Supplementary Information

Quinazoline-Dihydropyrimidinone Hybrids as Potent Antichagasic and Antileishmanial Agents: Synthesis, Biological Evaluation and Molecular Docking Studies

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Dose / response curves

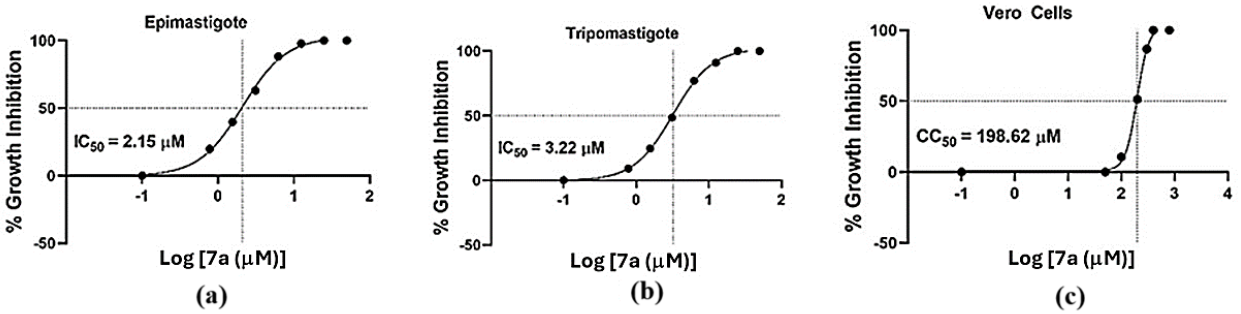

Figure S1. Dose-response curves of hybrid molecule **7a** on tripomastigotes (a) and epimastigotes (b) forms of *Trypanosoma cruzi* and, Vero cells (c).

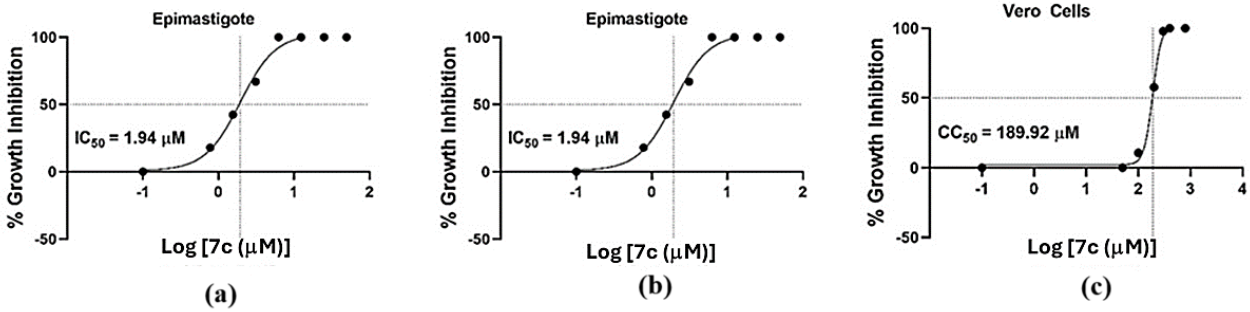

Figure S2. Dose-response curves of hybrid molecule **7c** on tripomastigotes (a) and epimastigotes (b) forms of *Trypanosoma cruzi* and, Vero cells (c).

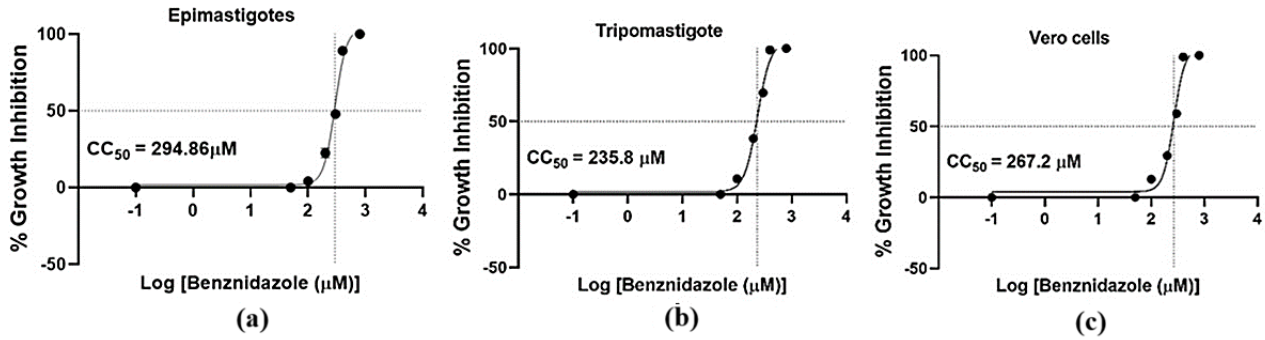

Figure S3. Dose-response curves of benznidazole treatment on tripomastigotes (a) and epimastigotes (b) forms of *Trypanosoma cruzi* and, Vero cells (c).

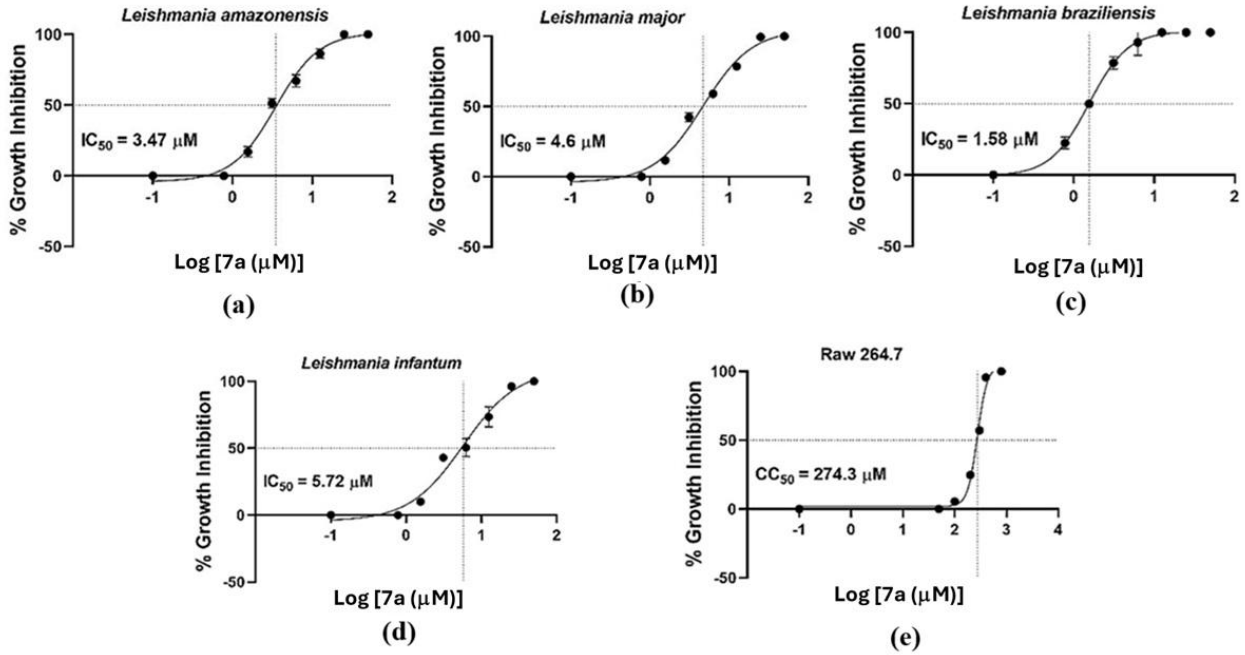

Figure S4. Dose-response curves of hybrid **7a** on promastigotes of *Leishmania* spp. and RAW 264.7 macrophages.

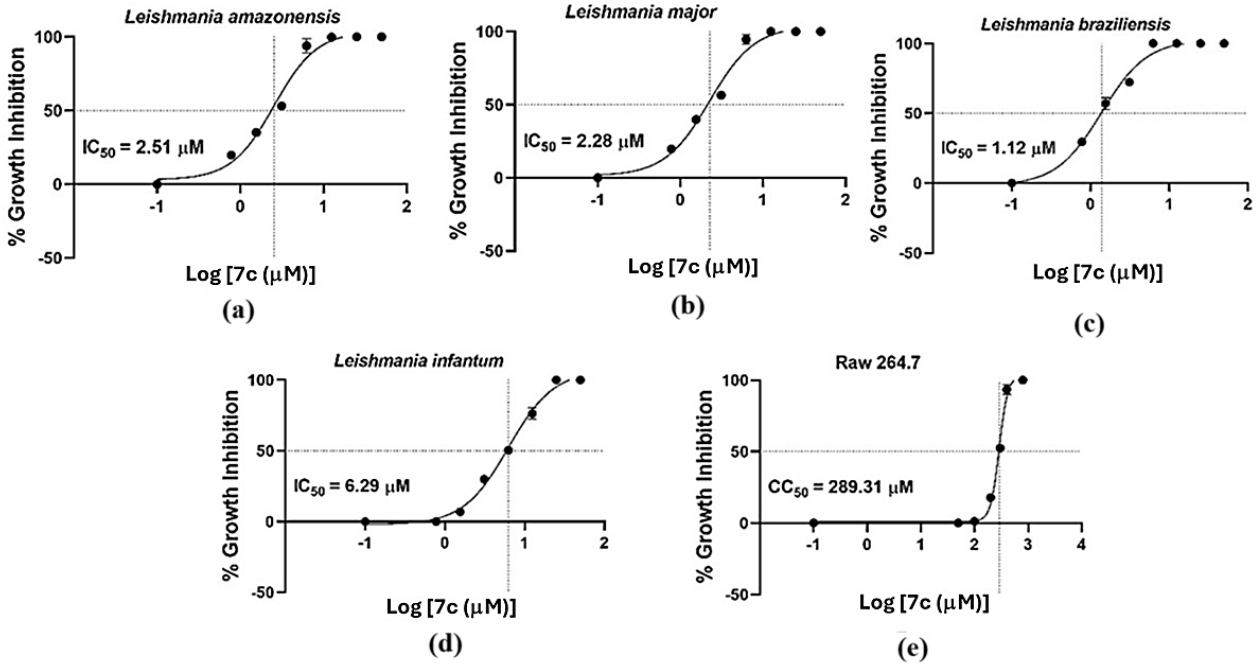

Figure S5. Dose-response curves of hybrid molecule **7c** on promastigotes of *Leishmania* spp. and RAW 264.7 macrophages.

