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Ten Papers: Briefly highlighting the contributions in them

I. Probing Endosomal Toll-like Receptors (TLRs).

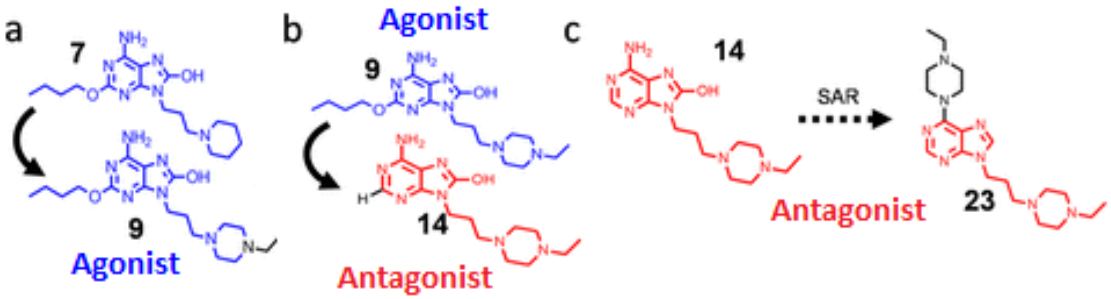
TLRs are members of the larger family of evolutionarily conserved pattern recognition receptors which are critical first line of defense for self-nonself discrimination by the host immune response. Aberrant endosomal TLR (TLR7 and TLR9) activation is implicated in autoreactive inflammation in different autoimmune diseases. Previous reported efforts in this context were mainly random in the *absence of human crystal structure of TLR7 and TLR9*. Many TLR9/TLR7 antagonists (especially oligonucleotides) are in different stages of clinical trial but yet to be available for clinical use. Small molecule candidates are always favorable for well-known reason as compared to oligonucleotides.

The goal of my lab is to rationally design selective antagonists for the nucleic acid-recognizing TLRs (TLR7 and TLR9) for devising novel therapeutic strategies in relevant clinical contexts. We have an exhaustive pipeline consisting of molecules from Purine, Quinazoline, Benzoxazole and Imidazopyridines. This platform was established through applying various rational design strategies described below. We also have two patents in the related field (US10662177-B2. Grant date: 26.05.2020 and WO/2019/092739).

<u>The rational design strategy is AGONIST to ANTAGONIST:</u>

1. **Purine Scaffold.** Our approach was to design TLR7/9 antagonist from TLR7 agonist. We hypothizes that both agonist and antagonist of TLR7/9 might bind at the similar site thus, might share similar structural feature for receptor affinity. We've mapped the path for transforming agonist to antagonist through a single-point change. We've established that a small structural modification 'Chemical Switch' in TLR7 ligand that can lead to reversal in their functional activity. The removal of the butoxy group at C2 position of the TLR7 purine agonist, transform the resulted compound into TLR7 antagonist. **This is a seminal contribution in the field of medicinal chemistry in general.**

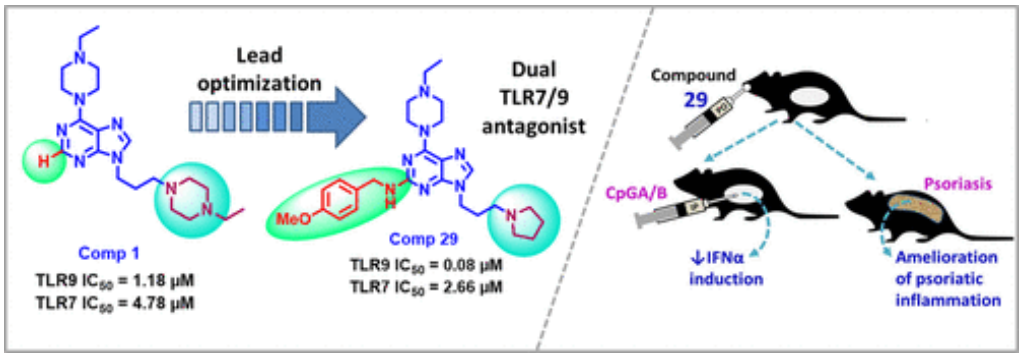
A Chemical Switch for Transforming a Purine Agonist for Toll-like Receptor 7 to a Clinically Relevant Antagonist. Mukherjee A, Raychaudhuri D, Sinha BP, Kundu B, Mitra M, Paul B, Bandopadhyay P, Ganguly D,* and **Talukdar A** ***. Journal of medicinal Chemistry**, **2020**, 63, 4776.



