Journal of Inorganic Biochemistry 141 (2014) 43–54

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

Synthesis and biological evaluation of 2-benzoylpyridine thiosemicarbazones in a dimeric system: Structure–activity relationship studies on their anti-proliferative and iron chelation efficacy

Adeline Y. Lukmantara [a,*], Danuta S. Kalinowski [b], Naresh Kumar [a,*], Des R. Richardson [b,**]

[a] School of Chemistry, University of New South Wales, Sydney, NSW 2052, Australia
[b] Molecular Pharmacology and Pathology Program, Department of Pathology and Bosch Institute, University of Sydney, Sydney, NSW, 2006, Australia

ARTICLE INFO

Article history:
Received 1 July 2014
Received in revised form 29 July 2014
Accepted 29 July 2014
Available online 7 August 2014

Keywords:
Thiosemicarbazone
Dimer
Lipophilicity
Iron

ABSTRACT

Thiosemicarbazone chelators represent an exciting class of biologically active compounds that show great potential as anti-tumor agents. Our previous studies demonstrated the potent anti-tumor activity of the 2′-benzoylpyridine thiosemicarbazone series. While extensive studies have been performed on monomeric thiosemicarbazone compounds, dimeric thiosemicarbazone chelators have received comparatively less attention. Thus, it was of interest to investigate the anti-proliferative activity and iron chelation efficacy of dimeric thiosemicarbazones. Two classes of dimeric thiosemicarbazones were designed and synthesized. The first class consisted of two benzoylpyridine-based thiosemicarbazone units connected *via* a hexane or dodecane alkyl bridge, while the second class of dimer consisted of two thiosemicarbazones attached to a 2,6-dibenzoylpyridine core. These dimeric ligands demonstrated greater anti-proliferative activity than the clinically used iron chelator, desferrioxamine. This study highlights the importance of optimal lipophilicity as a factor influencing the cytotoxicity and iron chelation efficacy of these chelators.

1. Introduction

Thiosemicarbazones have received significant attention from academia and the pharmaceutical industry over the past 50 years due to their rich biological activity [1]. These properties include potent anti-viral [2,3], anti-bacterial [4–6] and anti-cancer [7,8] efficacy. The α-(*N*)-heterocyclic class of thiosemicarbazones are derived from the Schiff base condensation reaction of thiosemicarbazides with aldehydes or ketones [1]. These ligands possess a conjugated *N*,*N*,*S* tridentate system that has been shown to be particularly important for their cytotoxic activity [9]. This effect is due to the fact that the N and S atoms are able to act as "soft" electron donors and chelate transition metal ions such as iron (Fe), copper and zinc to form cytotoxic metal complexes [5,9–11]. Such complexes can catalyze the formation of reactive oxygen species (ROS), including the hydroxyl radical, that can damage DNA and inhibit cellular proliferation [12–14].

Iron is an essential element for many cellular processes, including energy production, electron transport and DNA synthesis due to its role as a co-factor for proteins such as oxidases, cytochromes and ribonucleotide reductase [15–17]. While tumor cells and normal cells share highly similar biochemical processes, the higher requirement for Fe in cancerous tissues highlights the potential to target Fe and develop novel and selective chemotherapeutics [1,18–21]. The enhanced demand for Fe in tumor cells arises, at least in part, from the increased Fe requirement for enzymes that play critical roles in metabolism. These include ribonucleotide reductase (RR), an Fe-containing enzyme that catalyzes the conversion of ribonucleotides to deoxyribonucleotides, which is the rate-limiting step of DNA synthesis [1,15]. Hence, the inactivation of RR *via* Fe deprivation can inhibit DNA synthesis and cellular proliferation of tumor cells [22,23]. Furthermore, Fe chelation up-regulates the potent growth and metastasis suppressor, N-myc downstream regulated gene-1 (NDRG1), which suppresses multiple signaling pathways involved in tumorigenesis and metastasis [24–27].

