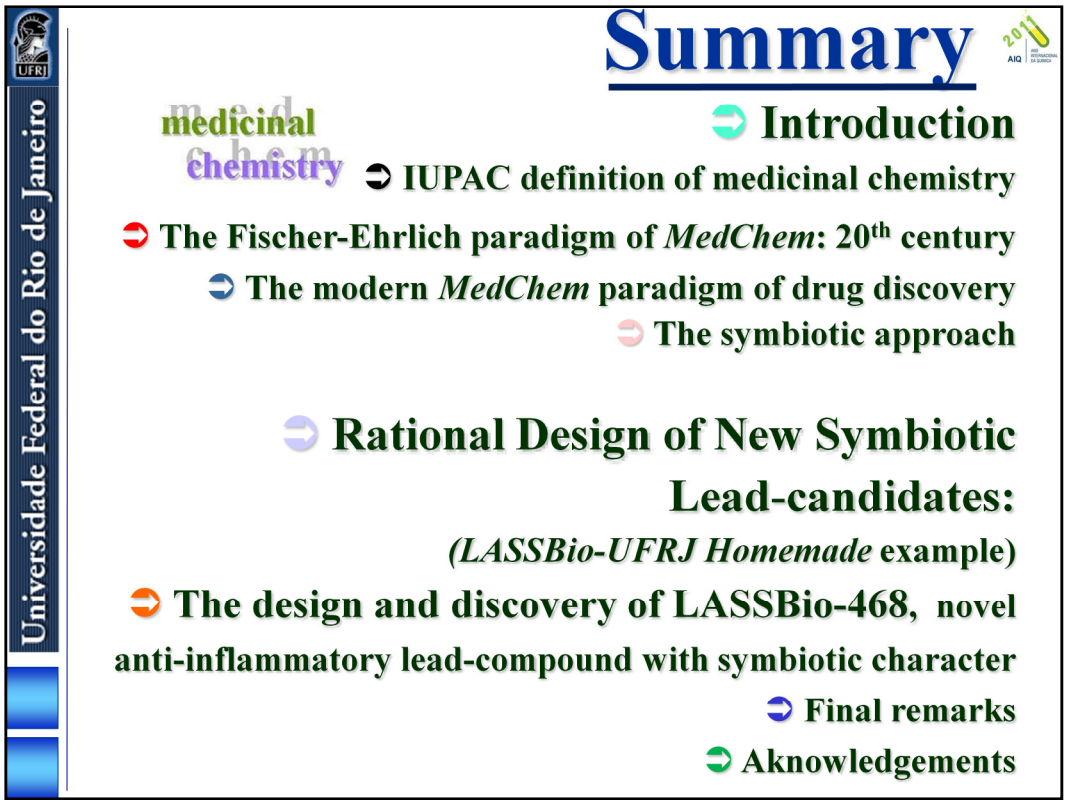
São Paulo Advanced School on Natural Products, Medicinal Chemistry and Organic Synthesis

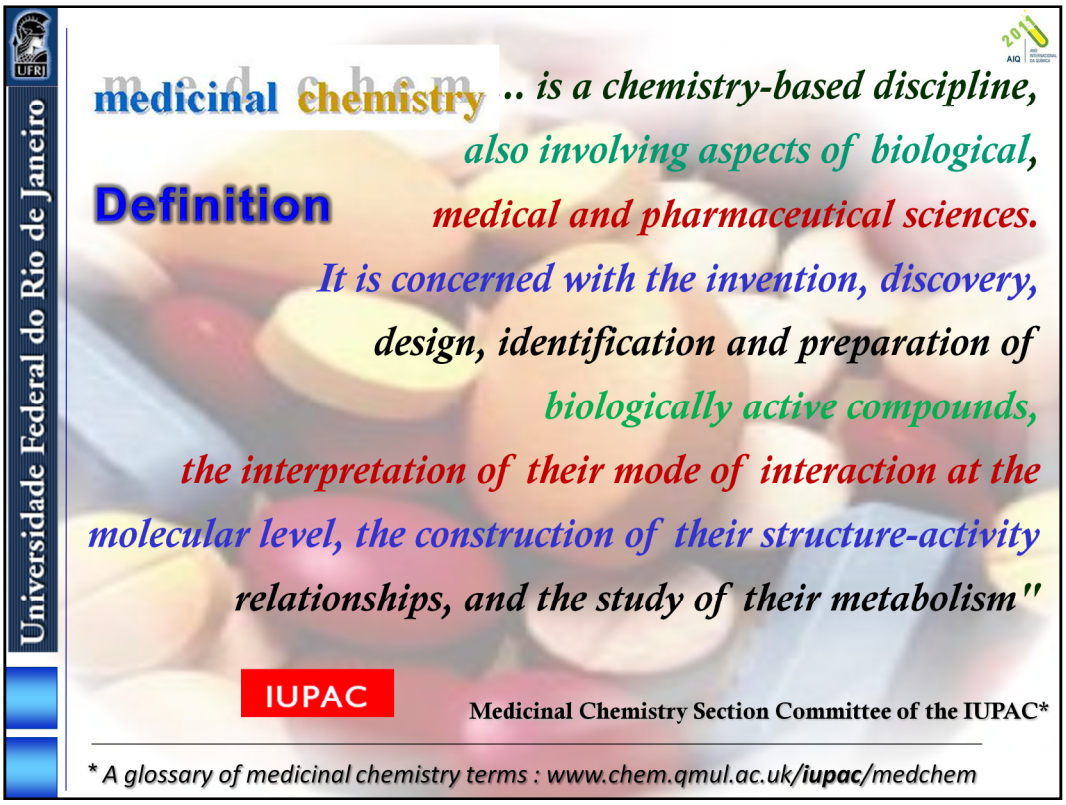
Integrated Solutions for Tomorrow's World

UNICAMP, S.P.

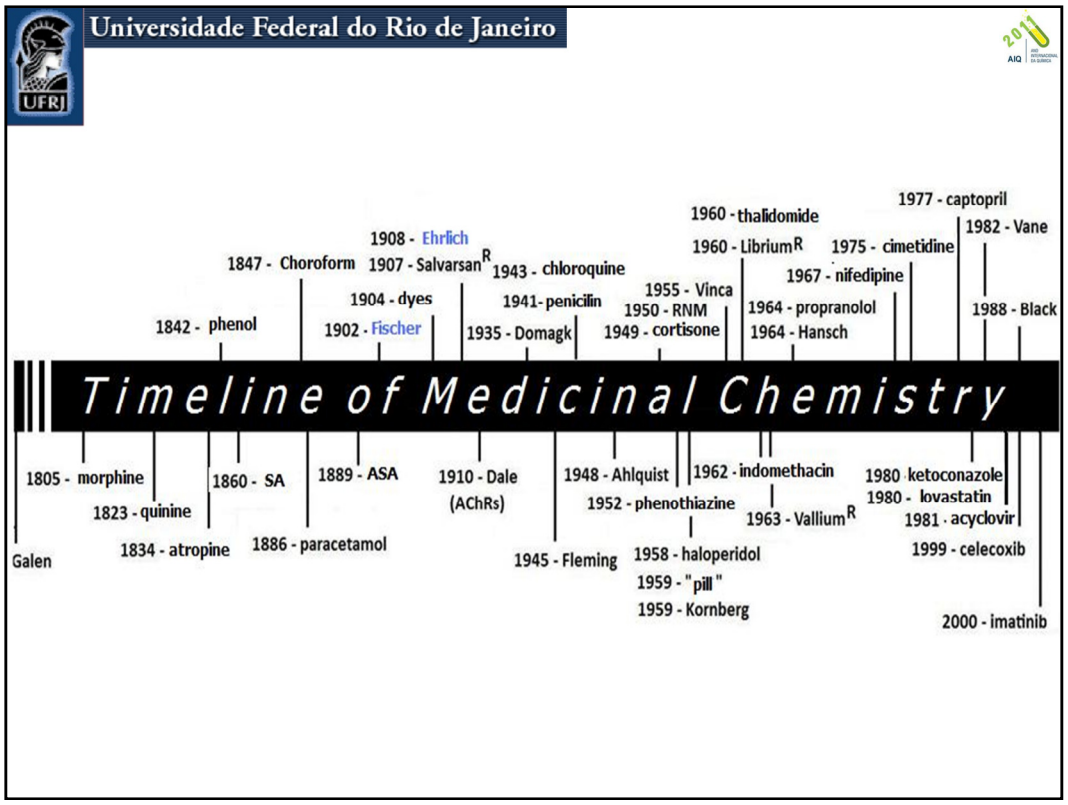
August 16, 2011



First of all, I would like to thank the organizers for inviting me to contribute to this important event . In my presentation – titled: New insights for multi-factorial disease therapy: the design of new symbiotic leads, I will take a closer look to two distinct paradigms of Medicinal Chemistry in the drug discovery process, concluding with a brief example of our research effort done at Laboratório de Avaliação e Síntese de Substâncias Bioativas (LASSBio; www.farmacia.ufrj.br/lassbio) of Universidade Federal do Rio de Janeiro (UFRJ) in the field of Medicinal Chemistry. I will divide my talk into two main parts: first, I will present the Medicinal Chemistry area and its definition from the Section Committee of MedChem of the International Union of Pure and Applied Chemistry (IUPAC). Next I will present what I call the first *MedChem* paradigm - The Fischer-Ehrlich paradigm - followed by actual one - the modern *MedChem* paradigm and I will end this part with what I call the symbiotic approach. Secondly, I will present part of our results aiming the design and discovery of a new anti-inflammatory drug candidate - LASSBio-468 - using the Symbiotic approach.



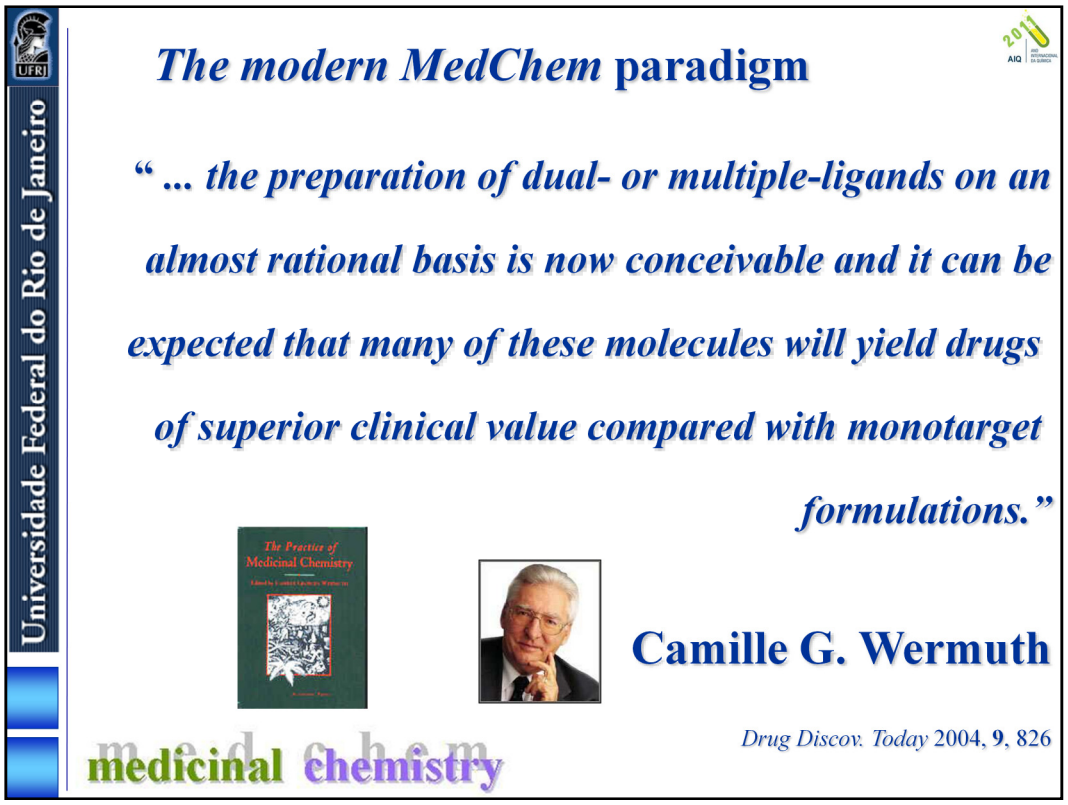
The IUPAC definition of medicinal chemistry was performed by the Section Committee, under presidency of Professor Topliss, in 1996, as follow.



