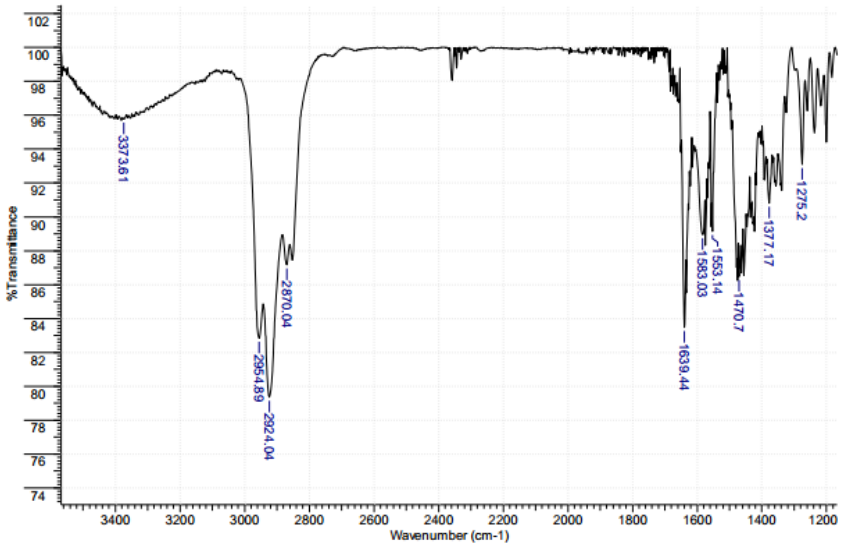


**Figure S1.** IR spectrum of compound **3**.

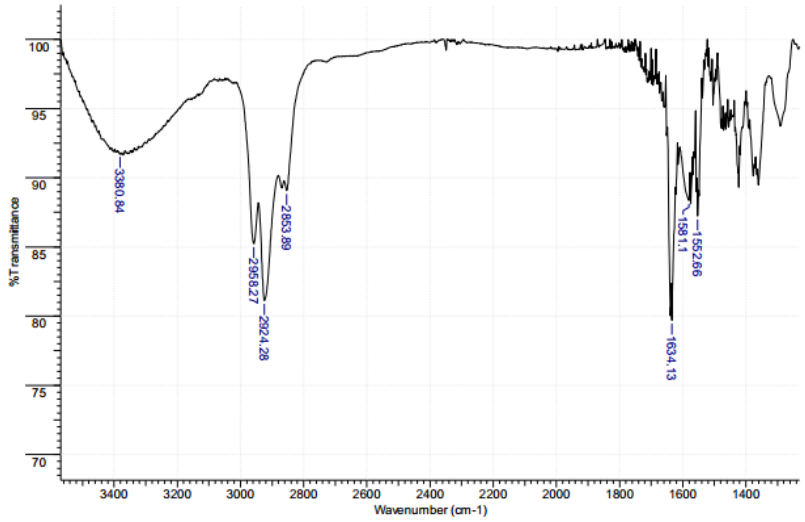


**Figure S2.** IR spectrum of compound **4**.

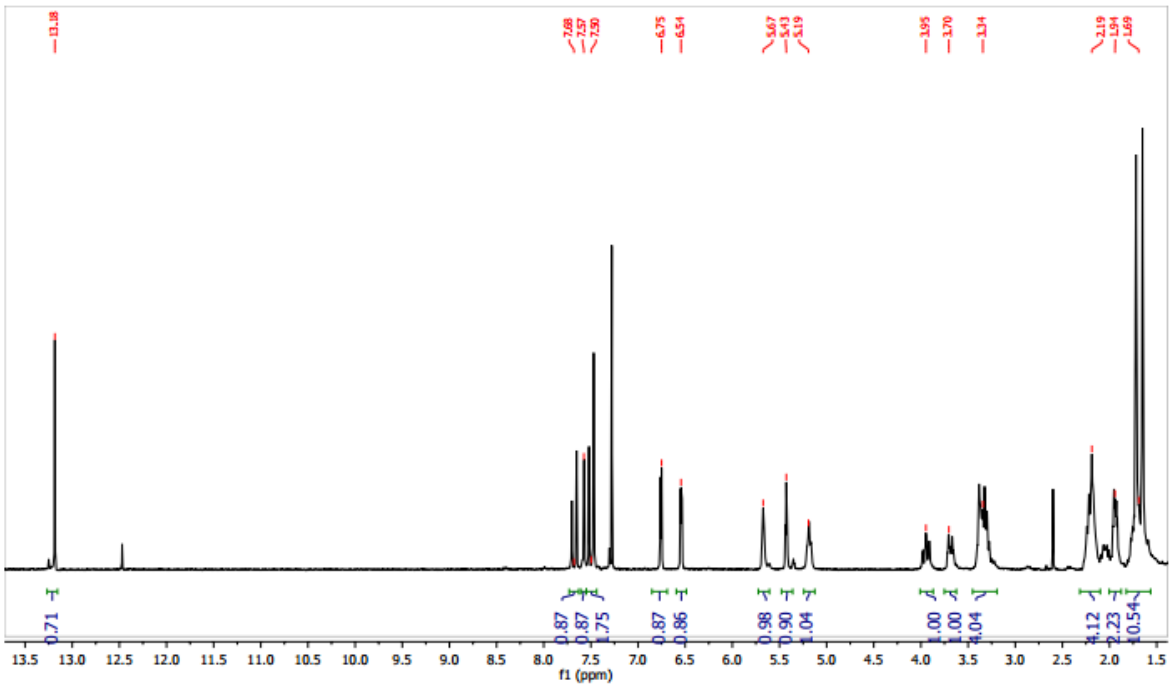


**Figure S3.** $^{1}$H NMR spectrum of compound **3**.

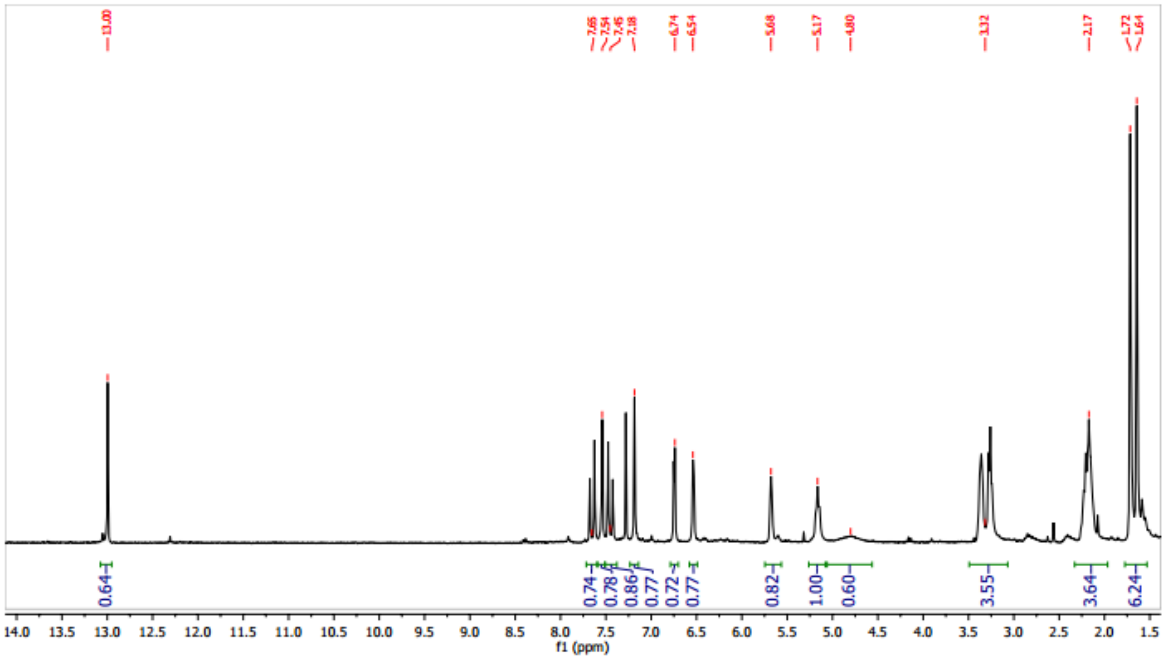


**Figure S4.** $^{1}$H NMR spectrum of compound **4**.

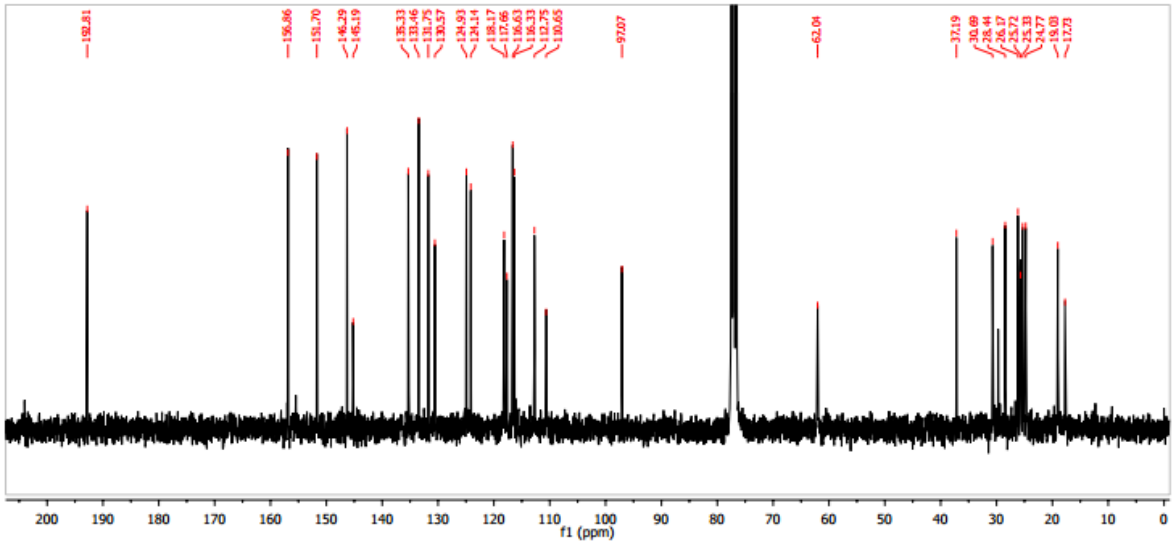


