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Patent Highlight
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PROTAC Degradation of IRAK4 for the Treatment of Neurodegenerative and Cardiovascular Diseases

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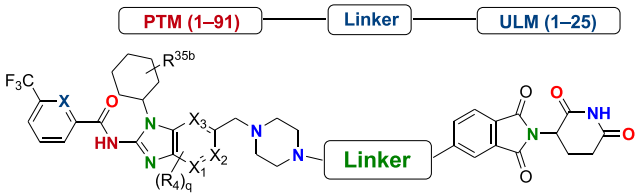
Important Compound Classes.



Title. Compounds and Methods for the Targeted Degradation of Interleukin-1 Receptor-Associated Kinase 4 Polypeptides

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Disease Area. Neurodegenerative and Cardiovascular Diseases

Biological Target. Interleukin-1 Receptor-Associated Kinase 4 (IRAK4)

Summary. Tumor protein kinases undergo frequent mutations in the human genome and provide essential function in essential cellular pathways, including cell apoptosis, signaling, signal transduction, and propagation of immune responses. For example, interleukin-1 receptor-associated kinase 4 (IRAK4) is a serine/threonine kinase that performs an important function of scaffolding and phosphorylation in toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling pathways. TLR and IL-1R are two large families of pattern recognition and cytokine receptors, respectively, that form a first line of response to infection in mammals and help to distinguish between self- and nonself-antigens. TLRs and IL-1R family of receptors enable cells to recognize molecular structures present in pathogens and respond to them by initiating and amplifying the pro-inflammatory cytokine response. The dysregulation of TLR signaling in particular has been implicated in autoinflammatory diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

Ligand binding to either IL-1R or TLR leads to dimerization and recruitment of adaptor molecules to a conserved toll/IL-1R (TIR) domain on the cytoplasm. The binding leads to recruitment of MyD88 and formation of the myddosome complex, which includes the interleukin-1 receptor activated kinase (IRAK) family. The first step in the pathway is autophosphorylation of IRAK4, which leads to IRAK1 phosphorylation and downstream signaling through multiple cascades. The IRAK kinase family consists of four subtypes: IRAK1 and IRAK4, which perform scaffolding functions, and IRAK2 and IRAK3, which are catalytically inactive. IRAK4 is the most upstream kinase that required IL-1R and TLR signaling. IRAK4 functions in a MyD88-dependent pathway and bears a huge therapeutic importance in anti-inflammatory and anticancer drug development. In humans, IRAK4 deficient phenotypes are highly susceptible to recurrent pyogenic bacterial infections and its inhibitors are considered valuable therapeutics in autoimmune and inflammatory disorders.

The X-ray crystal structure of human IRAK4 death domain (DD) revealed four distinct kinase specific structural regions: activation loop, ATP binding site, substrate binding site, and inhibitor binding region. The IRAK4 kinase domain (KD) consists of an N-terminal lobe with five antiparallel β-sheets and lacks a C-terminal extension, which is required by other IRAK members for TRAF6 interaction. IRAK4 as therapeutic target to treat inflammatory and autoimmune diseases is hampered by the challenge in identifying and developing selective inhibitors, due to in part the highly conserved structure of IRAK4 catalytic domain, which affects ligand selectivity. In addition, obtaining small molecules with properties suitable for animal trials has been scarce.

There are three major states of inhibitors for the IRAK4 target: (1) Type 1, the ATP-competitive structural rigid active or the inactive (type II) state; (2) allosteric state (type III) that displaces the ATP out of the catalytic site without competing for ATP; and (3) surface pockets that interact with kinase regulators (type IV) that target the surface and perform signal transduction. Rational drug design involves targeting the MyD88 binding surfaces on the DD and KD of IRAK4 by small, peptide-based inhibitors, which could be more specific and selective, as these sites are usually unique to a given kinase.

Protein−protein interactions are notoriously difficult to target using small molecules due to their large contact surfaces, shallow grooves, flat interfaces, and so forth. Since most small molecular drugs bind enzymes or receptors in tight and well-defined pockets, it has been challenging to successfully target protein−protein interactions using these small molecules. Recent drug development has focused on E3 ubiquitin ligases as more attractive therapeutic targets with specific ligands that could bind to these ligases, including the E3 ligases mouse double minute 2 homologue (MDM2) inhibitors and von Hippel−Lindau (VHL) tumor suppressor, which is the substrate recognition subunit of the E3 ligase complex VCB. Tumor suppressor gene p53 plays an important role in apoptosis that responds to DNA damage or stress and cell growth. The inactivation of p53 is one of the major pathways for tumor cell

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survival and about 50% of cancer patients were found to have p53 mutation. In normal cell condition, MDM2 keeps p53 at low concentration by down regulating it via protein−protein interaction. MDM2 binds to the N-terminal domain of p53 and blocks the expression of p53-responsive genes, and shuttles p53 from the nucleus to cytoplasm, which facilitates proteolytic degradation. Furthermore, MDM2 carries intrinsic E3 ligase activity by conjugating ubiquitin to p53 for degradation through the ubiquitin-dependent 26s proteasome system (UPS). Hypoxia Inducible Factor 1α (HIF-1α) is the primary substrate of VHL E3 ligase and a transcription factor that upregulates genes, including erythropoietin in response to low oxygen levels and proangiogenic growth factor VEGF.