Due to the importance of Fe in cancer cell proliferation, numerous Fe-chelating compounds have been investigated as potential anti-cancer agents [1]. For example, the thiosemicarbazone chelator, 3-aminopyridine 2-carboxaldehyde thiosemicarbazone (known as 3-AP or Triapine®), exhibited inhibition of L1210 leukemia cells *in vitro* and *in vivo* [15] and suppressed the growth of murine M109 lung carcinoma and human A2780 ovarian carcinoma xenografts in mice [20,28–31]. In fact, Triapine® was assessed in over 20 Phase I and II clinical trials and

* Corresponding authors at: Department of Chemistry, University of New South Wales, Australia. Tel.: +61 2 9385 4698.
** Correspondence to: D.R. Richardson, Department of Pathology and Bosch Institute, University of Sydney, Sydney 2006, Australia. Tel.: +61 2 9036 6548; fax: +61 2 9351 3429.
E-mail addresses: adeline.lukmantara@gmail.com (A.Y. Lukmantara), n.kumar@unsw.edu.au (N. Kumar), d.richardson@med.usyd.edu.au (D.R. Richardson).

http://dx.doi.org/10.1016/j.jinorgbio.2014.07.020
0162-0134/

recently has shown promising activity against cervical and vaginal cancers in combination with cisplatin and radiotherapy [32].

Other thiosemicarbazone-based chelators, including the 2′-benzoylpyridine thiosemicarbazone (BpT; **1**; Fig. 1), 2′-(3-nitrobenzoyl) pyridine thiosemicarbazone (NBpT; **2**; Fig. 1) and halogenated 2′-benzoylpyridine thiosemicarbazone (XBpT; **3**; Fig. 1) series have also shown potent and selective anti-tumor activity *in vitro* and *in vivo* (Fig. 1) [33,34]. In particular, 2′-benzoylpyridine 4,4-dimethyl-3-thiosemicarbazone (Bp44mT) showed potent anti-tumor activity upon oral administration to nude mice bearing human DMS-53 lung cancer xenografts and was very well tolerated [35]. Recently, we have also developed substituted 2′-benzoyl-6-methylpyridine thiosemicarbazones and 4-phenyl-substituted 2′-benzoylpyridine thiosemicarbazones that demonstrated selective anti-proliferative activity towards cancer cells *in vitro* [36,37].

While extensive studies have been performed on monomeric thiosemicarbazones compounds [1,33,36,37], dimeric thiosemicarbazone chelators have received comparatively less attention. Previous studies on the dimerization of thiazolones, quinones and aminopyridines, produced compounds with improved potency compared to the original monomers [38,39]. One of the most studied groups of dimeric thiosemicarbazones were the bis(thiosemicarbazone) series (**4**; Fig. 2). These ligands were composed of two thiosemicarbazone moieties connected by their imine nitrogens *via* a two-carbon bridge [40–42]. Earlier studies showed that bis(thiosemicarbazones) derived from dialdehydes, ketoaldehydes and diketones demonstrated anti-viral effects and anti-tumor activity against HeLa cells [42–45]. The copper(II), zinc(II), platinum(II) and palladium(II) complexes of bis(thiosemicarbazone) also possessed anti-neoplastic properties [43,45–54].

Due to the potential observed with bis(thiosemicarbazones), other dimeric structures were also investigated. Dithiosemicarbazones (**5**) are a class of dimeric thiosemicarbazones that consist of two thiosemicarbazone units connected by their amide nitrogen N(4) atoms *via* an aliphatic or aromatic spacer [41,42]. Notably, studies by Gingras et al. [55] have demonstrated that the dithiosemicarbazones (**5**) exhibited effective anti-fungal activity and *in vitro* anti-tumor activity [41,56]. Furthermore, the copper(II), platinum(II) and palladium(II) complexes of dithiosemicarbazones also showed anti-fungal, anti-bacterial and anti-tumor activities [40,41,56]. It was demonstrated that the dithiosemicarbazones formed 1:1 complexes with copper, in which the two thiocarbonyl groups of the symmetrical dithiosemicarbazone acted as donor atoms to a single copper ion [56].

