

Synergistic Investigation of Azo-Thiazole Derivatives Incorporating Thiazole Moieties: Comprehensive Exploration of Synthesis, Characterization, Computational Insights, Solvatochromism, and Multimodal Biological Activity Assessment

Abstract

In the present study, a novel series of azo-thiazole derivatives (**3a-c**) containing thiazole moiety unit was successfully synthesized. The structure of these derivatives was examined by spectroscopic techniques such as ^1H NMR, ^{13}C NMR, FT-IR, and HRMS. Further, the novel synthesized compounds were evaluated for their in-vitro biological activities such as antibacterial and anti-inflammatory as well as for *in silico* study. The antibacterial results demonstrated that compounds **3a** and **3c** (MIC=10 $\mu\text{g/mL}$) have notable potency against *Staphylococcus aureus* compared with azithromycin (MIC=40 $\mu\text{g/mL}$); Compound **3b** displayed a fourfold higher potency (24 recovery days, 1.83 mg/day) than HAMAZINE (28 recovery days, 4.14 mg/day) in promoting burn wound healing, and it also exhibited comparable inhibitory activity against screened bacterial pathogens when compared to reference drug. Docking on 1KZN, considering the excellent impact of the compounds on the Crystal Structure of E. coli 24kDa Domain in Complex with Clorobiocin, indicated a close binding of compounds 3a-c with the active site of protein 1KZN, in line with their observed biological activity. Additionally, we conducted molecular dynamics simulations on the docked complexes of compounds 3a-c with PDB: 1KZN to assess their stability and molecular interactions. Furthermore, we assessed the compounds' electrochemical characteristics via DFT calculations. Leveraging PASS and pkCSM platforms, we gained insights into controlling bioactivity and physicochemical features, highlighting these compounds as promising candidates for new active agents

