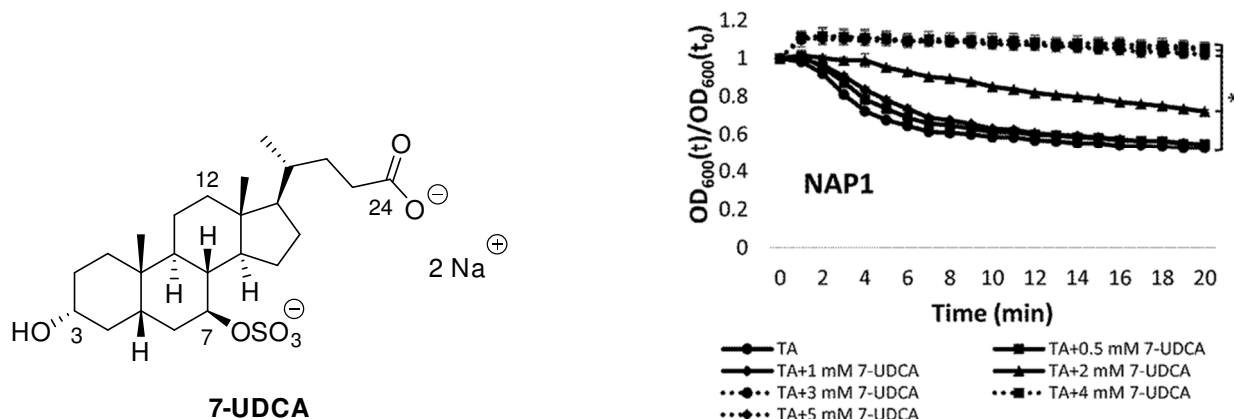**Supporting Information:**

Synthesis and Biological Evaluation of Bile Acid Analogs Inhibitory to

***Clostridium difficile* Spore Germination**

Kristen L. Stoltz,[†] Raymond Erickson,[§] Christopher Staley,[§] Alexa R. Weingarden,[§,‡] Erin Romens,[§] Clifford J. Steer,[♦] Alexander Khoruts,[*, §,⊥,◊] Michael J. Sadowsky,[*,§,ø] Peter I. Dosa[*,†]
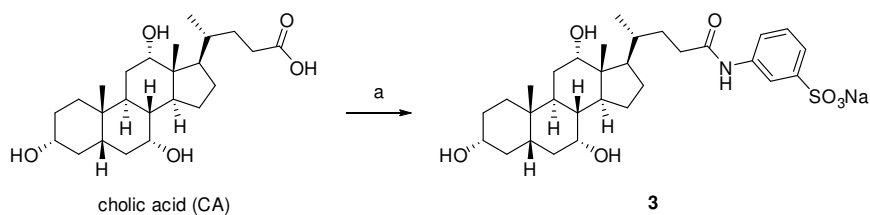
Optical Density Graph for 7-UDCA (4b)

Figure S1: The relative OD_{600} of spores after 20 min. exposure to 2 mM TCA with 0 mM, 0.5 mM, 1 mM, 2 mM, 3 mM, 4 mM, or 5 mM 7-UDCA. *$P < 0.01$. TCA indicates taurocholate; 7-UDCA, C7-sulfated UDCA.

# Synthesis of compound 3 and CDCA analog 11a.

Scheme S1: Synthesis of 3.[a]

[a]Reagents and conditions: HATU, TEA, 3-aminobenzenesulfonic acid, DCM, THF, then ion-exchange using Na⁺-Dowex resin.

Sodium 3-((R)-4-((3R,5S,7R,8R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamido)benzenesulfonate (3): To a solution of cholic acid (1.00 g, 2.45 mmol) in a mixture of DCM (5.4 mL) and THF

(2.7 mL) was added HATU (0.977 g, 2.57 mmol) and 3-aminobenzenesulfonic acid (0.424 g, 2.45 mmol). The reaction was stirred at room temperature for 18 h. and the solvent was removed under reduced pressure. A portion of the material was dissolved in DMSO (0.5 mL) and 1M triethylammonium acetate buffer (0.1 mL) and purified by flash column chromatography (5-100% 20 mM triethylammonium acetate buffer in acetonitrile in water as eluent, C_{18} column) to yield a white solid after lyophilization. To prepare the sodium salt of **3**, a 1 cm wide column was filled with 12 cm of Dowex-50 WX2 (50-100 mesh, strongly acidic) ion-exchange resin. The column was prepared by sequentially washing with 1:1 acetonitrile/water, ~1 M aqueous $NaHCO_3$ (caution: gas evolution), water, and finally 1:1 acetonitrile/water. The reaction product was dissolved in 1:1 acetonitrile/water and loaded onto the column, which was eluted with 1:1 acetonitrile/water. The fractions containing the product were lyophilized to furnish **3** as an off-white solid. ^1H NMR (400 MHz, CD_3OD) δ 7.98 (t, J = 1.9 Hz, 1H), 7.78 (dd, J = 8.2, 2.1 Hz, 1H), 7.56 (d, J = 7.7 Hz, 1H), 7.37 (t, J = 8.0 Hz, 1H), 3.97 (d, J = 3.1 Hz, 1H), 3.81 (q, J = 3.0 Hz, 1H), 3.38 (tt, J = 11.3, 4.4 Hz, 1H), 2.46 (ddd, J = 14.2, 9.8, 4.4 Hz, 1H), 2.30 (tdd, J = 18.2, 10.4, 6.2 Hz, 3H), 2.13 – 1.25 (m, 18H), 1.19 – 1.10 (m, 1H), 1.08 (d, J = 5.9 Hz, 3H), 1.04 – 0.94 (m, 1H), 0.93 (s, 3H), 0.73 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.3, 146.8, 140.0, 129.7, 122.7, 122.3, 118.6, 74.0, 72.9, 69.0, 48.0, 47.5, 43.2, 43.0, 41.0, 40.4, 36.9, 36.5, 35.9, 35.8, 35.0, 33.1, 31.2, 29.6, 28.7, 27.8, 24.2, 23.2, 17.8, 13.0. HRMS (ESI): m/z calcd. $C_{30}H_{44}NNa_2O_7S$ (M+Na)$^+$ 608.2634 found 608.2647.

Scheme S2: Synthesis of 11a.[a]
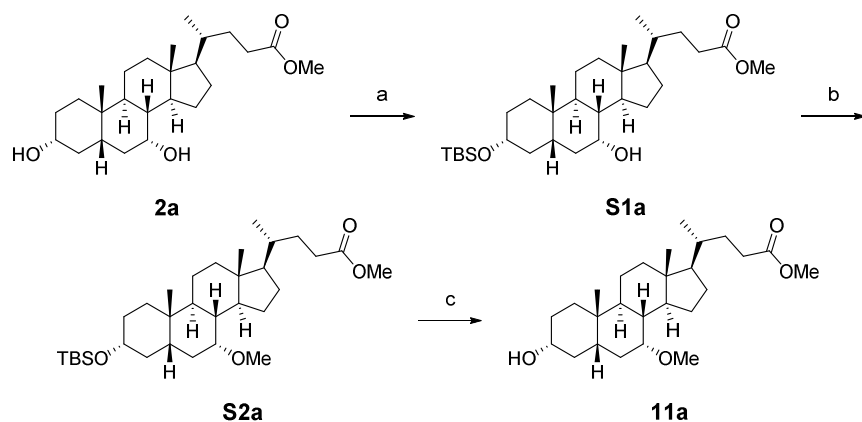
[a]Reagents and conditions: (a) TBSCl, imidazole, DMF; (b) MeOTf, 2,6-lutidine, DCM; (c) TBAF, THF.

Methyl (R)-4-((3R,5R,7R,8R,9S,10S,13R,14S,17R)-3-((tert-butyldimethylsilyl)oxy)-7-hydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (S1a): To a solution of **2a** (0.495 g, 1.21 mmol) in DMF (1 mL) was added imidazole (0.398 g, 5.84 mmol) and TBSCl (0.229 g, 1.52 mmol). The reaction mixture stirred at room temperature for 1 h. and was poured into a separatory funnel containing ice water. The aqueous layer was extracted with EtOAc (3 x), and the combined organic layers were washed with water (5 x), dried over MgSO$_4$, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (0-25% EtOAc in DCM as eluent) to obtain the silyl ether as a white foam (0.471 g, 74 % yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.84 (p, J = 3.2 Hz, 1H), 3.67 (s, 3H), 3.44 (tt, J = 10.9, 4.5 Hz, 1H), 2.36 (ddd, J = 15.2, 10.1, 5.1 Hz, 1H), 2.30 – 2.14 (m, 2H), 2.02 – 1.02 (m, 22H), 0.97 (dd, J = 14.4, 3.7 Hz, 1H), 0.93 (d, J = 6.4 Hz, 3H), 0.89 (s, 3H), 0.89 (s,9H), 0.66 (s, 3H), 0.05 (s, 6H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 174.9, 73.0, 68.7, 55.9, 51.6, 50.6, 42.8, 41.7, 40.2,

39.8, 39.6, 35.7, 35.5, 35.2, 34.8, 32.9, 31.2, 31.2, 31.1, 28.3, 26.1, 26.1, 26.1, 23.9, 23.0, 20.7, 18.4, 18.4, 11.9, -4.4, -4.5.

Methyl (R)-4-((3R,5R,7R,8R,9S,10S,13R,14S,17R)-3-((tert-butyldimethylsilyl)oxy)-7-methoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (S2a): To a solution of **S1α** (0.471 g, 0.904 mmol) and 2,6-lutidine (0.41 ml, 1.8 mmol) in DCM (9 ml) was added methyl triflate (0.11 ml, 0.95 mmol) and the reaction mixture stirred at room temperature for 12 h. The reaction mixture was quenched by the addition of water and stirred for 15 min. The aqueous layer was extracted with DCM (3 x 15 mL) and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel (0-20% EtOAc in DCM as eluent) to obtain **S2a** (0.057 g, 12% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (s, 3H), 3.42 (tt, J = 10.9, 4.5 Hz, 1H), 3.24 (s, 3H), 3.17 (q, J = 2.9 Hz, 1H), 2.35 (ddd, J = 15.2, 10.2, 5.0 Hz, 1H), 2.29 – 2.12 (m, 2H), 1.99 – 0.93 (m, 22H), 0.94 – 0. 92 (m, 1H), 0.91 (d, J = 6.3 Hz, 3H), 0.88 (s, 3H), 0.88 (s, 9H), 0.63 (s, 3H), 0.04 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 77.5, 77.4, 73.0, 55.9, 55.8, 51.6, 50.4, 42.6, 42.2, 39.7, 39.6, 38.8, 35.7, 35.5, 35.1, 33.8, 31.2, 31.2, 31.1, 28.3, 28.0, 26.1, 26.1, 26.1, 23.8, 23.1, 21.0, 18.4, 18.4, 11.8, -4.3.

Methyl (R)-4-((3R,5S,7R,8R,9S,10S,13R,14S,17R)-3-hydroxy-7-methoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (11a): To a solution of **S2α** (0.052 g, 0.097 mmol) in THF (1 mL) was added TBAF (0.11 ml, 0.11 mmol). The reaction stirred at room temperature for 24 h. and was concentrated under reduced pressure. The crude material was purified by flash column chromatography on silica gel (33% EtOAc in DCM) to obtain **11α** (0.030 g, 73% yield) as a colorless oil. ^{1}H NMR (400 MHz, DMSO-D_6) δ 4.34 (d, J = 4.8 Hz, 1H), 3.57 (s, 3H), 3.22 – 3.12 (m, 2H), 3.17 (s, 3H), 2.33 (ddd, J = 15.2, 9.6, 5.2 Hz, 1H),

2.20 (ddd, J = 15.8, 9.3, 6.9 Hz, 1H), 2.06 – 0.96 (m, 23H), 0.87 (d, J = 6.6 Hz, 3H), 0.85 (s, 3H), 0.60 (s, 3H). ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.67 (s, 3H), 3.44 (tt, J = 10.8, 4.6 Hz, 1H), 3.26 (s, 3H), 3.19 (d, J = 2.9 Hz, 1H), 2.35 (ddd, J = 15.3, 10.2, 5.0 Hz, 1H), 2.30 – 2.07 (m, 2H), 2.02 – 0.95 (m, 23H), 1.00 – 0.93 (m, 1H), 0.92 (d, J = 7.4 Hz, 3H), 0.91 (s, 3H), 0.64 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 174.9, 77.7, 77.4, 72.2, 56.1, 55.9, 51.6, 50.4, 42.6, 42.1, 39.7, 39.5, 38.7, 35.5, 35.5, 35.1, 33.9, 31.1, 31.0, 28.3, 28.0, 23.8, 23.0, 21.0, 18.4, 11.8. TLC-MS (ESI): m/z calcd. C$_{25}$H$_{40}$O$_3$ (M-CH$_3$OH)$^-$ 388.3, found 388.3.

Optical Density Graphs for Bile Acid Analogs

$OD_{600}(t)/OD_{600}(t_0) = OD_{600}$ normalized to the initial OD_{600} (relative OD_{600}). Data represent mean ± SEM for all graphs. Optical density data for **1b** was previously reported in Weingarden et al.[1] In some examples, compound **20b** was used as a positive control.
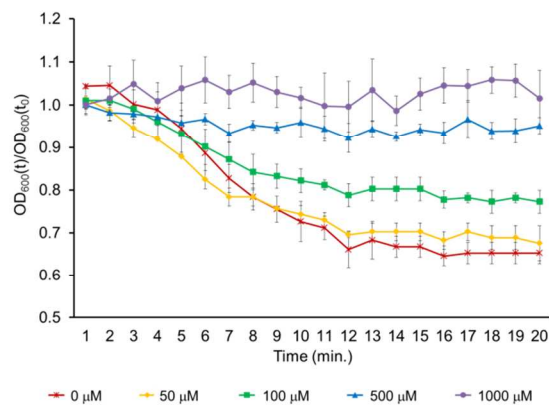

1a
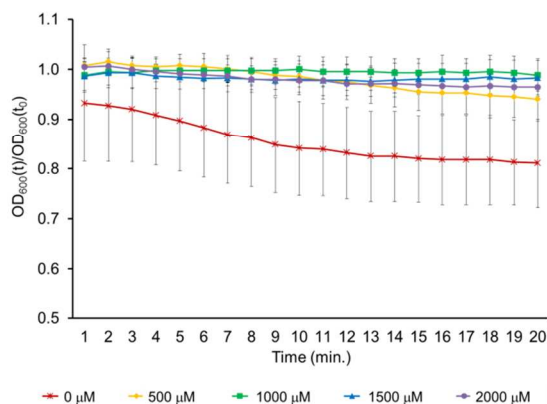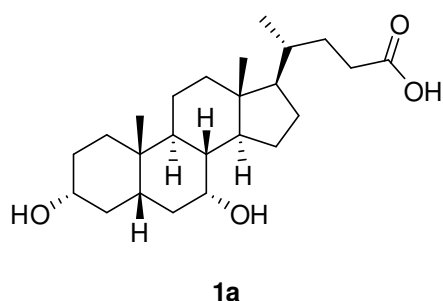
Figure S2: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 500 µM, 1000 µM, 1500 µM, or 2000 µM **1a**.
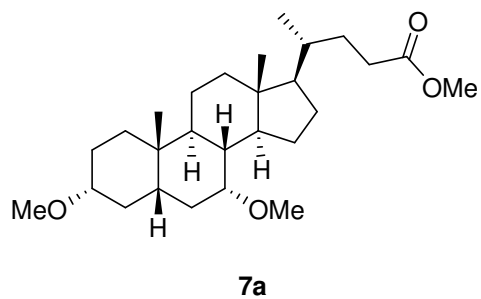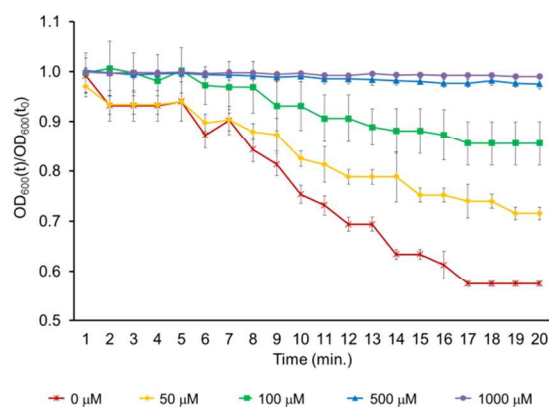
5a
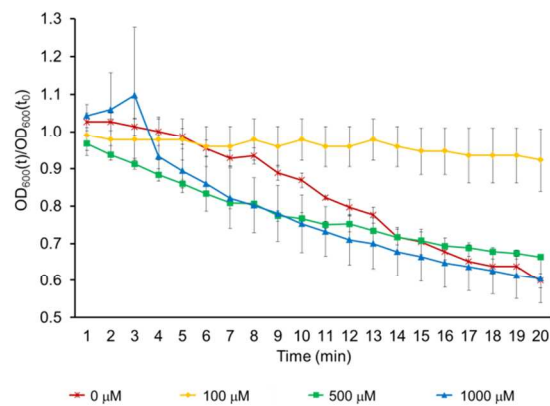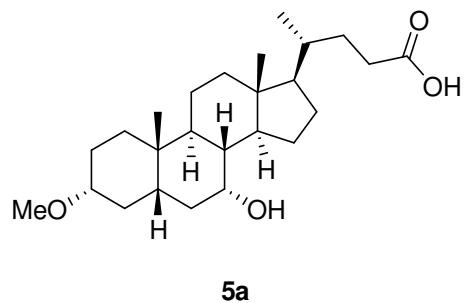
Figure S3: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 100 µM, 500 µM, or 1000 µM **5a**.

5b
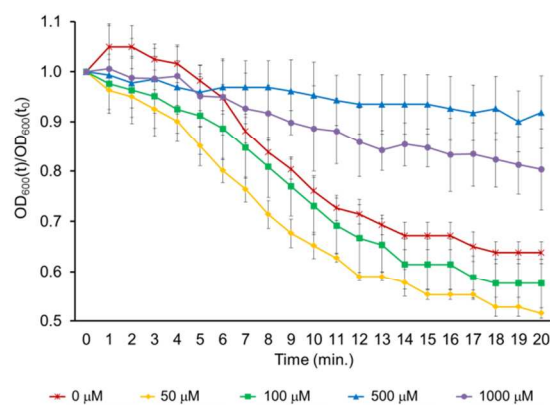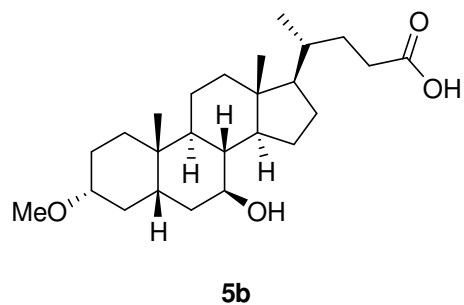
Figure S4: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM or 1000 µM **5b**.

