

Original article

Design, Synthesis, and In Silico Assessment of Phthalazine Derivatives using DFT, Molecular Docking, and ADMET Analysis

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Abstract

A series of phthalazine derivatives was synthesised, starting from 2-(1-oxo-4-phenylphthalazin-2(1H)-yl)acetohydrazide (1), which was efficiently converted into the oxadiazole phthalazine derivative (2) in excellent yield through a reaction with carbon disulfide under reflux. Subsequently, compound (2) served as a key intermediate to prepare a diverse range of new phthalazine derivatives (3–6) by reacting it with various alkyl halides and ethyl chloroacetate. All synthesised compounds were characterised using IR, NMR, and mass spectrometry. In addition, the molecular geometries of compounds (3–6) were optimised using density functional theory (DFT). For the in silico evaluation, molecular docking studies were conducted against two bacterial target proteins (PDB IDs: 6TLS and 4OD9) for the synthesised derivatives. All ligands exhibited significant binding affinity values, ranging from -6.24 to -7.92 kcal/mol. Finally, drug-likeness and ADME properties were predicted using SwissADME. These findings highlight the newly synthesised phthalazine derivatives as promising antibacterial candidates for further biological investigation.

Keywords. 1,3,4-Oxadiazol; Synthesis; Phthalazine; Molecular docking; Characterization; ADMET.

Introduction

Nitrogen-based heterocyclic frameworks are a cornerstone of medicinal chemistry, owing to their profound pharmacological utility [1, 2]. The incorporation of adjacent nitrogen atoms within these cyclic systems facilitates distinct physicochemical properties, which in turn promote high-affinity interactions with diverse biological substrates [3]. Consequently, N-heterocycles have become indispensable scaffolds in drug discovery, particularly in the development of antimicrobial, antiviral, and antineoplastic agents [3, 4]. Their inherent structural plasticity and chemical tunability make them premier candidates for identifying innovative therapeutic leads [5, 6]. Among these, phthalazine and its derivatives constitute a preeminent class of heterocycles that have garnered substantial interest. Structurally, phthalazine is defined by a bicyclic system where a benzene ring is ortho-fused to a pyridazine moiety [7, 8]. This configuration yields a rigid and stable architecture that serves as an ideal template for molecular modifications aimed at enhancing bioactivity [9]. The fusion of these aromatic systems not only ensures molecular stability but also provides a versatile platform for multi-target receptor binding [10]. Such structural advantages have positioned phthalazine derivatives as a focal point in contemporary pharmaceutical research [11-15].

The therapeutic landscape of phthalazine derivatives is remarkably broad. Systematic investigations have demonstrated their efficacy as anticancer [16, 17], antioxidant [18], and antimicrobial agents [19]. Furthermore, they have shown significant promise as anti-inflammatory [20], antifungal [21], anti-tubercular [22], and antidiabetic compounds [23], with recent studies even exploring their potential against SARS-CoV-2 [24] and HIV [25]. Their multi-target adaptability renders them highly effective candidates for addressing complex pathological conditions [26]. Nitrogen-containing heterocyclic compounds, such as phthalazine derivatives, have garnered significant interest in medicinal chemistry due to their ability to react with various biological targets [27]. In the modern era, computational chemistry and bioinformatics have become essential tools for the rational design and optimisation of pharmaceuticals. These in silico approaches provide predictive insights that significantly reduce the economic and temporal burdens associated with traditional experimental methods [28, 29]. Notably, molecular docking serves as a critical technique for elucidating the binding modalities between ligands and target receptors, thereby streamlining the identification of potent therapeutic agents [30, 31].

In the present study, a novel series of phthalazine-based scaffolds was synthesised starting from 2-(1-oxo-4-phenylphthalazin-2(1H)-yl)acetohydrazide. The structural integrity of the synthesised compounds was rigorously characterised using infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS). To further investigate their electronic properties and chemical stability, Density Functional Theory (DFT) calculations were employed to analyse HOMO-LUMO energy gaps and molecular electrostatic potential (MEP) surfaces. Additionally, molecular docking simulations were conducted to evaluate the binding affinity of compounds (2-6) against bacterial proteins 6TLS and 4OD9. Finally, ADME profiling was performed to assess the pharmacokinetic behaviour and drug-likeness of the target molecules.

Experimental

Materials and Methods

All reagents and solvents employed in this investigation were of analytical grade and procured from Aldrich Chemical Co. The specific chemical materials utilised were potassium hydroxide, dilute hydrochloric acid, carbon disulfide, anhydrous potassium carbonate, 2-chloro-N-phenylacetamide, methyl iodide, ethyl iodide, allyl bromide, dimethylformamide (DMF), methanol, and absolute ethanol.

Characterization Techniques

Melting points were measured using a Kofler Block apparatus and are presented without correction. Infrared (IR) spectra were recorded as KBr pellets on a Tensor 37 Bruker spectrophotometer within the 4000–400 cm^{-1} range at the Faculty of Science, Alexandria University. ^1H and ^{13}C NMR spectra were obtained at the same institution using a Bruker AC spectrometer at 500 MHz and 125 MHz, respectively, in DMSO-d_6 , with chemical shifts (Hz delta) reported in ppm relative to tetramethylsilane (TMS). Mass spectrometry (MS) was performed at 70 eV on a 5980 Series II GC coupled with a 5989 B spectrometer at the Faculty of Science, Cairo University. The progress of the reactions was monitored via thin-layer chromatography (TLC) using Merck Kieselgel 60-F254 plates, with spots visualised under UV illumination. All spectral data are found in the Supplementary data.

Synthesis of 5-[(1-Oxo-4-Phenylphthalazin-2(1H)-yl) Methyl]-2-(Mercapto)-1,3,4-Oxadiazole (2)

A mixture of 2-(1-oxo-4-phenylphthalazin-2(1H)-yl)acetohydrazide (1, 2.94g, 0.01mol) [32] and carbon disulfide (0.6 mL, 10 mmol) in methanolic potassium hydroxide (0.56 g KOH in 20 mL MeOH) was heated under reflux for 12 h. Upon completion, the reaction mixture was cooled and poured onto crushed ice (50 mL). The resulting solution was acidified with dilute HCl to pH 6, yielding a colourless precipitate. The crude product was collected by filtration and recrystallised from dimethylformamide (DMF) to afford the title compound (2) as colourless needles (2.9g, 86% yield). m.p. 238–241 °C; FT-IR (KBr) (cm^{-1}): 3055 (CH aromatic), 2974 (CH aliphatic), 1615 (CO, amide group of phthalazine ring) and 1570 cm^{-1} (C=N); ^1H NMR (DMSO-d_6): δ H = 5.50 (s, 2H, NCH_2), 7.52–8.34 (m, 9H, aromatic-H) and 14.66 (s, 1H, exchangeable by D_2O SH). ^{13}C NMR (DMSO-d_6 , 125 MHz, ppm) δ 46.2, 127.1, 127.5, 127.6, 129.1, 129.9, 132.9, 134.6, 147.8, 158.6, 160.0 and 178.4. MS (m/z, %): 336 (M^+ , 5%), 335 ($\text{M}^+ - \text{H}$, 26%), 262 ($\text{M}^+ - \text{CH}_2\text{N}_2\text{S}$, 53%) and 207 ($\text{M}^+ - \text{C}_3\text{H}_3\text{N}_3\text{OS}$, 100%).