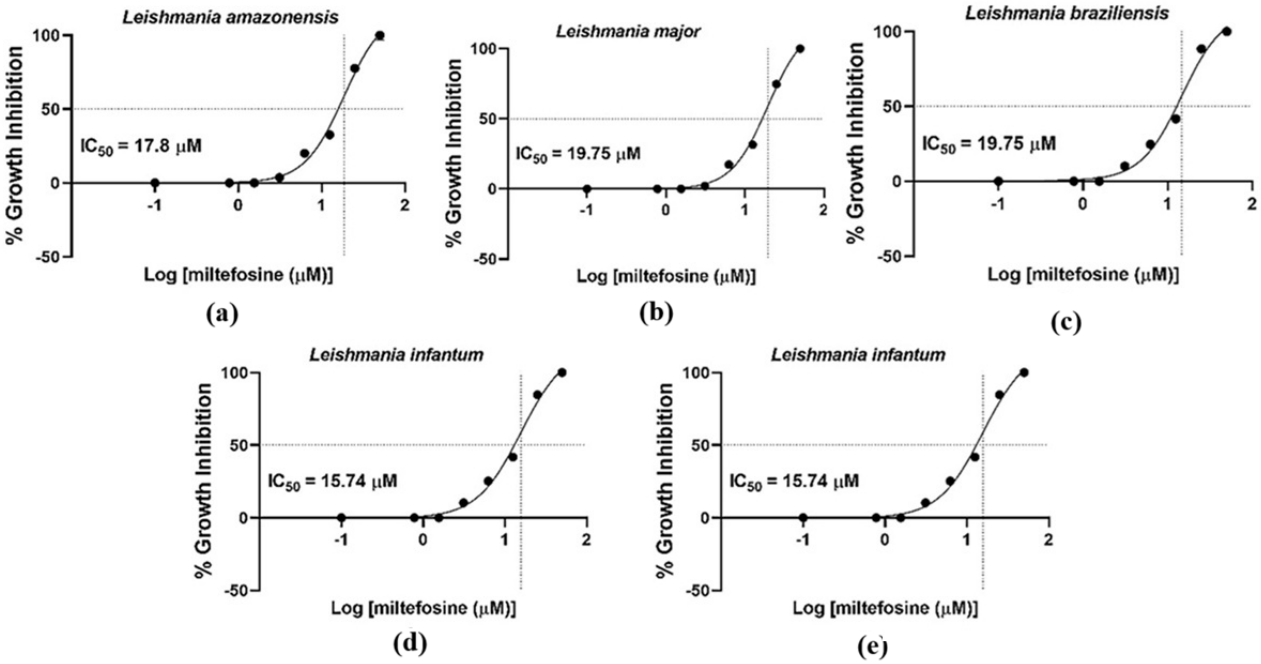

Figure S6. Dose-response curves of miltefosine treatment on promastigotes of *Leishmania* spp. and RAW 264.7 macrophages.

3D Molecular interaction's maps

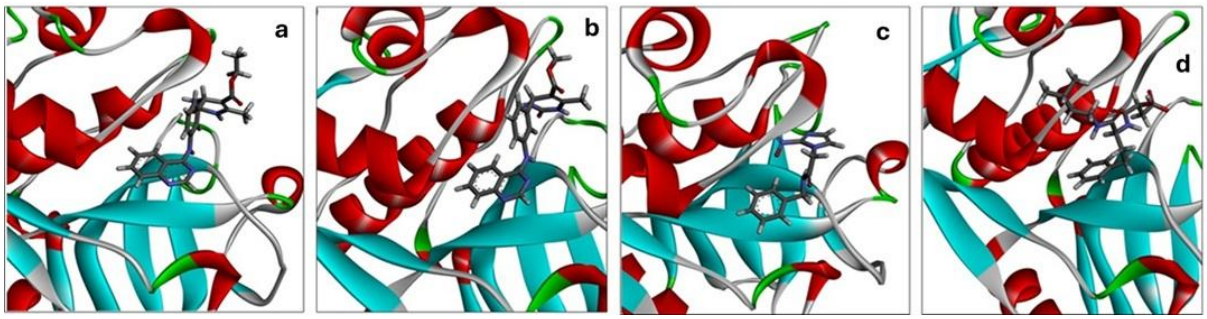

Figure S7. 3D molecular interaction maps of the complexes formed by compounds **7a** (a), **7c** (b), control compound benznidazole (c) and co-crystallized ligand homo phenyl-alanyl amino derivative (d), respectively, with the target cruzain from *Trypanosoma cruzi* (PDB ID: 1EWM).

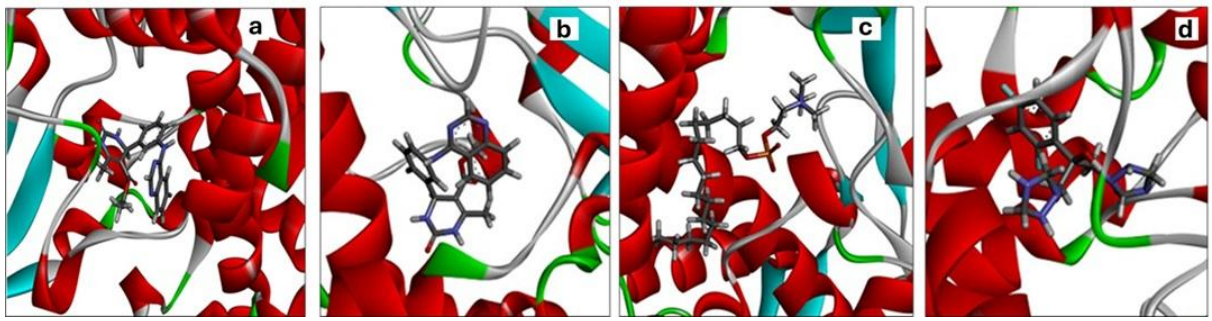

Figure S8. 3D molecular interaction maps of the complex formed by compound **7a** (a), **7b** (b), control compound miltefosine (c) and co-crystallized ligand difluoro-phenyl-triazole derivative (d), respectively, with the target sterol 14-alpha demethylase (CYP51) from *Leishmania infantum* (PDB: 3L4D).

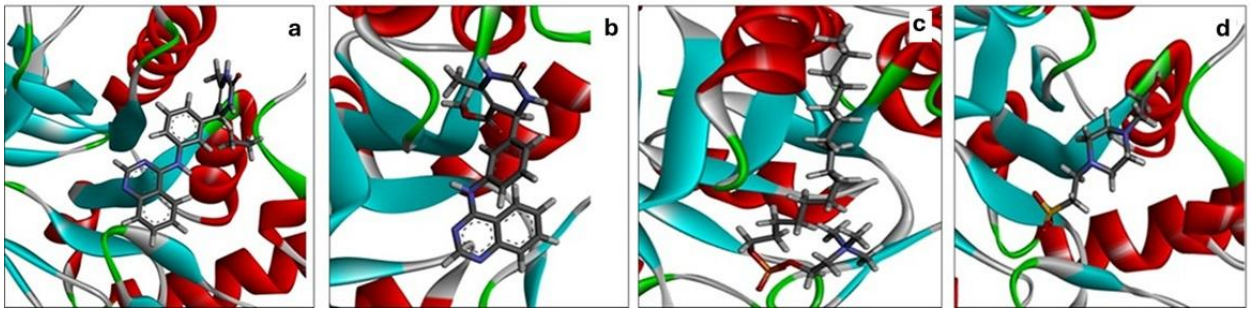

Figure S9. 3D molecular interaction maps of the complex formed by compound **7a** (a), **7c** (b), control compound miltefosine (c) and co-crystallized ligand piperazine derivative (d), respectively, with the target dihydroorotate dehydrogenase from *Leishmania braziliensis* (PDB: 4WZH).

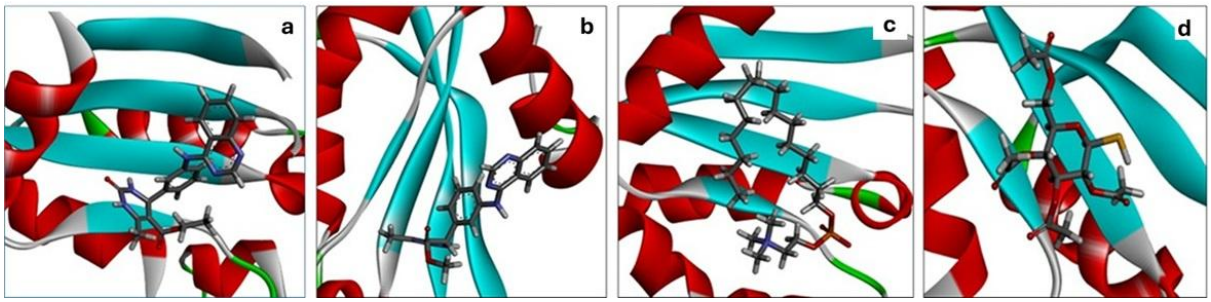

Figure S10. 3D molecular interaction maps of the complex formed by compound **7a** (a), **7c** (b), miltefosine (c) and reference control auranofin (d), respectively, with the target nucleoside diphosphatase kinase (NDK) protein from *Leishmania amazonensis* (PDB ID: 5GO1).

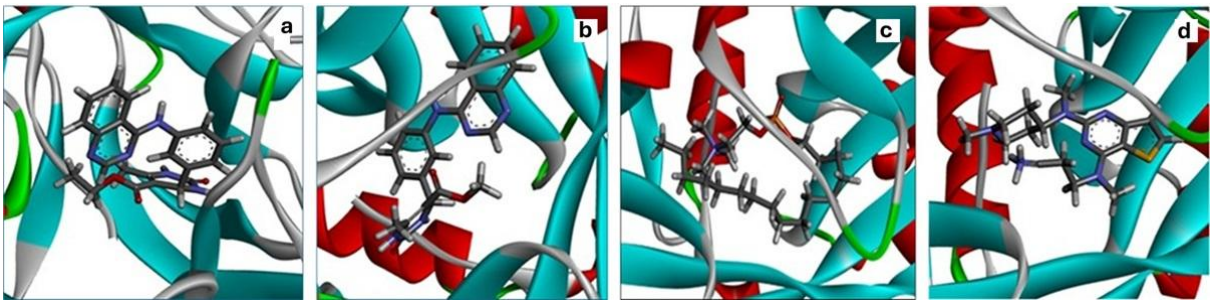

Figure S11. 3D molecular interaction maps of the complex formed by compound **7a** (a), **7c** (b), miltefosine (c) and PDB ligand thienopyrimidine derivative (d), respectively, with the target *N*-myristoyltransferase from *Leishmania major* (PDB ID: 4CGO).

Table S1. Binding energy values and normalized score (NS) according to the MolDock score and PLANTS score algorithms for the cruzain from *T. cruzi* (PDB: 1EWM)

ID	MolDock score	(NS) MolDock score	PLANTS score	(NS) PLANTS score	(NS) Total	RMSD
7a	–93.158	0.840	–440.769	0.816	0.828	0.446
7c	–84.432	0.761	–435.303	0.806	0.783	
Benznidazole	–86.776	0.782	–391.860	0.725	0.754	
PDB Ligand	–110.879	1	–540.025	1	1	

RMSD: root mean square deviation.

Table S2. Binding energy values and normalized score (NS) according to the MolDock score and PLANTS score algorithms for the protein sterol 14-alpha demethylase (CYP51) from *L. infantum* (PDB: 3L4D)

ID	MolDock score	(NS) MolDock score	PLANTS score	(NS) PLANTS score	(NS) Total	RMSD
7a	–85.157	0.790	–462.170	0.861	0.826	0.207
7c	–67.226	0.624	–460.375	0.858	0.741	
Miltefosine	–107.696	1	–219.457	0.409	0.704	
PDB Ligand	–72.461	0.672	–536.291	1	0.836	

RMSD: root mean square deviation.

