Supporting information for

Delivery of novel Coumarin-dihydropyrimidinone conjugates through mixed polymeric nanoparticle to potentiate therapeutic efficacy against triple-negative breast cancer

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General procedure for formation of ethyl 3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxopropanoate (**EDCO**)

To a mixture of 4-(Diethylamino)salicylaldehyde (1.92 gms,10 mmol) and Diethyl 1,3-acetonedicarboxylate (3.03 gms, 15 mmol) in 20 ml of dry ethanol, catalytic amount of Piperidine (0.049 ml, 0.5 mmol) was added drop wise. Then the reaction mixture was stirred bellow 25°C at argon atmosphere for overnight until completion of reaction, monitored by TLC. After that the solvent was evaporated in vacuum. The reaction mixture was quenched by addition of ice cold H_2O, and extracted with ethyl acetate. The combined organic phase was washed with brine and dried over Na_2SO_4. The organic phase was then reduced under vacuum and concentrated ethyl acetate extracts were chromatographed over a silica gel column (230-400). The product was obtained by elution with 40% ethyl acetate in petroleum ether.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27(1H, s), 7.20 (1H, d, J 9 Hz), 6.45 (1H, d, J 9 Hz), 6.26(1H, s), 4.04 (2H, m), 3.87 (2H, m), 3.28 (4H, m), 1.20 (9H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.0, 168.3, 160.8, 158.8, 153.3, 148.4, 132.1,114.5, 110.00, 108.2, 96.4, 60.8, 48.8, 45.1, 14.2, 12.3.

General procedure for synthesis of coumarin- dihydropyrimidinone derivatives (**CDHPs**)

A mixture of ethyl 3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxopropanoate (331 mg, 1 mmol), aldehyde (1 mmol) and urea (120 mg, 2 mmol) were taken in 10 ml of ethanol. Then (0.1 %) HCl and p-Toluenesulfonic acid (17.22 mg, 0.1 mmol) were added at the reaction mixture. Then the reaction mixture was heated at 90°C at argon atmosphere for overnight until completion of reaction, monitored by TLC. After that the solvent was evaporated in vacuum. The reaction mixture was quenched by addition of $NaHCO_3$ solution, and extracted with ethyl acetate. The combined organic phase was washed with brine and dried over Na_2SO_4. The organic phase was then reduced under vacuum and concentrated ethyl acetate extracts were chromatographed over a silica gel column (230-400). The product was obtained by elution with 40% acetone in petroleum ether.

Process for the preparation **CDHP-1**

A mixture of ethyl 3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxopropanoate (331 mg, 1 mmol), 4-Chloro-3-nitrobenzaldehyde (185 mg, 1 mmol) and urea (120 mg, 2 mmol) were taken in 10 ml of ethanol. Then 50µl of HCl and p-Toluenesulfonic acid (17.22 mg, 0.1 mmol) were added at the reaction mixture. Then the reaction mixture was heated at 90°C at argon atmosphere for 16h until completion of reaction, monitored by TLC. After that the solvent was evaporated in vacuum. The reaction mixture was quenched by addition of $NaHCO_3$ solution, and extracted with ethyl acetate. The combined organic phase was washed with brine and dried over Na_2SO_4. The organic phase was then reduced under vacuum and concentrated. The crude residue was purified by silica gel column chromatography (230-400) using 30% ethyl acetate in petroleum ether as eluent to obtain pure CDHP-1 as yellow solid (300 mg, 55% yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.82 (1H, s), 8.34(2H, m), 8.11 (3H, m), 7.73 (1H, d, J 9 Hz), 7.06(1H, d, J 9 Hz), 6.38 (1H, s), 5.58 (1H, s), 4.10 (2H, m), 3.72 (4H, m), 1.39 (6H, m), 1.15 (3H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.9, 159.8, 156.6, 152.2, 151.4, 147.9, 142.8, 132.4, 130.3, 124.4, 124.0, 109.6, 107.5, 96.6, 60.1, 53.4, 44.5, 14.0, 12.7. QTOF-MS -ESI (m/z) calculated for $C_{26}H_{26}ClN_4O_7^+$ $[M+H]^+$ 541.1484, found 541.4965.

Process for the preparation **CDHP-2**

A mixture of ethyl 3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxopropanoate (331 mg, 1 mmol), 4-Fluoro-3-nitrobenzaldehyde (169 mg, 1 mmol) and urea (120 mg, 2 mmol) were taken in 10 ml of ethanol. Then 30 µl of HCl and p-Toluenesulfonic acid (17.22 mg, 0.1 mmol) were added at the reaction mixture. Then the reaction mixture was heated at 90°C at argon atmosphere for 20 h until completion of reaction, monitored by TLC. After that the solvent was evaporated in vacuum. The reaction mixture was quenched by addition of $NaHCO_3$ solution, and extracted with ethyl acetate. The combined organic phase was washed with brine and dried over Na_2SO_4. The organic phase was then reduced under vacuum and concentrated. The crude

residue was purified by silica gel column chromatography (230-400) using 40 % acetone in petroleum ether as eluent to obtain pure CDHP-2 as orange yellow solid (320 mg, 62% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (1H), 8.16(1H), 7.87(1H, d, J 9 Hz), 7.64(1H, s), 7.27 (1H, d, J 9 Hz), 7.23(1H, s), 6.95 (1H, s), 6.58(1H, d, J 8.7 Hz), 6.47 (1H, s), 5.50 (1H, s), 3.98 (2H, m), 3.43(4H, m), 1.27 (6H, m), 1.01 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 160.6, 157.4, 153.7, 152.1, 143.3, 140.9, 137.8, 134.8, 134.8, 130.4, 125.4, 119.7, 115.5, 110.1, 108.4, 103.1, 98.0, 61.3, 54.9, 44.7, 14.5, 13.0; QTOF-MS -ESI (m/z) calculated for $C_{26}H_{26}FN_4O_7^+$ $[M+H]^+$ 525.178, found. 525.179

Process for the preparation **CDHP-3**

A mixture of ethyl 3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxopropanoate (331 mg, 1 mmol), 3-Nitro-4-(trifluoromethyl)benzaldehyde (219 mg, 1 mmol) and urea (120 mg, 2 mmol) were taken in 10 ml of ethanol. Then 40 µl of HCl and p-Toluenesulfonic acid (17.22 mg, 0.1 mmol) were added at the reaction mixture. Then the reaction mixture was heated at 90°C at argon atmosphere for 24h until completion of reaction, monitored by TLC. After that the solvent was evaporated in vacuum. The reaction mixture was quenched by addition of $NaHCO_3$ solution, and extracted with ethyl acetate. The combined organic phase was washed with brine and dried over Na_2SO_4. The organic phase was then reduced under vacuum and concentrated. The crude residue was purified by silica gel column chromatography (230-400) using 30% acetone in petroleum ether as eluent to obtain pure CDHP-3 as deep yellow solid (295 mg, 51% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64 (1H, s), 8.32 (1H), 8.20(1H, d, J 6.3 Hz), 8.04(1H, d, J 6.3 Hz), 7.97 (1H, s), 7.86(1H, s), 7.44 (1H, d J 6.9 Hz), 6.70 (1H, d, J 6.9 Hz), 6.56 (1H, s), 5.94 (1H, s), 3.72(2H, m), 3.44(4H, m), 1.12 (6H, m), 0.077 (3H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.9, 159.1, 156.1, 151.1, 150.9, 147.5, 145.0, 142.7, 142.2, 130.8, 121.5, 114.9, 109.1, 107.1, 99.9, 96.3, 59.5, 49.6, 44.1, 13.4, 12.2. QTOF-MS -ESI (m/z) calculated for $C_{27}H_{26}F_3N_4O_7^+$ $[M+H]^+$ 575.174, found. 575.184 ^{13}C (DEPT-135): 142.0, 130.5, 129.5, 121.3, 108.9, 96.1, 59.3, 49.3, 43.6, 13.2, 12.0.

Process for the preparation **CDHP-4**

A mixture of ethyl 3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxopropanoate (331 mg, 1 mmol), 3,4-Difluorobenzaldehyde (142 mg, 1 mmol) and urea (120 mg, 2 mmol) were taken in 10 ml of ethanol. Then 40 µl of HCl and p-Toluenesulfonic acid (17.22 mg, 0.1 mmol) were added at the reaction mixture. Then the reaction mixture was heated at 80°C at argon atmosphere for 24h until completion of reaction, monitored by TLC. After that the solvent was evaporated in vacuum. The reaction mixture was quenched by addition of $NaHCO_3$ solution, and extracted with ethyl acetate. The combined organic phase was washed with brine and dried over Na_2SO_4. The organic phase was then reduced under vacuum and concentrated. The crude residue was purified by silica gel column chromatography (230-400) using 25% acetone in petroleum ether as eluent to obtain pure CDHP-4 as bright yellow solid (360 mg, 72% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (1H, s), 7.46(1H, d, J 6Hz), 7.22(2H, m), 7.04 (2H, m), 6.46 (1H, d, J 6Hz), 6.37 (1H, s), 5.90(1H, s), 5.34 (1H, s), 3.86 (2H, m), 3.30(4H, m), 1.10 (6H, m), 0.8 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.8, 159.9, 157.0, 152.7, 151.7, 142.7, 142.0, 140.1, 129.7, 123.0, 117.8, 116.2, 115.0, 109.3, 107.7, 103.6, 97.4, 60.6, 55.1, 45.0, 29.4, 14.0, 12.5. QTOF-MS -ESI (m/z) calculated for $C_{26}H_{26}F_2N_3O_5^+$ $[M+H]^+$ 498.1835, found 498.1830. ^{13}C (DEPT-135): 142.5, 129.6, 122.8, 117.7, 117.5, 116.0, 115.9, 109.2, 97.2, 60.0, 55.0, 44.9, 13.8, 12.4. ^{19}F ($CDCl_3$) δ -136.52, -138. 57. HPLC purity> 96%.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.44(1H, s), 7.88(1H, s), 7.82 (1H, s), 7.38-7.35 (3H, m), 7.26(1H, s), 6.69 (1H, m), 6.53 (1H, s), 5.18 (1H, s), 3.78(2H, m), 3.78(4H, m), 1.10 (6H, m), 0.8 (3H, m) ^{13}C NMR (100 MHz, DMSO-d_6) 165.0, 159.8, 157.3, 156.6, 152.4, 151.4, 151.0, 150.9, 150.5, 148.6, 147.9, 144.8, 147.9, 144.8, 142.7, 142.1, 130.3,123.8, 118.2, 118.0, 116.1, 115.9, 115.8, 109.6, 107.7, 101.1, 96.8, 60.0, 59.6, 53.5, 44.6, 30.1, 14.2, 12.8.

Process for the preparation **CDHP-5**

A mixture of ethyl 3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxopropanoate (331 mg, 1 mmol), 4-Dimethylamino-2-nitrobenzaldehyde (194 mg, 2 mmol) and urea (120 mg, 2 mmol) were taken in 10 ml of ethanol. Then 60µl of HCl and p-Toluenesulfonic acid (17.22 mg, 0.1 mmol) were added at the reaction mixture. Then the reaction mixture was heated at 90°C at argon atmosphere for 24h until completion of reaction, monitored by TLC. After that the solvent was evaporated in vacuum. The reaction mixture was quenched by addition of $NaHCO_3$ solution, and extracted with ethyl acetate. The combined organic phase was washed with brine and dried over Na_2SO_4. The organic phase was then reduced under vacuum and concentrated. The crude residue was purified by silica gel column chromatography (230-400) using 40% acetone in petroleum ether as eluent to obtain pure CDHP-5 as deep yellow solid (380 mg, 69% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (1H, s), 7.81 (1H, s), 7.58(1H, d, J 6.6 Hz), 7.55(1H, s), 7.44 (1H, d, J 6.6 Hz), 7.06(2H, m), 6.7 (1H, m), 6.55 (1H, s), 5.94 (1H, s), 3.72(2H, m), 3.44(4H, m), 1.12 (6H, m), 0.081 (3H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0, 159.1, 156.1, 151.5, 150.8, 149.8, 148.4, 143.9, 141.8, 129.6, 124.4, 117.1, 115.5, 109.0, 107.2, 105.8, 101.0, 96.3, 59.3, 48.9, 44.0, 13.5, 12.2. QTOF-MS -ESI (m/z) calculated for $C_{28}H_{32}N_5O_7^+$ $[M+H]^+$ 550.2296, found 550.2278. ^{13}C (DEPT-135): 141.6, 129.4, 116.8, 108.7, 105.5, 96.0, 59.0, 48.6, 43.8, 13.2, 12.0.

