

1 **Table S1.** Mortality reduction, average APEC load reduction, and average lesion severity
 2 reduction in QSIs treated chickens

QSIs	Mortality rate % (Nr.)	Average APEC load (Log CFU/g of tissue)				Average lesion severity			
		Liver	Lung	Heart	Kidney	Liver	Lung	Heart	Airsac
QSI-1	50 (3/6)	5.30	5.92	5.63	6.28	2.17	0.50	3.00	1.83
QSI-2	33.3 (2/6)	4.20	5.15	4.87	5.07	1.00	0.17	1.67	0.92
QSI-5	0 (0/6)	0.72	0.75	0.58	0.67	0.33	0.17	0.33	0.17
QSI-6	66.6 (4/6)	4.62	5.17	4.87	5.35	0.83	0.50	1.50	1.17
QSI-7	66.6 (4/6)	5.95	6.10	6.13	6.13	1.33	0.17	1.33	1.00
QSI-8	33.3 (2/6)	3.48	4.60	3.60	3.65	0.50	0.17	0.50	0.50
QSI-10	16.6 (1/6)	2.50	3.08	2.50	2.47	0.00	0.00	0.33	0.17
PC	66.6 (4/6)	5.95	6.83	5.98	6.81	1.75	1.25	2.25	2.00

3 PC; positive control group (chickens infected with APEC and treated with 0.05% of DMSO). Lesions were scored as:
 4 air sacs, (1) slight opaque and/or thickened membranes ± slight amount of fibrin, (2) moderate opaque and/or
 5 thickened membranes ± moderate amount of fibrin, (3) severe opaque and/or thickened membranes ± severe amount
 6 of fibrin ± moderate to severe vascularization); Lung, (1) single small lesion, locally restricted (1/5 of the organ), (2)
 7 multiple, locally restricted small lesions and/or one bigger lesion (2/5 of the organ), (3) lesions cover about 1/2 of the
 8 organ, (4) lesions cover about 1/2 of the organ, (5) complete organ covered with lesions; Heart, (1) vascularization,
 9 opacity, (2) excessive or cloudy fluid in the pericardial cavity, (3) thickened pericardium, acute pericarditis; Liver, (1)
 10 decolorization and/or slight amounts of fibrinous exudates, (2) marked perihepatitis with high amounts of fibrinous
 11 exudates. The clinical signs and the daily mortality were recorded for 8 DPI.
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17 **Table S2.** Mortality reduction, average APEC load reduction and average lesion severity reduction
 18 in QSI-5 treated chickens at 1 mg/L, 5 mg/L, 10 mg/L, 20 mg/L.

QSI-5 dose	Mortality rate % (Nr.)	Average APEC load (Log CFU/ g of tissue)				Average lesion severity			
		Liver	Lung	Heart	Kidney	Liver	Lung	Heart	Airsac
1 mg/L	37.5 (3/8)	5.33	5.68	5.71	5.06	1.25	0.33	1.63	1.38
5 mg/L	66.6 (6/9)	6.03	5.86	6.09	6.10	1.33	0.38	1.89	1.33
10 mg/L	44.4 (4/9)	5.84	6.37	7.32	6.20	2.11	0.78	2.44	2.33
20 mg/L	66.6 (6/9)	6.53	6.58	6.69	6.56	1.22	0.44	2.11	1.67
PC	88.9 (8/9)	8.46	7.93	8.37	7.84	2.11	1.11	2.78	3.11

