Small molecule inhibitors of NOD2 signaling targeting the receptor-interacting protein kinase 2

A thesis submitted by

Bing Dai

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Advisor: Alexei Degterev Ph.D

Abstract

Receptor-interacting protein kinase 2 (RIPK2) mediates inflammatory signaling through the bacteria-sensing receptors nucleotide-binding oligomerization domain-containing protein 1 and 2 (NOD1 and NOD2). Designing kinase inhibitors target RIPK2 is a possible strategy to ameliorate NOD-mediated pathologies, such as Crohn's diseases, sarcoidosis and inflammatory arthritis. Discovering potent inhibitors is of importance for the further investigation of the RIPK2 function. The thesis describes characterization of the activity of a new series of RIPK2 inhibitors. To test their activity, three different methods were used to fully understand inhibition of NOD2 signaling by these molecules. Based on the HEK-BLUE assay, ADP-Glo assay, as well as the Nano-BRET assay, several CSLP compounds displayed potent, low nanomolar activity. Curiously, based on the residence time, we found there is no direct connection between the cellular IC_{50} of the molecules and the residence times. This study provides important data for further targeting RIPK2 kinase to attenuate pathologic inflammatory signaling.

Acknowledgements

I would like to thank my advisor Dr. Alexei Degterev for his teaching and all the knowledge I gained in his lab.

I can still remember at the 2nd month when he passed me a bottle of medium and said, “Hi, Bing, here is the first bottle of medium in your life.”

We had lots of discussions about the project. I made lots of mistakes and he fixed all of them.

Then I would like to thank Dr. David Greenblatt, it was the first time I spoke with a native speaker in English, I was so nervous at that time, he told me to relax and even played some relaxing music. Also I did my first rotation in his lab, he is such a kind and patient person that give me tremendous encouragement to present all my ideas.

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List of Abbreviations

ADP adenosine diphosphate

ATP adenosine triphosphate

BCR-ABL a fusion gene name

BRET Bioluminescence Resonance Energy Transfer

DNA deoxyribonucleic acid

FAS first apoptosis signal receptor

IAP inhibitor of apoptosis proteins

IC_{50} the half maximal inhibitory concentration

IKK NF-κB-activating IκB kinase

IκBs inhibitor of κB

LUBAC linear ubiquitin chain assembly complex

MAP mitogen-activated protein

MDP muramyl dipeptide

NEMO NF-kappa-B essential modulator

NF-κB nuclear factor kappa-light-chain-enhancer of activated B cells

NOD nucleotide-binding oligomerization domain-containing protein

PEI polyethylenimine

RHIM RIP homotypic interaction motif

RIPK receptor-interacting protein kinase

SEAP secreted embryonic alkaline phosphatase

TIE Tyrosine-protein kinase receptor

VEGFR vascular endothelial growth factor receptor

Chapter 1: Introduction

1.1 Background

NOD1 and NOD2 are important receptors for the innate immune system. It has been reported that NOD1 can be stimulated by a tripeptide motif found in peptidoglycan of Gram-negative bacteria[1], while NOD2 is triggered by MDP[2]. Multiple articles describe the function of NOD proteins in defenses against bacterial infections, such as Staphylococcus aureus[3] and Helicobacter pylori[4].

Receptor-interacting protein kinase 2 (RIPK2) is a threonine/serine protein kinase belonging to the RIPK family. This family possesses a relatively conserved kinase domain but distinct non-kinase regions[5]. It is a critical component of NOD1 and NOD2 signaling[6].

Stimulation of NOD2 recruits RIPK2 along with several ubiquitin ligases, including IAP[7] and LUBAC[8]. Additional ubiquitin-dependent signaling events activate MAP kinases and the IKK complex include IKKα, IKKβ, and NEMO (also termed IKKγ) [9]. This leads to the activation of NF-κB transcription factors controlling production of proinflammatory cytokines and chemokines[10].

Excessive NOD2 activation is associated with numerous diseases such as Crohn's diseases[11], sarcoidosis, and inflammatory arthritis[12]. Thus, RIPK2 inhibitors are sought as potential treatments for these diseases.

Several RIPK2 inhibitors have been developed, such as the commercial available drug ponatinib which primarily targets BCR-ABL, and regorafenib which has dual functions of VEGFR2-TIE2 tyrosine kinase inhibition. But these molecules lack specificity towards

RIPK2. We recently described a strategy targeting the activation loop of RIPK2[13]. A different class of molecules is exemplified by CSLP compound, as shown below.

