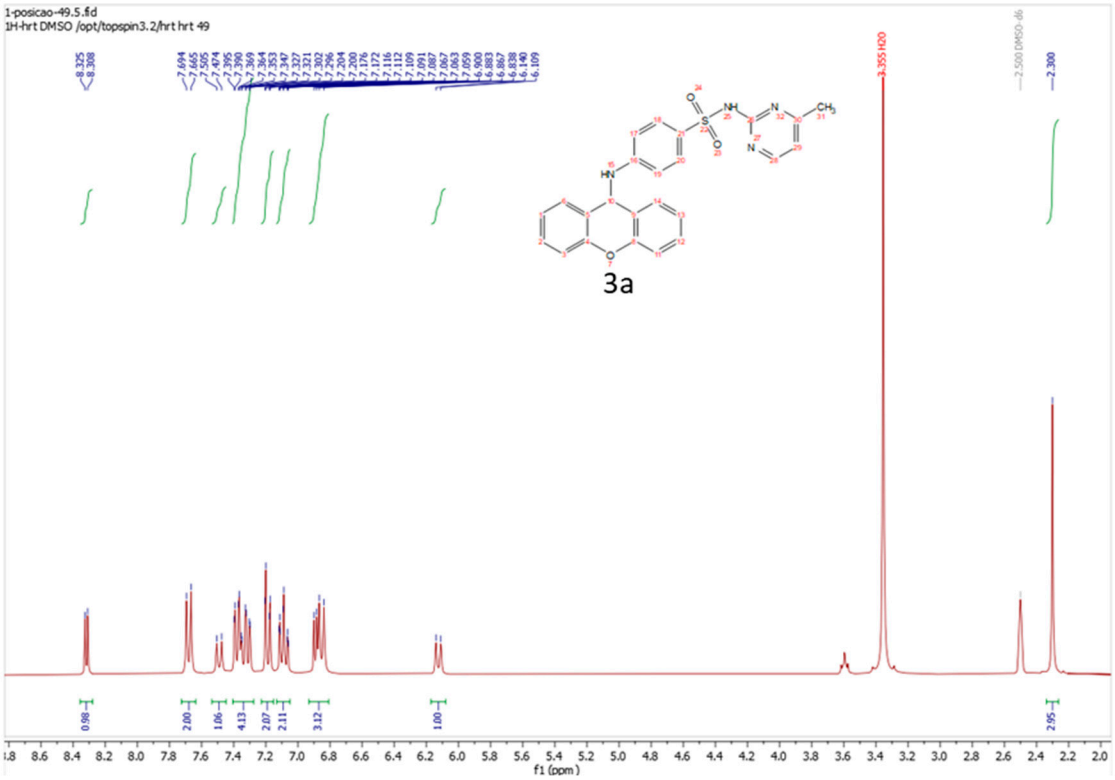
Supplementary Materials: Xanthene Derivatives Targeting Bacterial Efflux Pumps, Quorum-Sensing, and Biofilm Formation

Miguel Maia, Fernando Durães, Diana I. S. P. Resende, Nikoletta Szemerédi, Luís Gales, Paulo Martins-da-Costa, Madalena Pinto, Gabriella Spengler, and Emília Sousa