19 PC; positive control group (chickens infected with APEC and treated with 0.05% of DMSO). Lesions were scored as:
 20 air sacs, (1) slight opaque and/or thickened membranes ± slight amount of fibrin, (2) moderate opaque and/or
 21 thickened membranes ± moderate amount of fibrin, (3) severe opaque and/or thickened membranes ± severe amount
 22 of fibrin ± moderate to severe vascularization); Lung, (1) single small lesion, locally restricted (1/5 of the organ), (2)
 23 multiple, locally restricted small lesions and/or one bigger lesion (2/5 of the organ), (3) lesions cover about 1/2 of the
 24 organ, (4) lesions cover about 1/2 of the organ, (5) complete organ covered with lesions; Heart, (1) vascularization,
 25 opacity, (2) excessive or cloudy fluid in the pericardial cavity, (3) thickened pericardium, acute pericarditis; Liver, (1)
 26 decolorization and/or slight amounts of fibrinous exudates, (2) marked perihepatitis with high amounts of fibrinous
 27 exudates. The clinical signs and the daily mortality were recorded for 8 DPI.
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32 **Table S3.** Mortality reduction, average APEC load reduction and average lesion severity reduction
 33 in QSI-5 treated chickens compared to sulfadimethoxine (SDM).

QSI	Mortality rate% (Nr.)	Average APEC load (Log CFU/ g of tissue)				Average lesion severity			
		Liver	Lung	Heart	Kidney	Liver	Lung	Heart	Airsac
QSI-5	12.7 (8/63)	4.95	4.93	5.46	5.06	1.68	0.21	1.93	1.25
SDM	29.2 (19/65)	5.05	6.17	5.69	5.33	2.03	1.17	2.83	2.50
PC	45.6 (31/68)	7.24	7.68	7.92	7.73	2.52	1.85	3.58	3.36

34 PC; positive control group (chickens infected with APEC and treated with 0.05% of DMSO). Lesions were scored as:
 35 air sacs, (1) slight opaque and/or thickened membranes ± slight amount of fibrin, (2) moderate opaque and/or
 36 thickened membranes ± moderate amount of fibrin, (3) severe opaque and/or thickened membranes ± severe amount
 37 of fibrin ± moderate to severe vascularization); Lung, (1) single small lesion, locally restricted (1/5 of the organ), (2)
 38 multiple, locally restricted small lesions and/or one bigger lesion (2/5 of the organ), (3) lesions cover about 1/2 of the
 39 organ, (4) lesions cover about 1/2 of the organ, (5) complete organ covered with lesions; Heart, (1) vascularization,
 40 opacity, (2) excessive or cloudy fluid in the pericardial cavity, (3) thickened pericardium, acute pericarditis; Liver, (1)
 41 decolorization and/or slight amounts of fibrinous exudates, (2) marked perihepatitis with high amounts of fibrinous
 42 exudates. The mortality was recorded daily until the end of the experiment (42 days of age). Each group has a variable
 43 number of chickens as the weak chickens were removed from each group after setting up the experiment and before
 44 administering the first treatment.
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50 **Table S4:** Weekly and overall BWG in treated and control groups.

Groups	Week 2	Week 3	Week 4	Week 5	Week 6	Overall BWG*
QSI-5	106.12	454.27	519.41	668.96	910.3	2659.1 (P=0.85)
SDM	105.1	307.3	454.4	610.3	931	2408.1 (P=0.46)
PC	121.9	383.5	458.2	496.2	853.0	2312.8 (P=0.09)
NC	157.8	489.9	490.1	610.8	752.7	2501.2

51 *Average body weight of week 1 (QSI-5: 54.27, SDM (sulfadimethoxine): 53.86, PC: 52.19, and NC: 50.27) was used
 52 to calculate overall BWG. The BWG in QSI-5, SDM, and PC groups was statistically non-significant (Student's t-
 53 test) compared to NC group.
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61 **Table S5:** Weekly and overall FCR in treated and control groups.

Groups	Week 2	Week 3	Week 4	Week 5	Week 6	Overall FCR*
QSI-5	2.5	0.7	1.3	1.0	1.9	1.48 (P=0.94)
SDM	2.5	0.7	1.4	1.0	1.6	1.49 (P=0.64)
PC	2.2	0.9	1.9	1.3	1.4	1.51(P=0.43)
NC	2.0	0.7	1.4	1.1	2.0	1.45

62 * FCR was calculated by using formula: average overall feed intake/average BWG. FCR in QSI-5, sulfadimethoxine
 63 (SDM) and PC groups was statistically non-significant (Student's t-test) compared to NC group
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Supplemental Figure Legends

Figure S1: The survival rate of the chickens after treatment with; 1) different quorum sensing inhibitors, 2) different doses of QSI-5 (the best quorum sensing inhibitor), and 3) QSI-5 and compare its efficacy with the currently used antibiotic (sulfadimethoxine; SDM) in field-simulated conditions.

