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# Small Molecule Inhibitors of Inflammatory Caspases-1, -4, and -5

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**ABSTRACT** Inflammatory caspases-1, -4, and -5 play critical roles in mediating the innate immune response to pathogen- and damage-associated molecular patterns. Caspase-1 produces the cytokine interleukin-1β (IL-1β), while caspases-4 and -5 activate the NOD-like receptor protein 3 inflammasome via the non-canonical pathway. Overactivation of these enzymes is associated with an increased risk for inflammatory diseases such as lupus, psoriasis, and rheumatoid arthritis. Small molecule inhibitors (SMIs) have been developed to attenuate the production of IL-1β, but unfavorable bioavailability and toxicity have provided an insurmountable barrier in clinical trials. This work is concerned with delivering an overview of current progress in the synthetic design of SMIs of the inflammatory caspases.

## INTRODUCTION

Caspases-1, -4, and -5 are cysteine-dependent proteases that have essential roles in phagocytic cells of the innate immune system. Pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) are recognized by the NOD-like receptor protein 3 (NLRP3) inflammasome, which is responsible for cleaving the zymogen pro-caspase-1 into its active tetrameric form.[27] Once activated, caspase-1 produces the inflammatory cytokine interleukin-1β (IL-1β) from its precursor, which is secreted to recruit other circulating immune cells into the affected tissue. The inflammatory cascade is complicated by the involvement of caspases-4 and -5, which produce another inflammatory cytokine, interleukin-1α (IL-1α) and also indirectly activate pro-caspase-1.[1] These enzymes have been observed to be induced upon sensing pathogenic species like liposaccharides (LPS); though, like the canonical pathway, the mechanism of activation is not fully understood.[24]

Constitutive activity of the canonical NLRP3/caspase-1 pathway leads to harmful pyroptotic responses that are a characteristic of inflammatory diseases like lupus, psoriasis, and

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rheumatoid arthritis. Currently, there are several protein-based treatments that decrease the effects of IL-1β, such as anakinra, canakinumab, and rilonacept.[8] These proteins act as IL-1β antagonists, monoclonal antibodies, and soluble receptors, respectively (Figure 1). While anakinra blocks the cytokine's binding site on the IL-1β receptor protein, canakinumab is an antibody treatment that neutralizes IL-1β instead of competing with it at the binding site. Rilonacept is a receptor protein designed to bind IL-1β; however, since it is soluble in blood plasma and not integrated into the membrane of a leukocyte, any cytokines lured to this protein do not contribute to the inflammatory response. Although effective, these treatments are expensive to produce and must be administered via injection due to the harsh environment of the stomach, which prevents an optimal oral alternative. Additionally, the half-lives of these proteins are not ideal. For instance, anakinra degrades quickly and requires frequent injections, while the biostability of canakinumab confers a long half-life that leaves the individual at risk for infections.[4]

The disadvantages of protein-based treatment methods have made small molecule inhibitors (SMIs) of the inflammatory caspases attractive research prospects. This approach directly decreases the activation and secretion of IL-1β rather than merely disguising the cytokine's effects. Traditionally, potent inhibitors of caspases have employed an electrophilic functional group to trap the essential thiolate residue in the active site. However, SMIs have since broadened to target allosteric sites as well. Candidates that demonstrate compatibility with more than one caspase have also been discovered. Currently, all SMIs that have reached clinical trials have failed to pass them.[13] Herein, a review is presented of currently known SMIs of caspases-1, -4, and -5 with a focus on structural themes.

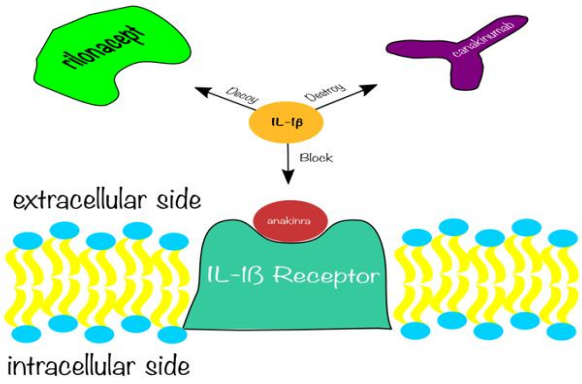


**Figure 1.** Decreased effects of IL-1β signaling via protein drugs. Displayed are the three approved treatments for targeting caspase-activated IL-1β: blocking the cytokine's receptor (anakinra), destroying the cytokine (canakinumab), and luring the cytokine to a soluble decoy receptor (rilonacept).

