Design, Synthesis, and Biological Evaluation of Novel Imidazole–Morpholinone Hybrids as Broad-Spectrum Antimicrobial Agents: Molecular Docking, DFT, and ADMET Studies

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Fig. S1: ^{1}H NMR spectrum of final compound 10a

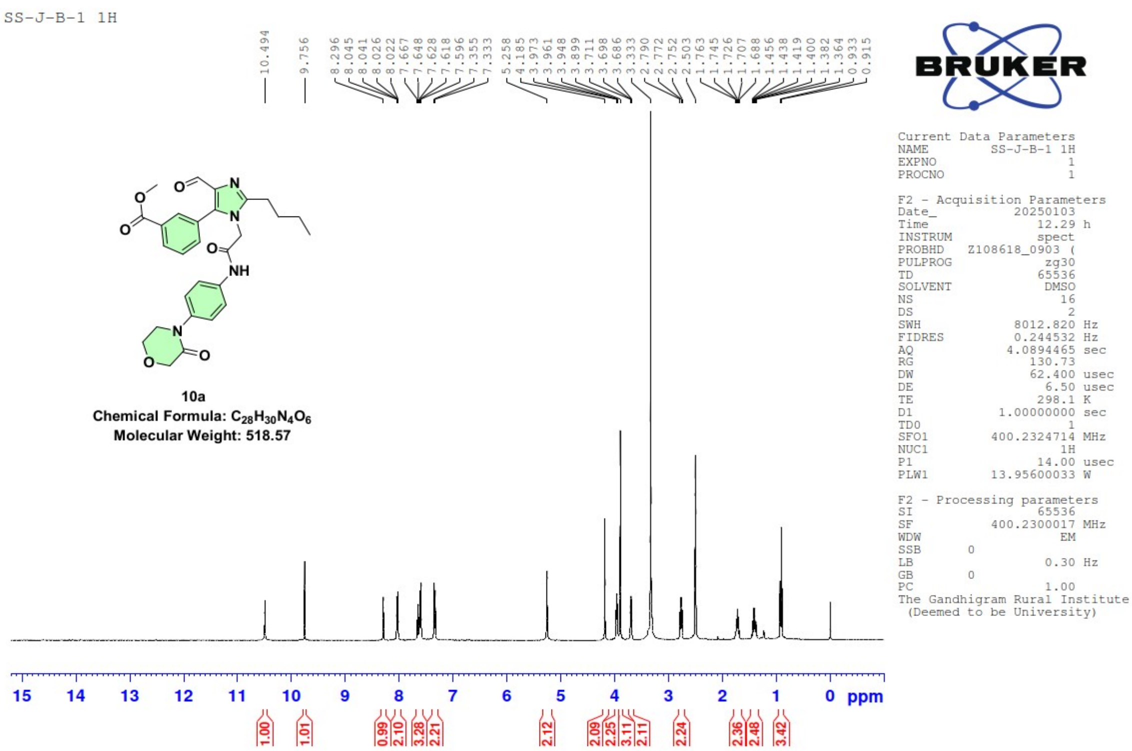


Fig. S2: ^{1}H NMR spectrum of final compound 10b

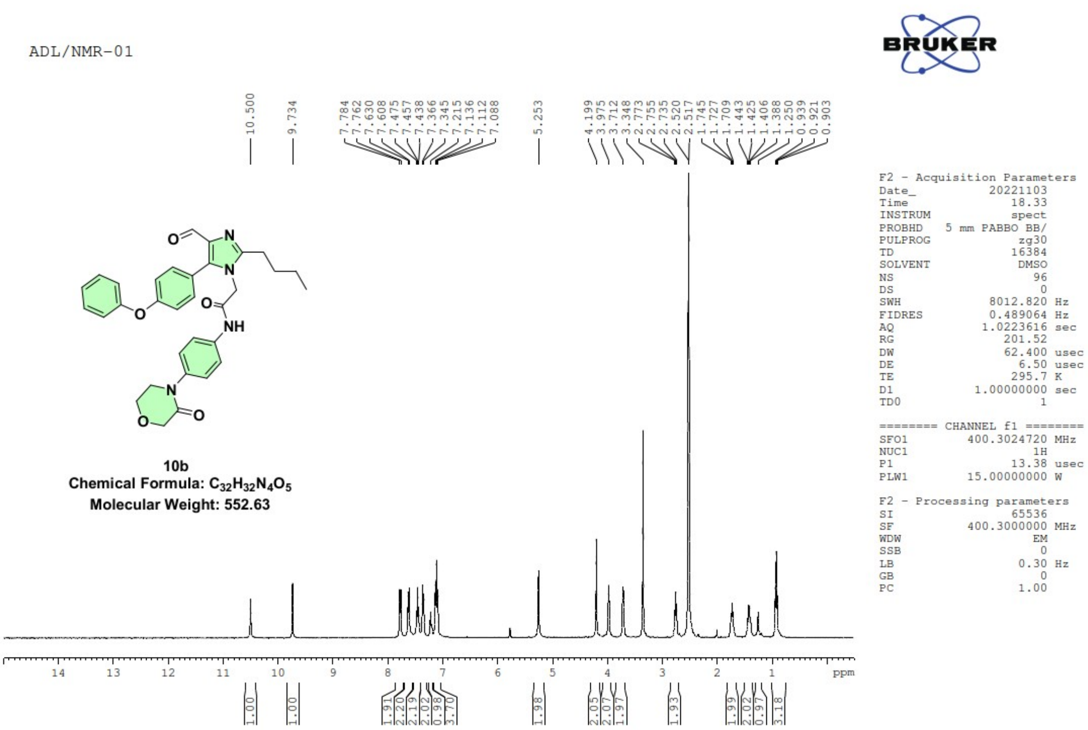


