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Design, Synthesis and Evaluation of Analogs of Initiation Factor 4E (eIF4E) Cap-Binding Antagonist Bn⁷-GMP

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Abstract

Aberrant regulation of cap-dependent translation has been frequently observed in the development of cancer. Association of the cap binding protein eIF4E with N⁷-methylated guanosine capped mRNA is the rate limiting step governing translation initiation; and therefore represents an attractive process for cancer drug discovery. Previously, replacement of the 7-Me group of the Me⁷-guanosine monophosphate with a benzyl group has been found to increase binding affinity to eIF4E. Recent X-ray crystallographic studies have revealed that the cap-dependent pocket undergoes a unique structural change in order to accommodate the benzyl group. To explore the structure activity relationships governing the affinity of N⁷-benzylated guanosine monophosphate (Bn⁷-GMP) for eIF4E, we virtually screened a library of 80 Bn⁷-GMP analogs utilizing CombiGlide as implemented in Schrodinger®. A subset library of substituted Bn⁷-GMP analogs was synthesized and their dissociation constants (K_d) were determined. Due to the poor correlation between docking/scoring results and experimental binding affinities, three-dimensional quantitative structure-activity relationship (3D-QSAR) calculations were performed. Two highly predictive and self-consistent CoMFA (comparative molecular field analysis) and CoMSIA (comparative molecular similarity indices analysis) models were derived and optimized. These models may be useful for the future design of eIF4E cap-binding antagonists.

Keywords

eIF4E; translation initiation; anticancer; antifibrotic; CoMFA; CoMSIA

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Supplementary Material

RP-HPLC traces of prepared compounds, a full list of the bromide analogs applied in the docking studies along with their corresponding Bn⁷-GMP derivatives' GScores, and additional nonlinear fitting results are given.

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1. Introduction

A major trait of all eukaryotic mRNA is the presence of a 5' cap structure and a polyA tail terminating their 3' end. The capped mRNA has a guanosine which is methylated at the N⁷ position, and connected to the first nucleotide of the mRNA through a triphosphate bridge. [1] The 5'-cap structure m⁷G (5')ppp(5')N (where N is the first transcribed nucleotide) serves as the assembly site for translation initiation factors and ribosomal recruitment during the initiation stage of translation.[2] In the initial phase of eukaryotic translation, eukaryotic initiation factor (eIF) 4E binds directly to 5' N⁷-methylated guanosine capped mRNA, followed by transport of the eIF4E-mRNA complex from the nucleus to the cytoplasm. eIF4E also has the ability to bind to a scaffolding protein eIF4G, which has binding sites for both eIF4A and eIF3. eIF3 is able to bind to both ribosomes and mRNA. The 5' mRNA, 40S ribosomal subunit, and the above initiation factors are assembled together in one complex and the 40S ribosomal subunit complex is then believed to scan along the mRNA for the start codon AUG, where translation starts.[2,3] Our studies' main focus of eIF4E is its role in translation despite it does show other functions in nuclear export and mRNA decay pathways[4].

Under homeostatic conditions, eIF4E is a key regulator of cell growth, proliferation and survival.[5] However, eIF4E functions as an oncogene when over expressed in target cells. [1,5–7] Studies have suggested that eIF4E levels are elevated in many solid tumors and tumor cell lines[8,9]. There is also evidence indicating that elevated eIF4E levels might contribute to the progression of tumors[3,10,11]. Under physiological conditions, the availability of eIF4E is tightly regulated. Control of eIF4E is exerted both by phosphorylation at Ser209[12,13] and by small inhibitory 4E-binding proteins (4E-BPs) that sequester eIF4E from its binding partner in the eIF4F complex, eIF4G.[6,14–17] Since eIF4E is the least abundant initiation factor [18], the recognition of capped mRNAs by eIF4E is the rate limiting step for translation initiation.[16] Consequently, the development of eIF4E antagonists could provide a novel avenue for cancer therapeutics and for probing the role of cap-dependent translation in normal biological processes.

Previously, Darzynkiewicz et al[19] have shown that mRNA capped with Bn⁷GpppG was able to enhance translation efficacy 1.8-fold greater than Me⁷-capped transcripts. In addition, Ghosh et al. have demonstrated that Bn⁷-GMP binds tightly to eIF4E ($K_d = 0.8 \mu\text{M}$) and that a corresponding tryptamine phosphoramidate pronucleotide is a potent inhibitor of not only reticulocyte translation, but also the epithelial-to-mesenchymal transition (EMT) in zebra fish. [20] A recent crystallographic study with co-crystals of Bn⁷-GMP and eIF4E revealed that one of the tryptophan side-chains involved in a critical cation-pi stacking interaction Me⁷-guanosine of capped mRNA had adopted a 180° ring flip conformation when bound to Bn⁷-GMP, allowing access by the benzyl moiety to a more pronounced hydrophobic pocket (Figure 1). [21] Consequently, since Bn⁷-GMP has been shown to have increased binding affinity relative to Me⁷-GMP, we carried out virtual screening of a series of *ortho*- *meta*- and *para*-Bn⁷-GMP substituents. The compounds were synthesized and their dissociation constant rank order determined and compared to the results of the modeling scoring results. Following that, 3D-QSAR calculations were performed to correlate eIF4E antagonists' chemical properties with their experimental binding affinity values. CoMFA[22] and CoMSIA[23] models were derived from a training set of eIF4E antagonists, generating the first reported 3D-QSAR models of eIF4E.

