Bioorganic & Medicinal Chemistry Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Carbamate substituted 2-amino-4,6-diphenylpyrimidines as adenosine receptor antagonists

Sarel J. Robinson [a,b], Jacobus P. Petzer [a,b], Amanda L. Rousseau [c], Gisella Terre'Blanche [a,b], Anél Petzer [b], Anna C. U. Lourens [a,b,*]

[a] Pharmaceutical Chemistry, School of Pharmacy, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa
[b] Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa
[c] Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, Private Bag 3, PO WITS 2050, South Africa

ARTICLE INFO

Article history:
Received 18 November 2015
Revised 3 January 2016
Accepted 4 January 2016
Available online xxxx

Keywords:
2-Aminopyrimidine
Adenosine A_1 and A_{2A} receptor
Dual
Antagonist

ABSTRACT

A novel series of carbamate substituted 2-amino-4,6-diphenylpyrimidines was evaluated as potential dual adenosine A_1 and A_{2A} receptor antagonists. The majority of the synthesised compounds exhibited promising dual affinities, with A_1K_i values ranging from 0.175 to 10.7 nM and $A_{2A}K_i$ values ranging from 1.58 to 451 nM. The in vivo activity illustrated for 3-(2-amino-6-phenylpyrimidin-4-yl)phenyl morpholine-4-carboxylate (**4c**) is indicative of the potential of these compounds as therapeutic agents in the treatment of Parkinson's disease, although physicochemical properties may require optimisation.

Parkinson's disease (PD) is a chronic neurodegenerative disease that affects over 1% of the world population. PD is an age related disease, and with life expectancy increasing worldwide, will continue to present a huge social and economic burden in the future.[1] Patients with PD mainly suffer from a progressive loss of motor function, but non-motor symptoms, such as cognitive impairment and depression often occur.[2] The motor symptoms of the disease can be attributed to the deterioration of dopaminergic neurons in the striatum, resulting in a significant loss of dopamine in this region.[3] There is still no cure for PD, but the dopaminergic therapies used clinically are reasonably effective in managing the symptoms during the early stages of the disease. Long-term treatment with dopaminergic therapies however, is associated with several undesirable side effects such as loss of drug efficacy, dyskinesia and depression.[4]

Adenosine is an endogenous ligand that acts as a neurotransmitter in the brain through the activation of its G-protein coupled receptors, namely the A_1, A_{2A}, A_{2B} and A_3 receptors.[5] Recently, dual targeted antagonism of adenosine A_1 and A_{2A} receptors has emerged as a promising non-dopaminergic alternative for the treatment of neurodegenerative diseases such as Parkinson's disease.[6,7] The appeal of adenosine A_{2A} receptors as a target in movement disorders is due to their distinct localisation in the striatum as well as their unique integrative action with dopamine D_2 receptors.[8] Adenosine A_{2A} receptors and dopamine D_2 receptors have a mutual antagonistic interaction, which means that antagonism of adenosine A_{2A} receptors would lead to enhanced D_2 signalling, providing a rationale for their use in the symptomatic treatment of PD.[9] The use of A_{2A} antagonists adjunctive to current dopaminergic therapy may be beneficial since the dosage of the dopaminergic drugs administered could potentially be lowered.[10] This may reduce the occurrence of dyskinesia and other side effects associated with dopaminergic drugs.[11] Furthermore, it has been suggested that A_{2A} antagonism may halt the progression of PD as preclinical evidence exists of its possible neuroprotective benefits.[12,13] Depression is a co-morbidity that often decreases quality of life in PD patients, especially in the later stages of the disease. A recent study illustrated the antidepressant effect of the known A_{2A} antagonist, KW6002, indicating an additional advantage of A_{2A} antagonism.[14]

