Bioorganic & Medicinal Chemistry Letters 28 (2018) 742–747

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Lysosomes-targeting imaging and anticancer properties of novel bis-naphthalimide derivatives

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ARTICLE INFO

Article history:
Received 17 November 2017
Revised 5 January 2018
Accepted 7 January 2018
Available online 9 January 2018

Keywords:
Naphthalimide
Lysosome
Anticancer
Fluorescence enhancement
Fluorescence imaging

ABSTRACT

A series of novel *N*,*N*-bis(3-aminopropyl)methylamine bridged bis-naphthalimide derivatives **NI1**–**NI8** containing saturated nitrogenous heterocycles were designed and synthesized, their cytotoxic activities against Hela, MCF-7, A549 and MGC-803 cells were investigated, Compounds **NI1**–**NI4** modified with piperidine and piperazine exhibited good and selective cytotoxic activity, for instance, compounds **NI1** and **NI4** showed potent cytotoxic activity against Hela cells and MGC-803 cells with the IC_{50} values of 2.89, 060, 2.73 and 1.60 μM, respectively, better than the control drug (Amonafide). However, compounds **NI5**–**NI8** conjugated with pyrrole derivatives showed weak cytotoxic activities against the all tested cell lines. Furthermore, their DNA binding properties, fluorescence imaging and cell cycle were investigated. Interestingly, compounds **NI1** and **NI4** showed fluorescence enhancement because of the strong binding with Ct-DNA, and exhibited fluorescence imaging with Hela cells on the lysosomes.

Naphthalimide derivatives are one of the most important and widely used molecules as the chemically therapeutic[1] and biological imaging agents,[2] and especially for recent years have been investigated as the fluorescent sensing probes[3] for targeting sensing of ion,[4] endogenous molecules[5] and cancer cells.[6]

In biological systems, it is well known that lysosome as one of the vital organelles[7] participates in many physiological processes, such as cell apoptosis, maintenance of cell-cycle progression, and supply of cellular energy,[8] etc. Recent research has suggested that abnormal lysosomal pH values were related to many diseases including cancer,[9] neuron degenerative disorders[10] and rheumatoid arthritis,[11] through facilitating the degradation of proteins in cellular metabolism.[12] Therefore, it is of great important to study real-time lysosomal pH changes for disease diagnosis.[13] In recent years, on the basis of the acidic feature of lysosome a series of lysosome-targeting probes of naphthalimide derivatives with the modification of morpholine or piperazine group have been designed and synthesized in order to enhance the targeting effects to the lysosomes through the electronic interactions.[14] For example, a methyl-piperazine modified naphthalimide derivative TNP showed lysosome-targeting fluorescence imaging and exhibited fluorescence turn on sensing of Fe (III) ion.[15] Ma and coworkers reported a lysosome-targeting fluorescence off-on probe for imaging of nitroreductase and hypoxia based on morpholine modified 4-nitro-1,8-naphthalimide derivative Lyso-NTR.[16]

It is well known that lysosomes are larger, less stable and more numerous in cancer cells than those in normal cells.[17] Furthermore, lysosomes also showed lower pH value (below 4.5) and exhibited greater cathepsin activity than that in normal cells (pH values of 4.5–6.5).[18] As a result, lysosomes are potential target for anticancer therapy.[19] The lysosome-targeting drugs might result in the lysosomal membrane permeabilization and the release of cathepsins into the cytosol, which triggered a variety of death pathways from classic apoptosis to necrosis.[20] On the other hand, lysosome-targeting drugs would impart the therapeutic efficacy and minimal side effects of the drugs through specific accumulations in the subcellular lysosomal organelle.[21] Recently, a lysosome-specific and ROS-dependent prodrug of aminoferrocene was reported by Mokhir and coworkers, which targeted lysosomes due to the conjugate of a piperidine group, and which exhibited high anticancer activity against a variety of cancer cell lines (IC_{50} = 3.5–7.2 μM) and in vivo (40 $mg{\cdot}kg^{-1}$, NK/Ly murine model).[22] A multifunctional nanoplatform for lysosome targeted delivery and photothermal therapy based on morpholine modified naphthalimide derivative and carbon dots was reported, which showed high anticancer efficacy.[21] Moreover, lysosome-targeting

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https://doi.org/10.1016/j.bmcl.2018.01.008
0960-894X/

metallodrugs have been widely studied.[23] For example, three highly positively charged ruthenium(II) polypyridyl complexes were reported by Chao and coworkers, which selectively localized in the lysosomes, and showed photodynamic therapy activity.[24] As we all known that naphthalimide derivatives as anticancer agents have been extensively studied for many years, and some of them (amonafide, mitonafide, elinafide, bisnafide, and UNBS5162, etc.) exhibited potent anticancer activities and have reached clinical trials.[25] However, the lysosome-targeting anticancer agent based on the naphthalimide derivatives was limited.[26] A series of naphthalimides were reported by Qian and coworkers, which proved to inhibit topoisomerase II, and induced lysosomal membrane permeabilization, and caused apoptosis and cell death.[26]

On the other hand, polyamines as the biological endogenous molecules play an important role in tumor cell growth and function.[27] Polyamines modified naphthalimide derivatives as anticancer agents have been widely constructed and have shown good anticancer activity.[28] Recently, a series of flavone-naphthalimide-polyamine conjugates were reported by Wang and co-workers, which showed mitochondria-targeted anticancer activity through harnessing a polyamine transporter for cell entrance.[29] To date, multimodal therapy has been widely developed on different biologically active species to obtain maximum therapeutic efficacy.[30]