1.2 CSLP compounds

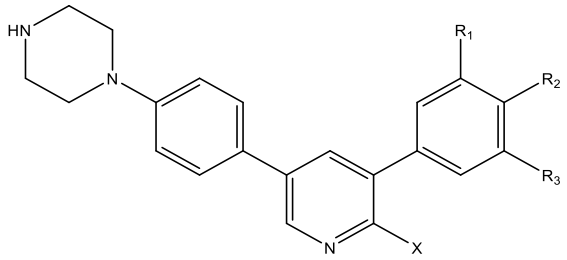

Figure 1.1: The molecular structure of CSLP compounds

Compounds	X	R1	R2	R3
CSLP 18	NH_2	H	OMe	$-NHSO_2{}^nPr$
CSLP 37	NH_2	F	OMe	$-NHSO_2{}^nPr$
CSLP 38	NH_2	F	H	$-NHSO_2{}^nPr$
CSLP 43	NH_2	OMe	OMe	$-NHSO_2{}^nPr$
CSLP 48	NH_2	H	OH	$-NHSO_2{}^nPr$
CSLP 51	NH_2	OMe	Me	$-NHSO_2Ph$
CSLP 52	NH_2	$-OCH_2CH_2O-$		$-NHSO_2{}^nPr$
CSLP 53	Me	F	OMe	$-NHSO_2{}^nPr$
CSLP 54	NH_2	OEt	Me	$-NHSO_2$ Bn
CSLP 55	NH_2	F	OMe	OMe

Table 1.1: The molecular structure of CSLP compounds

From previous studies we have already tested CSLP 1~50 activities using HEK-BLUE and ADP-Glo assay. Here we review some of these compounds as well as the CSLP51~55.

Chapter 2: Materials and Methods

2.1 HEK-Blue assay

HEK-Blue-hNOD2 cells were obtained from Invivogen. The cell line consists of HEK293 cells transfected with the human NOD2 gene and an optimized SEAP reporter gene.

The SEAP reporter cassette allows expression of secreted alkaline phosphatase following induction of NF-κB signaling pathway. Using SEAP substrate (HEK-BLUE Detection assay), allows monitoring NOD2-NF-κB activation.

For the HEK-Blue assays. HNOD2-HEK-Blue cells were obtained from Invivogen, cells were first thawed with DMEM medium cotaining 10% heat-inactivated FBS (Sigma), 1% antibiotic-antimycotic mix (ThermoFisher), and normocin 100μg/mL (InvivoGen). For further maintenance the medium is supplemented with extra 30 μg/ml Blasticidin and 100 μg/ml Zeocin, respectively. All antibiotics were from InvivoGen. After several passages, cells were seeded onto clear 96 well plates at $7.5*10^3$ cells/well and allowed to attach for 48 hr. On the day of the experiment, media was changed to 100 μL of HEK-Blue detection media (Invivogen). Inhibitors were diluted and added to 0.5 μL DMSO 15 min prior to the addition of 1 ng/ml L18-MDP (InvivoGen). After 8-9 hr, absorbance at 620 nm was measured using the Victor3V plate reader. Values of media-only wells were subtracted and %inhibition for each compound concentration relative to the DMSO/MDP-treated controls was calculated. Inhibition values plus StDev were fitted by non-linear regression using Prism software (GraphPad) to calculate IC_{50} values. The equation is called Sigmoidal dose response (variable slope) which is Y=Bottom + (Top-

Bottom)/(1+10^[(LogEC_{50}-X)*HillSlope)]. The Top is fixed as 100, Bottom is fixed as 0, EC_{50} and HillSlope is calculated by automatically.

2.2 ADP-Glo assay[14].

The ADP-Glo assay is based on the enzyme reaction between kinase and ATP. As ATP is converted by the kinase to generate ADP. ADP is then converted later back into ATP, which can be using for a luciferase reaction. Kinase inhibitor attenuates ADP generation, thus this assay can be used generally for many kinases.

For this assay (commercially available from Promega), 10 ng of recombinant RIPK2 was diluted in reaction buffer (40 mM Tris-HCl pH 7.5, 20 mM $MgCl_2$, 0.5 mM DTT, 0.01% BSA) supplemented with 50 μM ATP and varying concentrations of inhibitors. Reactions were performed at room temperature for 1 hour. In 5 μL total volume (5% final concentration of DMSO) and stopped by addition of 5 μL of ADP-Glo reagent for 40 min at room temperature. Luminescent signal was generated by addition of 10 μL of kinase detection reagent for 30 minutes at room temperature and determined using the Victor3V plate reader. Specific signal was calculated by subtracting values in the wells without protein and inhibitor from the values in the test wells. Inhibition, % = ((specific signal (DMSO control) - specific signal (inhibitor))/ (specific signal (DMSO control))) x 100%. Non-linear regression to determine IC_{50} values was performed using Prism software use the same equation showed before.

2.3 Nano-BRET assay

HEK-Blue or ADP-Glo assays report of the inhibition of NOD2 responses as well as the affinity of the inhibitors towards RIPK2. However, the ADP-Glo does not assess the binding between a kinase and inhibitors in the cells. This is achieved by using Bioluminescence Resonance Energy Transfer, an approach first developed by Promega. In this assay, the target protein is tagged with Nano Luciferase and acts as a BRET donor, while an ATP competitive inhibitor-based fluorescent tracer serves as a BRET acceptor. When the tracer binds the protein, energy transfer from the donor to the acceptor can be measured and is attenuated by an inhibitor.

