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Binding of the antibacterial drug clofoctol and analogues to the Cdc7/Dbf4 kinase complex. A computational study

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ABSTRACT: Drugs targeting the cell division cycle kinase 7 (Cdc7) are actively searched for the treatment of different pathologies such as amyotrophic lateral sclerosis and cancer. Cdc7 interacts with multiple protein partners, including protein Dbf4 to form the Dbf4-dependent kinase (DDK) complex which regulates DNA replication initiation. Cdc7 and its activator Dbf4 are over-expressed in some cancers. The antibacterial drug clofoctol (CFT), used to treat respiratory tract infections, has been shown to block Cdc7 kinase activity, acting as a non-ATP-competitive inhibitor, capable of arresting DNA synthesis in cancer cells. We have modeled the interaction of CFT with the DDK complex and identified four potential binding sites at the interface of the Cdc7/Dbf4 heterodimer: at T109 and D128 (Cdc7), V220 and I330 (Dbf4). CFT behaves as an interfacial protein-protein inhibitor of the Cdc7/Dbf4 complex, limiting drug access to the proximal kinase site. Six CFT analogues have been tested for binding to the kinase complex. Two potent binders were analyzed in detail. The CFT structure was modulated to replace the two chlorine atoms with hydroxyl groups. The empirical potential energy of interaction (ΔE) calculated with hydroxylated compounds points to a more favorable interaction with the DDK complex, in particular at D128 site with the compound bearing two *ortho*-OH groups. Our work contributes to the identification of novel DDK inhibitors.

Keywords: Clofoctol; Cdc7 kinase; Antibacterial drug; Cancer therapeutic; Drug-protein binding; Molecular modelling.

1. INTRODUCTION

The serine/threonine kinase Cdc7 (cell division cycle kinase 7) plays important roles in cells by directly phosphorylating a few key proteins implicated in different diseases. For examples, Cdc7 phosphorylates the nuclear protein TDP-43 implicated in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration [1, 2]. It also phosphorylates the Chk1-binding-domain (CKBD) of the claspin protein which plays a major role in cancer cells [3]. This conserved serine-threonine kinase supports major functions in DNA replication by facilitating the assembly of an initiation complex. It regulates many proteins, such as the transcription factor STAT3 [4], and contributes importantly to cell cycle progression [5]. The enzyme is essential for the activation of DNA replication origins, modulating the initiation and elongation

steps of DNA synthesis [6]. For these reasons, Cdc7 is viewed as an important molecular target for the design of drugs active against different diseases, such as ALS [2] and cancer [7-9].

A dozen of selective Cdc7 kinase inhibitors have been reported. They are mostly small heterocyclic molecules directed against the active kinase site of the protein. This is the case of the highly potent and selective thienopyrimidinone derivative TAK-931 which has revealed major anticancer activities and is currently undergoing clinical trials [10-12]. Another anticancer kinase inhibitor, PHA-767491, targets Cdc7 and a few other kinases [13-16]. Several other small molecules targeting the kinase active site of Cdc7 could be cited [17], in particular the benzofuropyrimidinone derivative XL413 with a nanomolar efficacy against Cdc7 [18-20]. The co-crystal structure of XL413 bound to Cdc7 has revealed that the drug fits into the active site of the enzyme, to compete with ATP [21]. As an orally bioavailable and selective Cdc7 inhibitor, XL413 (also known as BMS-863233), induces marked tumor growth inhibition in a colon (Colo-205) xenograft model [18]. It is also a useful tool to study the repair of double-strand DNA breaks and DNA replication [22, 23].

