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Synthesis and evaluation of a class of 1,4,7-triazacyclononane derivatives as iron depletion antitumor agents

Sheng Wang [a,d], Yongkang Gai [b,c,d], Shasha Zhang [a], Lei Ke [a], Xiang Ma [a,*], Guangya Xiang [a,*]

[a] School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China
[b] Department of Nuclear Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China
[c] Hubei Province Key Laboratory of Molecular Imaging, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

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ABSTRACT

Iron depletion has been confirmed as an efficient strategy for cancer treatment. In the current study, a series of 1,4,7-triazacyclononane derivatives HE-NO2A, HP-NO2A and NE2P2A, as well as the bifunctional chelators *p*-NO_2-PhPr-NE3TA and *p*-NH_2-PhPr-NE3TA were synthesized and evaluated as iron-depleting agents for the potential anti-cancer therapy against human hepatocellular carcinoma. The cytotoxicity of these chelators was measured using hepatocellular cancer cells and compared with the clinically available iron depletion agent DFO and the universal metal chelator DTPA. All these 1,4,7-triazacyclononane-based chelators exhibited much stronger antiproliferative activity than DFO and DTPA. Among them, chelators with phenylpropyl side chains, represented by *p*-NO_2-PhPr-NE3TA and *p*-NH_2-PhPr-NE3TA, displayed the highest antiproliferative activity against HepG2 cells. Hence, these compounds are attractive candidates for the advanced study as iron depletion agents for the potential anti-cancer therapy, and could be further in conjugation with a targeting moiety for the future development in targeted iron depletion therapy.

Iron is essential in many important cellular processes, such as oxygen delivery, electron transport, and DNA repair.[1] However, excessive or misplaced tissue iron may donate electrons to oxygen, thus giving rise to the production of cytotoxic reactive oxygen species (ROS). The formation of excessive ROS was known to cause serious tissue damage leading to iron overloading diseases and cancers.[2,3] Previous studies have shown that the overloaded iron in the body is correlated to the generation and the development of cancers.[4] Cancerous cells require larger quantities of iron than normal cells, which is reflected by the marked overexpression of transferrin receptor (TfR) for the uptake of iron into cells.[5] The overexpressed TfR on the cell surface was found in several types of cancers, including breast cancer, prostate cancer, liver cancer, leukemia and lymphoma.[6] The increased iron uptake played a pivotal role during the intensive DNA synthesis in neoplastic cells.[7] As the rate-limiting step in DNA synthesis, ribonucleotide reductase (RR) catalyzed the reduction of ribonucleotides to deoxyribonucleotides only in the presence of iron in its active site.[8]

The increased iron dependence of cancer cells suggested that iron depletion may be an effective strategy to inhibit the rapid proliferation of cancer cells.[9] Indeed, clinically available iron chelator desferrioxamine (DFO) for the treatment of iron overloading disease β-thalassemia and universal metal chelator diethylene triamine pentaacetic acid (DTPA) were shown to lead cellular iron deprivation and suppress the growth of aggressive cancer cells (Fig. 1).[1,6,10] Meanwhile, it was confirmed in many studies that DFO displayed both antiproliferative and pro-apoptotic effects on various cancer cell lines, including melanoma, breast carcinoma, prostate carcinoma, leukemia, lymphoma and hepatocellular carcinoma.[1,6,10] And among these cells, the antiproliferative effect of DFO on hepatocellular carcinoma cells were more pronounced.[11] On the contrary, normal hepatocytes exhibited the resistance to the antiproliferative activity of DFO when compared to various hepatoma cell lines.[12] The selectivity between hepatoma cells and normal hepatocytes suggests that DFO may be a potentially useful agent for treating hepatoma.[12] Moreover, mechanism studies confirmed that DFO inhibited the RR activity via the chelation of intracellular iron pool, thus preventing the iron from incorporating into the enzyme active site.[11] In addition, polyaminocarboxylate chelator DTPA was also extensively explored for the anticancer activity against neuroblastoma and ovarian carcinoma cell lines.[13] Unlike DFO, DTPA is a membrane impermeable iron chelator, thus

* Corresponding authors.
E-mail addresses: xiangma@hust.edu.cn (X. Ma), gyxiang1968@hotmail.com (G. Xiang).
[d] These authors contributed equally to this work.

