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Synthesis, characterization and tubulin-interaction profile of substituted cinnamoyl urea derivatives

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ABSTRACT

Substituted cinnamoyl ureas have been identified as novel compounds with their various biological activities. The novel cinnamoyl ureas were synthesized successfully by various substitutions, at the various position, which further were evaluated for their tubulin inhibitor activity.Thus in this research work, we aimed to use all these active moieties with urea and phenyl urea substitutions at carbonyl moiety. Molecular docking study was used for confirming their interaction with tubulin protein taking MSE137, ACO 201 and MSE148 as tubulin molecule for their antitumor activity. Through molecular docking study, the result showed that all the synthesized compounds act by inhibiting cell mitosis by binding to the protein tubulin in the mitotic spindle and preventing polymerization or depolymerization into the microtubules . Among the synthesized compounds 3a,3b, 3d showed higher no of interaction with amino acids of tubulin molecule , thus they were considered as good antitumor agents.

Keywords: Tubulin, Microtubules, Depolymerization, Antitumor, Molecular docking study,

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INTRODUCTION

Tubulin inhibitors are drugs that interfere directly with the tubulin system, which is in contrast to those drugs acting on DNA for cancer chemotherapy. Tubulin (*tubul- + -in*) in molecular biology can refer either to the tubulin protein superfamily of globular proteins, or one of the member proteins of that superfamily [1] . The tubulin superfamily contains six families of tubulins (alpha-, beta-, gamma-, delta-, epsilon and zeta-tubulins). Tubulin is also used to specifically refer to α-tubulin and β-tubulin, the proteins that make up microtubules in eukaryotic cells. Each has a molecular weight of approximately 50,000 Daltons [2] .

Tubulin binding drugs
Tubulin binding drugs have been classified on the basis of their mode of action and binding site [3]
I. Tubulin depolymerization inhibitors
a) **Palitaxel site ligands** - Paclitaxel, Epothilone, Docetaxel, Discodermolide Etc.

II. Tubulin polymerization inhibitors
a) **Colchicine binding site** - Colchicine, Combrestatin, 2-Methoxy Estradiol, Methoxy Benzenesulfonamides (E7010) Etc.

b) **Vinca alkaloids binding site** - Vinblastine, Vincristine, Vinorelbine, Vinfluine, Dolastatins, Halichondrins, Hemiasterlins, Cryptophysin 52, Etc.

Table 1 : Tubulin Inhibitors with their binding sites, therapeutic uses and stages of clinical development [4]

Classes of tubulin inhibitors	Binding domain	Related drugs or analogues	Therapeutic uses	Stage of clinical development
Polymerization inhibitors	**Vinca domain**	VINBLASTINE	Hodgkin's disease, testicular germ cell cancer	in clinical use; 22 combination trials in progress
		VINCRISTINE	Leukaemia, lymphomas	In clinical use; 108 combination trials in progress
		VINORELBINE	Solid tumours, lymphomas, lung cancer	In clinical use; 29 phase I–III clinical trials in progress (single and combination)
		VINFLUNINE	Bladder, non-small-cell lung cancer, breast cancer	Phase III
		CRYPTOPHYCIN 52	Solid tumours	Phase III finished
		HALICHONDRINS	-	Phase I
		DOLASTATINS	Potential vascular-targeting agent	Phase I; phase II completed
		HEMIASTERLINS	-	Phase I
	Colchicine domain	COLCHICINE	Non-neoplastic diseases (gout, familial mediterranean fever)	Appears to have failed trials, presumably because of toxicity
		COMBRETASTATINS	Potential vascular-targeting agent	Phase I
		2-METHOXY-ESTRADIOL	-	Phase I
		E7010	Solid tumours	Phase I, II
Depolymerization inhibitors	**Taxan site**	PACLITAXEL (TAXOL)	Ovarian, breast and lung tumours, Kaposi's sarcoma; trials with numerous other tumours	In clinical use; 207 Phase I–III trials in the United States; TL00139 is in Phase I trials
		DOCETAXEL (TAXOTERE)	Prostate, brain and lung tumours	8 trials in the United States (Phases I–III)
		EPOTHILON	Paclitaxel-resistant tumours	Phases I–III
		DISCODERMOLIDE	-	Phase I

Various Cinnamic acid derivatives have been discovered which acts as cytotoxic or microtubule destabilizing agents [5] . Most of cinnamic acid derivatives are substituted with electron donating hydroxy or methoxy groups at various positions [6]. This create the interest in the development of cinnamoyl derivatives as tubulin inhibitors, for the design and synthesis of novel antitumor agents with various substitution [7].