Discovery of TLR7 antagonist from known TLR7 agonist. (a) Conversion of TLR7 agonist **7** (EC_{50} = 2.6 μM) to a more potent TLR7 agonist **9** (EC_{50} = 0.9 μM) by the addition of ethylpiperazine group (b) Chemical Switch: Agonist **9** to weak TLR7 antagonist **14** (IC_{50} = 43.1 μM) by the elimination of C2 butoxyl group (c) Systematic SAR study to potent TLR7 antagonist **23** (IC_{50} = 4.7 μM).

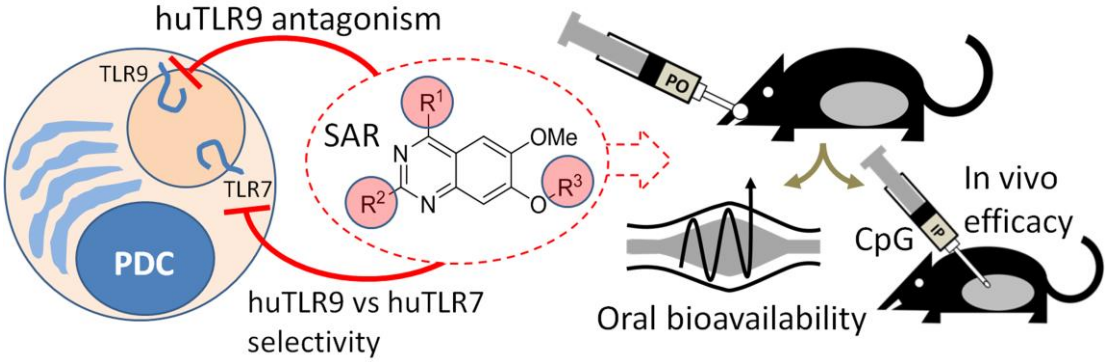
2. The subsequent publication was more of understanding the chemical spaces around our previously designed TLR7/9 antagonists with purine scaffold through extensive structure-activity relationship (SAR) and lead validation through systematically optimizing via in-vitro pharmacokinetic, in-vivo pharmacokinetics, in-vitro and in-vivo toxicity assessment, validation in in-house developed pharmacodynamic mouse model and finally establishing the efficacy of the lead candidate in a preclinical mouse model of the autoimmune disease psoriasis.

Systematic Optimization of Potent and Orally Bioavailable Purine Scaffold as a Dual Inhibitor of Toll-Like Receptors 7 and 9. Kundu B, Raychaudhuri D, Mukherjee A, Sinha BP, Sarkar D, Bandopadhyay P, Pal S, Das N, Dey D, Ramarao K, Nagireddy K, Ganguly D,* and **Talukdar A***. **Journal of Medicinal Chemistry**, **2021,** *64*, 9279–9301.



3. **<u>Activity Guided Rational Design: Quinazoline Scaffold.</u>** Through an activity-guided approach (based on hTLR9/7-inhibition in primary human immune cells and hTLR9/7 reporter cells), we have identified chemical features in quinazoline core that are not only essential for selective hTLR9 inhibition as well as dual TLR7 and TLR9 inhibition. We found that the substitution patterns at C-2, C-4 and C-7 of quinazoline ring act in concerted manner to influence the potency of hTLR9 inhibition as well as impart selectivity against the closely related receptor hTLR7. We have also optimized the lead candidate with favorable ADME properties along with favorable oral bioavailability, urinary excretion kinetics and in vivo TLR9 antagonism efficacy for one of the representative lead compound in a clinically relevant rodent model of aberrant TLR9 activation.

