

Design, Synthesis, Antimicrobial and Antioxidant Evaluation, Cytotoxicity Assessment, and Molecular Dynamics-Based Computational Studies of Novel PABA Analogues as Dual DHPS/DHFR Inhibitors

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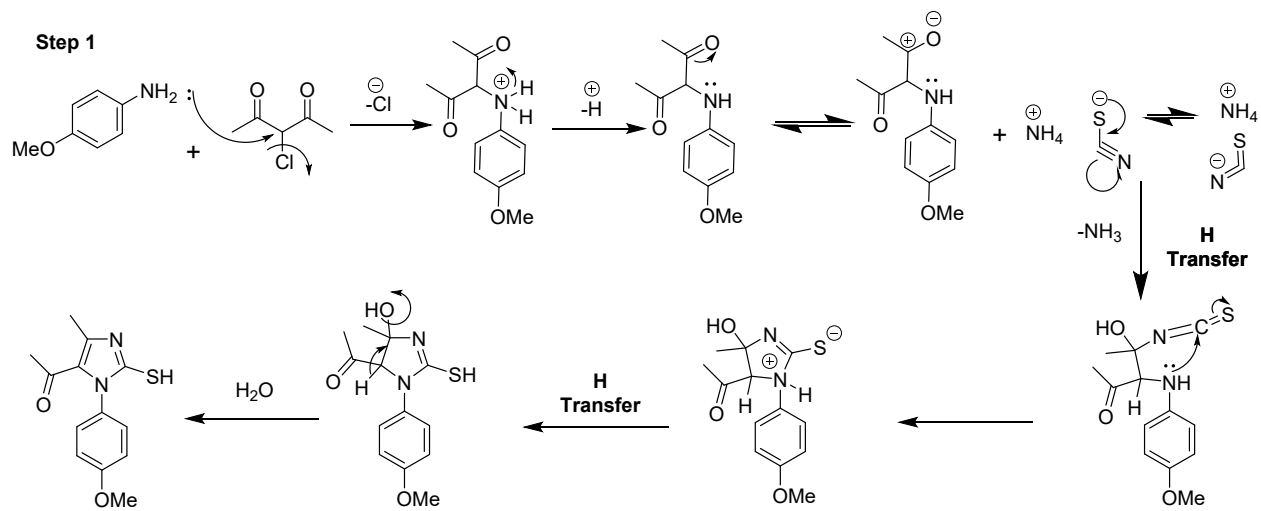
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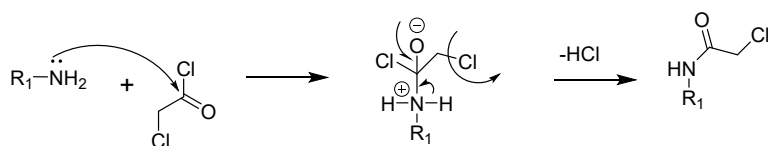
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Table S1. MIC Determination against MRSA and XDR *E.coli*

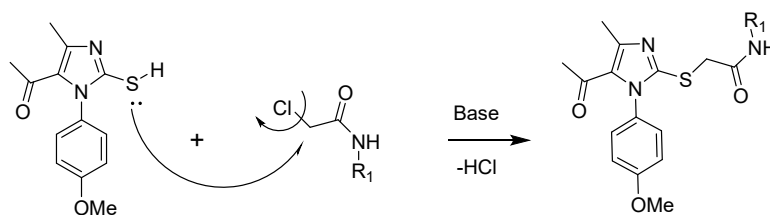
Figure S1: Reaction mechanism of synthesized compounds SB1-SB7



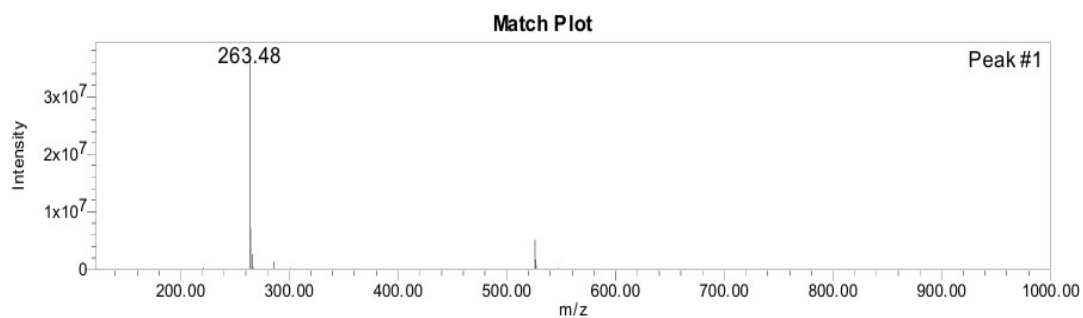
Step 2



Step 3



Complete Characterization Data



SampleName: DMInjection: 1 Name: Match1 Threshold: Base Peak 263.48 Channel Type 3D MS Channel
Description 1: 120.00-1000.00 ES+, Centroid, CV=10

Figure S2. ESI-MS spectrum of the lead molecule

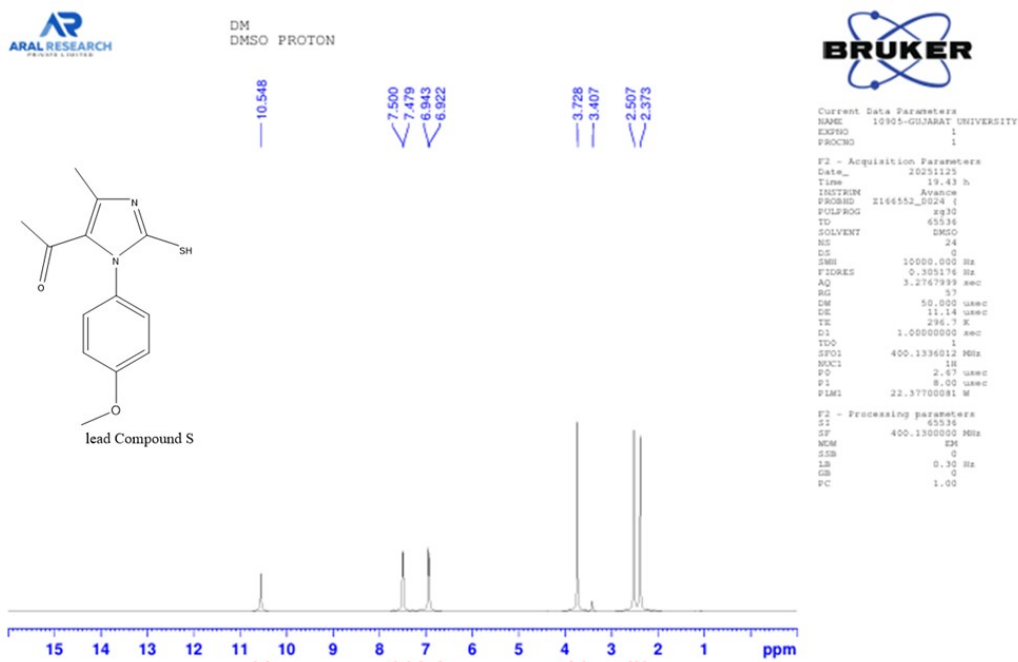


Figure S3. ¹H NMR spectrum of the lead molecule.

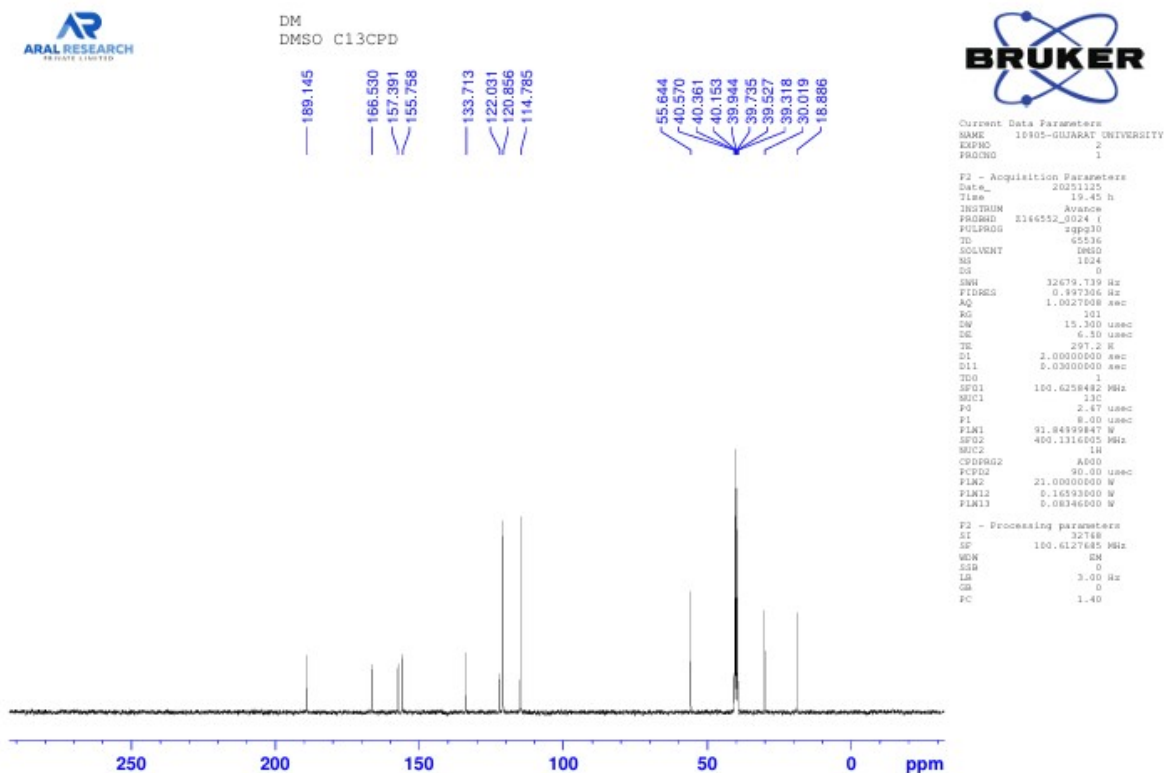


Figure S4. ¹³C NMR spectrum of the lead molecule.

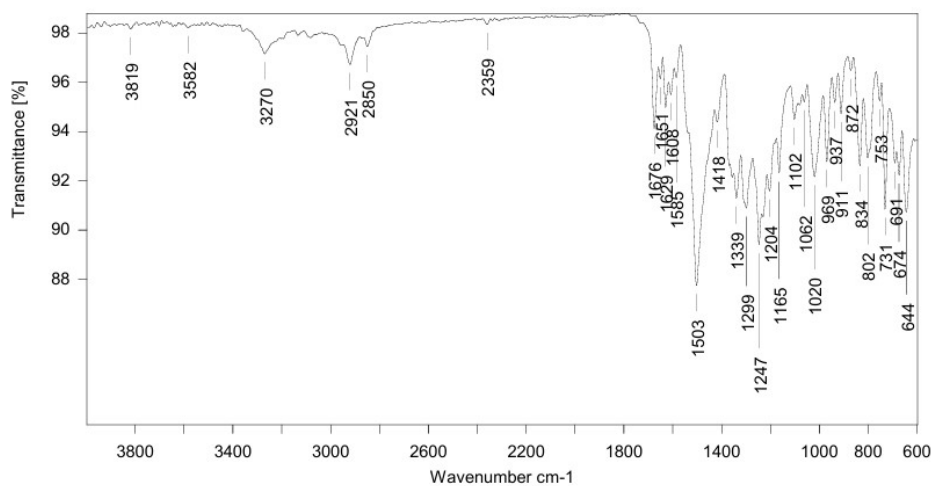


Figure S5. FTIR spectrum of SB1.

