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Computational Chemistry for some Novel Pyrimidine derivatives as Significant Antioxidants using cytochrome c peroxidase enzyme

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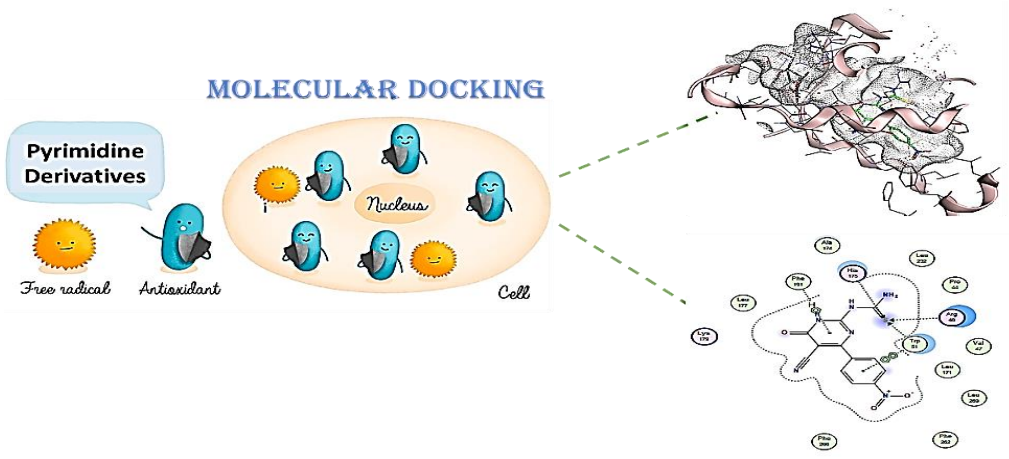
ABASTRACT : Reactive oxygen species (ROS) are common byproducts of cellular metabolism in plants. In humans, mitochondrial energy metabolism produces reactive oxygen species (ROS), mostly oxygen ions, as well as peroxides (both inorganic and organic). Some of these radicals are required for regular cellular activities, such as the transmission of neurological impulses. Under normal physiological conditions, most compounds develop slowly and are eliminated by intracellular antioxidant systems such as superoxide dismutase (SOD) and small molecules like vitamins C and E. However, high ROS levels demand the use of highly potent antioxidants to avoid biological injury. The current work provides an extremely simple and successful strategy to synthesize pyrimidine derivatives. Spectral and elemental analysis validated the structures of all produced substances. The computational chemistry program MOE (2022) was used to investigate molecular docking for antioxidant activity, using reference substances produced by the cytochrome c peroxidase enzyme in the database's molecular docking study to investigate the proposed mode of action (PDB code : 2X08, resolution: 2.01).

KEYWORDS : Pyrimidine, antioxidant; Molecular docking. Cytochrome c peroxidase

GRAPHICAL ABSTRACT



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I. INTRODICUCTION

Reactive oxygen species (ROS) are common byproducts of cellular metabolism in plants. In humans, mitochondrial energy metabolism produces ROS, mostly oxygen ions, in addition to peroxides (both inorganic and organic). Some of these radicals are required for regular cellular activities, such as the transmission of neural impulses **(Dayem, Choi, Kim, & Cho, 2010)**.

Moreover, excessive ROX production endangers the human body **(Chong, Li, & Maiese, 2005)**. Sufficient (ROS) and (RNS) can damage all biological macromolecules. ROS can destroy human cells by damaging the lipid in their membranes, causing permeability changes and DNA breakage **(Wang, Shum, Ho, & Wang, 2003)**. Oxidative stress causes a variety of health problems in humans, including diabetes, Alzheimer's **(Chong et al., 2005)**, neurodegenerative diseases, inflammatory sickness, ischemia-reperfusion damage, and aging **(Maiese, Daniela Morhan, & Zhong Chong, 2007)**.

Under typical physiological circumstances, most compounds develop slowly and are eliminated by intracellular antioxidant systems such as superoxide dismutase (SOD) and small molecules like vitamins C and E. **(Maiese et al., 2007)**. Nevertheless, high ROS levels demand the use of highly potent antioxidants to avoid biological injury.