6b
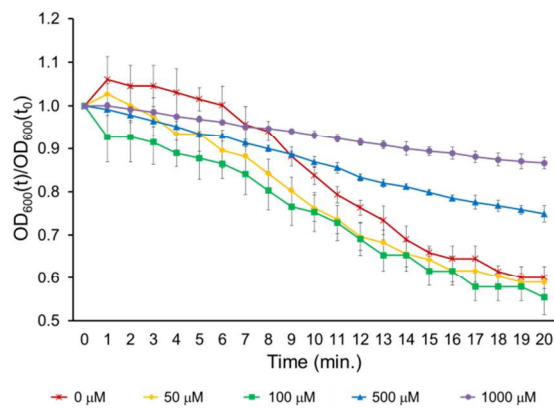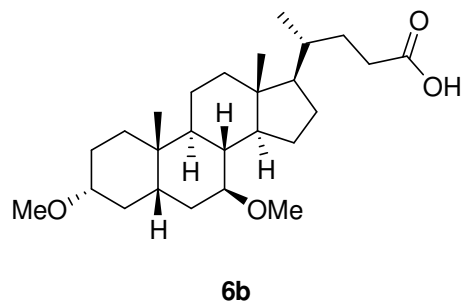
Figure S5: The relative OD$_{600}$ of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM or 1000 µM **6b**.
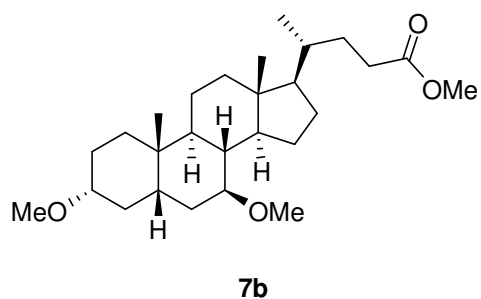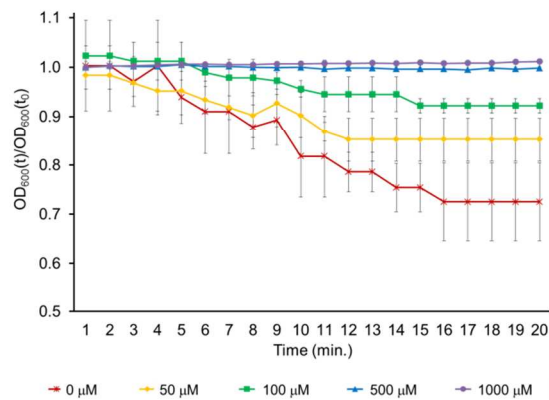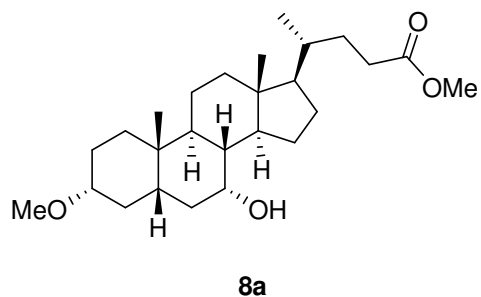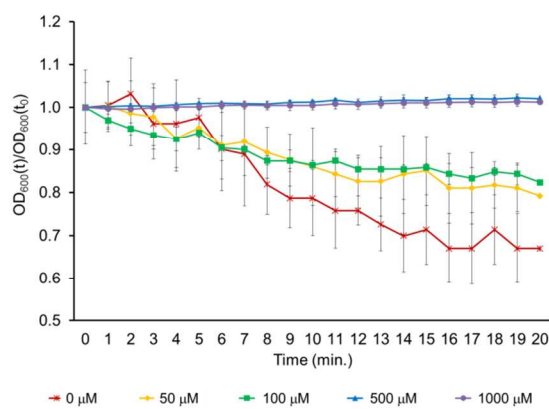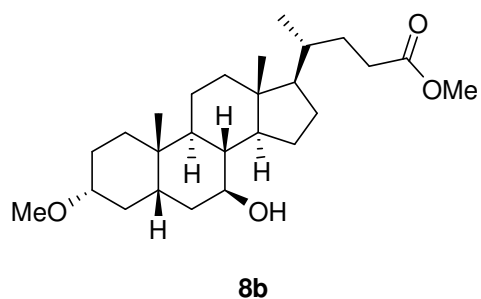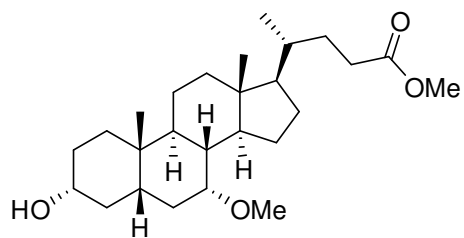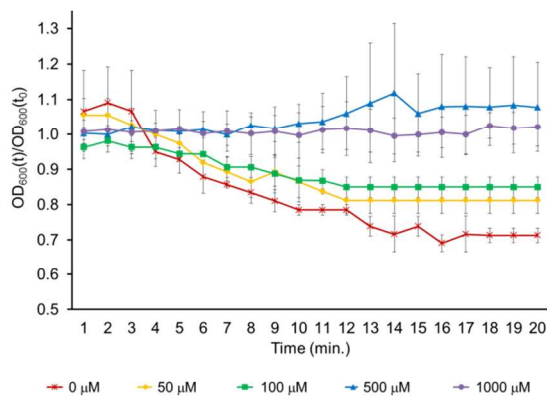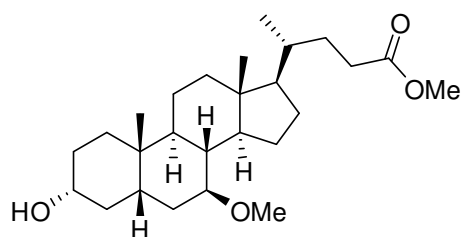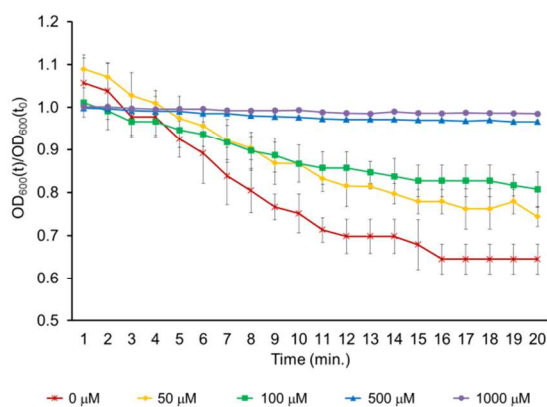

10b
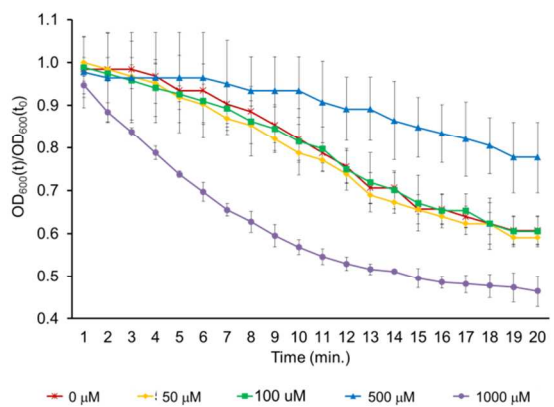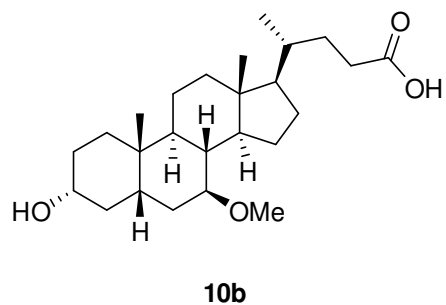
Figure S6: The relative OD$_{600}$ of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM or 1000 µM **10b**.
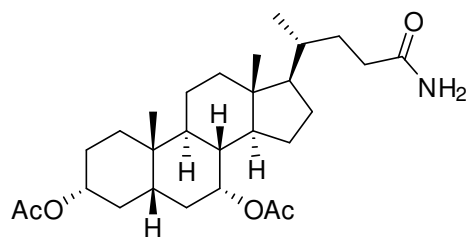
2a
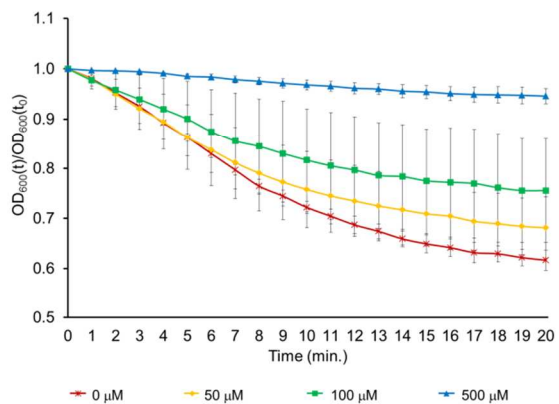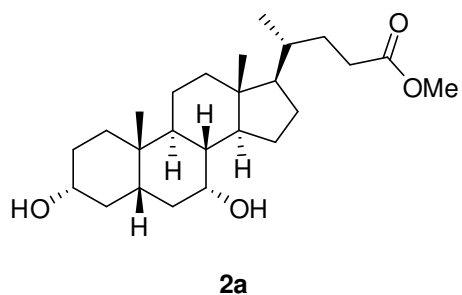
Figure S7: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, or 500 µM **2a**.

2b
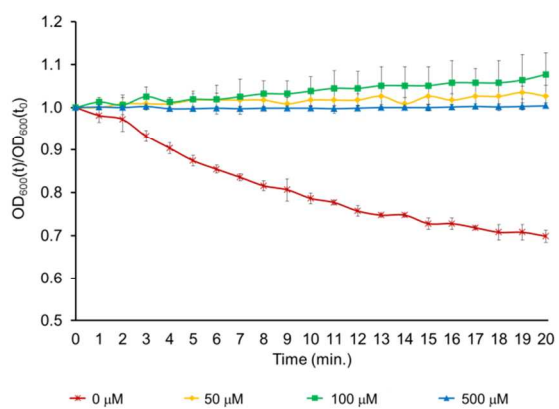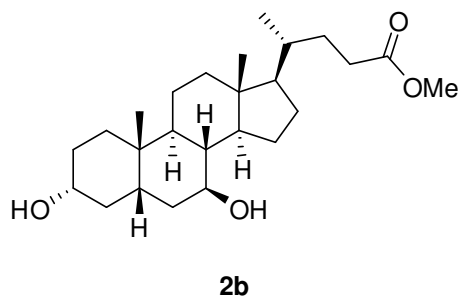
Figure S8: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, or 500 µM **2b**.

7a

Figure S9: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM, or 1000 µM **7a**.
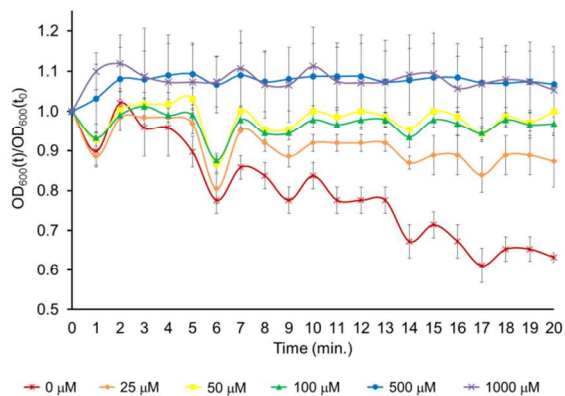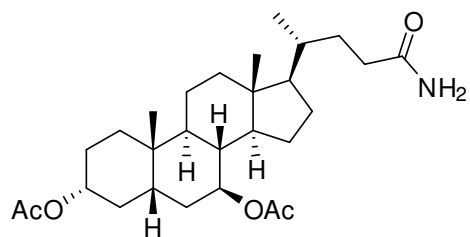

7b

Figure S10: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM, or 1000 µM **7b**.
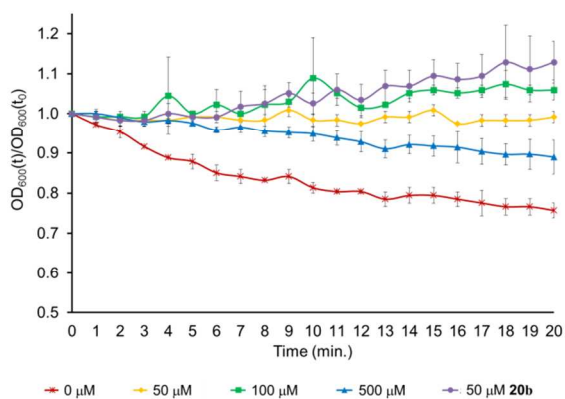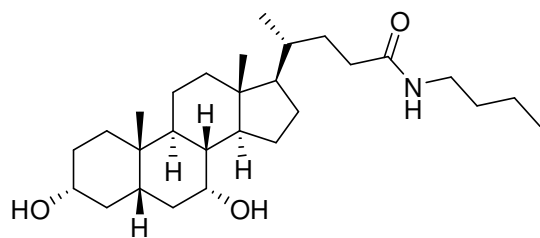
8a

Figure S11: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM, or 1000 µM **8a**.
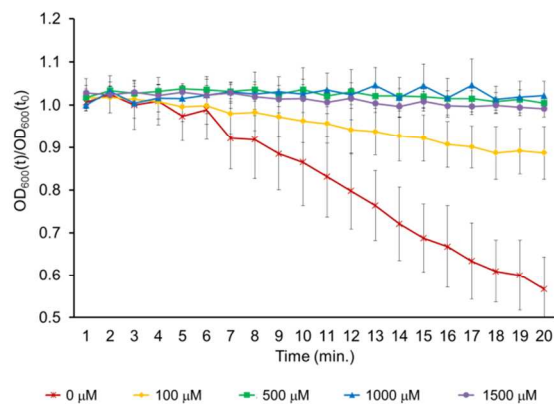

8b

Figure S12: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM, or 1000 µM **8b**.

11a

Figure S13: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM, or 1000 µM **11a**.
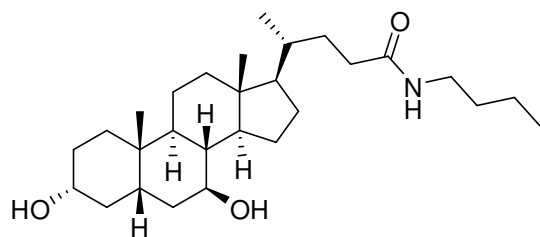

11b

Figure S14: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM, or 1000 µM **11b**.

16a

Figure S15: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 25 µM, 50 µM, 100 µM, 500 µM, or 1000 µM **16a**.

16b

Figure S16: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM **16b** 50 µM **16b**, 100 µM **16b**, 500 µM **16b**, or 50 µM **20b**.
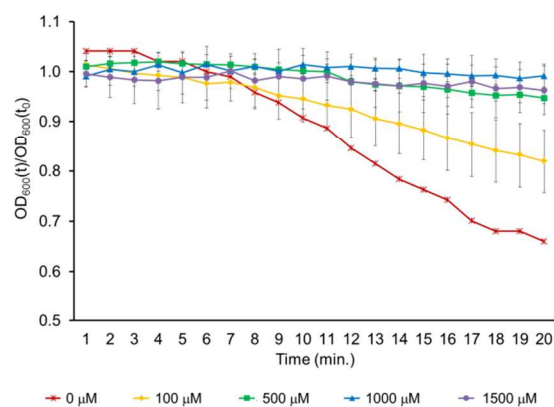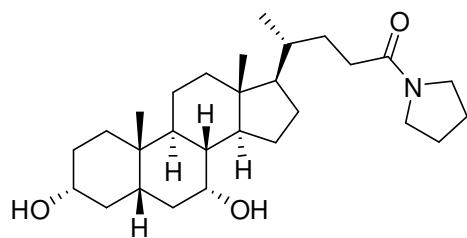
17a

Figure S17: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 100 µM, 500 µM, 1000 µM, or 1500 µM **17a**.
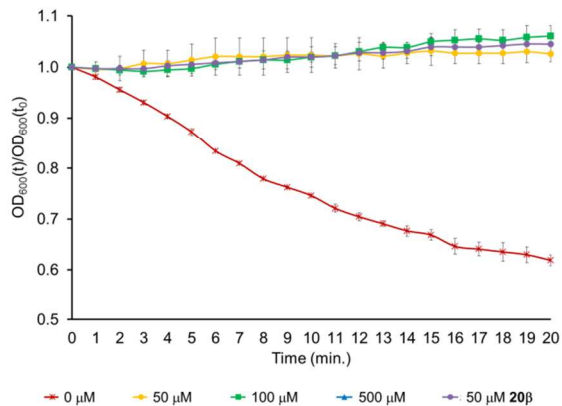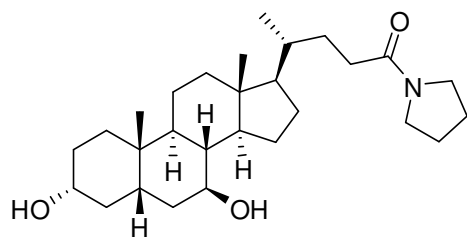

17b

Figure S18: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 100 µM, 500 µM, 1000 µM, or 1500 µM **17b**.
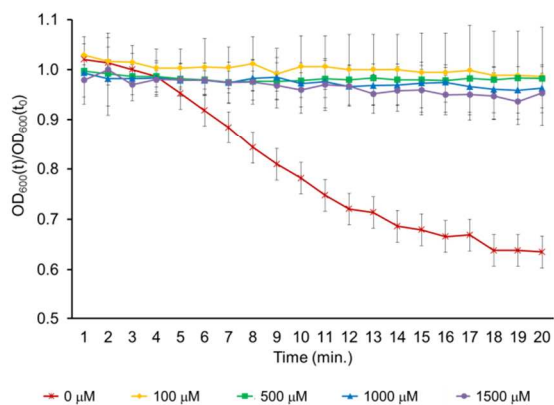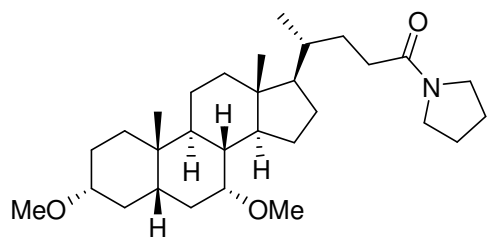
18a