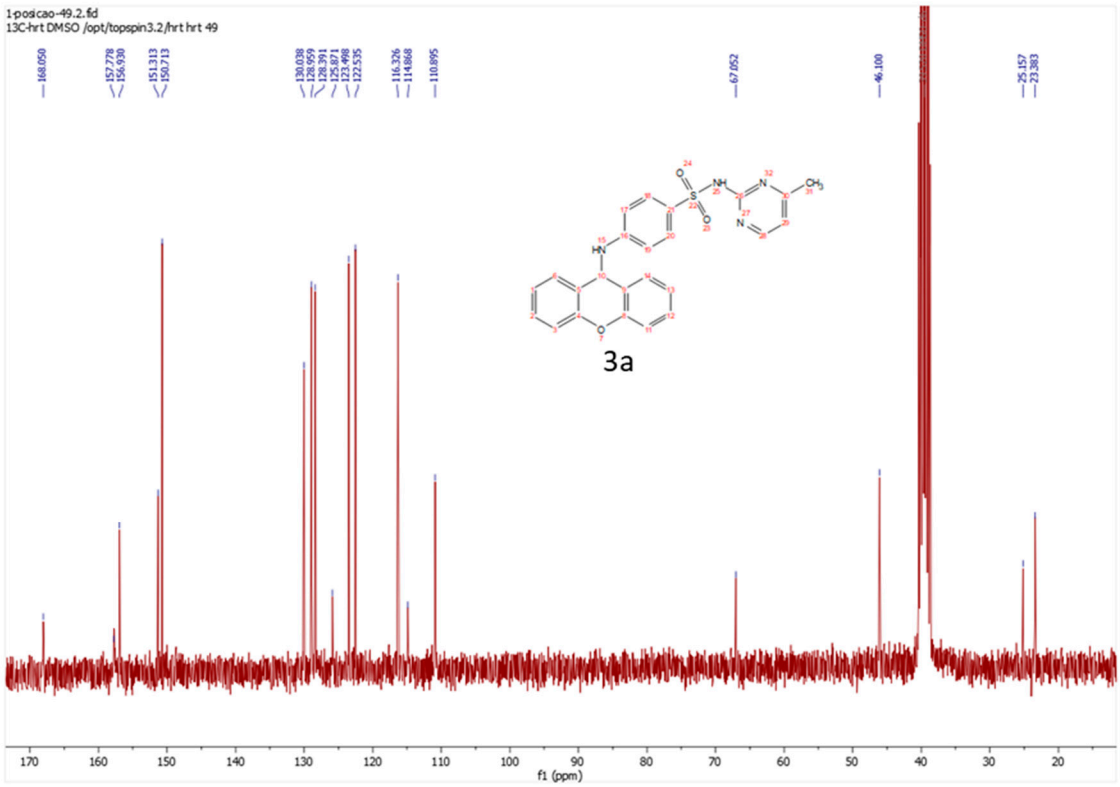


Figure S1. ^{1}H (top) and ^{13}C (bottom) NMR of compound **3a**.