Fig. S3: [1]H NMR spectrum of final compound 10c

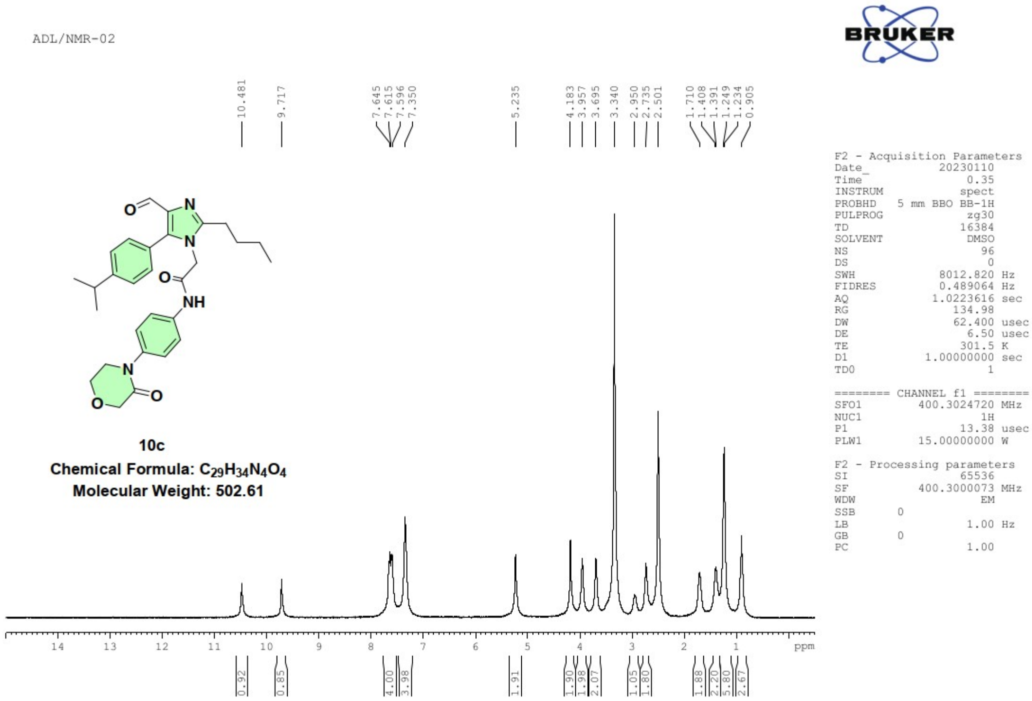


Fig. S4: [1]H NMR spectrum of final compound 10d

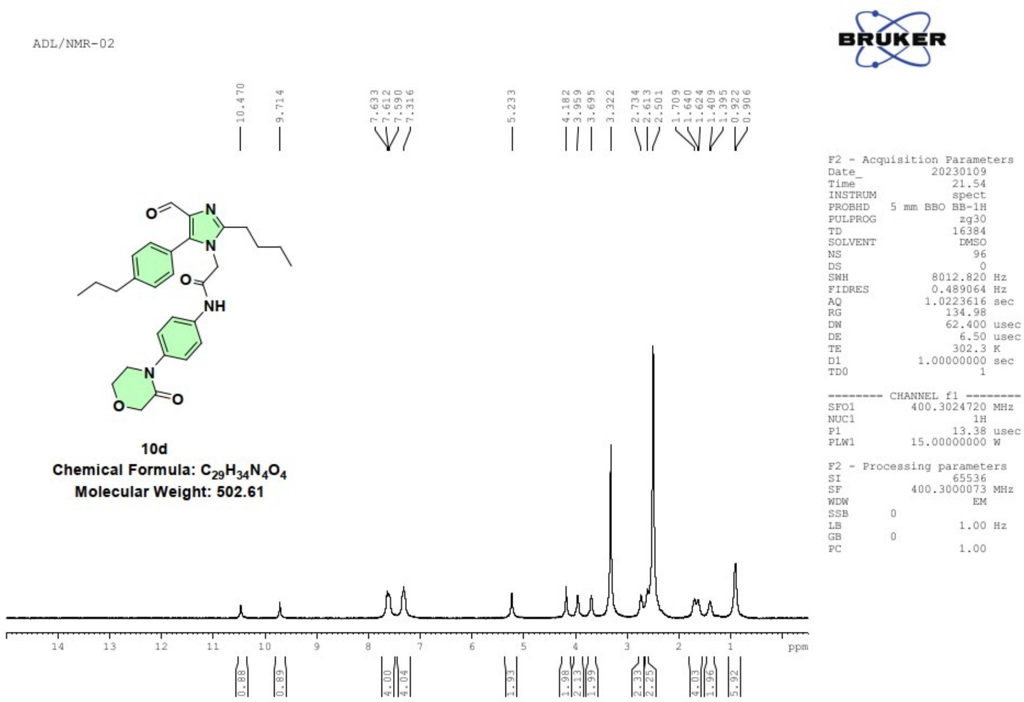


Fig. S5: [1]H NMR spectrum of final compound 10e

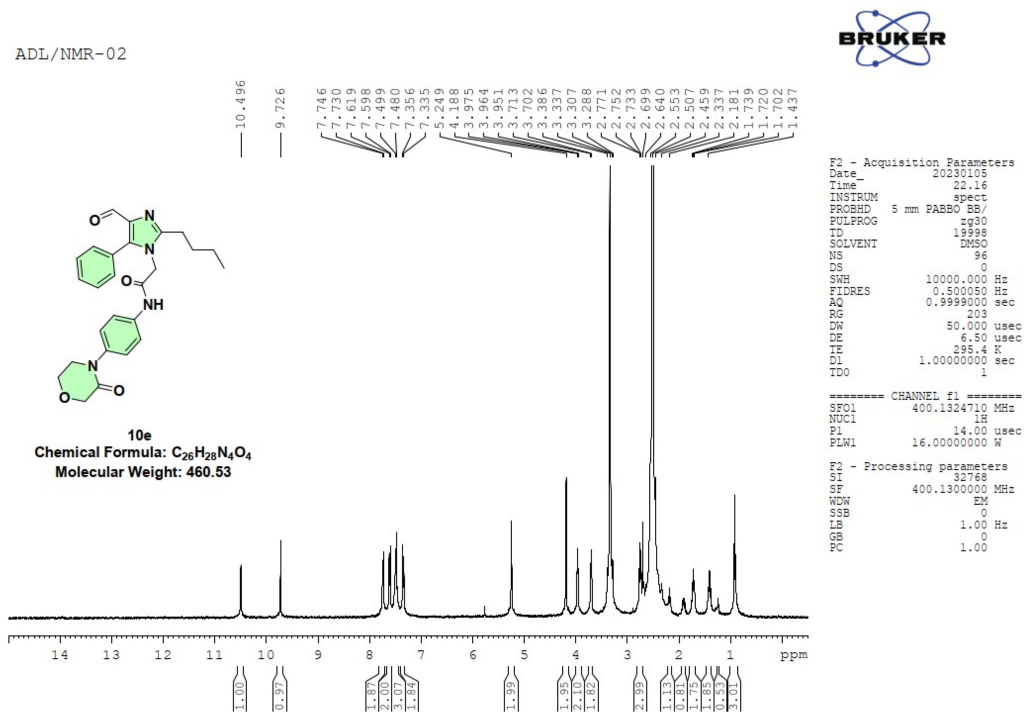


Fig. S6: [13]H NMR spectrum of final compound 10a

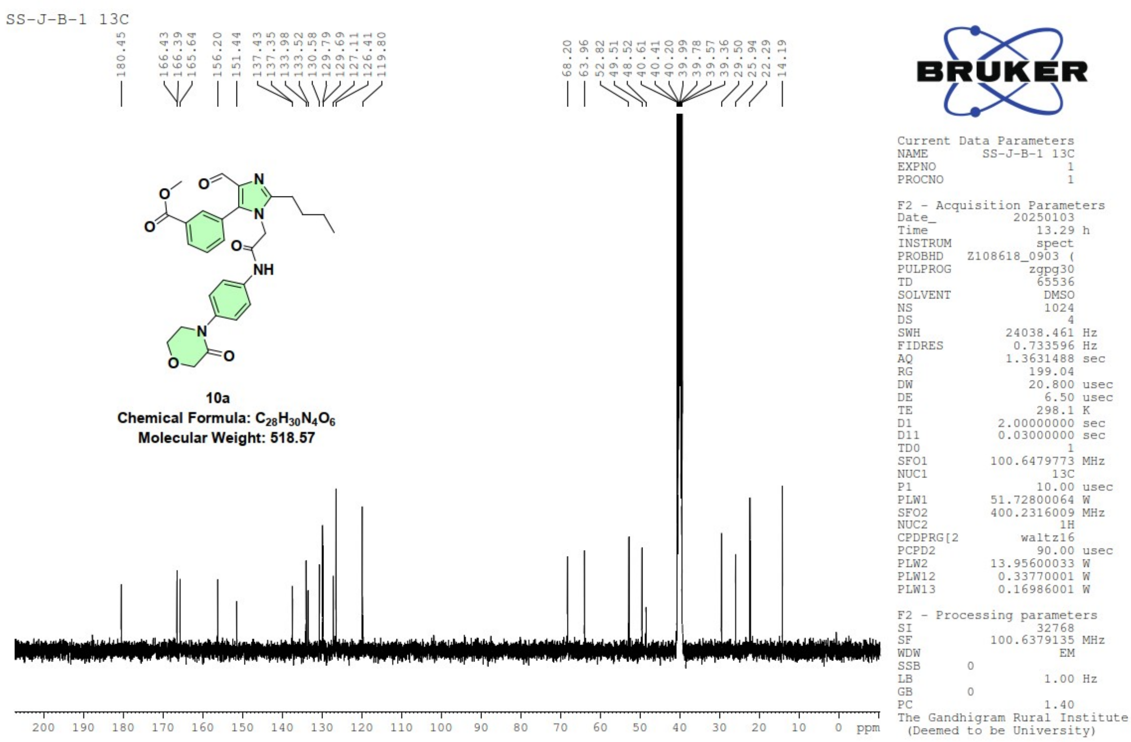