Table S3. Binding energy values and normalized score (NS) according to the MolDock score and PLANTS score algorithms for the protein dihydroorotate dehydrogenase from *L. braziliensis* (PDB: 4WZH)

ID	MolDock score	(NS) MolDock score	PLANTS score	(NS) PLANTS score	(NS) Total	RMSD
7a	–68.593	0.774	–512.989	0.972	0.873	0.238
7c	–64.640	0.729	–510.403	0.967	0.848	
Miltefosine	–88.582	1	–298.870	0.566	0.783	
PDB Ligand	–34.866	0.393	–527.756	1	0.696	

RMSD: root mean square deviation.

Table S4. Binding energy values and normalized score (NS) according to the MolDock score and PLANTS score algorithms for the nucleoside diphosphatase kinase (NDK) protein from *L. amazonensis* (PDB: 5GO1)

ID	MolDock score	(NS) MolDock score	PLANTS score	(NS) PLANTS score	(NS) Total	RMSD
7a	–99.186	0.863	–67.905	1	0.931	-
7c	–98.827	0.859	–65.930	0.970	0.915	
Miltefosine	–114.918	1	–65.005	0.957	0.978	
Control	–90.860	0.790	–44.975	0.662	0.726	

RMSD: root mean square deviation.

Table S5. Binding energy values and normalized score (NS) according to the MolDock score and PLANTS score algorithms for the protein *N*-myristoyltransferase from *L. major* (PDB: 4CGO)

ID	MolDock score	(NS) MolDock score	PLANTS score	(NS) PLANTS score	(NS) Total	RMSD
7a	–102.731	0.903	–542.852	0.955	0.929	0.178
7c	–96.238	0.846	–543.714	0.957	0.902	
Miltefosine	–111.112	0.977	–222.715	0.392	0.684	
PDB Ligand	–113.682	1	–567.843	1	1	

RMSD: root mean square deviation.

Coulomb and Lenard-Jones energies

Table S6. Contributions of Coulomb and Lennard-Jones energies for the compounds under study with the target cruzain from *T. cruzi* (PDB: 1EWM)

Compound	Coulomb energy / (kJ mol^{-1})	Lennard-Jones energy / (kJ mol^{-1})
7a	–44.504	–105.856
7c	–67.073	–83.710
Benznidazole	–52.482	–114.728
PDB Ligand	–68.267	–123.188

Table S7. Contributions of Coulomb and Lennard-Jones energies for the compounds under study with the target sterol 14-alpha demethylase (CYP51) from *L. infantum* (PDB: 3L4D)

Compound	Coulomb energy / (kJ mol^{-1})	Lennard-Jones energy / (kJ mol^{-1})
7a	–30.324	–199.667
7c	–92.268	–180.483
Miltefosine	–257.617	–205.112
PDB Ligand	–14.418	–154.189

Table S8. Contributions of Coulomb and Lennard-Jones energies for the compounds under study with the target dihydroorotate dehydrogenase from *L. braziliensis* (PDB: 4WZH)

Compound	Coulomb energy / (kJ mol^{-1})	Lennard-Jones energy / (kJ mol^{-1})
7a	–46.637	–135.453
7c	–62.466	–112.954
Miltefosine	–98.696	–125.121
PDB Ligand	–147.854	–81.223

Table S9. Contributions of Coulomb and Lennard-Jones energies for the compounds under study with the target nucleoside diphosphatase kinase (NDK) protein from *L. amazonensis* (PDB: 5GO1)

Compound	Coulomb energy / (kJ mol^{-1})	Lennard-Jones energy / (kJ mol^{-1})
7a	–34.319	–126.817
7c	–161.197	–134.809
Miltefosine	–71.485	–170.377
Auranofina	–214.472	–110.540

Table S10. Contributions of Coulomb and Lennard-Jones energies for the compounds under study with the target *N*-myristoyltransferase from *L. major* (PDB: 4CGO)

Compound	Coulomb energy / (kJ mol^{-1})	Lennard-Jones energy / (kJ mol^{-1})
7a	–48.861	–163.852
7c	–56.375	–128.375
Miltefosine	–142.246	–132.579
PDB Ligand	–40.879	–163.354

^{1}H NMR, ^{13}C NMR and HRMS spectra

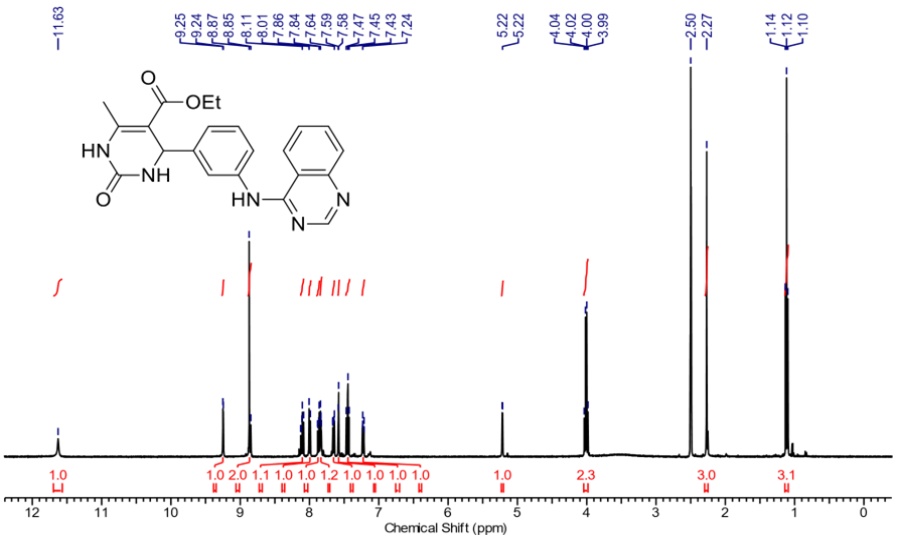

Figure S12. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7a**.

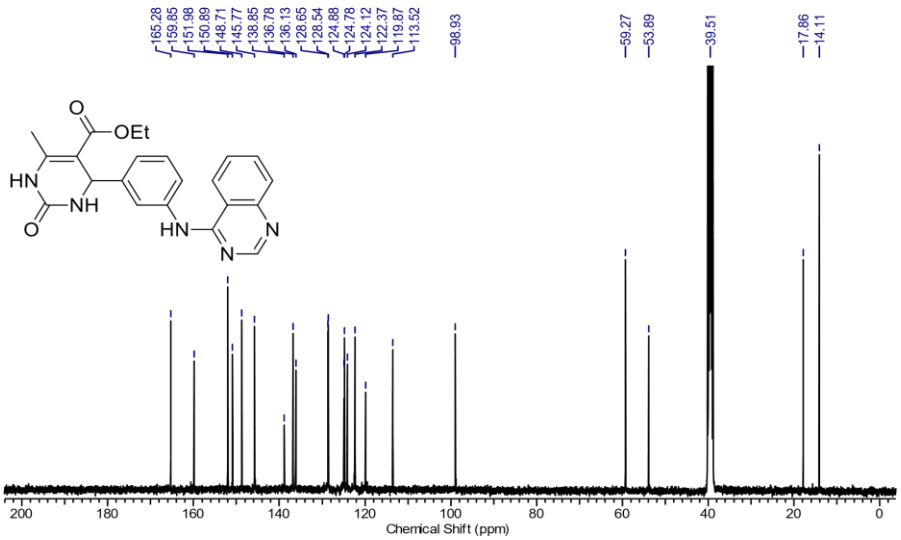

Figure S13. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7a**.

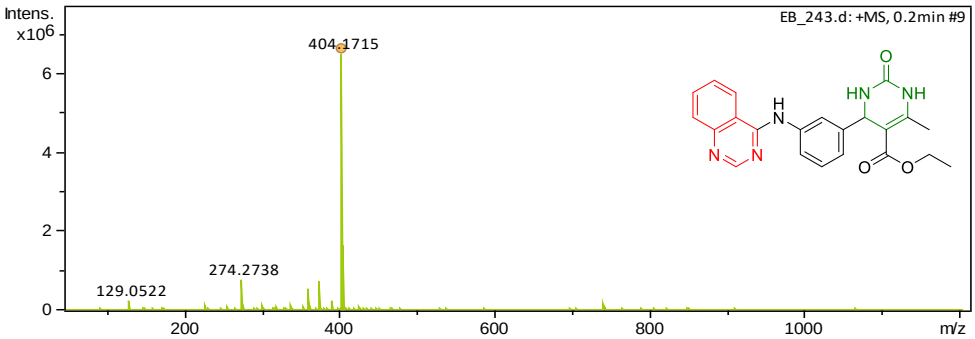

Figure S14. HRMS of compound **7a**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e$^-$ configuration	N-rule
404.1715	$C_{22}H_{22}N_5O_3$	404.1717	0.5	6.7	14.5	even	ok

rdb: ring and double bond.

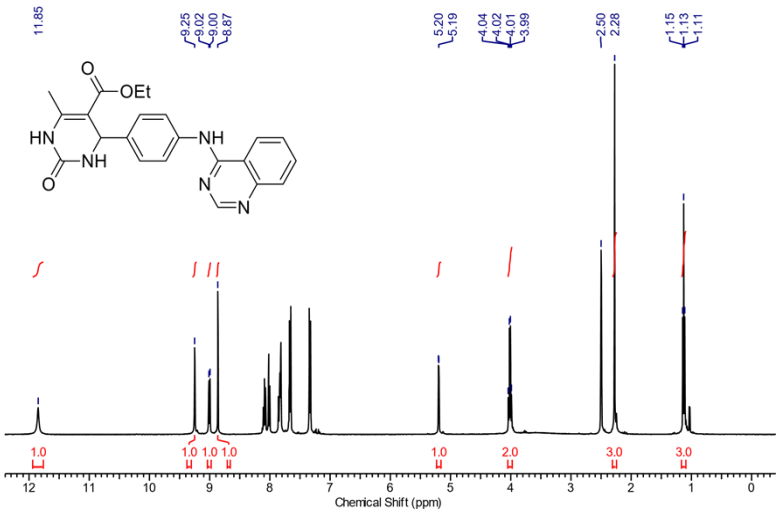

Figure S15. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7b**.

