Solid phase multicomponent synthesis of 3-substituted isoindolinones generates new cell penetrating probes as drug carriers

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Abstract

A modular solid phase multicomponent reaction for the synthesis of 3-substituted isoindolinone derivatives has been carried out. A mixture of a chiral β-keto lactam, an aldehyde, an isocyanide and a dienophile react to produce chiral 3-substituted isoindolinones in one pot. Modularity has been accomplished by using solid supported aldehydes and dienophiles. Optimization was achieved by using microwave as the source of energy. The reaction was also performed on a biologically relevant pro-apoptotic peptide $_D(KLAKLAK)_2$ on solid phase. The molecules show significant fluorescence with large Stokes shifts and fast cell penetration. The chimeric peptides can be tracked under microscope proving the potential of the probes as cell sensors. Additionally, they are efficiently internalized as compared to non-labeled peptide, with a concomitant induction of apoptosis proving their potential as drug carriers.

Synthetic Methods and Results

Multicomponent reactions (MCR's) are valuable tools for the facile one-pot synthesis of complex molecules. We have recently developed a new MCR4 useful for generating chiral analogs of 3-substituted isoindolinones, starting from tetramic acids as chiral precursors. Our strategy included the reaction in solution of substituted chiral tetramic acids which, together with an aldehyde, an isocyanide, a dienophile and a Lewis acid, produce 3-substituted isoindolinones in one pot. Briefly, the high acidity of the tetramic acid methylene **a**, reacts with an aldehyde **b** in the presence of $Ti(O\text{-}iPr)_4$, producing acondensation intermediate **c.** The latter reacts immediately with the isocyanide **d** to produce a fused bicycle [5+5] furan **e** that we have shown to be too reactive to be isolated. However, in the presence of a dieneophilef, furan **e** reacts to generate the Diels Alder intermediate **g**, which undergoes oxygen elimination and aromatization generating the 3-substituted isoindolinones **h** (Fig.1).

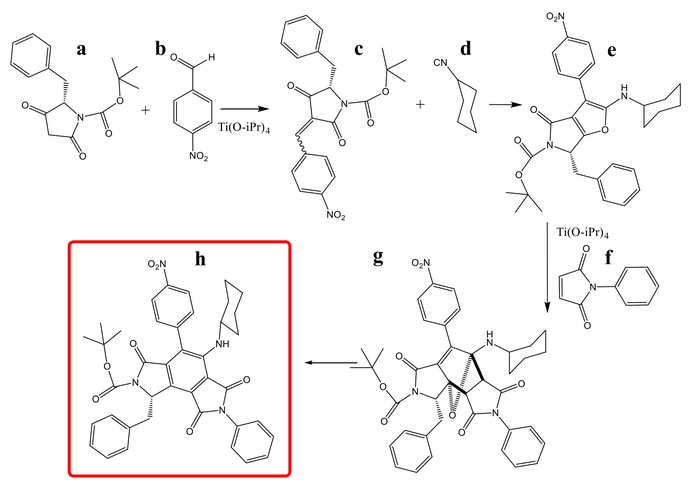

Figure 1: *MCR4 for generation of chiral 3-substituted isoindolinones*

Two anchoring strategies have been studied. 1- a bifunctional carboxylic acid-aldehyde (Fig. 2, method A) or, 2- a bifunctional carboxylic acid-dienophile (Fig. 2, method B) is anchored through the carboxylic acid to a Rink amide resin by acylation of a free amino group.

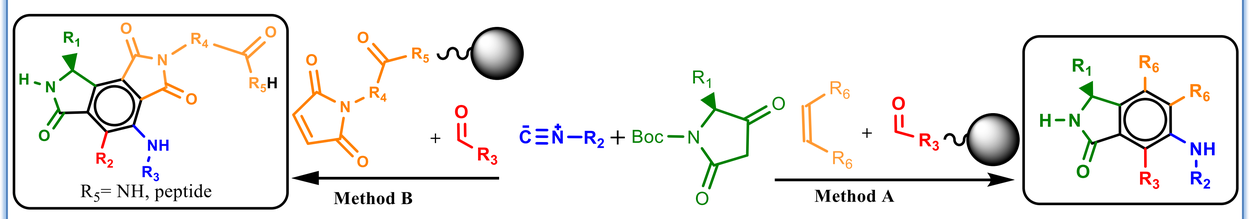

Figure 2: *Ugi like SPOS-MCR4 strategy*

Two series of materials were synthesized according to methods A and B. The usefulness of method B was confirmed by the synthesis of two molecules on a preinstalled peptide on the solid support (Fig.3).