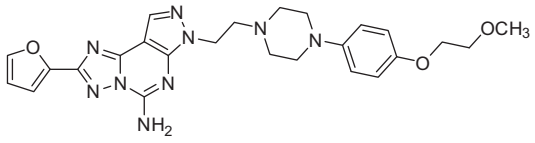
The brain distribution of the adenosine A_1 receptor on the other hand, is more widespread than that of the A_{2A} receptor, with high levels expressed in the striatum, hippocampus and neocortex.[15] Similar to adenosine A_{2A} antagonism, antagonism of A_1 receptors have been shown to result in activation of motor function in animals,[16,17] and may thus decrease motor deficiencies experienced in PD. It has also been reported that the antagonism of the A_1 receptor may enhance cognitive ability,[18–21] and since a decline in cognition is often observed in PD patients over time,

* Corresponding author. Tel.: +27 18 2992266; fax: +27 18 2994243.
E-mail address: arina.lourens@nwu.ac.za (A.C.U. Lourens).

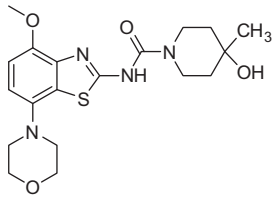
http://dx.doi.org/10.1016/j.bmcl.2016.01.004
0960-894X/

A_1 antagonism may thus be advantageous.[22] Dual antagonism of A_1 and A_{2A} receptors thus has the potential of addressing the multifactorial nature of PD symptoms with less dopaminergic side-effects than generally experienced with current therapies.

The best known adenosine receptor antagonists are the xanthines, such as caffeine and theophylline, which as a chemical class, have been reviewed extensively.[18] Several heterocyclic compounds have also progressed to clinical trials, and include compounds such as preladenant (**1**) and tozadenant (**2**).[23,24]

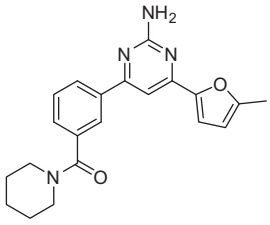


1

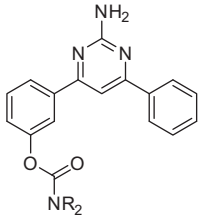


2

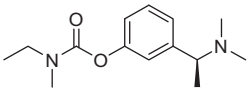
Of particular interest to our group was the fact that the 2-aminopyrimidine motif often occurred in heterocycles with adenosine A_{2A} and/or A_1 affinity.[7,25–29] Based on the aforementioned results, we set out to synthesise a small library of 2-aminopyrimidines to investigate the potential of these compounds as dual adenosine A_1 and A_{2A} antagonists. In a preliminary study reported recently, we synthesised amide derivative **3** which exhibited high dual affinity (A_1K_i = 9.54 nM; A_{2A} K_i = 6.34 nM) and in vivo activity.[30]



3



4



5

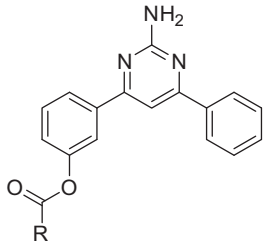
In order to further investigate the affinities of this class of compounds for adenosine receptors, we decided to synthesise a series of carbamate substituted 2-amino-4,6-diphenylpyrimidines (**4**). The carbamate moiety is often present in therapeutic agents, such as rivastigmine (**5**), an acetylcholinesterase inhibitor, which is used in the treatment of Alzheimer's disease. This amide-ester hybrid generally displays very good chemical and proteolytic stability and may increase permeability across cellular membranes.[31] The addition of the extra oxygen in structures such as **4**, alters the position of both the carbonyl and nitrogen groups and has the potential to change the hydrogen bonding between the compound and the receptor binding site, thus changing the affinity and possibly the selectivity of these compounds in comparison with the amide derivatives (**3**). It was also decided to replace the methyl furan substituent on position 4 with a phenyl ring, as this simplified the synthesis. Gratifyingly, preliminary results indicated that this change did not alter affinity to a significant degree.

