Design, synthesis, biological evaluation, and *in silico* studies of novel *N*-substituted-2-(3,4,5-trimethoxyphenyl)-1*H*-benzo[*d*]imidazole-6-carboxamides as promising anticancer agents

Supplementary data

Figure S31. The ^{1}H-NMR spectrum of **5o**.

Figure S32. The ^{13}C-NMR spectrum of **5o**.

Figure S33. Cytotoxicity of **5a-o**, doxorubicin, cisplatin, and etoposide against A549 cells. Cell viability was assessed by MTT assay. Cells were treated with increasing concentrations of investigated compounds for 72 h. Results are given as mean ± SD (n = 3-4) and IC_{50} values are indicated.

Figure S34. Cytotoxicity of **5a-o**, doxorubicin, cisplatin, and etoposide against SW480 cells. Cell viability was assessed by MTT assay. Cells were treated with increasing concentrations of investigated compounds for 72 h. Results are given as mean ± SD (n = 3-4) and IC_{50} values are indicated.

Figure S35. Cytotoxicity of **5e**, **5f**, **5m**, **5o**, doxorubicin, and cisplatin against MRC-5 cells. Cell viability was assessed by MTT assay. Cells were treated with increasing concentrations of investigated compounds for 72 h. Results are given as mean ± SD (n = 3-4) and IC_{50} values are indicated.

Figure S36. Representation of self-docking results. The co-crystallized inhibitors (cyan) docked into the binding sites and superimposed on co-crystallized inhibitors (yellow) in the crystal structures of topoisomerases: a) Human Topo I- DNA (PDB ID: 1T8I), b) Human Topo IIα ATPase-no DNA (PDB ID: 1ZXM), c) Human Topo IIα ATPase-no DNA (PDB ID: 1ZXN), d) Human Topo IIα- DNA (PDB ID: 5GWK), and e) Human Topo IIβ-DNA (PDB ID: 4G0V).

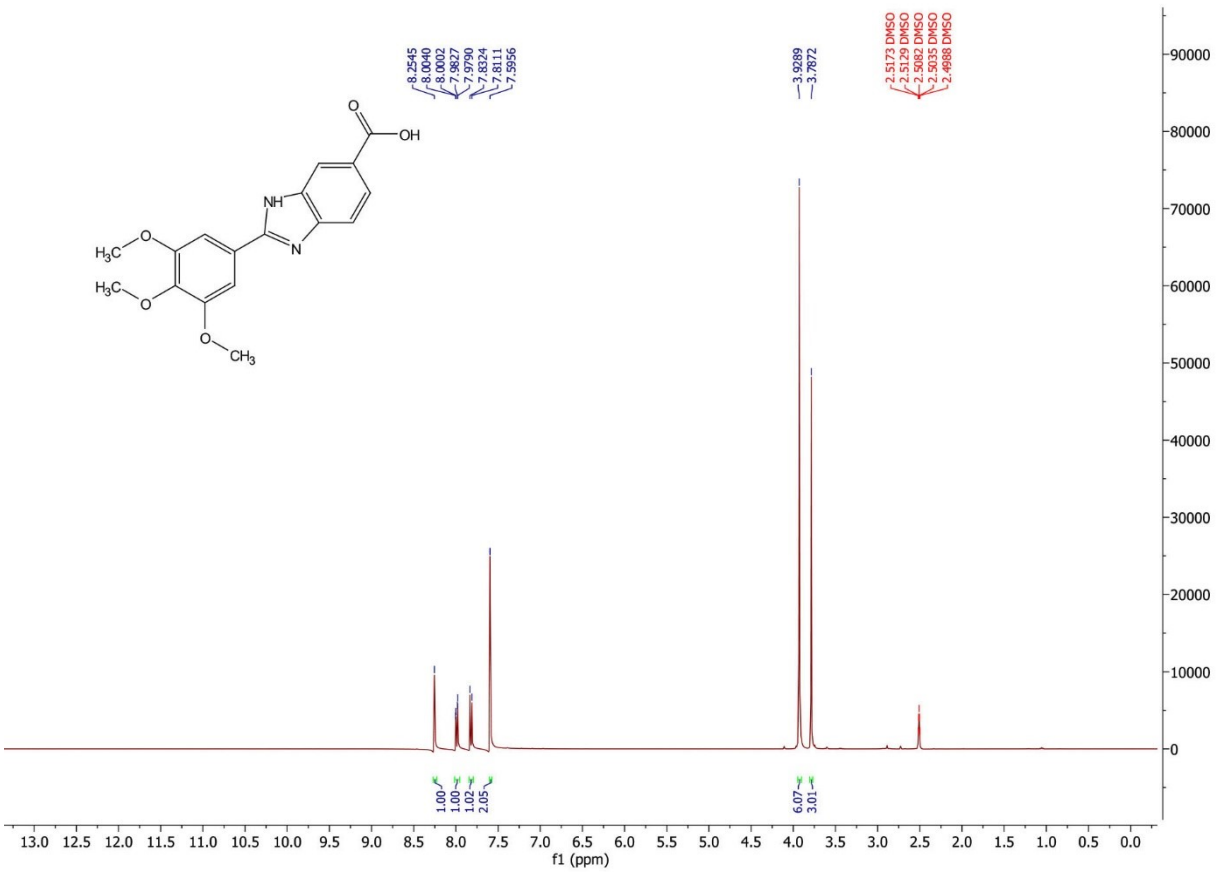


Figure S1. The ^{1}H-NMR spectrum of **3**.

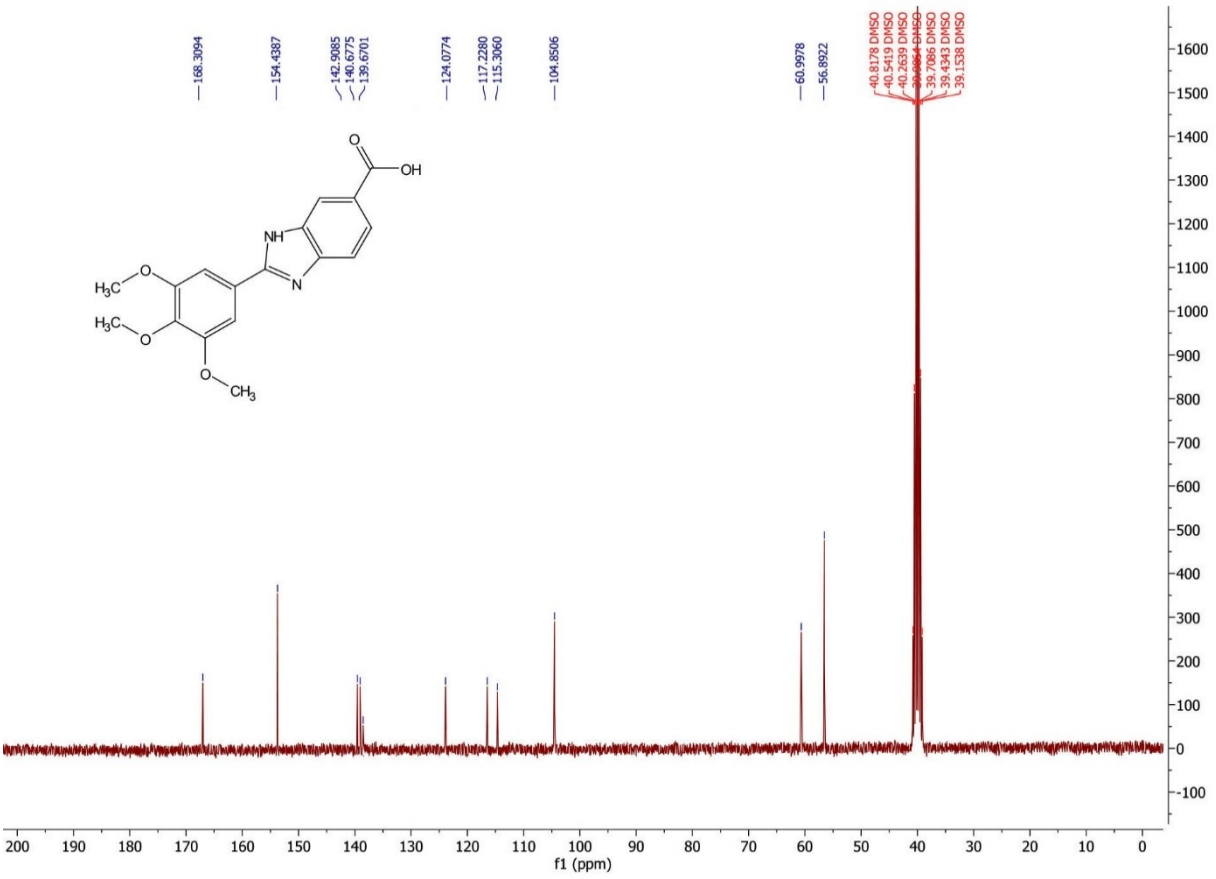


Figure S2. The ^{13}C-NMR spectrum of **3**.

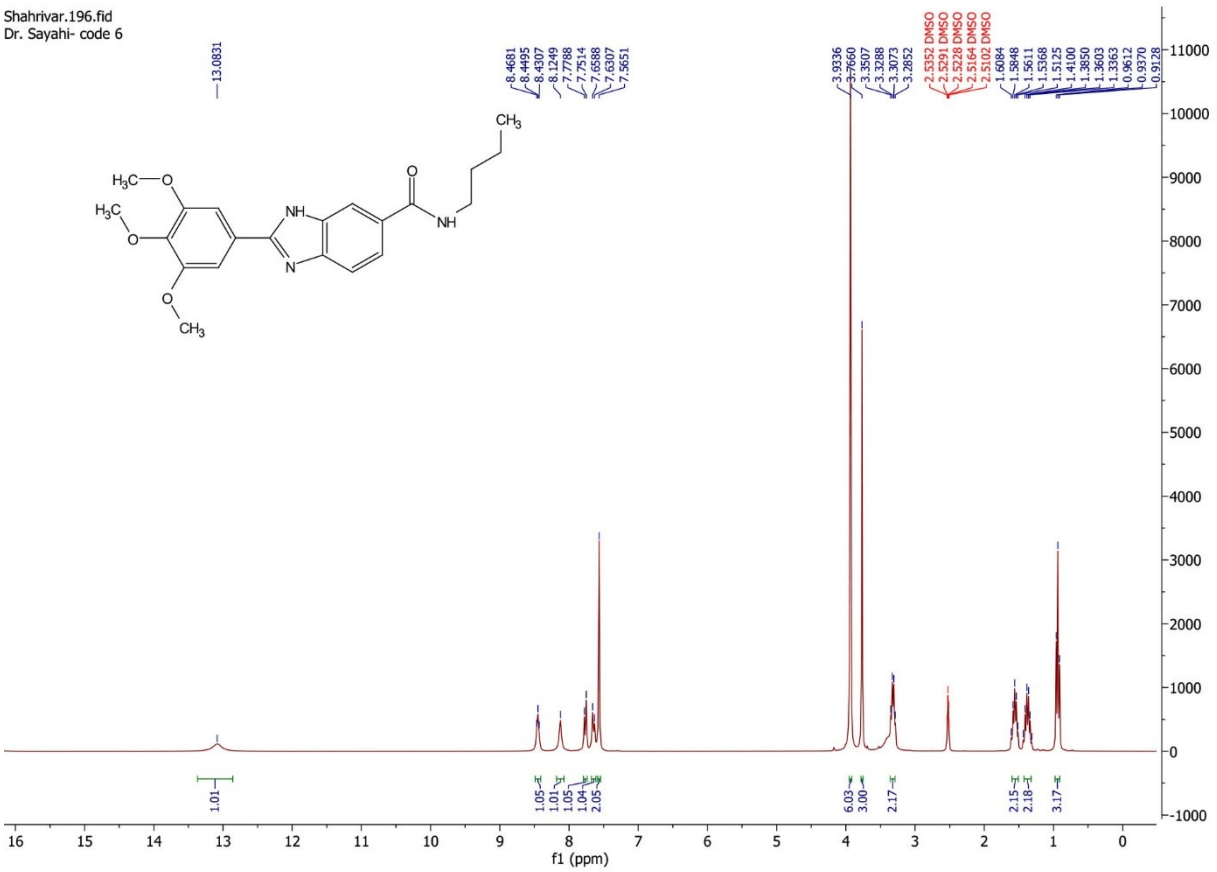


Figure S3. The ^{1}H-NMR spectrum of **5a**.

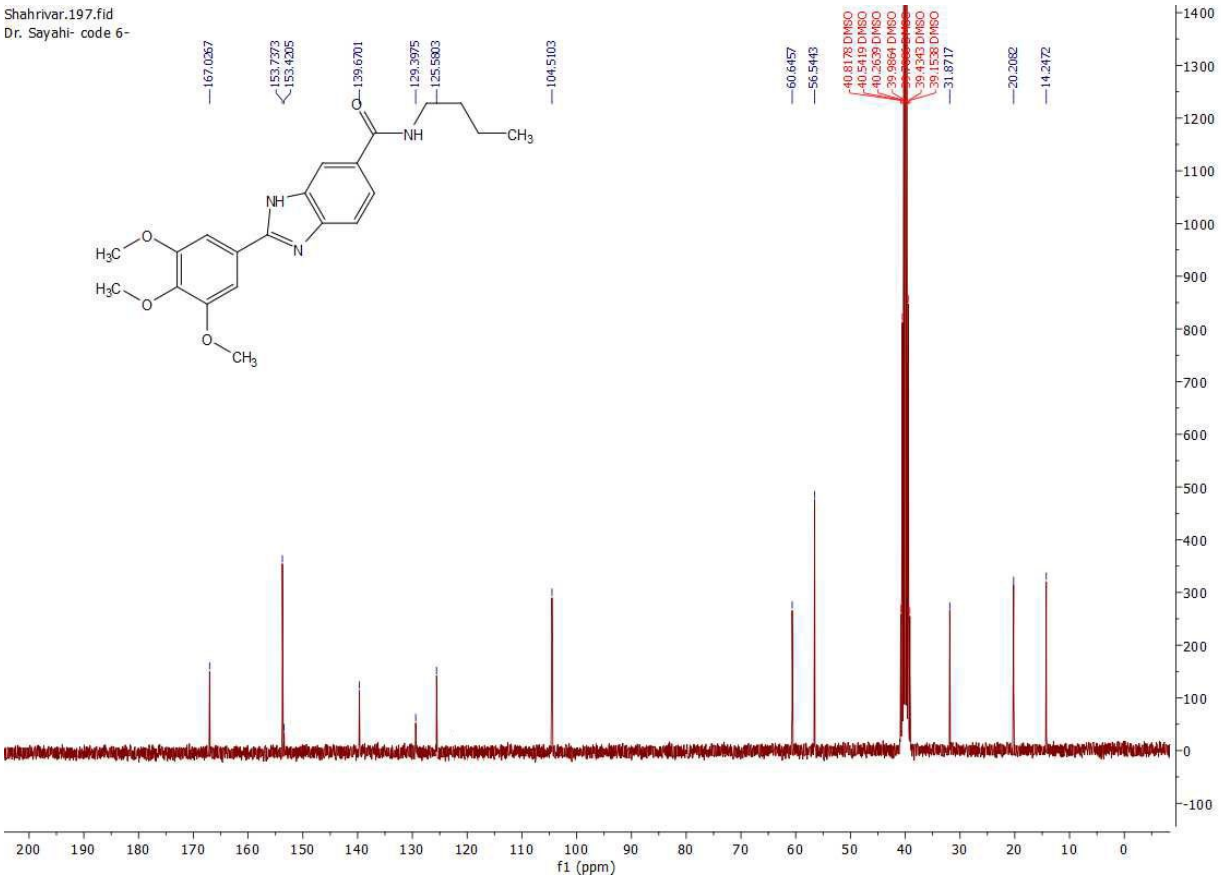


Figure S4. The ^{13}C-NMR spectrum of **5a**.

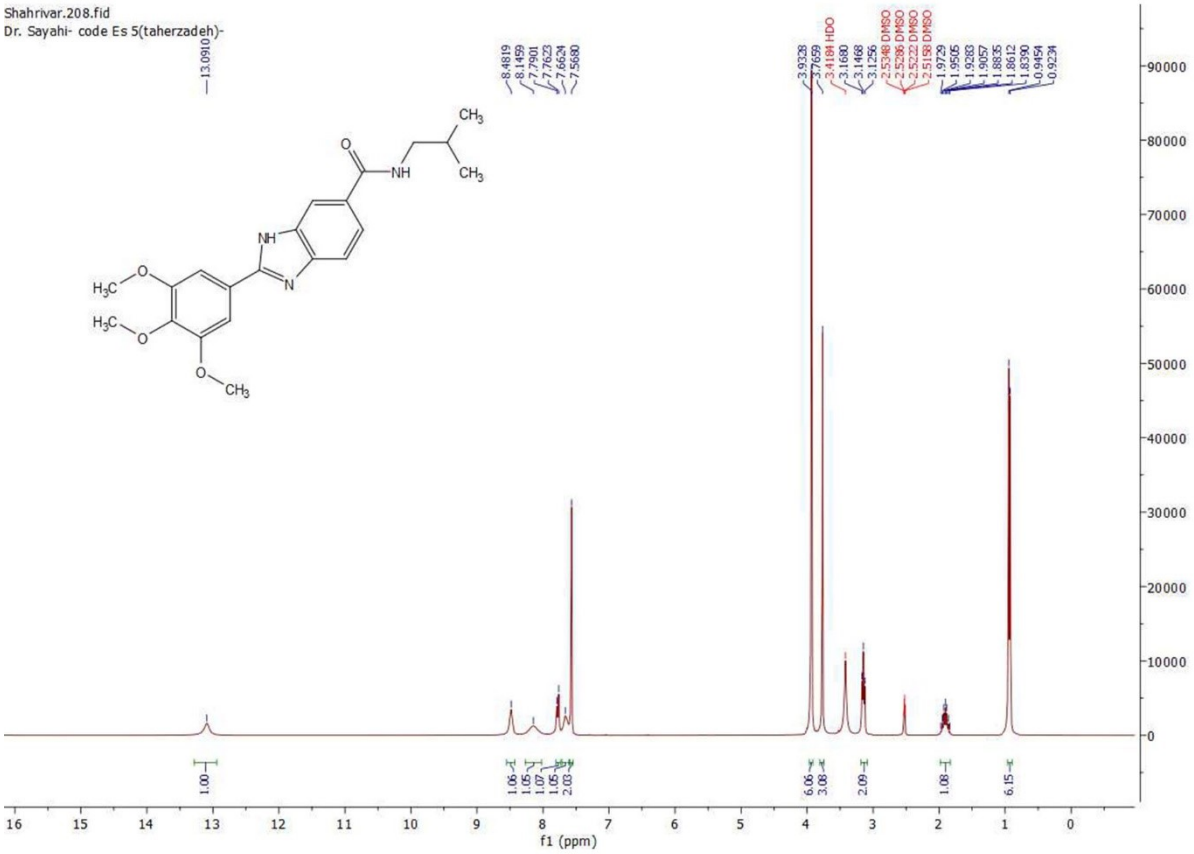


Figure S5. The ^{1}H-NMR spectrum of **5b**.

