1. Title of the Research Project: Design, synthesis and optimization of new multi-target compounds: melatonin agonists and MAGL inhibitors.
Keywords: melatonin agonists, MAGL inhibitors.

2. Research Area: CHIM/08 Pharmaceutical chemistry

3. General presentation of the project and state of the art

The endocannabinoid system (eCB) is composed by two G-coupled cannabinoid receptors, namely CB_1 and CB_2, their endogenous ligands, 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamine (AEA) and the enzymes involved in their biosynthesis and degradation. The eCB is prominent in both central and peripheral nervous systems. Its dysfunction is implicated in a wide range of pathological conditions, including pain, appetite, inflammation, memory and cognition, anxiety and depression, and cancer.[1]
Since direct activation of CB_1 receptors often occurs with several side effects such as substance abuse and loss of cognition and motor functions, recent drug discovery efforts moved to the regulation of the levels of endogenous endocannabinoids. AEA and 2-AG are produced through stimulus-dependent cleavage of membrane phospholipid precursors. After their release into the extracellular space, they are rapidly degraded by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively, belonging to the serine hydrolase superfamily.[1] Inhibition of these enzymes enhances the eCB tone at local levels exerting beneficial effects in animal models of pain, inflammation, cancer and neurodegeneration, while avoiding the classical drawbacks observed with exogenous CB1 agonists. For these reasons, remarkable efforts have been made both by academia and pharmaceutical companies to develop potent and selective FAAH or MAGL inhibitors.[2]
Another fascinating molecule which can reverse or prevent the course of several diseases is the neurohormone melatonin (MLT). MLT is primary secreted by the pineal gland at night and its functions are primarily mediated by the activation of two G-protein-coupled receptors, named MT_1 and MT_2. Modulation of melatoninergic system is considered an effective therapeutic strategy in several CNS-related pathologies, such as sleep and circadian rhythms disturbances, depression but also in neurodegenerative diseases, type 2 diabetes, stroke and cancer.[3] Recently, different series of dual-acting compounds, constituted by melatonin linked to another known neuroprotective agent, have been reported as novel potential therapeutic agents for the treatment of neurodegenerative disorders.[4]
Given the promising neuroprotective effects shown by melatonin receptor agonists and modulators of 2-AG endogenous level, the development of compounds able to modulate the eCB and melatonin system, also with multi-target mechanisms, may offer new therapeutic opportunities in the treatment of several neurodegenerative disease.

4. Research Objectives

The main scope of the present proposal is the design, synthesis and pharmacological characterization of an array of novel multitarget compounds, able to irreversibly inhibit MAGL enzyme and activate melatonin receptors, in order to develop new potential anti-inflammatory or neuroprotective agents.

5. Methodology and Expected Results

Structure-activity relationships (SARs) for MLT receptor ligands revealed that most of these compounds were structural analogues of MLT presenting three essential features: an aromatic ring bearing a methoxy group and an amide side chain in a relative arrangement similar to that present in MLT. On the other hand, most of reported MAGL inhibitors contain reactive carbamate, urea or azetidinyl amide moieties, which lead to inactivation of the enzyme by covalent modification of the active serine residue. Therefore, new

compounds potentially endowed with MAGL-inhibitor and melatoninergic activities will be designed and synthesized combining the pharmacophoric elements from known chemical classes of MAGL inhibitors and MLT receptor ligands agonists into a single molecule, taking also advantage of the availability of the X-ray structures for both targets. Examples of the proposed dual-target compounds are depicted in *Fig. 1*. In detail, the melatoninergic pharmacophore provided by an anilinoethylamide fragment (compounds A), a 2-acylaminomethyl tetrahydroquinoline (compounds B) or the indol-3-yl-ethylamide portion of melatonin (compounds C), was decorated with an azetidinyl substituent containing an appropriate urea or carbamate moiety to form covalent bonds with the catalytic Ser122 residue of the MAGL enzyme.

