UNIVERSIDADE FEDERAL DO AMAPÁ
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

RAIMUNDO DE ALMEIDA MIRA NETO

Avaliação da atividade antineoplasica de chalconas sintéticas utilizando modelos de glioblastomas *in vitro* e docking molecular para topoisomerase-II-α e tubulina

Macapá
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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade Federal do Amapá para obtenção do Título de Mestre em Ciências Farmacêuticas.

Orientador: Dr. Madson Ralide Fonseca Gomes

Macapá
2018

Dados Internacionais de Catalogação na Publicação (CIP)
Biblioteca Central da Universidade Federal do Amapá

615.1
M671a Mira Neto, Raimundo de Almeida

1.1.1. *Avaliação da atividade antineoplásica de chalconas sintéticas utilizando modelos de glioblastomas* In vitro *e docking molecular para Topoisomerase-II-α e Tubulina / Raimundo de Almeida Mira Neto; orientador, Madson Ralide Fonseca Gomes*. *– Macapá, 2018.*

55 f.

Dissertação (Mestrado) – Fundação Universidade Federal do Amapá, Programa de Pós-Graduação em Ciências Farmacêuticas.

1. Chalconas - Avaliação - Uso terapêutico. 2. Câncer -

Programa de Pós-Graduação em Ciências Farmacêuticas
da Universidade Federal do Amapá

BANCA EXAMINADORA

Aluno(a): Raimundo de Almeida Mira Neto

Orientador(a): Prof. Dr. Madson Ralide Fonseca Gomes

Co-Orientador(a):

Prof. Dr. Madson Ralide Fonseca Gomes / Presidente
Professor Titular do Curso de Farmácia da Universidade Federal do Amapá, UNIFAP.

Prof. Dra. Mayara Tânia Pinheiro / Membro Titular
Professor Titular do Curso de Farmácia da Universidade Federal do Amapá, UNIFAP.

Prof. Dra. Lorane Izabel da Silva Hage Melim / Membro Titular
Professor Titular do Curso de Farmácia da Universidade Federal do Amapá, UNIFAP.

Data: 23/04/2018

Dedico este trabalho a minha família.

AGRADECIMENTOS

Primeiramente agradeço a Deus pelo ingresso no programa de pós-graduação em Ciências Farmacêuticas, por todas as matérias cursadas e por todo o trabalho executado, pois tudo foi na graça deDeus.

Agradeço a minha esposa Thayse Alencar Mira, companheira fiel e paciente nessa jornada, jamais me permitiu desitir. Agradeço seu amor que muitas vezes me apoiou.

Agradeço a meus Pais Edson Pinheiro e Eliana Pinheiro pelo apoio e incentivo, que tambem jamais me permitiu desitir e sempre me fizeram acreditar nesse caminho acadêmico.

Agradeço ao meu orientador professor Madson Ralide, por sua pronta disposição em me orientar. Sempre foi um orientador presente e jamais deixou este estudo sem amparo.

Agradeço ao professor Irlom Maciel e sua equipe que nos forneceu as moléculas de chalconas. Agradeço o professor Breno Santos por sua orientação e ensinamento nos estudos in silico. Agradeço o professor Edivaldo Herculano e sua equipe que forneceu a estrutura de seu laboratório para execução dos testes in vitro, e por todo o conhecimento adquirido com sua equipe.

SUMÁRIO

Avaliação da atividade antineoplasica de chalconas sintéticas utilizando modelos de glioblastomas *in vitro* e docking molecular para topoisomerase-II-α e tubulina

RESUMO

Introdução: O glioblastoma é um tumor maligno com baixa taxa de sobrevivência, possui rápido crescimento e tem uma alta taxa de rotatividade celular. Estudos visando o desenvolvimento de novos fármacos anticancerígenos tem aumentado, a predição do modo de ancoragem de moléculas promissoras ao sitio de interesse é primordial no design racional de drogas. **Objetivo:** Esse estudo avaliou a atividade antineoplásica de chalconas sintéticas utilizando modelos de glioblastomas *in vitro* e propos por docking molecular o sitio de ação dessas moleculas. **Metodologia:** as chalconas foram sintetizadas e analisadas por cromatografia gasosa acoplada ao espectro de massas, a viabilidade celular foi investigada pelo teste do MTT e o estudo de docking molecular foi realizado no software GOLD. **Resultados e discussões:** Os resultados do ensaio MTT mostraram que as células AHOL1 e U87 tiveram sua viabilidade célular reduzida quando expostas as chalconas analisadas apresentando diferença significativa ($p<0,0001$) quando comparadas a linhagem sadia AN27. A análise comparativa das interações das moléculas com o alvo TOPIIA identificou interações em serina (SER148-149) e isoleucina (ILE125). A interação com o aminoácido serina estava presente tanto no docking dos ligantes mais promissores quanto nos de referencia, sugerindo sua importância no efeito inibitório do crescimento celular. A analise comparativa entre os ligantes de referência e as moleculas em estudos identificou o aminoácido LYS 352 presente em todos os encaixes, sugerindo que esse seja o principal aminoácido para a interação com a tubulina, observou-se tambem que a ausencia de interação com o aminoácido CYS 241 provoca redução no gold score. **Conclusões:** Os resultados obtidos no docking molecular corroboram com o observado no MTT, sugerindo que as moleculas em estudo possuem potencial antineoplásico em glioblastomas.

Palavras-Chave: *Chalconas; Citotoxicidade; Docking molecular; Topoisomerase II alfa; Tubulina.*

Evaluation of antineoplastic activity of synthetic chalcones using *in vitro* glioblastomas models and molecular docking for topoisomerase-II-α and tubulin

ABSTRACT

Introduction: Glioblastoma is a malignant tumor with low survival rate, has rapid growth and has a high turnover rate. Studies aiming at the development of new anticancer drugs have increased, predicting the mode of anchoring promising molecules to the site of interest is paramount in rational drug design. **Purpose:** This study evaluated the antineoplastic activity of synthetic chalcones using in vitro glioblastomas models and proposed by molecular docking the site of action of these molecules. **Methodology:** chalcones were synthesized and analyzed by gas chromatography coupled to the mass spectra, cell viability was investigated by the MTT test and the molecular docking study was performed in the GOLD software. **Results and discussion:** The results of the MTT assay showed that the AHOL1 and U87 cells had their cell viability reduced when the chalcones analyzed were exposed, presenting a significant difference ($p < 0.0001$) when compared to the AN27 healthy lineage. The comparative analysis of the interactions of the molecules with the TOPIIA target identified interactions in serine (SER148-149) and isoleucine (ILE125). Interaction with the serine amino acid was present in both the most promising and the reference binder docking, suggesting its importance in the inhibitory effect of cell growth. Comparative analysis between the reference ligands and the molecules in studies identified the amino acid LYS 352 present in all of the inserts, suggesting that this is the main amino acid for interaction with tubulin, it was also observed that the absence of interaction with the amino acid CYS 241 causes reduction in the gold score. **Conclusions:** The results obtained in the molecular docking corroborate with that observed in MTT, suggesting that the molecules under study have antineoplastic potential in glioblastomas.

Keywords: Chalconas; Cytotoxicity; Molecular docking; Topoisomerase II alpha; Tubulin.

1 INTRODUÇÃO

1.1 CÂNCER

As células somáticas acumulam mutações que podem conferir vantagem seletiva à célula, como o aumentando da sobrevivência ou proliferação, as que são seletivamente neutras e as mutações que são desvantagens para a célula e resultam em sua morte. O câncer é produto da evolução somática, em que uma única linhagem clonal adquire mutações que permitem a superação de restrições normais à proliferação celular, invadem os tecidos e se espalhem para outros órgãos (MARTINCORENA et al., 2017).

O sequenciamento de todo o genoma e as tecnologias massivamente paralelas permitiram entender que o câncer é uma doença causada principalmente por alterações genômicas, especialmente as mutações de células somáticas. O desenvolvimento do câncer é impulsionado pela acumulação de alterações que afetam a estrutura e a função do genoma. Estas alterações são frequentemente adquiridas como resultado da exposição a múltiplos agentes carcinogênicos, afetando não apenas o funcionamento das proteínas codificadas pelos genes alterados, mais também todo o circuito que controla o crescimento celular, o potencial replicativo, a sobrevivência e a resposta ao estresse. Dentre esses processos, incluem danos oxidativos, erros na ação das polimerases e recombinases, redução e reordenamento cromossômico. (SILVA; RIUL, 2011; ZHAO et al., 2015).

Desse modo, o termo câncer é designado a mais de uma centena de diferentes doenças heterogêneas do material genético que promovem alterações essenciais na fisiologia celular. A carcinogenese resulta de processos multifatoriais, que incluem a interação de fatores genéticos e agentes carcinógenos externos, físicos (ultravioleta e radiação ionizante), químicos (pesticidas, herbicidas, ou contaminantes de água e alimentos) e biológicos (determinados vírus, bactérias e parasitas), além do estilo de vida adotado por cada indivíduo. Proto-oncogenessão responsáveis pela divisão e crescimento celular em condições normais, podem se tornar oncogenes durante a mutação genética. Além disso, a falta de genes supressores de tumores desencadeia a divisão de células descontroladas (SILVA; MATTOS, 2011; HASSANNPOUR; DEHGHANI, 2017).

A tumorigênese tem sido considerada como um mecanismo autônomo da célula desencadeado pela acumulação de mutações capazes de conferir uma vantagem de

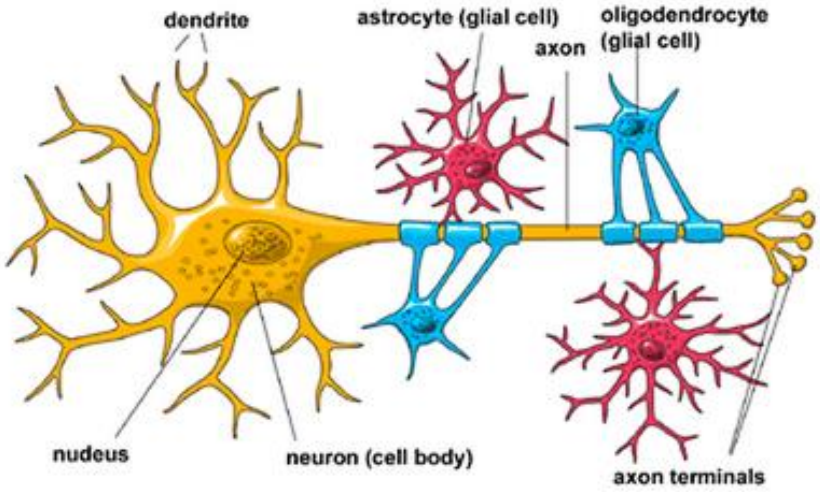
crescimento às células transformadas e a capacidade de invadir tecidos circundantes e eventualmente promover a metástase. A denominação do câncer é feita de acordo com o tipo celular de origem, o termo carcinoma é utilizado se o câncer se iniciar a partir de células epiteliais, sarcoma caso sua origem seja de linhagem celular mesodérmica, linfoma se a origem for células do sistema imunológico e, por fim, o termo leucemia é utilizado se o câncer for originado de células da medula óssea (SULTANA, 2014; GANDELLINI et al., 2015).

