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SYNTHESIS OF NOVEL 4-(2,5-DIPHENYL-3*H*-PYRROL-4-YL)-2-METHOXYPHENOL FROM THE β-CARBONYL COMPOUND AS DENGUE VIRUS RNA HELICASE INHIBITORS

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ABSTRACT

Dengue fever is a leading mosquito-transmitted viral infection. Dengue is a multifunctional protein. Serotype-specific DENV vaccine is long-term protection and more safety to be investigated. This study was conducted to design a new RNA Helicase inhibitor by in-silico method of ligand-receptor-based approaches. Pyrrole is a nitrogen-containing five-membered heterocyclic ring that owns biological and pharmaceutical various activities. In this study, a novel compound PL5 was synthesized and characterized by TLC, IR, NMR, and MASS spectral data. In-Silico studies using software to predict the physicochemical properties. Software is Molinspiration, Swiss ADME, and admetlab2.0. The Dengue virus RNA helicase protein is downloaded from the protein data bank website. Docking studies were conducted for the compounds on PDB ID: 2BHR by using AutoDock 1.5.7 software. To predict the binding affinity, the synthesized compounds are docked against the dengue virus RNA Helicase protein (2BHR). The synthesized compound is more binding affinity than the standard drug, so the compound PL5 is a potent inhibitor in RNA helicase protein.

Keywords: RNA helicase, DENV vaccine, Pyrrole derivatives, Spectral data, Potent inhibitor

INTRODUCTION

Dengue virus (DENV) is a single-stranded, RNA virus belonging to the Flaviviridae family. The carriers of DENV to humans are the mosquitoes Aedes aegypti. Dengue

infection is a rapidly growing health problem with an increase in the number of Infections in recent years **[1]**. The Infection causes a variety of illnesses, including dengue fever (DF) and dengue hemorrhagic fever (DHF). DENV has a positive-sense RNA genome, and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 proteins **[2]**. DENV NS3 is responsible for protease activity, a multifunctional protein, which contains helicase and protein. NS2B serves as a cofactor of NS3 protease and forms a complex with NS3. NS5 is the essential RNA-dependent RNA polymerase (RdRp) activity. The vector control efforts to stop the spread of the infection. Dengue fever disease is caused by four serologically distinct virus serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) **[3]**. The flavivirus NS2B-NS3 protease is essential for the virus replicative cycle, and thus constitutes an ideal target for antiviral drug development. An antiviral drug administered early during the course of infection inhibits viral replication and decreases the high viral load associated with the more severe forms of dengue disease. in the present study, we have focussed on the potential anti-DENV inhibitors by inhibitors of the NS5 RdRp polymerase **[4, 5]**. We developed new pyrrole derivatives as potential DENV NS3 protease and NS5 RdRp inhibitors.

Pyrrole is a five-membered, unsaturated, nitrogen-containing heterocyclic compound. Pyrrole rings are present in many natural and biologically active compounds. They are contained in the molecules of blood dye, hemoglobin, and plants -chlorophyll, vitamin B1, and many antibiotics. Various Pharmacological activities of anti-cancer activity, anti-viral activity, anti-inflammatory, anti-bacterial, and anti-fungal **[6]**. We have designed a novel pyrrole derivative compound. The synthesised compound (PL5) **4-(2,5-diphenyl-3*H*-pyrrol-4-yl)-2-methoxy phenol (Table 1)**. The synthesized compound is prepared by very simple reaction conditions and easily available reagents and solvents are used. They are a lot of pyrrole nucleus-based compounds are available in the market. Currently so much research on going into substituted pyrrole derivatives.

MATERIALS AND METHODS

All the chemicals were purchased from sigma Aldrich and used further without purification. The Melting points were determined using the melting point apparatus. The reaction and purity were followed by thin layer chromatography. ^{1}H and ^{13}C NMR Spectra were recorded on a 500 MHz Bruker Advance DPX250 spectrometer. IR spectra were recorded on an FT-IR spectrophotometer. Mass spectra were recorded on a Shimadzu Gas Chromatograph, GC-MS analysis is taken

