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(54) Title: PIFITHRIN ANALOGUES AND METHODS OF TREATING RETT SYNDROME

(57) Abstract: Disclosed herein are Pifithrin analogues and methods of treating Rett Syndrome with the Pifithrin analogues.

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PIFITHRIN ANALOGUES AND METHODS OF TREATING RETT SYNDROME

[0001] CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Patent Application No. 63/520,704, filed August 21, 2023, which is herein incorporated by reference in its entirety.

[0003] BACKGROUND OF THE INVENTION

[0004] 1. FIELD OF THE INVENTION

[0005] The field of the invention general relates to Pifithrin analogues and X-linked neurodevelopmental disorders such as Rett Syndrome and Down Syndrome.

[0006] 2. DESCRIPTION OF THE RELATED ART

[0007] Rett Syndrome is an X-linked neurodevelopmental disorder in which affected females exhibit motor delays, cognitive and neuropsychiatric disturbances, autism, and epilepsy. Rett Syndrome is typically caused by a mutation in the *MECP*2 gene on the X chromosome, and affected females exhibit symptoms as early as seven months of age. There is no known cure for Rett Syndrome. Treatments are directed at treating the symptoms, *e.g.*, anticonvulsants to reduce seizures.

[0008] SUMMARY OF THE INVENTION

[0009] In some embodiments, the present invention is directed to a Pifithrin analogue selected from the group consisting of: 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026); 3-(2-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL017); 3-(3-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL018); 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(*3H*)-imine hydrobromide (MXL019); 3-(4-Nitrobenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL020); 2-(2-Imino-4,5,6,7-tetrahydrobenzo[d]thiazol-3(2H)-yl)-1-(4-(pyrrolidin-1-yl)phenyl)ethan-1-one hydrogen bromide (MXL021); 3-(3-Methylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL022); 3-(3-Fluorobenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL023); 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL024); 3-(2-Methylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL025); 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-3(2H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL028); Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL029); 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole

(MXL030); Methyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL031); Methyl 2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL032); 2-Amino-N-benzyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL033); 2-Amino-N-(2-methoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL034); 2-Amino-N-(4-methoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL035); (2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)(2-aminophenyl)methanone (MXL036); 2-Amino-N-(pyridin-2-ylmethyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL037); 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL038); 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL040); 3-(4-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL041); 3-(3-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL042); 3-(3,5-Bis(trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL043); 3-(3,4-Dimethylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL044); 3-(3,4-Dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL045); 3-(3,5-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL046); 2-Amino-N-cyclopropyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL047); 2-Amino-N-(furan-2-ylmethyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL048); 3-(4-Methoxybenzyl)-3,4,5,6,7,8-hexahydro-2H-cyclohepta[d]thiazol-2-imine hydrogen bromide (MXL049); 3-(4-Methoxybenzyl)-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-2(3H)-imine hydrogen bromide (MXL050); 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-one hydrogen bromide (MXL051); 1-(3,5-Bis(trifluoromethyl)phenyl)-2-(2-imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)ethan-1-one hydrogen bromide (MXL052); 1-(3-Chlorophenyl)-2-(2-imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)ethan-1-one hydrogen bromide (MXL053); 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(4-(pyrrolidin-1-yl)phenyl)ethan-1-one hydrogen bromide (MXL054); and 2-Amino-N-(cyclopropylmethyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL055); and pharmaceutically acceptable salts, solvates, and prodrugs thereof. In some embodiments, the Pifithrin analogue is 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026); 3-(2-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide

(MXL017); 3-(3-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL018); 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(*3H*)-imine hydrobromide (MXL019); 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL024); 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-3(2H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL028); 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030); (2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)(2-aminophenyl)methanone (MXL036); 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL038); 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL040); 3-(4-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL041); 3-(3-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL042); 3-(3,5-Bis(trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL043); 3-(3,4-Dimethylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL044); 3-(3,4-Dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL045); 3-(3,5-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL046); 3-(4-Methoxybenzyl)-3,4,5,6,7,8-hexahydro-2H-cyclohepta[d]thiazol-2-imine hydrogen bromide (MXL049); 3-(4-Methoxybenzyl)-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-2(3H)-imine hydrogen bromide (MXL050); 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-one hydrogen bromide (MXL051); 1-(3,5-Bis(trifluoromethyl)phenyl)-2-(2-imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)ethan-1-one hydrogen bromide (MXL052); 1-(3-Chlorophenyl)-2-(2-imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)ethan-1-one hydrogen bromide (MXL053); or 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(4-(pyrrolidin-1-yl)phenyl)ethan-1-one hydrogen bromide (MXL054).

[0010] In some embodiments, the present invention is directed to a composition comprising one or more Pifithrin analogues as described above, and a pharmaceutically acceptable carrier.

[0011] In some embodiments, the present invention is directed to a method of treating a subject afflicted with Rett Syndrome, which comprises administering to the subject at least one Pifithrin analogue as described above or a composition thereof. In some embodiments, the present invention is directed to a method of treating one or more symptoms of Rett Syndrome in a subject afflicted with Rett Syndrome, which comprises

administering to the subject at least one Pifithrin analogue as described above or a composition thereof, wherein the one or more symptoms are selected from reduced mobility, dystonia, limb clasping, tremors, poor grooming, ataxia, learning delays, and abnormal anxiety/social behaviors.

[0012] In some embodiments, the present invention is directed to a method of treating a subject afflicted with Down Syndrome, which comprises administering to the subject at least one Pifithrin analogue as described above or a composition thereof.

[0013] In some embodiments, the present invention is directed to a method of treating neuronal dysfunction and/or neuronal senescence in a subject, which comprises administering to the subject at least one Pifithrin analogue as described above or a composition thereof.

[0014] In the treatment methods above, the at least one Pifithrin analogue are preferably: 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026); 3-(3-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL018); 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(*3H*)-imine hydrobromide (MXL019); 3-(3-Methylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL022); 3-(3-Fluorobenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL023); 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL024); 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-3(2H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL028); Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL029); 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030); Methyl 2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL032); 2-Amino-N-benzyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL033); 2-Amino-N-(2-methoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL034); 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL038); or 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL040).

[0015] In some embodiments, the present invention provides a method of administering a Pifithrin analogue to the brain of a subject, which comprises administering to the subject a compound selected from the group consisting of 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026); 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030); Methyl

2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL032); and pharmaceutically acceptable salts, solvates, and prodrugs thereof, or a composition thereof.

[0016] In some embodiments, the present invention is directed to a Pifithrin analogue for use as a medicament, wherein the Pifithrin analogue is: 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026); 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030); Methyl 2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL032); 3-(2-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL017); 3-(3-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL018); 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrobromide (MXL019); 3-(4-Nitrobenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL020); 2-(2-Imino-4,5,6,7-tetrahydrobenzo[d]thiazol-3(2H)-yl)-1-(4-(pyrrolidin-1-yl)phenyl)ethan-1-one hydrogen bromide (MXL021); 3-(3-Methylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL022); 3-(3-Fluorobenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL023); 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL024); 3-(2-Methylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL025); 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-3(2H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL028); Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL029); Methyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL031); 2-Amino-N-benzyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL033); 2-Amino-N-(2-methoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL034); 2-Amino-N-(4-methoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL035); (2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)(2-aminophenyl)methanone (MXL036); 2-Amino-N-(pyridin-2-ylmethyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL037); 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL038); 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL040); 3-(4-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL041); or 3-(3-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL042).

[0017] In some embodiments, the present invention is directed to a Pifithrin analogue for use as a medicament, wherein the Pifithrin analogue is: 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026); 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030); 3-(2-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL017); 3-(3-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL018); 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrobromide (MXL019); 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL024); 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-3(2H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL028); (2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)(2-aminophenyl)methanone (MXL036); 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL038); 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL040); 3-(4-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL041); 3-(3-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL042); 3-(3,5-Bis(trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL043); 3-(3,4-Dimethylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL044); 3-(3,4-Dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL045); 3-(3,5-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL046); 3-(4-Methoxybenzyl)-3,4,5,6,7,8-hexahydro-2H-cyclohepta[d]thiazol-2-imine hydrogen bromide (MXL049); 3-(4-Methoxybenzyl)-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-2(3H)-imine hydrogen bromide (MXL050); 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-one hydrogen bromide (MXL051); 1-(3,5-Bis(trifluoromethyl)phenyl)-2-(2-imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)ethan-1-one hydrogen bromide (MXL052); 1-(3-Chlorophenyl)-2-(2-imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)ethan-1-one hydrogen bromide (MXL053); or 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(4-(pyrrolidin-1-yl)phenyl)ethan-1-one hydrogen bromide (MXL054).

[0018] In some embodiments, the present invention is directed to a Pifithrin analogue for use as a medicament, wherein the Pifithrin analogue is: 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026); 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030); 3-(3-

Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL018); 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrobromide (MXL019); 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL024); 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-3(2H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL028); 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL038); or 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL040).

[0019] Both the foregoing general description and the following detailed description are exemplary and explanatory only and are intended to provide further explanation of the invention as claimed. The accompanying drawings are included to provide a further understanding of the invention and are incorporated in and constitute part of this specification, illustrate several embodiments of the invention, and together with the description explain the principles of the invention.

[0020] DESCRIPTION OF THE DRAWINGS

[0021] This invention is further understood by reference to the drawings wherein:

[0022] Figure 1, Figure 2, and Figure 3: Pifithrin α and Pifithrin analogues to block senescence in neurons. Cells were treated with UV irradiation to induce DNA damage and a senescence response. Cells were also pretreated with Pifithrin α (1) or a Pifithrin analogue (number denotes last digits of the given MXL compound, MXL007 was previously described in WO 2020247336). Compounds that block senescence in response to DNA damage show decreased expression of the indicated P53 targets relative to the treated control (below line). Wildtype IPSCs were treated with either the indicated Pifithrin analogue (10 μM) or equivalent amount of the vehicle (DMSO). 24 hours later cells were washed with PBS. Next, cells in PBS were treated with UV light at 100 mJ for 60 seconds to induce DNA damage and a p53 response. Fresh media with either indicated drug (10 μM) or equivalent amount of the vehicle (DMSO) was then applied to the cells. 4 hours post UV exposure cells were harvested and RNA was isolated. 500 ng of RNA were used to generate cDNA, followed by RT-PCR for the relative expression of the indicated genes, *i.e.*, DDIT4 (Figure 1), GADD45 (Figure 2), and DDB2 (Figure 3). Relative to untreated cells, one can see that these p53 target genes were induced by UV light. In addition, treatment with certain Pifithrin analogues show p53 target genes below the red bar indicating an effect on the p53 response.

[0023] Figure 4: BBB assay for MXL compounds. After dosing animals with the indicated compound, the animals were flushed with saline to remove latent compound. Then blood, brain, and liver were harvested and processed for mass spectrometry. After running standards (drugs alone), mass spectrometry was run to detect distribution throughout the body. Shown is the detection of MXL compounds in the brain, showing that MXL026, MXL030, and MXL032 were all abundant. These experiments (conducted in triplicate) show that animals can be dosed with Pifithrin analogues and that MXL026, MXL030, and MXL032 are stable, bio-available, and reach the brain. Note that molecules MXL028, MXL029, and MXL031 were not detected and are thereby presumed to not cross the blood brain barrier.

[0024] Figure 5 and Figure 6: MXL026 improves Rett symptoms in transgenic mice. Treatment of animals with loss of function for MECP2 with Pifithrin analogues. MXL026 showed the best *in vitro* activity and blood brain barrier permeability. Therefore, MXL026 was used to treat transgenic mice with loss of function alleles for MECP2. These male mice show phenotypes consistent with those seen in Rett patients and die of these symptoms within 10-16 weeks of age. To ensure consistent delivery of MXL026, mouse chow was formulated with MXL026 so that as animals feed themselves, they also dose themselves with the compound. Control chow (for control subjects) was prepared the same except it lacked any MXL compound. Experiments were performed with both control and transgenic mice starting at 6 weeks of age. Treatment of control mice was included to determine whether the Pifithrin analogue has any overt toxicity in normal animals and to ensure that any effect of the compound is specific to the mutation. The subjective health total scores are graphically represented in Figure 5. Figure 6 provides the details of the 3-way ANOVA (REML model). Shown are the results from a collection of distinct phenotypes (muscle tone, activity, weight, seizure, etc.) comprising an index of phenotype called the Subjective Health Index. The higher the score, the greater the progression of disease symptoms. Wildtype animals show a baseline score with minimal impairment (low score), and that this is not influenced by chow with compound. On the other hand, transgenic animals fed the chow with MXL026 show diminished effect of loss of MECP2 activity.

[0025] Figure 7: Down Syndrome neuronal senescence can be ameliorated with Pifithrin analogues. Neurons derived from hiPSCs of a subject having Down Syndrome were treated with DMSO, Pifithrin-α (Pifithrin), or Pifithrin analogues (MXL026 or MXL030). All three compounds appeared to decrease the percentage of senescent neurons.

