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Article

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Identification of eEF1A1 Gamendazole-Binding Site for Binding of 3-Hydroxy-4(1*H*)-Quinolinones as Novel Ligands with Anticancer Activity

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KEYWORDS. translation elongation factor, 3-hydroxy-4(1*H*)-quinolinones, molecular docking, affinity chromatography, isothermal titration calorimetry

ABSTRACT. Here, we have identified the interaction site of the contraceptive drug gamendazole using computational modeling. The drug was previously described as a ligand for eukaryotic translation elongation factor 1-alpha 1 (eEF1A1) and found to be a potential target site for derivatives of 2-phenyl-3-hydroxy-4(*1H*)-quinolinones (3-HQs) which exhibit anticancer activity. The interaction of this class of derivatives of 3HQs with eEF1A1inside the cancer cells was confirmed via pull-down assay. We designed and synthesized a new family of 3-HQs and subsequently applied isothermal titration calorimetry to show that these compounds strongly bind to eEF1A1. Further, we found some of these derivatives possess significant in vitro anticancer activity.

Introduction

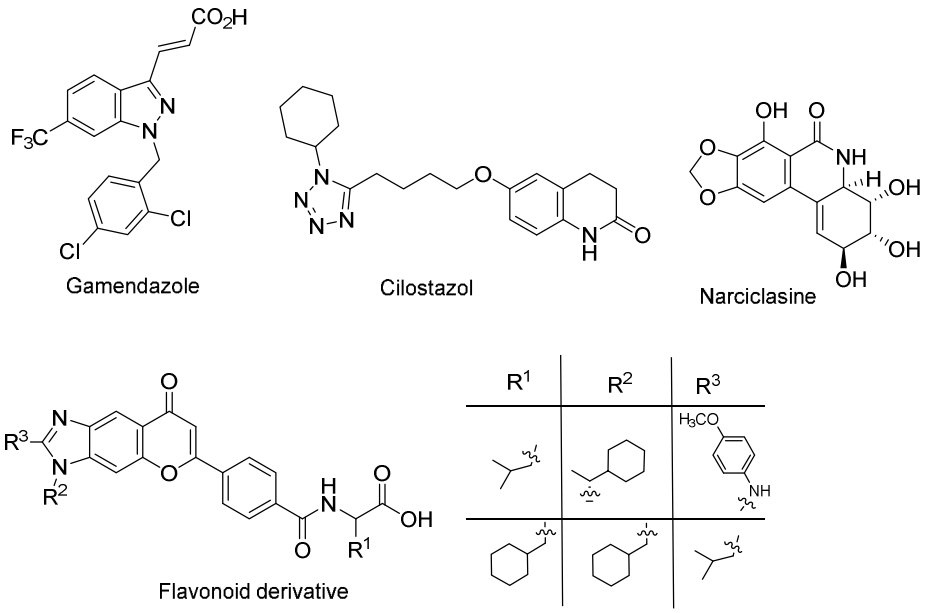
eEF1A1), the second most abundant eukaryotic protein, is responsible for ribosomal protein synthesis. It is also involved in cytoskeletal organization and other physiological activities.[1] Given its multifunctional property, eEF1A1 is considered as a moonlighting protein.[2] Moreover, eEF1A1 is associated with the development and progression of various cancers,[3–6] and has thus been proposed as a target for anticancer therapy.[7, 8] The overexpression and depletion of eEF1A1 have been shown to influence the cell apoptosis rate.[9, 10] Although eEF1A1was identified as a target a long time ago, only a few biological active compounds have been found to interact with eEF1A1. The male contraceptive agent gamendazole (Figure 1) binds directly to eEF1A1 but does not affect protein synthesis.[11] The phosphodiesterase inhibitor cilostazol (Figure 1) influences neurite outgrowth by binding to eEF1A1.[12] Narciclasine (Figure 1) and its analogs were reported to bind to eEF1A1 and overcome drug resistance by melanoma.[13]

However, the authors were not successful in the validation of the binding site. eEF1A1 has been described as a target for two natural flavonoid derivatives (Figure 1)[14], which exhibit high efficacy against breast cancer cells through their interaction with eEF1A1. However, despite this fact, a binding site has not been elucidated. Although an anticancer activity of such flavonoids caused by interaction with eEF1A1 has been shown (13,14), the systematic study of the description of binding site, in silico design, and synthesis of a set of compounds followed by verification of their biological activity against various types of cancer needs to be performed.

Several studies have reported that flavonoid aza-analogs – derivatives of 2-phenyl-3-hydroxy-4(1*H*)-quinolinone (3-HQ)– exhibit significant anticancer activities.[15-18] However, until now, no study has described their molecular target. We hypothesized that3-HQs due to its structural similarity to other flavonoid derivatives may serve as good ligands for interaction with eEF1A1.

In this report, we provide evidence of interaction of 3-HQ derivatives with eEF1A1 and the results of the rational design, synthesis, and *in vitro* biological evaluation of the new 3-HQ derivatives.

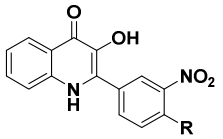
Figure 1. Structures targeting eEF1A1.[11-14]



Results and discussion

Interaction of 3-HQs with eEF1A1. The possible interaction of 3-HQs with eEF1A1 was first studied via docking experiments. Three ligands were chosen from the group of previously published active derivatives substituted by an amine at position *4′* and a nitro group at position *3′* of the 2-phenyl ring (Figure 2).[15, 19]

Figure 2. The reported IC_{50} (μM) of selected 3-HQs.[19]



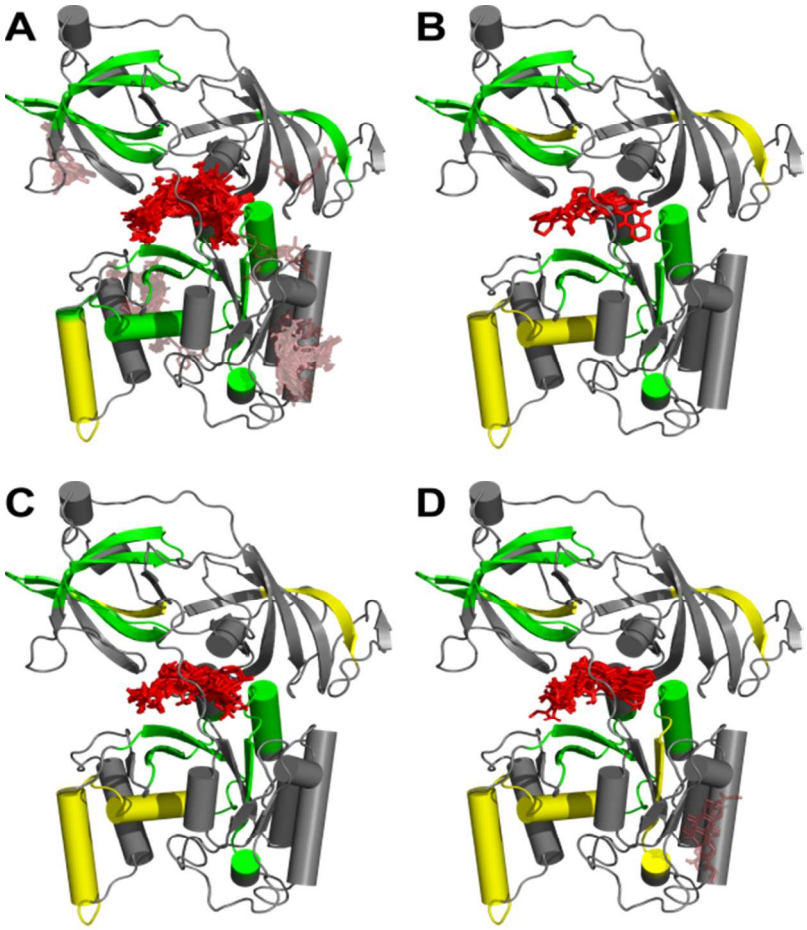
Comp. N°	R	CCRF-CEM	CEM-DNR-BULK	U2OS	K562	K562-Tax	A549	BJ
1		1.55	3.00	7.51	0.75	2.02	1.62	9.83
2		1.72	6.75	8.57	0.75	2.71	1.89	9.73
3		1.42	3.01	9.98	0.66	1.96	0.94	9.39

CCRF-CEM, acute lymphoblastic leukemia; CEM-DNR-BULK, CCRF-CEM cells, daunorubicin resistant; K562, acute myeloid leukemia; K562-TAX, K562 cells paclitaxel resistant; A549, lung adenocarcinoma; BJ, normal cycling fibroblasts cell line.

