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'Stitch' molecules ('TXA 2.0') for Potent Bleeding Control

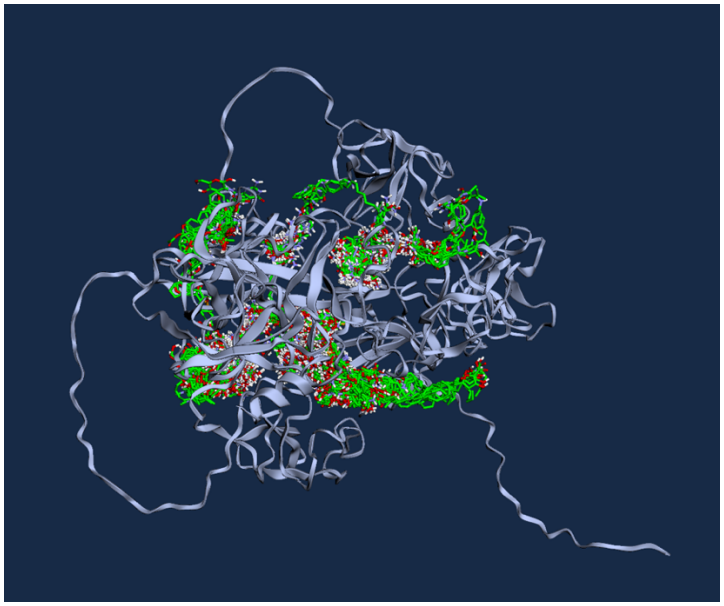
Summary: Several existing compounds (including FDA approved drugs, vitamins, and sugars) were discovered in this virtual drug screen as a potentially improved treatment for bleeding control, typically treated with TXA (Tranexamic acid). The new compounds show estimates of a **~5-33x improved dissociation constant** (Kd, binding stickiness, potency), and **~1.2-1.5x improved affinity** towards the Plasminogen protein target of TXA. The implications are that the new "Stitch" compounds may be **more potent** where a smaller dose could be used to achieve the same desired effect, or that larger doses (with minimal toxicity) could more rapidly control bleeding and reduce the risk of death in an extended time window.

Next Steps: Test and validate 'Stitch' Compounds, other list candidates or existing analogs in appropriate assays – maybe run more simulations/analysis in parallel too. (Methods on p.3)

Brief Background[1]**:** TXA (Tranexamic acid) is a synthetic analog of the lysine amino acid that is popularly used for controlling bleeding and hemorrhage following major trauma,
It works as an antifibrinolytic by binding ~5 sites in the plasminogen protein, in some sense slowing down or preventing the breakdown of blood clots and bleeding. TXA can decrease the risk of death due to significant bleeding and is most effective if taken withing 3 hours following major trauma and decreases risk of death from brain injury in that time window too.

TXA was synthesized in 1962 in Japan, can we create a better compound in 2024, 62 years later?

Figure1: Virtual drug screen against plasminogen (TXA target) crystal structure



100 ligands or drug candidates were generated from PocketFlow software against the Kringle2 domain of the human Plasminogen protein. Then, those 100 compounds were screened against the whole Plasminogen crystal structure (Alphafold) to get the hit rates and affinity in AutoDockVina software (data available separately). After, those synthetic compounds were similarity searched for existing and

commercially available analogs which were then re-screened virtually. The top compound structures seem nitrogenous (NH,NH2), with oxygens/hydroxyls(O, OH) and with bulky carbon rings or aromatics, seemingly similar features to TXA, which is potentially improving binding via more hydrogen bonds and sterics. The virtual screen also shows the molecules clustering in about 5 sites on the protein, similar to the expected number of TXA (control) binding sites. Some top hits were neurotoxins, so candidates were narrowed to safer options with higher affinity than TXA using AI language models/chats to filter out toxic candidates based on literature and clinical trials.

