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Synthesis and molecular modeling studies of novel *tert*-butyl 2, 4-disubstituted carboxamido phenylcarbamate derivatives and evaluation of their antimicrobial activity

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ABSTRACT

A new series of novel tert-butyl 2,4-disubstituted carboxamido phenylcarbamate derivatives were synthesized and evaluated for their biological activities against three gram positive bacteria viz; Bacillus licheniformis, Bacillus subtilis, Staphylococcus aureus, three gram negative bacteria viz; Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa & four fungi strains viz; Aspergilus niger, Candida albicance, Fusarium oxysporum, Fusarium solani. All compounds were characterized by IR , ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. Among all the title compounds ***6b, 6c, 6e, 6g & 6h*** *displayed the most potent antimicrobial activity. The compounds* ***6b & 6e*** *showed highest antibacterial effect with respect to standard ciprofloxacin. The remaining analogues were showed good to moderate antibacterial activity. The compounds* ***6b, 6c & 6e*** *showed promising antifungal activity with respect to standard Nystatin. The molecular docking studies were performed to newly synthesized carbamates* ***(6a-k)*** *into the active site of Mycobacterium tuberculosis enoyl reductase (InhA) complexed with 1-cyclohexyl-N-(3,5-dichlorophenyl)-5-oxopyrrolidine-3-carboxamide* ***(2H7M)****, Candida albicans dihydrofolate reductase complexed with NADPH and 6-methyl-5-[3-methyl-3-(3,4,5-trimethoxyphenyl)but-1-yn-1-yl]pyrimidine-2,4-diamine (UCP115A)* ***(3QLS)*** *to determine the probable binding modes.*

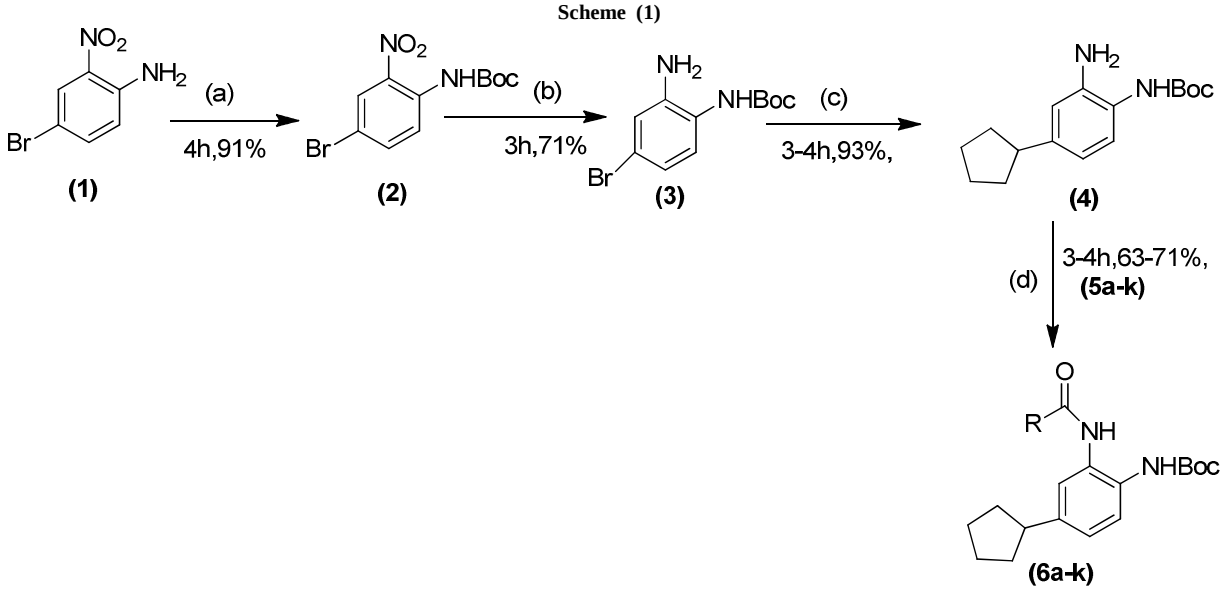
Keywords: Carbamate derivatives, Synthesis, Antimicrobial activity, Molecular modeling studies.

INTRODUCTION

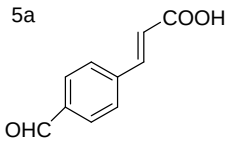
Approximately fifteen million people worldwide suffer a stroke each year resulting a death or sesorimoter and other defects. The phenyl carbamate is pharmaceutically acceptable salt for prevention of stroke [1]. Mono carbamates of optically pure form of halogenated & phenyl dicarbamates are also used in the treatment of disorder of central nerves systems specifically as anticonvulsive or anti epileptic agents[2]. The carbamate derivatives were associated with broad spectrum of biological activities including anticonvulsant [3], antituberculosis [4], analgesic, anti-inflammatory [5,6,7,8], insecticidal, antifungal ,antimicrobial[9] and antitumor properties. They were reported as intermediates in the synthesis of therapeutic agents [10] and showed anti-platelet activities. The amides with other aliphatic, aromatic and heterocyclic ring produce various type of biological activity. The 5-chloro quinoline 8-yl phenyl carbamates were reported as hits in a high throughput screened anti angiogenic agents [11]. A number of

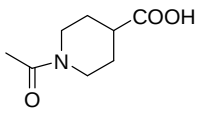
aromatic amides of aromatic and heterocyclic acids have been synthesized in search for new antagonists of excitatory amino acids receptors with anticonvulsant activity. Generally, benzyl amides were found to be more active than other amides. On the other hand, the most effective appeared amides of acids: picolinic, nicotinic, isonicotinic, nipecotic and isonipecotic. The amide derivatives can be synthesized very easily by the reaction of carboxylic acid group (-COOH) with amines. From the literature survey it was observed that there is a huge volume of research on synthesis of amides by using various coupling agents. The cyanuric chloride/triethyl amine[12] used as a coupling agent in large scale synthesis of amides, but due to side reactions like formation of some by-products, racemisation etc. are disadvantages. By using triphenyl trichloromethyl phosphonium chloride [13] as a coupling agent, separation of phosphorous by-products, toxicity and association of environmental risks are demerits. The cyanuric fluoride/pyridine[14], PPh3/N-bromosuccinimide[15], Diphenyl phosphonic azide (DPPA)[16,17], Carbonyl diimidazole (CDI)[18] etc. also reported for coupling of amides. The cyanuric fluoride is best reagent because it is less moisture sensitive, more reactive towards amines, the side reactions like racemisation etc. can be avoided. But it is very useful in peptide chemistry. The Diphenyl phosphonic azide (DPPA) reagent used in one pot process but only problem is due to occasional side reaction like Curtius rearrangement which leading to formation of unwanted corresponding isocyanate. The carbonyl diimidazole (CDI) reagent was also used in the formation of amide bond, but the reagent requires in large scale. By taking all the above considerations, here the researchers used O-(7-Azabenzotriazol-1-yl)-N,N,N′,N′-tetra methyl uronium hexa fluoro phosphate (HATU) derived from 1-hydroxy-7-azabenzotriazole (HOAt). The high coupling efficiencies and fast reaction rates associated with HATU coupling are thought to arise from a neighboring group effect brought about by the pyridine nitrogen atom, which stabilizes the incoming amine through a hydrogen-bonded 7-membered cyclic transition state [19]. Tempted by wide variety of coupling reagents, biological activities shown by amide derivatives and in continuation[20] of our efforts in search of potent molecules exhibiting antimicrobial activities, we report here the synthesis, antibacterial and antifungal activity evaluation of various amide derivatives.