Fig. S7: ^{13}H NMR spectrum of final compound 10b

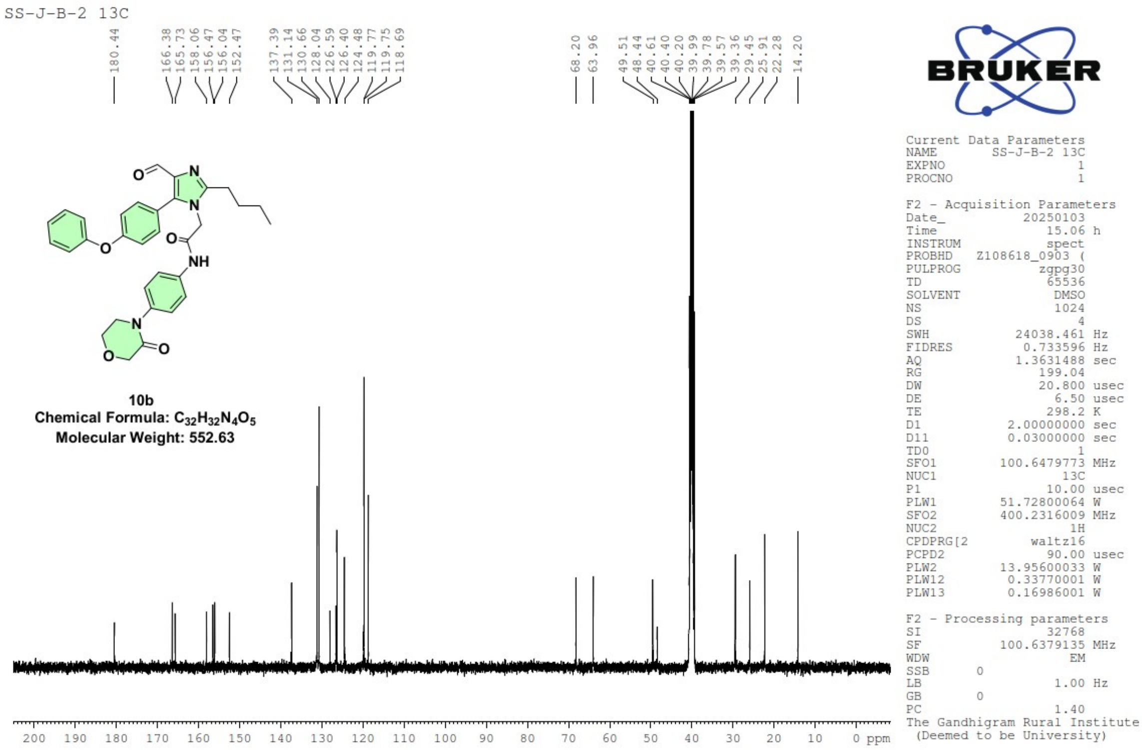


Fig. S8: ^{13}H NMR spectrum of final compound 10c

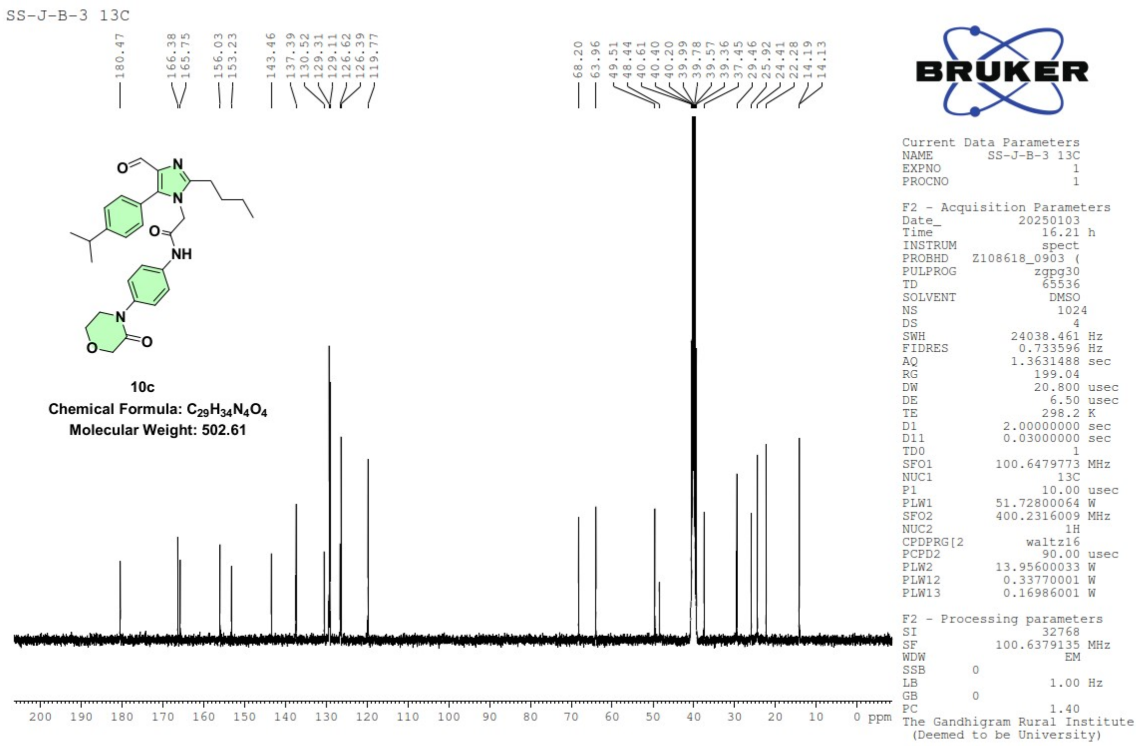


Fig. S9: [13]H NMR spectrum of final compound 10d

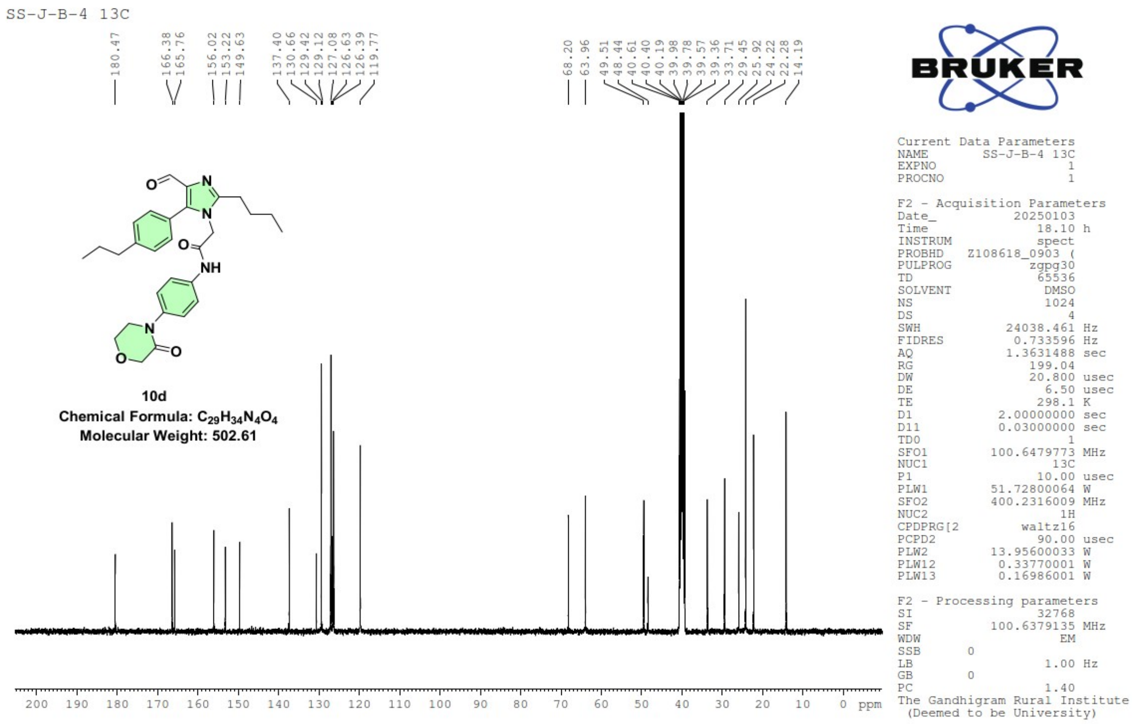


Fig. S10: [13]H NMR spectrum of final compound 10e

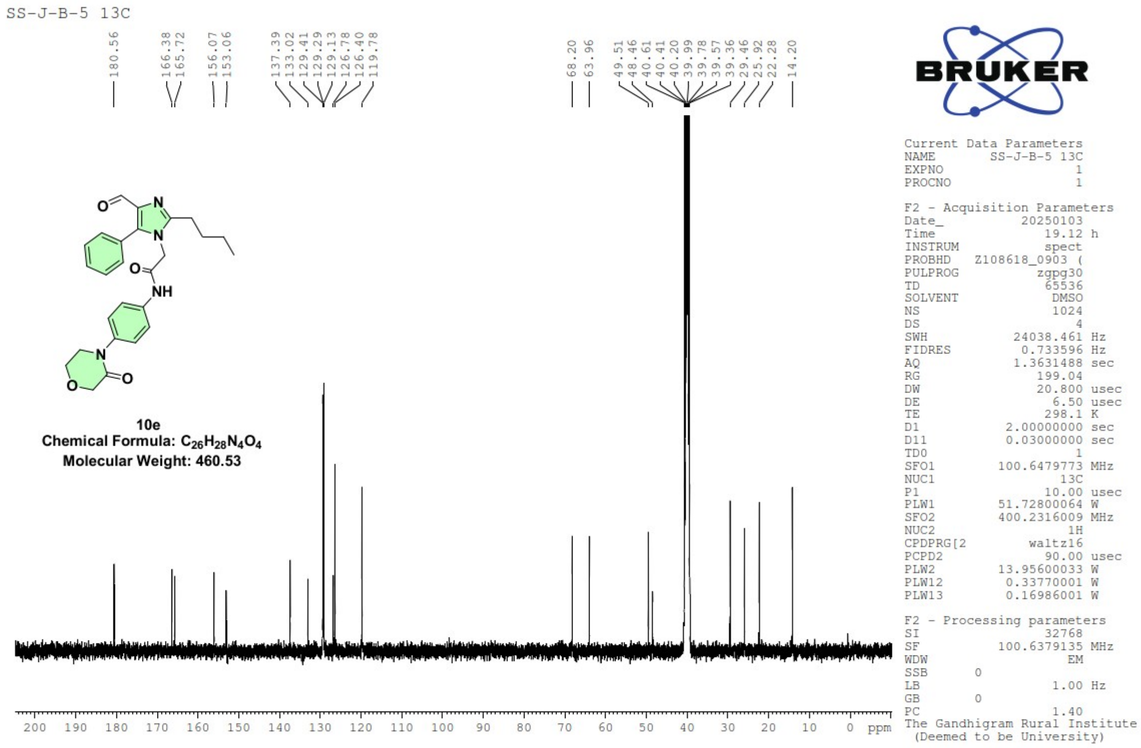
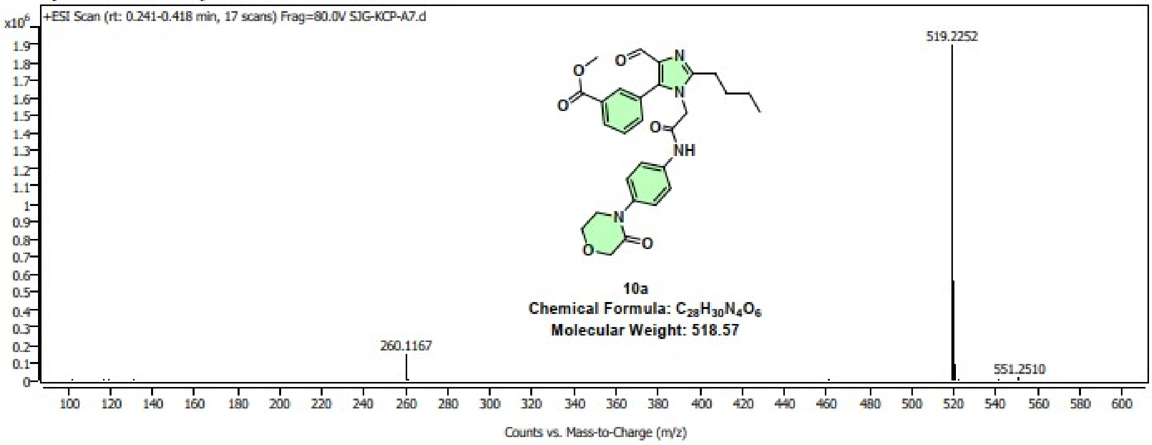