Many fruits and vegetables contain ascorbic acid (vitamin C), which is necessary for the nutrition of humans. Ascorbic acid, a "acidic carbohydrate," was found in the adrenal glands, lemons, cabbages, and oranges by Szent-Györgyi in 1928 **(Carpenter, 2012)**. Its chemistry and biology were first identified during the 1990s **(Gassmann, 1992)**. One of ascorbic acid's most important functions in the body is as an antioxidant. It is quite efficient in reducing ROS damage to body cells **(Saffi, Sonego, Varela, & Salvador, 2006)**. It is extremely efficient in removing peroxides and converting them to water **(Zámocký, Furtmüller, & Obinger, 2010)**. Ascorbate has a number of direct antioxidant activities in vitro that are all quite effective **(Elsayed, 2023; Halliwell & Gutteridge, 2015).**

We also evaluated the manufactured drugs using molecular docking, which can predict the most frequent binding mode(s) of a receptor to a protein in a three-dimensional geometry **(M. A. Aziz, W. S. Shehab, A. A. Al-Karmalawy, A. F. EL-Farargy, & M. H. Abdellattif, 2021; Kukol, 2008; Morris & Lim-Wilby, 2008)**. In this study, a number of Pyrimidine molecules were synthesized utilizing novel techniques. The compounds were found utilizing IR, ^{1}H-NMR and ^{13}C-NMR. The experimental data were obtained by computational investigations (molecular docking) using MOE (2022).

II. EXPERIMENTAL

Materials and methods

High-quality materials were used to accomplish this job. Sigma-Aldrich supplied all of the chemicals (Taufkirchen, Germany). El-Nasr Pharmaceutical Chemicals Company provided all solvents (analytical reagent grade, Egypt). The melting points were measured using a Cole-Parmer digital Electrothermal IA 9100 Series (Beacon Road, Stone, Staffordshire, ST15 OSA, UK) and are uncorrected. C, H, and N analyses were performed using a PerkinElmer CHN 2400. The FT-IR 460 PLUS was used to generate IR spectra (KBr disks). A Bruker 400, 100 MHz NMR Spectrometer was used to generate ^{1}H and ^{13}C-NMR spectra, with DMSO-d_6 as the solvent and chemical shifts expressed in (ppm) at the Main Laboratories of Chemical War, Nasr City, Egypt. The reactions were monitored using thin-layer chromatography (TLC) sheets covered with UV fluorescent silica gel Merck 60 F254 plates and a UV laser, with different solvents serving as mobile phases. MOE software (2022) was used to conduct molecular docking investigations.

synthesis

1-(5-Cyano-4-(4-nitrophenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thiourea (2):

To a solution of 6-(4-Nitrophenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **1** (0.55 g ,2mmol) and thiourea (0.15g, 2mmol) in absolute ethanol (20 ml) was heated under reflux for 5 h in the precence of 3- drops of glacial acetic acid. The reaction mixture was concentrated and the separated solid was filtered off, after cooling and recrystallized by ethanol to give compound **1** as yellow crystals, yield 45% mp. 260-262 °C. IR (KBr) spectrum, ν, cm^{-1}: 3517, 3459 and 3399 (2NH, NH_2), 2220 (C≡N), 1694 (C=O). ^{1}H NMR (DMSO-*d*6), δ (ppm): 8.24-8.26 (d, 2H, *J*=8 Hz, 2 Ar-H), 8.40-8.42 (d, 2H, *J*=8.4, 2Ar-H), 8.56 (s, 2H, NH_2, D_2O-exchangeable), 11.52 (s, 2H, 2NH, D_2O-exchangeable). Anal. Calcd for $C_{12}H_8N_6O_3S$ (316.3); C, 45.57; H, 2.55; N, 26.57. Found; C, 45.64; H, 2.51; N, 26.55.

