CATALYTIC EFFICACY AND COMPUTATIONAL DOCKING STUDIES OF NOVEL ISATIN SCHIFF BASE

[1*]R.Jayaprakash, [1] I. Ameeth Basha, Professor, [1]R.Shanmugavalli,
[1]Professor, Department of Chemistry, School of Arts and Science, AVIT campus, VMRF, Chennai-603104, TN, India
Corresponding Author*: **jayaprakashsangee1977@gmail.com**

ABSTRACT
Bioactive Isatin substituted highly stable Schiff base was prepared by the condensation of Isatin and 4, 4'-diamino benzanilide using various acid catalysts such as hydrochloric acid, sulphuric acid, acetic acid (pH -1.5) and natural lemon juice (pH-0.99) with different solvents. A simple and efficient method has been developed for the synthesis of dimeric Isatin Schiff base using catalytic amount of lemon juice in methanol as solvent and it was characterized by IR and ^{1}H NMR spectral studies. Molecular Docking study of the synthesized Schiff bases with twenty one known Homo Sepians and other proteins of microorganisms such as E.Coli, Salmonella, Bostaurus, Trypanosomabruceibrucei, EnterobacteriaphaseT7, HIV, Rattusnorvegius, MusMusculus, Rhodospirillumrubrum and covid 19 protein were also assayed for future drug research.
Keywords: *Isatin, catalysts, Schiff base, Docking Score*

1. INTRODUCTION

Schiff-bases have been explored as fine chemicals and medical substrates (Da Silva et.al, 2011; Kajal et.al, 2013) in various researches. Studies on the bioavailability of heterocyclic Schiff bases from natural sources are limited and hence synthetic heterocyclic Schiff bases have been received research interest in various fields. In addition to this secondary amine, carbonyl group, stable amide groups containing isatin derivatives are the active molecules (Valli et.al, 2011) in medicinal field. It is proposed to synthesize isatin Schiff bases using natural acids such as lemon juice and at refluxed conditions to avoid the inorganic chemical impurities. So, this work selected isatin and carried for the synthesis. At the same time, Computer-aided docking is an enormously beneficial tool to gain an understanding of protein–ligand interactions which is an important for the drug discovery (Rauf et.al, 2017). Molecular docking study (Hassanin et.al, 2018) against human disease causing proteins such as *E.Coli, Salmonella, Bostaurus, Trypanosomabruceibrucei, EnterobacteriaphaseT7,* HIV, *Rattusnorvegius, MusMusculus, Rhodospirillumrubrum* and covid-19 proteins was carried out using online mcule tool. Right now, covid-19 virus causing very big world shut down in all kind of activities. This simple test may useful for the future drug discovery to control bacterial, fungus and viral diseases. Best negative docking score for various poses of each protein was measured for the future experimental assays. The synthesized compound will be characterized by using IR and ^{1}H NMR spectra.

