

Synthesis of phthalimide-based piperazine conjugated novel analogs as anti-malarial agents

Meenakshi Bansal^{1,2}, Sumit Kumar^{1*}, Brijesh Rathi²

¹Department of Chemistry, Deenbandhu Chhotu Ram, University of Science & Technology, Murthal, Sonapat Haryana 131039 India. ²Laboratory for Translational Chemistry and Drug Discovery, Department of Chemistry, Hansraj College, University of Delhi, Delhi-110007 India.

*Corresponding Author:

Dr. Sumit Kumar, Ph.D. Email: sumitmalik.chem@dcrustm.org

Dr. Brijesh Rathi, PhD Email: brijeshrathi@hrc.du.ac.in

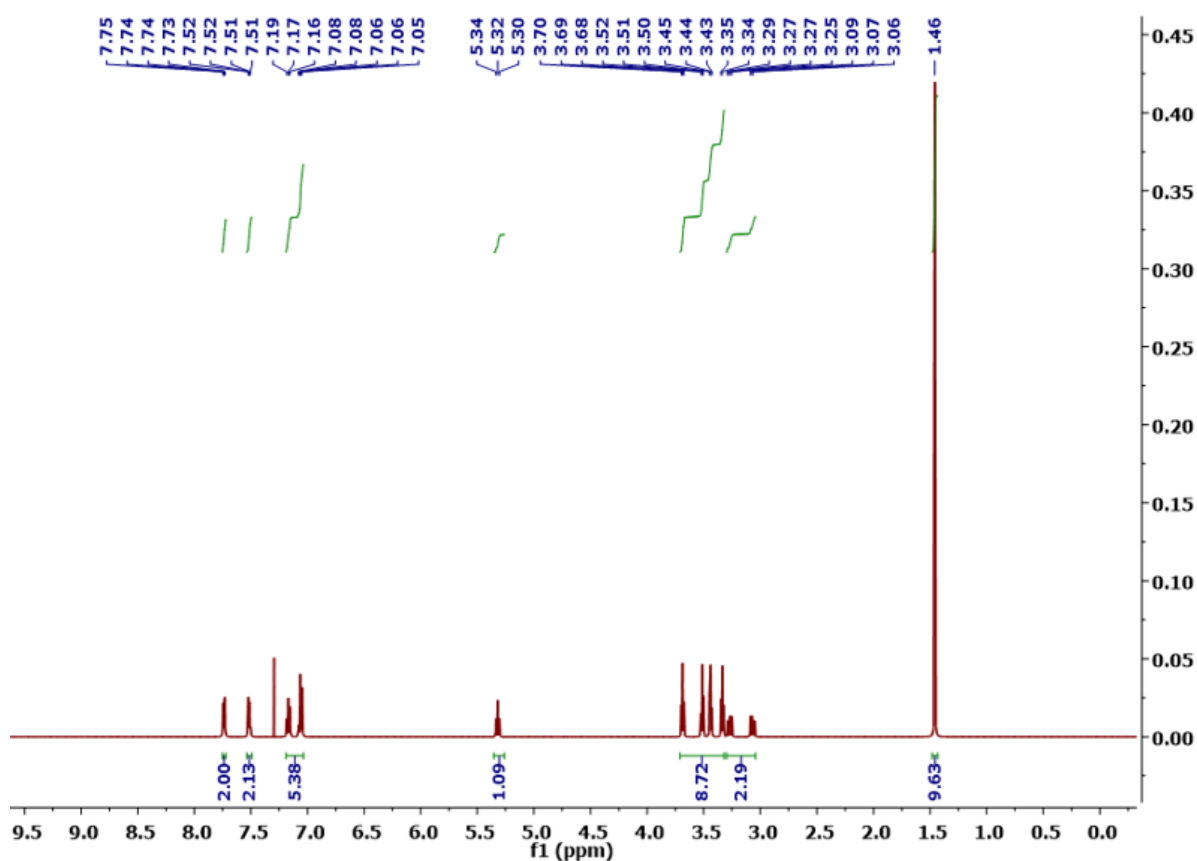
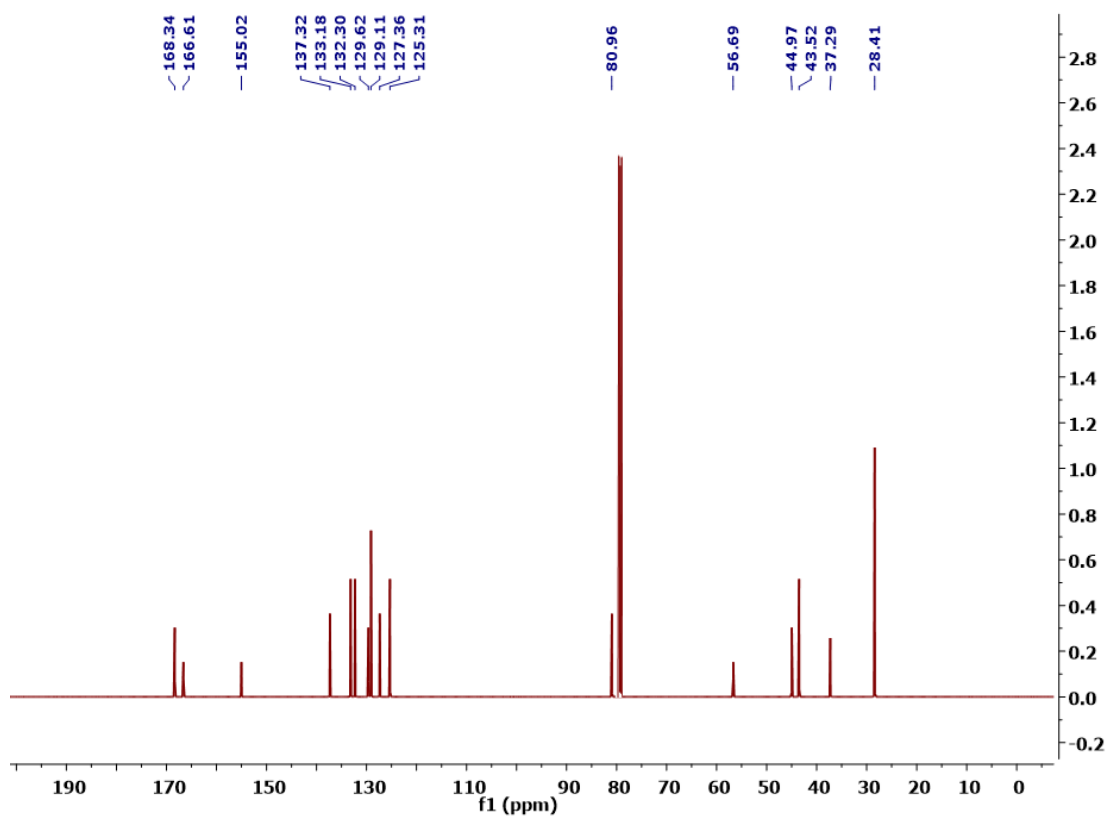
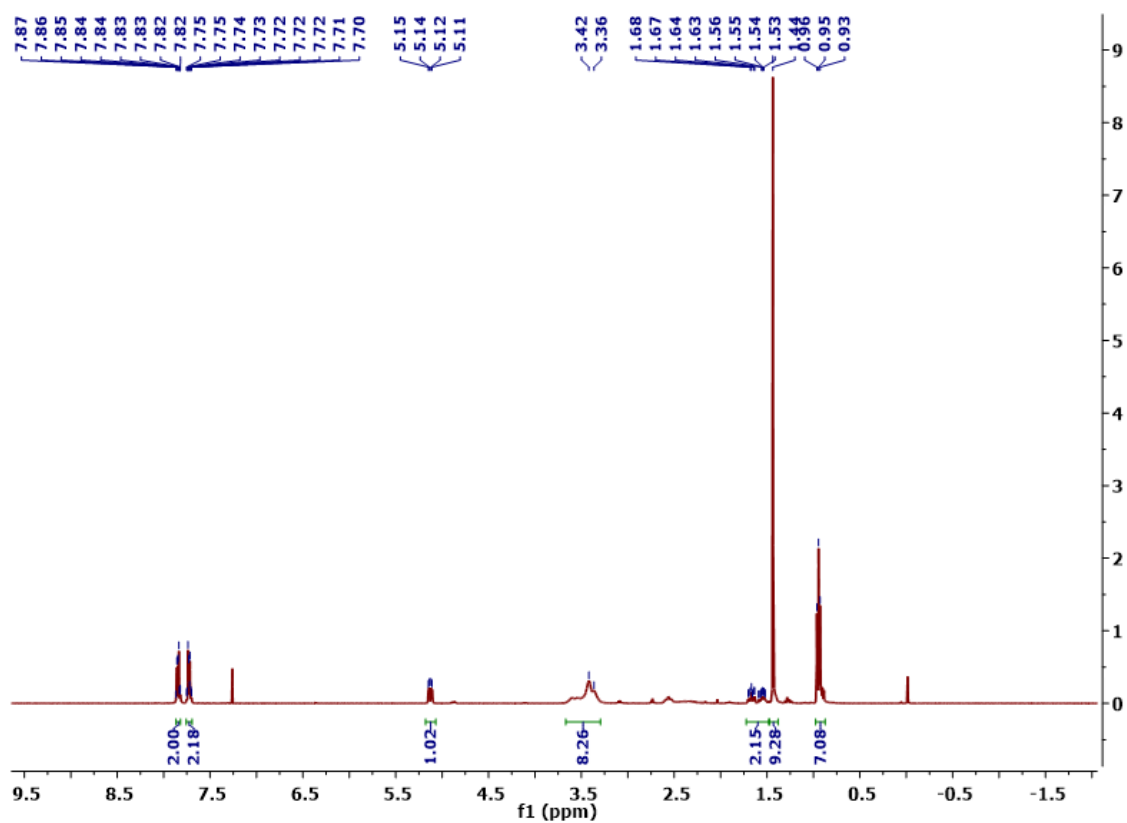
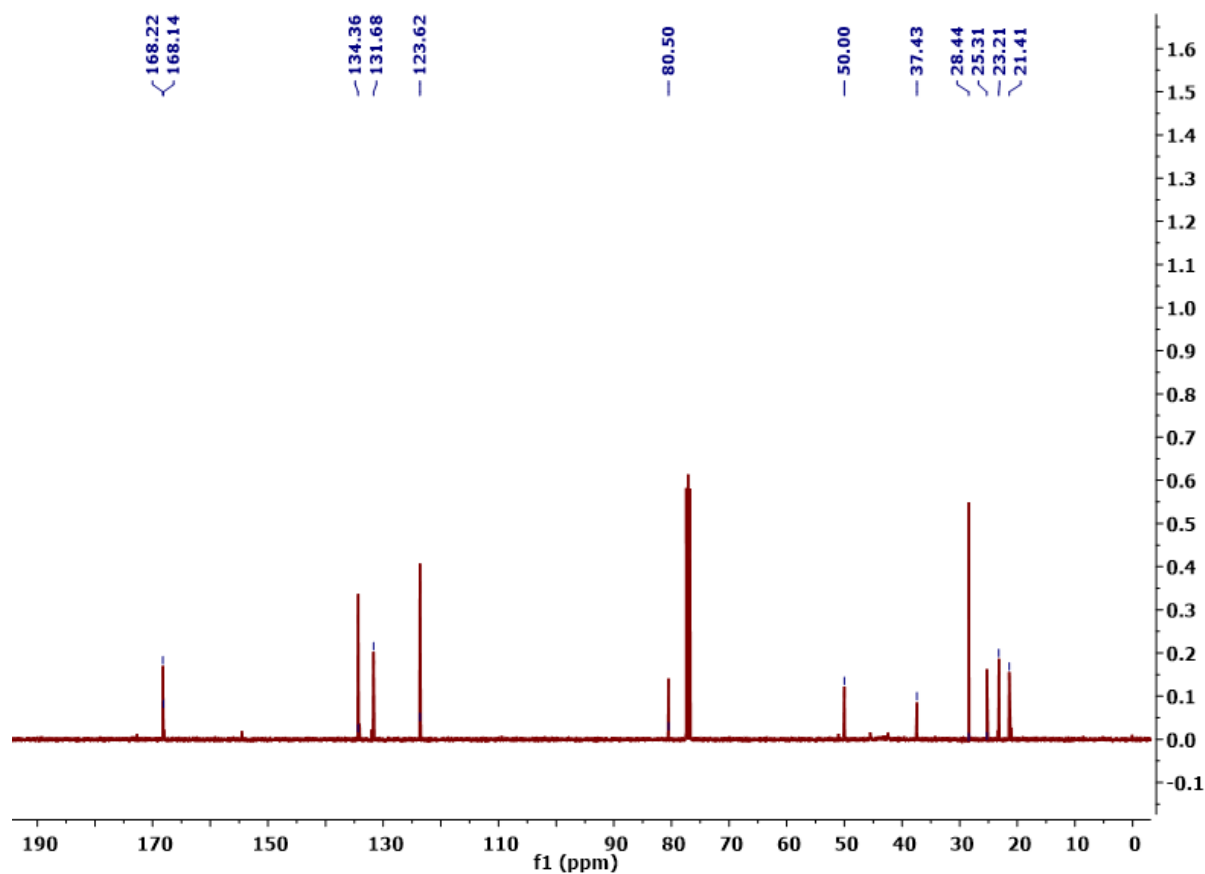
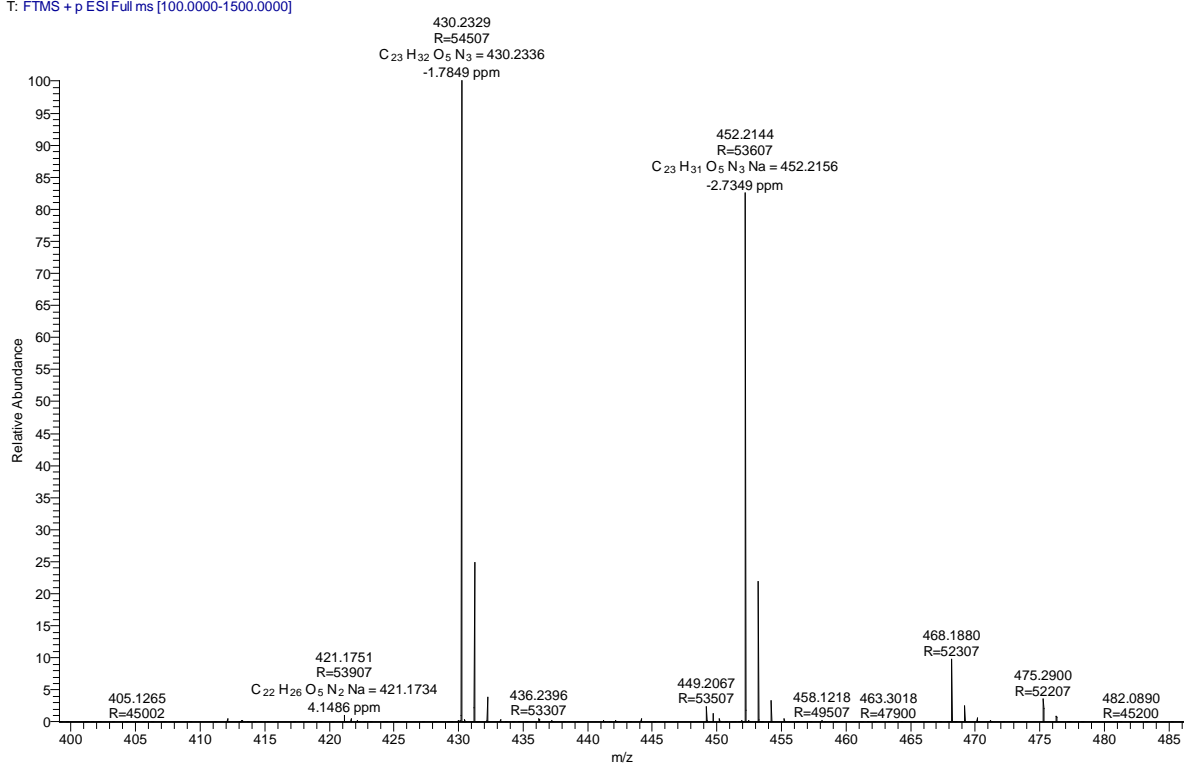