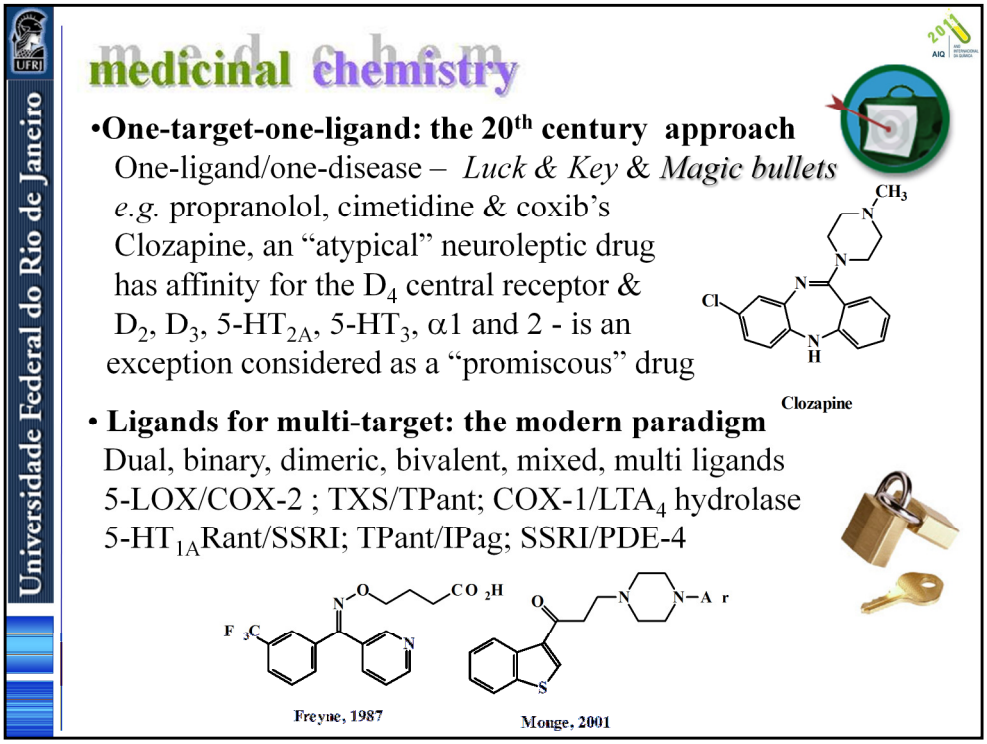
In this slide I show a possible Timeline of *MedChem* where we can see the principal scientists and achievements of this discipline, until 2000. For instance, morphine, that I consider one of the pioneer molecules, was first isolated from opium by Setürner, in 1805, and later originated the 4-phenylpiperidine hipno-analgesic class (*e.g*. meperidine or pethidine), in 1935. A second pioneer molecule is quinine, a quinoline alkaloid isolated from South America plant *Cinchona officinalis*, that furnish the framework for the first synthetic antimalarial compounds of 4-amino-quinoline class, *e.g.* chloroquine, discovery in 1947 and later mefloquine in 1970, from 4-hydroxymethyl quinoline class.

Many others drugs described until 2000, are illustrated in this figure and each of it represents an important therapeutic discovery as penicillin, propranolol, phenotiazine, indomethacin and cimetidine, for example. In this Timeline it is important to remark at the beginning of 20th century two Nobel winners: Emil Fischer and Paul Ehrlich. The first, won the Nobel Prize in Organic Chemistry, in 1902, and Ehrlich won Nobel Prize of Medicine, in 1908, both significantly influenced the thinking Medicinal Chemistry in the 20th century, as I will show in the next slide.

Emil Hermann Fischer studying carbohydrate chemistry was able to create the concept of Lock-Key in order to explain the different level of sweetness of very structurally similar compounds. For him, the small molecules (Key), represented by sugars, interact with macrobiomolecules (Lock) to give the taste of sweetness of a compound. This concept was the basis for the next molecular recognition pattern of a drug by a receptor. Ehrlich has a conviction that some particular compound could be developed to act as selective magic bullets, against a specific disease agent without harming the patient with a given disease. The resulting one-molecule, one-target philosophy has driven DD process in the pharmaceutical industry during the last century. With the increasing knowledge about receptors at molecular level and the discovery of molecular mechanism of action of drugs the DD process evolved to modern approach named structure-based drug discovery process or *lock-based DD* and ligand-based drug discovery or *key-based DD* process. Considering Ehrlich believe both approaches were based in the one-molecule, one-target philosophy. This approach, still valid nowadays, were responsible for most synthetic drugs shown in the previous slide. However, in complex or multi-factorial diseases, generally chronicles, as cancer, cardiovascular diseases, inflammatory diseases as rheumatoid arthritis, psoriasis and multiple sclerosis, CNS disorders as Alzheimer disease, and diabetes, all of them with polyetiological origin, it is now untenable the notion that they can be efficiently treated with drugs acting on single targets or supported by one-molecule, one-target philosophy.

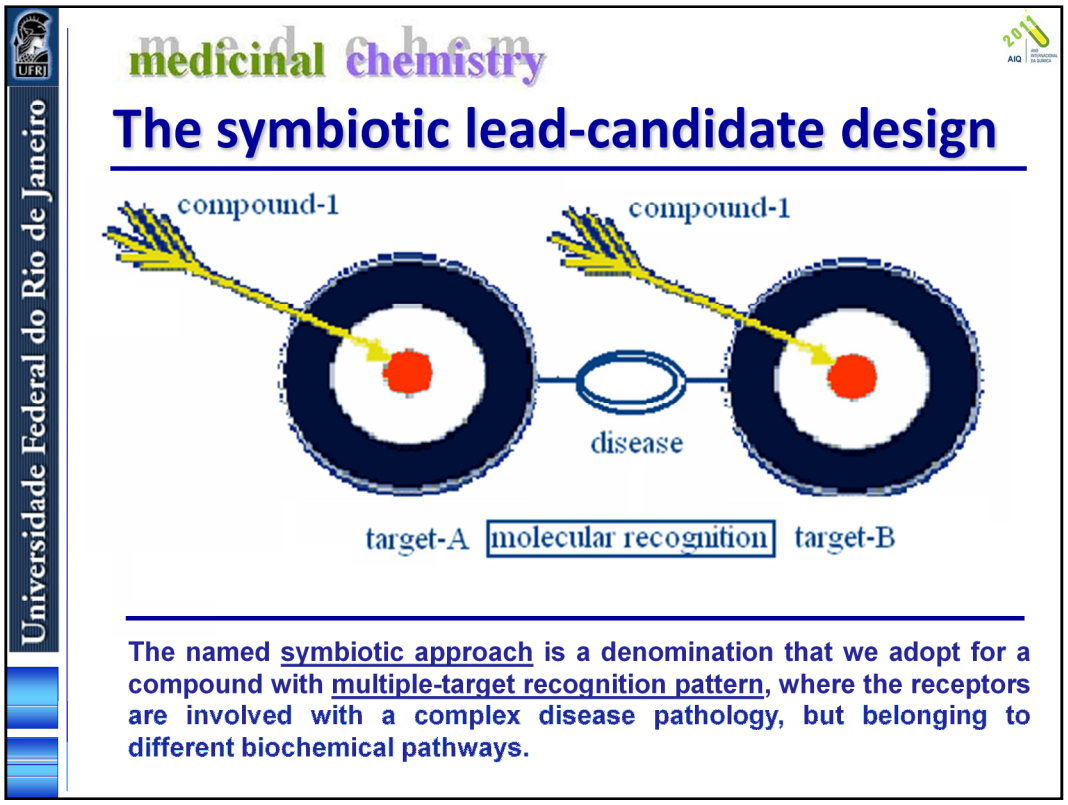


Camille Wermuth's published, few years ago, in *Drug Discovery Today* a sentence that I consider an excellent definition of the Modern MedChem paradigm, concern to rational discovery of multi-targeted drugs, useful for treatment of complex multi-factorial diseases.

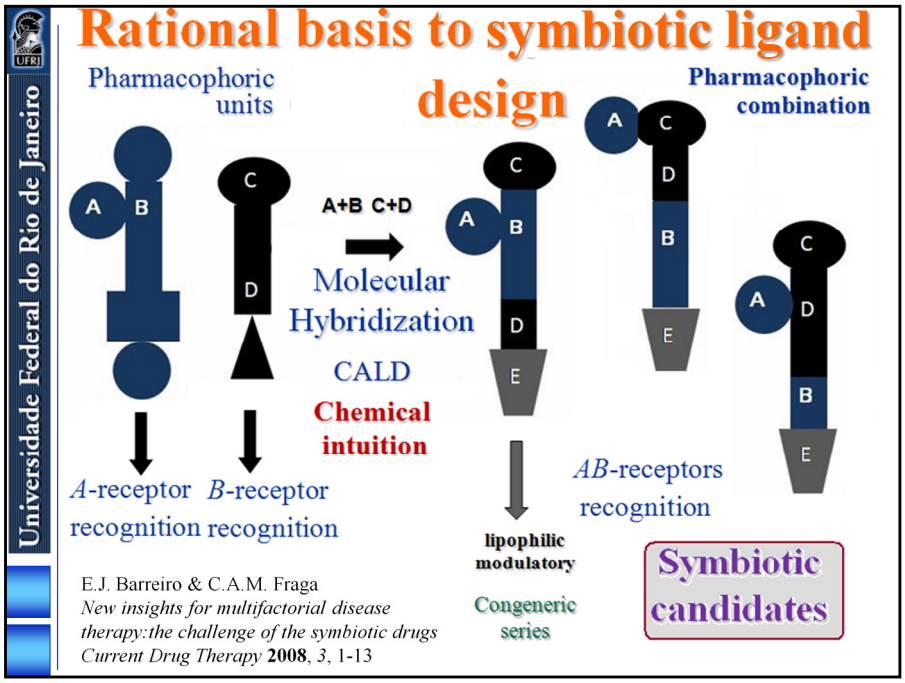


In this slide it is illustrated some important drugs, discovered under the <u>one-ligand/one-disease paradigm</u> as the first selective beta-blocker propranolol, the first selective histaminergic H-2 antagonist, cimetidine, both discovery by *Sir* James Black and co-workers. A third example was celecoxib, first selective inhibitor of COX-2, discovered in 1999, that represents perhaps last success achieved by *magic-bullet* phylosophy. Morever, clozapine, an important antipsychotic drug indicated for the treatment of schizophrenia, is one of the first drugs with multiple-target profile that present an attractive therapeutic profile. In fact, the therapeutic profile of clozapine, named "atypical" neuroactive drug, without extra-pyramidal effects, was associated with its "promiscous" or "dirty" character, acting at several neurotransmitter receptor systems as D2 (dopaminergic), D3, D4; serotonergic 5HT-2a, 5HT-3, 5HT-6 and 5HT-7; a-1 and 2-adrenergic receptors and histaminergic H1 and muscarinic m4 central receptors.

The multi-target modern paradigm appear in the literature with different denominations as dual, bivalent, mixed, binary, dimeric, and is illustrated by two simple small molecules with TPant/TXSi described by Freyne, in 1987, and the CNS active benzothiophene compound described by Monge, in 2001. Thus indicating that to obtain multi-target drug candidate it is not necessary to have complex molecules. Several others simple structures of dual agents could be mentioned herein.

