AGS incidence and severity in the Fmr1 KO on the C57BL6 and FVB backgrounds. Fmr1 KO and WT mice on the C57BL6 (Figures 3A-3F) or FVB

(Figures 3G-3L) backgrounds were treated as indicated, tested for AGS, and scored for wild running, clonic seizure, tonic seizure and death.

Figure 3A shows that injection of 30 mg/kg of Formula I acid significantly reduces AGS incidence.

5 Figures 3B and 3C show that injection of 30 mg/kg of Formula I acid attenuates AGS severity in Fmr1 KO mice.

Figure 3D shows that injection of 100 mg/kg Formula I significantly reduces AGS incidence and (Figures 3E and 3F) attenuates AGS severity in Fmr1 KO mice on the C57BL6 background.

10 Figures 3E and 3F show that injection of 100 mg/kg Formula I attenuates AGS severity in Fmr1 KO mice on the C57BL6 background.

Figure 3G shows that injection of 30 mg/kg Formula I does not significantly reduce AGS incidence or (Figures 3H and 3I) severity in Fmr1 KO mice on the FVB background.

15 Figures 3H and 3I show that injection of 30 mg/kg Formula I does not significantly reduce AGS severity in Fmr1 KO mice on the FVB background.

Figure 3J shows that injection of 100 mg/kg Formula I significantly reduces AGS incidence and (Figures 3K and 3L) severity in Fmr1 KO mice on the FVB background.

20 Figures 3K and 3L show that injection of 100 mg/kg Formula I significantly reduces AGS severity in Fmr1 KO mice on the FVB background.

Figures 4A-4D show that Formula I lactone and oral administration significantly reduces AGS incidence and severity in the Fmr1 KO. Fmr1 KO and WT mice (C57BL6) were treated as indicated and tested for AGS. The following
25 stages of AGS severity were scored: wild running, clonic seizure, tonic seizure, and death.

Figure 4A shows that injection of 30 mg/kg Formula I lactone significantly reduces AGS incidence and severity in Fmr1 KO mice.

30 Figures 4B and 4C show that injection of 30 mg/kg Formula I lactone significantly reduces AGS severity in Fmr1 KO mice.

Figure 4D shows that 48 h feeding of 0.1% Formula I chow significantly reduces AGS incidence in Fmr1 KO mice.

Figures 4E and 4F show that 48 h feeding of 0.1% Formula I chow significantly reduces AGS severity in Fmr1 KO mice.

DETAILED DESCRIPTION OF THE INVENTION

A description of example embodiments of the invention follows. The features and other details of the invention, either as steps of the invention or as combinations as parts of the invention, will now be particularly described and pointed out in the claims. It will be understood that the particular embodiments of the invention are shown by way of illustration and not as limitations of the invention. The principle features of this invention can be employed in various embodiments without departing from the scope of the invention.

In an embodiment, the invention is a method of treating a subject having a seizure disorder, comprising the step of administering a composition that includes at least one Ras inhibitor.

In another embodiment, the invention is a method of treating a subject having a seizure disorder, comprising the step of administering a composition that includes at least one 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor.

Ras (RAt Sarcoma) proteins (also referred to herein as “Ras”) are guanosine-nucleotide-binding proteins (G-proteins) involved in all signaling. Genes encoding Ras proteins were initially identified as oncogenes. Ras is associated with the plasma membrane of a cell because of prenylation and palmitoylation or the combination of prenylation and a polybasic sequence adjacent to the prenylation site. The carboxy-terminal CaaX amino acid sequence of Ras is initially farnesylated at a cysteine residue in the cytoplasm of the cell, which permits Ras to loosely insert into the endoplasmatic reticulum and other cellular membranes. The aaX tripeptide of Ras is then cleaved from the carboxy-terminus of Ras by a prenyl-protein specific endoprotease and the resulting carboxy-terminus methylated by a methyltransferase. Electrostatic interactions between the positively charged basic sequence of Ras with negative charges at the inner surface of the plasma membrane account for the predominant localization of Ras at the cell surface.

“Ras inhibitor,” as used herein, means a compound that prevents activation of a Ras protein. In an embodiment, a Ras inhibitor can bind Ras, thereby

preventing Ras from hydrolyzing GTP (guanosine triphosphate) and, thus, preventing activation of downstream signaling pathways.

The carboxy-terminal membrane targeting region of Ras has an aaX motif that is modified by farnesyl transferase resulting in the addition of a 15-carbon isoprenoid (farnesyl group). In an embodiment, the Ras inhibitor is a farnesyl transferase inhibitor.

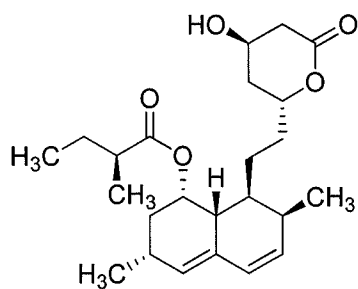
“Farnesyl transferase inhibitor,” as used herein, mean that the Ras inhibitor prevents the addition of a farnesyl group to Ras.

In an embodiment, the farnesyl transferase inhibitor is a 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor (also referred to herein as “statins”).

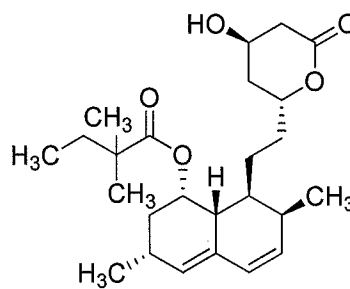
“A 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor,” as used herein, mean that the compound prevents the enzymatic activity of 3-hydroxy-3-methylglutaryl-Coenzyme A reductase. In an embodiment, the the 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor inhibits cell signaling via Ras. In another embodiment, the 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor prevents cell signaling through pathways other than Ras and is not a Ras inhibitor. For example, the 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor can inhibit glycogen synthase kinase 3beta (GSK3beta) signaling.

Statins are compounds that low cholesterol levels by inhibiting the enzyme HMG (3-hydroxy-3-methyl-glutaryl)-Coenzyme A (also referred to herein as “CoA”) reductase. HMG-CoA reductase is the rate-controlling enzyme of the mevalonate pathway, which produces cholesterol and other isoprenoids. In mammalian cells, HMG-CoA reductase is normally suppressed by cholesterol derived from the internalization and degradation of low density lipoprotein (LDL) by the LDL receptor and oxidized species of cholesterol. Competitive inhibitors of HMG-CoA reductase induce the expression of LDL receptors in the liver, which, in turn, increase the catabolism of plasma LDL and lower the plasma concentration of cholesterol. HMG-CoA reductase is anchored in the membrane of the endoplasmic reticulum in the cytoplasm of cells. In an embodiment, the 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor can include at least one member selected from the group consisting of Formulas I and II:

- 6 -



I; and



II.

In another embodiment, the invention is a method of treating a seizure disorder in a subject comprising administering a composition that includes at least one of a MEK inhibitor or an ERK (extracellular signal-regulated kinase) inhibitor (also referred to as a “MAPK (mitogen-activated protein kinase) inhibitor”).

Ras, when activated, activates RAF kinase, a protein kinase that phosphorylates and activates MEK (MEK1 and MEK2). Phosphorylated MEK activates ERK, which, in turn, alters cellular processes.

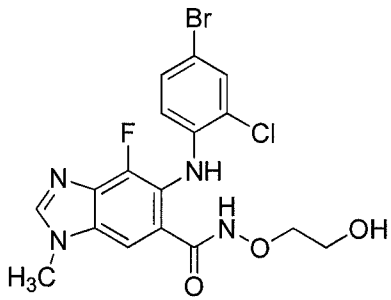
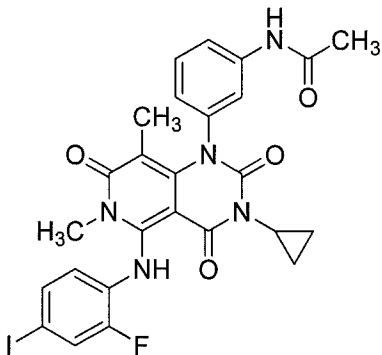
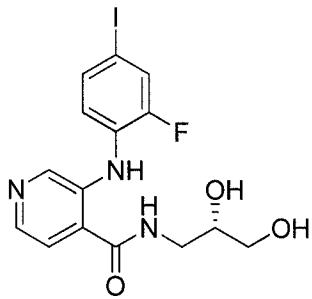
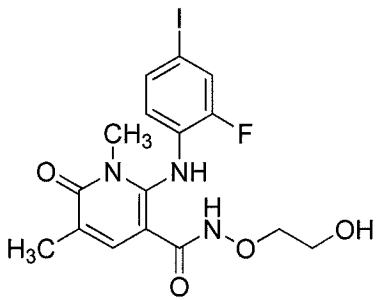
A “MEK inhibitor,” as used herein, means a compound that prevents activation of MEK. Exemplary MEK inhibitors for use in the methods include XL518 ([3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl][3-hydroxy-3-[(2R)-2-piperidinyl]-1-azetidiny]methane; EXELIXIS), CI-1040 (2-(2-chloro-4-iodophenylamino)-*N*-(cyclopropylmethoxy)-3,4-difluorobenzamide); CAS No: 212631-79-3; MEDKOO BIOSCIENCE), PD0325901 (*N*-[(2R)-2,3-dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-benzamide); CAS No: 391210-10-9, CAYMAN CHEMICAL), SELUMETINIB (C₁₇H₁₅BrClFN₄O₃, CAS No.: 606143-52-6, SELLECKCHEM), GSK1120212 (*N*-[3-[3-cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-3,4,6,7-tetrahydro-6,8-dimethyl-2,4,7-trioxypyrido[4,3-d]pyrimidin-1(2H)-yl]phenyl]acetamide); CAS No: 871700-17-3, ACTIVE BIOCHEM), AS703026 ((*S*)-*N*-(2,3-dihydroxypropyl)-3-((2-fluoro-4-iodophenyl)amino)isonicotinamide); CAS No: 1236699-92-5, SELLECKCHEM), AZD8330 (2-((2-fluoro-4-iodophenyl)amino)-*N*-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide); CAS No.: 869357-68-6, SELLECKCHEM), PD318088 (5-bromo-*N*-(2,3-dihydroxypropoxy)-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]benzamide); CAS

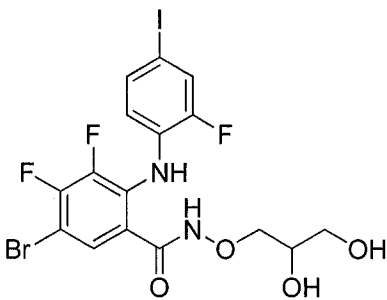
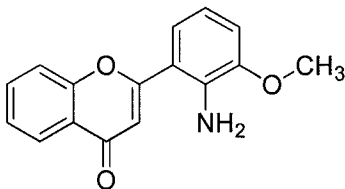
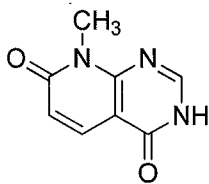
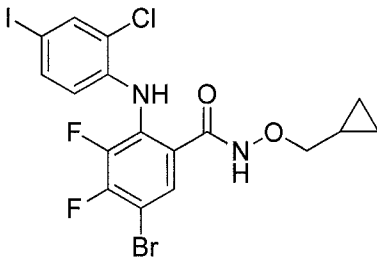
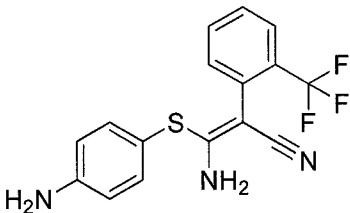
No: 391210-00-7; SELLECKCHEM), PD98059 (2-(2-amino-3-methoxyphenyl)-4*H*-1-benzopyran-4-one), CAS No: 167869-21-8, REAGENTS DIRECT), U0126 (PROMEGA), TAK-733 (8-methylpyrido[2,3-*d*]pyrimidine-4,7(3*H*,8*H*)-dione); CAS No.: 1035555-63-5, SELLECKCHEM), ARRY-162 (NOVARTIS), ARRY-300 (NOVARTIS), PD184161 (5-bromo-2-[(2-chloro-4-iodophenyl)amino]-*N*-(cyclopropylmethoxy)-3,4-difluoro-benzamide); CAS No: 212631-67-9, CAYMAN CHEMICAL), and SL327 (alpha-[amino[(4-aminophenyl)thio]methylene]-2-(trifluoromethyl)benzeneacetonitrile); CAS No. 305350-87-2, TOCRIS). The chemical structures of exemplary MEK inhibitors appear in Table 1.

10

Table 1. Chemical structures of exemplary MEK inhibitors.

