Supporting Information

Guanidinium Rich Dendron- Appended Hydnocarpin Executes Superior Anti-Neoplastic Effects Through Caspase Mediated Apoptosis

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1. Synthesis.

1.1 Materials and methods

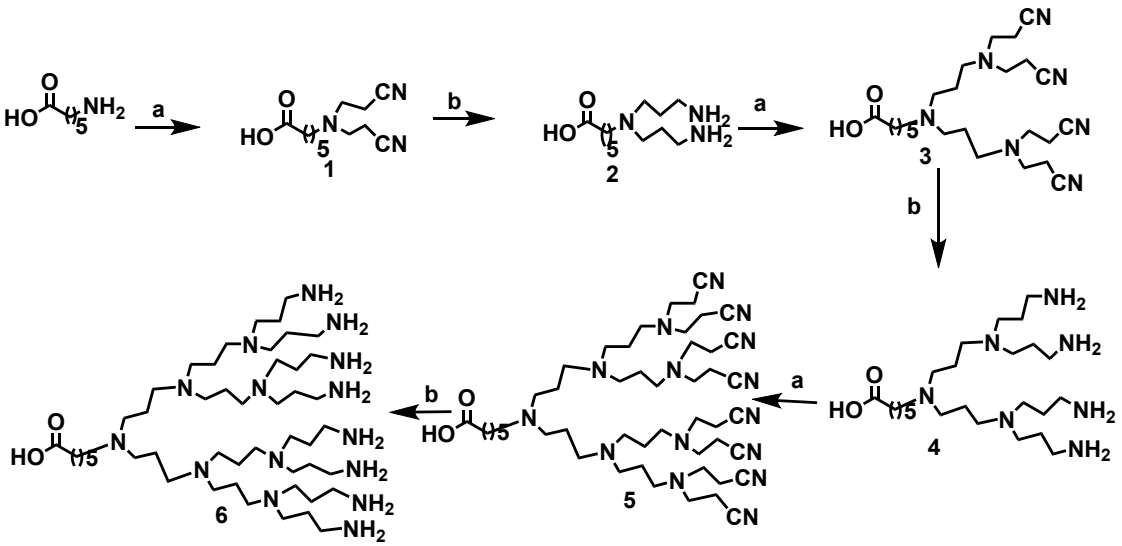
All non-hydrolytic chemical reactions for the preparation of drug carrier constructs, unless otherwise indicated, were carried out in oven-dried glassware under an inert atmosphere of dry argon or nitrogen. All the chemicals and solvents were purchased from Sigma Aldrich, Merk, and Specro chem and were used without further purification. Analytical TLC was performed on a Merck 60 F254 silica gel plate (0.25mm thickness), and visualization was done with UV light (254nm and 365nm), or by spraying with a 5% solution of phosphomolybdic acid or ninhydrine solution followed by charring with a heat gun. Column chromatography was performed on Merck 60 silica gel (60-120 or 100-200 mesh). HPLC was performed by Shimadzu HPLC system (Kyoto, Japan)consisting of SCL-10Avp system controller, two LC-8A solvent delivery units , SPD-M20A UV-vis photo diode array (PDA) detector, equipped with Multi PDA- LC solution software on a 250 mm x 4.6 mm i. d, 5µm, YMC-Pack R&D ODS analytical column (9YMC Co., Ltd. Japan). NMR spectra were recorded on a Bruker AMX 500 (1H-NMR at 500MHz) spectrometers (Falladen, Switzerland). Tetra methyl silane was used as reference for ^{1}H NMR, and the chemical shift were reported in ppm and the coupling constant in Hz. High resolution mass spectra were determined on a HR-EMI analysis of Thermo Scientific Exactive

system (Berman, Germany), and MALDI- TOF mass spectra on a Shimadzu Biotech, AXIMA-CFR PLUS system(Kyoto, Japan). Liophilization was performed with lyophilizer Tenesis Wizard 2 (Germany).

