Design, synthesis and biological evaluation of indanesulfonamides as carbonic anhydrase IX inhibitors

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1

Design and synthesis of new MET kinase inhibitors

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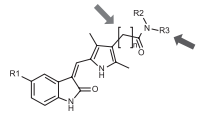
Protein kinases play a crucial role in signal transduction as well as in cell proliferation, differentiation and various regulatory mechanisms. Among them, the MET tyrosine kinase receptor (TKR) is involved in the development and progression of numerous human cancers and its expression correlates with poor patient prognosis. Therefore, the development of intracellular inhibitors for MET TKR appears as an attractive target for cancer therapy.

In a first time we designed a new series of pyrrole indolin-2-one derivatives as this backbone has been already identified as a key element for interacting at the ATP binding site of the MET TKR (figure 1). In order to optimise the pharmacological properties of pyrrole indolin-2-one derivatives, two strategies were considered: (i) Changes of type and positions of the lateral chains attached on the nucleus. (ii) Modification of pyrrole indolin-2-one nucleus by introduction of a new cycle and/or other heteroatoms.

In the second time, in order to identify original structures specific for MET TKR inhibition, we approached a rational design by an in silico virtual screening of several drug libraries. To do that, we use new classes of molecular descriptors (the fuzzy pharmacological triplets). They are adapted for the virtual screening by similarity and for the generation of the Structure-Activity Relations. Then the activity of these new structures is predicted thanks to an algorithm built with active and inactive molecules from the literature.

The confrontation of the two approaches should lead to original structures for MET TKR inhibition.



2

Novel photoactivatable analogues of glutathione

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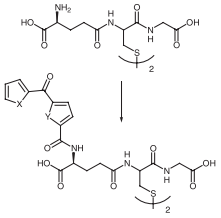
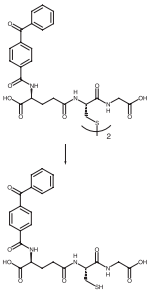
Glutathione (reduced or oxidized) is involved in many cellular processes such as disulphide exchange, detoxification of xenobiotics and maintenance of SH levels of proteins. Recently, Filomeni et al. [1] showed that the oxidized form of GSH (GSSG) also induces apoptosis which is a genetically controlled programmed cell death. To identify the proteins responsible for the apoptotic process, we synthesized photo-activatable probes based on glutathione structure. These probes bear benzophenone-like moiety as the benzophenone chromophore is one of the most efficient group for photolabeling.



X= S, Y= CH=CH (**a**) **4a**, 80%.
X= NH, Y= CH=CH (**b**) **4b**, 80%.
X= N-Me, Y= CH=CH (**c**) **4c**, 77%.
X= Y= CH=CH (**d**) **4d**, 85%.
X= CH=CH, Y= S (**e**) **4e**, 88%.

The synthesis of new photactivatable analogues of GSSG, as well as the synthesis of new benzophenone-like chromophores have been accomplished [2]. As expected, three of the new analogues of GSSG (4b-c, 4e) exhibit promising photochemical properties. This makes them putative photolabeling reagents for GSSG-utilizing systems.

Furthermore, the first N-acylated photo-activatable analogue of GSH was synthesized in a two-step procedure from GSSG, and was shown to be a photoaffinity label of glutathione *S*-transferases [3].



These new probes are currently used in the laboratory for the identification of new glutathione-binding proteins.

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3

Design, synthesis and pharmacological evaluation of potential cyclooxygenase inhibitors/NO-donors in prostate cancer therapy

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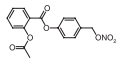
Even if the antiproliferative, proapoptotic and antiangiogenic mechanisms of Non Steroidal Anti Inflammatory Drugs (NSAID) [1] and COX-2 selective inhibitors (coxibs) remain to be fully identified, the potential of these compounds in chemoprevention and chemotherapy of several cancers is widely known. However, evidence of side effects on both gastric and vascular levels could hinder the long term use of these treatments.

Coupling a COX-2 selective cyclooxygenase inhibitor to preserve the gastroprotective property of COX-1, with a nitric oxide (NO) donor (for its gastroprotective, vasodilatating and proapoptotic potential) [2] seems attractive [3]. This strategy has mainly been used in the design of aspirin derivatives [4], usable in colorectal and bladder cancer.

We have chosen to modify specific profens (Ketoprofen, Suprofen, Carprofen), by adding a NO donor nitric ester through specific spacers. Those compounds are undergoing biological evaluation concerning their cyclooxygenase inhibiting and antiproliferative activities. NO releasing kinetics are also studied. Primary results are encouraging and give us the opportunity to design COX-2 selective inhibitors (overexpressed isoform in prostate cancer) based on a diarylpyrazole scaffold [5].



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4

Design and synthesis of potential topoisomerase I inhibitors derived from camptothecin

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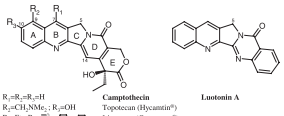
Camptothecin is a natural occurring compound that possesses a potent antitumor activity. It specifically inhibits the topoisomerase I enzyme by binding the binary complex DNA-topoisomerase. Some of its side effects are due to its low water solubility. Many derivatives have then been synthesized in order to increase both the solubility and the potency of camptothecins. Particularly, two molecules (Irinotecan, Topotecan) are actually used in clinic for the treatment of ovary, colon and lung cancers.

In the early pharmacomodulations on camptothecin, deep investigations on the lactone ring have lead to inactive or less potent compounds, inhibiting any modulation on this part of the molecule. Nevertheless, the dogma felt with the efficacy of homocamptothecins, whose lactone ring is a seven-membered ring. Moreover, it has been recently reported that luotonin A inhibits topoisomerase I. This heterocycle possesses a benzene ring as E ring, and also a nitrogen at position 14 [1].



Few studies [2] have been interested in A, B and C ring substitution, but almost no substitution of position 5 has been attempted [3]. The aim of this work is the design and the synthesis of some new derivatives of camptothecin, based on the luotonin A ring system. The synthesis of these derivatives has been realized starting from pyroglutamic acid, using Friedlander condensation as the final step. This pathway has then been applied with moderate to good yields to products also substituted on the aromatic rings.

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5

Regulation of tyrosine hydroxylase expression by CB_1 cannabinoid receptor: involvement of multiple signalling pathways

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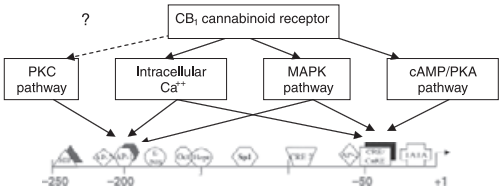
Regulation of central catecholamine transmission system by cannabinoid receptor has been recently reported and proposed to explain influences of natural cannabinoid derivatives such as Δ^9-THC on several central activities including modulation of motor and emotional behaviours. Regulation of activity and expression of tyrosine hydroxylase (TH), the major rate-limiting enzyme implicated in catecholamines biosynthesis, represents an effective mean to control this process. While modulations of TH activity and expression by prenatal exposure to natural cannabinoid extracts have been previously reported [1], little is known about influence of synthetic cannabinoid compounds on TH expression at a cellular level. Moreover, mechanisms implicated in this process have not been elucidated. Several signalling pathways (PKA, PKC, ERK1/2, Ca^{++}) have been described to induce TH gene expression through AP1 and CRE transcription factor binding sites. The same intracellular signalling pathways have also been identified as regulated by CB_1 cannabinoid receptor. Therefore, TH gene constitutes a potential target for the CB_1 cannabinoid receptor.



Regulation of TH expression was examined in murine N1E-115 neuroblastoma, a widely used model for the study of catecholamines synthesis expressing CB_1 cannabinoid receptors. TH promoter directed luciferase reporter gene assay was used to assess TH gene transcription; and TH protein level expression was determined by Western blot analysis. Effect of HU210, a synthetic analogue of Δ^9-THC, was evaluated respectively on a 250 and a 200 bp fragment of the proximal TH promoter. In addition, diverse kinase inhibitors were used to elucidated the different signalling pathways implicated in TH expression regulation.

For the first time, we report HU210-mediated reduction of TH protein level due to a CB_1 cannabinoid receptor dependent regulation of TH gene transcription. This effect requires regulation of transcription by the AP1 transcription factor binding site. PKA, ERK1/2 and PKC pathways seem to be involved in the regulation of TH gene transcription by acting on the AP1 and CRE consensus. Our results also suggest the ability of the CB_1 cannabinoid receptor to simultaneously regulate different signalling pathways involved in a unique response. In addition, the present data suggest that activation of CB_1 cannabinoid receptor by HU210 may operate an ''atypical'' cannabinoid signalling pathway involving PKC.

Reference

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6

Using iron oxide nanoparticles to magnetically label neurons and astrocytes

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Introduction Iron oxide nanoparticles are negative contrast agents for magnetic resonance imaging (MRI). To attempt to trace neuronal pathways in the brain by MRI, ultra small particles of iron oxide (USPIO) were coupled to wheat germ agglutinin (WGA), a lectin which is internalized by neurons and transported along axons after interaction with axon terminals [1, 2]. Monocrystalline iron oxide nanocompounds were coupled to WGA (MION-WGA) and a slow axonal transport of this contrast agent have been observed in peripheral nerves after intraneural injection [3, 4]. In this work, we compare the magnetic labeling of cultured neurons to the magnetic labeling of cultured astrocytes using WGA-coupled ultra small particles of iron oxide (USPIO), the USPIO-g-WGA, and evaluate their potential toxicity for these cells.

