

2,4,6-Trisubstituted Triazines as Protein A Mimetics for the Treatment of Autoimmune Diseases

Boulos Zacharie,* Shaun D. Abbott, Jean-François Bienvenu, Alan D. Cameron, Josée Cloutier, Jean-Simon Duceppe, Abdallah Ezzitouni, Daniel Fortin, Karine Houde, Caroline Lauzon, Nancie Moreau, Valérie Perron, Nicole Wilb, Michel Asselin, André Doucet, Marie-Ève Fafard, Dannyck Gaudreau, Brigitte Grouix, François Sarra-Bournet, Natalie St-Amant, Lyne Gagnon, and Christopher L. Penney

ProMetic BioSciences Inc., 500 Boulevard Cartier Ouest, Bureau 150, Laval, Québec H7V 5B7, Canada

Received September 21, 2009

A first-in-class series of low molecular weight trisubstituted triazines were synthesized and evaluated for their ability to mimic protein A binding to human IgG antibody. The structure–activity relationship (SAR) demonstrates that the 1,3-phenylenediamine component was essential for robust activity. Twenty-two compounds, represented by lead molecule **34**, displayed significant activity compared to protein A. These compounds may prove useful for the treatment of autoimmune disease.

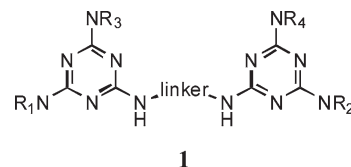
Introduction

Staphylococcal protein A (MW = 42 000) is displayed on the cell membrane of the Gram-positive bacterial pathogen *S. aureus*. This protein contains five domains that selectively bind with high affinity the tail (Fc^a) portion of human and mouse antibodies.¹ The interaction between protein A and immunoglobulin G (IgG) is one of the most studied protein–protein interactions.² The binding properties of protein A make it the standard tool for the purification of monoclonal antibodies. In general, antibodies are purified by ion-exchange or affinity column chromatography with protein A attached to a solid-phase support.³ However, purification with protein A affinity adsorbents is costly, as well as inadequate regarding purity and yield.⁴ Furthermore, these adsorbents have been shown to leak protein A and contaminate purified immunoglobulin products.⁵ Consequently, there is significant interest in developing a synthetic mimetic of protein A that is more economical and stable. However, few molecules have been reported in the literature that mimic bacterial protein A and can be used to bind IgG. These are small synthetic compounds,^{3a} larger peptides,⁶ or polypeptides⁷ covalently attached to a solid-phase support.^{3a,8}

Protein A also has therapeutic utility, but its toxicity and cost limit its use. Nonetheless, a protein A column from Cypress was approved by the U.S. FDA for the treatment of autoimmune diseases: immune thrombocytopenic purpura in 1987 and severe rheumatoid arthritis in 1999.⁹ This treatment

requires the use of protein A covalently linked to a silica matrix whereby the patient's blood is passed through an external device in a manner similar to kidney dialysis (apheresis). This treatment requires multiple visits to the doctor's office, and the outcome after treatment is often unpredictable.¹⁰ Indeed, the manufacture of this product was discontinued in 2008. Subsequently, there is need for a novel, safe, nontoxic small molecule mimetic of protein A¹¹ that can be administered as a drug.

1,3,5-Triazine compounds have been studied extensively and are the subject of many reviews.¹² The triazine scaffold provides the basis for the design of biologically relevant molecules with widespread application as therapeutics. For example, these compounds possess potent antiprotozoal,¹³ anticancer,¹⁴ anti-malarial,¹⁵ antiviral,¹⁶ and antimicrobial activity.¹⁷ As part of our autoimmune disease program, small molecules were examined as potential mimetics of protein A. It was reported^{3a} that the aromatic side chains of the hydrophobic core dipeptide Phe¹³²-Tyr¹³³ of protein A are able to bind the Fc portion of IgG. This structural feature was therefore taken into consideration when designing a protein A mimetic. Aniline and tyramine substituents may mimic the side chain of Phe¹³²-Tyr¹³³, while a triazine ring was used as a core structure to maintain optimal orientation of the two mimetic groups. In this paper, the synthesis and activities of trisubstituted triazines are reported as exemplified by the general structure **1**, where NR₁, NR₂, NR₃, and NR₄ represent primary or secondary amines.

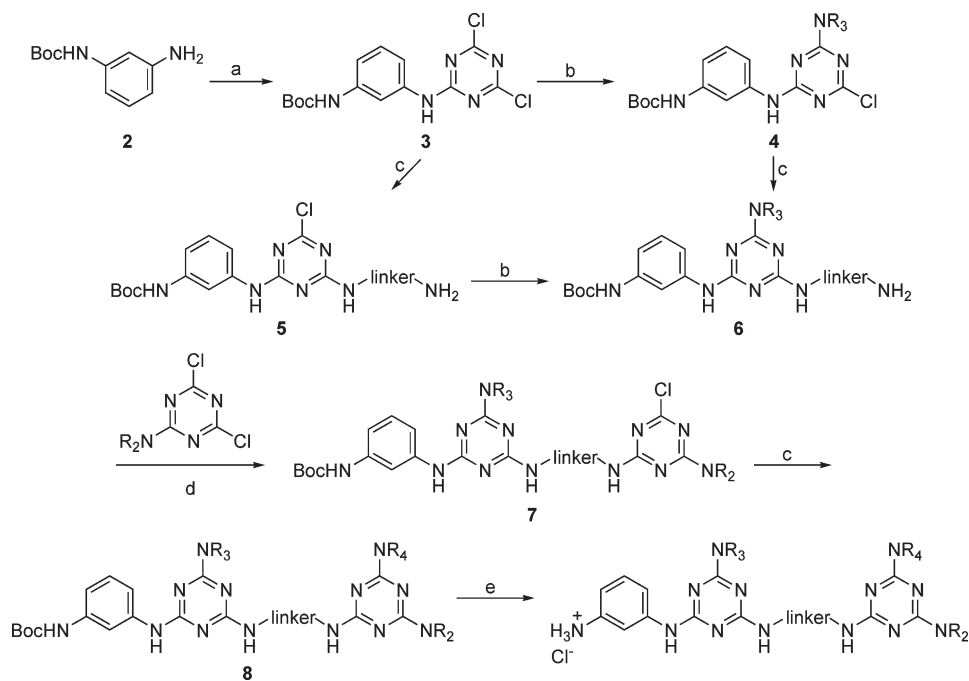


Chemistry

The starting material for these compounds is cyanuric chloride. This is an inexpensive commercially available reagent which makes its use attractive. The ease of displacement

*To whom correspondence should be addressed. Phone: (450) 781-1394, extension 2251. Fax: (450) 781-1403. E-mail: b.zacharie@prometic.com.

