Therapeutic Exploration of Metabotropic Glutamate Receptor Antagonists in Parkinson's Disease by Positron Emission Tomography

Rosario Sanchez-Pernaute, MD, PhD[1] and Anna-Liisa Brownell, PhD[2]

1. Neurologist and Neuroscientist, Parkinson Disease Program, Inbiomed Foundation, San Sebastian;
2. Associate Professor and Director, Experimental PET Laboratory, Department of Radiology, Massachusetts General Hospital

Abstract

Metabotropic glutamate receptors (mGluR)s are G-protein-coupled receptors that function as modulators of synaptic function and glutamate transmission. Post-synaptically localized subtype 5 mGlu5 receptors are co-localized with adenosine A2a, dopamine, and N-methyl-D-aspartate (NMDA) receptors and regulate local protein synthesis and messenger RNA (mRNA) translation at synapses, and are thus ideally positioned to control synaptic plasticity. Aberrant synaptic plasticity appears to be involved in a number of developmental and degenerative neuropsychiatric disorders, including Parkinson's disease. Pharmacological modulation of mGluR5 could potentially open new therapeutic avenues for the treatment of such disorders, for both symptomatic and neuroprotective purposes. In this review, we summarize a series of *in vivo* studies we performed in order to delineate the anatomical basis and functional role of mGluR5 antagonists in Parkinson's disease models, taking advantage of high-resolution positron emission tomography (PET) and the recent development of novel specific radiopharmaceuticals. Our findings of a prevalent distribution of mGluR5 in the striatum and limbic structures and a significant binding enhancement following dopamine lesions support the role of mGlu5 receptors in modulating dopamine- and glutamate-dependent signaling and synaptic plasticity within the basal ganglia cortico–subcortical loops.

Keywords

Metabotropic glutamate receptor (mGluR), mGluR5, dopamine, Parkinson's disease (PD), positron emission tomography (PET), FPEB, MPEP

Disclosure: This research was supported by National Institutes of Health (NIH) grants NIBIB-EB001850 and NINDS-NS39793. The authors have no conflicts of interest to declare.
Received: February 5, 2009 **Accepted:** July 17, 2009 *DOI: 10.17925/USN.2010.05.02.21*
Correspondence: Anna-Liisa Brownell, PhD, Associate Professor, Department of Radiology, Bartlett Hall 504R, Massachusetts General Hospital, Boston, MA 02114.
E: abrownell@partners.org

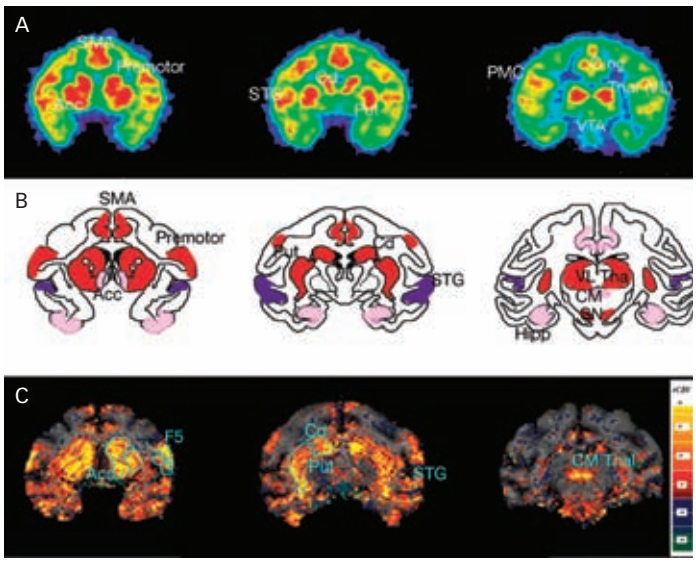
There is great interest in developing pharmacological compounds that function through metabotropic glutamate receptors (mGluRs) as modulators of synaptic function and that could consequently become effective therapeutic agents for several developmental and degenerative neuropsychiatric disorders. mGluRs are located perisynaptically, acting as sensors and modulators of glutamate transmission.[1] This functional specificity makes them attractive pharmacological targets. In particular, subtype 5 receptors (mGluR5) regulate local protein synthesis and messenger RNA (mRNA) translation at synapses, and are thus ideally positioned to control synaptic plasticity.[2–5] A number of studies have explored the therapeutic potential of drugs acting at the mGluR5 for a variety of conditions, including Parkinson's disease (PD), addiction, chronic pain, mood disorders, epilepsy, and fragile X, among others.[6,7] Despite their diversity, these disorders may share common mechanisms involving aberrant brain plasticity.

