Development Of Novel STING Pathway Agonists As Vaccine Adjuvants

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Overview

Adjuvants are essential components of subunit vaccines capable of promoting potent and persistent immune responses when combined with antigen or epitope vaccines. However, only few vaccine adjuvants have been approved by the FDA for human use so far. Therefore, there is still an urgent need to develop novel, effective and safe adjuvants for unmet medical needs.

Stimulator of Interferon Genes (STING) plays a central role in shaping both the innate and the adaptive immune response to microbial pathogens primarily by inducing the production of type I interferons (IFNs). STING-mediated processes therefore promote both antibody and cytotoxic T lymphocyte (CTL) immune responses against targeted antigens. Importantly, pharmacologic activation of STING-dependent phenotypes can be employed to greatly enhance vaccine-associated protective immunogenicity against viral, bacterial, and protozoal pathogens. These qualities have raised our interest in developing small molecule agonists of the STING pathway that can be used as adjuvants with protein-based vaccines. Many known STING agonists have shown activity as symmetrical or semi-symmetrical dimers or through binding of two molecules in the receptor pocket. Ramanjulu et al. developed a linking strategy to synergize the effect of two symmetry related amidobenzimidazile (ABZI)-based compounds to create linked ABZIs with enhanced binding to STING and cellular function. Herein, we describe the structure-activity relationship study and synthesis of a series of novel non-CDN small-molecule STING agonists including **compound 6**, **compound 10** and **compound 11** as highly potent human STING agonists with EC50 value of 1.2-1.8 µM. We also confirmed STING specificity of these novel agonists by abolishing STING signaling pathways in the STING knockout cells.

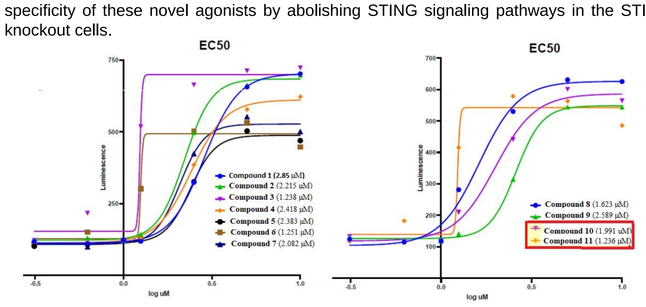
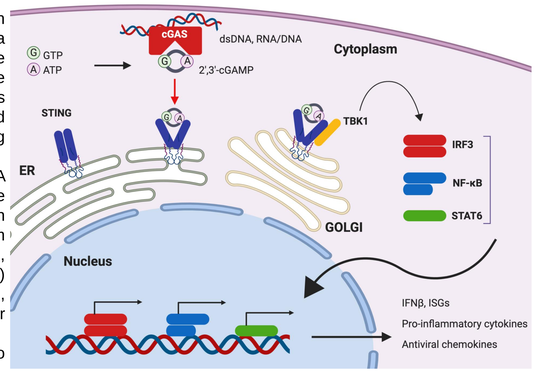


Figure 1. Dose-dependent activity of Inimmune synthesized leads on THF-ISRE cells. Indicated Inimmune synthesized compounds were exposed to reporter THF-ISRE cells in 96 well plates overnight. Luciferin substrate-containing buffer was added to cells and luminescence read on a plate reader. RLU was calculated relative to cells treated with 0.5% DMSO and EC50 calculated. Values presented are mean RLU based on duplicate observations.

Mechanism Of Action

Stimulator of interferon genes (STING) plays a crucial role in the human innate immune system, which is gradually concerned following the emerging immunotherapy.