Thus we aimed to synthesize some novel cinnamoyl ureas by substitutions at various position. Molecular docking study was used for confirming their interaction with tubulin protein for their antitumor activity.

MATERIALS AND METHODS

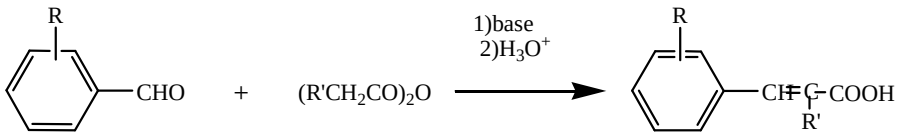
2.1 Materials and Methods

Most of the solvents used were of A. R grade and purified before use in different reactions. All the reactions were monitored on thin layer chromatography (TLC) prepared by using silica gel G, petroleum ether and ethyl acetate in various ratio were used as mobile phase.

General Route for the synthesis of substituted Cinnamoyl ureas

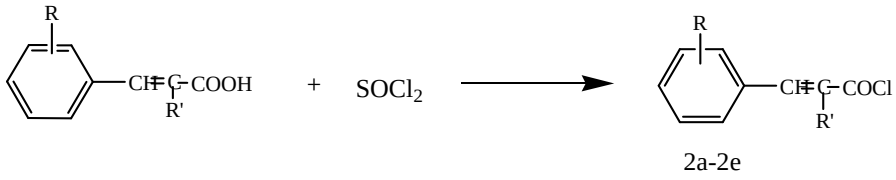
Synthesis takes place in 3 steps which were as follows:-

STEP I



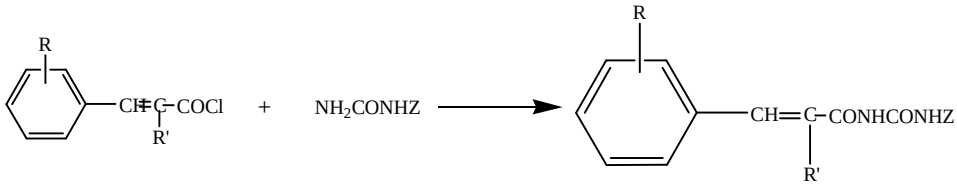
1a-1e

STEP II



2a-2e

STEP III



3a-3e

2.2.1 General Procedure for the Synthesis of Compounds (1a-1e)

Substituted benzaldehyde, propionic anhydride and freshly fused and finely powdered potassium acetate were heated in an oil bath at 160ºC for 1hr and at 180ºC for 3hrs. Mixture was then poured into 100 ml of water and steam distilled. Filtrate was acidified by conc. HCl until the evolution of carbon dioxide cease [8] . The solids so obtained were recrystallised from mixture of 3 vol. of water and 1 vol. of rectified spirit. The purity of compounds was checked by the TLC. The compounds prepared are shown in Table No-I

Table I- Physicochemical Parameters of synthesized compounds (1a-1e)

Compound code	R	R'	R_F Value	Molecular Formula	M.P (ºC)	% Yield
1a	p - NH_2	H	0.80	$C_9H_9NO_2$	110-111	78
1b	p- OH	H	0.72	$C_9H_8O_3$	132-133	66
1c	2,5 dichloro	H	0.75	$C_9H_6Cl_2O_2$	80-81	67
1d	2,4 diamino	H	0.69	$C_9H_{10}N_2O_2$	117-118	65
1e	3,5dimethoxy	CH_3	0.76	$C_{12}H_{14}O_4$	123-124	75

2.2.2 General Procedure for the Synthesis of Compounds (2a-2e)

These compounds were prepared by reacting initially formed acrylic acid derivatives with thionyl chloride. Mixture of 0.2 mol of substituted acrylic acid formed in the first step and 0.84mole of thionyl chloride was stirred under reflux until the disappearance of starting material for about 4 hrs [9]. After reaction the excess $SOCl_2$ was removed in vacuum and yellow residue was directly used for further reaction without any purification.