^a Abbreviations: dsDNA, double-standard deoxyribonucleic acid; DTH, delayed-type hypersensitivity; ELISA, enzyme-linked immunosorbent assay; F1, first generation; Fc, fragment crystallizable; FcγR, fragment crystallizable type γ receptor; Fab, fragment antigen-binding; FDA, Food and Drug Administration; HAG, heat aggregated human immunoglobulin G; IgG, immunoglobulin G; MRL/lpr, a substrain of Murphy Roths Large mice that is genetically predisposed to the development of systemic lupus erythematosus-like syndrome; NZB/NZW, cross-breed of New Zealand black and New Zealand white strains; PBS, phosphate buffered saline; SLE, systemic lupus erythematosus.

Scheme 1^a

^aFor intermediates **4a–8a**, NR₂ = 3-(Boc-amino)aniline, NR₃ = NR₄ = 2-aminoethanol, and H₂N-linker-NH₂ = 4-aminophenethylamine. Reagents and conditions: (a) cyanuric chloride, acetone/water, 0 °C; (b) hydroxyalkyl- or aminoarylamines, NaHCO₃, THF/acetone/water; (c) aminoalkyl- or aminoarylamines, THF, 60 °C; (d) NaHCO₃, THF/acetone/water; (e) CH₂Cl₂, 4 M HCl/dioxane.

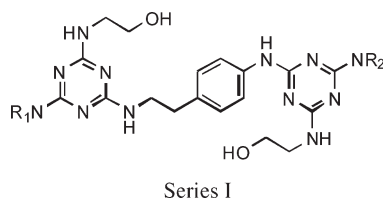
of chlorine atoms in cyanuric chloride by various nucleophiles enhances the utility of this reagent for the preparation of mono-, di-, and trisubstituted 1,3,5-triazines under controlled temperature.¹² The general synthetic procedure for triazine compounds is outlined in Scheme 1. It illustrates the route employed for the trisubstituted triazine compound **8** where the key intermediate is amino derivative **6**. This compound was prepared by two different approaches. The first method was the treatment of *m*-substituted aniline **2** with cyanuric chloride at 0 °C to give dichlorotriazine derivative **3**. This reaction proceeded in excellent yield (>99%) and is general for different aniline derivatives. The next step was the reaction of compound **3** with different hydroxyalkylamines or aminoarylamines in the presence of base to afford triazine **4** in high yield (>90%). This compound was then reacted with different aminoalkyl- or aminoarylamines to give the intermediate **6**. The latter was also prepared by converting dichlorotriazine **3** to the corresponding amine derivative **5** followed by reaction with hydroxyalkyl- or aminoarylamines. However, compound **5** was only isolated in low yield (40%) because of the formation of byproduct. Best results were obtained using the initial approach that gave high yield and purity of amino derivative **6**. The next step was linkage of the two triazine rings. This was achieved by slow addition of 4,6-dichlorotriazine bearing an aliphatic or aromatic amine on position 2 to amino derivative **6** in the presence of sodium bicarbonate to give the expected chloro derivative **7** in high yield (>80%). This reaction is general for different aminoalkyl-, aminoaryl-, or aminoalkylamines and can be used to generate linked bis-triazine analogues **7** with selected linkers. The final step was displacement of the last chlorine atom by various alkyl-, aryl-, or aralkylamines. Thus, treatment of compound **7** with different amines at 60 °C gave the final intermediate **8** in high yield (>80%). Subsequent acid-induced deprotection gave the final compound **1** in quantitative yield. The synthesis of a

representative example of compound **1** (compound **10**) is described in detail. This synthetic route is practical, amenable to scale-up, and offers a general procedure for the preparation of a variety of analogues of triazine compound **1**.

Results and Discussion

A number of triazine derivatives of structural type **1** were synthesized and evaluated as protein A mimetics by competitive ELISA. Twenty-two compounds (**10–12**, **14**, **19–23**, **26**, **29**, **31–36**, **40–44**) display significant activity comparable to protein A (Tables 1–3). As shown in Table 1 (series **I** compounds), analogues of **1** were synthesized in which NR₁ and NR₂ were different aliphatic or aromatic amines while maintaining the linker as 4-aminophenethylamine and NR₃ and NR₄ as ethanolamine. The most active triazine compounds are **10** and **15**. Clearly, the 3-amino function of the 3-aminoaniline group (NR₁) appears to be essential, and activity declines upon replacement of 3-aminoaniline with other groups such as 2-fluoro- (**9**) or 3-methylsulfonamidoaniline (**18**) or aminoethylamidine (**17**). Analogues were also synthesized in which 3-aminoaniline (NR₂) was replaced by other substituted anilines. In this case, 3-fluoro- (**11**), 4-fluoro- (**12**), 4-chloro- (**13**), and 3-methylsulfonylaniline (**14**) display less activity than compounds **10** and **15**. Replacement of aminoaniline (**10**) or ethanolamine (**15**) at NR₂ with ethylenediamine (**16**) results in a loss of activity. On the basis of this result, compound **10** was selected as a core structure upon which to define a structure–activity relationship for triazine scaffold **1**.