Fig. S11: HR-MS spectrum of final compound 10a

Sample Spectra

+ Scan (rt: 0.241-0.418 min) **Peak 1 from + TIC Scan**



Compound Details

Cpd. 1: C28 H30 N4 O6

Formula	m/z	Observed M/Z	Difference Da	Difference PPM	Score
C28 H30 N4 O6	519.2252	519.225167429387	1.32387840687898	2.5546819103201	95.81

Compound Spectra (Zoomed)

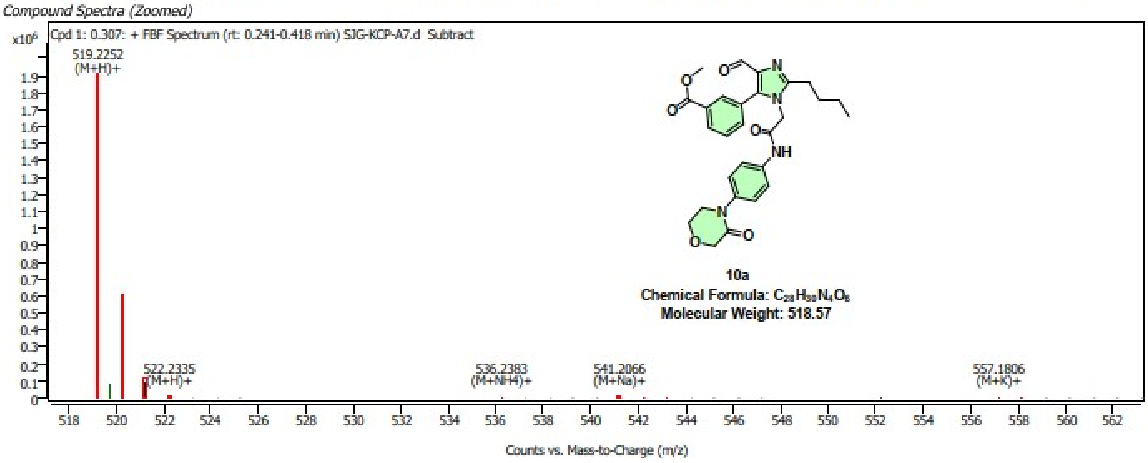
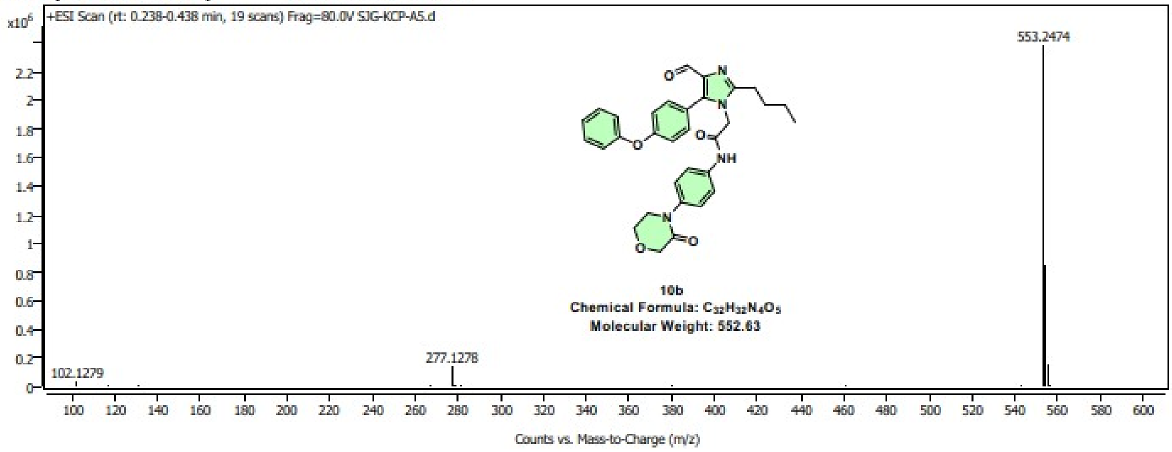


Fig. S12: HR-MS spectrum of final compound 10b

Sample Spectra

+ Scan (rt: 0.238-0.438 min) **Peak 1 from + TIC Scan**



Compound Details

Cpd. 1: C32 H32 N4 O5

Formula	m/z	Observed M/Z	Difference Da	Difference PPM	Score
C32 H32 N4 O5	575.2287	575.228700874384	2.74985842179376	4.97948720662693	89.84

Compound Spectra (Zoomed)

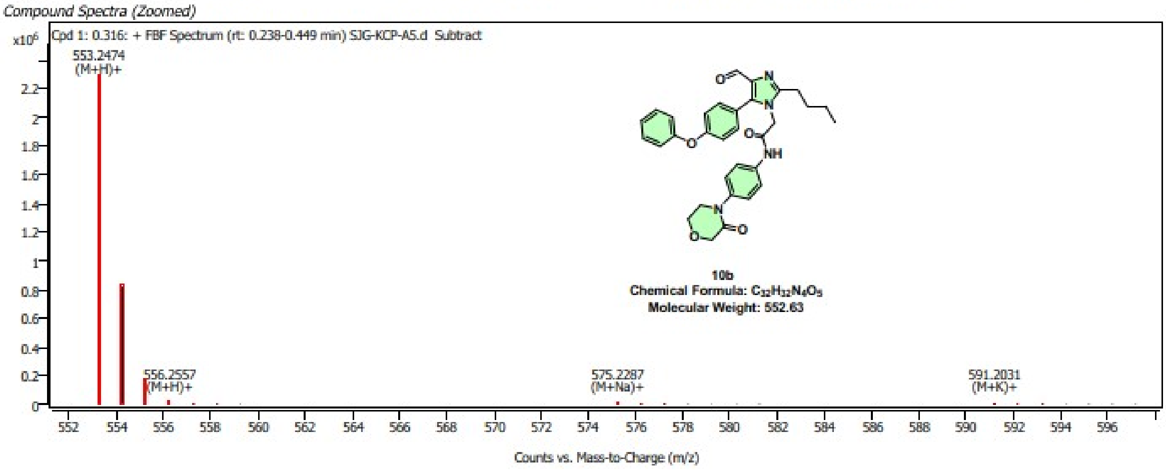
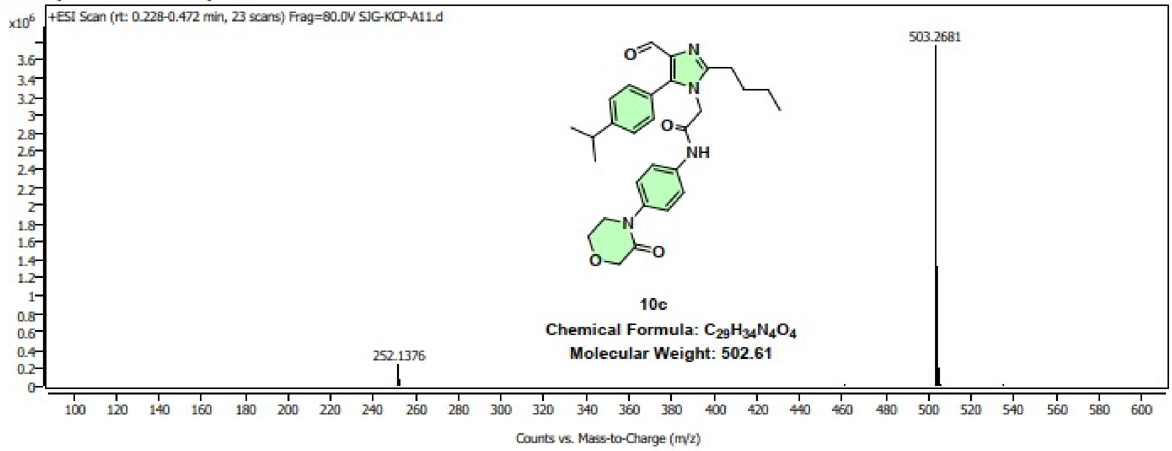


Fig. S13: HR-MS spectrum of final compound 10c

Sample Spectra

+ Scan (rt: 0.228-0.472 min) **Peak 1 from + TIC Scan**



Compound Details

Cpd. 1: C29 H34 N4 O4

Formula	m/z	Observed M/Z	Difference_Da	Difference_PPM	Score
C29 H34 N4 O4	525.2483	525.248317329607	2.52705892347649	5.03139600620311	89.56

Compound Spectra (Zoomed)