Reactions Of 5-[(1-Oxo-4-Phenylphthalazin-2(1H)-yl) Methyl]-2-(Mercapto)-1,3,4-Oxadiazole

5-[(1-oxo-4-phenylphthalazin-2(1H)-yl) methyl]-2-(methylthio)-1,3,4-oxadiazole (3)

A mixture of oxadiazole phthalazine derivative (2, 2.9g, 0.01 mol) and potassium hydroxide (0.56 g, 0.01 mol) in dimethylformamide (10 mL) was stirred at room temperature. To this solution, dimethyl sulfate (0.73 mL, 0.01 mol) was added dropwise. The reaction was maintained under constant stirring for 12 hours. Upon completion, the mixture was quenched by pouring it onto crushed ice. The resulting precipitate was collected by filtration, washed thoroughly with distilled water, and subsequently recrystallised from methanol to afford compound (3) as white needles (3.1g, 89 % yield); m. p. 174–176 °C; FT-IR (KBr) (cm^{-1}): 3058 (CH aromatic), 3030 (CH aliphatic), 1660 (CO, amide group of phthalazine ring) and 1583 cm^{-1} (C=N); ^1H NMR (DMSO-d_6): δ H = 2.66 (s, 3H, $-\text{SCH}_3$), 5.63 (s, 2H, $-\text{NCH}_2$), and 7.54–7.94 (m, 9H, aromatic-H). ^{13}C NMR (DMSO-d_6 , 125 MHz, ppm) δ 14.2, 45.4, 126.6, 126.9, 127.1, 128.6, 129.3, 132.3, 134.1, 134.2, 147.1, 158.0, 163.4 and 165.0. MS (m/z, %): 350 (M^+ , 5%), 349 ($\text{M}^+ - \text{H}$, 17%), 235 ($\text{M}^+ - \text{C}_3\text{H}_3\text{N}_2\text{OS}$, 13%), 207 ($\text{M}^+ - \text{C}_4\text{H}_5\text{N}_3\text{OS}$, 12%), 129 ($\text{M}^+ - \text{C}_{14}\text{H}_9\text{N}_2\text{O}$, 35%) and 76 ($\text{M}^+ - \text{C}_{12}\text{H}_{10}\text{N}_4\text{O}_2\text{S}$, 40%).

5-[(1-oxo-4-phenylphthalazin-2(1H)-yl) methyl]-2-(ethylthio)-1,3,4-oxadiazole (4)

To a solution of oxadiazole phthalazine derivative (2, 3.36 g, 0.01 mol) in DMF (20 mL), anhydrous potassium carbonate (1.3 g, 0.01 mol) and ethyl iodide (0.80 mL, 0.01 mol) were added. The reaction mixture was stirred at ambient temperature for 6 h. Upon completion, the mixture was poured onto crushed ice, and the resulting precipitate was filtered, washed thoroughly with water, and dried. Recrystallization from ethanol afforded compound (4) as colorless needles (3.0g, 83% yield); m.p. 126–128 °C; FT-IR (KBr) (cm^{-1}): 3064 (CH aromatic), 2971 (CH aliphatic), 1662 (CO, amide group of phthalazine ring) and 1582 cm^{-1} (C=N); ^1H NMR (DMSO-d_6): δ H 1.32 (t, 3H, $-\text{CH}_2\text{CH}_3$), 3.20 (q, 2H, $-\text{CH}_2\text{CH}_3$), 5.63 (s, 2H, CH_2), 7.55–8.37 (m, 9H, aromatic-H). ^{13}C NMR (DMSO-d_6 , 125 MHz, ppm) δ 14.7, 26.6, 45.5, 126.6, 126.9, 127.1, 128.6, 129.3, 132.3, 134.1, 134.2, 147.1, 158.0, 163.4 and 164.1. MS (m/z, %): 364 (M^+ , 4%), 363 ($\text{M}^+ - \text{H}$, 32%), 349 ($\text{M}^+ - \text{CH}_3$, 8%), 302 ($\text{M}^+ - \text{C}_2\text{H}_6\text{S}$, 48%), 235 ($\text{M}^+ - \text{C}_4\text{H}_5\text{N}_2\text{OS}$, 8%), 234 ($\text{M}^+ - \text{C}_4\text{H}_6\text{N}_2\text{OS}$, 54%), 207 ($\text{M}^+ - \text{C}_5\text{H}_7\text{N}_3\text{OS}$, 100%) and 129 ($\text{M}^+ - \text{C}_{15}\text{H}_{11}\text{N}_2\text{O}$, 64%).

5-[(1-Oxo-4-Phenylphthalazin-2(1H)-yl) Methyl]-2-(Allylthio)-1,3,4-Oxadiazole (5)

To a solution of oxadiazole phthalazine derivative (2, 3.36g, 0.01mol) in dry dimethylformamide (20 mL), anhydrous potassium hydroxide (0.56g, 0.01mol) and allyl bromide (0.86 mL, 0.01mol) was stirred at room temperature for 8 hrs. the solid mass poured onto crushed ice and the product was filtered, washed well with water, dried and crystallized from

ethanol yielded the titled compound (5) as colorless needles, (3.3g, 87% yield); m.p. 240-242 °C; FT-IR (KBr) (cm⁻¹): 3059 (CH aromatic), 3026(CH aliphatic), 1663 (CO, amide group of phthalazine ring) and 1583 cm⁻¹ (C=N); ¹H NMR (DMSO-d₆): δ 3.80 (d, 2H, -SCH₂), 4.99(d, 1H, -CH=CHS), 5.17 (d, 1H, -CH=CH₂cis), 5.60(s, 2H, -CH=CH₂ trans), 5.83(s, 1H, NCH₂-oxadiazole), 7.52-8.34(m, 9H, aromatic-H). ¹³C NMR (DMSO-d₆, 125 MHz, ppm) δ 35.1 9(SCH-oxadiazole), 45.9 (NCH-), 119.8 (CH=CHS), 127.1, 127.5, 127.7, 129.1, 129.9, 132.9, 133.0, 134.6, 134.7 (Ar-C), 147.6 (CH=CHS), 158.6 (C=N phthalazine), 164.0 (CO amide) and 164.2 (C-2, C5 oxadiazole). MS (m/z, %):376(M⁺,33%), 375(M⁺, -H, 100%), 260(M⁺-C₉H₁₀+1Hcap, 35%), 234(M⁺-C₅H₆N₂OS, 57%), 208(M⁺-C₆H₇N₃OS, 16%) and 129(M⁺-C₁₆H₁₂N₂O+Hcap, 34%).