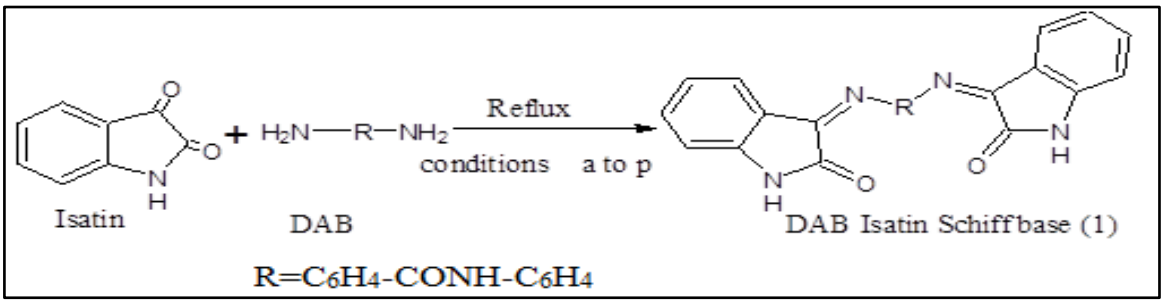
2. MATERIALS AND METHODS

All the solvents and inorganic acid catalysts were purchased from SRL chemicals and used as such. Isatin was purchased from Sigma Aldrich, USA. Fresh clear lemon fruit juice was isolated by centrifugation. Reaction monitoring and impurity profile were carried on Merck readymade silica gel thin layer chromatography plate. Melting point was measured by open capillary method using Sunsim apparatus. IR spectrum was recorded by Jasco FTIR spectrometer using ATR method. ^{1}H NMR spectra was taken on a Bruker NMR 400 spectrometer using DMSO as a solvent at a frequency of 500 MHz. Docking study was carried out through Mcule online tool, Covid-19 server, CLC drug discovery workbench -3 and poses documented through Molsoft ICM browser.

3. EXPERIMENTAL PROCEDURE

3.1. General procedure for the preparation of *(4z)-4-(2-Oxoindolin-3-Ylidene amino)-N-(4-(2-Oxoindolin-3-Ylidene Ami No) Phenyl) Benzamine* (1)

4, 4'-diamino benzanilide (DAB) was synthesized as per the literature (Georgeta et.al, 2004) method and recrystallized in methanol and DMF mixture. The compound was carried for the Schiff base preparation using **a-p** methods and shown in **Scheme.1**.



Scheme.1. preparation of isatin dab Schiff base

Conditions: a.Methanol/HCl , b. Methanol/ H_2SO_4 , c. Methanol/Acetic Acid, d. Methanol/ Lemon juice, e.Ethanol/HCl , f.Ethanol/ H_2SO_4 , g.Ethanol/ Acetic Acid, h.Ethanol/ Lemon juice, i. Water/HCl, j.water / H_2SO_4, k.water/ Acetic Acid, l.water/ Lemon juice, m. DMF/HCl, n.DMF / H_2SO_4, o.DMF / Acetic Acid, p.DMF / Lemon juice.

3.1.1. Methanol solvent and HCl catalyst

2mm of Isatin was taken in a 50ml RB flask with 20ml of methanol and 2ml of 1N hydrochloric acid (Sultan et.al, 2013). The reaction temperature was increased to 45^0C and maintained for 5 minutes then 10ml of 1mM 4, 4'-diamino benzanilide in methanol was added slowly over a period of 5 minutes. The clear solution was heated to reflux and the reaction was completed after 8 h. The Solid obtained was filtered at hot condition to avoid amine impurity. Yield 48%. MP->250^0C. The Same methodology was followed in other solvents such as ethanol (e), water (i) and DMF (m).

3.1.2. Methanol solvent and H_2SO_4 catalyst

2mm of Isatin was taken in a 50ml RB flask with 20ml of methanol and 2ml of 1N

sulphuric acid (Murhekar et.al, 2011). The reaction temperature was increased to 45^0C and maintained for 5 minutes then 10ml of 1mm 4, 4'-diamino benzanilide in methanol was added slowly over a period of 5 minutes. While the addition white solid was formed and the white turbid reaction mass was heated to reflux for 10 h and no product was formed. Same protocol was followed in ethanol (f), water (j) and DMF (n) solvents as well.

3.1.3. Methanol and Acetic Acid

2mm of Isatin was taken in a 50ml RB flask with 20ml of methanol and 2ml of acetic acid (Xavier et.al, 2014, Elemike et.al, 2018). The reaction temperature was raised to 45^0C and maintained for 5 minutes then 10ml of 1mm amine solution was added slowly over a period of 5 minutes. The clear orange solution was heated to reflux. Reaction was completed after 6 h. Solid obtained was filtered at 45^0C to avoid amine impurity. Yield 80%. MP- $>250^0$C. Same procedure was followed in ethanol (g), water (k) and DMF (o) solvents as well.