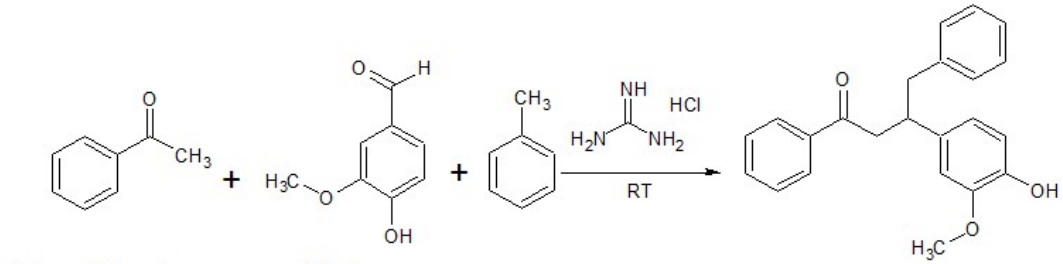
by Perkin Elmer, GC model: Clarus 680, Mass Spectrometer: Clarus 600(EI). Chemsketch was used to draw the 2D structure of molecules. Marvin's sketch was generate a 3d structure of PDB format The X-ray crystallography structure of Dengue Virus RNA Helicase [PDB ID: 2BHR] from the RCSB protein data bank. Physicochemical properties were calculated on Molinspiration and swiss ADME software. Docking was performed on AUTODOCK Tool 1.5.7. protein-ligand interactions were visualized on Ligplot. ADMET properties were calculated on Admetlab2.0 and Osiris property explorer.

EXPERIMENTAL METHODS

Procedure for the synthesis of compound PL5:

STEP 1:

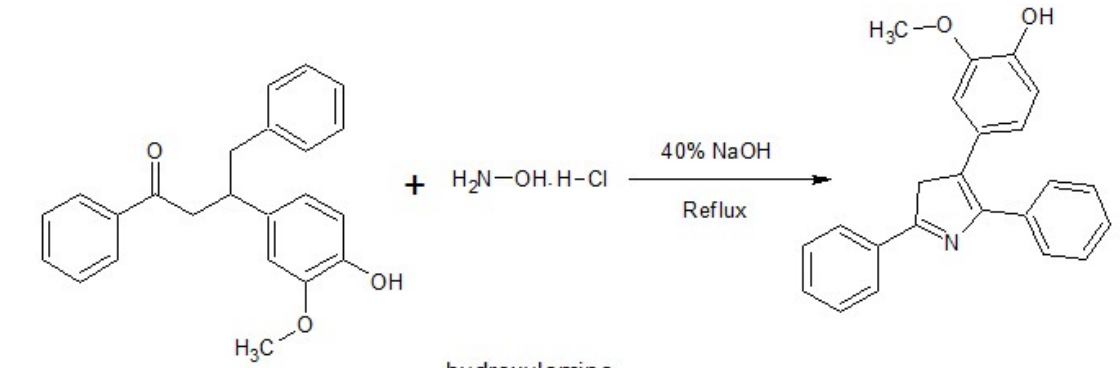
Guanidine hydrochloride (30 mmol) was added to a mixture of aromatic ketones (Acetophenone) (30 mmol), vanillin (30 mmol), and toluene (30 mmol) under the solvent-free condition at room temperature and the reaction mixture was stirred for (8-12 days) by using a magnetic stirrer. After completion of the reaction, as indicated by TLC, the precipitated solid was collected by filtration, and washed with water to remove the unreacted catalyst guanidine hydrochloride which was soluble in water. The crude mixture was purified by recrystallization from acetone: ethanol (2:3) to afford the pure products.



1-phenylethan-1-one | 4-hydroxy-3-methoxybenzaldehyde | toluene | 3-(4-hydroxy-3-methoxyphenyl)-1,4-diphenylbutan-1-one

STEP 2:

The 30mmol of intermediate and 30mmol of hydroxylamine hydrochloride were dissolved in ethanol (10- 15ml). To this 40% of NaOH solution(10ml) is to be added slowly with constant stirring. The above reaction mixture was allowed to reflux in a water bath for (7-10 days). The reaction process was monitored by TLC using Hexane: Ethyl Acetate (7:3). After the completion of the reaction mixture, the reaction mixture was cooled to room temperature and then poured into ice-cold water and neutralized by adding N/10 HCl. The precipitate was filtered and dried. The crude mixture was purified by recrystallization from acetone: ethanol (2:3) to afford the pure products.



3-(4-hydroxy-3-methoxyphenyl)-1,4-diphenylbutan-1-one

hydroxylamine hydrogen chloride

4-(2,5-diphenyl-3*H*-pyrrol-4-yl)-2-methoxyphenol

Table 1: Designed compound

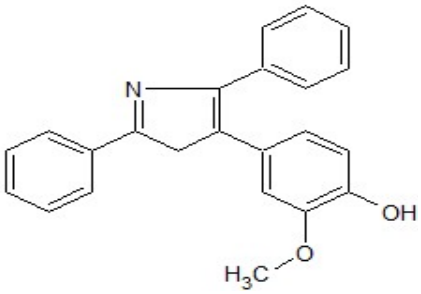
S.No	Compound Code	Designed Compounds
1	PL5	 **4-(2,5-diphenyl-3*H*-pyrrol-4-yl) -2-methoxy phenol**

