

[0318] Culture media was removed from cells and they were washed with 50 L/well of Ringer's Buffer (MgCl₂ 0.047 mg/mL, NaH₂PO₄ 0.18 mg/mL, Na₂HPO₄ 0.1 mg/mL, KCl 0.34 mg/mL, NaHCO₃ 1.26 mg/mL, D-glucose 1.8 mg/mL, NaCl 7 mg/mL; pH=7.4). Compounds suspended in DMSO were diluted in Ringer's Buffer containing 0.5% fatty acid free BSA plus 300 µM IBMX and incubated on cells for 45 min at 37° and 5% CO₂. After incubation cells were incubated for 10 min at room temp with Eu-cAMP tracer solution from a Perkin Elmer Lance HTRF Ultra cAMP assay kit (TRF0263). Then ULightTM-anti-cAMP solution from the Lance HTRF kit was added and incubated on a shaker at room temp for 1 hour prior to HTRF detection in a Perkin Elmer Envision plate reader.

[0319] EC₅₀ curves were generated with a four-parameter logistic equation using GraphPad Prism 5.03. Measured EC₅₀ value (µM) of example compounds in this assay is provided nM in the Table 1 below.

[0320] Table 1

Ex.	A.1 EC50	A.2 EC50	Ex.	A.1 EC50	A.2 EC50
1			18		6.8
2			19	50.5	
3			20		
4			21		50.3
5		32.7	22		30.2
6			23		172
7			24		48.2
8			25		242
9			26		72.3

10			27		161
11			28		77
12			29		184
13		49.3	30		30.2
14		38.7	31		18.0
15		36.9	32		23.5
16		123	33		13.7
17		23	34		62.5

[0321] Example B

[0322] Haloperidol-induced Catalepsy – in vivo rodent Parkinson's disease model

[0323] The motor symptoms of Parkinson's disease include akinesia, bradykinesia, rigidity, tremor and postural abnormalities and are associated with the loss of nigral dopaminergic cells and a decline in striatal dopamine levels. Administration of haloperidol to rodents leads to a transient parkinsonian-like state that is reversed by the administration of L-Dopa (Duty, S.; Jenner, P. Br. J. Pharmacol. (2011), 164, 1357–1391) and other drugs that have been clinically validated for the treatment of Parkinson's disease. Haloperidol antagonizes dopamine D2 and, to a lesser extent, D1 receptors in medium spiny neurons that comprise the indirect and direct pathways of the motor circuit respectively. The resultant block of striatal dopamine transmission results in abnormal downstream firing within the basal ganglia circuits that is manifest as symptoms of muscle rigidity and catalepsy. Catalepsy has been postulated to reflect the clinical features of Parkinson's disease, whereby patients experience an inability of to initiate movements.

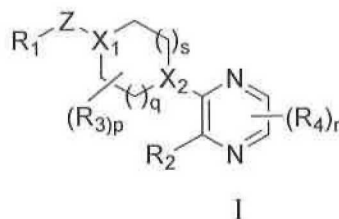
[0324] Male Sprague-Dawley rats (Charles River, Calco, Italy) weighing 175-200 g are used. Alternatively, male C57Bl6 mice weighing 25-35 g were used. The cataleptic state was

induced by the subcutaneous administration of the dopamine receptor antagonist haloperidol (0.3 mg/kg, sc), 90 min before testing the animals on the vertical grid test. For this test, the rats or mice were placed on the wire mesh cover of a 25 cm x43 cm plexiglass cage placed at an angle of about 70 degrees with the bench table. The subject was placed on the grid with all four legs abducted and extended (“frog posture”). The use of such an unnatural posture is essential for the specificity of this test for catalepsy. The time span from placement of the paws until the first complete removal of one paw (descent latency) was measured maximally for 120 sec for rats. For mice, the front paws of a mouse was placed on a horizontal metal bar raised 2” above a Plexiglas platform and time was recorded for up to 30 seconds per trial. The test ended when the animal’s front paws returned to the platform or after 30 seconds. The test was repeated three times and the average of the three trials was reported as the intensity index of catalepsy.

[0325] Catalepsy was measured 30 min, 120 min, and/or 240 min after dosing the subjects a 0.3 mg/kg i.p. dose of haloperidol along with the GPR6 modulator test compound. Test compound plasma and brain levels were determined by collected tissue samples at the end of the experiment, which was either at the 120 or 240 min time point. A representative number of compounds of the invention were administered in a dose range from 0.1 to 100 mg/kg i.p, sc or po in conjunction with haloperidol. The A2a antagonist KW6002 (istradefylline) was dosed at 0.6 mg/kg i.p. as a positive control.