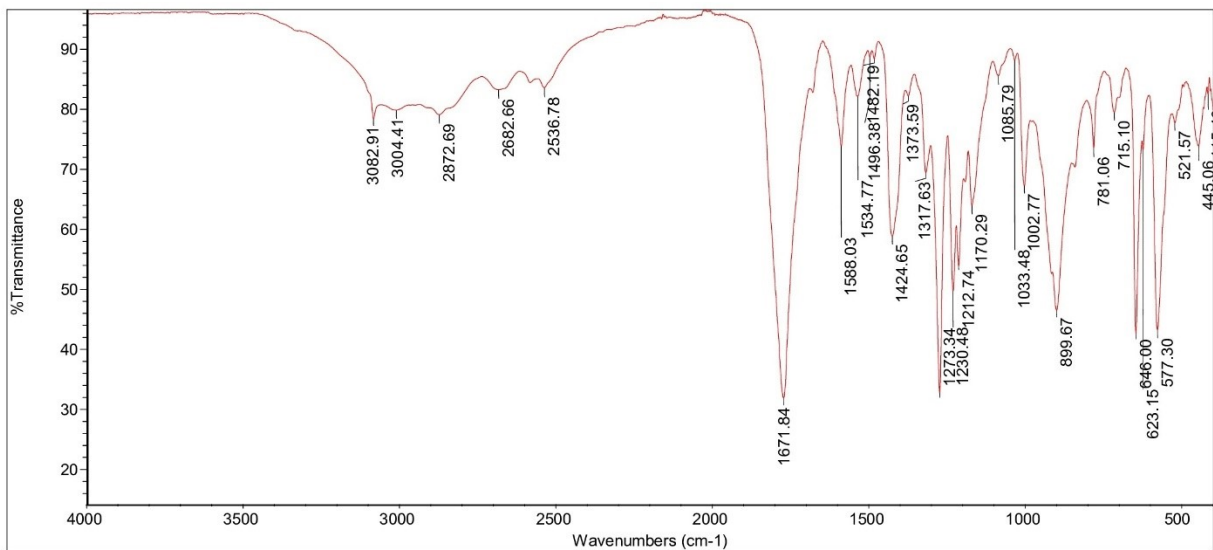


Fig .S1. Ftir spectrum of compound 3

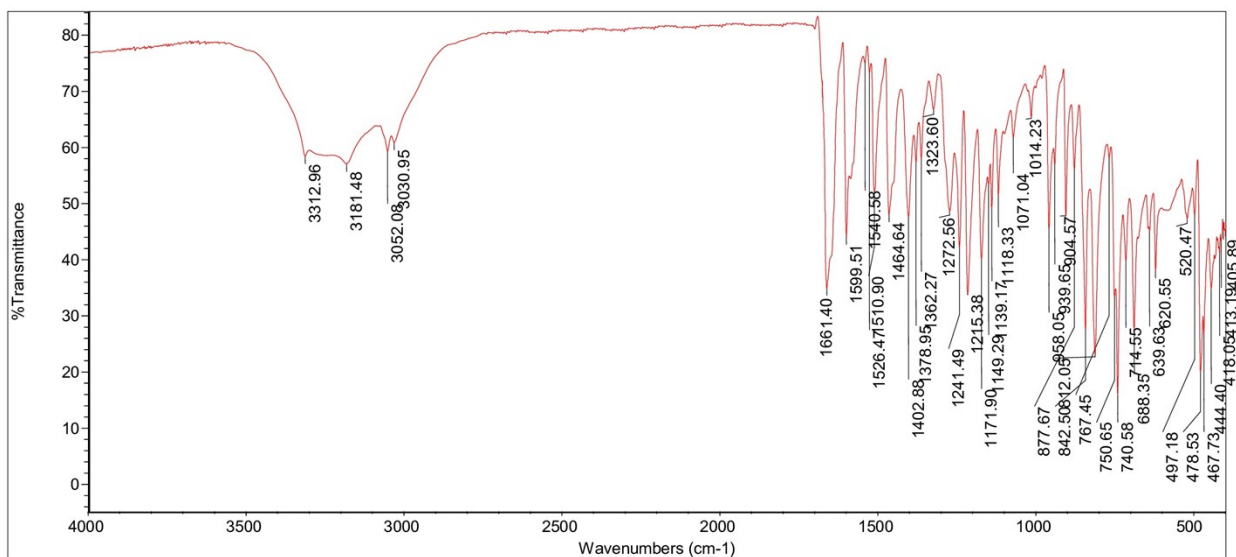


Fig .S2. Ftir spectrum of compound 3a

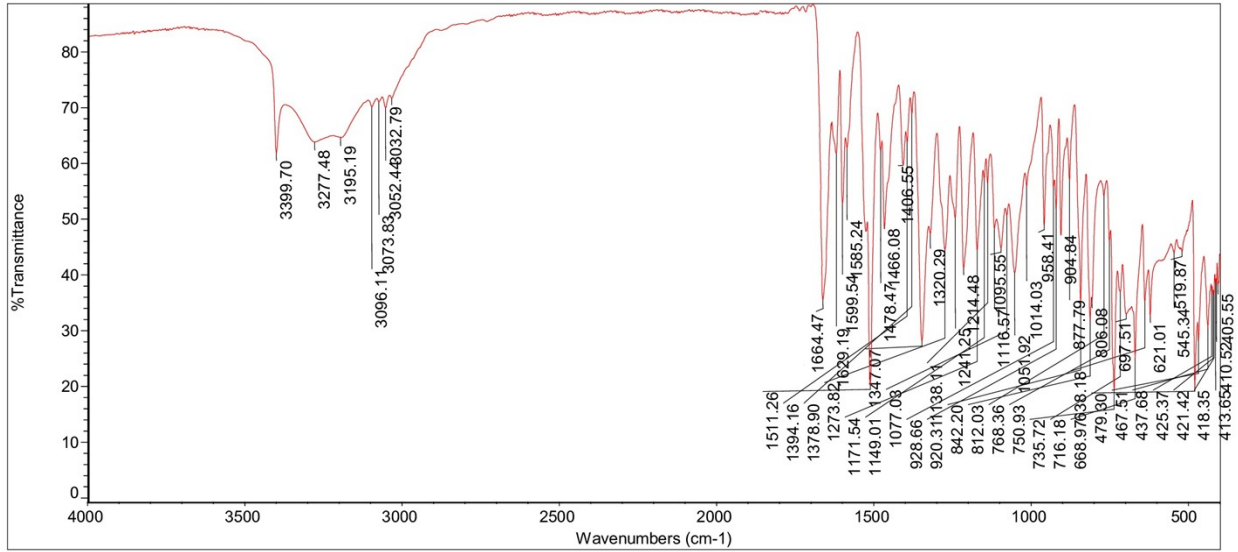


Fig .S3. Ftir spectrum of compound 3b

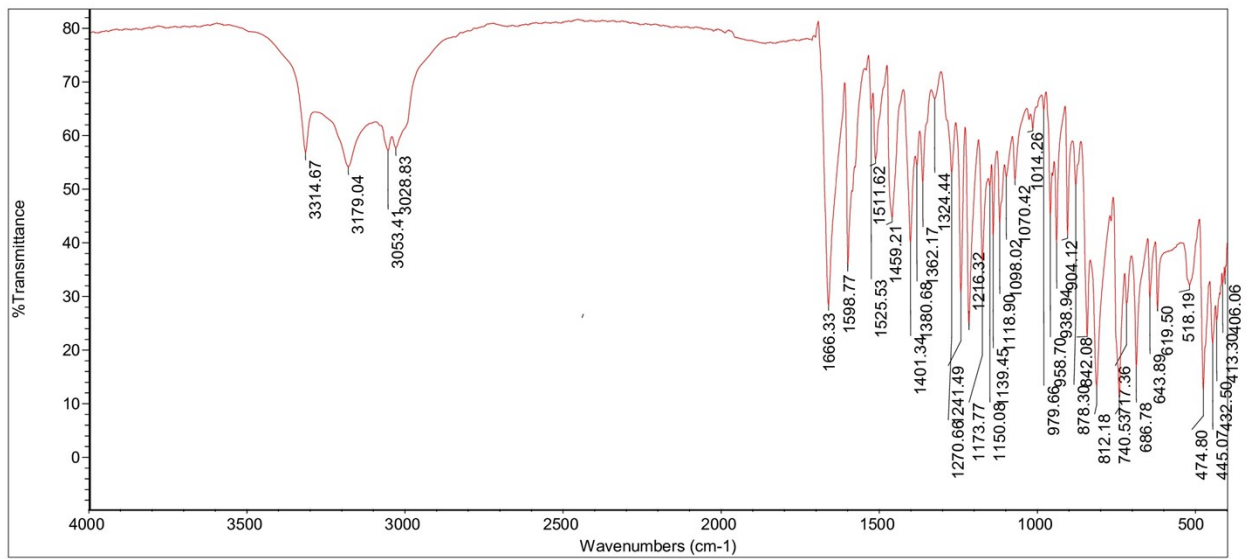
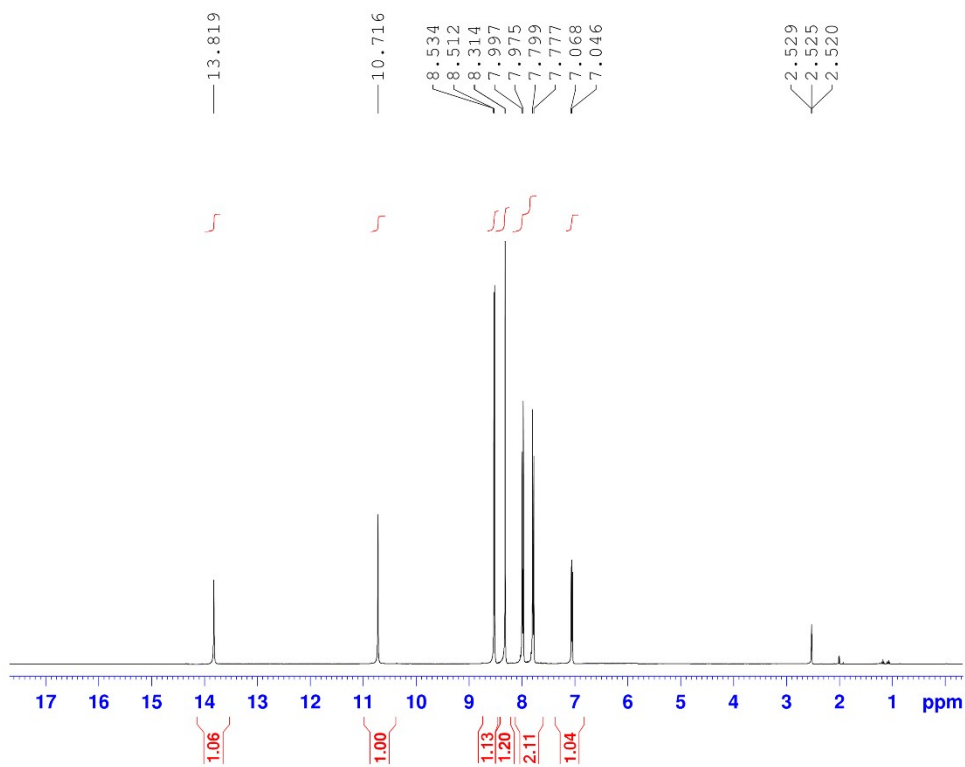


Fig .S4. Ftir spectrum of compound 3c

Sample Code: S-12- in DMSO (Mr.Sangar Hassaan)

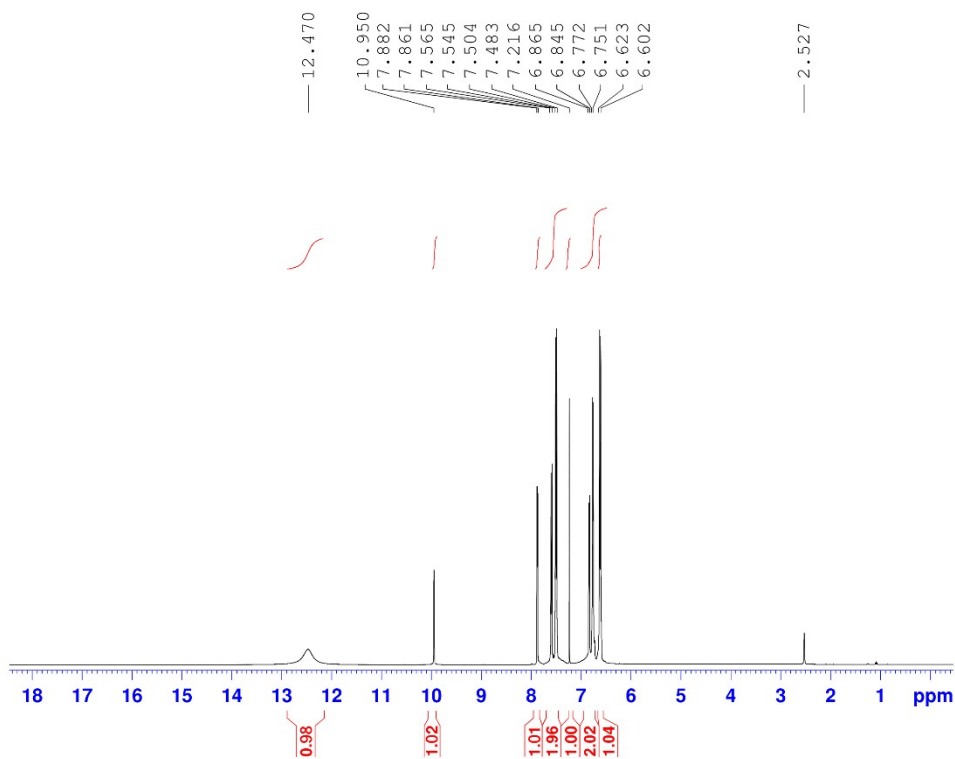


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FULPROG zg30
TD 65536
SOLVENT DMSO
NS 20
DS 0
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 101
DW 62.400 usec
DE 6.50 usec
TE 293.1 K
D1 4.0000000 sec
TD0 1

----- CHANNEL f1 -----
NUC1 1H
P1 14.00 usec
PL1 -2.00 dB
PLW 11.86359406 W
SFO1 400.2236020 MHz
SI 32768
SF 400.2200000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
FC 1.00

Fig.S5.hnmr spectrum of 3

Sample Code: S-2

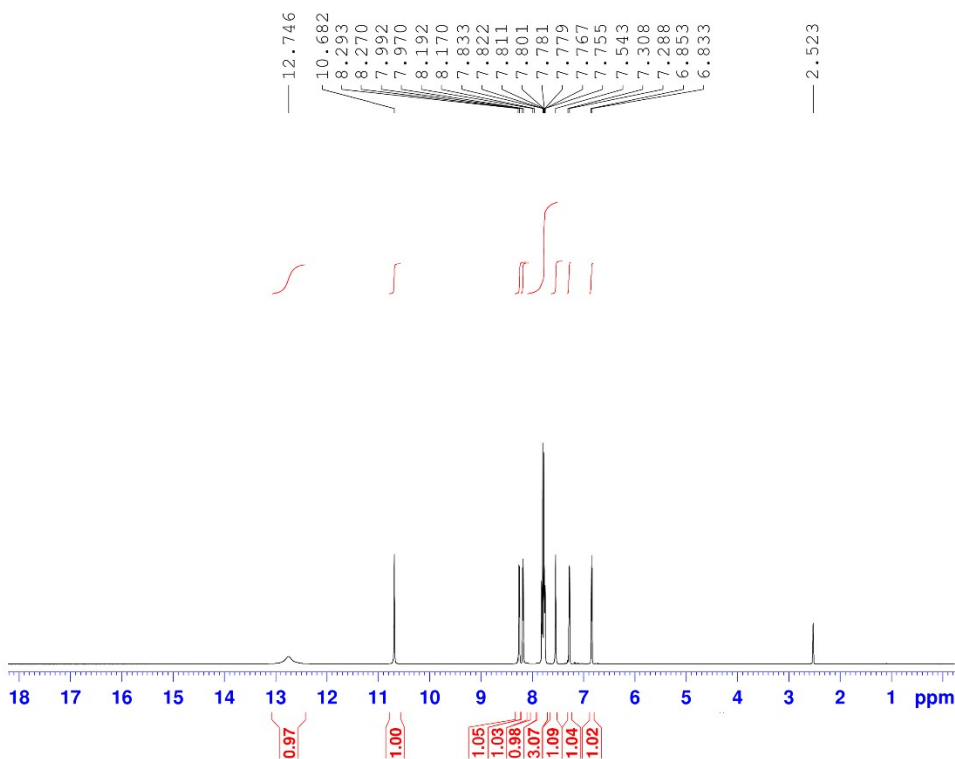


Sallahaddin-Kurdistan UN
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TD 65536
SOLVENT DMSO
NS 20
DS 0
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 57
DW 62.400 usec
DE 6.50 usec
TE 294.3 K
D1 4.0000000 sec
TDO 1

