Rational identification of small molecules derived from 9,10-dihydrophenanthrene as potential inhibitors of 3CL\(^{\text{pro}}\) enzyme for COVID-19 therapy: a computer-aided drug design approach

Ossama Daoui\(^1\) · Souad Elkhattabi\(^1\) · Samir Chtita\(^2\)

Abstract
Small molecules such as 9,10-dihydrophenanthrene derivatives have remarkable activity toward inhibition of SARS-CoV-2 3CL\(^{\text{pro}}\) and COVID-19 proliferation, which show a strong correlation between their structures and bioactivity. Therefore, these small compounds could be suitable for clinical pharmaceutical use against COVID-19. The objective of this study was to remodel the structures of 9,10-dihydrophenanthrene derivatives to achieve a powerful biological activity against 3CL\(^{\text{pro}}\) and favorable pharmacokinetic properties for drug design and discovery. Therefore, by the use of bioinformatics techniques, we developed robust 3D-QSAR models that are capable of describing the structure–activity relationship for 46 molecules based on 9,10-dihydrophenanthrene derivatives using CoMFA/SE (\(R^2=0.97, Q^2=0.81, R^2_{\text{pred}}=0.95, R^2_p=0.71\)) and CoMSIA/SEHDA (\(R^2=0.94, Q^2=0.76, R^2_{\text{pred}}=0.91, R^2_p=0.65\)) techniques. Accordingly, 96 lead compounds were generated based on a template molecule that showed the highest observed activity in vitro (\(\text{pIC}_{50}=5.81\)) and predicted their activities and bioavailability in silico. The rational screening outputs of 3D-QSAR, Molecular docking, ADMET, and MM-GBSA led to the identification of 9 novel modeled molecules as potent noncovalent drugs against SARS-CoV-2-3CL\(^{\text{pro}}\). Finally, by molecular dynamics simulations, the stability and structural dynamics of 3CL\(^{\text{pro}}\) free and complex (PDB code: 6LU7) were discussed in the presence of samples of 9,10-dihydrophenanthrene derivative in an aqueous environment. Overall, the retrosynthesis of the proposed drug compounds in this study and the evaluation of their bioactivity in vitro and in vivo may be interesting for designing and discovering a new drug effective against COVID-19.

Keywords SARS-CoV-2-3CL\(^{\text{pro}}\) · Non-covalent inhibitors · 9,10-dihydrophenanthrene · Potential drug

Introduction
The world is experiencing an unstable situation at all poles due to the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease 2019 (COVID-19), which has spread throughout the world and affected the majority of the community [1, 2]. Simultaneously, various strict precautionary measures have been taken to prevent the transmission and rapid spread of COVID-19 among people. Likewise, many efforts have been made worldwide to develop an effective drug to treat patients who are potentially infected with COVID-19, leading to the development of various vaccines that have been widely applied extensively to the public [3, 4]. However, SARS-CoV-2 is known for viral progression leading to the production of new mutants cloned from it. For example, alpha variant (B.1.1.7), beta variant (B.1.351), gamma variant (P.1), delta variant (B.1.617.2) [5, 6]. Moreover, there are several other mutations of this virus that have been monitored around the world. In this regard, several reports published by the World Health Organization have pointed out that the known SARS-CoV-2 mutations have little effect on the characteristics of COVID-19 represented in the propagation speed and the level of seriousness [7, 8]. Although
the vaccine proved successful against COVID-19 mutations, people who have been vaccinated are still at risk of reinfection with this virus [9]. There is a great need to find effective antiviral drugs related to coronavirus, such as COVID-19 as a model. Meanwhile, researchers are eager to discover and design suitable drugs or vaccine solutions that can be used successfully against diseases related to SARS-CoV-2 and its variants [10]. This is done by targeting different protein pathways such as coronavirus 3C-like protease (3CL\textsuperscript{pro}) [11–13], papain-like protease (PL\textsuperscript{pro}) [14], RNA-dependent RNA polymerase (RdRp) [15], viral spike glycoprotein (S protein) [16], transmembrane protease serine 2 (TMPRSS2) [17], angiotensin-converting enzyme 2 (ACE2) [18], angiotensin AT2 receptor [19], etc. In this work, we will focus our research efforts on identifying novel agents that can be able to inhibit and eliminate coronavirus life cycle processes by targeting the enzymatic activity of 3-chymotrypsin-like cysteine protease (3CL\textsuperscript{pro}) [20]. This is due to the enzymatic environment of 3CL\textsuperscript{pro} is highly suitable for coronavirus regeneration, it can cleave viral proteins into distinct functional fractions [21]. The critical importance of the 3CL\textsuperscript{pro} enzyme in the coronavirus life cycle makes it an ideal therapeutic target for the development of antiviral drugs with optimal activity against coronavirus [22]. The successful inhibition of 3CL\textsuperscript{pro} enzymatic activity may reduce the drug resistance potential associated with COVID-19 variants [23]. To date, there are two classes of 3CL\textsuperscript{pro} inhibitors for SARS-CoV-2, covalent and non-covalent inhibitors [24]. Their pattern of inhibition varies based on interaction mechanisms between the drug ligands and active amino acid residue sites in the 3CL\textsuperscript{pro} receptor pocket [25, 26]. Among many covalent 3CL\textsuperscript{pro} inhibitors, only PF-07321332 [27] and PF-00835231 [28] have reached the clinical trial stage [29–32]. On the other hand, several small molecules have been reported as non-covalent inhibitors of 3CL\textsuperscript{pro} such as sciadopitysin [33], 23R [34], CCF981 [35], and pyridine [36, 37]. Despite all these efforts, most of the proposed covalent and non-covalent SARS-CoV-2-3CL\textsuperscript{pro} inhibitors have drug-like properties and pharmacokinetics that are not compelling for appropriate drug use; the use of covalent peptide inhibitors exacerbates the adverse side effects of clinical use of these drugs [38, 39]. Therefore, it has become necessary to explore non-covalent 3CL\textsuperscript{pro} inhibitors with favorable drug properties, good pharmacokinetics, and high inhibitory activity against COVID-19. In this context, a recent study conducted by Zhang et al. [2], we collected 46 small molecules derived from 9,10-dihydrophenanthrene as non-covalent inhibitors of SARS-CoV-2 3CL\textsuperscript{pro}. Table 1 presents the structures of all 46 compounds as well as their corresponding half-maximal inhibitory concentration (pIC\textsubscript{50} = −Log IC\textsubscript{50}) data in the reference in the in vitro positive control [43, 44].

3D quantitative structure–activity relationship (3D-QSAR) analysis

Preparation of the database

Using SYBYL-X 2.1.1 software [45], we sketched the 3D structures of the 9,10-dihydrophenanthrene derivatives,
optimizing their energy structure to the minimum via the standard Tripos Powell force field (Gasteiger-Huckel partial atomic charge computation, spatial isolation function, RMSD of 0.01 kcal/mol, input grid spacing at 2 Å, 200 iterations). Then, due to the importance of molecular alignment in generating the pharmacological hypotheses

|   | pIC₅₀ |   | pIC₅₀ |   | pIC₅₀ |   | pIC₅₀ |   | pIC₅₀ |   | pIC₅₀ |
|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|
| 01 | 4.21  | 02 | 4.48  | 03 | 4.53  | 04 | 5.04  | 05 | 5.19  |
| 06 | 4.71  | 07 | 4.94  | 08 | 4.07  | 09 | 4.24  | 10 | 4.27  |
| 11 | 4.23  | 12 | 4.17  | 13 | 5.25  | 14 | 4.18  | 15 | 4.45  |
| 16 | 4.73  | 17 | 4.85  | 18 | 4.92  | 19 | 4.86  | 20 | 5.07  |
| 21 | 5.61  | 22 | 5.01  | 23 | 5.32  | 24 | 4.99  | 25 | 5.48  |
underlying the 3D molecular structures in the 3D-QSAR modeling, a molecular alignment of the database items was performed (Fig. 2) [40, 46].

