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CHALCONES LACK OF SPECIFICITY: TUBULINE, A MULTITARGETED MOLECULE

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ABSTRACT: Chalcones were considered promissing molecules. Compounds of this group present a large range of biological effects: antitumoral, antibacterial, antioxidand and so forth. However none of this compounds has a straight specific effect that can be exploited therapeutically in spite of chalcone excelent ADME characteristics. The aim of this study is to show computationally why chalcone have such a wide range of bioactivities, but fail as drugs. Hypothesis tested is that tubiline with was demonstrated as chalcone target has an abnormal affinity for chalcone molecules. Approach to this study was computational. A pdb model was choosen for tubuline. Docking studies were performed for 31 chalcones. Results showed that tubulin has an increas number of cavities for chalcone binding at each tubuline subunits. Docking energies are extremely favorable for ensuring tubuline–chalcone complexes. Compound 29 ((E)-3-4(dimethylamino) phenyl)-1-(4-hydroxyphenyl)-2-methylprop-2-en-1-one) has the most favorable binding affinity for tubuline.

KEYWORDS: binding affinity, chalcone, tubuline, multitarget.

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1.INTRODUCTION

The term cancer includes over 100 different types of diseases. After 50 years of research, it has been shown that in cancer are altered many genes[1]. It is a complex multi-stage process in which incipent cancer cell genomes acquire pro-oncogenic alleles, suppressor tumor genes and other genes that directly and indirecty control cellular proliferation. These different type of genes are involved in the development of human cancers by combining them differently [2]. The purpose of the anticancer activity is to kill the malignant cells without affecting the normal ones[3]. However, many therapeutic agents act non-selectively on both cancer and normal cells[4]. The characteristics of cancerous cells (uncontrolled proliferation, metastasis formation and the need of apoptosis) are the main elements that reduce the effectiveness of anticancer therapies[5]. The adverse effects of chemotherapy and the induction of resistance to the therapy are the causes of therapeutic failure. In this context, it is necessary to identify new compounds with antitumor activity [6]. Chalcones are privilegiated molecules that display of a simple typical chain in which the two aromatic nuclei are joined by a trans-enonic bridge [7]. The main method for syntesis of chalcones is the Claise-Schmidt condensation reaction of the aldehyde with acetophenone in basic or acidic catalysis [8]. The use of acidic catalysis in unfavorable due to the low yields and the negative environmental impact [9]. Chalcones are an important pharmacophore for many natural products such as cumarin, flavokawain, milepachin and xanthohumol. Milepachin, a new chalcone having 2,2-dimethyl-benzopyran subunit, exhibits significant cytotoxicity *in vitro* on various cell lies. *In vivo*, its antitumor activity is good [10]. Methoxychalcones are structurally similar to combrestatin A-4-5 and cholchicine due to their spatial orientation between the two aromatic subunits. Like combrestatin and cholchicine, methoxychalcones efficiently bind to the tubulin[11]. The antimitotic effect of chalcones is dependent on the aldehyde substituents, especially those from 2, 4 and 6 positions [12]. The chalcones from (*E*)-3-(4-dimethylamino)phenyl-1-(2,5-dimethoxyphenyl)-2-methylpropen-2-en-1-ones seria have the ability to inhibit the assembly of microtubules and are potent antimitotic agents. Podophyllotoxines and cholchicine have been shown to block the binding of chalcones to tubulin [13]. The aim of this study is to asses computationally tubulin interaction with 31 α-methyl chalcones previously synthetizated and biological evaluated for their anticervical cancer activity.

2. MATERIALS AND METHODS

In order to asses computationally tubulin interaction with chalcone 4O2A was consider[14].Tubuline as shown in Figure 1 is compose of 5 proteins, and cofactors. Protein A,B,C,D. Protein E mentaines the other structure together and is composed of a string of amino acid (see Figure2) [15] .