2-(4-nitrophenyl)-4,6-dioxo-6,11-dihydro-4*H*-pyrimido[2,1-*b*]quinazoline-3-carbonitrile (3):

To a solution of 6-(4-Nitrophenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **1** (0.27 g ,0.001 mole) and anthranilic acid (0.14 g ,0.001 mole) in 2% ethanolic sodium ethoxide (20 ml) was refluxed for

10 h. The reaction mixture was cooled then poured into ice –cold water and acidified with diluted hydrochloric acid. The resulting precipitate was filtered off, washed several times with water, dried, and recrystallized from ethanol to give brown powder compound **3**. Yield 55%. m.p: 220-222℃. IR (KBr) spectrum, ν, cm^{-1}: 3453, (NH), 2224 (C≡N), 1686, 1657 (2C=O). ^{1}H NMR (DMSO-*d6*), δ (ppm): 6.50-8.40 (m, 8H, Ar-H), 6.75 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (DMSO-*d6*), δ (ppm): 88.21, 110.06, 113.22, 115.03, 116.80, 123.30, 125.39, 130.93, 131.69, 134.71, 146.94, 151.38, 166.28 and 167.10. Anal. Calcd for $C_{18}H_9N_5O_4$ (359.3); C, 60.17; H, 2.52; N, 19.49. Found C, 60.20; H, 2.54; N, 19.45.

6-(4-nitrophenyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (4):

A mixture of 6-(4-Nitrophenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **1** (1g ,0.004 mole), ammonia solution (20 mL) and hydrogen peroxide (1.35 mL) were stirred at room temperature for 5 h. The reaction mixture was left over night ;the formed precipitate was filtered, and crystallized from ethanol to yield a white precipitate of compound **4**. Yield 80 %, m,p 218 -220℃. IR (KBr) spectrum, ν, cm^{-1}: 3298, 3158 (2NH), 2221 (C≡N), 1696,1675 (2C=O). ^{1}H NMR (DMSO-*d6*), δ (ppm): 8.21-8.23 (d, 2H, *J*=8 Hz, 2 Ar-H), 7.79-7.81 (d, 2H, *J*=8.4, 2Ar-H), 10.16 (s, 1H, NH, D_2O-exchangeable), 11.50 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (DMSO-*d6*), δ (ppm): 61.41, 64.84, 115.21, 123.95, 128.81, 141,25, 147.44, 165.34 and 167.31. Anal. Calcd for $C_{11}H_6N_4O_4$ (258.2); C, 51.17; H, 2.34; N, 21.70. Found; C, 51.20; H, 2.38; N, 21.74.

Computational Chemistry

Docking study:

Chemdraw 12.0 was utilized to develop computational techniques for the most bioactive compounds, which would be docked using Molecular Operating Environment software (2022). The London DG force and force field energy were utilized to analyze the results. All minimizations were conducted using MMFF 94 (Merck molecular force field 94) until a (RMSD) gradient of 0.1 kcalmol $^{-1}$ A $^{-1}$ was achieved. **(Abo Elmaaty et al., 2021; Alesawy et al., 2021; Hamed, Elsayed, Assy, & Shehab, 2022)**, Partial charges were approximated automatically. The dock function (S, Kcal/mol) of the MOE software was used to determine the ligand's binding capacity.

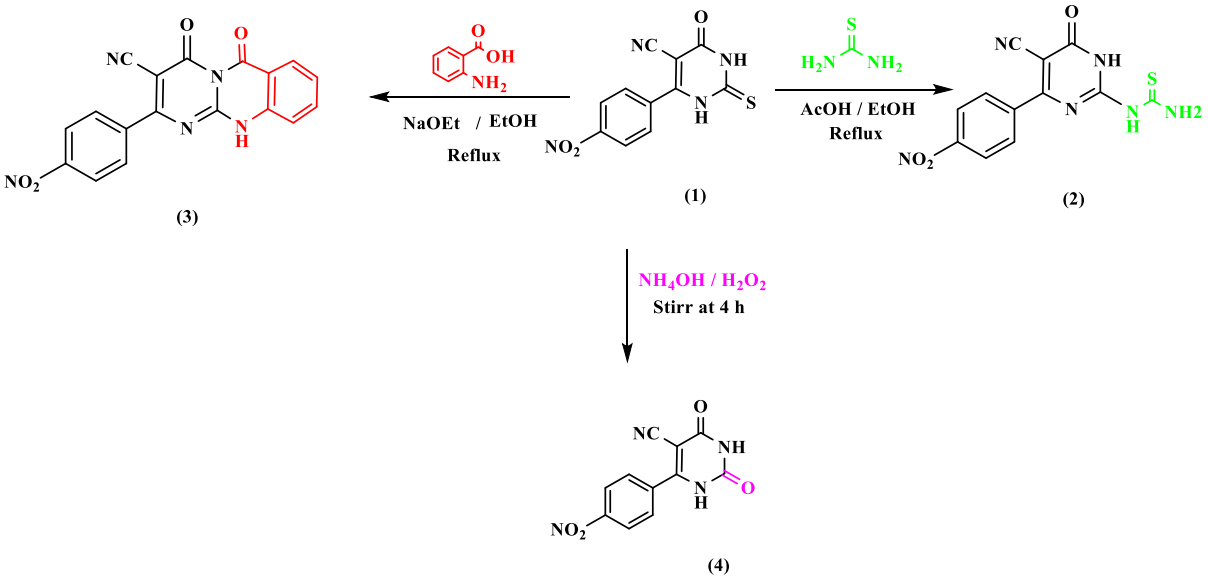
The enzyme's X-ray crystal structure in PDB format was obtained from the protein data bank (PDB ID: 2X08, resolution: 2.01; **(https://www.rcsb.org/structure/2X08)**. The enzyme was designed for docking studies by removing water, adding all hydrogen bonds, fixing potential, and creating artificial atoms from the resulting alpha spheres. **(Labute & Bioinformatics, 2009)**, The ligand's interaction with the amino acids in the active site is subsequently analyzed. The highest negative value for the active ligand leads to the best Docking Score **(M. A. Aziz, W. S. Shehab, A. A. Al-Karmalawy, A. F. El-Farargy, & M. H. J. I. j. o. m. s. Abdellattif, 2021; Hamed et al., 2022; Shehab et al., 2024; Singh et al., 2015)**.

