Supporting Information

Molecular insights into Mmpl3 leads to the development of novel indole-2-carboxamides as antitubercular agents

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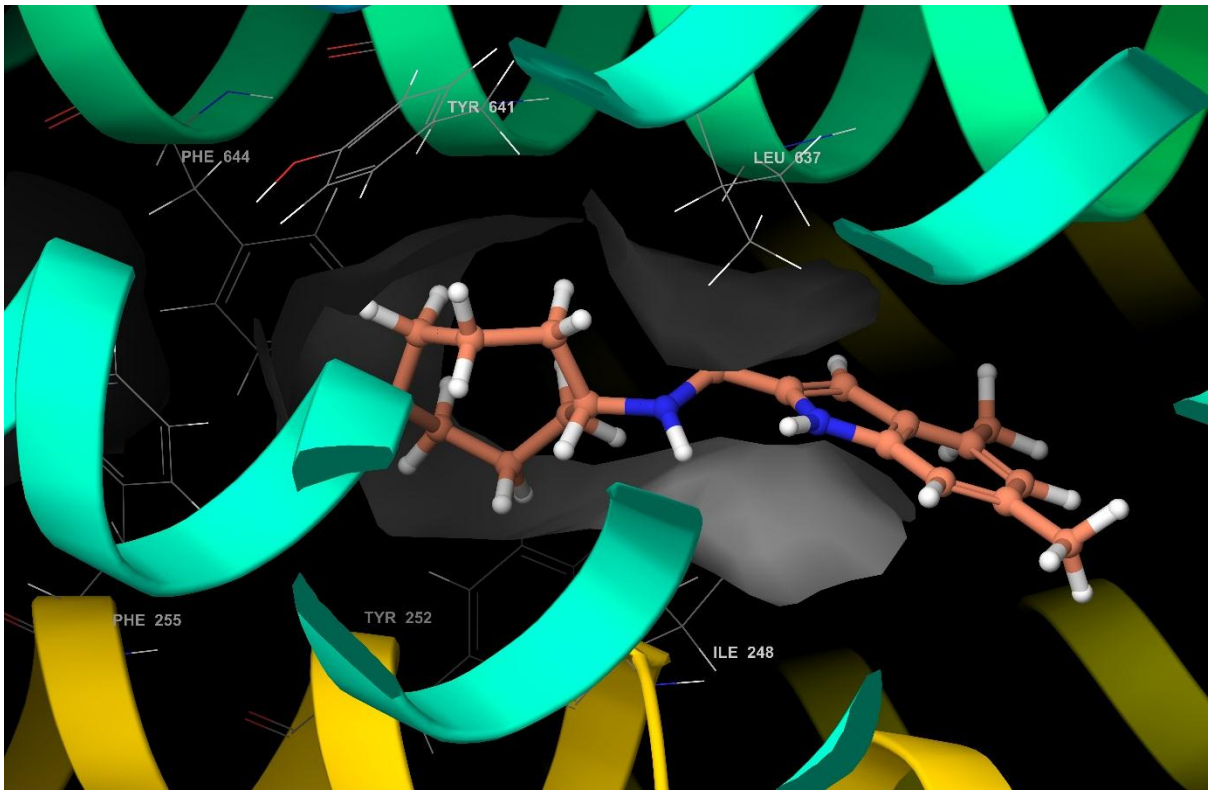
Figures and Tables



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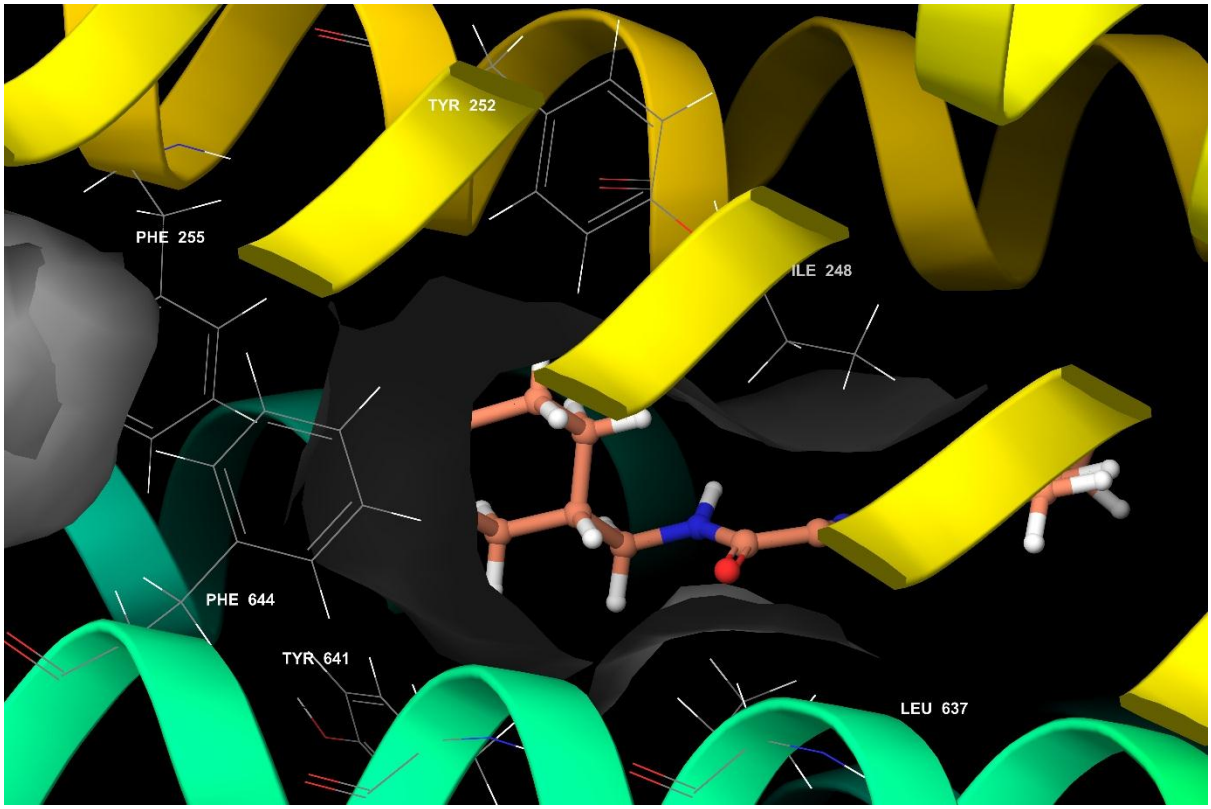
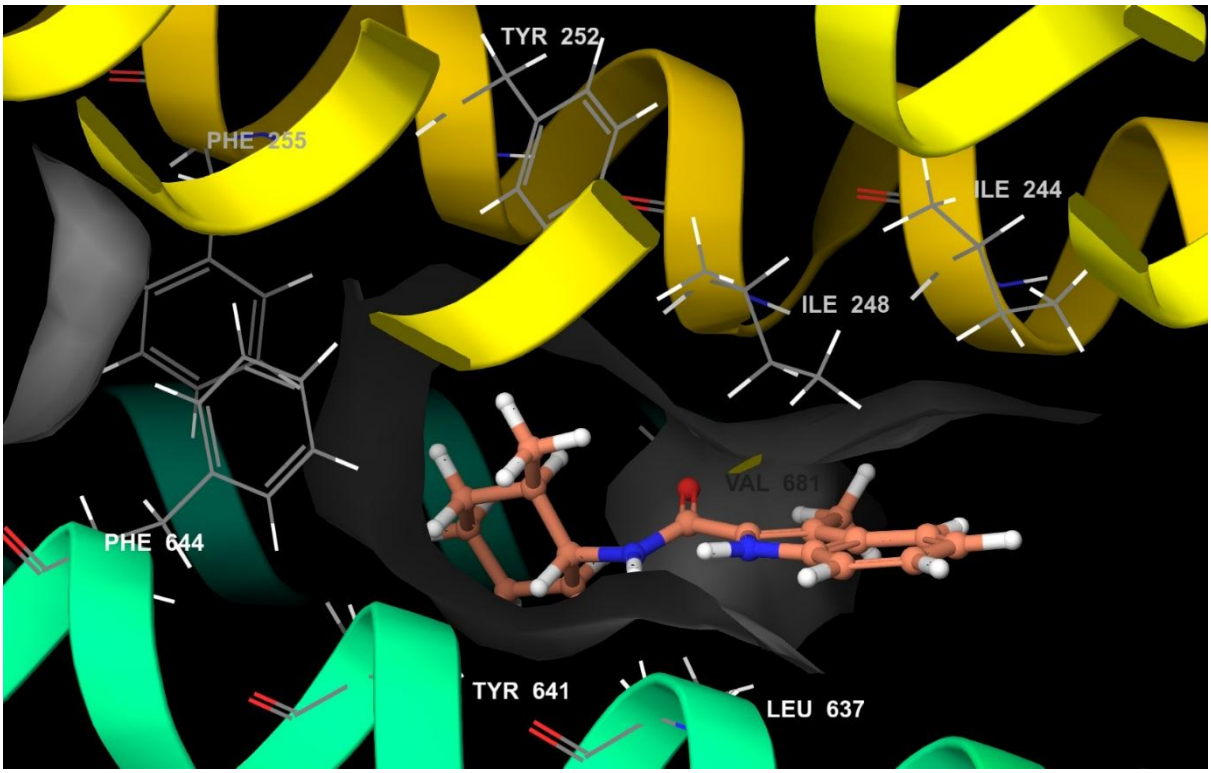


(b)

Fig. S1. (a) Protein-ligand complex of MmpL3 and A12. (b) Protein-ligand complex of MmpL3 and A16. In the figures, the green coloured ball and stick structures represent the ligand, the grey coloured ball and stick structures represent the residues at the binding site, and the grey coloured surface signify the hydrophobic pocket formed by the non-polar residues at the binding site.



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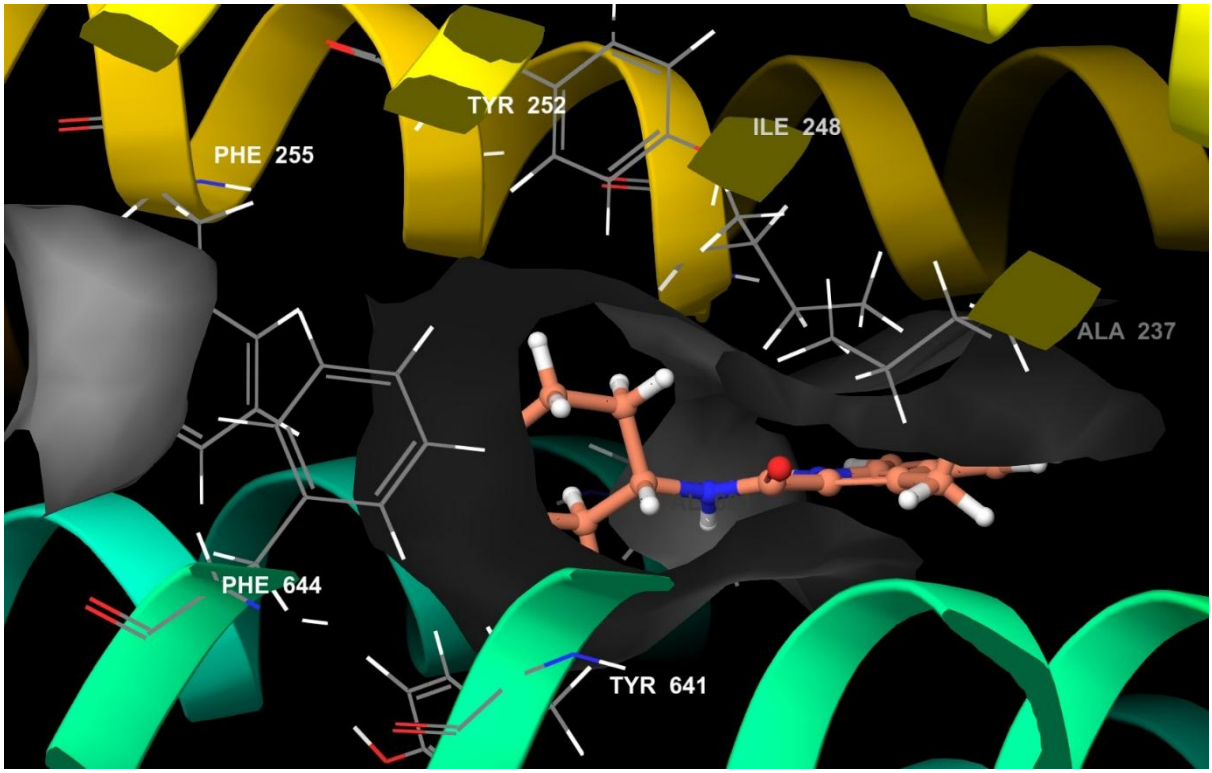

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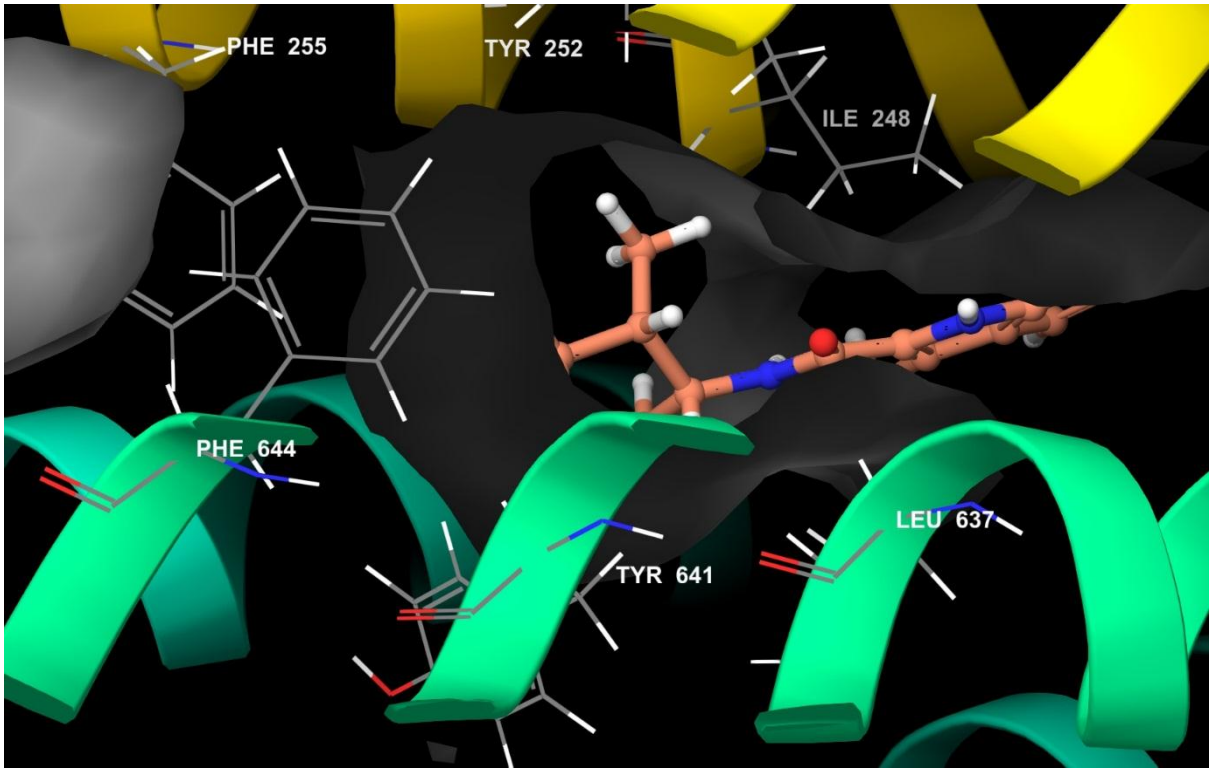
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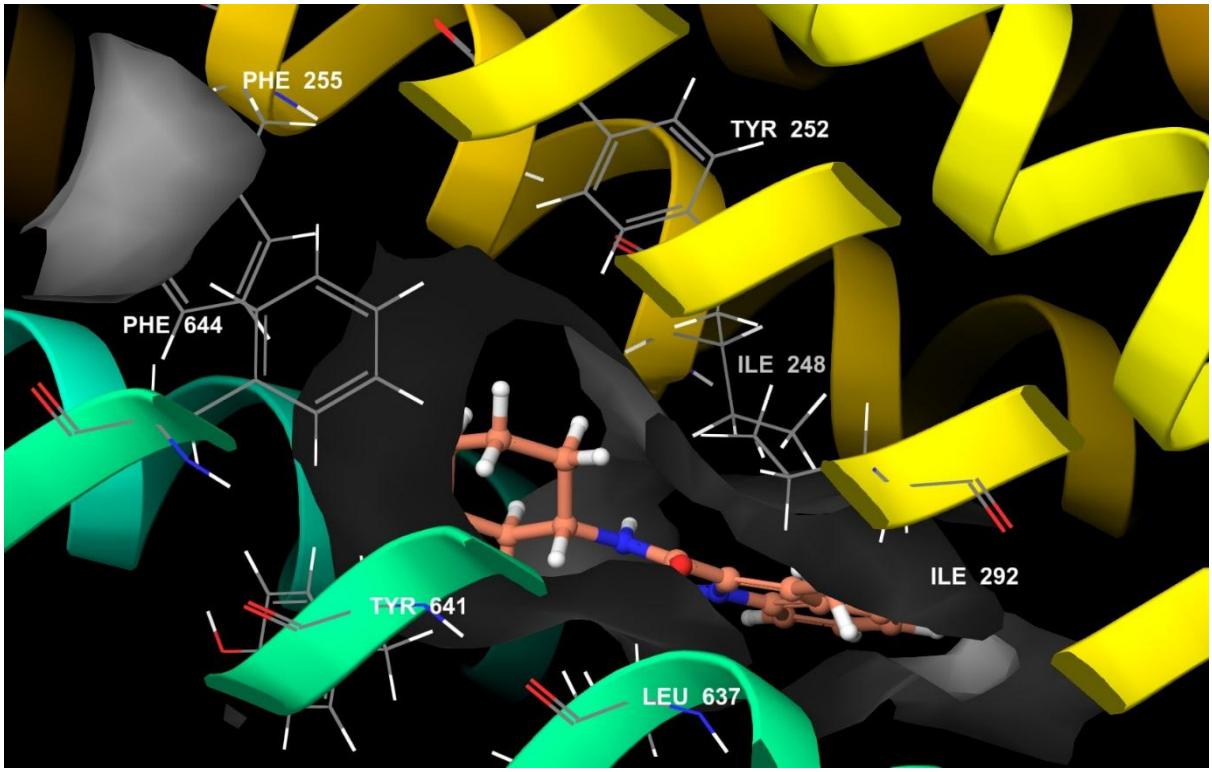
LEU 637
TYR 641

