BPS Report; Investigating the Adenosine A_1 Receptor Orthosteric Binding Pocket

I would like to thank the British Pharmacological Society and, my supervisor Professor Graham Ladds and the PhD candidate Ms. Xainglin Huang from the Department of Pharmacology in the University of Cambridge, for an incredible summer research opportunity. For my project, I have investigated the adenosine A_1 receptor (A_1R) hydrophobic binding sub-pocket, to attempt to decipher the binding mechanism of A_1R agonists.

The pocket as a key to deciphering the binding affinity at the A_1R?

The A_1R is the most characterised subtype of the G protein-coupled receptors, and is involved in the treatment of common illnesses such as glaucoma, type 2 diabetes mellitus or epilepsy[1,2]. However, there is currently a paucity of approved drugs that selectively and potently stimulate this receptor. Interestingly, a novel, very potent and highly A_1R selective agonist, compound **27**, has been shown to interact with a unique hydrophobic sub-pocket of the A_1R *in silico*, where the sub-pocket completely accommodates its 3-bromophenyl moiety.[3] This interaction may contribute to its high affinity to the receptor, making the hydrophobic sub-pocket of the A_1R a relevant and important research target. The fact that this pocket is absent in the other subtypes of the adenosine receptor, makes this statement more plausible. Contrastingly, **27**'s structural congener, compound **20**, which is unable to completely accommodate its 3-bromobenzyle group within this pocket, is also a much less potent and selective A_1R agonist (Figure 1).[3]

The project design

My project aimed to validate the *in silico* molecular dynamics (MD) findings of **27**, **20**, and their parent compound, **BnOCPA**, which was used as a reference as it is well characterised, as well as being a selective and potent A_1R agonist. [4,5] To determine if the hypotheses are reflective of *in vitro* data, the compounds' affinity to the A_1R was tested in cell lines differing by a single, mutated amino acid residue comprising the hydrophobic sub-pocket.

The amino acids of interest identified by the MD models were; $I69^{2.64}$, $N70^{2.65}$, and $Y271^{7.36}$.[4] These residues were mutated to alanine, a comparatively unreactive amino acid (ex; I69A) and expressed in HEK-293 cell lines, and were compared against the wild-type, and additionally, a $T257^{6.58}$ A cell line previously shown to successfully distinguish between A_1R agonists.[3] All of the receptors in the cell lines were tagged with N-luciferase, allowing for the use of the NanoBRET assay used to quantify the binding affinity of the compounds.[4] Briefly, it works by determining the compound's ability to competitively displace a fluorescent-tagged ligand (CA200645).[6]

Elucidating the unexpected interactions with the amino acids – Is it a matter of structure?

The results have shown that the MD simulations were largely accurate in reflecting the *in vitro*, experimental data. **20** showed reduced binding affinity to the wild-type compared to BnOCPA and **27** (Figure 2). Moreover, T257A as expected, allowed the differentiation of the agonists; it affected **27** and **BnOCPA**, but not **20**. Interestingly, although consistent with the lab's previous findings with **BnOCPA**, the effect of the mutagenesis of T257A was an increase, as opposed to a decrease, in binding affinity. It was proposed that this may be due to the change in the lipophilicity of the environment where T257 is located, under the ECL3 and surrounding the cyclopentyl groups of the compounds. As Thr is polar and lipophobic, while Ala is non-polar and lipophilic, following the mutation, this region of the receptor becomes more lipophilic. It therefore suggests that **27** and **BnOCPA** likely favour a relatively more lipophilic environment created by the mutation, whereas **20** is not significantly affected by this change in lipophilicity.

Regarding the amino acid residues located within the hydrophobic sub-pocket and more likely to be involved in the compounds' unique binding patterns, all of the compounds' binding affinity to the A_1R was significantly reduced in the cell lines expressing the I69A and Y271A mutants. This suggests that all of the tested compounds interact with residues I69 and Y271, and they are therefore likely involved in their binding pattern. Overall, as expected, **27** largely followed the trend of **BnOCPA** binding, and the reverse was true for **20**. However, the unexpected finding involved the binding pattern associated with the N70 residue. Although N70 is located within the hydrophobic sub-pocket, N70A did not affect the binding of the most potent compounds; **BnOCPA** and **27**. Whereas, it did affect the least potent, **20**. Following consultations with my supervisor, and our collaborators, the proposed reason for the N70 interaction, is that **20**'s purine ring features more interactions with the residue N70, compared to **27** or **BnOCPA**. As a result, if these interactions are lost due to the mutagenesis, this could explain the decreased binding affinity of **20**. Conversely, a low level of significant interactions between the residue and **27** or **BnOCPA**, could also explain the lack of effect that the mutagenesis of the residue had on these compounds' binding affinity.