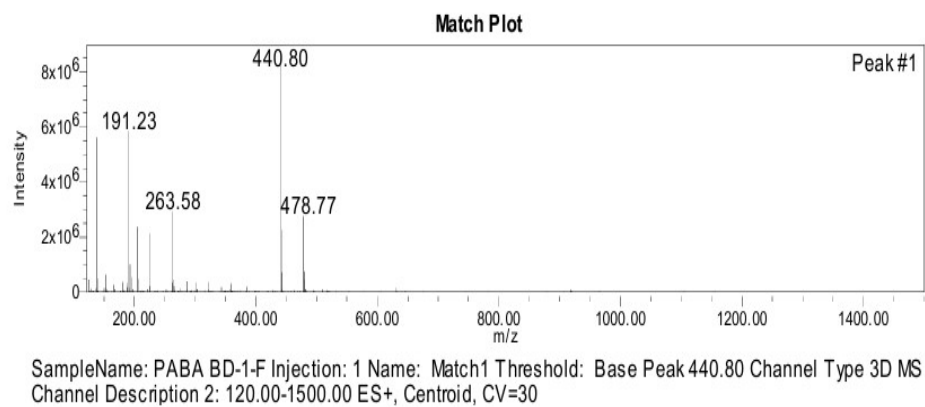
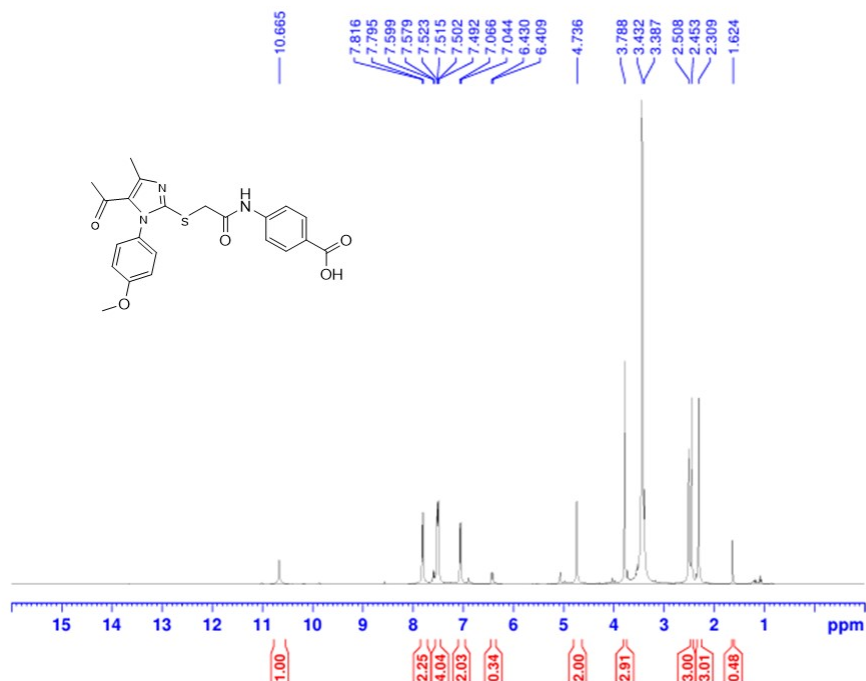


Figure S6. ESI-MS spectrum of SB1.

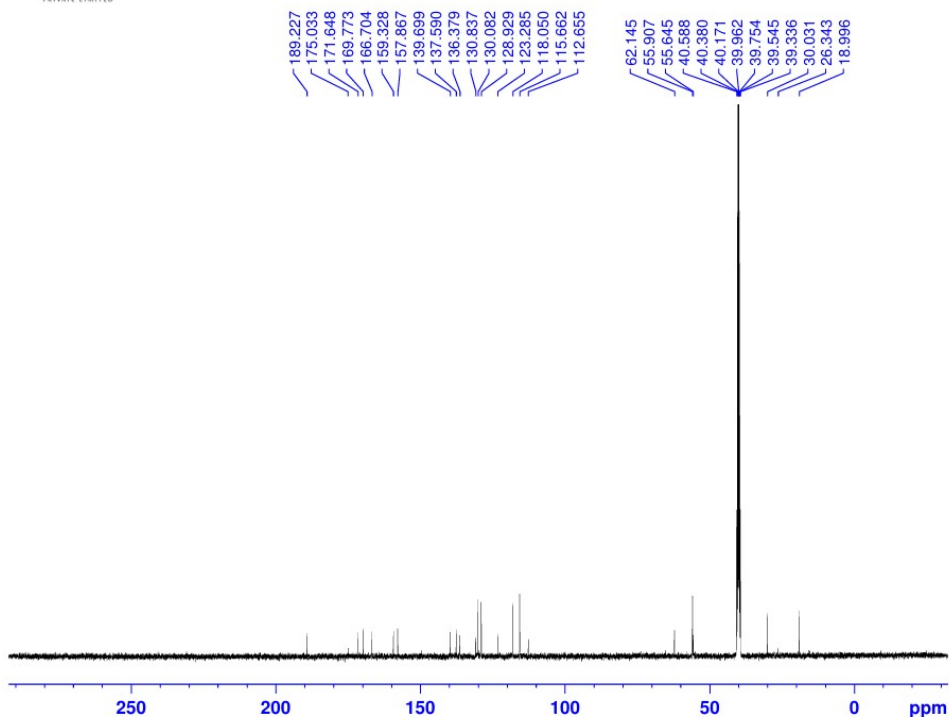


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PROCNO 1

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FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 101
DM 50.000 usec
DE 11.14 usec
TE 297.3 K
D1 1.0000000 sec
TDO 1
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NUC1 1H
PO 2.67 usec
P1 8.00 usec
PLW1 22.3770081 W

F2 - Processing parameters
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SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Figure S7. ¹H NMR spectrum of SB1.

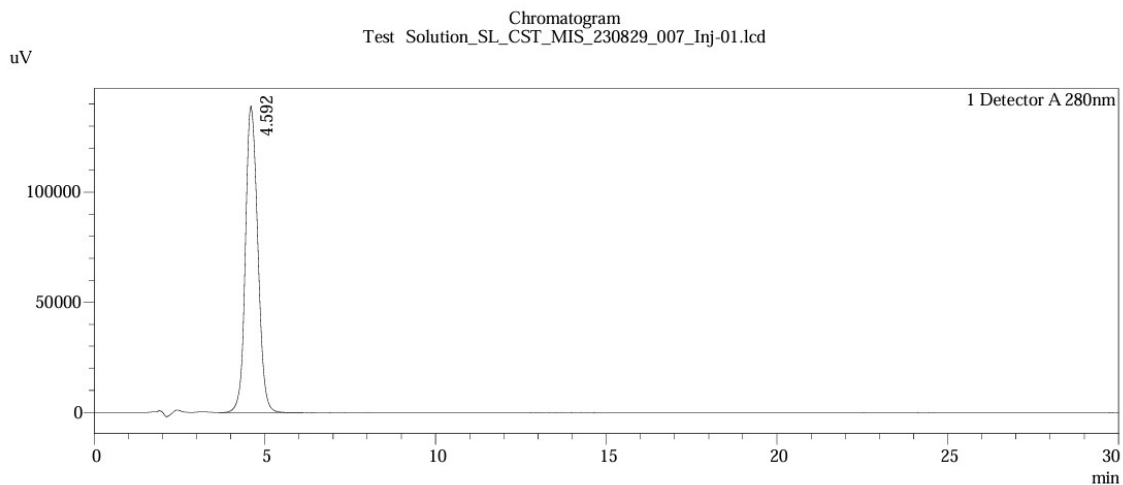


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PROCNO 1

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FIDRES 0.597306 Hz
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DE 6.50 usec
TE 298.0 K
D1 2.0000000 sec
D11 0.0300000 sec
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NUC1 13C
PO 2.67 usec
P1 8.00 usec
PLW1 104.5000000 W
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG2 A000
PCPD2 90.00 usec
PLW2 22.3770081 W
PLW12 0.17681000 W
PLW13 0.08893200 W

F2 - Processing parameters
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WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

Figure S8. ¹³C NMR spectrum of SB1.



Peak Table Test Solution_SL_CST_MIS_230829_007_Inj-01.lcd

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1	4.592	SB-1	3531599	100.000	1.126	5218
Total			3531599	100.000		

Figure S9: HPLC purity analysis of SB1

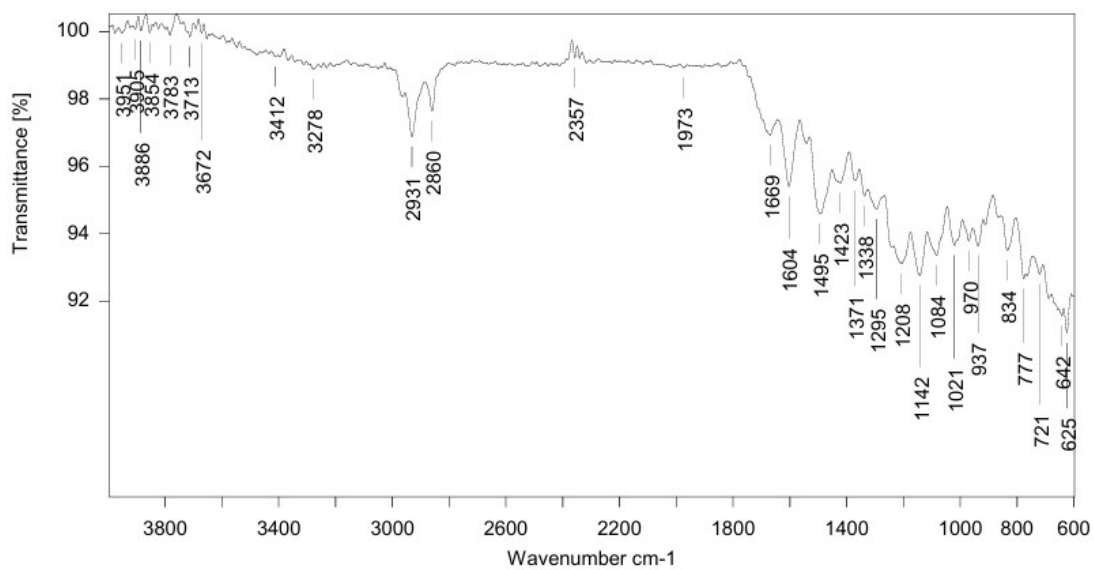
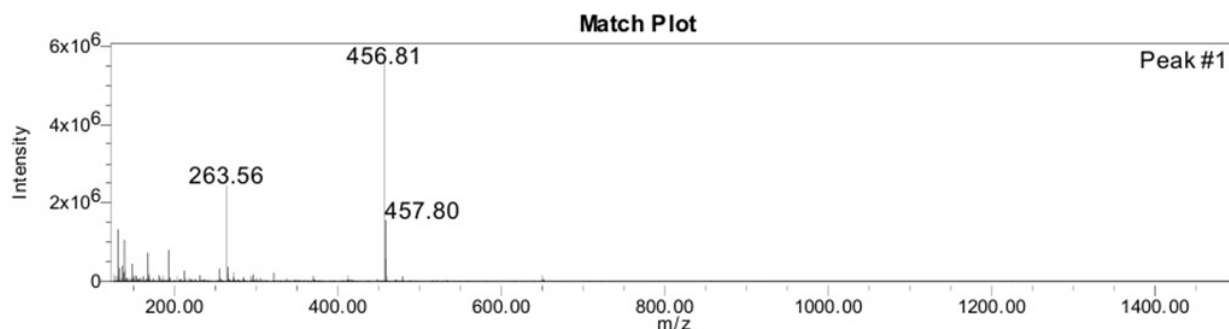


Figure S10. FTIR spectrum of SB2.



SampleName: SB-2 Injection: 1 Name: Match1 Threshold: Base Peak 456.81 Channel Type 3D MS Channel Description 2: 120.00-1500.00 ES+, Centroid, CV=30

Figure S11. ESI-MS spectrum of SB2.

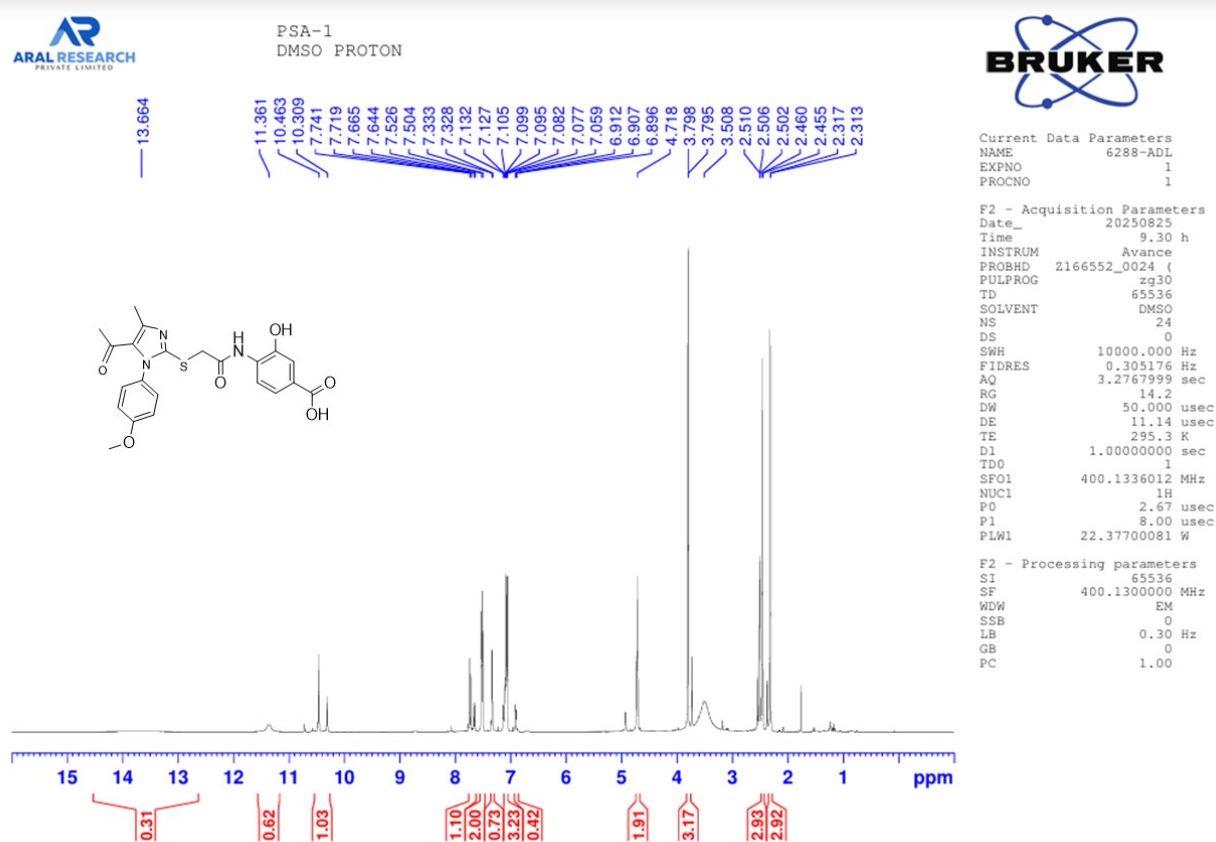


Figure S12. ¹H NMR spectrum of SB2.

