First-in-class non-carbohydrate inhibitors of sialic acid-binding immunomodulatory-type lectin-7 (Siglec-7) discovered from genetically encoded bicyclic peptide libraries

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Introduction

Sialic acid-binding immunoglobulin-type lectins (Siglecs) are a class of immunoinhibitory cell signaling proteins with significant implications in cancer.[1] Hypersialiation of cancer cells activates Siglecs, and this activation suppresses the immune recognition of these hyper-sialylated cells and promotes cancer-cell survival.[2–5] Although Siglecs' natural substrates are gangliosides (a class primarily composed of glycoprotein with terminal sialic acid residues), Siglec proteins have a low affinity for these substrates.[6,7] Hence, high-affinity inhibitors are a highly desirable focus of research.

Using genetically encoded libraries, we identified a group of bicyclic peptides with a strong affinity for Siglec-7 and Siglec-9. Specifically, we used bicyclic genetically encoded libraries modified by two-fold symmetric linkers (BiGEL2) to screen against these two targets, employing next-generation sequencing (NGS) analysis for hit nomination.

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SxC6C Bicyclic Genetically Encoded Library Panning Against Siglec-7

Fig. 1a

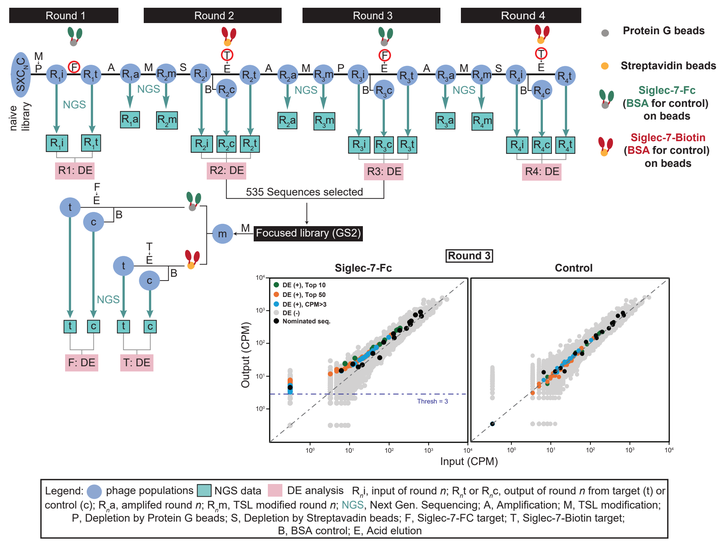

Fig. 1b

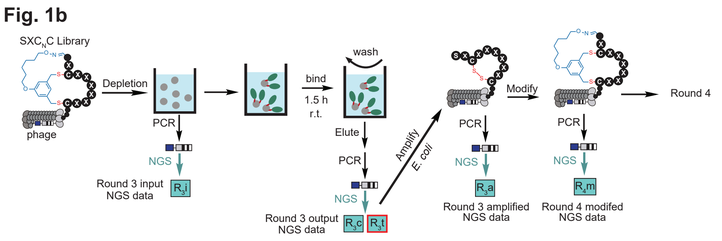

Figure 1. A) A flow chart of the sequencing and panning results for Siglec-7 including DE analysis of Round 3 panning against siglec-7-fc and its control counterparts B) Visual representation of a round 3 panning and steps involved.