Stationary phase used in TLC was silica gel and mobile phase used were acetone/petroleum ether or hexane/ethyl acetate in 3:1 ratio. The compounds prepared are shown in Table No- II

Table II- Physicochemical Parameters of synthesized compounds (2a-2e)

Compound name	R	R'	R_F Value	Molecular Formula	M.P.(ºC)	% Yield
2a	p - NH_2	H	0.82	C_9H_8ClNO	127-128	69
2b	p- OH	H	0.83	$C_9H_7ClO_2$	137-138	63
2c	2,5 dichloro	H	0.78	$C_9H_5Cl_3O$	97-98	71
2d	2,4 diamino	H	0.61	$C_9H_9ClN_2O$	121-122	70
2e	3,5dimethoxy	CH_3	0.80	$C_{12}H_{13}ClO_3$	125-126	73

2.2.3 General Procedure for the Synthesis of Compounds (3a-3e)

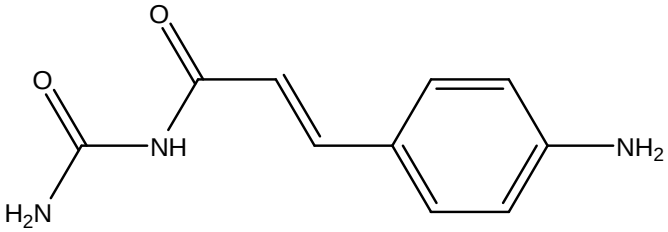
Acyl ureas were prepared by reacting various cinnamoyl chloride derivatives with urea. Commercially available urea was used for the reaction. Required amount of phenyl urea in 5% NaOH and small amount of cinnamoyl chloride prepared in previous step was added one at a time, with constant shaking and cooling in water (if necessary) until odor of cinnamoyl chloride had disappeared. It was made sure that the reaction was alkaline in nature [10]. The solid obtained was collected by filtration and washed with cold water. The product was

recrystallised from ethanol or dilute ethanol and purity of the compound was checked by TLC. The compounds thus synthesized are listed in table No- III

Table III- Physicochemical Parameters of synthesized compounds (3a-3e)

Compound name	R	R'	Z	R_F Value	Molecular Formula	M.P(°C)	%Yield
3a	p - NH_2	H	H	0.82	$C_{10}H_{11}N_3O_2$	129-130	90
3b	p- OH	H	H	0.83	$C_{10}H_{10}N_2O_3$	121-122	88
3c	2,5 dichloro	H	H	0.78	$C_{10}H_8Cl_2N_2O_2$	121-122	78
3d	2,4 diamino	H	H	0.61	$C_{10}H_{12}N_4O_2$	135-136	73
3e	3,5dimethoxy	CH_3	H	0.82	$C_{13}H_{16}N_2O_4$	129-130	81

2.2.3.1) **1-((E)-3-(4-aminophenyl)acryloyl)urea / 4- amino Cinnamoyl urea (3a)**

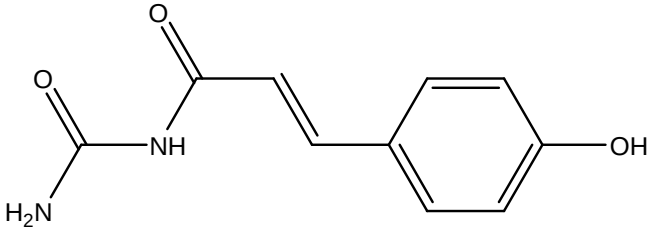


1-((*E*)-3-(4-aminophenyl)acryloyl)urea

White crystalline solid; **Molecularformula**-$C_{10}H_{11}N_3O_2$; **Yield**-90%; **M.P**-158-160°C;

IR (neat) n (cm^{-1}): 3406 , 2975, 1684 , 1627 ,1684 , 1220 ,3427 **^{1}HNMR(400MHz,$CDCl_3$)**: 9.32(1H,s), 5.58(2H,s), 3.89(2H,s) 6.98(2H,s),7.02(4H,s) **ESI-MS: *m/z*** 206 ($M+H^+$)

2.2.3.2) 1-((E)-3-(4-hydroxyphenyl)acryloyl)urea / 4- hydroxy cinnamoyl urea (3b)

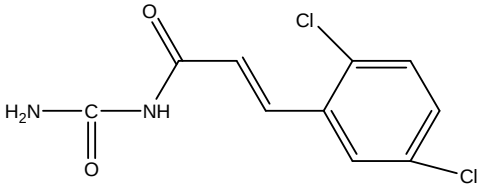


1-((*E*)-3-(4-hydroxyphenyl)acryloyl)urea

White crystalline solid; **Molecularformula**-$C_{10}H_{10}N_2O_3$; **Yield**-88%; **M.P**-155-156°C;

IR (neat) n (cm^{-1}): 3424,2963,1694, 1625 ,1694,1225,1225 **^{1}HNMR(400MHz,$CDCl_3$)**: 9.68(1H,s), 6.33(2H,s), 4.98(1H,s),7.20(4H,s), 7.53(2H,s),**ESI-MS: *m/z*** 206 ($M+H^+$)

2.2.3.3) 1-((E)-3-(2,5-dichlorophenyl)acryloyl)urea / 2,5 dichloro cinnamoyl urea (3c)

