Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Design, Synthesis and Characterization of Novel Inhibitors Against Mycobacterial β-Ketoacyl CoA Reductase FabG4

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Supporting Information

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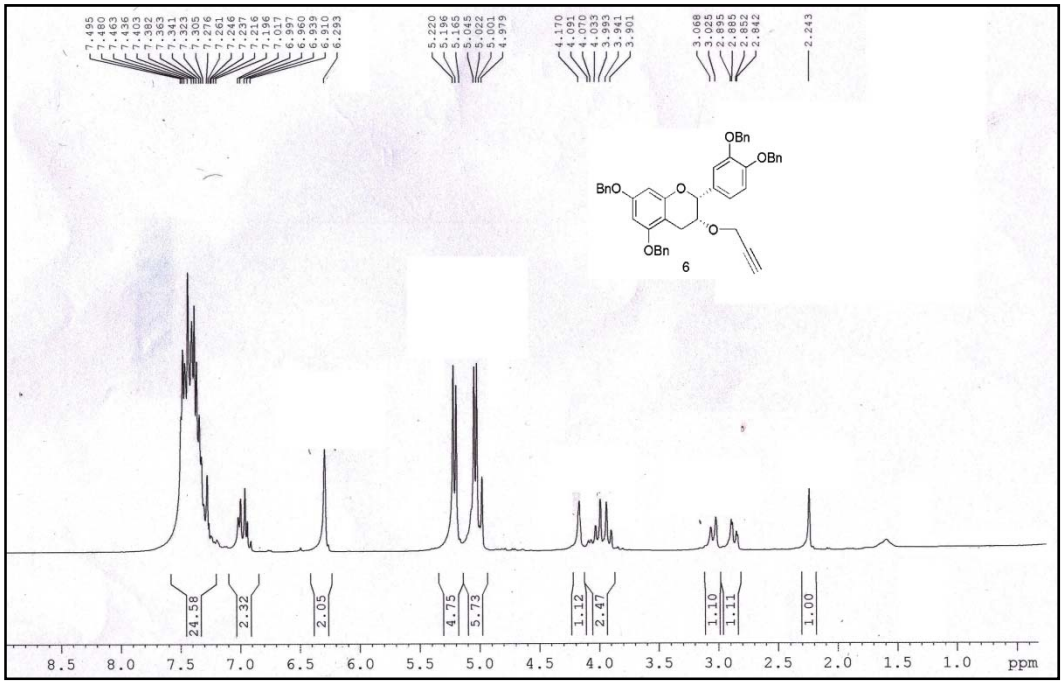
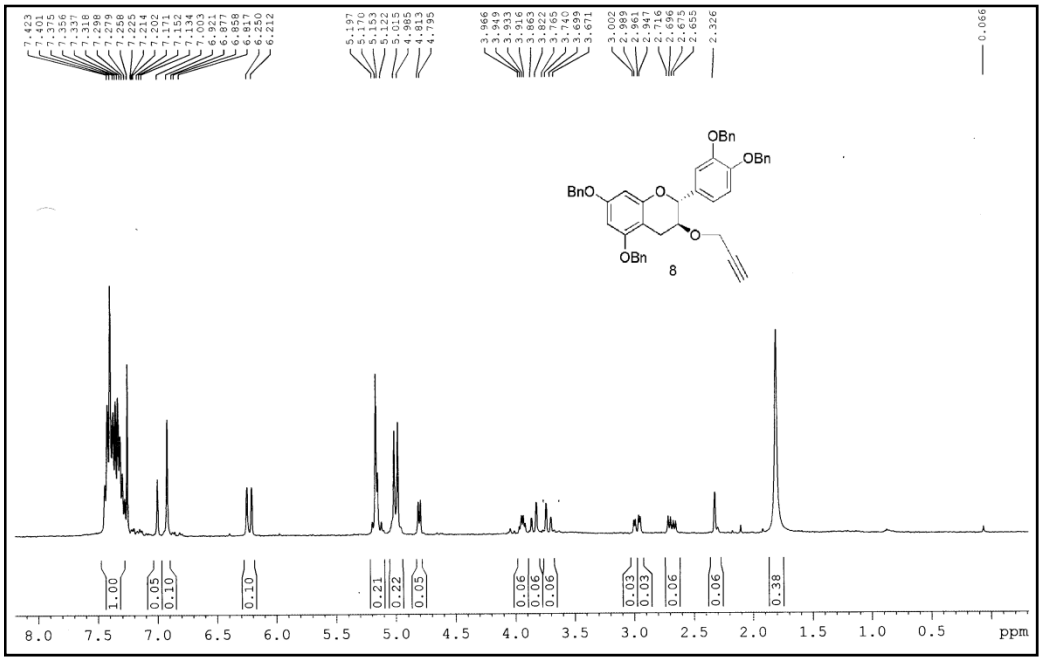
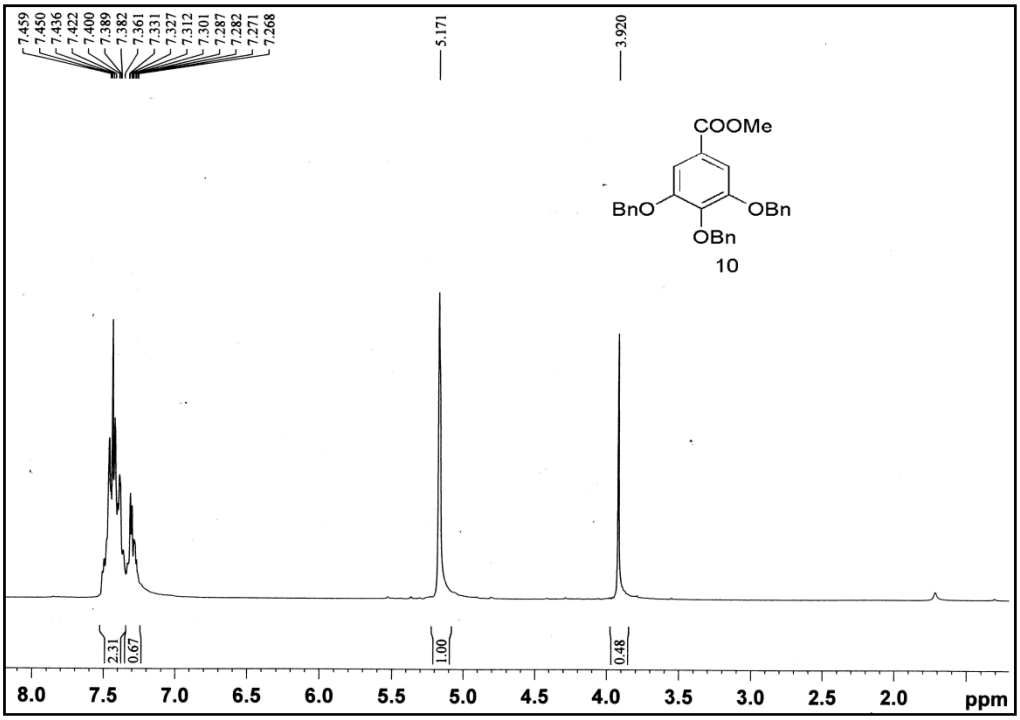
S1. NMR spectra of selected compounds



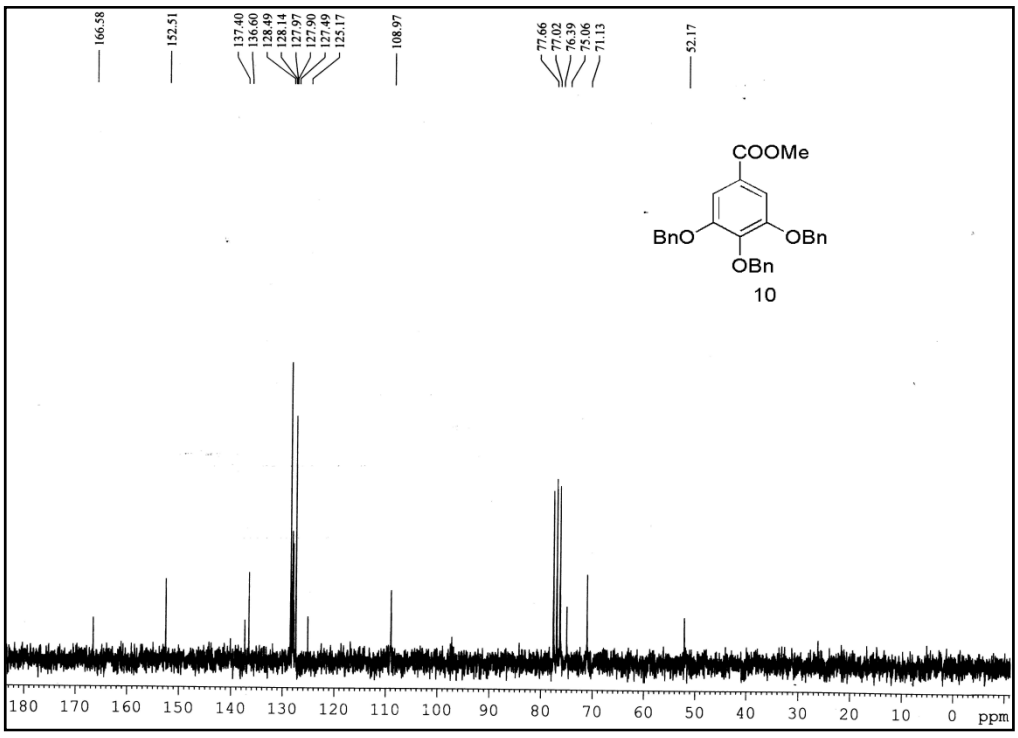
Compound 6: ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum



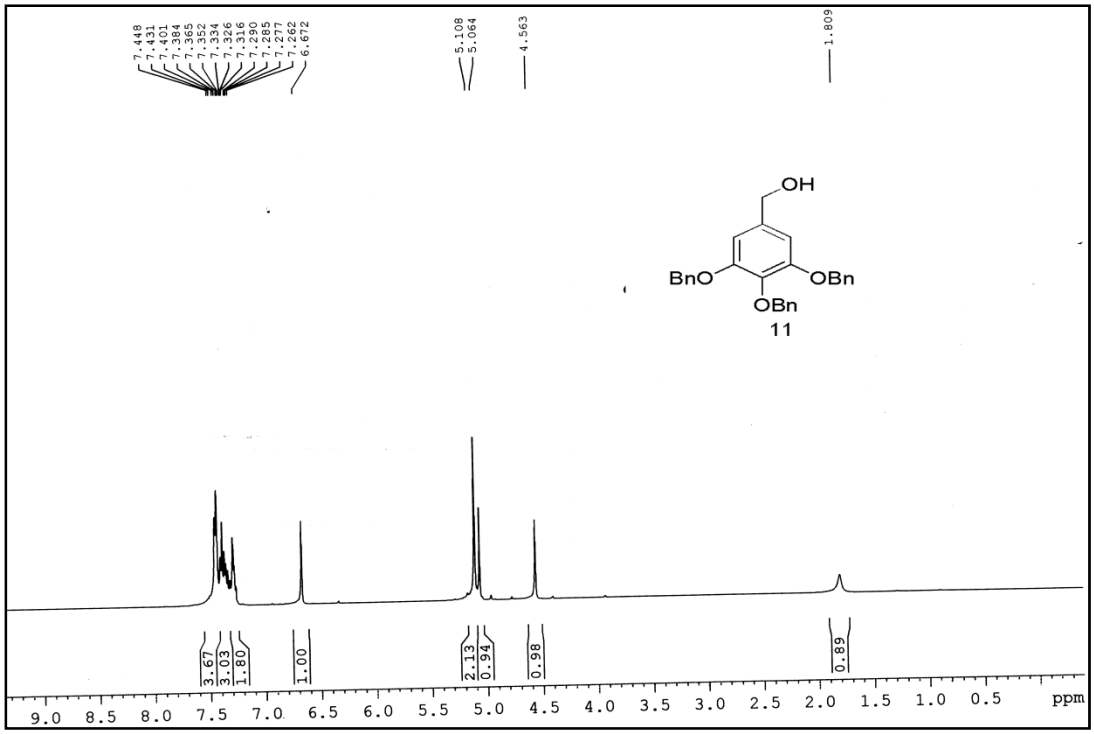
Compound 8: ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum



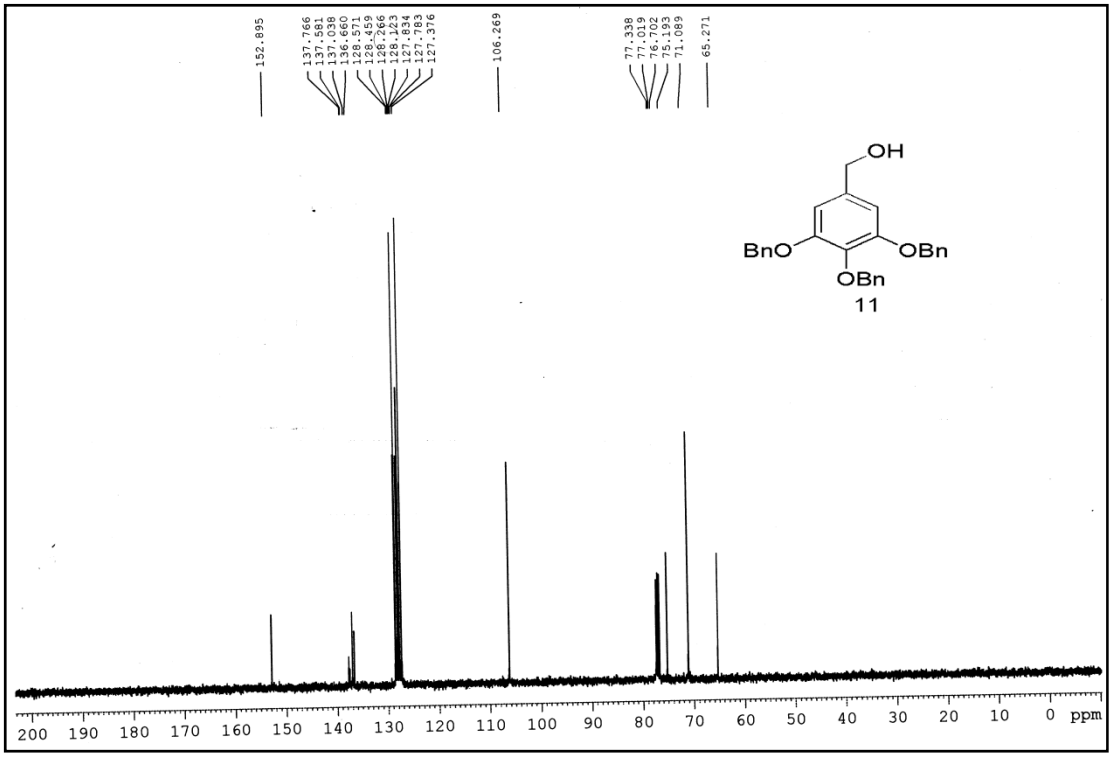
Compound 10: ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum



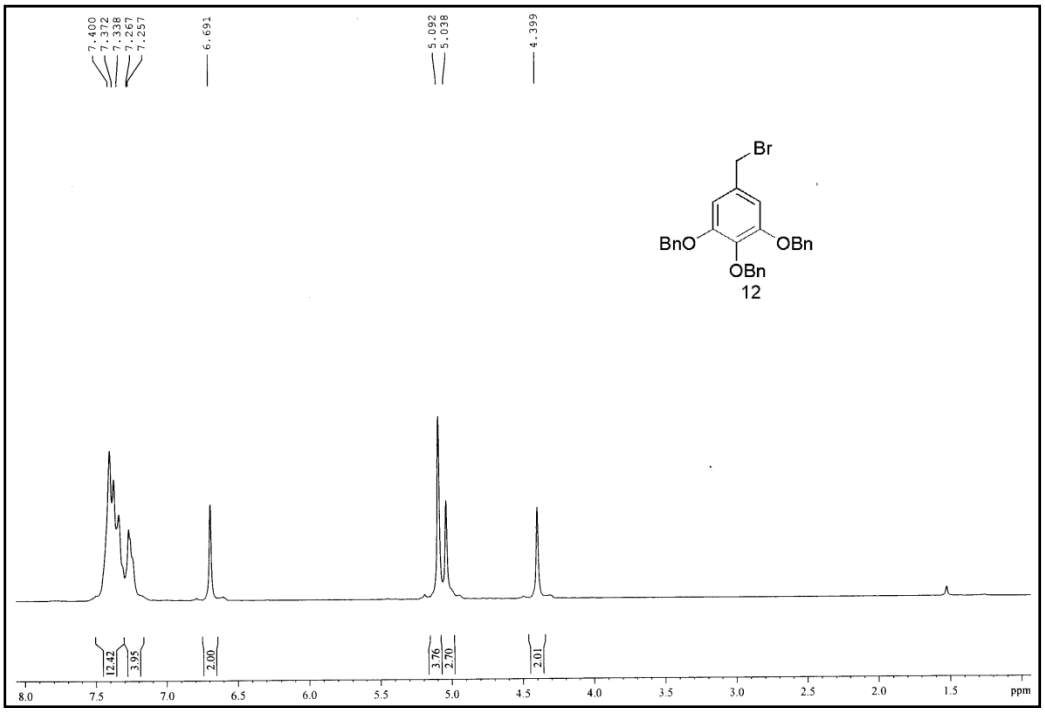
Compound 10: ^{13}C NMR ($CDCl_3$, 50 MHz) spectrum



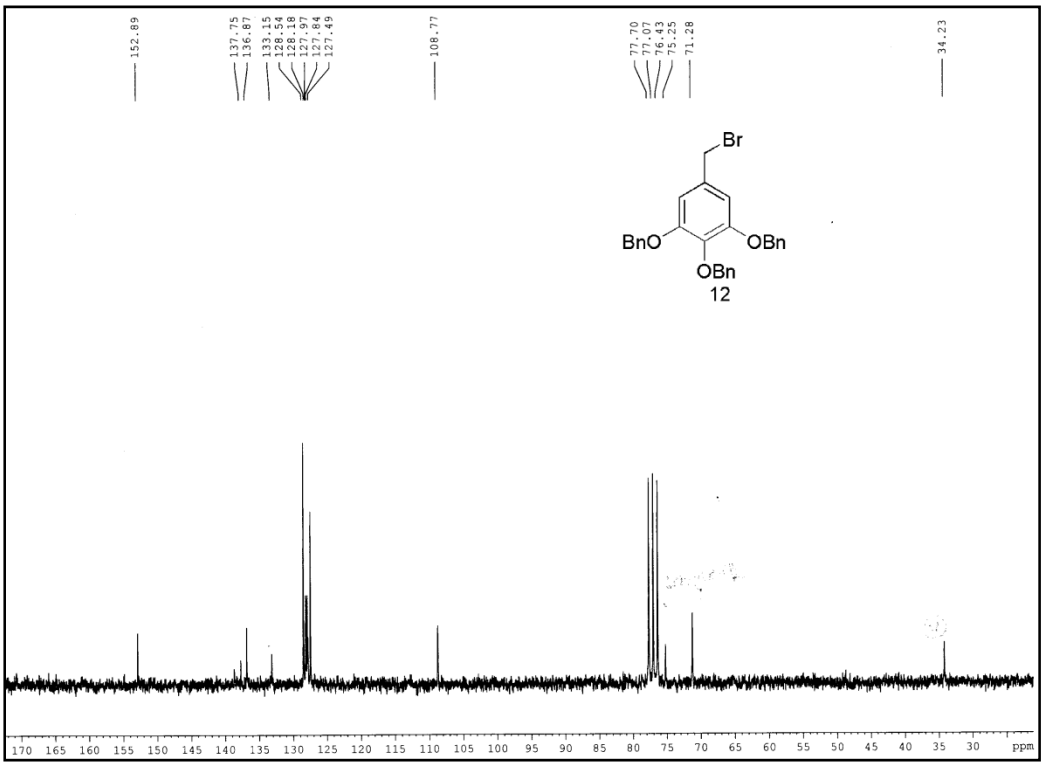
Compound 11: ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum



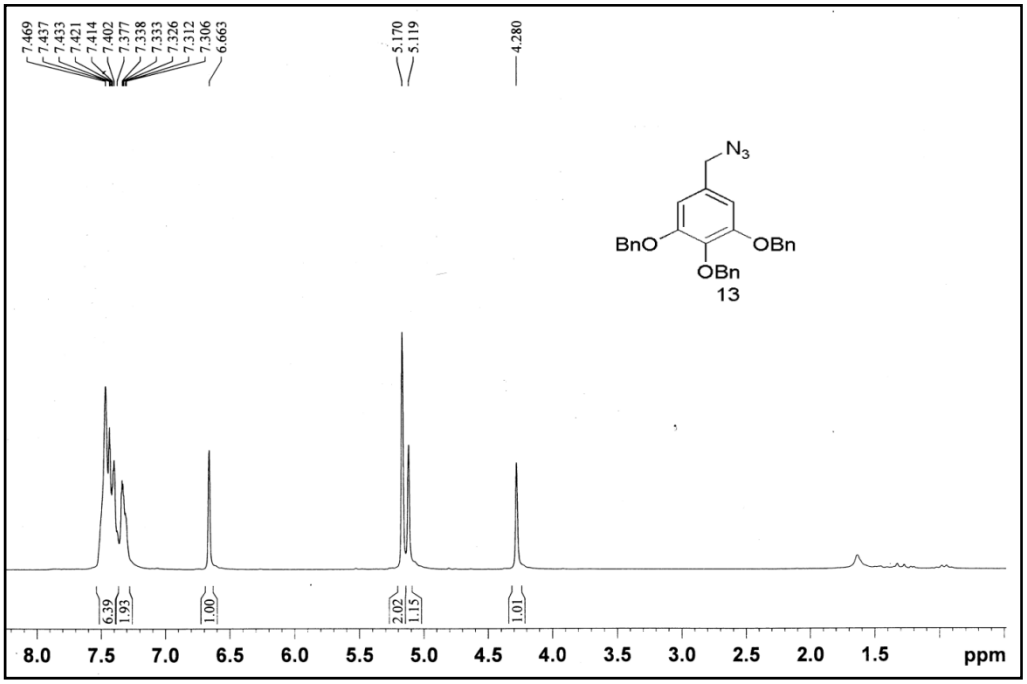
Compound 11: ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum



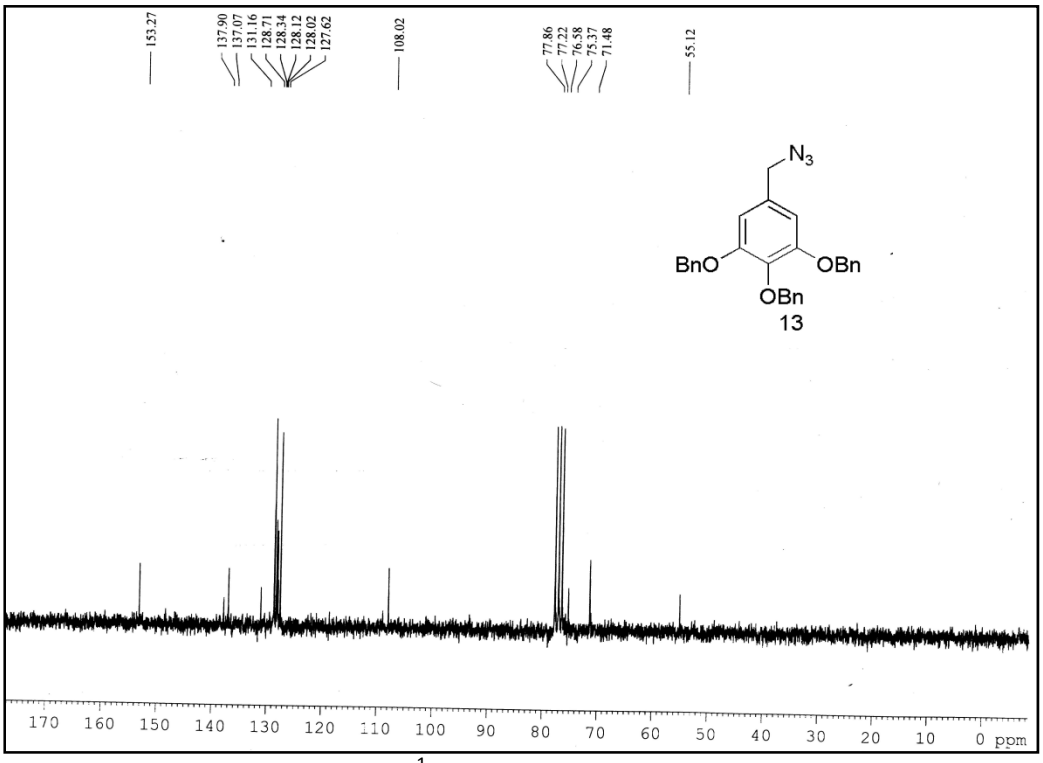
Compound 12: ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum



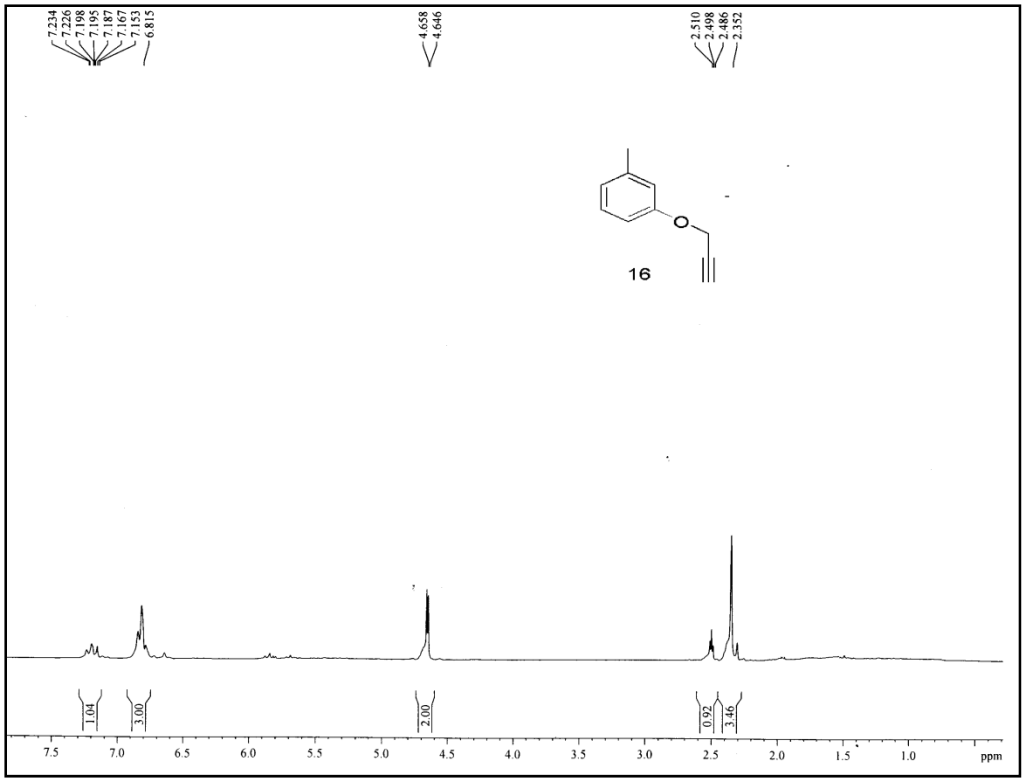
Compound 12: ^{13}C NMR ($CDCl_3$, 50 MHz) spectrum



