J Mol Model (2017) 23: 85
DOI 10.1007/s00894-017-3256-5

ORIGINAL PAPER

Theoretical and experimental study of polycyclic aromatic compounds as β-tubulin inhibitors

Fabian E. Olazarán[1] · Carlos A. García-Pérez[2] · Debasish Bandyopadhyay[3] · Isaias Balderas-Rentería[1] · Angel D. Reyes-Figueroa[4] · Lars Henschke[5] · Gildardo Rivera[2]

Received: 22 August 2016 / Accepted: 23 January 2017 / Published online: 18 February 2017

Abstract In this work, through a docking analysis of compounds from the ZINC chemical library on human β-tubulin using high performance computer cluster, we report new polycyclic aromatic compounds that bind with high energy on the colchicine binding site of β-tubulin, suggesting three new key amino acids. However, molecular dynamic analysis showed low stability in the interaction between ligand and receptor. Results were confirmed experimentally in in vitro and in vivo models that suggest that molecular dynamics simulation is the best option to find new potential β-tubulin inhibitors.

Keywords Virtual screening · Inhibitors · β-Tubulin · Anticancer · Colchicine

✉ Gildardo Rivera
gildardors@hotmail.com

[1] Facultad de Ciencias Químicas. Av. Universidad s/n, Ciudad Universitaria, San Nicolás de los Garza, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León 64451, México

[2] Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Boulevard del Maestro, s/n, Esq. Elías Piña, Reynosa, Tamualipas, Mexico 88710

[3] Department of Chemistry, The University of Texas Rio Grande Valley, 1201 West University Drive, Edinburg, TX 78539, USA

[4] Centro de Investigación y de Estudios Avanzados del Instituto Politecnico Nacional, Unidad Monterrey, Apodaca, Nuevo León 66600, México

[5] Department of Biology, University of Konstanz, Universitätsstraβe 10, 78457 Konstanz, Germany

Introduction

Cancer is currently a worldwide health problem and is among the leading causes of death according to the World Health Organization (WHO) [1]. Cancer cells are in constant cell division, triggering an increase in the activity of microtubules [2]. Microtubules consist of a dimer of α- and β-tubulin that bond to form the cytoskeleton [3]. Different drugs have been discovered with β-tubulin as a molecular target; for example, taxane and vinca derivatives [4]. Taxane derivatives (paclitaxel) are capable of permanently stabilizing microtubules by promoting microtubule assembly [5], whereas vinca derivatives (vinblastine) bind to tubulin, inhibiting the assembly of microtubules, both in different active sites on β-tubulin [6]. Additionally, Ravelli and co-workers [7] state that there is another active site on β-tubulin that binds to colchicine, which allows the development of new inhibitors; for example, the so-called compound combretastatin-A4. In recent years, the colchicine binding site of β-tubulin has gained more relevance for designing new anticancer drugs [8–11], because new compounds have shown more activity and efficient strategies to inhibit tubulin polymerization during mitosis and other essential cell processes [12].

The use of computation methods has acquired great importance in the discovery of new drugs [13]. A virtual screening of millions of compounds through computer systems provides the opportunity to discover new therapeutic alternatives in a relatively short time [14]. The in silico analysis of chemical and molecular interactions between proteins and drugs allows information to be obtained that could help determine their potential mechanisms of action [15]. Nowadays, Autodock [16], Maestro [17], MOE [18], QSAR-3D [19], among other software, are essential tools for drug discovery [20]. In the past, the search for molecules with biological activity using informatics software was limited due to a lack of computer equipment with a high capacity to process thousands of data

files. However, a virtual screening of millions of molecules in a relatively short time is now possible because academic organizations and pharmaceutical companies have advanced computer equipment known as a high performance computing cluster (HPCC) [21], which has a powerful processing capacity with data analysis in parallel [22]. Taking advantage of this technology, in the present study, the aim was to identify new compounds from a chemical library of 13,195,609 molecules as potential β-tubulin inhibitors by molecular docking and molecular dynamics (MD), confirmed experimentally in in vitro and in vivo models.

Material and methods

Analysis was performed at the University of Texas Rio Grande Valley High Performance Computing Center (UTRGV, HPCC) [23], which contains a head node, and 55 computing nodes. Each node has two dual core Xeon processors and 4GB RAM. The disk array has 13–146 GB drives that are active, with 1 spare. Altogether there is 1.9 TB disk space. Additionally, PyMOL Molecular Graphics System, Version 1.5.0.4, Maestro interface (version 10.1; Schrödinger LLC, New York) [24], Avogadro [25], UCSF Chimera [26], AutoDock 4 [27], and SOM [28, 29] software were used.

β-Tubulin homology

Currently, human β-tubulin has not been crystallized [7]. Previously, research works were performed with files from the Protein Data Bank (PDB) corresponding to structures of β-tubulin from *Bos taurus* (1SA0, 1Z2B and 1JFF) [30]; therefore, we considered working with a human β-tubulin obtained through the MODELLER program [31] based on its amino acid sequence reported in GenBank (NCBI record key NP_821080.1) [32].

ZINC chemistry library

ZINC is a chemistry library provided by the Shoichet Laboratory in the Department of Pharmaceutical Chemistry at the University of California, San Francisco (UCSF) [33]. From a standard subset (Clean Drug-Like) size of 13,195,609 molecules, Lipinski's rule of five (molecular weight < 500; partition coefficient log $P < 5$; number of H-bond donors <5; and number of H-bond acceptors <10), and about 100 rules that eliminate compounds with undesirable chemical groups, were applied. Additionally, inorganic compounds and compounds that had <30 atoms and >50 atoms were removed. Finally, we obtained 1,750,000 compounds that were analyzed by molecular docking on the colchicine binding site of human β-tubulin using HPCC. These were transformed from pdb to pdbqt format.