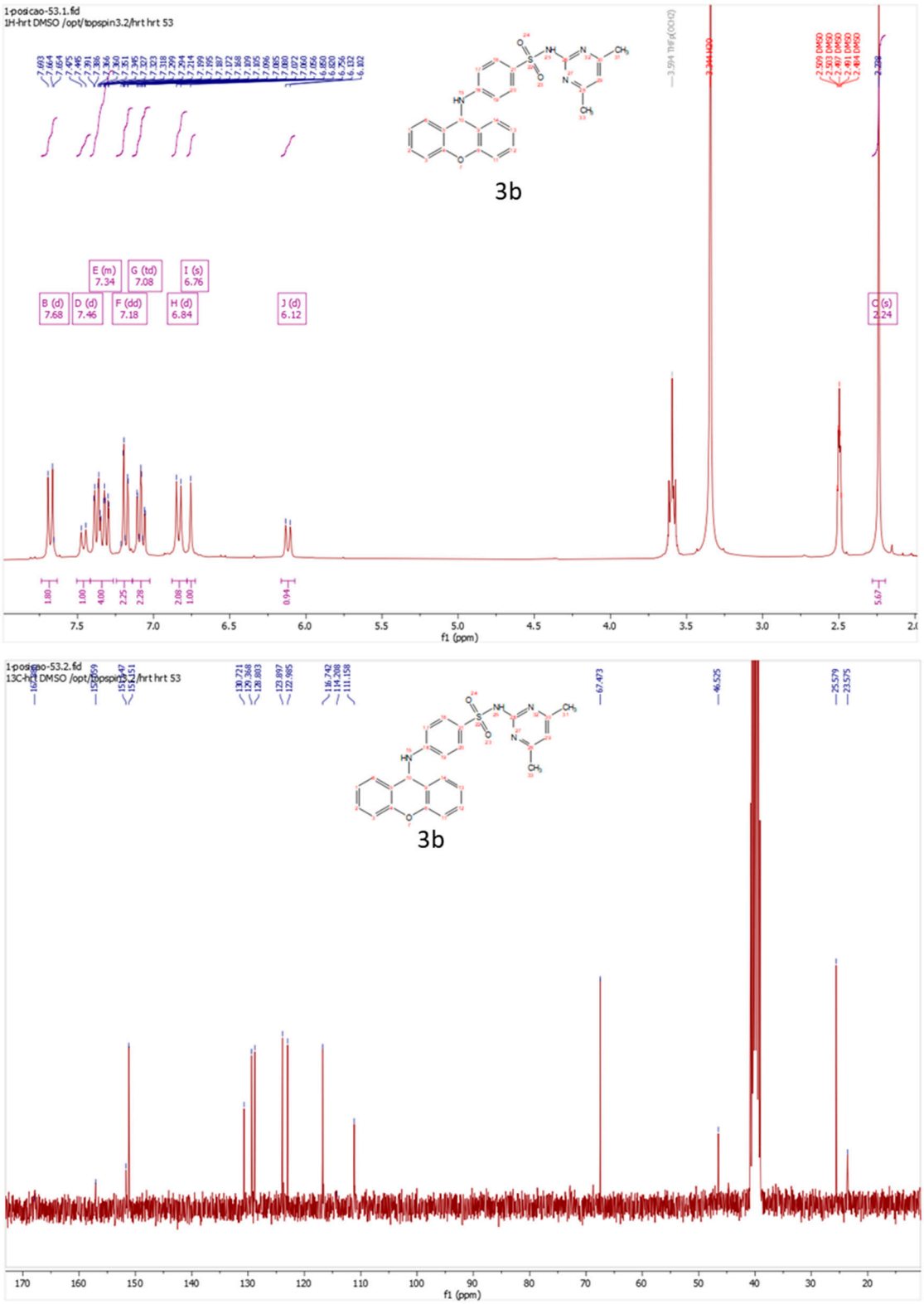


Figure S2. ^{1}H (top) and ^{13}C (bottom) NMR of compound **3b**.

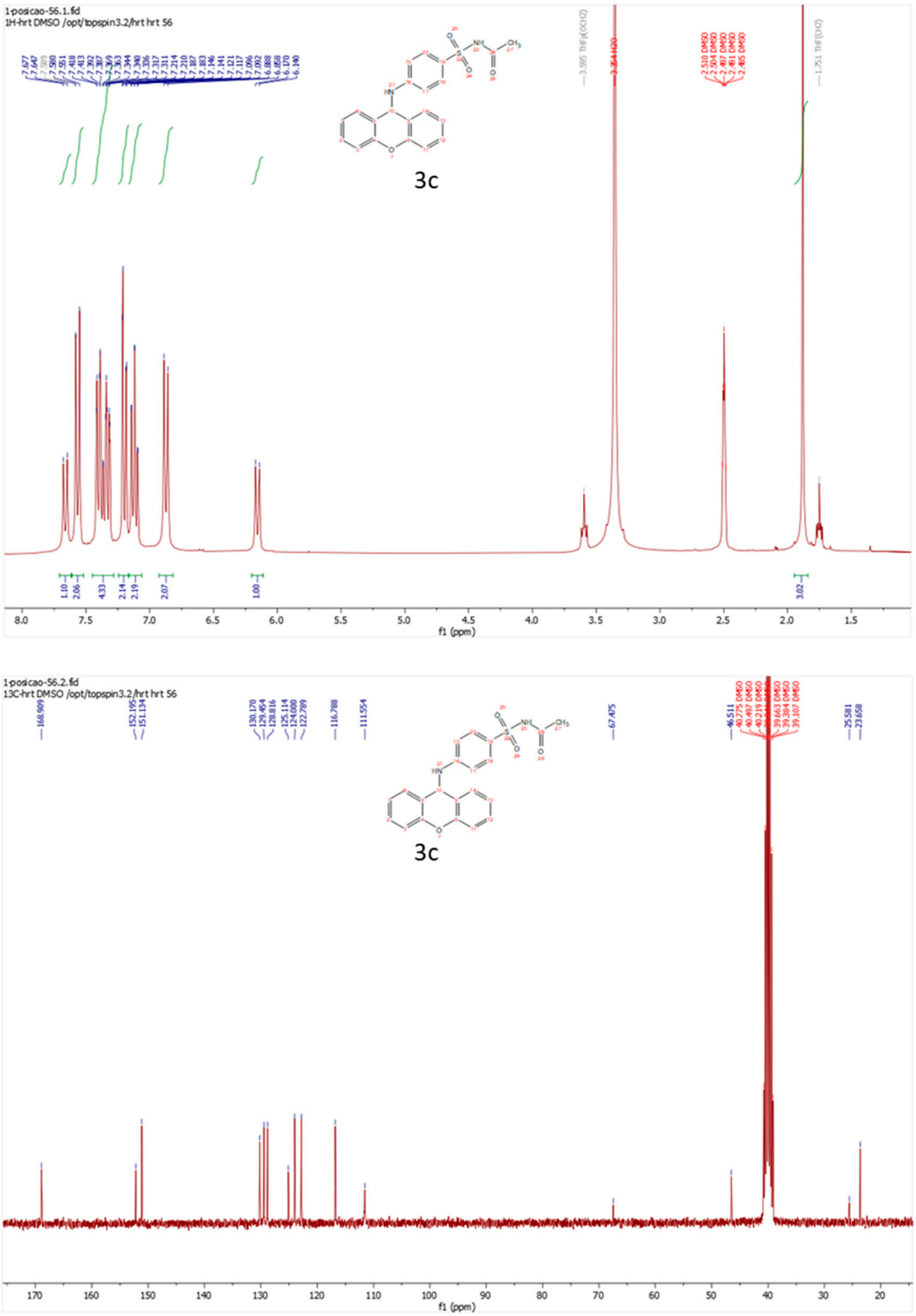


Figure S3. ^{1}H (top) and ^{13}C (bottom) NMR of compound **3c**.