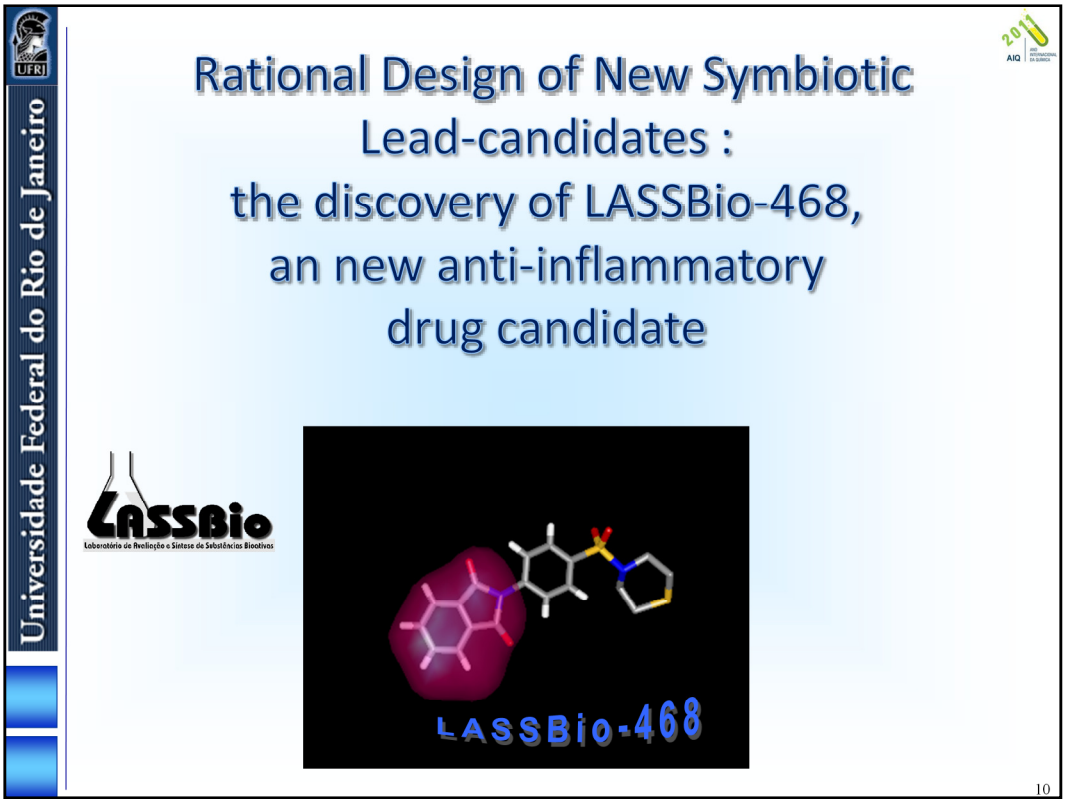


The named symbiotic approach is a denomination that we adopt for a compound with multiple-receptor recognition pattern, where these receptors are connected to a same complex disease pathology, but belonging to different biochemical windows. For example, a dual-inhibitor of COX-2 and LOX-5, with a desired anti-inflammatory profile will be not considered as a symbiotic candidate due the same biochemical cascade of the two receptors.

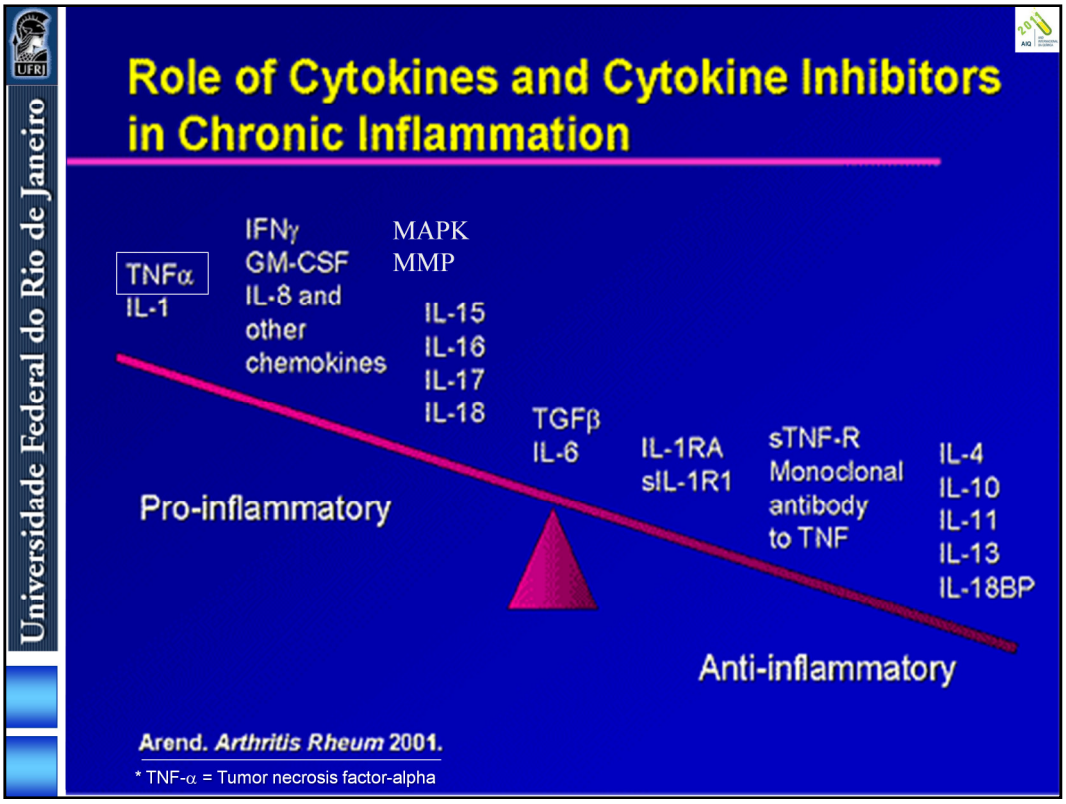


The rational basis to design <u>symbiotic-target-directed-ligand</u> can be considered as the same of any other <u>multi-target drug candidate</u>. For instance, it is necessary to identify the principals pharmacophoric fragments from a know ligand, or the principal pharmacophoric points of the natural agonist of the receptors A and B, separately. Next, to identify pharmacophoric units A- and B- and C- and D-, for receptors A and B, respectively and apply strategies of molecular design of Medicinal Chemistry, for instance the molecular hybridization on four molecular fragments, rising different new molecular framework. In addition, one can study the virtual compounds by molecular dynamics over each receptor A and B, separatly or on a chimeric double hybrid-receptor, constructed *in silico*. In addition, it is also possible to select among the new virtual planned compounds by measuring *in silico* its physical-chemical properties and conformational behavior in order to compare with the original natural ligand. In addition, in the design of symbiotic candidates it is possible to include several different auxophoric linkers in order to modulate its complete structural properties. By choosing the *better (top-one)* compound designed by this process the medicinal chemist construct next a congeneric series, formed by close structural related analogues that will be next biologically essayed. By this strategy it is possible to discover new useful drug candidates from a very small collection of molecules which can be eventually optimized next.

In the next slides I will present some results from our research effort in LASSBio UFRJ aiming the design and discovery of new symbiotic candidates.

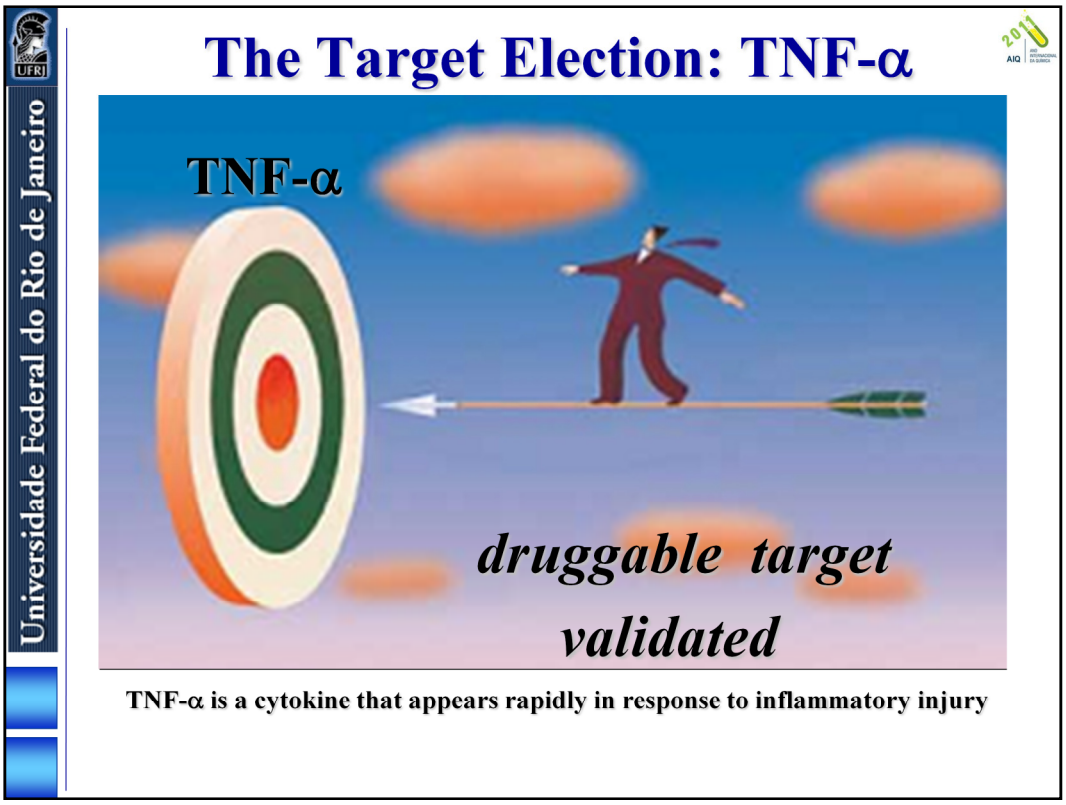


This work was performed at LASSBio in Federal University of Rio de Janeiro.

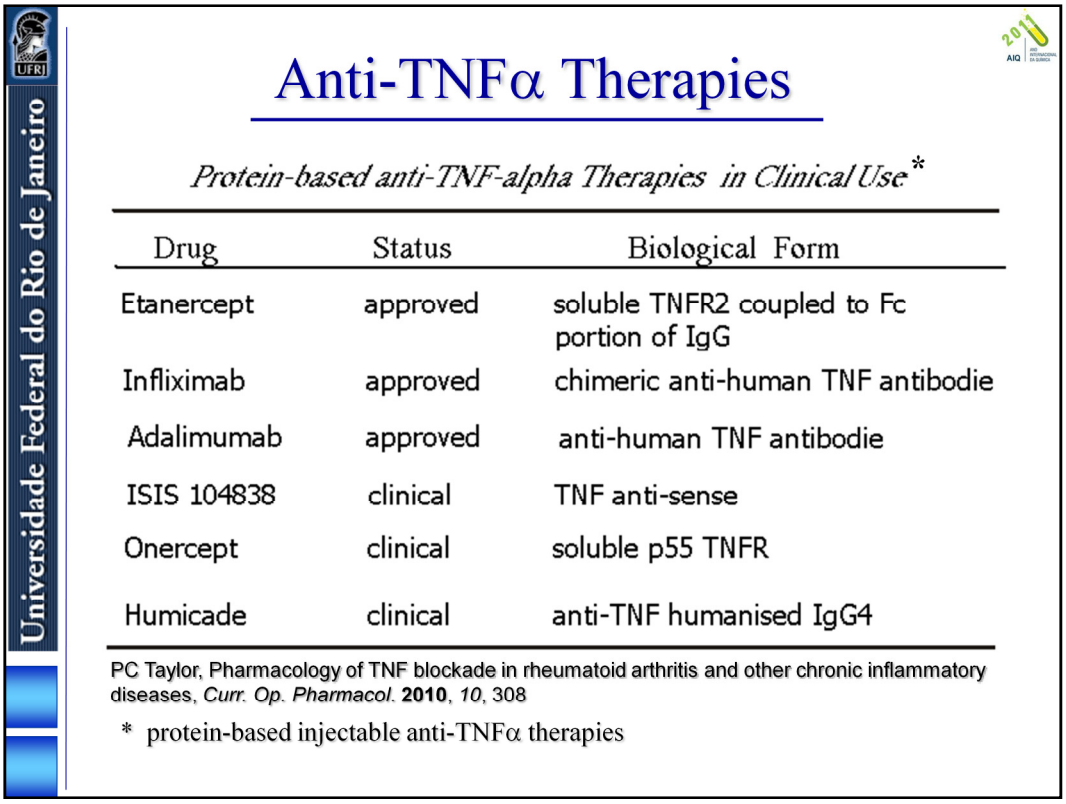


The complex inflammatory process is a biological response of the organism modulated by several mediators formed by different biochemical pathways: *e.g.* cytokines. eicosanoids, and enzymes belonging to GPCR class. The high morbidity level of several multi-factorial chronic inflammatory disorders as rheumatoid arthritis, psoriasis, Chron disease, and multiple sclerosis, with poor therapeutic resources can be circumvented by applying the symbiotic approach.