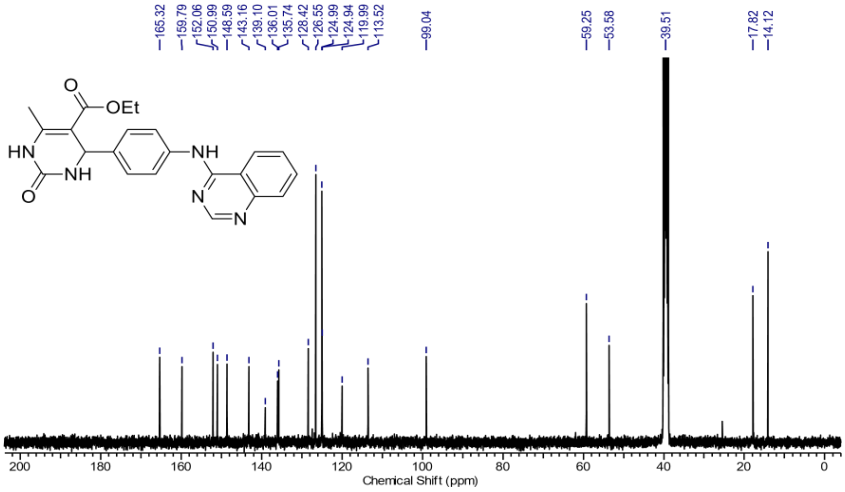

Figure S16. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7b**.

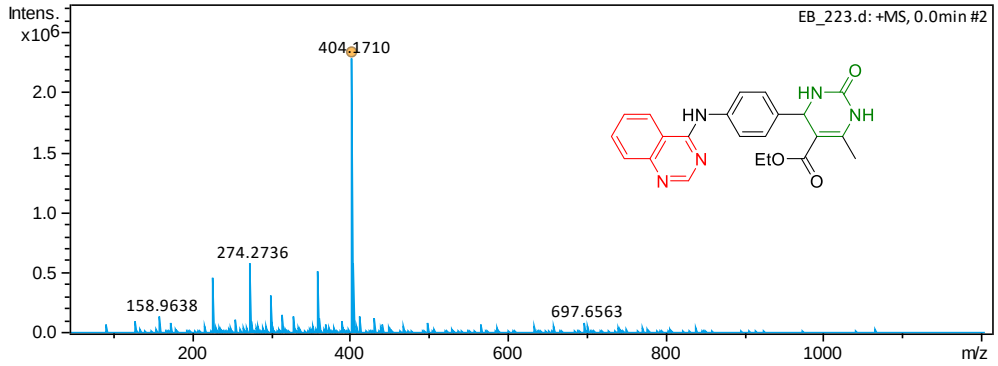

Figure S17. HRMS of compound **7b**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
404.1710	$C_{22}H_{22}N_5O_3$	404.1717	1.7	6.3	14.5	even	ok

rdb: ring and double bond.

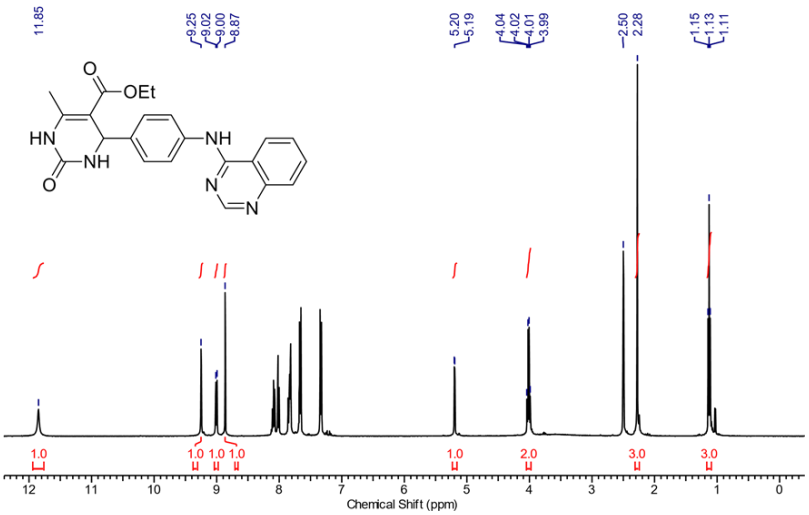

Figure S18. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7c**.

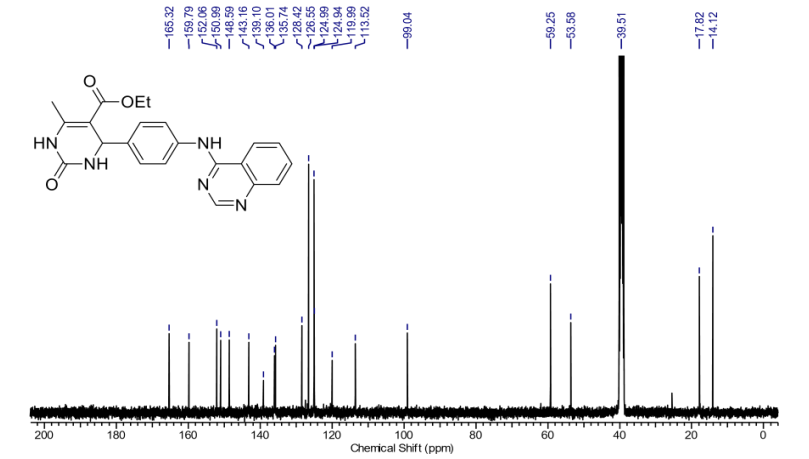

Figure S19. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7c**.

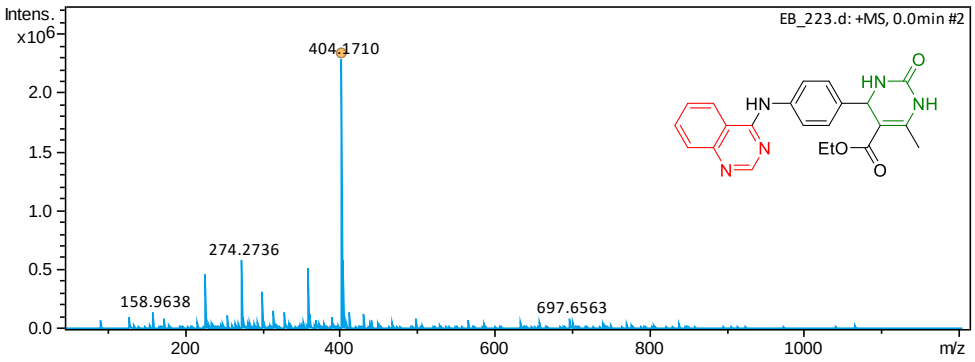

Figure S20. HRMS of compound **7c**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
404.1710	$C_{22}H_{22}N_5O_3$	404.1717	1.7	6.3	14.5	even	ok

rdb: ring and double bond.

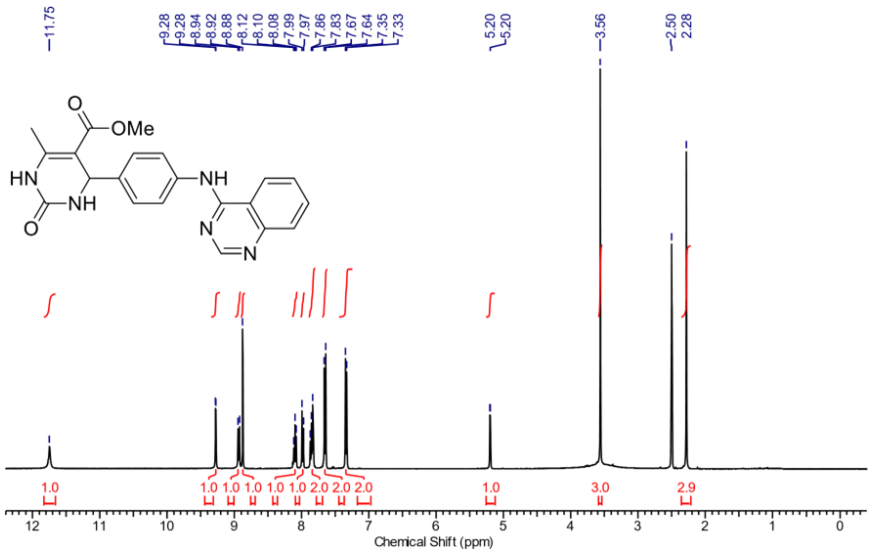

Figure S21. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7d**.

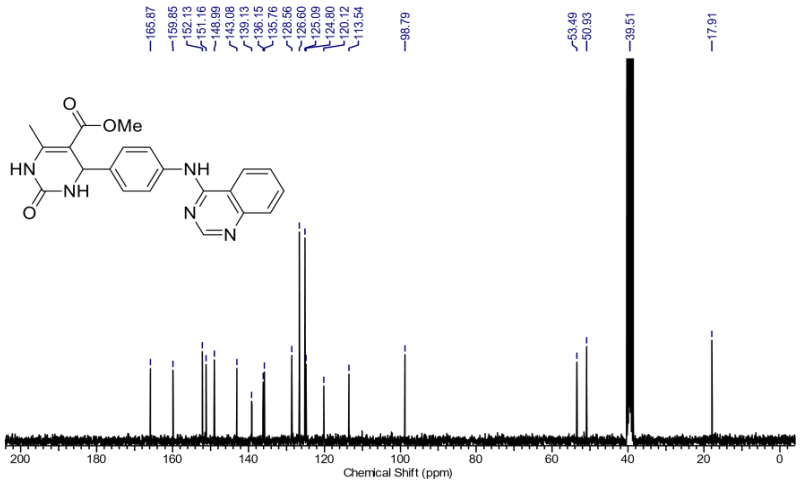

Figure S22. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7d**.

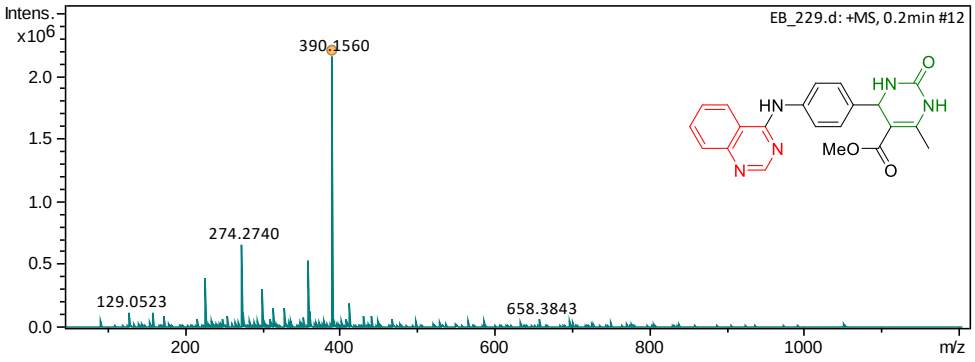

Figure S23. HRMS of compound **7d**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
390.1560	$C_{21}H_{20}N_5O_3$	390.1561	0.1	4.4	14.5	even	ok

rdb: ring and double bond.

