Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Electronic Supplementary Information

Experimental Design for Determining Quantitative Structure Activity Relationship for Antibacterial Chitosan Derivatives

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Table of Contents:

1. Synthesis of TBDMS-chitosan

Chitosan mesylate (7 g, 27.28 mmol on glucosamine unit basis) was dissolved in dry DMSO (100 mL). A solution of imidazole (31.5 g, 463.7 mmol) and TBDMS-Cl (20.5 g, 136.4mmol) in dry DMSO (100 mL) was added dropwise, and the resulting mixture was stirred at 25 °C for 24 h. The solid gel-type material obtained was filtered off and washed with H_2O (5×150 mL), and CH_3CN (3×100 mL). The material was then dried in vacuo at 40 °C for 18 h, to afford TBDMS chitosan (**3**) Yield: 9.6 g (89%).^{1}H NMR (400 MHz, $CDCl_3$): δ4.30 (br s, 1 H, H-1), 4.00–3.50 (m, 3 H, H-4,6), 3.50–3.10 (m, 2 H, H-3,5), 2.71 (brs, 1 H, H-2), 0.89 (br s, 18 H, $(CH_3)_3C$), 0.05 (br s, 12 H, $(CH_3)_2Si$) ppm. FT-IR (KBr): ν 3442 (br, N–H), 2956–2857 (C–H), 1705 (C=O amide I), 1573 (C=O amide II), 1472 (C–H), 1390–1361 (C–H *tert*-butyl), 1255 (Si–CH_3), 1105 –1050 (C–O), 837–778 (Si–CH_3) cm^{-1}.

2. Figure S1-S6. ^{1}H NMR spectra of chitosan derivatives

Figure S1. ^{1}H NMR (400 MHz, D_2O) spectrum of Mesylate salt of chitosan

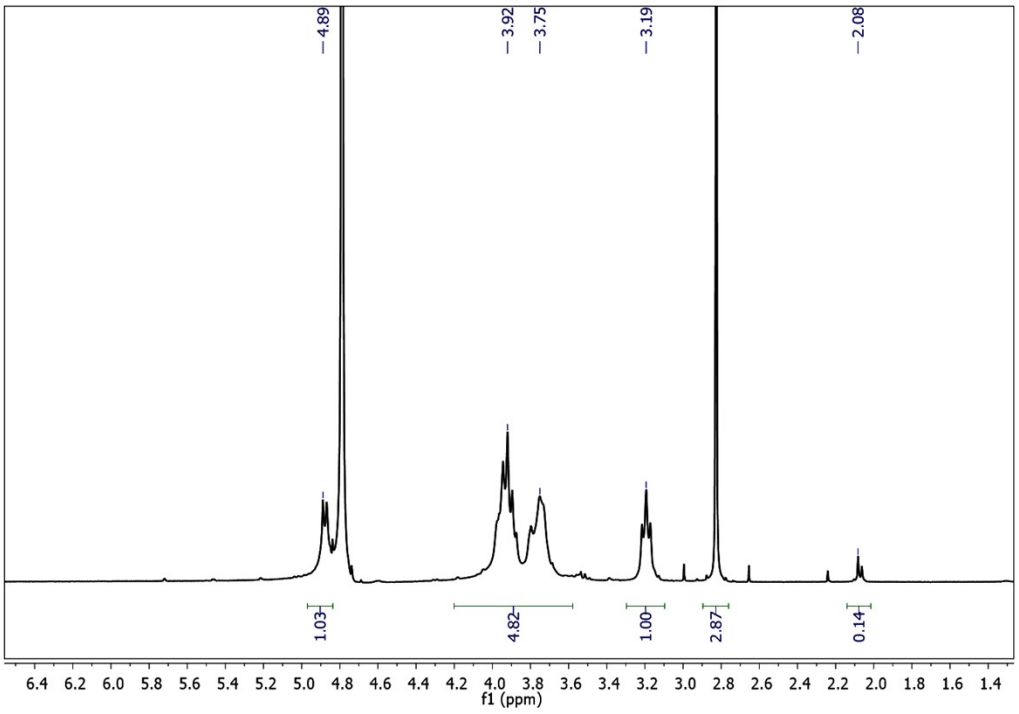


Figure S2. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of 3,6-*O*-diTBDMS chitosan