Fig 1. ¹H NMR Spectra of **6a**.

Fig 2. ^{13}C NMR Spectra of **6a**.Fig 3. ^1H NMR Spectra of **6b**.

Fig 4. ^{13}C Spectra of **6b**.

SS-6_210701131853 #314 RT: 1.67 AV: 1 NL: 8.17E8
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Fig 5. HRMS Spectra of **6b**.

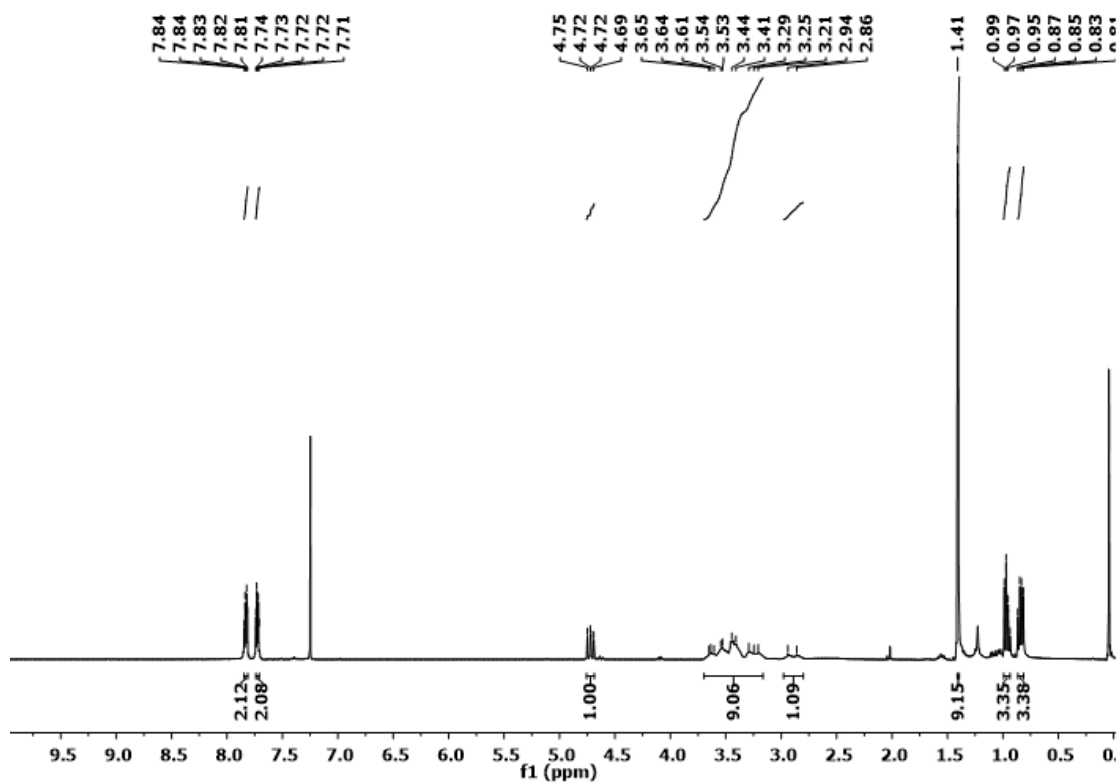


Fig 6. ¹H NMR Spectra of 6c.

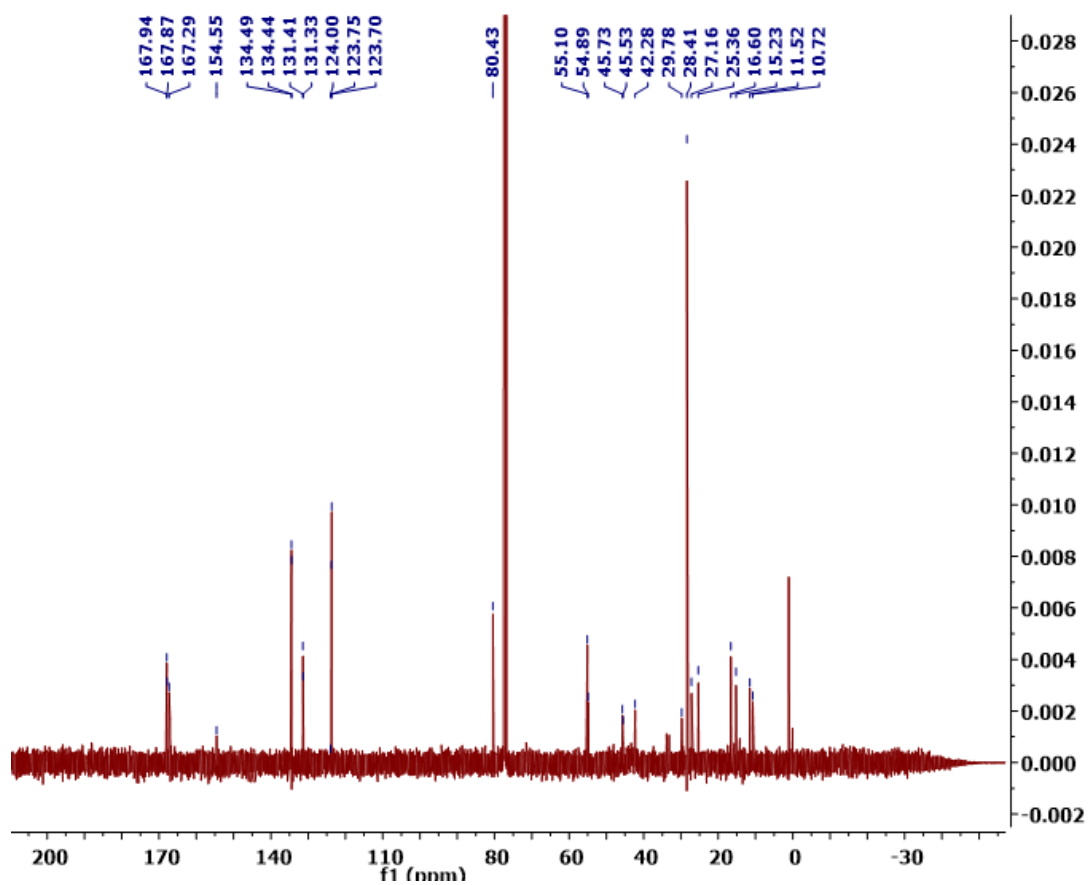


Fig 7. ¹³C NMR Spectra of 6c.

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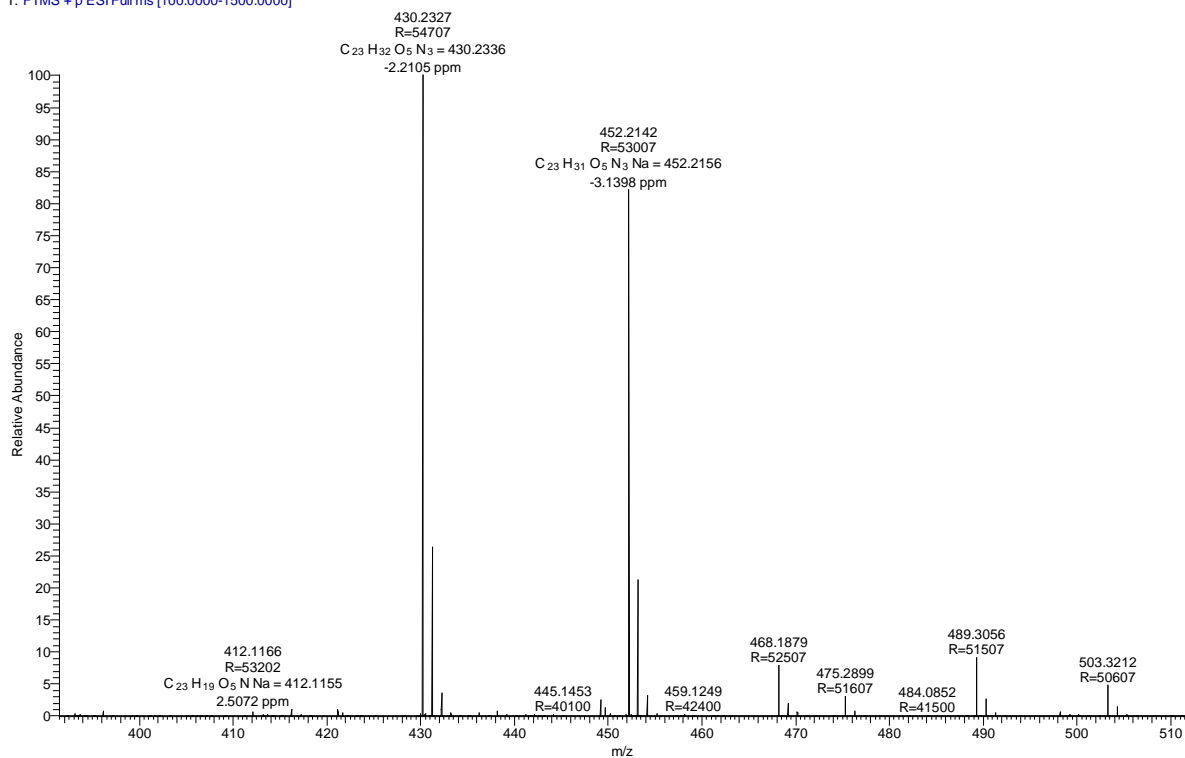


Fig 8. HRMS Spectra of **6c**.