No Brasil, os registros de câncer de base populacional, em conjunto com os registros hospitalares e com o sistema de informação sobre mortalidade do DataSUS (Departamento de Informática do Sistema Único de Saúde), formam o eixo estruturante para a vigilância e desenvolvimento de pesquisas sobre câncer, gerando condições necessáriass para o planejamento e avaliação das ações de prevenção e controle do câncer. No ano de 2016, estimou-se 5.440 novos casos de câncer do Sistema Nervoso Central (SNC) em homens e 4.830 em mulheres. Esses valores correspondem a um risco estimado de 5,50 novos casos a cada 100 mil homens e 4,68 para cada 100 mil mulheres. Sem considerar os tumores de pele não melanoma, o câncer do SNC em homens é o oitavo mais frequente nas Regiões Sul e Nordeste, ocupa a décima posição nas Regiões Centro-Oeste e Norte, e na Região Sudeste é o 11º mais frequente. Para as mulheres, é o sexto mais frequente na Região Sul, e oitavo mais frequente na Região Centro-Oeste, já na Região Norte, ocupa a décima posição. Enquanto, nas Regiões Sudeste e Nordeste é o 11º (BRASIL, 2015).

1.2 GLIOBLASTOMAS

As células gliais (glial radial, astrócitos, ependimócitos) são células não neuronais que desempenham funções essenciais para a homeostase do SNC (figura 1), incluindo manutenção dos níveis iônicos do meio extracelular, tendo um papel crítico no metabolismo do neurtransmissores, participação na formação da barreira hematoencefálica, secreção de fatores tróficos essenciais para a sobrevivência e diferenciação dos neurônios, direcionamento de axônios e formação e funcionamento das sinapses. Também estão envolvidos na regulação do fluxo sanguíneo cerebral e do acoplamento neurovascular, bem como no auxílio na defesa imune, por meio da síntese e secreção de diversas citocinas inflamatórias. Além disso, essas células têm grande impacto no controle energético cerebral, em razão do fornecimento de energia e metabólitos (FURNARI et al., 2007; GOMES; TORTELLI; DINIZ, 2013).

Figura 1 – Células Gliais



Fonte: FURNARI et al., 2007.

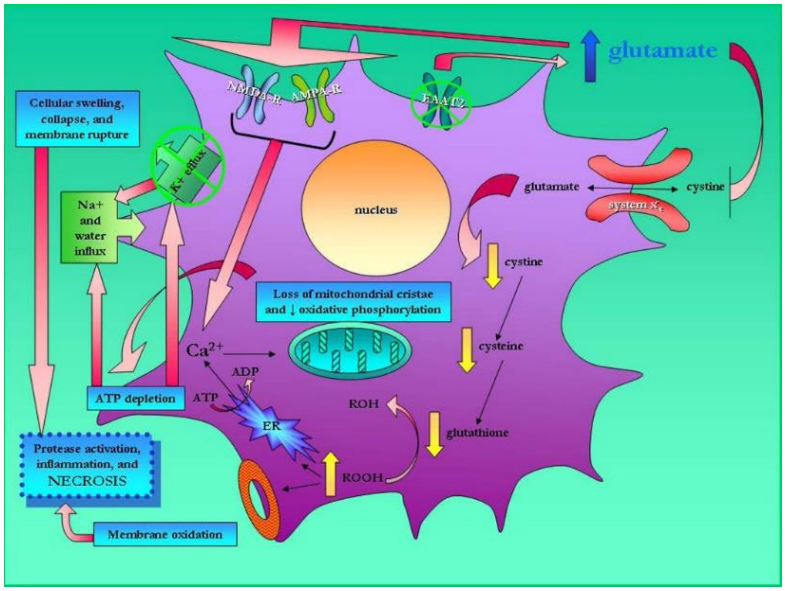
Os tumores cerebrais adultos de origem glial são divididos em astrocitomas, oligodendrogliomas e oligoastrocitomas com base em várias características histopatológicas chave. Além disso, esses tumores são classificados clinicamente como astrocitoma de classe I (pilocítico), astrocitoma grau II (difuso) da Organização Mundial de Saúde (OMS) benigno, astrocitoma maligno da OMS III (anaplásico) e astrocitoma de grau IV (glioblastoma), a forma mais maligna. Astrocitomas difusos de baixa qualidade seguem cursos clínicos longos se detectados precocemente; no entanto, os gliomas mais graves de Grau III carregam uma sobrevivência de cinco anos para 29,4% dos pacientes, e os glioblastomas carregam uma sobrevivência de cinco anos para apenas 3,4% dos casos (NOCH; KHALILI, 2009; JUNG et al., 2013).

O glioblastoma é um tumor maligno com baixa taxa de sobrevivência. É um tumor em rápido crescimento e tem uma alta taxa de rotatividade celular. O tumor supera sua necessidade metabólica por neovascularização, porém, a neovascularização não consegue acompanhar o rápido crescimento do tumor, ocasionando focos de necrose, que podem se unir e formar uma grande área de necrose central. Isso ocorre porque os vasos patológicos não possuem uma barreira hematoencefálica adequada e estão associados a um certo grau de vazamento capilar. O tumor interage com tecido cerebral adjacente, causando edema relativamente extenso (METWALI; ELHAKIM, 2017).

A necrose é uma característica marcante do glioblastoma em mais de 85% dos casos. O seu crescimento é fisicamente restringido pelo crânio e para superar esse desafio, ocorre aumento da liberação de glutamato que ocasionando a morte excitotóxica aos neurônios circundantes (figura 2). O mecanismo de necrose no glioblastoma começa

com a depleção aguda de ATP celular como resultado do colapso da cadeia de transporte de elétrons e subsequente diminuição da fosforilação oxidativa. Com essa diminuição canais e bombas iónicas dependentes de ATP começam a falhar, o que inicia um aumento maciço do volume celular através do influxo de Na^+, Ca^{2+} e diminuição do efluxo K^+. À medida que a membrana celular se rompe, os conteúdos da célula são liberados para o espaço extracelular e o estágio final de necrose envolve a ativação de proteases e uma resposta inflamatória localizada (SONTHEIMER, 2008; NOCH; KHALILI, 2009).

Figura 2 – Mecanismo de necrose por excitotóxicidade glutamatérgica em glioblastoma.



Fonte: SONTHEIMER, 2008.

A decisão cirúrgica para o manejo de tumores com edema perifocal, que corresponde a maioria dos casos de glioblastomas, é direta e implica em biopsia tumoral quando possível. O exame histopatológico fornece ao médico um diagnóstico seguro com a possibilidade de uma maior análise imuno-histoquímica, que pode determinar o gerenciamento quimioterapêutico adicional e também determinar o prognóstico (METWALI; ELHAKIM, 2017).

1.3 PLANEJAMENTO E DESENVOLVIMENTO DE FÁRMACOS ANTINEOPLÁSICAS

Estudos visando o desenvolvimento de novos fármacos anticancerígenos tem aumentado, sendo o objetivo primordial desenvolver tratamentos mais efetivos e seletivos, visando à descoberta de novas estratégias que impeçam o avanço da doença, possibilitando um melhor prognóstico. Os significativos avanços na biologia do câncer, possibilitaram direcionar as buscas por moléculas que atuem com mecanismos

específicos para cada tipo de enfermidade, como inibição da polimerização da tubulina, atuação no DNA, bloqueadores enzimáticos ou de microtúbulos celulares (BRANDÃO et al., 2010).

Métodos toxicológicos *in vitro* tornam-se uma opção na triagem em busca de novas moléculas que possuam efeitos citotóxicos em linhagens neoplasicas, além de diminuir custos, obter respostas rápidas e colaborar com o princípio dos 3R's (reduction, refinement e replacement). Dentre as vantagens desses métodos estão delimitação de variáveis analisadas, maior facilidade na obtenção de dados significativos, redução no período dos testes, possibilita maior número de repetições, além disso, requer menor quantidade da substância em análise (HARTUNG; DASTON, 2009; BEDNARCZUK et al., 2010).

Um dos ensaios mais utilizados é o testes do MTT (brometo de 3-(4,5-dimetiliazol-2- il) -2,5-difeniltetrazólio), onde células viáveis acumulam o sal amarelo MTT por endocitose e são capazes de transformá-lo em cristais de formazan. A redução do anel tetrazólico deste sal resulta na formação de cristais formazan de cor azul-purpura que se acumulam em compartimentos endossomais e/ou lisossomais. (FEKRAZAD et al., 2017).

A integração de estratégias computacionais e experimentais tem sido de grande valor na identificação e desenvolvimento de novos compostos promissores. Amplamente utilizado no design de fármacos modernos, os métodos de docking molecular exploram as conformações de ligantes adotadas nos locais de ligação aos alvos macromoleculares. Esta abordagem também estima a energia livre de ligação ligante-receptor, avaliando fenômenos críticos envolvidos no processo de reconhecimento intermolecular (FERREIRA et al., 2015).

Os avanços nos anos tornaram viável o uso de algoritmos intensivos em computação para triagem virtual de alta produtividade e triagem virtual inversa de interações moleculares. A primeira envolve o acoplamento de muitos ligantes contra um ou alguns receptores, enquanto que o segundo acopla muitos receptores contra um ou alguns ligantes. Uma combinação de algoritmos de identificação de poses e pontuação constituem a base dos mecanismos dos estudos de ancoragem. Os resultados de ancoragem molecular são avaliados por inspeção visual das poses do ligante ou quantitativamente usando um algoritmo de pontuação (JACOB; ANDERSEN; MCDOUGAL, 2012).

O software GOLD é um dos programas mais usados e adequados em vários estudos de ancoragem. É um programa flexível baseado em algoritmos genéticos para explorar a gama completa de flexibilidade conformacional do ligante com flexibilidade

parcial da proteína e satisfaz o requisito fundamental de que o ligante deve deslocar a água na ligação (JONES, 1997).

1.4 TOPOISOMERASE-II-α E TUBULINA

As topoisomerases são enzimas nucleares ubíquas que controlam o enovelamento do DNA. São essenciais durante a transcrição e a replicação e os inibidores da topoisomerase estão entre os fármacos anticancerígenos e antibacterianos mais eficazes e mais comumente usados, que se ligam e bloqueiam a atividade dessa enzima, seguindo-se a inibição da replicação do DNA (POMMIER, 2013; SADEGHI, 2015).

Os inibidores de Topoisomerase são eficazes, mas com limitada seletividade ao tumor. Seus efeitos colaterais e toxicidades limitantes da dose são devidas a toxicidade a células sadias, que, como as células cancerosas, precisam da topoisomerases para manutenção de sua homeostase. Os fármacos anticancerígenos topisomerase II alfa (TOP2A) minimiza os efeitos adversos de drogas não seletivas, como a leucemia mielóide aguda relacionada à terapia (T-AML) e cardiotoxicidade (RHAO. 2013, POMIER 2013).

Os microtúbulos são tambem importante alvo para terapias contra o câncer devido ao seu importante papel no citoesqueleto em células eucarióticas e suas funções na manutenção da forma celular, tráfico de proteínas, sinalização e segregação de cromossomos durante a mitose. Os agentes de segmentação de microtúbulos funcionam interferindo na dinâmica de controle do equilíbrio entre a montagem e desmontagem dos microtúbulos. Foram identificados quatro principais locais de ligação para esses agentes: o sitio do taxano e o sitio do laulimalídeo / peloreto A, para agentes de estabilização. E o local da vinca e da colchicina para agentes desestabilizadores de microtúbulos (DA et al., 2013; LI et al., 2017).