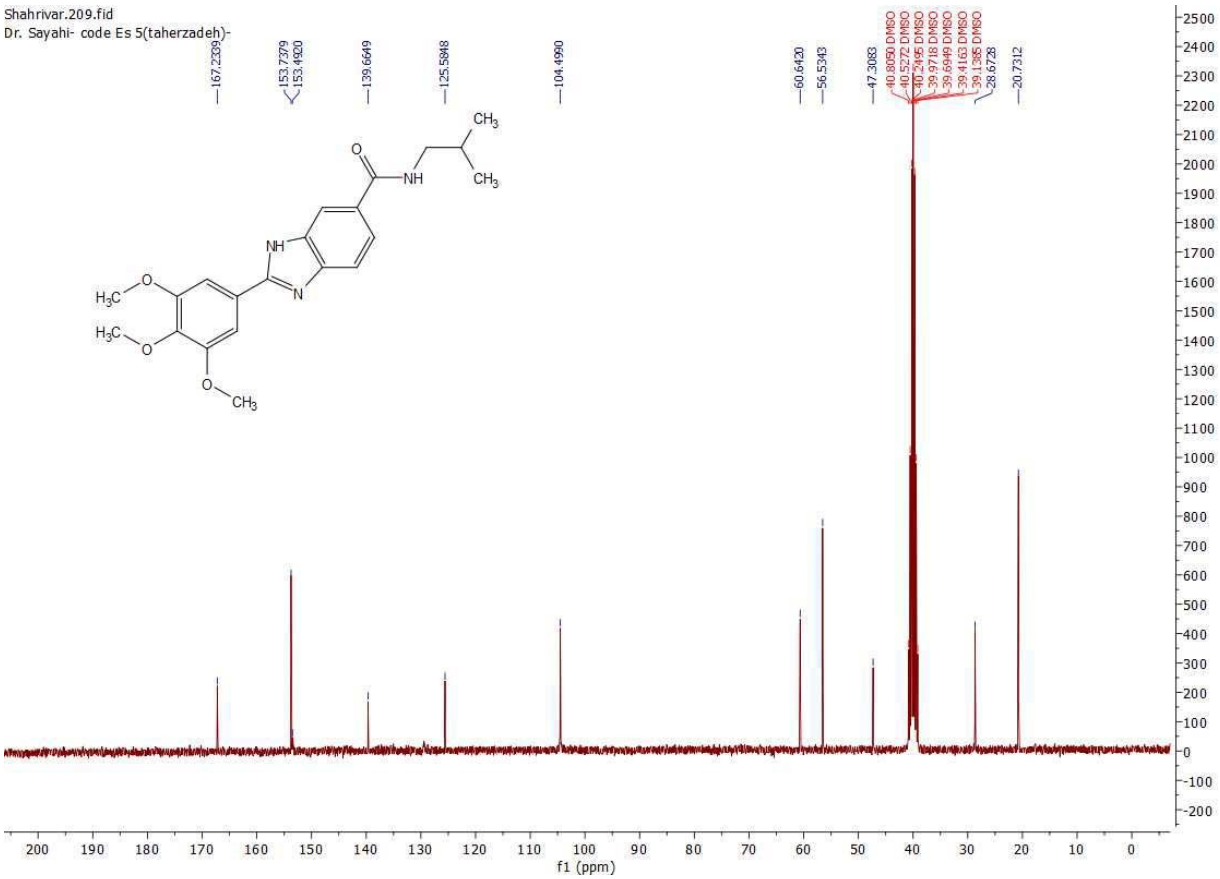


Figure S6. The ^{13}C-NMR spectrum of **5b**.

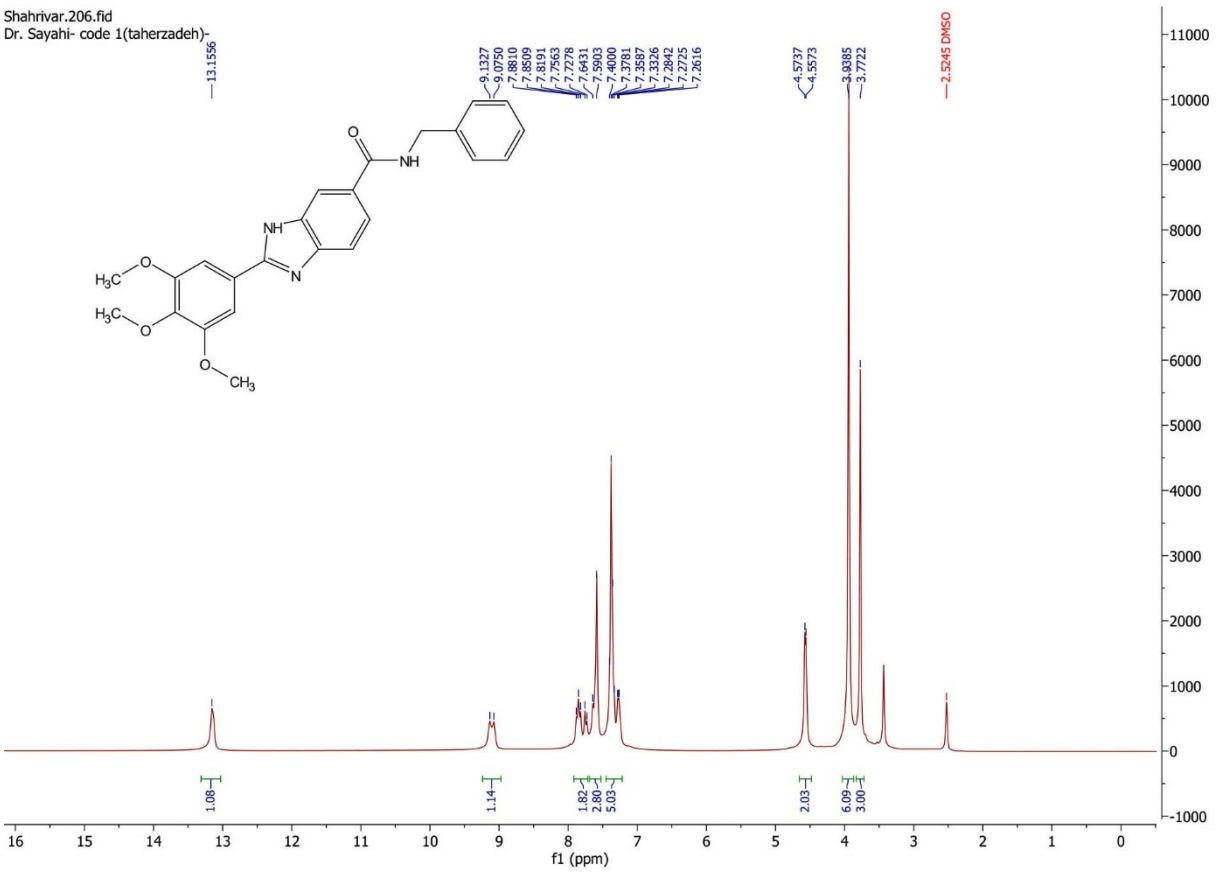


Figure S7. The ^{1}H-NMR spectrum of **5c**.

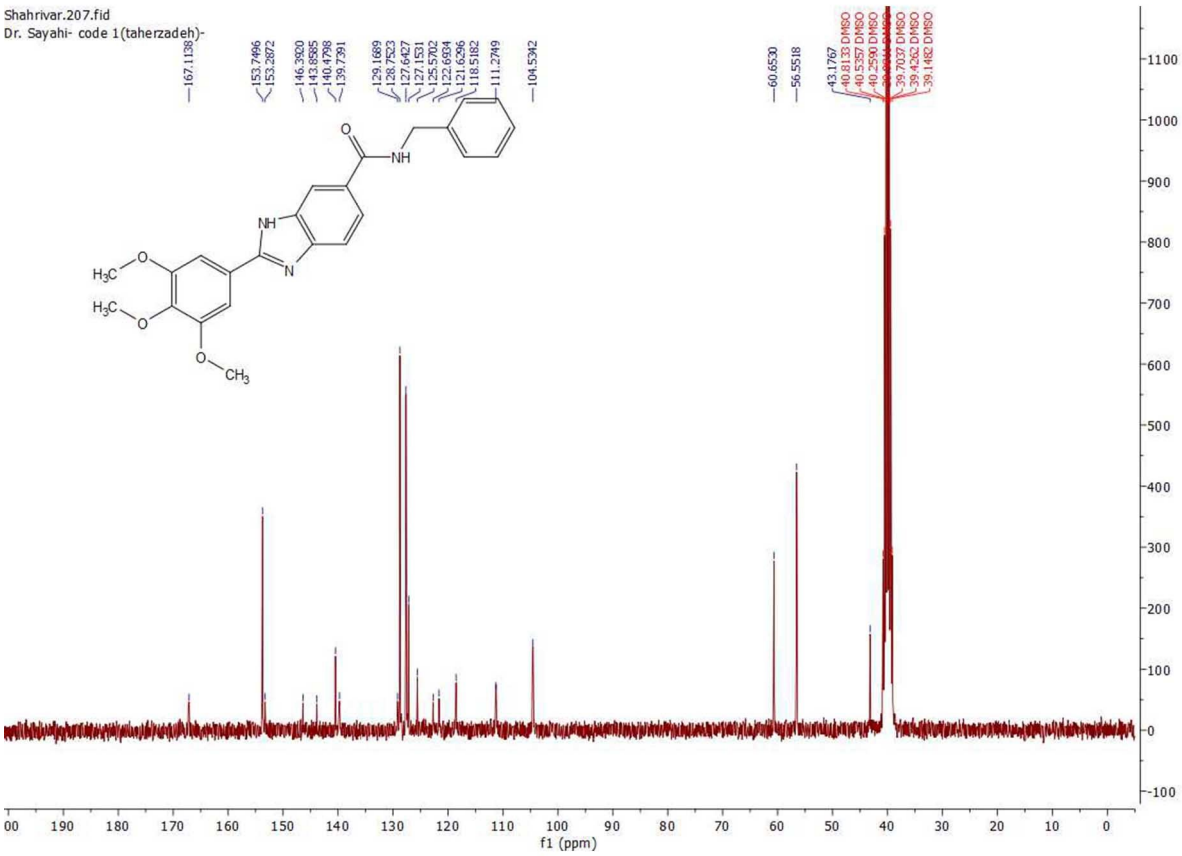


Figure S8. The ^{13}C-NMR spectrum of **5c**.

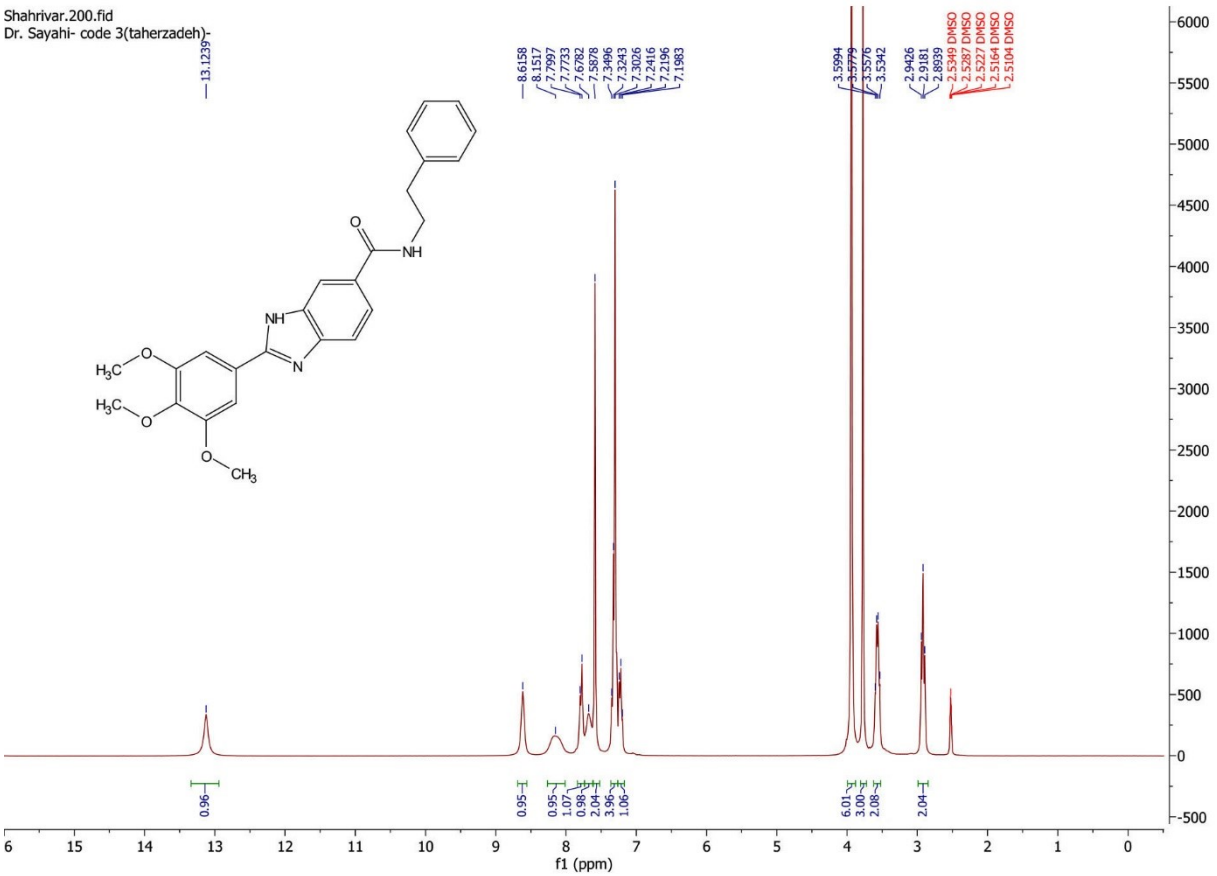


Figure S9. The ^{1}H-NMR spectrum of **5d**.

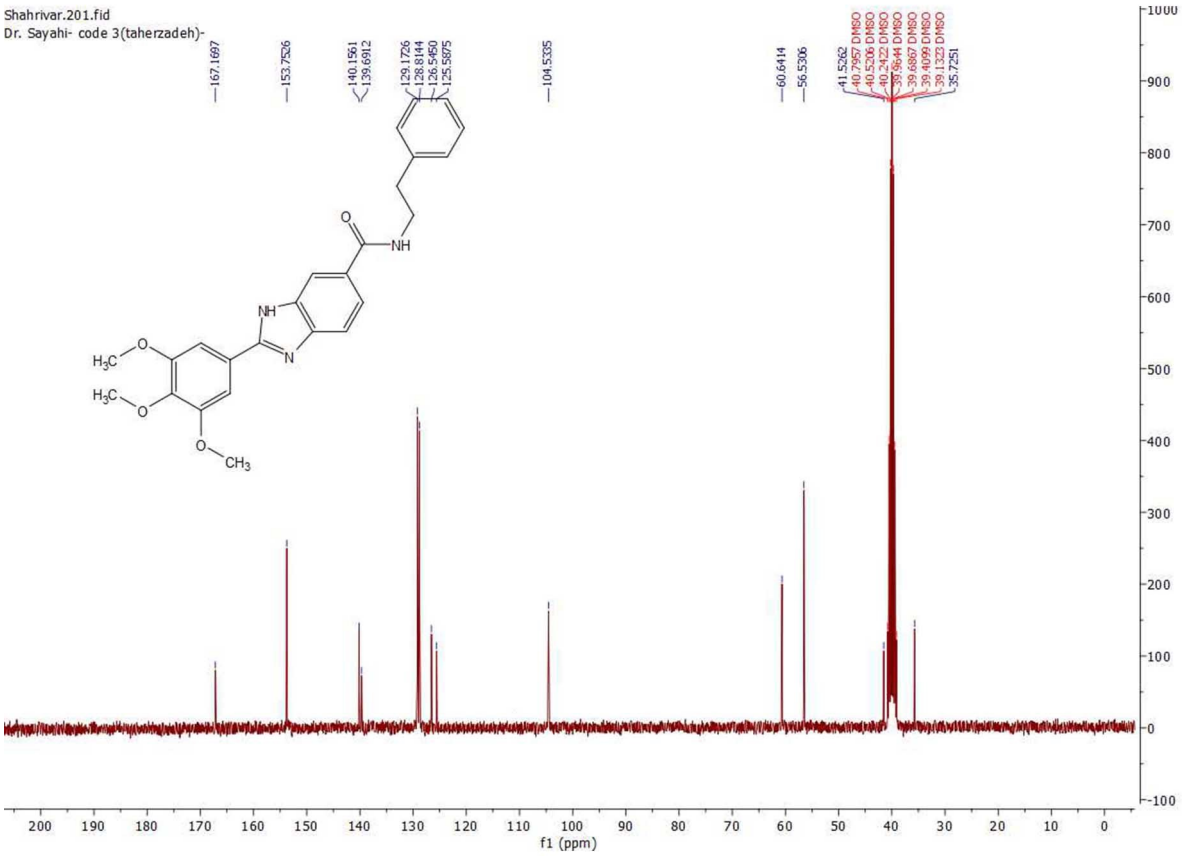


Figure S10. The ^{13}C-NMR spectrum of **5d**.