WHAT IS CLAIMED IS:

1. A compound of formula I



or a pharmaceutically acceptable salt thereof, wherein

R₁ is phenyl optionally substituted with 1 to 5 substituents independently selected from the group consisting of C₁₋₄ alkyl, C₁₋₄ alkoxy, cyano, halo, trifluoromethyl, and trifluoromethoxy;

X₁ is N and X₂ is CH; or

X₁ is CH and X₂ is N; or

X₁ is N and X₂ is N;

when X₁ is N, Z is selected from the group consisting of C₁₋₆ alkylene, C₁₋₆ haloalkylene, -C(O)-, and -S(O)₂-;

when X₁ is CH, Z is selected from the group consisting of C₁₋₆ alkylene, C₁₋₆ haloalkylene, -O-, -C(O)-, -NH-, -S-, -S(O)-, and -S(O)₂-;

q is 1;

s is 1;

R₂ is -NR₆R₇;

R₃, each time taken, is independently selected from the group consisting of C₁₋₆ alkyl, C₃₋₈ cycloalkyl, and trifluoromethyl;

p is 0, 1, or 2;

R₄, each time taken, is independently selected from the group consisting of C₁₋₆ alkyl, cyano, hydroxy, halo, C₃₋₆ heterocyclyl, -C(O)-R₈, -C(O)-N(R₉)(R₁₀), and -C(O)-OR₁₁;

r is 1 or 2;

R₆ is hydrogen;

R₇ is selected from the group consisting of C₁₋₆ alkyl, C₃₋₈ cycloalkyl, and C₃₋₆ heterocyclyl;

R₈ is selected from the group consisting of hydrogen, C₁₋₆ alkyl, C₃₋₈ cycloalkyl, C₆₋₁₀ aryl, C₁₋₁₀ heteroaryl, and C₃₋₆ heterocyclyl;

R₉ is selected from the group consisting of hydrogen and C₁₋₆ alkyl;

R₁₀ is selected from the group consisting of hydrogen, C₁₋₆ alkyl and C₃₋₈ cycloalkyl; or

R₉ and R₁₀ are taken together with the nitrogen to which they are attached form a 4 to 7 membered, saturated, ring optionally having 1 additional ring heteroatom selected from the group

N, O, and S and optionally substituted on any of the ring carbon atoms with 1 to 5 substituents independently selected from the group consisting of cyano, halo, hydroxy, amino, C₃₋₆ heterocyclyl, C₁₋₉ amide, C₁₋₆ alkyl, and C₁₋₄ alkoxy and substituted on any additional ring nitrogen by a substituent selected from the group consisting of hydrogen, C₃₋₈ cycloalkyl, and C₁₋₆ alkyl; and

R₁₁ is selected from the group consisting of hydrogen, C₁₋₆ alkyl, and C₃₋₈ cycloalkyl.

2. The compound or pharmaceutically acceptable salt thereof according to claim 1, wherein X₁ is CH and X₂ is N.
3. The compound or pharmaceutically acceptable salt thereof according to claim 1, wherein X₁ is N and X₂ is N.
4. The compound according to claim 3, wherein an R₄ is cyano.
5. The compound or pharmaceutically acceptable salt thereof according to any one of claims 1 to 4, wherein Z is C₁₋₆ alkylene.
6. The compound or pharmaceutically acceptable salt thereof according to any one of claims 1 to 2, wherein Z is -O-.
7. The compound or pharmaceutically acceptable salt thereof according to any one of claims 1 to 4, wherein Z is -C(O)-.
8. The compound according to claim 1, which is selected from the group consisting of:
5-(cyclopropylamino)-6-(4-(2,4-difluorobenzyl)piperazin-1-yl)pyrazine-2,3-dicarbonitrile;
5-(4-(2,4-difluorophenoxy)piperidin-1-yl)-6-(isopropylamino)pyrazine-2,3-dicarbonitrile;
5-(4-(5-chloro-2-fluorobenzoyl)piperidin-1-yl)-6-(isopropylamino)pyrazine-2,3-dicarbonitrile;
(R)-5-(4-((2,4-difluorophenyl)fluoromethyl)piperidin-1-yl)-6-(isopropylamino)pyrazine-2,3-dicarbonitrile; 5-(4-(4-chloro-2-fluorophenoxy)piperidin-1-yl)-6-(isopropylamino)pyrazine-2,3-dicarbonitrile; 5-(4-(4-chloro-2-fluorophenoxy)piperidin-1-yl)-3-cyano-6-(isopropylamino)pyrazine-2-carboxylic acid; 6-(4-(4-chloro-2-fluorophenoxy)piperidin-1-yl)-3-cyano-5-(isopropylamino)pyrazine-2-carboxylic acid; 3-cyano-6-(cyclopropylamino)-5-(4-(2,4-difluorobenzyl)piperazin-1-yl)pyrazine-2-carboxylic acid; 3-cyano-5-(cyclopropylamino)-6-(4-(2,4-difluorobenzyl)piperazin-1-yl)pyrazine-2-carboxylic acid; 3-cyano-5-(4-(2,4-difluorophenoxy)piperidin-1-yl)-6-(isopropylamino)pyrazine-2-carboxylic acid; 3-cyano-6-(4-(2,4-difluorophenoxy)piperidin-1-yl)-5-(isopropylamino)pyrazine-2-carboxylic acid; 5-(4-(5-chloro-2-fluorobenzoyl)piperidin-1-yl)-3-cyano-6-(isopropylamino)pyrazine-2-carboxylic acid; 6-(4-(5-chloro-2-fluorobenzoyl)piperidin-1-yl)-3-cyano-5-(isopropylamino)pyrazine-2-carboxylic acid; (R)-3-cyano-5-(4-((2,4-difluorophenyl)fluoromethyl)piperidin-1-yl)-6-(isopropylamino)pyrazine-2-carboxylic acid; (R)-3-cyano-6-(4-((2,4-difluorophenyl)fluoromethyl)piperidin-1-yl)-5-(isopropylamino)pyrazine-2-carboxylic acid; 6-(4-(2,4-difluorophenoxy)piperidin-1-yl)-5-(isopropylamino)pyrazine-2-carboxylic acid; 3-chloro-6-(4-(2,4-

