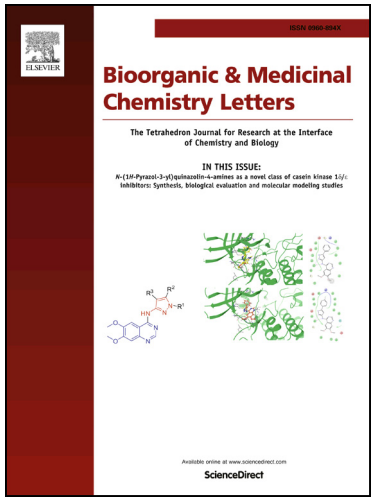
Accepted Manuscript



Imidazo[1,2-α]pyridines possess adenosine A_1 receptor affinity for the potential treatment of cognition in neurological disorders.

Roslyn Lefin, Mietha M van der Walt, Pieter J Milne, Gisella Terre'Blanche

PII: S0960-894X(17)30778-3
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.07.071
Reference: BMCL 25184

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 15 May 2017
Revised Date: 26 July 2017
Accepted Date: 27 July 2017

Please cite this article as: Lefin, R., van der Walt, M.M., Milne, P.J., Terre'Blanche, G., Imidazo[1,2-α]pyridines possess adenosine A_1 receptor affinity for the potential treatment of cognition in neurological disorders., *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.07.071

Imidazo[1,2-α]pyridines possess adenosine A_1 receptor affinity for the potential treatment of cognition in neurological disorders.

Roslyn Lefin [a], Mietha M van der Walt [b] Pieter J Milne [a,b] and Gisella Terre'Blanche [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, School of Pharmacy, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

Abstract

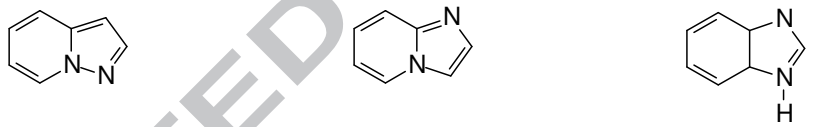
Previous research has shown that bicyclic 6:5-fused heteroaromatic compounds with two N-atoms have variable degrees of adenosine A_1 receptor antagonistic activity. Prompted by this imidazo[1,2-α]pyridine analogues were synthesized and evaluated for their adenosine A_1 and A_{2A} receptor affinity via radioligand binding studies and subjected to a GTP shift assay to determine its adenosine A_1 receptor agonistic or antagonistic functionality. Imidazo[1,2-α]pyridine, the parent scaffold, was found devoid of affinity for the adenosine A_1 and A_{2A} receptors. The influence of substitution on position C2 showed no improvement for either adenosine A_1 or A_{2A} receptor affinity. The addition of an amino or a cyclohexylamino group to position C3 also showed no improvement of adenosine A_1 or A_{2A} receptor affinity. Surprisingly *para*-substitution on the phenyl ring at position C2 in combination with a cyclohexylamino group at position C3 led to adenosine A_1 receptor affinity in the low micromolar range with compound **4d** showing: (1) the highest affinity for the adenosine A_1 receptor with a K_i value of 2.06 μM and (2) adenosine A_1 receptor antagonistic properties. This pilot study concludes that *para*-substituted 3-cyclohexylamino-2-phenyl-imidazo[1,2-α]pyridine analogues represent an interesting scaffold to investigate further structure-activity relationships in the design of novel imidazo[1,2-α]pyridine-based adenosine A_1 receptor antagonists for the treatment of neurodegenerative disorders.

Keywords: Alzheimer's disease, Parkinson's disease, imidazo[1,2-α]pyridine analogues, Adenosine A_1 and A_{2A} receptor antagonists

*Corresponding author. *Tel:* +27 18 2992264; *fax:* +27 18 2994243; *E-mail address*: Gisella.Terreblanche@nwu.ac.za (G. Terre'Blanche).

Adenosine has many physiological functions throughout the body as well as a role in numerous nervous system disorders including cognitive disorders, epilepsy, ischemia, stroke, Alzheimer's disease (AD) and Parkinson's disease (PD) [1]. The effects of adenosine in neurodegenerative disorders are predominately mediated by the A_1 adenosine receptors (ARs) expressed in the hippocampus and the A_{2A} ARs expressed in the striatum [2–4]. The A_{2A} ARs are important for motor function [5] and display neuroprotective [6] and antidepressant effects [7], whereas the A_1 ARs play an important role in cognitive function [8]. Antagonism of the A_1 AR represents a beneficial strategy in the treatment of AD and PD since they have cognitive enhancing abilities that are still inadequately treated with available treatment in both disorders. Furthermore, the A_1 and A_{2A} ARs exert a synergistic effect on motor function as the A_1 ARs enhance the presynaptic release of dopamine (DA) [9], while the A_{2A} ARs improve the postsynaptic DA activity [10,11]. For the abovementioned reasons A_1 and A_{2A} AR antagonists have valuable potential as treatment agents as it will synergistically improve motor function via A_1 and A_{2A} AR antagonism (PD), enhance cognitive function through A_1 AR antagonism (AD and PD), provide neuroprotective properties to sufferers by means of A_{2A} AR antagonism (AD and PD) and alleviate depressive symptoms via A_{2A} AR antagonism.

