

Identification and Characterization of Specific Caspase-1 and IL1R Inhibitors

by

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ABSTRACT

Autoinflammatory diseases occur when the body's innate immune system is abnormally activated. There are more than eighty types of autoinflammatory diseases which affect >5% of the population in Western countries. Elevated levels of Interleukin-1 (IL1) cytokines, especially IL1 β and IL1 α , are observed in many types of auto-inflammatory diseases including Behcet's Disease and Familial Mediterranean Fever (FMF). IL1 β binds Interleukin-1 Receptor (IL1R) and initiates NLRP3 (cryopyrin) inflammasome activation. Aberrant levels of IL1R signaling and inflammasome activation promote autoinflammatory diseases. With such a critical role there are several treatment options to reduce inflammasome activation including: caspase-1 inhibitors which are not FDA proved, IL1R antagonists and neutralizing α -IL1 β antibodies. Because the known therapies are protein-based, they are costly and require daily injections. However there are no identified IL1R small molecule inhibitors which can be less costly and overcome the injection barriers.

Therefore the goal of this thesis was to identify and characterize novel small molecule inflammasome inhibitors. For this, we utilized *in silico* modeling in combination with drug repurposing to develop small molecule inhibitors which target IL1R signaling pathway. We screened >300 indole derivatives, and 1280 FDA-approved drugs from the Prestwick Library in a cell-based assay. From this we identified several compounds with mid-nanomolar IC₅₀ and no obvious cellular toxicity. Further studies suggest that these are reversible inhibitors. To better understand the mechanism of inhibition, we developed a docking model to identify possible binding sites of small molecules and performed mutagenesis analysis with the predicted binding pocket. Our experimental data suggests that our small molecule inhibitors possibly inhibit some other protein/s in the down signaling mechanism of in the IL1R pathway. For future studies, we are going to try to

characterize this/these protein/s. In future experiments, animal models will be utilized for the biological analysis of our small molecule inhibitors.

ÖZET

Otoinflamatuvar hastalıklar doğuştan gelen bağışıklık sisteminin anormal olarak aktive olması sonucu ortaya çıkarlar. Bu hastalıklar batılı ülkelerdeki nüfusun yüzde beşinden daha fazlasını etkilemektedirler ve toplamda seksenden daha fazla çeşidi bulunmaktadır. Özellikle İnterleükin-1 alfa ve İnterleükin-1 beta başta olmak üzere, miktarı fazlaca yükselmiş İnterleükin-1 sitokinleri, Behçet Hastalığı ve Ailevi Akdeniz Ateşi gibi bir çok otoinflamatuvar hastalık çeşidinde görülmektedir. IL1 β sitokini İnterleükin-1 reseptörüne bağlandıktan sonra NLRP3 (Kriyopirin) inflamazomu aktive olur. Anormal derecede IL1R sinyalizasyonu ve akabindeki NLRP3 (Kriyopirin) inflamazom aktivasyonu otoinflamatuvar hastalıkları tetiklemektedir. Bu kritik rolü göz önüne alındığında inflamazom aktivasyonunu azaltmayı hedefleyen bir kaç tedavi seçeneği bulunmaktadır. Kaspaz-1 inhibitörleri (henüz FDA onayı alamamış), IL1R antagonistleri ve anti-IL1 β antikorları bu yöntemlerden bazılarıdır. Fakat bilinen tedavi yöntemleri protein bazlı olduğundan, pahalıdır ve günlük ya da haftalık enjeksiyon gerektirmektedirler. Küçük molekül inhibitörleri daha ucuz yöntemlerle üretilbilir ve hap yoluyla enjeksiyona ihtiyaç duymadan daha uygun koşullarda uygulanabilirler. Ancak şu ana kadar tanımlanmış herhangi bir IL1R küçük molekül inhibitörü bulunmamaktadır.

Bu yüzden, bu tezin amacı küçük molekül inflamazom inhibitörlerini keşfetmek, tanımlamak ve karakterize etmektir. IL1R sinyalizasyonunu hedefleyen küçük molekül inhibitörlerini geliştirebilmek için, bilgisayar modellemeleri ve bilinen ilaçların yeniden amaçlanması yöntemlerinden faydalandık. 300'ten daha fazla indol türevini ve 1280 tane FDA onayı almış ilacı hücre bazlı tetkiklerle analiz ettik. Bu analizlerin sonucunda, hücresel toksikliği olmayan ve orta nanomolar seviyede IC₅₀ değerine sahip bir çok molekül tanımladık. Daha sonraki çalışmalarla, bu inhibitörlerin tersinir olduğunu gösterdik. İnhibasyon mekanizmasını daha iyi anlayabilmek için bilgisayar yardımıyla

yanařtırma modeli geliřtirdik ve inhibitörlerin IL1 receptörüne tahmini bağlanma bölgesi üzerinde çeřitli mutasyonlarla analizler yaptık. Analizlerimiz, küçük molekül inhibitörlerimizin muhtemelen IL1R sinyalizasyonu yolağında başka bir protein ya da proteinleri inhibe ettiğini göstermiştir. Gelecek çalışmalarda, bu protein ya da proteinlerin neler olduğunu karakterize etmeye çalışacağız. Ayrıca, hayvan modelleri de küçük molekül inhibitörlerimizin biyolojik analizleri için kullanılacaktır.

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Chapter 1

INTRODUCTION

1. Autoinflammatory Diseases Overview

Autoinflammatory diseases occur when the body's innate immune system is abnormally activated [1]. They are quite common and affect >5% of the population in Western countries [2].

The underlying causes for different types of autoinflammatory diseases primarily arise from genetic susceptibility [3], and defects in cytokine production or signaling [4]. With such diversity it not surprising that there are many causes when the complexity and variety of these diseases are taken into consideration (Figure 1). Elevated levels of Interleukin-1 (IL1) cytokines, especially IL1 β and IL1 α , are observed in many types of auto-inflammatory diseases.

1.1.Behcet's Disease

Behcet's Disease is a rare autoimmune diseases that causes damage to blood vessels throughout the body, particularly veins (small-vessel systemic vasculitis) [5]. Mouth sores, genital sores, ocular inflammation and skin problems are major symptoms. The symptoms may include arthritis, blood clots, and inflammation in the central nervous system and digestive organs [6]. It is also known as 'Silk Road Disease' due to its prevalence in the Middle East and in Central Asia. It is a polygenic disease with unknown

etiology and the implications also include environmental factors which increase the mechanism of disease.

Because of the complexity of the Behcet's diseases, the therapies aim at decreasing the symptoms and reducing inflammation by controlling the immune system. There are numerous therapies including; tumor necrosis factor α (TNF- α) inhibitors which are infliximab [7], etanercept [8], adalimumab [9] and rituximab [10]; IL-1 blockage with canakinumab [11] or Anakinra [12]; Interferon α ; NSAID; corticosteroids and Colchicine [13] which is the most frequently prescribed medication. Colchicine is usually used for mucocutaneous manifestations of Behcet's Disease proved effective with the randomized controlled trials [14].

1.2.Familial Mediterranean fever (FMF)

FMF is a monogenic autosomal recessive inherited autoinflammatory disease caused by disruptions in the *MEFV* (Mediterranean fever) gene [15]. This gene affects expression of pyrin protein, which functions at the protein–protein interactions of different types of apoptotic pathways and inflammasomes. In the absence of proper pyrin protein, the control mechanism of inflammasomes is disrupted, causing inappropriate or prolonged inflammatory responses [16], [17]. The symptoms may include inflammation at skin, joints, peritoneum and pleura. [18]. Colchicine is the most common therapeutics for the treatment of FMF [19], [20]. In the case of Colchicine-resistant types of FMF, IL1R antagonist is used [21].

Inflammatory cause	Disease (MIM #)	Gene	Protein	Inheritance pattern/year of discovery/Refs	Disease onset	Flare/fever pattern	Specific organ inflammation	Treatment
IL-1 β	CAPS							
	FCAS #120100	<i>CIAS1</i> (1q44)	Cryopyrin	AD/(2001)/[4]	First 6 months of life, cold-induced	<24 h	Skin, eyes, joints, systemic	IL-1 blockade
	MWS #191900	<i>CIAS1</i> (1q44)	Cryopyrin	AD/(2001)/[4]	Infancy to adolescence	24–48 h	Skin, eyes, joints, inner ears, meninges (rare), systemic	IL-1 blockade
	NOMID #607115	<i>CIAS1</i> (1q44)	Cryopyrin	AD/ <i>de novo</i> (2002)/[5,6]	Neonatal or early infancy	Continuous with flares	Skin, eyes, joints, inner ears, meninges, bony epiphyseal hyperplasia, systemic	IL-1 blockade
IL-1 β and IL-1 α	DIRA #613852	<i>IL1RN</i> (2q14)	IL-1 receptor antagonist	AR/(2009)/[11]	Neonatal or early infancy	Continuous with flares	Skin, bones, lungs (rare), vasculitis (rare)	Anakinra
IL-1 α ...	FCAS2 #611762	<i>NLRP12</i> (19q13)	NLRP12	AD/(2008)/[13]	Neonatal or early infancy	Continuous with flares	Skin, eyes, joints, systemic	IL-1 blockade
IL-1 α ...	FMF #249100	<i>MEFV</i> (16p13)	pyrin	AR/(1997)/[1,2]	80% of the cases occur before the age of 20	1–3 days	Skin, joints, peritoneum, pleura	Colchicine, rarely IL-1 and TNF blockade or thalidomide if colchicine-resistant
IL-1 α ...	HIDS #260920	<i>MYK</i> (12q24)	Mevakinase kinase	AR/(1999)/[14,15]	Median age at onset 6 months	3–7 days	Skin, eyes, joints, prominent lymph nodes	NSAIDs, corticosteroids, TNF and IL-1 blockade
IL-1 α ...	Majeed's Syndrome #609628	<i>LPN2</i> (18p11)	Lipin2	AR/(2005)/[16]	Early infancy (1–19 months)	Weeks-months	Bones, peritoneum, anaemia	NSAIDs, corticosteroids, interferon- α , possibly IL-1 blockade
IL-1+TNF...	TRAPS #191190	<i>TNFRSF1A</i> (12p13)	TNF receptor 1	AD/(1999)/[3]	Median age at onset 3 years	1–4 weeks	Skin, eyes, joints, peritoneum, pleura	TNF blockade, steroids, IL-1 blockade, colchicine is ineffective
IL-1+TNF...	PAPA #604416	<i>C12orf7</i> (15q24)	PSTPIP1	AD/(2002)/[17]	Early childhood	Common	Skin, joints	Local and systemic corticosteroids, TNF or IL-1 blockade
Other pathways								
TNF, IL-1 α ...	PGA* #186580	<i>NOD2</i> (16q12)	Nod2	AD/ <i>de novo</i> (2001, 2005)/[18,19]	Early childhood	Uncommon	Skin, eyes, joints	NSAIDs, corticosteroids, methotrexate, cyclosporin, TNF or IL-1 blockade
TNF α ...	Chernblism #118400	<i>SH3BP2</i> (4p16)	SH3BP2	AD/(2001)/[20]	Childhood, spontaneous remission by 3rd decade	Uncommon	Jaws, eyes (rare)	NSAIDs, TNF inhibition, interferon- α , azithromycin, bisphosphonates
Lack of IL-10 signalling	Early-onset inflammatory bowel disease #613148	<i>IL10RA</i> (11q23)	IL-10 receptor, <i>IL10RB</i> also forms IL-22, -26, -28-29 receptors	AR/(2010)/[21]	Neonatal or early infancy	Continuous with flares	Colitis with fistula formation, folliculitis in patients with <i>IL10RB</i> mutations	Bone marrow transplantation
IL-36 α , IL-36 β , IL-36 γ (role of IL-1 unlikely but complete)	DITRA # 614204	<i>IL10RB</i> (21q22) <i>IL36RN/ILF5</i> (2q13-14)	IL-36	AR/ 2011/[22,23]	Carriable to adulthood, early onset at 2 weeks was reported in some cases	Flares of generalized and palmoplantar pustulosis	Skin	Actretin Topical steroids Limited use of biologics, i.e. adalimumab
Increase in IFN signalling	JMP, NNS, IASL, CANDIE #613732	<i>PSMB8</i> (6p21)	Inducible $\beta 5$ subunit of the immunoproteasome	AR/(2011)/[24–27]	Neonatal or early infancy	Continuous with flares	Skin, joints, lipodystrophy and muscle atrophy	Partial response to steroids, TNF inhibition, IL-6 α inhibition

CAPS: cryopyrin-associated periodic syndromes; FCAS: familial cold autoinflammatory syndrome; MWS: Muckle-Wells syndrome; NOMID: neonatal-onset multi-system inflammatory disease; DIRA: deficiency of the interleukin (IL)-1 receptor antagonist; DITRA: deficiency of the IL-36 α receptor antagonist; FMF: familial Mediterranean fever; HIDS: hyperimmunoglobulin D syndrome; PAPA: pyogenic arthritis, pyoderma gangrenosum and acne syndrome; TRAPS: tumour necrosis factor receptor-associated periodic syndrome; PGA: paediatric granulomatous arthritis. *including Blau syndrome (MIM 186580) and early-onset sarcoidosis (MIM 609464); JMP: joint contractures, muscle atrophy, paronychia-induced lipodystrophy; NNS: Nakajo-Nishimura syndrome; IASL: Japanese autoinflammatory syndrome with lipodystrophy; CANDIE: chronic atypical neutrophilic dermatitis with lipodystrophy and deviated temperatures; AR: autosomal recessive; AD: autosomal dominant; NSAID: non-steroidal inflammatory drug; TNF: tumour necrosis factor.

Figure 1: ‘Monogenic’ autoinflammatory syndromes [23].

2. Caspases (Cysteiny Aspartic Proteases) Overview

Caspases are cysteine proteases belonging to a unique class of cysteinyl aspartic proteases that are critical for apoptosis, necrosis and inflammation. In humans, there are twelve genes encoding caspases (caspase-1 to 10, caspase-14 and caspase-16). In mouse there are ten genes encoding caspases (caspase-1, 2, 3, 6, 7, 8, 9, 11, 12 and 14) [22]. Mouse caspase-11 and caspase-12 proteins are orthologs of human caspase-4 and caspase-5 with homologous functions [23], [24]. In addition, caspase-13 is found as a bovine homolog of human caspase-4 [25]. Caspases can be divided into two groups based on their major functions which are pro-apoptotic caspases such as caspase-2, 3, 6, 7, 8, 9, 10 [26] and pro-inflammatory caspases such as caspase-1, 4, 5, 11, and 12 [27], [28]. (Table 1).

However, classifying these hydrolytic enzymes according to their major functions is extremely simplistic. A more accurate description is based on their structure and domain organization. While the relative sizes of each protein are different, they assemble into three distinct structures. Moreover, the difference in the length of their structures of caspases corresponds to their hierarchy in the apoptotic signaling cascade [22], [29]. The first two structural groups are called initiator (upstream) caspases and have a longer protein domain which contains either a death effector domain (DED) or the caspase recruitment domain (CARD). (Figure 2A) [22], [30]. Caspase-1, -2, -4, -5, -9, -11, -12 have CARD domain whereas caspase-8 and caspase-9 have DED domain [31]. In contrast caspase-3, -6, -7 and -14 are defined as effector or downstream caspases and have shorter prodomains lacking DED or CARD prosequence [32]. Both DED and CARD prodomains consist of death domain fold (DD), which helps initiator caspases react to death signals. After activation of initiator caspases with death signals, those downstream caspases which do not have a DD fold will in turn be activated [33]. Afterwards, activated effector caspases start to cleave

other substrates that eventually lead to the cell death. The catalytic center of caspases are comprised of a series of loops L1, L2, L3 and L4 (Figure 2A) [22]. The active site cysteine is found in the catalytic loop 2. Another common feature among caspases is that they all contain a prodomain consisting of a large subunit (p20) and a small subunit (p10). Because caspases are firstly expressed as inactive precursors, they are typically activated by proteolytic cleavage into a large (p20) and a small subunit (p10) [34].

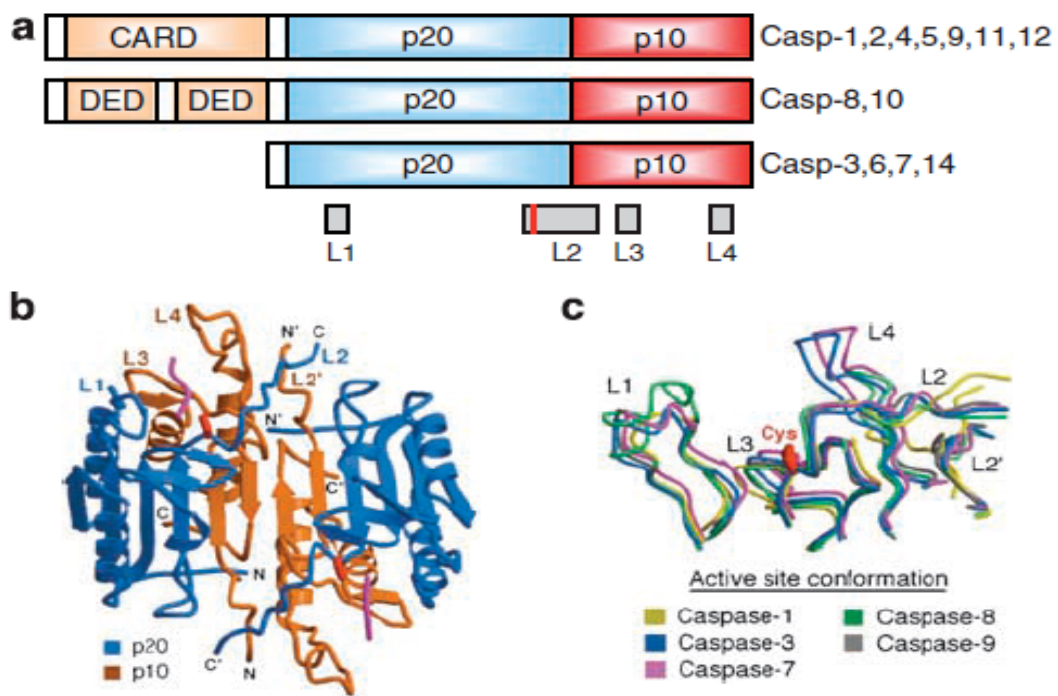


Figure 2: Structure and domain organization of mammalian caspases [22].

<i>Genotype</i>	<i>Apoptotic phenotype</i>	<i>Non-apoptotic phenotype</i>	<i>Reference</i>
Caspase-1 ^{-/-}	Viable. No defects in developmental cell death	Defects in pro-IL-1 β and pro-IL-18 processing. Resistant to LPS-induced septic shock	Kuida <i>et al.</i> (1995) and Li <i>et al.</i> (1995)
Caspase-2 ^{-/-}	Viable. No defects in developmental cell death except excess germ cells in the ovary of female mutants. Mutant oocytes and neurons show resistance to apoptosis <i>in vitro</i>	Fewer facial motor neurons at birth, which become normal by postnatal day 7	Bergeron <i>et al.</i> (1998) and Troy <i>et al.</i> (2000)
Caspase-3 ^{-/-}	Most mutants die postnatally in mixed 129/SvJ and C57BL/6 background but are viable in C57BL/6 background. Decreased apoptosis in brain		Kuida <i>et al.</i> (1996)
Caspase-7 ^{-/-}	Viable. Mutant MEFs show mild resistance to apoptosis		Lakhani <i>et al.</i> (2006)
Caspase-3 ^{-/-} and caspase-7 ^{-/-}	DKO die perinatally. About 10% DKO embryos displayed exencephaly.	Dilation of the atria and disorganization of the ventricular musculature	Lakhani <i>et al.</i> (2006)
Caspase-8 ^{-/-}	DKO MEFs are resistant to apoptosis. Prenatally lethal. Mutant MEFs are resistant to death receptor-mediated apoptosis	Impaired heart muscle development and congested accumulation of erythrocytes. Degeneration of yolk sac vasculature and circulatory failure. Defective hematopoietic progenitor function and macrophage differentiation	Kang <i>et al.</i> (2004) and Varfolomeev <i>et al.</i> (1998)
Caspase-9 ^{-/-}	Die perinatally. Defective brain development associated with decreased apoptosis. Mutant MEFs are resistant to apoptosis		Hakem <i>et al.</i> (1998) and Kuida <i>et al.</i> (1998)
Caspase-11 ^{-/-}	Viable. Defects in apoptosis induced by LPS and ischemic brain injury	Defects in IL-1 production. Resistant to LPS-induced septic shock. Reduced leukocyte motility	Kang <i>et al.</i> (2000), Li <i>et al.</i> (2007) and Wang <i>et al.</i> (1998)
Caspase-12 ^{-/-}	Viable. Reduced ER stress induced apoptosis	Enhanced bacterial clearance and sepsis resistance	Nakagawa <i>et al.</i> (2000) and Saleh <i>et al.</i> (2006)
Caspase-14 ^{-/-}		Defects in profilaggrin processing and skin epidermal barrier formation. Skin dehydration and sensitive to UV radiation	Denecker <i>et al.</i> (2007)

Abbreviations: DKO, double knockout; ER, endoplasmic reticulum; IL, interleukin; LPS, lipopolysaccharides; MEFs, mouse embryonic fibroblasts; UV, ultraviolet.

Table 1: The apoptotic and non-apoptotic phenotypes of caspase knockout mice. [24]

The cleavage of the linker results in rearrangement of the catalytic residues, thus revealing the proteolytic activity of caspases [35], [36]. An example can be seen in Figure 2B, with the active caspase-3 being comprised of a homodimer. Each monomer in this structure has a large (p20) and a small subunit (p10) [37]. This homodimer structure of active caspase-3 is also seen in caspase-1, -2, -7-, -8 and -9. Furthermore, loop 1 and loop 3 are more conserved than loop2 and loop4 between caspases (Figure 2C) [22]. Caspase enzymes are also critical to many inflammatory functions. In humans, caspase-1, -4, -5 and caspase-12 are involved in inflammatory reactions. They control the activation of pro-inflammatory cytokines such as proIL1 β and proIL-18 [38], [39]. Also, double knockout of caspase-11 in mice showed decreased IL-1 production. [40], [41].

2.1.Caspase-1

Caspase-1 is also known as interleukin-1 converting enzyme (ICE). It cleaves the pro-interleukin 1 β [42] and pro-interleukin 18 [43] to the active pro-inflammatory cytokines IL1 β and IL18 . Caspase-1 is expressed as an inactive zymogen that requires both cleavage and dimerization for activation. Then an autocatalytic reaction generates a homodimer which consists of two large and two small subunits like active caspase-3 in Figure 2B [44]. Double mutants or double knockouts of mice for caspase-1 gene demonstrate that caspase-1 is not involved in apoptosis [37], [45]. While, the over-expression of caspase-1 can cause apoptosis [46] this is likely due to the low specificity of caspase-1 at high concentrations causing the cleavage of apoptosis related proteins [47]. Caspase-1 is therefore generally accepted not to directly play a role in apoptosis, but instead is involved IL-1/IL-18 mediated inflammation [48], [49]. However, caspase-1 is not the only protein which cleaves proIL1 β and proIL-18. Studies with Caspase-1^{-/-} neutrophils and mast cells, demonstrated that other serine proteases (cathepsin G, neutrophil elastase,

proteinase 3, granzyme A and chymase) can cleave proIL1 β and proIL-18 in the absence of caspase-1 [50], [51].

3. The IL-1 Superfamily

The Interleukin-1 (IL-1) protein family consists of eleven cytokines; IL-1 α , IL-1 β , IL1Ra, IL-18, IL-36Ra, IL-36 α , IL-37, IL-36 β , IL-36 γ , IL-38 and IL-33 [52]. Because IL-1 α and IL-1 β shows dramatic pro-inflammatory effects, they are the most studied members. The cell surface receptor for IL-1 is IL-1RI which typically forms a heterodimer with an IL-1 RAcP (IL-1 receptor accessory protein) [53], [54]. The inactive form of pro-IL1 β is under the control of caspase-1 [52]. Given the importance of this pathway there are numerous control mechanisms to modulate the inflammatory response. Firstly, the Interleukin-1RII cell surface receptor serves as a delay receptor that binds IL-1 α or IL-1 β , but does not initiate the pathway [55]. This therefore increases the amount of IL-1 needed to trigger an inflammatory signal cascade. Moreover, there are natural IL-1R antagonists (IL-1 RA) [56] that can bind the IL-1 receptors and prevent interactions with IL-1 α or β cytokines [57]. Further single immunoglobulin IL-1 related receptor (SIGIRR) can limit inflammatory response to IL-1 cytokine [58].

4. The IL1R and TLR2 Pathways

As described earlier, the two IL1R have very different functions. Type I transmits the effects of IL1 cytokines with the help of the IL-1 receptor accessory protein (IL1RAP). The IL1 signal transduction requires both type I IL1R and IL1RAP [59].

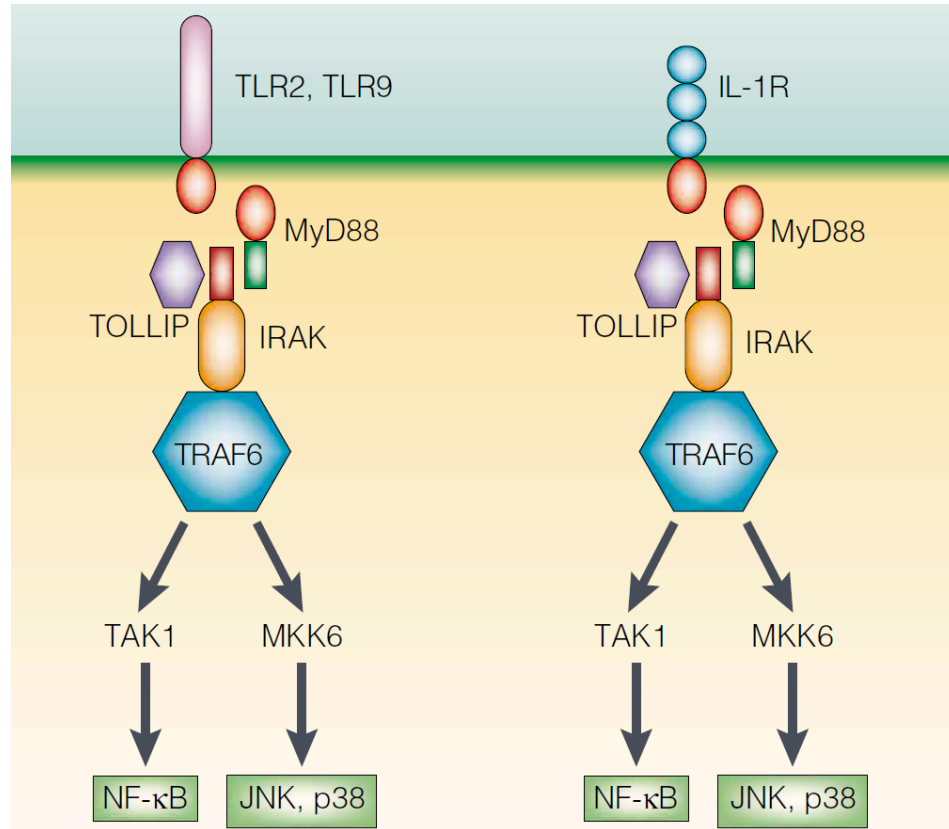


Figure 3: The Toll-like receptor (TLR) and interleukin-1 receptor (IL1R) family members [59].

Type II IL1R works as a decoy receptor and it does not have an intracellular domain for downstream signaling [54]. IL1RI consist of an extracellular Immunoglobulin-like domain (Ig-like) domain and an intracellular TIR domain. This TIR domain is lacking in IL1RII. Upon ligand binding, the TIR domains of IL1R and IL1RAP and MyD88 make oligamerization. This complex recruits Toll interacting protein (TOLLIP), IL-1R-associated kinase (IRAK) and TNF receptor-associated factor 6 (TRAF6) (Figure 3) [60].

The activated TRAF6 and TAK1 kinase forms a complex that phosphorylates the inhibitory molecule I κ B. Without this repression, NF κ B translocates to the nucleus, binds to regulatory DNA sequences and induces transcription of inflammatory genes. [61]. The over-activation of the IL1R pathway with various reasons causes the hyper activation of the signaling which results in auto-inflammatory disorders. Therefore, we focused on the inhibition of IL1R on our studies.

The TLR2 pathway is highly similar to the IL1R Pathway (Figure 3). The whole downstream signaling mechanism is identical. The only differences are the structure of the receptor and the ligands that activate this receptor [60], [62]. Moreover the co-receptor of the TLR2 is CD14 (cluster of differentiation 14) which is required for ligand binding and activation of signaling [63]. The TLR2 receptor recognizes PAMPs like LPS (lipopolysaccharide) [64]. We utilized the similarity between IL1R and TLR2 to analyze the specificity of our small molecule inhibitors.

5. Inflammasomes

Inflammasomes detect the presence of different PAMPs (pathogen-associated molecular patterns) or DAMPs (danger or damage-associated molecular patterns). [27]. The protein complex is comprised of Nod-Like Receptors NLRP3 (NALP3 or Cryopyrin), NLRP1 (NALP1), NLRC4 (ICE-protease activating factor), NLRP6, HIN200 (hematopoietic interferon-inducible nuclear antigens with 200 amino acid repeats), AIM2 (absent in melanoma 2) and IFI16 (interferon-inducible myeloid differentiation transcriptional activator). (Figure 4) [38] [65].

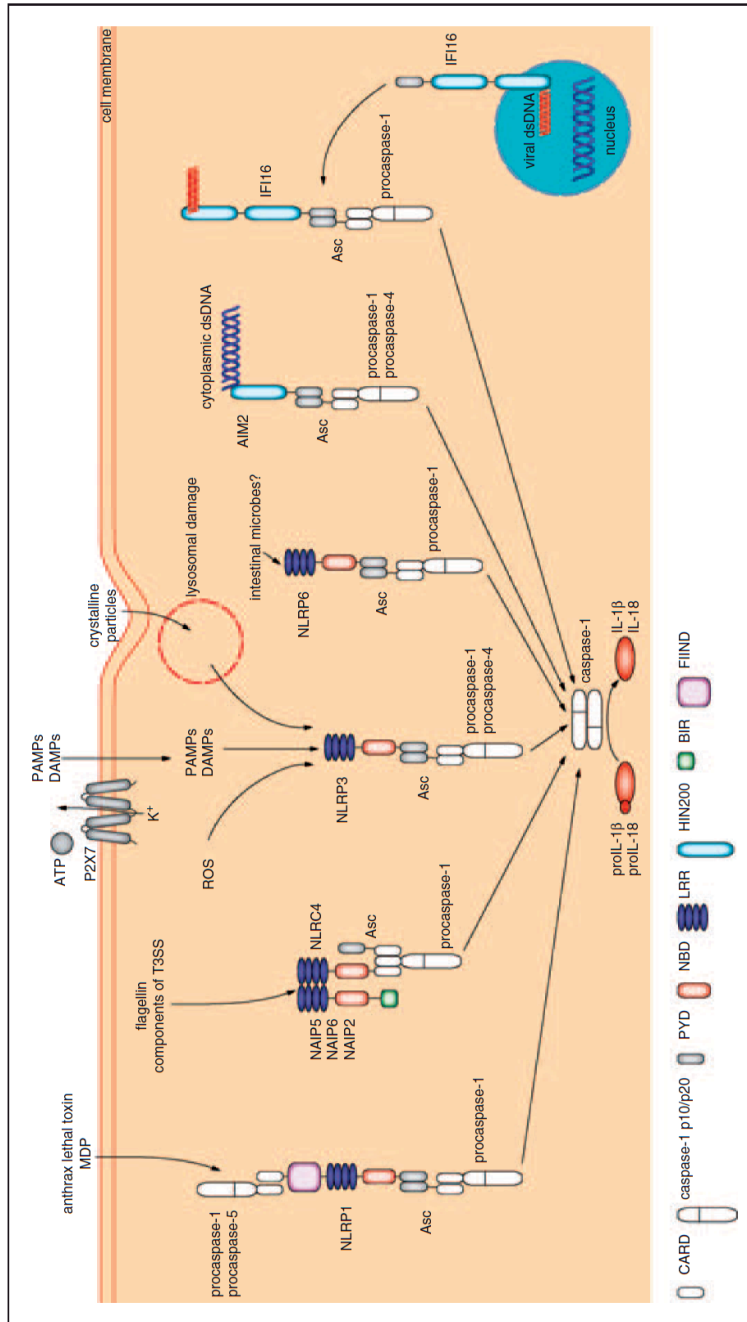


Figure 4: The different types of inflammasomes [27].

5.1.NLRP3 inflammasome

The NLRP3 inflammasome which is also known as NALP3 inflammasome or cryopyrin [66] (Figure 5) can be activated by various bacterial pathogens, fungal pathogens, viruses, parasites, reactive oxygen species, and efflux of potassium ion or cathepsin B release upon lysosomal damage. [67], [68]. Critically, the NLRP3 inflammasome can also be activated in gout by different types of crystals and particles [69], [70]. NALP3 protein consists of four domains including: Pyrin, NACHT, NAD (NALP-associated domain) and LRR (Leucine Rich Repeats) [71]. The CARD domain of pro-caspase-1 interacts with NLRP3 protein through ASC (Apoptosis-associated Speck-like protein). ASC makes the connection between caspase-1 and sensory proteins. Pyrin domains help to bridge ASC with NALP3 [72]. The Cardinal (CARD Inhibitor of NFkB-activating Ligands) protein has a role on oligomerization of the inflammasome [22]. After oligomerization, an autocatalytic event occurs by the reorganized active domains of caspase-1 resulting in the activation of this proteolytic enzyme. The activated caspase-1 then cleaves pro-IL1 β precursors into active IL1 β which exits the cell and triggers inflammasome events. (Figure 5) [27].

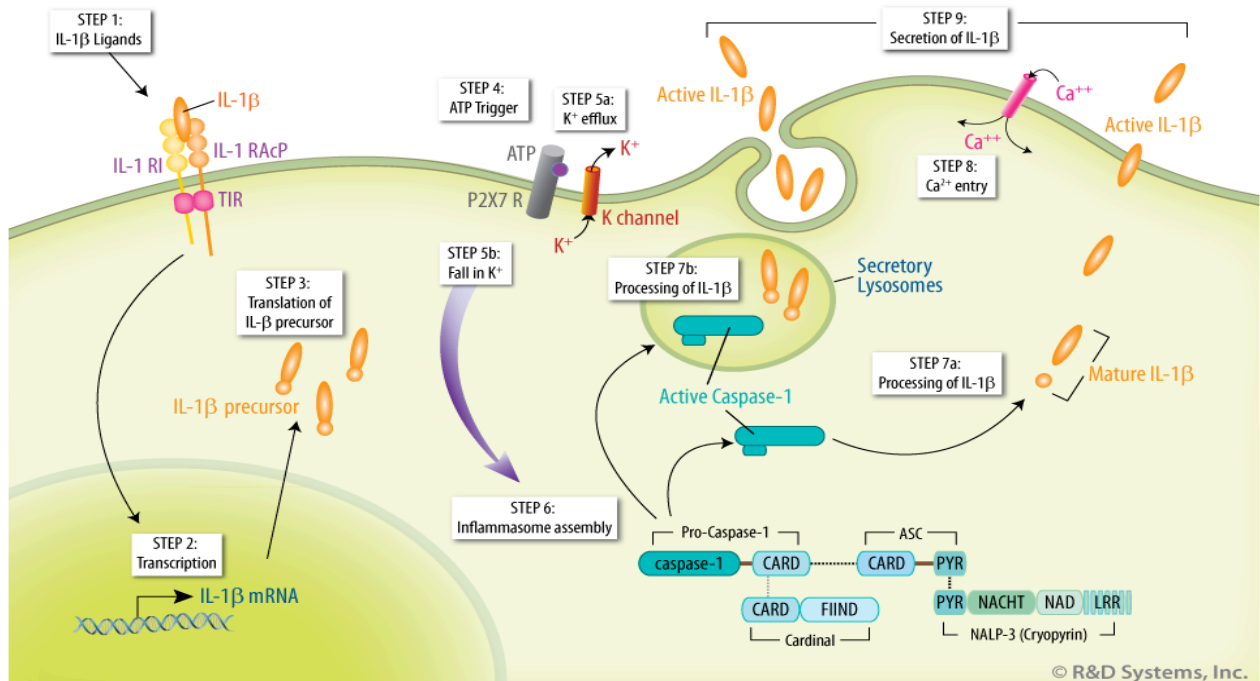


Figure 5: Activation of Caspase-1 by NALP3 and secretion of IL1β [73].

5.2. The other inflammasomes

Caspase 1 can also be activated by other inflammasomes including NLRP1, NLRC4, PYHIN and NLRP6 inflammasomes however these are considered to be minor pathways. The NLRP1 protein complex consists of NALP1, ASC, Caspase-1 and Caspase-5. NALP1 is similar to NALP3 protein with its PYD, NACHT, NAD and LRR domains [74]. In addition to these, NALP1 has a carboxyterminal CARD domain and FIIND (F-interacting domain) before CARD. Distinctly, NALP1 makes a connection with pro-Caspase-5 protein via its carboxyterminal CARD domain. In brief, NLRP1 inflammasome has a role on activation of Pro-Caspase-1 and also pro-Caspase-5. It triggers inflammation

by activation of Caspase-1 and respectively activation of pro-IL-1- β and pro-IL-18. [39], [75]. The NLRC4 inflammasome is another complex consisting of NLRC4 protein (called as NLR-family CARD domain-containing protein 4) or ICE-protease activating factor [76]. This inflammasome become activated by flagellin and by the type III secretion system (T3SS) rod protein from bacteria. [48], [77]. PYHIN (pyrin and HIN domain-containing protein) family inflammasomes include AIM2 (absent in melanoma 2) inflammasome and IFI16 (interferon-inducible myeloid differentiation transcriptional activator) inflammasome. The AIM2 inflammasome can directly bind double-stranded DNA which belongs to DNA viruses or cytosolic bacteria. [78]. Subsequently, ASC proteins can bind Pro-Caspase-1 protein via its CARD domain. After this recruitment, an autocatalytic reaction occurs and pro-Caspase-1 proteins turn into activated Caspase-1 and respectively activation of pro-IL1 β and pro-IL-18. [79]. The NLRP6 inflammasome is the last stated inflammasome which play a role on inflammation via Caspase-1 and IL1 β and IL-18. It is shown that the NLRP6 inflammasome is needed for a healthy microflora of the gastrointestinal tract in mice. NLRP6 protein becomes activated by intestinal microbes. [80]. The NLRP3 inflammasome is the most studied and the most important one among all of them. Therefore, we focused on the analyses on the NLRP3 inflammasome.

6. The Known Inhibitors

With such a wide spectrum characteristics of autoinflammatory diseases led wide spectrum of underlying causes and thus variety of treatment options shown in Figure 1. Interleukin-1 (IL-1) blockade [81], [82], Anakinra [83], [84], Colchicine [85], [86], Tumor necrosis factor (TNF) blockade [81], [87], non-steroidal inflammatory drugs (NSAID) [88], corticosteroids [89], interferon- α [90] and thalidomide [91] are major treatment options for many different disorders but the most common and effective treatment option is IL-1

blockage which is mentioned more detailed in next chapters. In brief, aberrant levels of IL-1 cytokines (especially IL1 β and IL1 α) are observed in many types of auto-inflammatory diseases. To reduce inflammasome activation, NLRP3 inflammasome is targeted by caspase-1 inhibitors or IL1R activity is targeted by IL1R antagonists and neutralizing α -IL1 β antibodies. [48], [82], [92] .

6.1. Anakinra

Anakinra is a recombinant human IL-1 receptor antagonist. It is the most common therapeutics in the clinical trials of autoinflammatory diseases such as PAPA syndrome, Sweet's syndrome, Muckle-Wells syndrome, familial Mediterranean fever, SAPHO syndrome and Behcet's Disease [84], [93], [94]. Because it is a protein, it has a short degradation time (ranged from 4 to 6 hours) in the body. It therefore requires daily injections.

6.2. Rilonacept

Rilonacept is an Interleukin-1 (IL) inhibitor also known as IL-1 Trap or Arcalyst [95]. It has a protein structure which consists of the extracellular ligand binding domains of type I IL1R and IL1RAP. It is FDA approved and used for the treatment of CAPS [including Familial Cold Autoinflammatory Syndrome (FCAS), Muckle-Wells Syndrome (MWS) and Neonatal-Onset Multisystem Inflammatory Disease (NOMID)] and gout [96], [97] [98]. It is also a protein-based therapeutics that requires injections.

6.3.Canakinumab

Canakinumab which is also known as Ilaris (previously ACZ885) is a human monoclonal antibody to target IL1 β [99]. FDA and EMA have approved Canakinumab for the treatment of CAPS (including FCAS, MWS and NOMID) [100].

6.4.Colchicine

Colchicine, originally extracted from plants of the genus *Colchicum*, is used to treat and decrease the symptoms of some autoinflammatory diseases such as gout, FMF, pericarditis, and Behçet's disease [86][13]. At high concentrations colchicine binds tubulin proteins, thus inhibits microtubule polymerization and results in mitotic arrest. However, at clinical concentrations the exact mechanism of action of Colchicine for anti-inflammatory effects is not fully understood. There are some proposed models for mechanism of action shown in Figure 6 [101], [102]. It is shown that the decrease in tubulin polymerization can decrease L-selectin expression on neutrophils [103]. It also inhibited the production of superoxide anion caused by MSU crystals [104]. It has been proposed that the decrease in tubulin polymerization can decrease NALP3 inflammasome activation [70]. Moreover, it is shown that the microtubule mediated NF κ B activation can be decreased by colchicine [105]. It is also hypothesized that colchicine inhibits the uncleaved form of caspase-1 or the polymerization of caspase-1 by inhibiting tubulin polymerization. However, almost all of these studies have been conducted at concentrations ~1000x higher than the serum concentration in patients.

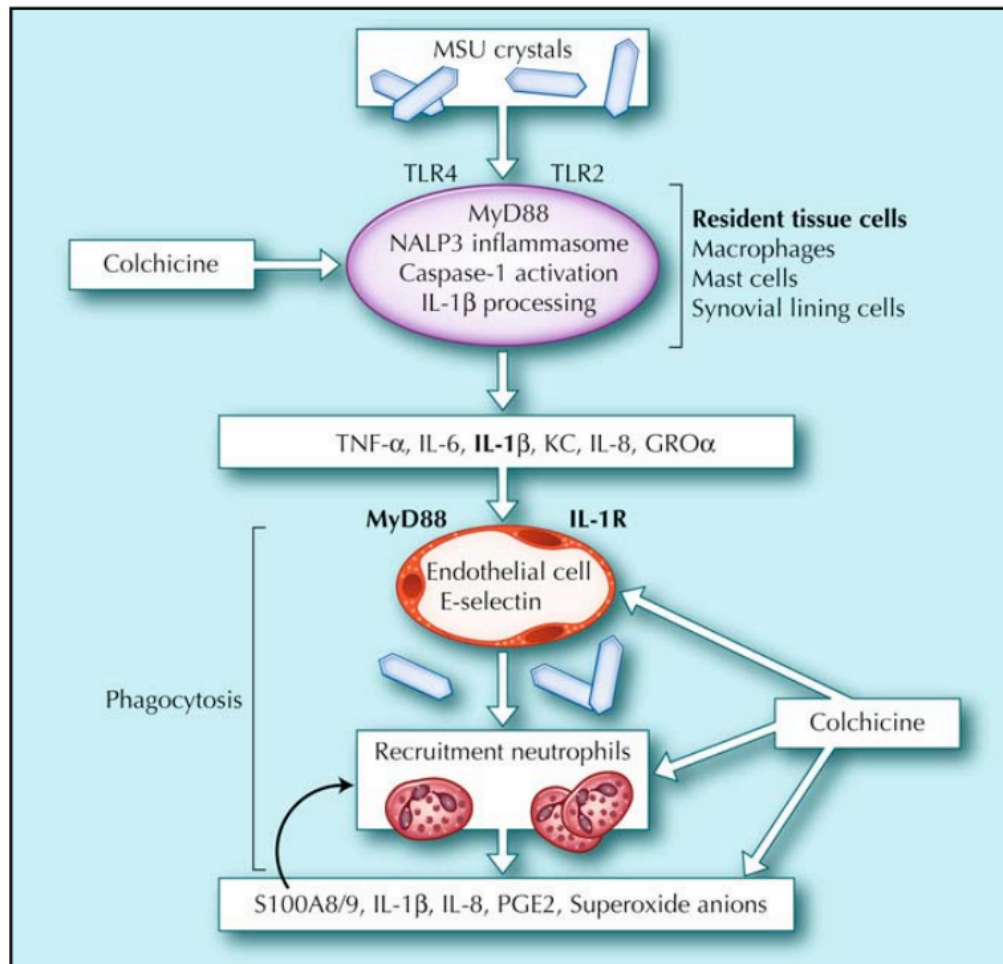


Figure 6: Mechanisms, mediators of crystal-induced inflammation and main sites of action of colchicine [102].

