One-pot synthesis, molecular docking, ADMET, and DFT studies of novel pyrazolines as promising SARS-CoV-2 main protease inhibitors

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Received: 19 April 2022 / Accepted: 1 September 2022 / Published online: 13 September 2022 © The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract
Pyrazoline and its derivatives have numerous prominent pharmacological effects. Focusing on its anti-viral property, we have designed and synthesized three novel pyrazoline derivatives (A1–A3) through one-pot three components and characterized them using different spectroscopic techniques (FT-IR, 1H NMR, 13C NMR, and UV). These compounds were evaluated against SARS-CoV-2 main protease utilizing in-silico molecular docking studies. The docking results displayed good inhibitory activity of the synthesized compounds. Among them, compound A2 was the most active against targeted protein. The drug-likeness and ADMET properties were predicted to have varied profiles but could still be developed, especially A2. DFT/TD-DFT calculations through B3LYP/6-311G++ level of theory were applied to provide comparable theoretical data along with MEP map and electronic energy gap of HOMO → LUMO.

Keywords Pyrazoline · One-pot synthesis · Molecular docking · ADMET · DFT
Introduction

Humanity is now plagued by the coronavirus disease 2019 (Covid-19) pandemic, which is caused by coronavirus 2 that causes severe acute respiratory syndrome (SARS-CoV-2) [1]. Even though COVID-19 is a viral infection, it can also affect a patient’s immune system. Therefore, secondary bacterial or fungal infections can take hold [2, 3]. Researchers worldwide have been attempting to investigate the potential treatment and discovery of effective therapeutic drug candidates for the management of COVID-19. Furthermore, some reports proposed potential inhibitory agents against COVID-19 targets. Currently, several molecules are being tested for their efficacy on COVID-19 disease, some of which have reached clinical trials, while others are still in the preclinical phase [1, 4, 5, 6, 7, 8, 9].

Pyrazoline is a five-membered heterocyclic having two adjacent nitrogen atoms within the ring [10, 11]. Nitrogen-containing heterocycles have a wide range of applications in medicine [12, 13, 14]. Therefore, the chemistry of pyrazoline compounds has attracted the attention of researchers due to their important biological activities such as anti-bacterial, anti-inflammatory, anti-cancer, anti-fungal, anti-oxidant, anti-depressant, anti-leishmanial, anti-convulsant, and anti-tumor properties [15, 16, 17, 18, 19, 20, 21, 22, 23], especially as anti-viral agent [24, 25, 26, 27].

Hence, we designed novel pyrazoline derivatives via one-pot three-component condensation reaction, as this became preferred protocol and was used extensively for synthesizing new heterocyclic compounds due to its simple set-up procedure, reduced reaction time, excellent yields and reduced pollutant production. Computationally (in silico) evaluated them as a potential agent against SARS-CoV-2 main protease (M\textsuperscript{pro}) with the help of molecular docking simulation. Moreover, the current study is going to investigate the different properties including drug-likeness and pharmacokinetics properties, structural characterization, spectroscopic, thermodynamic, and vibrational phenomena with stabilization energy of all the synthesized compounds. Based on the findings of this investigation, researchers will be able to discover an effective drug to treat COVID-19.

Experimental

Preparation of 1-(4-((4-chlorobenzyl)oxy)phenyl)ethan-1-one (3)

In 250-mL round bottom flask, a mixture of 4-hydroxyacetophenone (25 mmol), 4-chlorobenzylchloride (30 mmol) in the presence of catalytic anhydrous potassium carbonate (50 mmol) was refluxed for 6 h with stirring in absolute ethanol (50 mL). The reaction completion was monitored by changing the color, then poured into cold water. The obtained precipitate was filtered, washed with cold ethanol, dried in an oven, and recrystallized from ethanol to give compound 3. White powder; m.p.: 91.3–92.7 °C; yield; 97.0%; FT-IR (KBr) (\(\nu_{\text{max}}/\text{cm}^{-1}\)): 3008
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(C–H sp²), 2873 (C–H sp³), 1668 (C=O), 1597 (C=C); UV λ_max = 336 nm; ¹H NMR (400 MHz, CDCl₃) (ppm): δ 2.57 (3H, s, CH₃), 5.09 (2H, s, OCH₂), 6.95 (2H, d, J = 8.4 Hz, Ar–H), 7.35 (4H, m, Ar–H), 7.90 (2H, d, J = 8.4 Hz, Ar–H); ¹³C NMR (100 MHz, CDCl₃) (ppm): δ 196.70 (C=O), 162.30 (C-4), 134.60 (C-6), 134.00 (C-9), 130.70 (C-7 and C-7′), 130.60 (C-2 and C-2′), 128.80 (C-8 and C-8′), 128.7 (C-3), 114.50 (C-3 and C-3′), 69.30 (C-5), 26.30 (CH₃).