IUPAC Name	Chemical Structure
[3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl][3-hydroxy-3-[(2 <i>R</i>)-2-piperidinyl]-1-azetidiny]methane	
2-(2-chloro-4-iodophenylamino)- <i>N</i> -(cyclopropylmethoxy)-3,4-difluorobenzamide	
<i>N</i> -[(2 <i>R</i>)-2,3-dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-benzamide	

IUPAC Name	Chemical Structure
6-(4-bromo-2-chloroanilino)-7-fluoro-N-(2-hydroxyethoxy)-3-methylbenzimidazole-5-carboxamide	
N-[3-[3-cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-3,4,6,7-tetrahydro-6,8-dimethyl-2,4,7-trioxypyrido[4,3-d]pyrimidin-1(2H)-yl]phenyl]acetamide	
(S)-N-(2,3-dihydroxypropyl)-3-((2-fluoro-4-iodophenyl)amino)isonicotinamide	
2-((2-fluoro-4-iodophenyl)amino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide	

IUPAC Name	Chemical Structure
5-bromo- <i>N</i> -(2,3-dihydroxypropoxy)-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]benzamide	
2-(2-amino-3-methoxyphenyl)-4 <i>H</i> -1-benzopyran-4-one	
8-methylpyrido[2,3- <i>d</i>]pyrimidine-4,7(3 <i>H</i> ,8 <i>H</i>)-dione	
5-bromo-2-[(2-chloro-4-iodophenyl)amino]- <i>N</i> -(cyclopropylmethoxy)-3,4-difluorobenzamide	
alpha-[amino[(4-aminophenyl)thio]methylene]-2-(trifluoromethyl)benzeneacetonitrile	

The MEK inhibitor ARRY-162 (NOVARTIS) has a MEK Enzyme IC₅₀ = 12 nM; Malme-3M pERK IC₅₀ = 11 nM; Human Whole Blood TPA-induced TNF-α =

23 nM, IL-1 β and IL-6 = 21 nM. Safety data for ARRY-162 is as follows:

Genotoxicity: Negative; Cytochrome p450 Inhibition- IC₅₀: > 25 μ M (5 major isoforms); Kinase selectivity screen has no activity against ~200 other kinases at 1 μ M; and receptor screen is clean against 25 receptors. *In vivo* efficacy of ARRY-

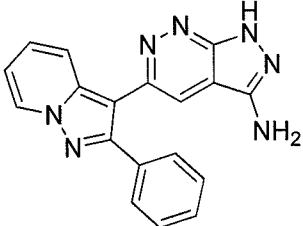
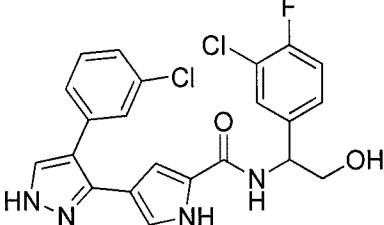
5 162 - Rat carrageenan paw edema: ED₅₀~ 7 mg/kg, PO, once; Rat Collagen-Induced Arthritis: ED₅₀= 3 mg/kg, PO, QD x 7; and rat adjuvant-induced arthritis: ED₅₀= 10 mg/kg, PO, QD x 7 (Winkler, J., *et al.*, *Inflamm. Res. Supplement 3*: FC11.4 (2007); Winkler, J., *et al.*, 9th Annual World Congress of Inflammation, July 2009, Tokyo, JP WS06-15).

10 An "ERK inhibitor," as used herein, means a compound that prevents activation of ERK. Exemplary ERK inhibitors for use in the methods of the invention include FR180204 (5-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)-1*H*-pyrazolo[3,4-c]pyridazin-3-amine), CAS No: 865362-74-9, SANTA CRUZ BIOTECHNOLOGY), and CAY10561 *N*-[1-(3-chloro-4-fluorophenyl)-2-

15 hydroxyethyl]-4-[4-(3-chlorophenyl)-1*H*-pyrazol-3-yl]-1*H*-pyrrole-2-carboxamide; CAS No: 933786-58-4, CAYMAN CHEMICAL), and AEZS-131 (AETERNA ZENTARIS). In another embodiment, the Ras inhibitor (e.g., farnesyl transferase inhibitor, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor) can be administered to a subject having a seizure disorder in combination with at least one

20 compound that down regulates Group I mGluR signaling. The compound that down regulates Group I mGluR signaling can be administered to the subject before, concomitant with or after administration of the Ras inhibitor. The chemical structures of exemplary ERK inhibitors appear in Table 2.

Table 2. Chemical structures of exemplary ERK inhibitors.

IUPAC Name	Chemical Structure
5-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)-1 <i>H</i> -pyrazolo[3,4-c]pyridazin-3-amine	
<i>N</i> -[1-(3-chloro-4-fluorophenyl)-2-hydroxyethyl]-4-[4-(3-chlorophenyl)-1 <i>H</i> -pyrazol-3-yl]-1 <i>H</i> -pyrrole-2-carboxamide	

In a further embodiment, at least one of a Ras inhibitor, a MEK inhibitor or an ERK (extracellular signal-regulated kinase) inhibitor (also referred to as a

5 “MAPK (mitogen-activated protein kinase) inhibitor”) can be administered to the subject having a seizure disorder in combination with at least one compound that down regulates Group I mGluR signaling.

“Down regulates mGluR signaling,” as used herein, refers to any decrease or any inhibition in a cellular process or a cellular event or intermediate in a cellular

10 event associated with any mechanism whereby metabotropic glutamate receptors (mGluRs) mediate a biological response. For example, down regulation can be the prevention or any decrease in binding of a signal external to a cell (a first messenger), such as a ligand (e.g., glutamate), to a Group I mGluR. Down regulation can be disruption of a cellular process following binding of an external

15 signal (e.g., ligand) to an mGluR, such as the prevention of activation of adenylyl cyclase or phospholipase C (PLC). Down regulation can also be disruption of a cellular processes following binding of an external signal to Group I mGluR, such as the prevention of activation of a G-protein (Gs, Gq), a decrease in a G-protein (Gs, Gq) activation, prevention of activation of second messengers activated by Group I

mGluR (e.g., cAMP, IP₃, diacylglycerol (DAG)) or a decrease in the activity of an intracellular effector, such as a cAMP-dependent protein kinase, protein kinase C (PKC) or calcium release.

Down regulation of Group I mGluR signaling can also be a decrease or inhibition in the release of glutamate. A decrease or inhibition of glutamate release can be through activation of presynaptic Group II and/or Group III mGluRs by, for example, agonists of Group II and Group III mGluRs. An "agonist," as used herein, is a compound that activates cell signaling. Exemplary Group II and Group III mGluR agonists for use in the invention include LY354740, L-AP4 (Capogna, M. *Eur. J. Neurosci.* 19: 2847-2858 (2004)) (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate ((2R,4R)-APDC), (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-1), *N*-Acetyl-L-aspartyl-L-glutamic acid (Spaglumeric acid), (S)-3-carboxy-4-hydroxyphenylglycine ((S)-3C4HPG), (S)-4-carboxy-3-hydroxyphenylglycine ((S)-4C3HPG) and AMN 082 dihydrochloride (Flor *et al.*, *Neuropharmacology* 49:244 (2005), the teachings of which are hereby incorporated by reference in its entirety).

Group I mGluR signaling also can be down regulated by decreasing or inhibiting protein synthesis in response to mGluR activation with, for example, rapamycin (Bierer *et al.*, *PNAS* 87:9231 (1990)). The protein synthesis in response to mGluR activation can be decreased or inhibited by decreasing or inhibiting the transcription, translation, posttranslational modifications, intracellular half-life and/or intracellular processing of signals mediated or activated by mGluR.

In a specific embodiment, suitable compounds that down regulate Group I mGluR can be Group I mGluR antagonists. An mGluR antagonist is a substance which diminishes or abolishes the effect of a ligand (or agonist) that activates an mGluR, for example GluR5 antagonists and mGluR1 antagonists. Exemplary suitable antagonists of mGluR5 are described in WO 01/66113, WO 01/32632, WO 01/14390, WO 01/08705, WO 01/05963, WO 01/02367, WO 01/02342, WO 01/02340, WO 00/20001, WO 00/73283, WO 00/69816, WO 00/63166, WO 00/26199, WO 00/26198, EP-A-0807621, US Patent No: 5,672,592, US Patent No: 5,795,877, US Patent No: 5,863,536, US Patent No: 5,880,112 and US Patent No: 5,902,817, all of which are incorporated by reference in their entirety.

In an embodiment, the subject treated by the methods of the invention has autism. The subject who has autism can further have fragile X syndrome.

In another embodiment, the subject treated by the methods of the invention can have fragile X syndrome. Fragile X mental retardation protein is a protein
5 encoded by the fragile X mental retardation gene 1 (FMR-1). Fragile X syndrome, the most common form of genetically inherited mental retardation, is associated with a fragile site expressed as an isochromatid gap in the metaphase chromosome at map position Xq 27.3. A mutation in the 5'-untranslated region of the FMR1 gene, located on the X chromosome, results in fragile X syndrome (FXS). FMRP is not
10 produced in fragile X syndrome. Genetic testing is available to provide a definitive diagnosis of fragile X syndrome by detecting the mutation in the FMR1 gene and its protein product, FMRP. Genetic testing can be performed on infants, children, adolescents and adults. One of ordinary skill in the art would be able to determine whether a subject has fragile X syndrome by employing established clinical criteria
15 and genetic testing.

In a further embodiment, the subject treated by the methods of the invention can have autism, also referred to herein as "autism spectrum disorder."

Autism spectrum disorder is a developmental disorder that affects an individual's ability to communicate, form relationships with others and respond
20 appropriately to the environment. Some individuals with autism spectrum disorder are high functioning, with speech and intelligence within normal range. Other individuals with autism spectrum disorder may be nonverbal and/or have varying degrees of mental retardation. Autism spectrum disorder can include Asperger's syndrome and idiopathic autism (e.g., autism of unknown origin). One of ordinary
25 skill in the art would be able to diagnosis an individual with autism spectrum disorder and determine whether the individual has idiopathic autism or Asperger's syndrome, employing well-known clinical criteria as described, for example, in Diagnostic and Statistical Manual of Mental Disorders (DSMMD) (4th ed., pp. 70-71) Washington, D.C., American Psychiatric, 1994.

30 For example, autism can be diagnosed by assessing the qualitative impairment in social interaction, such as marked impairment in the use of multiple nonverbal behaviors, such as eye-to-eye gaze, facial expression, body postures,

gestures to regulate social interaction, failure to develop peer relationships appropriate to developmental level and a lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g., by a lack of showing, bringing, or pointing out objects of interest). The development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime), a marked impairment in the ability to initiate or sustain a conversation with others and repetitive use of language or idiosyncratic language. Delayed or abnormal functioning in social interaction, language as used in social communication or symbolic or imaginative play prior to 3 years of age.

In an additional embodiment, the subject that has fragile X syndrome who is treated by the methods of the invention can have autism.

The subject treated by the methods of the invention can be a human subject that is treated as a child, an adolescent and an adult.

In another embodiment, subjects treated by the methods of the invention can have at least one condition selected from the group consisting of Angelman syndrome, Costello syndrome, cardio facio cutaneous syndrome, neurofibromatosis type I, Noonan syndrome and Coffin-Lowry syndrome. One of ordinary skill in the art would be able to diagnosis whether a subject has Angelman syndrome, Costello syndrome, cardio facio cutaneous syndrome, neurofibromatosis type I, Noonan syndrome and Coffin-Lowry syndrome.

Angelman Syndrome is a developmental disorder caused by loss of function of the maternal copy of the UBE3A gene. Symptoms of the disorder include developmental delay, intellectual disability, autism, sleep disturbances, movement disorders, and epilepsy (Mabb, A.M. *et al.*, *Trends in Neuroscience*, Jun 2011, volume 34(6), pp. 293-303, the teachings of which are incorporated by reference in their entirety).

Costello Syndrome is a rare disorder caused by mutations in HRAS which result in over-active Ras protein. Symptoms include developmental delay, intellectual disability, loose skin, flexible joints, heart defects, and short stature. Seizures are associated with this disorder (Kawame, H.M. *et al.*, *American Journal of Medical Genetics*, 2003, volume 118A(1), pp. 8-14; Krab, L.C. *et al.*, *Trends in*

Genetics, 2008, volume 24(10), pp. 498-510; Delrue, M.A. *et al*, *American Journal of Medical Genetics*, 2003, volume 123A(3), pp. 301-305, the teachings of all of which are incorporated by reference in their entirety).

5 Cardio Facio Cutaneous syndrome (CFC) syndrome is a disorder caused by mutations in the BRAF, MAP2K1 or MAP2K2 genes. It is characterized by abnormalities in the heart, skin and facial features, and is associated with developmental delay, intellectual disability, and seizures (Krab, L.C. *et al.*, *Trends in Genetics*, 2008, volume 24(10), pp. 498-510, the teachings of which are incorporated by reference in their entirety).