2.3.1 Nano-BRET target engagement assay

HEK-Blue cells were maintained as described above.

NanoLuc RIPK2 transfection: On day 1, HEK-Blue cells were seeded in a T-75 flask in 12 mL of media at a density of $2*10^5$ cells/mL. On day 2, 13.5 μg Transfection Carrier DNA (Promega), 1.5 μg NanoLuc-RIPK2 Fusion Vector (Promega), and 45 μL PEI(Sigma) were mixed in 1.2 ml of phenol red-free Opti-MEM media (ThermoFisher). After 30 min of room temperature incubation, the DNA mixture was added to the T-75 flask containing the cells. On day 3, cells were stimulated with 1 ng/ml L18-MDP for 1h, washed, trypsinized, and resuspended in Opti-MEM media. After a 5 min centrifugation at 1,400 rpm, cells were stored in aliquots at -80 ^{0}C.

Nano-BRET inhibition assay: HEK-Blue cells were thawed and density adjusted to $2*10^5$ cells/mL. 100X Nano-BRET In-cell Kinase Tracer (Promega) was diluted to 20X in

phenol red-free Opti-MEM supplemented with 12.5 mM HEPES (ThermoFisher) and 31.25% PEG-400 (Sigma). 10X inhibitor stock solutions in Opti-MEM were prepared by diluting DMSO stock solutions. For the assay, 11.9 mL cells/well were seeded onto a white low volume 384 well plate (Corning) and mixed with 0.7 ml 20X tracer and 1.4 ml 10X inhibitors, followed by incubation for 2 h at 37^0C. 3X substrate mix was prepared by adding Nano-BRET Nano-Glo substate (Promega) (1:166 dilution) and extracellular NanoLuc Inhibitor (Promega) (1:500 dilution) into Opti-MEM media. 7 ml of 3X substrate mix was added onto each well with the cells. Plates were mixed on a rotary shaker for 15 seconds at 500 rpm. Emission was determined using the Victor3V plate reader at 460 nm for donor (NanoLuc) and 610 nm for acceptor (tracer). Nano-BRET ratios were calculated as [(Acceptor sample / Donor sample) – (Acceptor no tracer control/Donor no tracer control)] * 1000, Affinity %=(sample Nano-BRET ratio / control Nano-BRET ratio)*100%, then values were used for non-linear regression to determine IC_{50} values was performed using Prism software use the same equation showed before.

2.3.2 Residence time measurements using RIPK2 Nano-BRET assay.

HEK-Blue cells transfected with NanoLuc-RIPK2 were adjusted to $2*10^5$ cells/mL and seeded onto 12 well plates at 1 ml cells/well. 1 μl of each compound was added to the test wells to achieve 5X IC_{50} concentration determined in previous Nano-BRET inhibition assay, and incubated for 2h at 37^0C. After incubation, cells were washed carefully with Opti-MEM, re-adjusted to $2*10^5$ cells/ml if needed and were added to a 384 well plate at 22.5 ml/well. As positive controls for maximal inhibition, samples were left with inhibitor without washes. 25 ml 2X substrate mix and 2.5 ml 20X tracer were added into

each well. 2X substrate mix was prepared by diluting Nano-BRET Nano-Glo substate (Promega) (1:250 dilution) and extracellular NanoLuc Inhibitor (Promega) (1:750 dilution) in Opti-MEM media. Plates were mixed for 10 second at 500 rpm in a rotary shaker and the emission signals were measured 60 times at 2 min intervals. The degree of inhibition at each time point was calculated based on samples with DMSO (no inhibition) and un-washed samples with inhibitor (maximal inhibition). Percent inhibition changes over time were used for non-linear regression to calculate T50% values the equation is the same as showed before.

