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Structure-Based Design of Caspase-1 Inhibitor Containing a Diphenyl Ether Sulfonamide

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Abstract—A series of compounds was designed and prepared as inhibitors of interleukin-1β converting enzyme (ICE), also known as caspase-1. These inhibitors, which employ a diphenyl ether sulfonamide, were designed to improve potency by forming favorable interactions between the diphenyl ether rings and the prime side hydrophobic region. An X-ray crystal structure of a representative member of the diphenyl ether sulfonamide series bound to the active site of caspase-1 was obtained.

Interleukin-1 (IL-1) appears to be a central mediator of the pathogenesis of acute and chronic inflammation.[1] Of the two IL-1 proteins that exist, α and β, it is the latter that is predominantly released from activated human monocytes.[2] IL-1β is synthesized as a cytosolic inactive precursor of 33K, which is released only upon cleavage to the mature 17.5K form. The intracellular protease (caspase-1), also referred to as interleukin-1β converting enzyme (ICE), responsible for this processing has been cloned[3] and the crystal structure elucidated.[4,5] Caspase-1 is a homodimer (consisting of two heterodimer subunits p10 and p20) cysteine protease that cleaves human proIL-1β between the Asp^{116}-Ala^{117}. Data from caspase-1 deficient mice show decreased levels of IL-1β upon lipopolysaccharide (LPS) stimulation, supporting the concept that caspase-1 inhibition would be beneficial in treatment of diseases in which IL-1β plays a role.[6] IL-1β has been shown to be important in the pathogenesis of a number of peripheral inflammatory disorders, including rheumatoid arthritis and inflammatory bowel disease. Caspase-1 might also be involved in unscheduled programmed cell death (apoptosis).[7] In addition, data in the literature suggest that the inhibition of IL-1β production by a caspase-1 inhibitor may be useful for treatment of brain damage in stroke[8] and myocardiac ischemia.[9]

The structure-based design and X-ray crystallographic analysis of a new class of active site caspase-1 inhibitors, which contain a biphenyl sulfonamide-aspartic acid aldehyde scaffold, have recently been reported from our laboratory.[10] Compound **1**, for example, is a reversible inhibitor of caspase-1. In this structure, a major conformational change has been observed in which the His237 side chain has rotated from a *gauche* to a *trans* orientation, creating a large hydrophobic pocket adjacent to the P1 site. Based on our modeling ideas, the biphenyl series was expanded to include a diphenyl ether sulfonamide derivative, which was predicted to bind with His237 in the *gauche* orientation. The diphenyl ether moiety is predicted to reach toward the previously recognized prime side hydrophobic pocket.[11]

The present report describes the structure-based design and the synthesis of potent compounds, which contain a diphenyl ether. Based on the initial model, a series of *o*-diphenyl ether derivatives was designed with the aim of maximizing favorable prime side hydrophobic interac-

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tions. Specifically, it was hoped that appended hydrophobic groups from the diphenyl ether sulfonamide would serve to further isolate the oxyanion from the solvent. As a first step the un-substituted diphenyl ether sulfonamide **2** was synthesized and was found to be equipotent ($IC_{50} = 24\,\mu M$) with **1**.

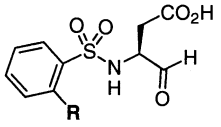
Initially, simple alkyl substituents on the remote phenyl ring were evaluated to probe the prime side hydrophobic pocket. As can be seen in Table 1, addition of these simple alkyl groups did provide improved potency when compared to compound **2**. Activity of the 3, 4, 5-trimethyl substituted compound **7** suggests that attaching branched alkyl groups to either the *meta* or *para* position may provide additional potency.

Specifically, appending a branched alkyl group like *meta*-isopropyl on the remote phenyl ring provided a more potent inhibitor **8** ($IC_{50} = 3.70\,\mu M$), with a 2-fold increase in potency relative to compound **7** (Table 2). A *para*-isopropyl substitution on the remote phenyl ring provides analogue, **9**, and is nearly as potent as **8** ($IC_{50} = 5.30\,\mu M$).

The crystal structure of **9** in the caspase-1 active site was determined to further guide our efforts exploring the hydrophobic prime side (Fig. 1). The aspartic acid aldehyde in **9** forms the standard covalent bond with catalytic Cys285 and orients the acidic side chain in the electropositive pocket formed by Arg179, Gln283, and Arg341. Additional polar interactions are formed between the SO_2NH group and the enzyme. The X-ray crystal structure shows that His237 is in the standard *gauche* orientation rather than *trans* as is observed in the caspase-1 structure bound with compound **1**. The isopropyl phenyl group is positioned in the hydrophobic region formed by Ile176, Pro177, and Ile239.