To develop 3D-QSAR pharmacophore models, the database of 46 molecules was divided into two sets (80% for training and 20% for testing) based on the structural diversity method and \( \text{pIC}_{50} \) scoring ranges. Table S1 presents the obtained division according to the \( \text{pIC}_{50} \) ranges of the investigated compounds (low active to high active). The training set included 37 molecules with inhibitory activity values (\( \text{pIC}_{50} \)) against 3CL\textsuperscript{pro} ranging from 4.07 to 5.81. These molecules were used as input to develop the 3D-QSAR pharmacophore models. Although the test set included nine items with activity values (\( \text{pIC}_{50} \)) against 3CL\textsuperscript{pro} ranging from 4.21 to 5.74, these molecules were used as samples to test the performance and predictive power of the developed 3D-QSAR pharmacophore models.

Table 1 (continued)

|  | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | DSF |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **pIC\textsubscript{50}** | 5.00 | 4.95 | 5.13 | 5.28 | 5.37 | 5.57 | 5.17 | 5.56 | 5.23 | 5.44 | 5.08 | 5.48 | 5.81 | 5.74 | 4.99 | 5.27 | 5.33 | 4.97 | 5.26 | 5.98 |

Fig. 2  
(a) template molecule T40,  
(b) common core, and  
(c) database aligned.
3D-QSAR modeling

Approaches include comparative molecular field analysis (CoMFA) and comparative molecular similarity index analysis (CoMSIA), which are the most recent and popular statistical methods used in 3D-QSAR modeling in drug design [46]. The main importance of these approaches is to provide valuable information on key molecular structural properties related to a biological response [47]. CoMFA analysis is used to characterize the effects of steric (S) and electrostatic (E) descriptors on the biological inhibitory activity, while CoMSIA is used to characterize the effects of steric (S), electrostatic (E), hydrophobic (H), hydrogen bond donor (D), and hydrogen bond acceptor (A) descriptors on biological inhibitory activity of the compounds. In this work, we perform 3D-QSAR modeling based on CoMFA and CoMSIA techniques to describe the relationship between the biological inhibitory activity of 9,10-dihydrophenanthrene derivatives and their 3D field descriptors (S, E, H, D, and A). During this process, the linear structure–activity relationship yielded by CoMFA and CoMSIA analysis is mapped out into contour maps, which are generated by the PLS algorithm (partial least squares) and the Tripos force field (2 Å spatial contour maps, which are generated by the PLS algorithm). During this process, the linear structure–activity relationship can be decoded and visualized as contour maps. Contour maps were generated based on the computation of field energies at each point in the grid as standard results of field properties of 9,10-dihydrophenanthrene derivatives and as a fraction in CoMFA and CoMSIA modeling equations [53]. Through the use of contour maps, which are generated by CoMFA and CoMSIA models, the favorable structural field properties of 9,10-dihydrophenanthrene derivatives can be rationalized to achieve a high level of inhibition against SARS-CoV-2-3CLpro. As a result, we can derive new molecular structures from the template molecule (T40, pIC50 = 5.81). The utilization of these models also allows us to predict the biological inhibitory activity of the designed molecules against SARS-CoV-2-3CLpro, which allowed us to select molecules with a higher activity compared to synthesized molecules as well as to disulfram used in the positive control in vitro (pIC50 = 5.98).

Generation of novel compounds, drug-like screening, and ADME-Tox

We use the molecule 1-(4-bromophenyl)-10-(hydroxymethyl)-8-(4-phenylpyridin-2-yl)-9,10-dihydrophenanthren-4-ol (T40) as a template to generate 100 new compounds via the LigDream toolkit (https://playmolecule.org/LigDream/). The new compound generation is powered through generative shape-based neural network decoding [54]. Novel compounds exhibit new scaffolds and multiple chemical moieties that cover a new site in the sample chemistry space that supports drug-like properties. The pKCSM online tool was used to analyze ADME (absorption, distribution, metabolism, and excretion) pharmacokinetics of proposed drug molecules that achieved high predicted inhibitory activity and binding affinity energies toward SARS-CoV-2-3CLpro, Osiris computations...
were used to predict the toxicity risks of the examined molecules [55, 56].

**Molecular docking**

We perform molecular docking simulations to predict potential non-covalent interaction profiles between active amino acid sites in the 3CL\textsuperscript{pro} receptor pocket (PDB code: 6LU7) to the proposed drug molecules. In this procedure, we consider active amino acid residues that interacted with the inhibitor N3 in 3CL\textsuperscript{pro} (PDB code: 6LU7) as references in inhibiting the enzymatic activity of SARS-CoV-2-3CL\textsuperscript{pro} by the generated small molecules possessing high pIC\textsubscript{50} activity and favorable drug-like properties. The crystal structure of the main protease COVID-19 in a complex with an inhibitor N3 (resolution: 2.16 Å, R-value work: 0.202, R-value observed: 0.204) [57] was adopted as a model to perform molecular docking simulation. Initially, the backbone structure of 3CL\textsuperscript{pro} and the ligands were prepared. Protein is prepared by the elimination of water molecules and nonprotein elements residing in the protein, the addition of polar hydrogen atoms and Gasteiger charges, and the setting up of the flexible molecular docking grid box (x = −10.75 Å, y = 12.46 Å, z = 68.92 Å, grid spacing 0.375 Å, size 40×60×40 Å\textsuperscript{3}), which contains amino acid residues (His41, Met49, Gln189, Cys145, Thr190, Ala191, Pro168, Leu167, Asn142, Glu166, His172, Met165, His164, Phe140, Glu143, Gln192, Thr26, Thr25, Thr24, Ser144, Gly143, Asn142, Leu141, His172, His164, and Asp187).

On the other hand, the ligands were prepared by optimizing the structures of the designed molecules as well as the inhibitors N3 and disulfiram via the three-parameter Becke hybrid method based on the Lee–Yang–Parr function (B3LYP) with 6-31G+(d,p) base in the solvent water based on the density functional theory (DFT), which is provided by the Gaussian 09 software [58]. This was done to ensure proper equilibration of the system and to check the proton and polar hydrogen atom states of the ligands in the aqueous environment [59]. In the present study, we performed molecular docking using AutoDockTools-1.5.7 and AutoDock vina [60, 61]. The molecular docking visualizations were displayed by Discovery Studio 2016 [62]. The molecular docking protocol is validated by the re-docking strategy based on the evaluation of the root mean square deviation (RMSD) between the original and re-docked N3 inhibitor [40]. After that, we evaluated the backbone stability of 3CL\textsuperscript{pro} protein complexed with N3 in an aqueous system by molecular dynamics simulations.

**Molecular mechanics-generalized Born surface area (MM-GBSA)**

MM-GBSA computations were used to re-examine novel binding modes in protein–ligand systems (screened by molecular docking and ADME-Tox predictions). This procedure aimed at selecting the most free-bound ligands to the 3CL\textsuperscript{pro} active pocket site, based on MM-GBSA, which generates parameters such as binding free energy (ΔG\textsubscript{bind}), hydrogen bond energy (ΔG\textsubscript{bind} H-bond), van der Waals energy (ΔG\textsubscript{bind} vdW), covalent energy (ΔG\textsubscript{bind} Covalent), coulomb energy (ΔG\textsubscript{bind} Coulomb), lipophilic energy (ΔG\textsubscript{bind} Lipo), generalized Born electrostatic solvation energy (ΔG\textsubscript{bind} Solv_\textsubscript{GB}), and packing energy (ΔG\textsubscript{bind} Packing). MM-GBSA computations performed using MM-GBSA Prime tool available in Schrödinger 2020–3 [63, 64]. Systems (protein–ligand) were prepared by minimizing their energy using the OPLS3e force field and V5GB solvent model at pH 7±2. Consequently, the energy of the protein ligands (E complex) and ligands (E ligands) is minimized, leading to the generation of binding free energy (ΔG\textsubscript{bind}) for complexes (Eq. 1) [65].

\[ \Delta G_{\text{bind}} = E_{\text{complex}}(\text{minimized}) + E_{\text{ligand}}(\text{minimized}) + E_{\text{receptor}}(\text{minimized}) \] (1)