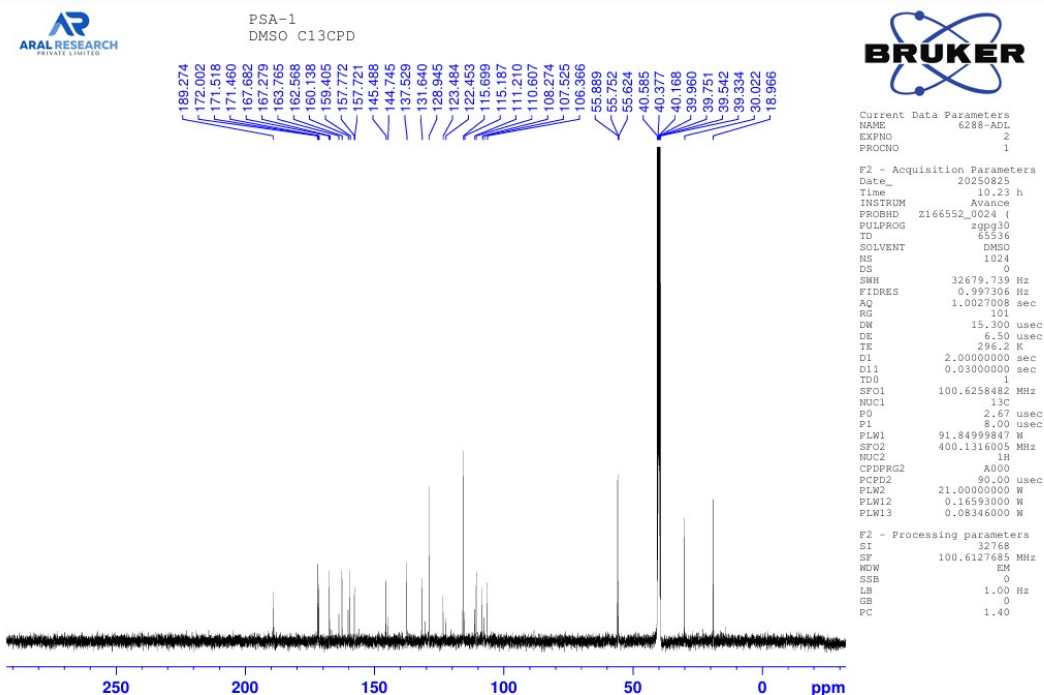


Figure S13. ¹³C NMR spectrum of SB2.

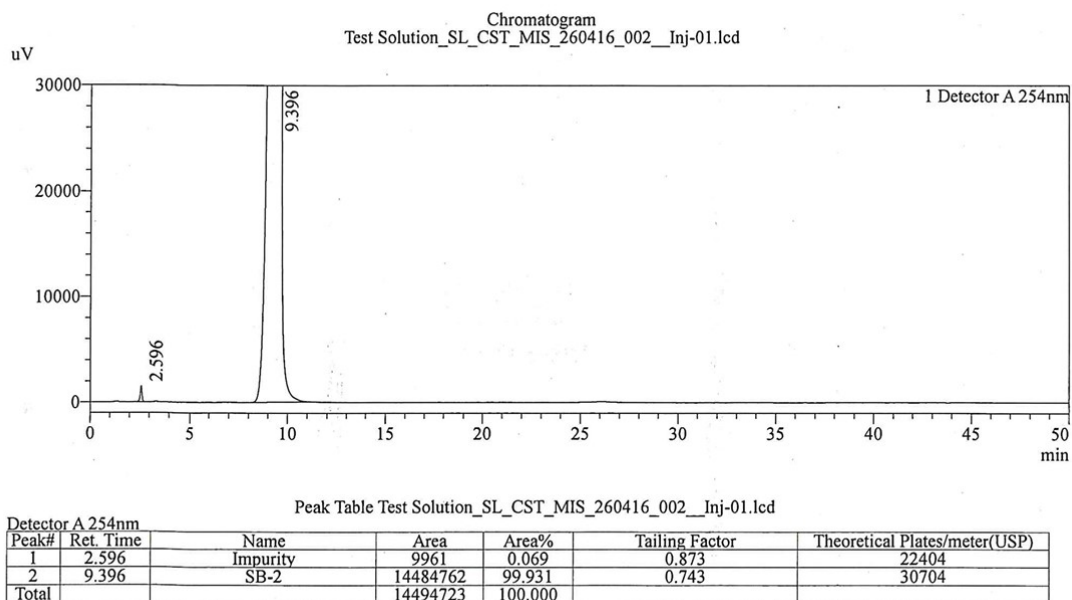


Figure S14: HPLC purity assay of SB2

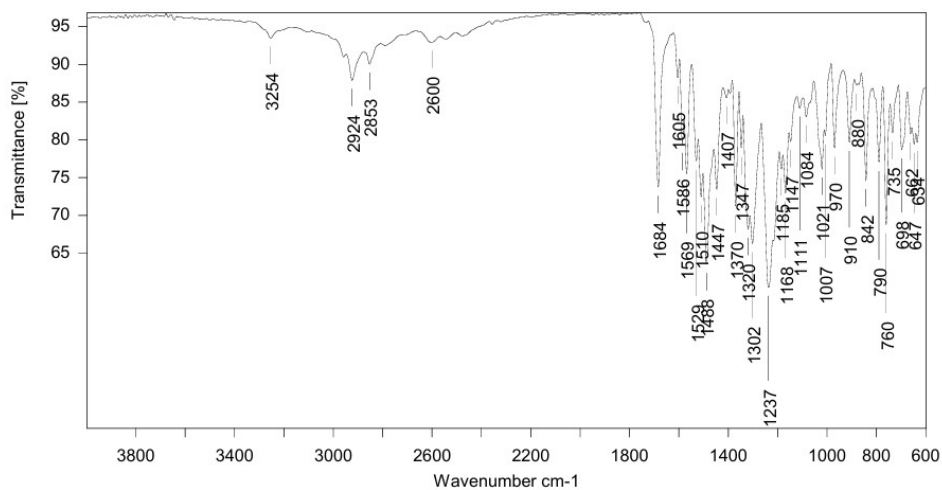


Figure S15. FTIR spectrum of SB3.

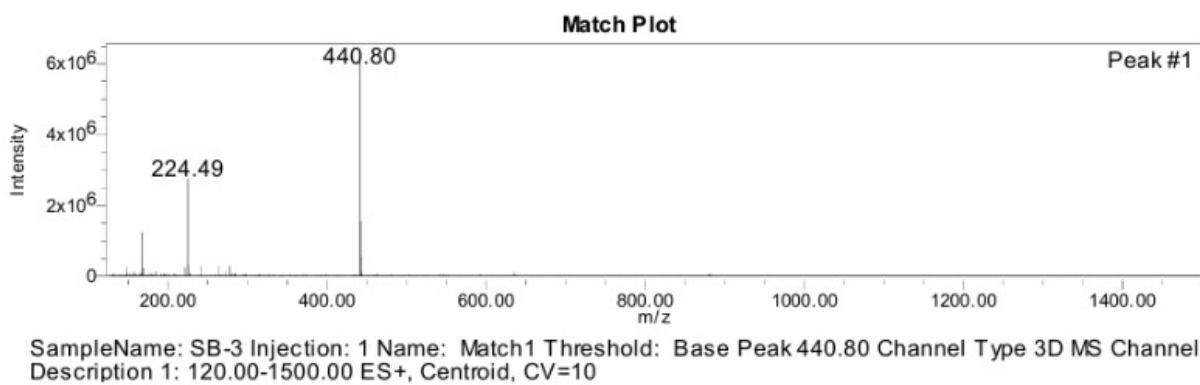


Figure S16. ESI-MS spectrum of SB3.

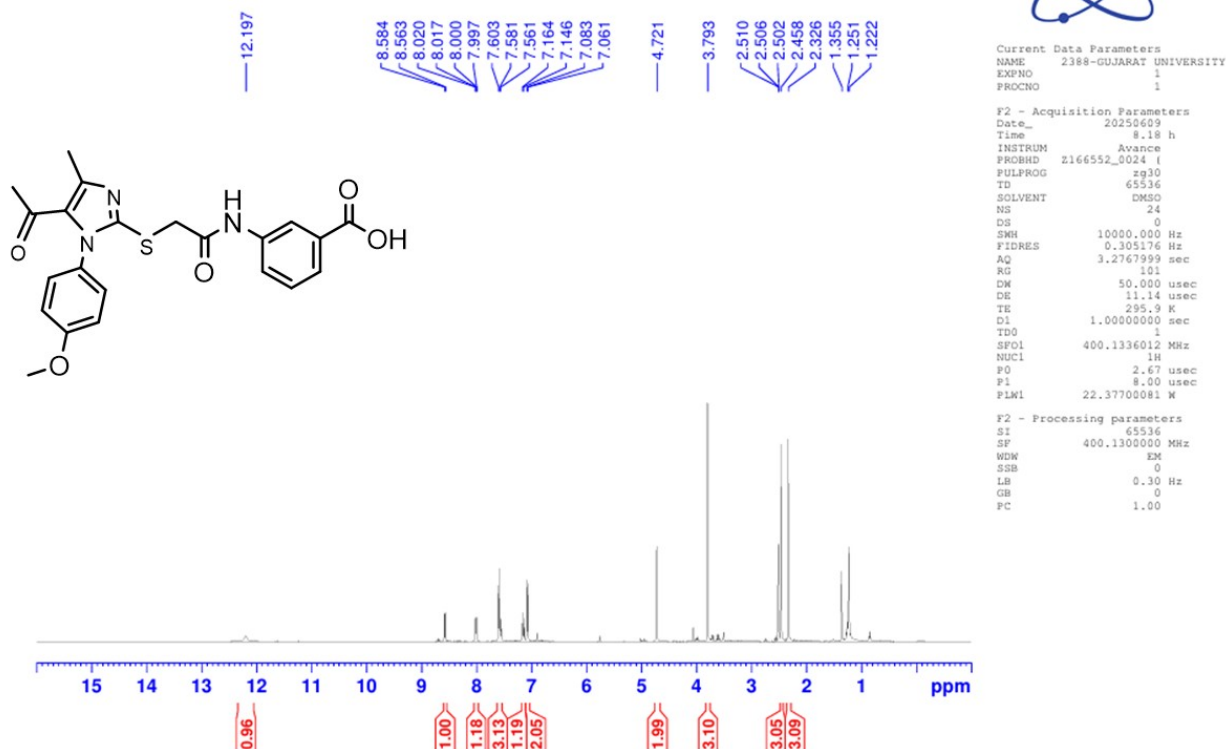


Figure S17. ¹H NMR spectrum of SB3.

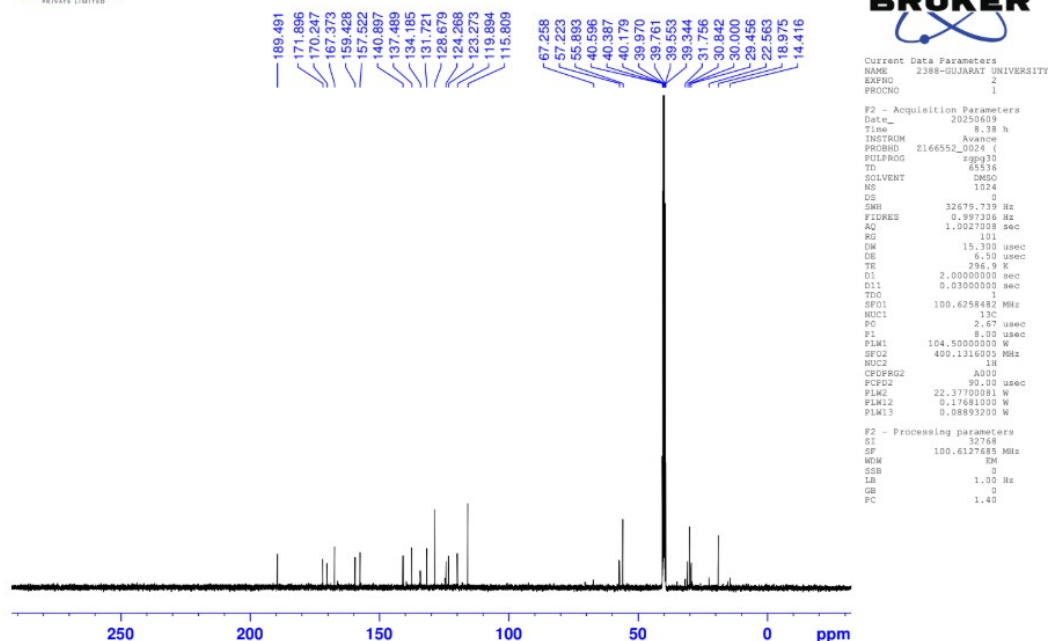


Figure S18. ¹³C NMR spectrum of SB3.