III. Results and Discussion

Chemistry

It was interesting to note that 2-thiopyrimidines-5-carbonitrile **1** was synthesized using a one-pot three-component reaction of 4-nitrobenzaldehyde with thiourea, ethyl cyanoacetate in ethanol under reflux conditions in presence of anhydrous potassium carbonate **(Singh et al., 2015)**. When thiourea was treated with pyrimidinethione **1** in refluxing ethanol/acetic acid, the pyrimidine derivative **2** obtained. The structure **2** was elucidated using infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR). The IR spectra exhibited an absorption band at 1694 cm^{-1} corresponding to the C=O group, an absorption band at 2220 cm^{-1} associated with the CN group and an absorption band across 3517, 3459 and 3399 cm^{-1} attributed to the 2 NH, NH_2 groups. The ^{1}H NMR spectra exhibited two doublet signals at 8.24-8.26 and 8.40-8.42 which were attributed to the presence of two aromatic hydrogen atoms. The spectra also indicated the presence of two singlet signals at 8.56 for NH_2 and 11.52 for 2 NH groups. When anthranilic acid was treated with pyrimidine thione 1 in ethanolic sodium ethoxide yielded pyrimido[2,1-b]quinazoline derivatives **3**. The structure **3** was elucidated using infrared (IR), proton, and carbon-13 nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR). The IR spectra exhibited an absorption band at 1686, 1657 cm^{-1} corresponding to 2 C=O group, an absorption band at 2224 cm^{-1} associated with the CN group and an absorption band across the 3453 cm^{-1} attributed to the 2 NH groups. The ^{1}H NMR spectra exhibited singlet signals at 6.75, which can be attributed to the existence of NH groups. In addition, the ^{13}C NMR spectrum showed the presence of SP and SP^2 carbons, which can be attributed to the C≡N, 2 C=O

functional groups at chemical shifts of 115.03, 166.28 and 167.10 ppm, respectively. Alkaline hydrolysis of tetrahydropyrimidine **1** using hydrogen peroxide in ammonium hydroxide gave dioxo tetrahydropyrimidine derivative **4**. Thus, IR spectrum of **4** showed two carbonyl groups signals at υ = 1696 and 1675.cm^{-1}, an absorption band across the 3298, 3158 cm^{-1} attributed to the 2 NH groups. The ^{1}H NMR spectra exhibited two singlet signals at 10.16 and 11.50, which can be attributed to the existence of 2 NH groups.



SCHEME (1) The synthesis of novel pyrimidine derivatives.