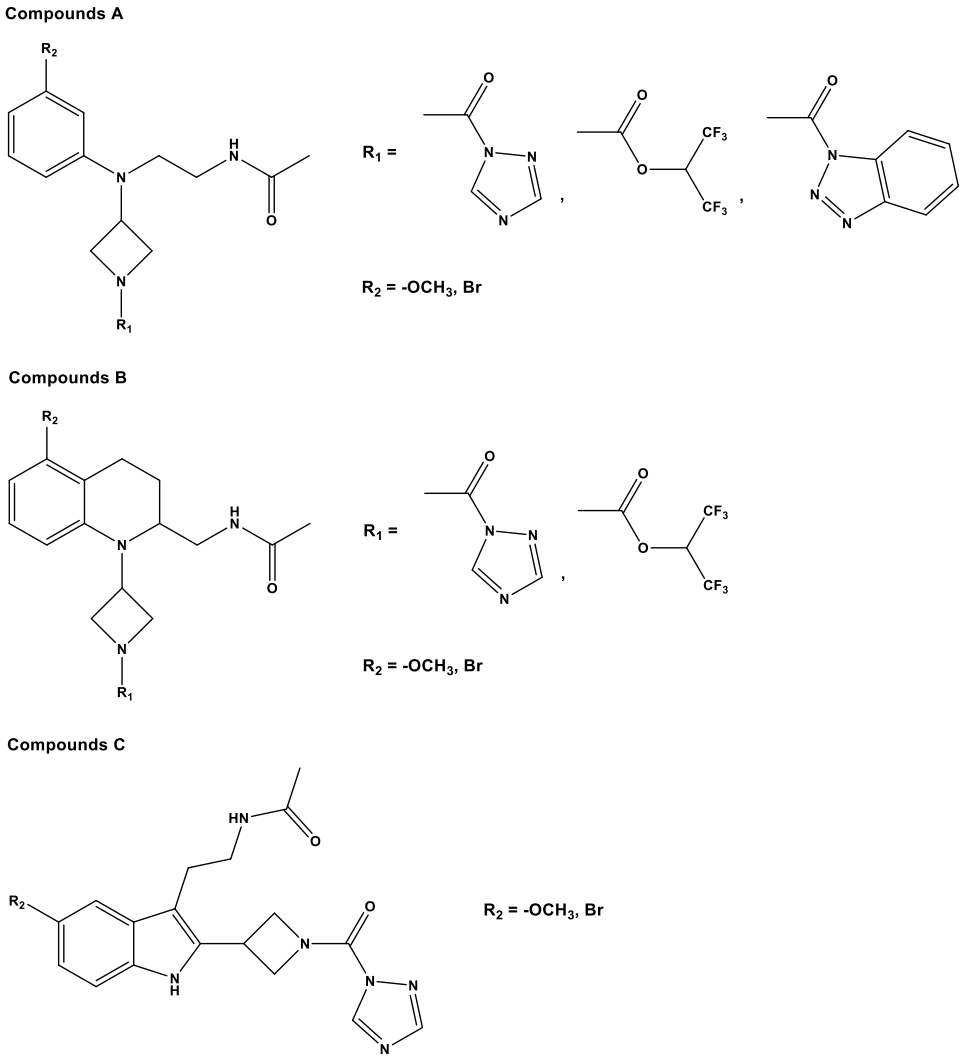

Fig. 1 Proposed novel multi-target compounds

Two possible retrosynthetic pathways for compounds A are shown in *Fig. 2*. The first hypothesis (arrow to the right) involves a reductive amination of commercially N-protected azetidin-3-one **4** with *N*-(2-aminoethyl)acetamide **3**, followed by a Pd-catalyzed N-arylation of the secondary amine **2** with 3-bromoanisole **1** and subsequent N-deprotection of the azetidine moiety. Finally, several approaches can be used for the introduction of different carbonyl-R^1 groups on the azetidine nitrogen, following experimental procedures already used for similar compound. For example, the combination of 1,1,1,3,3,3-hexafluoro-2-propanol, 4-nitrophenyl chloroformate, 4-dimethylaminopyridine (DMAP), pyridine, and Et_3N in CH_2Cl_2 can

be used to generate the hexafluoroisopropyl carbamates, whereas to introduce the triazolyl or benzotriazolyl carbonyls, triphosgene can be used as activating reagent. The second hypothesis (arrow to the bottom) starts from a reductive amination between the azetidinone **4** and anisidine **8**, followed by a second reductive amination with *N*-(2,2-dimethoxyethyl)acetamide **6**, a masked aldehyde, or in alternative the ethylamide chain can be introduced by N-cyanomethyl alkylation with bromoacetonitrile **7** and subsequent hydrogenation of the cyano group and contemporary N-acetylation of the crude intermediate amine. Substituent R_1 can be introduced following the synthetic steps illustrated above.

