Asian Journal of Chemistry; Vol. 28, No. 2 (2016), 410-414

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.19403

Synthesis, Characterization and Antitumor Potential of Cinnamoyl Urea Derivatives

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Received: 8 June 2015; *Accepted*: 3 August 2015; *Published online*: 3 November 2015; AJC-17619

Cinnamoyl ureas have been identified as novel compounds with their various biological activities. Some novel cinnamoyl ureas were synthesized successfully by these substitutions, at the phenyl ring, at α,β-unsaturated carbon and the substitution at the carbonyl functionality, which further were studied for their antitumor activity. Thus in this research work, we aimed to use all these active moieties with phenyl urea substitutions at carbonyl moiety. Molecular docking study was used for confirming their interaction with tubulin protein for their antitumor activity. Through *in silico* molecular docking study, the result showed that all the synthesized compounds (**3a-3e**) have minimum binding energy and good affinity toward their active pocket, thus they were considered as good antitumor agents.

Keywords: Cinnamoyl ureas, Tubulin, Antitumor, Molecular docking study.

INTRODUCTION

Tumor is an abnormal growth of tissue resulting from uncontrolled, progressive multiplication of cells and serving no physiological function. Various factors causing this uncontrolled multiplication are genetic factor, radiation chemicals/toxins, sun exposure and some causes are unknown [1]. The tumor cells division occurs due to complex cellular signaling involving hormones and growth factors. Latest evidences suggests that tubulin and microtubule-associated proteins may play an important role in a range of cellular stress responses, thus conferring survival advantage to tumor cells [2]. Tubulin is the target of some of the most widely used and time-honored anticancer tubulin-binding agents (TBAs). A major chemotherapeutic approach to the treatment of malignant tumors has been to disrupt the organization of microtubules in order to prevent mitotic spindle formation and hence progression to mitotic division [3].

Nowadays, docking methodology is used for finding the binding sites of synthesized derivatives to its biological receptor [4]. This technique can be used for the study of drug receptor interaction, design and synthesis of new molecules [5]. Along with binding site, the favourable conformation of molecule can also be identified by any bond interaction either hydrophilic or hydrophobic [6].

Various cinnamic acid analogs have been discovered which acts as cytotoxic or microtubule destabilizing agents [7]. Most of the cinnamic acid derivatives are substituted with electron donating hydroxy or methoxy groups at various positions [8]. 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 [9].

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.

EXPERIMENTAL

Most of the solvents used were of A.R. grade and purified before use in different reactions. Chemicals used were obtained from Merck, Central Drug House Pvt. Ltd. (CDH), India and Rankem Pvt. Ltd, India. Melting points of synthesized compounds were determined by melting point apparatus and uncorrected. All the infrared spectra were recorded on the Perkin Elmer and Shimadzu FTIR-spectrophotometer using KBR pellets. ^{1}H NMR spectra were recorded on 400 MHz spectrophotometer using DMSO as solvent and TMS as internal standard. The mass spectra were recorded on Waters, Q-TOF Micromass (LC-MS). All the reactions were monitored on thin

layer chromatography prepared by using silica gel G, petroleum ether and ethyl acetate in various ratio were used as mobile phase.

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 160 °C for 1 h and at 180 °C for 3 h. 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 [10]. The solids so obtained were recrystallized from mixture of 3 vol. of water and 1 vol. of rectified spirit. The purity of compounds was checked by the TLC.

3-(4-Aminophenyl)-2-methyl acrylic acid (1a): White crystalline solid; m.f.: $C_{10}H_{11}NO_2$; Yield: 82 %; R_f: 0.80; m.p.: 121-123 °C; IR (neat, ν_{max}, cm^{-1}): 1720, 2950, 3050, 1640, 2950, 3200 ^{1}H NMR (300 MHz, $CDCl_3$): 6.41 (2H, C-H aromatic), 7.0 (2H, C-H aromatic), 11 (1H, OH acid), 4 (2H, NH_2).

3-(4-Hydroxyphenyl)-2-methyl acrylic acid (1b): White crystalline solid; m.f.: $C_{10}H_{10}O_3$ Yield: 77 %; R_f: 0.72; m.p.: 137-138 °C; IR (neat, ν_{max}, cm^{-1}): 1713, 3100, 3144, 1637, 2850, 3380; ^{1}H NMR (300 MHz, $CDCl_3$): 6.68 (2H, C-H aromatic), 7.13 (2H, C-H aromatic), 10.93 (1H, OH acid), 5 (1H, OH).