**Molecular dynamics (MD)**

Using the simulated protein environment, we perform MD simulations to evaluate the stability level of the affinity of the 3CL\textsuperscript{pro} (PDB code: 6LU7) enzyme to candidate drug ligands. The MD computer simulations allow us to analyze the properties of molecular systems made up of many molecules, as well as to track the trajectories of atoms and molecules based on numerically solving Newton’s equations of motion of a system of interacting particles during a time frame [66, 67]. In this work, MD simulations were implemented using the Desmond package available in Schrödinger 2020–3 academic software [68]. The OPLS3e force field was used for modeling the complexes obtained by molecular docking. The docked protein–ligand systems (6LU7 uncomplexed and complexed to ligands) were solvated using the orthorhombic single point charge (SPC) explicit water model [69, 70]. Using the water model (SPC), an orthorhombic simulation box was prepared with the minimum distance between the edge surface of the protein and the protein boundary surface of 10 Å. Then, the charge of the solvated systems was neutralized to zero by adding Na\textsuperscript{+} and Cl\textsuperscript{−} counter ions, adjusting the physiological salt concentration to 0.15 M, optimizing the energies of the systems to a minimum of 2000 steps, using a Cylindrical reaction with 9 Å cutoffs and grid phase of 0.8 Å, as well as smooth particle mesh Ewald method with a tolerance of 1E–09 that were used to resolve long-range electrostatic interactions [71]. The Nose–Hoover thermal algorithm and the Martina-Tobias-Klein method were employed to generate slow heating of the systems under 300 K temperature and 1.01325 pressure bar, using 10,000 frames of each simulated
The results of the PLS analysis presented in Table 2 indicate the high power of both CoMFA and CoMSIA models to describe the quantitative relationship between the field descriptors (S, E, H, D, and A) and the inhibitory activity of 9,10-dihydrophenanthrene derivatives against SARS-CoV-2-3CL<sub>pro</sub>. This can be confirmed by the high values of the determination coefficients ($R^2 = 0.97$ and $R^2 = 0.94$), internal validation by (LOOCV) ($Q^2_{\text{loocv}} = 0.81$ and $Q^2_{\text{loocv}} = 0.76$), Fischer test ($F = 107.50$ and $F = 67.45$), Y-randomization test ($R^2_{\text{p}} = 0.71$ and $R^2_{\text{p}} = 0.65$, see Table S3), external test ($R^2_{\text{pred}} = 0.95$ and $R^2_{\text{pred}} = 0.91$) on the one hand, and the low SEE values (SEE = 0.105 and SEE = 0.131) on the other hand for CoMFA and CoMSIA models, respectively. The high correlation coefficients ($R^2$) for the CoMFA and CoMSIA models can be confirmed by the low residual values between the observed and predicted activities through the proposed 3D-QSAR models (Table S4). Also, we can notice that electrostatic (E) and steric (S) fields have a significant effect on the predictive performance of the inhibitory activity of SARS-CoV-2-3CL<sub>pro</sub> with fractions of 58% and 30% and 41.3% and 13.5% for the CoMFA and CoMSIA models, respectively. Furthermore, through the CoMSIA model, we can see the strong dependence of hydrophobic (H) and donor hydrogen bond (D) fields (27.5% and 21.2%) on the predictive power compared to hydrogen bond acceptor (A) fields (7.9%). By proportioning the contributions of the field descriptors (fractions), we can conclude that the biological inhibitory activity of 9,10-dihydrophenanthrene derivatives is influenced principally by their electrostatic (E), steric (S), hydrophobic (H), and hydrogen bond acceptor (A) properties. All statistical parameters obtained from CoMFA and CoMSIA analyses indicate the excellent performance of the CoMFA/SE and CoMSIA/SEHDA models in predicting inhibitory activity against 3CL<sub>pro</sub> based on the structure of 9,10-dihydrophenanthrene derivatives. Thus, these models can be reliably exploited to predict the bioactivity of new small molecules that can be modeled.

Analysis of CoMFA and CoMSIA contour maps

The 3D QSAR models can be interpreted at the molecular scale based on contour surface plots of the 3D structure–activity relationships generated by the proposed CoMFA and CoMSIA models (Figs. 3, 4). From Figs. 3a, b and 4a, b, we can notice a very similar spatial arrangement of the steric and electrostatic contours of CoMFA and CoMSIA on the structure of the template molecule (T40), which confirms the significant impact of the descriptors (S and E).

Table 2 Summary of statistical significance results for CoMFA and CoMSIA models

|   |   | $Q^2$ | $R^2$ | $R^2_{\text{pred}}$ | $R^2_{\text{p}}$ | $\text{SEE}$ | $F$ | $N$ | $\text{loocv}$ | Fractions | $S$ | $E$ | $H$ | $D$ | $A$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Threshold | >0.5 | >0.6 | Small | High | 1–6 | >0.6 | >0.5 | 0 | Fractions <1 | | | | | |
| CoMFA | 0.81 | 0.97 | 0.105 | 107.50 | 3 | 0.95 | 0.71 | 0.413 | 0.587 | - | - | - | - | - |
| CoMSIA | 0.76 | 0.94 | 0.131 | 67.45 | 4 | 0.91 | 0.65 | 0.135 | 0.299 | 0.275 | 0.079 | 0.212 | }

$Q^2$ coefficient of cross-validation correlation, $N$ optimal number of components identified by leave-one-out cross-validation (loocv), $\text{SEE}$ standard error of estimate, $R^2$ conventional coefficient of determination, $R^2_{\text{pred}}$ coefficient of determination according to the external test, $R^2_{\text{p}}$: Y-randomization test; fractions: contributions of steric (S), electrostatic (E), hydrophobic (H), donor (D), and acceptor (A) hydrogen bonds.
on the inhibitory biological activity ($pIC_{50}$). The predominance of yellow contour maps over green contour maps in the molecule **T40** backbone indicates that large ring radicals negatively affected the bioactivity of 9,10-dihydrophenanthrene derivatives against SARS-CoV-2 3CL$^\text{pro}$. Thus, the biological activity of the new molecules derived from molecule **T40** can be improved by introducing smaller moieties into the pharmacological sites covered by the yellow contours. Moreover, the predominance of blue contours over red contours along with the 4-phenylpyridin-2-yl moiety indicates that the enhancement of this site with electron-donating groups is favorable for the improved biological activity of (1-(4-bromophenyl)-10-(hydroxymethyl)-8-(4-phenylpyridin-2-yl)-9,10-dihydrophenanthren-4-ol) against SARS-CoV-2 3CL$^\text{pro}$.

On the other hand, the positioning of the white contours on the carbon sites C22 and C30 in the bromobenzene and phenyl rings indicates that these sites are unfavorable for hydrophobic moieties; so, the upgrade of C22 and C30 sites with hydrophilic moieties can be favorable to improve $pIC_{50}$ biological inhibitory activity (Fig. 4c). The dominance of the cyan over purple contour near the hydroxyl group indicates that this site is suitable for 80% of the hydrogen bond donor radicals (Fig. 4e), while the 4-phenylpyridin-2-yl region covered by 80% of the magenta contours is suitable to enhance the biological activity ($pIC_{50}$) through the insertion of hydrogen bond donor radicals at this site (Fig. 4e). Furthermore, the linear distribution of observed $pIC_{50}$ values versus those predicted by the CoMFA and CoMSIA models (Fig. 3c and Fig. 4f) confirms the hypotheses of a high pharmacological correlation between $pIC_{50}$ and the field properties (S, E) and (H, D, A) of 9,10-dihydrophenanthrene derivatives. As a result, the developed 3D-QSAR pharmacophore models (CoMSIA and CoMFA) can be exploited reliably.

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**Fig. 3** Contour maps generated by CoMFA model, a steric field interactions (green = 80% favorable/yellow = 20% unfavorable), b electrostatic field interactions (blue = 80% favorable/red = 20% unfavorable). c $pIC_{50}$ observed vs. $pIC_{50}$ predicted

**Fig. 4** Contour maps generated by the CoMSIA model, a steric field interactions (green = favorable/yellow = unfavorable), b electrostatic field interactions (blue = 80% favorable/red = 20% unfavorable), c hydrophobic field interactions (yellow = 80% favorable/white = 20% unfavorable), d hydrogen bond-donor field interactions (cyan = 80% favorable / purple = 20% unfavorable), e hydrogen bond-acceptor field interactions (magenta = 80% favorable/red = 20% unfavorable). f $pIC_{50}$ observed vs. $pIC_{50}$ predicted
to rationalize the generation of new molecules based on reference molecule T40 and predict their activities against SARS-CoV-2 3CLpro.