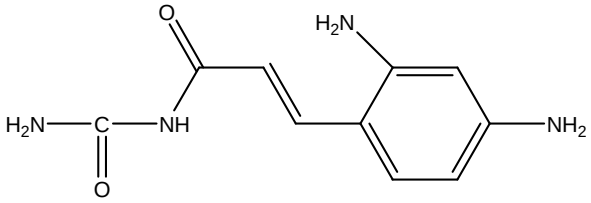


1-((*E*)-3-(2,5-dichlorophenyl)acryloyl)urea

White crystalline solid; **Molecularformula**-$C_{10}H_8Cl_2N_2O_2$; **Yield**-78%; **M.P**-148-150°C;

IR (neat) n (cm^{-1}): 3424,2963,1694,1625,1694,1225**^{1}HNMR(400MHz,CDCl$_3$)**:9.99(1H,s), 5.98(2H,s), 7.16(1H,d,J=4),7.09(1H,d,J=4) **ESI-MS: *m/z*** 258(M+H$^+$)

2.2.3.4) 1-((E)-3-(2,4-diaminophenyl)acryloyl)urea / 2,4 di-amino cinnamoyl urea (3d)

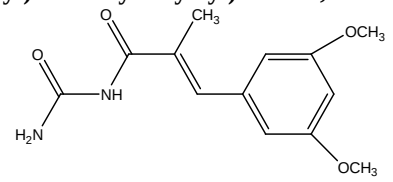


1-((*E*)-3-(2,4-diaminophenyl)acryloyl)urea

White crystalline solid; **Molecularformula**-$C_{10}H_{12}N_4O_2$; **Yield**-73%; **M.P**-167-168°C;

IR (neat) n (cm^{-1}):): 3406,2975,1684,1627,1684,1220 **^{1}HNMR(400MHz,CDCl$_3$)**:9.98(1H,s), 6.0(2H,s), 4(2H,s), 3.98(2H,s) **,**5.61-5.62(1H,m) **ESI-MS: *m/z*** 220(M+H$^+$)

2.2.3.5) 1-((E)-3-(3,5-dimethoxyphenyl)-2-methylacryloyl)urea / 3,5dimethoxy cinnamoyl urea (3e)



1-((*E*)-3-(3,5-dimethoxyphenyl)-2-methylacryloyl)urea

White crystalline solid; **Molecularformula**-$C_{13}H_{16}N_2O_4$; **Yield**-81%; **M.P**-188-189°C;

IR(neat)n(cm^1):3322,3032,1722,1624,1674,1345**^{1}HNMR(400MHz,CDCl$_3$)**:3.88(3H,s),3.98(3H,s),7.42(2H,m),9.88(1H,s),6.23(2H,s),1.99(3H,d,J=4) **ESI-MS: *m/z*** 264(M+H$^+$)

2.3 Tubulin Interaction Studies

The importance of tubulin and microtubules in chromosome segregation during cell division makes them attractive targets for anticancer drug design, i.e. in the development of anti-mitotic agents. Beneficially, the interference of tubulin / microtubule polymerization dynamics has two pivotal anticancer effects [11].

i) Inhibition of cancer cell proliferation through interruption of mitotic spindle formation, which leads to apoptosis.
ii) Disruption of cell signaling pathways involved in regulating and maintaining the cytoskeleton of endothelial cells in tumor vasculature.

The ligands were drawn in Marvin Sketch assigned with proper 2-D orientation [12] . The Auto Dock 4.0 suite molecular-docking tool was used and the methodology was followed *In silico* virtual screening of receptors is however, a daunting task, for both of the receptor based approaches (docking) and ligand based approaches. To perform the docking model, MSE137, ACO 201 and MSE148 used as tubulin reference molecule for their antitumor activity [13]. The synthesised compounds (3a-3e) were manually docked into sites of the enzymes and the docking energy was monitored to achieve a minimum value [14] .In the present study, the binding site was selected based on the amino acid residues, which are involved in binding with tubulin protein [15] .No of interactions of each synthesised molecules are noted along with bond energy.