10 Neurofibromatosis Type 1 is a developmental disorder caused by mutations in the NF1 gene. It is characterized by tumors in multiple organ systems and skin discoloration. Learning disabilities and epilepsy are associated with this disorder (Krab, L.C. *et al.*, *Trends in Genetics*, 2008, volume 24(10), pp. 498-510; Vivarelli, R.S. *et al.*, *Journal of Child Neurology*, 2003, volume 18(5), pp. 338-342, the
15 teachings of all of which are incorporated by reference in their entirety).

Noonan syndrome is a disorder caused by mutations in the PTPN11, SOS1, RAF1, KRAS, NRAS or BRAF genes. It is characterized by abnormalities in the heart, skeletal structure and circulatory system. Intellectual disability and seizures are associated with this disorder (Krab, L.C. *et al.*, *Trends in Genetics*, 2008, volume
20 24(10), pp. 498-510; Adachi, M. *et al.*, *Seizure*, 2011, epub ahead of print, the teachings of all of which are incorporated by reference in their entirety).

Coffin-Lowry syndrome is a disorder that is caused by mutations in the RPS6KA3 gene. It is characterized by disruptions in multiple organ systems, and is associated with intellectual disability, enhanced acoustic startle response, and
25 seizures (Touraine, R.L. *et al.*, *European Journal of Pediatrics*, 2002, volume 161(4), pp. 179-187; Krab, L.C. *et al.*, *Trends in Genetics*, 2008, volume 24(10), pp. 498-510, the teachings of all of which are incorporated by reference in their entirety).

The subject treated by the methods of the invention can have at least one
30 seizure disorder selected from the group consisting of an audiogenic seizure disorder and an epileptic seizure disorder. Seizures, including audiogenic seizures, can occur in individuals with at least one of fragile X syndrome, autism, Angelman syndrome,

Costello syndrome, cardio facio cutaneous syndrome, neurofibromatosis type I, Noonan syndrome and Coffin-Lowry syndrome. One of ordinary skill in the art would be able to diagnosis whether a subject has a seizure.

The Ras inhibitor (e.g., farnesyl transferase inhibitor, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor) can administered to the subject in a single dose or in multiple doses (i.e., more than one dose). Multiple doses can include multiple daily doses. Multiple doses can be 2, 3, 4, 5, 6, 7, 8, 9, 10 or more doses, which can be administered daily.

The Ras inhibitor (e.g., farnesyl transferase inhibitor, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor) can administered to the subject daily, weekly, monthly, yearly or for the lifetime of the subject.

The Ras inhibitor (e.g., farnesyl transferase inhibitor, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor), MEK inhibitor, ERK inhibitor, or Group I mGluR antagonist can be administered to the subject in at least one dose selected from the group consisting of a 1 mg dose, 5 mg dose, a 10 mg dose, a 20 mg dose, a 25 mg dose, a 40 mg dose, a 50 mg dose, a 80 mg dose, a 100 mg dose, a 120 mg dose, a 125 mg dose, a 160 mg dose, a 200 mg dose, a 400 mg dose and a 600 mg dose. The dose of the compound administered to the subject can be based on the weight of the subject, for example, a dose such as about 5 mg/kg, about 10 mg/kg, about 20 mg/kg, about 30 mg/kg, about 40 mg/kg, about 50 mg/kg, about 80 mg/kg, about 100 mg/kg, about 150 mg/kg or about 200 mg/kg.

The methods of the invention can be accomplished by the administration of the compounds of the invention (e.g., Ras inhibitors, MEK inhibitor, ERK inhibitor) to a subject (e.g., a human subject) by enteral or parenteral means. The route of administration can be by oral ingestion (e.g., tablet, capsule form) or intramuscular injection of the compound. Other routes of administration can include intravenous, intraarterial, intraperitoneal, or subcutaneous routes, nasal administration, suppositories and transdermal patches. The compounds employed in the methods of the invention can be administered in suitable excipients, including pharmaceutically acceptable salts.

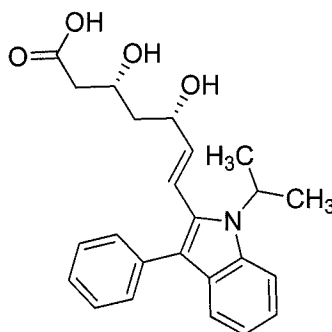
The Ras inhibitors employed in the methods of the invention can include farnesyl transferase inhibitors. Exemplary farnesyl transferase inhibitors can include

at least one member selected from the group consisting of a 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor (e.g., Formulas I-VII), a farnesyl pyrophosphate (FPP) analog (e.g., Formulas VIII-X), a CAAC peptidomimetic (e.g., Formulas XI-XXII), a bisubstrate inhibitor (e.g., Formulas XXIII-XXVI) and a nonpeptidomimetic inhibitor (e.g., Formulas XXVII-XXXII). The compounds of Formulas I-XXXII and exemplary doses for use in the methods of the invention are as described below.

LOVASTATIN (Formula I) (1*S*,3*R*,7*S*,8*S*,8*aR*)-8-{2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8*a*-hexahydronaphthalen-1-yl-(2*S*)-2-methylbutanoate; CAS No.: 75330-75-5 (MERCK & CO., INC., Germany) can be administered in a dose range of about 10 to about 100 mg/day.

SIMVASTATIN (Formula II) (1*S*,3*R*,7*S*,8*S*,8*aR*)-8-{2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8*a*-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate; CAS No.: 79902-63-9 (MERCK & CO., INC., Germany) can be administered in a dose range of about 5 to about 100 mg/day.

FLUVASTATIN (Formula III) (3*R*,5*S*,6*E*)-7-[3-(4-fluorophenyl)-1-(propan-2-yl)-1*H*-indol-2-yl]-3,5-dihydroxyhept-6-enoic acid; CAS No.: 93957-54-1 (NOVARTIS) can be administered in a dose range of about 20 to about 100 mg/day.

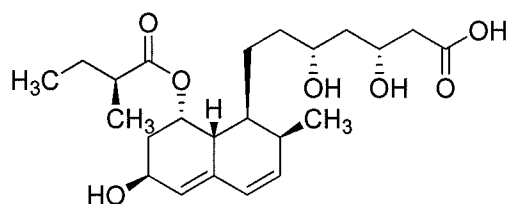


III

PRAVASTATIN (Formula IV) (3*R*,5*R*)-3,5-dihydroxy-7-((1*R*,2*S*,6*S*,8*R*,8*aR*)-6-hydroxy-2-methyl-8-{[(2*S*)-2-methylbutanoyl]oxy}-1,2,6,7,8,8*a*-hexahydronaphthalen-1-yl)-heptanoic acid; CAS No.: 81093-37-0

- 18 -

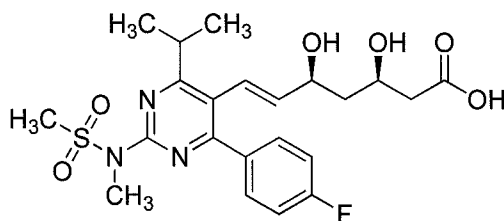
(BRISTOL-MYERS SQUIBB) can be administered in a dose range of about 20 to about 150 mg/day.



IV

5

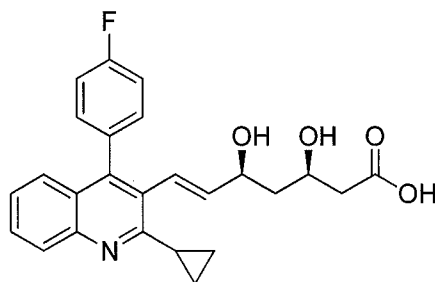
ROSUVASTATIN (Formula V) (3*R*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid; CAS No.: 287714-41-4 (ASTRAZENECA) can be administered in a dose range of about 5 to about 50 mg/day.



V

10

PITAVASTATIN (Formula VI) (3*R*,5*S*,6*E*)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3,5-dihydroxyhept-6-enoic acid; CAS No.: 147511-69-1 (ELI LILLY) can be administered in a dose range of about 1 to about 5 mg/day.



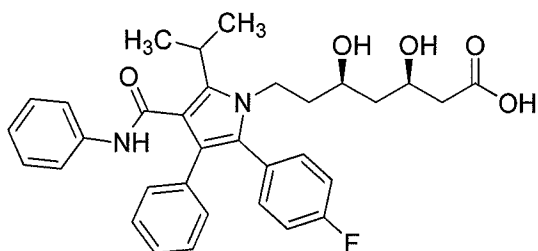
VI

15

ATORVASTATIN (Formula VII) (3*R*,5*R*)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid; CAS

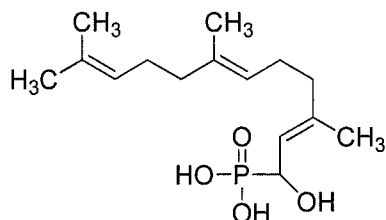
- 19 -

No.: 134523-00-5 (PFIZER) can be administered in a dose range of about 10 to about 100 mg/day.



VII

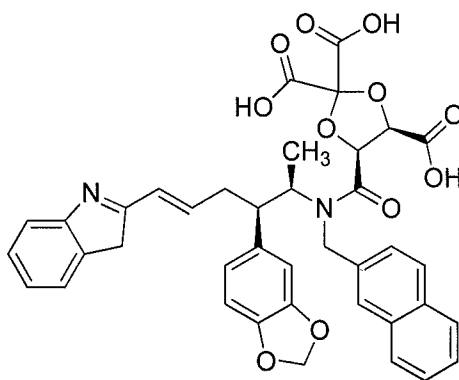
Alpha-hydroxyfarnesylphosphonic acid (Formula VIII)



VIII

5

J-104,871 (Formula IX) (4R*, 5S*)-5-{N-[(1R,2R,4E)-5-(2-benzoxazolyl)-1-methyl-2-(3,4-methylenedioxyphenyl)-4-pentenyl]-N-(2-naphthylmethyl)carbamoyl}-1,3-dioxolane-2,2,4-tricarboxylic acid can be administered in a dose range of about 1 to about 50 mg/kg/day.

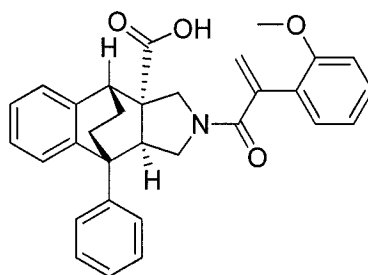


IX

10

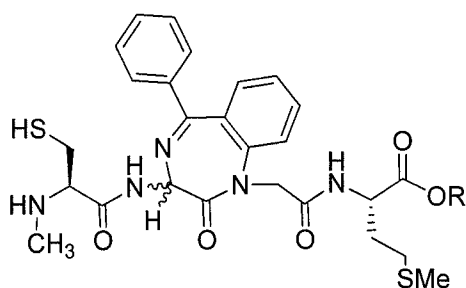
RPR-115135 (Formula X) (+/-)-11-[2-(2-methoxyphenyl)acryloyl]-1-phenyl-(1R,8R,9S,13S)-11-azatetracyclo[6.5.2.0.2,7.0.9,13]pentadeca-2,4,6-triene-9-carboxylic acid

- 20 -



X

BZA-5B (Formula XI) (Cys-(*N*-Me)-(BZA)-Met-COOMe); and BZA-2B (Cys-(*N*-Me)-(BZA)-Met-COOH)

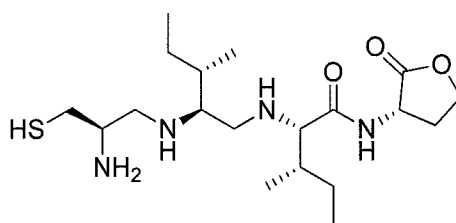


BZA-2B: R = H

BZA-5B: R = Me

XI

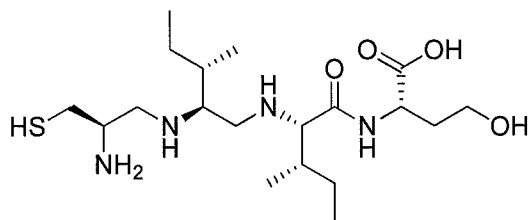
- 5 L-731,734 (Formula XII) (2*S*,3*S*)-2-(((2*S*,3*S*)-2-(((*R*)-2-amino-3-mercaptopropyl)amino)-3-methylpentyl)amino)-3-methyl-*N*-((*S*)-2-oxotetrahydrofuran-3-yl)pentanamide



XII

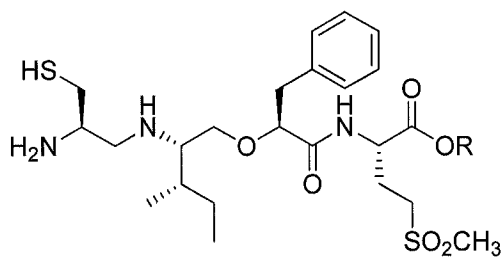
- 10 L-731,735 (Formula XIII) (*S*)-2-(((2*S*,3*S*)-2-(((2*S*,3*S*)-2-(((*R*)-2-amino-3-mercaptopropyl)amino)-3-methylpentyl)amino)-3-methylpentanamido)-4-hydroxybutanoic acid