Cdc7 has a regulatory protein partner named Dbf4, a cell cycle regulator. A heterodimer is formed between the Cdc7 kinase and its regulatory subunit Dbf4, providing the so-called the Dbf4-dependent kinase, DDK which is necessary to initiate DNA replication in eukaryotic cells by activating replicative helicases. The Cdc7/Dbf4 kinase complex is required to trigger initiation of DNA replication through the phosphorylation of minichromosome maintenance complex subunits 2-7 (MCM2-7) [19, 24]. The crystal structure of an active human Cdc7-Dbf4 construct has revealed the nature of the interface between the two interacting proteins and provided structural details to help the design of a novel class of non-competitive inhibitors [21]. In this context, Cheng and co-workers have recently developed a drug-screening platform to identify small molecules capable of interrupting the interaction between Cdc7 and Dbf4. They have discovered that two known drugs, dequalinium chloride and clofoctol, were able to inhibit Cdc7 in a non-ATP-competitive manner, leading to inhibition of DNA synthesis and anticancer effects [25]. The biphenyl compound clofoctol (CFT, Fig. 1) is interesting because it is a relatively simple small molecule, used for many years for the treatment of upper and lower respiratory tract infections in Europe (the drug was not approved in the US). CFT was developed in the late 1970s and marketed in France (trade name Octofene®) until 2005. CFT is still used in Italy (trade name Gramplus®). CFT inhibits the interaction between Cdc7 and Dbf4 *in vitro*, with a relatively good efficacy (IC_{50} = 11.9 mM) but CFT is less potent than dequalinium (IC_{50} = 2.0 mM). However, both compounds have shown anticancer effects and were able to sensitize the therapeutic effect of cisplatin and radiation in oral cancer cells [25]. We were interested in studying the well-established antibacterial drug CFT due to its good tolerance profile, known metabolism and relatively easy synthetic access. Moreover, the drug may be useful for the treatment of COVID-19, as discussed recently [26]. For these reasons, we have analyzed the interaction between CFT and the Cdc7-Dbf4 kinase complex using a computational approach, taking advantage of the crystallographic structure of the kinase inhibitor XL413 bound to the active site of Cdc7 interacting with its partner Dbf4 [21]. We have identified four potential binding sites for CFT at the interface of the two proteins. In a second step, we have investigated the compound structure-protein binding relationships, to identify analogue compounds with a potentially higher capacity of binding to DDK, based on a computational analysis.

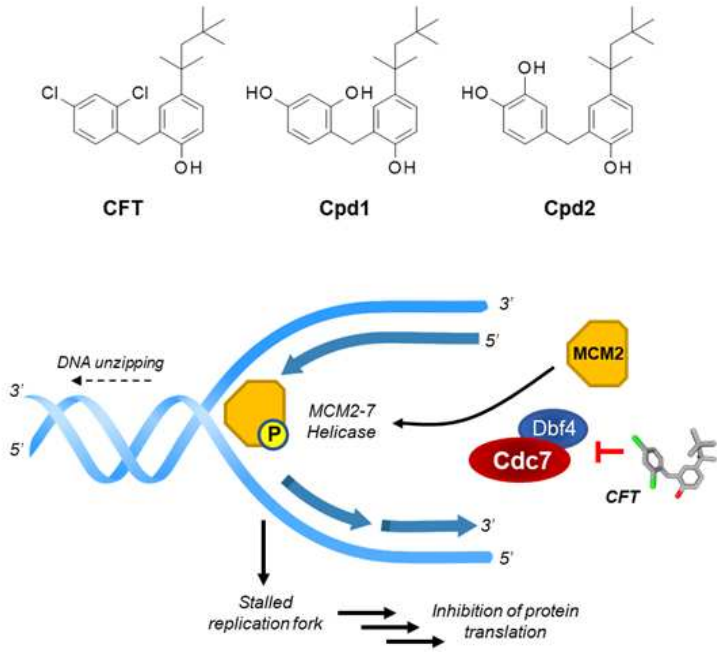

Figure 1. Chemical structures of the three compounds Clofoctol (CFT, PubChem CID# 2799), compound 1 (Cpd 1) and compound 2 (Cpd 2, PubChem CID# 53688744). Illustration of the inhibitory effect of CFT on the Cdc7/Dbf4 kinase complex, coupled to an inhibition of the phosphorylation of MCM2 helicase necessary for the progression of the replication fork. Inhibition of Cdc7 by CFT leads to protein synthesis inhibition.

2. MATERIALS AND METHODS

2.1. Preparation of the target protein and ligands

The three-dimensional structure of the Cdc7/Dbf4 kinase complex was retrieved from the Protein Data Bank (www.rcsb.org) under the PDB code 6YA6 (Minimal construct of Cdc7-Dbf4 bound to XL413) [21]. Docking experiments were performed with the GOLD software (GOLD 5.3 release, Cambridge Crystallographic Data Centre, Cambridge, UK). Before starting the docking procedure, the structure of the ligands has been optimized using a classical Monte Carlo conformational searching procedure as described in the BOSS software [27]. 6YA6 refers to a structure of the protein heterodimer with the drug XL413 bound to the kinase site.