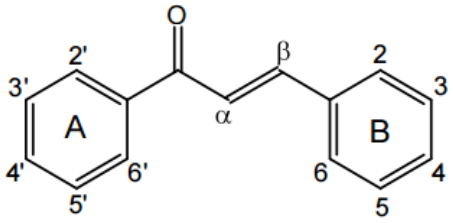
Os microtúbulos são importantes reguladores das células endoteliais, por isso há interesse em desenvolver agentes inibidores do sitio da colchicina para o tratamento do câncer, recentemente intensificou-se estudos com foco em inibição da angiogênese (prevenção da formação de novos vasos sanguíneos) e agentes desruptores vasculares (eliminação da vasculatura existente). Em particular, a família combretastatina estão progredindo através de ensaios clínicos para esse fim. Espera-se que novos agentes para o sitio da colchicina possam contornar a superexpressão da tuberculina βIII que compromete o uso clínico de taxanos e alcalóides da vinca (DA et al., 2013).

1.5 CHALCONAS

A maioria dos fármacos anticâncer introduzidas na terapêutica nas últimas décadas são originados ou derivados de produtos naturais (vimcristina, vindesina, paclitaxel), mostrando que essa fonte é muito importante nos estudos de desenvolvimento de novos medicamentos (BRANDÃO et al., 2010; LEITE; OLIVEIRA-FILHO, 2014).

Uma das classes mais estudadas de moléculas são as chalconas, que são cetonas α,β-insaturadas, com dois anéis aromáticos (figura - 2). O esqueleto molecular é caracterizado por porções de anéis aromáticos conectadas através de ponte de três carbonos com um grupo cetocarbonilo e uma α,β-insaturação. As chalconas são uma classe de flavonoides intermediários que apresenta importância farmacológica devido à sua presença em muitos compostos farmacêuticos (ARRUDA et al., 2016; CUSTODIO et al., 2017).

Figura 3 – Estrutura Fundamental das Chalconas



Fonte: CUSTODIO et al., 2017.

As chalconas e os derivados de chalconas são muitas vezes obtidos a partir de fontes naturais ou sintéticas. Devido à sua estrutura, estas moléculas são muito versáteis, pois podem conter diferentes anéis aromáticos, fundidos ou não, com heteroátomos na sua estrutura e diferentes substituintes. A reação de Claisen-Schmidt, que é a condensação de uma cetona aromática com um aldeído aromático na presença de um catalisador, é o método mais utilizado para a síntese de chalconas (RITTER et al., 2015; CUSTODIO et al., 2017).

Muitos estudos foram realizados com esta classe e uma gama de atividades foram relatadas, tais como anti-inflamatório, antifúngico, antibacteriano, leishimanicida, antiparasitário, antituberculoso, atividade antioxidante, antiulcerosos, citotóxicos, insecticidas e anticancerígenos (CUSTODIO et al., 2017).

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar a atividade antineoplásica de chalconas sintéticas utilizando modelos de glioblastomas *in vitro* e docking molecular.

2.2 OBJETIVOS ESPECIFICOS

a) Investigar a ação citotóxica de chalconas sintéticas frente a linhagem sadia AN27;
b) Investigar a atividade citotóxica de chalconas sintéticas em linhagens de glioblastoma (U87 e AHOL1);
c) Estudo das interações entre chalconas sintéticas e o alvo Topoisomerase-II-α;
d) Estudo das interações entre chalconas sintéticas e o alvo tubulina;

3 ARTIGO 1

Submetido para publicação no European Journal of Medicinal Chemistry.

EVALUATION OF ANTINEOPLASTIC ACTIVITY OF SYNTHETIC CHALCONES USING IN VITRO GLIOBLASTOMA AND MOLECULAR DOCKING MODELS FOR TOPOISOMERASE-II-α AND TUBULIN

Raimundo de Almeida Mira Neto[a*], Shayanne Vanessa Correa Henriques[a], Cleydson Breno Rodrigues dos Santos[b], Leonardo Bruno Federico[c], Edivaldo Herculano Corrêa de Oliveira[d], Michel Platini Caldas de Souza[d], Patrícia Natália B. da Silva[e], Irlon M. Ferreira[e], Madson Ralide Fonseca Gomes*[a].

Post graduate program in Pharmaceutical Sciences. Clinical Laboratory. Federal University of Amapá, Macapá, Brazil.[a]

Modeling and Chemistry Laboratory, Federal University of Amapá, Macapá, Brazil.[b]

Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of Sao Paulo, Ribeirão Preto, Brazil.[c]

Laboratory of Tissue Culture and Cytogenetics, SAMAM, Evandro Chagas Institute, Ananindeua, Para, Brazil.[d]

Group Biocatalysis and Biotransformation Group in Organic Chemistry, Collegiate of Chemistry Federal University of Amapá, Macapá, Brazil.[e]

ABSTRACT

Glioblastoma is a malignant tumor with a low patient survival rate and its incidence is increasing. The number of studies aiming at developing new anticancer drugs have increased, and predicting the mode of anchoring promising molecules to the site of interest is paramount in rational drug design. This study evaluated the antineoplastic activity of synthetic chalcones using in vitro glioblastoma models and proposed molecular docking at the site of these molecules' action. The MTT assay showed that AHOL1 and U87 cellular viability was significantly reduced compared to healthy AN27 cells when exposed to chalcones ($p<0.0001$). Comparative analysis of molecular interactions with the topoisomerase II-α target identified interactions with serine (SER148–149) and isoleucine (ILE125). Interaction with the serine amino acid was present in both the most promising and the reference binder docking, suggesting its importance inhibiting cell growth. Comparative analysis between the reference ligands and the molecules in this study showed that the amino acid LYS 352 present in all fittings, suggesting that this is the main amino acid for interaction with tubulin. It was also observed that the absence of interaction with the amino acid CYS 241 causes a reduction in the Gold score. The results obtained in molecular docking are consistent with those observed in MTT, suggesting that the molecules under study have antineoplastic potential in glioblastoma.

KEY WORDS: chalconas; cytotoxicity; molecular docking; topoisomerase II alpha; tubulin;

1. Introduction

Cancer is a disease that is caused by genomic changes, especially somatic cell mutations. Cancer development is driven by the accumulation of changes that affect genomic structure and function. These changes are often acquired as a result of exposure to multiple carcinogenic agents, affecting the functioning of the proteins encoded by the

altered genes and the whole circuit that controls cell growth, replicative potential, survival, and stress response. These processes include oxidative damage, errors in the action of polymerases and recombinases, and reduction and chromosomal rearrangement [1-4]. Adult brain tumors of glial origin are divided into astrocytomas, oligodendrogliomas, and oligoastrocytomas based on several key histopathological features. Diffuse, low-grade astrocytomas follow a long clinical course if detected early; however, the more severe Grade III gliomas carry a 5-year survival of 29.4%, and glioblastomas carry a 5-year survival of only 3.4% [5,6].

Glioblastoma is a malignant tumor with low survival rate, and it has rapid growth and a high turnover rate. The tumor overcomes its metabolic need for neovascularization, but neovascularization fails to keep up with its rapid growth, causing foci of necrosis that can unite and form a large area of central necrosis. This is because pathological vessels do not have an adequate blood-brain barrier and are associated with capillary leakage. The tumor interacts with adjacent brain tissue, causing relatively extensive edema [7,8].

The number of studies aimed at the development of new anticancer drugs have been increasing, with the objective of developing more effective and selective treatments. Significant advances in cancer biology have made it possible to direct the search for molecules that act with specific mechanisms, such as inhibition of tubulin (TUB) polymerization, DNA activation, enzymatic blockers, or cellular microtubules [9]. In vitro and in silico methods have become a valuable option to screen for new molecules. The advantages of these methods are the delimitation of the analyzed variables, reduced test duration, greater number of repetitions and require less quantity of the substance under analysis [10-12].

Predicting the mode of anchoring small molecules to macromolecules that have a known three-dimensional structure is a main problem in the rational design of drugs. Sequencing the human genome also generated an increase in the number of new

therapeutic targets for research. Additionally, high-throughput crystallographic and nuclear magnetic resonance methods were developed and these contributed to a more detailed understanding of protein and protein-ligand complex atomic structure, and molecular coupling studies are used as a tool to develop new drugs [13-15].

Therefore, the aim of this study is to evaluate the antineoplastic activity of synthetic chalcones using in vitro glioblastoma models and to use molecular docking to suggest the mechanism of action for these molecules.

2. Materials and methods

2.1 Chemicals

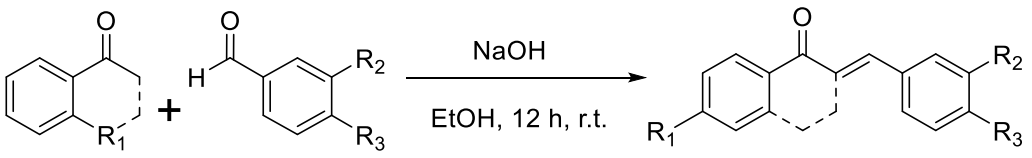
Acetophenone 1a (99%), 4-hidroxiacetophenone 1b (99%), cyclohexanone 1c (99%), benzaldehyde 2a (99%), 3-bromobenzaldehyde 2c (99%), 4-methylbenzaldehyde 2b (99%) and 3-nitrobenzaldehyde 2e (99%) were purchased from Sigma-Aldrich, 4-anisaldehyde 2b (98%) from Vetec and sodium hydroxide (97%) and hydrochloric acid (37%) from Quemis.

2.2 Spectroscopic Analysis

For gas chromatography–mass spectrometry, a Shimadzu GC2010 Plus gas chromatography system coupled to a mass-selective detector (Shimadzu MS2010 Plus) in electron ionization mode (70 eV) was used. ^{1}H NMR spectra were recorded on an Agilent Technologies 500/54 Premium Shielded or Agilent Technologies 400/54 Premium Shielded spectrometer, with $CDCl_3$ as the solvent and TMS as the internal standard unless otherwise noted. The chemical shifts are given in ppm and coupling constants (*J*) in Hz.