[0026] DETAILED DESCRIPTION OF THE INVENTION

[0027] Rett Syndrome is associated with loss of function mutations in the Methyl-CpG Binding Protein 2 (*MECP2*) gene, which results in abnormal neural activity. To study the effect Pifithrin analogues have on abnormal neural activity associated with Rett Syndrome, brain organoids as described in WO 2020247336 were used. Brain organoids are derived from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) (ESCs and iPSCs are collectively referred to as "PSCs", and human cells are referred to with a preceding "h", *e.g.*, hESCs, hiPSCs, and hPSCs) that self-organize into three-dimensional structures ("organoids") with broad cellular diversity that mimics the layered organization of human brain.

[0028] MXL018, MXL019, MXL022, MXL023, MXL024, MXL026, MXL028, MXL029, MXL030, MXL032, MXL033, MXL034, and MXL038 were selected and tested as being representative of the Pifithrin analogues disclosed herein. As shown in Figure 1, Figure 2, and Figure 3, the Pifithrin analogues were found to inhibit senescence and restore brainwaves and dendritic branching in organoids. Therefore, in some embodiments, the Pifithrin analogues disclosed herein may be used to treat Rett Syndrome in subjects. In some embodiments, Pifithrin analogues selected from MXL018, MXL019, MXL022, MXL023, MXL024, MXL026, MXL028, MXL029, MXL030, MXL032, MXL033, MXL034, and MXL038 are used to treat Rett Syndrome in subjects.

[0029] Pifithrin analogues MXL026, MXL030, and MXL032 were assayed in brain tissue of subjects to determine whether they cross the blood brain barrier (BBB). The results from subjects who were administered the Pifithrin analogues indicate that the Pifithrin analogues not only cross the BBB, but also remain bioavailable (Figure 4). Of the tested Pifithrin analogues, MXL026 showed the best combination of activity on senescence and p53 targets as well as the least toxicity through extensive cell culture. As such, MXL026 was selected for further *in vivo* experiments. Mice lacking expression of MECP2 were generated by molecular genetics. Male mice have only one X-chromosome and therefore are affected by loss of MECP2 in the hemizygous state. These mice do not survive beyond 20 weeks of age and show progressive induction of Rett-like phenotypes. These phenotypes can be quantified as the subjective health index (Figure 5, Figure 6). MXL026 was administered via mouse chow formulated with the MXL026 to ensure subjects were consistently administered MXL026 over the course of the experiment. Wildtype vs transgenic mice were compared to confirm the effect of the

loss of MECP2 in male mice, and indeed, these mice showed many hallmarks of Rett Syndrome within 2−4 weeks of age, which progressed significantly over time (Figure 5).

[0030] *Treatment of Rett Syndrome*

[0031] Mice that are models of Rett Syndrome and exhibit symptoms typical of those afflicted with Rett Syndrome were treated with MXL026 and exhibited amelioration of the symptoms as measured by a subjective index. The subjective index is an amalgamation of 8 phenotypes/symptoms (reduced mobility, dystonia, limb clasping, tremors, poor grooming, ataxia, learning delays, abnormal anxiety/social behaviors) that develop in mice lacking expression of a functional copy of MECP2 protein. The fact that the subjective index improved in mice fed chow formulated with MXL026 indicates that subjects suffering from MECP2 protein abnormalities (*e.g.*, mutations and/or abnormal amounts) may be treated with one or more Pifithrin analogues described herein, such as MXL026. Therefore, in some embodiments, one or more Pifithrin analogues may be used to treat subjects afflicted with Rett Syndrome. In some embodiments, one or more Pifithrin analogues may be used to treat or reduce the symptoms, *e.g.*, reduced mobility, ataxia, dystonia, tremors, seizures, learning deficiencies, and behavioral issues, associated with Rett Syndrome in subjects suffering from Rett Syndrome.

[0032] *Senescence Assay on Down Syndrome Neurons*

[0033] Cells with an extra copy of chromosome 21 are prone to cellular senescence. As described herein, neurons were created from hiPSCs derived from subjects afflicted with Down Syndrome ("Down Syndrome neurons"). Down Syndrome neurons exhibit elevated cellular senescence compared to control neurons (neurons created from hiPSCs derived from healthy, normal subjects). As shown in Figure 7, when treated with Pifithrin analogues, the levels of senescence in the Down Syndrome neurons were reduced to that observed for the control neurons. These results indicate that Pifithrin analogues can effectively reverse dysfunction in Down Syndrome neurons and may therefore improve neuronal function in subjects afflicted with Down Syndrome. Therefore, in some embodiments, one or more Pifithrin analogues may be used to treat neuronal dysfunction in subjects. In some embodiments, one or more Pifithrin analogues may be used to improve neuronal function in subjects who have Down Syndrome.

[0034] *Compositions*

[0035] Compositions, including pharmaceutical compositions, comprising one or more Pifithrin analogues are contemplated herein. The term "pharmaceutical composition" refers to a composition suitable for pharmaceutical use in a subject. A composition generally comprises an effective amount of an active agent and a diluent and/or carrier. A pharmaceutical composition generally comprises a therapeutically effective amount of an active agent and a pharmaceutically acceptable carrier.

[0036] As used herein, an "effective amount" refers to a dosage or amount sufficient to produce a desired result. The desired result may comprise an objective or subjective change as compared to a control in, for example, *in vitro* assays, and other laboratory experiments. As used herein, a "therapeutically effective amount" refers to an amount that may be used to treat, prevent, or inhibit a given disease or condition in a subject as compared to a control, such as a placebo. Again, the skilled artisan will appreciate that certain factors may influence the amount required to effectively treat a subject, including the degree of the condition or symptom to be treated, previous treatments, the general health and age of the subject, and the like. Nevertheless, effective amounts and therapeutically effective amounts may be readily determined by methods in the art.

[0037] The one or more Pifithrin analogues may be administered, preferably in the form of pharmaceutical compositions, to a subject. Preferably the subject is mammalian, more preferably, the subject is human. Preferred pharmaceutical compositions are those comprising at least one Pifithrin analogue in a therapeutically effective amount and a pharmaceutically acceptable vehicle. In some embodiments, a therapeutically effective amount of a Pifithrin analogue ranges from about 0.01 to about 10 mg/kg body weight, about 0.01 to about 3 mg/kg body weight, about 0.01 to about 2 mg/kg, about 0.01 to about 1 mg/kg, or about 0.01 to about 0.5 mg/kg body weight for parenteral formulations. Therapeutically effective amounts for oral administration may be up to about 10-fold higher. It should be noted that treatment of a subject with a therapeutically effective amount may be administered as a single dose or as a series of several doses. The dosages used for treatment may increase or decrease over the course of a given treatment. Optimal dosages for a given set of conditions may be ascertained by those skilled in the art using dosage-determination tests and/or diagnostic assays in the art. Dosage-determination tests and/or diagnostic assays may be used to monitor and adjust dosages during the course of treatment.

[0038] Pharmaceutical compositions may be formulated for the intended route of delivery, including intravenous, intramuscular, intra peritoneal, subcutaneous,

intraocular, intrathecal, intraarticular, intrasynovial, cisternal, intrahepatic, intralesional injection, intracranial injection, infusion, and/or inhaled routes of administration using methods known in the art. Pharmaceutical compositions may include one or more of the following: pH buffered solutions, adjuvants (*e.g.*, preservatives, wetting agents, emulsifying agents, and dispersing agents), liposomal formulations, nanoparticles, dispersions, suspensions, or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions. The compositions and formulations may be optimized for increased stability and efficacy using methods in the art. *See*, *e.g.*, Carra *et al.*, (2007) Vaccine 25:4149-4158.

[0039] The compositions may be administered to a subject by any suitable route including oral, transdermal, subcutaneous, intranasal, inhalation, intramuscular, and intravascular administration. It will be appreciated that the preferred route of administration and pharmaceutical formulation will vary with the condition and age of the subject, the nature of the condition to be treated, the therapeutic effect desired, and the particular Pifithrin analogue used.

[0040] As used herein, a "pharmaceutically acceptable vehicle" or "pharmaceutically acceptable carrier" are used interchangeably and refer to solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration and comply with the applicable standards and regulations, *e.g.*, the pharmacopeial standards set forth in the United States Pharmacopeia and the National Formulary (USP-NF) book, for pharmaceutical administration. Thus, for example, unsterile water is excluded as a pharmaceutically acceptable carrier for, at least, intravenous administration. Pharmaceutically acceptable vehicles include those known in the art. See, *e.g.*, Remington: The Science and Practice of Pharmacy 20th ed (2000) Lippincott Williams & Wilkins, Baltimore, MD.

[0041] A "pharmaceutically acceptable solvate" refers to a solvate form of a specified compound that retains the biological effectiveness of such compound. Examples of solvates include compounds of the invention in combination with water, isopropanol, ethanol, methanol, dimethyl sulfoxide, ethyl acetate, acetic acid, ethanolamine, or acetone. Those skilled in the art of organic chemistry will appreciate that many organic compounds can form complexes with solvents in which they are reacted or from which they are precipitated or crystallized. These complexes are known as "solvates". For example, a complex with water is known as a "hydrate". Solvates of compounds of formulas I and II are within the scope of the invention. It will also be appreciated by

those skilled in organic chemistry that many organic compounds can exist in more than one crystalline form. For example, crystalline form may vary from solvate to solvate. Thus, all crystalline forms of the compounds of formulas I and II or the pharmaceutically acceptable solvates thereof are contemplated herein.

[0042] The term "pharmaceutically acceptable salts" refers to salt forms that are pharmacologically acceptable and substantially non-toxic to the subject being treated with the compound of the invention. Pharmaceutically acceptable salts include conventional acid-addition salts or base-addition salts formed from suitable non-toxic organic or inorganic acids or inorganic bases. Exemplary acid-addition salts include those derived from inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, sulfamic acid, phosphoric acid, and nitric acid, and those derived from organic acids such as p-toluenesulfonic acid, methanesulfonic acid, ethane-disulfonic acid, isethionic acid, oxalic acid, p-bromophenylsulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, 2-acetoxybenzoic acid, acetic acid, phenylacetic acid, propionic acid, glycolic acid, stearic acid, lactic acid, malic acid, tartaric acid, ascorbic acid, maleic acid, hydroxymaleic acid, glutamic acid, salicylic acid, sulfanilic acid, and fumaric acid. Exemplary base-addition salts include those derived from ammonium hydroxides (e.g., a quaternary ammonium hydroxide such as tetramethylammonium hydroxide), those derived from inorganic bases such as alkali or alkaline earth-metal (e.g., sodium, potassium, lithium, calcium, or magnesium) hydroxides, and those derived from non-toxic organic bases such as basic amino acids.

[0043] "A pharmaceutically acceptable prodrug" is a compound that may be converted under physiological conditions or by solvolysis to the specified compound or to a pharmaceutically acceptable salt of such compound. "A pharmaceutically active metabolite" refers to a pharmacologically active product produced through metabolism in the body of a specified compound or salt thereof. Prodrugs and active metabolites of a compound may be identified using routine techniques known in the art. See, e.g., Bertolini, G. et al., (1997) J. Med. Chem. 40:2011-2016; Shan, D. et al., J. Pharm. Sci., 86(7):765-767; Bagshawe K., (1995) Drug Dev. Res. 34:220-230; Bodor, N., (1984) Advances in Drug Res. 13:224-331; Bundgaard, H., Design of Prodrugs (Elsevier Press, 1985) and Larsen, I. K., Design and Application of Prodrugs, Drug Design and Development (Krogsgaard-Larsen et al., eds., Harwood Academic Publishers, 1991).

[0044] The pharmaceutical compositions may be provided in dosage unit forms. As used herein, a "dosage unit form" refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of the

one or more Pifithrin analogue calculated to produce the desired therapeutic effect in association with the required pharmaceutically acceptable carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the given Pifithrin analogue and desired therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[0045] Toxicity and therapeutic efficacy of Pifithrin analogues according to the instant invention and compositions thereof can be determined using cell cultures and/or experimental animals and pharmaceutical procedures in the art. For example, one may determine the lethal dose, LC_{50} (the dose expressed as concentration x exposure time that is lethal to 50% of the population) or the LD_{50} (the dose lethal to 50% of the population), and the ED_{50} (the dose therapeutically effective in 50% of the population) by methods in the art. The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Pifithrin analogues which exhibit large therapeutic indices are preferred. While Pifithrin analogues that result in toxic side-effects may be used, care should be taken to design a delivery system that targets such compounds to the site of treatment to minimize potential damage to uninfected cells and, thereby, reduce side-effects.

[0046] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosages for use in humans. Preferred dosages provide a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary depending upon the dosage form employed and the route of administration utilized. Therapeutically effective amounts and dosages of one or more Pifithrin analogues can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography. Additionally, a dosage suitable for a given subject can be determined by an attending physician or qualified medical practitioner, based on various clinical factors.

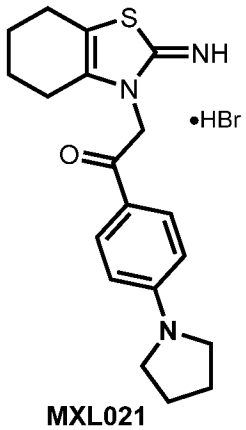
[0047] The following examples are intended to illustrate but not to limit the invention.