(c)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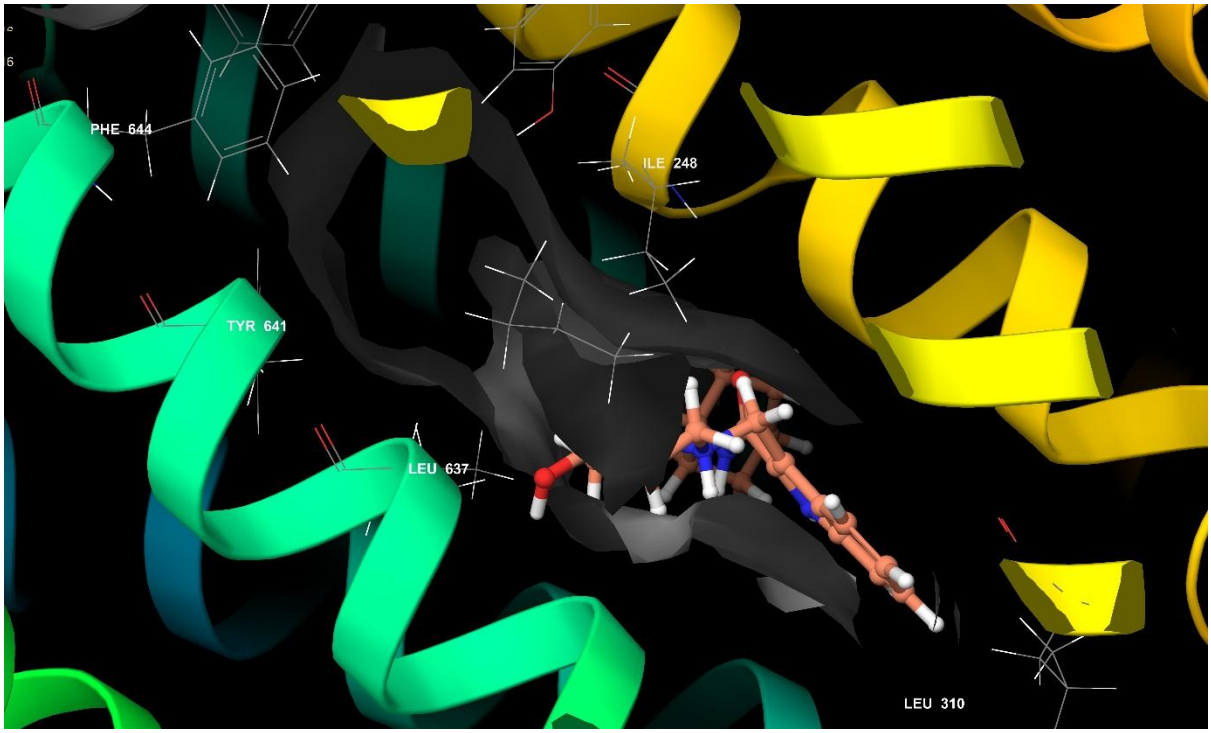
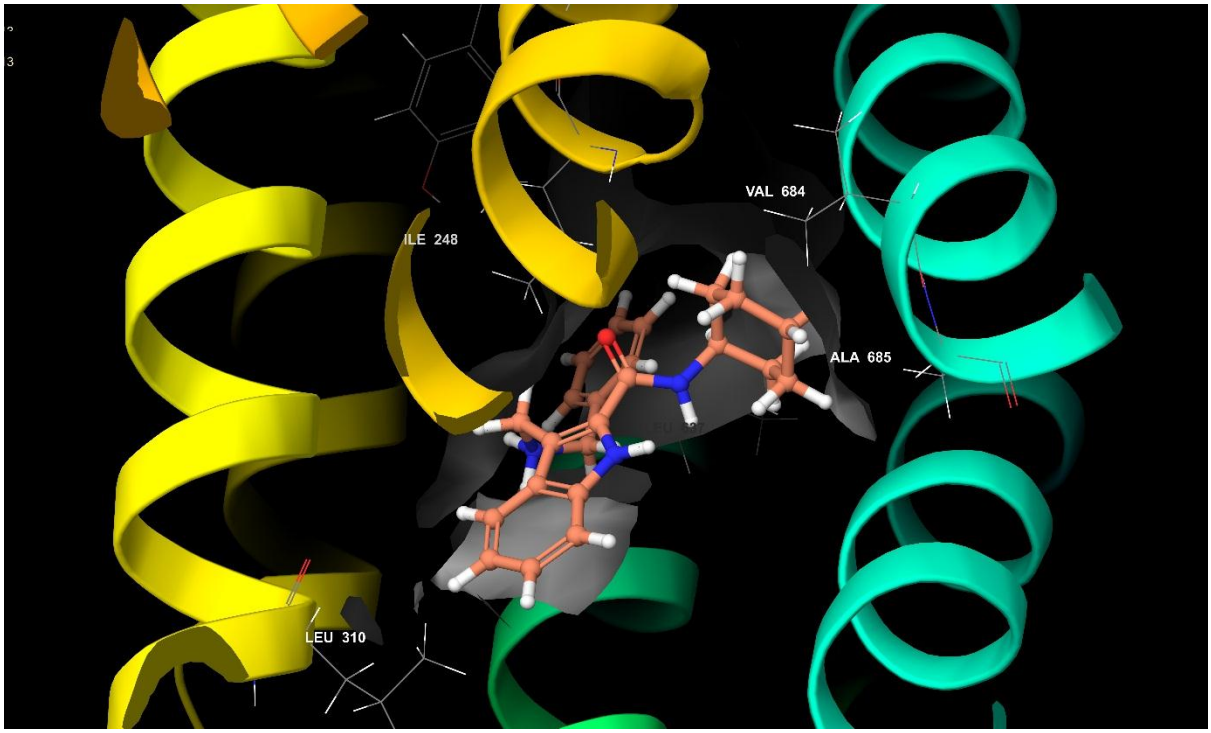
(d)



(e)

Fig. S2. (a) Protein-ligand complex of MmpL3 and 3a2T. (b) Protein-ligand complex of MmpL3 and 3a2'T. (c) Protein-ligand complex of MmpL3 and 3a2C. (d) Protein-ligand complex of MmpL3 and 3bC. (e) Protein-ligand complex of MmpL3 and 5eT. In the figures, the green coloured ball and stick structures represent the ligand, the grey coloured ball and stick structures represent the residues at the binding site, and the grey coloured surface signify the hydrophobic pocket formed by the non-polar residues at the binding site. 3a2T and 3a2'T represent the mirror image structures of the tran-isomeric forms of 3a. 3bC represents the cis-isomeric form of 3b and 5eT represent the trans-isomeric form of 5e.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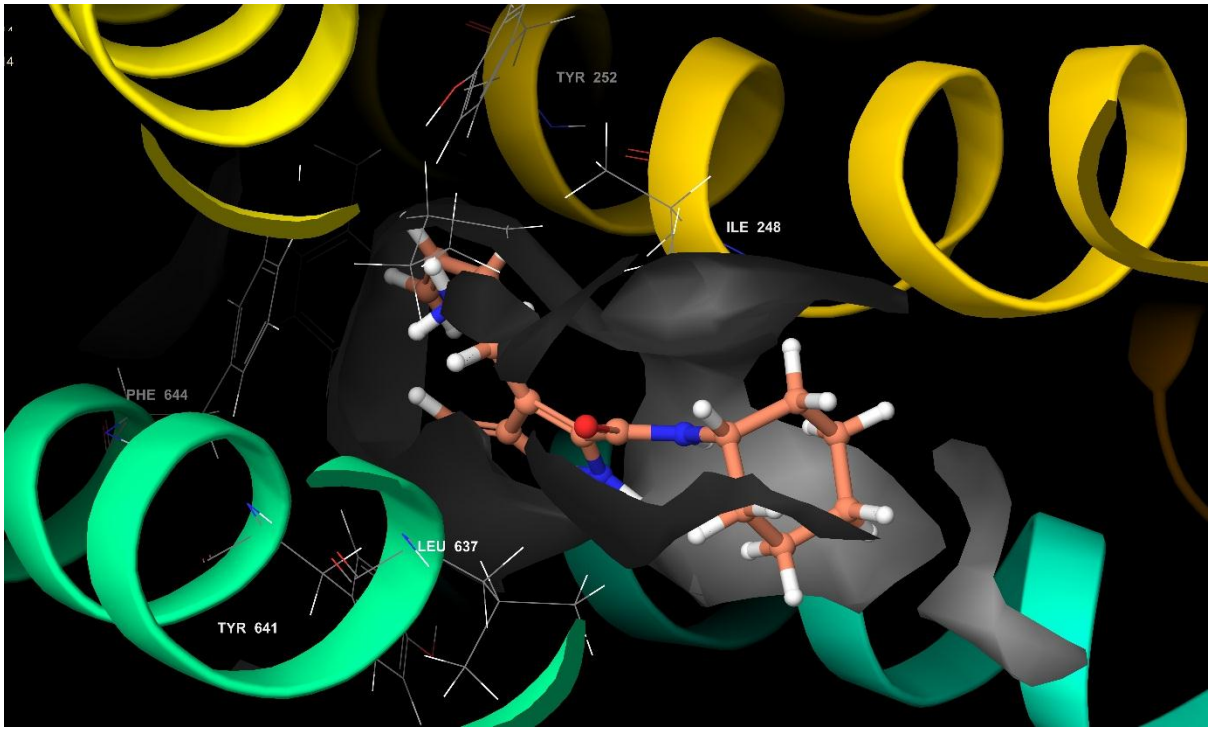
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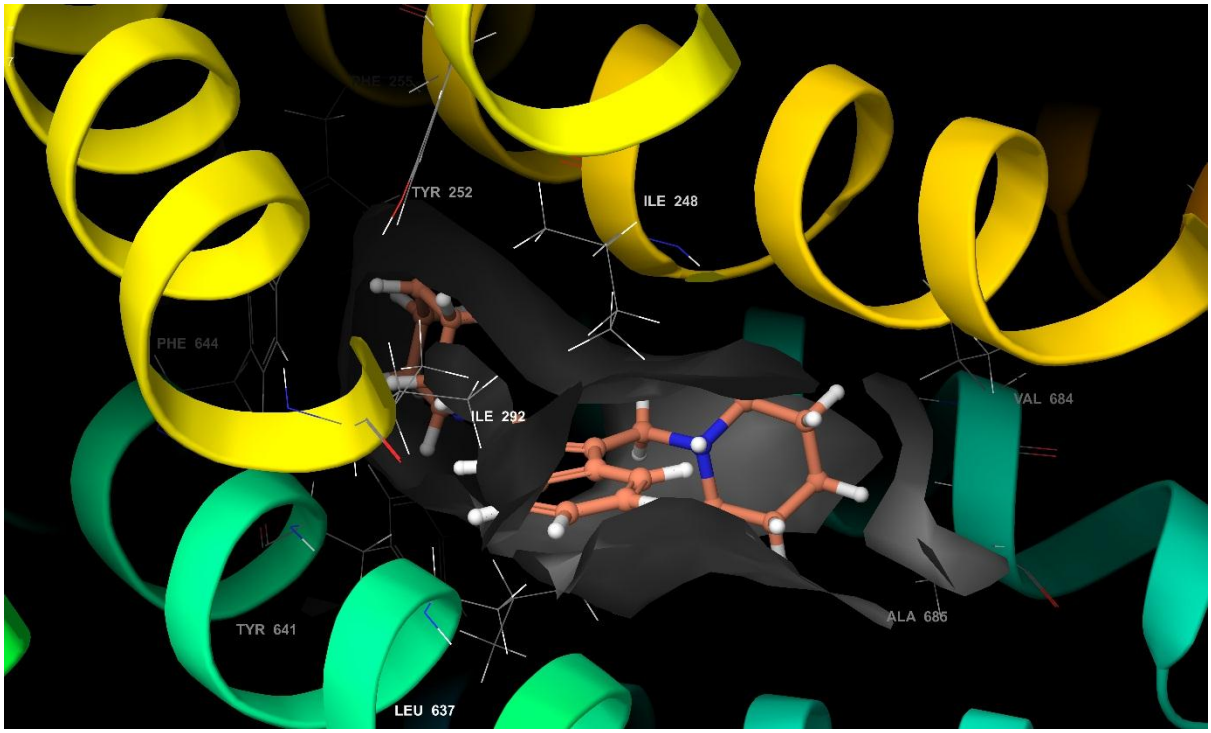
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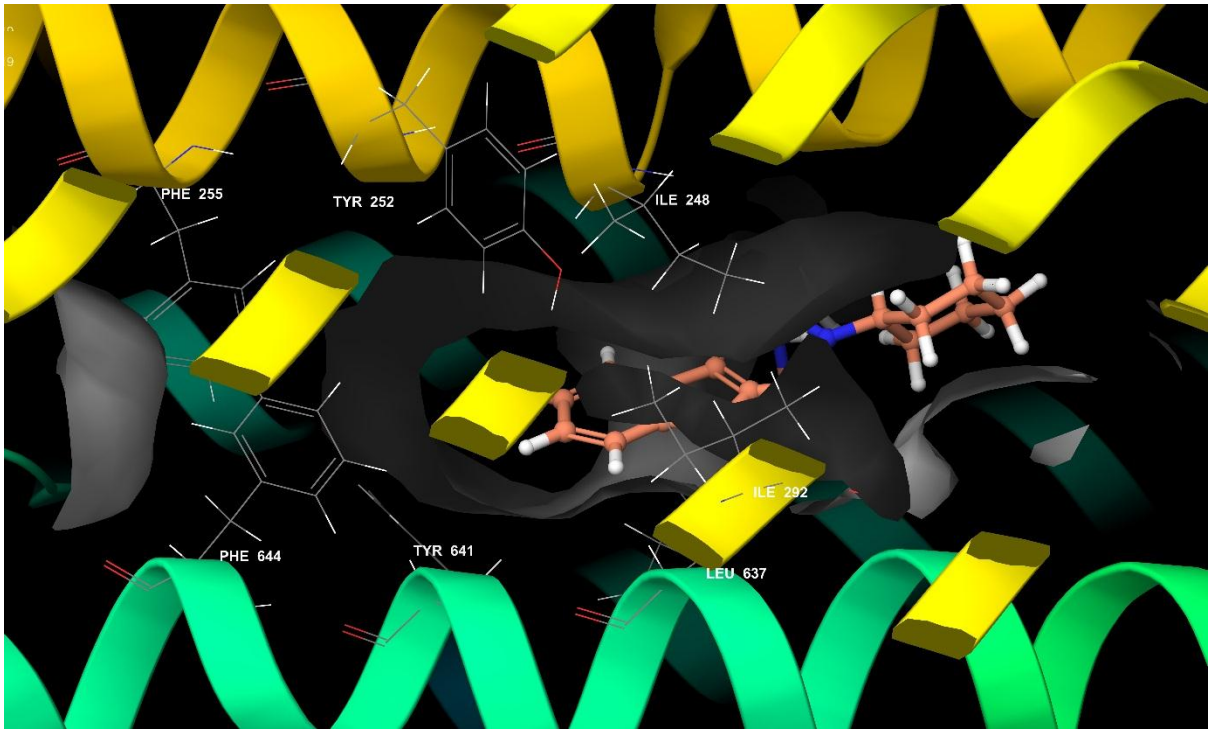
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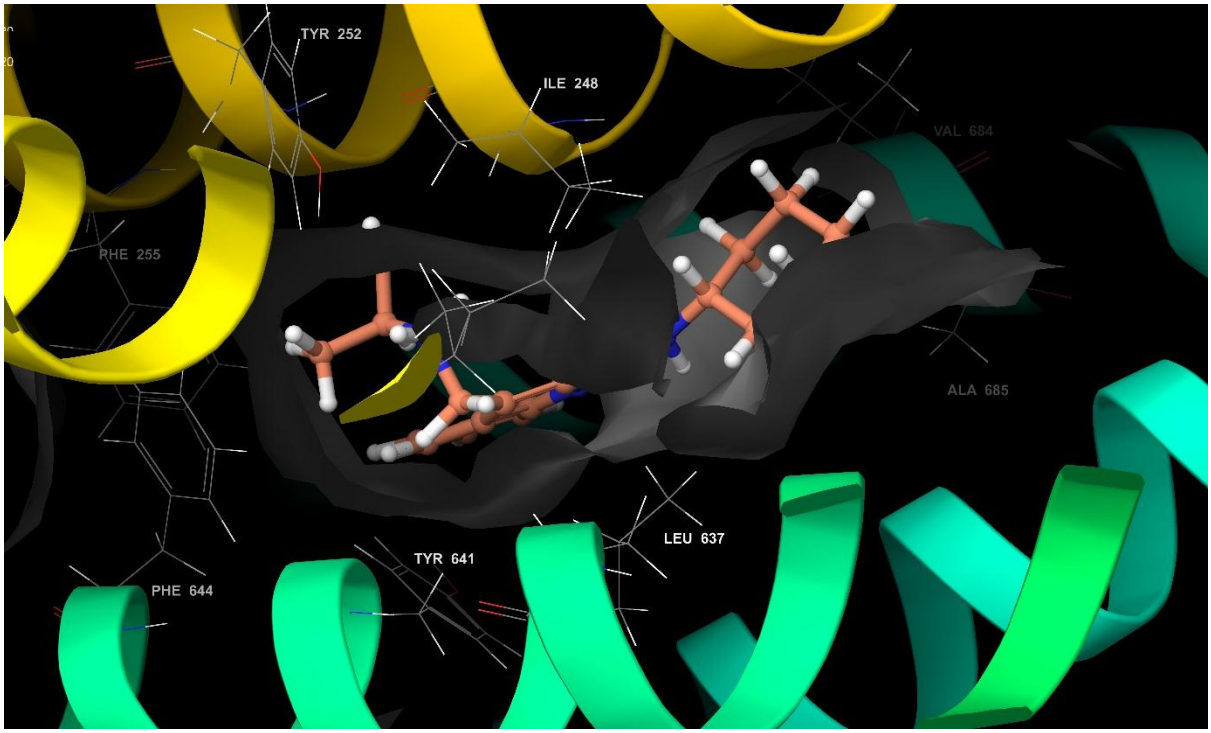

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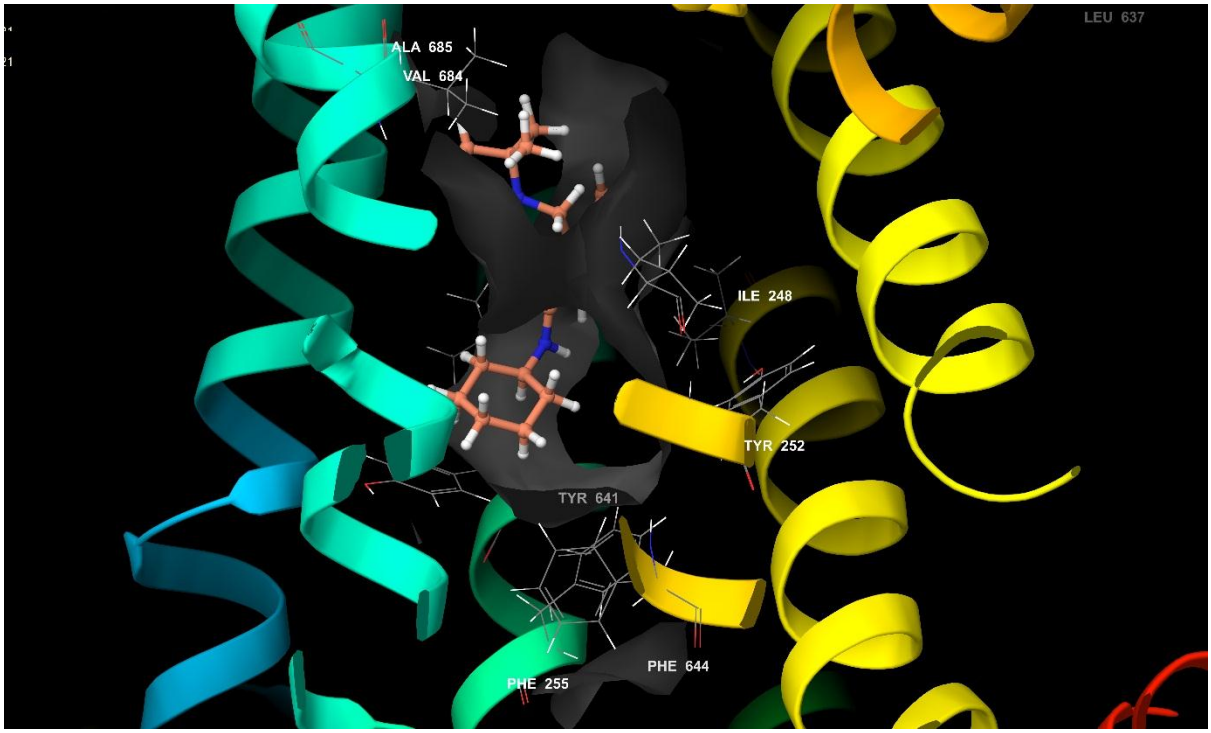


(f)

Fig. S3. (a) Protein-ligand complex of MmpL3 and 5a. (b) Protein-ligand complex of MmpL3 and 5c. (c) Protein-ligand complex of MmpL3 and 5d. (d) Protein-ligand complex of MmpL3 and 5f. (e) Protein-ligand complex of MmpL3 and 5g. (f) Protein-ligand complex of MmpL3 and 5g. In the figures, the green coloured ball and stick structures represent the ligand, the grey

coloured ball and stick structures represent the residues at the binding site, and the grey coloured surface signify the hydrophobic pocket formed by the non-polar residues at the binding site.