Process for the preparation **CDHP-6**

A mixture of ethyl 3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxopropanoate (331 mg, 1 mmol), 3,4,5-Trimethoxybenzaldehyde (196 mg, 1 mmol) and urea (120 mg, 2 mmol) were taken in 10 ml of ethanol. Then 50µl of HCl and p-Toluenesulfonic acid (17.22 mg, 0.1 mmol) were added at the reaction mixture. Then the reaction mixture was heated at 90°C at argon atmosphere for 20h until completion of reaction, monitored by TLC. After that the solvent was evaporated in vacuum. The reaction mixture was quenched by addition of $NaHCO_3$ solution, and extracted with ethyl acetate. The combined organic phase was washed with brine and dried

over Na_2SO_4. The organic phase was then reduced under vacuum and concentrated. The crude residue was purified by silica gel column chromatography (230-400) using 30% acetone in petroleum ether as eluent to obtain pure CDHP-6 as light yellow solid (400 mg, 73% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.33 (1H, s), 7.81 (2H, s), 7.46(1H, d, J 6.6 Hz), 6.82(2H, s), 6.70 (1H, d, J 6.6 Hz), 6.54(1H, m), 6.70 (1H, m), 6.55 (1H, s), 5.29 (1H, s), 5.14(1H, s), 3.86(2H, m), 3.76 (6H, s), 3.63(3H, m),3.41(4H, m), 1.23 (6H, m), 1.12 (3H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 159.4, 156.0, 152.8, 152.0, 150.8, 143.8, 141.8, 139.2, 136.7, 129.7, 115.7, 109.0, 107.2, 103.9, 100.8, 96.2, 62.5, 55.7, 53.8, 52.0, 44.0, 13.7, 12.2. QTOF-MS -ESI (m/z) calculated for $C_{29}H_{34}N_3O_8^+$ $[M+H]^+$ 552.2340, found 552.2349. ^{13}C (DEPT-135): 141.6, 129.5, 108.3, 103.6, 96.0, 62.3, 59.0, 55.5, 53.5, 51.7, 13.5, 12.0.

Process for the preparation **CDHP-7**

A mixture of ethyl 3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxopropanoate (331 mg, 1 mmol), 1-Naphthaldehyde (156 mg, 1 mmol) and urea (120 mg, 2 mmol) were taken in 10 ml of ethanol. Then 50µl of HCl and p-Toluenesulfonic acid (17.22 mg, 0.1 mmol) were added at the reaction mixture. Then the reaction mixture was heated at 90°C at argon atmosphere for 20h until completion of reaction, monitored by TLC. After that the solvent was evaporated in vacuum. The reaction mixture was quenched by addition of $NaHCO_3$ solution, and extracted with ethyl acetate. The combined organic phase was washed with brine and dried over Na_2SO_4. The organic phase was then reduced under vacuum and concentrated. The crude residue was purified by silica gel column chromatography (230-400) using 30% acetone in petroleum ether as eluent to obtain pure CDHP-7 as bright yellow solid (405 mg, 79% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (2H, m), 7.79 - 7.71 (3H, m), 7.56-7.49(2H, m), 7.39-7.36(2H, s), 7. 19-7.11 (1H, m), 6.44(2H, m), 6.38 (1H, s), 5.56 (1H, s), 3.86(2H, m), 3.32 (4H, s), 1.18 (6H, m), 0.9 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.7, 159.6, 156.6, 152.9, 151.2, 142.4, 141.4, 140.1, 133.2, 132.9, 129.4, 128.8, 128.2, 127.4, 125.9, 125.6, 124.7, 115.1, 108.9, 107.5, 103.7, 97.1, 60.1, 55.9,

44.7, 13.7, 12.3. QTOF-MS -ESI (m/z) calculated for $C_{30}H_{30}N_3O_5^+$ $[M+H]^+$ 512.2180, found 512.5515.

General procedure for synthesis of **CDHP@PEG$_{4000}$-PLGA-NPs**

Nanoparticles were prepared using a modified nanoprecipitation method. Briefly, 15 mg of Polyethylene glycol 4000 was dissolved in 10 ml of Milli-Q water at 37°C in Ultrasonic Bath Sonicator. Then 10ml of 4% polyvinyl alcohol (PVA) solution was added and the mixture was homogenized in a Probe Sonicator /Ultrasonic Homogenizer for 2min to obtain a clear solution. Next, 85 mg of poly (dl-lactide-coglycolide) PLGA (50:50) was dissolved in 4 ml of dichloromethane as an oil phase at room temperature to obtain uniform solution. The PLGA in dichloromethane was added drop wise by a syringe to the above PEG 4000 solution (containing PVA) at 40°C with continuous stirring at 5000 rpm. Then it was further emulsified in same Probe sonicator for additional 4 min. After then, the mixture was stirred at 2000 rpm (at 45°C) for 2h to remove organic solvent. In the next step, 10 mg of CDHPs dissolved in 2ml of dichloromethane was added by a 1ml syringe in spraying methods into the previous emulsion at 40°C with a flow rate of 0.2 ml/min for 30 min at continuous mode in Probe Sonicator. Increase in flow rate may result in precipitation/aggregations of yellow CDHP which can be clearly seen in the reaction container. After complete injection of CDHP by spraying, the organic solvent present in suspension was evaporated by room temperature stirring the whole solution at 1000 rpm for overnight at dark. All the above process is executed in dark. Finally, a light to deep yellow clear solution was observed. The nanoparticles were then collected by centrifugation at 10000g for 40 min at 4°C. The yellow color precipitate was washed with ethanol and Milli-Q water. Finally, the very light yellow nanoparticle were re-suspended in 4 ml of 3% sucrose solution as cryoprotectent and dried on a Lyophilizer. The nanoparticles can be stored at 4°C for long time use.