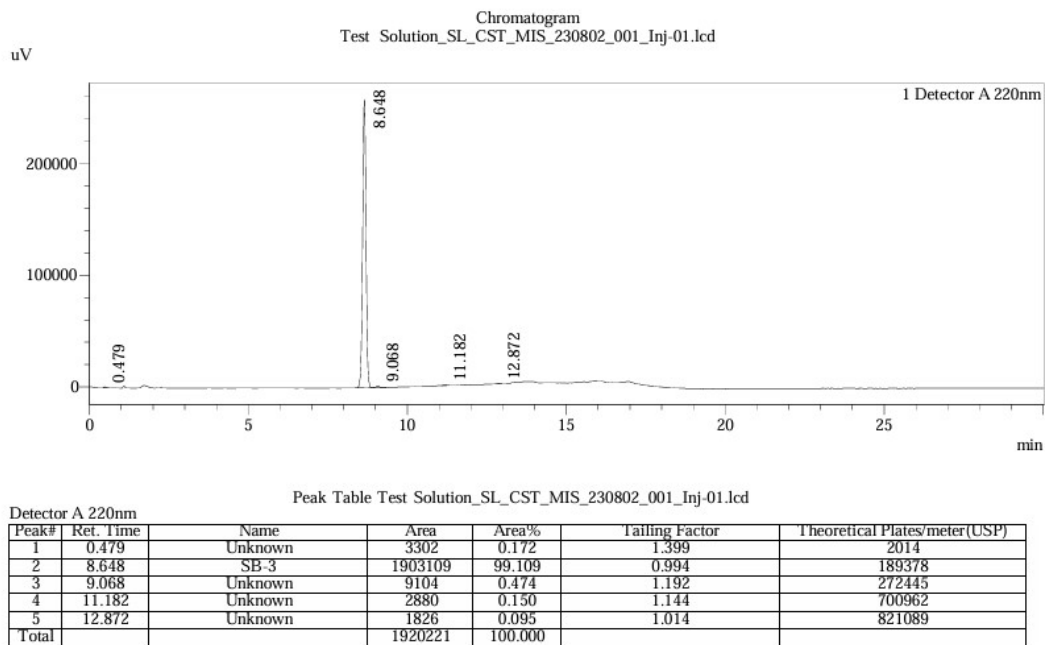


Figure S19. HPLC purity assay of SB3

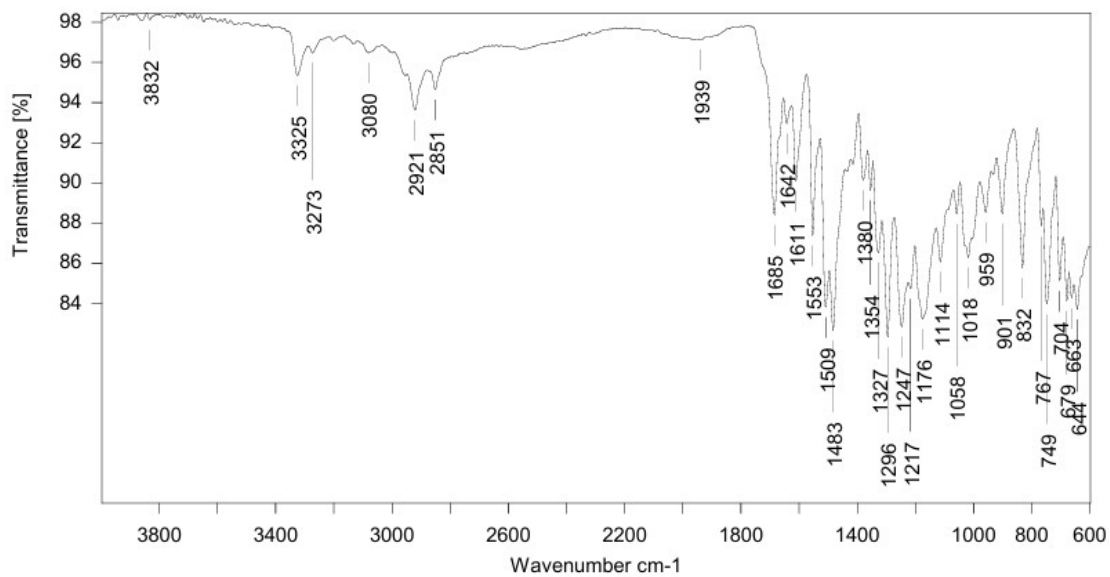


Figure S20. FTIR spectrum of SB4.

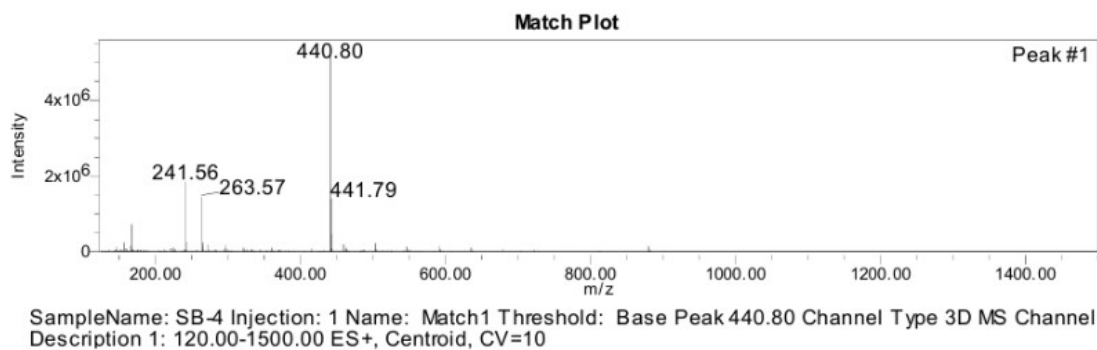


Figure S21. ESI-MS spectrum of SB4.

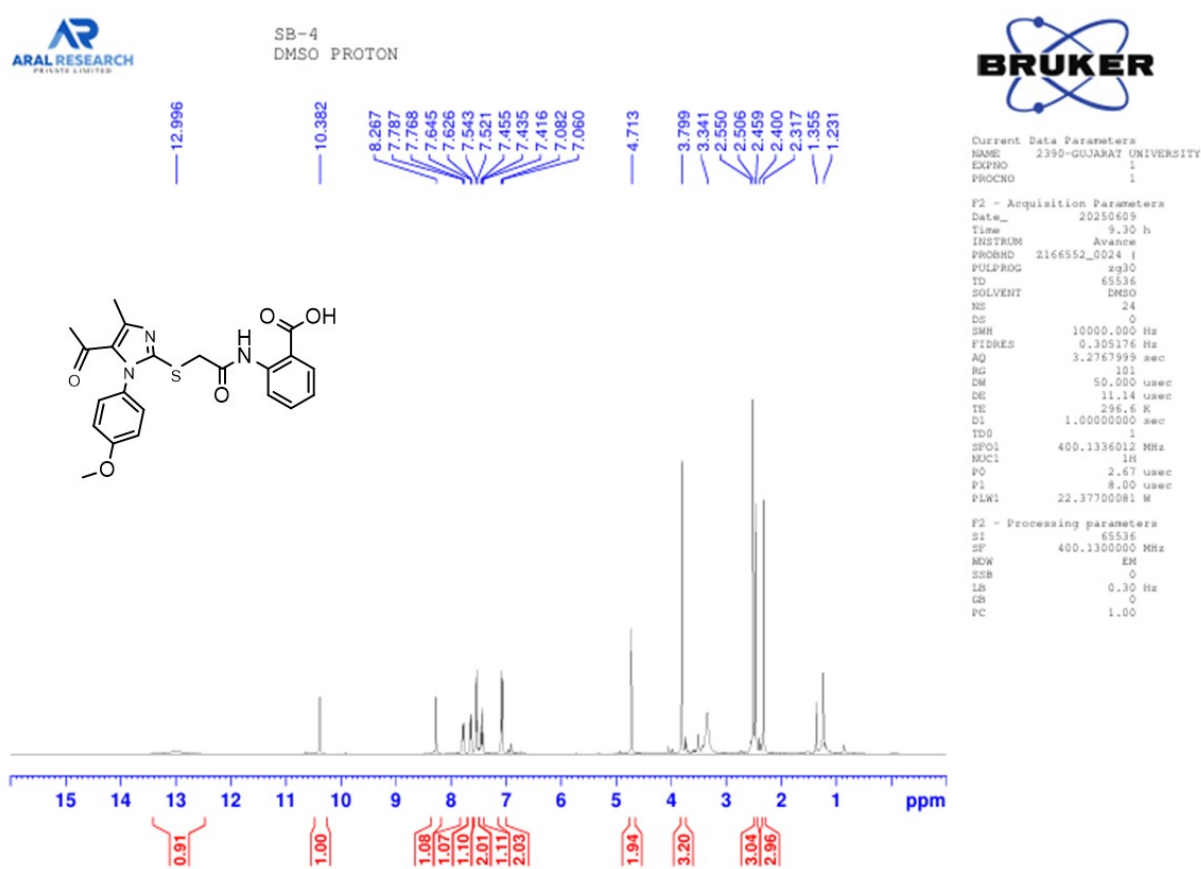


Figure S22. ¹H NMR spectrum of SB4.



SB-4
DMSO C13CPD

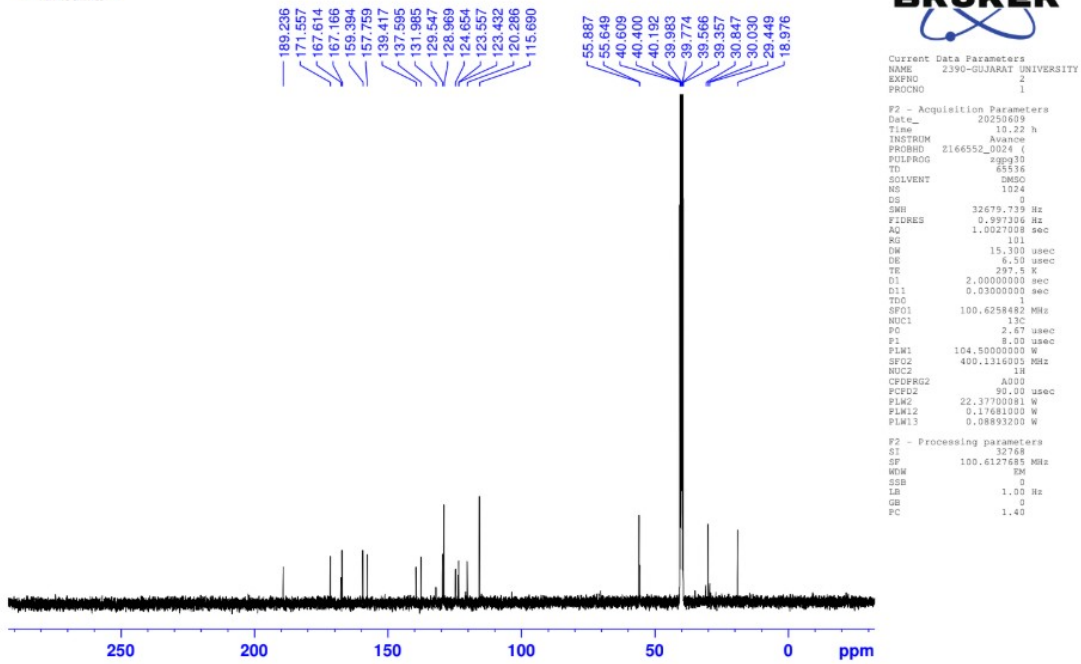
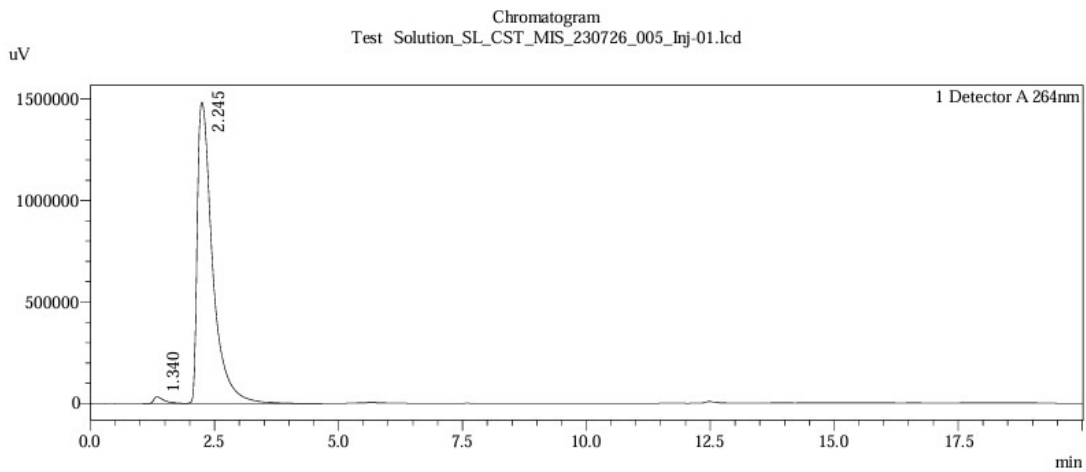


Figure S23. ¹³C NMR spectrum of SB4.