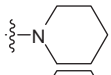
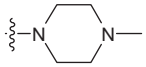
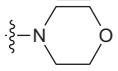
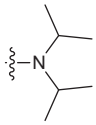
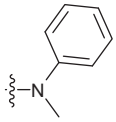
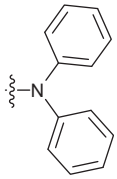
Nine carbamate substituted 2-amino-4,6-diphenylpyrimidines (Table 1) were successfully synthesised as indicated in Scheme 1. Firstly, 3-hydroxybenzaldehyde (**6**) was condensed with acetophenone (**7**), yielding chalcone (**8**), which was reacted with commercially available carbamoyl chlorides to obtain carbamates (**9a**–**i**).[32] Cyclisation was carried out with guanidine hydrochloride and sodium hydride in *N,N*-dimethylformamide yielding the desired 2-aminopyrimidines (**4a**–**i**) in low yields.[33] Initially, three equivalents of sodium hydride were used in the cyclisation step, but this resulted in cleavage of the carbamate group. This problem was overcome by reducing the number of molar equivalents of NaH used in the reaction. The structures of all synthesised compounds (Table 1) were confirmed by nuclear magnetic resonance spectroscopy and mass spectrometry, while purity was assessed by HPLC (see Supporting Information).

Radioligand binding assays were performed to assess the binding of synthesised compounds to adenosine receptors. The radioligands used were 1,3-[^{3}H]-dipropyl-8-cyclopentylxanthine ([^{3}H] DPCPX) for adenosine A_1 receptors, and [^{3}H]5'-*N*-ethylcarboxamide-adenosine ([^{3}H]NECA) for adenosine A_{2A} receptors. Striata from male Sprague–Dawley rats were used as receptor source for A_{2A} binding studies, while whole brains were utilised for A_1 binding studies (Ethics number: NWU-0035-10-A5). IC_{50} values were obtained from sigmoidal-dose response curves as generated by the Prism 5 software package (GraphPad) and the K_i values were calculated from the IC_{50} values using the Cheng–Prusoff equation.[34] Results from the receptor binding studies are presented in Table 1.

The results reveal that most of these compounds have potent dual affinity for both receptor subtypes, although affinities are generally higher for the adenosine A_1 receptor compared to the adenosine A_{2A} receptor. Compounds **4a**, **4b** and **4c** are the most promising candidates for dual antagonistic activity with both A_1K_i and $A_{2A}K_i$ values below 10 nM and selectivity indices of 4.6, 0.8 and 1.3, respectively. Compounds **4f** and **4g** in particular exhibit high affinities for the adenosine A_1 receptors, with K_i values of 0.468 and 0.175 nM, respectively. As exemplified by compounds **4a**, **4b** and **4c**, substitution with six-membered saturated cyclic carbamate substituents appears to yield optimal A_{2A} receptor affinity. Interestingly, although compound **4i** with diphenyl substitution still has high affinity for the adenosine A_1 receptor (K_i = 10.7 nM), its affinity for the adenosine A_{2A} receptor is comparatively weak with a K_i value of 451 nM. The size of the carbamate

Table 1
Adenosine receptor affinities (K_i) of the synthesised carbamates **4a–4i**

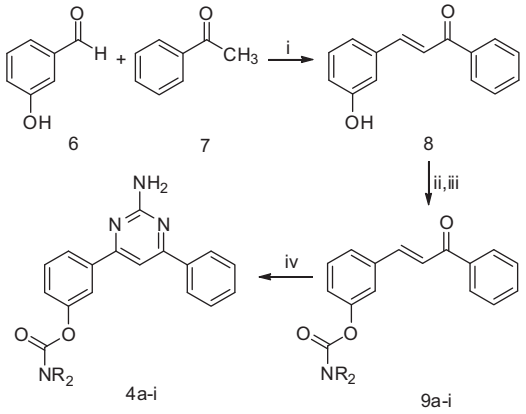


Compound	R	A_1[a] K_i (nM)	A_{2A}[a] K_i (nM)	SI[b] (A_{2A}/A_1)
4a		1.95 ± 0.278	8.94 ± 0.259	4.6
4b		2.04 ± 0.002	1.58 ± 0.311	0.8
4c		2.65 ± 0.059	3.50 ± 0.030	1.3
4d		0.835 ± 0.088	24.8 ± 6.61	30
4e		2.06 ± 0.135	51.9 ± 3.07	25
4f		0.468 ± 0.057	22.8 ± 1.75	49
4g		0.175 ± 0.017	12.1 ± 1.35	69
4h		1.07 ± 0.079	39.9 ± 4.77	37
4i		10.7 ± 0.431	451 ± 143	42
CPA[c]		8.48 ± 0.302		
ZM241385[d]			1.20 ± 0.387	