Considering the promising anti-proliferative properties exhibited by our monomeric thiosemicarbazone chelators [33,34], it was of interest to investigate the effect of dimerization of these compounds on their biological activity. In fact, chelators of higher denticity generally form more stable Fe complexes than those chelators of lower denticity [1]. Additionally, dimers have demonstrated greater Fe chelation efficacy *in vivo* than their corresponding monomer [1]. Thus, it was important to further explore the biological activity of dimeric thiosemicarbazones derived from the potent BpT series [33]. In this study, two types of dimerization strategies were explored. In the first approach, we investigated the synthesis of dithiosemicarbazones containing two benzoylpyridine moieties connected *via* an alkyl bridge. Previous studies have employed a variety of linkers to connect iron chelator moieties, ranging from 2 to 11 atoms in length [1,40,41]. Thus, we synthesized dithiosemicarbazones using both a short (hexyl) and long (dodecyl) alkyl bridge to investigate the effect of the linker length on anti-proliferative activity and iron chelation efficacy. The second strategy employed 2,6-dibenzoylpyridine (**6**) as a core scaffold to connect together two thiosemicarbazide "tails". We herein report the synthesis of two novel dithiosemicarbazones and five 2,6-dibenzoylpyridine thiosemicarbazones (2,6-diBpT), and their ability to mobilize cellular Fe and inhibit Fe uptake from the Fe transport protein, transferrin (Tf).

Notably, this investigation represents the first attempt to combine two thiosemicarbazide "tails" into a single molecule using dibenzoylpyridine. In addition, it is also the first study to investigate the anti-proliferative activity of dimeric dibenzoylpyridine thiosemicarbazones. The current research highlights important structure–activity relationships regarding the structural requirements necessary for dimeric thiosemicarbazone-based chelators with potent anti-cancer activity.

2. Experimental

2.1. Chemicals

All commercially available reagents were purchased from Fluka, Sigma Aldrich, Alfa Aesar and Lancaster, and used without further purification. Desferrioxamine (DFO) was purchased from Novartis, Basel, Switzerland. All reactions requiring anhydrous conditions were performed under an argon/nitrogen atmosphere. Anhydrous solvents were obtained using a PureSolv MD Solvent Purification System.

2.2. Physical measurements

^{1}H and ^{13}C NMR spectra were obtained in the selected solvent on a Bruker DPX 300 spectrometer at the designated frequency and were internally referenced to the solvent peaks. Chemical shifts (δ) are in parts per million (ppm) downfield from tetramethylsilane (TMS) and the observed coupling constant (*J*) is in Hertz (Hz). Multiplicities are recorded as singlet (s), doublet (d), doublet of doublet (dd), doublet of triplet (dt), triplet (t), quarter (q), multiplet (m) and broad singlet (bs), where appropriate. Melting points were measured using a Mel-Temp melting point apparatus and are uncorrected. Microanalysis was performed on a Carlo Erba Elemental Analyzer EA 1108 at the Campbell Microanalytical Laboratory, University of Otago, New Zealand. Infrared spectra were recorded with a Thermo Nicolet 370 FTIR spectrometer as KBr disks. Ultraviolet visible spectra were recorded using a Varian Cary 100 Scan spectrometer in the designated solvents and data reported as wavelength (λ) in nm and extinction coefficient (ε) in $cm^{-1}\,M^{-1}$. Gravity column chromatography was carried out using Grace Davison LC60A 40–63 μm silica gel. Flash chromatography was done implementing Grace Davison LC60A 6–35 μm silica gel. Reactions were monitored using thin layer chromatography, performed on Merck DC aluminum plates coated with silica gel GF_{254}. Compounds were detected by short and long wavelength ultraviolet light. The log P_{calc} values were calculated using ChemBio Draw Ultra 13.0.

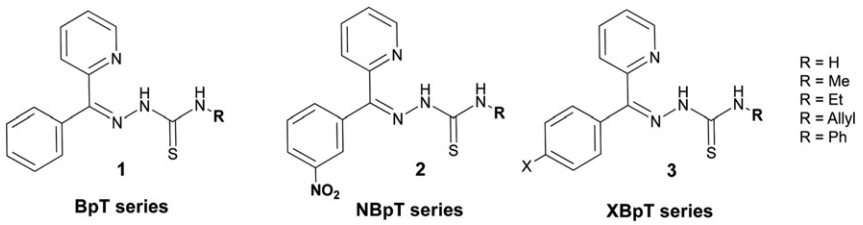

Fig. 1. General structures of the BpT, NBpT and XBpT series of thiosemicarbazone chelators.