Ethyl 2-((5-((1-Oxo-4-Phenylphthalazin-2(1H)-Yl) Methyl)-1,3,4-Oxadiazol-2-Yl) Thio) Acetate (6)

To the solution of oxadiazole phthalazine derivative (2, 3.3g, 0.01 mol) and anhydrous potassium carbonate (1.3g, 0.01 mol) in dry DMF (20ml), ethyl chloroacetate (1.06 ml, 0.01 mol) was added. The mixture was stirred at room temperature for 6 h, then poured into ice-cold water. The crude product was filtered, washed with water, and purified by recrystallization from ethanol to give compound (6) as colorless needles, (3.5g, 83% yield); m.p. 108-110 °C; FT-IR (KBr) (cm⁻¹): 3060 (CH aromatic), 3018(CH aliphatic), 1742 (CO, ester), 1646 cm⁻¹ (CO, amide group of phthalazine ring) and 1581 cm⁻¹ (C=N); ¹H NMR (DMSO-d₆): δ H 1.25 (t, 3H, -CH₂CH₃), 4.03 (s, 2H, -SCH₂), 4.20 (q, 2H, CH₂CH₃), 5.67(s, 2H, -NCH₂), 7.26-8.54 (m, 9H, aromatic-H). ¹³C NMR (DMSO-d₆, 125 MHz, ppm) δ 13.8, 33.7, 45.4, 61.5, 126.5, 126.9, 127.1, 128.5, 129.3, 129.4, 132.3, 134.0, 134.1, 147.1, 158.0, 163.4, 163.6 and 167.4. MS (m/z, %):422(M⁺, 11%), 421(M⁺-H, 44%), 303 (M⁺-C₄H₇O₂S, 22%), 235(M⁺-C₆H₇N₂O₃S, 19%), 235(M⁺-C₆H₇N₂O₃S, 19%), 207(M⁺-C₇H₉N₃O₃S, 100%) and 77 (M⁺-C₁₅H₁₃N₄O₄S, 45%).

DFT Calculation

Density Functional Theory (DFT) was employed as a robust in silico quantum mechanical approach to investigate the electronic structure and chemical reactivity of the synthesised compounds [33]. DFT offers an optimal balance between computational efficiency and chemical accuracy, making it a widely adopted method for determining molecular geometries, electronic spectra, and reaction pathways. In this study, geometry optimisations were performed using the B3LYP functional [34-37], in conjunction with the 6-311++G (d, p) basis set. All computational simulations were executed using the Gaussian 09 software package, while GaussView 05 was utilised for the visualisation and interpretation of the results [38]. The molecular and spectroscopic properties of the investigated compounds (2-6) were evaluated based on their fully optimised geometries. To ensure that the optimised structures correspond to true local minima on the potential energy surface, vibrational frequency analyses were conducted. The absence of imaginary frequencies confirmed the stability of the stationary points. Furthermore, several global reactivity descriptors were calculated based on the energies of the Highest Occupied Molecular Orbital E_{HOMO} and the Lowest Unoccupied Molecular Orbital ELUMO, according to the following equations:

$\Delta E = E_{LUMO} - E_{HOMO}$	(1) Energy Gap
$\chi = -(E_{HOMO} + E_{LUMO}) / 2$	(2) Absolute Electronegativity
$\eta = (E_{LUMO} - E_{HOMO}) / 2$	(3) Absolute Hardness
$\sigma = 1/\eta$	(4) Absolute Softness
$\mu = -\chi$	(5) Chemical Potentials
$S = 1/2\eta$	(6) Global Softness
$\omega = \mu/22\eta$	(7) Global Electrophilicity
$\Delta N = -\mu/\eta$	(8) Additional Electronic Charge

Molecular Docking

Molecular docking investigations were conducted to identify the optimal binding orientations and affinities of phthalazine derivatives within the active sites of target proteins (PDB IDs: 6TLS and 4OD9). To ensure an unbiased exploration of all potential binding cavities and structural interstices, a blind docking approach was implemented using MOE 2019 software [28]. The docking algorithms evaluated binding energy by integrating parameters such as molecular geometry, atomic configuration, and conformational internal energy. The most stable ligand-protein complexes were identified based on the lowest binding energy values, which signify the highest thermodynamic stability within the catalytic pocket [39].

Predicting ADME Properties

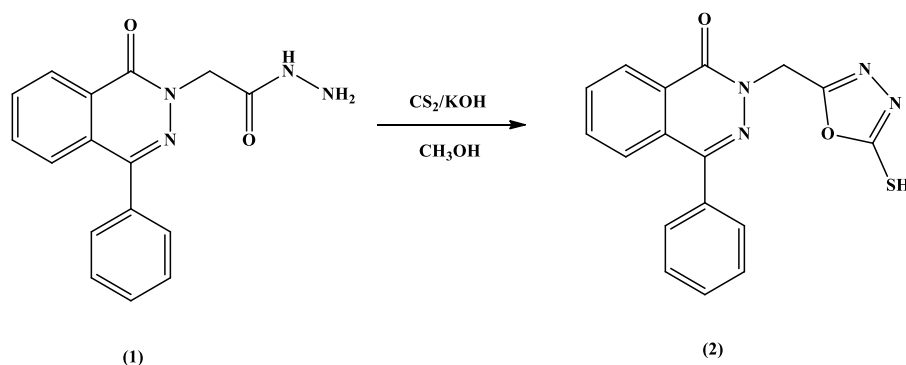
In contemporary drug discovery, computational methodologies serve as a pivotal preliminary phase, facilitating the assessment of candidate compounds before chemical synthesis or biological evaluation. This approach is particularly essential for characterising pharmacokinetic profiles, potential toxicity, and overall bioavailability [40]. The frequent disparity between in vitro observations and in vivo outcomes often underscores the high attrition rates of drug candidates. Such failures are typically attributed to suboptimal bioavailability, stemming from unfavourable structural attributes or poor pharmacokinetic properties. Furthermore, the inability to achieve therapeutic efficacy is frequently linked to adverse side

effects or unintended biological interactions. Therefore, to evaluate the therapeutic potential of the synthesised compounds, their ADME (Absorption, Distribution, Metabolism, and Excretion) parameters were analysed using the SwissADME platform. This computational tool provides critical insights into the pharmacokinetic and pharmacodynamic properties essential for the drug development process [41].

Results and Discussion

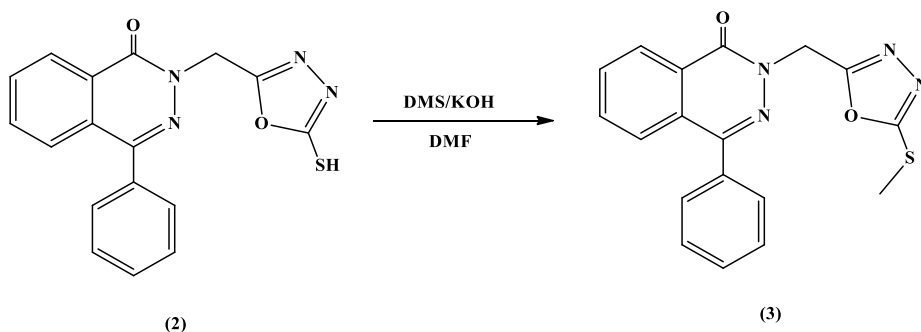
Chemistry

Thiol oxadiazole phthalazine (2) was obtained from the reaction of 2-(1-oxo-4-phenylphthalazin-2(1H)-yl)acetohydrazide (1) [32] with carbon disulfide in the presence of methanolic potassium hydroxide, yielding the key compound (2) in 86% yield (Scheme 1). The structure of (2) was deduced from its spectral analysis. Its IR spectrum (2) showed the disappearance of the carbonyl amide absorption. Its ^1H NMR spectrum revealed the absence of the NHNH_2 signal and the presence of the SH as a single signal at δH 14.66 in addition to a singlet at δH 5.50 ppm for the methylene bridge (NCH_2) group. Moreover, it showed multiple signals at δH 7.52- 8.34 (9H) for phenyl protons. The ^{13}C NMR spectrum of (2) exhibited several signals for aromatic carbons as well as a bridge methylene signal and 1 carbonyl signal at 46.2 and 158.6, respectively. The structure of (2) was also confirmed by its MS spectra, which showed a fragmentation process involving a sequential elimination of the $\text{C}_3\text{H}_3\text{N}_3\text{OS}$ molecule to give the most stable one as a base peak.