One-pot synthesis of pyrazoline derivatives (A1–A3)

In a round bottom flask, a mixture of compound 3 (7 mmol), substituted benzaldehydes (7 mmol), and thiosemicarbazide (10 mmol) were dissolved in absolute ethanol (30 mL). To this mixture a catalytic amount of sodium hydroxide (12 mmol) was added and refluxed with stirring. The completion of reaction was monitored by thin layer chromatography (TLC) in hexane/ethyl acetate as a solvent system. The solution was cooled to room temperature, filtered the obtained solid, washed with cold ethanol, and dried. The pure target compounds (A1–A3) were obtained by recrystallization from toluene.

5-(3-(Benzyloxy)phenyl)-3-(4-((4-chlorobenzyl)oxy)phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (A1)

Pale yellow; m.p.: 210.3–211.8 °C; yield: 75.5%; R_f = 0.36 in n-Hex:EtOAc (1:1); FT-IR (KBr) (υ max/cm⁻¹): 3487, 3360 (NH₂), 1604 (C=C), 1471 (C=N), 1355 (C=S); UV λ_max = 360 nm; ¹H NMR (400 MHz, CDCl₃) (ppm): δ 3.16 (1H, dd, J = 4.0 and 16.0 Hz, H_a-C 11), 3.78 (1H, dd, J = 4.0 and 16.0 Hz, H_b-C 11), 4.99 (2H, s, OCH₂), 5.09 (2H, s, OCH₂), 5.99 (1H, dd, J = 4.0 and 12.0 Hz, H-C 12), 6.16 (2H, s, NH₂), 6.82–7.77 (4H, m, Ar–H), 6.99 (2H, d, J = 8.0, H-7 and H-7′), 7.25–7.38 (16H, m, Ar–H), 7.65 (2H, d, J = 8.0 Hz, H-8 and H-8′); ¹³C NMR (100 MHz, CDCl₃) (ppm): δ 176.37 (C-24), 160.73 (C-6), 158.94 (C-15), 155.68 (C-10), 143.58 (C-13), 135.37 (C-20), 134.77 (C-4), 133.69 (C-5), 130.04 (C-1), 129.02 (C-3 and C-3′), 128.93 (C-21 and C-21′), 128.86 (C-17), 128.74 (C-22 and C-22′), 128.69 (C-2 and C-2′), 128.21 (C-8 and C-8′), 123.55 (C-9), 118.23 (C-18), 115.16 (C-7 and C-7′), 113.60 (C-16), 112.17 (C-14), 69.32 (C-5), 69.20 (C-19), 63.24 (C-12), 43.07 (C-11).

5-(3-(Benzyloxy)phenyl)-3-(4-((4-chlorobenzyl)oxy)phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (A2)

White powder; m.p.: 203.7–205.4 °C; yield: 73.3%; R_f = 0.43 in n-Hex:EtOAc (1:1); FT-IR (KBr) (υ max/cm⁻¹): 3423, 3236 (NH₂), 1591 (C=C), 1456 (C=N), 1382 (C=S); UV λ_max = 362 nm; ¹H NMR (400 MHz, CDCl₃) (ppm): δ 3.14 (1H, s, H_a-C 11), 3.77 (1H, s, H_b-C 11), 5.08 (2H, s, 2× OCH₂), 5.99 (1H, s, H_X-C 12), 6.84–7.65 (16H, m, Ar–H); ¹³C NMR (100 MHz, CDCl₃) (ppm): δ 176.41 (C-24), 160.70 (C-6), 158.94 (C-15), 155.68 (C-10), 143.58 (C-13), 135.37 (C-20), 134.77 (C-4), 133.69 (C-5), 130.04 (C-1), 129.02 (C-3 and C-3′), 128.93 (C-21 and C-21′), 128.86 (C-17), 128.74 (C-22 and C-22′), 128.69 (C-2 and C-2′), 128.21 (C-8 and C-8′), 123.55 (C-9), 118.23 (C-18), 115.16 (C-7 and C-7′), 113.60 (C-16), 112.17 (C-14), 69.32 (C-5), 69.20 (C-19), 63.24 (C-12), 43.07 (C-11).
128.17 (C-17), 127.93 (C-22 and C-22′), 127.63 (C-2 and C-2′), 125.25 (C-8 and C-8′), 123.58 (C-9), 117.97 (C-18), 115.11 (C-7 and C-7′), 113.45 (C-16), 112.24 (C-14), 70.10 (C-5), 69.30 (C-19), 63.25 (C-12), 43.06 (C-11).