Aim of the Thesis

Currently all approved drugs that inhibit the IL1R pathway are recombinant proteins. They are thereby extremely costly and require daily injections. There are no identified IL1R small molecule inhibitors that would be easier to produce and limit potential injections. Therefore the goal of this thesis is to identify and characterize novel small molecule inflammasome inhibitors. For this, we utilized *in silico* modeling in combination with drug repurposing to develop small molecule inhibitors that target IL1R signaling pathway.

CHAPTER 2

MATERIALS AND METHODS

1. Caspase-1 Inhibitor Drug Screening

The effects of the compounds on caspase-1 were assayed with the Fluorometric Caspase-1 Inhibitor Drug Screening Kit (BioVision, Catalog #K151-100). The kit utilizes synthetic peptide substrate YVAD-AFC (AFC, 7-amino-4-trifluoromethyl, coumarin) which is cleaved by active caspase-1 to release the free fluorescent AFC.

The assay was conducted following the manufacturers recommended procedures. Briefly, DTT was added to the 2X Reaction Buffer immediately before use (10 mM final concentration). The active purified Caspase-1 was then reconstituted in 550 μ l 2X Reaction Buffer and aliquoted. Samples were prepared in dH₂O to a final volume of 50 μ l/well with 1 unit of active Caspase-1. A Master Mix was then prepared with 45 μ l of the prepared 2X Reaction Buffer and 5 μ l of 1 mM YVAD-AFC substrate (50 μ M final concentration). To each well 50 μ l of the Master Mix was added to start the reaction. The plate was incubated at 37°C for 1-2 hour/s and then read in a fluorescence plate reader (Synergy H1 Multi-Mode Reader) at an excitation of 400nm and emission of 505nm.

A negative control (no caspase-1) and positive control (1 μ l of known caspase-1 inhibitor provided with the kit) were included in all experiments. The compounds were initially tested at 20 μ M concentration. Compounds that show >50% inhibition of Caspase-1 enzyme were tested by dose response curves (97nM-200 μ M).

2. Cellular Caspase-1 Detection with THP1 cells

Cellular inhibition of caspase-1 was assayed with a FAM-FLICA *in vitro* Caspase-1 Detection Kit from ImmunoChemistry Technologies (Cat# Caspase-1 655#97). THP1 cells were used with this FLICA assay to detect caspase-1 by utilizing a non-cytotoxic fluorescent labeled inhibitor of caspase-1. THP1 cells were grown in RPMI with 4.5 g/l glucose and 2 mM L-Glutamine (BioWhittaker, Cat#BE12-702F), 10% (v/v) heat-inactivated fetal bovine serum (Gibco Life Tech., Cat#10500), 50 U/ml penicillin, and 50 mg/ml streptomycin.

THP1 cells were centrifuged and re-suspended in fresh media to a concentration 5×10^5 cells/ml. A higher concentration can cause the natural apoptosis. A total of 2 ml/well of resuspended THP1 cells were seeded with 0.5 mM Adenosine triphosphate (Sigma, Cat#A2383) and 1 μ g/mL Lipopolysaccharide (Sigma, Cat#L6261) for caspase-1 inflammasome activation. Our compounds were included at 5 μ M. After 44-48 hours of incubation, cells were centrifuged at 1500 rpm for 5 minutes. Then, they were resuspended in 500 μ l/well fresh medium. From this, 290 μ l of cells were transferred into fresh tubes, and 10 μ l of 30x FLICA solution was added to each tube except the FLICA negative control. Then, the tubes were mixed with slight flicking and incubated at 37°C with aluminum cover to protect from light. Because cells can settle on the bottom of the tubes, they were resuspended by gentle swirling every 20 minutes. After incubation, the cells were washed 3x with Apoptosis Wash Buffer and analyzed by flow cytometer with FL1 filter at 488 nm.

3. IL1R Inhibitor Drug Screening

The effects of the compounds on IL1R inhibition were assayed with HEK-Blue IL-1 β cell line from InvivoGen (Catalog # hkb-il1b-kit) and homemade Secreted Embryonic Alkaline Phosphatase (SEAP) assay. HEK-Blue IL-1 β cells were grown in DMEM with 4.5 g/l glucose and 2 mM L-Glutamine (Gibco Life Technologies, Cat#41966-029), and 10% (v/v) heat-inactivated fetal bovine serum (Gibco Life Technologies, Cat#10500), 50 U/ml penicillin, and 50 mg/ml streptomycin. These cells can detect bioactive IL-1 β by monitoring the activation of the NF- κ B and AP-1 pathways. The cells respond specifically to IL-1 β which binds to IL-1R provoking a signaling cascade that leads to transcription of a SEAP reporter (Figure 7).

HEK-Blue IL-1 β cells were added to 96-well plates with 50,000 cells/well concentration. 1 ng/ml purified recombinant IL1 β (Peprotech, Cat#200-01B) is added exogenously for the test. In the primary screen, compounds were added at 5 μ M or 10 μ M. Compounds that showed >50% inhibition in the primary screen were tested by dose response curves from 10 nM to 40 μ M. Cells are incubated with both compound and purified recombinant IL1 β for 20-24 hours and then assayed for cellular activation using a colourmetric secreted alkaline phosphatase assay (SEAP). For the SEAP assay, 9.5 M Diethanolamine 99% (Acros Organics, Cat#113920025) was diluted to 2M with ddH₂O, and then 1 mM MgCl₂ and 0.5 mM ZnCl₂ were added. 4-Nitrophenyl phosphate disodium salt hexahydrate (Sigma, Cat#71768) was added to a 1 mg/ml final concentration. Simultaneously, 0.05% CHAPS (Sigma Ultra, Cat#N-2765) was prepared in PBS from 15% CHAPS stock in ddH₂O. 0.05% CHAPS was added to a new 96 well plates (20 μ l/well). Supernatant from the HEK-Blue IL-1 β plate (40-60 μ l) was added to the test plate. Then, 200 μ l of Diethanolamine/4-Nitrophenyl phosphate disodium salt mixture was

added. The plate was covered in foil and then incubated at room temperature for 15-45 minutes. The absorbance was read at 405 nm with Synergy H1 Multi-Mode Reader.

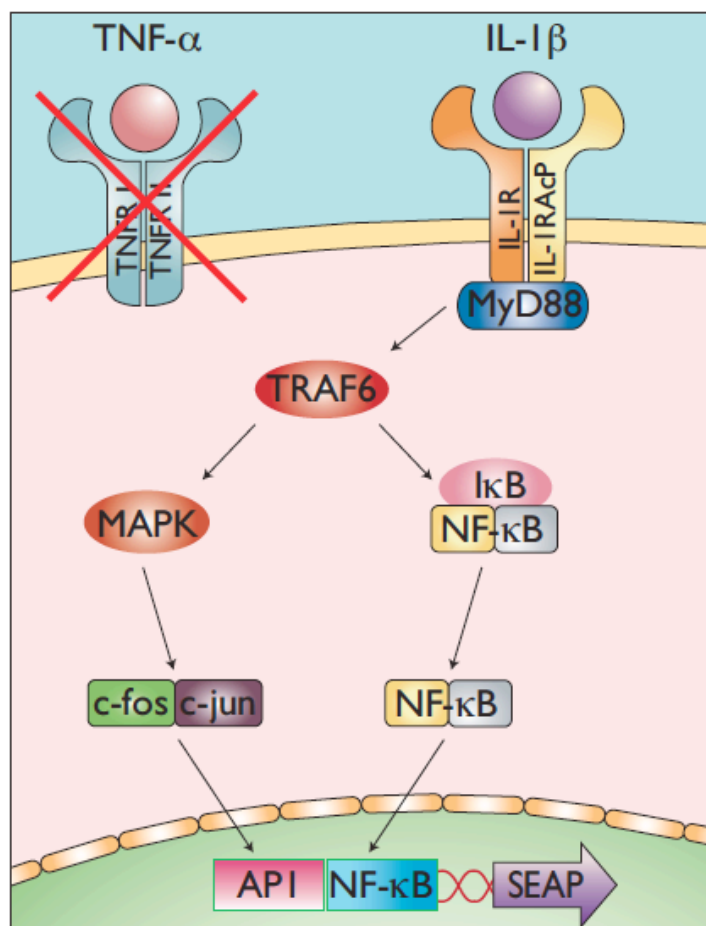


Figure 7: HEK Blue IL1β cell reporter assay

In the same dose response experiments the compounds were tested by for cytotoxicity using a non-radioactive cell proliferation assay (Promega, Cat# G5430). This prepared MTS reagent contains 2.0 mg/ml MTS tetrazolium solution in Dulbecco's phosphate

buffered saline with pH 6.0 and phenazinemethosulfate in Dulbecco's phosphate buffered saline with pH 7.3. The two components are mixed, aliquoted and frozen. For every experiment, fresh aliquots were used. Following removal of supernatant, 20 μ l/well MTS solution was added to each well. The solution was added directly in the same sample plate with HEK-Blue IL-1 β cells. After 2 hours of incubation, the absorbance was read at 490 nm with Synergy H1 Multi-Mode Reader. As a further step, the hits which have lower IC₅₀ value and do not show toxicity in short term are tested for long term toxicity with the same assay.

4. Cloning pcDNA4-IL1R plasmid

15151 plasmid obtained from Dr Ahmet Gul containing a human IL1R gene was amplified with Polymerase chain reaction (PCR) by using IL1R_ EcoRI forward and IL1R_ NotI reverse primers (Table 2), and High-Fidelity DNA Polymerase (Phusion, Cat#M0530S). The PCR was set up at 98°C initial denaturation for 30 seconds. Then, 30 cycles was performed at 98°C for 10 seconds, 60°C for 25 seconds and 72°C for 3 minutes for each cycle. Lastly, final extension was performed 72°C for 5 minutes. Following, the PCR product was purified with NucleoSpin Gel and PCR Clean-up kit (Macherey Nagel, Cat#740609). After purification, the product was double digested with NotI (New England Biolabs, Cat# R0189S) and EcoRI (New England Biolabs, Cat# R01015S) enzymes. Simultaneously, a pcDNA4 backbone was double digested with the same enzymes. The whole sample of digested pcDNA4 is loaded on 1% agarose gel prepared with 1x TAE (Tris base, acetic acid and EDTA) buffer. The gel electrophoresis is run at 90 V for 40 minutes. The digested pcDNA4 plasmid was cut from the agarose gel and purified with NucleoSpin Gel and PCR Clean-up kit. The double digested pcDNA4 backbone and IL1R insert were ligated with quick ligase kit (New England Biolabs, Cat# M2200S) in 15

minutes. The ligated constructs were transformed into chemically competent DH5 α *E. coli*. Colonies were selected; the plasmid was confirmed by restriction endonuclease digestion and then sent for sequencing to Macrogen. Following validation the plasmid was then amplified and isolated with a NucleoBond Xtra Midi Plus kit (Macherey Nagel, Cat#740412).

Gene	Type	Sequence	Size
IL1R	F	5'-GCCCGAATTCCATGAAAGTGTTACTCAGACTTA-3'	33 mer
IL1R	R	5'-AAAAGCGGCCGCCTACCCGAGAGGCACGT-3'	29 mer

Table 2: The list of forward and reverse primers for the cloning of pcDNA4-IL1R plasmid.

5. Luciferase Assay with Human Embryonic Kidney 293t Cells

Human Embryonic Kidney 293T cells were used to develop a transient IL1R reporter system. This homemade assay provides further verification of the results gained by HEK-Blue IL-1 β Kit showing the results can be replicated and not a false positive due to modulation of SEAP. HEK 293T cells are handled with the exact same medium used for HEK-Blue IL-1 β cells.

293T cells were plated in 48-well plates 25,000 cells/well. The seeded plates moved into cell incubator. These were then transfected with 250 ng per well pcDNA4-IL1R plasmid, and 250 ng per well NF κ B-TA-LUC-UBC-GFP-W vector (Addgene, Plasmid #49343) (Figure 8) and 3 μ l of FuGene 6 Transfection Reagent (Promega, Cat#E2691/2/3) for every μ g of DNA. Following, The DNA/FuGene mixture was

incubated for 30 minutes at room temperature. The positive control was a GFP-RelA (p65) plasmid (Figure 9) that will initiate activation of the NF κ B response element upstream of the SEAP. In the presence of GFP-RelA, NF- κ B gene becomes activated and luciferase signal can be detected [106]. Dose response curves of the compounds were prepared and added to HEK 293T cells at final concentration from 62 nM to 8 μ M. Following incubation, the transfection cocktails was added to HEK 293T cells. After 3 hours incubation at 37oC in humidified cell incubator, 1ng/ml purified recombinant IL1 β is added exogenously to the cells and then they were incubated for 20-24 hours and assayed for cellular activation using a Luciferase Assay.

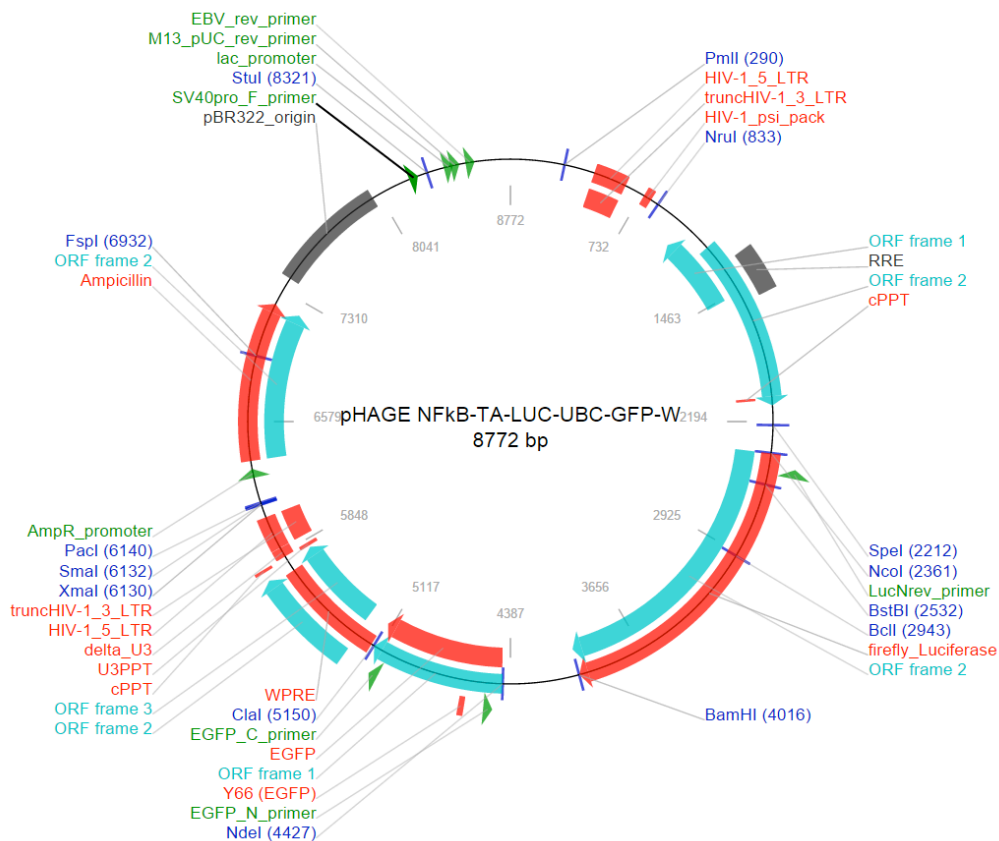


Figure 8: The map of pHAGE NF κ B-TA-LUC-UBC-GFP-W plasmids.

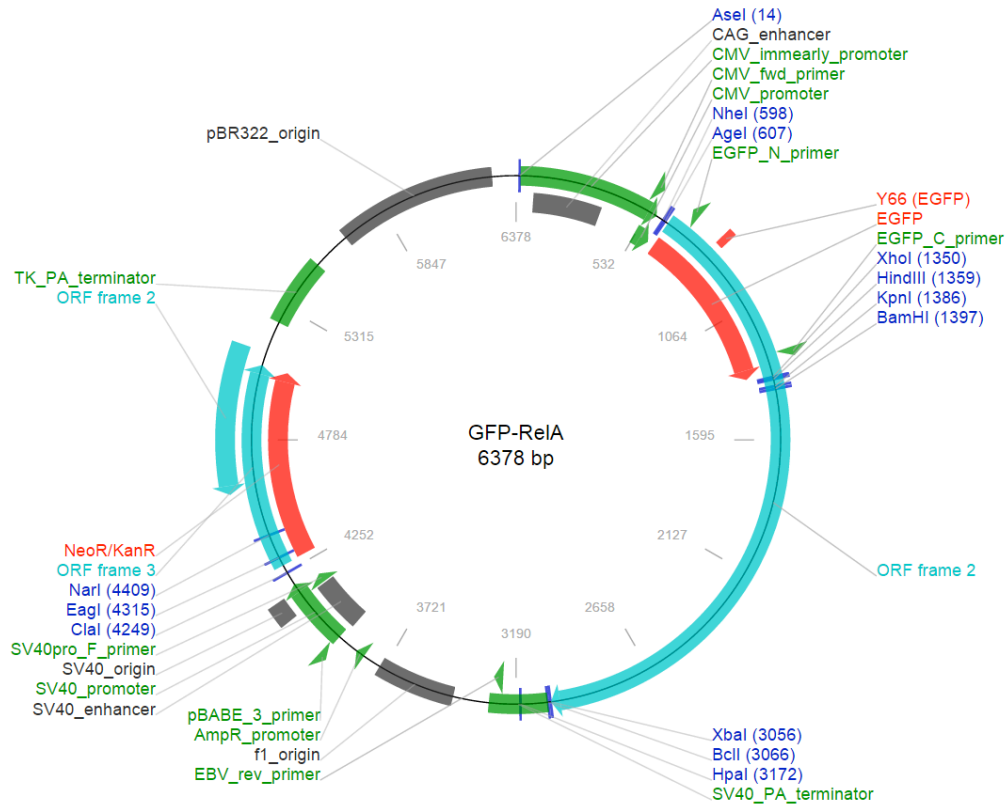


Figure 9: The map of GFP-RelA (p65) plasmid.

In the Luciferase Assay, 15 mg/ml D-luciferin Potassium Salt (Gold Biotechnology, Cat#LUCK-500) was dissolved in luciferase buffer (2 mM EDTA, 500 nM Tris pH7.8, 200 nM ATP, 20 mM MgSO₄, 100 mM DTT). Medium of transfected cells was discarded and the cells were then lysed with 30 µl/well 1x luciferase lysis buffer (NEB)). The plates were incubated for 5 minutes at room temperature with a plate shaker. The resulting lysate was transferred to a white 96 well plate (25 µl/well). Luciferin buffer (100 µl/well) was added to the plate using the automatic dispenser, mixed and then read using a Synergy H1 Multi-Mode Reader.

6. LPS Inhibitor Drug Screening

The effects of the compounds on TLR2 inhibition was assayed with mycoplasma detection kit from InvivoGen (Catalog# rep-pt1, Version# 15C04-MM) and the previously described SEAP assay. The mycoplasma detection kit has HEK-Blue-2 cells that express TLR2 (Figure 10). Thus the presence of mycoplasmas or LPS can activate NF- κ B transcription factor via a TLR2 signalling pathway. The maintenance and handling conditions for HEK-Blue™-2 cells are exactly same with HEK-Blue IL-1 β cells. The experiment is also set up with exact conditions except that HEK-Blue™-2 cells are incubated with 1 mg/ml LPS instead of 1ng/ml purified recombinant IL1 β . SEAP assay and short and long-term MTS assays are executed with same protocols as described in “IL1R Inhibitor Drug Screening”.

The maintenance and handling conditions for HEK-Blue-2 cells was exactly same with HEK-Blue IL-1 β cells. The experiment was also set up with exact conditions except that HEK-Blue-2 cells were incubated with 1 mg/ml LPS instead of 1ng/ml purified recombinant IL1 β . SEAP assay and short and long-term MTS assays are executed with same protocols as described in “IL1R Inhibitor Drug Screening”.

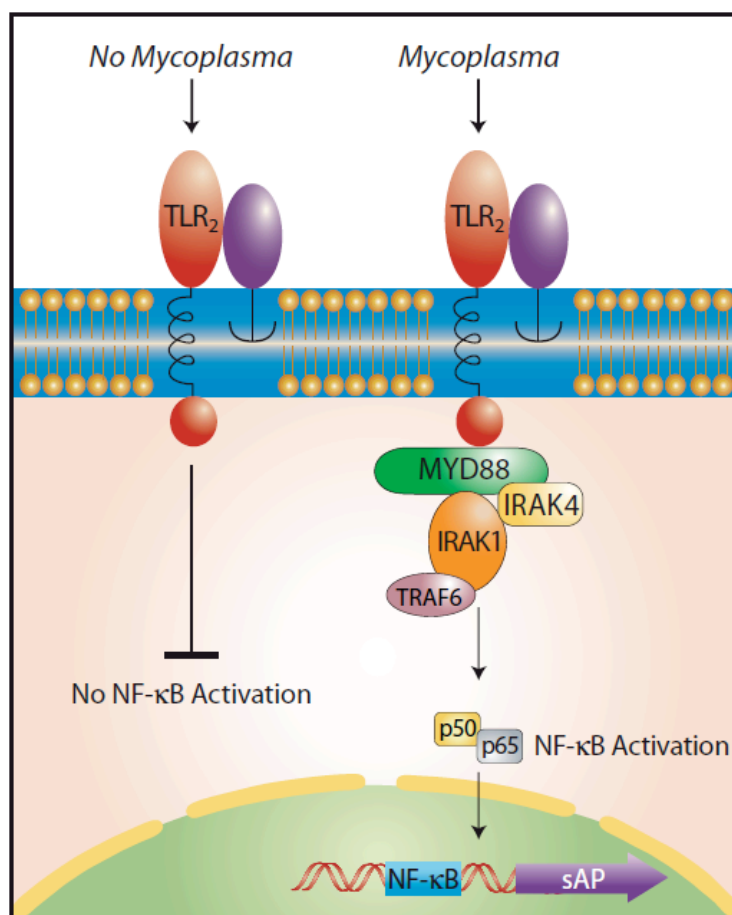


Figure 10: HEK-Blue-2 cell reporter assay

7. Lentiviral production

For the lentiviral production, 2.5 µg of transfer plasmid DNA, 2.25 µg of packaging vector which was 8.2dVPR, and 2.5 µg of envelope vector which was glycoprotein expressing plasmid VSVG were transfected to HEK 293T cells which were prepared 1 day before at 2.5×10^6 cells on 10 cm culture plates with 3 µl of FuGene 6 Transfection Reagent

(Promega, Cat#E2691/2/3) for every μg of DNA. After 48 hours, the medium was collected into 50 ml falcon tube and stored at 4°C , and then new medium was added to the cells. After 24 hour from first collection, the medium was again collected into the same 50 ml falcon tube and filtrated with a $0.45\ \mu\text{m}$ filter. The filtered viruses were aliquoted and stored at -80°C .

8. THP1, THP1-NF κ B-TA-LUC and THP1-NF κ B-TA-LUC/IL1R Cells

THP1 cells were transduced with lentiviruses containing pHAGE NF κ B-TA-LUC-UBC-GFP-W (Figure 8). Following recovery, they were sorted according based on their GFP expression in Yeditepe University with Fluorescence-Activated Cell Sorting (FACS). After sorting, they were grown to full confluency and transferred into multiple flasks.

To prepare transduction vector, pENT1a-IL1R plasmid (donor vector) was used in recombination reaction with pLenti-CMV-puro plasmid (destination vector) using a Gateway LR Clonase II Enzyme mix (Life Technologies, Cat#11791). The plasmids were incubated with LR clonase in TE buffer for 1 hour at room temperature. Then, $0.5\ \mu\text{l}$ Proteinase K was added to the mixture and incubated 15 minutes at 37°C . The final product was transformed into competent cells. Following validation by restriction endonuclease digest, the transformed bacteria were expanded and a plasmid was purified with a NucleoBond Xtra Midi Plus kit.

The pLenti-IL1R-CMV-puro plasmid was packaged into lentiviruses and then used to transduce THP1 cells stably expressing pHAGE NF κ B-TA-LUC-UBC-GFP-W. The double transduced THP1 cells were then selected with $0.5\ \mu\text{g}/\text{ml}$ puromycin. The resulting cells were expanded and frozen for subsequent experiments.

9. Luciferase Assay with THP1 cells

THP1-NFkB-TA-LUC or THP1-NFkB-TA-LUC/IL1R cells were plated into 12, 24 or 48 well plates with 1.5 million, 0.75 million or 0.38 million cells/ml respectively. Either LPS (1 µg/ml) or purified recombinant IL1β (5 ng/ml) was added to these cells with increasing concentration of the test compounds. The cells were also stimulated with 1.25 mM ATP. They were incubated for 20-24 hours and tested for luciferase activity as previously described.

10. Bradford Assay

Prior to western, protein concentration of samples were quantified with Bradford Assay. Nine different dilutions of BSA were prepared with 1X PBS. 5 µl of the standard solutions and the samples are added in triplicate to a 96 well-plate. 250 µl of Commasie Reagent was added to each well. After 10 minutes of incubation at room temperature, the absorbance was read at 595 nm with Synergy H1 Multi-Mode Reader to prepare a standard curve to use in calculation of protein concentrations.

11. SDS-PAGE and Western Blotting

SDS-PAGE gels were poured using a standard Laemmli discontinuous buffer. The separating gel consists of %10 Acrylamide, %0.01 SDS (sodium dodecyl sulfate), %0.01 APS (Ammonium Persulfate), 1.5 M Tris (pH 8.8) and Tetramethylethylenediamine (TEMED). After polymerization of the separating part, the stacking gel was prepared with %5 Acrylamide, %0.01 SDS (sodium dodecyl sulfate), %0.01 APS (Ammonium Persulfate), 1.0 M Tris (pH 6.8) and Tetramethylethylenediamine (TEMED).

Cells were lysed with 1x Passive Lysis Buffer at Room temperature. All lysate was stored at -80° for long-term storage or -20°C for short-term storage. Equal protein samples were mixed with 2X loading buffer containing 5% μ l β -mercaptoethanol (Biorad, USA) and 95% μ l Laemmli Buffer (Biorad, USA) and denatured at 95°C for 10 minutes. The resulting protein samples were loaded onto previously prepared SDS-PAGE gels mini gels with a protein marker in the first well (#1610374, BioRad).

The loaded gels were put into Mini-PROTEAN Tetra Cell device (#1658005EDU, BioRad) and the inner chamber is filled with 1x Reservoir Buffer (0.1%SDS). The device was run at a constant current (25 m x number of gels) for 45-50 minutes. After SDS-PAGE, the protein samples in the gel were transferred to PVDF membranes using a BioRad TransBlot Turbo Blotting System. Following transfer the membranes were blocked with 5% nonfat dry milk in TBS-T (20 mM TrisHCl, pH 7.8, 150 mM NaCl, 0.1%, v/v Tween-20) at room temperature for 1 hour. Then the primary antibodies were added and incubated either overnight at 4°C or 1 hour at room temperature on a shaker. Next the membrane was incubated with primary antibodies at different dilutions (Table 3). Secondary antibodies conjugated to HRP, anti-rabbit and anti-mouse were used. Visualization of of HRP was done using Clarity™ Western ECL Substrate (#170-5061, Biorad, USA). Membranes were exposed using CL-XPosure Film (Thermo-Scientific, USA).

Antibody	Catalog Number	Brand	Dilution
Caspase-1 (p10)	# sc-514	Santa Cruz Bio.	1/500
Caspase-1 (A-19)	# sc-622	Santa Cruz Bio.	1/100
IL-1R1	# sc-688	Santa Cruz Bio.	1/500
IL1 β	# sc-7884	Santa Cruz Bio.	1/500
Cleaved IL1 β	#sc-23459	Santa Cruz Bio.	1/100
GAPDH	#sc-25778	Santa Cruz Bio.	1/1500
Goat anti-rabbit 2 ^o	#sc-2004	Santa Cruz Bio.	1/4000
Goat anti-mouse 2 ^o	#sc-2055	Santa Cruz Bio.	1/4000

Table 3: List of primary and secondary antibodies with thier dilutions.

12.ELISA (enzyme-linked immune-sorbent assay)

Human IL1 β Platinum ELISA kit (eBioscience) was used to detect secreted IL1 β protein levels as per the manufacturer's instructions. THP1 cells were counted and added into 96-well plates (20,000 cells/ml) with 1 μ g/ml LPS and increasing concentrations of ZD0130 or ZD0196 from (30nM to 8 μ M). α -IL1 β antibody is used as positive control. The cells were incubated for 20-24 hours in 37°C. Next day, the microwell strips of the ELISA kit were washed twice with kit wash buffers. After washing, standard dilution curve of the reconstituted human IL1 β is done. Then the incubated 96-well plate was centrifuged at 1500 rpm for 5 minutes. THP1 supernatant (50 μ l) was transferred the ELISA plate with 50 μ l of sample diluent. After, 50 μ l of fresh prepared Biotin-Conjugate was added to the wells and incubated at room temperature for two hours on a microplate shaker at 400 rpm. The mixture was removed and wells were washed three times. Then 100 μ l of fresh

prepared Streptavidin-HRP was added to the wells and incubated at room temperature for one hour on a microplate shaker at 400 rpm. Again, the mixture was removed and wells were washed three times. Then, 100 μ l of TMB Substrate Solution is added to the wells. After 10 minutes incubation at room temperature, the reaction is stopped by the addition of 100 μ l of Stop Solution. The plate is read at 490 nm with Synergy H1 Multi-Mode Reader.

13.Site-Directed Mutagenesis

To investigate the binding dynamics of IL1R to our compounds, pcDNA4-IL1R plasmid was subjected to Site-Directed-Mutagenesis using the Q5 mutagenesis kit (New England Biolabs, Cat# E0554S) Ten amino acids were targeted for alanine mutagenesis (Table 4).

PCR (Polymerase chain reaction) mixes are set up in thin-walled PCR tubes with 25 ng of pcDNA4-IL1R plasmid, Q5 Hot Start High-Fidelity 2X Master Mix, 0.5 μ M forward and reverse primers. After PCR, Kinase Ligase and DpnI (KLD) treatment is done with PCR Product, 10X KLD Enzyme Mix, 2X KLD Reaction Buffer and Nuclease-free Water. The mixture as incubated at room temperature for 5 minutes. Then, the reaction was transformed into NEB 5-alpha Competent *E. coli* cells.

After obtaining site-directed mutagenized plasmids, they were sent to MacroGen Europe for sequencing to validate constructs. The validated constructs were used in the system which is described “Luciferase Assay with Human Embryonic Kidney 293t Cells”.

Mutation	Type	Sequence	Size
PHE128	F	5'-ACAAGCCATAGCTAAGCAGAAACTA-3'	25 mer
PHE128	R	5'-GCATTATAACATAAGTTAGGC-3'	21 mer
LYS129	F	5'-AGCCATATTTGCGCAGAAACTAC-3'	23 mer
LYS129	R	5'-TGTGCATTATAACATAAGTTAGG-3'	23 mer
GLN130	F	5'-CATATTTAAGGCGAAACTACCC-3'	22 mer
GLN130	R	5'-GCTTGTGCATTATAACATAAG-3'	21 mer
VAL141	F	5'-GAGGACTTGTGCGCCCTTATATG-3'	23 mer
VAL141	R	5'-CGTCTCCTGCAACGGGT-3'	18 mer
CYS142	F	5'-AGGACTTGTGGCCCTTATATG-3'	22 mer
CYS142	R	5'-CCGTCTCCTGCAACGGGT-3'	18 mer
PRO143	F	5'-ACTTGTGTGCGCTTATATGGAGTT-3'	24 mer
PRO143	R	5'-CCTCCGTCTCCTGCAACG-3'	18 mer
TYR144	F	5'-TGTGTGCCCTGCTATGGAGT-3'	20 mer
TYR144	R	5'-AGTCCTCCGTCTCCTGCA-3'	18 mer
MET145	F	5'-GTGCCCTTATGCGGAGTTTTT-3'	21 mer
MET145	R	5'-ACAAGTCCTCCGTCTCCTG-3'	19 mer
GLU146	F	5'-CCCTTATATGGCGTTTTTTAAAAA-3'	24 mer
GLU146	R	5'-CACACAAGTCCTCCGTCTC-3'	19 mer
LYS149	F	5'-GGAGTTTTTTGCAAATGAAAATAAT-3'	25 mer
LYS149	R	5'-ATATAAGGGCACACAAGTC-3'	19 mer
ASP179	F	5'-TGGAGTCAAAGCTAGGCTCATC-3'	22 mer
ASP179	R	5'-CTAAAGTGTATATTGTCAAGAAG-3'	23 mer

Table 4: List of forward and reverse primers for the mutagenesis of pcDNA4-IL1R.

14. Statistical analysis

Microsoft Excel 2010 and/or GraphPad Prism 6.1 were used for statistical analyses. The raw data are normalized to each plate's own control samples: positive inhibition and negative inhibition controls; also death controls are used for cytotoxicity assays (MTS). The dose response curves were prepared with GraphPad Prism 6.1 using non-linear regression curve fit.

CHAPTER 3

RESULTS

The results section of the thesis consists of two main sections. The first section of it contains the experiments and the analysis for caspase-1. The second main section includes the experiments and the analysis for human interleukin 1 receptor type I.

A Caspase-1

1. Effect of designed compounds on caspase-1

We focused on the inhibition of caspase-1, Interleukin-1 converting enzyme. The inhibition of caspase-1 protein would inhibit the activation of proIL1 proteins into active form. Thus it would prevent over-activation of inflammasomes which promote autoinflammatory diseases. With this aim, a series of compounds were designed and synthesized by Professor Nilgün Karalı. The effects of the compounds on purified Caspase-1 inhibition were assayed with a protein-based BioVision Fluorometric Caspase-1 Inhibitor Drug Screening Kit as previously described (Chapter 2, Section 1). A total of 34 compounds were tested in a primary preliminary screen at a 20 μ M concentration. Only one of the compounds, ZD0002, decreased the activity of purified caspase-1 enzyme >50%. The closest compound, ZD0005, did not inhibit the activity more than 50%. The other 32 compounds did not inhibit active caspase 1 (< 30% inhibition) (Figure 11).

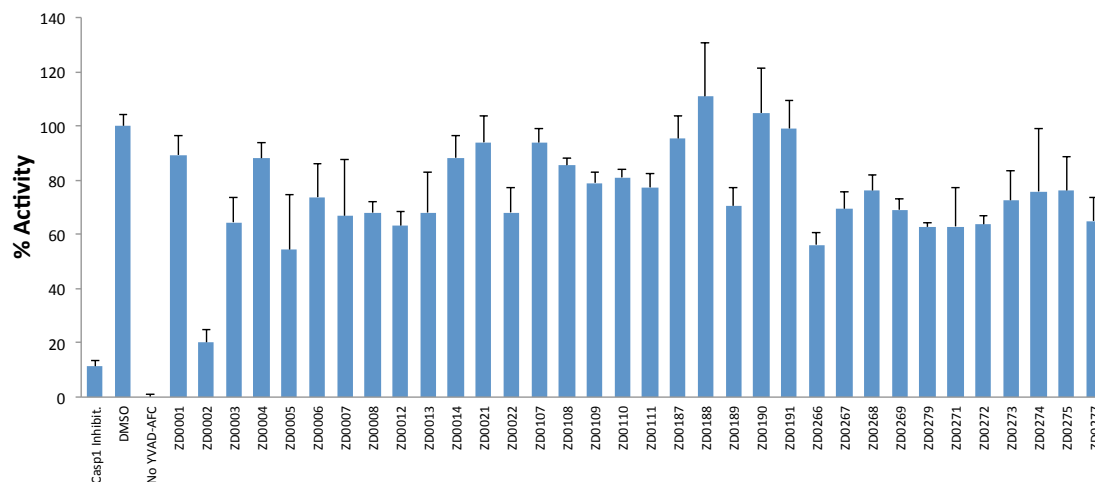


Figure 11: Primary screen with the BioVision Fluorometric Caspase-1 Inhibitor Drug Screening Kit. Compounds were tested at 20 μ M with the active purified caspase-1. The free fluorescent AFC, which is released by active caspase-1 following cleavage of YVAD-AFC, was measured at an excitation of 400nm and emission of 505nm following 1-2hr incubation at 37°C. The signals were normalized with a negative control (no caspase-1) and a positive control (1 μ l of known caspase-1 inhibitor).

To validate these results, ZD0002 and ZD0005 were tested with increasing concentrations (97nM to 200 μ M) to determine the IC_{50} . ZD0002 and ZD0005 were found to have an IC_{50} value of 6.3 and 38 μ M respectively (Figure 12).

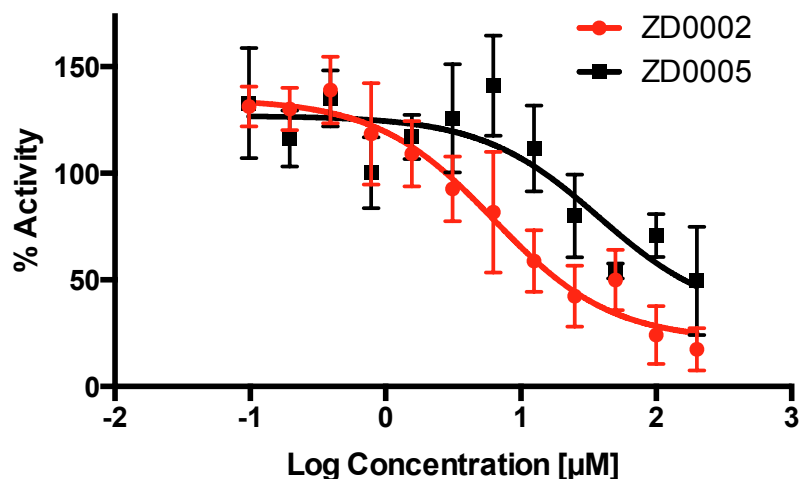


Figure 12: Dose response curves of the compounds ZD0002 and ZD0005. Proteins were incubated with compound (97nM – 200 μM) for 1-2 hours at 37°C. The free-fluorescent AFC was measured as previously described (Chapter 2, Section 1) and the data were processed with Graphpad 6.01. The IC_{50} of ZD0002 was 6.3 μM and ZD0005 was 38 μM.

2. Secondary screen for Caspase-1 inhibition did not reveal a better compound than ZD0002

Following the primary screen results, 6 additional compounds were synthesized to improve the efficiency to inhibit caspase-1. These compounds and a colchicine derivative, TK2, were assayed as previously described (Chapter 2, Section 1). Again, ZD0002 showed >50% inhibition of Caspase-1 enzyme at 20 μM but the inhibition was weaker than the previous screen (Figure 11). Furthermore, ZD0005 also showed a weaker inhibition that is around 20% inhibition (Figure 13). Only one of the new compounds, ZD0457, showed >80% inhibition of caspase-1. It was subsequently tested

with increasing concentrations of the compound (Figure 14). Despite the results from a single concentration the compound ZD0457 was found to have an IC_{50} value of 55 μ M.

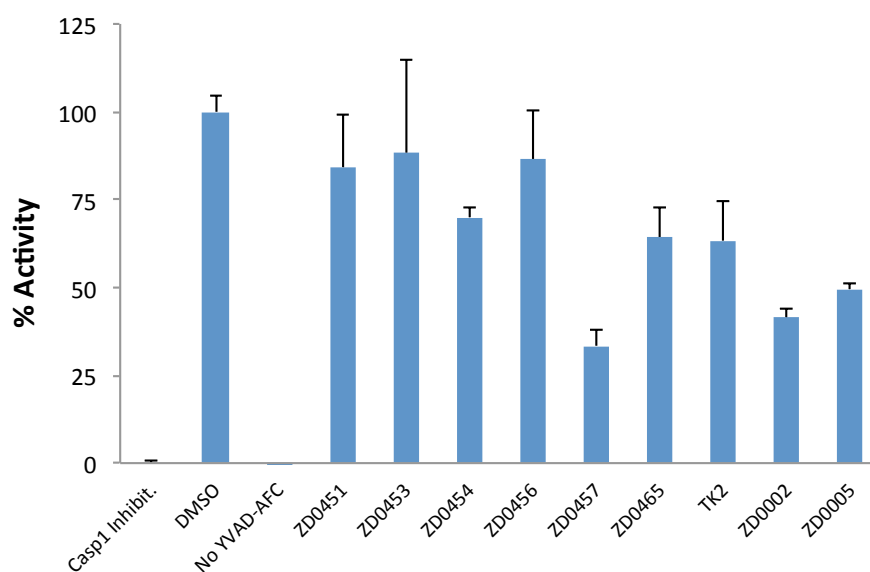


Figure 13: Secondary screen with the BioVision Fluorometric Caspase-1 Inhibitor Drug Screening Kit. Seven new compounds and ZD0002 and ZD0005 were tested at 20 μ M with the active purified Caspase-1 as previously described. After 1-2 hours of incubation at 37°C, the free-fluorescent AFC was measured as previously described (Chapter 2, Section 1).

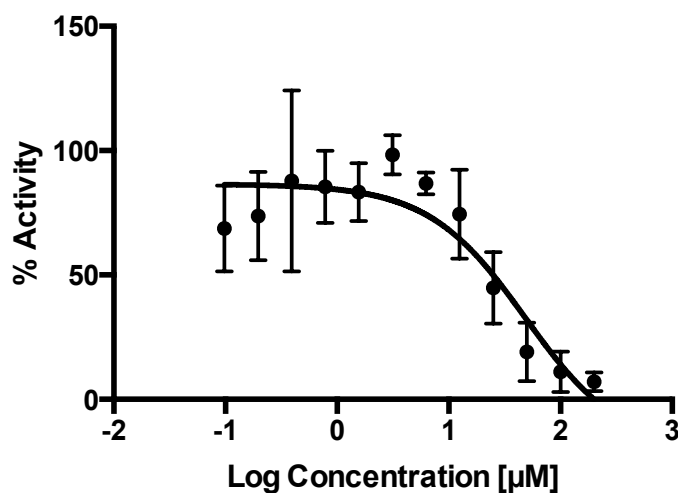


Figure 14: Dose response curve of the compound ZD0457. Increasing concentrations of the compound (97 nM – 200 μM) were assayed with purified caspase-1 as previously described. After 1-2 hours of incubation at 37°C, the free fluorescent AFC was measured and the data were processed with Graphpad 6.01 resulting that ZD0457 has an IC₅₀ value at 55 μM.