Fig1: Dengue virus RNA Helicase

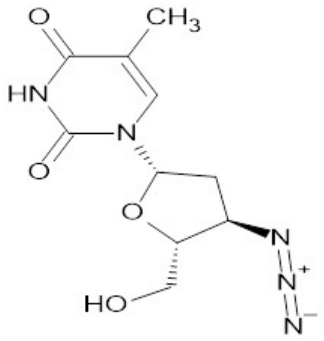


Fig2: Zidovudine (Azidothymidine)

RESULTS AND DISCUSSION

SPECTRAL DATA

The synthesized compound (PL5) **(4-(2,5-diphenyl-3*H*-pyrrol-4-yl)-2-methoxy phenol)**

IR spectra (cm^{-1}): 1599 (C=C), 1278(C-O), 1209 (O-H), 1430 (C-N)

^{1}H-NMR (500MHz) (CDcl3) δ(ppm): 2(CH2, Protons of methylene), 7.26(Ar-H),

7.12(CH,1-benzene), 7.6 (CH, benzylidenimin),

^{13}C-NMR: 37.1(CH2, aliphatic), 164.4(C,1-imine), 124.9(C,1-ethylene), 134.0(C,1-benzene), 129.0(C-H,1-benzene)

MASS (m/z, %); 341.14(100), 342.14(25)

Elem. analysis: C,80.92; H,5.61; N,4.10; O,9.37.

MOLECULAR DOCKING

The physicochemical properties of the compound PL5 and the standard drug zidovudine were compared with the observed values. The synthesized compound and standard drug are followed the Lipinski rule of five. To predict the observed values using different online software. The physicochemical properties and bioactivity values are calculated using Molinspiration software **(Tables 2 and 3)**. To predict toxicity using Osiris property explore software, ADMET properties were measured using admetlab2.0.the various rules are predicted using admetlab2.0 **(Table 4)**. The synthesized compound showed a reproductive effect is positive. The standard drug showed a mutagenic, tumorigenic, and reproductive effect **(Table 5)**.

Molecular docking was conducted on compound PL5 **[4-(2,5-diphenyl-3H-pyrrol-4-yl)-2-methoxy phenol]** against the dengue virus RNA helicase (**PDB ID: 2BHR**) **Figure 1**. The binding pose of RNA helicase with compound PL5 formed one hydrogen bond, LYS A:201 (3.27). The standard drug (**ZIDOVUDINE**) **Figure 2** docked against the RNA helicase protein. The binding pose of RNA helicase with zidovudine formed two hydrogen bonds, LYS A:357 (2.77) and GLU B:502 (3.29). The binding affinity of compound PL5 is -8.31 and the standard drug zidovudine is -7.33 **(Tables 6 & 7).** The compound PL5 is more binding affinity to compare the standard drug zidovudine. The docking pose is visualized in LIGPLOT.

Table 2: Molecular properties prediction of the PL5 compound using Molinspiration software

Compound Code	Molecular weight	milogP	n atoms	n OH	n OHNH	n rotb	volume
PL5	341.14	4.49	26	3	1	4	317.06
STD	267.25	-0.10	19	9	2	3	224.06

Table 3: Bioactivity Prediction of PL5 compound using Molinspiration software

Compound Code	GPCR Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
PL5	-0.00	-0.15	-0.14	0.00	-0.23	-0.08
STD	0.41	-0.08	-0.15	-0.79	-0.02	1.17

Table 4: Prediction of the rule using ADMET LAB 2.0

Compound Code	Lipinski Rule	Pfizer Rule	Gsk Rule	Golden Triangle
PL5	GREEN	RED	RED	GREEN
STD	GREEN	GREEN	GREEN	GREEN

Table 5: Toxicity prediction of PL5 compound using OSIRIS Property Explorer

Compound Code	Mutagenic	Tumorigenic	Irritant	Reproductive
PL5	GREEN	GREEN	GREEN	RED
STD	RED	RED	GREEN	RED

Table 6: Bioavailability prediction of the PL5 compound using Osiris property explorer

Compound code	Solubility	ClogP	TPSA	Density g/cm^3	Druglikness	Drug score
PL5	-4.73	4.59	41.82	1.15	3.32	0.35
STD	-1.44	-1.02	104.8	1.107	2.12	0.2

Table 7: Molecular docking interactions of PL5 compounds in the active site of RNA HELICASE protein (2BHR) by using AutoDock software

Compound Code	Amino acid involved in hydrogen bond interaction	Hydrogen bond distance (A°)	Binding energy (kcal/mol)
PL5	LYS201(A)	3.27	-8.31
STD	LYS357(A) GLU502(B)	2.77 3.29	-7.33

Table 8: Molecular docking reports of the PL5 compounds with RNA HELICASE protein (2BHR).