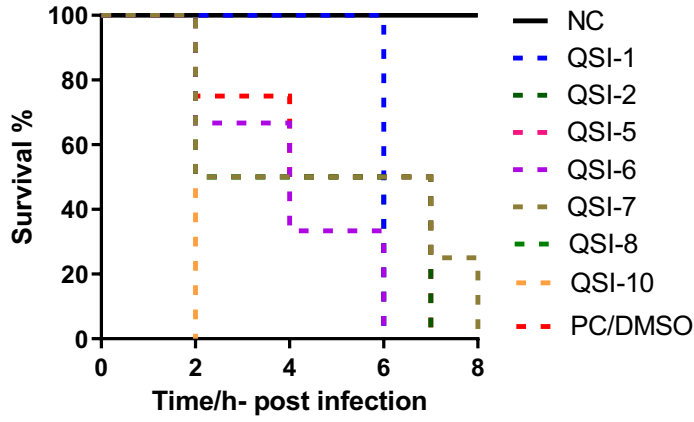
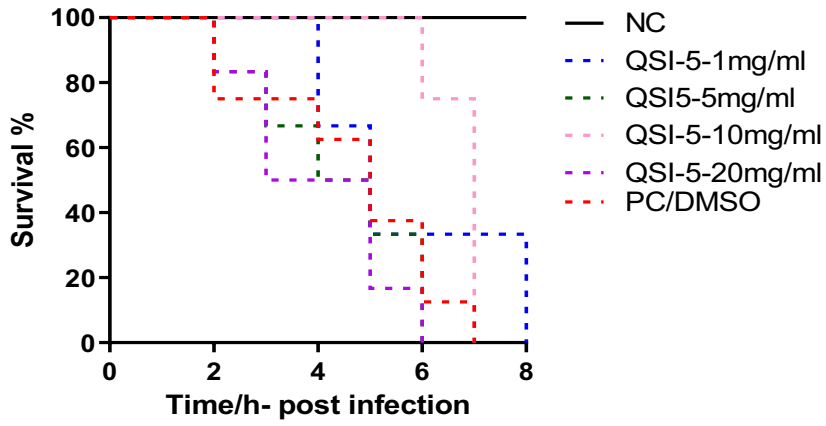
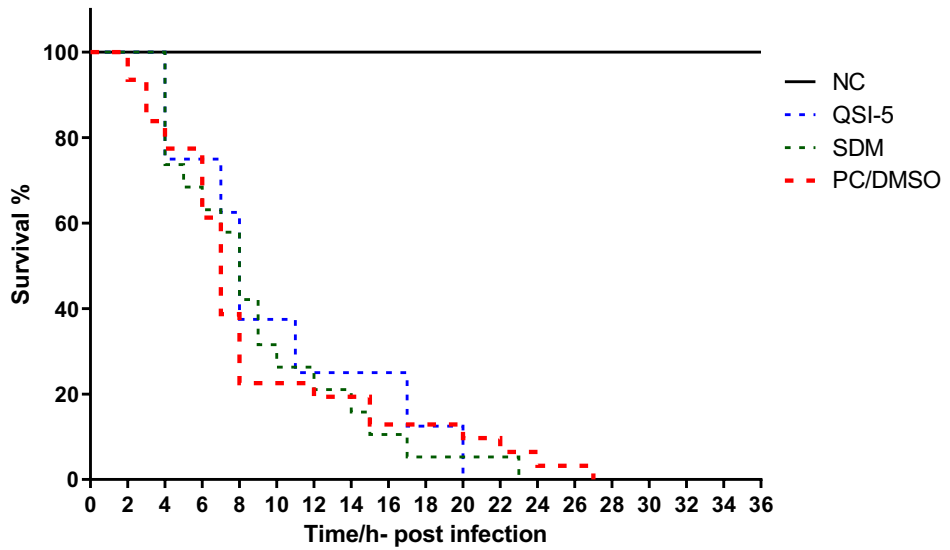
Figure S2: Principal component analysis (PCA) of metabolites clustering in serum of; (A) QSI-5 (PC3=6.78%, PC5=3.5%), and (B) QSI-8, and QSI-10 (PC1=30.5%, PC7=3.97%) treated chickens compared to non-infected and non-treated (NC) and DMSO treated and infected (PC) chicken groups.