- 21 -



XIII

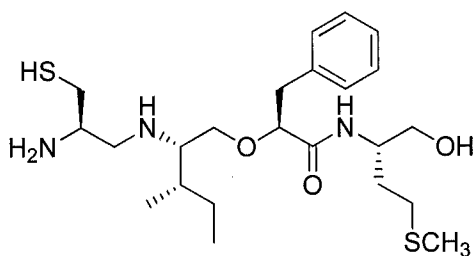
L-739,749 (Formula XIV) (S)-methyl 2-(((S)-2-(((2S,3S)-2-(((R)-2-amino-3-mercaptopropyl)amino)-3-methylpentyl)oxy)-3-phenylpropanamido)-4-(methylsulfonyl)butanoate; and L-739,750; (S)-2-(((S)-2-(((2S,3S)-2-(((R)-2-amino-3-mercaptopropyl)amino)-3-methylpentyl)oxy)-3-phenylpropanamido)-4-(methylsulfonyl)butanoic acid

L-739,749, R=CH₃

L-739,750, R=H

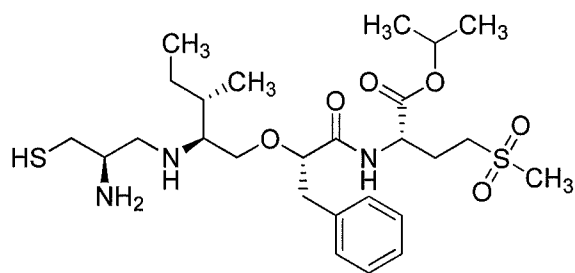
XIV

L-739,787 (Formula XV) (S)-2-(((2S,3S)-2-(((R)-2-amino-3-mercaptopropyl)amino)-3-methylpentyl)oxy)-N-((S)-1-hydroxy-4-(methylthio)butan-2-yl)-3-phenylpropanamide



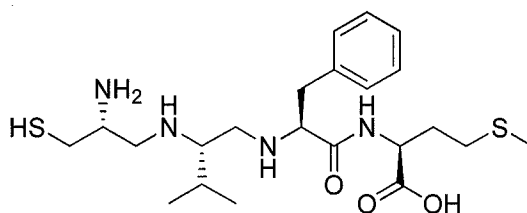
XV

L-744,832 (Formula XVI) (2S)-2-[[[(2S)-2-[[[(2S,3S)-2-[[[(2R)-2-amino-3-mercaptopropyl]amino]-3-methylpentyl]oxy]-1-oxo-3-phenylpropyl]amino]-4-(methylsulfonyl)-butanoic acid 1-methylethyl ester



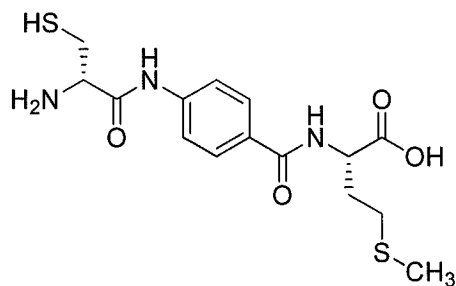
XVI

B581 (Formula XVII) (2S)-2-[[[(2S)-2-[[[(2S)-2-[[[(2R)-2-amino-3-sulfanylpropyl]amino]-3-methylbutyl]amino]-3-phenylpropanoyl]amino]-4-methylsulfanylbutanoic acid



XVII

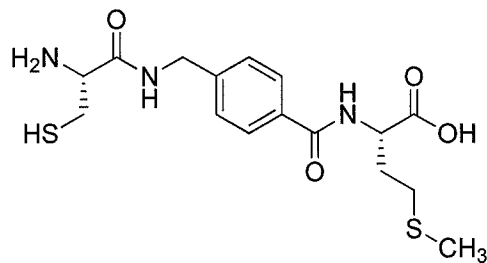
Cys-4-aminobenzoic-Met (Formula XVIII)



XVIII

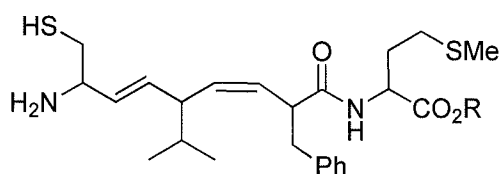
10

Cys-aminomethylbenzoic acid-Met (Formula XIX)



XIX

B956 and B1086



B956

R = H

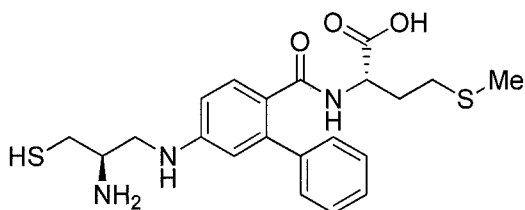
B1086

R = Me

XX

5

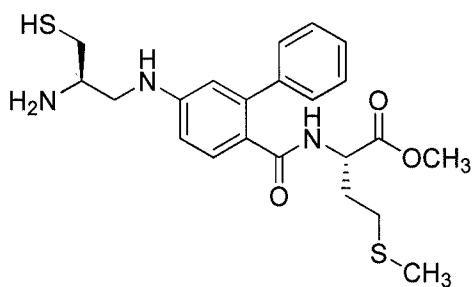
FTI-276 (Formula XXI) (S)-2-(5-(((R)-2-amino-3-mercaptopropyl)amino)-[1,1'-biphenyl]-2-ylcarboxamido)-4-(methylthio)butanoic acid



XXI

10

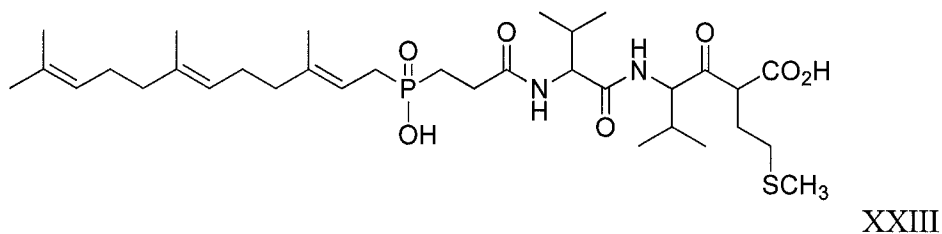
FTI-277 (Formula XXII) (S)-methyl 2-(5-(((R)-2-amino-3-mercaptopropyl)amino)-[1,1'-biphenyl]-2-ylcarboxamido)-4-(methylthio)butanoate



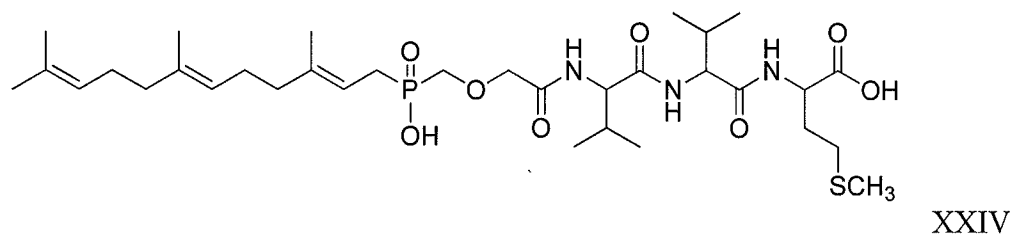
XXII

BMS-185878 (Formula XXIII)

- 24 -

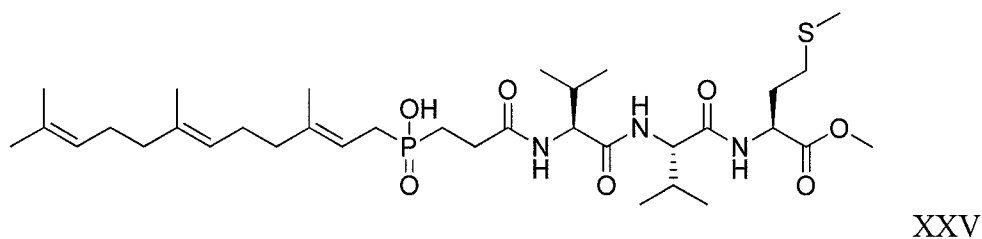


BMS-184467 (Formula XXIV)



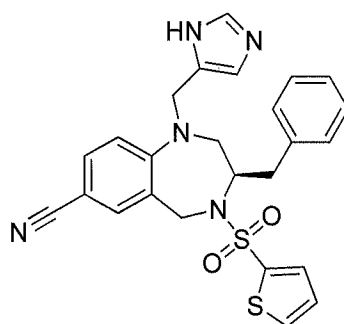
5

BMS (Formula XXV) 186511 [3-[[[(2S)-1-[[[(2S)-1-[[[(2S)-1-methoxy-4-methylsulfanyl-1-oxobutan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-3-oxopropyl]-[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyl]phosphinic acid



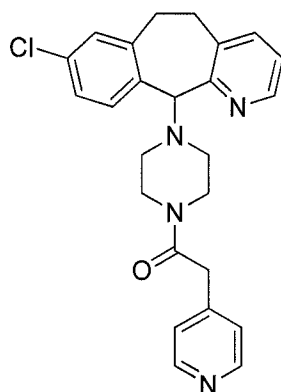
10

BMS-214662 (Formula XXVI) (3R)-3-benzyl-1-(1H-imidazol-5-ylmethyl)-4-thiophen-2-ylsulfonyl-3,5-dihydro-2H-1,4-benzodiazepine-7-carbonitrile can be administered in a dose range about 50 to about 200 mg/kg/week.



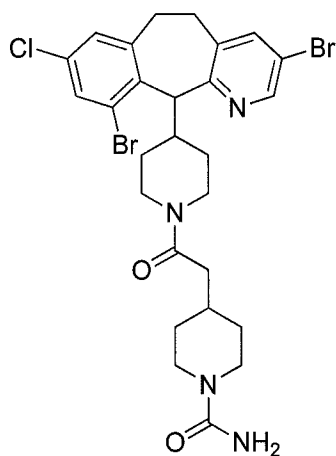
- 25 -

SCH54429 (Formula XXVII) 1-(8-chloro-6,11-dihydro-5*H*-benzo [5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-4-(4-pyridinylacetyl)piperazine can be administered in a dose range of about 1 to about 50 mg/kg/day.



XXVII

- 5 LONAFARNIB (Formula XXVIII) (SCH66336) 4-(2-(4-(8-chloro-3,10-dibromo-6,11-dihydro-5*H*-benzo(5,6)cyclohepta(1,2-*b*)pyridin-11-yl)-1-piperidiny)-2-oxoethyl)-1-piperidinecarboxamide can be administered in a dose range of about 25 to about 125 mg twice/day.

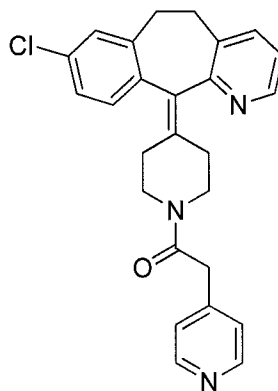


XXVIII

10

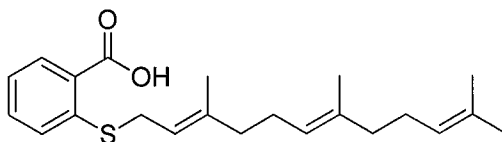
SCH 44342 (Formula XXIX) 1-(4-pyridylacetyl)- 4-(8-chloro-5,6-dihydro-11*H*benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)piperidine can be administered in a dose range of about 1 to about 50 mg/kg/day.

- 26 -



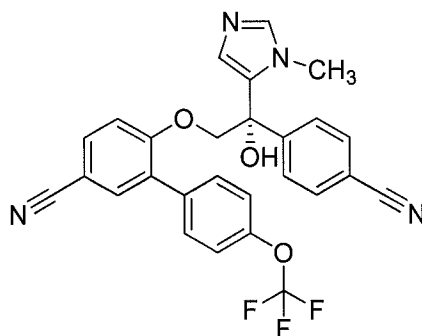
XXIX

SALIRASIB (Formula XXX) 2-[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyl]sulfanylbenzoic acid can be administered in a dose range about 1 to about 50
5 mg/kg/day.



XXX

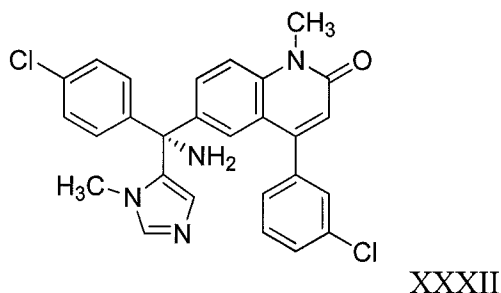
ABT-100 (Formula XXXI) 4-[(2S)-2-(4-cyanophenyl)-2-hydroxy-2-(3-methylimidazol-4-yl)ethoxy]-3-[4-(trifluoromethoxy)phenyl]benzonitrile can be
10 administered in a dose range of about 1 to about 50 mg/kg/day.



