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DESIGN, DEVELOPMENT AND SYNTHESIS OF NOVEL CHIRAL DERIVED MOLECULES, POTENTIALLY ACT AS RNA POLYMERASE NSP-12 AND NSP-6/10 COMPLEX INHIBITORS AGAINST COVID-19

Magendran Balaachari *, M. Menaga and S. Pooja Sri

Bioneemtec India Private Ltd, Women's Biotech Park, Siruseri - 603103, Tamil Nadu, India.

Keywords:

Di and Tridentate derived chiral molecules, Molecular docking, RNA polymerase, Covid-19, Remdesivir, Hydroxychloroquine

Correspondence to Author:
Magendran Balaachari

Director,
Bioneemtec India Private Ltd,
Women's Biotech Park, Siruseri - 603103, Tamil Nadu, India.

E-mail: magendran6@rediffmail.com

ABSTRACT: Novel Di and Tridentate chiral molecules and its analogues were designed and synthesized in that the chiral amino ester and 2-amino-3, 5-dibromobenzaldhehyde were condensed. Further, it has coupled with Cassic acid to form desired active molecules. The developed compounds are evaluated for molecular docking to check potency and may represent potential drug candidate inhibitors of RNA dependent RNA polymerase of Covid-19, compare to Remdesivir and Hydroxychloroquine. The present work is to identify and investigating the potential drug out of eight compounds that may be used to inhibit RNA-dependent RNA polymerase non-structural proteins complex of Covid-19 to prevent the replication of viral genome in host cells. The compounds BNT-202006, BNT-202008 & BNT-202009 showed and exhibited minimum binding energy when it bound with protein targets when compared with Remdesivir and Hydroxychloroquine as standard drug.

INTRODUCTION: There is an emergence of novel Coronavirus pandemic infections initially started in Wuhan, China in December 2019 [1] and it has spread across approximately in 208 countries with 1 million filed infective cases. On February 2020, the International Committee on Taxonomy of Viruses has announced it and renamed as SARS-Covid-19 [2]. Currently, SARS-CoV-2 infected patients were exposed to Remdesivir, a nucleotide analogue can act as a RdRp (RNA dependent RNA Polymerase) that block viral RNA replication. The active site of RdRp domain is composed by conserved motifs of A-G and resembled like other RNA polymerases structure.

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Once the drug get access to bind with a growing chain of viral protein cannot immediately stop replication process unless it counterpart to extend 3 to 4 nucleotides in the downstream position and thereby inhibiting the RdRp mechanism in lungs epithelial host cells.

MATERIALS AND METHODS:

RNA Dependent RNA Polymerase nsp Complex: The RNA dependent RNA polymerase nsp complex catalyzes a viral RNA replication and transcription machinery and a targeted molecules for antiviral drug such as Remdesivir. Here we used RNA dependent RNA polymerase nsp complex of nsp 12, 6/10 for molecular drug designing targets. These molecules acting as insight into a hotspot region for designing of new therapeutic anti-viral drugs targeting RdRp.

Chemistry: In the latest investigation into the structure and reactivity of tridentate Schiff base

ligands and complexes derived from L-tert-Leucine, were recently reported derived from ligand, which contained an unprecedented anti-skew carboxylate bridging group, previously some of the bi and tridentate amino alcohol ligands as Schiff bases were reported [3a-3b]. In the present study bromo substituted amino chiral compounds were synthesized by treating imino ester hydrochloride along with 2-amino-3,5-dibromo benzaldehyde to form schiff's bases and it has reduced C=N bond specifically using Tetrabutyl ammonium borohydride. Amine derived ester chiral molecules further react with cassic acid to form desired active amide molecules like BNT-202006 to BNT-202009. The schematic diagram of the molecules synthesized as below **Fig. 1** to **4**.

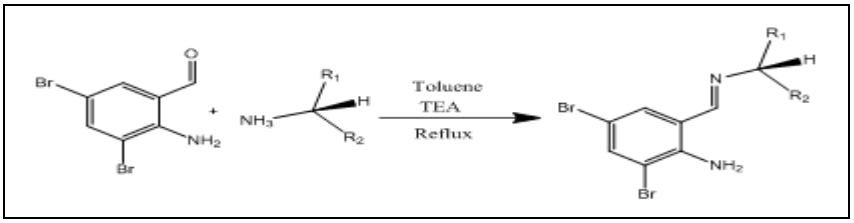


FIG. 1: SYNTHESIS OF COMPOUND 2 TO COMPOUND 4

S. no.	Compound #	R1	R2	Yield
1	Compound 2	$(CH_3)_2CH)$	$COOCH_3$	80%
2	Compound 3	$((C_6H_5)CH_2)$	$COOC_2H_5$	73%
3	Compound 4	$((HOC_6H_5)CH_2)$	$COOC_2H_5$	69%