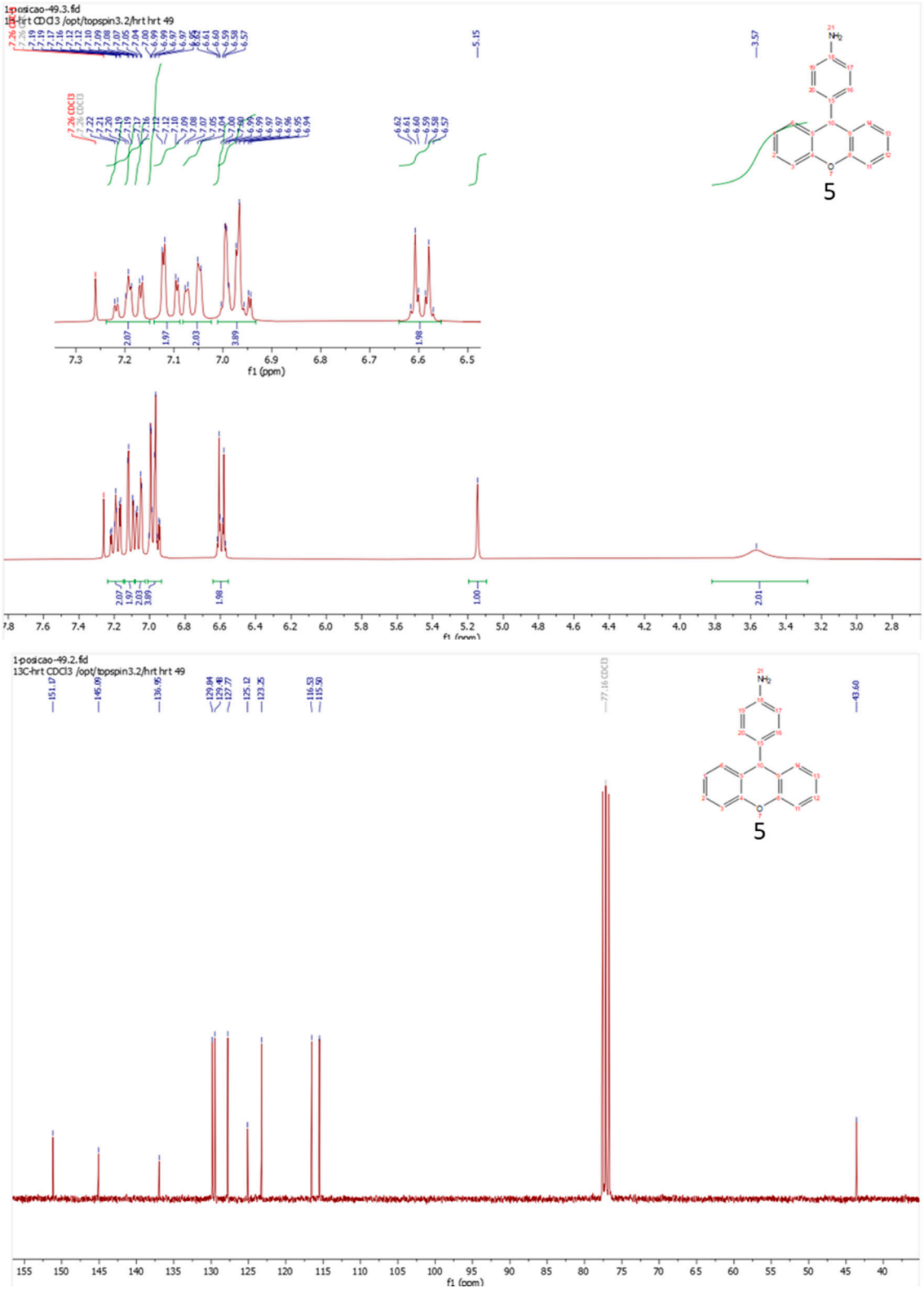


Figure S4. ^{1}H (top) and ^{13}C (bottom) NMR of compound **5**.