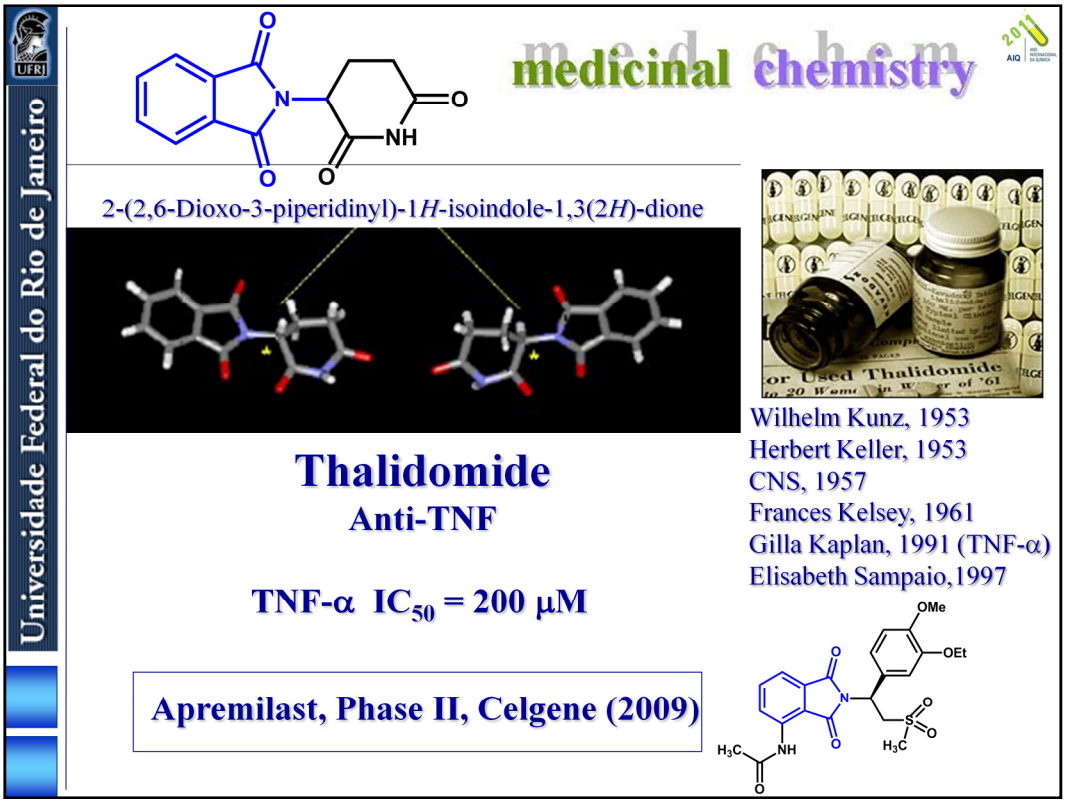
In this figure it is illustrated part of the complex process of inflammatory response and the principal pro-inflammatory cell mediators and the endogenous anti-inflammatory mediators. It is now well accepted the important pro-inflammatory role of the tumor necrosis factor-alpha.



Tumor necrosis factor-alpha (TNF-a) is a pleiotropic cytokine with both pro-inflammatory and immune-regulatory functions. These diverse activities are mediated via interaction with two distinct receptors, p55/TNFRI and p75/TNFRII expressed on most human cells, which activate separate transduction pathways resulting in distinct biologic effects. Elevated levels of TNF-alpha have been associated with a number of inflammatory diseases such as rheumatoid arthritis. The success of anti-TNF-alpha therapy of AI-chronicle diseases using biopharmaceuticals (biological agents such as antibodies or soluble receptors that target proteins) has validated the therapeutic importance of the blockade of TNF-alpha production in the treatment of patients with various of theses inflammatory diseases. Given the limitations of biopharmaceuticals, that presents some deleterious effects at immunologic system opening way for eventual opportunistic infections, reduce the therapeutic profile of these agents illustrated in the next slide.

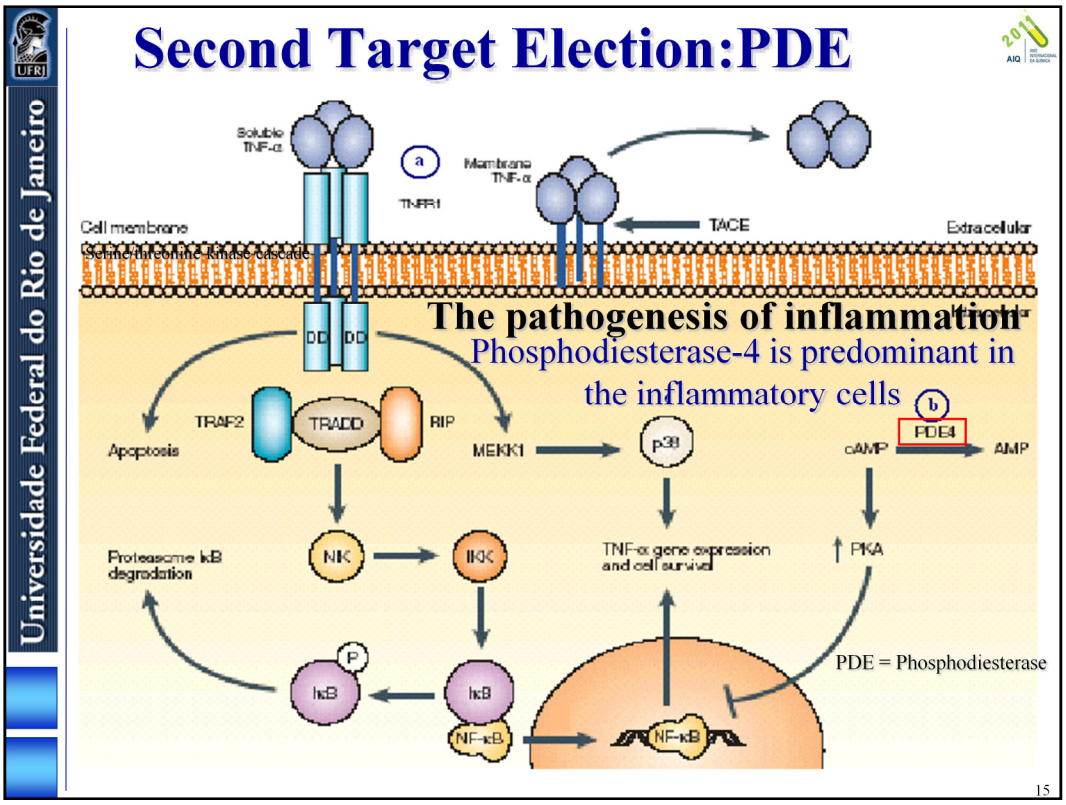


Biopharmaceuticals as anti-inflammatory acting at blockade of TNF

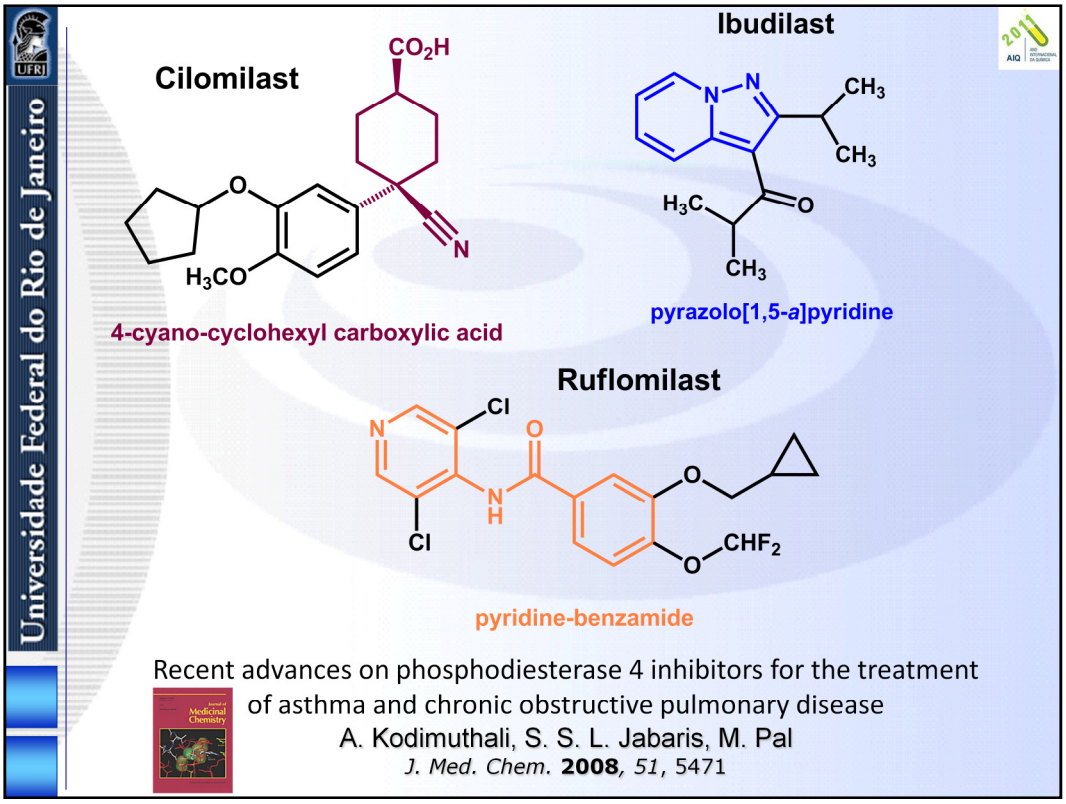


No small molecules drug are used to block TNF-alpha response. A drug that act by this mechanism might be attractive, and it could replace biopharmaceuticals if prove to be effective in specific clinical contexts without unacceptable side effects. The only small molecule that block the TNF-alpha response is thalidomide (THLD), a very simple molecule with two imides functions developed in the 50's. THLD was originally used as a sedative for the treatment of morning sickness, but was withdrawn in the early 1960s because of frequent teratogenicity (phocomelia). Some years after its mechanism of action was elucidated as acting at the TNF-alpha level. THLD has been approved by the Food and Drug Administration (FDA) of US, in July 1998, for treatment of erythema nodosum leprosum.