Examination of the X-ray structure of **9** reveals that incorporation of a methyl group adjacent to the isopropyl group, as in **10**, would result in improved potency. Indeed, compound **10** is 5-fold more potent than **9**. This increase in potency was attributed to the methyl group, which causes the isopropyl group to twist relative to aromatic ring, thereby allowing better complementarity of the isopropyl group with the enzyme hydrophobic surface. Replacement of the isopropyl group in **9** with a 2-butyl group to give **11** produced a 2-fold increase in IC_{50}. Addition of a methyl group to **11** enhanced potency by 2-fold providing **12**, which is equipotent to **10**. Compound **13** was synthesized in order to avoid the issue of the isobutyl stereochemistry. Molecular modeling[12] suggested caspase-1 could

Table 1. Evaluation of inhibitors against caspase-1



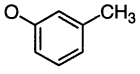
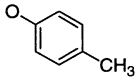
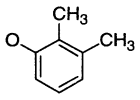
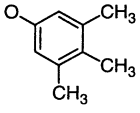
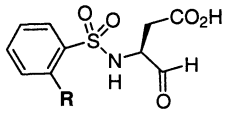
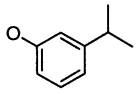
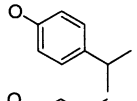
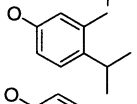
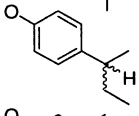
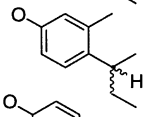
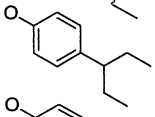
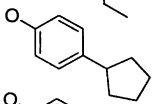
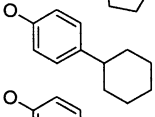
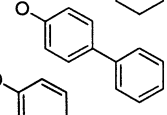
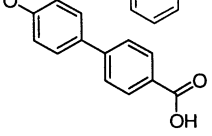
Compd	R	IC_{50} (μM)	K_i (μM)
1		20	1.6
2		24	2.4
3		14	3
4		11	0.43
5		9	1.1
6		13	1.0
7		7.8	0.45

Table 2. Evaluation of inhibitors against caspase-1



Compd	R	IC_{50} (μM)	K_i (μM)
8		3.7	0.41
9		5.3	0.62
10		1.0	0.11
11		2.2	0.49
12		1.0	0.10
13		3.5	0.44
14		4.8	0.88
15		4.3	0.90
16		4.4	0.29
17		58	4.2

accommodate a 3-pentyl substituent found in **13**. Furthermore, replacement of the isopropyl group in **9** with cyclic nonpolar groups resulted in **14** and **15**, which had binding affinities equipotent to **9**.

Replacing the cyclohexyl group in compound **15** with the phenyl group as in compound **16** provides a comparison between an aromatic and cyclic alkyl group binding in the prime side hydrophobic pocket. Although this substitution did not improve activity, this phenyl ring served as a template for the addition of polar groups which may interact favorably with the His248 side chain or Glu250 (NH). Unfortunately, compound **17** with a *para* carboxyl group exhibited poor binding affinity, suggesting a favorable interaction with His248 or Glu250 (NH) was not obtained (Table 2).

