Organometallic cobalamin anticancer derivatives for targeted prodrug delivery via transcobalamin-mediated uptake

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Cobalamin (Cbl, vitamin B12) is a water-soluble vitamin of primary importance to the metabolism of every cell in the human body. Once ingested, it is actively carried by proteins and ultimately internalized into the cells by a receptor-mediated endocytosis. Confined inside the cell, the cobalt center of Cbl undergoes a series of enzymatic reductions that triggers therelease of the β-axial ligand. Over the past few years, this feature has been explored in order to use vitamin B12 against cancer cells in the manner of a Trojan horse. Indeed, the ability of the cyanide ligand in CNCbl to bridge metal centers has been successfully used to attach metal complexes[1]. Based on recent advances in organometallic chemistry applied to Cbl[2,3], a new structural design was developed[4] and resumed herein. This approach offers the advantage of: 1. a better comparison between the cytotoxity of the complexes before their attachment to Cbl and after their release inside the cells; 2. a broader range of imaginable motifs; 3. an increased water solubility of the complexes.

Thus far, a series of fours vitamin derivatives of Pt (B12-1), Ru (B12-2 and B12-3) and Re (B12-4) were prepared and characterized (see Image). As a common structure, the attached complexes exhibit a bipyridine modified at the para position with a simple alkyne. The latter serves as a point of attachment between the cobalt of Cbl and the anticancer metal complexes. Chemical reduction using either cobaltocene or Zinc showed that the four complexes are released entirely from Cbl.

In term of serum stability, both ruthenium derivatives showed no evidence of human serum albumin binding after 24h. On the other hand, the free fraction of the Pt derivative was measured at 61% and 40% for the Re in the same period. B12-4 andB12-3 showed comparable/lower cytotoxicity to that of cisplatin, while B12-1 was less effective and B12-2 essentially non-cytotoxic. To measure the affinity of the derivatives with Cbl carrier proteins (TCII and IF), a fluorescent B12 (B12-CBC) [5] was prepared in order to perform competitive displacement assays which indicated that our derivatives are recognized by these transport proteins. Furthermore, fluorescence imaging show that the compound were internalized inside the cells and that they followed the natural cobalamin uptake.

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