3. Colchicine and Colchicine derivatives did not inhibit active Caspase-1

Colchicine, originally extracted from plants of the genus *Colchicum*, is used to treat and decrease the symptoms of gout. It is believed that Colchicine decreases the inflammosome activation to reduce the symptoms [107]. Thus, we expected Colchicine to inhibit active caspase-1 and maybe find out a better structure with the derivatives for the inhibition. Therefore, as a further screen for caspase-1 inhibition, seven colchicine derivatives were assayed with the protein-based *in vitro* assay at a single concentration (20

μM). Unfortunately none of colchicine derivatives showed any inhibition of the caspase-1 enzyme (Figure 15). The assay was repeated at a higher concentration ($40\ \mu\text{M}$) with additional compounds. Neither colchicine nor its derivatives show significant inhibition (Figure 16).

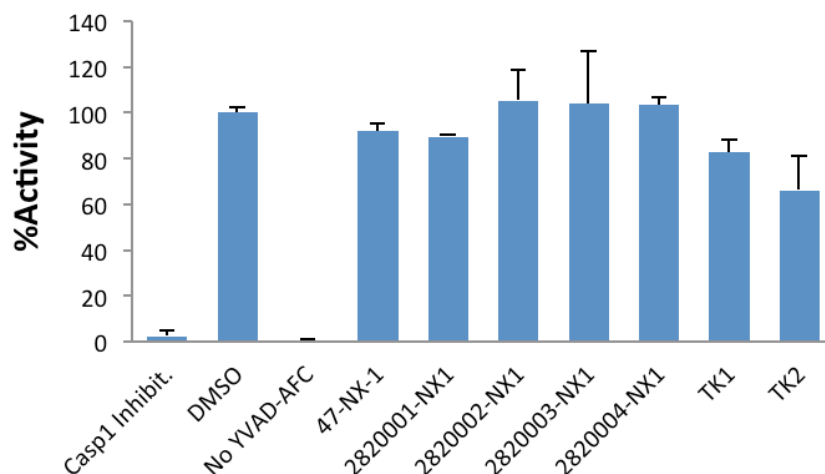


Figure 15: Effect of colchicine and its derivatives on purified caspase-1 ($20\ \mu\text{M}$). The compounds were tested at $20\ \mu\text{M}$ with the active purified Caspase-1 as previously described (Chapter 2, Section 1). After 1-2 hours of incubation at 37°C , the free fluorescent AFC was measured and the signals were normalized with controls to % Activity.

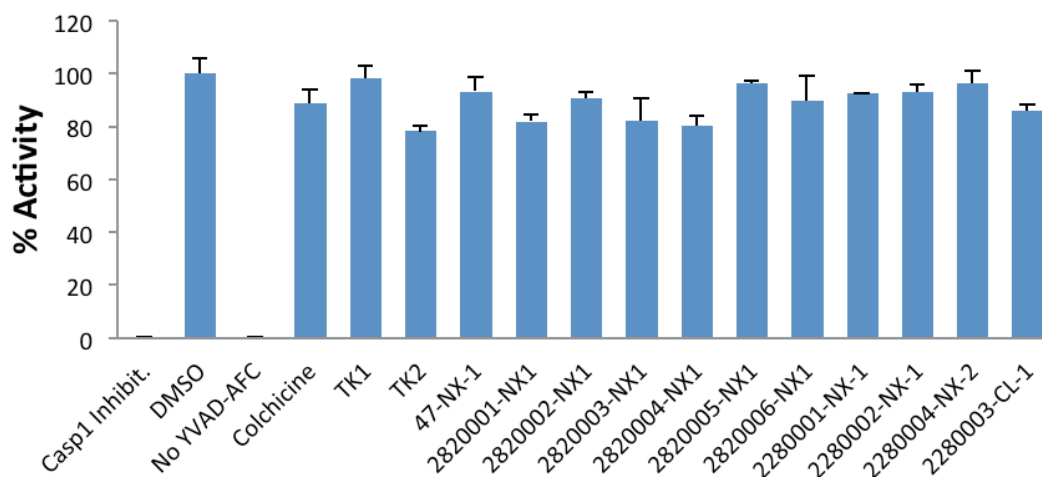


Figure 16: Effect of colchicine and its derivatives on purified caspase-1 (40 μ M). The compounds were tested at 40 μ M with the active purified Caspase-1 as previously described. After 1-2 hours of incubation at 37°C, the free fluorescent AFC was measured and the signals were normalized with a negative control and a positive control to %Activity.

4. Testing of potential caspase-1 inhibitors

From these results, Professor Nilgün Karalı synthesized an additional 10 derivatives. Unfortunately none of them could effectively inhibit caspase-1 (Figure 17).

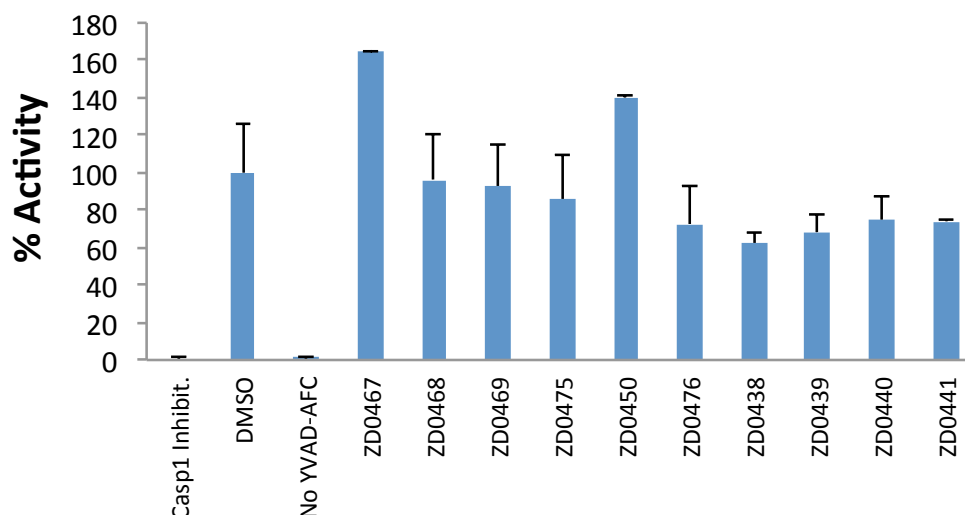


Figure 17: The screen of compounds against active caspase-1 protein. Ten new compounds were tested at 20 μ M with the active purified caspase-1. After 1-2 hours of incubation at 37°C, the free fluorescent AFC was measured and the signals were normalized with a negative control and a positive control to %Activity.

5. Colchicine and Colchicine derivatives did not inhibit activation of Caspase-1 on cellular detection system

Next, we tested Colchicine and colchicine derivatives on cellular detection system because some arguments state that colchicine inhibited the uncleaved form or polymerization of caspase-1. To extend from a cell free system that utilizes purified protein to a cellular detection system, a FAM-FLICA in vitro Caspase-1 Detection Kit from ImmunoChemistry Technologies is used. Cellular inhibition of caspase-1 was assayed by utilizing a fluorescently labeled substrate of caspase-1 that similar to the protein assay

(Section B1). However, the results did not validate this hypothesis. This FAM-FLICA substrate will only fluoresce following cleavage within the cell. To investigate inflammasome formation, a human leukemic cell line (THP1) was used for the assay [108]. It is a human monocytic cell line that expresses high levels of inflammasome proteins NLRP3, ASC and pro-caspase-1. They also express high levels of pattern-recognition receptors like TLR2, TLR4 and IL1R. [109] Firstly, ATP and LPS concentrations were optimized to increase the level of caspase-1 production and activation. Following optimization, 0.5 mM ATP and 1 $\mu\text{g/mL}$ LPS were found to effectively activate caspase-1 (Figure 18). With these conditions, colchicine and derivatives of colchicine were assayed at 5 μM . Surprisingly, Colchicine, TK1, TK2, 2820006-NX1 increased the level of active caspase-1, while 2820005-NX1 did not cause either an increase or decrease from LPS/ATP stimulated inflammasome activation (Figure 19).

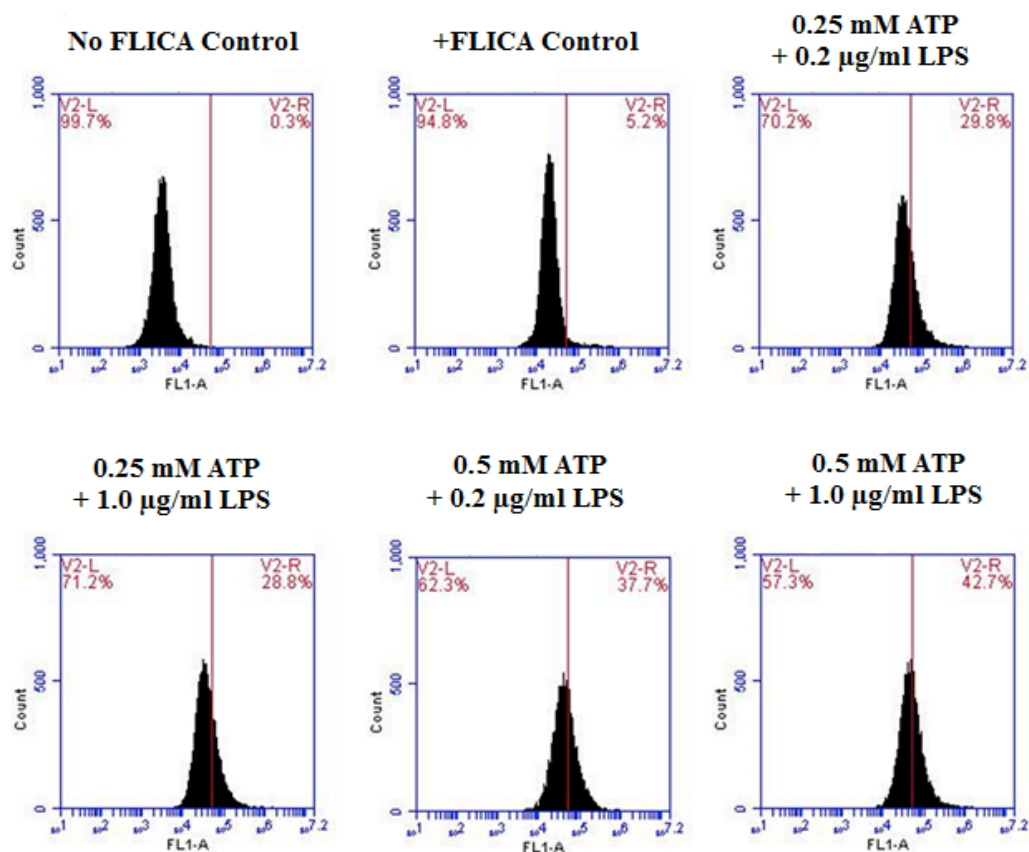


Figure 18: The optimization of FLICA assay. Different combinations of ATP and LPS concentrations were used to stimulate THP1 cells for 44-48 hours. The combination with 0.5 mM ATP and 1 µg/mL LPS are the most optimal conditions for the activation of inflammasome.

Based on the previous results it appeared that colchicine increased FAM-FLICA cleavage. This could be potentially due to high concentration of the compounds as colchicine is a toxic secondary metabolite and it is highly possible the derivatives have the toxic characteristic. Colchicine is a mitotic inhibitor which the disrupt microtubules [110]. To test if this was simply due to excessive levels of the compound several concentrations of colchicine were tested in the same FAM-FLICA assay. As before, the higher

concentrations of colchicine increased the level of caspase-1 activation significantly and the lowest concentration also increased but not significantly. Overall no caspase-1 inhibition was observed by colchicine (Figure 20).

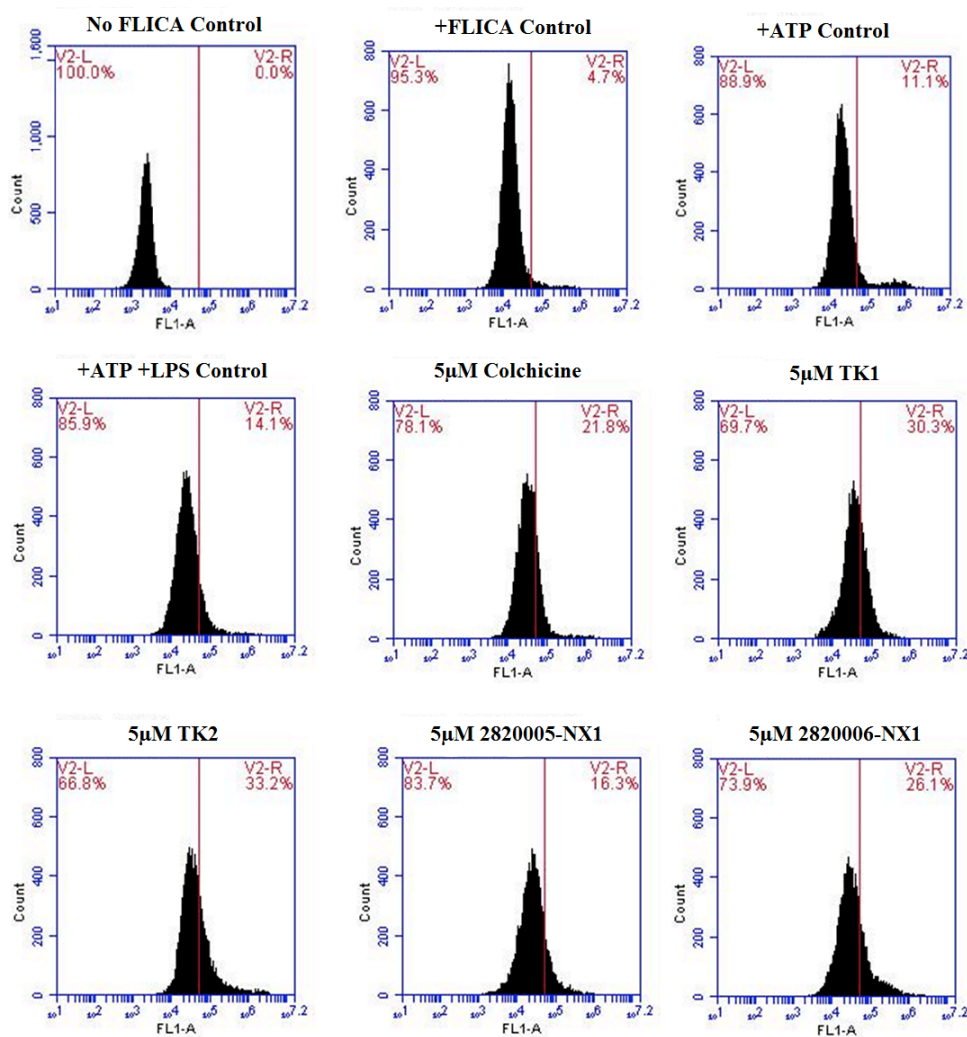


Figure 19: Colchicine and four colchicine derivatives did not inhibit cellular activation of caspase-1. THP1 cells were assayed with FAM-FLICA in vitro Caspase-1 Detection Kit following co-stimulated with 0.5 mM ATP and 1 μ g/mL LPS 44-48 hours. Compounds (5 μ M) were incubated over the induction period.

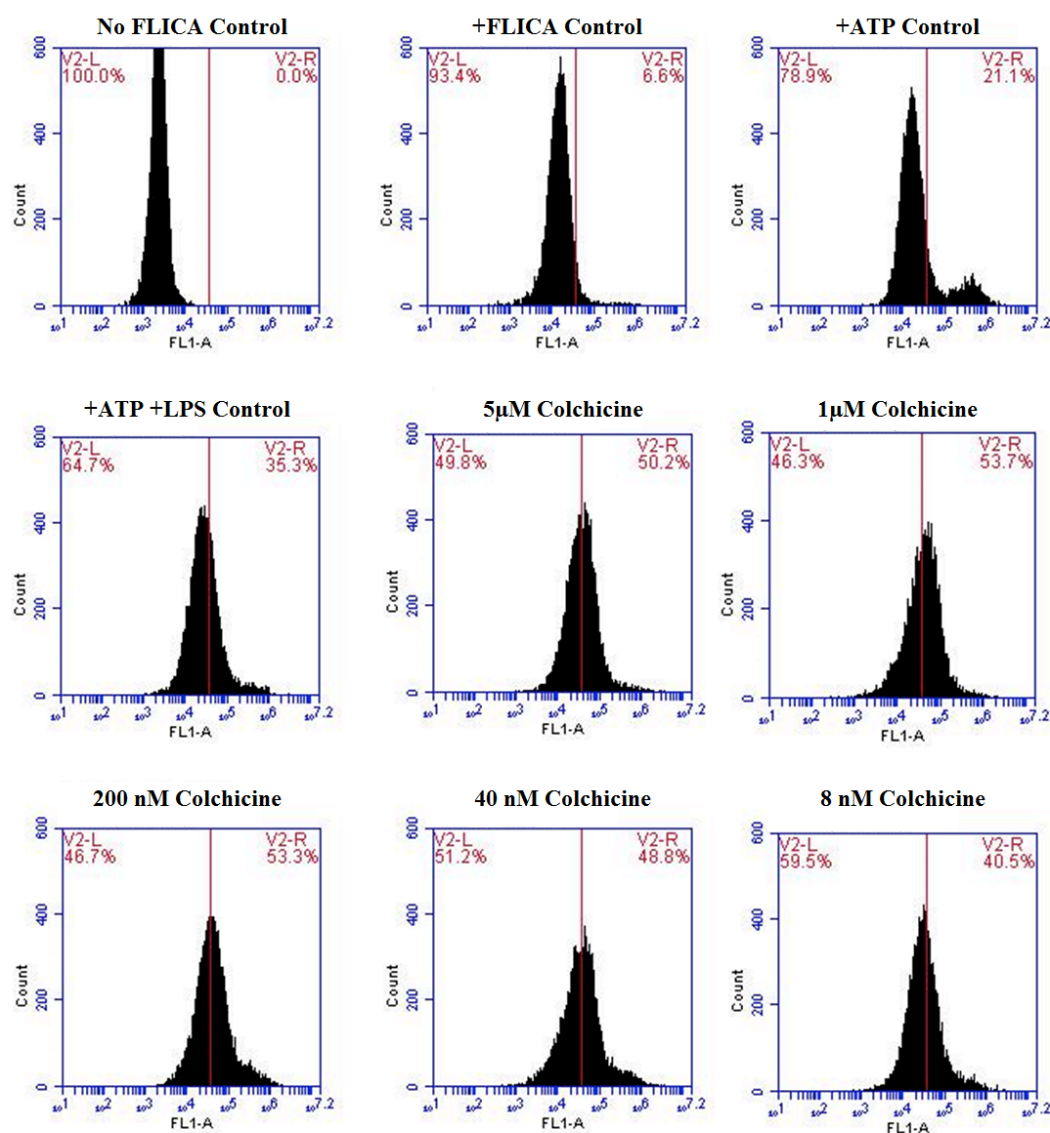


Figure 20: Dose response curve of colchicine in a cell based caspase-1 assay. THP1 cells were co-stimulated with 0.5 mM ATP and 1 µg/mL LPS and incubated with increasing concentrations of colchicine (8 nM to 5 µM) for 44-48 hrs. Following activation, caspase-1 activity was assayed as previously described with a FAM-FLICA reagent (Chapter 2, Section 2).

Based on these results, we proposed the inflammasome inducer ATP may activate a non-NLRP3 inflammasome. As a next experiment, monosodium urate crystals (MSU) were used to trigger the activation of caspase-1 activation with various concentrations of colchicine. MSU is another inflammasome inducer similar to ATP. As in earlier experiments, colchicine caused caspase-1 activation in this assay (Figure 21). Overall we showed that Colchicine and its derivatives did not cause caspase-1 inhibition.

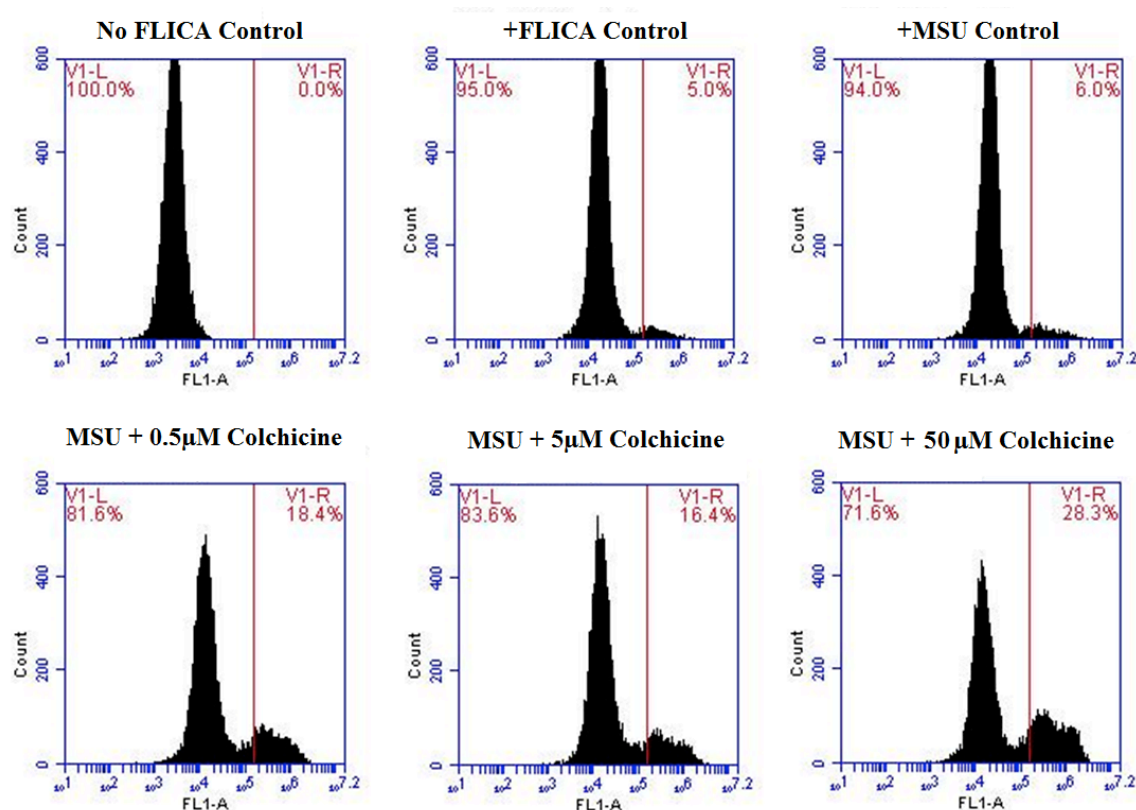


Figure 19: Effect of increasing concentrations of Colchicine on cellular caspase-1. THP1 cells were incubated with 0.5-50 μ M of Colchicine and then stimulated with MSU instead of LPS/ATP for 44-48 hours. Caspase-1 activity was assayed with FAM-FLICA as previously described (Chapter 2, Section 2).

B Human interleukin 1 receptor type I

Aberrant levels of IL1R activation are observed in many types of auto-inflammatory diseases. To reduce IL1R activation, IL1R activity is targeted by IL1R antagonists and neutralizing α -IL1 β antibodies. However, almost all therapeutics which inhibit the IL1R pathway are recombinant proteins. They are thereby extremely costly and require daily injections. There are no identified IL1R small molecule inhibitors that would be easier to produce and limit potential injections. To identify and characterize novel small molecule inflammasome inhibitors, we utilized *in silico* modeling in combination with drug repurposing to develop small molecule inhibitors that target IL1R signaling pathway.

1. The optimization of SEAP assay with HEK-Blue IL1 β cell line

The effects of the compounds on IL1R inhibition were tested with the HEK-Blue IL1 β cell line and assayed with a colourmetric reporter to detect Secreted Embryonic Alkaline Phosphatase (SEAP) assay. Firstly, the assay was optimized with varying amounts of purified recombinant IL1 β . From this 1 ng/ml of purified protein was found to be the lowest concentration of IL1 β to cause 95% of the reporter activation. As a positive control, experiments were validated with an Anti-IL1 β antibody (Figure 22). Moreover, we made a dose response curve with increasing concentrations of Anakinra, with a known inhibitor of IL1R. The results proved that Anakinra was highly potent and had an IC₅₀ value of 6.5 nM for the inhibition of IL1R (Figure 23).

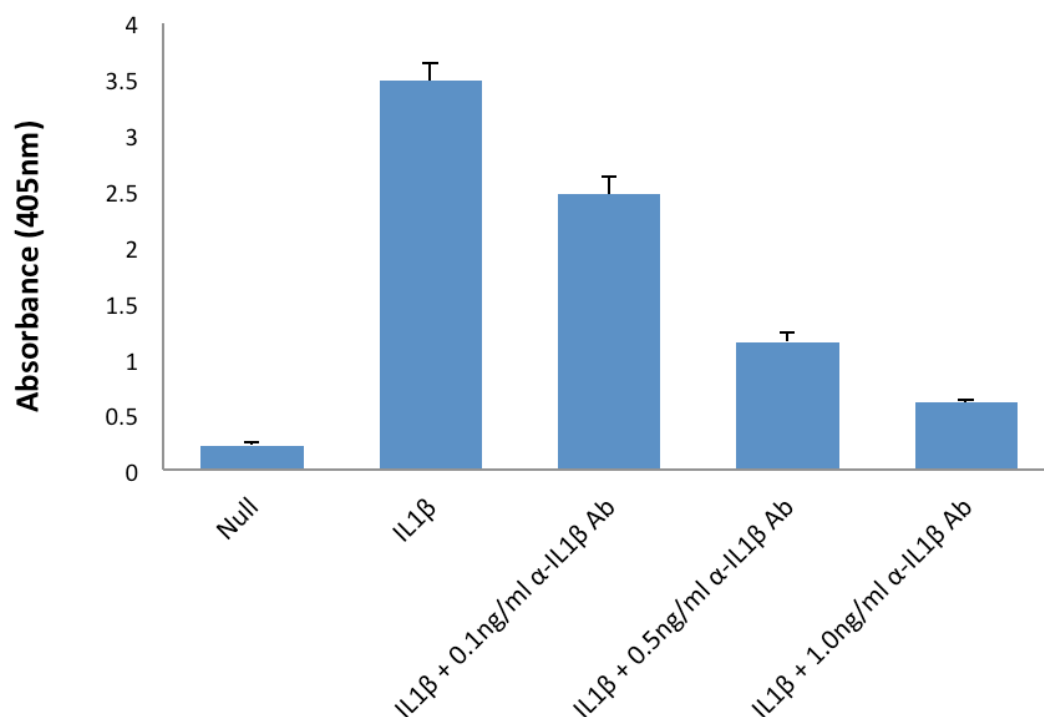


Figure 20: The optimization of SEAP assay. HEK-Blue IL1 β cells (50,000 per well) were incubated with 1 ng/ml purified recombinant IL1 β and various amounts of Anti-IL1 β antibody (0.1-1ng/ml). Following 20-24 hours incubation, 50 μ l of supernatant was transferred into a clear 96-well plate which contained 0.05% CHAPS (20 μ l/well). The colourmetric substrate (Chapter 2, Section2) was added to each well and then incubated at RT for 15-45 minutes. The absorbance was read at 405 nM.

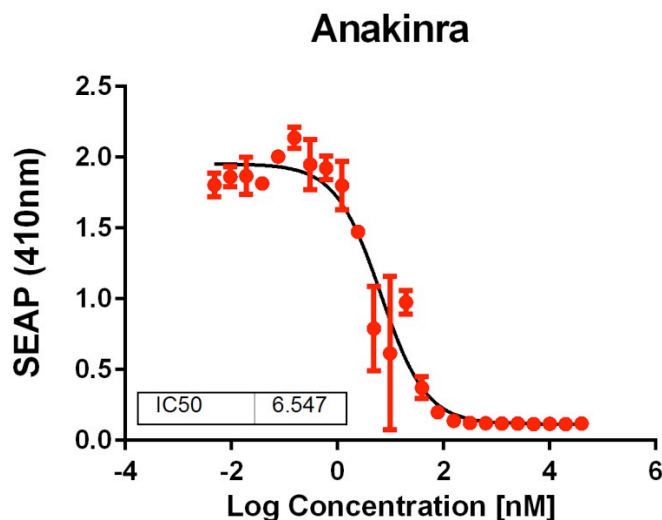


Figure 21: Dose-response curve of Anakinra. HEKBLUE IL1R cells were incubated with increasing concentrations of Anakinra (5pM - 40 μ M) and assayed as previously described (Section 2.3). IL1R activity (SEAP assay) results were analyzed with Graphpad 6.1.

2. The Prestwick Chemical Library Screen with SEAP and MTS assays with HEK-Blue IL1 β cell line

The Prestwick Chemical Library contains 1280 small molecules which are almost all clinically approved drugs (FDA, EMA and other agencies). In this screen, we utilized drug repurposing in which previously proved drugs are used to new indications. It is highly advantageous because the proved drugs have already analyzed necessary tests which are required for commercial purposes [111]. After optimization of the system, the Prestwick Chemical Library was screened at a single concentration (5 μ M). From this screen, 17 compounds showed >80% inhibition at 5 μ M (Figure 24). These were then assayed with viability (MTS) assay to remove any false positives (Figure 25). The eight chemicals with

low toxicity were analyzed with a dose-response curve to determine the IC_{50} . From this, six compounds showed variable levels of activity which could not be used to calculate an IC_{50} . The remaining two compounds Monensin sodium salt and Camptothecine (S,+) had an IC_{50} of 120nM and 5nM, respectively. However, reduction of SEAP appears to stems from their toxicity (Figure 26). Because the toxic effect of the drugs caused the death of cells, the SEAP production was decreased. In brief, the drug-profiling attempt with The Prestwick Chemical Library did not reveal drugs that could be profiled for the inhibition of the IL1R pathway.

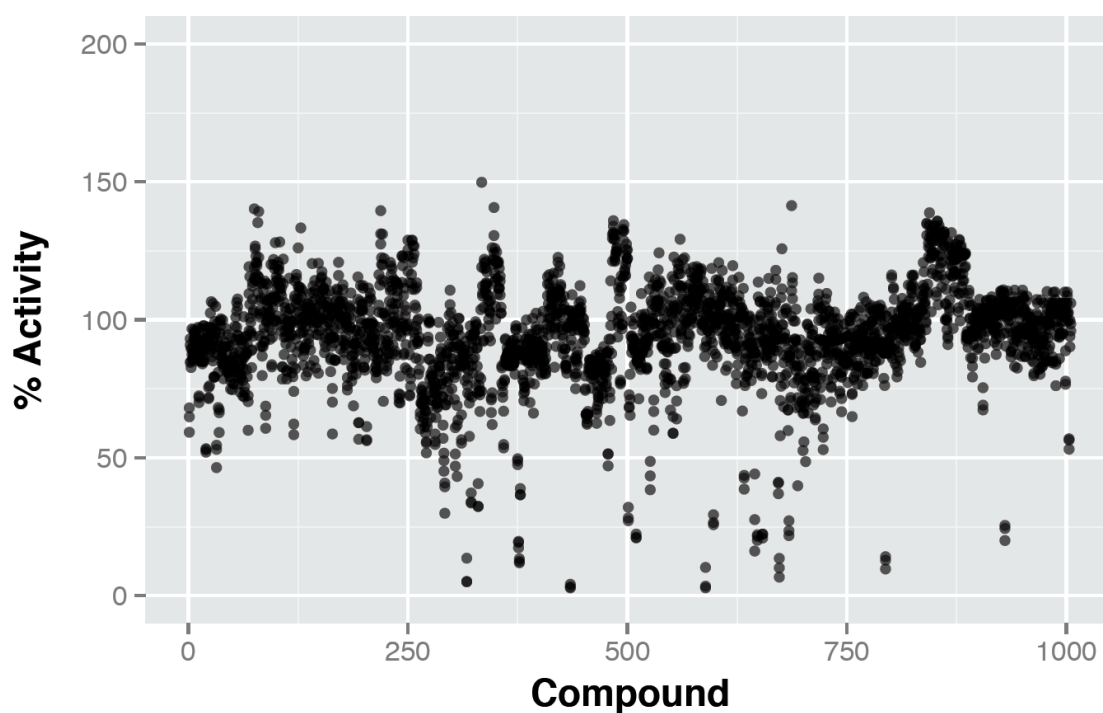


Figure 22: The Prestwick Chemical Library Screen at 5 μ M. IL1R activity analyses was done with SEAP assay as previously described (Chapter 2, Section 3). The absorbance data is normalized with negative control (+ α -IL1 β Ab) and positive control (+IL1 β) to %

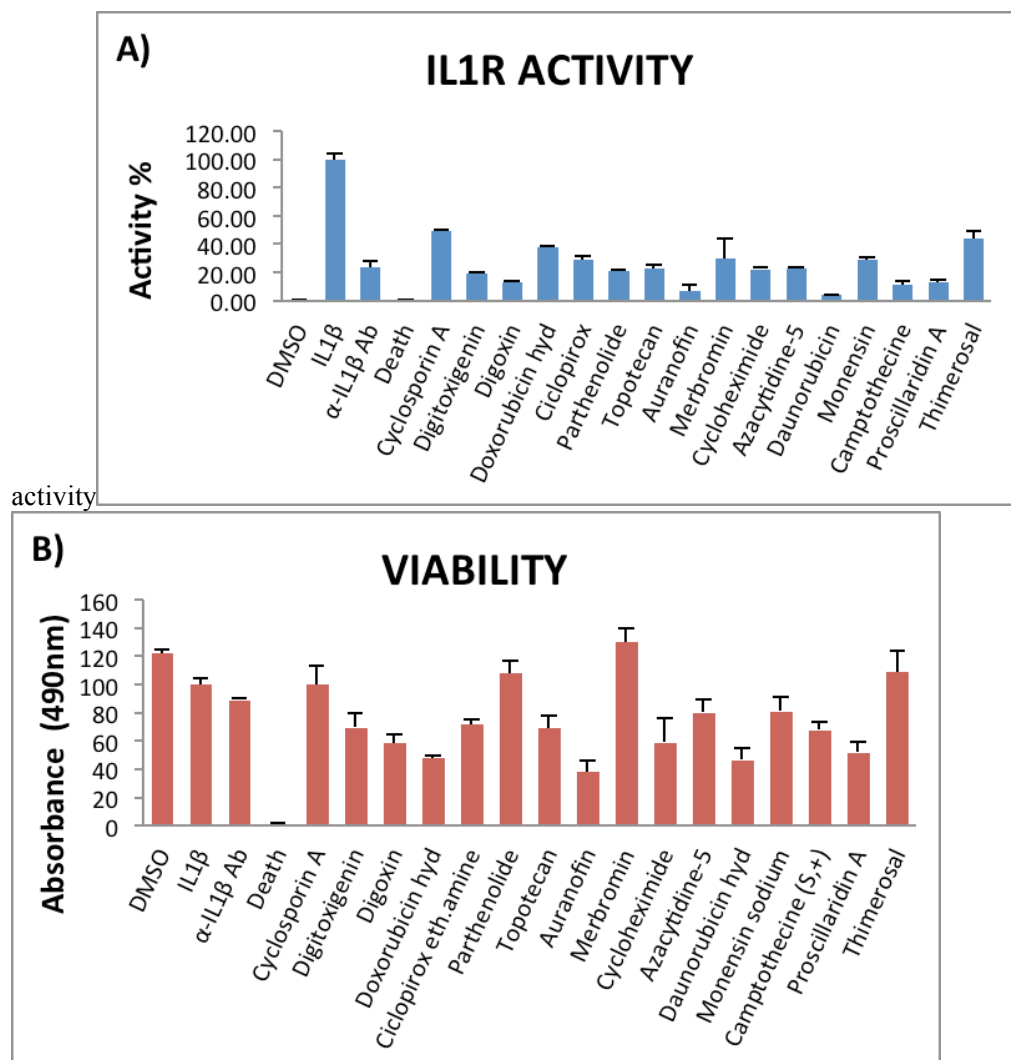
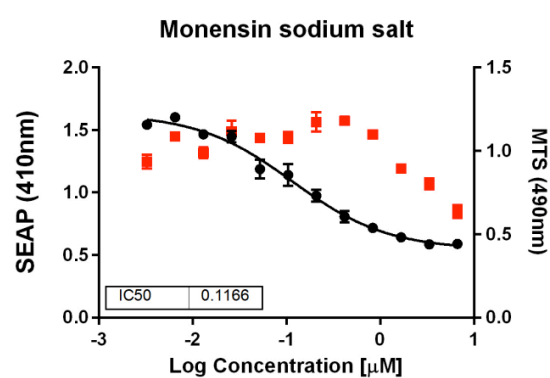
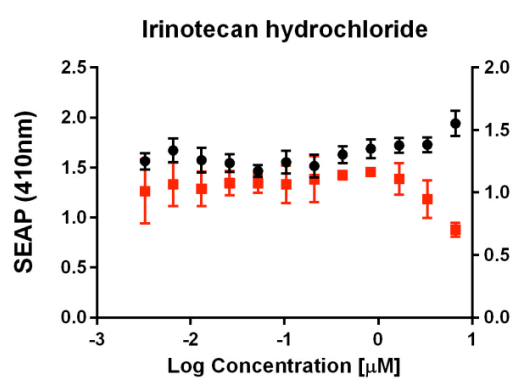
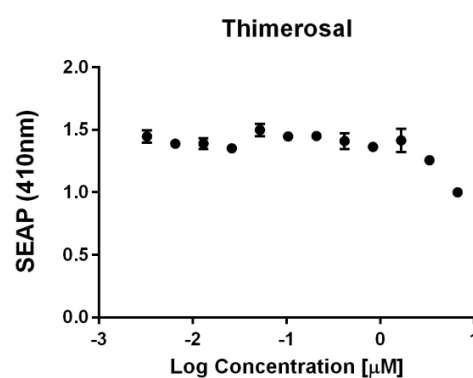
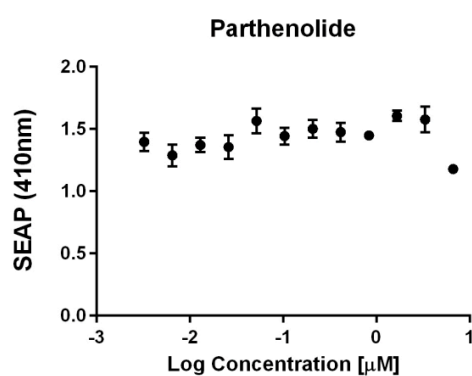


Figure 23: Prestwick Library screen with HEK-Blue IL1 β cell line. A) IL1R activity analysis with SEAP assay. HEK-Blue IL1 β cells (50,000/well) were incubated with 1 ng/ml purified IL1 β and the compounds (5 μ M). After 20-24 hours incubation, 50 μ l of the supernatant was transferred into a 96-well plate containing 20 μ l 0.05% CHAPS. Coloring substrate (Chapter 2, Section 3) was added over them and incubated at RT for 15-45 minutes. The absorbance was read at 405 nm. B) The toxicity of the compounds were analyzed with MTS assay. 20 μ l/well MTS solution was added to each well. The solution was added directly in the same sample plate with HEK-Blue IL-1 β cells. After 2 hours of incubation, the absorbance was read at 490 nm.



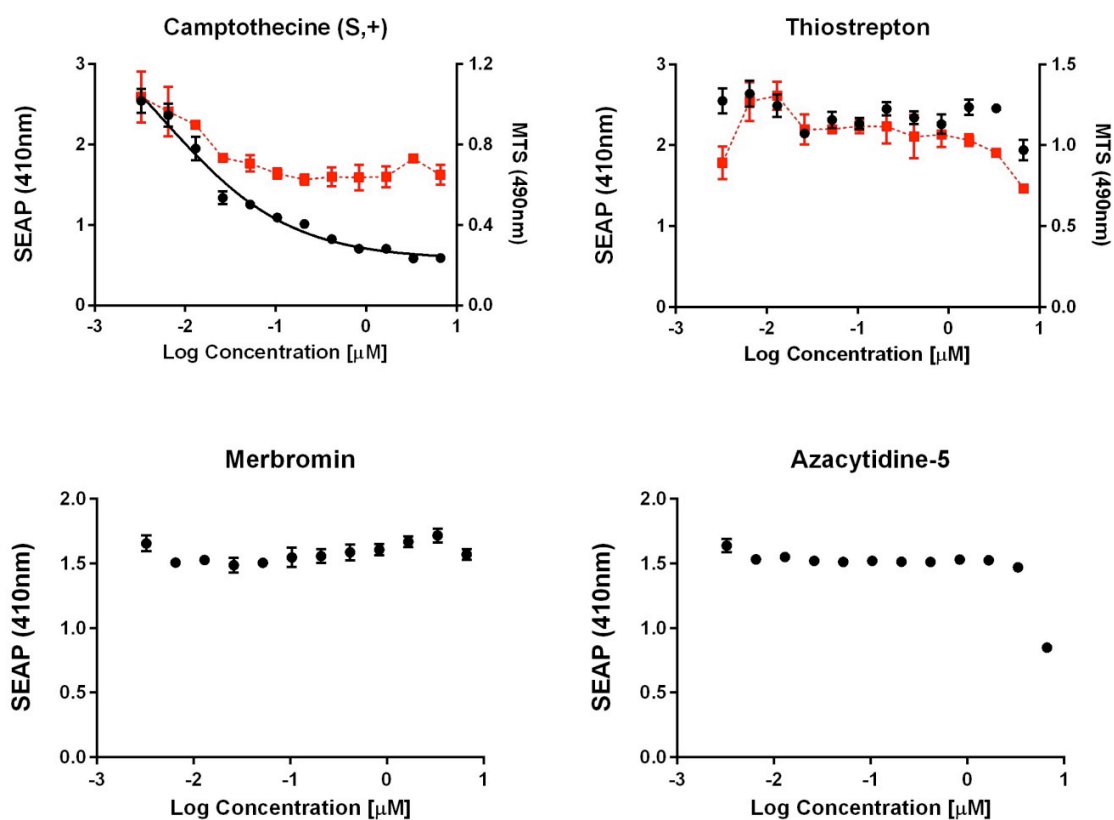


Figure 24: Dose-response curves of eight hits from Prestwick Library Screen. HEKBLUE IL1R cells were incubated with increasing concentrations of compound (10nM-40 μM) and assayed as previously described (Section 2.3). IL1R activity (SEAP assay) results are shown by black dots and lines. Viability (MTS) results are shown by red dots and dashed lines.

3. The primary screen of *in silico* compounds with SEAP and MTS assays with HEK-Blue IL1 β cell line

HEK-Blue IL-1 β cell lines were used to analyze the IL1R inhibition effects of *in silico* compounds. In our initial study, 45 compounds synthesized by Professor Nilgün Karali were tested at a single concentration (5 μ M). Given our previous results with the Prestwick library, all compounds were analyzed for toxicity with MTS assay following SEAP assay (Figure 27). Those compounds were either toxic or inactive were discarded (Figure 28). From the 45 compounds, 10 were defined as “hits” with greater than 75% inhibition and no obviously cytotoxicity. These compounds were further analyzed with a dose response curves from 10 nM to 40 μ M (Figure 29).

