

1,3,5-Triazine Derivatives as Protein A Mimetics for the Treatment of Autoimmune Diseases

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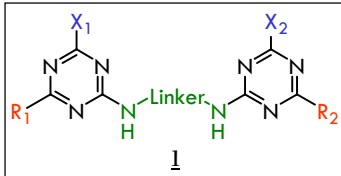
Introduction

Many autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosus, idiopathic thrombocytopenia purpura (ITP), glomerulonephritis or vasculitis) are related to the presence of pathogenic antibodies or immune complexes in the system. Cypress developed a protein A column to remove these autoantibodies and immune complexes during an apheresis procedure. The ProSORBA[®] column was approved by the US FDA in 1987 for ITP and in 1999 for rheumatoid arthritis. It has the advantage of not suppressing the immune system and is a therapeutic option for treatment-refractory patients.¹

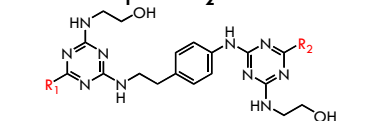
Protein A (MW = 42,000) is found on the surface of the bacteria *Staphylococcus aureus*. This protein contains five domains which are able to bind with high affinity to the tail portion of human and mouse antibodies. Protein A has therapeutic utility, but its toxicity and cost limit its therapeutic use. There is a definite need for a non-toxic small molecule mimetic of protein A which can be administered as a drug.

Abstract

We present herein the structure-activity relationships of a series of triazine dimers of general structure **1** that were developed as protein A mimetics. The triazine framework has been shown to form a very effective scaffold for the construction of protein binding ligands.² Three thousands compounds were synthesized and selected examples will be discussed. Some of these compounds were equipotent to protein A in a competitive IgG binding assay. The inhibition of *Staphylococcus aureus* Protein A binding to IgG by the biomimetic ligands was measured in a previously described binding assay.³ Due to solubility problems with some compounds, the IC₅₀ was determined in PBS in presence of 20% DMSO. A molecular binding study between IgG and our lead compound will also be presented. These compounds also demonstrate good *in vivo* activity in inflammation and autoimmune disease models.



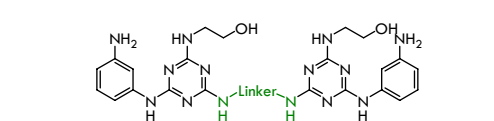
Effect of R₁ and R₂



Entry	R ₁	R ₂	IC ₅₀ (nM)
1			> 10 ⁵
2			117
3			209
4			204
5			208
6			192

IC₅₀ (Protein A) = 334 nM

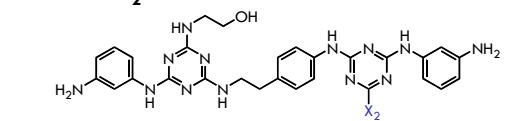
Effect of the diamine linker



Entry	Diamine Linker	IC ₅₀ (nM)	Entry	Diamine Linker	IC ₅₀ (nM)
1		321	7		617
2		424	8		248
3		165	9		1020
4		284	10		117
5		422	11		460
6		519	12		112

IC₅₀ (Protein A) = 334 nM

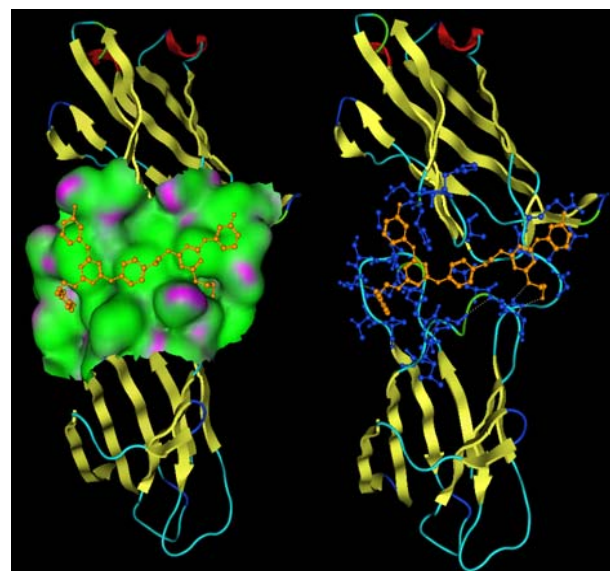
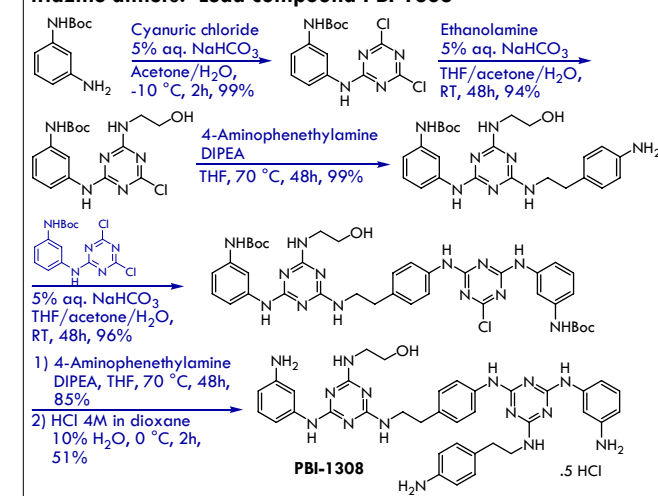
Effect of X₂



