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RESEARCH ARTICLE

IN SILICO SCREENING OF EFAVIRENZ AND EFAVIRENZ NICOTINAMIDE COCRYSTAL FOR ITS ACTIVITY AGAINST HIV AND SARS COV-2

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ABSTRACT

Recently many antiviral agents are under screening for the management of COVID-19 caused by SARS CoV-2. The *in silico* screening provides the insights of drug – receptor binding and efficacy of molecule to treat the diseases. In this work, the efficacy of an antiviral agent efavirenz and efavirenz nicotinamide cocrystal (ENCOC) against the SARS CoV-2 was determined using *in silico* techniques using AutoDock PyRx. Interestingly EFV shows the binding affinity with both HIV and SARS CoV-2 whereas newly synthesized ENCOC have prominent binding affinity with HIV and leading protease inhibitor of SARS CoV-2. The screening results confirms the activity of EFV and ENCOC against HIV-1 and SARS CoV-2 and warrants *in vivo* study for estimation of activity against SARS CoV-2

Key words: Efavirenz, Nicotinamide, Cocrystal, Co-formers, *in silico* screening, SARS CoV-2, acting antiviral agent.

INTRODUCTION

Human Immunodeficiency Virus (HIV) and viral infections like severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) have become a serious public health issue globally.

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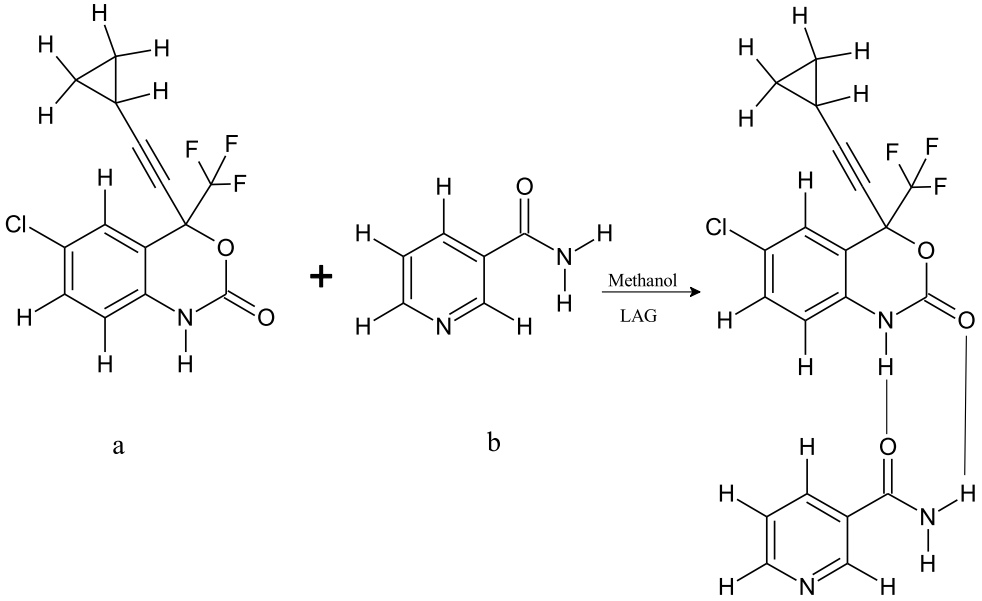
According to the World Health Organization (WHO), till 14 July 2021, 36.3 million people lost their lives, and 37.7 million individual were infected with HIV (1). In the current SARS CoV-2 pandemic 4.5 million human deaths and 217.55 million people were infected till the end of Aug 2021(2). To treat such deadly infections antiviral agents are the choice of treatment, Food and Drug administration (FDA) approved many antiviral agents like Veklury (Remdesivir) as therapeutic agents for treatment of SARS CoV-2. (3). The literature based evidences exhibits the utility of non-nucleoside reverse-transcriptase inhibitors (NNRTI) for blocking the RNA-dependent RNA polymerase (RdRp) and suppress the infection of SARS CoV-2 (4–9). Hence Efavirenz (EFV) a NNRTI used in the treatment of HIV in combination therapy was selected as model drug in this study. An approximate dose of EFV is 200 – 600 mg and it comes under BCS –II and having poor aqueous solubility, due to low aqueous solubility it exhibits low oral bioavailability (10–12). EFV is part of high activity antiretroviral therapy (HAART) and considered as best suitable API for treating HIV (13). This fact of EFV leads to be the suitable drug molecule for solubility enhancement. In this study nicotinamide (NICO) or vitamin B3 was used as coformer because it consisting both hydrogen bond donor (-NH2) and acceptor (C=O) group in its chemical structure which favors to formation of hydrogen bond. The expected molecular arrangement is depicted in Scheme 1.