2.2 *In silico* molecular docking procedure

Based on shape complementarity criteria, four possible binding sites for CFT have been defined around amino acid residues V220 and I330 of Dbf4 and T109 and D128 of Cdc7. These sites were identified using the software Discovery Studio Visualizer, to map the position of well-defined cavities susceptible to accommodate the ligand. Shape complementarity and geometry considerations are in favor of a docking grid centered in the volume defined by these amino acids. In each case, within the binding site, side chains of specific amino acids have been considered as fully flexible. The flexible amino acids are (i) for site V220, residues K451, S450, C449, S433, M428, V220, F219, K217, K216, K214, (ii) for site D128, residues D128, K126, H97, F124, H129, C298, C299, I101, R125, N127, (iii) for site T109, residues T109, R167, L108, C107, V110, I330, D329, V331, K333, S332, and (iv) for site I330, residues T109, L105, L108, V120, V110, I330, D329, V327, V326, V331. The ligand is always defined as flexible during the docking procedure. Up to 100 poses that are energetically reasonable were kept while searching for the correct binding mode of the

ligand. The decision to keep a trial pose is based on ranked poses, using the PLP fitness scoring function (which is the default in GOLD version 5.3 used here [28]). In addition, an empirical potential energy of interaction ΔE for the ranked complexes is evaluated using the simple expression ΔE(interaction) = E(complex) - (E(protein) + E(ligand)). For that purpose, the Spectroscopic Empirical Potential Energy function SPASIBA and the corresponding parameters were used [29, 30]. Molecular graphics and analysis were performed using Discovery Studio Visualizer, Biovia 2020 (Dassault Systèmes BIOVIA Discovery Studio Visualizer 2020, San Diego, Dassault Systèmes, 2020).

3. RESULTS

3.1. Interaction of CFT with the Cdc7/Dbf4 protein complex

The crystallographic structure of the kinase inhibitor XL413 bound to the active site of Cdc7 interacting with its partner Dbf4 (Protein Data Bank code: 6YA6) provides a solid basis to investigate the protein interaction with CFT. We analyzed the binding of CFT to the XL413-bound protein complex and identified four possible binding positions centered on amino acid residues V220 and I330 of Dbf4 and T109 and D128 of Cdc7, as represented in Fig. 2. Sites T109 and I330 have been mentioned by Cheng and co-workers when using their protein construct to identify small molecule binders [25]. Sites V220 and D128 are novel potential sites identified here; they both correspond to well-defined cavities susceptible to accommodate the CFT molecule. For each site, we calculated the empirical potential energy of interaction (DE) and energy of hydration (DG), as indicated in Table 1.