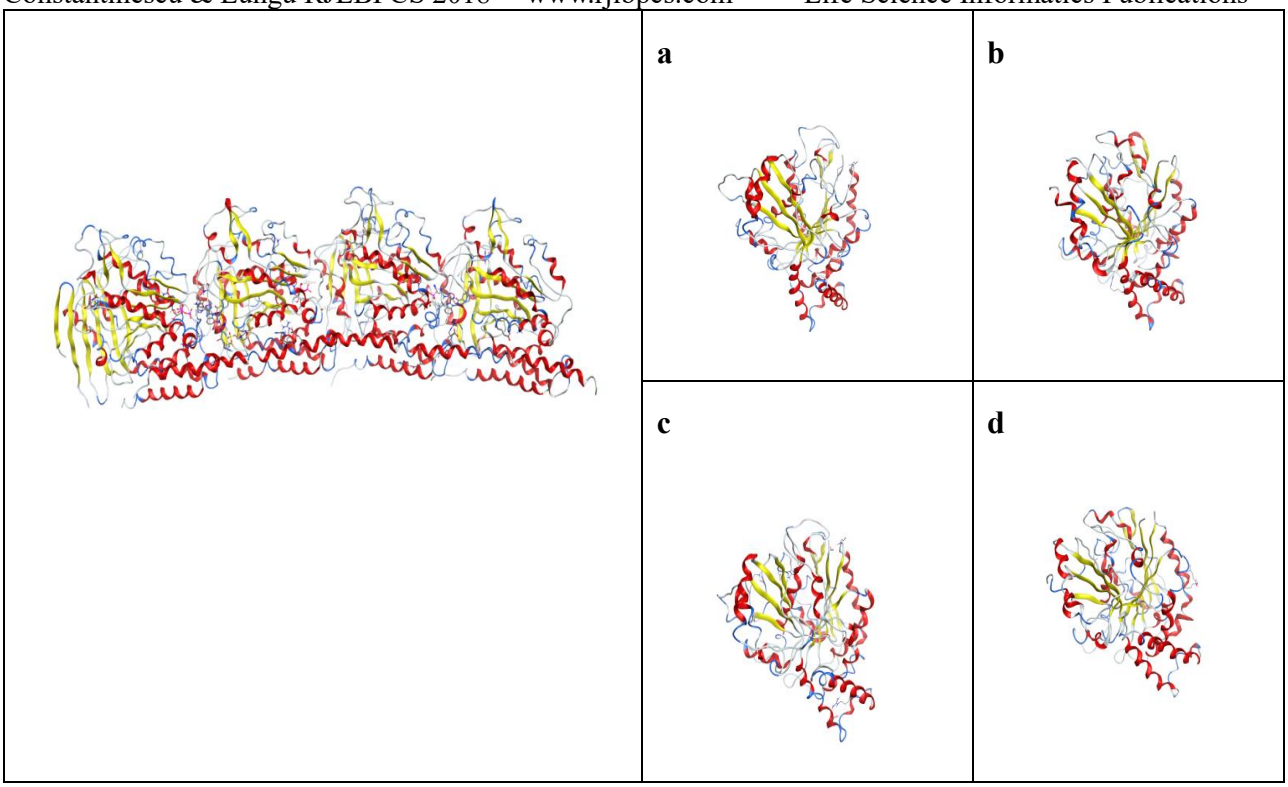

Figure 1 a,b,c,d : Tubulin and proteins A,B,C,D represented as ribbons

For entire tubulin molecule and for subunits A-D docking studies were performed . Compounds 1-31 were docked to these structures. Target structure was set as 4o2a. Protein was minimized, charges were corrected[16]. Force field used was Amber 10. For each 31 compounds a 3D structure was computed using 2D formulas. Ligands strucures were minimized using MM2 force field. Docking was perfomed using AutoDock 4.2 software package. Binding site detection was performed using same software [17]. Center grid coordinates are shown in Table 1. Box was set with a side of 15x15x15 Å. Furthermore for each unit binding were flooded with water molecules in order to obtauin a 3D model on witch surface and volume can be measured. Binding affinities were comuted for each complex. In order to assesed each tubulin subunit affinity for chalcone sum of binding affinities were calculated for each compound and compared with the sum of binding affinities for the hole tubulin (subunit A-E). Schrodinger and Pymol software packeges were used to represent the molecules[18].Results:Binding sites surface and volume are shown in Table1. Tubulin has the biggest binding site with a volume of 1121.79Å^3 followed by subunit C with a volume of 130.048Å^3. In Figure1 tubulin major binding site is shown in detailed. For subunits A-C five cavities were detect. Subunit D has three and subunit E has no cavity witch could serve as a potentially binding site.