3-(2,3,4-Trimethoxyphenyl)-2-methyl acrylic acid (1c): White crystalline solid; m.f.: $C_{13}H_{16}O_5$ Yield: 66 %; R_f: 0.64; m.p.: 119-120 °C; IR (neat, ν_{max}, cm^{-1}): 1717, 3026, 3039, 1648, 2854, 1012, 1018, 1038; ^{1}H NMR (300 MHz, $CDCl_3$): 6.17 (1H, C-H aromatic), 6.64 (1H, C-H aromatic), 11.10 (1H, OH acid), 4.73 (3H, OCH_3), 3.73 (3H, OCH_3), 3.73 (3H, OCH_3).

3-(3-Methoxyphenyl)-2-methyl acrylic acid (1d): White crystalline solid; m.f.: $C_{11}H_{12}O_3$ Yield: 70 %; R_f: 0.76; m.p.: 120-121 °C; IR (neat, ν_{max}, cm^{-1}): 1716, 3126, 3049, 1646, 2856, 1120; ^{1}H NMR (300 MHz, $CDCl_3$): 6.81 (1H, C-H aromatic), 6.65 (1H, C-H aromatic), 7.10 (1H, C-H aromatic), 10.90 (1H, OH acid), 3.73 (3H, OCH_3).

3-(2,3-Dihydroxyphenyl)-2-methyl acrylic acid (1e): White crystalline solid; m.f.: $C_{10}H_{10}O_4$ Yield: 75 %; R_f: 0.75; m.p.: 122-123 °C; IR (neat, ν_{max}, cm^{-1}): 1700, 3120, 3050, 1659, 2855, 1210, 1223, 3340, 3400; ^{1}H NMR (300 MHz, $CDCl_3$): 6.44 (1H, C-H aromatic), 6.60 (1H, C-H aromatic), 6.69 (1H, C-H aromatic) 10.80 (1H, OH acid), 4.73 (1H,OH), 5.00 (1H, OH).

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 0.84 mol of thionyl chloride was stirred under reflux until the disappearance of starting material for about 4 h [11]. After the reaction, 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.

3-(4-Aminophenyl)-2-methyl acryloyl chloride (2a): White crystalline solid; m.f.: $C_{10}H_{10}NOCl$; Yield: 72 %; R_f: 0.87; m.p.: 132-133 °C; IR (neat, ν_{max}, cm^{-1}): 1723, 3052, 1643, 2850, 3200; ^{1}H NMR (300 MHz, $CDCl_3$): 6.51 (2H, C-H aromatic), 7.05 (2H, C-H aromatic), 1.93 (3H, CH_3), 4.32 (2H, NH_2).

3-(4-Hydroxyphenyl)-2-methyl acryloyl chloride (2b): White crystalline solid; m.f.: $C_{10}H_9O_2Cl$; Yield: 67 %; R_f: 0.86; m.p.: 140-141 °C; IR (neat, ν_{max}, cm^{-1}): 1715, 3143, 1637, 2854, 3380; ^{1}H NMR (300 MHz, $CDCl_3$): 6.69 (2H, C-H aromatic), 7.23 (2H, C-H aromatic), 1.92 (1H, C-H), 4.79 (1H, OH).

3-(2,3,4-Trimethoxyphenyl)-2-methyl acryloyl chloride (2c): White crystalline solid; m.f.: $C_{13}H_{15}O_4Cl$; Yield: 69 %; R_f: 0.67; m.p.: 123-124 °C; IR (neat, ν_{max}, cm^{-1}): 1727 (C=O, acid) 3137 (CH, aromatic), 1658 (C=C, alkene), 2754 (C-H, methyl), 1028, 1036 (C-O, methoxy); ^{1}H NMR (300 MHz, $CDCl_3$): 6.27 (1H, C-H aromatic), 6.64 (1H, C-H aromatic), 4.73 (3H, OCH_3), 3.73 (3H, OCH_3), 3.73 (3H, OCH_3).