**Generation of novel compounds from 1-(4-bromophenyl)-10-(hydroxymethyl)-8-(4-phenylpyridin-2-yl)-9,10-dihydrophenanthren-4-ol (T40)**

Based on the pharmacological hypotheses obtained by the CoMFA and CoMSIA models (Fig. 5), new sets of (1-(4-bromophenyl)-10-(hydroxymethyl)-8-(4-phenylpyridin-2-yl)-9,10-dihydrophenanthren-4-ol) derivatives can be generated and their biological activity and pharmacokinetic properties can be predicted.

Through the use of LigDream and the artificial neural network tools in the PlayMolecule platform (https://playmolecule.org/LigDream/), 96 new molecules (D1–D96) were generated from the template compound (T40) based on auto-encoders and captioning networks approaches [75]. Once the new molecules are generated, we filter them based on the drug-likeness criteria (e.g., Lipinski RO5, Ghose violations, bioavailability, and synthetic accessibility) [76–79]. Table S5 shows the drug-like profile of the 96 new molecules generated from the structure of the reference molecule (T40), as well as the N3 inhibitor properties. The inflexible drug-like screening predicted that 47 new molecules did not violate any of Lipinski’s and Ghose’s rules (Table S5). Also, we can notice that the bioavailability and synthetic accessibility of the 47 screened small molecules were 0.55 and (2 < SA < 4), respectively. Template molecule (T40) and inhibitor N3 were observed to violate Lipinski’s (2 violations) and Ghose’s (3 violations), with poor bioavailability (0.17) and synthetic accessibility (4.07 and 6.43). This indicates that the 47 new molecules can act as an effective alternative oral drug against 3CLpro compared to the template molecule (T40) and inhibitor N3. To further validate these hypotheses, we apply the CoMFA and CoMSIA models to the new 47 candidate molecules and predict their inhibitory activity against SARS-CoV-2 3CLpro. The pIC50 of the novel designed molecules were predicted after preparing their 3D structures using the same strategy employed for 3D-QSAR modeling in this work (Fig. 6).

Thanks to the perfect alignment between the 47 filtered molecules and the template structure shown in Fig. 6, we can conclude that the structural improvements made to the structure of compound T40 were coherent with the pharmacological hypotheses extracted from this structure via the proposed CoMFA and CoMSIA models. Hence, the predictions of the biological inhibitory activity of new molecules against SARS-CoV-2 3CLpro by the CoMFA and CoMSIA models will be more stable and precise. Table 3 presents the predicted biological activities generated by CoMFA and CoMSIA models for all 47 candidate non-covalent SARS-CoV-2 3CLpro inhibitors.

From Table 3, we can notice that twenty new derived molecules (D02, D06, D07, D08, D12, D18, D23, D25, D26, D27, D28, D30, D38, D43, D44, D76, D87, D88, D92, D94) showed significantly better inhibitory activity (pIC50 > 6) than the reference inhibitors, which are molecule T40 (pIC50 = 5.8), DSF (pIC50 = 5.98) and N3 (pIC50 = 3.90). This means that the modular changes made to the structure of the template molecule (T40) were favorable to improving the biological activity of the proposed new class of non-covalent inhibitors of SARS-CoV-2 3CLpro. Thus, the 20 computer-based non-covalent inhibitors proposed in the current study (Fig. S1) can reach an excellent inhibitory activity against SARS-CoV-2 3CLpro and reach the therapeutic goal against COVID-19. To further confirm these hypotheses,
we perform molecular docking simulations and evaluate the level of binding energies of proposed molecules to the 3CL\textsuperscript{pro} enzyme.

**Molecular docking simulation**

**Molecular docking protocol validation**

First, we perform a re-docking of the covalent inhibitor N3 with the 3CL\textsuperscript{pro} receptor; this is done to validate the molecular docking protocol and also to get insight into the reference active amino acid residues involved in non-covalent interactions inside the 3CL\textsuperscript{pro} protein pocket (PDB code: 6LU7). Figure 7 displays 3D and 2D visualizations of the re-docking pathways of ligand N3 inside the 3CL\textsuperscript{pro} receptor pocket (6LU7).

The perfect superposition mode (RMSD = 0.131 < 2 Å) between the original and re-docked N3 ligand inside the 3CL\textsuperscript{pro} pocket (Fig. 7a) indicates the high precision of molecular docking predictions performed by AutoDock vina software. Also, Fig. 7b indicates that the N3 ligand (original and re-docked) interacted with the same active amino acid residues that are His41, Met49, Gln189, Thr190, Ala191, Pro168, Leu167, Cys145, Glu166, His172, Met165, His164, Phe140, and Glu143. Furthermore, we can see that most non-covalent interactions between the ligand N3 and the protein 6LU7 resulted from hydrophobic (Alkyl, Pi-Alkyl, Pi-Sigma), hydrogen bonds (conventional and carbon), and van der Waals interactions. Therefore, we consider the interactions of molecule T40 and its novel derivatives with certain of these residues as a reference mechanism for inhibiting 3CL\textsuperscript{pro} via non-covalent interactions. To complete the molecular docking validation protocol, we performed a molecular dynamics simulation on the crystalline complex (PDB:6LU7), this was done to evaluate the stability of the 3D structure of the 6LU7 receptor in the aqueous environment, as well as the stability of N3 in the active pocket of 3CL\textsuperscript{pro} (Fig. 8).

Figure 8a indicates that the 6LU7 backbone RMSD reached a remarkable equilibrium around the 1.8 Å range with some fluctuations that did not exceed approximately 2.4 Å throughout the MD simulation. The N3 ligand showed fluctuations in RMSD ranging from 2.4 to 4.8 Å from the onset of the simulation to around 35 ns, before stabilizing in the range of 1.2 to 3 Å until the end of the MD simulation time. From Fig. 8b and c, we can notice that N3 contacted the same active amino acid residues in the 6LU7 pocket predicted by the molecular re-docking protocol. The majority of the interactions were of the hydrogen bond and hydrophobic and water-bridge type. Most of the interactions formed with N3 inside the active pocket of 3CL\textsuperscript{pro} spanned from 25 to more than 100% of the MD simulation time, this is due to the large size of the N3 structure, which does not allow it to perform more flexible interactions inside the active pocket. Therefore, small molecules may be more flexible in terms of their interactions and stability in the 3CL\textsuperscript{pro} pocket.

**Table 3** The predicted pIC\textsubscript{50} activities of 47 screened molecules against SARS-CoV-2 3CL\textsuperscript{pro}

| Comp | Comp | Comp |
|------|------|------|
| CoMFA/SE | CoMSIA/SEHD | CoMFA/SE | CoMSIA/SEHD | CoMFA/SE | CoMSIA/SEHD |
| D02 | 6.302 | 6.938 | D25 | 6.144 | 6.878 | D76 | 6.300 | 6.986 |
| D03 | 6.146 | 5.918 | D26 | 6.290 | 6.913 | D78 | 5.180 | 4.789 |
| D04 | 6.080 | 5.929 | D27 | 6.149 | 6.988 | D79 | 5.167 | 4.781 |
| D05 | 6.123 | 5.877 | D28 | 6.238 | 6.858 | D80 | 5.160 | 4.827 |
| D06 | 6.261 | 6.967 | D30 | 6.105 | 6.847 | D81 | 5.163 | 5.013 |
| D07 | 6.187 | 6.893 | D38 | 6.165 | 6.736 | D82 | 5.179 | 4.994 |
| D08 | 6.259 | 6.852 | D41 | 5.125 | 5.007 | D84 | 5.172 | 5.997 |
| D12 | 6.240 | 6.826 | D42 | 5.195 | 4.918 | D87 | 6.221 | 6.005 |
| D13 | 6.161 | 5.931 | D43 | 6.246 | 6.860 | D88 | 6.268 | 6.015 |
| D14 | 6.107 | 5.950 | D44 | 6.159 | 6.724 | D92 | 6.239 | 6.969 |
| D17 | 6.143 | 5.963 | D45 | 5.809 | 4.924 | D94 | 6.226 | 6.850 |
| D18 | 6.218 | 6.857 | D46 | 5.278 | 4.842 | - | - | - |
| D19 | 6.190 | 5.947 | D49 | 3.930 | 4.675 | - | - | - |
| D20 | 5.891 | 4.937 | D54 | 5.227 | 4.965 | - | - | - |
| D21 | 6.106 | 5.983 | D60 | 5.250 | 5.029 | - | - | - |
| D22 | 6.369 | 5.994 | D62 | 5.060 | 4.893 | - | - | - |
| D23 | 6.104 | 6.231 | D70 | 4.997 | 4.885 | - | - | - |
| D24 | 6.145 | 5.830 | D73 | 4.861 | 4.767 | - | - | - |

Templates (compound T40: pIC\textsubscript{50} = 5.81), inhibitors (DSF: pIC\textsubscript{50} = 5.98), and (N3:pIC\textsubscript{50} = 3.90 [29]) Compounds with boldface values showed better inhibitory activity than the reference inhibitors (T40, DSF and N3)
compared to large molecules such as the peptide inhibitor N3 as a model.

