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NEW 5-DIMETHYL-3-HETEROARYL-1*H*-4-ONN-AZOXYCYANIDES: SYNTHESIS AND ANTIMYCOTIC ACTIVITY

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In the field of infectious diseases, in the last years an increasingly high importance is ascribed to mycosis as causes of illness and mortality in particularly susceptible patients: leukemia, organ transplant, AIDS and immunosuppressed patients, creating clinical and epidemiological problems. Although several drugs are available, antifungal spectrum is still limited especially for invasive infections, which often have fatal results. We previously reported the antifungal activity of a series of products, in which the 1,5-dimethyl-4-(cyano-NNO-azoxy) pyrazol-3-yl and 1,3-dimethyl-4-(cyano-NNO-azoxy) pyrazol-5-yl moieties were linked to pyridine, pyrazole, isoxazole, thiophene and the furan rings. All compounds displayed interesting antifungal activity against *Candida kruse*i and *Candida glabrata*, two fungal species resistant to azoles, is noteworthy. The presence of the cyano function appeared essential for activity. The need for novel antifungal agents lead us to synthesize and to investigate the antifungal activity of new ONN-azoxycyano derivatives in which the 3 position is replaced whit quinolinic and benzenic rings. The title compounds tested *in vitro* for antifungal activity against *C. glabrata* and *C. kruse*i, displayed remarkable antifungal activity, (MIC=0.25 and 0.5 lg/mL) that means that some of the title compounds were 16 and 32 fold more potent than Amphotericin B and Fluconazole, respectively. These results suggest that, depending on the heterocyclic molecule bound to the 3 position of 1*H*-4-ONN-azoxycianides, it is possible to modulate the antifungal activity of these new class of derivatives. Synthesis and *in vitro* biological test of title compounds will be reported.

IL-1β MAINTAINS THE DNA HYPERMETHYLATION OF ANTI-INFLAMMATORY *IL10* GENE IN A HUMAN INTESTINAL EPITHELIAL CELL LINE

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Intestinal inflammation is a natural process crucial to maintain gut integrity, but its deregulation is involved in the pathogenesis of severe intestinal disorders. Intestinal epithelial cells play a crucial role in the inflammatory response, modulating the immune cell exposure to antigens and by their ability to secrete many inflammatory mediators. IL-1β represents a pivotal player: secreted by infiltrated leucocytes, it induces the expression of several pro-inflammatory genes. Also the anti-inflammatory IL-10, whose function is to terminate the inflammatory process, modulates the intestinal physiology. Recent clinical reports showed that patients with ulcerative colitis in remission phase have significantly higher *IL10* gene expression in mucosa compared with active patients and controls. Moreover, in the latest years aberrant epigenetic mechanisms were put in binomial relationship with chronic inflammatory diseases. Previously, we described a demethylation of pro-inflammatory *IL6* and *IL8* genes in human colonic Caco-2 cells differentiated into an enterocyte-like phenotype and exposed to the inflammatory action of IL-1β. In the present study we evaluate whether the IL-1β treatment affected the methylation status of the anti-inflammatory *IL10* gene, in the same *in vitro* model. Our results showed that IL-1β treatment did not change the hypermethylation status of the *IL10* promoter. Moreover, in cell lysates from IL-1β-treated Caco-2 cells, we observed a dose-dependent increase of DNMTs activity and, surprisingly, a decrease of DNMT3b expression. These findings put in evidence the complexity of relationship between IL-1β and DNMTs, and may suggest a potential role of IL-1β as pleiotropic modulator of DNA methylation in Caco-2 cell line.

METHYLATION DECREASE OF *BECN1* GENE INDUCED BY PHYTOCHEMICAL INDICAXANTHIN IN CACO2 CELLS: AN EPIGENETIC HYPOTHESIS OF AUTOPHAGY

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Autophagy is a highly conserved catabolic process that degrades and recycles intracellular components through the lysosomes. The role of this process in tumor genesis and tumor progression is controversial: in the early stages, it can block tumor growth and conversely it can promote its progression in the later stages. The tumor suppressor *BECN1* gene, encodes the protein Beclin 1, a marker of autophagy down-regulated in several types of cancer, such as colorectal cancer. There are a lot of both genetic and environmental risk factors for colorectal cancer, including diet: for this reason, in accordance with epidemiological studies, consumption of foods rich in phytochemicals is widely promoted. The betalainindicaxantin (Ind) is a phytochemical from the Opuntia Ficus-Indica fruit having several biological activities, such as antioxidant, anti-inflammatory. It showed anti proliferative and proapoptotic effects in colorectal adenocarcinoma (Caco2) cells where was able to regulate gene expression through modulation of methylation state of DNA at CpG islands. For the first time, using Methylation-Sensitive Restriction Endonuclease PCR (MSRE-PCR), we report that Ind (50 e 100 μM) decreases the methylation of *BECN1* promoter in Caco2 cells, to the same extent as 5-azacytidine (Zcyd, positive control). Interestingly, colorimetric detection of DNA Methyl transferases activity, indicates that Ind reduced the activity of these enzymes, like Zcyd did. These preliminary data, indicating that Ind is able to decrease the methylation of *BECN1* gene, allow us to propose an epigenetic hypothesis of autophagy regulation in Caco2 cells.