Figure S19: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM **18a**, 50 µM **18a**, 100 µM **18a**, 500 µM **18a**, or 50 µM **20b**.
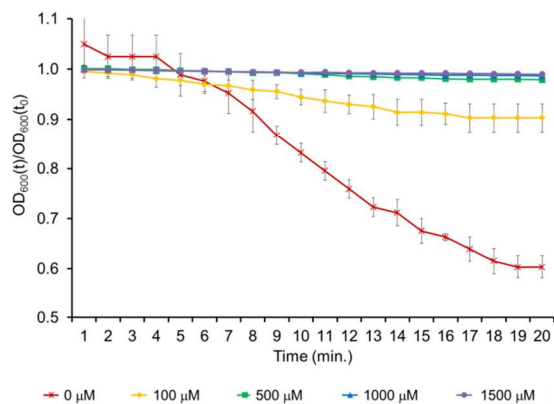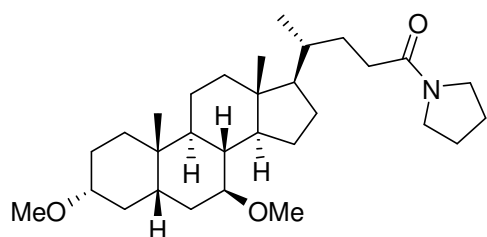

18b

Figure S20: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 100 µM, 500 µM, 1000 µM, or 1500 µM **18a**.
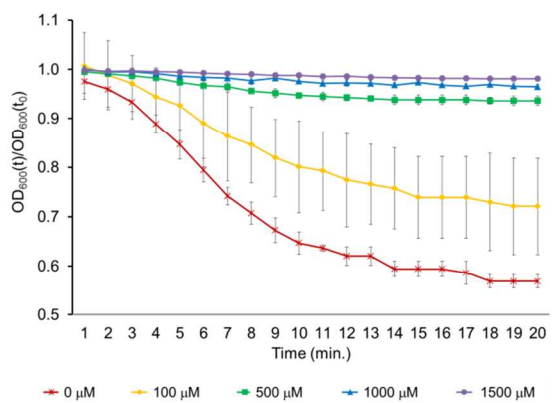
19a

Figure S21: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 100 µM, 500 µM, 1000 µM, or 1500 µM **19a**.
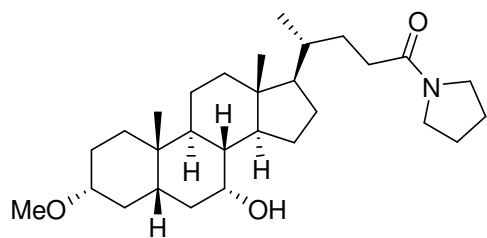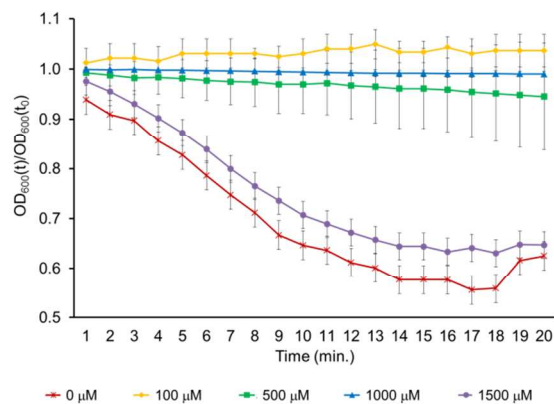

19b

Figure S22: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 100 µM, 500 µM, 1000 µM, or 1500 µM **19b**.

20a

Figure S23: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 100 µM, 500 µM, 1000 µM, or 1500 µM **20a**.
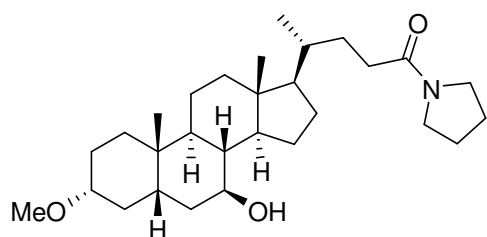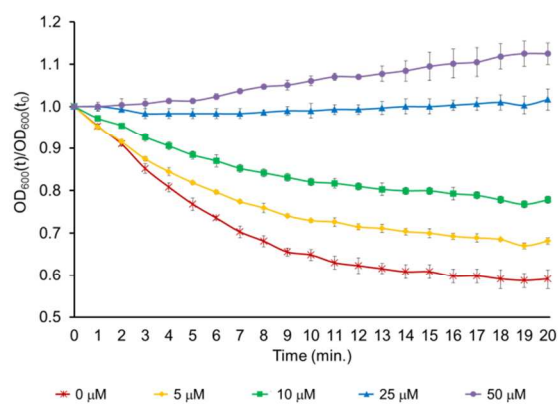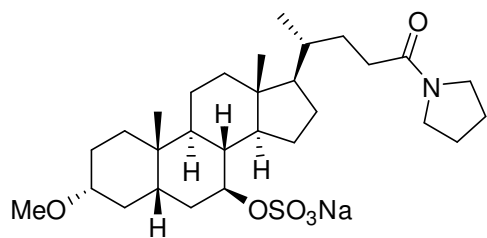

20b

Figure S24: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 5 µM, 10 µM, 25 µM, or 50 µM **20b**.

21b
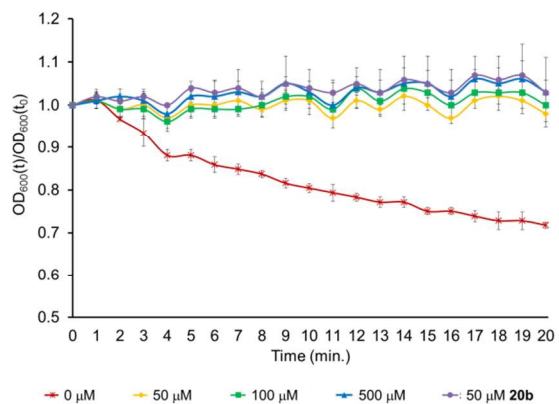
Figure S25: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM **21b**, 50 µM **21b**, 100 µM **21b**, 500 µM **21b**, or 50 µM **20b**.
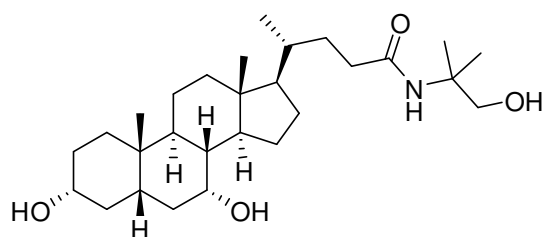
22a

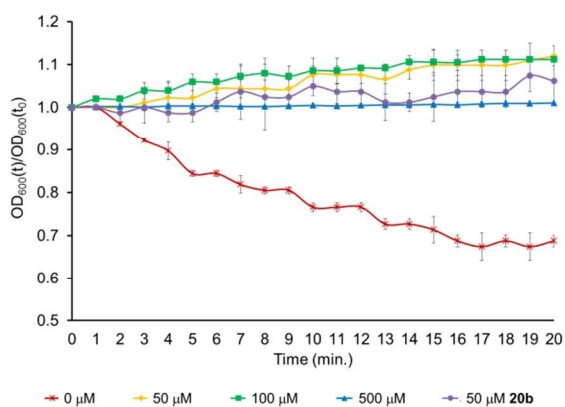**Figure S26:** The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM **22a**, 50 µM **22a**, 100 µM **22a**, 500 µM **22a**, or 50 µM **20b**.

24a

Figure S27: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM **24a**, 50 µM **24a**, 100 µM **24a**, 500 µM **24a**, or 50 µM **20b**.

24b

Figure S28: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM, or 1000 µM **24b**.
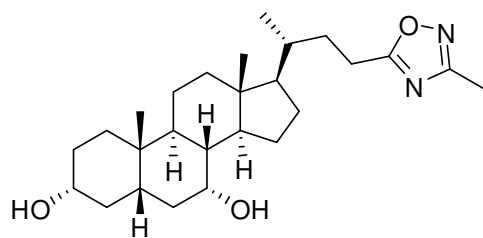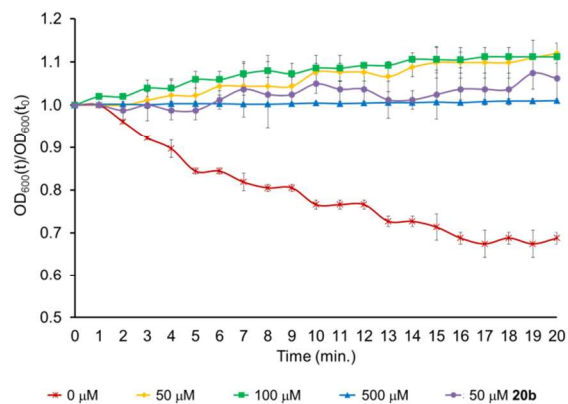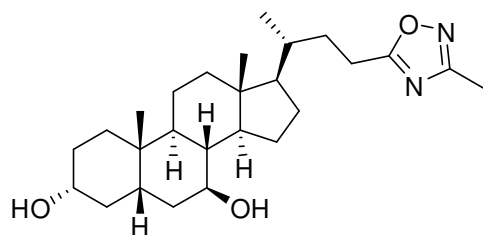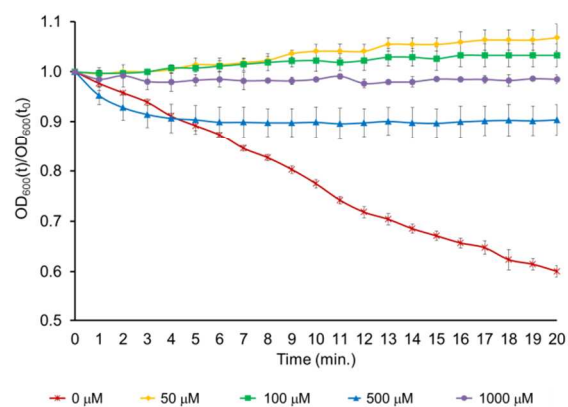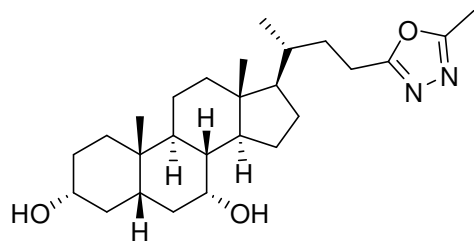
27a

Figure S29: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM **27a**, 50 µM **27a**, 100 µM **27a**, 500 µM **27a**, or 50 µM **20b**.

27b

Figure S30: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 50 µM, 100 µM, or 500 µM **27b**.
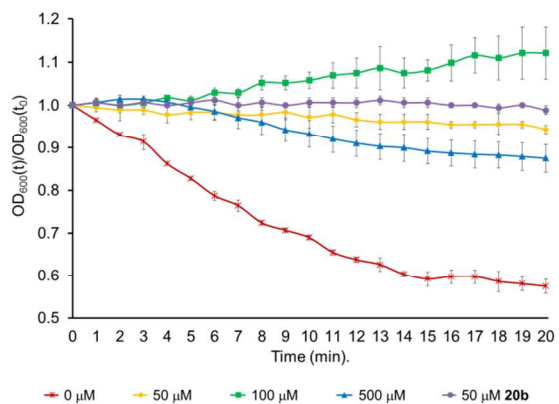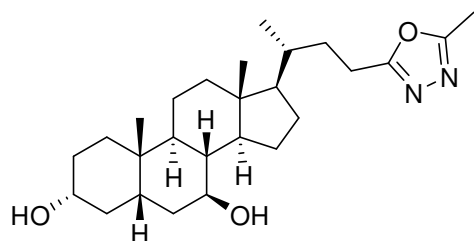
31b
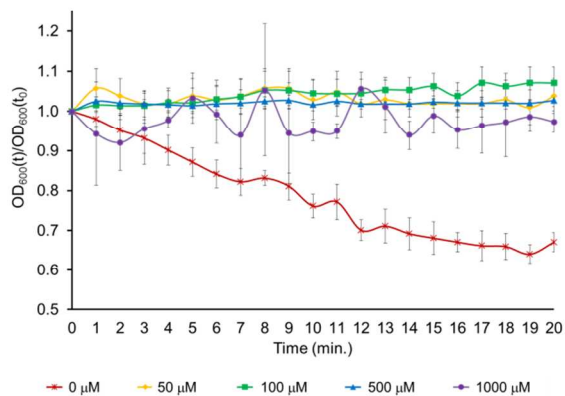
Figure S31: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM, or 1000 µM **31b**.
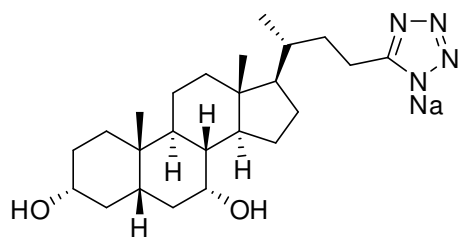
35a

Figure S32: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 5 µM, 10 µM, 25 µM, or 50 µM **35a**.

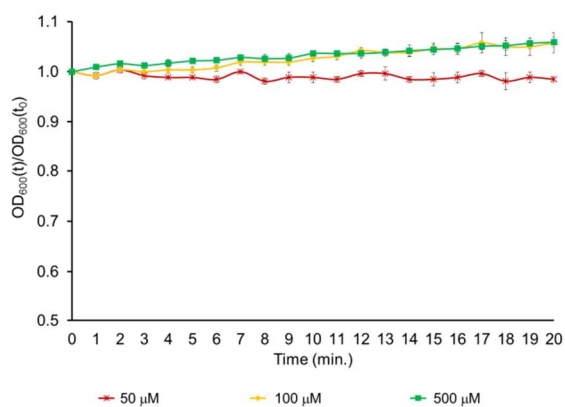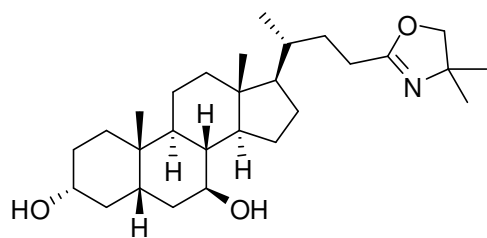**35b**
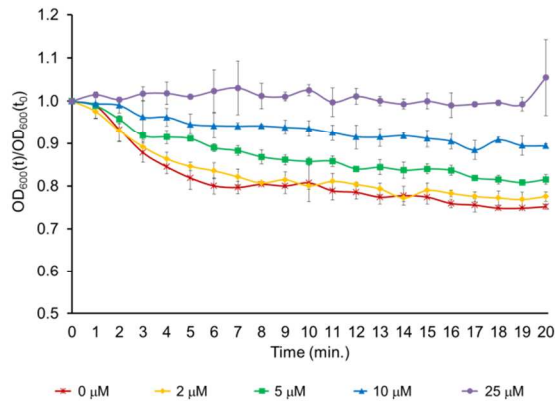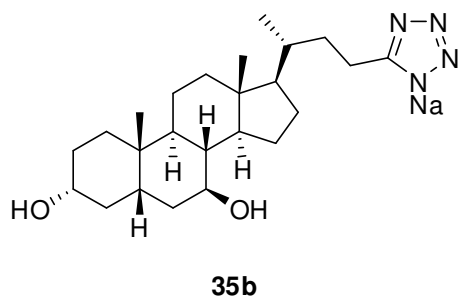
Figure S33: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 2 µM, 5 µM, 10 µM, or 25 µM **35b**.

37a
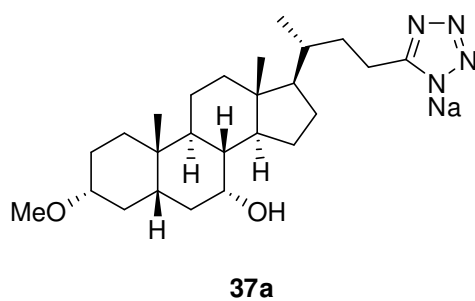
Figure S34: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 2 µM, 5 µM, 10 µM, or 25 µM **37a**.

37b
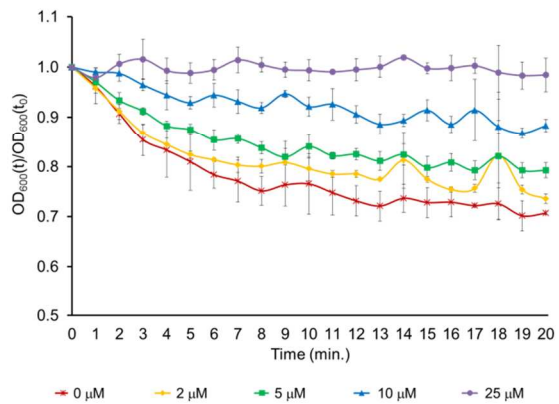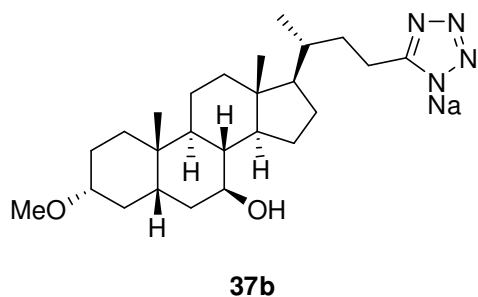
Figure S35: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 2 µM, 5 µM, 10 µM, or 25 µM **37b**.

38a
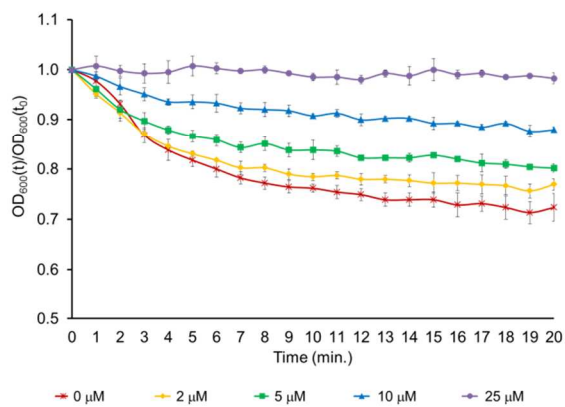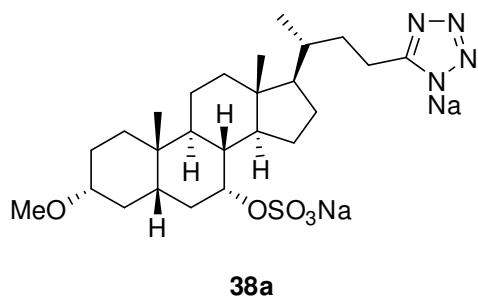
Figure S36: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 2 µM, 5 µM, 10 µM, or 25 µM **38a**.