Peak Table Test Solution_SL_CST_MIS_230726_005_Inj-01.lcd

Peak#	Ret. Time	Name	Area	Area%	Tailing Factor	Theoretical Plates/meter(USP)
1	1.340	Impurity	463051	1.431	2.088	1507
2	2.245	SB-4	31889161	98.569	2.374	2112
Total			32352212	100.000		

Figure S24. HPLC purity assay of SB4

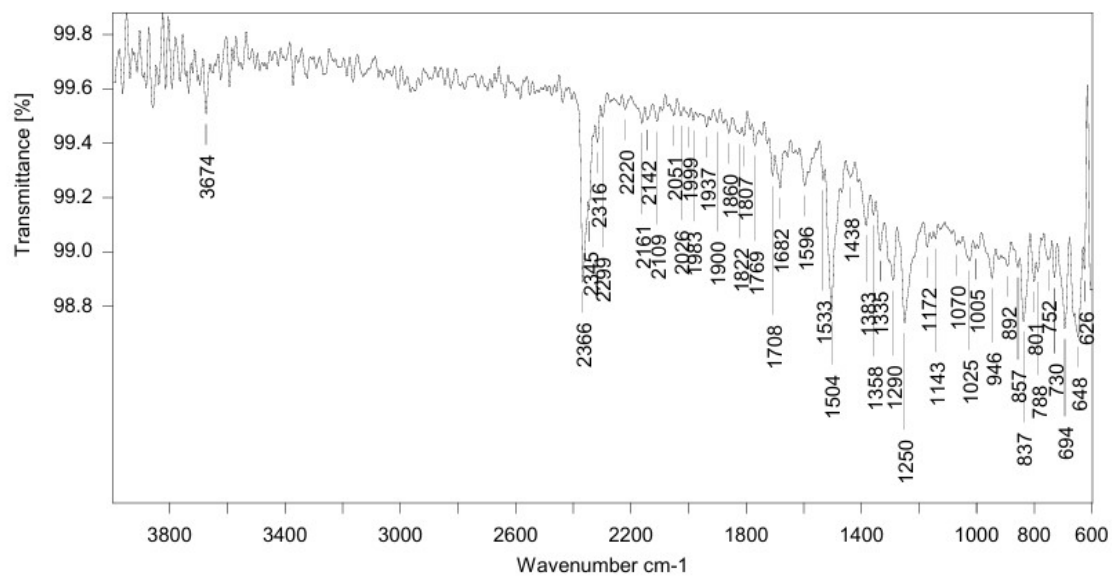
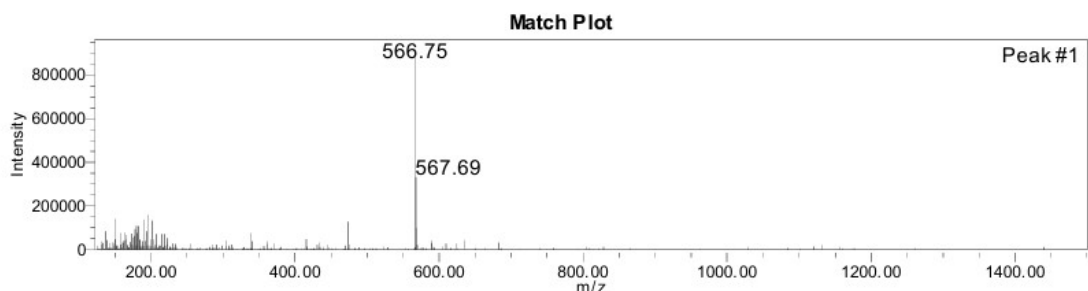
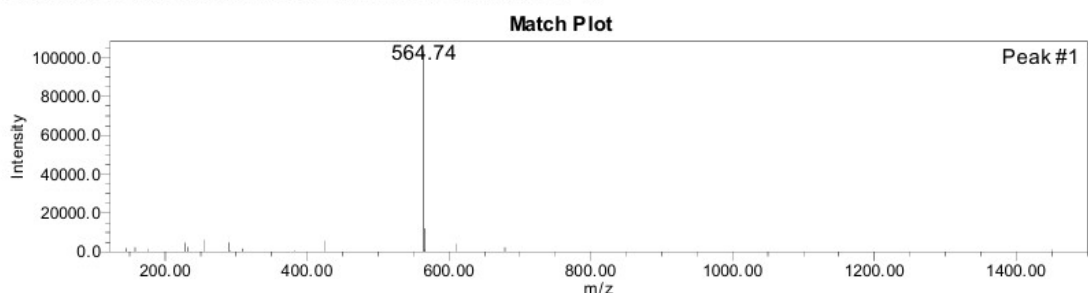


Figure S25. FTIR spectrum of SB5.



SampleName: COMPOUND_SB-8 Injection: 1 Name: Match1 Threshold: Base Peak 566.75 Channel Type 3D MS Channel Description 2: 120.00-1500.00 ES+, Centroid, CV=30



SampleName: COMPOUND_SB-8 Injection: 1 Name: Match1 Threshold: Base Peak 564.74 Channel Type 3D MS Channel Description 4: 120.00-1500.00 ES-, Centroid, CV=30

Figure S26. ESI-MS spectrum of SB5.

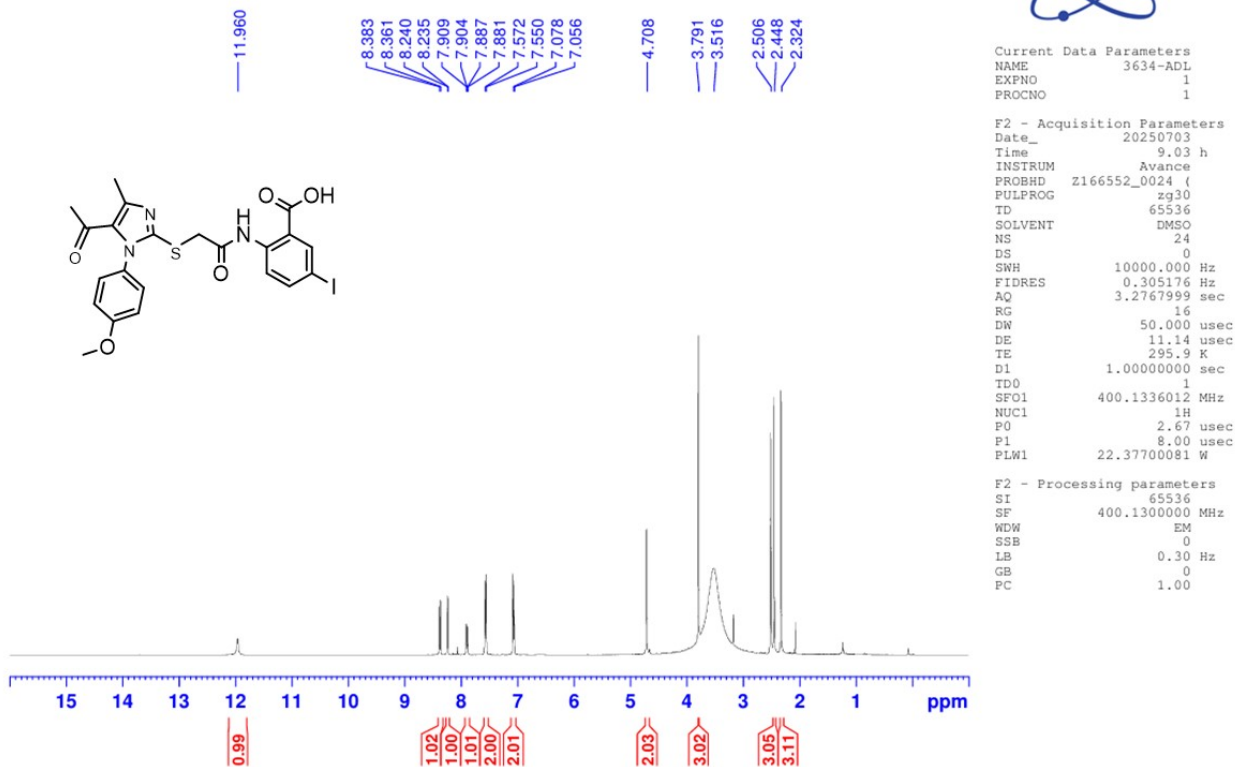


Figure S27. ¹H NMR spectrum of SB5.

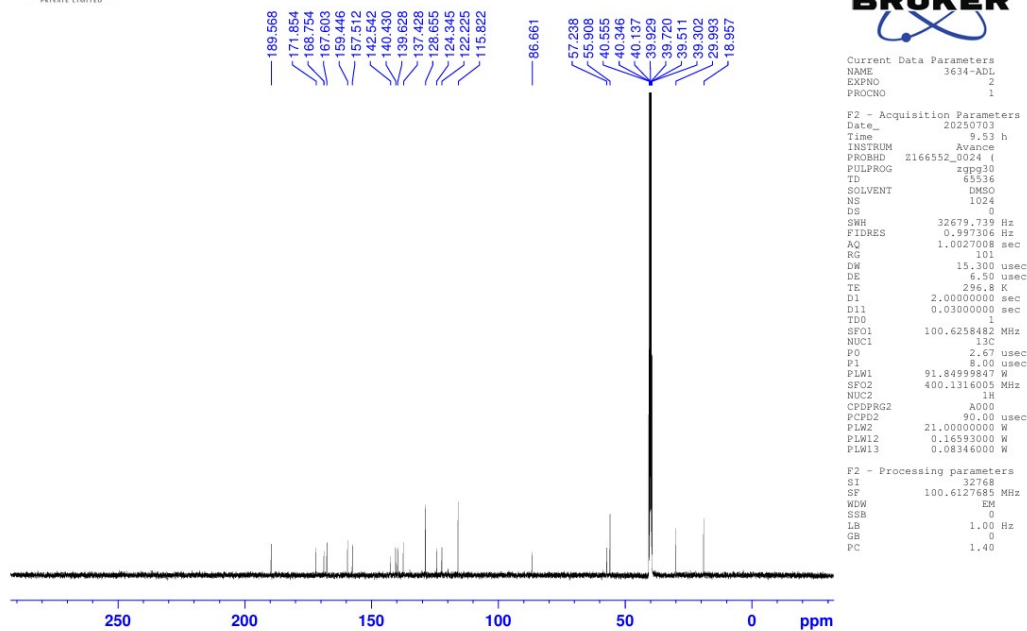
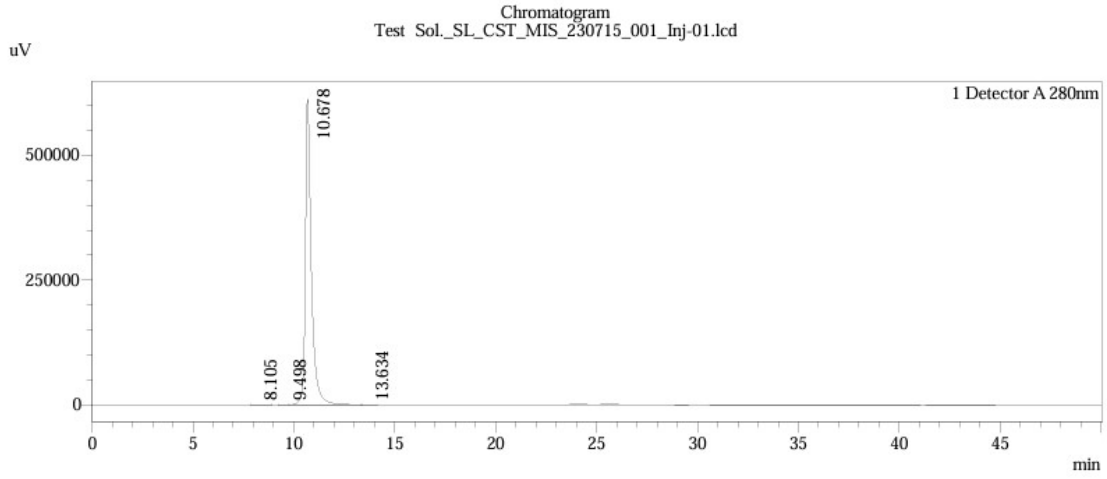


Figure S28. ¹³C NMR spectrum of SB5.