Vina analysis

From the list of 1,750,000 compounds, 60 groups were generated with approximately 30,000 compounds in each group. Configuration files of all the compounds from each group were generated through coordenadas.pl software (X = 116 735 center, Y = 89 925 center, Z = 6.724 size, X = 14, Y = 14 size, Z = 14 size). Once we obtained the configuration file of β-tubulin (TuCHOLB18_ph7.pdbqt), the AutoDock vina program [34] was run to obtain the values of free energy of each compound bonded to β-tubulin (kcal mol^{-1}).

SOM data analysis

In order to obtain groups according to their structural configuration in space and based on neural modeling, self-organizing maps (SOM) software was used [35].

Maestro and chimera software

Using Chimera software, chemical interactions (hydrogen bonds, Van der Waals forces, charge transfer, and others) between the compounds with different amino acids in the active site of β-tubulin were identified. Maestro software showed the key amino acids from the colchicine binding site of β-tubulin.

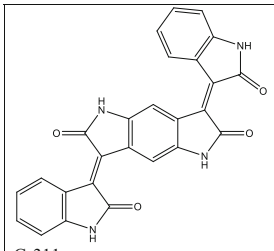
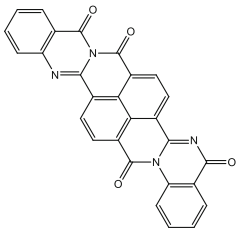
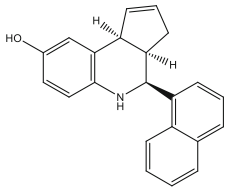
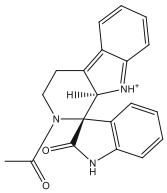
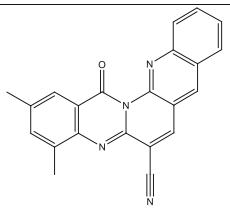
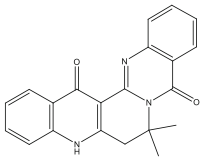
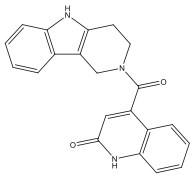
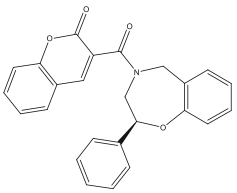
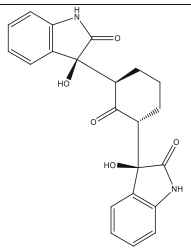
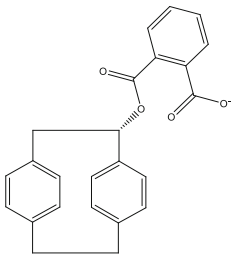
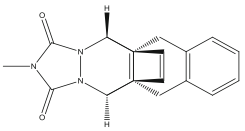
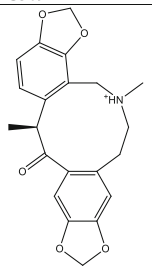
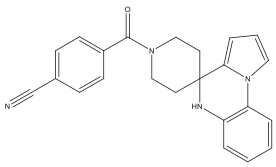
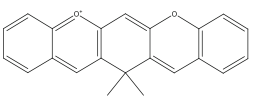
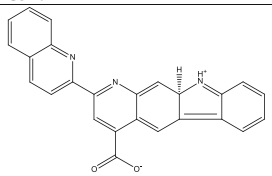
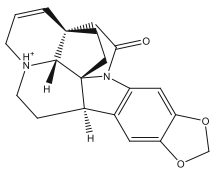
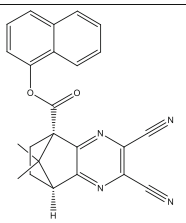
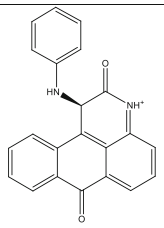
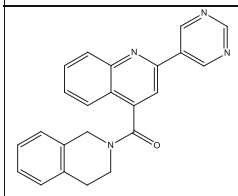
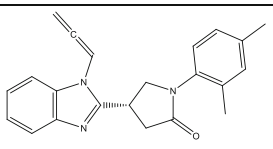
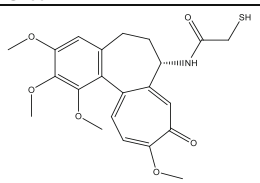
Molecular dynamics analysis

The starting complex was prepared by extraction of the ligands (G311 and G900), coordinates generated during the docking process. The protein coordinates were also processed to generate the topology files with the Amber99sb force field using the pdb2gms program from GROMACS 5.1 and the ligands with acpype tool. MD simulations were produced in GROMACS 5.1. The system was solvated using the spc216 water model in a cube with size (15×15×15), followed by the addition of Na^+ and Cl^- to neutralize the systems. Energy minimization consisted of 50,000 steps. The equilibration of the particles system for volume and temperature (NVT) as well as equilibration for pressure and temperature (NPT) were of 10 ns. Finally, the MD simulations were extended for 50 ns. Root mean square deviations (RMSD) and backbone atomic fluctuations (RMSF) in the docked complex were analyzed with GROMACS.

Free energy calculations

The set up for the free energy calculations with Bennett's acceptance ratio method (BAR) was as follows: $\lambda = 6$, temperature = 300 K.

Table 1 Structure of the best compounds of the top 20 groups with the highest binding value on the colchicine site of β-tubulin

 G-311	 G900	 G753
 G757	 G890	 G885
 G898	 G899	 G309
 G052	 G117	 G310
 G429	 G882	 G888
 G884	 G698	 G266
 G556	 G876	 Colchicine

Cell culture

HeLa S3 cells were cultured with DMEM (Gibco) supplemented with 10% FCS and antibiotics. For live cell experiments, CO_2-independent medium supplemented with 10% FCS, antibiotics, and Glutamax (1%), was used.