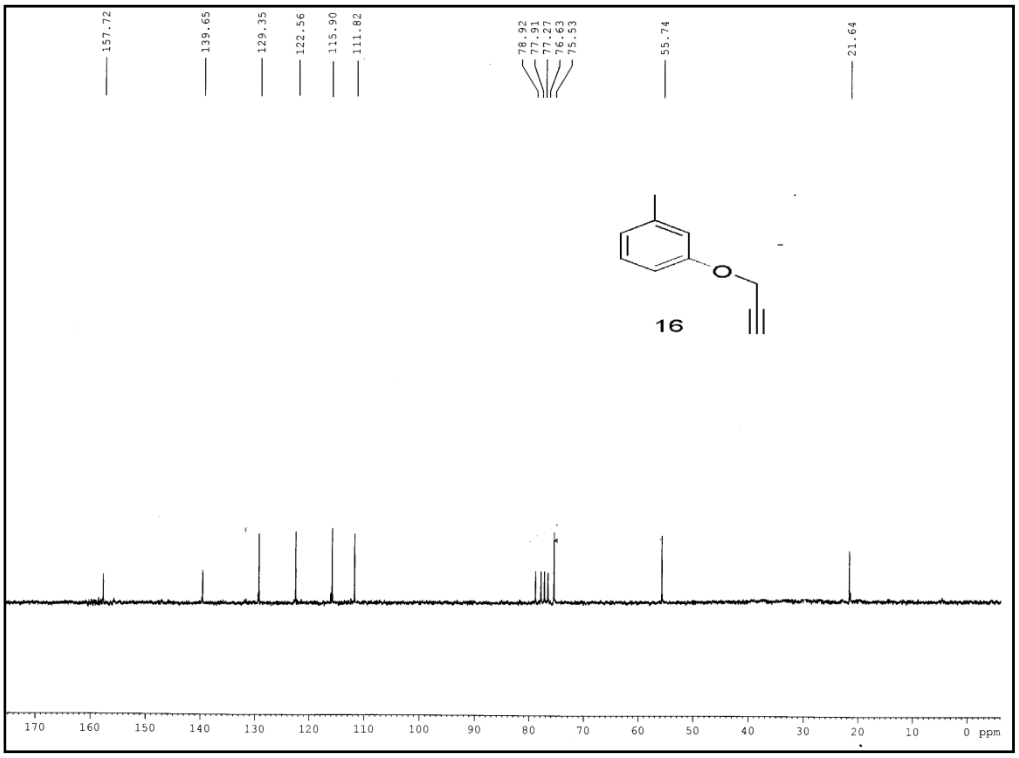
Compound 13: ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum



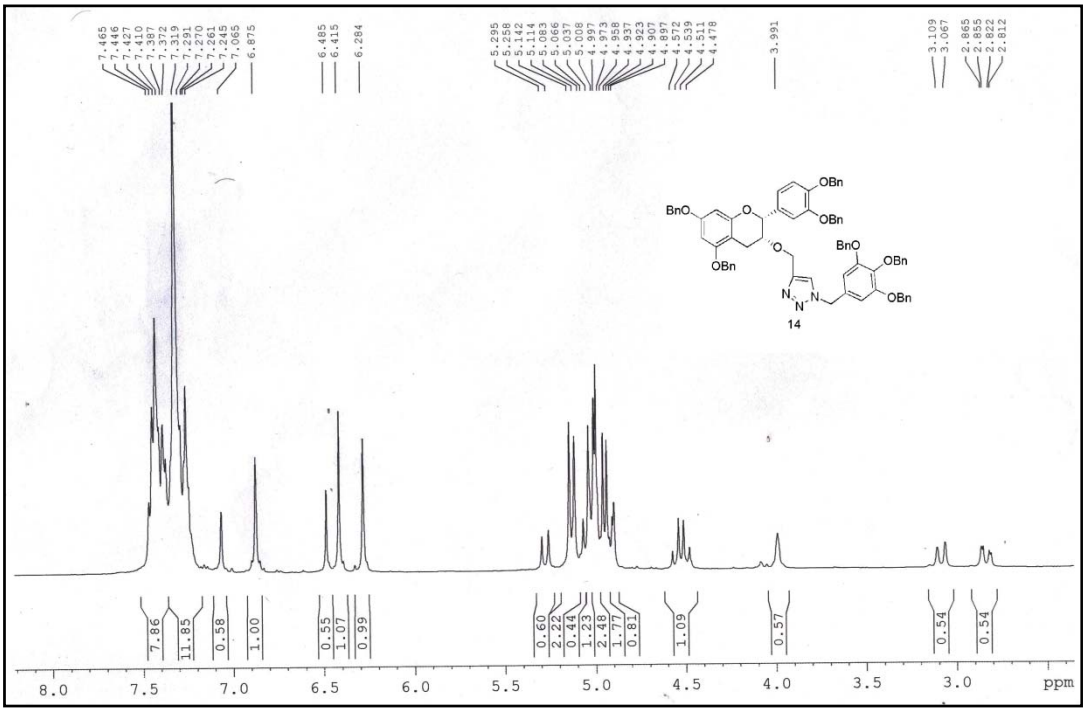
Compound 13: ^{1}H NMR ($CDCl_3$, 50 MHz) spectrum



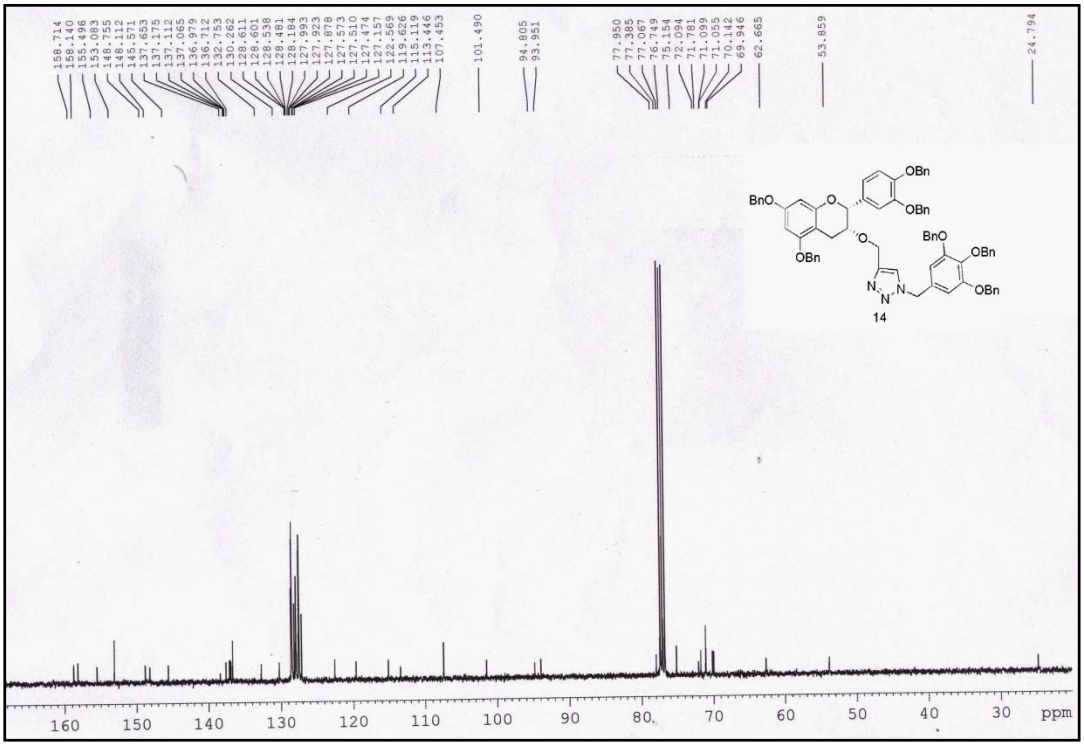
Compound 16: ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum



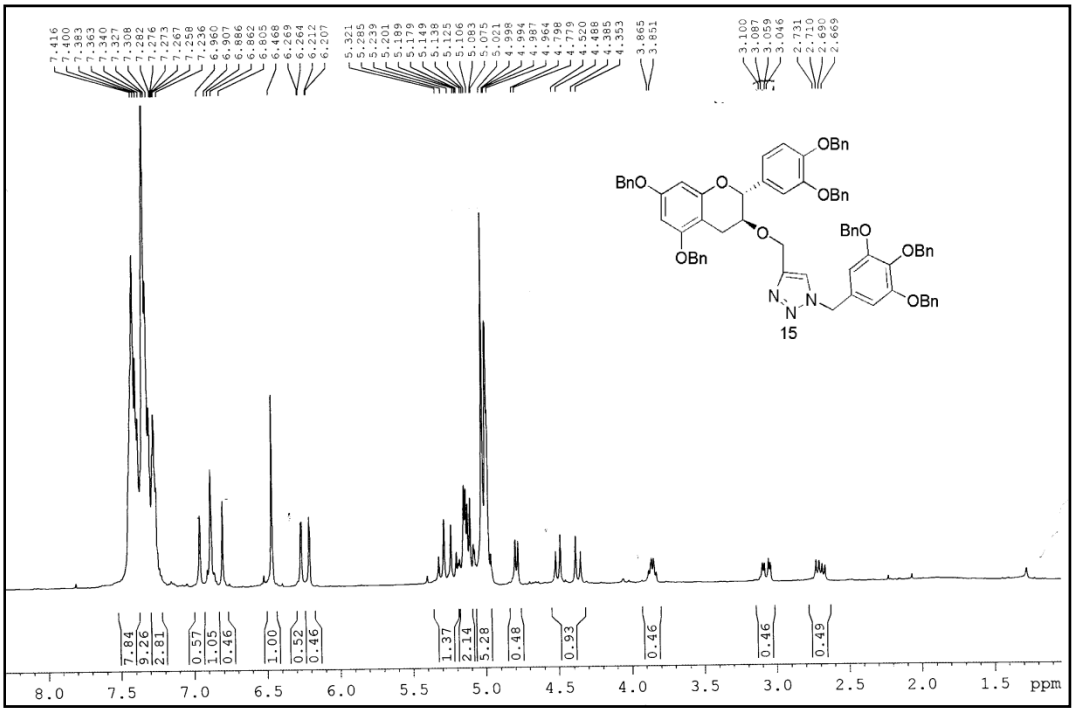
Compound 16: ^{13}C NMR ($CDCl_3$, 50 MHz) spectrum



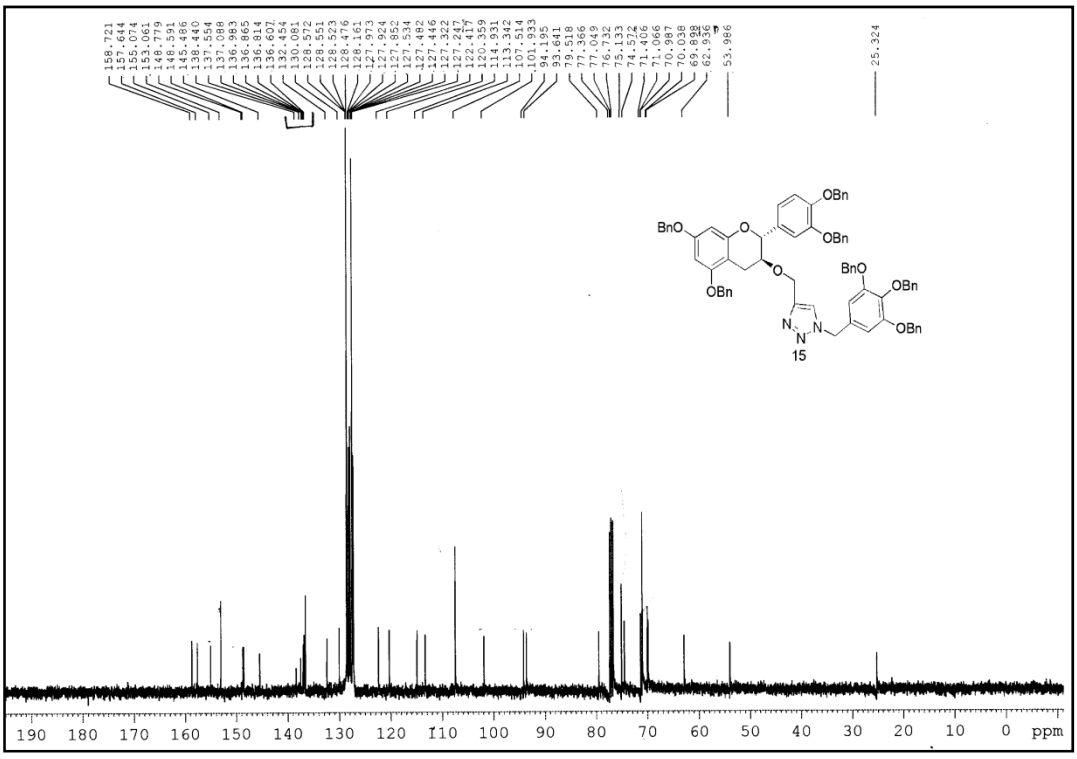
Compound 14: ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum



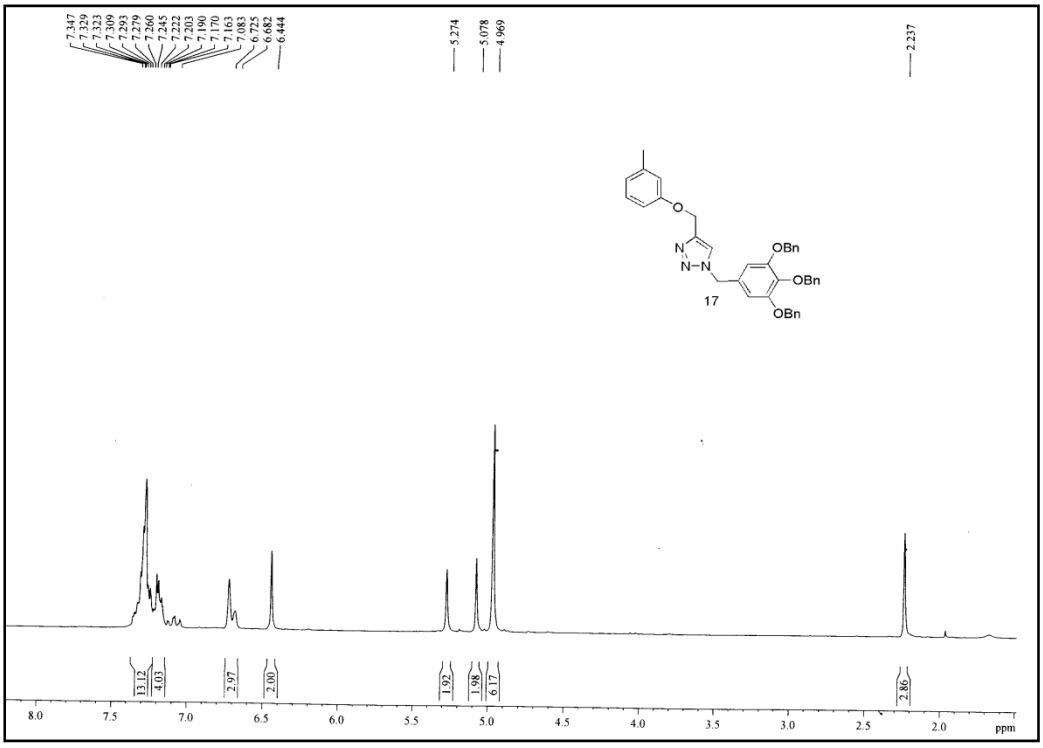
Compound 14: ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum



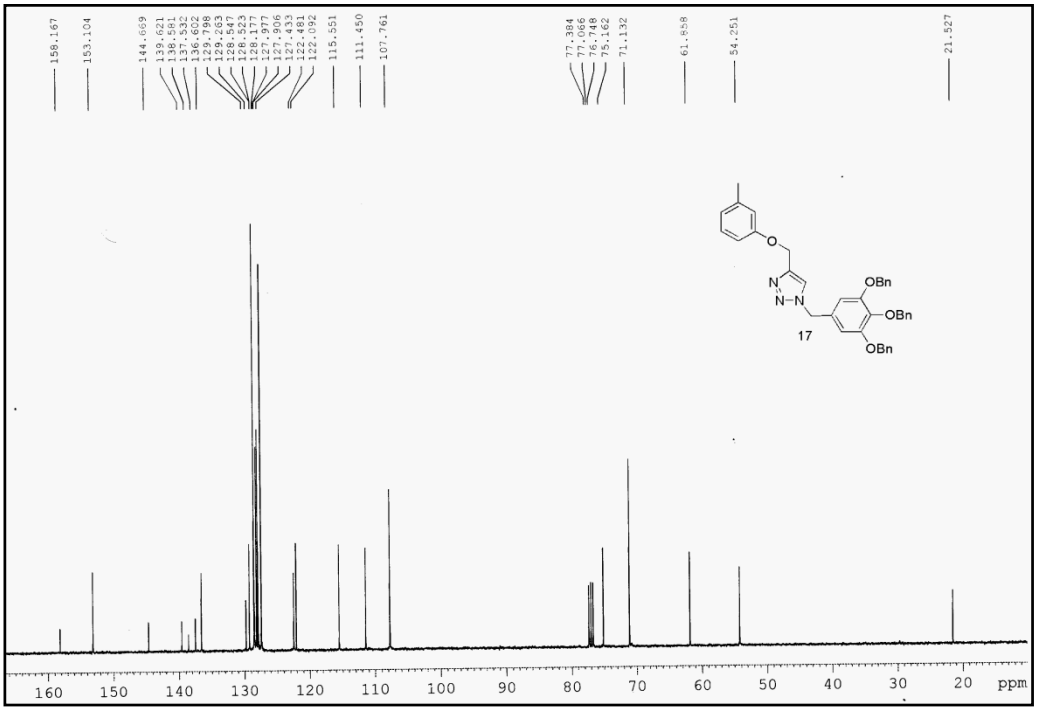
Compound 15: ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum



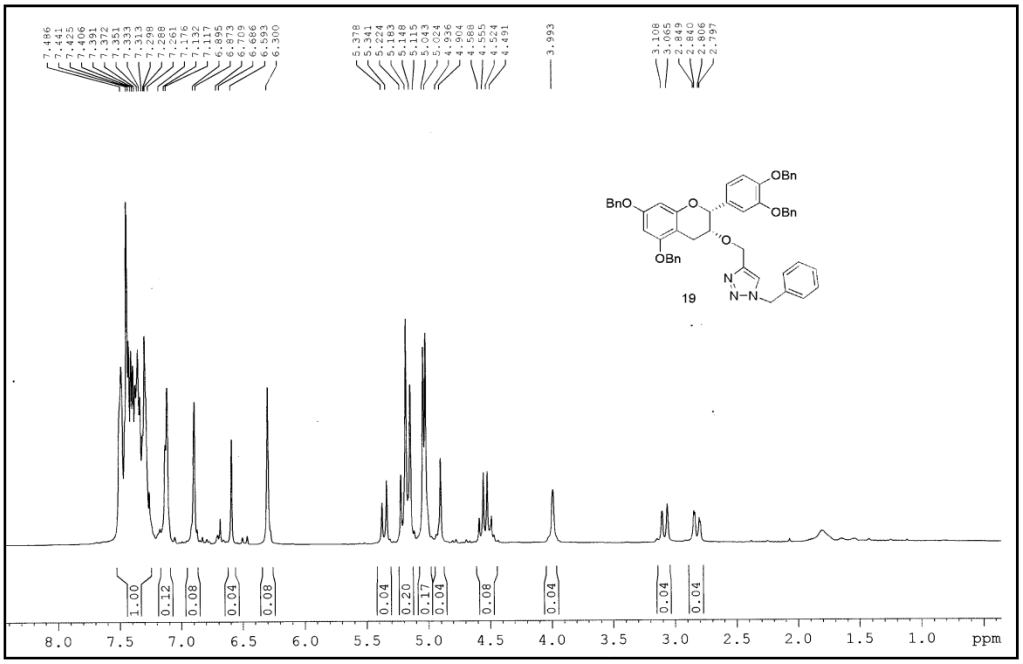
Compound 15: ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum



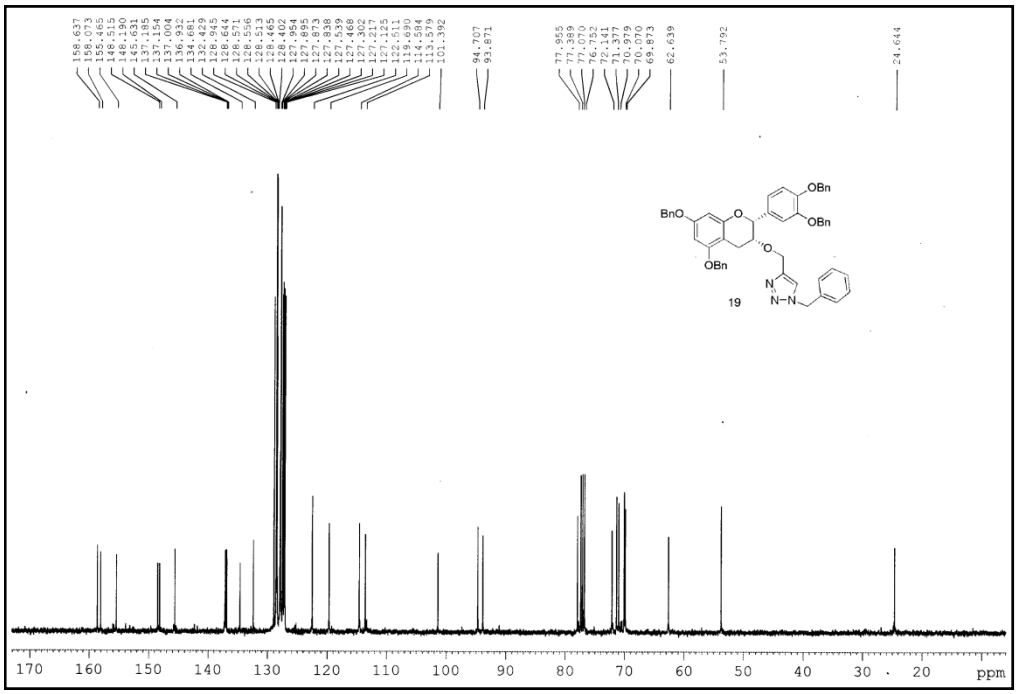
Compound 17: ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum



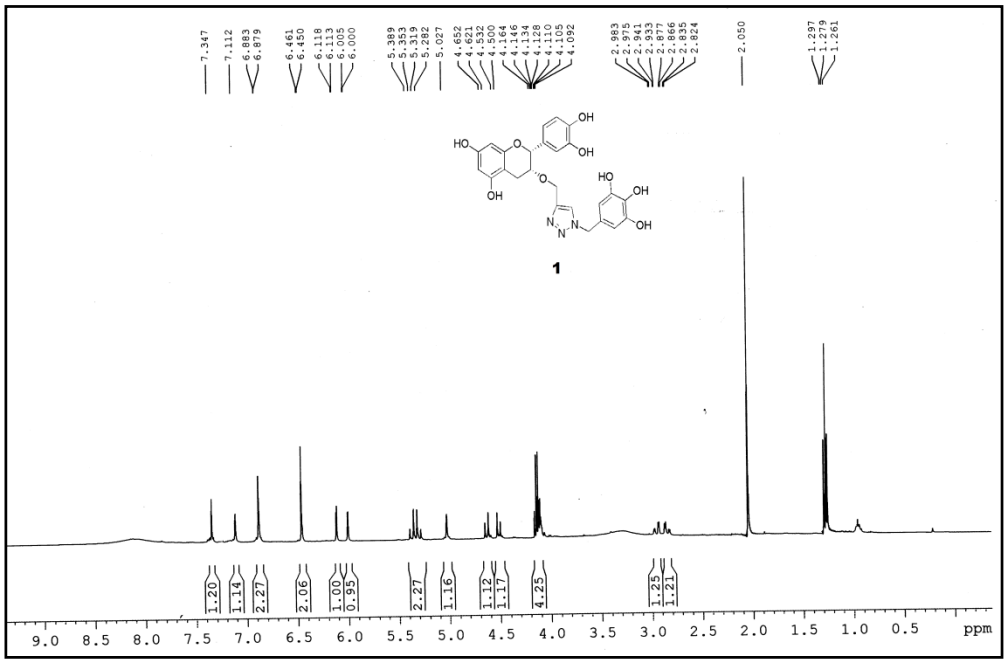
Compound 17: ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum



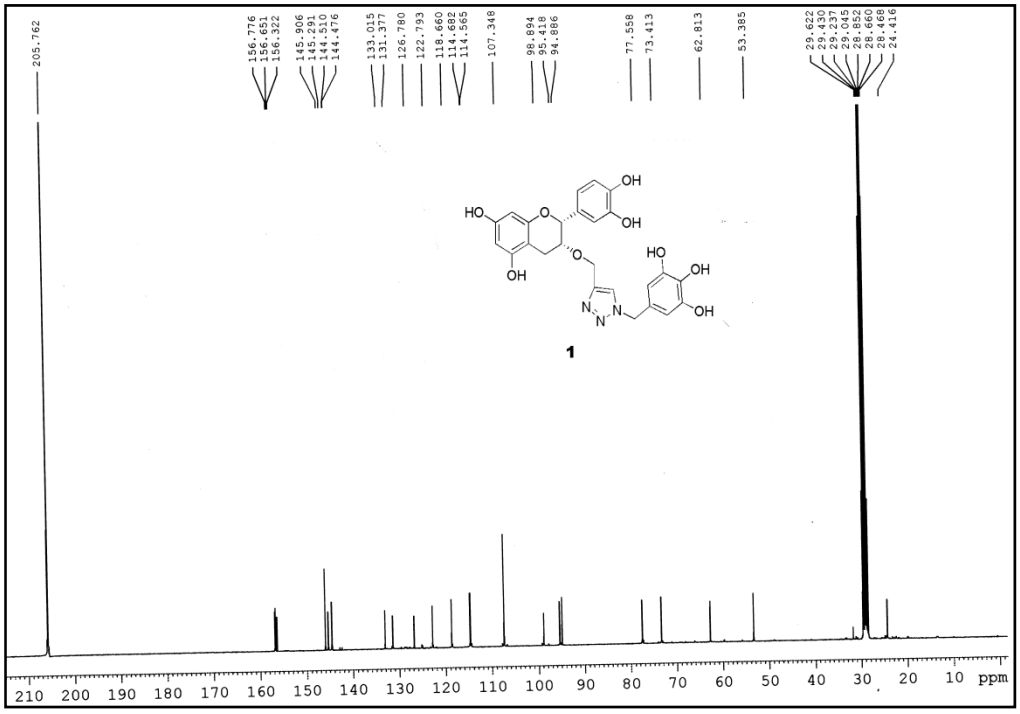
Compound 19: ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum



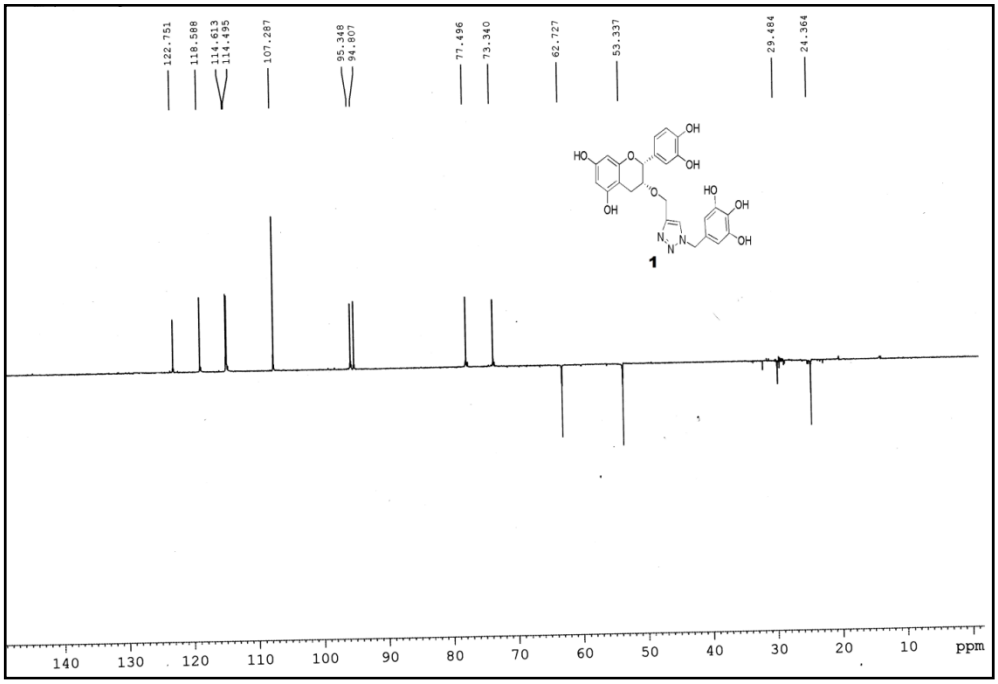
Compound 19: ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum



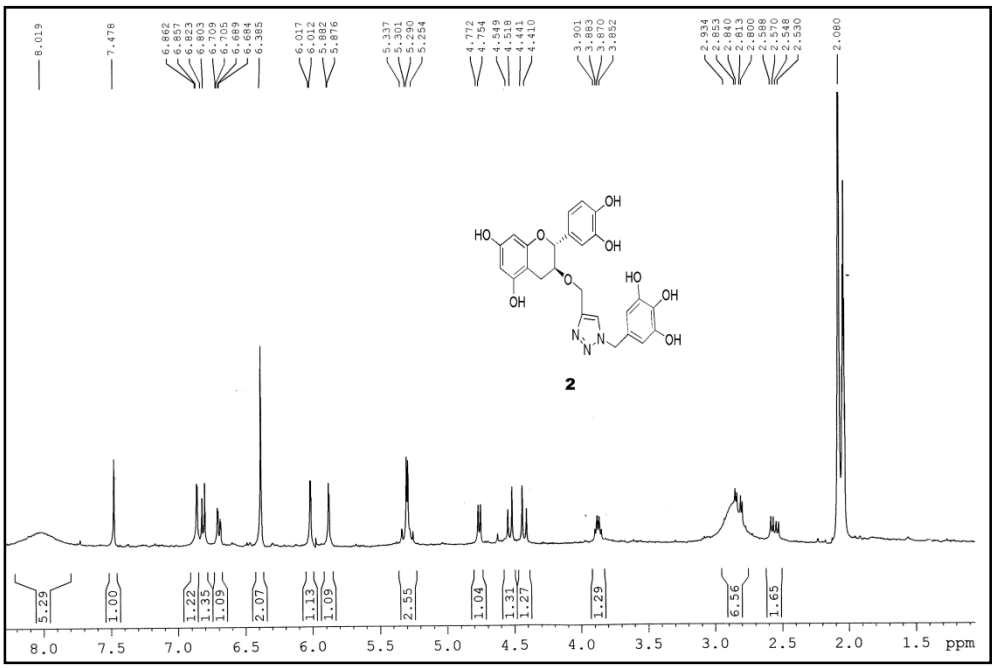
Compound 1: ^{1}H NMR (Acetone-d_6, 400 MHz) spectrum



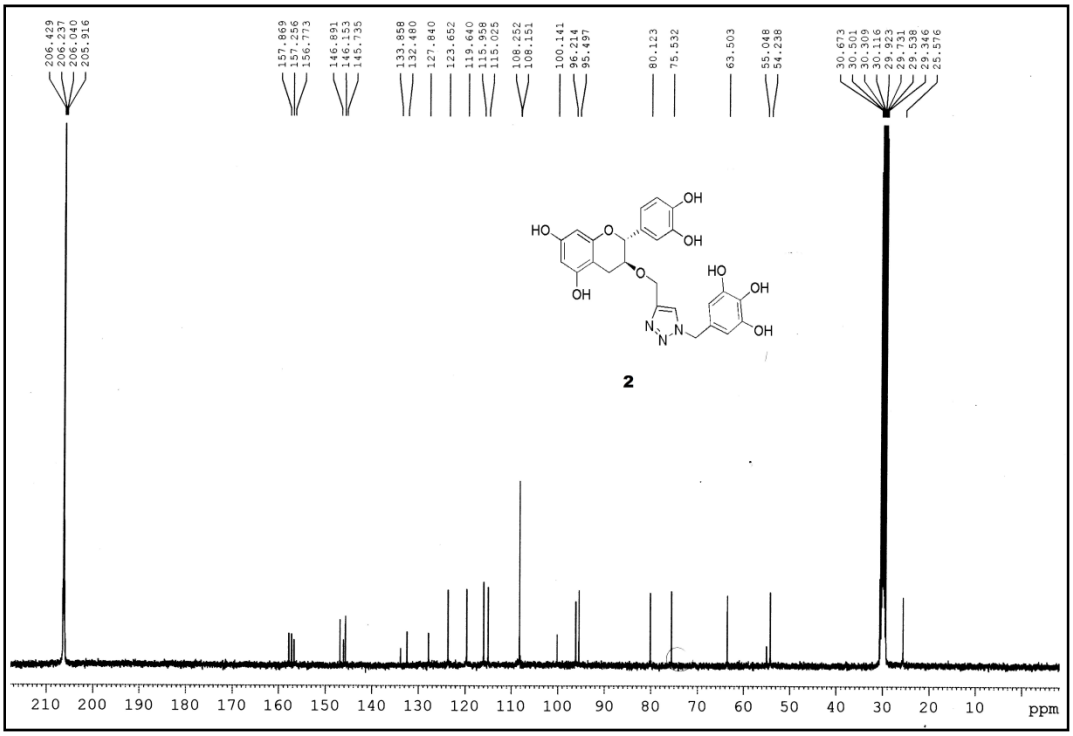
Compound 1: ^{13}C NMR (Acetone-d_6, 100 MHz) spectrum



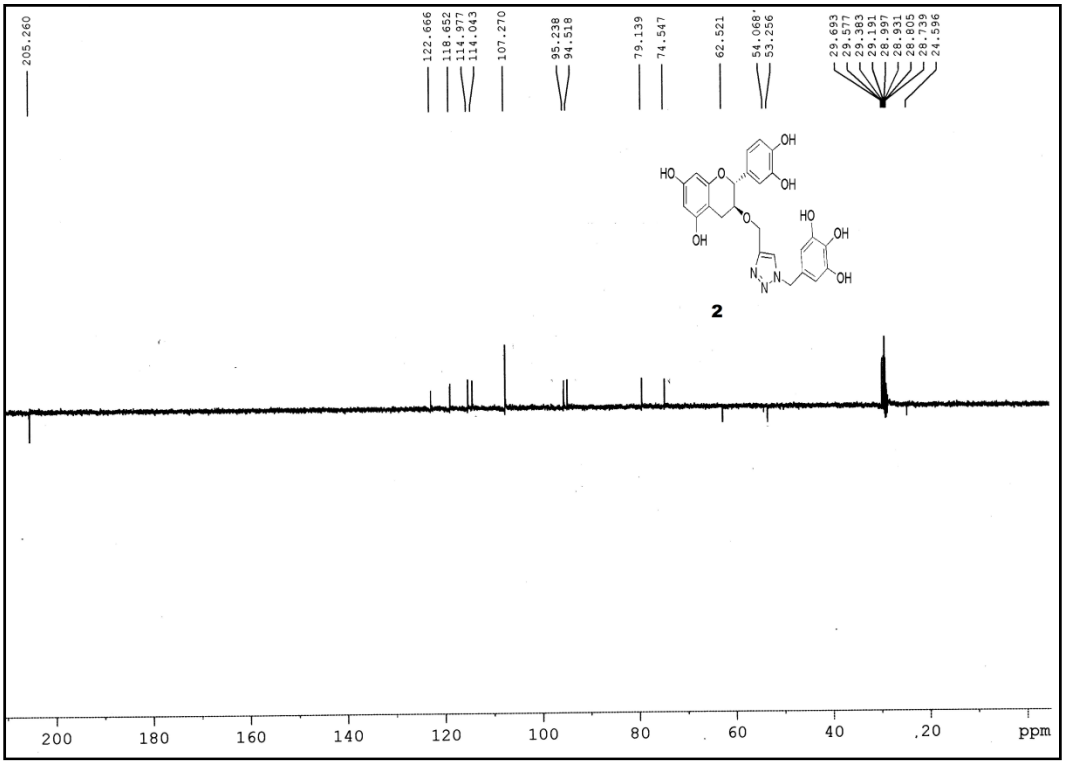
Compound 1: DEPT-135 NMR (Acetone-d_6, 100 MHz) spectrum



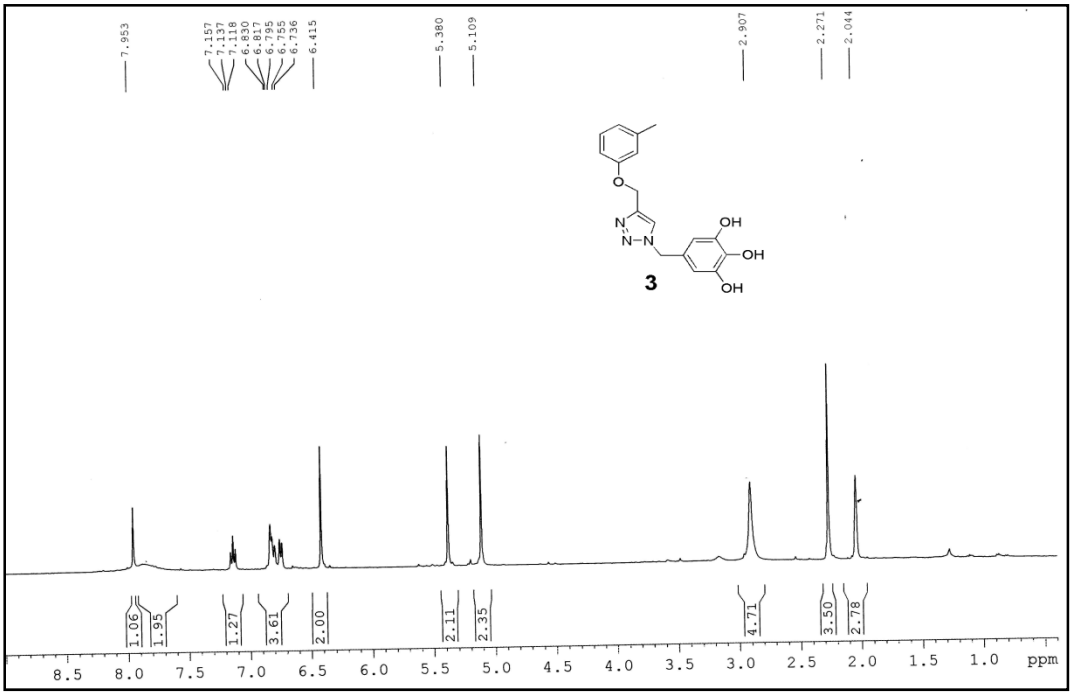
Compound 2: ^{1}H NMR (Acetone-d_6, 400 MHz) spectrum