Table 2 (series **II** compounds) illustrates the effect of the linker between the two triazine rings in compound **1**, where NR₁ and NR₂ are 3-aminoaniline and NR₃ and NR₄ are ethanolamine. Aliphatic linkers with a two- or three-carbon spacer (**19**, **20**, **22**, or **23**) demonstrate good activity compared to protein A. However, an increase in chain length of one

Table 1. IC₅₀ (nM) of Series I Compounds Relative to Protein A As Ascertained by a Protein A Human IgG Competitive ELISA

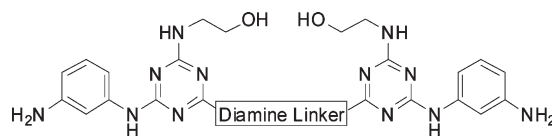
Compd No.	NR ₁	NR ₂	IC ₅₀ (nM) ^a	
			a	b
9			Inactive	Inactive
10			84	144
11			382	209
12			361	204
13			841	893
14			243	192
15			94	81
16			410	749
17			Inactive	Inactive
18			Inactive	Inactive
Protein A			189	334

^aCompounds were assayed in both PBS and 20% DMSO/PBS.

carbon (**21**) results in reduction of activity. In contrast, addition of an oxygen atom to the four-carbon spacer (**24**) led to reasonable activity. Selection of the linker is critical, since activity is dependent upon the distance between the two triazine rings. Replacement of the alkyl spacer by aromatic linkers gave different activities. For example, 4-aminoaniline (**26**) demonstrates better activity than 3-aminoaniline (**25**). The best linkers were 4-aminophenethylamine (**10**) or its cyclic structure (**29**). These analogues display the same activity as protein A. Prior work¹⁸ indicated the importance of 4-aminophenethylamine as a linker at this position. This moiety enhances good *in vitro* antibody binding activity, and compound **10** supports this finding. Interestingly, linkers such as 3-aminophenethylamine (**28**) or 4-amino- α -methylbenzylamine (isomeric attachment of the aminoethyl group) (**27**) displayed a

decrease in activity. Again, the distance and relative degrees of freedom are important at this position.

It was next decided to synthesize triazine **1** in which NR₁ and NR₂ were 3-aminoaniline, NR₃ was ethanolamine, and NR₄ was varied with different aromatic or aliphatic amines (Table 3, series **III** compounds). The phenethylamine group (**30**) results in a loss of activity. However, aminobenzylamine (**32**) or 2-, 3-, or 4-aminophenethylamine (**31**, **33**, or **34**) substituents display the same *in vitro* activity as protein A. Also, hydroxy substitution of the two-carbon spacer of 3-aminophenethylamine (**35**) retains activity comparable with that of compound **33**. Surprisingly, 3-hydroxyphenethylamine (**36**) possesses comparable activity with protein A while 4-hydroxy- (**37**), 4-fluoro- (**38**), and 4-dimethylaminophenethylamines (**39**) lose activity. Good activity is also observed with an aminoanilino substituent. For example, the

Table 2. IC₅₀ (nM) of Series II Compounds Relative to Protein A As Ascertained by a Protein A Human IgG Competitive ELISA

Series II

Compd No.	Linker	IC ₅₀ (nM) ^a	Compd No.	Linker	IC ₅₀ (nM) ^a
19		58	25		293
20		56	26		157
21		187	27		Not active
22		41	28		Not active
23		56	29		80
24		70	Protein A		189

^a Compounds were assayed in both PBS and 20% DMSO/PBS. Results for the PBS assay are reported. Results in 20% DMSO/PBS were consistent with results in PBS with the exception of compound **24** (5192 nM for 20% DMSO in PBS), probably because of solubility issues.

3-aminoaniline substituent (**40**) is more potent than protein A. When NR₄ is an aliphatic amine, the activities were variable and a rigid amine is preferred at this position. Piperazine (**44**) is the most effective substituent among these latter amines, e.g., *N,N*-dimethylaminoethylamine (**42**) and dihydropyrrole (**43**).

On the basis of the above results, triazine compound **34** was selected as the lead structure. It was of interest to demonstrate that this compound binds directly to IgG as in the case of protein A. Indeed, compound **34** linked to a solid-phase support was able to bind and purify IgG.¹⁹ Figure 1 shows a fitting^{20a} of compound **34** into the binding site of the B domain of protein A. The binding site is largely hydrophobic (green) and contains only a few sites for polar contacts (purple).^{20b} It is possible to explain the significant activity of compound **34** compared to the inactive compound **37**, perhaps because of a process of protonation or deprotonation implicated in the interaction of this compound and the binding site of protein A. Furthermore, **34** was able to inhibit the binding of heat aggregated human IgG to the FcγR-I (CD64, high affinity receptor for monomeric and aggregated IgG), FcγR-IIa (CD32a, low affinity receptor for aggregated IgG and immune complex), and FcγR-IIIb (CD16b, low affinity receptor for immune complex) receptors, as illustrated in Table 4. In the competitive binding ELISA, **34** demonstrated equipotent affinity for IgG relative to protein A. However, a difference in affinity for FcγR was observed relative to protein A. Both protein A and **34** displayed almost equipotent affinity for FcγR-I. A different affinity profile for FcγR-IIa and FcγR-IIIb was observed for protein A and **34**. This difference may be explained by inhibition of binding by a multimeric protein versus a low molecular weight compound.

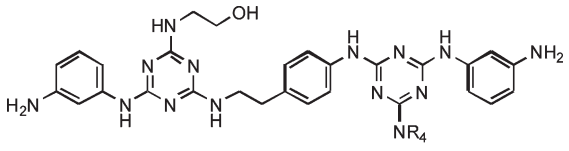
It was next desired to demonstrate that the binding activity of **34** would translate into a significant *in vivo* effect in a

relevant rodent model for autoimmune disease. For this purpose, use was made of two strains of mice that replicate the symptoms of systemic lupus erythematosus (SLE).²¹ Figure 2 illustrates the effect of weekly intravenous administration of **34** (50 mg/kg) on SLE in NZB/NZW-F1 mice. Treatment with **34** induces up to a 2-fold reduction of anti-dsDNA antibody without significant depletion of total IgG compared to vehicle.¹⁹ Most probably, compound **34** increases or facilitates the elimination of antibody immune complexes.