2.3 Preparation of the chalcones 3a–e

In two-necked round-bottomed flasks, mixtures of one of the ketones 1a–c (10 mmol), benzaldehydes 2a–e (11 mmol) and anhydrous EtOH (50 mL) were prepared. Each solution was stirred at room temperature for 5 min, after which 5 mL of NaOH was added (6 $molL^{-1}$). The reaction was stirred at room temperature for 12 h, and then stopped by adding HCl (5 mL, 10%), yielding a yellow precipitate. The precipitate was filtered off and recrystallized from EtOH. The products (3a–f) were obtained in good yields and identified by comparing spectroscopic data (1H NMR, GC–MS) with data in the literature Ferreira et. al. 2014. [16]



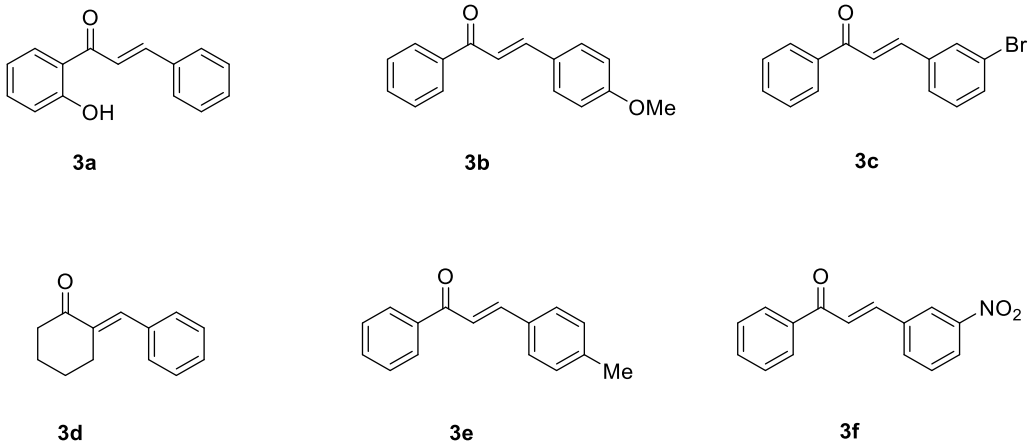
1

1a- R_1=OH
1b- R_1=H
1c- cyclohexanone

2

2a- R_2=H, R_3=H
2b- R_2=H, R_3=OCH_3
2c- R_2=H, R_3=Br
2d- R_2=R_3=H
2e- R_2=H, R_3=Me
2f- R_2=NO_2, R_3=H

3a-f



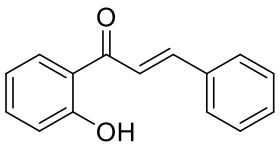
3a **3b** **3c**

3d **3e** **3f**

Figure 1 - Molecular structure of the synthesized chalcones

2.4 Spectral Data

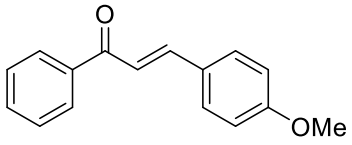
(E)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one (3a)



3a

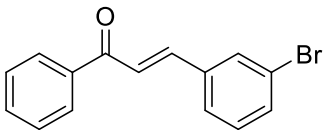
C15H12O2; 224 g.mol^{-1}; yellow solid; mp = 192 °C; ^{1}H NMR (500 MHz, Chloroform) δ 8.23 (d, *J* = 15.2 Hz, 1H), 7.63 – 7.54 (m, 4H), 7.45 – 7.38 (m, 3H), 7.34 (td, *J* = 7.5, 1.4 Hz, 1H), 7.01 (td, *J* = 7.5, 1.4 Hz, 1H), 6.94 (dd, *J* = 7.5, 1.4 Hz, 1H), 3.97 (s, 1H).

(E)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (3b)



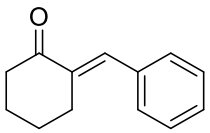
$C_{16}H_{14}O_2$; 238 g.mol^{-1}; yellow solid; mp = 77 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.90 (s, 3H), 6.97-7.01 (m, 2H), 7.39-7.44 (m, 3H), 7.55 (d, *J* = 15 Hz, 1H), 7.62-7.67 (m, 2H), 7.83-7.81 (d, *J* = 15.6 Hz, 1H); MS (EI, 70 eV) *m/z* = 238, 223, 161, 133, 108, 77.

(E)-3-(3-bromophenyl)-1-phenylprop-2-en-1-one (3c)



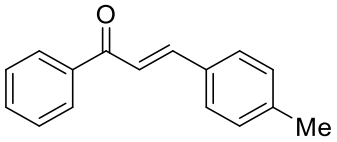
C15H11BrO; 287 g.mol^{-1}; yellow solid; mp = 154 °C; ^{1}H NMR (500 MHz, Chloroform) δ 8.24 (d, *J* = 15.0 Hz, 1H), 7.83 – 7.74 (m, 3H), 7.59 (ddd, *J* = 9.8, 8.3, 7.5 Hz, 2H), 7.56 – 7.40 (m, 4H), 7.31 (t, *J* = 7.4 Hz, 1H).

(E)-2-benzylidenecyclohexan-1-one (3d)



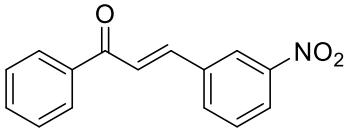
$C_{14}H_{16}O$; 200 $g.mol^{-1}$; yellow solid; mp= 192 °C; ^1H NMR (500 MHz, Chloroform) δ 7.50 (dt, *J* = 4.4, 2.3 Hz, 1H), 7.43 – 7.34 (m, 2H), 2.95 (t, *J* = 6.1 Hz, 1H), 2.40 (td, *J* = 5.5, 0.7 Hz, 1H), 1.62 (dp, *J* = 36.1, 5.5 Hz, 2H).

(E)-1-phenyl-3-(p-tolyl)prop-2-en-1-one (3e)



$C_{16}H_{14}O$; 222 $g.mol^{-1}$; yellow solid; mp = 107 °C; ^1H NMR (500 MHz, Chloroform) δ 8.22 (d, *J* = 15.2 Hz, 1H), 7.77 (dd, *J* = 7.5, 1.4 Hz, 2H), 7.59 (d, *J* = 15.2 Hz, 1H), 7.57 – 7.40 (m, 5H), 7.29 (d, *J* = 7.5 Hz, 2H), 2.36 (s, 3H).

(E)-3-(3-nitrophenyl)-1-phenylprop-2-en-1-one (3f)



$C_{15}H_{11}NO_2$; 253 $g.mol^{-1}$; yellow solid; mp = 108-109 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.55 (t, *J* = 7.5 Hz, 1H), 7.63 (dd, *J* = 7.7 Hz and 3.2 Hz, 1H), 7.67 (d, *J* = 15.6 Hz, 1H), 7.85 (d, *J* = 15.6 Hz, 1H), 7.94 (d, *J* = 7.7 Hz, 1H), 8.0 (s, 2H); MS (EI, 70 eV) *m/z* = 253, 236, 206, 178, 105, 77.

2.5 Determination of cytotoxicity using the MTT assay

AN27, AHOL1, and U87 cells were seeded at 0.7 × 10^5 cells/mL in 96-well plates. After 24 h, the tested compounds were added and incubated for 72 h at 37°C in a humid atmosphere containing 5% CO_2. After incubation, the medium was changed to a fresh medium containing 150 µL MTT (0.5 mg/mL) and incubated for 3 h. The plates were centrifuged again, the supernatants discarded, and the precipitate resuspended in 150 µL DMSO. To quantify Formazan using viable cells, the absorbances were read at 595 nm. A negative control was used when 0.5% DMSO medium (solvent) was added to the cells. Cell viability was expressed as the percentage of control cells and was calculated using the following equation: % CV = At / Ac × 100 equation, where % CV is cell viability in percent, At and Ac are the absorbances of treated and untreated cells, respectively, after subtracting the absorbance from MTT [17,18].

2.6 Molecular docking

Topoisomerase II alpha (TOPIIA) and tubulina (TUB) structures obtained using X-ray diffraction were downloaded from Protein Data Bank (PDB) with PDB codes 1ZXM (*Homo sapiens*) and 4O2B (*Rattus norvegicus*, *Bos taurus*. and *Gallus gallus*) with resolution of 1.87 and 2.3 Â, respectively [19-20]. The ligands used in the molecular docking study were six synthetic chalcones designed and pre-optimized in MM+ in the ACD / ChemSketch program and optimized in the HyperChem program using the semi-empirical AM1 method [21].

The protein/linker complex obtained in the PDB was inserted into the Gold program to validate the method, with **the** Phosphoaminophosphonic Acid-Adenylate Ester (ANP) structure bound to TOPIIA (*H. sapiens*, PDB 1ZXM) and the TUB bound colchicine structure (*R. norvegicus*, PDB 402B) and these were used as standard parameters by the genetic algorithm [22,23]. Docking studies for TOPIIA and TUB that targeted the

complexed test molecules were performed and visualization of the distances from the ligand to target interactions were performed using the Discovery Studio v17.2.0.16349 software.

2.7 Statistical analysis

The results were expressed as the mean and standard deviation, and data were analyzed using a two-way ANOVA, followed by Bonferroni's test. The inhibitory concentration to 50% of the sample (IC_{50}) was calculated using sigmoidal regression in a dose-dependent curve. The 95% confidence interval and $p<0.05$ were considered in all analyzes, using Graph Pad Prism 5.0 software.

3. Results and discussion

3.1. Cytotoxicity of MTT assay

The MTT test was the first widely accepted method to evaluate cytotoxicity and cell viability. The assay consists of the colorimetric determination performed on microtiter plates, where the absorbance measurements were obtained at the end of the test. Its main assumption depends on tetrazolium as an indicator of the potential for intracellular reduction, which, in turn, assesses overall cellular status and viability [24].

The strains AHOL1 and U87 were selected to evaluate the cytotoxic effect in neoplastic glioblastoma lines, compared to the AN27 line of healthy human fibroblasts. Cell viability was expressed as the percentage of viable cells relative to control. The main assumption depends on tetrazolium salt as an indicator of the intracellular reduction potential, which assesses the cell status and global viability. The enzymatic reduction of tetrazolium by cytosolic dehydrogenases and reducing agents results in the formation of water insoluble violet blue formazan products [24].

Chalcones (1,3-diaryl-2-propen-1-one) are natural intermediates of flavonoid compounds. The core structure of chalcones consists of two aromatic rings where are connected by a three carbon α, β-unsaturated carbonyl bridge. They are important compounds that show biological activity and have anticancer potential [25].

The MTT assay results showed that AHOL1 and U87 cells had reduced cell viability when exposed to molecule 3a, with an IC50 of 56.97 mg/mL and 67.96 mg/mL, respectively, and at a concentration of 75 mg/mL at ($p<0.0001$) compared to the AN27 lineage that did not show reduced viability (Figure 2A). For the second molecule (3b), viability reduction was also observed in AHOL1, with an IC50 of 36.32 mg/mL and U87 with an IC50 of 50.07 mg/mL. The AN27 line was not changed, showing a statistical difference compared to the other strains at concentrations of 50, 75, and 100 mg/mL ($p<0.0001$, Figure 2B).

Molecule 3c also promoted a reduction in the cellular viability of AHOL1 and U87, with an IC50 of 33.95 mg/mL and 50.15 mg/mL, respectively, which was significant compared with AN27 ($p<0.001$) at concentrations of 25, 50–75, and 100 mg/mL (Figure 2C). Molecule 3e reduced viability, with an IC50 27.43 mg/mL and 54.23 mg/mL for the AHOL1 and U87 lines, respectively, which was statistically significant at concentrations of 50, 75, and 100 mg/mL compared to strain AN27 (Figure 2D).

The MTT showed that molecule 3f was effective in reducing the cell viability of the three strains studied at concentrations above 25 mg/mL, and there was only a significant difference at concentrations of 7.5 and 10 mg/mL ($p<0.0001$) between AN27 and the glioblastoma lines AHOL1 and U87 (Figure 2F). The molecule 3d was not effective to reduce the cell viability of the studied strains (figure 2D).