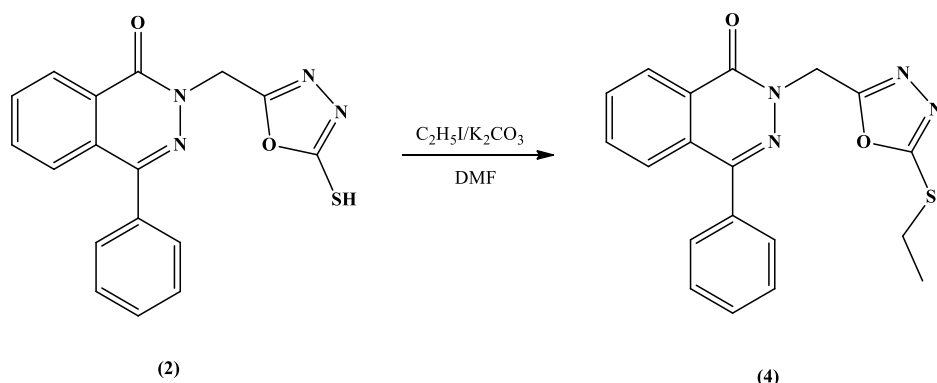
Scheme 1. 5-[(1-oxo-4-phenylphthalazin-2(1H)-yl) methyl]-2-(mercapto)-1,3,4-oxadiazole.

Methylation (2) with dimethylsulfoxide in dimethylformamide and potassium hydroxide afforded the corresponding methylthio oxadiazole derivative (3) (Scheme 2). Its ^1H NMR spectrum revealed the absence of the SH signal, as well as a new characteristic singlet at δH 2.66 ppm for the protons ($-\text{SCH}_3$). The ^{13}C NMR spectrum of (3) exhibited a signal for methyl at 14.2. Its mass spectrum exhibited the molecular ion peak at m/z 350.



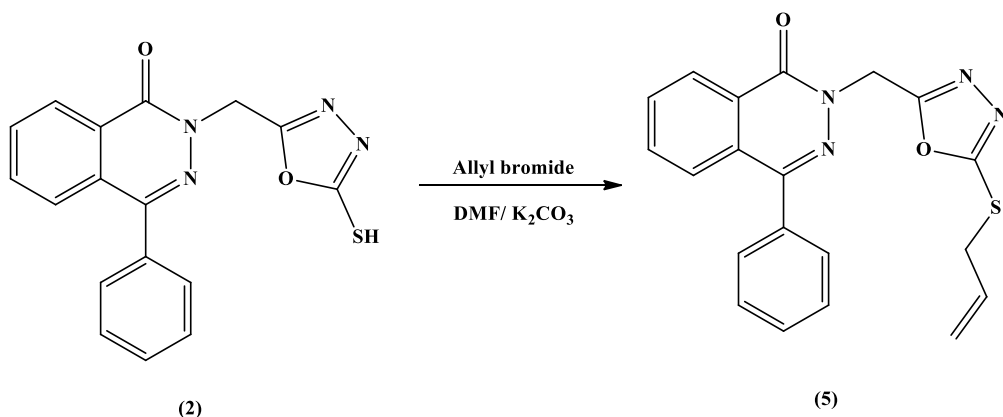
Scheme 2. 5-[(1-oxo-4-phenylphthalazin-2(1H)-yl) methyl]-2-(methylthio)-1,3,4-oxadiazole.

Stirring of (2) with ethyl iodide in ethanol afforded ethylthio oxadiazole phthalazine (Scheme 3). Its ^1H NMR spectrum showed new characteristic signals at δH 1.32 and 3.20 ppm for the protons ($-\text{SC}_2\text{H}_5$), respectively. The ^{13}C NMR spectrum of (4) showed the expected number of signals for the ethyl signals at 14.7 and 26.6, respectively. Its mass spectrum showed a peak corresponding to the molecular ion at m/z 364.



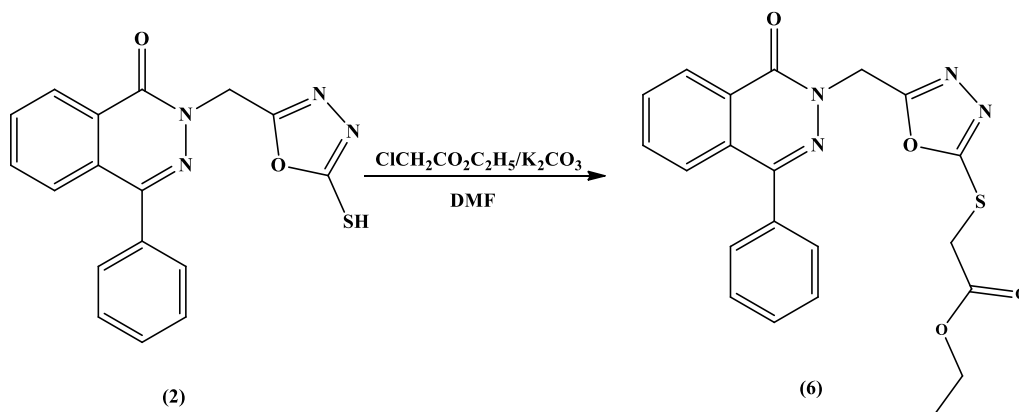
Scheme 3. 5-[(1-oxo-4-phenylphthalazin-2(1H)-yl) methyl]-2-(ethylthio)-1,3,4-oxadiazole.

Treatment of (2) with allyl bromide in DMF afforded allylthio oxadiazol phthalazine (5) (Scheme 4). Its ^1H NMR spectrum showed new characteristic signals at δ_{H} 3.80, 4.99, 5.60, 5.83 ppm for the protons ($\text{CH}_2\text{CH}_2\text{S}$), respectively. The ^{13}C NMR spectrum of (5) showed signals for ($\text{CH}_2\text{CH}_2\text{S}$) at 35.1, 119.8 and 133.0. Its mass spectrum shows the molecular ion peak at m/z 376.



Scheme 4. 5-[(1-oxo-4-phenylphthalazin-2(1H)-yl) methyl]-2-(allylthio)-1,3,4-oxadiazole

Finally, stirring of (2) with ethyl chloroacetate yielded the ester derivative (6) (Scheme 5). Its IR spectrum showed an absorption band at 1742 for the ester group. Also, its ^1H NMR spectrum revealed the absence of the SH signal and the presence of the ethyl ester group as a triplet at δ_{H} 1.25 ppm (CH_3) and a quartet at δ_{H} 4.20 ppm (CH_2), in addition to a singlet at δ_{H} 4.03 ppm for the (SCH_2) group. The ^{13}C NMR spectrum of (6) showed several ethyl signals at 13.8 and 33.7 and a further methylene signal at 61.5, in addition to a signal for the ester carbon at 167.4. Its mass spectrum shows the molecular ion peak at m/z 422.



Scheme 5. ethyl 2-[(5-[(1-oxo-4-phenylphthalazin-2(1H)-yl) methyl]-1,3,4-oxadiazol-2-yl) thio] acetate.

