BBA - General Subjects 1862 (2018) 615–629

Contents lists available at ScienceDirect

BBA - General Subjects

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

c-MYC G-quadruplex binding by the RNA polymerase I inhibitor BMH-21 and analogues revealed by a combined NMR and biochemical Approach

Loana Musso[a], Stefania Mazzini[a,*], Anna Rossini[c], Lorenzo Castagnoli[d], Leonardo Scaglioni[a], Roberto Artali[b], Massimo Di Nicola[c], Franco Zunino[c], Sabrina Dallavalle[a]

[a] Department of Food, Environmental and Nutritional Sciences, Division of Chemistry and Molecular Biology, Università degli Studi di Milano, via Celoria 2, I-20133 Milano, Italy
[b] Scientia Advice, di Roberto Artali, 20832 Desio, MB, Italy
[c] Fondazione IRCCS, Istituto Nazionale dei Tumori, via Venezian 1, 20133 Milano, Italy
[d] Molecular Targeting Unit, Department of Applied Research and Technological Development, Fondazione IRCCS Istituto Nazionale Tumori, via Venezian 1, 20133 Milano, Italy

ARTICLE INFO

Keywords:
c-MYC
G-quadruplex
BMH-21
Antitumor agents
NMR
Molecular modelling

ABSTRACT

Background: Pyridoquinazolinecarboxamides have been reported as RNA polymerase I inhibitors and represent a novel class of potential antitumor agents. BMH-21, was reported to intercalate with GC-rich rDNA, resulting in nucleolar stress as a primary mechanism of cytotoxicity.

Methods: The interaction of BMH-21 and analogues with DNA G-quadruplex structures was studied by NMR and molecular modelling. The cellular response was investigated in a panel of human tumor cell lines and protein expression was examined by Western Blot analysis.

Results and conclusions: We explored the ability of BMH-21 and its analogue **2** to bind to G-quadruplex present in the c-MYC promoter, by NMR and molecular modelling studies. We provide evidence that both compounds are not typical DNA intercalators but are effective binders of the tested G-quadruplex. The interaction with c-MYC G-quadruplex was reflected in down-regulation of c-Myc expression in human tumor cells. The inhibitory effect was almost complete in lymphoma cells SUDHL4 characterized by overexpression of c-Myc protein. This downregulation reflected an early and persistent modulation of cMyc mRNA. Given the relevance of c-MYC in regulation of ribosome biogenesis, it is conceivable that the inhibition of c-MYC contributes to the perturbation of nuclear functions and RNA polymerase I activity. Similar experiments with CX-5461, another RNA polymerase I transcription inhibitor, indicate the same behaviour in G-quadruplex stabilization.

General significance: Our results support the hypothesis that BMH-21 and analogue compounds share the same mechanism, i.e. G-quadruplex binding as a primary event of a cascade leading to inhibition of RNA polymerase I and apoptosis.

1. Introduction

DNA binding agents are still among the most effective antitumor agents. The cytotoxic effects of conventional DNA-interacting agents are ascribed to the direct or indirect induction of DNA damage. For this reason, the most relevant drawback of their use is the lack of tumor selectivity resulting in dose-limiting toxicity [1]. Several efforts have been devoted to the identification of novel DNA binding agents characterized by the ability to inhibit specific DNA functions relevant to malignant growth, independent of aspecific genotoxic stress. Some DNA-dependent functions have been identified as promising targets to exploit alterations related to the malignant behaviour.

Specifically, ribosome biogenesis, a multistep process that takes place in the nucleolus, is fundamental in supporting tumor cell growth. Thus, targeting the cancer-cell nucleolus in order to take advantage of enhanced ribosome biogenesis and protein synthesis with respect to normal cells, has recently attracted much interest [2,3].

Ribosome biogenesis requires ribosomal RNA (rRNA) transcription by RNA polymerase I (Pol I). In fast-replicating tumor cells, increase in rRNA transcription induces increase of the nucleolus size, which is more prominent when mutations of the tumor suppressing gene p53 are present [4]. Therefore, a way of decreasing the rapid proliferation could

* Corresponding author at: Università degli Studi di Milano, Department of Food, Environmental and Nutritional Sciences, Division of Chemistry and Molecular Biology, via Celoria 2, I-20133 Milano, Italy.
E-mail address: stefania.mazzini@unimi.it (S. Mazzini).

https://doi.org/10.1016/j.bbagen.2017.12.002
Received 23 June 2017; Received in revised form 3 November 2017; Accepted 7 December 2017
Available online 08 December 2017