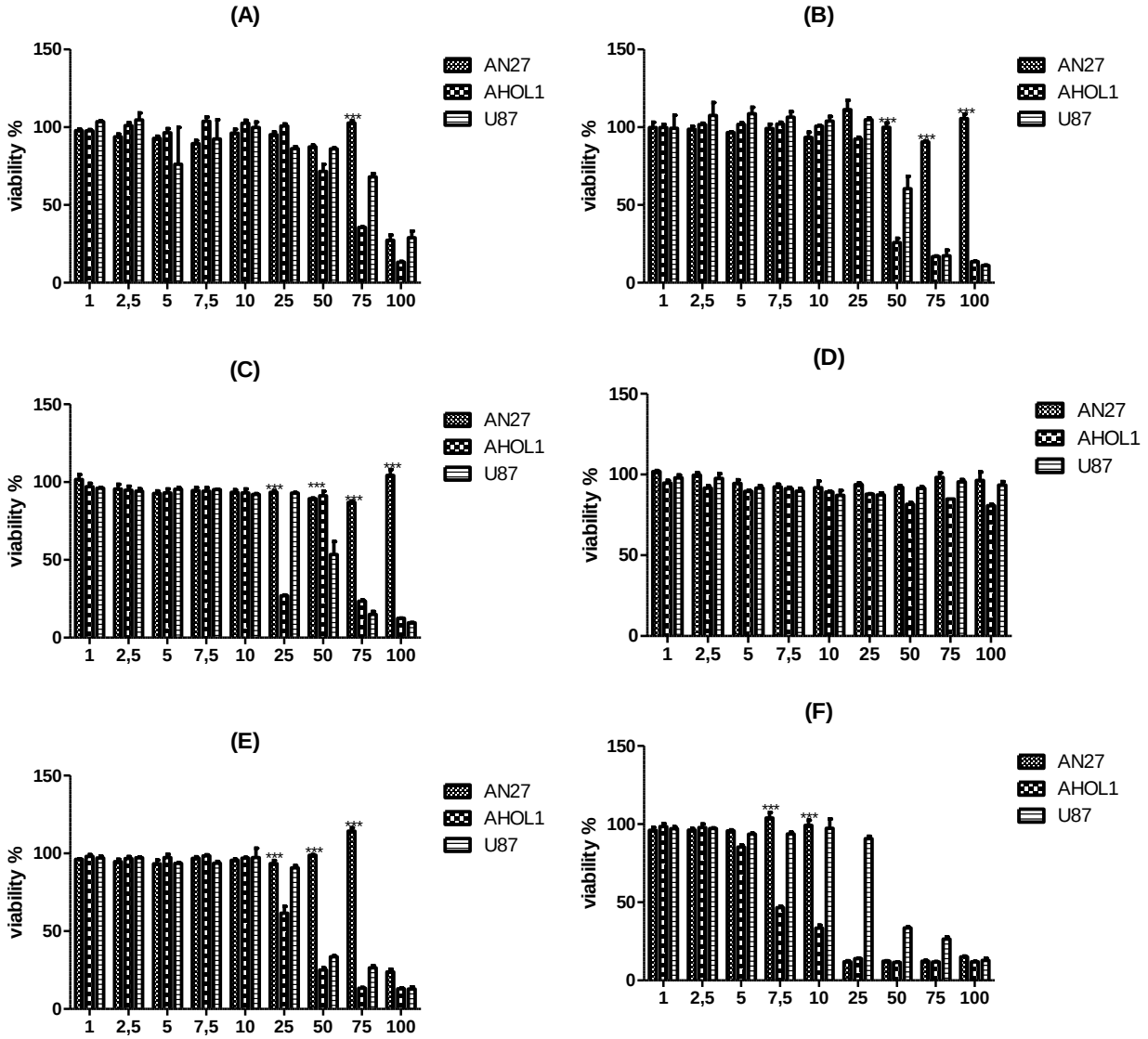


Figure 2 - Cell viability analysis of AN27, AHOL1 and U87 by the MTT assay. (A) 3a. (B) 3b. (C) 3c. (D) 3d. (E) 3e. (F) 3f. Two-way ANOVA; post-test of Bonferroni. *** p <0.0001.

3.2. Molecular docking study

To propose a mechanism of action for chalcones that had an effect on the reduction of cell viability using MMT, a molecular docking study was performed for TOPIIA and TUB targets, using the Gold software, which is one of the most frequently used and suitable programs for anchorage studies. It is a flexible program based on genetic algorithms to explore the full range of binder conformational flexibility with partial flexibility of the protein and it meets the fundamental requirement that the binder should displace the water during binding [13].

To validate the anchoring method, ANP and colchicine structures with crystallographic information were subjected to redocking until the results obtained by the software were similar to crystallographic information. Comparison between ANP and colchicine (green) crystallographic ligands and those resulting from redocking (red) are shown in Figure 4. The RMSD values obtained were 1.0706 and 0.3313, respectively, which is within the recommended range, as described below [26]. Thus, redocking reproduced the experimental linking modes, validating the ligand docking.

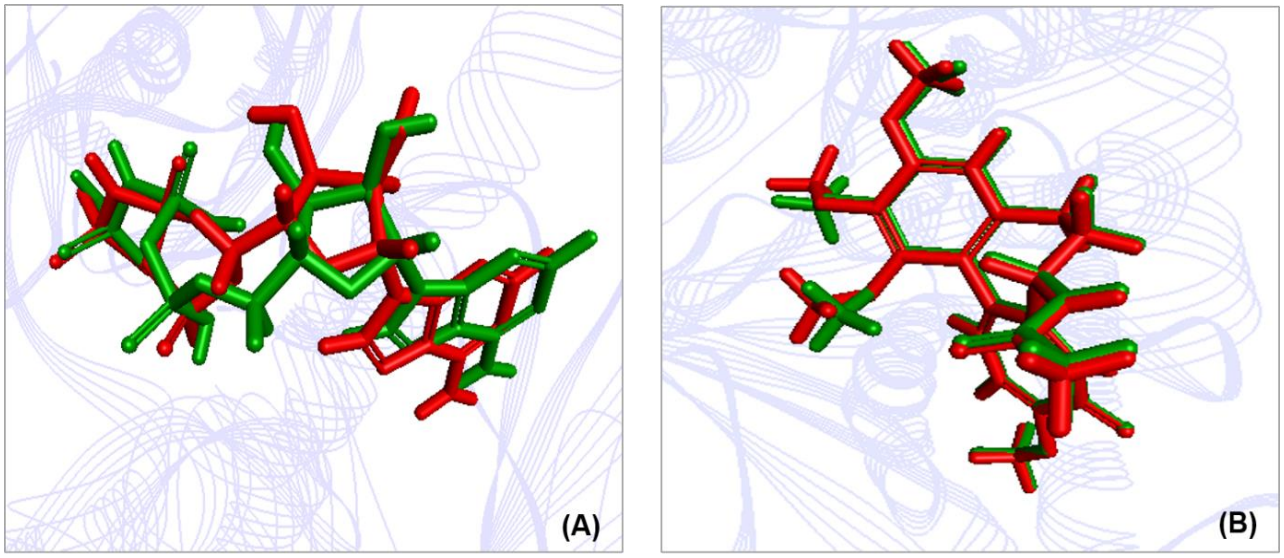


Figure 3 - Comparison between the crystallographic binder (green) and the best conformation obtained by molecular docking (red). (A) ANP and (B) Colchicine.

After the method was validated, molecular docking was used to predict the affinity of five synthetic chalcones with the targets under study. The docking method identified a conformation that allowed interaction with the ANP active sites (PDB 1ZXM) that occur between amino acid residues 87–95, 137–143, and 162–169 in the alpha helix, and between amino acid residues 116–125, 177–183, and 214–219 for the β leaf. The interaction sites for colchicine (PDB 402B) occur between amino acid residues 238–243 and 252–259 in the alpha helix, and between amino acid residues 135–137, 167–202, 314–318, 351–354, and 377–381 for the β sheet.

The redocking analysis for ANP identified hydrogen bonding with amino acids ASN 91, ASN 120, SER 148, ASN 163, TYR 165, GLY 166, and LYS 168. Pi-amide stacking

occurred at ALA 92, carbo-hydrogen bonding at ASN 95 and GLY 164, Pi-alkyl bonding was present at ILE 125, and acceptor and donor interaction occurred at SER 149 and ARG 162, respectively (Figure 4).

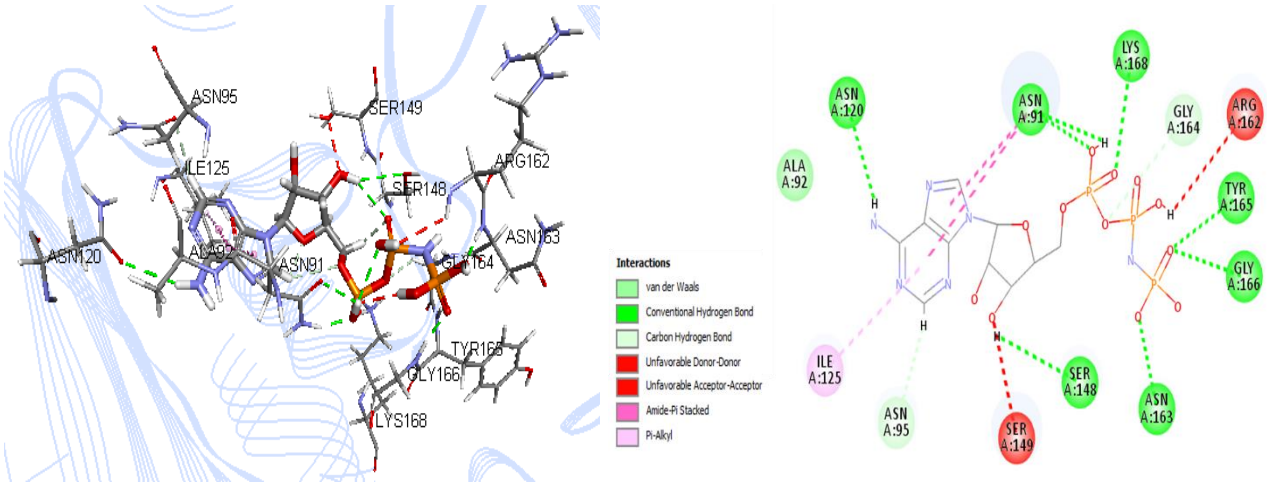


Figure 4 - Molecular redocking of ANP and interactions with Topoisomerase II-α residues

The redocking analysis for colchicine and tubulin revealed four Pi-alkyl interactions at CYS 241, LEU 255, ALA 316, and LYS 352. Additionally, two hydrogen-carbon bond-type interactions that occur at amino acids ASN 258 and VAL 315; an alkyl interaction at LEU 255; and a Pi-sulfur at the MET 259 inlet, totaling eight interactions with the molecular target (Figure 5).

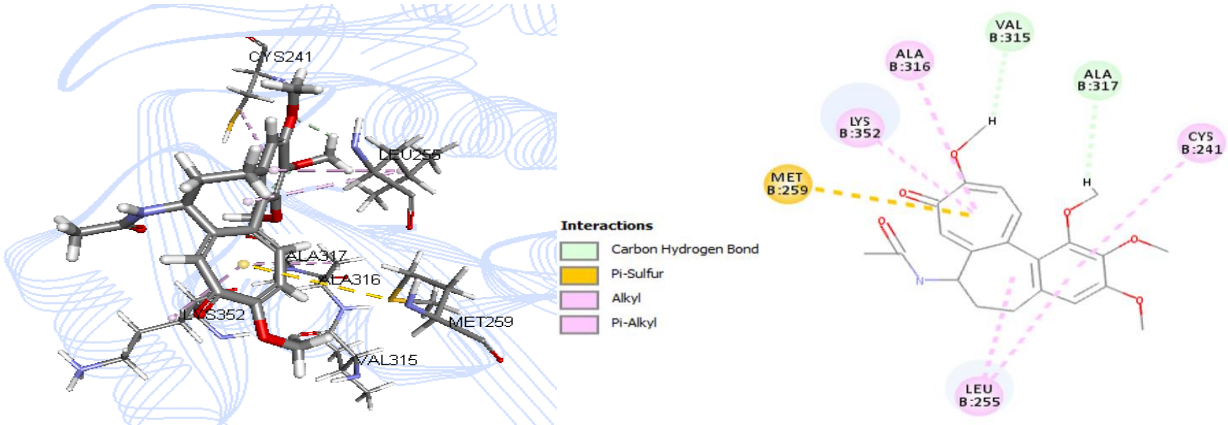


Figure 5 - Colchicine molecular docking and 2D diagram of the interactions with Tubulin residues.