3.1.4. Methanol and lemon juice

Fresh lemon juice was extracted manually and centrifuged. The clear upper layer was used for the reaction.2mm of Isatin was taken in a 50ml RB flask with 20ml of methanol and 2ml of lemon juice (Mohamed et.al, 2015, Sachdeva et.al, 2014) at room temperature. Reaction temperature was increased to 45ºC and maintained for 5 minutes then 10ml 1mm 4, 4'-diamino benzanilide solution added slowly over a period of 5 minutes. The clear orange solution was stirred for 3 h at reflux condition. Reaction was completed after 3 h. Solid obtained was filtered at 45ºC and washed by hot methanol to avoid amine impurity. Yield-90%. MP- $>250^0$C.Similarly the catalyst was used in ethanol (h), water (l) and DMF (p) solvents. The yield and their solubility were shown in **Table.1**.

Table.1. Solvent, Catalyst, yield, melting point, solubility and reaction conditions.

Solvent	Catalyst	% of yield	Time (hr)	M. P ^{O}C	Solubility
Methanol (Reflux)	**a**. HCl	48	8	>250	DMSO/ DMF
	b.H_2SO_4	White solid	10	-------	-------
	c.Acetic Acid	80	6	>250	DMSO/ DMF
	d.Lemon	90	3	>250	DMSO/ DMF
Ethanol (Reflux)	**e**.HCl	48	8	>250	DMSO/ DMF
	f.H_2SO_4	White solid	10	-----	-------
	g.Acetic Acid	60	6	>250	DMSO/ DMF
	h.Lemon	75	3	>250	DMSO/ DMF
Water (Reflux)	**i**.HCl	-----	8	-----	-----
	j.H_2SO_4	-----	10	-----	-----
	k.Acetic Acid	-----	6	-----	-----

	l.Lemon	-----	3	-----	-----
DMF (Reflux)	**m**.HCl	40	8	>250	DMSO/ DMF
	n.H_2SO_4	White solid	10	-----	--------
	o.Acetic Acid	56	6	>250	DMSO/ DMF
	p.Lemon	64	3	>250	DMSO/ DMF

3.2. Spectral characterization and Elemental analysis

All batch products were analyzed using FTIR spectral studies. Lemon juice catalyzed Isatin derivative exhibits IR frequencies at 3418 cm^{-1}, 2974 cm^{-1}, 3050 cm^{-1}, 1657 cm^{-1}, 1604 cm^{-1}, 1504 cm^{-1} deep-rooted the Schiff base . IR frequencies 3418 (-NH-), 2974 (Indole Hydrogens), 3050 (Ar-CH), 1657 (C=O), 1604(C=N), 1504 (-CONH-) cm^{-1}.