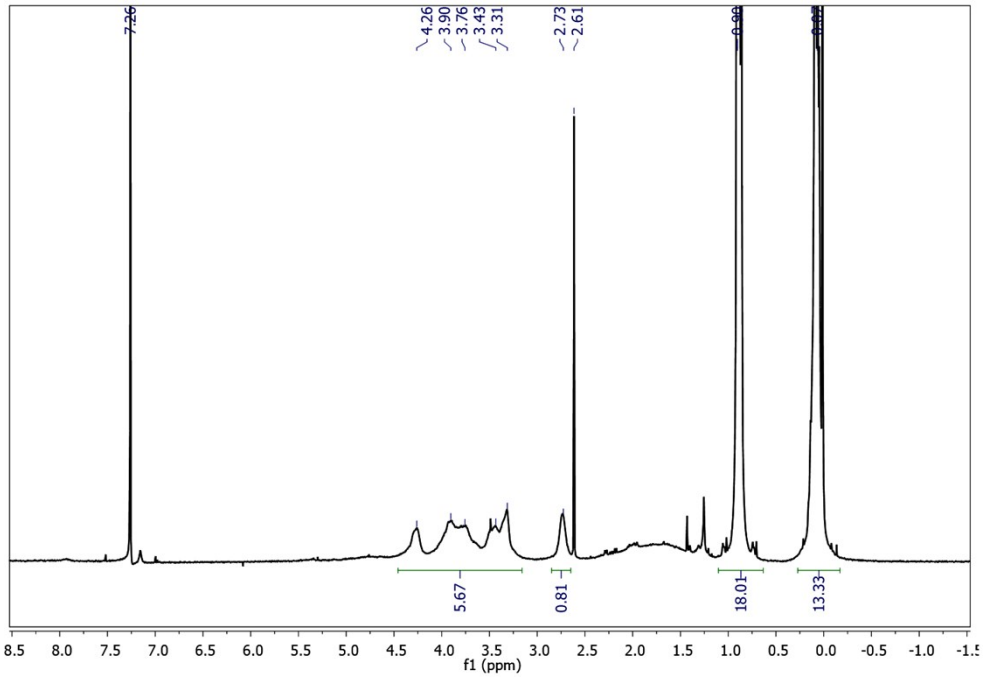


Figure S3. ^{1}H NMR (400 MHz, D_2O) spectrum of **3i**.

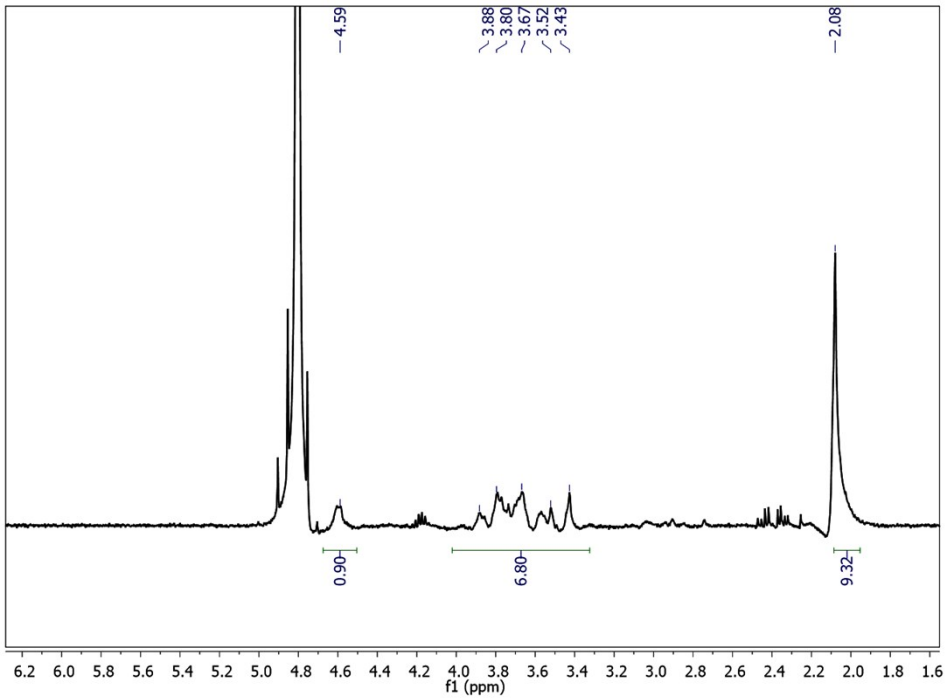


Figure S4. ^{1}H NMR (400 MHz, D_2O) spectrum of **3ii**.