In vitro and in vivo tubulin polymerization assays

The tubulin polymerization rate was determined by the change in absorbance at 340 nm over time. Purified pig brain tubulin was diluted in glutamate buffer (0.8 M, pH 6.6) to 10 μM, and supplemented with $MgCl_2$ (100 μM) and DMSO or compounds (with indicated concentrations) on ice. After preincubation at 30°C, reactions were put back on ice for 10 min, and GTP (0.4 mM) was added to the mixture. Polymerization was initiated by raising the temperature to 30°C, and absorbance (340 nm) was measured every 20 s over 60 min (TECAN Infinite F500 multimode reader).

The in vivo tubulin polymerization rate was determined in HeLa S3 cells. Cells were grown on cover slips overnight. After incubation on ice for 30 min, compounds G311, G900, nocodazole or DMSO were added to the cells and incubated for a further 30 min on ice. After transfer to a prewarmed plate at 37°C, cells were fixed after 5 min or immediately (0 min). Fixation was performed with 4% formaldehyde in PBS. To visualize microtubules, a FITC-conjugated anti-α-tubulin antibody (Sigma F2168) was used (dilution 1:500). Fluorescent signal was quantified using ImageJ.

Live cell imaging

HeLa S3 cells were seeded on a 12-well plate at a density of 1×10^5 cells/well and synchronized with thymidine (2 mM) for 18 h. Cells were treated with compounds G311, G900 or DMSO (final DMSO concentration 0.5%) 2 h after release. Time lapse microscopy ($t = 10$ min) was started 5 h after thymidine release.

Results and discussion

Virtual screening by molecular docking

To obtain new compounds with biological activity as potential β-tubulin inhibitors with optimal physicochemical properties for the development of new antitumor agents, 13,195,609 molecules from the ZINC chemical library were analyzed. Applying Lipinski's rule of five as the first inclusion factor, we obtained 1,750,000 molecules that obey the rules. Subsequently, all molecules were analyzed in the colchicine binding site of β-tubulin through the AutoDock vina program using a HPCC with a speed of 100 docking analyses per

Table 2 Physicochemical properties of the best 20 compounds

Group	Docking value (kcal mol^{-1})	Number of compounds	Log P	Mutagenic	Tumorogenic	H-bond donors	H-bond acceptors	Net charge	tPSA ($Å^2$)	Molecular weight (g/mol)	Rotable bonds
G311	−11.6	79	3.81	No	No	4	8	0	131	446.422	0
G900	−11.5	252,333	4.39	Yes	Yes	0	8	0	102	468.428	0
G753	−11.0	47,132	4.84	No	Yes	2	2	0	32	313.4	1
G757	−10.8	4587	2.01	No	No	2	5	1	63	332.383	0
G890	−10.8	31,612	4.50	No	No	0	5	0	71	350.381	0
G885	−10.7	67,131	3.12	No	No	1	5	0	67	343.386	0
G898	−10.7	19,812	2.19	No	No	2	5	0	69	343.386	1
G899	−10.7	121,102	3.76	No	No	0	5	0	60	397.43	2
G309	−10.6	10,497	1.16	No	No	4	7	0	116	392.411	2
G52	−10.5	2804	4.90	No	No	0	4	−1	66	371.412	4
G117	−10.5	700	1.42	No	No	0	5	0	49	319.364	0
G310	−10.5	89,322	4.44	No	No	1	6	0	83	440.908	2
G429	−10.5	24	3.09	No	No	1	5	0	61	368.44	1
G882	−10.5	5467	2.48	No	No	0	2	0	24	313.376	0
G888	−10.5	7895	3.91	No	No	1	5	0	80	389.414	2
G884	−10.5	41,135	1.53	No	No	1	5	1	43	337.399	0
G698	−10.4	38	4.26	No	No	0	6	0	100	394.434	3
G266	−10.4	16,516	4.51	No	No	2	4	1	60	339.374	2
G556	−10.4	717	3.99	No	No	1	4	0	37	377.875	1
G876	−10.4	9280	3.35	No	Yes	0	4	0	38	343.43	3
Colchicine	−7.4	1	1.10	No	No	1	7	0	83	399.443	5

minute. Results of ligand–receptor interaction allowed obtaining 732 groups using SOM software. From these groups, considering the higher energy value of interaction, the top 100 groups were selected; 40 groups were rejected because they showed a similar or equal scaffold with a chiral center. Finally, of the 60 remaining groups, only 20 with the highest value of interaction were selected. Table 1 shows the best compound from each group.

The results show that none of the top 20 compounds have a structural similarity with colchicine or other β-tubulin inhibitor [36]; however, compounds G900, G890, G885 and G882 have poly-aromatic groups such as naphthalene and anthracene, which are substituents known in compounds with anticancer activity. Only compounds G309, G310, G429, G753, G882 and G890 have been reported previously in studies of heterocyclic molecule synthesis, and G429 as a sodium-channel blocker [37–42]. Interestingly, protopine, a molecule with a structure highly similar to that of compound G310 has been reported as a paclitaxel binding site of β-tubulin [5].

In order to analyze the physicochemical properties of the 20 leader compounds versus colchicine, Table 2 was constructed with the help of data from the ZINC chemical library and Osiris Organic Chemistry [43]. Our reference colchicine compound showed no mutagenic or tumorigenic properties, with a binding value of −7.4 kcal mol^{-1} and 7 H-bond acceptor groups. All 20 compounds showed a better colchicine binding value. Compound leader G311 showed the highest docking energy (−11.6 kcal mol^{-1}), with no mutagenic and tumorigenic predictive properties, and 8 H-bond acceptor groups, which suggests that it is a good candidate as a β-tubulin inhibitor. Another 16 compounds also did not show either tumorigenic or mutagenic activity. Unfortunately, compound G900, with a high binding value (−11.5 kcal mol^{-1}) showed both toxic effects, and compounds G753 (−11.0 kcal mol^{-1}) and G876 (−10.4 kcal mol^{-1}) showed only a possible tumorigenic property. These results suggest discarding these three groups for the development of new β-tubulin inhibitors. We suggest that the compound leader G900 presents both tumorigenic and mutagenic effects because it has an excessive amount of aromatic rings [44]. In addition, this compound also shows a high capacity of H-bond acceptor groups; however, it is not capable of donating this kind of bond, and

