1138 *J. Med. Chem.* **2010,** *53,* 1138–1145
DOI: 10.1021/jm901403r

Journal of Medicinal Chemistry Article

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

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Received September 21, 2009

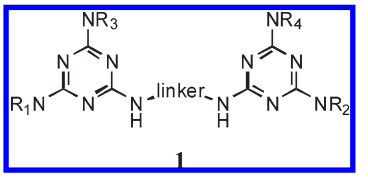
A first-in-class series of low molecular weight trisubstituted triazines were synthesized and evaluated for their ability to mimic protein A binding to human IgG antibody. The structure−activity relationship (SAR) demonstrates that the 1,3-phenylenediamine component was essential for robust activity. 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 polypeptides[7] 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 A[11] 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] antimalarial,[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 Phe[132]-Tyr[133] 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 Phe[132]-Tyr[133], 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 NR_1, NR_2, NR_3, and NR_4 represent primary or secondary amines.



1

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

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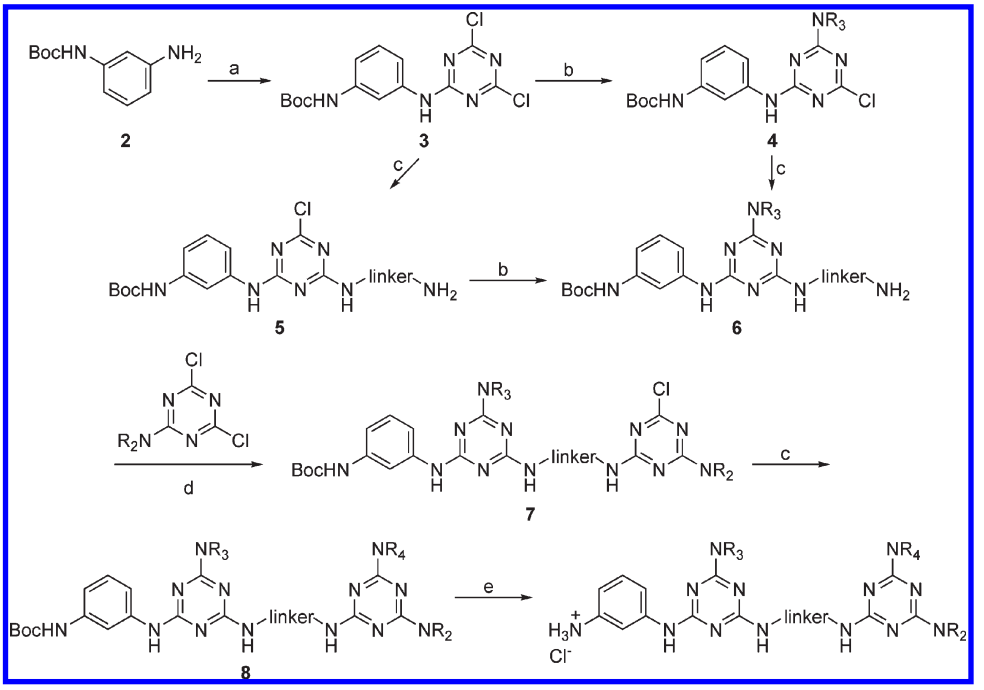
[a] Abbreviations: dsDNA, double-standard deoxyribunocleic acid; DTH, delayed-type hypersensitivity; ELISA, enzyme-linked immunosorbent assay; F1, first generation; Fc, fragment crystallizable; FcγR, fragment crystallizable type γ receptor; Fab, fragment antigen-binding; FDA, Food and Drug Administration; 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.

 Published on Web 01/04/2010

Scheme 1[a]



[a] For intermediates **4a**−**8a**, NR_2 = 3-(Boc-amino)aniline, NR_3 = NR_4 = 2-aminoethanol, and H_2N-linker-NH_2 = 4-aminophenethylamine. Reagents and conditions: (a) cyanuric chloride, acetone/water, 0 °C; (b) hydroxyalkyl- or aminoarylamines, $NaHCO_3$, THF/acetone/water; (c) aminoalkyl- or aminoarylamines, THF, 60 °C; (d) $NaHCO_3$, THF/acetone/water; (e) CH_2Cl_2, 4 M HCl/dioxane.