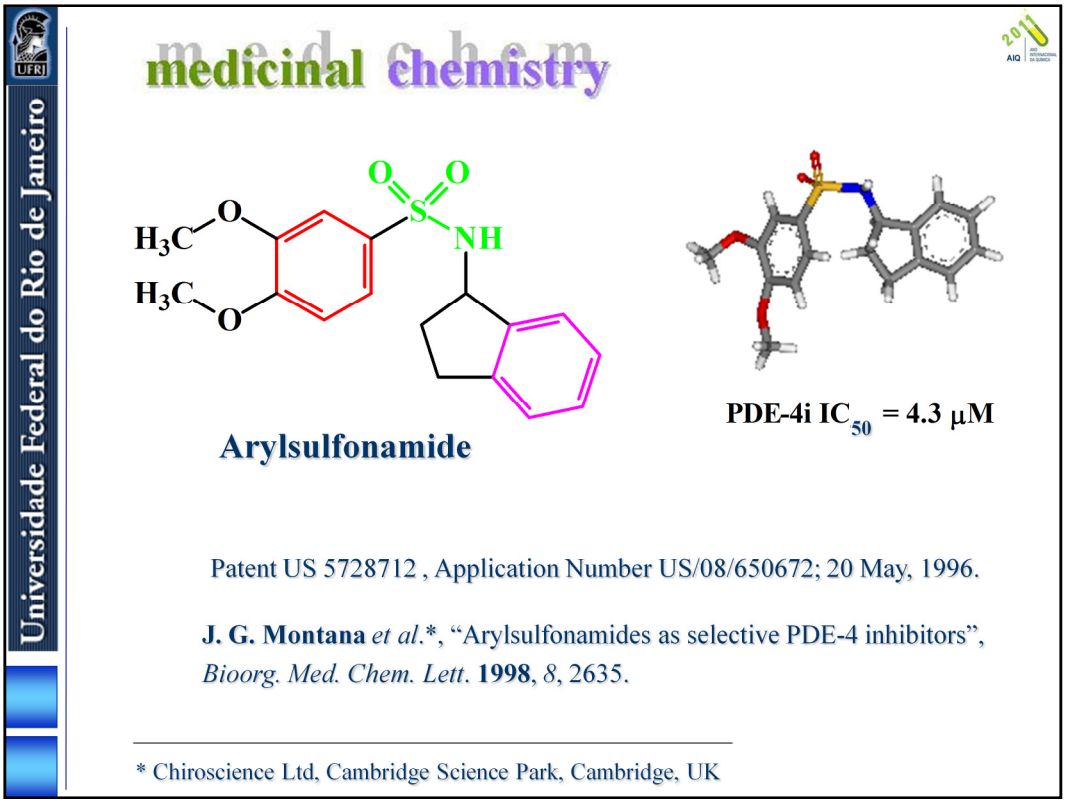
Several different analogues of THLD were described in the literature illustrating its use as useful scaffold for block the biological response of TNF-alpha. Recently, in 2009, Celgene Corporation describes the THLD modified chiral compound, named apremilast, that act blocking the response of this cytokine. This chemical entity is now in Phase II of clinical trials as anti-inflammatory drug.



Phosphodiesterases (PDEs) are intracellular enzymes that hydrolyze the phosphodiester bond of the second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). There are 11 distinct PDES, designated as PDE1 through PDE11. The phosphodiesterase 4 (PDE4) is the most important PDE family in the control of intra-cellular cAMP. and presents four isoforms PDE-4A, B, C and D. PDE4. These sub-types highly distributed in inflammatory responses.

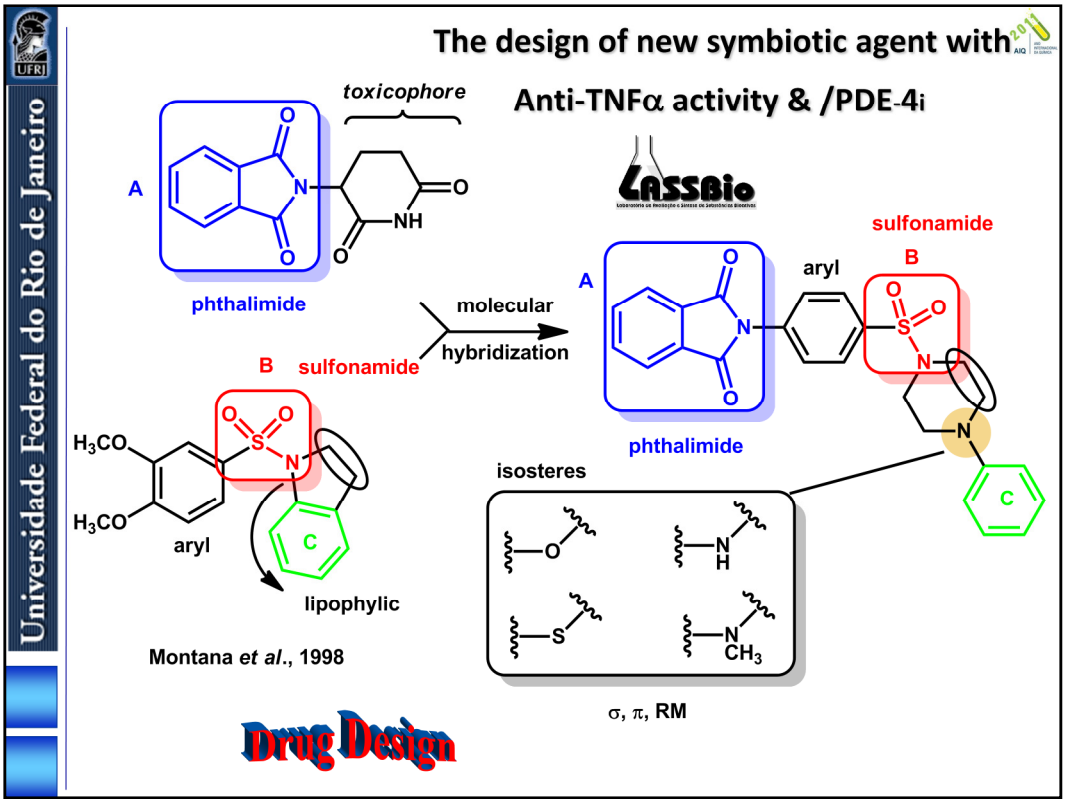


Cilomilast (SB-207,499, Aryflo™) is a PDE-4 inhibitor drug in clinic for treatment of Chronic Obstructive Pulmonary Disease (COPD) and asthma, approved by Food Drug Administration in 2003. Ibudilast (AV-411) is a second drug acting as PDE-4 inhibitor with central effects in Phase II-staged for use in multiple sclerosis. Due its central effect this drug is experimentally used in neuropathic pain. In last March, FDA-US authorize the use of roflumilast (Daxas™), a third PDE-4 inhibitor drug indicated also for asthma and treatment of severe COPD, associated with chronicle bronchitis and primary emphysema.

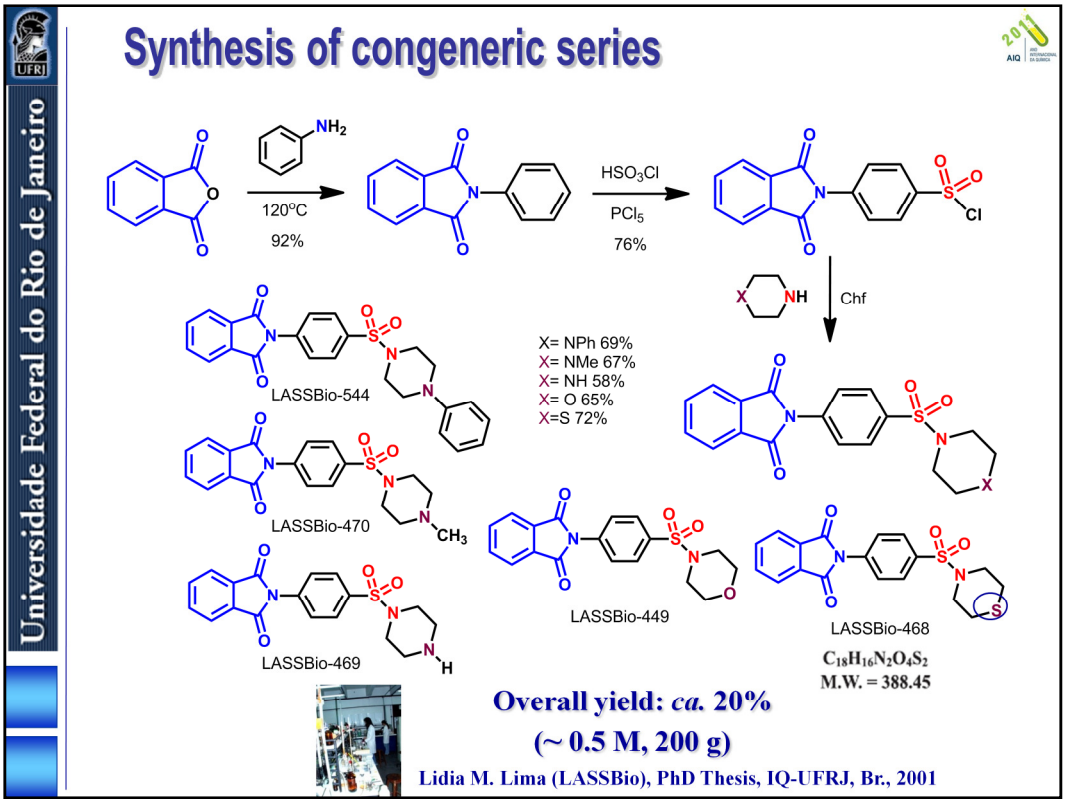


The dream of medicinal chemist is find a structurally simple molecule with a good biological profile. In 1996, Montana and co-workers at Chiroscience Ltd, Cambridge, UK, describe in an American patent a series of structurally simple arylsulfonamide with PDE-4 inhibitor properties. These results are published in *Bioorganic Medicinal Chemistry Letters*, two years after with emphasis to the illustrated compound. This compound presents an *ortho*-dimethoxyphenyl fragment, connected to the sulfonamide moiety with a lipophylic aromatic terminus.

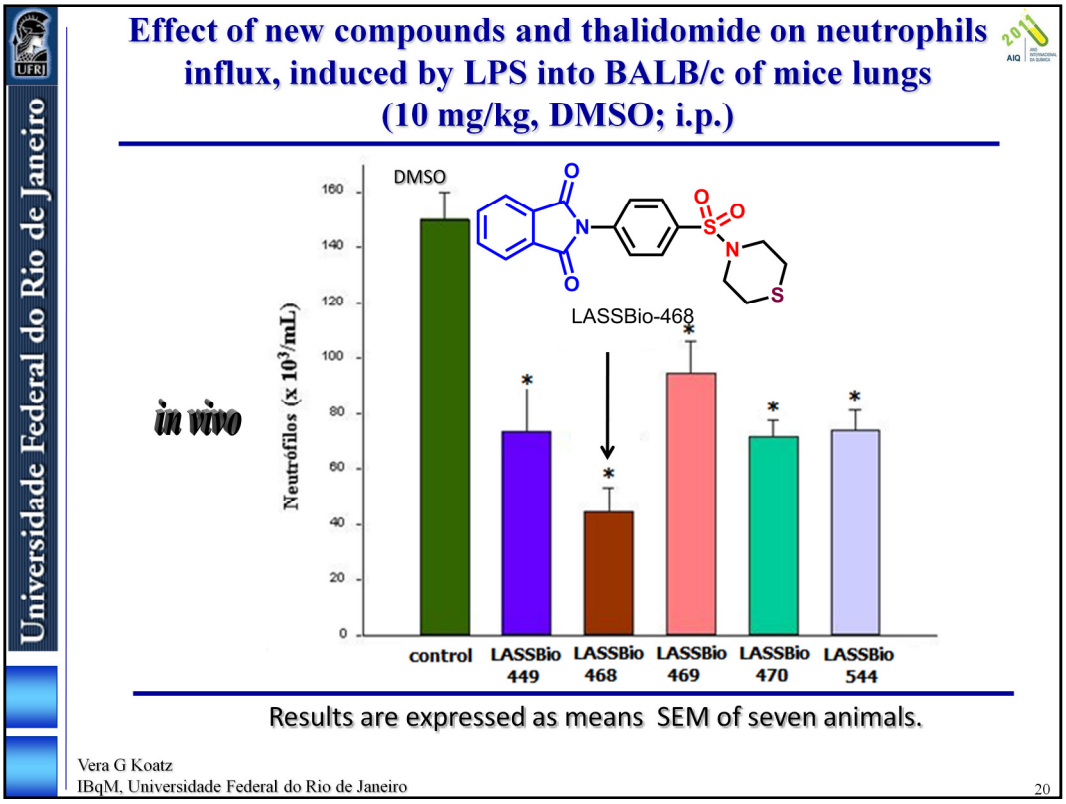
At this time, we were interested in LASSBio-UFRJ into identify new compounds with anti-inflammatory properties as dual symbiotic agent, blocking the TNF-alpha effect and inhibiting PDE-4 simultaneously. Thus an obvious idea was to construct an hybrid compound connecting THLD and Montana compound. The design basis of this new compound are described in the next slide.