   Activity-guided Development of Potent and Selective Toll-like Receptor 9 Antagonists. Paul B, Rahaman O, Roy S, Pal S, Satish S, Mukherjee A, Ghosh AR, Raychaudhuri D, Bhattacharya R, Goon S, Ganguly D* and **<u>Talukdar A</u>*. European Journal of Medicinal Chemistry**, **2018**, *159*, 187.



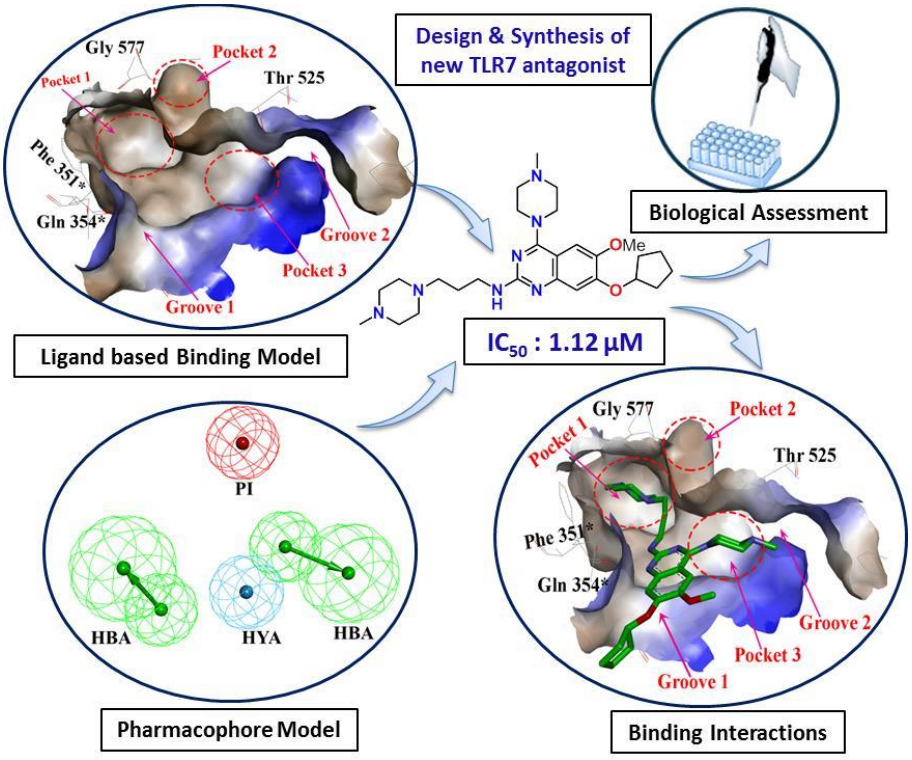
4. **<u>Chemical Optimization of existing antagonists: benzoxazole as model study.</u>** The initial study was based on structural exploration of the hTLR9/hTLR7 antagonism with the help of known E6446 hTLR9/hTLR7 antagonist having 2-phenylbenzoxazole core that can strongly inhibit TLR9/TLR7 signaling. From drug designing perspective our antagonists had lesser molecular flexibility. We provided a correlation between our binding mode hypothesis and hTLR9 antagonistic activity for future rational development.

   Design and Development of Benzoxazole Derivatives with Toll-like Receptor 9 Antagonism. Roy S, Mukherjee A, Paul B, Rahaman O, Roy S, Maithri G, Ramya B, Pal

S, Ganguly D,* and **Talukdar A***. **European Journal of Medicinal Chemistry**, **2017**, *134*, 334-347.