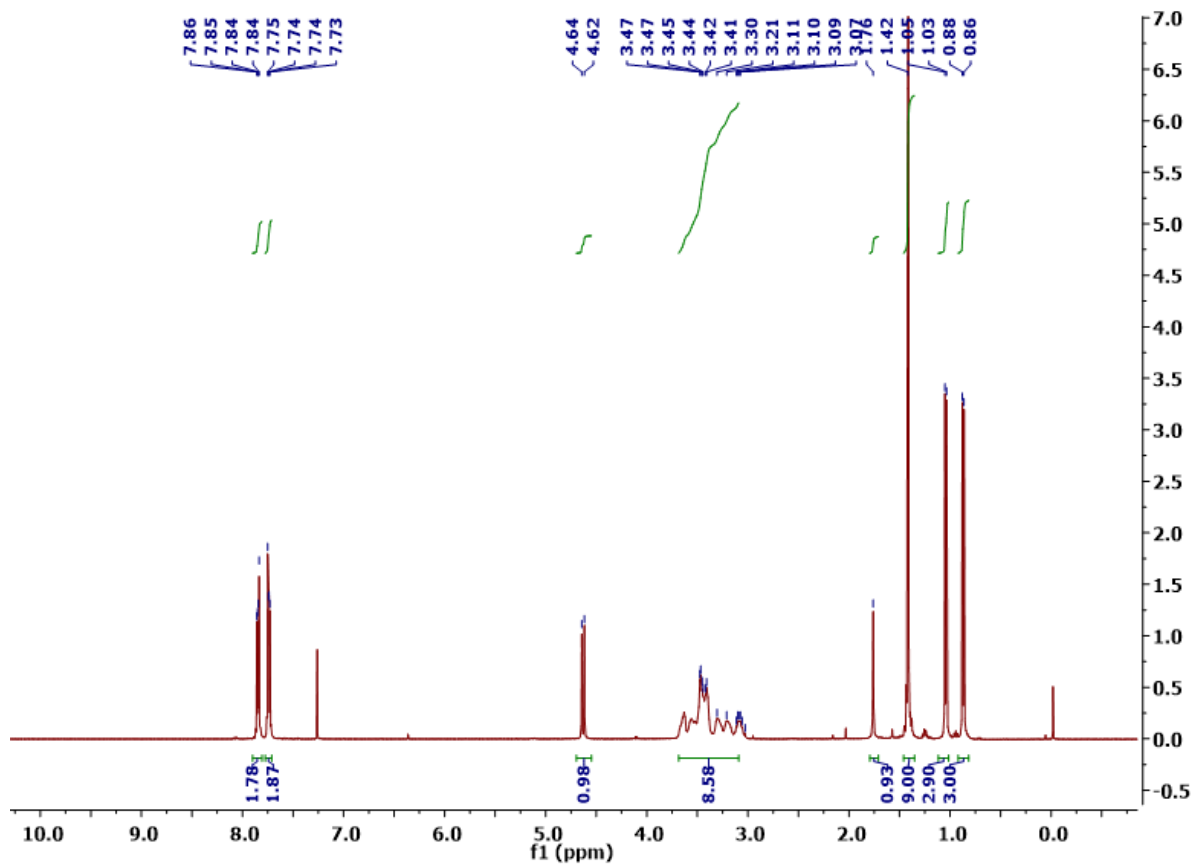
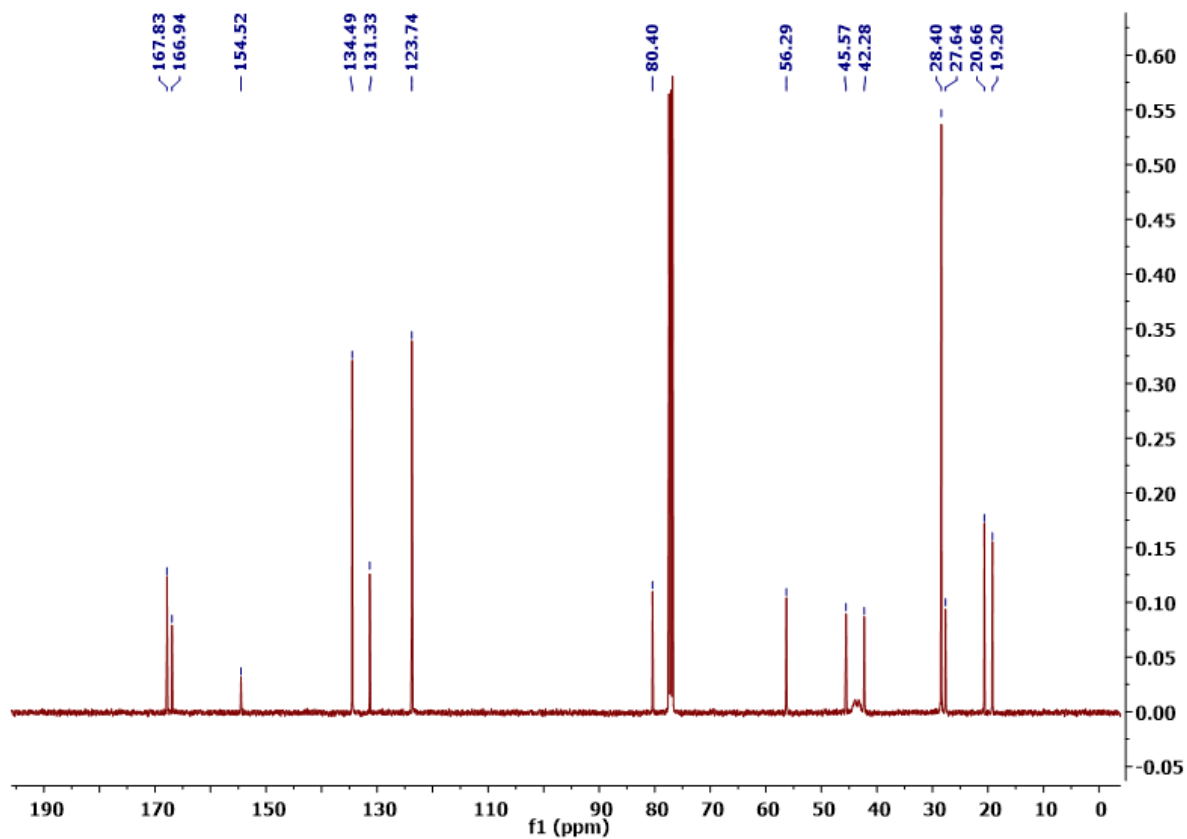
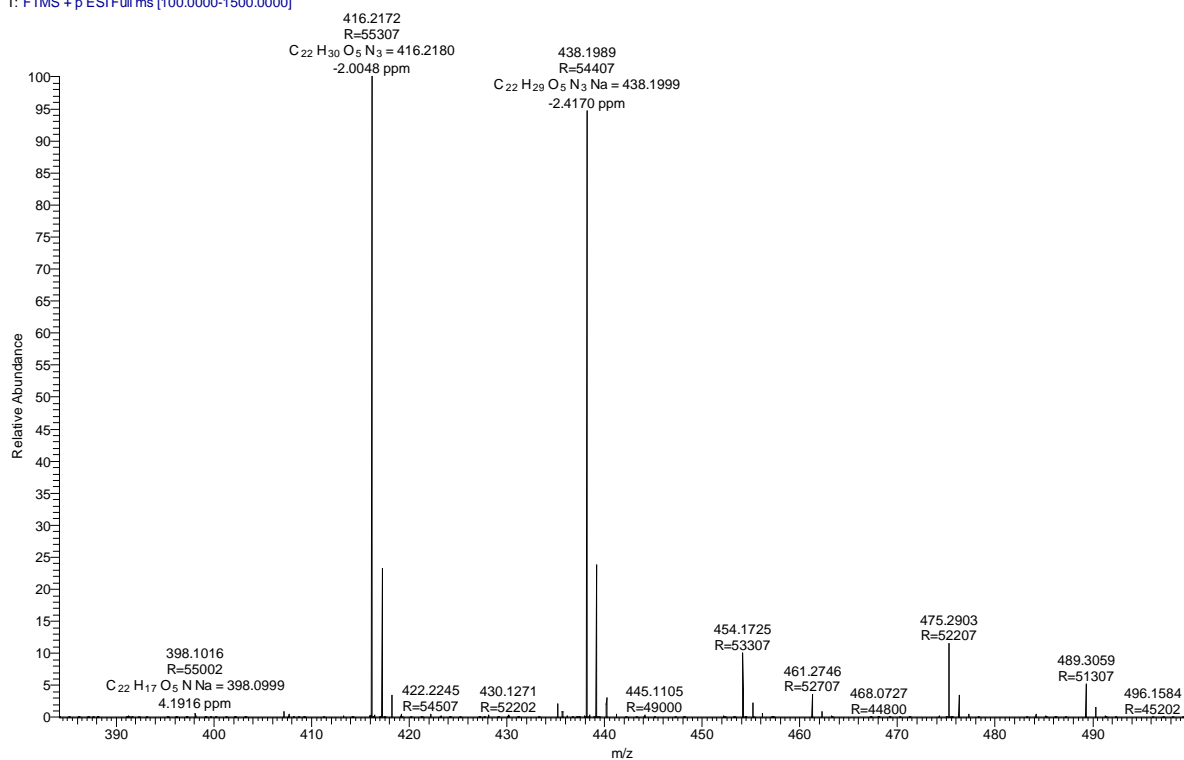
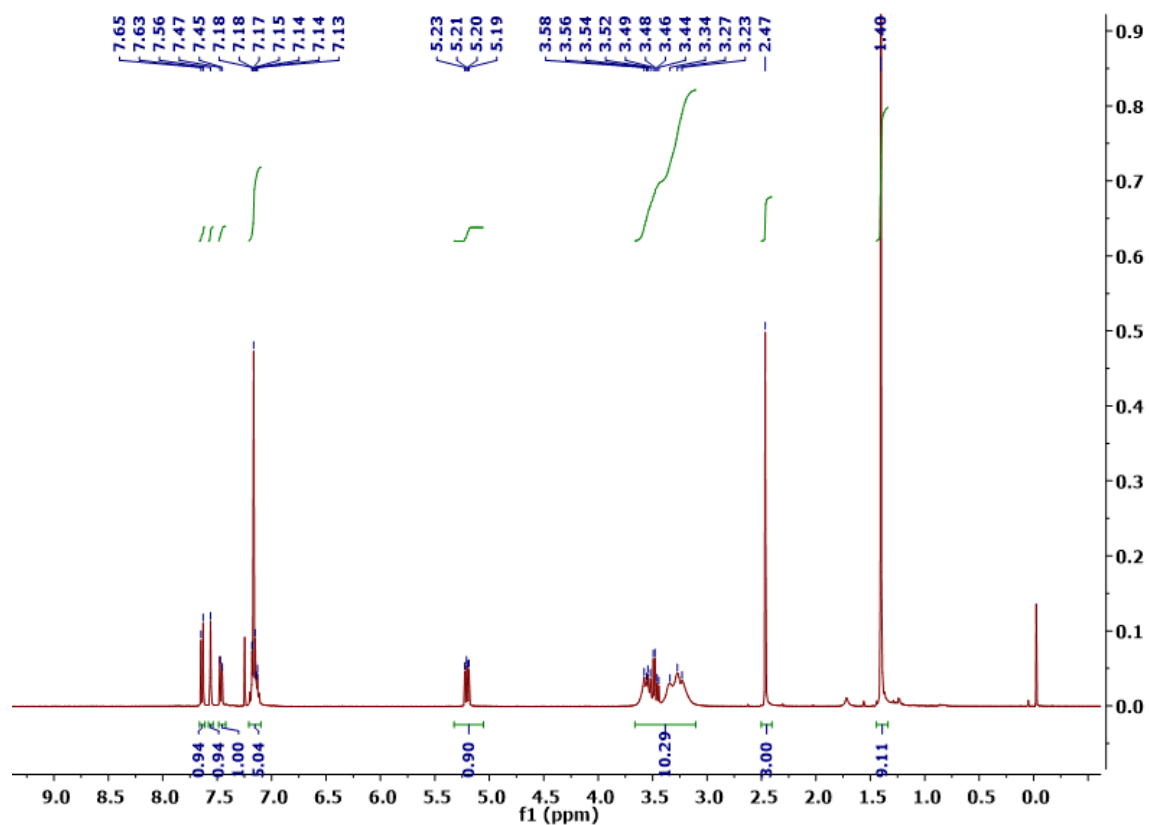
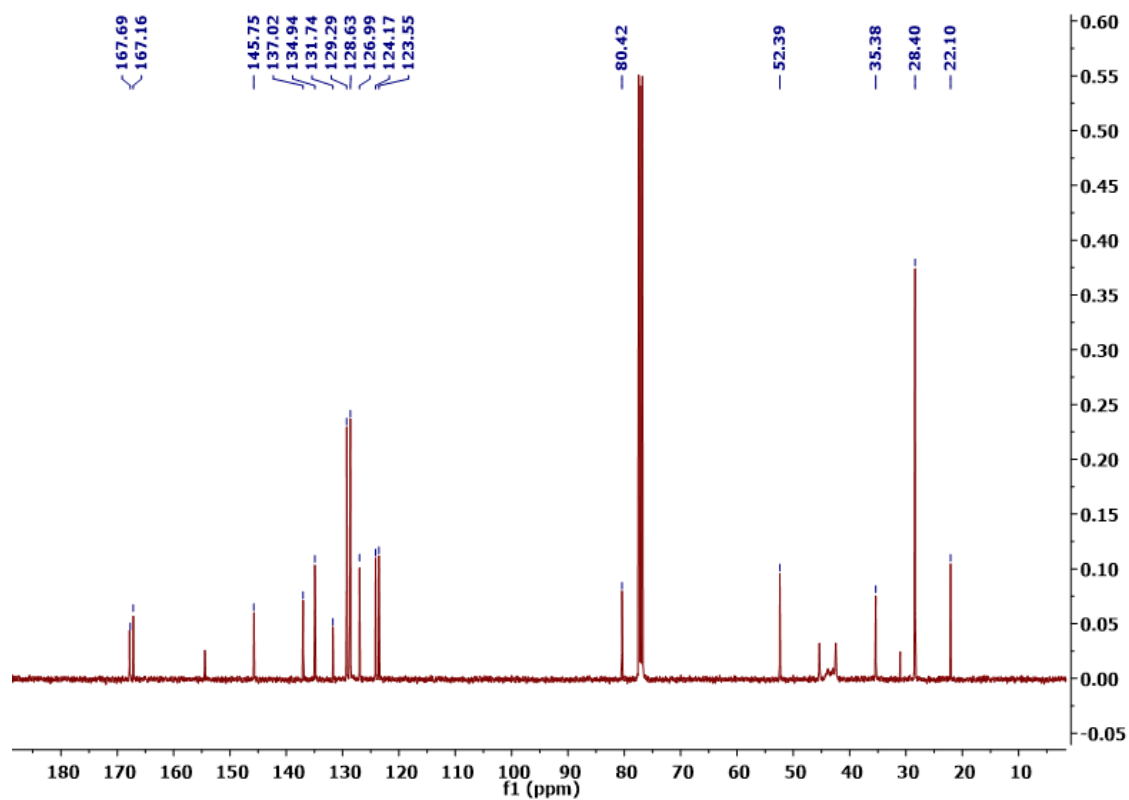