Compound Code	Electrostatic Energy (kcal/mol)	Inhibition Constant nM (nanomolar)	Intermolecular Energy (kcal/mol)	vdW + Hbond +desolv Energy (kcal/mol)
PL5	-0.23	812.39	-9.20	-8.97
STD	-0.39	4.25	-8.22	-7.83

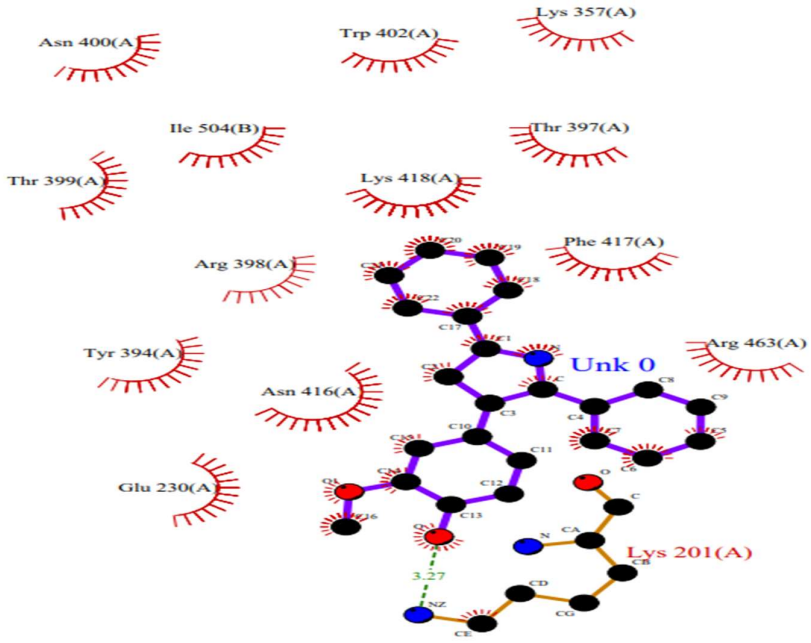


Figure 3: Docking pose of Ligand Pl5 with RNA Helicase Protein.

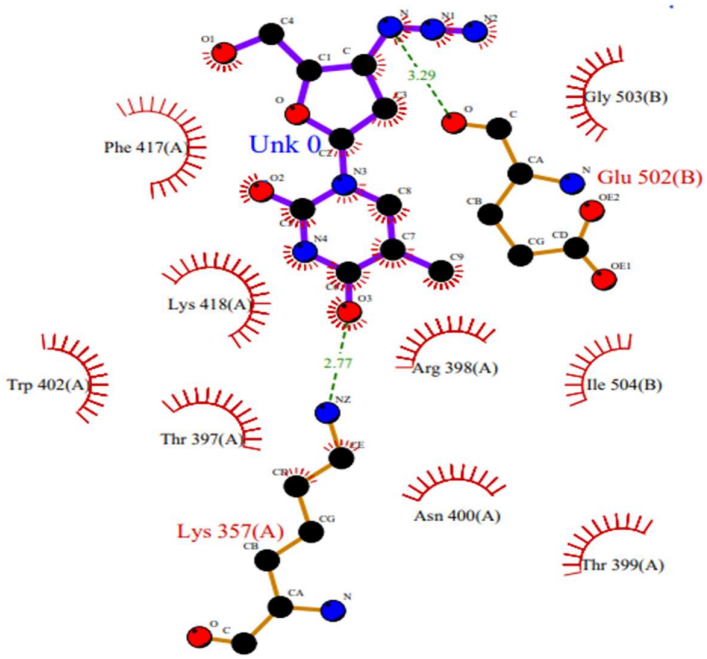


Figure 4: Docking pose of standard drug Zidovudine with RNA Helicase Protein.

CONCLUSION

In the present study, DENV RNA helicase is a potential drug target. A synthesized novel compound PL5 **[4-(2,5-diphenyl-3H-pyrrol-4-yl)-2-methoxyphenol]**. The synthesized compounds were confirmed by spectral data. *In-Silico* studies of the compounds studied using the software. ADMET prediction is essential to remove the toxic compound in a biological system. These PL5 compounds revealed good pharmacokinetic properties. The synthesized compounds and standard compounds are docked against PDB ID: 2BHR. The docked PL5 compounds are more binding affinity to the receptor. To compare the standard drug, and PL5 compound is a potent inhibitor against RNA helicase.

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