[0048] EXAMPLES

[0049] *Pifithrin-α and Pifithrin Analogues*

[0050] Pifithrin-α is an effective P53 inhibitor, but has a short half-life (degrades to Pifithrin β), and does not cross the blood-brain-barrier (BBB). Pifithrin β is more stable, but not predicted to cross BBB. Therefore, a variety of Pifithrin analogues were designed and tested. Pifithrin-α-Ac (MXL003) was designed to be a pro-drug to release Pifithrin-α once released into the brain. Pifithrin-TMS (MXL004) adds a silicon group to increase the lipophilicity to help it cross the BBB.

[0051] A. Pifithrin analogues MXL021, MXL026, MXL028, MXL051, MXL052, MXL053 and MXL54 were prepared using a similar synthetic route as that described in as described in WO 2020247336 for synthesizing 2-(2-imino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-3(2*H*)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (Pifithrin-α, MXL001).

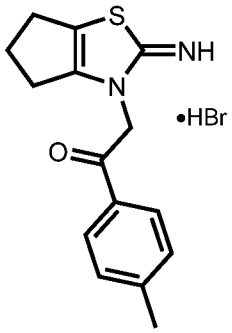


MXL021

[0052] 2-(2-Imino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-3(2*H*)-yl)-1-(4-(pyrrolidin-1-yl)phenyl)ethan-1-one hydrogen bromide (MXL021)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.42 (s, 2H), 7.83 (d, *J* = 8.9 Hz, 2H), 6.62 (d, *J* = 8.9 Hz, 2H), 5.55 (s, 2H), 3.34 (m, 4H), 2.52 (m, 2H), 2.26 (m, 2H), 1.97 (m, 4H), 1.70 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 187.5, 168.3, 152.0, 135.2, 131.1, 120.9, 114.8, 111.4, 51.5, 47.9, 25.4, 22.8, 22.6, 22.4, 21.3.

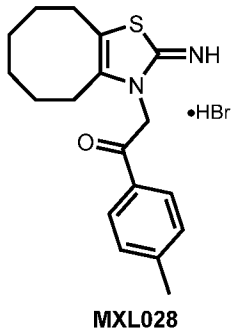


MXL026

[0053] 2-(2-Imino-5,6-dihydro-2H-cyclopenta[*d*]thiazol-3(4*H*)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.53 (s, 2H), 7.91 (d, *J* = 8.2 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 5.71 (s, 2H), 2.77 (t, *J* = 6.8 Hz, 2H), 2.58 (t, *J* = 6.9 Hz, 2H), 2.40 (s, 3H), 2.33 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 190.4, 173.6, 145.6, 144.3, 131.8, 129.9, 129.0, 118.1, 54.0, 28.2, 26.4, 25.5, 21.8.

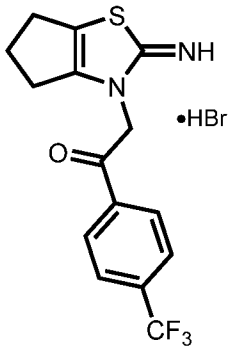


MXL028

[0054] 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[*d*]thiazol-3(2*H*)-yl)-1-(*p*-tolyl)ethan-1-one hydrogen bromide (MXL028)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.41 (s, 2H), 7.95 (d, *J* = 7.9 Hz, 2H), 7.42 (d, *J* = 7.9 Hz, 2H), 5.70 (s, 2H), 2.73 (m, 2H), 2.62 (m, 2H), 2.41 (s, 3H), 1.59 (m, 2H), 1.41 (m, 4H), 1.32 (m, 2H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 190.8, 168.3, 145.7, 136.8, 131.7, 129.9, 129.2, 117.0, 53.0, 30.7, 28.2, 25.5, 25.4, 24.7, 23.5, 21.8.

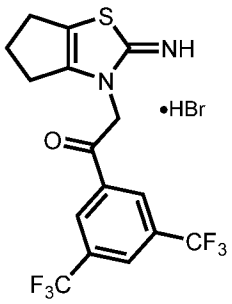


MXL051

[0055] 2-(2-Imino-5,6-dihydro-2H-cyclopenta[*d*]thiazol-3(4*H*)-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-one hydrogen bromide (MXL051)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.55 (s, 2H), 8.20 (d, J = 8.1 Hz, 2H), 8.00 (d, J = 8.1 Hz, 2H), 5.78 (s, 2H), 2.78 (t, J = 6.8 Hz, 2H), 2.62 (t, J = 7.0 Hz, 2H), 2.33 (m, 2H).

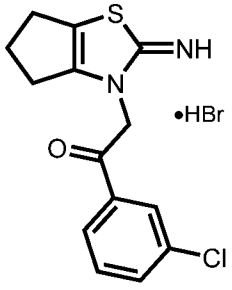
^{13}C NMR (126 MHz, DMSO-d$_6$) δ 190.6, 173.7, 144.3, 133.9 (q, J_{C-F} = 32.1 Hz), 129.8, 126.3, 125.3, 124.2 (q, J_{C-F} = 272.9 Hz),118.2, 54.5, 28.3, 26.4, 25.5.



MXL052

[0056] 1-(3,5-Bis(trifluoromethyl)phenyl)-2-(2-imino-5,6-dihydro-2*H*-cyclopenta[d]thiazol-3(4*H*)-yl)ethan-1-one hydrogen bromide (MXL052)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.53 (s, 2H), 8.56 (s, 2H), 8.55 (s, 1H), 5.87 (s, 2H), 2.79 (t, J = 6.9 Hz, 2H), 2.64 (t, J = 7.1 Hz, 2H), 2.34 (m, 2H).

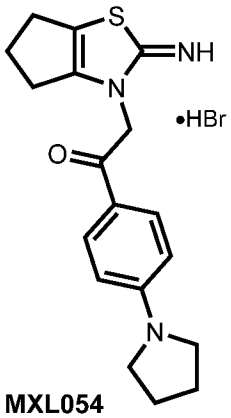


MXL053

[0057] 1-(3-Chlorophenyl)-2-(2-imino-5,6-dihydro-2*H*-cyclopenta[*d*]thiazol-3(4*H*)-yl)ethan-1-one hydrogen bromide (MXL053)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.51 (s, 2H), 8.04 (s, 1H), 7.95 (d, *J* = 7.8 Hz, 1H), 7.83 (m, 1H), 7.65 (app. t, *J* = 7.9 Hz, 1H), 5.73 (s, 2H), 2.78 (t, *J* = 6.9 Hz, 2H), 2.61 (t, *J* = 6.8 Hz, 2H), 2.33 (m, 2H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 190.1, 173.7, 144.3, 136.1, 134.5, 134.2, 131.4, 128.7, 127.5, 118.2, 54.3, 28.2, 26.4, 25.5.



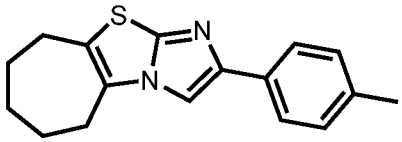
MXL054

[0058] 2-(2-Imino-5,6-dihydro-2H-cyclopenta[*d*]thiazol-3(4*H*)-yl)-1-(4-(pyrrolidin-1-yl)phenyl)ethan-1-one hydrogen bromide (MXL054)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.46 (s, 2H), 7.80 (d, *J* = 9.0 Hz, 2H), 6.62 (d, *J* = 9.0 Hz, 2H), 5.55 (s, 2H), 3.33 (m, 4H), 2.77 (t, *J* = 6.7 Hz, 2H), 2.54 (t, *J* = 6.8 Hz, 2H), 2.31 (m, 2H), 1.96 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 187.3, 173.6, 151.9, 144.5, 131.1, 121.0, 117.9, 111.4, 53.1, 47.9, 28.2, 26.3, 25.5, 25.4.

[0059] B. Pifithrin analogue MXL030 was prepared using a similar synthetic route as that described in as described in WO 2020247336 for synthesizing 2-(*p*-tolyl)-5,6,7,8-tetrahydroben-zo[*d*]imidazo[2,1-*b*]thiazole (Pifithrin β, MXL002).



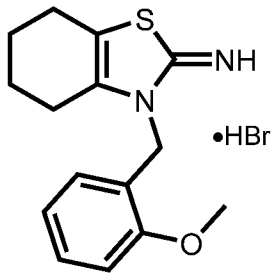
MXL030

2-(*p*-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[*d*]imidazo[2,1-*b*]thiazole (MXL030)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 7.70 (d, *J* = 8.0 Hz, 2H), 7.50 (s, 1H), 7.19 (d, *J* = 7.8 Hz, 2H), 2.83 (m, 2H), 2.73 (m, 2H), 2.36 (s, 3H), 1.86 (m, 6H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 147.5, 146.4, 136.8, 131.7, 129.3, 128.9, 124.9, 124.4, 105.6, 30.3, 28.6, 27.8, 27.7, 25.9, 21.3.

[0060] C. Pifithrin analogues MXL017, MXL018, MXL019, MXL020, MXL022, MXL023, MXL024, MXL025, MXL040, MXL041, MXL042, MXL043, MXL044, MXL045, MXL046, MXL049 and MXL050 were prepared using a similar synthetic route as that described in as described in WO 2020247336 for synthesizing 3-(4-bromobenzyl)-4,5,6,7-tetrahydro-benzo[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL005).

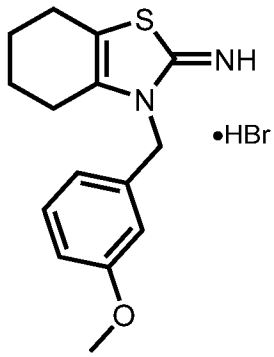


MXL017

[0061] 3-(2-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL017)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.46 (br. s, 2H), 7.34 (m, 1H), 7.08 (d, *J* = 7.7 Hz, 1H), 6.94 (m, 1H), 6.75 (dd, *J* = 7.7, 1.3 Hz, 1H), 5.12 (s, 2H), 3.82 (s, 3H), 2.50 (m, 2H), 2.27 (m, 2H), 1.66 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.8, 157.0, 135.2, 130.0, 126.6, 121.8, 121.2, 115.5, 111.7, 56.0, 44.9, 22.84, 22.78, 22.3, 21.4.

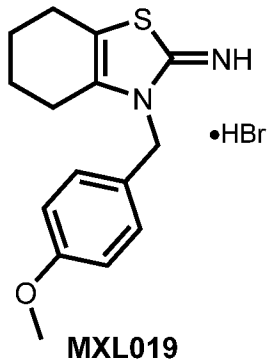


MXL018

[0062] 3-(3-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL018)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.56 (br. s, 2H), 7.31 (app. t, *J* = 8.0 Hz, 1H), 6.91 (dd, *J* = 8.0, 2.1 Hz, 1H), 6.71 (s, 1H), 6.62 (d, *J* = 7.6 Hz, 1H), 5.21 (s, 2H), 3.73 (s, 3H), 2.51 (m, 2H), 2.33 (m, 2H), 1.69 (m, 4H).

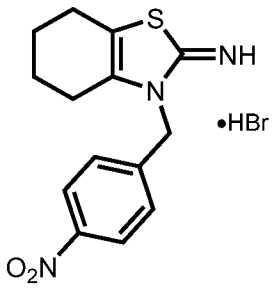
^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.9, 160.1, 136.0, 135.1, 130.8, 118.4, 115.8, 113.5, 113.0, 55.6, 48.1, 22.9, 22.8, 22.3, 21.4.



MXL019

[0063] 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(*3H*)-imine hydrobromide (MXL019)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.60 (s, 2H), 7.10 (d, J = 8.7 Hz, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 5.18 (s, 2H), 3.72 (s,3H), 2.43 (m, 1H), 2.36 (m, 2H), 1.71 (m, 1H), 1.67 (m, 4H).

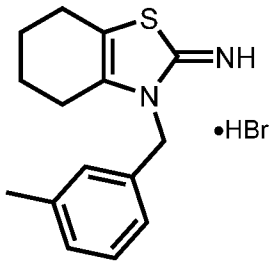


MXL020

[0064] 3-(4-Nitrobenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL020)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.65 (br. s, 2H), 8.25 (d, *J* = 8.2 Hz, 2H), 7.40 (d, *J* = 8.2 Hz, 2H), 5.41 (s, 2H), 2.52 (m, 2H), 2.30 (m, 2H), 1.69 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 168.1, 147.7, 142.0, 134.9, 128.0, 124.6, 116.0, 67.5, 47.8, 25.6, 22.9, 22.3, 21.3.

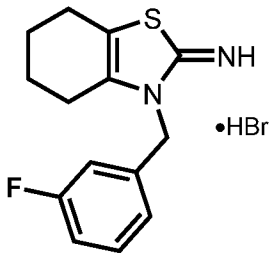


MXL022

[0065] 3-(3-Methylbenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL022)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.56 (br. s, 2H), 7.27 (app. t, J = 7.6 Hz, 1H), 7.14 (d, J = 7.5 Hz, 1H), 6.97 (s, 1H), 6.87 (d, J = 7.6 Hz, 1H), 5.21 (s, 2H), 2.51 (m, 2H), 2.33 (m, 2H), 2.28 (s, 3H), 1.68 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.8, 138.8, 135.1, 134.4, 129.5, 129.2, 127.3, 123.6, 115.7, 48.2, 23.0, 22.9, 22.3, 21.5, 21.4.