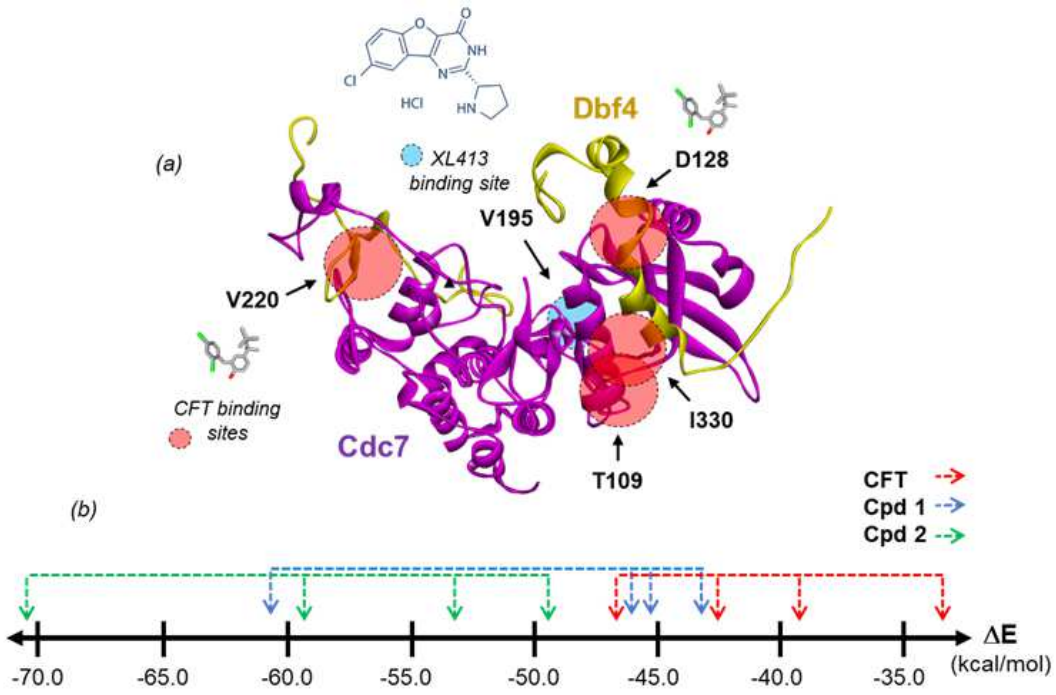

Figure 2. (a) Molecular model of the protein heterodimer, with Cdc7 (purple) and Dbf4 (yellow), and the position of the different drug binding sites identified. XL413 binds to the Cdc7 kinase site whereas as CFT binds at the interface of the Cdc7/Dbf4 complex, indirectly modulating the kinase activity of the enzyme. The four CFT-binding sites are centered on amino-acid residues V220 and I330 of Dbf4 and T109 and D128 of Cdc7. The structure of XL413 is shown (PubChem CID# 135564632). (b) Ranking of the drug binding sites in terms of empirical potential energy of interaction (DE values indicated in Table 1), for the three compounds.

Table 1. Calculated potential energy of interaction (ΔE) and free energy of hydration (ΔG) for the interaction of CFT and its two analogues with the Cdc7/Dbf4 kinase complex (kcal/mol).

Compound	**CFT**		**Cpd 1**		**Cpd 2**	
Binding site position[a]	ΔE	ΔG	ΔE	ΔG	ΔE	ΔG
D128	-42.7	-19.8	-60.90	-23.80	-70.60	-19.50
T109	-39.5	-18.2	-43.30	-22.90	-53.50	-19.50
V220	-46.9	-22.6	-46.70	-25.40	-59.70	-29.70
I330	-33.8	-17.1	-45.20	-20.00	-49.05	-16.10

[a]Drug interaction with the XL413-bound Cdc7/Dbf4 kinase complex (PDB code 6YA6).

The four sites rank in the order V220 > D128 > T109 > I330 in terms of binding energy (DE). CFT seems to form a more stable protein complex at site V220 which offers a well-adapted surface for binding. The drug can establish multiple molecular contacts at this V220 site, including H-bonds, p-stacking interactions and van der Waals contacts, stabilizing the drug-protein complex. However, the drug is essentially bound to the surface of the protein, bridging the two units of the heterodimer. A somewhat similar configuration was observed at sites I330 and T109. CFT binding to the T109 site is illustrated in Fig. 3, to show how the drug bridges two face-to-face a-helices and to present the different molecular contacts established between the small molecule and the proteins. The drug seems to be pasted on the outside surface of the protein interface, but well positioned to engage several H-bonds and van der Waals contact (Fig. 3c). The configuration is a little different at site D128 where there is a wider pocket between the turn of a b-sheet (D128) facing a helix fragment (Y324). The CFT molecule can fit into this cavity more easily and enters more deeply the structure, as shown in Fig. 4. The binding organization and the map of molecular contacts vary significantly from one site to another. For example, at sites V220 and I330 the two chlorine atoms of CFT seemed to play no role in the interaction with the protein, whereas at sites D128 and T109, a halogen bond was detected between the chlorine atom and the amino acid Glu-307 (at site D128) or Val-326 (at site T109) of protein Dbf4 (Figs. 3-4). But in all cases, the phenolic hydroxyl group of CFT is implicated in a H-bond interaction with the protein and multiple van der Waals contacts stabilize the drug-protein complex.