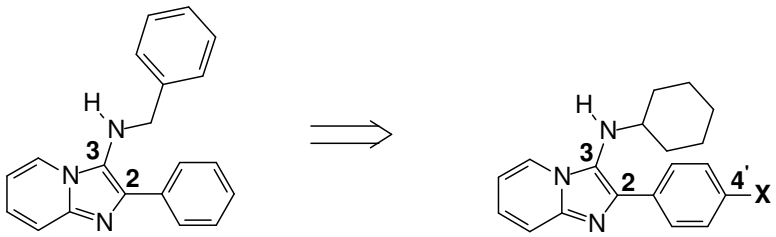
Compounds with affinity for the A_1 and A_{2A} ARs are generally divided into xanthine derivatives and the amino-substituted heterocyclic compounds (non-xanthine) [12]. The xanthine derivatives are one of the most researched AR classes and consist of a bicyclic fused 6:5-membered N-containing ring system. Various non-xanthine heterocyclic compounds have previously been documented to possess affinity for the A_1 AR with the majority of these compounds considered as fused bi-or-tri-heterocyclic systems that are extensions of the xanthine scaffold [13]. The pyrazolo[1,5-α]pyridines, imidazo[1,2-α]pyridines and benzimidazoles (Figure 1) currently constitutes the largest group of non-xanthine 6:5-fused heteroaromatic A_1 AR antagonists [13]



pyrazolo[1,5-α]pyridine **imidazo[1,2-α]pyridine** **benzimidazole**

Figure 1. The general scaffolds of bicyclic 6:5 fused N-containing heteraromatic compounds.

Compounds containing the imidazo[1,2-α]pyridine ring system have been shown to possess a broad range of useful pharmacological properties, including antibacterial, antifungal, anthelmintic, antiviral, antiprotozoal, anti-inflammatory, anticonvulsant, anxiolytic, hypnotic (e.g., zolpidem), gastrointestinal, antiulcer and immunomodulatory activities [14–17]. Until recently imidazo[1,2-α]pyridines have only been described in patent literature for their selective A_1 AR antagonistic properties [18]. However, a study conducted by Reutlinger and co-workers [19] identified N-benzyl-2-phenylimidazo[1,2-α]pyridin-3-amine as a potential A_1 AR ligand with 89% binding at 100 μM (Figure 2). Based on the latter finding, the imidazo[1,2-α]pyridines scaffold represent an interesting class of 6:5-fused bicyclic compounds and prompted the current pilot study to investigate the potential of several structurally related 3-cyclohexylamino-2-phenylimidazo[1,2-α]pyridine analogues possessing affinity for the A_1 and A_{2A} ARs. Furthermore, in order to find new insights into the structural requirements for AR binding, substitution at position C2 and C3 was explored (Figure 2).



N-benzyl-2-phenylimidazo[1,2-α]pyridin-3-amine **Imidazo[1,2-α]pyridine derivatives**

Figure 2. N-benzyl-2-phenylimidazo[1,2-α]pyridin-3-amine used as lead to investigate 3-cyclohexylamino-2-phenylimidazo[1,2-α]pyridine analogues.

The selected imidazo[1,2-α]pyridine analogues were either obtained commercially (**1**, **2a–d** and **3**) or synthesized (**4a–i**) according to literature procedures. Compound **1**, imidazo[1,2-α]pyridine, is seen as the parent compound of the study (Figure 1). The structure-activity relationships of the imidazo[1,2-α]pyridine scaffold was further explored via substitution at the C2 position alone (2-phenylimidazo[1,2-α]pyridines, **2a–d**) and in combination with position C3 (2-phenylimidazo[1,2-α]pyridin-3-amine, **3** and 3-cyclohexylamino-2-phenylimidazo[1,2-α]pyridines, **4a–i**). The C2 substitution explored the unsubstituted phenyl ring (**2a**) as well as *para*-substitution which included OH- (**2b**), OCH_3- (**2c**) and Br- (**2d**). The simultaneous substitution of C2 and C3 included C2 phenyl substitution combined with a C3 amino- (**3**) and C3 cyclohexyl amino-group (**4a**). The effect of *para*-substitution at the C2 phenyl ring was further explored with the C3 cyclohexyl amino series and included OH- (**4b**) OCH_3- (**4c**), CH_3- (**4d**), Br- (**4e**), Cl- (**4f**), F- (**4g**) CF_3- (**4h**) and NO_2- (**4i**) (Figure 3; Table 1)