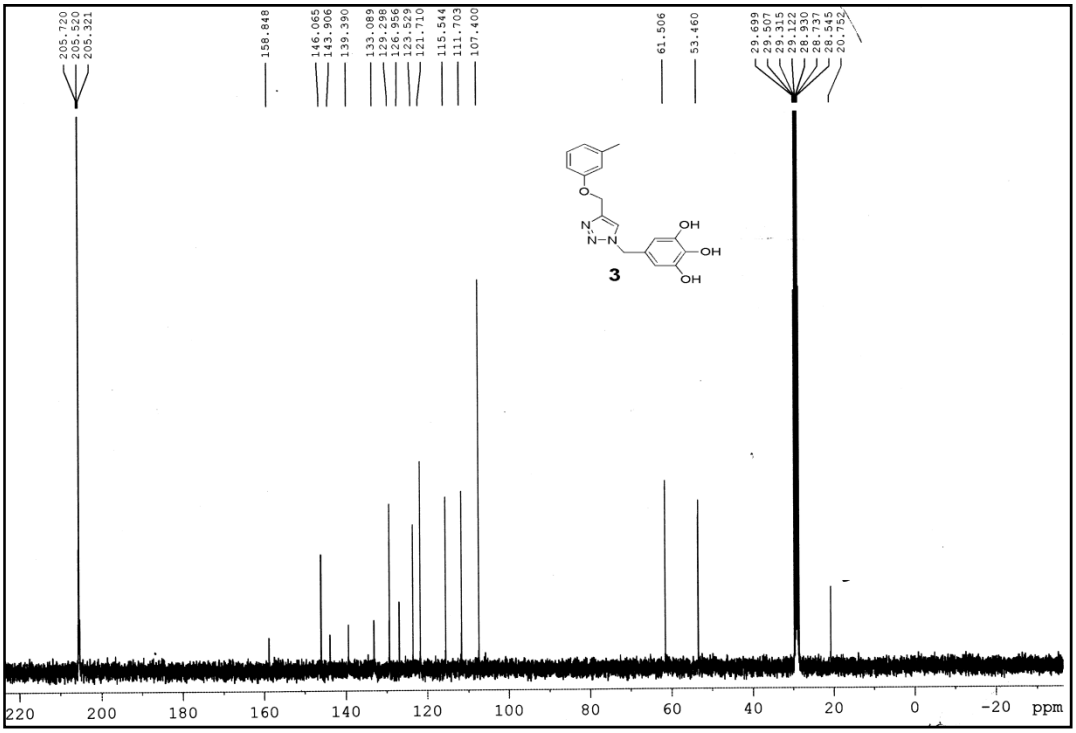
Compound 2: ^{13}C NMR (Acetone-d_6, 100 MHz) spectrum



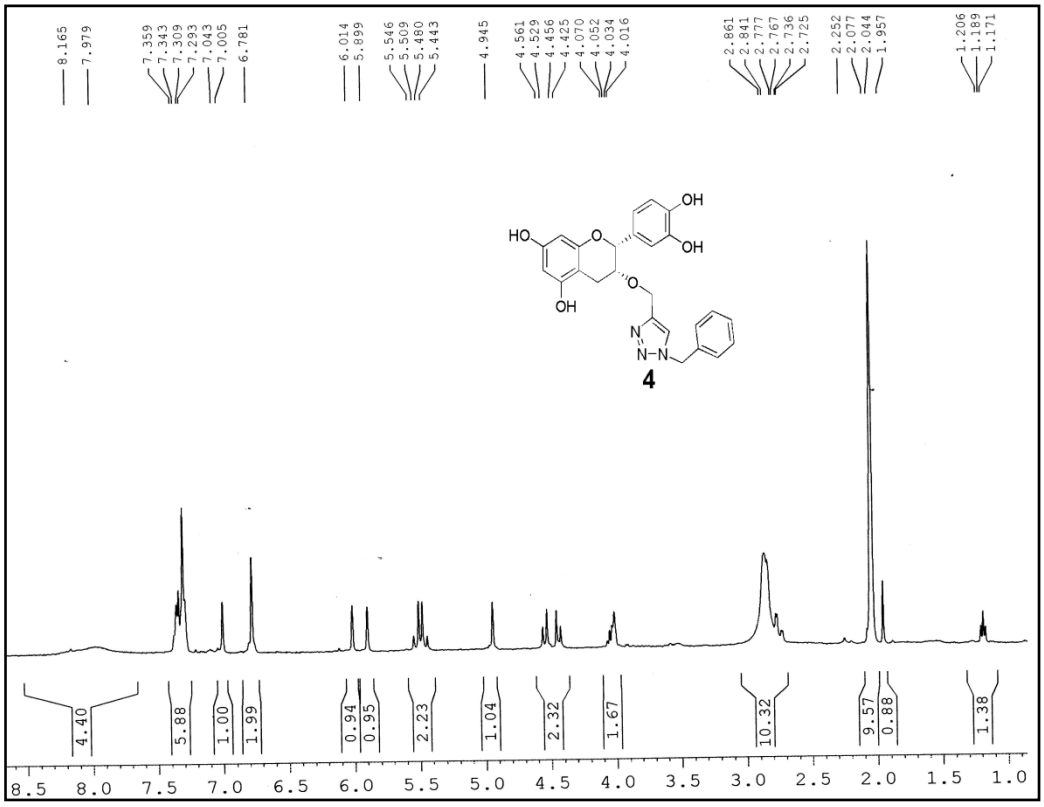
Compound 2: DEPT-135 NMR (Acetone-d_6, 100 MHz) spectrum



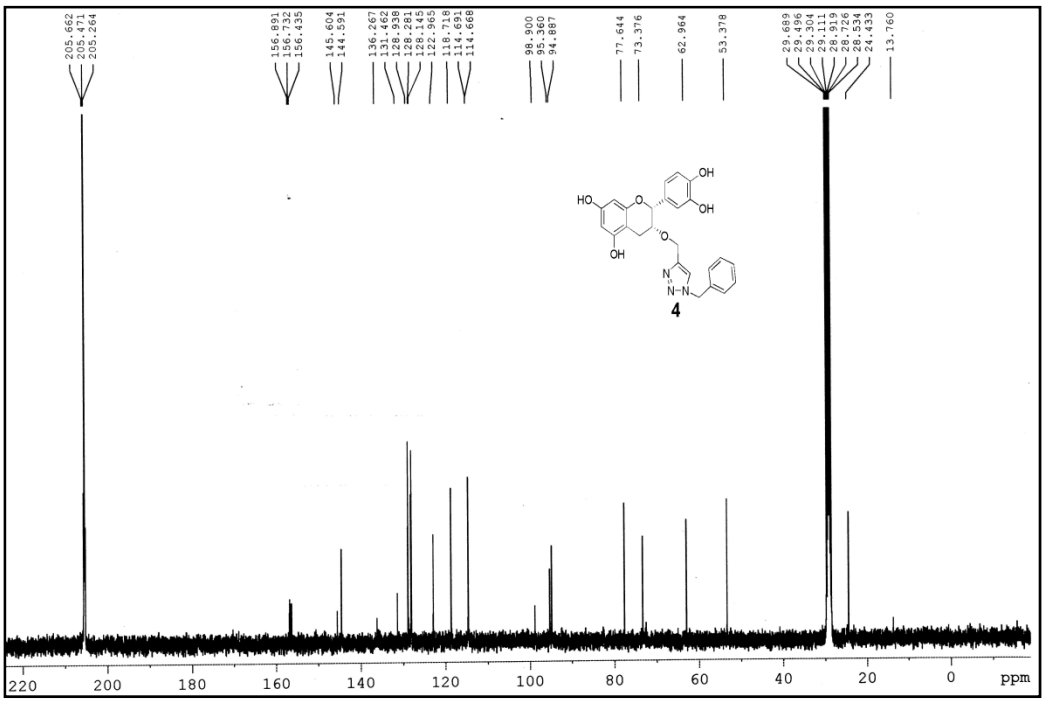
Compound 3: ^{1}H NMR (Acetone-d_6, 400 MHz) spectrum