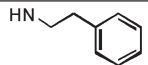
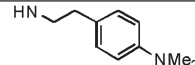
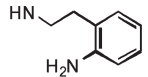
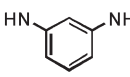
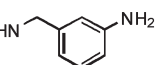

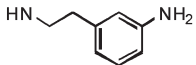
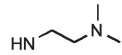
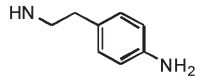
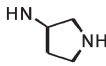
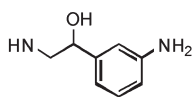

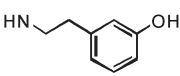
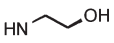
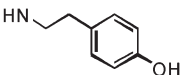
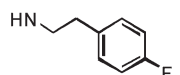
Another SLE experiment was performed using MRL/lpr mice. Mice were treated by intravenous injection of **34** (50 mg/kg, once a week) or vehicle once a week for 5 consecutive weeks (between weeks 18 and 22).²² Mice were sacrificed, and immune complex depositions were recorded in kidney tissue by electron microscopy. Micrographs A and B (Figure 3) represent kidney tissue from the control group (vehicle). In Figure 3A (4000×), many small and large immune complex depositions are observed in the epithelial (E) and mesangial (M) area. In Figure 3B (7000×), abundant and very large immune complex depositions are mostly observed in the mesangial area. Micrographs C and D (Figure 3) represent kidney tissue from the group treated with **34**. In Figure 3C (3500×), only a few immune complex depositions are observed in the mesangial area. In Figure 3D (7000×), only one smaller immune complex deposition is observed. However, mesangial cells appear to be very active, as demonstrated by the increase in lysosomes observed in an earlier stage of the pathology.

Conclusion

A first-in-class series of low molecular weight triazine dimers is described that mimic the ability of protein A to bind to murine and human IgG antibody. The SAR studies

Table 3. IC₅₀ (nM) of Series III Compounds Relative to Protein A As Ascertained by a Protein A Human IgG Competitive ELISA


Series III

Compd No.	NR ₄	IC ₅₀ (nM) ^a	Compd No.	NR ₄	IC ₅₀ (nM) ^a
30		449	39		695
31		185	40		56
32		141	41		89
33		119	42		89
34		54	43		233
35		130	44		59
36		118	10		84
37		Not active			
38		531	Protein A		189

^a Compounds were assayed in both PBS and 20% DMSO/PBS. Results for the PBS assay are reported. Results in 20% DMSO/PBS were consistent with results in PBS.

demonstrate the importance of the presence of a 1,3-phenylenediamine substituent. Lead structure **34** exhibits significant *in vivo* activity in a standard rodent model for autoimmune disease where antibody plays an important role in disease pathology. Additionally, **34** displays significant *in vivo* activity in other rodent models for immunological disease where antibody does not play a key role in disease pathology (e.g., DTH and adjuvant arthritis; results to be published elsewhere). In particular, compound **34** offers a novel approach to the treatment of autoimmune disease by virtue of a novel biochemical target coupled with significant *in vivo* activity.

Experimental Section

Melting points were obtained on an Electrothermal MEL-TEMP melting point apparatus. NMR spectra were recorded at 400 MHz for ¹H NMR, 377 MHz for ¹⁹F NMR, and 100 MHz for ¹³C NMR. All HPLC and mass spectra were recorded on a HP 1100 LC-MS Agilent instrument using a diode array detector, monitoring at 210 and 254 nm, and an analytical C18 column

Table 4. IC₅₀ (M) of Compound **34** Relative to Protein A As Ascertained by a Heat Aggregated Human IgG (HAG) Binding Assay

compd	binding assay, IC ₅₀ (M)			
	protein A competitive	FcγR-I-HAG	FcγR-IIa-HAG	FcγR-IIIb-HAG
protein A	1.6 × 10 ⁻⁷	4.5 × 10 ⁻⁷	1 × 10 ⁻¹⁰	> 1 × 10 ⁻⁶
34	8.3 × 10 ⁻⁸	2.9 × 10 ⁻⁶	1.2 × 10 ⁻⁵	5.7 × 10 ⁻⁵

(75 mm × 4.6 mm, 5 μm), with gradients of acetonitrile/water containing 0.01% TFA and a flow rate of 2 mL/min (30 °C). Gradients used were as follows: gradient A, 1–40%/6 min; gradient B, 15–99%/6 min; gradient C, 15–99%/10 min; gradient D, 10–40%/6 min; gradient E, 1–20%/6 min. The purity was ≥97% for all compounds and was determined by LC-MS.

2-([3-*tert*-Butoxycarbonylamino]phenylamino)-4,6-dichloro-[1,3,5]triazine (3). A solution of cyanuric chloride (2.37 g, 12.9 mmol) in acetone (16 mL) was cooled to 0 °C, and ice-water (6.6 mL) was added. The suspension was treated dropwise with a solution of **2** (2.65 g, 12.7 mmol) in acetone (7.7 mL). The pH of the resultant clear solution was adjusted to 7–8 by addition of

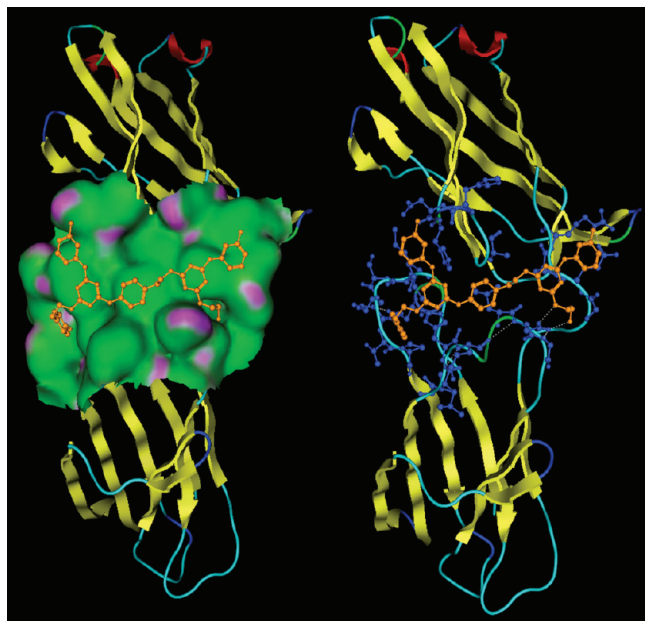


Figure 1. Postulated binding site of compound **34** (orange) onto the Fc portion of IgG.