Table 3 The best 20 compounds with the bind amino acids in colchicine binding site of β-tubulin

Amino acids	Colchicine	311	900	753	757	890	885	898	899	309	52	117	310	429	882	888	884	698	266	556	876	TOTAL
200 Tyr											1											1
235 Gly																	1					1
236 Val		**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**20**
237 Thr										1												1
239 Cys	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**20**
240 Leu	1				1			1		1				1				1	1	1	1	8
246 Leu	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**20**
247 Asn								1		1		1			1			1				5
248 Ala	1			1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17
250 Leu								1														1
252 Lys	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**20**
253 Leu	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**20**
255 Val		1														1						2
256 Asn	1	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	19
257 Met		1		1			1				1						1	1	1			7
314 Ala	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**20**
315 Ala		1	1	1	1	1	1	1		1	1	1	1	1		1	1	1	1	1	1	18
316 Val	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**20**
348 Asn	1																					0
350 Lys	1	1	1	1	1	1	1		1	1		1	1	1		1	1	1	1	1	1	17
351 Thr		1	1			1	1		1	1						1			1			8
352 Ala	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1		1	1	18
366 Thr			1		1				1				1	1	1	1	1	1	1	1	1	12
367 Phe				1																		1
368 Ile	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**20**
TOTAL	12	15	14	15	15	13	14	15	14	17	14	14	14	15	13	16	15	17	16	15	15	

presents a rigid structure with no chiral centers—an essential feature according to Lipinski's rule of five for a compound with possible biological activity. Another important consideration in the 17 compounds without toxic effects is the presence of routable bonds in 10 compounds that would be able to generate a different structural conformation that could either promote the dissemination of the molecule into the colchicine binding site of β-tubulin, or otherwise affect the activity due to the natural mobility of the chiral centers.

Although in other studies [45, 46], binding energy values of colchicine are different, it has been observed that such molecules have structural variations, different biological origins of the target protein (tubulin), and variable configurations, which highlight, among other factors, the importance of working with a human β-tubulin.

Analyzing the results obtained with the Maestro software, it can be seen, in Table 3 and Fig. 1a, the colchicine binding site with 13 amino acids (239 Cys, 240 Leu, 246 Leu, 248 Ala, 252 Lys, 253 Leu, 256 Asn, 314 Ala, 316 Val, 348 Asn, 350 Lys, 352 Ala and 368 Ile) of β-tubulin. From these amino acids, different research has been suggested, for example, nine amino acids (240 Leu, 248 Ala, 252 Lys, 256 Asn, 314 Ala, 316 Val, 348 Asn, 350 Lys and 352 Ala) for interaction with molecules in the colchicine binding site of β-tubulin. Additionally, O'Boyle et al., suggest three new key amino acids (239 Cys, 246 Leu and 253 Leu) [47]. These same amino acids were also found as binding sites of the colchicine molecule (except 368 Ile) and our 20 lead molecules (Table 3), except amino acid 348 Asn. Our results suggest new amino acids, 236 Val, 315 Ala and 368 Ile, as key in the colchicine binding site of β-tubulin.

MD analysis

MD simulation was performed to evaluate the effect of the best ranked compounds (G311 and G900) during their interaction with the β-tubulin protein. The RMSD analysis over time (Fig. 2) showed that most complexes did not reached equilibrium at ~50 ns, with three main fluctuation at 8 ns, 30 ns, and 45 ns for G900, and two fluctuation at 5 ns and 25 ns for G311.

The analysis of the root-mean-square fluctuation (RMSF) per residue for the five systems is shown in Fig. 3. The chief backbone fluctuations occurred in the active site for both compounds. Hence, both analyses suggested that both compounds did not reach stabilization inside the active site, which explains the low effectivity in the in vitro and in vivo analysis.

The free energy result obtained was: ΔG_{AB} of 1170.11 ± 2.75 kJ mol^{-1}. In Fig. 1, it can be seen clearly that the values of ΔG are repulsive and do not minimize, which correspond with the experimentally obtained results. However, it is necessary to take more simulation time to see whether the system becomes stable.

G311 and G900 reduce tubulin polymerization in vitro

To investigate whether compounds G311 and G900 perturb microtubule dynamics in vitro, the polymerization rate of purified pig brain tubulin was measured. Turbidity of polymerized microtubules was determined by absorbance at 340 nm over time. Compounds G311 and G900 both showed a concentration-dependent decrease in microtubule polymerization. In