**Figure S5.** $^{13}C$ NMR spectrum of compound **3**.

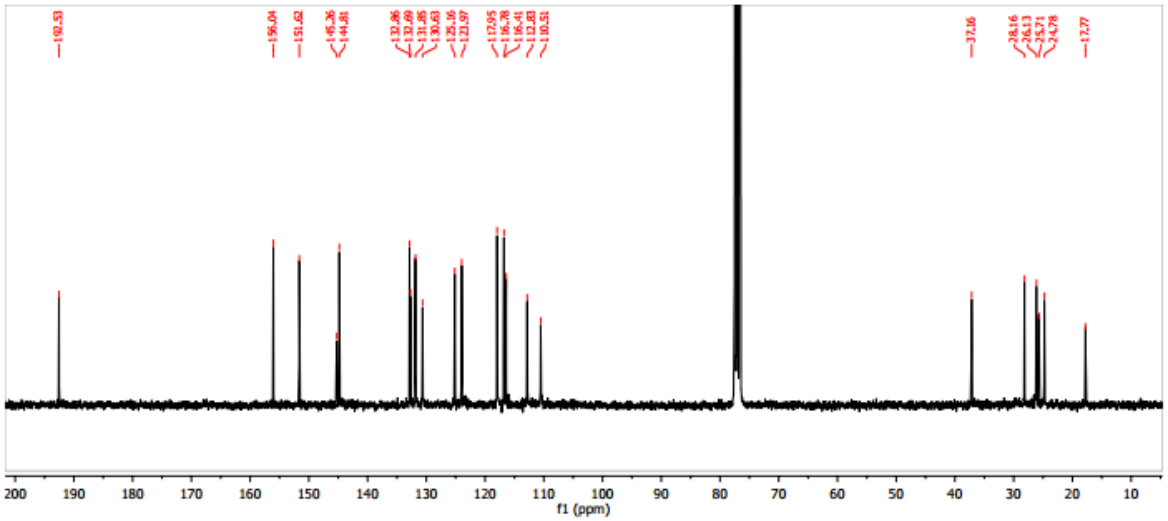


**Figure S6.** $^{13}C$ NMR spectrum of compound **4**.

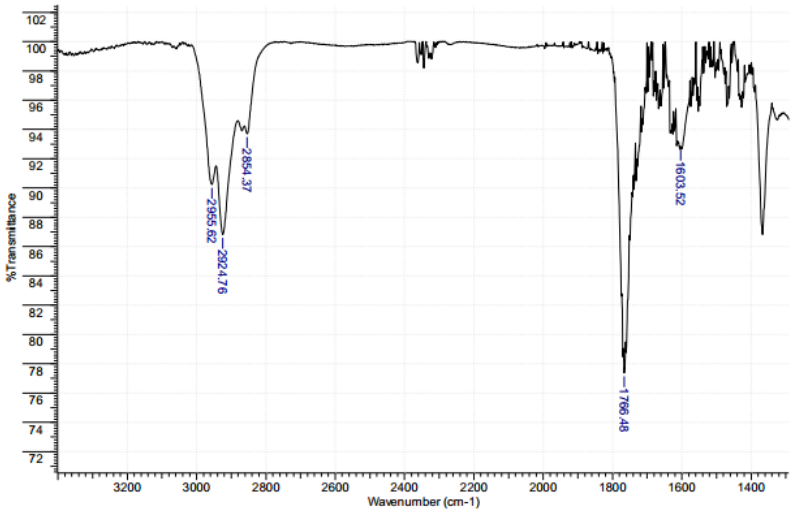


**Figure S7.** IR spectrum of compound **5**.

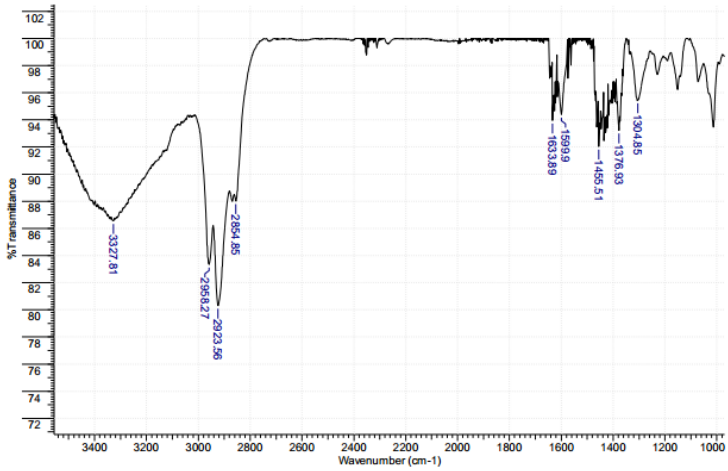


**Figure S8.** IR spectrum of compound **6**.

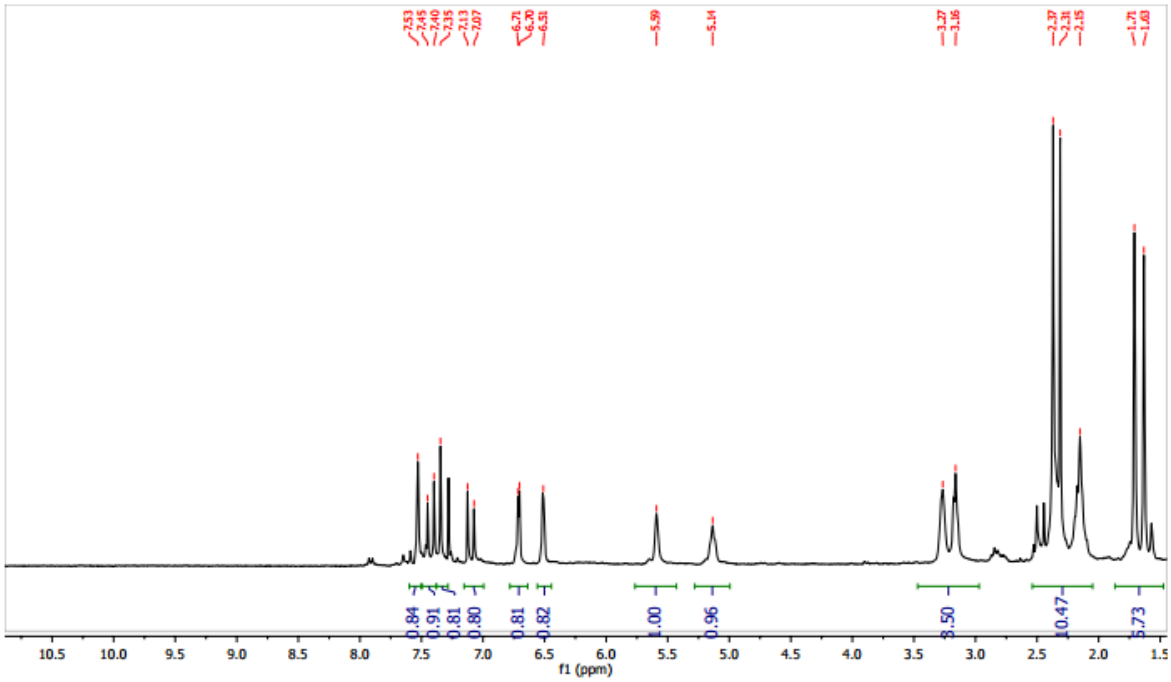