Since the crystallographic structure of human eEF1A1 is unavailable, a homology model was built using its known sequence (UniProt database ID: P68104[20]) and crystal structures of homologous eEF1A1from yeast and rabbit. The eEF1A1 protein structure consists of three domains connected by two coiled links in sequential order. The first domain is a combination of α-helices and β-sheets. The second and third domains consist primarily of β-sheets. First, we tried to verify the chosen docking method by using the known ligand, gamendazole as a standard, for which the protein binding site was previously identified by MALDI-TOF.[11] The results of this docking experiment presented in Figure 3-A and Table 2 show positive interaction of our model with gamendazole. Moreover, by comparing these results with the abovementioned binding site, we determined the amino acid sequence of the MALDI-TOF identified domain.

To better understand the binding site, we performed the docking experiments with the previously characterized crystal structures of the homologous proteins from yeast (1F60, 1G7C, 1IJE, 1IJF, 2B7B, and 2B7C[21-25]). All these docking experiments revealed the interaction of gamendazole with eEF1A1 with comparable docking energy in aa-tRNA binding site.[26, 27] A verification of binding site using known crystal structures of rabbit origin (4C0S[21-25]) could not be performed because of absence of crucial amino acids together with one artificially modified residue in the identified aa-tRNA binding site.[21-25]Among all the used crystal structures, 2B7C and 2B7B exhibited interaction with eEF1A1 also in proximity to the domain identified by MALDI-TOF and not confirmed by our docking to human eEF1A1 model (see Figure 3). The docking energy is comparable to the docking in the aa-tRNA binding site (see SI, Table S1). The only-MALDI-TOF identified domain [11] is known to be highly flexible.[26, 27]

Figure 3. The results of docking of gamendazole (A) and compounds **1-3** with the homology model of human eEF1A1 described in Figure 2 (B, C, and D, respectively)**.** Ligands in opaque red denote more favorable docking energy. The parts of the structures in green show the interaction sites identified by both MALDI-TOF [11] and our docking; those in yellow indicate sites identified by MALDI-TOF [11] only.

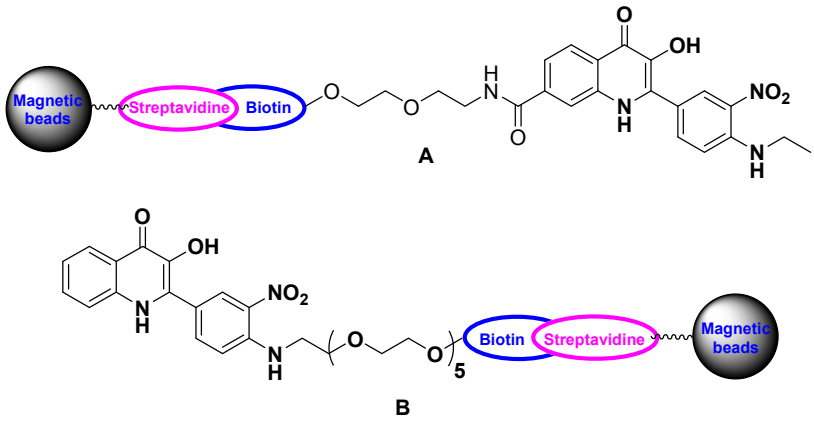


We found that the overall RMS between structures 2B7C and 1F60 is 1.13 Å (0.26 pm per residue), while RMS between the only-MALDI-TOF identified domains of these structures is 2.18 Å (4.24 pm per residue). The RMS between rest of the molecules in both protein structures

is 0.89 Å (0.24 pm per residue). We reasoned that due to the reason, no interaction of gamendazole in the only-MALDI-TOF domain was found with protein crystal structures 1G7C or 1IJF. Interaction in this domain is also less favorable in the case of 1IJE and 1F60 (see SI, Table S1). These facts led to the conclusion that our model for interaction with human eEF1A1 is valid. The 3-HQ derivatives **1-3** (Figure 2) were subjected to the docking study and these compounds docked into the same site as gamendazole (Figure 3-B,C,D). The conformation of the protein is less favorable for interaction with ligands in the highly flexible only-MALDI-TOF identified domain. This discrepancy is likely because our model does not address the loop and connecting domain flexibility.[11]

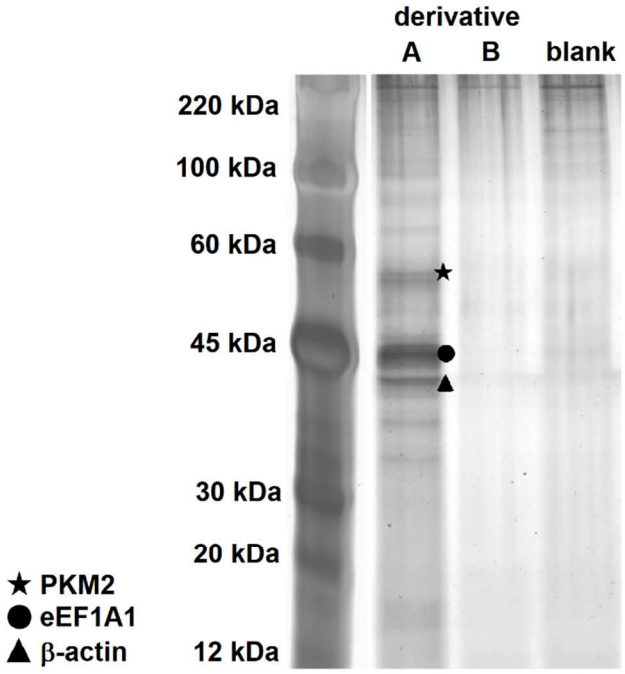
To provide evidence about the interaction of 3-HQs with eEF1A1, we performed pull-down assay with ligands **A** and **B** (Figure 4), derived from original compound **3** and immobilized on magnetic beads *via* streptavidin-biotin interaction.[28] Ligand **A** was attached to biotin *via* quinolinone benzene ring to enable interaction of proteins with 2-phenyl moiety, whereas ligand **B** was biotinylated *via* 2-phenyl ring to enable interaction of proteins with the opposite part of the 3-HQ scaffold. The synthesis of the biotinylated 3-HQs was performed using polystyrene resin with the combination of previously published protocols (see SI).[29]

Figure 4. Ligand **A** and **B** derived from active compound **3** used for pull-down assay.



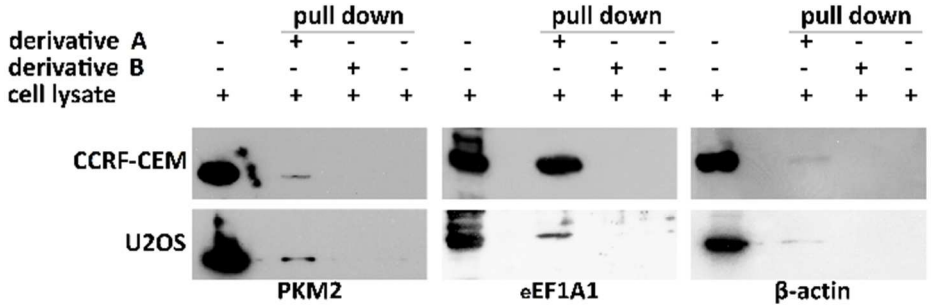
Pull-down assay for both derivatives was performed with total protein lysate prepared from CCRF-CEM or U2OS cells. Proteins released from the ligands were analyzed using SDS-PAGE (Figure 5) and then subjected to mass spectrometry characterization. The specific ligand-protein interaction was confirmed only for derivative **A**, suggesting the 2-phenyl moiety is the part of pharmacophore. In addition to the expected presence of eEF1A1 in the mixture of isolated proteins, we confirmed the presence of pyruvate kinase M2 (PKM2) and β-actin.

Figure 5. SDS-PAGE analysis of proteins isolated from pull-down assay of derivatives **A** and **B** incubated with CCRF-CEM protein lysate. The blank sample is represented by immobilization of biotin to streptavidin-coated magnetic beads.



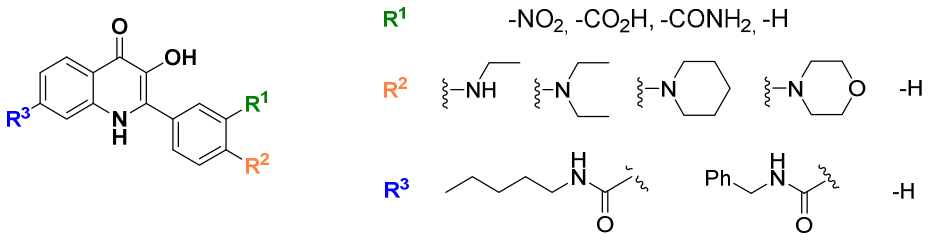
To validate identified targets, we performed western blot to visualize isolated proteins. We identified specific bands for PKM2, eEF1A1, and β-actin only for proteins captured by derivative **A;** while no signal for these proteins was detected for derivative **B** or biotinylated beads (Figure 6).

