

1,3,5-Triazine Derivatives with Improved Solubility for the Treatment of Inflammatory Diseases



B. Zacharie, D. Fortin, S.D. Abbott, J.-F. Bienvenu, A. Cameron, J. Cloutier, J.-S. Duceppe, A. Ezzitouni, K. Houde, C. Lauzon, J. Lechasseur, N. Moreau, V. Perron, N. Wilb, M.-È. Fafard, D. Gaudreau, L. Geerts, B. Grouix, F. Sarra-Bournet, L. Gagnon and C. Penney
ProMetic BioSciences Inc., 500 Cartier Blvd. W., Suite 150, Laval, Quebec, Canada H7V 5B7

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 potential 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.

We have presented previously a series of triazine dimers of general structure 1 that were developed as protein A mimetics.² The triazine framework has been shown to form a very effective scaffold for the construction of protein binding ligands.³ The inhibition of protein A binding to IgG by the biomimetic ligands was measured in a previously described binding assay.⁴ These compounds also demonstrate good *in vivo* activity in inflammation disease models. Described herein is a series of more soluble analogues that show improved oral activity. The structure-activity relationship of these compounds will be presented.

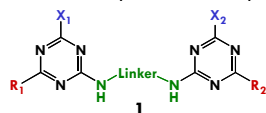


Table 1. Effect of the diamine linker

Entry	Diamine Linker	IC ₅₀ (nM)	Entry	Diamine Linker	IC ₅₀ (nM)
1		697	4		1250
2		1210	5		2190
3		631			

IC₅₀ (protein A) = 334 nM

Table 2. Effect of R₂

Entry	R ₂	IC ₅₀ (nM)	Entry	R ₂	IC ₅₀ (nM)
1		253	5		1250
2		444	6		1370
3		348	7		1470
4		1080	8		138
			9		2440

We have shown previously the importance of adding at least one 1,3-phenylenediamine substituent.²

IC₅₀ (protein A) = 334 nM

Table 3. Effect of X₁ and X₂

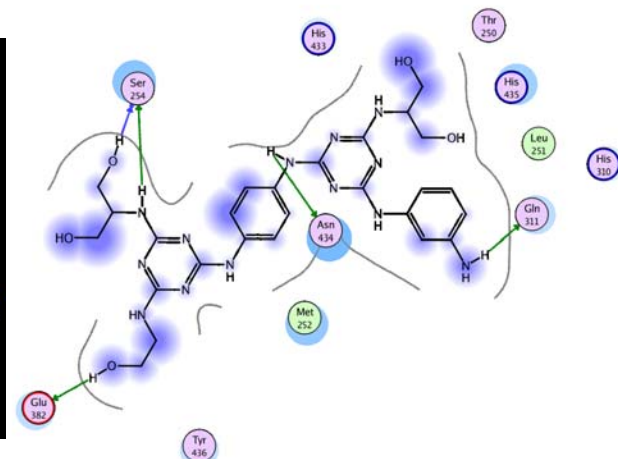
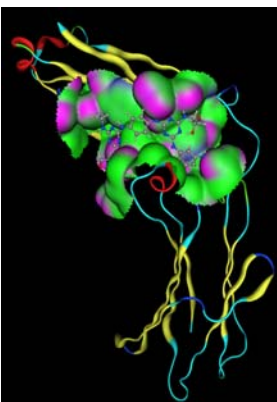
Entry	X ₁	X ₂	IC ₅₀ (nM)	Entry	X ₁	X ₂	IC ₅₀ (nM)
1			107	7			298
2			248	8			580
3			>10 ⁵	9			1250
4			501	10			834
5			868	11			398
6			430	12			791

IC₅₀ (protein A) = 334 nM

Possible binding site of PBI-3540 onto Fc portion of IgG

The active site was defined by the collection (300K, Heat:100 ps. Run: 1000 ps. Cool:100 ps.) of residues of IgG Fc within 5Å of bound Protein A (PDB: 1F2C.pdb.). MOE⁵ was used to generate several potential docking modes and a low energy one was chosen. Molecular dynamics

was used to explore the region and the residues defining the active site were also allowed to move. The structures below show the final bound complex. It is very possible that there are multiple binding modes.

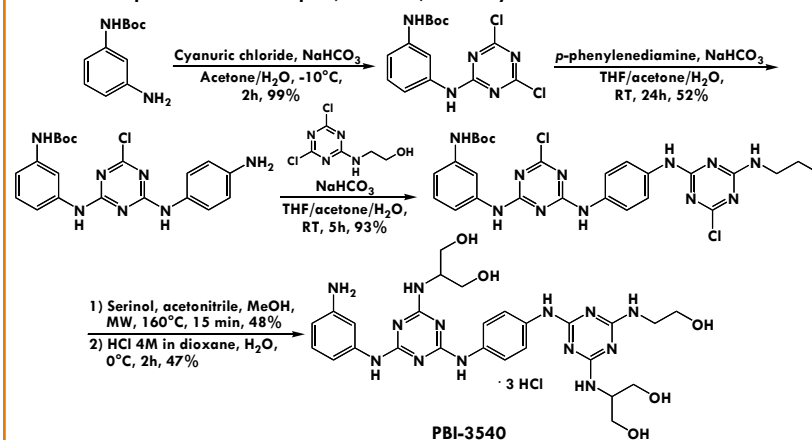


Physicochemical properties of PBI-3540

MW = 719 g/mol
LogD^{7.4} = -0.12; LogD^{6.2} = -0.21; LogD^{5.0} = -0.50
cLogP = 0.97

The compound showed no toxicity when given 50 mg/kg *i.v.* or oral.

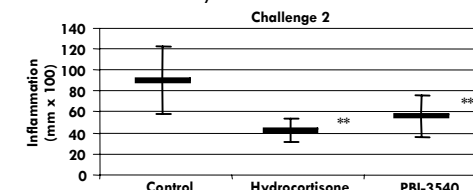
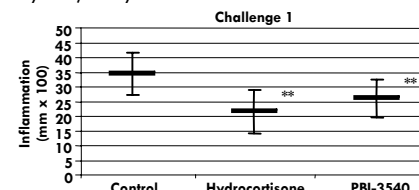
Representative example (PBI-3540) of the synthesis of a triazine dimer



Effect of oral administration of PBI-3540 on delayed-type hypersensitivity – *In vivo* models

The efficacy of these compounds was tested in known models of inflammation including contact hypersensitivity. The mice were sensitized with oxazolone at day -3 and challenged with an application of oxazolone on the surface of the right ear (Challenge 1, day 3; Challenge 2, day 10). Hydrocortisone and the test molecule were

orally administered daily at days 0 to 13 at a dose of 50 mg/kg. Ear thickness (inflammation) was measured on day 4 to day 7, and on day 11 to day 14. The measure for Challenge 1 is the mean inflammation at day 7, while Challenge 2 corresponds to the measure of inflammation at day 14.



** Significantly different from Control, p < 0.01

Conclusion

In summary, a 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 receptor site of the IgG Fc portion. These compounds show potent *in vivo* activity in an inflammation model, especially by oral route. Some derivatives of PBI-3540 are presently in preclinical development. These compounds offer a novel approach for the treatment of inflammatory diseases by virtue of their unique biochemical target.

References

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