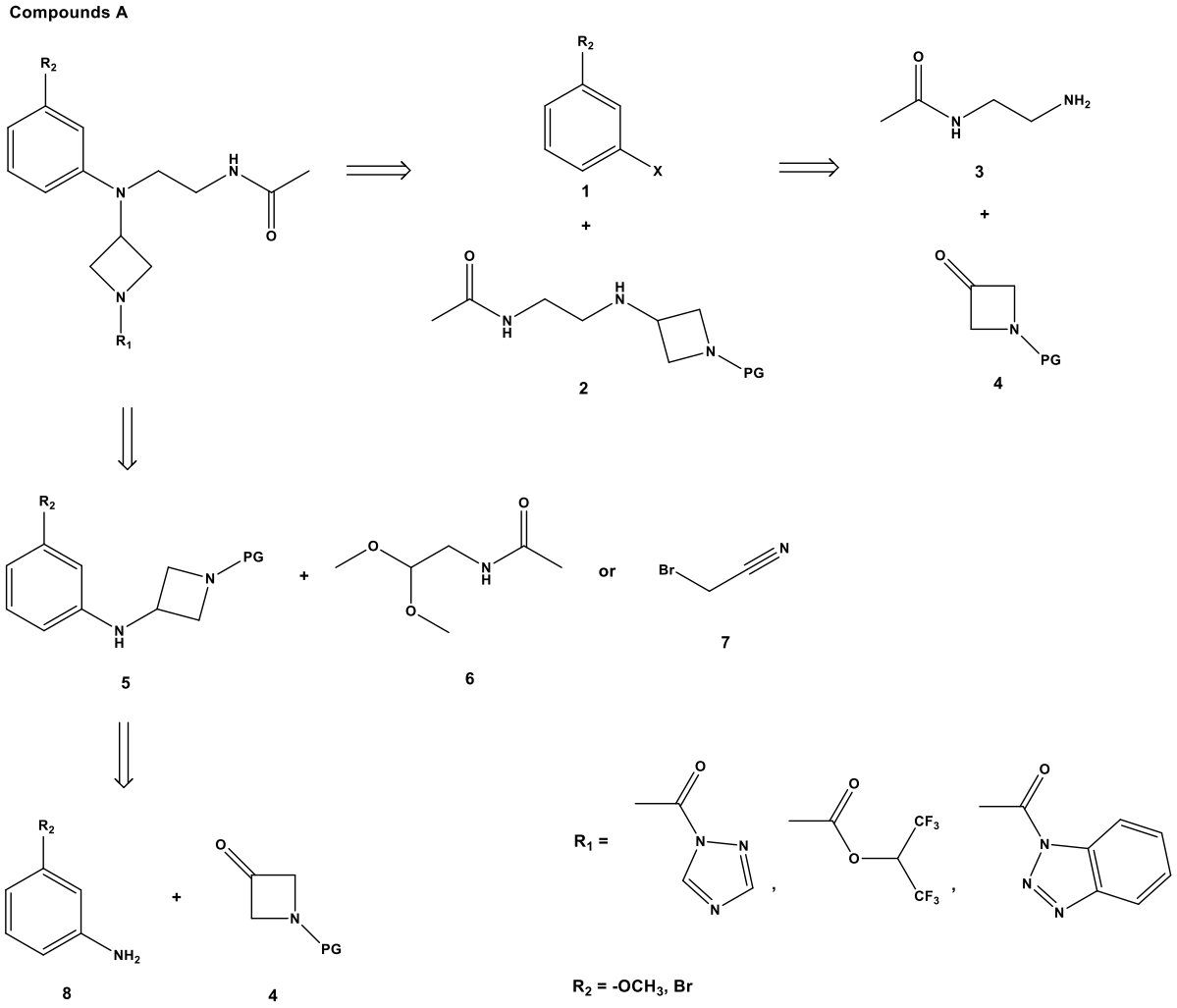

Fig. 2 Compounds A hypothetic retrosynthetic pathways

A different approach has been supposed for compounds B in *Fig.3*. Instead of a reductive amination, the azetidinyl moiety can be added by a nucleophilic substitution between N-((1,2,3,4-tetrahydroquinolin-2-yl)methyl)acetamide derivatives **9**, already known,[4] and N-protected azetidin-3-yl methanesulfonate **10**. Compound **10** can be obtained by reduction of N-protected azetidin-3-one **4** and subsequent mesilation of the intermediate alcohol. Introduction of different carbonyl-R^1 groups on the azetidine nitrogen can be achieved as reported for compounds A.