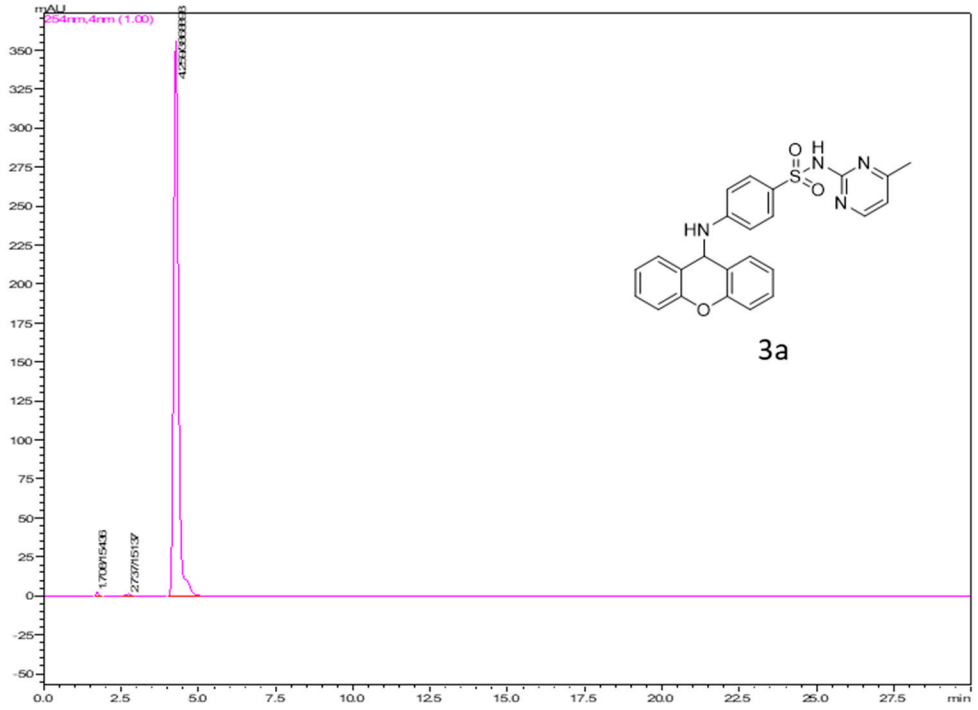


Figure S5. Chromatogram (HPLC) of compound **3a**. Purity (% a/a): 99.2%.

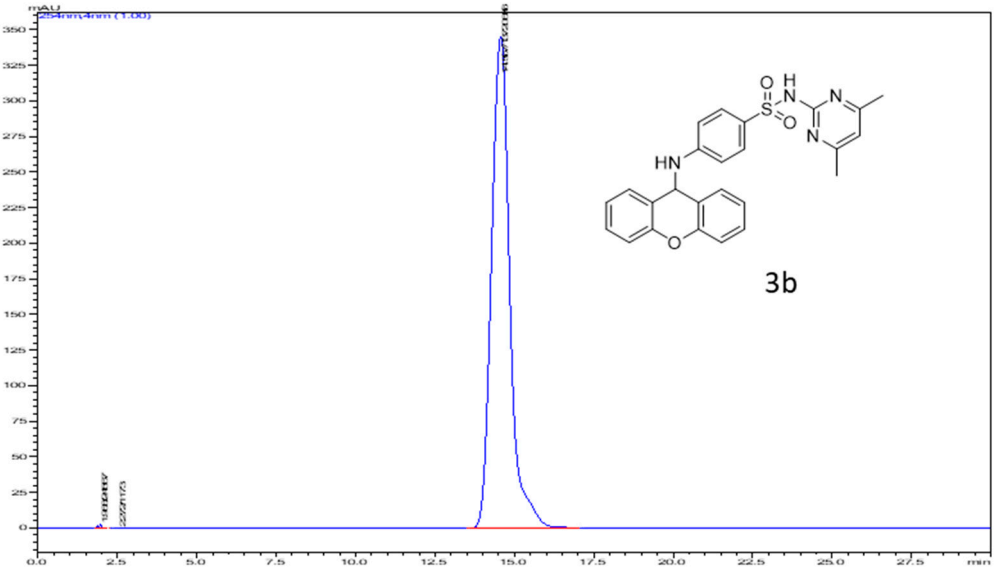


Figure S6. Chromatogram (HPLC) of compound **3b**. Purity (% a/a): 99.9%.