[a] All values are expressed as the mean ± SEM of duplicate determinations.
[b] Selectivity index ($A_{2A}K_i/A_1K_i$).
[c] N^6-Cyclopentyladenosine, a known adenosine A_1 agonist, used as positive control for A_1 receptor affinity – literature K_i = 7.9 nM.[35]
[d] A known adenosine A_{2A} receptor antagonist, used as positive control for A_{2A} affinity – literature K_i = 2 nM.[18]

substituent therefore appears to affect affinity to a greater degree for A_{2A} receptors, where substitution with groups that are either too small (e.g., **4e**) or too large (e.g., **4h** and **4i**) appears to be detrimental for affinity. By comparison, the A_1 receptors are able to accommodate larger carbamate substituents without loss of binding affinity.

Molecular modelling studies were performed in an attempt to rationalise the results obtained with the radioligand binding assays. The crystal structure of the human A_{2A} receptor crystallised with the known adenosine A_{2A} antagonist ZM241385 (PDB code: 3EML) was used as protein model. All compounds were successfully docked into the binding site using the C-DOCKER function of Discovery studio 3.1 (Accelrys). Hydrophobic (π–π) interactions were observed between the three-membered ring system and



Scheme 1. Synthesis. Reagents and conditions: (i) NaOH, 1 M (2 equiv), MeOH, 90 °C, 5 days (60%); (ii) K_2CO_3 (2 equiv), CH_3CN, rt, 30 min; (iii) carbamoyl chloride (1.2 equiv), reflux, 90 °C, overnight (70–90%); (iv) Guanidine hydrochloride (1.5 equiv), NaH (1.5 equiv), DMF, 110 °C, overnight (15–30%).

Phe168 for all compounds. Intermolecular hydrogen bonding interactions were also present between the exocyclic amino group of the pyrimidine ring and Glu169 as well as Asn253 (Fig. 1) for most derivatives. These interactions are believed to be responsible for anchoring the aminopyrimidine in the binding site and are similar to binding interactions previously determined for other 2-aminopyrimidine antagonists.[35] There was another noticeable hydrogen bond interaction between Glu169 and the carbamate carbonyl of most compounds. When ranking the compounds according to C-DOCKER- and C-DOCKER-interaction energies, it was observed that compound **4i** exhibited the least favourable values. This corresponds with the results obtained in the radioligand binding studies, where compound **4i** also exhibited the weakest affinity for the A_{2A} receptor among the compounds synthesised (K_i = 451 nM). Visual inspection of the lowest energy pose of compound **4i** reveals that the hydrogen bonding interactions with Glu169 and Asn253 are absent and that it has docked 'upside down' in the binding site when compared to the rest of the synthesised compounds (Fig. 2A), as well as ZM241385 (Fig. 2B). This supports the theory that A_{2A} receptor affinity is influenced by molecular size, with the receptor binding site unable to accommodate this large compound (**4i**). These molecular docking results provide, at least in part, some explanation for the results obtained with the radioligand binding assays.

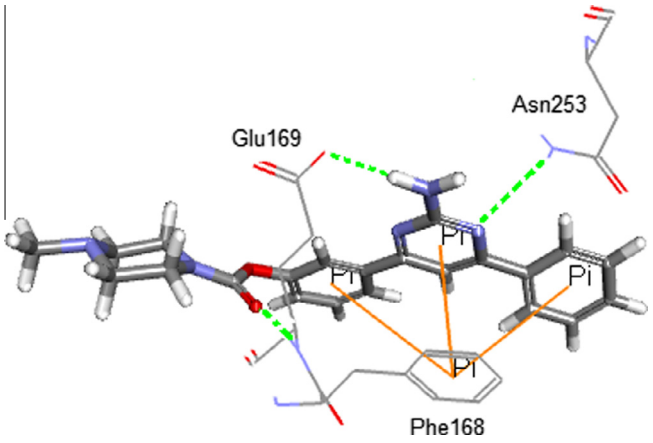


Figure 1. Compound **4b** docked in the binding site of the human A_{2A} receptor. Hydrophobic interactions are indicated in orange while intermolecular hydrogen bonding are indicated in green.

