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Design, synthesis and biological evaluation of Pontin ATPase inhibitors through a molecular docking approach

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ABSTRACT

A virtual screening strategy, through molecular docking, for the elaboration of an electronic library of Pontin inhibitors has resulted in the identification of two original scaffolds. The chemical synthesis of four candidates allowed extensive biological evaluations for their anticancer activity. Two compounds displayed an effect on Pontin ATPase activity, and one of them also exhibited a noticeable effect on cell growth. Further biological studies revealed that the most active compound induced apoptotic cell death together with necrosis, this latter effect being likely related to the cellular balance of ATP regulation.

Pontin and Reptin are ATPases that belong to the AAA+ (ATPases associated with diverse cellular activities) family.[1] As such, these proteins are implicated in multiple and very different cellular functions,[2] as reflected by the variety of names they are called, such as RuvBL1 (RuvB-like),[3] Rvb1,[4] TAP54a (TIP60 associated protein)[5] or TIP49 (TATA-box binding protein)[6–8] for Pontin, and RuvBL2, Rvb2, TAP54b or TIP48[9] for Reptin.[10] They contain characteristic Walker A and B domains, essential respectively for the binding and the hydrolysis of ATP and structural studies have highlighted their ability to form ring-shaped oligomeric complexes.[1,11,12] The crystallographic structure of Pontin alone was resolved as an hexamer in 2006,[13] and in 2011, a dodecameric assembly composed of two heterohexamers of alternating units of Pontin and Reptin has been published by the same team.[14] The structures display bound ADP for Pontin alone, or a mixture of bound ATP and ADP for the dodecamer of Pontin and Reptin. A similar heterododecameric structure has been reported by another group using cryo-electron microscopy.[15] The ATP/ADP binding site, which is located at the interface of two subunits, seems to involve residues from both chains.[13,14]

Recently, several reports have hinted at a link between Pontin and Reptin and various types of cancer.[16] We notably found that both proteins were overexpressed in human hepatocellular carcinoma where they are required for cell viability and proliferation.[17,18] They also interact with oncogenic transcription factors such as beta-catenin or c-Myc.[19,9] Finally, they are also required for the assembly and function of telomerase[20] and for the stability and function of mTOR.[21,22] As suggested by the use of mutants devoid of ATPase activity, most functions of these proteins require an ATPase activity, including those implied in cancer,[9,20–25] indicating that the inhibition of the ATPase activity of Pontin and/or Reptin could be of special interest for cancer therapy. In a previous Letter,[26] we have disclosed the discovery of the first four small molecules able to inhibit the ATPase activity of Pontin. This was achieved through the combination of molecular docking of compounds from commercially available libraries into the ATP binding site and in vitro ATPase assays. Further testing showed that one of these four molecules was competitive with ATP. Encouraged by these promising results we decided to look for Pontin inhibitors among original molecules from our laboratories. At this time, 4-hydroxy-2-pyridone and 4-hydroxy-2-quinolone moieties were of special interest to us because of their apparent similitude with quinones and napthoquinones. Starting from these scaffolds, simple chemical transformations were envisioned to introduce chemical diversity in an easy manner and prepare a set of innovative

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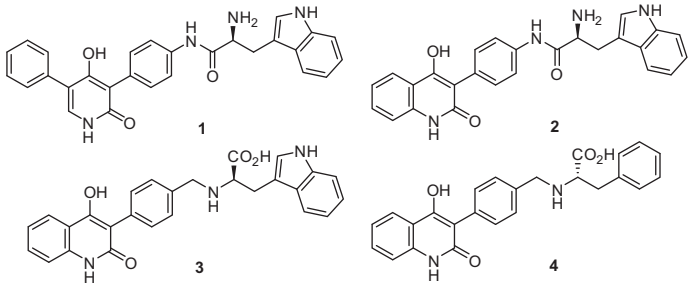
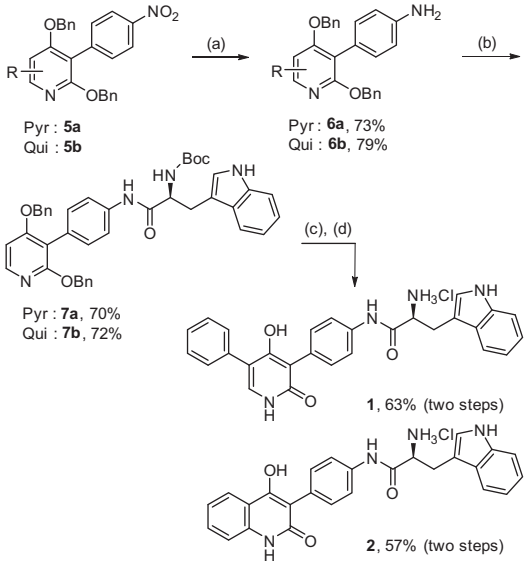
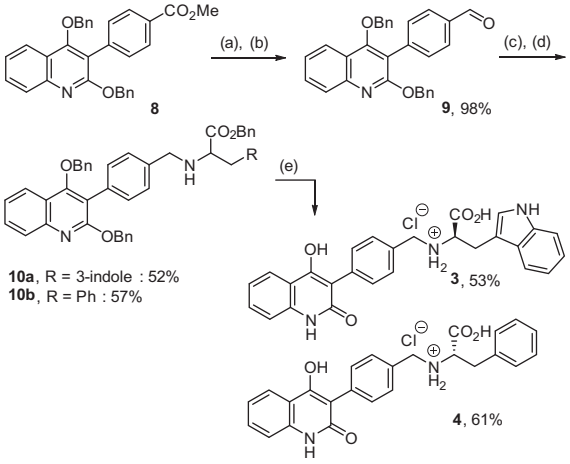