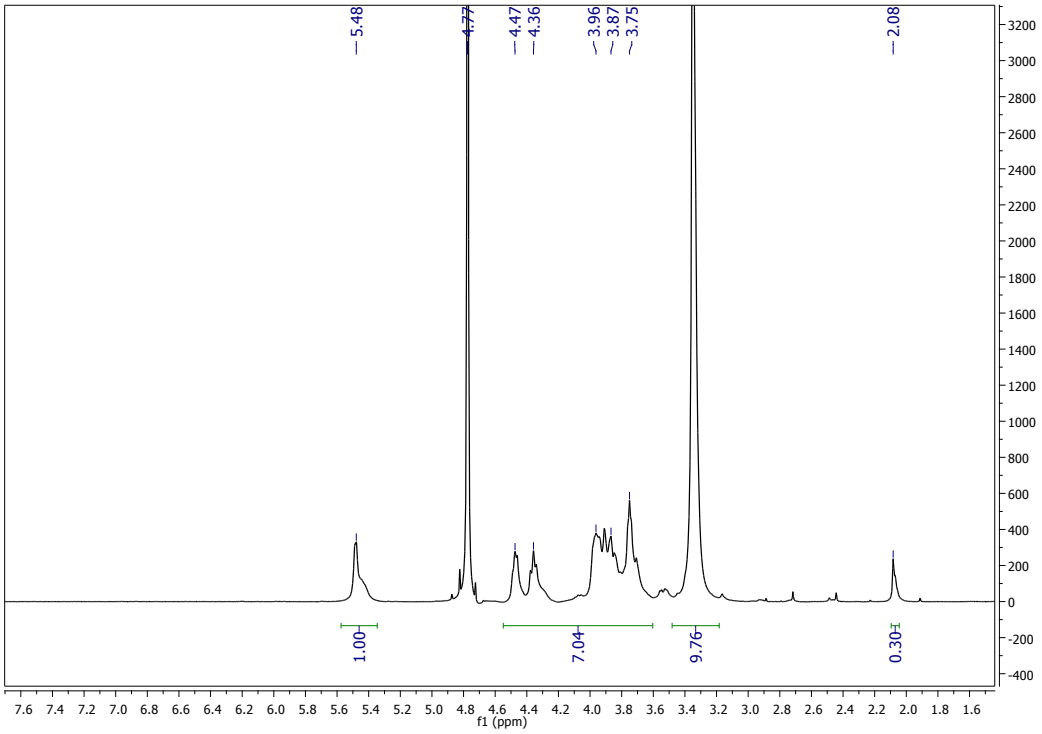


Figure S5. ^{1}H NMR (400 MHz, D_2O) spectrum of **3v**.

