Research Unit UMR 176 CNRS / Institut Curie
Group Medicinal Chemistry, Bioorganic Chemistry, Vectorisation (Paris)

Head of the group : Jean-Claude FLORENT

Permanent investigators:	6
Students and post-doctorants :	8
Technicians and administrative staff :	3,5

The main activity of the team of “Medicinal Chemistry, Bioorganic Chemistry, Vectorisation" is the pharmacomodulation and synthesis of small molecules for the study of living organisms. The synthesized molecules find multiple applications as probes for proteomics/genomics and/or as agents for therapeutic purposes. We continue our line of research on antitumour drug targeting, particularly through the use of Shiga toxin-drug conjugates (antitumour) or Shiga-peptide conjugates (for antitumour immunology). Our laboratory is also involved in another other important field, i.e. targeted therapy. Thus, we particularly develop flavonoid compounds as aminopeptidases inhibitors, potentially anti-angiogenic and anti-metastastic. Tubulin ligands for their potential antivascular activity, and hence analogs of Combretastatin A4, have been prepared. We conceive serine threonine kinases, inhibitors of CK2 and PIM1. We have organized, and continue to add small molecules, to the Curie-CNRS “chimiothèque”, a collection of small molecules, comprising the products synthesized at UMR 176. Some of the hits found by our biologists partners are being optimized as inhibitors of protein-protein interactions involved in cell migration and metastasis (L-lamiline/Syndecan3) and we develop a hit inhibitor of the serine-threonine phosphatase I (PP1). Furthermore, in a Curie “PIC” network in connection with a team of biologists from our Institute, we synthesize chemical probes to study molecular biology and mechanisms relating to the retrograde route taken by the Shiga toxin to the Golgi apparatus. This chemical biology programme has allowed us to synthesize chemical tools applied for the study of the Shiga toxin endocytosis by cell fluorescence microscopy on living cells or on Geant Unilamelar vesicles as cell models. We have undertaken a programme on the proteomic analysis of the cell surface proteins, which could follow this retrograde route of endocytosis.

I. Inhibition of tumour angiogenesis: synthesis of antivascular compounds

(R. Pontikis, J. C. Florent). Participants: N. Ty, M. Arthuis (PhD students).
(Collaboration : G. Chabot, INSERM U 640, UMR 8151 CNRS/Univ. Paris Descartes)

This project, previously supported by a “PIC” (Curie research programme), is the subject of an INCa contract (Institut National du Cancer)
Angiogenesis (i.e.: formation of new blood vessels from those pre-existing) is a fundamental process in tumour development. Thus, it constitutes a promising strategy in anticancer chemotherapy. We are interested in the antivascular approach, which involves the use of therapeutic agents that specifically destroy the established tumour vasculature.
Some inhibitors of tubulin depolymerization are able to induce a rapid tumour vascular collapse. Morphological and functional changes of endothelial cells of novel tumour blood vessels, cause a rapid interruption of the blood flow, leading to an extensive necrosis of the tumour cells. Clinical data with CA4P, a phosphate prodrug of combretastatin A4, confirmed this antivascular effect in humans.

The purpose of our work is to discover more efficient, more stable and less cytotoxic novel tubulin inhibitors, while trying to better understand the properties of these molecules in relation with the tubulin target.
CA4 is a natural *cis*-stilbene product. The *cis* orientation of the two aromatic rings is an essential requirement for potent biological activity. The structure-activity study of vinylogous CA4 analogues allowed us to select a compound, with a *cis-trans* butadiene bond and a simple phenyl ring. It displayed an greater inhibitory effect of tubulin polymerization than that of CA4, and less cytotoxicity, which represents an advantage for a potent anti-vascular agent. By its original structure, it can be considered as a new lead compound (J. Kaffy *et al*. *Org. Biomol. Chem*. **2005**, *3*, 2657). Furthermore, we have shown that the stabilization of these dienic structures by replacing the *cis* double bond by a *cis* cyclopropyl ring, can retain the antitubulin activity (Ty N. et al, *Bioorg. Med. Chem. Lett*. **2009**, in press). An enantioselective synthesis of novel chiral cyclopropyl derivatives is currently under investigation.
In order to reduce the conformational flexibility of CA4 and dienic derivatives, structures incorporating a 5 or 6-membered ring between the *cis* double-bond and one of the aromatic rings were considered (constrained analogues). Thus, various heterocyclic cores bearing a substituted exocyclic double bond have been prepared, stereoselectively, by a tandem Heck-Suzuki-Miyaura coupling reaction (M. Arthuis *et al*. *Tetrahedron Lett*. **2007**, *48*, 6397; M. Arthuis *et al*, *J. Org. Chem*. **2009**, in press).
The design of these molecules was guided by docking studies on the colchicine binding site of tubulin; coll. L. Morin-Allory, ICOA, UMR CNRS 6005, Orléans, France.
The synthesized compounds have been evaluated for their ability to inhibit tubulin polymerization and reduce tumour cell proliferation, but also for their effects on the morphology of human endothelial cells (*in vitro* test, predictive of an antivascular effect); coll. G. Chabot, U640 and UMR 8151, Univ. Paris 5, Paris.

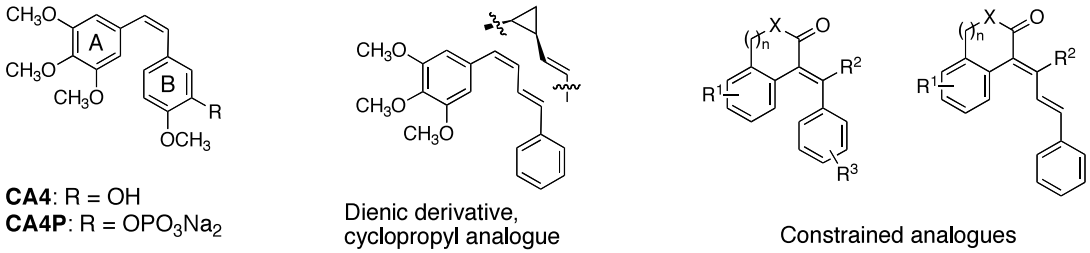

Figure 1: Targeted constrained analogues of CA4, as potential Vascular Disrupting Agents

II. *Vectorisation and activation of antitumor prodrugs by the B subunit of the Shiga toxin*

(F. Schmidt). Participants: G. Fillon, C. Smet-Gillardi (Curie temporaty staff).
(*Collaboration L. Johannes, UMR 144 CNRS/Institut Curie, Paris)*

In the field of targeting, we have initiated a collaboration with biologists (Ludger Johannes group at Curie) in order to use the B-subunit of Shiga toxin as a vectorisation agent.
The Shiga toxin is the agent of shigellosis, a form of dysentery. This bacterial protein consists of two subunits: the A subunit, the toxic one, inhibiting protein synthesis once in the cytoplasm. The pentameric B-subunit, the carrier, interacts with receptors on the cell surface, and targets the subunit A inside the cell to the Golgi apparatus. Its receptor is a cellular glycosphingolipid, the globotriosyl ceramide (Gb3), which is overexpressed in most cancers.
The cytotoxic agent is targeted as a prodrug, inactive in itself, but able to release the active compound by intracellular activation. The prodrug consists of 3 parts, the targeting moiety

(Shiga toxin B-subunit), a spacer and the cytotoxic agent. The advantage of such a strategy is due to both the recognition of tumour cells and internalization following the recognition step.