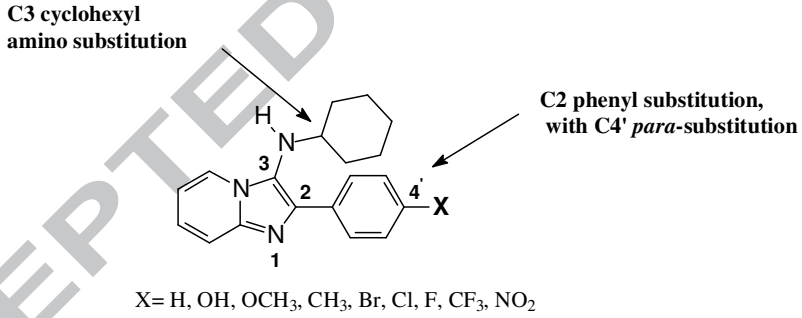
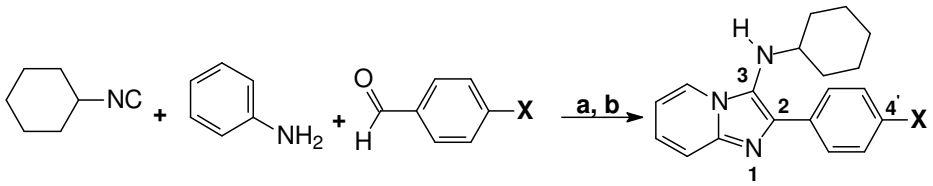


Figure 3. The imidazo[1,2-α]pyridine scaffold and suggested structural modifications.

Compounds **1**, **2a–d** and **3** were commercially available from Sigma-Aldrich® and subsequently used without any further purification. The synthetic route of the known synthesized imidazo[1,2-α]pyridine-based derivatives (**4a–i**) is outlined in Scheme 1. Compounds **4a–i** was successfully synthesized via a modified catalyst- and solvent-free three-compound procedure described in the literature [20]. Briefly, a mixture of 2-aminopyridine, the appropriate aldehyde and cyclohexylisocyanide was refluxed with no solvent at 120°C (**4a–g** and **4i**) or 60°C (**4h**) until completion of the reaction, as indicated by TLC. The crude compounds were purified by recrystallization from either hexane:ethyl acetate (**4a**, **4d**, **4e**, **4f**, **4g**, **4h**) or ethyl acetate:ethanol (**4b**, **4c**, **4i**) (Scheme 1). Compounds (**4a–i**) were successfully synthesized and the structures confirmed by NMR spectrometry and supported by MS results. Both the ^{1}H and ^{13}C NMR of each test compound corresponded with the proposed structures and the spectra reported in the literature [21].



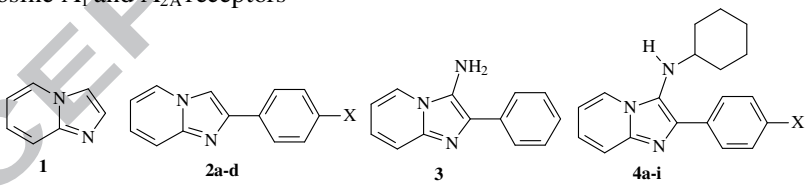
X= H, OH, OCH_3, CH_3, Br, Cl, F, CF_3, NO_2

Scheme 1: The catalyst- and solvent-free synthetic procedure that was utilized to obtain the corresponding imidazo[1,2-α]pyridines. Reagents and conditions: (a) heated at 120°C (**4a**, **4b**, **4c**, **4d**, **4e**, **4f**, **4g**, **4i**) or 60°C (**4h**), (b) reflux for the appropriate time.

The A_1 and A_{2A} AR affinity of all the test compounds (**1**, **2a–d**, **3** and **4a–i**) was determined via radioligand binding assays via a previously described procedure [22]. In short, the A_1 AR affinity was determined in the presence of the radioligand [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine ([^{3}H]DPCPX) with rat whole brain membranes expressing the A_1 AR, while the A_{2A} AR affinity was performed in the presence of 5'-N-[^{3}H]-ethylcarboxamideadenosine ([^{3}H]NECA) as radioligand with rat striatal membranes expressing the A_{2A} ARs. In order to minimize [^{3}H]NECA's binding to the A_1 AR the A_1 AR agonist N^6-cyclopentyladenosine (CPA) was also included in the A_{2A} AR radioligand binding experiment. Nonspecific binding was defined by the addition of 100 μM CPA. CPA was used as reference compound and its assay results confirmed validity of the radioligand binding assays. The K_i values were obtained by determining the IC_{50} values from sigmoidal-dose response curves by means of the Graphpad Software Inc. package. The corresponding K_i value for the competitive inhibition by the test compounds of [^{3}H]DPCPX (K_d = 0.36 nM) or [^{3}H]NECA (K_d = 15.3 nM) were subsequently calculated from the IC_{50} values [22]. The sigmoidal-dose response curves were obtained by plotting the specific binding (i.e. the eight concentrations of each test compound ranging between 0 μM and 100 μM) versus the logarithm of the test compound's concentrations [22]. The *in vitro* A_1 and A_{2A} AR affinity results of the test compounds and CPA are summarized in Table 1.