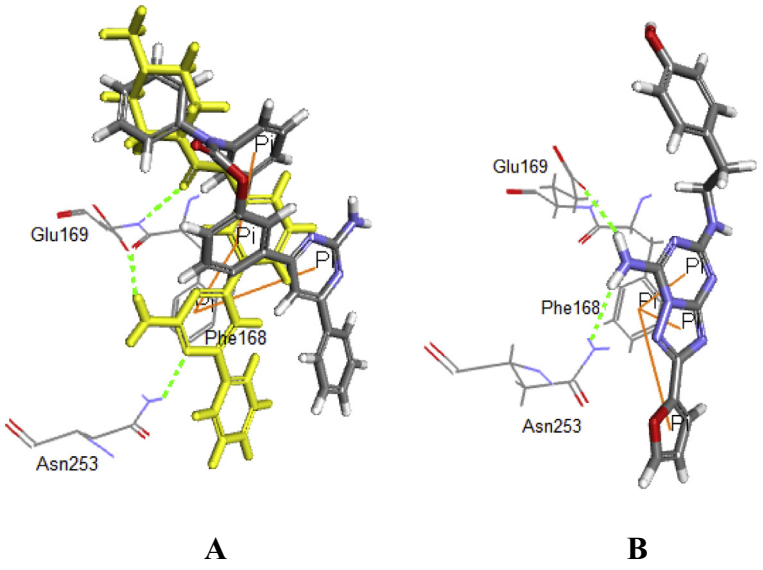


Figure 2. A. Compounds **4i** (in colour) and **4b** (in yellow) docked in the binding site of the human A_{2A} receptor. B. ZM241385 bound in the binding site of the human A_{2A} receptor.[36] Hydrophobic interactions are indicated in orange while intermolecular hydrogen bondings are indicated in green.

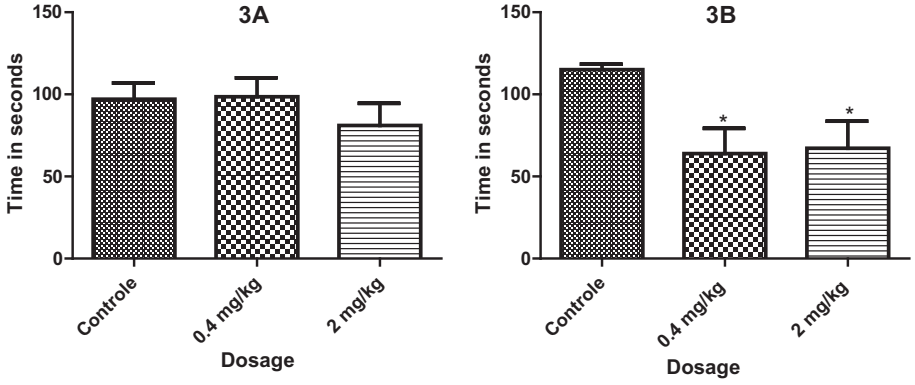


Figure 3. A. Graph illustrating no significant reduction of catalepsy for both concentrations of compound **4b**. B. Graph illustrating a significant attenuation of catalepsy with both concentrations of compound **4c**. (*Indicates significant differences compared with the haloperidol + vehicle control group as determined by one-way ANOVA [$F(2,24) = 3.97$ ($p = 0.032$) followed by Dunnet's post test with $p = 0.03–0.05$).

