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Research paper

Aroylhydrazone iron chelators: Tuning antioxidant and antiproliferative properties by hydrazide modifications

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ABSTRACT

Aroylhydrazones such as salicylaldehyde isonicotinoyl hydrazone (SIH) are tridentate iron chelators that may possess antioxidant and/or antineoplastic activities. Their main drawback, their low stability in plasma, has recently been partially overcome by exchanging the aldimine hydrogen for an unbranched alkyl group. In this study, ten analogs of methyl- and ethyl-substituted SIH derivatives with modified hydrazide scaffolds were synthesized to further explore their structure-activity relationships. Their iron-chelation efficiencies, anti- or pro-oxidant potentials, abilities to induce protection against model oxidative injury on the H9c2 cell line derived from rat embryonic cardiac tissue, cytotoxicities on the same H9c2 cells and antiproliferative activities on MCF-7 human breast adenocarcinoma and HL-60 human promyelotic leukemia cell lines were evaluated. Compounds derived from lipophilic naphthyl and biphenyl hydrazides displayed highly selective antiproliferative activities against both MCF-7 and HL-60 cell lines, and they showed markedly improved stabilities in plasma compared to SIH. Of particular interest is a hydrazone prepared from 2-hydroxypropiophenone and pyridazin-4-carbohydrazide that showed a considerable antiproliferative effect and protected cardiomyoblasts against oxidative stress with a five-fold higher selectivity compared to the parent compound SIH. Thus, this work highlighted new structure-activity relationships among antiproliferative and antioxidant aroylhydrazones and identified new lead compounds for further development.

1. Introduction

Iron plays an important role in various vital metabolic processes. It is a key element of heme proteins, which are responsible for oxygen transport, and a part of numerous enzymes that mediate redox reactions [1–4]. Iron can exist in two oxidative states, Fe^{2+} and Fe^{3+}, which can donate or accept an electron. This interconversion in the presence of oxygen can lead to the production of reactive oxygen species through Fenton-type chemistry [5], resulting in damage to proteins, lipid membranes and other cellular structures. Due to the potential toxicity of free iron ions, most of them are bound to storage or transport proteins, and only a small fraction is kept free as a labile iron pool, where iron is bound to small intracellular chelates and is accessible to strong chelators [3]. A major part of extracellular iron is bound in transferrin, a transport protein which carries the iron from the plasma into cells. Within the cells, iron is stored in ferritin, which removes the iron from the labile iron pool and maintains its low level [3,4].

The human body has no regulated means of excreting iron, which is therefore limited to a desquamation of epithelial cells or minor bleeding. The iron homeostasis is thus maintained by regulating its dietary uptake, which is small due to the ability of human organisms to recycle the vast majority of iron from senescent erythrocytes and other sources [6]. The accumulation of free iron ions in organs leads to their damage [5]. In diseases caused by an iron overload, the major cause of death is iron-induced cardiomyopathy [7,8]. Biocompatible iron chelators have been developed

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and introduced into clinical practice to accelerate iron excretion in iron-overload diseases such as thalassemia or hereditary hemochromatosis [9,10]. For decades, the chelator of choice to has been deferoxamine (DFO, Fig. 1) [11]. This naturally occurring siderophore is highly hydrophilic and thus can be administered solely via long-term subcutaneous infusions [10]. Deferiprone [12] (L1, Fig. 1) has been the first orally active iron chelator used in clinical practice. Although the oral administration route is associated with better patient compliance [13], deferiprone therapy may be associated with several adverse effects [14]. Another orally administered iron chelator is deferasirox [15,16] (ICL670A, Fig. 1).

As cardiomyopathy represents the most frequent and serious complication of iron overload diseases, there has been a particular focus on the protection of the cardiac tissue against oxidative stress with all newly developed chelators [17,18]. Pyridoxal isonicotinoyl hydrazone (PIH, Fig. 1) described by Ponka and colleagues is an orally active aroylhydrazone chelator with a chelating effectivity comparable to that of DFO [19]. The replacement of the pyridoxal moiety with salicylaldehyde led to the synthesis of (*E*)-*N*′-(2-hydroxybenzylidene)isonicotinohydrazide (salicylaldehyde isonicotinoyl hydrazone, SIH, Fig. 1) and its derivatives. Along with the increased lipophilicity of SIH compared to PIH, the binding affinity of SIH to iron ions increased as well [20], and the protective effect against oxidative stress was emphasized [21–23]. Unfortunately, SIH suffers from rapid hydrolysis in plasma and a short elimination half-life *in vivo* [24]; thus, the search for new iron chelators with improved properties is still ongoing.