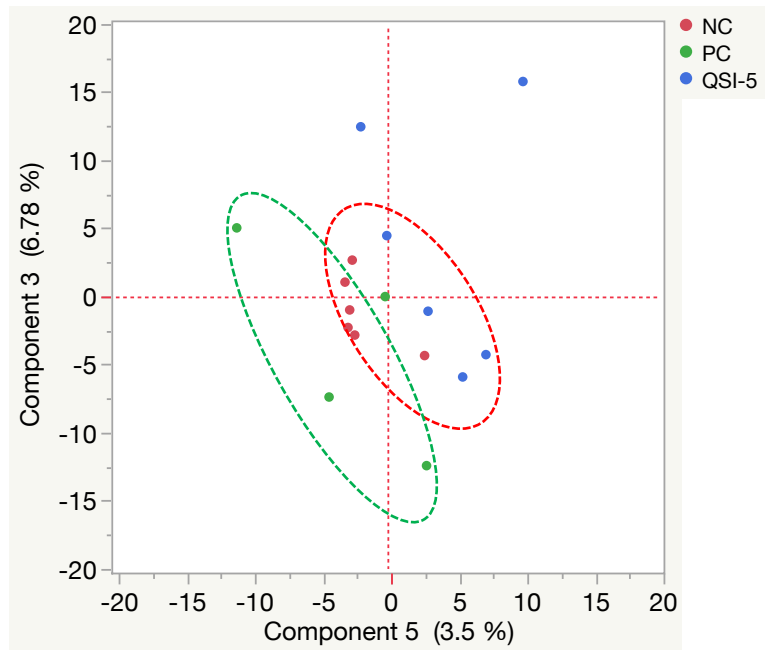
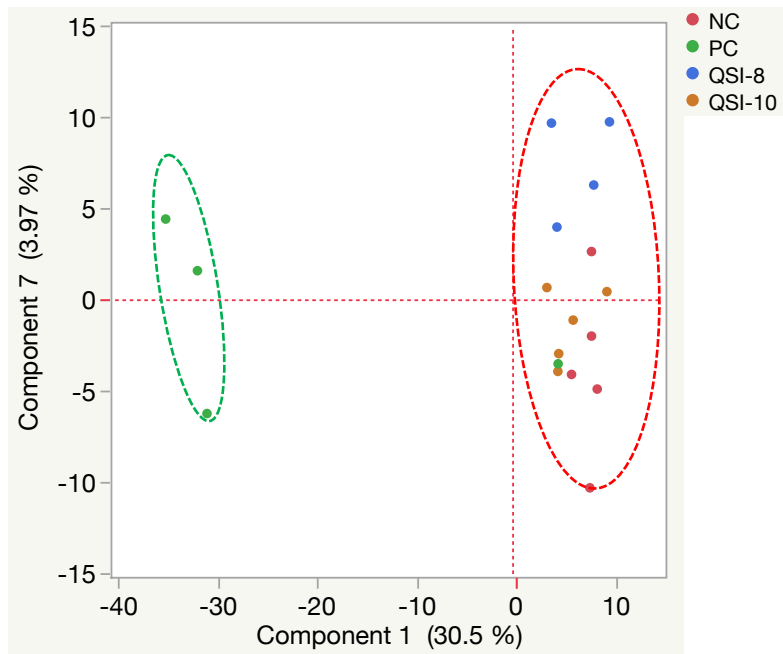
Figure S3: (A) Shannon's diversity index showing the microbial richness and evenness in QSIs treated groups compared to PC group. QSIs caused no significant differences in the richness and evenness of cecal microbiota compared to PC group. *P<0.05, Kruskal-Wallis test. (B) Impact of QSIs treatment on beta diversity of cecal microbiota of chickens. Beta diversity was evaluated using principal coordinates analysis of unweighted UniFrac values. There was no spatial separation observed in the cecal microbiota of the treated groups compared to PC and NC groups. Each dot represents cecum sample from each treatment groups.

