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2,6,9-Trisubstituted purine derivatives as protein A mimetics for the treatment of autoimmune diseases

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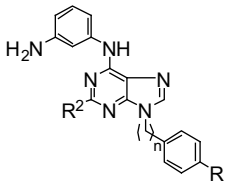
ABSTRACT

A series of 9-substituted and 2,9-disubstituted 6-(3-aminophenylamino) purines were synthesized and evaluated for their ability to mimic protein A binding to human IgG antibody. The structure–activity relationship (SAR) demonstrates that the 6-(3-aminoanilinyl) purine component was essential for activity. Purine **14** demonstrated significant activity, compared to protein A. These compounds may prove useful for the treatment of autoimmune diseases.

Staphylococcal protein A (SPA) is a cell-surface component of the bacterial pathogen *Staphylococcus aureus*. This protein, with a molecular weight of 42,000, contains five domains which selectively bind the tail (Fc) portion of human and mouse antibodies.[1] The interaction between SPA and immunoglobulin G (IgG) is one of the most studied protein–protein interactions.[2] The binding properties of protein A makes it commercially important for the purification of monoclonal antibodies. In general, antibodies are purified by classical (e.g., ion-exchange) column or affinity chromatography with protein A attached to a solid-phase support.[3] However, purification with SPA affinity absorbents is costly and these adsorbents have been shown to leak protein A and contaminate purified immunoglobulin products.[4] As a result, there is significant interest in developing a synthetic mimetic of SPA, or a protein A mimetic, which is more economical and stable. However, few molecules have been reported in the literature which mimic bacterial protein A and can be used to bind IgG. They are small synthetic compounds covalently attached to a solid-phase support[3a,5] or larger peptides[6] or polypeptides.[7] However, this approach is limited to molecules attached to solid-phase matrices.

Protein A also has therapeutic utility but its toxicity and cost limit its therapeutic use. Nonetheless, a protein A column (Prosorba®) from Cypress was approved by the US FDA for the treatment of autoimmune diseases; immune thrombocytopenic purpura in 1987 and severe rheumatoid arthritis in 1999.[8] This treatment requires the use of protein A covalently linked to an inert silica matrix whereby the patient's blood is passed through this column in a manner similar to kidney dialysis (apheresis). Unfortunately, this treatment requires multiple visits to the doctor's office and the outcome after treatment is often unpredictable.[9] Subsequently, there is a need for a novel, safe, non toxic small molecule mimetic of protein A which can be administered as a drug.

The purine scaffold has been exploited for designing biologically relevant molecules with broad biomedical value as therapeutics.[10] These compounds have attracted interest as molecular probes or lead molecules for drug development including inhibitors of kinase signaling.[10e] As part of our effort toward the discovery of therapeutics for the treatment of inflammatory conditions, we examined small molecules that are mimetics of protein A. In this letter, the synthesis and anti-inflammatory activities of di- and trisubstituted purines are reported as exemplified by the general structure **1**.



1

The general synthetic procedure for purine compounds is outlined in Schemes 1 and 2. Scheme 1 illustrates the two synthetic

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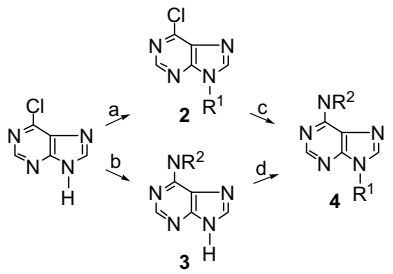
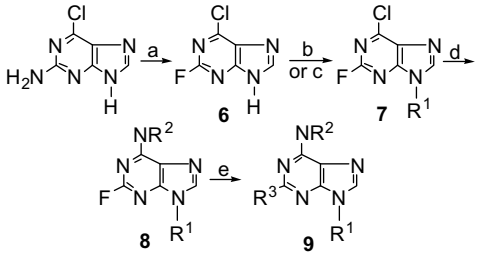


Scheme 1. Reagents and conditions: (a) aralkyl alcohol, DIAD, Ph_3P, THF or alkyl bromide, K_2CO_3, DMF, 25 °C, overnight; (b) arylamine or alkylamine, ethanol, 50 °C, 21 h; (c) arylamine, DIEA, *n*-butanol, 90 °C, overnight; (d) alkyl bromide, K_2CO_3, DMF, 100 °C, 20 min, microwave.