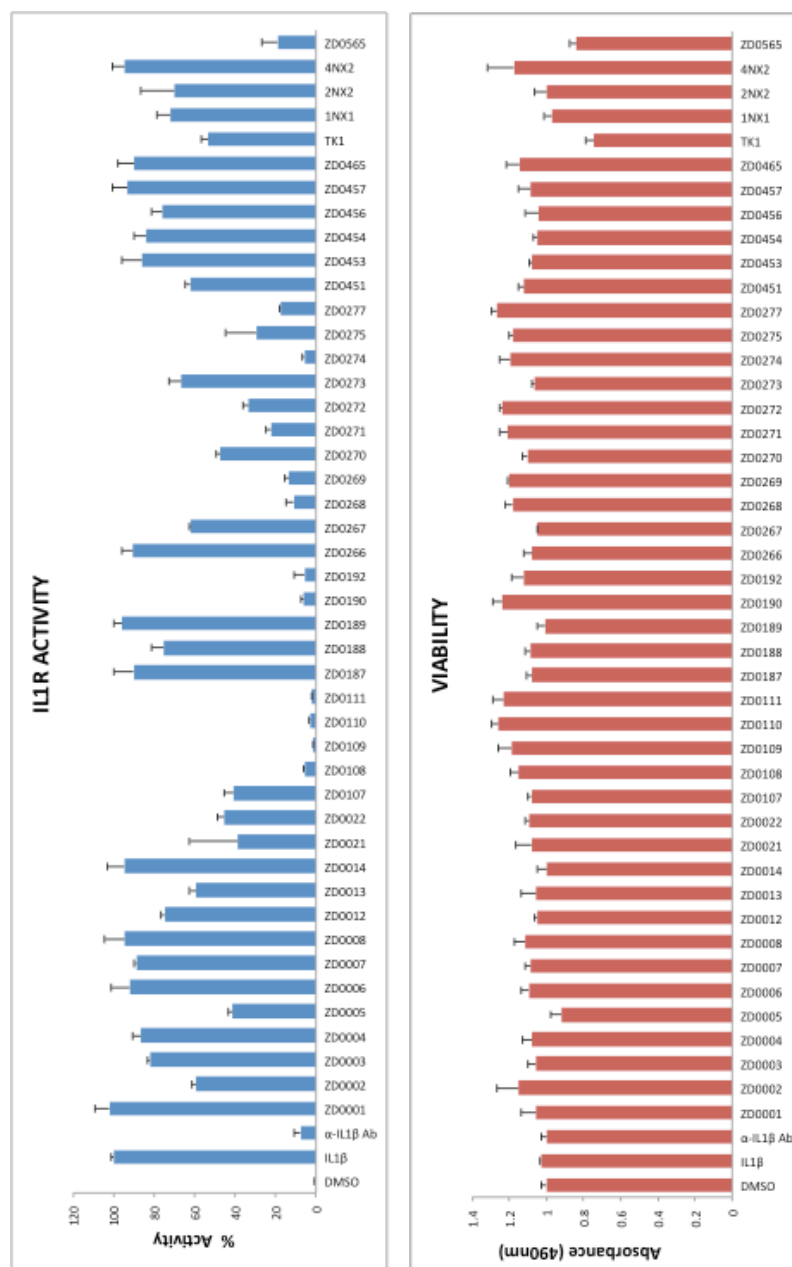


Figure 25: Primary screen of compounds at 5 μ M. IL1R activity analyses was done with SEAP assay as previously described (Chapter 2, Section 3). The absorbance data is normalized with negative control (+ α -IL1 β Ab) and positive control (+IL1 β) to % activity shown by blue bars. The toxicity of the compounds was analyzed with MTS assay shown by red bars.

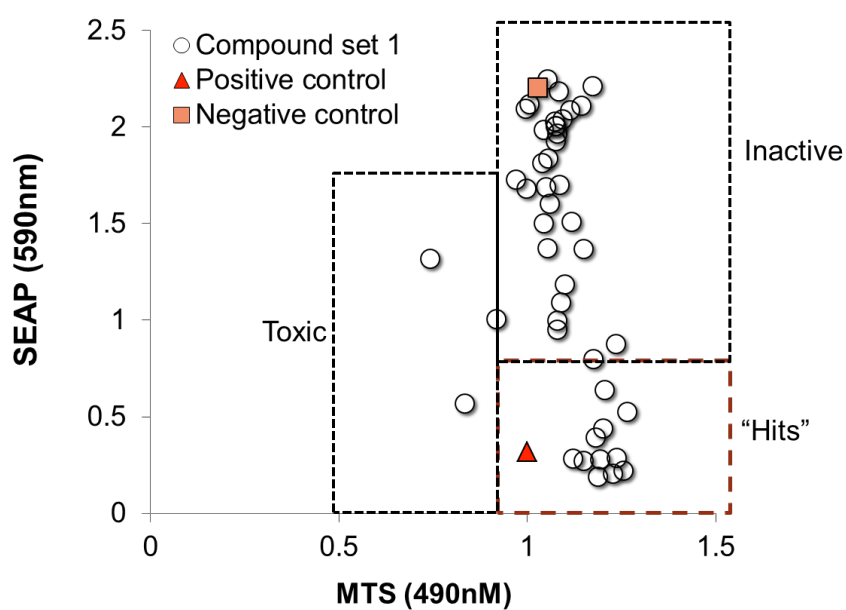
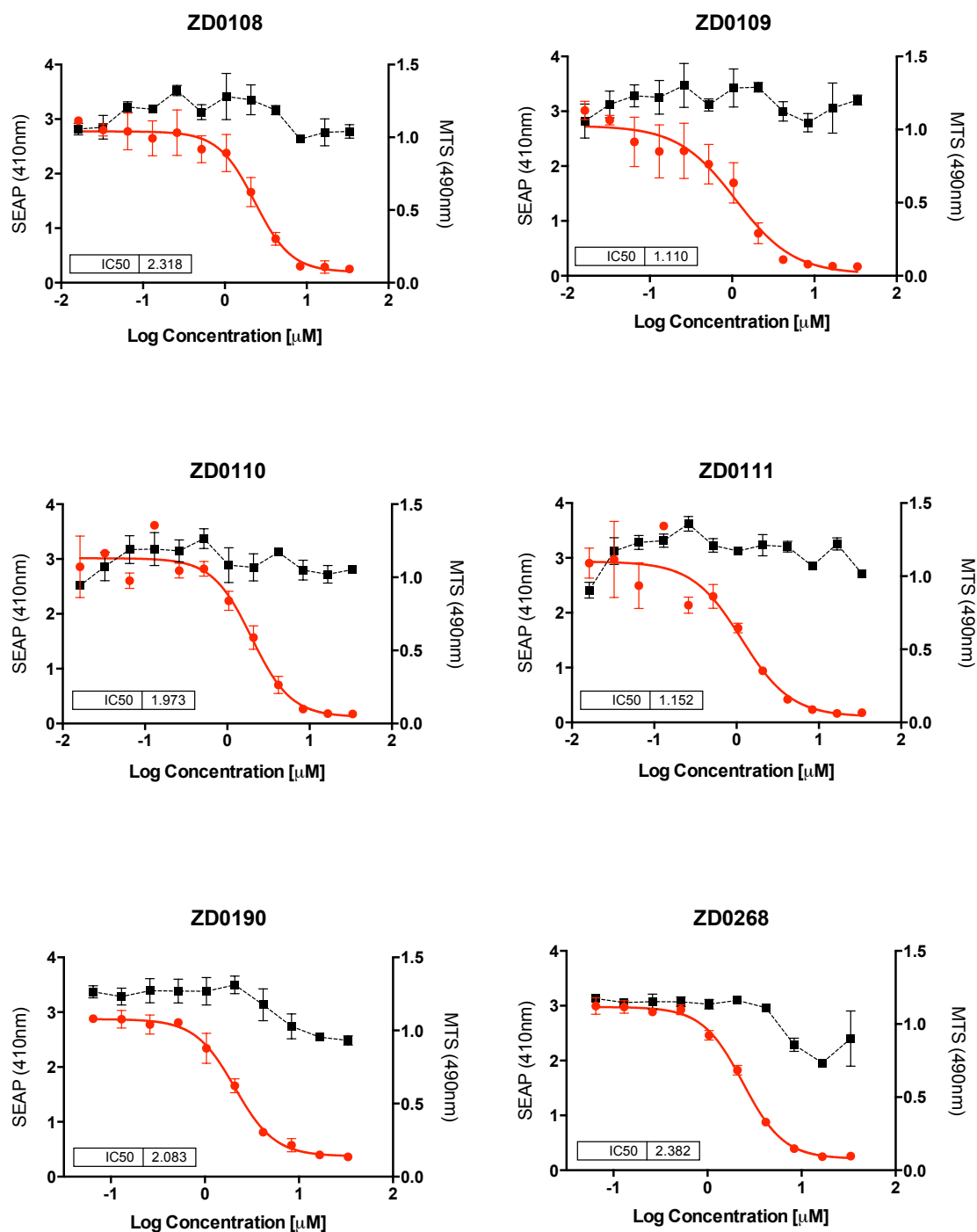


Figure 26: Classification of compounds from the primary screen. The 45 five compounds from the primary screen were plotted according to viability (MTS) and activity (SEAP). Compounds with lower IL1R activity and higher viability were labeled as hits.



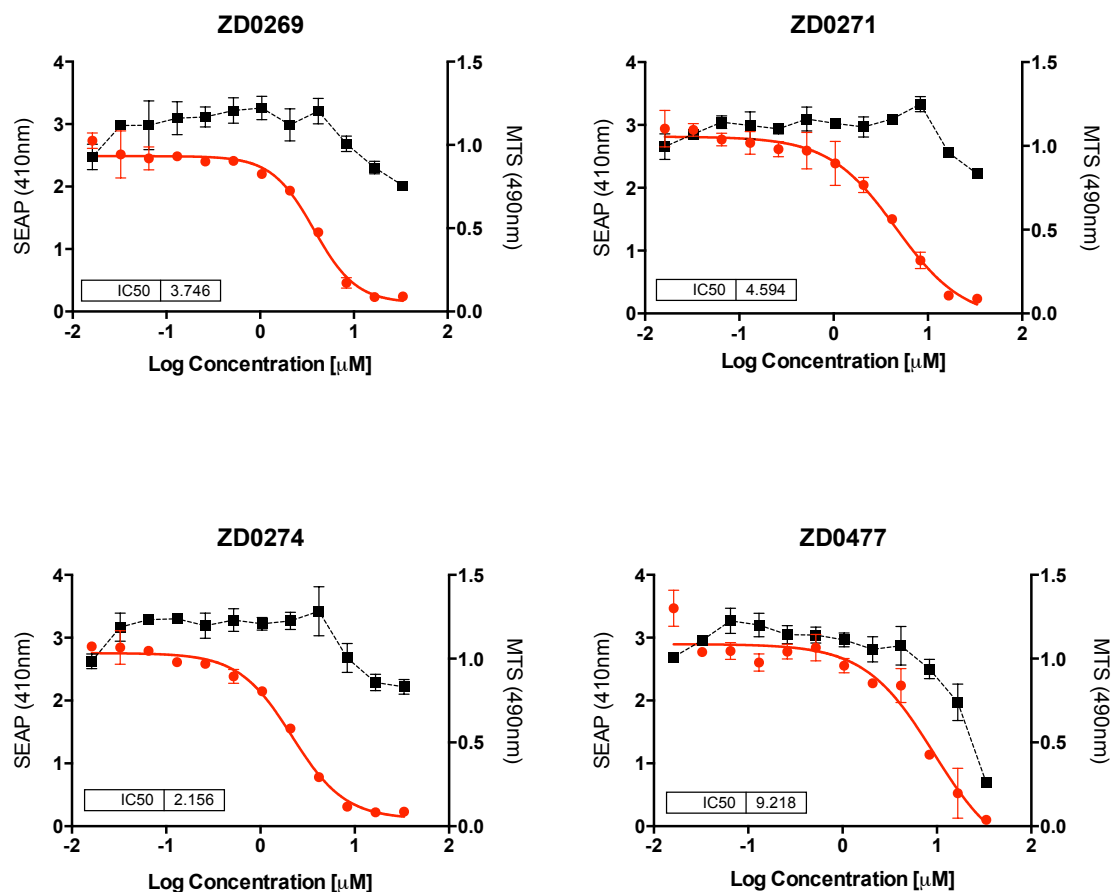


Figure 29: Dose-response curves of hits from the first screen of compounds. HEKBLUE cells were assayed with increasing concentrations of the compound (10 nM-40 μM) as previously described (Chapter 2, Section 3). IL1R activity (SEAP assay) results are shown by red dots and lines. Viability (MTS) results are shown by black dots and dashed lines. IC_{50} values are shown at the left bottom corner of the figures.

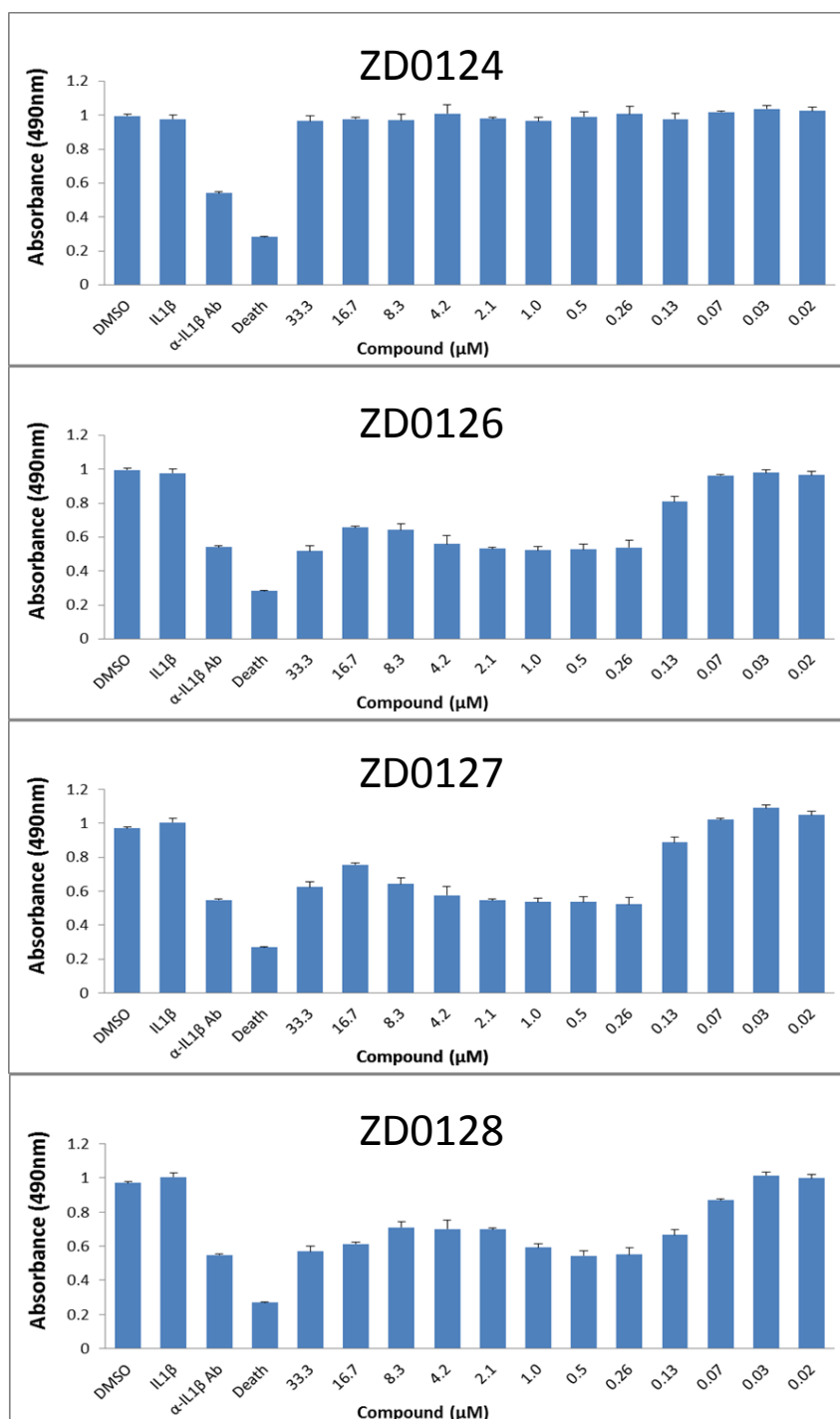
4. The secondary screen of *in silico* compounds with SEAP and MTS assays with HEK-Blue IL1 β cell line

From these results (Section B-3), an additional 55 derivatives were synthesized by Professor Nilgün Karali to develop the scaffold structure activity relationship (SAR). These compounds were first tested at 5 μ M for activity and toxicity. From this, 36 compounds were determined to be “hits” and characterized by dose response curves (Appendix II). Following these results, an additional 124 compounds were synthesized and tested at a single concentration. From this, a further 98 hits were analyzed with dose response curves (Appendix II).

These experiments provided valuable insight into the structure activity relationship of these small molecule inhibitors. The most potent inhibitors were found to have IC₅₀ values in the mid nano molar range.

5. Long term toxicity assays (MTS) with best hits from *in silico* screens and Anakinra

Preliminary studies of these compounds tested for cytotoxicity, but did not assay changes in cellular proliferation. To better characterize the effect of these compounds on cell growth the most potent derivatives were analyzed with longer proliferation assay. The cells were incubated with ZD0124, ZD0126, ZD0127, ZD0128, ZD0423, ZD0430, ZD0433 and Anakinra. The proliferation was then assayed by MTS following incubation for 4 days (Figure 30). ZD0124, ZD0430 and Anakinra did not show long-term toxicity with any of the concentrations.



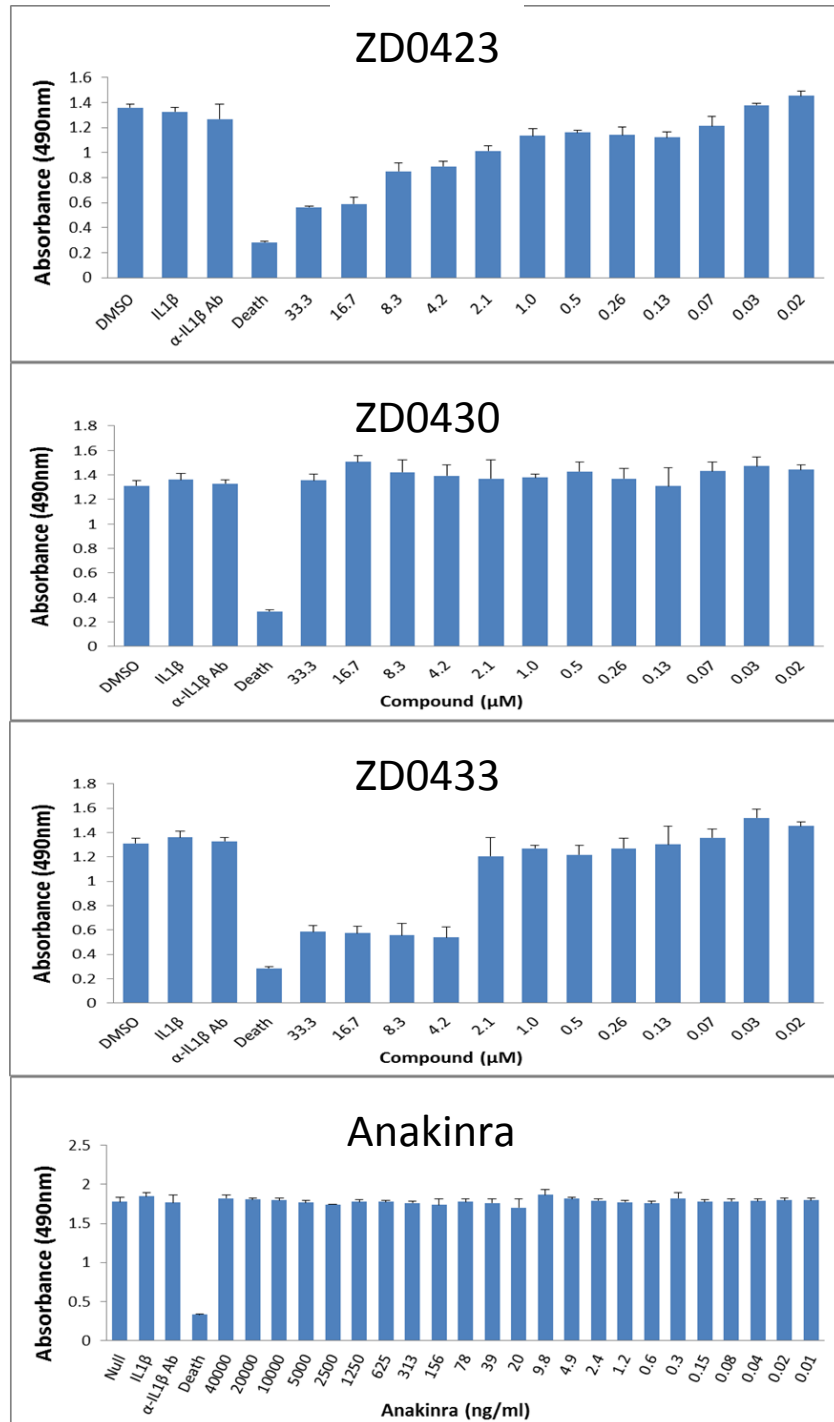


Figure 27: Effect of ZD0124, ZD0126, ZD0127, ZD0128, ZD0423, ZD0430, ZD0433 and Anakinra on cellular proliferation. The toxicity of the compounds was analyzed with MTS assay. All compounds except Anakinra were tested from 20nM to 33.3 μ M. Anakinra was tested from 10 pM to 40 μ M. After 4 days of incubation proliferation was assayed by MTS the absorbance was read at 490 nm.

6. Optimization and validation of the transfection of HEK 293t cells and the luciferase assay

To make a counter screen to confirm that the IL1R inhibition results were not SEAP specific we developed an IL1R luciferase assay. In this the NF κ B -TA-LUC-UBC-GFP-W vector was transfected into HEK 293t cells. The vector has an NF κ B binding site downstream of luciferase and when activated upon stimulation of IL1R by IL1 β leads to transcription of the reporter. A positive control GFP-RelA (p65) plasmid was transfected with the NF κ B -TA-LUC-UBC-GFP-W (Section 2, Chapter 5, Figure 8). The GFP-RelA (p65) plasmid (Section 2, Chapter 5, Figure 9) which makes the expression of RELA protein initiates activation of the NF κ B response element upstream of the Luciferase gene because the RELA protein functions in NF- κ B heterodimer formation [106]. Moreover, the cloned pcDNA4-IL1R plasmid (Section 2, Chapter 5) was used because HEK 293t cells did not have IL1R to respond IL1 β . The co-transfected HEK293t cells with NF κ B-TA-LUC-UBC-GFP-W vector and pcDNA4-IL1R plasmid produced a significant luciferase signal upon stimulation with purified recombinant IL1 β (Figure 31).

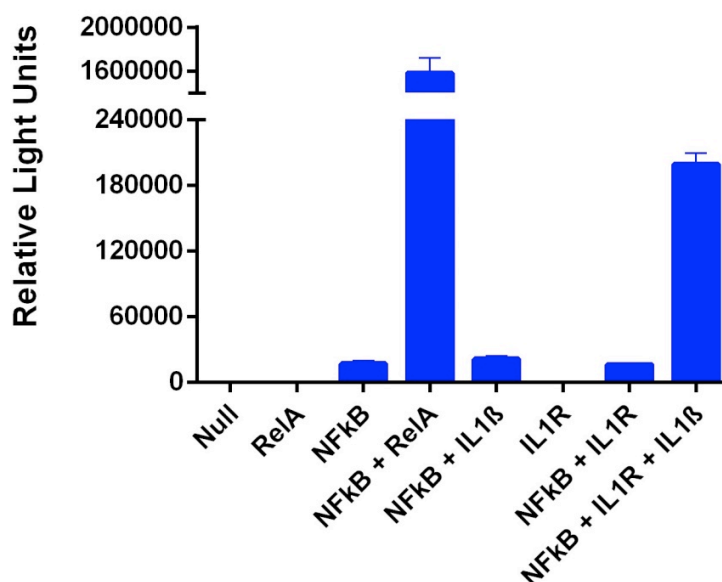


Figure 28: The optimization of the transfection of HEK 293t cells and the luciferase assay. GFP-RelA, only NFkB-TA-LUC, only pcDNA4-IL1R, NFkB-TA-LUC + IL1 β and NFkB-TA-LUC + pcDNA4-IL1R were negative controls. GFP-RelA + NFkB-TA-LUC was positive control. HEK 293t cells were transfected with given plasmids with transfection protocol defined at Chapter 2, Section 5. The luciferase assay was done with the Luciferase assay protocol defined at the same section.

After the optimization of the transfection conditions and the luciferase assay, the system was used with wild type pcDNA4-IL1R plasmid and ZD0130 to replicate the results of SEAP assay (Section B-3). Cells co-transfected with NFkB-TA-LUC-UBC-GFP-W vector and pcDNA4-IL1R plasmid were incubated with 1 ng/ml recombinant IL1 β and concentrations of ZD0130. Following overnight incubation the luciferase signals were quantified. As shown in Figure 32, the results with this luciferase reporter ($IC_{50} = 0.3 \mu M$) to that previously obtained with a SEAP reporter ($IC_{50} = 0.12 \mu M$).

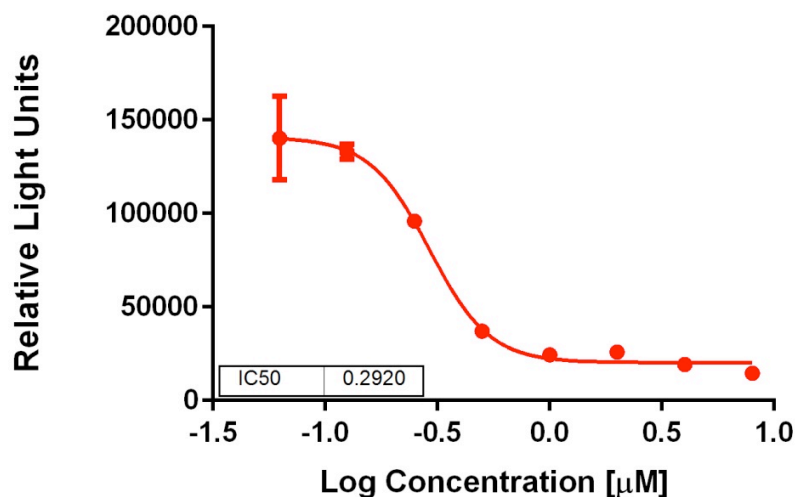


Figure 29: Effect of ZD0130 on IL1R luciferase reporter. HEK 293t cells were co-transfected with NFκB-TA-LUC-UBC-GFP-W vector and pcDNA4-IL1R plasmid. After incubation with 1 ng/ml recombinant IL1β, the luciferase assay was performed previously described.

7. Wash-out Analysis with ZD0162 and Anakinra

The small molecule inhibitors were also analyzed with washout experiment to investigate whether their inhibition effects of the compound were reversible or not. A control dose response curves was done as previously described in Section B3-B4. Simultaneously, a washout plates were prepared with same conditions of cells and compound. After 2 hours of incubation the cells were washed 3 times to remove the compound. Following incubation, 1 ng/ml recombinant IL1β was added to the washout plates and incubated overnight together with control plates. Next day, the SEAP assay was done to measure the activity of IL1R. The activity of ZD0162 was decreased by 1/10 with

wash-out resulting that ZD0162 was reversible. Moreover, the activity of Anakinra with wash-out was also decreased by 1/10 (Figure 33). Anakinra was also a reversible inhibitor.

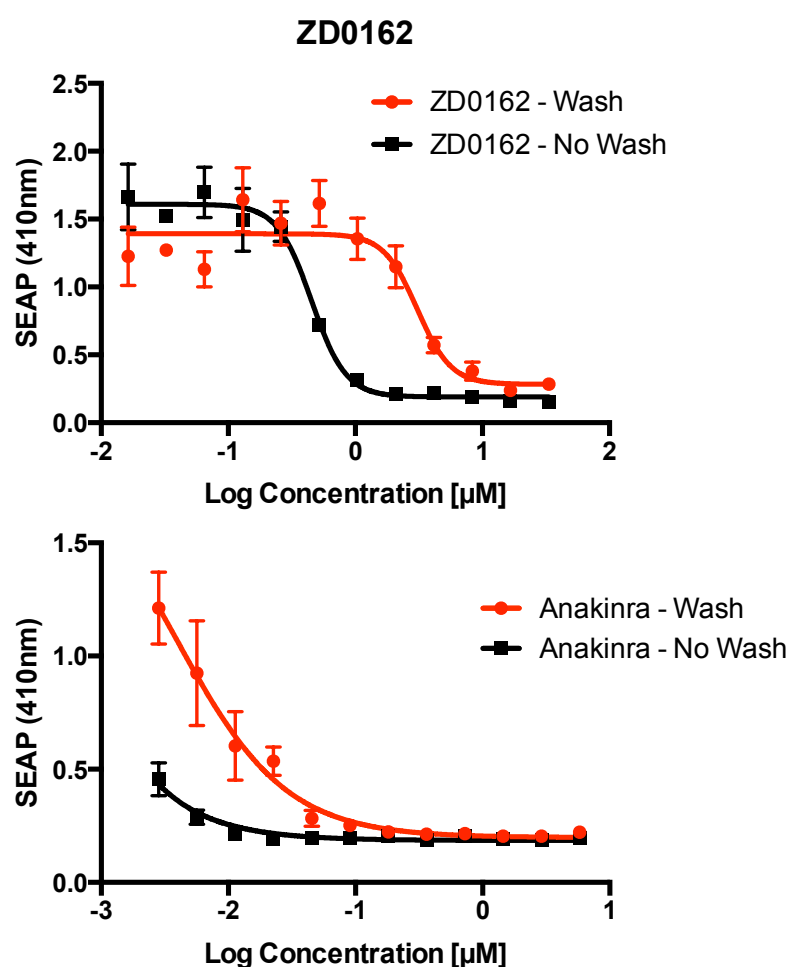


Figure 30: Wash-out experiment with ZD0162 and Anakinra. The samples were prepared with previously described conditions in Section B3. The medium of wash-out samples was renewed three times with fresh medium after 2 hours of incubation with ten minutes intervals. After 24 hours, the SEAP assay was done and the dose response curves were prepared with Graphpad 6.1.

8. Human IL1 β ELISA with ZD0130 and ZD0196

Previous experiments with HEK-Blue IL-1 β cell line demonstrated that several of the compounds could effectively inhibit IL1 β triggered activation of NF κ B mediated transcription (Section B4-B5). Moreover, short and long term MTS results showed that the inhibition of SEAP expression was not due to the toxicity which would reduce SEAP expression. However our experimental model could not distinguish if inhibition occurred directly through IL1R or something in the downstream activation pathway. The better characterize the mechanism of inhibition, we tested the effect of these compounds on toll-like receptor 2 (TLR2) mediated activation. Similar to IL1R this receptor utilizes a similar activation pathway mediated through (MyD88, IRAK, and TRAF). Therefore if the compound inhibits this pathway it is expected that TLR2 mediated activation will also be inhibited.

In these experiments the TLR2 receptor was activated with LPS to induce the release of IL1 β into the media. Secreted IL1 β protein was assayed by ELISA from supernatant of THP1 cell which treated with 1 μ g/ml LPS and ZD0130 or ZD0196. The addition variety of concentration of either ZD0130 (Figure 34) or ZD0196 (Figure 35) did not decrease the level of secreted IL1 β . Surprisingly, higher concentrations of ZD0130 actually increased the level of secreted IL1 β . As a possible explanation, the ELISA results were dependent on IL1 β secretion that could also be triggered with other mechanisms such as feedback loops. These results suggested that ZD0130 and ZD0196 did not inactivate any common downstream signaling elements between TLR2 pathway and IL1R pathway.

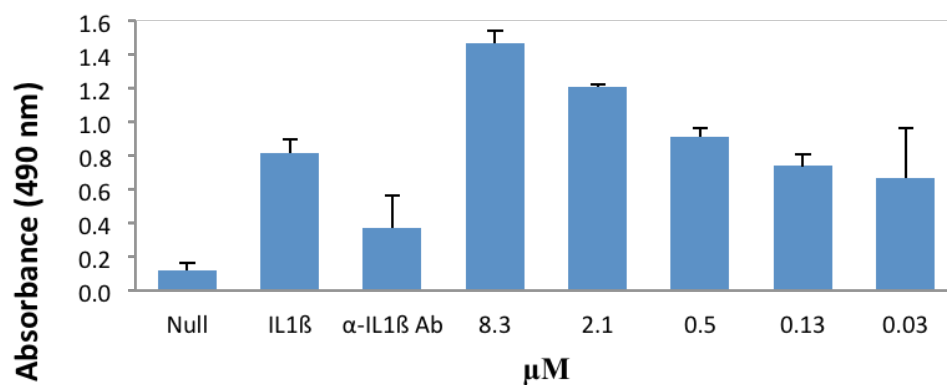


Figure 34: Human IL1 β ELISA with ZD0130. THP1 cells were incubated with 1 μ g/ml LPS with compound from 8 μ M to 30 nM for 20-24 hours in 37°C. Following incubation 50 μ l of supernatant was transferred into wells of ELISA plate and assayed as described (Chapter 2, Section 10).

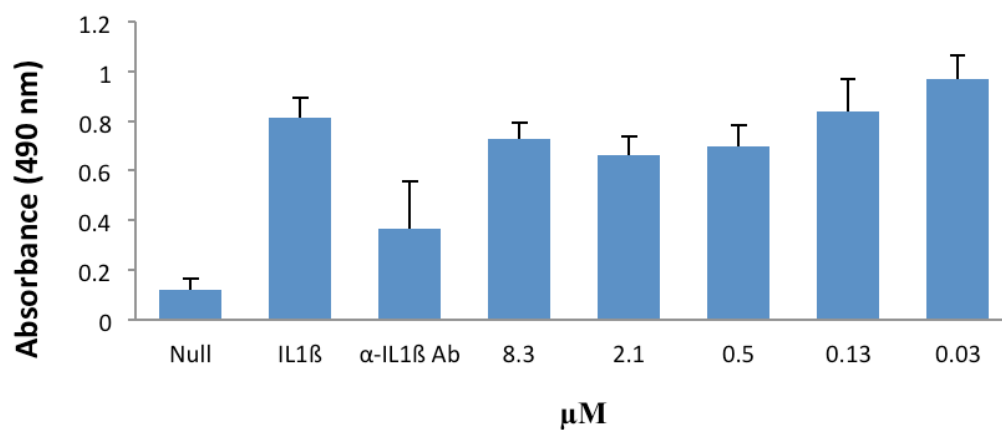


Figure 31: Human IL1 β ELISA with ZD0196. THP1 cells were incubated with 1 μ g/ml LPS with compound from 8 μ M to 30 nM for 20-24 hours in 37°C. 50 μ l of supernatant was transferred into wells of ELISA plate and assayed as described (Chapter 2, Section 10).

9. Potential binding sites of ZD0127 on IL1R

Following identification of the “hit” molecules, one of the most potent compounds (ZD0127) was docked throughout the crystal structure of IL1 β by Professor Burak Erman to identify a potential binding site on the IL1R (Figure 36). This was done with no *a priori* hypothesis and the compound allowed to bind anywhere on the protein. The best docking pose of ZD0127 in complex with IL1R is shown in Figure 36C. The location of binding closely matches previous X-ray crystal structure of a small antagonist peptide bound to the type-I IL1R (Figure 36B) as well as an essential region identified by site directed mutagenesis of IL1 β (Figure 36A).

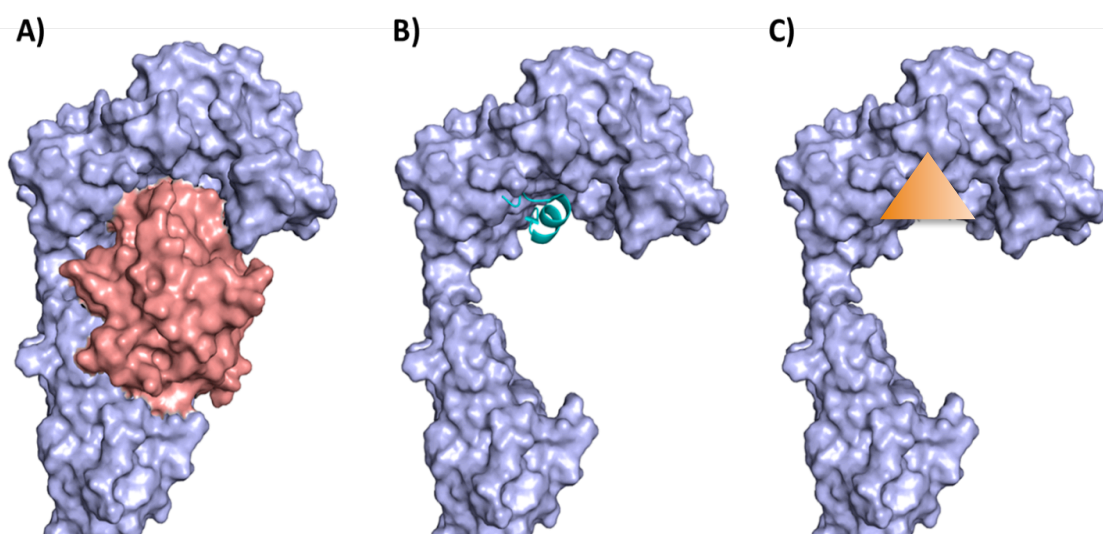


Figure 32: Predicted binding sites of ZD0127 on IL1R. Docking analysis proposed a potential binding site of our small molecule inhibitor (ZD0127) on the IL1R. Crystal structure of IL1R-1 with IL1 β ligand (A), of a small antagonist peptide bound to IL1R-1 (B) and of ZD0127 small molecule bound to IL1R-1 with the lowest binding energy (C).

Based off this predicted binding location, more than 90 compounds from the primary and the secondary screens (Section B3 and B4) were docked into the IL1R crystal structure by Professor Burak Erman. Interestingly, the compounds with the best experimental potency were calculated to be the most thermodynamically favored. Active compounds with $IC_{50} > 2 \mu M$ had a lower absolute free binding energies than those active compounds with an $IC_{50} < 2 \mu M$ (Figure 37).

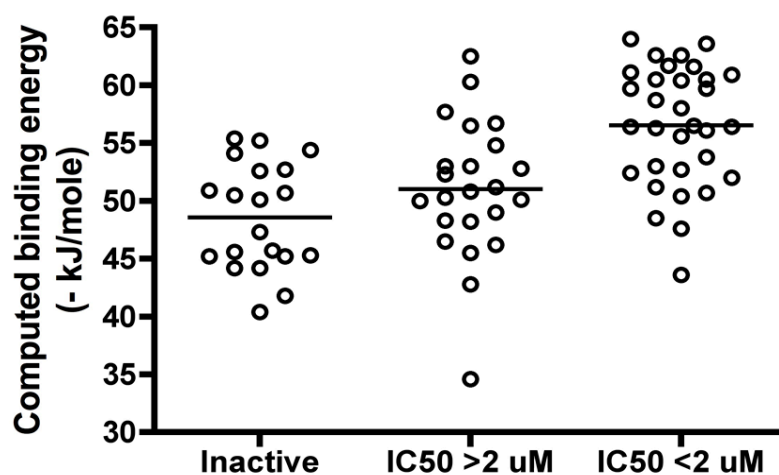


Figure 33: Docking of tested compounds into predicted cavity.

10. Mutagenesis analysis with HEK293t cells and the luciferase assay

To validate the binding pocket predicted from *in silico* docking we conducted scanning alanine mutagenesis. The 10 amino acids in the center of binding pocket of IL1R

were chosen including; lysine-129, glutamine-130, valine-141, cysteine-142, proline-143, tyrosine-144, methionine-145, glutamic acid-146, lysine-149, and asparagine-179 (Figure 38). These amino acids were mutated by site-directed mutagenesis into Alanine. The pcDNA4-IL1R plasmid was mutagenized with Site Directed Mutagenesis as previously described (Results Section 9).

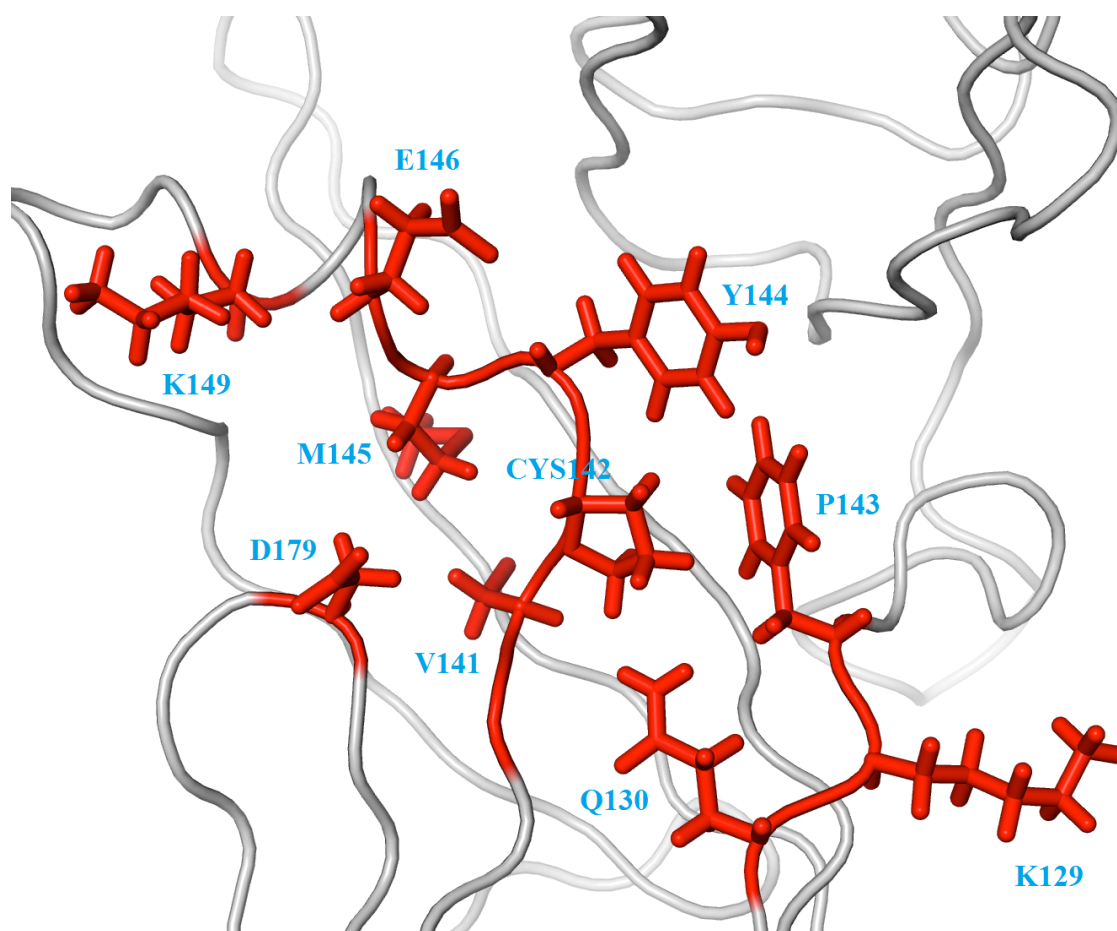


Figure 34: Predicted binding pocket of IL1R. The 10 critical amino acids are shown in red.

Following sequencing the mutated constructs were transfected into HEK 293t cells with a luciferase reporter to test for activity (Figure 39). F128A and V141A were found to totally inactivate IL1R and could not be used for further studies. However, nine of the mutations had sufficient activation to be tested with the IL1R small molecule inhibitors.