Figure S4: Synthesis of QSI- 5 (1-(4-methylbenzyl)-4-(3-phenylpropyl)piperazine) using reductive amination. 1-(4-Methylbenzyl)piperazine (50 mg, 0.26 mmol) and 3-phenylpropionaldehyde (35 mL, 0.26 mmol) were dissolved in DMF (1.32 mL) at room temperature with stirring. Acetic acid (15 mL, 0.26 mmol) was added to the mixture, followed by sodium triacetoxyborohydride (111.5 mg, 0.526 mmol). The resulting solution was allowed to stir for an additional 2.5 hours and then quenched with H₂O. The mixture was extracted three times with EtOAc and the combined layers were dried over sodium sulfate and concentrated in vacuo. The crude mixture was then purified using silica gel column chromatography (95:5 – 90:10 gradient, dichloromethane:methanol) to provide 36 mg (0.12 mmol, 44%) of the desired product. ¹H NMR (400 MHz, MeOD) δ 7.27 – 7.10 (m, 9H), 3.47 (s, 2H), 2.61 (t, J = 7.6 Hz, 2H), 2.49 (s, 8H), 2.39 – 2.33 (m, 2H), 2.31 (s, 3H), 1.86 – 1.76 (m, 2H); ¹³C NMR (101 MHz, MeOD) δ 141.76, 136.81, 133.63, 129.34, 128.51, 128.00, 127.96, 125.47, 62.21, 57.66, 52.41, 52.11, 33.27, 27.92, 19.72.

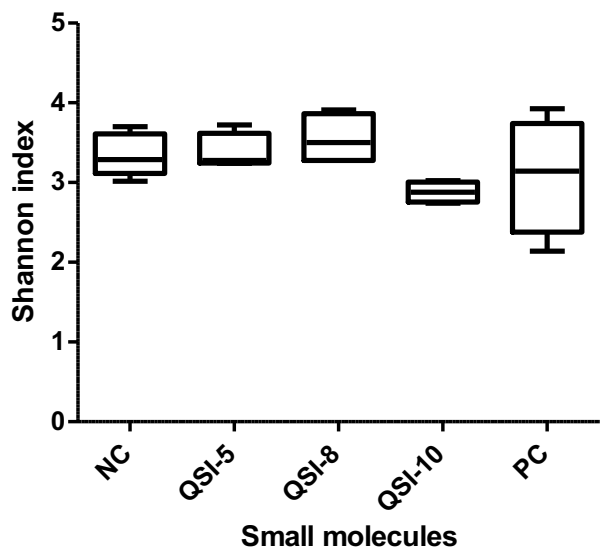
Figure S5: Calibration standard curves for determining the concentration of; A) QSI-5 and B) SDM in chicken's blood using LC-MS. For standard calibration, solutions of 0.0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, and 5 µg/mL of QSI-5 and SDM were used.

Figure S6: Calibration standard curves for determining the bioaccumulation of QSI-5 and SDM in chicken's; A) Kidney and B) liver and muscle using LC-MS.