For biological studies 2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid (HEPES) was purchased from Himedia (Mumbai, India). 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), Dulbecco's modified eagle's medium (DMEM), trypsin-EDTA and dimethylsulfoxide (DMSO) for cell culture solution were purchased from Sigma Aldrich. Penicillin-Streptomycin, neutravidin, alexafluor 568-carboxylic acid succinimidyl ester, fetal bovine serum (FBS) and Mito Tracker red CMX Ros were purchased from Invitrogen (Merelbeke, Belgium). Annexin V-FITC apoptosis detection kit was purchased fromBD Pharmingen (#556547, BD Biosciences, San Jose, CA) and TUNEL assay kit from Promega, USA. Caspase assay was performed with Apo Alert™ Caspase Profiling kit (Clontech, CA, USA). Bisbenzimide H 33258 (Hoechst) was purchased from Calbio chem (San Diego, CA, USA) and Lyso Tracker Deep Red was purchased from Molecular probes Life technologies

2 Synthesis of Carrier

2.1 Synthesis of G8-OA-PPI[1]



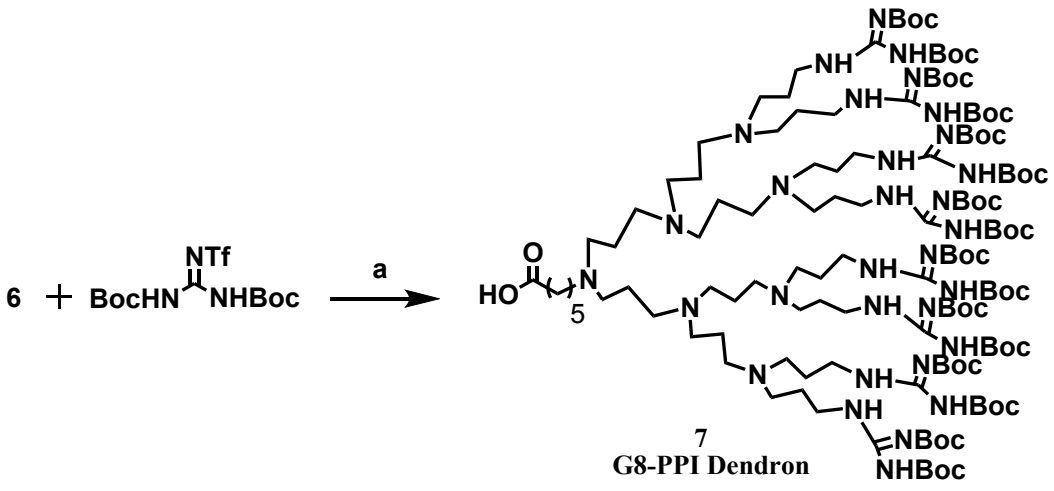
S I Scheme 1 : Synthesis of G8-OA -PPI a) Acrylonitrile, Glc-AcOH, Reflux, 30hrs, b) Raney Ni, EtOH, NaOH, 50Psi, 24Hrs

2.1.1:-Synthesis of compound 1

6-aminohexanoic acid (5g, 0.038mol), glacial acetic acid (43.7mL, 0.764mol) and acrylonitrile (188.58mL, 2.867mol) were added and kept in oil bath (105˚C), refluxed for 30h under N_2 atmosphere, the excess acrylonirile was concentrated under reduced pressure and co-evaporated repeatedly with toluene to remove residual acetic acid. Then the residue was dissolved in ethyl acetate washed several times with water, dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel column chromatography to obtain compound **1** as brownish yellow sticky solid, Yield 84%.

Synthesis of compound 2: To a solution of 1 (9.6g) in anhydrous ethanol (125ml) were added 1N NaOH solution 15ml and approximately 4g Raney-Ni catalyst. The mixture was hydrogenated in a Parr apparatus at 50 psi for 24 h at room temperature. The catalyst was filtered through celite column and eluted with ethanol. After the evaporation of ethanol under rotary evaporator to yield yellow sticky solid with 82 % yield.