## INHIBITORS OF CASPASE-1

Most known inhibitors of caspase-1 are peptidomimetic and bind at the aspartate-specific active site. The proteolytic residue Cys285 can be trapped by a carbonylic group in either a reversible or irreversible manner, stopping the cleavage of pro-IL-1β. The structure of a competitive caspase-1 inhibitor can be generalized as having a core, $P_4$-$P_2$, and prime side fragment (Figure 2).

The core fragment contains an aspartate moiety that acts as an electrophilic trap for the proteolytic thiolate. The prime side, which is not present in all inhibitors, adds additional stabilization in the active site by anchoring the substrate to the interior hydrophobic pocket of the enzyme. The

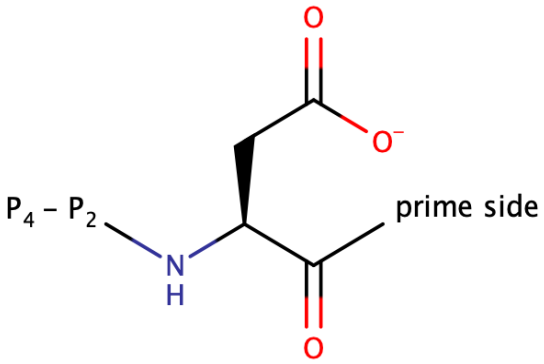


**Figure 2.** General structure of a competitive inhibitor of caspase-1. The scaffold contains an aspartate residue (core fragment) with extensions that interact with the $S_2$-$S_4$ region ($P_4$-$P_2$ fragment) and the interior hydrophobic pocket (prime side fragment) of caspase-1.

$P_4$-$P_2$ fragment describes the structure of one of the first-identified reversible inhibitors, Ac-YVAD-CHO.[14] The tyrosine ($P_4$), valine ($P_3$), and alanine ($P_2$) moieties appear to interact with caspase-1 through hydrophobic interactions of the side chains and hydrogen bonds of the backbone amides. (Figure 3). However, analysis via X-ray crystallography has demonstrated that the hydrogen bonds formed by the backbone amides of the $P_4$-$P_2$ fragment are not required for binding, prompting designs that involve less restricted peptidomimetic derivatives.[18]

A nearly invariable characteristic of all potent caspase-1 inhibitors is the presence of an electrophilic warhead. In both reversible and irreversible inhibition, the side chain of Cys285 undergoes a nucleophilic attack at the carbonylic carbon of the inhibitor. In reversible inhibition, the tetrahedral intermediate is momentarily stabilized by the oxyanion hole until collapsing and breaking its covalency with the inhibitor. An analogous intermediate characterizes the irreversible mode of inhibition. However, since the SMI is equipped with a leaving group, it can irreversibly trap the proteolytic cysteine residue by detaching its prime side.

Two of the first identified inhibitors of caspase-1 are the peptidic aldehydes **1** and **2** (Figure 4).[2, 21] These SMIs offer reversible interactions with the protease that closely resemble the natural substrate via an aspartate residue at the $P_1$ position, exhibiting $K_i$ values of 0.76 and 0.056 nM, respectively. The aromatic residues at the $P_4$ position are known to interact strongly with His342 and Pro343, two residues involved in inhibitor binding.[20] Despite significantly decreasing the levels of IL-1β in rats[17], neither of these SMIs demonstrate an acceptable cellular permeability in human cells.

Although inhibitors with tetrapeptide motifs like **1** and **2** are highly compatible with the active site of caspase-1, they are not promising drug candidates due to poor cell permeability, toxicity, stability, and selectivity.[3] Such a reality has prompted the design of less restricted peptidomimetic structures by using these two inhibitors as the epicenters of optimization. Since **1** and **2** are both potent inhibitors of caspase-1 despite having chemically different amino acid