Regarding PD, the loss of dopamine (DA)-mediated inhibition in the striatum results in excess excitatory transmission. It is therefore possible that mGluR5 modulation can improve PD symptoms directly[8–10] or through modulation of cholinergic interneurons,[11,12] although *in vivo* off-target effects of mGluR5 antagonists, particularly on N-methyl-D-aspartate (NMDA) and mGluR1s, have to be taken into account.[13] Furthermore, mGluR5 antagonists appear particularly promising for the treatment of levodopa-induced dyskinesia,[14–16] a frequent, invalidating complication of DA replacement therapy that represents an abnormal form of synaptic plasticity.[17] Finally, pharmacological manipulation of mGluR5s could open new modalities of neuroprotection for PD and other degenerative diseases of the nervous system, by decreasing excitotoxicity,[18] modulating signaling pathways, or, perhaps, through local translation of trophic factors. We have explored the anatomical basis and functional role of mGluR5 antagonists in models of PD, taking advantage of high-sensitivity positron emission tomography (PET) and the recent development of novel specific radiopharmaceuticals in a series of studies briefly reviewed here.

Metabotropic Glutamate Receptors

Glutamate is the most prevalent excitatory neurotransmitter in the brain, and a balanced glutamatergic transmission is required for normal brain function. Glutamate plays a role in a variety of physiological processes, such as long-term potentiation (learning and memory), the development of synaptic plasticity, motor control, respiration, cardiovascular regulation, emotional states, and sensory perception.[19] At the synaptic level glutamate is regulated by glutamate transporters and acts through two types of

Figure 1: Distribution of [18F]FPEB in a Parkinsonian Primate Brain



A: Distribution of [18F]FPEB in the brain of a primate rendered parkinsonian by systemic administration of MPTP reveals prominent binding over the DA-responsive and projection mesostriatal, mesolimbic, and mesocortical areas, which are schematically represented in B over the corresponding atlas coronal sections in red (motor), pink (limbic), and purple (associative). C: These areas match the areas that show a hemodynamic response to DA release induced by amphetamine, as we previously described using functional magnetic resonance imaging (fMRI).[30] Acc = nucleus accumbens; Cd = caudate nucleus; Cin = cingulate cortex; CM = centromedian/parafascicularis thalamic nuclei; F5 = ventral premotor; PMC = primary motor cortex; Put = putamen; SMA = supplementary motor area; SN = substantia nigra; STG = supratemporal gyrus; Thal = thalamus; VTA = ventral tegmental area.

receptor: the fast-ligand-binding receptors, which are directly coupled to the opening of cation channels in the cell membranes of the neurons and are termed 'ionotropic' (iGluR); and the second type, which are the slow G-protein-coupled 'metabotropic' receptors (mGluR),[20] and are associated with second messenger systems and lead to enhanced phosphoinositol hydrolysis (IP3), activation of phospholipase D, increases or decreases in adenyl cyclase and cyclic adenosine monophosphatase (cAMP) formation, and changes in ion channel function.[21] In the past several years, molecular cloning studies have revealed the existence of eight different subtypes of mGluRs.[6] mGluR subtypes can be divided into three groups according to their sequence similarities, signal transduction mechanisms, and pharmacological profiles.[22] The first group, comprising mGluR1 and mGluR5, is coupled to stimulation of IP3/Ca^{2+} signal transduction.[23] Group I mGluRs are especially effective at reducing GABA-inhibitory responses. The second group, consisting of mGluR2 and mGluR3, is negatively coupled through adenylate cyclase to cAMP formation.[24] The third group, including mGluR4, mGluR6, mGluR7, and mGluR8, is also negatively linked to adenylate cyclase activity but shows different agonist preference.[25]