Peak Table Test Sol_SL_CST_MIS_230715_001_Inj-01.lcd

Peak#	Ret. Time	Name	Area	Area%	Tailing Factor	Theoretical Plates/meter(USP)
1	8.105	Unknown	7435	0.059	2.360	25357
2	9.498	Unknown	2799	0.022	0.957	70927
3	10.678	SB-5	12593152	99.896	1.550	52140
4	13.634	Unknown	2824	0.022	1.618	110539
Total			12606211	100.000		

Figure S29. HPLC purity assay of SB5

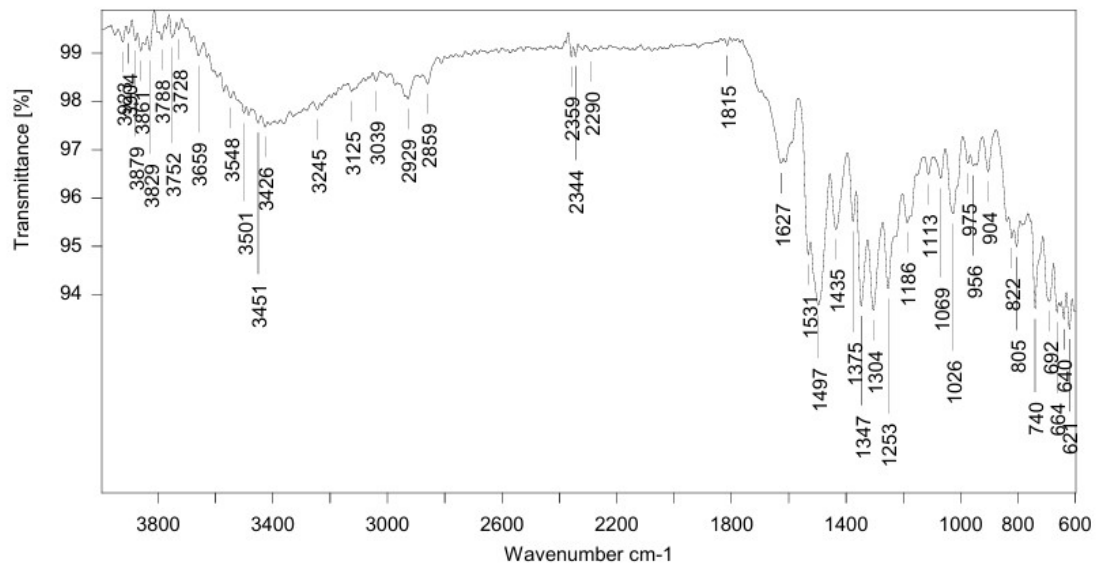


Figure S30. FTIR spectrum of SB6.

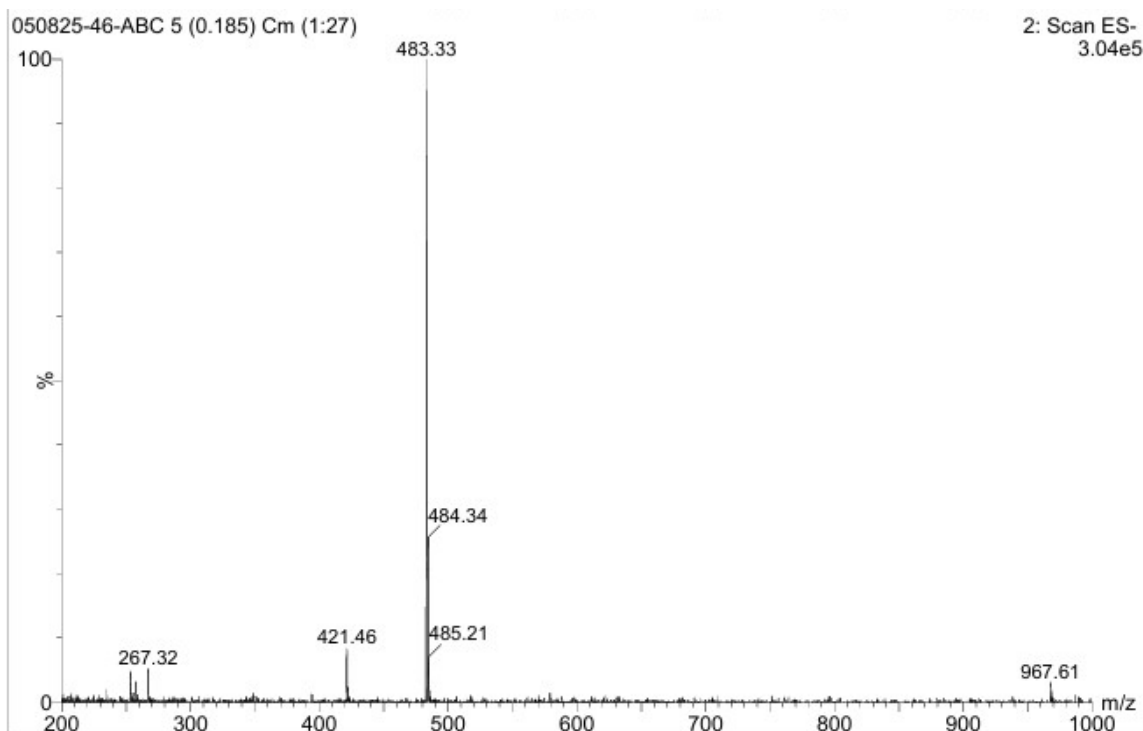


Figure S31. ESI-MS spectrum of SB6.

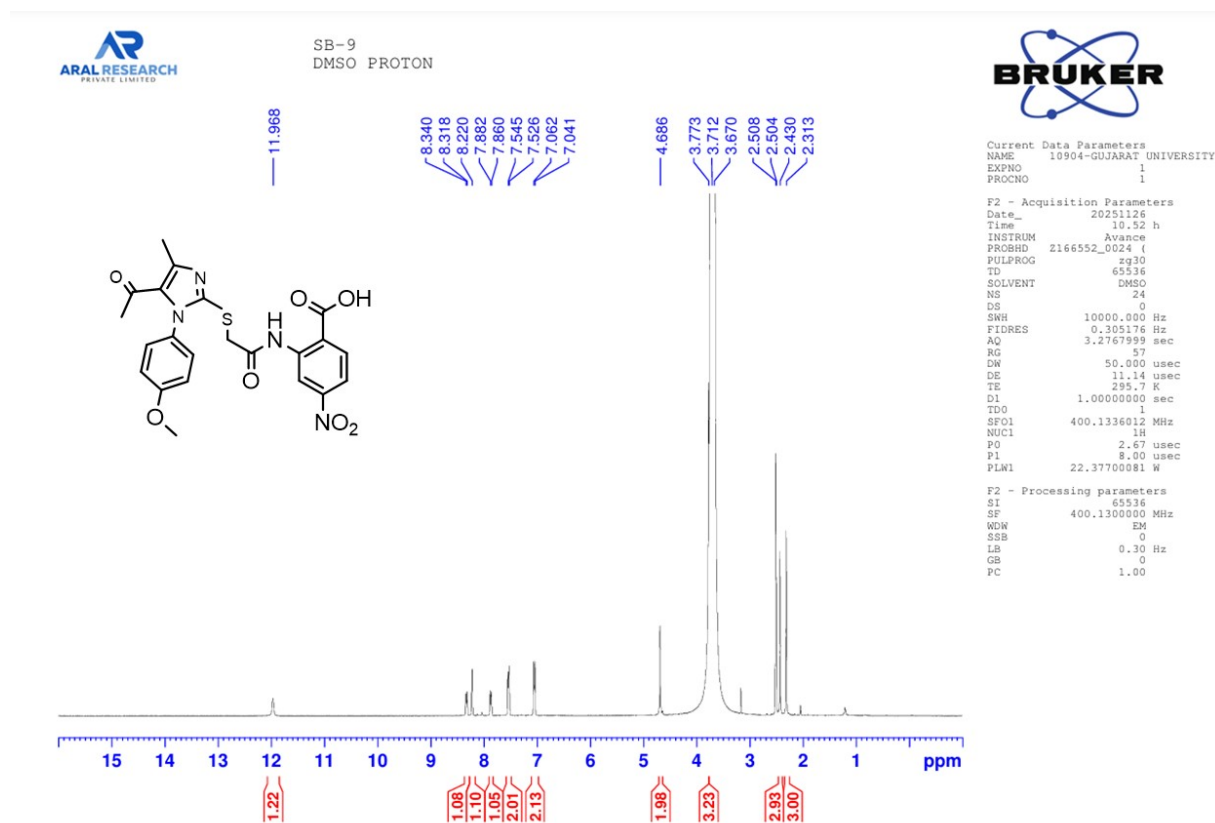


Figure S32. ¹H NMR spectrum of SB6.



SB-9
DMSO C13CPD

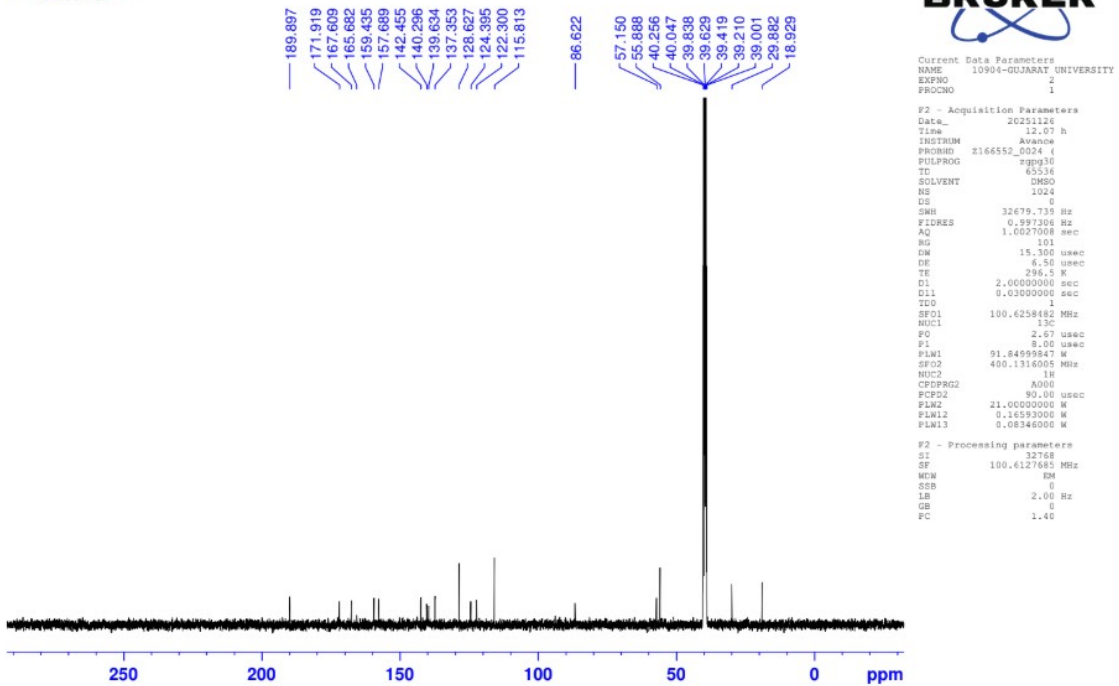
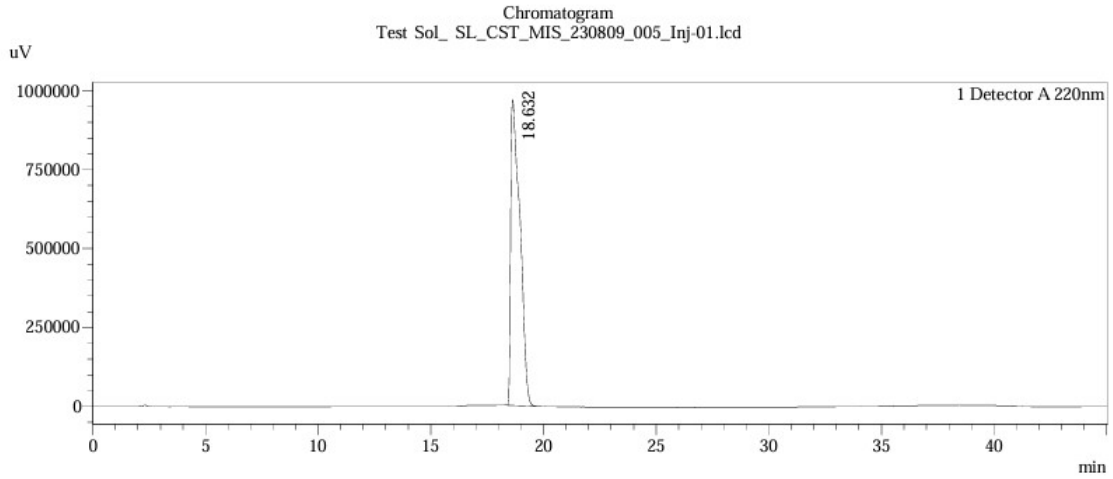


Figure S33. ¹³C NMR spectrum of SB6.