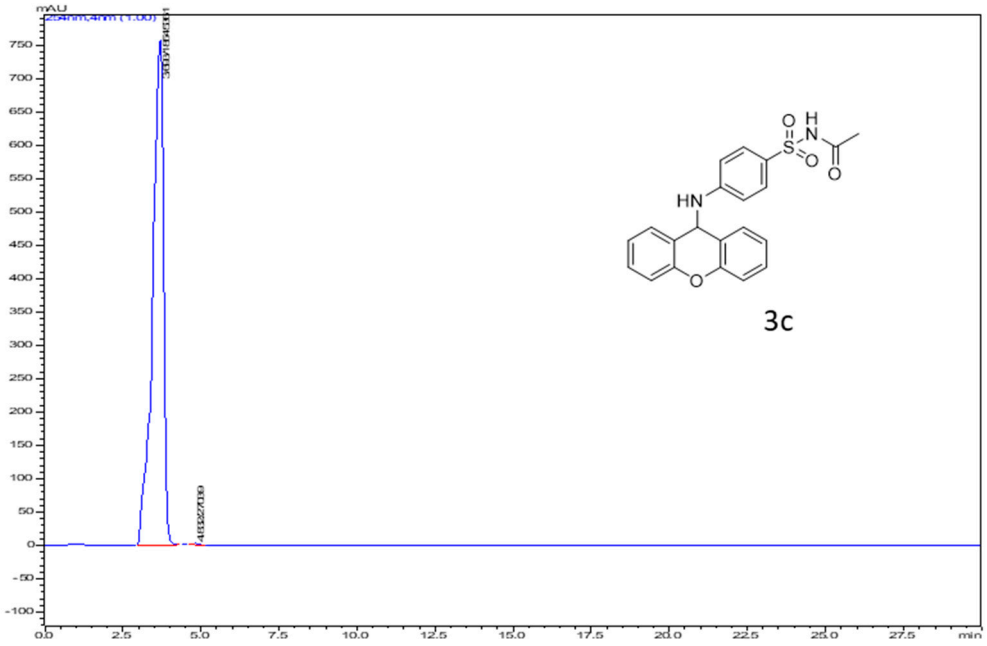


Figure S7. Chromatogram (HPLC) of compound **3c**. Purity (% a/a): 99.9%.

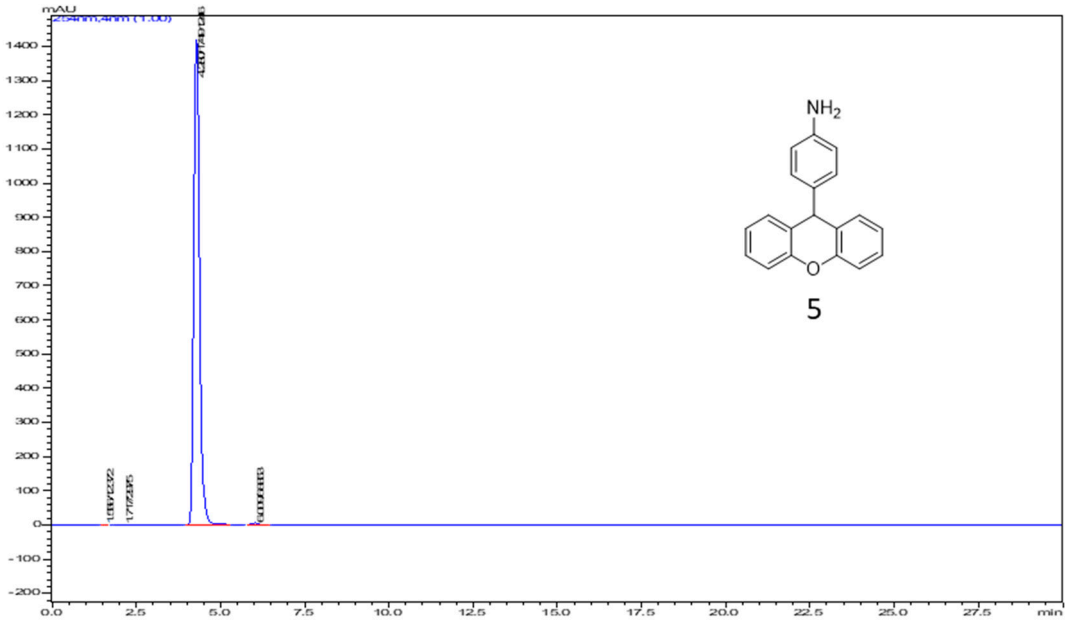


Figure S8. Chromatogram (HPLC) of compound **5**. Purity (% a/a): 99.5%.