Chapter 3: Results

3.1 HEK-BLUE assay

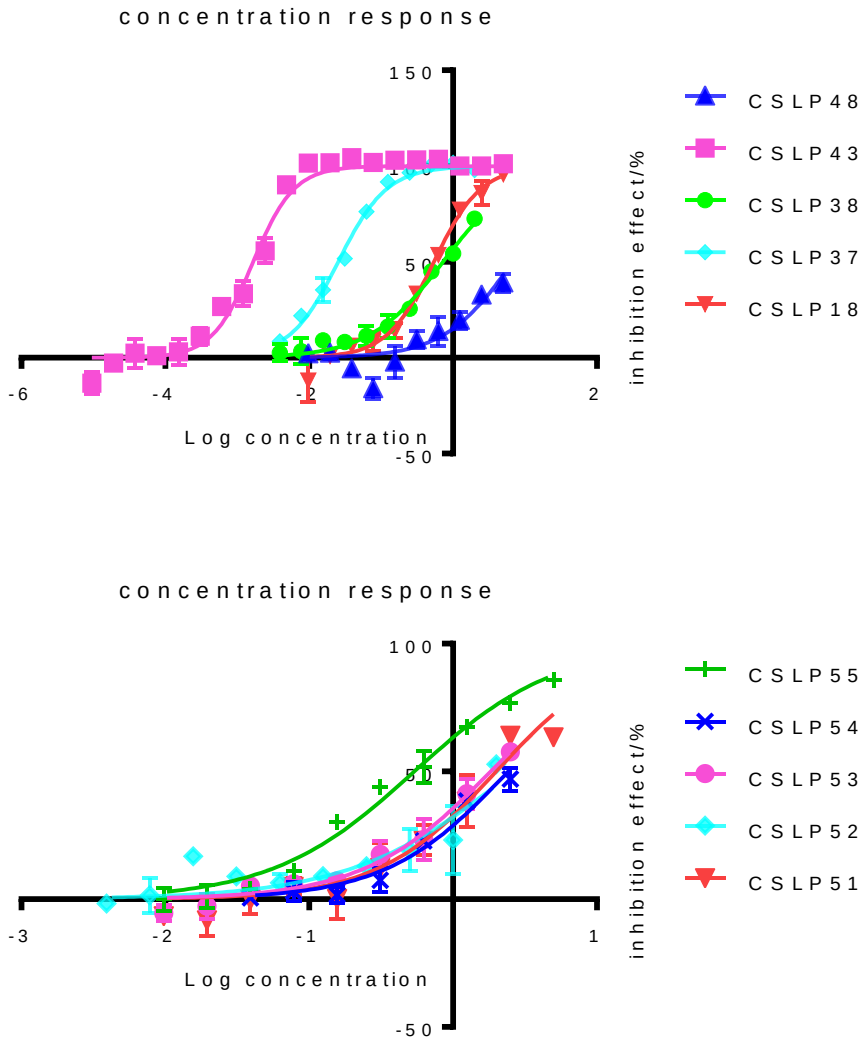

Figure 3.1: HEK BLUE concentration response curve.

The inhibition effect is normalized by the control. X axis is already transfer to log concentration, previous concentration is μM.

Compounds	IC_{50}/nM
CSLP 18	542.5
CSLP 37	25.3
CSLP 38	725.5
CSLP 43	1.7
CSLP 48	6766.0
CSLP 51	1995.0
CSLP 52	2647.0
CSLP 53	1830.0
CSLP 54	2417.0
CSLP 55	538.6

Table 3.1: IC_{50} of CSLP compounds in the HEK-Blue assay.