From the above points of views, we designed and synthesized herein novel polyamine-bridged bis-naphthalimide derivatives **NI1**–**NI8** (Fig. 1) modified with piperidine derivative (4-piperidinol, 4-piperidinemethanol, and 4-piperidineethanol), *N*-(2-hydroxyethyl)piperazine and pyrrolidine derivatives (L-prolinol, D-prolinol, (*R*)-3-hydroxypyrrolidine and (*S*)-3-hydroxypyrrolidine) at the 4-position in order to increase the lysosome-targeting affinity and anticancer activity for developing lysosome-targeting fluorescence imaging and anticancer agents. The preliminary evaluation of compounds **NI1**–**NI8** for cytotoxic activities against human epithelial cervicak cells (Hela), Michigan cancer foundation-7 cells (MCF-7), human gastric cancer cells (SGC-7901) and human lung adenocarcinoma epithelial cells (A549), revealed that **NI1** and **NI4** showed potent cytotoxic activity in Hela cells and MGC-803 cells with the IC_{50} values of 2.89, 0.60, 2.73 and 1.60 μM, respectively, better than the control drug (Amonafide). Furthermore, compounds **NI1** and **NI4** exhibited excellent lysosome-targeting fluorescence imaging.

As shown in Scheme S1, compounds **NI1**–**NI8** were synthesized from 4-bromo-1,8-naphthalic anhydride **1** by the condensation with *N*,*N*-bis(3-aminopropyl)ethylenediamine in toluene solvent, and then protection of the amine groups with Boc group. Further, substitution reaction of the 4-bromine with piperidine derivative (4-piperidinol, 4-piperidinemethanol, and 4-piperidineethanol), *N*-(2-hydroxyethyl)piperazine and pyrrole derivatives (L-prolinol, D-prolinol, (*R*)-3-hydroxypyrrolidine and (*S*)-3-hydroxypyrrolidine). At last, compounds **NI1**–**8** were obtained by removing of the Boc protecting group under concentrated hydrochloride acid condition. Their structures were fully characterized by NMR spectroscopy and HRMS spectrometry (Figs. S1–S51).

The cytotoxic activities of the novel bis-naphthalimide derivatives **NI1**–**NI8** against cancer cell lines (Hela, MCF-7, MGC-803 and A549) *in vitro* were measured using MTT method. As shown in Table 1, compounds **NI1**–**NI4** with piperidine and piperazine derivatives modification exhibited selectively cytotoxic activities in the tested cell lines, higher cytotoxic activities against the Hela and MGC-803 cells were found except compound **NI3**. However, compounds **NI5**–**NI8** with pyrrole derivatives conjugates showed weak anticancer activities against all tested cell lines. Compounds **NI1** and **NI4** showed potent cytotoxic activity in Hela cells and MGC-803 cells with the IC_{50} values of 2.89, 0.60, 2.73 and 1.60 μM, respectively, better than the control drug (Amonafide). All of the compounds **NI1**–**NI8** showed no anticancer activities against MCF-7 and A549 cells except compound **NI4**. The substituents of the 4-position on naphthalimide backbone exhibited important effects on the anticancer activities. Compounds **NI5**–**NI8** with pyrrole derivatives modification showed anticancer activities against A549 cells except for **NI8**, but no activities against the other tested cell lines. Considering the potent cytotoxicity of **NI1** and **NI4** against the Hela and MGC-803 cells, we investigated the DNA

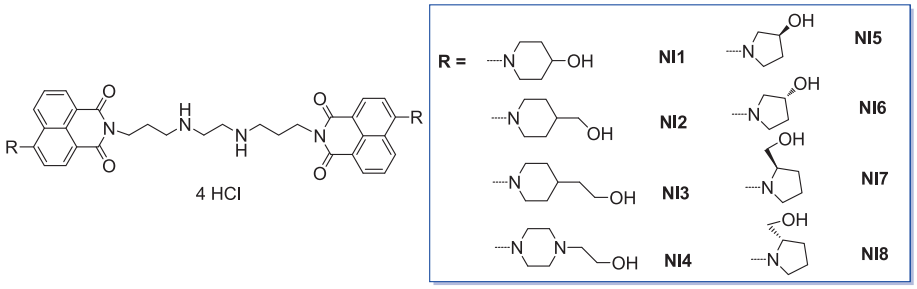

Fig. 1. The structures of compounds **NI1**–**NI8**.

Table 1
Cytotoxic activity of compounds **NI1**–**NI8** (IC_{50}, μM).

Compounds	IC_{50} (μM)			
	Hela	MCF-7	A549	MGC-803
NI1	2.89 ± 0.15	37.07 ± 0.04	46.21 ± 0.24	0.60 ± 0.10
NI2	4.03 ± 0.05	22.70 ± 0.02	13.96 ± 0.15	2.03 ± 0.25
NI3	6.88 ± 0.05	11.06 ± 0.00	43.46 ± 0.00	90.43 ± 0.2
NI4	2.73 ± 0.18	20.01 ± 0.0	2.59 ± 0.26	1.60 ± 0.37
NI5	35.44 ± 0.1	141.7 ± 0.02	83.59 ± 0.13	5.96 ± 0.08
NI6	45.37 ± 0.1	74.63 ± 0.05	22.99 ± 0.03	9.41 ± 0.27
NI7	89.17 ± 0.1	93.53 ± 0.08	72.62 ± 0.08	6.41 ± 0.34
NI8	85.15 ± 0.1	146.52 ± 0.0	471.62 ± 0.01	35.05 ± 0.13
Amonafide	3.41 ± 0.03	6.79 ± 0.02	2.00 ± 0.08	3.23 ± 0.43