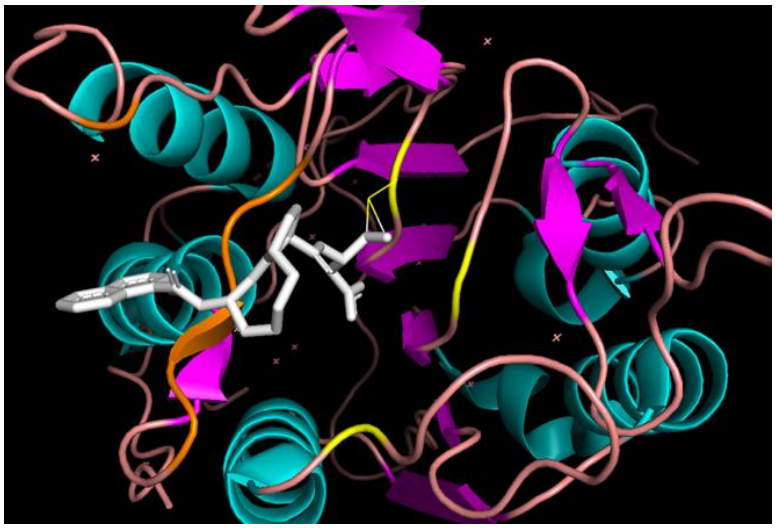

**Figure 3.** Peptidomimetic interactions with caspase-1. PyMOL entry 5mtk. Caspase-1 interacts with an inhibitor through its $S_1$ and $S_2$-$S_4$ regions, displayed in yellow and orange, respectively. The side chain of the putative catalytic residue Cys285 is showcased. The hydrophobic pocket is displayed in purple.

