Synthesis of Novel 4-(2-Chlorophenyl)-2,5-Diphenyl-3*H*-Pyrrole from the β-Carbonyl Compound as Isocitrate Dehydrogenase -2 Enzyme Inhibitors

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Abstract
Pyrroles are nitrogen-containing five-membered heterocyclic rings which own biological andPharmaceuticals influence due to their various activities like antiviral, anticancer, antimalarial, anti-inflammatory, antibacterial, and analgesic. Novel compounds of 4-(2-chlorophenyl)-2,5-diphenyl-3*H*-pyrrole were designed, synthesized, and undergo molecular docking, to calculate ADME properties. The designed compound has been evaluated *In-Silico* using Molinspiration, Osiris, and ADMETlab2.0 software to predict the physicochemical, ADME properties and toxicity. The synthesized compounds are to be characterized by melting point, TLC, IR, NMR, and MASS spectral data. The synthesized compounds are docked against Isocitrate Dehydrogenase-2 (IDH2) enzyme (PDB ID:6ADI) by using Autodock 1.5.7 software. The synthesized compounds act against the IDH2 receptor to predict binding affinity. These In-Silico studies signified that the compounds can act as a potent inhibitor of the IDH2 enzyme.
keywords: 1.Pyrrole, 2.IDH2 enzyme, 3.Potent inhibitor, 4.NMR, 5.Mass, 6.Spectral Data.

Introduction

Cancer is a leading cause of death worldwide, accounting for nearly 10 million Death in 2020. Blood cancer has been among one of the most common causes of death with an incidence of over one lakh people being diagnosed every year with a form of blood cancer such as lymphoma, leukemia, and multiple myeloma. Mutations of key enzyme genes involved in metabolic pathways are the main cause of abnormal metabolism by changing the expression and activity of a metabolic enzyme [(1)].

Isocitrate dehydrogenases (IDHs) play important roles in cellular metabolism. Isocitrate dehydrogenase-2 (IDH2) is a mitochondrial enzyme that catalyzes the metabolic conversion between isocitrate and alpha-ketoglutarate (α-KG) in the TCA cycle, mutated genes are associated with a variety of tumors, including acute myeloid leukemia (AML) [(2)]. The isocitrate dehydrogenase (IDH) enzymes are NADP-dependent molecules that normally function as homodimers to catalyze the oxidative decarboxylation of isocitrate to alpha-ketoglutarate (a-KG) with the concomitant production of NADPH. IDH2 mutation is an oncogenic event in acute myeloid leukemia (AML) due to the generation of 2-hydroxyglutarate. The roles of NADP-dependent IDH1 and 2 in normal cell and cancer metabolism are distinct from those of NAD-dependent IDH3.IDH2 mutation has the clinical characteristics of older age, lower white blood cell count; higher platelets, and NPM1 mutations in patients with AML [(3)]. In addition, researchers found that R172 IDH2 mutations had potential adverse prognostic significance and that R172 IDH2 mutations are mutually exclusive with any other prognosis-related mutations. IDH2 mutant AML patients are associated with resistance to treatment as illustrated by a low rate of CR and a high RR [(4)]. Inhibitors of mutant IDH2 may reduce the level of 2-HG to reverse cell differentiation and indirectly destroy the bone marrow microenvironment induced by 2-HG by blocking the proliferation of AML cells [(5)]. Enasidenib (AG-221) is the first IDH inhibitor approved by the FDA for the treatment of relapsed or refractory acute myeloid leukemia (RR-AML) with IDH2 mutations and achieved good therapeutic effects [(4)]

The currently reported mutation is the R172 mutation, and no R140 mutation has been found. TP53 and PI3K mutations in cancer patients with IDH2 mutations are common. Although IDH2-mutated SNUC was associated with a trend of improved free survival and overall survival, such a trend did not reach a significant level. A large number of preclinical studies have shown that IDH mutant inhibitors can

significantly reduce the level of 2-HG and have great effects on cell metabolism, growth, and tumorigenicity. Several of them have been used in clinical trials, and Enasidenib (AG-221) and Ivosidenib (AG-120) have been approved by FDA for human cancer treatment [6-8].