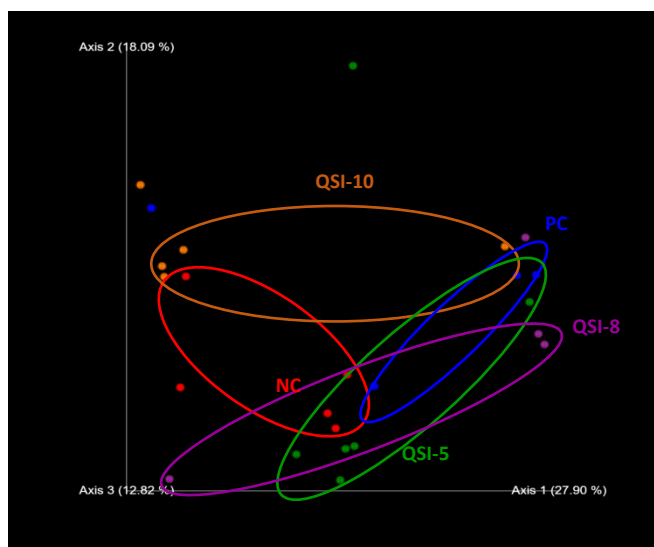
A**B****C**

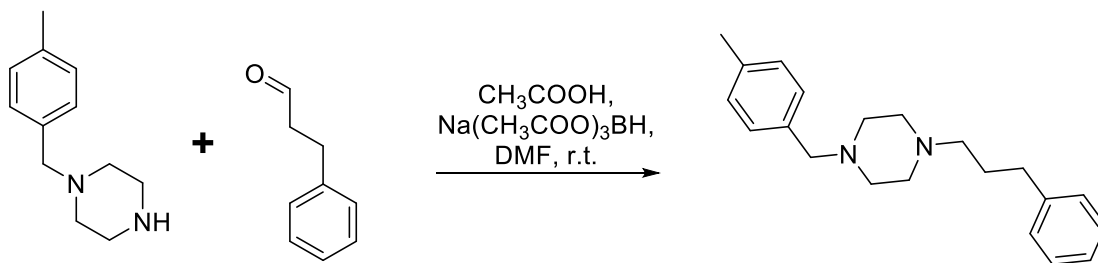
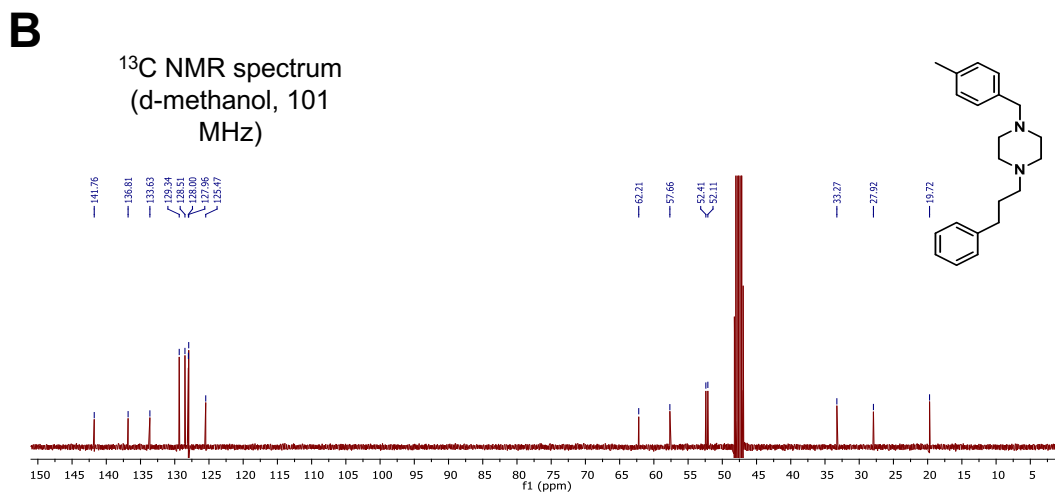