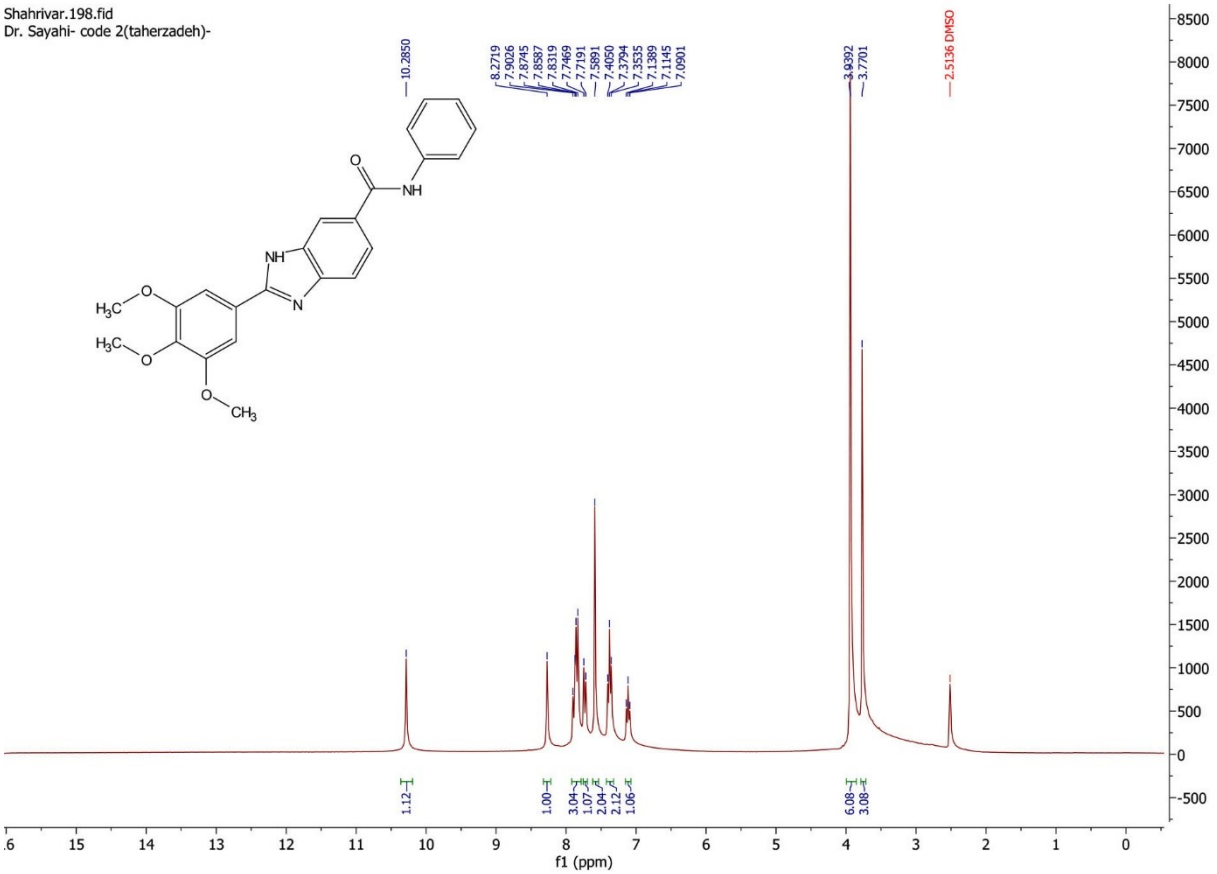


Figure S11. The ^{1}H-NMR spectrum of **5e**.

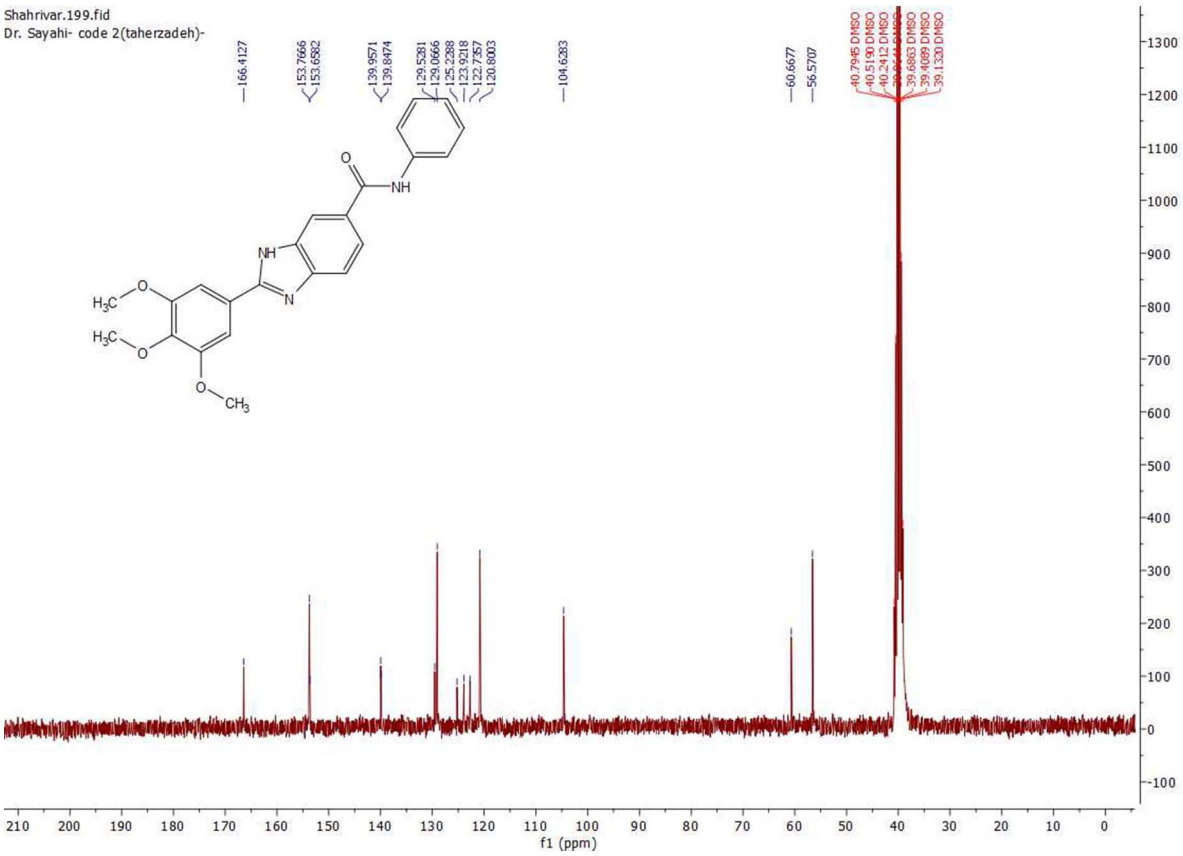


Figure S12. The ^{13}C-NMR spectrum of **5e**.

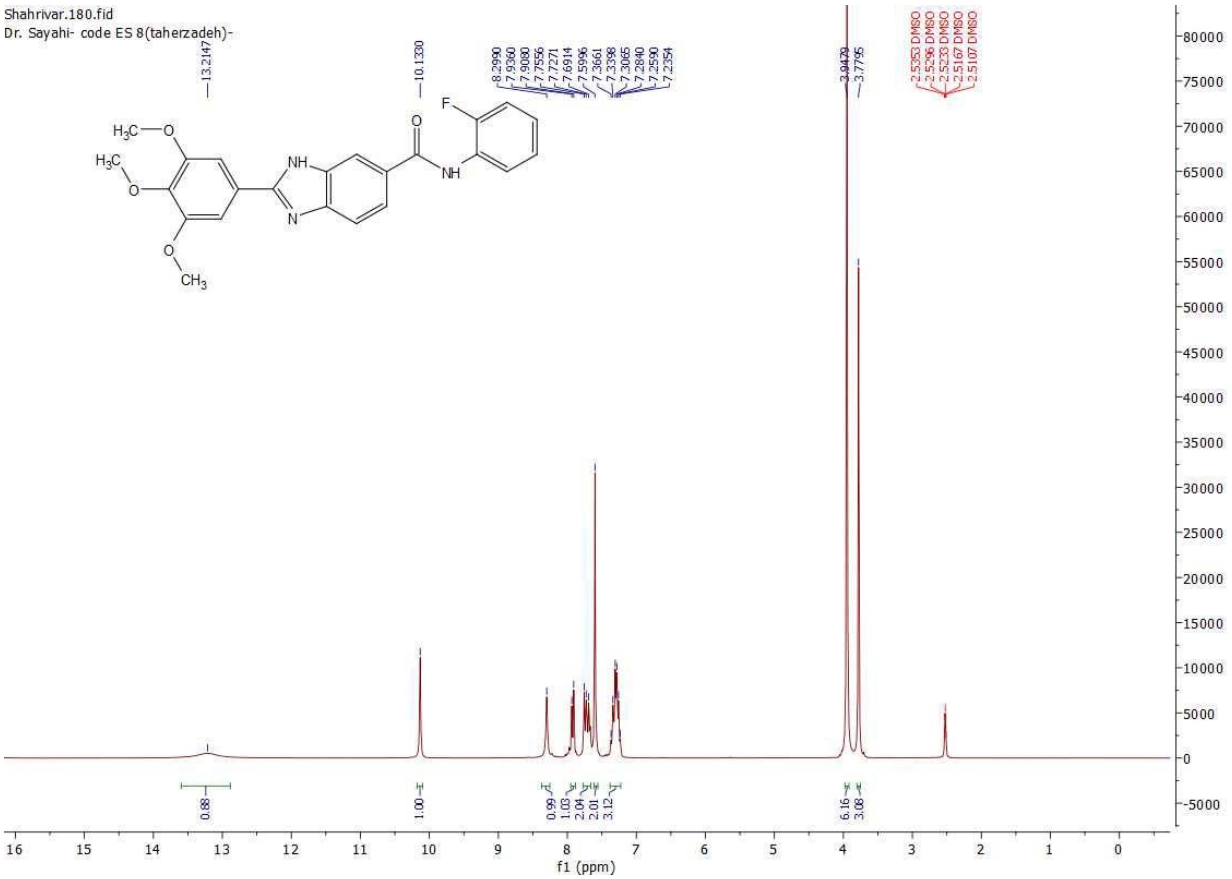


Figure S13. The ^{1}H-NMR spectrum of **5f**.

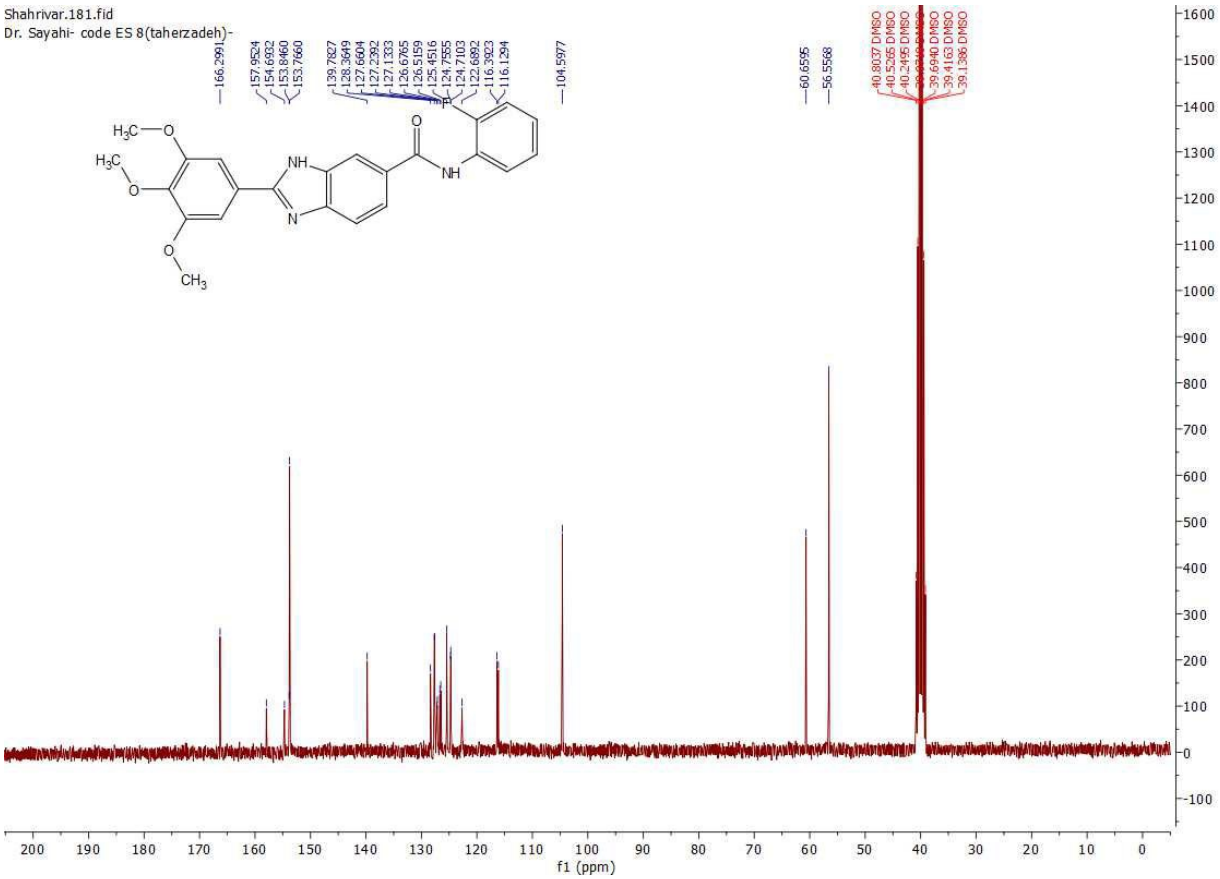


Figure S14. The ^{13}C-NMR spectrum of **5f**.

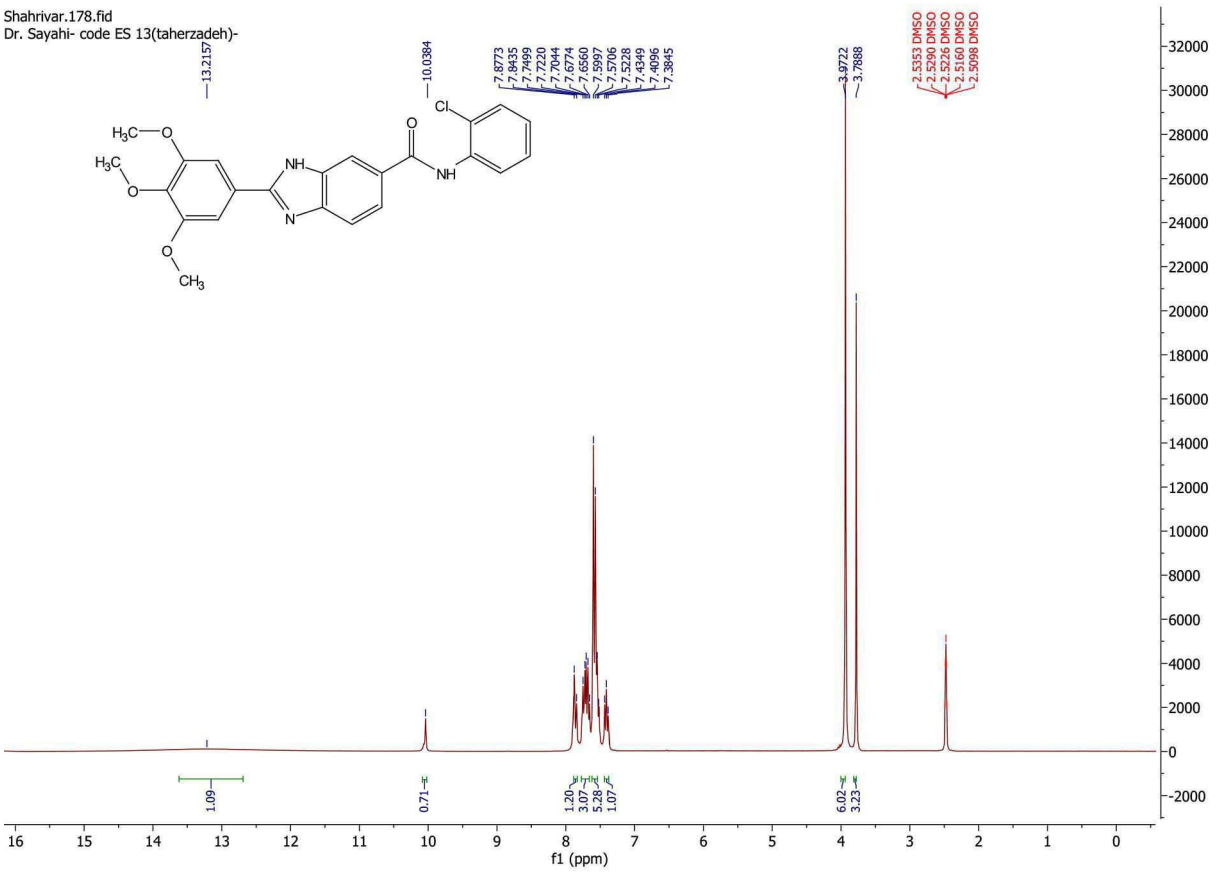


Figure S15. The ^{1}H-NMR spectrum of **5g**.

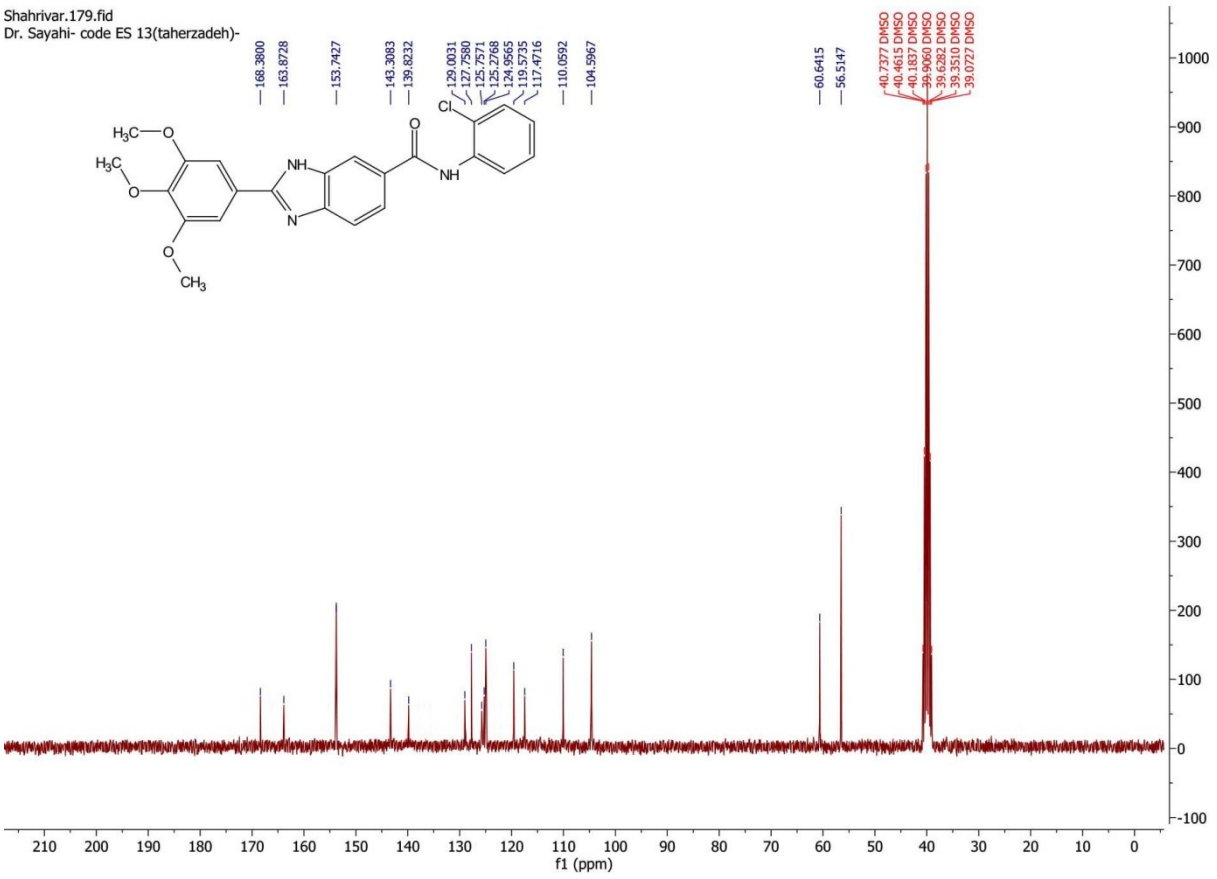


Figure S16. The ^{13}C-NMR spectrum of **5g**.

