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Research paper

Naphthalene diimide-polyamine hybrids as antiproliferative agents: Focus on the architecture of the polyamine chains

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ABSTRACT

Naphthalene diimides (NDIs) have been widely used as scaffold to design DNA-directed agents able to target peculiar DNA secondary arrangements endowed with relevant biochemical roles. Recently, we have reported disubstituted linear- and macrocyclic-NDIs that bind telomeric and non-telomeric G-quadruplex with high degree of affinity and selectivity. Herein, the synthesis, biological evaluation and molecular modelling studies of a series of asymmetrically substituted NDIs are reported. Among these, compound **9** emerges as the most interesting of the series being able to bind telomeric G-quadruplex ($\Delta Tm = 29\ ^{\circ}C$ at 2.5 μM), to inhibit the activity of DNA processing enzymes, such as topoisomerase II and TAQ-polymerase, and to exert antiproliferative effects in the NCI panel of cancer cell lines with GI_{50} values in the micro-to nanomolar concentration range (*i.e.* SR cell line, $GI_{50} = 76$ nM). Molecular mechanisms of cell death have been investigated and molecular modelling studies have been performed in order to shed light on the antiproliferative and DNA-recognition processes.

1. Introduction

Naphthalene diimides (NDIs) represent a class of electron-deficient aromatic compounds widely used in the field of supramolecular and material chemistry [1]. In the last years, this scaffold has been exploited in the design of biologically active compounds. In particular, published reports highlighted the use of NDI as optical imaging probe [2] and, in the medicinal chemistry field, as potential drug candidates. Indeed, several NDIs are able to inhibit enzymes, such as acetylcholinesterase [3], or to exert remarkable antiproliferative activities [4,5] through different mechanisms of action among which intercalation within double-stranded DNA (dsDNA) [6] and stabilization of higher order DNA secondary structures are worth of mention [7]. Over the years, dsDNA has represented the prominent target in the design of new anticancer agents; however, dsDNA-directed molecules suffer from different drawbacks, such as low selectivity towards non-cancerous cells [8]. Recently, other secondary DNA arrangements endowed with relevant biological roles have been discovered, notably the G-quadruplex structures [7]. Guanine-rich DNA sequences, located in distinct regions of the genome, such as oncogene promoters and telomeric regions, can assemble into G-quadruplex. Molecules able to stabilize these secondary structures, located at telomeric ends, prevent the binding of telomerase enzyme leading to senescence and cell death, while stabilization of G-quadruplex located in the promoter region of oncogenes results in a down-regulation of the expression of these genes [9,10]. Further, it is important to point out that G-quadruplex are significantly more prevalent in tumor tissues

Abbreviations: BASASA, Boltzmann Accessible Solvent Accessible Surface Area; BCL-2, B-cell lymphoma 2; dsDNA, double-stranded DNA; hTel, human telomeric; HSP90, heat shock protein 90; MD, Molecular Dynamics; MC, Monte Carlo method; NDI, Naphthalene Diimide; PARP, poly(ADP)ribose polymerase; SASA, Solvent Accessible Surface Area.

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compared to non-transformed cells [11].

A large number of molecules able to stabilize both telomeric and promoter G-quadruplex have been reported in the last years and a large number of them shares similar structural features, that is a large planar (hetero)aromatic core decorated with two or more harms carrying positively charged nitrogen(s) extremely favorable in the quadruplex recognition process [12]. NDIs have been extensively explored as scaffold for the design of G-quadruplex binders [13–16]. Indeed, it can be easily subjected to different types of core functionalization, thus allowing diverse structural modifications leading to di-, tri- and tetra-substituted derivatives [1,17]. In this context, a number of tetra-substituted NDIs have been developed by Neidle's group: in particular, compound **1** (Fig. 1) stabilizes several distinct DNA quadruplex sequences, such as hTel, HSP90 A and B, BCL-2 and k-RAS [18]. Moreover, **1** has significant *in vivo* antitumor activity in a MIA PaCa-2 pancreatic cancer xenograft model. Compound **2**, developed by Richter and co-workers, represents an example of tri-substituted NDI that displays stabilization of hTel and antiproliferative effects in colon (HT29), lung (A549) and melanoma (SKMel-5) tumor cells [19].