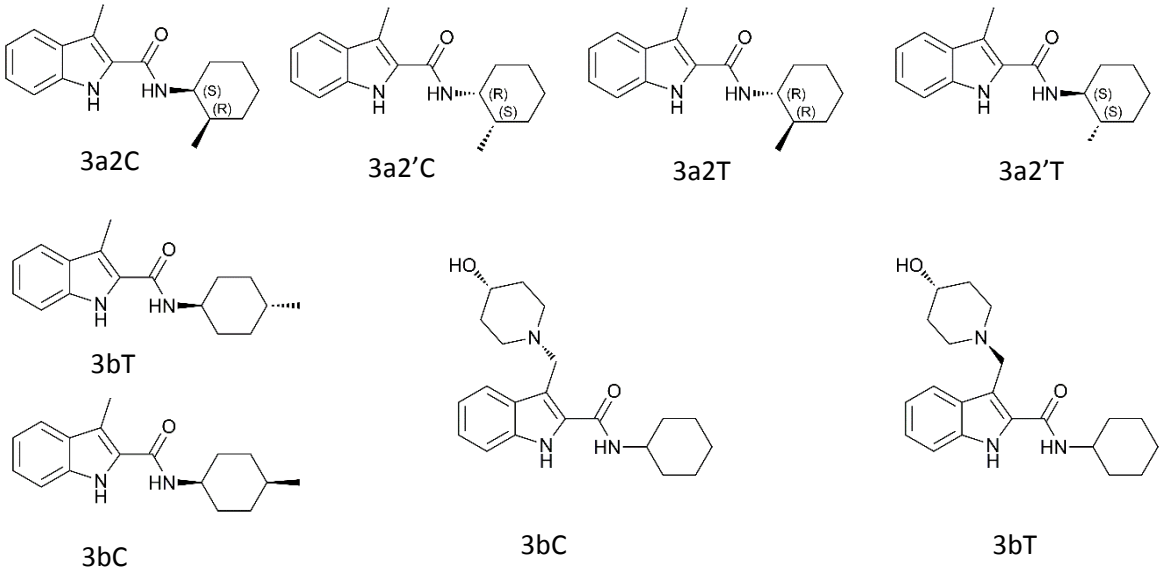
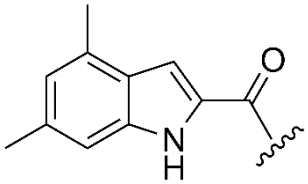
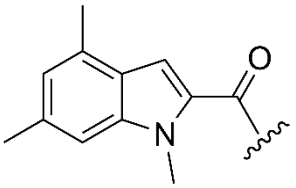


Figure S4. Stereoisomers of synthesized molecules 3a, 3b, and 5e. Molecules were labelled according to their cis or trans forms and the R/S configurations of their chiral centres.

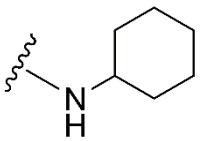
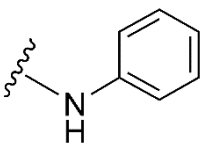
Table S1. Reported Indoles with their antitubercular activity.

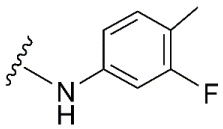
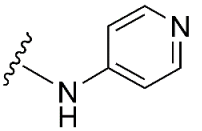
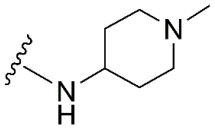
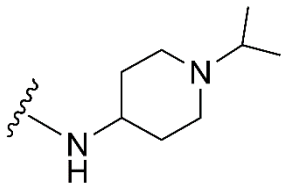
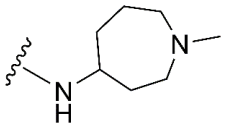
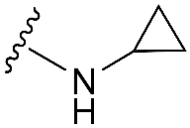
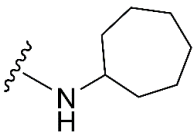
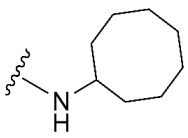
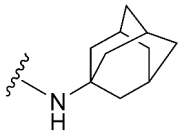


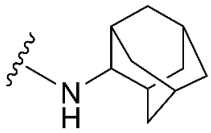
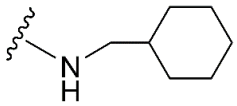
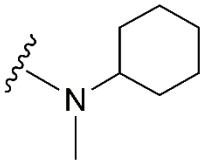
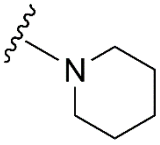
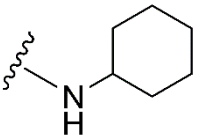
A3 - A17

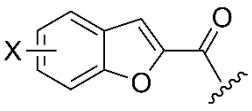


A18

Compound Name	MIC	X	R	dG Bind	pMIC**
A3 (*N-Cyclohexyl-4,6-dimethyl-1H-indole-2-carboxamide*)	0.93	-		-30.146	6.032
A4 (*N-Phenyl-4,6-dimethyl-1H-indole-2-carboxamide*)	3.8	-		-45.165	5.42

A5 (*N-(3-Fluoro-4-methylphenyl)-4,6-dimethyl-1H-indole-2-carboxamide*)	1.7	-		-49.617	5.77
A6 (*N-(4-Pyridinyl)-4,6-dimethyl-1H-indole-2-carboxamide*)	240	-		-34.199	3.62
A7 (*N-(1-Methyl-4-piperidinyl)-4,6-dimethyl-1H-indole-2-carboxamide*)	448	-		-61.306	3.349
A8 (*N-(1-Isopropyl-4-piperidinyl)-4,6-dimethyl-1H-indole-2-carboxamide*)	204	-		-75.494	3.69
A9 (*N-(1-Methyl-4-azepanyl)-4,6-dimethyl-1H-indole-2-carboxamide*)	428	-		-65.922	3.369
A10 (*N-Cyclopropyl-4,6-dimethyl-1H-indole-2-carboxamide*)	561	-		-33.608	3.251
A11 (*N-Cycloheptyl-4,6-dimethyl-1H-indole-2-carboxamide*)	0.055	-		-34.372	7.26
A12 (*N-Cyclooctyl-4,6-dimethyl-1H-indole-2-carboxamide*)	0.013	-		-41.156	7.886
A13 (*N-(1-Adamantyl)-4,6-dimethyl-1H-*	0.012	-		-24.645	7.921

indole-2-carboxamide)					
A14 (*N-(2-Adamantyl)-4,6-dimethyl-1H-indole-2-carboxamide*)	0.012	-		-41.459	7.921
A15 (*N-(Cyclohexylmethyl)-4,6-dimethyl-1H-indole-2-carboxamide*)	0.88	-		-38.389	6.056
A16 (*N-Cyclohexyl-N,4,6-trimethyl-1H-indole-2-carboxamide*)	450	-		-43.209	3.347
A17 ((*4,6-Dimethyl-1H-indol-2-yl)(piperidin-1-yl)methanone*)	>499	-		-31.34	-
A18 (*N-Cyclohexyl-1,4,6-trimethyl-1H-indole-2-carboxamide*)	450	-		-49.47	3.347



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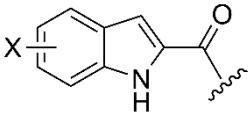

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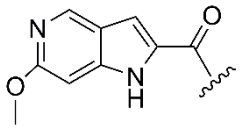

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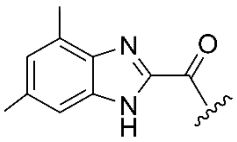
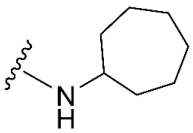
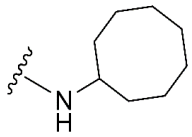
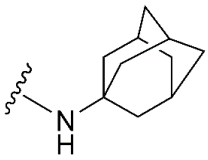
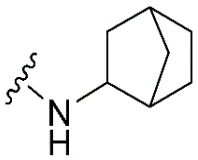
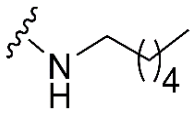
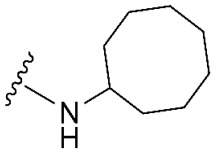
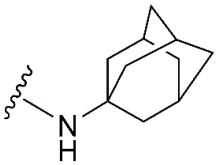
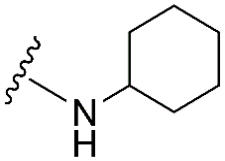
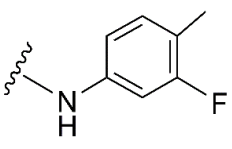
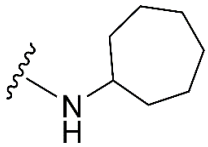
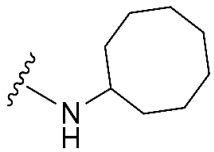
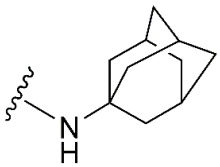
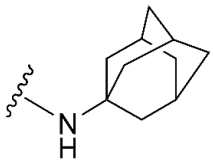
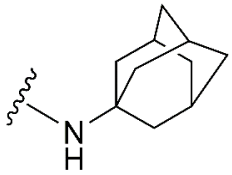
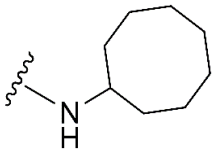
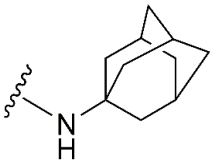
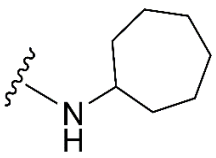
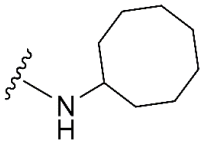
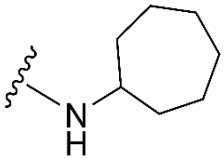
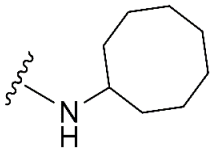
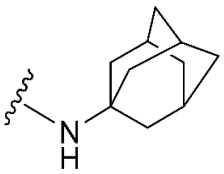
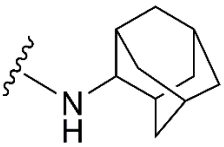


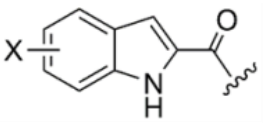
A37 – A40

Compound Name	**MIC**	**X**	**R**	**dG Bind (kcal/mol)**	**pMIC****
A19 (*N-Cycloheptyl-4,6-dimethylbenzofuran-2-carboxamide*)	56	4,6-dimethyl		-50.268	4.252

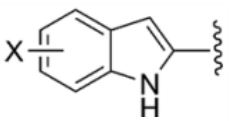
A20 (*N-Cyclooctyl-4,6-dimethylbenzofuran-2-carboxamide*)	27	4,6-dimethyl		-51.655	4.569
A21 (*N-(1-Adamantyl)-4,6-dimethylbenzofuran-2-carboxamide*)	3.1	4,6-dimethyl		-28.147	5.509
A22* (*N-(Bicyclo[2.2.1]-2-heptanyl)-4,6-dimethylbenzofuran-2-carboxamide*)	113	4,6-dimethyl		-42.033/-38.269	3.94
A23 (*N-Hexyl-4,6-dimethylbenzofuran-2-carboxamide*)	59	4,6-dimethyl		-48.692	4.229
A24 (*5-Chloro-N-cyclooctylbenzofuran-2-carboxamide*)	26	5-Cl		-43.748	4.585
A25 (*5-Chloro-N-(1-adamantyl)benzofuran-2-carboxamide*)	≥388	5-Cl		-29.327	-
A26 (*N-Cyclohexyl-1H-indole-2-carboxamide*)	>528	H		-33.358	-
A27 (*N-(3-Fluoro-4-methylphenyl)-1H-indole-2-carboxamide*)	477	H		-40.193	3.321

A28 (*N-Cycloheptyl-4,6-difluoro-1H-indole-2-carboxamide*)	0.86	4,6-difluro		-44.001	6.066
A29 (*N-Cyclooctyl-4,6-difluoro-1H-indole-2-carboxamide*)	0.1	4,6-difluro		-41.929	7
A30 (*N-(1-Adamantyl)-6-methoxy-1H-indole-2-carboxamide*)	0.77	6-OCH_3		-23.084	6.114
A31 (*N-(1-Adamantyl)-5-chloro-1H-indole-2-carboxamide*)	0.38	5-Cl		-22.11	6.42
A32 (*N-(1-Adamantyl)-6-hydroxy-1H-indole-2-carboxamide*)	13	6-OH		-22.848	4.886
A33 (*N-Cyclooctyl-6-methoxy-1H-pyrrolo[3,2-c]pyridine-2-carboxamide*)	6.6	-		-38.97	5.18
A34 (*N-(1-Adamantyl)-6-methoxy-1H-pyrrolo[3,2-c]pyridine-2-carboxamide*)	1.5	-		-25.807	5.824
A35 (*N-Cycloheptyl-4,6-bis(trifluoromethyl)-1H-indole-2-carboxamide*)	0.64	4,6-bis(CF_3)		-54.878	6.194

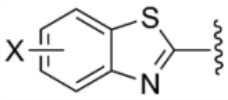
A36 (*N-Cyclooctyl-4,6-bis(trifluoromethyl)-1H-indole-2-carboxamide*)	0.04	4,6-bis(CF_3)		-56.092	7.398
A37 (*N-Cycloheptyl-4,6-dimethyl-1H-benzo[d]imidazole-2-carboxamide*)	>224	-		-20.23	-
A38 (*N-Cyclooctyl-4,6-dimethyl-1H-benzo[d]imidazole-2-carboxamide*)	1.7	-		-44.297	5.77
A39 (*N-(1-Adamantyl)-4,6-dimethyl-1H-benzo[d]imidazole-2-carboxamide*)	0.39	-		-26.591	6.409
A40 (*N-(2-Adamantyl)-4,6-dimethyl-1H-benzo[d]imidazole-2-carboxamide*)	1.5	-		-56.236	5.824



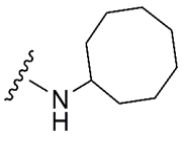
B3-B39

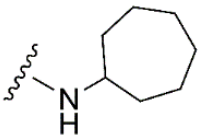
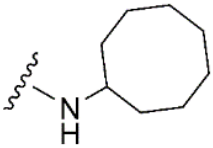
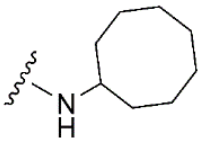
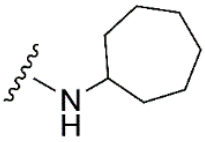
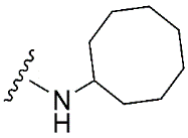
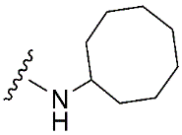
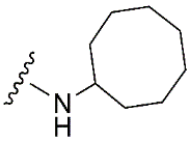
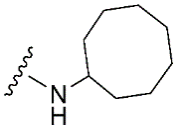


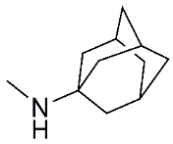
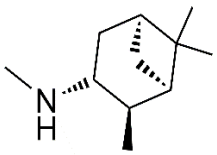
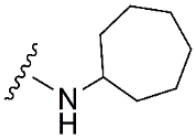
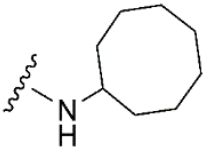
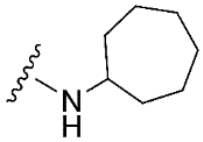
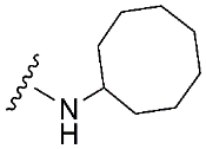
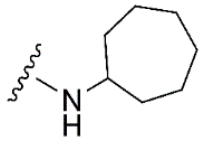
B40-B41

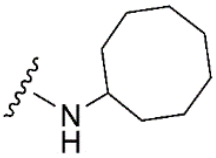
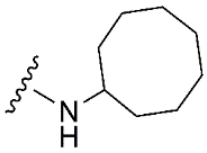
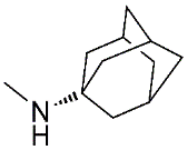
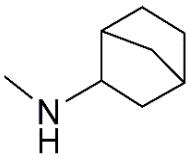
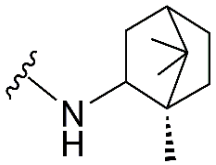
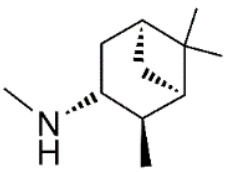