Figure 6. Characterization of proteins isolated from pull-down assay of derivatives **A** and **B** by western blot.



Structure design. Structural motifs of the compounds **1-3** were used to design a chemical analog set of potentially improved 3HQ inhibitors. The toxicity of these compounds against normal cells could be diminished by replacing the nitro group with its isosters such as carboxy or carboxamide motifs (R^1 in Figure 8). The amines substituted at position 4′ of the 2-phenyl ring were selected with respect to previous studies [19] (Figure 2) and were extended to a morpholine ring (R^2 in Figure 8). Moreover, the substitution of the benzene ring of the quinolinone skeleton by hydrophobic moieties was previously reported to positively influence its cytotoxicity and selectivity.[18] According to the proved interaction of derivative **A** with EF1A1, there is no negative effect on interaction when the moiety is bound *via* amidic bond at position 7. Therefore, the pentyl and benzylamide groups in this position were selected as representatives. By combining the three diverse positions, R^1, R^2 and R^3, an analog set of 3-HQs was compiled (Figure 8) and subjected to molecular docking simulations with eEF1A1.

Figure 8. Analog set of 3-HQs suggested for molecular docking.



The analog set of 3-HQ derivatives (Figure 8) was constructed using *ab initio* methods and then subjected to virtual screening. The derivatives with the most favorable docking energy (docking energy lower than -35589.5 $J.mol^{-1}$) were selected. The final selection of 3-HQ derivatives was

performed based on the ease of synthesis. As a result, seven 3-HQ derivatives were selected and synthesized (see **Table 1**).

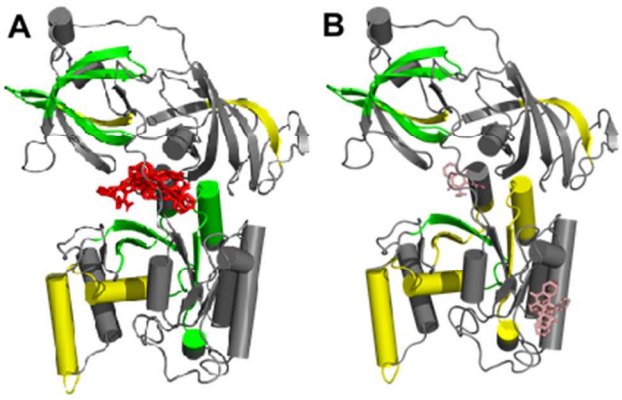
Table 1. Synthesized 3-HQ derivatives.

Comp. N^{o}	R^1	R^2	R^3
4	-CO_2H		-H
5	-CO_2H		-H
6	-$CONH_2$		-H
7	-$CONH_2$		-H
8	-$CONH_2$		-CONH-Pent
9	-$CONH_2$		-CONH-Bn
10	-H	-H	-H

The selected compounds included two cyclic amines (piperidine and morpholine) as R^2 substituents and substitution at position R^1 by the carboxylate and carboxamide functionalities. Further, unsubstituted derivative **10** was included as a control; it was previously described as inactive in cytotoxic assay.[16] In addition, molecular docking simulation also classified this compound as unsuitable for the target (Figure 9-B). The results of the molecular docking of the selected compounds to eEF1A1 are presented in **Table 2**. Most of the compounds bind to the same site as gamendazole, with binding energies ranging from -38520.4 to -41032.6 J.mol^{-1} (Table 2, Figure 9-A and SI). The results of compound **10** are in accordance with the expectation because it binds at a different location on the protein surface with the energy of -34752.1 J.mol^{-1} (Figure 9-B). Some weak interactions of compound **10** with the gamendazole docking site with the energy of -31821.2 J.mol^{-1} were also revealed. We identified that all studied compounds share same binding site which is located at the center of eEF1A (Table 2). The compounds

interacted with the loop between the first and second domains and was identified as part of the aa-tRNA binding site.[26, 27]. Moreover, this site is positioned opposite to that of the GTP, GDP, and GMP binding pockets as observed in the known PDB structures.[21, 22] These results suggest that the interaction between our compounds and eEF1A1 would not reduce eEF1A1´s activity by inhibiting the GTP binding site.

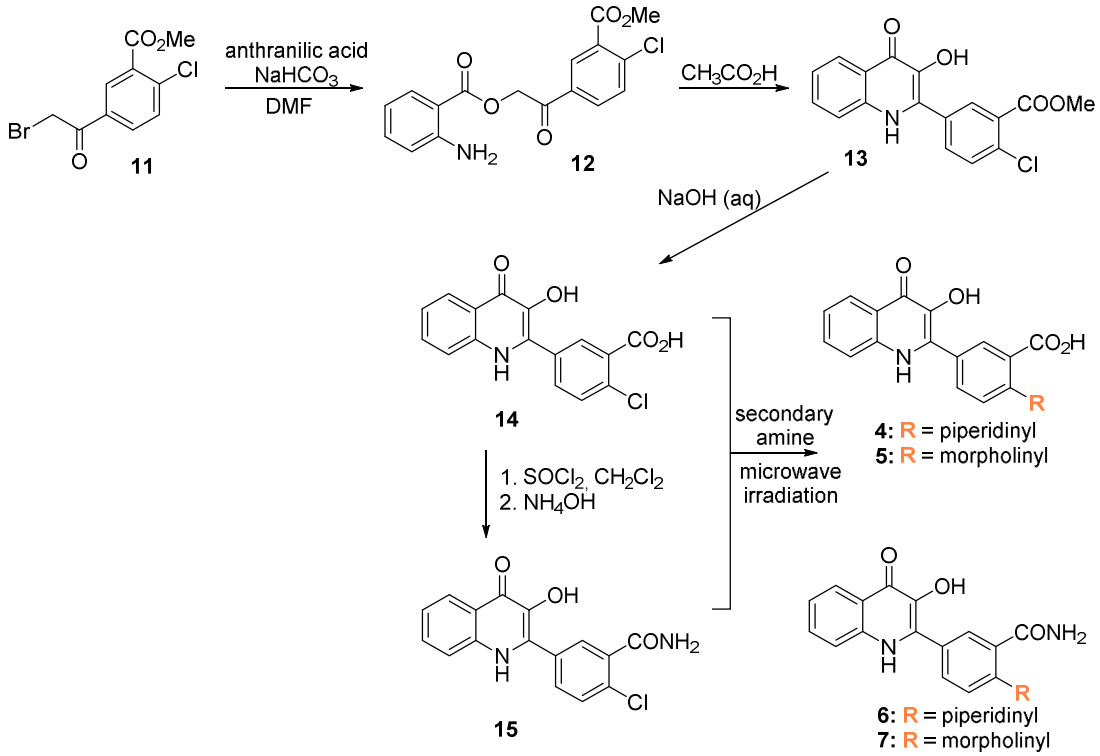
Figure 9. Docking of unsubstituted derivative **10 (B)** compared with active derivative **6 (A)** to an eEF1A1 model**.** Ligands shown in opaque red denote more favorable docking energy. The parts of the structures in green show the interactions identified by both MALDI-TOF[11] and our experiment; those in yellow indicate the interactions identified by MALDI-TOF.[11]



Synthesis. Derivatives **1-3** were synthesized according to a published protocol.[19] The synthesis of carboxylate and carboxamide analogs **4-7** is displayed in **Scheme 1**. Phenacyl ester **12**was prepared by the reaction of anthranilic acid with bromoketone**11**. The cyclization to 3-HQ **13**was inspired by a well-established method described previously.[30, 31]Hydrolysis of the methyl ester afforded carboxylate 3-HQ **14** and its following amidation, carboxamide 3-HQ **15**. These derivatives were subsequently transformed to the desired 3-HQ **4-5** and **6-7** by nucleophilic substitution with secondary amines (piperidine or morpholine) in a microwave reactor.