The most promising dual affinity compounds (**4b** and **4c**) were subsequently selected for in vivo screening, using the reversal of haloperidol induced catalepsy with the standard bar test as an indication of the potential of these compounds to act as antagonists at A_{2A} receptors.[16] Drug naïve Sprague–Dawley rats were divided into three groups each receiving intraperitoneal (i.p.) injections of haloperidol (5.0 mg/kg) to induce catalepsy. Thirty minutes later the animals from group 1 received DMSO (control group) and the other two groups received 0.4 and 2 mg/kg of the test compound, respectively. Catalepsy was measured 60 minutes after the first injection.

The results obtained after i.p. administration of the two selected compounds **4b** and **4c** are shown in Figure 3. Disappointingly, compound **4b** with the highest dual affinity in vitro, did not reverse catalepsy and appeared to be inactive in vivo (Fig. 3A). Contrastingly, after i.p. administration of compound **4c**, a significant reduction in catalepsy was observed when compared to the control group (Fig. 3B), which is indicative of adenosine A_{2A} antagonism. It is postulated that the negative results obtained with compound **4b** are due to unfavourable physicochemical properties, such as poor water solubility, resulting in the precipitation of the compound upon i.p. injection.

To obtain support for this theory, solubility and log *D* values were determined for compound **4b** in order to assess its suitability as a drug candidate. The octanol-buffer partition coefficient was determined with the shake flask method, while water solubility was determined by shaking an excessive amount of **4b** in water at 37 °C. For the Log *D* study potassium phosphate buffer (pH 7.4) was selected for the hydrophilic phase and *n*-octanol for the hydrophobic phase. Results are shown in Table 2.

It is generally accepted that the ideal log *D* value for a drug should be between 1 and 3,[37] thus allowing solubility in blood plasma while at the same time ensuring that the compound is lipophilic enough to cross the blood brain-barrier. A Log *D* value of 4.03 is thus rather lipophilic, and this combined with the low aqueous solubility may, at least in part, explain the lack of in vivo activity of **4b**. Other physicochemical and pharmacokinetic properties may however also contribute. For example, a high degree of plasma

Table 2
The Log *D* and solubility of compound **4b**

Log *D*	Solubility (μM)
4.03 ± 0.197	0.22 ± 0.072

and tissue binding of **4b** would limit exposure to the A_{2A} receptor in the brain, thus preventing a physiological response. A more detailed pharmacokinetic study is required to assess the exposure of this compound to the brain.

This study provides evidence that carbamate substitution of the 2-amino-4,6-diphenylpyrimidine scaffold results in compounds with potent dual affinity for both adenosine A_1 and A_{2A} receptors. Size of the substituent appears to affect adenosine A_{2A} affinity to a larger extent than A_1 affinity. Diphenyl substitution on the carbamate nitrogen in particular appears to be detrimental, partly due to a loss of important binding interactions as illustrated with molecular docking studies. The physicochemical properties of these compounds would have to be optimised in order to improve in vivo activity and applicability as therapeutic agents in the treatment of PD.

Acknowledgements

We gratefully acknowledge the assistance of Dr. Johan Jordaan and Mr. André Joubert of the SASOL Centre for Chemistry, North-West University for recording of NMR and mass spectra as well as Prof Jan du Preez for HPLC analyses. We are also thankful to Ms Madelein Geldenhuys for her assistance with the radioligand binding studies. The financial assistance of the North-West University, Medical Research Council (MRC) and the National Research Foundation (UID 76308) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.01.004.