XXXI

TIPIFARNIB (Formula XXXII) (R115777) 6-[(R)-amino-(4-chlorophenyl)-(3-methylimidazol-4-yl)methyl]-4-(3-chlorophenyl)-1-methylquinolin-2-one can be
15 administered in a dose range of about 50 to about 600 mg/day.

- 27 -



EXEMPLIFICATION

Example 1 – Reduction in Excessive Protein Synthesis and Audiogenic Seizures

The prevalence of autism spectrum disorders (ASDs) has risen sharply over the past decade [1]. ASDs are developmental disorders characterized by deficits in social interaction, language, communication, and behavior [2]. Fragile X syndrome (FXS) is the leading single-gene cause of autism [3], and the most common form of heritable mental retardation in humans [4]. Characterized by cognitive deficits, developmental delay, and behavioral abnormalities, FXS is caused by the loss of FMRP, the product of the *FMR1* gene [5, 6]. Evidence from both *in vitro* and *in vivo* studies suggests that FMRP is a repressor of translation [7-10].

Group 1 (Gp1) metabotropic glutamate receptors (mGluR1 and mGluR5) stimulate protein synthesis at synapses [11], and some lasting consequence of mGluR5 activation, such as long-term depression (LTD) of synaptic transmission in the CA1 region of hippocampus, are protein synthesis dependent [12-14]. Based on electrophysiological studies in *Fmr1* KO mice, it has been suggested that FMRP represses the synthesis of proteins required for mGluR-LTD [15-17]. These and related data led to the “mGluR theory of FXS”, which proposes that mGluR5 and FMRP act in functional opposition, with mGluR5 driving translation and FMRP repressing this translation [18]. Excessive Gp1 mGluR-mediated protein synthesis in the absence of FMRP is posited to cause the synaptic pathophysiology that gives rise to multiple symptoms of the disease [19].

The activation of Ras is dependent on its interaction with the plasma membrane, which requires posttranslational farnesylation [27-29]. Farnesyl transferase inhibitors (FTIs) prevent the farnesylation of Ras, and thereby reduce its

activity. HMG-CoA Reductase inhibitors, such as Formula I, have FTI activity. Formula I crosses the blood brain barrier, and can interfere with p21 Ras farnesylation in the brain [31, 32]. Formula I is well tolerated in humans, and is approved to treat hyperlipidemia in both adults and children [33].

5 Although some pharmacological interventions exist for the anxiety, epilepsy, and mood disorders associated with fragile X syndrome, there are currently few effective treatment strategies targeted at the core pathophysiological mechanisms underlying seizure disorders in fragile X syndrome and autism [34]. As described herein, an *in vitro* assay that models the core pathological phenotype of excessive
10 protein synthesis in the *Fmr1* KO mouse was employed.

Excessive Protein Synthesis

Materials and Methods

Dorsal hippocampal slices were prepared from juvenile (postnatal day 25-32)
15 WT and *Fmr1* KO mice using the same metabolic labeling procedure described in [35]. Briefly, 500 μ m slices were recovered in carbogenating, 32.5°C ACSF (in mM: NaCl: 124, KCl: 3, NaH₂PO₄: 1.25, NaHCO₃: 26, dextrose: 10, MgCl₂: 1, CaCl₂: 2, saturated with 95% O₂ and 5% CO₂), pre-incubated in actinomycin D (ActD, 25 μ M) for 30 min to prevent transcription, and protein synthesis measured
20 over 30 minutes via incorporation of a ³⁵S-labeled methionine/cysteine mix (³⁵S-Met/Cys).

Group I mGluR activate several signal pathways. To determine whether a MEK1/2-ERK1/2 inhibitor could rescue excessive protein synthesis in the *Fmr1* KO, hippocampal slices were incubated with $\pm$ 5 μ M U0126 (1,4-diamino-2,3-
25 dicyano-1,4-bis[2-aminophenylthio] butadiene) (TOCRIS BIOSCIENCE, Ellisville, Missouri) for 30 minutes, and performed metabolic labeling ($\pm$ 5 μ M U0126) for an additional 30 min.

Formula I (LOVASTATIN in lactone form; SIGMA) was evaluated to determine whether it can selectively reduce the excessive protein synthesis seen in
30 the *Fmr1* KO. Hippocampal slices were incubated with vehicle or 100 μ M Formula I (in lactone form) for 30 minutes, then protein synthesis measured $\pm$ 100 μ M Formula I for an additional 30 minutes.

Results

The data described herein show a significant elevation of basal protein synthesis in juvenile *Fmr1* KO hippocampal slices as compared to wild type (WT) controls (WT $100 \pm 2\%$, KO $111 \pm 4\%$; $n = 12$; $*p = 0.0267$) (Figures 1A and 1B),
 5 consistent with previous work in adult *Fmr1* KO mice [35, 36].

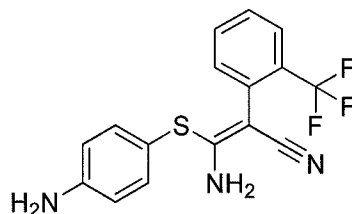
After 5 minutes of treatment with 50 μ M MPEP, an mGluR5 antagonist, protein synthesis in *Fmr1* KO hippocampal slices were reduced to WT levels (WT $100 \pm 1\%$, KO $117 \pm 2\%$, WT MPEP $104 \pm 2\%$, KO MPEP $95 \pm 2\%$; $n = 8$; ANOVA genotype x treatment $p < 0.05$; t-test $*p = 0.0248$) (Figure 1C).

10 U0126 significantly reduces protein synthesis in *Fmr1* KO, but not WT slices (WT: vehicle $100 \pm 6\%$, U0126 $94 \pm 6\%$; KO: vehicle $115 \pm 4\%$, U0126 $91 \pm 6\%$; $n = 9$; ANOVA treatment $p < 0.0009$, genotype x treatment $p < 0.03$; t-test: KO $*p = 0.0051$, WT $p = 0.153$) (Figures 1D and 1E). U0126 treatment significantly decreased ERK1/2 activation (WT: vehicle $100 \pm 15\%$, U0126 $15 \pm 3\%$; KO: vehicle
 15 $86 \pm 8\%$, U0126 $12 \pm 2\%$; $n = 4$; ANOVA treatment $p < 0.0001$, genotype x treatment $p = 0.606$; t-test: WT $*p < 0.01$, KO $*p < 0.005$) (Figures 1D and 1E). These data show that downregulation of the ERK1/2 pathway, for example, by compounds that inhibit ERK1/2 activation directly, or indirectly, by, for example, inhibiting activation of MEK signaling, is effective in correcting *Fmr1* KO
 20 phenotypes, which may be therapeutic in the treatment of seizure disorders of fragile X syndrome and autism.

These data show that Formula I, a Ras inhibitor, results in a significant reduction in ERK1/2 activation (WT: vehicle $100 \pm 5\%$, Formula I $87 \pm 6\%$; KO: vehicle $94 \pm 5\%$, Formula I $81 \pm 6\%$; $n = 13$; ANOVA genotype $p = 0.3900$,
 25 treatment $p = 0.0037$, genotype x treatment $p = 0.9266$; t-test: WT $*p = 0.0456$, KO $*p = 0.0382$), normalizes protein synthesis in the *Fmr1* KO down to WT levels (WT: vehicle $100 \pm 3\%$, Formula I $100 \pm 3\%$; KO: vehicle $116 \pm 4\%$, Formula I (LOVASTN) $104 \pm 2\%$; $n = 14$; ANOVA genotype $p = 0.0121$, treatment $p = 0.0660$, genotype x treatment $p = 0.0332$; t-test: KO $*p = 0.0129$, WT $p = 0.815$; $n =$
 30 14) (Figures 1F and 1G).

Formula I corrects Audiogenic Seizures in the *Fmr1* KO

The ability of SL 327 (alpha-[amino[(4-aminophenyl)thio]methylene]-2-(trifluoromethyl)benzeneacetonitrile) (TOCRIS BIOSCIENCE, Ellisville, Missouri), a brain-penetrant MEK1/2-ERK1/2 inhibitor, to correct AGS in the *Fmr1* KO mouse. The structural formula of SL 327 is depicted as follows:



Materials and Methods

Fmr1 KO and WT mice were injected intraperitoneally (i.p.) with 100 mg/kg SL 327 or vehicle (50% DMSO in ddH₂O). One hour later, mice were exposed to a seizure-inducing stimulus for 2 minutes, and scored for four stages of AGS: wild running (WR; pronounced, undirected running and thrashing), clonic seizure (violent spasms accompanied by loss of balance), tonic seizure (postural rigidity in limbs), and death [35, 37, 39]. All animals were injected and scored blind to genotype.

Results

Vehicle-treated *Fmr1* KO mice exhibited a 73% incidence of AGS, which was significantly elevated over the 4% incidence observed in vehicle-treated WT mice (Table 3). SL 327 Treatment with SL327 completely eliminated AGS in the *Fmr1* KO, dropping the incidence to 0% (Table 3).

To test whether Formula I could also correct AGS in the *Fmr1* KO, experiments were repeated using 30 mg/kg Formula I. Treatment with Formula I also corrects AGS in the *Fmr1* KO, lowering the incidence to 21% (Table 3). (Fisher's exact test (two-tail): WR: WT control vs. KO control ***p = 0.0007, KO control vs. KO SL 327 *p = 0.0101, KO control vs. KO Formula I *p = 0.0183, KO control vs. WT SL 327 *p = 0.0104, KO control vs. WT Formula I **p = 0.0076;

Clonic WT control vs. KO control *p = 0.0106, KO control vs. KO SL 327 *p = 0.0462, KO control vs. KO Formula I *p = 0.0269, KO control vs. WT SL 327 *p = 0.0448, KO control vs. WT Formula I *p = 0.0183; *Tonic* WT control vs. KO control *p = 0.0195, KO control vs. KO SL 327 *p = 0.0478, KO control vs. KO Formula I *p = 0.0125).

Table 3. Formula I corrects AGS in the *Fmr1* KO

	<i>WR</i>	<i>Clonic</i>	<i>Tonic</i>	<i>Death</i>	<i>Animals</i>
KO Control	73%	50%	42%	19%	26
KO SL 327	0%*	0%*	0%*	0%	11
KO Formula I	21%*	12%*	6%*	8%	33
WT Control	4%***	4%*	4%*	0%	23
WT SL 327	0%*	0%*	0%	0%	10
WT Formula I	13%**	8%*	8%	8%	24

Fisher's exact test (two-tail), compared to KO Control

*p < 0.05, **p < 0.01, ***p < 0.001

A significant challenge in the treatment of developmental seizure disorders, particularly in individuals with autism and fragile X syndrome, is finding a pharmaceutical agent that is safe for use in children. The data described herein show that treatment with the Ras inhibitor of Formula I after *in vivo* administration can decrease and prevent audiogenic seizures, which is a pathological condition observed in fragile X syndrome and autism.

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Example 2 – Reduction in Excessive Protein Synthesis and Audiogenic Seizures

Epilepsy is one of the most debilitating symptoms associated with autism spectrum disorders (ASD). The onset of epilepsy (epileptogenesis) within the first

20 few years of life is correlated with increased risk of developing ASD[1]. Despite this, there remains little understanding of the mechanisms linking epilepsy to autism, and no targeted treatments for preventing epileptogenesis[2]. The most prevalent single-gene cause of autism is fragile X syndrome (FXS), a developmental disorder associated with intellectual disability and a high incidence of epilepsy[3]. The

25 pathogenesis of FXS has been linked to excessive cerebral protein synthesis, which is downstream of constitutive activation of group I metabotropic glutamate receptor 5 (mGluR5)[4]. As described herein, Formula I (LOVASTATIN), a drug with an excellent safety profile that is prescribed and approved to treat hypercholesterolemia in children[9], decreases ERK1/2 signaling, normalizes excessive protein synthesis,

30 and corrects AGS in the *Fmr1* KO. LOVASTATIN, a Ras inhibitor, may be useful in the methods described herein as a treatment to block epileptogenesis in subjects, including humans with autism and humans with autism and fragile X syndrome.

Both autism and epilepsy are increasingly prevalent conditions, each affecting an estimated 1 in 100 individuals[10, 11]. About 30% of the ASD

35 population has epilepsy. Studies have found that the manifestation of seizures within the first 3 years of life correlate with an increased incidence of ASD in at-risk children[2]. Childhood epileptogenesis may contribute to the progression of autism,

underscoring the need for an effective prophylaxis for epilepsy that is safe for use during development. The genetic heterogeneity of autism, and of epilepsy, has made it difficult understand the molecular mechanisms linking the two disorders. To mitigate this complexity, a mouse model of FXS, a single-gene developmental disorder associated with about 30% incidence of both autism and epilepsy[13, 14] was employed in these studies. The *Fmr1* KO is a suitable model because it involves the loss of a single gene of known function, and thus the genetic contribution to the pathophysiology can be attributed to loss of FMRP function. In addition, robust epilepsy phenotypes occur both *in vitro*, as measured by ictal discharges in hippocampal slices, and *in vivo*, as measured by increased susceptibility to AGS[14, 15] in the fragile X knock out mouse.