Figure2: Compound hits table with notes and binding affinities relative to TXA (compound)

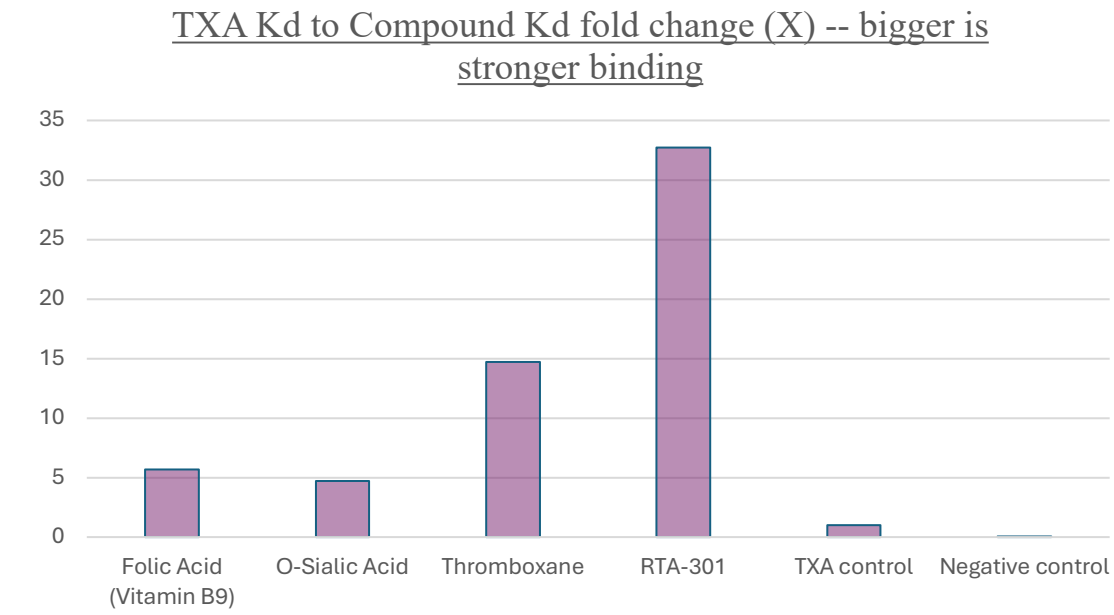
Compound Name	Average Affinity (kcal/mol)	~ Average Kd (uM)	Drug-likeness Score	Kd relative to TXA Kd (binding strength)	notes
Folic acid (Vitamin B9),	-5.8139989	**65**	-9.0275	**6x**	safer and more biocompatible, allows higher doses?
O-Sialic Acid/ UX-001	-5.7215389	**78**	-0.7991	**5x**	safer and more biocompatible, platelet sugars, relevant to thrombocytopenia disease
Thromboxane A2	-6.3204532	**5**	-9.5137	**15x**	strong but risk of cardio effects, increases thrombosis and blood clots, good and bad depending on dose, strokes or random clots
RTA-301/ Peloruside A	-6.8236078	**11**	0.14205	**33x**	side effect risk from tubulin binding, cancer cell division drug, cytotoxic at high doses?
TXA, +control, simulated	-4.7446211	**366**	-2.5918	**1x**	+ Control, slows clot break down
TXA, Literature [2,3]:	-4.2636	**750**	====	**0.5x**	Kd of 750uM for 4 low affinity sites --- same order of magnitude as simulated TXA --- and 1 high affinity site ~1uM.
Neg Control- large Bulky Compound	-2.531744	**30,364**	0.31496	**0.012x**	Negative Control