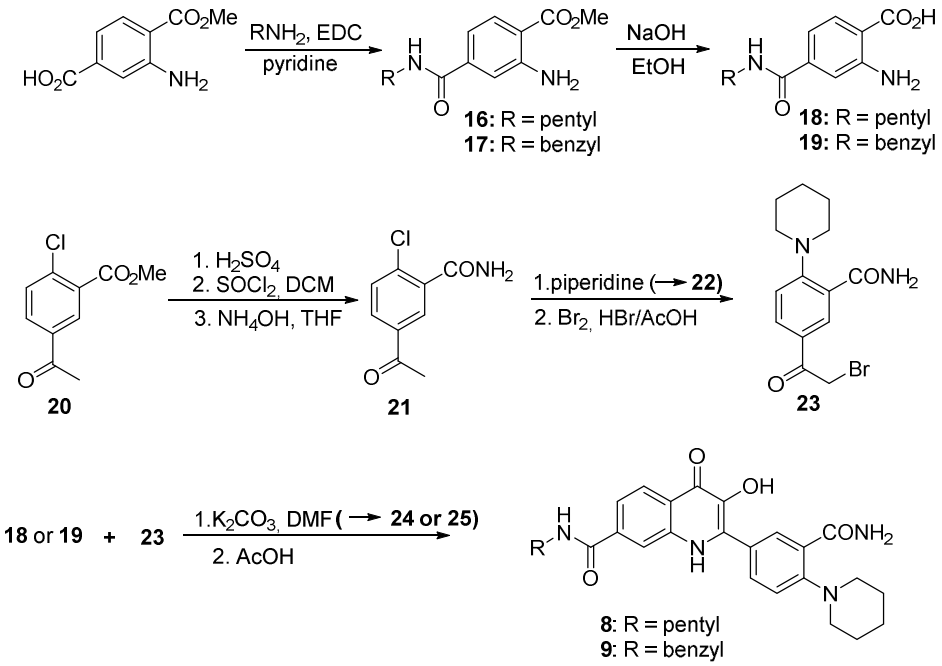
Introduction of carboxamide moiety into position 7 of the 3-HQ **8** and **9** was achieved in the first step of the synthesis (**Scheme 2**).

Scheme 1. Synthesis of selected 3-HQs.



Starting 2-amino-4-methylterephthalate was converted to amide **16**or **17** by reaction with pentyl- or benzylamine, respectively, in pyridine. Hydrolysis of ester group by sodium hydroxide to obtain the first building blocks **18** and **19** followed. Second building block **23** was gradually built up from ester **20** by its successive hydrolysis, chlorination, and amidation to form amide **21** followed by substitution of chlorine with piperidine and finally α-bromination (Scheme 2).

Scheme 2. Synthesis of designed 3-HQs substituted by 7-carboxamide group.



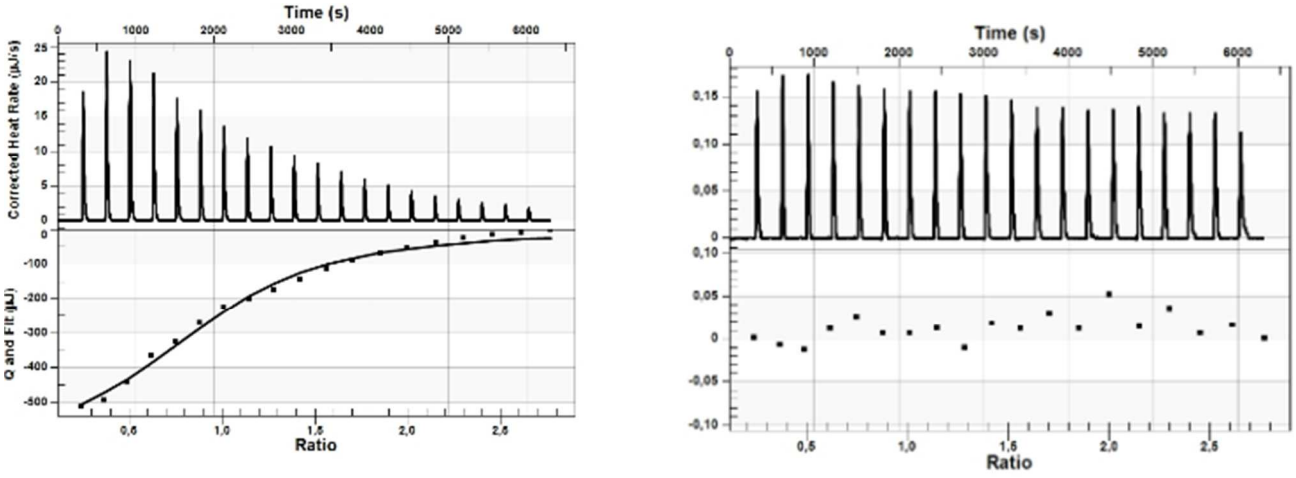
Biological evaluation. The interaction between the prepared compounds and eEF1A1 was studied using isothermal titration calorimetry (ITC). ITC measures the changes in heat during interaction and provides thermodynamic information about the binding affinity of a ligand to a protein. The measured enthalpies for the interactions of the synthesized 3-HQ derivatives with the protein and the K_a values suggest high affinities (**Table 2**). The only exception within the series is the non-substituted derivative **10**, used here as a negative control, which exhibited no interaction with the protein. This result is in accordance with the poor molecular docking results of the compound **10**. Similarly, gamendazole in the form of analytical standard [32] was subjected to the interaction studies as a positive control. As expected, binding of gamendazole with eEF1A was confirmed, although the interaction is much weaker than those of 3-HQs (see the titration profile in SI).

Table 2. Thermodynamic and binding parameters for the interaction of the 3-HQ compounds with eEF1A1 ($\Delta_r H_m^o$= enthalpy change of binding; K_a= association constant; n = stoichiometry; $\Delta_r S_m^o$= entropy change, $\Delta_r G_m^o$ = Gibbs energy change, Docking $\Delta_r G_m^o$ = Gibbs energy change determined by molecular modeling).

Compound	$\Delta_r H_m^o$(kJ mol$^-$	K_a^o(M^{-1})	n	$\Delta_r S_m^o$(J mol^{-1} K$^-$	$\Delta_r G_m^o$(J mol$^-$	Docking $\Delta_r G_m^o$(J mol$^-$
4	-11272	2.536 x	0.798	-37686	-35919	-38911
5	-1872	1.029 x	1.640	-6147	-39272	-38911
6	-15999	2.899 x	0.932	-53539	-36347	-38492
7	-7932	6.796 x	0.746	-26474	-38777	-38911
8	-9416	5.297 x	1.129	-31454	-37990	-38911
9	-9213	1.809 x	1.694	-30764	-40713	-43932
10			*no*			
Gamendazole	-1209	1,268 x	1,777	-3919	-40550	-36401

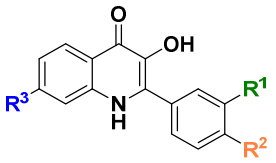
The value of the stoichiometric binding number (n) is approximately 1 for all the compounds. The negative changes in free energy and enthalpy confirm that the binding of the ligands to eEF1A1 is spontaneous and exothermic. An example of a calorimetric titration profile showing the ligand-protein interaction, i.e., that of derivative **6** in comparison with the non-interacting derivative**10**, is given in Figure 6 (for the titration profiles of other compounds, see SI).

Figure 6. Calorimetric titration profile for the interaction of 3-HQ **6** (left) and **10** (right) with eEF1A1.



Finally, we tested the *in vitro* activity of the prepared derivatives against cancer cell lines (Table 3). Compared to original compound **1**, the replacement of nitro group by carboxy moiety (derivatives **4** and **5**) caused the loss of activity. This could be caused by the carboxy group itself, which apparently restricts the penetration of the compounds through the cell membrane. Transformation of carboxy group to carboxamide (derivative **6**) significantly improved the activity in comparison with derivatives **4** and **5** and reached the similar values as observed for derivative **1,** except for CEM-DNR and U2OScell lines. The toxicity to normal cell line BJ was improved by one order. When the piperidine ring was replaced with morpholine (compare derivatives **6** and **7**), the activity was lost. As predicted, the improvement of toxicity against majority of the cancer cell lines was achieved by insertion of pentylamide moiety to position 7 (derivative **8**). In the case of improved toxicity,submicromolar IC_{50}values were achieved for two lines. Unfortunately, the toxicity against normal BJ cells was similar to that of original compound **1**. Replacement of pentylamide with benzylamide group (derivative **9**) led to IC_{50} values at micromolar range for adenocarcinoma A549 line, leukemia cell lines K562, CCRF-CEM, and colorectal tumor HCT-116. Similarly, the compound displayed submicromolarIC_{50} values for osteosarcoma cell lines (U2OS), whereas it did not exhibit activity against cancer cell lines (CEM-DNR and K562-TAX) resistant to chemotherapy. Most importantly, toxicity of **9** against normal cell line (BJ) was significantly higher than that of original compounds **1-3**.