Compound 3: ^{13}C NMR (Acetone-d_6, 100 MHz) spectrum

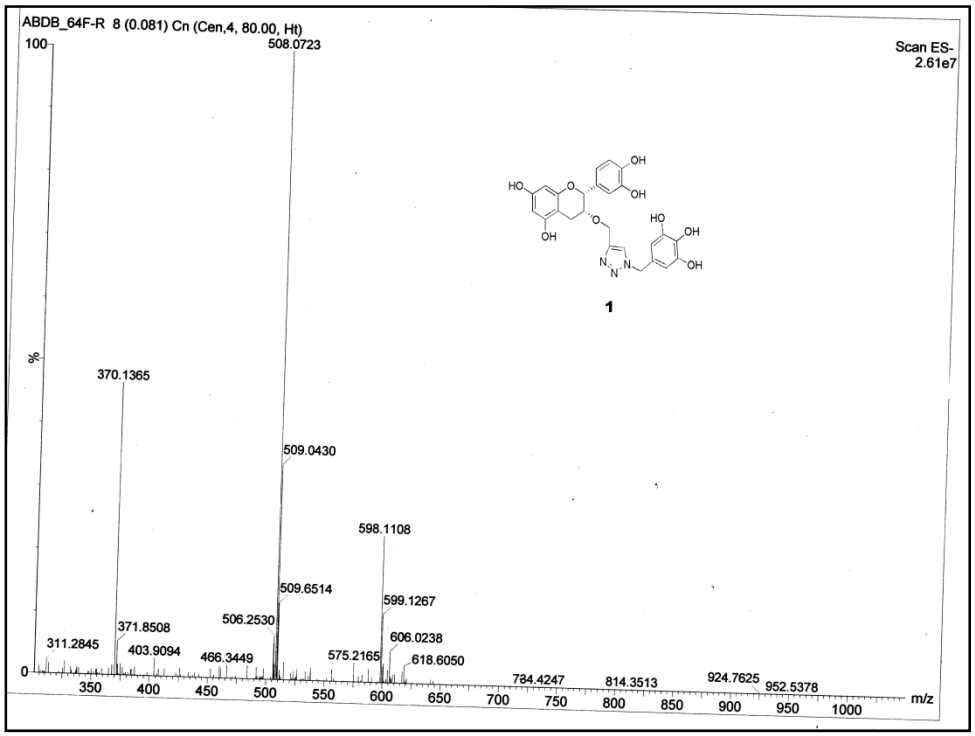


Compound 4: ^{1}H NMR (Acetone-d_6, 400 MHz) spectrum

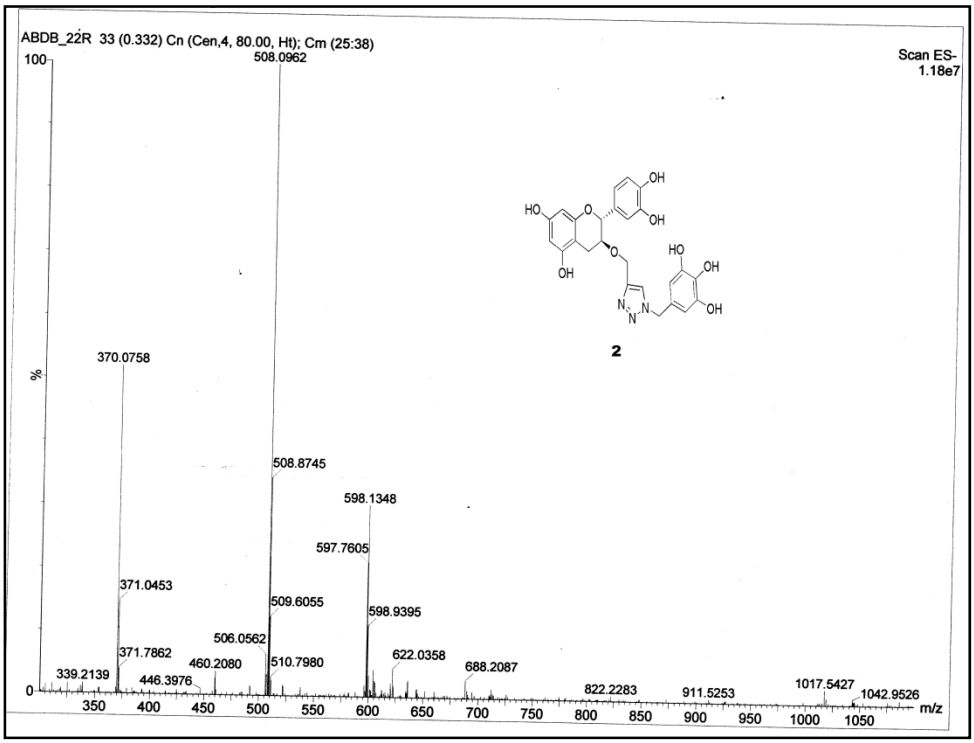


Compound 4: ^{13}C NMR (Acetone-d_6, 100 MHz) spectrum

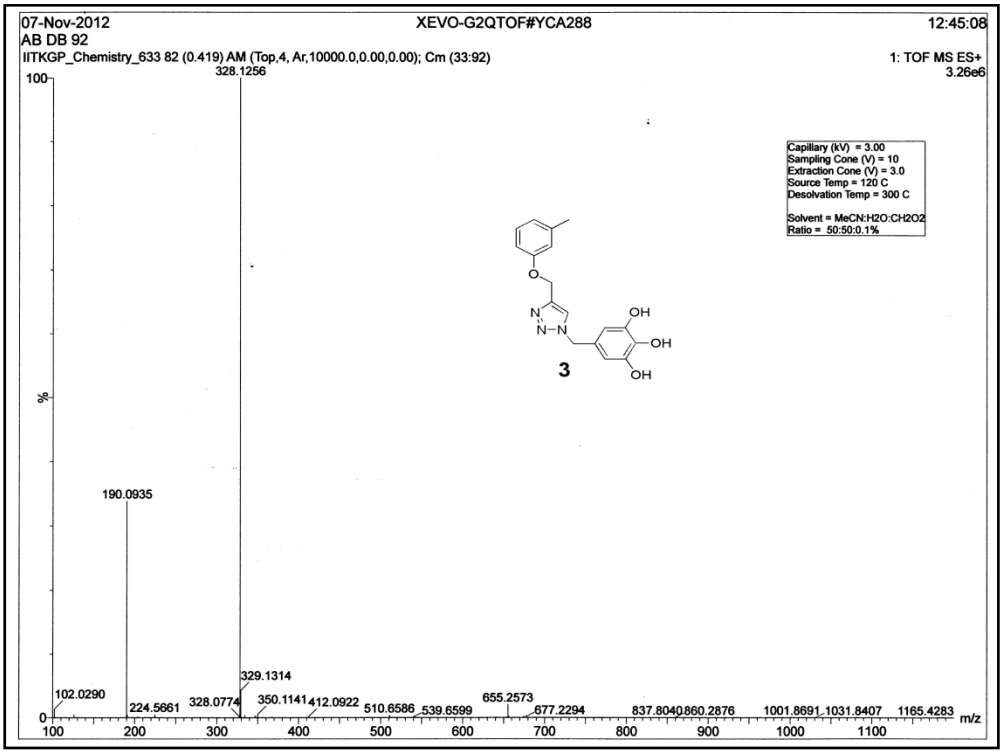
S2. Mass spectra of final compounds



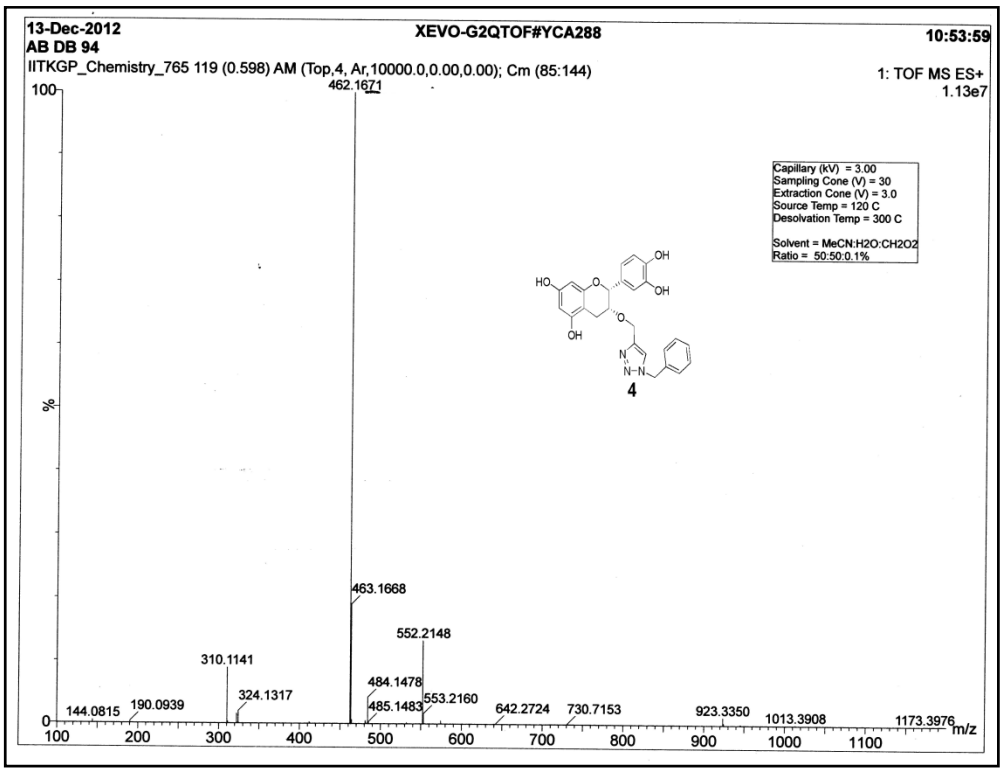
Compound 1: LCMS mass spectrum



Compound 2: LCMS mass spectrum

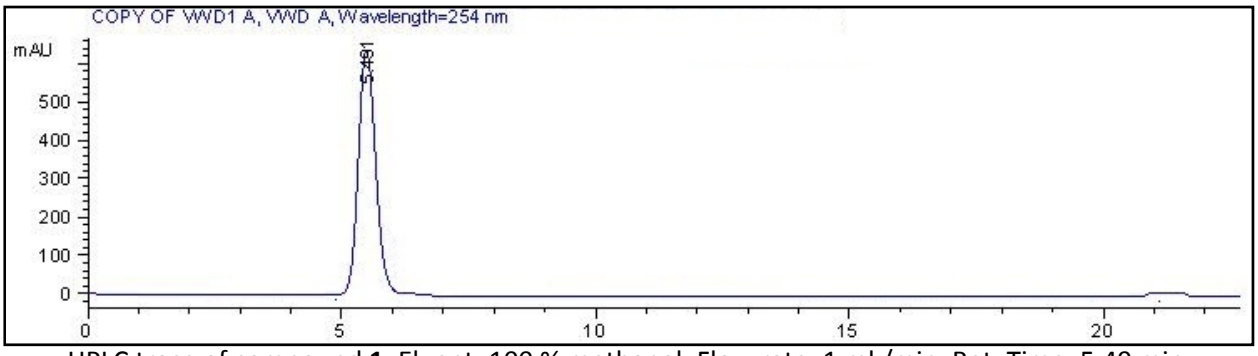


Compound 3: HRMS mass spectrum

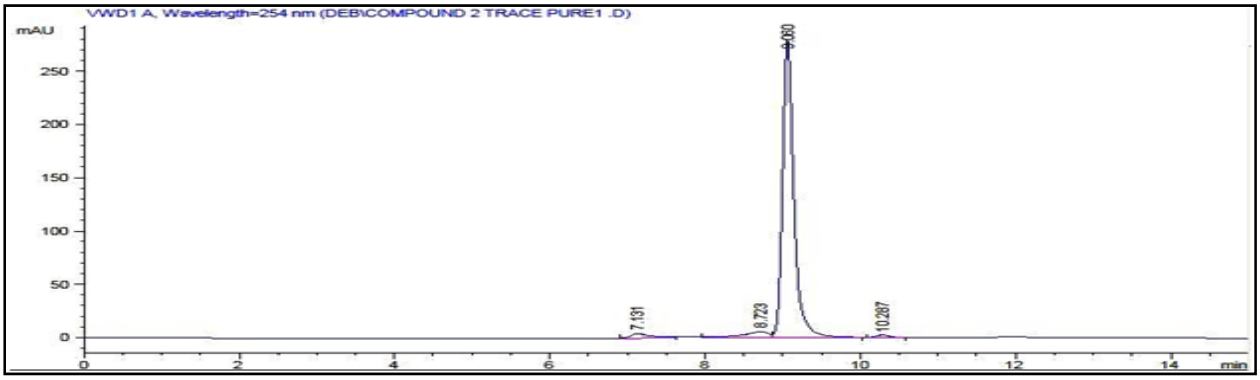


Compound 4: HRMS mass spectrum

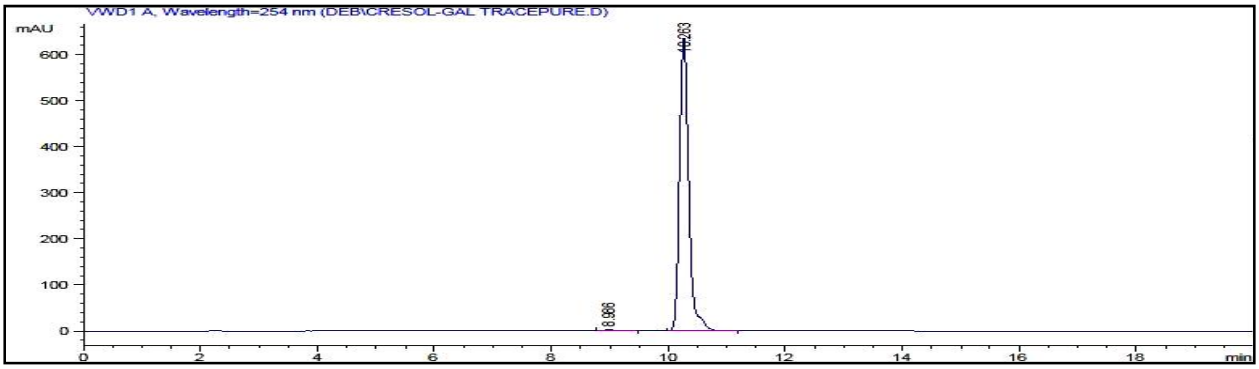
S3. HPLC traces of the synthesized final compounds



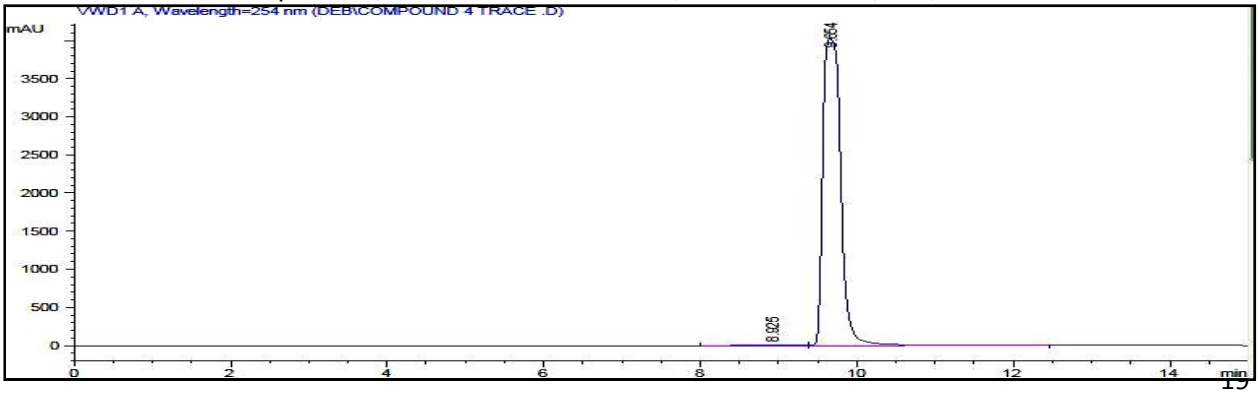
HPLC trace of compound **1,** Eluent: 100 % methanol, Flow rate: 1 ml /min, Ret. Time: 5.49 min



HPLC trace of compound **2,** Eluent: 100 % methanol, Flow rate: 0.5 ml /min, Ret. Time: 9.06 min



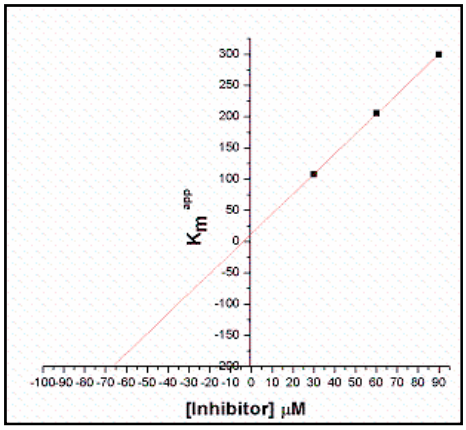
HPLC trace of compound **3,** Eluent: 100 % methanol, Flow rate: 1 ml /min, Ret. Time: 10.26 min



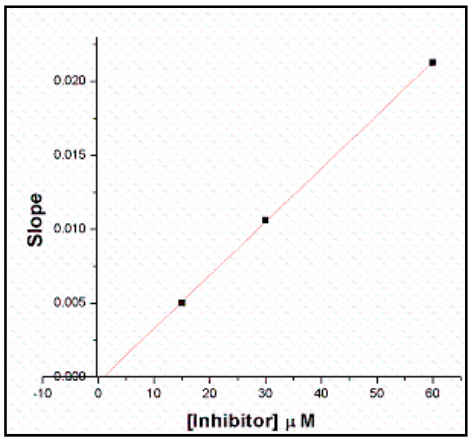
HPLC trace of c

HPLC trace of compound **4,** Eluent: 100 % methanol, Flow rate: 1 ml /min, Ret. Time: 9.66 min

S5. Secondary plots of inhibition kinetics

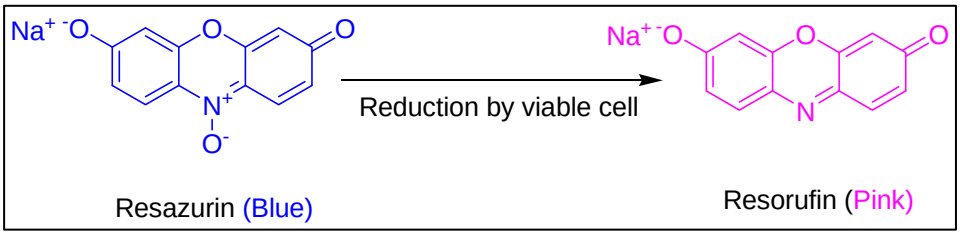


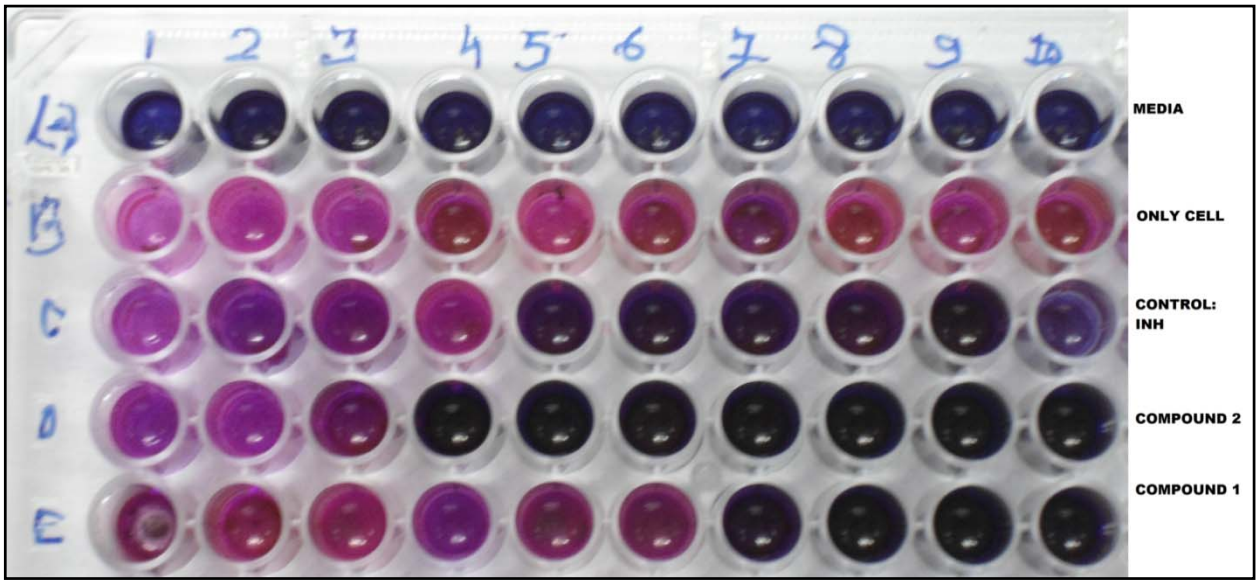
Compound **1** (Competitive Inhibitor)