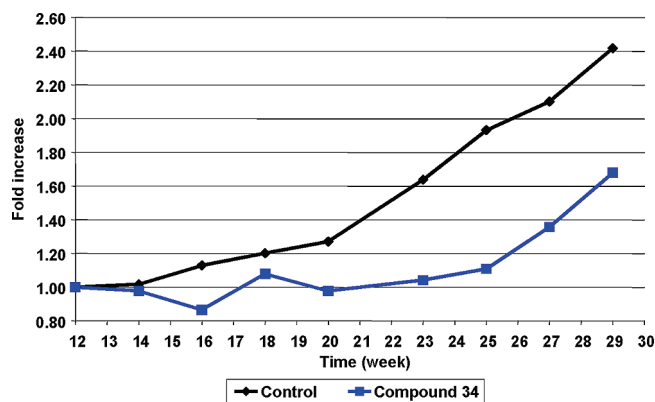


Figure 2. Inhibition of serum dsDNA autoantibodies by compound **34** in NZB/NZW mice.

5% w/v aqueous sodium bicarbonate (27 mL), and the mixture was stirred at 0 °C. The resultant precipitate was collected by filtration, washed with water (50 mL), and dried in vacuo to give **3** as a pale-brown solid (4.56 g, quantitative). Mp = 172–173 °C. ¹H NMR (CD₃OD, 400 MHz) δ: 7.69–7.73 (m, 1H), 7.14–7.30 (m, 3H), 1.52 (s, 1H). ¹³C NMR (CD₃OD, 100 MHz) δ: 170.32, 169.42, 164.52, 153.39, 140.72, 137.71, 129.53, 116.47, 115.78, 112.55, 79.78, 28.78. LRMS (ESI): *m/z* 356.0 (M(³⁵Cl)₂H⁺). HPLC: *t*_R = 4.4 min (gradient B, purity 100% at 254 nm).

2-[4-([3-*tert*-Butoxycarbonylamino]phenyl)amino]-6-chloro[1,3,5]-triazine-2-ylamino]ethanol (4a). A solution of **3** (210 mg, 0.59 mmol) in tetrahydrofuran/acetone/H₂O (4:1:1, 9 mL) was treated with ethanolamine (39 μL, 0.65 mmol) and 5% w/v aqueous sodium bicarbonate (1.3 mL), and the mixture was stirred at room temperature overnight. The mixture was treated with water (5 mL) and extracted with ethyl acetate (2 × 5 mL). Combined extracts were washed with saturated sodium chloride (10 mL), dried over sodium sulfate, and evaporated in vacuo to give the crude product. Purification on silica gel (elution with 5–50% ethyl acetate/hexanes) gave **4a** as an off-white solid (214 mg, 95%). Mp = 157–159 °C. ¹H NMR (CD₃OD, 400 MHz) δ: 8.20 (broad, 1H), 7.76 (broad, 1H), 7.24–7.30 (m, 1H), 7.08–7.22 (m, 3H), 6.96 (broad, 1H), 3.72 (t, *J* = 5.6 Hz, 1H),

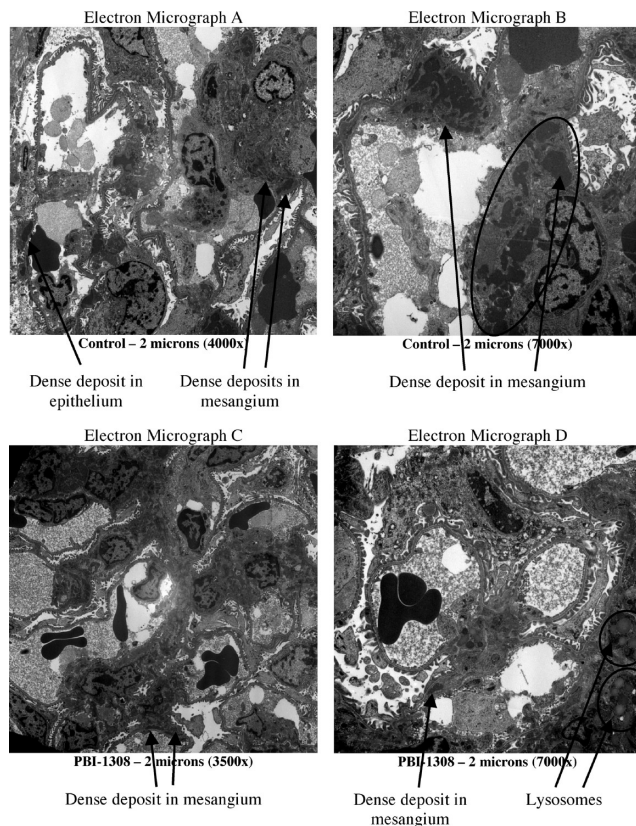


Figure 3. Electron micrographs of kidney tissue from SLE experimental in MRL/LPR mice. Micrographs A and B are from control group, and micrographs C and D are from the **34**-treated group.

3.67 (t, *J* = 5.6 Hz, 1H), 3.61 (broad, 1H), 3.46–3.52 (m, 1H), 1.51 (s, 9H). ¹³C NMR (CD₃OD, 100 MHz) δ: 169.37, 168.56, 166.36, 166.19, 164.12, 163.93, 154.06, 153.98, 139.85, 139.76, 139.62, 139.24, 139.15, 128.92, 128.68, 128.62, 115.23, 114.76, 113.88, 111.36, 111.03, 79.77, 79.64, 65.75, 60.51, 60.38, 43.28, 43.04, 27.79, 27.60 (mixture of rotamers). LRMS (ESI): *m/z* 381.2 (M(³⁵Cl)H⁺). HPLC: *t*_R = 3.4 min (gradient B, purity 100% at 254 nm).