Pyrrole is a five-membered aromatic ring, widely present in a variety of biological activities. Nitrogen heterocycles emerged as interesting scaffolds in organic and medicinal chemistry applications. Pyrrole is an important active chromophore with heterocyclic aromatic characteristics. The pyrrole nucleus could be present in synthetic products as single/ substituted/condensed rings, as a linker between different moieties, although it can be directly associated with biological targets [9-10]. The N-substituted pyrroles represent an extended family of derivatives, recognized as anticancer tools. Pyrrole derivatives show various pharmacological activities Anti-fungal and anti-bacterial activity, Anti-inflammatory activity and analgesic activity, Anti-cancer activity, Anti-viral activity, anti-malarial, antitubercular, anti-inflammatory, enzyme-inhibiting, and anticancer properties. Pyrrole is also found in several drugs atorvastatin, ketorolac, sunitinib, tolmetin, and licofelone [11].

Materials and Methods

All the chemicals (reagents and solvents) were purchased from commercial suppliers (Merck grade) Sigma Aldrich and used without further purification.The melting points of the synthesized compound were determined on the melting point apparatus and are uncorrected. Reactions and purity of the compounds were followed by thin-layer chromatography (TLC) on Merck (0.25 mm) precoated TLC plates and spots were detected by UV light. IR spectra of synthesized compounds were determined on FTIR at MTPG&RIHS, Puducherry.

Structure Elucidation:

Spectroscopic data were recorded by the following instruments.

Infrared (IR) spectra were recorded on a Shimadzu FT-IR-8201 PC spectrophotometer with only significant absorption band frequencies. ^{1}H NMR and ^{13}C NMR spectra were recorded on 500 MHz in CDCl3 or DMSO-d6 on a Bruker Avance DPX250 spectrometer (Bruker, Wissembourg, France). The chemical shift (δ) values are given in ppm (parts per million). The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets. The coupling constants (J) are reported in hertz (Hz).

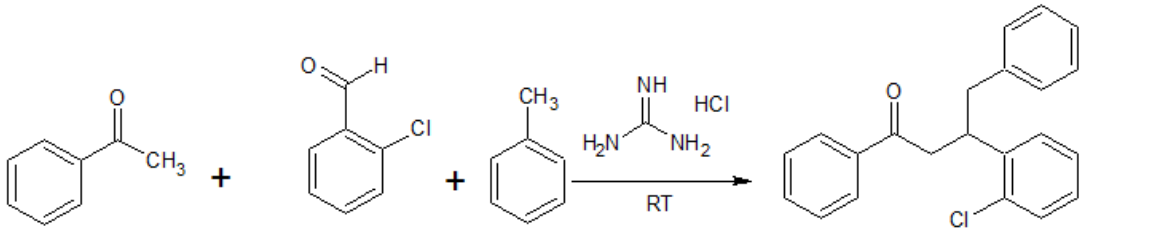
The GC-MS analysis is taken by Perkin Elmer, GC model: Clarus 680, Mass Spectrometer: Clarus 600(EI), software: TurboMass.

Experimental Methods:

Synthesis of 4-(2-chlorophenyl)-2,5-diphenyl-3H-pyrrole

Step 1:

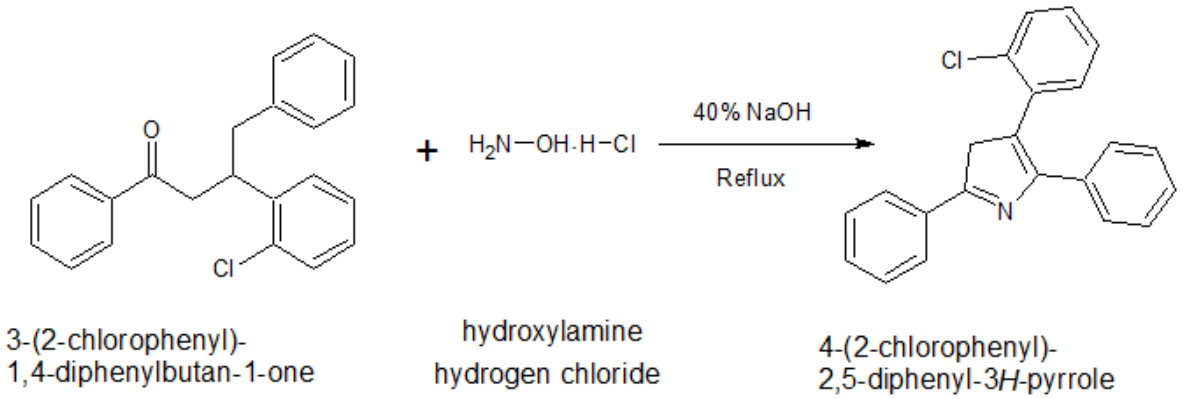
Guanidine hydrochloride (30 mmol) was added to a mixture of aromatic ketones (Acetophenone) (30 mmol), Ortho-chloro benzaldehyde (30 mmol), and toluene (30 mmol) under the solvent-free condition at room temperature and the reaction mixture was stirred for (8-12 days) by using a magnetic stirrer. After 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.