CHANNEL f1
NUC1 1H
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PL1 -2.00 dB
PL1W 11.8359408 W
SFO1 400.2236020 MHz
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GB 0
FC 1.00

Fig.s6.1hnmr spectrum of 3a

Sample Code: D10

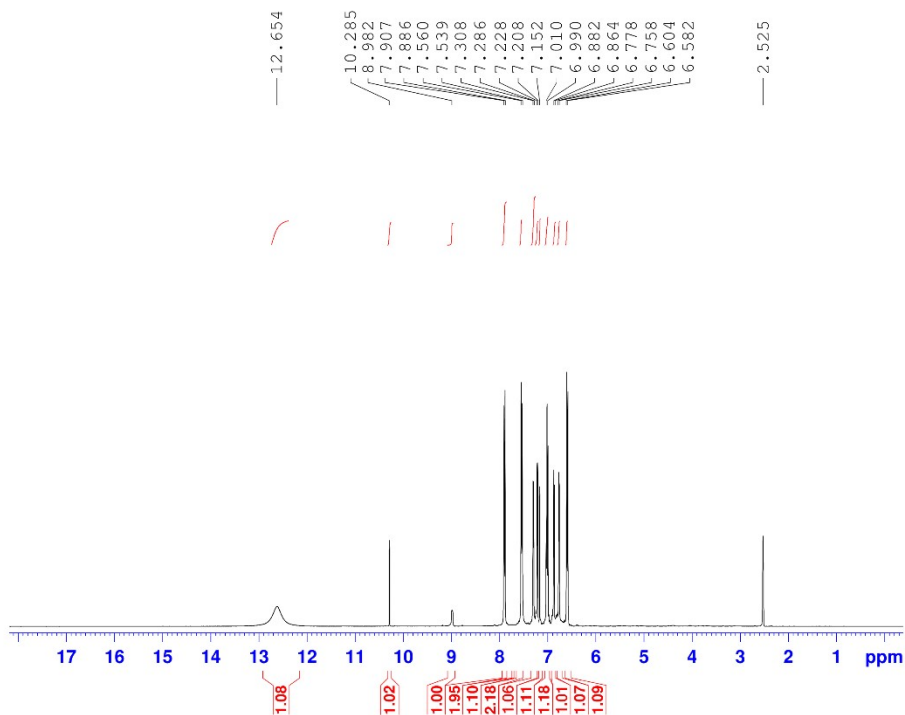


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SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 90.5
DW 62.400 usec
DE 6.50 usec
TE 294.8 K
D1 4.00000000 sec
TDO 1

----- CHANNEL f1 -----
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P1 14.00 usec
PL1 -2.00 dB
PL1W 11.86359406 W
SF01 400.2236020 MHz
SI 32768
SF 400.2200000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Fig.S7.1hnmr 3b

Sample Code: S-1-

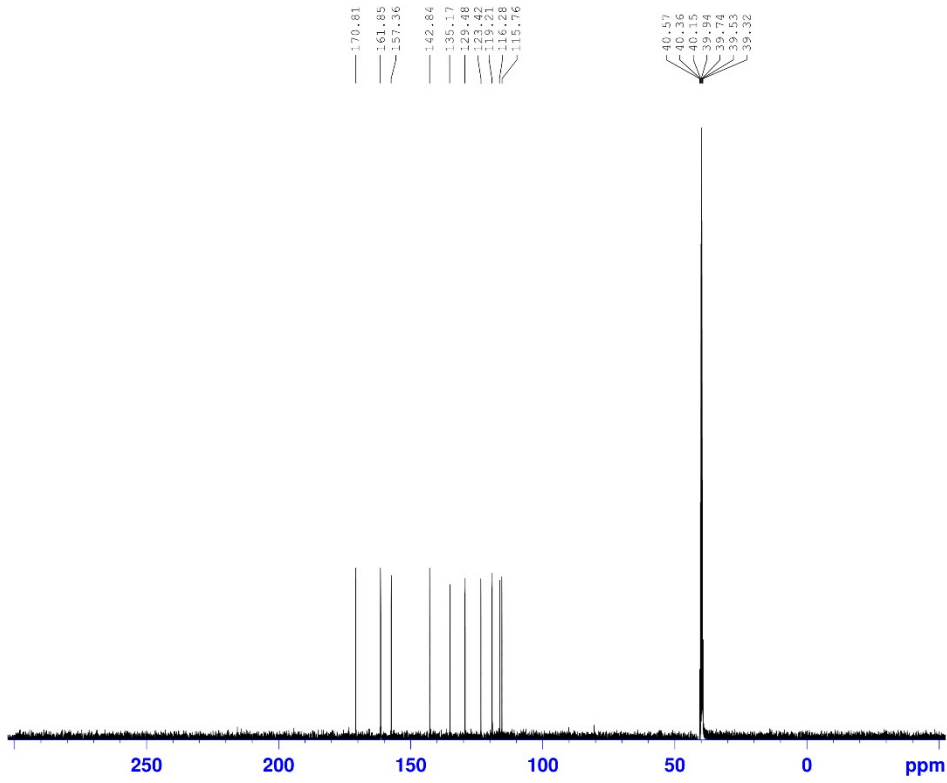


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SOLVENT DMSO
NS 20
DS 0
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894966 sec
RG 90.5
DW 62.400 usec
DE 6.50 usec
TE 294.8 K
D1 4.00000000 sec
TDO 1

----- CHANNEL f1 -----
NUC1 1H
P1 14.00 usec
PL1 -2.00 dB
PL1W 11.86359406 W
SF01 400.2236020 MHz
SI 32768
SF 400.2200000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Fig.S8.1hnmr spectrum of 3c

Sample Code: S-12- in DMSO (Mr.Sangar Hassaan)



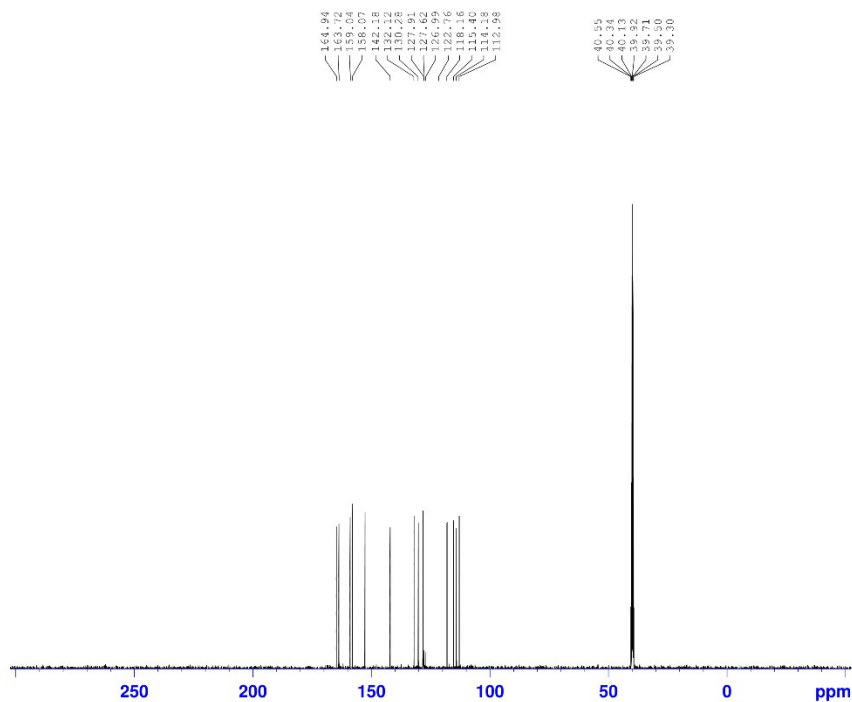
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TD 65536
SOLVENT DMSO
NS 76
DS 0
SWH 35714.285 Hz
FIDRES 0.544957 Hz
AQ 0.9175340 sec
RG 2050
DW 14.000 usec
DE 6.50 usec
TE 293.0 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1