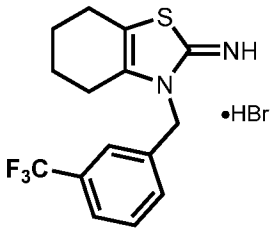


MXL023

[0066] 3-(3-Fluorobenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL023)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.60 (br. s, 2H), 7.45 (m, 1H), 7.18 (m, 1H), 7.03 (d, J = 9.8 Hz, 1H), 6.93 (d, J = 7.7 Hz, 1H), 5.27 (s, 2H), 2.51 (m, 2H), 2.33 (m, 2H),1.69 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 168.0, 162.8 (d, J = 244.8 Hz), 137.3 (d, J = 7.5 Hz), 134.9, 131.7 (d, J = 8.5 Hz), 122.6 (d, J = 2.6 Hz), 115.9, 115.5 (d, J = 20.9 Hz), 113.9 (d, J = 22.5 Hz), 47.7, 22.9, 22.8, 22.3, 21.4.

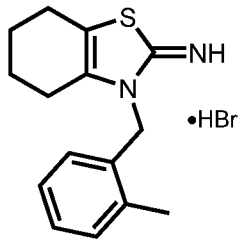


MXL024

[0067] 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL024)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.62 (s, 2H), 7.72 (d, J = 7.9 Hz, 1H), 7.64 (app. t, J = 7.8 Hz, 1H), 7.61 (s, 1H), 7.33 (d, J = 7.8 Hz, 1H), 5.36 (s, 2H), 2.52 (m, 2H), 2.33 (m, 2H), 1.69 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 168.0, 136.0, 134.9, 130.8, 130.4, 130.1 (q, $J_{C\text{-}F}$ = 31.8 Hz), 125.4, 124.5 (q, $J_{C\text{-}F}$ = 272.4 Hz), 123.9, 116.0, 47.7, 23.0, 22.9, 22.3, 21.4.

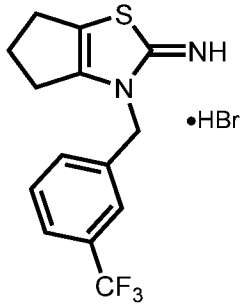


MXL025

[0068] 3-(2-Methylbenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL025)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.54 (s, 2H), 7.26 (d, *J* = 7.1 Hz, 1H), 7.22 (app. t, *J* = 7.4 Hz, 1H), 7.18 (t, *J* = 7.4 Hz, 1H), 6.43 (d, *J* = 7.4 Hz, 1H), 5.19 (s, 2H), 2.55 (m, 2H), 2.32 (s, 3H), 2.27 (m, 2H), 1.69 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.8, 135.9, 135.1, 132.3, 131.1, 128.1, 127.0, 123.2, 115.8, 46.8, 22.9, 22.6, 22.4, 21.3, 19.1.

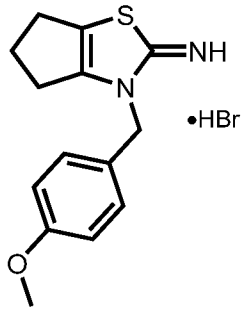


MXL040

[0069] 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2*H*-cyclopenta[*d*]thiazol-2-imine hydrogen bromide (MXL040)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.64 (s, 2H), 7.74 (d, *J* = 7.7 Hz, 1H), 7.68 (s, 1H), 7.65 (m, 1H), 7.44 (d, *J* = 7.7 Hz, 1H), 5.32 (s, 2H), 2.74 (m, 2H), 2.56 (m, 2H), 2.31 (m, 2H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 173.2, 143.8, 135.8, 131.3, 130.8, 130.1 (q, $J_{C\text{-}F}$ = 31.8 Hz), 125.6, 124.6, 124.5 (q, $J_{C\text{-}F}$ = 272.4 Hz), 119.4, 49.4, 28.1, 26.8, 25.7.

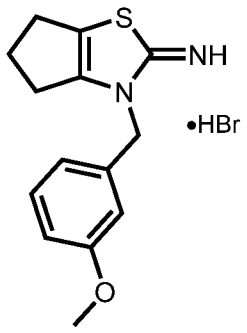


MXL041

[0070] 3-(4-Methoxybenzyl)-3,4,5,6-tetrahydro-2*H*-cyclopenta[*d*]thiazol-2-imine hydrogen bromide (MXL041)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.60 (s, 2H), 7.20 (d, *J* = 7.6 Hz, 2H), 6.95 (d, *J* = 7.6 Hz, 2H), 5.14 (s, 3H), 3.73 (s, 3H), 2.71 (m, 2H), 2.56 (m, 2H), 2.29 (m, 2H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 172.7, 159.7, 144.0, 129.4, 126.2, 119.2, 114.9, 55.7, 49.5, 28.0, 26.9, 25.7.

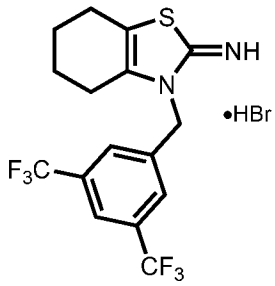


MXL042

[0071] 3-(3-Methoxybenzyl)-3,4,5,6-tetrahydro-2*H*-cyclopenta[*d*]thiazol-2-imine hydrogen bromide (MXL042)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.61 (s, 2H), 7.32 (app. t, *J* = 7.9 Hz, 1H), 6.92 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.80 (s, 1H), 6.73 (d, *J* = 7.6 Hz, 1H), 5.19 (s, 2H), 3.73 (s, 3H), 2.73 (t, *J* = 6.7 Hz, 2H), 2.56 (t, *J* = 6.8 Hz, 2H), 2.30 (m, 2H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 173.0, 160.0, 144.0, 135.8, 130.8, 119.2, 119.2, 113.8, 113.7, 55.7, 49.8, 28.0, 26.8, 25.7.

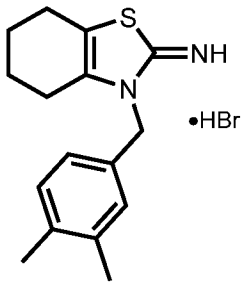


MXL043

[0072] 3-(3,5-Bis(trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL043)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.65 (s, 2H), 8.14 (s, 1H), 7.81 (s, 2H), 5.40 (s, 2H), 2.53 (m, 2H), 2.34 (m, 2H), 1.70 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 168.5, 138.0, 134.9, 130.2 (q, J_{C-F} = 33.0 Hz), 127.8, 123.6 (q, J_{C-F} = 272.9 Hz), 122.7, 116.1, 47.6, 23.0, 22.9, 22.3, 21.4.

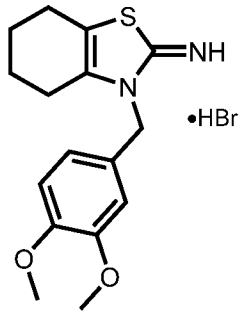


MXL044

[0073] 3-(3,4-Dimethylbenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL044)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.55 (s, 2H), 7.13 (d, *J* = 7.8 Hz, 1H), 6.94 (s, 1H), 6.80 (d, *J* = 7.8 Hz, 1H), 5.17 (s, 2H), 2.50 (m, 2H), 2.33 (m, 2H), 2.19 (s, 3H), 2.18 (s, 3H), 1.67 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.7, 137.5, 136.7, 135.2, 131.8, 130.5, 128.0, 124.0, 115.7, 48.1, 23.0, 22.9, 22.3, 21.4, 19.9, 19.5.

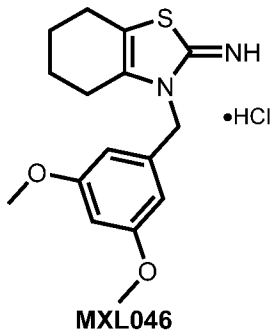


MXL045

[0074] 3-(3,4-Dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL045)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.53 (s, 2H), 6.93 (d, *J* = 8.3 Hz, 1H), 6.86 (s, 1H), 6.54 (d, *J* = 8.2 Hz, 1H), 5.14 (s, 2H), 3.73 (s, 3H), 3.71 (s, 3H), 2.51 (m, 2H), 2.37 (m, 2H), 1.69 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.7, 149.4, 149.1, 135.2, 126.6, 118.6, 115.7, 112.6, 111.3, 56.1, 48.0, 23.1, 22.9, 22.3, 21.4.

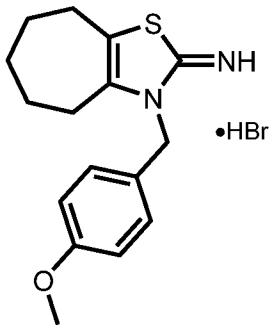


MXL046

[0075] 3-(3,5-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL046)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.55 (s, 2H), 6.47 (t, *J* = 2.1 Hz, 1H), 6.22 (d, *J* = 2.1 Hz, 2H), 5.16 (s, 2H), 3.71 (s, 6H), 2.51 (m, 2H), 2.34 (m, 2H), 1.69 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.9, 161.4, 136.7, 135.1, 115.7, 104.9, 99.4, 55.8, 48.1, 23.0, 22.9, 22.3, 21.4.

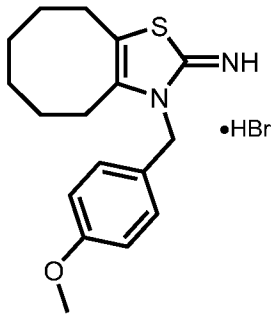


MXL049

[0076] 3-(4-Methoxybenzyl)-3,4,5,6,7,8-hexahydro-2*H*-cyclohepta[*d*]thiazol-2-imine hydrogen bromide (MXL049)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.43 (s, 2H), 7.06 (d, *J* = 8.7 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 5.21 (s, 2H), 3.72 (s, 3H), 2.63 (m, 2H), 2.54 (m, 2H), 1.69 (m, 2H), 1.61 (m, 2H), 1.47 (m, 2H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 166.8, 159.4, 138.9, 128.1. 126.4, 118.7, 114.9, 55.7, 48.4, 30.0, 26.9, 26.8, 25.8, 25.4.



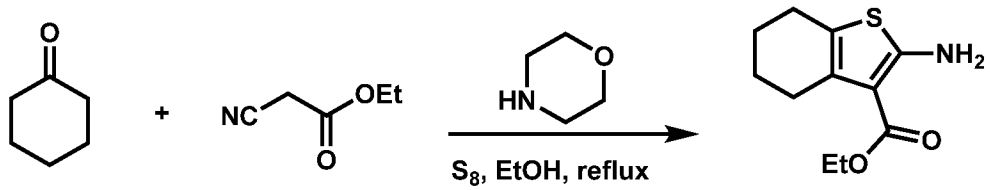
MXL050

[0077] 3-(4-Methoxybenzyl)-4,5,6,7,8,9-hexahydrocycloocta[*d*]thiazol-2(3*H*)-imine hydrogen bromide (MXL050)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.50 (s, 2H), 7.05 (d, *J* = 8.6 Hz, 2H), 6.94 (d, *J* = 8.6 Hz, 2H), 5.18 (s, 2H), 3.72 (s, 3H), 2.70 (m, 2H), 2.60 (m, 2H), 1.56 (m, 2H), 1.35 (m, 2H),1.29 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.7, 159.4, 136.8, 130.0, 126.4, 117.9, 114.9, 55.7, 48.3, 30.4, 28.4, 25.6, 25.3, 24.5, 23.9.

[0078] D. Synthesis of Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (MXL029):



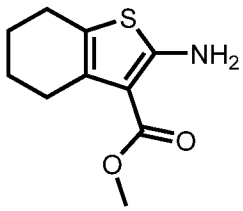
MXL029

[0079] The solution of cyclohexanone (4.91 g, 50 mmol), morpholine (4.4 g, 1 equiv.), ethyl cyanoacetate (5.65 g, 1 equiv.) and sulfur (1.92 g) in ethanol (35 mL) was heated to reflux for 3 h. After cooling down the mixture to room temperature, the precipitate was isolated by vacuum filtration, which provided the desired product, ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate, MXL029. Yield, 95%, 10.7 g.

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 7.17 (s, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 2.56 (t, *J* = 5.9 Hz, 2H), 2.39 (t, *J* = 5.8 Hz, 2H), 1.64 (m, 4H), 1.21 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 165.5, 163.3, 131.8, 115.9, 103.1, 59.1, 27.0, 24.4, 23.3, 22.9.

[0080] E. Methyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (MXL031) was prepared using a similar synthetic route as MXL029.

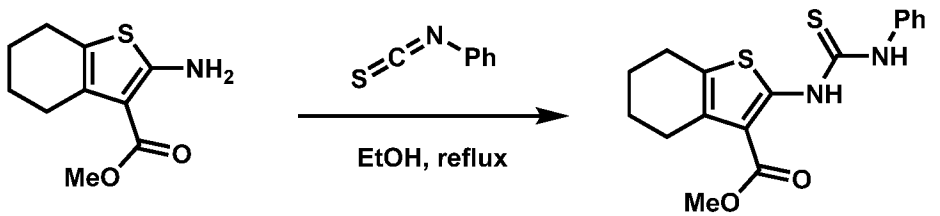


MXL031

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 3.78 (s, 3H), 2.68 (t, *J* = 5.9 Hz, 2H), 2.50 (t, *J* = 5.8 Hz, 2H), 1.75 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 166.5, 161.2, 132.5, 118.0, 106.0, 50.7, 26.9, 24.6, 23.3, 22.8.