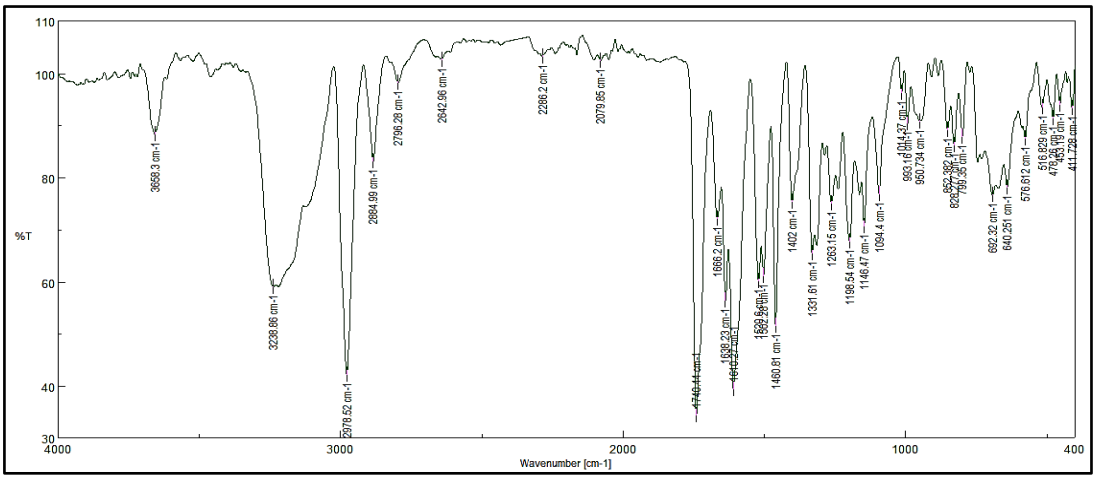


Figure.1. FTIR spectra of isatin Schiff base

Proton NMR showed three amide peaks at 10.61(lactam) ppm, 10.59(lactam) ppm and 10.24 ppm (–CONH). Elemental analysis confirmed the molecular formula as $C_{29}H_{19}N_5O_3$. ^1H NMR (500 MHz, DMSO-d_6) δ 11.01-10.90 (d, J = 13.6 Hz, 2H), 10.44 (s, 1H), 7.95 (d, J = 7.3 Hz, 2H), 7.73 (d, J = 7.9 Hz, 5H), 7.64 (d, J = 7.2 Hz, 2H), 7.56 – 7.33 (m, 5H), 7.30 (t, J = 7.4 Hz, 1H), 7.19 (d, J = 7.4 Hz, 1H) D_2O(3.383) , DMSO (2.5ppm). Theoretical Compositions of the molecule are C (71.74%), H (3.94%), N (14.43%), O (9.89%). Composition found was C-71.9%, H-3.95%, N-14.30%, O-9.85%. From the experimental value molecular formula of Schiff base was