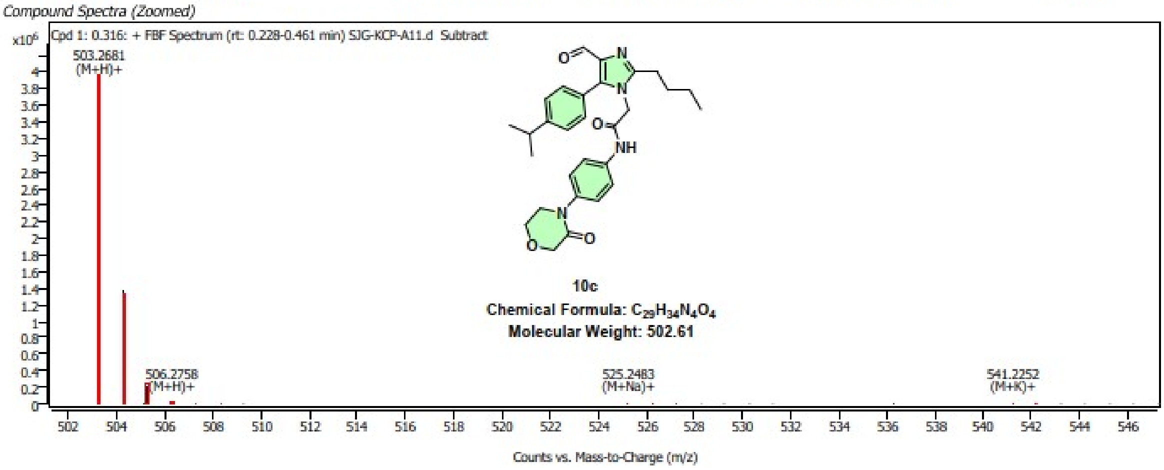
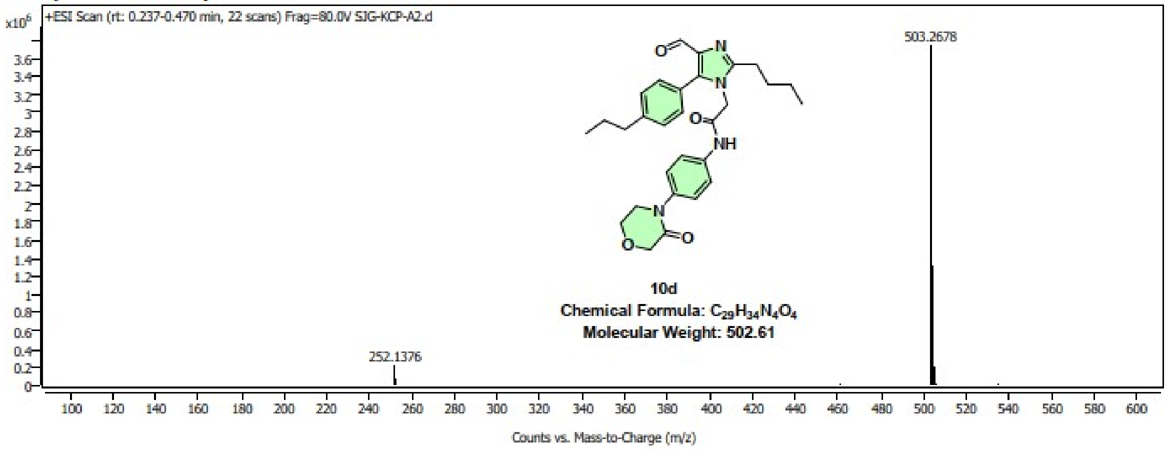


Fig. S14: HR-MS spectrum of final compound 10d

Sample Spectra

+ Scan (rt: 0.237-0.470 min) **Peak 1 from + TIC Scan**



Compound Details

Cpd. 1: C29 H34 N4 O4

Formula	m/z	Observed M/Z	Difference_Da	Difference_PPM	Score
C29 H34 N4 O4	525.2486	525.248607620822	2.31285181791918	4.60490782051464	94.73

Compound Spectra (Zoomed)

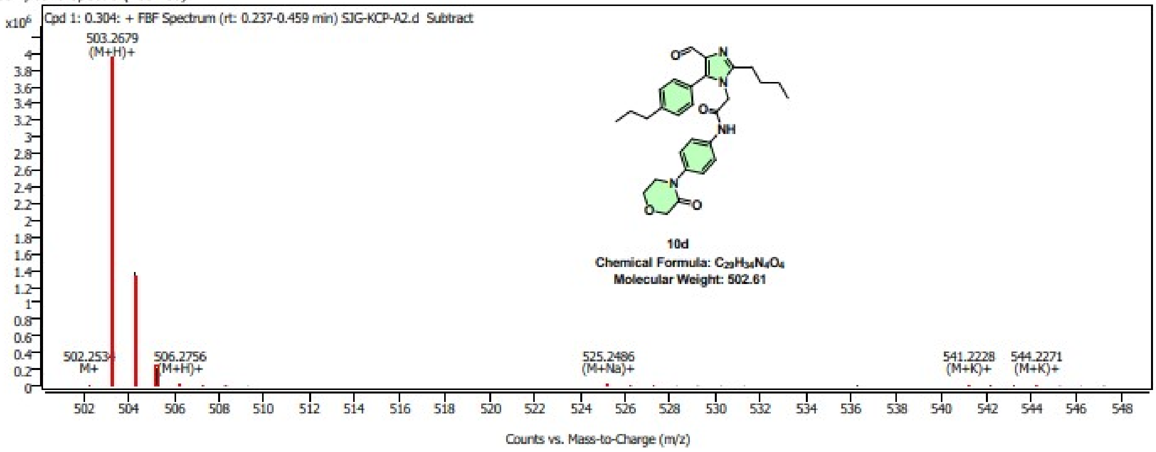
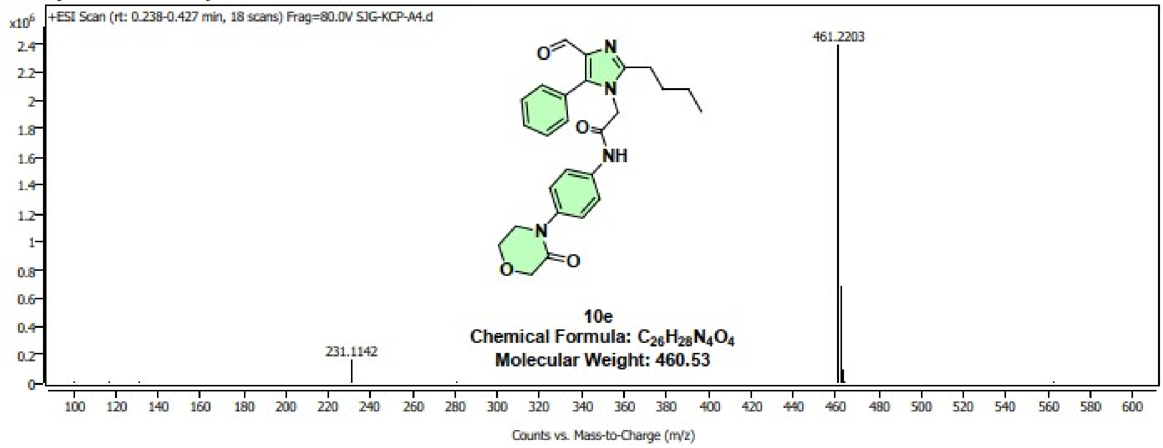


Fig. S15: HR-MS spectrum of final compound 10e

Sample Spectra

+ Scan (rt: 0.238-0.427 min) **Peak 1 from + TIC Scan**



Compound Details

Cpd. 1: C26 H28 N4 O4

Formula	m/z	Observed M/Z	Difference Da	Difference PPM	Score
C26 H28 N4 O4	461.2203	461.220330970966	1.94796542376707	4.2327653820119	91.83

Compound Spectra (Zoomed)