2. Methods

2.1 Computational Modeling - Docking

An *in silico* combinatorial library of 80 N⁷-benzyl modified GMP analogs was generated by CombiGlide (Schrodinger®, Inc.) using GMP as the core structure. This library of substituted

Bn⁷-GMP analogs was docked into the eIF4E binding site (2V8X.pdb[24]) obtained from the co-crystallization of eIF4E with Bn⁷-GMP,[19] using CombiGlide. GScore (Supplemental Figure 1S) represents the docking score. The GScore scoring function as implemented in CombiGlide is a modified and extended version of the empirically based ChemScore function [25]. More negative GScores correspond to more energetically favorable bound configurations for a particular compound.

2.2 Chemistry

A library of 16 compounds including Bn⁷-GMP was synthesized by reacting guanosine monophosphate disodium salt with substituted benzyl bromide in dimethyl sulfoxide (Scheme 1). Purification was carried out by DEAE Sephadex HCO₃⁻ column, followed by Dowex 80W–200W Na⁺ exchange column. Compounds were characterized by ¹H NMR, ³¹P NMR, ESI-MS and RP-HPLC. ¹H and ³¹P NMR were recorded on a Mercury 300 MHz spectrometer (Varian). HR-MS mass spectra were taken on a Bruker Bio-TOF-II mass spectrometer. Analytical RP-HPLC was performed on an Agilent 1100 Series by a LiChrospher® 100 RP-18 column with a solvent system of water/MeOH with 0.1% trifluoroacetic acid over 10 minutes.

2.3 Expression and Purification of eIF4E Protein

Our laboratory has previously purified eIF4E from the expression vector pPH70D-eIF4E, which encodes FLAG-DHFR (dihydrofolate reductase) followed by a thrombin sensitive linker and the mouse eIF4E.[26] This cap-free eIF4E had the advantage of avoiding the contamination of eIF4E purified from cap-affinity chromatography with cap-analog[15]. Generally, DHFR-eIF4E was overexpressed as a fusion protein in BL21 DE3 *E. coli* cells. This fusion protein was purified by methotrexate (MTX) affinity chromatography. After cleavage by human thrombin, eIF4E was purified by DEAE (diethylaminoethyl) Cellulose anion exchange column. The purified protein was aliquoted and stored in 10% glycerol at –80 °C immediately after purification and thawed on ice before use. The concentration of eIF4E for fluorescence titration assay was optimized to 200 nM and was used for all titration experiments.

2.4 Time-synchronized Fluorescence Titration Assay

To probe the effects of benzyl substitution on the binding affinities of Bn⁷-GMP analogs to eIF4E, dissociation constants of these analogs were determined by a fluorescence time-synchronized titration method[15,27]. Fluorescence titration has been widely used to study the binding of eIF4E and cap analogs due to the presence of four conserved tryptophan residues, which are quenched upon the association of cap analogs[27]. We utilized similar experimental procedures as previously reported[15] with minor modifications.

Fluorescence spectra were recorded on a Cary Eclipse fluorescence spectrophotometer (Varian, Inc.). Titration experiments were carried out at 20 °C in fresh prepared HEPES buffer (50 mM HEPES, 100 mM KCl, 1 mM dithiothreitol, 0.5 mM EDTA) at pH 7.2. Samples were mixed in a semi-micro cuvette with a magnetic stirrer inside. The nonlinear fitting was carried out by the statistics software *JMP IN 7.0* (SAS institute), in which the following equation was applied (Supplemental Figure 2S). Fluorescence quenching titration was performed in duplicate and two parallel correcting experiments were carried out. Each titration data was corrected by the intrinsic fluorescence from ligands and the decrease of eIF4E in buffer without ligands (The decrease is partially due to the degradation of eIF4E at 20 °C and partially due to the dilution factor.) In each titration, a new vial of eIF4E was thawed on ice before use to maintain the same initial activity of eIF4E. A series of ligand stocks, 20 μM, 50 μM, 100 μM, 200 μM, 500 μM, 1 mM and 2 mM, were prepared. Besides determining the dissociation constants of Bn⁷-GMP analogs to eIF4E, we also determined some representative analogs' dissociation constants in the presence of PD₂ (KKRYDREFLLGFQFIFA) which is an eIF4G-derived peptide containing the consensus binding region of eIF4G. In the experiments with PD₂, eIF4E

and PD₂ were mixed together first, followed by the titration with Bn⁷-GMP analogs. The final concentration of PD₂ is 1 μM.

Using the fluorescence titration assay, we have determined moderate binding affinities for our synthesized eIF4E antagonists (Table 1 compounds **1–16**), with K_d ranging from 1.32 to 69.13 μM. A typical titration curve is shown (Supplemental Figure 3S). The degree of fluorescence quenching by the analogs ranged from 20–70% (Supplemental Table 1S).

2.5 3D-QSAR

The dataset of 16 Bn⁷-GMP analogs, along with Me⁷-GTP, Me⁷-GDP and Me⁷-GMP was imported into the SYBYL 7.1 discovery suite[28] for subsequent CoMFA[22] and CoMSIA [23] model construction. Both CoMFA and CoMSIA are based on the assumptions that receptor-ligand interactions are primarily shape-dependent and noncovalent. CoMFA derives a QSAR model by systematically sampling steric and electrostatic fields around a compound training set, and correlating variations in these fields with experimental activities. CoMSIA models are derived by calculating similarity indices based on Gaussian functions representing steric, electrostatic, hydrophobic, hydrogen bond donor and hydrogen bond acceptor interactions and correlating those fields with experimental activities. These two QSAR methods together cover the primary contributions to ligand binding and can help to elucidate key ligand-receptor interactions.

