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P-Glycoprotein Targeted Nanoscale Drug Carriers

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RESEARCH ARTICLE

Multi-drug resistance (MDR) is a trend whereby tumor cells exposed to one cytotoxic agent develop cross-resistance to a range of structurally and functionally unrelated compounds. *P*-glycoprotein (*P*-gp) efflux pump is one of the mostly studied drug carrying processes that shuttle the drugs out of tumor cells. Thus, *P*-gp inhibitors have attracted a lot of attention as they can stop cancer drugs from being pumped out of target cells with the consumption of ATP. Using quantitive structure activity relationship (QSAR), we have successfully synthesized a series of novel *P*-gp inhibitors. The obtained dihydropyrroloquinoxalines series were fully characterized and then tested against bacterial and tumor assays with over-expressed *P*-gps. All compounds were bioactive especially *compound* 1*c* that had enhanced antibacterial activity. Furthermore, these compounds were utilized as targeting vectors to direct drug delivery vehicles such as silica nanoparticles (SNPs) to cancerous Hela cells with over expressed *P*-gps. Cell uptake studies showed a successful accumulation of these decorated SNPs in tumor cells compared to undecorated SNPs. The results obtained show that dihydropyrroloquinoxalines constitute a promising drug candidate for targeting cancers with MDR.

Keywords: *P*-Glycoprotein, Inhibitor, SNPs, Drug Delivery, Targeting.

1. INTRODUCTION

Multidrug resistance (MDR) is a trend whereby tumor cells exposed to one cytotoxic agent develop cross-resistance to a range of structurally and functionally unrelated compounds.[1–3] *P*-gp belongs to the ATP-binding cassette (ABC) family of transporters.[4] The expression of *P*-gp is higher in tumors than normal cells, resulting in the potential of resistance to some cytotoxic agents before chemotherapy is initiated.[5]

Inhibiting *P*-gp activity is a way of reversing MDR, and it has been extensively studied for more than two decades.[6] In order to overcome the disadvantages of low binding affinities of *P*-gp, as well as toxicity from high doses, people have developed 3 generations of *P*-gp inhibitors.[7,8] Using QSAR, we have successfully synthesized a series of novel *P*-gp inhibitors. The obtained molecules were fully characterized and subsequently assayed for their ability to target cancer cells that over-expressed *P*-gps.

Silica nanoparticles (SNPs) are attractive in the field of drug delivery and controlled release due to their high surface area, availability, as well as the ease of surface functionalization.[9,10] In the recent decade, polymer coating was used to increase the dispersability of SNPs in water, which also helps to enhance the biocompatibility. Published reports have focused on functionalizing the surface of SNPs with external mechanical interlocked molecules for controlled release.[11–14] Herein, we report the preparation of SNPs decorated with synthesized *P*-gp inhibitors and their potential to be used in*P*-gp targeted drug delivery.

2. EXPERIMENTAL DETAILS

2.1. Synthesis of *P*-Gp Inhibitors

Dichlorophenylenediamine and isatine were dissolved in acetic acid, and refluxed at 120 °C for 1 hour (Fig. 1). After cooling down, acetic acid was evaporated under vacuum. Flash column chromatography was ran to purify the product. The obtained products were fully characterized by ^{1}H NMR, ^{13}C NMR and high resolution mass spectroscopy.

2.2. Preparation of SNPs

SNPs were prepared by the Stöbermethod. Ethanol, water, andammonia solution (25%) was first thenstirred at 40 °C

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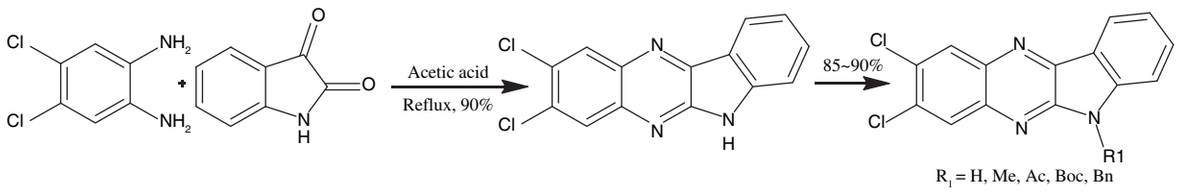