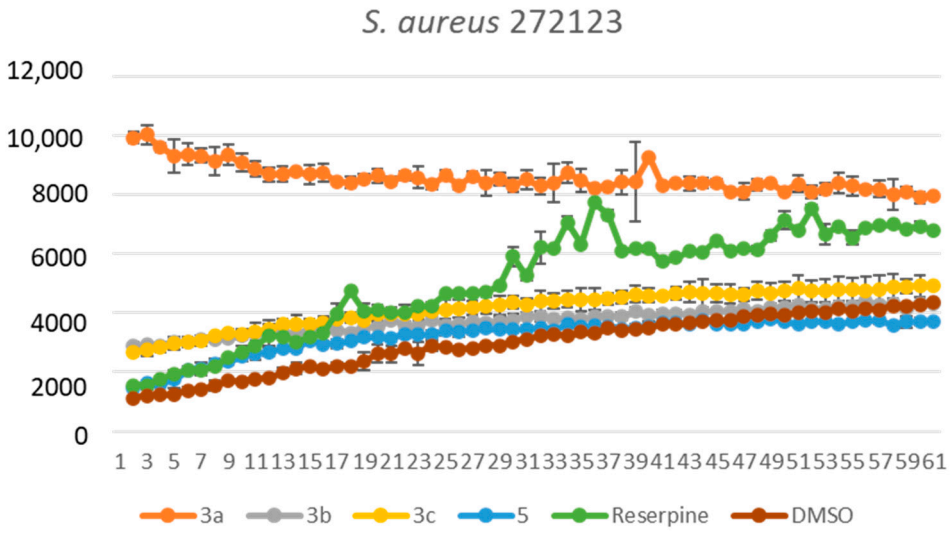


Figure S9. RFI curves of the tested compounds against *S. aureus* 272123.

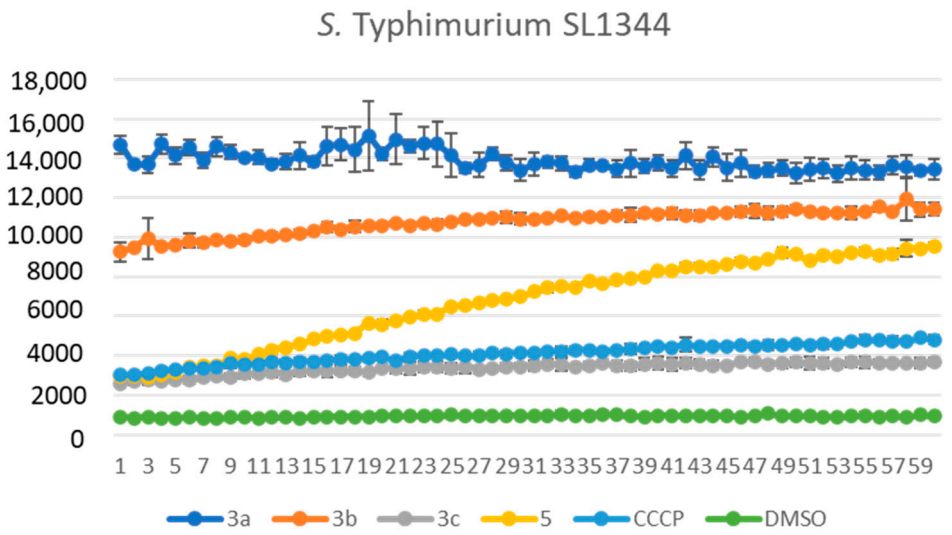


Figure S10. RFI curves of the tested compounds against *S.* Typhimurium SL1344.

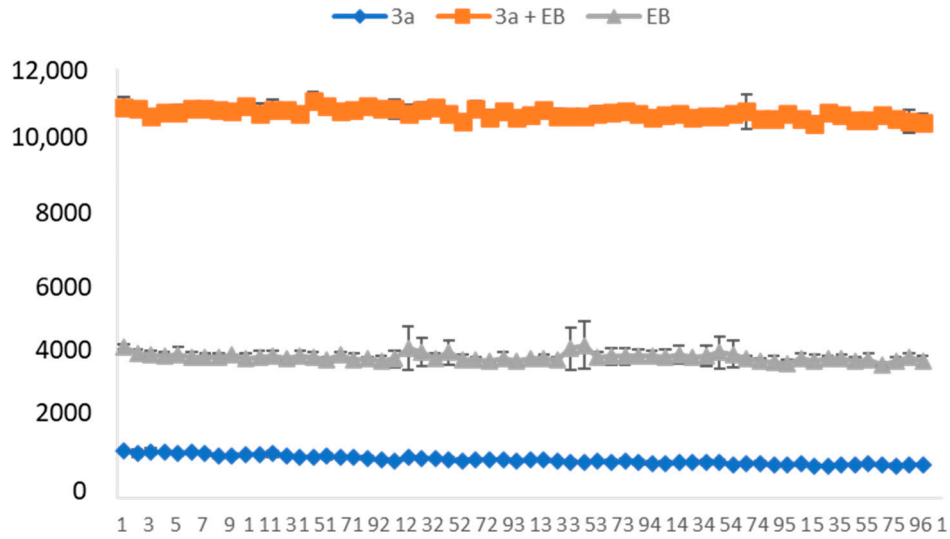


Figure S11. Fluorescence studies of compound **3a**.