mGluR5 was cloned in 1992[26] and, like the other seven subtypes, is a G-protein-coupled receptor with a large N-amino terminal domain. mGluR5 is expressed in limbic cortex, hippocampus, amygdala, basal ganglia, thalamus, and motor and premotor cortices. The receptor is localized mostly post-synaptically and co-localized with adenosine A2a,[27] DA, and NMDA receptors. Brain areas expressing mGluR5s are related to nociception, emotion, motivation, and motor control, leading to the assumption that mGluRs may have a critical role in pain, anxiety, depression, and neurodegenerative disorders, including PD. Interestingly, mGluR5 is enriched in cortical and subcortical areas and prominently activated in response to DA release (see *Figure 1*).[28]

mGluR5 Pharmacology

The diversity and heterogeneous distribution of mGluR subtypes through the central nervous system (CNS) provides an opportunity for developing compounds (or drugs) to selectively target a specific subsystem, aiming to ameliorate symptoms of distinct neurological disorders while limiting the disruptive effects of altered glutamate transmission on brain function. Such compounds represent both pharmacological tools to further investigate mGluR5 physiology and potential therapeutic agents for the treatment of several conditions that have been associated with abnormal activation of mGluR5 function.

mGluRs belong to class C of G-protein-coupled receptors and, in addition to the characteristic seven-strand transmembrane domain for G-protein activation, possess a large extracellular domain that is responsible for ligand recognition.[29] The mGluRs have a large bi-lobed extracellular N-terminus of ~560 amino acids that has been shown by mutagenesis studies to confer glutamate binding, agonist activation of the receptor, and subtype specificity for group-selective agonists.[30]

A large number of pharmacological agents active at mGluRs have been reported in the literature. According to the mode of binding, mGluR pharmacological agents can be classified into competitive and non-competitive agents and, based on the mode of action, they can be classified into agonists, antagonists, and positive/negative/neutral modulators or potentiators.[31] Competitive agonists and antagonists bind to the same orthosteric binding site as endogenous glutamate, which is a cleft between the two lobes in the extracellular N-terminus. Their binding ability depends on how much they could stabilize the closed conformation.[32] These ligands received the earliest research interest and they have been well developed. They are all glutamate analogs or substituted glycines, which implies that they have poor selectivity within their group. In addition, competitive agonists and antagonists have structural carboxyl and amino groups, which make them too polar to penetrate the blood–brain barrier.[30]

Starting from 1996,[33] a number of structural types of non-competitive negative, positive, and neutral allosteric modulators have been developed as mGluR ligands.[34] These ligands modulate mGlu receptor activity by binding to allosteric binding sites that are located in the seven-strand transmembrane domain. The allosteric binding sites are structurally distinct from the classic agonist, orthosteric, binding site.[35] Positive and negative modulators thus offer the potential for improved selectivity for individual mGluR family members compared with competitive agonists and antagonists at the glutamate site.[32] These ligands are not amino acid derivatives and are structurally diverse. They are lipophilic and have much better CNS penetrating capability. Thus, such positive and negative modulators with high binding affinity, high subtype selectivity, and appropriate lipophilicity are good candidates for mGluR radiotracer development.[36–38] These tracers do not show competitive binding with endogenous glutamate, which increases their sensitivity. In summary, classic mGluR5 agonists and antagonists are derived from endogenous

glutamate, thus lacking subtype selectivity and suitable lipophilicity to cross the blood–brain barrier. By contrast, allosteric modulators—negative, positive, neutral—are structurally diverse and amino-acid-unrelated, therefore possessing high binding affinity, high subtype selectivity, and appropriate lipophilicity.