The structure design of new series of symbiotic candidates that result in LASSBio-468, was a molecular-hybridization-based between THLD and Montana's compound. Electing the phthalimide fragment of THLD as A-fragment and connecting with modified arylsulfonamide fragment B from Montana's compound, including the displacing of the lipophylic unit C as N-substituent group in new piperazine ring, homologue of the *N*-cyclic sulfonamide structural unit of Montana series. In order to construct a congeneric series of analogues, it was applied the bioisosterism replacement to furnish morpholine and thiomorpholine ring, instead the piperazine system with *N*-phenyl and *N*-methyl analogues completing a small collection of only new five hybrid sulfonamide-phthalimide derivatives. The synthesis of the compounds are illustrated in the next slide.

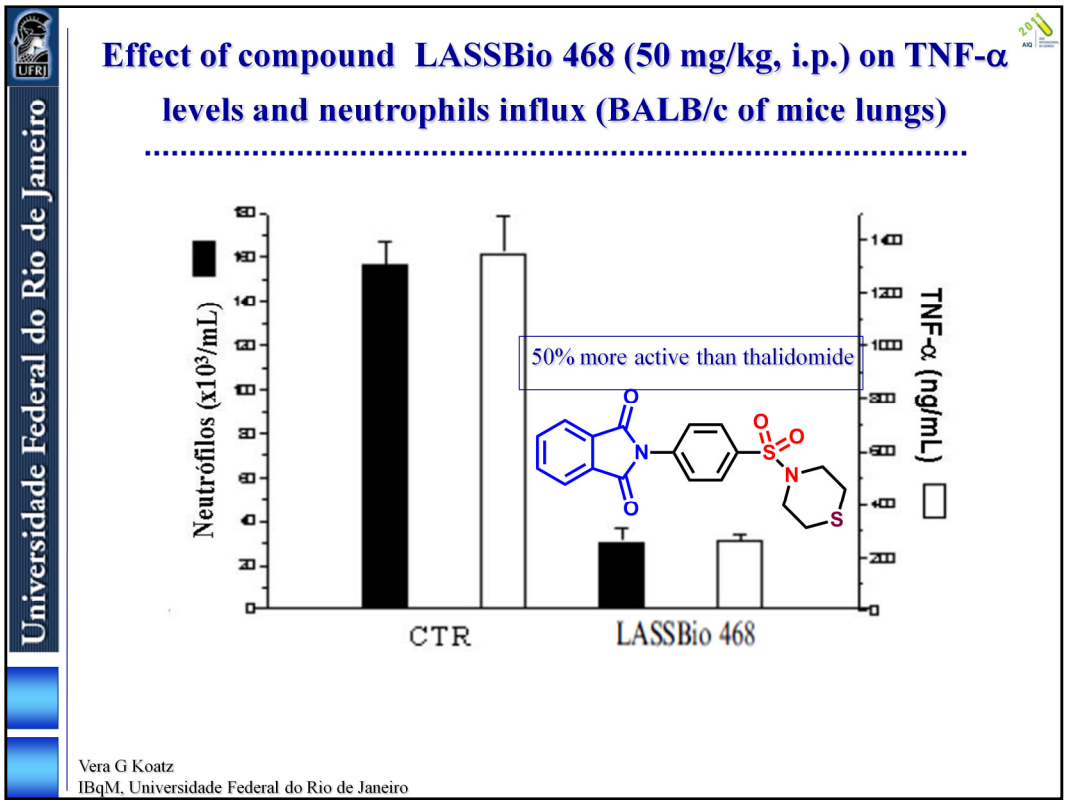


The synthetic route to these new compounds start from phthalic anhydride which was treated by aniline giving in appropriate yield the *N*-phenyl phthalimide derivative. The next step was the regioselective sulfonation of the *para*-position of *N*-phenyl ring followed by treatment of the sulfonyl-chroride with the appropriated cyclic amine to give the new series of sulfonamide derivatives, including LASSBio-468.obtained in *ca*. 20% of overall yield at *ca.* 0.5M scale. After purity determination and full structural confirmation, by using the classic spectroscopic methods, each compound was pharmacologically essayed as shown in the next slides.

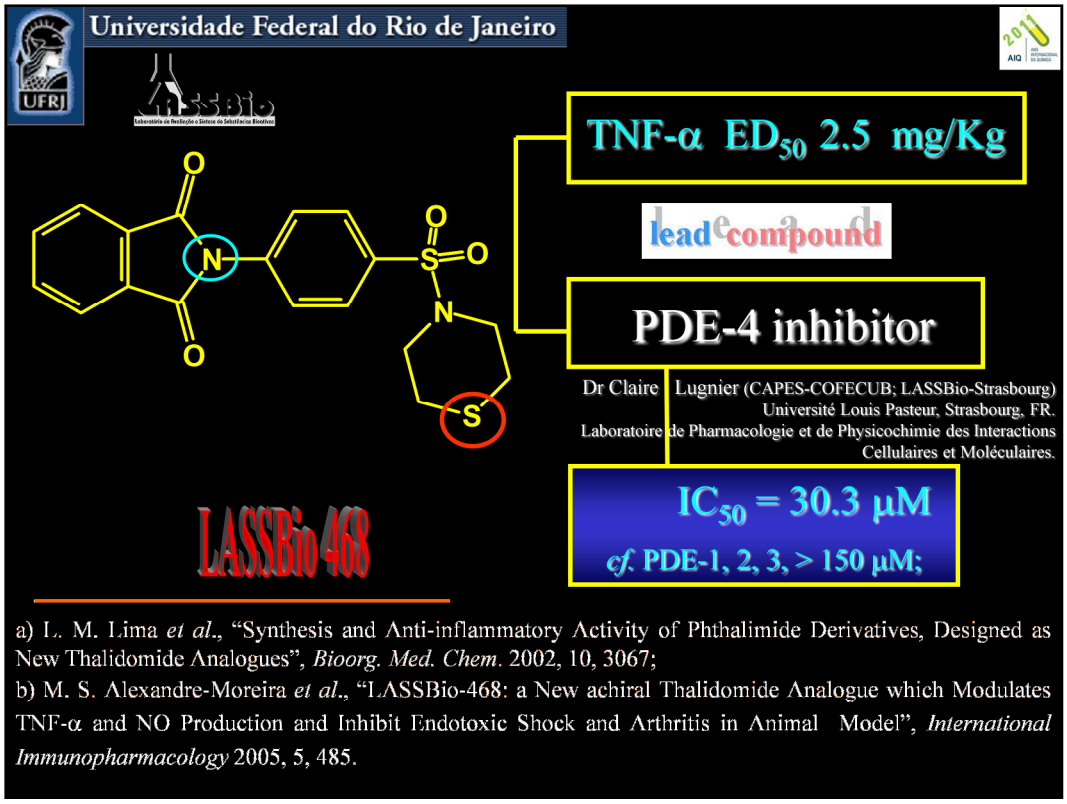


At lung level, THLD has been demonstrated to release TNF-alpha that favors the sequestration and migration of neutrophil which play a critical role in the pathogenesis of lung inflammation.

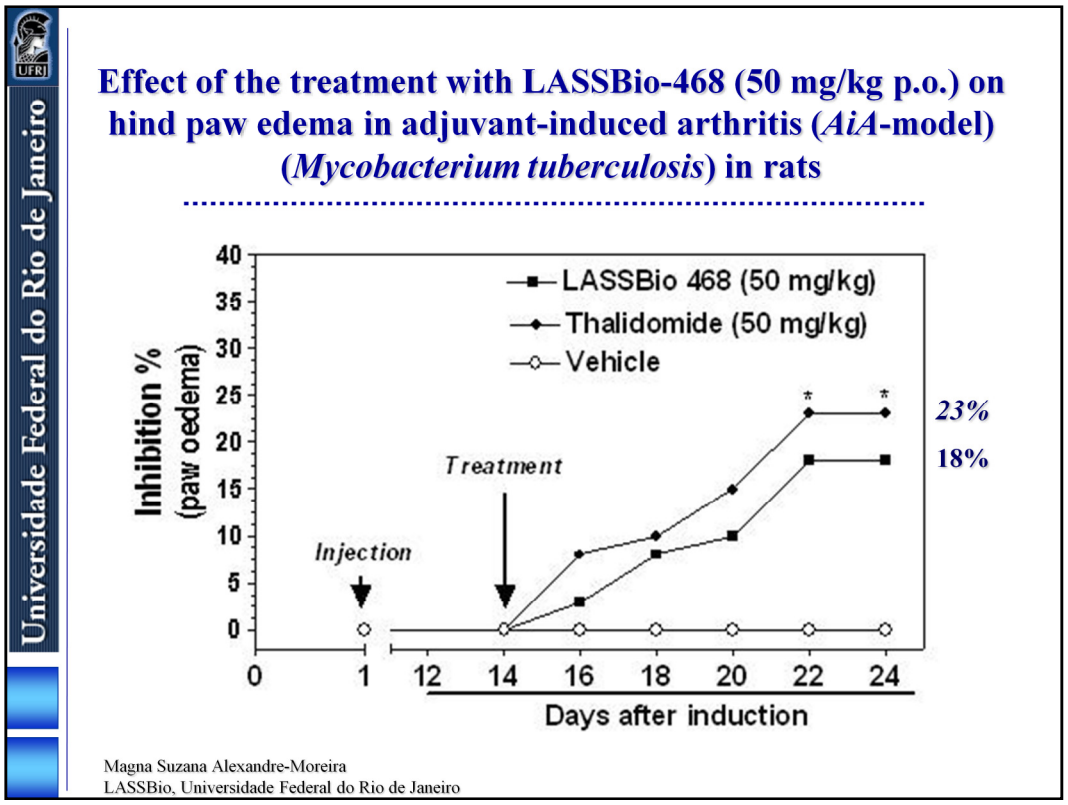
The new derivatives were screened for their ability to inhibit the acute inflammatory response, measured by inhibition of LPS-induced TNF-alpha and neutrophil infiltration into mice lungs. LASSBio 468 was the most active in this essay with ED_{50} 2.5 mg/kg.



To correlate the anti-inflammatory activity observed for LASSBio-468 with a possible effect on TNF-alpha production, the cytokine levels were evaluated as shown in the slide. <u>These results revealed the ability of LASSBio-468 to inhibit TNF-alpha levels in BALB of mice lungs treated with LPS</u>.

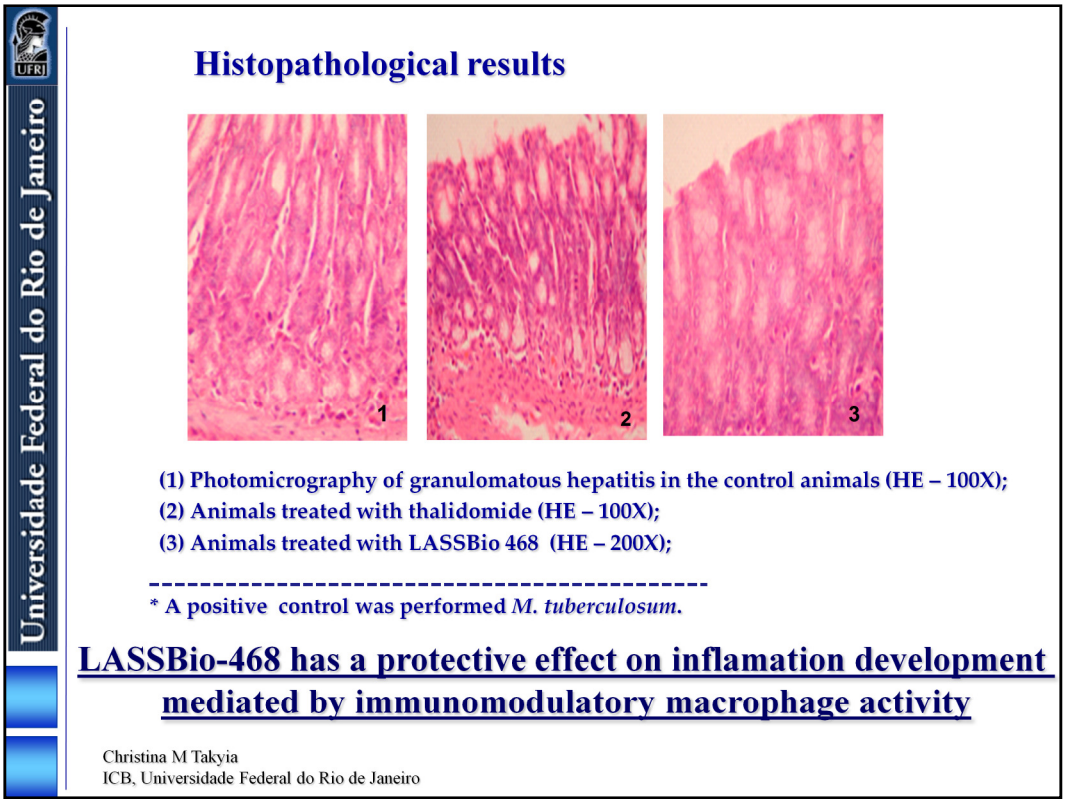


It is well-known that elevation of intracellular levels of cAMP in leukocytes is accompanied by inhibition of the production of TNF-alpha and is associated with inhibition of PDE-4 activity. Therefore, the new derivative 468 was also evaluated, *in vitro*, as PDE-4 and PDE-3 inhibitors, performed by Dr Claire Lugnier from Université Louis Pasteur, Strasbourg, FR. The results obtained with PDE's from bovine aorta assay indicated an inhibition effect at *ca*. 30 mM without action on PDE-1, 2, 3 and 5.