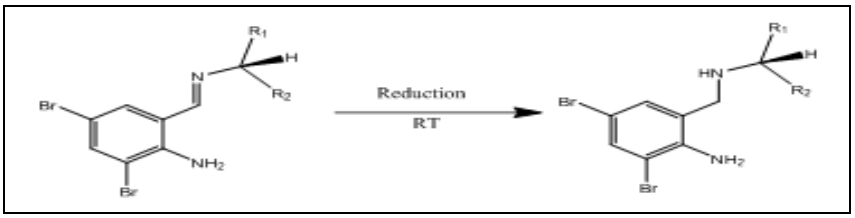


FIG. 2: SYNTHESIS OF COMPOUND 1

S. no.	Compound #	Reducing agent	R1	R2	Yield
1	Compound 1	TBAB / Methanol	$(CH_3)_2CH)$	$COOCH_3$	62%

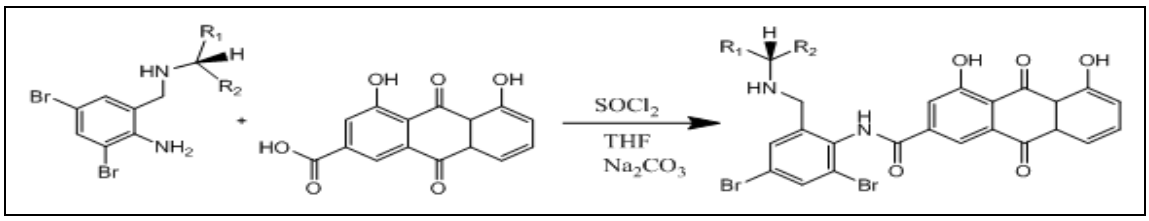


FIG. 3: SYNTHESIS OF COMPOUND BNT-202006, BNT-202008 AND BNT-202009

S. no.	Compound #	R1	R2	Crude Yield
1	BNT-202006	$(CH_3)_2CH)$	$COOCH_3$	68%
2	BNT-202008	$((HOC_6H_5)CH_2)$	$COOC_2H_5$	62%
3	BNT-202009	$((C_6H_5)CH_2)$	$COOC_2H_5$	60%

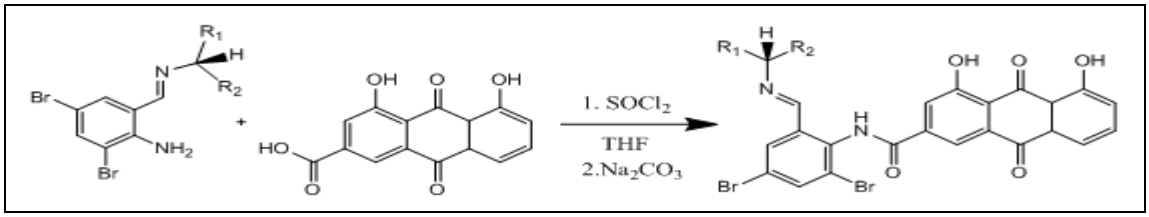


FIG. 4: SYNTHESIS OF COMPOUND BNT-202007

S. no.	Compound #	R1	R2	Crude Yield
1	BNT-202007	$(CH_3)_2CH)$	$COOCH_3$	71%

General Procedure for Synthesis of Compound 2 to 4: Amino ester hydrochloride (5.0g 0.03mol) taken with 100ml of Toluene and 2-amino-3,5-dibromobenzaldehyde (8.30g, 0.03mol) was added, to this triethylamine (1.0ml) was added dropwise at 30 ºC, slowly raise the temperature to reflux and started a collection of water using Dean stark apparatus, maintained the reflux for 4 h and cool to room temperature then formed triethylamine hydrochloride was removed by passing the reaction mass to silica pad column, the organic layer removed under vacuum, which affords desired product as yellow gel liquid.

Synthesis of Compound 1: Imino esters (5.0g, 0.0127mol) dissolved in 30ml of methanol and cool to 0-5 ºC and tetra butyl ammonium borohydride (1.2g, 0.005mol) added in a lot with the same temperature, the completion of the reaction was monitor by TLC (ethyl acetate: Hexane) 5:5, after completion the reaction quenched with 10ml of 1% dil. HCl solution and degassed the methanol completely then extracted with 50ml of ethyl acetate and washed with 50ml of water and removed the organic layer under vacuum afford the amino ester as crude material which is further purified by CC (ethyl acetate and Hexane as eluent) and isolated as pale yellow gel liquid.

General Procedure for Synthesis of BNT Compounds (BNT-202006 to BNT-202009): Cassic acid (1 mmol) in $SOCl_2$ (2 mmol) was stirred at reflux. After the completion of the reaction, the solvent was removed under reduced pressure. The crude acid chloride thus obtained was dissolved in 20 volumes of CH_2Cl_2. After the addition of the corresponding chiral derived imine or amine (1mmol) followed Na_2CO_3, the mixture was heated at reflux. After the completion of the reaction, the mixture was cooled to room temperature, filtered, and concentrated under vacuum to give a crude yellow semi-solid.

Targeted Protein Preparation: The crystalline structure of RNA dependent RNA polymerase complexed with non-structural proteins 12 (nsp 12) & nsp 6/10 [PDB ID: 6NUR & 6W4H] was retrieved from the public Protein Data Bank database in pdb format. Then targeted protein was prepared to remove the coordinates of other ligands hetero atom [HETATM], which is not a part of the native protein structure. Manually, Swiss PDB Viewer software was used for protein-energy minimization by the steepest energy minimization method. Now the energy minimized protein was used for the further docking computational studies.