**Figure S9.** $^{1}$H NMR spectrum of compound **5**.

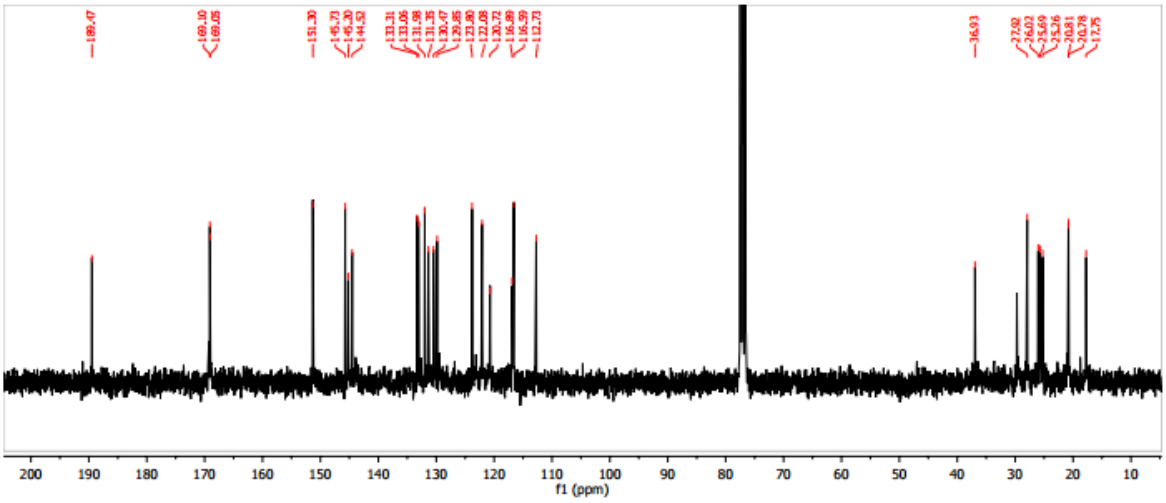


**Figure S10.** $^{13}C$ NMR spectrum of compound **5**.

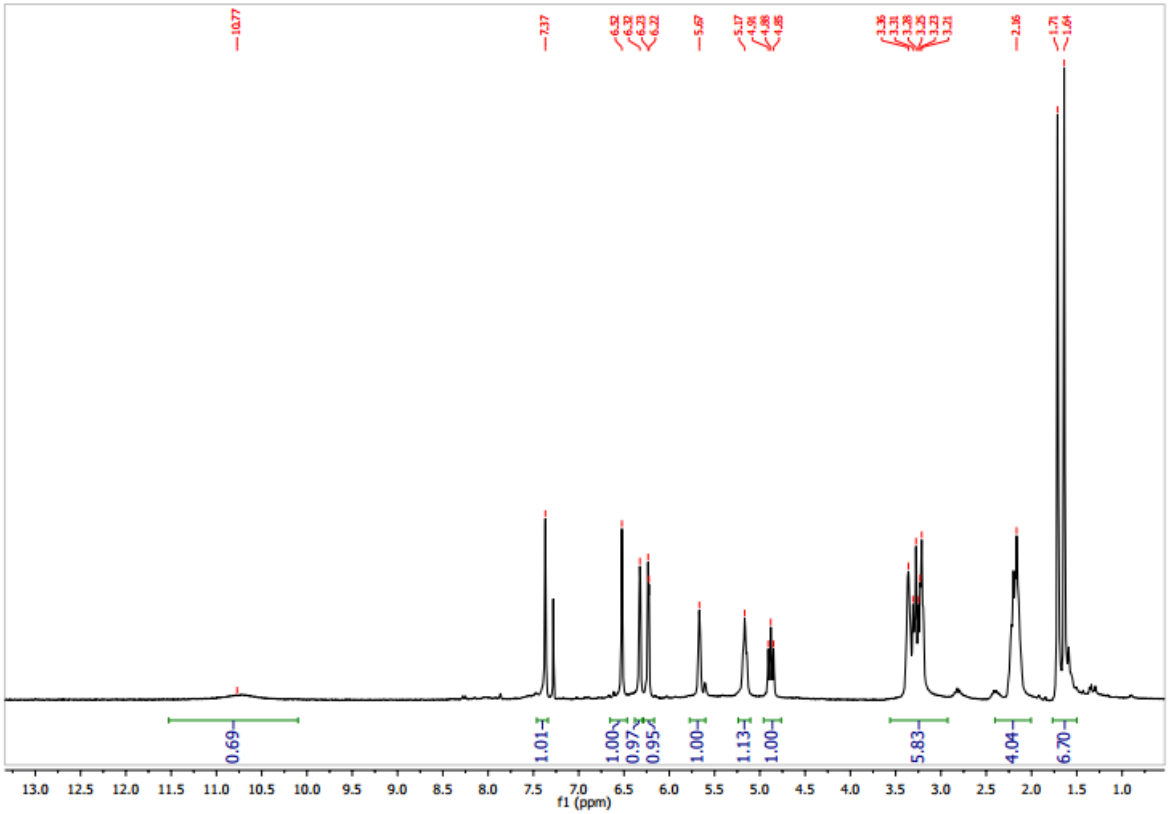


**Figure S11.** $^{1}$H NMR spectrum of compound **6**.

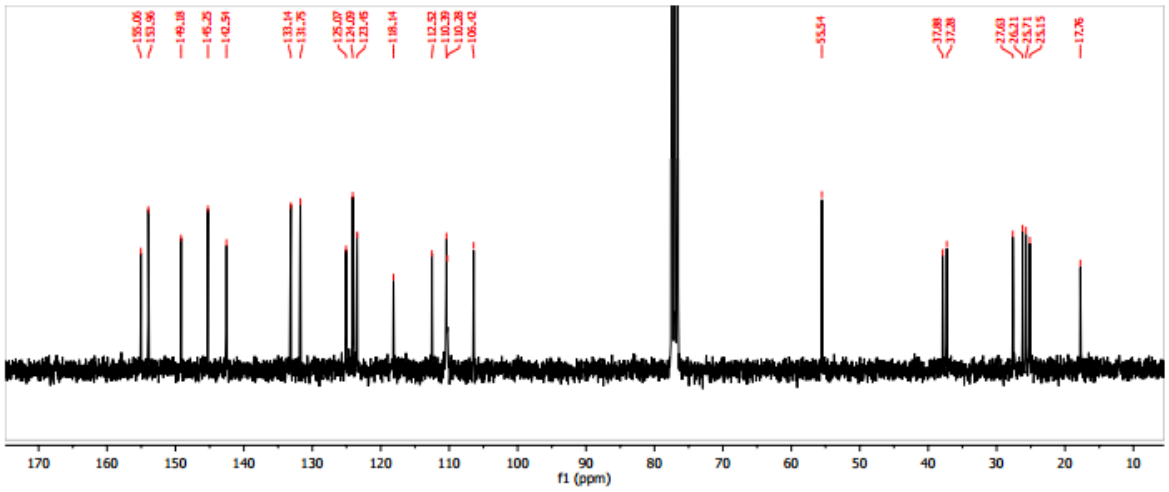


**Figure S12.** $^{13}$C NMR spectrum of compound **6**.

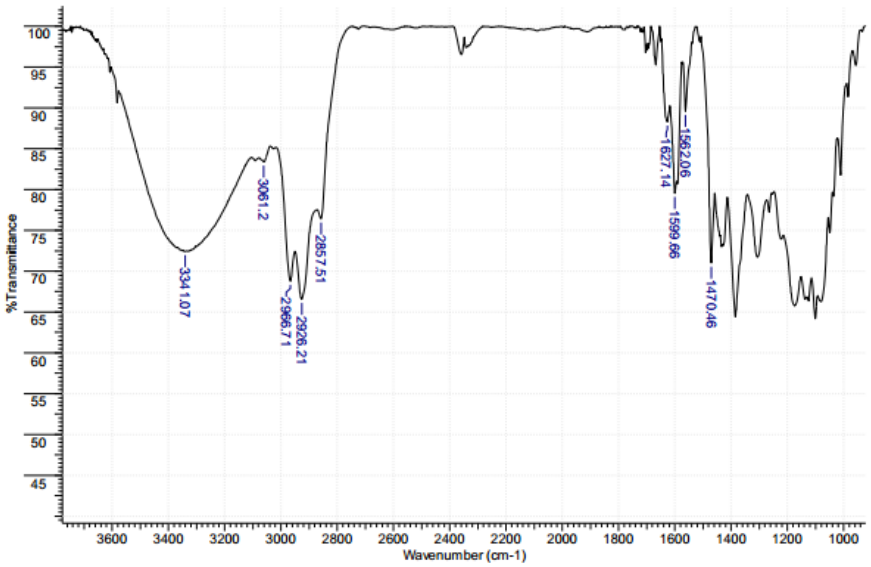


**Figure S13.** IR spectrum of compound **7**.

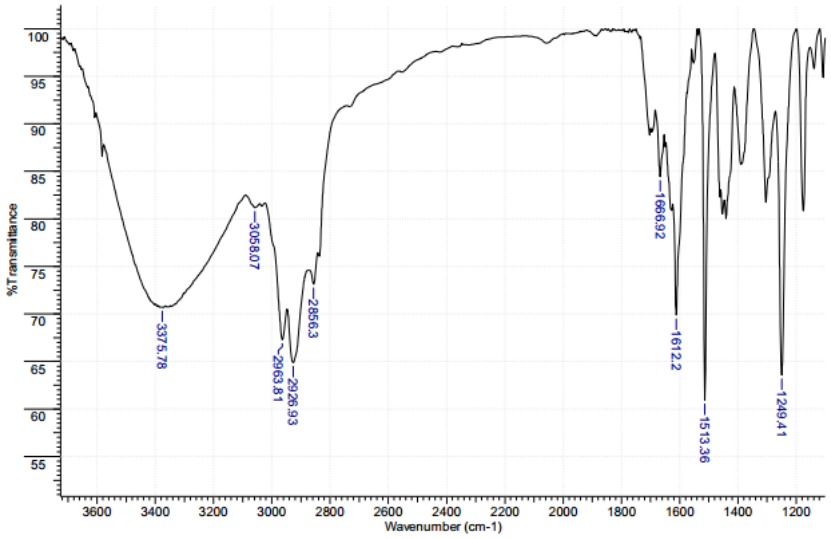