3-(4-((4-Chlorobenzyl)oxy)phenyl)-5-((3-nitrobenzyl)oxy)phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (A3)

Pale brown; m.p.: 147.4–148.7 °C; yield: 64.6%; \( R_f = 0.32 \) in \( n \)-Hex:EtOAc (1:1); FT-IR (KBr) (\( \nu_{\max} / \text{cm}^{-1} \)): 3471, 3354 (NH\(_2\)), 1597 (C=C), 1471 (C=N), 1346 (C=S); UV \( \lambda_{\max} = 294 \) nm; \(^1\)H NMR (400 MHz, CDCl\(_3\)) (ppm): \( \delta \) 3.15 (1H, d, \( J = 16.0 \) Hz, \( H_a-C_{11} \)), 3.72 (1H, dd, \( J = 16.0 \) Hz, \( H_b-C_{11} \)), 3.75 (1H, s, –CH-H X-C\(_{12}\) ), 5.07 (2H, s, –OCH\(_2\)), 5.08 (2H, s, –OCH\(_2\)), 6.38 (2H, s, NH\(_2\)), 5.99 (1H, d, \( J = 12.0 \) Hz, H-14), 6.85-7.73 (13H, m, Ar–H), 8.99 (1H, d, \( J = 8.0 \) Hz, H-23), 8.11 (1H, s, H-21); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) (ppm): \( \delta \) 179.27 (C-26), 160.74 (C-6), 158.56 (C-15), 155.73 (C-10), 148.29 (C-22), 143.83 (C-20), 139.08 (C-13), 134.82 (C-4), 133.95 (C-25), 133.32 (C-1), 130.20 (C-24), 129.54 (C-3 and C-3′), 129.05 (C-17), 128.23 (C-2 and C-2′), 125.31 (C-9), 123.47 (C-8 and C-8′), 122.83 (C-21), 118.53 (C-23), 115.15 (C7 and C-7′), 114.69 (C-18), 113.62 (C-16), 112.28 (C-14), 69.29 (C-5), 68.61 (C-19), 63.21 (C-12), 43.03 (C-11).

Molecular docking study

Ligand preparation

The 3D structures of titled compounds were drawn, and energy of each compound was minimized and saved in the pdb format by using Avogadro [28]. Subsequently, AutoDock Tools 4.2 [29] was used to open the pdb files, and torque adjustment was made by detecting roots and adjusting as desired. Then, the files were saved in the pdbqt format. Meantime, the structure data format (SDF) of the control lopinavir (CID: 92727), was downloaded from the PubChem database (www.pubchem.ncbi.nlm.nih.gov). The structure data file (SDF) format was subjected to the energy minimization process using MMFF94 (Merck Molecular Force Field 94) force field algorithm [30], the minimized structure was converted into PDBQT format using PyRx before performing molecular docking analysis.

Protein preparation

The 3D crystal structure of SARS-CoV-2 (M\(^{\text{PRO}}\)) was retrieved from the Protein Databank (http://www.rcsb.org) with PDB ID (6LU7). The co-crystallized ligand and water molecules were removed utilizing Discovery Studio Visualizer [31]. Afterward, polar hydrogens and Kollman charges were added. Partial charges of the
molecule were calculated using the Gasteiger method. The prepared file was saved as a pdbqt format for molecular docking using AutoDock Tools 4.2 [29].

**Molecular docking**

Molecular docking of the ligands was investigated by using the AutoDock Vina program [32]. Vina software was run in exhaustiveness = 8. The binding site was selected based on its co-crystallized ligand (N3) with the target protein [33]. Affinity scores (in kcal/mol) given by AutoDock Vina for all the compounds were obtained and ranked based on the free energy binding theory (more negative value means greater binding affinity). The resulting docking of ligand-receptor complexes with higher binding affinity was analyzed and drafted to 2D and 3D figures with the help of the DS Visualizer and UCSF Chimera.

**ADMET analysis**

According to the reported procedures, as described in the literature [34], prediction of drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the synthesized compounds was carried out utilizing online servers such as SwissADME, ProTox-II, and pkCSM.