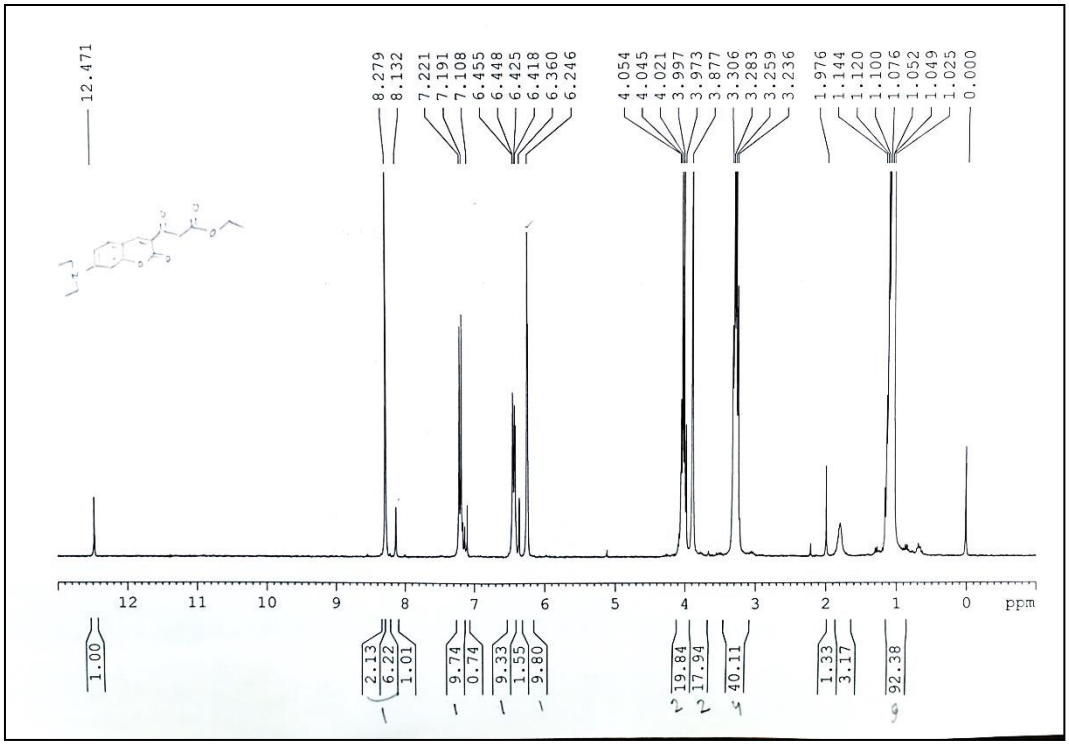

Figure S-1: ^{1}H NMR of EDCO

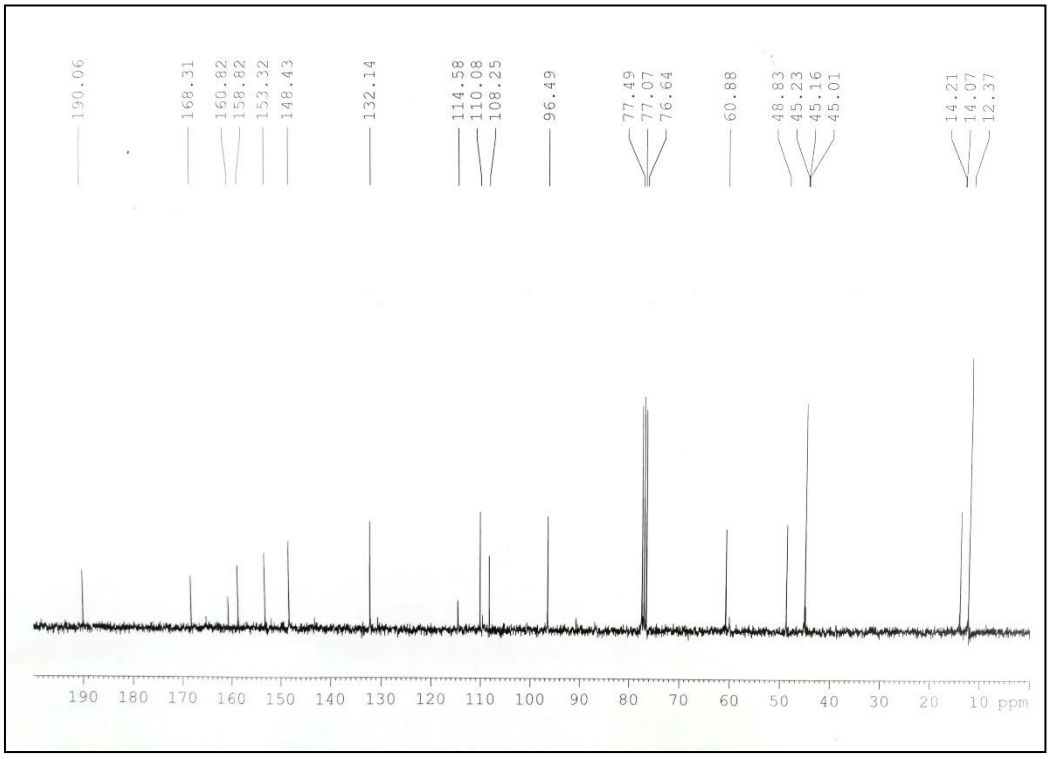

Figure S-2: ^{13}C NMR of EDCO

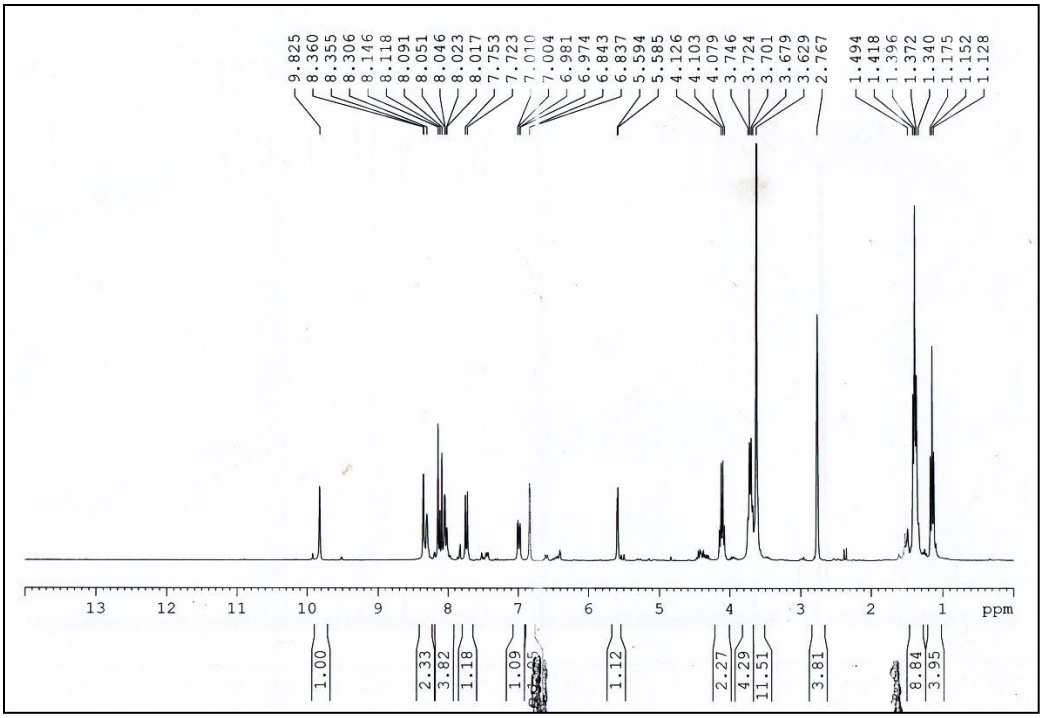

Figure S-3: ^{1}H NMR of CDHP-1

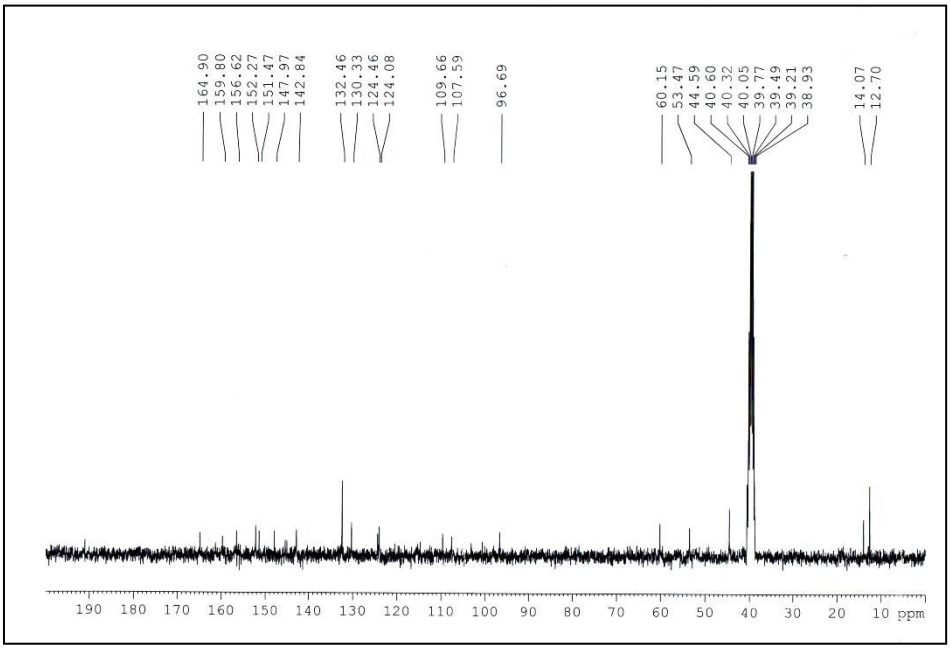

Figure S-4: ^{13}C NMR of CDHP-1

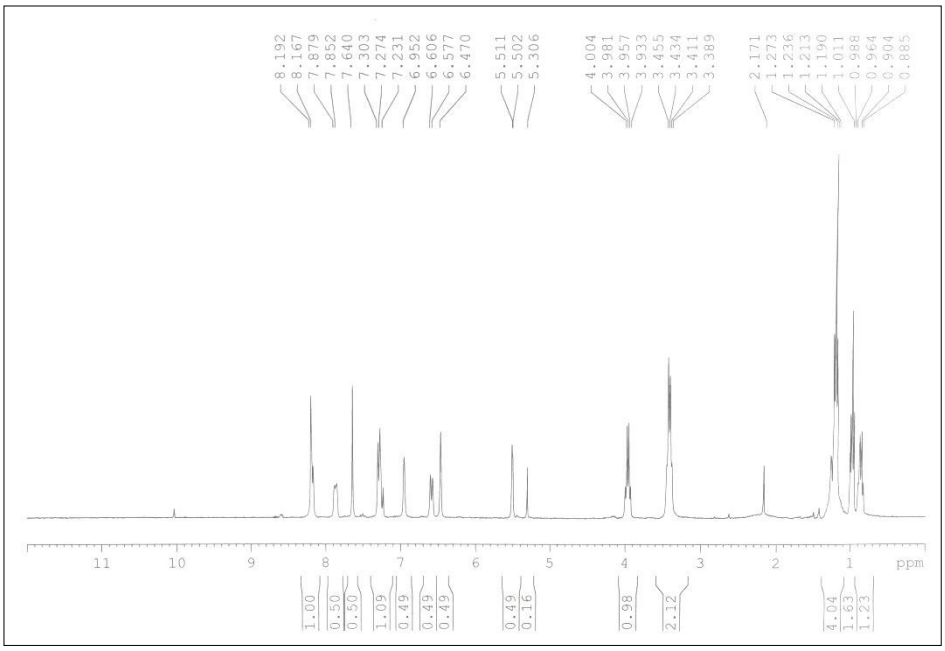

Figure S-5: ^{1}H NMR of CDHP-2

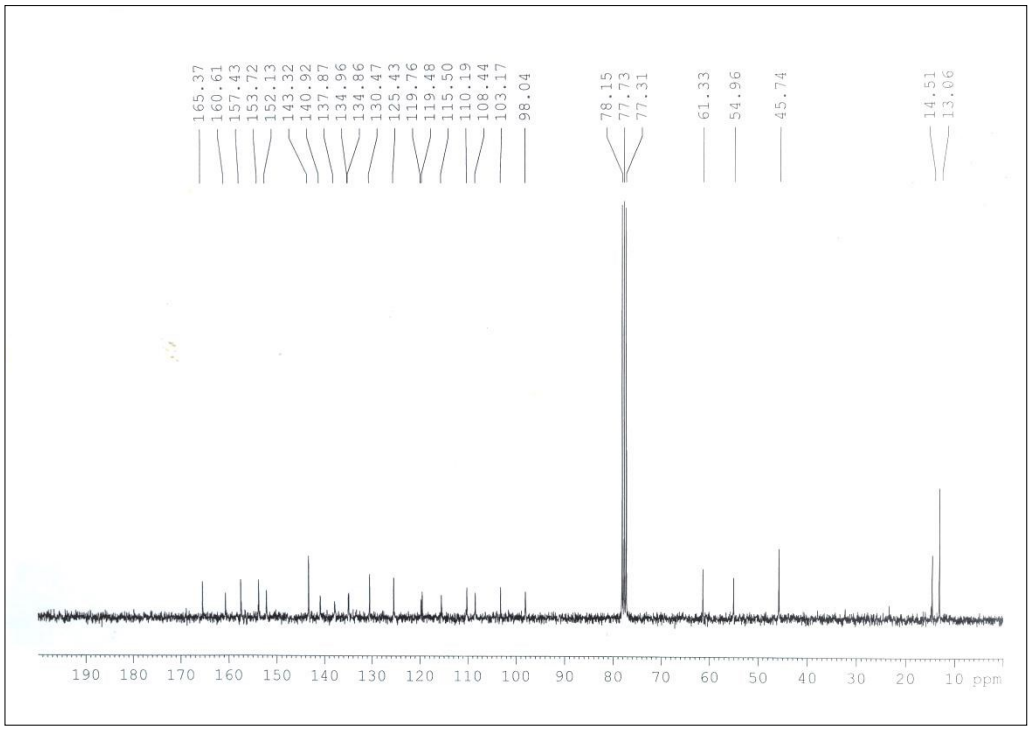

Figure S-6: ^{13}C NMR of CDHP-2

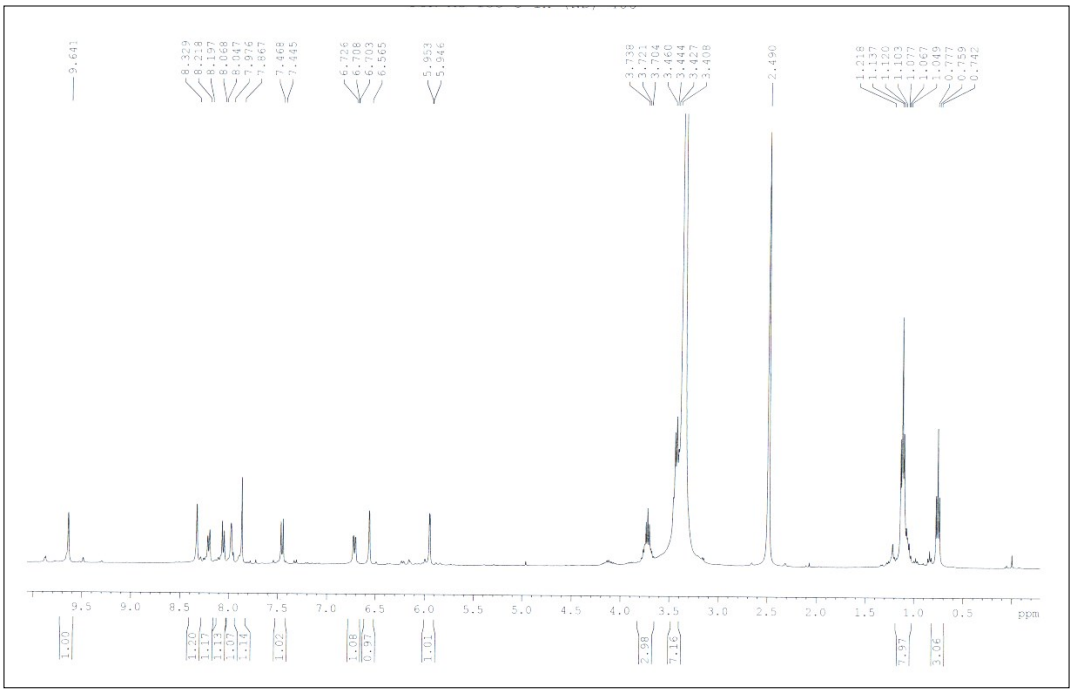

Figure S-7: ^{1}H NMR of CDHP-3