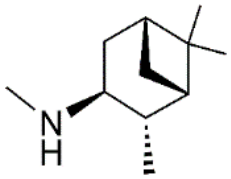
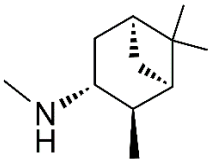
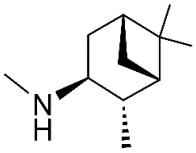
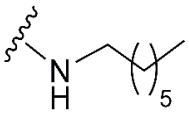
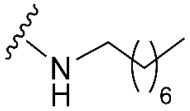
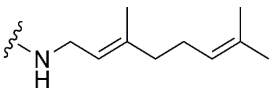
B42

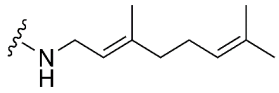
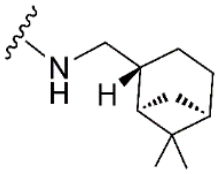
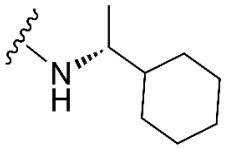
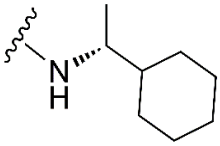
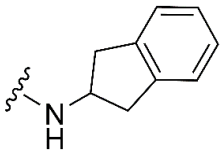
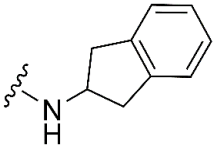
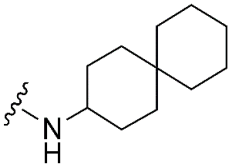
Compound Name	**MIC**	**X**	**R**	**dG Bind (kcal/mol)**	**pMIC****
B3 (*N-Cyclohexyl-4,6-dimethyl-1H-indole-2-carboxamide*)	0.013	4, 6-dimethyl		-44.645	7.886

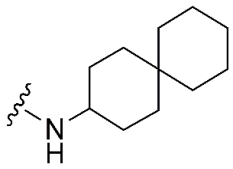
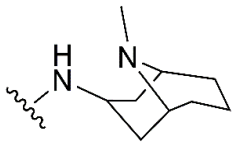
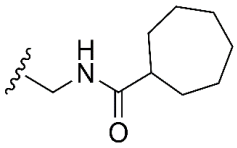
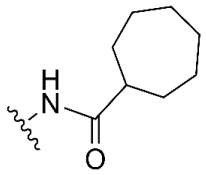
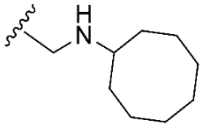
B4 (*N-Cycloheptyl-4-methyl-1H-indole-2-carboxamide*)	0.93	4-methyl		-51.154	6.032
B5 (*N-Cyclooctyl-4-methyl-1H-indole-2-carboxamide*)	0.11	4-methyl		-46.943	6.959
B6 (*4-Amino-N-cyclooctyl-1H-indole-2-carboxamide*)	20	4-amino		-39.861	4.699
B7 (*N-Cycloheptyl-5-methyl-1H-indole-2-carboxamide*)	7.4	5-methyl		-40.691	5.131
B8 (*N-Cyclooctyl-5-methyl-1H-indole-2-carboxamide*)	0.88	5-methyl		-40.691	6.056
B9 (*5-Amino-N-cyclooctyl-1H-indole-2-carboxamide*)	80	5-amino		-41.406	4.097
B10 (*N-Cyclooctyl-6-methyl-1H-indole-2-carboxamide*)	0.11	6-methyl		-41.571	6.959
B11 (*6-Bromo-N-cyclooctyl-1H-indole-2-carboxamide*)	0.09	6-Br		-43.103	7.046

B12 (*N-(1-Adamantanyl)-6-bromo-1H-indole-2-carboxamide*)	0.042	6-Br		-15.74	7.377
B13 (*6-Bromo-N-((1R,2R,3R,5S)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)-1H-indole-2-carboxamide*)	0.01	6-Br		-31.287	8
B14 (*N-Cycloheptyl-7-methyl-1H-indole-2-carboxamide*)	30	7-methyl		-40.826	4.523
B15 (*N-Cyclooctyl-7-methyl-1H-indole-2-carboxamide*)	3.5	7-methyl		-43.09	5.456
B16 (*N-Cycloheptyl-5,7-dimethyl-1H-indole-2-carboxamide*)	3.5	5,7-dimethyl		-15.214	5.456
B17 (*N-Cyclooctyl-5,7-dimethyl-1H-indole-2-carboxamide*)	0.21	5,7-dimethyl		-44.387	6.678
B18 (*N-Cycloheptyl-4,6-dimethoxy-1H-indole-2-carboxamide*)	3.2	4,6-dimethoxy		-41.776	5.495

B19 (*N-Cyclooctyl-4,6-dimethoxy-1H-indole-2-carboxamide*)	0.19	4,6-dimethoxy		-46.65	6.721
B20 (*4,6-Dichloro-N-cyclooctyl-1H-indole-2-carboxamide*)	0.011	4,6-dichloro		-48.404	7.959
B21 (*N-(1-Adamantanyl)-4,6-dichloro-1H-indole-2-carboxamide*)	0.011	4,6-dichloro		-29.464	7.959
B22* (*N-(exo-Bicyclo[2.2.1]heptan-2-yl)-4,6-dichloro-1H-indole-2-carboxamide*)	0.39	4,6-dichloro		-42.033/-38.269	6.409
B23 (*4,6-Dichloro-N-(endo-(1R)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)-1H-indole-2-carboxamide*)	0.043	4,6-dichloro		-41.988	7.367
B24 (*4,6-Dichloro-N-((1R,2R,3R,5S)-2,6,6-trimethylbicyclo[3.1.1]-heptan-3-yl)-1H-indole-2-carboxamide*)	0.021	4,6-dichloro		-45.39	7.678

B25 (*4,6-Dichloro-N-((1S,2S,3S,5R)-2,6,6-trimethylbicyclo[3.1.1]-heptan-3-yl)-1H-indole-2-carboxamide*)	0.086	4,6-dichloro		-60.342	7.066
B26 (*4,6-Difluoro-N-((1R,2R,3R,5S)-2,6,6-trimethylbicyclo[3.1.1]-heptan-3-yl)-1H-indole-2-carboxamide*)	0.012	4,6-difluro		-45.298	7.96
B27 (*4,6-Difluoro-N-((1S,2S,3S,5R)-2,6,6-trimethylbicyclo[3.1.1]-heptan-3-yl)-1H-indole-2-carboxamide*)	0.19	4,6-difluro		-50.138	6.721
B28 (*4,6-Dichloro-N-heptyl-1H-indole-2-carboxamide*)	>300	4,6-dichloro		-54.412	-
B29 (*4,6-Dichloro-N-octyl-1H-indole-2-carboxamide*)	>300	4,6-dichloro		-54.955	_
B30 *((E)-4,6-Dichloro-N-(3,7-dimethylocta-2,6-dien-1-yl)-1H-indole-2-Carboxamide*)	11	4,6-dichloro		-56.552	4.959

B31 *((E)-N-(3,7-Dimethylocta-2,6-dien-1-yl)-4,6-difluoro-1H-indole-2-Carboxamide*)	>192	4,6-difluro		-61.277	–
B32 (*4,6-Dichloro-N-[((1R,2R,5R)-6,6-dimethylbicyclo[3.1.1]heptan-2-yl)methyl]-1H-indole-2-carboxamide*)	175	4,6-dichloro		-43.342	3.757
B33 (*R)-N-(1-Cyclohexylethyl)-4,6-dichloro-1H-indole-2-carboxamide*)	5.9	4,6-dichloro		-57.106	5.229
B34 ((*R)-N-(1-Cyclohexylethyl)-4,6-difluoro-1H-indole-2-carboxamide*)	13	4,6-difluro		-48.334	4.886
B35 (*4,6-Dichloro-N-(2,3-dihydro-1H-inden-2-yl)-1H-indole-2-carboxamide*)	0.72	4,6-dichloro		-48.526	6.143
B36 (*N-4,6-Difluoro-(2,3-dihydro-1H-inden-2-yl)-1H-indole-2-carboxamide*)	102	4,6-difluro		-46.618	3.991
B37 (*4,6-Dichloro-N-(spiro[5.5]undecan-3-yl)-1H-indole-2-carboxamide*)	0.005	4,6-dichloro		-61.671	8.301

B38 (*4,6-Difluoro-N-(spiro[5.5]undecan-3-yl)-1H-indole-2-carboxamide*)	0.003	4,6-difluro		-57.453	8.523
B39* (*4,6-Dichloro-N-(9-methyl-9-azabicyclo[3.3.1]nonan-3-yl)-1H-indole-2-carboxamide*)	10.9	4,6-dichloro		-75.972/-69.899	4.963
B40 (*N-[(4, 6-Dimethyl-1H-indol-2-yl) methyl] - Cycloheptanecar-boxamide*)	54	4,6-dimethyl		-54.502	4.268
B41 (*N-[(4,6-Dimethyl-1H-indol-2-yl)methyl]cyclooctylamine*)	0.31	4,6-dimethyl		-73.543	6.509
B42 (*N-[6-(Trifluoromethoxy)benzothiazol-2-yl] - Cycloheptanecarboxamide*)	>300	5-triflurometh oxy		-50.598	_

Note: * mixture of isomeric forms; **pMIC= -log(10^{-6} X MIC);

Table S2. Calculation of Standard Deviation and Standard Error of MMGBSA dG bind of B38.

Sl No.	Compound Name	MD Time scale	Ensemble Average dG bind (kcal/mol) from MD snapshots	No. of snapshots	dG bind (kcal/mol) from minimized (docked) complex	Standard Deviation (kcal/mol)	Standard Error (kcal/mol)
1	B38	25 ns	-57.457	24	-57.453	0.239	0.107
2		25 ns	-57.138	16			
3		25 ns	-57.694	30			
4		25 ns	-57.108	40			
5		25 ns	-57.556	40			

^{1}H NMR

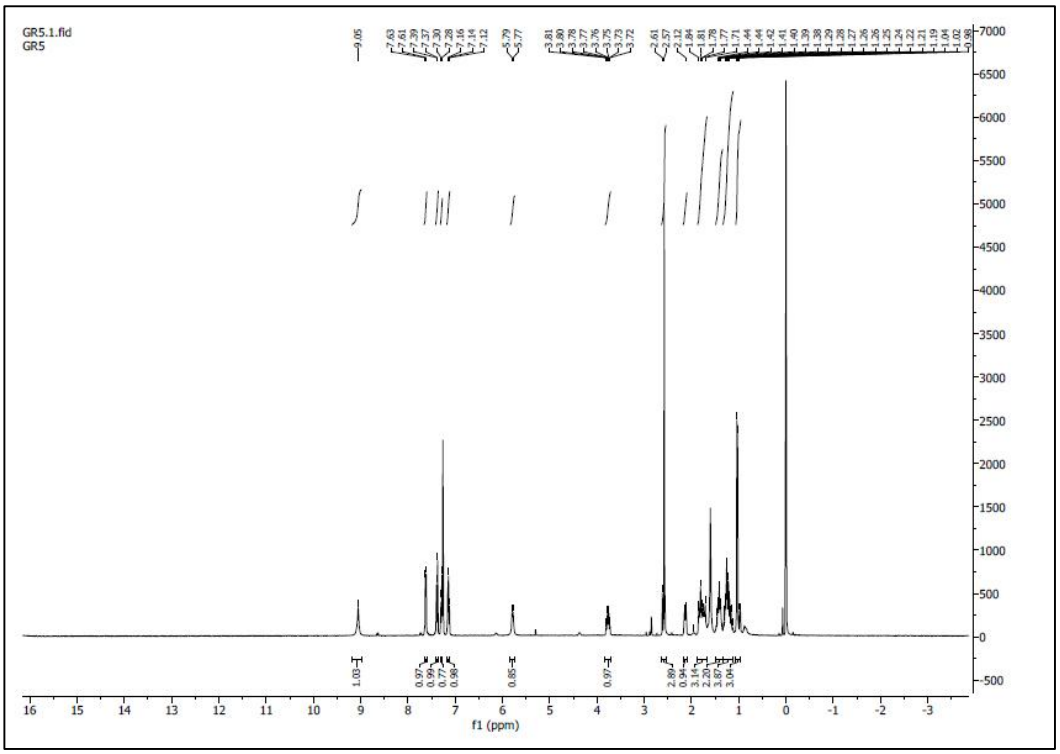


Figure S5: ^{1}H NMR of 3a in $CDCl_3$. Splitting of peaks at 2.61 to 2.57 ppm and at 1.24 to 1.19 ppm is due to isomeric mixture of 3a.

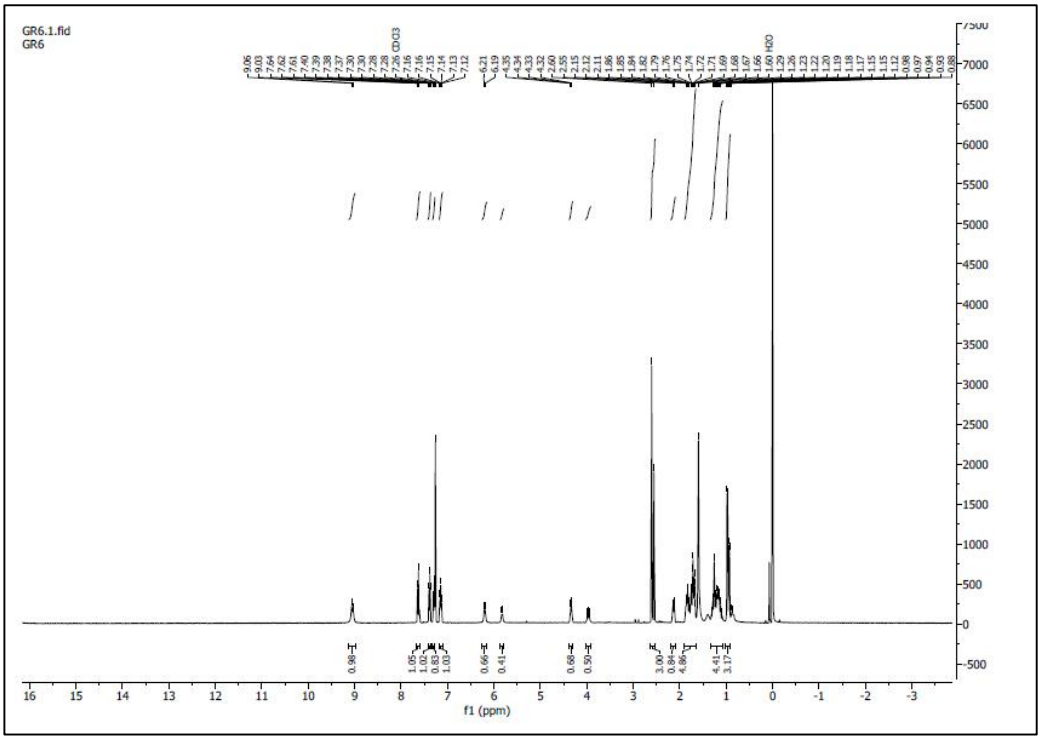


Figure S6: ^{1}H NMR of 3b in $CDCl_3$. Splitting of proton peaks into two at 6.21 to 6.19 ppm, 4.35 to 4.32 ppm, 2.60 to 2.55 ppm and 1.17 to 1.12 ppm is due to presence of isomeric mixture of 3b.

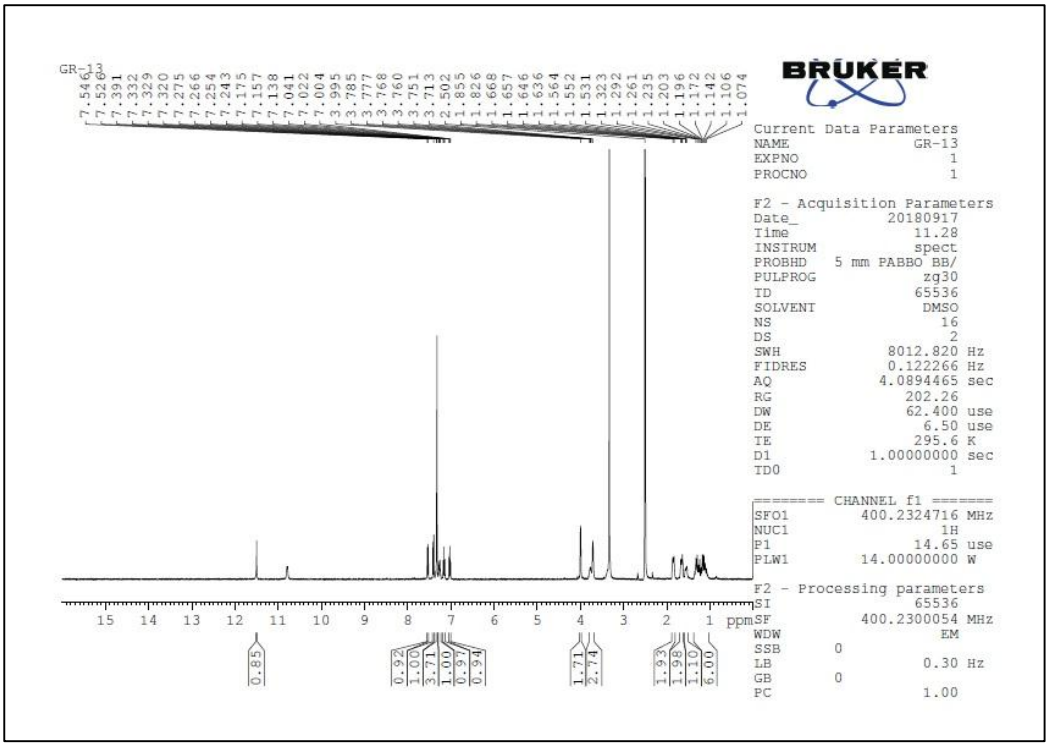


Figure S7: ^{1}H NMR of 5a in DMSO.