**Figure S14.** IR spectrum of compound **8**.

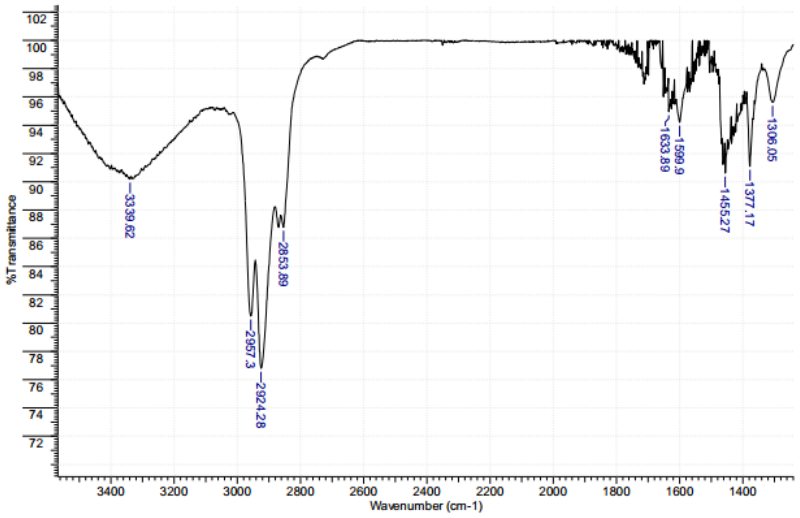


**Figure S15.** IR spectrum of compound **9**.

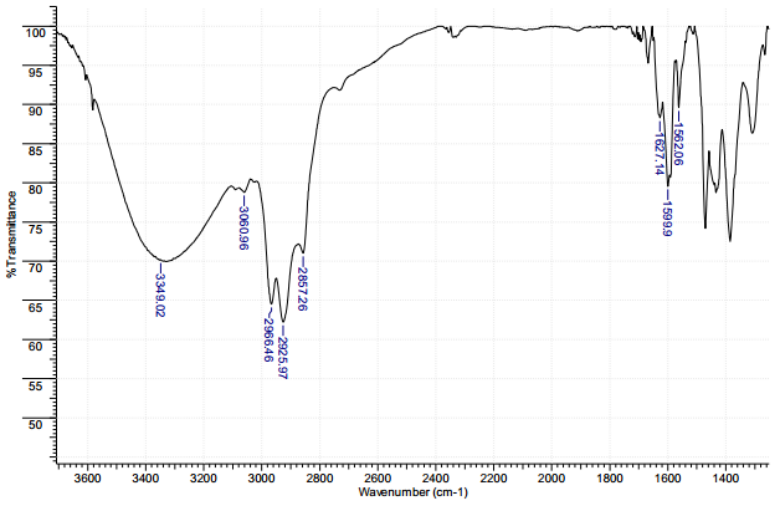


**Figure S16.** IR spectrum of compound **10**.

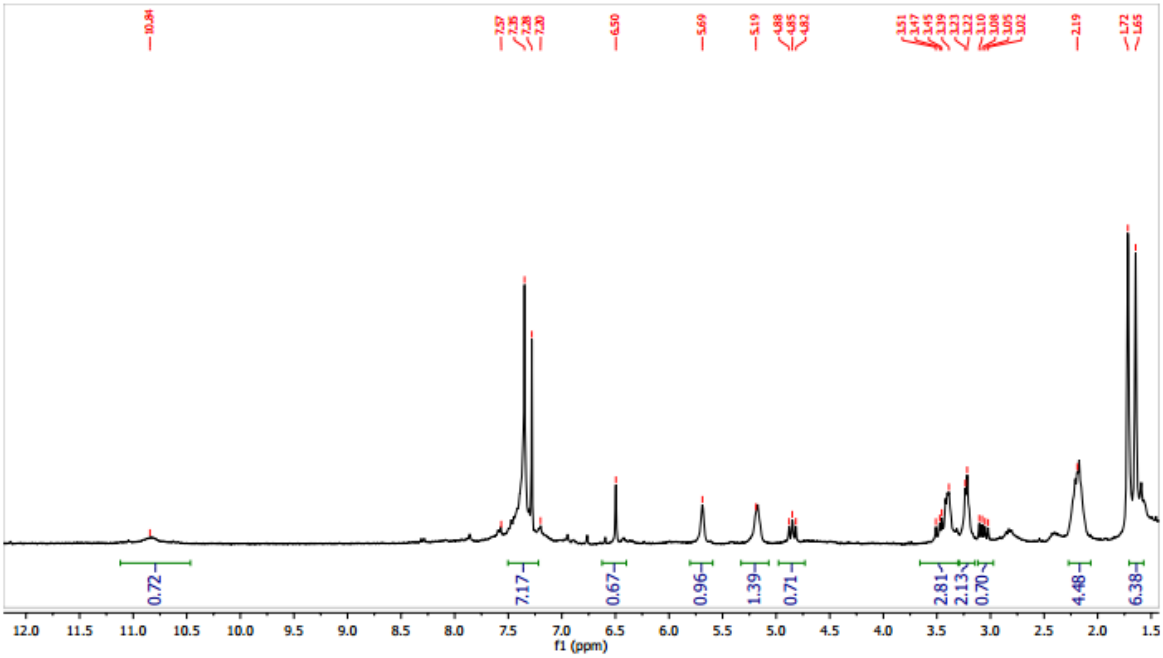


**Figure S17.** $^{1}$H NMR spectrum of compound **7**.

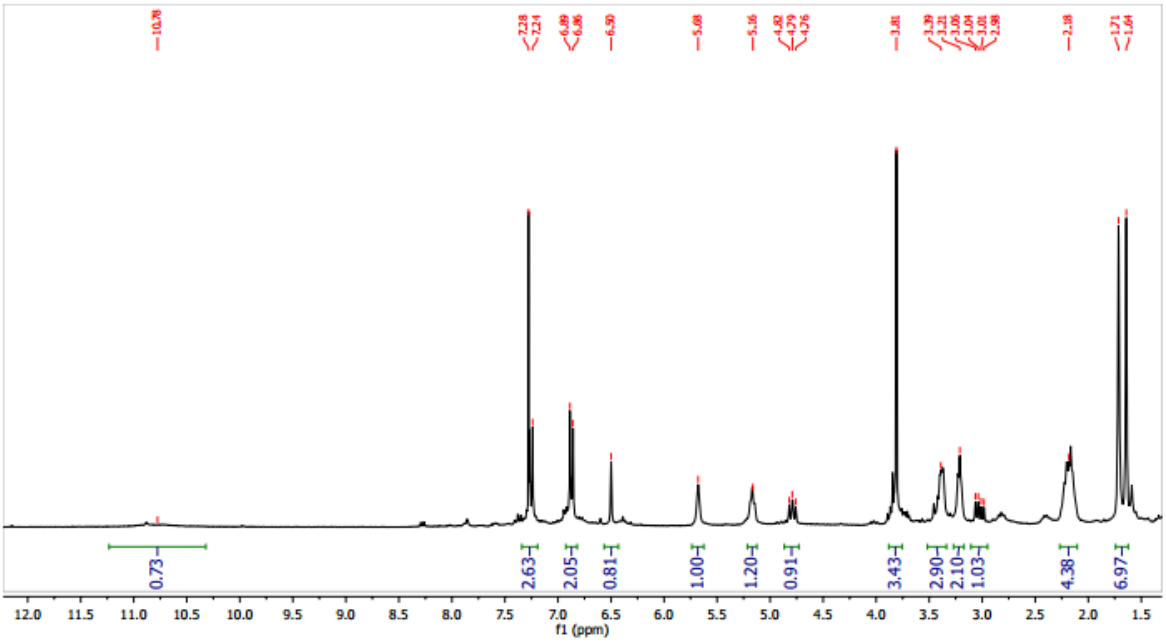