Table 1

The dissociation constant values (K_i values) for the binding of the imidazo[1,2-α]pyridine analogues to rat adenosine A_1 and A_{2A} receptors



		K_i ± SEM (μM)[a] / % displacement[b]			
Compd	**X**	A_1 vs [^{3}H]DPCPX	A_{2A} vs [^{3}H]NECA	A_1 + GTP[c] vs [^{3}H]DPCPX	GTP Shift[d]
1	-	> 100 (100%)[b]	> 100 (75%)[b]	-	-
2a	H	> 100 (49%)[b]	> 100 (52%)[b]	-	-
2b	OH	> 100 (47%)[b]	> 100 (85%)[b]	-	-
2c	OCH_3	> 100 (76%)[b]	> 100 (61%)[b]	-	-
2d	Br	> 100 (86%)[b]	> 100 (87%)[b]	-	-
3	-	> 100 (52%)[b]	> 100 (86%)[b]	-	-

4a	H	> 100 (57%)[b]	> 100 (77%)[b]	-	-
4b	OH	5.53 ± 0.86[a]	> 100 (27%)[b]	-	-
4c	OCH_3	7.61 ± 1.25[a]	> 100 (55%)[b]	-	-
4d	CH_3	2.06 ± 0.08[a]	> 100 (83%)[b]	2.00 ± 0.24[a]	0.97
4e	Br	3.90 ± 0.65[a]	> 100 (54%)[b]	-	-
4f	Cl	> 100 (61%)[b]	> 100 (64%)[b]	-	-
4g	F	> 100 (63%)[b]	> 100 (90%)[b]	-	-
4h	CF_3	> 100 (76%)[b]	> 100 (74%)[b]	-	-
4i	NO_2	> 100 (99%)[b]	> 100 (69%)[b]	-	-
CPA		0.015[e]	0.331[e]	0.99[e]	6.48[e]

[a] All K_i values determined in triplicate and expressed as mean ± SEM.
[b] Percentage displacement of the radioligand at the indicated concentration.
[c] GTP shift assay, where the 100 µM GTP was added to the A_1 AR radioligand binding assay.
[d] GTP shifts calculated by dividing the K_i in the presence of GTP by the K_i in the absence of GTP.
[e] Literature values obtained from references [22].

Compound **4d** was further evaluated via a GTP shift assay to determine the functionality of the test compounds towards the A_1 AR. The GTP shift assay was carried out as described previously with rat whole brain membranes and [^{3}H]DPCPX in the absence and presence of 100 µM GTP (Table 1; Figure 4). Nonspecific binding was defined by the addition of 10 µM DPCPX (unlabeled). A compound with a calculated GTP shift of approximately 1 is considered an antagonist, in turn the presence of GTP affects the competition curve of an agonist and shifts the curve to the right [22].

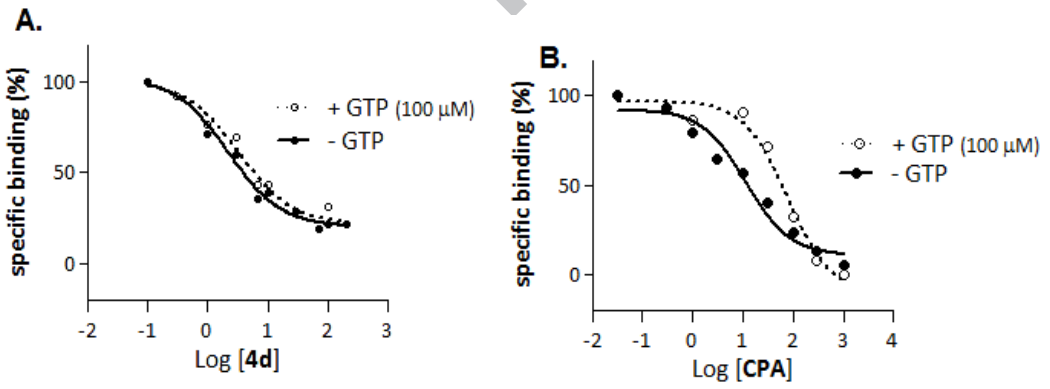


Figure 4. The sigmoidal-dose response curves of compound **4d** (Panel A) and CPA (Panel B, reference agonist) displaying the binding affinity to A_1 ARs in the absence (-) and presence (+) of GTP. (A) GTP shift of 0.97 calculated for the A_1 AR antagonist compound **4d**, and (B) GTP shift of 6.48 calculated for the A_1 AR agonist CPA.