assigned as $C_{29}H_{19}N_5O_3$ (Molecular Weight-485.5). The outcome spectral values almost coincidence with the reported values (Sezer et.al, 1999). Both FTIR and 1H-NMR spectra are displayed in **Figure.1, Figure.2** respectively.

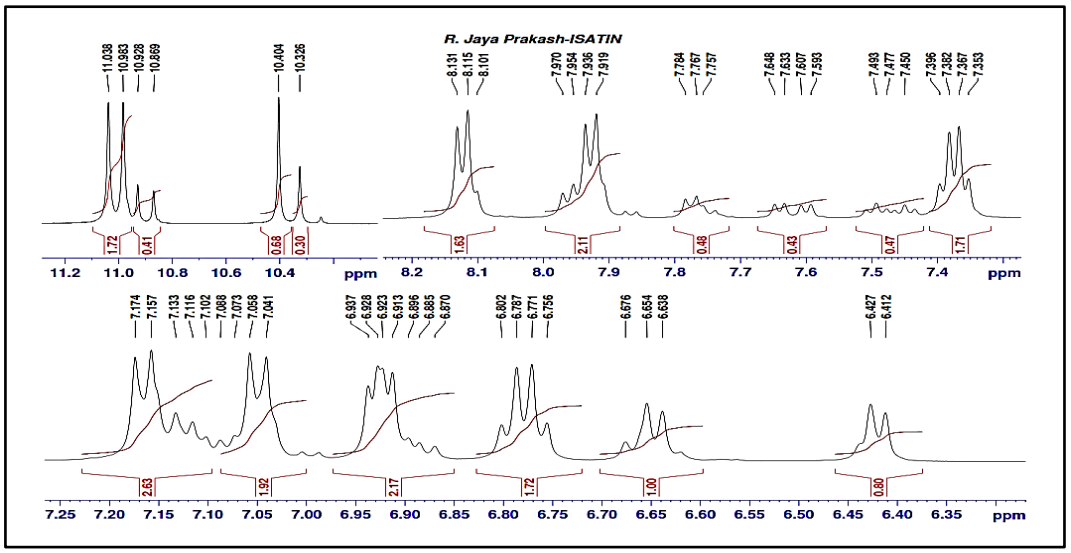


Figure.2. Processed ^{1}H-NMR spectra of isatin Schiff base

3.3. Active surface area study

The synthesized compound structure was analyzed by argus lab for the active area identification. The surface area of the molecule is exists near by the imine group and amide group of the molecule as shown in **Figure.3**. From the image, it was observed that the structure has more active area around the two imine, two cyclic imide and one amide groups in green colour.