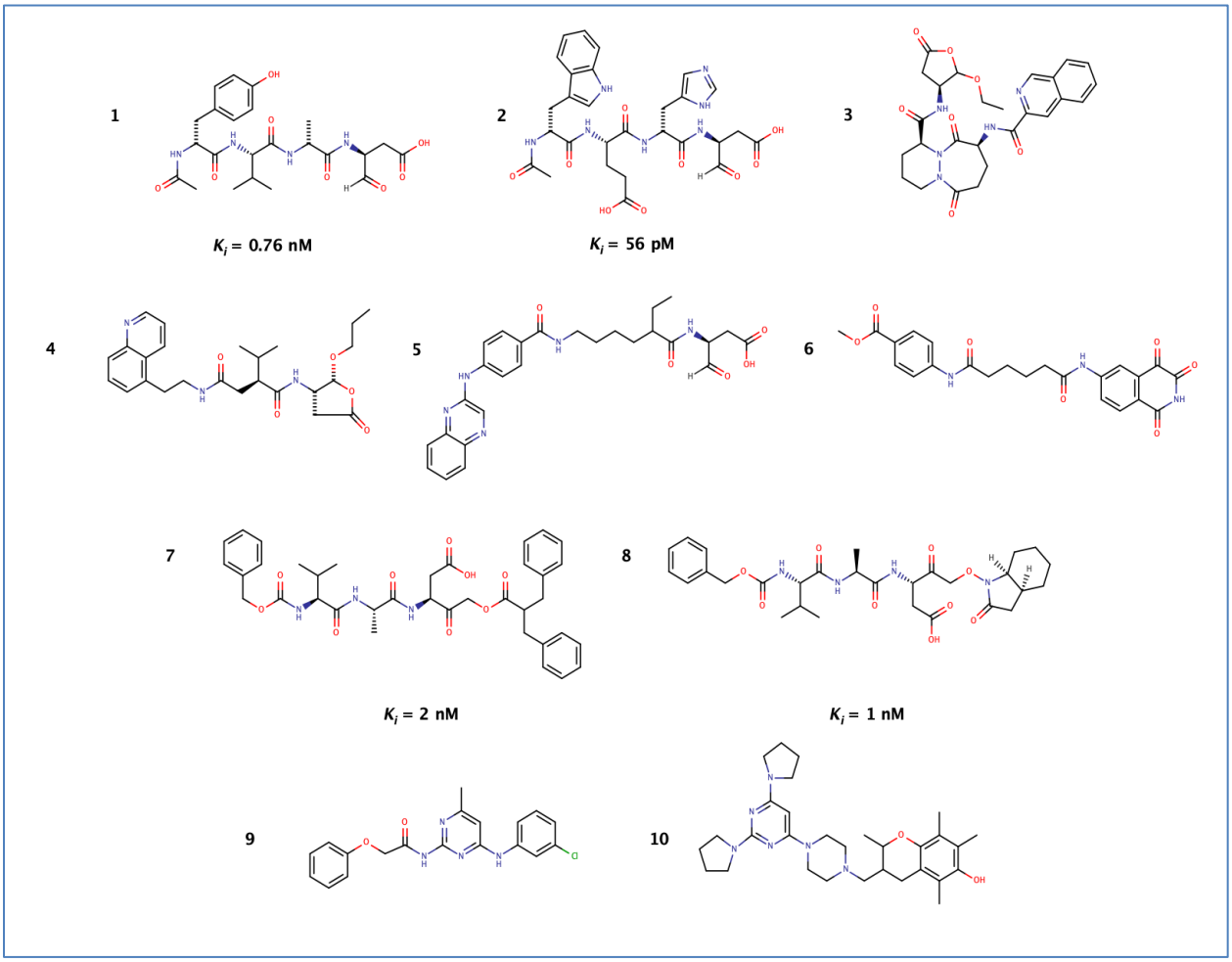


**Figure 4.** Known inhibitors of caspase-1.

residues at the $P_2$ and $P_3$ positions, this suggests that this part of the inhibitor can be varied to attain an improved pharmacological profile. The structure of the pro-drug pralnacasan **3** is one such variant. The nonpolar cyclic constraints in the $P_2$ and $P_3$ positions offer increased bioavailability and stability without affecting its potency. Indeed, **3** has caused decreased levels of IL-1β from mononuclear cells of LPS-challenged mice.[22] The drug advanced to late-stage clinical trials before its candidacy was withdrawn due to toxicological concerns present in the livers of the murine test subjects.[15]

The $P_2$-$P_3$ sites have also been designed with acyclic components. Succinic acid amides like **4** have shown promising bioavailability in addition to potent inhibition of caspase-1.[5] Like **1-3**, compound **4** has a truncated prime side fragment, possessing only an ethyl acylal pro-drug component. The pro-drug form was found to be essential for bioavailability. The ionization of the aspartate residue confers an inability to cross the lipophilic bilayer.

Interestingly, docking studies of **4** revealed that the succinic acid amide scaffold does not interact with the $S_3$ region of caspase-1 and instead prefers to stabilize itself through hydrogen bonding with the solvent. This finding provided further evidence that the $P_2$-$P_3$ components of a caspase-1 inhibitor are not essential for binding to the enzyme. Thus, they should be adjusted to optimize the alignment of the aromatic $P_4$ position in the active site. Analogs of **4** that employed cyclized $P_2$-$P_3$ components were substantially less potent. Since cyclization of the analogous positions in **3** did not cause a similar problem, it can be inferred that the effects of cyclization depend on the overall structure of the

inhibitor and cannot be generalized as having positive or negative effects on inhibitory potency. Compound **5** is another reversible inhibitor. The aromatic residues that interact with the $S_4$ region of caspase-1 are separated by several methylene groups. Cleverly, this SMI was optimized through a fragment-assembly approach. First, a $P_1$ fragment that contained a terminal thiol was bound to the active site; then, a disulfide bond formed with an analogously modified $P_3/P_4$ fragment only if this fragment was able to bind to the respective region of caspase-1.[19] Although neither fragment inhibits the protease by itself, a submicromolar potency is observed when they are combined. In the final structure of **5**, the disulfide bond is replaced with a hydrocarbon skeleton. The SMI shows appreciable inhibition of both caspases-1 and -5 with respective $K_i$ values of 0.016 and 2.03 μM and thus can be classified as a pan-inhibitor.

The compatibility of an inhibitor with two or more of the inflammatory caspases is no small feat. Indeed, compounds **1-4** have not been reported to have any effect on the activity of caspases-4 and -5, making compound **5** an attractive design for a reversible inhibitor. Broad inhibition of caspases-1, -4, and -5 is preferred, given that all three are influential in the production of IL-1β. By inhibiting two or more with one inhibitor, this SMI is more likely to show successful results in clinical trials. However, this challenge becomes increasingly difficult upon considering that the inhibitor must also be caspase-specific. If the SMI is promiscuous with all catalytic thiolates, including the apoptotic caspases, or attractive to other cellular nucleophiles, its utility will be compromised.

Unlike **1-5**, compound **6** is an irreversible inhibitor. This SMI is derived from isoquinoline-1,3,4-trione, which acts as the leaving group upon trapping the catalytic residue of caspase-1. Broad inhibition of the inflammatory caspases has been observed with the SMI, but it is selective for this family and does not act as a general protease inhibitor.[16] Remarkably, **6** does not possess the essential aspartic residue at $P_1$. The lack of this residue leads to slow binding to the aspartate-specific caspases, but since the binding is irreversible, it cannot be undone after doing so. The SMI also shows acceptable cellular penetration due to its largely hydrophobic scaffold. Structures like **5** and **6**, which link the $P_1$ and $P_4$ regions via methylene groups, may be useful in broadly inhibiting the caspase family. Currently, it does not appear that analogs of these structures have been extensively pursued. Therefore, optimization of these scaffolds could afford promising candidates.