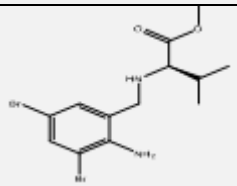
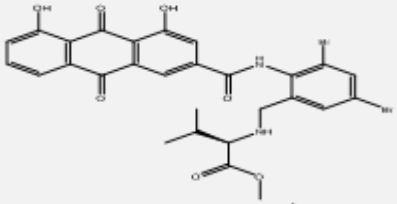
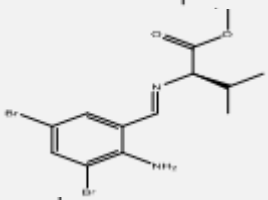
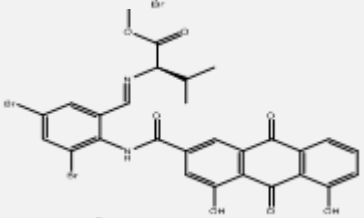
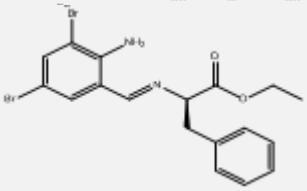
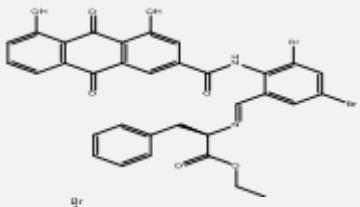
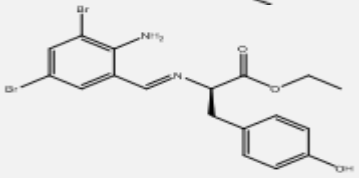
Compounds Library: The synthetically designed compounds were drawn using Chemsketch [3] and checked for Druglikeness scoring such as Lipinski, Ghose, Veber, Egan, Muegge rules to avoid late clinical trial faults. The designed compounds were saved as MDL Mol files and later converted into Autodock vina default pdb format using Open Babel software [4, 5]. Totally 8 compounds were designed and proceeded to virtual docking study for the screening of potent RNA polymerase inhibitors to combat, control, and reduce Covid-19 infections.

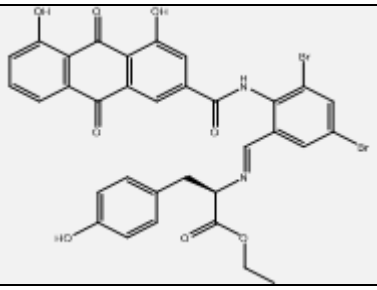
Molecular Docking Study Design: AutoDock 4.0 version was used in this virtual drug screening study. Target proteins were saved into PDBQT format using MGL Tools (version - 1.5.6rc3) throughout the docking procedure. The currently used standard drug Remdesivir and Hydroxy-chloroquine was taken as a positive control and reference for compound significant binding affinity comparison. All polar hydrogen atoms, kollman charges, and Assign AD4 type were manually added. An investigative analysis of compound binding poses generated by AutoDock showed accuracy achieved when the radius of gyration of a docking compound expanded to 2.9 times larger [6]. The grid size was automatically generated by installed MGL tools for increasing the docking accuracy of compounds with targeted proteins. The X, Y, and Z coordinates [149.984, 147.533 and 157.028] were used as grid box points. Lamarckian genetic algorithm was applied for better docking accuracy of compounds. Ten docking runs were performed by Cygwin command prompt software for RMSD data file generation as a dlg file format. The RMSD (Root Mean Square Deviation) scoring and ranking of the compounds was based on the AutoDock empirical scoring function as the minimum binding energy in kcal/mol and inhibition constant was noted. The best-docked poses of the protein-ligand complex were visualized by using three-dimensional visualizing UCSF Chimera software.

ADMET Properties Study: Prediction of Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) is an important step in drug development of process in pharmaceutical study. The best-bound compounds with significant minimum binding energy were checked for ADMET properties using web servers Swiss ADME[7] and ProTox-II - Prediction of Toxicity of chemicals [8, 9, 10]. ADMET computation allows to find physio-chemical properties, pharmacokinetic properties, drug likeness scoring and medicinal chemistry of compounds to investigate drug discovery study in an effective way.

RESULTS AND DISCUSSION: Molecular drug designing have been most useful rapid method of screening a potent inhibitors, modulators of disease causing proteins of microbial pathogens. Molecular docking study develops high impact in drug discovery process in a less time intervals with therapeutic index of new drug development in pharmaceutical industries.