References and notes

1. Dorsey, E. R.; Constantinescu, R.; Thompson, J. P.; Biglan, K. M.; Holloway, R. G.; Kieburtz, K. *Neurology* **2007**, *68*, 384.
2. Lazano, A. M.; Lang, A. E.; Hutchison, W. D.; Dostroysky, J. O. *Curr. Opin. Neurobiol.* **1998**, *8*, 783.
3. Schwarzschild, M. A.; Agnati, L.; Fuxe, K.; Chen, J. F.; Morelli, M. *Trends Neurosci.* **2006**, *29*, 647.
4. Dauer, W.; Predborski, S. *Neuron* **2003**, *39*, 889.
5. Abbracchio, M. P.; Cattabeni, F. *Ann. N.Y. Acad. Sci.* **1999**, *890*, 79.
6. Atack, J. R.; Shook, B. C.; Rassnick, S.; Jackson, P. F.; Rhodes, K.; Drinkenburg, W. H.; Agnaou, A.; TeRiele, P.; Langlois, X.; Hrupka, B.; De Haes, P.; Hendrickx, H.; Aerts, N.; Hens, K.; Wellens, A.; Vermeire, J.; Megens, A. A. *ACS Chem. Neurosci.* **2014**, *15*, 1005.
7. Shook, B. C.; Rassnick, S.; Hall, D.; Rupert, K. C.; Heintzelman, G. R.; Hansen, K.; Chakravarty, D.; Bullington, J. L.; Scannevin, R. H.; Magliaro, B.; Westover, L.; Carroll, K.; Lampron, L.; Russell, R.; Branum, S.; Wells, K.; Damon, S.; Youells, S.; Li, X.; Osbourne, M.; Demarest, K.; Tang, Y.; Rhodes, K.; Jackson, P. F. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2864.
8. Xu, K.; Bastia, E.; Schwarzschild, M. *Pharmacol. Ther.* **2005**, *105*, 267.
9. Pollack, A. E.; Fink, J. S. *Neuroscience* **1995**, *68*, 721.
10. Kase, H.; Mori, A.; Jenner, P. *Drug Discov. Today Ther. Strat.* **2004**, *1*, 51.
11. Bibbiani, F.; Oh, J. D.; Petzer, J. P.; Castagnoli, N., Jr.; Chen, J. F.; Schwarzschild, M. A.; Chase, T. N. *Exp. Neurol.* **2003**, *184*, 285.
12. Chen, J. F.; Xu, K.; Petzer, J. P.; Staal, R.; Xu, Y. H.; Beilstein, M.; Sonsalla, P. K.; Castagnoli, K.; Castagnoli, N., Jr.; Schwarzschild, M. A. J. *Neuroscience* **2001**, *21*, 143.
13. Ikeda, K.; Kurokawa, M.; Aoyama, S.; Kuwana, Y. J. *Neurochemistry* **2002**, *80*, 262.
14. Yamada, K.; Kobayashi, M.; Shiozaki, S.; Ohta, T.; Mori, A.; Jenner, P.; Kanda, T. *Psychopharmacology* **2014**, *14*, 2839.
15. Fredholm, B. B.; Ijzerman, A. P.; Jacobson, K. A.; Klotz, K. N.; Linden, J. M. *Pharmacol. Rev.* **2001**, *53*, 527.
16. Trevitt, J.; Vallance, C.; Harris, A.; Goode, T. *Pharmacol. Biochem. Behav.* **2009**, *92*, 521.
17. Antoniou, K.; Papadopoulou-Daifoti, Z.; Hyphantis, T.; Papathanasiou, G.; Bekris, E.; Marselos, M.; Panlilio, L.; Müller, C. E.; Goldberg, S. R.; Ferré, S. *Psychopharmacology* **2005**, *183*, 154.
18. Muller, C. E.; Scior, T. *Pharm. Acta Helv.* **1993**, *68*, 77.
19. DeNinno, M. P. *Annu. Rep. Med. Chem.* **1998**, *33*, 111.
20. Pires, V. A.; Pamplona, F. A.; Pandolfo, P.; Fernandes, D.; Prediger, R. D. S.; Takahashi, R. N. *Behav. Pharmacol.* **2009**, *20*, 134.
21. Davidson, D. A.; Weidley, E. *Life Sci.* **1976**, *18*, 1279.