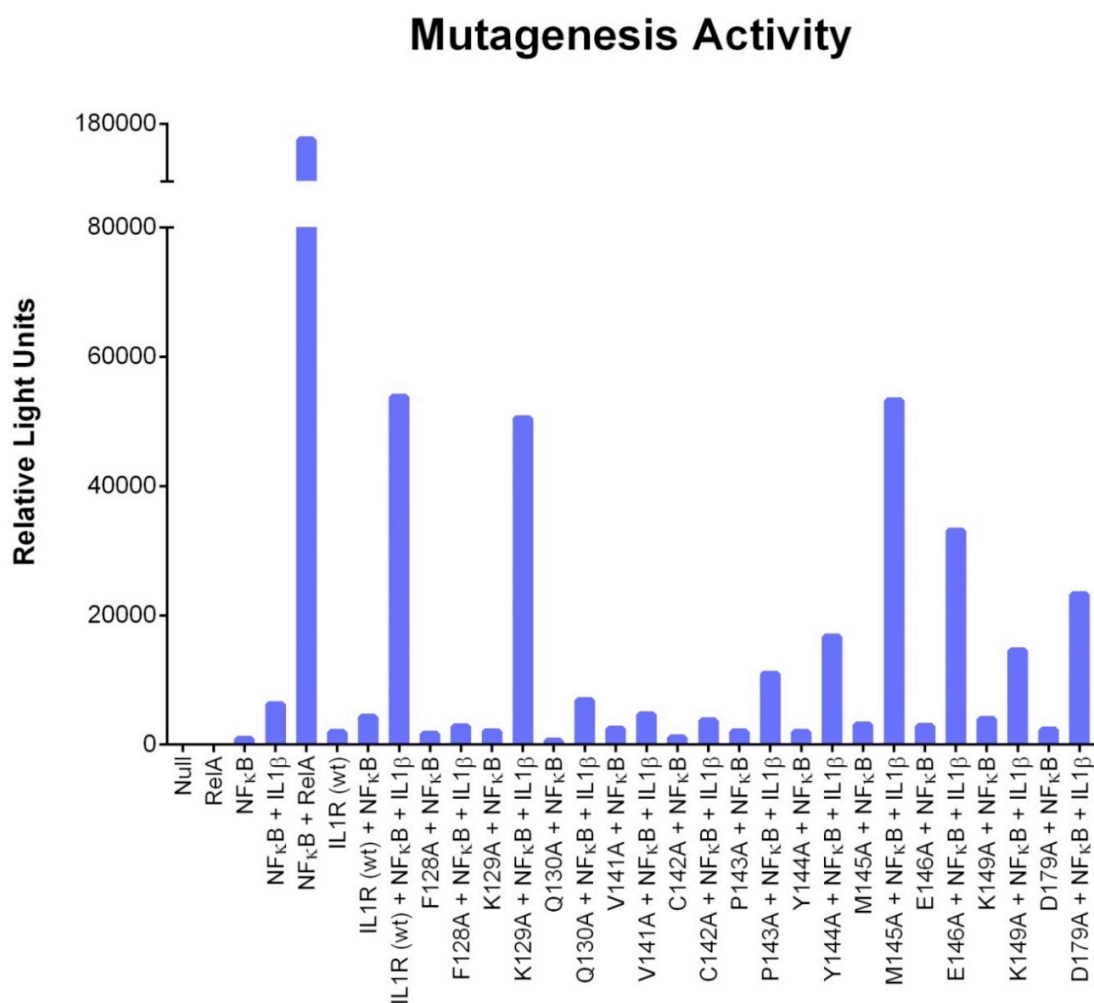
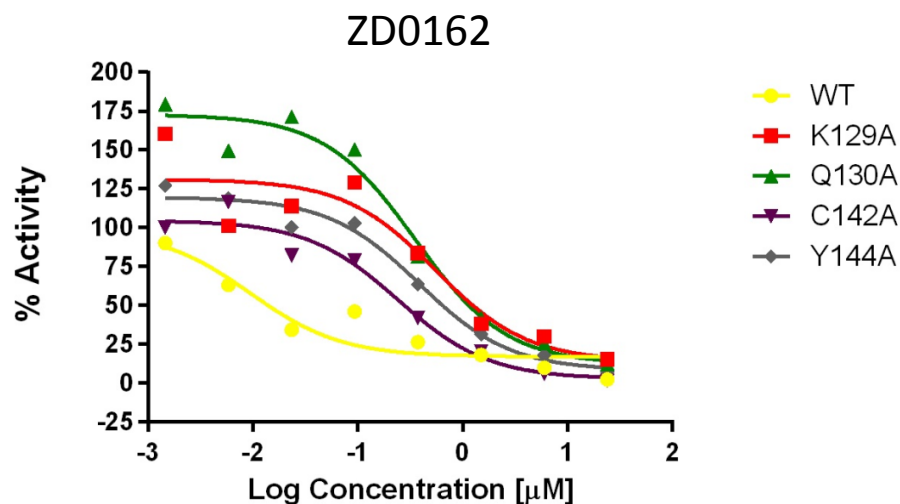


Figure 35: Mutagenesis analysis of IL1R. The 10 amino acids in the center of binding pocket of IL1R were mutagenized and the effect of each specific mutagenesis was assayed with HEK 293t cells and luciferase system.

Each mutated pcDNA4-IL1R plasmid was co-transfected with NF κ B-TA-LUC-UBC-GFP-W vector into HEK 293t cells and incubated with 1 ng/ml recombinant IL1 β and varying concentrations of ZD0162 or Anakinra. The wild type pcDNA4-IL1R plasmid was also used with same conditions as a control. ZD0162 was chosen because good IC₅₀ value (0.2 μ M). The assay was done as previously described (Results Section B-6). The first part of the screen revealed that the mutations did not affect the binding affinity of ZD0162 whose IC₅₀ value equals 0.2 μ M from SEAP assays. The IC₅₀ values of the mutations (K129A, Q130A, C142A and Y144A) for ZD0162 were 0.55, 0.35, 0.24 and 0.39 μ M respectively (Figure 40). On the other hand, the first half of the screen revealed that some of the mutations affected the binding affinity of Anakinra to IL1R. The IC₅₀ values of the mutations (K129A, Q130A, C142A and Y144A) for Anakinra were 21, 22, 0.6 and 5 μ M respectively. The IC₅₀ value of wild type IL1R for Anakinra was 23 nM in this screen (Figure 41). It is stated as 1-10nM in the literature.



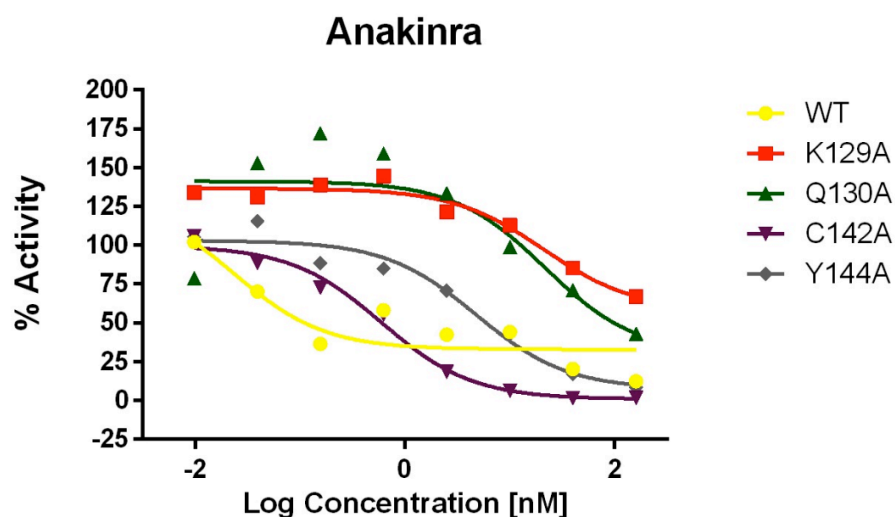


Figure 36: Dose-response curves of mutant IL1Rs. HEK 293t cells were co-transfected with NFkB-TA-LUC-UBC-GFP-W vector and pcDNA4-IL1R plasmids (WT, K129A, Q130A, C142A or Y144A). After incubation with 1 ng/ml recombinant IL1 β , the luciferase assay was performed previously described.

After these results the other mutants were experimented with the same way. The second part of the mutagenesis screen also supported that the mutations did not affect the binding affinity of ZD0162. The IC₅₀ values of ZD0162 with the mutations (P143A, M145A, E146A, K149A or D179A) were very similar to the IC₅₀ value of ZD0162 with wild type IL1R (Figure 41). All the values were between 1-200 nM. On the other hand, the second half of the screen revealed that some of the mutations affected the binding affinity of Anakinra to IL1R. The IC₅₀ values of the mutations (P143A, M145A, E146A, K149A or D179A) for Anakinra were 13, 5.5, 8, 18 and 10 nM respectively. However, the IC₅₀ value of wild type IL1R for Anakinra was also 9 nM that was literately stated. In brief, P143A,

M145A, E146A, K149A and D179A mutations did not affect the binding affinity of Anakinra to IL1R suggesting that these mutations did not play a critical role on the binding.

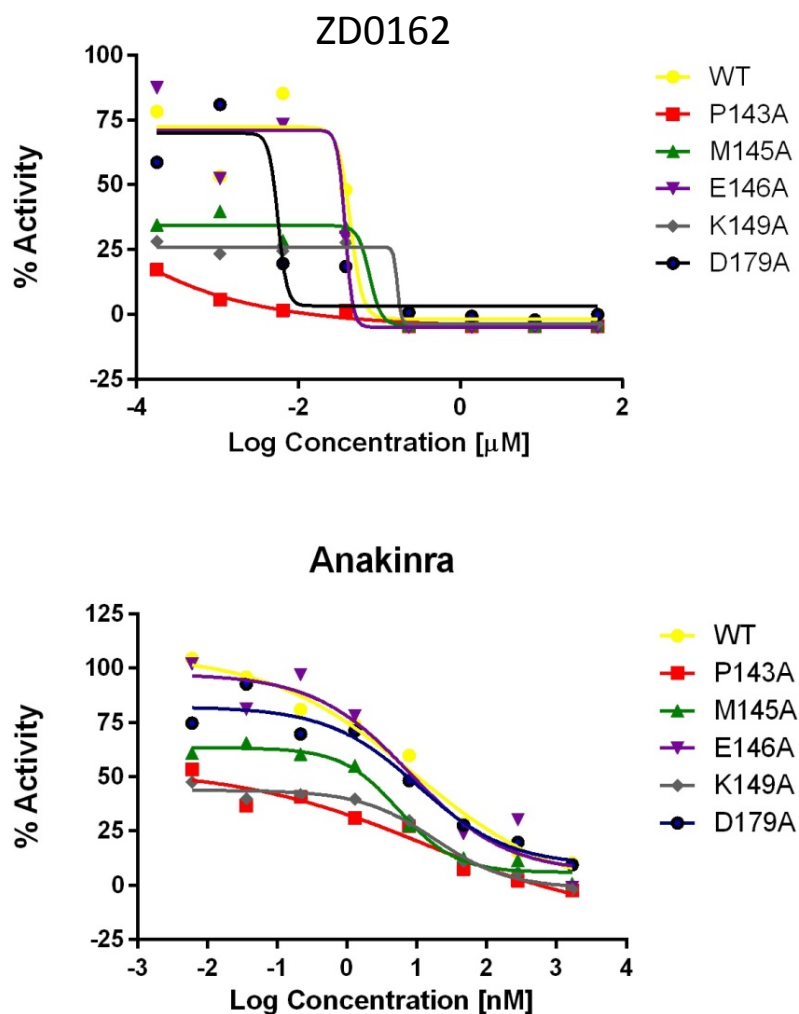


Figure 37: Dose-response curves of mutant IL1Rs. The HEK 293t cells were co-transfected with NFkB-TA-LUC-UBC-GFP-W vector and pcDNA4-IL1R plasmids (WT, P143A, M145A, E146A, K149A or D179A). After incubation with 1 ng/ml recombinant IL1 β , the luciferase assay was performed previously described.

11. SEAP and MTS assays with HEK-Blue-2 cells with *in silico* compounds

To confirm the specificity of these compounds (Section B3-4), the effects of the IL1R inhibitors on TLR2 inhibition was assayed with HEK-Blue-2 cells. The similarities between TLR2 pathway and IL1R pathway was described in Section B-8. Moreover, the HEK-Blue-2 cells and the HEK-Blue IL1 β cells share all the characteristics except the receptors on the cell surfaces. The cells were assayed with the same conditions as HEK-Blue IL1 β cells except these HEK-Blue-2 cells were stimulated with 1 mg/ml LPS instead of 1 ng/ml IL1 β . The most potent compounds (ZD0124, ZD0127, ZD0130, ZD0150, ZD0429 and ZD0430) were tested at multiple concentrations. Similar to previous work after SEAP assay, potential cytotoxicity was measured with MTS assay (Figure 42). Unfortunately these compounds inhibited the TLR2 pathway with the same level to IL1R cell line (Table 5). Anakinra was used as a control and as expected did not affect TLR2 activation (Figure 43).

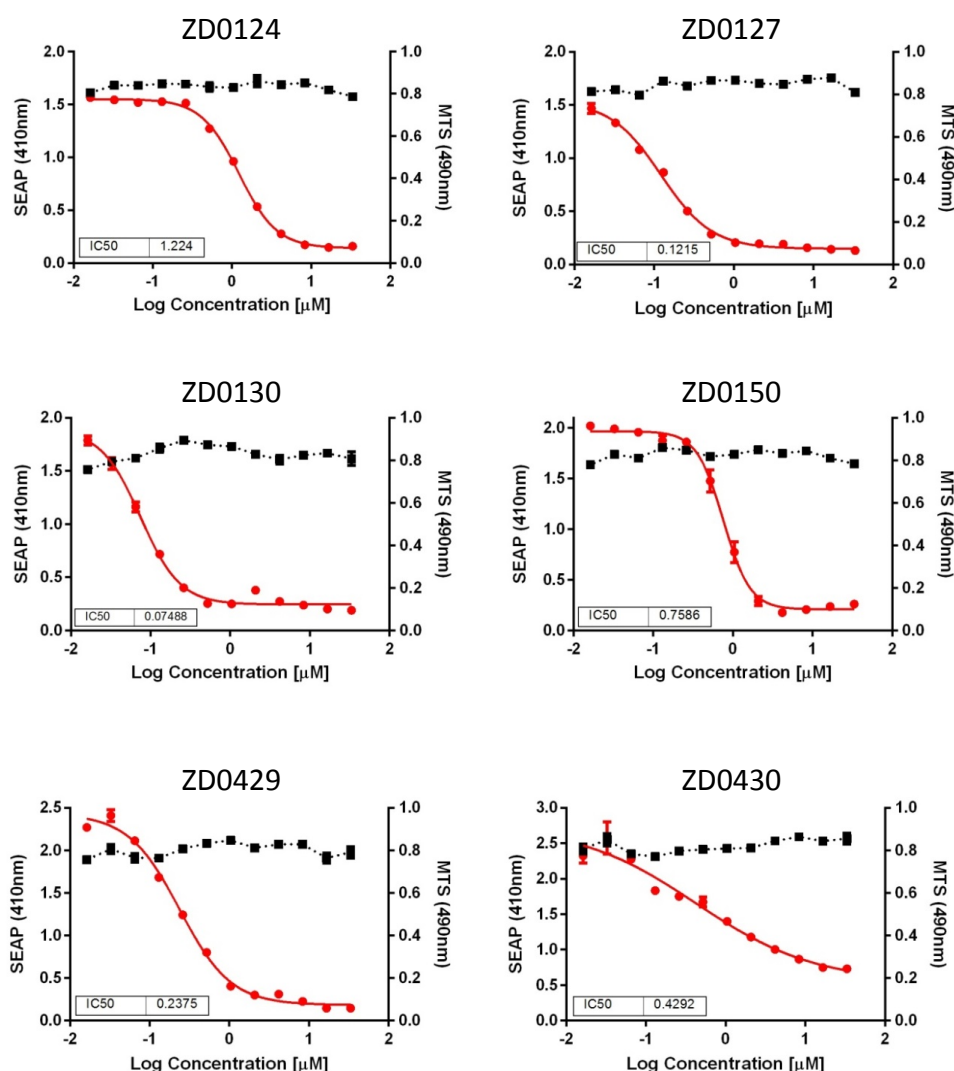


Figure 38: Dose-response curves of compounds with HEK-Blue-2 cells. Cells were stimulated with 1 mg/ml LPS in addition to compounds (10nM to 40 μM). After 20-24 hours incubation, SEAP assay was done previously described. TLR2 activity (SEAP assay) results are shown by red dots and lines. Viability (MTS) results are shown by black dots and dashed lines. IC_{50} values are shown at the left bottom corner of the figures.

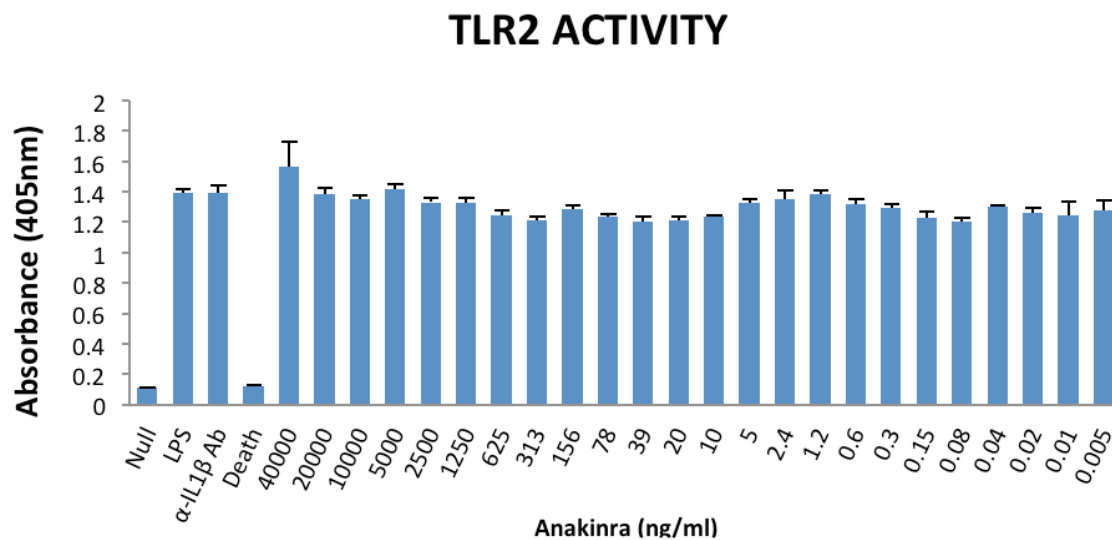


Figure 39: Dose-response curve of Anakinra with HEK-Blue-2 cells. Cells were stimulated with 1 mg/ml LPS in addition to Anakinra (5 pM to 40 μ M). After 20-24 hours incubation, SEAP assay was done as previously described.

Compound ID	IL1R Inhibition (IC ₅₀ in nM)	TLR2 Inhibition (IC ₅₀ in nM)
ZD0124	740	1224
ZD0127	88	121
ZD0130	115	75
ZD0150	1000	760
ZD0429	400	238
ZD0430	410	430
Anakinra	2	>40000

Table 5: The comparison of IC₅₀ values of compounds on the inhibition of IL1R and TLR2.

Given then similar level of inhibition between these two models, there two possible explanations. First, it is possible that these small molecule inhibitors affect both of the IL1R and TLR2 due to their structural similarities. However, this seems unlikely due to both the similar potency of these molecules as well as the results from our mutagenesis screen (Section 10). More likely, it is possible that these compounds inhibit some other protein/s in the down signaling. However, this did not explain our results with THP1 cells (Section 8). According to results of ELISA, ZD0130 and ZD0196 did not inactivate any common downstream signaling elements between TLR2 pathway and IL1R pathway because they did not decrease the secreted levels of LPS-induced IL1 β . As a possible explanation, THP1 cells secreted IL1 β via another mechanism because both mutagenesis screen and SEAP assay for TLR2 activation used highly similar cells (respectively HEK 293t cells and HEK-Blue-2 cells) to HEK-Blue IL1 β cells.

12. Analysis for off target effect of the small molecule inhibitors

To confirm that the effect of the compounds was not due to non-specific protein synthesis inhibition HEK 293t cells were transiently transfected a constitutively active GFP plasmid and increasing concentrations of ZD0162. After 48 hours of incubation with ZD0162, the GFP expressing HEK 293t cells were counted with a flow cytometer. The expression of GFP protein was identical between all the concentrations of ZD0162 and null control (Figure 44). These findings suggest that the compounds did not no-specifically inhibit protein transcription.

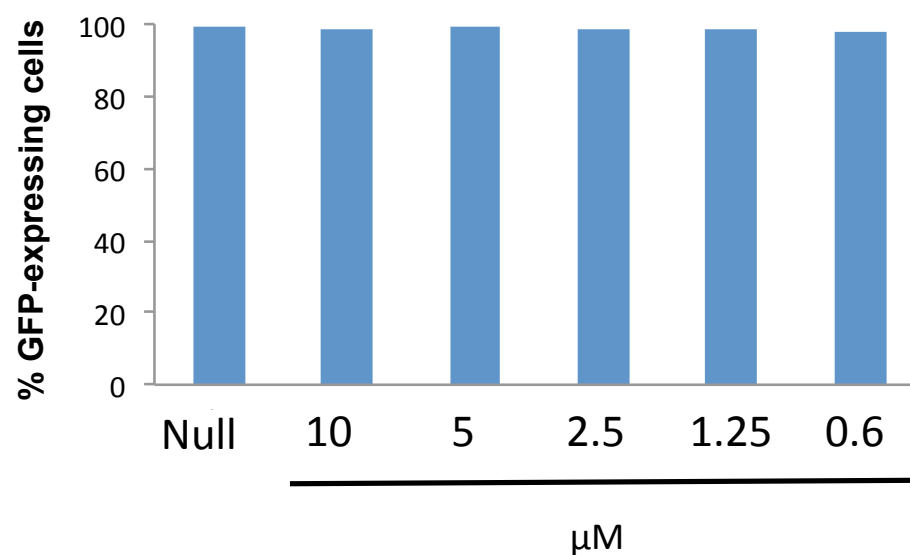


Figure 40: Transiently GFP-transfected HEK 293t cells incubated with varying concentrations of ZD0162. After transfection, the cells incubated with ZD0162 for 2 days. The GFP expressing cells were counted with a flow cytometer.

CHAPTER 4

DISCUSSION

There is a clinical need for effective small molecule inhibitors that can treat autoinflammatory disorders. While these diseases are complex, IL1R mediated activation of caspase-1 [48], [49] is a common driver of many diseases including Behcet's Disease [5], [6] and Familial Mediterranean fever (FMF) [20]. IL-1 blocking therapies [60], [61] therefore showed success against Cryopyrin-Associated Autoinflammatory Syndromes (CHAPS) [97], [112] and for the decrease of symptoms related with deficiency of the IL-1 receptor antagonist (DIRA) [82]. However, while there are a number of effective IL1 pathway inhibitors, almost all therapeutics are recombinant proteins and therefore very expensive to produce and generally have poor pharmacokinetics.

With these limitations the goal of this work was to identify novel small molecule inhibitors that inhibit IL1R or caspase-1. Based on previous work we first focused on caspase-1 inhibitors. A series of *in silico* derivatives were tested against active *in vitro* caspase-1. Only the compound ZD0002 had an IC_{50} value less than 10 μ M. Despite additional optimization, further derivatives did not show improved activity. Concurrent to these studies we analyzed colchicine and colchicine derivatives for caspase-1 inhibition. Colchicine is used to treat some IL1 mediated autoinflammatory diseases including gout [86] and Behcet's diseases [13] and thus believed to decrease the inflammasome activation [102]. Neither colchicine nor its derivatives showed significant inhibition for recombinant active caspase-1 *in vitro*.

We hypothesized that colchicine worked on the inactive polymerized form and therefore developed cellular detection system in THP1 cells. We found that Colchicine and

its derivatives (except 2820005-NX1) increased the level of active caspase-1 with either LPS/ATP stimulated or MSU stimulated inflammasomes. Because Colchicine is a toxic secondary metabolite [110], we wanted to show that the results were not due to high concentration of the compounds. A dose response analysis with Colchicine revealed that the higher concentrations of colchicine significantly and only the lowest concentration (8 nM) did not activate caspase-1. However, colchicine and its derivatives could not effectively inhibit caspase-1 at the concentrations tested. Our results suggest that Colchicine did not inhibit the uncleaved form or polymerization of caspase-1; conversely it increased the level of caspase-1 polymerization, thus resulted in the more activation of caspase-1. The results of decreasing concentrations of Colchicine probably indicated that it was due to toxic characteristic of it. A toxic compound could trigger immune system. Moreover the percentage of live cells was lower with addition of Colchicine in comparison to null controls, suggesting that Colchicine stimulation drove cells to cell death via apoptosis or pyroptosis. Our results showed Colchicine somehow increased caspase-1 activation; therefore, the over-activation of caspase-1 could result in pyroptosis [113].

To test for inhibitors of IL1R [60], [61], we initially screened 1280 FDA approved compounds in the Prestwick Library to inhibit SEAP production. Among all, only eight of them gave results for further analysis with dose response curves. However, the further analysis showed that none of the compounds in the Prestwick Library inhibited the IL1R pathway without toxicity. The unsuccess of this screen shows why there is no a known small molecule inhibitor for IL1R which could have a highly specific binding pocket to penetrate for inhibition.

Building on the methodologies developed, we initially tested a small series of compounds in an IL1R cell based assay. Interestingly, several potent inhibitors were identified. To increase activity and develop a SAR model, an additional 283 derivatives

were synthesized and tested. Several compounds were identified with a sub-nanomolar IC₅₀ and little cytotoxicity or decrease in cellular proliferation. Moreover, our work suggested that compound inhibition was reversible [114]. Further, the activity observed was not related to the colourmetric SEAP assay, as the compounds were also effective in a luciferase counter screen. In THP1 cells, our assay suggested that the compounds did not inactivate any common downstream signaling elements (MyD88, IRAK, and TRAF etc) between TLR2 pathway and IL1R pathway. Based on these results we then docked the compound throughout the IL1R to identify a potential binding site. After mutagenesis analysis of critical amino acid residues in the potential binding site, we showed that the mutations did not affect the binding affinity of ZD0162 but some of the mutations (K129A, Q130A, and Y144A) affected the binding affinity of Anakinra to IL1R. These results validated that Anakinra was highly specific to IL1R [115]; however suggested that our small molecules were not specific to IL1R. On the other hand other mutations were for neutral for the affinity of Anakinra (C142A, P143A, M145A, E146A, K149A or D179A). The results of mutagenesis screen and ELISA experiment contradicted.

To solve the contradiction between our ELISA results and the mutagenesis, we tested the compounds effect of TLR2 activation. We showed that our compounds inhibited the TLR2 pathway to a similar level as IL1R while Anakinra only affected IL1R activation. The results of ELISA were dependent on the release of IL1 β from THP1 cells. As a possible explanation, THP1 cells secreted IL1 β via another mechanism [45] because both mutagenesis screen and SEAP assay for TLR2 activation used highly similar cells (respectively HEK 293t cells and HEK-Blue-2 cells) to HEK-Blue IL1 β cells. Therefore, we proposed that our small molecule inhibitors inhibited some other protein/s in the down signaling (MyD88, IRAK, and TRAF etc) [61], [64].

For future studies, we are firstly going to try to characterize this/these protein/s. We are going to utilize the kinase activities of these intermediate relay with the help of phosphorylation specific antibodies which bind only the phosphorylated form of a protein [116]. Moreover, mutagenesis analyses of each specific intermediate relay can also be done as a counter analysis.

Appendix I – Prestwick Chemical Library

Prestwick Name	Chemical name	Chemical structure	MW (g/mol)	Therapeutic class
01A02	Azaguanine-8	C ₄ H ₄ N ₆ O	152.12	Oncology
01A03	Allantoin	C ₄ H ₆ N ₄ O ₃	158.12	Dermatology
01A04	Acetazolamide	C ₄ H ₆ N ₄ O ₃ S ₂	222.25	Metabolism
01A05	Metformin hydrochloride	C ₄ H ₁₂ CIN ₅	165.63	Endocrinology
01A06	Atracurium besylate	C ₆₅ H ₈₂ N ₂ O ₁₈ S ₂	1243.51	Neuromuscular
01A07	Isoflupredone acetate	C ₂₃ H ₂₉ FO ₆	420.48	Endocrinology
01A08	Amiloride hydrochloride dihydrate	C ₆ H ₁₃ Cl ₂ N ₇ O ₃	302.12	Metabolism
01A09	Amprolium hydrochloride	C ₁₄ H ₂₀ Cl ₂ N ₄	315.25	Infectiology
01A10	Hydrochlorothiazide	C ₇ H ₈ CIN ₃ O ₄ S ₂	297.74	Metabolism
01A11	Sulfaguanidine	C ₇ H ₁₀ N ₄ O ₂ S	214.25	Infectiology, Metabolism
01B02	Meticrane	C ₁₀ H ₁₃ NO ₄ S ₂	275.35	Metabolism
01B03	Benzonatate	C ₃₀ H ₅₃ NO ₁₁	603.76	Neuromuscular
01B04	Hydroflumethiazide	C ₈ H ₈ F ₃ N ₃ O ₄ S ₂	331.29	Metabolism
01B05	Sulfacetamide sodic hydrate	C ₈ H ₁₁ N ₂ NaO ₄ S	254.24	Infectiology, Metabolism
01B06	Heptaminol hydrochloride	C ₈ H ₂₀ CINO	181.71	Cardiovascular
01B07	Sulfathiazole	C ₉ H ₉ N ₃ O ₂ S ₂	255.32	Infectiology, Metabolism
01B08	Levodopa	C ₉ H ₁₁ NO ₄	197.19	Central Nervous System
01B09	Idoxuridine	C ₉ H ₁₁ IN ₂ O ₅	354.10	Infectiology
01B10	Captopril	C ₉ H ₁₅ NO ₃ S	217.29	Cardiovascular
01B11	Minoxidil	C ₉ H ₁₅ N ₅ O	209.25	Cardiovascular
01C02	Sulfaphenazole	C ₁₅ H ₁₄ N ₄ O ₂ S	314.37	Infectiology, Metabolism
01C03	Panthenol (D)	C ₉ H ₁₉ NO ₄	205.26	Metabolism
01C04	Sulfadiazine	C ₁₀ H ₁₀ N ₄ O ₂ S	250.28	Infectiology, Metabolism
01C05	Norethynodrel	C ₂₀ H ₂₆ O ₂	298.43	Endocrinology
01C06	Thiamphenicol	C ₁₂ H ₁₅ Cl ₂ NO ₅ S	356.23	Infectiology, Metabolism
01C07	Cimetidine	C ₁₀ H ₁₆ N ₆ S	252.34	Gastroenterology
01C08	Doxylamine succinate	C ₂₁ H ₂₈ N ₂ O ₅	388.47	Central Nervous System
01C09	Ethambutol dihydrochloride	C ₁₀ H ₂₆ Cl ₂ N ₂ O ₂	277.24	Infectiology
01C10	Antipyrine	C ₁₁ H ₁₂ N ₂ O	188.23	Central Nervous System
01C11	Antipyrine, 4-hydroxy	C ₁₁ H ₁₂ N ₂ O ₂	204.23	Metabolism
01D02	Chloramphenicol	C ₁₁ H ₁₂ Cl ₂ N ₂ O ₅	323.13	Infectiology, Metabolism
01D03	Epirizole	C ₁₁ H ₁₄ N ₄ O ₂	234.26	Central Nervous System
01D04	Diprophylline	C ₁₀ H ₁₄ N ₄ O ₄	254.25	Cardiovascular, Respiratory
01D05	Triamterene	C ₁₂ H ₁₁ N ₇	253.27	Metabolism
01D06	Dapsone	C ₁₂ H ₁₂ N ₂ O ₂ S	248.31	Infectiology
01D07	Troleandomycin	C ₄₁ H ₆₇ NO ₁₅	813.99	Infectiology

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01D08	Pyrimethamine	C12H13ClN4	248.72	Infectiology
01D09	Hexamethonium dibromide dihydrate	C12H34Br2N2O2	398.22	Cardiovascular
01D10	Diflunisal	C13H8F2O3	250.20	Central Nervous System
01D11	Niclosamide	C13H8Cl2N2O4	327.13	Infectiology
01E02	Procaine hydrochloride	C13H21ClN2O2	272.78	Neuromuscular
01E03	Moxisylyte hydrochloride	C16H26ClNO3	315.84	Cardiovascular
01E04	Betazole hydrochloride	C5H11Cl2N3	184.07	Gastroenterology
01E05	Isoxicam	C14H13N3O5S	335.34	Central Nervous System
01E06	Naproxen	C14H14O3	230.27	Central Nervous System
01E07	Naphazoline hydrochloride	C14H15ClN2	246.74	Cardiovascular
01E08	Ticlopidine hydrochloride	C14H15Cl2NS	300.25	Hematology
01E09	Dicyclomine hydrochloride	C19H36ClNO2	345.96	Gastroenterology
01E10	Amyleine hydrochloride	C14H22ClNO2	271.79	Neuromuscular
01E11	Lidocaine hydrochloride	C14H23ClN2O	270.81	Cardiovascular
01F02	Trichlorfon	C4H8Cl3O4P	257.44	Infectiology
01F03	Carbamazepine	C15H12N2O	236.28	Central Nervous System
01F04	Triflupromazine hydrochloride	C18H20ClF3N2S	388.89	Central Nervous System
01F05	Mefenamic acid	C15H15NO2	241.29	Central Nervous System
01F06	Acetohexamide	C15H20N2O4S	324.40	Endocrinology
01F07	Sulpiride	C15H23N3O4S	341.43	Central Nervous System
01F08	Benoxinate hydrochloride	C17H29ClN2O3	344.89	Neuromuscular
01F09	Oxethazaine	C28H41N3O3	467.66	Neuromuscular
01F10	Pheniramine maleate	C20H24N2O4	356.43	Allergology
01F11	Tolazoline hydrochloride	C10H13ClN2	196.68	Cardiovascular
01G02	Morantel tartrate	C16H22N2O6S	370.43	Infectiology
01G03	Homatropine hydrobromide (R,S)	C16H22BrNO3	356.26	Diagnostic, Ophthalmology
01G04	Nifedipine	C17H18N2O6	346.34	Cardiovascular
01G05	Chlorpromazine hydrochloride	C17H20Cl2N2S	355.33	Cardiovascular
01G06	Diphenhydramine hydrochloride	C17H22ClNO	291.82	Allergology
01G07	Minaprine dihydrochloride	C17H24Cl2N4O	371.31	Central Nervous System
01G08	Miconazole	C18H14Cl4N2O	416.14	Infectiology, Metabolism
01G09	Isoxsuprine hydrochloride	C18H24ClNO3	337.85	Cardiovascular
01G10	Acebutolol hydrochloride	C18H29ClN2O4	372.90	Cardiovascular
01G11	Tolnaftate	C19H17NOS	307.42	Infectiology
01H02	Todralazine hydrochloride	C11H13ClN4O2	268.70	Cardiovascular
01H03	Imipramine hydrochloride	C19H25ClN2	316.88	Central Nervous System
01H04	Sulindac	C20H17FO3S	356.42	Central Nervous System
01H05	Amitriptyline hydrochloride	C20H24ClN	313.87	Central Nervous System
01H06	Adiphenine hydrochloride	C20H26ClNO2	347.89	Neuromuscular
01H07	Dibucaine	C20H29N3O2	343.47	Neuromuscular
01H08	Prednisone	C21H26O5	358.44	Endocrinology

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01H09	Thioridazine hydrochloride	C21H27ClN2S2	407.04	Central Nervous System
01H10	Diphenamil methylsulfate	C21H27NO4S	389.52	Gastroenterology
01H11	Trimethobenzamide hydrochloride	C21H29ClN2O5	424.93	Central Nervous System
02A02	Metronidazole	C6H9N3O3	171.16	Infectiology, Metabolism
02A03	Fulvestrant	C32H47F5O3S	606.79	Endocrinology, Oncology
02A04	Edrophonium chloride	C10H16ClNO	201.70	Diagnostic, Neuromuscular
02A05	Moroxidine hydrochloride	C6H14ClN5O	207.66	Infectiology
02A06	Baclofen (R,S)	C10H12ClNO2	213.67	Central Nervous System
02A07	Acyclovir	C8H11N5O3	225.21	Metabolism
02A08	Diazoxide	C8H7ClN2O2S	230.67	Cardiovascular
02A09	Amidopyrine	C13H17N3O	231.30	Central Nervous System
02A10	Busulfan	C6H14O6S2	246.30	Oncology
02A11	Pindolol	C14H20N2O2	248.33	Cardiovascular
02B02	Khellin	C14H12O5	260.25	Cardiovascular
02B03	Zimelidine dihydrochloride monohydrate	C16H21BrCl2N2O	408.17	Central Nervous System
02B04	Azacyclonol	C18H21NO	267.37	Central Nervous System
02B05	Azathioprine	C9H7N7O2S	277.27	Oncology
02B06	Lynestrenol	C20H28O	284.45	Endocrinology
02B07	Guanabenz acetate	C10H12Cl2N4O2	291.14	Central Nervous System
02B08	Disulfiram	C10H20N2S4	296.54	Metabolism
02B09	Acetylsalicylsalicylic acid	C16H12O6	300.27	Central Nervous System
02B10	Mianserine hydrochloride	C18H21ClN2	300.83	Cardiovascular
02B11	Nocodazole	C14H11N3O3S	301.33	Oncology
02C02	R(-) Apomorphine hydrochloride hemihydrate	C34H38Cl2N2O5	625.60	Central Nervous System
02C03	Amoxapine	C17H16ClN3O	313.79	Central Nervous System
02C04	Cyproheptadine hydrochloride	C21H22ClN	323.87	Allergology, Central Nervous System
02C05	Famotidine	C8H15N7O2S3	337.45	Gastroenterology
02C06	Danazol	C22H27NO2	337.47	Endocrinology
02C07	Nicorandil	C8H9N3O4	211.18	Cardiovascular
02C08	Pioglitazone	C19H20N2O3S	356.45	Endocrinology
02C09	Nomifensine maleate	C20H22N2O4	354.41	Central Nervous System
02C10	Dizocilpine maleate	C20H19NO4	337.38	Central Nervous System
02C11	Oxandrolone	C19H30O3	306.45	Endocrinology
02D02	Naloxone hydrochloride	C19H22ClNO4	363.84	Central Nervous System
02D03	Metolazone	C16H16ClN3O3S	365.84	Cardiovascular
02D04	Ciprofloxacin hydrochloride monohydrate	C17H21ClFN3O4	385.83	Infectiology, Metabolism
02D05	Ampicillin trihydrate	C16H25N3O7S	403.46	Infectiology, Metabolism

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02D06	Haloperidol	C21H23ClFNO2	375.87	Central Nervous System
02D07	Naltrexone hydrochloride dihydrate	C20H28ClNO6	413.90	Central Nervous System
02D08	Chlorpheniramine maleate	C20H23ClN2O4	390.87	Allergology, Central Nervous System
02D09	Nalbuphine hydrochloride	C21H28ClNO4	393.91	Central Nervous System
02D10	Picotamide monohydrate	C21H22N4O4	394.43	Hematology
02D11	Triamcinolone	C21H27FO6	394.44	Endocrinology
02E02	Bromocryptine mesylate	C33H44BrN5O8S	750.72	Central Nervous System
02E03	Amfepramone hydrochloride	C13H20ClNO	241.76	Central Nervous System
02E04	Dehydrocholic acid	C24H34O5	402.54	Gastroenterology
02E05	Tioconazole	C16H13Cl3N2OS	387.72	Infectiology
02E06	Perphenazine	C21H26ClN3OS	403.98	Central Nervous System
02E07	Mefloquine hydrochloride	C17H17ClF6N2O	414.78	Infectiology
02E08	Isoconazole	C18H14Cl4N2O	416.14	Infectiology
02E09	Spirolactone	C24H32O4S	416.58	Endocrinology
02E10	Pirenzepine dihydrochloride	C19H23Cl2N5O2	424.33	Gastroenterology
02E11	Dexamethasone acetate	C24H31FO6	434.51	Endocrinology
02F02	Glipizide	C21H27N5O4S	445.54	Endocrinology
02F03	Loxapine succinate	C22H24ClN3O5	445.91	Central Nervous System
02F04	Hydroxyzine dihydrochloride	C21H29Cl3N2O2	447.84	Allergology
02F05	Diltiazem hydrochloride	C22H27ClN2O4S	450.99	Cardiovascular
02F06	Methotrexate	C20H22N8O5	454.45	Oncology
02F07	Astemizole	C28H31FN4O	458.58	Allergology
02F08	Clindamycin hydrochloride	C18H34Cl2N2O5S	461.45	Infectiology
02F09	Terfenadine	C32H41NO2	471.69	Allergology
02F10	Cefotaxime sodium salt	C16H16N5NaO7S2	477.45	Infectiology
02F11	Tetracycline hydrochloride	C22H25ClN2O8	480.91	Infectiology
02G02	Verapamil hydrochloride	C27H39ClN2O4	491.08	Cardiovascular
02G03	Dipyridamole	C24H40N8O4	504.64	Cardiovascular
02G04	Chlorhexidine	C22H30Cl2N10	505.46	Infectiology
02G05	Loperamide hydrochloride	C29H34Cl2N2O2	513.51	Gastroenterology
02G06	Chlortetracycline hydrochloride	C22H24Cl2N2O8	515.35	Infectiology
02G07	Tamoxifen citrate	C32H37NO8	563.65	Endocrinology, Oncology
02G08	Nicergoline	C24H26BrN3O3	484.40	Cardiovascular
02G09	Canrenoic acid potassium salt	C22H29KO4	396.58	Endocrinology
02G10	Thiopropazine dimesylate	C24H38N4O8S4	638.85	Central Nervous System
02G11	Dihydroergotamine tartrate	C70H80N10O16	1317.48	Central Nervous System
02H02	Erythromycin	C37H67NO13	733.95	Infectiology
02H03	Chloroxine	C9H5Cl2NO	214.05	Dermatology
02H04	Didanosine	C10H12N4O3	236.23	Infectiology
02H05	Josamycin	C42H69NO15	828.02	Infectiology

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02H06	Paclitaxel	C47H51NO14	853.93	Oncology
02H07	Ivermectin	C48H74O14	875.12	Infectiology
02H08	Gallamine triethiodide	C30H60I3N3O3	891.54	Neuromuscular
02H09	Neomycin sulfate	C23H48N6O17S	712.73	Infectiology
02H10	Dihydrostreptomycin sulfate	C42H88N14O36S3	1461.43	Infectiology
02H11	Gentamicine sulfate	C60H125N15O25S	1488.81	Infectiology
03A02	Isoniazid	C6H7N3O	137.14	Infectiology
03A03	Pentylenetetrazole	C6H10N4	138.17	Central Nervous System
03A04	Chlorzoxazone	C7H4ClNO2	169.57	Central Nervous System
03A05	Ornidazole	C7H10ClN3O3	219.63	Infectiology
03A06	Ethosuximide	C7H11NO2	141.17	Central Nervous System
03A07	Mafenide hydrochloride	C7H11ClN2O2S	222.69	Infectiology
03A08	Riluzole hydrochloride	C8H6ClF3N2OS	270.66	Central Nervous System
03A09	Nitrofurantoin	C8H6N4O5	238.16	Infectiology
03A10	Hydralazine hydrochloride	C8H9ClN4	196.64	Cardiovascular
03A11	Phenelzine sulfate	C8H14N2O4S	234.28	Central Nervous System
03B02	Tranexamic acid	C8H15NO2	157.21	Hematology
03B03	Etofylline	C9H12N4O3	224.22	Cardiovascular
03B04	Tranlycypromine hydrochloride	C9H12ClN	169.66	Central Nervous System
03B05	Alverine citrate salt	C26H35NO7	473.57	Neuromuscular
03B06	Aceclofenac	C16H13Cl2NO4	354.19	Central Nervous System
03B07	Iproniazide phosphate	C9H16N3O5P	277.22	Cardiovascular
03B08	Sulfamethoxazole	C10H11N3O3S	253.28	Infectiology
03B09	Mephesisin	C10H14O3	182.22	Central Nervous System
03B10	Phenformin hydrochloride	C10H16ClN5	241.73	Endocrinology
03B11	Flutamide	C11H11F3N2O3	276.22	Oncology
03C02	Ampyrone	C11H13N3O	203.25	Central Nervous System
03C03	Levamisole hydrochloride	C11H13ClN2S	240.76	Immunology
03C04	Pargyline hydrochloride	C11H14ClN	195.69	Cardiovascular
03C05	Methocarbamol	C11H15NO5	241.25	Central Nervous System
03C06	Aztreonam	C13H17N5O8S2	435.44	Infectiology
03C07	Cloxacillin sodium salt	C19H17ClN3NaO5S	457.87	Infectiology
03C08	Catharanthine	C21H24N2O2	336.44	Oncology
03C09	Pentolinium bitartrate	C23H42N2O12	538.60	Cardiovascular
03C10	Aminopurine, 6-benzyl	C12H11N5	225.25	Endocrinology
03C11	Tolbutamide	C12H18N2O3S	270.35	Endocrinology
03D02	Midodrine hydrochloride	C12H19ClN2O4	290.75	Cardiovascular
03D03	Thalidomide	C13H10N2O4	258.24	Central Nervous System
03D04	Oxolinic acid	C13H11NO5	261.24	Metabolism
03D05	Nimesulide	C13H12N2O5S	308.31	Metabolism
03D06	Asenapine maleate	C21H20ClNO5	401.85	Central Nervous System