Previous research [19] identified N-benzyl-2-phenylimidazo[1,2-α]pyridin-3-amine as a lead for designing compounds with A_1 AR affinity (Figure 2). Imidazo[1,2-α]pyridine (**1**), the parent scaffold of this pilot study, is unsubstituted at positions C2 and C3 and was devoid of A_1 and A_{2A} AR affinity. The influence of C2 substitution on A_1 and A_{2A} AR affinity was investigated by comparing compound

1 to compound **2a–d** containing a/an (un)substituted C2 phenyl ring. Although the specific binding percentages of these compounds improved compared to compound **1**, they did not display improved A_1 and A_{2A} AR affinity. The addition of an amino group at position C3 (**3** *vs*. **2a**) also showed no improvement for the A_1 and A_{2A} AR affinity. Alternatively the combination of a *para*-substituted phenyl ring at position C2 together with a cyclohexylamino group at position C3 displayed A_1 AR affinity in the low micromolar range (**4a** vs. **4b**, **4c**, **4d**, and **4e**). However, no significant A_{2A} AR affinity was obtained. It seems that the more bulky electron donating groups (-OH, $-OCH_3$, $-CH_3$) displayed good A_1 AR affinity, while the electron withdrawing groups (-Cl, -F, $-CF_3$, $-NO_2$) showed no A_1 AR affinity with the exception of the bromo group (**4e**) that possessed good A_1 AR affinity (Table 1).

The compound documented with the best A_1 AR affinity, was compound **4d** with a *para*-methyl substituent (K_i = 2.06 μM). Compound **4e** (*para*-bromo substituent) displayed the second highest A_1 AR affinity (K_i = 3.90 μM), followed by the *para*-hydroxy (**4b**) and *para*-methoxy (**4c**) analogues, exhibiting K_i values of 5.53 μM and 7.61 μM respectively for the A_1 AR. Unfortunately, none of these structural modifications favored A_{2A} AR affinity. Furthermore, selective A_1 AR affinity was only obtained with *para*-substitution of the phenyl ring at position C2 in combination with a cyclohexylamino substitution on position C3, thus requiring substitution of both position C2 and C3 to obtain A_1 AR affinity

In order to demonstrate if the compound possessing the highest A_1 AR binding affinity, compound **4d**, acted as an antagonist or agonist, a GTP shift experiment was performed. Generally, a rightward shift of the binding curve in the presence of GTP (due to an uncoupling of the A_1 AR from its G_i protein) is expected for an A_1 AR agonist. In the case of an A_1 AR antagonist, no significant shift is anticipated in the presence of GTP [22,23]. As expected compound **4d** showed no significant shift of the binding curve in the presence of GTP, thus compound **4d** may be considered an antagonist of the A_1 AR (Table 1**;** Figure 4). Based on the structural similarity of compound **4d** to compounds **4b**, **4c** and **4e**, the latter compounds are also expected to act as A_1 AR antagonists

For future optimization of the 3-cyclohexylamino-imidazo[1,2-α]pyridine scaffold the results of a study by Novellino and co-workers [24] gave valuable insight for future scaffold modifications. They explored the A_1 AR affinity of novel N-alkyl- and N-acyl-(7-substituted-2-phenylimidazo[1,2-α][1,3,5]triazin-4-yl)amines (ITAs). Their findings (Figure 5) highlighted the importance of a CH_2 spacer and CO linker. The CO linker enhanced A_1 AR affinity; however the affinity of the A_{2A} AR was diminished. When an acetyl group was introduced at the N4 position, it reduced the binding affinity of the A_1 AR, while the binding affinity of the A_{2A} AR remained unchanged. The A_1 AR affinity was improved when a CO-cyclohexyl and CO-phenyl group was introduced although the binding affinity of the A_{2A} AR showed no improvement. The addition of a methylene spacer between the N4 position and the acetyl group enhanced A_1 AR affinity. When a CO-cyclopentyl was introduced, it enhanced the affinity for the A_1 AR and affinity for the A_{2A} AR was gained. Substitution of the C7 position with a phenyl moiety rather than a methyl group combined with the CO-cyclopentyl maintained affinity for the A_1 AR, while A_{2A} AR affinity was further increased [24].