**Molecular docking test**

Molecular docking was performed to find potential active residue sites in the 3CL\textsuperscript{pro} enzyme pocket with which the designed and synthesized 9,10-dihydrophenanthrene derivatives can interact. Furthermore, we evaluated the binding affinity energies of the examined ligands and screened the best potential non-covalent inhibitors of 3CL\textsuperscript{pro}. This is done by selecting ligands that reach the lowest energy level when binding to the active pocket of the 3CL\textsuperscript{pro} receptor.

Figure 9 shows a 3D visualization of the optimal conformation positions of the new 20 derivatives of 9,10-dihydrophenanthrene, T40, inhibitors DSF and N3 in the active pocket of SARS-CoV-2 3CL\textsuperscript{pro} (PDB code:6LU7).

Figure 9 shows the optimal docking position of the investigated molecules in the active pocket of the 3CL\textsuperscript{pro}. This means that the small molecules of 9,10-dihydrophenanthrene derivatives can target the enzymatic activity of SARS-CoV-2 3CL\textsuperscript{pro} and achieve a potential inhibition of 3CL\textsuperscript{pro} through non-covalent interactions. Table S6 provides a detailed summary of the most important molecular docking results regarding the binding affinity energies and interactions of the studied ligands in the active pocket of 3CL\textsuperscript{pro}. From the molecular docking results presented in Table S6, we can notice that all new modeled molecules, as well as T40 and the DSF inhibitor, interacted with the majority of active residues in the 3CL\textsuperscript{pro} pocket with which inhibitor N3 interacted. Most non-covalent interactions that were formed between the investigated molecules and 3CL\textsuperscript{pro} were hydrophobic, hydrogen bonds (Pi-donor, carbon, and conventional hydrogen bonds), electrostatic, and van der Waals. This further confirms that the 9,10-dihydrophenanthrene-derivatives are flexible in terms of inhibiting the enzymatic activity of 3CL\textsuperscript{pro} by non-covalent mechanisms. Thus, active sites (His164, Cys145, Glu166, His41, His163, Gly143, Ser144, Asn142, Phe140, Leu141, Met165, Pro168, Leu167) in the 3CL\textsuperscript{pro} pocket, which interact with template molecule (T40) can consider those active sites as new potential keys involved in inhibition of SARS-CoV-2 3CL\textsuperscript{pro} enzymatic activity through non-covalent interactions.

**Analysis of binding affinity energies of ligands** From Table S6, we can notice that among twenty proposed molecules 11 molecules D06 (−10.5 kcal/mol), D07 (−10.7 kcal/mol), D08 (−10.8 kcal/mol), D12 (−10.6 kcal/mol), D18 (−10.4 kcal/mol), D23 (−10.9 kcal/mol), D25 (−10.6 kcal/mol), D26 (−10.5 kcal/mol), D27 (−10.5 kcal/mol), D30 (−10.5 kcal/mol), D76 (−11.1 kcal/mol) appeared to be more stable in terms of binding affinity energies compared to template molecules T40 (−10.2 kcal/mol) and DSF (−4.7 kcal/mol) inside the active site of the main protease.
Fig. 7 (continued)
Likewise, we can note that the eleven candidate molecules were able to interact with most of the active amino acid residues with which the reference inhibitor N3 (−13.5 kcal/mol) interacted within the active pocket of 3CL\textsuperscript{pro}. Thus, the high biological binding of the proposed small molecules to 6LU7 is likely to achieve excellent inhibition against SARS-CoV-2 3CL\textsuperscript{pro} compared to the template molecule T40.

**Non-covalent interaction analysis of examined ligands** Figure S2 displays a 2D visualization plot of key non-covalent interactions between the active reference residues in the 3CL\textsuperscript{pro} pocket and the eleven filtered molecules adjacent to templates (T40 and DSF). All non-covalent ligand–protein interactions identified in Fig. S2 are classified and summarized in Table 4.

From Fig. S2 (complex DSF-6LU7), we can observe that the structure of the DSF inhibitor used in the positive control interacts via a carbon-hydrogen bond with the active reference sites occupied by Gln189 (5.82 Å), three hydrophobic bonds (alkyl and pi-alkyl) with reference active sites occupied by residues Leu27 (4.27 Å), Cys145 (5.06 Å), and His41 (5.59 Å), as well as other interactions of the van der Waals class.

However, the non-covalent interactions generated between T40 (template) and the reference active sites in 3CL\textsuperscript{pro} (Fig. S2, complex T40-6LU7) indicate that the moiety (OH, R1) interacts with the active reference residues His164 (5.00 Å), Cys145 (6.77 Å) through two conventional hydrogen bonds. Moreover, the moiety (bromobenzyl, R2) interacted with one electrostatic bond with the reference site Glu166 (4.79 Å), and three hydrophobic bonds (stacked amide-Pi, alkyl, Pi-alkyl) between the moiety (4-phenylpyridine, R3) and the active reference sites Leu167 (6.54 Å), Pro18 (4.33 Å), Met165 (5.06 Å), as well as other interactions of van der Waals class. The overall non-covalent interactions and number of hydrogen
bonds generated in the complex (40-6LU7) led to higher binding affinity energy for template 40 (BAE = −10.2 kcal/mol) compared to DSF inhibitor (BAE = −4.7 kcal/mol). Also, from Fig. S2, (complex 40-6LU7), we can notice the main contribution of pharmacophore sites (R1, R2, R3) in the generation of most non-covalent inhibitory interactions. This implies that the 3D-QSAR pharmacophore modeling predictions were accurate in rationalizing the pharmacophore sites in the structure of template molecule T40 that are favorable to achieving high inhibitory activity against SARS-CoV-2 3CLpro. As a result, the structural modifications applied to the structure of T40 explain the significant improvement in the inhibitory activity and binding affinity energies of the eleven novel molecules (D06, D07, D08, D12, D18, D23, D25, D26, D27, D30, and D76) screened as promising inhibitors of 3CLpro enzymatic activity.

To sum up, the molecular docking simulation results indicated that the eleven novel small molecules achieved perfect docking poses in the active pocket of 3CLpro where the peptide inhibitor N3 interacts. The optimal binding modes, high binding affinity energies, and high predicted pIC50 biological activity of these molecules provide a strong theoretical basis for the nomination of these molecules as novel drug agents against COVID-19.

**ADME-Tox prediction**

The discovery of coronavirus protease inhibitors targeting 3CLpro has been a major innovation due to the poor pharmacokinetic properties of large peptide compounds such as the N3 (PubChem Compound CID: 6,323,191) covalent inhibitor model [80, 81]. Thus, the discovery of novel non-covalent 3CLpro inhibitors based on small molecule structures could be an effective therapeutic key against COVID-19 compared to covalent 3CLpro inhibitors. The evaluation of absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) properties is important in drug discovery and design to ensure effective and safe drugs. Based on this, we evaluate the pharmacokinetic properties (ADME-Tox) of eleven small molecules (D06, D07, D08, D12, D18, D23, D25, D26, D27, D30, and D76) candidates to reach the 3CLpro enzyme with sufficient inhibitory concentration, biological binding, and high drug response. Table S7 presents ADME-Tox predictions assessed in this work.