Cereblon, a protein that is highly conserved in plants and humans, forms an E3 ubiquitin ligase complex with damaged DNA binding protein 1 (DDB1), regulator of cullins 1 (ROC1), and Cullin-4A (CUL4A). This complex ubiquitinates a number of proteins, which results in increased levels of fibroblast growth factor 8 (FGF8) and fibroblast growth factor 10 (FGF10).

In this Patent Highlight, the bifunctional compounds are useful as modulators of targeted ubiquitination with respect to Interleukin-1 receptor-associated kinases-4, which is degraded or inhibited by these compounds. The bifunctional or PROTAC compounds comprise an E3 ubiquitin ligase binding moiety (ULM) and a moiety that binds a target protein such as protein/polypeptide targeting ligand (PTM group), which is in close proximity to the ubiquitin ligase to affect degradation of that protein.

Definitions. X_1 and X_2 are CH or N;

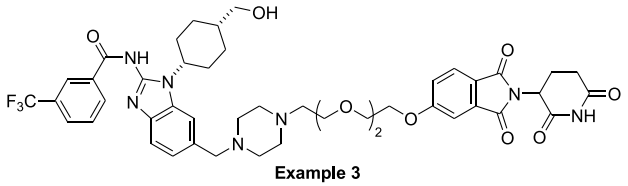
X_2 = CR_2 or N;

R_4 = H, halogen, alkyl, heterocycloalkyl, heteroaryl, and so forth;

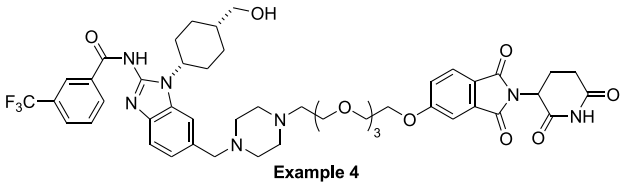
q = 0, 1, 2 or 3;

R^{35b} = H, halo, C_1−C_4alkoxy or hydroxy, etc.

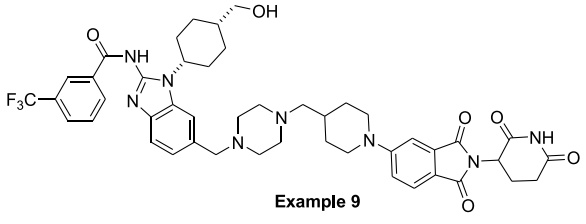
Key Structures.



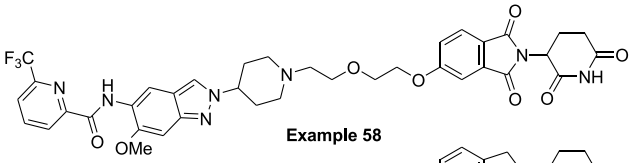
Example 3



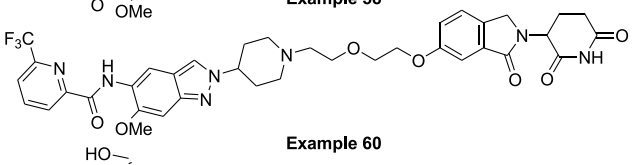
Example 4



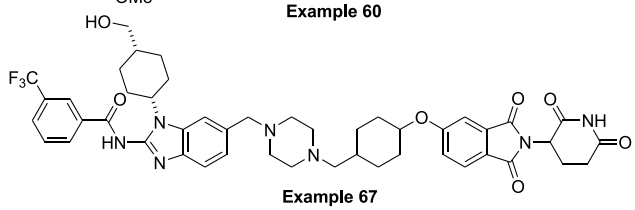
Example 9



Example 58



Example 60



Example 67

Biological Assay. Biochemical assay for IRAK4 kinase inhibition involves Z′-Lyte assay based on fluorescence resonance energy transfer (FRET) readout. Western Blot assay for assessing degradation of IRAK4 using MCF7 cells and enzyme-linked immunosorbent assay (ELISA) for IRAK4 degradation. Cell proliferation assay using CellTiter-Glo.

Biological Data. The compounds in this Patent Highlight shows biological activity for exemplified compounds and are shown in the Table below, where IRAK4 D_{max} ranges: A > 70, 70 ≥ B > 50; IRAK4 DC_{50} ranges: A < 30, 30 ≤ B < 100; IRAK4 IC_{50} ranges: A < 30, 30 ≤ B < 100.

Example #	IRAK4 Dmax (%)	IRAK4 DC_{50} (nM)	IRAK4 IC_{50} (nM)
3	A	A	A
4	A	A	A
9	A	A	A
58	B	A	A
60	B	A	A
67	A	B	A

Recent Review Articles.

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Notes

The author declares no competing financial interest.

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