3-(3-Methoxyphenyl)-2-methyl acryloyl chloride (2d): White crystalline solid; m.f.: $C_{11}H_{11}O_2Cl$; Yield: 65 %; R_f: 0.74; m.p.: 127-128 °C; IR (neat, ν_{max}, cm^{-1}): 1726 (C=O, acid), 3039 (C-H, aromatic), 1656 (C=C, alkenes), 2756 (C-H, methyl), 1122 (C-O, methoxy); ^{1}H NMR (300 MHz, $CDCl_3$): 6.81 (1H, C-H aromatic), 6.55 (1H, C-H aromatic), 7.30 (1H, C-H aromatic), 6.86 (1H, C-H aromatic), 7.81 (1H, C-H aromatic), 3.53 (3H, OCH_3).

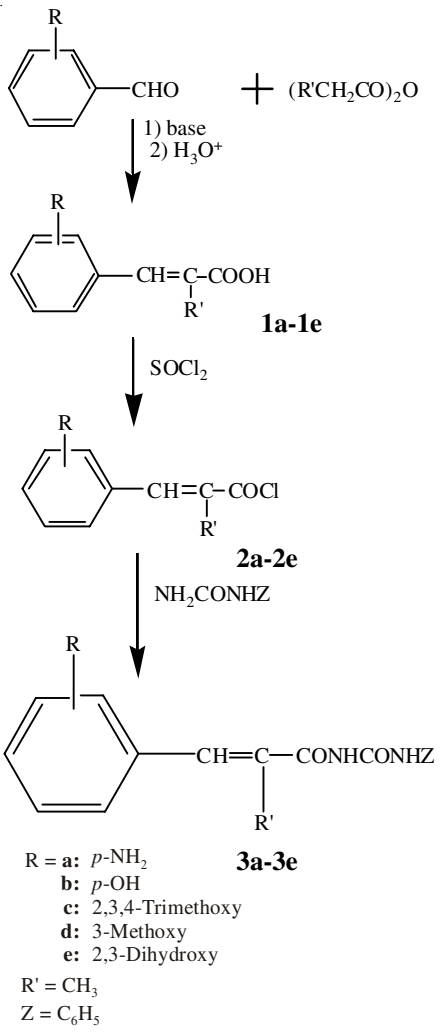
3-(2,3-Dihydroxyphenyl)-2-methyl acryloyl chloride (2e): White crystalline solid; m.f.: $C_{10}H_9O_3Cl$; Yield: 71 %; R_f: 0.66; m.p.: 136-137 °C; IR (neat, ν_{max}, cm^{-1}): 1710 (C=O, acid), 3049 (C-H, aromatic), 1669 (C=C, alkenes), 2845 (C-H, methyl), 1214 (C-O, hydroxy), 1225 (C-O, hydroxyl), 3339 (OH, alcohol), 3403 (OH, alcohol); ^{1}H NMR (300 MHz, $CDCl_3$): 6.69 (1H, C-H aromatic), 6.58 (1H, C-H aromatic), 6.44 (1H, C-H aromatic), 4.73 (1H, OH), 5.00 (1H, OH).

General procedure for the synthesis of compounds (3a-3e): Acyl ureas were prepared by reacting various cinnamoyl chloride derivatives with phenyl urea. Commercially available phenyl 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 [12]. The solid obtained was collected by filtration and washed with cold water (**Scheme-I**). The product was recrystallized from ethanol or dilute ethanol and purity of the compound was checked by TLC.

1-[(E)-3-(4-Aminophenyl)-2-methyl acryloyl]-3-phenylurea (3a): White crystalline solid; m.f.: $C_{17}H_{17}N_3O_2$; Yield: 72 %; R_f: 0.76; m.p.: 143-144 °C; IR (neat, ν_{max}, cm^{-1}): 1724, 1920, 3030, 1644, 2878, 3189, 3203; ^{1}H NMR (400 MHz, $CDCl_3$): 6.41 (2H, d, J = 14 Hz), 7.12 (2H, d, J = 13 Hz), 4 (2H, s), 1.78 (3H, d, J = 8.01), 5.98 (1H, s), 7.24 (5H, s), 10 (1H, s) ESI-MS: *m/z* 295.13 ($M+H^+$).

1-[(E)-3-(4-Hydroxyphenyl)-2-methyl acryloyl]-3-phenylurea (3b): White crystalline solid; m.f.: $C_{17}H_{16}N_2O_3$; Yield: 73 %; R_f: 0.88; m.p.: 140-141 °C; IR (neat, ν_{max}, cm^{-1}): 1688, 1927, 3055, 1643, 2949, 3196, 3327; ^{1}H NMR (400 MHz, $CDCl_3$) 6.68 (2H, d, J = 14 Hz), 7.13 (2H, d, J = 14 Hz), 5 (2H, s), 1.93 (3H, d, J = 8), 6 (1H, s), 7.64 (5H, s), 10 (1H, s); ESI-MS: *m/z* 296 ($M+H^+$).