The *FMR1* gene encodes for the translational repressor Fragile X Mental Retardation Protein (FMRP). Several pathological changes observed in FXS may stem from an elevation of basal protein synthesis in the nervous system due to a loss of FMRP. mGluR5-ERK1/2 pathway may be a contributor to the pathogenesis of FXS[16]. Inhibition of mGluR5 or ERK1/2, as described herein, corrects the AGS phenotype observed in the *Fmr1* KO mouse. ERK1/2 is a member of the larger MAP kinase (MAPK) signaling pathway, headed by the small GTPase Ras[17]. The HMG-CoA reductase inhibitor of Formula I has been shown to reduce ERK1/2 signaling in neurons *in vitro* and *in vivo* by limiting Ras activation. The maturation of Ras to its active conformation requires the addition of a farnesyl group, which facilitates interaction with the plasma membrane, and Formula I negatively regulates this process[20, 21]. Unlike many proposed therapies to treat seizure disorders, Formula I has an advantage because it has been approved by the Food and Drug Administration (FDA) to treat humans, including treatment of hypercholesterolemia in children [9, 22]. Formula I has an excellent safety profile, and is widely prescribed for long-term treatment in both adults and children.

Materials and Methods

Mice: *Fmr1* KO (Jackson Labs) and WT littermates, bred on the C57BL6 or FVB background strains, were group housed and maintained in a 12:12 h light:dark cycle. An *In vitro* hippocampal slice assay was used herein to recapitulate the

exaggerated protein synthesis phenotype seen in the *Fmr1* KO[6]. Hippocampal slices were prepared from juvenile *Fmr1* KO and wild type (WT) mice, pre-incubated in 10-50 μ M Formula I or vehicle for 30 minutes, and protein synthesis measured for another 30 min via incorporation of a 35 S-labeled methionine/cysteine mix.

Drugs: Formula I acid (EMD Biosciences), Formula I lactone (SIGMA), and actinomycin D (TOCRIS) were reconstituted according to manufacturer's instructions.

Metabolic labeling: Experiments were performed on male WT and *Fmr1* KO mice (postnatal day 25-32), blind to genotype, as described herein. Briefly, 500 μ m slices were recovered for 4 h in 32.5°C ACSF (in mM: 124 NaCl, 3 KCl, 1.25 NaH_2PO_4 , 26 NaHCO_3 , 10 dextrose, 1 MgCl_2 , 2 CaCl_2 , saturated with 95% O_2 and 5% CO_2), incubated for 30 min with 25 μ M ActD $\pm$ Formula I (10 μ M or 50 μ M), and transferred to fresh ACSF $\pm$ drug with 10 μ Ci/ml 35 S-Met/Cys (Perkin Elmer) for another 30 min. After labeling, slices were homogenized, and labeled proteins isolated by TCA precipitation. Samples were read with a scintillation counter and also subjected to a protein concentration assay (BIO-RAD). Final data were expressed as counts per minute (CPM) per μ g protein, normalized to the 35 S-Met/Cys ACSF used for incubation, and the average incorporation of all samples analyzed in that experiment.

Immunoblotting: Immunoblotting was performed, blind to genotype and treatment as described herein using primary antibodies to Ras (PIERCE), p-ERK1/2 (Thr202/Tyr204), or ERK1/2 (CELL SIGNALING TECHNOLOGY), and HRP-conjugated secondary antibodies (GE HEALTHCARE).

Ras activation assay: Slices (4-6 per animal) were prepared exactly as for metabolic labeling, and Ras-GTP isolated using the Active Ras Pull-Down and Detection kit (PIERCE) according to manufacturer's instructions.

AGS: Experiments were performed using male WT and *Fmr1* KO littermates (P17-25) as described herein. For acute exposure experiments, mice were weighed and injected i.p. with: (A) 30 mg/kg Formula I acid or vehicle (10% DMSO in saline), (B) 100 mg/kg Formula I acid or vehicle (33% DMSO in saline), or (C) 30 mg/kg Formula I lactone or vehicle (50% DMSO in saline), returned to their home

cages for 1 h. For oral administration experiments, mice were weaned onto standard rodent chow (BIO-SERV) formulated with 100 mg/kg IC Formula I (40 mg tablets, MYLAN INC.) or identical chow containing no Formula I, and allowed to feed ad libitum for 48 hours. Drug doses were chosen on based upon previous work [19, 23, 24]. Immediately prior to testing, mice were transferred to a quiet (about < 60 dB ambient sound) room for 1 hour. Mice were then transferred to a transparent plastic test chamber and, after 1 minute of habituation, exposed to a 130 dB stimulus (recorded sampling of a modified personal alarm, RADIOSHACK model 49-1010) for about 2 minutes. Each testing session contained mice from both genotype and treatment groups. For each group, incidence of the following stages of AGS was calculated: wild running (WR; pronounced, undirected running and thRashing), clonic seizure (violent spasms accompanied by loss of balance), tonic seizure (postural rigidity in limbs), and death. All aspects of the experiments were performed blind to genotype.

Statistics: For biochemistry experiments, outliers greater than 2 standard deviations from the mean were removed, and significance was determined by two-way repeated measures mixed model ANOVA. If significant effects were found by ANOVA, *post hoc* analyses were performed to compare individual groups using two-tailed paired or unpaired (for Ras activation assays) Student's t-tests. For AGS experiments, significance was determined by two-tailed Fisher's exact test (for overall incidence) or Kolmogorov-Smirnov test (for severity score distribution).

Results and Discussion

As described herein, 50 μ M Formula I normalizes protein synthesis in the *Fmr1* KO down to WT levels (WT: veh $100 \pm 5\%$, 10 μ M $103 \pm 4\%$; 50 μ M $88 \pm 5\%$; KO: veh $119 \pm 6\%$, 10 μ M $115 \pm 7\%$; 50 μ M $91 \pm 4\%$; $n = 11$; ANOVA genotype $*p = 0.0121$, treatment $p = 0.0660$, genotype x treatment $*p = 0.0332$; *post hoc* t-test: WT vs. KO veh $*p = 0.019$, KO veh vs. 50 μ M $*p = 0.011$) (Figures 2A-2C). These results show that Formula I is sufficient to normalize the excessive protein synthesis seen in the *Fmr1* KO.

To examine whether Formula I application significantly inhibits Ras-ERK1/2 activation, the amount of active Ras in Formula I-treated slices was measured.

Hippocampal slices were prepared from WT and *Fmr1* KO, then incubated with 10-50 μ M Formula I or vehicle as described above. Active (GTP-bound) Ras was isolated from homogenized slices using a GST-pull down assay, and compared to total Ras. These data show that 50 μ M Formula I significantly reduces the amount of active Ras in both WT and *Fmr1* KO hippocampal slices (WT: veh $100 \pm 11\%$, 10 μ M $87 \pm 14\%$; 50 μ M $61 \pm 12\%$; KO: veh $96 \pm 8\%$, 10 μ M $89 \pm 8\%$; 50 μ M $61 \pm 9\%$; ANOVA genotype x treatment $*p = 0.0462$; $n = 8$; *post hoc* t-test: WT veh vs. 50 μ M $*p = 0.039$, KO veh vs. 50 μ M $*p = 0.015$) (Figures 2D and 2E).

To determine whether the observed downregulation of active Ras was accompanied by a concomitant decrease in ERK1/2 signaling, ERK1/2 phosphorylation in hippocampal slices exposed to 10-50 μ M Formula I or vehicle for 1 hour was determined. Results from Western blot analysis reveal that 50 μ M Formula I significantly reduces ERK1/2 activation in both WT and *Fmr1* KO slices (WT: veh $100 \pm 7\%$, 10 μ M $94 \pm 8\%$; 50 μ M $84 \pm 5\%$; KO: veh $97 \pm 5\%$, 10 μ M $90 \pm 6\%$; 50 μ M $78 \pm 5\%$; ANOVA genotype $p = 0.3900$, treatment $*p = 0.0037$, genotype x treatment $p = 0.9266$; $n = 13$; *post hoc* t-test: WT veh vs. 50 μ M $*p = 0.035$, KO veh vs. 50 μ M $*p = 0.008$) (Figures 2F and 2G). These data show that Formula I effectively inhibits the Ras-ERK1/2 pathway in hippocampal slices. Excessive protein synthesis in the *Fmr1* KO may be due to a hypersensitivity to, not hyperactivation of, the ERK1/2 signaling pathway. The methods described herein inhibit activation of Ras signaling by inhibit Ras.

The *in vitro* studies indicated that Formula I is effective in correcting the pathological excess in protein synthesis seen in the *Fmr1* KO. To determine whether a reduction of epileptogenesis could be observed *in vivo*, AGS susceptibility in *Fmr1* KO mice acutely injected with Formula I was determined. Enhanced susceptibility to AGS is one of the most robust phenotypes observed in the *Fmr1* KO mouse, and is used to model the epilepsy observed in FXS patients [14, 15].

Fmr1 KO and WT mice were injected intraperitoneally (i.p.) with 30 mg/kg Formula I and, after one hour, exposed to a 130 dB seizure-inducing stimulus for 2 minutes (see Materials and Methods). For each mouse, four stages of increasing AGS severity were scored: wild running, clonic seizure, tonic seizure, or death[16]. Vehicle-treated *Fmr1* KO mice exhibited about 74% incidence of AGS, and no

seizures were observed in vehicle-treated WT mice (Fisher's exact test (FET): WT vs. KO *p = 0.0002; Figure 3A). Acute injection of Formula I reduced the incidence of AGS to about 28% in the *Fmr1* KO (FET: KO veh vs. lov *p = 0.009; n = 18-19) (Figure 3A). These results show that acute Formula I administration significantly
5 reduces seizure activity in the *Fmr1* KO. To assess whether Formula I affected the severity of seizures in the *Fmr1* KO, the distribution of AGS scores was compared between vehicle and Formula I treated groups. These data show that the significant difference in AGS severity between WT and *Fmr1* KO mice in the vehicle treated group (Kolmogorov-Smirnov test (K-S test): WT vs. KO veh *p = 0.002) is
10 eliminated with Formula I treatment (K-S test: WT vs. KO lov p = 0.999; KO veh vs. lov *p = 0.041) (Figures 3B and 3C). These results show that Formula I significantly lessens the severity of seizures observed in the *Fmr1* KO. Experiments using a higher dose of 100 mg/kg Formula I revealed a similar reduction in AGS incidence (KO veh vs. lov 85% to 23%; FET *p = 0.005; n = 13) and severity (K-S
15 test: KO veh vs. lov *p = 0.015; WT vs. KO veh. *p = 0.04; WT vs. KO lov p = 0.999) (Figures 3D-3F).

The *Fmr1* KO AGS phenotype is seen in multiple mouse strains[15]. The beneficial effects of Formula I on AGS susceptibility in different strains of mice, C57BL6 mice and *Fmr1* KO mice bred on the FVB background strain was
20 determined. About 85% incidence of AGS in vehicle treated *Fmr1* KO FVB mice[15] was observed, which was significantly higher than the 11% incidence observed in vehicle treated WT mice (FET: *p = 0.002). Interestingly, *Fmr1* KO FVB mice injected with 30 mg/kg Formula I exhibit about 64% incidence of AGS incidence, which is not significantly different from the vehicle treated group (FET: p
25 = 0.357; Figure 3G). The severity of AGS is similarly unaffected by this treatment (K-S test: KO veh vs. lov p = 0.862) (Figures 3H and 3I). Studies have shown a mouse strain-dependent difference in Formula I metabolism[23]. These experiments were repeated using a higher dose. Results from these experiments show that about 100 mg/kg Formula I significantly lowers AGS incidence (FET: KO veh 82%, KO
30 lov 21%, *p = 0.005; n = 11-14; Figure 3J) and reduces AGS severity (K-S test: KO veh vs. lov *p = 0.022; WT vs. KO veh. *p = 0.016; WT vs. KO lov p = 0.999) in

FVB *Fmr1* KO mice (Figures 3K and 3L). These results show that Formula I is effective in correcting AGS in *Fmr1* KO mice bred on multiple background strains.

In patients, Formula I is administered in its lactone form, which is metabolized into an active conformation (Formula I acid) in the body[9]. While our
5 *in vitro* studies required the use of Formula I acid, confirmation that the *in vivo* administration of Formula I lactone could reduce AGS incidence and severity in the *Fmr1* KO was made in AGS experiments using 30 mg/kg Formula I lactone. Formula I lactone significantly reduces AGS incidence from about 73% to about 20% (FET: *p = 0.009; n = 15; Figure 4A) and reduces AGS severity (K-S test: KO
10 veh vs. lov *p = 0.009; WT vs. KO veh. *p = 0.005; WT lov vs. lov p = 0.999) in the *Fmr1* KO (Figures 4B and 4C).