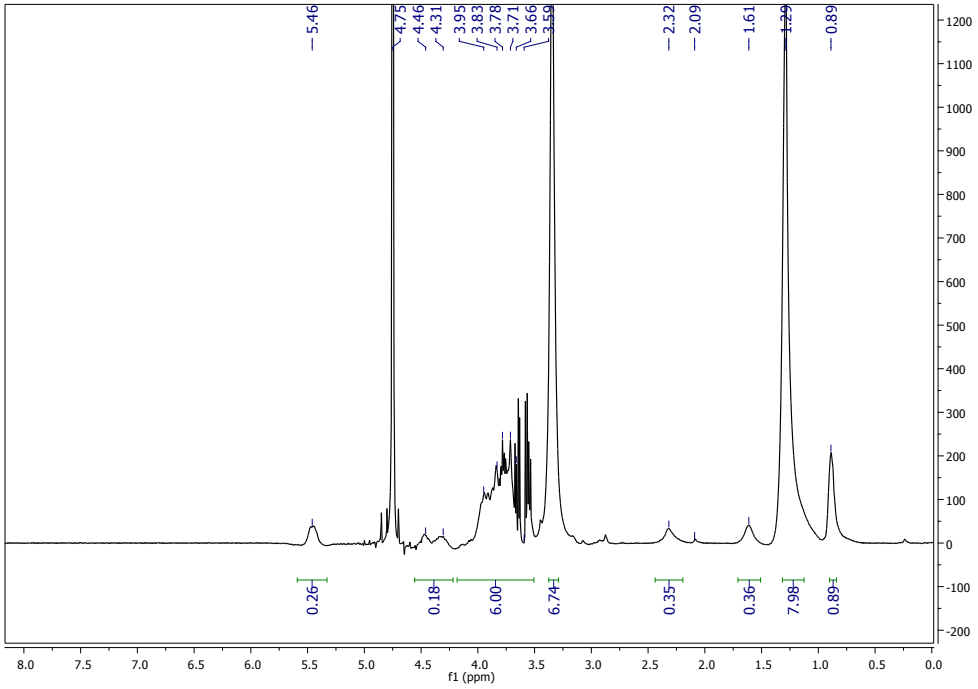
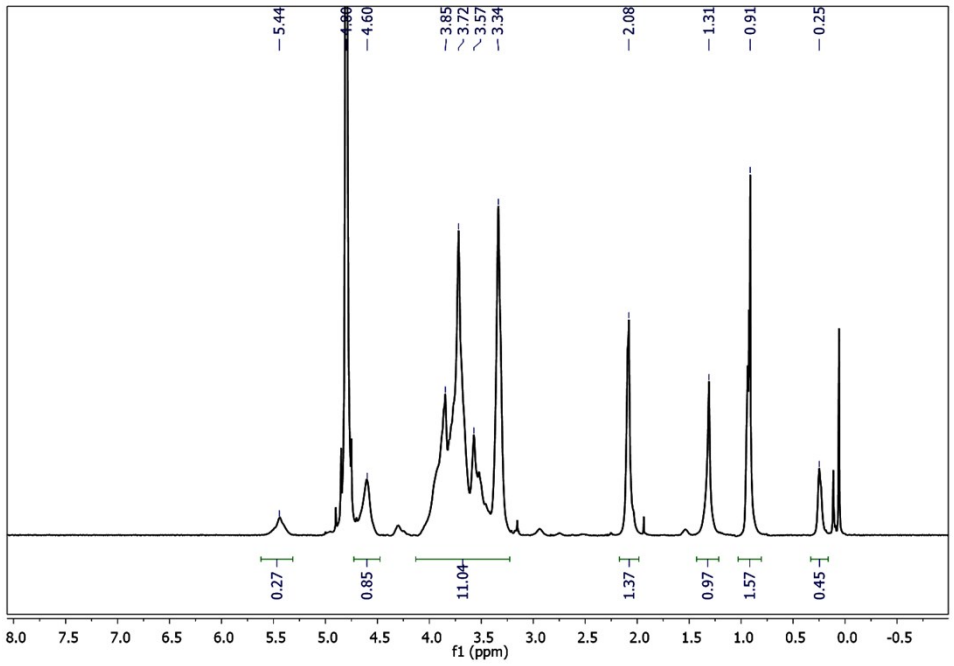


Figure S6. ^{1}H NMR (400 MHz, D_2O) spectrum of **3xiii**.



3. Figure S7-S9. COSY spectra of chitosan derivatives

Figure S7. COSY NMR spectrum of **3ii**.