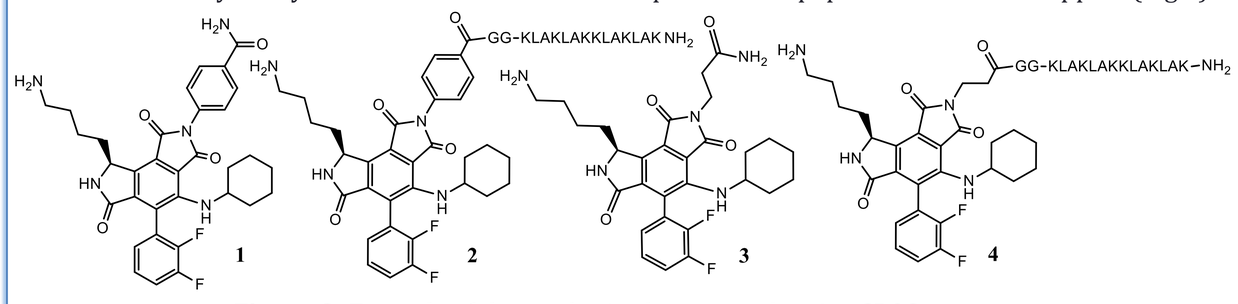

Figure 3: *Example of the synthesized compounds using SPOS strategy*

Results

Extending our new MCR4 reaction to SPOS represents the first example of the synthesis of 3-alkyl-isoindolinones with stereogenic control. Moreover, we demonstrate that the SPOS can be directly performed on a preinstalled peptide. The large excess of the components in the solutions together with the optimal selection of the component bound to the solid phase - allows obtaining highly pure products in excellent yields.

In addition, all the compounds display good fluorescence with excitation at 447nm and emission around 542nm, with excellent Stokes shifts of about 100 nm.

Biological Studies

Compounds were tested for XTT viability assays. In addition, real time fluorescent confocal microscopy studies and complementary flow cytometry (FACS) experiments where performed for better evaluation of the fast cell penetration ability of the compounds.

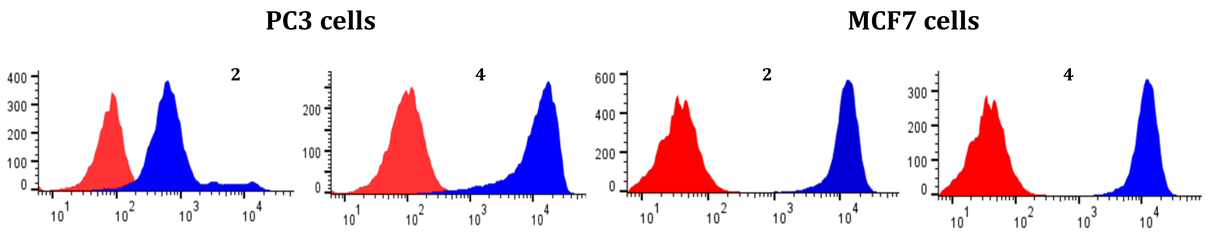

Figure 4: *Flow-cytometry analysis of PC-3 and MCF-7 cells treated with 2 and 4 at 20 mM immediately after addition of compounds. Red plots: control. Blue plots: treated cells. (ex= 405nm, em= 525 nm)*