1 - Shiga-SN-38 conjugate

One of our delivery systems was based on the cleavage of a disulphide by a reductor, such as intracellular glutathione, to release SN-38, a camptothecin derivative particularly active on colorectal cancer (El Alaoui *et al*. *Angewandte Chemie* (2007) 119, 6469-6472).

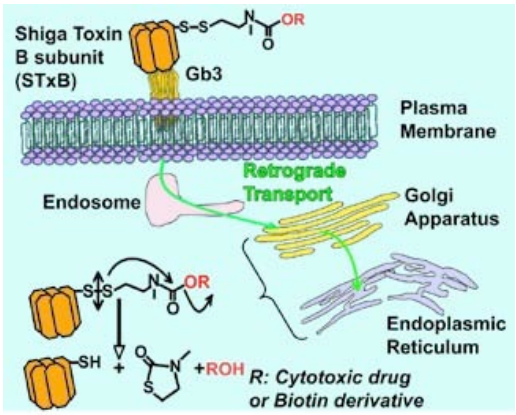

Figure 2: Mechanism of release

The conjugate is stable and releases the active compound within the cells. Cytotoxic measurements on the conjugate were carried out on HT29 cells (colorectal cancer). IC_{50} of the conjugate was better than that of the reference drug, irinotecan. Selectivity, for cells that express the receptor compared to those who do not express it, is very good. Tests on mice have shown a very good therapeutic effect inducing tumour regression.

2 - Shiga-pro-Benzodiazepine conjugate

In recent years, ligands of peripheral benzodiazepine receptors (PBR) have emerged as potential therapeutic agents for human cancers.

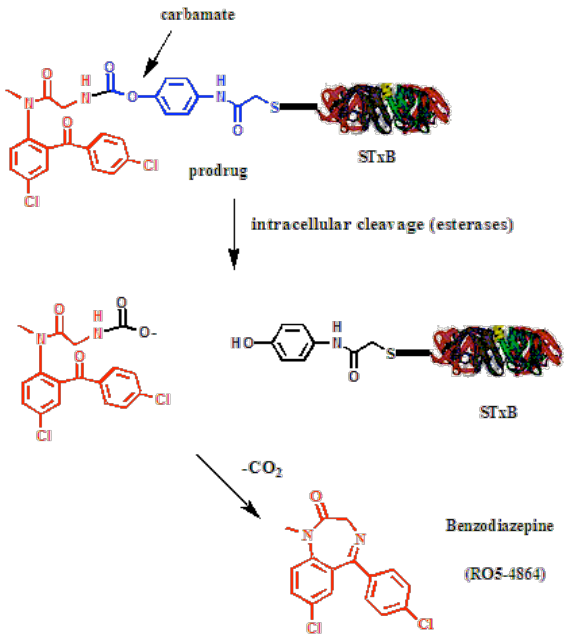

Figure 3: Mechanism of release.

RO5-4864 is a very good ligand of PBR, but there is no galenic form of the molecule - i.e. injectable in humans - due to the high insolubility of the compound. Therefore, it seemed interesting to explore a targeting approach of a benzodiazepine prodrug. In this view, we used the B-subunit of the Shiga toxin as the tumour targeting moiety. This targeting strategy is expected to increase both the solubility of the molecule and the selectivity of the product toward the tumor cells.
Our approach consisted in synthesizing acyclic pro- benzodiazepines linked to Shiga toxin by a spacer able to be cleaved intracellularly.
The prodrug was first obtained by an 11-step synthesis, in 4.7% global yield. A second scheme, more convergent, reduced the number of steps to 7, and global yield reached 15%. The coupling conditions with the thiol function of the modified B-subunit of Shiga toxin were optimized (pH 7.5).

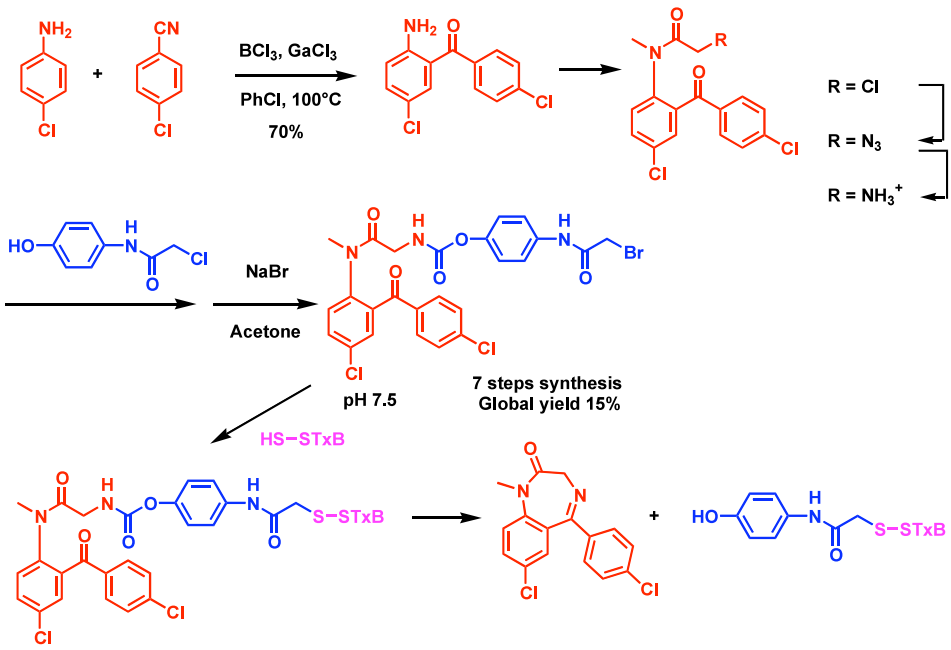

Figure 4: Synthetic scheme

The prodrug is much more soluble than R05-4864, stable and able to release the active benzodiazepine. Moreover, the conjugate is selective for cancer cells overexpressing the receptor for Shiga toxin (A. El Alaoui, *ChemMedChem*, 2008).