3D-QSAR calculations and visualizations were performed on a Linux SLED 10.2 operating system with two Intel Quad Core Xeon X5272 3.4 GHz CPUs, and an nVIDIA Quadro FX 4800 graphics system. Training-set compounds were constructed in Discovery Studio Visualizer 2.0 (Accelrys®) using the ligand (Bn⁷-GMP) extracted from 2V8X.pdb[24] as the construction template. The dataset was imported into the SYBYL 7.1 discovery suite[9] for subsequent CoMFA and CoMSIA model derivation.

The initial QSAR training set comprised 19 compounds (sixteen Bn⁷-GMP analogs along with Me⁷-GMP, Me⁷-GDP and Me⁷-GTP) with experimental binding affinities (K_d) for eIF4E ranging from 10 to ~70 000 nM. Three charge formalisms (MMFF94, Gasteiger-Hückel and Gasteiger-Marsili) were implemented in order to examine possible effects on improving the internal predictive ability of the models. Compound **1**, the original ligand bound in the crystal structure of 2V8X[24], was used as the alignment template. Compound **c**, which includes structural features common to all compounds, was used as the common substructure in the database alignment procedure.

Following standard CoMFA and CoMSIA methodologies, each training-set compound was placed into a three-dimensional lattice with grid points separated by 2 Å. For CoMFA, the steric (van der Waals) and electrostatic (Coulombic) field energies were calculated at each lattice point by summing the individual energy interactions between each atom of each training set molecule and a probe atom representing a sp³ carbon with a +1 charge. CoMSIA analyses were done focusing on hydrophobic and hydrogen bond donor/acceptor fields, with similarity indices computed using an sp³ carbon with a +1 charge, +1 radius and +1 hydrophobicity. Maximum field values were truncated in CoMFA to 30 kcal/mol for the steric fields and ± 30 kcal/mol for the electrostatic fields.

2.6 Regression Analysis

Linear regression equations were obtained by the partial least-squares (PLS) method, which correlates changes in the K_d values for the training set compounds with changes in steric and electrostatic fields (CoMFA) and similarity fields (CoMSIA). “Leave-one-out” cross-validation was implemented to assess the internal predictive ability of the CoMFA and

CoMSIA models. In this technique, compounds are systematically excluded from the training set, and the activity of each excluded compound is predicted by a new model derived from the remaining structures in the set. Cross-validation provided the optimum number of principal components (PCs) together with the highest cross-validated r^2 (r_{cv}^2 or q^2) value. The PLS analyses were then re-done without cross-validation using the optimum number of PCs, yielding final CoMFA and CoMSIA models from which conventional r^2 values, non-cross-validated standard errors of estimate, and F ratios were calculated. Model optimization was carried out by varying partial charge formalisms and column filtering levels (ranging from 0–8 kcal/mol) using the “divide and conquer” strategy developed and validated by Amin et al [29,30]. Contour maps were generated and visualized in order to graphically interpret each 3D-QSAR model.

3. Results

3.1 Computational Modeling- Docking

GScores generated from the virtual combinatorial library of Bn⁷-GMP analogs range from –12.12 to –5.61 (Supplemental Table 2S). Bn⁷-GMP exhibited a docked GScore of –10.35 while that of *para*-carboxylate Bn⁷-GMP was –12.12. With regard to the substitution effect, fluoro- and chloro-substituted GMPs demonstrated predicted binding modes similar to those observed in the crystal structure (2V8X.pdb[24]), incorporating the key π -cation interaction as well as hydrogen bonding to the phosphate group (Figure 1). Bn⁷-GMP analog fits perfectly on the concave surface of eIF4E, as shown in the secondary structure (Figure 2(a)). The *para*-halogenated analogs displayed minor differences in predicted binding modes, with the *para*-fluoro substituent oriented towards Trp166 and the *para*-chloro substituent oriented toward Lys162, most likely due to steric effects (Figure 2(b) and (c)). Regardless of substitution location, fluoro-substituted Bn⁷-GMPs exhibited a narrow GScore range from –10.44 to –10.05, while the GScores of chloro-substituted Bn⁷-GMPs ranged from –10.33 to –9.22. Interestingly, in the relatively bulky substituent, naphthyl Bn⁷-GMP, the guanine moiety was completely pushed away from the π -cation binding pocket while instead the naphthyl group was stacked in between Trp56 and Trp102 (Figure 2(d)).

3.2 Preparation of Bn⁷-GMP analogs

A series of Bn⁷-GMP analogs was prepared with minor modification according to a previously published method[19] in yields ranging from 50% to 90%. By using alkyl bromide compounds instead of alkyl chloride as starting materials, higher yields with less reaction times were observed. Most reactions were completed within 24 hours at R.T. For example, we prepared Bn⁷-GMP in a 70% yield, which is a significant improvement over the previously reported yield of 26%[19].