difluorophenoxy)piperidin-1-yl)-5-(isopropylamino)pyrazine-2-carbonitrile; 5-(4-(4-chloro-2-fluorophenoxy)piperidin-1-yl)-3-cyano-6-(isopropylamino)-N,N-dimethylpyrazine-2-carboxamide; 3-cyano-6-(cyclopropylamino)-5-(4-(2,4-difluorobenzyl) piperazin-1-yl)-N,N-dimethylpyrazine-2-carboxamide; 3-cyano-5-(4-(2,4-difluorophenoxy) piperidin-1-yl)-6-(isopropylamino)-N,N-dimethylpyrazine-2-carboxamide; 3-cyano-6-(4-(2,4-difluorophenoxy) piperidin-1-yl)-5-(isopropylamino)-N,N-dimethylpyrazine-2-carboxamide; 5-(4-(5-chloro-2-fluorobenzoyl)piperidin-1-yl)-3-cyano-6-(isopropylamino)-N,N-dimethylpyrazine-2-carboxamide; 6-(4-(2,4-difluorophenoxy) piperidin-1-yl)-5-(isopropylamino)-N,N-dimethylpyrazine-2-carboxamide; 6-(4-(5-chloro-2-fluorobenzoyl)piperidin-1-yl)-5-(isopropylamino)-N,N-dimethylpyrazine-2-carboxamide; (R)-6-(4-((2,4-difluorophenyl) fluoromethyl)piperidin-1-yl)-5-(isopropylamino)-N-methylpyrazine-2-carboxamide; (S)-6-(4-((2,4-difluorophenyl)fluoromethyl) piperidin-1-yl)-5-(isopropylamino)-N-methylpyrazine-2-carboxamide; (6-(4-(2,4-difluorophenoxy) piperidin-1-yl)-5-(isopropylamino) pyrazin-2-yl)(3-fluoroazetidin-1-yl)methanone; 5-(4-(2,4-difluorophenoxy)piperidin-1-yl)-6-(isopropylamino)-N,N-dimethylpyrazine-2-carboxamide; (R)-5-(cyclopropylamino)-6-(4-((2,4-difluorophenyl) fluoromethyl)piperidin-1-yl)pyrazine-2-carbonitrile; 6-(4-(2,4-difluorophenoxy) piperidin-1-yl)-5-(isopropylamino)pyrazine-2-carbonitrile; 5-(4-(2,4-difluorophenoxy) piperidin-1-yl)-6-(isopropylamino)pyrazine-2-carbonitrile; 5-(4-(4-chloro-2-fluorophenoxy)piperidin-1-yl)-6-(cyclopropylamino)pyrazine-2-carbonitrile; (S)-5-(4-((2,4-difluorophenyl)fluoromethyl)piperidin-1-yl)-6-(isopropylamino)pyrazine-2-carbonitrile; (R)-5-(4-((2,4-difluorophenyl)fluoromethyl)piperidin-1-yl)-6-(isopropylamino)pyrazine-2-carbonitrile; 6-(4-(2,4-difluorophenoxy)piperidin-1-yl)-5-(isopropylamino)-3-morpholinopyrazine-2-carbonitrile; or a pharmaceutically acceptable salt of any one of the above-mentioned compounds.

9. A pharmaceutical composition comprising a compound or pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 8, and a pharmaceutically acceptable excipient.

10. A compound or pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 8 for use as a medicament.

11. A compound or pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 8, for use in the treatment of a disorder selected from Parkinson's disease, levodopa induced dyskinesias, Huntington's disease, drug addiction, eating disorders, cognitive disorders, schizophrenia, bipolar disorders, and depression.

12. Use of a compound or pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 8 in the manufacture of a medicament for the treatment of a disorder selected from Parkinson's disease, levodopa induced dyskinesias, Huntington's disease, drug addiction, eating disorders, cognitive disorders, schizophrenia, bipolar disorders, and depression.