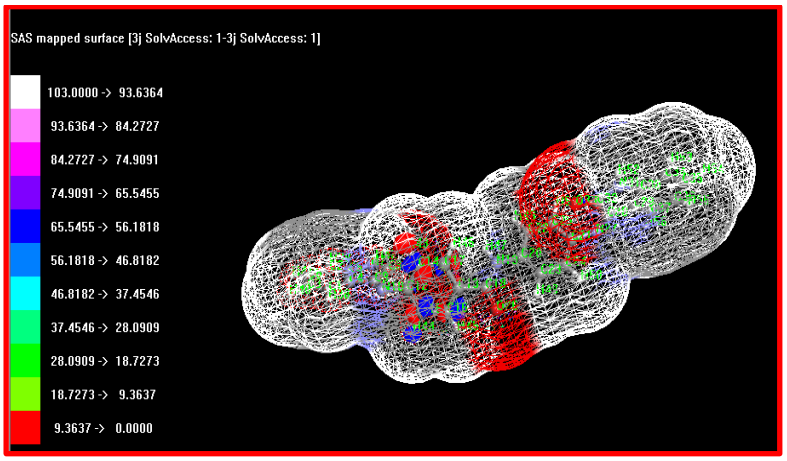


Figure.3. Active surface area of Schiff base

3.4. Ligand Protein Docking Study

Synthesized Schiff base molecular structure was used for preliminary ligand protein docking (Sheng et.al, 2010; Kaushik et.al, 2014) study by online mcule tool. Schiff base ligand smiles notation was submitted in mcule tool against the selected target protein. Various poses showed different negative and positive docking scores. This docking study carried against seminalribonuclease, GTPaseHRas, Phosphoglycerate Kinase, DNA ligase and covid-19 protease (6lu7) like twenty two important proteins of various organisms such as Homo Sapiens, *E.Coli*, *Salmonella*, *Bostaurus*, *Trypanosomabruceibrucei*, *EnterobacteriaphaseT7*, HIV, *Rattusnorvegius*, *MusMusculus*, *Rhodospirillumrubrum* and covid-19. Each protein

showed four poses and their docking scores were shown in **Table.2**. Out of four values best score noted and the images have shown in **Figure.4 (a-i).**

Table.2. Docking score against different proteins.

S.N	Name of the protein	ID No	Docking score for 4 poses				Best score	Organism
1	Seminal Ribonuclease	11ba	-8.3	-8.0	-7.1	-6.0	-8.3	*Bostaurus*
2	GTPaseHRas	121p	-10.2	-9.4	-9.3	-9.1	-10.2	*Homo sapiens*
3	Phosphoglycerate Kinase	13pk	-9.4	-8.3	-8.0	-7.9	-9.4	*Trypanosoma bruceibrucei*
4	DNA ligase	1a0i	-9.2	-8.9	-8.9	-8.8	-9.2	*Entero bacteriaphase*
5	Glutathione-s-transferase	18gs	-10.1	-9.7	-9.4	-9.3	-10.1	*Homo sapiens*
6	Proto oncogene tyrosine-protein kinase	1a08	-8.5	-8.3	-8.2	-7.4	-8.5	*Homo sapiens*
7	Progesterone receptor	1a28	15	17.1			15	*Homo sapiens*
8	Thrombin	1a2c	-8.5	-8.3	-8.3	-8.1	-8.5	*Homo sapiens*
9	UDP-N-acetylglucosamine 1-carboxy vinyl transferase	1a2n	-8.7	-8.4	-8.4	-7.6	-8.7	*E.Coli*
10	Protease	1a30	-10.7	-10.5	-10.4	-10.3	-10.7	*HIV Type-I*
11	Purine nucleoside phosphorylase	1a69	-9.0	-8.6	-8.6	-8.4	-9.0	*E.Coli*
12	ATP- dependent dethiobiotinsynthetese BioD-1	1a82	-10.1	-9.4	-8.7	-8.7	-10.1	*E.Coli*
13	GTP-cyclohydrolase-1	1a8r	-7.8	-7.7	-7.5	-7.3	-7.8	*E.Coli*
14	Neutrophil collagenase	1a86	-10.4	-10.2	-9.6	-9.4	-10.4	*Homo sapiens*
15	Cell division control protein 42 homolog	1ano	-9.2	-9.1	-8.0	-7.4	-9.2	*Homo sapiens*
16	Estrogen	1aqy	-13.2	-13.0	-12.0	-12.0	-13.2	*MusMusculus*
17	Protease	1aaq	-10.4	-10.3	-10.0	-9.9	-10.4	*HIV*
18	Periplasmicoligo peptide-binding protein	1b05	1.6	-----	------	-----	1.6	*Solmonella*