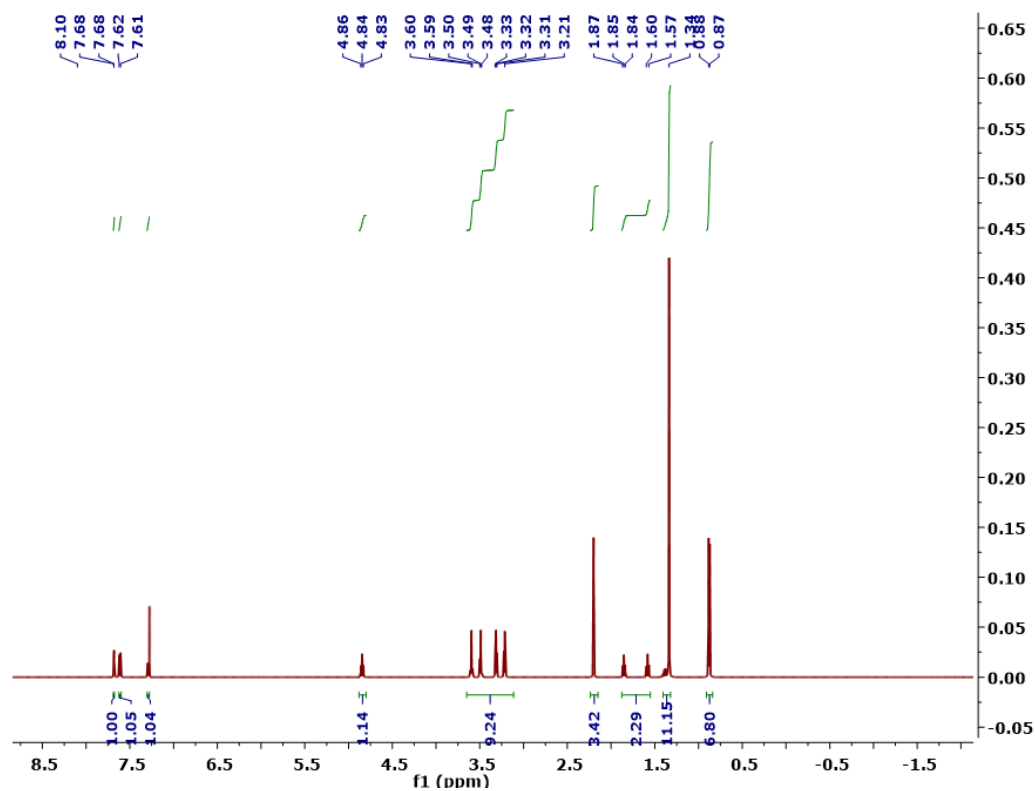
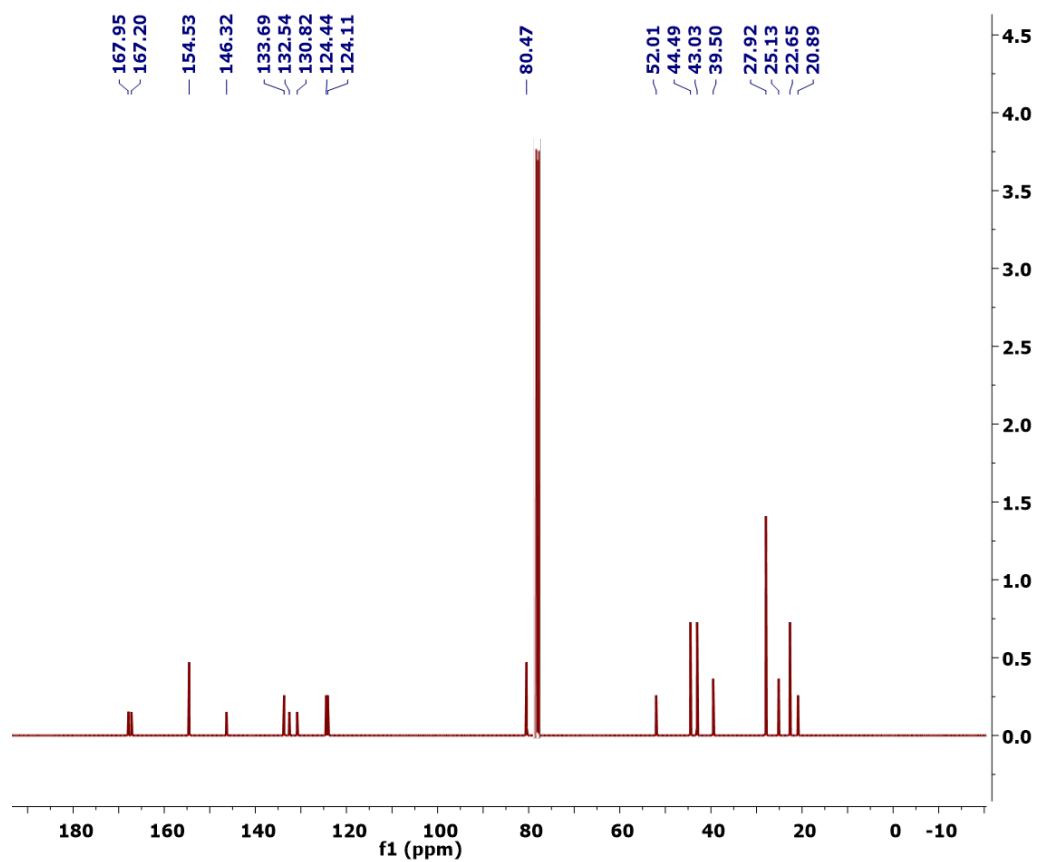
Fig 9. ^1H NMR Spectra of **6d**.

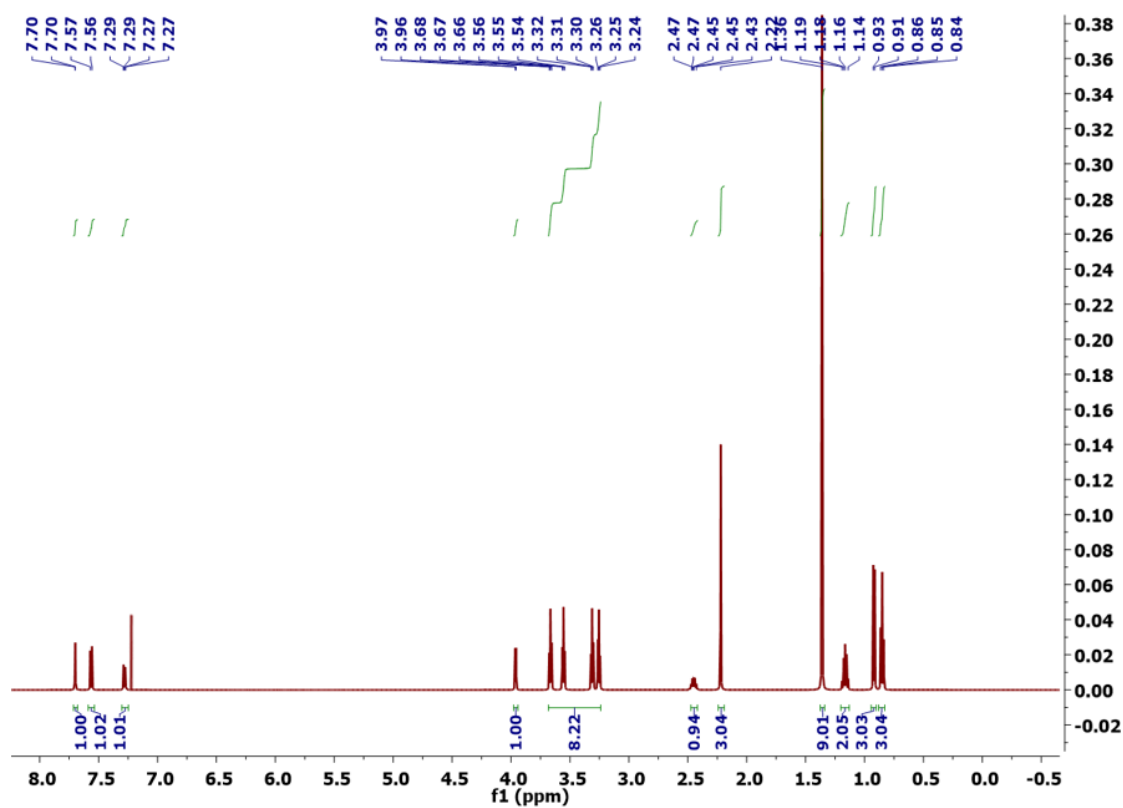
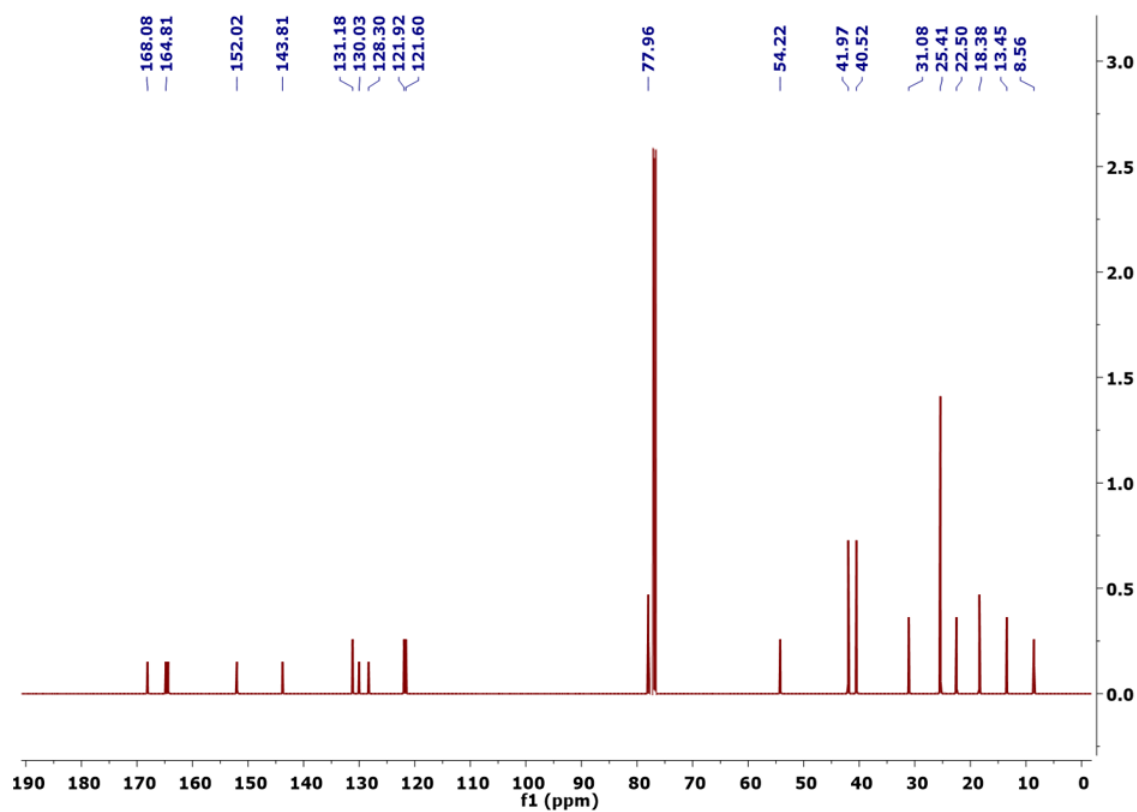
Fig 10. ^{13}C NMR Spectra of **6d**.