**Density functional theory**

In order to understand the spectral assignments, and to study the molecular geometry and electronic transitions of the synthesized compounds (A1, A2, and A3), a computational study was taken into account, to explore the molecular interactions in more detail. The Gaussian 09RevD.01 program [35] was used for theoretical studies. Gradient corrected correlation with Pople’s basis set B3LYP/6-311G++ was applied. The gauge independent atomic orbital (GIAO) and Integral Equation Formalism Polarizable Continuum Model (IEFPCM) with the same functional set were used for computing 1H-NMR chemical shifts in the liquid phase [36, 37, 38, 39, 40, 41, 42]. Optimized structure of A1, A2, and A3 was also obtained using the B3LYP/6-311G++ basis set. Highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), and electrostatic potential map (MEP) were calculated. The calculated IR frequencies through this method are found to be positive, indicating that the optimized structure is at a minimum on the potential energy surface. In the FTIR spectrum, bands that appeared were assigned with full accuracy, using animated modes of vibrations. The frontier molecular orbitals help in determining the chemical stability of the system. Moreover, structure-based molecular properties like atomic charges, total energy, stabilization energy, electronic properties, bond lengths, frontier molecular orbitals, and molecular electrostatic potential were calculated by this theory in the gas phase. For visualization of obtained DFT results, ChemCraft 1.5 software [43] was used [43].
Results and discussion

Chemistry

1-(4-((4-Chlorobenzyl)oxy)phenyl)ethan-1-one (3) was prepared in excellent yield (97%) based on of Williamson synthesis of ether by direct benzyla-
tion of para-hydroxy acetophenone with 4-chlorobenzylchloride, in the presence of anhydrous potassium carbonate in ethanol. The synthesis of pyrazoline derivatives (A1–A3) was accomplished via the condensation reaction of com-
ponent 3, substituted benzaldehydes with thiosemicarbazide in dilute ethanolic
sodium hydroxide solution (Scheme 1). The structures of synthesized pyrazo-
lines (A1–A3) were confirmed by FT-IR, 1H-NMR, 13C-NMR, and UV–Vis
spectroscopy. In FT-IR spectra of pyrazolines the disappearance of a band in
the range of 1653–1645 cm⁻¹ for carbonyl groups (C=O), were considered as
good evidence to produce pyrazoline, all spectra exhibited a characteristic two
bands at 3400–3200 cm⁻¹ corresponding to the (NH₂) stretching of carbothio-
amide group attached to pyrazoline ring, also the appearance of the strong band
at 1492–1456 cm⁻¹ for C=N stretching vibration, which indicates the presence
of pyrazoline ring, two bands at 1608–1597 cm⁻¹ and 1382–1340 cm⁻¹ referring
to (C=C) and (C=S) double bond, respectively. The 1H-NMR spectra of pyrazo-
lines display doublet to doublet (dd) signals corresponding to three protons (two
geriminal and one vicinal) of C₁₁ and C₁₂ of pyrazoline ring; they form a charac-
teristic ABX spin system explaining the inequivalence of protons at C₁₁.
Molecular docking study

Molecular docking was investigated by AutoDock Vina software due to its excellent speed and providing low root-mean-square deviation (RMSD) [32]. The binding affinity of titled compounds was compared with lopinavir which was used as a positive drug for comparison purposes which was recommended for clinical therapeutic [44]. The binding affinities of the synthesized compounds A1 and A2 (−8.2 and −8.3 kcal/mol, respectively) were higher than the binding affinity of the positive drug lopinavir (−7.8 kcal/mol). Thus, these two compounds could be effectively inhibiting the SARS-CoV-2 main protease (Mpro). The compound A3 has a slightly lower binding affinity (−7.5 kcal/mol) than the lopinavir. The docking results exhibited no significant differences in the binding affinities of compounds A1 (with chloro substituent) and A2 (without substituent), as shown in Table 1.

The A2-6LU7 complex established three hydrogen bonds with amino acid residues ASN238, LEU272, and LEU287. An alkyl interaction with LEU272 residue and π-alkyl interactions with TYR237 and LEU286 were also observed. Furthermore, it can form π-sigma interaction with LEU287. In contrast, compound A1 showed additional hydrophobic and electrostatic interactions with ASP197 residue of target receptor via π-sigma and π-anion bonds. Moreover, compound A3 (with nitro substituent) interacted with Mpro, formed five hydrogen bonds at residues THR199, ASN238, LEU271, ALA285, and LEU287. An alkyl and π-alkyl hydrophobic interactions were observed between this compound and the receptor. Additionally, amino acid residues ASP107 and LYS137 are involved in forming π-anion and π-cation electrostatic interactions, respectively. The docking interactions of synthesized compounds with the active site amino acid residues of SARS-CoV-2 (Mpro) are depicted in Fig. 1. The docking scores interaction modes of all docked ligands into the active site and the amino acid residues of Mpro are presented in Table 1.