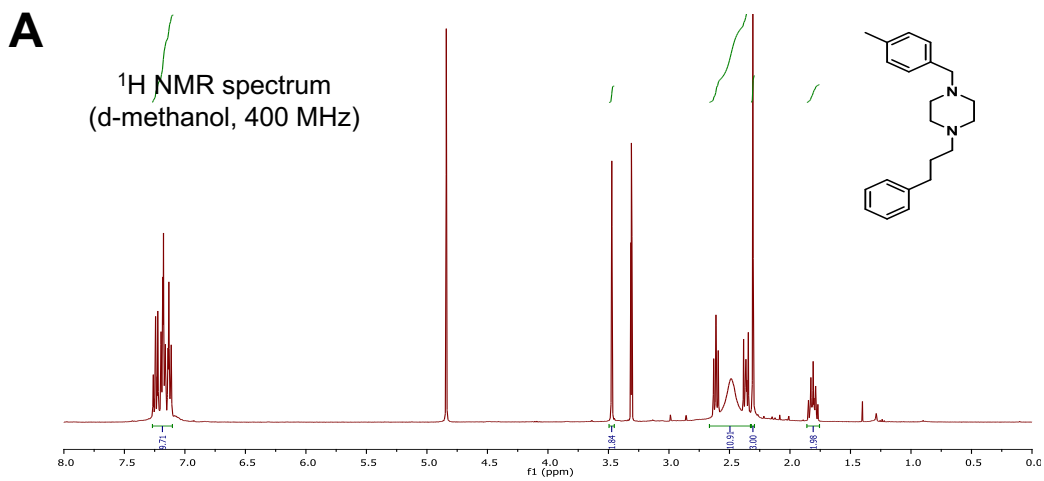
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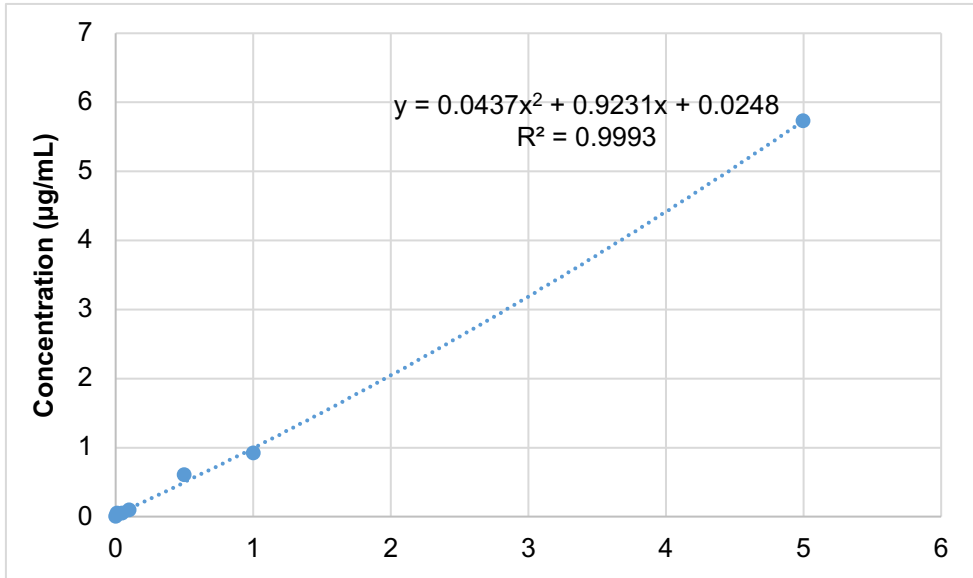