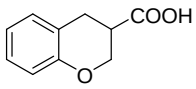
Scheme (1)

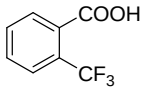


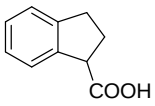
Where **5a-k** = substituted carboxylic acids(R):

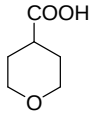
5a 

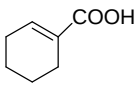
5b 

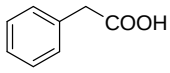
5c 

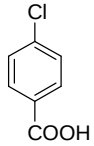
5d 

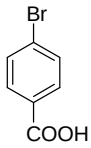
5e 

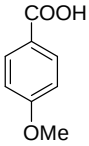
5f 

5g 

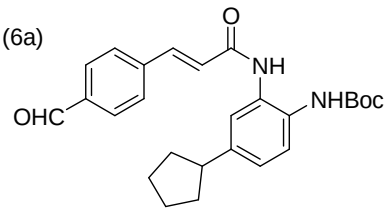
5h 

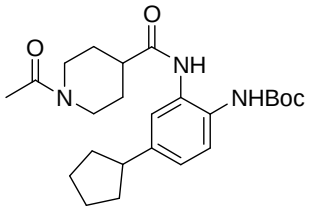
5i 

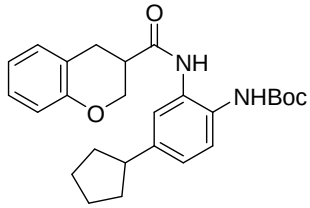
5j 

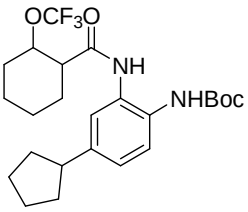
5k 

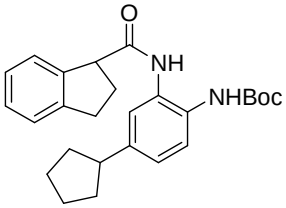
Reagents &Conditions:(a) *DCM,TEA,$(BOC)_2O$,* ***(b)****Methanol,$FeCl_3$,$N_2H_4.H_2O$,3h.Reflux,***(c)** *Cyclopentylboronicacid,Na_2CO_3,Pd(0),THF:H_2O* **,** ***(d)****DMF, HATU, R (R=different acids), DIPEA.*

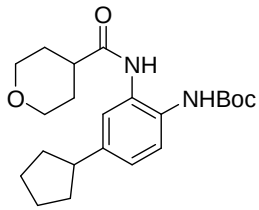
(6a) 

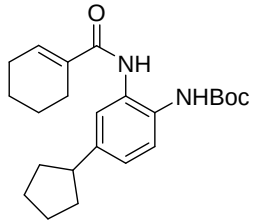
(6b) 

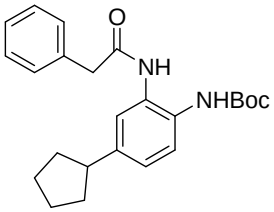
(6c) 

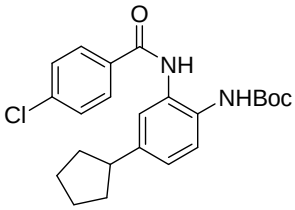
(6d) 

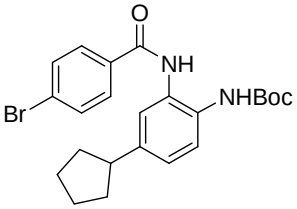
(6e) 

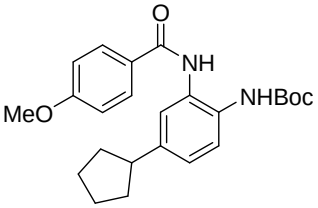
(6f) 

(6g) 

(6h) 

(6i) 

(6j) 

(6k) 

MATERIALS AND METHODS

Chemistry

Several substituted amide derivatives **(6a-k)** were synthesized from tert-butyl 2-amino 4-bromo phenylcarbamate **(4)** by dehydration reaction in presence of (HATU) with different carboxylic acids. The 2-nitro,4-bromo benzenamine **(1)** was allowed to undergo amine group protection via nucleophilic addition reaction by tertiary butoxy carbonic anhydride in presence of triethyl amine and produced tert-butyl 2-nitro,4-bromophenylcarbamate **(2)**. The resultant compound **(2)** underwent simple reduction with N_2H_4 in methanol and produced the tert-butyl 2-amino,4-bromophenyl carbamate **(3).** Furtherly it **(3)** underwent Suzuki coupling reaction with cyclopentylboronic acid in presence of sodium carbonate, Pd(0),THF:H_2O to obtain tert-butyl 2-amino,4-cyclopentyl phenylcarbamate **(4).** Finally the compound **(4)** individually allowed condensing with different carboxylic acids by using HATU/DMF in presence of DIPEA (N, N-Di isopropyl Ethylamine) to offer the carbamate analogues **(6a-k)**. The structures of the newly synthesized compounds **(6a-k),** intermediates **(2),(3)** & **(4)** have been characterized based on their spectral (IR, ^{1}H NMR, ^{13}C NMR & HRMS) and analytical data after methodical purification.

Biological Screening

Antimicrobial evaluation

The eleven newly synthesized target compounds were evaluated for their antimicrobial activity against three gram negative bacteria *viz; Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, three gram positive bacteria *viz; Bacillus licheniformis*, *Bacillus subtilis*, *Staphylococcus aureus* and four fungal strains *viz; Aspergilus niger*, *Candida albicance*, *Fusarium oxysporum*, *Fusarium solani*. Agar- diffusion method was taken for the determination of the preliminary antimicrobial activity.The ciprofloxacin and nystatin were used as reference drugs. The results were recorded for each tested compound diameter of inhibition zones of microbial growth around the disks in mm. The minimum inhibitory concentration (MIC) measurement was also determined for significant growth inhibition compounds. The inhibition zone diameter values and MIC (µg/ml) values were presented in **Table 1a, 1b**, **2a & 2b** respectively. The results revealed that most of tested compounds displayed variable inhibitory effects on the growth of the tested bacterial and fungal strains.

The compounds **6b, 6e, 6c, 6g** & **6h** showed good activities against (inhibitory zone ≥20 mm) bacterial and fungal strains. The MIC values of compounds **6b, 6e, 6c, 6g** & **6h** showed moderate to good inhibitory activity (300-25µg/ml) against bacterial and fungal strains, with respect to reference drugs.

Table -1a: Antibacterial activity of newly synthesized compounds

Organic compound	Zone of inhibition(mm)					
	Gram negative(-ve) bacteria			Gram positive (+ve) bacteria		
	Escherichia coli	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Bacillus licheniformis*	*Bacillus subtilis*	*Staphylococcus aureus*
6a	**17**	**20**	**15**	**16**	**19**	**20**
6b	**23**	**21**	**21**	**19**	**21**	**20**
6c	**19**	**24**	**21**	**22**	**21**	**20**
6d	**18**	**14**	**12**	**20**	**14**	**11**
6e	**22**	**20**	**19**	**24**	**19**	**19**
6f	**-**	**-**	**-**	**-**	**-**	**-**
6g	**21**	**19**	**23**	**25**	**24**	**19**
6h	**23**	**19**	**22**	**21**	**24**	**17**
6i	**-**	**-**	**-**	**-**	**-**	**-**
6j	**-**	**-**	**-**	**-**	**-**	**-**
6k	**-**	**-**	**-**	**-**	**-**	**-**
Ciprofloxacin	**25**	**24**	**26**	**24**	**21**	**25**
Control (1%DMSO)	-	-	-	-	-	-

- =No activity

Table- 1b: Antifungal activity of newly synthesized compounds

Organic compound	Zone of inhibition(mm) Fungi			
	Aspergilus niger	*Candida albicance*	*Fusarium oxysporum*	*Fusarium solani*
6a	**-**	**-**	**-**	**-**
6b	**24**	**21**	**22**	**21**
6c	**21**	**24**	**20**	**18**
6d	**-**	**-**	**-**	**-**
6e	**20**	**21**	**22**	**19**
6f	**-**	**-**	**-**	**-**
6g	**20**	**19**	**24**	**22**
6h	**19**	**21**	**23**	**20**
6i	**-**	**-**	**-**	**-**
6j	**-**	**-**	**-**	**-**
6k	**-**	**-**	**-**	**-**
Nystatin	**20**	**25**	**23**	**25**
Control (1%DMSO)	**-**	**-**	**-**	**-**

- =No activity

Table -2a: MICs of newly synthesized compounds for bacteria

Organic compound	MIC(µg/ml) Gram negative(-ve) bacteria			Gram positive (+ve) bacteria		
	Escherichia coli	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Bacillus licheniformis*	*Bacillus subtilis*	*Staphylococcus Aureus*
6b	**150**	**175**	**250**	**200**	**125**	**125**
6c	**100**	**50**	**150**	**100**	**75**	**75**
6e	**150**	**50**	**50**	**25**	**75**	**75**
6g	**100**	**125**	**175**	**200**	**250**	**125**
6h	**25**	**200**	**100**	**200**	**175**	**75**
Ciprofloxacin	**25**	**25**	**25**	**25**	**25**	**25**
Control (1%DMSO)	**-**	**-**	**-**	**-**	**-**	**-**

- =No activity

Table -2b: MICs of newly synthesized compounds for fungi

Organic compound	MIC(µg/ml) Fungi			
	Aspergilus niger	*Candida albicance*	*Fusarium oxysporum*	*Fusarium solani*
6b	**75**	**50**	**50**	**75**
6c	**75**	**50**	**25**	**50**
6e	**25**	**75**	**125**	**100**
6g	**50**	**25**	**75**	**25**
6h	**100**	**150**	**150**	**75**
Nystatin	**25**	**25**	**25**	**25**
Control (1%DMSO)	**-**	**-**	**-**	**-**

- =No activity

Antimicrobial evaluation

In vitro antimicrobial assay

Standard sterilized filter paper disks (5 mm diameter) impregnated with a solution of the test compound in DMSO (1 mg/ml) was placed on agar plate seeded with the appropriate test organism in triplicate. The ciprofloxacin was used as standard antibacterial agent and nystatin was used as antifungal agent, DMSO alone was used as control at the same above mentioned concentration. The plates were incubated at 37^0C for 1-5 days and antimicrobial activity was determined by measuring the diameter of the zone of inhibition surrounding microbial growth [21]. The target carbamates which showed significant growth inhibition zones were further evaluated for their MICs.