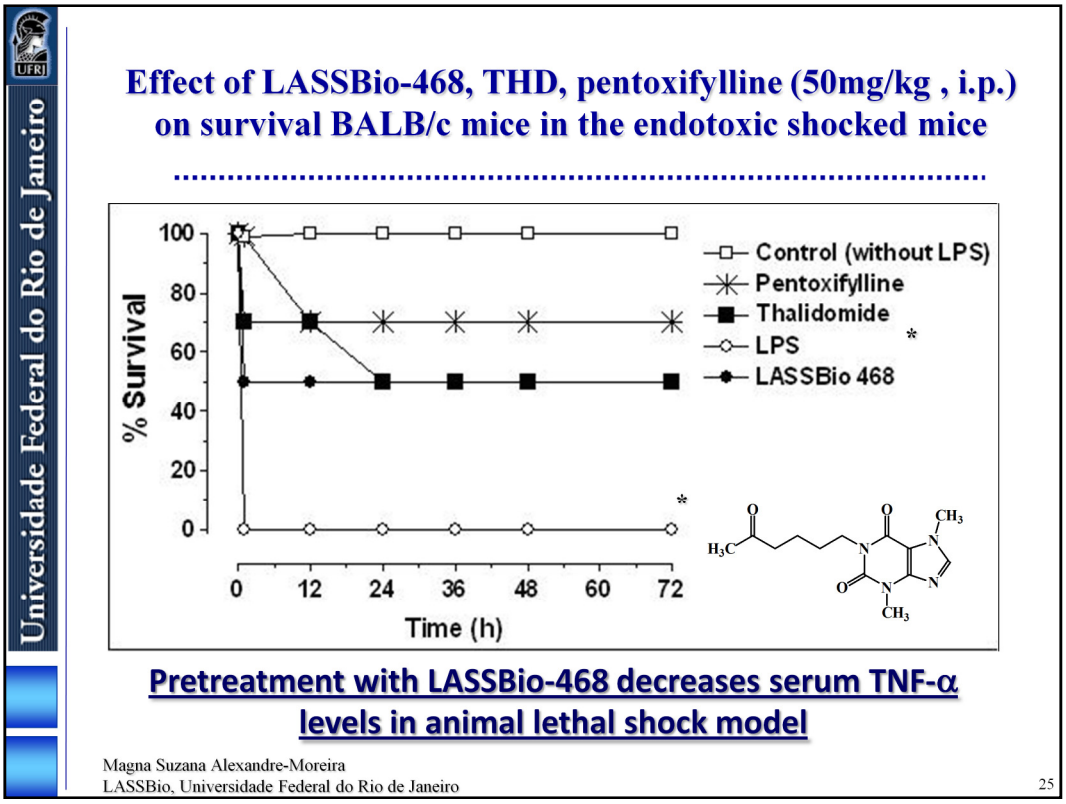
Considering that LASSBio-468 is a potent inhibitor of TNF-alpha and NO production *in vivo*, and that TNF-alpha exerts a predominant role on the animal model of rheumatoid arthritis, next we evaluated the effect of this compound on adjuvant-induced arthritis (AiA-model), induced by *M. tuber*culosum in rats.

LASSBio-468 effect was measured in the paw edema at the different day points. LASSBio-468 inhibits the paw edema in this model in *ca*. 18% almost the same effect of THLD (23%).



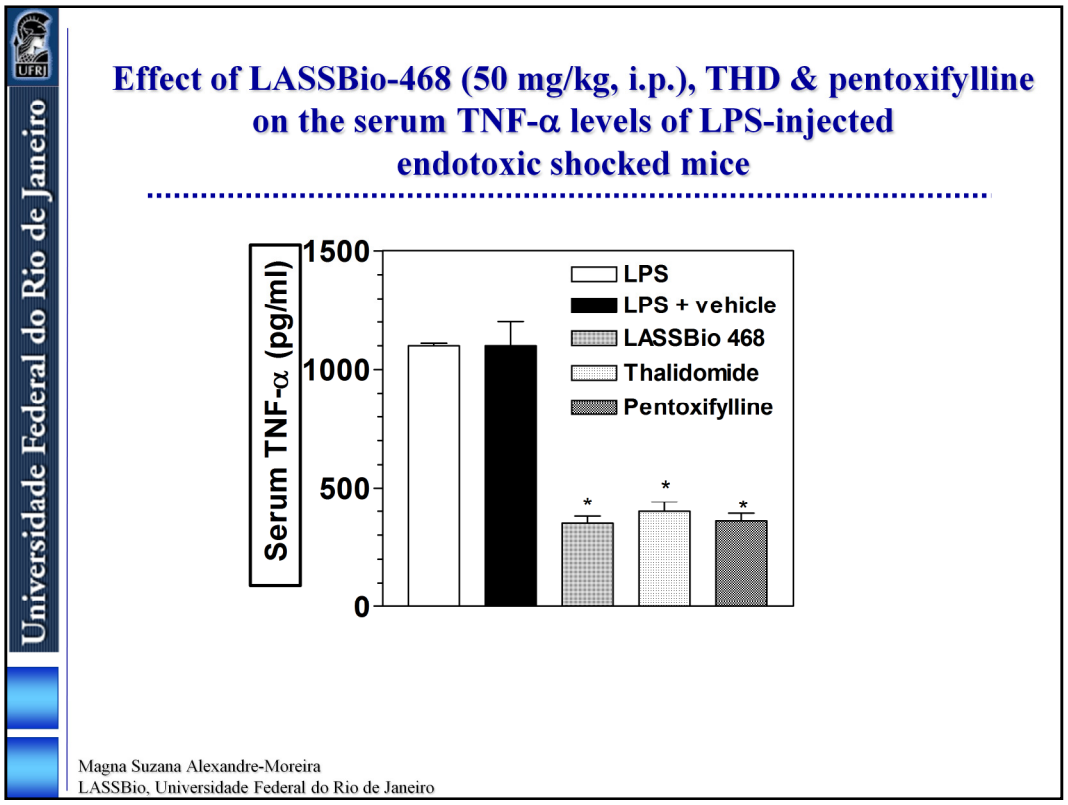
It is well established that AiA-model in rats exhibit a granulomatous hepatitis, constituted by large lobular and portal granulomas. In animals with administration of LASSBio-468 the granulomatous reaction was inhibited in liver, as illustrated by histopathological analysis in contrast to THLD-treated rats, which shows epithelioid and foamy cells granulomas still present.

These results show that LASSBio-468 has a protective effect on inflammation development mediated by imunomodulatory macrophage activity.

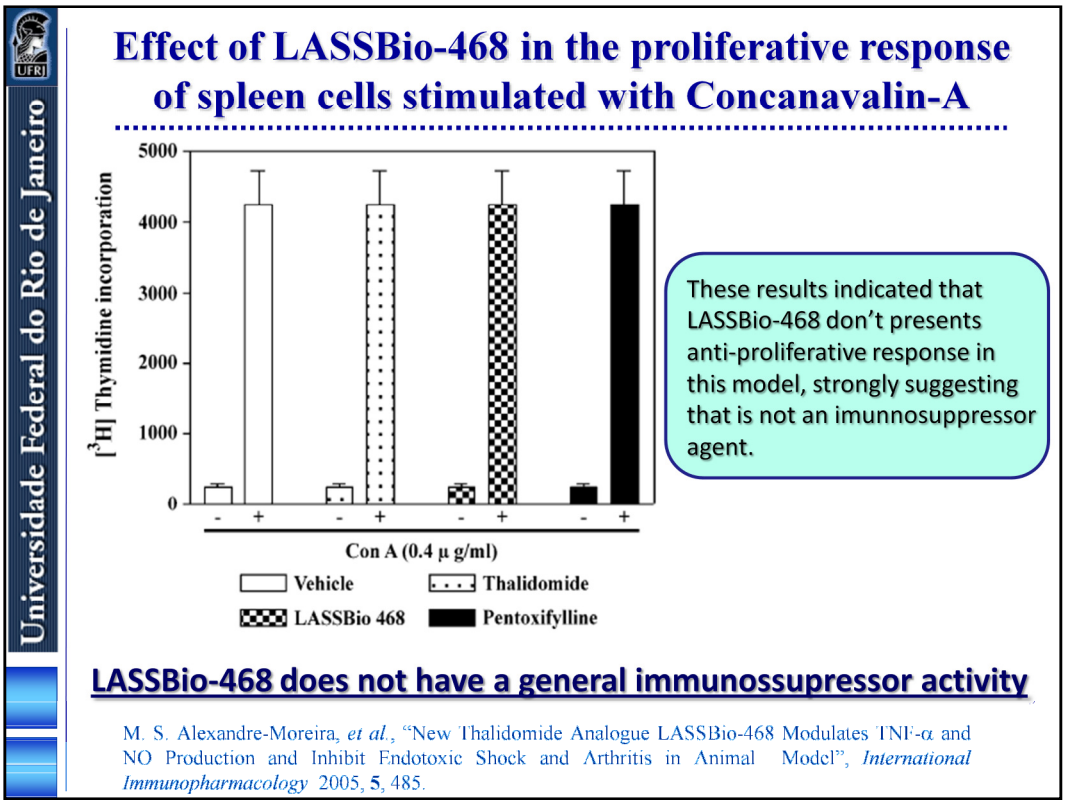


The effect of LASSBio-468 was also evaluated in shocked mouse model, using endotoxin, representing a functional pharmacological model. The results shows in the slide indicated that the pretreatment with LASSBio-468 (2 h before LPS challenges) significantly protected the mice (*ca*. 50%) from the lethal shock. Furthermore, the pretreatment with THLD (pentoxifylline) also prevented the endotoxin lethality, inducing survival rates at the same 50% (and 70% to pentoxifylline).