1-[(E)-3-(2,3,4-Trimethoxyphenyl)-2-methyl acryloyl]-3-phenylurea (3c): White crystalline solid; m.f.: $C_{20}H_{22}N_2O_5$; Yield: 70 %; R_f: 0.69; m.p.: 144-145 °C; IR (neat, ν_{max}, cm^{-1}): 1726, 1928, 3054, 1642, 2957, 3192, 3200; ^{1}H NMR (400 MHz,$CDCl_3$): 6.16-6.18 (1H, m), 6.63 (1H, d, J = 15 Hz), 3.70



Scheme-I

(3H, s), 3.73 (3H, s), 3.73 (3H, s), 1.93 (3H, d, *J* = 9), 5.97 (1H, s), 7.64 (5H, s), 10 (1H, s) ESI-MS: *m/z* 371 ($M+H^+$).

1-[(E)-3-(3-Methoxyphenyl)-2-methyl acryloyl]-3-phenylurea (3d): White crystalline solid; m.f.: $C_{18}H_{18}N_2O_3$; Yield: 72 %; R_f: 0.75; m.p.: 149-150 °C; IR (neat, ν_{max}, cm^{-1}): 1723, 1937, 3053, 1645, 2953, 3189, 3204; ^1H NMR (400 MHz, $CDCl_3$): 6.65-6.67 (1H, m), 6.80-6.81 (1H, m) 7.10 (1H, t, *J* = 14 Hz), 6.86 (1, H, d, *J* = 12 Hz), 3.73 (3H, s), 1.93 (3H, d, *J* = 8), 6 (1H, s), 7.24 (5H, s), 9.96 (1H, s) ESI-MS: *m/z* 310 ($M+H^+$).

1-[(E)-3-(2,3-Dihydroxyphenyl)-2-methyl acryloyl]-3-phenylurea (3e): White crystalline solid; m.f.: $C_{17}H_{16}N_2O_2$; Yield: 80 %; R_f: 0.67; m.p.: 141-142 °C; IR (neat, ν_{max}, cm^{-1}): 3160, 3204, 3040, 1727, 1653, 1932, 1223, 1255, 1255; ^1H NMR (400 MHz, $CDCl_3$) 5 (1H, s), 5 (1H, s), 6.44 (1H, t, *J* = 9), 6.69 (1H, d, *J* = 13 Hz), 1.90 (3H, d, *J* = 8), 6 (1H, s), 7.64 (5H, s), 10 (1H, s); ESI-MS: *m/z* 313 ($M+H^+$).

***in silico* Molecular docking studies:** The ligands were drawn in Marvin Sketch assigned with proper 2-D orientation [13] *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, the Auto Dock 4.0 suite molecular-docking tool was used and the methodology was followed [14]. The anticancer compounds were manually docked into sites of the enzymes and the docking energy was monitored to achieve a minimum value [5]. The default parameters of the automatic settings were used. Each docking experiment consisted of 10 docking runs and the search was conducted in a grid of 40 points per dimension. The binding position and bound conformation of the peptide and the rough estimate of its interactions were examined with the Auto Dock results [15]. To analyze the mode of binding docked conformation with minimum binding energy was selected. In the present study, the binding site was selected based on the amino acid residues, which are involved in binding with tubulin protein [14].

RESULTS AND DISCUSSION

Synthesis of cinnamoyl ureas derivatives and characterization: 3-Substituted-2-methyl acrylic acid (**1a-1e**) were synthesized by reaction of substituted aromatic aldehydes with propionic anhydride by refluxing in oil bath at 160 °C for 1 h and at 180 °C for 3 h. Further all substituted-2-methyl acrylic acid (**2a-2e**) were converted to corresponding acid chlorides by reaction with thionyl chlorides. Finally cinnamoyl ureas were obtained in good yield by reacting with phenyl urea in basic media. All the synthesized compounds (**1a-1e**, **2a-2e**) were characterized by IR, ^1H NMR analysis. Final compounds (**3a-3e**) were characterized and confirmed by recording their IR, ^1H NMR and mass spectra. All compounds were characterized after recrystallization from appropriate solvents. IR spectrum of most final compound showed absorption at 3200-3100 cm^{-1} which is due to the NH stretching. Bands near 1726, 1928, 3054, 1642, 2957 cm^{-1} are observed due to C=O, C=O (urea), C-H (aromatic), C=C and C-H (methyl) respectively in compounds **3a-3e**. The ^1H NMR spectrum of all synthesized compounds confirms their structure showing singlet near 6.00 due to 1H of NH and a doublet near 4.00.