[0081] F. Synthesis of Methyl 2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL032):



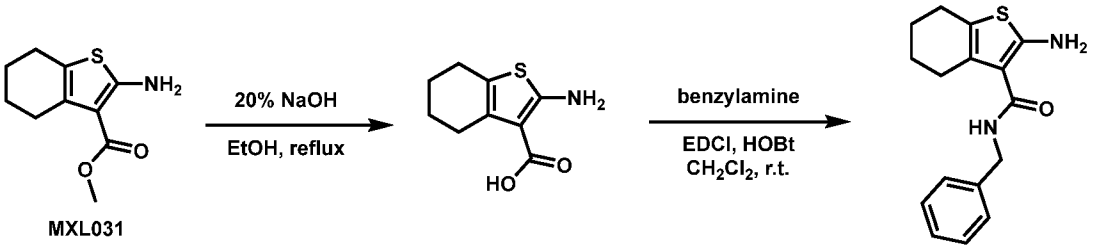
MXL031 MXL032

[0082] To the solution of MXL031 (422 mg, 2 mmol) in ethanol (3 mL) was added phenyl isothiocyanate (238 µL). The solution was heated to reflux for 24 h. After cooling down the mixture to room temperature, the precipitate was isolated by vacuum filtration, which provided the desired product, methyl 2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate, MXL032. Yield, 85%, 588 mg.

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 11.72 (s, 1H), 10.96 (s, 1H), 7.45 (d, *J* = 7.9 Hz, 2H), 7.39 (app. t, *J* = 7.7 Hz, 2H), 7.22 (t, *J* = 7.2 Hz, 1H), 3.72 (s, 3H), 2.66 (m, 2H), 2.56 (m, 2H), 1.68 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 176.0, 166.3, 149.9, 138.5, 130.4, 129.5, 126.3, 126.2, 124.9, 112.0, 52.0, 26.3, 24.0, 23.0, 22.8.

[0083] G. Synthesis of 2-Amino-N-benzyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL033):



MXL031 MXL033

[0084] To the solution of MXL031 (422 mg, 2 mmol) in ethanol (5 mL) was added 20% aq. NaOH solution (10 mL). The mixture was heated to reflux for 2 h. After cooling down the mixture to room temperature, 1 N HCl solution was used to acidify the reaction mixture to pH = 2. The resulting solution was extracted with ethyl acetate (3 × 20 mL). The organic phase was dried with anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography (6:1 hexanes : ethyl acetate), which generated the desired product, 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic acid.

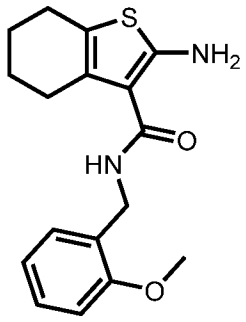
[0085] To the solution of 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic acid (50 mg, 0.2 mmol) in dichloromethane (10 mL) were added HOBt (54 mg, 3 equiv.) and EDCI (93 mg, 3 equiv.) and benzylamine (44 µL, 2 equiv.). The reaction was stirred

for 24 h and quenched by adding water (15 mL). The resulting solution was extracted with dichloromethane (3 × 20 mL). The organic phase was dried with anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography (10:1 hexanes : ethyl acetate), which generated the desired product, 2-amino-*N*-benzyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide, MXL033. Yield, 82%, 47 mg.

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.20 (s, 1H), 8.31 (s, 2H), 7.44 (m, 2H), 7.36 (m, 2H), 7.26 (m, 1H), 4.62 (s, 2H), 3.04 (t, J = 5.7 Hz, 2H), 2.72 (t, J = 5.7 Hz, 2H), 1.82 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 163.9, 156.3, 151.0, 138.7, 137.8, 134.9, 132.0, 131.8, 128.9, 128.8, 128.5, 127.4, 43.9, 29.7, 26.8, 25.8, 23.0, 22.7 (two isomers of the amide).

[0086] H. Pifithrin analogues MXL034, MXL035, MXL036, MXL037, MXL038, MXL047, MXL048 and MXL055 were prepared using a similar synthetic route as that described in as described for MXL033.

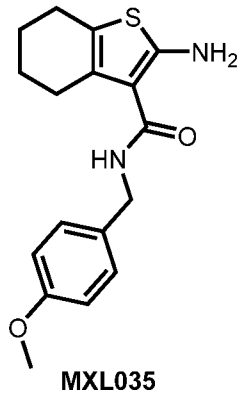


MXL034

[0087] 2-Amino-N-(2-methoxybenzyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (MXL034)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 7.30 (dd, J = 7.4, 1.6 Hz, 1H), 7.25 (m, 1H), 6.92 (m, 1H), 6.88 (d, J = 8.2 Hz, 1H), 4.55 (s, 2H), 3.86 (s, 3H), 2.59 (m, 2H), 2.51 (m, 2H), 1.78 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 166.2, 158.8, 157.5, 129.7, 128.9, 128.7, 126.8, 120.8, 118.7, 110.2, 108.9, 55.2, 39.3, 27.0, 24.6, 23.1, 22.9 (two isomers of the amide).

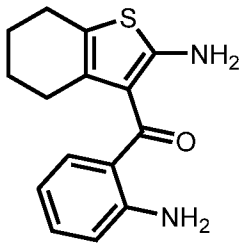


MXL035

[0088] 2-Amino-N-(4-methoxybenzyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (MXL035)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 7.24 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 4.50 (s, 2H), 3.79 (s, 3H), 2.55 (m, 2H), 2.52 (m, 2H), 1.76 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 166.4, 159.1, 158.9, 130.8, 128.9, 128.8, 118.9, 114.1, 108.6, 55.3, 42.8, 27.2, 24.6, 23.0, 22.9.

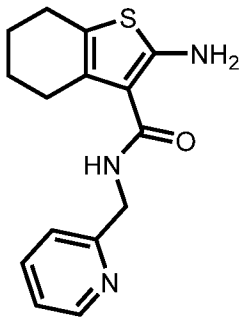


MXL036

[0089] (2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(2-aminophenyl)methanone (MXL036)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 8.05 (d, *J* = 8.5 Hz, 1H), 7.51 (m, 1H), 7.47 (d, *J* = 8.2 Hz, 1H), 7.40 (m, 1H), 6.38 (s, 2H), 2.90 (t, *J* = 4.8 Hz, 2H), 2.52 (t, *J* = 4.8 Hz, 2H), 1.81 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.6, 161.6, 143.5, 130.8, 129.2, 128.6, 124.7, 120.3, 118.9, 108.7, 99.5, 27.0, 24.6, 23.0, 22.7.

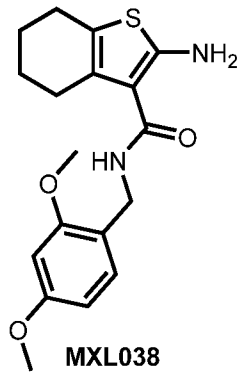


MXL037

[0090] 2-Amino-N-(pyridin-2-ylmethyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (MXL037)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 8.07 (d, *J* = 8.4 Hz, 1H), 7.53 (m, 1H), 7.48 (d, *J* = 8.3 Hz, 1H), 7.42 (m, 1H), 6.23 (s, 2H), 2.93 (t, *J* = 4.9 Hz, 2H), 2.55 (t, *J* = 4.8 Hz, 2H), 1.84 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.2, 161.7, 143.5, 130.9, 129.2, 128.5, 124.7, 120.4, 118.9, 108.6, 99.8, 27.1, 24.6, 23.1, 22.7.

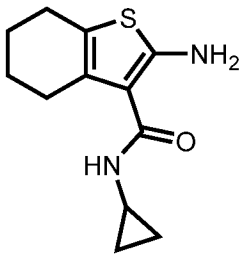


MXL038

[0091] 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (MXL038)

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 7.20 (d, *J* = 8.2 Hz, 1H), 6.46 (d, *J* = 2.4 Hz, 1H), 6.43 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.27 (s, 1H), 5.96 (s, 2H), 4.47 (s, 2H), 3.83 (s, 3H), 3.79 (s, 3H), 2.56 (m, 2H), 2.51 (m, 2H), 1.77 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d$_6$) δ 166.1, 160.4, 158.7, 158.5, 130.4, 129.0, 119.3, 118.7, 109.0, 103.9, 98.6, 55.4, 55.3, 38.8, 27.0, 24.6, 23.1, 22.9.

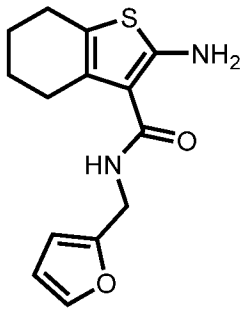


MXL047

[0092] 2-Amino-N-cyclopropyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (MXL047)

^{1}H NMR (500 MHz, CDCl$_3$) δ 6.02 (s, 2H), 5.80 (s, 1H), 2.77 (m, 1H), 2.51 (m, 4H), 1.77 (m, 4H), 0.79 (m, 2H), 0.51 (m, 2H).

^{13}C NMR (126 MHz, CDCl$_3$) δ 168.1, 159.2, 128.7, 118.8, 108.4, 27.2, 24.6, 23.0, 22.9, 22.4, 6.9.

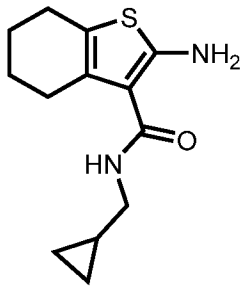


MXL048

[0093] 2-Amino-N-(furan-2-ylmethyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (MXL048)

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.36 (dd, *J* = 1.7, 0.7 Hz, 1H), 6.32 (dd, *J* = 3.1, 1.9 Hz, 1H), 6.24 (dd, *J* = 3.2, 0.6 Hz, 1H), 5.98 (s, 1H), 4.56 (s, 2H), 2.61 (m, 2H), 2.53 (m, 2H), 1.79 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 166.7, 159.3, 151.9, 142.1, 128.8, 118.9, 110.4, 108.5, 107.1, 36.2, 27.1, 24.6, 23.0, 22.9.



MXL055

[0094] 2-Amino-N-(cyclopropylmethyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (MXL055)

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.76 (m, 3H), 3.22 (m, 2H), 2.63 (m, 2H), 2.53 (m, 2H), 1.80 (m, 4H), 1.01 (m, 1H), 0.51 (m, 2H), 0.22 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5, 158.6, 128.9, 118.9, 108.9, 44.0, 27.2, 24.6, 23.0, 22.9, 10.9, 3.4.

[0095] *Blood Brain Barrier Assay*

[0096] Animals were injected with the indicated compounds resuspended in a mixture of DMSO and PBS. Each animal was injected with 50 μL of 10 mM solution. One hour after injection, the animals were sacrificed, and then perfused with PBS to remove any drug from circulation. The brain tissue was then harvested and subjected to tissue preparation for identification by mass spectrometry.

[0097] *Subjective Health Index – Treatment of Rett Syndrome*

[0098] The phenotype of *Mecp2*$^{tm1.1Bird}$/Y mice is first detected on the mixed 129/B6 genetic background at 4 weeks, when a neurological phenotype begins with a grasping or wringing of the forepaws, as well as a distinctive intermittent tremor. Subsequently, the mice become lethargic, lose muscle tone, develop severe limb clasping (now affecting the hindlimb), tremors increase in severity, and abnormal breathing develops by 6−8 weeks. Most males die by 8−10 weeks of age and normally all are dead by 12 weeks. Male mice tend to become obese on this genetic background. Home cage movement and social behaviors are abnormal. Finally, mice that live longer often develop eye inflammation or dermatitis. Animals are evaluated weekly using a standard health score that assesses activity, weight, muscle tone, limb clasping, tremors and grooming. Mice were given a score of 0 for asymptomatic similar to wild type, 1 for mildly affected or 2 for severely affected, per a subjective scoring system in the art (see, *e.g.*, Armstrong (2011)). A total health score is determined by adding the individual scores. Animals are first assessed and treated at four weeks postnatal (just after symptom onset) weekly until the age of 12 weeks. Mice with an improved phenotype showed a lower health score and lived longer. At 8 weeks of age, mice are assessed using: Rotarod – to test motor-coordination and learning skills (Stoelting Ugo Basile Mouse Rota-RodUgo Basile), and Open Field Activity – general locomotor activity and anxiety (Accuscan Instruments). At 10 weeks, animals are sacrificed by anesthesia followed by cardiac puncture to collect blood for PK studies. Whole lung, liver, and brain were necropsied and used for PK.

[0099] *Sensescence Assay on Down Syndrome Neurons*

[0100] Neurons were isolated from hiPSC lines with Trisomy or Disomy for chromosome 21 and plated onto coverslips. After two days of culture, the neurons were fixed briefly with 4% Paraformaldehyde, and then stained with X-gal reagent at pH 8, which reacts with endogenous b-galactosidase. Neurons that stain blue are considered to be senescent in this assay. The signal was acquired by Zeiss microscopy and quantified in ImageJ software.