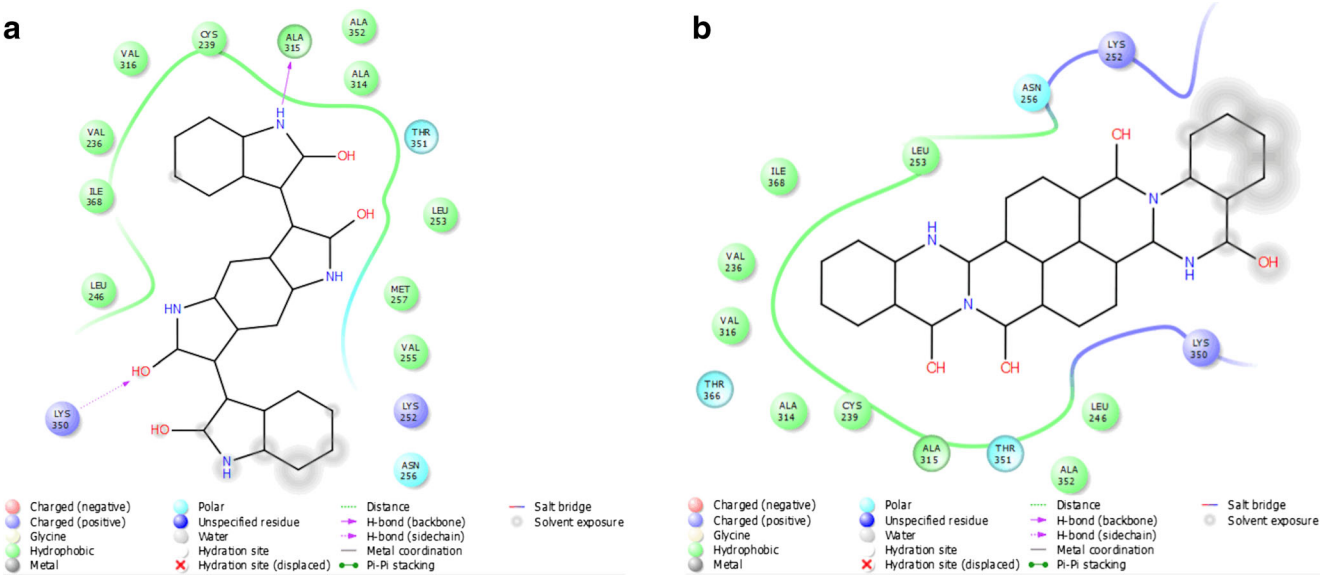


Fig. 1 A) Compound G311 in the binding site of β-tubulin; B) Compound G900 in the binding site of β-tubulin

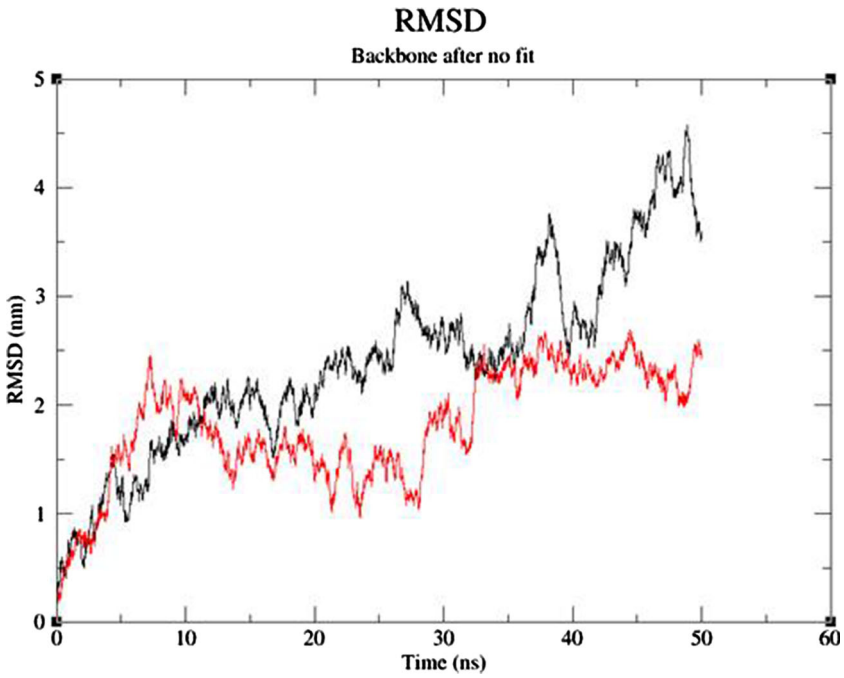


Fig. 2 Root mean square deviations (RMSD) for G311 and G900. *Black* G311, *Red* G900

comparison to the known inhibitors, nocodazole and colcemid, the inhibitory effect of G311 and G900 is minor (Fig. 4).

No perturbation of mitotic progression in HeLA S3 cells by G311 and G900

To test if compounds G311 and G900 have an effect on the cell cycle, mitotic duration of synchronized HeLa S3 cells was determined by live-cell imaging. The time period from nuclear envelope breakdown (cell rounding) was quantified until anaphase onset (first sign of a cleavage furrow). As shown in Fig. 5a (single experiment) and Fig. 5b (mean of three independent experiments), DMSO-treated cells had a mitotic duration of 45 min. Compounds G311 and G900 displayed very similar durations, and therefore there was no effect on mitotic

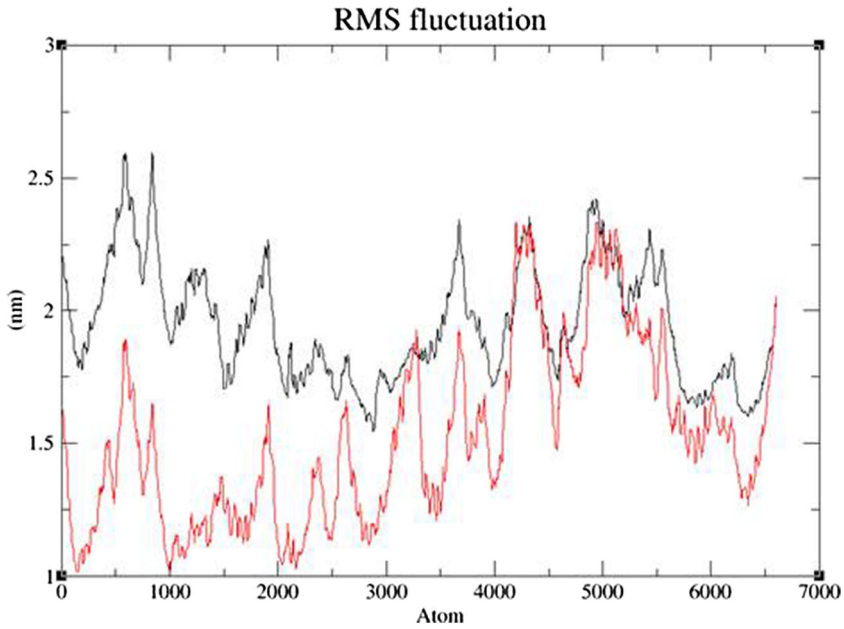


Fig. 3 Root mean square fluctuations (RMSF) for G311 and G900. *Black* G311, *Red* G900