3.2 ADP-glo assay

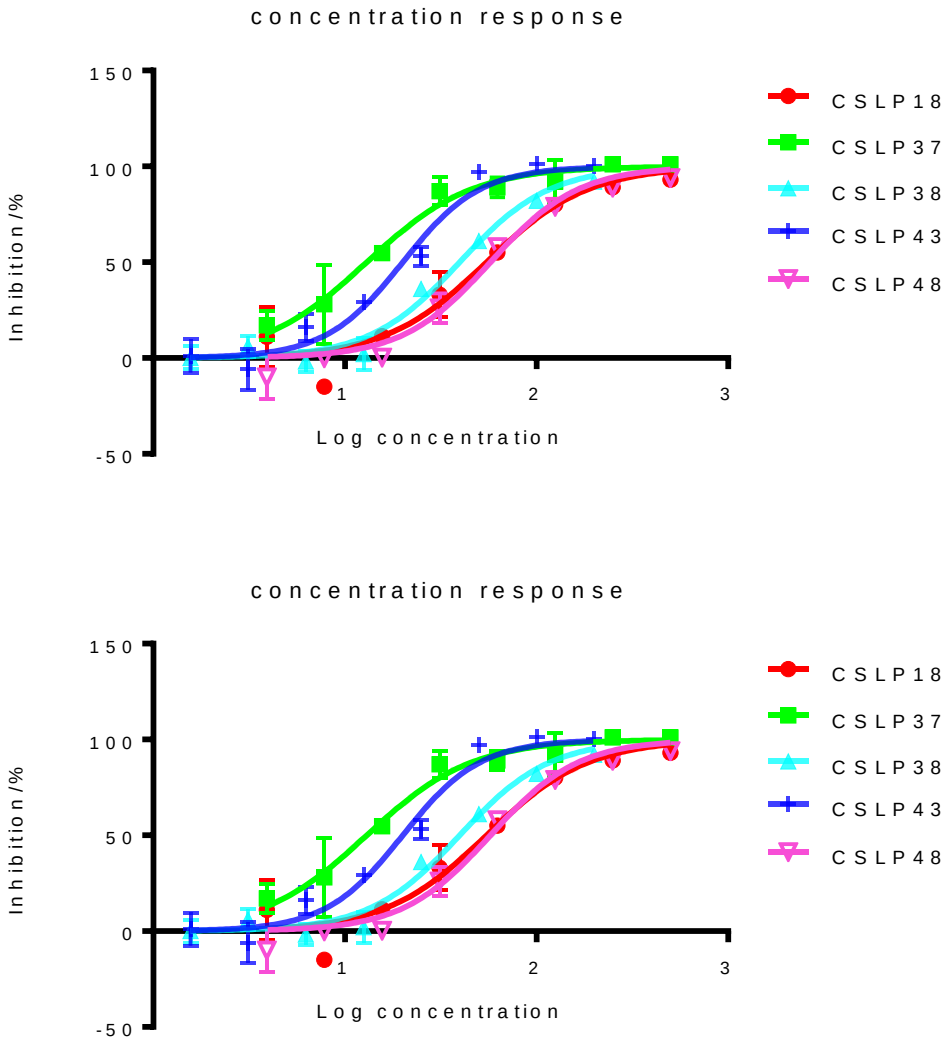

Figure 3.2: ADP-Glo concentration response curve.

The inhibition effect is normalized by the control. X axis is already transfer to log concentration, previous concentration is nM.

Compounds	IC_{50}/nM
CSLP 18	54.8
CSLP 37	13.0
CSLP 38	39.7
CSLP 43	20.0
CSLP 48	57.5
CSLP 51	No inhibition
CSLP 52	519.8
CSLP 53	3426.0
CSLP 54	5063.0
CSLP 55	39.19

Table 3.2: IC_{50} of CSLP compounds in the ADP-Glo assay.

From HEK-Blue and ADP-Glo assays, we observed some correlation between in vitro and in vivo activities. However, a number of outliers was identified including CSLP43 and CSLP48. Thus, we used a Nano-BRET assay method to examine whether aberrant cellular values may reflect differences in small molecules' binding to RIPK2 in the cells vs. recombinant RIPK2. Based on the HEK-Blue and ADP-Glo experiments, we choose CSLP18, 37, 38, 43, 48, 53, 55 for Nano-BRET assays, due to the broad range of their activities in ADP-Glo vs HEK-BLUE assays.