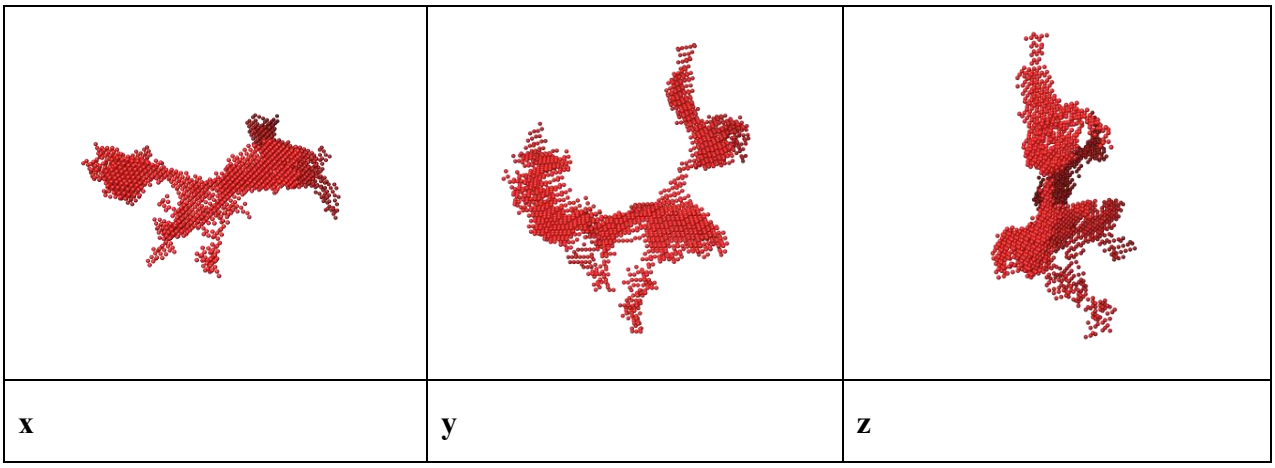

Figure 2: Major binding site of tubulin (4O2A)represented after x,y,z axes.

For subunit A compound 16 has the favorables binding affinity, for subunit B compound 27, for C compound 29 and for D compound 15 respectivelly. Molecule 29 (((E)-3-4(dimethylamino)phenyl)-1-(4-hydroxyphenyl)-2-methylprop-2-en-1-one) binds the most efficentelly to tubulin comparative to all compounds. In terms of total binding affinities tubulin as a hole is favorable of binding chalcone in comparison to each subunit (Figure3).

Table 1: Cavities of tubulin and its subunits along with there origin carthesian coordinates

Protein	Cavity	X(Å)	Y(Å)	Z (Å)	Volume ($Å^3$)	Surface($Å^2$)
A	1	22.149	74.5093	49.6081	109.056	309.76
	2	32.78349	99.5693	66.9188	36.864	116.48
	3	28.8945	83.4078	82.3992	22.016	97.28
	4	19.8278	91.4915	46.4277	15.36	57.6
	5	10.4305	75.1235	49.969	12.8	55.04
B	1	14.2999	48.4382	17.2122	115.712	362.24
	2	1.82867	63.1171	27.2059	49.664	180.48
	3	17.7924	64.3103	45.1801	39.936	142.08
	4	4.79128	45.5888	35.2533	11.776	51.2
	5	15.8422	62.9784	39.5845	10.752	48.64
C	1	15.2558	18.9855	-14.8529	130.048	395.52
	2	28.555	31.3152	7.01434	26.624	120.32
	3	6.355	29.2742	-17.8443	21.504	84.48
	4	23.6883	44.3613	-17.4574	19.968	79.36
	5	8.81214	11.8075	-2.9395	14.336	53.76
D	1	14.2867	7.51232	-19.2793	48.64	186.88
	2	3.28668	-1.01997	-37.7389	34.304	131.84

	3	14.5225	-1.014	-48.9425	10.24	46.08
A+B+C+D+E	1	16.1135	43.6399	11.5001	1121.79	2760.96
	2	12.2538	22.4787	-20.4674	430.592	1317.12
	3	18.6033	76.6592	44.8555	414.208	1254.4
	4	34.2519	58.8275	39.006	197.632	563.2
	5	10.1148	65.6662	53.3322	89.088	257.28

Table 2: Binding affinities for Tubulin and its subunits. In bolded shaded gray – favorable binding affinities for Tubulin and each subunit.