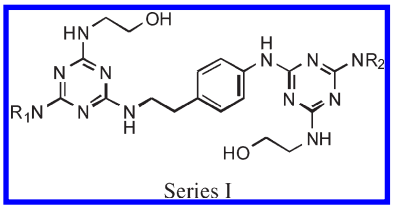
of chlorine atoms in cyanuric chloride by various nucleophiles enhances the utility of this reagent for the preparation of mono-, di-, and trisubstituted 1,3,5-triazines under controlled temperature.[12] The general synthetic procedure for triazine compounds is outlined in Scheme 1. It illustrates the 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 aminoaralkylamines 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

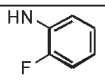
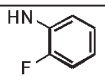
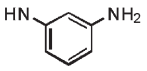
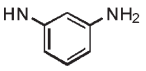
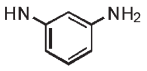
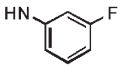
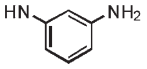
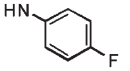
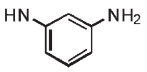
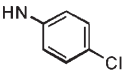
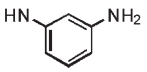
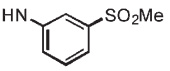
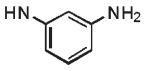
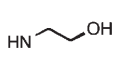
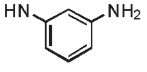
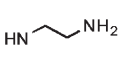
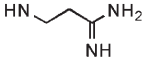
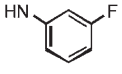
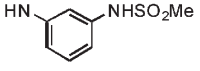
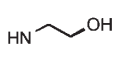
A number of triazine derivatives of structural type **1** were synthesized and evaluated as protein A mimetics by competitive ELISA. Twenty-two compounds (**10**−**12**, **14**, **19**−**23**, **26**, **29**, **31**−**36**, **40**−**44**) display significant activity comparable to protein A (Tables 1−3). As shown in Table 1 (series **I** compounds), analogues of **1** were synthesized in which NR_1 and NR_2 were different aliphatic or aromatic amines while maintaining the linker as 4-aminophenethylamine and NR_3 and NR_4 as ethanolamine. The most active triazine compounds are **10** and **15**. Clearly, the 3-amino function of the 3-aminoaniline group (NR_1) 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 (NR_2) 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 then compounds **10** and **15**. Replacement of aminoaniline (**10**) or ethanolamine (**15**) at NR_2 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 NR_1 and NR_2 are 3-aminoaniline and NR_3 and NR_4 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. IC_{50} (nM) of Series **I** Compounds Relative to Protein A As Ascertained by a Protein A Human IgG Competitive ELISA



Series I

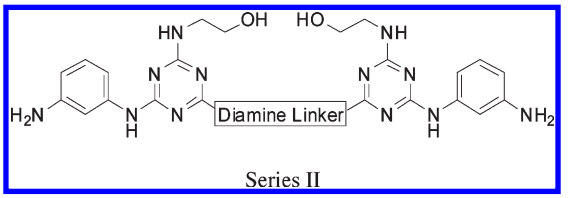
Compd No.	NR_1	NR_2	IC_{50} (nM)[a] a	b
9			Inactive	Inactive
10			84	144
11			382	209
12			361	204
13			841	893
14			243	192
15			94	81
16			410	749
17			Inactive	Inactive
18			Inactive	Inactive
Protein A			189	334

[a] Compounds were assayed in both PBS and 20% DMSO/PBS.

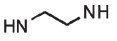
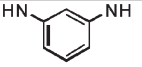
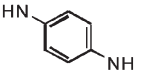
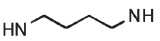
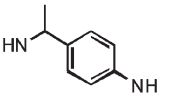
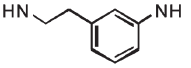
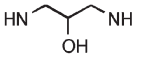
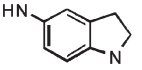
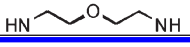
carbon (**21**) results in reduction of activity. In contrast, addition of an oxygen atom to the four-carbon spacer (**24**) led to reasonable activity. Selection of the linker is critical, since activity is dependent upon the distance between the two triazine rings. Replacement of the alkyl spacer by 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 work[18] 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 NR_1 and NR_2 were 3-aminoaniline, NR_3 was ethanolamine, and NR_4 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. IC_{50} (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 NR_4 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.