3.3 Nano-BRET target engagement assay

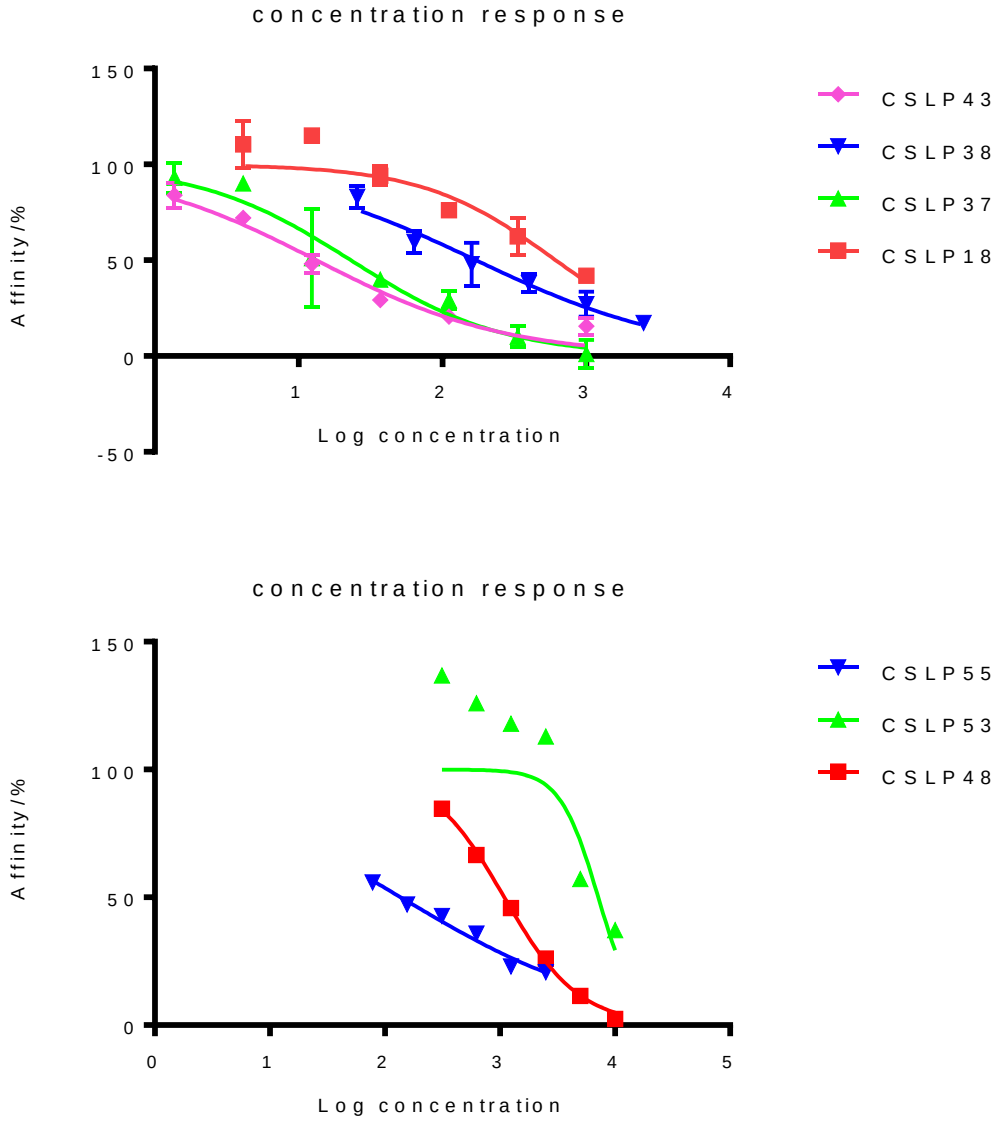

Figure 3.3: Nano-BRET concentration response curve.

Compounds	IC50/nM
CSLP 18	613.4
CSLP 37	23.1
CSLP 38	171.0
CSLP 43	13.3
CSLP 48	1091.0
CSLP 53	7149.0
CSLP 55	137.2

Table 3.3: IC_{50} of CSLP compounds in the Nano-BRET assay.

Comparison of the three methods indicate that Nano-BRET assay is closer indicator of changes in cellular responses (measured in HEK-BLUE assay) compared to the affinity of the compounds towards the recombinant RIPK2 (ADP-Glo assay). Because of major changes in cellular activities in CSLP analogs, we also sought to determine whether these molecules display differences in the residence times, which is an important parameter in the understanding tissue exposure to different drugs in vivo.

3.4 Nano-BRET residence time assay

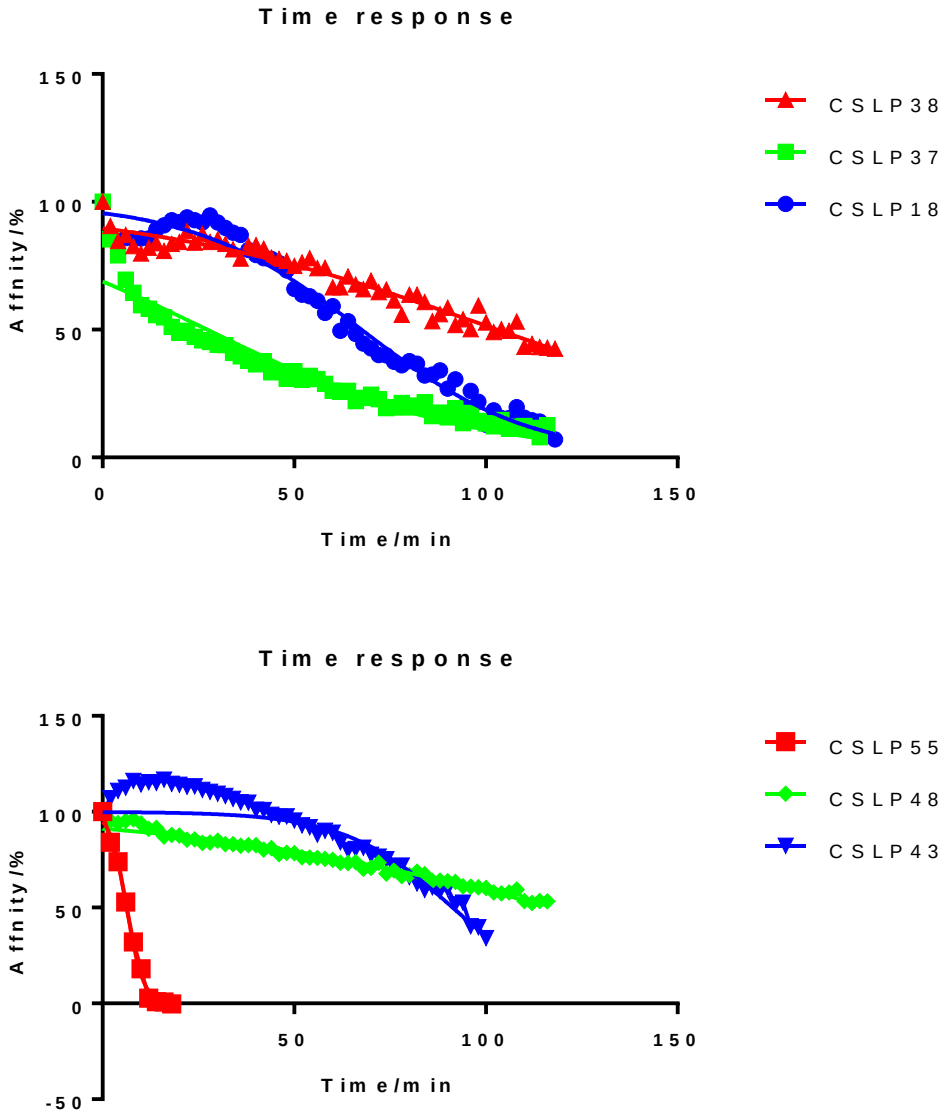

Figure 3.4 Nano-BRET residence time response curve.