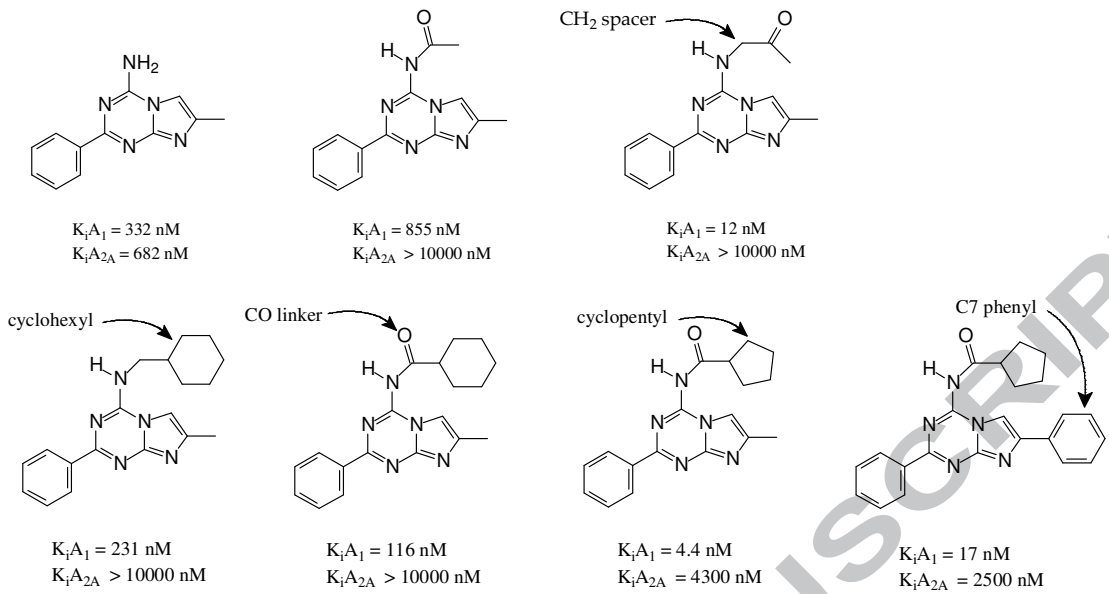


Figure 5. Structural modification to ITA analogues to gain A_1 and A_{2A} AR affinity [24].

A possible explanation for our low AR affinity can be ascribed, in part, to the research done by Gillespie and co-workers [25], where the increased number of nitrogens in the heterocyclic ring (from a pyridine to a pyrimidine) enhanced both A_1 and A_{2A} AR affinities. They compared the affinities of triazine, pyrimidine and pyridine scaffolds and observed that the pyridine scaffold was sevenfold less potent than the triazine and 45-fold less potent than the corresponding aminopyrimidine, thereby concluding that two nitrogens in the ring are optimum for both A_1 and A_{2A} AR affinity [25,26]. This trend was also observed in the ITA analogues synthesized by Novellino and co-workers [24], where the 6:5 fused bicyclic rings containing four nitrogen atoms, displayed better AR affinity in comparison to the current investigated imidazo[1,2-α]pyridine analogues containing only two nitrogens in the fused heterocyclic rings

Therefore we hypothesize that the introduction of additional nitrogens in the heterocyclic ring may increase both A_1 and A_{2A} AR affinity (Figure 6). Moreover the findings of Novellino and co-workers [24] can be used for further structural modifications (Figure 6) to 3-cyclohexylamino-2-(4'-methylphenyl)imidazo[1,2-α]pyridine (**4d**) to optimize the scaffold for improved A_1 and A_{2A} AR affinity (Figure 6). These modifications include the replacement of the cyclohexyl ring with a cyclopentyl ring to increase both A_1 and A_{2A} AR affinity and the insertion of a CO group and a methylene spacer between the NH and the CO group to increase A_1 AR affinity.

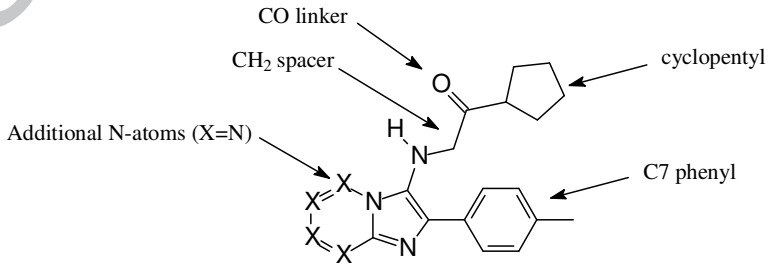


Figure 6. Proposed future structural modifications to compound **4d**.

In conclusion, the newly proposed structural optimization of the investigated imidazo[1,2-α]pyridine analogues may be implemented in future studies to potentially improve the A_1 AR affinity and possibly gain A_{2A} AR binding. Among the test compounds, **4d** possessing a *para*-substituted methyl functional group, was identified as the best selective A_1 AR antagonist (K_i = 2.06 μM) and may find therapeutic relevance to enhance PD-associated cognitive dysfunction. Since the study provided proof of concept that the imidazo[1,2-α]pyridine derivatives possess A_1 AR affinity, this scaffold can thus be used for further structure-activity relationship studies to design novel imidazo[1,2-α]pyridine-based A_1 AR antagonists for the potential treatment of cognition in neurological disorders.