3.3 Analog Dissociation Constants Determination

A halogen substituent in the benzyl ring of the lead compound Bn⁷-GMP (**1**) did not improve binding affinity in comparison to the unsubstituted Bn⁷-GMP (**1**). In the case of fluoro substituents, *meta*-F (**2**, $K_d = 1.59 \pm 0.07 \mu\text{M}$) was found to be the most potent while *ortho*-F (**7**, $K_d = 4.39 \pm 0.18 \mu\text{M}$) was 2.8- fold less potent and *para*-F (**11**, $K_d = 8.22 \pm 0.62 \mu\text{M}$) was 5.2- fold less potent. Increasing the size of the halogen by replacing F with a Cl atom (**3**, **4**, and **5**) reduced binding affinity nearly 2- fold ($K_d = 1.99 \pm 0.33 \mu\text{M}$, $2.20 \pm 0.41 \mu\text{M}$ and $2.24 \pm 0.60 \mu\text{M}$, respectively). Also the Cl substituted analogs showed no positional effects with the measured K_d values for the *ortho*-, *meta*- and *para*-substituted molecules all within the range of experimental error. Considering resonance and inductive effects, EWG *meta*-CF₃ (**10**, $K_d = 7.56 \pm 1.84 \mu\text{M}$) enhances potency by 2.1- fold compared to EDG *meta*-methyl (**14**, $K_d = 15.85 \pm 4.04 \mu\text{M}$). However, surprisingly a stronger EWG nitro group (**13**, $K_d = 14.36 \pm 4.32 \mu\text{M}$) actually resulted in a 3.1- fold decrease in potency in comparison to *para*-COOH

(**8**, $K_d = 4.56 \pm 1.04 \mu\text{M}$). These contradictory results suggest that resonance and inductive effects may not be the only factors that affect binding. In the series of *ortho*-substituents (**5**, **7**, and **16**), the chloro-substituted compound (**5**, $K_d = 2.24 \pm 0.60 \mu\text{M}$) was found to be the most potent with the fluoro-substituted compound (**7**, $K_d = 4.39 \pm 0.18 \mu\text{M}$) 2.0- fold less potent and the methyl-substituted compound (**16**, $K_d = 69.13 \pm 9.69 \mu\text{M}$) 15.7- fold weaker in binding to eIF4E. Considering the similar size of fluorine and methyl group, it seems that the larger chlorine atom fits better in the binding site of eIF4E. Additionally, the significant decrease in binding affinity resulting from the introduction of a methyl group at *ortho*- position suggests that the *ortho*-methyl substituent may interact unfavorably with the nearby amino acid residues. Consistent with our previous results, the introduction of a relatively bulky aryl group naphthyl (**12**, $K_d = 9.05 \pm 0.24 \mu\text{M}$) did not substantially attenuate binding affinity.[31]

Some analogs were chosen as representatives to study the effect of PD₂ on the binding of cap analogs to eIF4E. Their K_d values in the presence of PD₂ are also listed in Table 1 with the fitting results summarized in Supplemental Figure 4S. The binding affinity of Bn⁷-GMP to eIF4E increases less than 1- fold in the presence of PD₂ while there is no obvious effect on the other three fluoro substituents. It has been claimed that a conformational change occurs upon the binding of eIF4G to eIF4E[32,33]. However, our results are consistent with the most recent equilibrium binding and transient state fluorescence quench kinetic studies conducted by Rhoads et al[34,35], which found that a fragment of eIF4G containing the eIF4E-binding domain had little influence on the binding of m⁷GpppG to human eIF4E. Although the dissociation constants only increased slightly, a conformational change of eIF4E upon binding of PD₂ was suggested by the observed fluorescence emission spectrum shift (λ_{max} shifted from 340 nm to 335 nm; data not shown).

3.4 3D-QSAR

In the initial CoMFA model strategies, the full library of 19 compounds was used as the training set. The set was aligned using compound **1** as the template and compound **c** as the common substructure. In the initial leave-one-out cross-validation, a correlation coefficient of 0.480 was obtained with the optimum number of principal components at 6. A conventional r^2 of 0.954 was obtained in the subsequent PLS analysis, without cross-validation. This CoMFA model was visualized, and three outlier compounds were omitted to generate a new, significantly improved model with exceptionally high self-consistency ($r^2 = 0.992$, F-ratio = 189.609) and very good internal predictive ability ($r_{\text{cv}}^2 = 0.704$) (Supplemental Table 3S). A selection of charge formalisms and various column filtering levels were applied to further optimize the model. Three charge formalisms were implemented in turn: MMFF94, Gasteiger-Hückel and Gasteiger-Marsili. All three yielded very similar PLS analysis results (Supplemental Table 4S), with the MMFF94 formalism producing the best model. This observation is consistent with the most recent results published by Sorich and coworkers, which indicated that the MMFF94 method for computing partial charges resulted in statistically more predictive CoMFA and CoMSIA models than the Gasteiger charges[36]. Therefore, MMFF94 charges are recommended when possible to derive the most predictive CoMFA and CoMSIA models. Similarly, the best CoMSIA model was generated with exceptionally high self-consistency ($r^2 = 0.984$, F-ratio = 94.418) and very good internal predictive ability ($r_{\text{cv}}^2 = 0.623$) (Supplemental Table 5S). The experimental and CoMFA-predicted binding affinity values (Supplemental Table 6S) corresponding to the best predictive model in our case were plotted (Figure 3), and a contour map determined (Figure 4). For CoMSIA, parameter settings paralleled those for CoMFA: MMFF94 charges, six principal components, and column filtering of 5.6 kcal/mol. This model produced relatively high self-consistency ($r^2 = 0.984$, F-ratio = 94.418) and good internal predictive ability ($r_{\text{cv}}^2 = 0.623$). The experimental and CoMSIA-predicted binding affinity values corresponding to this model (Supplemental Table 7S) were plotted (Figure 5), and a contour map generated (Figure 6).