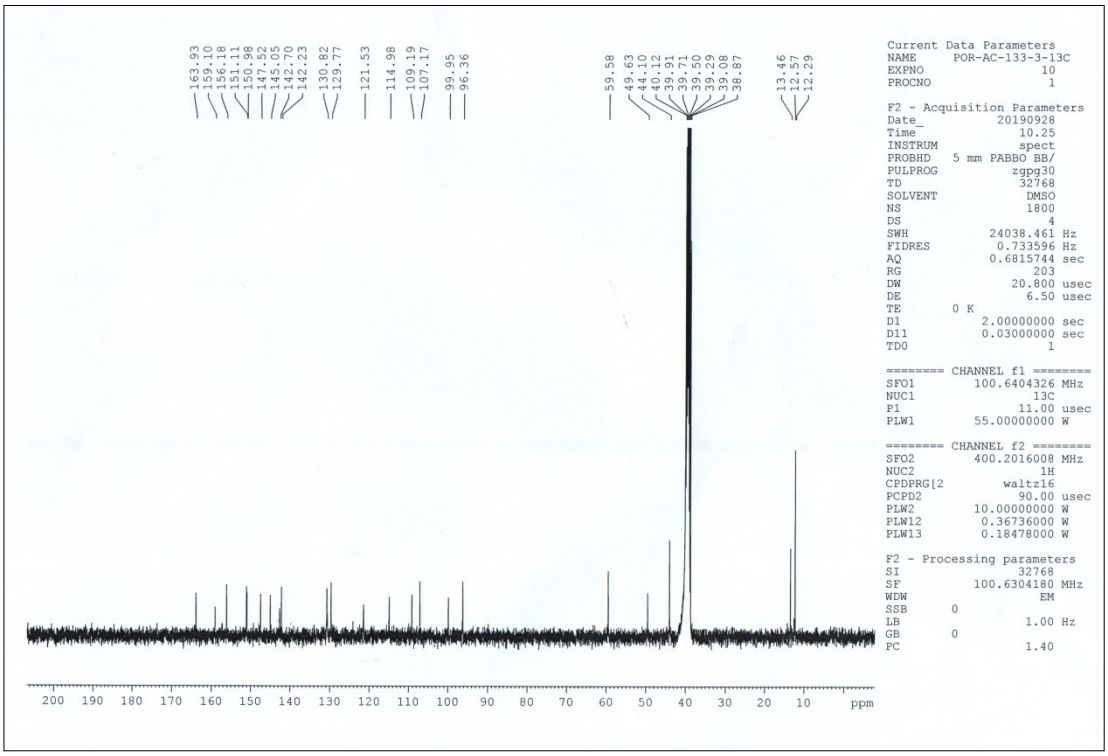

Figure S-8: ^{13}C NMR of CDHP-3

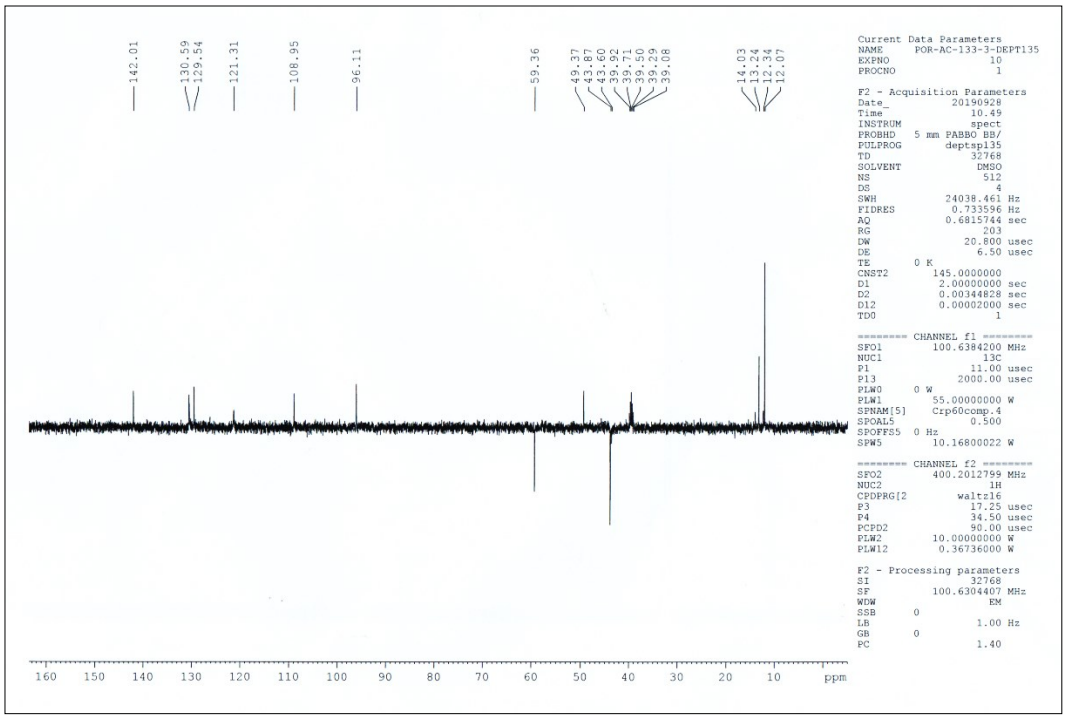

Figure S-9: ^{13}C (DEPT-135) CDHP-3

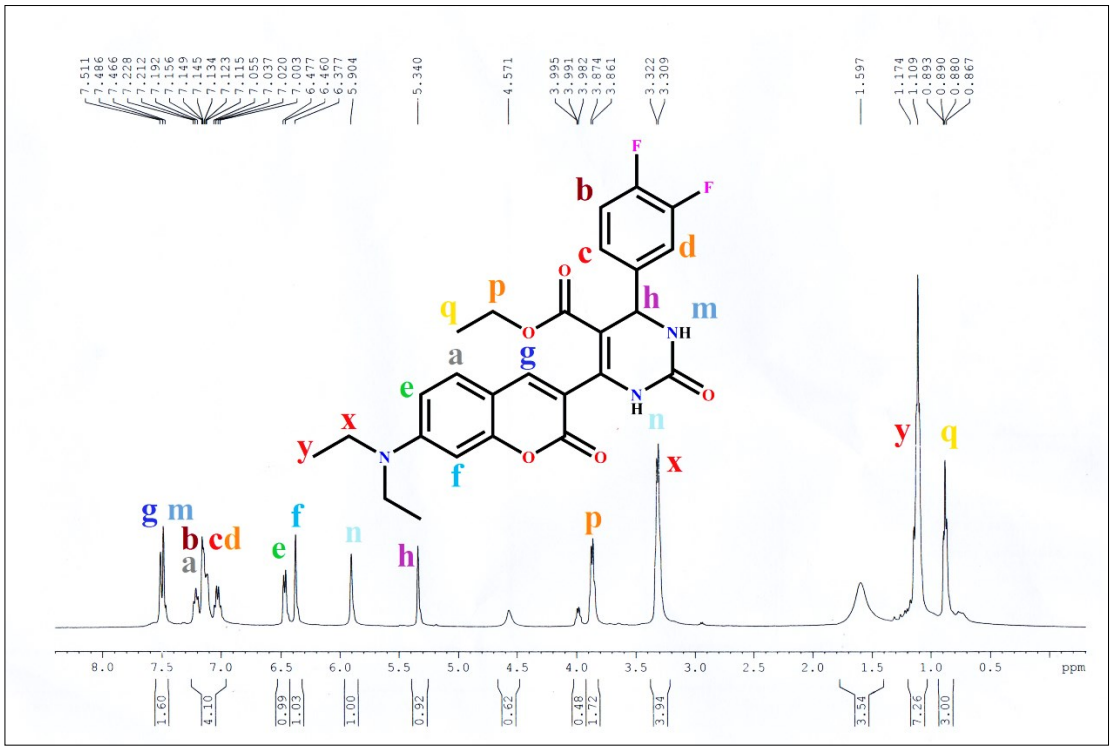

Figure S-10: ^{1}H NMR of CDHP-4

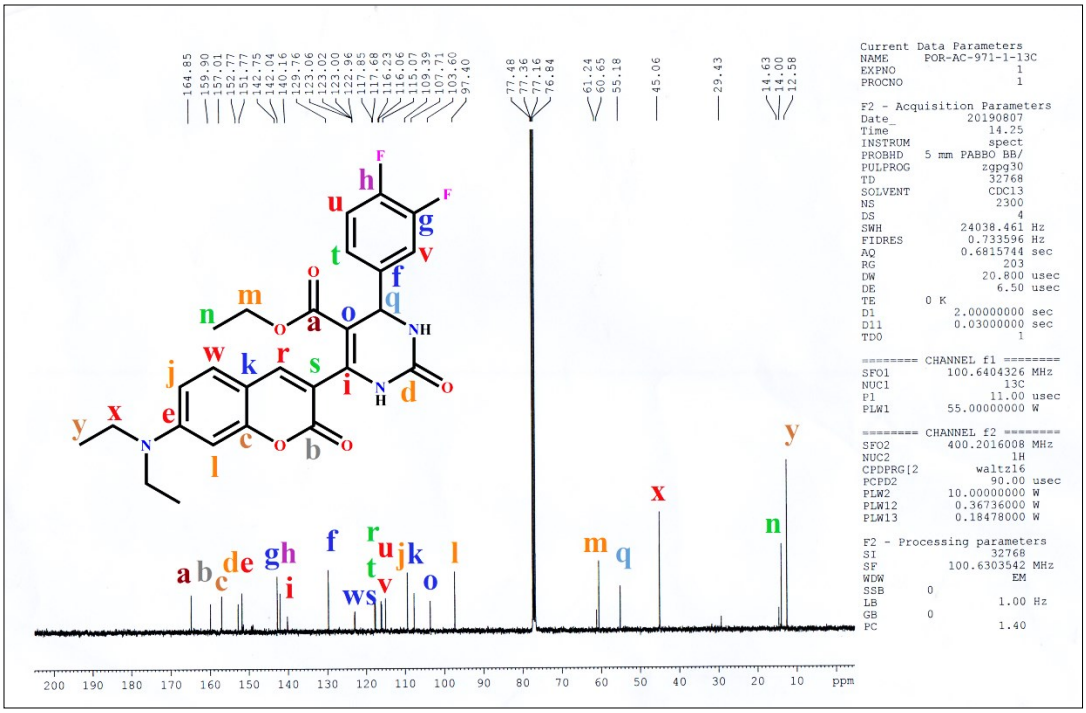

Figure S-11: ^{13}C CDHP-4

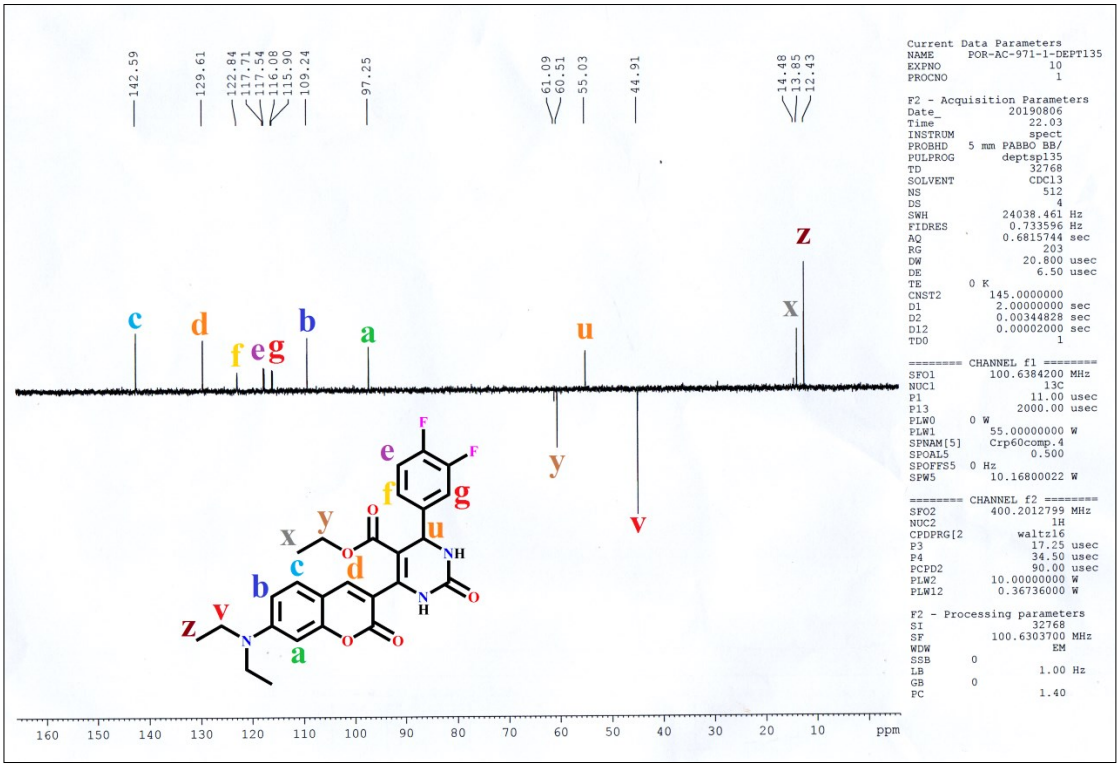

Figure S-12: ^{13}C (DEPT-135) NMR of CDHP-4

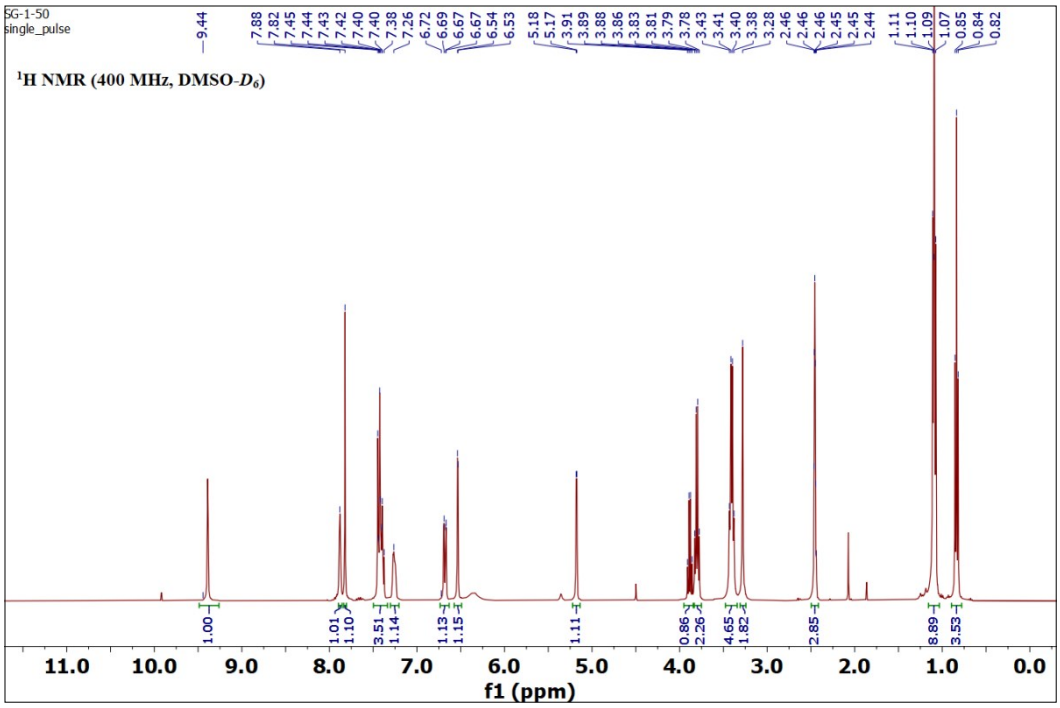

Figure S-13: ^{1}H NMR of CDHP-4 (DMSO-d_6)

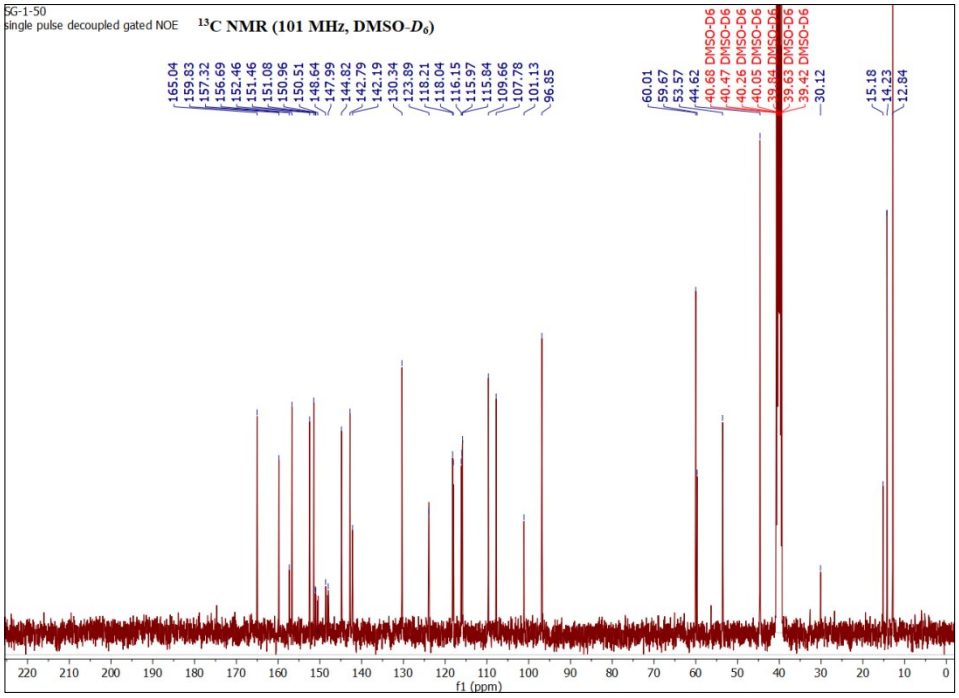

Figure S-14: ^{13}C NMR of CDHP-4(DMSO-d_6)