Table 3. Cytotoxic activity of prepared derivatives against cancer cell lines.



3-HQ	R^1	R^2	R^3	A549	U2OS	CCRF-CEM	CEM-DNR	HCT-116	K562	K562-TAX	BJ
4	-CO_2H	1-piperidinyl	H-	50	50	49.56	50	50	50	50	50
5	-CO_2H	4-morpholinyl	H-	50	50	49.56	50	50	50	50	50
6	-$CONH_2$	1-piperidinyl	H-	9.96	26.02	3.34	21.43	4.46	7.1	6.41	48.92
7	-$CONH_2$	4-morpholinyl	H-	44.37	50	16.24	50	30.6	50	50	50
8	-$CONH_2$	1-piperidinyl	$C_5H_{11}CONH$-	1.89	0.56	1.73	8.03	0.64	2.39	32.71	10.24
9	-$CONH_2$	1-piperidinyl	$PhCH_2CONH$-	3.77	0.58	1.73	>50	1.01	8.30	>50	>50
10	-H	-H	H-	>50	46.96	26.74	22.49	44.20	47.96	27.44	>50

Conclusion

We suggested previously reported cytotoxic derivatives of 2-phenyl-3-hydroxy-4(1*H*)-quinolinone as the ligands for eEF1A1 and *via* docking experiments we provided evidence of their interaction with this target. We further described in detail the binding site of these compounds which are similar to contraceptive agent gamendazole. The results obtained via computational study were corroborated by pull-down assay. The knowledge was used for the design of advanced derivatives of 3-HQs and their interaction with eEF1A1 was confirmed using micro calorimetric measurement. Some of these derivatives possess remarkable inhibitory activity against selected cancer cell lines and low toxicity against normal cell line, namely fibroblasts BJ. In summary, this study is the first report describing rational design and synthesis of anticancer compounds acting as eEF1A1 ligands using in silico technique and demonstrating the molecular target for 3-HQ derivatives, which have been reported as potent anticancer agents.

Detailed elucidation of binding site can serve for future design of new eEF1A1 ligands to inhibit this relatively unexplored molecular target and set up new strategy in anticancer treatment.

Experimental Section

Materials and Methods

For preparation and characterization of the compounds, LC/MS analyses were performed using UHPLC/MS with UHPLC chromatograph (Acquity) attached with a PDA detector, a single quadrupole mass spectrometer (Waters), an X-Select C18 column at 30 °C with a flow rate of 600 µl/min. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with a linear gradient over the course of 2.5 min; the final ratio was maintained for 1.5 min. The column was re-equilibrated with 10% B for 1 min. The APCI ionization operated at a discharge current of 5 µA, a vaporizer temperature of 350 °C, and a capillary temperature of 200 °C. Purity of the compounds was determined using the ratio of the appropriate peak area to sum of areas of all peaks of the mixture. Areas were determined by integration of the peaks from the PDA detector response. Purification was performed using semi-preparative HPLC with a Waters 1500 series HPLC equipped with a 2707 Autosampler, a 1525 binary HPLC pump, a 2998 Waters Photodiode Array Detector, and a Waters Fraction Collector III with a YMC C18 reverse phase column (20 × 100 mm, 5 µm particle size). The mobile phase consisted of acetonitrile and a 10 mM aqueous ammonium acetate gradient over 6 min. The purity of the tested compounds was determined by HPLC, which in all the cases was ≥96%.

NMR spectra were measured in DMSO-d_6 using a Jeol ECX-500 (500 MHz) spectrometer or a Bruker Avance (300 MHz) instrument. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (*J*) are reported in Hertz (Hz).

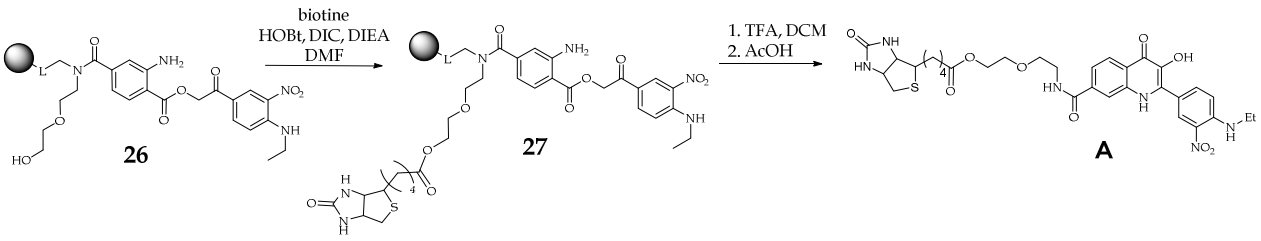
Solvents and chemicals were purchased from Sigma-Aldrich (Milwaukee, IL, www.sigmaaldrich.com).

All compounds tested on the biological activity were successfully filtered for PAINS.

Synthesis of studied compounds

2-(2-(2-(4-(Ethylamino)-3-nitrophenyl)-3-hydroxy-4-oxo-1,4-dihydroquinoline-7-carboxamido)ethoxy)ethyl 2-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)acetate(A)

Ligand **A** was prepared according to the following scheme:

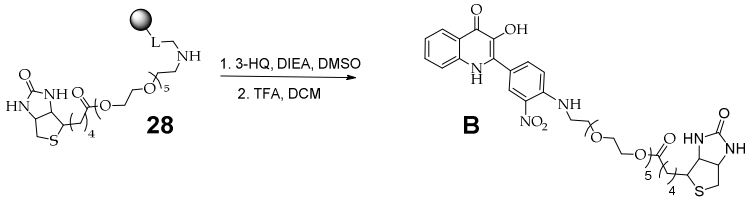


The compound **26**was prepared on solid support (BAL linker) according to the published procedure.[33] The resin **26**was then reacted with solution of biotin (0.24 g, 1.0 mmol), HOBt (135 mg, 1.0 mmol), *N,N*-diisopropylethylamine (DIEA) (0.05 ml, 0.3 mmol), and DIC (0.15 ml, 0.3 mmol) in DMF (2 ml) for 16 h at room temperature. The obtained resin **27**was then washed three times with DMF (3× 2 ml) and DCM (3 ×2 ml) and the compound was cleaved from the resin by 50% trifluoracetic acid (TFA) in DCMand dried undera stream of nitrogen. Final cyclization was performed by refluxing the cleaved compound in acetic acid (AcOH) for 2 h. The obtained ligand **A** was purified by HPLC, which resulted in~25%yield. MS (APCI) exact mass calculated for $C_{16}H_{11}ClNO_4$ $[M+H]^+$: 683.25; found: 683.70. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.70

(br, 1 H), 8.60 - 8.76 (m, 2 H), 8.39 (t, *J* = 5.58 Hz, 1 H), 8.24 (br, 1 H), 8.18 (d, *J* = 8.42 Hz, 1 H), 8.05 (d, *J* = 8.42 Hz, 1 H), 7.67 (d, *J* = 8.60 Hz, 1 H), 7.25 (d, *J* = 9.15 Hz, 1 H), 6.38 (d, *J* = 18.66 Hz, 2 H), 4.04 - 4.19 (m, 3 H), 3.18 - 3.69 (m, 15 H), 2.79 (dd, J_1= 12.26 Hz, J_2 = 4.94 Hz, 1 H), 2.55 (d, *J* = 12.44 Hz, 1 H), 2.25 (t, *J* = 7.41 Hz, 2 H), 1.37 - 1.65 (m, 3 H), 1.27 (t, *J* = 7.14 Hz, 3 H).

2-(2-((4-(3-Hydroxy-4-oxo-1,4-dihydroquinolin-2-yl)-2-nitrophenyl)amino)ethoxy)ethyl 2-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)acetate (B)

Ligand **B** was prepared according to the following scheme:



First, the polymer-supported biotinylated spacer **28**was prepared according to previously published procedure.[34] The resin **28**(300 mg) was then shaken in the solution of 2-(4-chloro-3-nitro)phenyl-3-hydroxyquinolin-4(*1H*)-one (3 mmol) and DIEA (0.15 ml, 1 mmol) in DMSO (9 ml) for 16 h at room temperature. Acid-mediated cleavage (50% TFA in DCM, 30 min, room temperature) and HPLC purification were performed to obtain final ligand **B**.

General procedure for preparation of compounds 4-7: Derivative **14** or **15** (0.28 mmol) was dissolved in corresponding amine (3 ml) and the mixture was stirred in microwave reactor for 6 h at 200 °C and 200 W. The precipitate formed was filtered, washed with water, and dried under vacuum.