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03D07	Pentoxifylline	C13H18N4O3	278.31	Cardiovascular
03D08	Metaraminol bitartrate	C17H25NO14	467.39	Cardiovascular
03D09	Salbutamol	C13H21NO3	239.32	Neuromuscular
03D10	Prilocaine hydrochloride	C13H21ClN2O	256.78	Neuromuscular
03D11	Camptothecine (S,+)	C20H16N2O4	348.36	Oncology
03E02	Ranitidine hydrochloride	C13H23ClN4O3S	350.87	Gastroenterology
03E03	Tiratricol, 3,3',5-triiodothyroacetic acid	C14H9I3O4	621.94	Endocrinology
03E04	Flufenamic acid	C14H10F3NO2	281.24	Central Nervous System
03E05	Flumequine	C14H12FNO3	261.26	Infectiology
03E06	Tolfenamic acid	C14H12ClNO2	261.71	Central Nervous System
03E07	Meclofenamic acid sodium salt monohydrate	C14H12Cl2NNaO3	336.15	Central Nervous System
03E08	Tibolone	C21H28O2	312.46	Endocrinology
03E09	Trimethoprim	C14H18N4O3	290.32	Infectiology
03E10	Metoclopramide monohydrochloride	C14H23Cl2N3O2	336.26	Central Nervous System
03E11	Fenbendazole	C15H13N3O2S	299.35	Infectiology
03F02	Piroxicam	C15H13N3O4S	331.35	Central Nervous System
03F03	Pyrantel tartrate	C15H20N2O6S	356.40	Infectiology
03F04	Fenspiride hydrochloride	C15H21ClN2O2	296.80	Respiratory
03F05	Gemfibrozil	C15H22O3	250.34	Metabolism
03F06	Mefexamide hydrochloride	C15H25ClN2O3	316.83	Central Nervous System
03F07	Tiaprime hydrochloride	C15H25ClN2O4S	364.89	Central Nervous System
03F08	Mebendazole	C16H13N3O3	295.30	Infectiology
03F09	Fenbufen	C16H14O3	254.29	Central Nervous System
03F10	Ketoprofen	C16H14O3	254.29	Central Nervous System
03F11	Indapamide	C16H16ClN3O3S	365.84	Metabolism
03G02	Norfloxacin	C16H18FN3O3	319.34	Infectiology
03G03	Antimycin A	C28H40N2O9	548.64	Infectiology
03G04	Xylometazoline hydrochloride	C16H25ClN2	280.84	Cardiovascular
03G05	Oxymetazoline hydrochloride	C16H25ClN2O	296.84	Respiratory
03G06	Nifenazone	C17H16N4O2	308.34	Central Nervous System
03G07	Griseofulvin	C17H17ClO6	352.77	Infectiology
03G08	Clemizole hydrochloride	C19H21Cl2N3	362.31	Allergology
03G09	Tropicamide	C17H20N2O2	284.36	Neuromuscular
03G10	Nefopam hydrochloride	C17H20ClNO	289.81	Central Nervous System
03G11	Phentolamine hydrochloride	C17H20ClN3O	317.82	Cardiovascular
03H02	Etodolac	C17H21NO3	287.36	Central Nervous System
03H03	Scopolamin-N-oxide hydrobromide	C17H22BrNO5	400.27	Neuromuscular
03H04	Hyoscyamine (L)	C17H23NO3	289.38	Central Nervous System
03H05	Chlorphensin carbamate	C10H12ClNO4	245.66	Central Nervous System

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03H06	Fadrozole hydrochloride	C14H14ClN3	259.74	Oncology
03H07	Dilazep dihydrochloride	C31H46Cl2N2O10	677.63	Cardiovascular
03H08	Ofloxacin	C18H20FN3O4	361.38	Infectiology
03H09	Lomefloxacin hydrochloride	C17H20ClF2N3O3	387.82	Infectiology
03H10	Orphenadrine hydrochloride	C18H24ClNO	305.85	Allergology
03H11	Proglumide	C18H26N2O4	334.42	Gastroenterology
04A02	Mexiletine hydrochloride	C11H18ClNO	215.73	Cardiovascular
04A03	Flavoxate hydrochloride	C24H26ClNO4	427.93	Metabolism
04A04	Bufexamac	C12H17NO3	223.27	Central Nervous System
04A05	Glutethimide, para-amino	C13H16N2O2	232.28	Oncology
04A06	Dropropizine (R,S)	C13H20N2O2	236.32	Respiratory
04A07	Pinacidil	C13H19N5	245.33	Cardiovascular
04A08	Albendazole	C12H15N3O2S	265.34	Metabolism
04A09	Clonidine hydrochloride	C9H10Cl3N3	266.56	Cardiovascular
04A10	Bupropion hydrochloride	C13H19Cl2NO	276.21	Central Nervous System
04A11	Alprenolol hydrochloride	C15H24ClNO2	285.82	Cardiovascular
04B02	Chlorothiazide	C7H6ClN3O4S2	295.72	Metabolism
04B03	Diphenidol hydrochloride	C21H28ClNO	345.92	Central Nervous System
04B04	Norethindrone	C20H26O2	298.43	Endocrinology
04B05	Nortriptyline hydrochloride	C19H22ClN	299.85	Central Nervous System
04B06	Niflumic acid	C13H9F3N2O2	282.22	Central Nervous System
04B07	Isotretinoin	C20H28O2	300.44	Dermatology
04B08	Retinoic acid	C20H28O2	300.44	Dermatology
04B09	Antazoline hydrochloride	C17H20ClN3	301.82	Allergology
04B10	Ethacrynic acid	C13H12Cl2O4	303.14	Metabolism
04B11	Praziquantel	C19H24N2O2	312.42	Infectiology
04C02	Ethisterone	C21H28O2	312.46	Endocrinology
04C03	Triprolidine hydrochloride	C19H23ClN2	314.86	Allergology
04C04	Doxepin hydrochloride	C19H22ClNO	315.85	Allergology
04C05	Dyclonine hydrochloride	C18H28ClNO2	325.88	Neuromuscular
04C06	Dimenhydrinate	C24H28ClN5O3	469.98	Allergology
04C07	Disopyramide	C21H29N3O	339.48	Cardiovascular
04C08	Clotrimazole	C22H17ClN2	344.85	Infectiology
04C09	Vinpocetine	C22H26N2O2	350.46	Cardiovascular
04C10	Clomipramine hydrochloride	C19H24Cl2N2	351.32	Central Nervous System
04C11	Fendiline hydrochloride	C23H26ClN	351.92	Cardiovascular
04D02	Vincamine	C21H26N2O3	354.45	Central Nervous System
04D03	Indomethacin	C19H16ClNO4	357.80	Central Nervous System
04D04	Cortisone	C21H28O5	360.45	Endocrinology
04D05	Prednisolone	C21H28O5	360.45	Endocrinology
04D06	Fenofibrate	C20H21ClO4	360.84	Metabolism

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04D07	Bumetanide	C17H20N2O5S	364.42	Metabolism
04D08	Labetalol hydrochloride	C19H25ClN2O3	364.88	Cardiovascular
04D09	Cinnarizine	C26H28N2	368.53	Allergology
04D10	Methylprednisolone, 6-alpha	C22H30O5	374.48	Endocrinology
04D11	Quinidine hydrochloride monohydrate	C20H27ClN2O3	378.90	Cardiovascular
04E02	Fludrocortisone acetate	C23H31FO6	422.50	Dermatology
04E03	Fenoterol hydrobromide	C17H22BrNO4	384.27	Neuromuscular
04E04	Homochlorcyclizine dihydrochloride	C19H25Cl3N2	387.78	Allergology
04E05	Diethylcarbamazine citrate	C16H29N3O8	391.42	Infectiology
04E06	Chenodioli	C24H40O4	392.58	Gastroenterology
04E07	Perhexiline maleate	C23H39NO4	393.57	Cardiovascular
04E08	Oxybutynin chloride	C22H32ClNO3	393.96	Neuromuscular
04E09	Spiperone	C23H26FN3O2	395.48	Central Nervous System
04E10	Pyrimidine maleate	C21H27N3O5	401.47	Allergology
04E11	Sulfinpyrazone	C23H20N2O3S	404.49	Hematology
04F02	Dantrolene sodium salt	C14H9N4NaO5	336.24	Neuromuscular
04F03	Trazodone hydrochloride	C19H23Cl2N5O	408.33	Central Nervous System
04F04	Glafenine hydrochloride	C19H18Cl2N2O4	409.27	Metabolism
04F05	Pimethixene maleate	C23H23NO4S	409.51	Allergology
04F06	Pergolide mesylate	C20H30N2O3S2	410.60	Central Nervous System
04F07	Acemetacin	C21H18ClNO6	415.83	Metabolism
04F08	Benzydamine hydrochloride	C19H24ClN3O	345.88	Central Nervous System
04F09	Fipexide hydrochloride	C20H22Cl2N2O4	425.32	Central Nervous System
04F10	Mifepristone	C29H35NO2	429.61	Endocrinology
04F11	Diperodon hydrochloride	C22H28ClN3O4	433.94	Neuromuscular
04G02	Lisinopril	C21H35N3O7	441.53	Cardiovascular
04G03	Lincomycin hydrochloride	C18H35ClN2O6S	443.01	Infectiology
04G04	Telenzepine dihydrochloride	C19H24Cl2N4O2S	443.40	Gastroenterology
04G05	Econazole nitrate	C18H16Cl3N3O4	444.70	Infectiology
04G06	Bupivacaine hydrochloride	C18H29ClN2O	324.90	Neuromuscular
04G07	Clemastine fumarate	C25H30ClNO5	459.97	Allergology
04G08	Oxytetracycline dihydrate	C22H28N2O11	496.48	Infectiology
04G09	Pimozide	C28H29F2N3O	461.56	Central Nervous System
04G10	Amodiaquin dihydrochloride dihydrate	C20H28Cl3N3O3	464.82	Infectiology
04G11	Mebeverine hydrochloride	C25H36ClNO5	466.02	Neuromuscular
04H02	Ifenprodil tartrate	C25H33NO8	475.54	Cardiovascular
04H03	Flunarizine dihydrochloride	C26H28Cl2F2N2	477.43	Central Nervous System
04H04	Trifluoperazine dihydrochloride	C21H26Cl2F3N3S	480.43	Central Nervous System
04H05	Enalapril maleate	C24H32N2O9	492.53	Cardiovascular
04H06	Minocycline hydrochloride	C23H28ClN3O7	493.95	Infectiology
04H07	Glibenclamide	C23H28ClN3O5S	494.01	Endocrinology

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04H08	Guanethidine sulfate	C20H46N8O4S	494.70	Central Nervous System
04H09	Quinacrine dihydrochloride dihydrate	C23H36Cl3N3O3	508.92	Infectiology
04H10	Clofilium tosylate	C28H44ClNO3S	510.18	Cardiovascular
04H11	Fluphenazine dihydrochloride	C22H28Cl2F3N3OS	510.45	Central Nervous System
05A02	Streptomycin sulfate	C42H84N14O36S3	1457.40	Infectiology
05A03	Alfuzosin hydrochloride	C19H28ClN5O4	425.92	Cardiovascular
05A04	Chlorpropamide	C10H13ClN2O3S	276.74	Endocrinology
05A05	Phenylpropanolamine hydrochloride	C9H14ClNO	187.67	Respiratory
05A06	Ascorbic acid	C6H8O6	176.13	Metabolism
05A07	Methyldopa (L,-)	C10H13NO4	211.22	Cardiovascular
05A08	Cefoperazone dihydrate	C25H31N9O10S2	681.71	Infectiology
05A09	Zoxazolamine	C7H5ClN2O	168.58	Metabolism
05A10	Tacrine hydrochloride	C13H15ClN2	234.73	Central Nervous System
05A11	Bisoprolol fumarate	C40H66N2O12	766.98	Cardiovascular
05B02	Tremorine dihydrochloride	C12H22Cl2N2	265.23	Central Nervous System
05B03	Practolol	C14H22N2O3	266.34	Cardiovascular
05B04	Zidovudine, AZT	C10H13N5O4	267.25	Infectiology
05B05	Sulfisoxazole	C11H13N3O3S	267.31	Infectiology
05B06	Zaprinast	C13H13N5O2	271.28	Cardiovascular
05B07	Chlormezanone	C11H12ClNO3S	273.74	Central Nervous System
05B08	Procainamide hydrochloride	C13H22ClN3O	271.79	Cardiovascular
05B09	N6-methyladenosine	C11H15N5O4	281.27	Oncology
05B10	Guanfacine hydrochloride	C9H10Cl3N3O	282.56	Cardiovascular
05B11	Domperidone	C22H24ClN5O2	425.92	Central Nervous System
05C02	Furosemide	C12H11ClN2O5S	330.75	Metabolism
05C03	Methapyrilene hydrochloride	C14H20ClN3S	297.85	Allergology
05C04	Desipramine hydrochloride	C18H23ClN2	302.85	Central Nervous System
05C05	Clorgyline hydrochloride	C13H16Cl3NO	308.64	Central Nervous System
05C06	Clenbuterol hydrochloride	C12H19Cl3N2O	313.66	Neuromuscular
05C07	Maprotiline hydrochloride	C20H24ClN	313.87	Central Nervous System
05C08	Thioguanosine	C10H13N5O4S	299.31	Metabolism
05C09	Chlorprothixene hydrochloride	C18H19Cl2NS	352.33	Central Nervous System
05C10	Ritodrine hydrochloride	C17H22ClNO3	323.82	Neuromuscular
05C11	Clozapine	C18H19ClN4	326.83	Central Nervous System
05D02	Chlorthalidone	C14H11ClN2O4S	338.77	Metabolism
05D03	Dobutamine hydrochloride	C18H24ClNO3	337.85	Cardiovascular
05D04	Moclobemide	C13H17ClN2O2	268.75	Central Nervous System
05D05	Clopamide	C14H20ClN3O3S	345.85	Metabolism
05D06	Hycanthone	C20H24N2O2S	356.49	Infectiology
05D07	Adenosine 5'-monophosphate monohydrate	C10H16N5O8P	365.24	Cardiovascular

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05D08	Amoxicillin	C16H19N3O5S	365.41	Metabolism
05D09	Pemirolast potassium	C10H7KN6O	266.31	Ophthalmology
05D10	Dextromethorphan hydrobromide monohydrate	C18H28BrNO2	370.33	Central Nervous System
05D11	Droperidol	C22H22FN3O2	379.44	Central Nervous System
05E02	Bambuterol hydrochloride	C18H30ClN3O5	403.91	Neuromuscular
05E03	Betamethasone	C22H29FO5	392.47	Endocrinology
05E04	Colchicine	C22H25NO6	399.45	Metabolism
05E05	Metergoline	C25H29N3O2	403.53	Central Nervous System
05E06	Brinzolamide	C12H21N3O5S3	383.51	Metabolism
05E07	Ambroxol hydrochloride	C13H19Br2ClN2O	414.57	Respiratory
05E08	Benfluorex hydrochloride	C19H21ClF3NO2	387.83	Central Nervous System
05E09	Bepidil hydrochloride	C24H35ClN2O	403.01	Cardiovascular
05E10	Meloxicam	C14H13N3O4S2	351.41	Metabolism
05E11	Benzbromarone	C17H12Br2O3	424.09	Cardiovascular
05F02	Ketotifen fumarate	C23H23NO5S	425.51	Allergology
05F03	Debrisoquin sulfate	C20H28N6O4S	448.55	Cardiovascular
05F04	Amethopterin (R,S)	C20H22N8O5	454.45	Immunology
05F05	Methylergometrine maleate	C24H29N3O6	455.52	Neuromuscular
05F06	Methiothepin maleate	C24H28N2O4S2	472.63	Central Nervous System
05F07	Clofazimine	C27H22Cl2N4	473.41	Infectiology
05F08	Nafronyl oxalate	C26H35NO7	473.57	Cardiovascular
05F09	Bezafibrate	C19H20ClNO4	361.83	Metabolism
05F10	Nefazodone HCl	C25H33Cl2N5O2	506.48	Central Nervous System
05F11	Clebopride maleate	C24H28ClN3O6	489.96	Central Nervous System
05G02	Lidoflazine	C30H35F2N3O	491.63	Cardiovascular
05G03	Betaxolol hydrochloride	C18H30ClNO3	343.90	Cardiovascular
05G04	Nicardipine hydrochloride	C26H30ClN3O6	516.00	Cardiovascular
05G05	Probucol	C31H48O2S2	516.86	Metabolism
05G06	Mitoxantrone dihydrochloride	C22H30Cl2N4O6	517.41	Oncology
05G07	GBR 12909 dihydrochloride	C28H34Cl2F2N2O	523.50	Central Nervous System
05G08	Carbetapentane citrate	C26H39NO10	525.60	Central Nervous System
05G09	Dequalinium dichloride	C30H40Cl2N4	527.59	Infectiology
05G10	Ketoconazole	C26H28Cl2N4O4	531.44	Infectiology
05G11	Fusidic acid sodium salt	C31H47NaO6	538.71	Infectiology
05H02	Terbutaline hemisulfate	C24H40N2O10S	548.66	Respiratory
05H03	Ketanserin tartrate hydrate	C26H30FN3O10	563.54	Cardiovascular
05H04	Hemicholinium bromide	C24H34Br2N2O4	574.36	Neuromuscular
05H05	Kanamycin A sulfate	C18H38N4O15S	582.59	Infectiology
05H06	Amikacin hydrate	C22H47N5O15	621.64	Infectiology
05H07	Etoposide	C29H32O13	588.57	Oncology

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05H08	Clomiphene citrate (Z,E)	C32H36ClNO8	598.10	Endocrinology
05H09	Oxantel pamoate	C49H48N4O8	820.95	Infectiology
05H10	Prochlorperazine dimaleate	C28H32ClN3O8S	606.10	Central Nervous System
05H11	Hesperidin	C28H34O15	610.57	Oncology
06A02	Testosterone propionate	C22H32O3	344.50	Endocrinology
06A03	Haloprogyn	C9H4Cl3IO	361.40	Central Nervous System
06A04	Thyroxine (L)	C15H11I4NO4	776.88	Endocrinology
06A05	Idebenone	C19H30O5	338.45	Oncology
06A06	Pepstatin A	C34H63N5O9	685.91	Infectiology
06A07	Morpholinoethylamino-3-benzocyclohepta-(5,6-c)-pyridazine dihydrochloride	C19H26Cl2N4O	397.35	
06A08	Adamantamine fumarate	C24H38N2O4	418.58	Infectiology
06A09	Butoconazole nitrate	C19H18Cl3N3O3S	474.80	Infectiology
06A10	Amiodarone hydrochloride	C25H30ClI2NO3	681.78	Cardiovascular
06A11	Amphotericin B	C47H73NO17	924.10	Infectiology
06B02	Androsterone	C19H30O2	290.45	Endocrinology
06B03	Amifostine	C5H15N2O3PS	214.22	Diagnostic
06B04	Carbarsone	C7H9AsN2O4	260.08	Infectiology
06B05	Amlodipine	C20H25ClN2O5	408.89	Cardiovascular
06B06	Modafinil	C15H15NO2S	273.36	Central Nervous System
06B07	Bacampicillin hydrochloride	C21H28ClN3O7S	501.99	Infectiology
06B08	Lamivudine	C8H11N3O3S	229.26	Infectiology
06B09	Biotin	C10H16N2O3S	244.31	Metabolism
06B10	Bisacodyl	C22H19NO4	361.40	Gastroenterology
06B11	Erlotinib	C22H23N3O4	393.45	Oncology
06C02	Suloctidil	C20H35NOS	337.57	Neuromuscular
06C03	Zotepine	C18H18ClNOS	331.87	Central Nervous System
06C04	Carisoprodol	C12H24N2O4	260.34	Central Nervous System
06C05	Cephalosporanic acid, 7-amino	C10H12N2O5S	272.28	Infectiology
06C06	Chicago sky blue 6B	C34H24N6Na4O16S4	992.82	Central Nervous System
06C07	Buflomedil hydrochloride	C17H26ClNO4	343.85	Cardiovascular
06C08	Dibenzepine hydrochloride	C18H22ClN3O	331.85	Central Nervous System
06C09	Roxatidine Acetate HCl	C19H29ClN2O4	384.91	Gastroenterology
06C10	Valacyclovir hydrochloride	C13H21ClN6O4	360.80	Infectiology
06C11	Cisapride	C23H29ClFN3O4	465.96	Gastroenterology
06D02	Pefloxacin	C17H20FN3O3	333.37	Infectiology
06D03	Corticosterone	C21H30O4	346.47	Endocrinology
06D04	Cyanocobalamin	C63H88CoN14O14P	1355.40	Metabolism
06D05	Cefadroxil	C16H17N3O5S	363.39	Infectiology
06D06	Cyclosporin A	C62H111N11O12	1202.64	Immunology

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06D07	Digitoxigenin	C23H34O4	374.53	Cardiovascular
06D08	Digoxin	C41H64O14	780.96	Cardiovascular
06D09	Doxorubicin hydrochloride	C27H30ClNO11	579.99	Infectiology
06D10	Carbimazole	C7H10N2O2S	186.23	Metabolism
06D11	Epiandrosterone	C19H30O2	290.45	Endocrinology
06E02	Estradiol-17 beta	C18H24O2	272.39	Endocrinology
06E03	Clobutinol hydrochloride	C14H23Cl2NO	292.25	Central Nervous System
06E04	Gabazine bromide	C15H18BrN3O3	368.23	Central Nervous System
06E05	Oxcarbazepine	C15H12N2O2	252.28	Central Nervous System
06E06	Cyclobenzaprine hydrochloride	C20H22ClN	311.86	Neuromuscular
06E07	Carteolol hydrochloride	C16H25ClN2O3	328.84	Cardiovascular
06E08	Hydrocortisone base	C21H30O5	362.47	Endocrinology
06E09	Hydroxytacrine maleate (R,S)	C17H18N2O5	330.34	Central Nervous System
06E10	Pilocarpine nitrate	C11H17N3O5	271.28	Ophthalmology
06E11	Dicloxacillin sodium salt hydrate	C19H18Cl2N3NaO6S	510.33	Infectiology
06F02	Alizapride HCl	C16H22ClN5O2	351.84	Central Nervous System
06F03	Stanozolol	C21H32N2O	328.50	Endocrinology
06F04	Calcipotriene	C27H40O3	412.62	Dermatology
06F05	Linezolid	C16H20FN3O4	337.35	Infectiology
06F06	Mebhydroline 1,5-naphthalenedisulfonate	C48H48N4O6S2	841.07	Allergology
06F07	Meclocycline sulfosalicylate	C29H27ClN2O14S	695.06	Infectiology
06F08	Meclozine dihydrochloride	C25H29Cl3N2	463.88	Allergology
06F09	Melatonin	C13H16N2O2	232.28	Central Nervous System
06F10	Butalbital	C11H16N2O3	224.26	Central Nervous System
06F11	Dinoprost trometamol	C24H45NO8	475.63	Endocrinology
06G02	Tropisetron HCl	C17H21ClN2O2	320.82	Central Nervous System
06G03	Cefixime	C16H15N5O7S2	453.46	Infectiology
06G04	Metrizamide	C18H22I3N3O8	789.10	Diagnostic
06G05	Quetiapine hemifumarate	C46H54N6O8S2	883.11	Central Nervous System
06G06	Tosufloxacin hydrochloride	C19H16ClF3N4O3	440.81	Infectiology
06G07	Efavirenz	C14H9ClF3NO2	315.68	Infectiology
06G08	Rifapentine	C47H64N4O12	877.05	Infectiology
06G09	Neostigmine bromide	C12H19BrN2O2	303.20	Diagnostic
06G10	Niridazole	C6H6N4O3S	214.20	Infectiology
06G11	Ceforanide	C20H21N7O6S2	519.56	Infectiology
06H02	Vatalanib	C20H15ClN4	346.82	Oncology
06H03	Itopride	C20H26N2O4	358.44	Metabolism
06H04	Cefotetan	C17H17N7O8S4	575.62	Infectiology
06H05	Fentiazac	C17H12ClNO2S	329.81	Metabolism
06H06	Brompheniramine maleate	C20H23BrN2O4	435.32	Allergology

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06H07	Primaquine diphosphate	C15H27N3O9P2	455.34	Infectiology
06H08	Progesterone	C21H30O2	314.47	Endocrinology
06H09	Felodipine	C18H19Cl2NO4	384.26	Neuromuscular
06H10	Raclopride	C15H20Cl2N2O3	347.24	Central Nervous System
06H11	Closantel	C22H14Cl2I2N2O2	663.08	Infectiology
07A02	Serotonin hydrochloride	C10H13ClN2O	212.68	Central Nervous System
07A03	Cefotiam hydrochloride	C18H24ClN9O4S3	562.09	Infectiology
07A04	Rofecoxib	C17H14O4S	314.36	Metabolism
07A05	Benperidol	C22H24FN3O2	381.45	Central Nervous System
07A06	Cefaclor hydrate	C15H16ClN3O5S	385.83	Infectiology
07A07	Colistin sulfate	C52H100N16O17S	1253.54	Infectiology
07A08	Daunorubicin hydrochloride	C27H30ClNO10	563.99	Infectiology
07A09	Dosulepin hydrochloride	C19H22ClNS	331.91	Central Nervous System
07A10	Ceftazidime pentahydrate	C22H32N6O12S2	636.66	Infectiology
07A11	Iobenguane sulfate	C8H12IN3O4S	373.17	Oncology
07B02	Metixene hydrochloride	C20H24ClNS	345.94	Central Nervous System
07B03	Nitrofuril	C6H6N4O4	198.14	Infectiology
07B04	Omeprazole	C17H19N3O3S	345.42	Gastroenterology
07B05	Propylthiouracil	C7H10N2OS	170.23	Metabolism
07B06	Terconazole	C26H31Cl2N5O3	532.47	Infectiology
07B07	Tiaprofenic acid	C14H12O3S	260.31	Central Nervous System
07B08	Vancomycin hydrochloride	C66H76Cl3N9O24	1485.75	Infectiology
07B09	Artemisinin	C15H22O5	282.34	Infectiology
07B10	Propafenone hydrochloride	C21H28ClNO3	377.92	Cardiovascular
07B11	Ethamivan	C12H17NO3	223.27	Central Nervous System
07C02	Vigabatrin	C6H11NO2	129.16	Central Nervous System
07C03	Biperiden hydrochloride	C21H30ClNO	347.93	Central Nervous System
07C04	Cetirizine dihydrochloride	C21H27Cl3N2O3	461.82	Allergology
07C05	Etifenin	C16H22N2O5	322.36	Diagnostic
07C06	Metaproterenol sulfate, orciprenaline sulfate	C22H36N2O10S	520.60	Respiratory
07C07	Sisomicin sulfate	C19H39N5O11S	545.61	Infectiology
07C08	Sibutramine HCl	C17H27Cl2N	316.32	Central Nervous System
07C09	Acenocoumarol	C19H15NO6	353.33	Hematology
07C10	Bromperidol	C21H23BrFNO2	420.33	Central Nervous System
07C11	Cyclizine hydrochloride	C18H23ClN2	302.85	Allergology
07D02	Fluoxetine hydrochloride	C17H19ClF3NO	345.80	Central Nervous System
07D03	Iohexol	C19H26I3N3O9	821.15	Diagnostic
07D04	Norcyclobenzaprine	C19H19N	261.37	Gastroenterology
07D05	Pyrazinamide	C5H5N3O	123.12	Infectiology
07D06	Trimethadione	C6H9NO3	143.14	Central Nervous System

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07D07	Lovastatin	C ₂₄ H ₃₆ O ₅	404.55	Metabolism
07D08	Nystatine	C ₄₇ H ₇₅ NO ₁₇	926.12	Infectiology
07D09	Budesonide	C ₂₅ H ₃₄ O ₆	430.55	Endocrinology
07D10	Imipenem	C ₁₂ H ₁₇ N ₃ O ₄ S	299.35	Infectiology
07D11	Sulfasalazine	C ₁₈ H ₁₄ N ₄ O ₅ S	398.40	Infectiology
07E02	Lofexidine	C ₁₁ H ₁₂ Cl ₂ N ₂ O	259.14	Cardiovascular
07E03	Thiostrepton	C ₇₂ H ₈₅ N ₁₉ O ₁₈ S ₅	1664.92	Infectiology
07E04	Miglitol	C ₈ H ₁₇ NO ₅	207.23	Endocrinology
07E05	Tiabendazole	C ₁₀ H ₇ N ₃ S	201.25	Infectiology
07E06	Rifampicin	C ₄₃ H ₅₈ N ₄ O ₁₂	822.96	Infectiology
07E07	Ethionamide	C ₈ H ₁₀ N ₂ S	166.25	Infectiology
07E08	Tenoxicam	C ₁₃ H ₁₁ N ₃ O ₄ S ₂	337.38	Central Nervous System
07E09	Triflusal	C ₁₀ H ₇ F ₃ O ₄	248.16	Hematology
07E10	Mesoridazine besylate	C ₂₇ H ₃₂ N ₂ O ₄ S ₃	544.76	Central Nervous System
07E11	Trolox	C ₁₄ H ₁₈ O ₄	250.30	Metabolism
07F02	Pirenperone	C ₂₃ H ₂₄ FN ₃ O ₂	393.47	Central Nervous System
07F03	Isoquinoline, 6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydro, hydrochloride	C ₁₂ H ₁₈ ClNO ₂	243.74	
07F04	Phenacetin	C ₁₀ H ₁₃ NO ₂	179.22	Central Nervous System
07F05	Atovaquone	C ₂₂ H ₁₉ ClO ₃	366.85	Infectiology
07F06	Methoxamine hydrochloride	C ₁₁ H ₁₈ ClNO ₃	247.72	Cardiovascular
07F07	(S)-(-)-Atenolol	C ₁₄ H ₂₂ N ₂ O ₃	266.34	Cardiovascular
07F08	Piracetam	C ₆ H ₁₀ N ₂ O ₂	142.16	Central Nervous System
07F09	Phenindione	C ₁₅ H ₁₀ O ₂	222.25	Hematology
07F10	Thiocolchicoside	C ₂₇ H ₃₃ NO ₁₀ S	563.63	Central Nervous System
07F11	Clorsulon	C ₈ H ₈ Cl ₃ N ₃ O ₄ S ₂	380.66	Infectiology
07G02	Ciclopirox ethanolamine	C ₁₄ H ₂₄ N ₂ O ₃	268.36	Infectiology
07G03	Probenecid	C ₁₃ H ₁₉ NO ₄ S	285.36	Metabolism
07G04	Betahistine mesylate	C ₁₀ H ₂₀ N ₂ O ₆ S ₂	328.41	Allergology
07G05	Tobramycin	C ₁₈ H ₃₇ N ₅ O ₉	467.52	Infectiology
07G06	Tetramisole hydrochloride	C ₁₁ H ₁₃ ClN ₂ S	240.76	Immunology
07G07	Pregnenolone	C ₂₁ H ₃₂ O ₂	316.49	Endocrinology
07G08	Molsidomine	C ₉ H ₁₄ N ₄ O ₄	242.24	Cardiovascular
07G09	Chloroquine diphosphate	C ₁₈ H ₃₂ ClN ₃ O ₈ P ₂	515.87	Metabolism
07G10	Trimetazidine dihydrochloride	C ₁₄ H ₂₄ Cl ₂ N ₂ O ₃	339.26	Cardiovascular
07G11	Parthenolide	C ₁₅ H ₂₀ O ₃	248.32	Metabolism
07H02	Hexetidine	C ₂₁ H ₄₅ N ₃	339.61	Infectiology
07H03	Selegiline hydrochloride	C ₁₃ H ₁₈ ClN	223.75	Central Nervous System
07H04	Pentamidine isethionate	C ₂₃ H ₃₆ N ₄ O ₁₀ S ₂	592.69	Infectiology
07H05	Tolazamide	C ₁₄ H ₂₁ N ₃ O ₃ S	311.41	Metabolism
07H06	Nifuroxazide	C ₁₂ H ₉ N ₃ O ₅	275.22	Infectiology

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07H07	Mirtazapine	C17H19N3	265.36	Central Nervous System
07H08	Dirithromycin	C42H78N2O14	835.09	Infectiology
07H09	Gliclazide	C15H21N3O3S	323.42	Metabolism
07H10	DO 897/99	C26H33Cl2N3O2	490.48	Central Nervous System
07H11	Prenylamine lactate	C27H33NO3	419.57	Cardiovascular
08A02	Ziprasidone Hydrochloride	C21H22Cl2N4OS	449.41	Central Nervous System
08A03	Mevastatin	C23H34O5	390.52	Cardiovascular
08A04	Pyridostigmine iodide	C9H13IN2O2	308.12	Central Nervous System
08A05	Pentobarbital	C11H18N2O3	226.28	Central Nervous System
08A06	Atropine sulfate monohydrate	C34H50N2O11S	694.85	Ophthalmology
08A07	Eserine hemisulfate salt	C30H44N6O8S	648.78	Central Nervous System
08A08	Itraconazole	C35H38Cl2N8O4	705.65	Infectiology
08A09	Acarbose	C25H43NO18	645.62	Endocrinology
08A10	Entacapone	C14H15N3O5	305.29	Central Nervous System
08A11	Nicotinamide	C6H6N2O	122.13	Dermatology
08B02	Tetracaine hydrochloride	C15H25ClN2O2	300.83	Neuromuscular
08B03	Mometasone furoate	C27H30Cl2O6	521.44	Endocrinology
08B04	Troglitazone	C24H27NO5S	441.55	Metabolism
08B05	Dacarbazine	C6H10N6O	182.19	Oncology
08B06	Tenatoprazole	C16H18N4O3S	346.41	Metabolism
08B07	Acetopromazine maleate salt	C23H26N2O5S	442.54	Central Nervous System
08B08	Escitalopram	C22H23FN2O5	414.44	Central Nervous System
08B09	Ropinirole HCl	C16H25ClN2O	296.84	Central Nervous System
08B10	Lacidipine	C26H33NO6	455.56	Cardiovascular
08B11	Argatroban	C23H36N6O5S	508.64	Hematology
08C02	Reboxetine mesylate	C20H27NO6S	409.51	Central Nervous System
08C03	Camylofine chlorhydrate	C19H34Cl2N2O2	393.40	
08C04	Papaverine hydrochloride	C20H22ClNO4	375.86	Cardiovascular
08C05	Yohimbine hydrochloride	C21H27ClN2O3	390.91	Cardiovascular
08C06	Voriconazole	C16H14F3N5O	349.32	Infectiology
08C07	Alfacalcidol	C27H44O2	400.65	Metabolism
08C08	Cilostazol	C20H27N5O2	369.47	Hematology
08C09	Galanthamine hydrobromide	C17H22BrNO3	368.27	Central Nervous System
08C10	Azelastine HCl	C22H25Cl2N3O	418.37	Immunology
08C11	Etretinate	C23H30O3	354.49	Dermatology
08D02	Emedastine	C25H34N4O9	534.57	Allergology
08D03	Etofenamate	C18H18F3NO4	369.34	Metabolism
08D04	Zaleplon	C17H15N5O	305.34	Central Nervous System
08D05	Diclofenac sodium	C14H10Cl2NNaO2	318.14	Central Nervous System
08D06	Exemestane	C20H24O2	296.41	Endocrinology
08D07	Fomepizole	C4H6N2	82.11	Metabolism

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08D08	Temozolomide	C ₆ H ₆ N ₆ O ₂	194.15	Oncology
08D09	Xylazine	C ₁₂ H ₁₆ N ₂ S	220.34	Central Nervous System
08D10	Celiprolol HCl	C ₂₀ H ₃₄ CIN ₃ O ₄	415.96	Cardiovascular
08D11	Zopiclone	C ₁₇ H ₁₇ CIN ₆ O ₃	388.82	Central Nervous System
08E02	Tranilast	C ₁₈ H ₁₇ NO ₅	327.34	Allergology
08E03	Tizanidine HCl	C ₉ H ₉ CIN ₂ N ₅ S	290.18	Metabolism
08E04	Zafirlukast	C ₃₁ H ₃₃ N ₃ O ₆ S	575.69	Respiratory
08E05	Butenafine Hydrochloride	C ₂₃ H ₂₈ CIN	353.94	Infectiology
08E06	Carbadox	C ₁₁ H ₁₀ N ₄ O ₄	262.23	Infectiology
08E07	Rimantadine Hydrochloride	C ₁₂ H ₂₂ CIN	215.77	Infectiology
08E08	Eburnamonine (-)	C ₁₉ H ₂₂ N ₂ O	294.40	Central Nervous System
08E09	Oxibendazol	C ₁₂ H ₁₅ N ₃ O ₃	249.27	Metabolism
08E10	Ipsapirone	C ₁₉ H ₂₃ N ₅ O ₃ S	401.49	Central Nervous System
08E11	Hydroxychloroquine sulfate	C ₁₈ H ₂₈ CIN ₃ O ₅ S	433.96	Metabolism
08F02	Loracarbef	C ₁₆ H ₁₆ CIN ₃ O ₄	349.78	Infectiology
08F03	Fenipentol	C ₁₁ H ₁₆ O	164.25	Metabolism
08F04	Diosmin	C ₂₈ H ₃₂ O ₁₅	608.56	Cardiovascular
08F05	Carbidopa	C ₁₀ H ₁₄ N ₂ O ₄	226.23	Central Nervous System
08F06	(-)-Emtricitabine	C ₈ H ₁₀ FN ₃ O ₃ S	247.25	Infectiology
08F07	Demecarium bromide	C ₃₂ H ₅₂ Br ₂ N ₄ O ₄	716.60	Ophthalmology
08F08	Quipazine dimaleate salt	C ₂₁ H ₂₃ N ₃ O ₈	445.43	Central Nervous System
08F09	Acipimox	C ₆ H ₆ N ₂ O ₃	154.13	Metabolism
08F10	Diflorasone Diacetate	C ₂₆ H ₃₂ F ₂ O ₇	494.54	Endocrinology
08F11	Acamprosate calcium	C ₁₀ H ₂₀ CaN ₂ O ₈ S ₂	400.49	Central Nervous System
08G02	Mizolastine	C ₂₄ H ₂₅ FN ₆ O	432.50	Allergology
08G03	Amisulpride	C ₁₇ H ₂₇ N ₃ O ₄ S	369.49	Central Nervous System
08G04	Pyridoxine hydrochloride	C ₈ H ₁₂ CINO ₃	205.64	Metabolism
08G05	Mercaptopurine	C ₅ H ₄ N ₄ S	152.18	Immunology
08G06	Cytarabine	C ₉ H ₁₃ N ₃ O ₅	243.22	Oncology
08G07	Racecadotril	C ₂₁ H ₂₃ N ₃ O ₄ S	385.49	Gastroenterology
08G08	Folic acid	C ₁₉ H ₁₉ N ₇ O ₆	441.41	Metabolism
08G09	Benazepril HCl	C ₂₄ H ₂₉ CIN ₂ O ₅	460.96	Cardiovascular
08G10	Aniracetam	C ₁₂ H ₁₃ NO ₃	219.24	Central Nervous System
08G11	Dimethisoquin hydrochloride	C ₁₇ H ₂₅ CIN ₂ O	308.85	Neuromuscular
08H02	Alendronate sodium	C ₄ H ₁₂ NNaO ₇ P ₂	271.08	Metabolism
08H03	Dipivefrin hydrochloride	C ₁₉ H ₃₀ CINO ₅	387.91	Ophthalmology
08H04	Thiorphan	C ₁₂ H ₁₅ NO ₃ S	253.32	Gastroenterology
08H05	Tomoxetine hydrochloride	C ₁₇ H ₂₂ CINO	291.82	Central Nervous System
08H06	Aceclidine Hydrochloride	C ₉ H ₁₆ CINO ₂	205.69	Ophthalmology
08H07	Penciclovir	C ₁₀ H ₁₅ N ₅ O ₃	253.26	Infectiology
08H08	Levetiracetam	C ₈ H ₁₄ N ₂ O ₂	170.21	Central Nervous System

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08H09	Dexfenfluramine hydrochloride	C12H17ClF3N	267.72	Central Nervous System
08H10	Etoricoxib	C18H15ClN2O2S	358.85	Central Nervous System
08H11	Sertindole	C24H26ClFN4O	440.95	Central Nervous System
09A02	Sulmazole	C14H13N3O2S	287.34	Cardiovascular
09A03	Gefitinib	C22H24ClFN4O3	446.91	Oncology
09A04	Flunisolide	C24H31FO6	434.51	Endocrinology
09A05	N-Acetyl-DL-homocysteine Thiolactone	C6H9NO2S	159.21	Respiratory
09A06	Flurandrenolide	C24H33FO6	436.53	Dermatology
09A07	Oxiconazole Nitrate	C18H14Cl4N4O4	492.15	Infectiology
09A08	Rebamipide	C19H15ClN2O4	370.80	Metabolism
09A09	Nilvadipine	C19H19N3O6	385.38	Cardiovascular
09A10	Etanidazole	C7H10N4O4	214.18	Oncology
09A11	Pinaverium bromide	C26H41Br2NO4	591.43	Neuromuscular
09B02	Glimepiride	C24H34N4O5S	490.63	Endocrinology
09B03	Picrotoxinin	C15H16O6	292.29	Central Nervous System
09B04	Mepenzolate bromide	C21H26BrNO3	420.35	Neuromuscular
09B05	Benfotiamine	C19H23N4O6PS	466.46	Metabolism
09B06	Halcinonide	C24H32ClFO5	454.97	Dermatology
09B07	Lanatoside C	C49H76O20	985.14	Cardiovascular
09B08	Benzamil hydrochloride	C13H15Cl2N7O	356.22	Metabolism
09B09	Suxibuzone	C24H26N2O6	438.48	Central Nervous System
09B10	6-Furfurylaminopurine	C10H9N5O	215.22	Dermatology
09B11	Avermectin B1a	C48H72O14	873.10	Infectiology
09C02	Pranlukast	C27H23N5O4	481.52	Respiratory
09C03	Penicillamine	C5H11NO2S	149.21	Central Nervous System
09C04	Zileuton	C11H12N2O2S	236.29	Respiratory
09C05	Loratadine	C22H23ClN2O2	382.89	Allergology
09C06	Tetraethylenepentamine pentahydrochloride	C8H28Cl5N5	371.61	Metabolism
09C07	Nisoldipine	C20H24N2O6	388.42	Cardiovascular
09C08	Acefylline	C9H10N4O4	238.20	Central Nervous System
09C09	Acitretin	C21H26O3	326.44	Dermatology
09C10	Zonisamide	C8H8N2O3S	212.23	Central Nervous System
09C11	Irsogladine maleate	C13H11Cl2N5O4	372.17	Metabolism
09D02	Dydrogesterone	C21H28O2	312.46	Endocrinology
09D03	Sumatriptan succinate	C18H27N3O6S	413.50	Central Nervous System
09D04	Opipramol dihydrochloride	C23H31Cl2N3O	436.43	Central Nervous System
09D05	Nalidixic acid sodium salt	C12H11N2NaO3	254.22	Infectiology
09D06	Oxacillin sodium	C19H18N3NaO5S	423.43	Infectiology
09D07	Beta-Escin	C55H86O24	1131.28	Metabolism