22. Mihara, T.; Mihara, K.; Yarimizu, J.; Mitani, Y.; Matsuda, R.; Yamamoto, H.; Aoki, S.; Akahane, A.; Iwashita, A.; Matsuoka, N. *J. Pharmacol. Exp. Ther.* **2007**, *323*, 708.
23. Hauser, R. A.; Cantillon, M.; Pourcher, E.; Micheli, F.; Mok, V.; Onofrj, M.; Huyck, S.; Wolski, K. *Lancet Neurol.* **2011**, *10*, 221.
24. Hauser, R. A.; Olanow, C. W.; Kieburtz, K. D.; Pourcher, E.; Docu-Axelerad, A.; Lew, M.; Kozyolkin, O.; Neale, A.; Resburg, C.; Meya, U.; Kenney, C.; Bandak, S. *Lancet Neurol.* **2014**, *13*, 767.
25. Van Veldhoven, J. P. D.; Chang, L. C. W.; von Frijtag, J. K.; Kunzel, D.; Mulder-Krieger, T.; Struensee-Link, R.; Beukers, M. W.; Brussee, J.; IJzerman, A. P. *Bioorg. Med. Chem.* **2008**, 2741.
26. Matasi, J. J.; Caldwell, P.; Hao, J.; Neustadt, B.; Larik, L.; Foster, C. J.; Lachowicz, J.; Tulshian, D. B. *Bioorg. Med. Chem.* **2005**, *15*, 1333.
27. Shook, B. C.; Rassnick, S.; Chakravarty, D.; Wallace, N.; Ault, M.; Crooke, J.; Barbay, J. K.; Wang, A.; Leonard, K.; Powell, M. T.; Alford, V.; Hall, D.; Rupert, K. C.; Heintzelman, G. R.; Hansen, K.; Bullington, J. L.; Scannevin, R. H.; Carroll, K.; Lampron, L.; Westover, L.; Russell, R.; Branum, S.; Wells, K.; Damon, S.; Youells, S.; Beauchamp, D.; Li, X.; Rhodes, K.; Jackson, P. F. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2868.
28. Shook, B. C.; Rassnick, S.; Osborne, M. C.; Davis, S.; Westover, L.; Boulet, J.; Hall, D.; Rupert, K. C.; Heintzelman, K.; Hansen, D.; Chakravarty, J. L.; Bullington, R.; Russell, S.; Branum, K. M.; Wells, S.; Damon, G. R.; Youells, S.; Li, X.; Beauchamp, D. A.; Palmer, D.; Reyes, M.; Demarest, K.; Tang, Y.; Rhodes, K.; Jackson, P. F. *J. Med. Chem.* **2010**, *53*, 8104.
29. Shook, B. C.; Rassnick, S.; Wallace, N.; Crooke, J.; Ault, M.; Chakravarty, D.; Barbay, J. K.; Wang, A.; Powell, M. T.; Leonard, K.; Alford, V.; Scannevin, R. H.; Carroll, K.; Lampron, L.; Westover, L.; Lim, H. K.; Russell, R.; Branum, S.; Wells, K. M.; Damon, S.; Youells, S.; Li, X.; Beauchamp, D. A.; Rhodes, K.; Jackson, P. F. *J. Med. Chem.* **2012**, *55*, 1402.
30. Robinson, S. J.; Petzer, J. P.; Rousseau, A. L.; Terre'Blanche, G.; Petzer, A.; Lourens, A. C. U. *Eur. J. Med. Chem.* **2015**, *104*, 177.
31. Ghosh, A. K.; Brindisi, M. *J. Med. Chem.* **2015**, *58*, 2895.
32. Bacon, E.R.; Singh, B. Patent, US5736548 A, 1998.
33. Sharma, M.; Chaturvedi, V.; Manju, Y. K.; Bhatnagar, S.; Srivastava, K.; Puri, S. K.; Chauhan, P. M. S. *Eur. J. Med. Chem.* **2009**, *44*, 2081.
34. Bruns, R. F.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331.
35. Zhang, L.; Liu, T.; Wang, X.; Li, G.; Li, Y.; Yang, L.; Wang, Y. *BioSystems* **2014**, *115*, 13.
36. Jaakola, V. P.; Griffith, M. T.; Hanson, M. A.; Cherezov, V.; Chien, E. Y. T. *Science* **2008**, *322*, 1211.
37. Kerns, E. H.; Di, L. *Maryland* **2008**, *3*, 45.