As previously stated, it is not uncommon for the prime side of a caspase inhibitor to be missing or simply used as a leaving group. Compounds **7** and **8** are unique in that they are inhibitors that were developed by optimizing the prime side interactions with caspase-1.[6] Both are remarkably potent, recording $K_i$ values of 2 and 1 pM, respectively. The former is an acyloxyalkyl ketone that possesses a bulky hydrophobic and aromatic prime side, which anchors the SMI to the protease's hydrophobic interior. SMI **7** also exhibits bimodal inhibition, decreasing caspase-1 activity via reversible and irreversible inhibition. Interestingly, SAR studies revealed that optimizing the potency of the ester favored the irreversible mechanism. Compound **8** is a hydroxamate analog that exhibits only reversible inhibition regardless of the potency.

Although diverse in structure, compounds **1-8** can be described using the general structure of caspase-1 inhibitors (with the exception of **6**). They contain an aspartate residue at the $P_1$ position and have modifications in the $P_2$-$P_4$ and prime side regions. Recently, there has been a focus on caspase-1 SMIs that significantly deviate from the traditional structure. Indeed, inhibitors that occupy similar spatial areas as **1** and **2** but are not peptidomimetic are attractive candidates, given the poor biostability associated with hydrolysable species. Compounds **9** and **10** are examples of such candidates, as neither have an aspartate residue nor many enticing peptide bonds that can allure cellular nucleophiles. SMI **9** was optimized for the active site through the variation of the substituents on the diaminopyrimidine derivative.[9] No covalent interactions with Cys285 were reported despite the binding of **9** to the active site. Similarly, LC-MS analysis of caspase-1 after treatment with

compound **10** did not suggest any covalent interactions either.[10] Notably, this compound inhibits caspase-1 ($K_i$ = 48 nM) even with the addition of excess substrate[14], suggesting that it likely operates via an allosteric mechanism. Though allosteric inhibitors of caspase-1 do exist, they are rare.

Compounds **9** and **10** likely offer insight into the future structure of a successful drug candidate for caspase-1 inhibition. These SMIs have a scaffold that should be able to readily permeate the cellular membrane and evade the attention of cellular nucleophiles. Therefore, optimizing **9** and **10** and further elucidating their mechanisms of inhibition are useful research targets.

## INHIBITORS OF CASPASES-4 AND -5

Of the SMIs discussed so far, most have not been pan-caspase inhibitors. With the exception of **5** and **6**, the target of inhibition has been exclusively caspase-1. Impeding the activity of this enzyme has received significantly more attention than its inflammatory counterparts, caspases-4 and -5. The reason for this trend is that the pathway that converts pro-IL-1β into its active cytokine form is the prototypical innate immune response. Additionally, the crystallized structure of caspase-4 has only recently been deposited on online databases, limiting the use of docking studies to model interactions. The crystallized structure of caspase-5 remains outstanding; though, sequence alignment with caspase-1 has been performed to predict its native form.

Both caspases-4 and -5 are known to activate the NLRP3 inflammasome through the noncanonical pathway and also regulate the secretion of another cytokine, IL-1α.[26] Therefore, from a therapeutic perspective, it may be impractical to decrease inflammation by only inhibiting caspase-1. Fortunately, the genetic sequence among the inflammatory caspases is highly conserved, which allows for shared characteristics among putative SMIs, such as aspartate-specificity and hydrophobicity in the $S_4$ region.[25]

When caspases-4 and -5 were discovered, it was ascertained that the proteases must have similar substrate preferences due to pan-inhibition via the cowpox virus-derived inhibitor, CrmA.[11] Shortly after, peptidic SMIs that are analogous to the putative caspase-1 inhibitor Ac-YVAD-CHO were identified. Compounds **11** and **12** are inhibitors of caspases-4 and -5, respectively; though, both show a certain degree of pan-inhibition (Figure 5). The structures of these SMIs are Z-LEVD-FMK and Z-WEHD-FMK. These SMIs diffuse across the lipid bilayer of the cell membrane more easily than **1** and **2** due to the esterification of the hydrophilic carboxy groups on glutamate and aspartate. It should be noted that peptidic aldehyde versions of **11** and **12**, like **1** and **2**, have also been identified as possessing inhibitory effects on their respective caspase.[12] It is not surprising that, like caspase-1, the activity