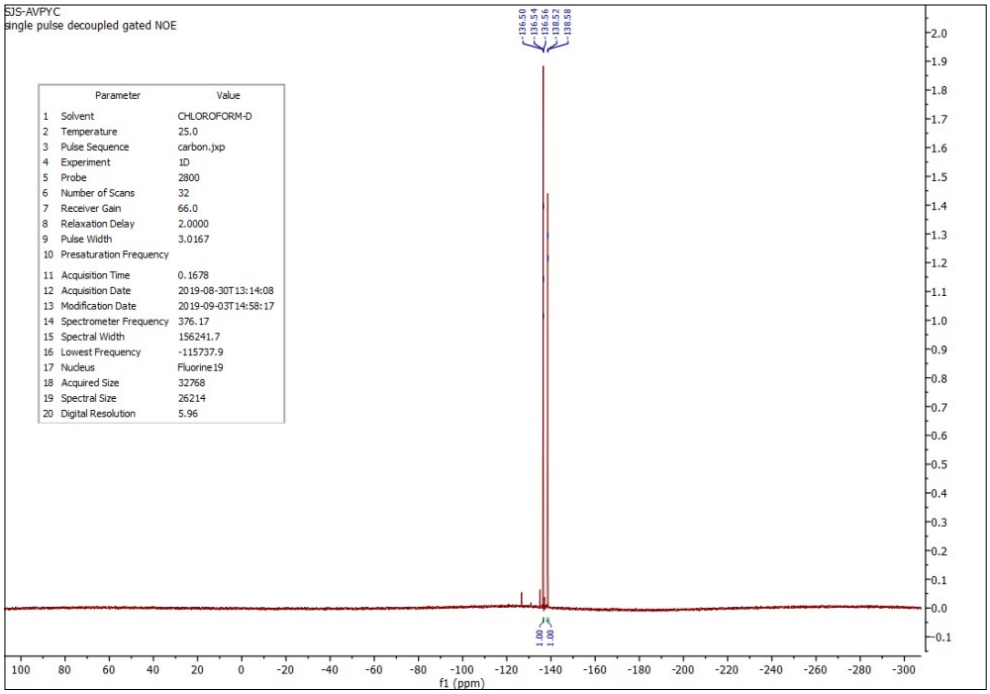

Figure S-15: ^{19}F NMR of CDHP-4

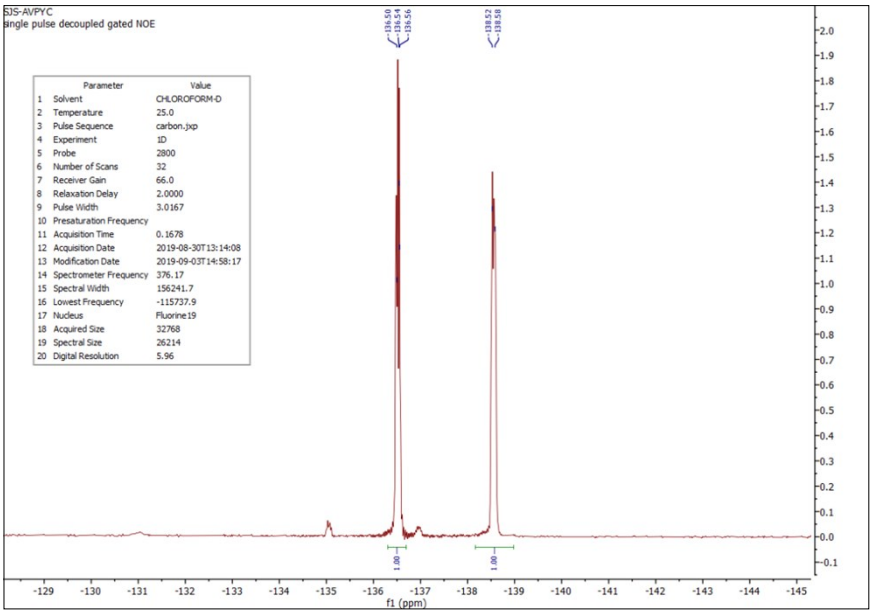

Figure S-16: ^{19}F NMR of CDHP-4 (expanded)

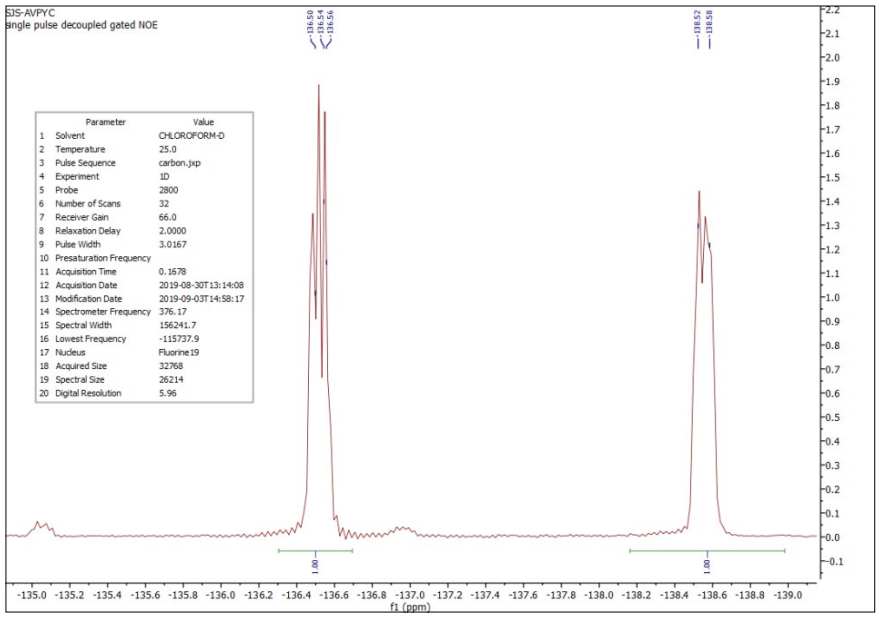

Figure S-17: ^{19}F NMR of CDHP-4 (expanded)