Minimal inhibitory concentration (MIC) measurement

The microorganism's susceptibility tests in nutrient and potato dextrose broths were used for the determination of MIC. The stock solutions of the tested compounds, ciprofloxacin and nystatin were prepared in DMSO at

concentration of 1000 µg/ml followed by dilutions at concentrations of (250-25µg/ml). The microorganism suspensions were inoculated into the different concentrations of corresponding compounds and control experiments. These were incubated at 37^0C for 1–5 days for MIC determination.

Table- 3a: Molinspiration properties of (6a-k):

Comp	Gpcr ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor	milogp[a]	TPSA[b]	n violations[c]	M.wt[d]	nON[e]	nOHNH[f]
6a	0.09	0.12	0.02	0.21	0.12	0.15	5.93	84.5	1	434.53	6	2
6b	0.24	0.12	0.05	0.05	0.38	0.14	3.95	87.73	0	429.56	7	2
6c	0.08	-0.04	-0.12	0.06	0.27	0.11	5.95	76.66	1	454.59	6	2
6d	0.27	0.22	0.01	0.32	0.47	0.21	6.45	76.66	1	470.53	6	2
6e	0.29	0.12	0.02	0.35	0.36	0.24	5.65	67.43	1	420.55	5	2
6f	0.19	0.11	0.05	0.13	0.38	0.17	4.54	76.66	0	388.50	6	2
6g	0.22	0.22	0.04	0.26	0.31	0.30	5.89	67.43	0	384.52	5	2
6h	0.22	0.15	0.08	0.17	0.36	0.19	5.59	67.43	1	394.51	5	2
6i	0.19	0.13	0.12	0.12	0.29	0.16	6.18	67.43	1	380.12	5	2
6j	0.10	0.07	0.10	0.04	0.23	0.12	6.31	67.43	1	459.38	5	2
6k	0.14	0.07	0.10	0.12	0.27	0.14	5.56	76.66	0	410.51	6	2

TPSA and calculated Lipinski's rule of five for compounds under biological investigations
a:calculated lipophilicity, **b**:Total polar surface area, **c**:no of violations from Lipinski's rule of five, **d:**Molecular weight **e:**no. of hydrogen bond acceptors, **f:**no. of hydrogen bond donors

We calculated the compliance of compounds to the Lipinski's rule of five[22]. Briefly, the rule is based upon the observation that most biological active drugs have a molecular weight (MWt of 500 or less, a log P no higher than 5 five or fewer hydrogen bond donor sites and ten or fewer hydrogen acceptor sites (N or O atoms).These results displayed in T**able 3a** and showed that all the synthesized compounds obey with this rule. Among all the compounds, the **(6b)** (*invitro* best active) has lipophilicity ($m_i$logp) 3.95, higher TPSA value.

General chemistry aspects
Thin Layer Chromatography (TLC) was performed on E.Merk AL Silica gel 60 F254 plates and visualized under UV light. The infrared (IR) spectra were determined in a perkin-Elmer Fourier transform (FDIR spectrum). ^{1}H-NMR spectra were recorded on Varian EM-360 (400MHz mercury plus) spectrometer in DMSOd6 and calibrated using solvent signals 2.50(DMSO-d6)]. All chemical shifts recorded in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Agilent ion trap MS. Spectrometer at energy of ionizing electron equal to 70ev.The most of the reagents were purchased from Aldrich chemical company, Fluka and Merck Company.

General Synthetic Procedure
Preparation of tert-butyl 2-nitro 4-bromo phenyl carbamate (2). The 2-nitro,4-bromoaniline (**1**) (0.0362 mol,1.0eq) was dissolved in DCM (30 ml) and added triethylamine (0.0543 mol,1.5 eq).The reaction mixture was cooled to 0^0C and Boc anhydride (0.04706 mol,1.3 eq) added drop wise to the reaction mixture . After completion of the addition, the reaction mixture was stirred at room temperature for 1hr to obtain compound **(2).** The completion of the reaction was monitored by TLC, then water was added to the reaction mass, extracted with DCM, the organic layer was separated, washed with water, brine solution and dried over Na_2SO_4. The organic layer was filtered and concentrated under vacuum to affor crude residue of compound **(2)**. It was purified on column chromatography, eluting with 30% ethyl acetate in hexane to obtain compound **(2)** as a yellow solid.

Yellow solid, Yield**:** 91%, ^{1}H-NMR(400MHz, DMSO-d6)**:** δ 9.15 (s, 1H); 8.53 (d,J=7.2Hz, 1H); 7.78-7.82 (2H, m); 8.24(d, J=7.2Hz, 1H),1.38(s,9H),^{13}C-NMR(400MHz, DMSO-d_6): δ152.5(Ar-CNO_2),142.4 (NHBoc C=O),131.2(Ar-C),131.1(Ar-C),125.3(Ar-C),125.2(Ar-C),118.4(Ar-C),79.5(NHBoc-C),28.4(NHBoc-CH_3).HRMS(EI) Calcd. for $C_{11}H_{14}N_2O_4$ (M^+)238.24: Found:238.23.

Preparation of tert-butyl 2-amino4-bromo phenyl carbamate (3). The compound **(2)** (0.021 mol,1.0 eq)) was dissolved in methanol (50 ml) and $FeCl_3$ (0.0013 mol,0.00625 eq),$N_2H_4.H_2O$(20 ml) were added at room temperature. The reaction mixture was heated under reflux conditions for 3hrs. The completion of the reaction was monitored by TLC. The contents were cooled to room temperature, concentrated under reduced pressure to remove the solvent. Then it was basified with sat.$NaHCO_3$ solution and diluted with DCM, then washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to obtain crude solid which

was purified by column chromatography eluting with 20% ethyl acetate in hexane, to obtain compound **(3)** as a white solid.

white solid, Yield: 71%, ^{1}H-NMR(400MHz, DMSO-d_6)**:** δ 9.15(s,1H);8.00(d, J=7.2Hz,1H); 7.40(dd, J=7.5Hz, J=3.1Hz, 1H,);6.79(dd, J=7.52Hz, J=3.1Hz, 1H),6.27(s,2H),1.38(s,9H).; ^{13}C-NMR(400MHz, DMSO-d_6): 152.5 (NHBoc C=O), δ152.5(Ar-CNH_2),125.5(Ar-C),125.1(Ar-C),122.8(Ar-C),118.9(Ar-C),114.5(Ar-C),79.5(NHBoc-C),28.4(NHBoc-CH_3)**.**HRMS(EI) Calcd. for $C_{11}H_{16}N_2O_2$ (M^+)208.26: Found:208.24.

Preparation of tert-butyl 2-amino4-cyclopentyl phenyl carbamate (4). The compound **(3) (**0.02 mol,1.0 eq) was dissolved in THF/H_2O (82.5 ml).To the above solution Pd(0) (0.002 mol,0.1 eq),cyclopentylboronicacid (0.03 mol,1.5 eq),and Na_2CO_3 (0.03 mol,1.5 eq) were added at room temperature. Then the reaction mixture was allowed to refluxing condition and stirred for 12h.After completion of the reaction, the contents were filtered through celite bed fallowed by washing with ethyl acetate, separated the organic layer .Then organic layer was washed with water, brine and dried over anhydrous Na_2SO_4.The organic layer was filtered and concentrated under vacuum. The crude residue was purified by column chromatography eluting with 20% EtOAc in n- hexane. The desired compound **(4)** was obtained as light brick red colour solid.