Compound **2** (Mixed Inhibitor)

S6. Images of REMA assay

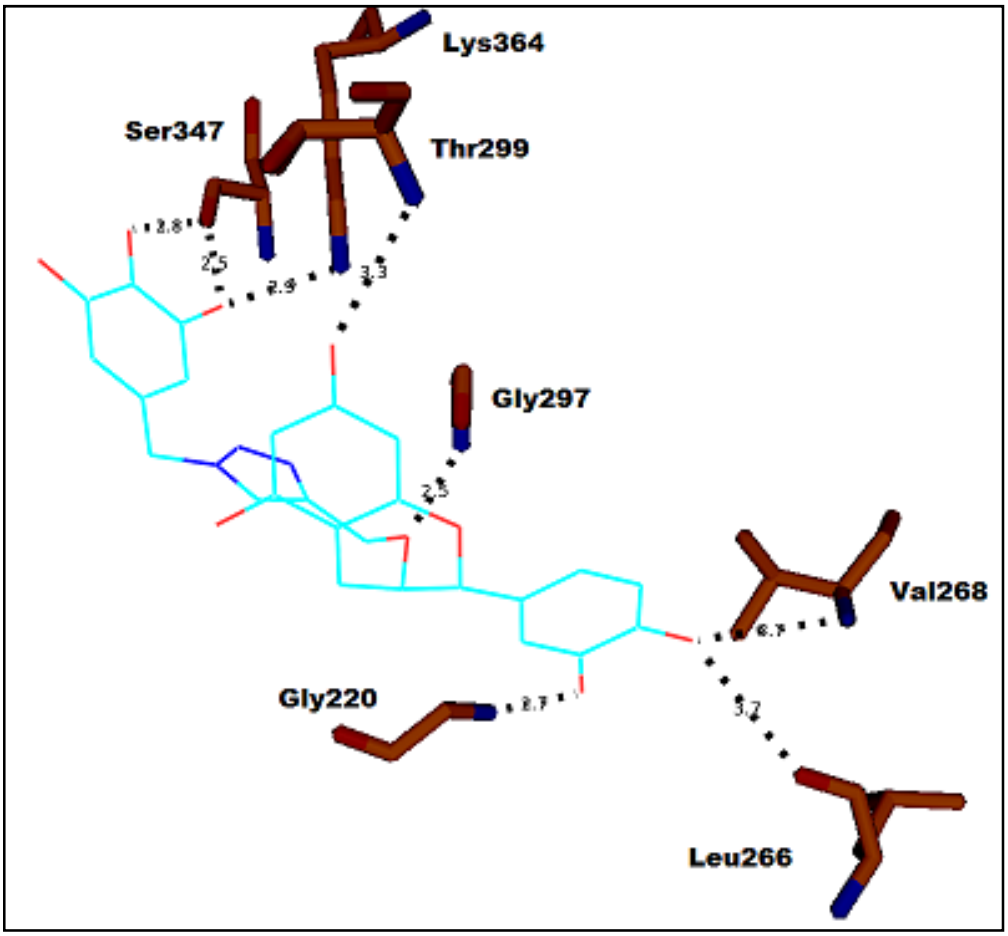




Resazurin assay plate. Pink colour indicates growth and blue indicates inhibition. Row A = only media, negative control; Row B = only culture, growth control; Row C = culture + INH, positive control; Row D = culture + compound **2**; Row E = culture + compound **1.**

S7. Details interaction from docking studies with distances

Compound **1**:

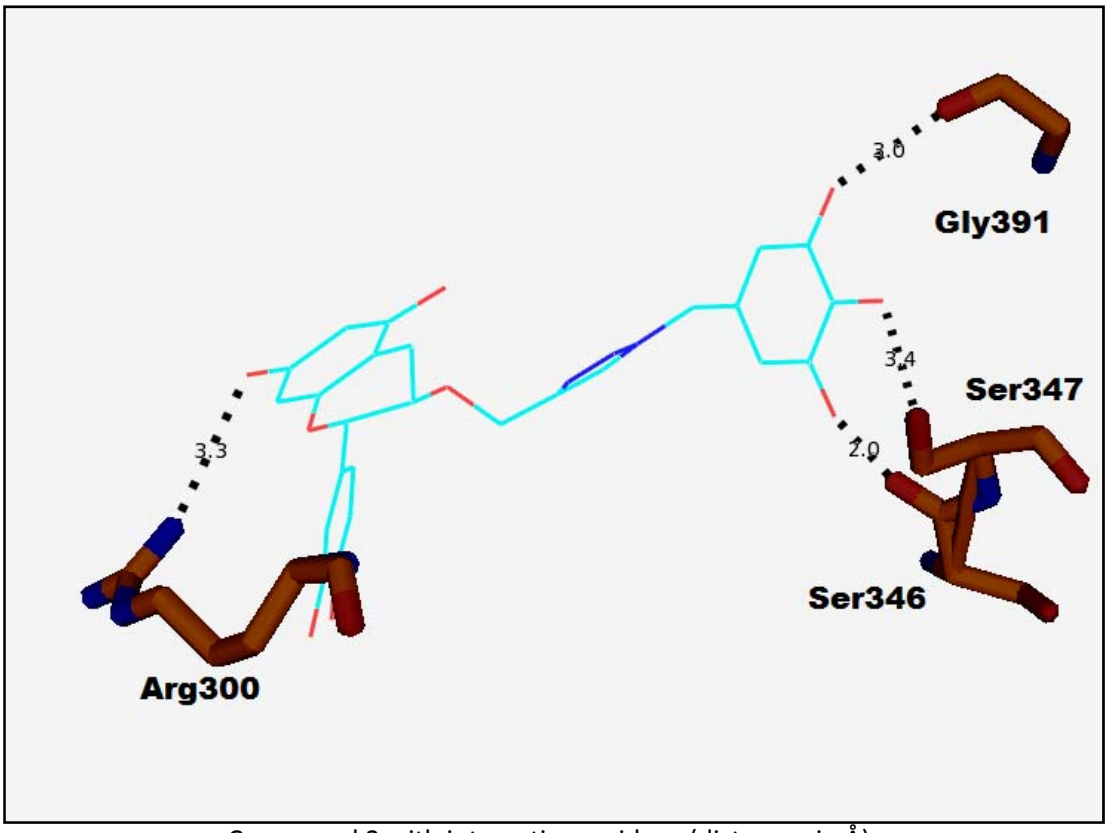


Compound 1 with interacting residues (distances in Å)

A-subsite of NADH binding region	**P-subsite of NADH binding region**	**N-subsite of NADH binding region**	**Catalytic tetrad**
Val268 (H-bond, 3.7 Å)	Gly297 (H-bond, 2.5 Å)	Ser346 (H-bond, 2.8 Å)	Lys364 (H-bond, 2.9 Å)
Leu26 (H-bond, 3.7 Å)	Thr299 (H-bond, 3.3 Å)	Ser346 (H-bond, 2.6 Å)	
Gly220 (H-bond, 2.7 Å)			

Compound **1** totally competes at all three binding subsites of NADH binding region; supports that compound 1 is a competitive inhibitor.

Compound **2**:

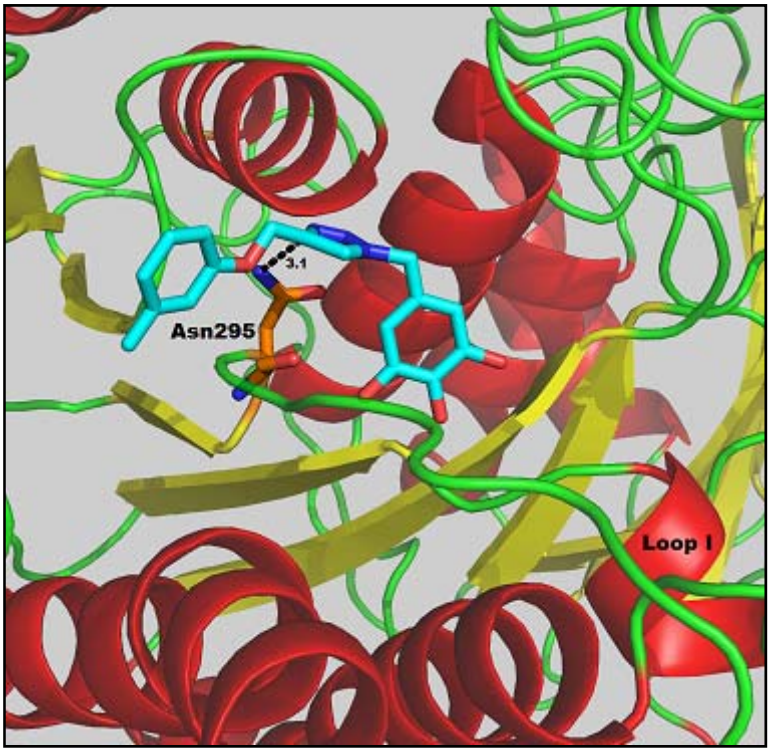


Compound 2 with interacting residues (distances in Å)

A-subsite of NADH binding region	P-subsite of NADH binding region	N-subsite of NADH binding region	Loop I	Catalytic tetrad
X	X	Ser346 (H-bond, 2.0 Å)	Arg300 (H-bond, 3.3 Å)	Ser347 (H-bond, 3.4 Å)
X	X	Gly391 (H-bond, 3.0 Å)		

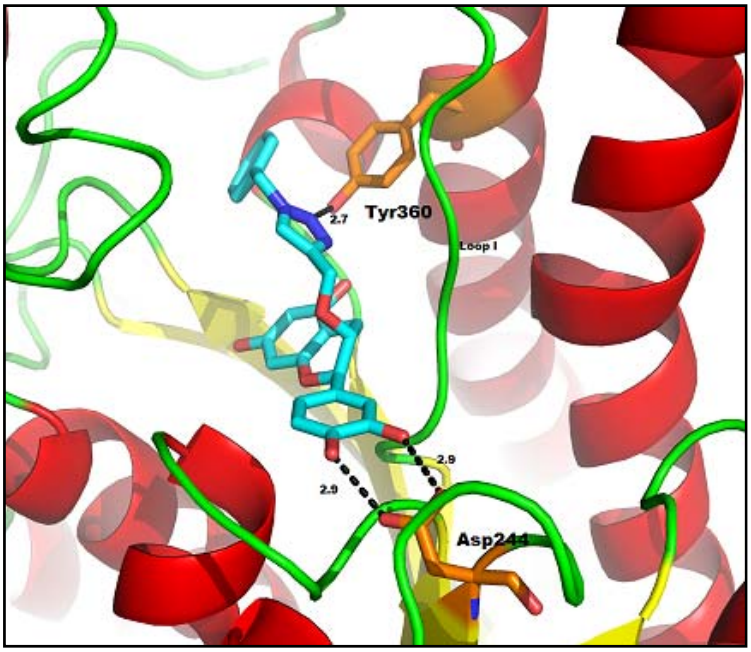
Compound **2** mainly interacts with catalytic tetrad and loop I; It can bind with free enzyme as well as enzyme-NADH complex resulting mixed inhibition.

Compound **3**:

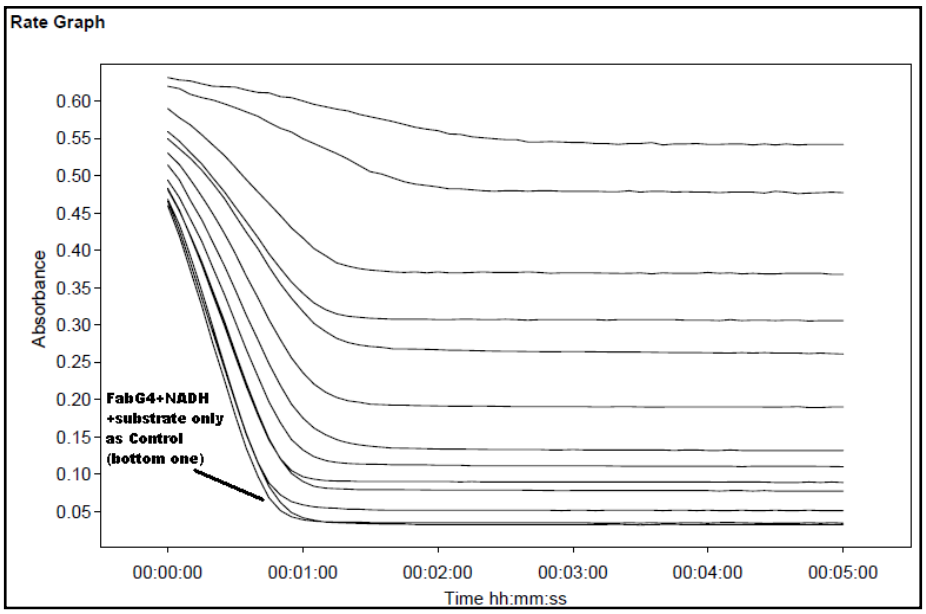


Compound **3** with interacting residues (distance in Å)

Compound **4**:



Compound **4** with interacting residues (distances in Å)



A typical kinetic plot of absorbance vs time with increasing concentration of inhibitor (compound 4) in presence of substrate acetoacetyl CoA (positive control) .