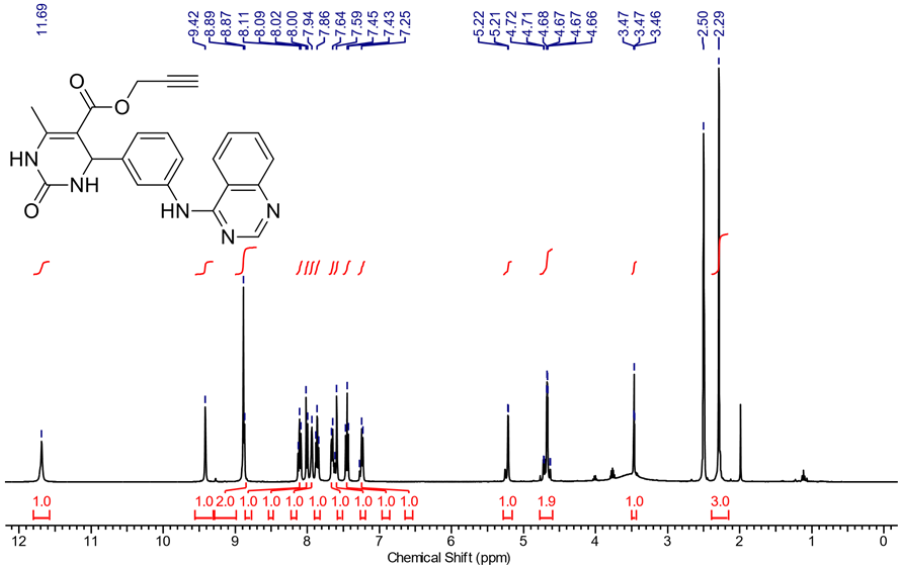

Figure S24. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7e**.

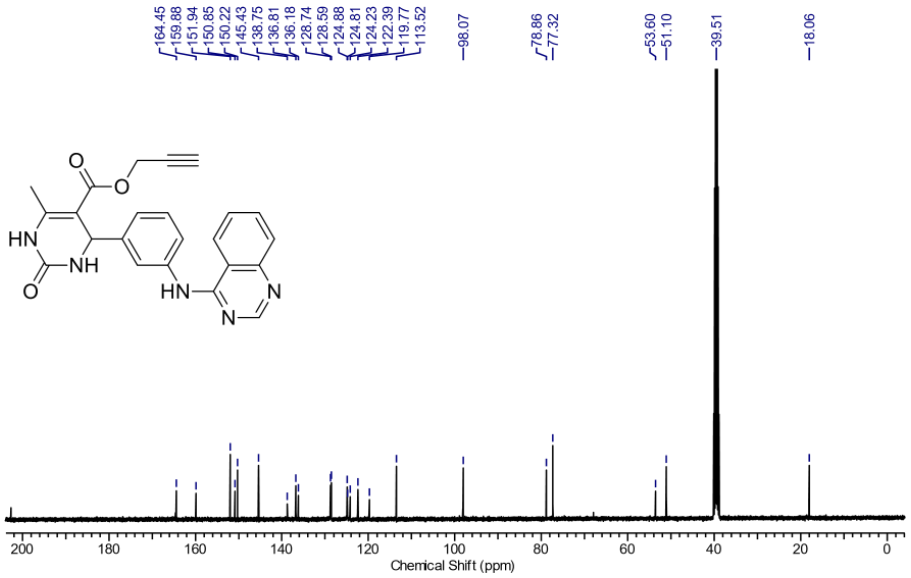

Figure S25. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7e**.

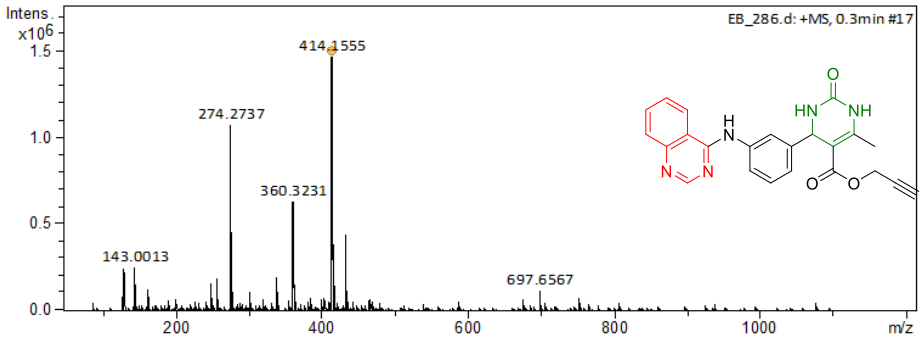

Figure S26. HRMS of compound **7e**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
414.1555	$C_{23}H_{20}N_5O_3$	414.1561	1.3	5.5	16.5	even	ok

rdb: ring and double bond.

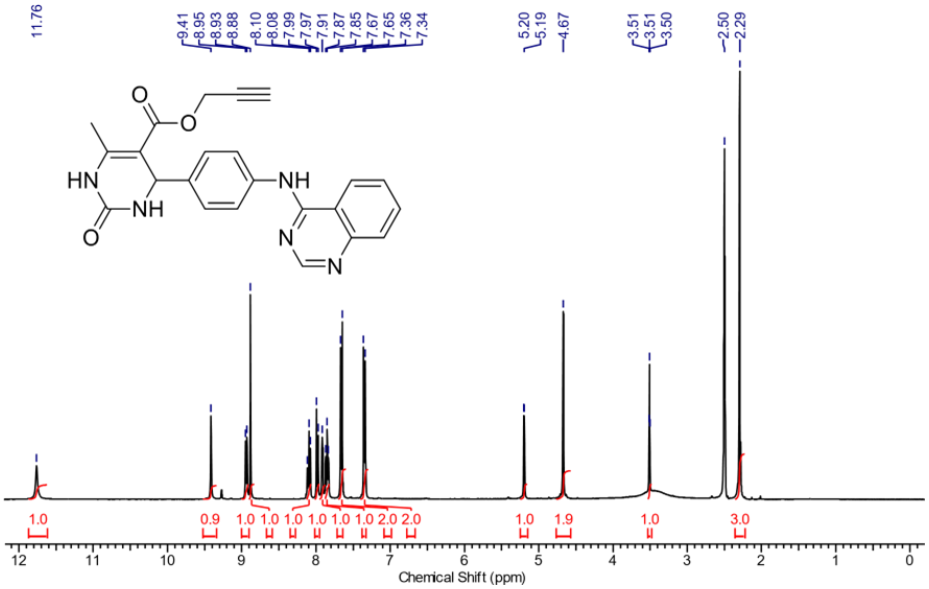

Figure S27. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7f**.

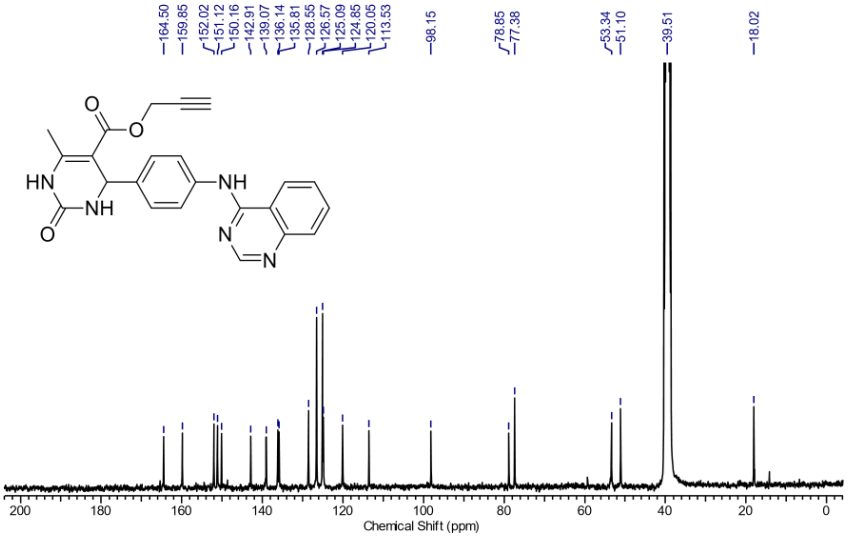

Figure S28. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7f**.

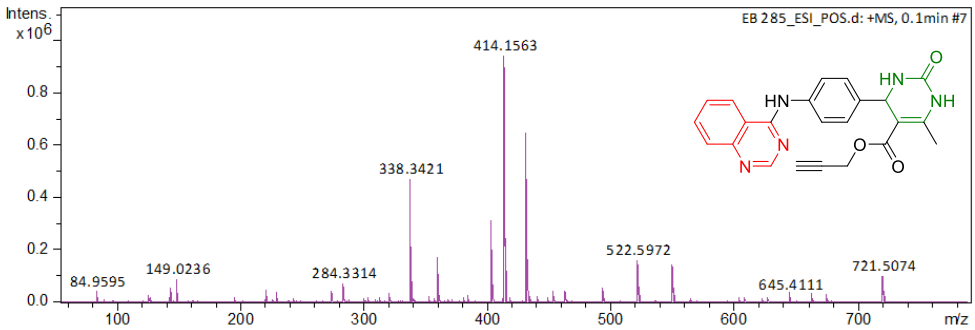

Figure S29. HRMS of compound **7f**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
414.1563	$C_{23}H_{20}N_5O_3$	414.1561	–0.7	6.7	16.5	even	ok

rdb: ring and double bond.

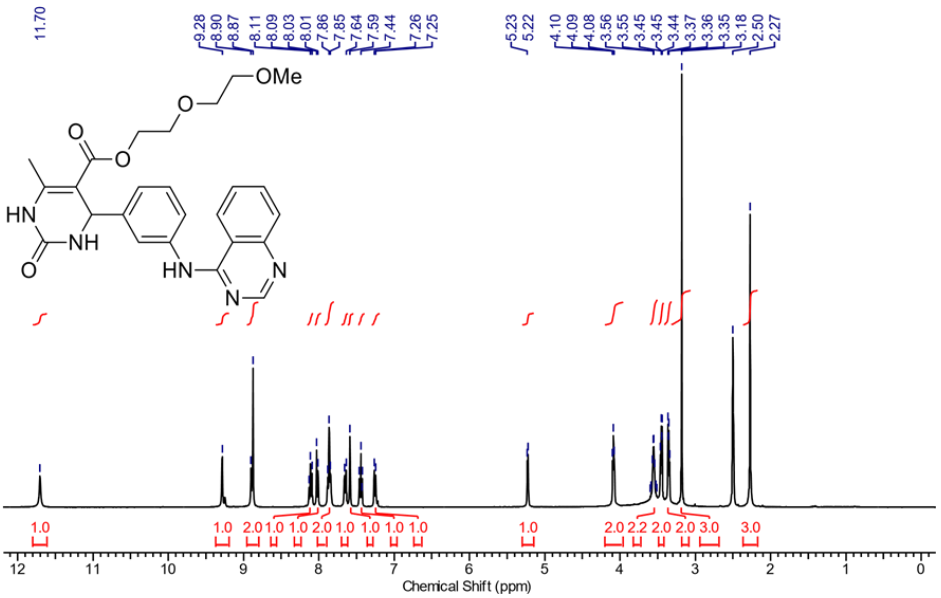

Figure S30. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7g**.