===== CHANNEL f1 =====
NUC1 13C
P1 9.00 usec
PL1 -0.50 dB
PL1W 42.02801895 W
SFO1 100.6479784 MHz

===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PF2 -2.00 dB
PL12 14.16 dB
PL13 17.90 dB
PL2W 11.86359406 W
PL12W 0.28722104 W
PL13W 0.12139934 W
SFO2 400.2216009 MHz
SI 32768
SF 100.6353990 MHz
WDW EM
SBB 0
LB 1.00 Hz
GB 0
PC 1.40
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Fig.S9. 13CNMR spectrum of 3

Sample Code:S-2



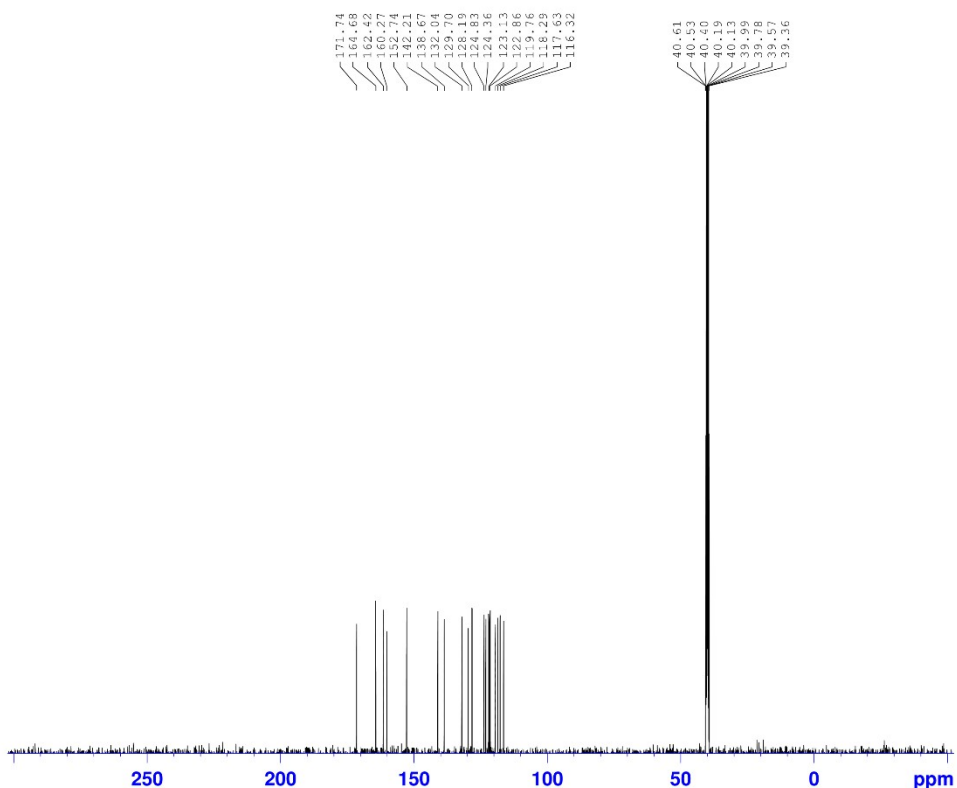
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ID 65536
SOLVENT DMSO
NS 144
DS 0
SWH 35714.285 Hz
FIDRES 0.544957 Hz
AQ 0.9175540 sec
RG 2050
DW 14.000 usec
DE 6.50 usec
TE 295.7 K
D1 1.00000000 sec
D11 0.03000000 sec
TDO 1

===== CHANNEL f1 =====
NUC1 13C
P1 9.00 usec
PL1 -0.90 dB
PL1W 42.02801895 W
SFO1 100.6479784 MHz

===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -2.00 dB
PL12 14.16 dB
PL13 17.90 dB
PL2W 11.86359406 W
PL12W 0.28722104 W
PL13W 0.12139934 W
SFO2 400.2216009 MHz
SI 32768
SF 100.6353990 MHz
WDW EM
SBB 0
LB 1.00 Hz
GB 0
PC 1.40
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Fig.S10. 13CNMR spectrum of 3a

Sample Code:D10



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EXPNO 272
PROCNO 1
Date_ 20230420
Time 10.07
INSTRUM spect
PROBHD 5 mm PABBO B3-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 551
DS 0
SWH 35714.285 Hz
FIDRES 0.544957 Hz
AQ 0.9175540 sec
RG 2050
DW 14.000 usec
DE 6.50 usec
TE 295.7 K
D1 1.00000000 sec
D11 0.03000000 sec
TDO 1

===== CHANNEL f1 =====
NUC1 13C
P1 9.00 usec
PL1 -0.90 dB
PL1W 42.02801895 W
SFO1 100.6479784 MHz

===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -2.00 dB
PL12 14.16 dB
PL13 17.90 dB
PL2W 11.86359406 W
PL12W 0.28722104 W
PL13W 0.12139934 W
SFO2 400.2216009 MHz
SI 32768
SF 100.6353990 MHz
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SBB 0
LB 1.00 Hz
GB 0
PC 1.40
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Fig.S11. ¹³CNMR spectrum of 3b

Sample Code:S-1-

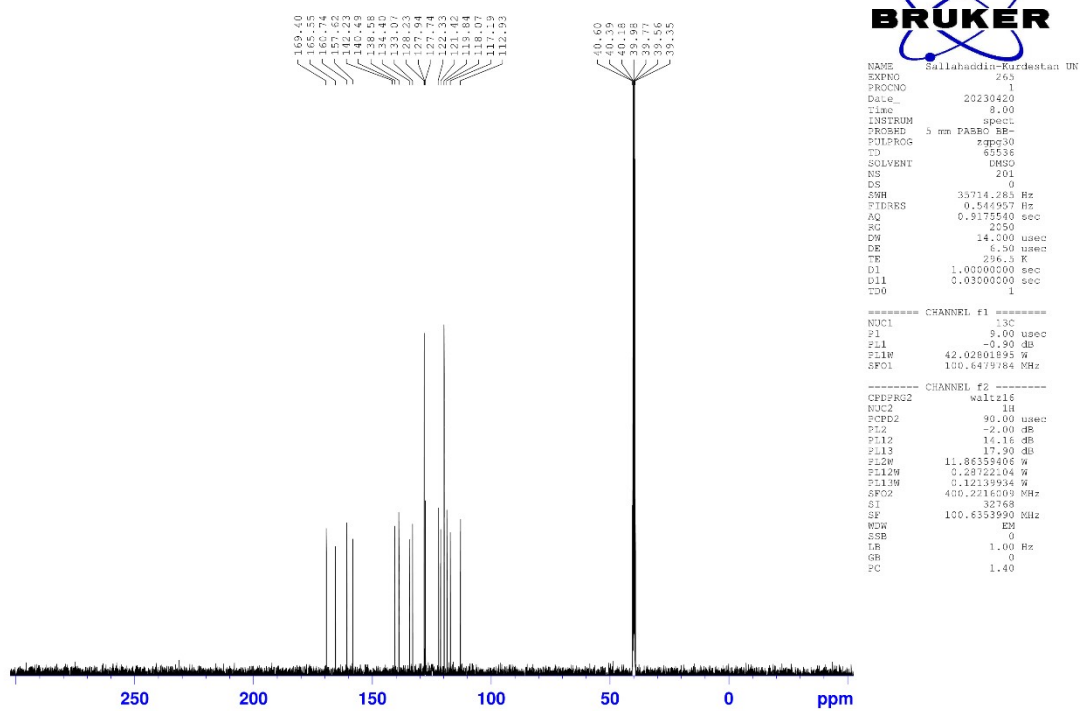


Fig.S12. ¹³CNMR spectrum of 3c

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	4.4 psi
Focus	Not active	Set Capillary	3800 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	600 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