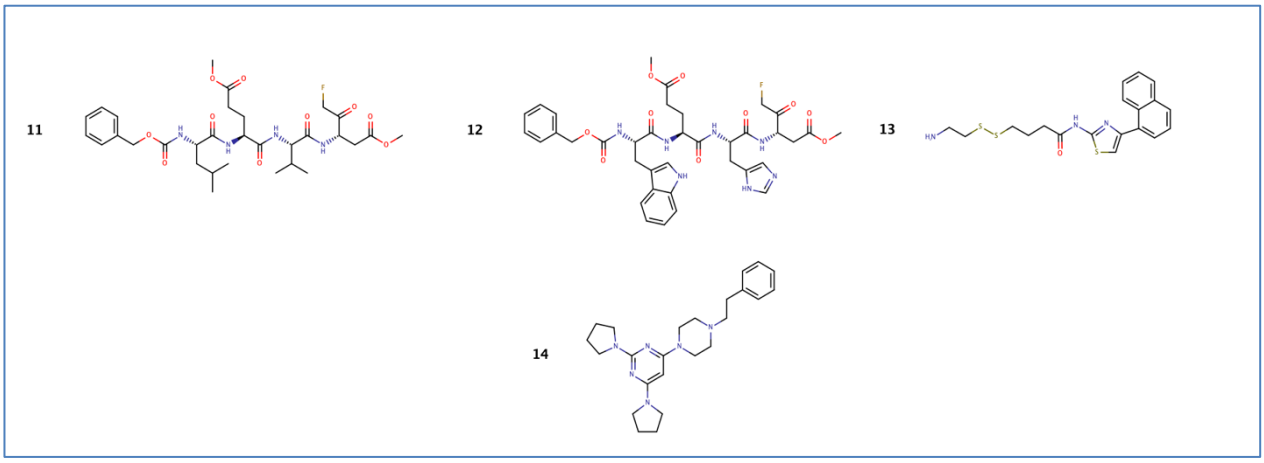


**Figure 5.** Known inhibitors of caspases-4 and -5.

of caspases-4 and -5 can be suppressed by species that closely mimic a natural peptide. The active sites are highly conserved and thus readily bind to these structures. Such a luxury comes at the cost of biostability and other problems, as previously stated.

SMIs like **13** and **14** are not as obvious as the substrate-based **11** and **12** but are remarkably promising allosteric prospects. Caspases, including both the inflammatory and apoptotic players, have a cavity at the dimeric interface of the heterodimers, which is located 15 Å away from the active site. In regard to caspase-1, Cys331 is found within this region and can be allosterically trapped.[23] When such process occurs, a salt bridge between Arg286 and Glu390 that aligns the active site for catalysis is broken. Fortunately, all three of these residues are conserved throughout the caspase family, denoting a common regulatory mechanism. It should be noted that this mechanism is likely responsible for the allosteric inhibition of caspase-1 by **10**.

Compound **13** is a naphthyl-thiazole-containing SMI. Upon treatment of caspase-5 with **13**, both the turnover rate and binding affinity of the substrate decreased. Such an observation is consistent with a mechanism of allosteric regulation. This SMI therefore likely acts as a negative regulator molecule and causes a conformational change in caspase-5.[7] No effects were observed on caspase-1, indicating an appreciable selectivity. Thus far, SMIs **1-13** have shown no or partial pan-inhibition of the inflammatory caspases. Compound **14** deviates from this trend, demonstrating $K_i$ values of 16, 8, and 16 nM for caspases-1, -4, and -5, respectively.[10] This triamino pyrimidine derivative is an alluring prospect for future optimization.

## SUMMARY

The known inhibitors of caspases-1, -4, and -5 have evolved from solely substrate-based molecules into less restricted peptidomimetic structures and, recently, allosteric regulators. It is thus clear from the trends in current research on the inflammatory caspases that the eventual successful therapeutic candidate will not closely mirror a natural peptide. While these SMIs are useful for research purposes, they are not viable pharmaceutic candidates due to a lack of stability and selectivity. Therefore, SMIs that attract less undesirable side reactions will likely be critical to surpassing clinical trials. This hurdle favors allosteric regulators, like compounds **10**, **13**, and **14**. The conserved dimer interface among the caspases could allow for selective pan-inhibition that would effectively shut off the innate immune response, temporarily alleviating the pain of the discussed pathologies. Although further work should be performed to optimize the allosteric regulators, a challenge will be the compact pocket of the dimer interface that may limit the extent of structural modifications.