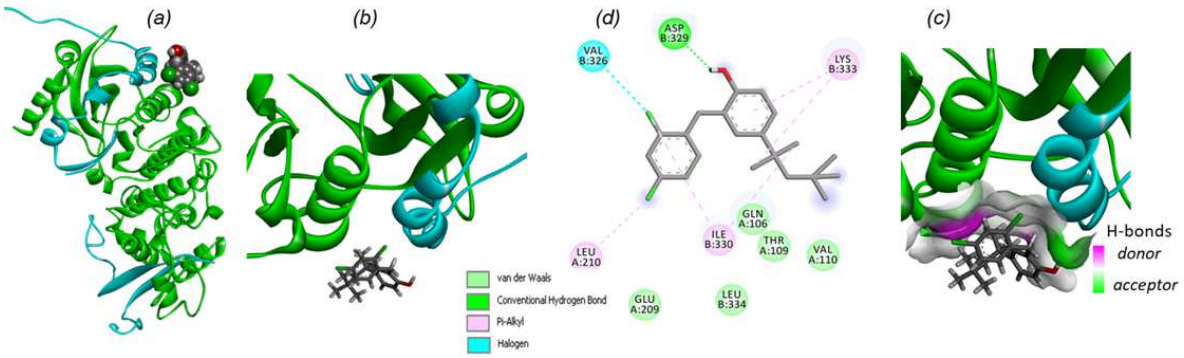

Figure 3. Molecular model for the binding of CFT to site T109 within the Cdc7/Dbf4 kinase complex. The structure of the Cdc7/Dbf4 complex used to model CFT binding derives from the crystal structure of Cdc7/Dbf4 (PDB code: 6YA6). (a) A ribbon model of the protein complex (Cdc7 in green, Dbf4 in blue) with the drug inserted in the cavity centered around residue T109. (b) Close-up view of the drug CFT facing two a-helices of the Cdc7/Dbf4 complex. (c) Contact binding map with the indicated color code. (d) a close view of the drug-proteins interface, with the H-bond donor/acceptor sites indicated.

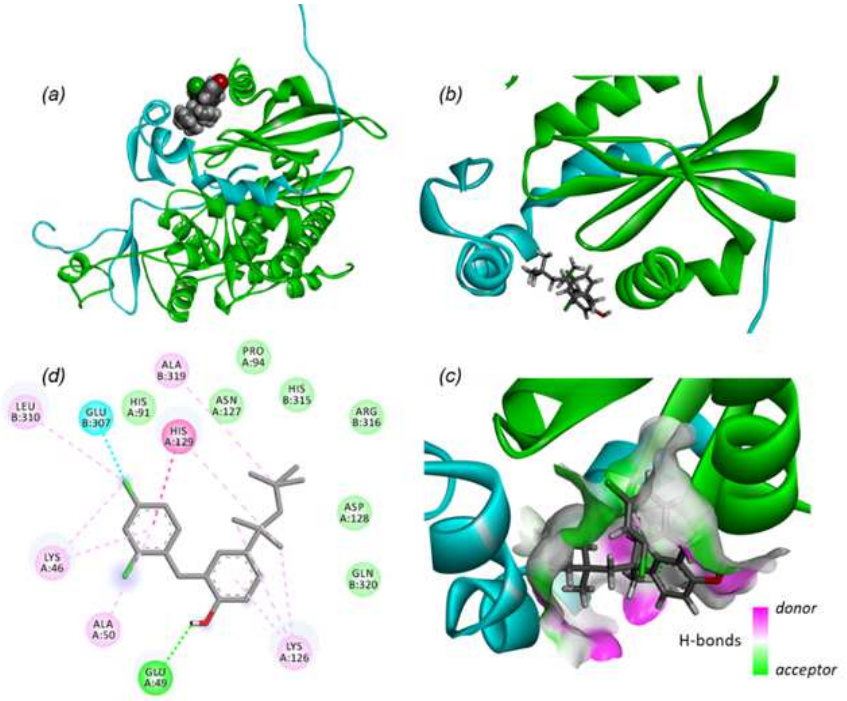

Figure 4. Molecular model for the binding of CFT to site D128 within the Cdc7/Dbf4 kinase complex. Note the deeper insertion of the drug in the protein cavity, between two a-helices of the Cdc7/Dbf4 complex. Other details as for Fig. 3.

The four potential binding sites for CFT were identified using the crystal structure of the XL413-bound protein complex, but the XL413 molecule has no influence of CFT binding. We compared the binding of CFT to the V220 site on the Cdc7/Dbf4 heterodimer, with and without XL413 and found no difference (ΔE = -46.919 and -46.936 kcal/mol, with and without XL413 bound, respectively). Binding of XL413 to the kinase site (centered on residue Val-195) has no effect on CFT binding. In sharp contrast, binding of CFT to each site markedly reduces the interaction of XL413 at the kinase site. The calculated ΔE value was -53.70 kcal/mol for XL413 bound to the CFT-free Cdc7/Dbf4 complex and this value increased to -50.40, -44.60, -44.40 and -40.8 kcal/mol, in the presence of CFT bound to site D128, I330, V220 and T109, respectively. Binding of CFT to the T109 site exerted a major negative influence on the interaction of XL413 with the Val-195 kinase site of the Cdc7/Dbf4 complex. The interaction of XL413 with the protein complex is stabilized by 22 molecular contacts between the drug and Cdc7, in particular 3 conventional H-bonds and 4 p-stacking interactions (plus 7 alkyl interactions and 8 van der Waals contacts). In the presence of CFT, the number of molecular interactions between XL413 and Cdc7 was reduced to 19 contacts, including only 2 conventional H-bonds and 2 p-stacking interactions. For example, the H-bond distance between the C=O group of XL413 and residue Lys-90 of Cdc7 increased from 2.769 Å to 3.545 Å in the presence of CFT. There is no doubt that CFT destabilizes the XL413-Cdc7 interaction. Collectively, our analysis indicates that CFT behaves as an interfacial protein-protein inhibitor of the Cdc7/Dbf4 complex, limiting drug access to the proximal kinase site. This configuration can explain the indirect kinase inhibitory action of CFT.