[0101] REFERENCES

[0102] The following references are herein incorporated by reference in their entirety with the exception that, should the scope and meaning of a term conflict with a definition explicitly set forth herein, the definition explicitly set forth herein controls:

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32. WO 2020247336, which is herein incorporated by reference in its entirety.

[0103] All scientific and technical terms used in this application have meanings commonly used in the art unless otherwise specified.

[0104] Except when specifically indicated, peptides are indicated with the N-terminus on the left and the sequences are written from the N-terminus to the C-terminus. Similarly, except when specifically indicated, nucleic acid sequences are indicated with the 5' end on the left and the sequences are written from 5' to 3'.

[0105] As used herein, a "Pifithrin analogue" refers to MXL017, MXL018, MXL019, MXL020, MXL021, MXL022, MXL023, MXL024, MXL025, MXL026, MXL027, MXL028, MXL029, MXL030, MXL031, MXL032, MXL033, MXL034, MXL035, MXL036, MXL037, MXL038, MXL040, MXL041, MXL042, MXL043, MXL044,

MXL045, MXL046, MXL047, MXL048, MXL049, MXL050, MXL051, MXL052, MXL053, MXL054, and MXL055 as described herein. Preferred Pifithrin analogues are MXL017, MXL018, MXL019, MXL024, MXL026, MXL028, MXL030, MXL036, MXL038, MXL040, MXL041, MXL042, MXL043, MXL044, MXL045, MXL046, MXL049, MXL050, MXL051, MXL052, MXL053, and MXL054, and even more preferred are MXL026 and MXL030.

[0106] As used herein, the terms "subject", "patient", and "individual" are used interchangeably to refer to humans and non-human animals. The terms "non-human animal" and "animal" refer to all non-human vertebrates, *e.g.*, non-human mammals and non-mammals, such as non-human primates, horses, sheep, dogs, cows, pigs, chickens, and other veterinary subjects and test animals. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

[0107] The use of the singular can include the plural unless specifically stated otherwise. As used in the specification and the appended claims, the singular forms "a", "an", and "the" can include plural referents unless the context clearly dictates otherwise.

[0108] As used herein, "and/or" means "and" or "or". For example, "A and/or B" means "A, B, or both A and B" and "A, B, C, and/or D" means "A, B, C, D, or a combination thereof" and said "A, B, C, D, or a combination thereof" means any subset of A, B, C, and D, for example, a single member subset (*e.g.*, A or B or C or D), a two-member subset (*e.g.*, A and B; A and C; *etc.*), or a three-member subset (*e.g.*, A, B, and C; or A, B, and D; *etc.*), or all four members (*e.g.*, A, B, C, and D).

[0109] As used herein, the phrase "one or more of", *e.g.*, "one or more of A, B, and/or C" means "one or more of A", "one or more of B", "one or more of C", "one or more of A and one or more of B", "one or more of B and one or more of C", "one or more of A and one or more of C" and "one or more of A, one or more of B, and one or more of C".

[0110] As used herein, the phrase "consists essentially of" in the context of neural cells having a loss of function mutation in the Methyl-CpG Binding Protein 2 (MECP2) gene means that the neural cells may have other genetic mutations so long as the mutations do not affect the phenotype of the *MECP2*$^{-}$ mutation. In the context of a given ingredient in a composition, "consists essentially of" means that the composition may include additional ingredients so long as the additional ingredients do not adversely impact the activity, *e.g.*, biological or pharmaceutical function, of the given ingredient.

[0111] The phrase "comprises, consists essentially of, or consists of A" is used as a tool to avoid excess page and translation fees and means that in some embodiments the given thing at issue: comprises A, consists essentially of A, or consists of A. For example, the

sentence "In some embodiments, the composition comprises, consists essentially of, or consists of A" is to be interpreted as if written as the following three separate sentences: "In some embodiments, the composition comprises A. In some embodiments, the composition consists essentially of A. In some embodiments, the composition consists of A."

[0112] Similarly, a sentence reciting a string of alternates is to be interpreted as if a string of sentences were provided such that each given alternate was provided in a sentence by itself. For example, the sentence "In some embodiments, the composition comprises A, B, or C" is to be interpreted as if written as the following three separate sentences: "In some embodiments, the composition comprises A. In some embodiments, the composition comprises B. In some embodiments, the composition comprises C." As another example, the sentence "In some embodiments, the composition comprises at least A, B, or C" is to be interpreted as if written as the following three separate sentences: "In some embodiments, the composition comprises at least A. In some embodiments, the composition comprises at least B. In some embodiments, the composition comprises at least C."

[0113] To the extent necessary to understand or complete the disclosure of the present invention, all publications, patents, and patent applications mentioned herein are expressly incorporated by reference therein to the same extent as though each were individually so incorporated.

[0114] Having thus described exemplary embodiments of the present invention, it should be noted by those skilled in the art that the within disclosures are exemplary only and that various other alternatives, adaptations, and modifications may be made within the scope of the present invention. Accordingly, the present invention is not limited to the specific embodiments as illustrated herein, but is only limited by the following claims.

What is claimed is:

1. A Pifithrin analogue selected from the group consisting of:
   - 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026);
   - 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030);
   - Methyl 2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL032);
   - 3-(2-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL017);
   - 3-(3-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL018);
   - 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(*3H*)-imine hydrobromide (MXL019);
   - 3-(4-Nitrobenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL020);
   - 2-(2-Imino-4,5,6,7-tetrahydrobenzo[d]thiazol-3(2H)-yl)-1-(4-(pyrrolidin-1-yl)phenyl)ethan-1-one hydrogen bromide (MXL021);
   - 3-(3-Methylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL022);
   - 3-(3-Fluorobenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL023);
   - 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL024);
   - 3-(2-Methylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL025);
   - 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-3(2H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL028);
   - Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL029);
   - Methyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL031);
   - 2-Amino-N-benzyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL033);
   - 2-Amino-N-(2-methoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL034);
   - 2-Amino-N-(4-methoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL035);

- (2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)(2-aminophenyl)methanone (MXL036);
- 2-Amino-N-(pyridin-2-ylmethyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL037);
- 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL038);
- 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL040);
- 3-(4-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL041);
- 3-(3-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL042);
- 3-(3,5-Bis(trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL043);
- 3-(3,4-Dimethylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL044);
- 3-(3,4-Dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL045);
- 3-(3,5-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL046);
- 2-Amino-N-cyclopropyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL047);
- 2-Amino-N-(furan-2-ylmethyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL048);
- 3-(4-Methoxybenzyl)-3,4,5,6,7,8-hexahydro-2H-cyclohepta[d]thiazol-2-imine hydrogen bromide (MXL049);
- 3-(4-Methoxybenzyl)-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-2(3H)-imine hydrogen bromide (MXL050);
- 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-one hydrogen bromide (MXL051);
- 1-(3,5-Bis(trifluoromethyl)phenyl)-2-(2-imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)ethan-1-one hydrogen bromide (MXL052);
- 1-(3-Chlorophenyl)-2-(2-imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)ethan-1-one hydrogen bromide (MXL053);

- 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(4-(pyrrolidin-1-yl)phenyl)ethan-1-one hydrogen bromide (MXL054); and
- 2-Amino-N-(cyclopropylmethyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL055);

and pharmaceutically acceptable salts, solvates, and prodrugs thereof.

2. The Pifithrin analogue according to claim 1, wherein the Pifithrin analogue is
   - 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026);
   - 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030);
   - 3-(2-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL017);
   - 3-(3-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL018);
   - 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(*3H*)-imine hydrobromide (MXL019)
   - 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL024);
   - 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-3(2H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL028);
   - (2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)(2-aminophenyl)methanone (MXL036);
   - 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL038);
   - 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL040);
   - 3-(4-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL041);
   - 3-(3-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL042);
   - 3-(3,5-Bis(trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL043);
   - 3-(3,4-Dimethylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL044);

- 3-(3,4-Dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL045);
- 3-(3,5-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL046);
- 3-(4-Methoxybenzyl)-3,4,5,6,7,8-hexahydro-2H-cyclohepta[d]thiazol-2-imine hydrogen bromide (MXL049);
- 3-(4-Methoxybenzyl)-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-2(3H)-imine hydrogen bromide (MXL050);
- 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-one hydrogen bromide (MXL051);
- 1-(3,5-Bis(trifluoromethyl)phenyl)-2-(2-imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)ethan-1-one hydrogen bromide (MXL052);
- 1-(3-Chlorophenyl)-2-(2-imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)ethan-1-one hydrogen bromide (MXL053); or
- 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(4-(pyrrolidin-1-yl)phenyl)ethan-1-one hydrogen bromide (MXL054).

3. A composition comprising one or more Pifithrin analogues according to claim 1 or claim 2, and a pharmaceutically acceptable carrier.

4. The composition according to claim 3, wherein the one or more Pifithrin analogues is 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026); or 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030).

5. A method of treating a subject afflicted with Rett Syndrome, which comprises administering to the subject at least one Pifithrin analogue according to claim 1 or claim 2 or a composition thereof.

6. A method of treating a subject afflicted with Down Syndrome, which comprises administering to the subject at least one Pifithrin analogue according to claim 1 or claim 2 or a composition thereof.

7. A method of treating neuronal dysfunction and/or neuronal senescence in a subject, which comprises administering to the subject at least one Pifithrin analogue according to claim 1 or claim 2 or a composition thereof.

8. The method according to claim 7, wherein the subject is afflicted with Rett Syndrome or Down Syndrome.

9. The method according to any one of claims 5 – 8, wherein the at least one Pifithrin analogue is:
   - 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026);
   - 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030);
   - Methyl 2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL032);
   - 3-(3-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL018);
   - 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2(*3H*)-imine hydrobromide (MXL019)
   - 3-(3-Methylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL022);
   - 3-(3-Fluorobenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL023);
   - 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL024);
   - 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-3(2H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL028);
   - Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL029);
   - 2-Amino-N-benzyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL033);
   - 2-Amino-N-(2-methoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL034);
   - 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL038); or
   - 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL040).

10. The method according to any one of claims 5 − 8, wherein the at least one Pifithrin analogue is selected from the group consisting of 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026); 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030); Methyl 2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL032); and pharmaceutically acceptable salts, solvates, and prodrugs thereof, or a composition thereof.

11. A method of administering a Pifithrin analogue to the brain of a subject, which comprises administering to the subject a compound selected from the group consisting of 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026); 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030); Methyl 2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL032); and pharmaceutically acceptable salts, solvates, and prodrugs thereof, or a composition thereof.

12. A Pifithrin analogue for use as a medicament, wherein the Pifithrin analogue is:
    - 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026);
    - 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030);
    - Methyl 2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL032);
    - 3-(2-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL017);
    - 3-(3-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL018);
    - 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrobromide (MXL019);
    - 3-(4-Nitrobenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL020);
    - 2-(2-Imino-4,5,6,7-tetrahydrobenzo[d]thiazol-3(2H)-yl)-1-(4-(pyrrolidin-1-yl)phenyl)ethan-1-one hydrogen bromide (MXL021);
    - 3-(3-Methylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL022);

- 3-(3-Fluorobenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL023);
- 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL024);
- 3-(2-Methylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL025);
- 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-3(2H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL028);
- Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL029);
- Methyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (MXL031);
- 2-Amino-N-benzyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL033);
- 2-Amino-N-(2-methoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL034);
- 2-Amino-N-(4-methoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL035);
- (2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)(2-aminophenyl)methanone (MXL036);
- 2-Amino-N-(pyridin-2-ylmethyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL037);
- 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL038);
- 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL040);
- 3-(4-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL041); or
- 3-(3-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL042).

13. A Pifithrin analogue for use as a medicament, wherein the Pifithrin analogue is:
    - 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026);
    - 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030);
    - 3-(2-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL017);

- 3-(3-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL018);
- 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrobromide (MXL019);
- 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL024);
- 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-3(2H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL028);
- (2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)(2-aminophenyl)methanone (MXL036);
- 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL038);
- 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL040);
- 3-(4-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL041);
- 3-(3-Methoxybenzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL042);
- 3-(3,5-Bis(trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL043);
- 3-(3,4-Dimethylbenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL044);
- 3-(3,4-Dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL045);
- 3-(3,5-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL046);
- 3-(4-Methoxybenzyl)-3,4,5,6,7,8-hexahydro-2H-cyclohepta[d]thiazol-2-imine hydrogen bromide (MXL049);
- 3-(4-Methoxybenzyl)-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-2(3H)-imine hydrogen bromide (MXL050);
- 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-one hydrogen bromide (MXL051);
- 1-(3,5-Bis(trifluoromethyl)phenyl)-2-(2-imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)ethan-1-one hydrogen bromide (MXL052);

- 1-(3-Chlorophenyl)-2-(2-imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)ethan-1-one hydrogen bromide (MXL053); or
- 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(4-(pyrrolidin-1-yl)phenyl)ethan-1-one hydrogen bromide (MXL054).