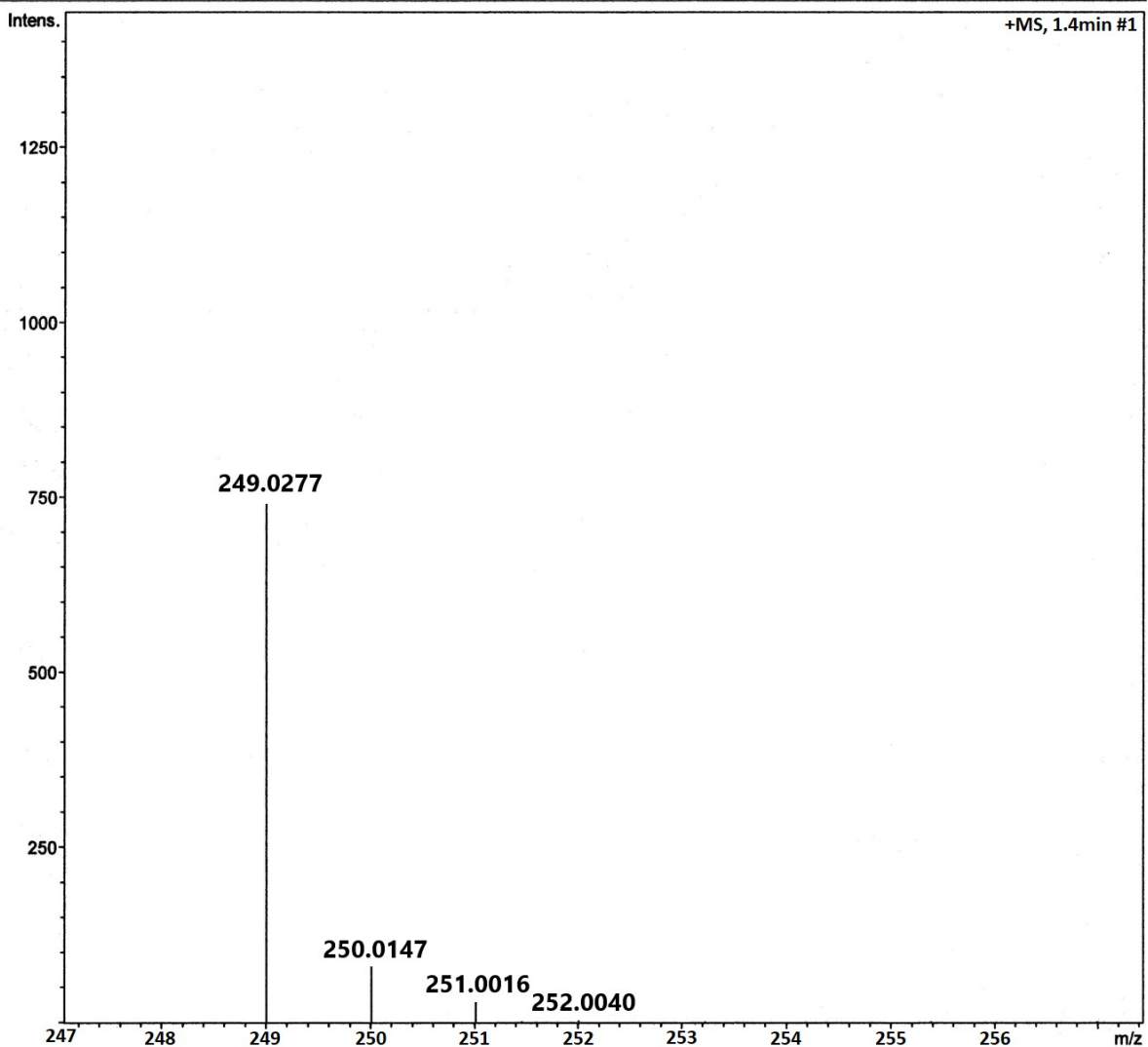


Fig.S13. HRMS spectrum of 3

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	4.4 psi
Focus	Not active	Set Capillary	3800 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	600 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

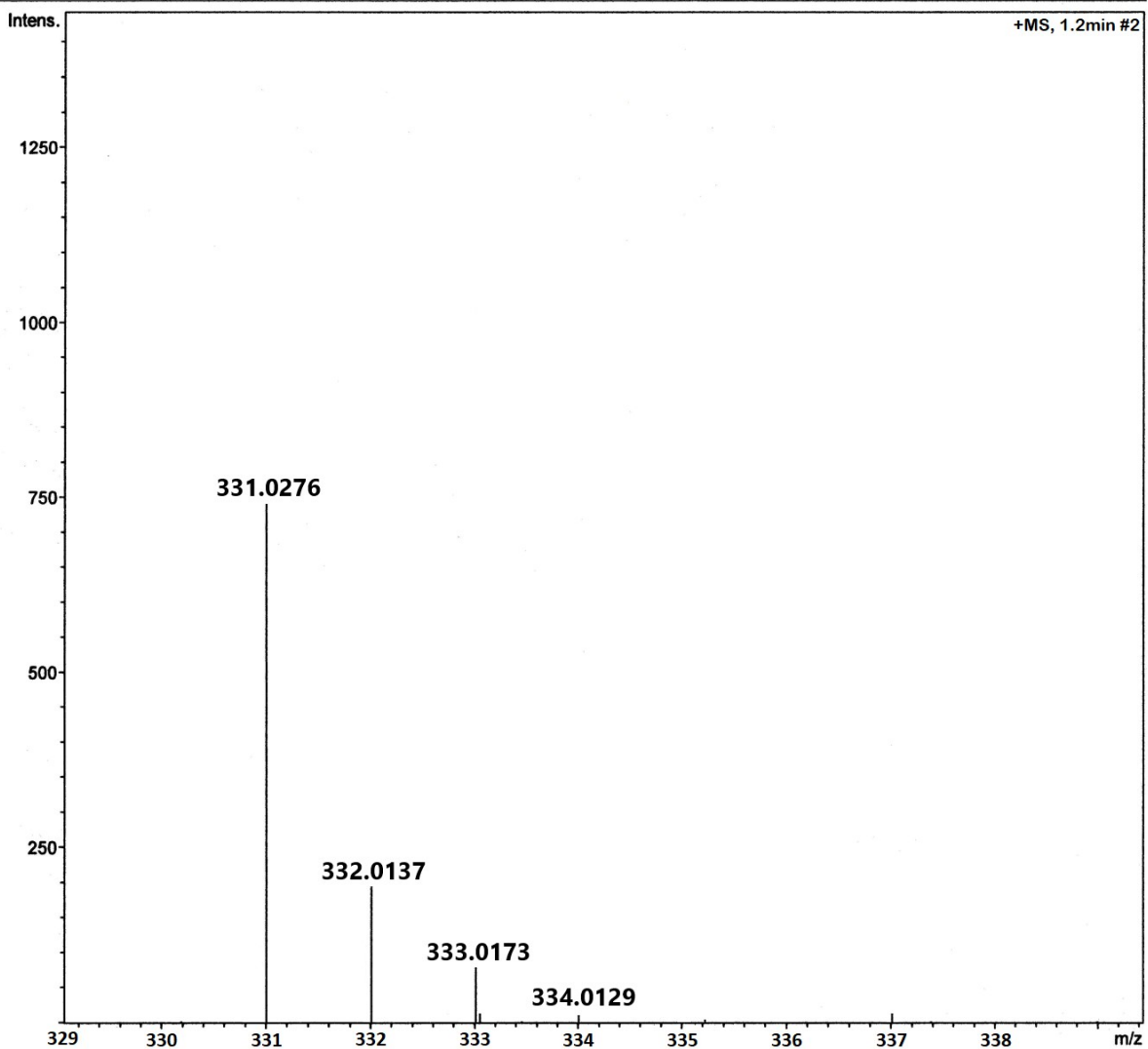


Fig.S14. HRMS spectrum of 3a

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	4.4 psi
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Scan End	600 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