Finally, although my project did not fully delve into this phenomenon, it is interesting to note that despite the similarity of the trends observed with **27** and **BnOCPA**, which was mostly expected, these two compounds are also the most structurally dissimilar. This suggests that the binding affinity to this hydrophobic sub-pocket is likely related to the length of the atom linker between the N^6-cyclopentane and the phenyl rings, the presence or lack of the Br moiety, and therefore the ultimate size of the compounds tested.

Final thoughts on the research experience

To conclude, this has been a greatly satisfying and enjoyable, as well as humbling and enriching experience, thanks to the Prof Ladds' laboratory. I was fortunate enough to work alongside very talented people, enabling me to have my small contribution and name, featured alongside the lab members and collaborators, on a paper that has been accepted for publication in Journal of Medicinal Chemistry. Moreover, apart from gathering interesting data, I have learned laboratory techniques related to tissue culture and various assays, as well as improved my soft skills such as patience, critical analysis and public speaking. I believe this will greatly help me in my work on my final year capstone project in my university, and in my post-graduate academic journey.

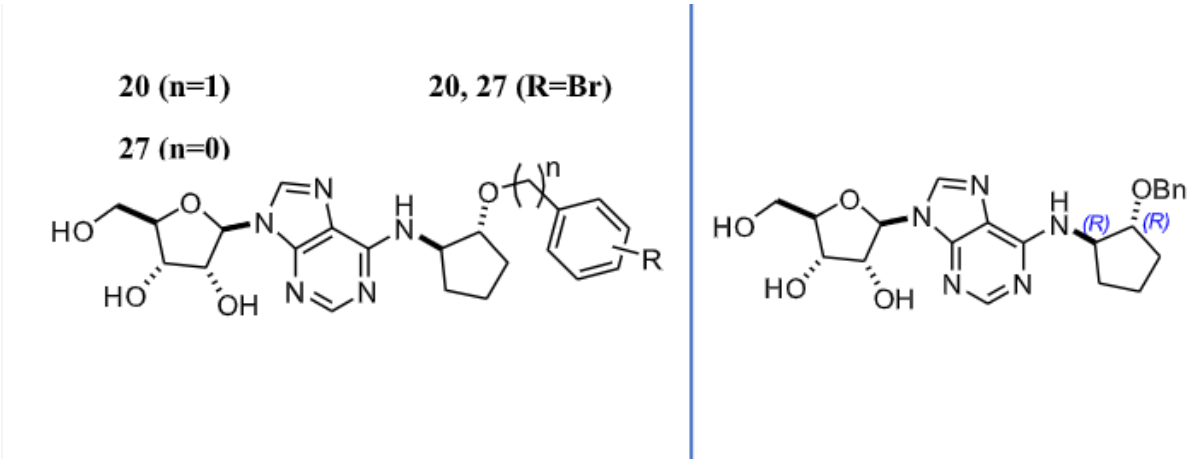

Figure 1; The Chemical Structure of cmpd27, a phenoxycyclopentyl adenosine derivative, its benzyloxycyclopentyl congener cmpd20, and BnOCPA. The main structural differences between the compounds, are a 3-bromophenyl moiety in cmpd27, 3-bromobenzyl moiety in cmpd20, and a 3-benzyl moiety in BnOCPA.

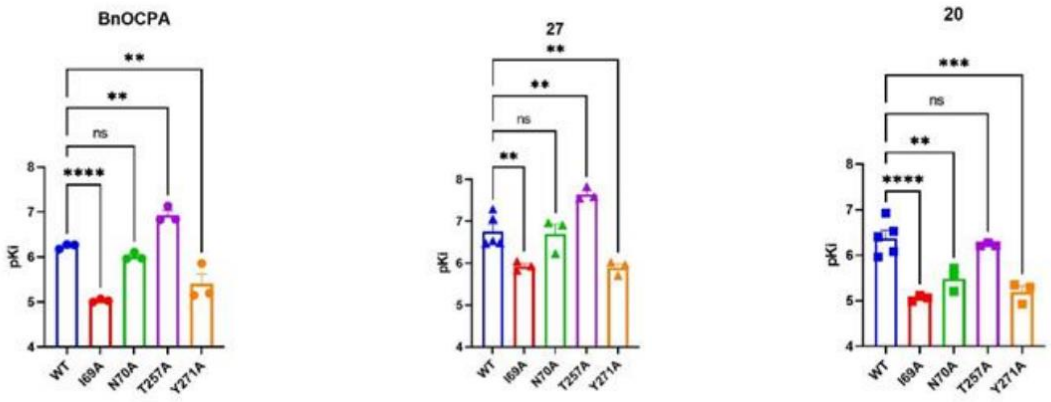

Figure 2; Bar charts depicting the pKi values that determine whether the binding affinity of the compounds is different across the mutant variants. Statistical significance (* $p < 0.05$; **$p<0.01$;*** $p<0.001$;****$p<0.0001$) between the populations was carried out using the one-way ANOVA and Dunnett's post-test.

References;

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