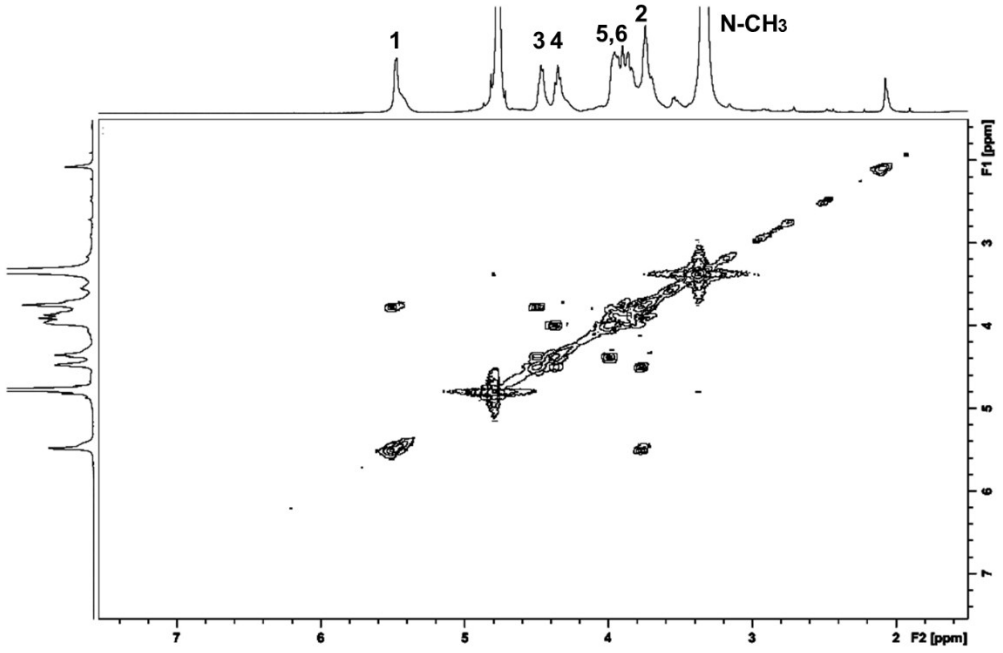


Figure S8. COSY NMR spectrum of **3v**.

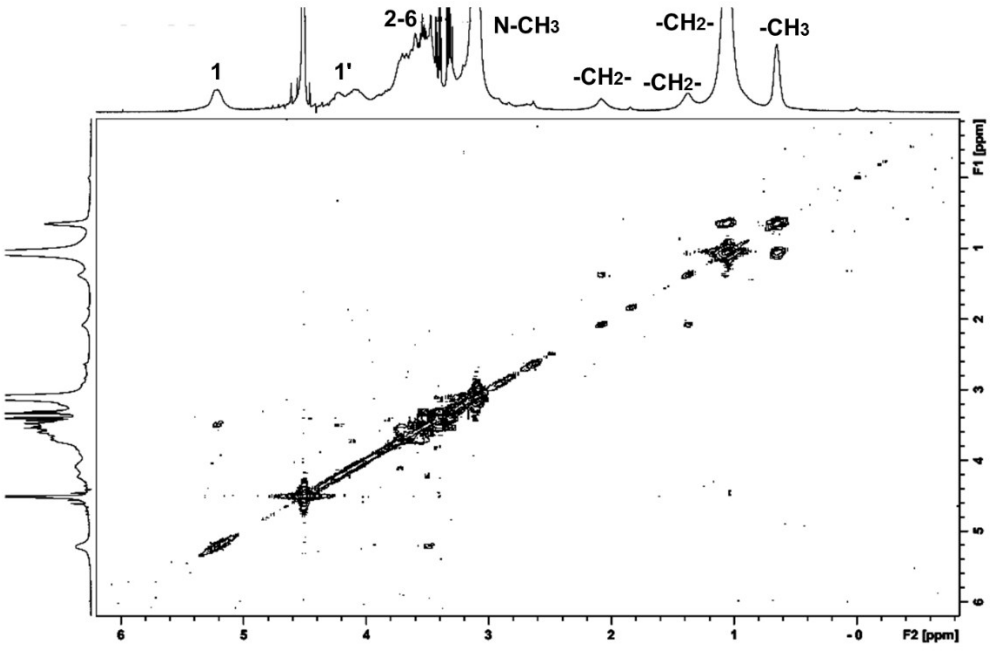
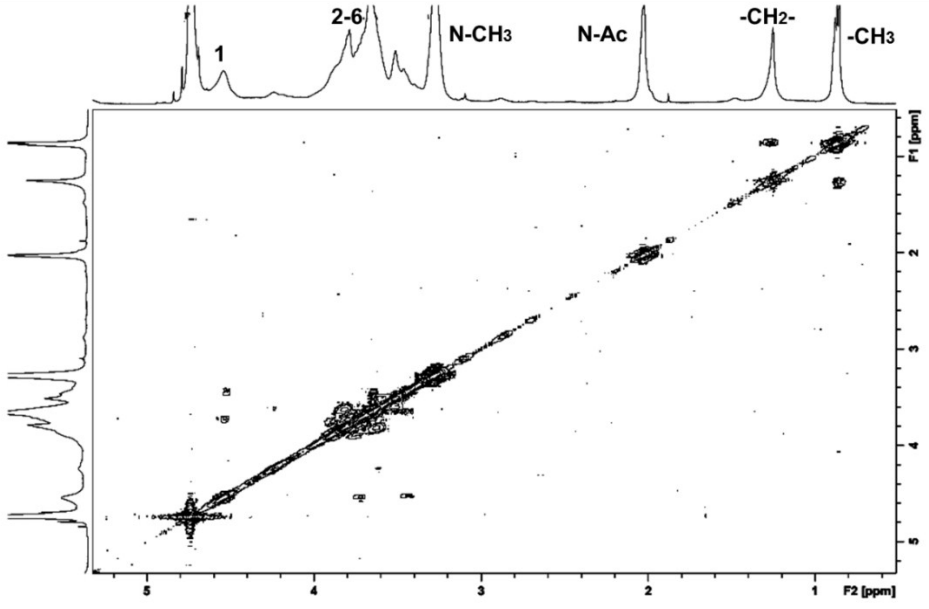