Double-stranded DNA (dsDNA) in the cytoplasm, which can be derived from pathogens (e.g., bacteria or viruses) and "self" cells (e.g., cancers, dead cells, or damaged mitochondria), binds to and activates cyclic. guanosine monophosphate-adenosine monophosphate synthase (cGAS). The activated DNA-cGAS then catalyzes the synthesis of 2',3'-cyclic GMP-AMP (2',3'-cGAMP) using cytosolic GTP and ATP as substrates. After the binding of 2',3'-cGAMP to STING, activated STING translocates from the endoplasmic reticulum to the perinuclear vesicles via the Golgi apparatus. Consequently, TBK1 is recruited to the STING signalosome, resulting in TBK1 autophosphorylation and the subsequent phosphorylation of STING tails. Then, IRF3 is recruited onto STING signalosome which is subsequently phosphorylated by TBK1. The phosphorylated IRF3 dimer ultimately induces the expression of type I IFNs and pro inflammatory cytokines which could be used for treatment of infection, inflammation, and tumorigenesis

Structure-Based Virtual Screening

The STING receptor is a "V-shaped" symmetrical dimer that opens and closes. Some STING agonists are bound in a dimer conformation. We applied MolSoft's dimeric docking method to determine whether some potential lead STING agonists in this study can dimerize in the pocket. Initially, we docked known STING agonist, G10 molecule to the STING binding pocket using MolSoft's ICM-Pro software (v3.8-7c MolSoft LLC, San Diego, CA). The crystal structure with PDB code 6dxg was used and the pocket was defined using MolSoft's ICM Pocket Finder. G10 gave a "good" dimeric binding pose with a good ligand docking score in this model.

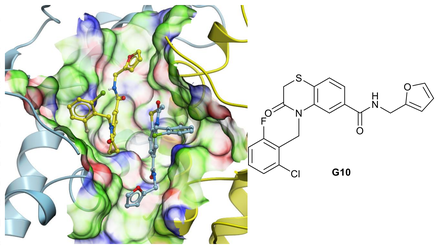


Figure 2. G10 (yellow and blue stick) docked to the STING crystal structure (ribbon representation). The surface of the ligand-binding pocket is displayed, colored by binding property (red- hydrogen bond donor, blue acceptor, green hydrophobic and white lipophilic) .

Optimization of core molecule

In order to evaluate the structural features responsible for the remarkable activity of G10 molecule, we decided to optimize the core molecule. Studies included change of position of the oxo group, change of position of the carbonyl group on the aromatic ring, change of position of the amide linkage and insertion of carbon instead of sulfur. About 50 analogues were synthesized and biologically tested for better SAR understanding.

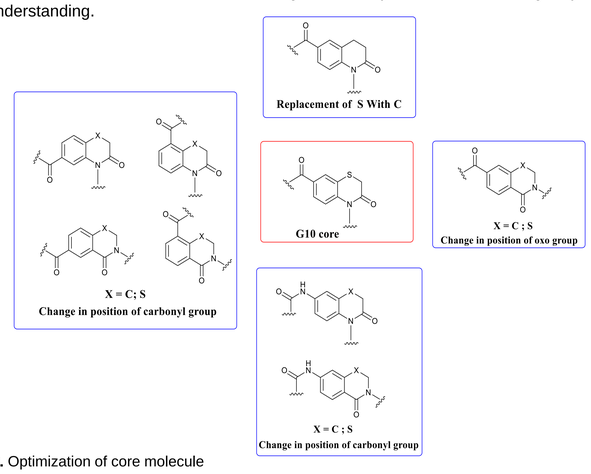
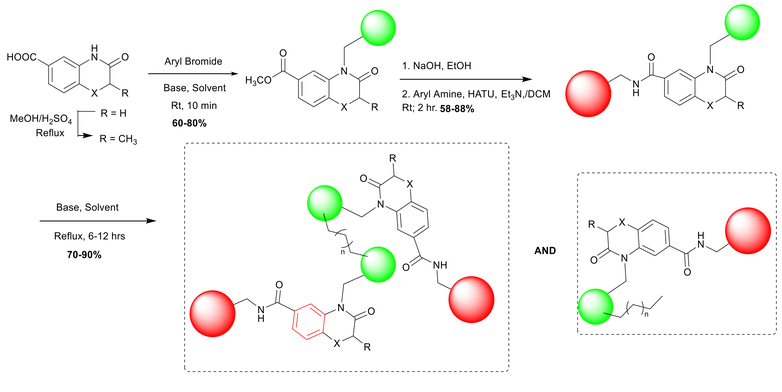