The first selective mGlu5 receptor antagonists were identified in 1999[39] through random screening and have been followed by a large number of potent, subtype-selective, and structurally diverse allosteric modulators, mostly developed by the pharmaceutical industry. Indeed, in search of alternative chemical structures for orthosteric antagonists, Novartis AG developed the first selective antagonist, SIB-1757 (6-methyl-2-(phenylazo)-3-pyridinol),[39] which inhibits glutamate-induced receptor activation without affecting the affinity for glutamate, i.e. in a non-competitive manner. Subsequent optimization and replacement of the trans-olefinic tether with a carbon triple bond led to the synthesis of 2-methyl-6-(phenylethylyn) pyridine (MPEP),[40] which displays highly improved mGluR5 antagonist activity and has been considered a prototypical mGluR5 antagonist, although it is also active at the NMDA receptor.[13,30] Structure–activity relationship studies performed on MPEP, in which chemical modifications were made to each of the three regions of the original molecule, led to identification of MTEP ((2-methyl-1,3-thiazo-4-yl)ethynyl pyridine) and other highly potent and selective diaryl (heteroaryl) acetylenes as mGluR5 non-competitive antagonists.[41] With the assumption that the (2-methyl-1,3-thiazo-4-yl)ethynyl group is an optimal chemical structure to confer mGluR5 antagonist activity to a compound, further structure–activity relationship studies on MTEP have identified other ligands containing thiazole moieties as mGluR5 non-competitive antagonists with improved pharmacological profiles. Although most of the ligands are MPEP- or MTEP-derived, some compounds lacking the acetylenic tether have been delineated as potent and selective mGluR5 non-competitive antagonists as well.

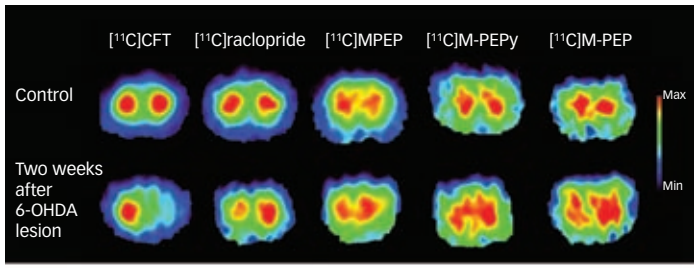
Compared with available negative modulators, potent and selective positive allosteric modulators of mGluR5 are less well developed. Since the discovery of the first mGluR5-positive modulator,[42] 3,3'-difluorobenzaldazine, Merck has reported three series of positive allosteric modulators for mGlu5 receptor, which are the benzaldazine series, benzamide series, and pyrazole series.[42–44] The discovery of non-competitive allosteric modulators with high binding affinity and subtype selectivity facilitates the exploration of the physiological and pathological functions of mGluR5 in normal and diseased states.

mGluR5 Binding in the Normal and Parkinsonian Brain

DA exerts a complex regulation of glutamate neurotransmission in the basal ganglia circuitry through differential effects on striatopallidal and striatonigral neurons mediated by DA receptors D1 and D2.[45] In PD, loss of DA results in an imbalance in other neurotransmitters, mostly excitatory. Taking advantage of new PET tracers, we have examined the distribution of mGluR5 in the rodent and primate brain and the effects of DA denervation in parkinsonian animals. In our recent studies,[28,38,46] we have synthesized and radiolabeled five non-competitive antagonists for mGluR5—[^{11}C]M-MPEP (2-[(3-methoxyphenyl)ethynyl]-6-methylpyridine), [^{11}C]M-PEPy (3-methoxy-5-[(2-pyridyl)ethynyl]pyridine,), [^{11}C]MPEP (2-methyl-6-(2-phenylethynyl) pyridine), [^{18}F]FMTEP (2-fluoro-5-(2-(2-methylthiazol-4-yl)ethynyl)pyridine), and [^{18}F]FPEB (3-fluoro-5-(2-pyridyl-ethynyl)benzonitrile)—and conducted *in vivo* PET imaging studies in different disease models to investigate mGluR5 expression and function. All of the compounds showed prominent binding in the striatum and limbic regions of the brain. Using [^{11}C]MPEP in rats with a unilateral lesion of the DA system, we have described a small enhancement on the side of the lesion in the striatum, cortex and hippocampus.[46] *Figure 2* illustrates the enhancement observed in the striatum, on the side of DA lesion, with three mGluR5 compounds in the 6-hydroxy-DA rat model of PD.