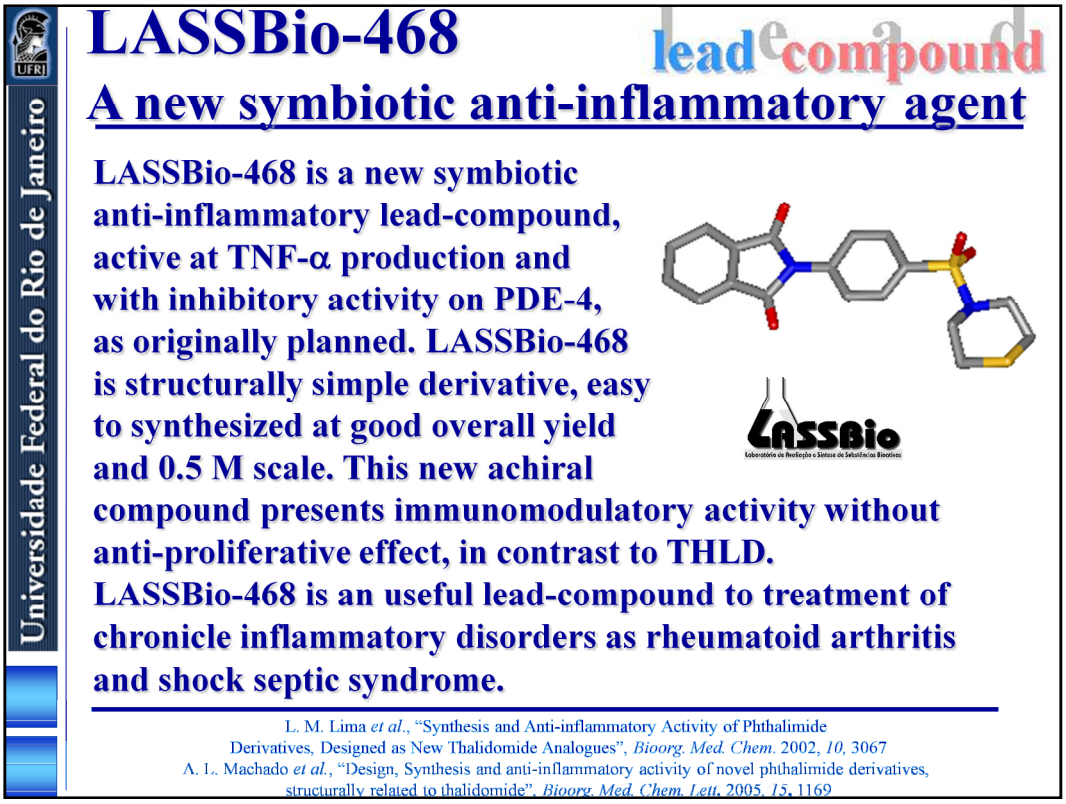
Pretreatment with LASSBio-468 decreases serum TNF-alpha (and NO) levels in lethal shock.



This effect is TNF-alpha related.

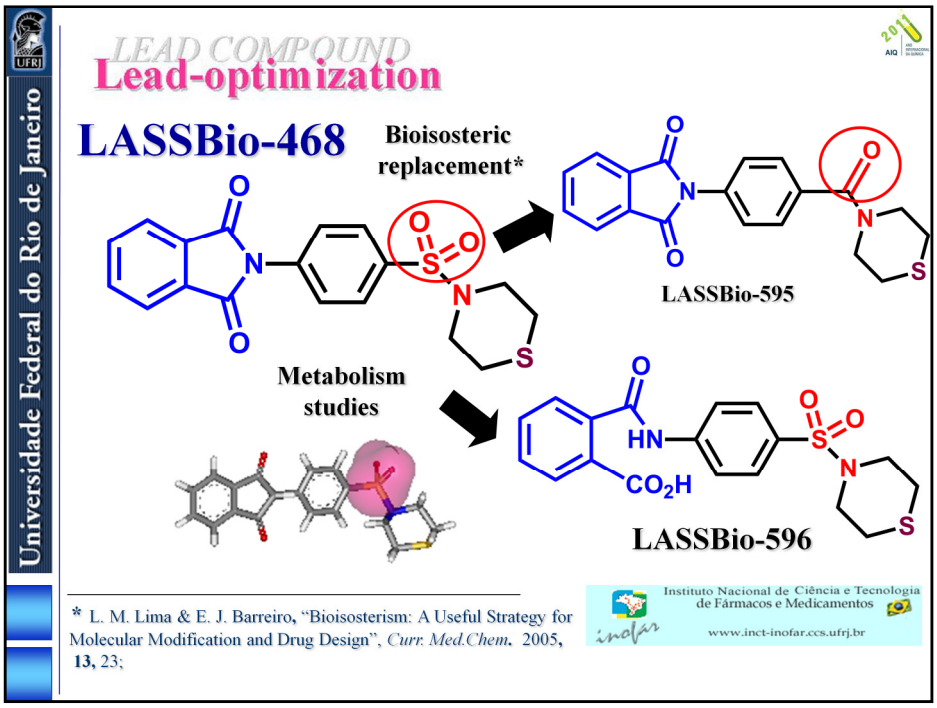


In order to discard the possibility that LASSBio-468 has a nonspecific immunossupressive activity, we examined its effect on the proliferative activity of splenocytes obtained from mice immunized with ovalbumine (OVA). We did not observe any suppression of T-cell proliferation in response to Concavalin A mitogen, clearly demonstrating that LASSBio-468 does not have broadly affected immune system cells, as THLD does.

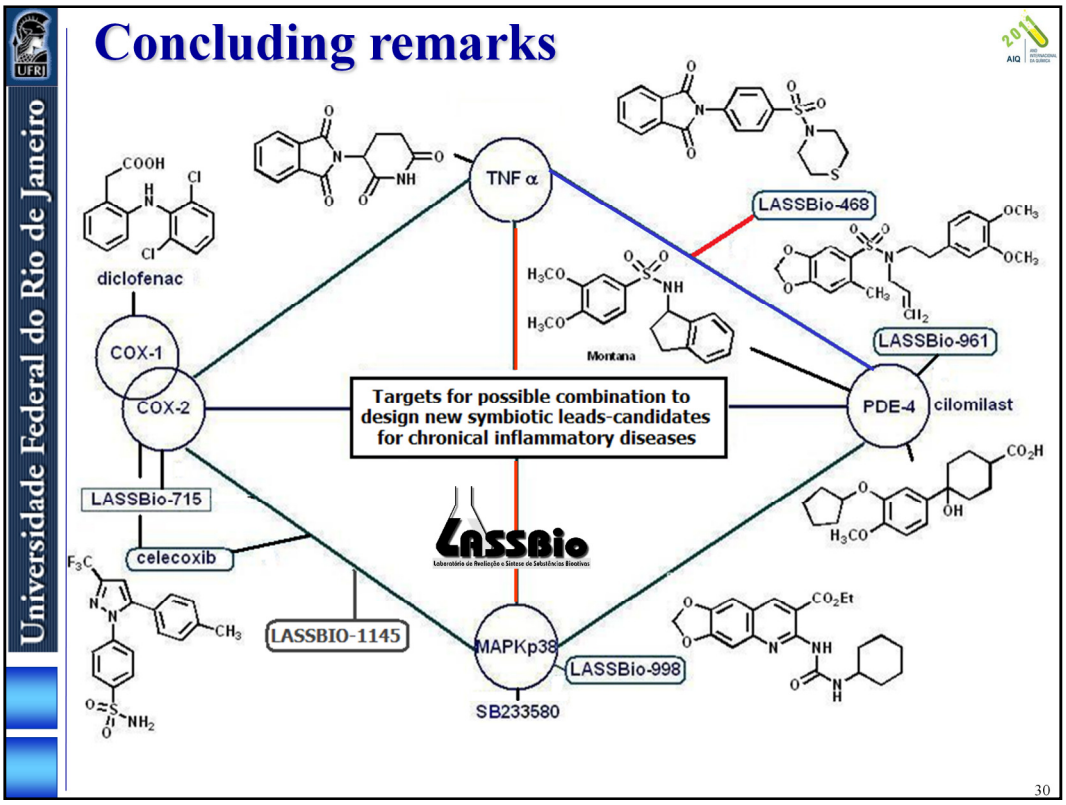


Resumé. In this work we was able to discover a new symbiotic anti-inflammatory lead compound, active at TNF-alpha production (and NO down modulation) with inhibitory activity on PDE-4, as originally planned.

It is a useful new lead-compound with important activity on rheumatoid arthritis and shock septic animal models.



In medicinal chemistry the discovery of a new lead-compound is always followed by molecular optimization studies. This slide shows some of studies performed in order to optimize the biological profile LASSBio-468. Bioisosteric replacement of the sulphone unit by a carbonyl gives an very poor active amide analogue. Studies on metabolic stability of LASSBio-468 with rat microssomes indicated a principal metabolite identified as the imide hydrolysis product.This compound was prepared in the lab (treatment of LASSBio-468 with lithium hydroxide in a mixture of THF/MeOH/H_2O; 77% yield) LASSBio-596 that presents a remarkable profile of anti-inflammatory activity. This compound is now under investigation at the Instituto Nacional de Ciência e Tecnologia de Fármacos e Medicamentos (INCT-INOFAR), a research project supported by CNPq (MCT-BR) a Brazilian MedChem network. For more information's visit the project home-page at www.inct-inofar.ccs.ufrj.br



As final remarks it is illustrated the symbiotic-projects tree performed at LASSBio-UFRJ searching anti-inflammatory activity. It was possible to discover the compound LASSBio-1145 a dual symbiotic drug candidate acting at MAPK p38 & COX-2 and LASSBio-998 an inhibitor of MAKP p38 that is a new structural pattern which are used as template to include molecular modification to include the PDE-4 inhibitory activity. LASSBio-1145 as LASSBio-468 represents original lead-compounds useful as drugs candidates to treat anti-inflammatory chronicle degenerative multi-factorial diseases.

Universidade Federal do Rio de Janeiro

UFRJ

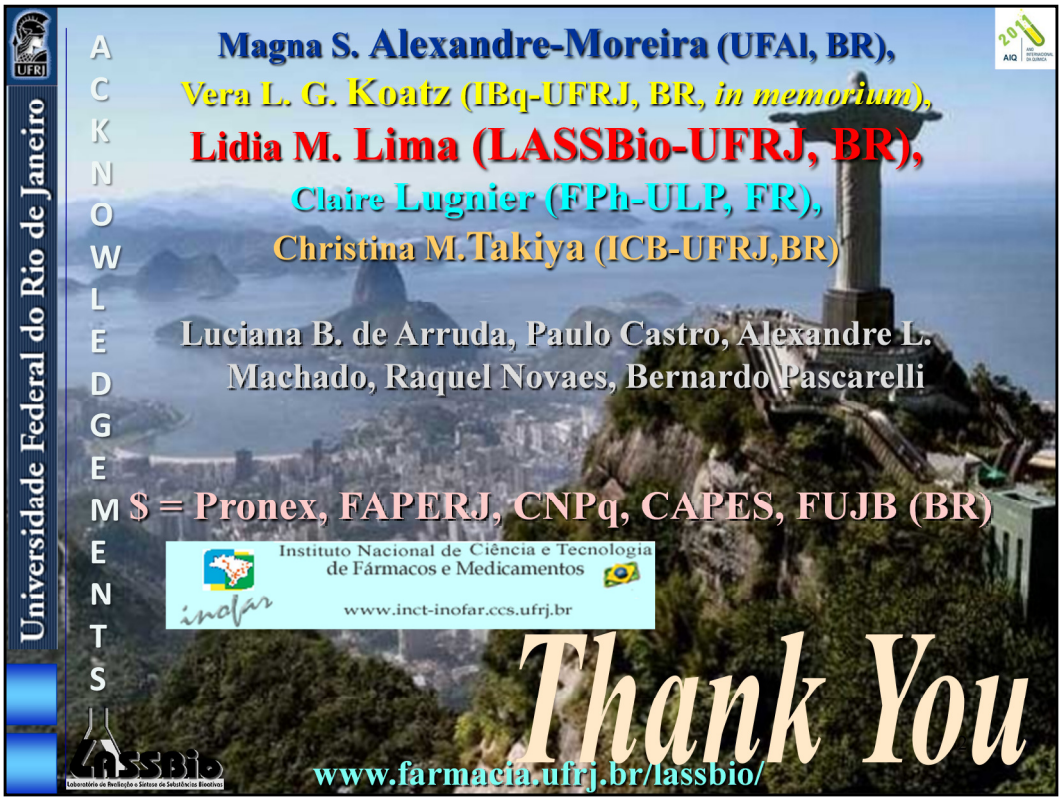
medicinal chemistry

“ ...medicinal chemists today live in exciting times... their work can have a beneficial effect on millions of suffering patients – surely an important motivating factor for any scientist...”

J. G. Lombardino

The Role of the Medicinal Chemist in Drug Discovery – Then and Now,
Nature Rev. Drug Disc. 2004, **3**, 853.

To close this presentation I show a sentence of Joseph Lombardino, the discover of piroxican in Pfizer, about the role of medicinal chemist in the drug discovery process.



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