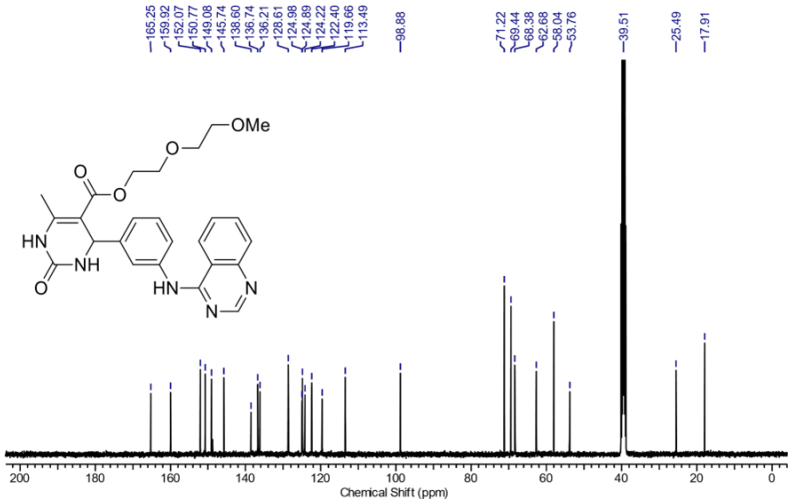

Figure S31. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7g**.

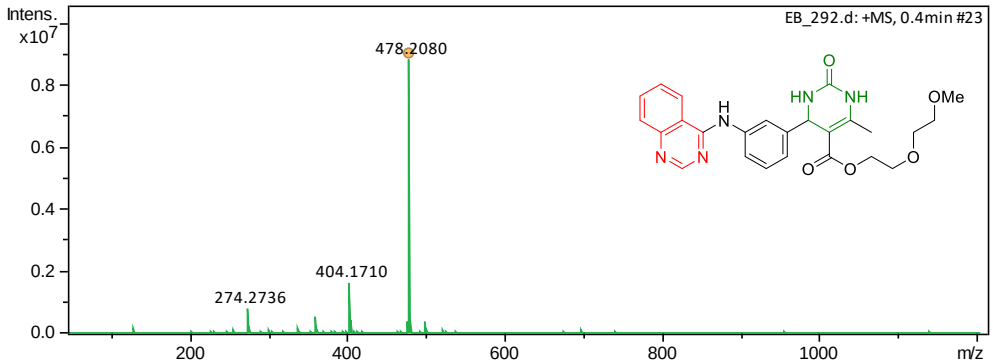

Figure S32. HRMS of compound **7g**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
478.2080	$C_{25}H_{28}N_5O_5$	478.2085	1.0	5.9	14.5	even	ok

rdb: ring and double bond.

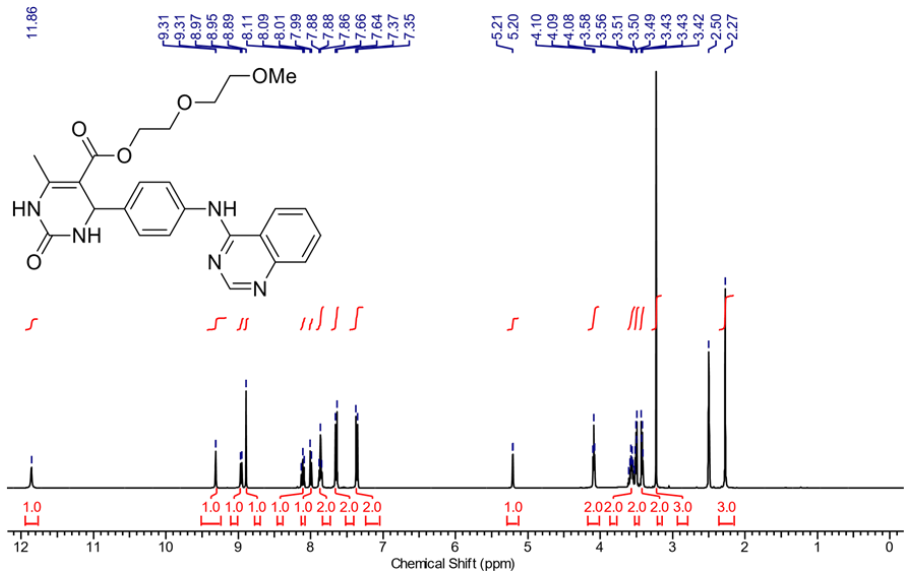

Figure S33. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7h**.

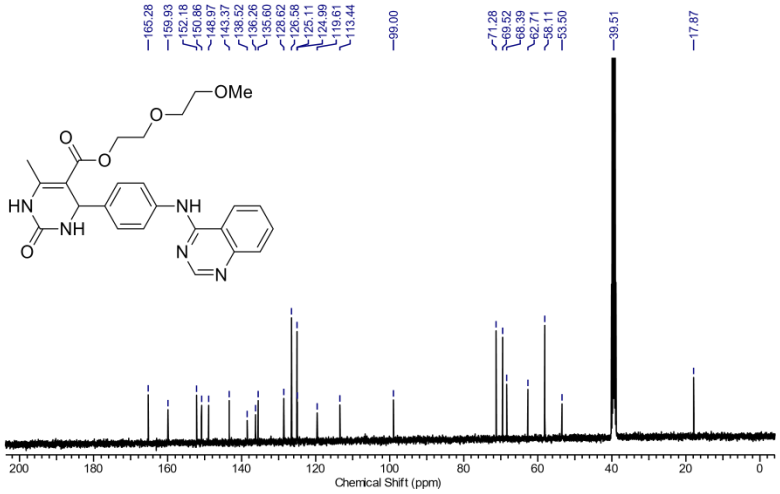

Figure S34. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7h**.

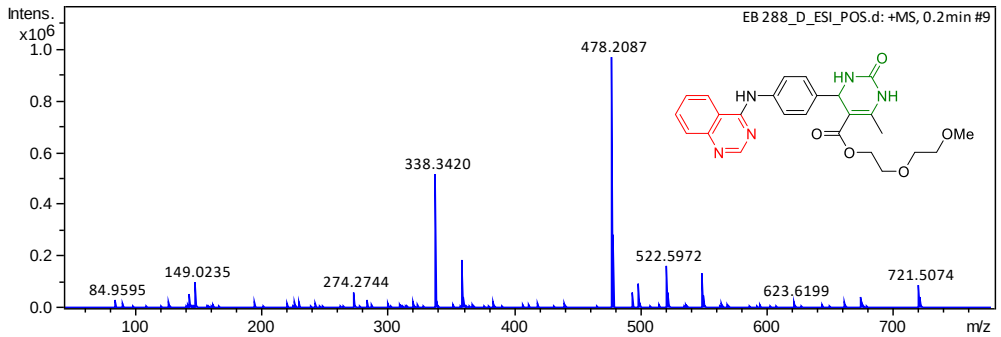

Figure S35. HRMS of compound **7h**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
478.2087	$C_{25}H_{28}N_5O_5$	478.2085	–0.4	3.8	14.5	even	ok

rdb: ring and double bond.

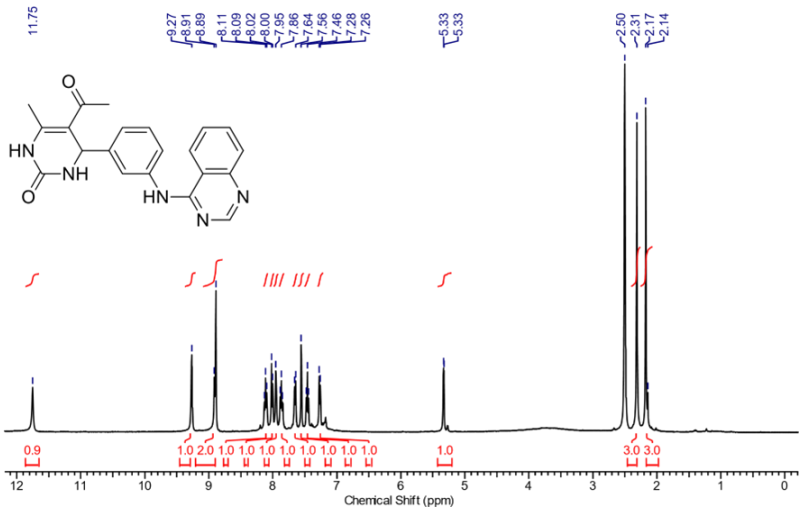

Figure S36. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7i**.

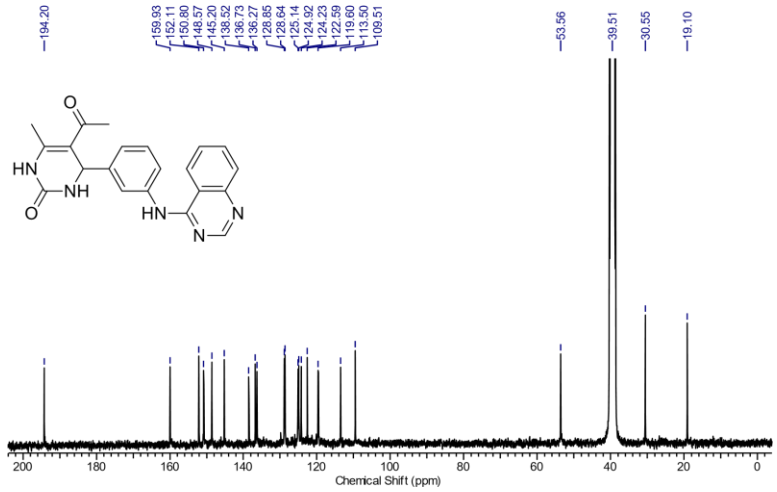

Figure S37. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7i**.

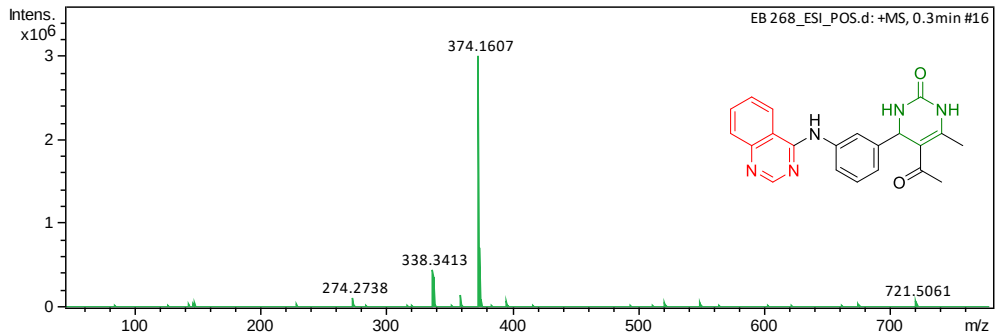

Figure S38. HRMS of compound **7i**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
374.1607	$C_{21}H_{20}N_5O_2$	374.1612	1.2	7.3	14.5	even	ok

rdb: ring and double bond.