S23

38b
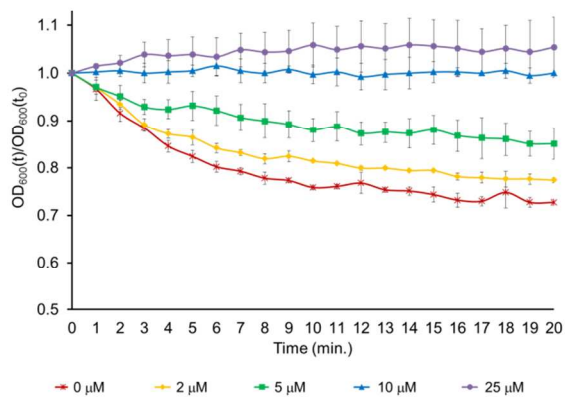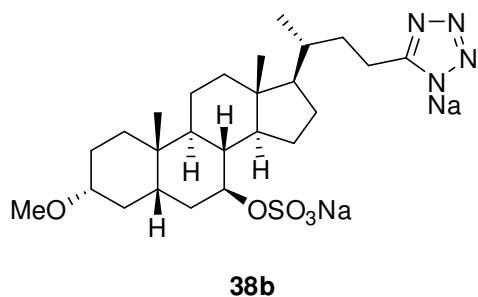
Figure S37: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 2 µM, 5 µM, 10 µM, or 25 µM **38b**.

3
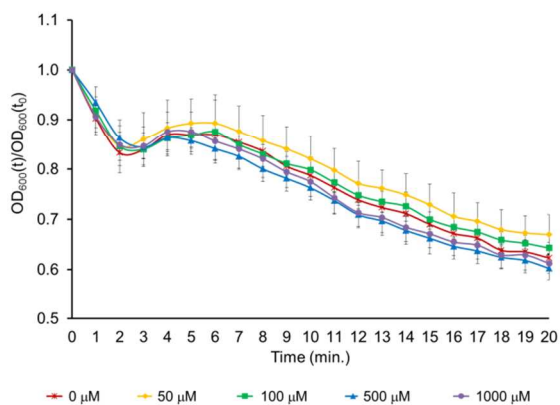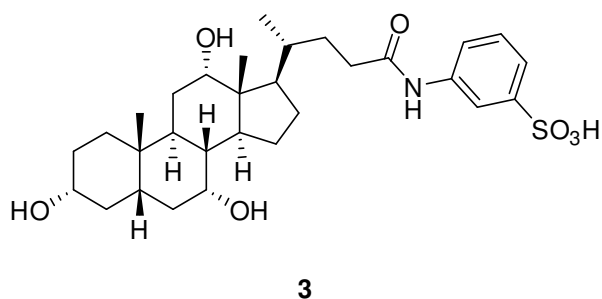
Figure S38: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM, or 1000 µM **3**.
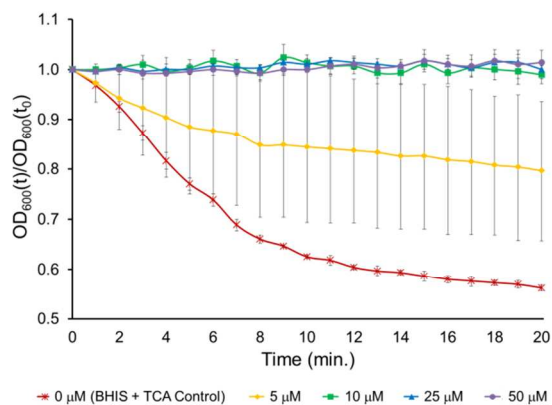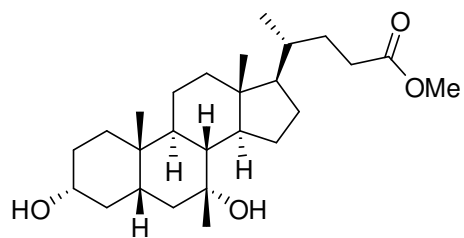
13
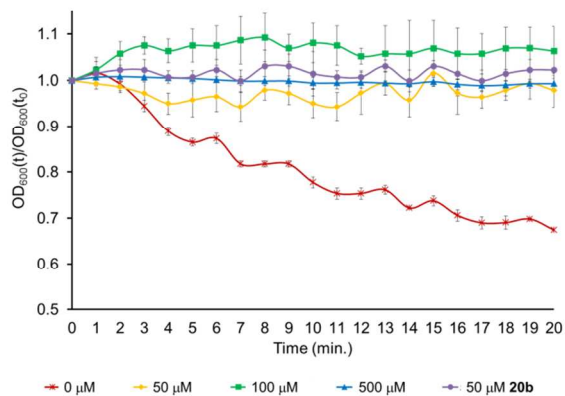
Figure S39: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM **13**, 50 µM **13**, 100 µM **13**, 500 µM **13**, or 50 µM **20b**.
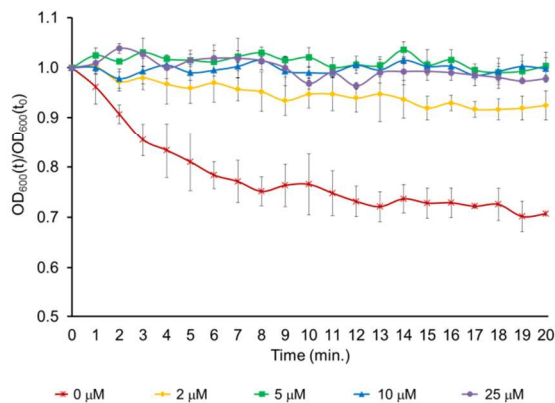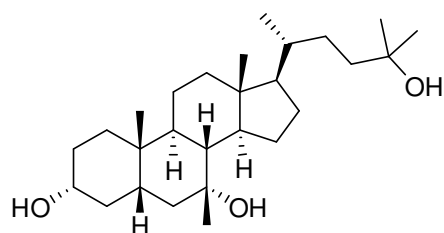

14
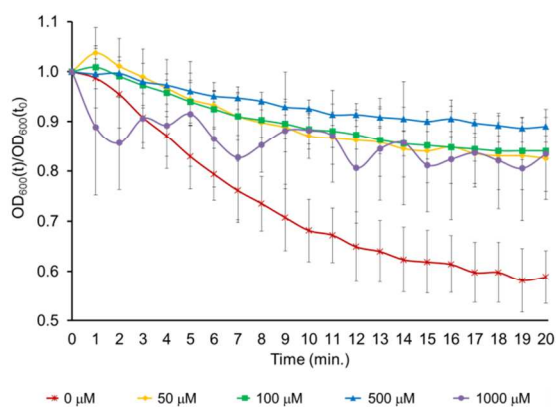
Figure S40: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 50 µM, 100 µM, 500 µM, or 1000 µM **14**.

39
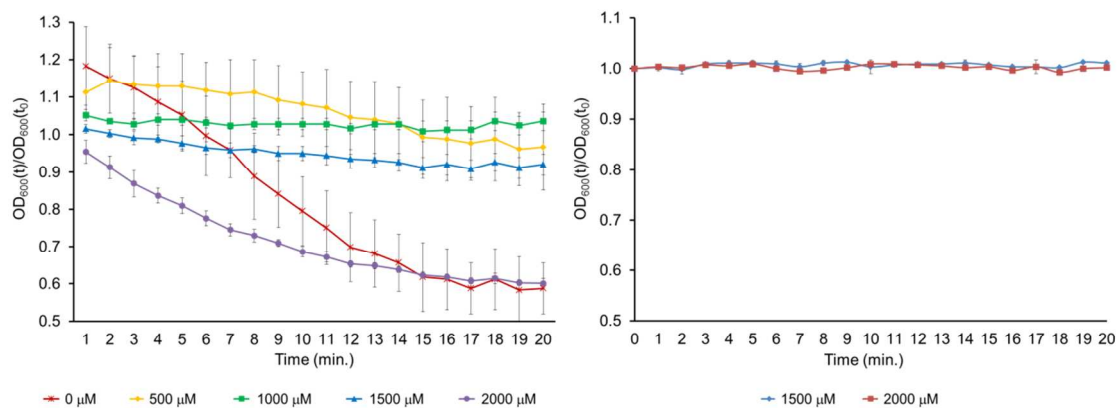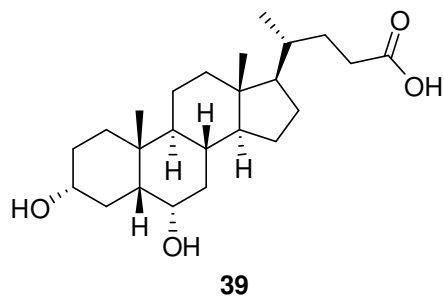
Figure S41: (Left): The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 500 µM, 1000 µM, 1500 µM, or 2000 µM **39**. (Right): The relative OD_{600} of spores in BHIS after 20 min. exposure 1500 µM or 2000 µM **39**.

40
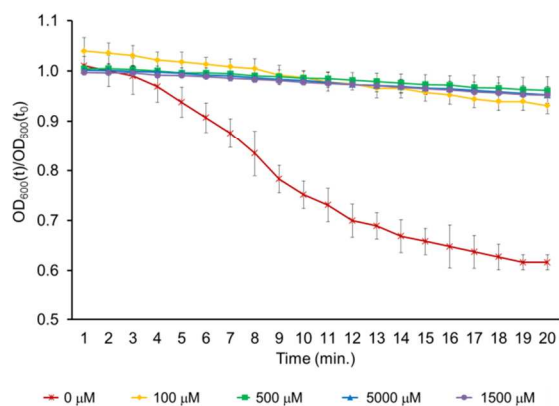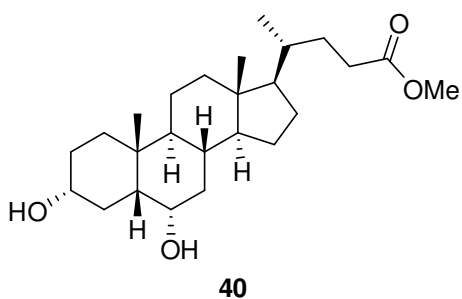
Figure S42: The relative OD_{600} of spores in BHIS after 20 min. exposure to 2000 µM TCA and 0 µM, 500 µM, 1000 µM, 1500 µM, or 2000 µM **40**.

Phase-Contrast Microscopy Assay Data for Compounds at 10 µM

Example graphs of one spore count experiment for each compound listed in Table 2. Each experiment was repeated three times.
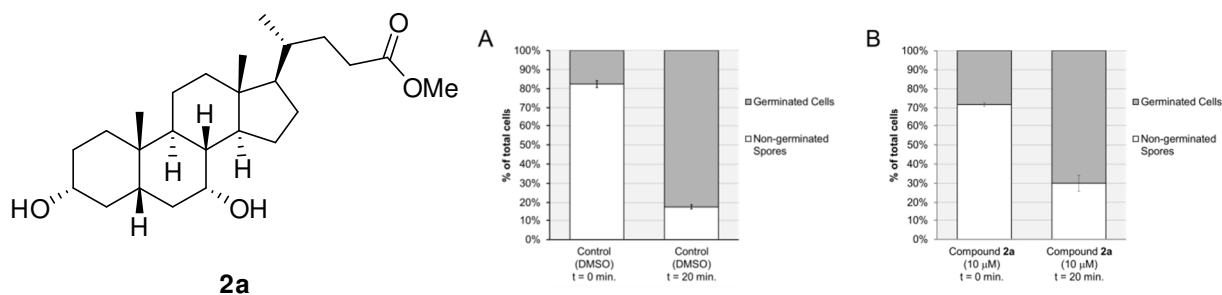
Figure S43: A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 18% to 82% over 20 min. B) The number of germinated spores rose from 28% to 70% over 20 min. in the presence of 10 µM of **2a**.
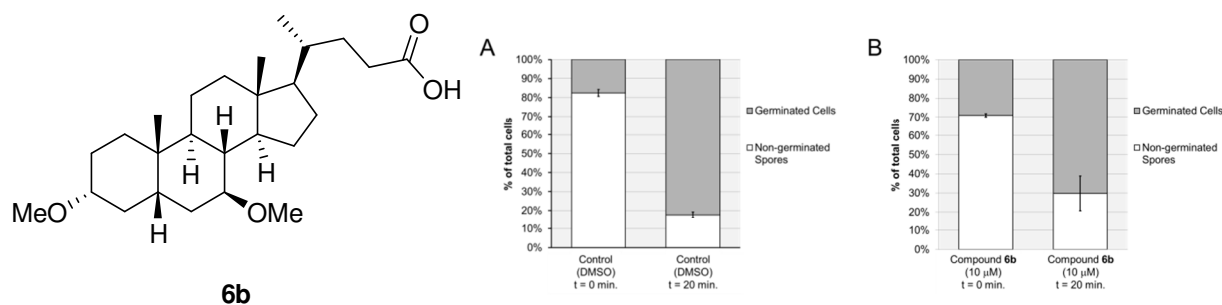
Figure S44: A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 18% to 82% over 20 min. B) The number of germinated spores rose from 29% to 70% over 20 min. in the presence of 10 µM of **6b**.

20b
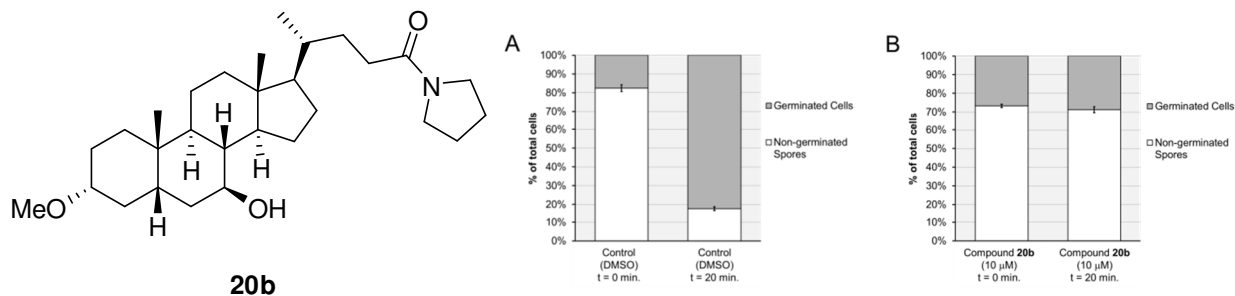
Figure S45: A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 18% to 82% over 20 min. B) The number of germinated spores rose from 28% to 29% over 20 min. in the presence of 10 µM of **20b**.

21b

Figure S46: A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 18% to 82% over 20 min. B) The number of germinated spores rose from 27% to 31% over 20 min. in the presence of 10 µM of **21b**.

Figure S47: A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 20% to 71% over 20 min. B) The number of germinated spores rose from 18% to 22% over 20 min. in the presence of 10 µM of **35a**.

Figure S48: A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 20% to 71% over 20 min. B) The number of germinated spores rose from 25% to 32% over 20 min. in the presence of 10 µM of **35b**.

37a

Figure S49: A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 20% to 71% over 20 min. B) The number of germinated spores rose from 20% to 25% over 20 min. in the presence of 10 µM of **37a**.

37b

Figure S50: A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 20% to 71% over 20 min. B) The number of germinated spores rose from 19% to 30% over 20 min. in the presence of 10 µM of **37b**.

Figure S51: A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 20% to 71% over 20 min. B) The number of germinated spores rose from 20% to 42% over 20 min. in the presence of 10 µM of **38a**.
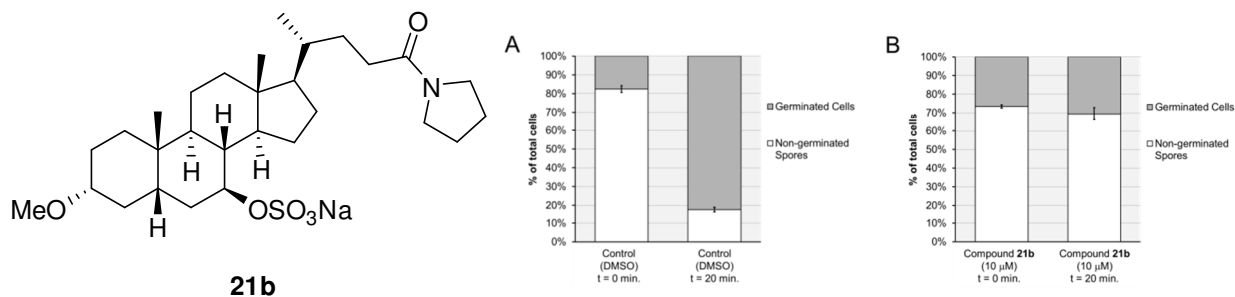

Figure S52: A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 20% to 71% over 20 min. B) The number of germinated spores rose from 22% to 45% over 20 min. in the presence of 10 µM of **38b**.
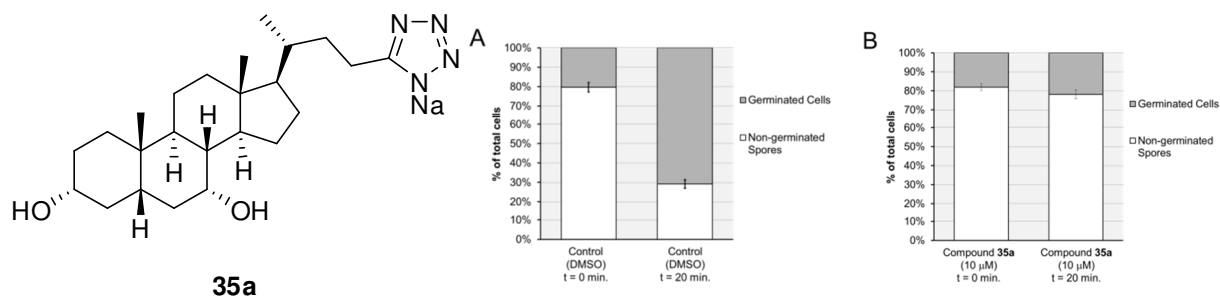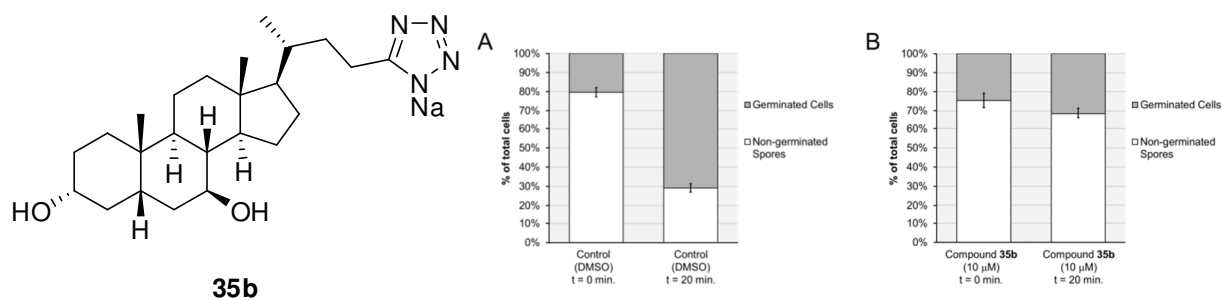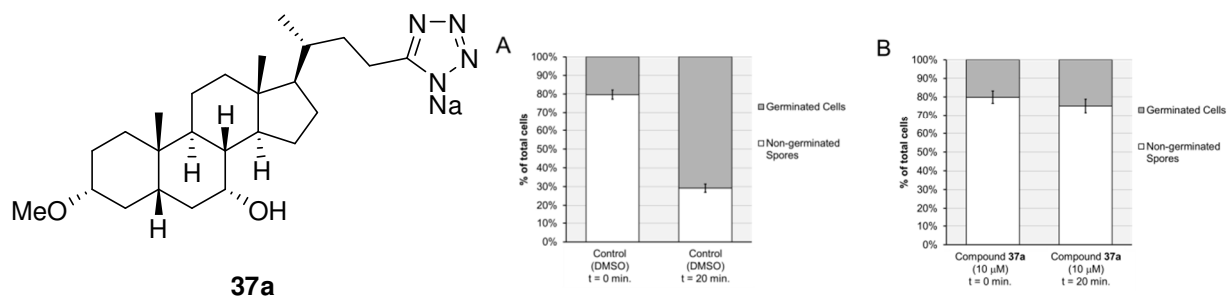
Figure S53: A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 18% to 81% over 20 min. B) The number of germinated spores rose from 25% to 74% over 20 min. in the presence of 10 µM of **3**.