Compounds B

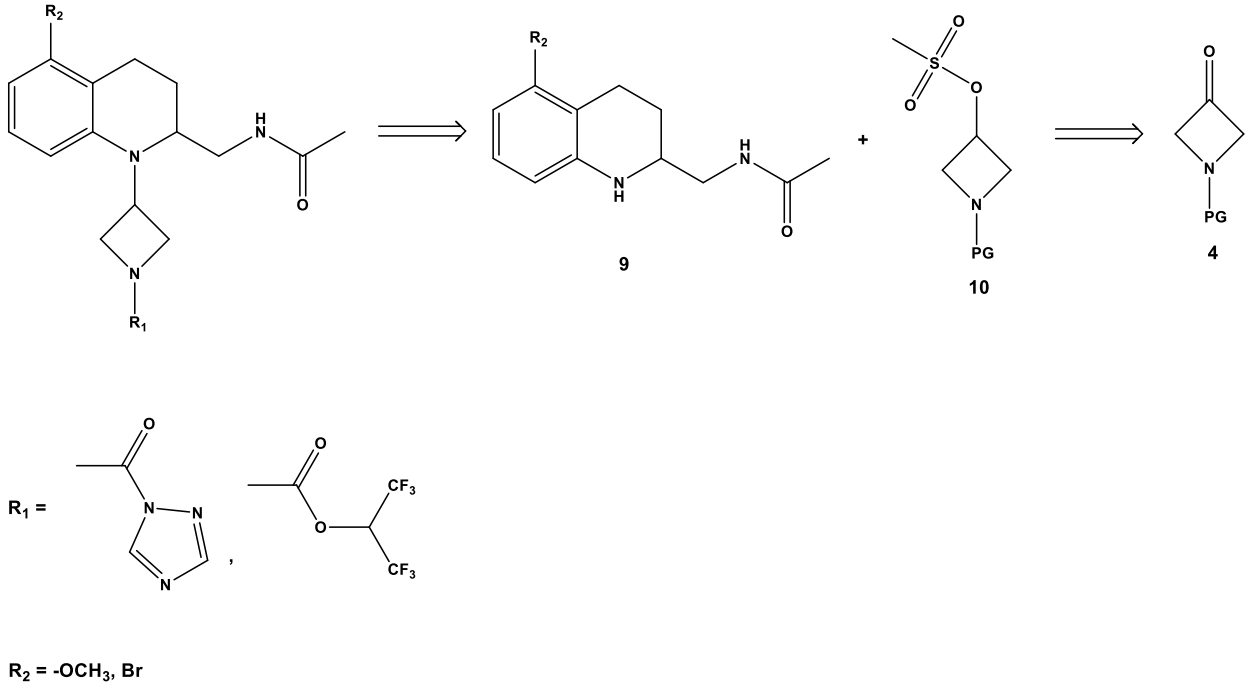

Fig. 3 Compounds B hypothetic retrosynthetic pathway

A possible synthesis of compounds C is shown in *Fig. 4* and involves a C_2-H-alkylation of MLT with N-protected 3-bromo-azetidine **11**[5] mediated by Pd(II) and norbornene or in alternative it can be applied a C_2-H-alkylation between **11** and a 5-sbstituted indole **13**, followed by C_3 reductive alkylation of the intermediate indole with **6** in presence of TES and TFA. After deprotection of the azetidine moiety, triazoyl carbonyl can be introduced as shown in the previous examples.