Computational Study

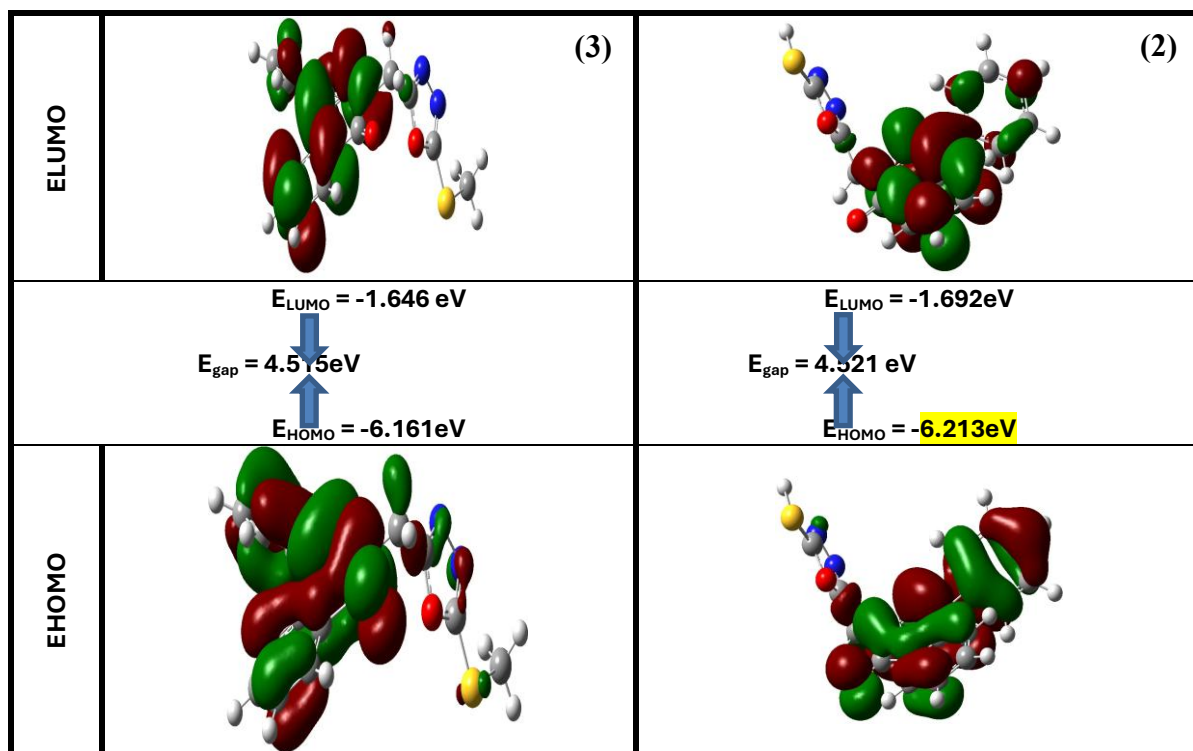
DFT Calculation

Frontier Molecular Orbital (FMO) Analysis

The electronic structures of the studied compounds (2, 3, 4, 5, and 6) were investigated using DFT calculations at the B3LYP/6-311++G (d, p) level. The HOMO and LUMO energies and derived quantum chemical parameters are summarised in Table 1. The HOMO-LUMO gap (ΔE) serves as an indicator of chemical reactivity and kinetic stability of the molecules. All compounds exhibit moderate HOMO-LUMO gaps (4.503–4.521 eV), indicating a balance between chemical stability and reactivity. Compound (4) has the smallest gap (4.503 eV), suggesting slightly higher reactivity, whereas (2) shows the largest gap (4.521 eV), reflecting higher kinetic stability. Electronegativity (χ) and hardness (η) values are comparable across the series, confirming similar electron-attracting tendencies and resistance to electronic deformation. Softness (σ) values (~ 0.442 – 0.444 eV⁻¹) indicate a consistent reactivity profile, while the electrophilicity index (ω) is highest for (4) (3.492 eV), correlating with its increased susceptibility to nucleophilic attack. ΔN_{max} values further support this, with (4) showing the largest charge-transfer capacity, as shown in (Fig. 1).

Table1. Quantum parameters of study compounds (2-6)

Compound	2	3	4	5	6
HOMO	-6.213	-6.161	-6.217	-6.209	-6.156
LUMO	-1.692	-1.646	-1.714	-1.697	-1.643
Gap	4.521	4.515	4.503	4.512	4.513
χ	3.953	3.904	3.966	3.953	3.900
η	2.261	2.258	2.252	2.256	2.257
σ	0.442	0.443	0.444	0.443	0.443
Pi	-3.953	-3.904	-3.966	-3.953	-3.900
S	0.221	0.221	0.222	0.222	0.222
ω	3.455	3.375	3.492	3.463	3.369
ΔN_{max}	1.749	1.729	1.761	1.752	1.728