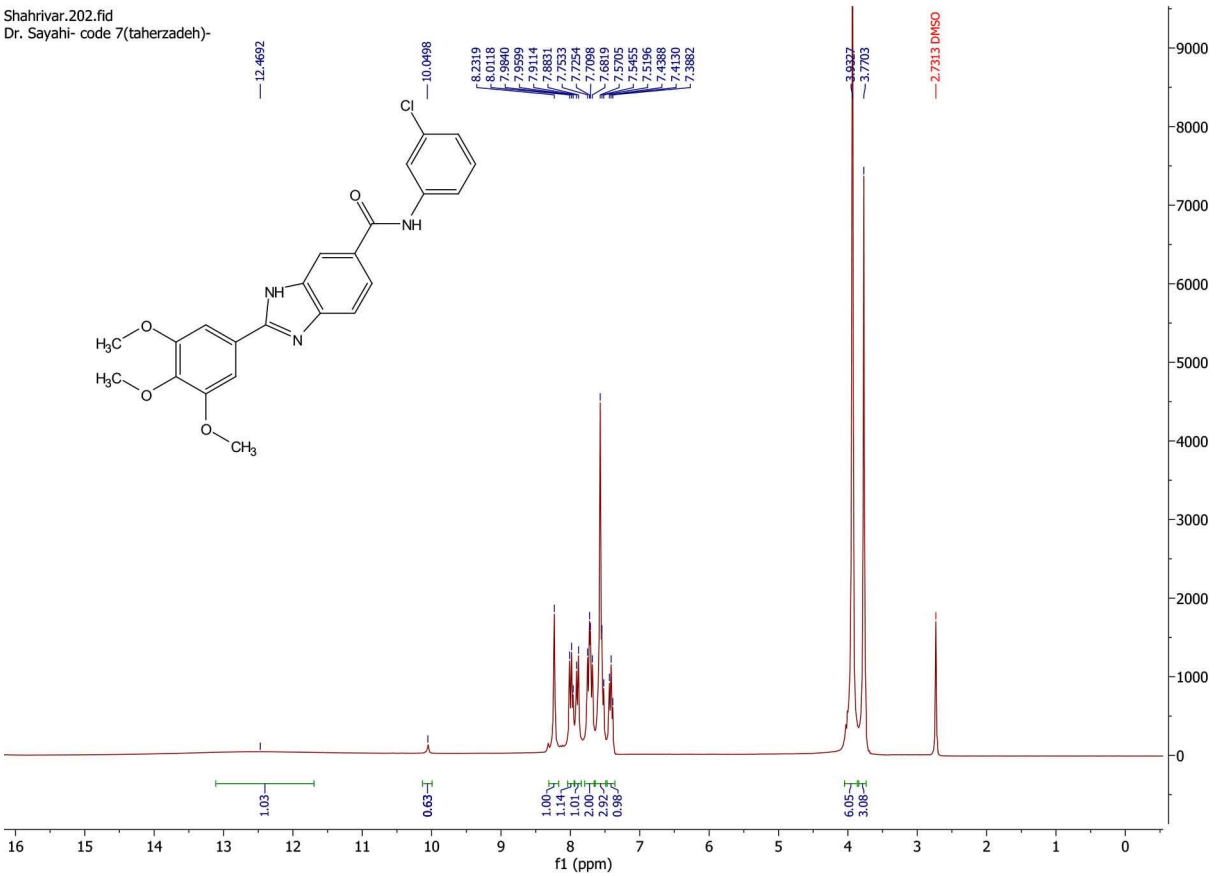


Figure S17. The ^{1}H-NMR spectrum of **5h**.

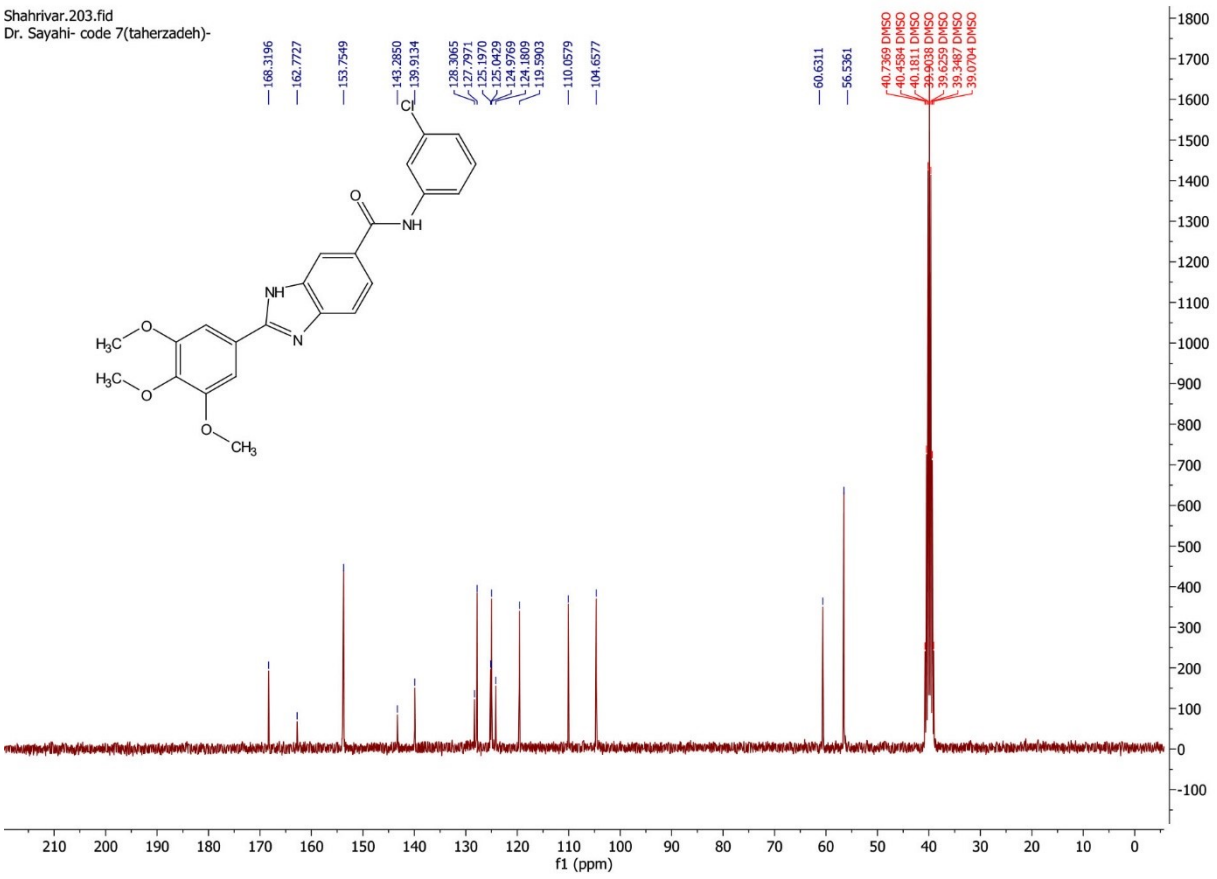


Figure S18. The ^{13}C-NMR spectrum of **5h**.

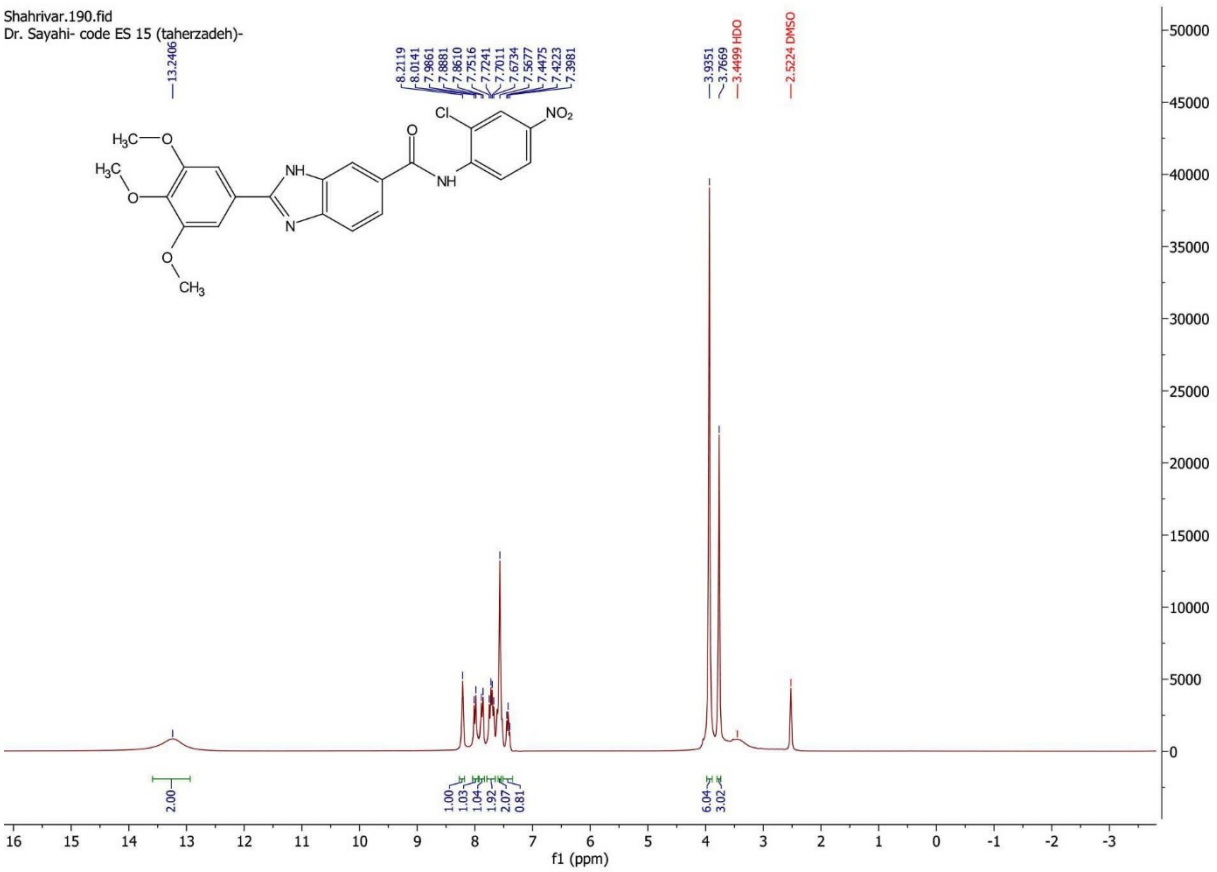


Figure S19. The ^{1}H-NMR spectrum of **5i**.

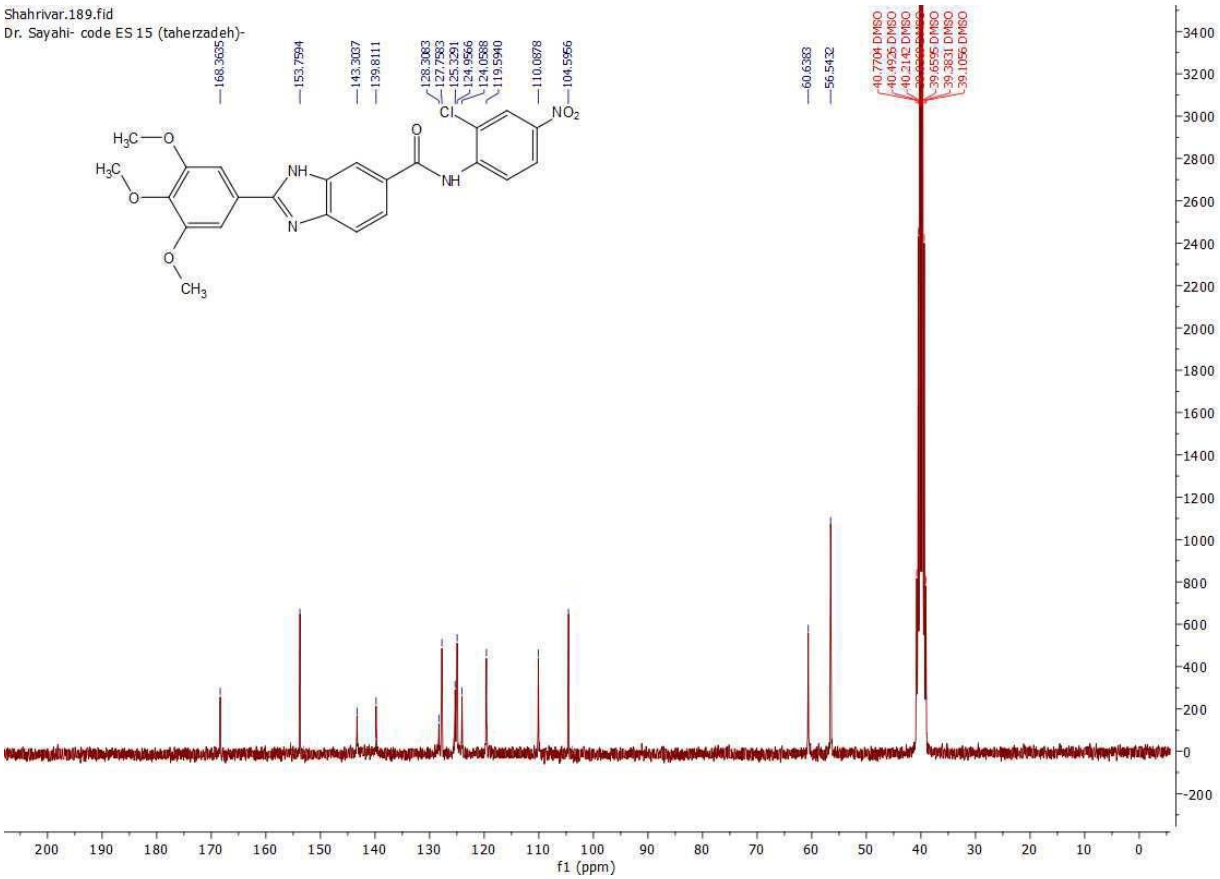


Figure S20. The ^{13}C-NMR spectrum of **5i**.

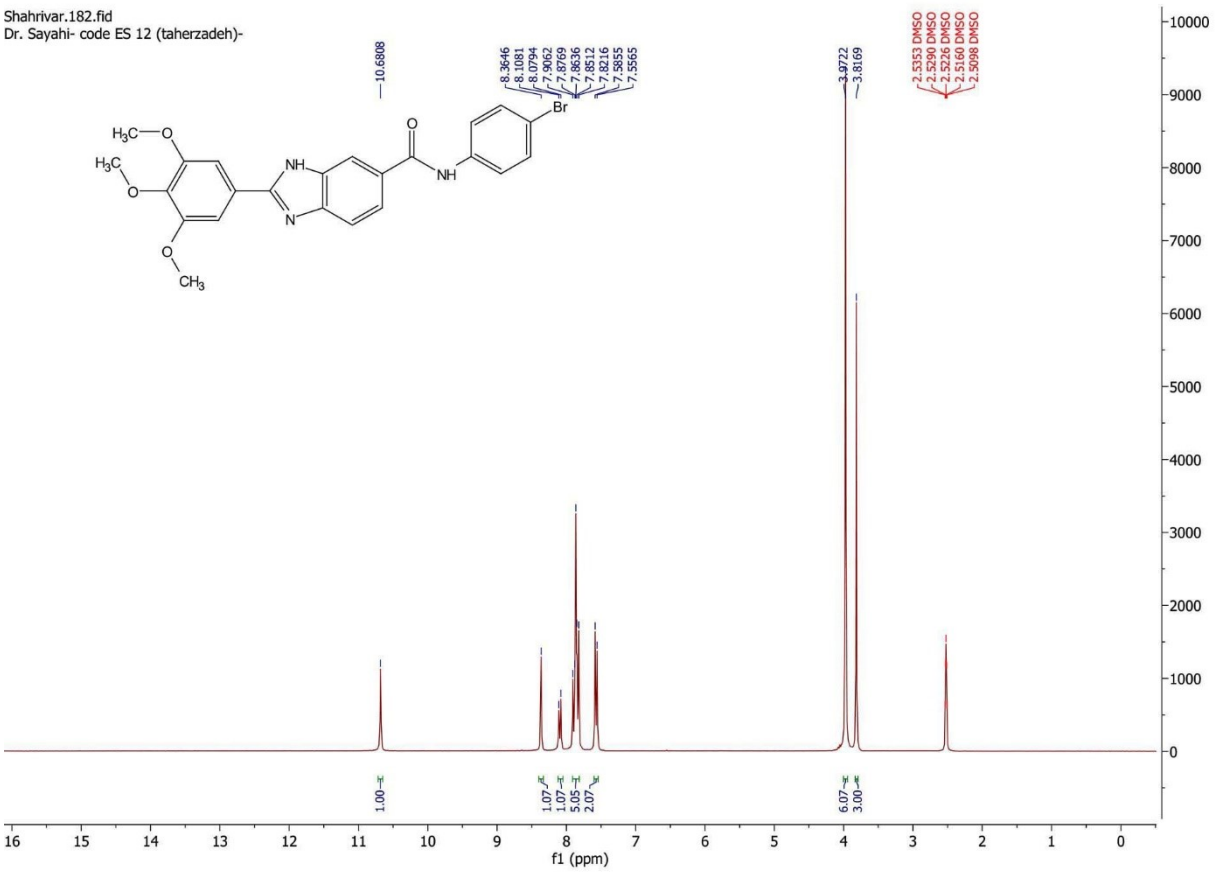


Figure S21. The ^{1}H-NMR spectrum of **5j**.