As part of our drug discovery program, we have recently reported some symmetrically di-substituted NDIs endowed with remarkable antiproliferative activities. Compound **3** (Fig. 1), bearing 2-methoxybenzylpropylendiamine side chains, strongly binds DNA and induces apoptotic cell death. Compound **3** was subject to an optimization campaign aimed at improving its pharmacological profile [16,20]. Among the new derivatives, compounds **4** and **5** (Fig. 1) emerged as potent G-quadruplex binders. Compound **4**, bearing 2,3,4-trimethoxybenzylpropylendiamine chains, is cytotoxic in a large panel of cancer cell lines thank to a simultaneous interaction with different biological targets among which the interaction with telomeric G-quadruplex is worth of mention [20]. Macrocyclic analogue **5** strongly stabilizes hTel and c-Kit quadruplex sequences with a good level of selectivity for G-quadruplex over dsDNA [16]. Unfortunately, in spite of these encouraging properties, compound **5** has weaker antiproliferative effects compared to compounds **3** and **4**.

From biophysical analysis and molecular modelling studies performed on these disubstituted and macrocyclic NDIs emerged that G-quadruplex binding property strongly depend on a) the length of the polymethylene linker separating the nitrogen atom(s) of the lateral chains mounted on the NDI nucleus, b) the number of the nitrogen atoms of the lateral chains and c) the nature of the substituent on aromatic rings of the lateral chains. Furthermore, molecular modelling studies point out that the NDI-DNA recognition process is driven by the establishment of both electrostatic and hydrophobic interactions involving both NDI core and peripheral substituted aromatic ring.

Based on these premises, we attempted to increase the cytotoxic activity of NDIs towards cancer cells by merging the best features of the compounds obtained so far focusing on G-quadruplex. To this aim, we designed asymmetric substituted NDIs where one arm was constituted by a substituted-benzylpropylendiamine and the other harm was constituted by a polyaminechain. Indeed, it is well known that polyamines are able to establish strong interactions with biological counterparts due to their positively charged nitrogen atoms. The interaction of polyamines (*i. e.* spermine) on G-quadruplex has been recently confirmed, evidencing different effects on the tetrahelical stability, according to their concentration [21,22]. In addition, it is well established that the polyamine's uptake by the polyamine transport system, which is overactive in cancer cells, contributes to the increased antiproliferative activity of some anticancer agents conjugate with polyamines [23].

In order to identify the most favorable substituent on the aromatic ring of the side chain, in terms of G-quadruplex recognition, 2-methoxybenzylpropylendiamine-NDI-spermine (**6**) and 2,3,4-trimethoxybenzylpropylendiamine-NDI-spermine (**7**) were designed as prototypes (Fig. 2). After a preliminary evaluation, the most efficient hit for the stabilization of the G-quadruplex form of hTel (compound **6**, see later), was modified in the polyamine chain by varying the length of the chain, the number of nitrogen atoms and the distance between them. To this aim, both natural and non-natural polyamines were employed.

In particular, we synthesized derivatives: *i*) **8**–**10** characterized

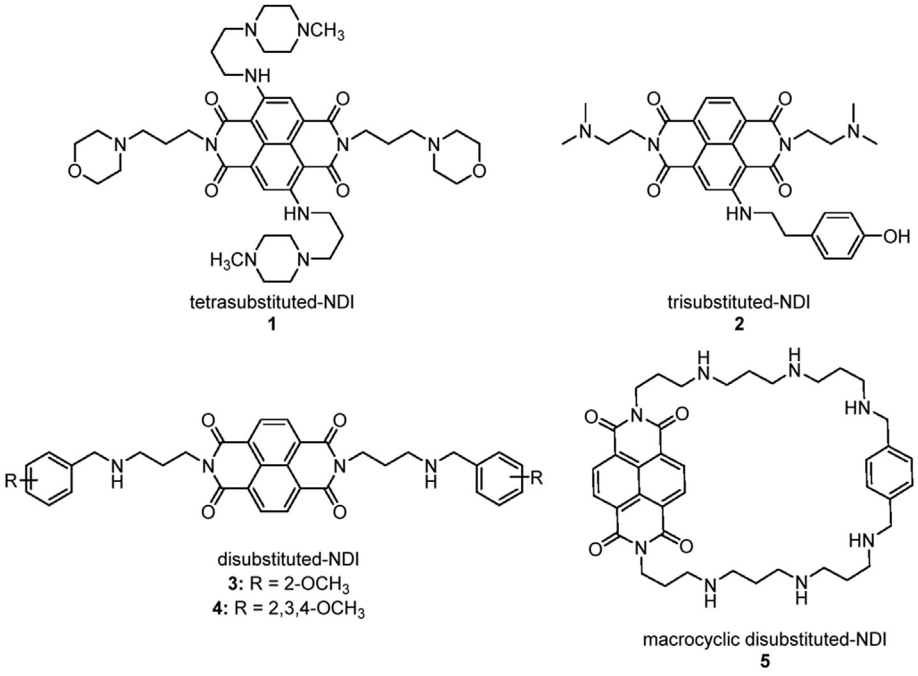

Fig. 1. Selected NDI-based G-quadruplex binders reported in literature.