Methods USPIO-g-WGA were obtained by grafting the lectin WGA onto the dextran coating of USPIO. The specificity of these iron oxide nanoparticles for neurons was assessed using the MRI method, by comparing their ability to magnetically label cultured neurons and astrocytes. Primary cultures of neurons and astrocytes were respectively prepared from 18 days fetal rat brains and from 2 days newborn rat brains. For magnetic labeling experiments, cells were incubated for 30 min with increasing iron concentrations (0.5–4 mM) of USPIO-g-WGA or ungrafted USPIO. They were then washed, transferred in PCR tubes, and finally resuspended in gelatin for MR imaging. The potential toxicity of USPIO and USPIO-g-WGA for neurons and astrocytes was evaluated with a lactate dehydrogenase (LDH) release assay (LDH activity is a marker of cell damage) after incubation with various concentrations of USPIO-g-WGA or USPIO (0.5–4 mM).

Results Transversal relaxation rate (R_2) measurements were performed on T_2-weighted MR images of neuron or astrocyte samples after incubation with USPIO-g-WGA or USPIO. These measurements showed that the R_2 enhancement of neuronal samples after incubation with increasing concentrations of USPIO-g-WGA was significantly higher than the R_2 enhancement induced on astrocytes after incubation with the same contrast agent. It was also demonstrated that the incubation with uncoupled USPIO did not allow to obtain a significantly different magnetic labeling between both cell types. LDH release assay did not show any significant toxicity of USPIO or USPIO-g-WGA for neurons or astrocytes after 30 min of incubation at each concentration used.

Conclusion This work shows that USPIO-g-WGA allows for a magnetic labeling of neuron primary cultures *in vitro* and a subsequent T_2 effect detectable in MRI. Moreover, in our experimental conditions, we observed no significant toxicity associated to contrast agents as compared to control neurons or astrocytes, which were not exposed to USPIO or USPIO-g-WGA. The WGA lectin can be transported along neuronal pathways. Slow axonal transport (1–7 mm/day) was reported after intraneural injection of MION-WGA in facial or sciatic nerve (3, 4). The intracerebral behavior of the USPIO and USPIO-g-WGA will be studied by MRI and histology after stereotaxic injection in the mouse brain. Attempts of *in vivo* neuronal tract tracing will be performed with these particles.

Acknowledgements This work has been supported by the IUAP program (phase V, P5/04) of the Federal State of Belgium. The authors thank Ms Marlène Genlain for her precious help.

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7

Recursive partitioning for the prediction of cytochromes P450 2D6 and 1A2 inhibition

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Recursive Partitioning (RP) has been used to build decision trees for the prediction of the inhibition of two cytochromes: CYP2D6 and CYP1A2. The main characteristic of the work is the quality of the datasets. Data have been extracted from AUREUS-PHARMA highly structured databases which contain precise measures and experimental protocol concerning the inhibition of the two cytochromes. This abundance of information could lead to the constitution of pertinent datasets for each case studied. The building of decision trees was preceded by a successful evaluation of the chemical space covered by the datasets. Three main sets of descriptors were used: 2D, P_VSA, and 3D descriptors, all calculated by the MOE software suite. The models reached 90% of global *Accuracy* and often exceeded this percentage for other parameters such as *Sensitivity* (recall), *Specificity*, and *Precision*. CYP2D6 datasets provided 11 models with *Accuracy* over 80%, while CYP1A2 datasets counted only 4 high-accuracy models. Models based on K_i measures gave better models than those based on IC_{50} measures. Comparing P_VSA and 3D descriptors, models have similar *Accuracy* parameters but P_VSA seemed to be more efficient considering that 3D descriptors needed an energy minimization of the molecules and longer computing time.

8

Synthesis and pharmacological characterization of new nicotinic ligands

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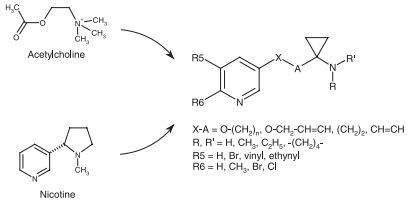
Nicotine has been shown in a variety of studies to improve aspects of cognitive performances in humans and animals. Recently, nicotinic acetylcholine receptor (nAChRs) subtypes and their role in disease and therapy have received a particular attention. However, the clinical use of nicotinic ligands as therapeutic agents is severely limited by their cardiovascular and neuromuscular side effects mainly resulting from a non selective activation of different nAChRs subtypes. To obtain novel selective nAChR ligands with marked effects on cognition but devoid of nicotine-like side effects, our own efforts have been concentrated on the synthesis of new compounds including elements from both acetylcholine and nicotine and characterized by a restricted conformational mobility provided by a cyclopropane ring.



Binding studies using rat membranes indicated that most of these molecules are specific ligands for α4β2 nAChR *vs* muscarinic receptors and α7 nAChR. Influence of the nature of the linker between the cyclopropane ring and the pyridine moiety, as well as the variation of the substitutions of the amino group and of the pyridine ring have been investigated. Preparation from an unique key cylopropane alcohol intermediate and pharmacological evaluation will be presented and discussed.

9

Allylstannation: polymer-supported reagents and synthesis of iminosugars

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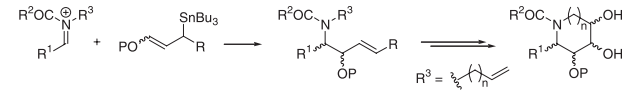
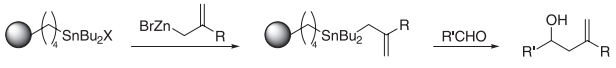
The versatility of allyltins for achievement of selective organic syntheses in mild experimental conditions has been often used with special emphasis on chemio-, diastereo- or enantio-selective reactions.

We report here the diastereoselective synthesis of iminosugars in a sequence combining an allylstannation of an *N*-alkenyl acyl iminium salt with a ring-closing metathesis and a dihydroxylation reaction [1]. This strategy was efficient for 6- or 7-membered rings and was successfully extended to fused heterocycles as exemplified by the synthesis of (±)-1-deoxy-6,8a-di-*epi*-castanospermine [2].



In spite of their remarkable possibilities, the use of organotin reagents was often limited by the difficulty to remove correctly organotin residues from the products at the end of the work-up, a point which is of crucial importance when bioactive molecules are involved. We recently reported a strategy using supported organotins which has been proved to be highly efficient to avoid this problem in a regioselective synthesis of bromo- and iodo-anilines [3]. We report here also an efficient approach using supported allyltins [4].



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10

Design and preclinical evaluation of melanoma targeting agents for internal radionuclide therapy.

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Background Targeted internal radionuclide therapy would be an effective alternative for disseminated melanoma treatment. *N*-(2-diethylaminoethyl)-4-iodobenzamide (BZA) and compounds of the series exhibit a specific affinity for melanoma tissue giving them a potent application for gamma imaging (^{123}I) or radionuclide therapy (^{131}I or ^{125}I) [1,2]. The *ortho*-iodinated analog (BZA_2) is developed as a specific melanoma imaging agent for SPECT [3]. The melanoma affinity is due to a cellular internalisation and a binding to melanin pigment [4,5].

Aim A pharmacochemical study has been done in order to find potent derivatives with a longer retention time in the tumour of melanoma B16 bearing mice and so, promising agents for the targeting of radionuclides in melanoma for therapy. New molecules synthesized were BZA analogs varying by (i) alkyldiamino chain and amino alkyl substituents, (ii) aromatic ring.

Methods Design of compounds, synthesis, labelling by ^{125}I and study of their biodistribution in B 16 F0 melanoma bearing C57BL6 mice after intravenous injection (0.1 µmol, 0.74–0.92 MBq/animal; 10 animals/compound). Mice were sacrificed, quickly frozen in liquid nitrogen at different times after administration (1, 3, 6, 24 or 72 hours) and cryosectioned into slices of 40 µm at −22 °C. The radioactivity contained in the slices was analyzed using an AMBIS 4000 detector. The radioactivity was quantified in different organs including tumour and expressed as percentage of injected dose/g of tissue (%ID/g). For comparison of tumour (T) uptake with other tissues, ratios of radioactive concentrations (T/organ) were determined illustrating the image contrast. Dosimetry parameters for a ^{131}I utilisation were extrapolated using MIRD program. Finally, the effects of ^{131}I-UMR12 on the tumour growth were evaluated on melanoma bearing mice.

Results For number of the studied molecules, a tumour fixation was observed and four compounds BZ18, UMR3, UMR7 and UMR12 exhibited an original pharmacokinetic profile: high, specific and durable tumour concentration with a rapid clearance from non-target organs. For BZ18, UMR3, UMR7 and UMR12 respectively, the tumour concentration after 72 h was 4, 3.4, 7.4 and 15.6 fold compared to BZA and in term of dosimetry, for ^{131}I labelled, the tumoural absorbed dose was 1.9, 1.4, 3.7, and 4.9 fold. Such profiles made these compounds promising for an application to internal radionuclide therapy and particularly UMR12, which was first selected.

Moreover, i.v. administration of ^{131}I-UMR12 to melanoma bearing mice showed a net decrease in tumoural growth comparatively to controls and a lengthening of median survival time.

Conclusions These data are promising for an application to internal radionuclide therapy, namely ^{131}I-UMR12. Our study of antitumoural efficacy is undergoing with different experimental protocols and on different melanoma animal models (grafted tumour and/or metastases). The cellular internalisation of UMR 12 has to be confirmed and the evaluation of ^{125}I-UMR 12, Auger electrons emitting is planned.