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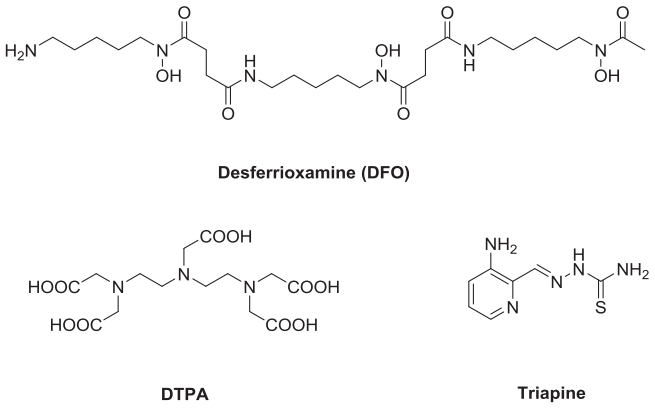

Fig. 1. Structures of iron chelators for clinical anticancer treatment.

the antiproliferative activity was probably produced by the chelating effect on the extracellular pool of iron.[10] More recently, a promising iron depletion agent, triapine (Fig. 1) has shown remarkable antitumor activity on several types of cancers in clinical trials.[14] And combined with a range of chemotherapeutics, triapine has also demonstrated promising results in several phase II clinical trials.[15]

During the past few years, 1,4,7-triazacyclononane (TACN) derivatives have attracted our attention, such as NETA, and its bifunctional version C-NETA (Fig. 2).[16] The origin of interest in the macrocycle TACN was from the property that TACN can coordinate facially to Fe^{3+} with the metal lying out of the plane defined by three nitrogen atoms to form exclusively five-membered chelate rings. Therefore, TACN appears to be an excellent basic platform to start with for the development of novel iron chelators as antitumor agents. In this study, a class of TACN-based chelators, HE-NO2A, HP-NO2A and NE2P2A, as well as the bifunctional versions of C-NETA, *p*-NO_2-PhPr-NE3TA and *p*-NH_2-PhPr-NE3TA (Fig. 2) were synthesized. Antiproliferative activity of the above chelators together with reported chelators C-NETA was evaluated against HepG2 cancer cells in vitro.

The structures of TACN-based chelators HE-NO2A, HP-NO2A, NE2P2A, C-NETA, *p*-NO_2-PhPr-NE3TA and *p*-NH_2-PhPr-NE3TA are shown in Fig. 2. Previously, we reported the synthesis of HE-NO2A and its analogue HP-NO2A, both of which featured a TACN platform combining with one hydroxypyridinonate and two carboxylic acid pendant arms.[17] Recently, a new bifunctional version of C-NETA, denoted as *p*-NO_2-PhPr-NE3TA, was designed and synthesized in our laboratory.[18] Both C-NETA and *p*-NO_2-PhPr-NE3TA possess a nitro group which can be further converted to an amino (NH_2) or isothiocyanate (NCS) group for conjugation with a receptor-targeting molecule. In particular, *p*-NO_2-PhPr-NE3TA contains a *p*-nitro-phenylpropyl group on nitrogen in the pendant arm, and the long propyl chain in the structure was designed to reduce potential steric hindrance during the formation of iron complex. And *p*-NO_2-PhPr-NE3TA possesses seven coordinating groups, which may be more effective in binding to the hexacoordinate iron than eight coordination groups in C-NETA. In current study, ligand *p*-NO_2-PhPr-NE3TA was synthesized according to a reported method with slight modification.[18] In the meantime, the reduction version of *p*-NO_2-PhPr-NE3TA, denoted as *p*-NH_2-PhPr-NE3TA, was also prepared.