Entry	X ₂	IC ₅₀ (nM)	Entry	X ₂	IC ₅₀ (nM)
1		64	7		77
2		130	8		2560
3		111	9		117
4		51	10		408
5		57	11		196
6		9900	12		69

IC₅₀ (Protein A) = 334 nM

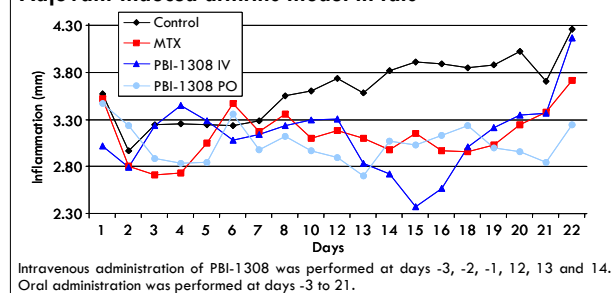
Representative example of the synthesis of unsymmetrical triazine dimers: Lead compound PBI-1308



Possible binding site of PBI-1308 onto Fc portion of IgG

The active site was defined by the collection residues of IgG Fc within 5A of bound Protein A (PDB: 1F2C.pdb). Moe was used to generate several potential docking modes and a low energy one was chosen. Molecular dynamic (500K, Heat:100 ps. Run:200 ps. Cool:100 ps.) was used to explore the region and the residues defining the active site were also allowed to move. The structures above show the final bound complex. It is likely that there are multiple binding modes.

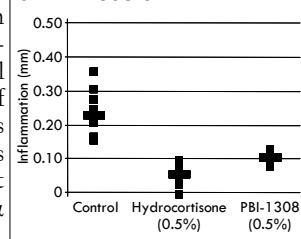
Inhibition of inflammation by oral (PO) and intravenous (IV) administration of PBI-1308 in a Complete Freund's Adjuvant-induced arthritis model in rats



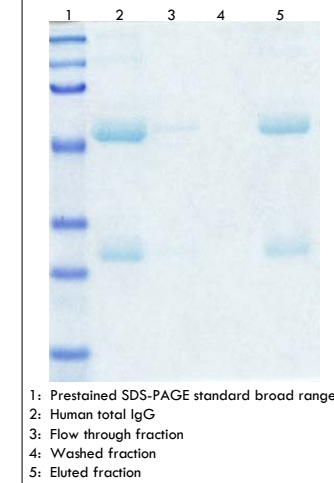
In vivo models

The efficacy of these compounds was tested in several known models of inflammation including Delayed-Type Hypersensitivity (DTH) and adjuvant-induced arthritis. In DTH, topical administration of PBI-1308 induced a significant reduction of the inflammation and is equipotent to hydrocortisone (positive control). In a Complete Freund's Adjuvant-induced arthritis (AIA) model in rats, significant inhibition of AIA by oral and intravenous administration of PBI-1308 was accompanied by significant reduction of PGE₂ and TNF-α in the inflamed tissue.

Effect of topical application of PBI-1308 on DTH



SDS-PAGE of human total IgG bound and purified by PBI-1308 linked on a solid support



Specific assay

A derivative of PBI-1308 was covalently linked to agarose beads to test its ability as an affinity ligand to bind immunoglobulins (Ig).² A chromatography experiment proved that this derivative selectively binds human, mouse and rat IgG subclasses.

Conclusion

A first in class series of low molecular weight synthetic molecules is described that mimic the ability of protein A to bind to human IgG antibody. The SAR studies demonstrate the importance of the presence of at least one 1,3-phenylenediamine substituent. The hydrophobicity of these 1,3,5-triazine dimers is important for binding to the IgG Fc portion. These compounds show potent *in vivo* activity in several inflammation models, by intravenous, oral or topical routes. A second generation series of compounds has also been developed and will be presented in due course. These compounds offer a unique approach for the treatment of autoimmune diseases by virtue of their novel biochemical target.

References

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- C.R. Lowe, K. Sproule, R. Li, D.J. Stewart, J.C. Pearson, S.J. Burton Patent US6117996A September 12th 2000: Triazine based ligands and use thereof.
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