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11

Antibacterial and antifungal activities of phenothiazine salts derivatives

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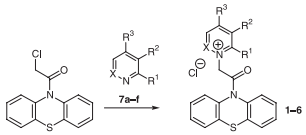
Phenothiazine derivatives have long been known to display interesting biological activities [1] as witnessed by recent articles emphasizing their antibacterial properties [2].

In our ongoing program aimed at synthesizing new phenothiazine derivatives [3] we have recently developed a convenient method for the assembly of cycloimmonium salts 1-6 by the N-alkylation of the appropriate azaheterocyclic models (pyridine, α- and γ-picoline, 3- and 4-hydroxypyridine, pyridazine) with *N*-(2-chloroacetyl)-phenothiazine.



		X	R^1	R^2	R^3
7a	1	CH	H	H	H
7b	2	CH	CH_3	H	H
7c	3	CH	H	H	CH_3
7d	4	CH	H	OH	H
7e	5	CH	H	H	OH
7f	6	N	H	H	H

Scheme 1 1-[2-Oxo-2(10*H*-phenothiazin-10-yl)ethyl]cycloimmonium chlorides (1–6) prepared.

The main objective of this study was to reveal the antimicrobial properties of these models incorporating differentially substituted azaheterocyclic units (1-6) on a variety of micro-organisms, e.g. fungi and bacteria. For this purpose four varieties of fungi have been tested: *Aspergillus niger, Penicillium frequentans, Alternaria alternata, Trichoderma* viride. Three bacterial species, namely: *Clostridium, Pseudomonas, Bacillus*, were also selected upon extraction from objets d'art (polychrome wood, paper, frescos, man-maid fibres or ceramics). The phenothiazine derivatives synthesized were tested in vitro in stationary liquid culture, Sabouroud for fungi and gelose for bacteria. From values obtained one could reasonably assume that the antifungal activity was superior to the antibacterial activity.

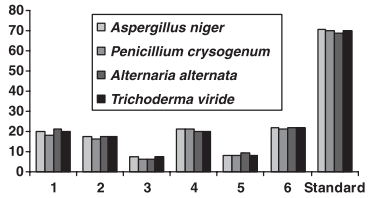


Figure 1 Inhibition of fungi growing by 1–6 at 2% for 144 h.

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12

Preparative enantioseparations of aromatase inhibitors, imidazole derivatives, by HPLC using polysaccharide chiral stationary phases

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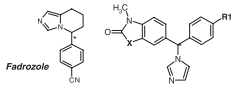
The studied N-imidazole derivatives, analogues of fadrozole, constitute new potent nonsteroidal inhibitors of aromatase (P450 arom.), useful in second line therapy of estrogen dependent breast cancer in postmenopausal women. These compounds having one chiral center, the resolution of their racemic mixtures was necessary to investigate the pharmacological properties of each enantiomer. Chiral HPLC has been recognized as a useful method to furnish milligram amounts of both enantiomers.

Analytical HPLC methods for the enantioseparation of the 6-[1(imidazol-1-yl)-1-phenylmethyl]-3-methyl-1,3-benzoxazol-2(3H)-one and 6-[1(imidazol-1-yl)-1-phenylmethyl]-3-methyl-1,3-benzothiazol-2(3H)-one derivatives



Compound	X	R1
1	S	H
2	O	H
3	S	CN
4	O	CN

were successfully developed using polysaccharide-based stationary phases (cellulose Chiralcel® and amylose Chiralpak® columns) [1–2]. The next step was the preparative resolution of the most active racemic mixtures 1–4 to test the inhibitory activity of each enantiomer.

In the analytical scale, the choice of optimal experimental conditions was achieved after screening various parameters: type of the CSP (Chiralcel® OJ, OD-H or Chiralpak® AS, AD), nature of the hexane-alcohol mobile phase (ethanol, 1-propanol or 2-propanol in the range 5–65% (v/v)) and temperature (in the range 15–45 °C).

Before the preparative resolution, the semi-preparative scale using columns with enlarged diameter and enlarged granulometry was necessary to determine the effect of increased sample load on the separation. The selected semi-preparative conditions were transposed to the preparative scale with a scale-up factor calculated to account for the geometrical parameters of the semi-preparative and preparative columns. In the preparative conditions, one injection permitted to enantioresolve between 50 and 130 mg of the racemic mixtures (the injected mass depends on the nature of the compound). After evaporation and recristallization, the yields of the preparative enantioresolution were higher than 65% for the eight resolved enantiomers [3].

Analytical enantioseparation methods using both UV and Evaporative Light-Scattering Detection (ELSD) were validated (in terms of repeatability and linearity) to determine their enantiomeric purity. The limits of detection (LD) and quantification (LQ) were determined: LD varied, for the various solutes, from 1 to 80 µg/L and from 2.05 to 10.05 mg/L with the UV detection and ELSD, respectively. Single-crystal X-ray analysis was successful to determine the absolute configuration of the individual enantiomers. A relation between the retention order and the absolute configuration of the enantiomers was established. The pharmacological tests are under run to determine the enantioselective activity.

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13

New oligopyrrole carboxamides: DNA binding activities and topoisomerase I inhibitory effects

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In the course of finding new compounds specifically interacting with DNA, combilexins represent an interesting family of molecules which are able to interfere with the regulation of gene expression and consequently play a great role in the treatment of associated pathologies such as cancer.

Based on our previous results, a new series of combilexins was synthesized coupling a DNA intercalating element (acridone or naphtalimide) to a minor groove DNA binding structure by a flexible linker or spacer of different length. This minor groove binding element could either be a methyl-pyrrole, a methyl-thiophene, a methyl-thiazole or a methyl-imidazole coupled to a methyl-pyrrole-carboxamide element.

The DNA binding ability of the miscellaneous derivatives was studied by absorbance spectroscopy of the compounds in the presence and in the absence of CT-DNA and by measuring the variation of the melting temperature of DNA in dependence on the tested compounds. Regarding these experiments we were able to classify the various combilexins into different sub-groups depending on their DNA binding potency. The different structural elements essential for an efficient DNA binding of those combilexins will be discussed.

The DNA binding mode of the combilexins (DNA minor groove binding or intercalation process between adjacent base pairs) was evidenced using either spectrometric (circular dichroism) or biochemical (topoisomerase I-induced relaxation of the DNA) studies and clearly revealed the groove binding of most compounds associated with an intercalation of the chromophore ring for some of them. The selectivity of sequence recognition was analyzed by DNase I footprinting studies in comparison to netropsin, an AT-rich minor groove DNA binder used as reference.

We showed that none of those compounds are topoisomerase I or II poisoning agents but that some of them act as suppressors of the topoisomerase I activity. Indeed, following the effect of the topoisomerase I suppressor agent F11782, some combilexins (such as the methyl-thiophene derivatives KB32, KB47 and KB52) inhibit the poisoning effect of topoisomerase I induced by camptothecin.

In conclusion, we defined the DNA binding mode of action and the profile of topoisomerase I inhibition of this new series of combilexins and established several structure/activity relationships useful for the future rational drug design of more specific and selective compounds.

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DNA alkylation and destabilisation by S23906-1: importance of the presence and position of the aromatic ring fused to the core acronycine

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Understanding molecular and cellular mechanisms of action actively contributes to the rational design of more potent compounds that still retains their peculiar functions. In this way, S23906-1 was selected from a series of ester derivatives of benzo-[*b*]-acronycine for its alkylation potency (Thi Mai et al., 2003, J. Med. Chem, 46, 3072) and anti-tumor properties on a wide variety of pre-clinical models such justifying its entry in phase I clinical trials.

On the molecular point of view, S23906-1 presents the original property to alkylate DNA through a covalent link to the exocyclic NH_2 group in the minor groove part of guanines (David-Cordonnier et al., 2002, Biochemistry, 41, 9911). During the alkylation process, this diacetylated derivative looses an acetoxyl group to form a carbocation. This reactive intermediate then covalently bonds to the NH_2 group of guanines or to the thiol group of glutathion (David-Cordonnier et al., 2004, Current Medicinal Chemistry-Anti-Cancer Agents, 4, 83). However, by contrast with ecteinascidine 743 which also alkylates DNA at the NH_2 position of guanines, S23906-1 presents the unique property to locally destabilize the double helix of DNA (David-Cordonnier et al., 2005, Mol. Cancer Ther., **4**, 71).

Therefore, we attempted to evaluate the influence of the addition at different point of fusion of an aromatic group fused to the core acronycine on the DNA alkylation potency and the local opening of the DNA helix. In this view, the diol, diacetatylated and carbonate cyclic derivatives of the benzo-[*a*], -[*b*], -[*c*] series were synthesized and compared with the acronycine series using various spectrometric and biochemical properties.

At the DNA alkylation level, gel shift experiments using a radio-labeled 117 bp DNA fragment incubated with the different compounds bearing the aromatic ring fused on the *c*-position regarding to the axis of the core acronycine are unable to alkylate DNA. DNA melting temperature studies (ΔTm) and gel shift assays using a short double stranded DNA fragment revealed DNA helix destabilization using the various di-acetylated versions of benzo-[*a*] (S71344-1), benzo-[*b*] (S23906-1) et acronycine (S17887-1) series. Compounds S23906-1 and S71344-1 present the same efficiency to destabilize the DNA helix ($\Delta Tm \sim 10$ °C) suggesting that the position of the aromatic ring has weak influence on the efficiency of the local opening of the helix. However, S17887-1 is more potent in destabilizing the DNA helix than the benzo-[*a*] or -[*b*] derivatives and decreases the melting temperature of alkylated DNA versus non-alkylated DNA of more than 20 °C.