#	∑ABCD-ABCDE	∑ABCD	Prot A	Prot B	Prot C	Prot D	Prot ABCDE
1	-491.022	-385.93	-99.14	-106.60	-96.63	-83.56	-105.09
2	-477.41	-382.08	-102.31	-103.17	-94.25	-82.35	-95.33
3	-528.95	-422.07	-113.55	-113.18	-101.09	-94.25	-106.88
4	-502.91	-404.97	-111.02	-108.96	-99.09	-85.9	-97.94
5	-557.8	-434.54	-117.07	-123.77	-100.89	-92.81	-123.26
6	-576.19	-463.74	-114.79	-128.72	-115.14	-105.09	-112.45
7	-558.86	-434.77	-107.9	-130.07	-106.35	-90.45	-124.09
8	-557.34	-444.12	-108.12	-125.24	-108.55	-102.21	-113.22
9	-531.55	-414.69	-104.44	-114.74	-100.91	-94.6	-116.86
10	-531.57	-419.54	-100.23	-123.65	-107.97	-87.69	-112.03
11	-549.97	-425.25	-112.79	-125.93	-99.27	-87.26	-124.72
12	-518.75	-400.94	-96.44	-116.11	-96.11	-92.28	-117.81
13	-515.53	-397.00	-111.43	-116.03	-84.85	-84.69	-118.53
14	-546.53	-430.37	-117.01	-119.58	-102.29	-91.49	-116.16
15	-584.88	-465.79	-125.51	-124.88	-106.79	**-108.61**	-119.09
16	-582.75	-459.93	**-127.53**	-128.41	-109.5	-94.49	-122.82
17	-590.75	-465.74	-124.14	-127.77	-122.09	-91.74	-125.01
18	-560.64	-434.67	-102.77	-127.44	-106.86	-97.6	-125.97
19	-586.9	-452.95	-122.27	-126.57	-108.81	-95.3	-133.95
20	-516.43	-395.93	-97.94	-119.9	-88.87	-89.22	-120.5
21	-559.54	-433.38	-114.37	-118.03	-106.69	-94.29	-126.16
22	-562.5	-434.08	-116.53	-119.44	-108.89	-89.22	-128.42
23	-526.87	-421.82	-114.33	-121.39	-95.57	-90.53	-105.05
24	-584.61	-461.06	-123.53	-140.25	-110.07	-87.21	-123.55
25	-497.86	-388.88	-103.89	-109.12	-85.95	-89.92	-108.98

26	-546.05	-432.83	-110.45	-126.67	-99.13	-96.58	-113.22
27	-595.15	-462.18	-120.79	**-130.5**	-116.96	-93.93	-132.97
28	-553.53	-442.56	-107.48	-129.14	-107.57	-98.37	-110.97
29	**-611.15**	**-469.18**	-126.24	-125.79	**-130.63**	-86.52	**-141.97**
30	-517.04	-409.00	-113.24	-116.01	-93.60	-86.15	-108.04
31	-558.53	-448.93	-110.21	-124.37	-112.11	-102.24	-109.60
∑colum	-16979.56	**-13338.92**	-3477.46	**-3771.43**	-3223.48	-2866.55	-3640.64

