Biochimie 95 (2013) 1437–1449

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Synthesis and evaluation of the antioxidative potential of minoxidil–polyamine conjugates

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ARTICLE INFO

Article history:
Received 23 November 2012
Accepted 19 March 2013
Available online 28 March 2013

Keywords:
Minoxidil
Polyamines
Conjugates
Antioxidant activities
Anti-inflammatory activities
Lipoxygenase inhibitors
Lipid peroxidation
Cytotoxicity

ABSTRACT

A series of conjugates (MNX–CO–PA) of minoxidil (MNX) with the polyamines (PAs) putrescine (PUT), spermidine (SPD) and spermine (SPM) as well as dopamine were produced through activation of MNX with *N,N′*-carbonyldiimidazole, followed by reaction with dopamine or selectively protected PAs and acid-mediated deprotection. These conjugates together with conjugates of the general type MNX–PA or PA–MNX–PA, readily produced using literature protocols, were tested as antioxidants. The most potent inhibitors of lipid peroxidation were the conjugates MNX–SPM (**2**, 94%), SPM–MNX–SPM (**4**, 94%) and MNX–N^4-SPD (**7**, 91%) and MNX (91%). The most powerful lipoxygenase (LOX) inhibitors were MNX (IC_{50} = 20 μM) and the conjugates MNX–N^8-SPD (**9**, IC_{50} = 22.1 μM), MNX–CO–dopamine (**11**, IC_{50} = 28 μM) and MNX–N^1-SPD (**8**, IC_{50} = 30 μM). The most interesting conjugates **2**, MNX–CO–PUT (**5**), **8** and **11** as well as MNX were generally found to exhibit weaker (22–36.5%) or no (conjugate **8**) anti-inflammatory activity than indomethacin (47%) with the exception of MNX which showed almost equal potency (49%) to indomethacin. The cytocompatibility of conjugates and MNX at the highest concentration of 100 μM showed a survival percentage of 87–107%, with the exception of conjugates with SPM (compound **2**) and MNX–CO–SPM (**6**), which showed considerable cytotoxicity (survival percentage 8–14%). Molecular docking studies were carried on conjugate **9** and the parent compound MNX and were found to be in accordance with our experimental biological results.

1. Introduction

Minoxidil (MNX, Fig. 1) is an antihypertensive agent and a peripheral vasodilator. MNX exerts its antihypertensive effect through its sulphated metabolite (MNXS), known to cause opening of the K_{ATP} channels [1,2]. More importantly, MNX is currently the drug of choice in the clinical management of male pattern baldness (MPB), also known as androgenic alopecia. The exact mechanism through which MNX stimulates hair growth is not known although several *in vitro* effects of MNX have been associated with this activity [3]. Among the established effects of MNX, which is of relevance to the present study, is its ability to stimulate prostaglandin endoperoxide synthase (PGHS), also known as cyclooxygenase (COX), and in particular its PGHS-1 isoform and to increase production of PGE2 and leukotriene B4. In addition, MNX was found to inhibit prostacyclin synthesis, but these properties could not be directly

Abbreviations: AAPH, 2,2-azo-bis(2-amidinopropane) dihydrochloride; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); CDI, *N,N′*-carbonyldiimidazole; DMPO, 5,5-dimethyl-4,5-dihydro-3*H*-pyrrole N-oxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; ET, electron transfer; HAT, hydrogen atom transfer; HOSu, *N*-hydroxysuccinimide; LOX, Lipoxygenase; NDGA, nordihydroguaiaretic acid; PA(s), polyamine(s); ROS, Reactive Oxygen Species; PUT, putrescine; SPD, spermidine; SPM, spermine; DMPO 5, 5-Dimethyl-1-pyrroline-N-oxide; TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxyl radical; TFA, trifluoroacetic acid; Trt, trityl (triphenylmethyl).

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http://dx.doi.org/10.1016/j.biochi.2013.03.009