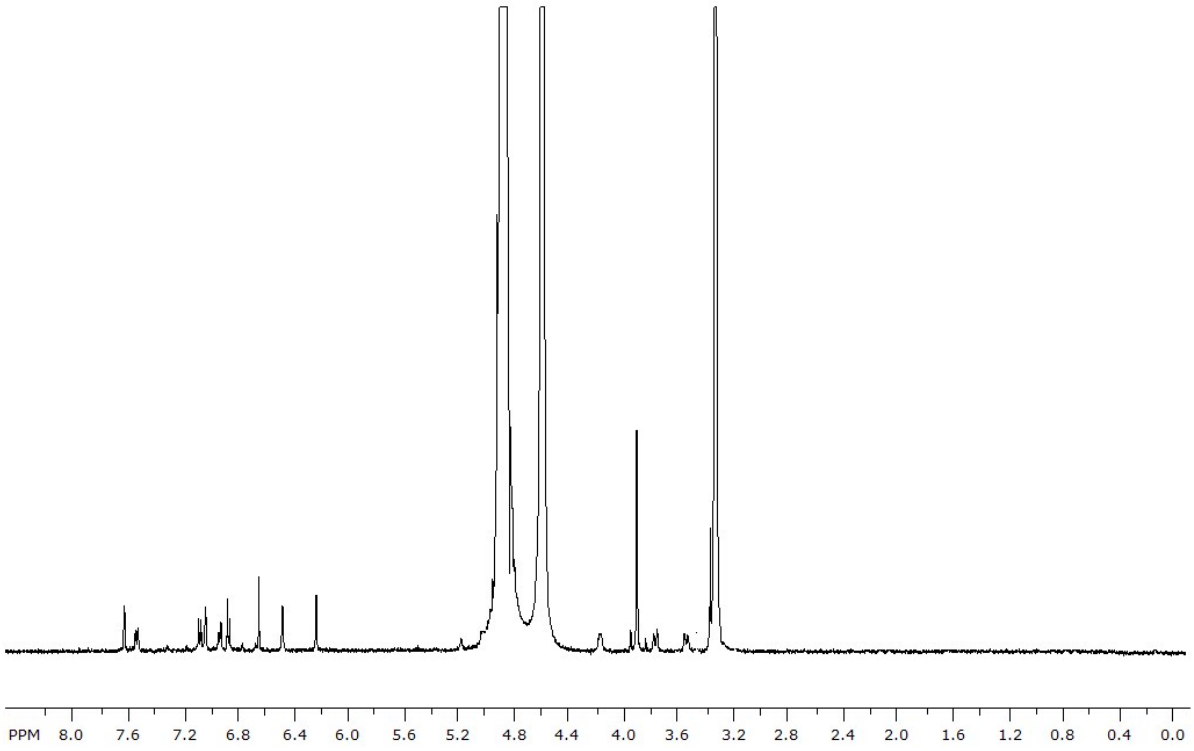
2.2 Synthesis of G8-PPI



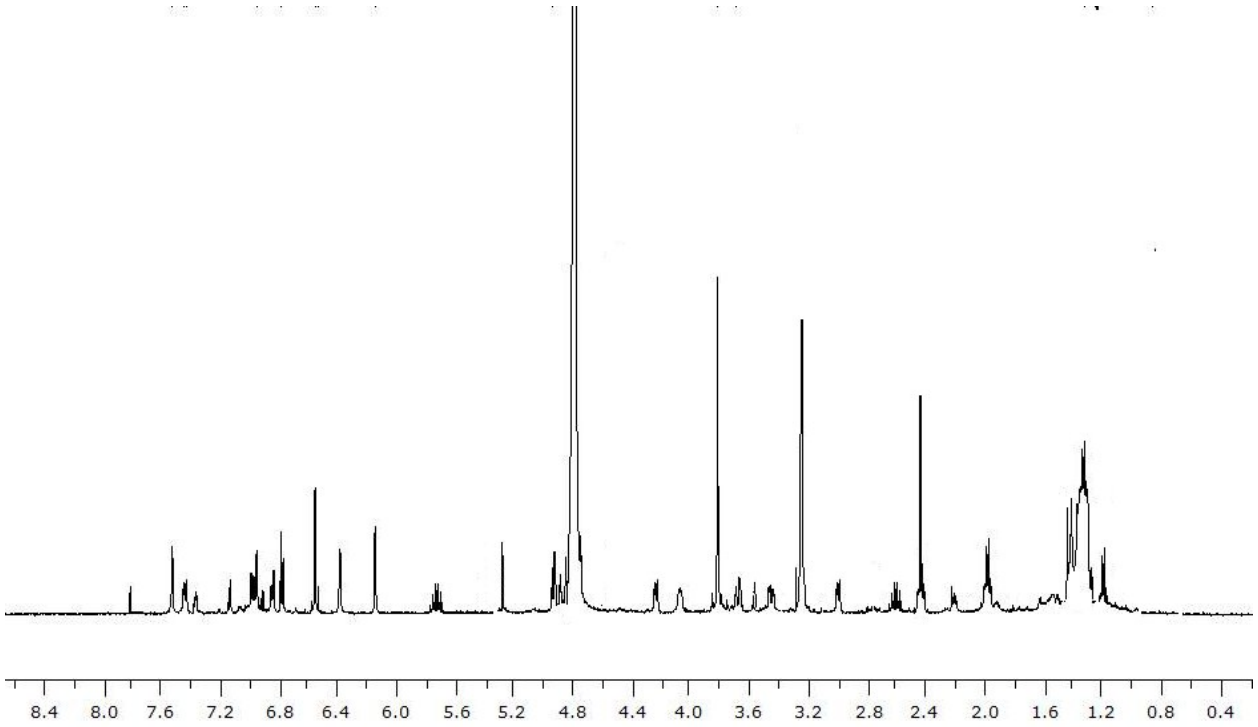
SI Sheme 2: Reagents and conditions: a)N,N'-Di Boc -N"-trifluoro methane sulfonyl Guanidine TEA in Dioxane /water.