**Figure S18.** $^{1}$H NMR spectrum of compound **8**.

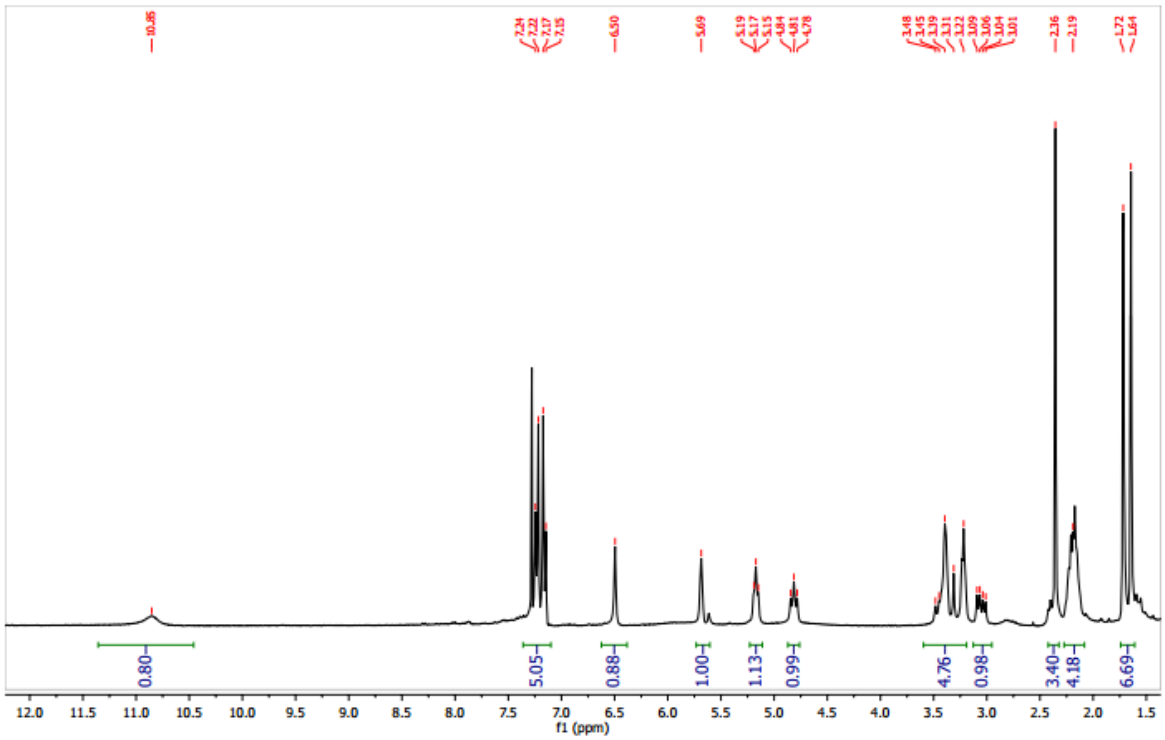


**Figure S19.** $^{1}$H NMR spectrum of compound **9**.

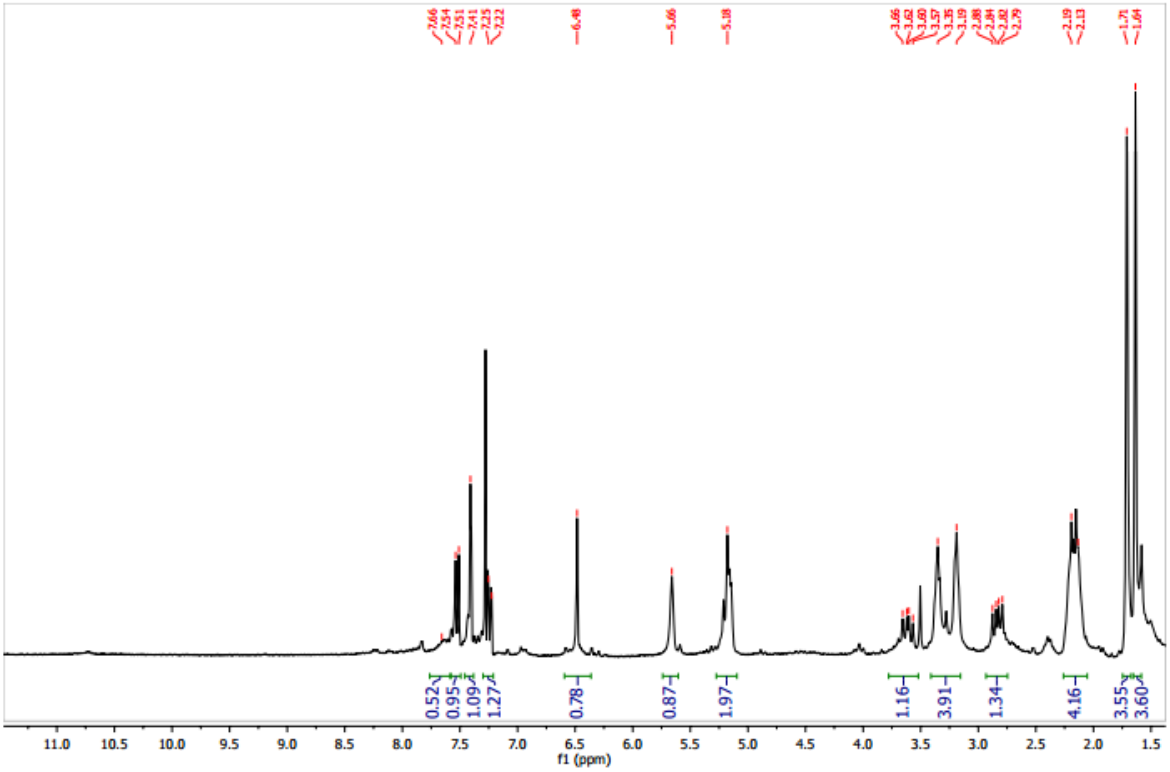


**Figure S20.** $^{1}$H NMR spectrum of compound **10**.

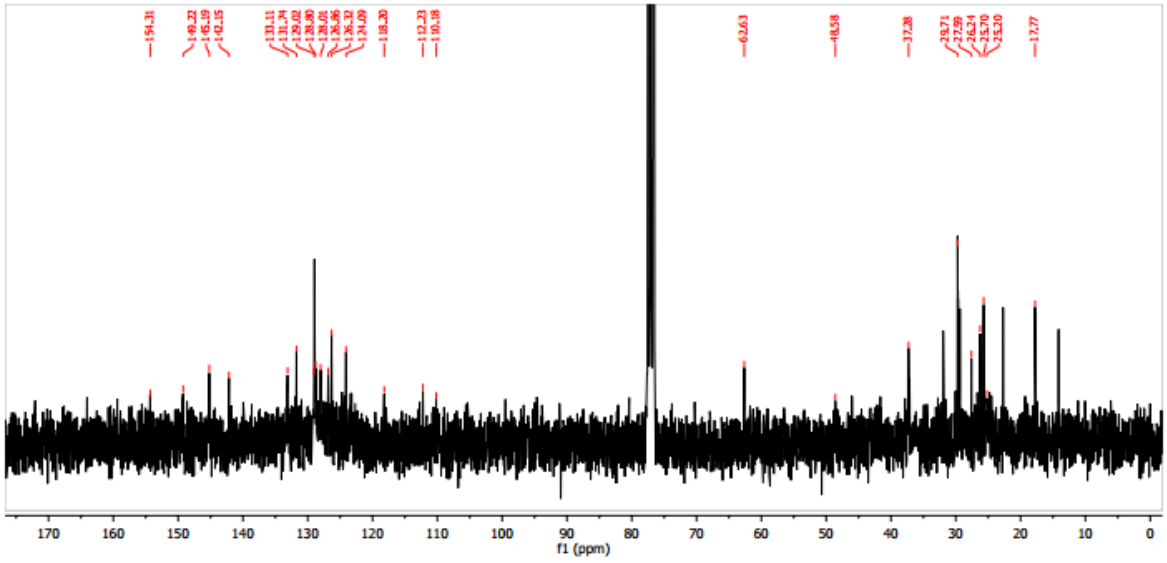


**Figure S21.** $^{13}C$ NMR spectrum of compound **7**.

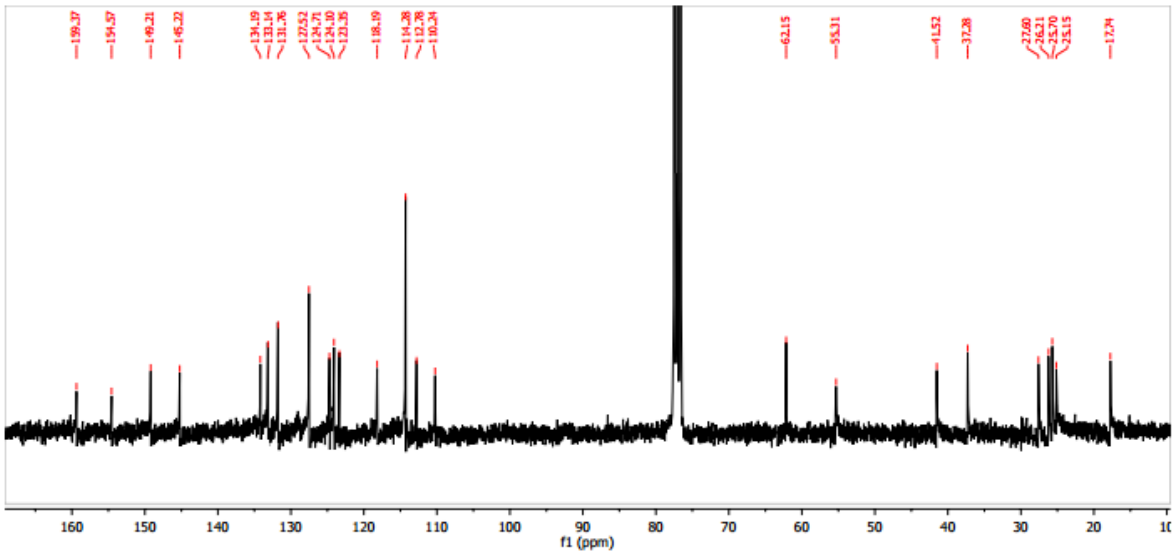