In order to qualitatively evaluate this DNA helix destabilization process, nuclease S1 digestion of the various alkylated forms of the 117 bp radio-labeled DNA fragment were performed and revealed different sites of digestion. Indeed, the presence and position of the additional aromatic group of S23906-1 modulates the alkylation potency and the local opening of the DNA helix. Complementary studies are in due course to precisely localize the various alkylation sites obtained with this series of drug candidates.

15

Design, synthesis and pharmacological evaluation of potential telomerase inhibitors in cancer treatment

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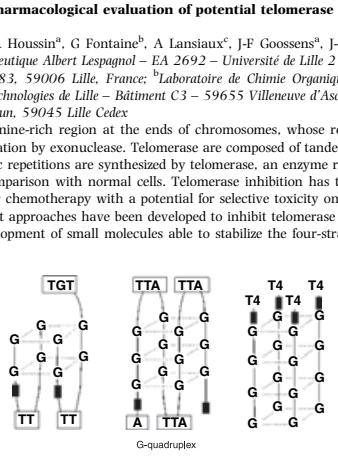
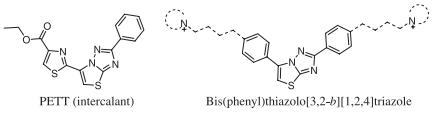
Human telomeres are guanine-rich region at the ends of chromosomes, whose role is to prevent aberrant recombination and degradation by exonuclease. Telomerase are composed of tandem repeats of the sequence [TTAGGG]. These telomeric repetitions are synthesized by telomerase, an enzyme reaching up to 85–90% in human cancer cells in comparison with normal cells. Telomerase inhibition has thus been identified as an attractive target for cancer chemotherapy with a potential for selective toxicity on cancer cells over normal ones. A number of different approaches have been developed to inhibit telomerase activity in human cancer cells. One of it is the development of small molecules able to stabilize the four-stranded DNA G-quadruplex structures.



G-quadruplex

Accordingly, we planned to develop new G-quadruplex ligands presenting an original thiazolotriazole bicycle issued from the PETT molecule. We have substituted the heterocycle by phenyl groups to reinforce the contact with orbitals of bases pairs and we have positioned positively charged substituents to interact with the phosphates of DNA.


PETT (intercalant) Bis(phenyl)thiazolo[3,2-*b*][1,2,4]triazole

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Study of secondary products during an synthesis of camptothecin analogues

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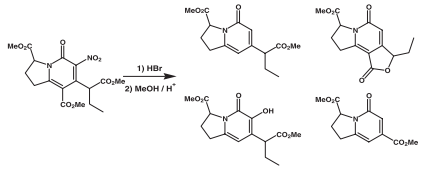
Obtaining secondary products in the chemical reactions cannot be avoided. Systematic study of these by-products has proven to be interesting from a chemical [1] point of view but also for the design of innovating structures with therapeutic aim. Within the laboratory, we develop molecules able to interfere with the cellular proliferation mainly via the inhibition of the topoisomerases I and II [2]. During a step of decarboxylation to obtain a key precursor to CTP analogues, we did not obtain the desired compound but a series of secondary products.



Purification of these products by preparative chromatography (direct and reverse phase) allowed their separation and their characterization. Elucidation of these structures was carried out by NMR (^1H, ^{13}C, DEPT, COSY and NOESY), mass spectrometry (LC/MS) and infrared spectroscopy. Even if the reaction mechanisms are still under discussion, these molecules leads to interesting new structures which will be tested on the same therapeutic targets.

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17

Design, synthesis and pharmacological evaluation of pyrrolic derivatives as new PDK-1 potent inhibitors

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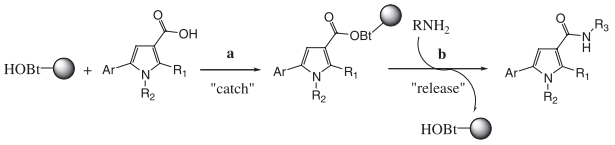
Conventional chemotherapies are frequently strongly limited by drug resistance and high toxicity. There is also a continuing effort to find new compounds that might be more effective and selective. In this context, it has been shown that the PI3K/Akt signaling pathway regulated a lot of fundamental cellular functions such as survival, growth, metabolism, differenciation. As a result, this finding made it particularly attractive to design new anticancer drugs. In prostate cancer cell lines, many alterations of the components of the PI3K/Akt enzymatic pathway lead to a permanent and inappropriate activation of the cascade. Since PDK-1 regulates the activity of a large number of AGC kinases (such Akt) in the presence of phosphatidylinositol-3,4,5-trisphosphate (PIP3) and also plays a critical role in the PI3K/Akt pathway, we hypothetize that direct inhibition of the PDK-1 enzyme may contribute to reduce proliferation and survival of prostate cancer cell.

To date, only a few PDK-1 inhibitors have been reported. OSU-2067 [1], a derivative of the COX-2 inhibitor Celecoxib, was shown to exert an efficient enzyme inhibition and to possess interesting antitumor effects. Using a ligand based approach, a small library of pyrrolic derivatives was synthesized using a solid-phase chemistry on a Quest205® synthesiser.



All the compounds were evaluated against the prostate cancer cell line PC-3. First results revealed four compounds with an interesting growth inhibitory potency (<10 μM). The inhibitory effect on the PDK-1 recombinant enzyme is still in progress.

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18

Synthesis and antimalarial activity of ferrocenic triterpenoids derivatives

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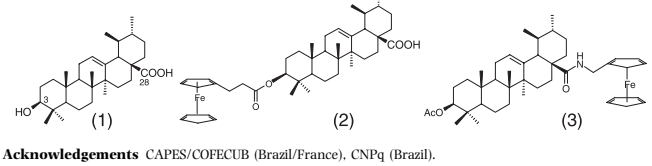
Malaria is one of the ten more prevailing and fatal infectious diseases of the world. It is a problem of public health in 90 countries, whereas approximately 40% of the world population is under risk of contamination. It causes between 1.2 and 2.7 millions deaths each year [1]. The majority of the drugs used for the therapeutics was developed starting from natural sources, like quinine, artemisinin, and their derivatives. Furthermore, there is a scaling problem of resistance to commonly used chemotherapeutic agents. In the course of our search for new antimalarials compounds we have primilary extracted the aglycons from *Ilex paraguariensis*. One of them, ursolic acid (1) was found to exhibit *in vitro* inhibitory activity on strain *Plasmodium falciparum* ghana (IC_{50} = 7 μM). Considering that ferroquine, a 4-aminoquinoline antimalarial drug that present a ferrocenic group in its side chain, exhibits notable activity against both chloroquine sensitive and resistant strains of *P. falciparum* [2], we have lead the introduction of a ferric moiety in order to evaluate its effect in our molecule. The synthesis of two triterpenoids with a ferrocenic linker at C_3 (2) and C_{28} (3) has been undertaken. The pharmacologic assay of these potential antimalarial compounds was accomplished by Pr. Grellier group from *Museum National d'Histoire Naturelle*, Paris.



Acknowledgements CAPES/COFECUB (Brazil/France), CNPq (Brazil).

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19

Enantiomeric resolution of antitumoral homocamptothecin compounds, topoisomerase I inhibitors, by capillary electrophoresis, using fluorescence detection

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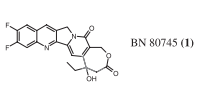
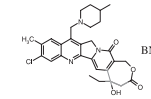
Camptothecin derivatives represent a well established class of anticancer agents which exert their cytotoxic action *via* an inhibition of the nuclear enzyme topoisomerase I. To enhance stability in plasma, a second generation of CPT analogues, christened homocamptothecins (hCPTs), were designed by insertion of a methylene spacer between the alcohol and the carbonyl functions of CPT. Their activity is related to the *R*-absolute configuration of the stereogenic center at position 20 on the E-ring. Among this family, two halogenated derivatives, codeveloped by BEAUFOUR-IPSEN and ROCHE, have been selected for clinical trials. The determination of enantiomeric excesses of this optically active compounds is necessary to validate the biological studies.

The work reported here is concerned with the baseline separation of the enantiomers of two pairs homocamptothecin derivatives using cyclodextrin capillary zone electrophoresis (CD-CZE) on a BECKMAN P/ACE MDQ: (i) BN80745 (1)



BN 80745 (**1**)



BN 80875 (**2**)

corresponding to the racemic mixture of BN80914 ((*S*)-1) and BN80915 ((*R*)-1), neutral compounds in the classical EC pH range, and (ii) BN80875 (2) which represents the racemic mixture of BN80927 ((*R*)-2) and BN80928 ((*S*)-2), cationic compounds at acidic pH.

A method for enantiomeric resolution of these compounds and determination of their enantiomeric purity was developed using neutral CDs (native and hydroxypropylated α, β, γ CDs) or anionic CDs (highly Sulfated α, β, γ CDs, carboxymethyl-β-CD), as chiral selectors according to ionization properties of the solutes, and capillaries dynamically coated with polyethylene oxide (PEO). Operational parameters such as the nature and the concentration of chiral selectors and organic modifiers, the temperature and the applied voltage were investigated. The highly Sulfated-β-CD from BECKMAN-COULTER (France) was found to be the most effective complexing agent, allowing good enantiomeric resolutions. UV diode array detector and fluorescence detector were compared in term of detection performances. Whereas results obtained with the UV detector are in accordance with the ICH acceptance criteria for 2, those obtained with compound 1 require to use a fluorescence detection that leads to improved limit of detection. The optimized methods were then validated using the adequate detector, in the goal to analyze the galenic forms. These validations are relative to precision and accuracy, linearity, limits of detection and quantification. These methods were compared to the chiral HPLC methods previously described [1].

