ABSTRACT

Histone lysine methyltransferases have crucial roles in a number of biological processes and human diseases by controlling gene expression and chromatin state. Within this family, the lysine methyltransferase G9a has emerged as critical player in several pathologic states, particularly because of its important role in the silencing of tumor suppressor genes and in the regulation of other chromatin events. The low number of G9a chemical probes suitable for cell-based and animal studies, as well as the limited chemical diversity demand for the development of new modulators.

In this thesis, two different approaches aimed at the identification of novel chemotypes for the modulation of G9a are presented. On one hand, from a medicinal chemistry prospective, we considered the modification of the central core of the potent and selective inhibitor UNC0638, resulting in a 1,4-benzodiazepine derivative **EML741**. To validate the approach, we designed and synthesized a small set of ring-expanded derivatives and tested their activity *in vitro*. Peptide-based biochemical assays (AlphaLISA) validated our design, as compound **EML741** preserves the activity of the parent compound. In fact, **EML741** is a G9a competitive inhibitor with respect to substrate endowed with potent activity and selectivity. In addition, **EML741** showed favorable physico-chemical properties as it is quite soluble and chemical stable in aqueous media, and exhibited a membrane permeability profile (PAMPA and PAMPA-BBB) better than the parent compound UNC0638.

The second approach was aimed to explore a high-diversity chemical space, generating a reporter cell line that enhance the expression of a fluorescent protein as result of chromatin changes provoked by G9a inhibition. By means of lentiviral infection, a construct encoding for a blue fluorescent protein (BFP) was integrated in the background of the human KBM7 cell line, in genomic *loci* whose chromatin organization is regulated by G9a activity. Two cell lines were selected and their specificity to detect G9a inhibition was preliminary evaluated. The validation of

these cell lines is still ongoing. When completed, this reporter could be used both in chemical and genetic screens.