	R_1	Novel	Yield (%)	M.P. (°C)	MS	1H NMR shifts (400 MHz, CDCl3)
1*a*	H	N	92	N/A	288.01	N/A
1*b*	Me	N	88	N/A	302.02	N/A
1*c*	Ac	Y	90	223.5–227.4	330.02	8.45–8.44 (*d*, 1 H, $J = 0.9$ Hz), 8.43 (*s*, 1 H), 8.25 (*s*, 1 H), 7.66–7.62 (*m*, 1 H), 7.40–7.37 (*m*, 2 H), 7.34–7.27 (*m*, 5 H), 5.67 (*s*, 2 H)
1*d*	Bn	Y	85	381.2–384.0	378.06	8.40–8.35 (*m*, 2 H), 8.37 (*s*, 2 H), 7.76–7.71 (*m*, 1 H), 7.54–7.50 (*m*, 1 H), 1.81 (*s*, 9 H)
1*e*	Boc	Y	86	239.5–241.2	388.06	8.79–8.77 (*d*, 1 H, $J = 8.4$ Hz), 8.38–8.36 (*d*, 1 H, $J = 5.1$ Hz), 8.36 (*s*, 1 H), 8.25 (*s*, 1 H), 7.77–7.72 (*m*, 1 H), 7.56–7.52 (*m*, 1 H), 3.20 (*s*, 3 H)

Fig. 1. Synthetic scheme and characterization of the *P*-gp inhibitors.

for 2 hr followed by addition of tetraethoxylsilane (TEOS). The mixture was stirred at 40 °C overnight. After drying, SNPs were dispersed in toluene, heated to 95 °C, with vigorous stirring. And 3-aminopropyltriethoxylsilane was added drop wise into the mixture. The amino-functionalized SNPs (ASNPs) were washed and collected after stirring overnight. The product was characterized by high resolution TEM.

2.3. Functionalization of SNPs

Ullmanncoupling reaction was usedfor the functionalization of silica nanoparticles. ASNPs were first dispersed in dimethyl sulfoxide (DMSO) followed by the addition of functionalized *P*gp inhibitors, *N*,*N*-dimethylethylenediamine, K_2CO_3, and CuI. The mixture was heated at 80 °C for 20 hr. The product was then cooled down to room temperature and washed 5 times with *N*,*N*-dimethylformide (DMF). The particles were dried at 80 °C under vacuum.

2.4. Cellular Test

HeLa cells were chosen as a model cell line for these targeting assays using the inhibitor decorated SNPs. HeLa cells express higher *P*-gps population than normal cells.[15] Confocal microscopic images were taken after incubating HeLa cells for 24 with either the decorated SNPs or ASNP. β-actin (cytoplasmic structures) and DAPI (nuclear stain) were used to stain the cells.

3. RESULTS AND DISCUSSION

A series of *P*gp inhibitors 1*a*–*e* (Fig. 1) were successfully synthesized and characterized by NMR and HRMS. The products were then assayed for anti-bacterial activity. Compound1*c* demonstrated the greatest anti-bacterial potential. One major challenge when performing the anti-tumor assays was that none of these compounds was soluble in 5% DMSO solution. To overcome this obstacle, we decided to prepare the silica nanoparticles (SNPs) and directly functionalize them with these compounds for the cell uptake tests.

The Stöber method was successfully used to prepare ASNPs with a diameter around 120 nm (Fig. 2). Subsequently we used Ullmann coupling to crosslink the inhibitors directly to ASNPs. As illustrated in Figure 3, the inhibitor (1*c*) has an excitation wavelength at around

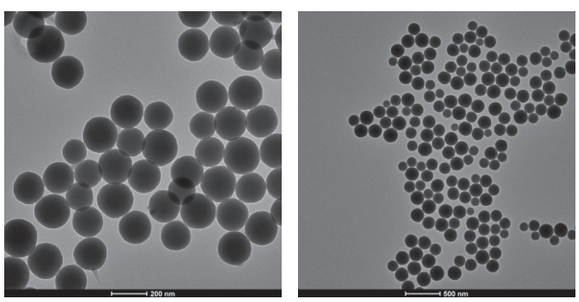

Fig. 2. TEM of the silica nanoparticles synthesized.