Acknowledgements

We are grateful to Dr. J. Jordaan and Mr. A. Joubert of the SASOL Centre for Chemistry, North-West University, for recording the NMR and MS spectra, respectively. The financial assistance of the North-West University, the South African Medical Research Council and the National Research Foundation 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.

References

1. Gomes CV, Kaster MP, Tomé AR, Agostinho PM, Cunha RA. Adenosine receptors and brain diseases: neuroprotection and neurodegeneration. *Biochim Biophys Acta.* 2011;1808:1380-99.

2. Fredholm BB, Ijzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev.* 2001;53:527-552.

3. Rivkees SA, Price SL, Zhou FC. Immunohistochemical detection of A_1 adenosine receptors in rat brain with emphasis on localization in the hippocampal formation, cerebral cortex, cerebellum, and basal ganglia. *Brain Res.* 1995;677:193-203.

4. Sebastiao AM, Ribeiro JA. Adenosine A_{2A} receptor-mediated excitatory actions on the nervous system. *Prog Neurobiol***.** 1996;48:167-189.

5. Kuwana Y, Shiozaki S, Kanda T, Kurokawa M, Koga K, Ochi M, Ikeda K, Kase H, Jackson MJ, Smith LA, Pearce RK. Antiparkinsonian activity of adenosine A_{2A} antagonists in experimental models. *Adv Neurol.* 1998;80:121-123.

6. Monopoli A, Lozza G, Forlani A, Mattavelli A, Ongini E. Blockade of adenosine A_{2A} receptors by SCH 58261 results in neuroprotective effects in cerebral ischaemia in rats. *Neuroreport*. 1998;9*:*3955-3958.

7. Yacoubi ME, Ledent C, Parmentier M, Bertorelli R, Ongini E, Costentin J, Vaugeois JM. Adenosine A_{2A} receptor antagonists are potential antidepressants: evidence based on pharmacology and A_{2A} receptor knockout mice. *Br J Pharmacol.* 2001;134:68-77.

8. Mihara T, Mihara K, Yarimizu J, Mitani Y, Matsuda R, Yamamoto H, Aoki S, Akahane A, Iwashita A, Matsuoka N. Pharmacological characterization of a novel, potent adenosine A_1 and A_{2A} receptor dual antagonist, 5-[5-amino-3-(4-fluorophenyl) pyrazin-2-yl]-1-isopropylpyridine-2(1H)-one (ASP5854), in models of Parkinson's disease and cognition. *J Pharmacol Exp Ther.* 2007;323:708-719.

9. Fuxe K, Ferré S, Genedani S, Franco R, Agnati LF. Adenosine receptor–dopamine receptor interactions in the basal ganglia and their relevance for brain function. *Physiol Behav.* 2007;92:210-217.

10. Fuxe K, Marcellino D, Genedani S, Agnati L. Adenosine A_{2A} receptors, dopamine D_2 receptors and their interactions in Parkinson's disease. *Mov Disord.* 2007;22:1990-2017.

11. Schiffmann SN, Fisone G, Moresco R, Cunha RA, Ferré S. Adenosine A_{2A} receptors and basal ganglia physiology. *Prog Neurobiol.* 2007;83:277-292.

12. Müller CE, Stein B. Adenosine receptor antagonists: structures and potential therapeutic applications. *Current Pharmaceut Design.* 1996;2:501-530.

13. Chang LC, Brussee J, Ijzerman AP. Non-Xanthine Antagonists for the Adenosine A_1 Receptor. *Chem Biodivers.* 2004;1:1591-1626.

14. Gueiffier A, Mavel S, Lhassani M, Elhakmaoui A, Snoeck R, Andrei G, Chavignon O, Teulade JC, Witvrouw M, Balzarini J, De Clercq E. Synthesis of imidazo [1, 2-α] pyridines as antiviral agents. *J Med Chem.* 1998;41:5108-5112.

15. Al-Tel TH, Al-Qawasmeh RA, Zaarour R. Design, synthesis and *in vitro* antimicrobial evaluation of novel Imidazo [1, 2-α] pyridine and imidazo [2, 1-β][1, 3] benzothiazole motifs. *Eur J Med Chem.* 2011;46:1874-1881.

16. Abignente E, De Caprariis P, Rimoli MG, Capasso F. Research on heterocyclic compounds, XXIX. Synthesis and antiinflammatory activity of imidazo [1, 2-α] pyrazine derivatives. *Farmaco.* 1992;47:919-930.

17. Lange J, Karolak-Wojciechowska J, Wejroch K, Rump S. A structure-activity relationship study of the affinity of selected imidazo [1, 2-α] pyridine derivatives, congeners of zolpidem, for the omega 1-subtype of the benzodiazepine receptor. *Acta Pol Pharm.* 2001;58:43-52.