19	Glutathione S-transferase	1b4p	-7.8	-7.7	-7.4	-7.0	-7.8	*Rattusnorvegius*
20	Thymidylate synthase	1bid	-11.0	-11.0	-10.6	-10.4	-11	*E.Coli*
22	Covid-19	6lu7	-8.7	-8.7	-8.4	-8.2	-8.7	*Protease protein*

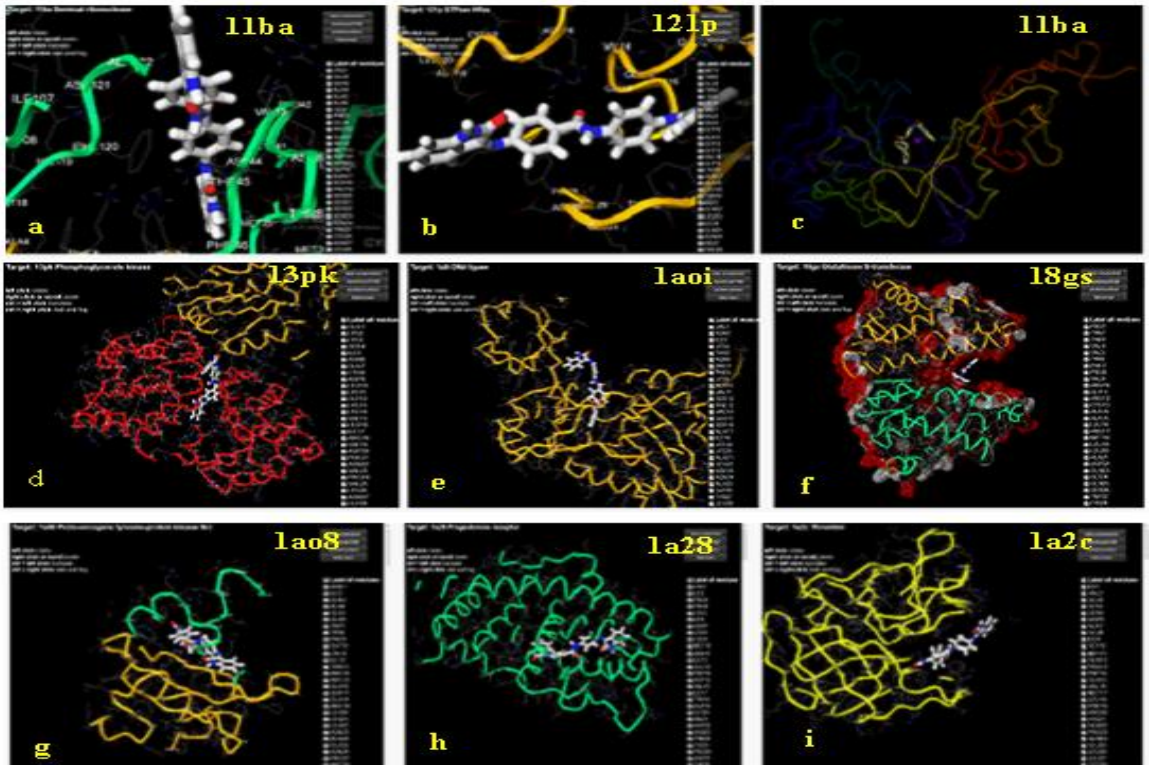


Figure.4. Some of the docked images

3.5. Docking study using covid-19 server

The prepared structure mol2 file and the covid-19 protease protein 6lu7 are updated in https://ncov.schanglab.org.cn/index.php server. The docking image has shown in **Figure.5.** The docking score of the compound exists between -8.20 and -7.70 kcal/mol. Top 10 images and the ligand position was analyzed for further research.

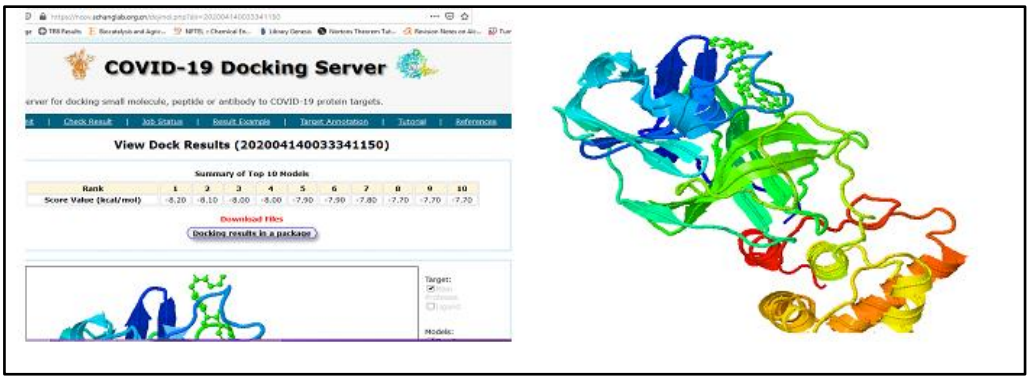


Figure.5. Docking image of isatin-DAB Schiff base against covid-19 protein

3.6. Docking study using CLC drug discovery workbench-3

The updated covid-19 6lu7 protein sequences were analyzed using CLC drug discovery Workbench -3 and have shown in **Figure.6**. Then the ligand structure was updated and

docked with the radius of 15A°. The docking score obtained through this CLC drug discovery software is -69.25kcal/mol and displayed in **Figure.7**.