Computational Chemistry

Molecular docking study:

To test the predicted mode of action, the newly created and synthesized therapeutic targets were compared to ascorbic acid, a reference substance generated by the cytochrome c peroxidase enzyme, using the database's molecular docking research (PDB code: 2X08). The purpose of this study was to learn more about how the chemicals designed to connect to the protein-binding site of the cytochrome c peroxidase enzyme worked. To validate the results of the present docking experiment at the active site, the co-crystallized ligand ascorbic acid was docked again with the same number of factors. The root mean square deviation (RMSD) and energy score for the best-docked location were 0.892 and -5.32 Kcal/mol, respectively, confirming the docking research conducted with MOE software. Ascorbic acid created four hydrogen connections with His181, Leu177, (two hydrogen bond) and Lys179 **Figure 1**.

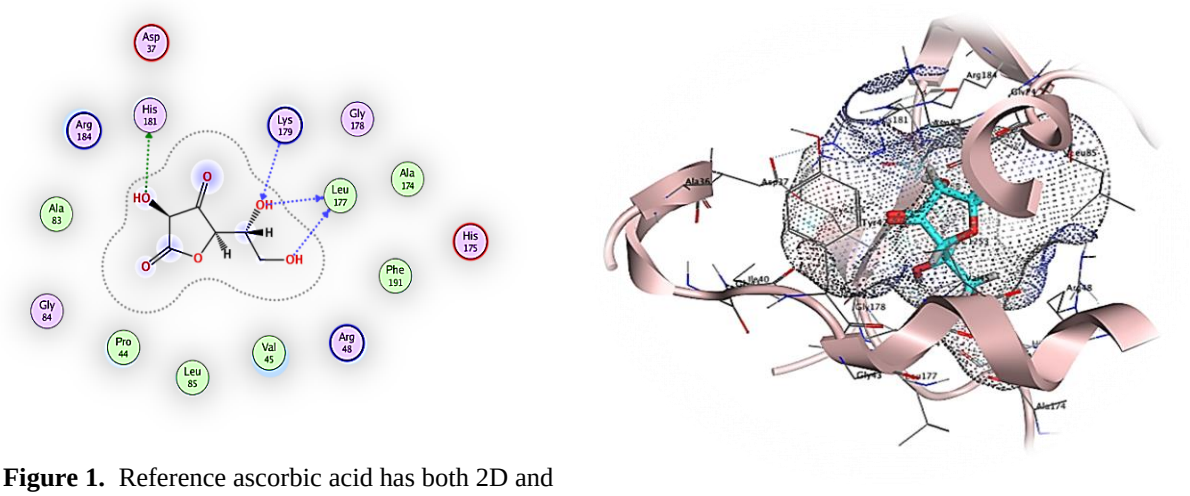


Figure 1. Reference ascorbic acid has both 2D and 3D receptor interactions.

Compounds **2** suggested binding mode has energy score of -7.809 kcal/mol. Compound **3** has an energy value of -8.406 kcal/mol while Compound **4** showed score -6.686 kcal/mol, as shown in **Table (1) (Figure2)**. The score of these compounds sure that this compound is very stabling the protein-binding site of the cytochrome c peroxidase enzyme.

Table 1. The binding scores, RMSD values, distance, and receptor contacts of the top three potential compounds (1,2 and 3) were compared to the docked reference ascorbic acid for antioxidant activity.

Comp.	Score (Kcal/mol)	RMSD	Interacting residues		
			Receptor interactions	Distance (Å)	E (Kcal/mol)
Ascorbic acid	-5.324	0.892	His181/H-donor	3.15	-3.10
			Leu177/H-donor	3.13	-.70
			Leu177/H-donor	3.04	-1.70
			Lys179 H-acceptor	3.15	0.3
			ARG 48/ H-acceptor	3.85	-0.5
			ARG 48/ H-acceptor	3.40	-1.0
2	-7.809	0.580	TRP 51/ H-acceptor	4.07	-2.4
			HIS 175/ H-acceptor	3.35	-3.0
			PHE 191/ pi-H	4.42	-0.5
			TRP 51/ pi-pi	3.77	-0.0
			PHE 191/ pi-H	4.52	-0.7
3	-8.4067	0.497	PHE 191/ pi-H	4.09	-0.6
			TRP 51/ pi-pi	3.83	-0.0
4	-6.686	1.447	TRP 51/ pi-pi	3.79	-0.0

