Selection and Characterization of hSTING-Agonists *via In Silico* Modeling and SPR

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Cayman Chemical

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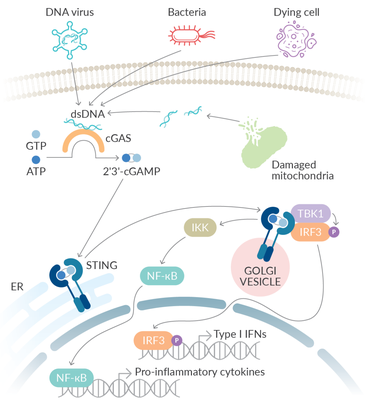
Innate Immunity: The cGAS-STING Pathway



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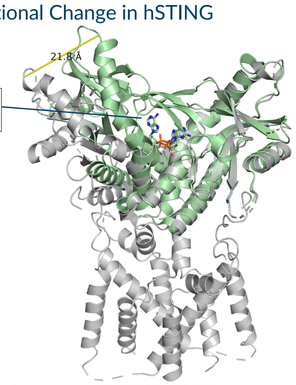
Ligand-Induced Conformational Change in hSTING

Ligand Binding Domain (LBD), which undergoes significant conformational change upon binding agonists. (~20 Å)



Open conformation FL-hSTING (PDB ID 6NT5) in grey, closed conformation 2'3'-cGAMP (Item No. 19887)-bound hSTING, LBD (PDB 4KSY) in green

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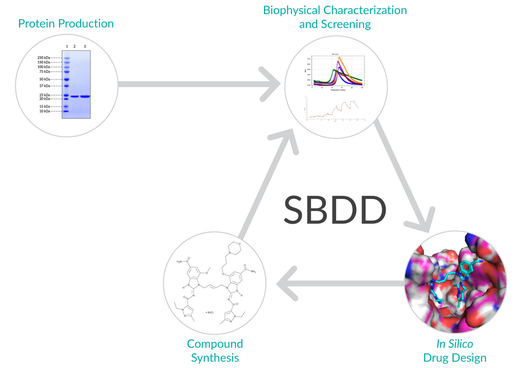
Investigating hSTING Agonist Binding Workflow



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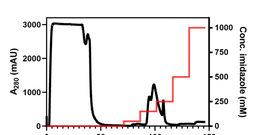
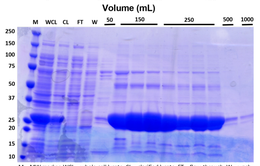
Purification of hSTING Variants

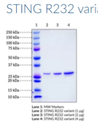
- R232 and A162 hSTING were overexpressed in *E. coli*
- Each purified using an N-terminal His tag, yields >90% purity from affinity chromatography alone.
- Verified that proteins are well-folded and monodispersed by TSA and SEC, respectively.





M = MW marker, WCL = whole cell lysate, CL = clarified lysate, FT = flow-through, W = wash

STING R232 variant (human, recombinant)



BULK & CUSTOM | BOOKMARK

100 µg
$485
Ready to ship

Active, pure human recombinant enzyme

Item No. 22816

Purity: ≥80% estimated by SDS-PAGE

STING A162 variant (human, recombinant)

BULK & CUSTOM | BOOKMARK

100 µg
$484
Contact for status

Pure human recombinant enzyme

Item No. 25306

Purity: ≥75%

STING Agonist Catalog from Cayman Chemical

- Cayman offers a variety of STING agonists

Item No.	Product Name	Structure
32520	STING agonist 1a	
27886	STING Agonist C11	
22353	G10	
24206	ML RR-S2 CDA (ammonium salt)	

Item No.	Product Name	Structure
28054	diABZI STING Agonist 3 (hydrochloride)	
32521	STING agonist 12b	
30140	MSA-2	

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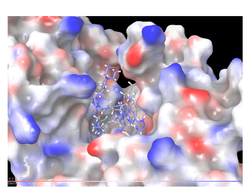
In Silico Screening of hSTING Agonists

- Performed our established computer-aided screening techniques using Schrödinger software to select hSTING agonists offered in our catalog for further characterization *via* Surface Plasmon Resonance (SPR).
  - Glide and Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) calculations were performed against R232, H232, and A162 hSTING variants
  - ADME (Absorption, Distribution, Metabolism, Excretion) and toxicity predictions were computed using QikProp.