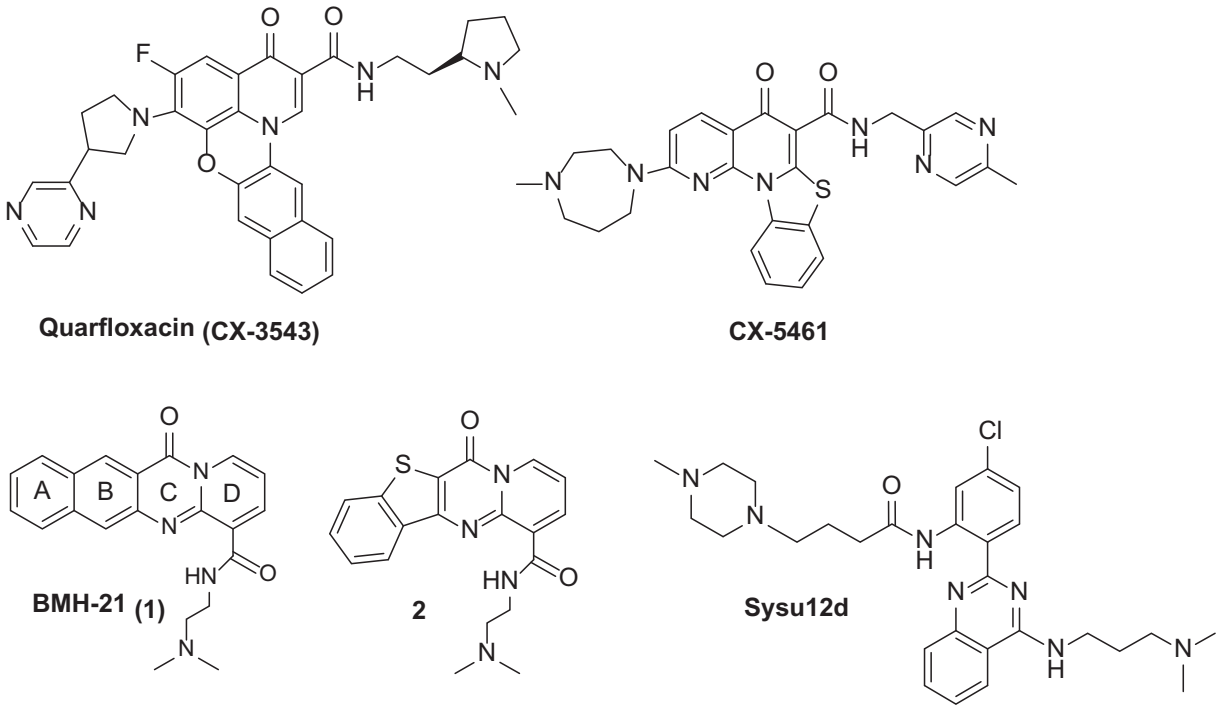

Chart 1. Structure of rRNA transcription inhibitors and new compound **2**.

be the down-modulation of rRNA synthesis ("ribosome starvation"), by inhibiting Pol I.

A number of DNA interacting agents are known to affect the nucleolar function at various levels [5], most of them however inducing extensive DNA damage, as is the case for Actinomycin D, a rRNA inhibitor in clinical use that binds duplex DNA by intercalation [6]. In this context, recent interest has been dedicated to the discovery of agents that selectively target the nucleolar stress pathway independently of DNA damage.

Among new molecules that apparently exert their antiproliferative activity by inhibiting rDNA transcription and inducing apoptosis in cancer cells, there are planar heterocyclic molecules such as CX-3543 (quarfloxacin) [7], which reached phase II of clinical development, CX-5461 [8,9], BMH-21 (**1**) [10–12] and its congeners [13,14], and Sysu12d [15] (Chart 1).

Bywater et al. [8] demonstrated that the apoptotic death induced in a lymphoma model by reduction in Pol I transcription after treatment with CX-5461 occurred rapidly as the consequence of activation of p53, following perturbations of the nucleolus. Similar conclusions were reached in the study of the other above mentioned compounds.

We were particularly intrigued by the results of a study on BMH-21 [12] because the compound significantly inhibits Pol I and is deemed to intercalate into double strand DNA with binding preference toward GC-rich DNA sequences [10,11]. However, its intercalating ability is quite unconventional, as it does not cause DNA damage. In fact, it was reported that BMH-21 does not induce phosphorylation of H2AX, a key biomarker of DNA damage stress [12]. The evidences for intercalation of **1** were hypo- and bathochromic shifts in the UV/VIS spectrum in the presence of DNA, and unwinding of plasmid DNA [10]. Molecular modelling supported this hypothesis, showing that BMH-21 can stack flatly between GC bases and that its positively charged side chain potentially interacts with the DNA backbone [11].

To further investigate the mode of interaction of BMH-21 with DNA, we undertook a NMR study of the interaction of **1** with some duplex and quadruplex DNA oligomers. On the basis of the structural features of BMH-21 and its peculiar mode of action, we hypothesized that this compound might interact with G-quadruplex. Intramolecular G-quadruplex structures are present in the guanine rich regions of human telomeres [16], in ribosomal DNA and in gene promoters c-MYC, bcl-2, c-kit [17–24]. As the overexpression of the c-MYC oncogene is one of the most common aberrations found in a wide range of human tumors [25], the stabilization of G-quadruplex by small molecules in the c-MYC promoter has been proposed as a promising antitumor strategy [17,24].

The results of this study support the binding of the lead compound BMH-21 (**1**) to c-MYC G-quadruplex, a finding which is consistent with downregulation of protein expression in tumor cells. This downregulation reflected an early and persistent modulation of cMyc mRNA.

To ensure that this feature was not restricted to BMH-21 itself and to support a common mechanism of actions of compounds containing the same scaffold, we synthesized and tested a new analogue of BMH-21 (compound **2**) with a thiophene in place of a benzene ring in the tetracyclic system and made a similar investigation of compound CX-5461, a reported Pol 1 inhibitor [7], now in phase I of clinical trials [26].

2. Materials and methods

2.1. Chemistry. General methods

All reagents and solvents were reagent grade or were purified by standard methods before use. Melting points were determined in open capillaries. NMR spectra were recorded on Varian Mercury 300 MHz and Bruker AV600 spectrometers. The accurate mass spectra were recorded using a Bruker Daltonic model ICR-FTMS APEX II. Solvents were routinely distilled prior to use; dry methylene chloride was obtained by distillation from phosphorus pentoxide and toluene from $CaCl_2$. All reactions requiring anhydrous conditions were performed under a positive nitrogen flow, and all glassware were oven dried. Isolation and purification of the compounds were performed by flash column chromatography on silica gel 60 (230–400 mesh). Analytical thin-layer chromatography (TLC) was conducted on TLC plates (silica gel 60 F254, aluminium foil) and spots were visualized by UV light and/or by means of dyeing reagents.

Compound BHM-21 was synthesized following a procedure