To further investigate the viability of Formula I as a treatment in humans, Formula I was orally administrated to correct AGS incidence and severity in the *Fmr1* KO. WT and *Fmr1* KO mice were fed standard rodent chow supplemented
15 with about 0.1% Formula I, which corresponds to a dose of about 10 mg/kg/day[24]. After a 48 hour exposure to either Formula I chow or control chow with the same formulation, mice were tested for AGS. Oral administration of Formula I significantly reduces AGS incidence (FET: KO con 71%, KO lov 25%, *p = 0.003; n = 21-24; Figure 4D) and severity (K-S test: KO veh vs. lov *p = 0.016; WT vs.
20 KO veh. *p = 0.0001; WT vs. KO lov p = 0.461) in the *Fmr1* KO mouse (Figures 4E and 4F). These results show that oral administration of pharmaceutical grade Formula I is effective in correcting AGS susceptibility in the *Fmr1* KO.

Preventing epileptogenesis in ASD patients could have a dramatic impact on the progression and severity of humans with epilepsy, including humans with autism
25 and humans with fragile X syndrome who are autistic. Given the increasing prevalence of ASD, identifying an effective strategy for preventing epileptogenesis is of particular urgency. Ras inhibitors, such as Formulas I-XXXII may correct excessive protein synthesis and significantly reduces the incidence and severity of AGS in the *Fmr1* KO. Ras inhibitors may be useful to treat seizures in FXS and
30 other autism-linked developmental disorders.

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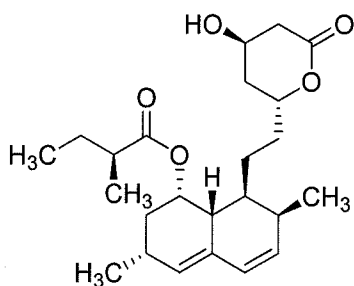
The teachings of all patents, published applications and references cited herein are incorporated by reference in their entirety.

While this invention has been particularly shown and described with references to example embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

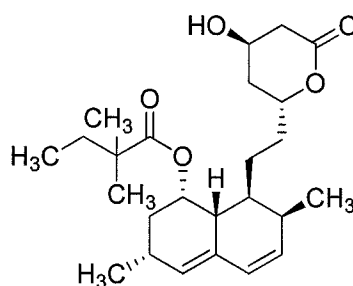
CLAIMS

What is claimed is:

1. A method of treating a subject having a seizure disorder, comprising the step of administering a composition that includes at least one Ras inhibitor.
- 5 2. The method of Claim 1, wherein the Ras inhibitor includes a farnesyl transferase inhibitor.
3. The method of Claim 2, wherein the farnesyl transferase inhibitor includes a 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor.
4. The method of Claim 3, wherein the 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor includes at least one member selected from the group consisting of Formulas I and II:
- 10



I; and



II.

5. The method of Claim 1, wherein the subject has autism spectrum disorder.
- 15 6. The method of Claim 5, wherein the subject further has fragile X syndrome.
7. The method of Claim 1, wherein the subject has fragile X syndrome.
8. The method of Claim 1, wherein the Ras inhibitor is administered to the subject in a single dose.
9. The method of Claim 1, wherein the Ras inhibitor is administered to the subject in multiple doses.
- 20

10. The method of Claim 4, wherein the 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor is administered to the subject in a single dose.
11. The method of Claim 4, wherein the 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor is administered to the subject in multiple doses.
- 5 12. The method of Claim 4, wherein the 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor is administered daily in at least one dose selected from the group consisting of a 1 mg dose, 5 mg dose, a 10 mg dose, a 20 mg dose, a 25 mg dose, a 40 mg dose, a 50 mg dose, a 80 mg dose, a 100 mg dose, a 120 mg dose, a 125 mg dose, a 160 mg dose, a 200 mg dose, a 400 mg dose
10 and a 600 mg dose.
13. The method of Claim 1, wherein the seizure disorder is an audiogenic seizure disorder.
14. The method of Claim 1, wherein the seizure disorder is an epileptic seizure disorder.
- 15 15. The method of Claim 1, wherein the subject is a human.
16. The method of Claim 1, wherein the subject has at least one condition selected from the group consisting of Angelman syndrome, Costello syndrome, cardio facio cutaneous syndrome, neurofibromatosis type I, Noonan syndrome and Coffin-Lowry syndrome.
- 20 17. A method of treating a subject having a seizure disorder, comprising the step of administering a composition that includes at least one 3-hydroxy-3-methylglutaryl-Coenzyme A reductase inhibitor.

Figure 1A

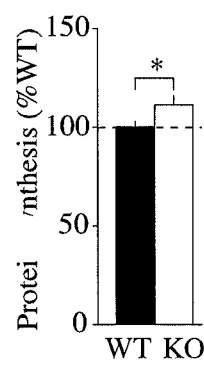


Figure 1B

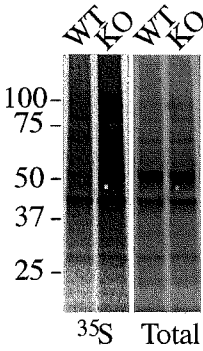
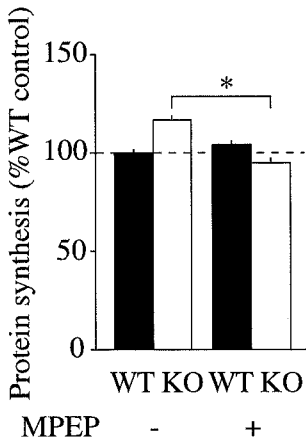


Figure 1C



FIGURES 1A-1C

Figure 1D

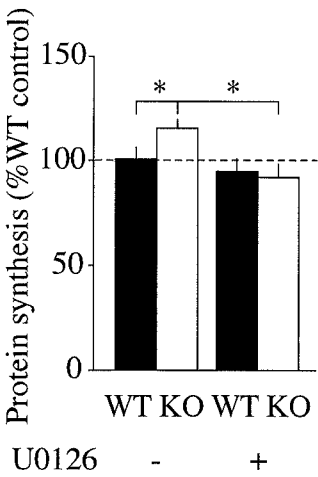
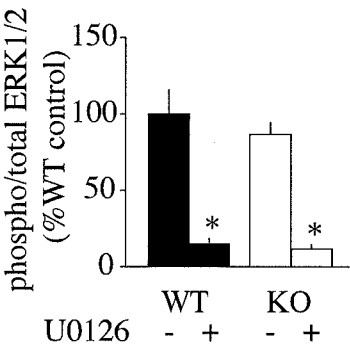


Figure 1E



Figure 1F



FIGURES 1D-1F

Figure 1G

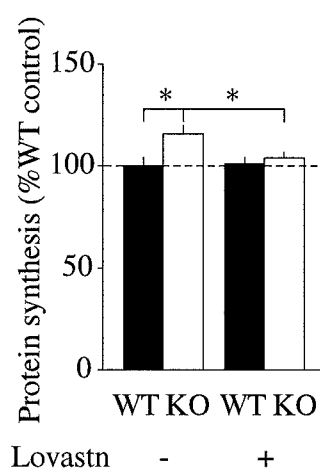
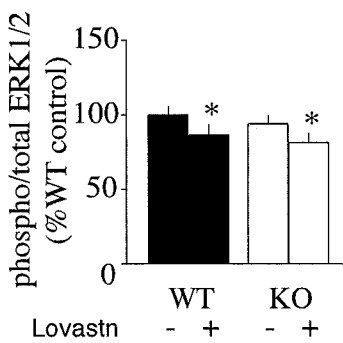


Figure 1H



Figure 1I



FIGURES 1G-1I

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Figure 2A

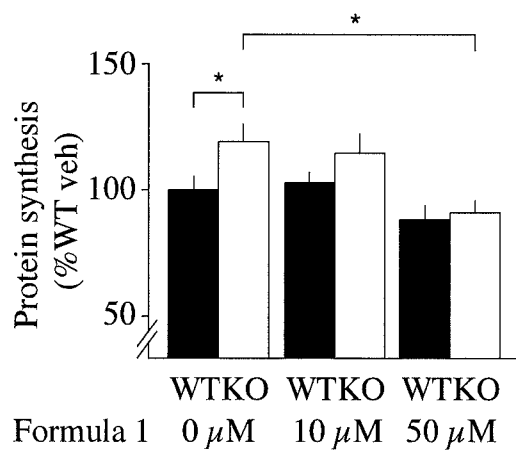


Figure 2B

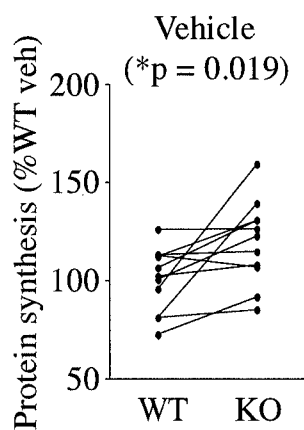
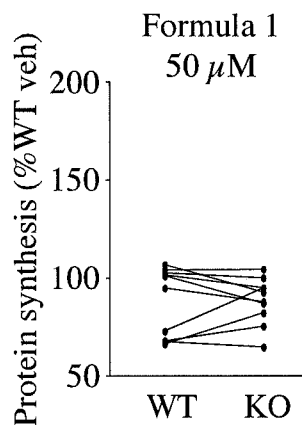


Figure 2C



FIGURES 2A-2C

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Figure 2D

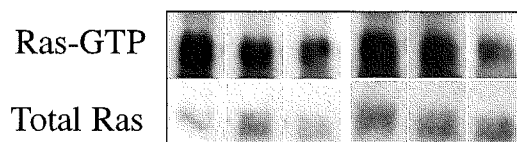
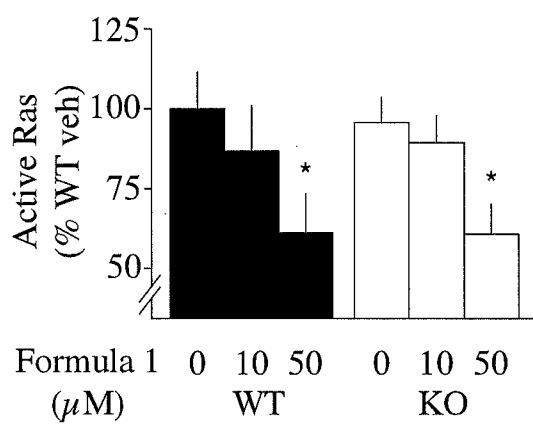


Figure 2E



FIGURES 2D-2E

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Figure 2F

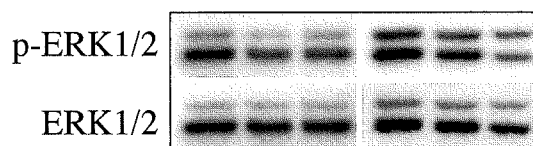
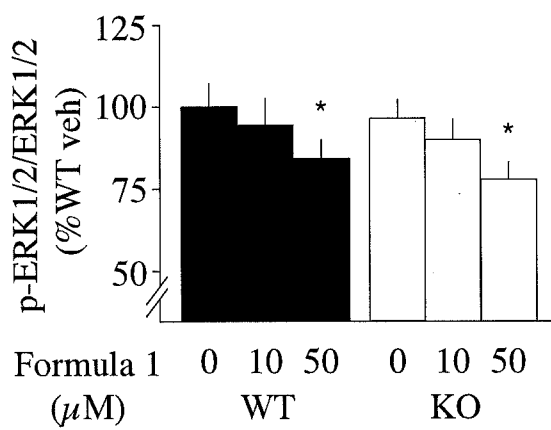


Figure 2G



FIGURES 2F-2G

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Figure 3A

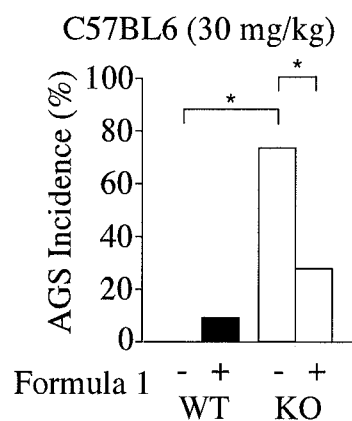


Figure 3B

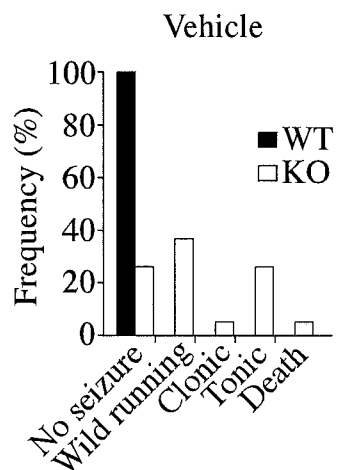
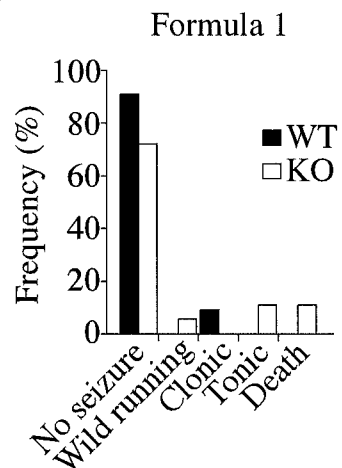