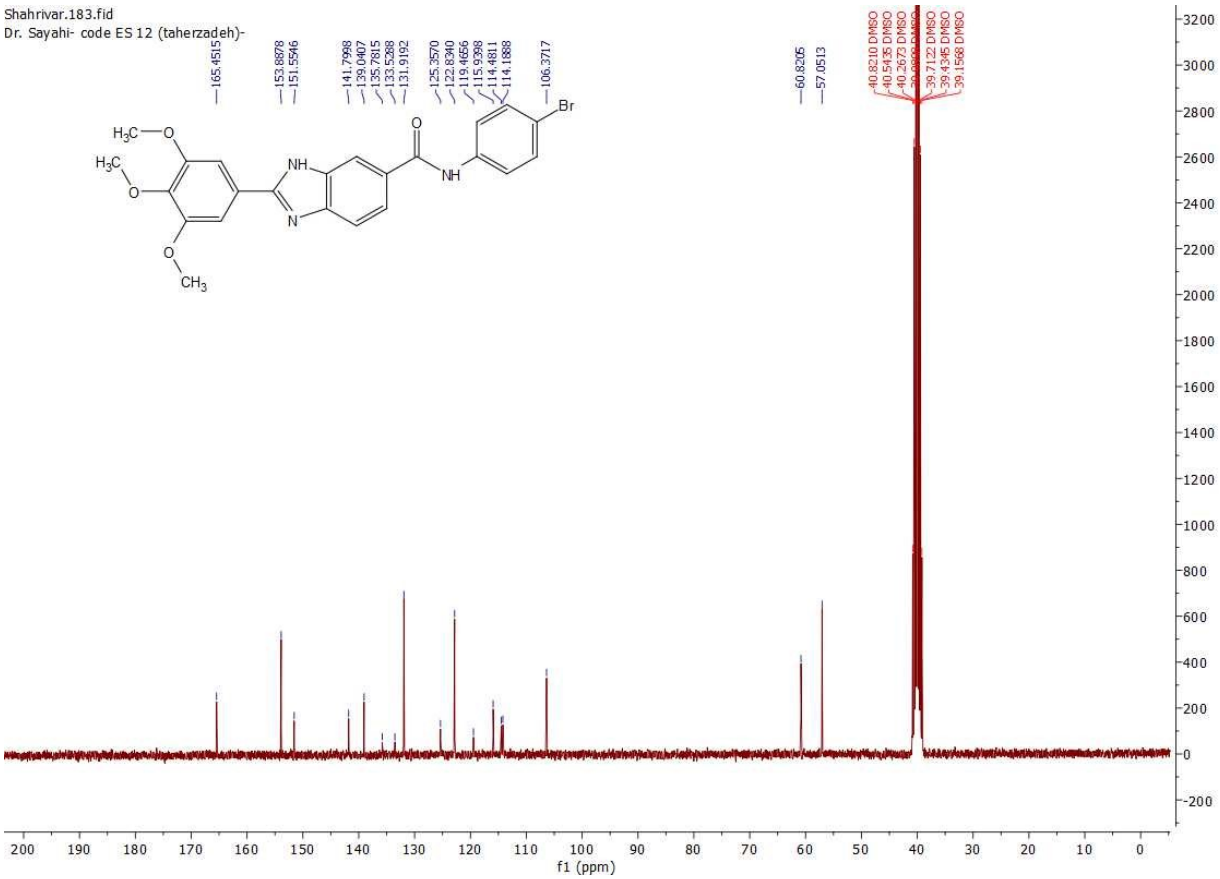


Figure S22. The ^{13}C-NMR spectrum of **5j**.

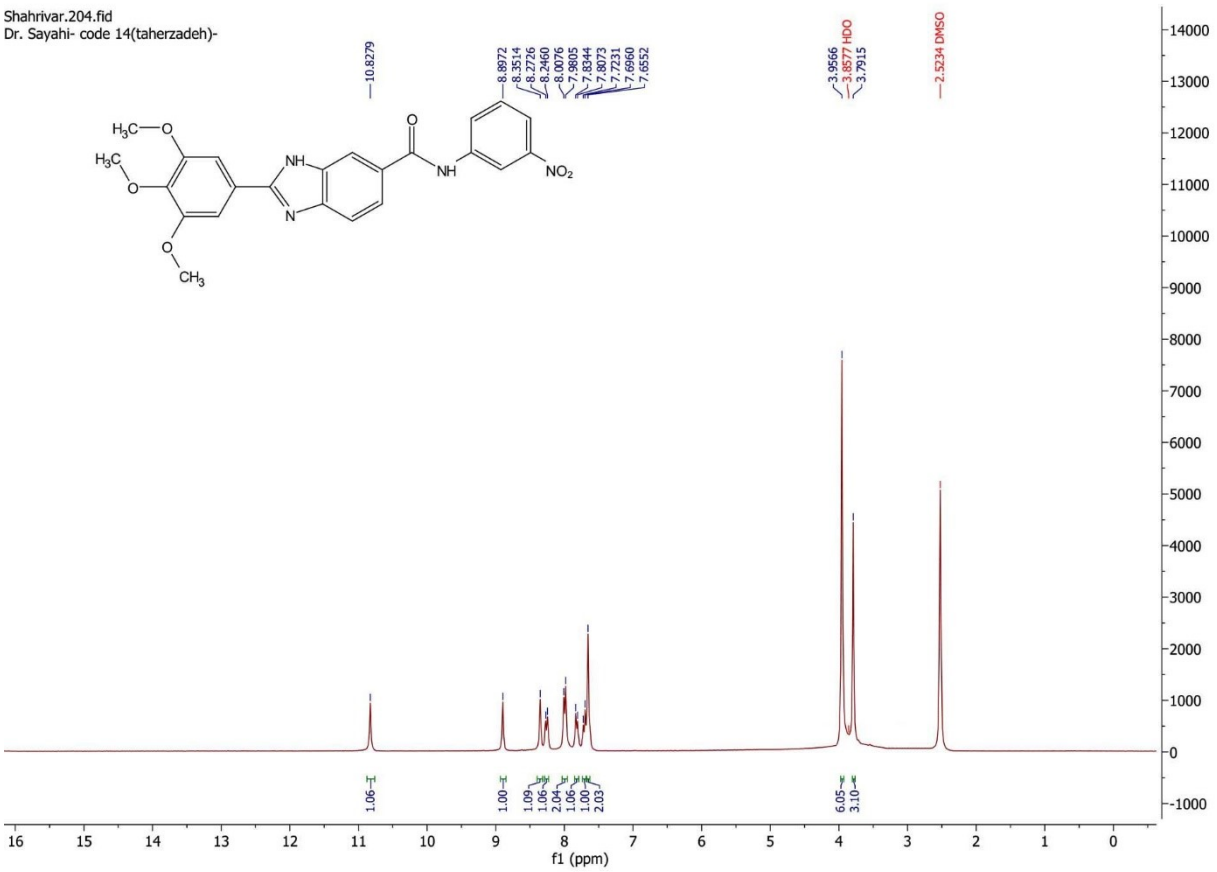


Figure S23. The ^{1}H-NMR spectrum of **5k**.

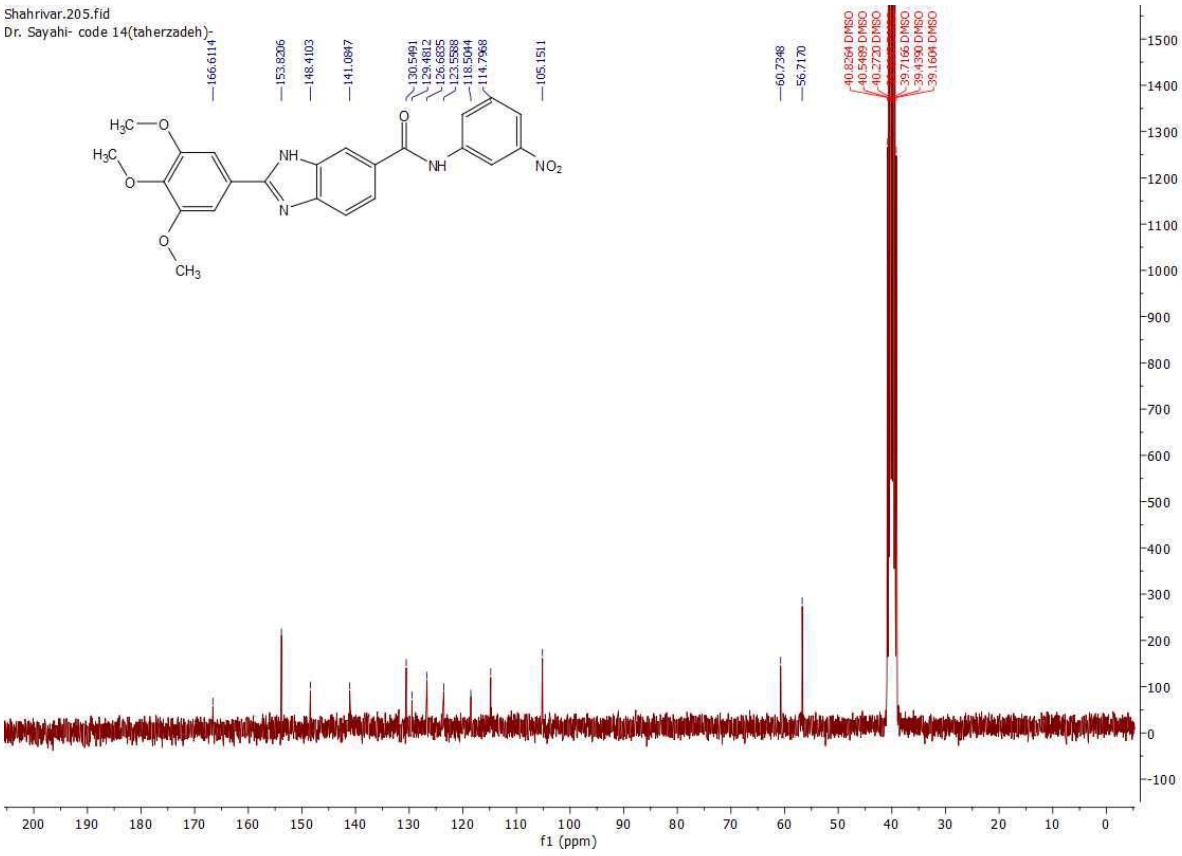


Figure S24. The ^{13}C-NMR spectrum of **5k**.

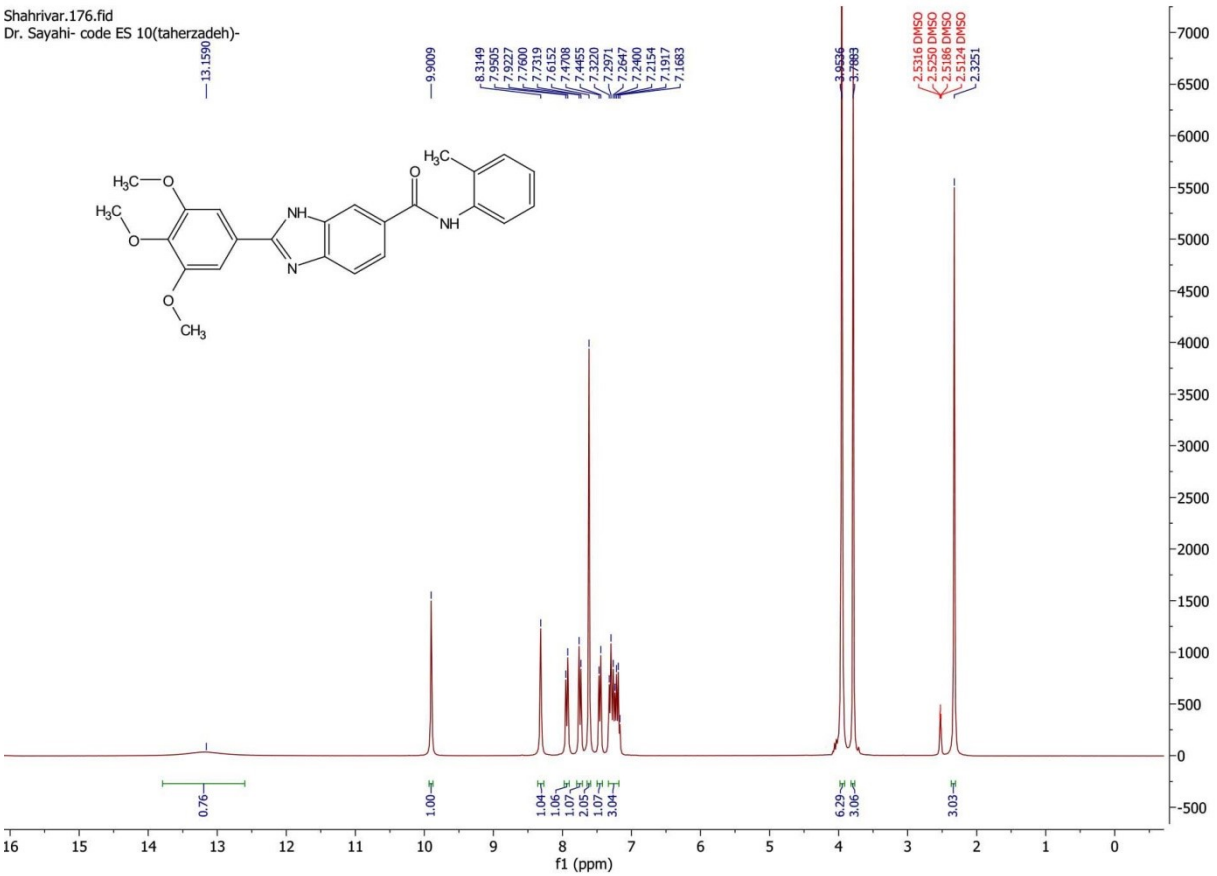


Figure S25. The ^{1}H-NMR spectrum of **5l**.

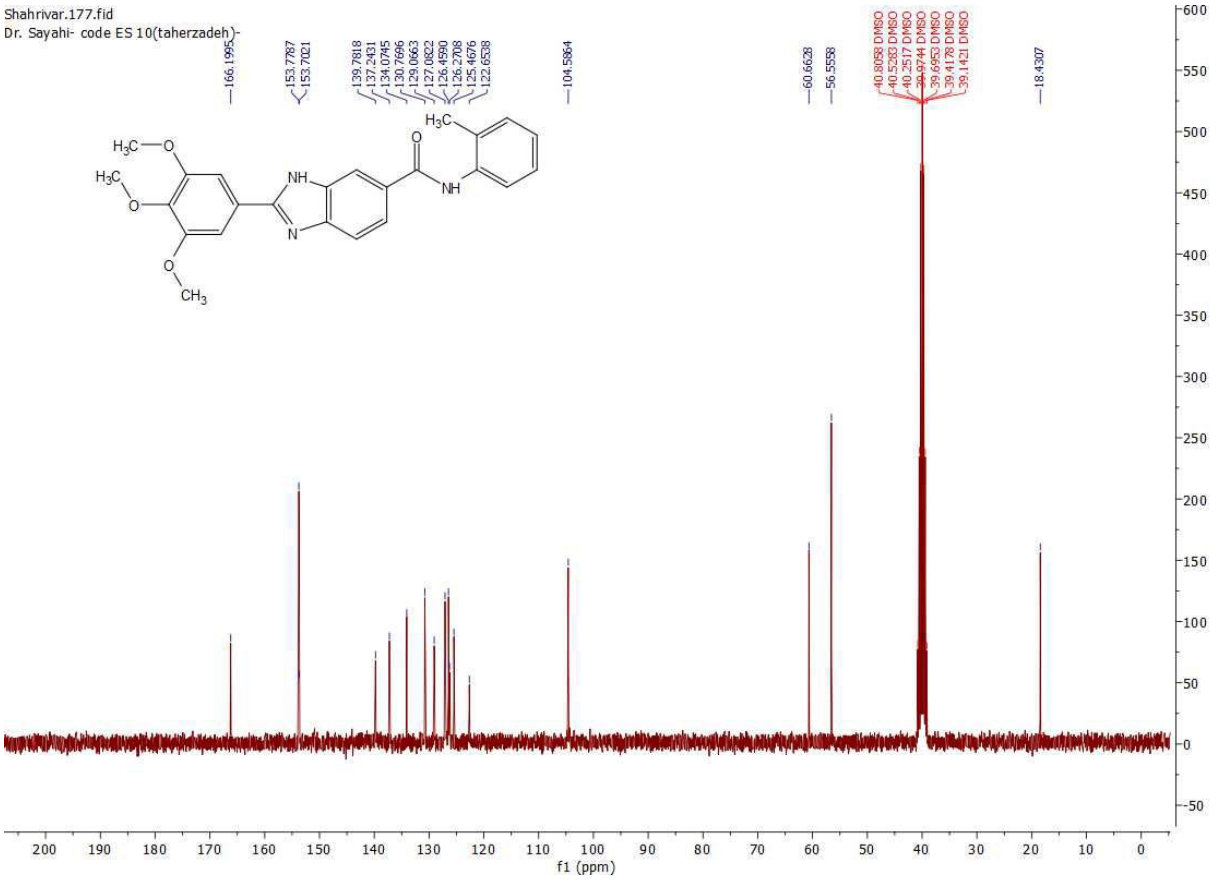


Figure S26. The ^{13}C-NMR spectrum of **5l**.