Yield: 93%. m.p. 128.8-131.4^{0}C. Rf = 0.62 (EtOAc / n-hexane = 1:2, v/v). ^{1}H-NMR(400MHz,DMSO-d_6): δ8.17(s, 1H), 7.03-7.01(dd, J=7.52,3.52Hz, 1H), 6.57(s, 1H), 6.42-6.39(dd, J=7.5,3.52Hz, 1H), 4.70(s, 2H),2.83-2.75(m,1H),1.96-1.89(m,2H), 1.74-1.68(m,2H), 1.65-1.58(m, 2H),1.43(m,2H),^{13}C NMR (400MHz, DMSO-d6): δ 153.6(NHBoc C=O), 142.6(Ar-C), 141.0(Ar-c-NH_2), 124.5(Ar-C), 121.4(Ar-C), 114.9(Ar-C), 114.1(Ar-C), 78.4 (NHBoc-C), 44.8(CH),34.0(CH_2), 28.1(NHBoc-CH_3),24.9(CH_2). HRMS(EI) Calcd. for $C_{16}H_{24}N_2O_2$ (M^+)276.37: Found:276.35.

General procedure for the preparation of carbamate derivatives (6a-k). The compound **(4)** (0.9615 m.mol,1 .0 eq) was dissolved in DMF (10 ml) and added HATU (1.9230m.mol,2.0 eq), DIPEA(N,N-diisopropylethylamine) (2.4038m.mol,2.5eq) and allowed to react with different carboxylic acids (0.9615m.mol,1.0 eq) individually at 0^{o}C temperature. The reaction mixture was stirred for 30 min and it was kept at room temperature for 3hrs to complete the reaction. Completion of the reaction was monitored by TLC. The product mixture diluted with water, extracted with diethyl ether & separated the organic layer, washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to obtain crude solid which was purified by column chromatography eluting with 30%- 40% ethyl acetate in hexane, to obtain corresponding title compounds **(6a-k)** as solids.Yields:63-71%.

Spectral data of carbamate analogues (6a-k).

Tert-butyl 2-((E)-3-(4-formylphenyl) acrylamido)-4-cyclopentylphenylcarbamate(6a)

White solide, Yield: 63%. m.p. 203.5^{0}C. Rf = 0.62 (EtOAc / n-hexane = 1:2, v/v). ^{1}H-NMR(400MHz,DMSO-d_6): δ9.60(S, 1H), 8.13(S, 1H), 8.03-8.01(d, J=7.6Hz, 1H), 7.90-7.89(d,J=7.5Hz, 2H), 7.78-7.70(t, J=8.0Hz, 4H), 7.17(S, 1H), 7.13-7.11(d,8.8Hz,1H), 6.79-6.75(d,J=7.3Hz,1H), 3.32-2.90(m, 1H), 2.02-2.01(t, J = 8.1Hz, 2H), 1.77(s, 2H), 1.66-1.53(m, 4H), 1.44(s, 9H). ^{13}C NMR (400MHz, DMSO-d6): δ 192.0 (CHO), 168.0(Amide C=O),152.5(NHBoc C=O),141.8(Ar-C),141.1(=CH),141.0(Ar-C),136.1(Ar-C),129.8(Ar-C), 129.6(Ar-C),129.3(Ar-C),125.2(Ar-C),122.6(Ar-C),121.4(Ar-C),118.8(=CH),79.9(NHBoc-C),46.1(CH), 34.6(CH_2), 28.4(NHBoc-CH_3),25.5(CH_2). HRMS(EI) Calcd. for $C_{26}H_{30}N_2O_4$ (M^+)434.53: Found:434.52

Tert-butyl2-(1-acetylpiperidine-4-carboxamido)-4-cyclopentylphenylcarbamate(6b)

Light brown solid, Yield: 66%. m.p. 208.5^{0}C. R*f*= 0.53 (EtOAc / *n*-hexane = 1:2, v/v). ^{1}H- NMR (400MHz, DMSO-d6): δ9.33 (s, 1H), 8.27 (s,1H), 7.34-7.33(d,J=7.2Hz,2H), 7.01-6.99(d,J=7.1Hz, 1H), 4.37-4.34(d,J=7.2Hz, 1H), 3.87-3.83(d,J=7.2Hz, 1H), 3.11-3.05(t,J=7.5Hz, 1H), 2.93-2.88(t,J=7.5Hz, 1H), 2.66-2.59(t,J=7.4Hz,2H), 2.53(s,3H), 1.82-1.79(d, J=5.2Hz,5H), 1.74(s,4H), 1.64-1.49(m, 3H), 1.44(s, 9H).

^{13}C NMR (400MHz, DMSO-d6): δ172.5 (NAc-C=O),155.5(AmideC=O),152.5(NHBoc C=O),141.8(Ar-C), 130.3(Ar-C),125.2(Ar-C),122.6(Ar-C),121.4(Ar-C),120.3(Ar-C), 79.5(NHBoc-C),46.1(CH),43.7(CH_2), 38.4(CH), 34.6(CH_2), 29.6(CH_2),28.4(NHBoc-CH_3),25.5(CH_2),21.1(NAc-CH_3). HRMS (EI) Calcd. for $C_{24}H_{35}N_3O_4$ (M^+)429.55: Found:429.54.

Tert-butyl 2-(chroman-3-carboxamido)4-cyclopentylphenylcarbamate(6c)
White solide,Yield: 65%. m.p. 213.3^{0}C. R*f*= 0.63 (EtOAc / *n*-hexane = 1:2, v/v). ^{1}H NMR (400MHz,DMSO-d6) δ: 9.56 (s,1H), 8.32(s, 3H), 6.87-6.77(m, 2H),4.46-4.43(d,J=7.2Hz, 1H), 4.01-3.96(t,J=8.0Hz, 1H), 3.11-2.89(m, 4H), 1.96(bs, 2H), 1.74(s, 2H), 1.52(s, 4H), 1.46(s, 9H).

^{13}C NMR (400MHz, DMSO-d6): δ174.5(Amide C=O), 161.0(Ar-C), 152.6(NHBoc C=O), 141.8(Ar-C),135.5(Ar-C),130.1(Ar-C),126.6(Ar-C),125.0(Ar-C), 122.6(Ar-C), 122.3(Ar-C) , 121.4(Ar-C), 121.1(Ar-C), 120.4(Ar-C), 120.1(Ar-C),79.5(NHBoc-C),65.3(CH_2),47.8(CH), 46.1(CH),34.6(CH_2), 28.8(CH_2),28.3(NHBoc-CH_3),25.5(CH_2). HRMS (EI) Calcd. for $C_{26}H_{32}N_2O_4$ (M^+)436.53: Found:436.52.

Tert-butyl 2-(2-(trifluoromethoxy)cyclohexanecarboxamido)-4 Cyclopentylphenylcarbamate(6d)
Pale yellow solid,Yield: 69%. m.p. 317.6^{0}C. R*f*= 0.53 (EtOAc / *n*-hexane = 1:2, v/v). ^{1}H NMR (400MHz,DMSO-d6): δ9.89 (s, 1H), 8.51(s, 1H), 8.09(d, J=7.2Hz, 2H), 7.63(t,J=7.5Hz, 2H), 7.43(m, 2H), 7.23(s, 1H), 2.93(m, 1H),2.13(bs, 2H), 1.79(m, 2H), 1.69(m, 2H), 1.49(m, 2H), 1.43(s, 9H).

^{13}C NMR (400MHz, DMSO-d6): δ173.0(Amide C=O), 153.2(NHBoc C=O), 141.8(Ar-C),130.3(Ar-C),125.6(-CF_3),125.3(Ar-C),122.6(Ar-C), 121.5(Ar-C), 119.3(Ar-C) , 79.5(NHBoc-C), 64.1(CH), 46.1(CH), 42.4(CH), 34.6(CH_2), 30.8(CH_2), 28.4(NHBoc-CH_3),25.5(CH_2), 25.1(CH_2), 23.8(CH_2). HRMS (EI) Calcd. for $C_{24}H_{33}F_3N_2O_4$ (M^+)470.53: Found:470.52.

Tert-butyl 2-(2,3-dihydro-1H-indene-1-carboxamido)-4-cyclopentylphenylcarbamate(6e)
Brick red solid, Yield: 64%. m.p. 323.67^{0}C. R*f*= 0.68 (EtOAc / *n*-hexane = 1:2, v/v). ^{1}H NMR (500MHz,DMSO-d6): δ9.71 (s, 1H), 8.12(s, 1H), 7.37-7.35(d,J=7.2Hz, 3H), 7.32-7.31(d, J=7.2Hz,1H), 7.26-7.13(m, 2H),7.03-7.01(d, J=7.2Hz, 1H), 4.14-4.11(t, J=8.0Hz, 1H), 3.06-3.00(m, 1H), 2.92-2.85(m, 2H), 2.32-2.28(m, 2H), 1.96-1.95(d, J=7.0Hz 2H), 1.76-1.64(m, 2H), 1.65-1.46(m, 4H), 1.42(s, 9H).

^{13}C NMR (400MHz, DMSO-d6): δ172.0(Amide C=O), 152.5(NHBoc C=O), 145.7(Ar-C),143.1(Ar-C),143.1(Ar-C),141.5(Ar-C), 129.7(Ar-C), 128.3(Ar-C) , 126.1(Ar-C), 125.2(Ar-C), 122.6(Ar-C), 121.3(Ar-C), 119.5(Ar-C),79.5(NHBoc-C), 49.0(CH),46.1(CH), 34.6(CH), 33.7(CH_2), 29.8(CH_2),28.4(NHBoc-CH_3),25.5(CH_2). HRMS (EI) Calcd. for $C_{26}H_{32}N_2O_3$ (M^+)420.54: Found:420.52.

Tert-butyl 4-cyclopentyl-2-(tetrahydro-2H-pyran-4carboxamido) phenylcarbamate(6f)
Block solid, Yield: 66%, m.p. 363.17^{0}C; R*f*= 0.45 (EtOAc / *n*-hexane = 1:2, v/v).^{1}H NMR (500MHz,DMSO-d6): δ9.27 (s, 1H), 8.23(s, 1H), 7.33-7.32(d, J=7.2Hz, 2H),6.99-6.98(d, J=7.2Hz, 1H), 3.89-3.87(d, J=7.2Hz, 2H),3.36-3.32(t, J=7.5Hz, 2H), 2.91-2.88(t, J=8.0Hz, 1H), 2.63-2.59(t, J=8.0Hz ,1H), 1.96-1.95(d, J=3.2Hz ,4H), 1.76-1.46(m, 8H), 1.42(s, 9H).

^{13}C NMR (400MHz, DMSO-d6): δ172.9(Amide C=O), 152.5(NHBoc C=O), 141.8(Ar-C),130.3(Ar-C),125.2(Ar-C),122.6(Ar-C), 121.4(Ar-C), 119.1(Ar-C), 79.5(NHBoc-C), 66.0(CH_2),46.1(CH), 37.4(CH), 34.7(CH_2), 28.4(NHBoc-CH_3),28.2(CH_2), 25.5(CH_2). HRMS (EI) Calcd. for $C_{22}H_{32}N_2O_4$ (M^+)388.52: Found:388.51.

Tert-butyl 2-(cyclohex-1-enecarboxamido)-4-cyclopentylphenylcarbamate(6g)
Light red solid, Yield: 68%. m.p. 323.67^{0}C; . R*f*= 0.68 (EtOAc / *n*-hexane = 1:2, v/v). ^{1}H NMR (500MHz,DMSO-d6): δ9.09 (s, 1H), 8.57(s, 1H), 7.40(s, 1H), 7.28-7.26(d,J=7.2Hz, 1H), 7.02-7.00(d, J=7.2Hz, 1H), 6.71(s, 1H), 2.94-2.91 (t, J=8.0Hz, 1H), 2.25-2.18(t, J=5.2Hz, 4H), 1.99-1.98(d, J=7.2Hz ,2H), 1.78-1.75(t, J=7.5Hz, 2H), 1.64-1.48(m, 8H), 1.45(s, 9H).

^{13}C NMR (400MHz, DMSO-d6): δ163.2(Amide C=O), 152.5(NHBoc C=O), 141.8(Ar-C), 139.9(=CH_2), 132.5(=CH_2), 129.3(Ar-C), 125.5(Ar-C), 122.6(Ar-C), 121.4(Ar-C), 119.8(Ar-C), 79.5(NHBoc-C), 46.3(CH), 34.6(CH_2), 28.4(NHBoc-CH_3),25.5(CH_2), 25.0(CH_2), 23.2(CH_2), 22.0(CH_2), 21.3(CH_2). HRMS (EI) Calcd. for $C_{23}H_{32}N_2O_3$ (M^+)385.53: Found:385.52.

Tert-butyl 2-(2-phenylacetamido-4-cyclopentylcarbamate(6h)
Pink solid, Yield: 69%, m.p. 347.05^{0}C;. R*f*= 0.57 (EtOAc / *n*-hexane = 1:2, v/v). ^{1}H NMR (400MHz,DMSO-d_6): δ9.64(s, 1H), 8.14(s, 1H), 7.39-7.25(m, 7H), 7.03-7.01(d, J=7.2Hz, 1H), 3.67(s, 2H), 2.94-2.86(m, 1H), 1.98-1.95(t, J=5.2Hz, 2H), 1.48-1.46(t, J=5.2Hz, 2H), 1.43(s, 9H).

^{13}C NMR (400MHz, DMSO-d6): δ168.9(Amide C=O), 152.5(NHBoc C=O), 141.8(Ar-C), 135.6(Ar-C),134.0(Ar-C), 129.6(Ar-C), 129.2(Ar-C), 127.6(Ar-C), 125.2(Ar-C), 122.6(Ar-C), 121.3(Ar-C), 119.3(Ar-C), 79.5(NHBoc-C), 46.1(CH),44.2(Ar-CH_2), 34.6(CH_2), 28.4(NHBoc-CH_3),25.5(CH_2), HRMS (EI) Calcd. for $C_{24}H_{30}N_2O_3$ (M^+)394.51: Found:394.50.

Tert-butyl 2-(4-chlorobenzamido)-4-cyclopentylphenylcarbamate(6i)
Light pink solid, Yield: 71%. m.p. 289.35^{0}C. R*f*= 0.55 (EtOAc / *n*-hexane = 1:2, v/v). IR (KBr,cm-1) ^{1}H NMR (500MHz,DMSO-d6) : δ9.87 (s, 1H), 8.52(s, 1H), 7.91-7.89(d,J=7.2Hz, 2H), 7.77-7.75(d, J=7.2Hz, 2H), 7.43-7.39(t, J=5.2Hz, 2H), 7.10-7.08(m, 1H), 2.99-2.92(m, 1H), 2.01-1.99(t, J=5.2Hz 2H), 1.79-1.72(m, 2H), 1.68-1.60(m, 2H), 1.55-1.46(m, 2H), 1.43(s, 9H).

^{13}C NMR (400MHz, DMSO-d6): δ172.5(Amide C=O), 152.5(NHBoc C=O), 141.8(Ar-C), 137.7(Ar-Cl),130.1(Ar-C), 128.9(Ar-C), 127.5(Ar-C), 125.2(Ar-C), 122.5(Ar-C), 121.3(Ar-C), 119.5(Ar-C), 79.5(NHBoc-C), 46.1(CH), 34.6(CH_2), 28.4(NHBoc-CH_3),25.5(CH_2). HRMS (EI) Calcd. for $C_{23}H_{27}ClN_2O_3$ (M^+)414.92: Found:414.90.

Tert-butyl 2-(4-bromobenzamido)-4-cyclopentylphenylcarbamate(6j)
Brown solid, Yield: 70%, m.p. 347.05^{0}C; R*f*= 0.57 (EtOAc / *n*-hexane = 1:2, v/v). ^{1}H NMR (400MHz, DMSO-d6): δ9.80 (s, 1H), 8.50(s, 1H), 8.09(d,J=7.2Hz, 2H), 7.63(t, J=5.2Hz, 2H), 7.43(m, 2H), 7.23(s, 1H), 2.93(m, 1H),2.13(bs, 2H), 1.79(m, 2H), 1.69(m, 2H), 1.49(m, 2H), 1.43(s, 9H).

^{13}C NMR (400MHz, DMSO-d6): δ172.3(Amide C=O), 152.2(NHBoc C=O), 141.5(Ar-C), 137.4(Ar-C-Cl),130.1(Ar-C), 128.5(Ar-C), 127.3(Ar-C), 126.5(Ar-Br), 122.3(Ar-C), 120.1(Ar-C), 119.3(Ar-C), 79.5(NHBoc-C), 46.1(CH), 34.6(CH_2), 28.4(NHBoc-CH_3),25.5(CH_2). HRMS (EI) Calcd. for $C_{23}H_{27}BrN_2O_3$ (M^+)459.32: Found:459.31.

Tert-butyl 2-(4-methoxybenzamido)-4-cyclopentylphenylcarbamate(6k)
Red solid, Yield: 71%, m.p. 307.09^{0}C; R*f*= 0.58 (EtOAc / *n*-hexane = 1:2, v/v).^{1}H NMR (400MHz,DMSO-d6): δ9.64(s, 1H), 8.14(s, 1H), 7.39-7.25(m, 6H), 7.03-7.01(d,J=7.2Hz, 1H), 3.67(s, 3H), 2.94-2.86(m, 1H), 1.98-1.95(t, J=5.2Hz 2H), 1.48-1.46(t, J=5.2Hz, 2H), 1.43(s, 9H).

^{13}C NMR (400MHz, DMSO-d6): δ173.2(Amide C=O), 164.0(Ar-C), 152.5(NHBoc C=O), 141.8(Ar-C), 130.1(Ar-C), 128.5(Ar-C), 127.2(Ar-C), 126.4(Ar-C-Br), 124.3(Ar-C), 121.5(Ar-C), 119.4(Ar-C), 79.5(NHBoc-C),55.8(Ar-O-CH_3), 46.1(CH), 34.6(CH_2), 28.4(NHBoc-CH_3),25.5(CH_2). HRMS (EI) Calcd. for $C_{24}H_{30}N_2O_4$ (M^+)410.51: Found:410.50.

MOLECULAR MODELING STUDIES

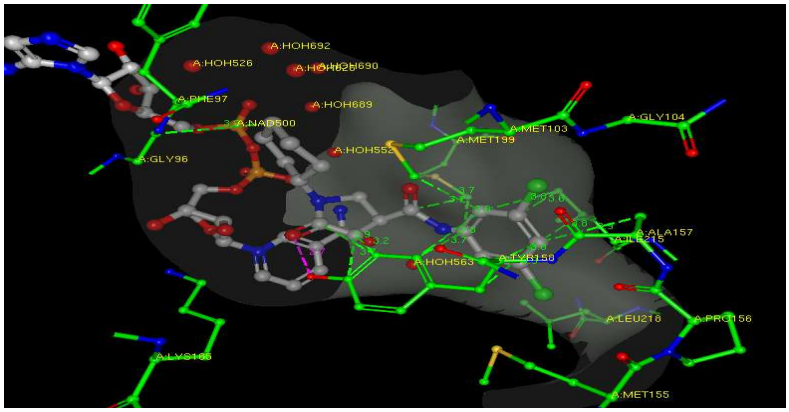


Figure 01: Active site amino acids of crystallographic protein (2H7M)

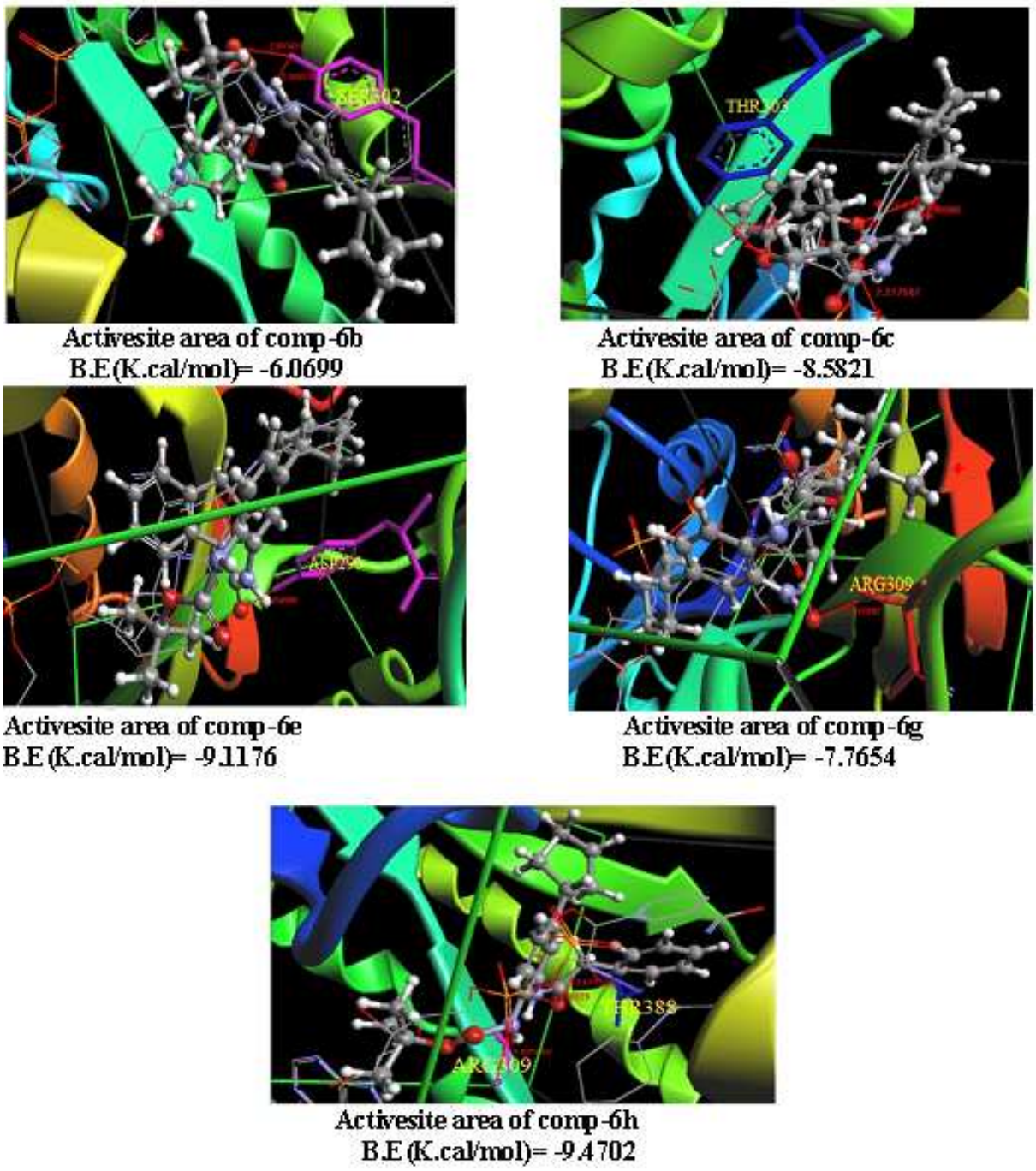


Figure 02: Binding orientations of database Ball cylinder low model compounds 6b, 6c, 6e, 6g & 6h crystallographic conformation of active site (PDB ID 2H7M)

Hydrogen bonds are shown in red colour dotted lines

Table- 3b: Chemscore of antibacterial activity of amide derivatives (6a-k)

Comp	Score	DG	S(hbond)	S(metal)	S(lipo)	DE(clash)	DE(int)
6a	19.78	-42.04	0.91	0.00	314.16	3.23	19.03
6b	34.79	-46.31	2.70	0.00	302.61	1.37	10.16
6c	27.16	-43.09	0.99	0.00	322.57	1.63	14.29
6d	9.10	-40.28	0.71	0.00	317.77	0.83	30.35
6e	31.13	-45.05	0.96	0.00	339.22	0.25	13.67
6f	0.52	-33.05	0.00	0.00	265.43	0.07	32.46
6g	23.45	-42.44	0.73	0.00	321.65	0.64	18.35
6h	21.69	-42.40	0.99	0.00	318.15	0.07	20.64
6i	-1.94	-46.88	1.93	0.00	325.55	1.77	47.05
6j	4.29	-45.96	1.92	0.00	318.11	1.85	39.83
6k	-8.08	-46.71	1.78	0.00	332.31	2.08	52.72

ChemScore = $\Delta G_{binding}$+ P_{clash}+$C_{internal}P_{internal}$ + ($C_{covalent}P_{covalent}$+$p_{constraint}$)

Score = -(DG + DE(clash) + DE(int))

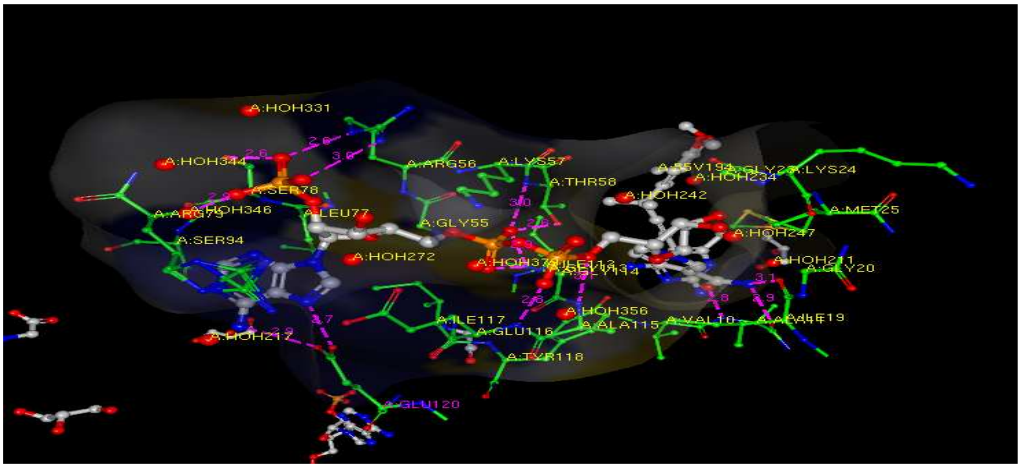


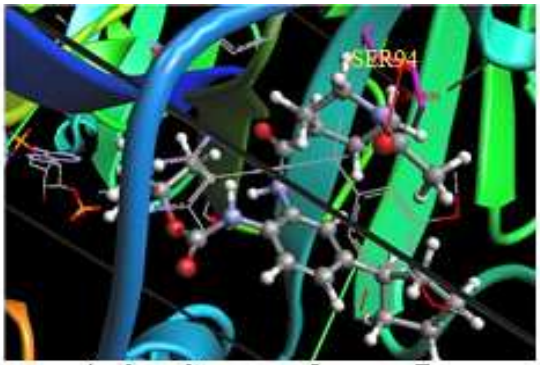
Figure 03: Active site amino acids of crystallographic protein (3QLS)

Table -4: Chem score of antifungal activity of amide derivatives (6a-k).

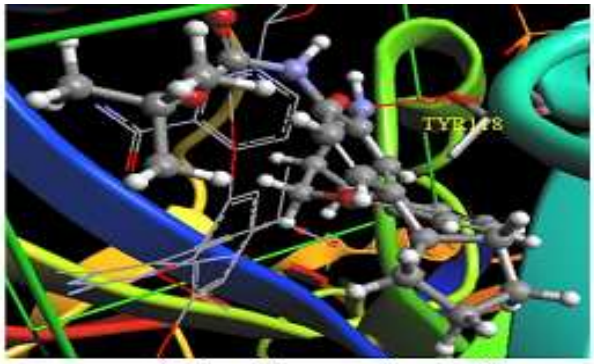
Comp	Score	DG	S(hbond)	S(metal)	S(lipo)	DE(clash)	DE(int)
6a	13.80	-35.84	1.05	0.00	257.08	1.64	20.40
6b	26.61	-34.39	0.98	0.00	249.86	0.04	7.74
6c	22.09	-40.69	1.27	0.00	293.89	5.42	13.18
6d	4.28	-34.02	0.97	0.00	256.81	0.19	29.56
6e	21.87	-39.31	0.97	0.00	289.82	0.92	16.52
6f	1.13	-35.39	1.48	0.00	243.10	0.59	33.66
6g	19.22	-37.49	0.94	0.00	273.29	0.18	18.09
6h	16.26	-41.55	0.62	0.00	321.45	3.03	22.25
6i	-8.95	-35.99	0.00	0.00	287.64	0.12	44.82
6j	-2.95	-37.76	0.93	0.00	276.26	0.25	40.47
6k	-18.50	-35.08	0.00	0.00	283.55	1.27	52.31

Chemscore = $\Delta G_{binding}$+ P_{clash}+$C_{internal}P_{internal}$ + ($C_{covalent}P_{covalent}$+$p_{constraint}$)

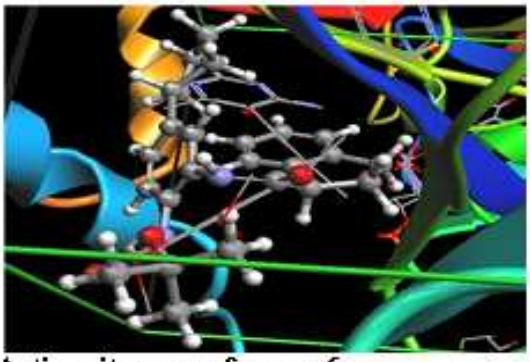
Score = -(DG + DE(clash) + DE(int))



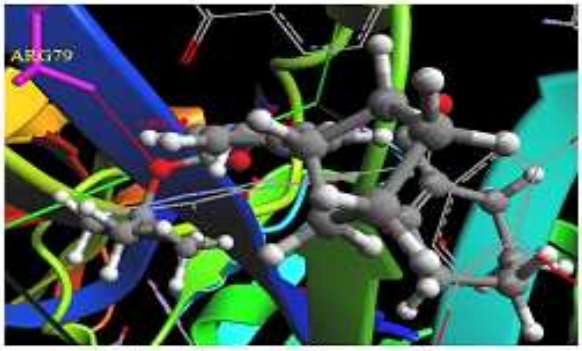
Active site area of comp-6b
B.E(K.cal/mol)= -9.7529



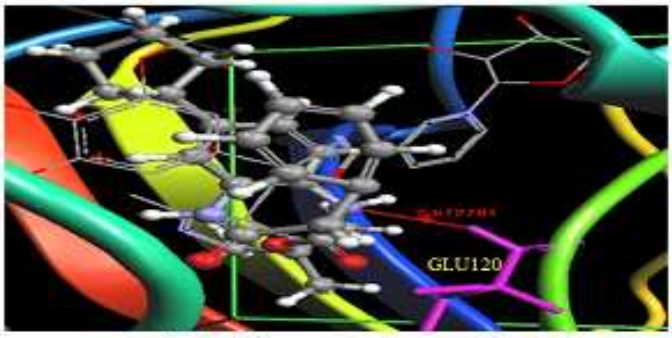
Active site area of comp-6c
B.E(K.cal/mol)= -11.35



Active site area of comp-6e
B.E(K.cal/mol)= -12.2305



Active site area of comp-6g
B.E(K.cal/mol)= -11.6769



Active site area of comp-6h
B.E(K.cal/mol)= -10.404

Figure 04: Binding orientations of database Ball cylinder low model compounds 6b, 6c, 6e, 6g & 6h crystallographic conformation of active site (pdb id 3QLS)

Hydrogen bonds are shown in red colour dotted lines.

Table-5: Binding energy values of amide derivatives (PDB ID 2H7M)

S.NO	compound	Argus B. E(K.cal/mol)	Elapsed time(seconds)
1	**6a**	-9.8499	14
2	**6b**	-6.0699	13
3	**6c**	-8.5821	14
4	**6d**	-7.1544	14
5	**6e**	-9.1176	14
6	**6f**	-8.1413	12
7	**6g**	-7.7654	13
8	**6h**	-9.4702	14
9	**6i**	-7.8462	12
10	**6j**	-6.6706	12
11	**6k**	-6.4983	13

Table- 6: Binding energy values of amide derivatives (with PDB ID 3QLS)

S.NO	compound	Argus B. E (K.cal/mol)	Elapsed time (seconds)	Ga dock energy (K.cal/mol)	Elapsed time (seconds)
1	**6a**	-9.9788	5	-7.9054	15
2	**6b**	-9.7529	5	-8.1065	14
3	**6c**	-11.3558	4	-8.3382	13
4	**6d**	-10.2375	3	-4.5774	15
5	**6e**	-12.2305	4	-3.6571	16
6	**6f**	-9.7127	4	-5.2726	12
7	**6g**	-11.6769	3	-0.7067	14
8	**6h**	-10.404	4	-9.9450	13
9	**6i**	-10.778	3	-9.4377	13
10	**6j**	-11.5455	3	-5.1095	14
11	**6k**	10.2845	4	-5.5439	13

In addition to the synthetic work, an exploration of the docking studies of tested tert-butyl 2, 4-disubstituted carboxamido phenylcarbamate derivatives was made to explain the observed variance in their antimicrobial activity. This predicts the best drug candidate providing an insight into the substitutional and configurational requirements for optimum receptor pit which leads to the development of best pharmacophore activity. In the present study, version 2.0 of the GOLD (Genetic optimization for ligand docking) docking program was used. The Gold program uses a genetic algorithm (GA) to explore the full range of ligand flexibility and the rotational flexibility of selected receptor hydrogens[23].The interaction of the ligand with the receptor in the modeled complexes is investigated and observed the fitness function ability of oxidoreductase protein by different inhibitors. The 3D structures of selected Proteins [Escherichia coli **(2H7M)**], [Candida albicance **(3QLS)**] were selected from PDB Bank RCSB [24] with an X-ray resolution in the ranges of 1.62Å, 1.72Å.Among the above proteins **(2H7M)** was screened based on the fitness score. All docking runs were carried out using standard default settings with a population size of 100, a selection pressure of 1.1, a maximum of 100000 operations, number of islands as 5, a niche size of 2, and a mutation and cross over rate of 95. These protein-ligand complexes were prepared for docking studies by adding hydrogen atoms, removing water molecules and co-crystallized inhibitors and refined by using the Deep View/SwissPdbViewer3.7 (SP5)[25].The Successful docking has been performed for the selected set of *viz;* **(6a-k)** inhibitors and their corresponding chemscore, binding energy values with their respective RMSD have been produced in the **Table3b**, **Table 4**, **Table 5 & Table 6**. Argus Lab 4.0.1[26]is molecular modeling and docking software. Argus lab was used to visualize the binding conformations of these analogs within the active site of **2H7M,3QLS** proteins and details are displayed in (**Fig.(2),(Fig (4)**.In the active site region (15Å) of **(2H7M)** protein Ile194, Lys165, Tyr158, Ser20, Ile95, Asp64, Ile21, Phe149, Gly96, Arg195, Thr196, Phe41, Asp14, Val 65 amino acids can play important role, in (3QLS) Lys192, Glu120, Tyr118, Glu116, Ala115, Gly114, Ile112, Arg79,Ser78,Thr58,Arg56,Glu32 amino acids play important role and are shown in (F**ig 1), (Fig 3).** The molecules which are exhibiting optimum lipophilic, maximum hydrogen bonding with low clash requirements are important for good fit in the active site of the target. The chemscore estimated by Gold Software was found to have a good correlation with the experimental inhibitory activity (MIC values). Docking results revealed that all the molecules have good score values with comparable binding energy values. Among these compounds, **6b, 6c, 6e, 6g & 6h** showed best docking scores with appreciable binding energy values which also exactly correlated with the experimental antibacterial biological activity. The compounds **6b, 6e** showed threshold antibacterial activity which also supported by the docking results. The compounds **6c, 6g, 6h** showed good antibacterial activity, **6a** compound showed moderate antibacterial activity which also supported by the docking results. In view of antifungal activity,

compounds **6b, 6c, 6e** showed highest activity. **6g, 6h** compounds showed good antifungal activity,**6a** compound showed moderate activity which also supported by the docking results. The tert-butyl 2,4-disubstituted carboxamido phenylcarbamate analogues **6b, 6c, 6e, 6g & 6h** showed best antifungal activity which also exactly correlated with the docking scores, binding energy values calculated with the Candida albicance**(3QLS).**Binding orientations of database ball cylinder low model with colored amino acid residues compounds **6b,6c,6e,6g,6h** with crystallographic conformation of active site **(PDB ID 2H7M,3QLS)**. Hydrogen bonds are shown in red color dotted lines. As observed in (F**ig 2), (Fig 4)** these compounds were docked in the active site of protein, with a significant different binding mode. The binding energy values of these analogues **(6a-k)** was estimated by Argus Lab 4.0.1 and values was shown in **Table 5**, T**able 6.**

Binding affinities of the amide derivatives with Autodockvina 4.0:

***Insilico* molecular docking**

The 3D structure of selected Proteins [Escherichia coli **(2H7M)**], [Candida albicance **(3QLS)**] were selected from protein data bank RCSB. The synthesized compounds which are derivatives of tert-butyl 2,4-disubstituted carboxamido phenylcarbamate **(6a-k)** were taken for prediction of 3D structure and energy was minimized for flexible docking using Argus lab. In addition, to the docking studies of GOLD, ArgusLab 4.0.1, The Autodockvina 4.0 [27] was also performed. . In this docking study receptor was treated as a rigid body and a grid potential was used to evaluate the scoring function. Here 3Dstructure of proteins [Escherichia coli **(2H7M)**], [Candida albicance **(3QLS)**] were used as receptors and all the synthesized compounds **(6a-k)** were used as ligands. In Autodockvina 4.0, non polar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. The grid calculation were set up, 3.18, 36.913, 52.02A° grid originating at 40, 40, 40with resolution of 0.375 A°, respectively, was generated around the compound. The molecular docking studies showed a good correlation between their MIC and Autodock binding free energy. Almost all the compounds(**Table 7**) used for docking showed best fit Root mean square difference (RMSD) with [Escherichia coli **(2H7M)**], [Candida albicance **(3QLS)**].Among the compounds tested for docking study, compounds **6b, 6c, 6e, 6g & 6h** showed best affinities with employed protein **(2H7M)**,compounds **6b, 6c, 6e, 6g & 6h** with **(3QLS).**

Table- 7: AutoDock4.0.1 with pdb id (3V2B),(3QLS).

Docking(K.cal/mol)	Affinity(K.cal/mol)	Docking(K.cal/mol)	Docking(K.cal/mol)
2H7M vs. **6a**	-6.95	3QLS vs. **6a**	-6.55
2H7M vs. **6b**	-7.48	3QLS vs. **6b**	-5.69
2H7M vs. **6c**	-7.18	3QLS vs. **6c**	-6.01
2H7M vs. **6d**	-5.84	3QLS vs. **6d**	-5.44
2H7M vs. **6e**	-7.11	3QLS vs. **6e**	-5.18
2H7M vs. **6f**	-6.00	3QLS vs. **6f**	-6.91
2H7M vs. **6g**	-7.15	3QLS vs. **6g**	-8.17
2H7M vs. **6h**	-6.11	3QLS vs. **6h**	-6.51
2H7M vs. **6i**	-6.14	3QLS vs. **6i**	-6.01
2H7M vs. **6j**	-	3QLS vs. **6j**	-
2H7M vs. **6k**	-5.45	3QLS vs. **6k**	-5.40

RESULTS AND DISSCUSSION

The newly synthesized tert-butyl 2, 4-disubstituted carboxamido phenylcarbamate derivatives **(6a-k)** were screened for their antibacterial and antifungal activity by Agar-diffusion method. Six bacterial strains *viz*; *Escherichia coli*, *Klebsiella pneumonia, Pseudomonas aeruginosa*,*Bacillus licheniformis*, *Bacillus subtilis, Staphylococcus aureus* and four fungal strains *viz; Aspergilus niger*, *Candida albicance*, *Fusarium oxysporum*, *Fusarium solani* were employed,the results were presented in **Table (1a, 1b,2a & 2b)**.The molecular docking studies were performed for the analysis with training set composed of newly synthesized compounds **(6a-k)** whose inhibitory activity is known, for find out the molecular facilities responsible for biological activities. The results revealed that most of tested compounds displayed variable inhibitory effects on the growth of the tested bacterial and fungal strains. The compounds **6b, 6e, 6c, 6g** & **6h** showed good activities against (inhibitory zone ≥20 mm) bacterial and fungal strains. The compounds **6b & 6e** showed highest antibacterial activity with respect to reference drug. This may be attributed that the presence of piperidine, indene moieties attached to carbonyl linkage of amide functional group. The compounds with chromene **(6c)**, cyclohexene **(6g)** and benzyl **(6h)** molecular entities which attached to carbonyl linkage of amide functional group showed promising antibacterial activity. However the compound with vinyl benzene moiety **(6a)** at amide linkage showed moderate antibacterial activity. In view of antifungal activity, compounds **6b, 6c, 6e** showed highest activity, compounds **6a 6g & 6h** showed moderate to good antifungal

activity. It is observed that the molecular entities for fungal activities same as that of the bacterial activities. The compounds **(6a-k)** used for docking showed best fit [Escherichia coli **(2H7M)**], [Candida albicance **(3QLS)**], evaluate the best GOLD score and evaluate the antimicrobial activity. In order to determine the ability of auto dock 4.0 and to reproduce the orientation and also position of compounds **(6a-k)** in (2H7M),(3QLS) proteins the tert-butyl 2,4-disubstituted carboxamido phenylcarbamate derivatives were extracted and docked back into the corresponding binding pocket. The compounds **6b, 6c, 6e, 6g & 6h** performed for docking study and showed best affinities with low energy of **-7.48,-7.18,-7.11,-7.15,-6.11**.kcal/mol against employed protein **(2H7M)**,**-5.69,-6.01,-5.18,-8.17,-6.51** kcal/mol against **(3QLS)** which was also correlated by experimental antibacterial activity. The results were correlated with *invitro* antimicrobial activity and it was supported by docking studies.

CONCLUSION

In conclusion a number of **(6a–k)** carbamate derivatives have been synthesized and characterized by spectroscopic data. On antibacterial and antifungal screening of **(6a-k)** respectively, compounds **6b & 6e** exhibited good antibacterial activity and compounds **6b,6c,6e** exhibited good antifungal activity.MIC determination experiment concluded that the compounds **6b & 6e** are more potent. The molecular modeling studies were applied to newly synthesized tert-butyl 2,4-disubstituted carboxamido phenylcarbamate derivatives **(6a-k)**.From the modeling studies we concluded that experimental biological data was correlated with docking studies.

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