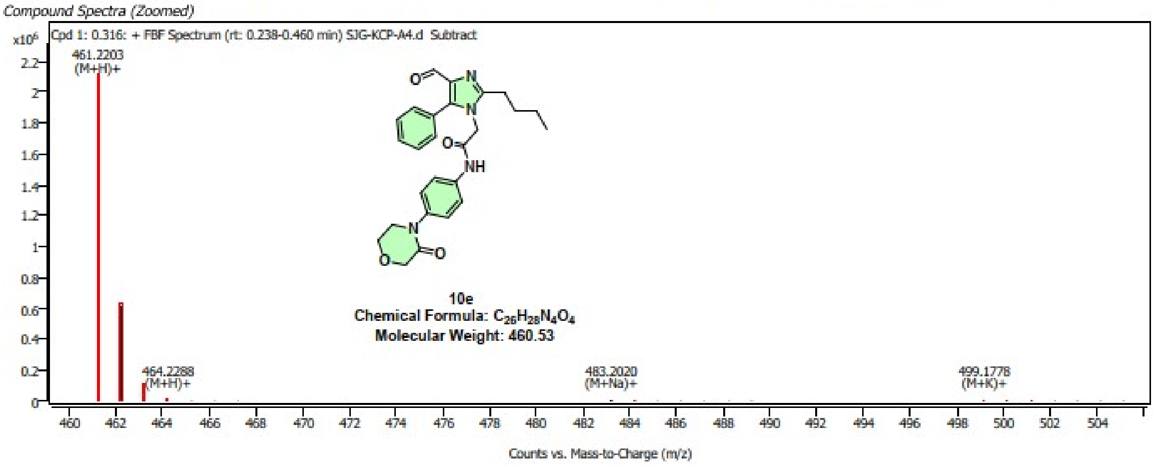


Fig. S16: IR spectrum of final compound 10a

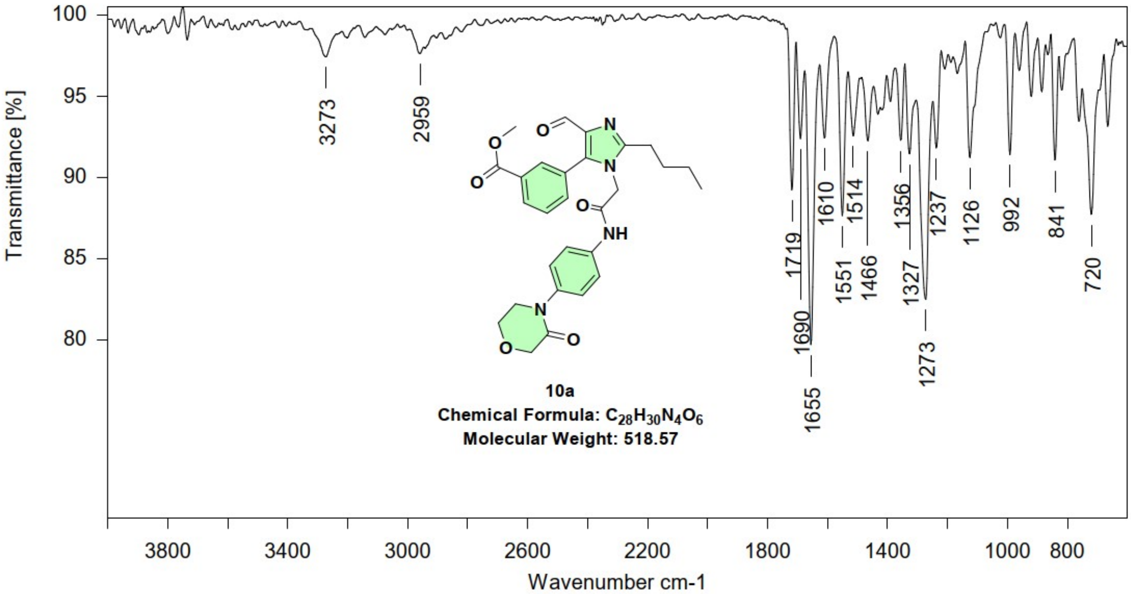


Fig. S17: IR spectrum of final compound 10b

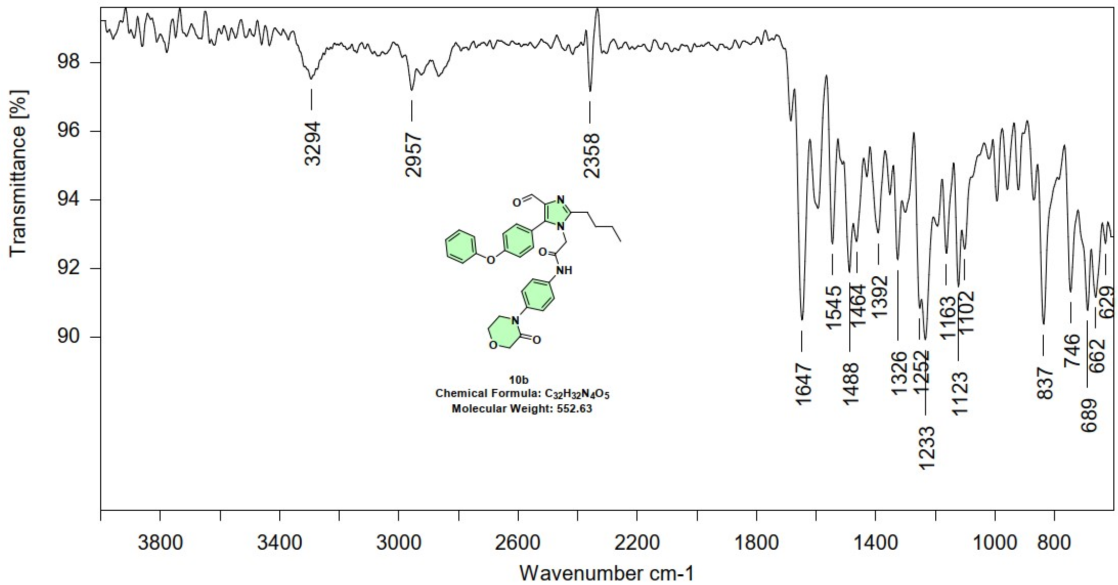


Fig. S18: IR spectrum of final compound 10c

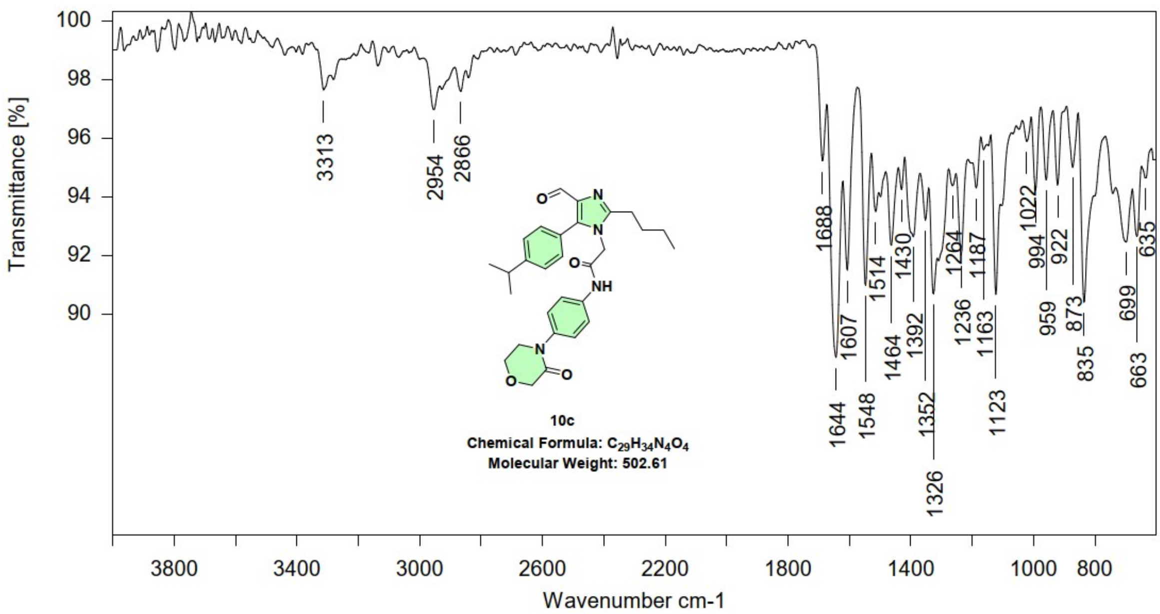


Fig. S19: IR spectrum of final compound 10d

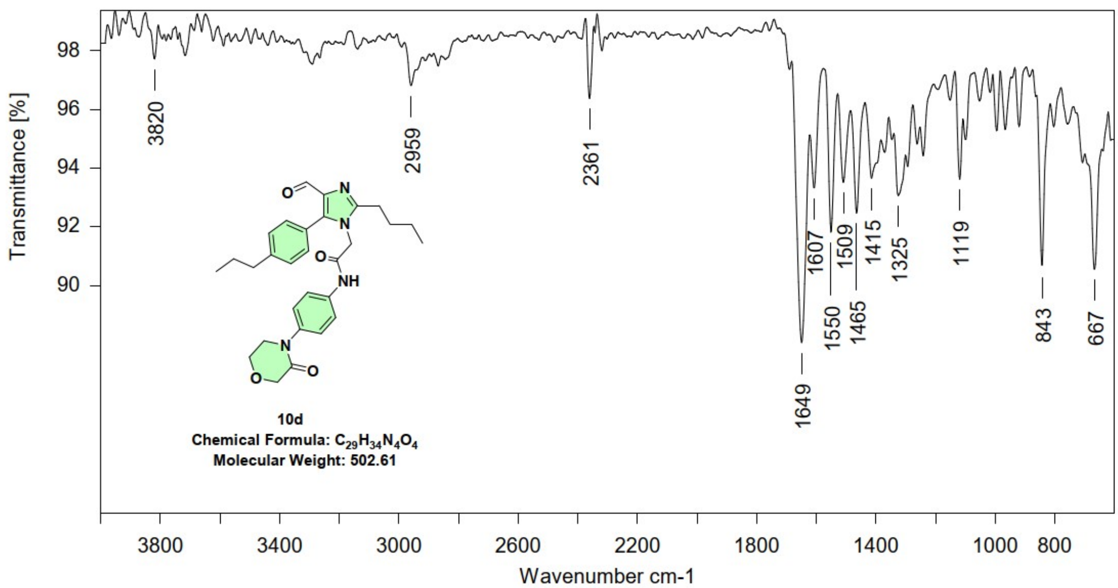


Fig. S20: IR spectrum of final compound 10e

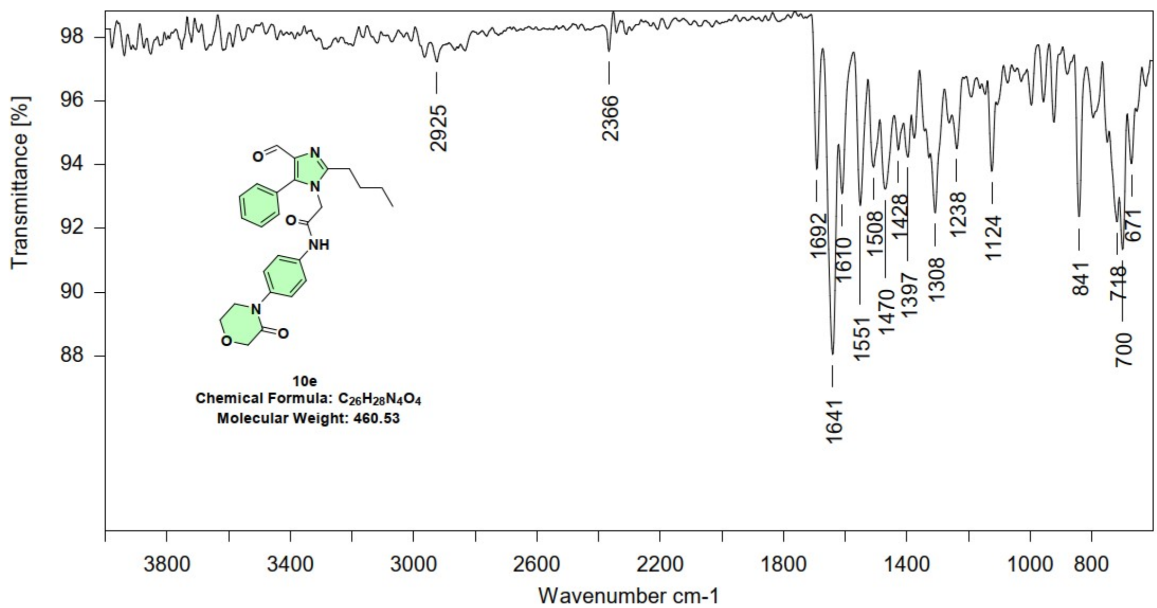
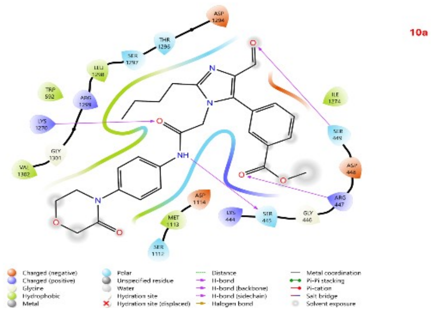
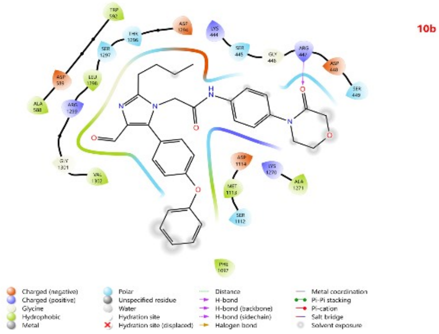
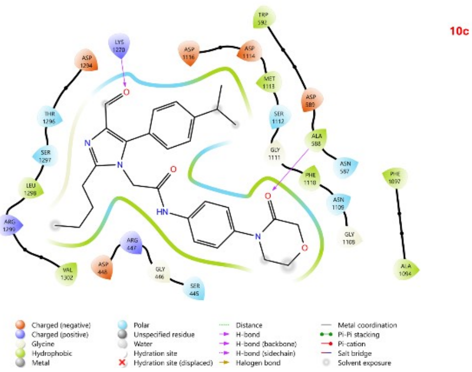


Table S1. Molecular docking scores (kcal/mol), binding poses, hydrophobic/polar interactions, hydrogen-bond interactions, and charged/glycine backbone contacts of compounds 10a–e and reference drug Novobiocin against *Staphylococcus aureus* DNA gyrase (PDB: 2XCT).

Staphylococcus aureus DNA gyrase (2XCT)					
Compound Code	**Docking Score (kcal/mol)**	**Docking Image**	**Hydrophobic Interactions / Polar Interactions**	**H-bond Interactions**	**Others (Charged / GLY)**
10a	**−5.308**		TRP 592, LEU 1298, VAL 1302, MET 1113, ILE 1274, THR 1296, SER 1297, SER 449, SER 445, SER 1112	LYS 1270 (backbone), ARG 1299 (backbone), ARG 447 (sidechain), SER 445 (backbone)	ARG 1299, ARG 447, LYS 1270 (charged+); ASP 1294, ASP 448, ASP 1114 (charged−); GLY 1301, GLY 446
10b	**−5.842**		TRP 592, LEU 1298, VAL 1302, MET 1113, ALA 588, ALA 1271, PHE 1097, THR 1296, SER 1297, SER 449, SER 445, SER 1112	ARG 447 (sidechain)	ARG 1299, ARG 447, LYS 1270 (charged+); ASP 1294, ASP 589, ASP 448, ASP 1114 (charged−); GLY 1301, GLY 446