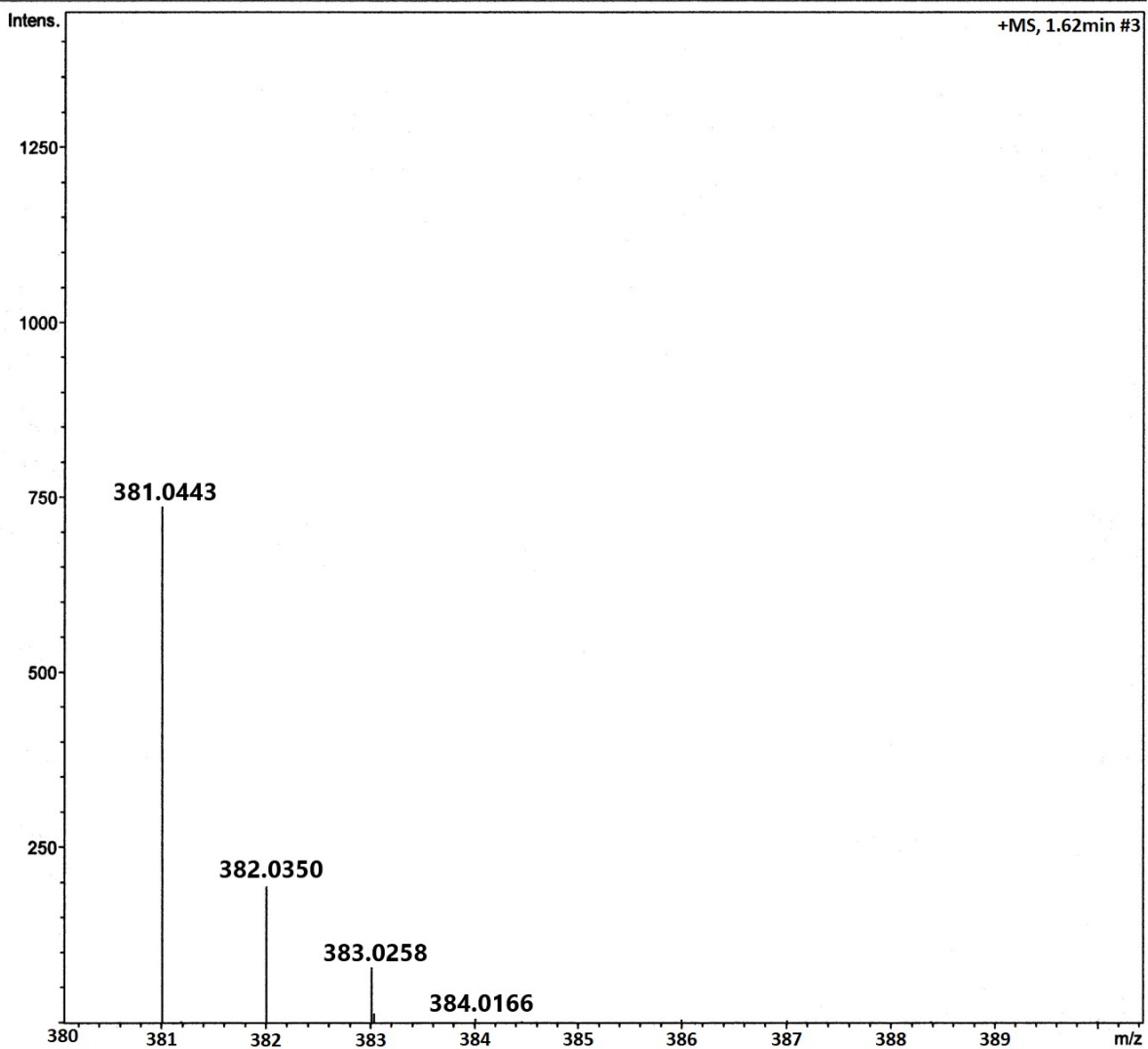


Fig.S15. HRMS spectrum of 3b

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	4.4 psi
Focus	Not active	Set Capillary	3800 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	600 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