Inhibitors of caspase-1 have been the focus of recent research. While there are over one-hundred known SMIs of caspase-1, there is a small list for both caspases-4 and -5. As more SMIs of these caspases are discovered, optimization will be facilitated. Until recently, little information has been available on public databases regarding the quaternary structures of these proteases. Although homology modeling is useful for docking studies, it is not as helpful as a crystallized model. Therefore, it stands to reason that inhibitors or regulators of caspases-4 and -5 will become more available in the coming years. With some good fortune, a therapeutic SMI of the inflammatory caspases can be expected within the next decade. Although the same was said a decade prior, a focus on optimizing a non-traditional structure, like an allosteric regulator, was not actively pursued at the time.

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**REFERENCES**

(1) Baker, P. J.; Boucher, D.; Bierschenk, D.; Tebartz, C.; Whitney, P. G.; D'Silva, D. B.; Tanzer, M. C.; Monteleone, M.; Robertson, A. A. B.; Cooper, M. A.; Alvarez-Diaz, S.; Herold, M. J.; Bedoui, S.; Schroder, K.; Masters, S. L., NLRP3 inflammasome activation downstream of cytoplasmic LPS recognition by both caspase-4 and caspase-5. European Journal of Immunology 2015, 45 (10), 2918-2926.

(2) Chapman, K. T., Synthesis of a potent, reversible inhibitor of interleukin-1β converting enzyme. Bioorganic & Medicinal Chemistry Letters 1992, 2 (6), 613-618.

(3) Degterev, A.; Boyce, M.; Yuan, J., A decade of caspases. Oncogene 2003, 22 (53), 8543-8567.

(4) de Koning, H. D.; Schalkwijk, J.; van der Ven-Jongekrijg, J.; Stoffels, M.; van der Meer, J. W.; Simon, A., Sustained efficacy of the monoclonal anti-interleukin-1 beta antibody canakinumab in a 9-month trial in Schnitzler's syndrome. Ann Rheum Dis 2013, 72 (10), 1634-8.

(5) Galatsis, P.; Caprathe, B.; Gilmore, J.; Thomas, A.; Linn, K.; Sheehan, S.; Harter, W.; Kostlan, C.; Lunney, E.; Stankovic, C.; Rubin, J.; Brady, K.; Allen, H.; Talanian, R., Succinic acid amides as P2–P3 replacements for inhibitors of interleukin-1β converting enzyme (ICE or caspase 1). Bioorganic & Medicinal Chemistry Letters 2010, 20 (17), 5184-5190.

(6) Galatsis, P.; Caprathe, B.; Downing, D.; Gilmore, J.; Harter, W.; Hays, S.; Kostlan, C.; Linn, K.; Lunney, E.; Para, K.; Thomas, A.; Warmus, J.; Allen, H.; Brady, K.; Talanian, R.; Walker, N., Inhibition of interleukin-1β converting enzyme (ICE or caspase 1) by aspartyl acyloxyalkyl ketones and aspartyl amidooxyalkyl ketones. Bioorganic & Medicinal Chemistry Letters 2010, 20 (17), 5089-5094.

(7) Gao, J.; Wells, J. A., Identification of Specific Tethered Inhibitors for Caspase-5. Chemical Biology & Drug Design 2012, 79 (2), 209-215.

(8) Goh, A. X.; Bertin-Maghit, S.; Ping Yeo, S.; Ho, A. W.; Derks, H.; Mortellaro, A.; Wang, C. I., A novel human anti-interleukin-1beta neutralizing monoclonal antibody showing in vivo efficacy. MAbs 2014, 6 (3), 765-73.

(9) Kawarski, M.; Hagerman, T. K.; Karver, C. E., Lazaroids U83836E and U74389G are Potent, Time-Dependent Inhibitors of Caspase-1. Chemical Biology & Drug Design 2015, 86 (5), 1049-1054.

(10) Kent, C. R.; Bryja, M.; Gustafson, H. A.; Kawarski, M. Y.; Lenti, G.; Pierce, E. N.; Knopp, R. C.; Ceja, V.; Pati, B.; Walters, D. E.; Karver, C. E., Variation of the aryl substituent on the piperazine ring within the 4-(piperazin-1-yl)-2,6-di(pyrrolidin-1-yl)pyrimidine scaffold unveils potent, non-

competitive inhibitors of the inflammatory caspases. Bioorganic & Medicinal Chemistry Letters 2016, 26 (22), 5476-5480.

(11) Kamada, S.; Funahashi, Y.; Tsujimoto, Y., Caspase-4 and caspase-5, members of the ICE/CED-3 family of cysteine proteases, are CrmA-inhibitable proteases. Cell Death & Differentiation 1997, 4 (6), 473-478.