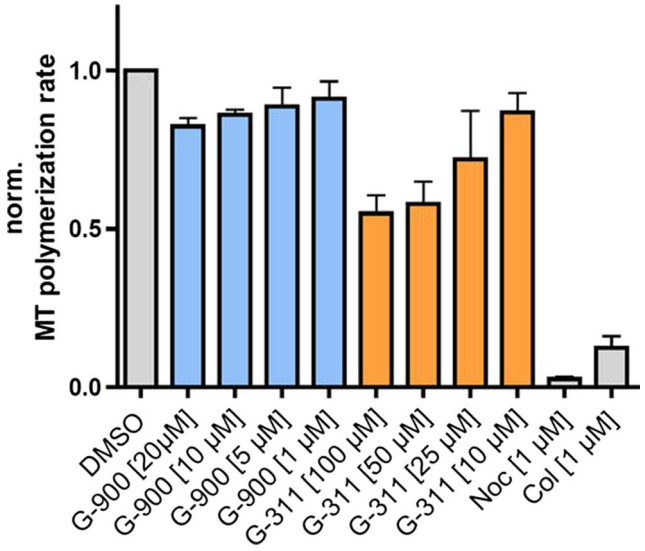


Fig. 4 Normalized microtubule polymerization rate with compound G311, compound G900, nocodazole and colcemid at indicated concentrations. Mean and SD are shown from three independent experiments

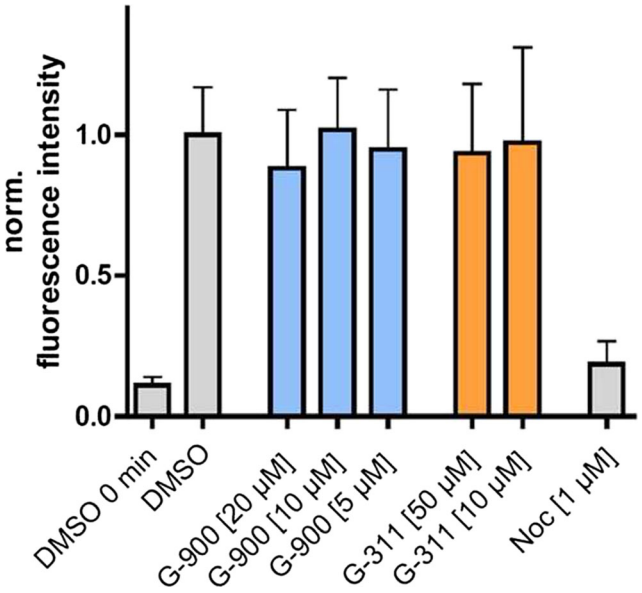


Fig. 6 Quantification of immunofluorescent labelling of tubulin. Treatment with compound G311, compound G900 and nocodazole at indicated concentrations, as well as a control in which cells were fixed before incubation at 37°C. At least 25 cells were quantified for each condition

progression. Cells treated with nocodazole and colcemid arrested in mitosis and were not quantified.

G311 and G900 show no effect on microtubule re-polymerization in HeLa S3 cells

To verify the inhibitory effect of G311 and G900 in live cells, a microtubule regrowth assay was performed. Microtubules were completely depolymerized in HeLa S3 cells by incubation on ice for 30 min. After adding DMSO, nocodazole, compound G311 or G900, the cells were returned to 37°C to restore the microtubule network. Cells were either fixed directly (0 min) or after 5 min. Quantification of immunostained microtubules by anti-α-tubulin-FITC is shown in Fig. 6. Compounds G311 and G900 did not show a significant decrease in microtubule re-polymerization compared to nocodazole (Fig. 6).

Conclusions

In this work, we reported new compounds as potential β-tubulin inhibitors by virtual screening, suggesting three new key amino acids (236 Val, 315 Ala and 368 Ile) in the

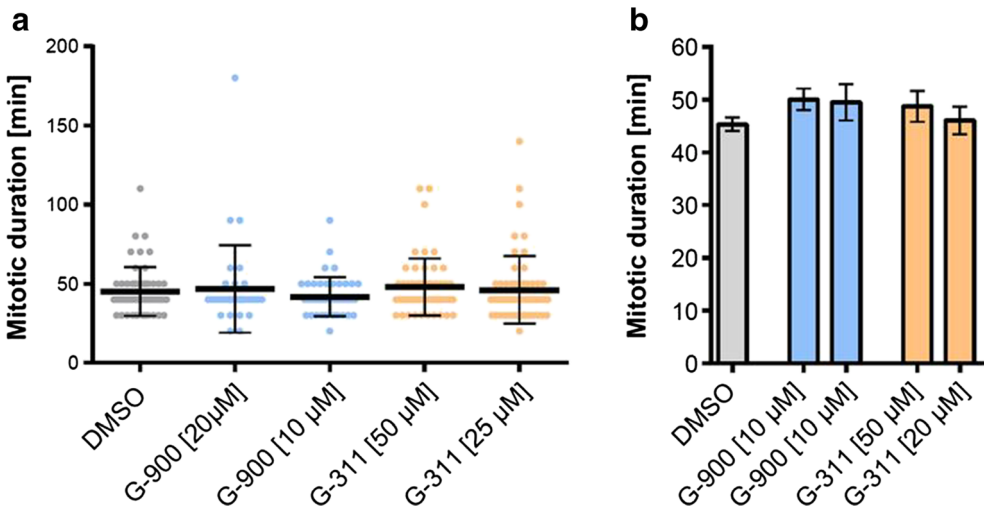


Fig. 5a,b Mitotic duration of HeLa S3 cells with dimethylsulfoxide (DMSO), compound G311 and compound G900 at indicated concentrations. **a** *Dot plot* of a single experiment. **b** Mean and SD of three independent experiments

colchicine binding site of β-tubulin. However, MD analysis showed a low stability in the binding site of β-colchicine. These results were confirmed in in vitro and in vivo models on β-tubulin without positive results, suggesting that MD is a more predictive computational tool than molecular docking, which could help in the search for more selective new inhibitor drugs.