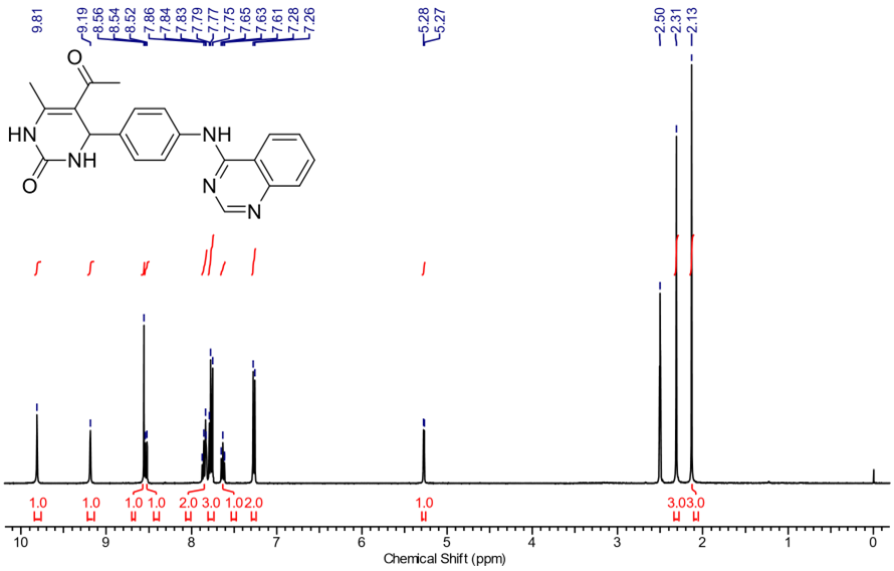

Figure S39. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7j**.

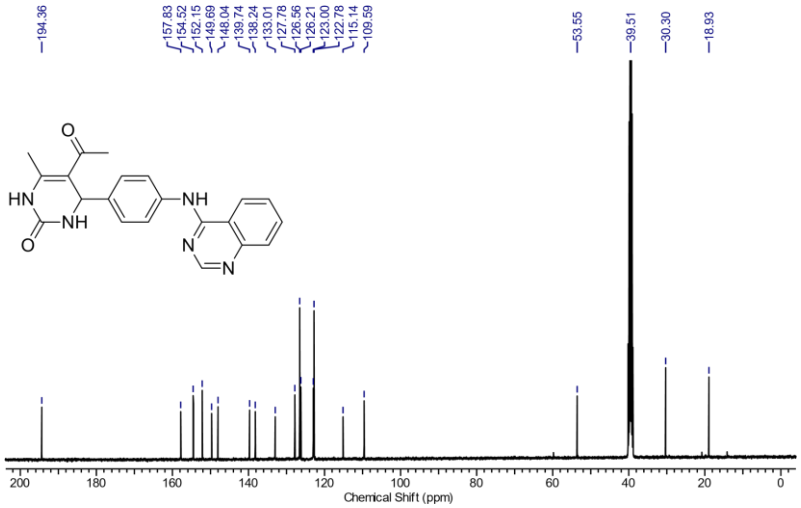

Figure S40. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7j**.

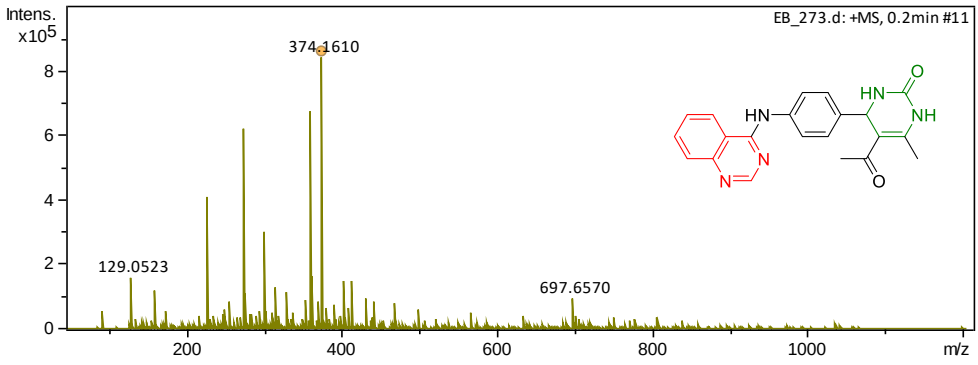

Figure S41. HRMS of compound **7j**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
374.1610	$C_{21}H_{20}N_5O_2$	374.1612	0.4	7.4	14.5	even	ok

rdb: ring and double bond.

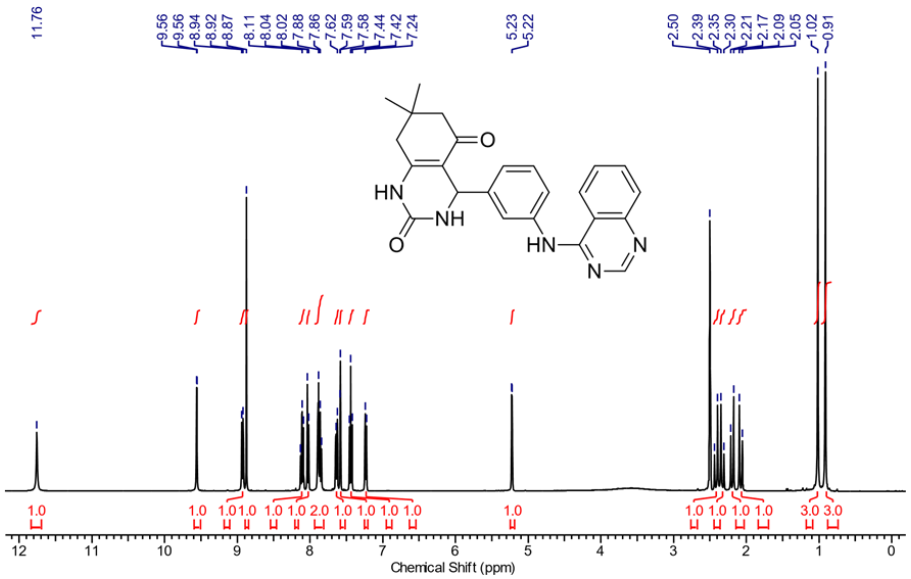

Figure S42. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7k**.

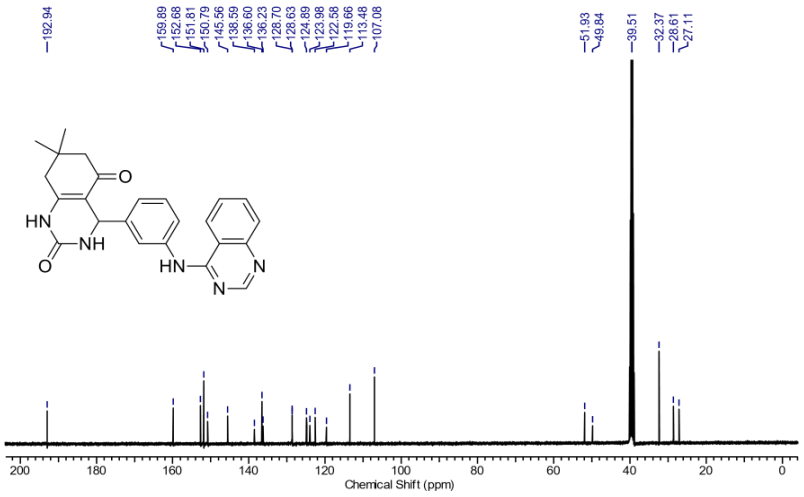

Figure S43. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7k**.

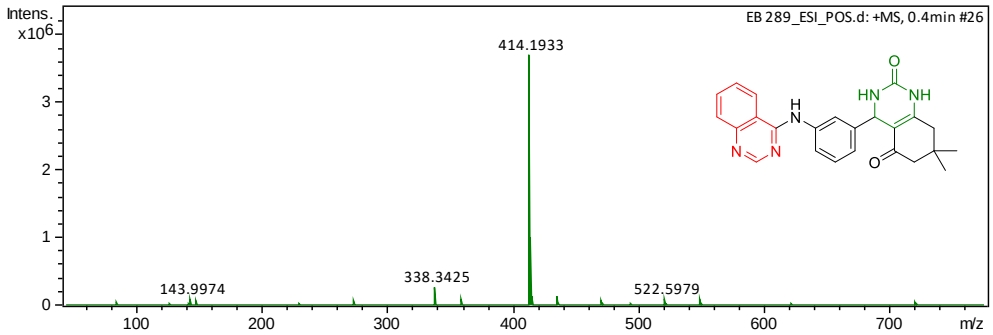

Figure S44. HRMS of compound **7k**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
414.1933	$C_{24}H_{24}N_5O_2$	414.1925	–2	5.7	15.5	even	ok

rdb: ring and double bond.

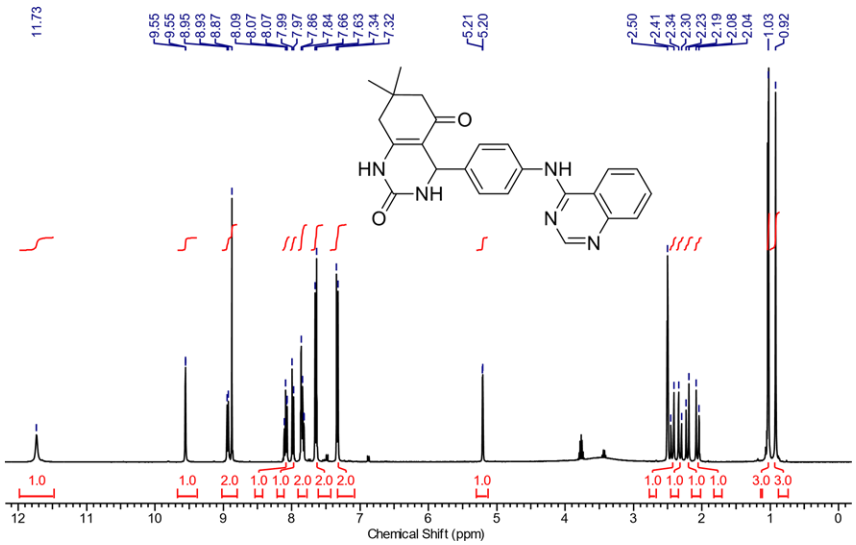

Figure S45. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7l**.

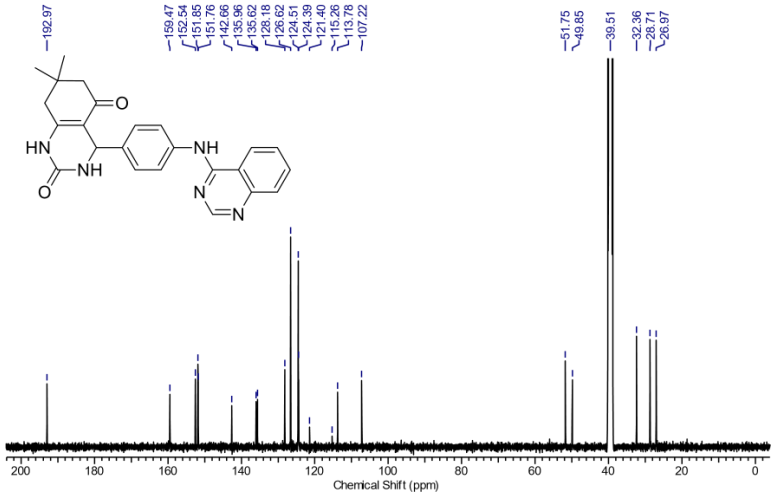

Figure S46. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7l**.