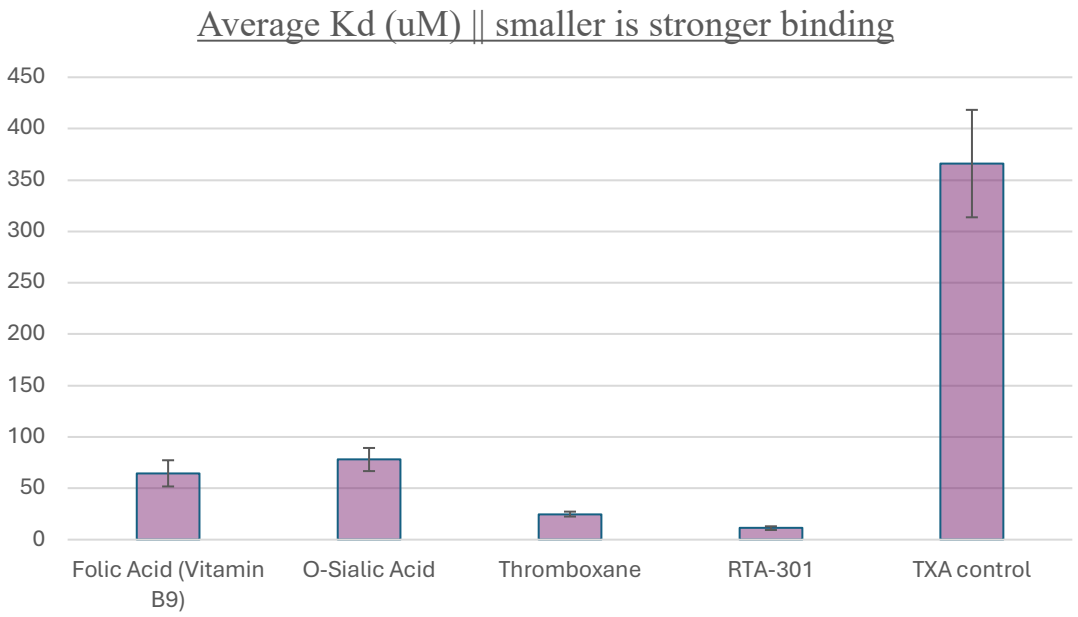
Stitch" compound simulations show a ~**5-33x improved dissociation constant** (Kd, binding stickiness, dissociation constant) compared to TXA simulations. And "shows ~**1.2-1.5x improved affinity** from simulations. There are more candidate analogs, but these seem like the top intriguing candidates for now. D- arginine amino acid also seems to bind as well as TXA (not shown), maybe an alternative. Perhaps convenient drug delivery methods can be explored if doses can be low enough, such as nicotine patch-type delivery or microneedles for emergency situations, instead of IV.

A smaller Kd dissociation constant (micromolar uM) and more negative affinity suggests stronger binding to the protein target and potency. Kd estimates were calculated by converting simulated affinity

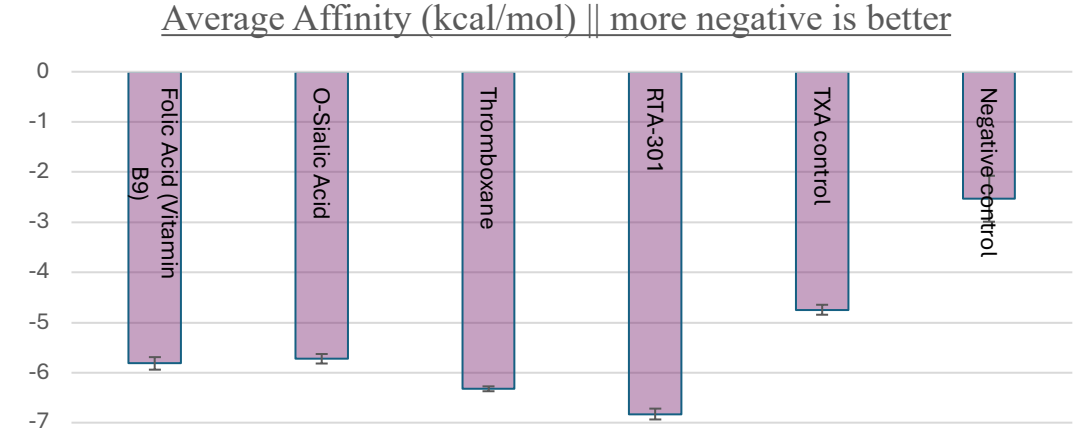
values (ΔG, Gibbs free energy, kcal/mol) using the formula ---- ΔG=RTlnKd.. P-values and error bars in below plots demonstrate statistical significance. P < 0.001

Figure3: Kd and affinity plots for "Stitch" Compounds





	Affinity P-Value	Kd P-Value
Folic Acid (Vitamin B9	6.53581E-06	0.00034032
O-Sialic Acid	5.08348E-07	0.00048563
Thromboxane	5.09723E-09	0.00018101
RTA-301	1.98227E-10	0.00013899
TXA control	1	1
Negative control	0.01042693	0.07077672