Drug-likeness and ADMET prediction

The pharmacokinetic properties and toxicity profile of the pyrazolines (A1–A3) were predicted in-silico, as presented in Table 2. The obtained results are in agreement with parameters reported in the literature [45], as well as referring to Lipinski’s rule of five (LRO5) [46]. The first parameter observed was molecular weight (Mol. Wt.), where all compounds met the LRO5 criteria, which were ≤500 g/mol. However, the Mol. Wt. of all ligands still met the criteria set by Chander et al. [45]. Then, all compounds showed a TPA value of less than 140 A2, thus fulfilling the criteria in LRO5 [46], even though the compound A3 showed a value that was close to the threshold. The number of hydrogen donor and acceptor groups of all compounds meets the LRO5 criteria, with no more than 5 and 10 groups, respectively. All compounds did not meet the criteria for Log P based on LRO5 [46], especially compound A1, whose Log P values were huge (>7.5). The high Log P value can affect the absorption and distribution of the compound, so it is necessary to consider ways to handle it [47]. Only compound A3 showed
| Ligand   | Kcal/mol | Interaction | H-bond     | Hydrophobic  | Electrostatic |
|----------|----------|-------------|------------|--------------|---------------|
| A2       | −8.3     | H-bond      | ASN238, LEU272, LEU287 | LEU272, LEU286 | TYR237, LEU287 |
| A1       | −8.2     | H-bond      | ASN238, LEU272, LEU287 | LEU272, LEU286 | ASP197, LEU287 |
| A3       | −7.5     | H-bond      | THR199, ASN238, LEU271 | LEU286, LEU287 | LYS137 |
| Lopinavir| −7.8     |             |             |              |               |
Fig. 1 Three-dimensional and two-dimensional diagrams illustrating the various interaction modes of (A) A1, (B) A2, and (C) A3 within active site of SARS-CoV-2 (6LU7)
optimal Log $S$ values, although all of them had good CaCO$_2$. The compound A3 again is the only one that shows a relatively low Log $BB$ value outside the optimal range, and also shows the highest amount of Rot, even though it is still in the optimal range.

Finally, all compounds are predicted to have moderate toxicity (harmful if swallowed) with an LD$_{50}$ of 1000 mg/kg and are in class IV of the globally harmonized system of classification of labeling of chemicals [48]. However, compound A3 was predicted to be a mutagen with a positive Ames test result. Further analysis of the target toxicity of each compound with ProTox-II is presented in Fig. 2. Among synthesized compounds, only A3 showed the probability of toxicity to several targets such as carcinogenicity (carcino) and mutagenicity (mutagen), although with a low probability (<0.7) [48]. Overall, compound A1 shows absorption and distribution deficiencies, while A3 compounds tend to be more toxic. Thus, compound A2 has the most ideal ADMET properties compared to the two.

### Table 2 ADMET properties of synthesized compounds

| Compounds | A1        | A2        | A3        |
|-----------|-----------|-----------|-----------|
| Molecular weight (g/mol) | 528.077   | 562.522   | 573.074   |
| Topological polar surface area (Å$^2$) | 92.17     | 92.17     | 137.99    |
| Hydrogen bond donor | 1         | 1         | 1         |
| Hydrogen bond acceptor | 8         | 8         | 9         |
| Aqueous solubility(log [mol/L]) | −6.613    | −6.621    | −5.956    |
| Predicted apparent Caco-2 cell permeability (10$^{-6}$ cm/s) | 1.021     | 1.008     | 0.604     |
| Acute toxicity | IV        | IV        | IV        |
| Predicted LD$_{50}$ (mg/kg) | 1000      | 1000      | 1000      |
| Ames | NO        | NO        | YES       |

![Fig. 2 Prediction of toxicity parameters with ProTox-II](image)
DFT studies

The total SCF energy of the synthesized compounds A1, A2, and A3 after 8, 16, and 40 optimization steps was obtained to be $-2791.37$, $-2331.77$, and $-2387.12$ a.u., respectively. The stabilization energy for A1, A2, and A3 was calculated to be $-288.81 \times 10^3$, $-407.88$, and $-351.40 \times 10^2$ kcal/mol, respectively, which endorsed the stability of the compounds. The optimization of all compounds (A1, A2, and A3) was carried out through the B3LYP/6-311G++ calculations. Figure 3 shows the optimized structures of A1, A2, and A3 having ground state minimum energy, strain-free lattice constants, and atomic coordinates. From the optimized structures, the bond lengths were also obtained, which are represented in Fig. 4 and Table 3. The obtained Mulliken charges of the titled compounds were compared, which showed the shift in Mulliken charges, indicating the compound formation has different structural values. Figure 3 and Table 4 represent the Mulliken atomic numbers scheme and atomic charges, respectively.