Peak Table Test Sol_ SL_CST_MIS_230809_005_Inj-01.lcd

Peak#	Ret. Time	Name	Area	Area%	Tailing Factor	Theoretical Plates/meter(USP)
1	18.632	SB-6	27547040	100.000	2.582	59916
Total			27547040	100.000		

Figure S34. HPLC purity assay of SB6

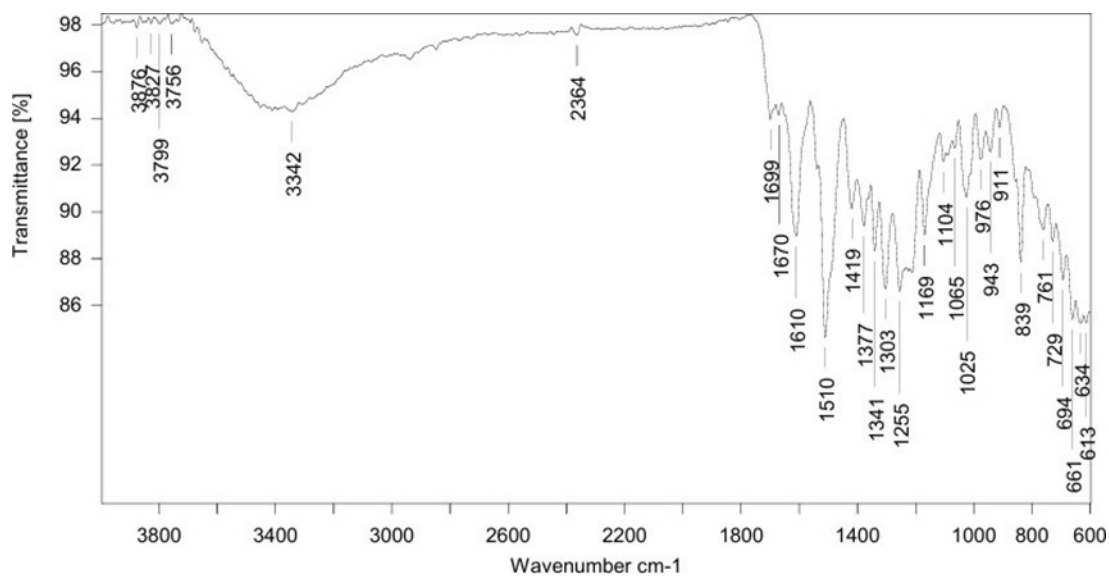


Figure S35. FTIR spectrum of SB7.

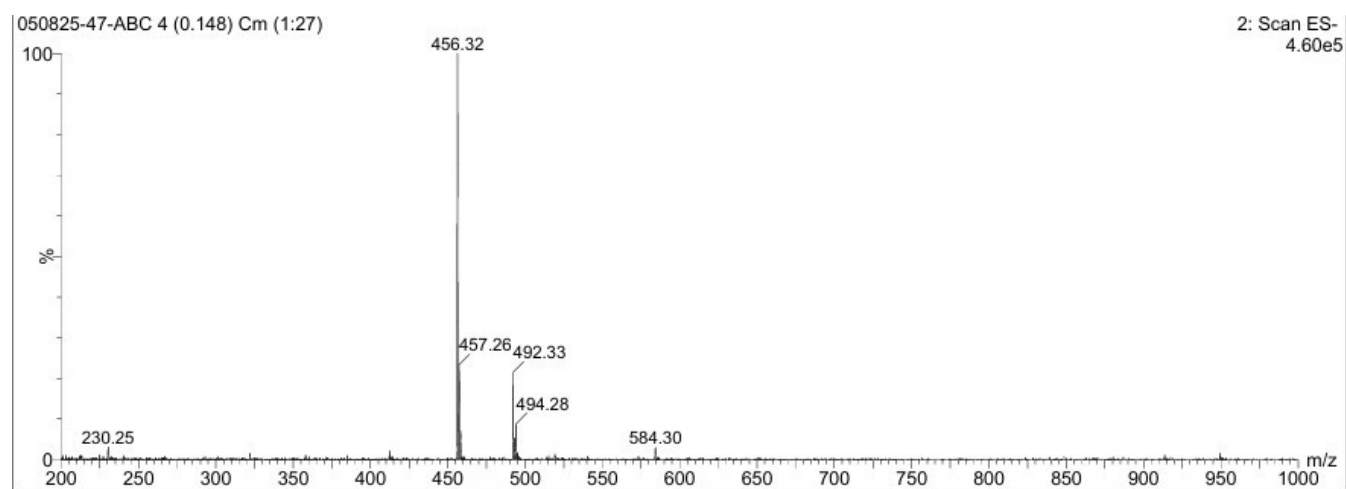
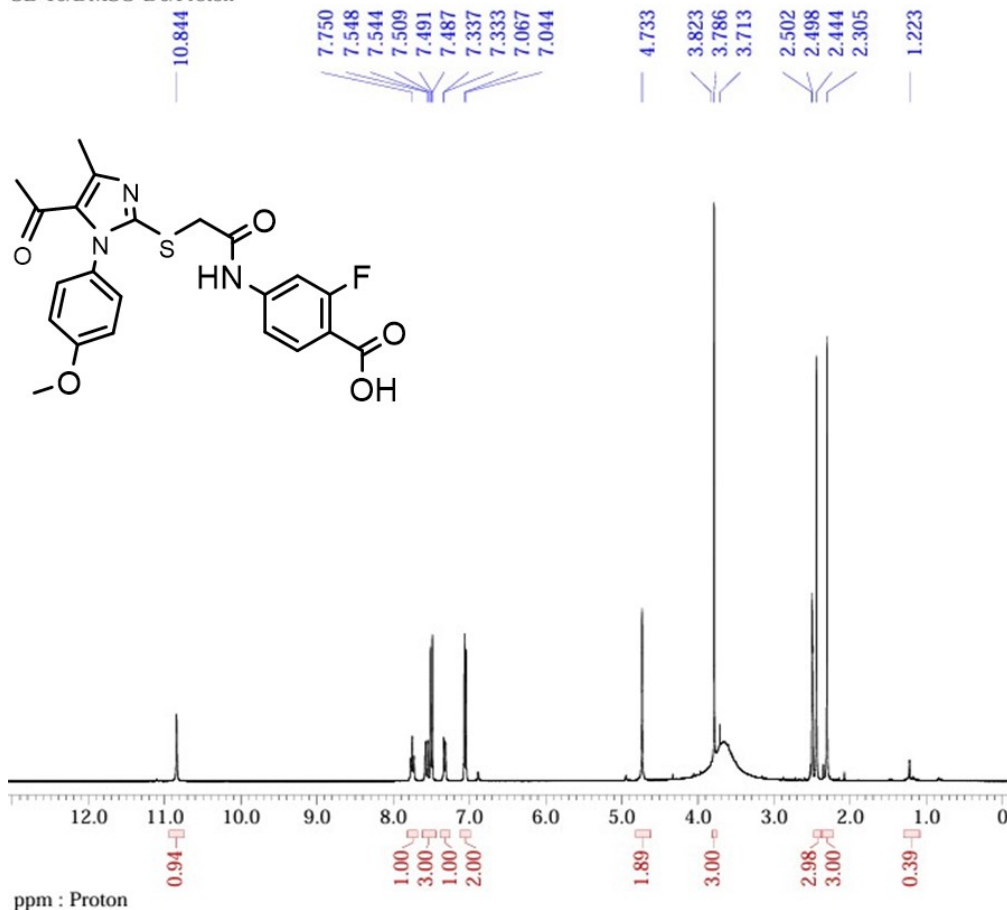


Figure S36. ESI-MS spectrum of SB7.

SB-10/DMSO-D6/Proton



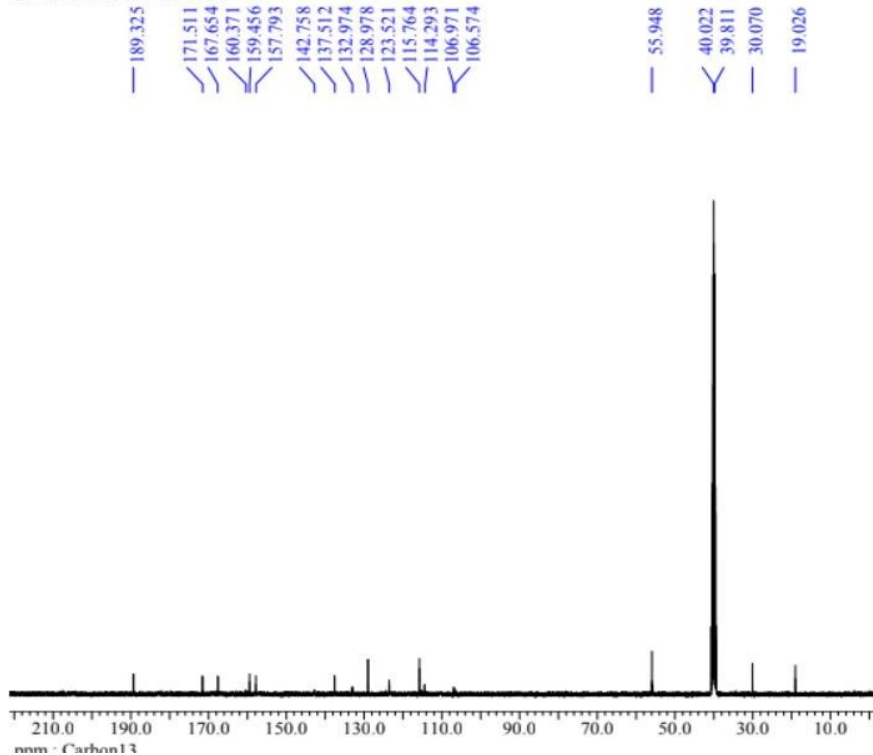
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fft( 1, TRUE, TRUE )
machinphase
ppm
auto_reference( 5[%], TRUE )
reference( 2.49189[ppm], 2.5[ppm] )

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Author        = zasya
Solvent       = DMSO-D6
Experiment    = single_pulse_jxp
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Revision_Time = 6-AUG-2025 09:47:35
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X_Angle      = 45[deg]
X_Atn        = 1.5[dB]
X_Pulse      = 2.625[us]
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Temp_Get     = 25[dC]
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Figure S37. ¹H NMR spectrum of SB7.

SB-10 1/DMSO-D6/Carbon



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ppm
thresh( 5[%], 1 )
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Author        = zasya
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Dia_Units    = [ppm]
Scans        = 512
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X_Offset     = 100[ppm]
X_Points     = 31415
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Irr_Offset   = 5[ppm]
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X_Angle      = 30[deg]
X_Atn        = 4.5[dB]
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X_90_Width   = 9.925[us]
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Figure S38. ¹³C NMR spectrum of SB7.

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PROCNO 1

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DS 0
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FIDRES 2.243940 Hz
AQ 0.4456448 sec
RG 101
DW 3.400 usec
DE 6.50 usec
TE 295.4 K
D1 1.0000000 sec
D11 0.0300000 sec
TD0 1
SFO1 376.4607164 MHz
NUC1 19F
P1 12.00 usec
PLM1 33.05300140 W
SFO2 400.1316005 MHz
NUC2 1H
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PLW2 20.65200043 W
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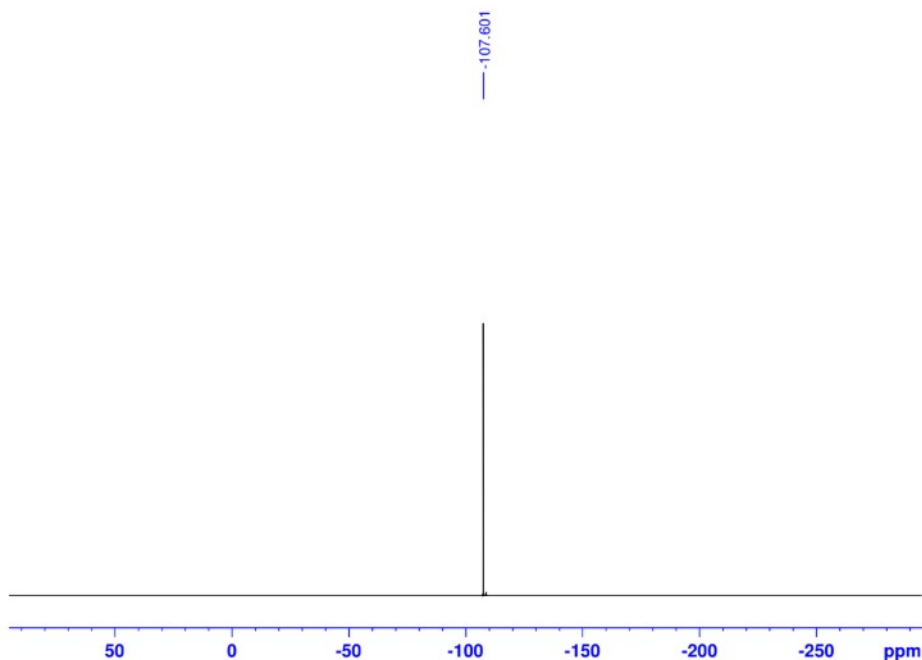
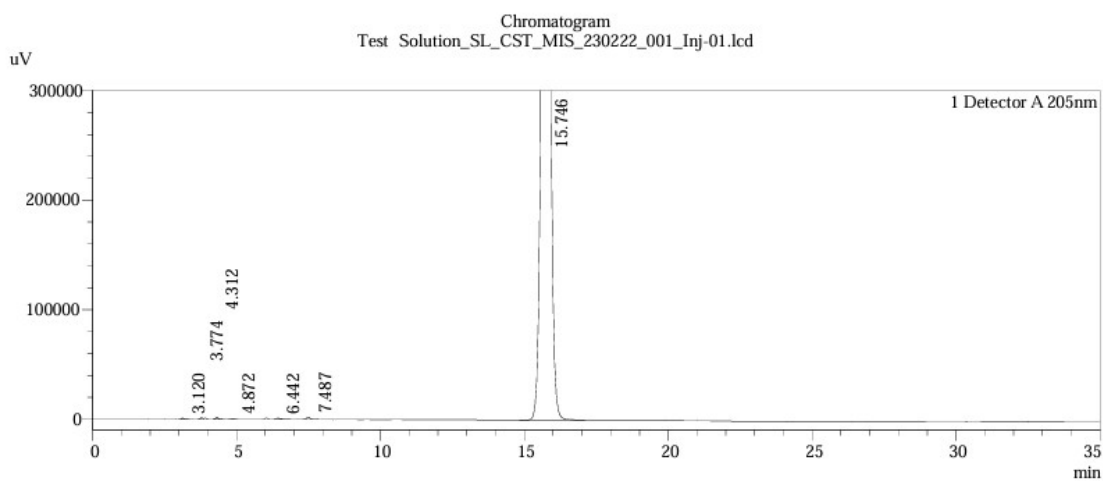