3.2. Drug design of CFT-derived interfacial inhibitors of the Cdc7/Dbf4 complex

CFT is a small molecule composed of a 2-(dichlorophenyl-methyl)phenol aromatic unit substituted at position 4 with a tert-octyl group (2,4,4-trimethyl-pentanyl side chain). The molecule can be easily synthesized, and several analogues can be found in the PubChem data bank. We have tested different

derivatives of CFT for their capacity to interact with the Cdc7/Dbf4 complex. The interaction of the best six compounds with the protein Cold Shock Domain-containing E1 (CSDE1) has been reported recently [26]. Here we used the same compounds to test their binding capacity to the Cdc7/Dbf4 complex. We observed that the removal of the tert-octyl group or its replacement with a methyl or a tert-butyl group reduced the binding capacity (data not shown). Apparently, this group plays an important role in the drug-protein interaction and should be preserved. In sharp contrast, the replacement of the two chlorine atoms of CFT with two hydroxyl groups afforded a compound (Cpd 1 in Fig. 1) with an enhanced capacity of interaction with the Cdc7/Dbf4 complex. Apparently, this molecule has never been described (we could not find it in PubMed and the literature) but the computational analysis suggests that it should bind more tightly to the Cdc7/Dbf4 complex. The empirical potential energy of interaction (ΔE) calculated for each site indicates that the compound presents a much better binding to site D128, with a gain of energy of 45% (ΔE varied from -42.7 to -60.9 kcal/mol). Its binding to sites T109 and I330 is also improved, but to a lesser extent compared to site D128, whereas the Cl -> OH replacement showed no effect for binding to site V220 (Table 1). This Cpd 1 is well adapted for binding to site D128, as illustrated in Fig. 5. The compound inserts deeply into the protein cavity (Fig. 5a), using each of its three polar hydroxyl groups to connect with the protein interface (Fig. 5b). Several molecular contacts stabilize the drug-protein interaction (Fig. 5c). This more polar compound (and less water-insoluble, Table 2) is much better adapted than CFT for the interaction at the D128 site.

Table 2. Molecular properties of CFT and its two analogues.

Compound	CFT	Cpd 1	Cpd 2
Molecular Weight	365.3	328.5	328.5
Dipole moment (D)	3.1	1.4	3.1
SASA[a] (Å^2)	586.7	565.0	567.6
Hydrophobic SASA	294.7	305.0	299.0
Hydrophilic SASA	34.7	103.9	120.8
Molecular Volume (Å^3)	1123.5	1082.0	1082.3
Donor Hydrogen Bonds	1	3	3
Acceptor Hydrogen Bonds	1	3	3
log P (octanol/water)	6.4	4.1	4.0
log S (aqueous solubility)	-6.2	-3.9	-4.0

[a]Total Solvent Accessible Surface Area (SASA), calculated with a probe of 1.4Å radius. Drug properties were calculated with the BOSS 4.9 software [27] according to published procedures [29, 30].

Finally, we varied the relative position of the two hydroxyl groups on the phenyl ring. In Cpd 1, the two -OH groups are in the *meta* position whereas they are in the *ortho* position in Cpd 2 (Fig. 1). This molecule (CID#: 53688744) is included in an old patent on a series of compounds active against the Herpes virus in combination with propolis [31] but its mechanism of action is unknown. The modeling analysis of this second analogue revealed that it is even more adapted for binding to the D128 site of the Cdc7/Dbf4 complex than Cpd 1. The empirical potential energy of interaction at site D128 reached -70 kcal/mol, which is considerably superior to the value calculated with CFT and Cpd 1 (Table 1). The simple relocation of the OH groups from the *meta* to the *ortho* position improved the interaction by about 18%. This molecule bridges the two a-helices of the protein complex, via hydrophilic interactions (Fig. 6a/b). Different H-bonds between Cpd 2 and the protein complex stabilize the molecular structure. Notably a stacking interaction between the phenol

ring of the drug and residue His-129 of Cdc7 can be seen with both Cpd 1 and Cpd 2. H-bonds with Cdc7 residues Ala-50 and Lys-126 can be observed also with both compounds, but they implicate distinct OH groups. There are more potential drug-protein contacts with Cpd 2 *vs*. Cpd 1 (compare Figs 5 and 6). Thus, we have identified a new molecule which is predicted to form more stable complexes with the Cdc7/Dbf4 heterodimer than CFT. Not only Cpd 2 presents a more favorable binding interaction at site D128, but it binds well also to the other three sites V220, T109 and I330 (Fig 2b). Globally, its binding capacity to the Cdc7/Dbf4 complex is much more favorable than that of CFT (Table 1). Our *in silico* analysis augurs well for this compound as a Cdc7 inhibitor. It would be interesting to investigate further the biological properties of this antiviral molecule.