The molecular electrostatic potential (MEP) map (Fig. 5) represents the electrostatic potentials strength of reactants and synthesized moieties. The red color represents the electronegative area, and the blue color is for the electropositive.
region. The interaction between the reactants can be studied by MEP, as the electron-deficient region lies at C and H of reactant and electron-rich at O atom. These results show the preferential binding sites over the molecule for the electrophilic and nucleophilic attacks [49, 50]. MEP map of the designed compounds is plotted from deep red to deep blue color scale as $-5.159 \text{ e}^{-2}$ to $+5.159 \text{ e}^{-2}$, $-4.970 \text{ e}^{-2}$ to $+4.970 \text{ e}^{-2}$, and $-5.148 \text{ e}^{-2}$ to $+5.148 \text{ e}^{-2}$ for $A_1$, $A_2$, and $A_3$, respectively, as shown in Fig. 5. B3LYP/6-311G++ basis set was also used to investigate the IR spectrum theoretically. The comparison of experimental FTIR spectra of the synthesized compounds with the simulated spectra for $A_1$, $A_2$, and $A_3$ scaled at 0.9785, 0.9200, and 0.9190, respectively, are shown in Figs. 6, 7 and 8. The computed IR spectrum in animated modes was found to be compatible with few significant experimental vibrational signals in the FTIR spectra of the compounds [51]. The slight difference between theoretical and experimental frequencies is due to theoretical values being acquired in gas phase and experimental values being obtained in solid phase and also due to neglecting the incompleteness and anharmonicity of the basis set. Therefore, to reduce the differences, the scaling factor is used. The nature of the electronic transitions has been explored in the gas phase through TD-DFT method as shown in Fig. 9. Two absorption bands at 375 and 450 nm can be seen through TD-DFT calculations for $A_1$. The simulated absorption bands at 450 and 375 nm are assigned to HOMO to LUMO and HOMO to LUMO + 1. Similar results were obtained for $A_2$ and $A_3$ from TD-DFT calculations.

![Diagram](image-url)
Table 3: The bond lengths of the synthesized compounds obtained through DFT

| S. no. | Atoms/bond length (Å) | A1   | A2   | A3   |
|--------|-----------------------|------|------|------|
| 1      | R(1–2)                | 1.396| 1.396| 1.398|
| 2      | R(1–6)                | 1.401| 1.401| 1.404|
| 3      | R(1–7)                | 1.082| 1.082| 1.086|
| 4      | R(2–3)                | 1.39  | 1.390| 1.394|
| 5      | R(2–8)                | 1.079| 1.079| 1.083|
| 6      | R(3–4)                | 1.39  | 1.390| 1.393|
| 7      | R(3–11)               | 1.828 | 1.829| 1.826|
| 8      | R(4–5)                | 1.396 | 1.396| 1.399|
| 9      | R(4–9)                | 1.079 | 1.079| 1.083|
| 10     | R(5–6)                | 1.401 | 1.401| 1.403|
| 11     | R(5–10)               | 1.082 | 1.082| 1.086|
| 12     | R(6–12)               | 1.501 | 1.501| 1.502|
| 13     | R(12–13)              | 1.474 | 1.474| 1.472|
| 14     | R(12–62)              | 1.092 | 1.092| 1.096|
| 15     | R(12–63)              | 1.092 | 1.092| 1.097|
| 16     | R(13–14)              | 1.386 | 1.387| 1.385|
| 17     | R(14–15)              | 1.405 | 1.405| 1.407|
| 18     | R(14–16)              | 1.403 | 1.403| 1.406|
| 19     | R(15–17)              | 1.389 | 1.389| 1.391|
| 20     | R(15–18)              | 1.079 | 1.079| 1.083|
| 21     | R(16–19)              | 1.384 | 1.384| 1.386|
| 22     | R(16–20)              | 1.08  | 1.080| 1.083|
| 23     | R(17–21)              | 1.417 | 1.417| 1.419|
| 24     | R(17–22)              | 1.082 | 1.082| 1.086|
| 25     | R(19–21)              | 1.421 | 1.421| 1.423|
| 26     | R(19–23)              | 1.08  | 1.080| 1.084|
| 27     | R(21–28)              | 1.432 | 1.431| 1.431|
| 28     | R(24–25)              | 1.525 | 1.525| 1.524|
| 29     | R(24–26)              | 1.099 | 1.099| 1.103|
| 30     | R(24–28)              | 1.522 | 1.522| 1.522|
| 31     | R(24–31)              | 1.105 | 1.105| 1.112|
| 32     | R(25–30)              | 1.421 | 1.421| 1.417|
| 33     | R(25–37)              | 1.426 | 1.427| 1.426|
| 34     | R(27–29)              | 1.017 | 1.016| 1.020|
| 35     | R(28–29)              | 1.338 | 1.339| 1.342|
| 36     | R(29–30)              | 1.391 | 1.390| 1.390|
| 37     | R(30–32)              | 1.384 | 1.384| 1.385|
| 38     | R(32–33)              | 1.745 | 1.745| 1.749|
| 39     | R(32–34)              | 1.347 | 1.347| 1.349|
| 40     | R(34–35)              | 1.008 | 1.008| 1.011|
| 41     | R(34–36)              | 1.003 | 1.003| 1.007|
| 42     | R(37–38)              | 1.422 | 1.422| 1.423|
The $^1\text{H}$-NMR spectra for all the compounds were also obtained through GIAO calculation, as shown in Figs. 10, 11 and 12 [51]. Frontier molecular orbitals (FMOs) are significant factors for explaining chemical behavior; hence, they were also computed theoretically. LUMO are generally electron acceptors, while HOMO are electron donors. The chemical stability of molecules is related to the energy gap between the HOMO and LUMO. According to the literature, compounds with a small energy gap have low kinetic stability, more chemical reactivity, and are soft in nature, whereas compounds with a large energy gap have high kinetic stability, less chemical reactivity, and are hard in nature [52]. Figure 13 represents the spatial arrangements of HOMO and LUMO along with their gap and associated energies. The HOMO–LUMO gap ($\Delta E$) for compounds A1, A2, and A3 were calculated to be 3.216, 3.156, and 2.622 eV, respectively [53]. On the basis of $\Delta E$, the chemical reactivity of the compounds is arranged in the following sequence—A1 > A2 > A3.
Table 4  Mulliken atomic charges on the atoms of the optimized structure of the titled compounds