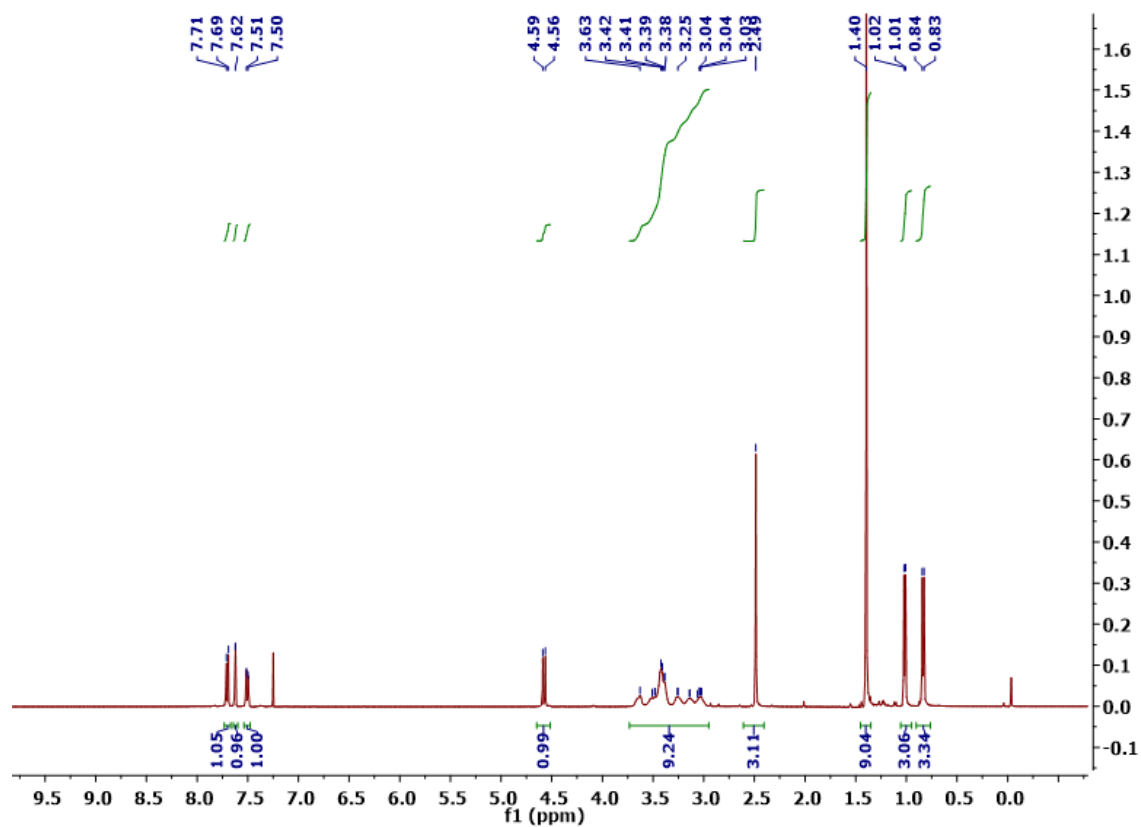
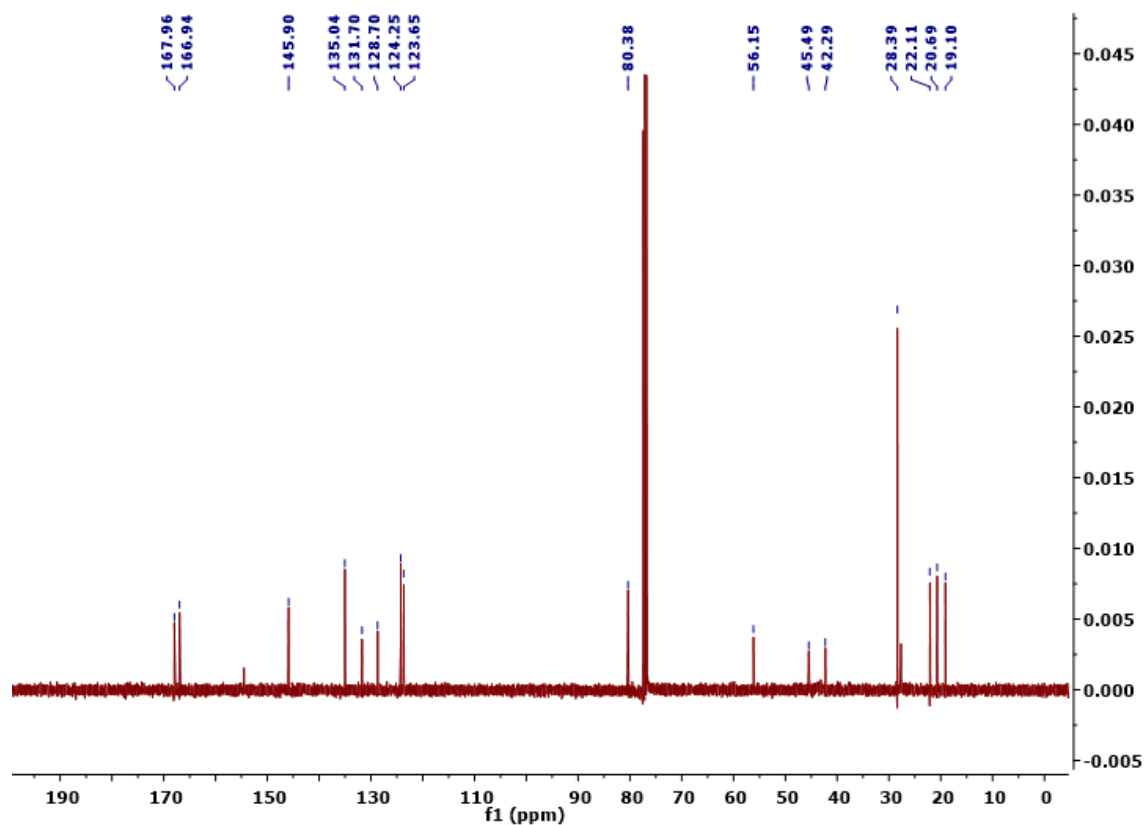
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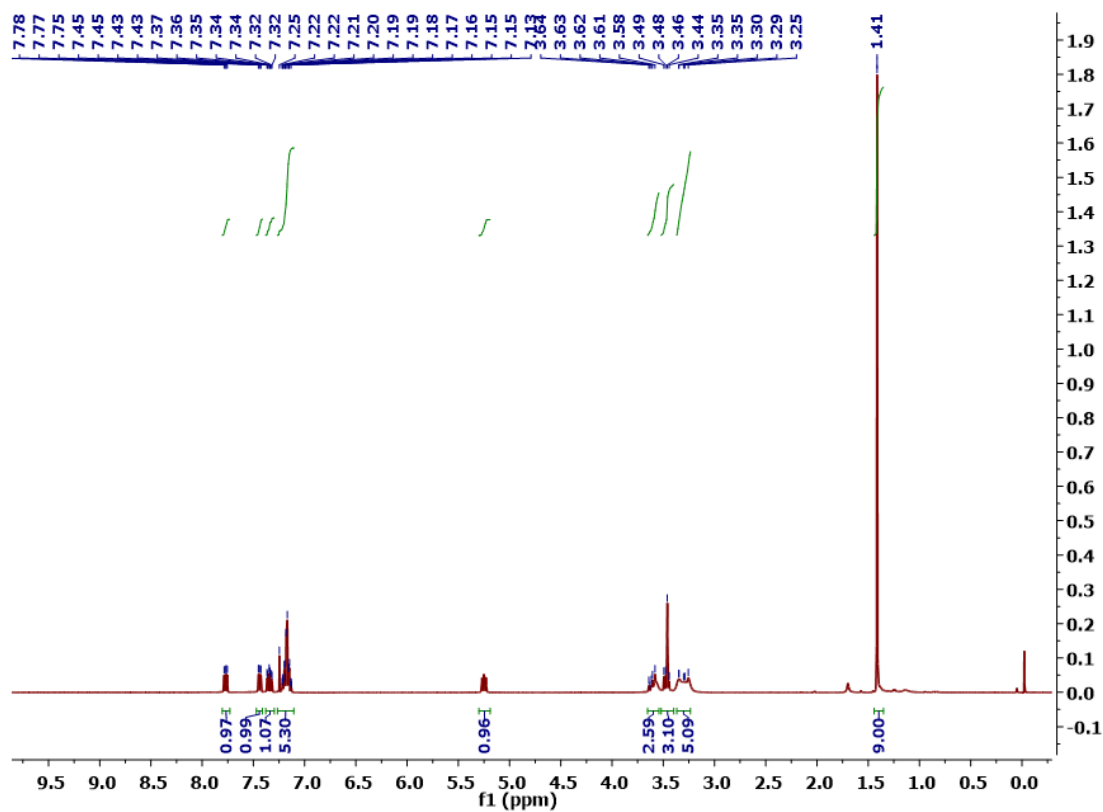
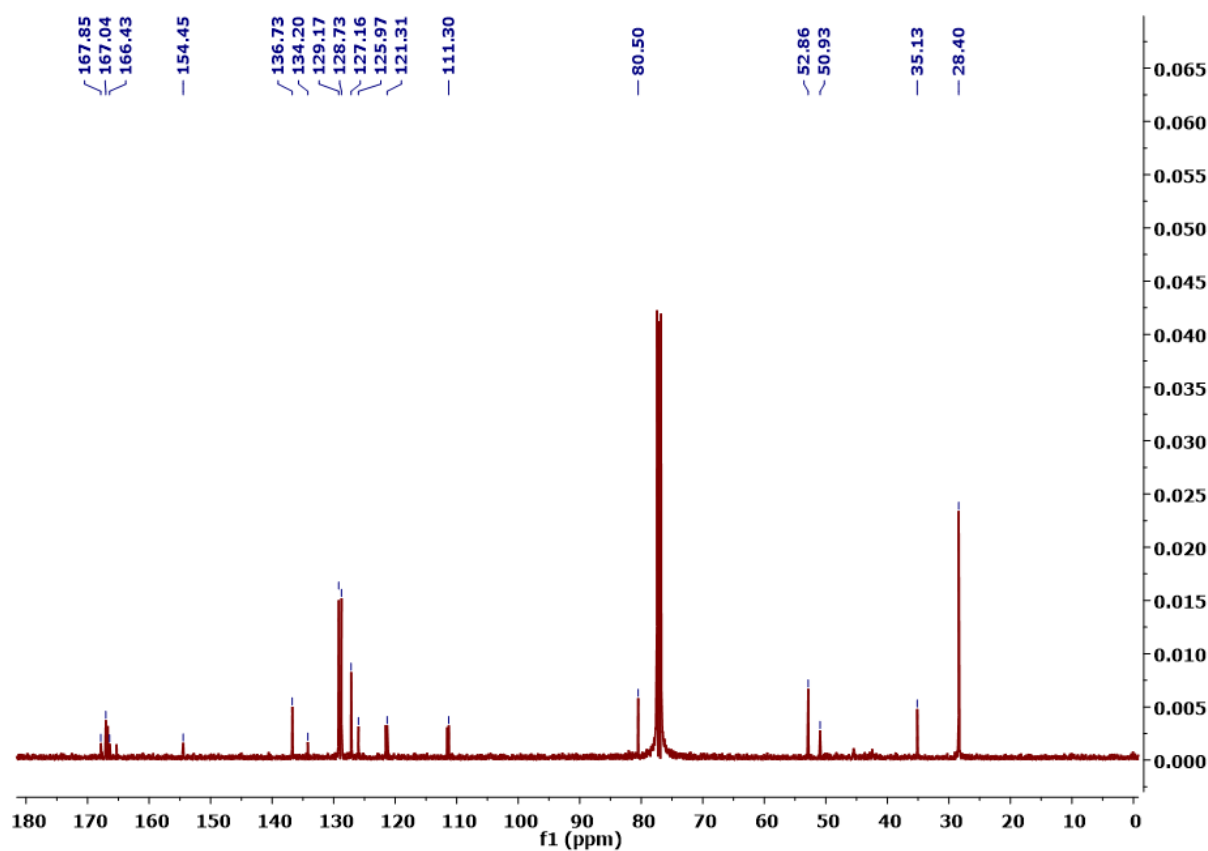
Fig 11. HRMS Spectra of **6d**.

