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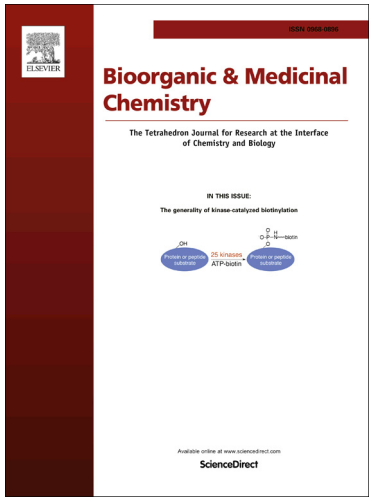

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Towards the development of activity-based probes for detection of Lysine-Specific Demethylase-1 activity

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ABSTRACT

The implications of Lysine-Specific Demethylase-1 (LSD1) in tumorigenesis have urged scientists to develop diagnostic tools in order to explore the function of this enzyme. In this work, we present our efforts on the development of tranylcypromine (TCP)-based functionalized probes for activity-based protein profiling (ABPP) of LSD1 activity. Biotinylated forms of selected compounds enabled dose-dependent enzyme labeling of recombinant LSD1. However, treatment with LSD1 inhibitors did not clearly reduce the LSD1 labeling efficiency thus indicating that labelling using these probes in not activity dependent. This calls for alternative strategies to develop probes for ABPP of the enzyme LSD1.

1. Introduction

Among post-translational modifications, histone lysine methylation has gained increasing attention as a major regulator of the transcriptional potential of eukaryotic cells. Lysine residues in histone tails (mainly of histones H3 and H4) can be mono-, di- or trimethylated on their ε-amino groups by lysine methyltransferases (KMTs) resulting in different functional outputs.[1,2] Lysine demethylases (KDMs) act as erasers of these marks and, depending on the enzymatic mechanism, they are categorized to lysine-specific demethylases (LSDs) and JumonjiC (JmjC) demethylases.[3] The first class are FAD (flavin adenine dinucleotide)-dependent amine oxidases that demethylate mono- or dimethylated lysine residues producing formaldehyde and hydrogen peroxide, whereas the second class utilizes Fe(II) and α-ketoglutarate as cofactors and can also act on trimethylated lysine side chains *via* its dioxygenase function.

Two mammalian flavin-containing demethylases have been identified: LSD1 (also known as KDM1A, AOF2, BHC110 or KIAA0601) and LSD2 (KDM1B or AOF1).[4,5] The best studied is LSD1 which is now well documented to represent an important player in developmental processes, embryogenesis and ~~differenti~~ation of numerous cellular types.[6-8] According to its substrates and interactions with other proteins, LSD1 is known to mediate transcriptional activation or repression.[9,10] Accumulating data suggest that any imbalance of the dynamic regulation of lysine methylation due to aberrant expression of LSD1 can cause dramatic alterations in gene transcription and, consequently, in the development and progression of various cancer types.[11-14] Nevertheless, several studies demonstrate that, in coordination with other proteins, LSD1 affects the growth of breast cancer cells negatively,[15-17] while promoting effects have been described for viral infections.[18,19]

Due to its significant role in pathogenesis, LSD1 has been an emerging pharmacological target and thus, the development of potent inhibitors has attracted increasing research interest. Up to date, a wide variety of compounds has been reported to inactivate LSD1 in a reversible[20-24] or irreversible way,[25-27] which have been evaluated mainly for their antiproliferative effects. The majority of them was inspired by several anti-MAO (monoamine oxidase) agents found to inhibit LSD1 with tranylcypromine (*trans*-2-phenylcyclopropyl-1-amine, TCP) taking center stage. TCP is a non-selective irreversible MAO A/B inhibitor with a reported potency over LSD1 ranging from 20-400 μM.[25,28] Structural and kinetic studies revealed a suicide-mechanism of time-dependent

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