Acknowledgments F.E.O.S. is the recipient of a scholarship (No. 419250) from Consejo Nacional de Ciencia y Tecnología (CONACyT), Mexico. We wish to express our gratitude to the Universidad Autonoma de Nuevo Leon (UANL), Instituto Politecnico Nacional (IPN), University of Texas Rio Grande Valley (UTRGV) and the University of Konstanz (UK) for their technical and financial support. In particular to Thomas U. Mayer for his support in the in vitro and in vivo assays, and Robert C. Jackson from UTRGV. G.R. holds a scholarship from the "Comision de Operacion y Fomento de Actividades Academicas" (COFAA-IPN) and the "Programa de Estimulos al Desempeño de los Investigadores" (EDI-IPN). All participants declare no conflict of interest.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2015) Global cancer statistics, 2012. CA Cancer J Clin 65(2):87–108
2. Abolhasani H, Zarghi A, Hamzeh-Mivehroud M, Alizadeh AA, Shahbazi Mojarrad J, Dastmalchi S (2015) In-silico investigation of tubulin binding modes of a series of novel antiproliferative spiroisoxazoline compounds using docking studies. Iran J Pharm Res 14(1):141–147
3. Ayaz P, Ye X, Huddleston P, Brautigam CA, Rice LM (2012) A TOG: αβ-tubulin complex structure reveals conformation-based mechanisms for a microtubule polymerase. Science 337(6096): 857–860
4. Lippert JW 3rd (2007) Vascular disrupting agents. Bioorg Med Chem 15(2):605–615
5. Abro A, Kulsoom S, Riaz N (2013) Pharmacophore model generation for microtubule-stabilizing anti-mitotic agents (MSAAs) against ovarian cancer. Med Chem Res 22(9):4322–4330
6. Kars MD, Işeri ÖD, Gündüz U (2011) A microarray based expression profiling of paclitaxel and vincristine resistant MCF-7 cells. Eur J Pharmacol 657(1):4–9
7. Ravelli RB, Gigant B, Curmi PA, Jourdain I, Lachkar S, Sobel A, Knossow M (2004) Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. Nature 428(6979): 198–202
8. Greene LM, Nathwani SM, Bright SA, Fayne D, Croke A, Gagliardi M, Zisterer DM (2010) The vascular targeting agent combretastatin-A4 and a novel cis-restricted β-lactam analogue, CA-432, induce apoptosis in human chronic myeloid leukemia cells and ex vivo patient samples including those displaying multidrug resistance. J Pharmacol Exp Ther 335(2):302–313
9. Greene LM, Carr M, Keeley NO, Lawler M, Meegan MJ, Zisterer DM (2011) BubR1 is required for the mitotic block induced by combretastatin-A4 and a novel cis-restricted ß-lactam analogue in human cancer cells. Int J Mol Med 27(5):715–723
10. O'Boyle NM, Carr M, Greene LM, Bergin O, Nathwani SM, McCabe T, Meegan MJ (2010) Synthesis and evaluation of azetidinone analogues of combretastatin A-4 as tubulin targeting agents. J Med Chem 53(24):8569–8584
11. O'Boyle NM, Knox AJ, Price TT, Williams DC, Zisterer DM, Lloyd DG, Meegan MJ (2011) Lead identification of β-lactam and related imine inhibitors of the molecular chaperone heat shock protein 90. Bioorg Med Chem 19(20):6055–6068
12. Loong HH, Yeo W (2014) Microtubule-targeting agents in oncology and therapeutic potential in hepatocellular carcinoma. OncoTargets Ther 7:575–585
13. Kumar A, Zhang KY (2015) Hierarchical virtual screening approaches in small molecule drug discovery. Methods 71:26–37
14. Segura-Cabrera A, Rodríguez-Pérez MA (2008) Structure-based prediction of Mycobacterium tuberculosis shikimate kinase inhibitors by high-throughput virtual screening. Bioorg Med Chem Lett 18(11):3152–3157
15. Kumar V, Krishna S, Siddiqi MI (2015) Virtual screening strategies: recent advances in the identification and design of anti-cancer agents. Methods 71:64–70
16. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ (2009) AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem 30(16):2785–2791
17. Mahaddalkar T, Suri C, Naik PK, Lopus M (2015) Biochemical characterization and molecular dynamic simulation of β-sitosterol as a tubulin-binding anticancer agent. Eur J Pharmacol 760:154–162
18. Vilar S, Cozza G, Moro S (2008) Medicinal chemistry and the molecular operating environment (MOE): application of QSAR and molecular docking to drug discovery. Curr Top Med Chem 8(18):1555–1572
19. Gupta AK, Varshney K, Saxena AK (2012) Toward the identification of a reliable 3D QSAR pharmacophore model for the CCK2 receptor antagonism. J Chem Inf Model 52(5):1376–1390
20. Penthala NR, Zong H, Ketkar A, Madadi NR, Janganati V, Eoff RL, Crooks PA (2015) Synthesis, anticancer activity and molecular docking studies on a series of heterocyclic trans-cyanocombretastatin analogues as antitubulin agents. Eur J Med Chem 92:212–220
21. Kendrick I, Kumari D, Yakaboski A, Dimakis N, Smotkin ES (2010) Elucidating the ionomer-electrified metal interface. J Am Chem Soc 132(49):17611–17616
22. Chopra A, Anderson A, Giardina C (2014) Novel piperazine-based compounds inhibit microtubule dynamics and sensitize colon cancer cells to tumor necrosis factor-induced apoptosis. J Biol Chem 289(5):2978–2991
23. Webber M, Dimakis N, Kumari D, Fuccillo M, Smotkin ES (2010) Mechanically coupled internal coordinates of ionomer vibrational modes. Macromolecules 43(13):5500–5502
24. DeLano WL (2002) The PyMOL molecular graphics system. https://www.pymol.org/. Accessed: 5 April 2016
25. Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, Hutchison GR (2012) Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. J Cheminform 4(1):17
26. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera—a visualization system for exploratory research and analysis. J Comput Chem 25(13): 1605–1612
27. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 31(2):455–461
28. Guthikonda SM (2005) Kohonen Self-Organizing Maps. Wittenberg University. http://www.shy.am/wp-content/uploads/2009/01/kohonen-self-organizing-maps-shyam-guthikonda.pdf. Accessed: 5 April 2016
29. Kohonen T (1998) The self-organizing map. Neurocomputing 21(1):1–6
30. Chen K, Huzil JT, Freedman H, Ramachandran P, Antoniou A, Tuszynski JA, Kurgan L (2008) Identification of tubulin drug binding sites and prediction of relative differences in binding affinities to

