Adenosine analogs have a dual immunomodulatory and antiviral function and are potential therapeutic agents against SARS-CoV-2

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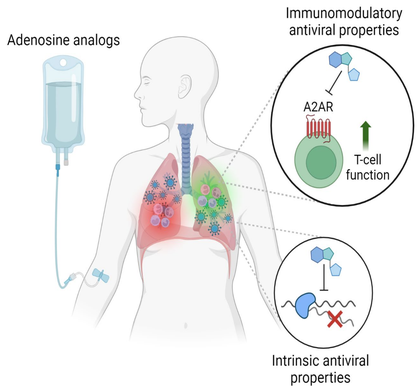
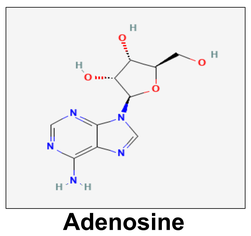
Introduction

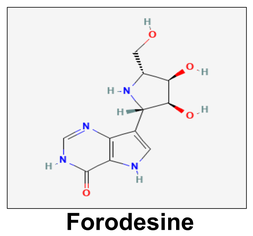


SARS-CoV-2 is a contagious virus that causes COVID-19, a disease that has affected millions of people worldwide and claimed over six million lives. The pandemic has unveiled an urgent need for new antivirals to control emerging infectious diseases and potential future pandemics.

Classic antivirals are currently designed to directly interfere with pathogens. Adenosine analogs are antiviral drugs which resemble the structure of adenosine (1), a metabolite that derives from the breakdown of ATP and can suppress the immune response through activation of the Adenosine A2A Receptor (A2AR) in immune cells.



Adenosine



Forodesine

Adenosine is overproduced in some infectious diseases, like COVID-19, where it restricts protective antiviral immune responses. Previous work from our lab showed that adenosine-mediated activation of A2AR causes suppression of CD8+ T-cells effector functions in COVID-19 mouse models (2,3). Importantly, our group showed that adenosine analogs could restore antiviral T-cell responses in COVID-19 models, through blocking the A2AR (2,3). Therefore, we propose that adenosine analogs that interact with A2AR may be endowed with dual – immunomodulatory and intrinsic - antiviral functions. In this study, adenosine analogs were screened using molecular docking to predict whether they would bind to A2AR and CD8+ T-cell proliferation was used as a functional assay to test the action of adenosine analogs on A2AR.

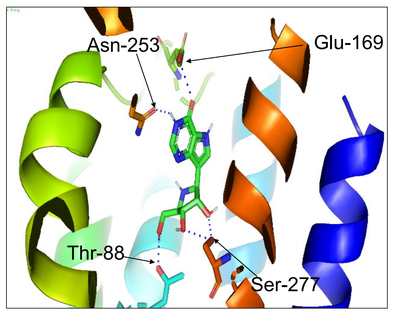
Screening of adenosine analogs

Adenosine Analog	ΔG	Interacting residues
Forodesine	**-7.3**	**Glu-169, Asn-253, Ser-277, Thr-88**
Riboprine	-8.2	His-278, Ser-277
8-chloroadenosine	-7.2	His-278
Maribavir	-7.2	Tyr-9
Vidarabine	-7.0	Ser-277
Aristeromycin	-7.0	Glu-169, Asn-253, Ser-277
Decoyinine	-6.4	Ser-277
Galidesivir	-7.4	Ser-277, Thr-88
Adenosine	-7.3	Glu-169, Asn-253, Ser-277, His-278

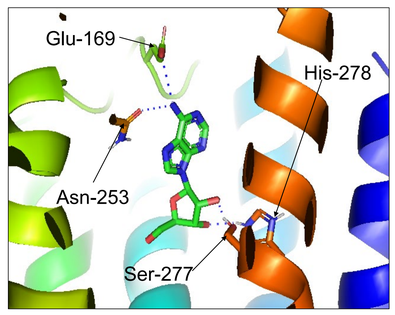
We selected eight adenosine analogs to screen for binding to A2AR. These analogs were known or predicted to inhibit the replication of SARS-CoV-2 and were used in clinical settings (1). As shown in the table, using molecular docking - AutodockVina software, we calculated the affinity for binding to A2AR (ΔG) and the residues of interaction between A2AR and the analogs. Forodesine, Riboprine and 8-chloroadenosine showed the lowest ΔG and similar interacting residues as adenosine, suggesting they likely bind to A2AR.

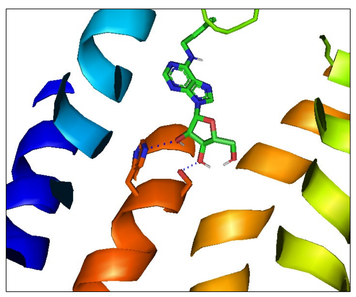
Molecular Docking of the adenosine analogs