**Figure S22.** $^{13}$C NMR spectrum of compound **8**.

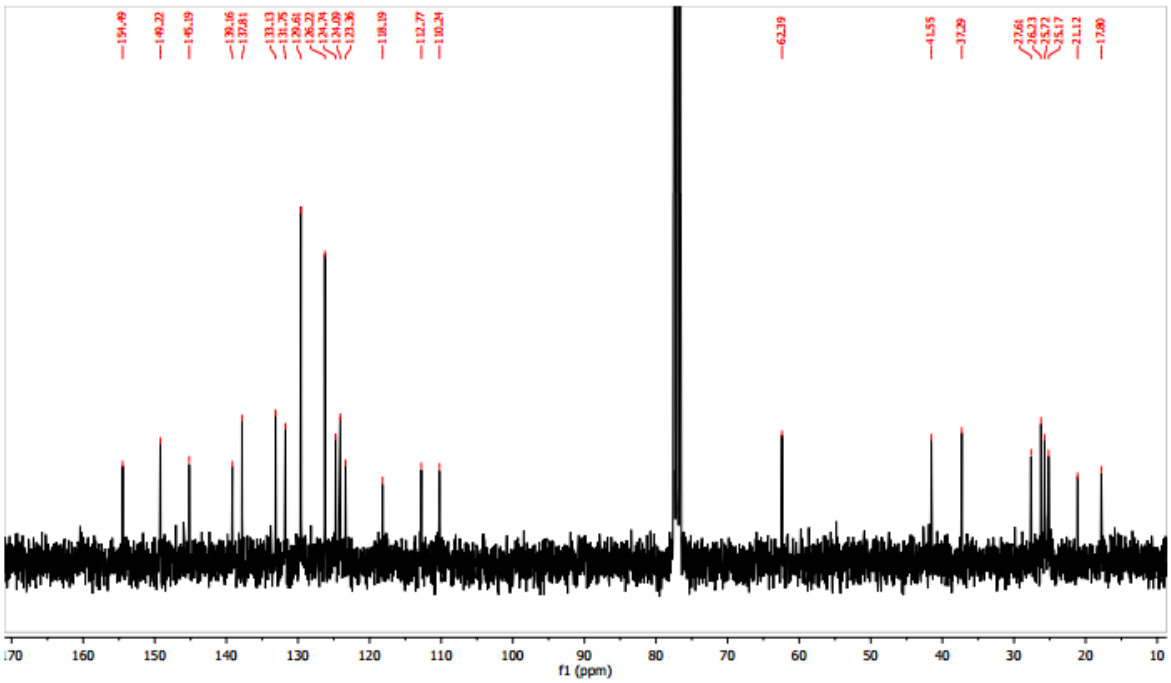


**Figure S23.** $^{13}C$ NMR spectrum of compound **9**.

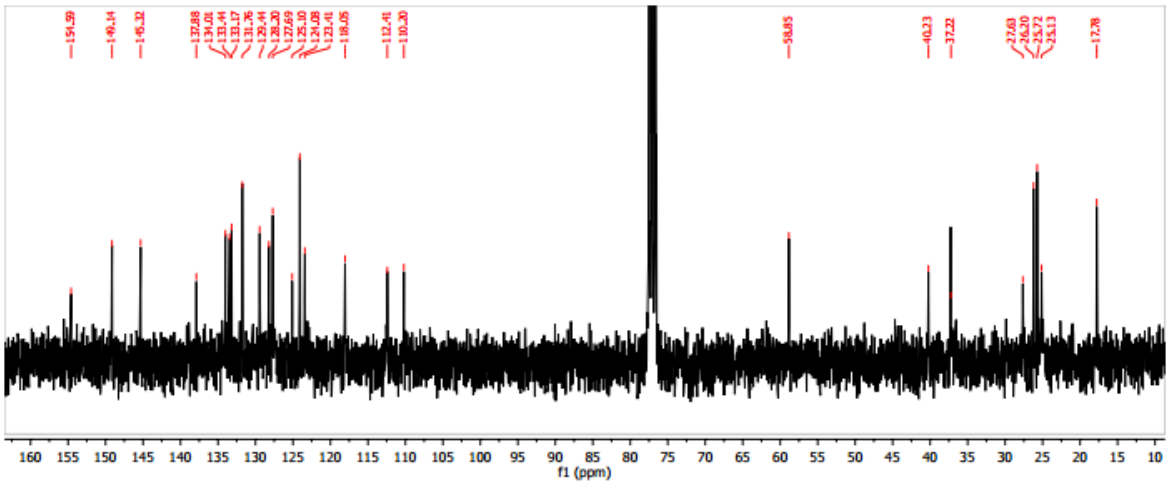


**Figure S24.** $^{13}C$ NMR spectrum of compound **10**.

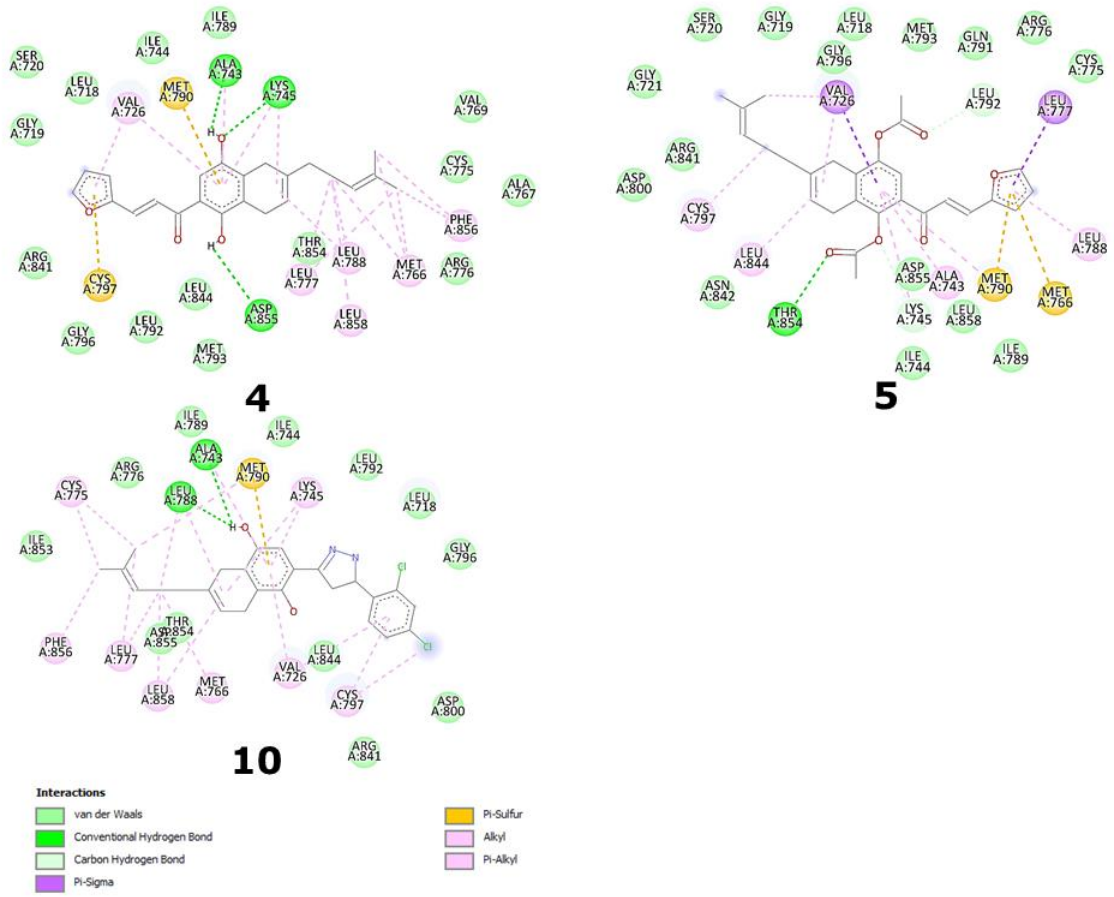


**Figure S25.** Plotted 2D maps of H-bonds and hydrophobic interactions of CHBQs 4 and 5, and PIBHQ 10 with EGFR residues.