From the ADME-Tox predictions presented in Table S7, it can be observed that all examined molecules can be a substrate of P-glycoprotein, which explains the high absorption rates of these molecules in the human intestine (>90%). In contrast, the N3 inhibitor showed a low absorption of 57.88%; so, the small intestine may not be able to absorb enough N3 peptide, which may lead to rapid clearance before reaching the therapeutic target. Regarding distribution property, the negative VDss values for all molecules indicate that the distribution of these molecules is in the bloodstream of the body. The significantly low fraction unbound (FU) values of the examined molecules suggest that these molecules are more likely to bind the protease than to the plasma. Furthermore, all molecules were unable to penetrate the central nervous system (CNS) and the blood–brain barrier (BBB), except molecule D12 (logBB = 0.29), which means that the proposed molecules have no potential impact on the brain. In terms of metabolism, all examined molecules could be substrates for the enzyme cytochrome 3A4 responsible for drug metabolism in the human body; so, the drug compounds will not be rapidly excreted and metabolized by the human body. Regarding the excretion property, the low total clearance values of all molecules mean that the 11 proposed drug molecules as well as the template molecule T40, have significant half-life and stability in the body. This may allow them to reach their therapeutic target before excretion, unlike
| Ligands | Binding energy (kcal/mol) | Hydrogen-binding interactions | Electrostatic interactions | van der Waals interactions | Hydrophobic interactions |
|--------|--------------------------|------------------------------|---------------------------|---------------------------|------------------------|
| DSF    | −4.7                     | Gln189 (3.50 Å), Asn142 (3.51 Å) | -                         | Thr25, Thr26, Met49, Gly143, Glu166, Met165 | Leu27 (4.06 Å), Cys145 (4.63 Å), His41 (4.99 Å) |
| T40    | −10.2                    | Cys145(3.02 Å), His164 (2.72 Å) | Cys145(5.24 Å), Glu166(3.95 Å) | His41, His163, Gln143, Ser144, Asn142, Phel40, Leu141, | Met165 (4.89, 5.38, 5.41 Å), Pro168 (4.04 Å), Leu167 (4.50 Å) |
| D06    | −10.5                    | Gln189 (3.35 Å)                | Met49(4.92 Å), His41(4.54 Å), Cys145(5.20 Å) | Tyr54, Pro52, His164, Glu166, Gln192, Thr190, Asp187, Arg188, Leu167 | Met165 (5.50 Å), Pro168 (4.72 Å) |
| D07    | −10.7                    | Ser144 (3.53 Å), Asn142 (2.81 Å), Gly143 (2.83 Å) | -                         | Glu166, Met165, His163, Phe140, Leu141, Met49, Ser46, Pro168 | Cys145 (5.06, 4.56 Å) |
| D08    | −10.8                    | Ser144 (2.54 Å), Gly143 (2.82 Å), Gly166 (3.06 Å), Thr25 (2.81 Å), Thr24 (2.69 Å) | -                         | Met165, His163, Leu141, Asn142, His41, Leu27, Thr45, Met49, Gln189 | Cys145 (4.87, 5.06 Å) |
| D12    | −10.6                    | Ser144 (2.42 Å), Asn142 (2.64 Å), Gly143 (2.64 Å) | -                         | Glu166, Met165, His163, Phe140, Leu141, Leu27, Ser46, Met49 | Cys145 (4.94, 5.13 Å) |
| D18    | −10.4                    | Ser144 (2.33 Å), Gly143 (2.80 Å), Asn142 (2.48 Å) | -                         | Glu166, Met165, His163, Phe140, Leu141, Leu41, Thr24, Met49 | Cys145 (5.13, 4.82 Å) |
| D23    | −10.9                    | Gln192 (2.61 Å), Arg188 (2.55 Å), Met165 (2.84 Å) | -                         | Leu67, Thr190, Asp187, His164, His41, Met49, Leu27, Cys145, Thr26, Glu166, Gly143 | Met165 (5.32, 4.22 Å), Gln189 (3.48 Å), Pro168 (5.13, 5.23 Å) |
| D25    | −10.6                    | Asn142 (2.71 Å), Ser144 (2.36 Å), Gly143 (2.65 Å) | -                         | Pro168, Glu166, Met165, His163, Leu41, Phe140, Leu27, Met49, Ser46, Met49 | Cys145 (5.07, 5.10 Å) |
| D26    | −10.5                    | Arg188 (2.54 Å), Cys145(3.95 Å), Gln189 (3.42 Å), Gln192 (unfavorable donor-donor, 2.14 Å) | His41(3.49 Å)              | Pro168, Thr190, Asp187, His164, Met49, Gly143, Glu166 | Met165 (5.16, 4.36 Å) |
| D27    | −10.5                    | Thr190 (3.09 Å), Gln192 (2.97 Å), Arg188 (2.53 Å) | -                         | Leu67, Gln189, His164, Gly143, Asn142, Glu166 | Pro168 (5.42 Å), Met165 (4.40, 4.92 Å), Cys145 (5.07 Å) |
| D30    | −10.5                    | Asn142 (2.68), Ser144 (2.43), Gly143 (2.67) | -                         | Glu166, Met165, His163, Phe140, Leu41, Leu27, Ser46, Met49 | Cys145 (4.96, 5.17 Å) |
| D76    | −11.1                    | Arg188 (2.46 Å), Met165 (3.02 Å), Glu166 (2.17 Å), Gln189 (3.22 Å) | -                         | Gln192, His164, Asn142, Ser144, Gly143, Leu141, Leu167 | Met165 (4.78, 5.44 Å), Pro168 (5.30 Å), Cys145 (5.12 Å) |
MM-GBSA computations

Table 5 shows the binding free energy values obtained by MM-GBSA for the selected ligands (D07, D08, D12, D18, D23, D25, D26, D27, and D76) and also for references (T40 and N3). Although the ligands (T40 and N3) did not show a good fit for drug-like features, their presence as references were necessary during the in silico validation routines. The MM-GBSA computations presented in Table 6 indicate that the binding energies of the designed ligands range from the lowest value of $-47.83$ kcal/mol (D27) to the highest level of $-59.54$ kcal/mol (D23). The proposed drug ligands showed relatively higher binding energies than the reference molecule T40 ($-47.77$ kcal/mol) and less than the N3 peptide inhibitor ($-83.84$ kcal/mol). Among the ligand/protein binding patterns evaluated via MM-GBSA, we can notice the significant contribution of ($\Delta G_{\text{bind Vdw}}$, $\Delta G_{\text{bind H-bond}}$, $\Delta G_{\text{bind Coulomb}}$, $\Delta G_{\text{bind Lipo}}$, and $\Delta G_{\text{bind Packing}}$) energies to the average binding energy ($\Delta G_{\text{bind}}$) of the nine proposed drug molecules and also for the references (T40 and N3). The positive energy contribution of ($\Delta G_{\text{bind Solv_GB}}$) and ($\Delta G_{\text{bind Covalent}}$) was not favorable to ($\Delta G_{\text{bind}}$), which can lead to resistance against the binding. This means that the effect of the energies of non-binding interactions is much more favorable to achieving high and equilibrium stability of the examined ligands in the active pocket of 3CL$^{\text{pro}}$ compared to potential covalent interactions.

In brief, the obtained MM-GBSA computations confirm the molecular docking results related to the high binding affinity energies of the proposed small molecule (D07, D08, D12, D18, D23, D25, D26, D2, and D76) toward the active site inside the 3CL$^{\text{pro}}$ pocket.

Molecular dynamics simulations

Molecular dynamics simulations were performed to examine the stability level of potential non-covalent interactions between the active residues of the 3CL$^{\text{pro}}$ (6LU7) pocket and investigated 9,10-dihydrophenanthrene derivatives. For this purpose, we consider the samples D08-6LU7($\Delta G_{\text{bind}}=-55.15$ kcal/mol), D23-6LU7 ($\Delta G_{\text{bind}}=-59.54$ kcal/mol), and D76-6LU7 ($\Delta G_{\text{bind}}=-57.30$ kcal/mol) as test items and sample T40-6LU7 (\Delta G_{\text{bind}}=-47.77 kcal/mol) as a reference in MD simulations protocol.

RMSD and RMSF analysis

The root mean square deviation (RMSD) and fluctuation (RMSF) parameters were used to estimate the range of potential fluctuations in the backbone of 6LU7 proteases (uncomplexed and complexed). The RMSD and RMSF indices express the average $\alpha$-carbon backbone for all atoms of amino acid residues that formed the 6LU7 systems. In this work, RMSD and RMSF variations of examined 6LU7 systems were evaluated based on the first frame of the uncomplexed 6LU7 backbone (6LU7 free). Figure 10 shows the RMSD and RMSF time scales obtained after 100 ns of MD simulation trajectory.