Compounds	T50%/min
CSLP 18	67.6
CSLP 37	27.3
CSLP 38	103
CSLP 43	91.3
CSLP 48	118.2
CSLP 55	6.3

Table 3.4 Nano-BRET T50% of CSLP compounds.

Curiously, we find no correlation between residence times and cellular affinities or activities. When used at the comparable 5X IC_{50} concentrations, molecules again display a broad range of residence times. However, while CSLP 37 is a potent NOD2/RIPK2 inhibitor, its residence time is short, while CSLP 43 is also a potent inhibitor, but its residence time is long.

Chapter 4: Discussion.

Targeting the NOD1 and NOD2 pathway using the RIPK2 inhibitors could be a useful approach. Testing the IC_{50} against recombinant target proteins is the first step to understand an inhibitor properties. Unexpectedly, multiple CSLP analogs displayed potent inhibition of RIPK2 in our ADP-Glo RIPK2 assay. However, only a small subset of these molecules displayed potent inhibition of RIPK2 responses in the cells, based on our HEK-BLUE data and other assays, which were performed by other lab members. To address this important discrepancy, we implemented a cellular target engagement method, recently developed by Promega. We applied this approach both to evaluate cellular affinity of inhibitors and their residence times. Strikingly, these data clearly suggested that binding of the inhibitors to the full length RIPK2 protein in the endogenous environment is very different from interactions between the inhibitors and the isolated recombinant RIPK2 kinase domain. This is a very important conclusion suggesting an intrinsic weakness of in vitro inhibitor characterization in some cases.

We are currently pursuing further characterization of these molecules. We have recently established ability of CSLP37 to block responses to MDP in vivo and this work is currently under review for publication. Additionally, further optimization of these molecules to achieve better accumulation in the central nervous system is currently ongoing. Ultimately, we plan to test these inhibitors as a new approach to inhibition of neuroinflammation in mouse models of multiple sclerosis.

Furthermore, RIPK family has several members. RIPK1 contains a C-terminal death domain which was originally found to interact with FAS receptor. That's why it is called "receptor-interacting protein kinase"[15]. It can also bind other FAS family members such

as TNF receptor 1, TNF-related apoptosis-inducing ligand receptors 1 and 2, and TNF-receptor-related apoptosis-mediating protein[16-18]. RIPK3 is another important RIPK family member. This protein forms a complex with RIPK1 through their RHIM domains, which mediates both apoptosis and necroptosis depending on a cellular context. Conversely, RIPK2 plays a critical role in NF-kappa-B pathway, which mediates inhibition of cell death and pro-inflammatory gene expression[19]. Thus, understanding selectivity of the CSLP series is another important direction of our future work. Given structural similarities between RIPKs, it will be very informative to further elucidate molecular basis for selectivity is it is observed in further analyses of RIPK1/RIPK3. Conversely, combined inhibition of multiple RIPK family members offer additional benefits against inflammatory pathologies, involving a combination of cell death and persistent inflammation.

Chapter 5: References

1. Stephen E. Girardin, Ivo G. Boneca, Leticia A. M. Carneiro, Aude Antignac, Muguette Jéhanno, Jérôme Viala, Karsten Tedin, Muhamed-Kheir Taha, Agnès Labigne, Ulrich Zäthringer, Anthony J. Coyle, Peter S. DiStefano, John Bertin, Philippe J. Sansonetti, Dana J. Philpott. Nod1 Detects a Unique Muropeptide from Gram-Negative Bacterial Peptidoglycan. Science 06 Jun 2003: Vol. 300, Issue 5625, pp. 1584-1587.

2. Stephen E. Girardin, Ivo G. Boneca, Jérôme Viala, Mathias Chamaillard, Agnès Labigne, Gilles Thomas, Dana J. Philpott and Philippe J. Sansonetti. Nod2 Is a General Sensor of Peptidoglycan through Muramyl Dipeptide (MDP) Detection. The journal of biological chemistry Vol. 278, No. 11, Issue of March 14, pp. 8869–8872, 2003.

3. Petr Hruz, Annelies S. Zinkernagel, Gabriela Jenikova, Gregory J. Botwin, Jean-Pierre Hugot, Michael Karin, Victor Nizet and Lars Eckmann. NOD2 contributes to cutaneous defense against Staphylococcus aureus through α-toxin-dependent innate immune activation. PNAS August 4, 2009. 106 (31) 12873-12878.

4. Zhe-Xuan Li, Yu-Mei Wang, Fu-Bing Tang, Lian Zhang, Yang Zhang, Jun-Ling Ma, Tong Zhou, and Wei-Cheng You, Kai-Feng Pan. NOD1 and NOD2 Genetic Variants in Association with Risk of Gastric Cancer and Its Precursors in a Chinese Population. PLOS ONE DOI:10.1371/journal.pone.0124949 May 1, 2015.