To enhance the solubility, stability and pharmacokinetics various pharmaceutical approaches like Solid dispersion(14), Co-crystallization (15,16) Hot melt extrusion(17), nanotechnology(18), cyclodextrin based tactics (19,20) are utilized. In this study attempt was made for solubility enhancement of an antiviral agent EFV through pharmaceutical cocrystallization technique.

The pharmaceutical cocrystal approach is gaining attention from pharmaceutical industries because of its scalability, uninterrupted manufacturing process, economy and applicability(21).

In this work we synthesized pharmaceutical cocrystal (ENCOC) of EFV and NICO through liquid assisted grinding (LAG) method to enhance the solubility and physicochemical properties. Further we performed *in silico* screening of EFV and ENCOC for screening of its antiviral activity and study the effectiveness against SARS Co-2. The AutoDock PyRx, Auto dock Vina, open Bable and Discovery Studio software were utilized for molecular modeling and computational studies. The main focus of present work is to study the effect of pharmaceutical cocrystallization on solubility of an antiviral agent EFV and it's *in silico* screening for anti- HIV and SARS CoV-2 activity.



a b c

Scheme I. Schematically representation of formation of EFV (a), NICO (b) Pharmaceutical cocrystal (ENCOC) (c)

MATERIALS AND METHODS

EFV was received as a gift sample from the Emcure R&D center Gandhinagar, Ahmedabad. NICO was purchased from the central drug house, solvents such as Methanol, water (HPLC) were purchased from Finar chemicals.

Synthesis of EFV Nico Cocrystal

The cocrystal of EFV was prepared through the Liquid Assisted Grinding (LAG) method. Accurately weighed EFV and NICO were triturated for 1.30 hrs. with slow addition of few drops of methanol. The formulated cocrystals were kept in a vacuum desiccator for 12 hrs. at room temperature and further used for characterization (22,23)

EXPERIMENTAL

In Silico Molecular Interaction

Platform For Molecular Modelling

Auto Dock python prescription 0.8 (PyRx)(24) were used for computational studies of EFV and ENCOC with HIV-1 reverse transcriptase protein (PDB ID: 1FK9)(25) and SARS CoV-2 main protease inhibiter (PDB ID: 5R81 and 5R82)(26). The Protein data base (PDB) file of proteins were downloaded from RCSB PDB (https://www.rcsb.org/). Discovery Studio Visualizer (v 21.1. 0.20298) was used for preparation of input

file for PyRx and post docking analysis (molecular interaction, Ligand- protein binding, H- bond , hydrophobicity, charges, ionizability and Aromaticity). The ChemDraw ultra v 12.0.2.1076 2D was used for elucidation of structure of ligand (EFV and ENCOC). The protein molecule file was uploaded to PyRx software and macromolecule was prepared through AutoDock and converted in to pdbqt format. Whereas the 3D ligand was uploaded through Open Bable and converted in to pdbqt file after energy minimization with uff force field. Further for the selection of binding site at HIV Protein (1fk9) macromolecule at reported residues(25) and its interaction with ligand after preparation of grid was studied through Auto Dock Vina software. Further the stability of EFV and ENCOC in the active site of concern macromolecule was determined through Root-Mean-Square Deviation (RMSD) and binding affinity was observed through AutoDock Vina(27).To study the intermolecular interaction and selection of amino acid residue of SARS CoV-2 main protease inhibitor the blind docking was performed.

Docking of Sars Cov-2 Using Autodock Vina

The main protease inhibitor of SARS CoV-2 5r81 and 5r82 were reported binding affinity with EFV with docking score -6.5 and -6.6 respectively(28). To confirm the binding affinity and selection of interacting residue the blind run of AutoDock Vina was carried out at Vina search space at center (x, y, z) 12.02, 0.67,4.52, and dimensions (Å) (x, y, z) 37.36, 64.30, 61.26.(29). The residues were selected based on the interaction between the macromolecule and ligand (Table2) and same residues were selected for docking of newly synthesized formulation ENCOC.