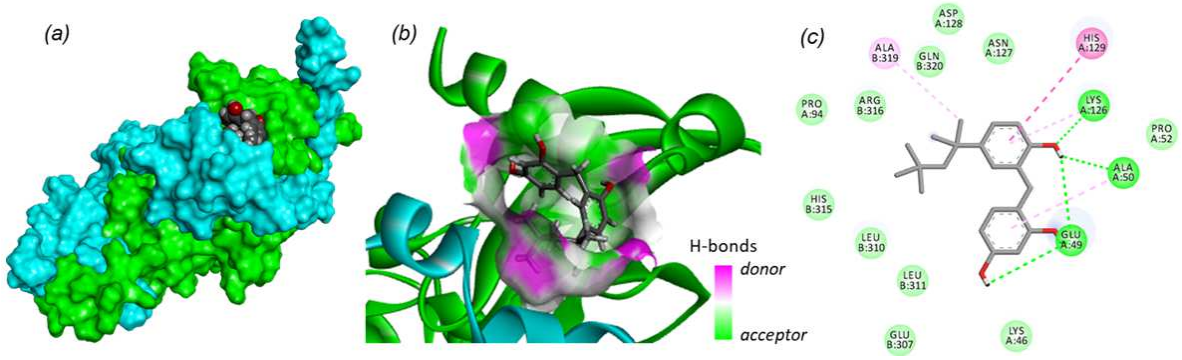

Figure 5. Binding of Cpd 1 to site D128 of the Cdc7/Dbf4 kinase complex. (a) The protein surface is shown in green (Cdc7) and blue (Dbf4), with a CPK model of drug. (b) Close-up view of Cpd 1 binding site D128, with the H-bond donor/acceptor surfaces. (c) Contact binding map (amino acids A refer to Cdc7, amino acids B refer to Dbf4).

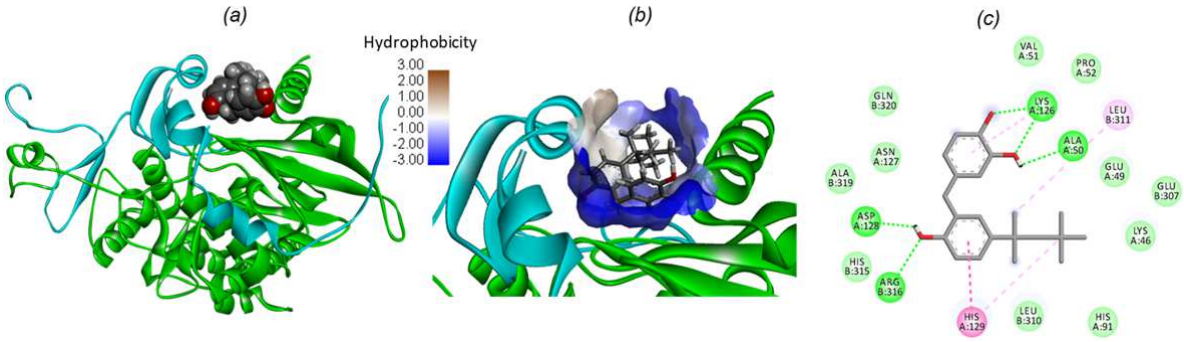

Figure 6. Binding of Cpd 2 to site D128 of the Cdc7/Dbf4 kinase complex. (a) A ribbon model of the protein complex (Cdc7 in green, Dbf4 in blue) with the drug (CPK model) inserted in the cavity centered around residue D128. (b) Close-up view of Cpd 2 binding site D128, with the hydrophobic/hydrophilic surfaces. (c) Contact binding map (amino acids A refer to Cdc7, amino acids B refer to Dbf4).