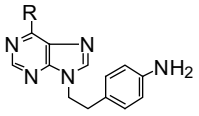
Scheme 2. Reagents and conditions: (a) $NaNO_2$, HBF_4; (b) aralkyl alcohol, DIAD, Ph_3P, THF, 25 °C, overnight; (c) alkyl iodide, Cs_2CO_3, dioxane, 25 °C, overnight; (d) arylamine, DIEA, *n*-butanol, 65 °C, overnight; (e) ammonia or alkylamine, DIEA, *n*-butanol, 110 °C, overnight.

routes employed for the disubstituted purine derivative **4**. The first approach was the Mitsunobu reaction[11] of 6-chloropurine with the aralkyl alcohol to give 9-substituted aralkyl derivative **2**. This reaction proceeded in high yield (>80%) and is general for different aralkyl alcohols. In addition, the desired *N*-9 adduct was the major product with only traces of *N*-7 adduct. Compound **2** was also prepared by reacting 6-chloropurine with alkyl bromide in the presence of potassium carbonate.[12] The chloro intermediate **2** was then treated with different alkyl or arylamines to give the final product **4**. Other routes were also examined for the preparation of disubstituted purine **4**. For example, 6-chloropurine was treated with arylamine in ethanol at 50 °C to give 6-substituted purine **3** in respectable yield. The latter was treated with alkyl bromide in presence of a base at 100 °C for 20 min by microwave to afford compound **4**. Both procedures were practical and used to generate 6,9-disubstituted analogues of **4**. Scheme 2 demonstrates the synthetic method for the trisubstituted purine derivative **9** where the key intermediates is the 2-fluoro derivative **7**. This compound was prepared by converting 2-amino-6-chloropurine to the corresponding fluoro derivative **6**[13] followed by a Mitsunobu reaction with the aralkyl alcohol to give compound **7** in high yield (>85%). Again**,** the *N*-9 adduct was the major product. The chloro derivative **7** was then reacted with aryl amines in hot butanol in the presence of base to give the expected fluoro derivative **8** in good yield. Displacement of fluorine with different alkyl or aralkyl amines gave the trisubstituted product **9**. The reaction is practical and amenable to scale-up starting from commercially available 2-amino-6-chloropurine. Also, it offers the advantage of a general procedure for the preparation of a variety of analogues of 2,6,9-trisubstituted purines.

A number of purine derivatives of **4** and **9** were synthesized[14] and evaluated as SPA mimetics in a competitive protein A binding ELISA assay. Six compounds (**17**, **22**, **23**, **32**, **34** and **49**) display significant activity comparable to protein A (Tables 1–3). As shown in Table 1, analogues of disubstituted purine **4** were synthesized in which different aliphatic (**10**–**13**) or aromatic (**14**–**16**) amines were varied at position 6 while maintaining the 9-(4-aminophenethylamino)purine component. Only 3-aminoaniline **14** showed activity. Clearly, the 3-amino function of the aniline appears to be essential and activity declines upon substitution with other groups (3-OMe **15** or 3-fluoro **16**). On the basis of this result, **14** was selected as a core structure upon which to define a structure–activity relationship for a trisubstituted purine scaffold. Table 2 illustrates the variation at the 9 position (R^1) on the purine ring. Prior work[15] indicated the importance of 4-aminophenethyl amine at this position. This fragment enhances good in vitro antibody binding activity and compound **14** supports this finding. Our results showed that 4-carboxymethylbenzyl (**23**) or 4-aminobenzyl (**17**) substituents display the same in vitro activity as protein A. Also, the corresponding analogues with halogens demonstrate reasonable activity. For example, the 3-chloro (**28**) and the 4-chloro (**27**) benzyl analogues display as good activity as **23** and **17** while activity is reduced with 4-fluoro (**20**) and 3-fluoro (**21**) benzyl derivatives. Homologation of the chain with a one-carbon spacer between the 9-substituted purine ring and the phenyl (**29**) led to a drop in activity. Substitution of the phenyl ring of the 9-phenethyl group (R^1) with polar functions gave different activities. For example, 4-amino (**14**), 3-fluoro (**25**) and 4-fluorophenethyl (**24**) analogues demonstrate good activity. However, replacement of the 4-fluoro with 4-chloro (**26**) or 3-amino groups (**19**) results in a loss of activity. The corresponding analogue with a 4-methyl substituent (**30**) retains the activity of compound **14**. Interestingly, methyl substitution of the two-carbon spacer of the 9-phenethyl group (**22**) showed a significant increase in activity; equipotent with SPA. Also, analogues were synthesized in which a heteroatom was added to the two-carbon spacer of **29**. As a result, the oxygen derivatives (**32**–**34**) demonstrated good activity. Introduction of rigidity, for example, **31**, diminishes the activity relative to the 9-phenethyl derivative (**29**). Surprisingly, 9-substituted alkyl (**36**) possesses comparable activity with SPA.