Synthesis of compound 7: To a solution of 6 (176 mg, 0.19 $x10^{-3}$ mol) in 10ml of water ,1N HCl solution was added drop wise to a neutral pH .To this solution dioxane were added (40 mL) and then N,N-di-Boc-N-trifluorometanesulfonylguanidine [2](600 mg, 1.5 $x10^{-3}$ mol) and triethylamine (0.13 ml, 0.95 $x10^{-3}$ mol). After stirring for 72 hrs at RT, the reaction mixture was evaporated and residue was diluted with ethyl acetate, water and brine. The organic layer was dried over Na_2SO_4 and concentrated.

3. Spectral Data



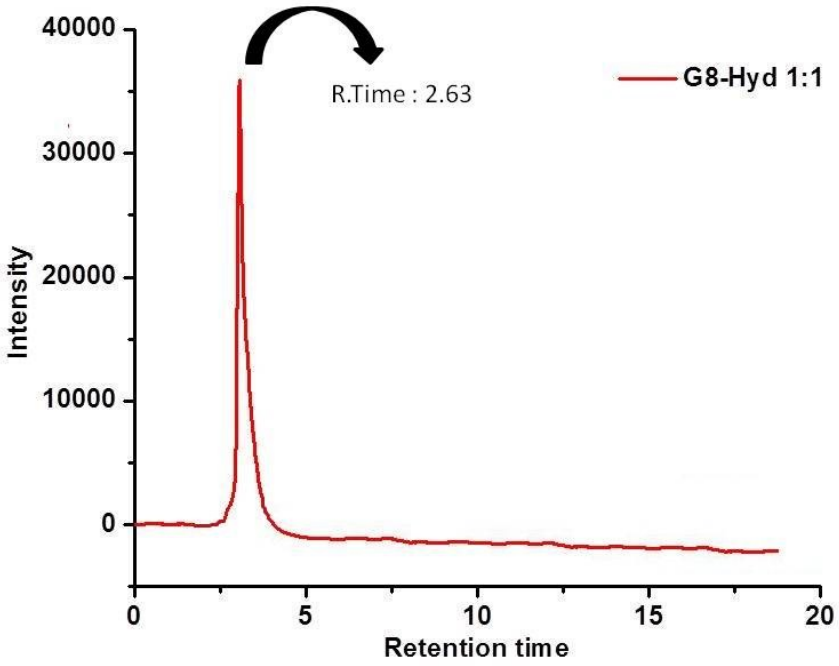
SI Fig1: ^{1}H NMR (500 MHz, CD_3OD) spectrum of HY