14. A Pifithrin analogue for use as a medicament, wherein the Pifithrin analogue is:
- 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL026);
- 2-(p-Tolyl)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole (MXL030);
- 3-(3-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL018);
- 3-(4-Methoxybenzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrobromide (MXL019);
- 3-(3-(Trifluoromethyl)benzyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2(3H)-imine hydrogen bromide (MXL024);
- 2-(2-Imino-4,5,6,7,8,9-hexahydrocycloocta[d]thiazol-3(2H)-yl)-1-(p-tolyl)ethan-1-one hydrogen bromide (MXL028);
- 2-Amino-N-(2,4-dimethoxybenzyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (MXL038); or
- 3-(3-(Trifluoromethyl)benzyl)-3,4,5,6-tetrahydro-2H-cyclopenta[d]thiazol-2-imine hydrogen bromide (MXL040).

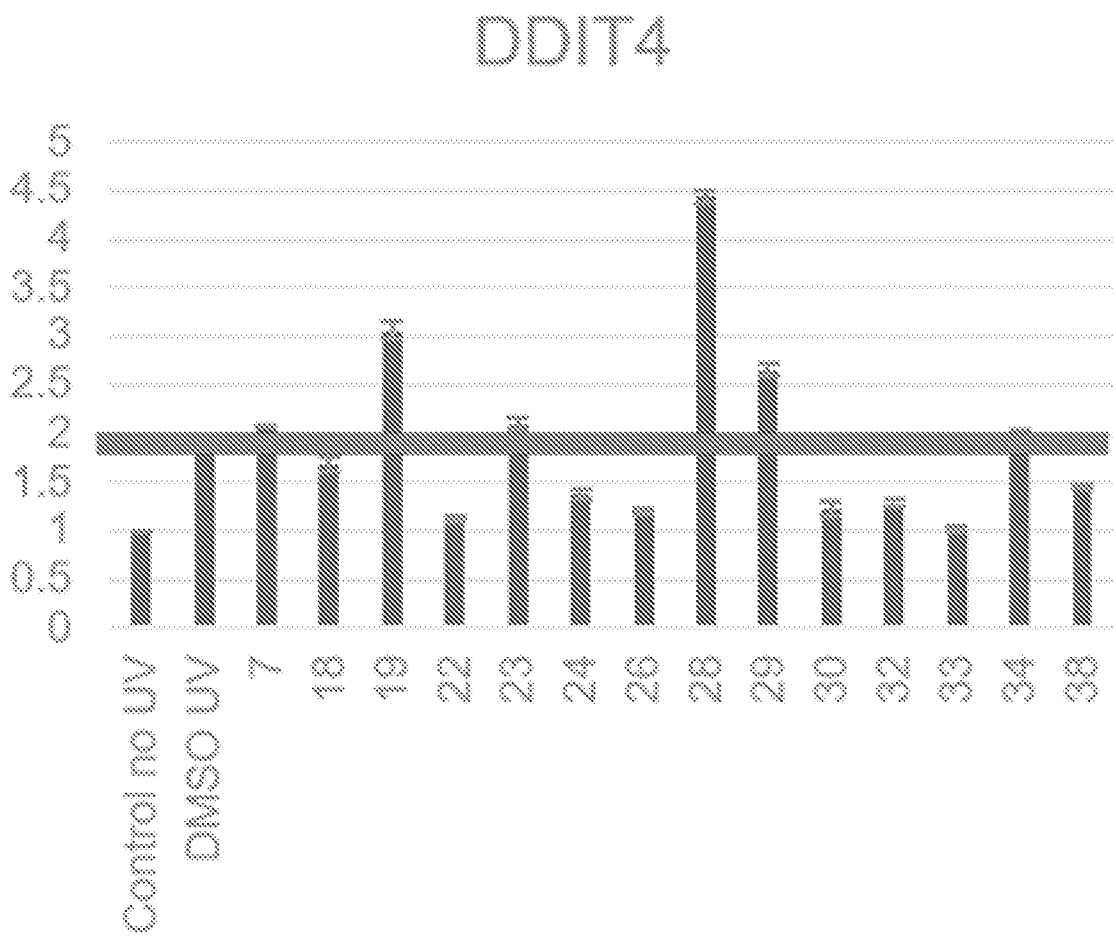
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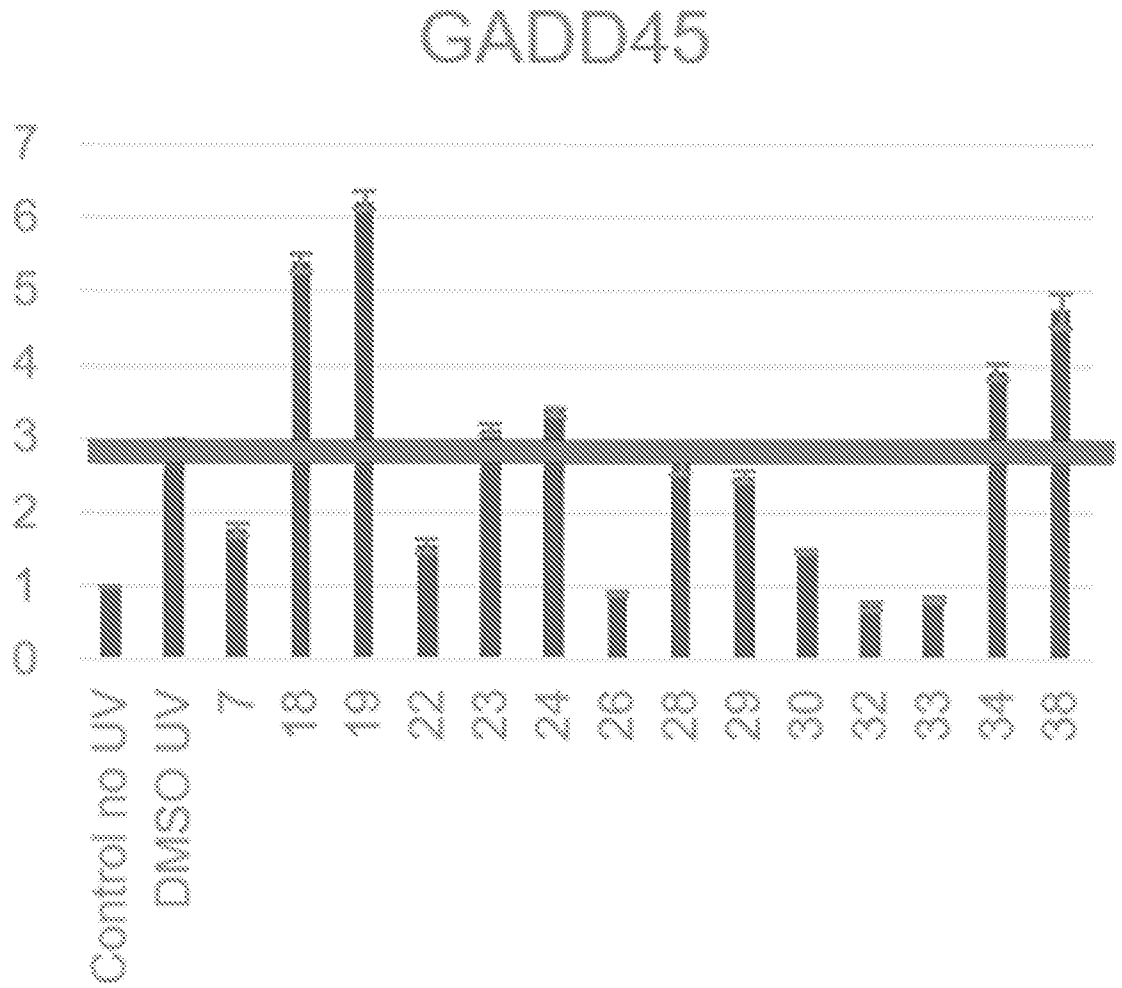
Figure 1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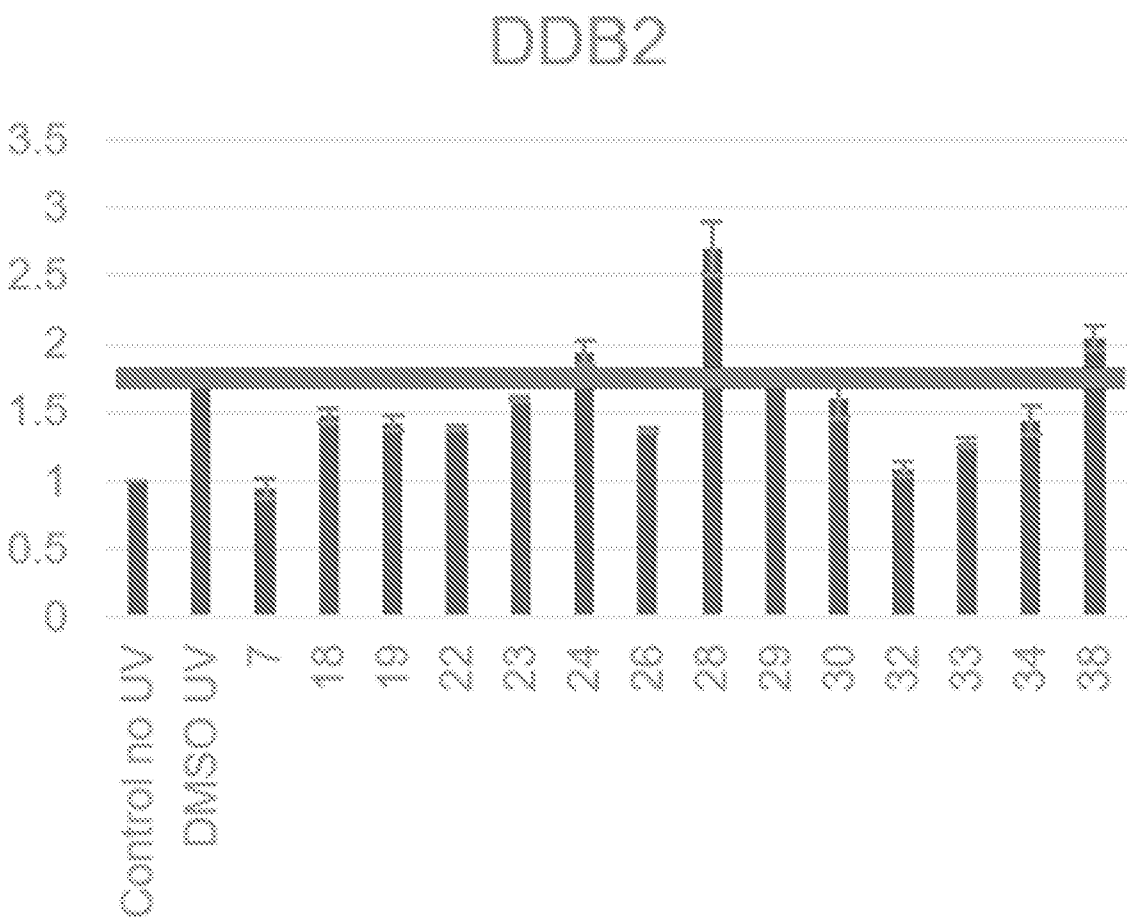
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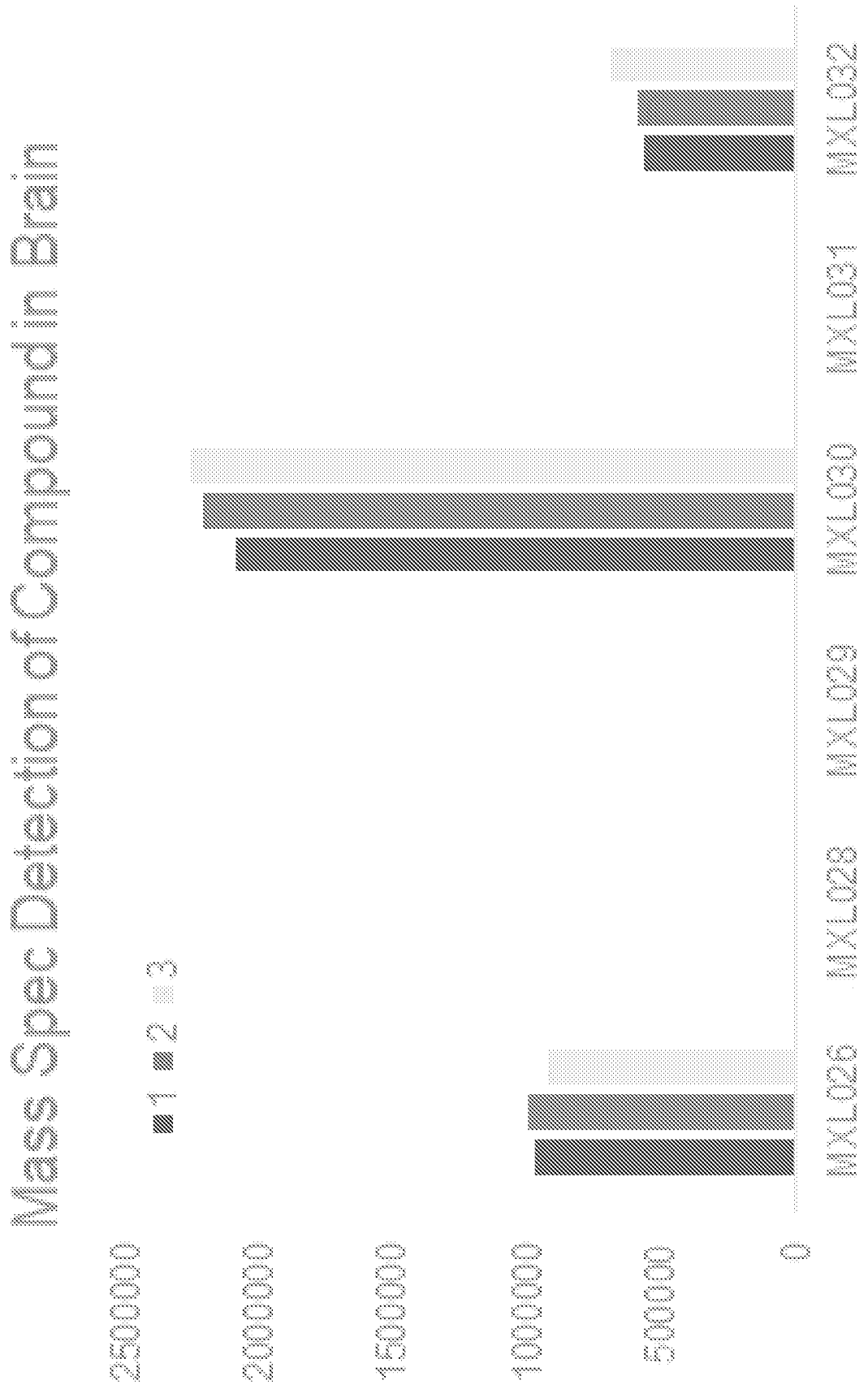
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MXL032