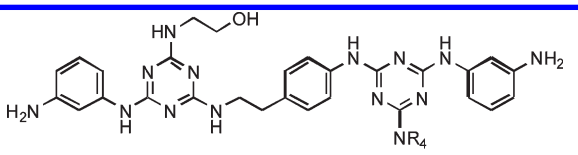
It was next desired to demonstrate that the binding activity of **34** would translate into a significant in vivo effect in a relevant rodent model for autoimmune disease. For this purpose, use was made of two strains of mice that replicate the symptoms of systemic lupus erythematosus (SLE).[21] Figure 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/IPR 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.

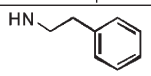
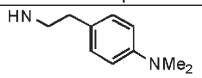
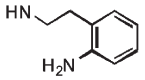
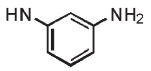
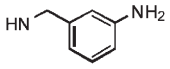
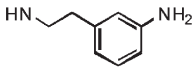
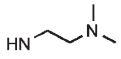
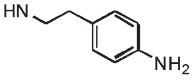
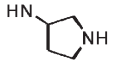
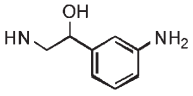
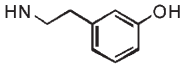
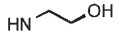
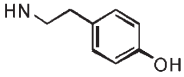
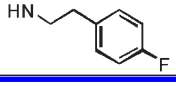
Conclusion

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. IC_{50} (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**	NMe_2	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 ^{1}H NMR, 377 MHz for ^{19}F NMR, and 100 MHz for ^{13}C 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 (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.

Table 4. IC_{50} (M) of Compound **34** Relative to Protein A As Ascertained by a Heat Aggregated Human IgG (HAG) Binding Assay

	binding assay, IC_{50} (M)			
compd	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}	$>1 \times 10^{-6}$
34	8.3×10^{-8}	2.9×10^{-6}	1.2×10^{-5}	5.7×10^{-5}

2-([3-*tert*-Butoxycarbonylaminophenyl]amino)-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

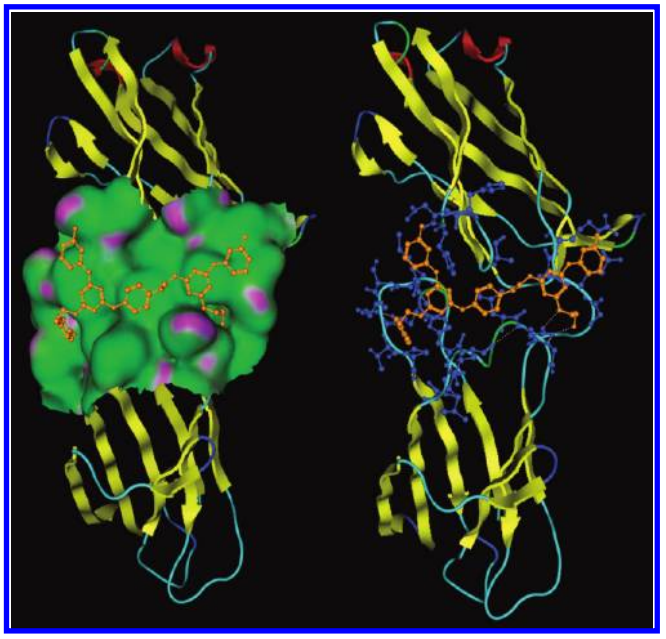


Figure 1. Postulated binding site of compound **34** (orange) onto the Fc portion of IgG.

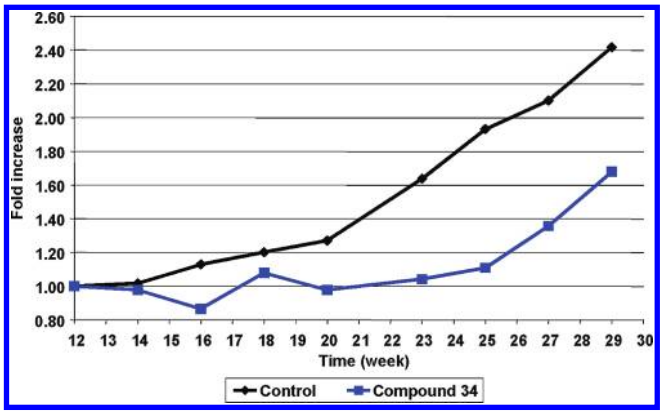


Figure 2. Inhibition of serum dsDNA autoantibodies by compound **34** in NZB/NZW mice.

