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Discovery and structure–activity relationships of small molecules that block the human immunoglobulin G–human neonatal Fc receptor (hIgG–hFcRn) protein–protein interaction

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ABSTRACT

The neonatal Fc receptor, FcRn, prolongs the half-life of IgG in the serum and represents a potential therapeutic target for the treatment of autoimmune disease. Small molecules that block the protein–protein interactions of human IgG–human FcRn may lower pathogenic autoantibodies and provide effective treatment. A novel class of quinoxalines has been discovered as antagonists of the IgG:FcRn protein–protein interaction through optimization of a hit derived from a virtual ligand-based screen.

The neonatal Fc receptor, FcRn, is a 52 kDa heterodimeric glycoprotein widely expressed in many different tissues and cell types and is the key regulatory protein for IgG homeostasis in animals and humans.[1] FcRn extends the half-life of IgG in the serum by binding to IgG in endosomal compartments and shuttling the protein back to the cell surface for release and recirculation.[2] In this process, FcRn diverts IgG from lysosomal degradation.[3] As a result, IgG has a serum half-life of three weeks in humans while other proteins of similar size may only have a half-life on the order of days.

The fact that FcRn can regulate the half-life of IgG has led to the investigation of this receptor as a potential therapeutic target in autoimmune disease.[4] Reduction of the serum levels of pathogenic antibodies may alleviate symptoms of diseases in which IgG is the pathogenic agent. Indeed, it has been shown that FcRn-deficient mice are protected against models of arthritis and models of various skin blistering diseases.[5] A monoclonal antibody targeting FcRn reduced symptoms of experimental autoimmune myasthenia gravis in rats.[6] Recently it was reported that chemically modified 3.4 kDa peptide can bind to human FcRn and block its interaction with human IgG both in vitro and in vivo.[7,8]

The inhibition of protein–protein interactions is a major challenge for small molecular drug discovery because such interactions generally cover large protein surfaces.[9] For example, the rat Fc:FcRn protein–protein interface is approximately 1870 Å^2.[10] However, we were encouraged by the fact that a small peptide inhibitor of FcRn was capable of blocking IgG binding to FcRn using only 360 Å^2 of buried surface area.[11] This suggested that only key FcRn surface 'hot spots' need to be targeted for effective inhibition of the IgG–FcRn interaction.

Here we report our discovery and optimization of small molecules that block the protein–protein interaction between human IgG (hIgG) and the soluble extracellular domain of human FcRn (shFcRn). Using the coordinates from a crystal structure of shFcRn and an antagonist peptide,[11] a ligand-based virtual screen of 2.5 million compounds generated a ranked list of virtual hits. The top 500 compounds were tested in an IgG–FcRn competition assay (vide infra) to identify compounds that could interfere with this protein–protein interaction. Using this methodology, we identified quinoxaline hit compound **1** (Fig. 1) with an IC_{50} of more than 150 μM.

The synthesis of various quinoxaline analogs (**2**) is depicted in Scheme 1. In general, the condensation of R^1-substituted 2,3-dichloroquinoxaline **3** with a various cyanoacetamides **4** generated **5**. Displacement of the chlorine atom of **5** with various amines provided the modified quinoxaline analogs **2**. Cyanoacetamides **4** were

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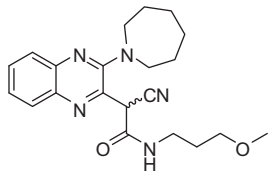


1

IC_{50} >150 μM

Figure 1. Chemical structure of quinoxaline hit compound **1**.

generally obtained by coupling of commercially available cyanoacetic acid with various amines using PyBOP and standard conditions. In cases where the substituted 2,3-dichloroquinoxaline **3** was not commercially available, the preparation of the requisite R^1-substituted quinoxalines is described in Scheme 2. Condensation of R^1-substituted benzene-1,2-diamine (**6**) with diethyl oxalate (**7**) gave the corresponding quinoxaline dione **8**. Reflux of **8** in phosphorus oxychloride generated the various 2,3-dichloroquinoxaline analogs **3**.