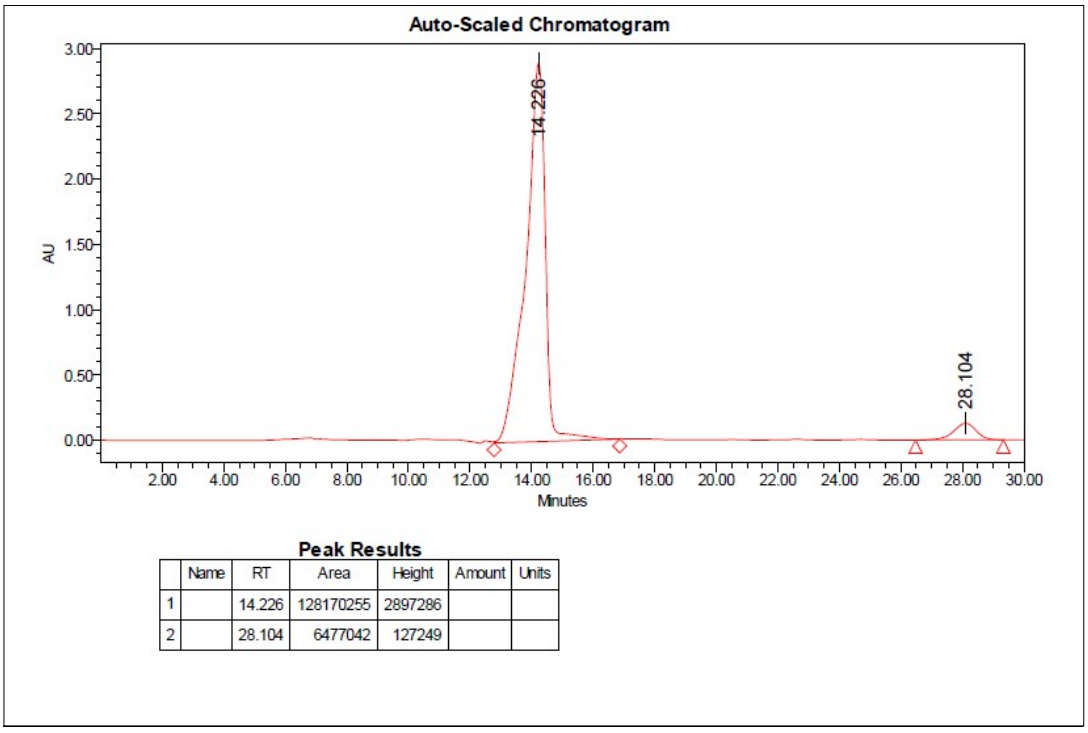

Figure S-18: HPLC purity of CDHP-4

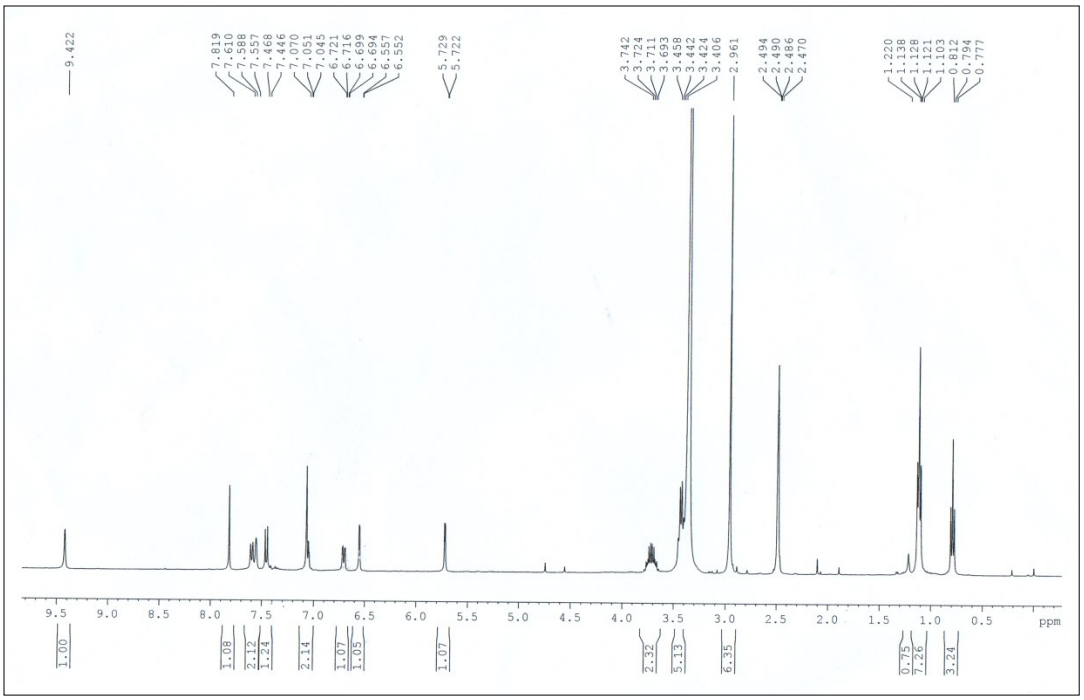

Figure S-19: ^{1}H NMR of CDHP-5

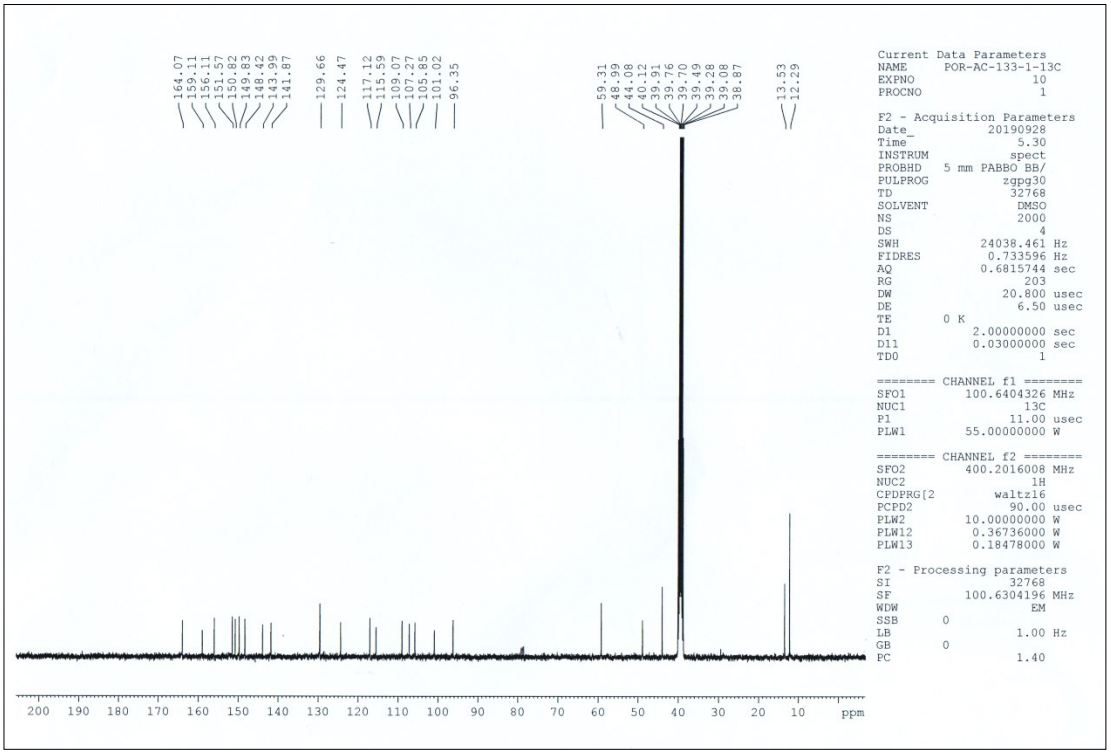

Figure S-20: ^{13}C NMR of CDHP-5

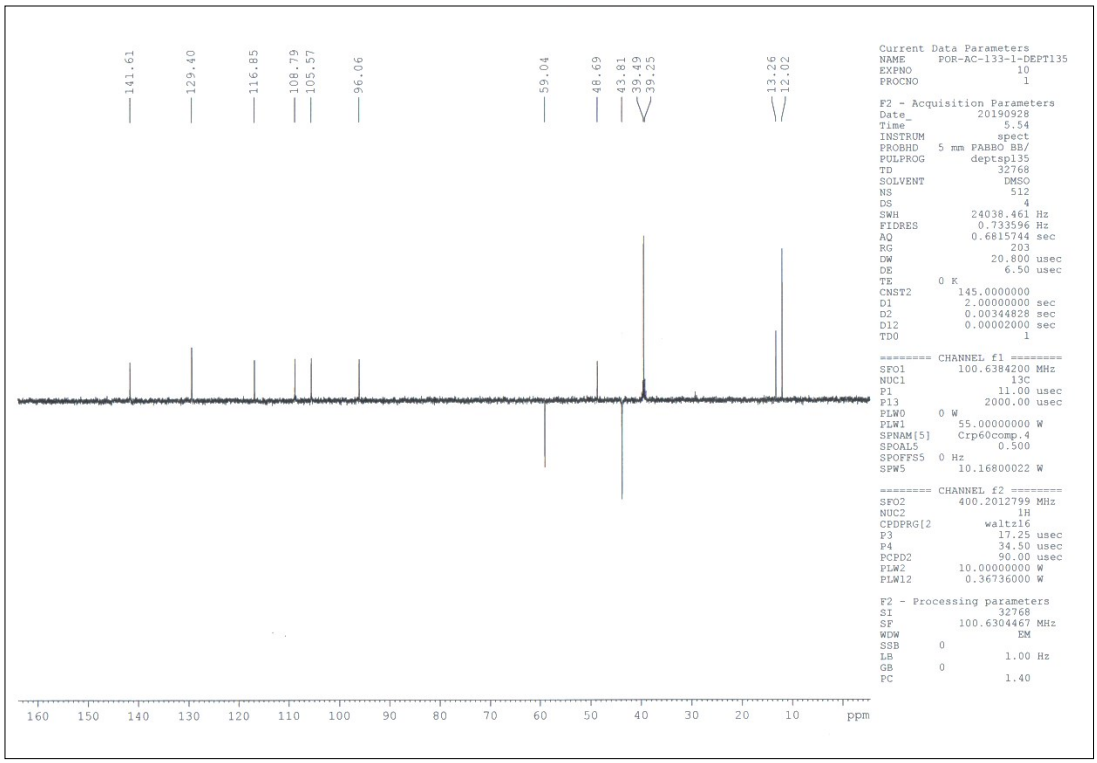

Figure S-21: ^{13}C (DEPT-135) NMR of CDHP-5

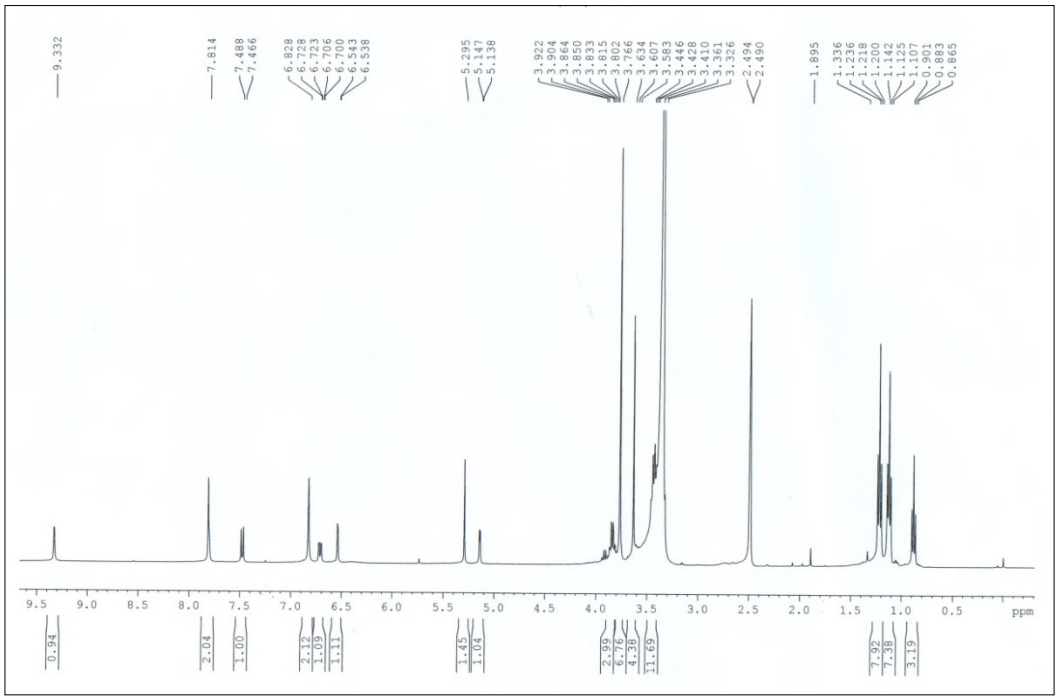

Figure S-22: ^{1}H NMR of CDHP-6 (DMSO-d_6)

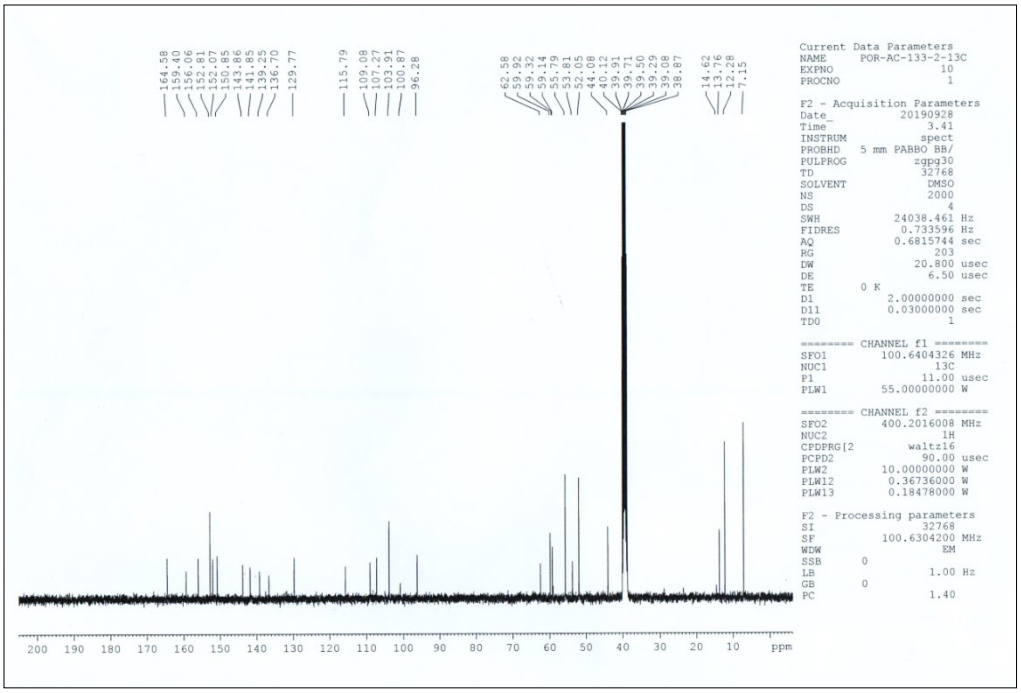

Figure S-23: ^{13}C NMR of CDHP-6(DMSO-d_6)