Table IV: Docking study data showing tubulin interaction of synthesised Cinnamoyl ureas

Docking of protein (Tubulin) with ligands						
S.No	Compound Name	No of Interactions	Protein residue name	Compound residue name	Bond Length	Bond Energy
1	3a	6	Phe(118)	N(12)	2.86065	-2.5
			Ser(154)	O(14)	2.78873	-2.5
			Phe(118)	N(10)	3.25137	0.27589
			Ser(154)	O(13)	3.09957	-2.5
			Asp(151)	O(6)	3.10001	-2.49997
			Val(115)	O(6)	3.10001	-2.49994
2	3b	6	Phe(118)	N(11)	2.88065	-2.5
			Ser(154)	O(12)	3.09991	-2.5
			Phe(118)	N(9)	3.25537	0.26664
			Ser(154)	O(13)	2.79341	-2.5
			Asp(151)	N(14)	3.10011	-2.49945
			Val(115)	N(14)	3.10008	-2.49959
3	3c	4	Ser(154)	O(13)	2.81595	-2.5
			Ser(154)	O(12)	2.75591	-2.5
			Phe(118)	N(11)	2.9999	-2.5
			Ser(154)	O(13)	3.10055	-2.49726
4	3d	6	Phe(118)	N(11)	2.77452	-2.5
			Ser(154)	O(13)	2.6377	-2.5
			Ser(154)	O(12)	2.61329	-2.5
			Asp(151)	N(15)	2.97479	-2.5
			Val(115)	N(15)	2.65274	-2.5
			Ser(154)	O(13)	3.17478	-2.12609
			Phe(118)	O(12)	3.09098	-2.5
			Pro(153)	N(11)	3.48488	-0.575618
			Ser(154)	O(14)	3.4258	-0.870995
			Asp(151)	O(12)	3.10053	-1.08435
5	3e	4	Leu(138)	O(13)	3.56924	-0.1537
			Leu(138)	O(12)	3.08974	-2.5
			Phe(134)	N(11)	2.97397	-2.5
			Tyr(150)	O(16)	3.13753	-2.31235
			Phe(118)	N(11)	3.10001	-1.19571
			Phe(118)	N(9)	3.13483	-0.619163
6)	ACO201(A)	8	Arg(152)	N(39)	3.34554	-0.645553
			Arg(152)	O(36)	2.84055	-2.5
			Gln(53)	N(39)	3.57519	0.370312
			Gln(53)	O(36)	3.0643	-2.5
			Ser(49)	O(24)	2.40575	-0.881262
			Ser(154)	O(16)	3.22297	-1.88515
			Lys(156)	O(12)	1.96771	-2.88947
			Gln(125)	O(12)	2.96183	-2.32728
7)	MES(137)	5	Glu(144)	N(0)	3.07233	-2.5
			Pro(145)	N(0)	3.10014	-2.49931
			Val(115)	O(3)	3.26753	-0.75441
			Leu(113)	O(3)	2.83785	-2.5
			Ala(149)	O(3)	3.10025	-2.49876
			Ala(149)	O(3)	2.62258	-2.5
8)	MES(148)	6	Glu(144)	N(0)	3.09915	-2.5
			Pro(145)	N(0)	3.08851	-2.5
			Val(115)	O(3)	2.87091	-2.5
			Leu(113)	O(3)	3.25601	-0.79729
			Ala(149)	O(3)	3.10042	-2.4979
			Ala(149)	O(3)	2.59975	-2.49792

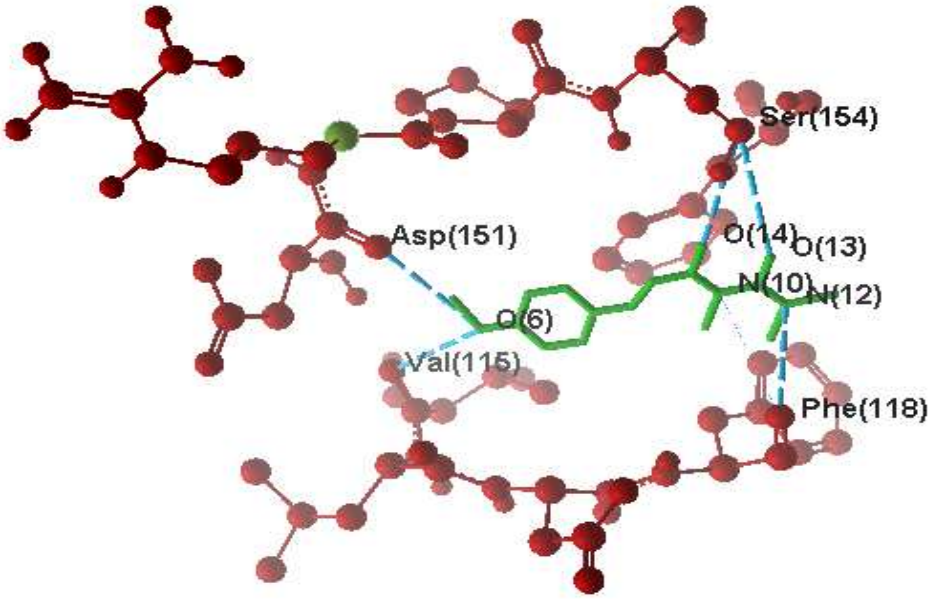


FIG.I *In silico* binding of 3a with Tubulin. Receptor contacts- Phe(118), Ser(154), Phe(118), Val(115), Asp(151)