Concerning the current developments of the project, our goal is to improve the *in vivo* efficacy without increasing toxicity, which means increasing the cytotoxic agent/ Shiga toxin B-subunit ratio. In this view, spacers for linking multiple drug units to the Shiga toxin are being synthesised.

III. Synthesis of contrast agents targeted by the Shiga toxin

(F. Schmidt). Participant: N. Bogliotti, post-doctorant (P.G. de Gennes grant)
(Coll. L. Johannes, UMR 144 CNRS/Institut Curie, Paris; V. Semetey, UMR 168 CNRS/Institut Curie, Paris; M. Tanter, C. Boccara, ESPCI, Paris)

This project is conducted within a collaboration between our laboratory, a biology laboratory (L. Johannes group), a laboratory for physical chemistry and surface materials (V. Semetey group) at Institut Curie, and two laboratories of physicists at ESPCI (Mr. Tanter, C. Boccara).

It is possible to destroy tumours by using ultrasounds powerful enough to heat cells and kill them. The problem is to focus the beam in order to heat only the targeted area. Indeed, the non-homogeneity of the surrounding tissue makes it difficult to obtain a good definition of the area to be irradiated. To remedy this defect, a technique called ultrasonic time reversal is used, which consists in recovering an ultrasonic signal emitted by the tumor, analyze the signal and send it back with a destructive intensity to the target area, taking into account the signature of the ultrasonic signal.
The whole would thus be a multiple-step protocole. First, the injection of a contrast agent targeted by the Shiga toxin (STxB) that recognizes the tumor area and a laser irradiation permits to obtain an ultrasound image taking into account the distortions due to the environment of the tumour. Then, a phase of recovery and analysis of the signal takes place permitting to tailor an ultrasonic emission for targeted area. This emission will be strong enough to achieve the destruction of the tumour by heating.
As the contrast agent, we chose gold particles (nanorods), that give rise to a photoacoustic effect compatible with *in vivo* use.

IV. Towards a New Method for Functional Proteomics for Identification of Proteins Involved in the Retrograde Transport

(J.-C. Florent, M. Azoulay). Participant : R. Christiano (PhD student, ARC grant) *(Collaboration : L. Johannes, UMR 144 CNRS, Institut Curie)*

In this project, we develop an approach of functional proteomics to identify proteins that use the retrograde transport route. Recently discovered, this channel allows molecules to avoid degradation and recycling to other compartments of the cell. Proteins and lipids are directly driven from the early endosome to the trans-golgi (TGN), bypassing the late endosomes. The path of retrograde transport is clearly necessary for cell entry of pathogens such as toxins (STxB, CTxB, ...) or viruses (HIV, Herpes, ...) and the list of cellular proteins which utilize this route is growing steadily. Recent studies suggest a role of retrograde transport in many cell functions such as antigen presentation, transport of glucose and copper and transport of certain receptors signal (IFN, EGFR,). The elucidation of the mechanisms involved, but also the identification of new proteins of this pathway, are therefore of major interest both in basic knowledge and in the therapeutic area.
In a first step, we modified all surface proteins with a sulfatation signal peptide linked to a biotin. Among the proteins as amended, those that will be directed to the acceptor compartment of the retrograde pathway (Golgi, ...) are sulphated on the peptide tag by an sulfotransferase enzyme localised in the Golgi /TGN area. The biotinylated proteins, isolated with streptavidin beads, are then characterized by gel electrophoresis, and characterized if radioactive as those following the retrograde route, and identified by mass spectrometry MaldiToff.

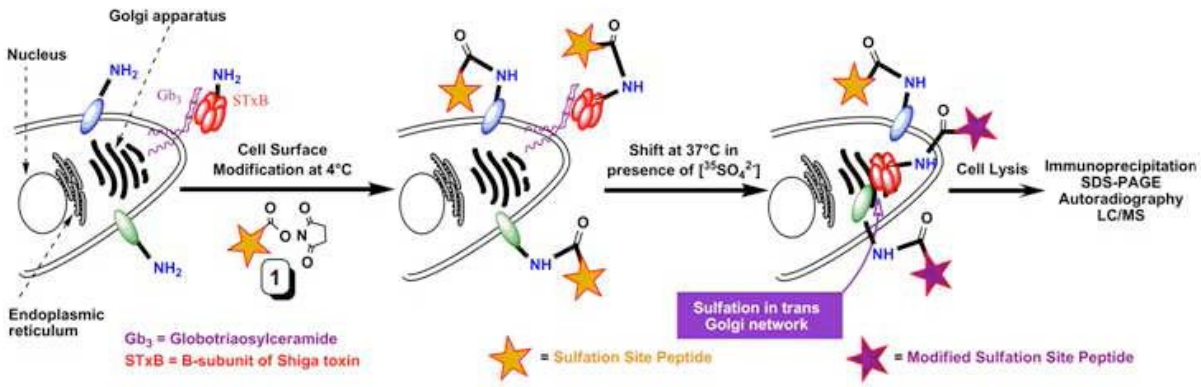

Figure 5 : Principle of the method of proteomics

This approach was therefore carried out on a protein model using the B-subunit of Shiga toxin (STxB), anchored to the glycolipid receptor Gb3 naturally present on the surface of Hela cells.
By comparison with a Shiga-B genetically modified bearing the tag fusion gene as a reference, we could evaluate the effectiveness of the method. We have shown that, presently, the method is not sufficiently effective and we are working to optimize it (see diagram below).

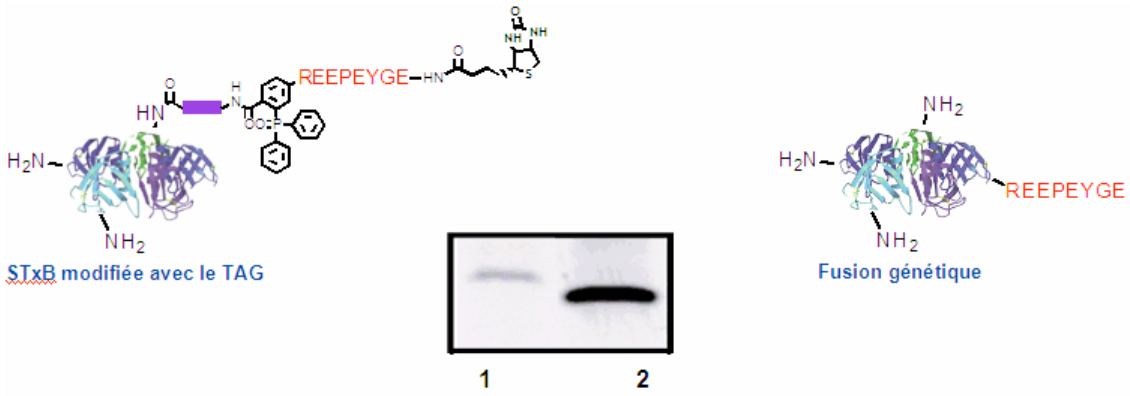

Figure 6 : Comparison of the results obtained by measuring the radioactivity after gel migration (Lane 1), after ligation with the peptide tag of the protein Shiga already anchored on the surface compared to Lane 2, which uses the Shiga with engineered Tag.