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. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.69−7.73 (m, 1H), 7.14−7.30 (m, 3H), 1.52 (s, 1H). ^{13}C NMR (CD_3OD, 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 ($M(^{35}Cl_2)H^+$). HPLC: t_R = 4.4 min (gradient B, purity 100% at 254 nm).

2-[4-([3-*tert*-Butoxycarbonylaminophenyl]amino)-6-chloro[1,3,5]-triazine-2-ylamino]ethanol (4a). A solution of **3** (210 mg, 0.59 mmol) in tetrahydrofuran/acetone/H_2O (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. ^{1}H NMR (CD_3OD, 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), 3.67 (t, J = 5.6 Hz, 1H), 3.61 (broad, 1H), 3.46−3.52 (m, 1H), 1.51 (s, 9H). ^{13}C NMR (CD_3OD, 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 ($M(^{35}Cl)H^+$). HPLC: t_R = 3.4 min (gradient B, purity 100% at 254 nm).

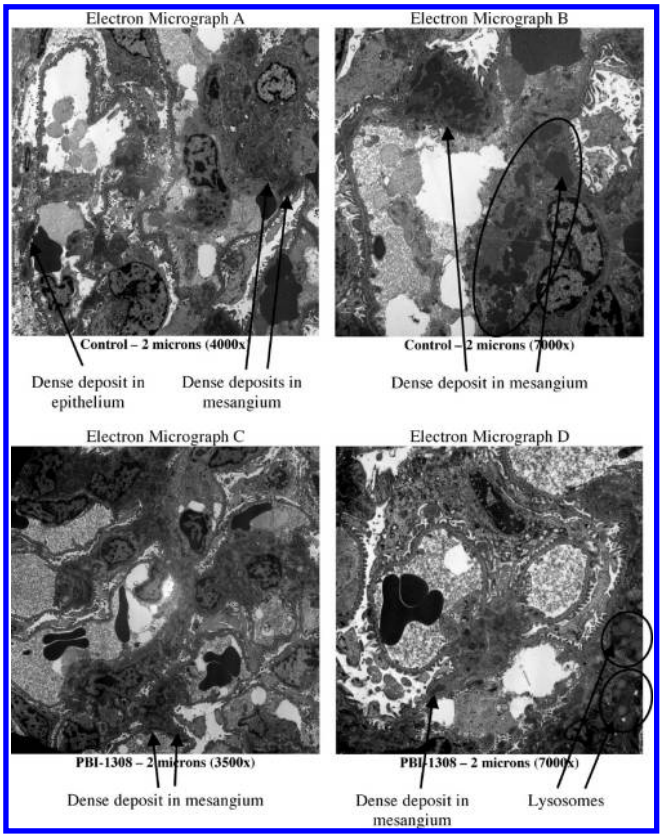


Figure 3. Electron micrographs of kidney tissue from SLE experiment in MRL/LPR mice. Micrographs A and B are from control group, and micrographs C and D are from the **34**-treated group.

2-[4-[2-[4-Aminophenyl]ethylamino]-6-([3-*tert*-butoxycarbonylaminophenyl]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. ^{1}H NMR (CD_3OD, 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, 2 H), 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*-Butoxycarbonylaminophenyl]amino)-6-[2-[4-[4-([3-*tert*-butoxycarbonylaminophenyl]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. ^{1}H NMR (CD_3OD, 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). ^{13}C NMR (CD_3OD, 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 ($M(^{35}Cl)H^+$). HPLC: t_R = 3.2 min (gradient B, purity 100% at 254 nm).

2-(4-([3-*tert*-Butoxycarbonylaminophenyl]amino)-6-(4-(2-(4-([3-*tert*-butoxycarbonylaminophenyl]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. ^{1}H NMR (CD_3OD, 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 (IC_{50}) 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.

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