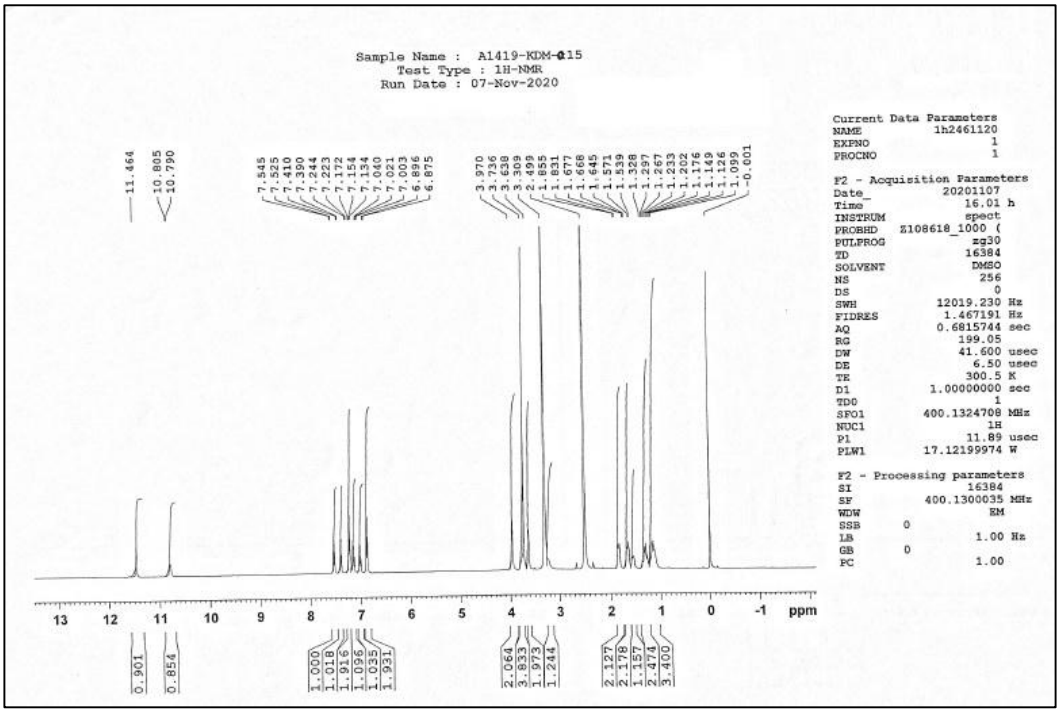


Figure S8: ^{1}H NMR of 5b in DMSO.

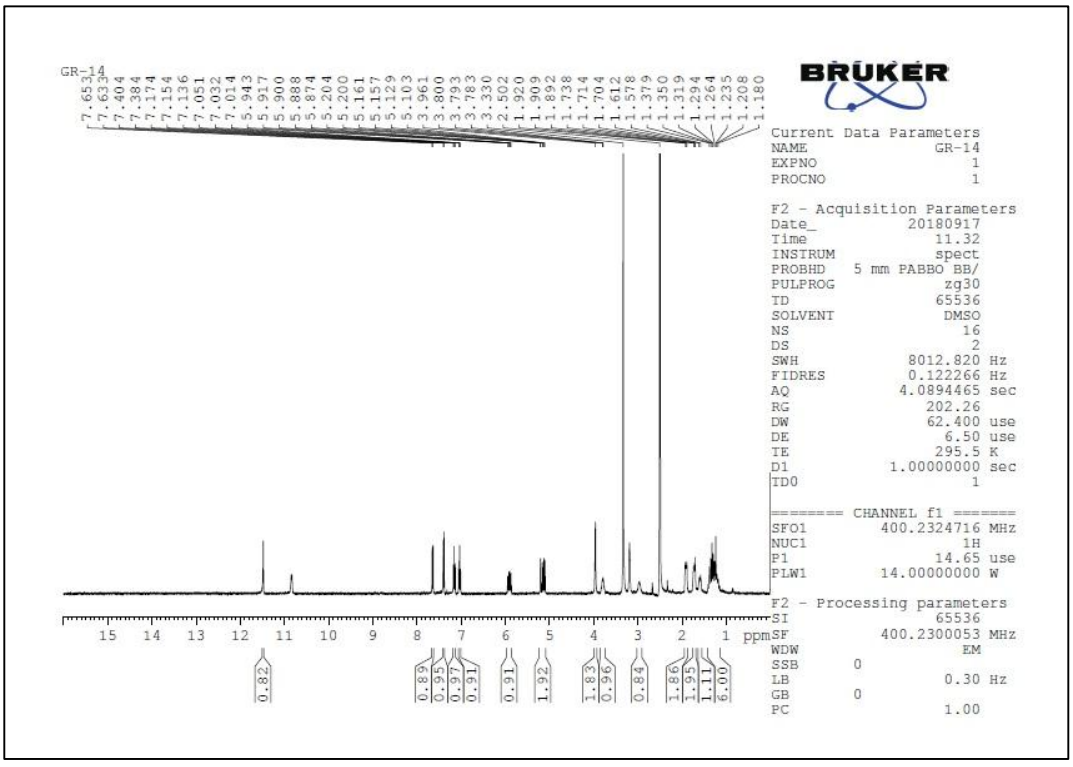


Figure S9: ^{1}H NMR of 5c in DMSO.

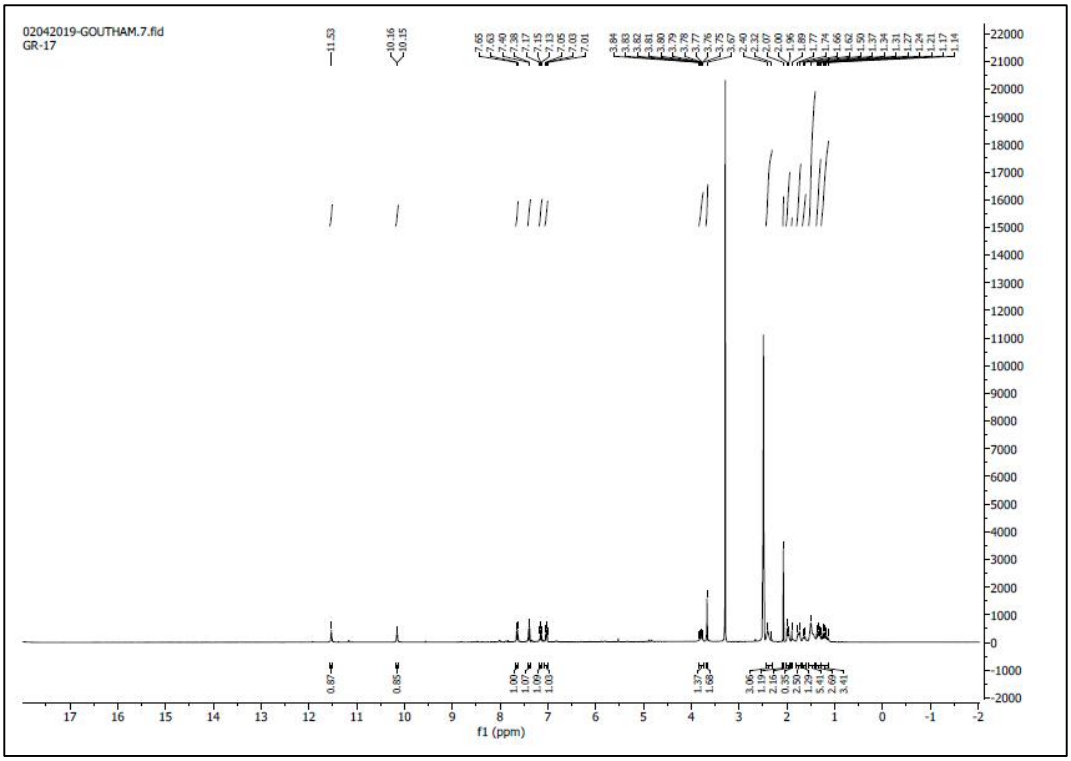


Figure S10: ^{1}H NMR of 5d in DMSO.

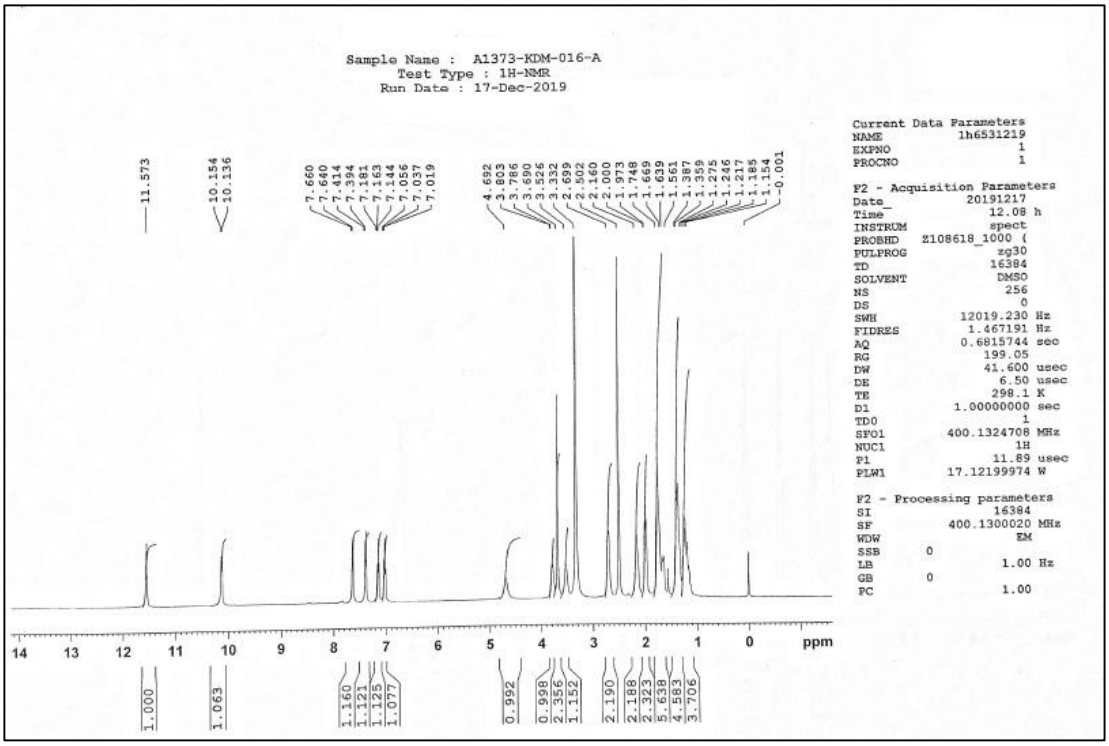


Figure S11: ^{1}H NMR of 5e in DMSO.

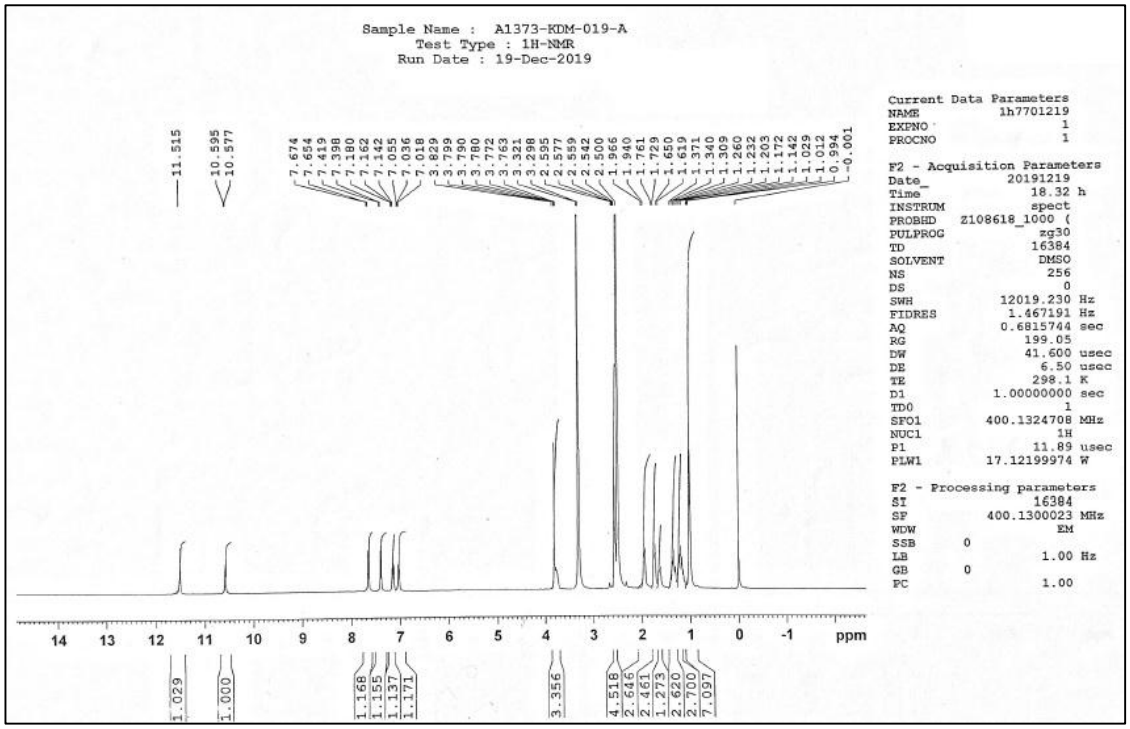


Figure S12: ^{1}H NMR of 5f in DMSO.

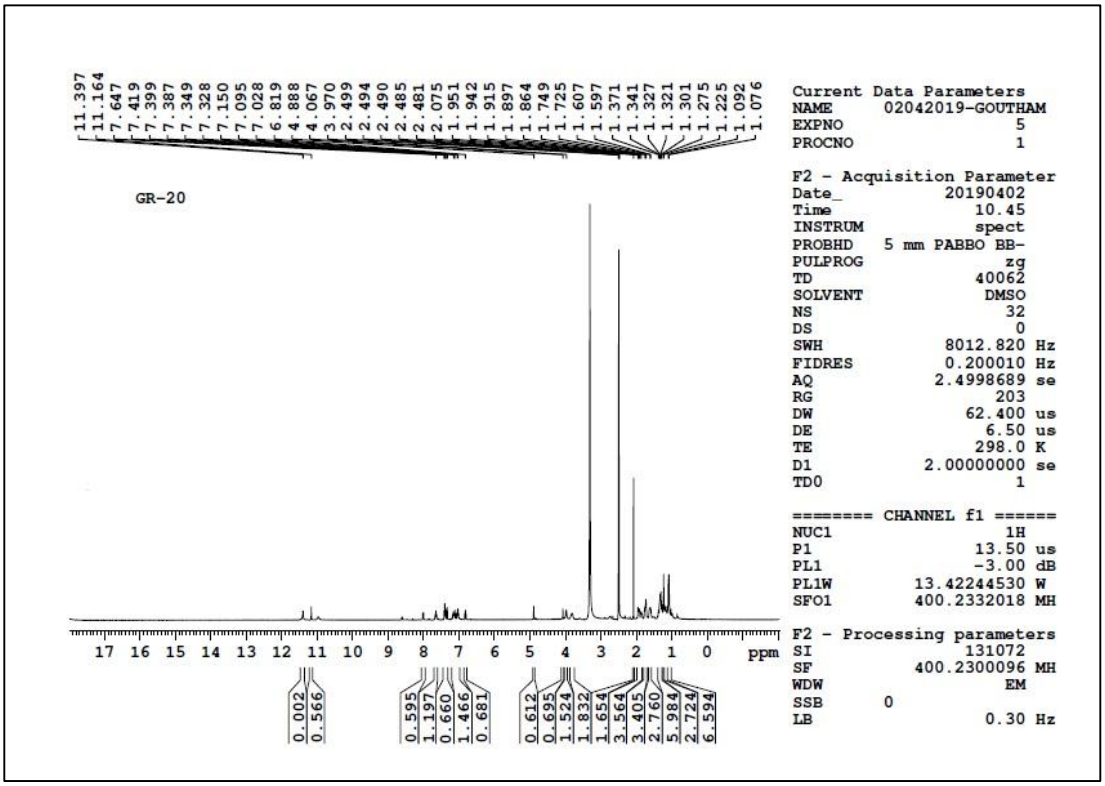


Figure S13: ^{1}H NMR of 5g in DMSO.

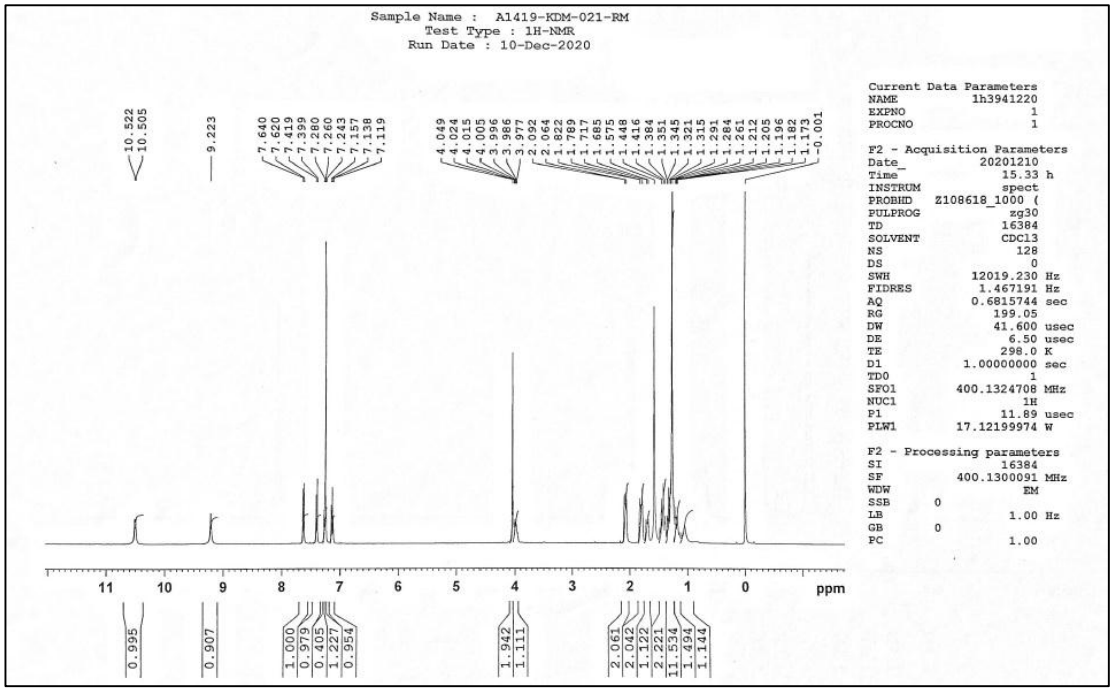


Figure S14: ^{1}H NMR of 5h in $CDCl_3$.