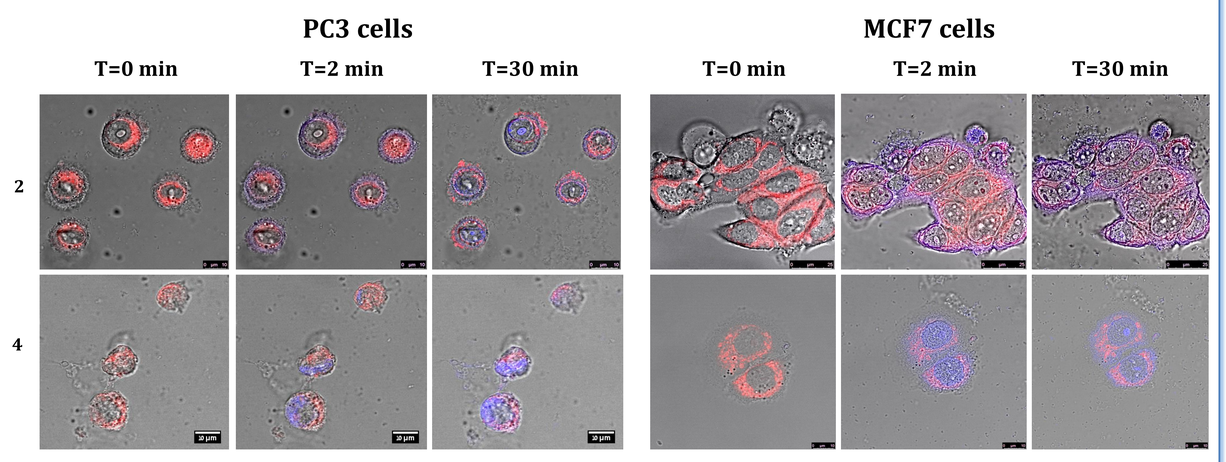

Figure 5: *Cell penetration of compounds [5μM] into MCF-7 and PC-3 cells by confocal microscopy. Overlapped channels: Ex = 405nm, Em= 542nm (2 and 4), Ex = 552nm, Em= 600nm (MitoTracker).*

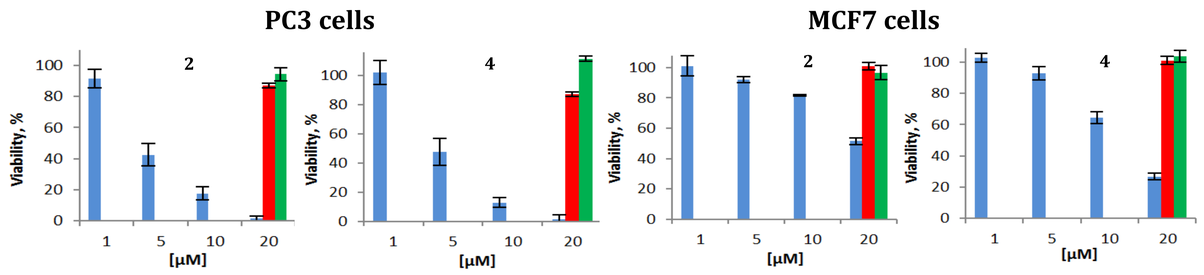

Figure 6: *XTT viability assay of 2 and 4(blue). Controls: Ac-$_D(KLAKLAK)_2$ (red), fluorescent probe 1 and 3 (green)*

Results

Compounds 2 and 4 were submitted to flow cytometry and microscopy analyses. Both compounds readily penetrate into the tested cells as demonstrated by flow cytometry (Fig. 4) and could be tracked under confocal microscopy (Fig. 5) proving that both probes can transport the peptides into the cells.

In addition, in order to assess a biological activity of the carried peptide, 2 and 4 were tested for cell toxicity using XTT viability assays and compared to relevant controls (Fig. 6). It was shown that compound 2 displays IC_{50} of 5μM in PC-3 cells and 20 μM in MCF-7 cells and compound 4 displays IC_{50} of 5μM in PC3 cells and 15 μM in MCF-7 cells, while none of the isolated elements $_D$(Ac-KLAKLAK)$_2$ or compounds 1 and 3 displayed any toxic activity at these concentrations.

Overall, the synthesis of compounds 1 and 3 directly on a biologically relevant peptide proved to be efficient for inducing cell penetration of the peptide followed by a concomitant intracellular activity such as apoptosis mediated by the cargo peptide and allowed tracking the hybrids under the microscope.

Conclusion

We have developed a solid phase methodology for the synthesis of complex 3-substituted isoindolinones in good yields by a multicomponent reaction mediated by microwave energy.

The solid supported component can be an aldehyde or a dienophile. Both strategies lead to the desired products in good yields. The products display significant fluorescence with excellent Stokes shifts.

We have demonstrated a fast cell penetration of some of the non-toxic compounds using microscopy and flow cytometry which make the molecules attractive as molecular probes for cell sensing and as carriers for cell penetration applications. As proof of concept, we have synthesized two probes directly on the pro-apoptotic peptide $_D(KLAKLAK)_2$ and demonstrated the ability of the probes not only to facilitate penetration into the cells, but also allowed tracking the material and induced a concomitant biological activity exerted by the transported peptide.

Acknowledgments

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References

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