4. Discussion

The development of inhibitors of cap-dependent translation has focused several protein that either constituent or facilitate assembly of the eIF4F complex.[37] In particular, several attempts have been made to target the cap-binding protein, eIF4E, by either inhibition of eIF4G [38] or cap binding[19,39]. Bn⁷-GMP has been shown to be an antagonist of eIF4E cap binding [20,34,35] and recently an x-ray structure of the Bn⁷-GMP complex[24] has become determined. Therefore, aided by computer modeling, we have attempted to explore the importance of the benzyl moiety on the binding of Bn⁷-GMP to the cap-binding protein, eIF4E. A combinatorial library of 80 compounds was generated and docked into the binding site of eIF4E (2V8X.pdb[24]) using CombiGlide. Based upon the docking results, sixteen compounds were selected for synthesis and characterized. Their binding affinity values were determined by fluorescence titration experiments. Seven of the Bn⁷-GMP derivatives showed similar binding affinity to eIF4E, within 2- to 5- fold.

Among the fluoro substituted Bn⁷-GMPs, differences in binding affinity were observed. The K_d value for *para*-Fluoro Bn⁷-GMP (**11**, $K_d = 8.22 \pm 0.62 \mu\text{M}$) was found to be more than 5-fold greater than the value for *meta*-Fluoro Bn⁷-GMP (**2**, $K_d = 1.59 \pm 0.07 \mu\text{M}$), which is consistent with previous reported values[19]. For chloro substituted Bn⁷-GMPs, the position of the halogen had little effect on the K_d values. Recently, Walkinshaw et al. determined K_d values of 7 μM and 2 μM for Bn⁷-GMP and *para*-F Bn⁷GMP, respectively, with a novel mass spectroscopy method. [24] In contrast, we observed an 8- fold reduction in the binding affinity for *para*-fluoro substituted Bn⁷-GMP (**11**, $K_d = 8.22 \pm 0.62 \mu\text{M}$) when compared to Bn⁷-GMP. While it can be argued that the values are not significantly different, we did not find that the introduction of a fluoro atom at the *para* position of the benzyl moiety enhanced binding affinity.

Similar to binding studies with Me⁷-GTP[35], the presence of PD₂, an eIF4G-derived peptide with nanomolar affinity, had little effect on the binding affinities of Bn⁷-GMP analogs to eIF4E. For example, the K_d for Bn⁷-GMP to eIF4E decreased from $1.32 \pm 0.062 \mu\text{M}$ to $0.79 \pm 0.18 \mu\text{M}$. In the case of three fluoro-substituted Bn⁷-GMP analogues, no significant difference of binding affinity was observed in the presence or in the absence of PD₂.

When the docking scores from CombiGlide were compared to the experimental results, little correlation was found. There are several possible reasons for this finding. Rigid-receptor docking procedures such as that implemented in CombiGlide cannot take into account changes in receptor configuration that occur upon ligand binding[40]. Moreover, docking and scoring in general does not account for all possible contributions to ligand binding, and is best used to examine potential binding modes, prioritize compounds for synthesis and increase the number of active compounds that are experimentally evaluated. The fact that cap binding can induce long-range conformational changes is likely to make docking even more problematic[41]. In the crystal structure of Bn⁷-GMP and eIF4E complex[24], W103 adopts a 180° ring flips to accommodate the bulky benzyl group[24]. Naphthyl (compound **12**, $K_d = 9.05 \pm 0.24 \mu\text{M}$) is selected to represent a bulky substitution. The moderate binding affinity observed showed that a cap analog with a bulky substitution, like naphthyl, can still fit well into the binding site, which is not predicted from computational modeling. Molecular modeling predicted that the purine moiety, which contributes to the key π -cation interaction with W102 and W56, would extend out of the cap binding site. Meanwhile, the naphthyl group forms a parallel stack with the two binding site tryptophan residues (Figure 2(d)). If W103 can flip 180° to accommodate a bulky benzyl group, other conformational changes may also occur to accommodate the bulkier naphthyl group, thus explaining why the bulky sized naphthyl Bn⁷-GMP still exhibited moderate binding to eIF4E. Ongoing X-ray crystallography studies should reveal whether the

cap binding site of eIF4E accommodates the naphthyl moiety of compound **12** via a new binding mode.

To further gain insights into how our newly synthesized antagonists could contribute to the binding to eIF4E, we performed 3D-QSAR calculations to correlate the molecular properties of eIF4E antagonists with the experimentally determined binding affinity values. Two highly predictive and self-consistent CoMFA and CoMSIA models were derived for Bn⁷-GMP analogs; these models may prove useful for guiding the design of structurally similar compounds. The best CoMFA model indicated that a negative charge is favored in the ligand region of the phosphate group, and that various benzyl substitutions are tolerated in regions of favored steric bulk (Figure 4). The best CoMSIA model revealed the importance of key hydrophobic and hydrogen-bonding interactions for successful eIF4E antagonists (Figure 6). This model in particular pinpointed one small hydrophobic region (yellow) close to the *ortho*- side of benzyl group, along with one large hydrogen-bond acceptor disfavored region (red) close to the *para*- side of the benzyl group, and two medium-sized hydrogen-bond donor disfavored regions (purple) close to the *meta*- side of the benzyl group. The CoMSIA model also indicated that hydrogen-bond acceptors are favored on the ligand at the phosphate region, which parallels experimental X-ray binding modes.