As shown in Scheme 1, the key step for the preparation of the target ligands *p*-NO_2-PhPr-NE3TA and *p*-NH_2-PhPr-NE3TA is the coupling reaction of fragment **4** with **5** in the presence of anhydrous K_2CO_3 in acetonitrile (MeCN).[17] Specifically, fragment **4** was prepared starting from commercially available material 1-(3-bromopropyl)-4-nitrobenzene (**1**). Reaction of **1** with excess

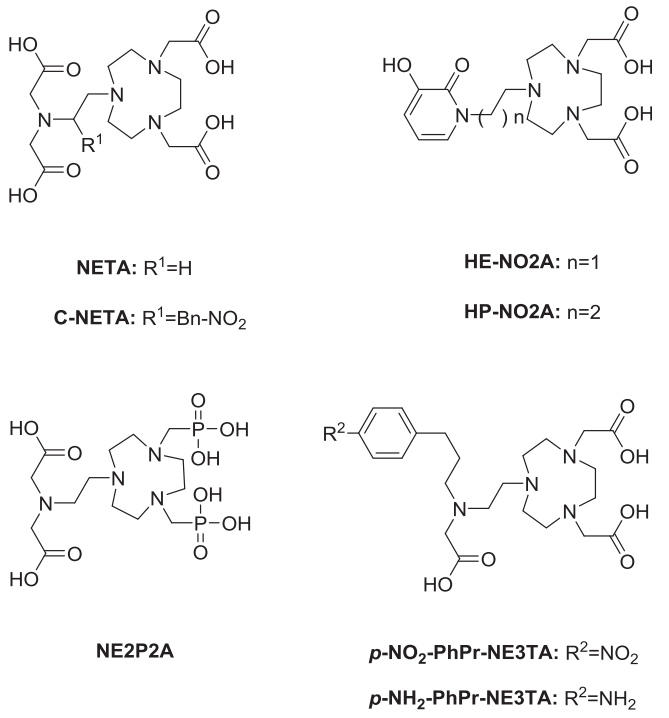

Fig. 2. Structures of TACN-based iron-depleting antitumor agents.

amount of 2-aminoethanol in the presence of triethylamine (TEA) in MeCN gave *N*-*p*-nitro-phenylpropyl ethanol amine **2**. Subsequently, compound **2** was alkylated by *tert*-butyl bromoacetate to provide **3**, which was further reacted with *N*-bromosuccinimide (NBS) and triphenylphosphine (PPh_3) to yield corresponding bromide **4**. The crucial intermediate **6** was obtained in the coupling reaction of **4** and **5**, which was isolated by column chromatography in good yield (63.2%). The *tert*-butyl groups in precursor **6** were then successfully removed by treatment with trifluoroacetic acid (TFA) in methylene dichloride (DCM) to afford desired chelator *p*-NO_2-PhPr-NE3TA (**7**) in an excellent yield (99.0%). Reaction of intermediate **6** in 10% Pd/C in methanol under H_2 gas at room temperature provided the aniline **8**, which was then subjected to deprotection of *tert*-butyl groups, thereby affording the desired chelator *p*-NH_2-PhPr-NE3TA (**9**) in 93.5% yield.

Chelators synthesized above are based on the TACN derivatives with acetate pendant arms. However, little has been explored for other suitable pendent donor groups which is one of the decisive factors determining the effectiveness of iron chelator.[19] Recently, many reports indicate that replacing carboxylate pendant arms in chelators with phosphonate pendant arms can accelerate the metal-binding kinetics.[20] In this context, the di-methylphosphonate pendant armed TACN derivative NE2P2A was designed and synthesized. Particularly, NE2P2A contains an additional carboxylate pendant arm in combination with TACN platform which is similar with the coordination groups in DTPA, thus promoting to form stable complexes with iron in fast thermodynamic kinetics. An efficient and convenient synthetic route of NE2P2A was developed as illustrated in Scheme 2. The key step is the coupling reaction of the fragment **11** with **12** in the presence of anhydrous K_2CO_3 in MeCN to yield crucial intermediate **13**.[21] Fragment **11** was synthesized according to a known procedure as reported previously.[22] Briefly, reaction of 2-aminoethanol with excess amount of benzyl bromoacetate in the presence of anhydrous $KHCO_3$ in dimethyl formamide (DMF) gave the dialkylated product **10**, which was subsequently converted to bromide **11**. A coupling reaction of **11** with **12** gave intermediate **13** in 39.1% yield. The ethyl and ben-