Scale-up of an Antiviral Polyamide Comprising Pyrrole and Imidazole Amino Acids

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Introduction

Cervical cancer is caused by the human papillomavirus (HPV) [1,2]. The existence of traditional “druggable” targets for HPV is minimal at best because a very small number of proteins is encoded by the small HPV genome (see below). Our nontraditional approach to HPV16 antivirals is to target viral DNA sequences, including those at or near the viral origin of replication (ori), using DNA-binding drug candidates. The compounds we used are polyamides (PAs) derived from N-methylpyrrole (Py) and N-methylimidazole (Im); they are selective DNA binding agents that can physically block access to target DNA sequences by proteins or alter the DNA local structure to prevent protein binding. Previous studies from other labs [3] have shown that PAs cause physical changes in DNA structure, widening the minor groove, narrowing of the major groove, raising the Tm, etc.

HPV16 and 18 are the most common cancer-causing strains [2]. Viral proteins E1 and E2 bind to HPV DNA, at the ori and recruit human DNA polymerase; they control viral replication and some viral gene expression. We proposed that HPV16 replication could be stopped by designing DNA-binding polyamide molecules to bind viral DNA sites with possible targets, including the E1 and E2 binding sites and other regions of the HPV16 genome. The polyamides we chose to work with have a hairpin structure [2,4]. Armed with the HPV16 sequence and the PA binding rules [3], polyamides can be synthesized [5,6] to selectively bind to specific regions of the HPV16 genome, which is a circular dsDNA molecule.

By testing in human keratinocyte monolayers, we discovered significant antiviral activity for certain polyamides; e.g. NV-HPV16-1 has an apparent IC50 of 0.10 ± 0.02 µM in human cell culture. The sequence of NV-HPV16-1 is Im-Py-Py-β-Py-Py-γ-Py-Py-β-Py-Py-Py-β-Ta in which β, γ and Ta refer to β-alanine, γ-aminobutyric acid and N-N-((dimethylamio)propyl)amine respectively. To further advance candidate NV-HPV16-1, we needed to scale-up the synthesis using a solid-phase method.

Results and Discussion

NV-HPV16-1 was successfully synthesized by the solid-phase method with 10g Boc-β-alanine PAM resin. The reactions were monitored by LC/MS. (Figure 1A and B). Figure 1A shows two product peaks, which is evidence of aggregation under HPLC conditions, even at 40°C: the peaks at 1.6 and 4.4 min both show desired compound by ESI+ ms. The isolated yield of the tris(TFA) salt following synthesis (16 cycles) and HPLC was 17%. Although standard peptide methodology is applicable to the general synthetic process, the nucleophilicity of the aromatic amines of the pyrrole and imidazole-based building blocks is poor. Prolonged coupling time and/or double coupling are often necessary when the synthesis is near completion. Fmoc reagents became unstable during long reactions in the presence of DIEA, making tBoc methods preferred in this case (Boc is also preferred for atom economy).

Conclusions

The target antiviral polyamide has been synthesized by manual solid-phase methods on 10 g of Boc-β-alanine PAM resin with a 17% isolated yield.

Statement: Drs. Fisher and Bashkin are co-founders and owners of NanoVir, LLC.
Fig. 1. A. RP HPLC, HPLC/MS of crude NV-HPV16-1. B. ESI mass spec of NV-HPV16-1 showing [M+H]+ and [M+2H]2+ at m/z = 1894.5 and 948.

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References

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