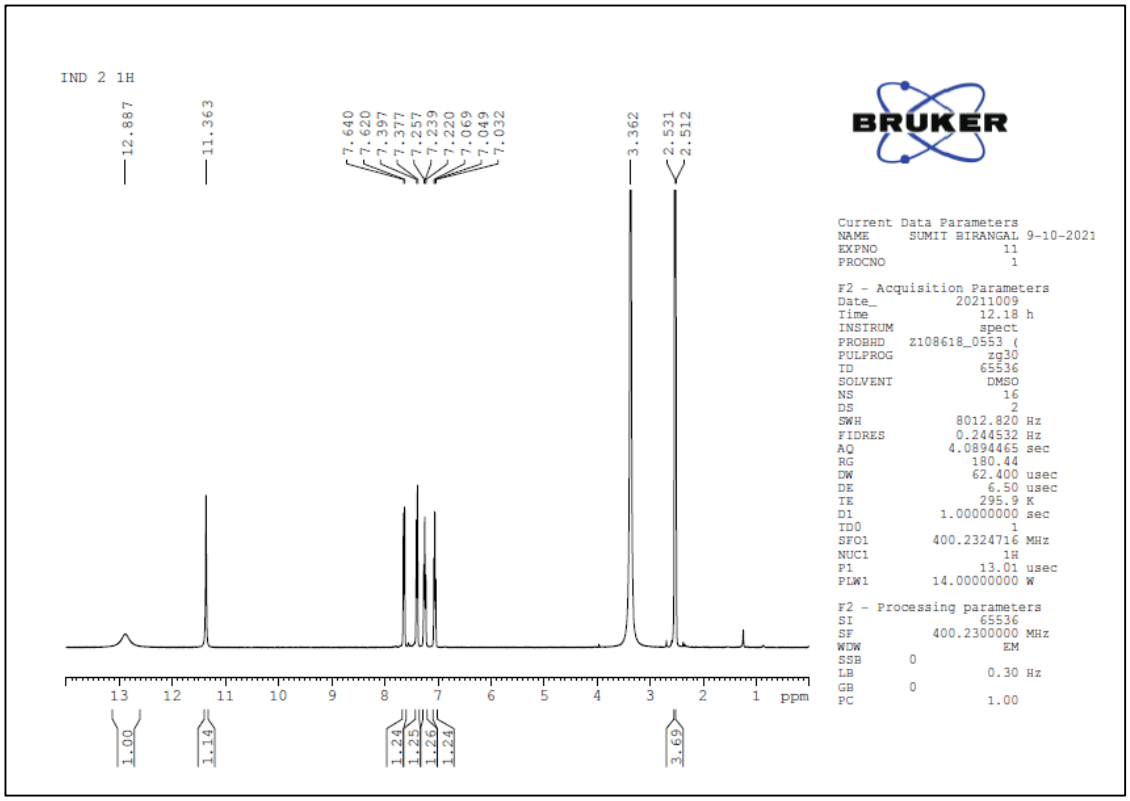


Figure S15: ^{1}H NMR of intermediate 2 in DMSO. 1H NMR (400 Mhz, DMSO-d) δ 12.887 (s, 1H), 11.36 (s, 1H), 7.63 (d, J = 8 Hz, 1H), 7.39 (d, J = 8, 1H), 7.24 (t, J = 7.4, 1H), 7.049 (t, J = 7.4, 1H), 2.53 (s, 3H).

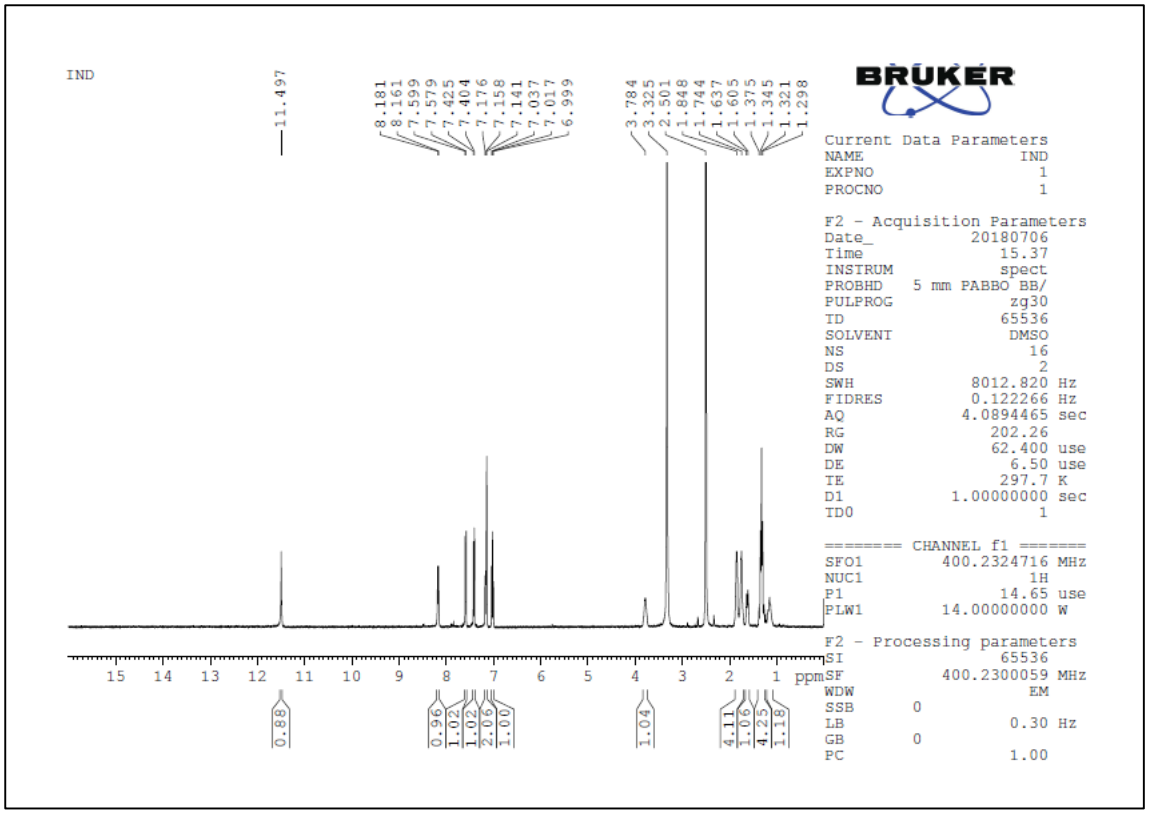


Figure S16: ^{1}H NMR of intermediate 4 in DMSO. 1H NMR (400 Mhz, DMSO-d) δ 11.50 (s, 1H), 8.17 (d, J = 8, 1H), 7.59 (d, J = 8 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.16 (t, J = 7.4 Hz, 1H), 7.02 (t, J = 7.6, 1H), 3.78 (m, 1H), 1.85 – 1.74 (m, 4H), 1.62 (d, J= 12.8, 2H), 1.38 – 1.27 (m, 4H), 1.18 – 1.15 (m, 1H)

^{13}C NMR

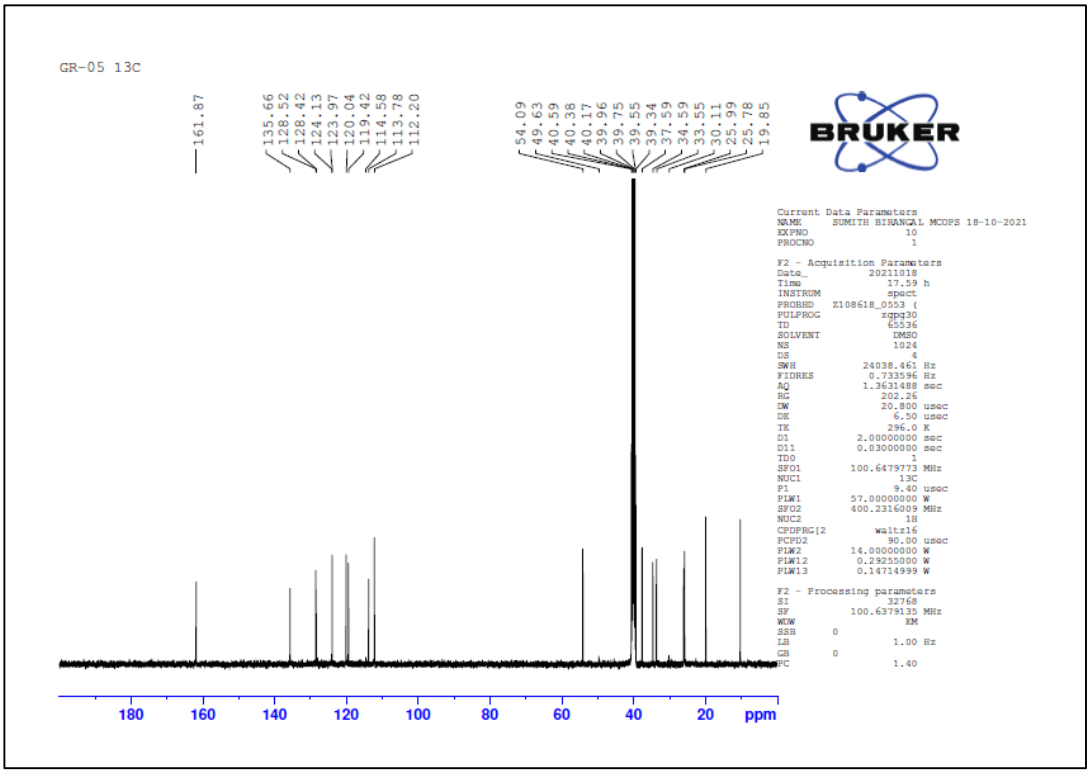


Figure S17: ^{13}C NMR of 3a in DMSO. Splitting of peaks caused due to the presence of trans and cis-isomeric forms of 3a.

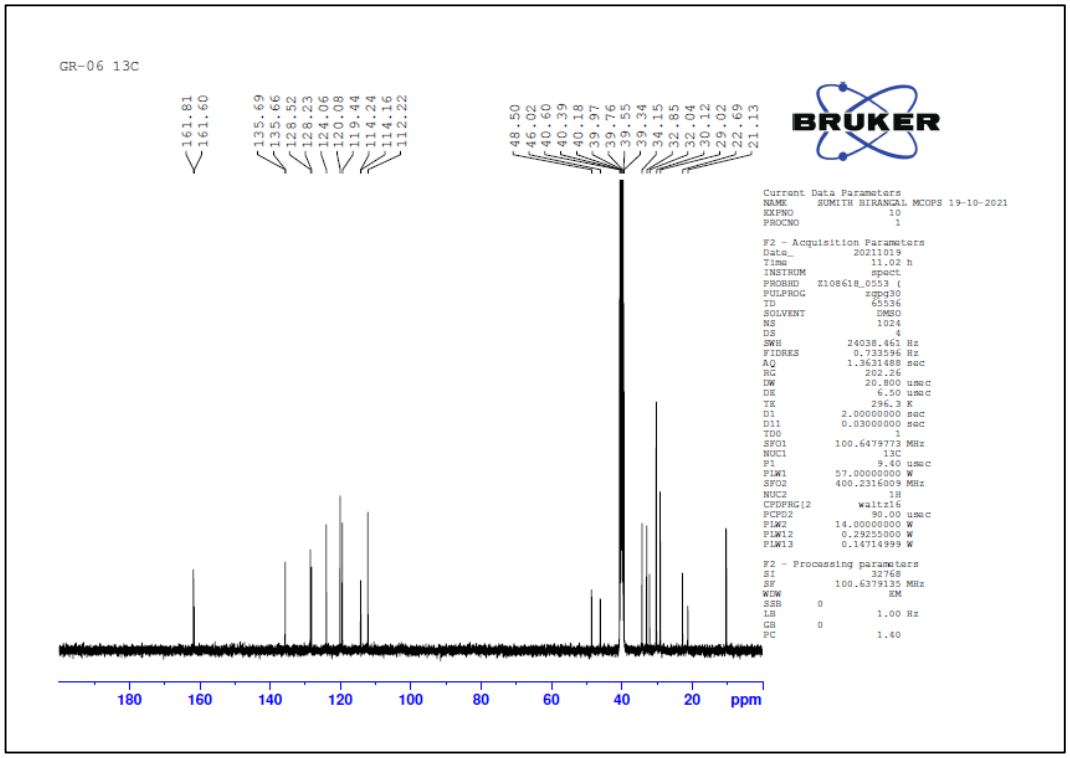


Figure S18: ^{13}C NMR of 3b in DMSO. Splitting of peaks caused due to the presence of trans and cis-isomeric forms of 3b.

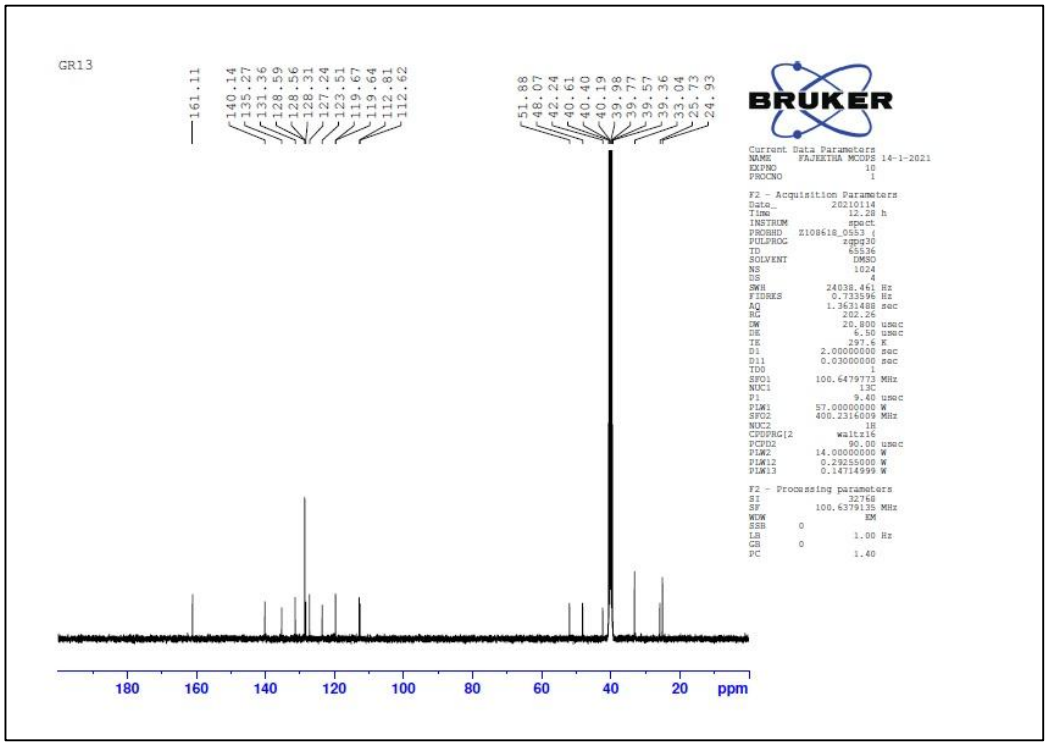


Figure S19: ^{13}C NMR of 5a in DMSO.

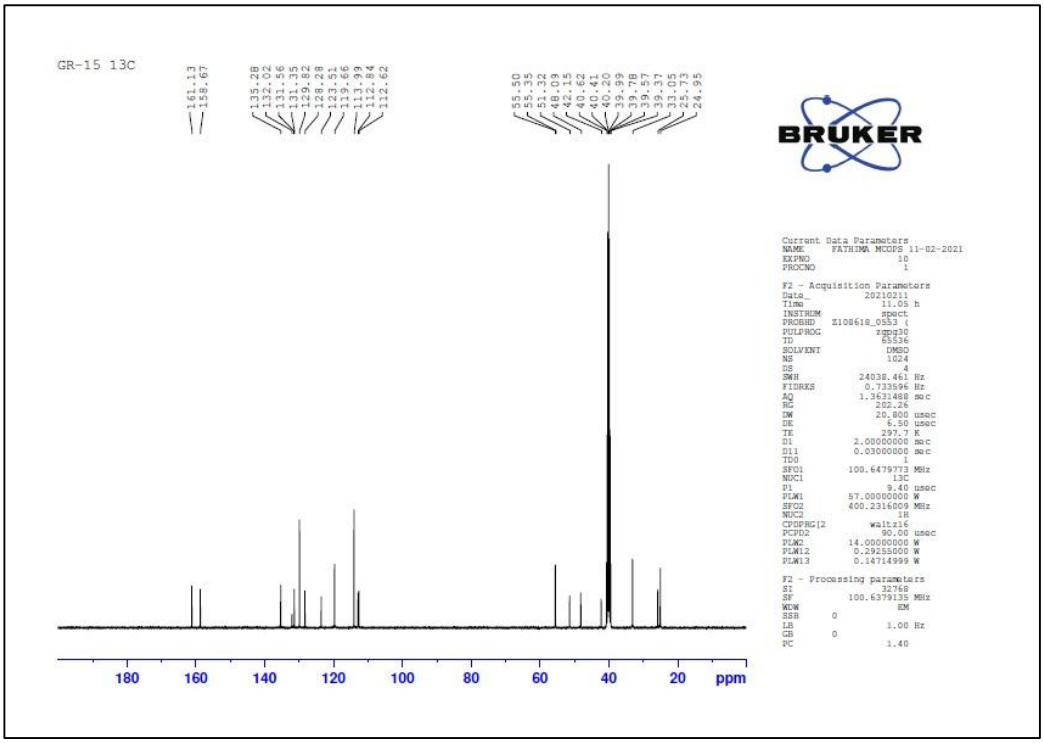


Figure S20: ^{13}C NMR of 5b in DMSO.

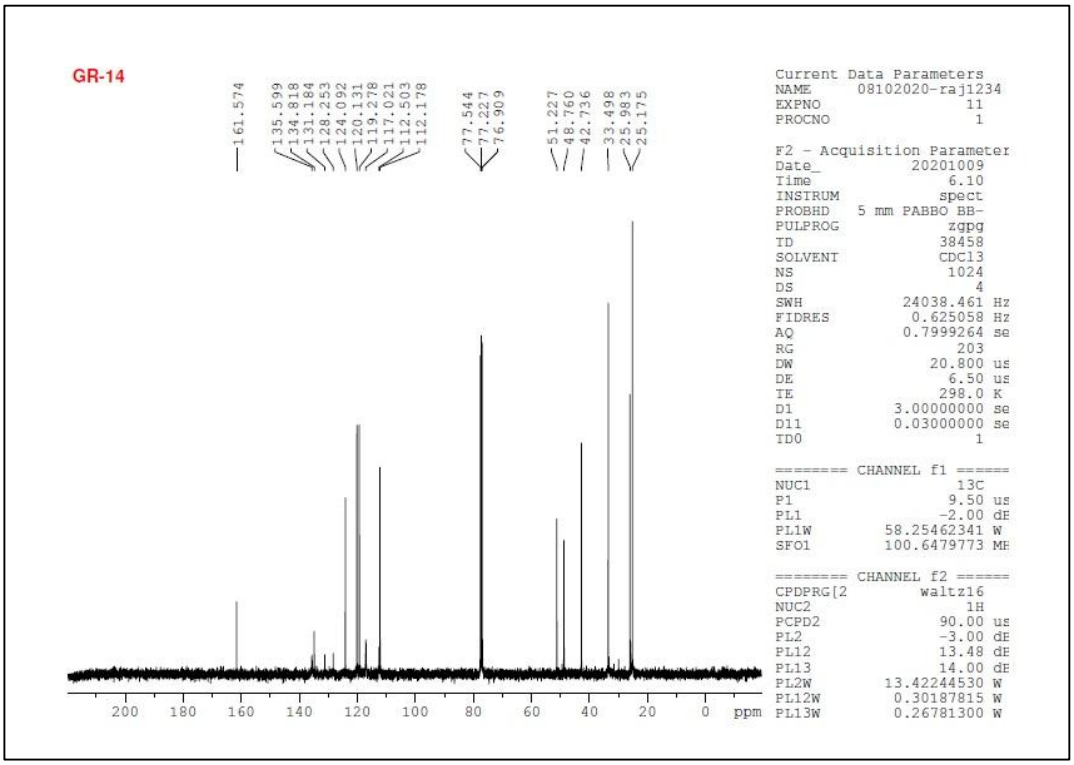


Figure S21: ^{13}C NMR of 5c in $CDCl_3$.

