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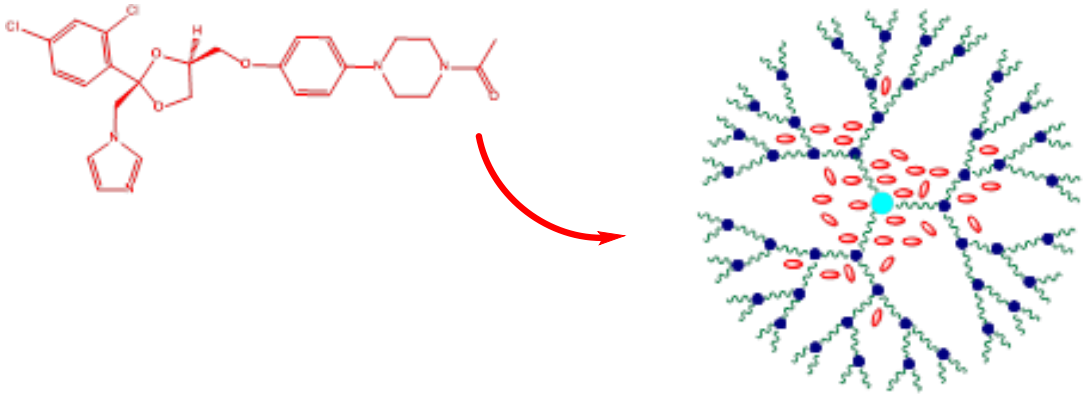
# KETOCONAZOLE: FROM FUNDAMENTALS TO NEW ANTIFUNGAL FORMULATIONS BASED ON PAMAM DENDRIMERS WITH IMPROVED BIODISPONIBILITY

## (KET-IN-PAMAM)

## No. Contract TE122/2022



**KTZ-PAMAM-G5-$NH_2$**
supramolecular complex

***The goal of the project*** is to create a young, multidisciplinary research team capable to promote new solid forms/formulations of active pharmaceutical ingredient **Ketoconazole** (**KTZ**) with improved solubility and bioavailability. The team consists of five young researchers (project leader - chemical engineer, 2 chemists, 1 biotehnologist, 2 dermatologist), and one postdoc (physicist). Obtaining new solid forms/formulations of KTZ may form the basis of a better product in order to maximize the therapeutic efficacy of the drug.

Mycotic infections represent a worldwide medical problem due to: (i) the increasing frequency, (ii) the development of resistance of various pathogenic strains to commercial antifungal products, (iii) the need for long-term treatment in case of deep infections or infection of the fascia (hair and nails), which causes (iv) accumulation and, as a result, toxicity at the liver level.

KTZ is the first FDA-approved broad-spectrum antifungal used in the treatment of superficial and systemic mycoses, belonging to class II (BCS), with low aqueous solubility and high permeability, pronounced hydrophobicity and weak basicity. The low bioavailability at pH>3 represents the major drawback of its effectiveness in the case of oral administration, being eliminated from the gastrointestinal tract before being completely dissolved, reducing its absorption into the bloodstream. The increase in bioavailability has the effect of using a lower concentration of the active substance to obtain the desired therapeutic effect, at the same time reducing the hepatotoxicity manifested after long-term treatment.

The research team will evaluate the market potential for already obtained co-crystal solid forms of KTZ with Adipic Acid (**KTZ-AA**) and with Sorbic Acid (**KTZ-SA**) with 100- and 40-fold improved solubility *vs.* commercial KTZ and also, for the supramolecular complex between KTZ and PAMAM dendrimer, as active pharmaceutical compounds in optimized oral/topical products. For the co-crystal type forms, the preliminary informations of preformulation stage will be completed with specific studies/evaluations: compatibility with pharmaceutical excipients, *in vitro* and *in vivo* biocompatibility tests, respectively protocol for the co-crystallization process on a laboratory scale of the order of grams, essential for technology transfer regarding the development of a successful industrial co-crystallization process.

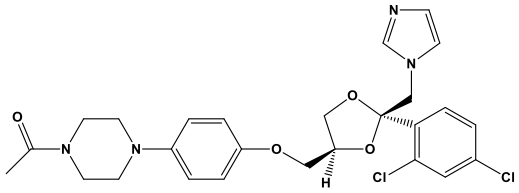
***Specific Objectives***:

(I) Obtaining and characterization of the carrier system KTZ-PAMAM complex for topical use.

(II) Optimization of solid forms of KTZ co-crystals with Adipic and Sorbic Acids for oral drug therapy through preformulations, antifungal activity and biocompatibilities studies.

