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Research paper

Design of nucleotide-mimetic and non-nucleotide inhibitors of the translation initiation factor eIF4E: Synthesis, structural and functional characterisation

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ABSTRACT

Eukaryotic translation initiation factor 4E (eIF4E) is considered as the corner stone in the cap-dependent translation initiation machinery. Its role is to recruit mRNA to the ribosome through recognition of the 5′-terminal mRNA cap structure (m^7GpppN, where G is guanosine, N is any nucleotide). eIF4E is implicated in cell transformation, tumourigenesis, and angiogenesis by facilitating translation of oncogenic mRNAs; it is thus regarded as an attractive anticancer drug target. We have used two approaches to design cap-binding inhibitors of eIF4E by modifying the N^7-substituent of m^7GMP and replacing the phosphate group with isosteres such as squaramides, sulfonamides, and tetrazoles, as well as by structure-based virtual screening aimed at identifying non-nucleotide cap-binding antagonists. Phosphomimetic nucleotide derivatives and highly ranking virtual hits were evaluated in a series of *in vitro* and cell-based assays to identify the first non-nucleotide eIF4E cap-binding inhibitor with activities in cell-based assays, *N*-[(5,6-dihydro-6-oxo-1,3-dioxolo[4,5-*g*]quinolin-7-yl)methyl]-*N*′-(2-methyl-propyl)-*N*-(phenylmethyl)thiourea (**14**), including down-regulation of oncogenic proteins and suppression of RNA incorporation into polysomes. Although we did not observe cellular activity with any of our modified m^7GMP phosphate isostere compounds, we obtained X-ray crystallography structures of three such compounds in complex with eIF4E, 5′-deoxy-5′-(1,2-dioxo-3-hydroxycyclobut-3-en-4-yl)amino-N^7-methyl-guanosine (**4a**), N^7-3-chlorobenzyl-5′-deoxy-5′-(1,2-dioxo-3-hydroxy-cyclobut-3-en-4-yl)amino-guanosine (**4f**), and N^7-benzyl-5′-deoxy-5′-(trifluoromethyl-sulfamoyl)guanosine (**7a**). Collectively, the data we present on structure-based design of eIF4E cap-binding inhibitors should facilitate the optimisation of such compounds as potential anticancer agents.

1. Introduction

In eukaryotes, most mRNAs are translated using a cap-dependent mechanism, which consists of three stages: initiation, elongation, and termination. The initiation step requires a number of eukaryotic translation initiation factors (eIFs) [1]. Initially, the 40S ribosomal subunit associates with Met-tRNAi and a group of initiation factors, including eIF1, eIF1A, eIF2, eIF3, and eIF5 to form the 43S pre-initiation complex [2]. This complex is then recruited to the 5′-end of capped mRNA by another complex of factors (known as eIF4F) consisting of eIF4E, eIF4G, and eIF4A. eIF4E is an indispensable element for cap-dependent translation initiation and plays a major role in recognition of the mRNA cap structure (m^7GpppN, where N is any nucleotide). It binds to eIF4G, which serves as a scaffolding protein to gather eIF4A (ATP-dependent RNA

Abbreviations: eIF, eukaryotic translation initiation factor; 4E-BP, eF4E-binding protein; FP, fluorescence polarisation; m^7G, N^7-methyl guanosine; RRL, rabbit reticulocyte lysate.

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helicase), eIF4B, eIF3, and poly-A-binding protein to form the 48S complex [3,4]. This complex then scans the mRNA for an initiation codon and, once this is located, the 60S ribosomal subunit joins to form the elongation-competent fully functional 80S.

Under typical circumstances, eIF4E is the least abundant of the cellular translation initiation components [5] and it represents the rate-limiting element for the initiation process [6,7]. Under resting conditions, eIF4E is kept in an inactive form bound to 4E-binding proteins (4E-BPs); once the latter have been phosphorylated through the PI3K-AKT-mTOR signalling pathway, eIF4E is released and can associate with eIF4G to launch translation.

eIF4E is overexpressed in numerous human tumours, and it contributes to transformation, tumourigenesis, and progression of cancers [8,9]. eIF4E overexpression facilitates translation of weak and highly structured mRNAs that typically encode proteins involved in cancer pathology, such as proto-oncoproteins (*e.g.* cyclin D1, ornithine decarboxylase), angiogenesis factors (*e.g.* FGF-2, VEGF), and factors related to tumour invasiveness, such as MMP-9 [10], due to the ability of eIF4E to stimulate eIF4A [11]. For these reasons, eIF4E represents an attractive cancer drug target [12]. Moreover, a recent study suggests that targeting eIF4E for cancer treatment has minimal effects on growth of –and protein synthesis in– healthy cells [13].

Strategies to design eIF4E–mRNA cap-binding antagonists have been based on nucleoside (purine N^7-substitutions) [14–17] and nucleotide (altered phosphate groups) [17–19] modifications of 7-methylguanosine (m^7G) nucleotides (Fig. 1) [12], including recent work affording for the first time m^7G monophosphate (m^7GMP) nucleotide mimetic compounds with high affinity for eIF4E [20]. However, to date no cell-permeable small-molecule eIF4E cap-binding antagonists have been reported. As with antiviral agents derived from nucleotides, prodrugs that mask the ionic nature of phosphate groups, such as phosphate esters of phosphoramidates, may offer an avenue for the design of cell-permeable eIF4E inhibitors, but this strategy has not progressed very far as yet [21].