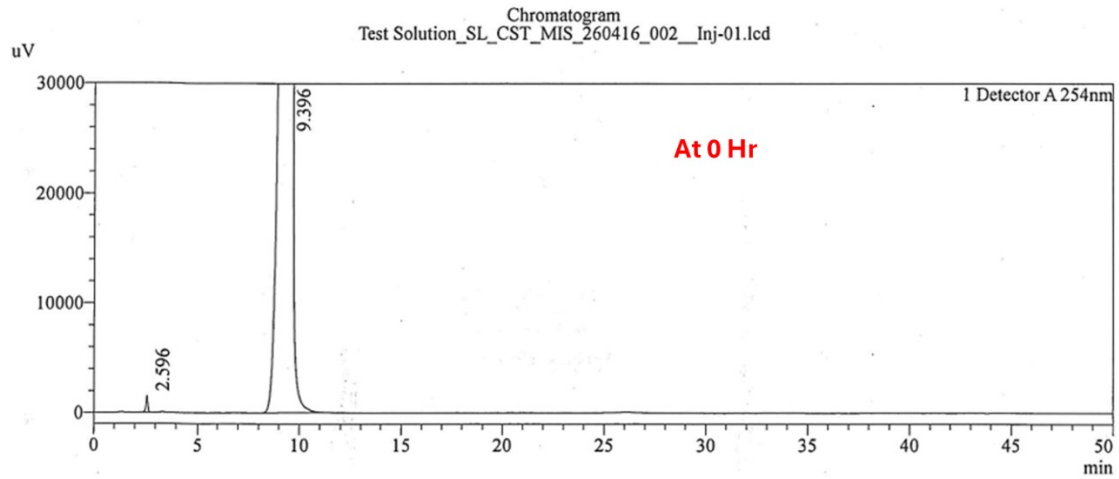
Figure S39. ¹⁹F NMR spectrum of SB7



Peak Table Test Solution_SL_CST_MIS_230222_001_Inj-01.lcd

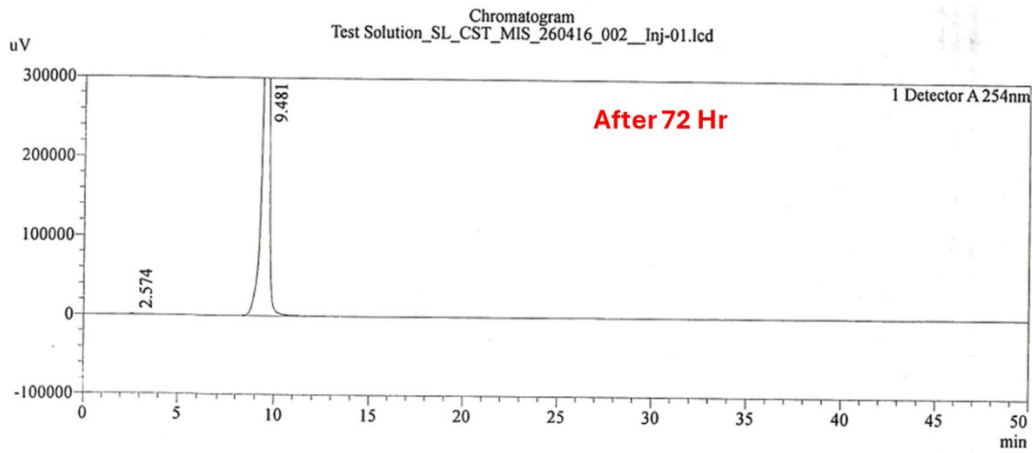
Peak#	Ret. Time	Name	Area	Area%	Tailing Factor	Theoretical Plates/meter(USP)
1	3.120	Unknown Impurity	5847	0.035	1.367	43893
2	3.774	Unknown Impurity	5497	0.033	1.013	79346
3	4.312	Unknown Impurity	11209	0.068	1.401	65100
4	4.872	Unknown Impurity	4534	0.027	1.050	72315
5	6.442	Unknown Impurity	5691	0.034	1.093	114580
6	7.487	Unknown Impurity	16140	0.097	1.102	117552
7	15.746	SB-7	16527980	99.705	1.021	109606
Total			16576898	100.000		

Figure S40. HPLC purity assay of SB7



Peak Table Test Solution_SL_CST_MIS_260416_002_Inj-01.lcd

Detector A 254nm						
Peak#	Ret. Time	Name	Area	Area%	Tailing Factor	Theoretical Plates/meter(USP)
1	2.596	Impurity	9961	0.069	0.873	22404
2	9.396	SB-2	14484762	99.931	0.743	30704
Total			14494723	100.000		



Peak Table Test Solution_SL_CST_MIS_260416_002_Inj-01.lcd

Detector A 254nm						
Peak#	Ret. Time	Name	Area	Area%	Tailing Factor	Theoretical Plates/meter(USP)
1	2.574	Impurity	9578	0.067	0.956	22506
2	9.481	SB-2	14372988	99.933	0.837	34338
Total			14382566	100.000		

Figure S41: HPLC stability assay for SB2

Antimicrobial Evolution against MRSA (60 ug/ml)

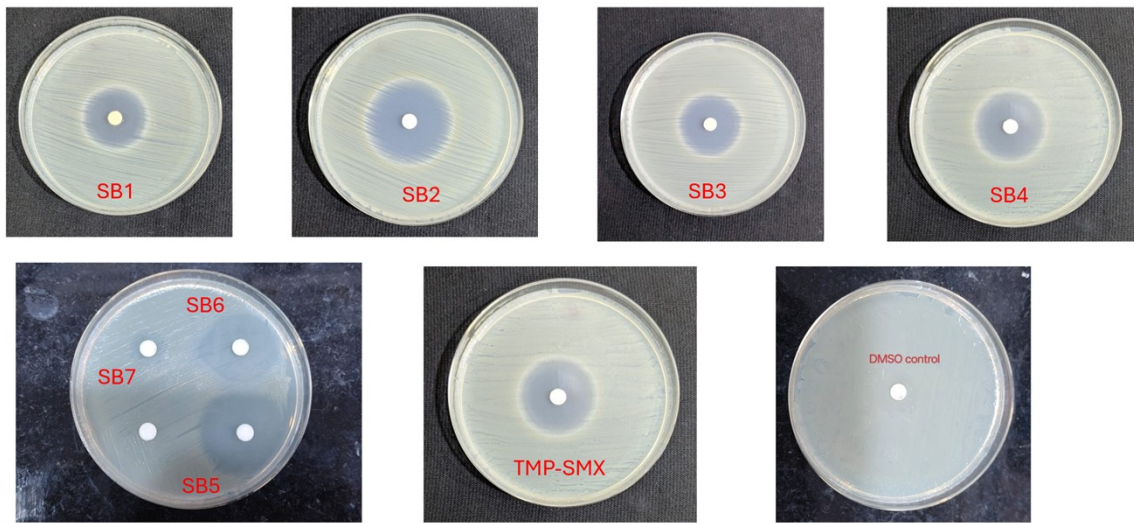


Figure S42. Images of inhibition zone of compound SB1-SB7 in comparison with SUL-TMP against MRSA

Antimicrobial Evolution against XDR E.coli (60 ug/ml)

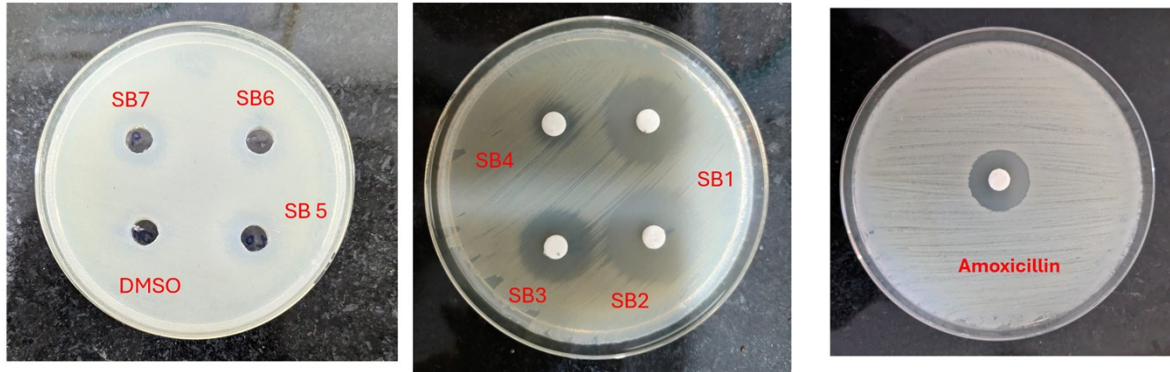


Figure S43. Images of inhibition zone of compound SB1-SB7 in comparison with Amoxicillin against XDR E.coli

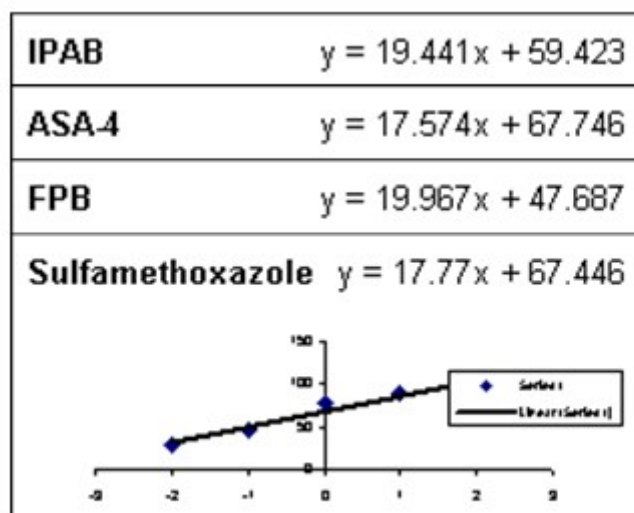


Figure S44. Dose–response curve of compounds against DHPS

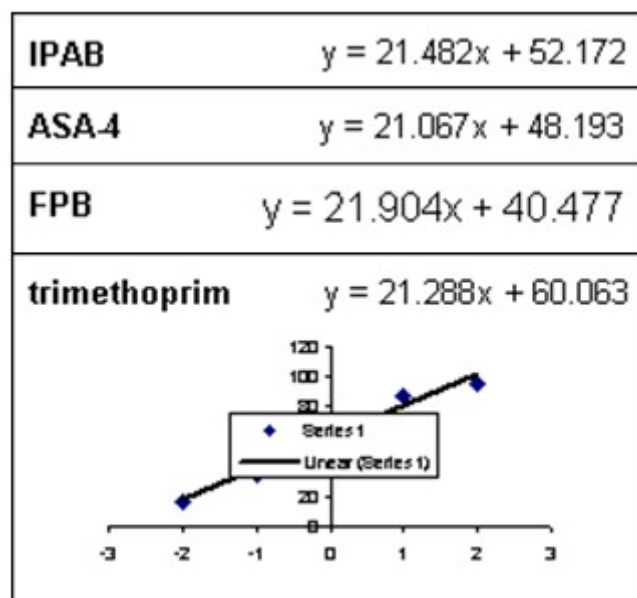


Figure S45. Dose–response curve of compounds against DHFR

Table S1. MIC Determination against MRSA and XDR *E.coli*

Compound SB2	MRSA (µg/mL)	XDR <i>E.coli</i> (µg/mL)	Compound SB5(µg/mL)	MRSA (µg/mL)	XDR <i>E.coli</i> (µg/mL)
Replicate 1	2	8	Replicate 1	2	16
Replicate 2	2	8	Replicate 2	2	16
Replicate 3	2	8	Replicate 3	2	16
Reported MIC	2	8	Reported MIC	2	16