| Mulliken atomic numbers/ Mulliken atomic charges | A1 | A2 | A3 |
|------------------------------------------------|----|----|----|
| 1 C −0.09663 1 C −0.09799 1 C −0.0889 |    |    |    |
| 2 C −0.05658 2 C −0.05673 2 C −0.05779 |    |    |    |
| 3 C −0.28835 3 C −0.28837 3 C −0.28679 |    |    |    |
| 4 C −0.05667 4 C −0.05665 4 C −0.05844 |    |    |    |
| 5 C −0.09767 5 C −0.09638 5 C −0.10434 |    |    |    |
| 6 C 0.0289 6 C 0.02878 6 C 0.02876 |    |    |    |
| 7 H 0.16969 H 0.16965 H 0.16993 |    |    |    |
| 8 H 0.19152 8 H 0.19128 8 H 0.19084 |    |    |    |
| 9 H 0.1915 9 H 0.19122 9 H 0.19036 |    |    |    |
| 10 H 0.1695 10 H 0.16945 10 H 0.16975 |    |    |    |
| 11 Cl −0.01301 11 Cl −0.01384 11 Cl −0.01308 |    |    |    |
| 12 C −0.24677 12 C −0.24594 12 C −0.24858 |    |    |    |
| 13 O −0.5044 13 O −0.5051 13 O −0.50338 |    |    |    |
| 14 C 0.2788 14 C 0.27778 14 C 0.27679 |    |    |    |
| 15 C −0.19588 15 C −0.19584 15 C −0.19768 |    |    |    |
| 16 C −0.19079 16 C −0.19078 16 C −0.18968 |    |    |    |
| 17 C −0.05001 17 C −0.05052 17 C −0.04908 |    |    |    |
| 18 H 0.18117 18 H 0.18037 18 H 0.18005 |    |    |    |
| 19 C −0.08283 19 C −0.08414 19 C −0.08639 |    |    |    |
| 20 H 0.18216 20 H 0.18129 20 H 0.18032 |    |    |    |
| 21 C −0.04972 21 C −0.04866 21 C −0.04486 |    |    |    |
| 22 H 0.16939 22 H 0.16865 22 H 0.1684 |    |    |    |
| 23 H 0.18185 23 H 0.18177 23 H 0.18121 |    |    |    |
| 24 C −0.63412 24 C −0.63298 24 C −0.61926 |    |    |    |
| 25 C 0.31144 25 C 0.31268 25 C 0.32061 |    |    |    |
| 26 H 0.21514 26 H 0.21506 26 H 0.21667 |    |    |    |
| 27 H 0.39089 27 H 0.39072 27 H 0.39142 |    |    |    |
| 28 C 0.36127 28 C 0.3591 28 C 0.34802 |    |    |    |
| 29 N −0.469401 29 N −0.49453 29 N −0.4963 |    |    |    |
| 30 N −0.4569 30 N −0.45762 30 N −0.46603 |    |    |    |
| 31 H 0.20027 31 H 0.1993 31 H 0.19416 |    |    |    |
| 32 C 0.19441 32 C 0.19545 32 C 0.19612 |    |    |    |
| 33 S −0.15294 33 S −0.1548 33 S −0.15819 |    |    |    |
| 34 N −0.72191 34 N −0.72185 34 N −0.72204 |    |    |    |
| 35 H 0.37171 35 H 0.37201 35 H 0.37329 |    |    |    |
| 36 H 0.37122 36 H 0.3708 36 H 0.37094 |    |    |    |
| 37 C −0.04855 37 C −0.05053 37 C −0.04191 |    |    |    |
| 38 C −0.19693 38 C −0.1974 38 C −0.20431 |    |    |    |
| 39 C −0.15232 39 C −0.1517 39 C −0.16724 |    |    |    |
| 40 C 0.24021 40 C 0.24059 40 C 0.24655 |    |    |    |
| 41 H 0.1626 41 H 0.16281 41 H 0.16176 |    |    |    |
Certain molecular properties in the gas phase related to chemical reactivity for the compounds are shown in Table 5 based on the optimized structure and HOMO–LUMO.