Phase-contrast Microscopy Assay Data for Selected Compounds at 50 µM

Table S1: Percent germination of NAP1 spores in the presence of 2000 µM TCA and 50 µM bile acid analogs after 20 min.

Number	R^1	R^2	R^3	Percent Spore Germination[a]		
				t_0	t_{20}	Relative to control[b]
20β		-OMe	-βOH	16 ± 2	19 ± 2	6 ± 2
21β		-OMe	-βOSO_3Na	17 ± 6	16 ± 6	-1 ± 6

20b
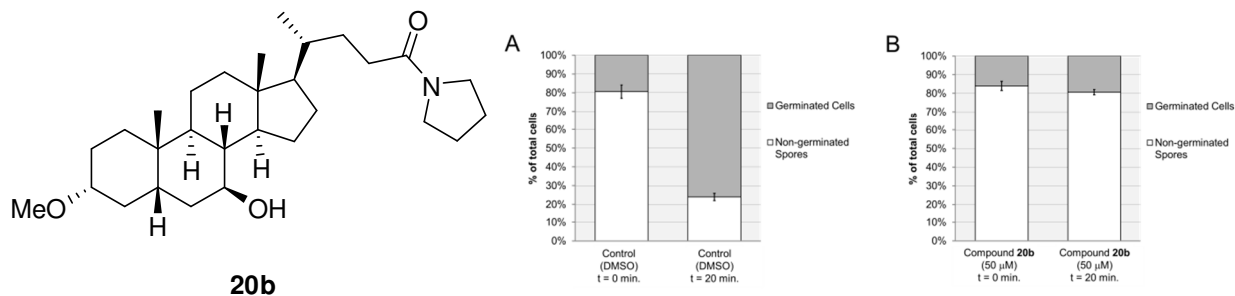
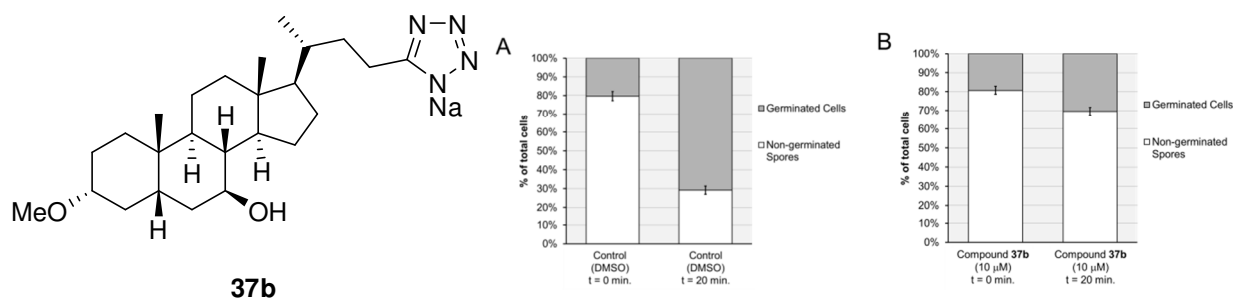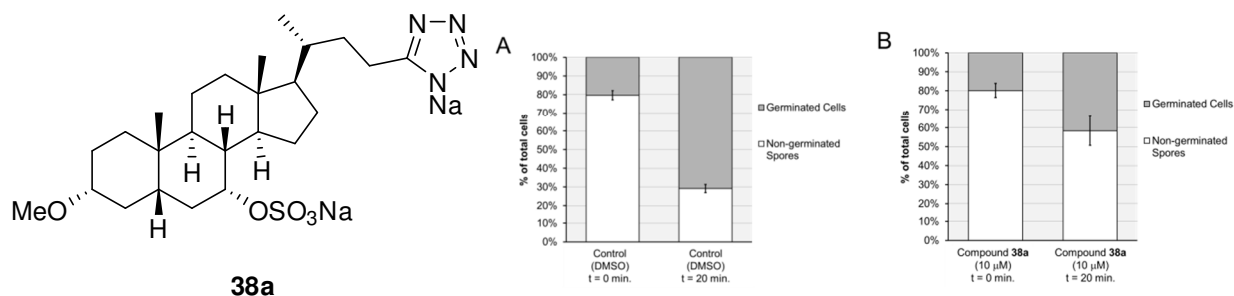**Figure S54:** A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 19% to 76% over 20 min. B) The number of germinated spores rose from 16% to 19% over 20 min. in the presence of 50 µM of **20b**. Data average of three runs.

21b
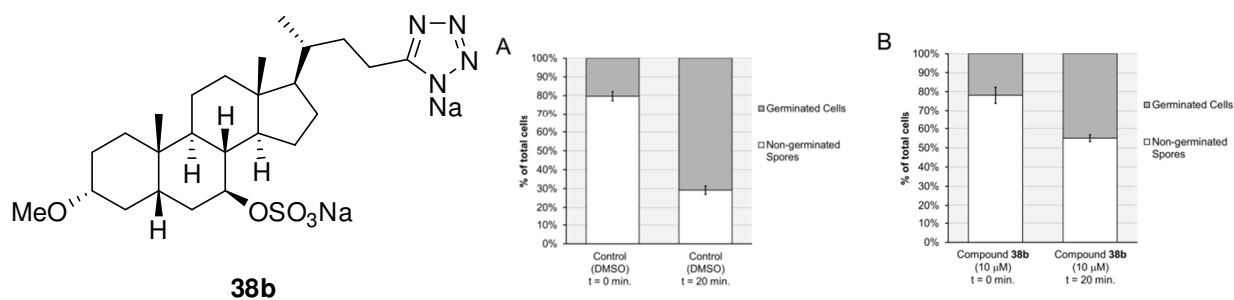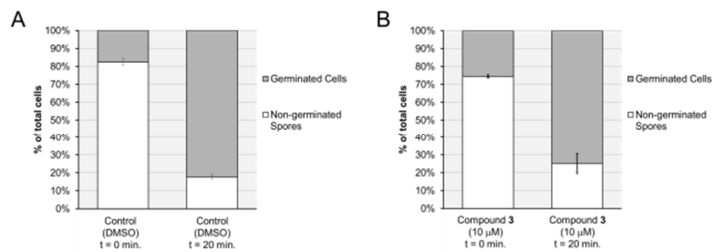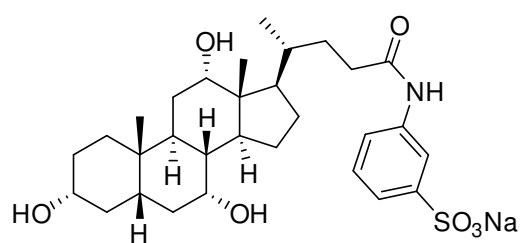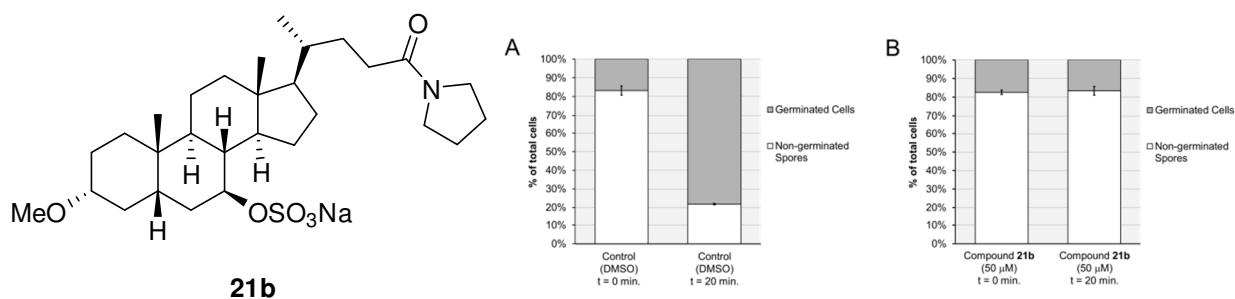
Figure S55: A) Under the conditions of the control experiment (BHIS, 2000 µM TCA, and DMSO), the number of germinated spores rose from 17% to 78% over 20 min. B) The number of germinated spores changed from 17% to 16% over 20 min. in the presence of 50 µM of **21b**. Data average of three runs.

Kinetic Analysis of Spore Germination in the Presence of Selected Compounds
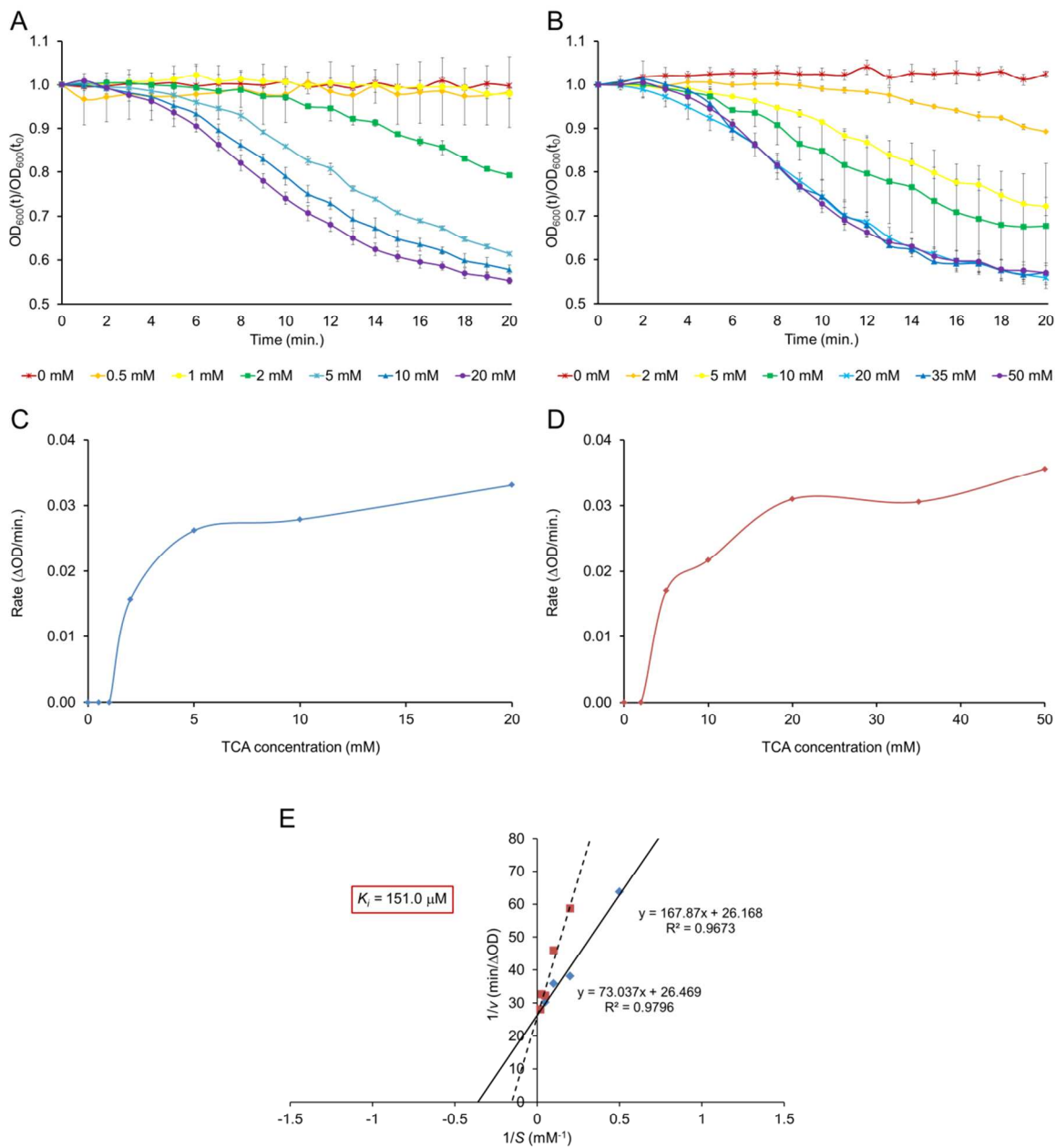
Figure S56a: Experiment 1. A) The relative OD_{600} of spores in BHIS after 20 min. exposure to 0 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, 10 mM, or 20 mM TCA. B) The relative OD_{600} of spores in

BHIS after 20 min. exposure to 0.2 mM **2a** and 0 mM, 2 mM, 5 mM, 10 mM, 20 mM, 35 mM, 50 mM TCA. C) The linear portion of each curve in A was used to determine the maximum rate of germination for each concentration. The maximum rate of germination was plotted versus TCA concentration. D) The linear portion of each curve in B was used to determine the maximum rate of germination for each concentration. The maximum rate of germination was plotted versus TCA concentration. E) The inverse rate ($1/v$ [min./ΔOD_{600}], where v = maximum rate) versus the inverse TCA concentration ($1/S$ [mM^{-1}], where S = inhibitor concentration) was plotted. The linear best fit line was generated and used to determine the apparent K_m for TCA and V_{max} for germination. The K_i value for **2a** was determined using the equation K_i = [inhibitor]/($[K_{m\ inhibitor}/K_{m\ TCA}] - 1$).
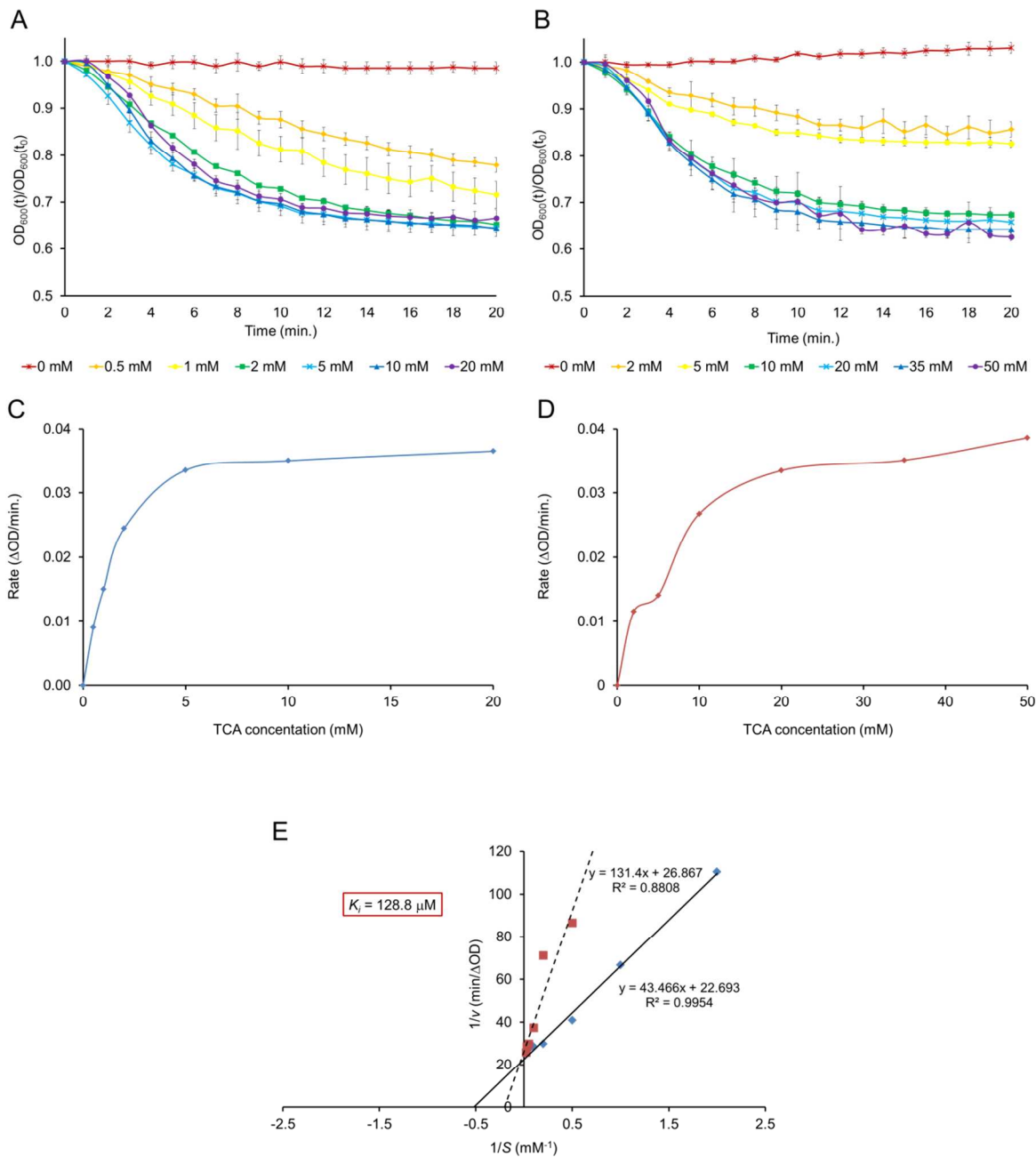
Figure S56b: Experiment 2.