RESULTS AND DISCUSSION

In silico Molecular Interaction

AutoDock Vina reproduced the ten orientation for intermolecular interaction between macromolecule and ligand, Amongst that the orientation with higher negative binding affinity was selected(30) and further results of binding of EFV and ENCOC with HIV and SARS CoV-2 protein were mentioned in table no 2 and 3 and shown in fig 9 to 14. Approved antiviral agent EFV shows the intermolecular H- bonding (Lys-101) and Pi –Alkyl interaction (Leu-100, Val-106) with amino acid residue of HIV protein with binding score -6.7 kcal/mol. The newly synthesized ENCOC exhibits the conventional H- Bonding (Thr-139, Glu-28, and Lys 32) and Pi- Hydrogen interaction (Ile 31) with amino acid residues with bond length 3.22 to 3.69 Å. This molecular simulation results indicates the better *in silico* interaction of ENCOC with HIV protein (PDB ID 1fk9) and exhibits the enhanced anti HIV activity than EFV. Furthermore, we did the *in-silico* screening of EFV for the activity against the SARS CoV-2 and we reported here EFV exhibits the binding affinity with main protease inhibitor of SARS CoV-2 and exhibits the potential activity (*in*

silico) against SARS CoV-2. The EFV binds with amino acid residue of main protease inhibitor of SARS CoV-2 (PDB ID 5r81 and 5r82) with binding affinity -7.1 and -6.6 and forms the intermolecular H-bond (Thr-111, Arg-298), halogen (Asn-151) and Pi- Alkyl (Phe-294) bond. We also studied *in silico* binding affinity of ENCOC with main protease inhibitor of SARS CoV-2 and found that ENCOC exhibits the better intermolecular interaction with binding affinity -7.2 and -7.7 kcal/mol. (Table 2, Fig 14). ENCOC forms the H- bond (Asp-289, Leu-287, Tyr-239, Pro-108, Asn-203 and His-246 with amino acid residues.