3.2.1. Molecular docking for topoisomerase II-α

Topoisomerases are nuclear enzymes that control DNA folding and are essential during transcription and replication, and topoisomerase inhibitors are among the most

commonly used and effective anti-cancer and antibacterial drugs that bind and block the activity of that enzyme, followed by inhibition of DNA replication [27,28].

Analysis of doxorubicin (Figure 6) indicated the presence of three hydrogen bonds, two at ASN 91 and one at ASN 120; and two Pi-alkyl interactions at ILE 125, one at VAL 137, and two at ILE 141. There is also a hydrogen bond-carbon at SER 148, an alkyl interaction at ALA 167, and a donor-donor bond at LYS 168, totaling 11 interactions with the receptor.

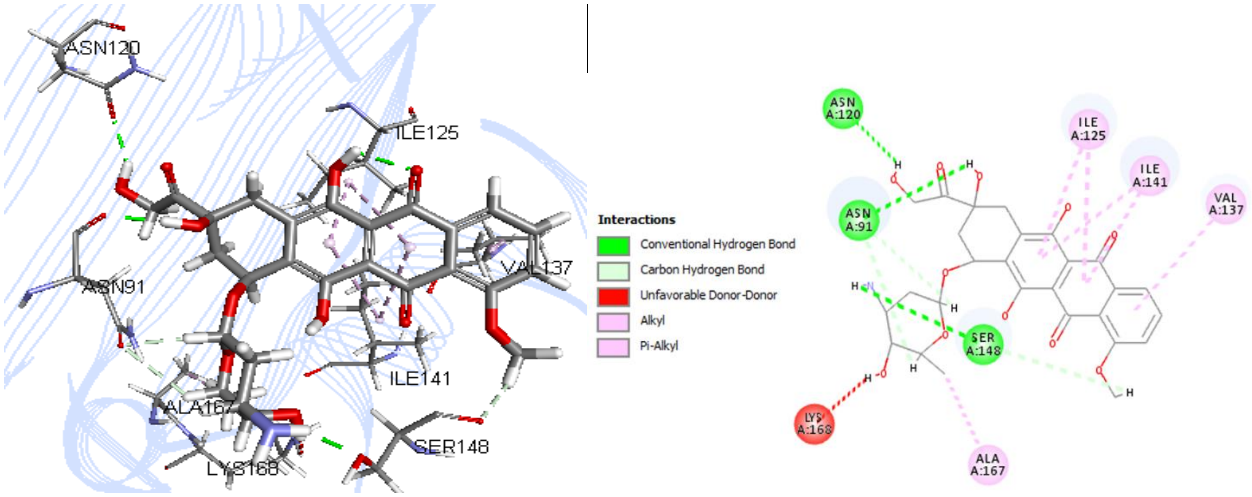


Figure 6 - Molecular docking and 2D diagram of doxorubicin and Topoisomerase II-α binding pocket interactions

For molecule 3a, two interactions with the active site were identified: a hydrogen-carbon bond at SER 148 and a hydrogen interaction at SER 149. Molecule 3b also presented two target sites with a hydrophobic pi-alkyl at ILE 125, and a hydrogen bond at SER 149. Analysis of molecule 3c showed two interactions per carbon-hydrogen bond at SER 148 and SER 149, but in the amino acid SER 148, there was an unfavorable acceptor-acceptor interaction, a Pi-alkyl at PHE 142, and an alkyl interaction with the ILE 217 amino acid, totaling five interactions with the target (Figures 7 and 8).

Were seven interactions between molecule 3d and the target under study. Two Amide-Pi Stacked interactions in ASN 91 and ALA 92, two Pi-alkyl interactions in ILE 125 and PHE 142, two alkyl interactions in ILE 142 and ALA 157, and a carbon-hydrogen bond

in SER 149. Molecule 3e showed a carbon-hydrogen bond at the amino acid SER 148, and a hydrogen bond at SER 149, totaling two interactions with the molecular target (Figure 11). Molecule 3f analysis revealed two charge-load interactions in GLU 87, and four hydrogen bonds at ARG 162, ASN 163, TYR 165, and GLY 166, totaling six interactions with TOPIIA (Figures 7 and 8).

Comparative analysis of the molecular interactions with the target identified two repeated interactions in serine (SER148–149) and isoleucine (ILE125). The amino acid SER was present in the interactions of the ANP ligand with TOPIIA in doxorubicin and molecules of chalcones a,b, c, e, and f, suggesting its fundamental importance in the inhibitory effect on cell growth. Additionally, molecule 3f in the MTT test showed the lowest selectivity between healthy and neoplastic cells, suggesting the importance of the amino acid SER in selectivity for TOPIIA and neoplastic cells.

The drug doxorubicin targets TOPIIA and topoisomerase 2 beta (TOPIIB), and is associated with several adverse effects and cardiotoxicity. TOPIIβ is present in most tissues, including tumors, and TOPIIα is absent in cardiac tissue; thus, selective antineoplastic drugs for TOPIIA present more selectivity for tumoral tissue and lower cardiotoxicity [28].

Topoisomerase inhibitors are effective, but they have limited tumor selectivity. Their side effects and dose-limiting toxicities cause toxicity to healthy cells, which, like cancer cells, need topoisomerases to maintain their homeostasis. TOPIIA anticancer drugs minimize the adverse effects of non-selective drugs, such as therapy-related acute myeloid leukemia (T-AML) and cardiotoxicity [27,29].

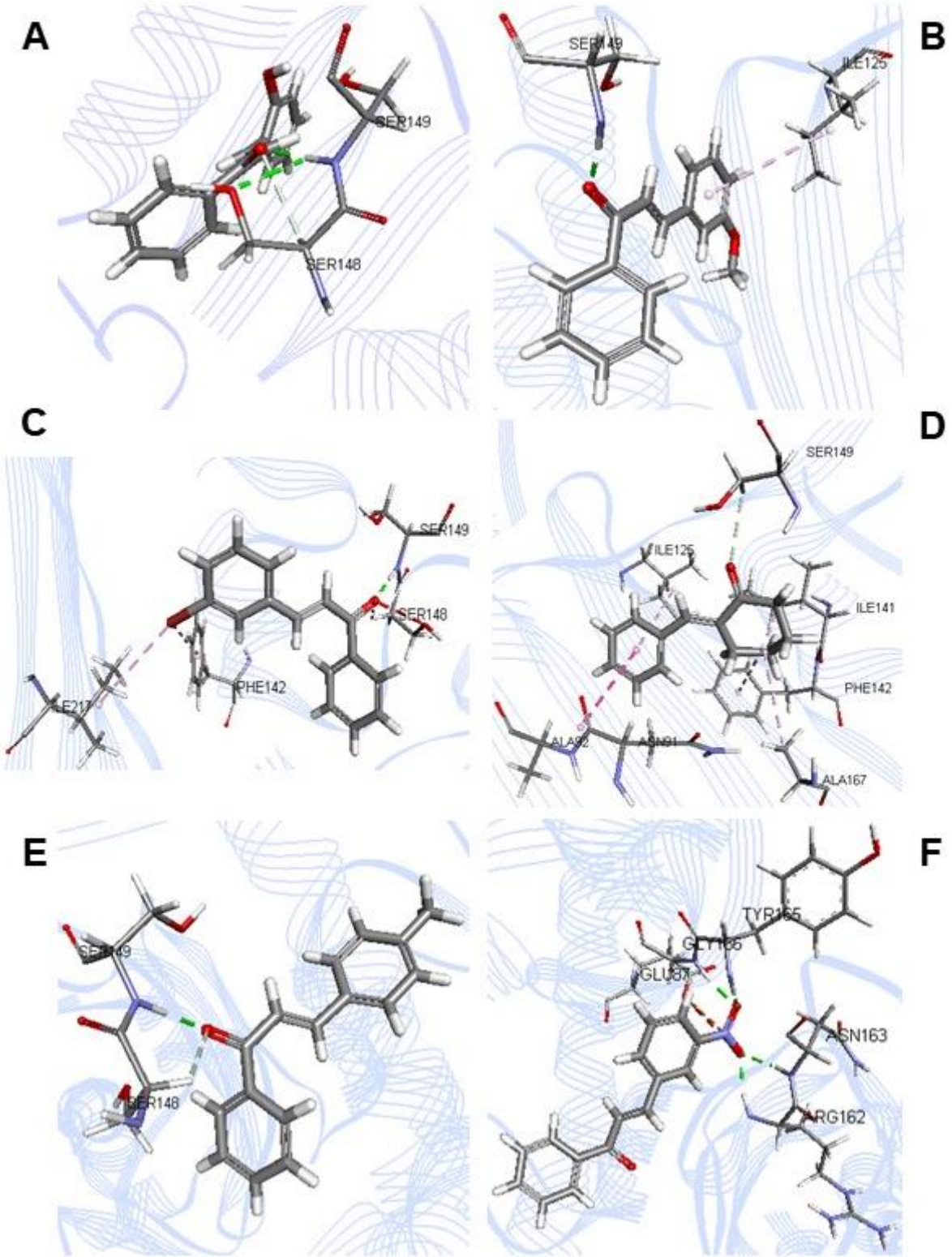


Figure 7 - Molecular docking and interactions with Topoisomerase II-α residues. (A) 3a. (B) 3b. (C) 3c. (D) 3d. (E) 3e. (F) 3f.