TABLE 1: COMPOUNDS LIBRARY USED FOR DOCKING STUDY

Compound #	Structure	Atoms composition
Compound 1		C(39.62%) H(4.60%) Br(40.55%) N (7.11%) O (8.12%)
BNT-202007		C(50.93%) H(3.66%) Br(24.20%) N(4.24%) O(16.96%)
Compound 2		C(39.82%) H(4.11%) Br(40.76%) N(7.14%) O(8.16%)
BNT-202006		C(51.09%) H(3.37%) Br(24.28%) N(4.26%) O(17.01%)
Compound 3		C(47.60%) H(3.99%) Br(35.19%) N(6.17%) O(7.05%)
BNT-202009		C(55.02%) H(3.36%) Br(22.18%) N(3.89%) O(15.55%)
Compound 4		C(45.98%) H(3.86%) Br(33.99%) N(5.96%) O(10.21%)

BNT-202008		C(53.83%) H(3.29%) Br(21.70%) N(3.80%) O(17.38%)

Molecular drug designing have been the most useful rapid method of screening potent inhibitors, modulators of disease-causing proteins of microbial pathogens. Molecular docking study develops high impact in the drug discovery process in a less time intervals with a therapeutic index of new drug development in pharmaceutical industries.

Molecular Docking & ADMET Properties Analysis: Molecular docking of the protein-ligand complex is a time-consuming method in new drug development before drug formulations and their inhibiting interaction investigations against a target protein molecules of viral pathogens. All the AMDE properties were predicted with the help of the Swiss ADME web tool for a better understanding of newly designed drug candidates. The compounds highlighted shown a minimum binding affinity with Covid-19 targeted proteins. Hence, this study supports to aid new drug development against SARS-Covid-19 infections in the human population. New drug development was important when the virus showed resistance to existing drugs such as *Remdesivir* and many other candidates.

From the docking study, we found that compounds BNT-202006, BNT-202008 & BNT-202009 exhibited the highest inhibition (minimum binding energy) against targeted proteins.

TABLE 2: RMSD (ROOT MEAN SQUARE DEVIATIONS) DATA WITH MINIMUM BINDING ENERGIES OF DESIGNED 8 DRUG CANDIDATES AGAINST COVID-19 RNA POLYMERASE nsp 12 & nsp 6/10 COMPLEX

S. no.	Compounds #	Nsp 12 complex Minimum binding energy in (kcal/mol)	Nsp 6/10 complex Minimum binding energy in (kcal/mol)
1	Compound 1	-7.54	-6.76
2	Compound 2	-6.64	-6.78
3	Compound 3	-4.89	-5.33
4	Compound 4	-7.14	-8.17
5	BNT-202006	-9.23	-12.37
6	BNT-202007	-9.60	-8.57
7	BNT-202008	-9.92	-9.81
8	BNT-202009	-10.55	-10.77
9	Remdesivir	-7.25	-7.64
10	Hydroxychloroquine	-5.68	-6.98

Most docked poses of BNT-202006, BNT-202008 and BNT-202009:

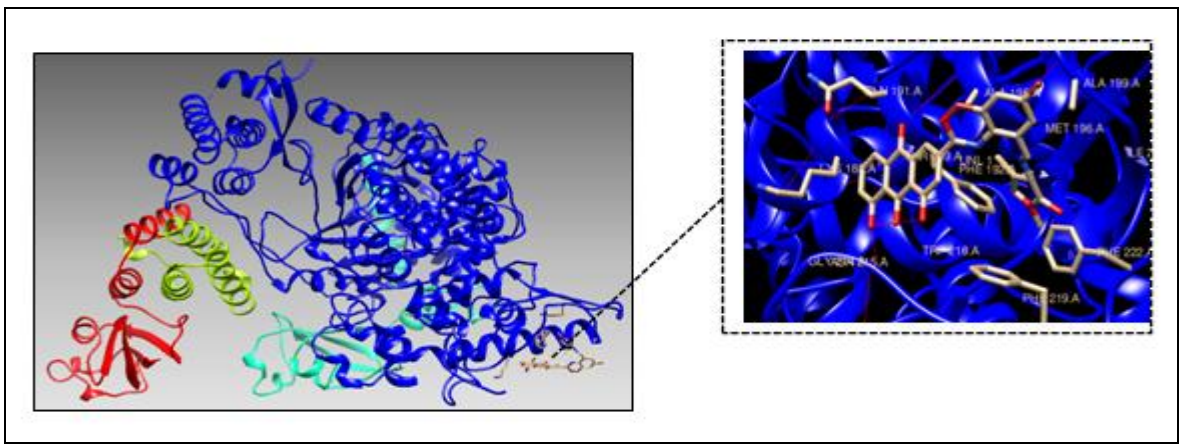


FIG. 5: RNA DEPENDENT RNA POLYMERASE nsp12 COMPLEX BOUND WITH BNT-202006

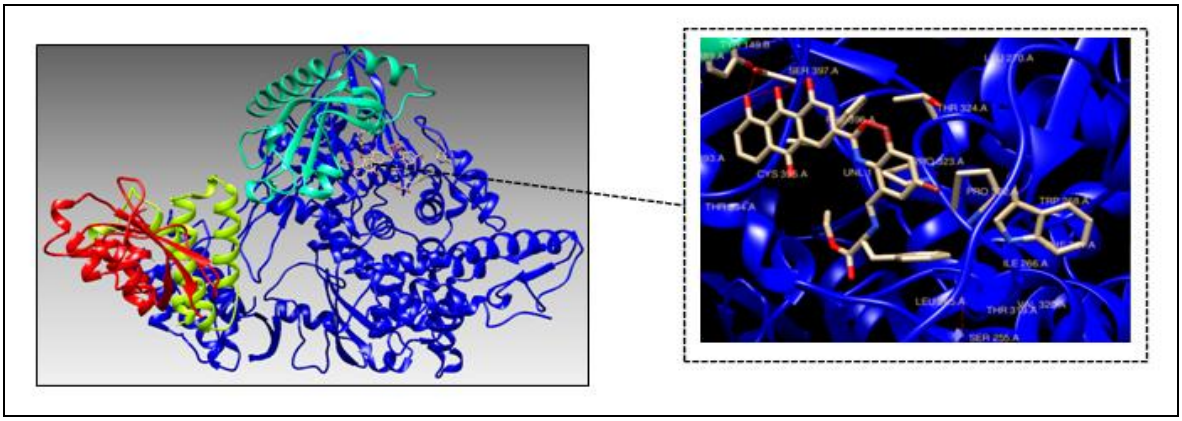


FIG. 6: RNA DEPENDENT RNA POLYMERASE nsp 12 COMPLEX BOUND WITH BNT-202008

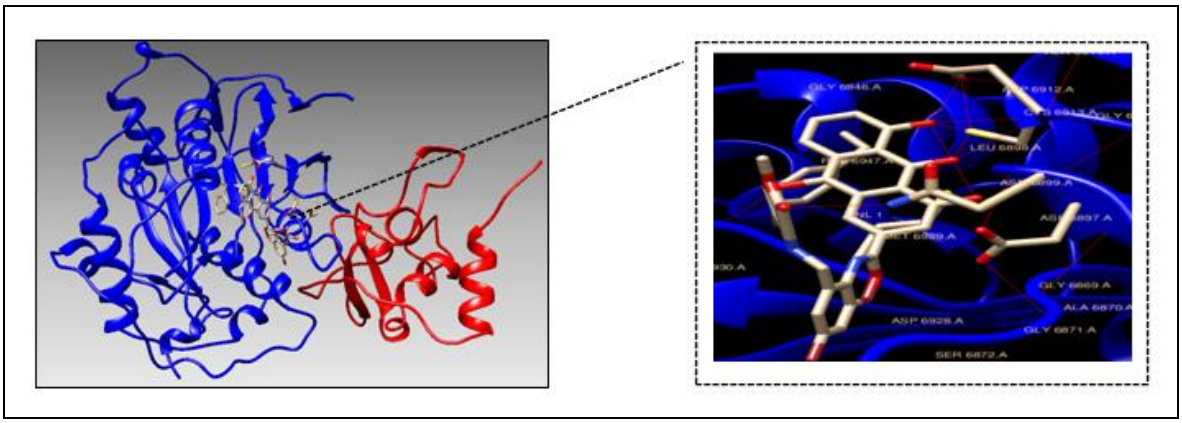


FIG. 7: RNA DEPENDENT RNA POLYMERASE nsp 12 COMPLEX BOUND WITH BNT-202009

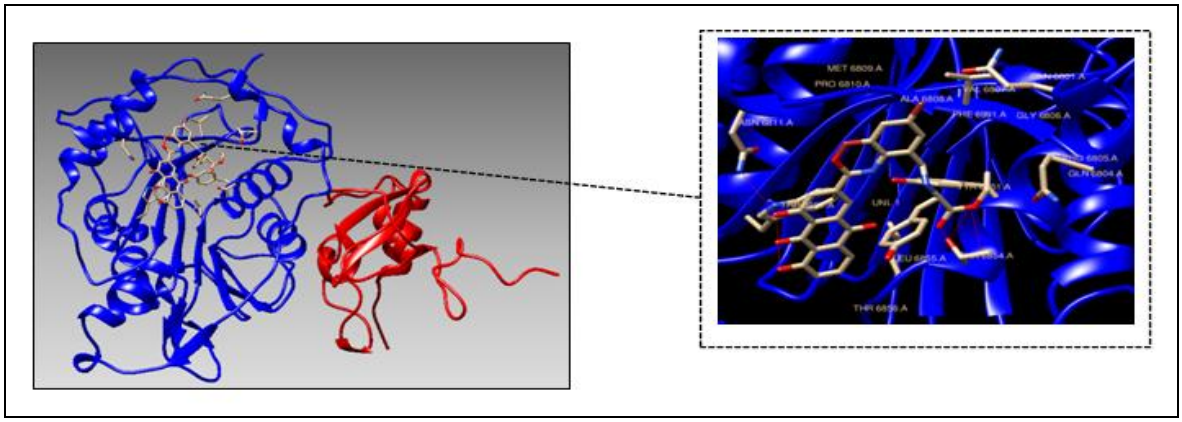


FIG. 8: RNA DEPENDENT RNA POLYMERASE nsp 6/10 COMPLEX BOUND WITH BNT-202006

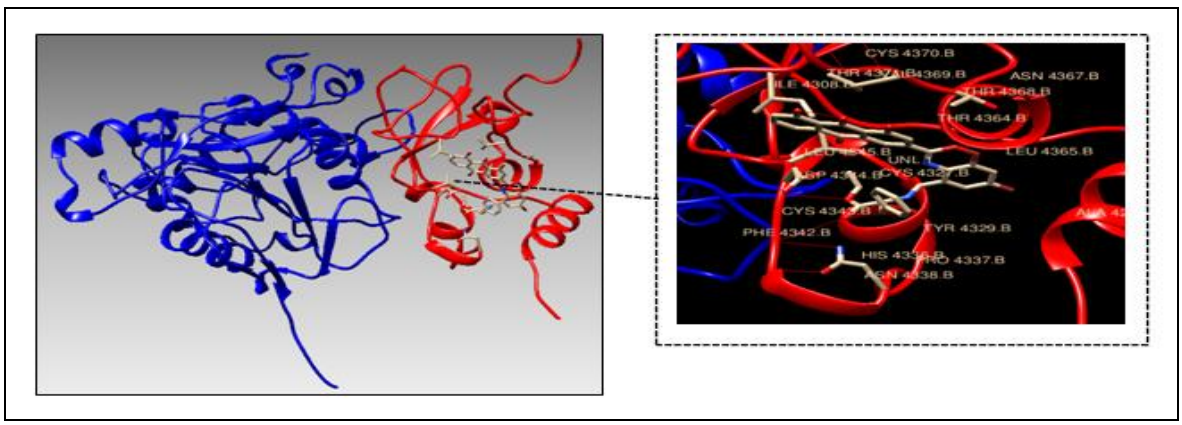