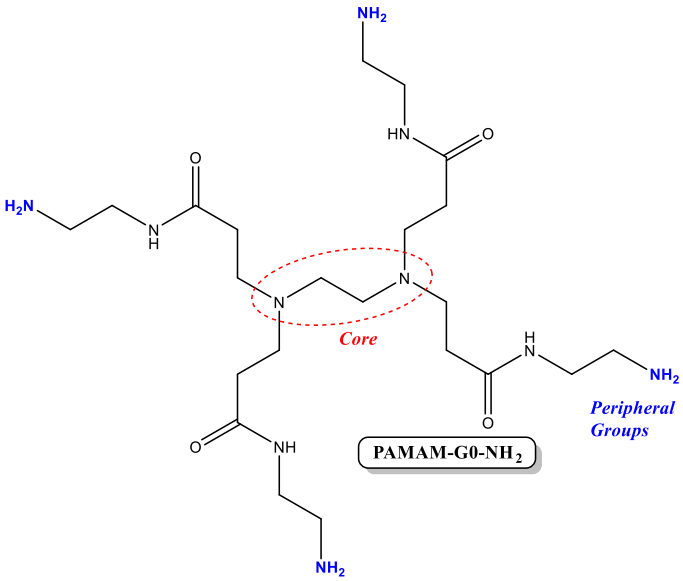
All the ***Research Activities*** proposed within the project, associated with these Specific Objectives, have been successfully completed, their degree of fulfillment being 100%.

In order to improve the solubility/bioavailability of the antifungal active substance Ketoconazole we developed a drug-carrier product based on PAMAM dendrimer of G5 generation with peripheral amino groups, **KTZ-PAMAM-G5-$NH_2$**. The encapsulation of KTZ in the PAMAM dendrimer is based on non-covalent, electrostatic and hydrogen bonding interactions. The obtained drug-delivery system is of supramolecular complex type, with an amorphous structure, and the higher generation PAMAM-NH2 dendrimer G5 allows the encapsulation in its cavities, respectively the transport, of a larger amount of the active pharmaceutical substance KTZ.



**Ketoconazole (KTZ)**

$C_{26}H_{28}Cl_2N_4O_4$
Molecular Weight: 531.43 g/mol

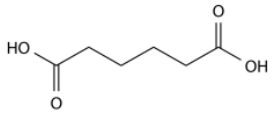



**PAMAM-G5-$NH_2$**
128 $NH_2$ peripheral groups
$C_{1264}H_{2532}N_{506}$
Molecular Weight: 28824.81 g/mol

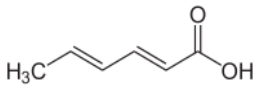
The **KTZ-PAMAM-G5-$NH_2$** formulation was physico-chemically characterized by specific analyzes for solid forms: X-ray powder diffraction, thermal behavior by DSC, FTIR and NMR on liquids spectroscopic methods, respectively purity by HPLC. The solubility in aqueous solution was studied by nephelometry, thus determining the concentration of KTZ in the complex *vs.* the concentration of the commercial KTZ corresponding to the precipitation process in water. The solid-state time stability of the complex was evaluated by exposure to ambient conditions and low temperature (4oC), the test indicating its stability. The *in vitro* release investigated by the dialysis method of the KTZ encapsulated in the PAMAM-G5-$NH_2$

dendrimer, in bidistilled water, indicated a slow, controlled process and the stability of the complex in solution. The lack of toxicity on human fibroblast cultures shows that the formulation is well tolerated by human skin cells, making it possible to use it safely. The epicutaneous auricular sensitization test on an animal model (MEST-mouse ear sensitization test) indicated the absence of allergic skin sensitivity. The obtained product **KTZ-PAMAM-G5-$NH_2$** is thus indicated for ***topical administration*** and can be used for the local therapy of cutaneous mycosis.

The crystalline forms of co-crystal type **Ketoconazole-Sorbic Acid** (**KTZ-SA**) and **Ketoconazole-Adipic Acid** (**KTZ-AA**), identified before the project, with improved physico-chemical properties *vs.* pure KTZ active substance, were evaluated for their potential as ***oral antifungal products***.




**Adipic Acid (AA)**




**Sorbic Acid (SA)**

In the preformulation stage, the following were evaluated: i) compatibility with pharmaceutical excipients, ii) the metastable crystallization zone, particularly important for iii) the scaling of the cocrystallization process on a large laboratory scale of the order of grams. This step provides an indication of how robust the cocrystallization process is and what the chances of transfer to industrial scale are (in the order of kilograms). The biocompatibility of the co-crystal formulations was investigated *in vitro* on human dermal fibroblast cultures, proving their tolerance on human skin cells. The *in vivo* biocompatibility testing step on animal model is also essential in order to assess the general acute toxicity in the case of oral administration. Acute toxicity tests performed on Wistar rats showed a lack of both biochemical and histological hepatic and general toxicity compared to pure KTZ, thus indicating improved biocompatibility of both co-crystal products. An increase in the bioavailability of KTZ results in the use of a lower concentration of the active substance to obtain the desired therapeutic effect, at the same time reducing the manifested hepatotoxicity caused especially by long-term treatment in the case of deep infections.

Project leader,
Dr. Eng. Martin Flavia-Adina