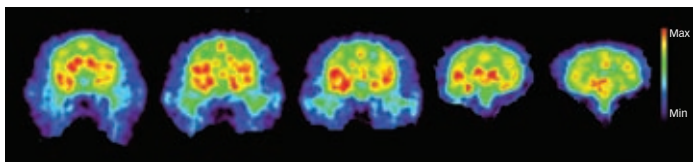
In naïve primates, the mGluR5 tracer [^{11}C]MPEPy rapidly accumulates in discrete cortical and subcortical regions (see *Figure 3*), including the premotor and cingulate cortices, superior temporal gyrus and limbic (paraentorhinal/amygdala/hippocampal) cortex, the nucleus accumbens, caudate, and putamen, the ventral thalamus, and the midbrain. This distribution matches the areas that have high mGluR5 mRNA expression in the rodent brain.[47] Following systemic administration of a neurotoxin, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), we found a significant enhancement of mGluR5 binding (~20%) in the motor regions of the striatum.[28] In the primate brain, the distribution of mGluR5 radiotracers corresponds well with DA-responsive areas (see *Figure 1*).

Figure 2: Color-coded Illustration of Dopaminergic and Glutamatergic Receptor Function in Normal (Spague Dawley) Rat Brain and 6-hydroxydopamine Lesioned Rat Model of Parkinson's Disease



[^{11}C]CFT (2β-carbomethoxy-3β-(4-fluorophenyl)tropane) is a sensitive marker for dopamine transporter; [^{11}C]raclopride is a marker for dopamine D2 receptor; [^{11}C]MPEP, [^{11}C]M-MPEP and [^{11}C]M-PEPy are markers for mGluR5. Note that after the unilateral administration of 6-OHDA into the right middle forebrain bundle there is a significant decrease in [^{11}C]CFT accumulation on the lesioned side. By contrast, [^{11}C]raclopride shows enhanced accumulation on the same side as an indication of compensatory dopamine receptor supersensitivity. Also, mGluR5 ligands show enhanced accumulation on the lesioned side as an indication of functionally enhanced glutamate release. Administered radioactivity (intravenously) was 0.8±0.2mCi. [^{11}C]CFT images illustrate accumulation at 25–40 minutes after administration of the radioligand, [^{11}C]raclopride at 10–25 minutes, and mGluR5 ligands correspondingly at 2–15 minutes.

Figure 3: Distribution of [^{11}C]M-PEPy Binding in a Normal Primate Brain (*Macaca fascicularis*) Shown at Five Coronal Levels



[^{11}C]M-PEPy binds to the nucleus caudate, putamen, amygdala, hippocampus, and thalamus. The cerebellar area does not show enhanced binding of [^{11}C]M-PEPy. Administered radioactivity (IV) was 9.5mCi and the images illustrate accumulation at five to 20 minutes after administration. Slice thickness is 1.25mm.