It was next decided to synthesize trisubstituted purines. Therefore, analogues of the lead compound (**14**) were made in which different groups were introduced at position 2 of the purine ring. Table 3 summarizes the results. In general, it can be seen that a rigid amine at R^2 is preferred since activity is dependent upon the distance and relative degrees of freedom

Table 1
IC_{50} (μM) of 6-substituted-9-(4-aminophenylethyl)-purine relative to protein A as ascertained by a protein A human IgG competitive ELISA



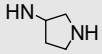
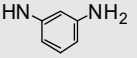
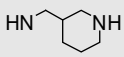
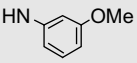
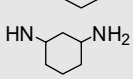
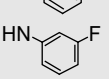
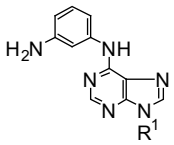
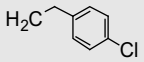
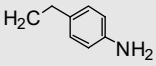
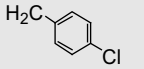
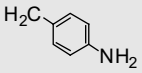
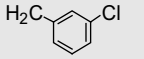
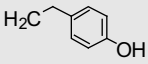
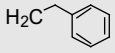
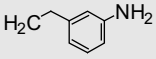
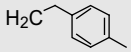
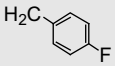
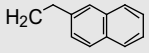
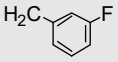
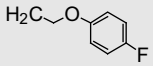
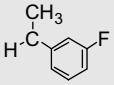
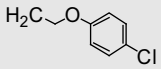
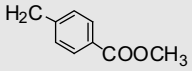
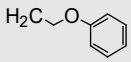
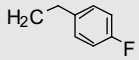
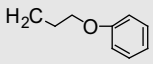
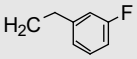
Compound	R	IC_{50} (μM) in PBS	Compound	R	IC_{50} (μM) in PBS
Protein A		0.2	**13**		Inactive
10		Inactive	**14**		1.0
11		Inactive	**15**		Inactive
12		Inactive	**16**		Inactive

Table 2
IC_{50} (μM) of 9-substituted-6-(3-aminophenylamino)-purine relative to protein A as ascertained by a protein A human IgG competitive ELISA



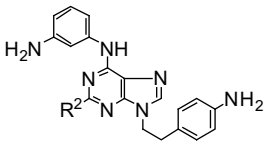
Compound	R^1	IC_{50} (μM) in PBS	Compound	R^1	IC_{50} (μM) in PBS
Protein A		0.2	**26**		3.6
14		1	**27**		0.5
17		0.2	**28**		0.4
18		Inactive	**29**		2.3
19		5.1	**30**		1.5
20		1.7	**31**		12.5
21		1.8	**32**		0.3
22		0.2	**33**		0.5
23		0.3	**34**		0.2
24		0.6	**35**		0.6
25		0.5	**36**		0.4

at this position. Cyclopentylamine (**49**) is the most effective substituent among rigid amines evaluated such as isopropyl (**45**) and cyclobutyl (**48**). Activity was not improved, relative to **14**, in the case of benzyl amine derivatives (**51**) and (**52**). While hydrogen substitution, compound **14**, results in a reasonable activity, the 2-amino (**37**) and the 2-fluoro (**38**) analogues are less active.