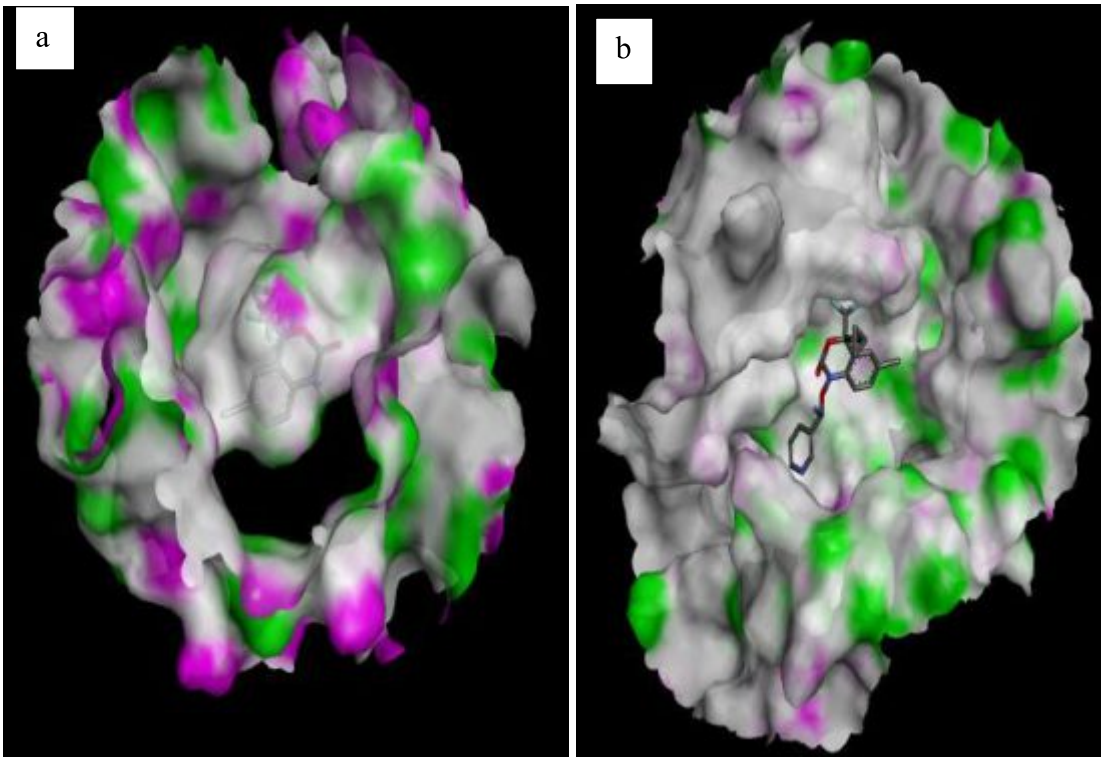


Fig.1. Binding Pocket of EFV (a) and ENCOC (b) in active site of HIV protein 1k9

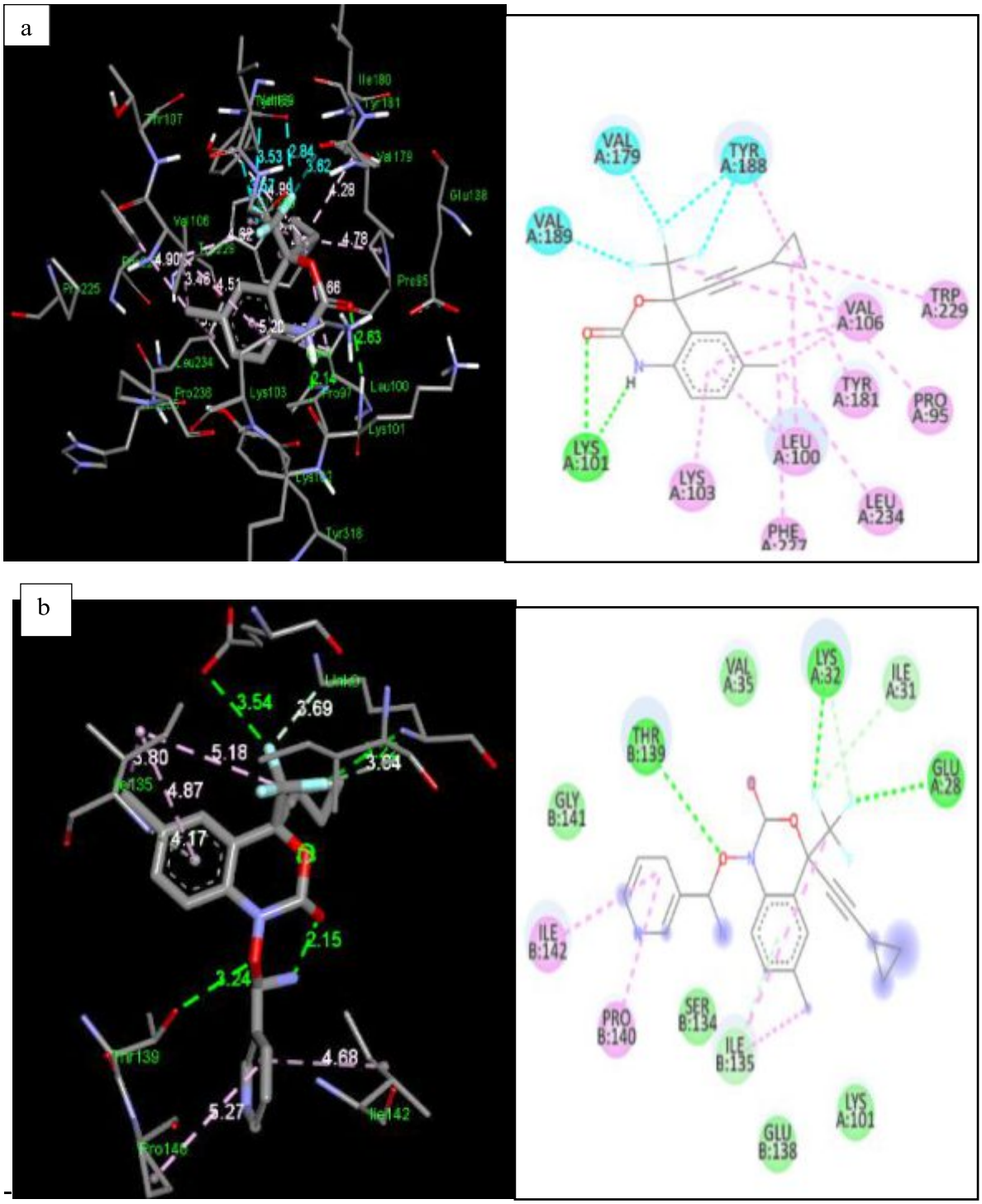


Fig.2. Docked poses of EFV (a) and newly synthesised pharmaceutical cocrystal ENCOC (b) in active site of HIV protein (1fk9)