tubulin isotypes using digital signal processing. J Mol Graph Model 27(4):497–505
31. Webb B, Sali A (2002) Comparative Protein Structure Modeling Using MODELLER. In: Current Protocols in Bioinformatics. Wiley, New York. doi:10.1002/cpbi.3
32. National Center for Biotechnology Information. tubulin beta-2B chain [Homo sapiens] http://www.ncbi.nlm.nih.gov/protein/29788768/may 2015. Accessed: 5 March 2016
33. Irwin JJ, Shoichet BK (2005) ZINC-a free database of commercially available compounds for virtual screening. J Chem Inf Model 45(1):177–182
34. Bañuelos-Hernández AE, Mendoza-Espinoza JA, Pereda-Miranda R, Cerda-García-Rojas CM (2014) Studies of (−)-Pironetin Binding to α-Tubulin: conformation, docking, and molecular dynamics. J Org Chem 79(9):3752–3764
35. Patel B, Jajoo A, Tibrewal Y, Joshi A (2015) An Efficient Parallel Algorithm for Self-Organizing Maps using MPI-OpenMP based Cluster. In: IJCA Proceedings on International Conference on Advanced Computing and Communication Techniques for High Performance Applications ICACCTHPA 2014 (2):5–9
36. Bhattacharyya B, Panda D, Gupta S, Banerjee M (2008) Anti-mitotic activity of colchicine and the structural basis for its interaction with tubulin. Med Res Rev 28(1):155–183
37. Iwasa K, Kamigauchi M, Takao N, Cushman M, Wong WC, J-k C (1988) Formation of benzo [c] phenanthridines by oxidative C-N bond fission of protoberberines followed by intramolecular recyclization in cell cultures of corydalisincisa. Tetrahedron Lett 29(49):6457–6460
38. Khilya O, Volovnenko T, Volovenko YM (2006) The reaction of 2-hetarylacetonitriles with heterocyclic haloaldehydes. Chem Heterocycl Compd 42(10):1311–1324
39. Kulkarni AR, Thakur GA (2013) Microwave-assisted expeditious and efficient synthesis of cyclopentene ring-fused tetrahydroquinoline derivatives using three-component Povarov reaction. Tetrahedron Lett 54(48):6592–6595
40. Becerra D, Insuasty B, Cobo J, Glidewell C (2013) 3, 3′-[(1RS, 3SR)-2-Oxocyclohexane-1, 3-diyl] bis [(3RS, 3′ SR)-3-hydroxyindolin-2-one] dihydrate: organic layers of R22 (8), R22 (16) and R66 (40) rings linked by tetrameric water aggregates. Acta Cryst C 69(9):1081–1084
41. Yang SW, Ho GD, Tulshian D, Bercovici A, Tan Z, Hanisak J, Brumfield S, Matasi J, Sun X, Sakwa SA, Herr RJ, Zhou X, Bridal T, Urban M, Vivian J, Rindgen D, Sorota S (2014) Bioavailable pyrrolo-benzo-1,4-diazines as Na(v)1.7 sodium channel blockers for the treatment of pain. Bioorg Med Chem Lett 24(21):4958–4962
42. Olekhnovich E, Boroshko S, Korobka I, Metelitsa A, Olekhnovich L (2001) Acetals and vinyl ethers of unsaturated aldehydes and ketones in the new syntheses of heterocyclic compounds: XII. New alternatives of acid condensation of cyclohexane-1, 4-diones with hydroxyarylaldehydes under dehydration conditions. Fluorecence spectra of the products. Russ J Org Chem 37(4):527–538
43. Sander T (2001) OSIRIS property explorer. Actelion Pharmaceuticals, Allschwil, Switzerland
44. Dipple A, Khan QA, Page JE, Ponten I, Szeliga J (1999) DNA reactions, mutagenic action and stealth properties of polycyclic aromatic hydrocarbon carcinogens (review). Int J Oncol 14(1):103–112
45. Pineda O, Farras J, Maccari L, Manetti F, Botta M, Vilarrasa J (2004) Computational comparison of microtubule-stabilising agents laulimalide and peloruside with taxol and colchicine. Bioorg Med Chem Lett 14(19):4825–4829
46. Naik PK, Santoshi S, Rai A, Joshi HC (2011) Molecular modelling and competition binding study of Br-noscapine and colchicine provide insight into noscapinoid-tubulin binding site. J Mol Graph Model 29(7):947–955
47. O'Boyle NM, Pollock JK, Carr M, Knox AJ, Nathwani SM, Wang S, Caboni L, Zisterer DM, Meegan MJ (2014) beta-Lactam estrogen receptor antagonists and a dual-targeting estrogen receptor/tubulin ligand. J Med Chem 57(22):9370–9382