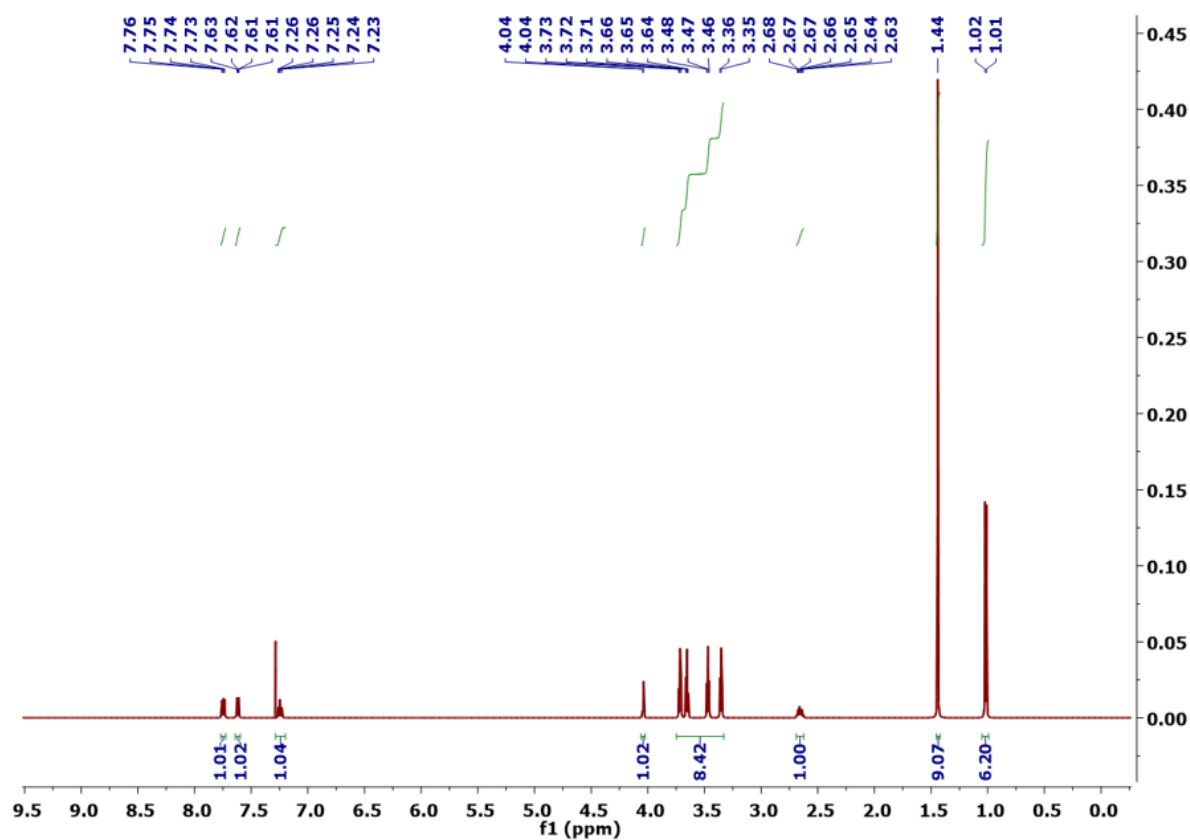
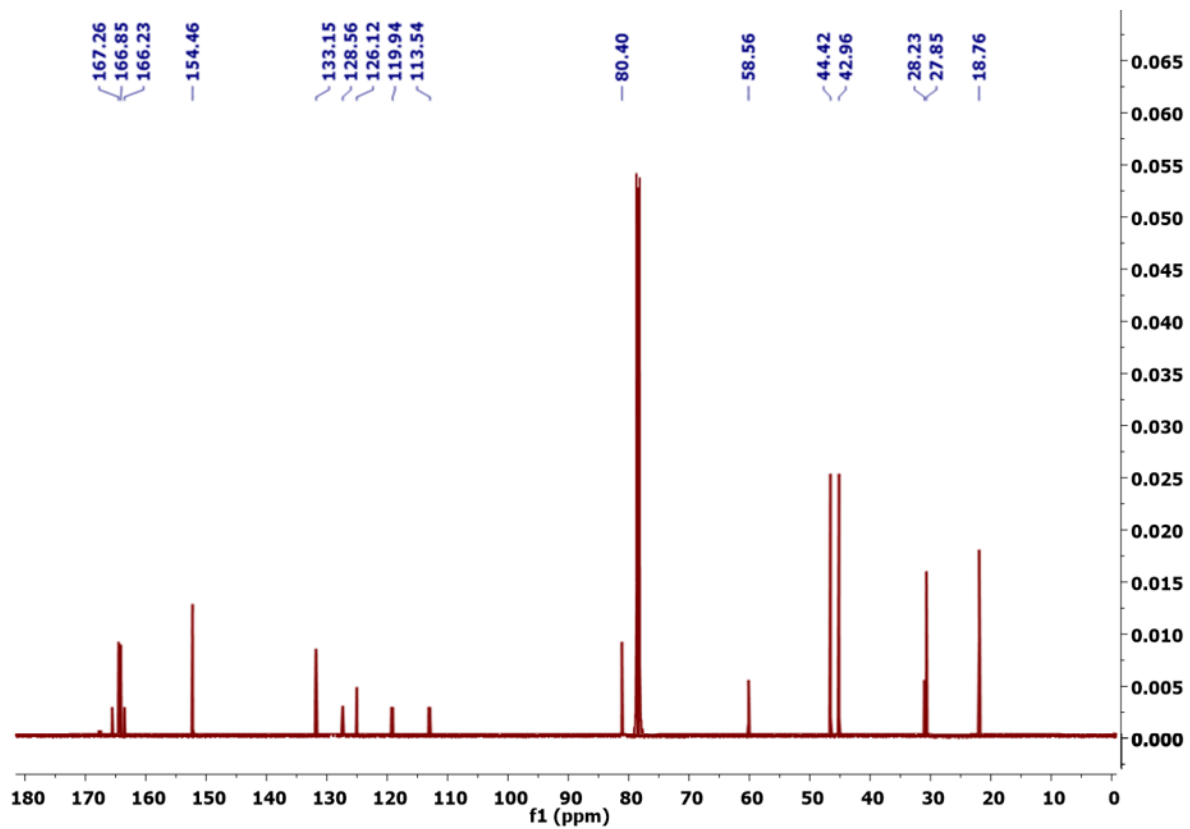
Fig 12. ^1H NMR Spectra of 7a.Fig 13. ^{13}C NMR Spectra of 7a.

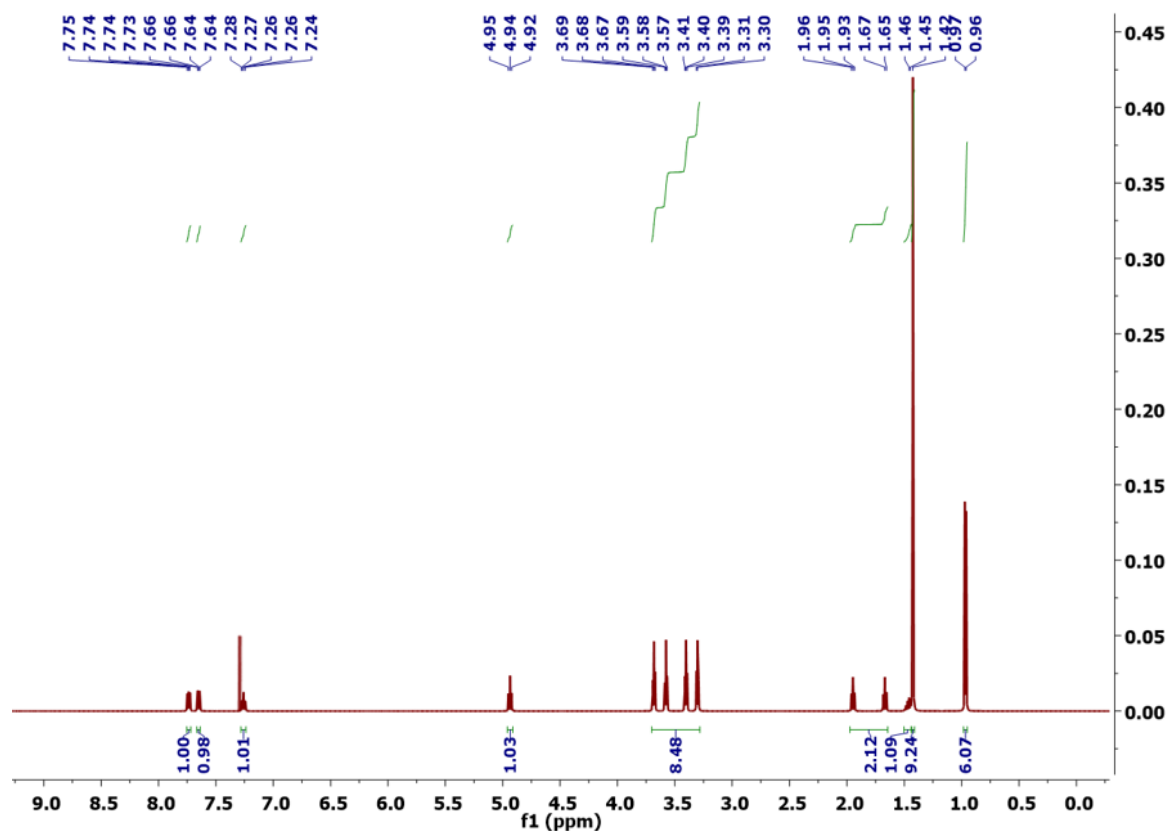
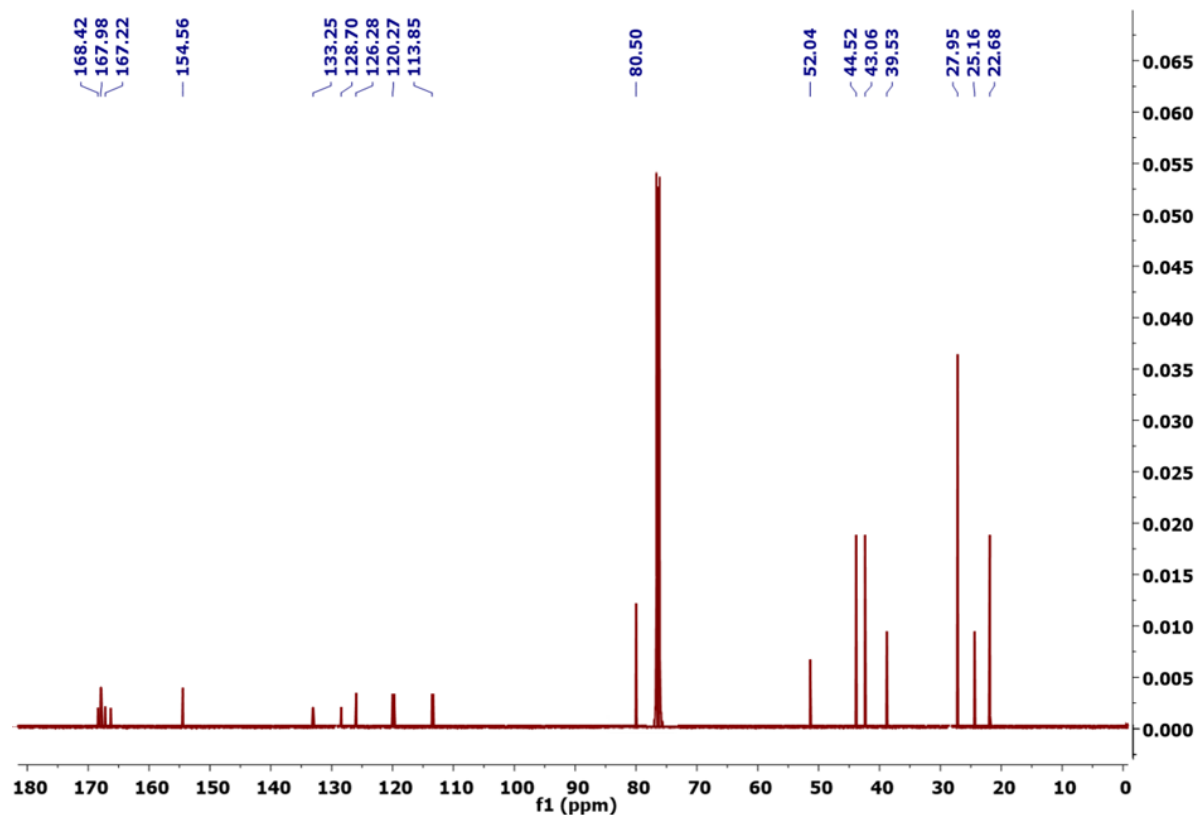
Fig 14. ^1H NMR Spectra of **7b**.Fig 15. ^{13}C NMR Spectra of **7b**.