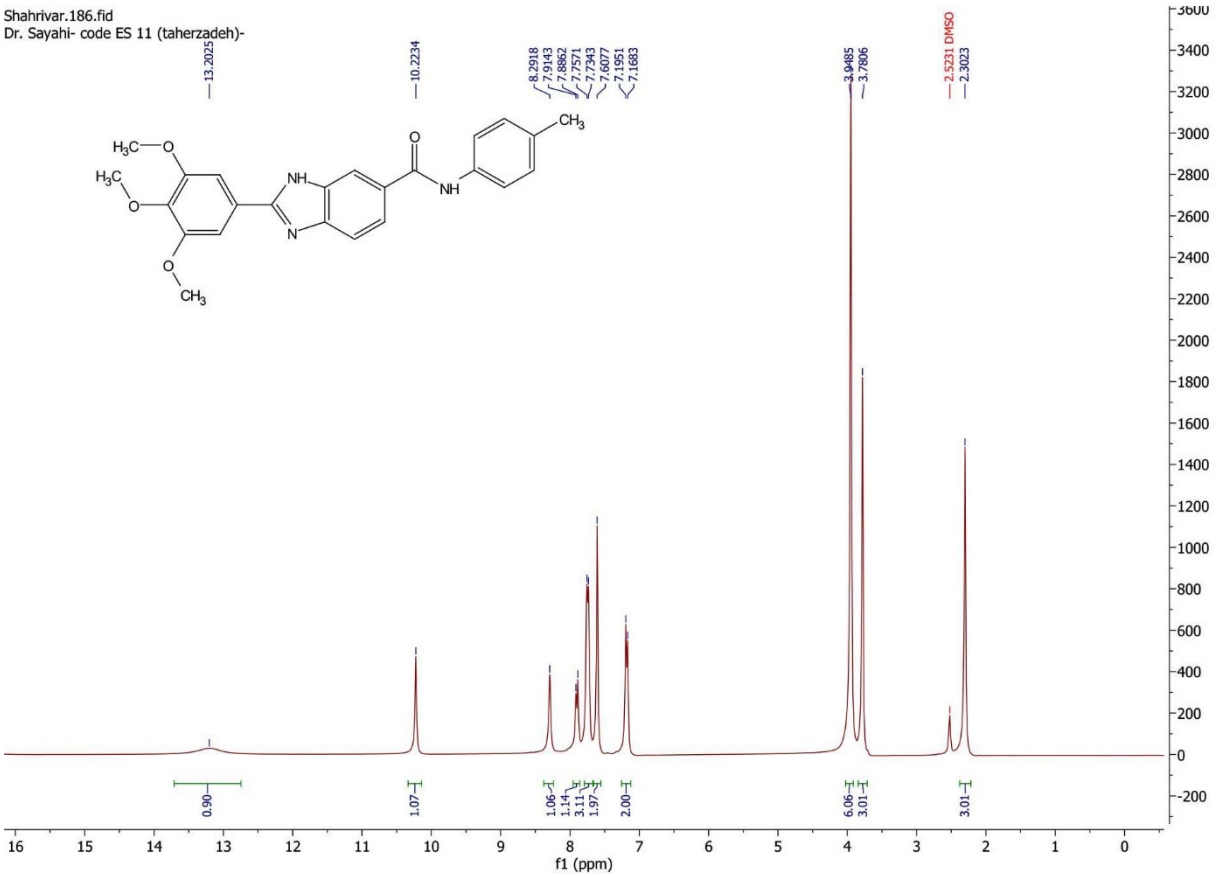


Figure S27. The ^{1}H-NMR spectrum of **5m**.

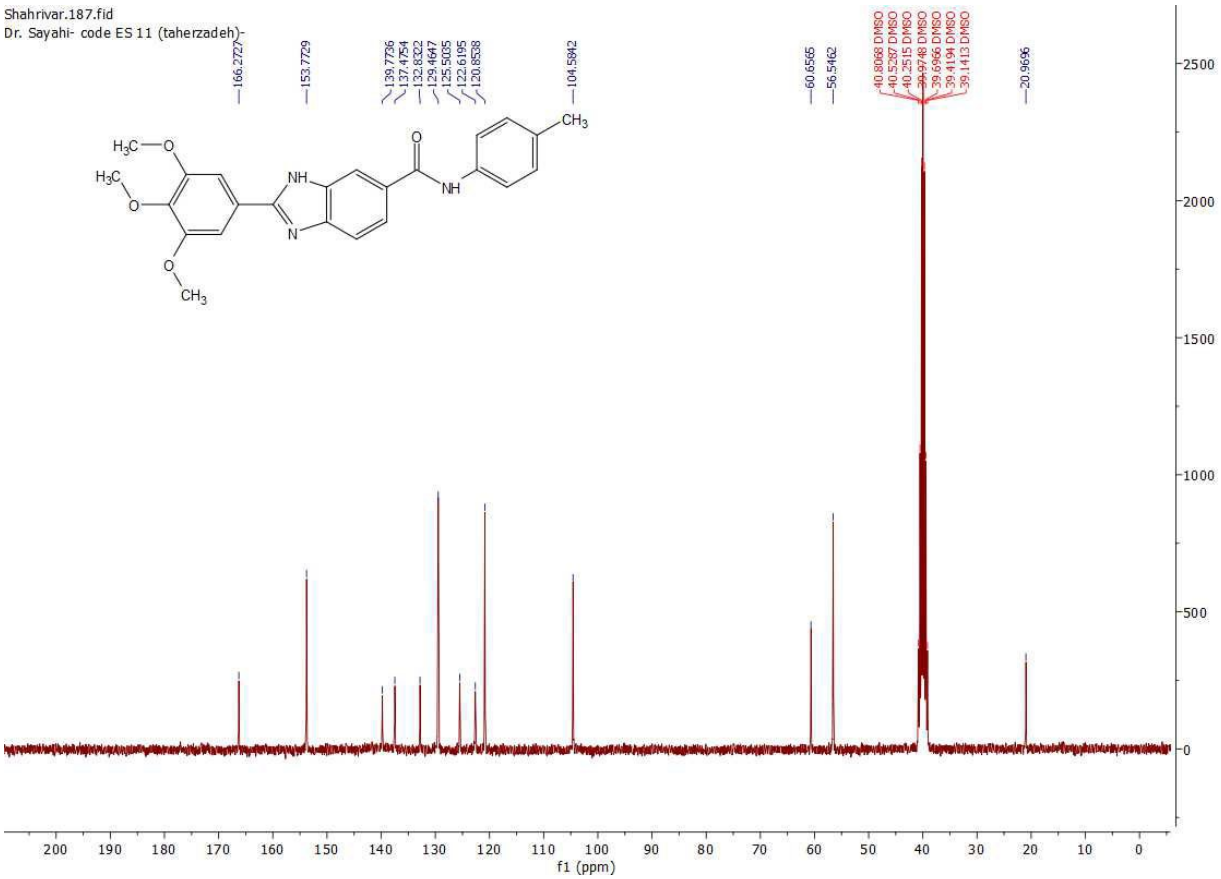


Figure S28. The ^{13}C-NMR spectrum of **5m**.

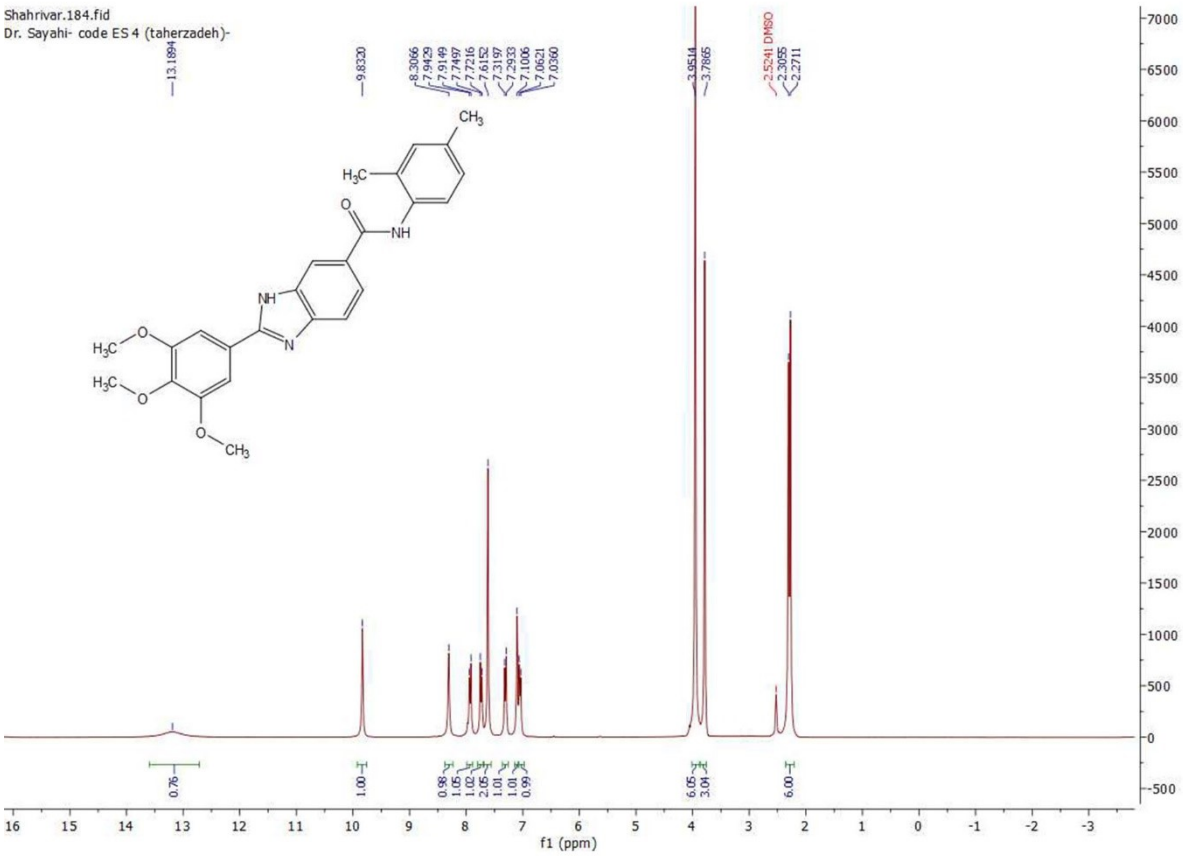


Figure S29. The ^{1}H-NMR spectrum of **5n**.

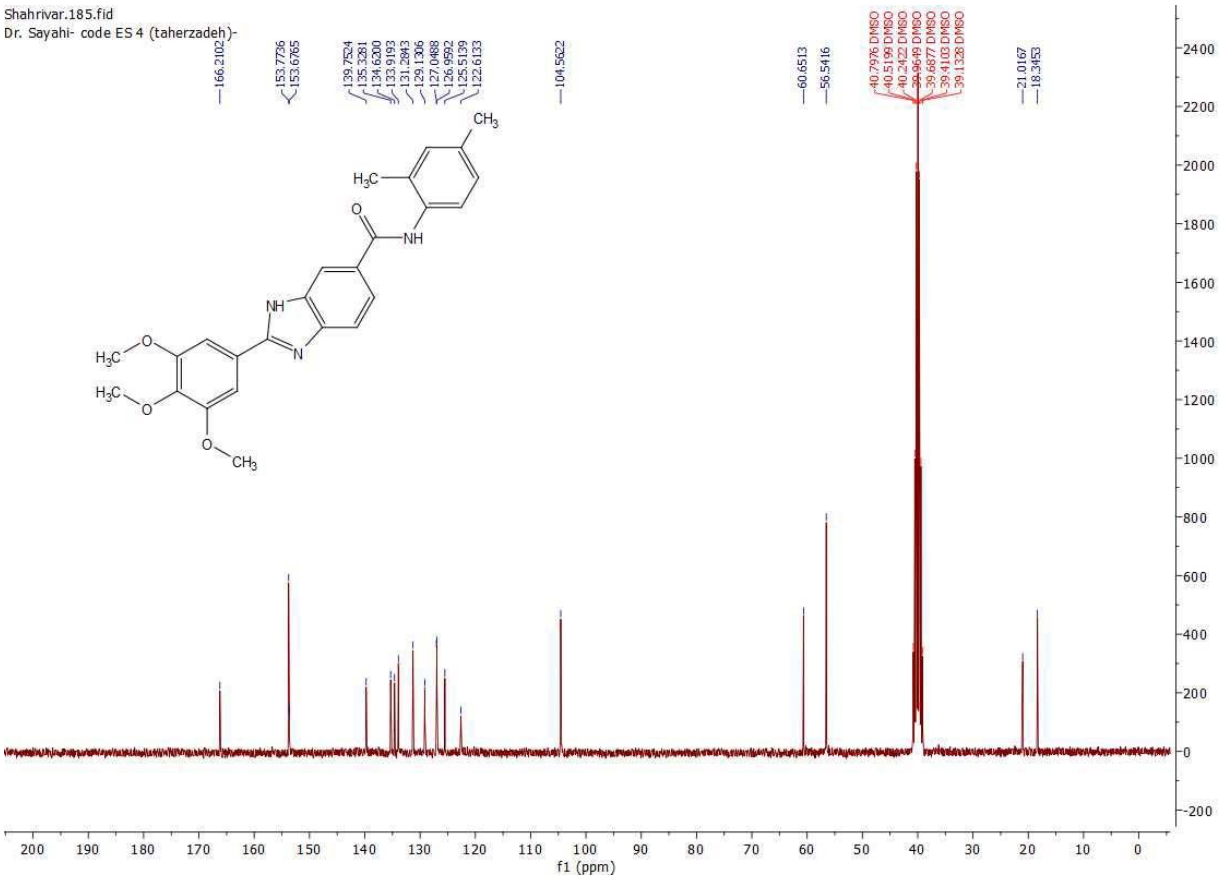


Figure S30. The ^{13}C-NMR spectrum of **5n**.

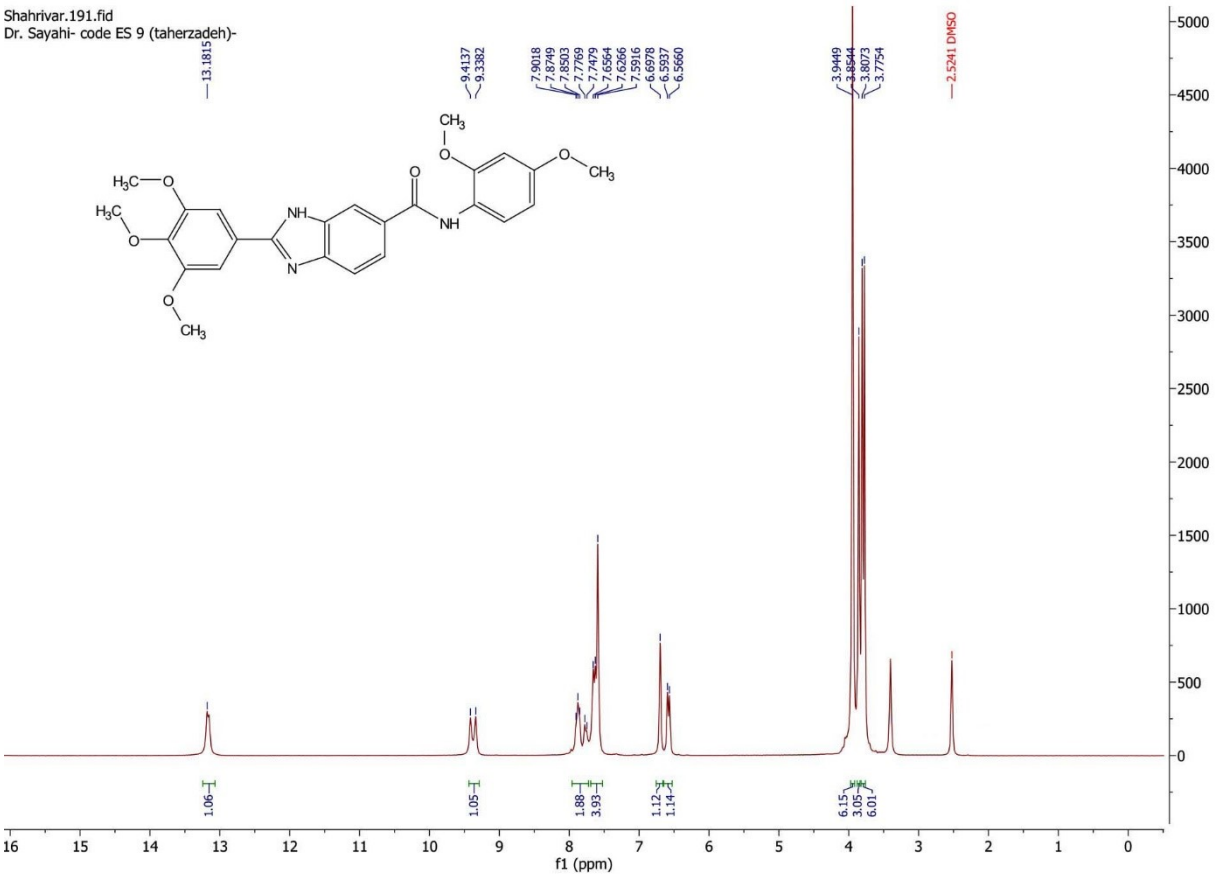


Figure S31. The ^{1}H-NMR spectrum of **5o**.