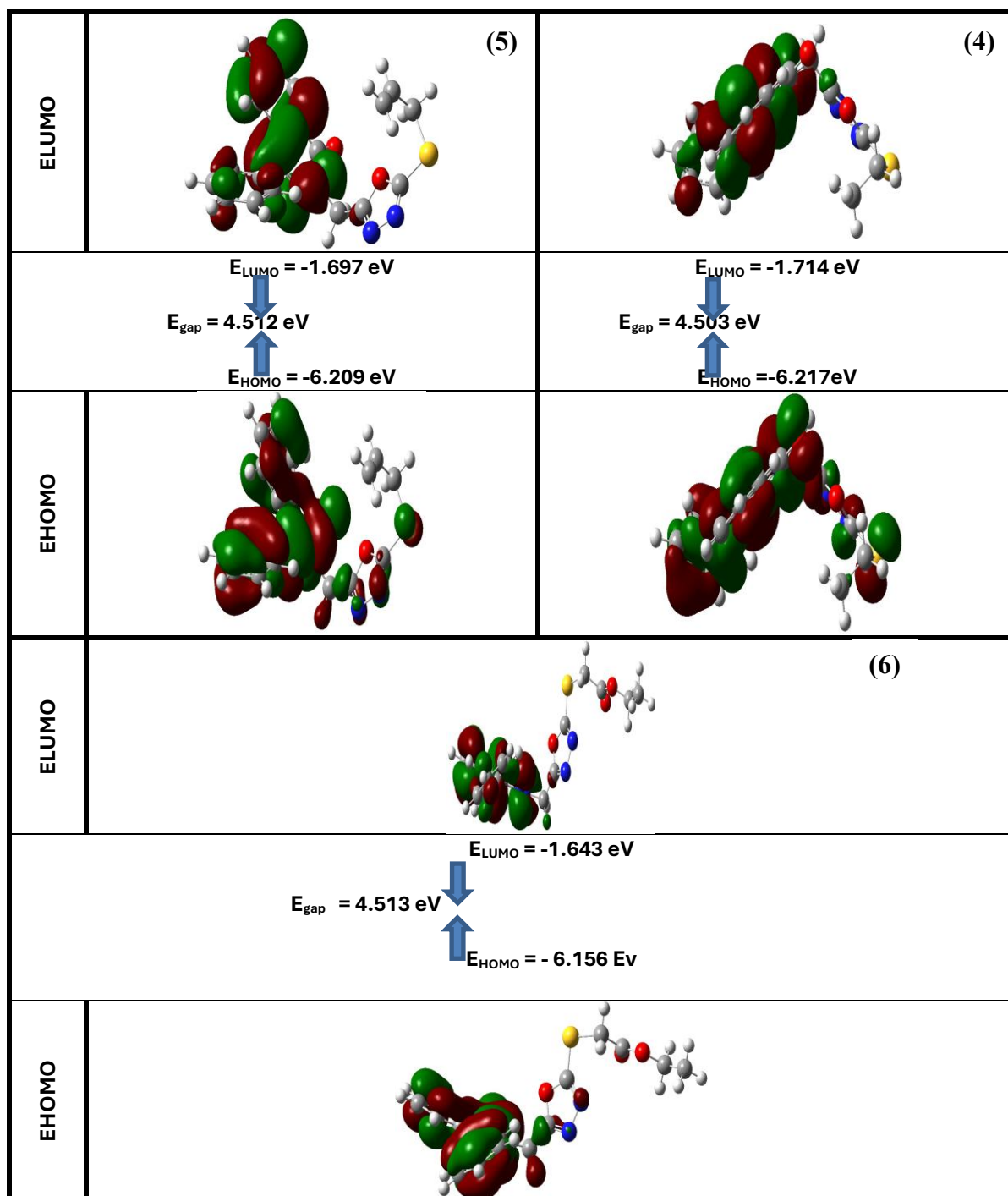


Fig. 1. HOMO and LUMO surface plots of compounds (2- 6) with their corresponding HOMO–LUMO energy gaps (ΔE , in eV).

Electronic Potential Surface Analysis

The Molecular Electrostatic Potential (MESP) surfaces of compounds (2,3,4,5 and 6) were analysed to delineate the nucleophilic (electron-rich) and electrophilic (electron-poor) areas (Fig. 2) [29, 42]. Molecular Electrostatic Potential (MESP) maps reveal that electron-rich regions (red) are localised on the oxadiazole ring and sulfur substituents, identifying potential nucleophilic sites. In contrast, electron-deficient regions (blue) are concentrated over the phthalazinone cores, highlighting electrophilic sites. The HOMO orbitals are mainly distributed over the oxadiazole-thio moieties, while LUMO orbitals are localised on the aromatic cores, in agreement with the MESP patterns and supporting the observed electronic behaviour. In summary, (4) is the most reactive among the studied compounds, followed by (5 and 6), while (2) is the most stable. The combined FMO and MESP analyses provide a coherent picture of reactivity trends and potential active sites, offering valuable insights for further molecular design.

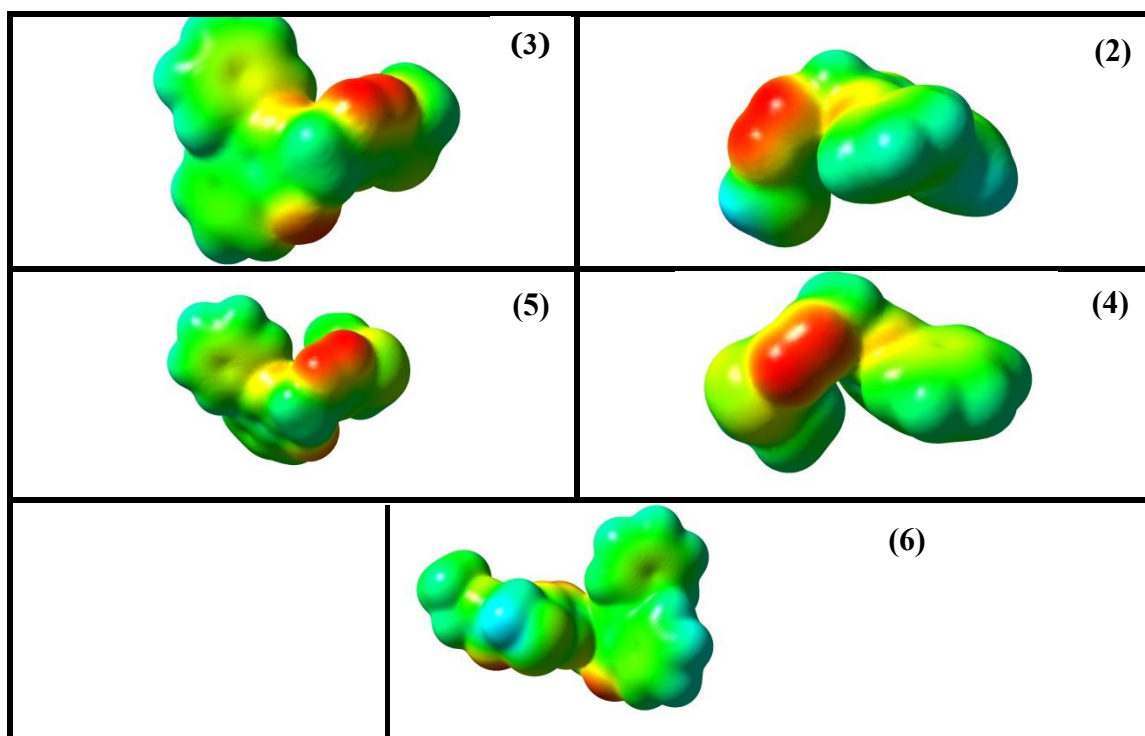


Fig. 2. Electrostatic potential (ESP) surfaces of compounds (2-6)