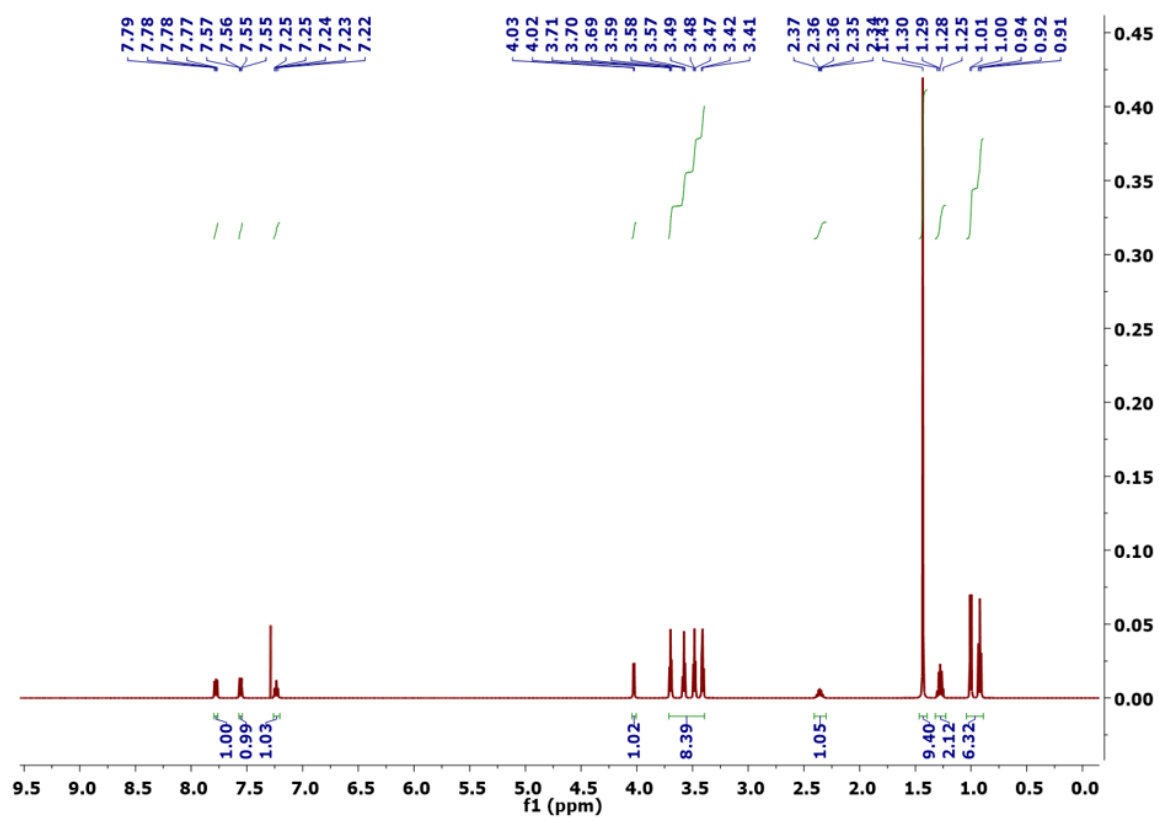
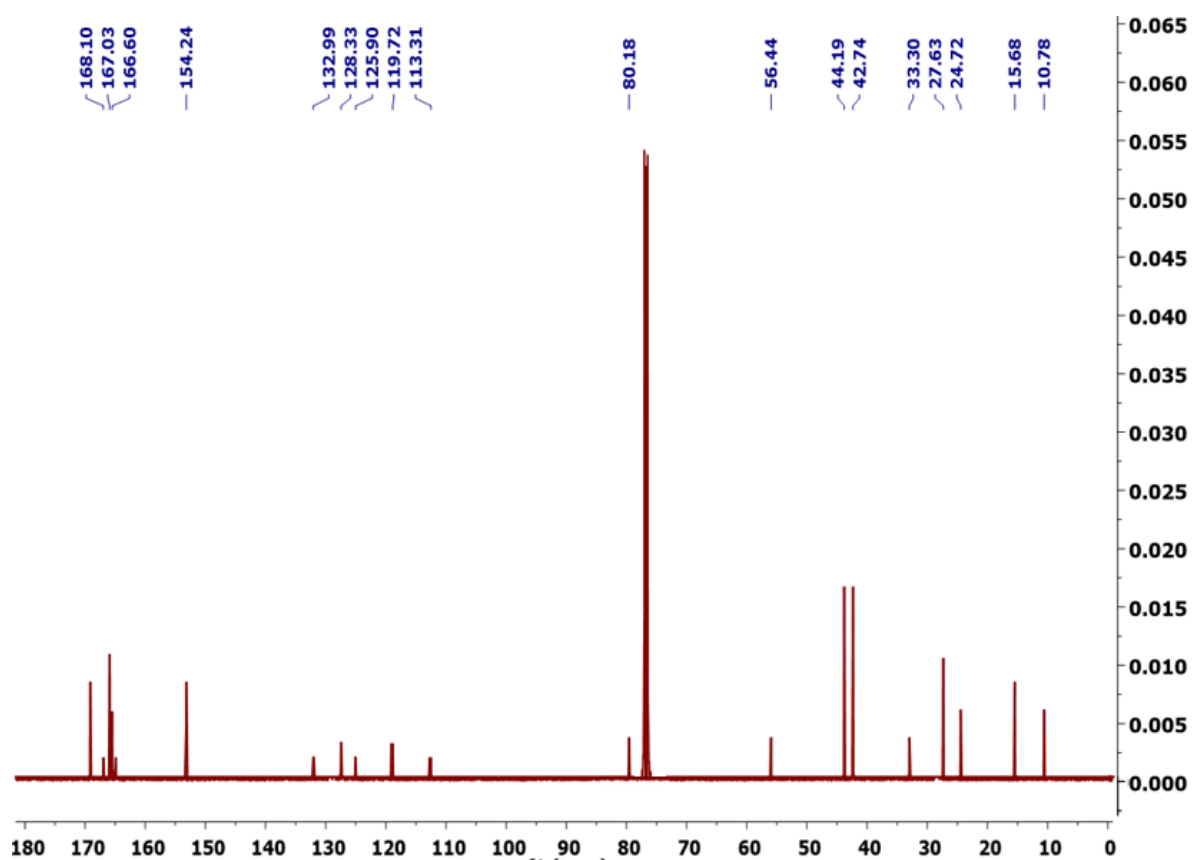
Fig 16. ^1H NMR Spectra of **7c**.Fig 17. ^{13}C NMR Spectra of **7c**.

Fig 18. ^1H NMR Spectra of **7d**.Fig 19. ^{13}C NMR Spectra of **7d**.

Fig 20. ^1H NMR Spectra of **8a**.Fig 21. ^{13}C NMR Spectra of **8a**.

Fig 22. ^1H NMR Spectra of **8b**.Fig 23. ^{13}C NMR Spectra of **8b**.

Fig 24. ^1H NMR Spectra of **8c**.Fig 25. ^{13}C NMR Spectra of **8c**.

Fig 26. ^1H NMR Spectra of **8d**.Fig 27. ^{13}C NMR Spectra of **8d**.

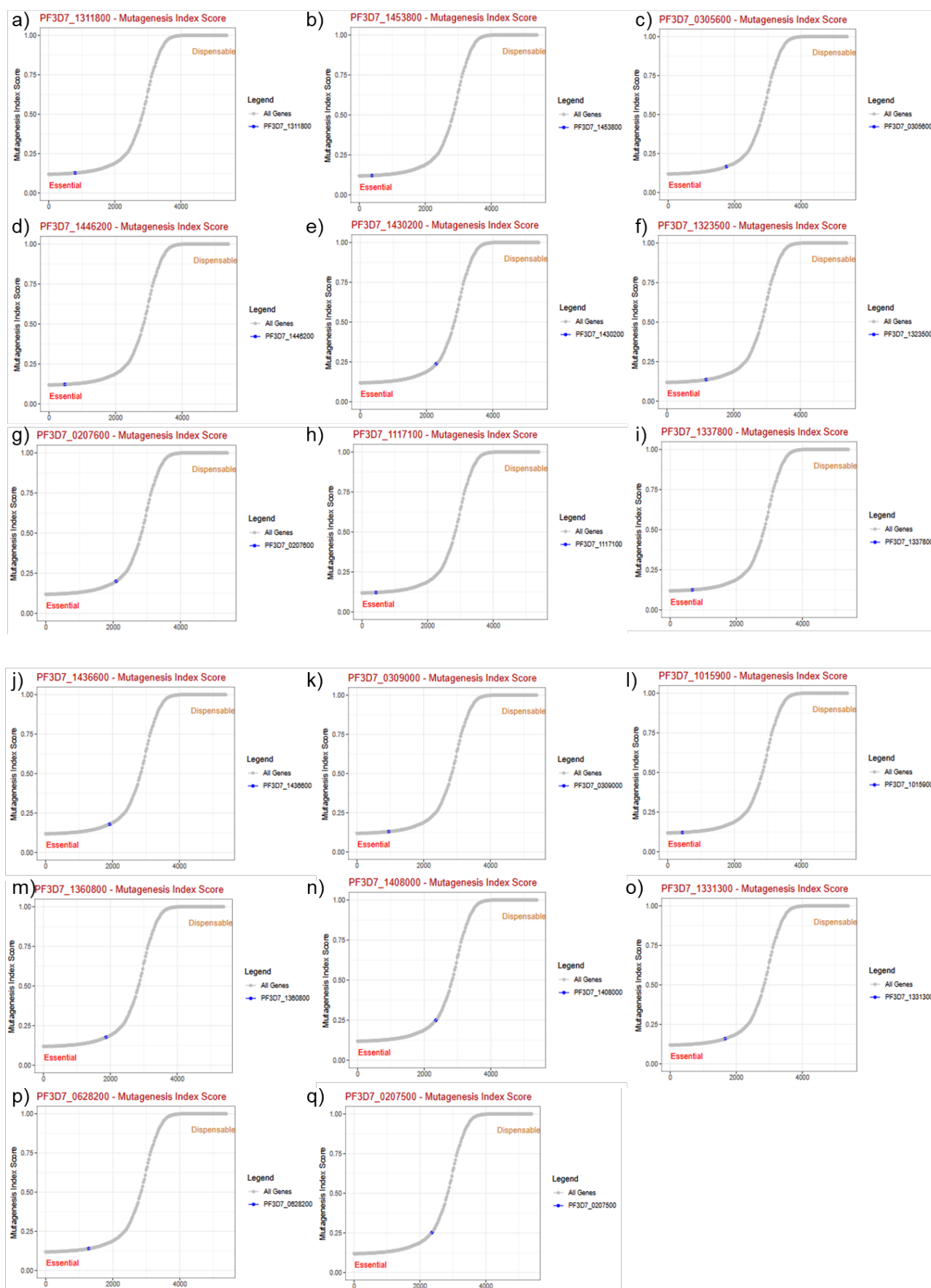


Figure S28. Plot showing essential genes; **a)** Aminopeptidase N, **b)** Bifunctional glucose-6-phosphate 1-dehydrogenase/6-phosphogluconolactonase, **c)** DNA-endonuclease, **d)** Leucine

aminopeptidase, e) PlmIX, f) PlmV, g) Serine-repeat antigen protein 5, h) Ubiquitin carboxyl-terminal hydrolase UCH54, i) Calcium-dependent protein kinase 5, j) cGMP-dependent protein kinase, k) Dual specificity protein phosphatase YVH1, l) Enolase, m) Falcilysin, n) PlmII o) Signal peptidase complex catalytic subunit SEC11, p) Eukaryotic translation initiation factor 2-alpha kinase PK4, and q) Serine-repeat antigen protein 6.