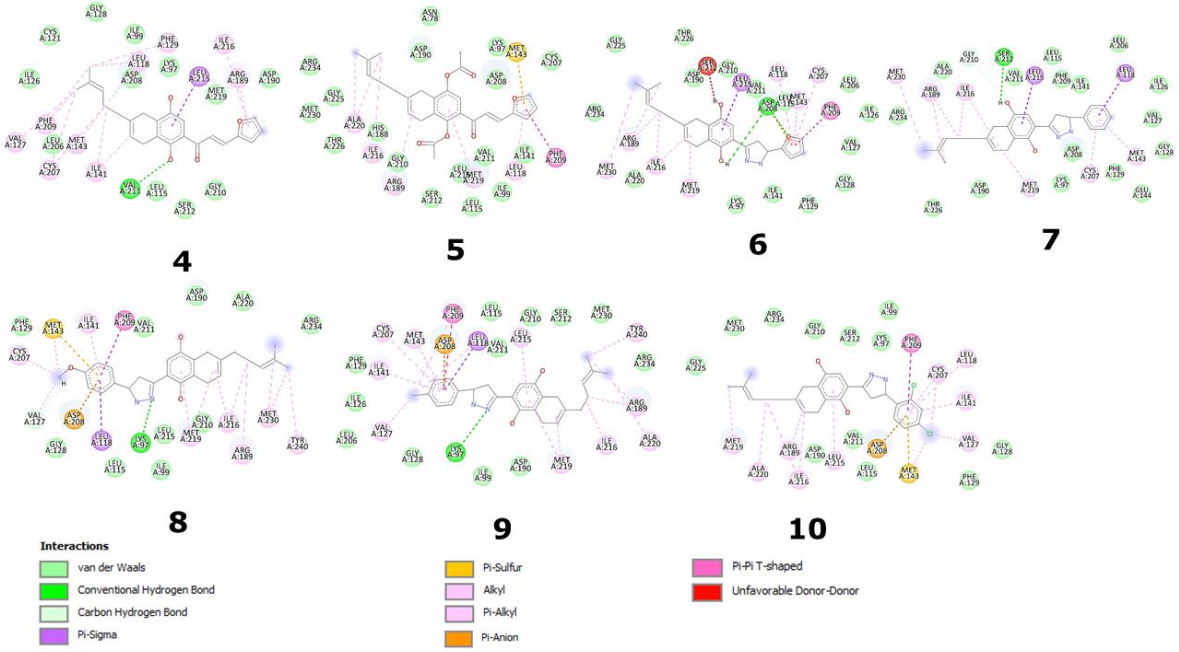


**Figure S26.** Plotted 2D maps of H-bonds and hydrophobic interactions of CHBQs **4** and **5**, and PIBHQs **6–10** with MEK1 residues.

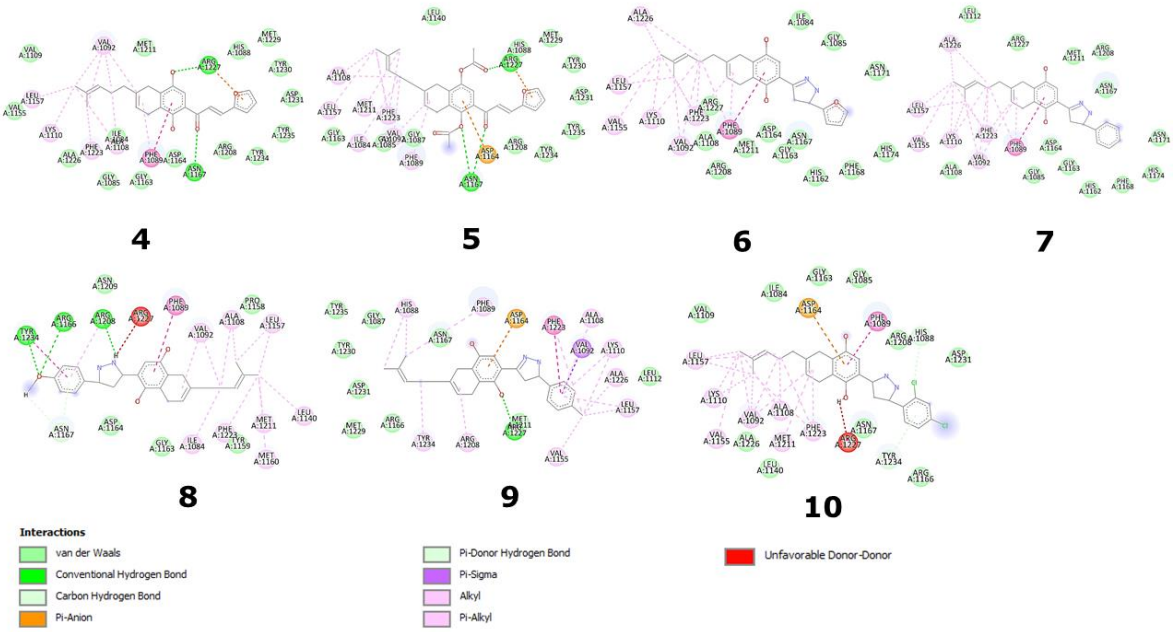


**Figure S27.** Plotted 2D maps of H-bonds and hydrophobic interactions of CHBQs **4** and **5**, and PIBHQs **6–10** with c-MET residues.

**Table S1.** Predicted binding free energy values ($\Delta G_{bin}$ kcal/mol) of chalcone/pyrazoline-1,4-benzohydroquinone hybrids with selected proteins overexpressed in cancer.

| Compounds | Target proteins | | | | | | | | | | | | | | | | Total avge. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DHFR | COX-2 | FGFR-2 | VEGRF-2 | NR3A1 | EGFR | HER2 | NR3A2 | c-MET | TRKA | ERK2 | MEK1 | CK4 | TPK | TopoII | TUB | |
| **4** | -9.1 | -9.2 | -8.9 | -9.9 | -8.3 | -10.7 | -7.8 | -9.0 | -9.7 | -9.2 | -9.2 | -9.6 | -8.4 | -8.6 | -8.3 | -8.7 | -9.11 |
| **5** | -7.2 | -8.3 | -8.7 | -7.4 | -8.3 | -8.5 | -7.6 | -6.9 | -9.5 | -9.2 | -8.8 | -8.5 | -8.3 | -8.5 | -7.5 | -8.6 | -8.26 |
| **6** | -8.7 | -9-0 | -9.1 | -10.2 | -8.7 | -11.3 | -9.1 | -9.4 | -10.3 | -9.5 | -9.9 | -10.0 | -7.9 | -10.0 | -8.6 | -9.0 | -9.55 |
| **7** | -9.5 | -8.6 | -9.6 | -10.2 | -8.8 | -11.4 | -9.0 | -10.0 | -9.8 | -10.0 | -9.5 | -10.7 | -8.7 | -10.4 | -8.5 | -9.5 | -9.82 |
| **8** | -9.5 | -8.5 | -9.3 | -8.7 | -8.5 | -11.0 | -7.8 | -9.9 | -9.7 | -9.3 | -9.5 | -10.3 | -8.8 | -10.2 | -8.5 | -9.2 | -9.42 |
| **9** | -9.5 | -8.9 | -9.7 | -10 | -8.9 | -11.2 | -9.2 | -10.3 | -9.9 | -10.1 | -9.9 | -10.6 | -9.0 | -10.6 | -9.8 | -9.6 | -9.92 |
| **10** | -9.6 | -8.7 | -9.8 | -9.9 | -8.8 | -11.4 | -8.5 | -9.9 | -9.9 | -9.9 | -9.9 | -10.4 | -9,2 | -10.6 | -9.4 | -8.8 | -9.83 |
| **P avge.** | -9.01 | -8.74 | -9.30 | -9.47 | -8.61 | **-10.79** | -8.43 | -9.34 | **-9.83** | -9.60 | -9.53 | **-10.01** | -8.61 | **-9.84** | -8.66 | -9.06 | |
| **Doxorubicin** | -7.7 | -8.3 | -9.0 | -9.1 | -7.7 | -8.9 | -6.2 | -8.2 | -8.7 | -9.0 | -9.2 | -9.1 | -7.4 | -10.1 | -7.7 | -8.1 | |