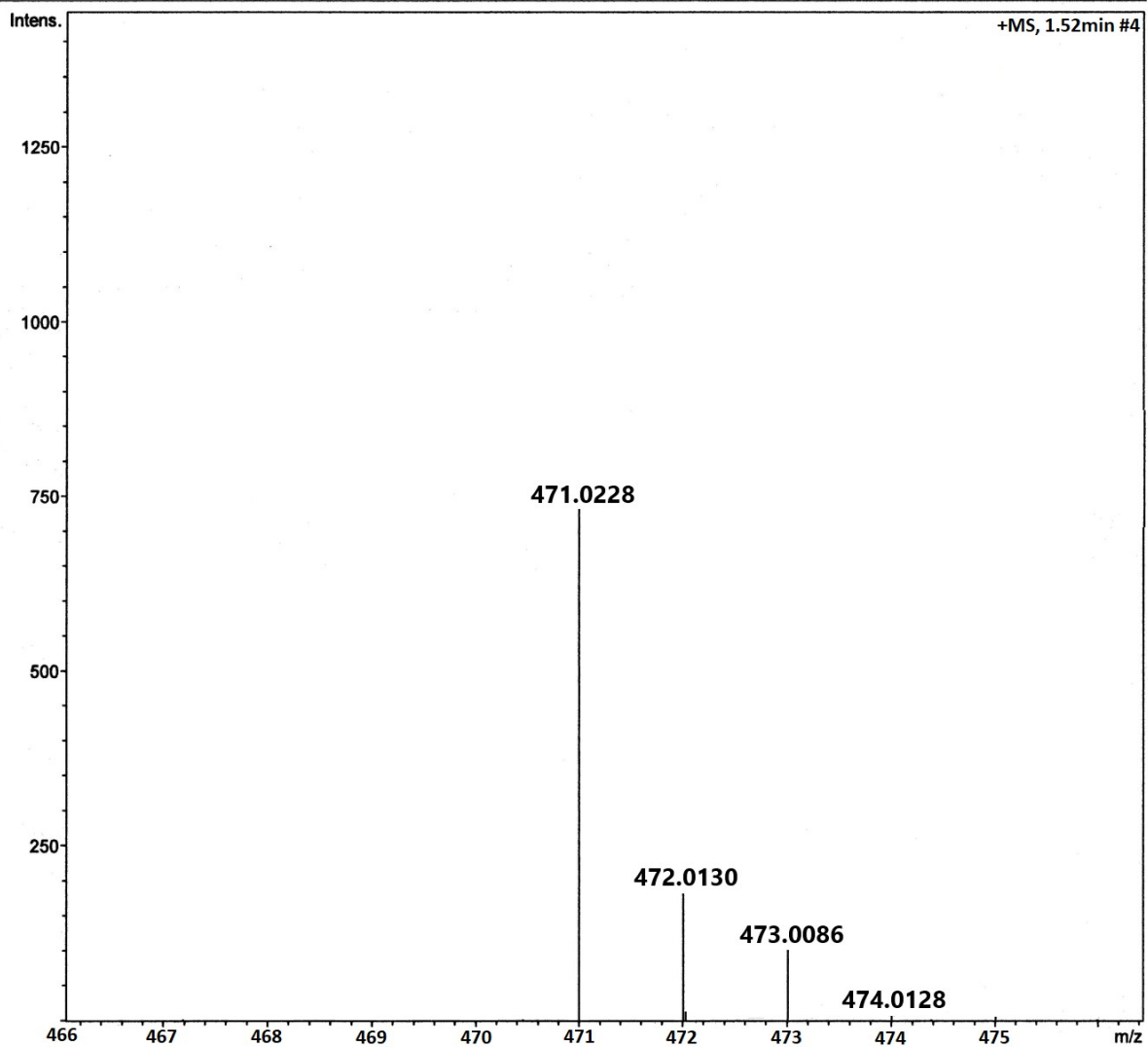


Fig.S16. HRMS spectrum of 3c

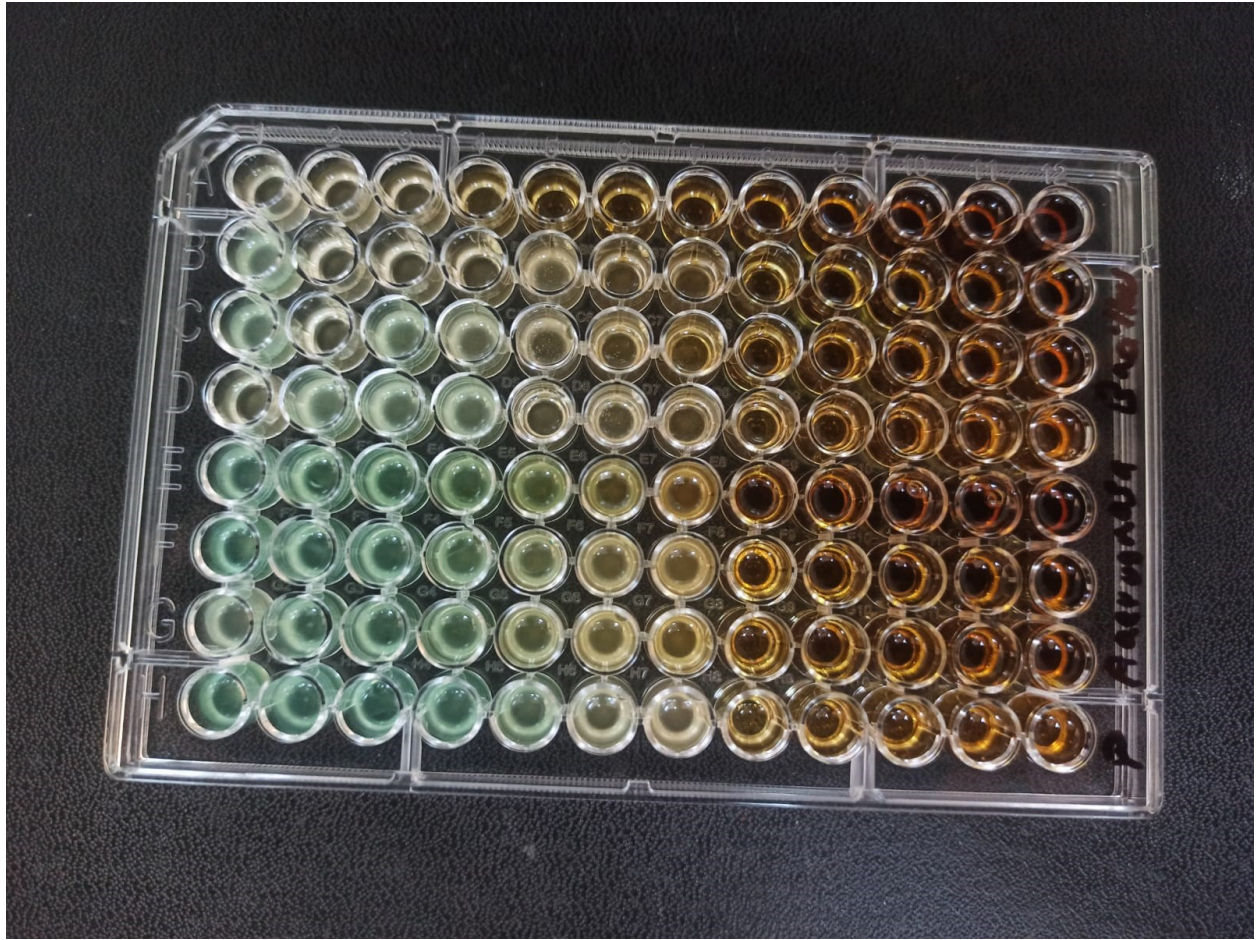


Fig.S17.MIC OF 3 and 3a-c against *Pseudomonas aeruginosa* and *Bacillus cereus*

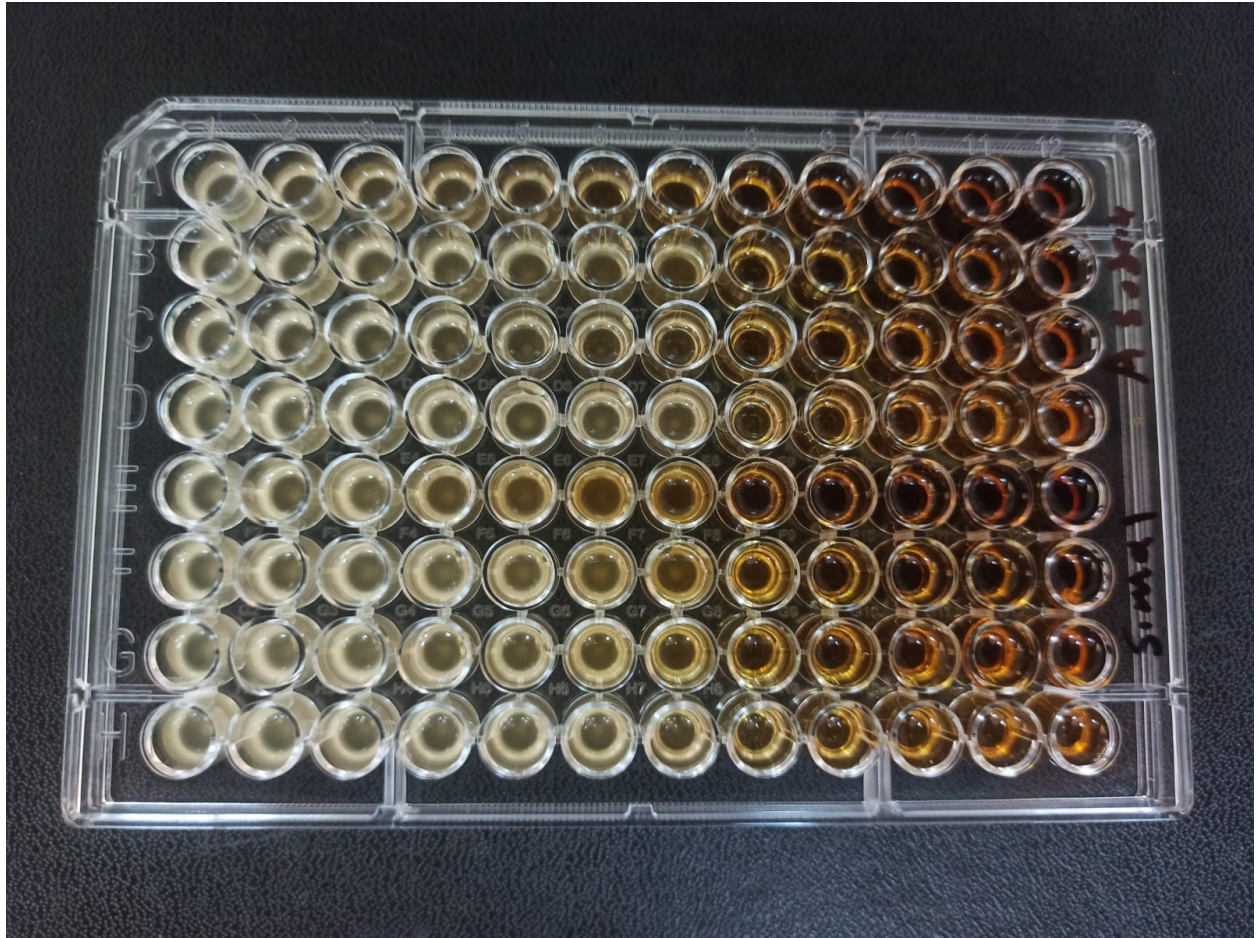


Fig.S18.MIC OF 3 and 3a-c against *Stenotrophomonas maltophilia* and *Aeromonas sobria*

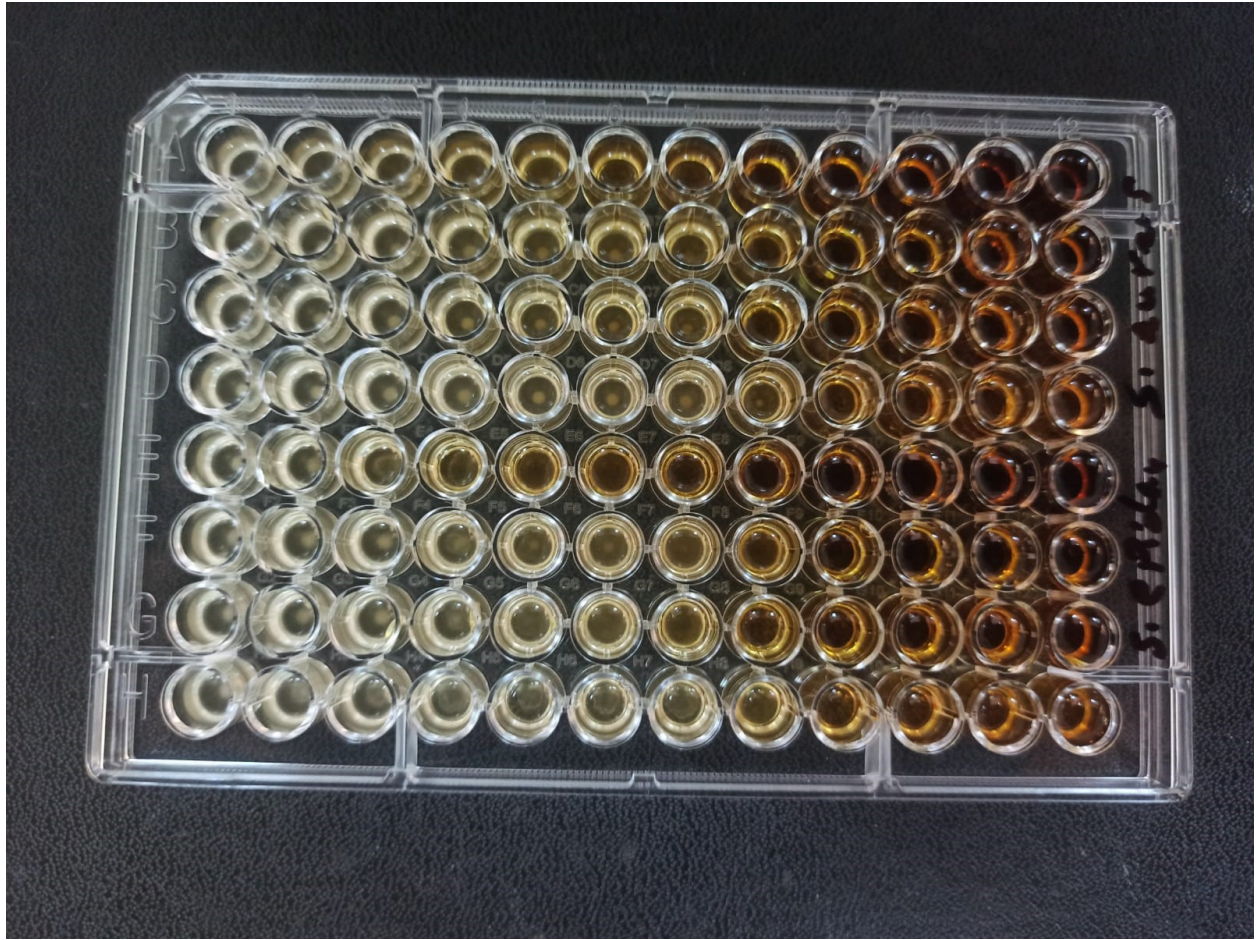


Fig.S19.MIC OF 3 and 3a-c against Staphylococcus aureus and Staphylococcus epidermidis