Forodesine

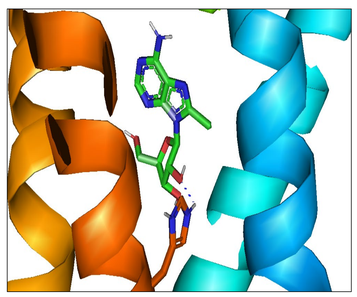


Adenosine

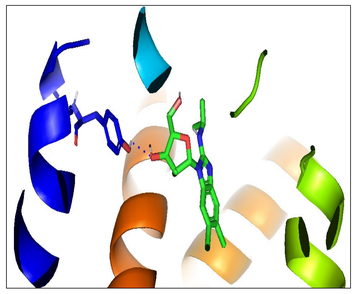




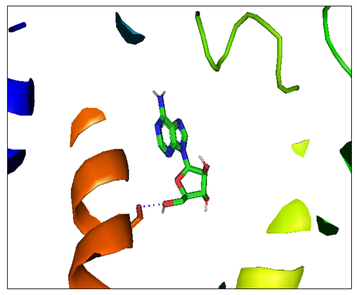
Riboprine



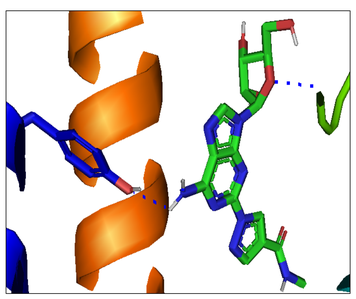
8-chloroadenosine



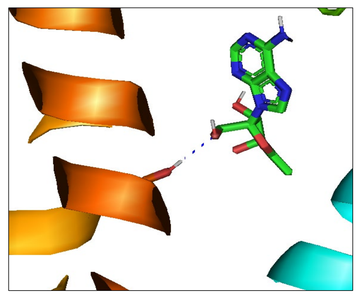
Maribavir



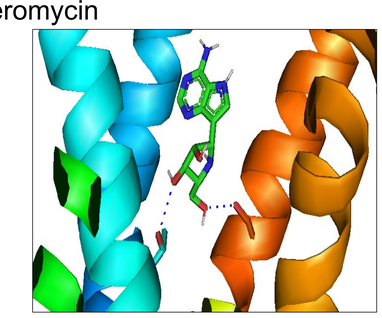
Vidarabine



Aristeromycin



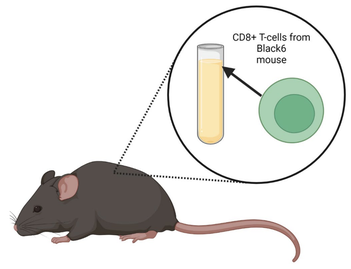
Decoyinine

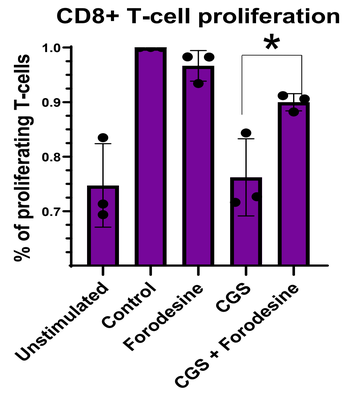


Galidesivir

We displayed molecular docking results using Pymol and analyzed the interaction between the analogs and A2AR compared with adenosine. Among all analyzed analogs, forodesine was ultimately selected because it had the most residues in common with adenosine-A2AR complex and a low ΔG, thus is predicted to bind A2AR.

Forodesine shows immunomodulatory properties





To investigate whether forodesine has immunomodulatory properties through A2AR, we measured the proliferation of CD8+ T-cells treated with forodesine or the A2AR agonist CGS-21680 (CGS) or the combination of the two drugs. To do this, we isolated CD8+ T-cells from C57BL/6 mice and stained with CFSE dye to measure proliferation using flow cytometry. We found that the adenosine agonist CGS suppresses the proliferation of CD8+ T-cells whereas forodesine rescues the proliferation from CGS. These results suggest that forodesine can counteract CGS biding to A2AR, suggesting that forodesine binds to and has immunomodulatory properties through A2AR. In this figure, we show the results of three independent experiments +/- standard deviation. Statistical significance was measured by Student's *t*-test (P values ≤ 0.05 were considered significant).

Conclusion

We found that forodesine treatment restored T-cell proliferation from the A2AR agonist CGS in CD8+ T-cells, suggesting that forodesine binda and blocks A2AR. Our results indicate that adenosine analogs, like forodesine, may be endowed with immunomodulatory properties through A2AR, in addition to their intrinsic antiviral properties. In conclusion, adenosine analogs, like forodesine, could be used as novel dual – immunomodulatory and intrinsic – antiviral drugs. These compounds could represent game-changing therapies, not only to control COVID-19, but also other emerging viral diseases and future pandemics.

Future work: Future work will focus on: characterizing forodesine using *in vitro* and *in vivo* functional assays, including testing the drug in vivo in non-infected and SARS-CoV-2 infected mice; testing the other adenosine analogs *in vitro* and *in vivo*, like we did for forodesine.

References

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2. Monticone G., Huang Z., Hewins P., Cook T., Mirzalieva O., King B., Larter K., Sanchez-Pino M.D., Foster T. P., Nichols O. V., Ramsay A. J., Majumder S., Wyczechowska D., Tauzier D., Gravois E., Crabtree J. S., Garai J., Li L., Zabaleta J.4, Barbier M. T., Del Valle L., Jurado K.A. and Miele L. Novel immunomodulatory activity of nucleoside analog medications (in press, EMBO Reports, 2023)
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