Structure-Based Virtual Screening

System Preparation
Docking (Glide)
Scoring Functions
MM/GBSA, FEP
Ranking
ADME/Tox Predictions
Experimental Testing (such as SPR and DSF)



Docked diABZI agonist-3 in R232 hSTING binding site (open conformation, PDB ID 6DXL)

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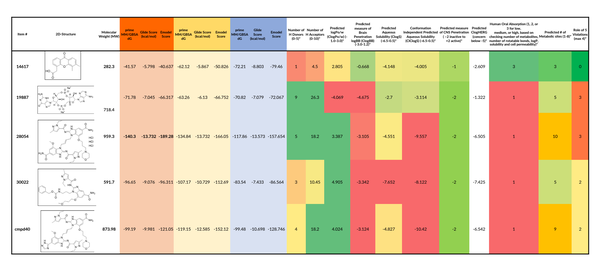
In silico Screening of hSTING Agonists

- Almost 30 hSTING agonists docked against R232, H232, and A162 variants using Glide docking.
- ADME/tox properties were predicted for all the compounds using QikProp.
- MM/GBSA calculations were also performed to obtain better enrichment and estimate relative binding affinity.



- Highlighted orange, yellow, and blue values are related to docking scores against R232, H232, and A162 hSTING variants, respectively.
- * Recommended values for drug-like molecules.

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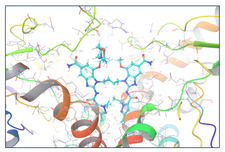
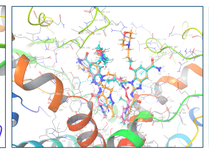
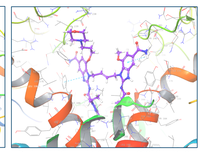
In silico Screening of hSTING Agonists



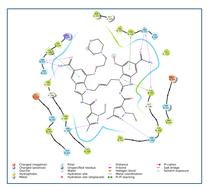
Docked diABZI agonist-3 (Item No. 28054) in R232 hSTING binding site (open conformation, PDB ID 6DXL) is shown in cyan sticks.



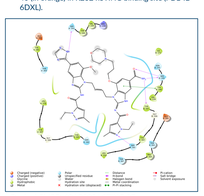
Overlay of docked diABZI agonist-3 (Item No. 28054 in cyan), CAY10748 (Item No. 30022, in pink) and CMPD-40 (in orange) in R232 hSTING binding site (PDB ID 6DXL).



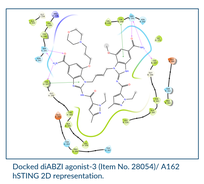
Docked diABZI agonist-3 (Item No. 28054) in A162 hSTING binding site (open conformation, PDB ID 6DXL) is shown in purple sticks.



Docked diABZI agonist-3 (Item No. 28054)/ R232 hSTING 2D representation.



Docked CMPD-40/ R232 hSTING 2D representation.



Docked diABZI agonist-3 (Item No. 28054)/ A162 hSTING 2D representation.

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In silico Screening of hSTING Agonists

- Based on our modeling results, diABZI agonist-3 compound (Item No. 28054) was ranked top amongst the other agonists with the highest estimated relative affinity towards R232, H232, and A162 hSTING variants (lowest MM/GBSA dG Bind: -140, -134, and -117, respectively, prediction of ligand binding free energy).
  - Hydrogen bond interaction was disrupted between agonist and Ser in Ala162 variant.
  - Therefore, this compound was selected for further characterization *via* SPR analysis.

- CAY10748 (Item No. 30022) and CMPD-40 (currently in our R&D synthesis pipeline) were the second top-ranked compounds (MM/GBSA dG Bind: -96 and -99, respectively).
  - No significant differences in interactions with protein binding pocket was observed compared with diABZI agonist-3 (Item No. 28054).
  - In CMPD-40, 1,2,4,triazole ring (instead of amide) is making hydrogen bond interaction to Ser241.

- DMXAA (Item No. 14617) had slightly improved docking scores against A162 variant (MM/GBSA dG Bind: -72.21) compared to R232 and H232 hSTING, which correlates with the previously published data*.
  - Item Nos. 14617 and 30022 compounds couldn't be tested further for SPR due to the solubility issue.
  - Salt versions of both of these compounds are in the synthesis progress, which could potentially help the solubility issue.

*Liu, Y., Lu, X., Qin, N., *et al.* STING, a promising target for small molecular immune modulator: A review. *Eur. J. Med. Chem.* **211**, 113113 (2021).