V. Using bioorthogonal ligation reactions as tools in biology

(J.-C. Florent, M. Azoulay). *(Collaborations : L. Johannes UMR 144 CNRS/Institut-Curie)*

Dendritic cells called "antigen presenting" derived from bone marrow are present in all tissues. They play a key role in triggering the immune response. They capture foreign antigens and stimulate cytotoxic T-lymphocytes that are specialized in the recognition and destruction of cells bearing the antigens presented. In addition, dendritic cells express the Gb3 receptor of the Shiga STxB subunit, which allows the entry of this protein into the cell. Since the principle of antitumour vaccination is to use a vector to deliver tumour antigens to dendritic cells, we chose to use STxB as an alternative vector to viral vectors currently studied.
Preliminary assays have shown that coupling the immunogen E7 protein with modified STxB using the conventional bi-specific reagent SMPB (4 [4-maleimidophenyl] butyric acid N-

hydroxuccinimide) induced high precipitation of the E7 protein. This lack of specificity creates problems with the coupling of STxB and non-reproducibility of the quality of coupled protein obtained.
To circumvent the difficulties often encountered using the techniques of protein-protein ligation or protein-RNA/or DNA, we used the reaction of "click chemistry" using the Huisgen reaction. We tested a method of convergent coupling protein in two stages: the first by the functionalization of STxB with an alkyne and the protein of interest with an azide, the second time by the coupling of proteins functionalized via cycloaddition [3 +2] Huisgen. We showed, by immunofluorescence, that this method enables convergent ligation and preserves the integrity of both proteins or the ability of recognition and transport of the STxB by Gb3. Thereafter, other proteins such as Bovine Serum Albumin (BSA) or Her2/neu protein were coupled to STxB following the same method, which led to an European patent.

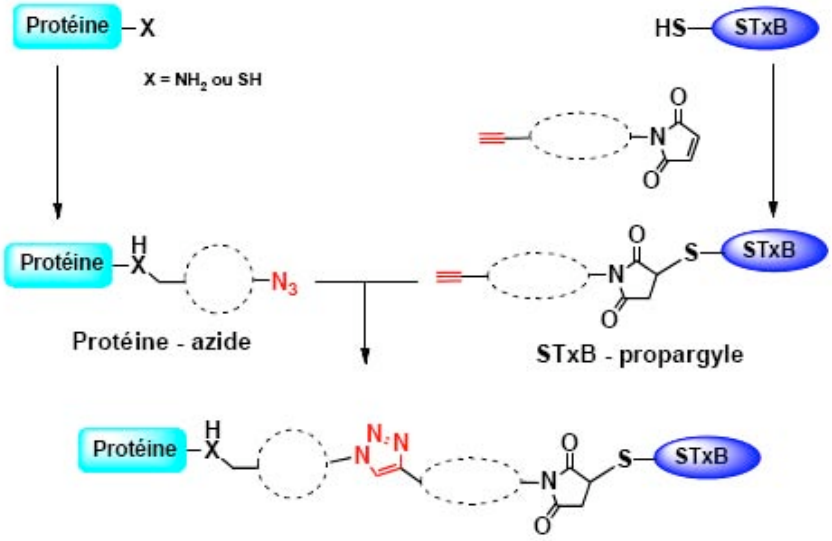

Figure 7 : Ligation of the Shiga protein by Huisgen reaction

VI Design of chemical tools for studying the cell biology of the retrograde path.

(J.-C. Florent) Participant : R. Christiano (PhD student, ARC grant). *(Collaborations : L. Johannes, UMR 144 CNRS/Institut Curie ; P. Bassereau, UMR 168 CNRS/Institut Curie)*

We have developed an access road to the Gb3 trisaccharide Globotriose receptor of the Shiga toxin. The synthesis of glycosphingolipids to examine how the cell membrane captures pathogens associated to its surface has been completed successful (W. Römer *et al*, *Nature*, 450, 670-675, 2007).
With the help of glycolipids synthesized by our group, W. Römer (P. Bassereau team, UMR 168 CNRS/Institut Curie) has reproduced the mechanism of endocytosis by connecting the unit B of the Shiga recognition when it is in contact with giant vesicles (GUV) incorporating the glycosphingolipid Gb3, mimicking a lipid bi-layer. The formation of intense spots has been observed in imaging the surface of the GUV, from which tubes are formed. Using variants of the receptor Gb3 of diverse lengths that we have synthesized, it was shown that the receptor itself was involved in the process of invagination, i.e. the creation of micro-domains, induced by the toxin, followed by depression of the membrane, ultimately leading to the formation of microtubules. The mechanism was thus demonstrated as being purely physical.

Pursuing this study, we have built a globosphingolipid carrying fluorescent tags. Our goal will be to preserve the Shiga- Gb3 fluorescent recognition.

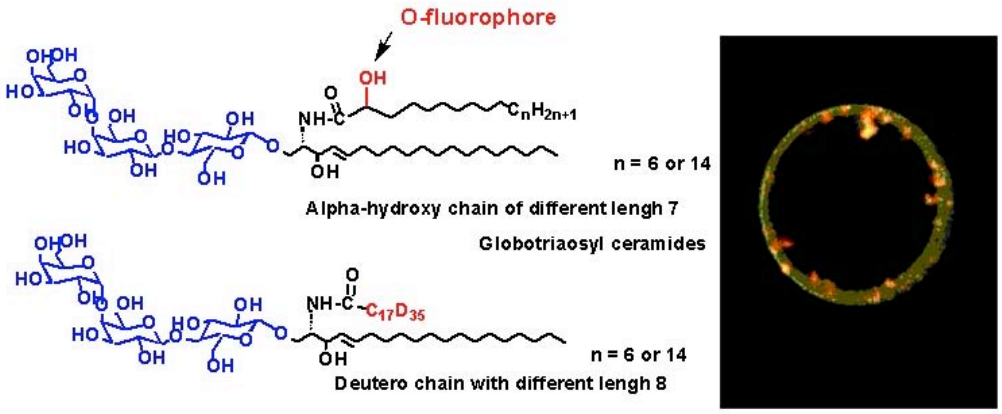

Figure 8 : Glycerolipid probes prepared and visualization of invaginations caused by the binding of Shiga on Gb3 anchored on a model membrane (GUV).