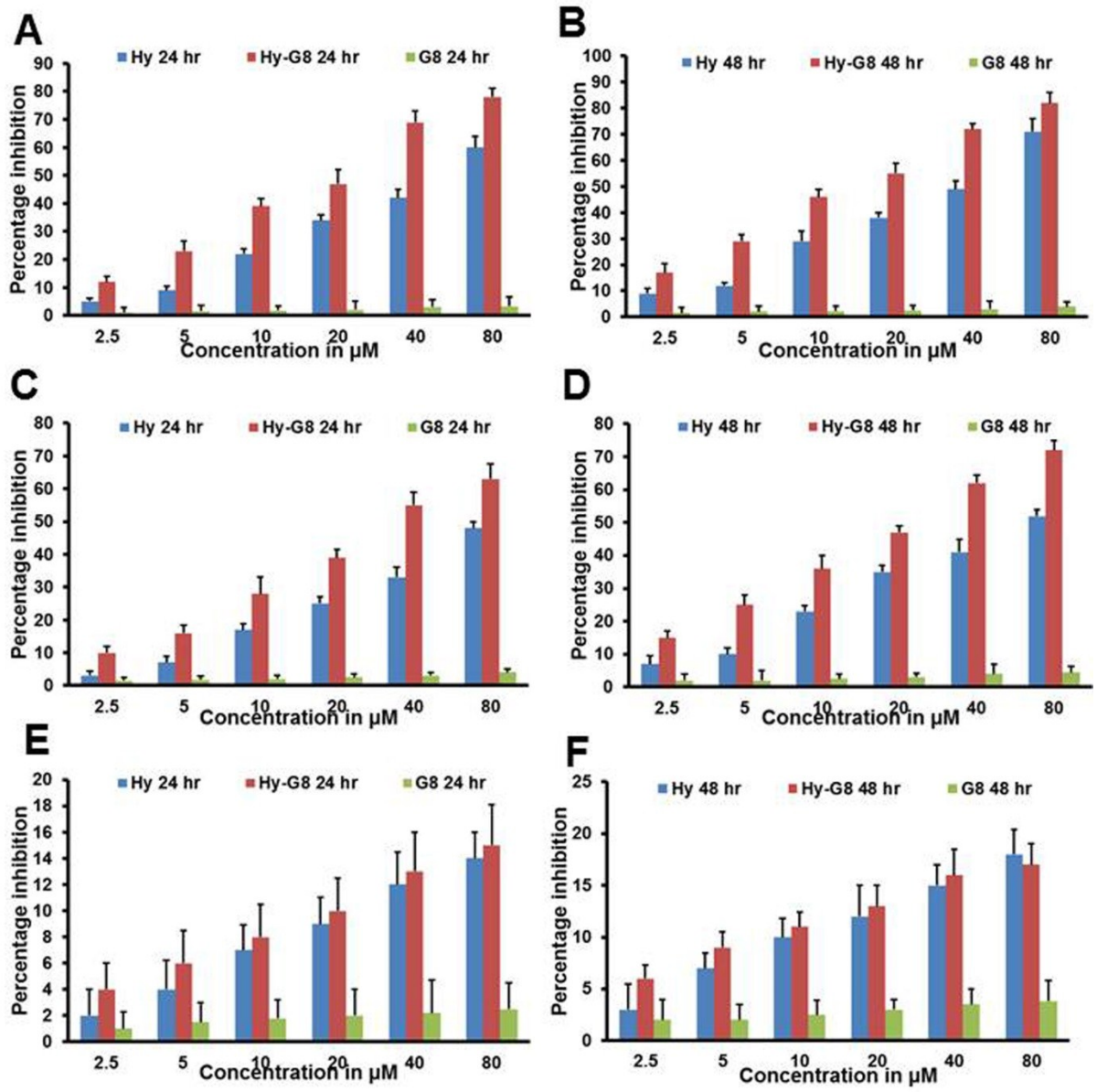
SI Fig2: ^{1}H NMR (500 MHz, CD_3OD) spectrum of HY-G8

3. HPLC purity

The HPLC analysis was conducted using Shimadzu RP- HPLC ODS column with mobile phase consisting of in Methanol (A) and in water (B). The gradient was linearly increased from 0% to 80% B for 35 minutes at the flow rate of 1 mL/min at ambient temperature. UV-VIS detection was monitored simultaneously at 254 nm and 274 nm wavelengths. Compound retention time is found at 2.63 min.



SI Fig3: HPLC profile of HY-G8



SI Fig4*: Assay for cytotoxicity in cancer cells and normal cells treated with Hy, Hy-G8 and G8 after 24 and 48 hrs of administration.* ***(A)*** *A375 cells 24 hr,* ***(B)*** *48 hr ,* ***(C)*** *A549 cells 24 hr,* ***(D)*** *48 hr ,* ***(E)*** *3T3-L1 cells 24 hr and* ***(F)*** *48 hr.*

References

1. J. B. Nair, S. Mohapatra,S.Ghosh and K. K. Maiti, *Chem. Commun.*, 2015, **51**, 2403–2406.

2. K. K. Maiti, W. S. Lee, T. Takeuchi, C. Watkins, M. Fretz, D.-C. Kim, S. Futaki, A. Jones, K.-T. Kim, and S.-K. Chung, *Angew. Chem. Int. Ed. Engl.*, 2007, **46**, 5880–4.