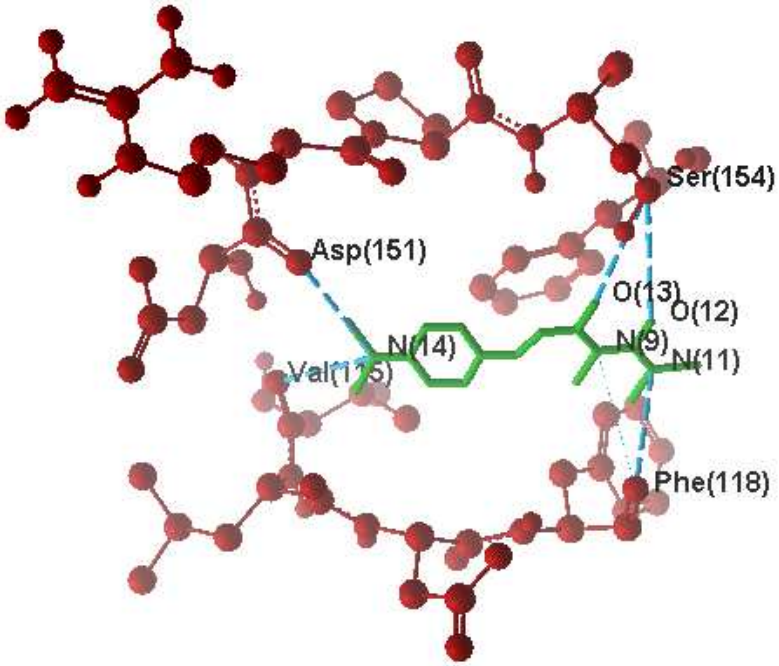


Fig. II *In silico* binding of 3b with Tubulin. Receptor contacts- Phe(118), Ser(154), Asp(151), Val(115)

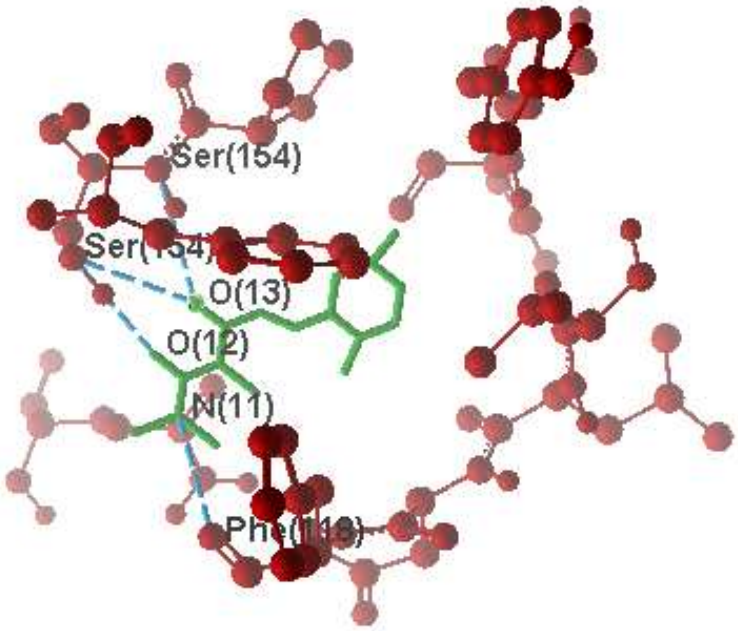


Fig. III *In silico* Binding of 3c with Tubulin. Receptor contacts- Ser(154), Phe(118))