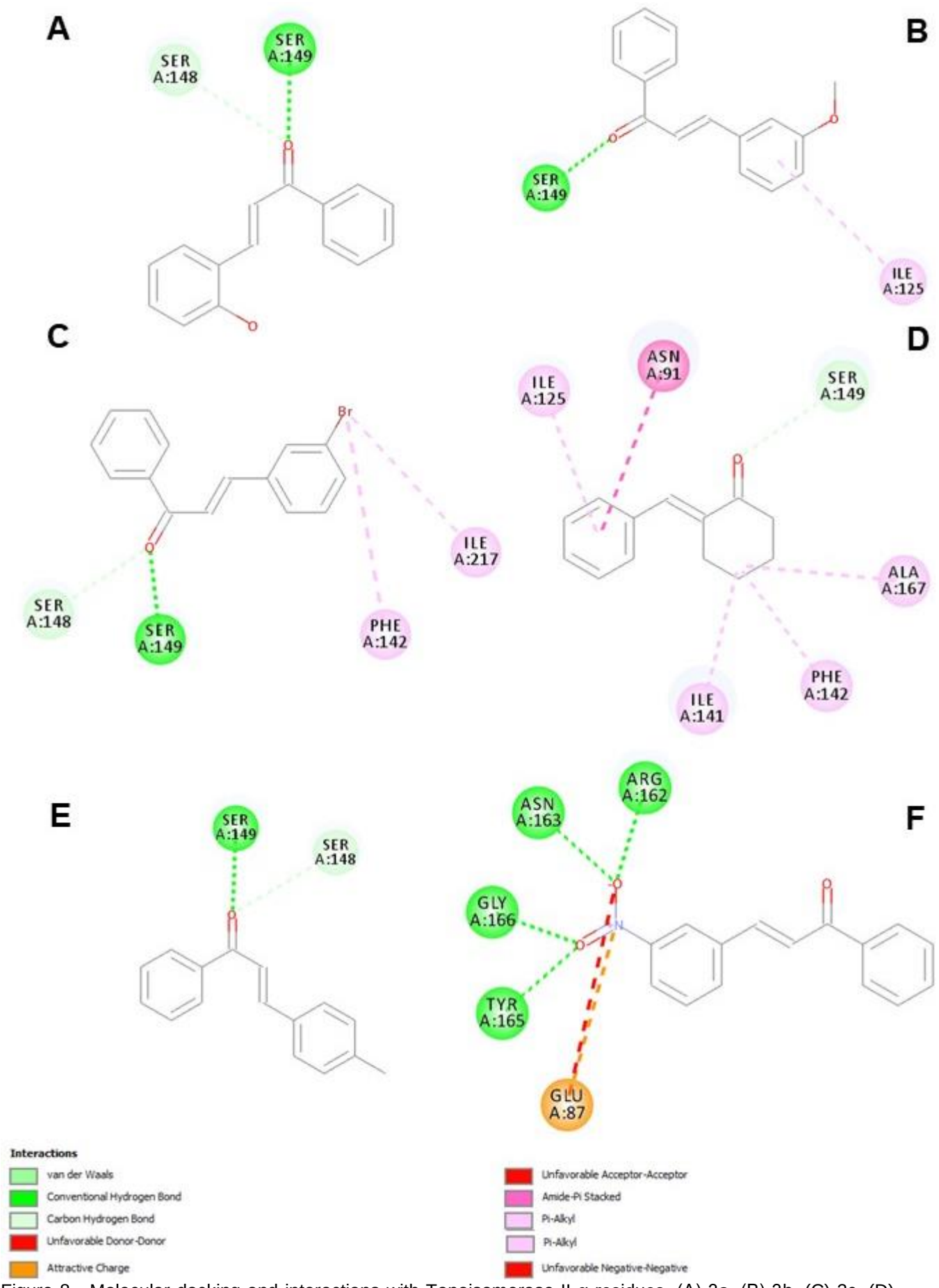


Figure 8 - Molecular docking and interactions with Topoisomerase II-α residues. (A) 3a. (B) 3b. (C) 3c. (D) 3d. (E) 3e. (F) 3f.

Table 2 - Distances, types of interaction and gold score between binders and Topoisomera II alpha

Structure	Aminoacides	Distance (Â)	Interaction Type	Gold Score
Phosphoaminophosphonic Acid-Adenylate Ester	ASN 91	1.75	Hydrogen bond	76.2451
	ASN 91	2.41	Hydrogen bond	
	ALA 92	4.82	Amide-Pi stacked	
	ALA 92	5.30	Amide-Pi stacked	
	ASN 95	2.60	Hydrogen-carbon bond	
	ASN 120	2.69	Hydrogen bond	
	ILE 125	5.35	Pi-alkyl	
	SER 148	2.88	Hydrogen bond	
	SER 149	2.60	Acceptor-acceptor	
	ARG 162	2.65	Donor-donor	
	ASN 163	1.79	Hydrogen bond	
	GLY 164	2.94	Hydrogen-carbon bond	
	TYR 165	1.91	Hydrogen bond	
	GLY 166	1.74	Hydrogen bond	
	LYS 168	2.10	Hydrogen bond	
Doxorrubicina	ASN 91	2.14	Hydrogen bond	73.6892
	ASN 91	2.96	Hydrogen bond	
	ASN 120	2.01	Hydrogen bond	
	ILE 125	4.45	Pi-alkyl	
	ILE 125	4.96	Pi-alkyl	
	VAL137	5.00	Pi-alkyl	
	ILE 141	4.78	Pi-alkyl	
	ILE 141	5.01	Pi-alkyl	
	SER 148	2.43	Hydrogen-carbon bond	
	ALA 167	3.21	Alkyl	
	LYS 168	2.62	Donor-Donor	
3a	SER 148	3.03	Hydrogen-carbon bond	52.7719
	SER 149	1.83	Hydrogen bond	
3b	ILE 125	5.17	Pi-alkyl hydrophobic	54.7246
	SER 149	1.80	Hydrogen bond	
3c	PHE 142	4.79	Pi-alkyl	54.9591
	SER 148	2.88	Unfavorable acceptor	
	SER 148	3.02	Hydrogen-carbon bond	
	SER 149	1.85	Hydrogen-carbon bond	
	ILE 217	4.92	Alkyl	
3d	ASN 91	4.87	Amide-Pi stacked	42.7550
	ALA 92	4.87	Amide-Pi stacked	
	ILE 125	5.31	Pi-alkyl	
	ILE 142	3.98	Alkyl	
	PHE 142	4.65	Pi-alkyl	
	SER 149	3.03	Hydrogen-carbon bond	
	ALA 167	4.53	Alkyl	
3e	SER 148	3.07	Hydrogen-carbon bond	55.6680
	SER 149	4.90	Hydrogen bonding	

3f	GLU 87 GLU 87 ARG 162 ASN 163 TYR 165 GLY 166	4.73 5.02 1.92 2.04 2.02 1.74	Attractive Charge Unfavorable negative-negative Hydrogen bond Hydrogen bond Hydrogen bond Hydrogen bond	65.3370

3.2.2. Molecular docking for tubulin

Microtubules are formed by the association of α- and β-TUB heterodimers and serve as important components of the cytoskeleton in eukaryotic cells. They are important in the process of cell division, which makes them an important target for anticancer drugs. Target molecules in the microtubules can act by stabilizing or destabilizing, interfering in their dynamics, which can lead to mitotic block and cellular apoptosis. Four major binding sites for these agents have been identified: the taxane site and the laulimalide site for stabilizing agents, and the vinca and colchicine sites for microtubule destabilizing agents [30,31].

Doxorubicin analysis identified six interactions: four conventional bonds, three with LYS 254 and one with THR 353; one alkyl linkage with LYS 352; and one carbon-hydroxyl linkage with amino acid LYS 254 (Figure 9).

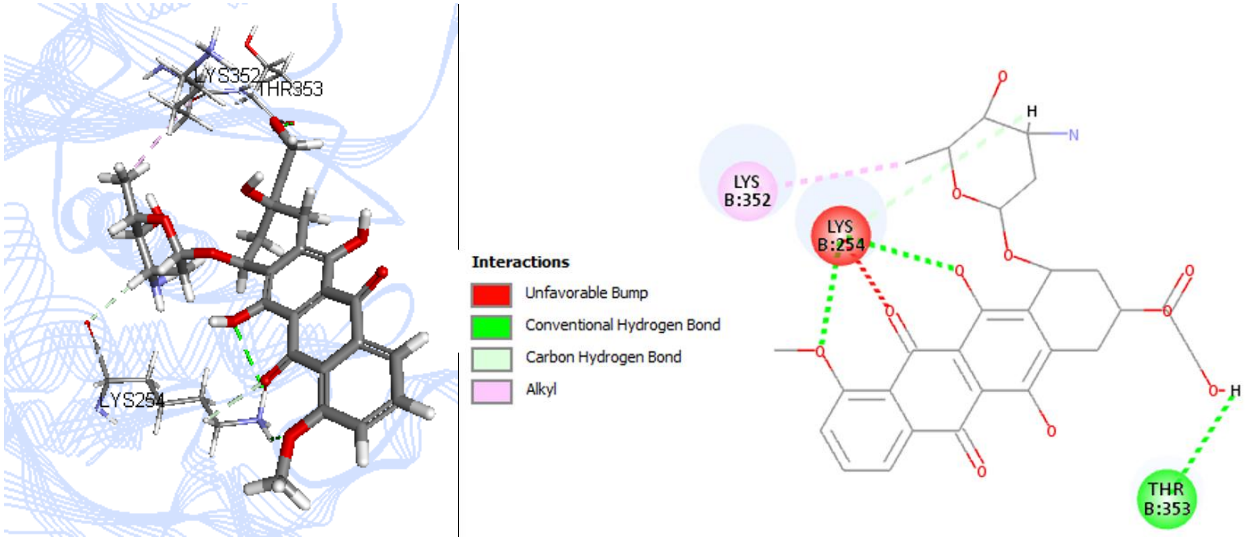


Figure 9 - Molecular docking and 2D diagram of doxorubicin and Tubulin binding pocket interactions

Molecule 3a showed five interactions with the tubulin site, three Pi-alkyl interactions with LEU 242, LEU 255, and LYS 352; a Pi-sulfur bond with MET 259; and a Pi-sigma bond with CYS 241. For molecule 3b, there were four Pi-alkyl interactions at ALA 316, LYS 352, LYS 352, and ALA 354; and a LYS 254 carbon-hydrogen bond, totaling five interactions. molecule 3c showed three PI-alkyl interactions at CYS 241, LEU 255, and LYS 352, two alkyl interactions at CYS 241 and ILE 318; an amide-Pi stacked ASN 258 interaction; and a Pi-sulfur bond MET 259 (Figures 10 and 11).

Three Pi-alkyl interactions were identified between molecule 3d and tubulin site in amino acids CYS 241, LEU 242 and LEU 255, and three alkyl interactions in LEU 255, ALA 316 and LYS 352. Analysis of the docking between molecule 3e and tubulin showed two alkyl interactions at CYS 241 and LEU 242, four Pi-alkyl bonds at MET 259, ALA 316, CYS 241, and LYS 352; a Pi-sigma bond at LEU 255; and a hydrogen bond at ASN 258. Molecule 3f showed two Pi-alkyl interactions at LYS 352, and two hydrogen bonds at ASN 258 and THR 353 (Figures 10 and 11).

Comparative analysis of the colchicine binder; doxorubicin, which is used clinically; and the molecules in this study identified the amino acid LYS 352 that was present at all interactions. This suggests that LYS 352 is the main amino acid that interacts with tubulin, and a second amino acid, CYS 241, may also be related to the reduction in the Gold score for interactions in which it is not present (doxorubicin, molecule 3b, and molecule 3f).

The microtubule plays an important role in fundamental cellular processes such as cell division; maintaining cellular integrity; and regulating motility, cell signaling, cellular secretion, and intracellular transport. Therefore, inhibition of microtubule function seems to be a strong approach for anticancer therapy. The unique feature of microtubule binding agents in contrast to other anticancer drug categories is their complexity and structural diversity, which offers many possibilities for scaffold optimization and design [32].