2-[4-[2-[4-Aminophenyl]ethylamino]-6-([3-*tert*-butoxycarbonylamino]phenyl)amino][1,3,5]triazine-2-ylamino]ethanol (6a). A solution of **4a** (214 mg, 0.56 mmol) in anhydrous tetrahydrofuran (6 mL) was added to 4-aminophenethylamine (230 mg, 1.69 mmol), and the mixture was stirred at 60 °C overnight. The suspension was dissolved by addition of methanol and was evaporated onto silica gel. Purification on silica gel (elution with 10–75% ethyl acetate in hexanes) gave **5a** as a white solid (220 mg, 81%). Mp = 154–155 °C. ¹H NMR (CD₃OD, 400 MHz) δ: 7.26 (broad, 2H), 7.13 (t, *J* = 7.8 Hz, 2H), 6.98 (d, *J* = 8.2 Hz, 4H), 6.68 (d, *J* = 2.5 Hz, 2H), 3.68 (broad, 2H), 3.52 (broad, 4H), 2.72 (t, *J* = 7.4 Hz, 2H), 1.49 (s, 9H). LRMS (ESI): *m/z* 481.4 (MH⁺). HPLC: *t*_R = 1.7 min (gradient B, purity 100% at 254 nm).

2-[4-([3-*tert*-Butoxycarbonylamino]phenyl)amino]-6-[2-[4-[4-([3-*tert*-butoxycarbonylamino]phenyl)amino]-6-chloro[1,3,5]triazine-2-ylamino]phenyl]ethylamino][1,3,5]triazine-2-ylamino]ethanol (7a). A solution of **6a** (220 mg, 0.46 mmol) in tetrahydrofuran/acetone/water (4:1:1, 6 mL) was treated with **3** (179 mg, 0.50 mmol) and 5% w/v aqueous sodium bicarbonate (0.4 mL), and the mixture was stirred at room temperature overnight. The mixture was treated with water (5 mL) and extracted with ethyl acetate (2 × 5 mL). Combined extracts were washed with saturated sodium chloride (10 mL), dried over sodium sulfate, and evaporated in vacuo to give the crude product. Purification on silica gel (elution with 10–100% ethyl acetate/hexanes) gave **7a** as an off-white solid (303 mg, 83%).

Mp = 146–149 °C. ¹H NMR (CD₃OD, 400 MHz) δ: 7.75–7.72 (m, 3H), 6.88–7.32 (m, 9H), 3.68 (broad, 2H), 3.60 (broad, 2H), 3.54 (broad, 2H), 2.84 (t, *J* = 7.2 Hz, 2H), 1.49 (s, 18H). ¹³C NMR (CD₃OD, 100 MHz) δ: 171.83, 165.94, 164.52, 154.02, 140.50, 139.55, 136.57, 135.22, 128.96, 128.71, 128.50, 120.94, 114.54, 112.85, 110.90, 79.85, 79.57, 61.35, 60.38, 42.85, 42.11, 35.35, 27.59, 27.58, 19.71, 13.30. LRMS (ESI): *m/z* 800.4 (M(³⁵Cl)H⁺). HPLC: *t*_R = 3.2 min (gradient B, purity 100% at 254 nm).

2-(4-([3-*tert*-butoxycarbonylamino]amino)-6-(4-(2-(4-([3-*tert*-butoxycarbonylamino]amino)-6-(2-hydroxyethylamino)[1,3,5]triazin-2-ylamino)ethyl)phenylamino)[1,3,5]triazin-2-ylamino)ethanol (8a). A solution of **7a** (46 mg, 0.057 mmol) in anhydrous tetrahydrofuran (1 mL) was treated with ethanolamine (10 μL, 0.17 mmol), and the mixture was stirred at 60 °C overnight. The suspension was dissolved by addition of methanol and evaporated onto silica gel. Purification on silica gel (elution with 10–100% ethyl acetate/hexanes) gave **8a** as a white solid (39 mg, 82%). LRMS (ESI): *m/z* 825.4 (MH⁺), 847.4 (MNa⁺). HPLC: *t*_R = 2.5 min (gradient B, purity 100% at 254 nm).

2-(4-(3-aminophenylamino)-6-(4-(2-(4-(3-aminophenylamino)-6-(2-hydroxyethylamino)[1,3,5]triazin-2-ylamino)ethyl)phenylamino)-[1,3,5]triazin-2-ylamino)ethanol (10). A solution of **8a** (39 mg, 0.047 mmol) in anhydrous dichloromethane (0.79 mL) was cooled to 0 °C and treated with a 4 M solution of hydrochloric acid in 1,4-dioxane (2.36 mL, 9.44 mmol). The solution was stirred for 5.5 h, with slow warming to ambient temperature. 1,2-Dichloroethane (2 mL) was then added, and solvents were evaporated in vacuo. The residue was triturated with diethyl ether/hexanes (3:1, 4 mL) with sonication. This process was repeated two more times, and the solid was dried in vacuo to give **10** as a white solid (41 mg, 99.9%). Mp = 199–203 °C. ¹H NMR (CD₃OD, 400 MHz) δ: 7.87–8.01 (m, 1H), 7.62–7.77 (m, 2H), 7.48–7.60 (m, 5H), 7.26–7.40 (m, 2H), 7.15–7.20 (m, 2H), 3.67–3.78 (m, 6H), 3.58–3.66 (m, 4H), 2.95–3.03 (m, 2H). LRMS (ESI): *m/z* 625.4 (MH⁺). HPLC: *t*_R = 3.3 min (gradient A, purity 100% at 254 nm).

Protein A Human IgG Competitive ELISA. This assay evaluates the ability of compounds to mimic protein A by inhibition of binding to IgG. The competitive protein A binding assay was performed on a 96-well plate Maxisorp surface to enhance the binding of protein A to the bottom of the plate. The wells were coated with 100 μL of protein A (0.8 μg) and incubated overnight at 4 °C. After incubation, unbound protein A was removed by three washes with phosphate buffered saline (PBS). The plate was then incubated with 100 μL/well of a 2% solution of bovine serum albumin (BSA) for 1 h at 37 °C to block nonspecific protein binding. After incubation, the plate was washed three times with PBS. An amount of 50 μL of test compounds or protein A, diluted in PBS or PBS–20% DMSO at appropriate concentration, was added to the wells followed by addition of 50 μL of peroxidase-conjugated human IgG (HRP-IgG). After 1 h of incubation at 37 °C, the plate was washed three times with PBS to remove unbound HRP-IgG. Bound HRP-IgG was detected by incubation with 100 μL of 2,2′-azino-di[3-ethylbenzthiazoline sulfonate]diammonium salt (ABTS) solution for 20 min in the dark at room temperature. The plate was then read at 405 nm on an EL 800 universal Microplate reader (Bio-Tek). Data were analyzed using Microsoft Excel, and the concentration of compound that inhibits 50% binding of protein A (IC₅₀) was calculated using the Prism software.