5-(3-Hydroxy-4-oxo-1,4-dihydroquinolin-2-yl)-2-(piperidin-1-yl)benzoic acid (4): Yellow powder. HPLC purity: 98%. Yield: 0.14 g (40%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.64 (br, 1H), 8.43 (s, 1 H), 8.15 (d, J = 8.0 Hz, 1H), 8.06 (d, J = 7.5 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.60 (t, J = 7.0 Hz, 1H), 7.28 (t, J = 7.5 Hz, 1H), 3.12 (t, J = 5.0 Hz, 4H), 1.81 – 1.74 (m, 4H), 1.68 – 1.60 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 170.1, 166.6, 151.1, 138.1, 138.0, 134.2, 131.5, 130.7, 130.3, 130.0, 124.7, 124.5, 122.6, 121.9 (2C), 118.4, 53.5, 25.6, 22.3. HRMS (ESI) calculated for $[M+H]^+$ $C_{21}H_{20}N_2O_4$ 365.1496; found: 365.1495.

5-(3-Hydroxy-4-oxo-1,4-dihydroquinolin-2-yl)-2-morpholinobenzoic acid (5): Yellow powder. HPLC purity: 98%. Yield: 0.05 g (47%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.58 (s, 1H), 8.29 (d, J = 2.0 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 8.00 (dd, J_1= 8.5 Hz, J_2 = 2.0 Hz, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.62 - 7.58 (m, 2H) 7.27 (t, J = 7.5 Hz, 1H), 3.81 (t, J = 4.5 Hz, 4 H), 3.13 (t, J = 4.5 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 172.0, 170.0, 167.3, 151.2, 138.1, 137.9, 133.9, 131.7, 130.6, 130.2, 127.8, 124.5, 124.3, 121.9, 121.8, 120.9, 119.5, 118.4, 66.2, 52.2. HRMS (ESI) calculated for $[M+H]^+$ $C_{20}H_{19}N_2O_5$ 367.1288; found: 367.1288.

5-(3-Hydroxy-4-oxo-1,4-dihydroquinolin-2-yl)-2-(piperidin-1-yl)benzamide (6): Yellow powder. HPLC purity: 98%. Yield: 0.14 g (48%);^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.49 (br, 1H), 8.18 (s, 1H), 8.14 (d, J = 8.0 Hz, 1H), 7.88 (br, 1H), 7.72 (br, 1H), 7.66 (s, 1H), 7.58 (t, J = 7.0 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.26 (t, J = 7.5 Hz, 1H), 2.97 (t, J = 4.5 Hz, 4H), 1.73 – 1.66 (m, 4H), 1.57 – 1.50 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 169.8, 167.9, 152.5, 138.0, 137.9, 132.3, 131.0, 130.9, 130.4, 128.1, 126.3, 124.4, 121.8, 119.3, 118.4, 53.6, 25.8, 23.3. HRMS (ESI) calcd for $[M+H]^+$ $C_{21}H_{22}N_3O_3$ 364.1656; found: 364.1654.

5-(3-Hydroxy-4-oxo-1,4-dihydroquinolin-2-yl)-2-morpholinobenzamide (7): Yellow powder. HPLC purity: 99%. Yield: 0.10 g (56%);^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.53 (br, 1H), 8.26 (s, 1H), 8.14 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 2.5 Hz, 1H), 7.88 (dd, J_1= 2.0 Hz, J_2 = 8.0 Hz, 1H), 7.72 (d, J = 8.6 Hz, 1H), 7.63 (s, 1H), 7.58 (td, J_1= 1.5 Hz, J_2 = 7.0 Hz, 1H), 7.32 – 7.23 (m, 2H), 3.78 (t, J = 3.5 Hz, 4H), 3.03 (t, J = 3.5 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 169.9, 168.4, 151.0, 138.0, 137.8, 132.2, 130.9, 130.8, 130.5, 128.6, 126.1, 124.4, 121.8 (2C), 118.6, 118.4, 66.2, 52.3. HRMS (ESI) calculated for $[M+H]^+$ $C_{20}H_{20}N_3O_4$ 366.1448; found: 366.1447.

2-(3-Carbamoyl-4-(piperidin-1-yl)phenyl)-3-hydroxy-4-oxo-N-pentyl-1,4-dihydroquinoline-7-carboxamide (8)

The phenacylester**24** (0.08 g, 0.2 mmol) was dissolved in glacial acetic acid (3 ml) and the mixture was refluxed for 2 h. After cooling down to room temperature, the mixture was poured into ice-cold water. The precipitate was filtered off, washed with 10% solution of $NaHCO_3$ and dried under the vacuum. Green powder. HPLC purity: 99%. Yield: 0.07 g (92%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.71 (s, 1H), 8.71-8.53 (m, 1H), 8.48 (s, 1H), 8.27-8.08 (m, 3H), 7.89 (d, J = 7.4 Hz, 1H), 7.72-7.52 (m, 2H), 7.33 (d, J = 8.6 Hz, 1H), 3.33-3.21 (m, 2H), 3.03-2.92 (m, 4H), 1.75-1.62 (m, 4H), 1.62-1.48 (m, 4H), 1.37- 1.26 (m, 4H), 0.89 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 169.5, 168.0, 165.8, 152.6, 138.4, 137.5, 136.6, 132.3, 131.6, 131.1, 128.1, 125.8, 124.6, 122.8, 119.7, 119.2, 118.4, 53.6, 40.0 (*overlay with solvent*), 28.7 (2C), 25.8, 23.3, 21.9, 13.9. HRMS (APCI) calculated for $[M+H]^+$ $C_{27}H_{32}N_4O_4$ 477.2496; found: 477.2503.

N-Benzyl-2-(3-carbamoyl-4-(piperidin-1-yl)phenyl)-3-hydroxy-4-oxo-1,4-dihydroquinoline-7-carboxamide (9)

The compound was prepared from phenacylester **25** according to the procedure described for compound **8.** Green powder. HPLC purity: 99%. Yield: 0.06 g (88%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.26-9.13 (m, 1H), 8.50 (s, 1H), 8.28 (s, 1H), 8.23-8.17 (m, 2H), 7.91 (d, J = 8.0 Hz, 1H), 7.72-7.66 (m, 2H), 7.38-7.29 (m, 5H) 7.29-7.55 (m, 1H), 4.51 (d, J = 5.7 Hz, 2H), 3.01-2.94 (m, 4H), 1.76-1.63 (m, 4H), 1.60-1.51 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 172.2, 168.0, 166.0, 152.5, 139.5, 138.6, 137.7, 136.0, 132.3, 132.0, 131.1, 128.3, 128.1, 127.2, 126.8, 126.2, 124.6, 123.0, 119.6, 119.2, 119.04, 53.6, 42.7, 25.8, 23.4. HRMS (APCI) calculated for $[M-H]^-$ $C_{29}H_{28}N_4O_4$ 495.2027; found: 495.2022.

Methyl 5-(2-((2-aminobenzoyl)oxy)acetyl)-2-chlorobenzoate (12): Anthranilic acid (0.97 g, 7.1 mmol) and $NaHCO_3$ (0.78 g, 9.3 mmol) were dissolved in DMF (50 ml) and stirred for 30 min at 100 °C. The mixture was cooled down to room temperature and bromoacetophenone **11** (2.05 g, 7.1 mmol) was added. The reaction mixture was stirred for 16 h at room temperature. The product was extracted to EtOAc (three times), the organic layer was dried over Na_2SO_4 and evaporated to dryness. White powder. LC purity: 99%. Yield: 1.06 g (91%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.37 (d, J = 2.5 Hz, 1H), 8.18 (dd, J_1= 8.5 Hz, J_2 = 2.0 Hz, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.81 (dd, J_1= 8.0 Hz, J_2 = 1.5 Hz, 1H), 7.31 – 7.28 (m, 1H), 6.80 (d, J = 8.5 Hz, 1H), 6.64 (br, 2H), 6.59 – 6.54 (m, 1H), 5.68 (s, 2H), 3.91 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 192.1, 166.6, 164.8, 151.6, 137.1, 134.5, 132.8, 132.0, 131.6, 130.8, 130.7, 130.2, 166.6, 114.8, 108.0, 66.4, 52.9.