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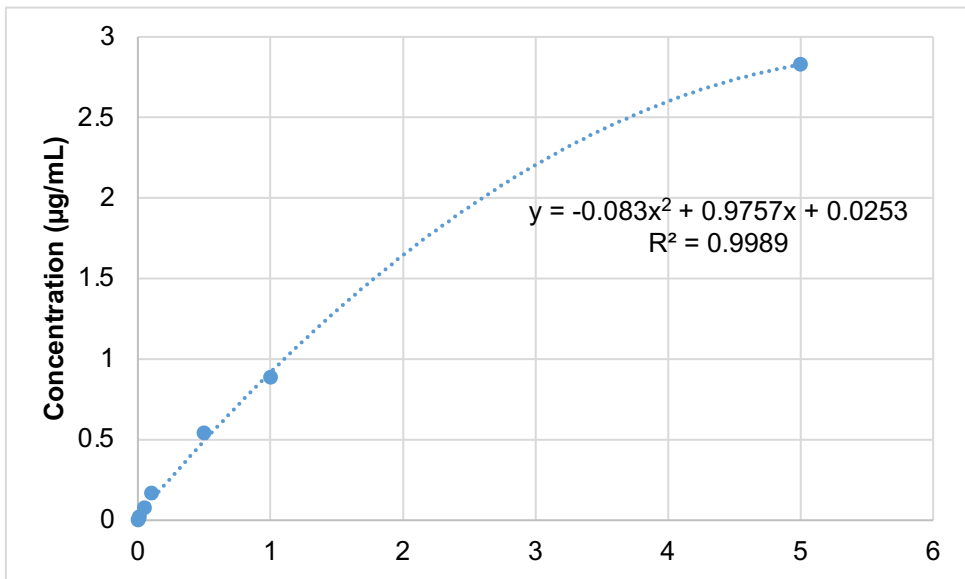




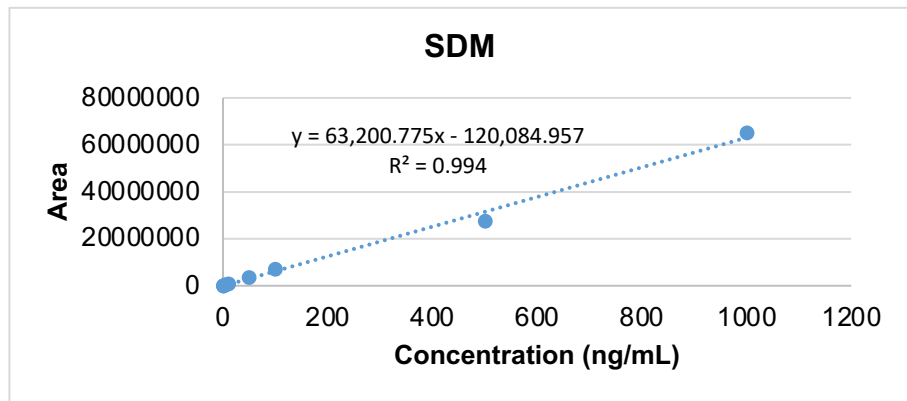
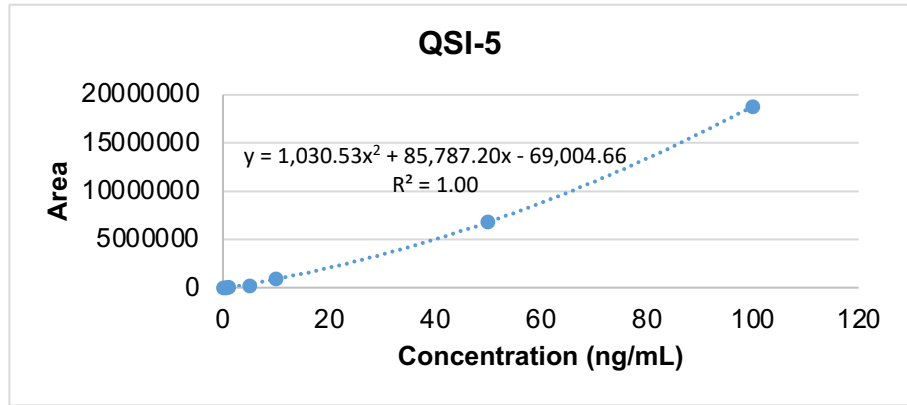
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