Figure 3C



FIGURES 3A-3C

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Figure 3D

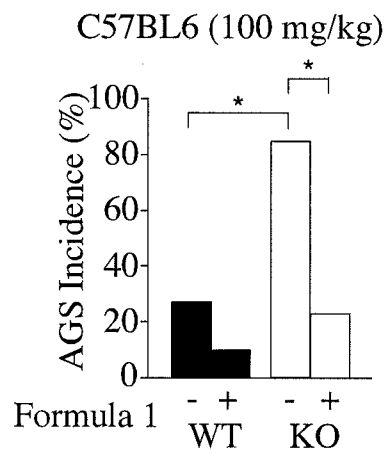


Figure 3E

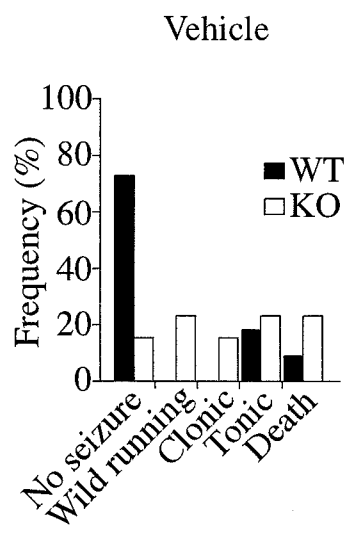
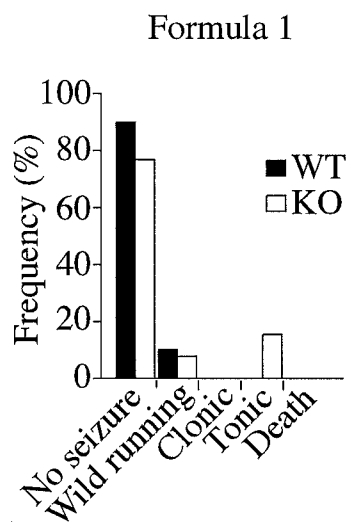


Figure 3F



FIGURES 3D-3F

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Figure 3G

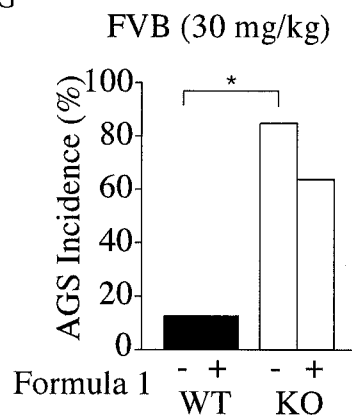


Figure 3H

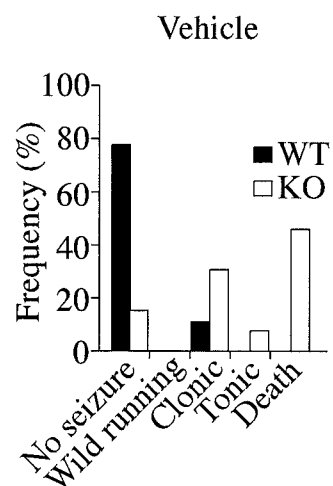
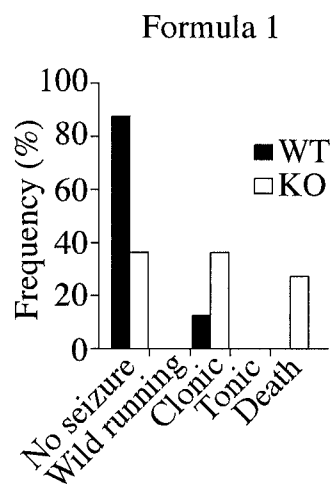
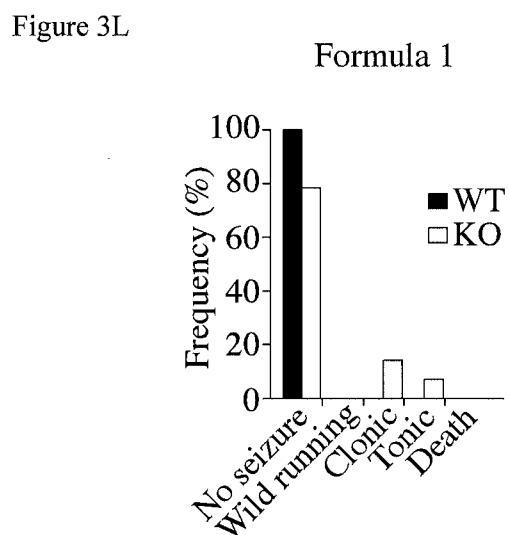
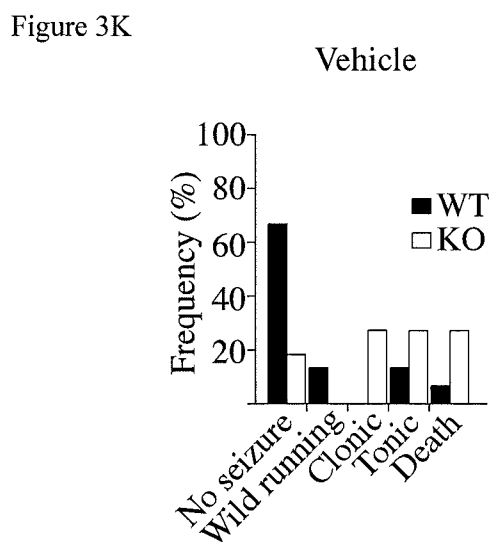
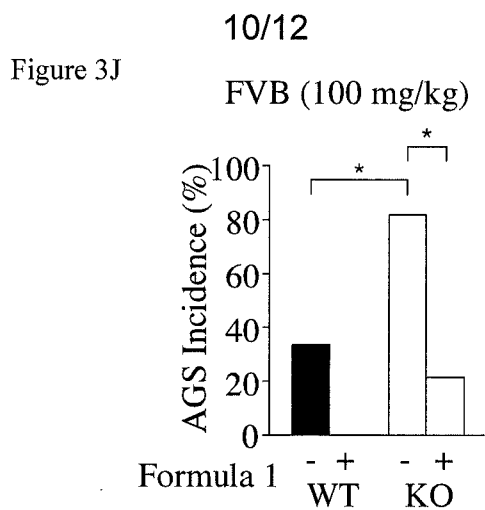


Figure 3I



FIGURES 3G-3I



FIGURES 3J-3L

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Figure 4A

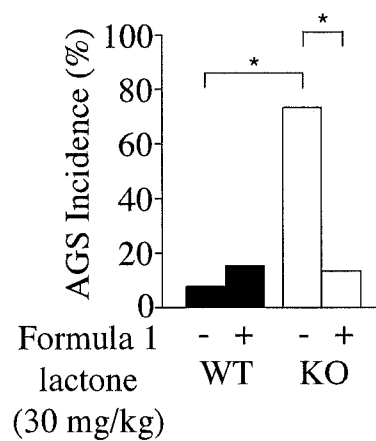


Figure 4B

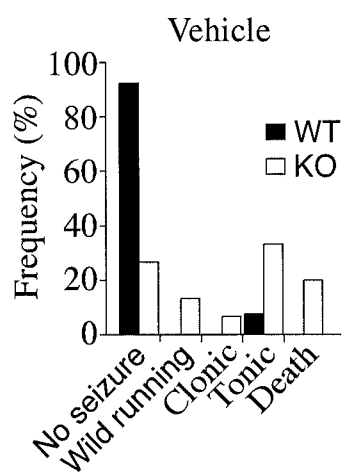
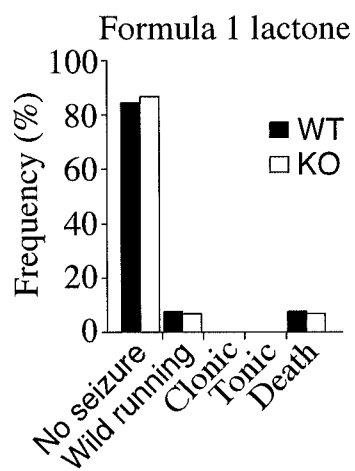


Figure 4C



FIGURES 4A-4C

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Figure 4D

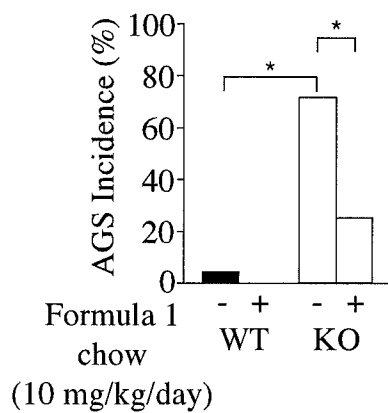


Figure 4E

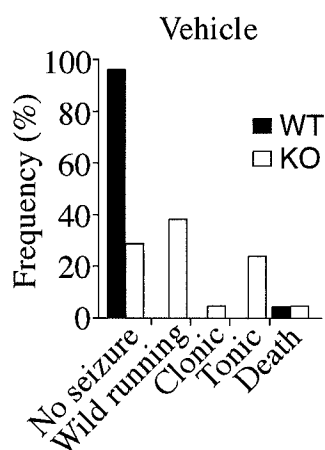
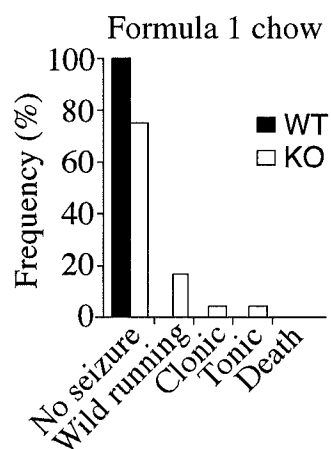


Figure 4F



FIGURES 4D-4F

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/057099

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/366 A61P25/08 A61P25/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/147275 A1 (NEURON BIOPHARMA S A [ES]; BURGOS MUNOZ JAVIER SANTOS [ES]; RAMIREZ MO) 10 December 2009 (2009-12-10) page 2, line 32 - page 3, line 9 examples 1,10 claims	1-17
X	WO 2005/120496 A2 (UNIV CALIFORNIA [US]; SILVA ALCINO J [US]) 22 December 2005 (2005-12-22) pages 3 and 4, paragraphs [0009] and [0010] pages 14, paragraph [0039] pages 26 and 27, paragraphs [0063] and [0064] figure 3 and 4 claims 1-3, 4, 8-12, 22 ----- -/--	1-17

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2011/057099

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LI ET AL: "The HMG-CoA Reductase Inhibitor Lovastatin Reverses the Learning and Attention Deficits in a Mouse Model of Neurofibromatosis Type 1", CURRENT BIOLOGY, CURRENT SCIENCE, GB, vol. 15, no. 21, 8 November 2005 (2005-11-08), pages 1961-1967, XP005175775, ISSN: 0960-9822, DOI: 10.1016/J.CUB.2005.09.043 cited in the application abstract figures 2, 3 page 1965, column 1, paragraph 2	1-4, 8-12, 14-17
X	BENNETT E ET AL: "Neurofibromatosis type 1: Its association with the Ras/MAPK pathway syndromes", JOURNAL OF PEDIATRIC NEUROLOGY,, vol. 7, no. 2, 1 January 2009 (2009-01-01) , pages 105-115, XP009155612, ISSN: 1875-9041, DOI: 10.3233/JPN-2009-0276 page 113, column 1, paragraph 2 - page 114, column 1, paragraph 1	1-4, 8-12, 14-17
X	JOSHI V A ET AL: "Towards a Treatment for RAS-MAPK Pathway Disorders", MONOGRAPHS IN HUMAN GENETICS,, vol. 17, 1 January 2009 (2009-01-01), pages 151-164, XP009155557, ISSN: 0077-0876 abstract page 156; table 1	1,2,8,9, 15,16
A	YAN ET AL: "Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP", NEUROPHARMACOLOGY, PERGAMON PRESS, OXFORD, GB, vol. 49, no. 7, 1 December 2005 (2005-12-01), pages 1053-1066, XP005125740, ISSN: 0028-3908, DOI: 10.1016/J.NEUROPHARM.2005.06.004 cited in the application abstract	1-17

INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BEAR M F ET AL: "The mGluR theory of fragile X mental retardation", TRENDS IN NEUROSCIENCE, ELSEVIER, AMSTERDAM, NL, vol. 27, no. 7, 1 July 2004 (2004-07-01), pages 370-377, XP004516797, ISSN: 0166-2236, DOI: 10.1016/J.TINS.2004.04.009 cited in the application pages 373 and 374, "The mGluR theory of fragile X mental retardation" -----	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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		ES 2330184 A1	04-12-2009
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