

Identification and validation of novel molecules obtained by integrated computational and experimental approaches for the read-through of PTCs in CF cells

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Background. Cystic Fibrosis patients with nonsense-mutation in the CFTR gene generally make virtually no CFTR protein and thus often have a more severe form of CF. Recently, Ataluren (formerly PTC124) was suggested to induce the read-through of premature termination codons (PTCs) mainly the UGA codon. However, despite promising results there is not a general consensus of Ataluren efficacy and mechanism of action.

Hypothesis and objectives. The design of new small molecules (PTC124 related) together with the understanding of their mechanism of action could lead to new pharmacologic approaches for the cure of CF caused by nonsense mutations in the CFTR gene. This project was aimed to evaluate the activity of some PTC124 analogues identified by a virtual screening, by different orthogonal assays with vectors containing PTCs in reporter genes and in CF bronchial epithelial cells.

Methods. Design and synthesis of the new PTC's read-through promoters was based on a virtual screening approach (1) and starting from the results obtained in our precedent study (2). We used the FLuc assay, the green fluorescent protein assay (GFP) and the IB3 cell line to test the new identified products (3-4). In order to understand their mechanism of action computational studies were done to model the interaction between the bioactive synthesized compounds and the cellular target.

Results. We synthesized 18 analogues of Ataluren (PTC124) and tested them in three different biological models. Ten of these new compounds showed high read-through capacity (unpublished results).

Spin off for research & clinical purposes. Identification of molecules displaying readthrough activity higher than Ataluren

(PTC124). Understanding the mechanism of action of readthrough promoting molecules.

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