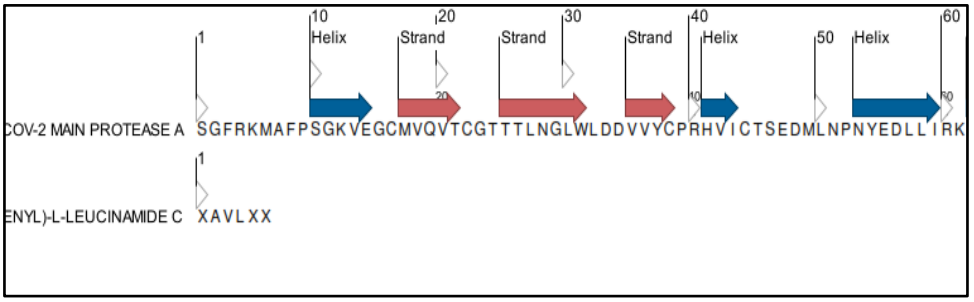


Figure.6. covid-19 6lu7 protein sequence image

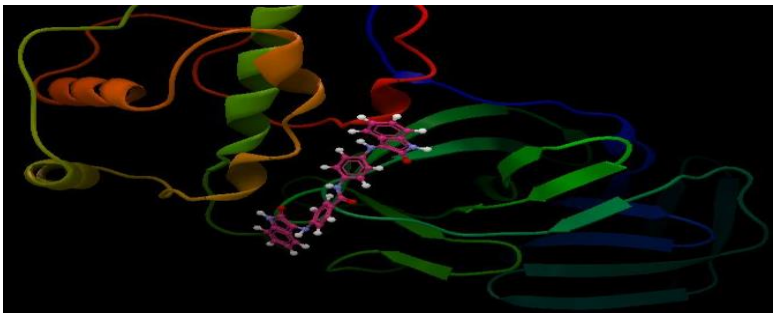


Figure.7. covid-19 6lu7 protein docked image

4. CONCLUSION

Isatin Schiff base have been synthesized successfully for the theoretical computational studies for the future drug discovery. This research used naturally available lemon juice extract as catalyst for the preparation of isatin Schiff base in methanol at reflux condition. Simple and efficient method has been developed for the synthesis of dimeric Isatin Schiff base in presence of natural catalyst due to the pH difference. Pure clear lemon juice exhibited pH-0.99 and acetic acid showed pH- 1.5. Initially, we tried the reaction of equimolar quantities of isatin and DAB but the reaction was not completed and the un-reacted DAB was existing .The product obtained from various conditions were tested for their solubility and surprisingly the product is soluble only in DMSO, DMF respectively. Based on TLC, Melting point, dark-orange and solubility, the product is identified and the FTIR is compared. Then the theoretical breakdown of the compound is identified as shown in **Figure.12**. When the compound decomposed into different compounds like in **Figure.12**, then they can be act as a good inhibitor of various pathogenic proteins.

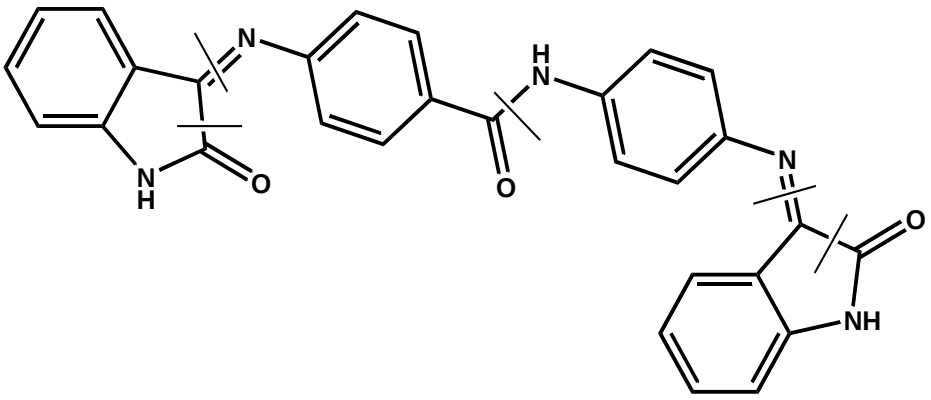


Figure.12. various breakage of the isatin derivative

Hence, the computational molecule-protein docking study was done through mcule online tool, covid19 and offline CLC drug discovery work bench-3. Total twenty two proteins were docked against the Schiff base and they showed best score between -13.2 and +15 respectively. Positive docking score of Schiff base showed lower energy than the proteins and weak binding. Negative score exposed the good binding ability with the proteins, good locking and flexibility. This computer aided outcomes are useful for the future drug discovery. The synthesized novel compound exhibited the negative score against the covid-19 protein and may be the reason of decomposition of the molecule in body fluid conditions. Apart from the different strategies of the drug design for the covid-19 such as coating on virus using simple molecules, reacting with ACE-2 using protein, and addition of antibody to enhance the immunity. This theoretical strategy may follow that the formed decomposed compounds may coat the pathogens surface and may protect the body.

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