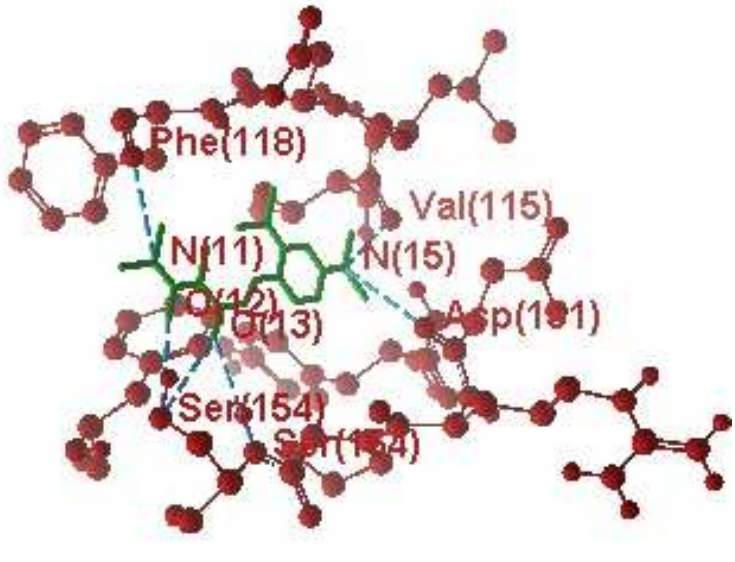


Fig. IV *In silico* Binding of 3d with Tubulin. Receptor contacts- Phe(118), Ser(154), Val(115), Pro(153), Asp(151)

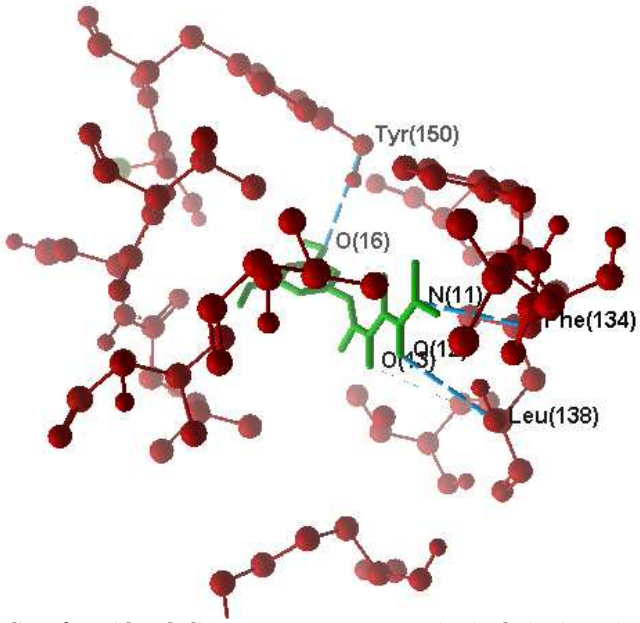


Fig.V *In silico* Binding of 3e with Tubulin. Receptor contacts- Leu(138), Phe(134), Tyr(150), Phe(118)

PROTEIN (TUBULIN) LIGANDS

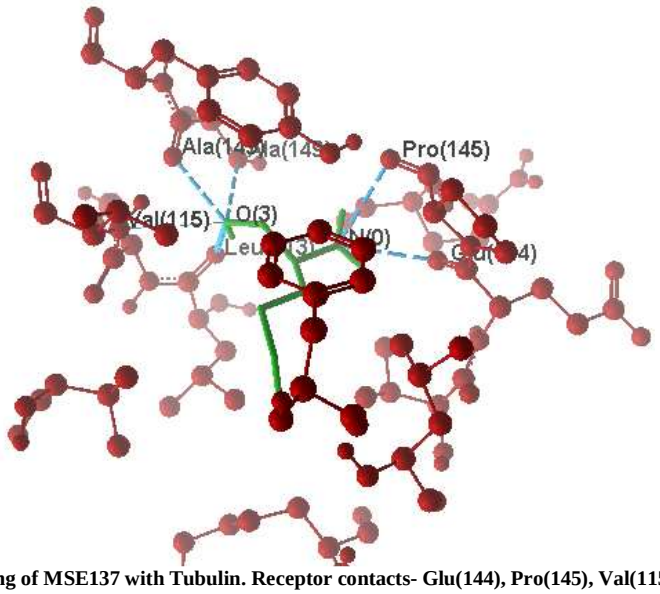


Fig. VI *In silico* binding of MSE137 with Tubulin. Receptor contacts- Glu(144), Pro(145), Val(115), Leu(113), Ala(149)