The ability of these analogs to block hIgG binding to shFcRn was assessed using a FcRn–IgG competition ELISA assay as described previously.[7] Briefly, various concentrations of small molecules were each mixed with 3 nM hIgG and incubated with shFcRn-coated plates. The concentration required to inhibit 50% of the IgG–FcRn interaction was determined and reported as its IC_{50}.

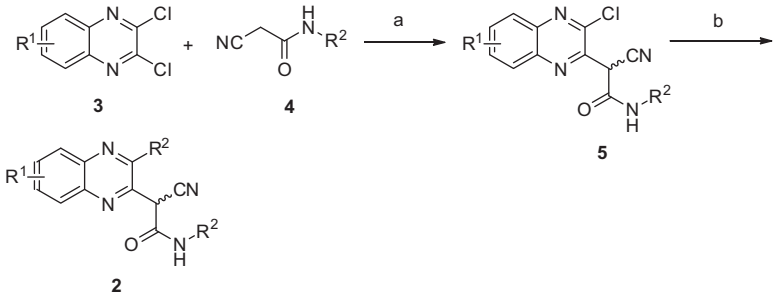
Starting from the initial hit **1**, we first explored the amide position and the effect of various alkyl groups on activity (**9**–**13**, Table 1). The alkyl ether chain of compound **9** with one less methylene had similar activity to **1** while compounds with other alkyl groups (**10**–**12**) showed no activity which suggested a possible role for the methoxy functional group of compound **1**. Next we sought to replace the alkyl ether group with aryl groups as illustrated in Table 1. The unsubstituted phenyl and heteroaryl analogs (**13**–**17**) showed no activity. However, introduction of ether a *para* or a *meta*-substituent on the phenyl ring had significant effects on the activity. For example, the 3-F-substituted phenyl substituent (**18**) possessed an IC_{50} of 150 μM and recapitulated the activity of the original hit compound **1**. The 4-meoxyphenyl compound **22** increased the activity to 50 μM, which is threefold higher than compound **1**. Notably, the 3-methoxy phenyl compound **21** was 15-fold more active than hit compound **1**. In contrast, methoxy substitutions on the benzyl ring exemplified by compounds **28** and **29** resulted in a reduction in activity as compared to the phenyl substituted compounds **21** and **22**. The activity of disubstituted methoxy phenyl compound **27** is much lower than the corresponding mono-substituted compounds. Interestingly, the conversion of a methoxy group in compound **22** to a hydroxyl group (**23**) abolished its activity demonstrating selectivity for the methoxy substituent.

A preliminary SAR study of compound **21** using compounds in Figure 2 indicated the important role of the amide NH and the quinoxaline ring. Methylation of the amide NH resulted in no measurable activity up to 500 μM as shown by compounds **32**. Similarly, inclusion of a smaller pyrazine ring instead of the quinoxaline ring in compound **33** also showed no activity up to 500 μM.

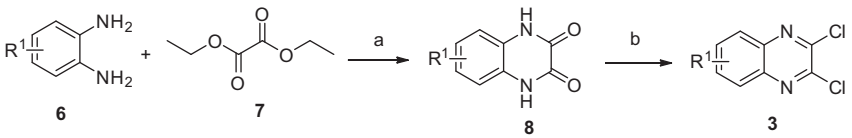
With the optimized methoxy phenyl compound **21**, we next turned our attention to examining the effect of substitution at the 6 or 7 positions on the quinoxaline ring, as shown by compounds **35–38** (Table 2). Both electron withdrawing and donating groups were attached to the quinoxaline ring in compound **21** but both changes resulted in reductions in activity. Further extension from the 6 position of the quinoxaline ring by an amide linkage, as shown in compounds **39**–**42**, also resulted in a loss of activity. 6,7-Dichloro-quinoxaline analog **38** showed only a small threefold reduction in activity. This may suggest that only small substitutions at the 6 and 7 positions on the quinoxaline ring are somewhat tolerated.

We also investigated ring size and modifications at the azepane moiety (Table 3). For example, the 7-membered ring analogs **43–48** were aimed at modulating the activity and solubility but were inactive. However, compound **50** with an 8-membered alkyl ring showed similar activity as compound **21**. Interestingly, 6-membered piperidine analogs **51–53** were also inactive. Connection to the quinoxaline of various phenyl or alkyl groups by either a secondary amine or thioether (compounds **54–58)** also abolished the activity. Our SAR suggested that the unsubstituted 7 and 8 membered-alkyl rings azepane and azocane provide optimal activity.