(12) Kang, S. J.; Lee, Y. J.; Kang, S. G.; Cho, S.; Yoon, W.; Lim, J. H.; Min, S.-H.; Lee, T. H.; Kim, B. M., Caspase-4 is essential for saikosaponin a-induced apoptosis acting upstream of caspase-2 and γ-H2AX in colon cancer cells. Oncotarget 2017, 8 (59), 100433-100448.

(13) Kudelova, J.; Fleischmannova, J.; Adamova, E.; Matalova, E., Pharmacological caspase inhibitors: research towards therapeutic perspectives. J Physiol Pharmacol 2015, 66 (4), 473-82.

(14) Le, G. T.; Abbenante, G., Inhibitors of TACE and Caspase-1 as anti-inflammatory drugs. Curr Med Chem 2005, 12 (25), 2963-2977.

(15) MacKenzie, S. H.; Schipper, J. L.; Clark, A. C., The potential for caspases in drug discovery. Curr Opin Drug Discov Devel 2010, 13 (5), 568-576.

(16) Ma, X.-Q.; Zhang, H.-J.; Zhang, Y.-H.; Chen, Y.-H.; Wu, F.; Du, J.-Q.; Yu, H.-P.; Zhou, Z.-L.; Li, J.-Y.; Nan, F.-J.; Li, J., Novel irreversible caspase-1 inhibitor attenuates the maturation of intracellular interleukin-1β. Biochemistry and Cell Biology 2007, 85 (1), 56-65.

(17) Miller, D. K., The role of the Caspase family of cysteine proteases in apoptosis. Seminars in Immunology 1997, 9 (1), 35-49.

(18) Mullican, M. D.; Lauffer, D. J.; Gillespie, R. J.; Matharu, S. S.; Kay, D.; Porritt, G. M.; Evans, P. L.; Golec, J. M. C.; Murcko, M. A.; Luong, Y.-P.; Raybuck, S. A.; Livingston, D. J., The synthesis and evaluation of peptidyl aspartyl aldehydes as inhibitors of ice. Bioorganic & Medicinal Chemistry Letters 1994, 4 (19), 2359-2364.

(19) O'Brien, T.; Fahr, B. T.; Sopko, M. M.; Lam, J. W.; Waal, N. D.; Raimundo, B. C.; Purkey, H. E.; Pham, P.; Romanowski, M. J., Structural analysis of caspase-1 inhibitors derived from Tethering. Acta Crystallogr Sect F Struct Biol Cryst Commun 2005, 61 (Pt 5), 451-458.

(20) Patel, S.; Modi, P.; Ranjan, V.; Chhabria, M., Structure-based design, synthesis and evaluation of 2,4-diaminopyrimidine derivatives as novel caspase-1 inhibitors. Bioorganic Chemistry 2018, 78, 258-268.

(21) Rano, T. A.; Timkey, T.; Peterson, E. P.; Rotonda, J.; Nicholson, D. W.; Becker, J. W.; Chapman, K. T.; Thornberry, N. A., A combinatorial approach for determining protease specificities: application to interleukin-1beta converting enzyme (ICE). Chem Biol 1997, 4 (2), 149-55.

(22) Rudolphi, K.; Gerwin, N.; Verzijl, N.; van der Kraan, P.; van den Berg, W., Pralnacasan, an inhibitor of interleukin-1β converting enzyme, reduces joint damage in two murine models of osteoarthritis. Osteoarthritis and Cartilage 2003, 11 (10), 738-746.

(23) Scheer, J. M.; Romanowski, M. J.; Wells, J. A., A common allosteric site and mechanism in caspases. Proceedings of the National Academy of Sciences 2006, 103 (20), 7595.

(24) Shi, J., Zhao, Y., Wang, Y. et al., Inflammatory caspases are innate immune receptors for intracellular LPS. Nature 2014, 514, 187–192.

(25) Thornberry, N. A.; Chapman, K. T.; Nicholson, D. W., Determination of caspase specificities using a peptide combinatorial library. Methods Enzymol 2000, 322, 100-110.

(26) Viganò, E.; Diamond, C. E.; Spreafico, R.; Balachander, A.; Sobota, R. M.; Mortellaro, A., Human caspase-4 and caspase-5 regulate the one-step non-canonical inflammasome activation in monocytes. Nature Communications 2015, 6 (1), 8761.

(27) Yamin, T.-T.; Ayala, J. M.; Miller, D. K., Activation of the Native 45-kDa Precursor Form of Interleukin-1-converting Enzyme. Journal of Biological Chemistry 1996, 271 (22), 13273-13282.