FIG. 9: RNA DEPENDENT RNA POLYMERASE nsp 6/10 COMPLEX BOUND WITH BNT-202008

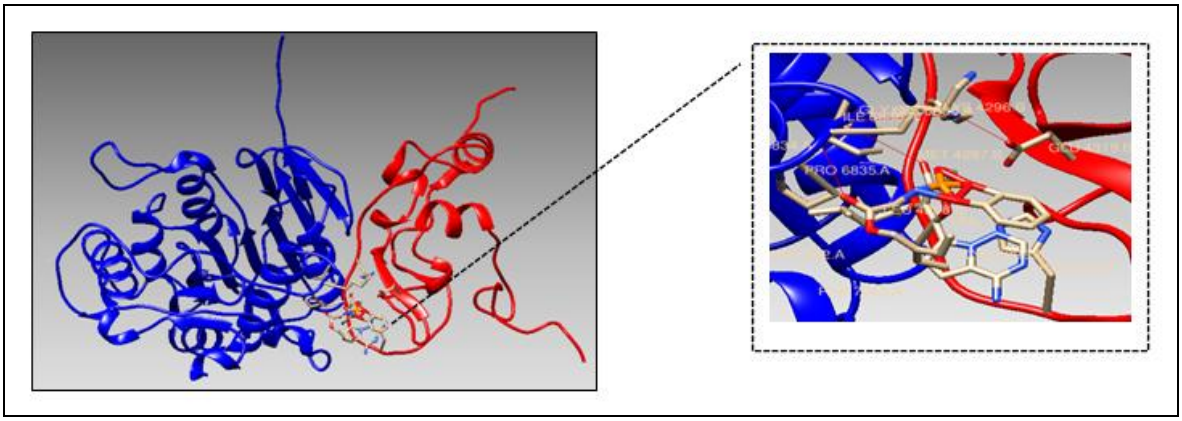


FIG. 10: RNA DEPENDENT RNA POLYMERASE nsp 6/10 COMPLEX BOUND WITH BNT-202009

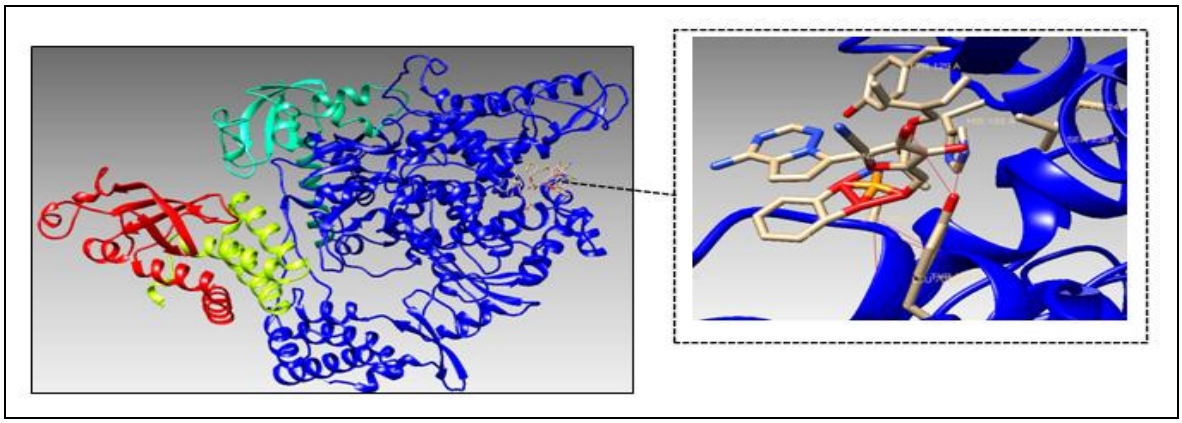


FIG. 11: RNA DEPENDENT RNA POLYMERASE nsp 6/10 COMPLEX BOUND WITH REMDESIVIR

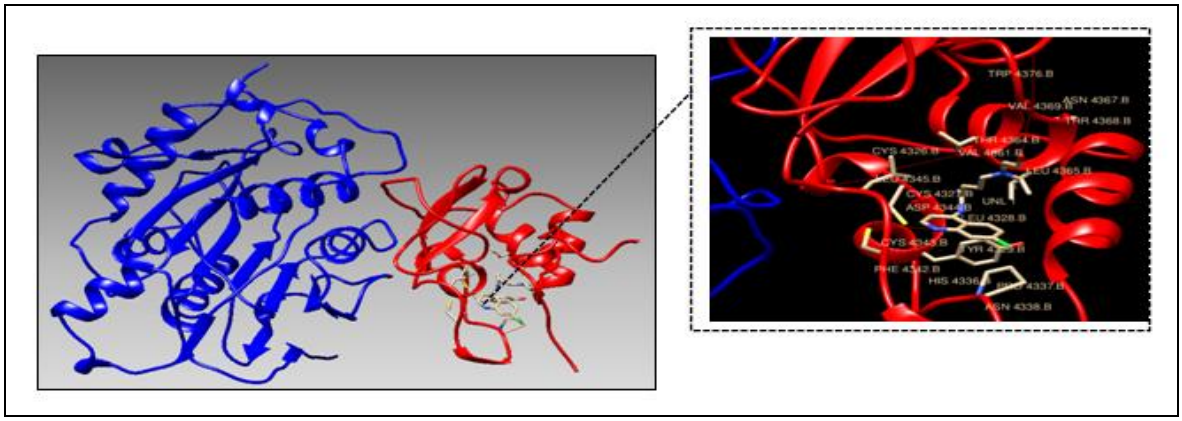


FIG. 12: RNA DEPENDENT RNA POLYMERASE nsp 12 COMPLEX BOUND WITH REMDESIVIR

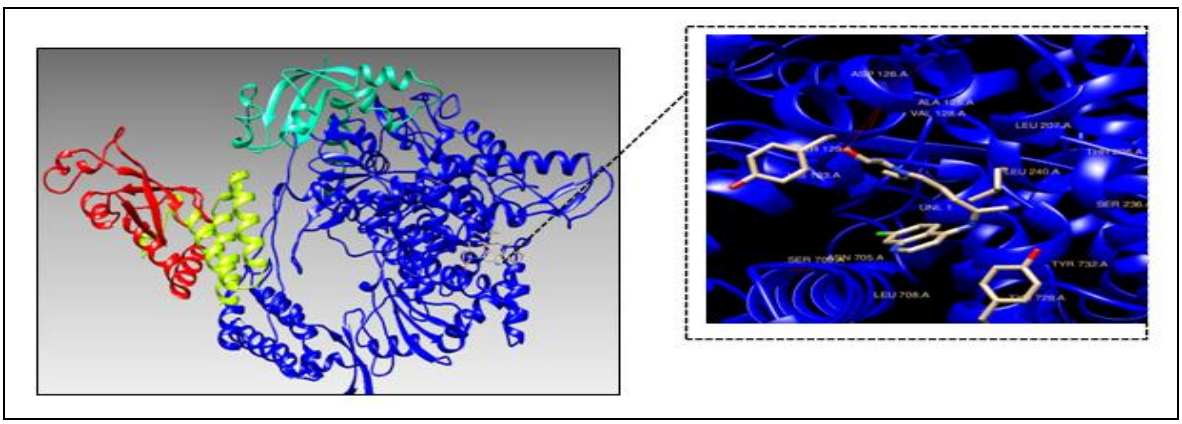


FIG. 13: RNA DEPENDENT RNA POLYMERASE nsp 6/10 COMPLEX BOUND WITH HYDROXYCHLOROQUINE DRUG

Ligand-Protein Complex Interacting Profiles: The Protein-Ligand Interaction Profiler (PLIP) web bioinformatic tool. PLIP is entirely working based on a python command-line application [11]. Best lowest minimum binding energy exhibited compounds were analyzed and investigating their interacting chain with bound compounds, interacting amino acids in hydrophobic interaction with compounds (ligands) and protein atoms, respectively.