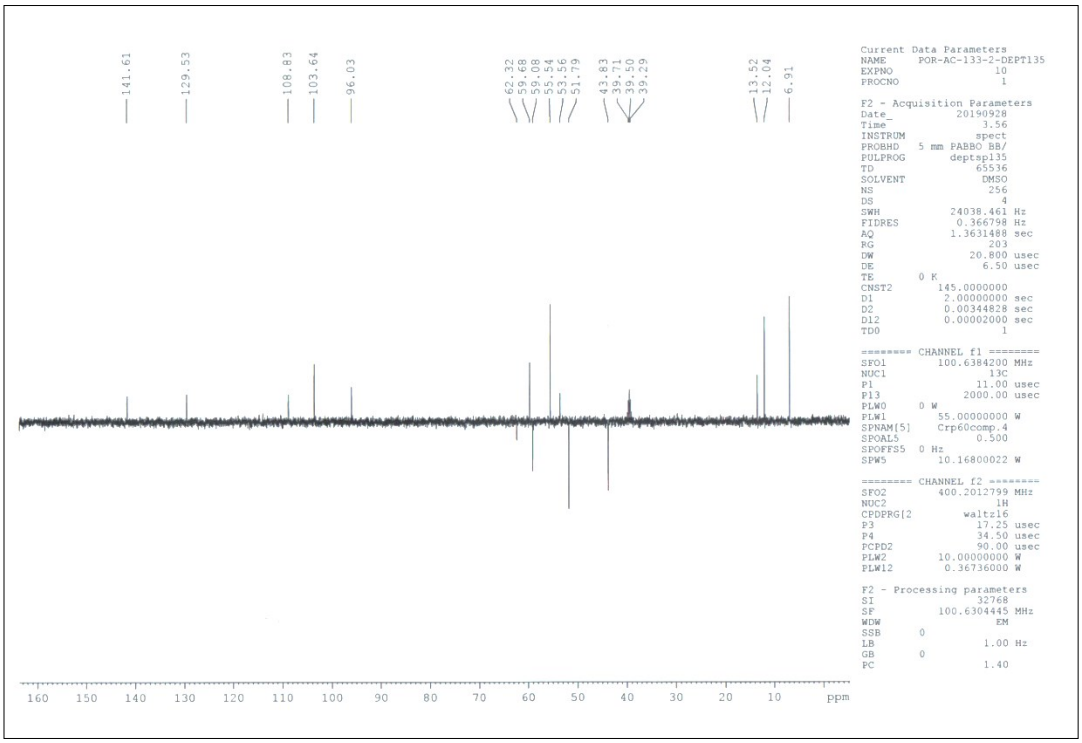

Figure S-24: ^{13}C (DEPT-135) NMR of CDHP-6

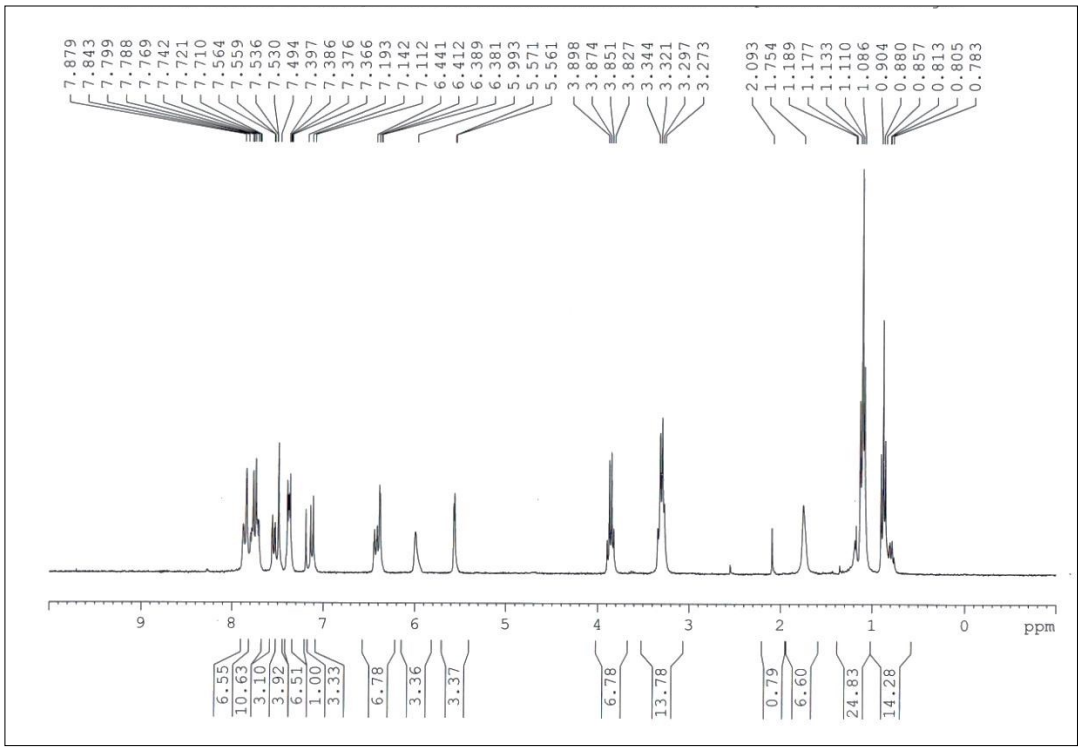

Figure S-25: ^{1}H NMR of CDHP-7

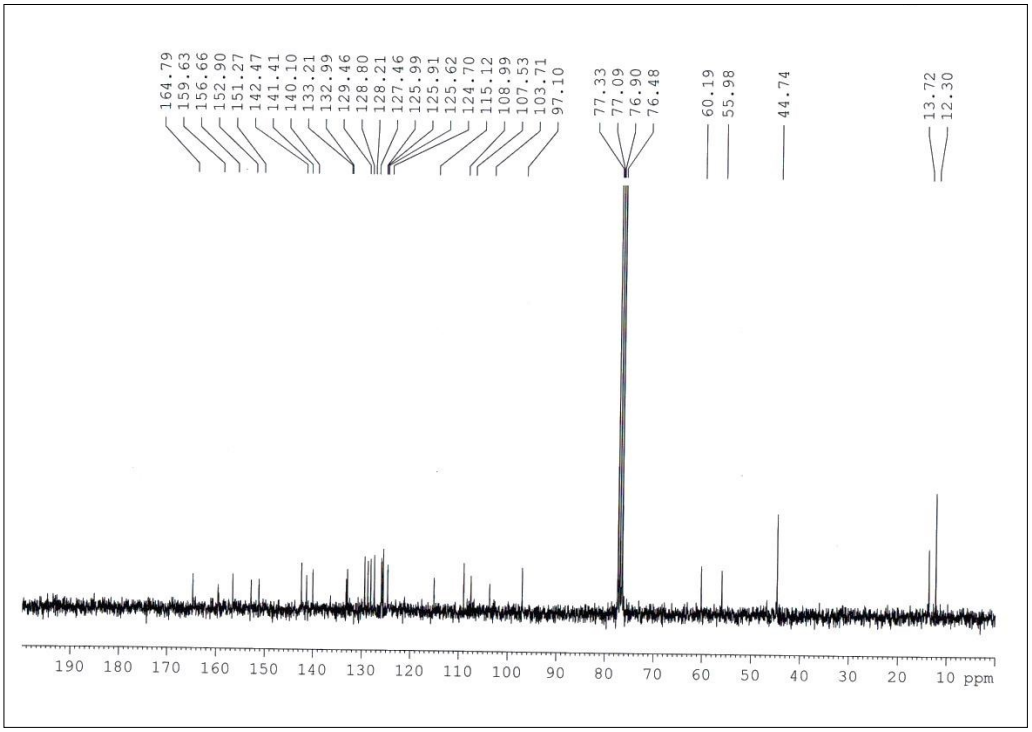

Figure S-26: ^{13}C NMR of CDHP-7

***In vitro* drug release**

In order to evaluate the CDHP-4 release pattern from CDHP-4@PP-NPs *in vitro* drug release was examined. Briefly, 20 mg of CDHP-4@PP-NPs were dispersed in 5ml phosphate buffer mixed with FBS (at 10 % v/v concentration) with pH7.4 and pH5.3 respectively. Then these solutions were transferred inside a dialysis membrane whose MWCO is 2KDa (Sigma, USA). The membrane was placed against 100 ml of respective phosphate buffer (pH 7.4 or pH 5.3 containing 1% Tween 80) in a beaker at a stirring rate of 150 rpm at 37°C. At various time intervals, 3 mL of the dialyzed buffer was withdrawn and the absorbance at ~398 nm was measured to determine the quantity of the CDHP-4 released and 3 mL of fresh phosphate buffer was added to the dialysis solution to maintain sink volume.

Table S-1: Physicochemical properties of CDHP-4-loaded mixed polymeric nanoparticles

Name	CDHP-4: PLGA-PEG (W:W)	PLGA:PEG	%EE[a] (W/W)	%DL[a] (W/W)	%Yield[c]	Size[d] (in nm)	Zeta Potential(mV)
CDHP-4$_{1:5}$@-PLGA-Nps	1:5	PLGA 0% PEG	64.5 ± 1.5	14.93 ± 0.02	72±4	246.3 ± 4.2	-26.1±0.90
CDHP-4$_{1:5}$@-PP$_5$-Nps		PLGA 5% PEG	66.1 ± 1.4	15.48 ± 0.23	71±3	250.4 ± 2.5	-22.3±1.09
CDHP-4$_{1:5}$@-PP$_{10}$-Nps		PLGA 10% PEG	65.2 ± 1.7	15.52 ± 0.50	70±3	253.2 ± 3.7	-18.4±1.24
CDHP-4$_{1:5}$@-PP$_{15}$-Nps		PLGA 15% PEG	63.1 ± 2.0	15.02 ± 0.45	70±2	230.7 ± 5.8	-14.3±0.89
CDHP-4$_{1:5}$@-PP$_{20}$-Nps		PLGA 20% PEG	62.2 ± 1.5	14.80 ± 0.45	70±4	225.4 ± 3.1	-16.1±0.23
CDHP-4$_{1:5}$@-PP$_{30}$-Nps		PLGA 30% PEG	59.5 ± 2.5	14.16 ± 0.35	70±2	228.6 ± 3.5	-18.2±1.01
CDHP-4$_{1:5}$@-		PLGA	55.1 ± 1.5	13.91 ± 0.50	66±4	223.3 ± 3.9	-24.2±1.23

PEG_{50}-Nps		50% PEG					
CDHP-$4_{1:10}$@-PLGA-Nps	1:10	PLGA 0% PEG	79.9 ± 1.4	9.95 ± 0.18	*73*±2	213.4 ± 2.2	-27.4±0.95
CDHP-$4_{1:10}$@-PP_5-Nps		PLGA 5% PEG	80.5 ± 1.5	9.62±0.35	76±2	*212.0 ± 3.4*	*-23.9*±1.10
CDHP-$4_{1:10}$@-PP_{10}-Nps		PLGA 10% PEG	85.7 ± 1.3	10.52 ± 0.45	74±3	190.9 ± 2.4	-19.7±0.75
CDHP-$4_{1:10}$@-PP_{15}-Nps		**PLGA 15% PEG**	***87.7 ± 1.7***	***10.77 ± 0.25***	**74±2**	**182.4 ± 3.4**	**-14.5±0.69 -15.8±0.88 (blank)**
CDHP-$4_{1:10}$@-PP_{20}-Nps		PLGA 20% PEG	75.3 ± 1.5	9.77 ± 0.20	70±2	174.1 ± 2.5	-18.2±1.23
CDHP-$4_{1:10}$@-PP_{30}-Nps		PLGA 30% PEG	73.2 ± 2.7	9.78 ± 0.21	68±4	163.3 ± 2.5	-19.1±1.08
CDHP-$4_{1:10}$@-PEG_{50}-Nps		PLGA 50% PEG	70.8 ± 3.5	9.46 ± 0.32	68±2	158.4 ± 3.4	-23.7±0.20

From the table S-1 it could be observed that 1:10 (W/W) CDHP-4: polymer ratio attends the most satisfactory encapsulation efficiency of 87.7%. In this polymer content the ratio of PLGA: PEG was found to be 85:15. Hence, it is also obvious that PEG_{4000} is necessary to achieve most favorable nanoparticle. However, increasing PEG above that decreases the encapsulation efficiency. On the other hand, increasing the PLGA amount had a beneficial impact on encapsulation efficiency of CDHP-4. However, optimum PLGA quantity was found to be 85%. It is important to note that increasing the concentration of PVA diminished entrapment efficiency considerably. Hence we have used 4% PVA in synthesis of the nanoparticle. The drug loading content of CDHP-4@PP-NPs is found to be 10.77%. Moreover, the yield of the reaction was found to 74% in this selected polymer ratio. The size of the nanoparticle could be adjusted by synthetic procedure. However, increasing the polymer ratio generally decreases the size of the nanoparticle. The zeta potential of blank PEG-PLGA NPs was found to be -15.8 which become -14.5 upon encapsulation with CDHP-4. It is generally observed that with increasing PVA the value of zeta potential decreases.

Determination of stability of nanoparticles

The stability of aqueous solutions of nanoparticles at 37°C and freezer temperature (~4°C) was investigated up to 60 days after their synthesis. To examine this, two nanoparticle solutions in Millipore water having same concentration were stored at 4°C and 37°C respectively. At different time intervals, 6 ml aqueous solution was directly taken out from each container and collected into a clean centrifuge tube followed by centrifugation at 14,000 rpm for 30 minutes at room temperature. The supernatant of each tube was subjected to UV-Visible spectroscopy at ~398 nm wavelength for measuring the CDHP-4 concentration of the respective aqueous solution. From this percentage drug retention of that solution was calculated according to the following formula:

$$\%drug\ retention = \frac{The\ final\ concentration\ of\ drug\ in\ nanoparticles\ in\ nth\ day\ after\ its\ synthesis}{The\ initial\ concentration\ of\ drug\ in\ nanoparticles\ after\ synthesis} \times 100$$

Furthermore, the hydrodynamic diameters were also measured in this regard. For that, another 6ml aqueous solution was collected from each glass vials and directly subjected to DLS measurement. The entire investigation was performed thrice and individual measurements were done in triplicate. The values were expressed as mean ± standard error plotted against their respective months.

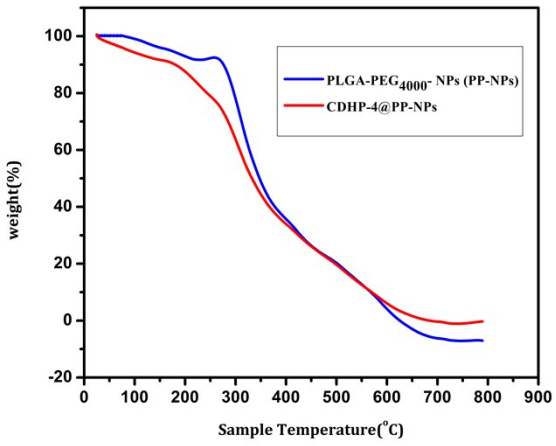

Figure S-27: TGA thermogram of CDHP-4@PP-NPs

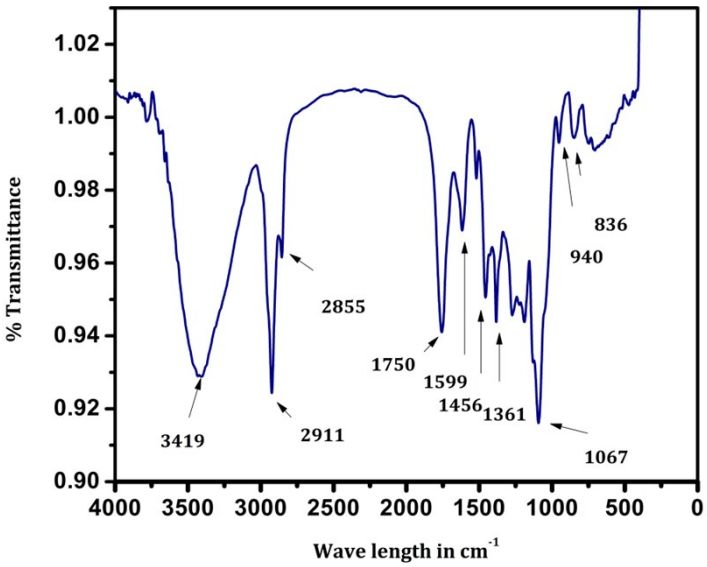

Figure S-28: FTIR of CDHP-4@PP-NPs

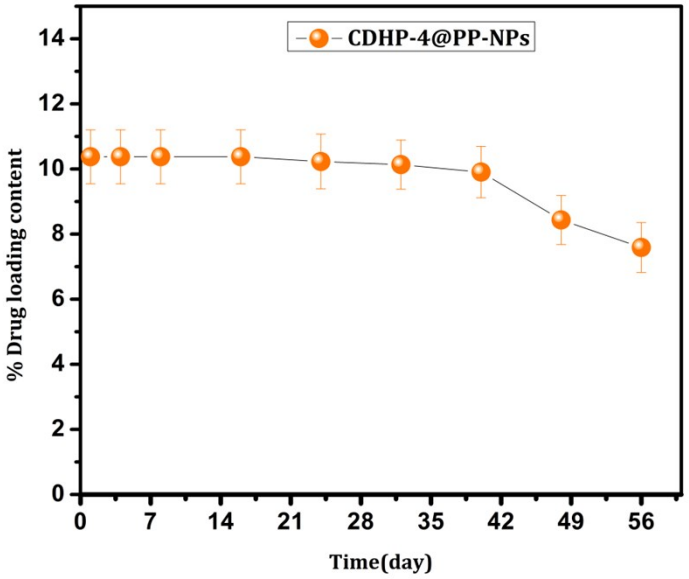

Figure S-29: Percentage drug (CDHP-4) loading content of CDHP-4@PP-NPs at different time intervals