Table S1. Physicochemical properties (ADME), Drug-likeness model score (DrugL Score), and Blood-Brain Barrier (BBB) Score of all the synthesized compounds **6-8(a-d)**.

Entry No.	Cmpd.	MW	n ON	N OHNH	Nrot	TPSA	m LogP	DrugL Score	BBB Score	N Vio.
1	6a	463.21	5	0	8	87.23	2.45	-0.23	3.24	0
2	6b	415.48	5	0	7	87.23	1.84	-0.92	3.10	0
3	6c	429.51	5	0	8	87.23	2.05	-0.59	3.10	0
4	6d	429.51	5	0	8	87.23	2.05	-0.62	3.10	0
5	7a	477.55	5	0	8	87.23	2.65	-0.34	3.23	0
6	7b	429.51	5	0	7	87.23	2.05	-0.73	3.10	0
7	7c	443.54	5	0	8	87.23	2.27	-0.47	3.09	0
8	7d	443.54	5	0	8	87.23	2.27	-0.50	3.09	0
9	8a	481.52	6	0	8	87.23	2.82	0.09	3.24	0
10	8b	433.47	6	0	7	87.23	2.22	-0.41	3.11	0
11	8c	447.50	6	0	8	87.23	2.43	-0.15	3.10	0
12	8d	447.50	6	0	8	87.23	2.43	-0.19	3.10	0

Cmpd. = Compound; MW = molecular weight (g/mol); nON = no. of hydrogen bond acceptor; nOHNH = no. of hydrogen bond donors; Nrot = no. of rotatable bonds; TPSA = total polar surface area; MLogP = Predicted octanol/water partition coefficient; DrugL. Score = Drug-likeness model score (0-2); BBB Score = The Blood-Brain Barrier (BBB) Score (6-High,0-Low); nVio. = no. of Lipinski violation

Table S2. Protein Details to be docked by 12 compounds

S. No.	Entry	Protein names	Gene names or gene model	Length	Alignment	PDB	Essential (piggyBac insertion mutagenesis)	Remarks
1	O96935	Aminopeptidase N	M1AAP MAL13P1.56 PF3D7_1311800	1085	Pass	3EBH	Essential	
2	Q8IKU0	Bifunctional glucose-6-phosphate 1-dehydrogenase/6-phosphogluconolactonase	GluPho PF14_0511 PF3D7_1453800	910	Pass	Alphafold model	Essential	
3	A0A5K1K8H0	Calcium-dependent protein kinase 5	CDPK5 PF3D7_1337800	568	Pass	Alphafold model	Essential	
4	O97240	DNA-endonuclease	APE1 PF3D7_0305600	617	Pass	Alphafold model	Essential	
5	O77334	Dual specificity protein phosphatase YVH1	YVH1 PF3D7_0309000	575	Pass	Alphafold model	Essential	
6	Q76NL8	Falcilysin	FLN PF3D7_1360800	1193	Pass	7DI7	Essential	
7	Q8IL11	Leucine aminopeptidase	LAP PF3D7_1446200	605	Pass	3KR4	Essential	
8	Q8ILG2	Plasmepsin IX	PMIX PF3D7_1430200	627	Pass	Alphafold model	Essential	
9	Q8I6Z5	Plasmepsin V	PMV PF3D7_1323500	590	Pass	Alphafold model	Essential	
10	Q9TY95	Serine-repeat antigen protein 5	PF3D7_0207600	997	Pass	6X42	Essential	
11	Q9TY96	Serine-repeat antigen protein 6	SERA6 PF3D7_0207500	1031	Pass	Alphafold model	Essential	
12	Q8IIJ6	Ubiquitin carboxyl-terminal hydrolase UCH54	UCH54 UCH37 PF3D7_1117100	465	Pass	Alphafold model	Essential	
13	C6KTB8	Eukaryotic translation initiation factor 2-alpha kinase PK4	PK4 PF3D7_0628200 PFF1370w	3072	Pass		Essential	No alpha fold predicted protein model structure
14	Q8I719	cGMP-dependent protein kinase	PKG PF3D7_1436600	853			Essential	Similar to Human

15	Q8IJN7	Enolase	ENO PF10_0155 PF3D7_10159 00	446			Essential	Similar to Human
16	Q8I6V3	Plasmepsin II	PMII PF3D7_14080 00	453		5YIC	Essential	Similar to Human
17	Q8IE14	Signal peptidase complex catalytic subunit SEC11	SEC11 SPC21 PF3D7_13313 00	184			Essential	Similar to Human
18	Q8IOV0	Subtilisin-like protease 1	SUB1 PF3D7_05075 00	688			Essential	This is a substrate for Plasmepsi n X
19	Q8IHZ5	Subtilisin-like protease 2	SUB2 PF3D7_11369 00	1341			Essential	This is a substrate for Plasmepsi n X
20	Q8IAS0	Plasmepsin X	PMX PF3D7_08082 00	573		8DSR	Non-Essential	
21	Q8I3X4	Purine nucleoside phosphorylase	PNP PF3D7_05133 00	245			Non-Essential	
22	Q8I295	4-hydroxy-3- methylbut-2-enyl diphosphate reductase, apicoplast	LytB IspH PF3D7_01044 00	535			Non-Essential	
23	Q8IDF6	Adenylosuccinate synthetase	Adss PF13_0287	442			Non-Essential	
24	Q8I1T8	ATPase ASNA1 homolog	PFD0725c	379			Non-Essential	
25	P62344	Calcium-dependent protein kinase 1	CDPK1 CPK1 PF3D7_02175 00 PFB0815w	524			Non-Essential	
26	Q8ICR0	Calcium-dependent protein kinase 2	CDPK2 CPK2 MAL6P1.108 PF3D7_06106 00 PFF0520w	513			Non-Essential	
27	Q9NJU9	Calcium-dependent protein kinase 3	CDPK3 CPK3 MAL3P3.17 PF3D7_03101 00 PFC0420w	562			Non-Essential	
28	Q8IBS5	Calcium-dependent protein kinase 4	CDPK4 CPK4 PF3D7_07175 00	528			Non-Essential	
29	Q8IHZ9	Casein kinase I	CK1 PF11_0377 PF3D7_11365 00	324			Non-Essential	

30	Q8I5V4	cGMP-specific 3',5'-cyclic phosphodiesterase alpha	PDEalpha PDE1 PF3D7_1209500.1 PFL0475w	954			Non-Essential	
31	Q8IKD3	cGMP-specific 3',5'-cyclic phosphodiesterase delta	PDEdelta PDE4 PF3D7_1470500	815			Non-Essential	
32	P61075	Cyclin-dependent kinase 2 homolog	CRK2 PK5 MAL13P1.279 PF3D7_1356900	288			Non-Essential	
33	Q8IIS0	D-aminoacyl-tRNA deacylase	DTD PF11_0095 PF3D7_1108200	164			Non-Essential	
34	Q08210	Dihydroorotate dehydrogenase	PFF0160c	569			Non-Essential	
35	Q8IIJ9	Dipeptidyl aminopeptidase 1	DPAP1 PF11_0174 PF3D7_1116700	700			Non-Essential	
36	Q7KQM1	DNA primase small subunit	PF14_0366 PF3D7_1438700	452			Non-Essential	
37	Q8I6Z7	Dual 3',5'-cyclic-AMP and -GMP phosphodiesterase beta	PDEbeta PDE2 PF3D7_1321500.1	1139			Non-Essential	
38	Q8IL26	Eukaryotic translation initiation factor 2-alpha kinase 1	eIK1 PF14_0423 PF3D7_1444500	1558			Non-Essential	
39	Q8I265	Eukaryotic translation initiation factor 2-alpha kinase 2	eIK2 PF3D7_0107600	1595			Non-Essential	
40	Q7KQL9	Fructose-bisphosphate aldolase	FBPA PF14_0425 PF3D7_1444800	369			Non-Essential	
41	O15770	Glutathione reductase	GR GR3 PF14_0192 PF3D7_1419800	546			Non-Essential	
42	Q8IJR9	GMP synthase [glutamine-hydrolyzing]	GMPS PF10_0123 PF3D7_1012600	555			Non-Essential	
43	A0A144A134	IMP-specific 5'-nucleotidase 1	ISN1 PF3D7_1206100	444			Non-Essential	
44	Q8IDF3	Metacaspase-1	MCA1 PF3D7_1354800	613			Non-Essential	

45	Q8IKW2	NAD-dependent protein deacetylase Sir2B	Sir2B PF14_0489 PF3D7_1451400	1304			Non-Essential	
46	Q8IE47	NAD-dependent protein deacylase Sir2A	Sir2A Sir2 PF13_0152 PF3D7_1328800	273			Non-Essential	
47	Q7KQM4	Plasmepsin I	PMI PF14_0076 PF3D7_1407900	452			Non-Essential	
48	Q8IM16	Plasmepsin IV	PMIV PF3D7_1407800	449			Non-Essential	
49	C6KTB7	Putative E3 ubiquitin-protein ligase protein PFF1365c	PFF1365c	10287			Non-Essential	
50	Q8I2A6	Putative zinc carboxypeptidase	PF3D7_0103400 PFA0170c	1620			Non-Essential	
51	C6KT50	Pyridoxal 5'-phosphate synthase subunit Pdx1	pdx1 PFF1025c	301			Non-Essential	
52	Q8IIK4	Pyridoxal 5'-phosphate synthase subunit PDX2	PDX2 PF11_0169 PF3D7_1116200	219			Non-Essential	
53	Q8I3W2	Ribose-5-phosphate isomerase	PF3D7_0514600	236			Non-Essential	
54	Q8IIU6	RNA-splicing ligase RtcB homolog	RTCB PF11_0068 PF3D7_1105700	506			Non-Essential	
55	Q8IAR5	Serine protease DegP homolog	DegP PF3D7_0807700	870			Non-Essential	
56	Q8I430	Subtilisin-like protease 3	SUB3 PF3D7_0507200	769			Non-Essential	
57	Q7KQL8	Thioredoxin 1	TRX1 PF14_0545 PF3D7_1457200	104			Non-Essential	
58	Q8IDP4	Thioredoxin 2	TRX2 PF3D7_1345100	157			Non-Essential	
59	Q4VWQ3	Thioredoxin 3	TRX3 PF3D7_0916100	180			Non-Essential	
60	P61076	Thioredoxin reductase	TRXR PF3D7_0923800 PFI1170c	617			Non-Essential	
61	Q7KQM0	Triosephosphate isomerase	TPI PF14_0378	248			Non-Essential	

				PF3D7_14399 00				
62	Q8IKM8	Ubiquitin terminal UCHL3	carboxyl- hydrolase	UCHL3 PF3D7_14604 00	232			Non-Essential