Iron chelators have also been systematically studied for their antiproliferative effect. The rationale behind this research is that cancer cells have a higher iron uptake demand to ensure their rapid growth [25]. This makes them sensitive to iron depletion caused by iron chelation as demonstrated by both *in vitro* and *in vivo* studies [26–28]. Nevertheless, the actual mechanism of the antiproliferative action of iron chelators may be more complex. The inhibition of the iron-containing enzyme ribonucleotide reductase can contribute to the overall antiproliferative effect because it affects a rate-limiting step in DNA synthesis [29]. Thiosemicarbazones (*e.g.*, Dp44mT, Fig. 1), a class of novel potent antitumor agents, form redox-active complexes with metals [30] and induce oxidative stress [31]. Additionally, some SIH analogs have shown considerable antiproliferative activities against MCF-7 human breast adenocarcinoma and HL-60 human promyelocytic leukemia cell lines [32], although the exact mechanism of their action remains unknown.

In our previous study, we were able to overcome the rapid hydrolysis of SIH by replacing the parent salicylaldehyde with a ketone, which greatly improved the stabilities of the new compounds in plasma [33]. The methyl- and ethyl-analogs of SIH, especially (*E*)-*N*′-[1-(2-hydroxyphenyl)ethylidene]isonicotinoylhydrazide (HAPI; Fig. 1) and (*E*)-*N*′-[1-(2-hydroxyphenyl)propylidene]isonicotinoylhydrazide (HPPI; Fig. 1), displayed comparable iron chelation and cardioprotective abilities to the parent SIH and even higher and more specific antiproliferative activities against MCF-7 human breast adenocarcinoma and HL-60 human promyelocytic leukemia cell lines compared to those of SIH [32,33].

In the present study we prepared derivatives of HAPI and HPPI with modified hydrazide parts (Fig. 2) to possibly improve their cytoprotective and/or antiproliferative activities as well as to further investigate the aroylhydrazone structure-activity relationships. In the first series, the 4-pyridyl cycle of HAPI or HPPI was replaced with the more lipophilic aromate, namely biphenyl-4-yl (Fig. 2, compounds **4a** and **5a**) or 1-naphthyl (Fig. 2, compounds **4b** and **5b**). The rationale behind this modification is that increased lipophilicity is connected with enhanced transport through biological membranes and may enhance the target activity of aroylhydrazones as previously described [34,35]. In addition, this

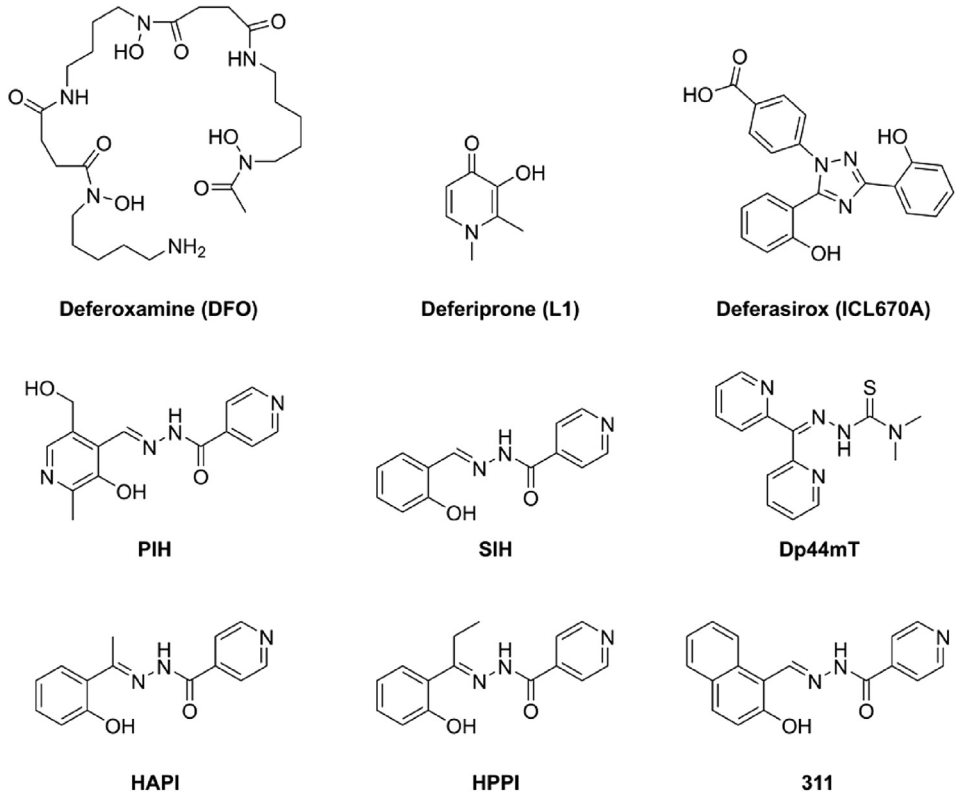

Fig. 1. Chemical structures of previously described iron chelators.