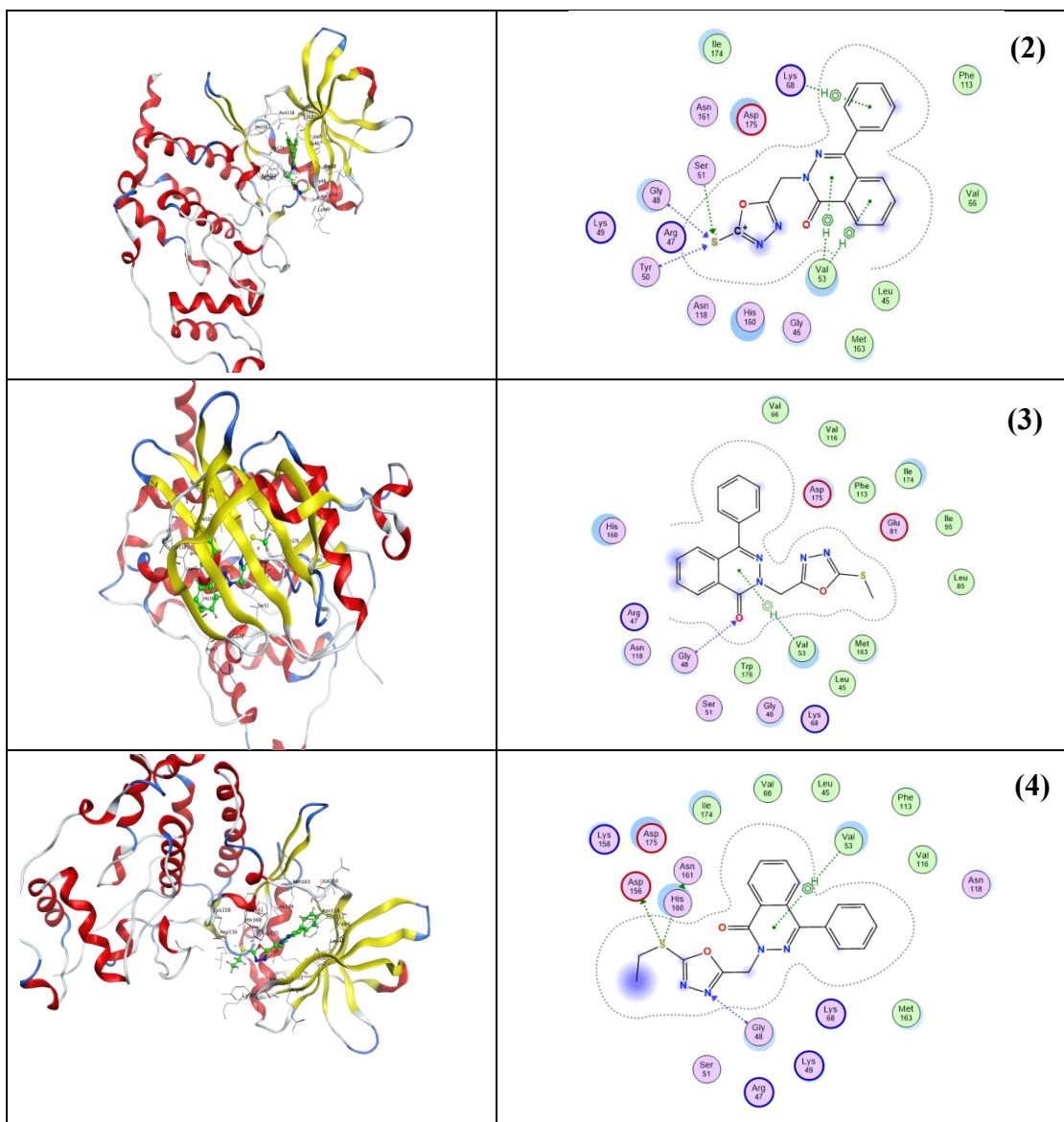
Molecular Docking Studies

The molecular docking analysis of the synthesised compounds against the 6TLS and 4OD9 receptors demonstrated marked variations in binding affinity and stability (Table 2 and Table S1). Compounds (6 and 3) showed the most favourable binding energies toward the 6TLS receptor (-7.92 and -7.66 kcal/mol, respectively), accompanied by low RMSD values, which indicate excellent geometric complementarity and stable accommodation within the active site. For compound (6), the predominant interactions included hydrogen bond donor and acceptor interactions with SER51, in addition to π -H and π -cation interactions involving VAL66 and VAL53. These interactions collectively enhanced ligand stabilisation within the binding pocket (Fig. 3 and Fig. S1). The intermolecular distances between the ligand and the interacting amino acid residues ranged from 2.73 to 4.26 Å, which are consistent with stable noncovalent interactions without inducing significant conformational distortion. Regarding the 4OD9 receptor, compounds (6 and 5) exhibited the most favourable docking scores. The ester functionality of compound 5001 formed strong hydrogen-bond acceptor interactions with ASP33 and PHE32, resulting in a highly negative binding energy (-7.43 kcal/mol), while compound (5) displayed a binding energy of -7.17 kcal/mol, further supporting stable complex formation. Additional π -H interactions with aromatic residues such as VAL30 and TRP40 contributed to the overall stabilisation of the ligand-receptor complex. These results suggest that specific structural modifications, including hydroxyl, methyl, and aromatic ring substitutions, play a critical role in strengthening interactions with key amino acid residues such as LYS, MET, and VAL. Accordingly, compounds (6 and 3) (against 6TLS) and compound (6) (against 4OD9) can be considered promising candidates for further in vitro and in vivo investigations, as their ability to establish multiple stabilising interactions may correlate with enhanced biological activity.

Table 2. Molecular docking energy scores of 6TLS and compounds studied

Compounds	Docking score(kcal/mol)	RMSD(Å)	Receptor interaction	Distance	Energy (kcal/mol)
2	-6.74	1.50	GLY48/H-acceptor	3.63	-3.0
			TYR50/H-acceptor	3.3	-7.7
			TYR50/H-acceptor	3.94	-0.9
			TYR50/H-acceptor	3.72	-0.9
			SER51/H-acceptor	3.5	-3.7
			VAL53/pi-H	4.21	-0.7
			VAL53/pi-H	4.26	-0.5

			LYS68/pi-H	4.11	-0.6
3	-7.66	2.37	GLY48/H-acceptor	3.67	-0.7
			VAL53/pi-H	3.75	-0.8
4	-6.96	1.52	ASP156/H-donor	3.33	-4.6
			ASN161/H-donor	3.54	-1.1
			GLY48/H-acceptor	3.31	-1.3
			VAL53/pi-H	3.87	-0.7
5	-7.19	1.38	ASN161/H-donor	3.86	-0.5
			LYS68/H-acceptor	2.73	-6
			LYS158/H-acceptor	3.52	-0.5
			MET163/pi-H	3.51	-0.5
			ILE174/pi-H	3.98	-0.8
6			ILE174/pi-H	3.58	-0.5
	-7.92	1.47	SER51/H-donor	2.99	-1.4
			SER51/H-acceptor	3.08	-1.6
			VAL66/pi-H	4.1	-0.8
			VAL53/pi-H	3.99	-0.6



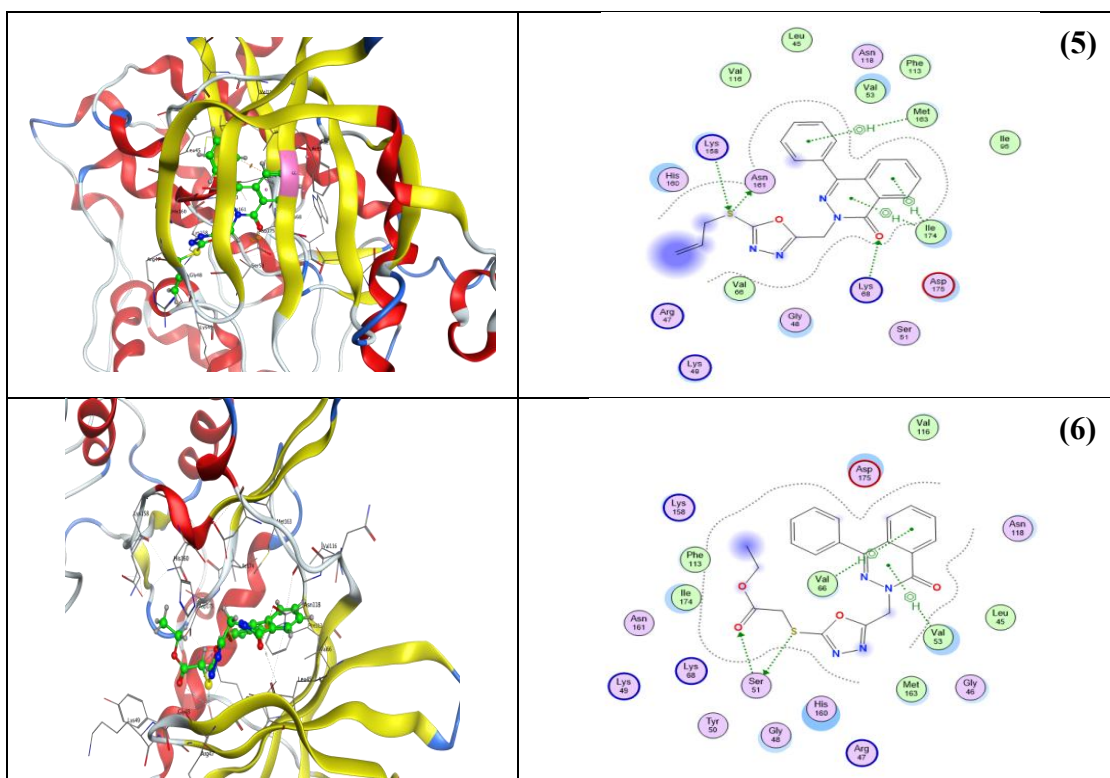


Fig. 3. The molecular docking of compounds (2-6) with protein 6TLS; (a) 3D and b) 2D