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20

Identification by phage display and in vitro characterisation of heptapeptides targeting the amyloid-β peptide that will allow the detection by molecular imaging of senile plaques in Alzheimer's disease

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Purpose and introduction Alzheimer's disease (AD) is an incurable neurodegenerative disorder that is the fourth cause of mortality, after cardiac disease, cancer, and stroke, and the first cause of dementia in elderly in the developed countries. Currently, AD is only diagnosed by cognitive examinations and eventually by an autopsy revealing the presence of amyloid deposition (i.e. senile plaques, SPs) and of neurofibrilary tangles, as well as the brain atrophy. The SPs, which are essentially composed of amyloid-β peptide ($A\beta_{42}$), are intracellular lesions and the principal hallmark of AD.

An early, noninvasive and rigorous AD diagnosis would allow the development of new and more efficient therapeutic tools, and would make easier the understanding of its molecular mechanisms. Molecular imaging, and in particular MRI that offers an excellent anatomical resolution, would allow the detection of PS, whose size is often less than 200 micrometers, and consequently the AD differentiation. In the present work, we have used the technology of phage display to identify heptapeptides that, after grafting to a magnetic reporter molecule, will allow the AD diagnosis by MRI.

Methods In order to identify peptides with high affinity for $A\beta_{42}$, a disulfide constrained heptapeptide phage display library (New England Biolabs, Leusden, The Netherlands) was screened against $A\beta_{42}$ (Bachem, Suisse) immobilized by hydrophobic interactions on a polystyrene surface. After four rounds of biopanning, 72 phage clone candidates were arbitrarily isolated and their affinity for the target were evaluated by ELISA. Then, the insert structure of the 22 best clones was determined by their genome sequencing. Finally, their dissociation constant (K_d) for $A\beta_{42}$ were estimated with the aim to identify the more efficient peptides. The K_d of one of these peptides was measured after synthesis and biotinylation. This peptide was grafted to USPIO (Ultra Small Particles of Iron Oxide). A first *in vitro* characterization of this specific MRI contrast agent was done by histology on AD mouse (eighteen months old double transgenic mice expressing APP[V717I] and PS1-A246E [1]) brain sections stained with Prussian Blue (Perl's iron staining method).

Results The analysis of the selected peptide sequences show that the amino acids Leu, Pro, His, and Phe are better represented, which suggests hydrophobic interactions with the target. The K_d values of the 22 phage clones range between 2.2×10^{-10} M and 2×10^{-9} M. The synthesized and biotinylated peptide have a K_d for $A\beta_{42}$ of 4×10^{-7} M. The specific contrast agent allowed the PS staining on histological sections.

Conclusion The contrast agent seem to be specific of PS and will be investigated *in vivo* by MRI on animal models of AD.

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21

Design, synthesis and pharmacological evaluation of new inhibitors of FAAH

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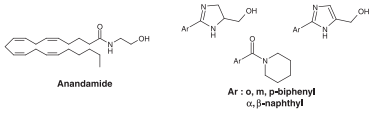
With nearly 10 000 deaths each year in France, the prostate cancer is the second cause of death per cancer after that of the lung. To obtain a significant survival of the patients, it is necessary to have molecules able to bring the cancerous prostatic cells towards a programmed death and more particularly when the tumoral cells have become gradually androgeno-independent. The endocannabinoid system represents an original therapeutic target for the development of such compounds.

The principal endocannabinoid, anandamide, seems to play an important part in the control of tumoral prostatic cell proliferation and apoptosis [1]. Anandamide is an important endogenous agonist for the cannabinoid CB1 receptor, binding to the same site as Δ^9-tetrahydrocannabinol. Recent research has shown that CB1 cannabinoid receptor is coupled to the generation of the lipid second messenger ceramide via two different pathways: sphingomyelin hydrolysis and ceramide synthesis de novo. Substained ceramide accumulation in tumor cells mediates cannabinoid induced apoptosis. This effect seems to be due to the impact of ceramide on key cell signalling systems such as the extracellular signal-regulated kinase cascade and the Akt pathway. However, the pharmacological activity of anandamide, of short duration, is regulated by an enzyme, the fatty acid amide hydrolase (FAAH).

Thus, we attempted to synthesize inhibitors of FAAH, in order to increase and prolong the anti-proliferative and/or pro-apoptotic activity of anandamide in human prostatic cancer cell lines. We based our work on molecular modeling studies highlighting a conformational analogy between the fatty chain of anandamide and suitably substituted aromatic moieties [2]. Therefore, we propose the synthesis of new biphenyl type derivatives carrying an imidazole, imidazoline or cyclohexylamide group potentially inhibiting the FAAH.



Anandamide

Ar : o, m, p-biphenyl
α,β-naphthyl

The inhibiting activities of these compounds were determined by measurement of the [^{3}H]-anandamide hydrolysis by FAAH. The first results are encouraging for the cyclohexylamide series (IC_{50} = 5 μM) and indicate that these structures can constitute hits for the design of inhibitors of FAAH.

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22

Separation of four insaturated nucleosides aromatic analogs of stavudine, two techniques on testing: high performance liquid chromatography and capillary electrophoresis

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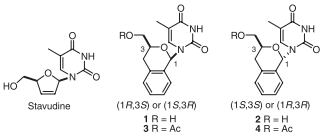
Chemotherapy for the treatment of Acquired Immuno-Deficiency Syndrome (AIDS) began 20 years ago and since then, interest in the synthesis of new therapeutic agents has continued to grow. Today, a number of 2',3'-dideoxynucleosides (ddNs) such as AZT (3'-azido-3'-deoxythymidine – Zidovudine, Retrovir®) and ddI (2',3'-dideoxyinosine – Didanosine, Videx®) or 2',3'-didehydro-2',3'-dideoxynucleosides (d4Ns) corresponding to the introduction of a double bond at the 2',3' position, such as d4G (2',3'-didehydro-2',3'-dideoxyguanosine – Carbovir), d4C (2',3'-didehydro-2',3'-dideoxycytosine), d4T (2',3'-didehydro-2',3'-dideoxythymidine – Stavudine, Zerit®) (Figure 1), possessing β-D configuration, have been approved by the US Food and Drug Administration for Human Immuno-deficiency Virus (HIV) therapy. In order to promote our study of the structure-activity relationship and to obtain compounds with a higher therapeutic index, we have developed routes to two diastereoisomeric pairs of novel analogues of d4T having an isochroman glycon moiety.



Stavudine (1*R*,3*S*) or (1*S*,3*R*) **1** R = H **3** R = Ac (1*S*,3*S*) or (1*R*,3*R*) **2** R = H **4** R = Ac

Figure 1

We now report and compare two methods of determining the enantiomeric purity of these new potential antiviral agents by direct analytical HPLC and CE. These agents are nucleoside analogues with two chiral centres. The acetylated derivatives 3 and 4 were synthesised as a pair of enantiomers (50/50) by a classical pathway previously described [1] and the free nucleosides 1 and 2 were obtained by the removal of the acetyl group of 3 and 4. Enzymatic transesterification of nucleoside analogues 1–4 (incubated with lipase) gave stereoselected pairs of enantiomers (90/10). The enantioseparation was developed using amylose tris-3,5-dimethylphenylcarbamate (Chiralpak AD), and (tris-(*S*)-1-phenylethylcarbamate (Chiralpak AS), cellulose tris-3,5-dimethylphenylcarbamate (Chiralcel OD-H), or tris-methylbenzoate (Chiralcel OJ) and cyclodextrin (Cyclobond I 2000 and Cyclobond I 2000 RSP) chiral stationary phases for HPLC and anionic α, β and γ-cyclodextrins (highly S-CD) as chiral selectors in CE. All the chromatographic and electrophoretic parameters were optimized and validated (linearity, repeatability, LOD and LOQ). The HPLC method was found to be superior in sensitivity to the CE method.

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23

A new expeditious synthesis of variously n-functionalized isoindolinones. application to the construction of biologically active compounds

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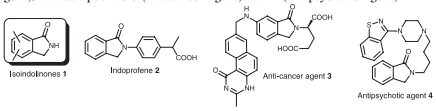
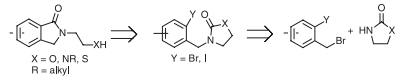
Isoindolinones 1 are a class of fused heterobicyclic systems incorporating a five-membered lactam unit and represent the basic skeleton of a wide variety of synthetic and naturally occurring bioactive molecules. These include indoprofene 2 (anti-inflammatory agent), and compounds 3 (anticancer agent) and 4 (antipsychotic agent).



Isoindolinones **1** Indoprofene **2** Anti-cancer agent **3** Antipsychotic agent **4**

Due to the increasing medicinal interest in this family of compounds several syntheses of these bicyclic lactams have appeared but their applicability is generally unsatisfactory because mainly of restrictions in the choice of substituents namely in their nature, their number and their position on the aromatic nucleus. We have then developed a new synthetic approach to these compounds that is based on the Parham annulation technique, i.e. creation of an aryllithiated species by metal halogen interconversion and subsequent trapping with an internal electrophile. The hallmark of this conceptually new approach was the incorporation of the internal electrophile, i.e. a (thio)carbamate or an urea, in a five-membered heterocyclic system which then provided the potential for a direct access to an isoindolinone with the concomitant formation of a functionalized *N*-alkyl chain.