Given the emerging importance of eIF4E as a validated anti-cancer target, our development of the first 3D-QSAR model for inhibitor binding to the eIF4E cap-binding site should facilitate the design of new and more potent inhibitors.

5. Experimental

5.1. ((2R,3S,4R,5R)-5-(2-amino-7-benzyl-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, **1**

Yield: 70%; ¹H NMR (D₂O, 300 MHz): δ 9.42 (s, 1H), δ 6.92–7.21 (m, 5H), δ 5.92 (s, 1H), δ 5.41 (s, 2H), δ 3.92–4.63 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 4.81, RP-HPLC RT: 1.69 min, HR- MS (ESI neg) C₁₇H₁₉N₅OP, m/z: calcd 452.0977, found 452.0946, Err 6.84 ppm

5.2. ((2R,3S,4R,5R)-5-(2-amino-7-(3-fluorobenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, **2**

Yield: 32%, ¹H NMR (D₂O, 300 MHz): δ 9.42 (s, 1H), δ 6.92–7.21 (m, 5H), δ 5.92 (s, 1H), δ 5.41 (d, 2H), δ 3.92–4.63 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 4.81, RP-HPLC RT: 1.70 min, HR- MS (ESI neg) C₁₇H₁₈FN₅O₈P, m/z: calcd 470.0883, found 470.0884, Err -0.34 ppm

5.3. ((2R,3S,4R,5R)-5-(2-amino-7-(4-chlorobenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, **3**

Yield: 75%, ¹H NMR (D₂O, 300 MHz): δ 7.24–7.35 (m, 4H), δ 5.95 (d, 1H), δ 5.52 (s, 2H), δ 3.62–4.35 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 5.511, RP-HPLC RT: 2.30 min, HR- MS (ESI neg) C₁₇H₁₈ClN₅O₈P, m/z: calcd 486.0587, found 486.0564, Err 4.75 ppm

5.4. ((2R,3S,4R,5R)-5-(2-amino-7-(3-chlorobenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, **4**

Yield: 33%, ¹H NMR (D₂O, 300 MHz): δ 7.19–7.25 (m, 4H), δ 5.92 (d, 1H), δ 5.49 (s, 2H), δ 3.71–4.50 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 1.889, RP-HPLC RT: 1.73 min, HR- MS (ESI neg) C₁₇H₁₈ClN₅O₈P, m/z: calcd 486.0587, found 486.0574, Err 2.60 ppm

5.5. ((2R,3S,4R,5R)-5-(2-amino-7-(2-chlorobenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, 5

Yield: 67%, ¹H NMR (D₂O, 300 MHz): δ 9.11 (s, 1H), δ 7.12–7.31 (m, 4H), δ 5.91 (d, 1H), δ 5.61 (s, 2H), δ 3.82–4.31 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 3.182, RP-HPLC RT: 1.67 min, HR- MS (ESI neg) C₁₇H₁₈ClN₅O₈P, m/z: calcd 486.0587, found 486.0605, Err –3.73 ppm

5.6. ((2R,3S,4R,5R)-5-(2-amino-7-(2-chloro-5-fluorobenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, 6

Yield: 41%, ¹H NMR (D₂O, 300 MHz): δ 7.38 (m, 1H), δ 6.81–6.92 (m, 2H), δ 5.91 (d, 1H), δ 5.60 (s, 2H), δ 3.92–4.29 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 1.259, RP-HPLC RT: 1.92 min, HR- MS (ESI neg) C₁₇H₁₇ClFN₅O₈P, m/z: calcd 504.0493, found 504.0504, Err –2.21 ppm

5.7. ((2R,3S,4R,5R)-5-(2-amino-7-(2-fluorobenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, 7

Yield: 48%, ¹H NMR (D₂O, 300 MHz): δ 6.89–7.14 (m, 4H), δ 5.79 (d, 1H), δ 5.42 (s, 2H), δ 3.63–4.46 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 4.723, RP-HPLC RT: 1.92 min, HR- MS (ESI neg) C₁₇H₁₈FN₅O₈P, m/z: calcd 470.0883, found 470.0884, Err –0.34 ppm

5.8. ((2R,3S,4R,5R)-5-(2-amino-7-(4-carboxybenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, 8

Yield: 62%, ¹H NMR (D₂O, 300 MHz): δ 9.21 (s, 1H), δ 7.71 (d, 2H), δ 7.35 (d, 2H), δ 5.91 (d, 1H), δ 5.52 (s, 2H), δ 3.96–4.31 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 1.376, RP-HPLC RT: 1.67 min, HR- MS (ESI neg) C₁₈H₁₉N₅O₁₀P, m/z: calcd 496.0875, found 496.0877, Err –0.45 ppm

5.9. ((2R,3S,4R,5R)-5-(2-amino-7-(3-methoxybenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, 9