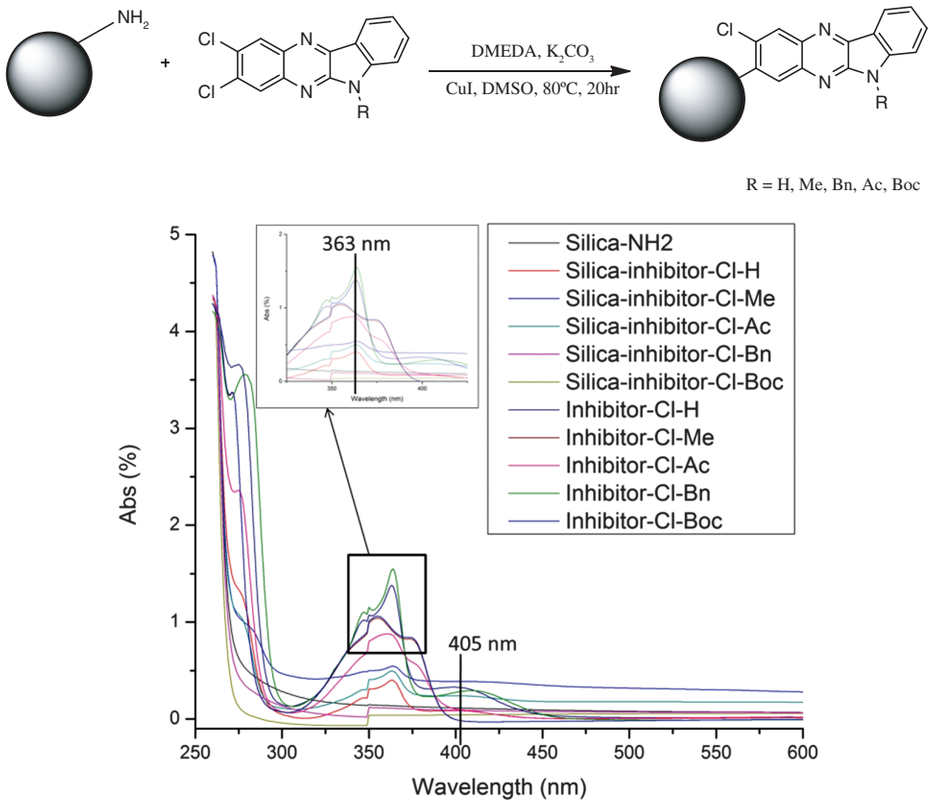

Fig. 3. Ullmann coupling of inhibitors onto the surface of ASNPs (above); UV-vis spectrum of SNPs, *P*-gp inhibitors and SNPs decorated with inhibitors in DMF (below).

363 nm and 400 nm, while for SNPs, the peak lies below 300 nm. When they were re-dispersed in DMF, there was an obvious UV-vis absorption peak at 363 nm, which is also the character peak for the inhibitor, indicating that the coupling reaction was successful. However, for 1*d* (R_1 = Bn) and 1*e* (R_1 = Boc), there was no peak at 363 nm. It is possible that steric hinderance and hydrophobic character of the side groups push the molecules away from the hydrophilic ASNPs and thus dramatically slowing down or even stopping the reaction.

We used the three decorated SNPs (1*a–c*) and ANPS as a control, in cellular assays. Confocal studies using an excitation wavelength of 405 nm was used for the analysis of the SNPs with HeLa cells. It is apparent from Figure 4 thatthe emission of the inhibitor decorated SNPs almost overlapped with the HeLa cell structures outlined with beta-actin and DAPI staining, implying that the *P*-gp-inhibitor decorated SNPs were targeted to the *P*-gp expressing HeLa cells whereas the ASNPs were not.

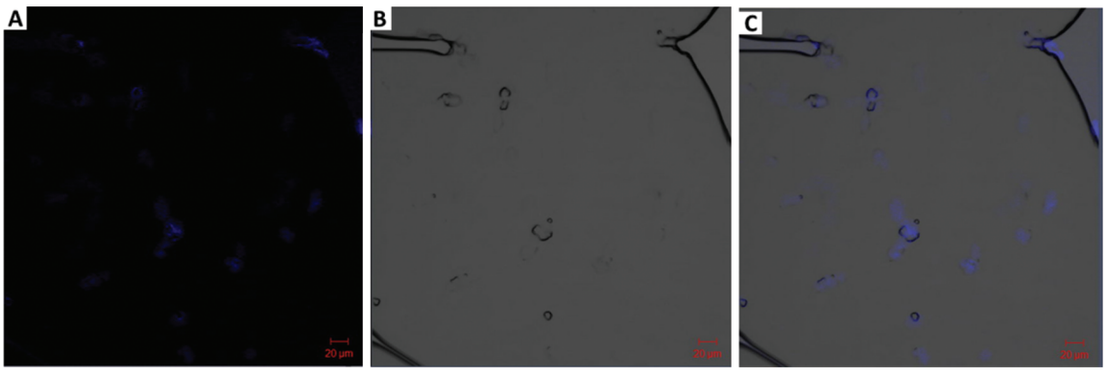

Fig. 4. Confocal microscope image of Dapi stained HeLa cells with decorated SNPs (1*b*) with HeLa: (A) Fluorescence images of the decorated SNPs at 405 nm; (B) Bright field images of HeLa cells; (C) Overlap of A and B.

4. CONCLUSIONS

We have successfully synthesized a series of compounds with a high potential of inhibiting *P*-gp efflux pumps. Ullmann coupling was used to get three of the compounds crosslinked to amino group decorated silica nanoparticles. After performing the *in-vitro* testing with HeLa cell line, we found that the decorated SNPs preferentially accumulated in tumor cells compared to undecorated SNPs that were not taken up by the cells. Thus, targeting *P*-gp can be a new method for improving the operation of drug delivery vehiclesand increasing the retention of drug in diseased cells versus healthy ones.

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