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Novel polyamine analogues: From substrates towards potential inhibitors of monoamine oxidases

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ABSTRACT

New polyamine derivatives **1**–**8**, related to the previously reported N^1,N^{12}-dibenzyldodecane-1,12-diamine (Bis-Bza-Diado) and N^1-benzyl-spermine (BD6), have been synthesized and used as "probes" (potential substrates or inhibitors) of the human monoamine oxidases (MAO A and MAO B) and Vascular-Adhesion-protein −1 (VAP-1). Compound **8**, the most effective inhibitor of the series, is characterized by a 12-methylene carbon chain ending with an isothiocyanate (ITC) group. Interestingly, it behaves as competitive inhibitor of MAO B and as irreversible inhibitor of MAO A. Compound **3**, an asymmetric spermine analogue bearing a thiophene ring, acts as a reversible mixed inhibitor, selective for MAO B (K_{IE} = 23 μM). Docking studies performed using the available Protein Data Bank (PDB) structures of MAO A and MAO B, suggested that the different mode of inhibition of **8** may be explained by the different binding poses of **8** into the active site cavities of the two MAO isoforms. The ε-amino group of Lys 305 of MAO A is proposed as possible target of the ITC group of the inhibitor. Further studies are in progress to confirm this hypothesis.

These results indicate a potential use of the polyamine scaffold for the development of new MAO inhibitors for application in human pathologies involving these enzymes.

1. Introduction

Monoamine oxidase A and B (MAO A and MAO B) are FAD-containing enzymes bound to the mitochondrial outer membrane, which play essential roles in the catabolism of amine neurotransmitters in the brain and peripheral tissues. MAO A is more specific for serotonin, MAO B for benzylamine (BZA) and β-phenylethylamine, while both MAO isoforms are active against dopamine, adrenaline and noradrenaline. An increase of oxidative deamination catalysed by MAOs may lead both to a depletion of the neurotransmitter levels and to an overproduction of their by-products (hydrogen peroxide and aldehydes), which contribute to the development of an inflammatory and "oxidative stress" condition, generally associated to the initial stage and to the progression of neurodegeneration [1–4].

For these reasons, MAO A and MAO B are well-known targets of anti-neurodegenerative and anti-depressant therapies: MAO B mainly in Parkinson's and Alzheimer's diseases, while MAO A mainly in affective disorders [2,4].

Many MAO inhibitors have been developed and some of them are "old" drugs in clinical use, such as the anti-depressant,

Abbreviations: BZA, benzylamine; tyramine, *p*-tyramine; MAO, monoamine oxidase; VAP-1, semicarbazide-sensitive amine oxidase/vascular-adhesion-protein-1; Bis-Bza-Diado, N^1,N^{12}-dibenzyl-dodecane-1,12-diamine; BD6, N^1-(3-aminopropyl)-N^4-(3-(benzylamino)propyl)butane-1,4-diamine/N^1-benzyl-spermine; ITC, isothiocyanate; AITC, allyl isothiocyanate; K_{IE}, enzyme–inhibitor dissociation constant/inhibition constant; K_{IES}, enzyme–substrate complex-inhibitor dissociation constant; K_m, Michaelis–Menten constant; V_{max}, maximum velocity.

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irreversible MAO A inhibitor clorgyline and the antiparkinson, MAO-B irreversible inhibitors selegiline and rasagiline [2,5]. Most of the irreversible inhibitors are characterized by a propargylamine moiety, able to inactivate the FAD cofactor, and may cause side effects. To overcome this problem, new generations of inhibitors, such as the reversible MAO A inhibitors moclobemide and toloxatone were developed [5,6]. Additionally, recent findings on neuroprotective properties of some MAO inhibitors [2,6–8], have been a spur to develop novel MAO inhibitors. Although the drug discovery in this field has been focused mainly to treat neurological disorders, MAO A inhibitors might find a new application in treating different pathological conditions, such as prostate cancer; actually, it was identified high expression of MAO A in normal basal prostatic epithelium and high-grade primary prostate cancer [9,10].

In the last years, the renewed interest for MAO inhibitors has produced many patents: novel structures, such as pyrazole derivatives, hydrazine-based compounds and other heterocycles, such as chromones and chalcones, have been used as scaffolds and proposed for potential pharmacological applications, in particular in neurodegenerative diseases [5,9,11–14].

Little information is available about the effect of polyamine derivatives as MAO inhibitors. In our recent paper, we reported the MAO inhibitory activities of N^1,N^{12}-dibenzyldodecane-1,12-diamine (Bis-Bza-Diado) and of N^1-benzyl-spermine (BD6) [15]. In particular, we found that Bis-Bza-Diado behaved as a reversible, although weak MAO B inhibitor (K_I = 130 μM [15];). The polycationic structure of polyamines allows them to interact with a variety of cellular targets, which may be either activated or inhibited, suggesting that polyamine research is an important field for drug development with great potential for identifying new molecules for different diseases. Polyamine-based analogues have shown potential as antiproliferative [16], antiparasitic, antibacterial, and anti neurodegenerative [17] agents and could also be used as probes to investigate the role of cellular processes regulated by enzymes or transcription [18].