Here we report on new methods to design cap antagonists and we show a group of nucleotide mimetic compounds with phosphate group isosteres and various purine N^7-substituents. The biological activities of these compounds were assessed using a range of techniques and the eIF4E-binding modes of three compounds were determined experimentally using X-ray crystallography. However, despite phosphate group modifications, these nucleotide mimetics still exhibit poor cellular bioactivity. Therefore, a computer-aided drug design method was exploited to design a set of non-nucleotide compounds to provide the first small-molecule eIF4E inhibitor possessing cellular activity consistent with blocking of eIF4E-mediated initiation of translation.

2. Results and discussion

2.1. Design and synthesis of nucleotide monophosphate mimetic eIF4E inhibitors

One of the main challenges in the design of inhibitors of eIF4E cap binding has been to achieve membrane permeability and thus cellular bioavailability [20]. This is due to the fact that the affinity of eIF4E for the RNA m^7GpppN cap structure derives in large part from polar interactions between the ligand triphosphate group and the receptor protein (Fig. 1a) [15,25]. In order to address the permeability problem, as well as the intrinsic hydrolytic and enzymatic lability of nucleotides, we aimed to design nucleoside monophosphate mimetics [25,26]. Several phosphate group replacements were investigated, such as squaramides, sulfonamides, and tetrazoles. Since *e.g.* m^7GMP has much lower affinity for eIF4E than m^7GTP [25], we expected to compensate the loss in affinity with nucleoside monophosphate mimetics through optimal purine N^7-substituent modifications, based on previous studies demonstrating that replacing the N^7-methyl group with bulkier groups contributed significantly to ligand affinity (Fig. 1b) [27,28].

Squaric acid possesses similar charge distribution, polarity, and acidic properties as phosphoric acid, hence the squaramide group could represent an isostere for the phosphate group [29,30]. Similarly, sulfonamide derivatives have been reported as phosphate mimics in the design of tyrosine phosphatase inhibitors [31]. The third candidate we considered for phosphate group replacement was a tetrazole [32], as this system mimics the acidic features of the phosphate group, provided the proton attached to one of the tetrazole ring nitrogens is not replaced [33].

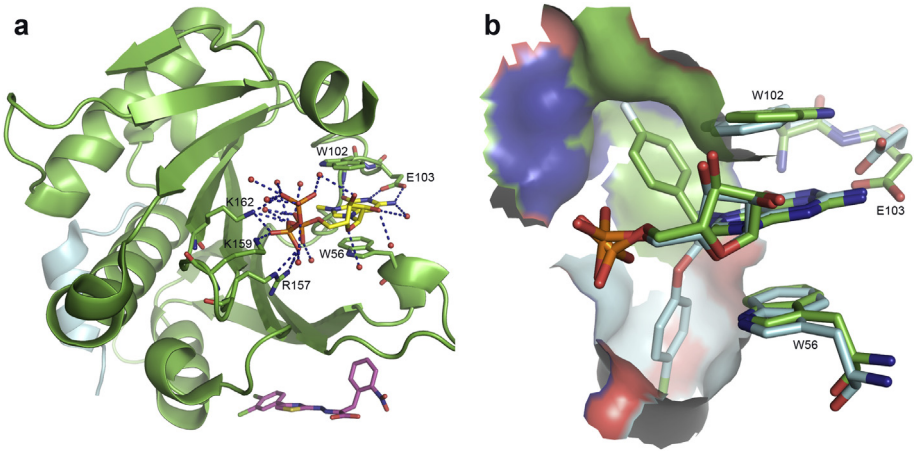

Fig. 1. (**a**) mRNA cap recognition (represented by m^7GTP shown as yellow CPK sticks) and binding of eIF4G or 4E-BPs (represented by a peptide derived from 4E-BP1 shown as a cyan cartoon) occurs on opposite faces of eIF4E (green cartoon). Apart from direct cap-binding antagonists, allosteric inhibitors (binding pose of 4-EGI1 [22] shown as magenta sticks) and inhibitors derived from eIF4G and 4E-BPs [23] are being developed. Whereas m^7GTP-binding is dominated by polar interactions between the cationic N-methylpurine system and eIF4E residues W56, W102, and E103 (cation–π interaction and H-bonds), as well as the phosphate groups with residues R157, K159, and K162, (**b**) GMP derivatives with N^7-substituents other than methyl, such as 4-fluorobenzyl [24] (green) or (4-chlorophenoxy)ethyl [20] (cyan), also make hydrophobic contacts with two concave lipophilic pockets (surface representations) behind the W56–W102 stack. Figure constructed from PDB entries 2V8W, 2V8Y, 4DT6, and 4TPW. 3D-Structure illustrations in this and subsequent Figures were prepared using MacPyMOL (The PyMOL Molecular Graphics System, Version 1.2, Schrödinger, LLC). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)