Figure 1. Selected structures from in silico studies to be prepared by chemical synthesis.



Scheme 1. Preparation of compounds **1**–**2**. Reagents and conditions: (a) Fe, acetic acid, reflux, 0.5 h; (b) Boc-(L)-Trp-OH, EDC, HOBt, di-isopropyl ethyl amine, DMF, 25 °C, 12 h; (c) TFA, CH_2Cl_2, 0–25 °C, 0.5 h; (d) H_2, Pd/C, MeOH, 25 °C, 12 h, then HCl.



Scheme 2. Preparation of compounds **3**–**4**. Reagents and conditions: (a) $LiAlH_4$, THF, 0–25 °C, 0.5 h; (b) stabilized 2-iodoxy benzoic acid, THF, 60 °C, 0.5 h; (c) L-phenylalanine benzyl ester or D-tryptophan benzyl ester, MS 4 Å, CH_2Cl_2, MeOH, 25 °C, 2 h; (d) $NaBH_4$, −5 °C, 3 h; (e) H_2, Pd/C, MeOH, 25 °C, 12 h, then HCl.

small compounds. Parallel to the organic chemistry effort, an in-house virtual chemical database based on the chosen scaffolds was simultaneously prepared, then extended along with the syntheses progress. This database contains all the compounds believed to be accessible through synthetic chemistry.

The already published[26] procedure was repeated in this study. Briefly, the crystal structure of the hexamer of Pontin alone with bound ADP was obtained from the Protein Data Bank, code 2C9O.[13] Two adjacent subunits, forming a complete ATP/ADP binding site, were extracted from the ring-shaped hexamer. The resulting homodimer was used as the receptor for docking. It was formed of one subunit as the main docking area, while the second sub-unit was positioned on top of the cavity, as if to forbid access to the exterior, as observed in the published crystal structure. Two missing loops in the crystal structure (residues 142–155 and 248–276) were not completed since they were far enough from the nucleotide binding site and did not impact the docking results. Water molecules were removed from the receptor, then missing hydrogen atoms were added while applying Charmm Forcefield in Discovery Studio 3.1 (Accelrys). The structure with bound ADP was minimized while the backbone was fixed, using a Steepest Descent algorithm (2000 step, gradient 0.01).

The virtual chemical database was prepared with Discovery Studio 3.1. Two original moieties of special interest within our group were chosen: pyridone[27] and quinolone.[28] Several modifications and implementations were proposed and introduced on the original scaffolds. The changes were oriented either by the experience of the organic chemists in the synthesis of this family of compounds, or by the potential activity increase brought by some functional groups, as suggested by previous virtual screening. If the envisioned chemical modifications were not judged feasible, they were not incorporated. The final database contained more than 800 original molecules designed for their easy access. All docking calculations were conducted using Autodock Vina 1.0.2.[29] The docking grid was a box with the following dimensions: $20 \times 22 \times 20$ Å, in order to incorporate all residues of the cavity with a margin of 3 Å in all directions. As already stated, 74 amino acids from the first sub-unit, plus 6 from the second sub-unit were included, at least partially, in the docking box. The whole receptor was kept rigid during the docking, while all the ligands were fully flexible. The poses obtained were rescored with DrugScore[30,31] and XScore[32] then consensus scoring determined the most probable ligands as already published.[26] A short list of 15 compounds was proposed to the chemists and 4 were immediately chosen to be prepared, because of their synthetic accessibility.

Molecular docking of molecules from the virtual database led us to the selection of several compounds, among which molecules **1**–**4**, depicted in Figure 1, were the most easily accessible for the campaign of chemical synthesis.

We recently described the preparation of 3-aryl-2,4-oxypyridines and 3-aryl-2,4-oxyquinolines through a practical Pd/C-catalyzed Suzuki–Miyaura reaction.[33] Thereby, with the aid of this efficient methodology, we prepared the 4-hydroxy-2-pyridone (Pyr) and 4-hydroxy-2-quinolone (Qui) cores of our targets **1**–**4**.