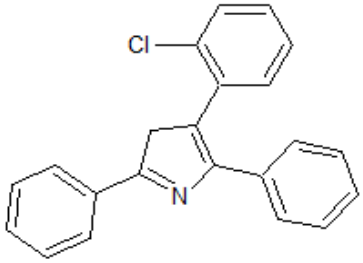


1-phenylethan-1-one 2-chlorobenzaldehyde toluene 3-(2-chlorophenyl)-1,4-diphenylbutan-1-one

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 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.



S.No	Compound Code	Designed Compounds
1	PL3	 4-(2-chlorophenyl)-2,5-diphenyl-3*H*-pyrrole

Results and Discussion:

Spectral data:

4-(2-chlorophenyl)-2,5-diphenyl-3*H*-pyrrole (PL3) **(**92%w/w);

The structure of the PL3 compounds was elucidated by spectral data.

IR (KBr) (cm^{-1}):1680(C=C),1402(C-N),3076(C-H),759(C-Cl).

^{1}H-NMR (500 MHz) (CDcl3) δ(ppm):2 (CH2, protons of methylene), 7.6(CH, benzylidenimin),7.30(Ar-H)

^{13}C-NMR:37.1(CH2,aliphatic), 164.6(C,1-imine), 132(C,1-ethylene),128(C-H,1-benzene).

MS (m/z, %);329.10(100), 331.09(32).

Anal. Calc. for $C_{22}H_{16}ClN$ (329.1)

Found: C,80.11; H,4.89; Cl,10.75;N,4.25.

Molecular Properties:

Molecular properties are mainly hydrophobicity, molecular size, and the presence of various pharmacophoric features that influence the pharmacokinetics and pharmacodynamics behavior of molecules in the living organism, including bioavailability. The calculated value of the predicted molecular properties and bioactivity for compounds PL3 is given in (Table 2 and Table 3). The synthesized compounds PL3 are satisfying the Lipinski rule and golden triangle rule and the standard compounds

(Enasidenib) are satisfying the Lipinski rule, Pfizer rule, and golden triangle rule (Table 4). The toxicity of the compounds was also predicted using Osiris property explorer. The toxicity prediction would be useful for the selection of compounds to test in animal models [20]. The Osiris calculation (Table 5) and (Table 6)

Molecular Docking:

Molecular docking studies of compound 4-(2-chlorophenyl)-2,5-diphenyl-3*H*-pyrrolealong with standard drug Enasidenib (figure 2) were carried out with the protein target IDH2 R140Q (PDB ID: 6ADI) (figure 1) using Auto Dock 1.5.7 software. The docking studies investigate the compound binding pose of the molecule. PL3 compound formed hydrogen bonding interaction. The compound PL3 (-11.81) showed a nearer binding score respectively compared to the standard ENASIDENIB (-6.80 kcal/mol). Compound PL3 formed one hydrogen bond with Gln 316 with a show distance of 2.99 A° respectively compared to the standard Enasidenib formed three hydrogen bonds Gln 343 (A), Ala 347 (A), and Ser 332 (A) 3.04, 3.26 and 2.62 A°. The docking pose is viewed in LIGPLOT. The molecular interaction (Table 7) and (Table 8). The docking pose of compound PL3 and standard drug Enasidenib is shown in (figure 3)