Additional optimization efforts were directed towards extension of the methoxy phenyl ring which proved active in compounds **21** and **22** (Table 4). Substitutions extended from

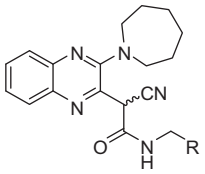


Scheme 1. Reagents and conditions: (a) *t*-BuOK, THF, reflux, 3 h (60–71%); (b) amines, Et_3N, *i*PrOH, 80 °C, 4 h (80–90%).

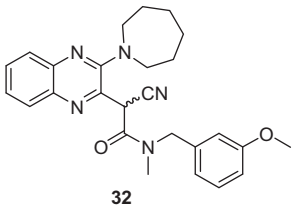


Scheme 2. Reagents and conditions: (a) EtOH, reflux, 12 h (80–90%); (b) $POCl_3$, reflux, 12 h (90–95%).

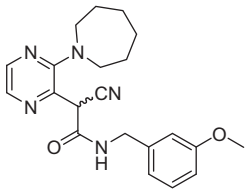
Table 1
SAR of the amide substituent of compound **1**



Entry	R	IC_{50} (μM)
1	Methoxyethyl	>150
9	Methoxymethyl	120
10	Allyl	>250
11	*n*-Propyl	>500
12	Cyclohexanyl	>500
13	Ph	>500
14	2-Pyridine	>1000
15	3-Pyridine	>1000
16	2-Furan	>1000
17	2-Thiophen	>1000
18	3-F-Ph	150
19	3-Cl-Ph	100
20	3-CF_3O-Ph	80
21	3-MeO-Ph	10
22	4-MeO-Ph	50
23	4-OH-Ph	>500
24	4-CN-Ph	40
25	4-$N(Me)_2$-Ph	>500
26	2-MeO-Ph	150
27	3,4-$(MeO)_2$-Ph	250
28	3-MeO-Bn	100
29	4-MeO-Bn	120
30	4-Amino-Bn	>500
31	4-Acetamide-Bn	>500



32
IC_{50} > 500 μM

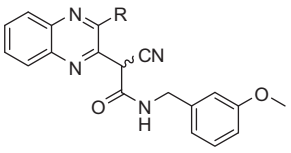


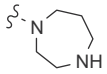
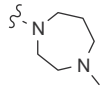
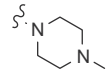
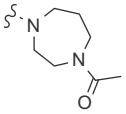
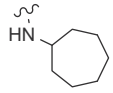
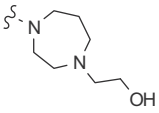
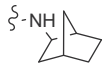
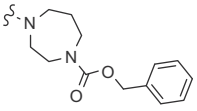
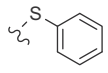
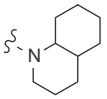
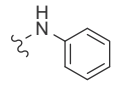
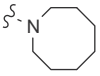
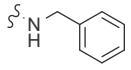
33
IC_{50} > 500 μM

Figure 2. Chemical structures of compounds **32** and **33**.

meta-position (**59–62**) of the phenyl ring resulted in a reduction in activity. Addition of a morpholine group at the *para*-methoxy phenyl (**63**) also reduced the activity. From our limited data set, it appears that only a small methoxy group is tolerated at the *meta* and *para*-position of the phenyl ring in compounds **21** and **22**. Introduction of a methyl group to the benzyl group in **22** produced

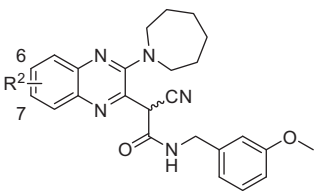
Table 3
SAR of ring size

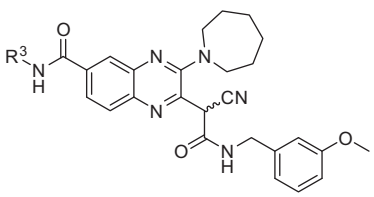


R		IC_{50} (μM)	R		IC_{50} (μM)
43		>500	**51**		>500
44		>500	**52**		>500
45		>500	**53**		>500
46		>500	**54**		>500
47		>500	**55**		>500
48		>500	**56**		>500
49		>500	**57**		>500
50		6	**58**		>500