VII. Synthesis and biological evaluation of flavonoids for antitumour activity

1. Identification of active flavone-8-acetic acid (FAA) metabolites, compounds targeting tumoral vascularization.

(D. Dauzonne) (Collaboration : G. *Chabot, INSERM U640, UMR8151 CNRS/Université Paris Descartes).*

Flavonoids exert a very broad spectrum of biological and pharmaceutical activities including antitumour activities. Among flavonoids that have been tested in cancer therapy, flavone-8-acetic acid (FAA) emerged as an interesting lead because it has demonstrated a spectacular activity in murine and transplanted human tumours in mice. However, FAA has not shown anticancer activity in humans. This interspecies difference in FAA anticancer activity has been hypothesized to be due to metabolic activation of this flavonoid *in vivo* in mice. The aim of this work was to compare the metabolic pathways in the two species.

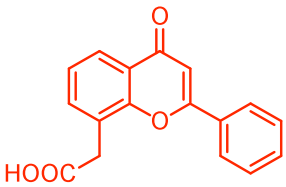

Figure 9 : Flavone-8-acetic acid

We have shown than the FAA can be metabolized by mouse microsomes, thus forming six new metabolites which have been characterized : 3',4'-dihydrodiol-FAA, 5,6-epoxy-FAA, 4'-OH-FAA, 3'-OH-FAA, 3',4'-epoxy-FAA and 6-OH-FAA. Compared to human microsomal preparations, we have observed that metabolization of FAA is much more efficient using murine microsomes. We have also identified the P450 microsomes and the epoxide hydrolase involved in the *in vitro* FAA's metabolism by these murine microsomes. In mice, after *in vivo* administration, several FAA's metabolites have been identified in urine and plasma.

In order to evaluate the cytotoxicity of FAA's metabolites, we have tested the incubation extract in the presence of murine microsomes. We have also separately tested its monohydroxylated derivatives on murine cells of B16 melanoma. FAA was proved to be more cytotoxic in the presence of mouse microsomes. We have noticed, too, that one of the murine metabolites, 4'-OH-FAA, was able to modify the morphology of endothelial cells *in vitro* at low concentrations. These results as a whole strongly suggest that the FAA metabolism could be involved in its anticancer activity as observed in mice (M. H. Pham *et al. Drug. Metab. Dispos.* **2007**. *35,* 2023-34). In order to evaluate their *in vivo* activity, the gram scale unequivocal synthesis of some murine metabolites (6-OH-FAA, 4'OH-FAA) is currently carried out. This work is part of a selected INCA project.

2. *Design and synthesis of flavone derivatives as novel inhibitors of the aminopeptidase-N/CD13 of leukemic cells. Biological evaluation in proliferation and migration associated to angiogenesis.*

(D. Dauzonne) *(Collaboration : B. Bauvois, J.-P. Kolb, Centre de Recherches Biomédicales des Cordeliers UMR-S872, Paris).*

The cell surface metalloproteinase aminopeptidase N (APN/CD13) is overexpressed in several solid and hematological tumours (renal and prostatic cancers, melanoma, acute and chronic myeloid leukemias), and on the human endothelial cells of angiogenic, but not normal, vasculature. *In vitro* and *in vivo* experiments suggest that the APN from endothelial cells and tumour cells contributes to tumour process and to angiogenesis, associated with the progression of the tumour by increasing their rate of proliferation and/or favouring their motility and invasion ability.

Several commercial APN inhibitors (bestatin, actinonin, betulinic acid, amastatin...) exist. However current limitations in the use of these inhibitors include their toxicity and/or their low specificity. Moreover, there is still no information about the crystallized structure of APN, therefore impeding the development of APN inhibitors through a computer-assisted optimization process. We recently reported the synthesis of one non-cytotoxic flavone-8-acetic acid derivative (the 2',3-dinitroflavone-8-acetic acid) and its ability to selectively inhibit the enzymatic activity of APN in human leukemic myeloid cells. This molecule is now provided by EMD Biosciences Inc (San Diego, CA) (supplied by Calbiochem n°164602).

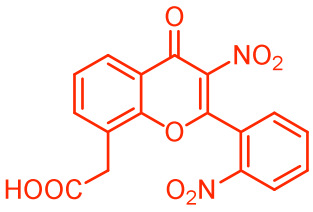

Figure 10 : 2',3-dinitroflavone-8-acetic acid

In this context, and considering 2',3-dinitroflavone-8-acetic acid as a model, we have undertaken the synthesis of novel flavone derivatives in view of selecting new non-cytotoxic molecules as highly specific inhibitors of APN capable of blocking tumour process. Tests are performed on human leukemic myeloid (NB4, HL-60, U937, THP1) and endothelial (HBMEC) cell lines, and also primary blood cells from patients with leukemia (Dept. of Haematology, Hôtel-Dieu, Paris). Our current experiments indicate that APN from leukemic cell lines confers (i) proliferative ability, and (ii) motility (chemokinesis and invasion through a Matrigel coat in transwell) which are dependent on the level of APN expression.

Identification and use of new non-cytotoxic molecules capable to efficiently inhibit the enzymatic activity of APN and its related actions on tumour cells should allow the opening of new therapeutic perspectives at short term in preclinical studies on experimental animals (mouse cancer models) and, at middle term, in clinical trials on patients with cancer.

VIII. DNA methyltransferase inhibitors (DNMT inhibitors)

(D. Dauzonne). Participant : R. Ait Sarkouh (Master 2 student) *(Collaboration : P. A. Arimondo, USM 0503 MNHN, UMR 5153 CNRS, U 565 INSERM, Muséum National d'Histoire Naturelle, Paris).*

DNA methylation is an epigenetic modification involved in the regulation of gene expression. This alkylation has several roles in the functioning of the cell (chromosome stability, cell differentiation, genomic imprinting). The enzymes responsible for this methylation in humans are DNA methyltransferases (DNMT) that catalyze the transfer of a 5-methyl position of certain 2'-deoxycytidines to give 5-methyl-2'-deoxycytidine. These enzymes recognize CpG sequences highly represented in the promoters of genes, and methylation of these sequences caused suppression of the transcript.
Disturbances of methylation are associated with certain cancers often presenting local hypermethylation of the promotors of tumour suppressor genes, leading to genomic instability. Therefore, the search for inhibitors of DNMTs is a promising area of investigation for the research of new cancer therapies. Molecules inhibiting this class of enzymes have been recently found among the compounds of the the Curie Institute's chemical library by a team from the Museum National d'Histoire Naturelle (MNHN USM 0503, UMR 5153 CNRS, INSERM U 565). They compounds belong to the class of flavonoids (general formula shown below):

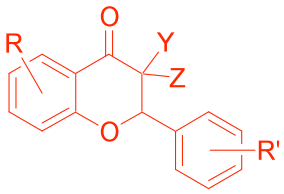

Figure 11 : DNMT Hit development

Several novel molecules belonging to this series are currently synthesised.