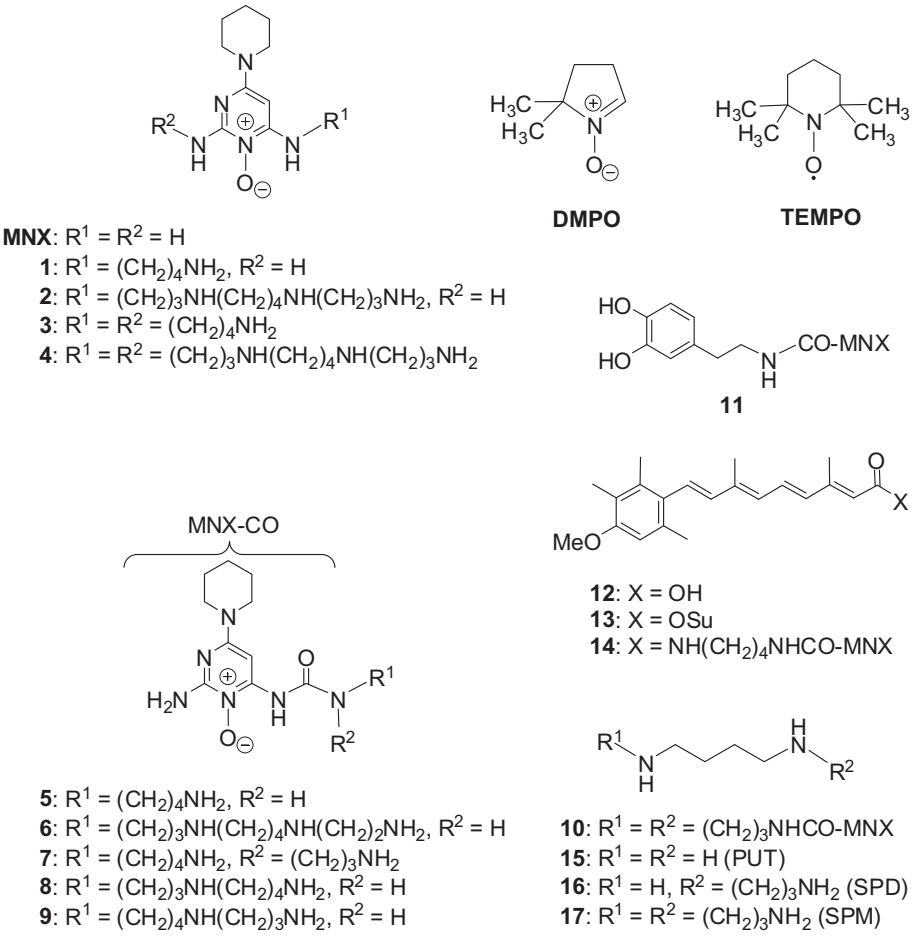

Fig. 1. Structures of compounds encountered in the present work.

correlated to hair growth [1]. MNX structurally resembles the spin traps nitrones, such as DMPO, and the spin labels, such as TEMPO (Fig. 1) [4]. Nitrones efficiently trap reactive oxygen-centred radicals (ROS), generating relatively stable and therefore long-lived nitroxyl radicals, thus associated with potential antioxidative activity [4,5].

On the other hand naturally occurring PAs, such as PUT, SPD and SPM, present antioxidative activity which is correlated to the number of the amino groups in the molecule (e.g. SPM more efficient than SPD) [6]. SPM is a natural antioxidant, which is found in all living organisms. Research shows that SPM has an important function in those areas of the body that are exposed to a high level of oxygen usage. This pertains to the skin, the brain, sperm cells and the lungs in particular. SPM's role is that of protecting cells against free radicals and their destructive effects [7]. Persistently high levels of ROS can modify essentially biological molecules, such as lipids, proteins and DNA. It is consistent that rates of ROS production are increased in most diseases. Oxidative stress has been associated with several human diseases such as cancer, neurodegenerative syndromes and inflammation. There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs.

Today, there is an increase interest in the combination of two pharmacophores on the same scaffold. This procedure leads to hybrid molecules or conjugates. Hybrid drugs, just as their name implies, combine two drugs in a single molecule with the goal of creating a chemical entity more medically effective than its individual components. These combo drugs can indeed be more powerful than either of their precursors [8].

Thus, we thought of interest to evaluate the antioxidative/anti-inflammatory activity of MNX and combine MNX with PAs in an attempt to produce MNX–PA conjugates with improved antioxidative/anti-inflammatory activity. Examples of such conjugates are the asymmetric conjugates **1**–**2** and **5**–**9** and the symmetric conjugates **3**–**4** and **10**. With the exception of conjugates **8** and **9**, the synthesis of all other conjugates has been recently disclosed by our group [9]. For the sake of the present study, the two linear SPD conjugates **8** and **9** were synthesized as well as two other novel types of MNX conjugates, namely the MNX–dopamine conjugate **11** and MNX–PA–drug hybrid conjugates, as exemplified with the MNX–PUT–ACI conjugate **14** (Fig. 1, fully extended structures of the MNX conjugates referred to above may be seen in the Supplementary data). The design of MNX–dopamine conjugate was based on previous studies which showed that incorporation of dopamine into antioxidant molecules leads in general to an improvement of the antioxidative properties of the latter [10]. On the other hand, the design of the hybrid conjugate **14** was based on the facts that (a) the retinoid acitretin (ACI) is a potent anti-inflammatory agent [10], (b) retinoids, such as *all-trans*-retinoic acid, have been used together with minoxidil for the promotion of