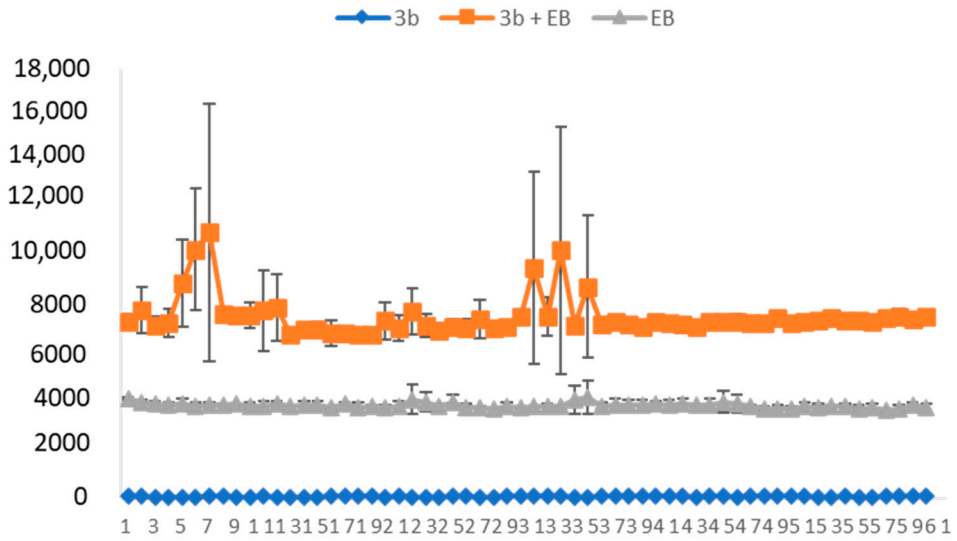


Figure S12. Fluorescence studies of compound **3b**.

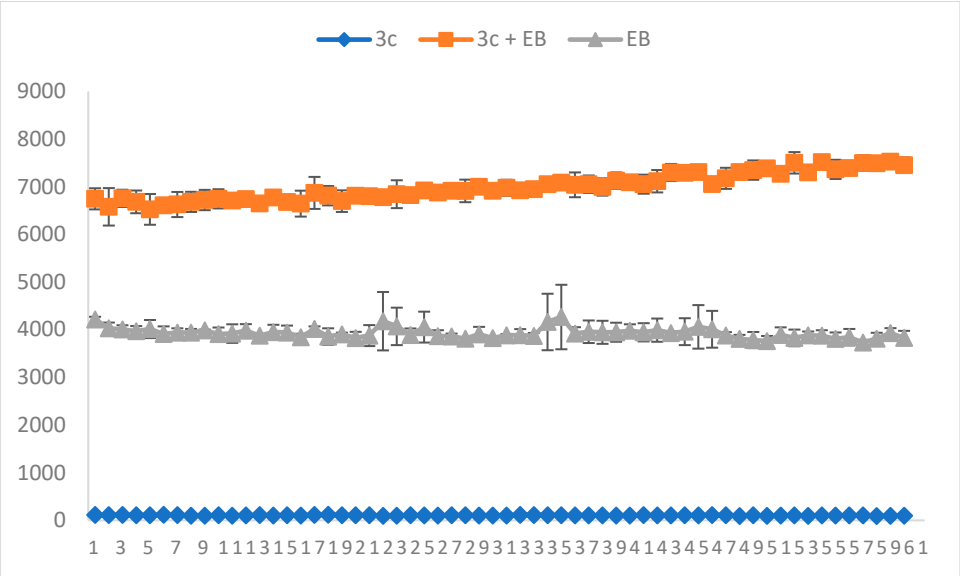


Figure S13. Fluorescence studies of compound **3c**.

Table S1. Docking scores (kcal/mol) for the xanthenes and reference compounds against bacterial efflux pumps.

Compound	AcrB		AcrA		TolC
	SBS	HT	HH	LD	
3a	−9.5	−4.6	−8.3	−7.7	−9.0
3b	−9.5	−0.8	−8.1	−7.7	−9.2
3c	−8.2	3.2	−7.0	−6.8	−8.7
5	−7.1	−6.2	−6.8	−6.5	−6.9
D13-9001	−9.7	26.5	−6.2	−5.1	−7.4
Doxorubicin	−8.9	15.4	−7.2	−5.6	−7.2
MBX-3132	−7.9	2.9	−7.9	−6.2	−7.7
Minocycline	−8.7	26.7	−6.2	−5.4	−7.7
PAβN	−7.1	−4.7	−5.8	−4.9	−7.1
Reserpine	−8.7	10.9	5.6	4.6	−7.5

SBS: Substrate-binding site; **HT**: Hydrophobic trap; **HH**: Helical hairpin; **LD**: Lipoyl domain; **BCR**: Binding core region; **CS**: Cytoplasmic side; **TMD**: Transmembrane domain; **NBD**: Nucleotide binding domain; **PAβN**: Phneyl-arginine β-naphthylimide.