From Fig. 10a, it appears that the $\alpha$ atoms of the 6LU7 protease backbone were not affected in their stability after D08, D23, and D76 ligands docked into the active pocket of 6LU7 during 100 ns of the MD trajectory. Some slight fluctuations in the amino acid side chains were observed in the
D08-6LU7 complex from 70 to 80 ns; these fluctuations did not exceed 1.5 Å after which D08-6LU7 returned to equilibrium until the end of the MD simulation time set at 100 ns. The average RMSD values obtained for 6LU7 free and complexed with the ligands D08, D23, D76, and T40, respectively, were 1.239 Å, 1.179 Å, 1.876 Å, 1.305 Å, 1.284 Å, and 1.400 Å. The low values of fluctuations observed in the RMSD indicate that the proposed drug molecules reached a good equilibrium in the 6LU6 protease pocket.

The mean RMSF values of the α-carbon residues of the examined systems 6LU7, 6LU7-D08, 6LU7-D23, 6LU7-D76, and 6LU7-T40, respectively, were 0.821 Å, 0.961 Å, 0.886 Å, 0.873 Å, and 0.788 Å (Fig. 10b). Despite the presence of some fluctuations related to extreme residue sequences (SER_47, LEU_50, ASN_51, ASP_155, THR_304, PHE_305, and GLN_306), the average values of these fluctuations did not exceed 4.5 Å, indicating the expected high stability of the examined ligands in the active pocket of 6LU7.

The RMSF data presented in Fig. 10c indicate the presence of some fluctuations in the structures of the D08, D23, D76, and T40 ligands. The observed fluctuations could be due to some structural properties of the ligands, such as rotation angles, torsion, and flexible interactions between the ligands and their binding sites in the active 6LU7 pocket.

Dynamics of protein–ligand interactions (PL-contacts)

As previously mentioned in Fig. 7b, the active site in the 6LU7 protease pocket contains the polar amino acids threonine (Thr190, Thr24), glutamine (Gln189, Gln192), aspartagine (Asn142) and aspartic (Asp187), serine (Ser144), and the negatively charged glutamic acid (Glu166), positively charged amino acids such as histidine (His163, His172, His41, His164) and nonpolar amino acids such as phenylalanine (Phe140), methionine (Met165, Met49), leucine (Leu146), proline (Pro168), alanine (Ala191), and the hydrophobic amino acid cysteine (Cys145). Interactions of the tested ligands with these residues and not moving away from the active pocket containing them can be considered a mechanism of inhibition of the enzymatic activity of 3CL^pr^-pp. Figures 11 and 12 show synthetic diagrams of the key contacts that occur between the protein and the examined ligands during the MD simulations.

From Fig. 11 and 12, we can see that the examined ligands were able to maintain most interactions with the residues predicted by molecular docking, meaning that the small designed molecules were able to dock into the active 3CL^pr^-pp pocket throughout the MD simulation and did not move away from the target active site. Table 6 presents a summary of the key protein–ligand contacts for the examined complexes 6LU7-D08, 6LU7-D23, 6LU7-D76, and 6LU7-T40, as well as the contact ratios identified at all 100 ns of MD simulations.

| Complex | Protein–ligand contacts | H-bonds | Hydrophobic | Water bridges |
|---------|--------------------------|---------|-------------|--------------|
| 6LU7-D08 | Thr24 (12%), Thr25 (18%), His41 (15%), Gln192 (13%), Ser144 (60%), Gln189 (20%), Thr45 (18%), Cys145 (10%), Glu166 (25%), and Met65 (38%) | Thr24 (12%), Thr25 (18%), His41 (15%), Gln192 (13%), Ser144 (60%), Gln189 (20%), Thr45 (18%), Cys145 (10%), Glu166 (25%), and Met65 (38%) | Thr24 (12%), Thr25 (18%), His41 (15%), Gln192 (13%), Ser144 (60%), Gln189 (20%), Thr45 (18%), Cys145 (10%), Glu166 (25%), and Met65 (38%) | Thr24 (12%), Thr25 (18%), His41 (15%), Gln192 (13%), Ser144 (60%), Gln189 (20%), Thr45 (18%), Cys145 (10%), Glu166 (25%), and Met65 (38%) |
From Table 6, we can notice that ligand D8 was able to contact by conventional hydrogen bonds with the residues Thr24 (12%), Thr25 (18%), His41 (15%), Ser144 (60%), Gln189 (20%) Cy145 (10%), Thr45 (8%), Asn142 (3%), Glu166 (4%), and Thr190 (5%). Additionally, a hydrogen bond formed through a water bridge with the amino acid Thr26 (16%). D08 is also contacted by hydrophobic interactions with Leu27 (6%), His41 (56%), Met49 (40%), Cys145 (10%), Met165 (37%), and Ala191 (3%).

For ligand D23, it was able to bind via hydrogen bonds to amino acid residues His41, Asn142 (ion bridge and water), and Val186 for periods less than 10%, as well as bind to Glu166 (25%), Asp187 (54%), and Gln189 (18%). Hydrophobic interactions were also formed with His41 (16%), Met49 (18%), Cy145 (10%), Met165 (38%), and with Pro168 and Leu166 (<10%).

For ligand D76, there is a hydrogen bond contact with residues Asp (83%), His164 (42% water-bridge-aided), Glu166 (23%), Gln189 (13% water-bridge aided), and Val186 (5%). Additionally, hydrophobic interactions were formed with Met49 (5%), Met165 (22%), Leu167 (6%), and Pro168 (26%).

Ligand T40 was contacted by hydrogen bonds with the amino acid residues Thr16 (<3%), His41(22%), Glu166(41%), and Gln189(18%). In addition, hydrophobic interactions were formed between T40 and the amino acid

**Fig. 10** a) RMSD of free protease 6LU7, complexed with ligands D08, D23, D76, and T40. b) RMSF of backbone atoms in free 6LU7, complexed with the ligands D08, D23, D76, and T40. c) RMSF of ligands D08, D23, D76, and T40 complexed with 6LU7.

**Fig. 11** Contact histogram of 6LU7-D08, 6LU7-D23, 6LU7-D76, and 6LU7-T40 along the MD time course.
residues His41 (20%), Met49 (12%), Met165 (40%), Leu167 (50%), Pro168 (52%), and Ala191 (< 10%).

Moreover, from Table 6, we can also notice that the water bridge interactions contributed to the stability of ligands D8, D23, D76, and T40 inside the 3CL\textsuperscript{pro} active pocket. The medium to low contact ratios of non-covalent interactions formed between ligands D08, D23, D76, and T40 with active amino acid residues in the 6LU7 pocket can be explained by their high $\Delta G_{\text{bind}}$ (from $-47.77$ to $-57.30$ kcal/mol). This means that the structures of the proposed drug molecules have a flexible structure and good structural properties that allow them to make many contacts in the active pocket through different interactional modes.

From the summary of protein–ligand interactions presented in Figs. 11, 12, and Table 6, it can be concluded that weak H-bonds, hydrophobic bonds, and water bridges strongly contribute to the stability of the drug ligands (D8, D23, D76, and T40) with 3CL\textsuperscript{pro}. These weak non-covalent interactions are very appropriate for drug ligands to achieve protein binding compatibility and reach the desired therapeutic target, as well as to facilitate the removal of drug compounds after reaching the therapy. This is because strong covalent interactions between the ligand and the receptor are difficult to remove and can result in the opposite effect of covalent drug compounds. Therefore, non-covalent small molecule drugs may have very comparable therapeutic and pharmacokinetic activity against SARS-CoV-2 3CL\textsuperscript{pro} compared to covalent drug molecules such as covalent Michael inhibitor (N3).

**Properties of ligands**

Figure 13 presents the properties of D08, D23, D76, and T40 ligands estimated at over 100 ns of MD simulation. A total of six properties of ligands were evaluated: ligand RMSD, radius of gyration (rGyr), intramolecular hydrogen bonds (intra-HB), molecular surface area (MolSA), solvent accessible surface area (SASA), and polar surface area (PSA).