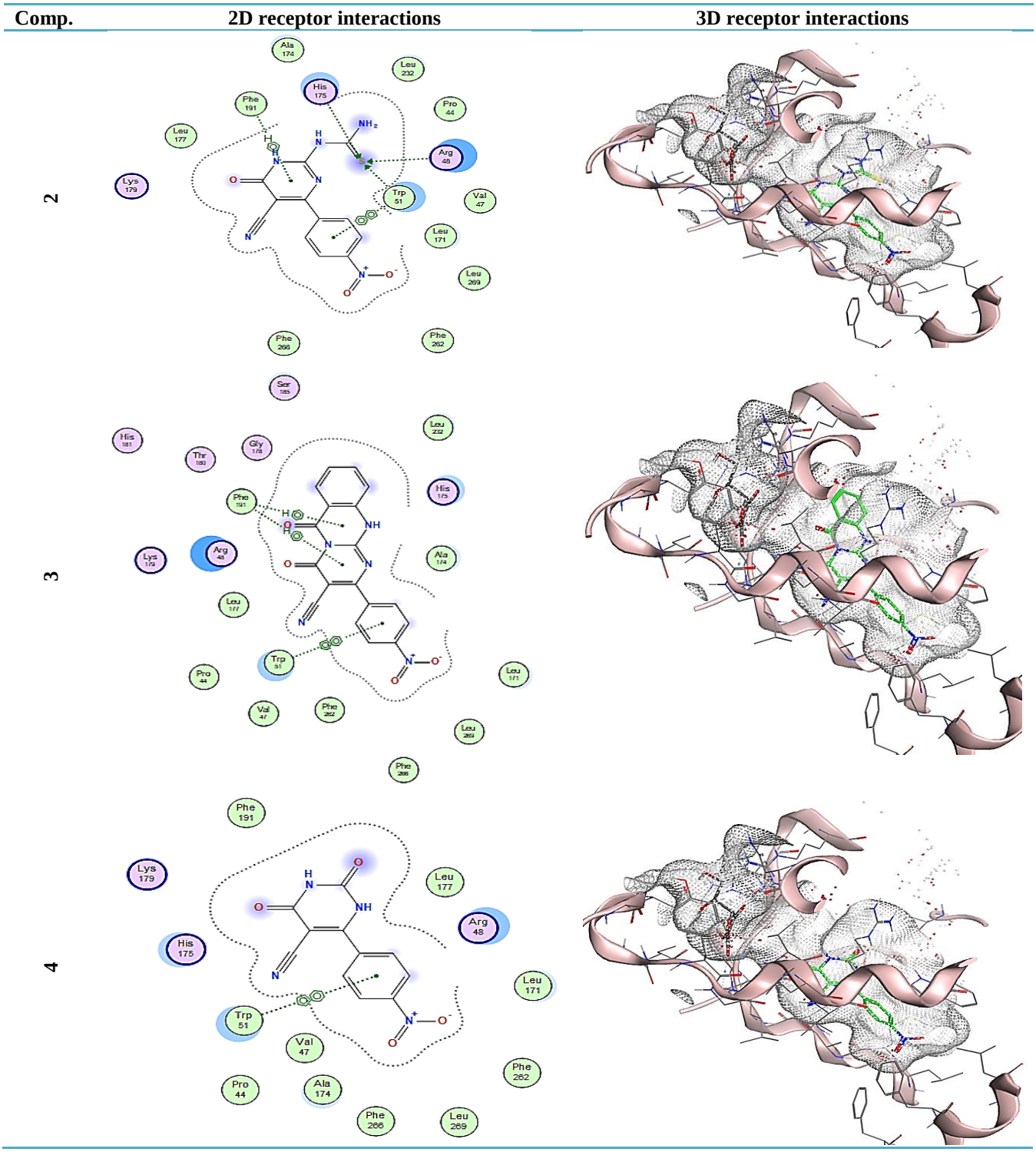


Figure 2. 2D receptor interactions and 3D receptor interactions of synthesized compounds.

VI. CONCLUSION

The current work offers a relatively simple and successful method for synthesizing pyrimidine derivatives. The compounds were identified by IR, ^{1}H-NMR, and ^{13}C-NMR. Finally, the authors developed a preliminary molecular docking investigation for the produced compounds 1,2 and 3 with the cytochrome c peroxidase enzyme (PDB ID: 2X08; resolution: 2.01).

V. REFERENCE

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