10c	**−6.533**		TRP 592, LEU 1298, VAL 1302, MET 1113, ALA 588, PHE 1097, PHE 1110, ALA 1094, THR 1296, SER 1297, SER 445, SER 1112, ASN 587, ASN 1109	LYS 1270 (backbone), PHE 1110 (sidechain)	ARG 1299, ARG 447, LYS 1270 (charged+); ASP 1294, ASP 589, ASP 448, ASP 1114, ASP 1116 (charged−); GLY 1301, GLY 446, GLY 1111, GLY 1108

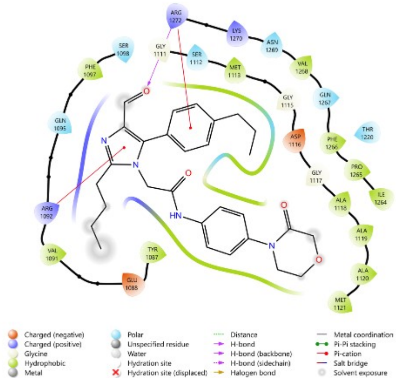
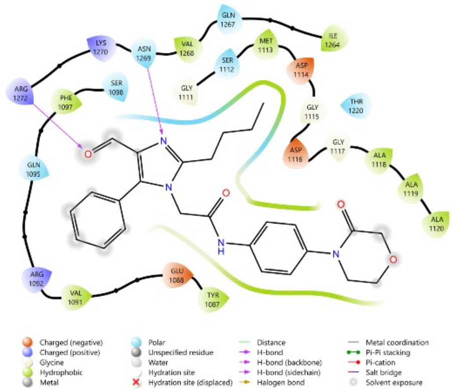
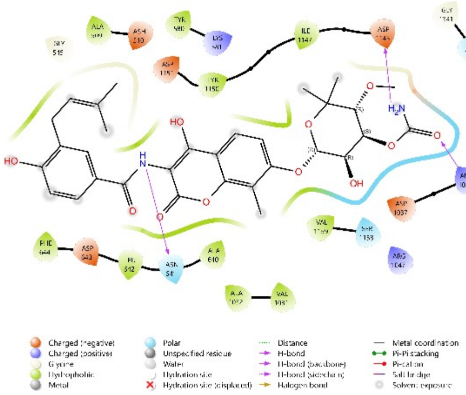
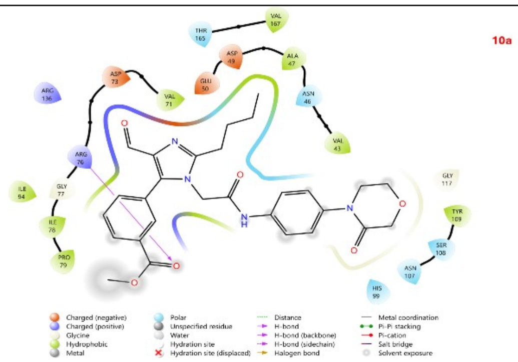
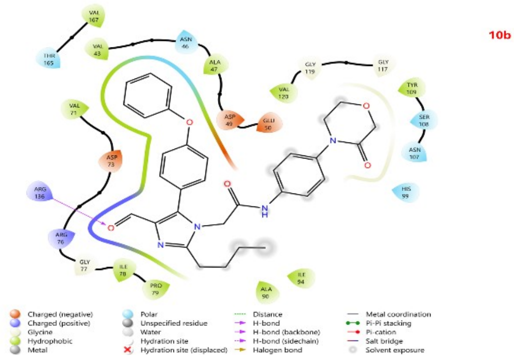
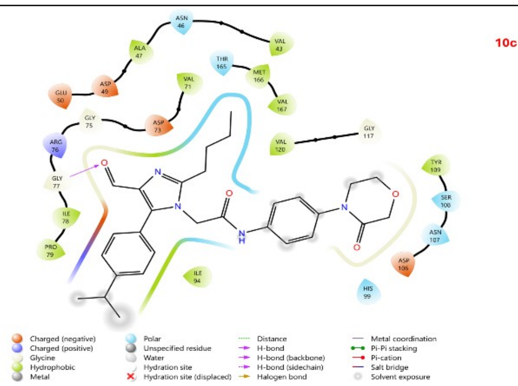
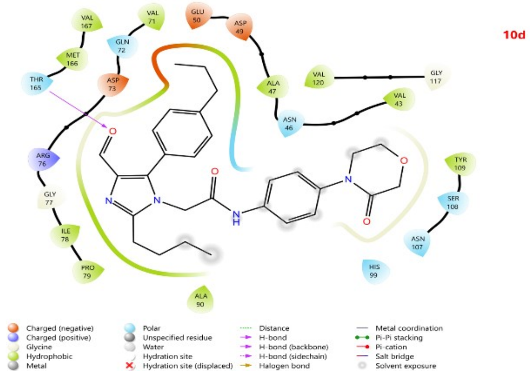
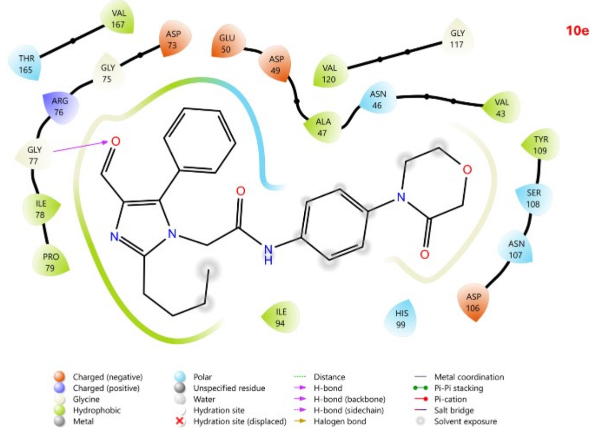
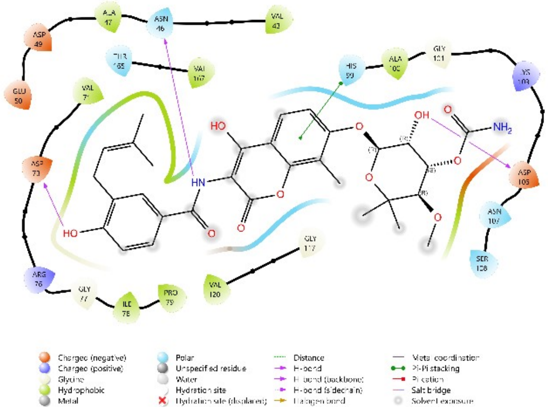
10d	**−5.712**		PHE 1097, VAL 1091, MET 1113, TYR 1087, PHE 1266, ILE 1264, ALA 1118, ALA 1119, ALA 1120, MET 1121, VAL 1268, PRO 1265, SER 1098, SER 1112, GLN 1095, GLN 1267,	ARG 1272 (backbone); Pi-cation: ARG 1092	ARG 1272, ARG 1092, LYS 1270 (charged+) ; ASP 1116, GLU 1088 (charged−) ; GLY 1111, GLY 1115, GLY 1117
10e	**−5.658**		VAL 1268, MET 1113, ILE 1264, PHE 1097, VAL 1091, TYR 1087, ALA 1118, ALA 1119, ALA 1120, SER 1098, SER 1112, GLN 1267, ASN 1269, THR 1220	ASN 1269 (backbone), ARG 1272 (sidechain)	ARG 1272, ARG 1092, LYS 1270 (charged+) ; ASP 1114, ASP 1116, GLU 1088 (charged−) ; GLY 1111, GLY 1115, GLY 1117
Novobiocin	**−5.7223**		ALA 509, TYR 580, ALA 640, LEU 642, PHE 644, ALA 1032, VAL 1031, ILE 1147, TYR 1150, VAL 1159	ASN 641 backbone), ARG 1039 (backbone), ASP 1145 (backbone),	LYS 581, ARG 1039, ARG 1047, (charged+) ; ASH 510, ASP 643, ASP 1037, ASP 1151, ASP 1145, (charged−) ;