Based on our previous results, the aim of this study was to investigate the structural features required to improve the interactions of a polyamine scaffold with the MAO active sites. It was verified that it is possible to modulate both affinity and selectivity for diverse biological targets by inserting different groups onto a polymethylene backbone, as well as appropriate spacers separating the amine functions [19,20]. Thus, using BD6 and Bis-Bza-Diado as lead compounds, eight novel polyamine derivatives (**1**–**8**) were designed and synthesized to be tested as substrates or inhibitors of different types of amine oxidases, as potential therapeutic targets.

Firstly, the compounds were evaluated as potential substrates of both human MAOs and semicarbazide-sensitive amine oxidase named vascular-adhesion-protein-1 (VAP-1). VAP-1 is a human AO involved in the inflammatory processes and various disorders [21], and whose circulating form was found increased in plasma of patients with Alzheimer's disease [22,23]. The compounds which did not act as substrates were then tested as potential inhibitors.

The drug design and molecular structures of polyamines **1**–**8** are shown in Fig. 1.

The structure modifications applied to BD6 and Bis-Bza-Diado concerned both aromatic rings and nitrogen atoms, in order to improve the negligible activity of MAOs on spermine and the weak inhibitory effect of the two polyamine derivatives BD6 and Bis-Bza-Diado on MAO B [15].

To this aim we replaced the benzyl group of BD6 by different aromatic ring, such as naphthalene, pyridine and thiophene, providing compounds **1**, **2**, and **3**, respectively. By a kinetic

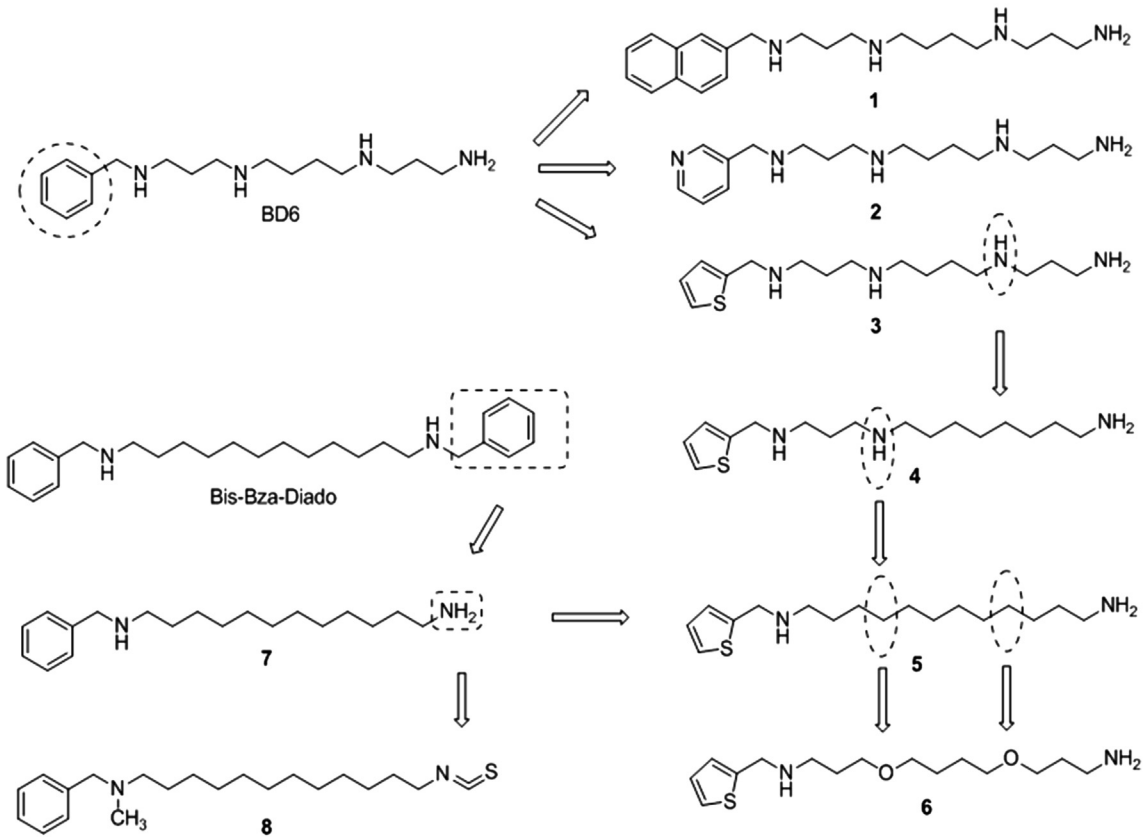

Fig. 1. Drug design of compounds **1**–**8**, starting from BD6 and Bis-Bza-Diado.