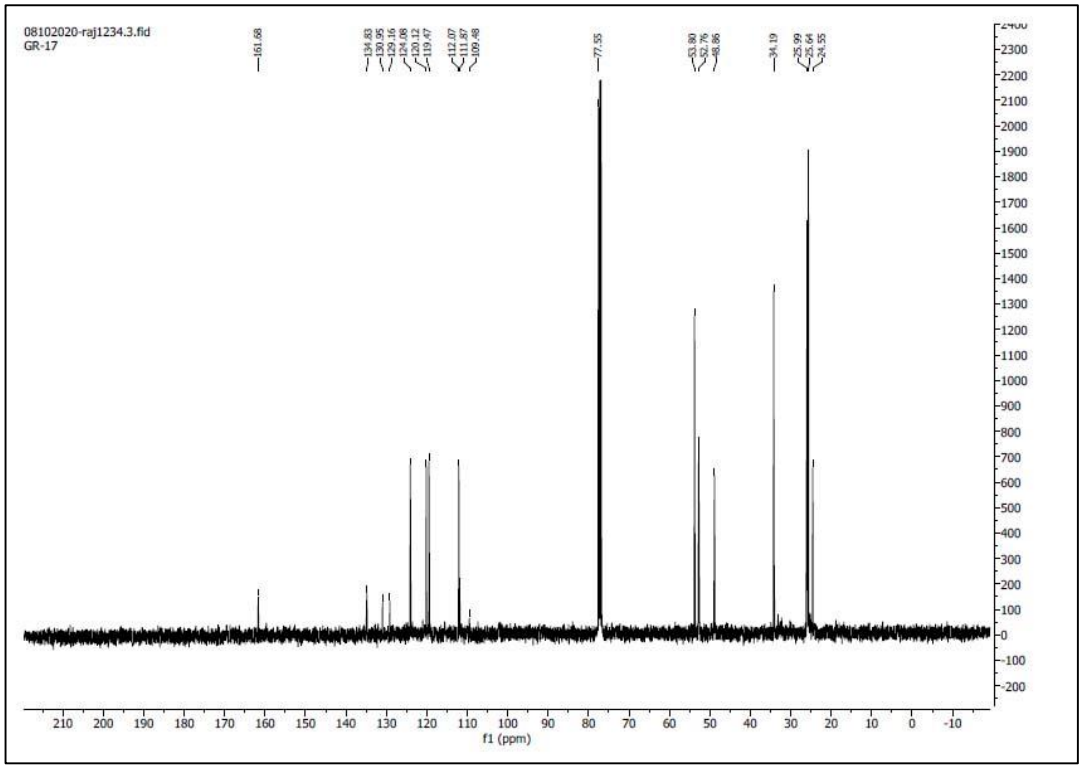


Figure S22: ^{13}C NMR of 5d in $CDCl_3$.

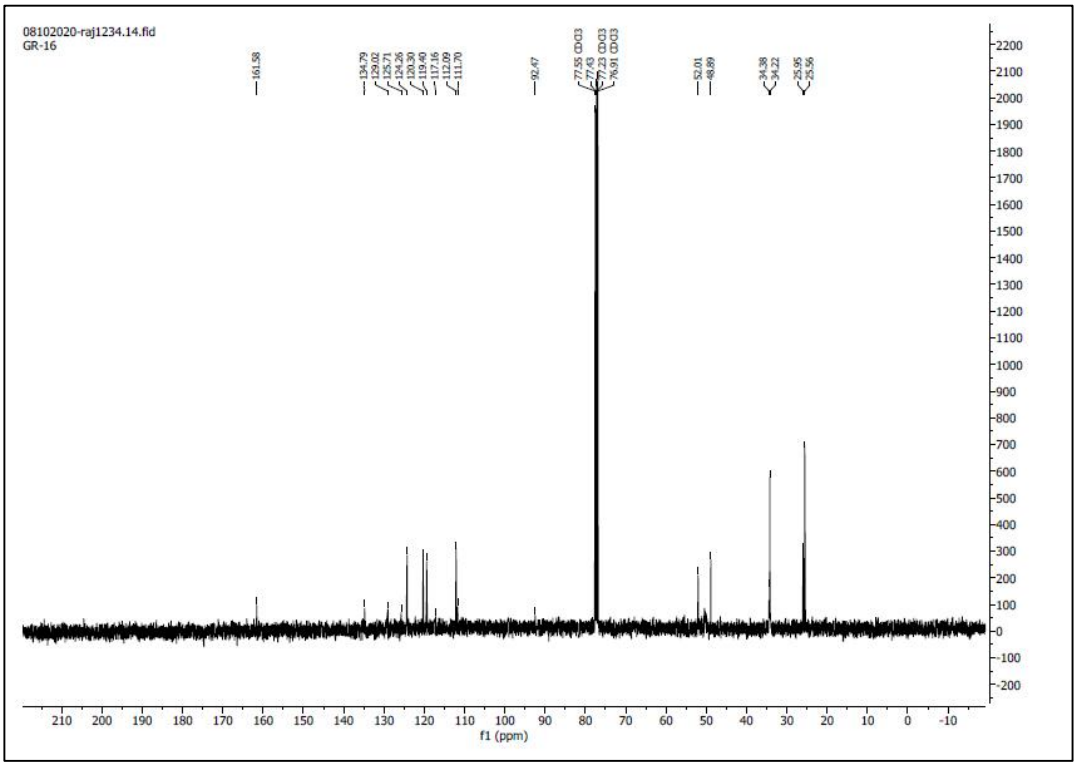


Figure S23: ^{13}C NMR of 5e in $CDCl_3$.

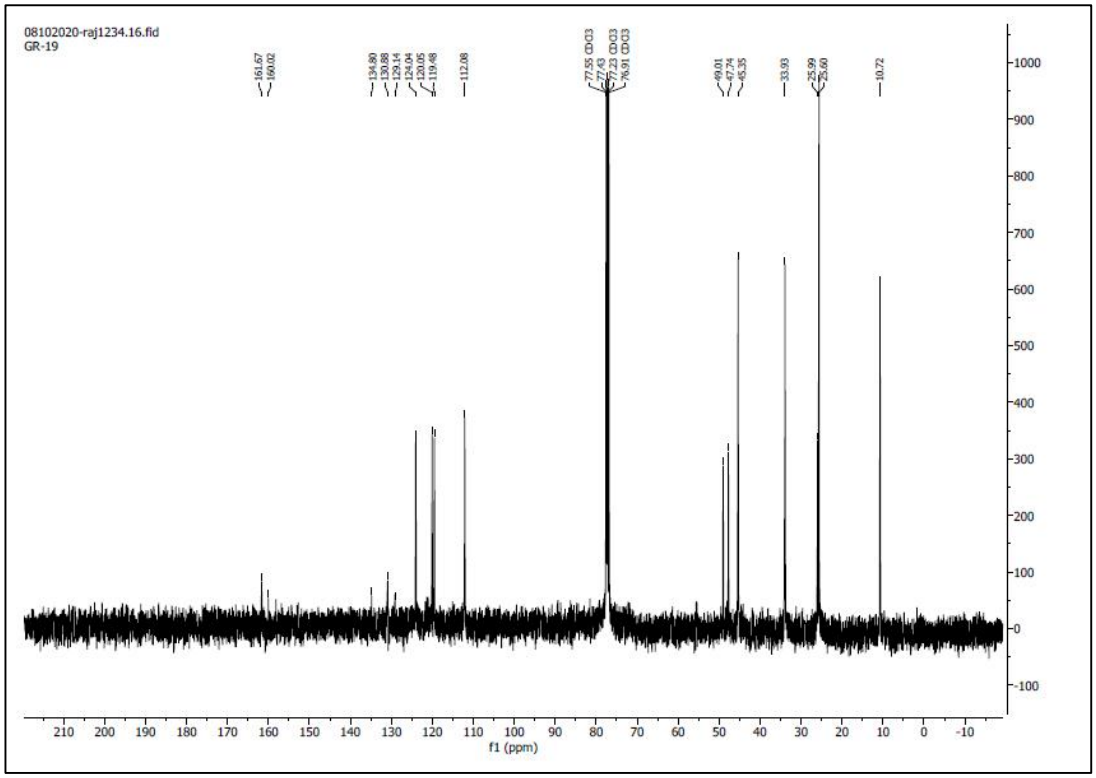


Figure S24: ^{13}C NMR of 5f in $CDCl_3$.

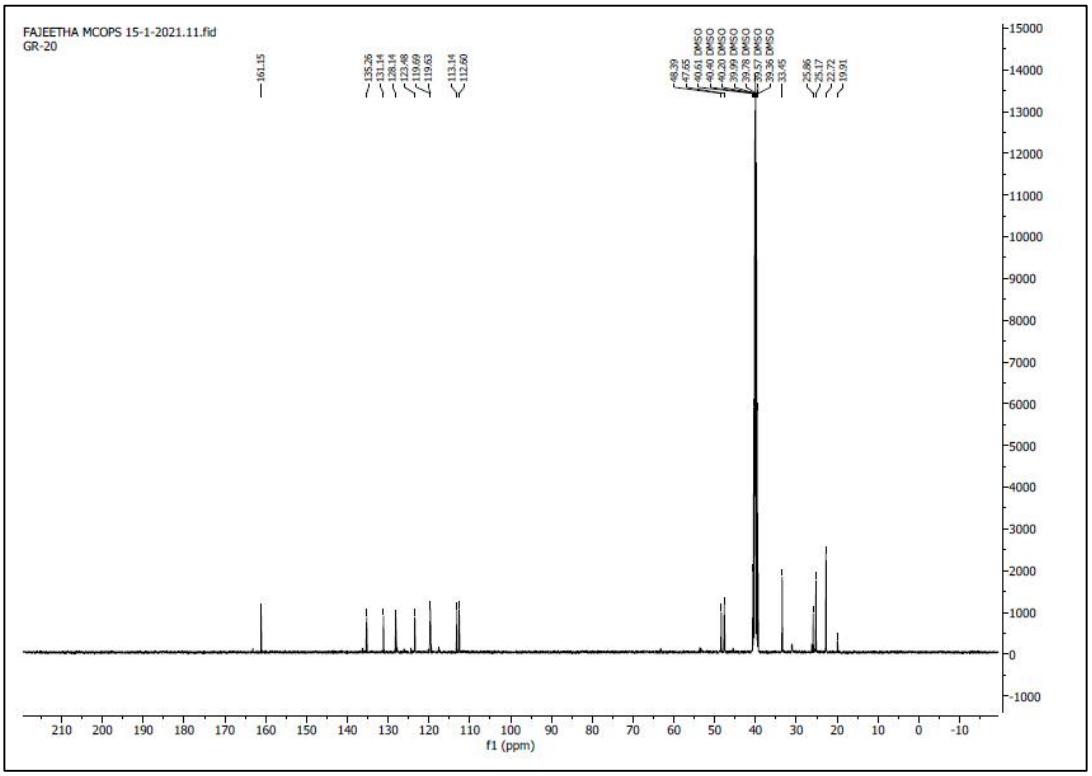


Figure S25: ^{13}C NMR of 5g in $CDCl_3$.

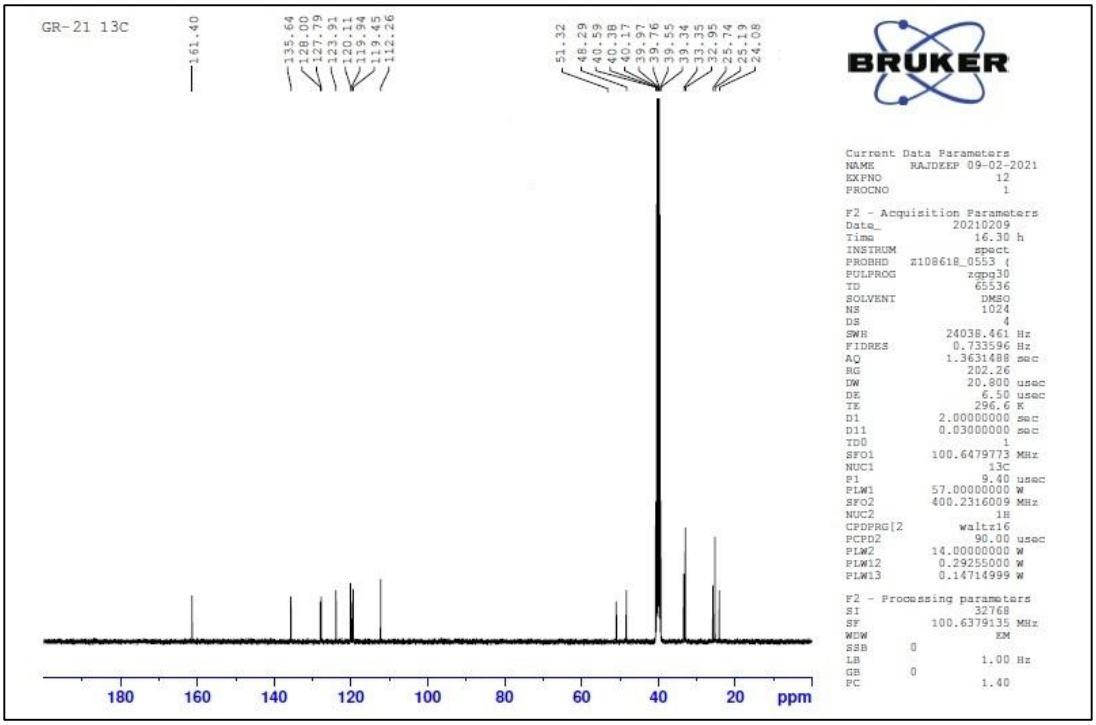


Figure S26: ^{13}C NMR of 5h in DMSO.