Table S2. Molecular docking scores (kcal/mol), binding poses, hydrophobic/polar interactions, hydrogen-bond interactions, and charged/glycine backbone contacts of compounds 10a–e and reference drug Novobiocin against *Escherichia coli* GyrB24 (PDB: 7P2M).

Escherichia coli DNA gyrase subunit B (GyrB24) (7P2M)					
Compound Code	**Docking Score (kcal/mol)**	**Docking Image**	**Hydrophobic Interactions/ Polar Interactions**	**H-bond Interactions**	**Others (Charged / GLY)**
10a	**−4.582**		VAL 167, ALA 47, VAL 43, ILE 94, ILE 78, PRO 79, TYR 109, THR 165, ASN 46, HIS 99, SER 108, ASN 107	ARG 76 (backbone)	ARG 136, ARG 76 (charged+) ; ASP 49, ASP 73, GLU 50 (charged−) ; GLY 77, GLY 117
10b	**−4.918**		VAL 167, VAL 43, ALA 47, VAL 71, ILE 78, PRO 79, ILE 94, ALA 90, TYR 109, VAL 120, THR 165, ASN 46, HIS 99, SER 108, ASN 107	ARG 76 (backbone)	ARG 136, ARG 76 (charged+) ; ASP 49, ASP 73, GLU 50 (charged−) ; GLY 77, GLY 117, GLY 119
10c	**−5.821**		VAL 43, ALA 47, VAL 71, ILE 78, PRO 79, ILE 94, MET 166, VAL 167, VAL 120, TYR 109, ASN 46, THR 165, HIS 99, SER 108, ASN 107	ARG 76 (backbone)	ARG 76 (charged+) ; ASP 49, ASP 73, GLU 50, ASP 106 (charged−) ; GLY 75, GLY 77, GLY 117

10d	−5.546		VAL 71, VAL 43, ALA 47, MET 166, VAL 167, ILE 78, PRO 79, ALA 90, VAL 120, TYR 109, THR 165, ASN 46, GLN 72, HIS 99, SER 108, ASN 107	THR 165 (backbone)	ARG 76 (charged+) ; ASP 49, ASP 73, GLU 50 (charged−) ; GLY 77, GLY 117
10e	−5.382		VAL 167, VAL 43, ALA 47, VAL 120, ILE 78, PRO 79, ILE 94, TYR 109, THR 165, ASN 46, HIS 99, SER 108, ASN 107	ARG 76 (backbone)	ARG 76 (charged+) ; ASP 49, ASP 73, GLU 50, ASP 106 (charged−) ; GLY 75, GLY 77, GLY 117
Novobiocin	−5.192		VAL 43, ALA 47, VAL 71, ILE 78, PRO 79, ALA 100, VAL 120, VAL 167,	ASN 46 (sidechain), ASP 73 (side chain) ASP 106 (sidechain)	ARG 76, LYS 103 (charged+) ; ASP 49, GLU 50, ASP 73, (charged−)

In Vitro Antimicrobial Activity

Microorganisms and Culture Conditions

Antimicrobial activity of compounds 10a–e was evaluated against four bacterial strains, *Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442, *Escherichia coli* MTCC 443, and *Pseudomonas aeruginosa* MTCC 1688 and two fungal strains, *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 282. All microbial strains were procured from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India [1]. Bacterial strains were maintained on nutrient agar (NA) and fungal strains on Sabouraud dextrose agar (SDA) at appropriate temperatures (37 °C for bacteria; 28 °C for fungi) [3].

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of all synthesised compounds was determined by the broth microdilution method following the Clinical and Laboratory Standards Institute (CLSI) guidelines [1,2]. Stock solutions of test compounds were prepared in dimethyl sulfoxide (DMSO) at a concentration of 1000 µg/mL and subsequently diluted in Mueller–Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi to achieve the required concentration range (3.90–250 µg/mL) [3]. Serial two-fold dilutions were performed in 96-well microtitre plates [3]. Each well was inoculated with a standardised microbial suspension (approximately 5×10^5 CFU/mL for bacteria; 0.5×10^3 to 2.5×10^3 CFU/mL for fungi) and incubated at 37 °C for 24 h (bacteria) or 48–72 h at 28 °C (fungi) [1,2]. Chloramphenicol and griseofulvin were used as reference standards for antibacterial and antifungal activity, respectively [3]. DMSO at the highest concentration used served as the negative control. The MIC was defined as the lowest concentration of compound that visually inhibited microbial growth [1,2,3]. All assays were performed in triplicate, and results are expressed as mean ± standard deviation (SD).

References

[1] Clinical and Laboratory Standards Institute (CLSI). (2022). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically (12th ed.; CLSI standard M07). CLSI.

[2] Clinical and Laboratory Standards Institute (CLSI). (2022). Reference method for broth dilution antifungal susceptibility testing of yeasts (4th ed.; CLSI standard M27). CLSI.

[3] Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Table S3. In vitro antimicrobial activity (MIC, µg/mL) of compounds 10a–e against bacterial strains (*S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*) and fungal strains (*C. albicans*, *A. niger*); chloramphenicol and griseofulvin used as reference standards.

Compound	*S. aureus* MTCC 96 (µg/mL)	*S. pyogenes* MTCC 442 (µg/mL)	*E. coli* MTCC 443 (µg/mL)	*P. aeruginosa* MTCC 1688 (µg/mL)	*C. albicans* MTCC 227 (µg/mL)	*A. niger* MTCC 282 (µg/mL)
10a	15.62 ± 0.3	62.5 ± 0.5	31.25 ± 0.6	7.82 ± 0.4	31.25 ± 0.1	62.5 ± 0.3
10b	31.25 ± 0.4	15.62 ± 0.5	62.5 ± 0.5	31.25 ± 0.4	62.5 ± 0.1	31.25 ± 0.2
10c	7.81 ± 0.2	7.82 ± 0.7	15.62 ± 0.3	15.62 ± 0.3	15.62 ± 0.3	31.25 ± 0.5
10d	15.62 ± 0.6	15.62 ± 0.1	31.25 ± 0.3	31.25 ± 0.5	62.5 ± 0.7	62.5 ± 0.1
10e	15.62 ± 0.8	31.25 ± 0.3	31.25 ± 0.2	62.5 ± 0.5	62.5 ± 0.2	62.5 ± 0.3
Chloramphenicol	7.8 ± 0.1	7.8 ± 0.1	7.8 ± 0.2	7.8 ± 0.2	-	-
Griseofulvin	-	-	-	-	15.62 ± 0.4	15.62 ± 0.2