Figure 3. Optimization of core molecule

Synthetic Scheme



In Vitro data

The synthesized compounds were tested for luciferase activity in THF-ISRE (human) cells and RAW-Lucia ISG (mouse) cells. Compounds were also screened for STING specificity using THF-ISRE ΔSTING and THF-ISRE ΔTRIF/MAVs cells. Data for the initial compound library are presented below.

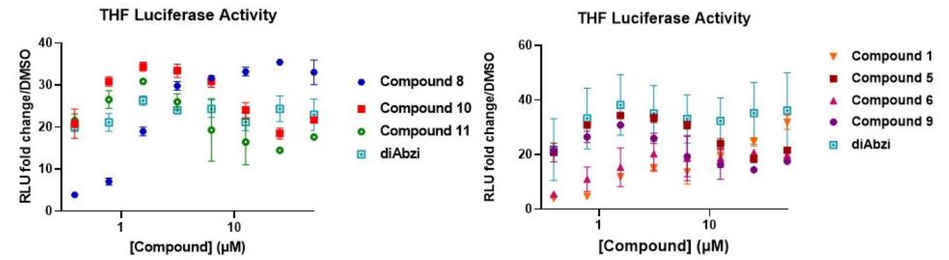


Figure 4. Compounds were diluted to a 2 mg/mL final concentration with 100% DMSO. Treatment was added after cells were cultured overnight at 15,000 cells/well. Compounds, serially diluted by 2, started at 50 µM and ended at 0.3906 µM concentration. Treated cells incubated overnight before testing for luciferase activity. Steady-Glo (Promega) was added 1:1 to cell suspensions in an opaque, flat-bottom 96 well plate. After 5 minutes, the plate was read on Molecular Devices iD5 plate reader.

Zika VLP Prime-Boost Vaccination

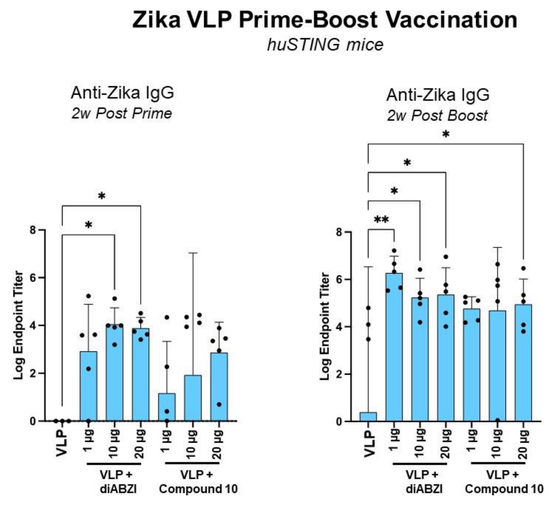


Figure 5. Zika IgG antibody titer after HuSTING mice vaccination. Mice were dosed on day 0 and day 14 with ZIKA VLP with or without adjuvant. Blood was collected on day 14 (dp1) and at the end of the study (dp2).

Conclusion

1- A library of derivatives has been synthesized by following the established optimized reaction conditions, in good to excellent yield.
2- The studies resulted in a new class of relatively small and potent STING agonists.
3- Investigations to formulate the lead compounds for in vivo studies are underway.
4- These new potent STING agonists are easy to prepare and may find application in future efforts to advance vaccine discovery and development in the fight to circumvent alphaviral infection and treat cancer.

Acknowledgement and References

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