Proteins with their respective (PDB) entries: **DHFR**: Dihydrofolate reductase (1DLS); **COX-2**: Cyclooxygenase 2 (3LN2); **FGFR-2**: Fibroblast growth factor receptor 2 (1GJO); **VEGRF-2**: Vascular endothelial growth factor receptor 2 (3VHE); **NR3A1**: Estrogen receptor α (3ERT); **EGFR**: Epidermal growth factor receptor (5GTY); **HER2**: Epidermal growth factor receptor 2 (7JXH); **TRKA**: Tropomyosin receptor kinase A (6PL2); **NR3A2**: Estrogen receptor beta (2QTU); **c-MET**: Mesenchymal-epithelial transition factor (3RHK); **TRKA**: Tropomyosin receptor kinase A (6PL2); **ERK2**: Extra-cellular signal-regulated kinase 2 (2OJG); **MEK1**: MAPK/ERK kinase (4AN3); **CK4**: Cyclin-dependent kinase 4 (1PXL); **TPK**: Tyrosine-protein kinase (4EHZ); TopoII: Topoisomerase II (5GWK); **TUB**: alpha tubulin (6WSL). P avge.: Protein average. mean of the $\Delta G_{bin}$ values for the interactions of each protein with all the hybrids; the two proteins with the highest global chalcone/pyrazoline-1,4-benzohydroquinone affinity are highlighted on red color.

**Table S2.** Predicted binding free energy values ($\Delta G_{bin}$, kcal/mol) and binding site contacts of synthesized cytotoxic hybrids with amino acids of TPK, TRKA and CK4.

| Compounds | $\Delta G_{bin}$ | H-Bonds and Hydrophobic Contacts in the Binding Site* |
|---|---|---|
| | | **TPK** (mean $\Delta G_{bin}$ = -9.72 kcal/mol) |
| **4** | -8.9 | Leu881, Gly882, Glu883, **Gly884**, His885, Phe886, Gly887, Lys888, Val889* Ala906, Lys908 *, Met956*, **Asp1003**, **Arg1007**, Asn1008, Leu1010, Gly1020, Asp1021 *, Gly1023, Leu1024, Asp1042 |
| **5** | -8.5 | Gly882, Glu883, Gly884, His885, Phe886, Gly887, Lys888, Val889, Lys908, Leu910, His918, Asp921, Leu922, Lys924, Glu925, Arg1002, Asp1003, Arg1007, Asn1008, Gly1020, Asp1021, Gly1023, Leu1024 *, Lys1026, Val1037, Asp1039*, Asp1042, Pro1044 |
| **6** | -10.0 | Leu881, Gly882, Glu883, **Gly884**, His885*, Phe886*, Gly887, Lys888, Val889, Ala906, Lys908*, Leu910, His918, Asp921, Leu922, Met956, Ser963, Asp1003, **Arg1007**, **Asn1008**, Leu1010, Gly1020, Asp1021*, Gly1023, Leu1024 |
| **7** | -10.4 | Leu881, Gly882, Glu883, Gly884, His885, Phe886, **Gly887**, Lys888, Val889, Ala906, Lys908*, Leu910, His918, Asp921, Leu922, Glu925, Met956, Ser963, **Asp1003**, **Arg1007**, Asn1008, Leu1010, Gly1020, **Asp1021***, Gly1023, Leu1024 |
| **8** | -10.2 | Leu881, Gly882, Glu883, Gly884, His885, Phe886*, **Gly887**, Lys888, Val889, Ala906, Lys908*, His918, Met956, Ser963, Arg1002, **Asp1003**, Arg1007, Asn1008, Leu1010, Gly1020, Asp1021*, Leu1024, Asp1039, Asp1042 |
| **9** | -10.6 | Leu881*, Gly882, Glu883, Gly884, His885, Phe886, Gly887, Lys888, Val889*, Ala906, Lys908*, Leu910, His918, Asp921, Leu922, Phe958, Leu959, Gly962, Ser963, Glu966, Asp1003, **Arg1007**, **Asn1008**, Leu1010*, Asp1021*, Gly1023, Leu1024 |
| | | **TRKA** (mean $\Delta G_{bin}$ = -9.55 kcal/mol) |
| **4** | -9.2 | Leu516*, Gly517, Val524*, Ala542, Lys544, Glu560, Leu564*, Ile572, Val573*, Phe589, **Glu590**, Tyr591, Met592, Gly595, Asp596, Arg599, Leu657, Arg673, Ile666, Gly667, Asp668 *, Phe669 |
| **5** | -9.2 | Leu516, Gly517, Glu518, Gly519, Val524*, Ala542, Lys544, Glu560, Leu564*, Val573*, Phe589*, Glu590, Tyr591, Met592, Gly595, Asp596, Leu657, Gly667, Asp668, Phe669*, Arg673, Ile675, Tyr676 |
| **6** | -9.5 | Leu516, Gly517, Glu518, Val524, Ala542, Lys544, Glu560, Glu564, Val573, Phe589, Glu590, Tyr591, Met592, Gly595, **Asp596**, Leu657, Gly667, Asp668, Phe669*, **Arg673**, Ile675*, Tyr676 |
| **7** | -10.0 | Leu516, Gly517, Val524, Ala542, Lys544, Glu560, Leu564, Val573, Phe589, Glu590, Tyr591, Gly595, **Asp596**, Arg599, Leu657, Leu667, Asp668, Ile675*, Arg673, Tyr676, Asp668, Phe669* |
| **8** | -9.3 | Leu516, Gly517, Val524*, Ala542*, Lys544, Glu560, Leu564*, Val573*, Phe589*, Tyr591, Met592, His594, Gly595, Asp596, Arg599, Leu657, Gly667, Asp668, Phe669 *, Arg673 |
| **9** | -10.1 | Leu516*, Val524, Ala542, Lys544, Glu560, Leu564, Val573*, Phe589*, Tyr591, Gly595, Asp596, Arg599, Leu657*, Gly667, Asp668, Phe669 *, Arg673, Ile675, Tyr676 |
| | | **CK4** (mean $\Delta G_{bin}$ = -8.52 kcal/mol) |
| **4** | -8.4 | Ile10, Gly11, Glu12, Gly13, Thr14, Val18, Lys33, Phe82, Leu83, His84, Gln85, Asp86, Lys89, Lys129, **Gln131**, **Asn132**, Leu134, Ala144, Asp145, Glu162, Val163, Val164 |
| **5** | -8.3 | Glu8, Ile10, Gly11, **Glu12**, Gly13, Thr14, Val18, Lys20, Ala31, **Lys33**, Phe82, Leu83, His84, Gln85, Asp86*, Lys88, Lys89, Lys129*, Gln131, **Asn132**, Leu134, Ala144, Asp145, Leu298 |
| **6** | -7.9 | **Ile10**, Gly11, Glu12, Glu13, Thr14, Tyr15, Val18, Lys33, Phe82, Leu83, His84, Gln85, Asp86, Lys89, Asp127, Lys129, Gln131, **Asn132***, Leu134, **Asp145** |
| **7** | -8.7 | Ile10*, Gly11, Glu12, Gly13, Thr14, Val18, Lys20, Lys33*, Phe82, **Leu83**, His84, Gln85, Asp86, Lys89, Lys129, Gln131, Asn132, Leu134, Ala144, Asp145, Thr160, Glu162, Val163, Val164 |
| **8** | -8.8 | Glu8, Ile10*, Gly11, Glu12, Gly13, Thr14, Val18, Lys20, Lys33*, Phe82, **Leu83**, His84, Gln85, Asp86, Lys89, Lys129, Gln131, Asn132, Leu134, Ala144, **Asp145**, Thr160, Glu162, Val163, Val164 |
| **9** | -9.0 | Glu8, Ile10*, Gly11, Glu12, Gly13, Thr14, Val18, Lys20, Lys33, Phe82, **Leu83**, His84, Gln85, Asp86, Lys89, Lys129, Gln131, Asn132, Leu134, Ala144, **Asp145**, Thr160, Glu162, Val163, Val164 |

Bolded names correspond to those amino acids involved in H-bonds with the corresponding CBHQs and PIBHQs.
Residues with * correspond to amino acids that interact with the ligand by any type of Pi-interactions