4. DISCUSSION

The planar tricyclic compound XL413 is a potent inhibitor of the serine/threonine kinase Cdc7 which is frequently over-expressed in breast cancer cells [19]. It has revealed Cdc7-dependent cell cycle arrest and *in vivo* tumor growth inhibition in a xenograft model of colorectal cancer [18]. This Cdc7-selective ATP-competitive inhibitor displays anticancer and promotes the antifibrotic properties of pirfenidone, used to slow down the progression of pulmonary fibrosis [20]. In human breast cells, the kinase activity of Cdc7 is required for proliferation, and a full and sustained inhibition of the kinase is necessary to block the cell-cycle

progression. This is difficult to achieve with an ATP-competitor like XL413, because DNA replication and cell proliferation can occur even with a reduced Cdc7 activity [32]. This compound (BMS-863233) was advanced to Phase 1 clinical trial in patients with refractory hematological cancer (NCT00838890) and advanced solid tumors (NCT00886782), but the development was not pursued. The compound is useful to study gene modification in cells [23] but more potent and better tolerated inhibitors of the Dbf4-dependent kinase (DDK) are needed to treat cancers. This enzyme, which plays important roles in the regulation of the initiation of DNA replication, represents an attractive target to treat breast cancer but also oral squamous cell carcinoma [9,33]. The knowledge of its mode of binding to the kinase site of Cdc7 is useful to guide the development of novel compounds [21].

In the frame of a drug screening process, the antibacterial drug CFT has been identified as a blocker of the interaction between Cdc7 and Dbf4. Moreover, the compound has revealed anticancer effects and a capacity to promote the activity of cisplatin and radiation in oral cancer cells [25]. As a well-established antibacterial drug used for more than 30 years in Human, CFT can be considered as a good candidate for a repositioning approach. These considerations prompted us to analyze the interaction between CFT and the Cdc7/Dbf4 kinase complex using molecular modeling. We identified four potential binding sites for CFT, around positions T109, D128, V220 and I330. Our calculations suggest that the best site for CFT is V220, a site identified for the first time, but it appears as a relatively "superficial", solvent-exposed site on the protein heterodimer. In this case, CFT binds to an open cavity, a wide depression on the surface of the Cdc7/Dbf4 complex. In contrast, the deeper site D128 allows a better anchorage of the drug molecule. In this case, the drug can penetrate more deeply into the protein cavity, so as to bridge two face-to-face a-helices of Dbf4 and Cdc7, truly as an interfacial protein inhibitor. This is one of the preferred sites for CFT, although the drug can also bind to sites T109 and I330 previously proposed [25].

Molecular modeling is a useful tool to study structure-binding relationships. Our modulation of the structure of CFT led to the identification of two compounds with superior binding capacity to the Cdc7/Dbf4 complex compared to CFT, at least *in silico*. The replacement of the two chlorine atoms with more polar hydroxyl groups provides a compound (Cpd 1) better adapted to interact with the D128 site. The binding capacity is markedly improved with the double Cl -> OH substitution. Interestingly, the relocation of the OH groups in the *ortho* position further reinforced the binding capacity of the drug to the D128 site and to the other sites identified with CFT. The modeling analysis indicates that this Cpd 2 behaves as a robust blocker of the Cdc7/Dbf4 interface. The prediction now requires an experimental validation. Our molecular docking study opens the door toward the rational design of a novel class of CFT-based Cdc7 modulators.

Different drug scaffolds can be used to design Cdc7 inhibitors, such as peptidomimetic that mimic pharmacophoric properties of DBF4 [34]. Potent Cdc7 inhibitors have been elaborated, such as the kinase inhibitor simurosertib (TAK-931), a quinuclidine-containing specific inhibitor of Cdc7 considered as a clinical candidate for the treatment of cancer [11,12]. Other ATP-competitive inhibitors of Cdc7 have been identified [35-37]. The use of CFT as a non-ATP competitive inhibitor is important to consider because it is a well-established drug, with a known safety profile and a good tolerance in Human. A repositioning of CFT may be envisioned [25] or the design of more potent analogues, taking into consideration the work reported here.

5. CONCLUSION

Molecular modeling has been instrumental to identify potential binding sites of CFT to the Cdc7/Dbf4 kinase complex. Four possible sites of drug interaction were located and one position, D128 of

Cdc7, provided an optimized site. The computational study also led to the identification of two CFT analogues with an improved Cdc7/Dbf4-binding capacity. This *in silico* approach can be useful to guide the design and synthesis of CFT analogues targeting Cdc7 and acting as anticancer agents.

Authors' Contributions: GV: investigation; visualization; software. CB: conceptualization; visualization; writing - original draft; writing - review & editing. Both authors are read and approved the final manuscript.

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