Methyl 2-chloro-5-(3-hydroxy-4-oxo-1,4-dihydroquinolin-2-yl)benzoate (13): Derivative **12** (1.04 g, 3.0 mmol) was dissolved in AcOH (25 ml) and the mixture was boiled for 4 h. It was then cooled down to room temperature and poured into ice-cold water. The precipitate formed was filtered off, washed with water and dried under vacuum. Yellow powder. LC purity: 99%. Yield: 0.86 g (87%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.64 (s, 1H), 8.67 (br, 1H) 8.23 (d, J = 1.7 Hz, 1H), 8.12 (d, J = 7.4 Hz, 1 H), 8.00 (dd, J_1= 8.3 Hz, J_2 = 2.0 Hz, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.61 (t, J = 8.0 Hz, 1H), 7.28 (t, J = 7.7 Hz, 1 H), 3.91 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 170.2, 165.2, 138.1 (2C), 133.7, 132.4, 131.5, 131.4, 130.8, 130.0, 129.1, 124.5, 122.0, 121.9, 118.4, 52.8, 21.1. HRMS (ESI) calculated for $[M+H]^+$ $C_{17}H_{13}ClNO_4$ 330.0528; found: 330.0527.

2-Chloro-5-(3-hydroxy-4-oxo-1,4-dihydroquinolin-2-yl)benzoic acid (14): Derivative **13** (0.49 g, 1.5 mmol) was treated with 10% aqueous NaOH (20 ml) for 1 h at 50 °C. The reaction mixture was then cooled down to room temperature, acidified with 1M HCl and extracted three times to ethylacetate. The combined organic layers were washed with brine and dried over $MgSO_4$. The solvent was evaporated and the residue dried under vacuum. Yellow powder. LC purity: 99%. Yield: 0.25 g (90%). LC/MS analysis: MS (ESI) exact mass calculated for $C_{16}H_{11}ClNO_4$ $[M+H]^+$: 316.04; found: 316.35, purity: 99%. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 13.64 (br, 1H) 11.64 (s, 1H) 8.23 (d, J = 2.5 Hz, 1H), 8.15 (d, J = 10.5 Hz, 1H), 7.97 (dd, J_1= 2.5 Hz, J_2 = 10.5 Hz, 1H), 7.76 – 7.70 (m, 2H), 7.61 (t, J = 10.5 Hz, 1 H), 7.28 (t, J = 9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 170.2, 166.4, 138.2, 138.1, 133.2, 132.4, 131.5, 131.4, 131.2, 130.8, 130.7, 129.3, 124.5, 122.0, 121.9, 118.5.

2-Chloro-5-(3-hydroxy-4-oxo-1,4-dihydroquinolin-2-yl)benzamide (15): The compound was prepared from corresponding acid **14**(0.49 g, 1.5 mmol) which was stirred in $SOCl_2$ (10 ml) for 2

h at room temperature. The solvent was evaporated, and the residue dissolved in DCM(10 ml). NH_4OH (10 ml) was added and the mixture was stirred 16 h at room temperature. It was then washed with 1M HCl, water and brine and dried over Na_2SO_4. The inorganic salt was filtered off, the solvent evaporated, and the residue dried under vacuum. Yellow powder. LC purity: 99%. Yield: 0.37 g (76%). LC/MS analysis: MS (ESI) exact mass calculated for $C_{16}H_{12}ClN_2O_3$ $[M+H]^+$: 315.73; found: 315.34, purity: 98%. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.61 (br, 1H), 8.15 (d, J = 10.5 Hz, 1H), 7.99 (s, 1H), 7.92 - 7.89 (m, 2H), 7.74 - 7.67 (m, 3H), 7.60 (t, J = 9.0 Hz, 1H), 7.28 (t, J = 9.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 170.2, 167.7, 138.1, 136.9, 131.4, 131.3, 131.0, 130.7, 130.5, 129.6, 129.4, 129.1, 124.5, 122.0, 121.8, 118.5.

Methyl 2-amino-4-(pentylcarbamoyl)benzoate (16)

2-aminomethylterephthalate (1.00 g, 5.1 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.87 g, 7.5 mmol), and pentylamine (0.85 ml, 5.1 mmol) in pyridine (20 ml) were stirred for 16 h at room temperature. The solvents were then evaporated, the residue dissolved in EtOAc, and washed three times with 1M HCl and saturated solution of $NaHCO_3$, and dried over $MgSO_4$. The solvent was evaporated and the residue was dried under the vacuum. Yellow powder. HPLC purity: 99%. Yield: 1.30 g (97%). ^{1}H NMR(500 MHz, $CDCl_3$) δ ppm 7.85 (d, J = 8.6 Hz, 1H), 7.11 (d, J = 1.1 Hz, 1H), 6.86 (dd, J = 8.0, 1.7 Hz, 1H), 6.24 (s, 1H), 5.85 (s, 2H), 3.87 (s, 3H), 3.44-3.35 (m, 2H), 1.63-1.53 (m, 2H), 1.39-1.28 (m, 4H), 0.89 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6)δ ppm 168.1, 167.0, 150.6, 140.0, 131.8, 115.9, 113.6, 112.6, 51.9, 40.3, 29.4, 29.2, 22.5, 14.1. HRMS (APCI) calculated for $[M+H]^+$ $C_{14}H_{20}N_2O_3$ 265.1547; found: 265.1548.

Methyl 2-amino-4-(benzylcarbamoyl)benzoate (17)

The compound was prepared according to the procedure described for compound **16**with the use of benzylamine. Yellow oil. HPLC purity: 99%. Yield: 1.39 g (96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 9.02-8.94 (m, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.37-7.19 (m, 6H), 6.96 (dd, *J* = 8.6, 1.7 Hz, 1H), 6.76 (s, 2H), 4.43 (d, *J* = 6.3 Hz, 2H), 3.81 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 167.4, 166.0, 151.0, 139.6, 130.8, 128.3, 127,2, 126.7, 116.0, 112.9, 110.4, 51.6, 42.6. HRMS (ESI) calculated for $[M+H]^+$ $C_{16}H_{16}N_2O_3$ 285.1234; found: 285.1235.

2-Amino-4-(pentylcarbamoyl)benzoic acid (18)

Ester **16**(0.75 mg, 2.8 mmol) was dissolved in EtOH (17 ml). Aqueous solution of NaOH (0.55 ml, 1M) was added and the reaction mixture was stirred for 2 h at 40 °C. The solvents were then removed under the vacuum, water (10 ml) was added and the pH was adjusted to 7 by addition of AcOH. The precipitate formed was filtered off, washed with water and dried under the vacuum. Pink powder. HPLC purity: 99%. Yield: 605 mg (80%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.37-8.29 (m, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.15 (d, *J* = 1.7 Hz, 1H), 6.86 (dd, *J* = 8.6, 1.7 Hz, 1H), 3.24-3.14 (m, 2H), 1.54-1.44 (m, 2H), 1.35-1.21 (m, 4H), 0.86 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 169.5, 166.1, 151.1, 139.4, 131.2, 115.5, 112.7, 112.3, 40.0 (overlay with solvent), 28.7 (2C), 21.9, 13.9. HRMS (APCI) calculated for $[M+H]^+$ $C_{13}H_{18}N_2O_3$ 251.1390; found: 251.1388.

2-Amino-4-(benzylcarbamoyl)benzoic acid (19)

The compound was prepared according to the procedure described for compound **18** from derivative **17**. Yellow powder. HPLC purity: 99%. Yield: 0.7 g (75%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.06-8.88 (m, 1H), 7.75 (d, *J* = 8.6 Hz, 1H), 7.42-7.29 (m, 4H), 7.29-7.21 (m, 2H), 6.96 (dd, *J* = 8.6, 1.7 Hz, 1H), 4.46 (d, *J* = 5.7 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ

ppm 169.2, 166.2, 151.3, 139.6, 139.3, 131.3, 128.3, 127.2, 126.7, 115.7, 112.7, 111.4, 52.6. HRMS: (ESI) calculated for $[M-H]^-$ $C_{15}H_{14}N_2O_3$ 269.0921; found: 269.0920

5-Acetyl-2-chlorobenzamide (21)

Starting ester **20** (0.68 g, 3.4 mmol), which had been prepared according to the published procedure,[35]was stirred in the mixture of concentrated acetic acid and concentrated sulfuric acid (8.5/1.5, w/v, 32 ml) for 2 h at 80 °C. The reaction mixture was then allowed to cool down to room temperature and neutralized to pH 3 with saturated solution of K_2CO_3. The mixture was then extracted to DCM (3 ×50 ml), the organic layers were washed with saturated solution of $NaHCO_3$, and dried over Na_2SO_4. After the filtration and evaporation of the solvent, the obtained yellow powder was immediately used in the next step. The powder was dissolved in DCM (10 ml) under inert atmosphere. The suspension was stirred at 0 °C and $SOCl_2$ (2 ml, 0.4 mmol) was added drop-wise. The mixture was stirred for 1.5 h at 50 °C. During this time, the insoluble material was dissolved. It was then allowed to cool down to room temperature and the solvent was removed under the stream of nitrogen. Resulting brown solid was dissolved in THF (10 ml), cooled down to 0 °C, and NH_4OH solution (25%, 2 ml) was added. This mixture was stirred for 30 min at room temperature, followed by extraction to EtOAc (3 × 50 ml). Combined organic layers were washed with brine and dried over $MgSO_4$. The solvent was finally evaporated under the vacuum yielding amide **21**. Yellow powder. HPLC purity: 99%. Yield: 0.53 mg (78%). ^{1}H NMR(500 MHz, DMSO-d_6) δ ppm 8.04 (s, 1H), 8.02-7.93 (m, 2H), 7.72 (s, 1H), 7.64 (dd, J = 8.6, 1.1, 1H), 2.60 (s, 3H). 13C NMR (126 MHz, DMSO-d_6)δ ppm 196.8, 167.4, 137.3, 135.3, 134.7, 130.2, 130.0, 128.3, 26.8. HRMS (APCI) calculated for $[M+H]^+$ $C_9H_8ClNO_2$ 198.0316; found: 198.0319.