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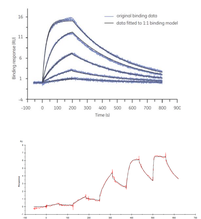
hSTING/Agonists Binding Analysis Studied by SPR

- Established a SPR platform to evaluate binding kinetics and affinity measurements for the hSTING agonists through Single Cycle Kinetics (SCK).
- Synthesized canonical CDN, 2',3'-cGAMP (Item No. 19887) and amidobenzimidazoles, diABZI agonist-3 (Item No. 28054) and purified recombinant hSTING variants were used for SPR analysis using a Biacore T-200 instrument.



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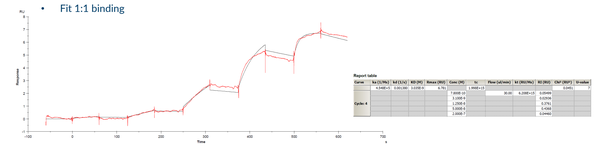
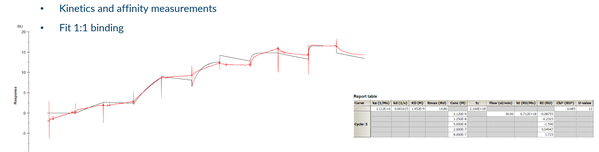
SPR Experiments

- 2',3'-cGAMP (Item No.19887)/ R232 hSTING, K_d = 3.05 nM
  - Kinetics and affinity measurements
  - Fit 1:1 binding



- 2',3'-cGAMP (Item No. 19887)/ A162 hSTING, K_d = 1.45 nM
  - Kinetics and affinity measurements
  - Fit 1:1 binding



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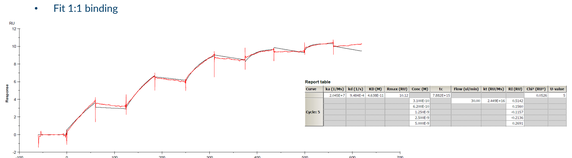
SPR Experiments

- diABZI agonist-3 (Item No. 28054)/ R232 hSTING, K_d = 0.05 nM
  - Kinetics and affinity measurements
  - Fit 1:1 binding



- Both tested agonists (Item Nos. 19887 and 28054) are tight binders for hSTING.
  - Item No. 28054 did not bind A162 variant as well (data not shown).
- Measured affinity for canonical CDN (Item No. 19887) was slightly improved compared to the previously reported data for A162 (~4nM from ITC experiments).
- First reported kinetics and affinity (K_d) values for diABZI agonist-3 (Item No. 28054) towards hSTING (R232). IC_{50} value was reported previously (0.37 µM).

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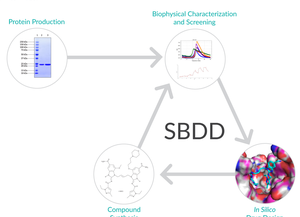
Conclusions

- STING agonists have the potential to enhance the initial anticancer response.
- Cayman Chemical offers a dedicated tool set to study human STING (hSTING) ligand-binding interactions.
- We conducted our established virtual screening techniques to select hSTING agonists for further characterization *via* SPR.
- SPR platform was stablished to evaluate binding kinetics and affinity measurements for the selected agonists using our purified hSTING variants.
- Correlation between SPR results and our *in silico* scoring and ranking.
- First reported kinetics and affinity (K_d) values for diABZI agonist-3 (Item No. 28054) towards hSTING (R232).
- Canonical CDN agonist 2',3'-cGAMP (Item No. 19887) and diABZI agonist-3 (Item No. 28054) could bind tight to both R232 and A162 variants (K_d = 3.05 and 1.45 nM, respectively).
- These findings could provide guidelines for the design and development of hSTING agonists research.



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STING AGONISTS

Item No.	Product Name
30022	CAY10748
28054	diABZI STING Agonist 3
30156	DSDP
22353	G10
24106	ML RR-S2 CDA
30140	MSA-2
31759	SR 717
32520	STING Agonist 1a
27886	STING Agonist C11
32521	STING Agonist 12b

Available at www.caymanchem.com

hSTING VARIANTS

Item No.	Variant/Mutation
22816	R232 (wild-type)
22815	R232H (less dominant allele)
15139	R232H, SUMO-tagged
23592	G230A, R293Q (reduced IFN response)
23594	R284M (constitutively active)
23593	K224R (reduced ubiquitination)
25306	S162A (binds DMXAA)

Learn more at www.caymanchem.com/STINGvariants

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- Biophysical Characterization
- Protein Crystallization
- Structure Determination by X-Ray Crystallography
- SPR Binding Analysis
- *In Silico* Docking and Homology Modeling

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