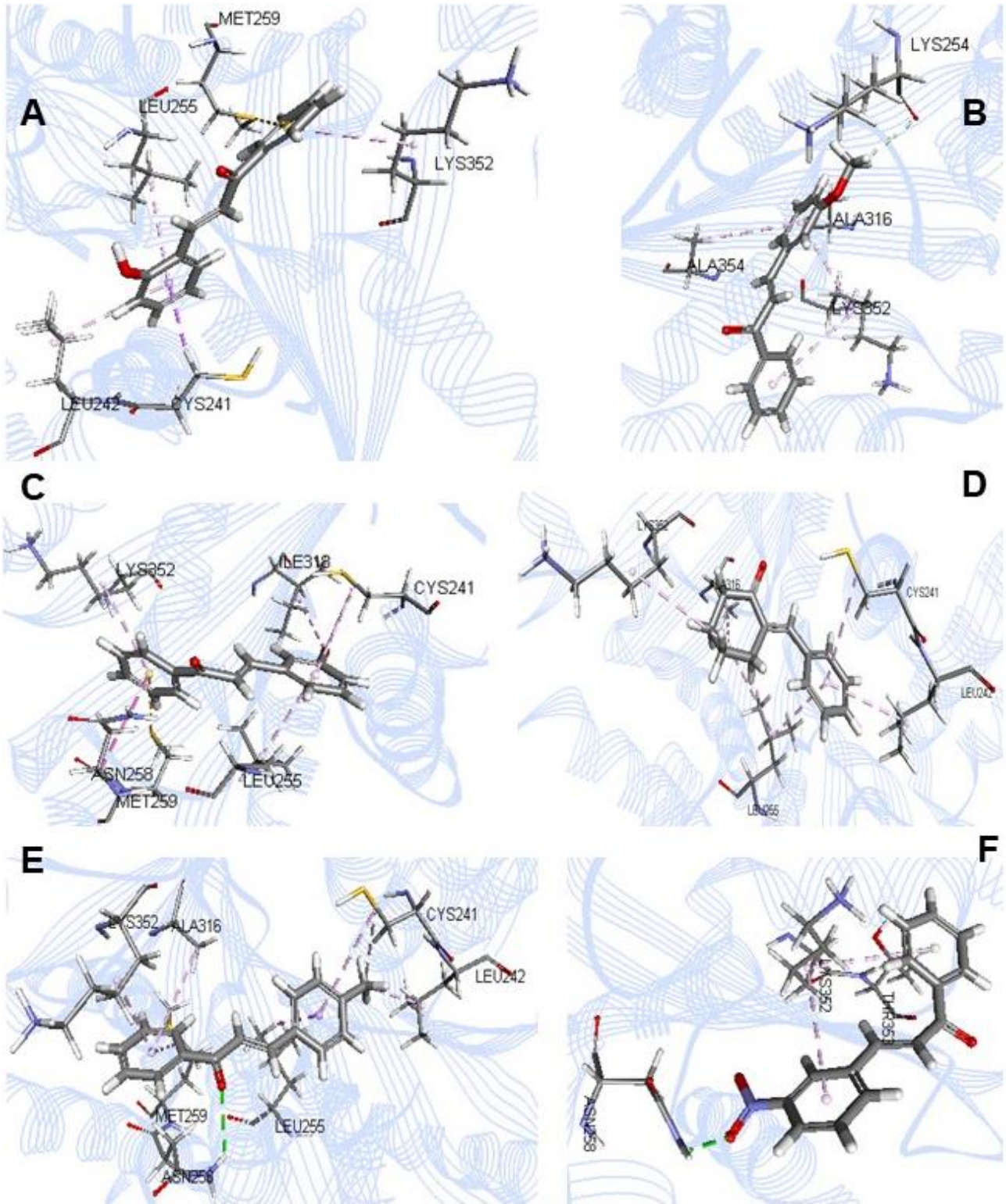


Figure 10 - Molecular docking and interactions with Tubulin residues. (A) 3a. (B) 3b. (C) 3c. (D) 3d. (E) 3e. (F) 3f.

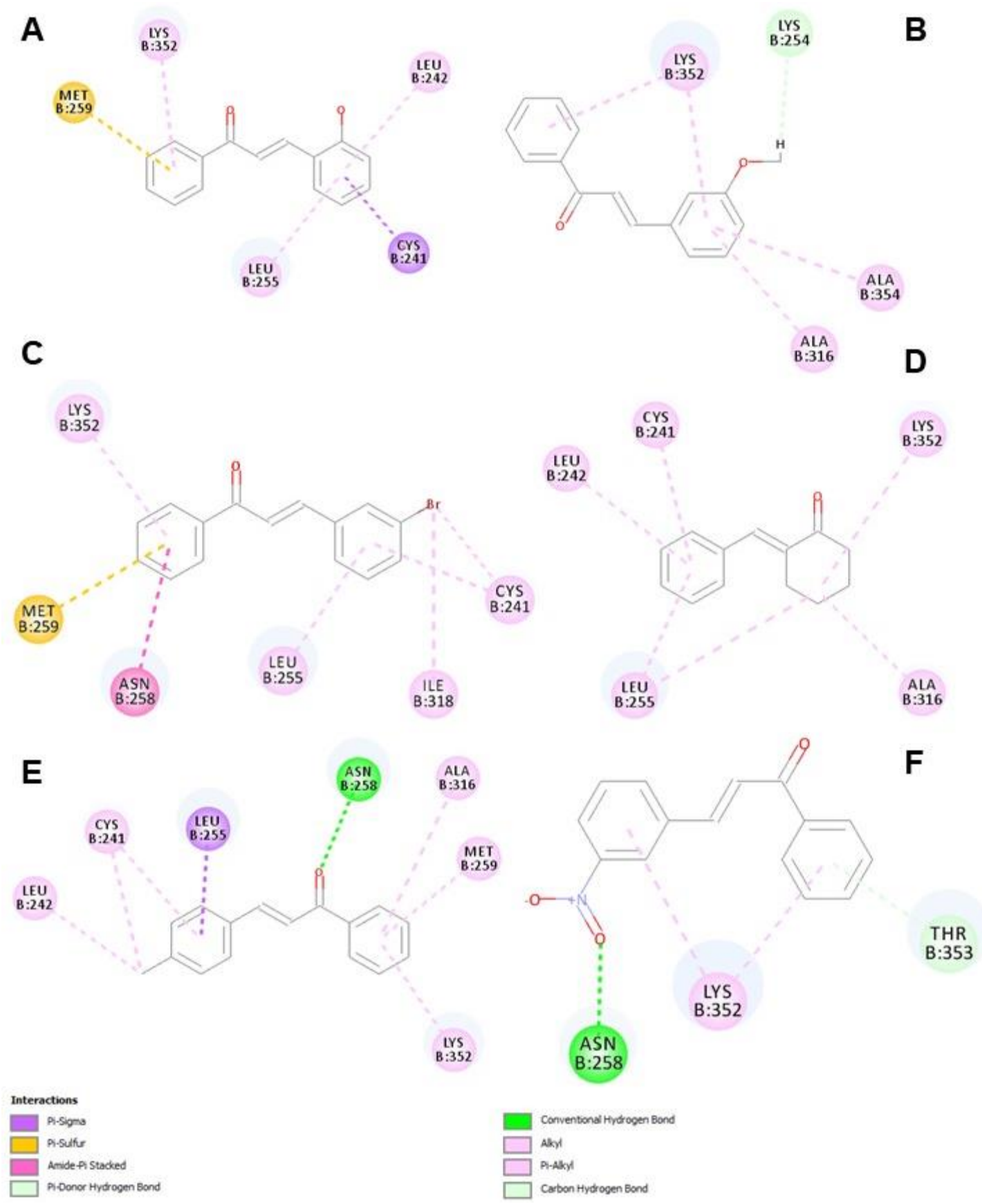


Figure 11 - Diagram 2D of the interactions with Tubulin residues. (A) 3a. (B) 3b. (C) 3c. (D) 3d. (E) 3e. (F) 3f.

Table 3 - Distances, types of interaction and gold score between binders and TUB

Structure	Aminoacides	Distance (Â)	Interaction Type	Gold Score
Colchicina	CYS 241	5.50	Pi-Alkyl	80.0201
	LEU 255	4.21	Pi-Alkyl	
	LEU 255	5.00	Alkyl	
	ASN 258	3.04	Hydrogen-carbon bond	
	MET 259	5.23	Pi-Sulfur	
	VAL 315	2.49	Hydrogen-carbon bond	
	ALA 316	5.45	Pi-Alkyl	
	LYS 352	4.20	Pi-Alkyl	
Doxorrubicina	LYS 254	1.47	Conventional Connection	54.0183
	LYS 254	2.78	Conventional	
	LYS 254	2.59	Hydrogen-carbon bond	
	LYS 254	2.81	Conventional	
	LYS 352	3.91	Alkyl	
	THR3 353	2.02	Conventional	
3a	CYS 241	2.80	Pi-Sigma	57.6683
	LEU 242	5.15	Pi-Alkyl	
	LEU 255	4.43	Pi-Alkyl	
	MET 259	5.13	Pi-Sulfur	
	LYS 352	4.65	Pi-Alkyl	
3b	LYS 254	2.63	Hydrogen-carbon bond	52.9918
	ALA 316	5.10	Pi-Alkyl	
	LYS 352	4.80	Pi-Alkyl	
	LYS 352	4.95	Pi-Alkyl	
	ALA 354	5.15	Pi-Alkyl	
3c	CYS 241	3.55	Alkyl	59.1093
	CYS 241	4.58	Pi-Alkyl	
	LEU 255	4.20	Pi-Alkyl	
	ASN 258	4.74	Amide-Pi stacked	
	MET 259	4.96	Pi-Sulfur	
	ILE 318	4.35	Alkyl	
	LYS 352	4.81	Pi-alkyl	
3d	CYS 241	4.45	Pi-Alkyl	43.5491
	LEU 242	4.81	Pi-Alkyl	
	LEU 255	5.35	Alkyl	
	LEU 255	4.35	Pi-Alkyl	
	ALA 316	4.56	Alkyl	
	LYS 352	5.40	Alkyl	
3e	CYS 241	4.10	Alkyl	59.2652
	CYS 241	4.91	Pi-alkyl	
	LEU 242	3.74	Alkyl	
	LEU 255	2.68	Pi-sigma	
	ASN 258	2.80	Hydrogen bond	
	MET 259	4.99	Pi-Alkyl	
	ALA 316	5.42	Pi-Alkyl	
	LYS 352	4.09	Pi-Alkyl	
3f	ASN 258	2.11	Hydrogen bond	53.8388
	LYS 352	4.87	Pi-Alkyl	
	LYS 352	4.97	Pi-Alkyl	
	THR 353	2.78	Pi-Donor Hydrogen bond	

Conclusions

The antineoplastic activity of the six molecules was tested using AHOL1 and U87 glioblastoma lines, and a significant reduction of cell viability was observed compared with the AN27 healthy lineage. MTT results are consistent with predictions of molecular docking, where the molecules under analysis coupled to the target in a manner similar to doxorubicin and to the ligand that was coupled to the PDB. The amino acids serine (SER148–149) and isoleucine (ILE125) are potentially the most important for interaction with TOPIIA. For TUB, the amino acid LYS 352 is present in all the moieties, suggesting that LYS 352 is the main amino acid that interacts with the target. Additionally, the interactions occur mainly by alkylation of these amino acids. More studies are required.

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5 CONSIDERAÇÕES FINAIS E PERSPECTIVAS

- Ocorreu redução da viabilidade celular das linhagens de glioblastoma AHOL1 e U87, expostas as chalconas, quando comparadas a viabilidade celular da linhagem AN27 saudável;

- Os resultados do MTT são consistentes com as previsões de docking molecular, onde as moléculas analisadas se acoplaram ao alvo de maneira similar à doxorrubicina e ao ligante que estava acoplado ao PDB;

- Os aminoácidos serina (SER148–149) e isoleucina (ILE125) são potencialmente os mais importantes para interação com TOPIIA;

- Para TUB, o aminoácido LYS 352 está presente em todas as frações, sugerindo que o LYS 352 é o principal aminoácido que interage com o alvo. Além disso, as interações ocorrem principalmente por alquilação desses aminoácidos. Mais estudos são necessários;

- São necessários estudos complementares *in silico* e *in vivo*, para garantir efetividade e segurança do uso dessas moléculas. Além de, melhor elucidar os mecanismos envolvidos na ação antineoplásica.

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Anexo 1 – Normas de publicação dos respectivos periódicos

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