Mass Spectra

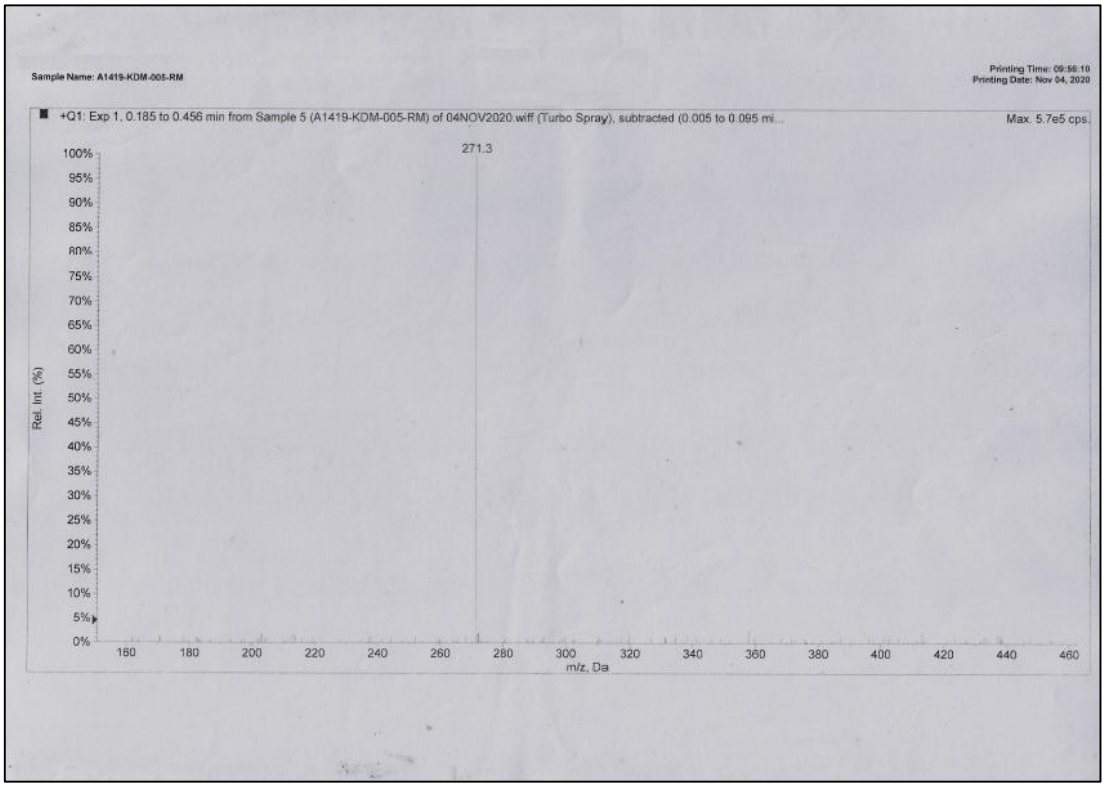


Figure S27: M.S. of 3a

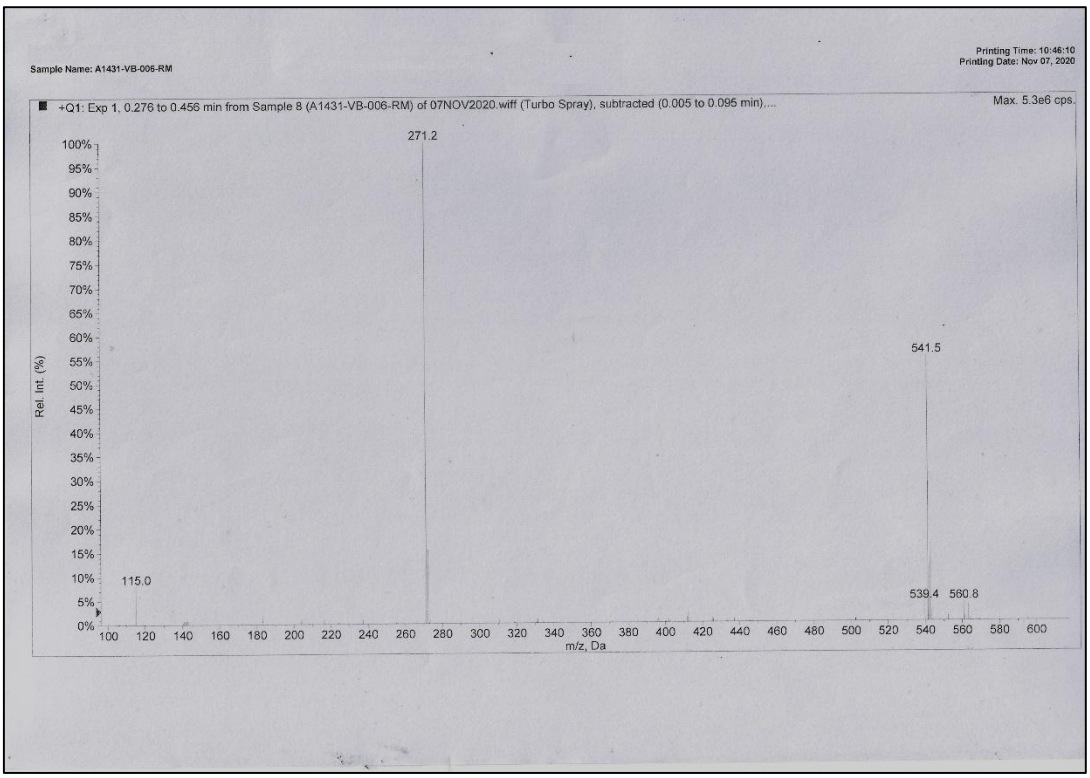


Figure S28: M.S. of 3b

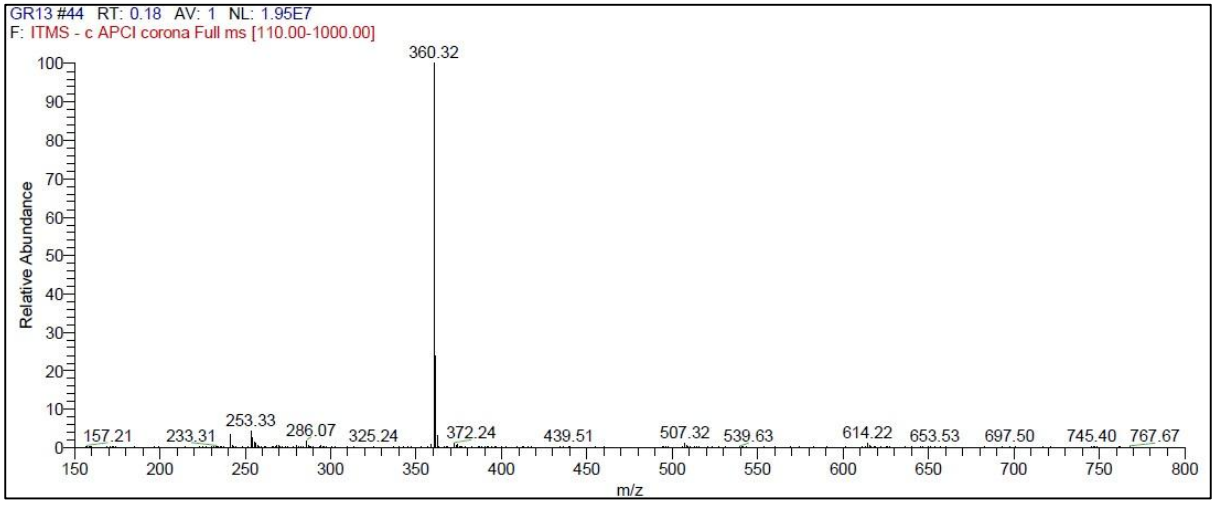


Figure S29: M.S. of 5a

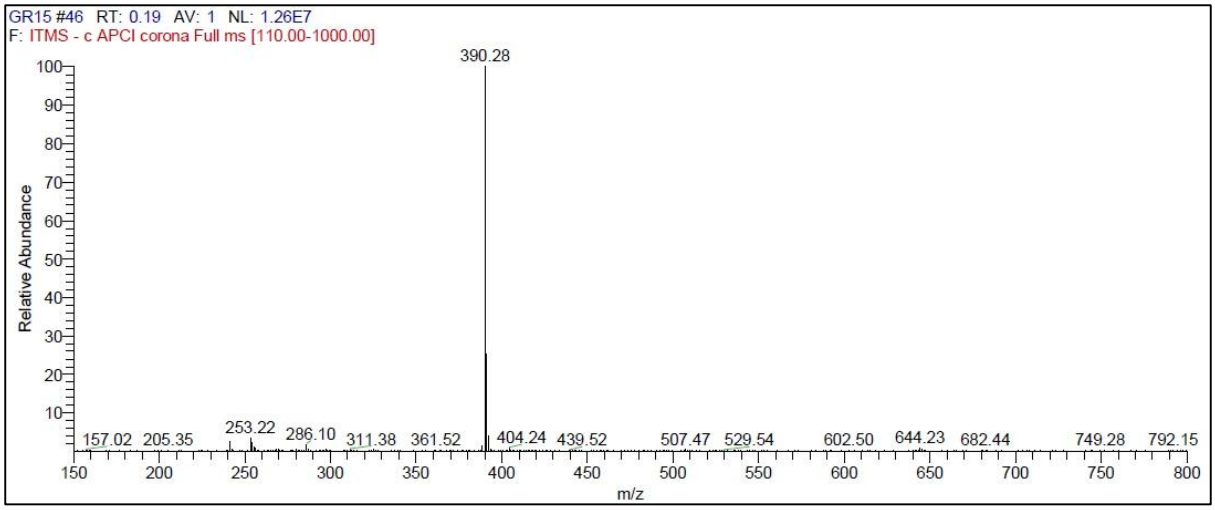


Figure S30: M.S. of 5b

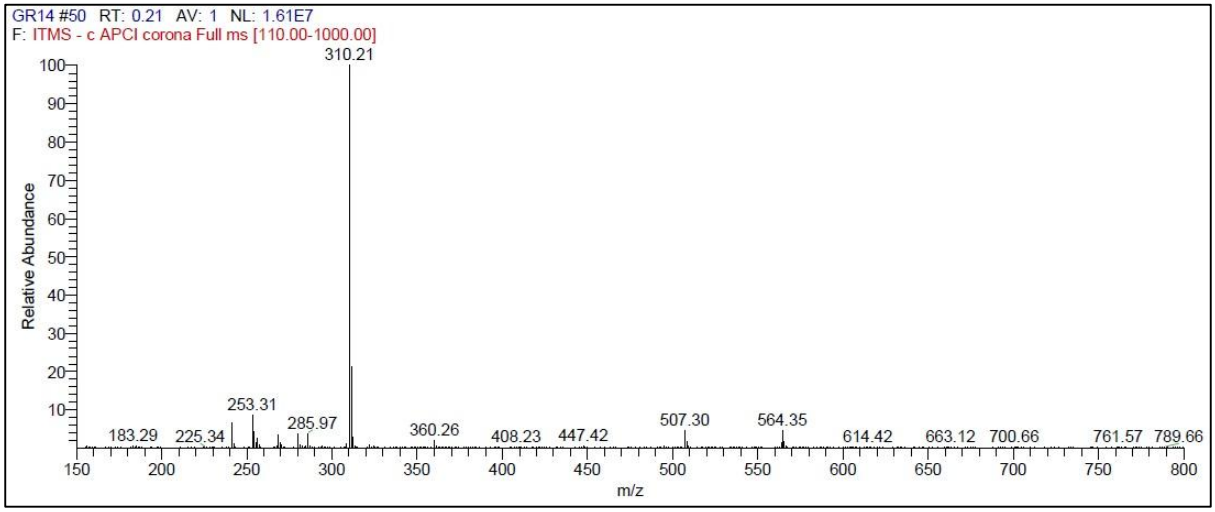


Figure S31: M.S. of 5c

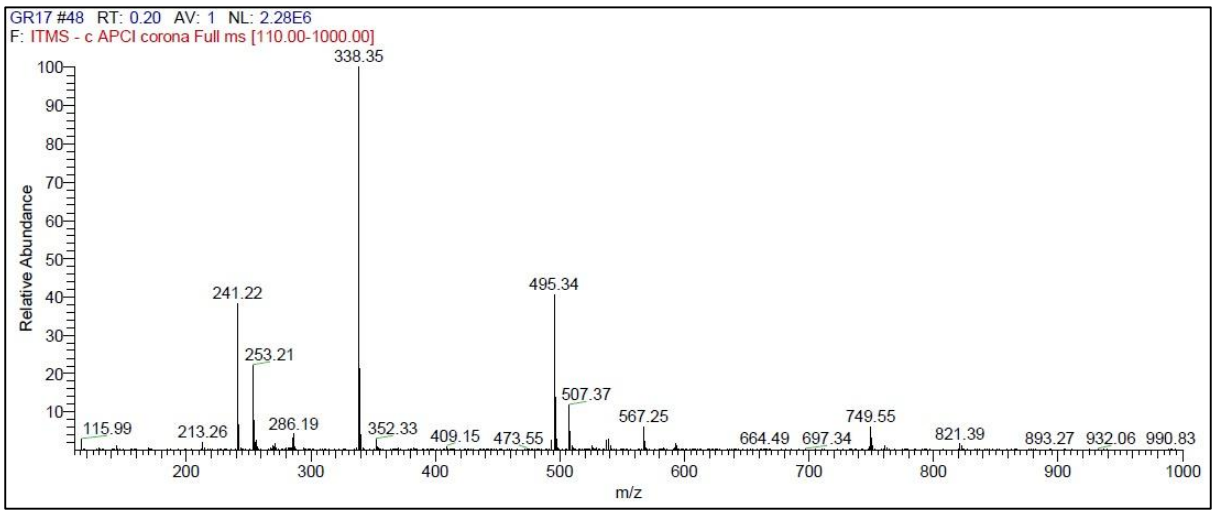


Figure S32: M.S. of 5d

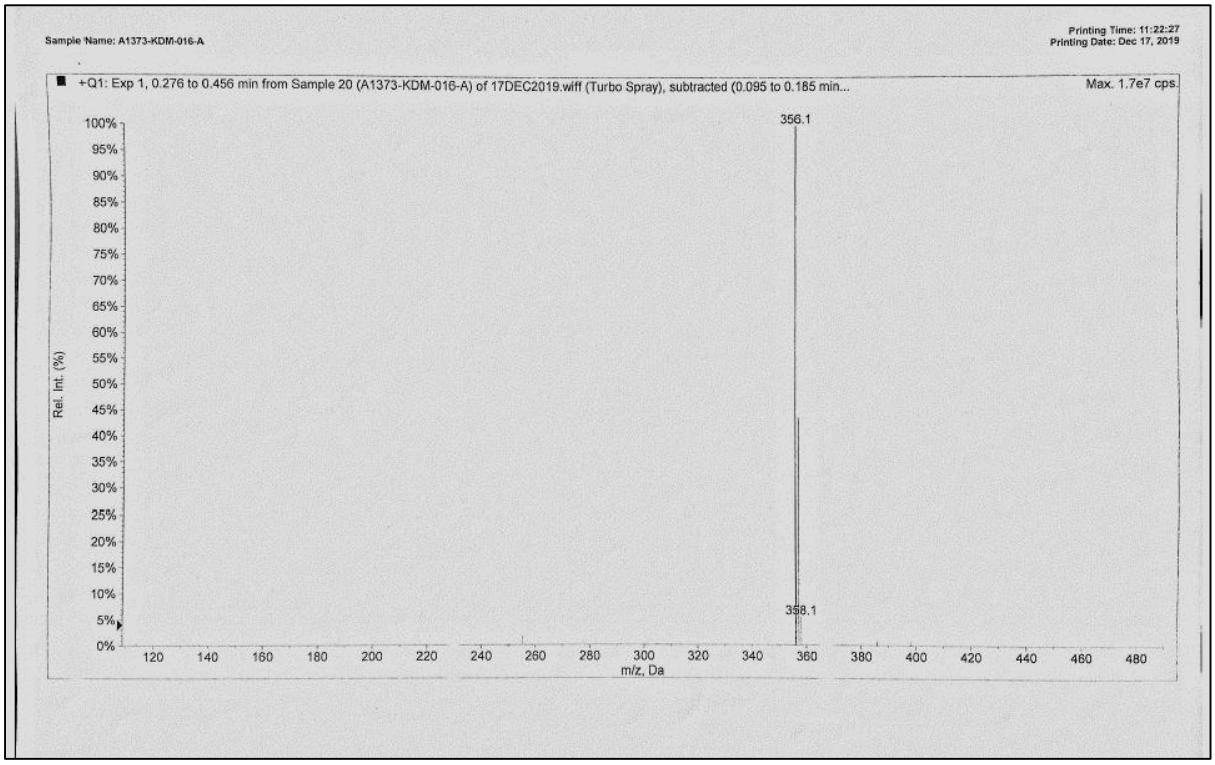


Figure S33: M.S. of 5e

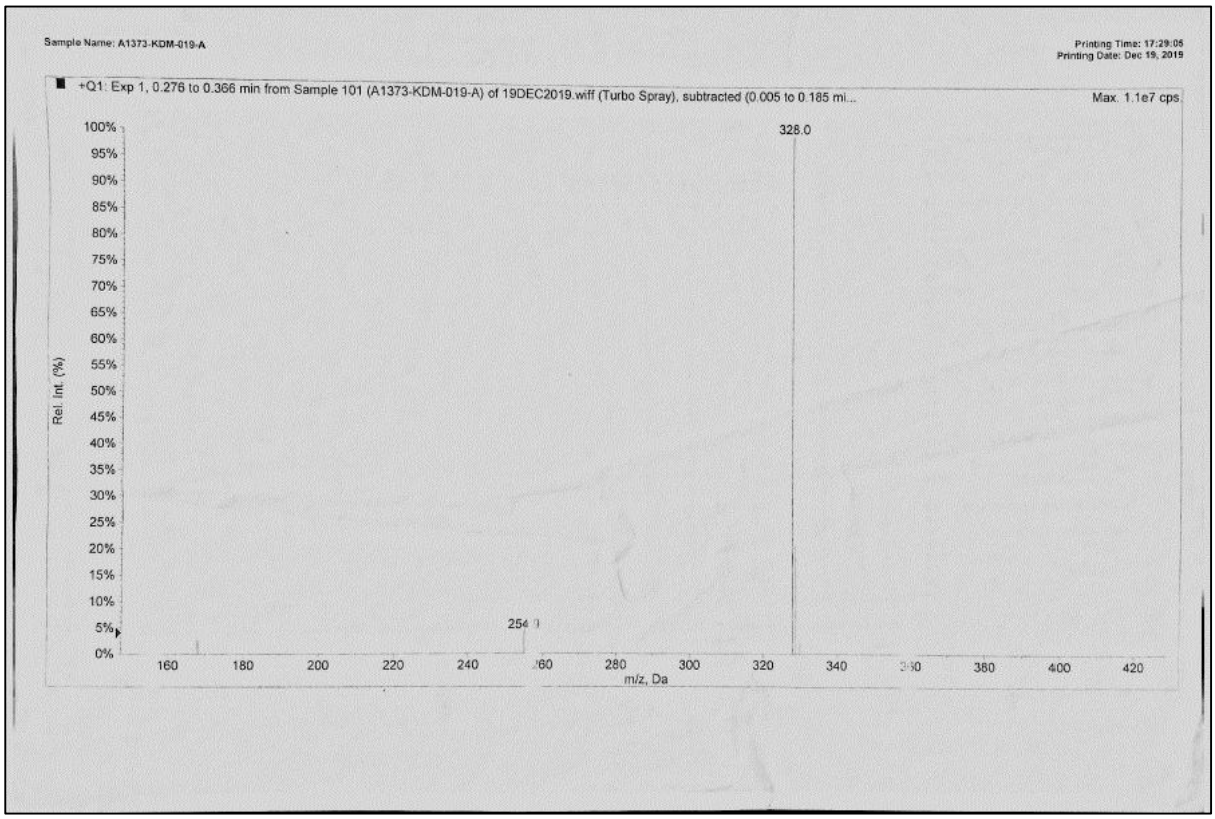


Figure S34: M.S. of 5f

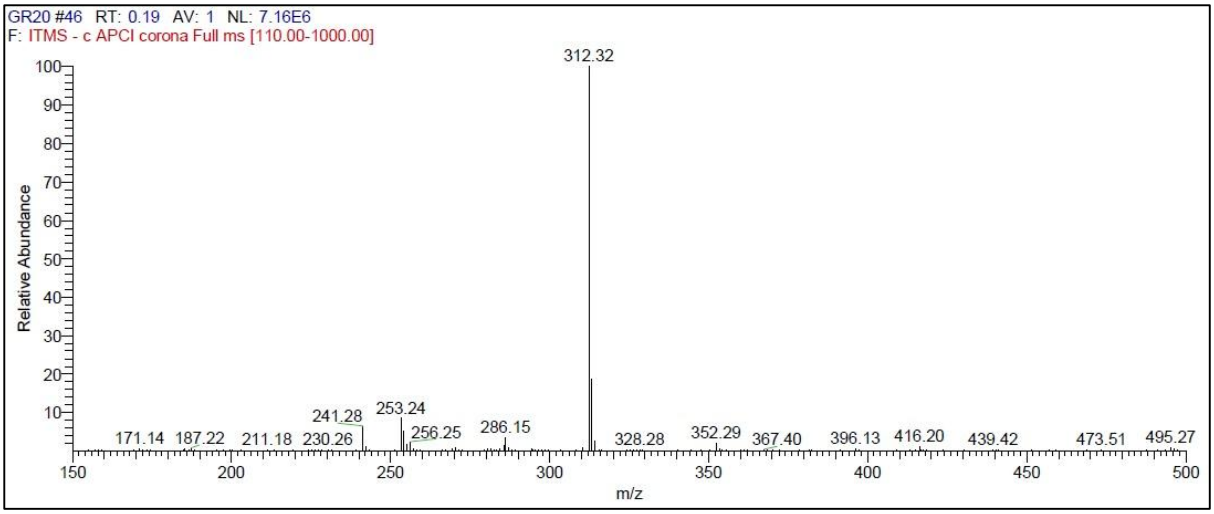


Figure S35: M.S. of 5g

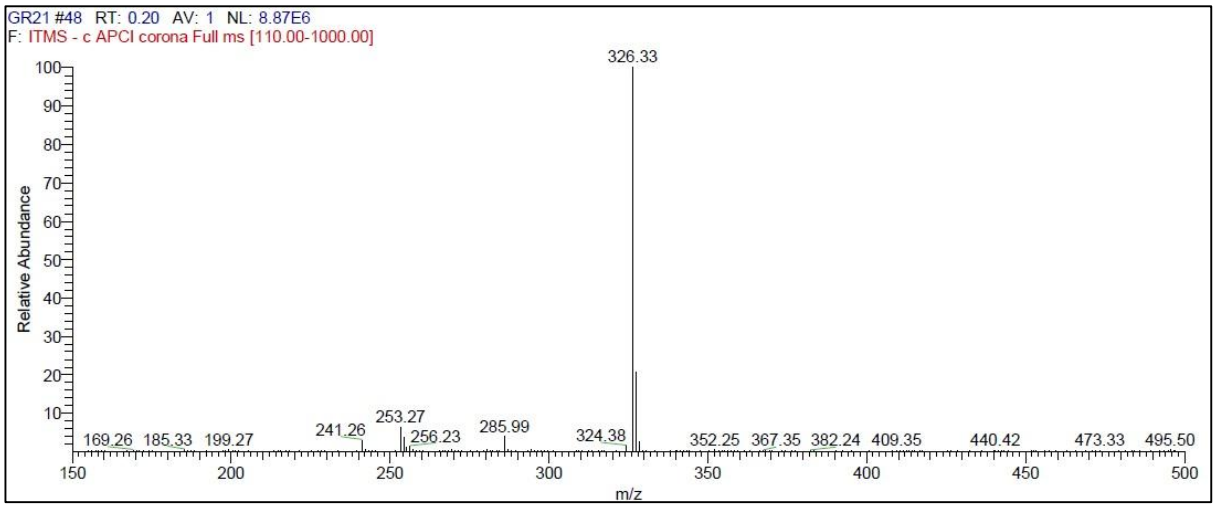


Figure S36: M.S. of 5h