Heat Aggregated Human IgG Binding Assay. In vitro binding assay on human Fcγ receptors was conducted on the basis of an assay described in the literature.²³

SLE Models. (1) New Zealand mice of the F1 hybrid cross NZB/NZW develop most of the autoimmune abnormalities seen in human SLE and die from SLE-like immune complex (IC)-mediated glomerulonephritis. The mice develop high titers of anti-DNA (double-strand and single-strand) and nuclear

extract (NE) antibodies, as well as SLE-related clinical manifestations including leukopenia, thrombocytopenia, proteinuria, and glomerulonephritis. These mice develop anti-DNA antibodies after the age of 3 months, with a peak of anti-DNA antibody response occurring at 7 months. Mice were treated by intravenous injection of compound **34** or vehicle once a week from week 12. Serum dsDNA autoantibodies were measured as described by Marino et al.²⁴

(2) MRL/lpr mice also spontaneously develop SLE-like syndrome. MRL/lpr mice have a homozygous *fas* mutation, which accelerates autoimmunity. Mice were treated by intravenous injection of compound **34** or vehicle once a week for 5 consecutive weeks. Mice were sacrificed, and immune complex deposition was recorded in kidney tissue by electron microscopy.

Acknowledgment. The authors thank Mona Saleh for data management and administrative support, Marc-Alexandre Duceppe, Joël Lechasseur, and Valérie Leduc for valuable technical support, and Lyne Marcil for the preparation of this manuscript.

Supporting Information Available: Characterization data for compounds **9** and **11–44**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) (a) Langone, J. J. Protein A of *Staphylococcus aureus* and related immunoglobulin receptors produced by streptococci and pneumococci. *Adv. Immunol.* **1982**, *32*, 157–252. (b) Nilsson, B.; Abrahamén, L. Fusions to staphylococcal protein A. *Methods Enzymol.* **1990**, *185*, 144–161. (c) Moks, T.; Abrahamén, L.; Nilsson, B.; Hellman, U.; Sjoquist, J.; Uhlén, M. Staphylococcal protein A consists of five IgG-binding domains. *Eur. J. Biochem.* **1986**, *156*, 637–643.
- (2) Delano, W.; Ultsch, M.; de Vos, A. M.; Wells, J. A. Convergent solutions to binding at a protein–protein interface. *Science* **2000**, *287*, 1279–1283 and references cited therein.
- (3) (a) Li, R.; Dowd, V.; Stewart, D. J.; Burton, J.; Lowe, C. R. Design, Synthesis, and application of a protein A mimetic. *Nat. Biotechnol.* **1998**, *16*, 190–195. (b) Fassina, G.; Ruvo, M.; Palombo, G.; Verdoliva, A.; Marino, M. Novel ligands for the affinity-chromatographic purification of antibodies. *J. Biochem. Biophys. Methods* **2001**, *49*, 481–490.
- (4) McCormick, D. Bioseparations look ahead to the past. *Pharm. Technol.* **2005**, *29*, 36–44.
- (5) Godfrey, M. A.; Kwasowski, P.; Clift, R.; Marks, V. Assessment of the suitability of commercially available SpA affinity solid phases for the purification of murine monoclonal antibodies at process scale. *J. Immunol. Methods* **1993**, *160*, 97–105.
- (6) Teng, S.; Sproule, K.; Hussain, A.; Lowe, C. R. A strategy for the generation of biomimetic ligands for affinity chromatography. Combinatorial synthesis and biological evaluation of an IgG binding ligand. *J. Mol. Recognit.* **1999**, *12*, 67–75.
- (7) (a) Delano, W. L.; Ultsch, M. H.; de Vos, A. M.; Wells, J. A. Convergent solutions to binding at a protein–protein interface. *Science* **2000**, *287*, 1279–1283. (b) Ehrlich, G. K.; Bailon, P. Identification of model peptides as affinity ligands for the purification of humanized monoclonal antibodies by means of phage display. *J. Biochem. Biophys. Methods* **2001**, *49*, 443–454. (c) Fassina, G.; Verdoliva, A.; Odera, M. R.; Ruvo, M.; Cassini, G. Protein A mimetic peptide ligand for affinity purification of antibodies. *J. Mol. Recognit.* **1996**, *9*, 564–569.
- (8) Johnson, C. P.; Jensen, I. E.; Prakasam, A.; Vijayendran, R.; Leckband, D. Engineered Protein A for the orientational control of immobilized proteins. *Bioconjugate Chem.* **2003**, *14*, 974–978.
- (9) (a) Sasso, E. H.; Merrill, C.; Furst, D. E. Immunoglobulin binding properties of the prosorba immunoadsorption column in treatment of rheumatoid arthritis. *Ther. Aphereseis* **2001**, *5*, 84–91. (b) Brunner, J.; Kern, P. M.; Gaipl, U. S.; Voll, R. E.; Kalden, J. R.; Wiesenhuber, C. W.; Hermann, M. The low-throughput protein A adsorber: an immune modulatory device. Hypothesis for the mechanism of action in the treatment of rheumatoid arthritis. *Mod. Rheumatol.* **2005**, *15*, 9–18.
- (10) Silverman, G.; Goodyear, C. S.; Siegel, D. L. On the mechanism of staphylococcal protein A immunomodulation. *Transfusion* **2005**, *45*, 274–280.