Figure S56c: Experiment 3.

Figure S57a: Experiment 1. A) The relative OD_{600} of spores in BHIS after 20 min. exposure to 0 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, 10 mM, or 20 mM TCA. B) The relative OD_{600} of spores in BHIS after 20 min. exposure to 0.2 mM **20b** and 0 mM, 1 mM, 2 mM, 3 mM, 5 mM, 10 mM, or 20 mM TCA. C) The linear portion of each curve in A was used to determine the maximum rate of germination for each concentration. The maximum rate of germination was plotted versus

TCA concentration. D) The linear portion of each curve in B was used to determine the maximum rate of germination for each concentration. The maximum rate of germination was plotted versus TCA concentration. E) The inverse rate ($1/v$ [min./ΔOD_{600}], where v = maximum rate) versus the inverse TCA concentration ($1/S$ [mM^{-1}], where S = inhibitor concentration) was plotted. The linear best fit line was generated and used to determine the apparent K_m for TCA and V_{max} for germination. The K_i value for **20b** was determined using the equation K_i = [inhibitor]/([$K_{m\ inhibitor}$/$K_{m\ TCA}$] − 1).
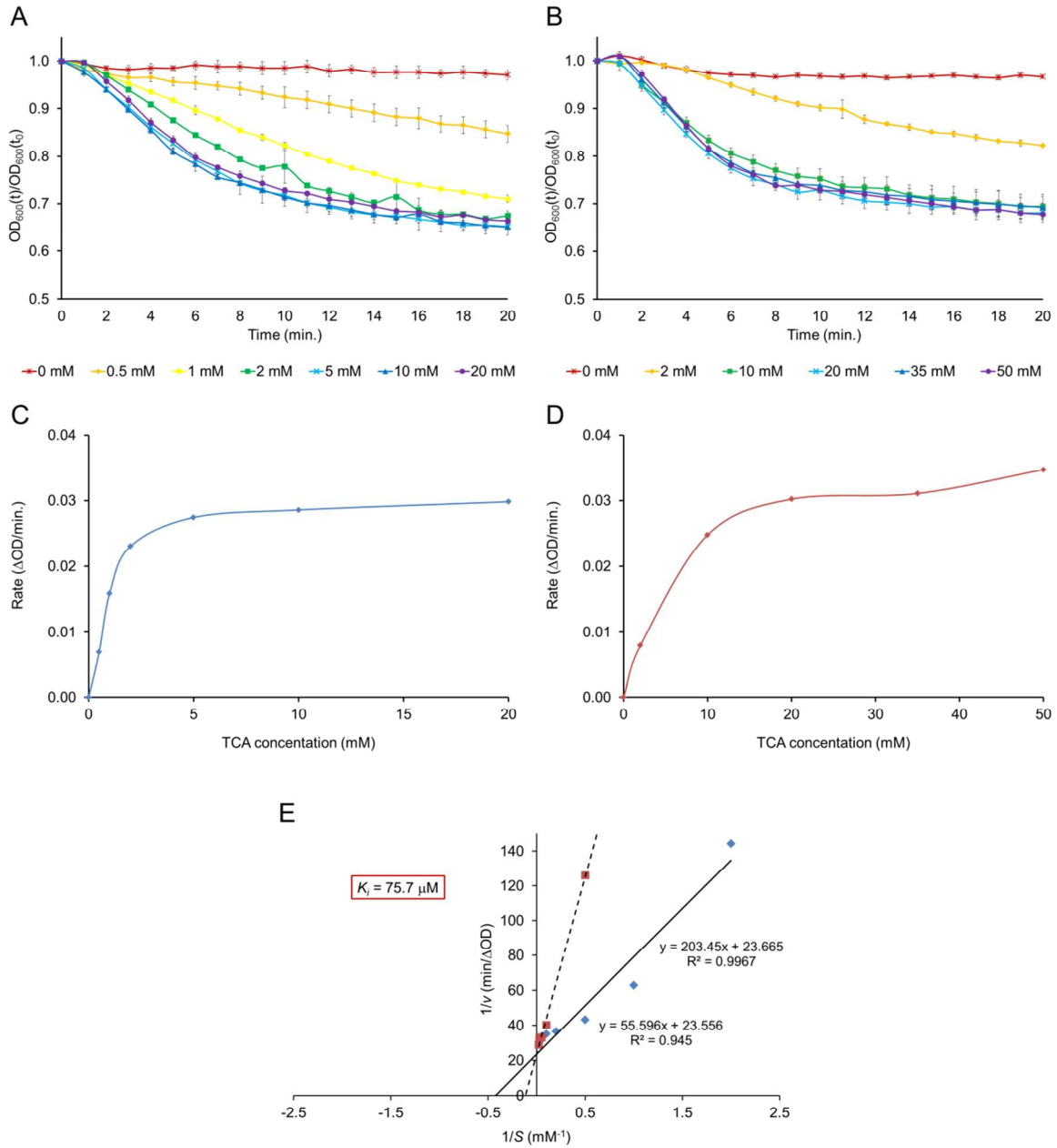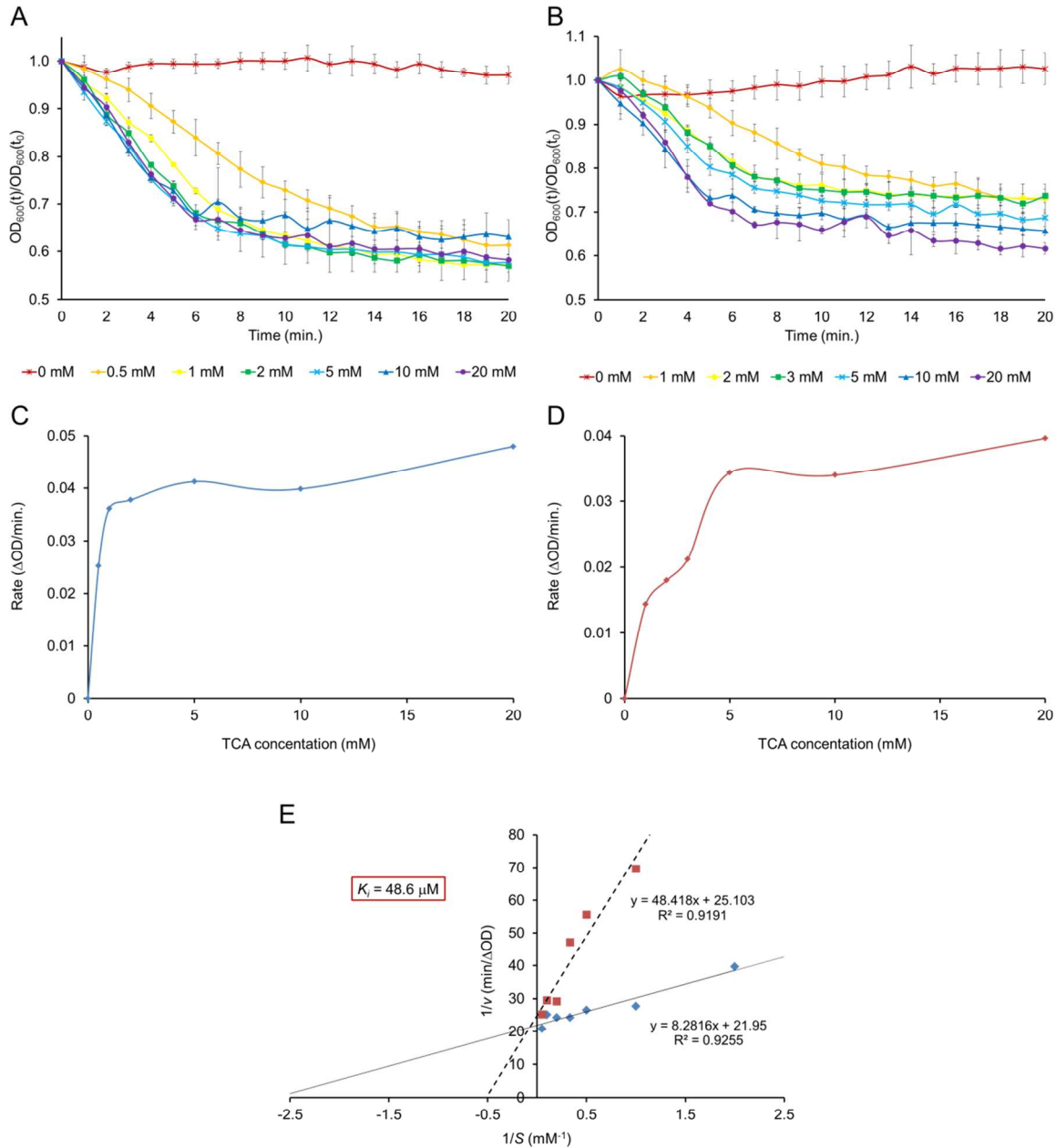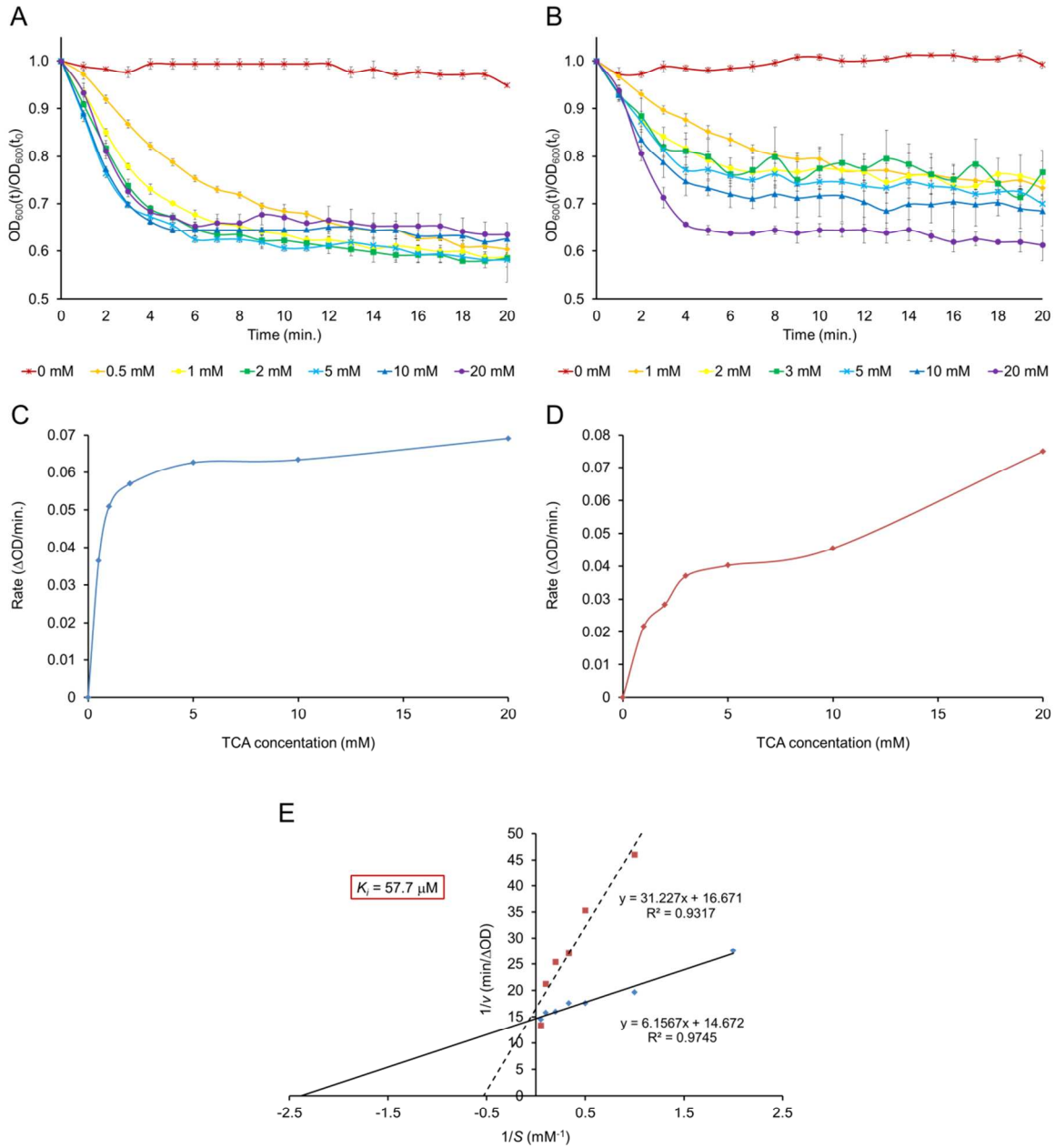
Figure S57b: Experiment 2.

Figure S57c: Experiment 3.

Figure S58a: Experiment 1. A) The relative OD_{600} of spores in BHIS after 20 min. exposure to 0

mM, 1 mM, 2 mM, 5 mM, 10 mM, or 20 mM TCA. B) The relative OD_{600} of spores in BHIS

after 20 min. exposure to 0.2 mM **21b** and 0 mM, 1 mM, 2 mM, 5 mM, 10 mM, 20 mM, or 35

mM TCA. C) The linear portion of each curve in A was used to determine the maximum rate of

germination for each concentration. The maximum rate of germination was plotted versus TCA

concentration. D) The linear portion of each curve in B was used to determine the maximum rate of germination for each concentration. The maximum rate of germination was plotted versus TCA concentration. E) The inverse rate ($1/v$ [min./ΔOD_{600}], where v = maximum rate) versus the inverse TCA concentration ($1/S$ [mM^{-1}], where S = inhibitor concentration) was plotted. The linear best fit line was generated and used to determine the apparent K_m for TCA and V_{max} for germination. The K_i value for **21b** was determined using the equation K_i = [inhibitor]/([K_m $_{inhibitor}$/$K_{m\ TCA}$] − 1).

Figure S58b: Experiment 2.