From an technical imaging point of view, we found that the accumulation of pyridine analogs into the brain mGluR5s is very fast (one to five minutes) and is followed by a fast washout, which is problematic, causing poor image quality because of statistically low counts. This makes them unfavorable for development for human studies in spite of their excellent *in vitro* pharmacological characteristics. Instead, benzonitrile derivatives have turned out to be promising ligands to target mGluR5 *in vivo* and are good candidates for human applications.[38,48]

mGluR5 Antagonists in Parkinson's Disease Therapy

From our *in vivo* studies we can conclude that the prevalent distribution of mGluR5 in the striatum and limbic structures supports their role in modulating DA- and glutamate-dependent signaling and synaptic plasticity within the basal ganglia cortico–subcortical loops. In PD, the death of DA neurons in the substantia nigra pars compacta causes a loss of DA in the basal ganglia. DA modulation of neurotransmission in the striatum and other basal ganglia structures is crucial to gate cortical and thalamic excitatory input through the direct and indirect pathways. We have found an upregulation of mGluR5 following DA denervation in animal models of PD,[28,46] which probably represents a local compensatory mechanism, directed to dampen an excessive excitability of striatopallidal neurons. Drugs targeting the mGluR5 might provide new approaches by selectively reducing glutamate transmission in the areas where it is abnormally enhanced. While current surgical approaches in PD aimed at reducing or interrupting transmission in the indirect pathway are quite effective, they are invasive and expensive, and mGluR5 antagonists could provide an alternative approach.

In addition, we and others have found enhanced mGluR5 expression in several brain areas related to the indirect pathway in models of L-3,4-dihydrophenylalanine (L-DOPA)-induced dyskinesias, and some studies have shown promising therapeutic results after using mGluR5 antagonists.[15,49] At present is unclear whether pharmacological normalization of DA levels in PD patients is capable of modifying the adaptive post-synaptic changes and enhancement of mGluR5. If changes in striatal mGluR5 are sustained or evolve independently, it may be necessary to target specifically this pathway in order to obtain full reversal of PD symptomatology and L-DOPA-induced dyskinesias.

Lastly, the possibility of modifying the progression of PD with these compounds is appealing, but more work needs to be undertaken, with more selective modulators, to validate mGluR5s in the basal ganglia circuitry as targets for neuroprotective purposes. ■

Rosario Sanchez-Pernaute, MD, PhD, is a neurologist, neuroscientist, and Director of the Cell Therapy Program for Parkinson's Disease (PD) at Inbiomed Foundation in San Sebastian in Spain, where she is working on the development of novel cellular and molecular therapies for PD. Her research is funded by competitive grants from the Spanish Ministry of Science and Innovation (MICINN) and the Basque government and is focused on stem cells for cell replacement in PD and the molecular modeling of dyskinesias. Dr Sanchez-Pernaute has a long-standing research interest in functional neuroimaging (positron emission tomography [PET] and functional magnetic resonance imaging [fMRI]) for *in vivo* evaluation of novel therapeutic approaches in experimental models of PD and for the study of disease mechanisms and plasticity.

Anna-Liisa Brownell, PhD, is an Associate Professor and Director of the Experimental Positron Emission Tomography (PET) Laboratory in the Department of Radiology at Massachusetts General Hospital and Harvard University, where she is also a medical physicist and neuroscientist working on the development of novel functional imaging techniques for neurodegenerative diseases. Her research is primarily focused on Parkinson's disease (PD) and she has developed several experimental animal models, imaging ligands, and techniques, especially using PET to investigate neural degeneration and regeneration. Dr Brownell's recent activities include the development of several PET imaging ligands for metabotropic glutamate receptors to investigate their role in glutamatergic neurotransmission. All of her work has been supported by research grants from the National Institutes of Health (NIH), Department of Energy, and Department of Defense.