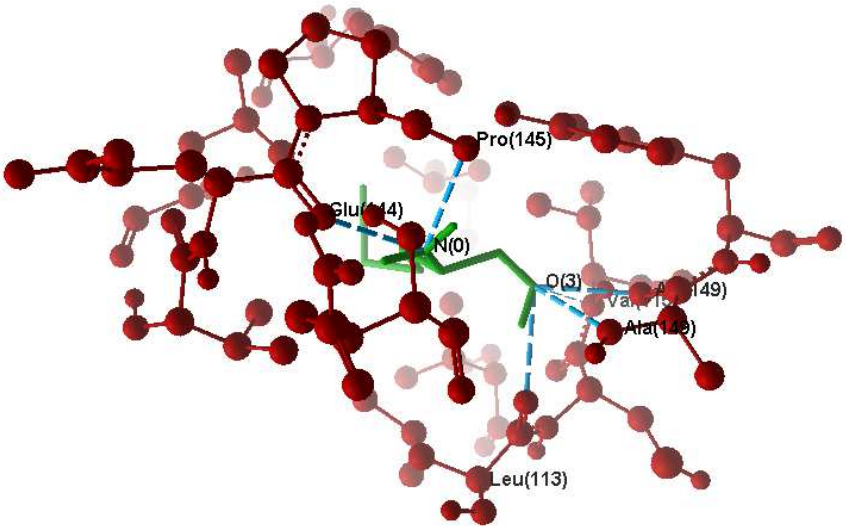


Fig.VII *In silico* binding of MSE148 with Tubulin. Receptor contacts- Glu(144), Pro(145), Val(115), Leu(113), Ala(149

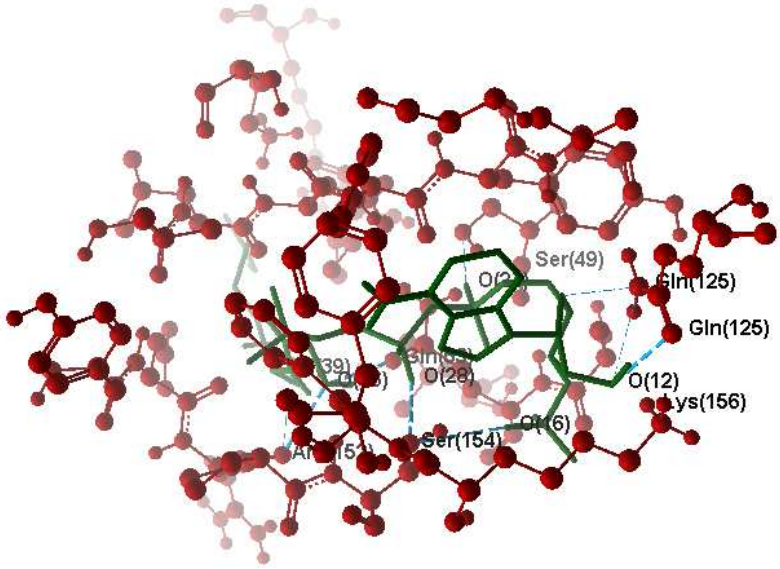


Fig.VIII *In silico* binding of ACO 201 with Tubulin. Receptor contacts- Arg(152), Gln(53), Ser(49), Ser(154), Lys(156), Gln(125)

RESULTS AND DISCUSSION

3.1 Synthesis of cinnamoyl ureas derivatives and characterization

Cinnamoyl ureas are prepared in three steps. In the first step substituted cinnamic acid (1a-1e)are prepared which further are converted to their chloride derivatives. Chloride derivatives (2a-2e) in the second step are prepared by reaction of substituted cinnamic acid with thionyl chloride. In the last and final step Cinnamoyl chlorides are converted to Cinnamoyl urea (3a-3e) by the reaction with simple urea. The reaction pathway has been summarized in Scheme. All the synthesized compounds were characterized and confirmed by recording their IR, ^{1}HNMR analysis and mass spectra. All compounds were characterized after recrystallization from appropriate solvents.

Molecular docking studies

The synthesized ligand molecules having 2D structure were converted to energy minimized 3D structures.Structure of tubulin protein was obtained from Protein Data Bank. Considering tubulin protein as the target receptor, automated docking studies with newly synthesized candidates lead compounds was performed to determine the best

in silico conformation. The docking of tubulin with newly synthesized candidates ligands (3a-3e) exhibited well established bonds with one or more amino acids in the receptor active pocket. All the six synthesized molecules were docked. Fig.1-3 shows the docked images of MSE137, ACO 201 and MSE148 and Fig.4-9 shows the docked images of selected candidate ligands with tubulin protein. Table 4 shows the No of interactions, protein residue name, compound residue name , bond length and bond energy of all synthsised compounds and of MSE137, ACO 201 and MSE148. *In silico* studies revealed all the synthesized molecules showed good no of interactions ranging from 4-6.

CONCLUSION

These new cinnamoyl urea derivatives were synthesized and docked for their tubulin interaction studies. All the synthesized compounds were purified by recrystallization using appropriate solvents and monitored by TLC They were further characterized by spectral analysis, physicochemical properties and and elemental analyses. Molecular docking studies of the synthesized compounds were carried out and the results shows that among the synthesized Cinnamoyl urea derivatives, compounds 3d showed high affinity with low energy of (-2.5) K.cal/mol with employed tubulin protein Hence this study has widened the scope of developing these cinnamoyl urea derivatives as promising antitumor agents.

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