Table 1. Ligand Protein binding interaction of EFV and ENCOC with HIV protein

Name of Ligand	HIV Protein	Binding affinity (kcal/mol)	Interacting Moieties	Type of Bond/ Interaction	Amino acid residue	Bond length (Å)
EFV	1FK9	-6.7	C=O	Conventional H-Bond	Lys 101	2.63
			NH_2	Conventional H-Bond	Lys 101	2.14
			CF_3	Halogen (F)	Val 189	3.57
			Ar-Ring	Pi- Alkyl	Leu-100 Val-106	4.75 4.51
ENCOC	1FK9	-7.6	C=O	Conventional H-Bond	Thr-139	3.24
			CF_3	Conventional H-Bond Pi donor H- Bond	Glu-28 Lys-32 Ile -31	3.22 3.54 3.69
			Ar-Ring	Pi- Donor H- Bond Pi- Alkyl Alkyl	Ile-135 Ile-135 Ile -142	4.17 4.87 4.68

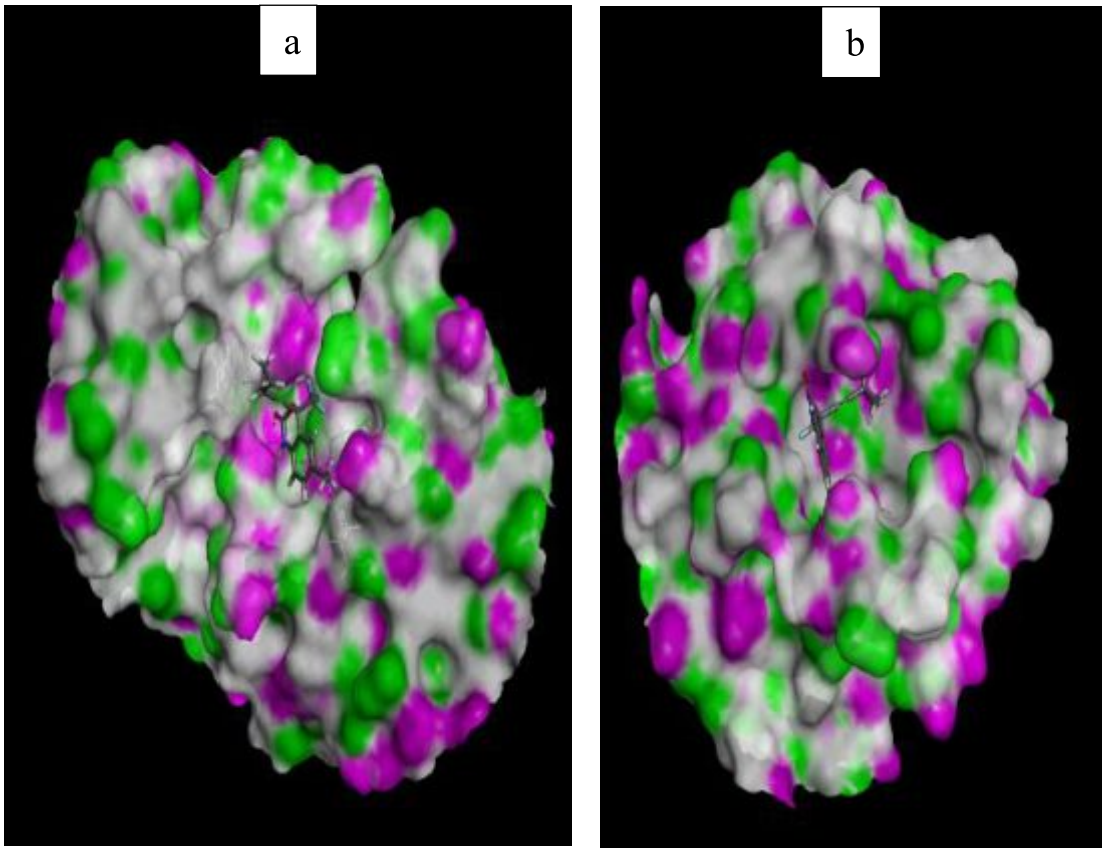


Fig.3 Binding pockets of EFV with SARS CoV-2 protein (a) 5r81 and (b) 5r82

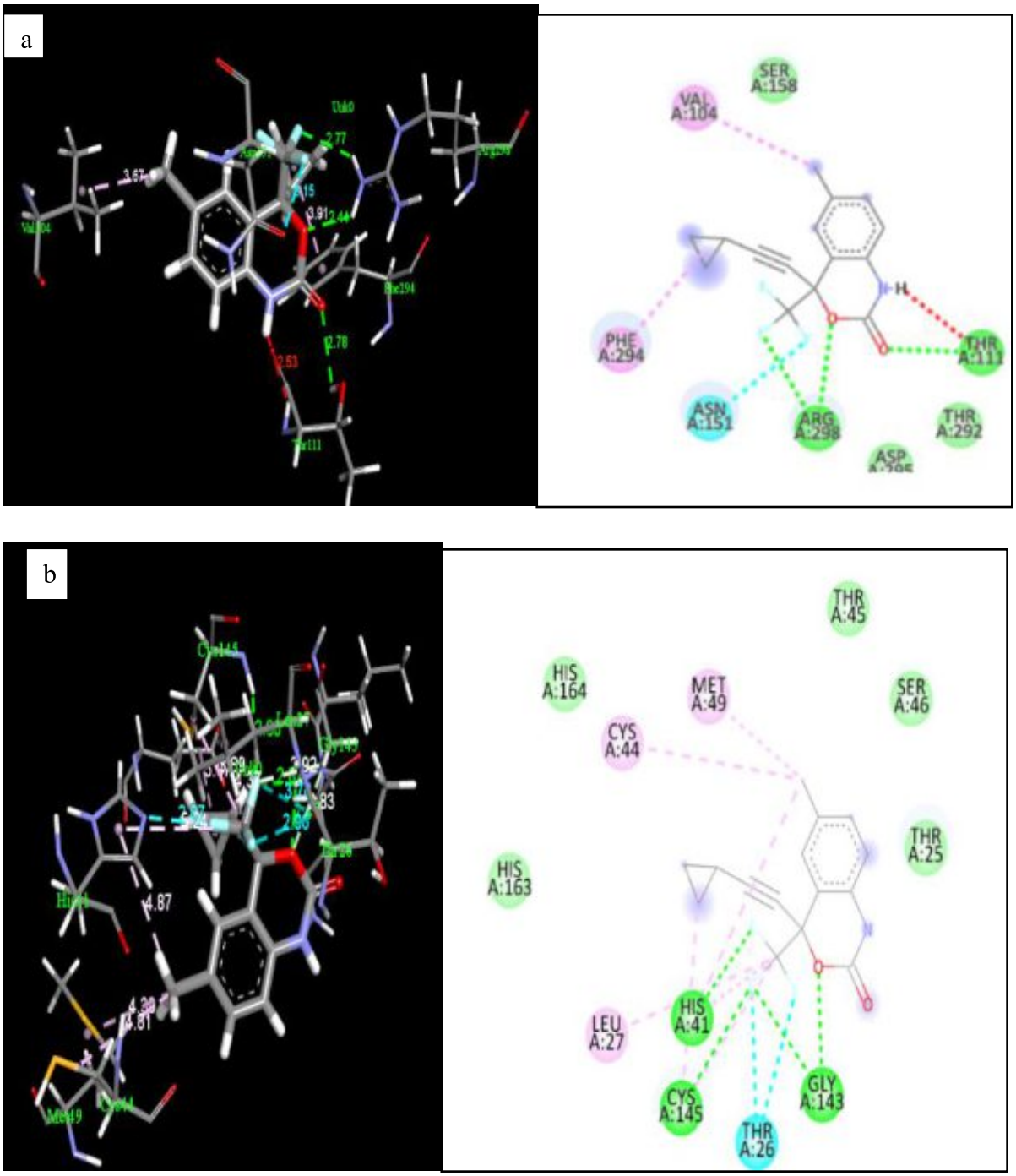


Fig.4. Docking poses of EFV in active site of SARS CoV-2 protein 5r81 (a) and 5r82 (b)