5-Acetyl-2-(piperidin-1-yl)benzamide (22)

Amide **21** (0.70 g, 3.5 mmol) and piperidine (0.79 ml, 8.0 mmol) were dissolved in DMSO (3 ml). The mixture was stirred at 120 °C for 1.5 h. After cooling down to room temperature, the mixture was poured into water and extracted to EtOAc (3 × 40 ml). Combined organic layers were then washed with water (3 ×30 ml), brine and dried over Na_2SO_4. The solvent was removed under the vacuum and the residue was filtered through silica-gel with EtOAc as the eluent. White powder. HPLC purity: 99%. Yield: 0.66 g (76%). ^{1}H NMR(500 MHz, DMSO-d_6) δ ppm 8.08 (d, J = 2.3 Hz, 1H), 8.01 (s, 1H), 7.92 (dd, J = 8.6, 2.3 Hz, 1H), 7.58 (s, 1H), 7.12 (d, J = 8.6 Hz, 1H), 3.04-2.02 (m, 4H), 2.51 (s, 3H, *overlay with solvent*), 1.68-1.63 (m, 4H), 1.57-1.49 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 196.1, 168.9, 154.9, 131.2, 130.5, 129.2, 127.6, 118.1, 52.6, 26.4, 25.4, 23.4. HRMS (APCI) calculated for $[M-H]^-$ $C_{14}H_{18}N_2O_2$ 247.1441; found: 247.1444.

5-(2-Bromoacetyl)-2-(piperidin-1-yl)benzamide (23)

Acetophenone**22**(0.50 g, 2.0 mmol) was dissolved in THF (10 ml). Catalytic amount of 33% HBr in AcOH was added followed by drop-wise addition of Br_2 (0.21 ml, 4.1 mmol) in THF (20 ml). The reaction was followed by TLC analysis. After the consumption of the starting material, the precipitate formed was filtered off and washed several times with diethyl ether. White powder. HPLC purity: 99%. Yield: 0.43 mg (65%). ^{1}H NMR(500 MHz, DMSO-d_6) δ ppm 8.05 (d, J = 2.3 Hz, 1H), 7.99-7.84 (m, 2H), 7.59 (s, 1H), 7.13 (d, J = 8.6 Hz, 1H), 4.77 (s, 2H), 3.15-3.05 (m, 4H), 1.70-1.61 (m, 4H), 1.58-1.50 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 190.0, 169.0, 155.0, 131.5, 130.7, 129.3, 126.7, 118.9, 52.8, 33.6, 25.2, 23.0. HRMS (APCI) calculated for $[M+H]^+$ $C_{14}H_{17}BrN_2O_2$ 325.0546; found: 325.0543.

2-(3-Carbamoyl-4-(piperidin-1-yl)phenyl)-2-oxoethyl 2-amino-4-(pentylcarbamoyl)benzoate (24)

Aminoterephthalate18 (0.70 g, 0.3 mmol) and K_2CO_3 (0.38 g, 0.3 mmol) were suspended in DMF (20 ml). The reaction mixture was stirred for 1 h at 90 °C. It was then allowed to cool down to room temperature and 2-bromacetophenone **23**was added. The reaction was stirred for 1 h at room temperature and subsequently poured into the ice-cold aqueous solution of $NaHCO_3$ (10%). The precipitate formed was filtered off, washed with water and dried under the vacuum. Green-yellow powder. HPLC purity: 99%. Yield: 0.11 g (80%). ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ ppm 8.43 (t, *J* = 5.7 Hz, 1H), 8.07 (d, *J* = 1.7 Hz, 1H), 7.99-7.94 (m, 2H), 7.84 (d, *J* = 8.6 Hz, 1H), 7.60 (s, 1H), 7.24 (d, *J* = 1.1 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 1H), 6.95 (dd, *J* = 8.4, 1.8 Hz, 1H), 6.76 (s, 1H), 5.62 (s, 2H), 3.26-3.17 (m, 2H), 3.13-3.02 (m, 4H), 1.68-1.63 (m, 4H), 1.59-1.44 (m, 4H), 1.36-1.23 (m, 4H), 0.88 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$)δ ppm 191.0, 169.0, 166.4, 165.9, 155.0, 151,2, 140.2, 130.9, 130.7, 130.0, 127.5, 125.7, 118.0, 115.9, 112.9, 109.9, 66.3, 52.3, 40.0 (*overlay with solvent*), 28.7 (2C), 25.3, 23.4, 21.9, 13.9. HRMS (APCI) calculated for $[M+H]^+$ $C_{27}H_{34}N_4O_5$ 495.2602; found: 495.2604.

2-(3-Carbamoyl-4-(piperidin-1-yl)phenyl)-2-oxoethyl 2-amino-4-(benzylcarbamoyl)benzoate (25)

The compound was prepared according to the same procedure as compound **24** with the use ofaminoterephthalate**19**. Green-yellow powder. HPLC purity: 99%. Yield: 0.79 g (70%). ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ ppm 9.05-9.01 (m, 1H), 8.07 (d, *J* = 2.3 Hz, 1H), 7.99-7.91 (m, 2H), 7.87 (d, *J* = 8.3 Hz, 1H), 7.59 (s, 1H), 7.37-7.27 (m, 5H), 7.27-7.19 (m, 1H), 7.14 (d, *J* = 8.9 Hz, 1H), 7.01 (dd, *J* = 8.3, 1.4 Hz, 1H), 6.78 (s, 2H), 5.62 (s, 2H), 4.46 (d, *J* = 6.0 Hz, 2H), 3.16-3.02 (m, 4H), 1.68-1.63 (m, 4H), 1.59-1.52 (m, 2H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ ppm 191.0, 169.0, 166.4, 166.1, 155.1, 151.3, 139.9, 139.6, 131.1, 130.7, 130.0, 128.3, 127.6,

127.2, 126.8, 125.6, 117.9, 116.0, 113.0, 110.0, 66.3, 52.3, 42.6, 25.4, 23.5. HRMS (APCI) calculated for $[M+H]^+$ $C_{29}H_{30}N_4O_5$ 515.2289; found: 515.2293.

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge on the website of ACS Publications with DOI:… and contains materials and methods description, the alignment used for homology modeling, docking studies and calorimetric titration profiles of studied compounds, and separate file with molecular formula strings (CSV).

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ABBREVIATIONS USED

A549, lung adenocarcinoma; APCI, atmospheric pressure chemical ionization; BJ, normal cycling fibroblasts cell line; CEM-DNR-BULK, CEM cells daunorubicin resistant; CCRF-CEM, acute lymphoblastic leukemia; 4C0S, mammalian translation elongation factor eEF1A2; DCM, dichloromethane; DIC, *N,N′*-diisopropylcarbodiimide; DIEA, *N,N*-diisopropylethylamine; EDC, *N*-ethyl-*N′*-(3-dimethylaminopropyl)carbodiimide hydrochloride; eEF1A1, eukaryotic translation elongation actor 1-alpha 1; 3-HQ, 2-phenyl-3-hydroxy-4(1H)-quinolinones; HOBt, 1-hydroxybenzotriazole hydrate; IC_{50}, half maximal inhibitory concentration; ITC, isothermal titration calorimetry; K562, acute myeloid leukemia; K562-Tax, K562 cells paclitaxel resistant;

PDA, photodiode array; PKM2, pyruvate kinase M2; RMS, residual mean square; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TFA, trifluoracetic acid; THF, tetrahydrofuran; UHPLC, ultra-high performance liquid chromatography

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