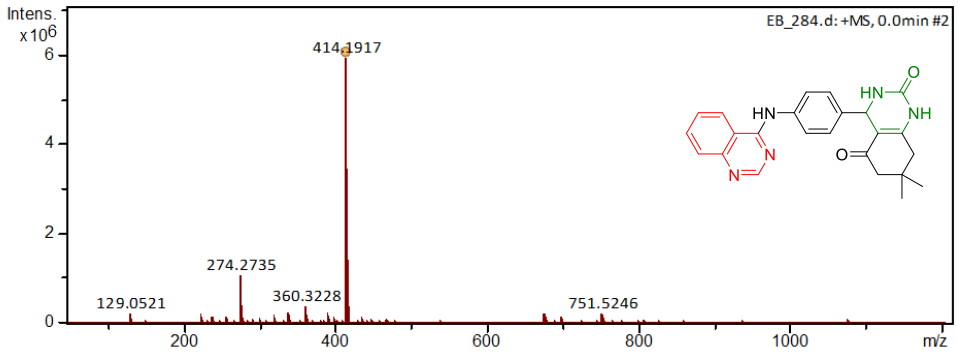

Figure S47. HRMS of compound **7l**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
414.1917	$C_{24}H_{24}N_5O_2$	414.1925	1.8	9.4	15.5	even	ok

rdb: ring and double bond.

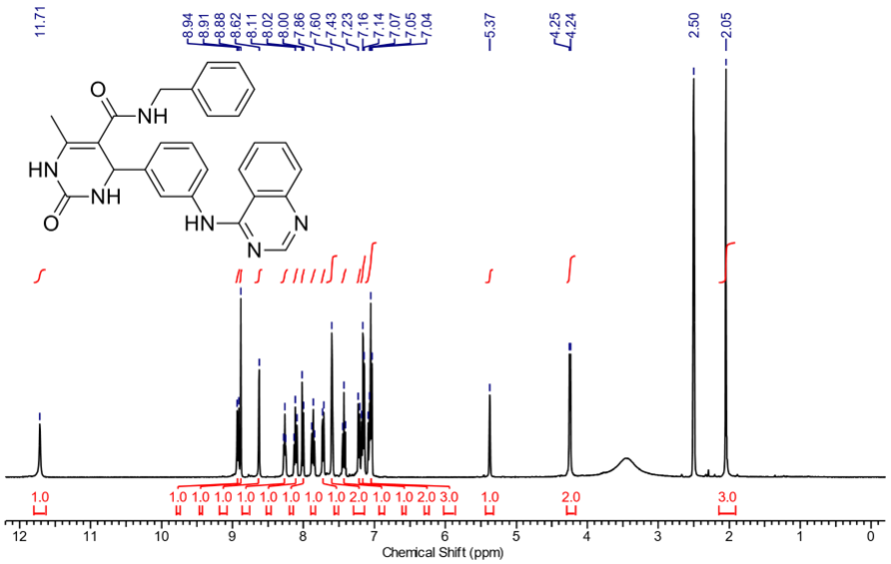

Figure S48. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7m**.

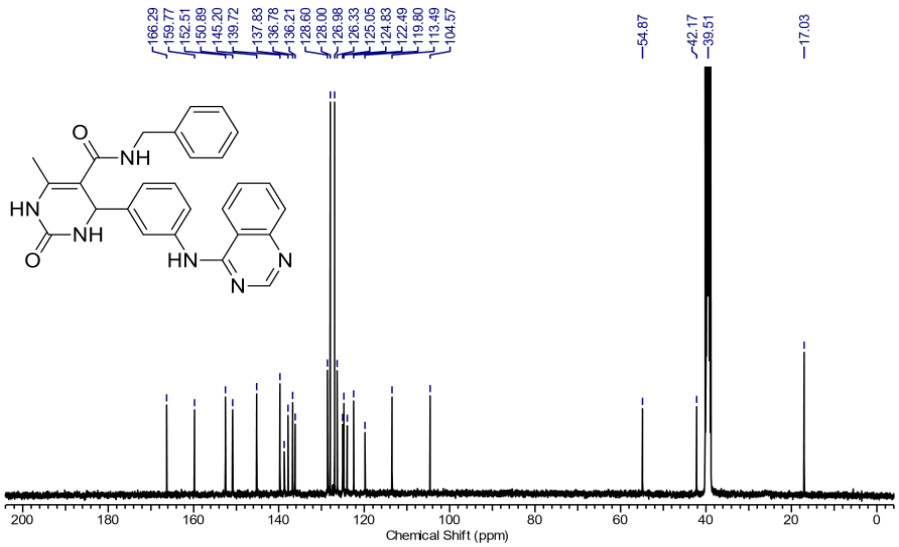

Figure S49. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7m**.

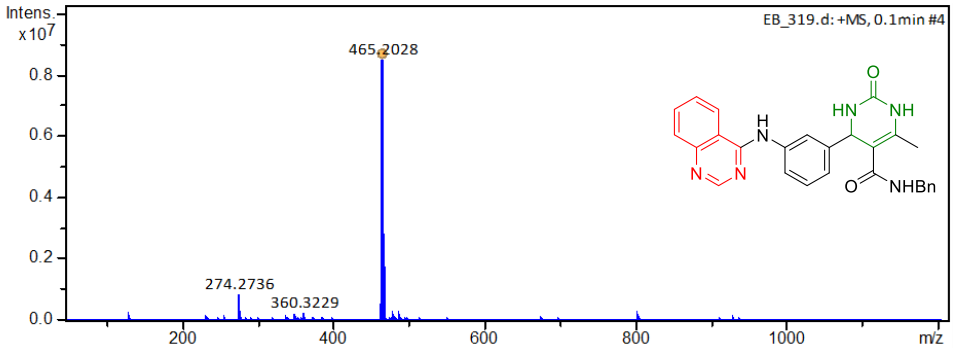

Figure S50. HRMS of compound **7m**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
465.2028	$C_{27}H_{25}N_6O_2$	465.2034	1.2	5.9	18.5	even	ok

rdb: ring and double bond.

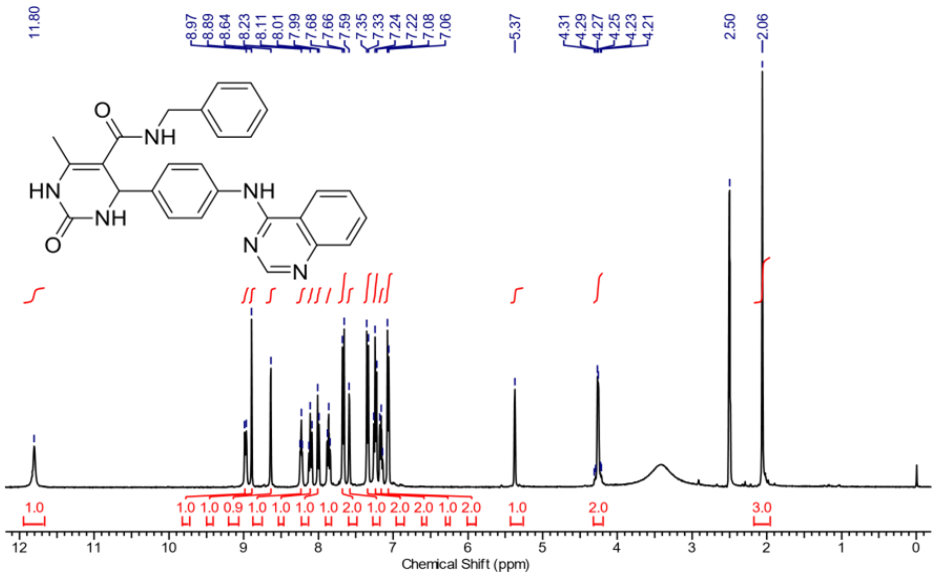

Figure S51. ^{1}H NMR (400 MHz, DMSO-d_6) of compound **7n**.

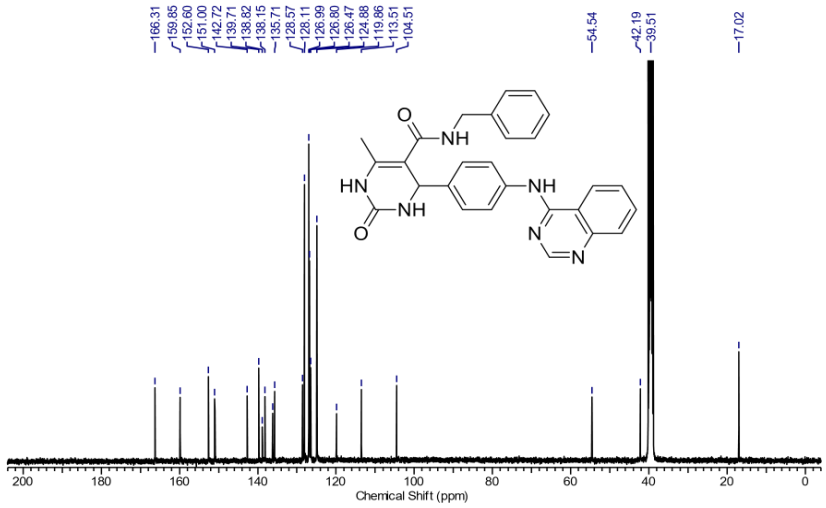

Figure S52. ^{13}C NMR (100 MHz, DMSO-d_6) of compound **7n**.

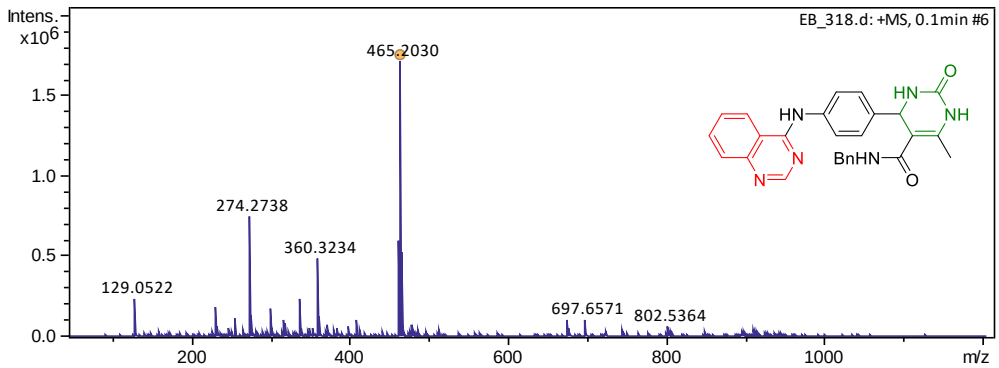

Figure S53. HRMS of compound **7n**.

Measured *m/z*	Ion formula	*m/z*	error /ppm	mSigma	rdb	e⁻ configuration	N-rule
465.2030	$C_{27}H_{25}N_6O_2$	465.2034	0.7	6.1	18.5	even	ok

rdb: ring and double bond.