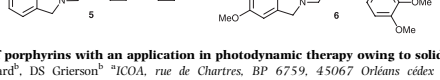


X = O, NR, S
R = alkyl

Y = Br, I

The synthetic potentiality of this new synthetic route was emphasized by the assembly of the architecturally sophisticated models 5 and 6 endowed with promising pharmaceutical properties:

- The 2-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-2,3-dihydro-1*H*-isoindol-1-one 5 displays a good affinity for 5-HT_{1A} receptors;
- The 2-(3-{[1-(3,4-dimethoxyphenyl)ethyl]methylamino}propyl)-5,6-dimethoxy-2,3-dihydro-1*H*-isoindol-1-one 6 has a negative chronotrope effect on human beings.



5 **6**

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Deymetrization of porphyrins with an application in photodynamic therapy owing to solid phase chemistry

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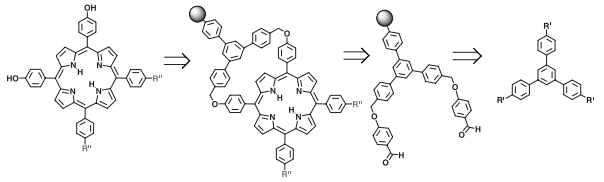
PDT (PhotoDynamic Therapy) is a relatively new therapeutic approach for the treatment of localized malignant tumours, directly accessible to light or by endoscopy way (retinoblastoma, skin cancer…) Treatment is based upon non-toxic photoactivated molecules, ''photosensitizers'', which are preferentially retained in tumour cells and light at an appropriate wave length, allowing to target tumour cells [1,2].

At the Institut Curie, research works is focalized on dissymmetrical porphyrins, substituted by carbohydrates [3], which seem to be recognised by tumour cells. A structure/activity study showed a planar structure and an amphiphilic character are important factors for good photodynamic activity. Moreover, porphyrins substituted by two or three sugars are the most phototoxic known molecules. The objective of this thesis is the optimization of the *meso cis*-disubstituted glycoconjugated porphyrins using to solid phase chemistry. The classical solution phase synthesis of these compounds leads to a statistical mixture of molecules which are difficult to separate and purify. Solid phase chemistry is an effective method allowing to avoid purification and to ''dessymetrize'' a symmetrical macrocycle by a selective mono or diprotection. In this way, we can produce *meso cis*-disubstitued porphyrins from a resin allowing the diprotection of the macrocycle. The attachment of a linker, like a triarylbenzene precursor of porphyrin, on the resin followed by its transformation into a porphyrin and its cleavage can lead to these target compounds.

We have prepared different linkers and used them to carry out reactions in solution phase leading to a symmetrical porphyrin. We have also grafted a dissymmetrical linker onto a resin and a dissymmetrical porphyrin has been synthesized. The principal difficulty is the selective synthesis of the tripyranne, intermediate of the porphyrin.



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25

Effects on anti-gad antibodies on extra-cellular concentrations of glumatate in cerebellar nuclei of rats

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Background In human, anti-gad antibodies (GAD-Ab) are associated with stiff-person syndrome and cerebellar ataxia. In vitro experiments using GAD-Ab from patients with neurological syndromes have shown a suppression of gamma-amino-butyric acid release.

Aims To demonstrate a possible pathogenic role of GAD-Ab in neurological diseases using an in vivo model.

Methods Sera were collected from five patients with GAD-Ab and neurological syndromes. None of them had diabetes mellitus. All experiments were performed with a purified IgG fraction. The animals underwent cerebellar surgery on the left side. We injected GAD-Ab locally in cerebellar nuclei of rats (coordinates were AP: −11.6, L: +2.3, V: −4.8) and we assessed the synaptic regulation of glutamate using microdialysis. Probes (CMA, Sweden) were inserted in lateral nuclei (group I: controls $n = 6$ rats; group II: injection of Gad-Ab $n = 5$ rats). Flow rate was 2 μL/min. We applied NMDA (10 mM) in loco.

Results In group I, local infusion of NMDA was associated with a marked reduction of glutamate (mean +/− SEM: 25.29 +/− 9.97% of baseline at 20 min, 13.71 +/− 5.9% at 40 min). In group II, the reduction of glutamate contents following NMDA infusion was absent at 20 min and was mild at 40 min (respectively 100.67 +/− 14.26% and 70.63 +/− 18.57%, respectively; Group by time interaction: $P < 0.001$).

Conclusion This is the first in vivo demonstration that GAD-Ab extracted from neurological patients impair synaptic regulation of glutamate in cerebellar nuclei of rats.

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Binding mode of new (thio)hydantoin inhibitors of fatty acid amide hydrolase (FAAH)

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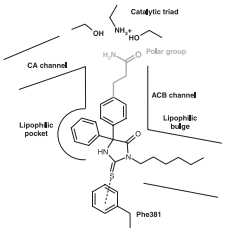
Fatty acid amide hydrolase (FAAH) is a membrane enzyme, responsible for the hydrolysis of signaling lipids, including endocannabinoids like anandamide and 2-arachidonoylglycerol [1]. FAAH rat isoform was crystallized and is the only characterized mammalian member of the amidase signature superfamily of serine hydrolase bearing a unique catalytic mechanism (Ser-Ser-Lys) [2]. Anandamide exhibits a range of biological properties having clinical implications in the treatment of sleep disorders, anxiety, epilepsy, cancer and neurodegenerative disorders. Inhibition of FAAH is therefore an interesting and very promising therapeutic target [3].

Recently, (thio)hydantoins (thioxoimidazolidinones and imidazolidinediones) were identified as potential FAAH inhibitors [4]



In order to understand the SAR of this series, characterize their binding mode in the FAAH and propose potentially more active analogues, structural studies were performed on five selected compounds. Their alkyl chain lies in the Acyl Chain Binding channel (ACB) and one of the phenyl rings points toward the catalytic triad. The other phenyl group fills a lipophilic pocket often unoccupied by the reference inhibitors. Polar groups para-substituted to the phenyl would enhance their inhibitory potency by gaining interactions with the hydrophilic catalytic triad.

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27

Design and synthesis of 4,6-dimethoxy-1,3,5-triazines as potential anticancer derivatives

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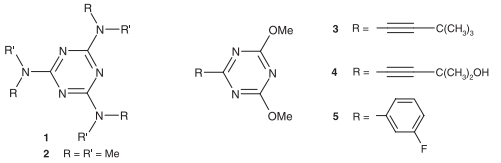
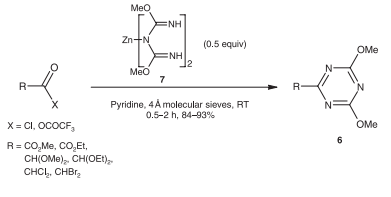
A series of 2,4,6-tris(N,N-dialkylamino)-1,3,5-triazines 1 has been studied for their antitumoral activity [1–3]. Among these derivatives, the hexamethylmelamine 2, an alkylating agent, is very efficient against ovarian, breast, and lung cancers but it generates side effects limiting its use in clinic. Thereafter, similar compounds such as 2-alkyl-4,6-diheteroalkyl-1,3,5-triazines [3–5] demonstrated a significant cytotoxicity towards various tumor cell lines in vitro, but their mechanism of action is still unknown.



The cytotoxic properties of these triazines led us to develop an easy and efficient method of synthesis of 4,6-dimethoxy-1,3,5-triazines 6 by using an activated form of a carboxylic acid with a stoichiometric amount of zinc dimethyl imidodicarbonimidate 7 according to the following scheme: 5



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28

Design, synthesis and antimalarial activity of some new amodiaquine and amopyroquine analogues

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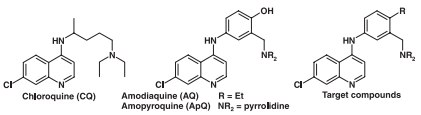
The use of chloroquine (CQ), the most prescribed antimalarial drug for more then 60 years, decrease in importance because of the development of CQ-resistant strains of *Plasmodium falciparum*. CQ and others 4-aminoquinolein drugs mode of action is based on their accumulation in the food vacuole and inhibition of hem degradation. Clinical use of amodiaquine (AQ), active against CQ-resistant strains, has been restricted for prophylactic use because of observed side effects (as hepatotoxicity and agranulocytosis) reported in some cases. The observed drug toxicity is believed to be related to the formation of a quinine-imine metabolite [1].

Although considered responsible for the AQ toxicity, the presence of the 4'-hydroxyl group has been considered necessary for the antimalarial activity. However evaluation of the 4'-dehydroamodiaquine derivative (4'-deOHAQ) pointed out a similar activity, though a little inferior to that of AQ[2]. In order to explain the structure-activity relationships, two hypotheses were considered: 4'-deOHAQ posses its own antimalarial activity or it is oxidized *in vivo* to AQ which would be responsible for the measured activity.

In order to block the 4' position and avoid the possible oxidation *in vivo*, to optimise the activity against CQ-resistant strains, but also to explain the mechanism responsible for the 4'-deOHAQ antimalarial activity, a series of 4'-substituted new derivatives of 4'-deOHAQ have been designed. The products were synthesized using aryl-aryl and aryl-alkyl Suzuki coupling reactions. We were also interested in determining the effects of the change of the side chain diethylamino function with a pyrrolidine cycle. This allowed us to develop a parallel 4'-substituted series of amopyroquine (ApQ). Nanomolar activities have been measured against all the tested CQ-resistant strains. Low cytotoxicity levels were determined on MRC-5 cell lines.



