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Original article

Novel imidazole derivatives as heme oxygenase-1 (HO-1) and heme oxygenase-2 (HO-2) inhibitors and their cytotoxic activity in human-derived cancer cell lines

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ABSTRACT

Heme oxygenase (HO) is a cytoprotective enzyme that can be overexpressed in some pathological conditions, including certain cancers. In this work, novel imidazole derivatives were designed and synthesized as inhibitors of heme oxygenase-1 (HO-1) and heme oxygenase-2 (HO-2). In these compounds the imidazole ring, crucial for the activity, is connected to a hydrophobic group, represented by aryloxy, benzothiazole, or benzoxazole moieties, by means of alkyl or thioalkyl chains of different length. Many of the tested compounds were potent and/or selective against one of the two isoforms of HO. Furthermore, most of the pentyl derivatives showed to be better inhibitors of HO-2 with respect to HO-1, revealing a critical role of the alkyl chain in discriminating between the two isoenzymes. Compounds which showed the better profile of HO inhibition were selected and tested to evaluate their cytotoxic properties in prostate and breast cancer cell lines (DU-145, PC3, LnCap, MDA-MB-231, and MCF-7). In these assays, aryloxyalkyl derivatives resulted more cytotoxic than benzothiazolethioalkyl ones; in particular compound **31** was active against all the cell lines tested, confirming the anti-proliferative properties of HO inhibitors and their potential use in the treatment of specific cancers.

1. Introduction

Heme oxygenase (HO) is the rate-limiting enzyme that catalyzes the metabolism of the pro-oxidant agent heme into equimolar amounts of carbon monoxide (CO), free iron, and the bile pigment biliverdin, which in turn is reduced to bilirubin by biliverdin reductase [1]. Each of these metabolites has regulatory functions: CO is a cell-signaling molecule with anti-inflammatory, anti-proliferative, and antiapoptotic effects [2], biliverdin and bilirubin possess antioxidant properties [3].

Three isoforms of HO have been identified so far, namely HO-1, HO-2, and HO-3 [4], but enzymatic activity is attributable to two functional isoforms only: HO-1, which is highly inducible and HO-2, which is constitutive. HO-1 is a 32 kDa heat shock protein that is expressed in tissues rich in reticuloendothelial cells such as spleen. In other tissues its expression is induced by numerous stimuli, including its substrate heme, heavy metals, reactive oxygen species, hypoxia, NO, ultraviolet radiations, and xenobiotics. HO-2 is a 36 kDa protein constitutively present in brain and testis, but also in endothelium, distal nephron segment, liver, and gut myenteric plexus.

The biological functions of HO are mainly associated with a basic adaptive and defensive response against oxidative and cellular stress and with a maintaining of cellular homeostasis [5]. Consequently, HO-1 up-regulation might have therapeutic applications in many oxidative stress-associated diseases, such as diabetes, obesity, cardiovascular and eye diseases, atherosclerosis, inflammation, and vascular injury. However, over-activity of the HO

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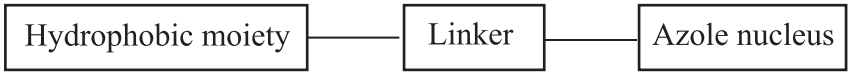
system may not exert only cytoprotective effects, but may contribute to tissue injury under certain unfavorable cases. HO-1 down-regulation or direct inhibition may ameliorate diseases in which HO over-activity is observed, for example hyperbilirubinemia and neonatal jaundice [6], and various bacterial or fungal infectious diseases [7]. Moreover, an increased expression of HO-1 has been observed in several cancers, suggesting that one of the main therapeutic applications of HO-1 inhibitors could be the treatment of specific cancers. Multiple studies confirm implications of HO in various tumors [8,9] and the effectiveness of treatment with HO inhibitors [10,11].