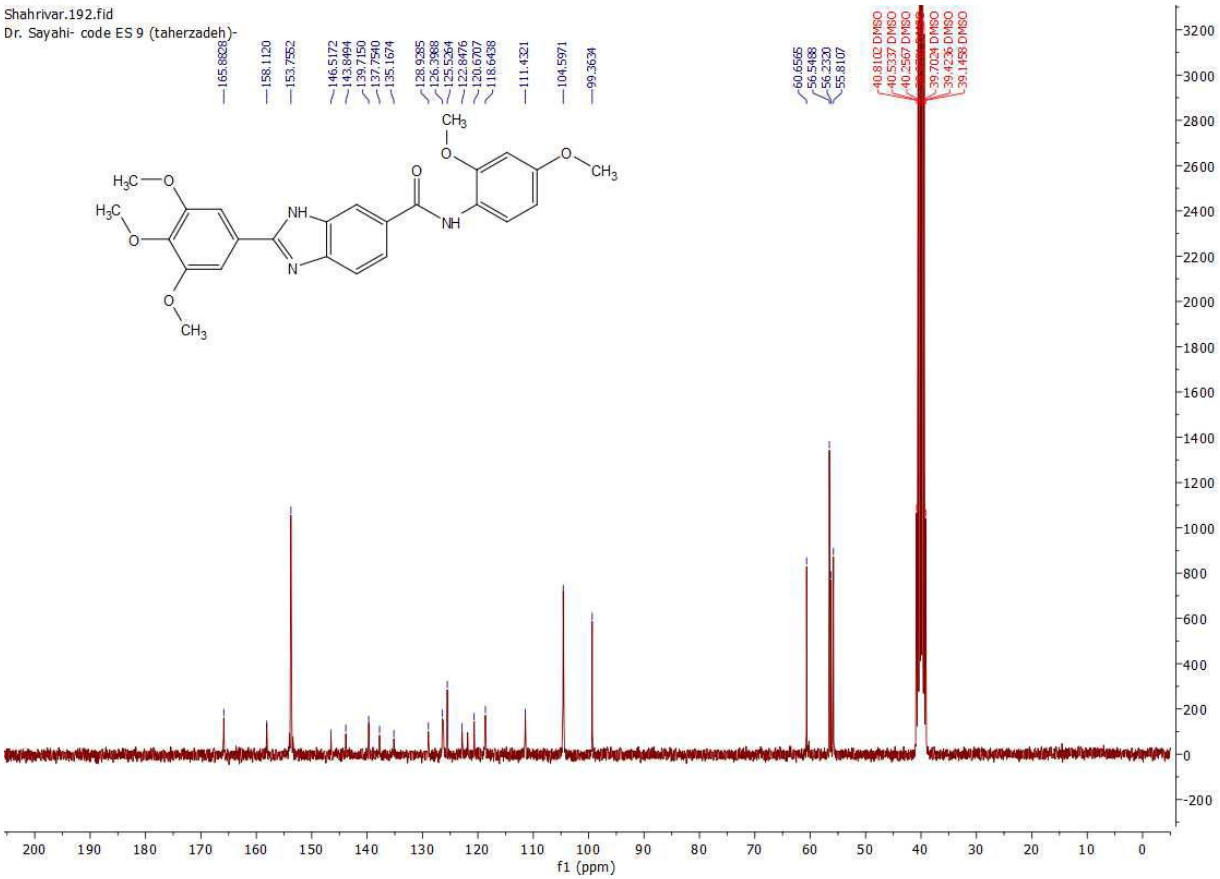
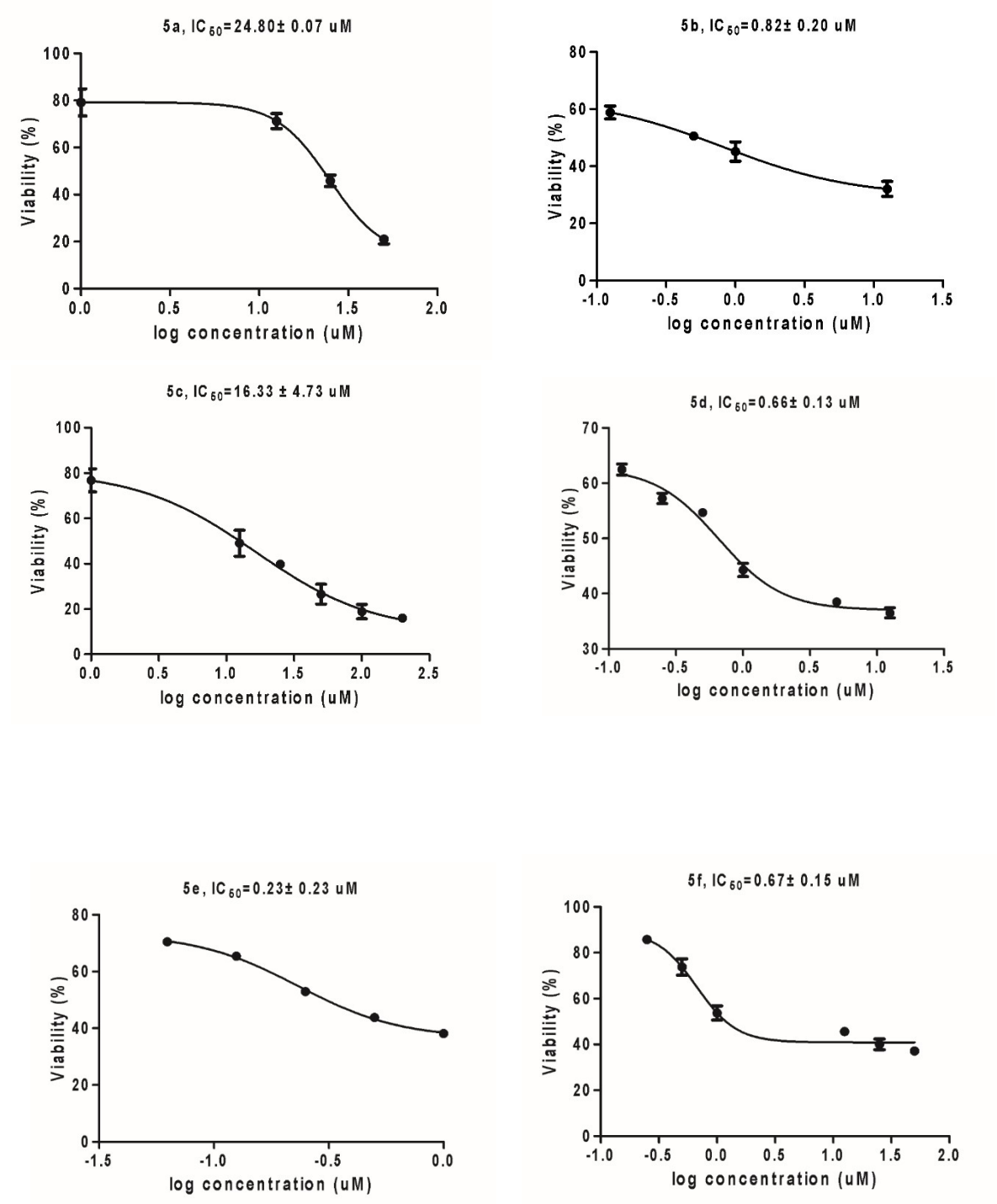
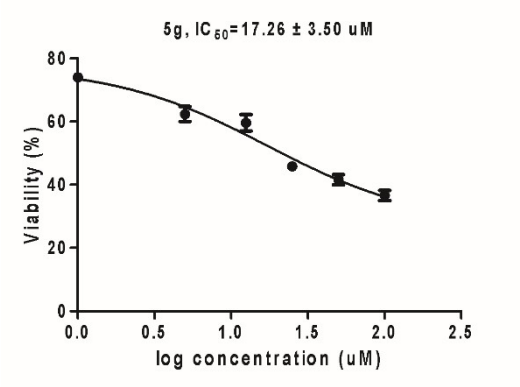
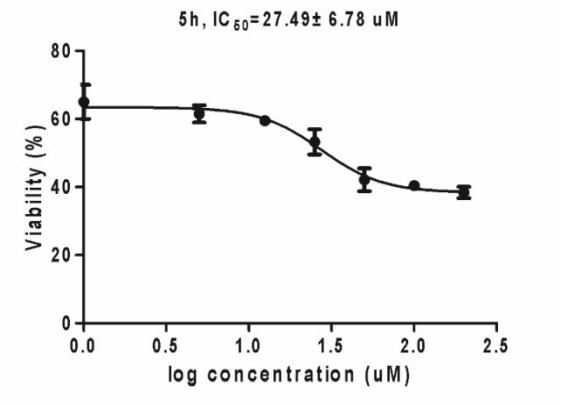


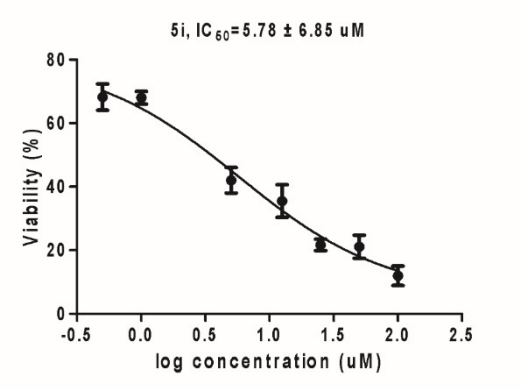
Figure S32. The ^{13}C-NMR spectrum of **5o**.

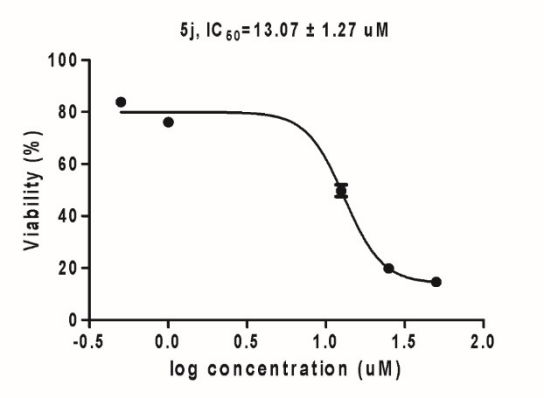
Figure S33. Cytotoxicity of **5a-o**, doxorubicin, cisplatin, and etoposide against A549 cells.

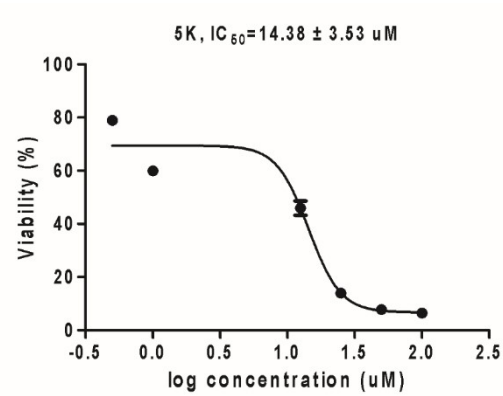


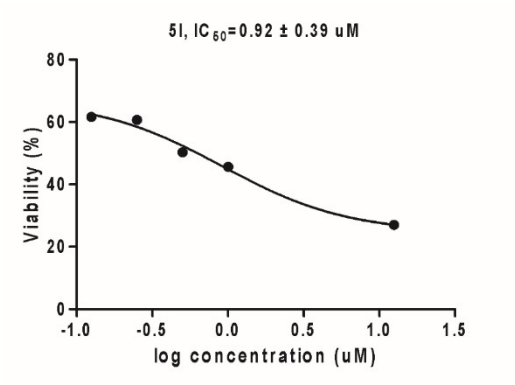

5g, IC50=17.26 ± 3.50 uM
Viability (%)
log concentration (uM)

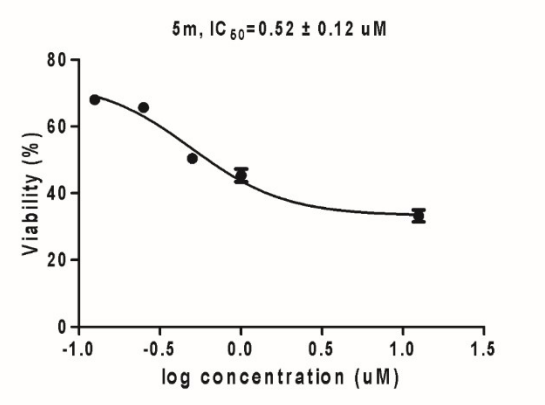

5h, IC50=27.49± 6.78 uM
Viability (%)
log concentration (uM)

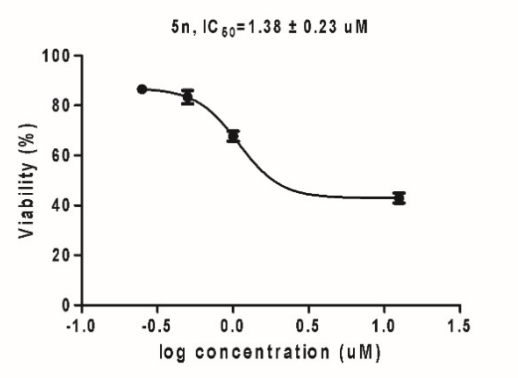

5i, IC50=5.78 ± 6.85 uM
Viability (%)
log concentration (uM)

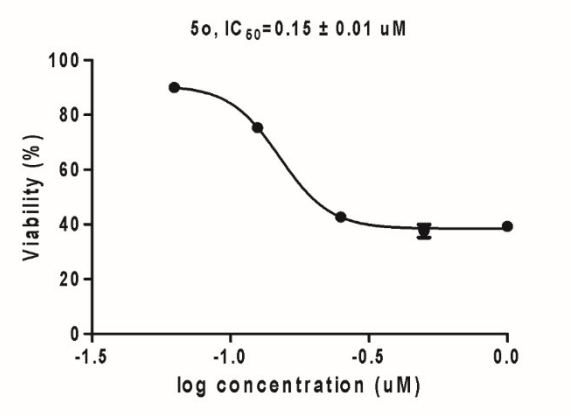

5j, IC50=13.07 ± 1.27 uM
Viability (%)
log concentration (uM)

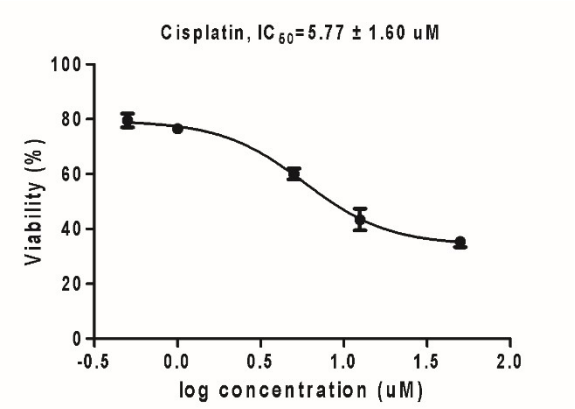

5K, IC50=14.38 ± 3.53 uM
Viability (%)
log concentration (uM)

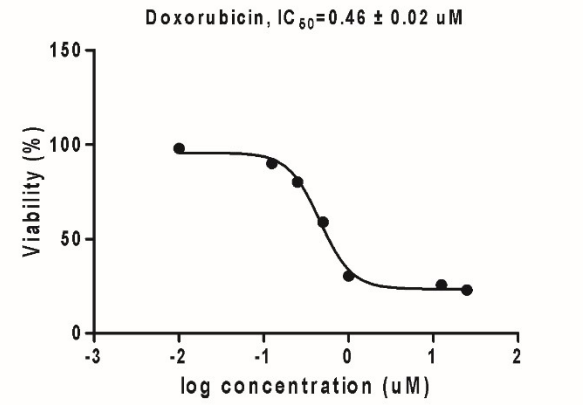

5l, IC50=0.92 ± 0.39 uM
Viability (%)
log concentration (uM)


5m, IC50=0.52 ± 0.12 uM
Viability (%)
log concentration (uM)


5n, IC50=1.38 ± 0.23 uM
Viability (%)
log concentration (uM)


5o, IC50=0.15 ± 0.01 uM
Viability (%)
log concentration (uM)


Cisplatin, IC50=5.77 ± 1.60 uM
Viability (%)
log concentration (uM)


Doxorubicin, IC50=0.46 ± 0.02 uM
Viability (%)
log concentration (uM)

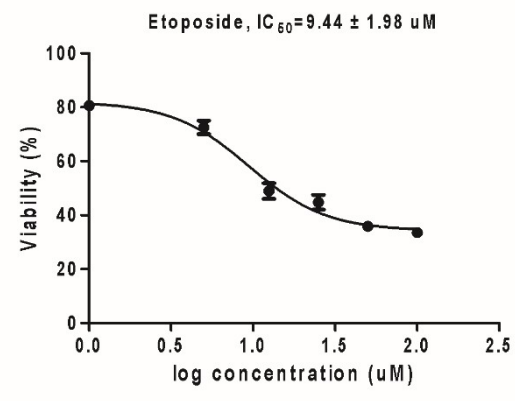
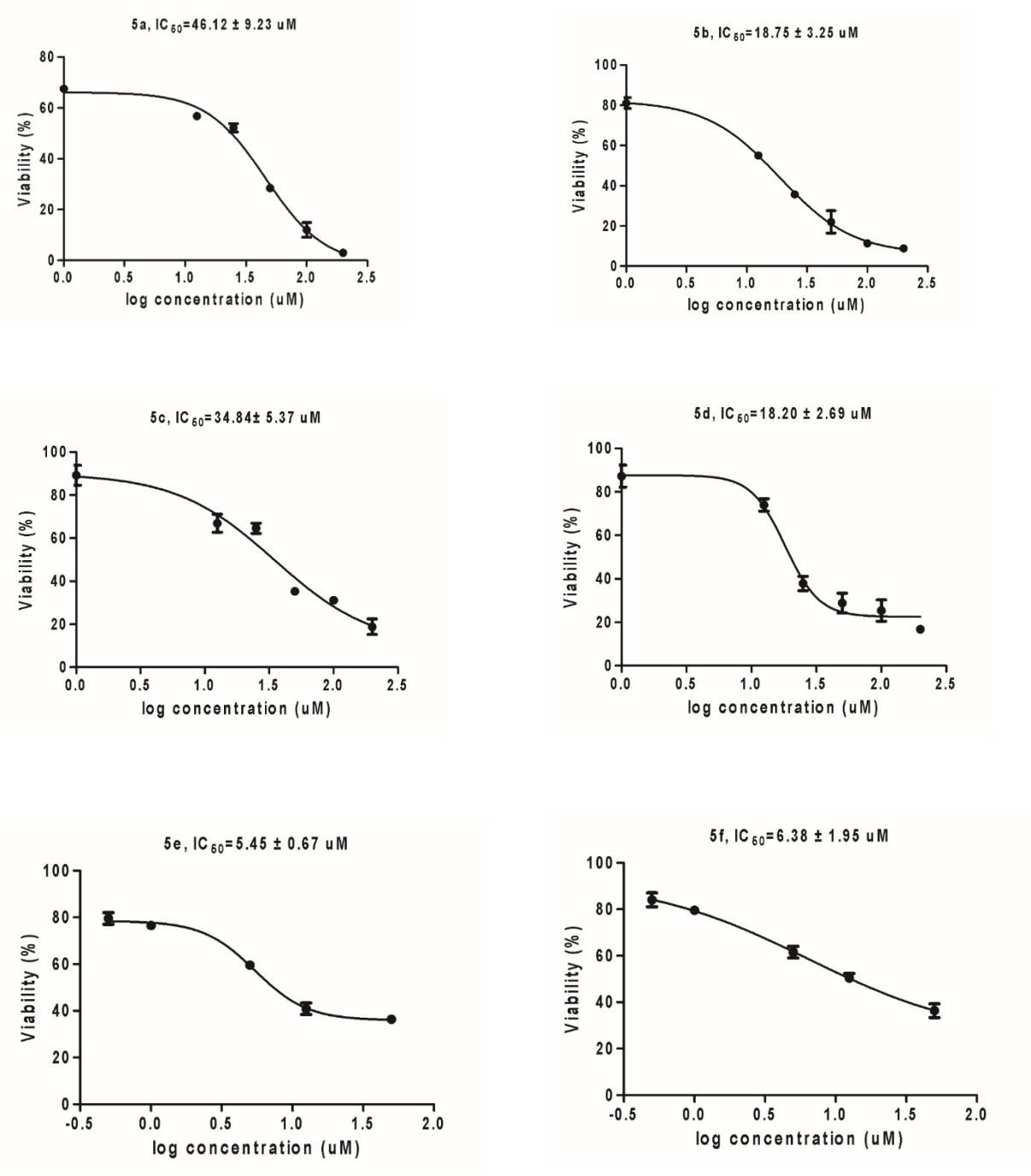
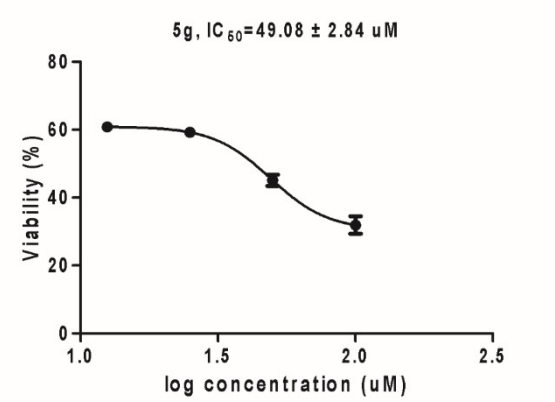
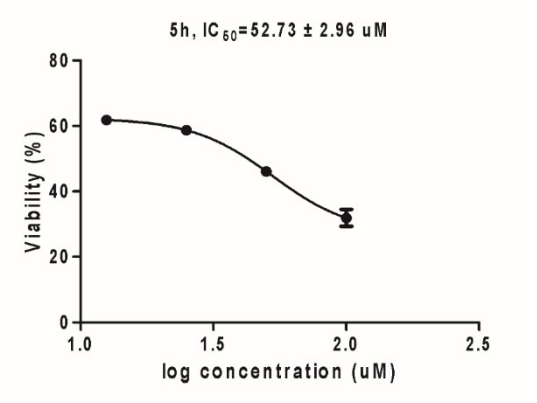
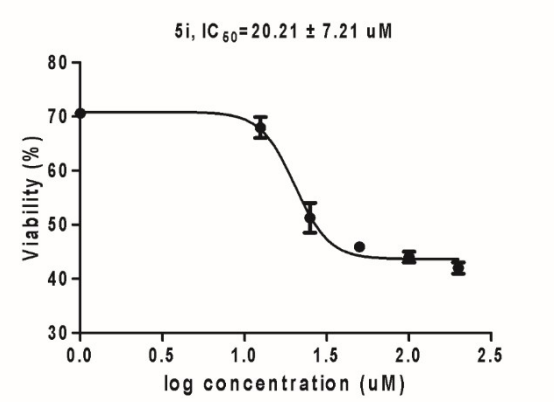