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29

DNA binding and topoisomerase i inhibition by a new series of aza derivatives of rebeccamycine

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Indolocarbazoles represent an important family of agents, some of them bearing highly potent anti-tumor activities. They are classified in two sub-families depending on the bilateral (homologues of staurosporine, a none-selective kinase inhibitor) or monolateral (deriving from rebeccamycin, a DNA binding compound acting as a topoisomerase I poisoning agent) link of the sugar ring to the nitrogen group of the indole ring. Numerous indolocarbazole derivatives were synthesized to select specific topoisomerase I poisons such as NB-506 or J-107088 and NSC 655649 tested in various clinical trials.

More recently, a new series of derivatives bearing a nitrogen on ring A were synthesized and further characterized for their profile of cytotoxicity different from that observed using other rebeccamycin derivatives (Messaoudi et al., 2005, Eur J Med Chem, **40**, 961).

Here, we evaluated the DNA interaction properties of the various derivatives in term of their relative affinity to nucleic acids and their mode of interaction with the DNA helix. Using various spectral approaches (UV/visible absorption, melting temperature studies, circular dichroism) and biochemical experiments (topoisomerase induced relaxation of plasmid DNA, DNase I footprinting), we identified one compound as a very strong DNA binder, several compounds as weaker DNA binding agents whereas the later sub-group failed to interact with nucleic acids. All of the compounds designed as DNA binders intercalate between adjacent base pairs of the helix.

The propensity of the various derivatives to block the topoisomerase I/DNA cleavable complex was then evaluated using either biochemical and cellular approaches that identified some compounds as potent topoisomerase I poisons. The topoisomerase I-induced cleavage of the DNA occurs at nucleotide sites different from those usually observed using camptothecin, the reference drug in topoisomerase I studies structurally different from indolocarbazoles, or using NB-506, as the reference indolocarbazole inhibitor of topoisomerase I. Using cellular models, the topoisomerase I poisoning derivatives present a greater cytotoxicity on cells with potent topoisomerase I activity (CEM) than on cells mutated on topoisomerase I (CEM-C2).

In conclusion, we selected a strong DNA binding compound but lacking topoisomerase I poisoning effect and several other aza-indolocarbazole derivatives with potent topoisomerase I poisoning effect acting at new cleavage sites, different from that of the reference drugs camptothecin and NB-506.

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Synthesis and structural study of peptides with an anti-thrombosis activity profile

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Fibrillar type I and type III collagens are among the most thrombogenic components of the subendothelial layer [1]. The KBGEBGPK octapeptide sequence is located in the CB4 fragment of the type III collagen α1-chain (residues 655–662 of the α1-chain precursor). This octapeptide sequence inhibits human platelet aggregation induced by type III collagen in both static and flow conditions. This inhibition is specific to type III collagen and does not affect platelet interactions with type I collagen [2]. This sequence would act like an antagonist of the protein *Type III Collagen Binding Protein* (TIIICBP) [3].

Aiming at a better understanding of the role of the tertiary structure of the KBGEBGPK octapeptide in the recognition by TIIICBP receptor, firstly, we synthesized the octapeptide of reference. Secondly, we incorporated this sequence in a more structured (simple trimer) peptide which do not have a triple helix structure (demonstrated by studies of CD and polarimetry). Finally, we incorporated this same octapeptidic sequence in the peptides that have an alpha triple-helix structure. Structuration which is found in the collagen (confirmed by CD and polarimetry).

The peptides were synthesized on an Applied Biosystems Model 433A peptide synthesizer, using standard automated continuous-flow solid-phase peptide synthesis methods. The structural study of those compounds has shown that KBGEBGPK reference octapeptide has a β-turn structure, stabilized by β-sheet. The biological essays have shown that the octapeptide sequence has a strong antithrombotic activity *in vitro* and *in vivo*. Moreover, the incorporation of this sequence in the simple trimer involves a significant increase in the antithrombotic activity. This can be explained by the structural studies which have shown that the simple trimer is particularly more strongly structured than the monomer of octapeptide KBGEBGPK.

However, when this octapeptide sequence is incorporated in a peptide mimant the triple-helix of the collagen of the type polyproline II, the antithrombotic activity is suppressed in favor of a prothrombotic activity. Thus, this octapeptidic sequence (structured β-turn) seems to act like an antagonist of TIIICBP, by preventing the recognition by the latter of this same sequence when it is built-in in the collagen of the type III. On the other hand, when the octapeptide of reference is built-in in a structure of the type polyproline II, it is recognized by TIIICBP like an analogue of the collagen of the type III. This phenomenon of recognition can be at the origin of the phenomenon of thrombosis observed.

Acknowledgements CNPq (Brazil).

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Rational exploration of structural databases : the case of H_3 Ligands

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The histamine H_3 receptor has been portended as a drug target in the central nervous system (CNS) in different pathological disorders such as dementia, cognition impairments, obesity and ADHD.

In connection with our drug discovery program using rational exploration of structural databases, we wish to report in this poster the emergence of a new antagonist H_3 series. We will briefly describe first the strategy for database mining, then the application to our in-house database leading to the identification of a hit. Finally, we will highlight the different pharmacomodulations giving rise to the lead candidate (Figure 1).

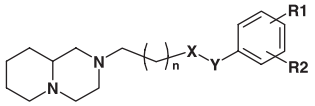

Figure 1 Synthesis, biological and pharmacokinetic data will be presented

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Design and synthesis of matrix metalloproteinases inhibitors inspired from patterns resulting from coumarins and iridoids isolated from two plants endemic to madagascar

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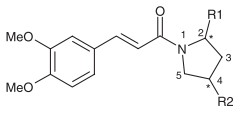
An interdisciplinary scientific approach, ethnopharmacology, deals with the empirical knowledge of populations concerning the uses of traditional medicinal remedies. This concept can be a tool devoted to an improvement of sanitary situation in countries where Western medicine is strictly inaccessible, but also in order to select plants with interesting pharmacological potential. Within the framework of our research, we were interested in the traditional medicine of Northern Madagascar, through ethnobotanical investigations led with groups of women, in association with the University of sciences of Antsiranana and association ''Jardins du Monde''. From this field research, one endemic Bignoniaceae has been selected according to several criteria: Perichlaena richardii Baill.

We conducted phytochemical and pharmacological tests to justify or not traditional uses of this plant. From the extracts, compounds have been isolated : caffeic acid, an iridoid glycoside substituted by a caffeic acid (verminoside), and a flavonol glycoside by a disaccharide (rutin). Another compound isolated is still in the identification process. From an Apiaceae, endemic to Madagascar, Phellolophium madagascariense Baker, three coumarins were also extracted, one identified as osthol [1]. These various molecules have been tested for these potential inhibition of cellular proliferation and activity of enzymes involved in cancer such as COX, LOX, tyrosine-kinase and matrix metalloproteinases (MMP-9), the last ones are more particularly involved in the tumoral progress.

From these natural models, in our search for anti-cancer agents, we then considered the design, synthesis and pharmacological study of pseudopeptidic compounds inspired from caffeoyl prolines showing an inhibiting activity with respect to matrix metalloproteinases. We kept caffeic acid from the verminosid and inserted an isopentenoyl group from the osthol because of its interesting antiproliferative properties. Various structures have been synthetised; initially, we chose to insert the zinc-binding group (carboxylic acid) in position 2 and a long hydrocarbon chain likely to interact with the S'1 pocket of the enzyme. The works of Li Y.L. et al. [2] and our first pharmacological results obtained, made us modify our pharmacomodulation, by different substitutions in position 2 and in position 4 of the proline.



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33

Novel 4-Oxo-1,4-dihydroquinoline-3-carboxamide derivatives as new CB_2 cannabinoid receptors agonists

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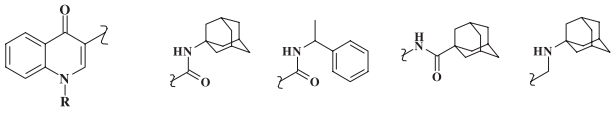
Since the Δ^8-tetrahydrocannabinol (Δ^9-THC) characterization in the sixties, as the major psychoactive component of *Cannabis Sativa L.* [1], many improvements have been made in cannabinoid pharmacology knowledge, particularly, in the nineties with the discovery of two G-protein coupled receptors: the CB_1 receptor [2], which is mainly located in the central nervous system (CNS), and the CB_2 receptor which is principally present in the immune system [3].

Thanks to the development of a great variety of new synthetic ligands, the study of these receptors revealed a large therapeutical potency. Actually Δ^9-THC is currently marketed to reduce emesis and/or prevent cachexia in AIDS or cancer patients. But recent data indicate that stimulation of both cannabinoid receptors participate in the control of cellular proliferation [4,5] CB_1 receptors stimulation could induce undesirable effects due to its CNS localization, so we proposed to study the anticancer potency of novel CB_2-selective agonists, and more particularly in prostate cancer.

In this way, a set of 4-oxo-1,4-dihydroquinoline derivatives, characterized by the presence of some important structural requirements exhibited by other cannabinoid ligands such as WIN 55,212-2, SR 144528 and JTE-907, was synthesized [6].