Figure S9. COSY NMR spectrum of **3xiii**.



4. Table S1. Average molecular weight of the chitosan derivatives:

Compound No.	Average Molecular weight (Mw)	Viscosity-average molecular weight (Mv)	Dispersity (Đ)
3i	7020	7052	1.23
3ii	5076	5321	1.20
3iii	11364	11386	1.56
3iv	5269	5296	1.35
3v	8695	8869	1.38
3vi	6350	6450	1.23
3vii	6789	6856	1.28
3viii	9569	9572	1.42
3ix	9896	9902	1.32
3x	8923	8996	1.36
3xi	12563	12763	1.67
3xii	12035	12092	1.63
3xiii	6523	6589	1.32
3xiv	8963	9201	1.58

5. Table S2. MIC, MLC and Hemolytic activity (HC_{50}) values for the chitosan derivatives:

Compound No.	*S. aureus* (μg/mL)		*E. coli* (μg/mL)		Hemolytic activity (HC_{50}) (μg/mL)
	MIC	MLC	MIC	MLC	
3i	16384	32768	32768	32768	8598
3ii	16	16	512	512	192
3iii	32	32	1024	2048	192
3iv	512	1024	8192	8192	3072
3v	64	64	2048	4098	1536
3vi	128	512	512	512	1288
3vii	8192	8192	32768	32768	6144
3viii	2048	2048	32768	32768	6144
3ix	32	64	2048	8192	1024
3x	128	256	16384	16384	3072
3xi	2048	8192	16384	16384	597
3xii	2048	4096	16384	32768	1136
3xiii	2048	4096	16384	32768	597
3xiv	2048	4096	16384	16384	1136