On the basis of the above results, it was of interest to determine if this activity would translate into an in vivo effect. Therefore, the lead compound **14** was tested in two inflammatory disease models. Table 4 shows the effect of intravenous administration of **14** on oxazolone-induced delayed-type hypersensitivity (DTH) in mice.[16] This compound induces a significant reduction of inflammation as seen by reduced ear thickness. It displays similar potency to methotrexate and reduces redness, crust formation and ear swelling. Also, the effect of oral administration of **14** was studied on DTH. Figure 1 represents the activity of this compound in comparison with hydrocortisone as the positive control.[17] Again, **14** induces a significant reduction in inflammation ($p = 0.002$, day 14) as seen by reduced ear thickness in both challenges 1 and 2. Encouraged by these results, it was of interest to determine the effect of **14** on Freund's adjuvant-induced arthritis.[18] As illustrated in Figure 2 (100% of the animals rapidly developed a synovitis. A significant reduction (20%, day 21) in the severity of arthritis (inflammatory index) was observed by intravenous injection of methotrexate (positive control) by day 13 and over. A significant reduction (up to 25%) of the inflammatory index was also observed with intravenous injection of **14** from day 1 to day 21 at a dose of 25 mg/kg. Compound **14** also displayed reduction (up to 30%) in inflammation when the experiment was repeated with compound administered orally at a dose of 50 mg/kg (data not shown). Animals treated with **14** showed less inflammation than the control group and the activity of this compound is similar to methotrexate.

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 a 1,3-phenylenediamine substituent. The hydrophobicity of these purines is important for binding to the IgG tail portion. These compounds, represented by lead compound **14**, show good in vivo activity in standard models of inflammation when administered by oral and intravenous routes. They offer a unique approach for the treatment of autoimmune diseases by virtue of their novel biochemical target.

Table 3
IC_{50} (μM) of 2-substituted-6-(3-aminophenylamino)-9-(4-aminophenylethyl)-purine relative to protein A as ascertained by a protein A human IgG competitive ELISA



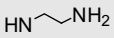
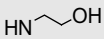
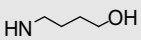
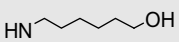
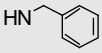
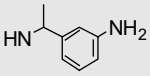
Compound	R^2	IC_{50} (μM) in PBS	Compound	R^2	IC_{50} (μM) in PBS
Protein A		0.2	**44**		6
14	H	1	**45**		1.1
37	NH_2	13	**46**		13
38	F	Inactive	**47**		0.6
39		Inactive	**48**		2.3
40		64	**49**		0.2
41		Inactive	**50**		9
42		4.8	**51**		1.8
43		22	**52**		14

Table 4
Effect of intravenous administration of compound **14** on DTH in mice

	Inflammation/ear thickness (mm)			
	Challenge 1		Challenge 2	
Control	0.29 ± 0.10		0.83 ± 0.46	
Methotrexate	0.17 ± 0.04	$p = 0.001$	0.46 ± 0.17	$p = 0.02$
Compound **14**	0.19 ± 0.06	$p = 0.008$	0.56 ± 0.12	$p = 0.05$

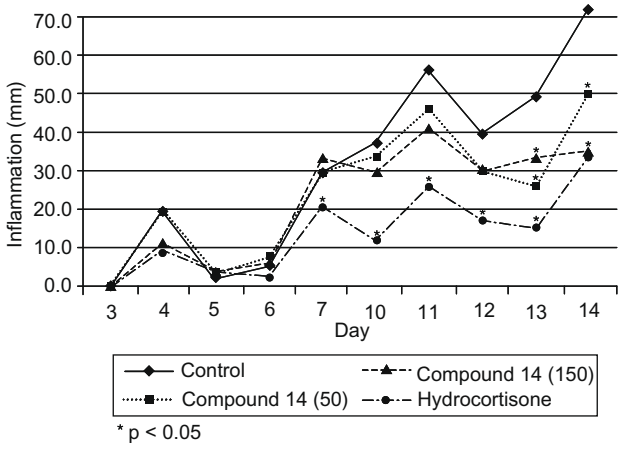


Figure 1. Effect of oral administration of compound **14** on DTH in mice.

Registry numbers: **14**, 917460-18-5; **17**, 917381-70-5; **19**, 917381-75-0; **24**, 917381-77-2; **37**, 917381-65-8; **40**, 917381-71-6; **42**, 917381-67-0; **43**, 917381-74-9; **44**, 917381-73-8; **46**, 917381-68-1; **47**, 917381-66-9; **49**, 917381-76-1.

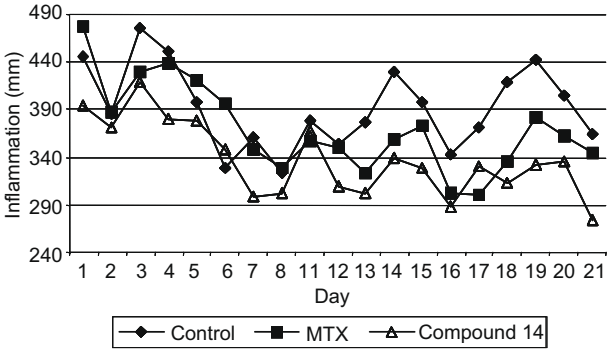


Figure 2. Effect of intravenous administration of compound **14** on adjuvant-induced arthritis in rats.