Aliphatic or aromatic substituent in position 3 have been selected on the basis of other cannabinoid pharmacophores such as those present both in the quinolone derivative JTE-907 and in the biarylpyrazole SR-144528 and a hydrophobic substituent in position 1 as in the aminoalkylindole derivatives. Introduction of chiral substituents in position 3 shown that these kind of ligands bind stereoselectively to the *h*CB_2 receptor. These compounds were obtained by enantioselective-synthesis or by preparative chiral HPLC.

These quinoline derivatives were synthesized and assayed to measure their respective affinity for both human CB1 and CB2 cannabinoid receptors. The results indicate that these quinoline derivatives exhibited a strong CB2 receptor selectivity. Moreover, in the [^{35}S]-GTPγS binding assay, all the compounds behaved as CB2 receptor agonists. Molecular modeling studies showed that 4-oxo-1,4-dihydro-quinoline derivatives interacts with the CB2 receptor through a combination of hydrogen bond and aromatic/hydrophobic interactions. In conclusion, 4-oxo-1,4-dihydroquinoline derivatives constitute a new class of potent and selective CB2 cannabinoid receptors agonists.

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Design, synthesis and biological evaluation of indanesulfonamides as carbonic anhydrase IX inhibitors

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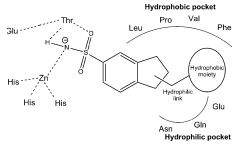
Tumor cells exhibit an extracellular pH (pH_e) more acidic than normal cells. This acidification contributes to the acquisition of metastatic phenotypes [1] and is probably related to a strong activity of carbonic anhydrase IX (CA IX). This enzyme is overexpressed in tumoral tissues but is not present in their normal counterparts [2]. A drug which can selectively inhibit CA IX could on one hand, decrease the tumoral progression and on the other hand, enhance the uptake of some chemotherapeutics by modulating the pH_e.

In a previous study, derivatives of 2-amino-indanesulfonamide were synthetised and showed a good inhibition against the human carbonic anhydrase I and II [3]. On the basis of these preliminary results, we designed other original indanesulfonamides based on the ''tail approach'' [4]. A general pharmacophore has been drawn from the analysis of CAs active site and from the structure of inhibitors described in the literature. Different hydrophobic side chains, which target the hydrophobic pocket, were incorporated in the indanesulfonamide scaffold with an amide linker to interact with the hydrophilic part of the active site.



The inhibitory activities of indanesulfonamides were evaluated against the hCA IX and against two other biologically relevant isozymes (hCA I and II). In order to establish preliminary hypothesis for the design of new selective CA IX inhibitors, we conducted molecular modeling studies. We describe here the first hCA IX model built by homology with another CA isozyme previously crystallized. Docking studies were performed to explore the binding mode of our indanesulfonamide derivatives [5].

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Synthesis and evaluation of tetrahydroquinoline-hydantoine derivatives as σ1 receptor selective ligands

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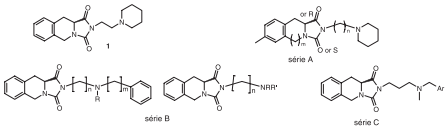
σ_1 receptors are known to be involved in the regulation of numerous central neurotransmitters. Therefore, the synthesis of specific σ_1 receptors ligands appears to be interesting in the treatment of many neurologic and motor diseases.

Di- and trisubstituted hydantoins have been widely used in biological screenings resulting in numerous pharmaceutical application. Our strategy was to design a new series of more constrained derivatives. Indeed, the tetrahydroisoquinoleine ring was selected as a pharmacophoric moiety frequently found in our screenings of combinatorial libraries and a convenient and efficient method to prepare Tic-hydantoins was described [1].

When evaluated in a profiling screening, compound 1 revealed to be a ligand of guinea pig σ_1 receptor with IC_{50} of 16 nM. Binding of this compound at the receptor σ_1 could be explained by the association of an aromatic moiety and a nitrogen atom previously proved to be a pharmacophoric element in a series of phenylalkylpiperidines and phenylalkylpiperazines [2].

The aim of our work was to study on the one hand, the impact of Tic-hydantoin moiety (series A), on the other hand the nature and the length of the chain on both sides of the nitrogen atom (series B). These studies showed powerful selective ligands of σ_1 with IC_{50} in the order of nM [3]. These compounds show a low cytotoxicity. Indeed most of tested compounds have a CC_{50} superior to 10 μM. To complete the structure-activity relationship of this family of compounds, we are studying the role of the phenyl core in the affinity and specificity of σ_1 receptors (series C) by introducing various hydrophobic groups (aromatic or not). Therefore, a new strategy of convergent synthesis was elaborated.



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Chiral capillary electrophoresis: resolution of baclofen enantiomers and aromatic aminoacids analogs, with highly sulfated cyclodextrins

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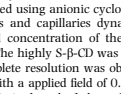
The neutral aminoacid, γ-aminobutyric acid (GABA) is an inhibitory neurotransmitter that intervenes in the control of neuronal activity in the mammalian central nervous system and in the regulation of many physiological mechanisms. Baclofen, 4-amino-3-(4-chlorophenyl) butyric acid, is one of the selective agonist for the $GABA_B$ receptor and is still marketed as Baclofene-Irex® and Lioresal® for the treatment of spasticity.



Baclofen

Baclofen has a chiral center and its enantiomers were found to have quite different pharmacodynamics properties: (*R*)-(−) enantiomer being 100 times more active than (*S*)-(+) enantiomer. Chiral capillary electrophoresis (CE) is an alternative method of choice for rapidity and efficiency [2] to chiral HPLC [1] to determine enantiomeric purity.

Using cyclodextrin capillary zone electrophoresis (CD-CZE), on a BECKMAN P/ACE MDQ coupled with UV-on line detection, baseline separation of baclofen was shortly achieved. A method for the enantioresolution of this compound and determination of enantiomeric purity was developed using anionic cyclodextrins (highly sulfated-CD or highly S-CD from BECKMAN COULTER, France) as chiral selectors and capillaries dynamically coated with polyethylene oxide (PEO). Operational parameters such as the nature and concentration of the chiral selectors, buffer concentration, organic modifiers and applied voltage were investigated. The highly S-β-CD was found to be the most effective complexing agent, allowing good enantiomeric resolution. The complete resolution was obtained using 25 mM phosphate buffer at pH 2.5 containing 3% (w/v) of highly S-β-CD at 25 °C with a applied field of 0.40 kV/cm.The apparent association constants of the inclusion complexes were calculated. This optimised method showed a strong resolving power (R_s = 3.40) in a short run time (8.5 min) and was validated in terms of repeatability and limits of detection (0.13 $\mu g.mL^{-1}$) and quantification. The migration order was determined [3]. The use of highly S-CDs leads to far better results, in comparaison of neutral CDs [2], in term of time, resolution and limit of detection. This optimization of the chiral resolution of baclofen makes this method suitable for routine analysis of its enantiomers in raw material and/or in formulations.

This method was also extended to aromatic aminoacids analogs of baclofen to probe the structure and function relatioship of host-guest interaction with regard to their affinity and chiral resolution [4].

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Synthesis and evaluation of novel bisbenzamidines with a possible efficacy for the treatment of malaria

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Recently we demonstrated [1] that 4,4'-(piperazine-1,4-diyl)bisbenzamidine (1) constitutes a possible antimalarial drug characterized by a *in vitro* antiplasmodial activity in the nanomolar range. That compound could exert its effects [2] by complexing haem in the food vacuole of the parasite, thus acting in the same way as chloroquine [3].

In order to better understand that mode of action, and more particularly to assess the influence of the rigidity of the piperazine ring, we now describe the synthesis and the evaluation of novel anologs of 1. In those analogs the heterocyclic system has been replaced by an ethylene-1,2-diamine moiety, eventually substituted on one or both nitrogen atoms.

1

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From design to in vivo pharmacological evaluation of farnesyltransferase inhibitors in cancer

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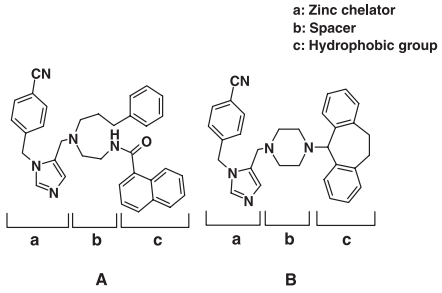
Nowadays, it is necessary for treating cancer, to discover new compounds with great activity and selectivity for deregulated cells. The strategy explored here resides in the inactivation of Ras, a frequently mutated oncoprotein (approximately 30% of cancers) implicated in the regulation of cell growth. The activation of Ras oncoproteins requires a post-translational modification allowing its anchorage by an isoprenoic moiety into the plasma membrane. This discovery has led to the development of specific inhibitors of the zinc-metalloenzyme farnesyltransferase (FTase) responsible for such post-translational modifications.

The FTase recognizes specifically the C-terminal sequence of farnesylated proteins, called the «CA_1A_2X box» (C = cysteine, X = one amino acid which induces selectivity of the prenylation enzymes, farnesyltransferase and geranylgeranyltransferase-I, and A_1A_2 = a dipeptide linker). One of the strategies to inhibit this enzyme consists in the design of peptidomimetics of the C-terminal sequence. On the basis of this model, we designed a focused library [1,2] to complete the structure-activity relationships.

From the pharmacophoric model established by our team, compounds A and B were selected. Beside the enzymatic assay (FTase and GGTase-I), the activity of these new compounds was screened on a panel of six cell lines. The lack of alternative treatment in colon cancer and the attractiveness of the concept of blockade of the Ras function by FTase inhibition led us to investigate on in vivo models (xenograft mice) these two FTIs on this type of cancer.



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