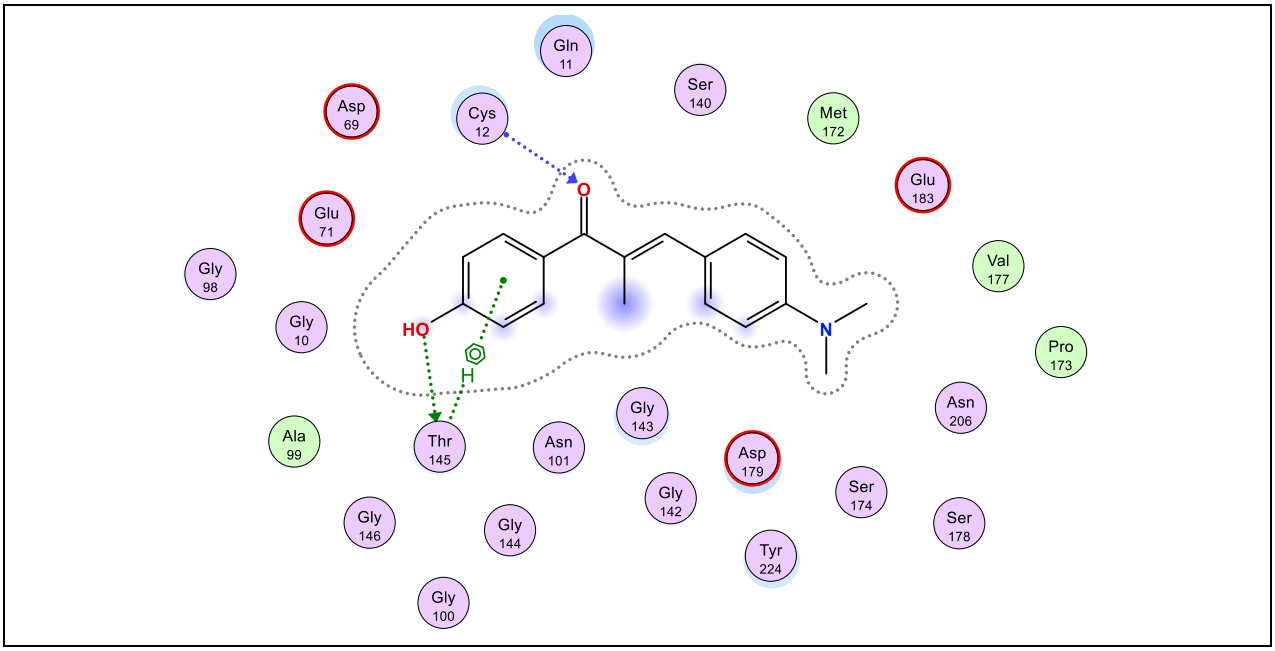

Figure 3: Compound 29 interactions with Tubulin major binding site. Blue arrow H bound , vilot- positive charge, violet halo blue- basic, green-hydrofobic

3. RESULTS AND DISCUSSION

In general, the binding affinity term stands for the capacity of ligands to form coordination bonds with a receptor[19]. The binding affinity of a ligand with a receptor depends upon the interaction force of attraction between the ligands and their receptor binding sites[20]. Selective ligands bind to a very limited kinds of receptors [21]. Non-selective ligands bind to several types of receptors. This play an important role in pharmacology, where drugs that are non-selective tend to have more adverse effects, because they bind to several other receptors in addition to one generating the desired effect [22]. Microtubules are cellular structures present in eukaryotic organisms and are involved in mitosis, motility, cytoskeletal arhitecture, intracellular transport and secretion[23]. The structural component of the microtubules is tubulin, a dimeric protein molecule. It has two similar subunits (α and β) and a molecular weight of 55.000 Da [24].It is known that microtubules represent an important therapeutic target for anticancer therapy[25]. There are four tubulin-binding sites that

influence microtubulin dynamics[26]. Classical antitubulinic agents exhibit dose-dependent toxicity and multidrug resistance due to overexpression of p-glycoprotein or multidrug resistance-associated proteins [27].Chalcones has antitubulinic properties by reversible binding to the colchicine site[28]. Although this compound class presents this important pharmacological property, there are no chalcones with antitubulin activity in clinical and preclinical studies. This is due to their metabolic instability in vivo. The phenolic group can be easily metabolized by a phase II reaction, and the enonic system participates in Michael-type additions with biological nucleophiles (e.g., glutathione). Other weaknesses of the chalcones are: a) they are compounds with numerous biological activities and implicitly lacking in selectivity, and b) have a low permeability. For these reasons, chalcones are intermediate for stable analogs with antimitotic properties, which show a favorable balance / safety balance [29, 30].For the 31 methylchalcones studied, affinity for the five tubulin binding sites is very good. Due to the tubulin's ability to bind the chalcones at multiple sites, they show a low selectivity. The best binding affinity is represented by the substituted in the para position of acetophenone with a hydroxy group and in the para position of the aldehyde with a dimethylamine.

4. CONCLUSION

Chalcones has antitubulinic properties by binding to the colchicine site. From the 31 methyl chalcones analyzed,(E)-3-4(dimethylamino)phenyl)-1-(4-hydroxyphenyl)-2-methylprop-2-en-1-oneit shows the best binding activity. Due to the tubulin's ability to bind chalcones to multiple sites, chalcones are low selectivity antimitotic agents.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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