IX. Search for active molecules on mitochondrial diseases

(D. Dauzonne). *(Collaboration : M. Blondel, CNRS, UMR-S613, Université de Brest, France).*

This project is devoted to the research of therapeutic molecules to treat certain mitochondrial diseases (*e. g.*: muscular dystrophies, encephalopathies…). The team led by Pr. Marc Blondel have developed a simple and fast screening test that uses a model of mitochondrial disease in the yeast *Saccharomyces cerevisiae*. The examination of the Institut Curie's chemical library has revealed that some compounds, belonging to the class of flavonoids, are proving effective in the activity test studied (restoration of respiratory growth of mutant yeast, thus indicating a correction of the mitochondrial activity).
Based on the already collected data, further studies are ongoing and re-synthesis of active compounds, as well as preparation of new derivatives, are underway.

X. Understanding and fighting metastasis: Inhibition of syndecan-1 mediated cell adhesion

(E. Bertounesque). Participants : A. Carrër (Doctorant, Institut Curie-CNRS) and a post-doctoral ANR researcher). *Collaboration : P. Rousselle, Institut de Biologie et Chimie des Protéines (UMR 5086), Lyon; I. García-Sáez, Institut de Biologie Structurale (UMR 5075), Grenoble, France)*.

This research project, called CHEMISPIKE, financed by the French National Agency for Research (ANR), is coordinated by Patricia Rousselle (IBCP, UMR 5086).
Approximately 90% of all cancer deaths arise from the metastatic spread of primary tumours (e.g. colorectal and breast cancers). Of all the processes involved in carcinogenesis, local invasion and the formation of metastases are clinically the most relevant, but they are poorly understood at the molecular level. During progression from tumour growth to metastasis, cellular invasive and migratory behaviour is governed at both extracellular and intracellular levels and depends on the carefully balanced dynamic interaction of the cell with its extracellular matrix. The cell surface receptors EGFR (epidermal growth factor receptor), syndecans (e.g. syndecan-1) and integrins (a6b4) are thought to play key roles in regulating tumour growth, metastasis and tumour angiogenesis (see Figure below). Laminin 332 (also named laminin 5) is a major adhesion substrate for epithelial cells. Through interactions with these receptors, laminin 332 drives tumorigenesis via PI3K and RAC1 activation, promoting carcinoma cell migration, notably those with malignant characteristics, and may act as a ligand for invasiveness.

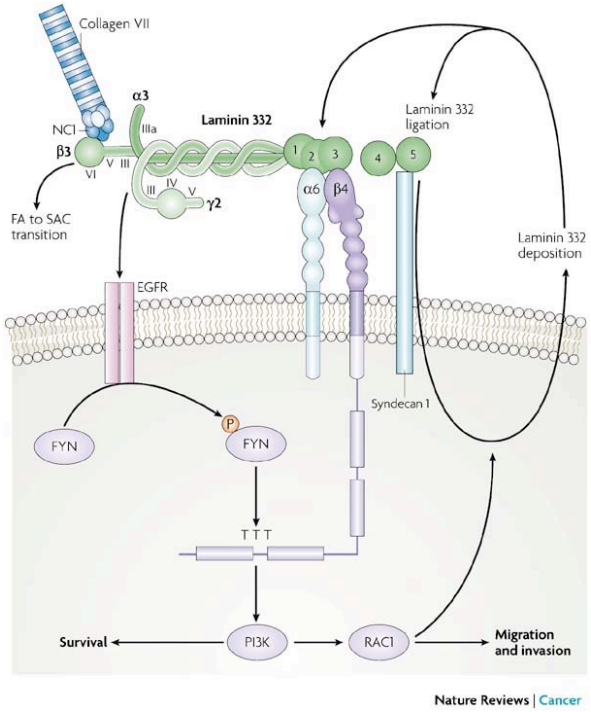

Figure 12: From "Laminin 332 in squamous-cell carcinoma", M. Peter Marinkovich, *Nat. Rev. Cancer* (2007), *7*, 370-380.

P. Rousselle and co-workers have recently identified a region in laminin 332 (the LG4/5 domain), which, by interacting with syndecan-1, may strongly influence epithelial cell behaviour (see above Figure). Indeed, they have shown that the syndecan-1 interaction with the LG4/5 domain in laminin 332 is essential for epithelial cell migration and they are currently

studying the triggered intracellular signalling cascade. The fact that syndecan-1 is predominantly expressed by different carcinomas and plays multiple roles in the regulation of cell-matrix interactions reinforces the notion that the interaction between syndecan-1 and LG4/5 participates in the malignant process.
In order to develop inhibitors of the LG4/5 domain that specifically block the syndecan-1 mediated interaction with this domain in laminin 332, P. Rousselle and co-workers have developed a specific syndecan-1 mediated cell adhesion assay successfully used for the screening of a large library of chemical agents at the "Centre for Bio-Active Molecules Screening (CMBA)" CEA Grenoble (Laurence Lafanechère). They have found a significant number of hits, from the CNRS UMR 176-Institut Curie chemical library, conferring a 50 to 100 % inhibition of cell adhesion to LG4/5 but not to the rest of the laminin 332 molecule. Among the identified structural classes are the benzofuran (**1**) and 7-deazapurine (**2**) series (Figure 10), which have been selected for the CHEMISPIKE collaborative research programme.

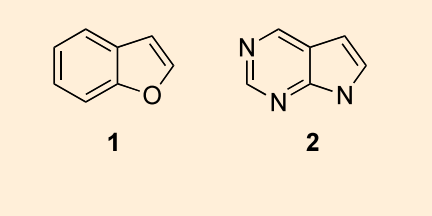

Figure 13. Medicinal chemistry research in lead generation and optimization from the hits based on the benzofuran or 7-deazapurine scaffolds.