Fig. 2a

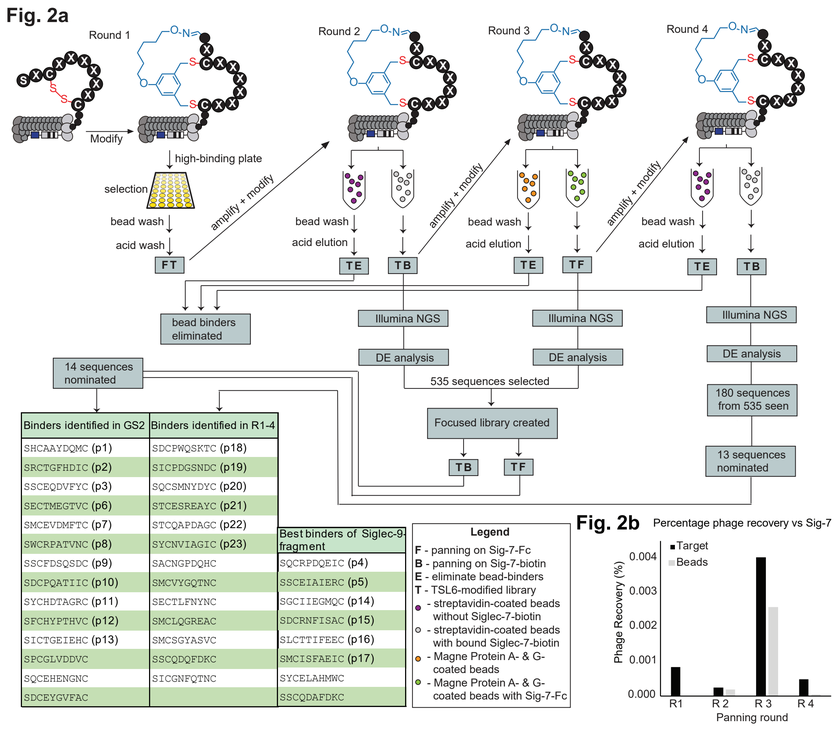

Fig. 2b Percentage phage recovery vs Sig-7

Figure 2. A) The overall workflow of the panning experiments that led to the 27 nominated binding sequences against Siglec-7. The targets are the result of panning against the TSL6_SxC6C library for four rounds alternating between Siglec 7-Fc and Siglec-7-biotin. B) Percentage of the phage recovered after each panning round. *NGS = next-generation sequencing

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SPR & ELISA Results of Top BInders - Against Competitve Binder GD3

Fig. 3a

		K_D (μM) i	ii	iii	IC_{50} (μM) iv
p1	SHCAAYDQMC	155	----	----	----
p2	SRCTGFHDIC	2850	----	N/F	----
p3	SSCEQDVFYC	3.0	2.6	1.1	>1000
p4	SQCRPDQEIC	N/F/15	----	N/F	----
p5	SSCEIAIERC	17	7	30	26
p6	SECTMEGTVC	N/F	N/F	N/F	----
p7	SMCEVDMFTC	N/F	N/F	N/F	215
p8	SWCRPATVNC	1.5	2.5	3.1	3.8
p9	SSCFDSQSDC	N/F	N/F	N/F	----
p10	SDCPQATIIC		Insoluble		Insol
p11	SYCHDTAGRC	N/F	N/F	N/F	50
p12	SFCHYPTHVC	1.7	1.5	59	11
p13	SICTGEIEHC	N/F	N/F	N/F	----
p14	SGCIIEGMQC	N/F	N/F	N/F	----
p15	SDCRNFISAC	N/F	N/F	N/F	----
p16	SLCTTIFEEC	N/F	N/F	N/F	----
p17	SSCQDAFDKC	N/F	N/F	N/F	----
p18	SDCPWQSKTC	865	N/F	N/F	103
p19	SICPDGSNDC	N/F	N/F	N/F	>1000
p20	SQCSMNYDYC	N/F	N/F	N/F	>1000
p21	STCESREAYC	N/F	N/F	N/F	>1000
p22	STCQAPDAGC	N/F	N/F	N/F	125
p23	SYCNVIAGIC		Insoluble		Insol
p24	SACAWHQHAC	N/F	N/F	N/F	----
p25	SACSDTGLQC	N/F	N/F	N/F	264

Fig. 3b

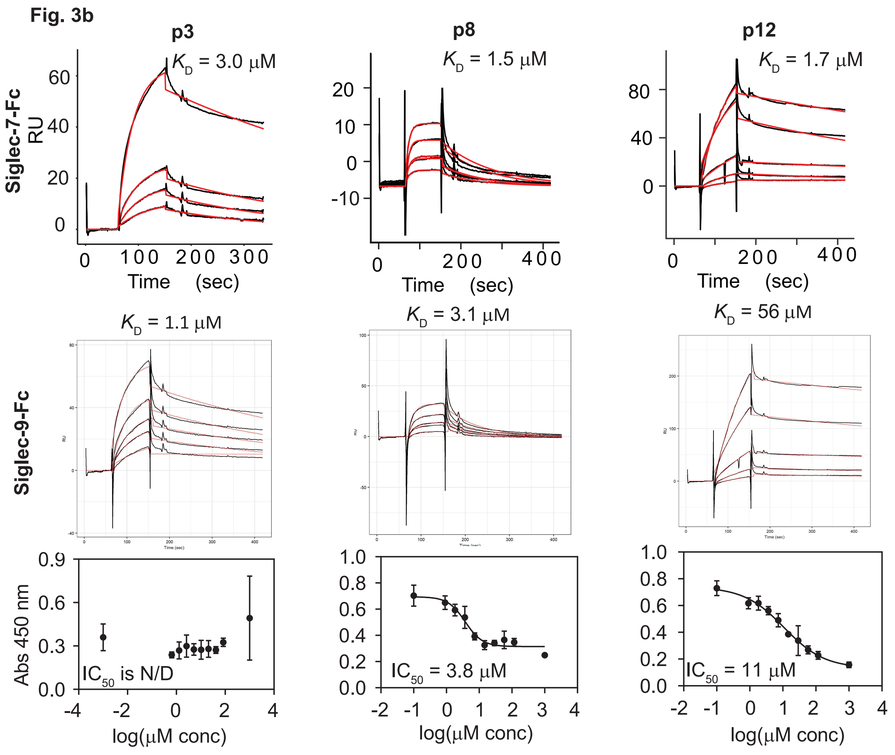
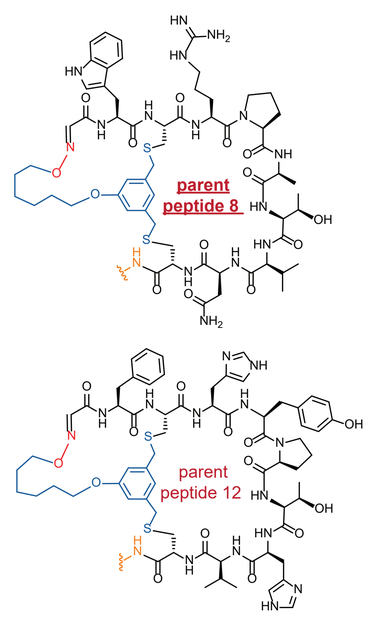


Figure 3. A) SPR evaluation of binding of 25 peptides to (i) Siglec-7-Fc; (ii) Siglec-7; (iii) Siglec-9 and (iv) inhibition of GD3:Siglec-7 interaction measured by ELISA. B) Surface plasmon resonance and ELISA binding traces of the three most active macrobicycles, p3, p8 and p12.

Fig. 4a

	PRIMARY LEAD Sequence	linker	IC_{50} (μM)
8a	SWCRPATVNC-NH_2	TSL6	3.8
8b	SWCRPATVNC-NH_2	TSL1	14
8c	SACRPATVNC-NH_2	TSL6	26
8d	SWCAPATVNC-NH_2	TSL6	>100
8e	SWCRAATVNC-NH_2	TSL6	16
8f	SWCRPGTVNC-NH_2	TSL6	29
8g	SWCRPAAVNC-NH_2	TSL6	69
8h	SWCRPATANC-NH_2	TSL6	3.8
8i	SWCRPATVAC-NH_2	TSL6	28
8j	SWCRPATVNC-NH_2	MBX	37
8k	SACRPATVNC-NH_2	MBX	>100
8l	SWCAPATVNC-NH_2	MBX	>100
8m	SWCRAATVNC-NH_2	MBX	34
8n	SWCRPGTVNC-NH_2	MBX	>100
8o	SWCRPAAVNC-NH_2	MBX	>100
8p	SWCRPATANC-NH_2	MBX	>100
8q	SWCRPATVAC-NH_2	MBX	>100
8r	sWCRPATVNCGGGZ-NH_2	S-S	>100
8s	SWCRPATVNCGGGZ-NH_2	TSL6	31
8t	SWCRPATVNCGGGZ-NH_2	TSL3	67
8u	SWCRPATVNCGGGZ-NH_2	TSL1	118
8v	SWCRPATVNCGGGZ-NH_2	MBX	51
8w	sAAAAAAWCRPATVNCGGGZ-NH_2	TSL1	155



	SECONDARY LEAD Sequence	linker	IC_{50} (μM)
12a	SFCHYPTHVC-NH_2	TSL6	11
12b	SACHYPTHVC-NH_2	TSL6	11
12c	SFCAYPTHVC-NH_2	TSL6	>100
12d	SFCHAPTHVC-NH_2	TSL6	9.5
12e	SFCHYATHVC-NH_2	TSL6	4.0
12f	SFCHYPAHVC-NH_2	TSL6	6.1
12g	SFCHYPTAVC-NH_2	TSL6	12
12h	SFCHYPTHAC-NH_2	TSL6	10
12i	SFCHAAAHVC-NH_2	TSL6	22
12j	SFCHYPTHVC-NH_2	MBX	77
12k	SACHYPTHVC-NH_2	MBX	113
12l	SFCAYPTHVC-NH_2	MBX	>100
12m	SFCHAPTHVC-NH_2	MBX	>100
12n	SFCHYATHVC-NH_2	MBX	6.1
12o	SFCHYPAHVC-NH_2	MBX	107
12p	SFCHYPTAVC-NH_2	MBX	51
12q	SFCHYPTHAC-NH_2	MBX	51
12r	SFCHYPTHVCGGGZ-NH_2	TSL6	4.6
12s	SFCHYPTHVCGGGZ-NH_2	TSL3	5.8
12t	SFCHYPTHVCGGGZ-NH_2	TSL1	9.5
12u	SFCHYPTHVCGGGZ-NH_2	MBX	132
12v	SAAAAAAFCHYPTHVCGGGZ-NH_2	TSL1	24

Fig. 4b

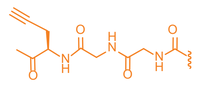
	p5 IC_{50} (μM)	p5 K_D (μM)	p8 IC_{50} (μM)	p8 K_D (μM)	p12 IC_{50} (μM)	p12 K_D (μM)
	26	N/A	31	N/A	4.6	N/A

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Figure 4. A) IC50 values of the alanine-scanned and linker-modified variants of our lead binders, p8 and p12, were obtained to build an SAR profile. Z = L-propargylglycine. B) C-terminal elongation

Conclusion

We employed bicyclic genetically encoded libraries (BiGEL) to discover bicyclic inhibitors that block the interaction of the immunomodulatory protein Siglec-7 with its glycan ligand GD3. BiGEL were produced by chemical ligation of two-fold symmetric linchpin (TSL-6) to the N-terminus and two Cys residues in phage-displayed libraries.

Panning of BiGEL against Siglec-7 and Siglec-9 proteins combined with next-generation sequencing (NGS) and differential enrichment analysis yielded 815 candidates. Further refinement in a focused library resulted in nomination of 34 sequences, from which 23 were tested by surface plasmon resonance (SPR) to yield KD in 1–1000 μM range. Competitive ELISA further identified a subset of the leads that disrupted Siglec-7:GD3 interaction with the IC_{50} values ranging from 3 to 300 μM.

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48Hour Discovery
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