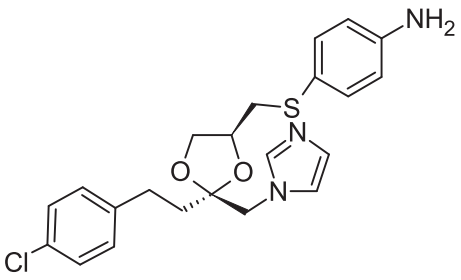
Most of the actual knowledge on HO system is related to HO-1 isoform, whereas spare information is available on HO-2 isoform. Recent works report significant tumor regression mediated by a down regulation of both HO-1 and HO-2 system [12]. Therefore, selective HO-1 or HO-2 inhibitors may provide useful tools for the elucidation of the physiological roles of HO system and may have important clinical applications.

In the recent years, a number of imidazole-based compounds derived from structural modifications of the first described non-porphyrin HO-inhibitor azalanstat **1** (Fig. 1) [13] have been reported as HO inhibitors. Their chemistry and the meaningful structural insights into human HO-1 inhibition have been recently reviewed [14]. In brief, three key chemical moieties are necessary for inhibition of HO: an azolyl nucleus, a hydrophobic portion, and a central alkyl linker. This linker may have different length and may incorporate a dioxolane ring, a ketone or an alcohol function and heteroatoms such as sulfur or oxygen. Some representative compounds **1**–**7**, possessing the above-mentioned chemical features, are depicted in Fig. 1 [15–18].

Selected compounds have been more deeply studied and have shown to be effective also in intact cells and in *in vivo* models. For example, it has been demonstrated that compound **5** inhibits cell proliferation *in vitro* and tumor growth *in vivo* when tested in a model of hormone-refractory prostate cancer [19].

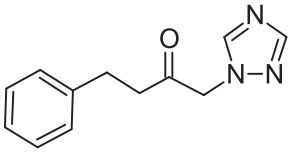
Continuing our study on imidazole-based compounds as enzymatic inhibitors [20–22], we recently investigated a series of aryloxyalkyl imidazoles as HO-1 and HO-2 inhibitors [17,18]. Among them, 1-[4-(3-bromophenoxy)butyl]-1*H*-imidazole **6** and 1-[4-(4-iodophenoxy)butyl]-1*H*-imidazole **7** (Fig. 1) showed the highest inhibitory potency. HO-1 overexpression, together with a variety of molecular mechanisms, is responsible of resistance to imatinib





azalanstat (1)

IC_{50} HO-1 = 5.5 μM, IC_{50} HO-2 = 24.5 μM



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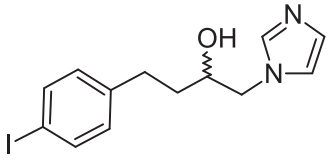
IC_{50} HO-1 = 2.6 μM, IC_{50} HO-2 = 34 μM



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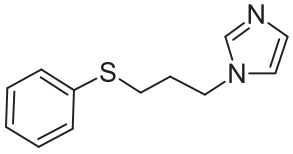
IC_{50} HO-1 = 0.06 μM, IC_{50} HO-2 = 1.8 μM



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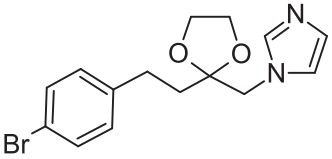
IC_{50} HO-1 = 2.4 μM, IC_{50} HO-2 = 16 μM



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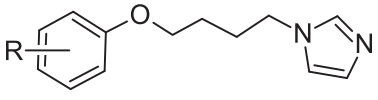
IC_{50} HO-1 = 1.9 μM, IC_{50} HO-2 = >100 μM



6, 7

6 R = 3-Br, IC_{50} HO-1 = 2.1 μM, IC_{50} HO-2 = 2.2 μM

7 R = 4-I, IC_{50} HO-1 = 1 μM, IC_{50} HO-2 = 10 μM

Fig. 1. Chemical structures and IC_{50} values of HO inhibitors **1**–**7**.