Table 2: Molecular properties prediction of the PL3 compound using Molinspiration software

Compound Code	Molecular weight	milogP	logD	n OH	n OHNH	n rotb	volume
PL3	329.83	5.79	4.73	1	0	3	297.40
STD	473.38	4.39	2.60	8	3	8	367.92

Table 3: Bioactivity Prediction studies of PL3 compound using Molinspiration software

Compound Code	GPCR Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
PL3	-0.02	-0.18	-0.22	-0.04	-0.28	-0.20
STD	0.34	0.25	0.71	0.04	0.05	0.38

Table 4: Prediction of the rule using ADMET LAB2.0

Compound Code	Lipinski Rule	Pfizer Rule	Gsk Rule	Golden Triangle
PL3	GREEN	RED	RED	GREEN
STD	GREEN	GREEN	RED	GREEN

Table 5: Toxicity prediction of PL3 compound using OSIRIS Property Explorer

Compound Code	Mutagenic	Tumorigenic	Irritant	Reproductive
PL3	GREEN	GREEN	GREEN	RED
STD	GREEN	GREEN	RED	GREEN

Table 6: Bioavailability prediction of the PL3 compound using Osiris property explorer

Compound code	Solubility	ClogP	TPSA	Density g/cm^3	Druglikness	Drug score
PL3	-5.74	5.56	12.36	1.15	3.27	0.25
STD	-5.3	3.65	108.7	1.47	-7.49	0.15

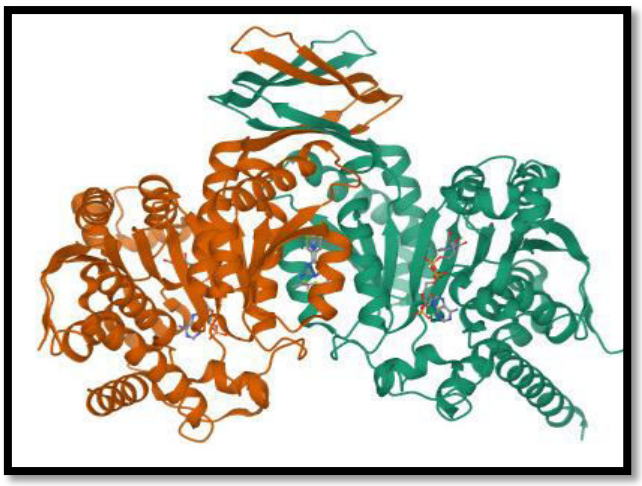


Figure 1: Crystal structure of IDH2 R140Q in complex with AG-881 (PDB ID: 6ADI)