TABLE 3: PROTEIN-LIGAND INTERACTION PROFILES OF nsp12 COMPLEX

Compounds	Interacting Chain	Interacting amino acids (Hydrophobic interaction)	Ligand atom	Protein atom
BNT-202006	A	LYS, GLN, PHE, ALA, MET, ALA, TRP	8673, 8669, 8669,8664, 8678, 8656,	604, 627, 636, 661, 666, 693
BNT-202008	B	PRO, PRO, PRO, TRP	8690, 8669, 8673, 8658	6810, 7163, 7164, 7192
BNT-202009	A	THR	8658	1704

TABLE 4: PROTEIN-LIGAND INTERACTION PROFILES OF nsp6/10 COMPLEX

Compounds	Interacting Chain	Interacting amino acids (Hydrophobic interaction)	Ligand atom	Protein atom
BNT-202006	A	MET, PHE	3219, 3228	1005, 1149
BNT-202008	A	PRO, ALA, TYR, TYR, LEU, TRP	3247, 3212, 3247, 3237, 3226, 3221	60, 77, 419, 421, 455, 1475
BNT-202009	B	ILE, ASP, LEU, THR, VAL	3226, 3246, 3246, 3215, 3223	2618, 2886, 2895, 3071, 3078

TABLE 5: HYDROGEN BONDS ANALYSIS OF nsp12 COMPLEX OF BNT-202006

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor	Side chain	Donor Atom	Acceptor Atom
1	216A	TRP	1.91	2.89	176.73	✓	✗	808	8672 [O3]
2	216A	TRP	3.46	4.07	124.78	✗	✗	8672 [O3]	811 [O2]

TABLE 6: HYDROGEN BONDS ANALYSIS OF nsp12 COMPLEX OF BNT-202008

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor	Side chain	Donor Atom	Acceptor Atom
1	177B	SER	2.66	3.04	103.69	✓	✓	7158 [O3]	8674 [O3]
2	182B	TRP	2.94	3.85	153.89	✓	✗	7187	8654 [O2]
3	447A	ASN	2.74	3.55	139.97	✓	✓	2671	8676 [N2]

TABLE 7: HYDROGEN BONDS ANALYSIS OF nsp12 COMPLEX OF BNT-202009

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor	Side chain	Donor Atom	Acceptor Atom
1	149B	TYR	2.71	3.25	117.22	✓	✓	6942 [O3]	8674 [O3]
2	388A	LEU	3.29	3.74	111.53	✗	✗	8672 [O3]	2197 [O2]
3	397A	SER	3.00	3.80	138.85	✓	✗	2269	8672 [O3]

TABLE 8: HYDROGEN BONDS ANALYSIS OF nsp6/10 COMPLEX OF BNT-202006

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor	Side chain	Donor Atom	Acceptor Atom
1	6869A	GLY	3.26	3.86	121.63	✓	✗	565	3217 [O3]
2	6897A	ASP	3.50	4.04	119.79	✗	✓	3217 [O3]	766 [O2]
3	6898A	LEU	2.57	3.26	126.92	✓	✗	767	3217 [O3]
4	6899A	ASN	2.79	3.74	160.60	✓	✓	782	3238 [O3]
5	6913A	CYS	2.01	2.93	154.48	✓	✗	877	3229 [O3]
6	6930A	TYR	3.28	3.90	122.58	✓	✗	1009	3211 [O2]
7	6930A	TYR	2.15	3.05	160.65	✗	✗	3224 [O3]	1012 [O2]

TABLE 9: HYDROGEN BONDS ANALYSIS OF nsp6/10 COMPLEX OF BNT-202008

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor	Side chain	Donor Atom	Acceptor Atom
1	6811A	ASN	2.58	3.49	153.06	✓	✓	100	3217 [O3]
2	6851A	TYR	3.25	3.69	110.86	✓	✓	424 [O3]	3224 [O3]

TABLE 10: HYDROGEN BONDS ANALYSIS OF nsp6/10 COMPLEX OF BNT-202009

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor	Side chain	Donor Atom	Acceptor Atom
1	4338B	ASN	2.33	2.80	108.25	✓	✓	2844	3236 [O2]
2	4344B	ASP	2.30	3.28	171.21	✓	✗	2882	3244 [O3]
3	4369B	VAL	3.20	3.95	134.34	✓	✗	3072	3217 [O3]
4	4369B	VAL	2.85	3.25	106.91	✗	✗	3217 [O3]	3075 [O2]

Toxicity Prediction: Hepatotoxicity, carcino-genicity, cytotoxicity, and mutagenicity probability endpoints were predicted using toxicity model computation. Compounds BNT-202006, BNT-202008 &, BNT-202009 were showed no active toxicity properties and supported to use of our compounds for Covid-19 drug compounds.

CONCLUSION: Presently, Eight di and tridentate derived chiral molecules were synthesized, and a docking study was carried against Covid-19. The genome of Covid-19 expressing the genomic characteristics of the HIV virus, and hence it was showing drug response to Remdesivir (a standard anti-RNA polymerase used in HIV treatment).

The present focus to identify and investigate the potential drug out of eight compounds that may be used to inhibit RNA-dependent RNA polymerase non-structural proteins complex of Covid-19 to prevent the replication of viral genome in host cells. The compounds BNT-202006, BNT-202008 & BNT-202009 showed and exhibited minimum binding energy when it bound with protein targets when compared with Remdesivir and Hydroxy chloroquine as standard drug.

We took RNA dependent RNA polymerase non-structural proteins 12 complex and RNA dependent RNA polymerase non-structural proteins 6/10 complex as protein targets. Based on the above docking score, we recommended that our chemically synthesized compounds *i.e.*, BNT-202006, BNT-202008, and BNT-202009, may represent potential drug candidates, inhibitors of RNA-dependent RNA polymerase of Covid-19.

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CONFLICTS OF INTEREST: Nil

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