A

B

C

D

E

K_i = 14.3 µM

y = 215.68x + 9.966
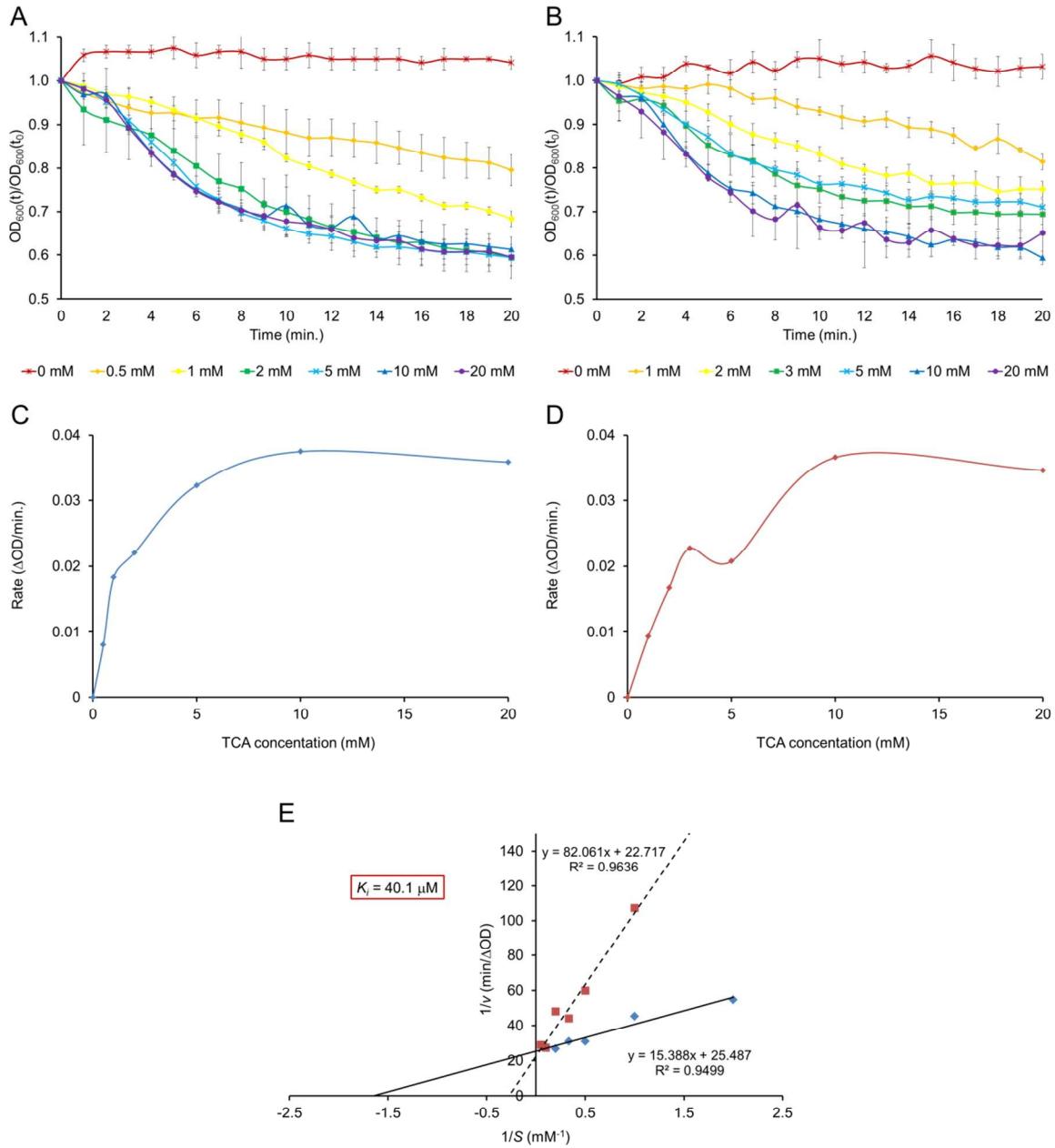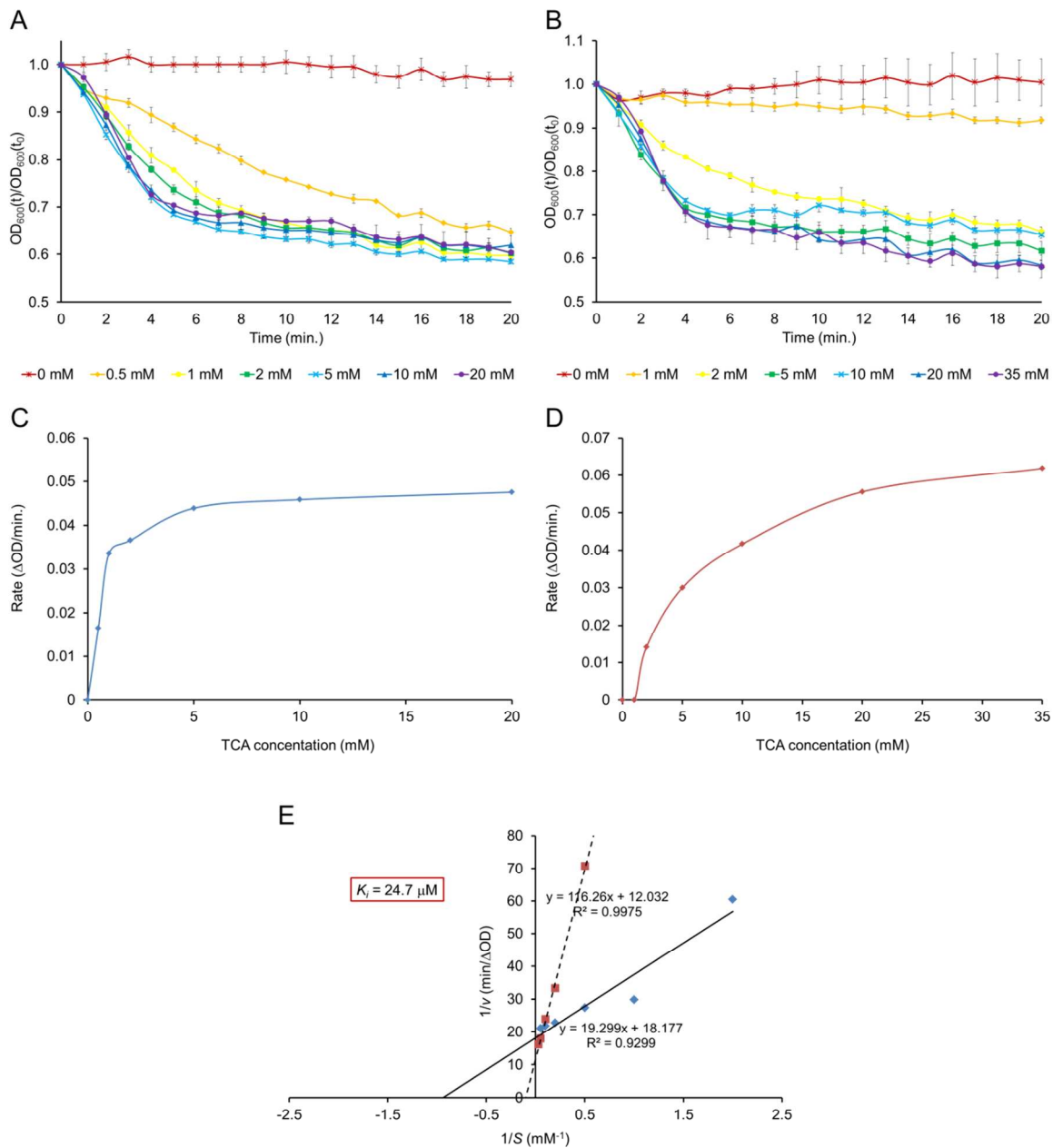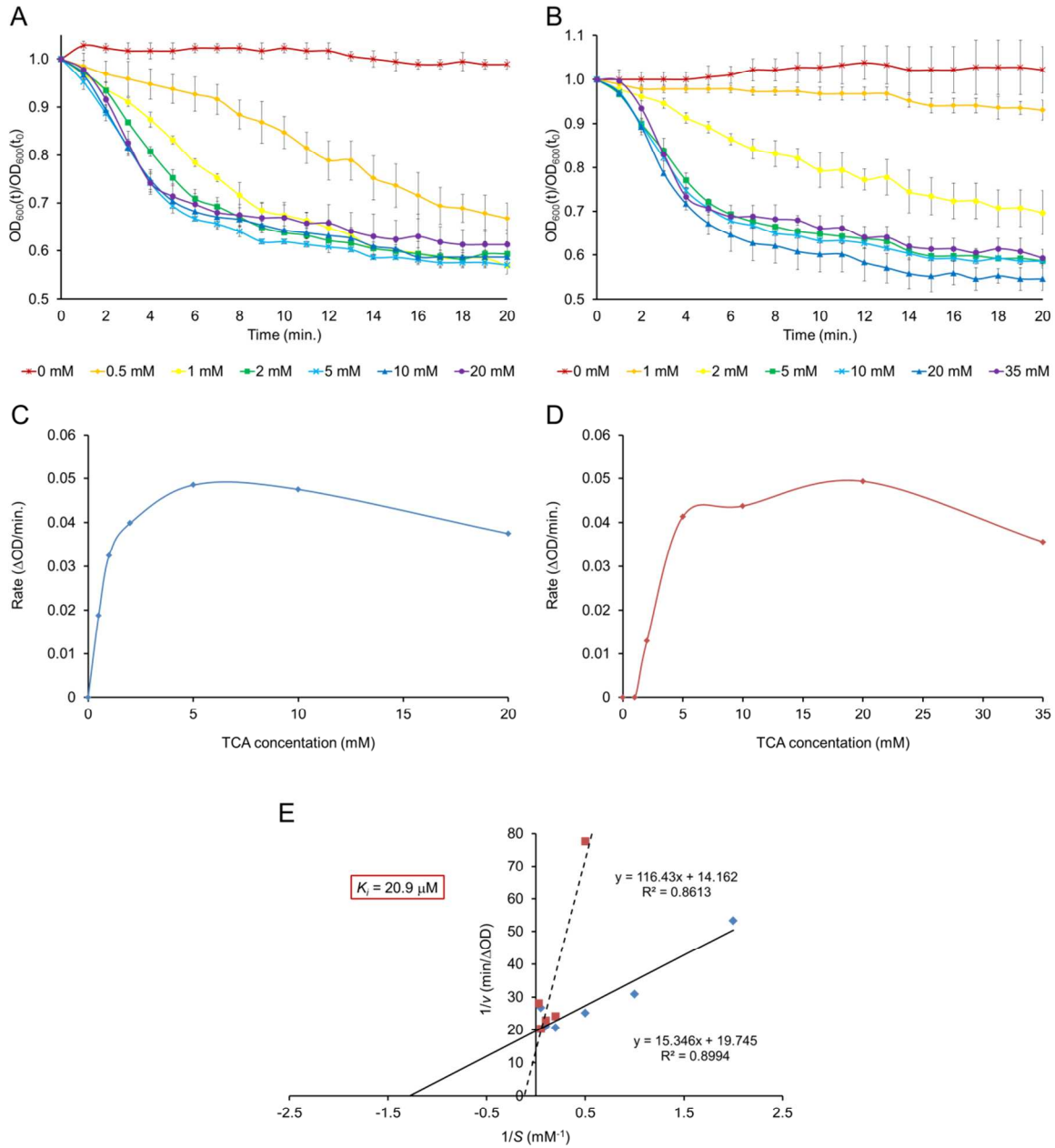R² = 0.975

y = 25.072x + 17.415
R² = 0.9835

Figure S58c: Experiment 3.

Figure S59a: Experiment 1. A) The relative OD_{600} of spores in BHIS after 20 min. exposure to 0

mM, 1 mM, 2 mM, 5 mM, 10 mM, or 20 mM TCA. B) The relative OD_{600} of spores in BHIS

after 20 min. exposure to 0.2 mM **37a** and 0 mM, 2 mM, 5 mM, 10 mM, 20 mM, 35 mM, or 50

mM TCA. C) The linear portion of each curve in A was used to determine the maximum rate of

germination for each concentration. The maximum rate of germination was plotted versus TCA

concentration. D) The linear portion of each curve in B was used to determine the maximum rate of germination for each concentration. The maximum rate of germination was plotted versus TCA concentration. E) The inverse rate ($1/v$ [min./ΔOD_{600}], where v = maximum rate) versus the inverse TCA concentration ($1/S$ [mM^{-1}], where S = inhibitor concentration) was plotted. The linear best fit line was generated and used to determine the apparent K_m for TCA and V_{max} for germination. The K_i value for **37a** was determined using the equation K_i = [inhibitor]/([$K_{m\ inhibitor}$/$K_{m\ TCA}$] − 1).
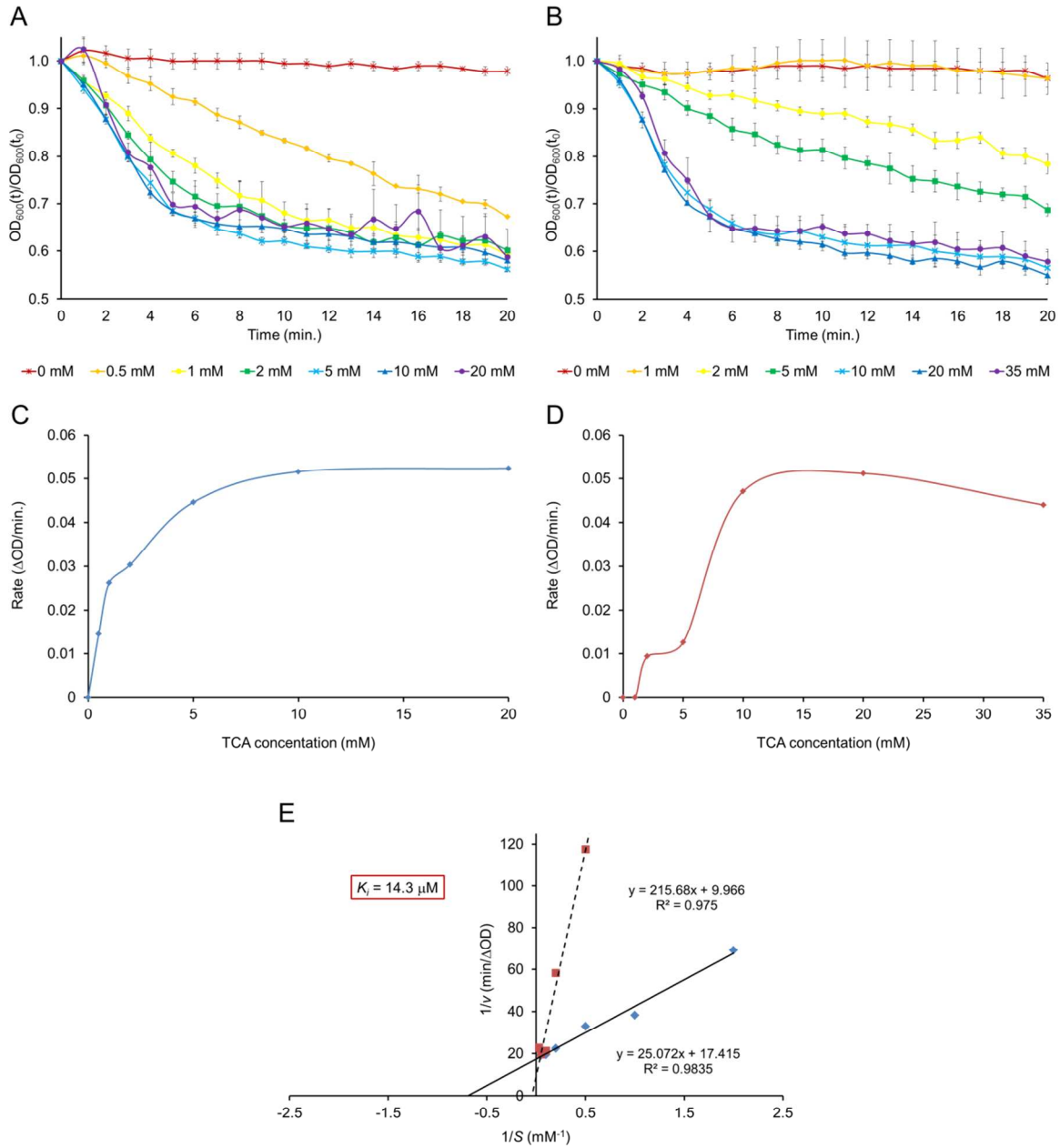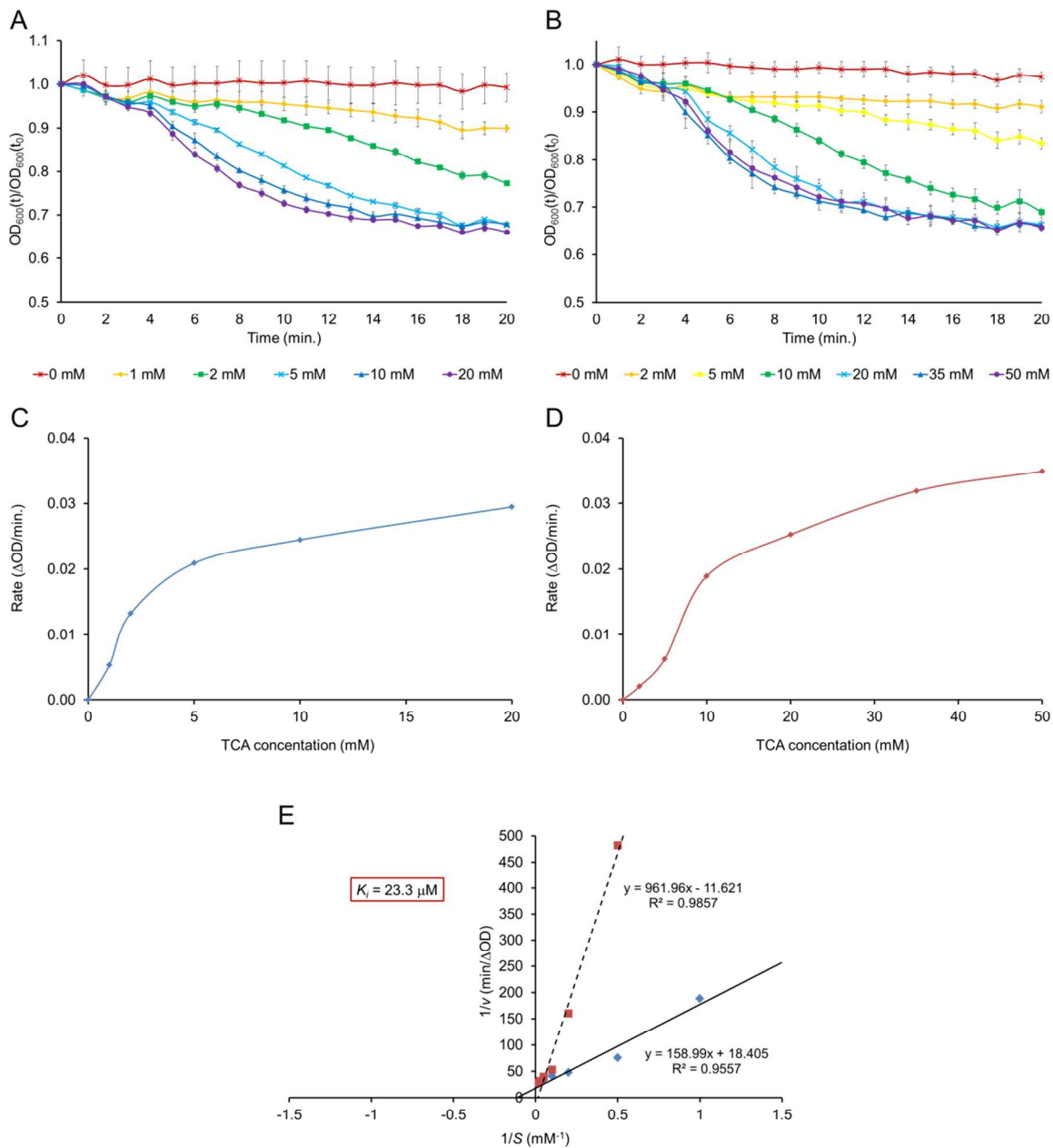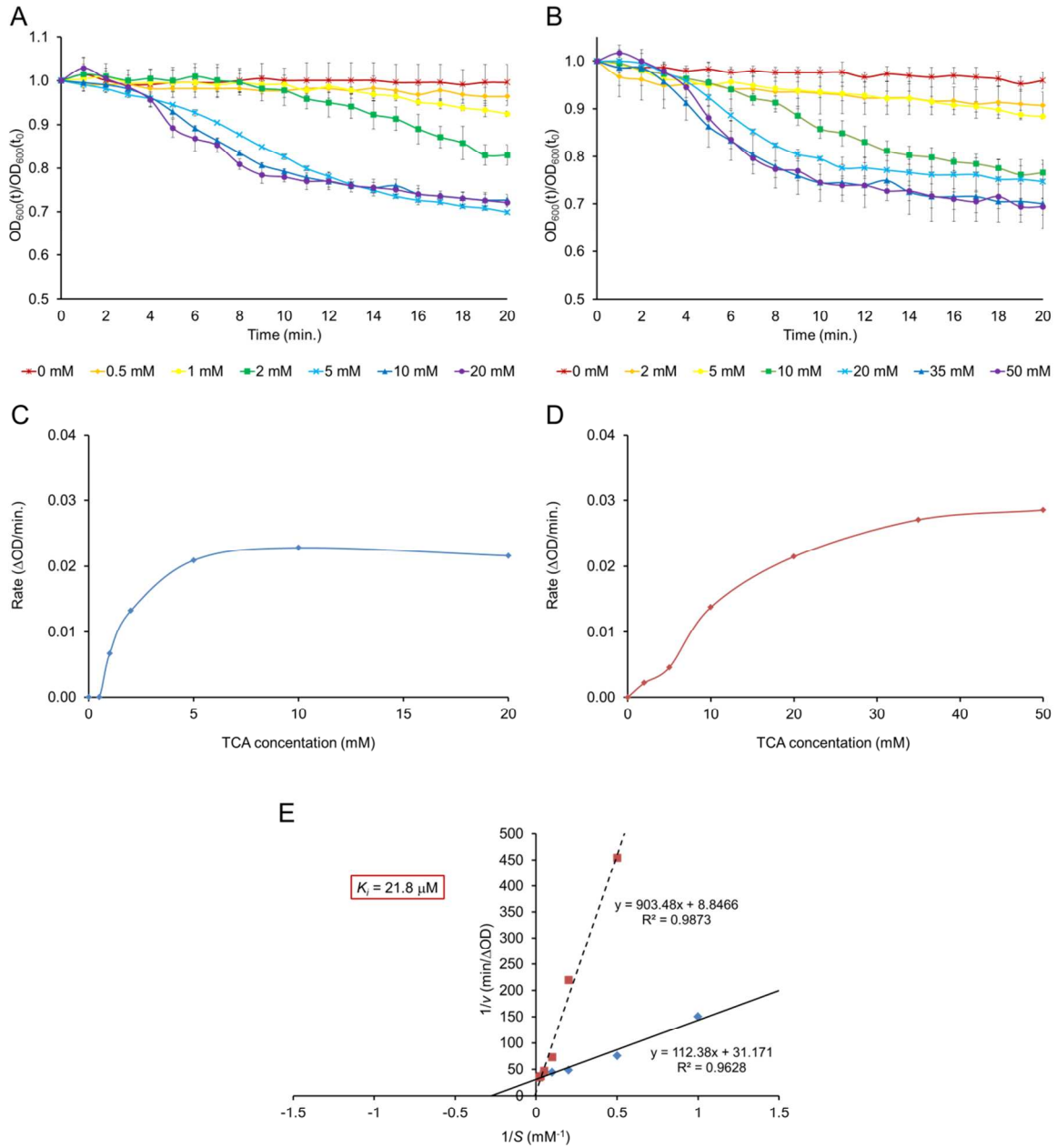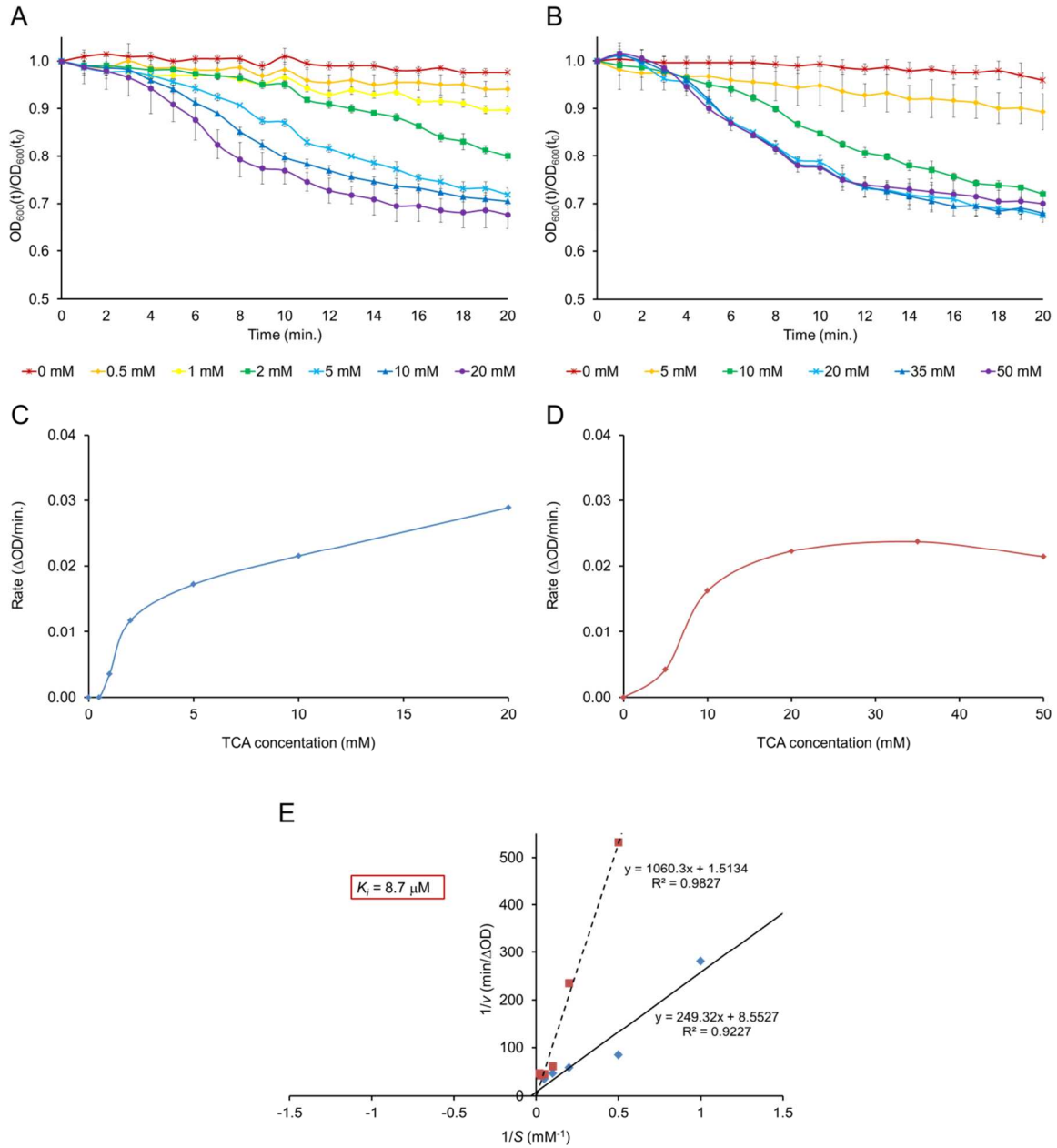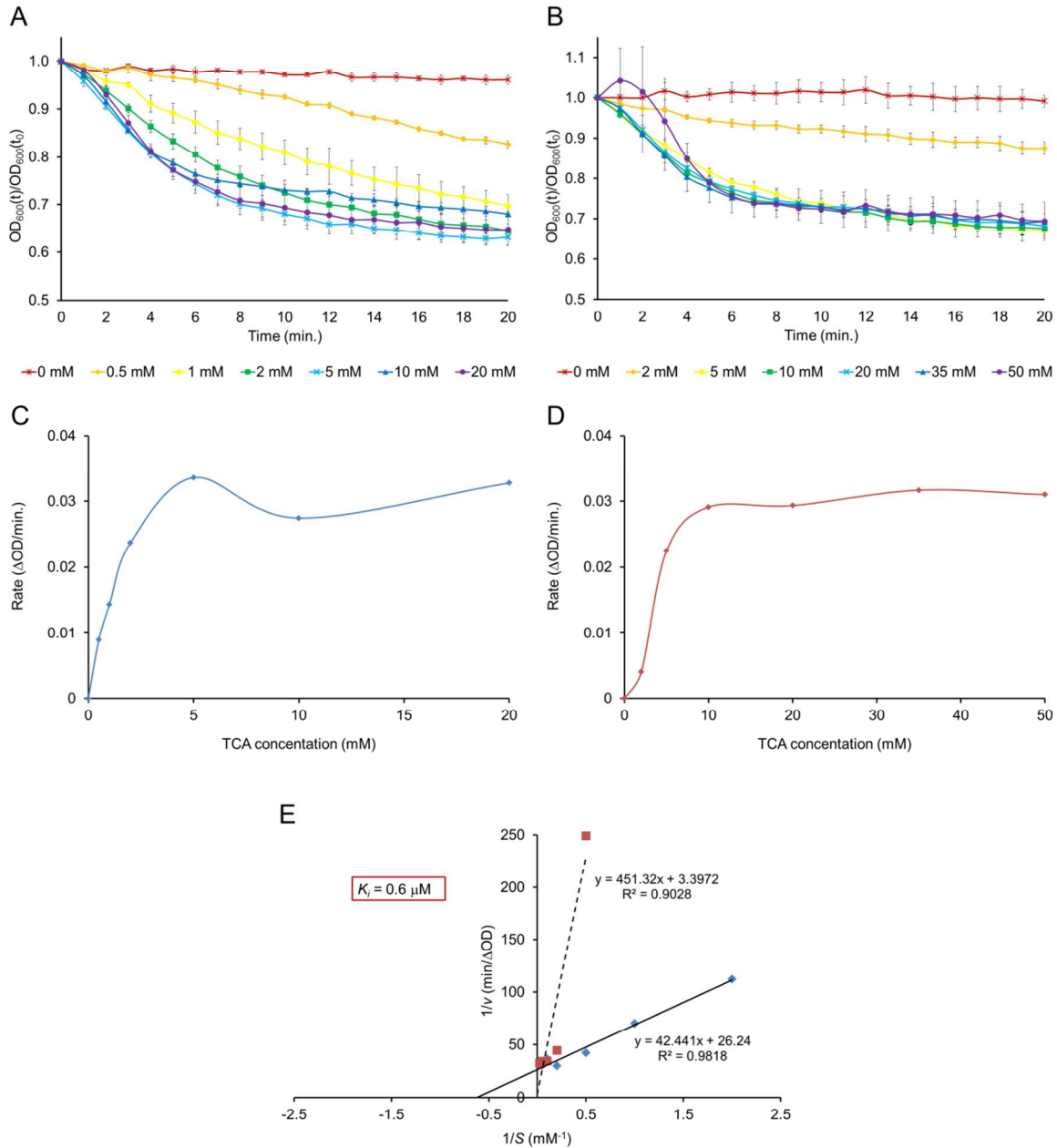
Figure S59b: Experiment 2.