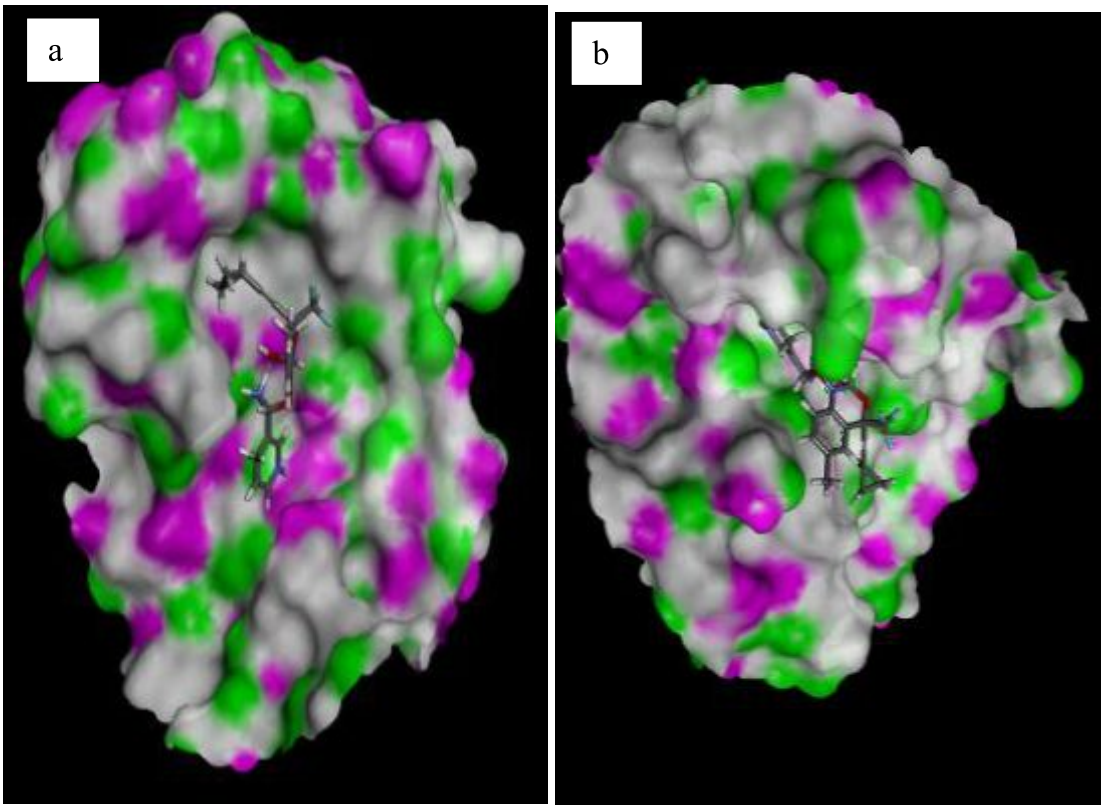


Fig.5. Binding pockets of newly synthesised pharmaceutical cocrystal ENCOC with SARS CoV-2 protein (a) 5r81 and (b) 5r82