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14. Zacharie, B. et al. WO 2006/136005. The purity and identity of all the products were monitored by LC/MS at 210 or 250 nm (Agilent 1100) using an analytical C18 column (75×4.6 mm, 5 μm) with different gradients of CH_3CN–H_2O containing 0.01% TFA as the eluant. All compounds demonstrated >98% purity. ^{1}H NMR and ESI-MS characterization data were consistent with the expected structure. For example: **14** brown solid; mp: 167 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 8.50 (s, 1H), 8.30 (s, 1H), 8.20–8.15 (m, 1H), 7.80–7.70 (m, 1H), 7.58 (t, J = 8.24 Hz, 1H), 7.40–7.20 (m, 5H), 4.63 (t, J = 7.04 Hz, 2H), 3.31 (m, 2H); LRMS (ESI): m/z 346 (MH^+), 368 (MNa^+). Data for **17** brown solid; mp: 225 °C (decomposition); ^{1}H NMR (400 MHz, CD_3OD): δ 8.75 (s, 1H), 8.53 (s, 1H), 7.98 (s, 1H), 7.80–7.20 (m, 7H), 7.51 (t, J = 8.02 Hz, 1H), 5.66 (s, 2H); LRMS (ESI): m/z 332 (MH^+), 354 (MNa^+). Data for **22** brown solid ^{1}H NMR (400 MHz, D_2O): δ 8.26 (s, 1H), 8.09 (s, 1H), 7.49–7.48 (m, 1H), 7.40–7.36 (m, 2H), 7.23–7.18 (m, 1H), 7.08–7.06 (m, 1H), 6.95–6.86 (m, 3H), 5.73 (dd, J = 7.04 and 14.28 Hz, 1H), 1.81 (d, J = 7.04 Hz, 3H); LRMS (ESI): m/z 349 (MH^+). Data for **49** brown solid; mp: 250 °C (decomposition); ^{1}H NMR (400 MHz, CD_3OD): δ 8.45 (s, 1H), 8.13 (s, 1H), 7.90 (m, 1H), 7.57 (t, J = 8.22 Hz, 1H), 7.40 (m, 4H), 7.20 (m, 1H), 4.57 (t, J = 7.04 Hz, 2H), 4.35 (q, J = 6.65 Hz, 1H), 3.30 (m, 2H), 2.10 (m, 2H), 1.75 (m, 6H); LRMS (ESI): m/z 429 (MH^+), 451 (MNa^+).
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16. On day 0, mice were sensitized with 100 μl of oxazolone in 5% acetone. On day 0, 1 and 2, mice were treated by intravenous administration of the vehicle (control) or methotrexate (MTX; positive control) or the compound at 50 mg/kg. Mice were challenged with an application of 50 μl of oxazolone on the surface of the right ear (first challenge, day 3; second challenge, day 10). Ear thickness was measured on day 4 to day 7, and on day 11 to 14. Redness and crust formation was also observed. Mice were sacrificed on day 14. T_{DTH} (CD4) cells play an important role in regulating the intensity of the DTH response.
17. Oral administration of compound **14** was undertaken following the same protocol as Ref. 15 with the exception that compound **14** was orally administered at 50 or 150 mg/kg from day 0 to day 13.
18. Adjuvant-induced arthritis was induced in female Lewis rats by the injection of lyophilized *Mycobacterium butyricum* suspended in mineral oil into the footpad. The development of arthritis was monitored over a 3-week period post-adjuvant injection. Inflammation peaks at day 3 following the adjuvant administration. Immune activation appears around day 14. Compounds were injected iv day −3, −2 and −1 pre-adjuvant injection and at day 10, 11 and 12 post-adjuvant injection. Body weight was recorded. The arthritis index, which is a measure of inflammation (edema), redness, and stiffness of the articulations, was used to monitor the development of the disease. The degree of arthritis was determined by measuring two perpendicular diameters of the ankles in the mediolateral and dorsoventral planes using a caliper. Joint circumference in millimeters is then calculated using a geometric formula. Both the incidence and severity of the arthritis was evaluated. Incidence is defined as the number of rats with clinical evidence of joint inflammation during the study period.