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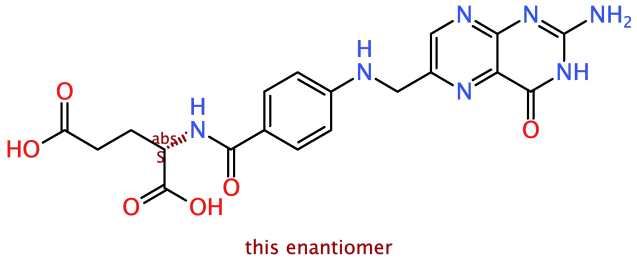
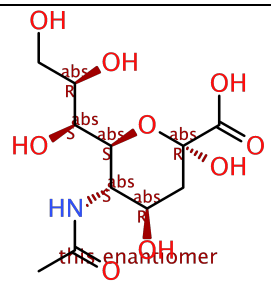
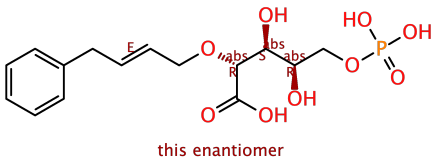
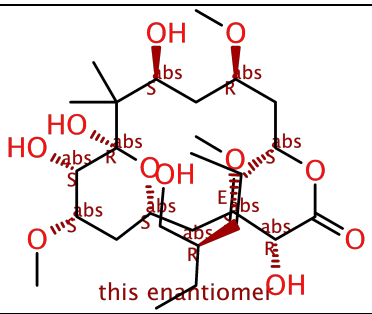
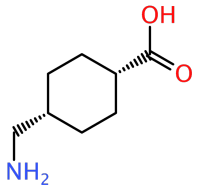
Compound Name	Structure	MW (g/mol)
Folic acid (Vitamin B9)		441.403
O-Sialic Acid/ UX-001		309.27
Thromboxane A2		376.297
RTA-301/ Peloruside A		548.667
TXA, + control		157.212

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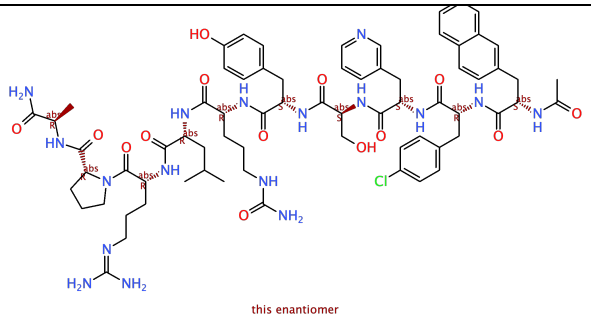
Negative Control, large Bulky Compound		1431.06

Methods and References

"Stitch" compounds info: Smiles code, chemical formula, other analogs available upon request

Links for software, generates ligands and affinity tests:
https://neurosnap.ai/
https://neurosnap.ai/service/PocketFlow (PocketFlow)
https://neurosnap.ai/service/AutoDock%20Vina%20(smina) (Autodock)
Data Warrior (Visualizing chemical structures and affinity rankings, reading ligand output files)
Perplexity, ScholarGPT/ChatGPT (AI drug info searches and narrowing)
Drug analog search: https://idrblab.net/ttd/ttd-search/drug-similarity

PocketFlow for candidate ligand generation against 1 plasminogen Kringle2 domain binding site, and AutodockVina for affinity tests of the 100 generated ligand candidates against the whole Plasminogen protein (AlphaFold), ~5 expected binding sites.

Protein and domain targets:
Plasminogen protein Kringle2 domain, a lysine and TXA binding site (1 of 5)
https://www.rcsb.org/structure/1b2i

AlphaFold whole Plasminogen (PLG) crystal structure – AI generated and cross referenced
https://alphafold.ebi.ac.uk/entry/P00747

Affinity to dissociation rate, Kd conversion:
https://www.novoprolabs.com/tools/deltag2kd
https://www.unitconverters.net/prefixes-converter.html

Kd has a quantitative relationship with ΔG(molar Gibbs free energy) :
ΔG=RTlnKd the relation between ΔG and Kd at 298K（25℃）, R=0.001987 kcal/mol
Kd = e^(ΔG / RT)

ΔG(kcal/mol)- Affinity	Kd(mol/L)
-6.5	1.70E-05
-6	3.95E-05

-5.5	9.20E-05
-5	2.14E-04
-4.5 (~TXA, simulation and literature in range)	4.99E-04
-4	1.16E-03

Citations:

1. https://en.wikipedia.org/wiki/Tranexamic_acid
2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6418500/
3. https://www.pfizermedicalinformation.com/cyklokapron/clinical-pharmacology