Etoposide, IC50=9.44 ± 1.98 uM
Viability (%)
log concentration (uM)

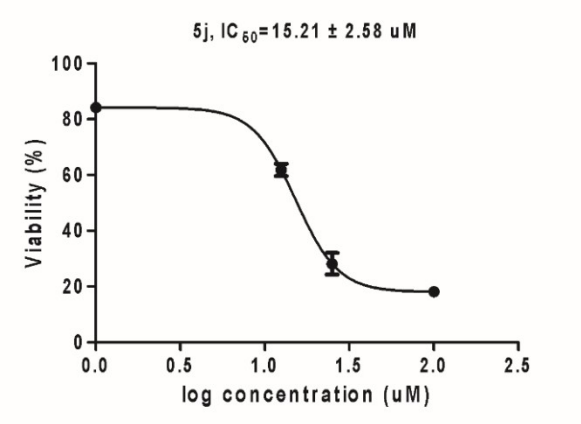
Figure S34. Cytotoxicity of **5a-o**, doxorubicin, cisplatin, and etoposide against SW480 cells.

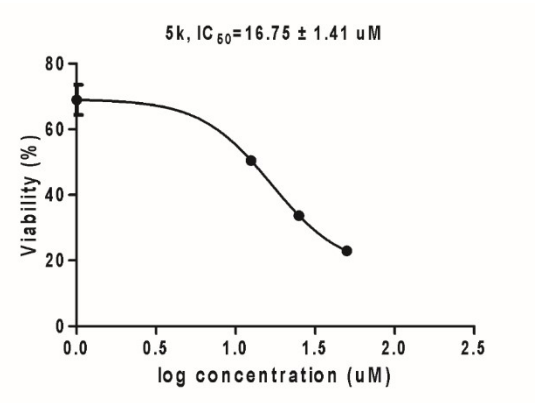


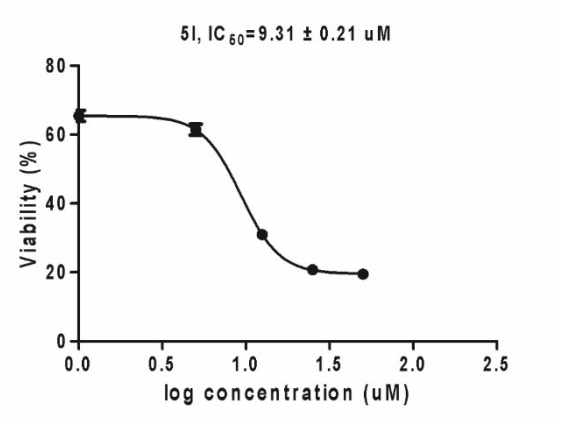

5g, IC60=49.08 ± 2.84 uM
Viability (%)
log concentration (uM)

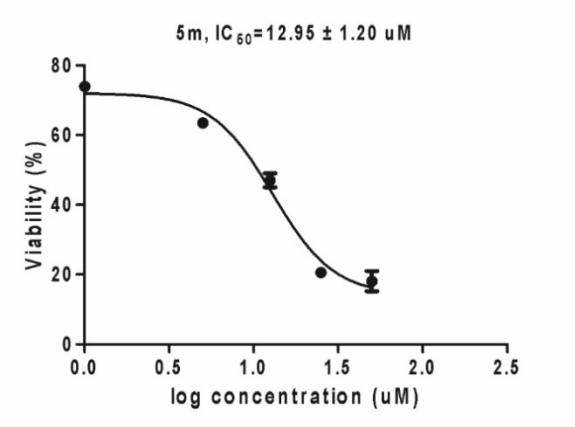

5h, IC60=52.73 ± 2.96 uM
Viability (%)
log concentration (uM)

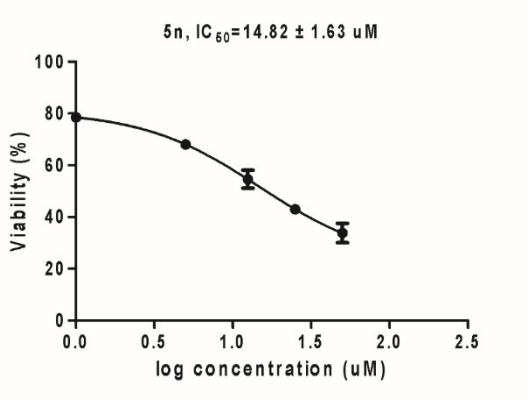

5i, IC60=20.21 ± 7.21 uM
Viability (%)
log concentration (uM)

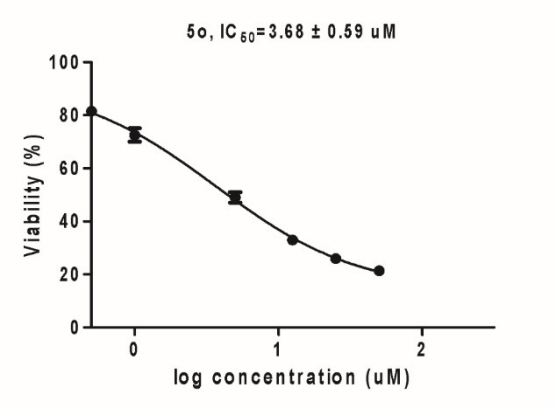

5j, IC60=15.21 ± 2.58 uM
Viability (%)
log concentration (uM)

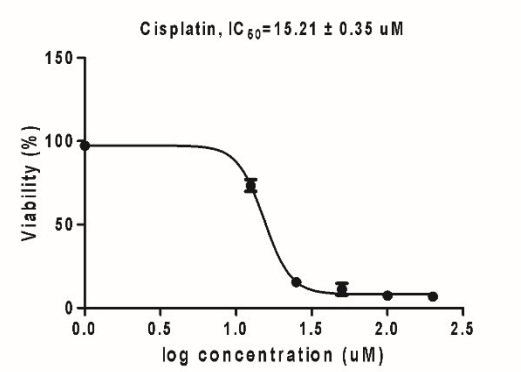

5k, IC60=16.75 ± 1.41 uM
Viability (%)
log concentration (uM)

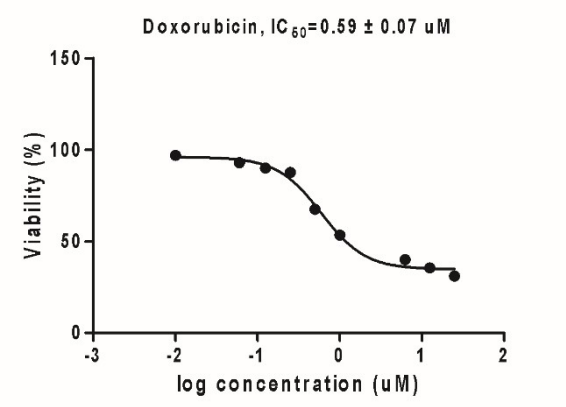

5l, IC60=9.31 ± 0.21 uM
Viability (%)
log concentration (uM)


5m, IC50=12.95 ± 1.20 uM
Viability (%)
log concentration (uM)


5n, IC50=14.82 ± 1.63 uM
Viability (%)
log concentration (uM)


5o, IC50=3.68 ± 0.59 uM
Viability (%)
log concentration (uM)


Cisplatin, IC50=15.21 ± 0.35 uM
Viability (%)
log concentration (uM)


Doxorubicin, IC50=0.59 ± 0.07 uM
Viability (%)
log concentration (uM)

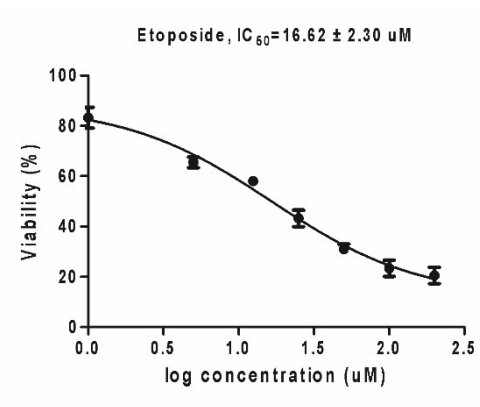
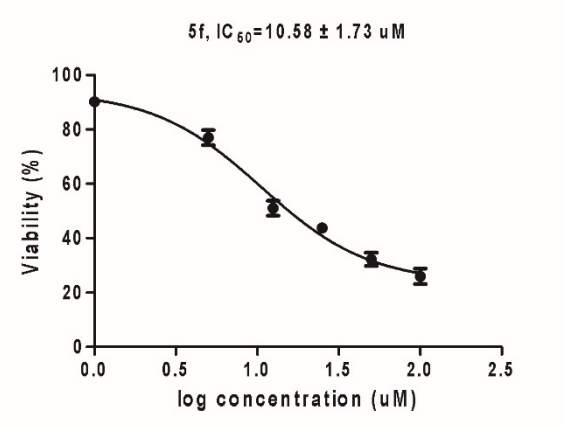
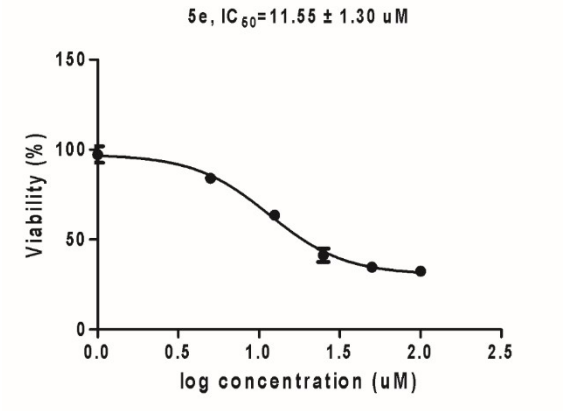
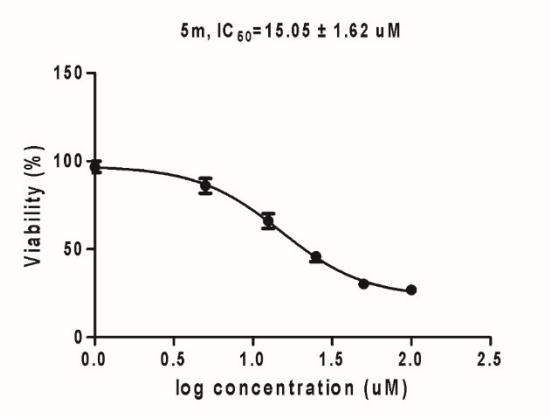
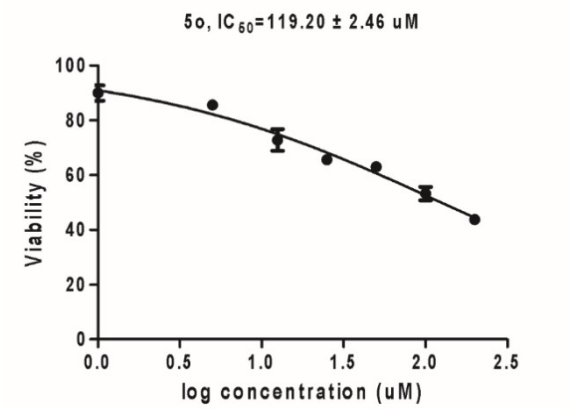

Etoposide, IC50=16.62 ± 2.30 uM
Viability (%)
log concentration (uM)

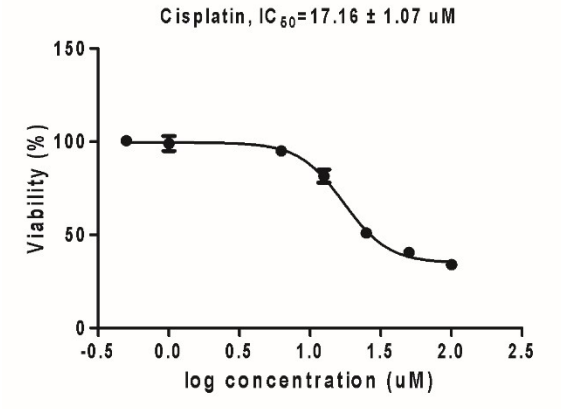
Figure S35. Cytotoxicity of **5e**, **5m**, **5o**, doxorubicin, and cisplatin against MRC-5 cells.











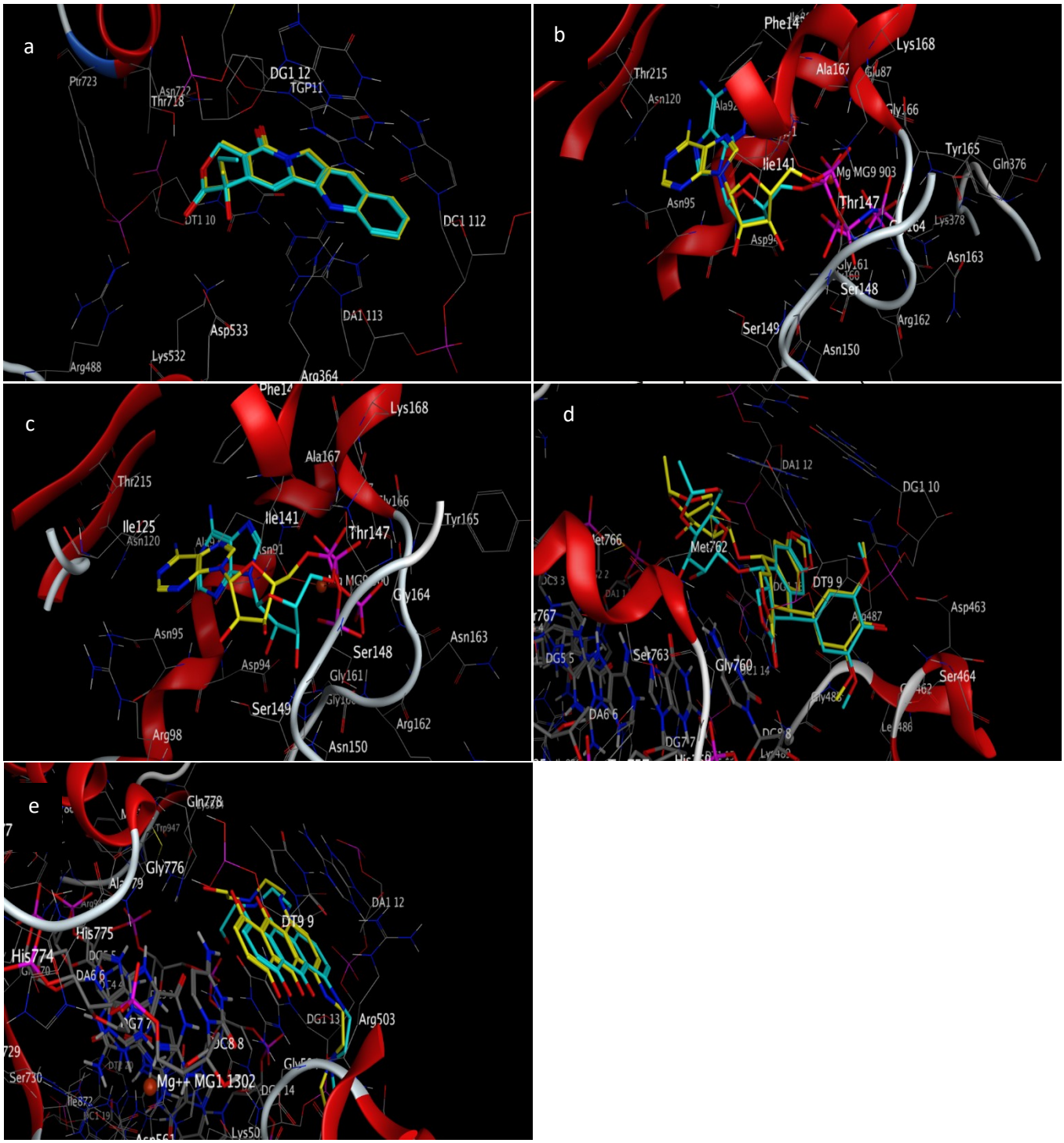


Figure S36. Representation of self-docking results. The co-crystallized inhibitors (cyan) docked into the binding sites and superimposed on co-crystallized inhibitors (yellow) in the

crystal structures of topoisomerases: a) Human Topo I- DNA (PDB ID: 1T8I), b) Human Topo IIα ATPase-no DNA (PDB ID: 1ZXM), c) Human Topo IIα ATPase-no DNA (PDB ID: 1ZXN), d) Human Topo IIα- DNA (PDB ID: 5GWK), and e) Human Topo IIβ-DNA (PDB ID: 4G0V).