18. Beresis R, Colletti S, Doherty J, Zaller D. Phenyl substituted imidazopyridines and phenyl substituted benzimidazoles. 2003; Patent: US 2050165232 A1.

19. Reutlinger M, Rodrigues T, Schneider P, Schneider G. Combining On-Chip Synthesis of a Focused Combinatorial Library with Computational Target Prediction Reveals Imidazopyridine GPCR Ligands. *Angew Chem Int Ed.* 2014;*53:*582-585.

20. Adib M, Mahdavi M, Noghani MA, Mirzaei P. Catalyst-free three-compound reaction between aminopyridines (or 2-aminothiazoles), aldehydes, and isocyanides in water. *Tetrahedron Lett.* 2007;48*:*7263-7265.

21. Bode ML, Gravestock D, Moleele SS, Van der Westhuyzen CW, Pelly SC, Steenkamp PA, Hoppe HC, Khan T, Nkabinde LA. Imidazo[1,2-α]pyridin-3-amines as potential HIV-1 non-nucleoside reverse transcriptase inhibitors. *Bioorg Med Chem.* 2011;19:4227-4237.

22. Van der Walt MM, Terre'Blanche G. 1,3,7-Triethyl-substituted xanthines—possess nanomolar affinity for the adenosine A_1 receptor. *Bioorg Med Chem.* 2015;23:6641-6649.

23. Gütschow M, Schlenk M, Gäb J, Paskaleva M, Alnouri MW, Scolari S, Iqbal J, Müller CE. Benzothiazinones: A novel class of adenosine receptor antagonists structurally unrelated to xanthine and adenine derivatives. *J Med Chem.* 2012;55:3331-3341.

24. Novellino E, Abignente E, Cosimelli B, Greco G, Iadanza M, Laneri S, Lavecchia A, Rimoli MG, Settimo FD, Primofiore G, Tuscano,D. Design, synthesis and biological evaluation of novel N-alkyl-

and N-acyl-(7-substituted-2-phenylimidazo [1, 2-α][1, 3, 5] triazin-4-yl) amines (ITAs) as novel A_1 adenosine receptor antagonists. *J Med Chem.* 2002;45:5030-5036.

25. Gillespie RJ, Bamford SJ, Gaur S, Jordan AM, Lerpiniere J, Mansell HL, Stratton GC. Antagonists of the human A_{2A} receptor. Part 5: Highly bio-available pyrimidine-4-carboxamides. *Bioorg Med Chem Lett.* 2009;19:2664-2667.

26. Gillespie RJ, Bamford SJ, Clay A, Gaur S, Haymes T, Jackson PS, Jordan AM, Klenke B, Leonardi S, Liu J, Mansell HL. Antagonists of the human A_{2A} receptor. Part 6: Further optimization of pyrimidine-4-carboxamides. *Bioorg Med Chem.* 2009;17:6590-6605.

Imidazo[1,2-α]pyridines possess adenosine A_1 receptor affinity for the potential treatment of cognition in neurological disorders.

R Lefin, MM van der Walt PJ Milne and G Terre'Blanche

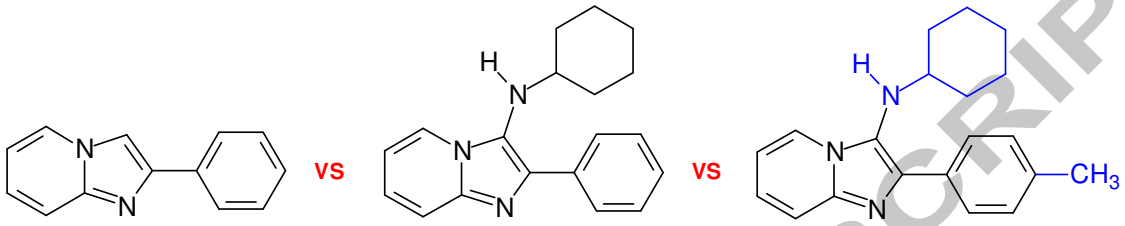
Highlights:

- A_1 receptors are considered drug targets for neurological disorders.
- Known 3-cyclohexylamino-2-phenyl-imidazo[1,2-α]pyridines were synthesized.
- *Para*-substituted analogs favor A_1 affinity with diminished A_{2A} affinity.
- *Para*-methyl-substituted compound 4d possess highest K_i value of 2.06 µM.

Imidazo[1,2-α]pyridines possess adenosine A_1 receptor affinity for the potential treatment of cognition in neurological disorders.

R Lefin, MM van der Walt PJ Milne and G Terre'Blanche

Graphical abstract



K_iA_1 > 100 µM
K_iA_{2A} > 100 µM

K_iA_1 > 100 µM
K_iA_{2A} > 100 µM

K_iA_1 = 2.06 µM
K_iA_{2A} > 100 µM