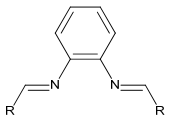
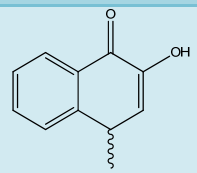
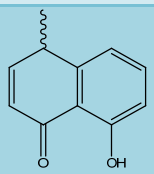
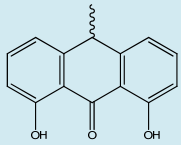
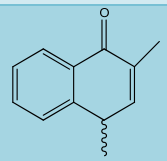
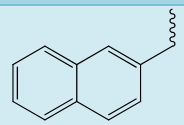
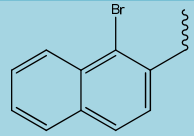
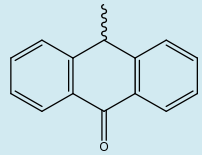
Table 1. Various energies in the binding process of *ortho*-phenylenediamine derivatives, N3 and Lopinavir with COVID-19 protease enzymes (6LU7, 6M03) obtained from molecular docking. The unit of all energies (ΔG) is kcal/mol.



Substituent (R)	Compounds	Log P* Calculated	Hydrogen bonds**		Binding energy (ΔG) kcal/mol.	
			donors	acceptors	6LU7	6M03
N3 Inhibitor	**680.79**	4.37	6	9	-7.9	-7.8
Lopinavir	**628.8**	5.92	4	5	-7.6	-7.0
	**NHM1 420.42 g/mol**	4.36	2	6	-9.0	-8.9
	**NHM2 420.42 g/mol**	5.04	2	6	-9.0	-8.4
	**NHM3 552.53 g/mol**	7.17	2	8	-8.6	-9.2
	**NHM4 416.47 g/mol**	5.4	0	4	-9.5	-8.1
	**NHM5 384.47 g/mol**	7.02	0	2	-8.7	-8.4
	**NHM6 542.26 g/moL**	8.4	0	2	-9	-9.2
	**NHM7 488.53 g/mol**	7.49	0	4	-10.5	-11.0

The modeling studies indicate *van der Waals*, hydrogen bonding **(Table 1)** and electrostatic interactions between *ortho*-phenylenediamine derivatives with protease enzymes (6LU7 and 6M03). The contribution of van der Waals and hydrogen bonding interaction is much greater than that of the electrostatic interaction because the sum of van der Waals energy, hydrogen bonding energy and desolvation free energy is larger than the electrostatic energy, [26-27]. The *ortho*-phenylenediamine derivatives, and protease enzymes (6LU7 and 6M03) interactions are shown in **Figure 2**. *Ortho*-phenylenediamine derivatives provide higher binding energy (-8.1 to -11.0 kcal/mol) compared to standard 6LU7 and 6M03 (-7.0 to -7.9 kcal/mol) **(Table 1)**. **Figure 2** indicates four hydrogen bonds between NHM7 and 6LU7. In addition, NHM7 showed good docking interaction of -11.0 kcal/mol with the 6LU7 binding site (GLU166, VAL3, GLU166 and LEU4) **(Figure 2).** Compound NHM7 has the highest binding energy of the series. This compound has an extra phenyl moiety attached to the naphthyl analogue of the phenylenediamine Schiff`s base derivative with a log P value of 7.49 indicating the importance of the lipophilicity for the interaction with the active site. The interaction of similar Schiff's base *ortho*-phenylenediamine derivatives with the proteases binding site of the enzyme is essential for effective inhibition as previously reported [28-31]. Therefore, *ortho*-phenylenediamine derivatives may be considered the most effective NHM7 and 6LU7 proteases inhibitors.

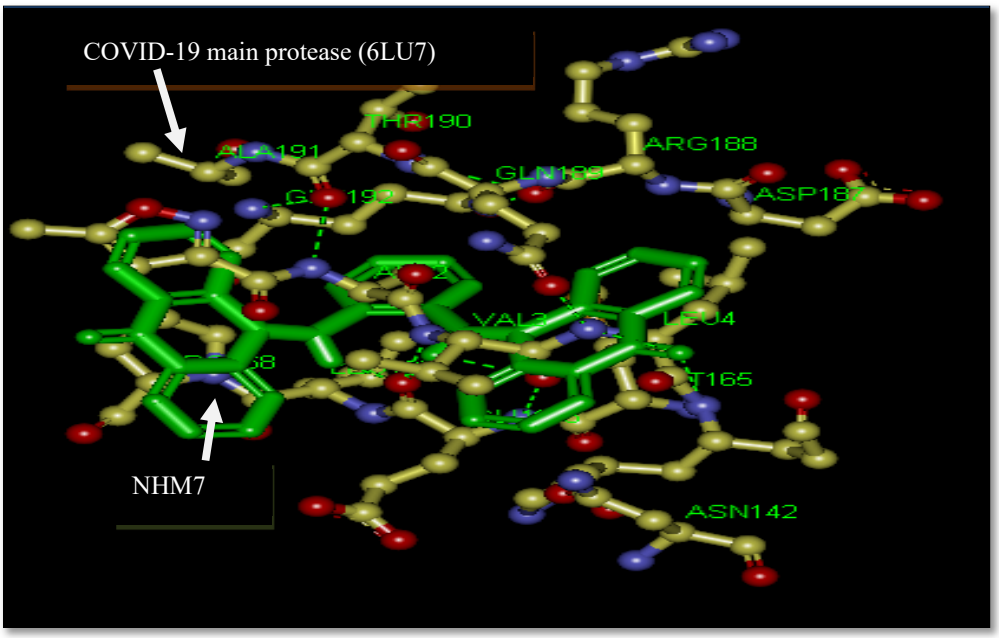


Figure 2. Interaction model between NHM7 with COVID-19 main protease (6LU7) active site. NHM7 l green colour. Hydrogen bonds green broken line.

The obtained results using computational drug repurposing is an efficient way to find novel applications for already known drugs [32]. Molecular docking and binding free energy calculations for *ortho*- phenylenediamine derivatives can be used to forecast drug-target interactions and binding affinity. The appearance of resistance to existing antiviral drugs or vaccines is a major challenge in antiviral drug development. The drug repurposing technique allows finding novel antiviral agents within a short period in order to overcome the challenges in antiviral therapy. Computational drug repurposing has previously been used to recognize drug candidates for viral infectious diseases like ZIKA, Ebola, influenza and dengue infections. These methods were also utilized to recognize possible drugs against MERS-CoV and SARS-CoV [33,34] and following the COVID-19 outbreak, computational repurposing has been and are used for COVID-19.

CONCLUSION

In spite of the economic and societal shock of COVID-19 infections and the probability of future outbreaks of even more stern pathogenic COVID-19 in humans, there is still a lack of efficient antiviral strategies to treat COVID-19 and only few options are available to prevent COVID-19 infections. Rapid development and use of a broad-spectrum protease inhibitor alone or in combination with other potent inhibitors of proteases might fill the therapeutic gap spanning quarantine and hospital setting.

Further elaborative work is necessary for better understanding the mechanisms of protease inhibition. According to modeling studies *ortho*-phenylenediamine derivatives may have the ability to inhibit COVID-19 proteases making them reasonable candidates for consideration of clinical trials and warrant further examination. Results presented in this study shall motivate future efforts in finding potent *ortho*-phenylenediamine derivatives that can be used for COVID-19 protease inhibition *in vivo.*

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