In particular, the CHEMISPIKE programme aims at (1) dissecting the molecular mechanism underlying the LG4/5-syndecan-1 interaction and the associated signalling cascade, (2) identifying the most specific inhibitors among the Hits derived from the screening of a chemical library, and determine their respective targets, (3) performing medicinal chemistry research through Hit-to-Lead and Lead optimization studies, with the design and synthesis of candidate drugs, (4) testing the anti-tumour potential of the identified LG4/5 inhibitors in a pre-clinical model of colon carcinoma, and (5) solving the structure of the a3 LG4/5 fragment with the most relevant identified inhibitors using X-ray crystallography for rational drug design.

In conclusion, we focus on studying fundamental biology of syndecan-1 mediated cell adhesion and developing a new therapeutic strategy in oncology for the treatment of carcinomas, with a pharmaceutical partner. In chemistry, special emphasis is given upon the development of strategies and methods that allow the preparation of novel compounds based on molecular diversity.

XI. CK2 (casein kinase 2) Inhibitors

(F. Schmidt) Participant: M. López Ramos, G. Calvet (*Collaboration: C. Cochet, L. Lafanechère, U 366 INSERM, CEA, Grenoble; L. Mouawad, INSERM U 759, Institut Curie, Orsay; J.-B. Reiser, ISB, Grenoble*).

The serine/threonine kinases CK2 and PIM-1, apoptosis suppressors, are overexpressed in tumour cells, particularly in prostate cancer. CK2 has been validated for the treatment of cancer through the use of antisense RNA. Recent results show that the family of PIM kinases plays a role in the development and progression of prostate cancer.
To date, several types of CK2 inhibitors have been described, but there are relatively few known PIM-1 inhibitors.

The small molecules library of the Curie Institute was screened by Claude Cochet (INSERM U244) and Laurence Lafanechère (INSERM U366) at the CEA, Grenoble. Among thousands of compounds tested, several molecules have emerged, such as the inhibiting kinase CK2. Some of these compounds have also shown inhibition of PIM-1. Selectivity against a panel of other kinases is very good. Since the structures are different from those previously described, we can expect to develop a new family of inhibitors of these kinases.

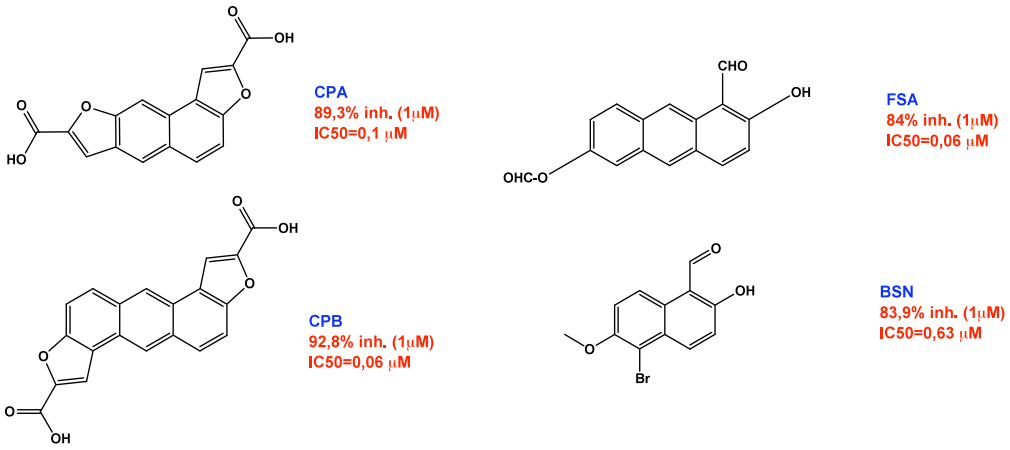

Figure 14: Residual CK2 activity at 1 mM in % and IC_{50}

Analogues were synthesized to determine the prerequisites for an active compound. Along with chemical synthesis to establish the structure activity relationships, modeling studies, i.e.: docking (coll. Liliane Mouawad) have determined the geometry of fixation of our most active compounds inside the ATP site of CK2.
The molecules contain three elements of recognition, a polar element interacting at the bottom of the active site, in particular the residues Lys68 and Asp175, a relatively flat hydrophobic, and a polar group that can be placed at the entrance to the cavity. The validity of these docking calculations was confirmed by the X-ray results of one of our inhibitor co-crystallized with CK2 (J.-B. Reiser, IBS, Grenoble)

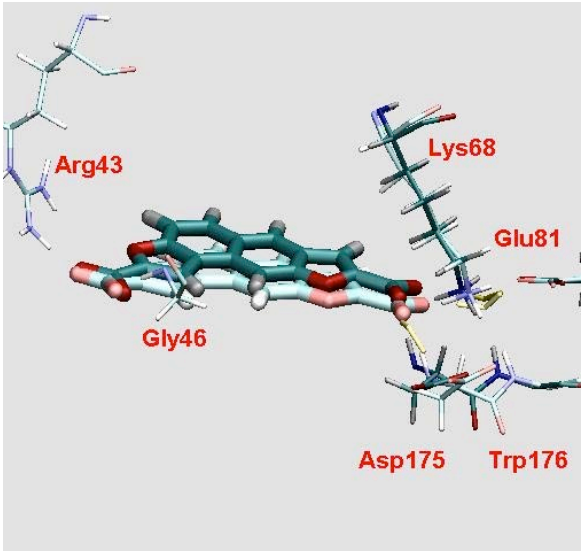

Figure 15: Comparison between radiocrystallographic structure and docking.
The main objective of the project is therapeutic. Our goal is to find the best candidate with pro-apoptotic antitumour properties for further development.

XII. UMR 176 CNRS/Institut Curie library of small compounds (“Chimiothèque”)

(Manager: F. Mahuteau-Betzer). Participants : M. Bombled, L. Charles, S. Dubruille-Thirot

1. Origins and purpose

The main motivations and occupations of the Medicinal Chemistry Laboratory of the Curie Institute during the last fifty years have been the discovery of cancer-related molecules. A direct consequence of this activity is the existence of samples of a large number of compounds prepared (often in significant quantities).
Moreover, advances in proteomics and genomics have led to the discovery of a growing number of new protein targets with therapeutic potential.
By screening small molecules, either individually or, preferably, in collections called "libraries of small molecules” (chimiothèque), numerous opportunities will be offered to discover or identify biologically active molecules, which are tools in Chemical Biology or drugs of the future.
The discovery of molecules for therapeutic "drug discovery" implies a close collaboration between chemists, biologists and, whenever possible, chemoinformaticians and structural biologists. It is commonly accepted that out of 10,000 molecules synthesized only one will enter into clinical phase. In absolute terms, this can be regarded as an enormous waste of molecules. However, in general, the application that a molecule has in biology is often not the one for which it was originally synthesized. Therefore, the probability is not insignificant that the therapeutic potential of the 9999 other molecules will be discovered by continuous screening against new targets.
This reality is behind the creation of the “Chimiothèque” Curie-CNRS in June 2001, which comprises 6720 compounds formatted in 96-well microplates.
In 2004, our chemical library was updated to 7440 compounds. In July 2006, a final update was made and 8560 compounds are now available in the form of microplates.