1. Zhang H, Sulzer D, *J Neurosci*, 2003;23(33):10585–92.
2. Bassell GJ, Warren ST, *Neuron*, 2008;60(2):201–14.
3. Muddashetty RS, et al., *J Neurosci*, 2007;27(20):5338–48.
4. Repicky SE, Broadie K, *J Neurophysiol*, 2009;101(2):627–87.
5. Waung MW, et al., *Neuron*, 2008;59(1):84–97.
6. Bordi F, Ugolini A, *Prog Neurobiol*, 1999;59(1):55–79.
7. Slassi A, et al., *Curr Topics Med Chem*, 2005;5(9):897–911.
8. Armentero MT, et al., *Neurobiol Dis*, 2006;22(1):1–9.
9. Breysse N, et al., *J Neurosci*, 2003;23(23):8302–9.
10. Breysse N, et al., *J Neurosci*, 2002;22(13):5669–78.
11. Gubellini P, et al., *Prog Neurobiol*, 2004;74(5):271–300.
12. Pisani A, et al., *Neuropharmacology*, 2003;45(1):45–56.
13. Lea PMt, Faden AI, *CNS Drug Reviews*, 2006;12(2):149–66.
14. Dekundy A, et al., *Brain Res Bull*, 2006;69(3):318–26.
15. Mela F, et al., *J Neurochem*, 2007;101(2):483–97.
16. Samadi P, et al., *Neurobiol Aging*, 2008;29(7):1040–51.
17. Picconi B, et al., *Nat Neurosci*, 2003;6(5):501–6.
18. Vernon AC, et al., *J Neurochem*, 2007;103(3):1075–91.
19. Bliss TVP, Collingridge GL, *Nature*, 1993;361:31–9.
20. Fonnum F, *J Neurochem*, 1984;42(1):1–11.
21. Nicholls DG, *Prog Brain Res*, 1998;116:15–22.
22. Pin J-P, Duvoisin R, *Neuropharmacology*, 1995;34:1–26.
23. Schoepp DD, et al., *J Neurochem*, 1994;63:769–72.
24. Tanabe Y, et al., *J Neurosci*, 1997;13:1372–8.
25. Conn P, Pin JP, *Annu Rev Pharmacol Toxicol*, 1997;37:205–38.
26. Abe T, et al., *J Biol Chem*, 1992;267(19):13361–8.
27. Morelli M, et al., *Prog Neurobiol*, 2007;83(5):293–309.
28. Sanchez-Pernaute R, et al., *Neuro Image*, 2008;42(1):248–51.
29. Schoepp D, et al., *Neuropharmacology*, 1999;38:1431–76.
30. Carroll FI, *Ann New York Acad Sci*, 2008;1141:221–32.
31. Layton ME, *Curr Topics Med Chem*, 2005;5(9):859–67.
32. Kew JN, *Pharmacol Ther*, 2004;104(3):233–44.
33. Annoura H, et al., *Bioorg Med Chem Lett*, 1996;6:763–6.
34. Ritzen A, et al., *Basic Clin Pharmacol Toxicol*, 2005;97(4):202–13.
35. Williams DL Jr, Lindsley CW, et al., *Curr Topics Med Chem*, 2005;5(9):825–46.
36. Hamill TG, et al., *Synapse*, 2005;56(4):205–16.
37. Patel S, et al., *Nuc Med Biol*, 2007;34(8):1009–17.
38. Wang JQ, et al., *Synapse*, 2007;61(12):951–61.
39. Varney MA, et al., *J Pharmacol Exp Ther*, 1999;290(1):170–81.
40. Gasparini F, et al., *Neuropharmacology*, 1999;38(10):1493–1503.
41. Cosford N, et al., *Bioor Med Chem Lett*, 2003;13:351–4.
42. O'Brien JA, et al., *J Pharmacol Exp Ther*, 2004;309(2):568–77.
43. Kinney GG, et al., *J Pharmacol Exp Ther*, 2005;313(1):199–206.
44. Lindsley CW, et al., *J Med Chem*, 2004;47(24):5825–8.
45. Gerfen CR, et al., *Science*, 1990;250(4986):1429–32.
46. Pellegrino D, et al., *J Nucl Med*, 2007;48(7):1147–53.
47. Messenger MJ, et al., *Neuropharmacology*, 2002;43(2):261–71.
48. Brown AK, et al., *J Nucl Med*, 2008;49(12):2042–8.
49. Levandis G, et al., *Neurobiol Dis*, 2008;29(1):161–8.
50. Jenkins BG, et al., *J Neurosci*, 2004;24(43):9553–60.