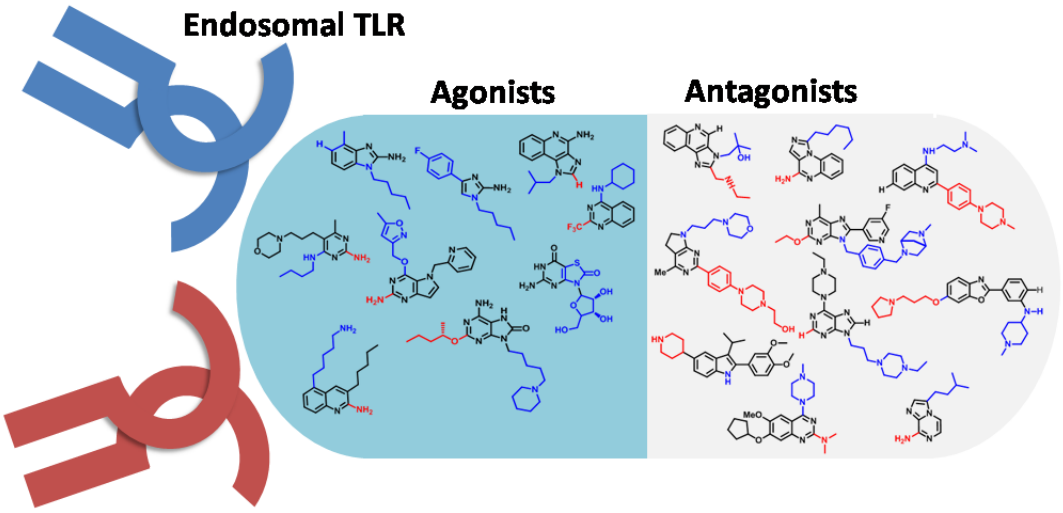
5. **Universal Binding Model.** Due to the unusual topology of the ligand binding surface of TLR9 and TLR7 lacking conventional pockets, the functional mechanism of potential TLR9 antagonism by small molecules is not understood, consequently small molecule TLR9 antagonists have so far been developed by empirical screening. Our lab has proposed hypothetical binding model to design TLR9 and TLR7 antagonists. The proposed ligand-receptor interaction could be which correlate with TLR7 antagonistic activity thus paving the way for rational design using varied chemotypes. **Later our hypothetical model was validated through X-ray co-crystal structure of TLR7 published in Nature Comm. by Tojo et. al. doi: https://doi.org/10.1038/s41467-020-19025-z**

Synthesis and characterization of new potent TLR7 antagonists based on analysis of the binding mode using biomolecular simulations. Pal S, Paul B, Bandopadhyay P, Preethy N, Sarkar D, Rahaman O, Goon S, Roy S, Ganguly D,* **Talukdar A *. European Journal of Medicinal Chemistry, 2021,** 210, 112978.



6. Perspective. Recently, we have published a Perspective in J. Med. Chemistry highlighting rational medicinal chemistry approaches to elucidate the structural attributes of small molecules capable of agonism or antagonism or of elegantly switching between the two. The structural evolution of different chemotypes can provide the framework for the future development of endosomal TLR agonists and antagonists.

Structural Evolution and Translational Potential for Agonists and Antagonists of Endosomal Toll-like Receptors. **Talukdar A**,* Ganguly D, Roy S, Das N, Sarkar D. **Journal of Medicinal Chemistry**, **2021,** 64, 12, 8010.