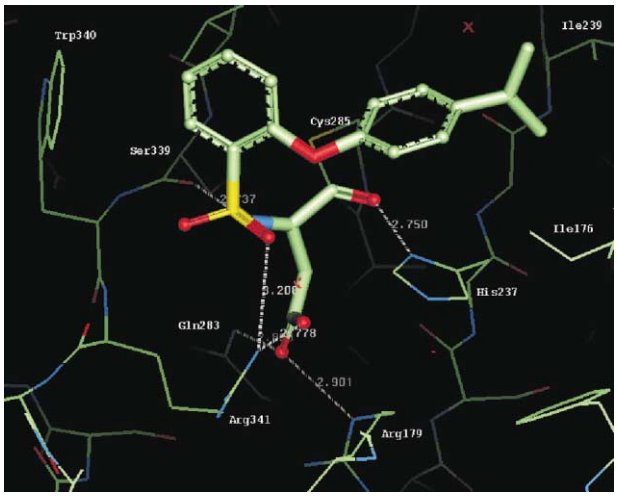
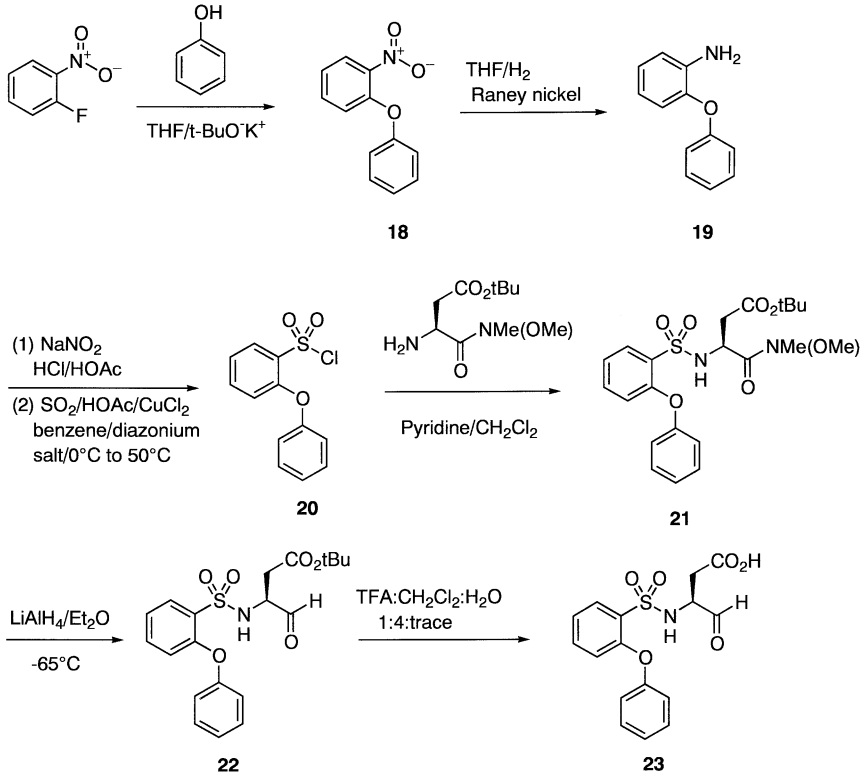


Figure 1. Crystal structure of **9** in complex with caspase-1. The catalytic residue (Cys285) forms the standard covalent bond with the aspartic acid aldehyde and orients the acidic side chain in the electropositive pocket formed by residues (Arg179, Gln283, Arg341) and inhibitor **9** is shown with thicker bonds. Protein and inhibitor atoms are shown with atoms in green (carbon), blue (nitrogen), red (oxygen), and yellow (sulfur).

The synthetic route to compounds of type **2** is shown in Scheme 1. The phenol was treated with potassium *t*-butoxide in dry THF for 1 h. The *o*-fluoro-nitrobenzene was added to afford the diphenyl ether **18**. The nitro group was subsequently reduced by catalytic hydrogenation using Raney nickel to give aniline **19**. The resulting aniline was treated with sodium nitrite/HCl or isoamyl nitrite/boron trifluoride etherate to form the diazonium salt which was treated with a mixture of sulfur dioxide/HOAc/$CuCl_2$ to produce the sulfonyl chloride **20**.[13] The resulting sulfonyl chloride was used to acylate Asp–N(OCH_3)CH_3/pyridine/CH_2Cl_2, giving the diphenyl ether sulfonamide **21**. The Weinreb amide was reduced with lithium aluminum hydride in Et_2O at −65 °C yielded the aldehyde **22**. The *tert*-butyl ester protecting group was then removed with 15% TFA in CH_2Cl_2 to provide the acid **23** as a final product.

In conclusion, the diphenyl ether sulfonamides described above provide a novel lead template for the development of caspase-1 inhibitors. This series differs from previously described biphenyl sulfonamide small molecule inhibitors of caspase-1, in that His237 binds in a



Scheme 1. Synthesis of diphenyl ether sulfonamide **2**.

gauche orientation and the distal substituted phenyl ring binds in the prime side hydrophobic pocket. The 20-fold increase in binding affinity over biphenyl sulfonamide series[10] observed in this diphenyl ether series is encouraging, suggesting that caspase-1 inhibitors built on this novel structure have the potential for future drug development.

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