2. Contents

Historically, the activity of the laboratory on the development of anti-cancer agents specifically designed was particularly targeted to the synthesis of DNA interacting. Within this framework, 6 cytotoxic molecules have being entered into clinical trials.
At the origin of the “Chimiothèque”, there are a large number of polyheterocyclic compounds (mono- to polycyclic) belonging to a wide variety of families (merged, linear or connected polycycles).
During the past 4 years, the “chimiotheque” has grown steadily, and comprehends now over 8560 substances. These compounds are synthetic analogues obtained during optimization programmes against different therapeutic targets (anti-HIV, anti-kinases,…). Targeted libraries have also been built from "scaffolds" with drug-like properties, such as purines.

3. Value of the “Chimiothèque” of the Curie-CNRS

An important question concerning the “chimiothèque” is the value of the compounds that comprise it.
First, the results of screening carried out on the chimiothèque have shown that our collection is diverse enough to enable us to find active molecules against different therapeutic targets.
Insofar as the chemical library contains molecules that were designed to interact with the living, it is largely composed of heterocyclic compounds. Such compounds are known for their "drug-like properties".

A relatively large number of molecules present in the chemical library possess cytotoxic properties more or less (often less) important. Indeed, the flatness and intercalating properties of a compound is not sufficient for it to be cytotoxic: it must also interact with or inhibit an enzyme (e.g.: topoisomerase). Anyway, the cytotoxic properties should not be perceived as prohibitive. In the initial phase of drug discovery, screening is often performed *in vitro* in the absence of nucleic acids (DNA and RNA). Their cytotoxic properties have no impact on the objective of the primary screens. The buttons are identified real hits and, therefore, the starting point of a new research programme. To quantify the cytotoxicity of compounds in our collection, a screening will be conducted (Coll. Thierry Cresteil, ICSN, Gif/Yvette, France).
The chemical library was established by collecting (almost) all the molecules prepared in the laboratory. With much hindsight, we identified a number of products such as "frequent hitters" or too reactive and therefore not relevant to the screening.
Among them are molecules containing nitrofuran, aldehydes or enones, sub-structures to be avoided *a priori* for their too high reactivity. It was therefore proposed to remove them from the collection. However, there are a significant number of drugs or compounds in development that contain these motifs. These include compound CI1033 (irreversible inhibitor of EGF, Phase III; Pfizer), which contains a ground acrylamide in its structure. In the same way, some nitrofurans are mutagenic but nitrofuroxide, an intestinal disinfectant, is a marketed drug. Finally, although aldehydes are highly reactive, benzaldehyde is present in certain foods.
Thus, although these fragments or sub-structures are to be avoided *a priori*, there are interesting exceptions to this rule. Therefore, we still have not decided what to keep or eliminate from our chemical library. If one considers that the primary screening allows us to identify a key which is the starting point for an optimization process to a more "drug like" molecule, then it seems reasonable to retain these compounds.
In order to assess the contents of our chemical library, we established a collaboration with Luc Morin-Allory (Orléans, France). His team analyzed the contents of our chemical library and initial results indicate that 6234 molecules out of 7680 would be "drug-like" (80%) using the score and 4950 CFMS compounds would be "lead-like" (65%).

In conclusion, the analysis suggests that the chemical library contains a vast majority of compounds of great interest for research in "drug discovery".

3. Collaborations

Our chemical library was screened on over forty biological tests through 23 collaborations.
The most successful collaborations over the past four years are:

- Aurora kinase A (cancer) - A. Molla (Grenoble)
- Malaria - Ph. Grellier (Muséum National d'Histoire Naturelle, Paris)
- Stabilizer tubulin (cancer) - L. Lafanechère (CEA Platform, Grenoble)
- CK2 - C. Cochet (CEA, Grenoble)
- Amphiregulin (lung cancer) – M.C. Favrot (via ECA Platform)
- DNA Marker BENA435 - A. Popov (Grenoble)
- Stabilizer tubulin via MAPs (cancer) - A. Popov (Grenoble)
- Protein-Protein Interaction P66-PVNA (cancer) - G. Baldacci (Institut Curie)
- Alternative Splicing and NMD (HIV disease and Duchenne) - J. Tazi (Montpellier)
- Illness of Dengue, West Nile, Hepatitis C - B. Canard (Marseilles)
- Inhibition of metastasis inhibitors interaction lamine5 / syndecan proteoglycans. P. Rousselle (IBCP, Lyon)
- Bilharzia; target NAD + glycohydrolase. F. Schuber (UMR 7175, Strasbourg)

- Tuberculosis. UMP kinase from M. tuberculosis, and antiviral drugs - H. Munier-Lehman (CNRS URA 2128, Institut Pasteur, Paris)
- Neuropathies, yeast tests - M. Blondel (Université de Brest)

The results of these screenings are many! Because of the primary screening, a large number of hits, belonging to different chemical families, have been identified. The question that arises is how to organize our research to meet the needs of biologists who wish that an optimization program be set for the biggest hits. From the chemical point of view, the question is how to deploy our forces to work on each of these projects.

4. Developments from the "screens" of the biological chimiothèque

The Institut Curie has built a laboratory for rapid robotic synthesis at UMR 176, on the site of Orsay, under the responsibility of F. Mahuteau-Betzer. This platform is designed to optimize some hits when we deem it useful. We have clusterized our efforts to be more effective. The results from these screens have led to new fields of research, especially focusing on CK2 in our Paris laboratory (F. Schmidt, Florent group) and a second cluster organized on a family of molecules with an unfused polyheterocyclic system for these various applications (DNA Marker, Alternative Splicing - NMD, PCNA-p66) (F. Mahuteau-Betzer, Orsay), and Dengue virus (S. Piguel, Orsay). Metastasis inhibition (E. Bertounesque, Paris). This organization enables us to harmonize our efforts with regard to our objectives.

KEY PUBLICATIONS

2009

M. ARTHUIS, R. PONTIKIS, J.-C. FLORENT M. ARTHUIS, R. PONTIKIS, J.-C. FLORENT. Stereoselective Synthesis of Novel Highly Substituted Isochromanone and Isoquinolinone-Containing Exocyclic Tetrasubstituted. Alkenes. *J. Org. Chem*., in press.

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