5. Duanwu Zhang, Juan Lin, and Jiahuai Han. Receptor-interacting protein (RIP) kinase family. Cell Mol Immunol. 2010 Jul; 7(4): 243–249.

6. Jong-Hwan Park, Yun-Gi Kim, Christine McDonald, Thirumala-Devi Kanneganti, Mizuho Hasegawa, Mathilde Body-Malapel, Naohiro Inohara and Gabriel Núñez. RICK/RIP2 Mediates Innate Immune Responses Induced through Nod1 and Nod2 but Not TLRs. J Immunol February 15, 2007, 178 (4) 2380-2386.

7. Bertrand MJ, Doiron K, Labbé K, Korneluk RG, Barker PA, Saleh M. Cellular inhibitors of apoptosis cIAP1 and cIAP2 are required for innate immunity signaling by the pattern recognition receptors NOD1 and NOD2. Immunity 30, 789–801.

8. Rune Busk Damgaard, Ueli Nachbur, Monica Yabal, Wendy Wei-Lynn Wong, Berthe Katrine Fiil, Mischa Kastirr, Eva Rieser, James Arthur Rickard, Aleksandra Bankovacki, Christian Peschel, Juergen Ruland, Simon Bekker-Jensen, Niels Mailand, Thomas Kaufmann, Andreas Strasser, Henning Walczak, John Silke, Philipp J. Jost. The Ubiquitin Ligase XIAP Recruits LUBAC for NOD2 Signaling in Inflammation and Innate Immunity. Molecular Cell 46, 746–758

9. Chen G, Shaw MH, Kim YG, Nuñez G. NOD-like receptors: role in innate immunity and inflammatory disease. Annu Rev Pathol. 2009; 4: 365-98.

10. Hasegawa M, Fujimoto Y, Lucas PC, Nakano H, Fukase K, Núñez G, Inohara N. A critical role of RICK/RIP2 polyubiquitination in Nod-induced NF-kappaB activation. EMBO J. 2008 Jan 23; 27 (2):373-83. Epub 2007 Dec 13.

11. Van Limbergen J, Wilson DC, Satsangi J. The genetics of Crohn's disease. Annu Rev Genomics Hum Genet. 2009; 10: 89-116.

12. Ota M, Tahara T, Otsuka T, Jing W, Nomura T, Hayashi R, Shimasaki T, Nakamura M, Shibata T, Arisawa T. Association between receptor interacting serine/threonine kinase 2 polymorphisms and gastric cancer susceptibility. Oncol Lett. 2018 Mar; 15(3):3772-3778.

13. Chalada Suebsuwong, Daniel M. Pinkas,b Soumya S. Ray, Joshua C. Bufton, Bing Dai, Alex N. Bullock, Alexei Degterev, and Gregory D. Cunye. Activation loop targeting strategy for design of receptor-interacting protein kinase 2 (RIPK2) inhibitors. Bioorg Med Chem Lett. 2018 Feb 15; 28(4): 577–583.

14. Peter Canning, Qui Ruan, Tobias Schwerd, Matous Hrdinka, Jenny L. Maki, Danish Saleh, Chalada Suebsuwong, Soumya Ray, Paul E. Brennan, Gregory D. Cuny, Holm H. Uhlig, Mads Gyrd-Hansen, Alexei Degterev, and Alex N. Bullock. Inflammatory Signaling by NOD-RIPK2 Is Inhibited by Clinically Relevant Type II Kinase Inhibitors. Chem Biol. 2015 Sep 17; 22(9): 1174–1184.

15. Stanger BZ, Leder P, Lee TH, Kim E, Seed B. RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. Cell 1995; 81: 513–523.

16 Stanger BZ, Leder P, Lee TH, Kim E, Seed B. RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. Cell 1995; 81: 513–523.

17 Chaudhary PM, Eby M, Jasmin A, Bookwalter A, Murray J, Hood L. Death receptor 5, a new member of the TNFR family, and DR4 induce FADD-dependent apoptosis and activate the NF-kappaB pathway. Immunity 1997; 7: 821–830.

18 Wen L, Zhuang L, Luo X, Wei P. TL1A-induced NF-kappaB activation and c-IAP2 production prevent DR3-mediated apoptosis in TF-1 cells. J Biol Chem 2003; 278: 39251–39258

19 McCarthy JV, Ni J, Dixit VM. RIP2 is a novel NF-kappaB-activating and cell deathinducing kinase. J Biol Chem 1998; 273: 16968–16975.

20. Sun X, Yin J, Starovasnik MA, Fairbrother WJ, Dixit VM. Identification of a novel homotypic interaction motif required for the phosphorylation of receptor-interacting protein (RIP) by RIP3. J Biol Chem 2002; 277: 9505–9511.