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09D08	Thiamine hydrochloride	C12H18Cl2N4OS	337.27	Immunology
09D09	Tazobactam	C10H12N4O5S	300.29	Infectiology
09D10	Ibandronate sodium	C9H22NNaO7P2	341.22	Metabolism
09D11	Warfarin	C19H16O4	308.34	Hematology
09E02	Pranoprofen	C15H13NO3	255.28	Metabolism
09E03	Secnidazole	C7H11N3O3	185.18	Infectiology
09E04	Pempidine tartrate	C14H27NO6	305.37	Cardiovascular
09E05	Clodronate	CH2Cl2Na2O6P2	288.86	Metabolism
09E06	Ibutilide fumarate	C44H76N4O10S2	885.25	Cardiovascular
09E07	Thimerosal	C9H9HgNaO2S	404.81	Infectiology
09E08	Tramadol hydrochloride	C16H26ClNO2	299.84	Central Nervous System
09E09	Estropipate	C22H32N2O5S	436.57	Endocrinology
09E10	Butylscopolammonium (n-) bromide	C21H30BrNO4	440.38	Central Nervous System
09E11	Irinotecan hydrochloride trihydrate	C33H45ClN4O9	677.20	Oncology
09F02	Tylosin	C46H77NO17	916.12	Infectiology
09F03	Citalopram Hydrobromide	C20H22BrFN2O	405.31	Central Nervous System
09F04	Promazine hydrochloride	C17H21ClN2S	320.89	Central Nervous System
09F05	Sulfamerazine	C11H12N4O2S	264.31	Infectiology
09F06	Venlafaxine	C17H27NO2	277.41	Central Nervous System
09F07	Ethotoin	C11H12N2O2	204.23	Central Nervous System
09F08	3-alpha-Hydroxy-5-beta-androstan-17-one	C19H30O2	290.45	Endocrinology
09F09	Tetrahydrozoline hydrochloride	C13H17ClN2	236.75	Cardiovascular
09F10	Hexestrol	C18H22O2	270.37	Endocrinology
09F11	Cefmetazole sodium salt	C15H16N7NaO5S3	493.52	Infectiology
09G02	Trihexyphenidyl-D,L Hydrochloride	C20H32ClNO	337.94	Central Nervous System
09G03	Succinylsulfathiazole	C13H13N3O5S2	355.39	Infectiology
09G04	Famprofazone	C24H31N3O	377.53	Central Nervous System
09G05	Bromopride	C14H22BrN3O2	344.25	Central Nervous System
09G06	Methyl benzethonium chloride	C28H44ClNO2	462.12	Infectiology
09G07	Chlorcyclizine hydrochloride	C18H22Cl2N2	337.30	Allergology
09G08	Diphenylpyraline hydrochloride	C19H24ClNO	317.86	Allergology
09G09	Benzethonium chloride	C27H42ClNO2	448.09	Infectiology
09G10	Trioxsalen	C14H12O3	228.25	Dermatology
09G11	Doxofylline	C11H14N4O4	266.26	Respiratory
09H02	Sulfabenzamide	C13H12N2O3S	276.32	Infectiology
09H03	Benzocaine	C9H11NO2	165.19	Neuromuscular
09H04	Dipyrone	C13H16N3NaO4S	333.34	Central Nervous System
09H05	Isosorbide dinitrate	C6H8N2O8	236.14	Cardiovascular
09H06	Sulfachloropyridazine	C10H9ClN4O2S	284.73	Infectiology
09H07	Pramoxine hydrochloride	C17H28ClNO3	329.87	Neuromuscular

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09H08	Finasteride	C23H36N2O2	372.56	Endocrinology
09H09	Fluorometholone	C22H29FO4	376.47	Endocrinology
09H10	Cephalothin sodium salt	C16H15N2NaO6S2	418.43	Infectiology
09H11	Cefuroxime sodium salt	C16H15N4NaO8S	446.37	Infectiology
10A02	Althiazide	C11H14ClN3O4S3	383.90	Metabolism
10A03	Isopyrin hydrochloride	C14H20ClN3O	281.79	Central Nervous System
10A04	Phenethicillin potassium salt	C17H19KN2O5S	402.52	Infectiology
10A05	Sulfamethoxypyridazine	C11H12N4O3S	280.31	Infectiology
10A06	Deferoxamine mesylate	C26H52N6O11S	656.80	Diagnostic
10A07	Mephentermine hemisulfate	C22H36N2O4S	424.61	Cardiovascular
10A08	Liranaftate	C18H20N2O2S	328.44	Infectiology
10A09	Sulfadimethoxine	C12H14N4O4S	310.33	Infectiology
10A10	Sulfanilamide	C6H8N2O2S	172.21	Infectiology
10A11	Balsalazide Sodium	C17H13N3Na2O6	401.29	Gastroenterology
10B02	Sulfaquinoxaline sodium salt	C14H11N4NaO2S	322.32	Infectiology
10B03	Streptozotocin	C8H15N3O7	265.22	Oncology
10B04	Metoprolol-(+,-) (+)-tartrate salt	C34H56N2O12	684.83	Cardiovascular
10B05	Flumethasone	C22H28F2O5	410.46	Metabolism
10B06	Flecainide acetate	C19H24F6N2O5	474.40	Cardiovascular
10B07	Cefazolin sodium salt	C14H13N8NaO4S3	476.49	Metabolism
10B08	Atractyloside potassium salt	C30H44K2O16S2	803.01	Oncology
10B09	Folinic acid calcium salt	C20H21CaN7O7	511.51	Hematology
10B10	Levonordefrin	C9H13NO3	183.21	Cardiovascular
10B11	Ebselen	C13H9NOSe	274.18	Metabolism
10C02	Nadide	C21H27N7O14P2	663.44	Metabolism
10C03	Sulfamethizole	C9H10N4O2S2	270.33	Metabolism
10C04	Medrysone	C22H32O3	344.50	Metabolism
10C05	Flunixin meglumine	C21H28F3N3O7	491.47	Central Nervous System
10C06	Spiramycin	C43H73N2O14R	842.07	Metabolism
10C07	Glycopyrrolate	C19H28BrNO3	398.34	Gastroenterology
10C08	Aprepitant	C23H21F7N4O3	534.44	Metabolism
10C09	Monensin sodium salt	C36H61NaO11	692.87	Infectiology
10C10	Isoetharine mesylate salt	C14H25NO6S	335.42	Respiratory
10C11	Mevalonic-D, L acid lactone	C6H10O3	130.14	Cardiovascular
10D02	Terazosin hydrochloride	C19H26ClN5O4	423.90	Cardiovascular
10D03	Phenazopyridine hydrochloride	C11H12ClN5	249.70	Central Nervous System
10D04	Demeclocycline hydrochloride	C21H22Cl2N2O8	501.32	Metabolism
10D05	Fenoprofen calcium salt dihydrate	C30H30CaO8	558.65	Metabolism
10D06	Piperacillin sodium salt	C23H26N5NaO7S	539.55	Metabolism
10D07	Diethylstilbestrol	C18H20O2	268.36	Endocrinology
10D08	Chlorotrianisene	C23H21ClO3	380.88	Endocrinology

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10D09	Ribostamycin sulfate salt	C17H36N4O14S	552.56	Metabolism
10D10	Methacholine chloride	C8H18ClNO2	195.69	Diagnostic
10D11	Pipenzolate bromide	C22H28BrNO3	434.38	Gastroenterology
10E02	Butamben	C11H15NO2	193.25	Central Nervous System
10E03	Sulfapyridine	C11H11N3O2S	249.29	Metabolism
10E04	Meclofenoxate hydrochloride	C12H17Cl2NO3	294.18	Central Nervous System
10E05	Furaltadone hydrochloride	C13H17ClN4O6	360.76	Infectiology
10E06	Ethoxyquin	C14H19NO	217.31	Metabolism
10E07	Tinidazole	C8H13N3O4S	247.27	Infectiology
10E08	Guanadrel sulfate	C20H40N6O8S	524.64	Cardiovascular
10E09	Vidarabine	C10H13N5O4	267.25	Metabolism
10E10	Sulfameter	C11H12N4O3S	280.31	Metabolism
10E11	Isopropamide iodide	C23H33IN2O	480.44	Metabolism
10F02	Alclometasone dipropionate	C28H37ClO7	521.06	Metabolism
10F03	Leflunomide	C12H9F3N2O2	270.21	Immunology
10F04	Norgestrel(-)-D	C21H28O2	312.46	Endocrinology
10F05	Fluocinonide	C26H32F2O7	494.54	Metabolism
10F06	Sulfamethazine sodium salt	C12H13N4NaO2S	300.32	Metabolism
10F07	Guaifenesin	C10H14O4	198.22	Respiratory
10F08	Alexidine dihydrochloride	C26H58Cl2N10	581.73	Infectiology
10F09	Proadifen hydrochloride	C23H32ClNO2	389.97	Neuromuscular
10F10	Zomepirac sodium salt	C15H13ClNNaO3	313.72	Metabolism
10F11	Cinoxacin	C12H10N2O5	262.22	Metabolism
10G02	Clobetasol propionate	C25H32ClFO5	466.98	Metabolism
10G03	Podophyllotoxin	C22H22O8	414.42	Metabolism
10G04	Clofibric acid	C10H11ClO3	214.65	Metabolism
10G05	Bendroflumethiazide	C15H14F3N3O4S2	421.42	Cardiovascular
10G06	Dicumarol	C19H12O6	336.30	Hematology
10G07	Methimazole	C4H6N2S	114.17	Endocrinology
10G08	Merbromin	C20H8Br2HgNa2O6	750.66	Infectiology
10G09	Hexylcaine hydrochloride	C16H24ClNO2	297.83	Dermatology
10G10	Drofenine hydrochloride	C20H32ClNO2	353.94	Neuromuscular
10G11	Cycloheximide	C15H23NO4	281.35	Infectiology
10H02	(R) -Naproxen sodium salt	C14H13NaO3	252.25	Metabolism
10H03	Propidium iodide	C27H34I2N4	668.41	Infectiology
10H04	Cloperastine hydrochloride	C20H25Cl2NO	366.33	Respiratory
10H05	Eucatropine hydrochloride	C17H26ClNO3	327.85	Neuromuscular
10H06	Isocarboxazid	C12H13N3O2	231.26	Central Nervous System
10H07	Lithocholic acid	C24H40O3	376.58	Metabolism
10H08	Methotrimeprazine maleat salt	C23H28N2O5S	444.55	Central Nervous System
10H09	Dienestrol	C18H18O2	266.34	Endocrinology

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10H10	Pridinol methanesulfonate salt	C21H29NO4S	391.53	Central Nervous System
10H11	Amrinone	C10H9N3O	187.20	Cardiovascular
11A02	Carbinoxamine maleate salt	C20H23ClN2O5	406.87	Allergology
11A03	Methazolamide	C5H8N4O3S2	236.27	Metabolism
11A04	Pyrithyldione	C9H13NO2	167.21	Central Nervous System
11A05	Spectinomycin dihydrochloride	C14H26Cl2N2O7	405.28	Metabolism
11A06	Piromidic acid	C14H16N4O3	288.31	Metabolism
11A07	Trimipramine maleate salt	C24H30N2O4	410.52	Central Nervous System
11A08	Chloropyramine hydrochloride	C16H21Cl2N3	326.27	Allergology
11A09	Furazolidone	C8H7N3O5	225.16	Metabolism
11A10	Dichlorphenamide	C6H6Cl2N2O4S2	305.16	Ophthalmology
11A11	Sulconazole nitrate	C18H16Cl3N3O3S	460.77	Metabolism
11B02	Auranofin	C20H34AuO9PS	678.49	Metabolism
11B03	Cromolyn disodium salt	C23H14Na2O11	512.34	Allergology
11B04	Bucladesine sodium salt	C18H23N5NaO8P	491.38	Cardiovascular
11B05	Cefsulodin sodium salt	C22H19N4NaO8S2	554.54	Metabolism
11B06	Fosfosal	C7H7O6P	218.10	Central Nervous System
11B07	Suprofen	C14H12O3S	260.31	Central Nervous System
11B08	Deflazacort	C25H31NO6	441.53	Immunology
11B09	Nadolol	C17H27NO4	309.41	Cardiovascular
11B10	Moxalactam disodium salt	C20H18N6Na2O9S	564.44	Metabolism
11B11	Aminophylline	C16H24N10O4	420.43	Cardiovascular
11C02	Azlocillin sodium salt	C20H22N5NaO6S	483.48	Metabolism
11C03	Clidinium bromide	C22H26BrNO3	432.36	Neuromuscular
11C04	Sulfamonomethoxine	C11H12N4O3S	280.31	Metabolism
11C05	Benzthiazide	C15H14ClN3O4S3	431.94	Cardiovascular
11C06	Trichlormethiazide	C8H8Cl3N3O4S2	380.66	Cardiovascular
11C07	Oxalamine citrate salt	C20H27N3O8	437.45	Central Nervous System
11C08	Propantheline bromide	C23H30BrNO3	448.40	Neuromuscular
11C09	Viloxazine hydrochloride	C13H20ClNO3	273.76	Central Nervous System
11C10	Dimethadione	C5H7NO3	129.12	Central Nervous System
11C11	Ethaverine hydrochloride	C24H30ClNO4	431.96	Central Nervous System
11D02	Butacaine	C18H30N2O2	306.45	Dermatology
11D03	Cefoxitin sodium salt	C16H16N3NaO7S2	449.44	Metabolism
11D04	Ifosfamide	C7H15Cl2N2O2P	261.09	Oncology
11D05	Novobiocin sodium salt	C31H35N2NaO11	634.62	Metabolism
11D06	Tetrahydroxy-1,4-quinone monohydrate	C6H6O7	190.11	Dermatology
11D07	Indoprofen	C17H15NO3	281.31	Central Nervous System
11D08	Carbenoxolone disodium salt	C34H48Na2O7	614.74	Metabolism
11D09	Iocetamic acid	C12H13I3N2O3	613.96	Diagnostic

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11D10	Ganciclovir	C ₉ H ₁₃ N ₅ O ₄	255.24	Metabolism
11D11	Ethopropazine hydrochloride	C ₁₉ H ₂₅ ClN ₂ S	348.94	Central Nervous System
11E02	Olanzapine	C ₁₇ H ₂₀ N ₄ S	312.44	Central Nervous System
11E03	Trimeprazine tartrate	C ₄₀ H ₅₀ N ₄ O ₆ S ₂	747.00	Allergology
11E04	Nafcillin sodium salt monohydrate	C ₂₁ H ₂₃ N ₂ NaO ₆ S	454.48	Metabolism
11E05	Procyclidine hydrochloride	C ₁₉ H ₃₀ ClNO	323.91	Central Nervous System
11E06	Amiprilose hydrochloride	C ₁₄ H ₂₈ ClNO ₆	341.84	Immunology
11E07	Ethynylestradiol 3-methyl ether	C ₂₁ H ₂₆ O ₂	310.44	Endocrinology
11E08	(-) -Levobunolol hydrochloride	C ₁₇ H ₂₆ ClNO ₃	327.85	Ophthalmology
11E09	Iodixanol	C ₃₅ H ₄₄ I ₆ N ₆ O ₁₅	1550.20	Diagnostic
11E10	Clinafloxacin	C ₁₇ H ₁₇ ClF ₃ N ₃ O ₃	365.79	Infectiology
11E11	Equilin	C ₁₈ H ₂₀ O ₂	268.36	Endocrinology
11F02	Paroxetine Hydrochloride	C ₁₉ H ₂₁ ClFNO ₃	365.84	Central Nervous System
11F03	Nylidrin	C ₁₉ H ₂₅ NO ₂	299.42	Cardiovascular
11F04	Liothyronine	C ₁₅ H ₁₂ I ₃ NO ₄	650.98	Endocrinology
11F05	Roxithromycin	C ₄₁ H ₇₆ N ₂ O ₁₅	837.07	Metabolism
11F06	Beclomethasone dipropionate	C ₂₈ H ₃₇ ClO ₇	521.06	Metabolism
11F07	Tolmetin sodium salt dihydrate	C ₁₅ H ₁₈ NNaO ₅	315.30	Metabolism
11F08	(+) -Levobunolol hydrochloride	C ₁₇ H ₂₆ ClNO ₃	327.85	Ophthalmology
11F09	Doxazosin mesylate	C ₂₄ H ₂₉ N ₅ O ₈ S	547.59	Cardiovascular
11F10	Fluvastatin sodium salt	C ₂₄ H ₂₅ FNNaO ₄	433.46	Cardiovascular
11F11	Methylhydantoin-5-(L)	C ₄ H ₆ N ₂ O ₂	114.10	Central Nervous System
11G02	Gabapentin	C ₉ H ₁₇ NO ₂	171.24	Central Nervous System
11G03	Raloxifene hydrochloride	C ₂₈ H ₂₈ ClNO ₄ S	510.06	Endocrinology
11G04	Etidronic acid, disodium salt	C ₂ H ₆ Na ₂ O ₇ P ₂	249.99	Metabolism
11G05	Methylhydantoin-5-(D)	C ₄ H ₆ N ₂ O ₂	114.10	
11G06	Simvastatin	C ₂₅ H ₃₈ O ₅	418.58	Cardiovascular
11G07	Azacytidine-5	C ₈ H ₁₂ N ₄ O ₅	244.21	Oncology
11G08	Paromomycin sulfate	C ₂₃ H ₄₇ N ₅ O ₁₈ S	713.72	Metabolism
11G09	Acetaminophen	C ₈ H ₉ NO ₂	151.17	Central Nervous System
11G10	Phthalylsulfathiazole	C ₁₇ H ₁₃ N ₃ O ₅ S ₂	403.44	Metabolism
11G11	Luteolin	C ₁₅ H ₁₀ O ₆	286.24	Respiratory
11H02	Iopamidol	C ₁₇ H ₂₂ I ₃ N ₃ O ₈	777.09	Diagnostic
11H03	Iopromide	C ₁₈ H ₂₄ I ₃ N ₃ O ₈	791.12	Diagnostic
11H04	Theophylline monohydrate	C ₇ H ₁₀ N ₄ O ₃	198.18	Cardiovascular
11H05	Theobromine	C ₇ H ₈ N ₄ O ₂	180.17	Cardiovascular
11H06	Reserpine	C ₃₃ H ₄₀ N ₂ O ₉	608.69	Central Nervous System
11H07	Bicalutamide	C ₁₈ H ₁₄ F ₄ N ₂ O ₄ S	430.38	Endocrinology
11H08	Scopolamine hydrochloride	C ₁₇ H ₂₂ ClNO ₄	339.82	Central Nervous System
11H09	Ioversol	C ₁₈ H ₂₄ I ₃ N ₃ O ₉	807.12	Diagnostic
11H10	Rabeprazole Sodium salt	C ₁₈ H ₂₁ N ₃ NaO ₃ S	382.44	Metabolism

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11H11	Carbachol	C6H15ClN2O2	182.65	Cardiovascular
12A02	Niacin	C6H5NO2	123.11	Cardiovascular
12A03	Bemegride	C8H13NO2	155.20	Central Nervous System
12A04	Digoxigenin	C23H34O5	390.52	Diagnostic
12A05	Meglumine	C7H17NO5	195.22	Metabolism
12A06	Dolasetron mesilate	C20H26N2O7S	438.50	Central Nervous System
12A07	Clioquinol	C9H5ClINO	305.50	Metabolism
12A08	Oxybenzone	C14H12O3	228.25	Dermatology
12A09	Promethazine hydrochloride	C17H21ClN2S	320.89	Allergology
12A10	Diacerein	C19H12O8	368.30	Immunology
12A11	Esmolol hydrochloride	C16H26ClNO4	331.84	Cardiovascular
12B02	Cortisol acetate	C23H32O6	404.51	Metabolism
12B03	Flubendazol	C16H12FN3O3	313.29	Metabolism
12B04	Felbinac	C14H12O2	212.25	Central Nervous System
12B05	Butylparaben	C11H14O3	194.23	Metabolism
12B06	Aminohippuric acid	C9H10N2O3	194.19	Diagnostic
12B07	N-Acetyl-L-leucine	C8H15NO3	173.21	Central Nervous System
12B08	Pipemidic acid	C14H17N5O3	303.32	Metabolism
12B09	Dioxybenzone	C14H12O4	244.25	Dermatology
12B10	Adrenosterone	C19H24O3	300.40	Endocrinology
12B11	Methylatropine nitrate	C18H26N2O6	366.42	Neuromuscular
12C02	Hymecromone	C10H8O3	176.17	Metabolism
12C03	Abacavir Sulfate	C28H38N12O6S	670.76	Infectiology
12C04	Diloxanide furoate	C14H11Cl2NO4	328.15	Metabolism
12C05	Metyrapone	C14H14N2O	226.28	Endocrinology
12C06	Urapidil hydrochloride	C20H30ClN5O3	423.95	Cardiovascular
12C07	Fluspirilen	C29H31F2N3O	475.59	Central Nervous System
12C08	S-(+)-ibuprofen	C13H18O2	206.29	Central Nervous System
12C09	Ethinodiol diacetate	C24H32O4	384.52	Endocrinology
12C10	Nabumetone	C15H16O2	228.29	Central Nervous System
12C11	Nisoxetine hydrochloride	C17H22ClNO2	307.82	Central Nervous System
12D02	(+)-Isoproterenol (+)-bitartrate salt	C15H23NO9	361.35	Respiratory
12D03	Monobenzone	C13H12O2	200.24	Dermatology
12D04	2-Aminobenzenesulfonamide	C6H8N2O2S	172.21	Metabolism
12D05	Estrone	C18H22O2	270.37	Endocrinology
12D06	Lorglumide sodium salt	C22H31Cl2N2NaO4	481.40	Metabolism
12D07	Nitrendipine	C18H20N2O6	360.37	Cardiovascular
12D08	Flurbiprofen	C15H13FO2	244.27	Central Nervous System
12D09	Nimodipine	C21H26N2O7	418.45	Cardiovascular
12D10	Bacitracin	C66H103N17O16S	1422.73	Metabolism
12D11	L(-)-vesamicol hydrochloride	C17H26ClNO	295.86	Neuromuscular

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12E02	Nizatidine	C12H21N5O2S2	331.46	Metabolism
12E03	Thiopiperamide maleate	C19H28N4O4S	408.52	Central Nervous System
12E04	Xamoterol hemifumarate	C36H54N6O14	794.86	Cardiovascular
12E05	Rolipram	C16H21NO3	275.35	Central Nervous System
12E06	Thonzonium bromide	C32H55BrN4O	591.73	Dermatology
12E07	Idazoxan hydrochloride	C11H13ClN2O2	240.69	Central Nervous System
12E08	Quinapril HCl	C25H31ClN2O5	474.99	Cardiovascular
12E09	Nilutamide	C12H10F3N3O4	317.23	Oncology
12E10	Ketorolac tromethamine	C19H24N2O6	376.41	Central Nervous System
12E11	Protriptyline hydrochloride	C19H22ClN	299.85	Central Nervous System
12F02	Propofol	C12H18O	178.28	Central Nervous System
12F03	S(-)Eticlopride hydrochloride	C17H26Cl2N2O3	377.31	Central Nervous System
12F04	Primidone	C12H14N2O2	218.26	Central Nervous System
12F05	Flucytosine	C4H4FN3O	129.09	Metabolism
12F06	(-)-MK 801 hydrogen maleate	C20H19NO4	337.38	Central Nervous System
12F07	Bephenium hydroxynaphthoate	C28H29NO4	443.55	Metabolism
12F08	Dehydroisoandosterone 3-acetate	C21H30O3	330.47	Endocrinology
12F09	Benserazide hydrochloride	C10H16ClN3O5	293.71	Central Nervous System
12F10	Iodipamide	C20H14I6N2O6	1139.77	Diagnostic
12F11	Allopurinol	C5H4N4O	136.11	Metabolism
12G02	Pentetic acid	C14H23N3O10	393.35	Oncology
12G03	Bretylum tosylate	C18H24BrN3O3S	414.36	Cardiovascular
12G04	Pralidoxime chloride	C7H9ClN2O	172.62	Neuromuscular
12G05	Phenoxybenzamine hydrochloride	C18H23Cl2NO	340.30	Cardiovascular
12G06	Salmeterol	C25H37NO4	415.58	Respiratory
12G07	Altretamine	C9H18N6	210.28	Oncology
12G08	Prazosin hydrochloride	C19H22ClN5O4	419.87	Cardiovascular
12G09	Timolol maleate salt	C17H28N4O7S	432.50	Cardiovascular
12G10	(+,-)-Octopamine hydrochloride	C8H12ClNO2	189.64	Cardiovascular
12G11	Stavudine	C10H12N2O4	224.22	Infectiology
12H02	Crotamiton	C13H17NO	203.29	Dermatology
12H03	Toremifene	C26H28ClNO	405.97	Endocrinology
12H04	(R)-(+)-Atenolol	C14H22N2O3	266.34	Cardiovascular
12H05	Tyloxapol	C59H96O12	997.42	Respiratory
12H06	Florfenicol	C12H14Cl2FNO4S	358.22	Metabolism
12H07	Megestrol acetate	C24H32O4	384.52	Endocrinology
12H08	Deoxycorticosterone	C21H30O3	330.47	Endocrinology
12H09	Urosiol	C24H40O4	392.58	Metabolism
12H10	Proparacaine hydrochloride	C16H27ClN2O3	330.86	Central Nervous System
12H11	Aminocaproic acid	C6H13NO2	131.18	Allergology
13A02	Denatonium benzoate	C28H34N2O3	446.59	Neuromuscular

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13A03	Canrenone	C22H28O3	340.47	Endocrinology
13A04	Enilconazole	C14H14Cl2N2O	297.19	Metabolism
13A05	Methacycline hydrochloride	C22H23ClN2O8	478.89	Metabolism
13A06	Floxuridine	C9H11FN2O5	246.20	Oncology
13A07	Sotalol hydrochloride	C12H21ClN2O3S	308.83	Cardiovascular
13A08	Gestrinone	C21H24O2	308.42	Endocrinology
13A09	Decamethonium bromide	C16H38Br2N2	418.30	Neuromuscular
13A10	Darifenacin hydrobromide	C28H31BrN2O2	507.48	Neuromuscular
13A11	Indatraline hydrochloride	C16H16Cl3N	328.67	Central Nervous System
13B02	Remoxipride Hydrochloride	C16H24BrClN2O3	407.74	Central Nervous System
13B03	THIP Hydrochloride	C6H9ClN2O2	176.60	Central Nervous System
13B04	Pirlindole mesylate	C16H22N2O3S	322.43	Central Nervous System
13B05	Pronethalol hydrochloride	C15H20ClNO	265.79	Cardiovascular
13B06	Naftopidil dihydrochloride	C24H30Cl2N2O3	465.42	Cardiovascular
13B07	Tracazolate hydrochloride	C16H25ClN4O2	340.86	Central Nervous System
13B08	Zardaverine	C12H10F2N2O3	268.22	Respiratory
13B09	Memantine Hydrochloride	C12H22ClN	215.77	Central Nervous System
13B10	Ozagrel hydrochloride	C13H13ClN2O2	264.71	Cardiovascular
13B11	Piribedil hydrochloride	C16H19ClN4O2	334.81	Cardiovascular
13C02	Nitrocaramiphen hydrochloride	C18H27ClN2O4	370.88	Central Nervous System
13C03	Nandrolone	C18H26O2	274.41	Endocrinology
13C04	Dimaprit dihydrochloride	C6H17Cl2N3S	234.19	Metabolism
13C05	Oxfendazol	C15H13N3O3S	315.35	Metabolism
13C06	Guaiacol	C7H8O2	124.14	Respiratory
13C07	Proscillaridin A	C30H42O8	530.66	Cardiovascular
13C08	Pramipexole	C10H17N3S	211.33	Central Nervous System
13C09	Norgestimate	C23H31NO3	369.51	Endocrinology
13C10	Chlormadinone acetate	C23H29ClO4	404.94	Endocrinology
13C11	Phenylbutazone	C19H20N2O2	308.38	Metabolism
13D02	Gliquidone	C27H33N3O6S	527.64	Endocrinology
13D03	Pizotifen malate	C23H27NO5S	429.54	Allergology
13D04	Ribavirin	C8H12N4O5	244.21	Metabolism
13D05	Cyclopenthiazide	C13H18ClN3O4S2	379.89	Cardiovascular
13D06	Fluvoxamine maleate	C19H25F3N2O6	434.42	Central Nervous System
13D07	Prothionamide	C9H12N2S	180.27	Infectiology
13D08	Fluticasone propionate	C25H31F3O5S	500.58	Cardiovascular
13D09	Zuclopenthixol dihydrochloride	C22H27Cl3N2OS	473.90	Central Nervous System
13D10	Proguanil hydrochloride	C11H17Cl2N5	290.20	Metabolism
13D11	Lymecycline	C29H38N4O10	602.65	Metabolism
13E02	Alfadolone acetate	C23H34O5	390.52	Central Nervous System
13E03	Alfaxalone	C21H32O3	332.49	Central Nervous System

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13E04	Azapropazone	C16H20N4O2	300.36	Central Nervous System
13E05	Meptazinol hydrochloride	C15H24ClNO	269.82	Central Nervous System
13E06	Apramycin	C21H41N5O11	539.59	Metabolism
13E07	Epitiostanol	C19H30OS	306.51	Oncology
13E08	Fursultiamine Hydrochloride	C17H27ClN4O3S2	435.01	Metabolism
13E09	Gabexate mesilate	C17H27N3O7S	417.48	Hematology
13E10	Pivampicillin	C22H29N3O6S	463.56	Metabolism
13E11	Talampicillin hydrochloride	C24H24ClN3O6S	517.99	Metabolism
13F02	Flucloxacillin sodium	C19H16ClFN3NaO5S	475.86	Metabolism
13F03	Trapidil	C10H15N5	205.26	Cardiovascular
13F04	Deptropine citrate	C29H35NO8	525.60	Allergology
13F05	Sertraline	C17H17Cl2N	306.24	Central Nervous System
13F06	Ethamsylate	C10H17NO5S	263.31	Cardiovascular
13F07	Moxonidine	C9H12ClN5O	241.68	Cardiovascular
13F08	Etilefrine hydrochloride	C10H16ClNO2	217.70	Cardiovascular
13F09	Alprostadil	C20H34O5	354.49	Cardiovascular
13F10	Tribenoside	C29H34O6	478.59	Cardiovascular
13F11	Rimexolone	C24H34O3	370.54	Metabolism
13G02	Isradipine	C19H21N3O5	371.40	Cardiovascular
13G03	Tiletamine hydrochloride	C12H18ClNOS	259.80	Central Nervous System
13G04	Isometheptene mucate	C24H48N2O8	492.66	Cardiovascular
13G05	Nifurtimox	C10H13N3O5S	287.30	Metabolism
13G06	Letrozole	C17H11N5	285.31	Oncology
13G07	Arbutin	C12H16O7	272.26	Metabolism
13G08	Tocainide hydrochloride	C11H17ClN2O	228.72	Cardiovascular
13G09	Benzathine benzylpenicillin	C48H56N6O10S2	941.14	Metabolism
13G10	Risperidone	C23H27FN4O2	410.50	Central Nervous System
13G11	Torsemide	C16H20N4O3S	348.43	Cardiovascular
13H02	Halofantrine hydrochloride	C26H31Cl3F3NO	536.90	Metabolism
13H03	Articaine hydrochloride	C13H21ClN2O3S	320.84	Central Nervous System
13H04	Nomegestrol acetate	C23H30O4	370.49	Endocrinology
13H05	Pancuronium bromide	C35H60Br2N2O4	732.69	Neuromuscular
13H06	Molindone hydrochloride	C16H25ClN2O2	312.84	Central Nervous System
13H07	Alcuronium chloride	C44H50Cl2N4O2	737.82	Neuromuscular
13H08	Zalcitabine	C9H13N3O3	211.22	Metabolism
13H09	Methyldopate hydrochloride	C12H18ClNO4	275.73	Cardiovascular
13H10	Levocabastine hydrochloride	C26H30ClFN2O2	456.99	Allergology
13H11	Pyruvium pamoate	C75H70N6O6	1151.43	Metabolism
14A02	Etomidate	C14H16N2O2	244.30	Central Nervous System
14A03	Tridihexethyl chloride	C21H36ClNO	353.98	Neuromuscular
14A04	Penbutolol sulfate	C36H60N2O8S	680.95	Cardiovascular

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14A05	Prednicarbate	C27H36O8	488.58	Metabolism
14A06	Sertaconazole nitrate	C20H16Cl3N3O4S	500.79	Metabolism
14A07	Repaglinide	C27H36N2O4	452.60	Endocrinology
14A08	Piretanide	C17H18N2O5S	362.41	Cardiovascular
14A09	Piperacetazine	C24H30N2O2S	410.58	Central Nervous System
14A10	Oxyphenbutazone	C19H20N2O3	324.38	Metabolism
14A11	Quinethazone	C10H12ClN3O3S	289.74	Cardiovascular
14B02	Moricizine hydrochloride	C22H26ClN3O4S	463.99	Cardiovascular
14B03	Iopanoic acid	C11H12I3NO2	570.94	Diagnostic
14B04	Pivmecillinam hydrochloride	C21H34ClN3O5S	476.04	Metabolism
14B05	Levopropoxyphene napsylate	C32H37NO5S	547.72	Central Nervous System
14B06	Piperidolate hydrochloride	C21H26ClNO2	359.90	Neuromuscular
14B07	Trifluridine	C10H11F3N2O5	296.20	Metabolism
14B08	Oxprenolol hydrochloride	C15H24ClNO3	301.82	Cardiovascular
14B09	Ondansetron Hydrochloride	C18H20ClN3O	329.83	Central Nervous System
14B10	Propoxycaine hydrochloride	C16H27ClN2O3	330.86	Central Nervous System
14B11	Oxaprozin	C18H15NO3	293.33	Central Nervous System
14C02	Phensuximide	C11H11NO2	189.22	Central Nervous System
14C03	Ioxaglic acid	C24H21I6N5O8	1268.89	Diagnostic
14C04	Naftifine hydrochloride	C21H22ClN	323.87	Infectiology
14C05	Meprylcaine hydrochloride	C14H22ClNO2	271.79	Neuromuscular
14C06	Milrinone	C12H9N3O	211.23	Cardiovascular
14C07	Methantheline bromide	C21H26BrNO3	420.35	Neuromuscular
14C08	Ticarcillin sodium	C15H15N2NaO6S2	406.41	Infectiology
14C09	Thiethylperazine dimalate	C30H41N3O10S2	667.80	Central Nervous System
14C10	Mesalamine	C7H7NO3	153.14	Metabolism
14C11	Vorinostat	C14H20N2O3	264.33	Oncology
14D02	Imidurea	C11H16N8O8	388.30	Infectiology
14D03	Lansoprazole	C16H14F3N3O2S	369.37	Metabolism
14D04	Bethanechol chloride	C7H17ClN2O2	196.68	Metabolism
14D05	Cyproterone acetate	C24H29ClO4	416.95	Endocrinology
14D06	(R)-Propranolol hydrochloride	C16H22ClNO2	295.81	Cardiovascular
14D07	Ciprofibrate	C13H14Cl2O3	289.16	Metabolism
14D08	Formestane	C19H26O3	302.42	Endocrinology
14D09	Benzylpenicillin sodium	C16H17N2NaO4S	356.38	Infectiology
14D10	Chlorambucil	C14H19Cl2NO2	304.22	Oncology
14D11	Methiazole	C12H15N3O2S	265.34	Infectiology
14E02	(S)-propranolol hydrochloride	C16H22ClNO2	295.81	Cardiovascular
14E03	(-)-Eseroline fumarate salt	C17H22N2O5	334.38	Central Nervous System
14E04	Isosorbide mononitrate	C6H9NO6	191.14	Cardiovascular
14E05	Levalbuterol hydrochloride	C13H22ClNO3	275.78	Respiratory

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14E06	Topiramate	C12H21NO8S	339.37	Central Nervous System
14E07	D-cycloserine	C3H6N2O2	102.09	Infectiology
14E08	2-Chloropyrazine	C4H3ClN2	114.53	Cardiovascular
14E09	(+,-)-Synephrine	C9H13NO2	167.21	Cardiovascular
14E10	(S)-(-)-Cycloserine	C3H6N2O2	102.09	Infectiology
14E11	Homosalate	C16H22O3	262.35	Dermatology
14F02	Spaglumic acid	C11H16N2O8	304.26	Allergology
14F03	Ranolazine	C24H33N3O4	427.55	Cardiovascular
14F04	Misoprostol	C22H38O5	382.55	Metabolism
14F05	Sulfadoxine	C12H14N4O4S	310.33	Infectiology
14F06	Cyclopentolate hydrochloride	C17H26ClNO3	327.85	Metabolism
14F07	Estriol	C18H24O3	288.39	Endocrinology
14F08	(-)-Isoproterenol hydrochloride	C11H18ClNO3	247.72	Cardiovascular
14F09	Sarafloxacin	C20H17F2N3O3	385.37	Infectiology
14F10	Nialamide	C16H18N4O2	298.35	Central Nervous System
14F11	Toltrazuril	C18H14F3N3O4S	425.39	Infectiology
14G02	Perindopril	C19H32N2O5	368.48	Cardiovascular
14G03	Fexofenadine HCl	C32H40ClNO4	538.13	Allergology
14G04	4-aminosalicylic acid	C7H7NO3	153.14	Infectiology
14G05	Clonixin Lysinate	C19H25ClN4O4	408.89	Central Nervous System
14G06	Verteporfin	C41H42N4O8	718.81	Ophthalmology
14G07	Meropenem	C17H25N3O5S	383.47	Infectiology
14G08	Ramipril	C23H32N2O5	416.52	Cardiovascular
14G09	Mephenytoin	C12H14N2O2	218.26	Central Nervous System
14G10	Rifabutin	C46H62N4O11	847.03	Infectiology
14G11	Parbendazole	C13H17N3O2	247.30	Infectiology
14H02	Mecamylamine hydrochloride	C11H22ClN	203.76	Cardiovascular
14H03	Procarbazine hydrochloride	C12H20ClN3O	257.77	Oncology
14H04	Viomycin sulfate	C25H45N13O14S	783.78	Infectiology
14H05	Saquinavir mesylate	C39H54N6O8S	766.96	Immunology
14H06	Ronidazole	C6H8N4O4	200.16	Infectiology
14H07	Dorzolamide hydrochloride	C10H17ClN2O4S3	360.90	Cardiovascular
14H08	Azaperone	C19H22FN3O	327.41	Central Nervous System
14H09	Cefepime hydrochloride	C19H25ClN6O5S2	517.03	Infectiology
14H10	Clocortolone pivalate	C27H36ClFO5	495.04	Endocrinology
14H11	Nadifloxacin	C19H21FN2O4	360.39	Infectiology
15A02	Buspirone hydrochloride	C21H32ClN5O2	421.97	Central Nervous System
15A03	Anastrozole	C17H19N5	293.37	Oncology
15A04	Doxycycline hydrochloride	C22H25ClN2O8	480.91	Metabolism
15A05	Sulbactam	C8H11NO5S	233.24	Infectiology
15A06	Fleroxacin	C17H18F3N3O3	369.35	Infectiology

Chapter 5: Appendix

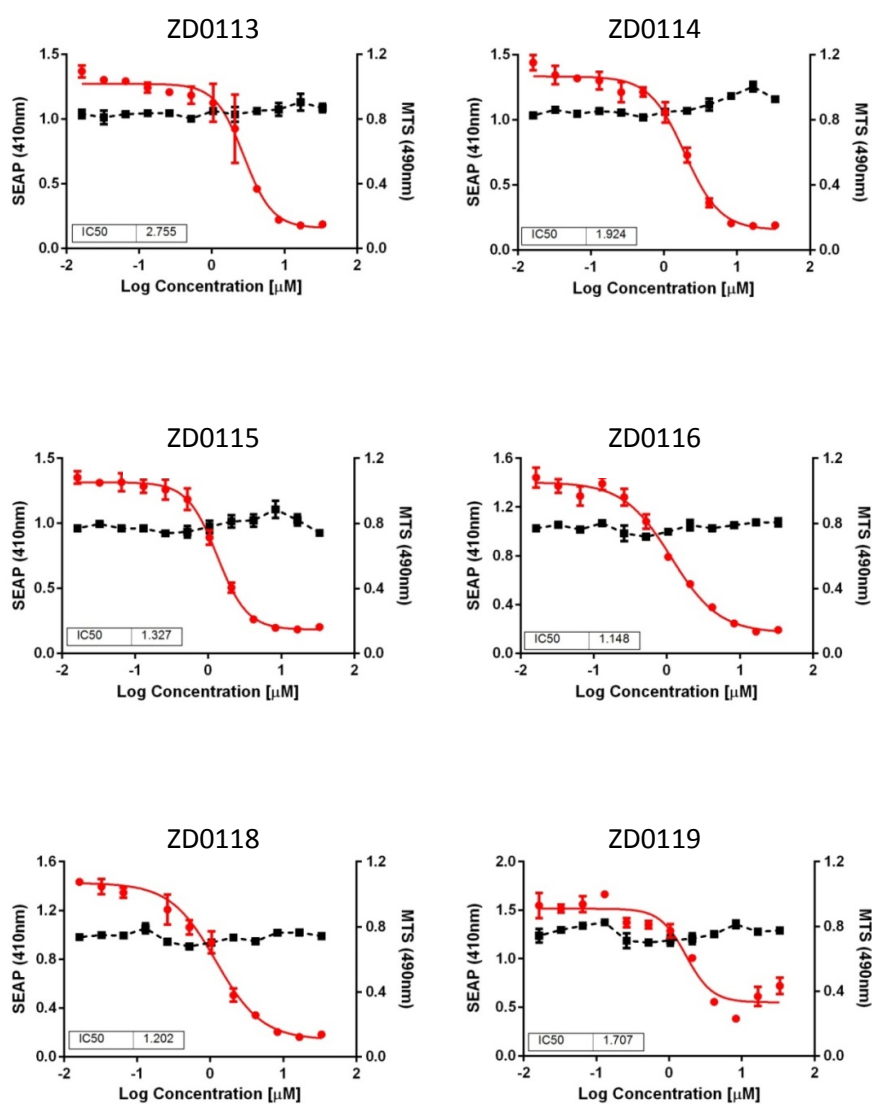
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15A09	Mepivacaine hydrochloride	C15H23ClN2O	282.82	Neuromuscular
15A10	Rifaximin	C43H51N3O11	785.90	Infectiology
15A11	Estradiol Valerate	C23H32O3	356.51	Endocrinology
15B02	Acetylcysteine	C5H9NO3S	163.20	Metabolism
15B03	Melengestrol acetate	C25H32O4	396.53	Endocrinology
15B04	Bromhexine hydrochloride	C14H21Br2ClN2	412.60	Respiratory
15B05	Anethole-trithione	C10H8OS3	240.37	Metabolism
15B06	Amcinonide	C28H35FO7	502.59	Metabolism
15B07	Caffeine	C8H10N4O2	194.19	Central Nervous System
15B08	Carvedilol	C24H26N2O4	406.49	Cardiovascular
15B09	Methenamine	C6H12N4	140.19	Infectiology
15B10	Phentermine hydrochloride	C10H16ClN	185.70	Central Nervous System
15B11	Diclazuril	C17H9Cl3N4O2	407.65	Metabolism
15C02	Famciclovir	C14H19N5O4	321.34	Infectiology
15C03	Dopamine hydrochloride	C8H12ClNO2	189.64	Cardiovascular
15C04	Cefdinir	C14H13N5O5S2	395.42	Infectiology
15C05	Carprofen	C15H12ClNO2	273.72	Metabolism
15C06	Celecoxib	C17H14F3N3O2S	381.38	Metabolism
15C07	Candesartan	C24H20N6O3	440.47	Cardiovascular
15C08	Fludarabine	C10H12FN5O4	285.24	Oncology
15C09	Cladribine	C10H12ClN5O3	285.69	Oncology
15C10	Vardenafil	C23H32N6O4S	488.61	Cardiovascular
15C11	Fluconazole	C13H12F2N6O	306.28	Metabolism
15D02	5-fluorouracil	C4H3FN2O2	130.08	Oncology
15D03	Mesna	C2H5NaO3S2	164.18	Oncology
15D04	Mitotane	C14H10Cl4	320.05	Endocrinology
15D05	Ambrisentan	C22H22N2O4	378.43	Cardiovascular
15D06	Triclosan	C12H7Cl3O2	289.55	Infectiology
15D07	Enoxacin	C15H17FN4O3	320.33	Infectiology
15D08	Olopatadine hydrochloride	C21H24ClNO3	373.88	Allergology
15D09	Granisetron	C18H24N4O	312.42	Endocrinology
15D10	Anthralin	C14H10O3	226.23	Dermatology
15D11	Lamotrigine	C9H7Cl2N5	256.10	Central Nervous System
15E02	Clofibrate	C12H15ClO3	242.70	Metabolism
15E03	Cyclophosphamide	C7H15Cl2N2O2P	261.09	Immunology
15E04	Aripiprazole	C23H27Cl2N3O2	448.40	Central Nervous System
15E05	Ethinylestradiol	C20H24O2	296.41	Endocrinology
15E06	Fluocinolone acetonide	C24H30F2O6	452.50	Metabolism
15E07	Sparfloxacin	C19H22F2N4O3	392.41	Infectiology