Yield: 81%, ¹H NMR (D₂O, 300 MHz): δ 7.11 (t, 1H), δ 6.62–6.81 (m, 3H), δ 5.82 (d, 1H), δ 5.44 (s, 2H), δ 3.73–4.56 (m, 6H, ribose), δ 2.58 (s, 3H), ³¹P NMR (D₂O, 300 MHz): δ 4.728, RP-HPLC RT: 1.89 min, HR- MS (ESI neg) C₁₈H₂₁N₅O₉P, m/z: calcd 482.1082, found 482.1089, Err –1.38 ppm

5.10 ((2R,3S,4R,5R)-5-(2-amino-6-oxo-7-(3-(trifluoromethyl)benzyl)-1H-purin-1-ium- 9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, 10

Yield: 34%, ¹H NMR (D₂O, 300 MHz): δ 9.21 (s, 1H), δ 7.41–7.51 (m, 4H), δ 5.92 (d, 1H), δ 5.53 (s, 2H), δ 3.93–4.29 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 3.094, RP-HPLC RT: 2.13 min, HR- MS (ESI neg) C₁₈H₁₈F₃N₅O₈P, m/z: calcd 520.0851, found 520.0860, Err –1.82 ppm

5.11 ((2R,3S,4R,5R)-5-(2-amino-7-(4-fluorobenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, 11

Yield: 56%, ¹H NMR (D₂O, 300 MHz): δ 6.92–7.22 (m, 4H), δ 5.81 (d, 1H), δ 5.49 (s, 2H), δ 3.92–4.55 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 4.601, RP-HPLC RT: 1.87 min, HR- MS (ESI neg) C₁₇H₁₈FN₅O₈P, m/z: calcd 470.0883, found 470.0883, Err 0 ppm

5.12. ((2R,3S,4R,5R)-5-(2-amino-7-(naphthalen-2-ylmethyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, 12

Yield: 68%, ¹H NMR (D₂O, 300 MHz): δ 7.57–7.63 (m, 5H), δ 7.25–7.28 (m, 3H), δ 5.80 (s, 1H), δ 5.57 (s, 2H), δ 3.82–4.53 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 4.92, RP-HPLC RT: 2.30 min, HR- MS (ESI neg) C₂₁H₂₁N₅O₈P, m/z: calcd 502.1133, found 502.1124, Err 1.9 ppm

5.13. ((2R,3S,4R,5R)-5-(2-amino-7-(3-nitrobenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, 13

Yield: 37%, ¹H NMR (D₂O, 300 MHz): δ 7.19–7.93 (m, 5H), δ 5.31 (s, 1H), δ 5.19 (s, 2H), δ 3.42–3.98 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 3.802, RP-HPLC RT: 1.90 min, HR- MS (ESI neg) C₁₇H₁₈N₆O₁₀P, m/z: calcd 497.0828, found 497.0807, Err 4.17 ppm

5.14. ((2R,3S,4R,5R)-5-(2-amino-7-(3-methylbenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, 14

Yield: 51%, ¹H NMR (D₂O, 300 MHz): δ 7.13–7.22 (m, 4H), δ 5.92 (d, 1H), δ 5.51 (s, 2H), δ 3.91–4.51 (m, 6H, ribose), δ 2.21 (s, 3H), ³¹P NMR (D₂O, 300 MHz): δ 4.306, RP-HPLC RT: 1.89 min, HR- MS (ESI neg) C₁₈H₂₁N₅O₈P, m/z: calcd 466.1133, found 466.1154, Err –4.51 ppm

5.15. ((2R,3S,4R,5R)-5-(2-amino-7-(2,5-dichlorobenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, 15

Yield: 64%, ¹H NMR (D₂O, 300 MHz): δ 6.81–7.12 (m, 5H), δ 5.75 (s, 1H), δ 5.39 (s, 2H), δ 3.92–4.63 (m, 6H, ribose), ³¹P NMR (D₂O, 300 MHz): δ 3.935, RP-HPLC RT: 2.42 min, HR- MS (ESI neg) C₁₇H₁₇Cl₂N₅O₈P, m/z: calcd 520.0197, found 520.0179, Err –4.06 ppm

5.16. ((2R,3S,4R,5R)-5-(2-amino-7-(2-methylbenzyl)-6-oxo-1H-purin-1-ium-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl phosphate, 16

Yield: 71%, ¹H NMR (D₂O, 300 MHz): δ 6.82–7.11 (m, 5H), δ 5.81 (s, 1H), δ 5.52 (s, 2H), δ 3.76–4.81 (m, 6H, ribose), δ 2.19 (s, 3H), ³¹P NMR (D₂O, 300 MHz): δ 4.774, RP-HPLC RT: 2.31 min, HR- MS (ESI neg) C₁₈H₂₁N₅O₈P, m/z: calcd 466.1133, found 466.1173, Err –8.62 ppm

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Bn ⁷ -GMP	N ⁷ - benzylated guanosine monophosphate
GDP	guanosine diphosphate
GTP	guanosine triphosphate
EWG	electron withdrawing group
EDG	electron donating group

ESI-MS	electrospray ionization mass spectrometry
HPLC	high performance liquid chromatography
HEPES	<i>N</i> -(2-hydroxyethyl)-piperazine- <i>N'</i> -2-ethanesulfonic acid
CoMFA	Comparative Molecular Field Analysis
CoMSIA	Comparative Molecular Similarity Indices Analysis

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Figure 1. Docked configuration of *meta*-F Bn⁷-GMP, with MOLCAD electron density surface (electrostatic potential mapping) of the eIF4E active site
Red = positive electrostatic potential; blue = negative electrostatic potential.

