Inserm U761

Conception of protein-protein interactions inhibitors : screening and libraries synthesis

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INTRODUCTION

Targeting the interfaces between proteins has a huge therapeutic potential, but discovering small-molecule drugs that disrupt protein–protein interactions is an enormous challenge. Several recent success stories, however, indicate that protein–protein interfaces might be more tractable than has been thought. These studies discovered small molecules that bind with drug-like potencies to 'hotspots' on the contact surfaces involved in protein–protein interactions. Some of these small molecules are now making their way through clinical trials, so this highhanging fruit might not be far out of reach.[1]

p53 / HDM2

The p53 tumor suppressor protein plays a central role in the coordination of the cellular response to stress through the initiation of growth arrest and/or the induction of apoptosis. (**Figure 1**) There are also instances of human cancers in which wild type p53 is present, but is inactivated through alternate means such as overexpression or amplification of hdm2. The hdm2 oncogene product (HDM2) suppresses the transcriptional activity of p53.(**Figure 2**)
The disruption of the protein–protein interaction between p53 and HDM2 is an attractive approach for cancer therapy, because it offers the possibility to up-regulate the p53 response. Within the last few years there have been several reports of small molecule HDM2 antagonists and three recent reports describe more potent molecules. (**Figure 3**)[2]

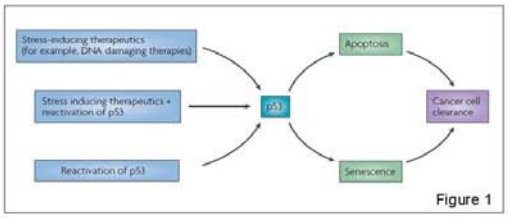


Figure 1

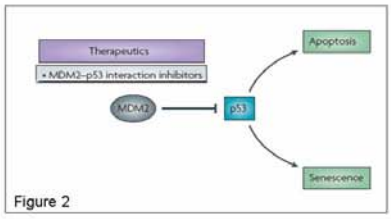


Figure 2

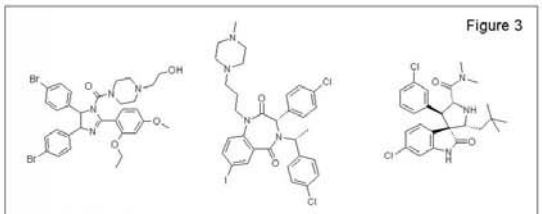
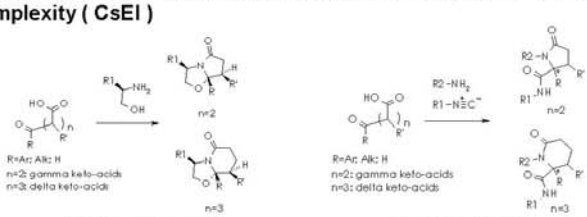


Figure 3
Nutline-3, benzodiazepinedione , spiro-oxindole

CHEMISTRY PROJECT

Libraries usually lack templates whose structure are closed to natural products.
The synthesis of privileged structures will enhance the value of libraries and also increase the success rate in test campaign. Indeed, some biological systems such as protein-protein interactions need the screening of privileged molecules (**Figure 4**).
Our strategy to access to privileged structures consist in the synthesis in one step of diversified molecules presenting quaternary carbon included in a cycle like in natural products. For this purpose, two types of reaction are appropriate : BiNu-BiE reactions and IMCRs (Isonitrile Multi-Component Reactions) [3]. (**Figure 5**)

Chemistry : condensation reactions that efficiently create structural complexity (CsEI)



Meyers reaction (BiE-BiNu) **Ugi reaction (IMCR)**
Figure 5

Privileged structures:
semi-rigid-scaffold, direction in a 3-D space

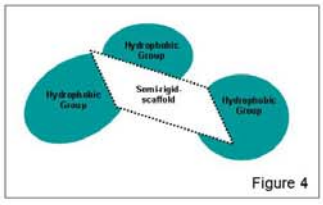


Figure 4

SCREENING & RESULTS

8800 privileged compounds from our laboratory library (42000 compounds) were screened using a fluorescence polarization assay : 8000 molecules with molecular weight slightly higher than recommended by Lipinski that target protein-protein interactions (CER library) and 800 molecules designed in our laboratory (HTS library).

Fluorescence polarization principle : The FP methodology allows the direct measurement of the ratio between bound and free labelled ligand in solution without any separation steps. It is based on the principle that small molecules have more rotational freedom than large molecules and a binding event results in an increase in polarization by restricting the small fluorescent peptide's rotational mobility.

Fluorescence polarization assay : Human recombinant HDM2 (final concentration 0.1 µg/mL, expressed in *E. coli* and purified by U761, Inserm, C-Dithem) was first incubated with compounds, then the labelled p53 peptide (final concentration 10 nM) was added. The CER library compounds were tested at 30 µM, and the HTS library compounds were tested at 10 µM. (**Figure 6**)

Peptide p53 : FAM-RFMDYWEGL [4]
Emission wavelength: 420nm
Excitation wavelength: 535nm

From the results of the screening, two hits have been identified. (**Figure 7&8**) These molecules will be synthesized in order to confirm the values of pIC50 and to make a co-crystallisation with MDM2. The small fragment is very interesting for a development in medicinal chemistry.

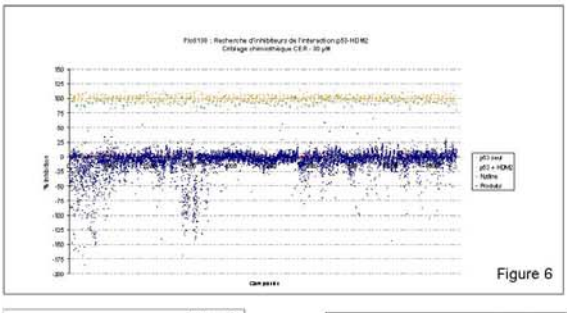


Figure 6

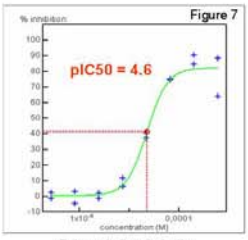


Figure 7
Beta-carboline derivative
MW = 436

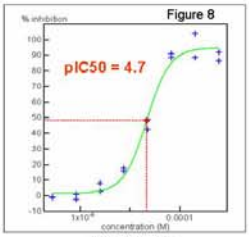


Figure 8
Small fragment
MW = 209

[1] Wells J.A.& McClendon C.L.; Nature, 2007 Dec, 450, 1001-1009
[2] Vazquez A. et al.;Nature Reviews Drug Discovery, 2008 Dec, 7, 979-987
[3]Dömling A.; Chem.Rev.,2006,106,17-89
[4] Parks et al. (Johnson & Johnson) Bioorg Med Chem Lett. 2005 Feb 1;15(3):765-70