- (11) Zacharie, B.; Fortin, F.; Wilb, N.; Bienvenu, J.-F.; Asselin, M.; Grouix, B.; Penney, C. 2,6,9-Trisubstituted purine derivatives as protein A mimetics for the treatment of autoimmune diseases. *Bioorg. Med. Chem.* **2009**, *19*, 242–246.
- (12) Blotny, G. Recent applications of 2,4,6-trichloro-1,3,5-triazine and its derivatives in organic synthesis. *Tetrahedron* **2006**, *62*, 9507–9522 and references cited therein.
- (13) Baliani, A.; Bueno, G. J.; Stewart, M. L.; Yardlev, V.; Brun, R.; Barrett, M. P.; Gilbert, I. H. Design and synthesis of a series of melamine-based nitroheterocycles with activity against Trypanosomatid parasites. *J. Med. Chem.* **2005**, *48*, 5570–5579.
- (14) (a) Menicagli, R.; Samaritani, S.; Signore, G.; Vaglini, F.; Via, L. D. In vitro cytotoxic activities of 2-alkyl-4,6-diheteroalkyl-1,3,5-triazines: new molecules in anticancer research. *J. Med. Chem.* **2004**, *47*, 4649–4652. (b) Kawashima, S.; Matsuno, T.; Yaguchi, S.; Sasahara, H.; Watanabe, T. Heterocyclic Compound and Antitumor Agent Containing the Same as Active Ingredient U.S. Patent 7,071,189, **2006**. (c) Matsuno, T.; Kato, M.; Sasahara, H.; Watanabe, T.; Inaba, M.; Takahashi, M.; Yaguchi, S.-I.; Yoshioka, K.; Sakato, M.; Kawashima, S. Synthesis and antitumor activity of benzimidazolyl-1,3,5-triazine and benzimidazolylpyrimidine derivatives. *Chem. Pharm. Bull.* **2000**, *48*, 1778–1781. (d) Moon, H.-S.; Jacobson, E. M.; Khersonsky, S. M.; Luzung, M. R.; Walsh, D. P.; Xiong, W.; Lee, J. W.; Parikh, P. B.; Lam, J. C.; Kang, T.-W.; Rosania, G. R.; Schier, A. F.; Chang, Y.-T. A novel microtubule destabilizing entity from orthogonal synthesis of triazine library and zebrafish embryo screening. *J. Am. Chem. Soc.* **2002**, *124*, 11608–11609. (e) Arya, K.; Dandia, A. Synthesis and cytotoxic activity of trisubstituted-1,3,5-triazines. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3298–3304.
- (15) Melato, S.; Prosperi, D.; Coghi, P.; Basilico, B.; Monti, D. A combinatorial approach to 2,4,6-trisubstituted triazines with potent antimalarial activity: combining conventional synthesis and microwave-assistance. *ChemMedChem* **2008**, *3*, 873–876 and references cited therein.
- (16) Xiong, Y.-Z.; Chen, F.-E.; Balzarini, J.; De clerq, E.; Pannecouque, C. Non-nucleoside HIV-1 reverse transcriptase inhibitors. Part 11: structural modulations of diaryl triazines with potent anti-HIV activity. *Eur. J. Med. Chem.* **2008**, *43*, 1230–1236 and references cited therein.
- (17) (a) Zhou, C.; Min, J.; Liu, Z.; Young, Z.; Deshazer, H.; Gao, T.; Chang, Y.-T.; Kallenbach, R. Synthesis and biological evaluation of novel 1,3,5-triazine derivatives as antimicrobial agents. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1308–1311. (b) Srinivas, K.; Srinivas, U.; Bhanuprakash, K.; Harakishore, K.; Murthy, U. S. N.; Rao, V. J. Synthesis and antibacterial activity of various substituted triazines. *Eur. J. Med. Chem.* **2006**, *41*, 1240–1246.
- (18) Penney, C. Zacharie, B.; Abbott, S. D.; Bienvenu, J.-F.; Cameron, A. D.; Duceppe, J.-S.; Ezzitouni, A.; Fortin, D.; Houde, K.; Moreau, N.; Wilb, N.; Grouix, B.; Gagnon, L. Triazines Dimmers for the Treatment of Autoimmune Diseases. WO 2005/049607, **2005**.
- (19) Unpublished results.
- (20) (a) The active site was defined by the collection residues of IgG Fc within 5 Å of bound protein A (PDB code 1FC2). MOE was used to generate several potential docking modes, and a low energy one was chosen. Molecular dynamics (500 K; 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. (b) The binding site was specified to be the same as for protein A: Deisenhofer, J. Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment-b of protein A from *Staphylococcus aureus* at 2.9 Å and 2.8 Å resolution. *Biochemistry* **1981**, *20* (9), 2361–2370.
- (21) Werwitzke, S.; Trick, D.; Sondermann, P.; Kamino, K.; Schlegelberger, B.; Kniesch, K.; Tiede, A.; Jacob, U.; Schmidt, R. E.; Witte, T. Treatment of lupus-probe NZB/NZW F₁ mice with recombinant soluble Fcγ receptor II (CD32). *Ann. Rheum. Dis.* **2008**, *67*, 154–161.
- (22) Passwell, J.; Schreiner, G. F.; Nonaka, M.; Beuscher, H. U.; Colten, H. R. Local extrahepatic expression of complement genes C3, factor B, C2, and C4 is increased in murine lupus nephritis. *J. Clin. Invest.* **1988**, *82*, 1676–1684.
- (23) (a) Shields, R. L.; Namenuk, A. K.; Hong, K.; Meng, Y. G.; Rae, J.; Briggs, J.; Xie, D.; Lai, J.; Stadlen, A.; Li, B.; Fox, J. A.; Presta, L. G. High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR. *J. Biol. Chem.* **2001**, *276*, 6591–6604. (b) Wines, B. D.; Gavin, A.; Powell, M. S.; Steinitz, M.; Buchanan, R. R. C.; Hogarth, P. M. Soluble FcγRIIIa inhibits rheumatoid factor binding to immune complexes. *Immunology* **2003**, *109*, 246–254.
- (24) Marino, M.; Ruvo, M.; De Falco, S.; Fassina, G. Prevention of systemic lupus erythematosus in MRL/lpr mice by administration of an immunoglobulin-binding peptide. *Nat. Biotechnol.* **2000**, *18*, 735–739.