Molecular docking studies: Considering the well obtained experimental results, it was thought worthy to perform molecular docking studies, hence screening the compounds, inculcating the compounds for *in silico.* Native crystal structure of tubulin protein was obtained from Protein Data Bank. Considering tubulin protein as the target receptor, automated

TABLE-1
DOCKING STUDY DATA SHOWING BINDING AFFINITY BETWEEN SYNTHESIZED COMPOUNDS AND TUBULIN PROTEIN

S. No.	Compound no.	No. of conformation	Binding affinity (kcal/mol)	Protein residue name, number and distance (Å)
1	**3a**	First	-8.00	Gly 134 (3.5), Leu 122 (2.7), Arg 158 (3.0) and Ser 160 (3.2)
2	**3b**	First	-8.60	Ile 121 (3.1), Leu 122 (2.8), Phe 124 (2.6), Asp 157 (2.5) and Ser 160 (3.4)
3	**3c**	Second	-8.60	Arg 132 (3.4), Gly 136 ((3.4), Arg 137 (3.4)
4	**3d**	Second	-7.90	Gly 134 (3.4)
5	**3e**	First	-7.40	Arg 132 (3.5), Lys 165 (2.5)

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. The synthesized ligand molecules having 2D structure were converted to energy minimized 3D structures. All the five synthesized molecules were docked. Figs. 1-5 shows the docked images of selected candidate ligands with tubulin protein. Table-1 shows the binding affinity, protein residue name, number and distance covered by binding to ligands. *in silico* studies revealed all the synthesized molecules showed good binding energy toward the target protein ranging from -8.60 to -7.40 kcal mol^{-1}.

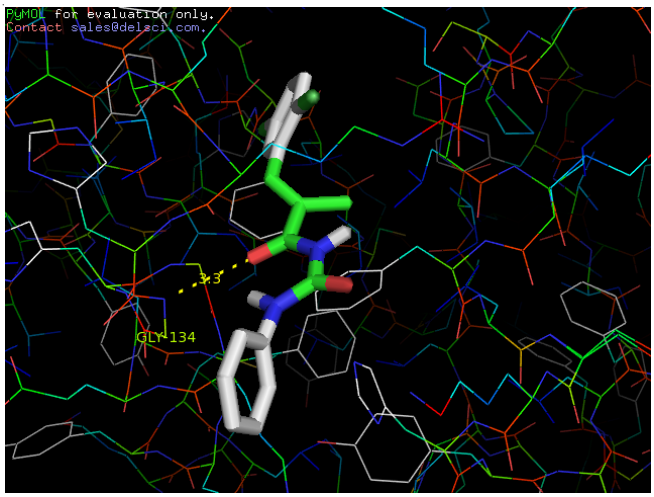


Fig. 1. *in silico* Binding of **3a** with Tubulin (Binding energy = -8 kcal/mole, receptor contacts- Gly-134, Leu-122, Arg-158 and Ser-160)

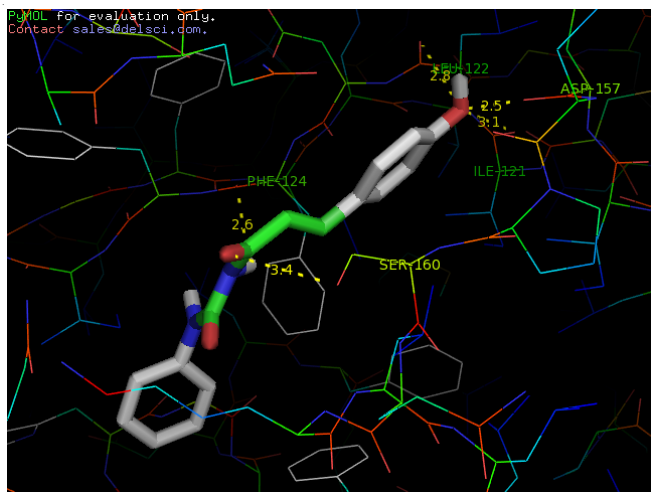


Fig. 2. *in silico* Binding of **3b** with Tubulin (Binding energy = -8.60 kcal/mole, receptor contacts- Ile 121, Leu 122, Phe 124, Asp 157 and Ser 160