ADME Predictions

The in silico ADMET evaluation of compounds (2–6) revealed distinct pharmacokinetic and toxicological patterns correlated with their structural features [28]. Molecular weights ranged from 334–422 Da, with TPSA values of 73.8–111.9 Å², indicating sufficient polarity for oral absorption while maintaining moderate lipophilicity (logP 1.6–3.6) (Fig. 4 and Table S2). Compound (6) showed the highest flexibility (0.333) and esterification, enhancing its predicted intestinal absorption (F30% = 0.098) but also increasing reactive alerts [43]. All compounds satisfied Lipinski's criteria, supporting drug-likeness [44]. Variations in Pfizer and GSK rules highlighted that (4, 5 and 6) deviate slightly from optimal physicochemical limits [45]. High plasma protein binding (>95%) was observed across the series, whereas BBB penetration varied, with (3) showing the highest CNS exposure (0.506) and (5) the lowest (0.049), reflecting the impact of bulky or polar substituents [46]. Metabolic predictions indicated strong inhibition potential for CYP2C9/2C19 across all derivatives, with variable CYP3A4 inhibition (0.197–0.891), suggesting possible metabolic interactions. Clearance and half-life values were generally low, consistent with rapid systemic elimination [47]. Toxicity assessments showed low hERG inhibition (<0.2), moderate hepatotoxicity and DILI risk, and minor genotoxic alerts, particularly in (6) [48]. Structural variations, such as thioalkyl and ester substituents, correlated with subtle increases in skin sensitisation and AMES toxicity. Environmental toxicity predictions were minimal, indicating low bioaccumulation potential. Overall, compounds (2, 3 and 4) combined favourable absorption, distribution, and low toxicity, representing the most promising pharmacokinetic profiles, while (6), despite higher oral bioavailability, exhibited increased flexibility and reactive alerts, warranting further optimisation [49].

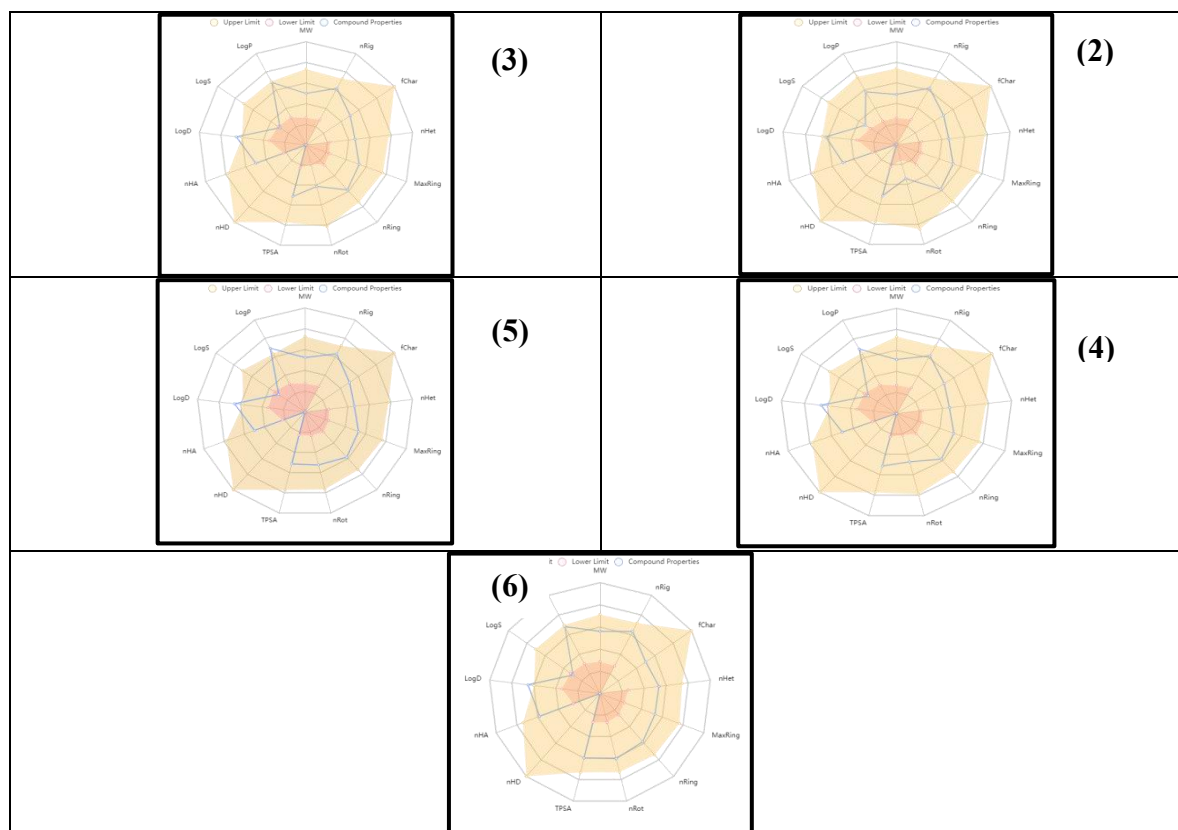


Fig.4. Bioavailability illustrations of the phthalazine derivatives.

Conclusion

In this study, five phthalazine-containing compounds were successfully synthesised and characterised by FT-IR, ^1H NMR, ^{13}C NMR, and mass spectroscopy. Further, the DFT calculations demonstrated that all compounds possess moderate energy gaps (4.503–4.521 eV), indicating a favourable balance between kinetic stability and chemical reactivity. Compound (4) exhibited the lowest energy gap and the highest electrophilicity index and charge transfer capacity, marking it as the most reactive member of the series toward nucleophilic attacks. Conversely, compound (2) showed the highest stability due to its larger energy gap. Overall, the close similarity in electronegativity and hardness values across the series suggests that these compounds share comparable electronic demands and resistance to deformation, providing a consistent baseline for further property optimisation in future applications. In addition, molecular docking results revealed that compounds (6 and 3) are the most promising candidates. Specifically, compound (6) demonstrated superior binding affinities toward both 6TLS (–7.92 kcal/mol) and 4OD9 (–7.43 kcal/mol) receptors, stabilised by a robust network of hydrogen bonds and π interactions with key residues such as SER51, ASP33, and PHE32. The low RMSD values and optimal intermolecular distances (2.73–4.26 Å) confirm the structural stability of these complexes. These results highlight the critical role of specific functional substitutions such as hydroxyl, methyl, and ester groups in enhancing ligand-receptor complementarity. Consequently, compounds (6 and 3) emerge as strong leads for further in vitro and in vivo pharmacological evaluations, potentially serving as a basis for developing a highly effective therapeutic agent. Finally, drug-likeness predictions for all synthesised compounds were conducted using SwissADME.

Acknowledgements

All authors express their sincere gratitude to Dr Saleh Bufarwa saleh.bufarwa@omu.edu.ly, Chemistry Department, Faculty of Science, Omar Al-Mukhtar University, El-Beida, Libya, for providing the characterisation facilities and support to complete this work.

Disclosure of Interest

The authors report there are no competing interests to declare.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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