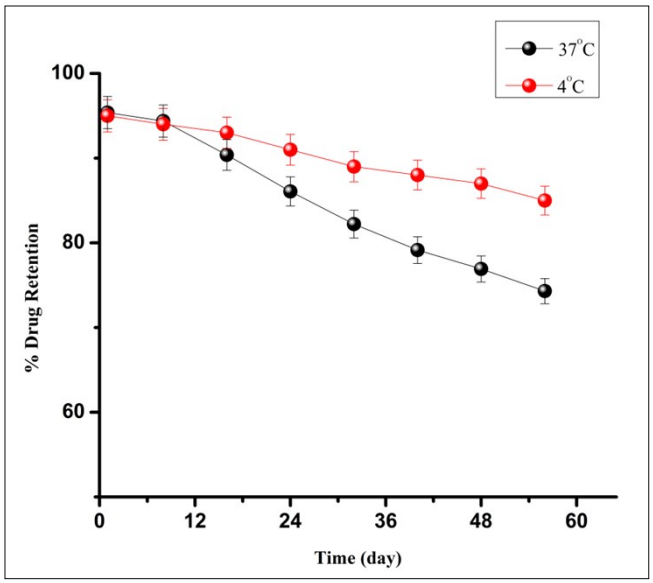

Figure S-30: Percentage drug (CDHP-4) retention of CDHP-4@PP-NPs at different temperature

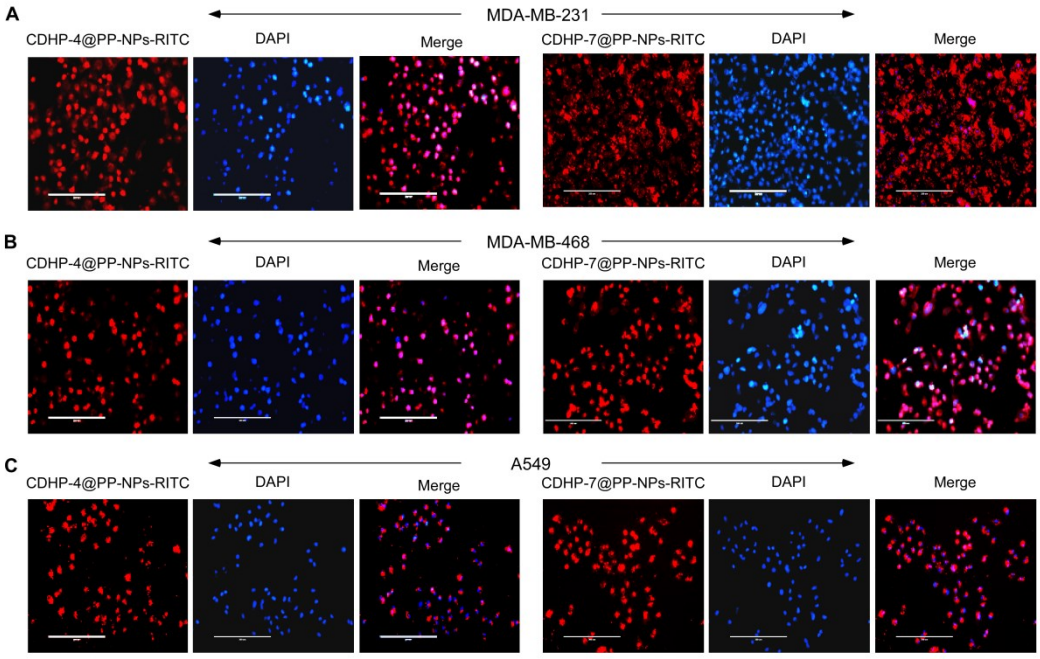

Figure S-31: A comparative study to evaluate the cellular uptake efficiency of RITC- CDHP-4@PP-NPs and RITC-CDHP-4@PP-NPs in different cell lines.

Validation of anti-cancer effect of CDHP-4@PP-NPs in Mouse mammary carcinoma cells (4T1)

Next we investigated the anticancer property of CDHP-4 and CDHP-4@PP-NPs in Mouse mammary carcinoma (4T1) cells. To begin with, MTT assay was performed and assessed the percentage cell death in 4T1 cells. The results obtained indicated that CDHP-4@PP-NPs induced significantly higher cell death by than CDHP-4 alone (Figure S-32A).

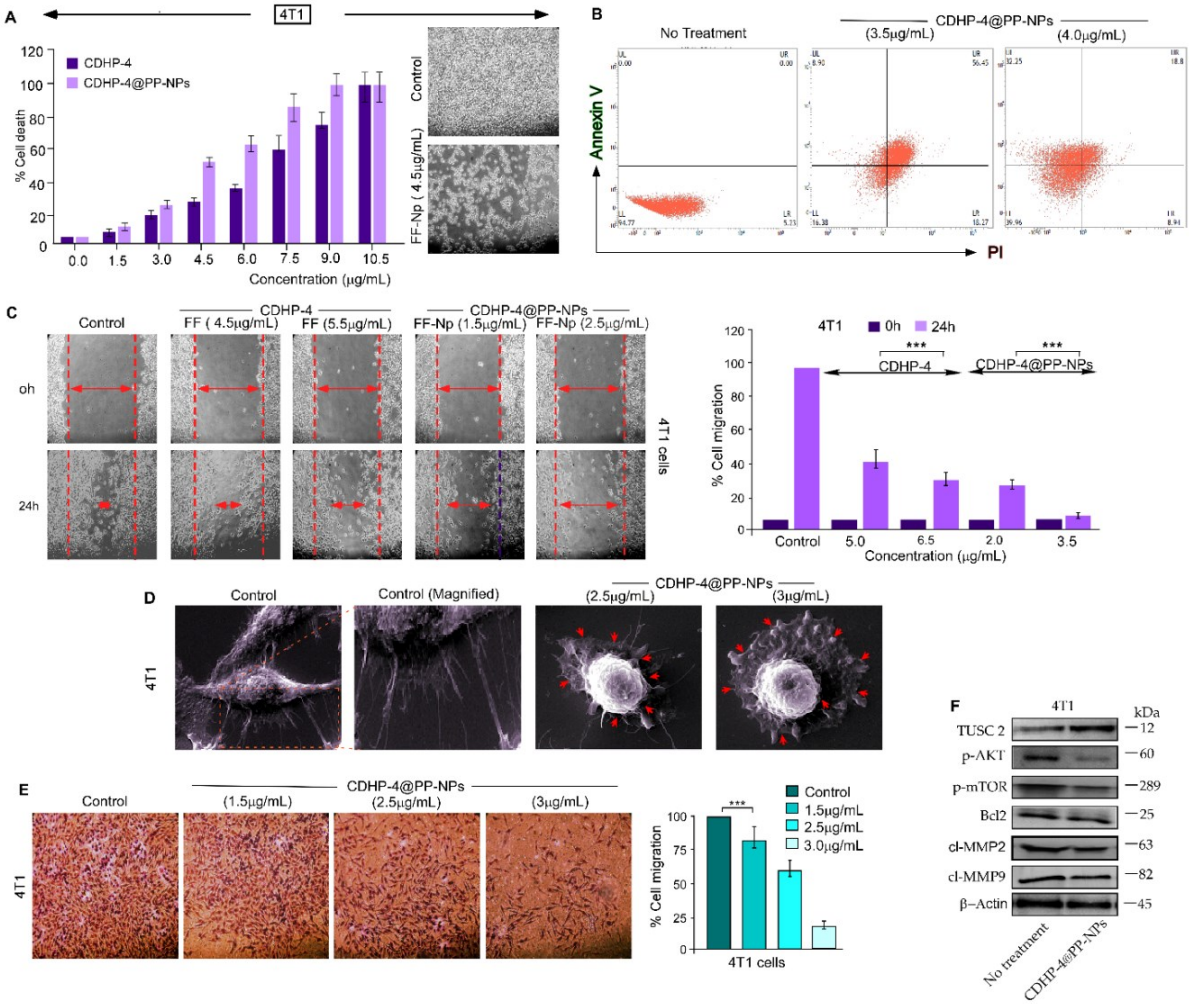

Figure S-32: **CDHP-4@PP-NPs attenuates migration in 4T1 cells in-vitro. (A)** Cell death analysis in variously treated sets of experiment with respect to control in 4T1 cells; (B) The percentage apoptotic cell death in different sets of experiment has been represented by Flow cytometer; (C, left panel) Phase-contrast images of wound healing assay showing pattern of migration in 4T1 cells on treatment with or without CDHP-4@PP-NPs overnight; (C, right

panel) The bar graph, derived from wound healing assay, depicting the corresponding percentage of cell migration of differently treated sets of experiment; (D) The Bio-SEM images of differently treated 4T1 cells evidenced for irregular and distorted cytoskeleton along with membrane blabbing in treated cells as compared to control cells, bearing large number of intact protrusions; (E, left panel) Transwell migration assay evidenced for maximum number of untreated 4T1 cells to be migrated to undersurface unlike the treated cells; (E, right panel) In indifferent set of experiment, the bar graph is synchronously showing the percentage non-migratory cells with respect to untreated cells; (F) Western blot analysis in 4T1 cells treated with CDHP-4@PP-NPs depicting increase in expression of TUSC2 protein and decrease in expression of p-Akt, p-Mtor, Bcl2, cl-MMP2 and cl-MMP9 with respect to untreated set. Values are mean ±SEM of three independent experiments in each case or representative of typical experiment. ***P<0.001.

Next, flowcytometric analysis revealed that CDHP-4@PP-NPs could induce apoptosis in approximately 75% of population in 4T1 cells (Figure S-32B). After then, we examined the anti-migratory effect of CDHP-4@PP-NPs and CDHP-4 in 4T1 cells by Bi-directional wound healing assay. The observation clearly indicated that even though CDHP-4 could impair the migration of 4T1 cells, but CDHP-4@PP-NPs inhibited cell migration significantly at very low concentration. The corresponding percentage cell migration of 4T1 cells under the effect of CDHP-4 and CDHP-4@PP-NPs was represented in Figure S-32C (right panel). The ultrastructure of 4T1 cells treated with CDHP-4@PP-NPs revealed smooth surface with distorted cytoskeleton, membrane blabbing and tiny apoptotic bodies, unlike the control cells bearing large number of intact lamella protrusions (Figure S-32D). Treatment of 4T1 cells for 24 h, allowed very few number of 4T1 cells to have migrated through Trans well inserts to the undersurface, when compared with control cells left untreated (Figure S-32E, left and right panel). Next, we investigated the expression profile of different proteins like TUSC2, p-Akt, p-mTOR, Bcl2, cl-MMP2, and cl-MMP9 in CDHP-4@PP-NPs treated 4T1 cells through western blot analysis (Fig. S-32 F). Interestingly, the observation led us to conclude that CDHP-4@PP-NPs potentially down-regulated p-Akt, p-mTOR, Bcl2, cl-MMP2, and cl-MMP9 and up-regulated TUSC2 in 4T1 cells.

Table S-2: m-RNA Primers sequence

Primer Name	Primer Sequence
Human GAPDH Forward	5′- CTT TGG TAT CGT GGA AGG ACT C - 3′
Human GAPDH Reverse	5′- GTA GAG GCA GGG ATG ATG TTC - 3′
Chick GAPDH Forward	5′ - GAG GAA AGG TCG CCT GGT GGA TCG - 3′
Chick GAPDH Reverse	5′ - GGT GAG GAC AAG CAG TGA GGA ACG - 3′
Human Alu Forward	5′ - ACG CCT GTA ATC CCA GCA CTT - 3′
Human Alu Reverse	5′ - TCG CCC AGG CTG GAG TGC A - 3′

Table S-3: mi-RNA Primers sequence

Primer Name	Primer Sequence
Has-miR -U6 RT	5′ - AAA ATA TGG AAC GCT TCA CGA ATT TG - 3′
Has-miR -U6 Forward	5 -′ CTG GCT TCG GCA GCA CAT A - 3′
Has-miR- U6 Reverse	5′- CAC GAA TT GCG TGT CAT CC -3′
Universal Reverse	5′- CCA GTG CAG GGT CCG AGG TA - 3′
Has-miR -138 Stem	5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTG - 3′
Has-miR -138 Forward	5′-ACACTCCAGCTGGGAGCTGGTGTTG - 3′