Figure 4

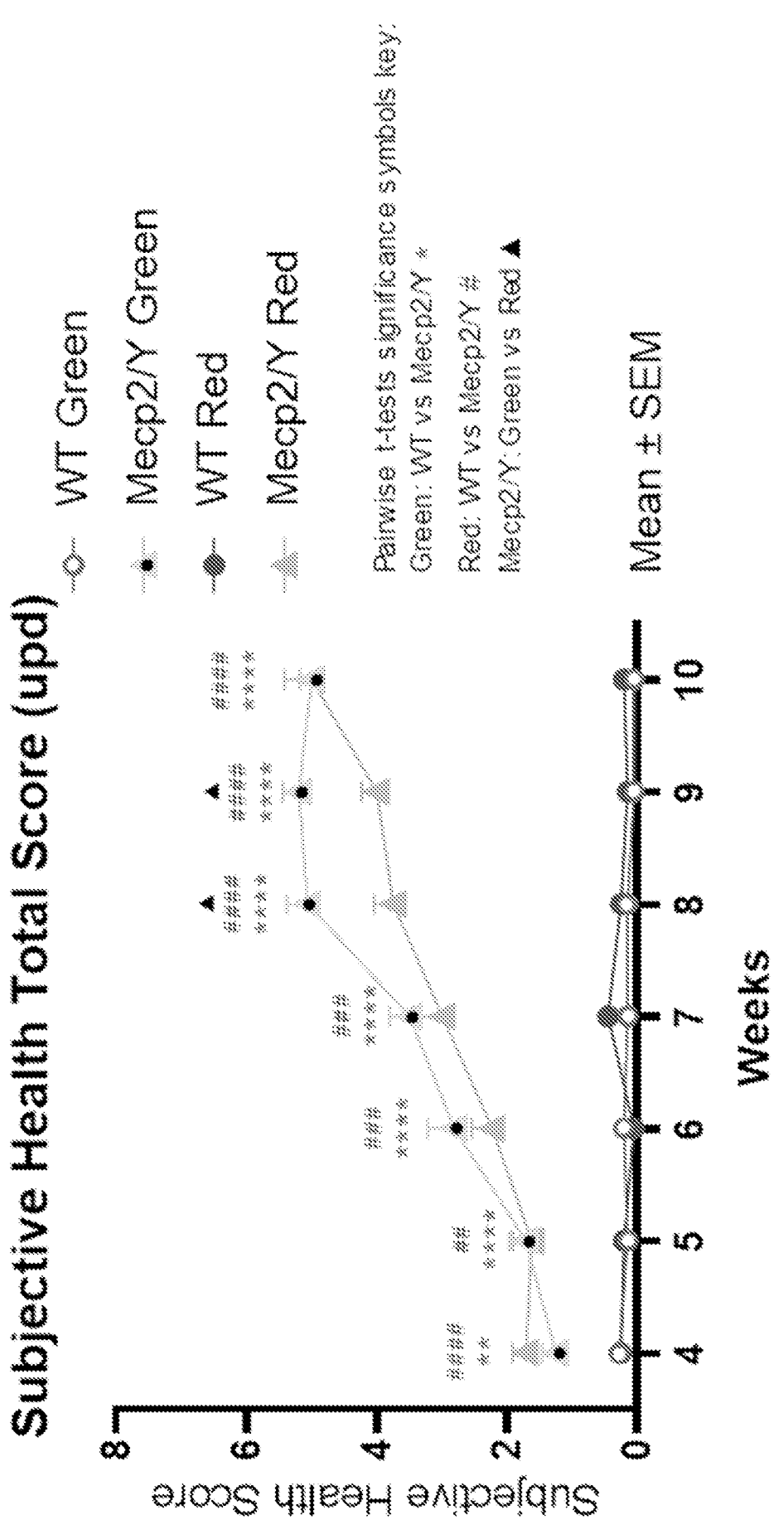
Subjective Health Total Score (upd)
WT Green
Mecp2/Y Green
WT Red
Mecp2/Y Red
Pairwise t-tests significance symbols key:
Green: WT vs Mecp2/Y *
Red: WT vs Mecp2/Y #
Mecp2/Y: Green vs Red ▲
Mean ± SEM
Subjective Health Score
Weeks
0
2
4
6
8
4
5
6
7
8
9
10

Figure 5

3-way ANOVA (REML model)

Mixed-effects model (REML)	Matching by factor: Age (weeks)			
Assume sphericity?	No			
Alpha	0.05			
Fixed effects (type III)	P value	P value summary	Statistically significant (P < 0.05)?	F (DFn, DFd)
Age (weeks)	<0.0001	****	Yes	F (4.606, 241.0) = 59.65
Food	0.1716	ns	No	F (1, 54) = 1.919
Genotype	<0.0001	****	Yes	F (1, 54) = 520.8
Age (weeks) x Food	0.0096	**	Yes	F (6, 314) = 2.880
Age (weeks) x Genotype	<0.0001	****	Yes	F (6, 314) = 65.17
Food x Genotype	0.0580	ns	No	F (1, 54) = 3.752
Age (weeks) x Food x Genotype	0.0036	**	Yes	F (6, 314) = 3.302

Figure 6

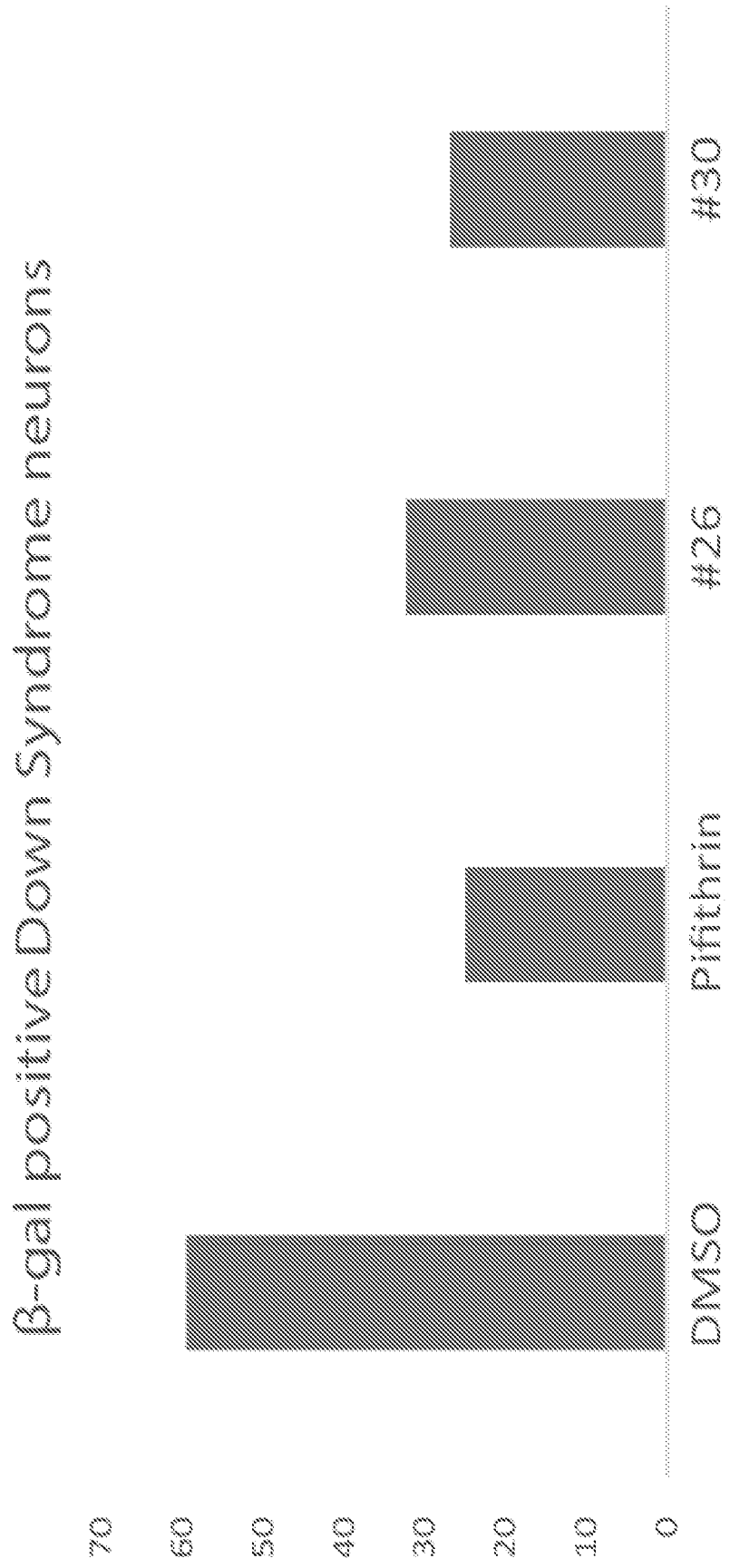
β-gal positive Down Syndrome neurons
70
60
50
40
30
20
10
0
DMSO
Pifithrin
#26
#30

Figure 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/041929

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 31/395*** (2024.01); ***C07D 277/60*** (2024.01); ***C07D 277/62*** (2024.01); ***C07D 277/82*** (2024.01); *A61K 31/33* (2024.01)

CPC: ***A61K 31/395; C07D 277/60; C07D 277/62; C07D 277/82;*** *A61K 31/33*

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)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2022/0251054 A1 (The University of California) 11 August 2022 (11.08.2022) para [0004]; [0009]	1-8, and 11-14
A	US 2006/0122178 A1 (Cottam et al.) 08 June 2006 (08.06.2006) para [0003], [0009], [0016], abstract	1-8, and 11-14
A	CA 2706586 A1 (Abbott Laboratories) 28 May 2009 (28.05.2009) entire document	1-8, and 11-14
A	"PubChem CID 130644079", create date: 10 September 2017 page 2 formula	1-8, and 11-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 December 2024 (01.12.2024)	**17 December 2024 (17.12.2024)**

Name and mailing address of the ISA/US	Authorized officer
COMMISSIONER FOR PATENTS **MAIL STOP PCT, ATTN: ISA/US** **P.O. Box 1450** **Alexandria, VA 22313-1450** **UNITED STATES OF AMERICA**	**KARI RODRIQUEZ**
Facsimile No. **571-273-8300**	Telephone No. **PCT Help Desk: 571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/041929

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☑ Claims Nos.: **9-10**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: Claims 1-8, and 11-14 directed to a Pifithrin analogue and the method of administering. Claim 1 will be searched to the extent that it encompasses the first species of claim 1, represented by 2-(2-Imino-5,6-dihydro-2H-cyclopenta[d]thiazol-3(4H)-y1)-1-(p-tolypethan-1-one hydrogen bromide (MXL026). It is believed that claims 1-8, and 11-14 read on this first named invention, and thus these claims will be searched without fee. This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Applicant is invited to elect additional compounds of claim 1, wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be a second species of claim 1, represented by 2-(p-Toly1)-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1 - b]thiazol e (MXL030) (i.e, claims 1-8, and 11-14)

The groups of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Each invention in Group I+ includes the technical feature of a unique compound of Formula (I), which is not required by any other invention of Group I+

Common Technical Features:

The inventions of Group I+ share the technical feature of a compound of Formula Pifithrin analogue.

These shared technical features, however, do not provide a contribution over the prior art as being anticipated by US 2022/0251054 A1 to The Reagent of the University of California which discloses the Pifithrin analogue (para [0004], compound of formula)

Accordingly, the inventions listed as Groups I+, above lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature providing contribution over prior art.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/041929

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-8, and 11-14**

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.