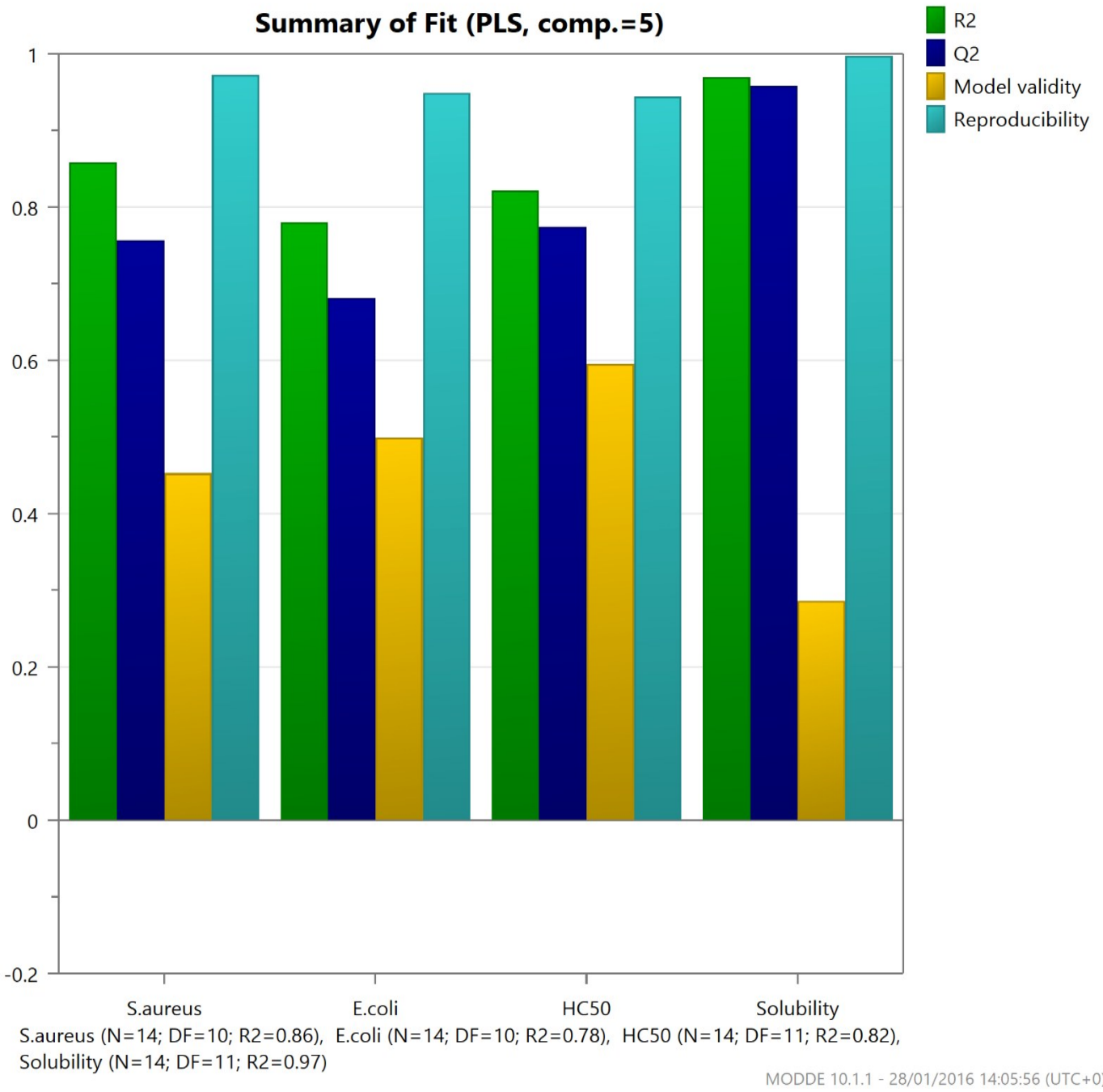
6. **Table S3. Showing the difference in proposed and observed values for different substitutions**

Proposed values			**Observed values**			**Percentage difference**		
TRI	**ACE**	**STE**	**TRI**	**ACE**	**STE**	**TRI**	**ACE**	**STE**
0.05	0.95	0	0.03	0.97	0	40.00	-2.11	0
0.95	0.05	0	0.95	0.05	0	0	0	0
0.95	0.05	0	0.95	0.05	0	0	0	0
0.05	0.65	0.3	0.08	0.66	0.26	-60.00	-1.54	13.33
0.65	0.05	0.3	0.67	0.05	0.28	-3.08	0	6.67
0.35	0.65	0	0.39	0.61	0	-11.43	6.15	0
0.05	0.85	0.1	0.07	0.82	0.11	-40.00	3.53	-10.00
0.05	0.75	0.2	0.08	0.74	0.18	-60.00	1.33	10.00
0.75	0.05	0.2	0.72	0.05	0.23	4.00	0	-15.00
0.45	0.25	0.3	0.4	0.35	0.25	11.11	-40.00	16.67
0.425	0.425	0.15	0.43	0.45	0.12	-1.18	-5.88	20.00
0.425	0.425	0.15	0.43	0.43	0.14	-1.18	-1.18	6.67
0.425	0.425	0.15	0.46	0.39	0.15	-8.24	8.24	0
0.425	0.425	0.15	0.43	0.43	0.14	-1.18	-1.18	6.67
				Average		**-9.37**	**-2.33**	**3.93**