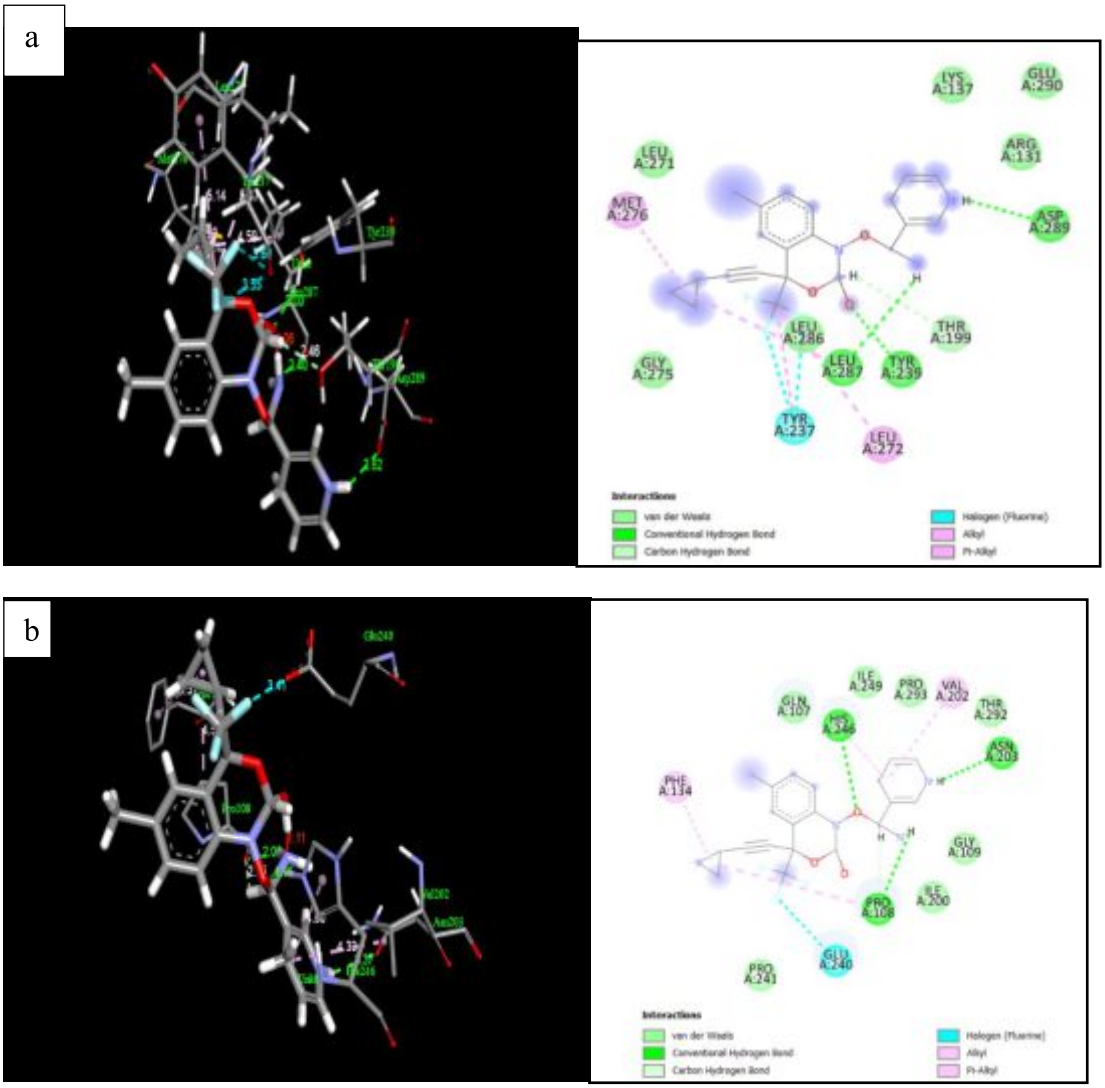


Fig.6. Docking poses of newly synthesised pharmaceutical cocrystal ENCOC with SARS CoV-2 protein (a) 5r81 and (b) 5r82

Table.2. Ligand Protein binding interaction of EFV and ENCOC with SARS CoV-2 protein

Name of Ligand	COVID 19 protein	Binding affinity (kcal/mol)	Interacting Moieties	Type of Bond/ Interaction	Amino acid residue	Bond length (Å)
EFV	5r81	-7.1	C=O	H- Bond	Thr-111	2.78
			C-O	H- Bond	Arg-298	2.44
			C-F	H- Bond Halogen (F)	Arg-298 Asn-151	2.77 3.15
			C-H	Pi- Alkyl	Phe-294	3.99
	5r82	-6.6	C-O	H- Bond	Gly-143	2.47
			CF_3	H- Bond Halogen (F) Halogen (F)	Cys-145 Gly-143 His-41 Thr-26	2.80 2.87 2.87 2.90
			C-H	Pi- Alkyl Alkyl	His-41 Met-49 Cys-44	4.87 4.39 4.81
ENCOC	5r81	-7.2	NH	H-Bond	Asp-289	2.62
			NH_2	H- Bond	Leu-287	2.40
			C=O	H- Bond	Tyr-239	2.00
			CH	Van der Waals	Thr-199	2.46
			CF_3	Halogen (F)	Tyr-237	3.53 3.60
			CH	Alkyl Alkyl	Leu-287 Met-276	4.59 4.93
	5r82	-7.7	NH	H- Bond	Pro-108 Asn-203	2.06 2.37
			C=O	H- Bond	His-246	1.95
			Ar- Ring	Alkyl Pi- Alkyl	Val-202 His-246	4.33 4.90
			CF_3	Halogen (F)	Glu-240	3.41
			CH	Alkyl	Pro-108	4.55

The *in silico* screening of EFV and ENCOC shows the potential activity against both HIV and SARS CoV-2. The carboxylic acid (C=O) and amino (NH_2) group of coformer NICO used in synthesis of ENCOC plays the crucial role in H-bonding of ENCOC with HIV and SARSCoV-2 protein. Hence, we recommended further studies on the application of pharmaceutical co-crystallization for enhanced *in vivo* pharmacokinetic activity of approved antiviral agent against SARS CoV-2.

CONCLUSION

The antiviral drug EFV's cocrystal was created in the current work employing liquid assisted grinding with methanol as the solvent. In order to increase the solubility of EFV, the vitamin B3 nicotinamide, which is easily water-soluble, was utilized as a coformer. *in silico* screening we determined the strong binding affinity of EFV and ENCOC with both HIV-1 reverse transcriptase and main protease of SARS CoV-2 that confirms the activity of EFV and ENCOC are highly active against HIV-1 and SARS CoV-2 and warrants *in vivo* study for estimation of activity against SARS CoV-2.

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