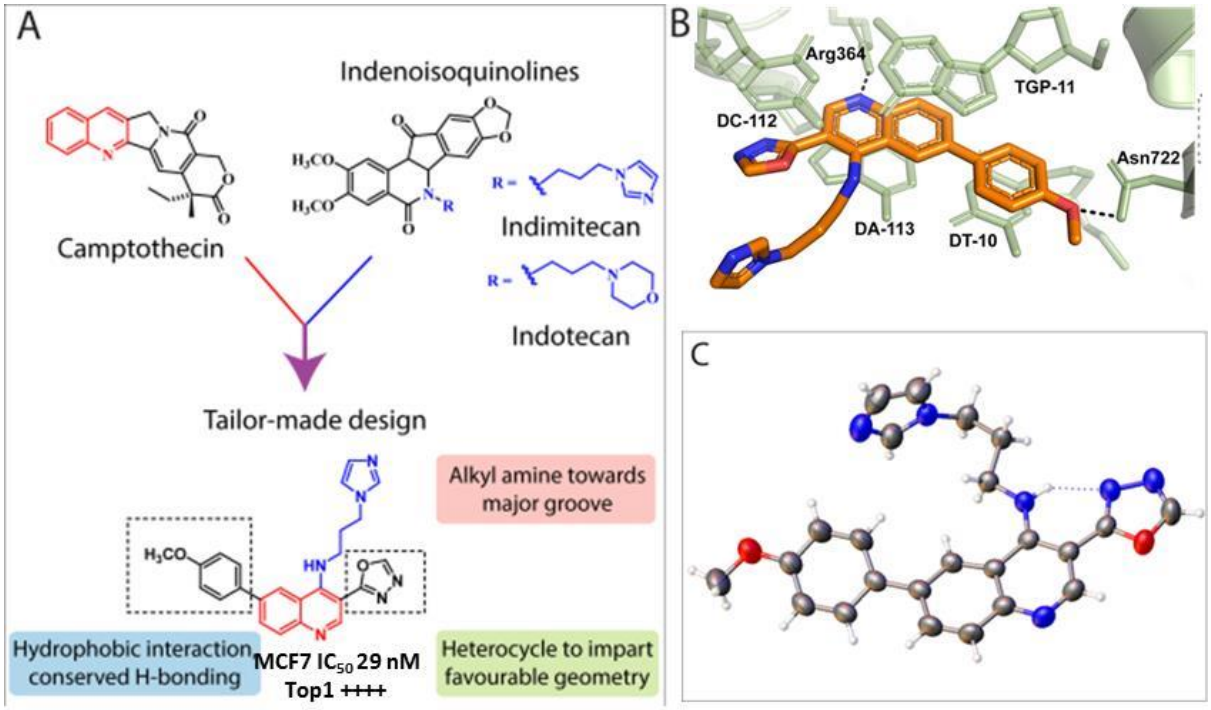
II. Design and Development of *non-camptothecin'* topoisomerase I (Top1) inhibitors for cancer chemotherapy

In various tumor cells, Top1 is over-expressed than the normal cells; hence, modulating the Top1 activity in tumor cells to block DNA replication and cell division has made Top1 an attractive drug target for anticancer therapy. Anticancer drugs like camptothecin (CPT) and its FDA approved derivatives (Topotecan and Irinotecan) selectively trap Top1-DNA cleavable complexes, which can account for the killing of dividing malignant cells by 'Top1 poisons'. Topotecan and irinotecan are used for advanced colorectal carcinomas and ovarian cancers, which emphasizes the significance of Top1 as a drug target. However, CPTs are not ideal drug molecules due to their toxicity, inherently unstable chemical structure which rapidly inactivated in plasma due to hydrolysis of

lactone E-ring and suffer from rapid cellular efflux via membrane pumps (Pgp). As a result there is great interest in the development of '*non-camptothecin*' Top1 inhibitors as anticancer agents.

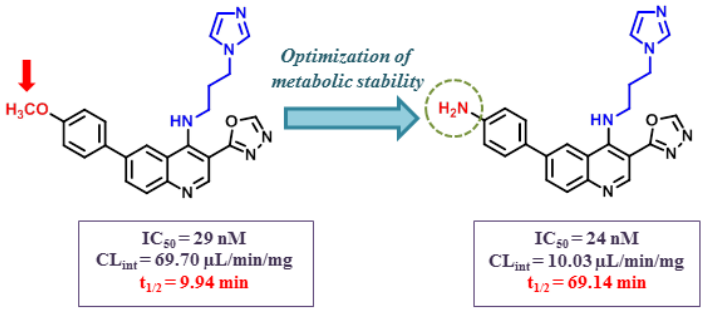
7. **Hypothesis driven Conceptual Design.** *Here we have successfully developed and patented (WO2019229765) a new class of potent and selective Top1 poison based on the quinoline core with improved physicochemical properties as well as potency than camptothecin.* The design was initiated based on the structural features of known ligands/poison that bind through the network of interactions in the active site of human Top1 enzyme. *Our selective lead Top1 poison is bicyclic in nature unlike polycyclic camptothecins or indenoisoquinolines.* Strategically placed C4 nitrogen atom along with a heterocycle at the C-3 position would form an intramolecular hydrogen bonding (proved through crystal study), which impart requisite polycyclic geometry and suitable curvature essential for stabilizing the Top1−DNA cleavage complex. Our Top1 poison does not intercalate with DNA nor react with Top1 enzyme but have the ability to stabilize covalent Top1-DNA intermediate to form ternary complex. We have also provided mechanistic insight of Top1 inhibition through live cancer cell imaging and through mutation study. Unlike CPTs, they can stabilize Top1−DNA cleavage complexes even after 5 h proved by gamma-H2AX assay.