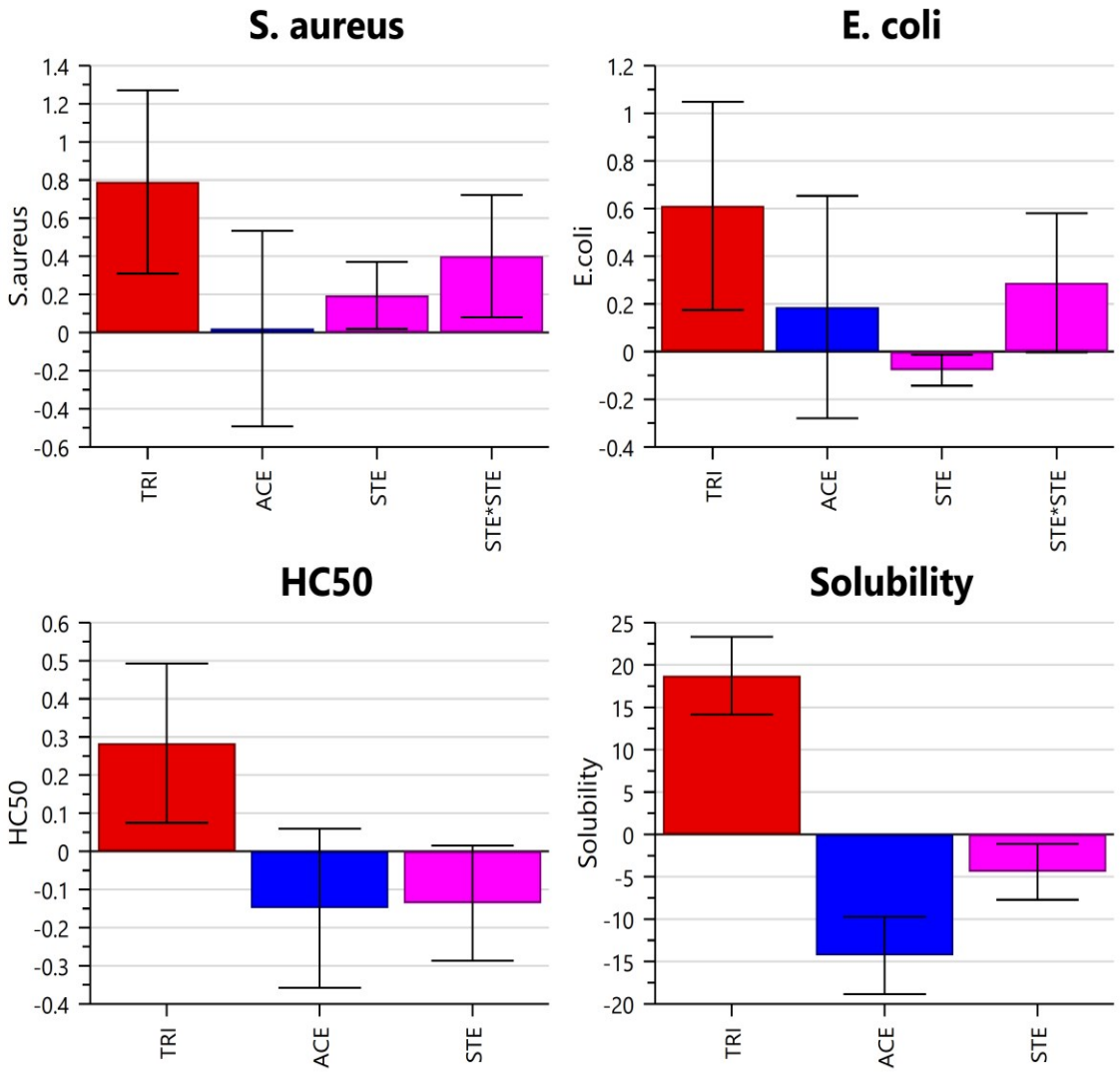
When comparing the models obtained from the proposed values and the observed values, it can be seen that in the former model the model validity is not good and the Q2 (y1=0.74, y2=0.59, y3=0.73 and y4=0.95) and R2 (y1=0.83, y2=0.72, y3=0.78 and y4=0.97) values are lower when the proposed factor values were used. On the other hand, when the observed values were used as factors, it resulted in a better model validity with an improved Q2 (y1=0.76, y2=0.68, y3=0.77 and y4=0.95) and R2 (y1=0.89, y2=0.82, y3=0.93 and y4=0.98) values. The two co-efficient plots and response contour plots have been included to show the small difference in the significance of the individual and the interaction terms and the resulting shift in the positioning of the response values. The difference in the proposed and observed values for the different substituents can be seen in table S3.

(Where, y represents the responses, y1= *S. aureus*; y2= *E. coli*; y3= HC_{50} and y4= Solubility)

7. **Summary of Fit for the model (observed values) after refinement:**



8. Coefficient Plot obtained from the proposed factors in the design matrix:



9. Response Contour Plot obtained from the proposed factors in the design matrix: