1-(2-(METHYLAMINO)PHENYL) UNDECAN – 1 – ONE : A POTENT DRUG MOLECULE FOR PEPTIC ULCER?

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ABSTRACT

1-(2-(methylamino)phenyl) undecan – 1 – one was isolated from leaves an Indian traditional medicinal plant *Pajanelia longifolia* (Wild) K. Schuman belonging to the family Bignoniaceae. Based on the traditional report of medicinal utility, the leaves of the plant were subjected to phytochemical analysis. Using Thin Layer Chromatography (TLC) and Column Chromatography (CC), bioactivity guided fractionation was done and it was observed that fraction I possesses potential microbial agent against *Staphyllococcus sp., Klebsiella sp.* and *Candida albicans.*

Applying Spectroscopic techniques, structure of fraction I was determined as 1-(2-(methylamino)phenyl) undecan – 1 – one.

In silico screening of the compound for determination of possible mechanism of action against microbes failed to locate any specific receptor but the compound randomly docked with undecaprenyl pyrophosphate synthase of *Helicobacter pylori*, a causal agent of peptic ulcer. Interestingly, this enzyme is responsible for synthesis of cell wall material in this bacteria. 1-(2-(methylamino)phenyl) undecan – 1 – one may therefore, be used to block synthesis of cell wall material in *H. pylori* and thus may be a potential drug molecule for treatment of peptic ulcer. However, ADME Tox software prediction and confirmatory studies from wet lab are still under progress.

Key words: 1-(2-(methylamino)phenyl) undecan – 1 – one, undecaprenyl pyrophosphate synthase, *Helicobacter pylori*, peptic ulcer.

INTRODUCTION

In the era of post genomic scientific research after completion of Human Genome Project, the concept of drug discovery has been changed drastically. Recent advances in disciplines like biology, chemistry, pharmacology and computer aided drug designing and their integration has changed the face of drug discovery research. Undoubtedly these advances have facilitated the translation of vast information of human body in developing target oriented drugs.

Computer aided drug discovery is a complex process and generally starts with identification of a compound (ligand) that binds with a target (receptor) on a simple computer screen. (Lang et al., 2009). The molecules that show good affinity to any receptor are called “hits” and the compounds which show low toxicity, and sufficient aqueous solubility to be orally active are often called “lead”. Generally hits are identified by screening while the leads are developed from natural products or chemical synthesis. Screening involves large number of compounds either of natural product origin or synthesized or even collected from databases. For targets enzymes are generally used during screening and the best compounds are moved forward in a process aimed at modifying their chemical structure to improve their potency, specificity and *in vivo* activity. Synthetic methods include combinatorial chemistry and library synthesis (Lang et al., 2007).

The use of plant extracts or compounds of natural product origin in treatment of diseases is not new but still remains significant even in the era of post genomic research. Various plants have been used for centuries in the treatment of various infectious diseases and plants with antimicrobial activity are also known to be numerous (Walsh and Groll, 1999; Fleming et al., 2002). The search for novel antimicrobial drug has been influenced by ethnobotanical information and such studies have been successful with a high degree of correlation between

traditional knowledge and laboratory analysis (Mc Cutcheon et al., 1994; Bergeron et al., 1996; Jones et al., 2000).
In silico drug discovery process involves identification of a proper lead compound for a given molecular target which is critical and analysis of a compound to be probable drug which is fundamental and in this process of identification of proper lead compound, the plant kingdom is considered to be a vast repository.
In the present work, *Pajanelia longifolia* (Wild) K. Schuman a traditional medicinal plant of North East India whose leaves and fruits are often consumed as vegetables has been considered for isolation and identification of bioactive molecules present in it. This study also aimed at antimicrobial activity assessment of the isolated compound and *in silico* screening of the compound(s) isolated to predict suitable receptor for the ligand(s).

MATERIAL AND METHODS

As per the accepted international protocol, 5 kg of the shade dried and milled leaf material was extracted with petroleum ether (60-80°C) followed by extraction with ethyl acetate and 70% (v/v) ethanol using a Soxhlet Apparatus. The fractions were evaporated in a Rotary Evaporator to obtain final crude extract. The crude extract was subjected to column chromatography (CC) to obtain Fraction 1. The purity of the fraction was tested by Thin Layer Chromatography (TLC) and confirmed by using Gas Chromatography (GC). The CC fraction (Fraction 1) was further subjected to Preparatory Thin Layer Chromatography (PTLC) to obtain pure form.
5 mm filter paper discs were prepared with pure fraction by keeping them in the fraction overnight. The discs were then applied over pure culture of test pathogen *Staphyllococcus sp, E. coli, Klebsela sp* and *Candida albicans* and incubated at ± 28°C. After 48 hours of incubation, zone of inhibition formed by each disc was measured in mm. Part of the pure faction was subjected to spectroscopic study (FT-IR, NMR and GC-MS) for structure elucidation. CHN analysis of the compound was also done for determination of percentages of carbon, hydrogen, nitrogen and sulphur.
For *in silico* screening three dimensional structure of the ligand was obtained using software Chem Office. Receptors were downloaded from Protein Data Bank (PDB) and docking was performed using software Autodoc 4. Active site of the receptor was visualized using UCSF Chimera.

RESULTS AND DISCUSSION

Wet lab results of antimicrobial activity test of fraction I i.e. compound **1-(2-(methylamino)phenyl) undecan – 1 – one** has been represented in Table I and Fig. I. Sensitivity of the same pathogen against some standard antibiotics is represented in Fig. II. It is seen that the compound is active against all the tested pathogens *Staphyllococcus sp, Klebsiella sp* and *Candida albicans* in varying degrees except *E. coli*. However, sensitivity of pure compound was not greater than standard anibiotics viz. Amikacin, Gentamicin and Cephalexin used as control.

Table1

Name of Pathgen	Average Zone of Inhibition (mm)			
	Ethyl Acetate Extract	Amikacin	Gentamicin	Cephalexin
1. *Staphyllococcus sp*	09	22	18	23
2. *Candida albicans*	8.2	32	38	20
3. *E. coli*	00	00	25	22
4. *Klebsiella sp*	8.2	38	30	30

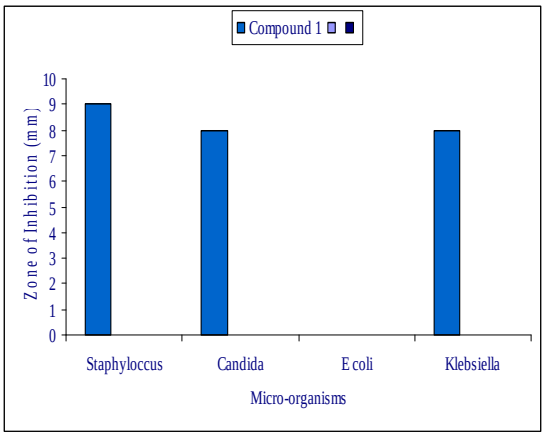


Figure 1

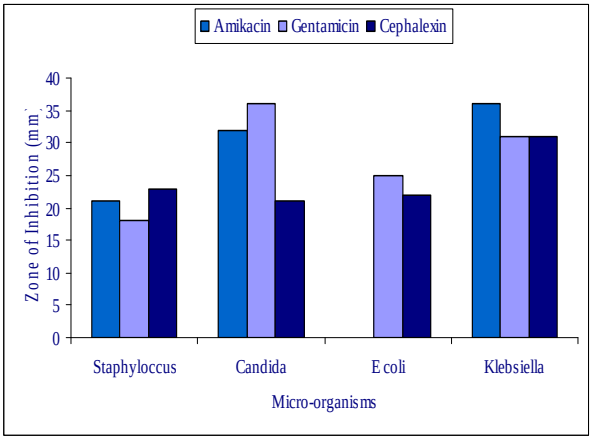


Figure 2

Fig. 1. shows the anti-microbial activity of the Compound 1 showed depicting high anti-microbial activity. Highest activity was recorded in case of *Klebsiella sp.* followed by *Candida* and *Staphylococcus sp.* No activity was recorded for *E. coli.* Fig. 2. illustrates the zone of inhibition by standard antibiotics.

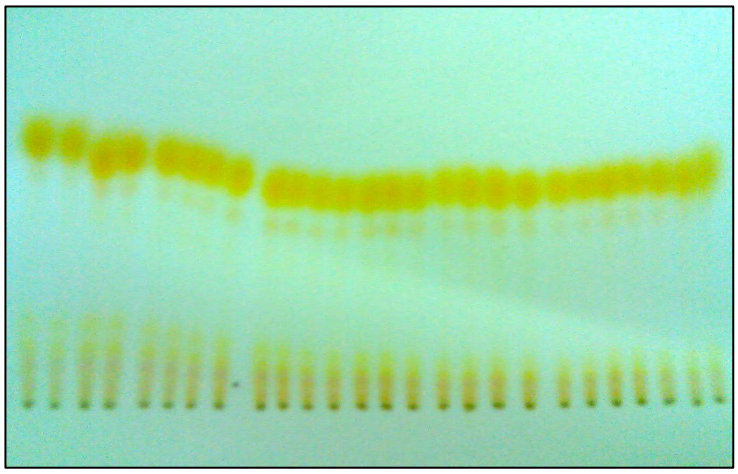


Figure 3 PTLC Separation of Compound 1 using hexane as eluent

Physical feature of the pure compound after PTLC separation was recorded as follows:

Compound 1
State: Semi-solid
Colour: Wine Red
Solubility: Ethyl acetate, methanol
TMS, DMSO and CHCl3

The EIMS of the Compound showed the molecular ion peak at m/z = 275. From the CHN analysis of the compound (C = 78.5%, H = 10.68%, N = 5.11% and O = 5.71%) correlated with the mass corresponding to the molecular formula of the compound **C18H29NO.** Band assignment of FT-IR spectra of the compound I was done as follows:

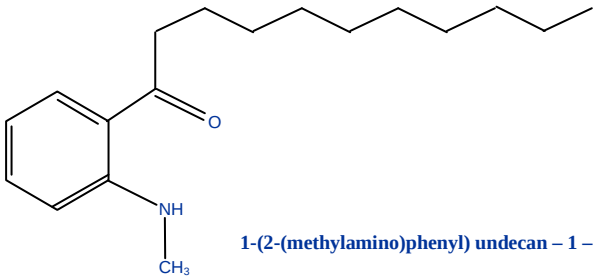
Bands (cm-1)	Assignments
3320	20 aromatic amine (N-H)
~3008	Benzene Ring
1713	C = O of ester group
1461	- CH2 sesioring
2995	Aliphatic –CH structure asymmetric for CH3
2925	Aliphatic – CH structure asymmetric for CH2
2854	Aliphatic – CH structure symmetric for CH2
1272	C-N (20 aromatic amine)
742	C-H blending for 1,2-disubstituted benzene ring

The ^{13}C NMR spectrum showed the signal for carbon of 14 different environments. The presence of lesser number of peaks in ^{13}C NMR spectrum (compared to number of carbons in the molecular formula) indicated some of the carbons having chemical shift. The peaks at δ 115.4 ppm, 130.0 ppm, 117.8 ppm, 134.2 ppm, 114.0 ppm and 147.1 ppm attributed to the six carbon of the aromatic system, where as the peaks at further downfield region (298.7 ppm) to ketonic carbon.

Carbon	δ (ppm)	Carbon	δ (ppm)
1	115.4	10	29.0
2	130.0	11	29.5
3	117.8	12	29.6
4	134.2	13	29.2
5	114.0	14	29.5
6	147.1	15	30.9
7	200.1	16	22.3
8	38.8	17	14.0
9	24.8	18	28.7

Based on ^{13}C NMR and FT-IR band assignment and on Molecular Mass given by Mass Spectrometer, tentative structure of the compound in fraction I is determined as follows--

Compound 1



1-(2-(methylamino)phenyl) undecan – 1 – one

Compound 1: Wine red semi soild material; EIMS m/z of 275; IR (KBr) *Vmax* (cm^{-1}): 3320; ~3008, 1713; 1416; 2995; 2925; 1272; 742; ^{1}H NMR (400 MHz $CDCl_3$) δ (ppm) 0.8, 1-2, 2.3, 5.4, 7.3-7.4; ^{13}C NMR (400 MHz $CDCl_3$) δ (ppm) 115.4, 130.0, 117.8, 134.2, 114.0, 147.1, 200.1, 38.8, 24.8, 29.0, 29.5, 29.6, 29.6, 29.2, 29.5, 30.9, 22.3, 14.0, 28.7

Visualization of binding of undecaprenyl pyrophosphate synthase of *Helicobacter pyroli* with ligand has been shown in Fig. IV and V. Fig. VI represents the involvement of undecaprenyl pyrophosphate synthase enzyme in biosynthesis of peptidoglycan – the precursor for synthesis of cell wall material in *Helicobacter pulori.* Fig. VII shows the different amino acids of the binding sites of undecaprenyl pyrophosphate synthase of *Helicobacter pyroli* after binding of the ligand. The data presented are the results of Docking with AutoDock 4.

Estimated Free Energy of Binding during docking was -1.51 kcal/mol. This indicates that the compound 1-(2-(methylamino)phenyl) undecan – 1 – one possesses good binding affinity with undecaprenyl pyrophosphate synthase.

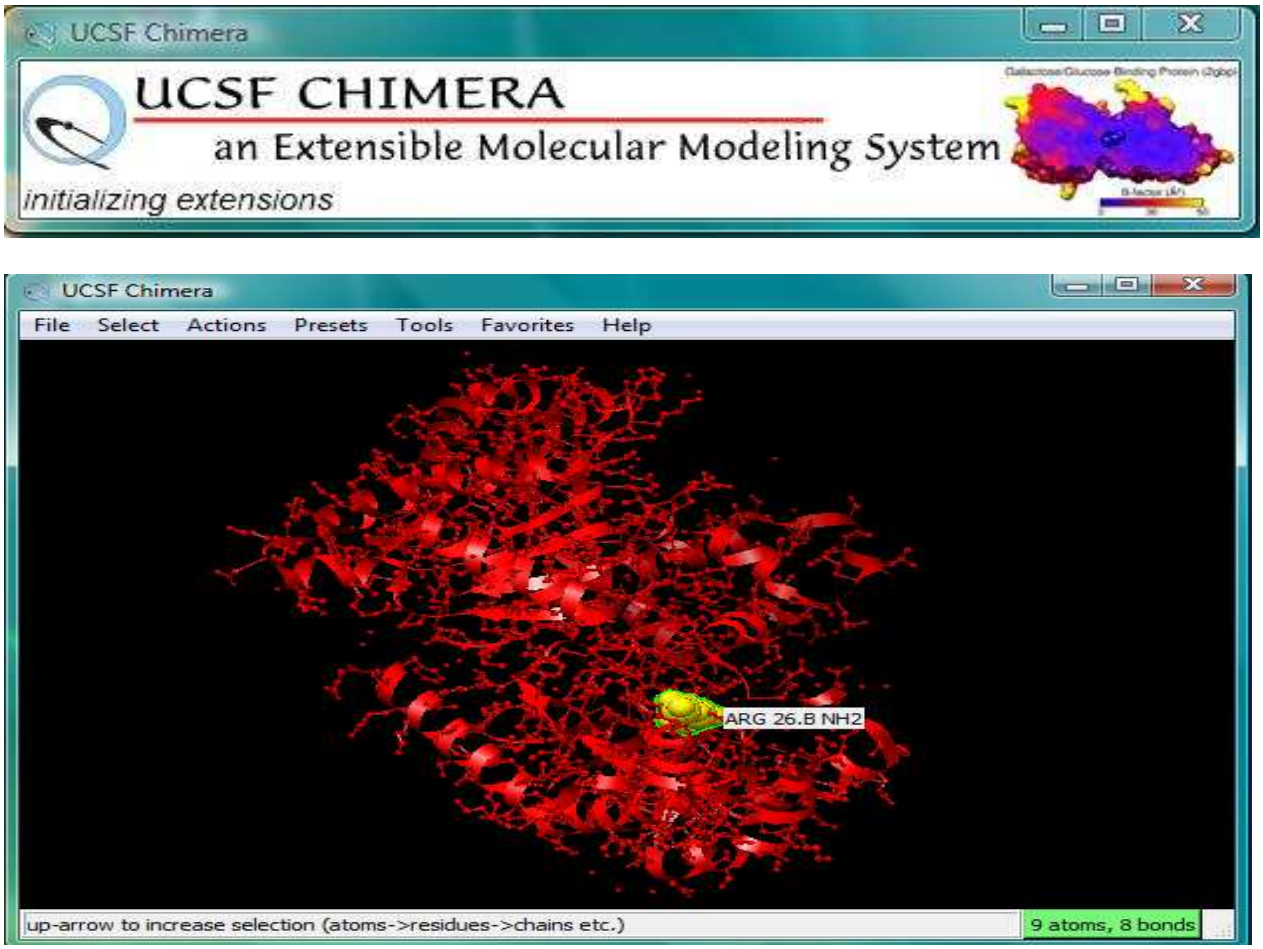
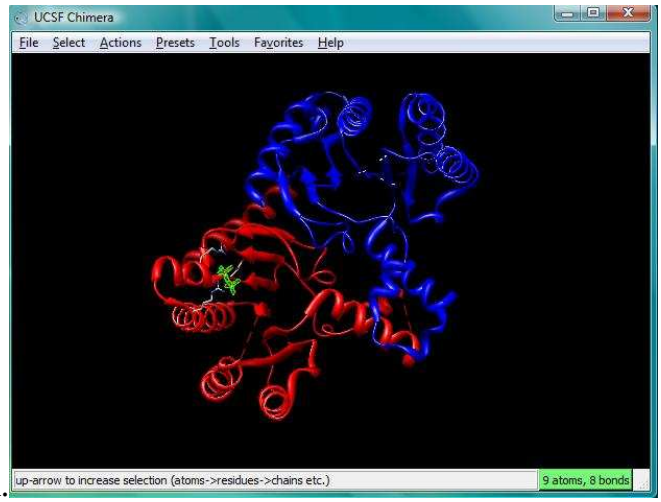
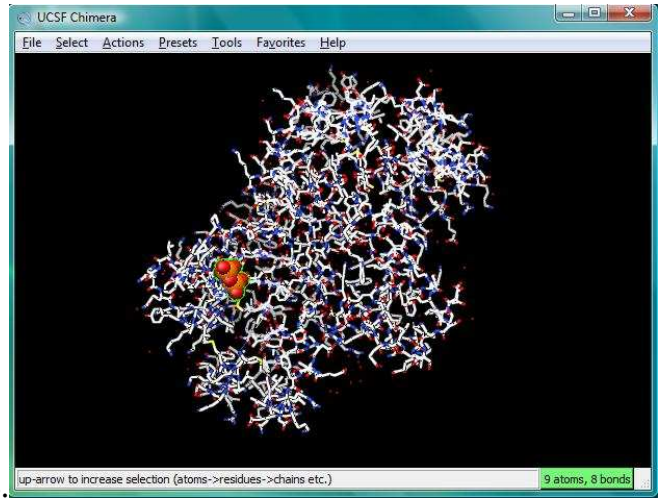


Figure 4 Visualization of undecaprenyl pyrophosphate synthase of *Helicobacter pylori* complexed with ligand in SCSF CHIMERA molecular modeling system. The ligand bound to the receptor is depicted in yellow with florescent green boundary.

A.



B.



C.

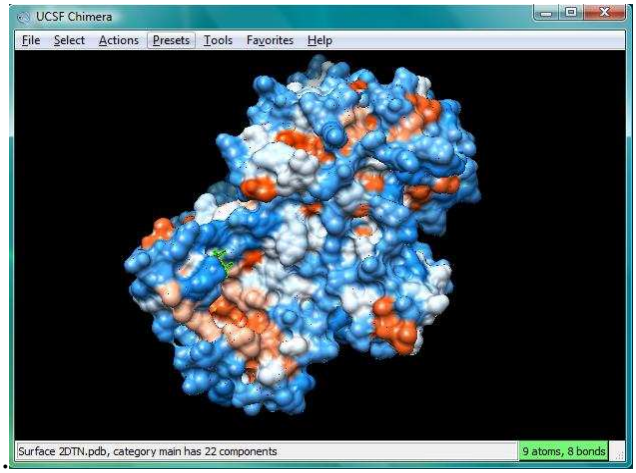


Figure 5. Visualization of undecaprenyl pyrophosphate synthase of *Helicobacter pyroli* complexed with ligand in UCSF CHIMERA molecular modeling system. The Receptor and the ligand interaction has been shown in various forms.
(A). Ribbon (B). All atom and (C). Hydrophobicity surface. The points marked by the arrows are the ligands bound to the receptor.

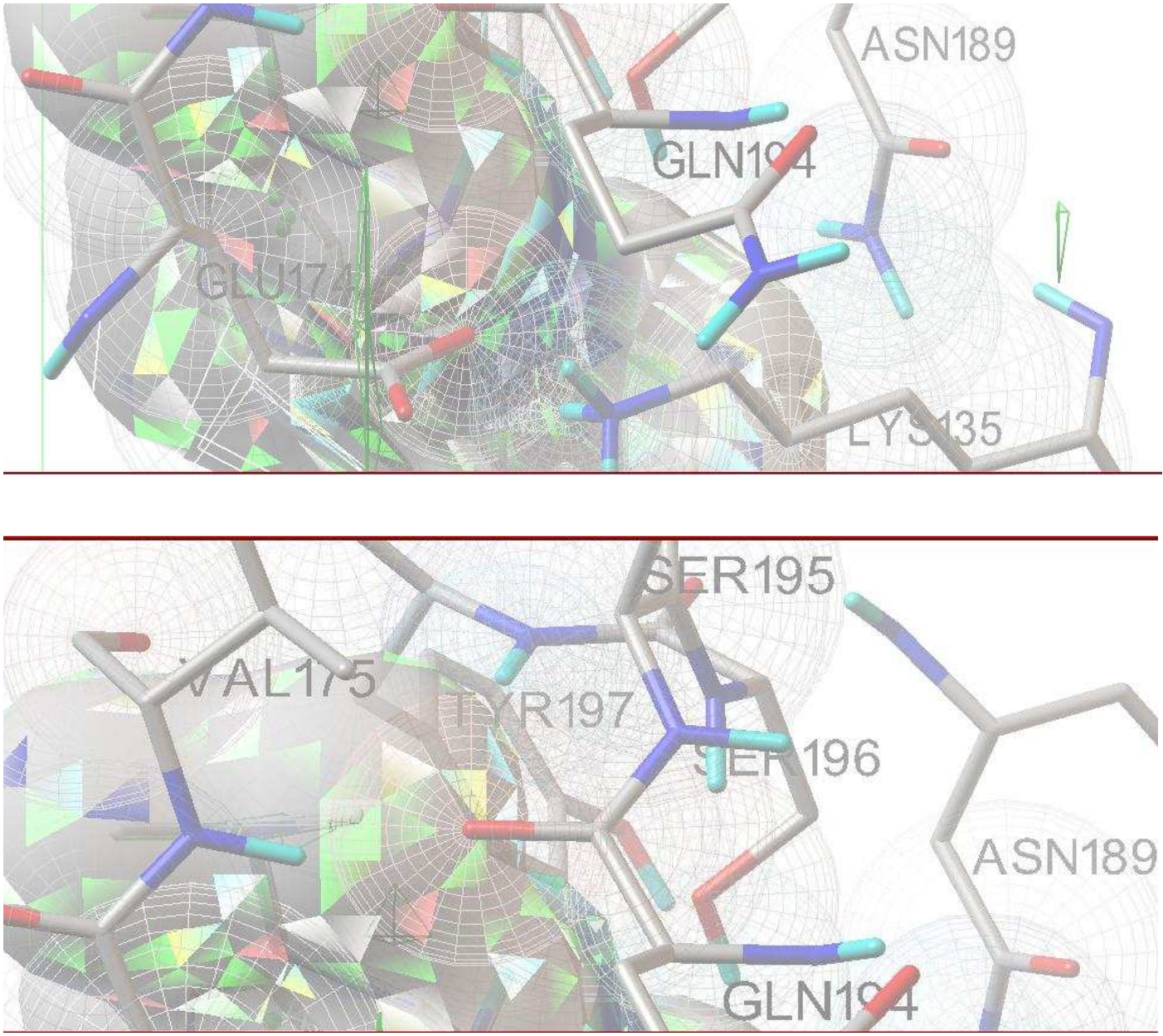


Figure 6 Amino acids of the binding sites of undecaprenyl pyrophosphate synthase of *Helicobacter pyroli* after binding of the ligand. The data presented are the results of Docking with AutoDock 4.

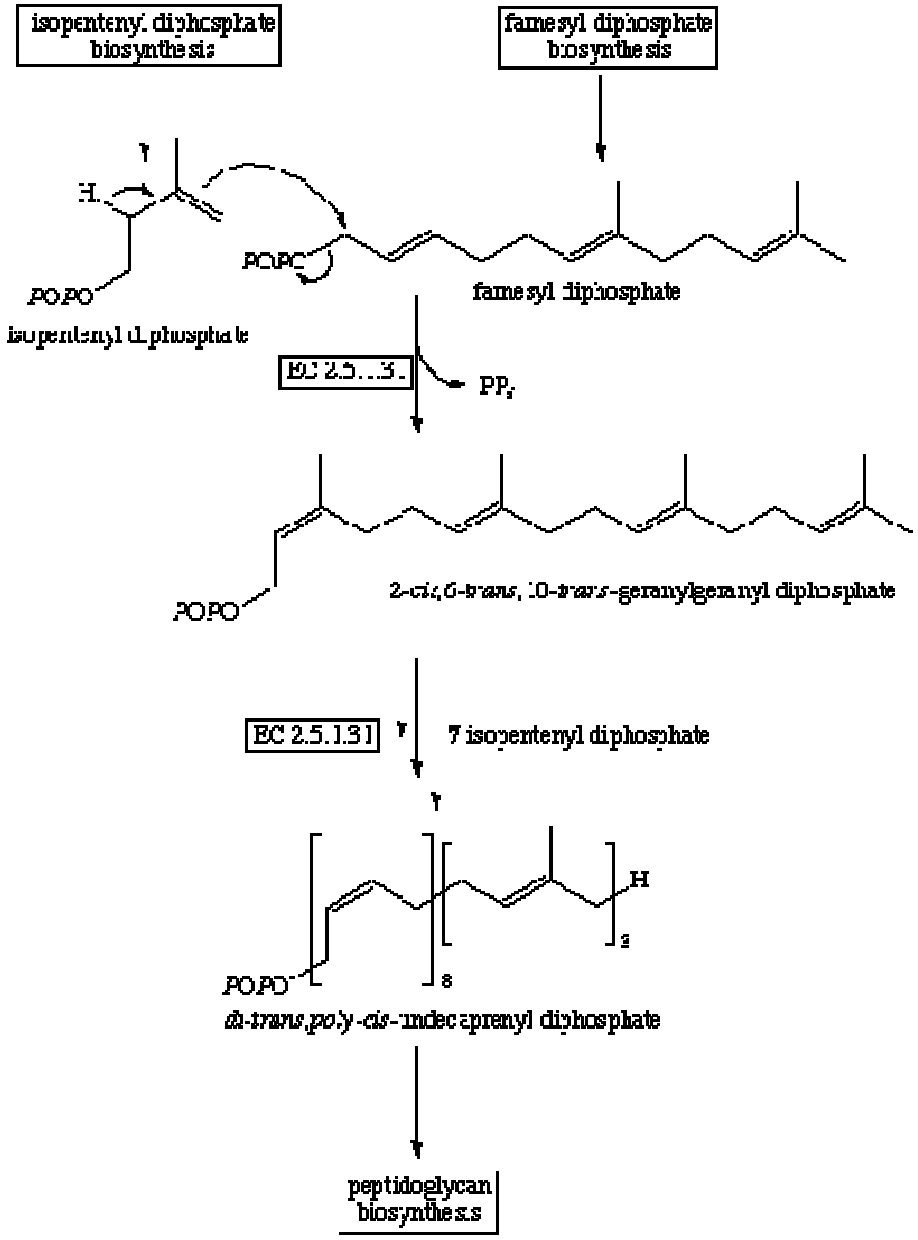


Fig. VII. Pathway of undecaprenyl pyrophosphate synthase involved Peptidoglycan Biosynthesis.

CONCLUSIONS

Although **1-(2-(methylamino)phenyl) undecan – 1 – one** showed sufficient sensitivity against microbes like *Staphyllococcus sp, Klebsela sp* and *Candida albicans* in the wet lab work, *in silico* screening suggested that the same compound may be a potent drug molecule to block biosynthesis of peptidoglycans -a precursor for synthesis of cell wall material in *Helicobacter pylori,* the causal organism of peptic ulcer. Thereby, the compound **1-(2-(methylamino)phenyl) undecan – 1 – one** may serve as a good drug for the treatment of peptic ulcer. However, this finding needs confirmation from wet lab and presence of alternative biosynthetic pathway for synthesis of peptidoglycans, if any, need to be investigated. At the same time, searching the receptor for the bacteria against which the compound showed activity is also necessary for understanding the possible mechanism of action of the compound on pathogenic microbes. Moreover, ADME Tox prediction for the compound is also necessary to assess other drug characteristics of the compound.

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