two isomers, **64** and **65**, with the same activity indicating that the stereochemistry at the benzyl position had little effect on activity. Addition of a methyl group in this position also resulted in a minor improvement in activity as compared to compound **22**. Encouraged by these findings, we re-positioned the solubilizing morpholine group to the benzyl carbon to generate compound **66** which improved the IC_{50} to 2 μM (Fig. 3). This represents a 25-fold increase in activity as compared to compound **22**, and at least a 75-fold increase in activity from the original hit compound **1**. It should be noted from Figure 3 that the Hill slopes of compounds 1, 21 and

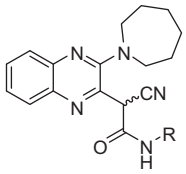
Table 2
SAR of quinoxaline 6 and 7 positions

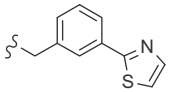
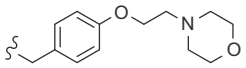
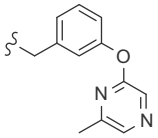
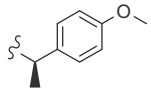
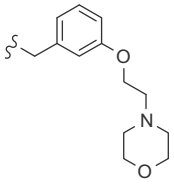
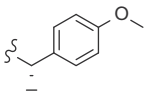
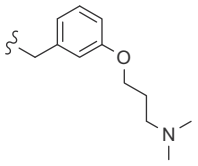
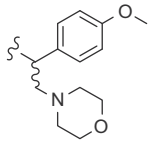




Entry	R^2	IC_{50} (μM)	Entry	R^3	IC_{50} (μM)
35	6-CN	100	**39**	Ph	>500
36	6-NO2	500	**40**	Pyridin-3ylmethyl	>500
37	7-OMe	>500	**41**	Pyridin-3ylethyl	>500
38	6,7-Cl, Cl	30	**42**	Dimethylaminopropyl	400

Table 4
SAR of phenyl ring substitutions



R		IC_{50} (μM)	R		IC_{50} (μM)
59		40	**63**		>500
60		200	**64**		40
61		400	**65**		40
62		200	**66**		2

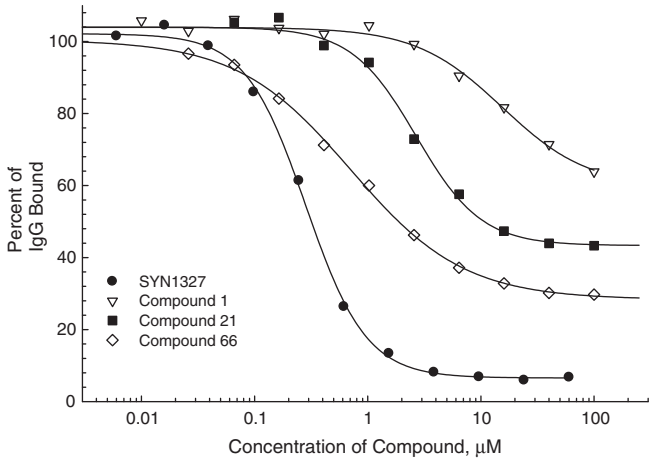


Figure 3. Representative inhibition curves for compounds **1**, **21**, **66** and control peptide SYN1327[7] using the FcRn–IgG competition ELISA assay.

66 vary among themselves despite their similar chemical structures. In addition, the slopes vary in comparison to control peptide SYN1327 when assayed in the same experiment. This phenomenon suggests some form of negative cooperativity for the small molecule binding, or heterogeneous binding and is not fully understood. Further work will be required to elucidate the cause of this effect and their mode of binding and inhibition.

In summary, a series of novel quinoxaline analogs were prepared and found to inhibit the human IgG:human FcRn protein–protein interaction. Chemical optimization of the initial hit compound **1** to compound **66** resulted in a 75-fold improvement in in vitro activity. This family of compounds may serve as useful tools in the study of FcRn biology, as well as starting points for the further development of orally available small molecule inhibitors of FcRn for therapeutic use.

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