For ligand D08 in 6LU7-D08, the RMSD ranged from 0.5 to 1.5 Å, and its equilibrium was approximately at 1 Å. rGyr was limited to the range (4.25–4.75 Å), and its equilibrium was around 4.5 Å. The intra-HB was high throughout the MD simulation. MolSA was in the range (376–400 Å\textsuperscript{2}), and its equilibrium was about 390 Å\textsuperscript{2}. SASA showed strong fluctuations ranging from about (200–600 Å\textsuperscript{2}) over the time interval (40–80 ns), then stabilized at around 400 Å\textsuperscript{2}. PSA was in the range (120–180 Å\textsuperscript{2}), and its equilibrium was around 160 Å\textsuperscript{2}.

In the 6LU7-D23 complex, the ligand D23 showed many fluctuations in RMSD ranging from 0.8–2.4 Å and stabilized at about 1.6 Å. rGyr ranged from 5.6 to 4.4 Å and stabilized at about 5 Å. Intra-HB was low throughout the MD simulation. MolSA was ranged between 375 and 420 Å\textsuperscript{2} and stabilized perfectly along the simulation time at about 405 Å\textsuperscript{2}. SASA showed strong fluctuations around the range 200–400 Å\textsuperscript{2} and later stabilized at around 200 Å\textsuperscript{2}. PSA was found limited in the range 80–140 Å\textsuperscript{2} and stabilized at around 120 Å\textsuperscript{2}.

In the 6LU7-D76 complex, the ligand D76 showed perfect stability in terms of RMSD in the range 0.5–1.2 Å over the simulation period with some slight fluctuations at about 80 ns. rGyr was in the range 4.8–4.5 Å and stabilized at about 4.65 Å. Intra-HB was not detected in the D76 ligand. MolSA was in the range 376–392 Å\textsuperscript{2} and stabilized at approximately 384 Å\textsuperscript{2}. The SASA was in the range 180–300 Å\textsuperscript{2} and almost stabilized at 240 Å\textsuperscript{2}. PSA was in the range of 75–105 Å\textsuperscript{2} and stabilized at about 90 Å\textsuperscript{2}.

In the 6LU7-T40 complex, the RMSD of the ligand T40 was in the range 0.5–1.8 Å and stabilized at about 1.2 Å.
rGyr was in the range 4.8–5.7 Å and stabilized after 40 ns at about 5.4 Å. Intra-HB was not detected in the T40 ligand. MolSA was in the range 424–448 Å² and stabilized at about 440 Å². SASA was in the range of about 160–320 Å²; it stabilizes at about 240 Å² after 40 ns of the MD trajectory. PSA was in the range 70–100 Å² and stabilized at about 80 Å².

Fig. 13  Timeline of the properties of the ligands D08, D23, D76, and T40 complexed with 6LU7 during 100 ns of MD trajectory

Fig. 14  The thermodynamic properties of the 6LU7 systems (6LU7 Free, 6LU7-D08, 6LU7-D23, 6LU7-D76, and 6LU7-T40)
Thermodynamic properties analysis

The summary of the quality of the MD simulation is analyzed through the computation of the stability profile for the investigated systems in terms of the variation in total energy (E), potential energy (E_P), temperature (T), pressure (P), and volume (V) over the 100 ns of MD simulation trajectory. Figure 14 shows the thermodynamic properties diagrams generated for the 6LU7, 6LU7-D08, 6LU7-D23, 6LU7-D76, and 6LU7-T40 systems.

The generated thermodynamic properties show that the scores (E, EP, T, P, and V) of the complexes (6LU7-D08, 6LU7-D23, 6LU7-D76, and 6LU7-T40) remained stable and close to those of free 6LU7. This finding can be confirmed by the average values (E, EP, T, P, and V) of the 6LU7 complexes that are very close to those of free 6LU7 (Table 7). These results further prove that the structures of small 9,10-dihydrophenanthrene compounds can reach perfect stability in the active pocket of 3CL\textsuperscript{Pro}.

All in all, molecular dynamics analyses show the high stability of the samples (D08, D23, D76, and T40) inside the 3CL\textsuperscript{Pro} active pocket. Therefore, the choice of the nine molecules (D07, D08, D12, D18, D23, D25, D26, D27, and D76) screened through 3D-QSAR, molecular docking, drug-like, ADMET, and MM-GBSA studies can be validated as promising non-covalent inhibitors of SARS-CoV 3CL\textsuperscript{Pro}.

**Conclusion**

The outbreak of coronavirus 2019 (COVID-19) has negatively impacted daily life in all regions of the world. Due to the severity of COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), it is necessary to advance the search for an appropriate and effective drug against COVID-19. In this context, 3-chymotrypsin-like cysteine protease (3CL\textsuperscript{Pro}) is an indispensable input to viral replication. Therefore, inhibition of 3CL\textsuperscript{Pro} enzymatic activity becomes an attractive target against COVID-19. To search for novel pharmacological compounds against COVID-19 through computer-aided drug design, we used a series of 46 small molecules derived from 9,10-dihydrophenanthrene as potential inhibitors of SARS-CoV-2 3CL\textsuperscript{Pro} in the computational analysis and pharmaceutical parameters screening. In this study, the 3D quantitative structure–activity relationship (3D-QSAR) for 9,10-dihydrophenanthrene derivatives was carefully analyzed and described using CoMFA and CoMSIA techniques. As a result, based on the structure of the 9,10-dihydrophenanthrenes derivatives, two models CoMFA/SE and CoMSIA/SEHDA were developed. Both models showed a high ability to predict the biological activity of pIC\textsubscript{50} against SARS-CoV-2 3CL\textsuperscript{Pro}, the pharmacological sites were rationalized, and the most important features favorable for modeling and improving the biological activity of the studied molecules were identified. Accordingly, 96 new drug molecules were generated based on the structure of the synthesized template molecule T40 exhibiting the highest biological activity pIC\textsubscript{50} observed in vitro, followed by bioavailability parameter screening to select candidate drug compounds.

Then, the bioactivity (pIC\textsubscript{50}) of the modeled molecules was predicted by 3D-QSAR models, their non-covalent interaction to 3CL\textsuperscript{Pro} (PDB code:6LU7) was investigated via molecular docking, and in silico pharmacokinetics, ADME properties, and toxicity were evaluated, as well as free binding energies (\(\Delta G_{\text{bind}}\)) were scored by MM-GBSA computations. The results of this study demonstrated that the generated nine compounds generated D07, D08, D12, D18, D23, D25, D26, D27, and D76 have high biological inhibitory activity (pIC\textsubscript{50}), excellent non-covalent binding to 3CL\textsuperscript{Pro}, good pharmacokinetic suitability and less potential toxicity compared to the template synthesized compound T40 and N3 peptidic inhibitor. The results obtained were confirmed by molecular dynamics simulations of the tested systems (6LU7 uncomplexed and complex). For this purpose, the structural stability and dynamics of free and complexed with the tested ligands (D08, D23, D76, and T40) in an aqueous environment were discussed.

Finally, we have shown that nine small molecules modeled on 9,10-dihydrophenanthrene structures have the potential to act as a promising non-covalent drug candidate against COVID-19 by inhibiting the enzymatic activity of 3CL\textsuperscript{Pro}.

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**Table 7** Scores average of the thermodynamics properties of analyzed systems

| Systems  | Average property values | Potential energy (kcal/mol) | Temperature (K) | Pressure (bar) | Volume (Å\textsuperscript{3}) |
|----------|-------------------------|----------------------------|-----------------|---------------|----------------|
| 6LU7-Free | 99,905.370              | 122,063.495               | 298.705         | 1.409         | 362,814.329    |
| 6LU7-D08  | 99,756.824              | 121,927.605               | 298.714         | 1.565         | 362,806.668    |
| 6LU7-D23  | 99,661.259              | 121,823.752               | 298.702         | 1.193         | 362,702.992    |
| 6LU7-D76  | 99,649.019              | 121,807.582               | 298.709         | 1.247         | 362,706.347    |
| 6LU7-T40  | 99,603.363              | 121,760.057               | 298.704         | 1.885         | 362,615.184    |
Therefore, the adoption of the small molecule structures proposed in this study will be useful as a key starting point for the development of therapy against COVID-19. Thus, the retrosynthesis of these molecules and the evaluation of their bioactivity in vitro and in vivo may be of interest in the context of SARS-CoV-2 3CL\textsuperscript{pro} drug design and discovery. Also, the potential activity of the proposed small molecules against other protein pathways of coronaviruses can be investigated.

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Data availability All data used in this work are private.

Code availability The codes used in this work are not available.

Declarations

Conflicts of interest The authors declare no competing interests.

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