**Conclusion**

We have successfully synthesized three new pyrazoline analogues and evaluated their in silico inhibitory activity against SARS-CoV-2 M\(^{\text{pro}}\). The results exhibited that all of them have potential inhibitory. Compound A\(_2\) \((-8.3\ \text{kcal/mol})\) was found to be the most potent SARS-CoV-2 M\(^{\text{pro}}\) inhibitor among the docked compounds. Furthermore, compound A\(_2\) also showed more ideal ADMET properties compared to the other designed compounds. The optimized structures alone with the molecular properties were obtained for the synthesized compounds through theoretical DFT calculations, which supports the experimental
One-pot synthesis, molecular docking, ADMET, and DFT studies…

Fig. 5 MEP surface map of (a), reactant-a (b), reactant-b (c) A1, (d) A2, and (e) A3 with respective color scales

Fig. 6 FTIR spectra of A1, experimental (green) and computational (black)
Fig. 7 FTIR spectra of A2, experimental (green) and computational (black)

Fig. 8 FTIR spectra of A3, experimental (green) and computational (black)
Fig. 9 UV-visible spectra of (a) A1, (b) A2, and (c) A3 obtained through TD-DFT calculations

Fig. 10. $^1$H NMR spectrum for A1 calculated through DFT (GIAO) calculations
data. Various other theoretical molecular parameters calculated through DFT and in vitro evaluation against SARS-CoV-2 MPro will help researchers for future investigations.

Fig. 11. $^1$H NMR spectrum of A2 calculated through DFT (GIAO) calculations

Fig. 12. $^1$H NMR spectrum of A3 calculated through DFT (GIAO) calculations
Fig. 13  Spatial plot of HOMO and LUMO with their energy gap

| Parameters                                      | RB3LYP/6-311G++ | A1                | A2                | A3                |
|------------------------------------------------|-----------------|-------------------|-------------------|-------------------|
| Minimum SCF energy (a.u.)                      |                 | −2791.25089341    | −2331.79962255    | −2386.73822668    |
| Stabilization energy (kcal/mol)                |                 | −288.81 × 10³     | −407.88           | −351.40 × 10²     |
| Polarizability (α) (a.u.)                      |                 | 559.311624        | 546.570683        | 535.285008        |
| Dipole moment (debye)                          |                 | 3.907789          | 2.630545          | 3.317812          |
| Zero point vibrational energy (kcal/mol)       |                 | 305.38410         | 311.69885         | 324.56856         |
| Total thermal energy (kcal/mol)                |                 | 326.740           | 332.206           | 345.938           |
| Electronic spatial extent (a.u.)               |                 | 62,027.6922       | 46,602.7123       | 49,737.2080       |
| Frontier MO energies (eV)                      |                 | −1.3007           | −1.2687           | −1.2632           |
| LUMO + 1                                       |                 | −2.8166           | −2.7478           | −2.7168           |
| HOMO                                           |                 | −6.0333           | −5.9041           | −5.3389           |
| Gap (LUMO–HOMO)                                |                 | 3.2167            | 3.1563            | 2.6221            |
Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s11164-022-04831-5.

Acknowledgements  The authors wish to thank Universiti Teknologi Malaysia and the Ministry of Higher Education (MOHE) Malaysia for funding this research under the Fundamental Research Grant Scheme (FRGS/1/2019/STG01/UTM/02/7).

Declarations

Conflict of interest  The authors have no conflict of interest to declare.

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