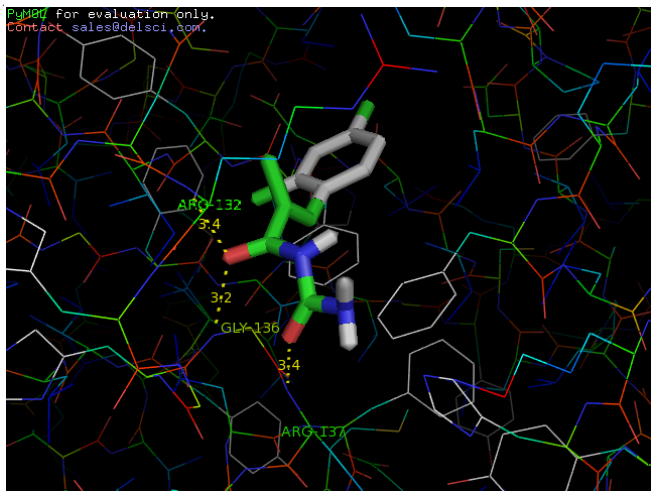


Fig. 3. *in silico* Binding of **3c** with Tubulin (Binding energy = -8.60 kcal/mole, receptor contacts- Arg 132, Gly 136, Arg 137

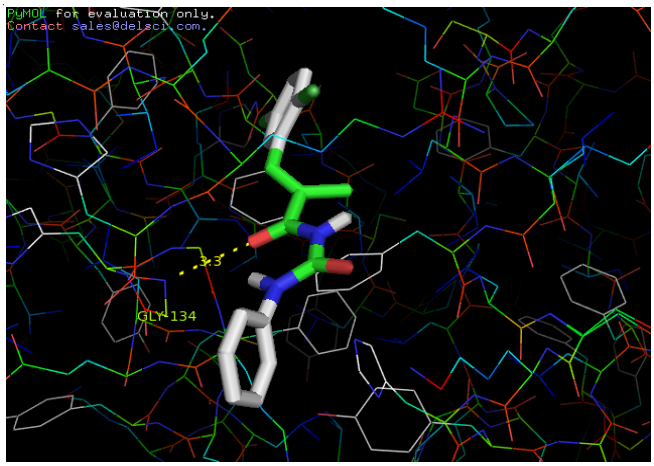


Fig. 4. *in silico* Binding of **3d** with Tubulin (Binding energy= -7.90 kcal/mole, receptor contacts- Gly 134

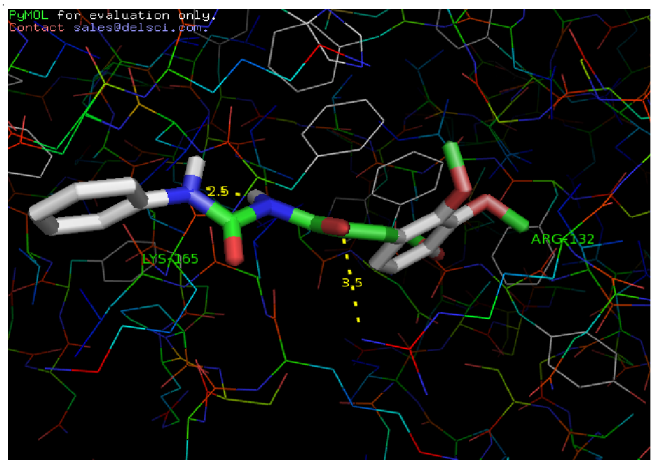


Fig. 5. *in silico* Binding of **3e** with Tubulin (Binding energy= -7.40 kcal/mole, receptor contacts- Arg 132, Lys 165

Conclusion

These new cinnamoyl urea derivatives were synthesized in reasonably good yields. All the synthesized compounds were purified by recrystallization using appropriate solvents and monitored by TLC They were further characterized by ^{1}H NMR, Mass spectrometry, IR studies and elemental analyses. All the newly synthesized compounds were tested for antitumor activity. Finally the molecular docking studies of the synthesized compounds were carried out and the results of such studies were reported by *in silico* docking studies. *in silico* studies revealed that among the synthesized compounds **3b** and **3c** showed high affinity with low energy of (-8.60) Kcal/

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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