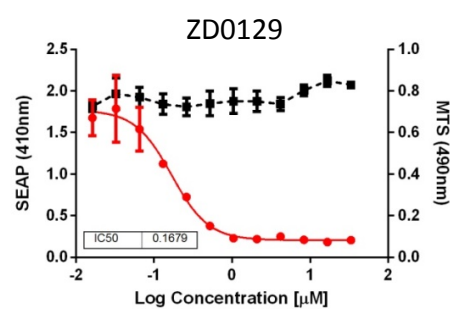
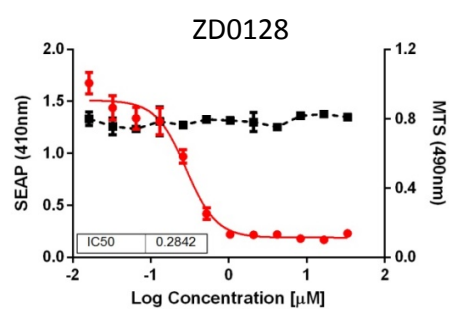
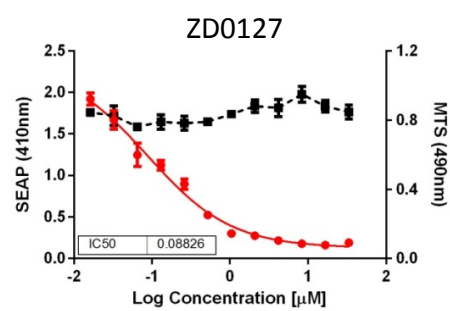
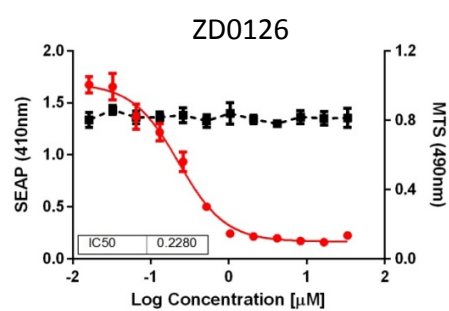
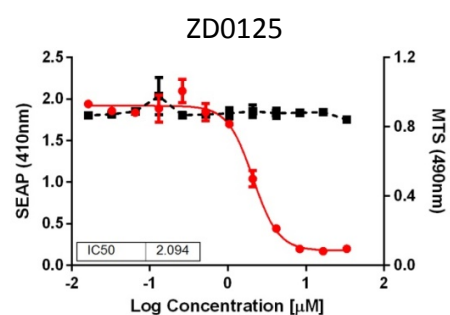
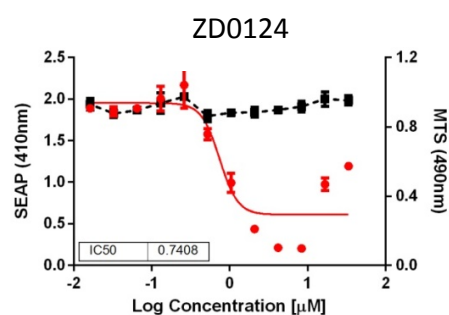
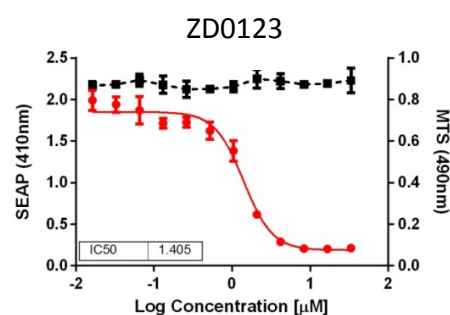
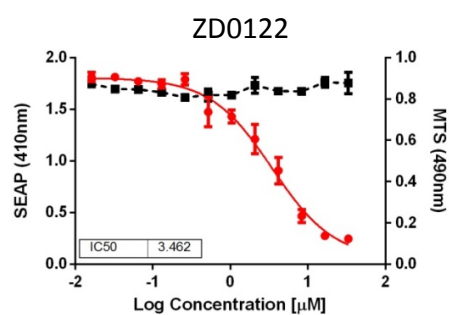
Chapter 5: Appendix

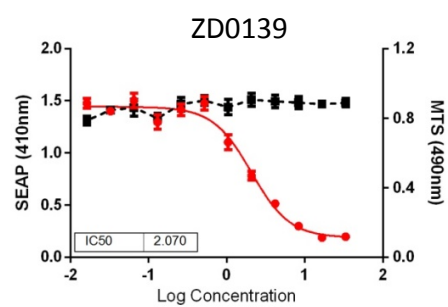
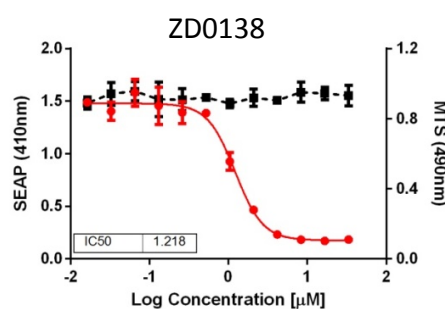
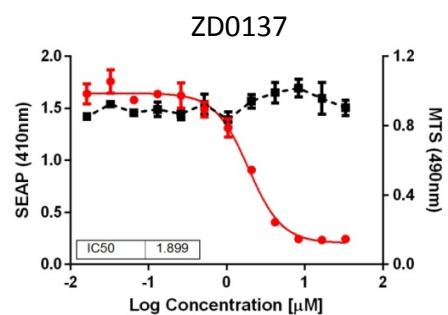
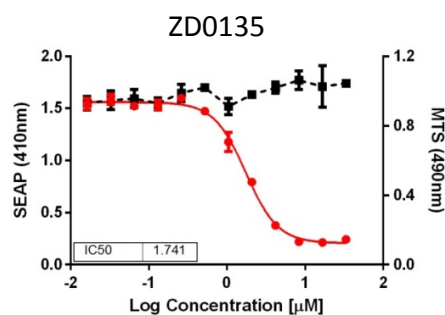
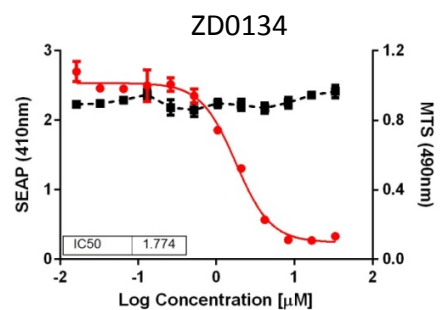
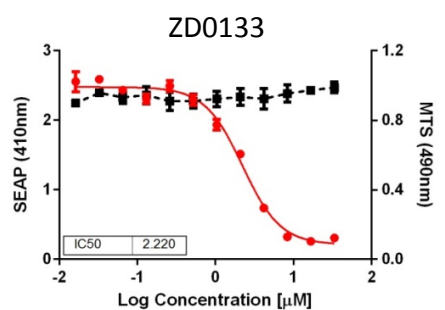
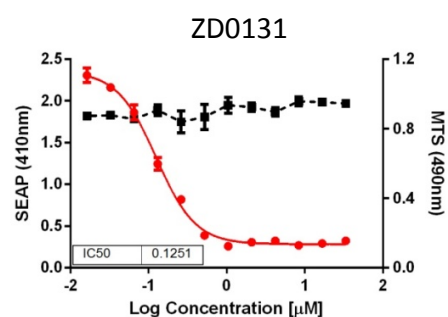
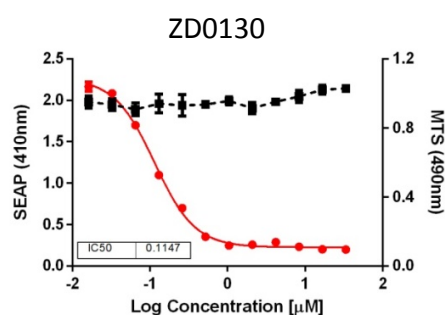
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15E09	Clarithromycin	C38H69NO13	747.97	Infectiology
15E10	Tripelennamine hydrochloride	C16H22ClN3	291.83	Allergology
15E11	Tulobuterol	C12H18ClNO	227.74	Respiratory
15F02	Topotecan	C23H23N3O5	421.46	Oncology
15F03	Atorvastatin	C33H35FN2O5	558.66	Metabolism
15F04	Azithromycin	C38H72N2O12	749.00	Infectiology
15F05	Ibudilast	C14H18N2O	230.31	Metabolism
15F06	Losartan	C22H23ClN6O	422.92	Cardiovascular
15F07	Benzotropine mesylate	C22H29NO4S	403.54	Central Nervous System
15F08	Vecuronium bromide	C34H57BrN2O4	637.75	Metabolism
15F09	Telmisartan	C33H30N4O2	514.63	Cardiovascular
15F10	Nalmefene hydrochloride	C21H26ClNO3	375.90	Central Nervous System
15F11	Bifonazole	C22H18N2	310.40	Infectiology
15G02	Gatifloxacin	C19H22FN3O4	375.40	Infectiology
15G03	Bosentan	C27H29N5O6S	551.63	Cardiovascular
15G04	Gemcitabine	C9H11F2N3O4	263.20	Oncology
15G05	Olmesartan	C29H30N6O6	558.60	Cardiovascular
15G06	Racepinephrine HCl	C9H14ClNO3	219.67	Cardiovascular
15G07	Montelukast	C35H36ClNO3S	586.20	Respiratory
15G08	Docetaxel	C43H53NO14	807.90	Oncology
15G09	Cilnidipine	C27H28N2O7	492.53	Cardiovascular
15G10	Imiquimod	C14H16N4	240.31	Dermatology
15G11	Fosinopril	C30H46NO7P	563.68	Cardiovascular
15H02	Imatinib	C29H31N7O	493.62	Oncology
15H03	Moxifloxacin	C21H24FN3O4	401.44	Infectiology
15H04	Formoterol fumarate	C42H52N4O12	804.90	Respiratory
15H05	Rufloxacin	C17H18FN3O3S	363.41	Infectiology
15H06	Pravastatin	C23H36O7	424.54	Metabolism
15H07	Rosiglitazone Hydrochloride	C18H20ClN3O3S	393.90	Metabolism
15H08	Rivastigmine	C14H22N2O2	250.34	Central Nervous System
15H09	Sildenafil	C22H30N6O4S	474.59	Cardiovascular
15H10	Acetylsalicylic acid	C9H8O4	180.16	Central Nervous System
15H11	Hexachlorophene	C13H6Cl6O2	406.91	Infectiology

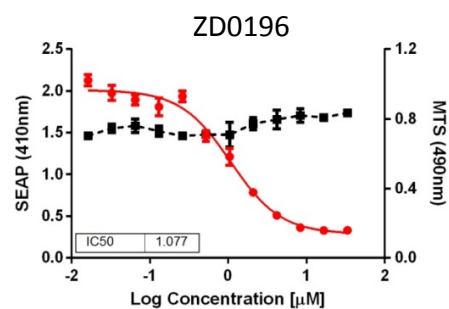
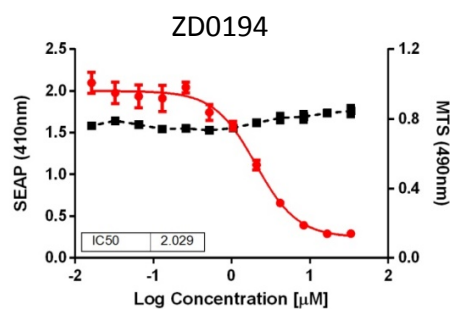
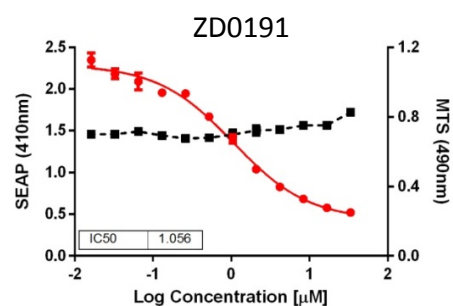
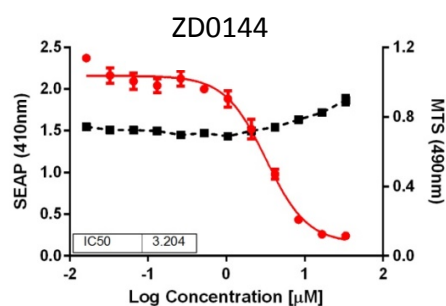
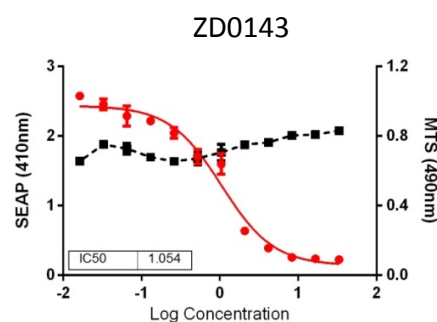
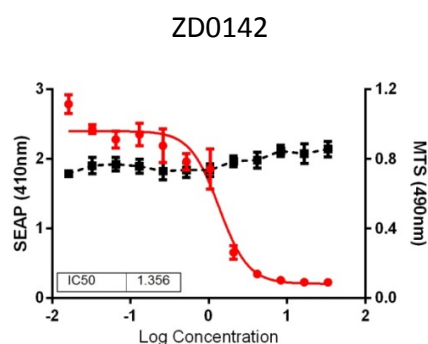
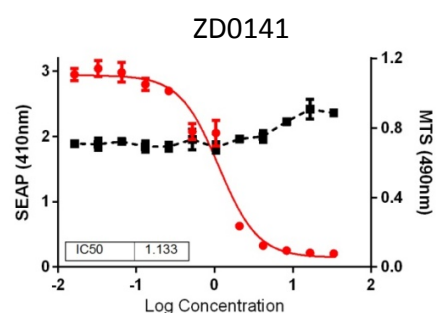
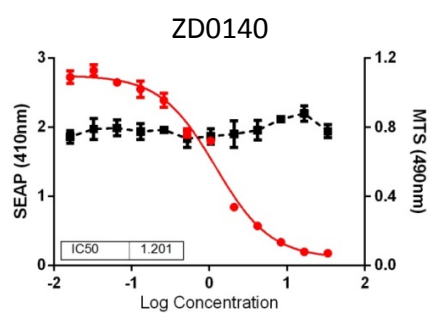
Appendix II

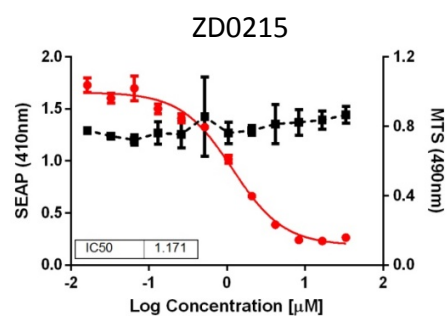
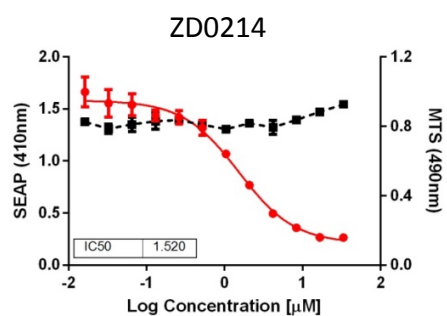
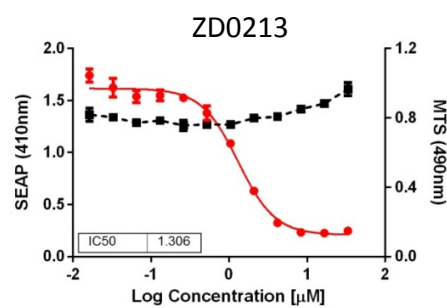
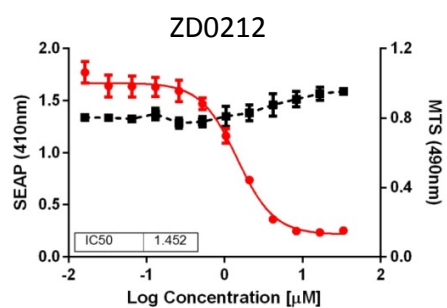
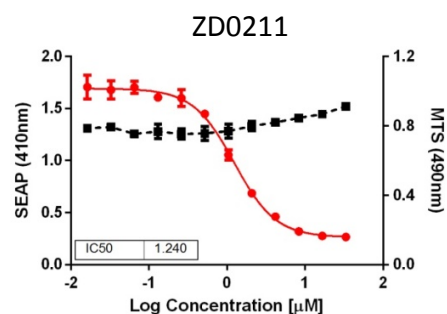
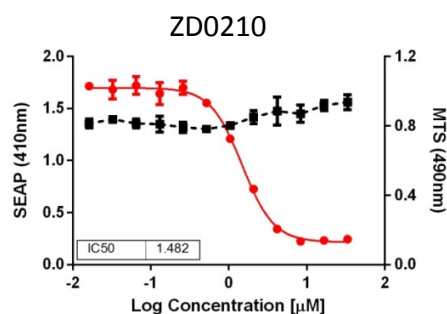
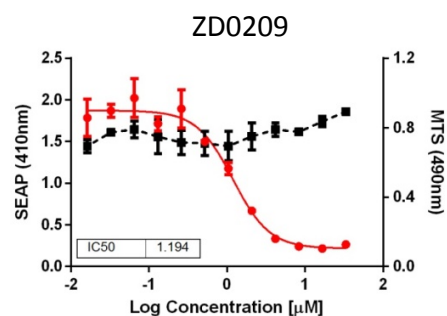
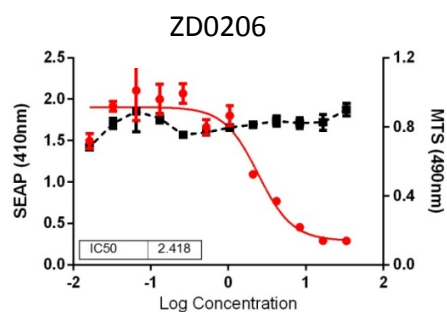
Dose Response Curves

1. The second screen with HEK-Blue IL1 β Cells

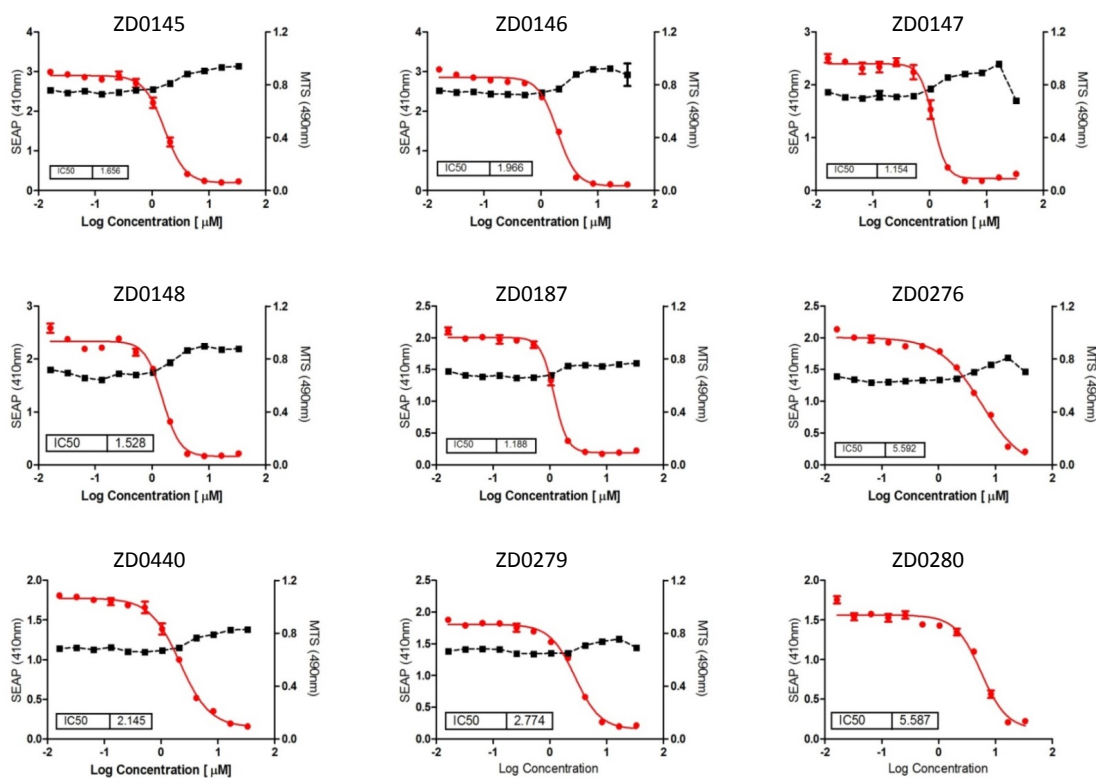


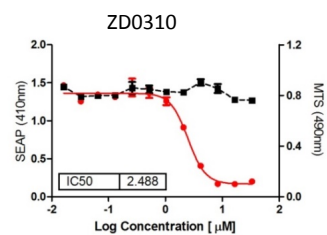
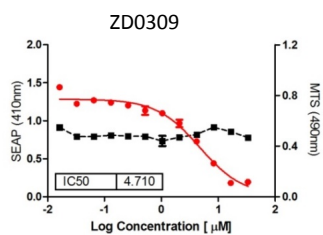
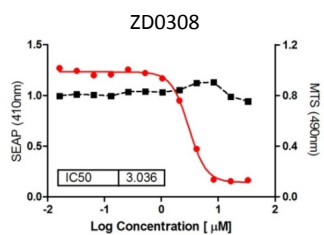
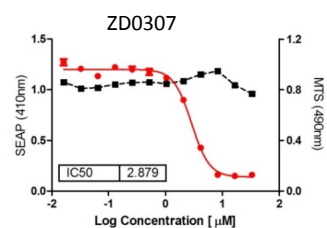
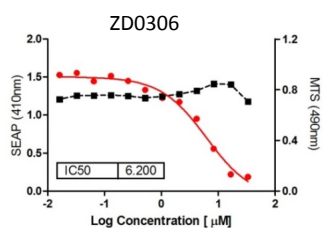
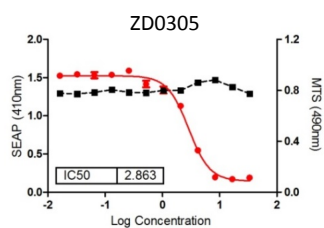
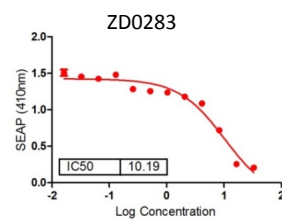
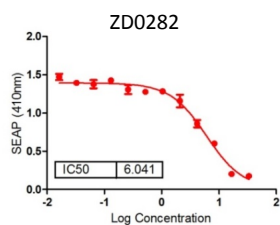
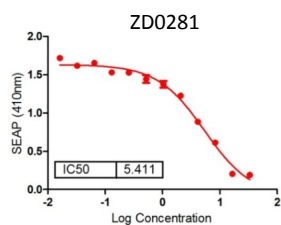


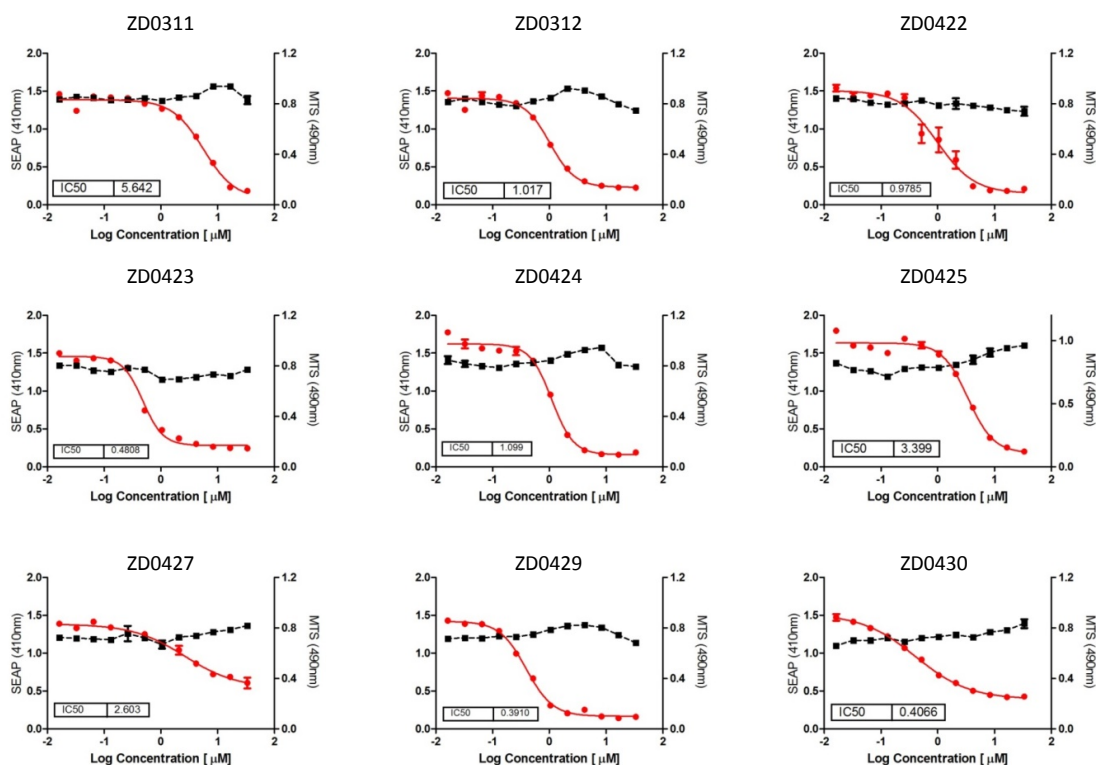




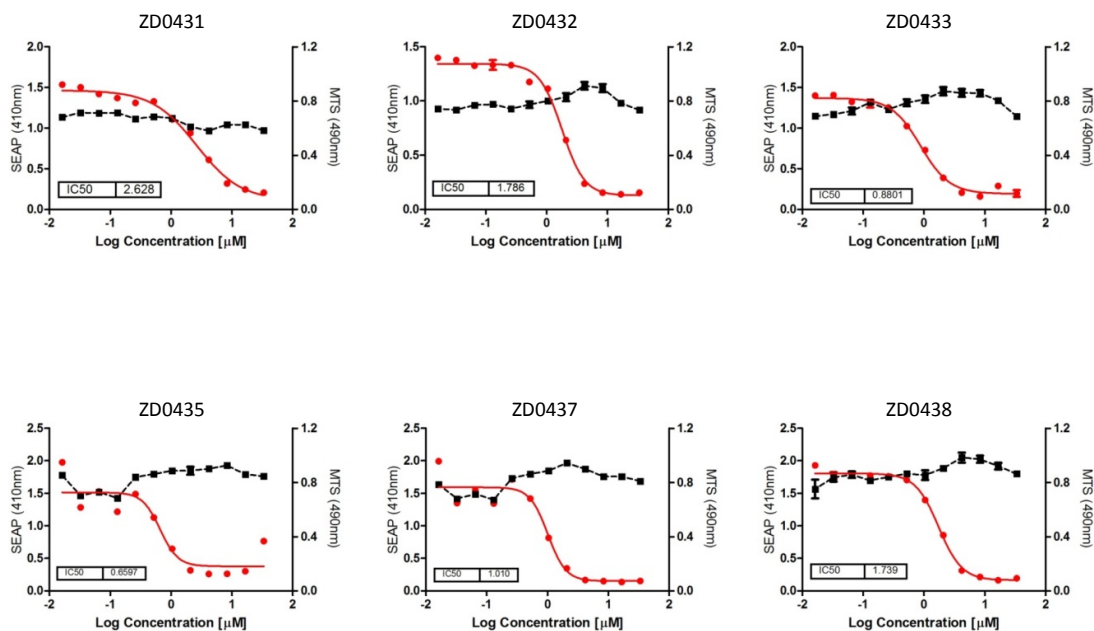
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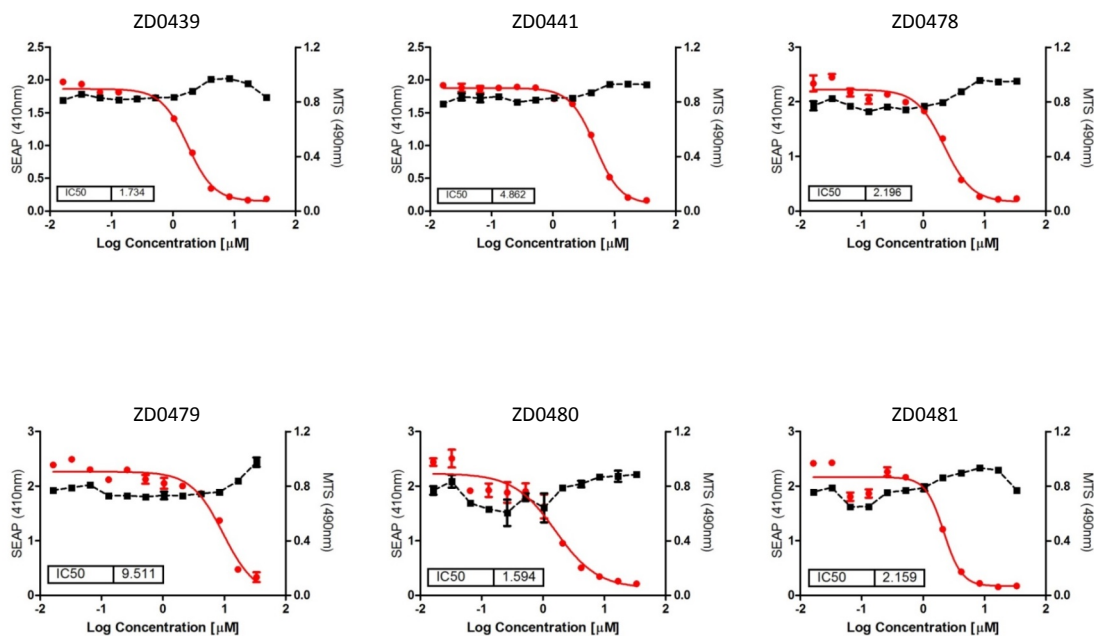




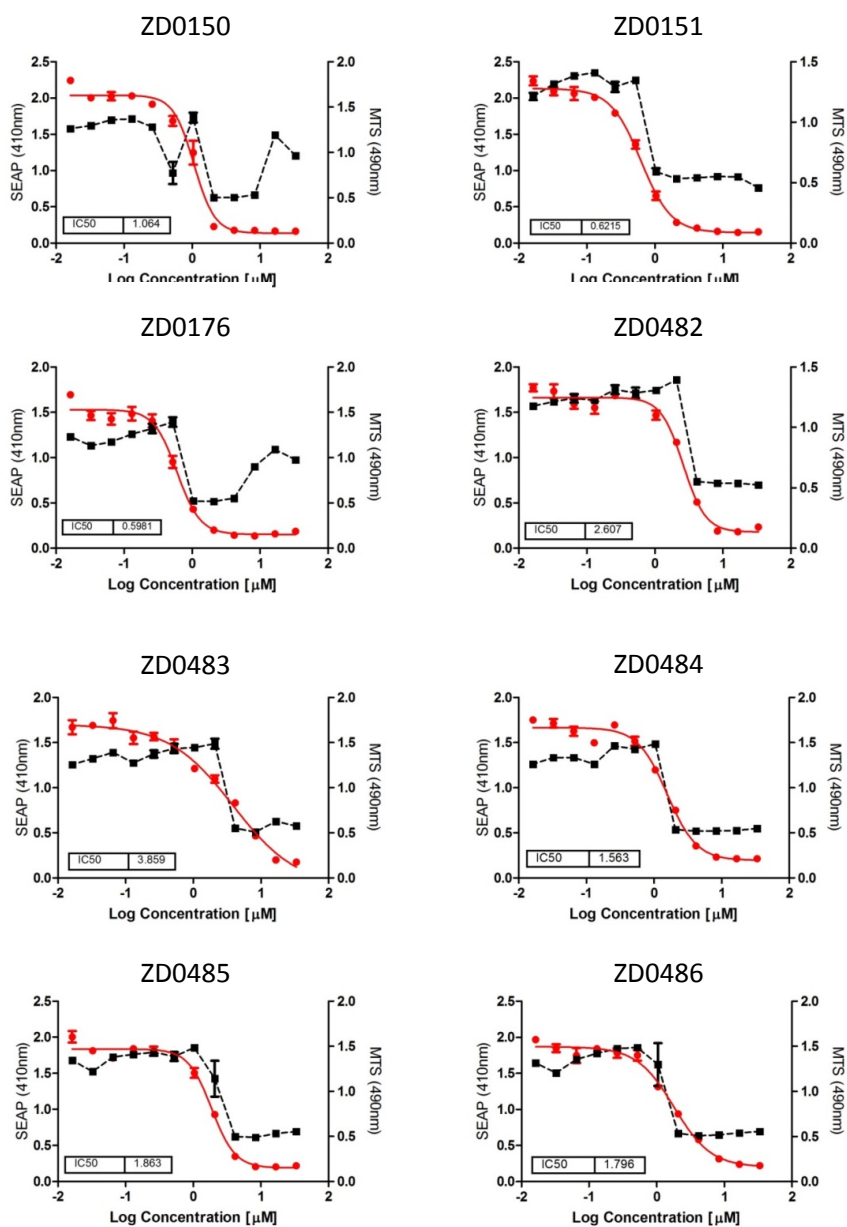


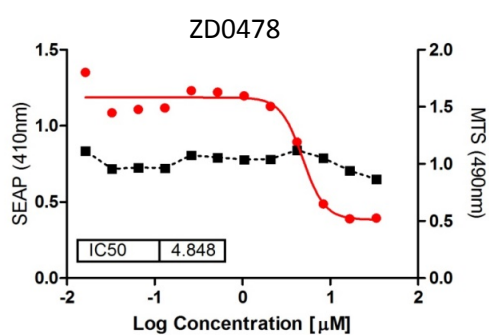
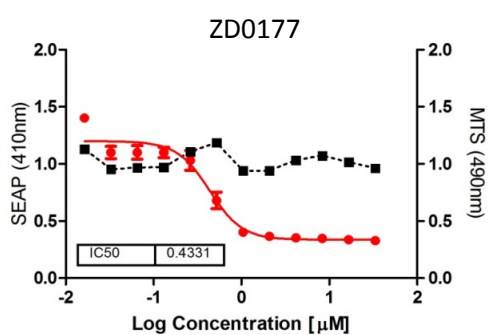
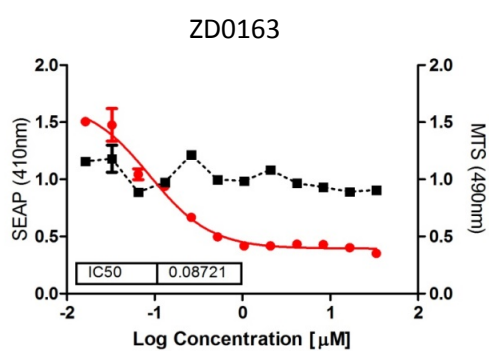
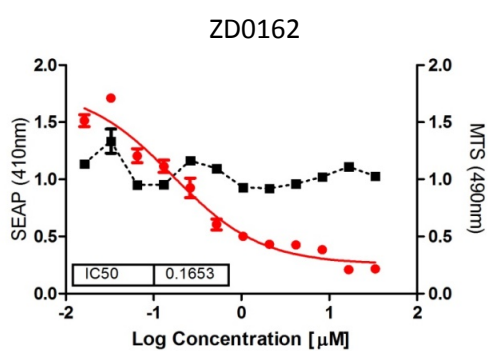
3. The Forth screen with HEK-Blue IL1 β Cells



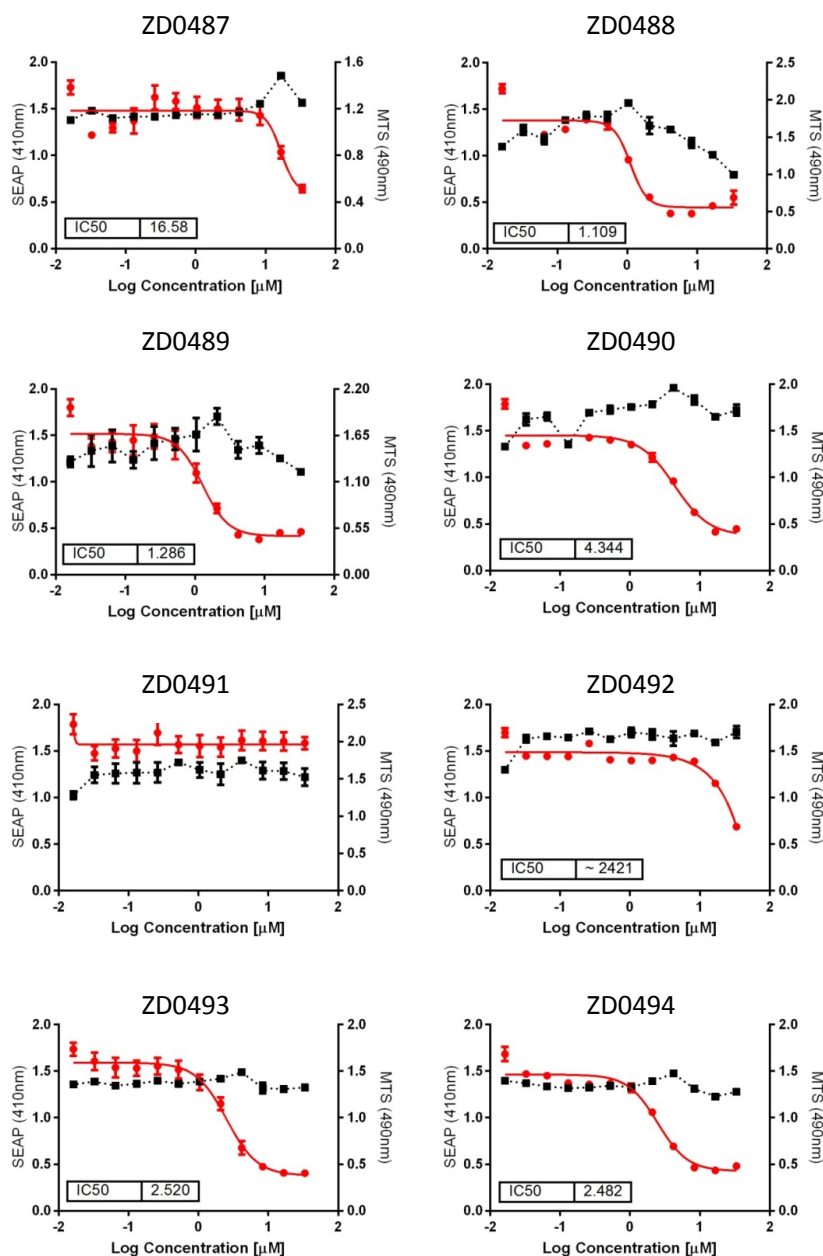


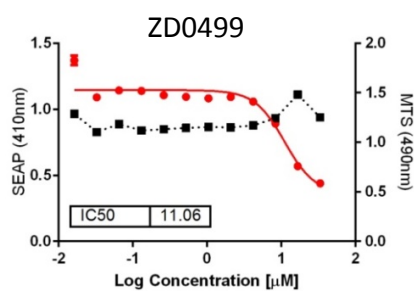
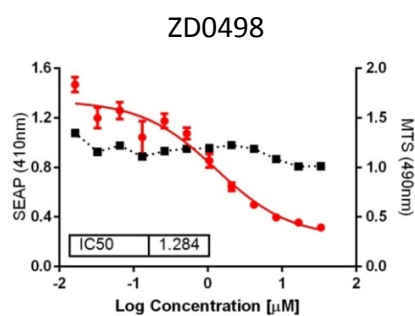
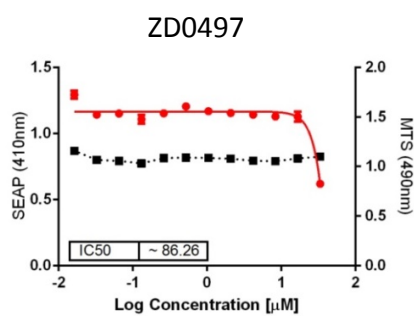
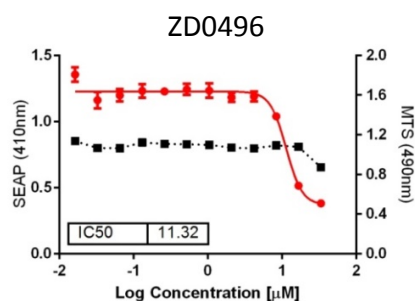
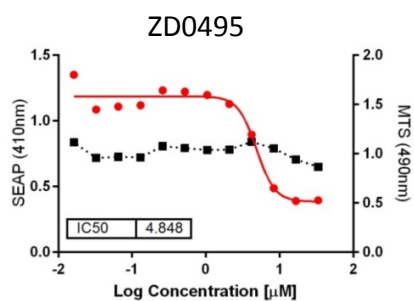
4. The Fifth screen with HEK-Blue IL1 β Cells





5. The Six Screen with HEK-Blue IL1 β Cells





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