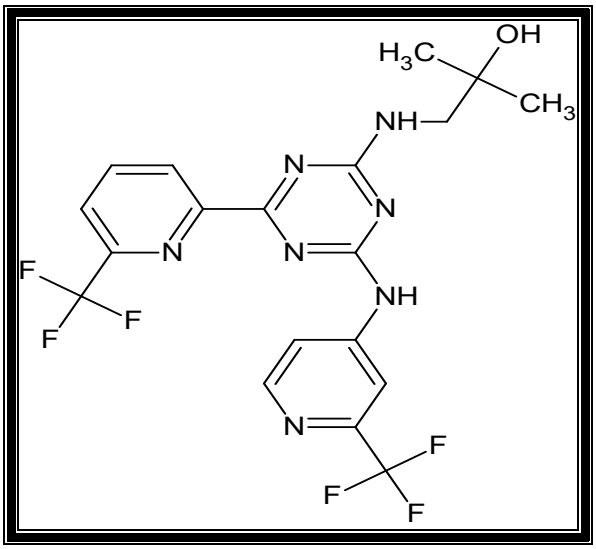


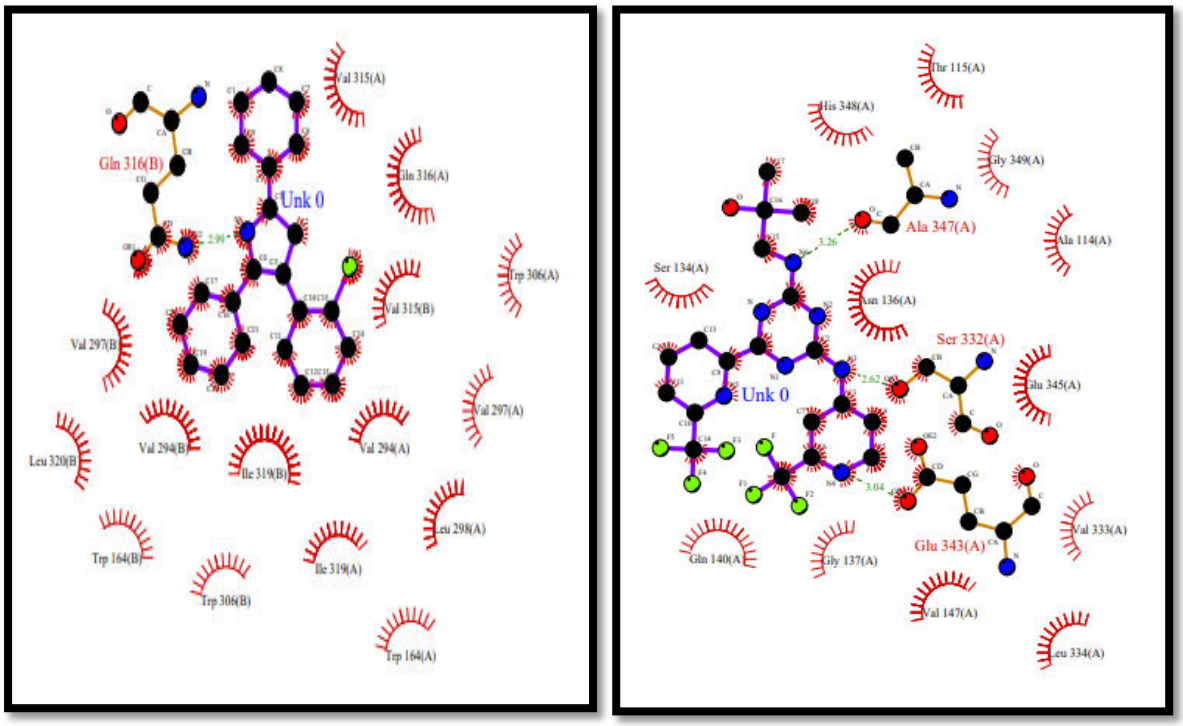
Figure 2: Standard drug (ENASIDENIB)

Table 7: Molecular docking interactions of PL3 compounds in the active site of IDH2 enzyme (6adi) by using auto dock software.

Compound Code	Amino acid involved in hydrogen bond interaction	Hydrogen bond distance (A°)	Binding energy (kcal/mol)
PL3	GLN316 (B)	2.99	-11.85
STD	GLN343 (A) ALA347 (A) SER332 (A)	3.04 3.26 2.62	-6.80

Table 8: Molecular docking reports of the PL3 compounds with IDH2 protein (6ADI)

Compound Code	Electrostatic Energy (kcal/mol)	Inhibition Constant nM (nanomolar)	Intermolecular Energy (kcal/mol)	vdW + Hbond + desolv Energy (kcal/mol)
PL3	-0.01	2.06	-12.45	-12.44
STD	-0.71	10.42	-9.18	-8.47



PL3STANDARD ENASIDENIB

Figure 3: Docking pose of compound PL3 and STANDARD ENASIDENIB in the active site of the protein IDH2 enzyme (6ADI).

Conclusion

In the research, Novel compounds of 4-(2-chlorophenyl)-2,5-diphenyl-3*H*-pyrrolewas synthesized using simple reaction condition and are easily available reagent and solvents. The synthesized compound was confirmed by IR,NMR,and MASS Spectroscopy. *In-Silico* studies of the designed compounds using Molinspiration and Admetlab2.0. PL3 compounds obey Lipinski's rule of five and showed good oral bioavailability. Toxicity prediction studies of PL3 compound using Osiris property explorer software. The docked PL3 compound was found to possess good interaction with the receptor. It haspotent activity against the IDH2 enzyme. It showed stronger binding affinity compared to the standard drug(Enasedinib). Compound PL3 is a potent inhibitor against the IDH2 enzyme.

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