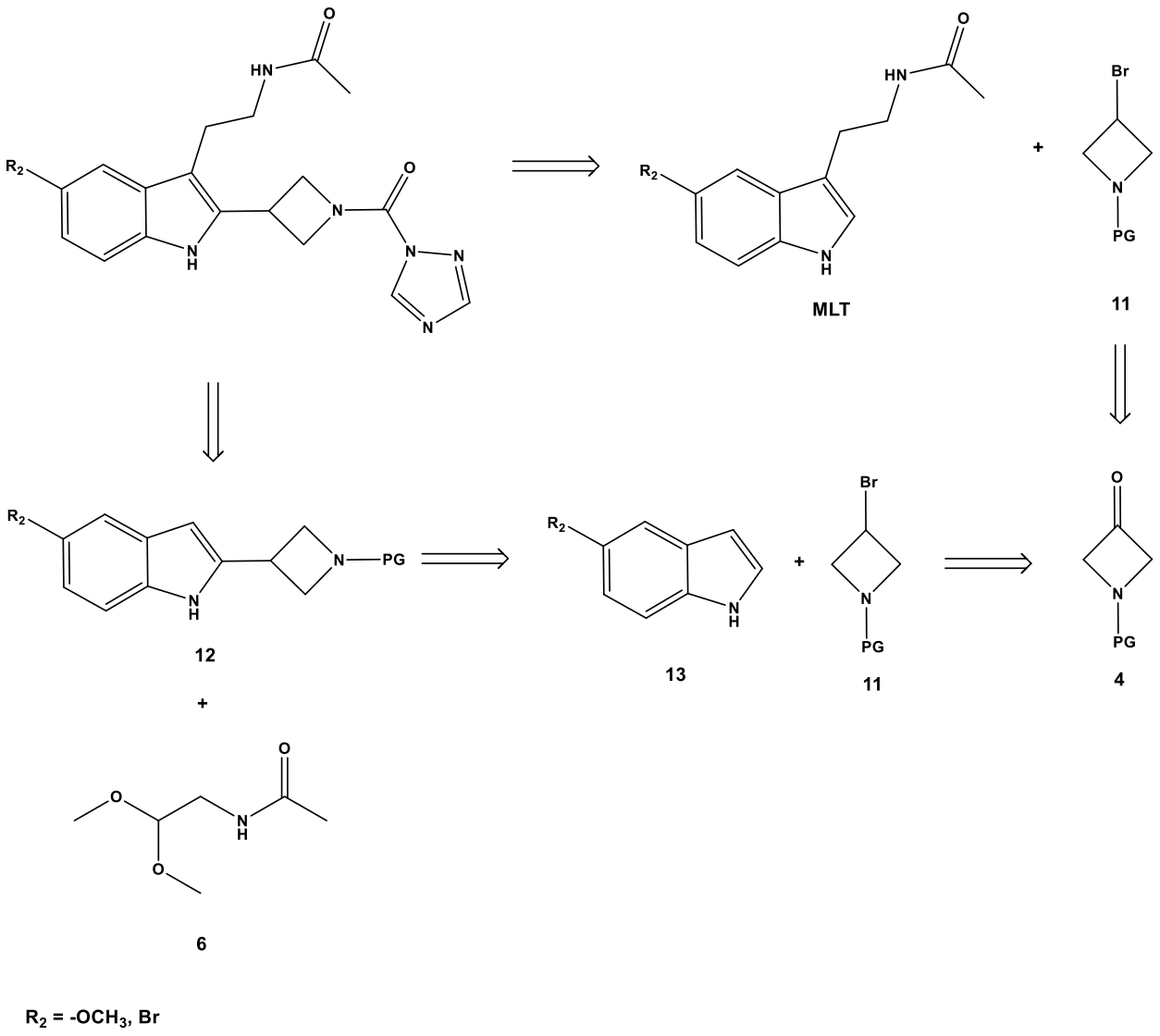

Fig. 4 Compounds C hypothetic retrosynthetic pathways

6. Bibliography

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7. Description of the research in the three-year period (feasibility)

The current proposal is based on different tasks with well-defined chemical and biological/pharmacological aims.

First year: Design and synthesis of a first series of potential dual-acting compound able to irreversibly inhibit MAGL enzyme and activate melatonin receptors. The affinity and selectivity of the new compounds will be evaluated in competition binding experiments in cloned human MT1 and MT2 receptors using 2-[^{125}I]iodomelatonin as the labeled ligand. The new compounds will be also investigated for their in vitro ability to inhibit MAGL enzyme.

Second year: Synthesis and structure-activity relationships of new compounds. The compounds endowed with the most promising activity on MAGL enzyme and MLT receptors will be tested in appropriate inflammation and neuroprotection experimental models.

Third year: Drug design and synthetic strategies to optimize the metabolic stability of selected compound, providing derivatives to be used as pharmacological tools to evaluate the role of MT1, MT2 receptors and MAGL enzyme in inflammation and neuroprotection. Writing of the final dissertation.