Figure 2. Docking of Bn⁷-GMP analogs in the eIF4E binding site

- (a) Secondary structure of eIF4E bound to *meta*-F Bn⁷-GMP
 (b) Docked configuration of Cl-substituted Bn⁷-GMP analogs
 (c) Docked configuration of F-substituted Bn⁷-GMP analogs
 (d) Docked configuration of naphthyl Bn⁷-GMP
 Hydrogen bonds are shown as yellow dotted lines.

Figure 3. A plot of the CoMFA-predicted vs experimental binding affinity values for Bn⁷-GMP Analogs
(Compounds 5, 11, 15 Omitted, MMFF94 Charges and Column Filtering = 5.6 kcal/mol)

(a)

(b)

(c)

Figure 4. CoMFA contour map for Bn⁷-GMP Analogs

(Compounds 5, 11, 15 Omitted) from best model with MMFF94 charges, shown with compound **16** ($pK_d = 3.160$). Colored polyhedra represent areas on or near the ligand where properties correlate strongly (contribution level of 80%) with binding affinity. (a) top (b) side (c) bottom views. Green = positive steric potential; yellow = negative steric potential, red = negative electrostatic potential, blue = positive electrostatic potential.

Figure 5. A plot of the CoMSIA-predicted vs experimental binding affinity values for Bn⁷-GMP Analogs
(Compounds 5, 11, 15 Omitted, MMFF94 Charges and Column Filtering = 5.6 kcal/mol)

(a)

(b)

Figure 6. CoMSIA contour map for Bn⁷-GMP Analogs (Compounds 5, 11, 15 Omitted), best model with MMFF94 charges, shown with compound **16** ($pK_d = 3.160$). Colored polyhedra represent areas on or near the ligand where properties correlate strongly (contribution level of 80%) with binding affinity. (a) top (b) side (c) bottom views. Yellow=hydrophobic functionalities favored, cyan = H-bond donors favored, purple = H-bond donors disfavored, magenta = H-bond acceptors favored and red = H-bond acceptors disfavored

Scheme 1.
General Synthetic Route of Bn⁷-GMP Analogs

Table 1

Summary of Gscores and K_d values for eIF4E Antagonists

No.	R ₁	R ₂	GScore	K_d μ M (w/o PD ₂)	K_d μ M (w/PD ₂)	
a	-CH ₃	(PO ₃ ⁻) ₃	..*	0.01 $\pm$ 0.003	ND	ND
b	-CH ₃	(PO ₃ ⁻) ₂	..	0.045 $\pm$ 0.01	ND	ND
c	-CH ₃	PO ₃ ²⁻	..	7.50 $\pm$ 0.40	ND	ND
1	-Bn	PO ₃ ²⁻	-10.35	1.32 $\pm$ 0.062	0.79 $\pm$ 0.18	
2	- <i>meta</i> -F Bn	PO ₃ ²⁻	-10.44	1.59 $\pm$ 0.07	2.23 $\pm$ 0.20	
3	- <i>para</i> -Cl Bn	PO ₃ ²⁻	-9.22	1.99 $\pm$ 0.33	ND	ND
4	- <i>meta</i> -Cl Bn	PO ₃ ²⁻	-10.33	2.20 $\pm$ 0.41	ND	ND
5	- <i>ortho</i> -Cl Bn	PO ₃ ²⁻	-10.02	2.24 $\pm$ 0.60	ND	ND
6	-2-Cl, 5-F Bn	PO ₃ ²⁻	-9.63	3.10 $\pm$ 0.37	ND	ND
7	- <i>ortho</i> -F Bn	PO ₃ ²⁻	-10.39	4.39 $\pm$ 0.18	3.36 $\pm$ 0.26	
8	- <i>para</i> -COOH Bn	PO ₃ ²⁻	-12.12	4.59 $\pm$ 1.04	ND	ND
9	- <i>meta</i> -OMe Bn	PO ₃ ²⁻	-9.77	6.60 $\pm$ 0.98	ND	ND
10	- <i>meta</i> -CF ₃ Bn	PO ₃ ²⁻	-9.69	7.56 $\pm$ 1.84	ND	ND
11	- <i>para</i> -F Bn	PO ₃ ²⁻	-10.05	8.22 $\pm$ 0.62	7.51 $\pm$ 1.83	
12	-Naphthyl Bn	PO ₃ ²⁻	-7.42	9.05 $\pm$ 0.24	ND	ND

No.	R ₁	R ₂	GScore	K _d μM (w/o PD ₂)	K _d μM (w/PD ₂)
13	-meta-NO ₂ Bn	PO ₃ ²⁻	-9.73	14.4 ± 4.32	ND
14	-meta-CH ₃ Bn	PO ₃ ²⁻	-10.33	15.8 ± 4.04	ND
15	-2,5-DiCl Bn	PO ₃ ²⁻	-9.63	17.3 ± 4.98	ND
16	-ortho-CH ₃ Bn	PO ₃ ²⁻	-10.06	69.1 ± 9.69	ND

* GScores of Me⁷-GTP, Me⁷-GDP and Me⁷-GMP were not calculated since the three dimensional structure of eIF4E they bind to is different from that of eIF4E to which Bn⁷-GMP analogs bind, as indicated in PDB files.

** ND: Not Determined