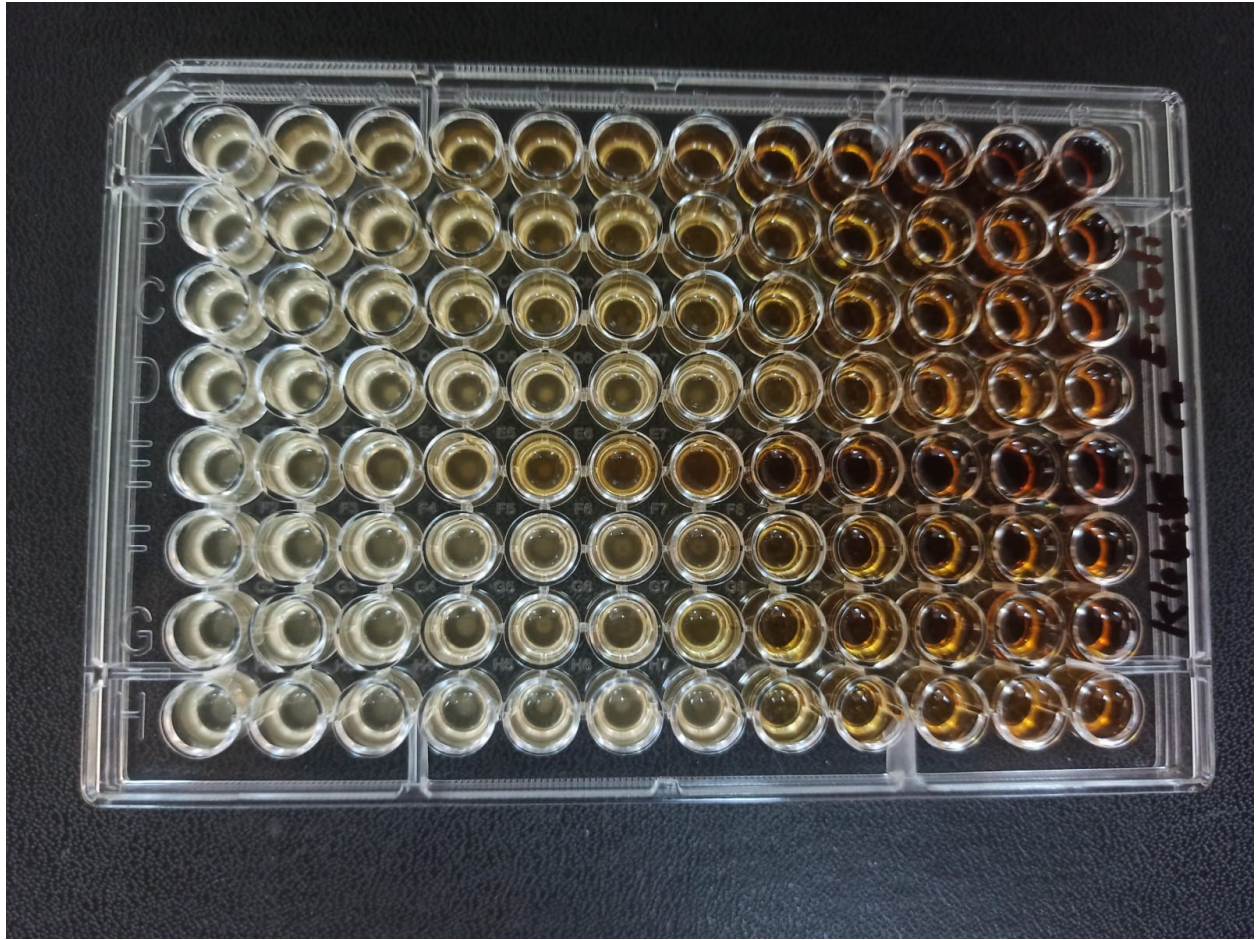


Fig.S20.MIC OF 3 and 3a-c against klebsiella pneumoniae. and Escherichia coli









Fig.S21-S26: Standard drug and -ve control















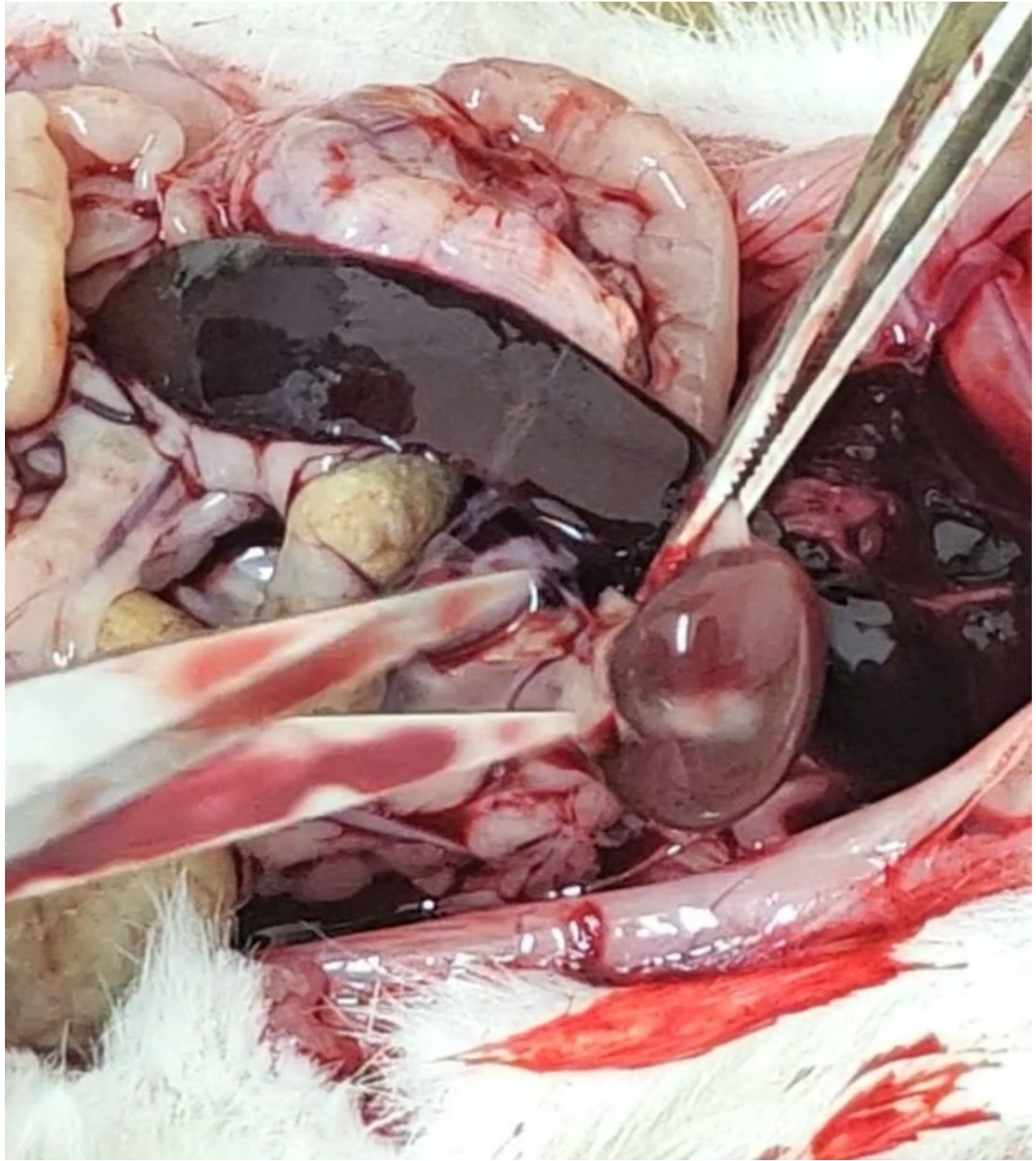




Fig.S27-S35: Standard drug and -ve control acute toxicity measurement

Table S1. Prediction in silico of **Metabolism** of 3a-c derivatives

Entry	Metabolism						
	CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
3a	NO	NO	YES	YES	YES	NO	NO
3b	NO	YES	YES	YES	YES	NO	YES
3c	NO	YES	YES	YES	YES	NO	YES

Table S2. Prediction in silico of **Toxicity** of 3a-c derivatives.

Entry	Toxicity									
	AMES toxicity	Max. tolerated dose (human)	hERG I inhibitor	hERG II inhibitor	Oral Rat Acute Toxicity (LD50)	Oral Rat Chronic Toxicity (LOAEL)	Hepatotoxicity	Skin Sensitisation	<i>T.Pyriformis</i> toxicity	<i>Minnow</i> toxicity
3a	NO	0.042	NO	YES	2.546	0.806	YES	NO	0.532	1.933
3b	NO	0.243	NO	YES	2.324	0.788	YES	NO	0.371	0.05
3c	NO	0.562	NO	YES	2.346	0.417	YES	NO	0.289	-0.154