Figure S59c: Experiment 3.

Figure S60a: Experiment 1. A) The relative OD_{600} of spores in BHIS after 20 min. exposure to 0 mM, 1 mM, 2 mM, 5 mM, 10 mM, or 20 mM TCA. B) The relative OD_{600} of spores in BHIS after 20 min. exposure to 0.05 mM **38b** and 0 mM, 2 mM, 5 mM, 10 mM, 20 mM, 35 mM, or 50 mM TCA. C) The linear portion of each curve in A was used to determine the maximum rate of germination for each concentration. The maximum rate of germination was plotted versus TCA

concentration. D) The linear portion of each curve in B was used to determine the maximum rate of germination for each concentration. The maximum rate of germination was plotted versus TCA concentration. E) The inverse rate ($1/v$ [min./ΔOD_{600}], where v = maximum rate) versus the inverse TCA concentration ($1/S$ [mM^{-1}], where S = inhibitor concentration) was plotted. The linear best fit line was generated and used to determine the apparent K_m for TCA and V_{max} for germination. The K_i value for **38b** was determined using the equation K_i = [inhibitor]/([$K_{m\ inhibitor}$/$K_{m\ TCA}$] − 1).
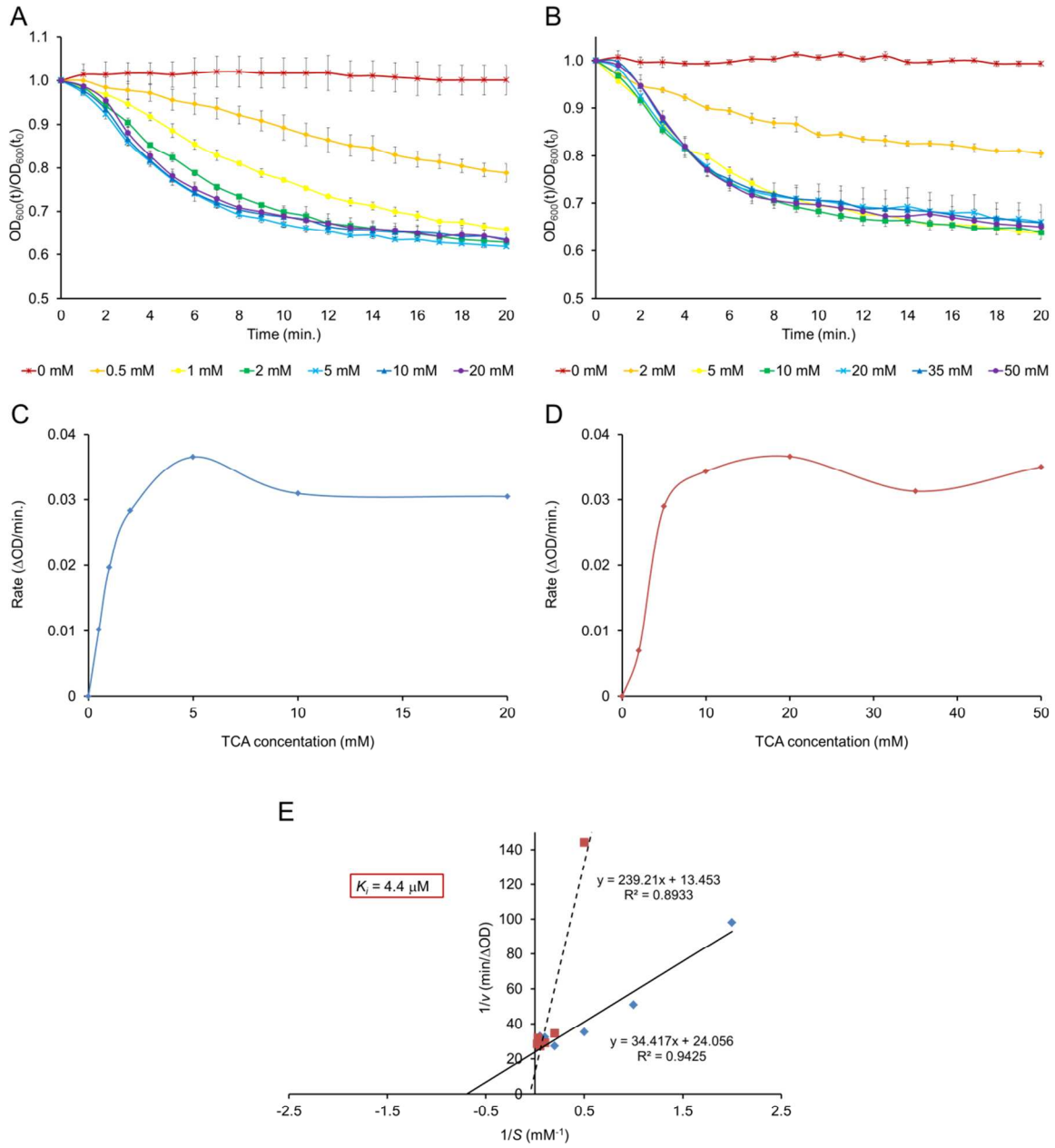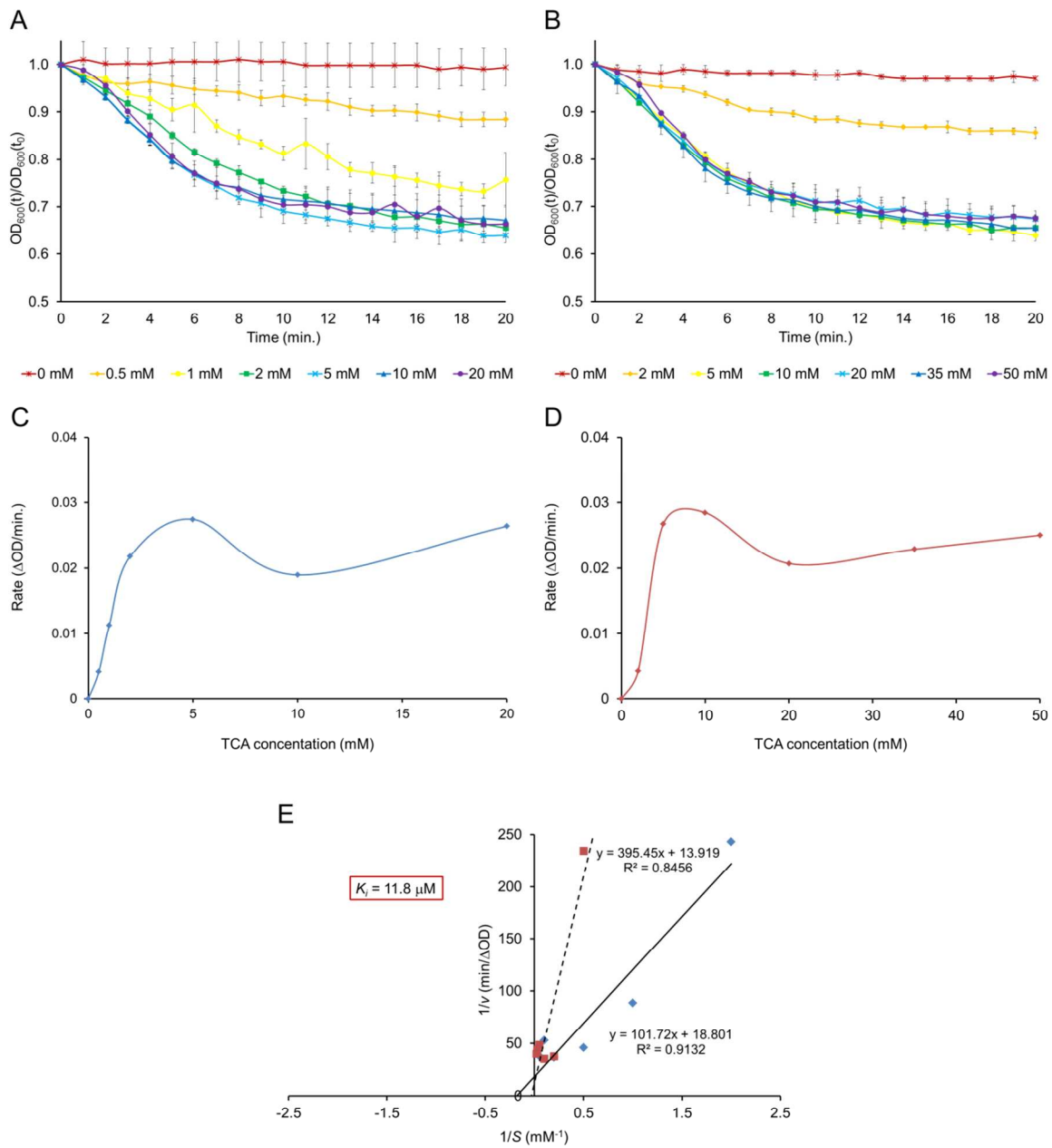
Figure S60b: Experiment 2.

Figure S60c: Experiment 3.

References

1.   Weingarden, A. R.; Chen, C.; Zhang, N.; Graiziger, C. T.; Dosa, P. I.; Steer, C. J.; Shaughnessy, M. K.; Johnson, J. R.; Sadowsky, M. J.; Khoruts, A., Ursodeoxycholic Acid Inhibits *Clostridium difficile* Spore Germination and Vegetative Growth, and Prevents the Recurrence of Ileal Pouchitis Associated With the Infection. *J. Clin. Gastroenterol.* **2016**, *50*, 624-630.