   Discovery and Mechanistic Study of Tailor-Made Quinoline Derivatives as Topoisomerase 1 Poisons with Potent Anticancer Activity. Kundu B, Das SK, Paul Chowdhuri S, Pal S, Sarkar D, Ghosh A, Mukherjee A, Bhattacharya D, Das BB,* and **Talukdar A*. Journal of Medicinal Chemistry, 2019**, *62*, 3428.



8. **Lead Optimization**. The developed Top1 poison with quinoline core was further optimized for greater metabolic stability and efficacy. For lead optimization various in-vitro pharmacokinetic assays were performed such as solubility, Caco2 permiability, metabolic stability, plasma stability, identification of metabolities etc.

   Development of a metabolically stable topoisomerase I poison as anticancer agent. Kundu B, Sarkar D, Chowdhuri SP, Pal S, Das SK, Das BB, and **Talukdar A*. European Journal of Medicinal Chemistry**, **2020**, *202*, 112551.

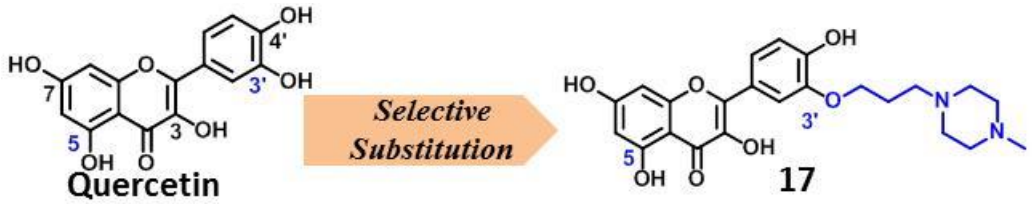


9. **Ligand-based Design**. In order to envisage structurally diverse novel chemical entity as Top1 poison with better efficacy, Ligand-based-pharmacophore model was developed using 3D QSAR pharmacophore generation (HypoGen algorithm) methodology to identify hit molecules which are now being modified in my lab for designing future class of potential topoisomerase I inhibitor.

Ligand-based Pharmacophore Modeling, Virtual Screening and Molecular Docking Studies for Discovery of Potential Topoisomerase I Inhibitors. Pal S, Kumar V, Kundu B, Bhattacharya D, Preethy N, Reddy MP, **Talukdar A*. Computational and Structural Biotechnology Journal, 2019**, *17*, 291.

10. **Natural product Based drug design through modulating pharmacokinetics**. Regioselective substitution of specific hydroxyl group quercetin to modulate its log D value and aqueous solubility through the attachment facilitator moieties led to ~100-fold increase in the cytotoxic activity of our semisynthetic derivatives in colon cancer cells as compared to quercetin. We have also validated the lead derivative in in-vivo CT-26 tumor-bearing mice in a colon cancer model. We believe that the study has an immense potential toward the systemic development of natural products which has poor bioavailability.

Semisynthetic Quercetin Derivatives with Potent Antitumor Activity in Colon Carcinoma. A Mukherjee, S Mishra, NK Kotla, K Manna, S Roy, B Kundu, Bhattacharya D, Saha KD, **Talukdar A*. ACS Omega, 2019,** *4*, 7285.



Compd	Solubility pH=7.4 (µg/mL)	$logD_{7.4}$	IC_{50} HCT116 (µM)	In Vivo Efficacy (CT 26) (Dose: 50 mg/kg)	
				Tumor reduction	Survival (30 days)
Quercetin	0.1	0.76	45.3	15%	0%
17	**41.5**	**2.53**	**0.48**	**60%**	**67%**